CHEMICAL PROBES FOR HISTONE LYSINE DEMETHYLYASES

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of the

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This thesis is dedicated to my father, Dr Heiko Gerken. Thank you for your encouragement, your sense of humor, and for providing me with the best possible opportunities in life.
DECLARATION

This dissertation describes work carried out in the Chemistry Research Laboratory and the Target Discovery Institute (Nuffield Department of Medicine) at the University of Oxford between October 2013 and December 2016. The dissertation is the product of my own work. It includes results that were obtained through collaboration, which are specifically indicated in the text.

Philip A. Gerken
ACKNOWLEDGEMENTS

In the words of perhaps the most eminent Austro-American philosopher of all time:

Life is continuously being hungry. The meaning of life is not simply to exist, to survive, but to go ahead, to go up, to achieve, to conquer.*

I feel incredibly privileged to have spent the last three years under the supervision of Prof. Martin D. Smith, who has continuously encouraged me to be “hungry” and strive towards ambitious goals. I am tremendously grateful for his advice, insights, and for all the knowledge and skills that I have acquired during the time I have spent working in his research group.

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* Arnold Schwarzenegger on the meaning of life.
proteins required for this project (I am sorry I still have no idea who you are) and the mass spectrometry staff for maintaining the protein MS equipment. Within the CRL, I am grateful to the NMR and MS support staff for their analytical assistance and to Dr Barbara Odell in particular for running so many variable-temperature NMR experiments.

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My family has always been incredibly supportive and keen to glean some information from me regarding the nature of my work. I hope you will find this thesis enlightening! Mom, whatever it is, please don’t worry. Dad, it’s never too late to learn how to use an iphone. Laura and Mona, you definitely deserve better birthday and Christmas presents from me.

My final acknowledgement goes to my girlfriend Rebecca. Thank you for putting up with my constant tardiness, my lack of social grace, and my appalling taste in music. You are wonderful, and I look forward to our many adventures together.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>2-OG</td>
<td>2-oxoglutarate</td>
</tr>
<tr>
<td>3-D</td>
<td>three-dimensional</td>
</tr>
<tr>
<td>Å</td>
<td>Ångstöms</td>
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<tr>
<td>Ac</td>
<td>acetyl</td>
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<tr>
<td>aq.</td>
<td>aqueous</td>
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<tr>
<td>Ar</td>
<td>denotes aromatic system</td>
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<tr>
<td>Asp</td>
<td>aspartic acid</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>Bu</td>
<td>n-butyl</td>
</tr>
<tr>
<td>c</td>
<td>centi-</td>
</tr>
<tr>
<td>cat.</td>
<td>catalyst</td>
</tr>
<tr>
<td>COSY</td>
<td>correlation spectroscopy</td>
</tr>
<tr>
<td>cyc</td>
<td>cyclohexyl</td>
</tr>
<tr>
<td>d</td>
<td>deci-</td>
</tr>
<tr>
<td>d.r.</td>
<td>diastereomeric ratio</td>
</tr>
<tr>
<td>dba</td>
<td>dibenzylideneacetone</td>
</tr>
<tr>
<td>DIPEA</td>
<td>diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>EIC</td>
<td>extracted ion chromatogram</td>
</tr>
<tr>
<td>e.r.</td>
<td>enantiomeric ratio</td>
</tr>
<tr>
<td>eq.</td>
<td>equivalents</td>
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<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>f</td>
<td>frequency</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
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<td>g</td>
<td>grams</td>
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</table>
Glu  glutamic acid
h  hours
h  Planck’s constant
H  histone
HMBC  heteronuclear multiple-bond correlation spectroscopy
HMDS  hexamethyldisilazide
HPLC  high-performance liquid chromatography
HRMS  high-resolution mass spectrometry
HSQC  heteronuclear single-quantum correlation spectroscopy
Hz  hertz
i  iso
IC_{50}  inhibitor concentration corresponding to 50% of the maximal response
IR  infra-red
IUPAC  International Union of Pure and Applied Chemistry
JmjC  Jumanji-C
k  kilo-
K  Kelvin
K  lysine
KDM  histone lysine demethylase
K_M  Michaelis-Menten constant
L  liters
LDA  lithium diisopropylamide
LRMS  low-resolution mass spectrometry
Lys  lysine
m  milli-
M  moles per liter
m  meta-
m  meters
Me  methyl
min  minutes
mol  moles
MP  melting point
MS  mass spectrometry
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>MS/MS</td>
<td>tandem mass spectrometry</td>
</tr>
<tr>
<td>n</td>
<td>denotes generic integer</td>
</tr>
<tr>
<td>n</td>
<td>nano-</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NMP</td>
<td>1-methyl-2-pyrrolidinone</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>nOe</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>o</td>
<td>ortho-</td>
</tr>
<tr>
<td>p</td>
<td>para-</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>Pr</td>
<td>n-propyl</td>
</tr>
<tr>
<td>PTC</td>
<td>phase-transfer catalysis</td>
</tr>
<tr>
<td>Py</td>
<td>pyridyl</td>
</tr>
<tr>
<td>quant.</td>
<td>quantitative</td>
</tr>
<tr>
<td>R</td>
<td>denotes generic substituent</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>s.</td>
<td>solid</td>
</tr>
<tr>
<td>SnAr</td>
<td>nucleophilic aromatic substitution</td>
</tr>
<tr>
<td>t</td>
<td>tertiary</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilyl</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>λ</td>
<td>wavelength</td>
</tr>
<tr>
<td>μ</td>
<td>micro-</td>
</tr>
<tr>
<td>v</td>
<td>rate</td>
</tr>
<tr>
<td>v&lt;sub&gt;max&lt;/sub&gt;</td>
<td>maximum rate</td>
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</table>
**ABSTRACT**

The primary objective of this DPhil research project was to develop selective and cell-active inhibitors of the histone lysine demethylase KDM2A, which could potentially lead to the discovery of a novel chemical probe. Chapter one of this thesis introduces the role of histone lysine demethylases (KDMs) in the epigenetic regulation of gene expression and discusses the value of chemical probes as tools to study these enzymes. Chapter two describes the synthesis of a library of indoline-based KDM2A inhibitors using a modular synthetic approach to explore key structure-activity relationships and a chiral counterion-mediated strategy to synthesize lead candidates enantioselectively (Figure 1).

![Figure 1](image1.png)

**Figure 1** – Modular synthetic strategy towards library of indoline-based KDM2A inhibitors.

Chapter three discusses investigations into the cellular activity of lead compounds and explores strategies to address limitations associated with cytotoxicity and promiscuity. Chapter four describes the application of a variety of experimental techniques to identify the mode of target inhibition. Finally, chapter five focuses on the development of an enantioselective C-acylation reaction to access spirocyclic fragments asymmetrically (Figure 2).

![Figure 2](image2.png)

**Figure 2** – Enantioselective synthesis of spirocyclic scaffolds using chiral phase-transfer catalysis.
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1. **INTRODUCTION**

1.1 **Epigenetics**

1.1.1 *Chromatin and Gene Expression*

In 1974, Roger Kornberg proposed that the structure of chromatin is based on a repeating unit of histone oligomers closely associated with DNA.\(^1\,^2\) This discovery revealed the molecular framework for the packaging of genetic information in eukaryotic cells. Over the following decades, advances in experimental techniques helped identify the broad spectrum of chemical modifications of both histones and DNA and thus provided an insight into the mechanisms by which gene expression is regulated.\(^3\) Together, the structural organization of chromatin and the dynamic nature of its chemical transformation constitute the foundation of epigenetics.

The complete human genome contains approximately 3 billion base pairs.\(^4\) Crucially, this genetic code is broadly conserved within all cells of an individual. Therefore, the differentiation of cells, their ability to perform specialized functions, and their requirement to respond to environmental stimuli relies on the precise regulation of gene expression. The modulation of chromatin structure is an essential aspect of this. The basic repeating unit of chromatin is the nucleosome, which consists of 146 DNA base pairs wrapped around an octameric histone protein core (Figure 1.1).\(^5\,^6\) An additional linker histone (H1) stabilizes the nucleosome complex and is crucial for the packaging of chromatin.\(^7\) The amino-terminal regions of the histones (frequently referred to as \(N\)-terminal “tails”) extend beyond the nucleosome core. This makes them accessible to a
variety of enzymes, which catalyze a range of chemical modifications including phosphorylation, ubiquitination, acetylation, and methylation. The precise combination of chemical groups decorating the histone N-terminal tails influences the compactness of chromatin either directly or indirectly via the recruitment of proteins that control chromatin structure. This, in turn, affects the accessibility of genes to the transcription apparatus and hence the extent to which they are transcribed.

Figure 1.1 – X-ray crystal structure of the human nucleosome complex (PDB ID: 2CV5). DNA wraps around a core of histone proteins consisting of 2 copies of 4 histones: H2A, H2B, H3, and H4. The histone N-terminal regions (tails) protrude beyond the nucleosome core and may be subjected to a variety of post-translational modifications.

1.1.2 Writers, Readers, and Erasers

The proteins involved in the epigenetic regulation of gene expression can be broadly divided into 3 categories: writers, readers, and erasers. Writers are enzymes that catalyze the addition of chemical groups to the histone N-terminal tails. The most widely-studied writers are histone acetyltransferases (HATs) and histone methyltransferases (HMTs),
which catalyze the acetylation of lysine residues and the methylation of either lysine or arginine residues respectively. Histone acetylation is broadly associated with activation of gene expression, as acetyl groups neutralize the positive charge on lysine residues. This reduces the affinity of histones for DNA, which contains a negatively-charged phosphate backbone, causing chromatin to adopt a more open, unfolded structure. Histone methylation is arguably the most complex form of post-translational histone modification, as multiple methylation states are possible for both lysine and arginine residues. Methyl marks are recognized by a variety of regulatory proteins that either increase or decrease the extent of transcription, so that histone methylation may be associated with both activation and repression of gene expression. The observation that particular combinations of acetylation and methylation states often collaborate to bring about a particular change of gene expression has given rise to the “histone code hypothesis”.

Readers are domains that selectively bind to epigenetic marks on the histone N-terminal tails and typically exist within complexes of other domains or proteins that influence chromatin structure and transcription. Bromodomains, for example, recognize acetylated lysine residues and are often found in proteins that also contain enzymatic domains that add or remove epigenetic marks. Methylated lysine residues, in turn, are recognized by a variety of readers including plant homeodomains (PHDs), tudor domains, and malignant brain tumor (MBT) domains. A common feature of all of these domains is the presence of an “aromatic cage”, which forms a binding pocket for the methylated lysine residues. The resulting complex is stabilized by cation-π interactions involving the charged methyl amino-group of methylated lysine and the aromatic residues of the reader domain.
Finally, erasers catalyze the removal of the chemical groups from the histone \(N\)‐terminal tails and are therefore a counterbalance to the writers. Histone deacetylases (HDACs) and histone lysine demethylases (KDMs) remove the post‐translational modifications introduced by HATs and HMTs respectively. The reversibility of both histone acetylation and methylation is an essential requirement for the dynamic nature of the epigenetic regulation of gene expression.\(^\text{17}\)

1.1.3 Chemical Probes for Epigenetics

Regulation of gene expression via the chemical transformation of chromatin and DNA has a profound impact on a number of fundamental biological processes, including cellular differentiation,\(^\text{18}\) embryonic development,\(^\text{19}\) and ageing.\(^\text{20}\) However, the complex and interwoven character of epigenetics often makes elucidating the precise downstream effects of individual epigenetic components difficult to determine. Traditional approaches relying on gene knockdown using siRNA\(^\text{21}\) can provide some insight, but such strategies frequently give rise to ambiguous results. It is reasoned that the partial or complete subdual of expression of a specific writer, reader, or eraser not only removes it from the cellular system, but also perturbs the activities of other epigenetic proteins and domains that usually interact with it. For example, the histone lysine demethylases KDM2A has been shown to associate with the HP1 family of heterochromatin‐stabilizing proteins, which are essential for the maintenance of the heterochromatin‐rich regions around the centromeres and telomeres of chromosomes.\(^\text{22, 23}\) Knocking down the entire KDM2A gene would therefore not only attenuate the level of histone lysine demethylation, but also influence the HP1‐mediated regulation of chromatin structure. Consequently, a more subtle approach that enables researchers to selectively target the individual functional roles of
specific epigenetic proteins and domains is required. The use of selective chemical probes represents one such strategy.

In general, the term chemical probe refers to a small molecule that facilitates the study of the mechanistic roles and phenotypic impacts of a protein or domain. Although chemical probes can interact with their targets in a variety of ways, including inhibition, activation, and fluorescent binding, this thesis will largely focus on chemical probes that act as inhibitors. A particular challenge associated with the development of chemical probes for epigenetics is achieving selectivity between the structurally similar members of each protein family, which is essential if a chemical probe is to be a useful tool for elucidating the downstream effects of the individual writers, readers, and erasers of epigenetics. In addition, targeting epigenetics represents a pivotal approach to addressing disease at the transcription level, and well-characterized, readily available chemical probes have the potential to accelerate the development of novel pharmaceuticals. In particular, epigenetic chemical probes may be used to validate and invalidate potential targets for drug discovery research programs. This is perhaps best illustrated by the discovery of the BET-bromodomain inhibitor JQ1 (Figure 1.2). Within the first 4 years of the initial disclosure of its biological activity, five clinical trials for unique BET bromodomain inhibitors were initiated.

![Chemical structure of JQ1](image)

**Figure 1.2** – The BET bromodomain chemical probe JQ1 and its measured dissociation constants with respect to the four (full length) members of the BET bromodomain family.
1.2 Histone Lysine Demethylases

1.2.1 Phylogeny and Mechanisms of Catalysis

Histone lysine demethylases (KDMs) are divided into 2 subfamilies based on their sequence homologies and catalytic mechanisms: The flavin-dependent lysine-specific demethylases (LSD or KDM1 subfamily) and the Jumanji-C (JmjC) domain containing 2-oxoglutarate dependent lysine demethylases. KDMs belonging to the KDM1 subfamily are structurally closely related to the monoamine oxidases (MOAs) and require a flavin adenine dinucleotide (FAD) cofactor for demethylation. A possible mechanism of catalysis involving a single electron transfer from the methylated lysine sidechain to the flavin cofactor is illustrated in Figure 1.3, although alternative mechanistic pathways involving hydrogen atom transfer, nucleophilic attack, and hydride transfer have also been proposed. The chemical requirements of all of these mechanisms dictate the substrate scope of FAD-dependent demethylases, and the 3 members of this subfamily (KDM1A, KDM1B-1, and KDM1B-2) do not display activity towards trimethylated lysine residues, which would be unable to form the required iminium ion intermediate.

Figure 1.3 – A possible catalytic mechanism of demethylation by KDMs belonging to the KDM1 subfamily: SET from the methylated lysine sidechain to the FAD cofactor produces an intermediate iminium ion, which reacts with water to release formaldehyde as a byproduct.
Figure 1.4 – Phylogenetic tree of the JmjC subfamily of histone lysine demethylases, showing reported substrate selectivities.

The JmjC subfamily of KDMs is significantly larger and comprises around 20 enzymes, which can be grouped broadly into 7 further subfamilies based on sequence homology and substrate specificity (Figure 1.4). They belong to the 2-oxoglutarate (2-OG) dependent oxygenase family of enzymes, which contain a catalytically-active Fe$^{ll}$ ion and oxidize their substrates via a radical mechanism that involves the concomitant oxidative decarboxylation of 2-OG to succinate. The mechanistic details of this reaction have been probed extensively and are summarized in Figure 1.5. In the majority of enzymes, the Fe$^{ll}$ ion is coordinated by 2 histidines and 1 acidic residue. 2-OG coordinates in a bidentate manner, and its C5 carboxylate group interacts electrostatically with a basic residue such as lysine or arginine. Oxygen binds and initiates the oxidative decarboxylation of 2-OG to generate succinate, CO$_2$, and an intermediate Fe$^{IV}$-oxo species, for which there is spectroscopic evidence. This reactive Fe$^{IV}$-oxo intermediate
effectively inserts an oxygen atom into the C-H bond of one of the methyl groups of the methylated lysine substrate, presumably via a radical rebound mechanism. As with the FAD-dependent demethylases, the initial reaction product collapses to release the demethylated lysine residue and formaldehyde as a byproduct. However, unlike enzymes in the KDM1 subfamily, many of the JmjC demethylases show activity towards trimethylated lysine residues, and in several cases arginine demethylation has been observed as well. This enhanced substrate scope is enabled by their distinct mechanism of catalysis.

**Figure 1.5** – Catalytic cycle of JmjC KDMs: Oxidative decarboxylation of bound 2-OG generates succinate and a Fe(IV)-oxo intermediate, which reacts with one of the methyl groups of the methyllysine substrate. The X-ray crystal structure of the KDM2A active site (Ni\textsuperscript{II} instead of Fe\textsuperscript{II} and N-OG instead of 2-OG) is depicted at the center of the cycle (PDB ID: 4QXC).
1.2.2  **Inhibitors of KDMs**

The fundamental importance of KDMs in maintaining the dynamic equilibrium of histone methylation and their connection to a variety of diseases\textsuperscript{13, 30, 39} has led to the development of a range of selective inhibitors. Molecules based on the scaffold of the known monoamine oxidase inhibitor tranylcypromine (1) form the largest class of KDM1 inhibitors (Figure 1.6).\textsuperscript{40} Structural studies have shown that these compounds are irreversible inhibitors that form covalent adducts with the FAD cofactor and that the substituents on the phenyl ring of the tranylcypromine core occupy the substrate binding site.\textsuperscript{41, 42} Recently, A. Mai and co-workers developed the bifunctional pan-KDM inhibitors 4 and 5 by linking the KDM1-selective tranylcypromine motif with JmjC-KDM inhibitory motifs based on bipyridine and 8-hydroxyquinoline respectively.\textsuperscript{43}

![Figure 1.6](image.png)

**Figure 1.6** – Inhibitors of the KDM1 subfamily of lysine demethylases: tranylcypromine (1) is a known monoamine oxidase (MOA) inhibitor and its scaffold forms the basis of many selective KDM1 inhibitors such as 2. These compounds are irreversible inhibitors and react with the FAD cofactor in the enzyme active site (3).
Inhibitors of the JmjC subfamily of KDMs based on iron-chelating 2-OG mimics: These inhibitors tend to be small, polar acidic compounds with variable degrees of selectivity. GSK-J1 (11) is a widely distributed chemical probe for the KDM5 and KDM6 demethylases.

The majority of inhibitors of the JmjC lysine demethylases are iron-chelating 2-OG mimics and include a variety of small, polar molecules such as N-oxalylglycine (N-OG 6), daminozide (7), and IOX1 (8), a well-studied broad spectrum 2-OG oxygenase inhibitor (Figure 1.7). One strategy for improving the potency and selectivity of such scaffolds is based on combining iron-chelating motifs with fragments that extend into the histone binding pocket. This approach led to the discovery of the potent yet non-selective inhibitor methylstat (9), in which a hydroxamic acid “head group” is linked to a methyllysine mimic. The methyl ester prodrug version of 9 was found to inhibit KDM4C-catalyzed demethylation of H3K9me3 in HeLa cells. Similarly, the bifunctional KDM4 inhibitor 10 combines a 2-OG-competitive 8-hydroxyquinoline unit with a fragment resembling the dimethyllysine sidechain. In 2012, GSK-J1 (11) was reported as a potent and selective inhibitor of members of the KDM6 subfamily. Structural studies revealed that the pyridyl-pyrimidine core chelates Fe^{II} in a bidentate fashion, with the acidic sidechain occupying the 2-OG
binding pocket. The ethyl ester prodrug version of 11 was shown to inhibit proinflammatory gene activation in human macrophages, demonstrating the potential of KDM inhibitors in drug discovery.\textsuperscript{49} Since this initial report, however, further investigations revealed that 11 is also a potent inhibitor of the KDM5 demethylases.\textsuperscript{50} This illustrates the importance of extensively characterizing the selectivity profile of a chemical probe candidate before it should be used as a tool to elucidate connections between a subfamily of epigenetic proteins and a specific biological process. Finally, recent examples of highly potent 2-OG-competitive KDM inhibitors include a series of KDM4/5-selective pyridopyrimidinones\textsuperscript{51} and a series of KDM5 inhibitors based on a cyanopyrazole scaffold.\textsuperscript{52}

Figure 1.8 – Peptide inhibitors of JmjC KDMs: Modified linear peptides based on the relevant H3 tail sequence such as 12 and 13 occupy the histone binding site. The cyclic peptide inhibitor 14 was found to interact with several allosteric sites on KDM4C.
An alternative approach to inhibiting the JmjC lysine demethylases relies on linear and cyclic peptides as mimics of the histone substrate or as allosteric binders (Figure 1.8). In 2011, R. P. Clausen and J. L. Kristensen described a strategy for discovering peptides that selectively inhibit PHF8, KDM4A, and KDM4C based on truncating and modifying the relevant histone 3 (H3) tail sequence (12). Shortly afterwards, C. J. Schofield and co-workers used a rational design approach relying on non-denaturing mass spectrometry and x-ray crystallography to identify a reasonably potent and selective inhibitor of KDM4A and KDM4E. In this study, modified peptides were covalently linked to N-oxalyl-cysteine to generate adducts that occupy both the 2-OG and substrate binding sites (13). The discovery of cyclic peptide inhibitors such as 14 was facilitated by the creation of a phage display library. Hydrogen/deuterium exchange mass spectrometry suggests an allosteric mode of inhibition with respect to KDM4C.

Although the strategies described above have led to the discovery of a variety of reasonably potent and in some cases even selective inhibitors of the JmjC KDMs, the majority of these 2-OG competitors and peptides are generally not suitable as chemical probes. In particular, the molecules derived from either approach are usually characterized by poor cellular permeability and can therefore not be used to explore the impact of KDM inhibition in a cellular context. The research described in this thesis attempts to address the need for novel classes of inhibitors of the JmjC KDMs. Specifically, it focuses on the development and characterization of a highly selective, cell-active inhibitor and potential chemical probe for the histone lysine demethylase KDM2A.
1.3 KDM2A

1.3.1 Structural Features and Substrate Selectivity

KDM2A selectively catalyzes the demethylation of both mono- and di-methylated lysine 36 on histone 3 (H3K36). Like most of the JmjC KDMs, it has several aliases, notably FBXL11 (F-box and leucine-rich repeat protein 11) and JHDM1A (JmjC domain containing histone demethylase 1A). In its entirety, KDM2A consists of 1161 amino acid residues and contains 9 domains (Figure 1.9-A). A truncated version of KDM2A without the CXXC, PHD, Fbox, and LRR repeat domains retains the full enzyme’s catalytic activity, although loss of the PHD domain in particular reduces turnover. The CXXC domain recognizes and binds to non-methylated CpG DNA islands, thereby directing demethylation to segments of chromatin in the vicinity of these genetic markers. The PHD domain is atypical in that it does not contain an aromatic cage for recognizing methylated lysine residues and does not appear to bind to any known epigenetic marks. Similarly, the precise functional roles of the JmjN, Fbox, and LRR repeat domains are still under investigation.

A detailed study exploring the structural origins of the enzyme’s substrate selectivity using a 13-residue peptide to replicate the H3 tail was reported by the D. J. Patel and O. Gozani. It revealed that molecular recognition of the peptide by KDM2A involves the JmjC, hairpin, mixed, and C-terminal domains. The peptide’s G33, G34, and P38 residues enable the necessary U-shaped conformation in the active complex, and the aromatic sidechain of Y41 occupies a pocket outside the active site (Figure 1.9-B). These residues and their relative locations are unique to the H3K36 sequence. The lack of activity of KDM2A towards trimethylated H3K36 may be explained by a potential steric clash between the lysine sidechain’s additional CH$_3$ group and 2-OG.
Figure 1.9 – A. Complete domain architecture of KDM2A: The CXXC, PHD, FBox, and LRR repeat domains are not essential for catalytic activity. B. An x-ray crystal structure (PDB ID: 4QXC)$^{38}$ showing the peptide substrate (yellow) inside KDM2A’s JmjC domain active site (grey): Important peptide residues for substrate recognition are highlighted.

### 1.3.2 Biological Importance and Implications in Disease

Due to its role in regulating the methylation dynamics of H3K36, which impact gene expression patterns across the entire genome, KDM2A has been implicated in a variety of fundamental biological processes and diseases. For example, it has been shown to be involved in the regulation of NF-κB, which coordinates cytokine production and the immune response.$^{59}$ Both KDM2A and the closely related demethylase KDM2B are involved
in the vitamin-C-dependent reprogramming of somatic cells to induced pluripotent stem cells.\textsuperscript{60} In addition, gene knockdown experiments suggest that KDM2A may play a crucial role in embryonic development.\textsuperscript{61} Studies have shown that the methylation state of H3K36 may be connected to specific oncogenic consequences.\textsuperscript{62-64} As a result, KDM2A has been linked to various cancers. Notably, the enzyme is found to be overexpressed in gastric cancer cells,\textsuperscript{65} and its knockdown reduces the proliferation of non-small cell lung cancer (NSCLC) cells.\textsuperscript{66} Together, these findings suggest that inhibiting KDM2A may represent a strategy for targeting certain cancers at the transcription level. However, in the absence of a selective, cell-permeable chemical probe for KDM2A, target validation remains a challenge.

1.3.3  Inhibitors of KDM2A

Several of the relatively broad spectrum 2-OG competitors described in subsection 1.2.2 show inhibitory activity towards KDM2A. For example, the plant growth regulator daminozide (7) appears to be remarkably selective for the KDM2/7 subfamily relative to representative members of most of the other KDM subfamilies as well as other 2-OG dependent oxygenases.\textsuperscript{45} In addition, initial studies suggest that both methylstat (9) and the fluorescent analogue methylstat\textsuperscript{fluor} (15) are potent (sub-μM) inhibitors of KDM2A.\textsuperscript{47, 67} However, to our knowledge, the only highly-selective KDM2A inhibitor disclosed in the literature to date is the triazolopyridine-based compound (16) reported by C. J. Schofield and P. E. Brennan in 2014, which is also thought to occupy the 2-OG binding site (Figure 10).\textsuperscript{68} As a single enantiomer, 16 has an IC\textsubscript{50} of 63 nM towards KDM2A (using the RapidFire assay – see subsection 2.1.2) and greater than 30-fold selectivity over representative members of most of the other KDM subfamilies. Unfortunately, 16 does not inhibit
demethylation of H3K36me2 in cells, and development of a cell-active pro-drug has proven to be challenging. A similar imidazole-pyridine scaffold has recently been developed by Quanticel Pharmaceuticals (17). Derivatives display inhibitory activity towards KDM2A, KDM2B, and PHF8.

![Chemical structures](image)

Figure 1.10 – Inhibitors of KDM2A: Methylstatfluor (15) is a fluorescent analogue of methylstat (9). The triazolopyridine-based inhibitor 16 displays greater than 30-fold selectivity towards KDM2A but is unable to penetrate cells. Imidazole-pyrimidines (17) may be a potent new class of KDM2 inhibitors.

1.4 Project Background

1.4.1 Rationale and Hit Identification

Due to the limitations of both 2-OG mimics and peptides as KDM inhibitors, we envisaged an alternative approach towards the development of a novel selective inhibitor and chemical probe candidate for KDM2A. Specifically, we aimed to develop a small, non-peptidic molecule that would compete with the enzyme’s histone substrate and therefore be more likely to possess the physicochemical properties required for cellular permeability. In order to identify a starting point, a library of known binders to methyl-lysine reading domains and HMTs was screened for inhibitory activity against a select panel of KDMs using
an AlphaScreen (see subsection 2.1.1).* We reasoned that such a specialized library would be more likely to contain molecules that also interact with demethylases. Samples of many of the tested compounds were generously provided by Prof. Stephen Frye, University of North Carolina. From this initial screen, indole 18 was identified as a promising hit for further optimization, due to its reasonably low IC50 and apparent selectivity for KDM2A (Table 1.1). The N-alkyl pyrrolidine group is a common motif in inhibitors of methyl-lysine reading domains and HMTs, as it has been shown to mimic the dimethylated lysine sidechain.71, 72 It was therefore initially proposed that 18 might be acting as a peptide/histone competitor.

![Image of compound 18]

**Table 1.1** – Inhibitory activity of initial hit 17 against a panel of demethylases.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>% Inhibition ([18] = 20 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDM2A</td>
<td>93</td>
</tr>
<tr>
<td>KDM3A</td>
<td>37</td>
</tr>
<tr>
<td>JMJD4C</td>
<td>0</td>
</tr>
<tr>
<td>KDM5B</td>
<td>36</td>
</tr>
<tr>
<td>KDM6B</td>
<td>0</td>
</tr>
</tbody>
</table>

After confirming its potency using the orthogonal RapidFire activity assay (see subsection 2.1.2), a small library of close analogues of 18 was synthesized and tested to explore structure-activity relationships (SARs) around the indole core.† Key results of this preliminary investigation are illustrated in Figure 1.11. Replacing the methoxy substituent at the C5 position with a trifluoromethoxy group led to a drop in inhibitory activity (18 vs. 19 and 20 vs. 21). Functionalization at the indole NH position to generate a tert-butylocarbamate (20) and a S-phenylsulfonamide (22) resulted in an increase in potency, while the other sulfonamide (23) and cyclohexyl amide (24) examples were found to be less active than the original hit.

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* This work was done by Dr Anthony Tumber, Target Discovery Institute.
† This work was done by Dr Tamas Szommer, Target Discovery Institute.
Figure 1.11 – Initial SAR studies around the indole core of 18, including IC_{50} values for KDM2A inhibition (determined by RapidFire activity assay*).

1.4.2 Pharmacophore Design and Synthetic Approach

We hypothesized that both the inhibitory activity and selectivity of 18 might be further improved by replacing the original indole scaffold with an indoline ring system, which would potentially enable a more efficient exploration of chemical space. Therefore, pharmacophore 25 was proposed, and we aimed to generate a library of potential KDM2A inhibitors based on this structure (Figure 1.12). A modular synthetic approach would be

* IC_{50}-determination experiments were performed in duplicate by Dr Anthony Tumber. The values presented here correspond to the arithmetic mean of the 2 obtained measurements displayed to 2 significant figures. This approach for presenting inhibitory activities will be adopted throughout this thesis. For a more statistically-rigorous summary of all RapidFire results, see section 8.1 in the appendix.
employed to expedite SAR studies, and we anticipated being able to apply recently-developed methodology to synthesize lead compounds asymmetrically.

**Figure 1.12** – Indoline-containing pharmacophore.

Previous research in the Smith group has focused on the development of cation-directed enantioselective cyclization reactions to generate various heterocyclic scaffolds, including indolines\(^{73,74}\) and aza-indolines,\(^{75}\) indolenines,\(^{76}\) pyrroloindolines,\(^{77}\) and indanes\(^{78}\) (Figure 1.13). These reactions rely on the formation of a tight-ion pair involving a chiral quaternary ammonium catalyst and the deprotonated substrate. The stereocontrolled 5-endo-trig cyclization of aldimines under basic conditions and in the presence of a cinchona-alkaloid catalyst (26 to 27) is most relevant to this project.

**Figure 1.13** – Examples of cation-directed cyclization reactions developed in the Smith group.
Our general synthetic strategy towards the generation of a diverse library of functionalized indolines is illustrated in Scheme 1.1. We anticipated that the modular nature of this synthetic approach would provide access to a variety of compounds containing different substituents at the indoline C2 and C3 positions. In addition, we envisaged making use of enantioselective phase-transfer catalysis to control both the relative and the absolute stereochemistry of the indoline core (34 to 35). N-acylation would stabilize this core towards oxidation and provide an additional handle for functionalization. Finally, metal-catalyzed cross coupling reactions would be used to link the indoline ring system to the N-alkyl pyrrolidine “head-group” via a choice of linkers.

Scheme 1.1 – General synthetic approach to the creation of a library of potential KDM2A inhibitors based on the structure of pharmacophore 25.

This thesis will explore the details, scope, and limitations of this synthetic approach in the context of developing novel KDM2A inhibitors and chemical probe candidates. Subsequently, it will describe investigations into the mode of inhibition of lead compounds, drawing on a variety of experimental techniques. The insights attained in the course of these studies demonstrate the successful application and further development of asymmetric organocatalysis to a topical challenge in chemical biology.
2. **Synthesis of a Library of KDM2A Inhibitors**

2.1 Biochemical Activity Assays

2.1.1 *AlphaScreen™*

The availability of robust biological assays is essential for investigating structure-activity relationships effectively. At the outset of this project, two biochemical activity assays, the AlphaScreen™ and the RapidFire assay, could be relied upon to provide reproducible results for KDM inhibition. These assays have been applied widely within the Structural Genomics Consortium (SGC). Unless otherwise stated, all of the IC₅₀ values presented in this thesis were obtained by Dr Anthony Tumber at the Target Discovery Institute (TDI).

The application of Amplified Luminescence Proximity Homogenous Assay (ALPHA) technology to the study of JmjC-KDMs was first reported by A. Kawamura and C. J. Schofield in 2010. The principle features of this assay are illustrated in Figure 2.1. Biotinylated peptide oligomers containing a specific mono-, di-, or trimethyl-lysine residue are immobilized on streptavidin-coated “donor” beads, which contain a covalently-attached photosensitizer such as phthalocyanine. Antibodies that selectively recognize a particular methylation state of the peptide are immobilized on streptavidin-coated “acceptor” beads, which contain covalently-attached thioxene and one or multiple fluorophores. Upon illumination of this system with a 680 nm laser, the photosensitizer on the donor beads is
electronically excited and causes the excitation of ambient oxygen to its more reactive singlet state via collision-mediated energy transfer. Provided that the antibody is bound to the peptide and the donor and acceptor beads are therefore in close proximity (within approx. 200 nm), this singlet oxygen has a distinct likelihood of reacting with the thioxene on the acceptor beads. The reactive intermediates that are formed can undergo chemiluminescence and thereby excite the fluorophores on the acceptor beads. This generates a measurable fluorescence signal.

**Figure 2.1** – General principle of the AlphaScreen™: 1) A 680 nm laser excites photosensitizers immobilized on the surface of the donor beads, resulting in the formation of singlet oxygen. 2) Singlet oxygen reacts with thioxene immobilized on the surface of the acceptor beads. The decomposition of the resulting intermediates generates chemiluminescence, which excites immobilized fluorophores. 3) Fluorescence is only detected if the beads are in sufficient proximity to enable the efficient transfer of energy between them.
In the presence of the relevant catalytically-active KDM, the methyl-lysine residues on the peptide will be demethylated, and consequently the antibody will not bind. As a result, the donor and acceptor beads are likely to be too far apart to enable the efficient transfer of energy from the photosensitizer to thioxene via singlet oxygen. Instead, the singlet oxygen that is generated is more likely to be consumed by various quenching processes. This ultimately results in a reduction of the fluorescence signal. The extent of this signal reduction can be attenuated by adding a KDM inhibitor.

The principle strength of the AlphaScreen™ is its high degree of signal amplification, as each donor bead is able to emit up to 60,000 molecules of singlet oxygen per second. Therefore, only small quantities of peptide and protein are required, making it an ideal platform for high-throughput screening (HTS). Principal drawbacks of the AlphaScreen™ include potential fluorescence and fluorescence-quenching by compounds containing aromatic functional groups as well as quenching of singlet oxygen by inhibitors. Both of these events distort the measured signal and may generate either false-positive or false-negative results. Therefore, it is essential to have an independent, non-luminescence-based assay to verify inhibitory activities.

2.1.2 RapidFire Activity Assay

The RapidFire activity assay provides a relatively direct insight into the catalytic activity of KDMs. It relies on a high-throughput mass spectrometer to monitor the progress of demethylation of an approximately 20-meric peptide substrate. The peaks corresponding to peptides in different methylation states may be integrated, and the ratio of the integration areas reveals the conversion of the reaction. The addition of an

* Peptide sequence: SAPATGGVK(Me2)KPHRYRPGTVAL
enzymatic inhibitor reduces the conversion at a given time point in a concentration-dependent manner. Typical spectra generated by this assay and a time-course activity plot are illustrated in Figure 2.2.

![Figure 2.2](image)

**Figure 2.2** – A) The KDM2A-catalyzed conversion of the H3K36me2 peptide to H3K36me1 and H3K36me0 peptides may be monitored over time using the RapidFire MS assay. B) Time-course activity plot of KDM2A-catalyzed demethylation of H3K36me2 peptide.

Thanks to its simplicity, the RapidFire assay is generally regarded as a robust and reliable approach to determining inhibitory activities. Its principle drawbacks are the requirement for a costly and elaborate mass-spectrometry platform, a relatively low level of sensitivity so that larger quantities of peptide and enzyme are required, and a long sample run-time. These factors make the RapidFire assay less suitable for HTS or selectivity screening, and it is generally applied to lead optimization instead.

### 2.2 SAR Studies with Racemic Indolines

#### 2.2.1 Criteria for Chemical Probe Development

In order to devise a clear project goal, we decided to adopt the SGC guidelines for
chemical probe development, which outline the criteria that an inhibitor needs to satisfy before being considered a true chemical probe: 85

1) *In vitro* potency (i.e. IC$_{50}$, K$_i$ or K$_D$) $\leq$ 100 nM.

2) > 30-fold selectivity relative to other proteins in the same family (i.e. members of the KDM3, KDM4, KDM5, KDM6, and KDM7 subfamilies in our case).

3) Significant on-target cellular activity at 1 µM.

We envisaged using both the AlphaScreen™ and RapidFire activity assays to assess *in vitro* potency of synthesized compounds and provide guidance for SAR investigations. For lead compounds, we would also obtain AlphaScreen™ IC$_{50}$-values with respect to representative members of the various KDM subfamilies in order to evaluate selectivity. It is important to note that all KDM assays of the same type (i.e. AlphaScreen™ or RapidFire) contain analogous components at identical concentrations, measure the same output, and operate according to the same principles, and we therefore reasoned that comparing IC$_{50}$ values would provide a reliable insight into selectivity. Finally, cellular activities of lead compounds would be determined using an immunofluorescence-based assay to monitor histone demethylation in a cellular context (subsection 3.1.1).

2.2.2 Substituents at the Indoline C3 Position

We began our methodical exploration of SARs around the indoline core of pharmacophore 25 by investigating the effect of various substituents at the indoline C3-position. To a certain extent, the scope of potential functional group combinations was limited by our chosen synthetic approach, which required the C3-substituents to be sufficiently electron-withdrawing to enable deprotonation and subsequent cyclization of
imine 42 to generate the desired indoline core (Scheme 2.1). In most cases, we envisaged introducing these groups \textit{via} a nucleophilic aromatic substitution on 1-fluoro-2-nitrobenzene 37. Reduction of the nitro group, followed by condensation with 5-bromo-3-pyridinecarboxaldehyde would afford imine 42. Under racemic reaction conditions, we anticipated that imine 42 would cyclize to afford a mixture of separable diastereomers. Acylation with a simple acetyl group in the first instance would stabilize the indoline core towards oxidation, and a Stille-Migita cross-coupling would introduce the $N$-alkyl pyrrolidine function connected to the indoline core by a \textit{trans}-alkene linker.

Scheme 2.1 – Synthetic approach towards the exploration of SARs around the indoline C3 position.

To begin with, nitrile 46 (R$^1$,R$^2$ = Ph,CN) was synthesized using a $S_{N}Ar$ reaction, involving treatment of a mixture of 1-fluoro-2-nitrobenzene and benzyl cyanide with aqueous NaOH in the presence of stoichiometric tetrabutylammonium bisulfate. The use of tetrabutylammonium salts in reactions of this type and the significance of their stoichiometry has been discussed previously by M. Mąkosza.\textsuperscript{86} The reaction could be
performed on a multi-gram scale and reproducibly afforded nitrile 46 in moderate yield. Reduction of the nitro group to generate aniline 47 was accomplished using zinc powder in the presence of NH₄Cl. No reduction of the nitrile function under these conditions was observed. Aniline 47 was subsequently condensed with 5-bromo-3-pyridinecarboxaldehyde 41 using excess MgSO₄ as a desiccant to afford imine 48 as a single geometrical isomer (Scheme 2.2).

Scheme 2.2 – Synthesis of imine 48 via Sₐr, followed by nitro-group reduction and condensation.

To form the indoline core, imine 48 was treated with 2 equivalents of sublimed KO'Bu. At room temperature and after a reaction time of 1 h, imine 48 was consumed completely to afford a 1:1.3 mixture of indoline diastereomers 49 and 50. Lowering the temperature to 0 °C and reducing the reaction time to 20 minutes increased this ratio to 1:1.7, while maintaining complete conversion (Scheme 2.3). In order to determine whether the KO'Bu-induced cyclization of imine 48 is reversible, both diastereomers were re-subjected to the reaction conditions. Indeed, 49 and 50 were found to interconvert under these conditions.

Indolines have a propensity to undergo oxidation to indolenines (3H-indoles) on standing, and N-protection of the indoline core was therefore necessary to minimize this complication. Both diastereomers were found to be moderately unreactive to a variety of acetylation reagents including acetic anhydride and di-tert-butyl dicarbonate. However, the use of acetyl chloride and pyridine gave an excellent yield of both desired protected products 52 and 53 in a rapid manner (Scheme 2.4).
Scheme 2.3 – Cyclization of imine 48 to afford a diastereomeric mixture of indolines 49 and 50. The relative stereochemistries of both products were determined by nOe spectroscopy.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>49:50</th>
<th>Isolated yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 min, rt</td>
<td>1:1.3</td>
<td>75%</td>
</tr>
<tr>
<td>20 min, 0 °C</td>
<td>1:1.7</td>
<td>84%</td>
</tr>
</tbody>
</table>

Scheme 2.4 – N-Acetylation of the indoline core.

We envisaged using the bromopyridine moiety on the indoline C2 position as a functional handle for coupling to the indoline core via metal-catalyzed cross-coupling methodologies. Scheme 2.5 illustrates the synthesis of E-7-tributylstannyl-hept-6-en-1-pyrrolidine 57, which we anticipated could be utilized as a cross-coupling partner via a Stille-Migita reaction. J. M. Chong and co-workers had previously demonstrated the impact of different Pd(0) catalysts and phosphine ligands on the regioselectivity of hydrostannylation reactions involving terminal alkynes. In the course of their investigations, bulkier phosphine ligands were generally found to improve regioselectivity, and, for most substrates, the combination of Pd$_2$(dba)$_3$ with Cyc$_3$PH$^+$BF$_4$ in $^{i}$Pr$_2$NEt maximized the yield of the desired E-vinylstannane product (Figure 2.3). Application of
these reaction conditions to the hydrostannylation of 7-hetyne-1-ol 54 with tributyltin hydride afforded vinylstannane 55 in good yield and as a single regio- and stereoisomer. Furthermore, this reaction could be performed reliably on a gram-scale. The primary alcohol group of 55 was converted to an alkyl bromide using an Appel reaction, and substitution with pyrrolidine generated vinylstannane 57 in moderate yield.

Scheme 2.5 – Synthesis of vinylstannane 57.

Figure 2.3 – Mechanistic rational for the observed regioselectivity of hydrostannylation of terminal alkynes, as proposed by J. M. Chong. The reaction is suggested to proceed via initial hydropalladation rather than stannylpalladation. Pathway A is preferred over pathway B, as it avoids steric clash involving the bulky phosphine ligands.

A variety of catalysts and reaction conditions for the Pd(0)-catalyzed cross-coupling of aryl halides and vinylstannanes have been reported. Fortunately, we were able to draw
on the extensive experience of Smith group member Dr Craig Johnston to decide which of these conditions would be most applicable to our combination of coupling partners. The Stille-Migita cross-coupling of indolines 52 and 53 with vinylstannane 57 was ultimately carried out in the presence of catalytic Pd(PPh₃)₄ in NMP, according to a procedure previously described by D. M. Berger. Under these conditions, indolines 58 and 59 were obtained in moderate yield with complete retention of the E-alkene geometry (Scheme 2.6).

\[ \text{Scheme 2.6 – Stille-Migita cross coupling to afford indolines 58 and 59. The E-geometry of the alkene linker was confirmed by analysis of the alkene-proton coupling constants.} \]

We decided to apply this six-step synthetic route to the synthesis of analogues of 58 and 59, bearing different functional groups on the indoline C3 position. Scheme 2.7 illustrates the synthesis of indolines 69 and 70, in which the nitrile group of 58 and 59 is replaced by a methyl ester. Different reagents and reaction conditions to those discussed previously were used to effect the S_N_Ar reaction between 1-fluoro-2-nitrobenzene 37 and methyl phenylacetate 61. This transformation proved to be substantially less reliable than the analogous reaction with benzyl cyanide due to the formation of a variety of unidentified side products. The nitro group of ester 62 was reduced by hydrogenation over a palladium on charcoal catalyst, as reduction using Zn powder and NH₄Cl resulted in \textit{in situ} cyclization.
of the initial aniline product to form a lactam. Aniline 63 was also found to undergo lactamization during silica-gel column chromatography, and it was therefore necessary to use unpurified material in the condensation with 5-bromo-3-carboxaldehyde 41 to generate imine 64. Imine 64 was cyclized under identical reaction conditions to those applied to imine 48 to afford the diastereomeric indolines 65 and 66 in essentially equal proportions. Indolines 65 and 66 were acetylated using acetyl chloride and pyridine, and the resulting products underwent cross-coupling with vinylstannane 57 to afford 69 and 70 in moderate yield.

Scheme 2.7 – Synthesis of indolines 69 and 70: The relative stereochemistries of indolines 65 and 66 were assigned by nOe spectroscopy.
In order to have an example that did not contain a phenyl substituent on the indoline C3 position, we investigated the possibility of a nitrile-ester combination of functional groups on this site. Ethyl cyanoacetate 72 and 1-fluoro-2-nitrobenzene 37 underwent a SnAr reaction to afford ester 73. As had been observed with the conversion of nitro-ester 62 to aniline 63, aniline 74 was susceptible to in situ lactamization when Zn-powder and NH₄Cl were used for reduction of the nitro group. A hydrogenation reaction was therefore performed instead. We anticipated that treatment of the unpurified aniline 74 with 5-bromo-3-carboxaldehyde 41 in the presence of excess MgSO₄ as a desiccant would afford imine 75. However, we identified the isolated product of this reaction as indoline 77, obtained as a 97:3 mixture of diastereomers. The relative stereochemistry of the major product was determined by X-ray crystallography (Figure 2.4). We postulate that the spontaneous cyclization of imine 75 is a result of the facile formation of a reactive zwitterionic form (76). Acetylation of indoline 77 and Stille-Migita cross-coupling with vinylstannane 57 afforded indoline 79 (Scheme 2.8).

![Chemical reaction diagram]

**Scheme 2.8** – Synthesis of indoline 79. Imine 75 could not be isolated, and underwent spontaneous cyclization to indoline 77.
As a final example, we investigated the possibility of an ester-alkyl combination of substituents on the indoline C3 position. Condensation of methyl-2-nitrophenylacetic acid 82 with paraformaldehyde afforded ester 83 in excellent yield. Conveniently, both the alkene and the nitro group could be reduced by hydrogenation over a palladium on charcoal catalyst to give aniline 84, which was condensed without further purification with 5-bromo-3-pyridinecarboxaldehyde 41, yielding imine 85 (Scheme 2.9).

\[
\begin{align*}
81 & \xrightarrow{H_2SO_4, MeOH, 60 °C, 16 h} 82 \\
& \xrightarrow{2 \text{ h}} \text{quant.} \\
83 & \xrightarrow{(\text{CH}_3O)_3, K_2CO_3, TBAI, PhMe, 50 °C, 5h} \text{84} \\
& \xrightarrow{41, \text{MgSO}_4, \text{PhMe, rt, 1 h}} \text{58% (2 steps)} \quad \text{85}
\end{align*}
\]

Scheme 2.9 – Synthesis of imine 85.

Attempts to induce the cyclization of imine 85 with a variety of bases including KO\textsuperscript{t}Bu, NaH, and KHMDS in either toluene or THF proved unfruitful. Given the stark colour change of the reaction mixture upon addition of these bases, we considered it likely that

* X-ray diffraction data was collected and the structure solved by John Joliffe.
deprotonation to generate a delocalized anionic intermediate was occurring. The absence of reactivity was therefore attributed to a higher activation energy barrier of cyclization. Unfortunately, heating the reaction mixture to address this potential issue also failed to induce the desired reaction, suggesting that an alternative synthetic approach would be needed to obtain this particular substitution pattern on the indoline ring system (Scheme 2.10).

Scheme 2.10 – Unsuccessful cyclization of imine 85.

Aware of the potential assay interference issues associated with organotin or palladium contaminants, we eluted all final KDM2A inhibitor candidates through a short column of K$_2$CO$_3$-silica, previously reported as a highly effective method for removing organotin impurities,$^9$ and filtered the eluent through a pad of Celite™ as a means of removing any remaining palladium salts. The compounds were subsequently tested for inhibitory activity using the RapidFire activity assay (Figure 2.5).

Excitingly, indolines 58 and 59 showed substantially improved potency relative to the original indole hit 18. Indolines 69 and 70 appeared to be marginally more potent, and indoline 79 was similarly effective to 18. For both of the investigated diastereomeric pairs (58, 59 and 69, 70), the syn diastereomers (59 and 70) were found to be more potent than their respective anti analogues (58 and 69). Given these promising results, we decided to focus on indoline scaffolds containing the Ph,CN substitution pattern on the C3 position for the remainder of our SAR investigations.
Figure 2.5 – Effect of C3 substituents on inhibitory activity. IC$_{50}$ values were determined by RapidFire activity assay. pIC$_{50}$ values and errors are shown in parentheses.

2.2.3 Head Group and Linker

We had initially hypothesized that the N-alkyl pyrrolidine motif in the original hit 18 was acting as a dimethyllysine mimic and that 18 and its derivatives might therefore occupy the histone binding site. To explore the significance of the pyrrolidine “head-group” with respect to inhibition, a series of indoline analogues was synthesized by Dr Jamie Wolstenhulme and tested for inhibitory activity using the AlphaScreen™ (Figure 2.6). For practical synthetic reasons, this series is based on a slightly different indoline core to the one described in the previous subsection. The pyridyl ring at the indoline C2 position is replaced by a phenyl ring, and an ether linker is used instead of an E-alkene. In general, the combination of these 2 modifications yielded weaker KDM2A inhibitors (Figure 2.6).

* For a statistical error analysis of inhibitory activities, see section 8.1 in the appendix.
Replacing the pyrrolidine head-group with piperidine did not have a significant effect on potency (86 vs. 87), while using a morpholine head-group was found to diminish inhibitory activity (88). Substituting pyrrolidine with diethylamine (89) or cyclopentane (90) resulted in a dramatic decrease in KDM2A inhibition, suggesting that a cyclic tertiary amine “head-group” is critical for achieving a high level of potency.

\[ \text{IC}_{50} \text{ 0.89} \mu M (6.0 \pm 0.05) \]

\[ \text{IC}_{50} \text{ 3.3} \mu M (5.5 \pm 0.06) \]

\[ \text{IC}_{50} \text{ 17} \mu M (4.8 \pm 0.06) \]

\[ \text{IC}_{50} \text{ 29} \mu M (4.5 \pm 0.22) \]

*Figure 2.6* – Effect of “head-group” on inhibitory activity. *IC*\textsubscript{50} values were determined by RapidFire activity assay. pIC\textsubscript{50} values and errors are shown in parentheses.

Next, we decided to explore the effect of varying the length of the alkyl chain on inhibitory activity. A series of indolines with alkyl chains of different lengths was synthesized by Dr Jamie Wolstenhulme and evaluated using the AlphaScreen™ (Table 2.1). A chain length of seven or eight atoms (including oxygen) was found to be optimal,

*Compounds synthesized by Dr Jamie Wolstenhulme.*
although we were surprised that there was not a more substantial drop in inhibitory activity for compounds with much shorter or longer alkyl chains.

<table>
<thead>
<tr>
<th>n</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0 (5.7 ± 0.12)</td>
</tr>
<tr>
<td>2</td>
<td>1.3 (5.9 ± 0.13)</td>
</tr>
<tr>
<td>3</td>
<td>1.0 (6.0 ± 0.06)</td>
</tr>
<tr>
<td>4</td>
<td>0.89 (6.0 ± 0.05)</td>
</tr>
<tr>
<td>5</td>
<td>1.4 (5.9 ± 0.07)</td>
</tr>
<tr>
<td>6</td>
<td>1.5 (5.8 ± 0.17)</td>
</tr>
</tbody>
</table>

Table 2.1 – Effect of alkyl chain length on inhibitory activity. *IC$_{50}$ values were determined by RapidFire activity assay. pIC$_{50}$ values and errors are shown in parentheses.

Having identified the optimal chain length and head-group, we wanted to investigate a range of linkers between the indoline core and the N-alkyl pyrrolidine motif. Synthesis of the diastereomeric pyridyl analogues of ether-linked indoline 86 is illustrated in Scheme 2.11. Condensation of aniline 47 with 5-(benzyloxy)nicotinaldehyde 91 * afforded imine 92 in good yield, and KO$_t$Bu-induced cyclization of this substrate afforded an inseparable mixture of diastereomers. Fortunately, the acetylated indolines 94 and 95 were separable by silica-gel column chromatography. In both cases, the benzyl group was removed by hydrogenation over a palladium on charcoal catalyst, and O-alkylation of the resulting products with 1,7-dibromoheptane 97, followed by substitution with pyrrolidine, afforded the final KDM2A inhibitor candidates 99 and 102.

We rationalized that using more configurationally-constrained linkers might improve potency, as this would reduce conformational flexibility overall and consequently lower the entropic “cost” of binding to KDM2A. Alkyne 105 was synthesized in two steps from heptyn-1-ol 103 and was used as a substrate for Sonogashira coupling* to indolines 52 and 53, generating the alkyne-linked products 106 and 107 (Scheme 2.12).

* Compounds synthesized by Dr Jamie Wolstenhulme.
Scheme 2.11 – Synthesis of ether-linked indolines 99 and 102. Indoline 93 was obtained as an inseparable mixture of diastereomers.

Scheme 2.12 – Synthesis of alkyne 105 and indolines 106 and 107.

In order to be able to make a direct comparison to a more conformationally-flexible analogue, the syn-diastereomer 107 was subsequently converted to the fully-saturated
alkane-linked compound 108 by hydrogenation over a palladium on charcoal catalyst (Scheme 2.13).

Scheme 2.13 – Hydrogenation of alkyne-linked indoline 107.

Synthesis of a Z-alkene-linked analogue posed an interesting challenge. Partial hydrogenation of the alkyne linker of 107 in a syn-selective manner using Lindlar’s catalyst was considered as a possible option. However, given the challenges frequently associated with over-reduction in this reaction, we envisaged using a stereoselective coupling instead. Fortunately, K. Oshima and co-workers had reported a hydroindation-coupling sequence to access functionalized Z-alkenes (Scheme 2.14), and within the Smith group, this reaction was applied to the total synthesis of the polycyclic alkaloid gephyrotoxin by fellow group member Shuyu Chu. Mechanistically, this transformation is based on the in situ generation of HInCl₂ from InCl₃ and DIBAL-H, which is thought to undergo radical trans-hydrometallation to the alkyne substrate in the presence of a radical initiator such as BEt₃ (Figure 2.7). The resulting Z-vinylindane can subsequently undergo Pd(0)-catalyzed Suzuki-type coupling to aryl iodides.

Scheme 2.14 – Trans-hydroindation of terminal alkynes, followed by Suzuki-type coupling to generate Z-alkenes as reported by K. Oshima.
Initially, we attempted to perform hydroindation using alkyne 105, followed by coupling to indoline 53. However, a complex mixture of products containing only a trace of the desired coupling adduct was obtained. We postulated that the presence of the basic pyrrolidine group was responsible for inducing a variety of side reactions, and alkyne 105 was therefore protonated with 1 equivalent of acetic acid before addition to the hydroindation reaction mixture (Scheme 2.15). Unfortunately, this failed to improve the outcome, and we therefore sought an alternative route to indoline 109.

Given the apparent incompatibility of alkyne 105 with this methodology, we decided to attempt the same hydroindation-coupling sequence using 6-heptyn-1-ol 103 as the alkyne component. Pleasingly, we were able to isolate the Z-alkene-linked indoline 110 as the major reaction product. The yield of this transformation could be improved by using
Pd(PPh₃)₄ as the catalyst instead of Pd₂(dba)₃/P(2-furyl)₃ (Scheme 2.16). The primary alcohol of indoline 110 was subsequently converted to a tosylate leaving group, which was displaced by pyrrolidine to afford indoline 109 (Scheme 2.17).

Scheme 2.16 – Hydroindation of 6-heptyn-1-ol 103, followed by coupling to indoline 53. The Z-geometry of the alkene linker was confirmed by analysis of the alkene-proton coupling constants.

Scheme 2.17 – Tosylation of indoline 110, followed by displacement of tosylate by pyrrolidine.

In order to have a final example of a configurationally-constrained linker, we envisaged using a copper-catalyzed click reaction to connect the indoline core and N-alkyl pyrrolidine motif through a triazole ring. The azide component of this reaction (114) was synthesized in two steps from 1,5-dibromopentane 112, and the alkyne components (118 and 119) were synthesized by Sonogashira coupling of indolines 52 and 53 with trimethylsilyl-acetylene 115, followed by TMS-deprotection with TBAF.
The combination of CuSO$_4$ and sodium-L-ascorbate has been used extensively as a reliable method of generating Cu$^+$ in situ.$^{99}$ We therefore adopted a procedure that uses this combination of reagents to carry out click reactions of indolines 118 and 119 with azide 114 (Scheme 2.20). In spite of the relatively modest yields of these transformations, we obtained sufficient material for an evaluation of biological activity.

Scheme 2.20 – Cu-catalyzed click reactions of azide 114 with indolines 118 and 119.
The impact of varying the linker on KDM2A inhibitory activity was assessed using the AlphaScreen™ and RapidFire activity assay (Figure 2.8). As for the E-alkene-linked indolines 58 and 59, the syn-diastereomers (102, 107, and 122) were all found to be more potent than their respective anti-analogues (99, 106, and 121). The observation that the ether-linked compounds (99 and 102) and the saturated alkane compound 108 displayed higher inhibitory activities than analogues with more conformationally-rigid linkers such as alkynes 106 and 107 and triazoles 121 and 122 suggests that a reasonably high degree of linker flexibility is desirable. Finally, the observed IC₅₀ value of the Z-alkene-linked indoline 109 was found to be greater than that of its E-alkene equivalent 59.

**Figure 2.8** – Effect of linker on inhibitory activity. IC₅₀ values were determined by RapidFire activity assay. pIC₅₀ values and errors are shown in parentheses.
2.2.4 Aromatic Rings at the Indoline C2 Position

Having explored the substituents at the indoline C3 position, linker, alkyl chain, and head-group, we wanted to investigate the significance of the aryl ring at the indoline C2 position. The majority of examples described so far contain a pyridyl ring at this site. In order to gauge the contribution of the pyridyl-nitrogen to inhibition, examples containing an analogously-substituted phenyl ring were synthesized. Cyclization of imine 123 afforded indolines 124 and 125 in an approximately 1:2 ratio. Both products were acetylated and underwent Stille-Migita cross-coupling with vinylstannane 57 (Scheme 2.21).

![Scheme 2.21 – Synthesis of C2-phenyl examples 128 and 129. The relative stereochemistries of indolines 124 and 125 was assigned by analogy to previously-reported similar compounds.](#)

We anticipated that a wide range of aldehydes could be condensed with aniline 47, providing access to a diverse library of indolines with different C2 substituents. However, we were limited by the low commercial availability of halogenated aromatic aldehydes and their precursors, and we therefore focused on the synthesis of indolines containing regioisomeric furyl rings at the indoline C2 position.
Condensation of aniline 47 with 4-bromo-2-furaldehyde 131 was found to proceed very slowly, even when pyrrolidine was used as a nucleophilic catalyst. Curiously, cyclization of imine 132 afforded indoline 133 as a single diastereomer, while cyclization of imine 138 gave an inseparable mixture of diastereomers. The acetylated indolines 139 and 140 could be separated by silica-gel column chromatography, and Sonogashira coupling of all acetylated products with alkyne 105, followed by hydrogenation, afforded indolines 136, 142, and 144 (Scheme 2.22).

Scheme 2.22 – Synthesis of furyl-containing indolines with both alkyne and alkane linkers.
The impact of varying the C2 aryl ring on inhibitory activity was assessed using the RapidFire assay. The diastereomeric phenyl indolines 128 and 129 had very similar IC\textsubscript{50} values, and both were found to be substantially weaker inhibitors than their pyridyl equivalents, implying that the pyridyl nitrogen plays an important role in the inhibitor-KDM2A interaction. Of the furyl analogues, the syn 5´-alkane-linked diastereomer 144 was more potent than both the anti 5´-alkane-linked diastereomer 142 and the syn 4´-alkane-linked diastereomer 136. Finally, the alkyne-linked indolines 135 and 143 were found to be weaker than their fully saturated linker equivalents 136 and 144, confirming the importance of linker flexibility for inhibitory activity (Figure 2.9).

**Figure 2.9** – Effect of C2 aryl ring on inhibitory activity. IC\textsubscript{50} values were determined by RapidFire activity assay. pIC\textsubscript{50} values and errors are shown in parantheses.
2.2.5 Selectivity

In order to determine their selectivity towards KDM2A, several indolines were tested for inhibitory activity against a representative panel of KDMs belonging to different subfamilies.* Excitingly, all compounds displayed exceptionally high selectivity towards KDM2A based on AlphaScreen™ IC\textsubscript{50} values obtained under identical conditions (Table 2.2). The high potency and selectivity displayed by indoline 59 in particular led us to pursue this compound as a promising lead for chemical probe development.

<table>
<thead>
<tr>
<th>Compound</th>
<th>KDM2A</th>
<th>KDM3A</th>
<th>KDM4A</th>
<th>KDM4C</th>
<th>KDM5B</th>
<th>KDM5C</th>
<th>KDM6B</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>0.25</td>
<td>115</td>
<td>&gt;1000</td>
<td>219</td>
<td>&gt;100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>144</td>
<td>0.24\textsuperscript{a}</td>
<td>11</td>
<td>17</td>
<td>36</td>
<td>14</td>
<td>9.9</td>
<td>14</td>
</tr>
<tr>
<td>58</td>
<td>0.28</td>
<td>&gt;1000</td>
<td>112</td>
<td>242</td>
<td>&gt;100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>0.82</td>
<td></td>
<td>39</td>
<td>36</td>
<td>247</td>
<td></td>
<td></td>
</tr>
<tr>
<td>121</td>
<td>1.8</td>
<td>&gt;1000</td>
<td>72</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2 – AlphaScreen™ IC\textsubscript{50} values (µM) under identical conditions for a panel of KDMs. \textsuperscript{[a]} IC\textsubscript{50} determined by RapidFrire activity assay.

2.3 Enantioselective Synthesis

2.3.1 A Resolution Approach

So far, all indoline-based KDM2A inhibitors had been synthesized and tested as racemic mixtures. In order to obtain optically-pure samples of both enantiomers, we wanted to carry out a chemical resolution of the indoline core. Chloroformate 147 was synthesized in 2 steps from L-(+)-mandelic acid,\textsuperscript{101} and N-acylation of indoline 50 using this chiral resolving agent afforded a mixture of diastereomers that were (just about) separable by silica-gel column chromatography (Scheme 2.23).

\textsuperscript{*} Selectivity profiling was conducted by Dr Anthony Tumber. For a statistical analysis of these results, see section 8.1 in the appendix.
We initially attempted to remove the carbamate resolving group with SmI$_2$ as a single electron source. The group of D. Proctor has demonstrated the application of SmI$_2$ as a selective reducing agent for a range of functional groups including isopropyl esters, and we anticipated that the alcohol product of this reduction would rapidly collapse to afford the deprotected, optically-pure indoline cores. Unfortunately, in spite of numerous attempts using both commercial and freshly-prepared SmI$_2$, the desired transformation was not accomplished (Scheme 2.24).

We reasoned that hydrogenolysis of the chiral resolving groups might be a possible alternative to SmI$_2$-deprotection. Indeed, Sonogashira coupling of indolines 148 and 149 with alkyne 105, followed by hydrogenolysis, concomitant reduction of the alkyne linker, and N-acetylation afforded sufficient amounts of the optically pure indolines (R,R)-108 and
(S,S)-108 for biological testing. The intermediates of this sequence were used without purification in order to expedite synthesis of the final products (Scheme 2.25). Ultimately, both indoline enantiomers were obtained with e.r. > 99:1 and were tested for inhibitory activity using the RapidFire activity assay (Table 2.3). Although there is a significant difference in potency between the enantiomers, the magnitude of this difference is smaller than what is commonly observed for many chiral chemical probes (e.g. JQ1 – 49-190 nM vs. inactive,26 LP99 – 99 nM vs. inactive,103 SGC-CBP30 – 79 nM vs. 501 nM104).

Scheme 2.25 – Telescoped synthesis of resolved, optically pure indolines (R,R)-108 and (S,S)-108.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>108</td>
<td>0.25 (6.6 ± 0.09)</td>
</tr>
<tr>
<td>(R,R)-108</td>
<td>0.39 (6.4 ± 0.04)</td>
</tr>
<tr>
<td>(S,S)-108</td>
<td>0.16 (6.8 ± 0.03)</td>
</tr>
</tbody>
</table>

Table 2.3 – Inhibitory activities of enantiomers of indoline 108. Absolute stereochemistry is assigned retrospectively (see subsection 2.3.3). pIC50 values and errors are shown in parentheses.
2.3.2 **Enantioselective PTC – Effect of Substrate Structure**

Given the limitations of chiral resolution, we envisaged an alternative approach to the enantioselective synthesis of lead compounds that would be based on asymmetric catalysis. As described previously, a particular area of interest within the Smith group is the use of chiral, cationic phase-transfer catalysts to influence the stereochemical outcome of cyclization reactions (see Subsection 1.4.2). At the outset of this project, a substantial amount of research into the enantioselective cyclization of imines based on 2-phenylacetonitrile-anilines had already been carried out (Scheme 2.26).

![Scheme 2.26](image)

**Scheme 2.26** – Chiral cation controlled enantioselective cyclization of imines to form indolines. Use of the pseudoenantiomeric phase-transfer catalyst affords the opposite enantiomer.\(^{74}\)

A detailed analysis of the kinetics of this reaction by group members Krishna Sharma and Dr Jamie Wolstenhulme revealed a complex catalytic mechanism. It was proposed that the quininium phase-transfer catalyst both initiates and participates in an autocatalytic reaction cycle (Figure 2.10). Initially, deprotonation of the imine substrate occurs at the interfacial boundary and the resulting anion is extracted into the organic phase by the catalyst, where it undergoes enantioselective cyclization to form an indolinyl anion. This indolinyl anion can deprotonate the catalyst to generate a zwitterionic species in a rapid and irreversible manner. As a zwitterion, the catalyst can deprotonate another molecule of substrate in the organic phase and thereby continue the reaction cycle. Finally, regarding the imine cyclization itself, quantum calculations based on density functional theory (DFT)
suggest that the transformation proceeds via a formally-disfavored 5-endo-trig\textsuperscript{105} ring-closure as opposed to a 6π-electrocyclization.

![Proposed catalytic cycle of imine cyclization](image)

**Figure 2.10** – Proposed catalytic cycle of imine cyclization, based on reaction kinetics. This figure is conceptually reproduced from M. D. Smith et al. JACS. 2015, 137 (41).

We initially attempted to apply the optimized reaction conditions for these 2-phenylacetonitrile-aniline-derived aldimines to the cyclization of imine 48. Unfortunately, only a very modest e.r. was attained with this substrate (Scheme 2.27). A limited screen of bases, reaction conditions, and catalysts failed to improve the outcome.

**Scheme 2.27** – Application of previously discovered optimized conditions to the cyclization of imine 48 resulted in only a minor enantiomeric excess.
In order to investigate the extent to which pyridyl substituents and regiochemistry might affect enantioselectivity, we attempted the cyclization of imines 153 and 155 under similar reaction conditions. In both cases, a marginal improvement in enantioselectivity was observed (Scheme 2.28 – A,B). Although these encouraging results did not address the fundamental synthetic issue at hand, we concluded that modifying the pyridyl ring would be the most promising approach to achieving higher levels of asymmetric induction. This hypothesis was ultimately confirmed by the cyclization of the O\textit{Bn}-pyridyl imine 92 to afford indoline 93 with a very promising e.r. (Scheme 2.28 – C). We anticipated that indoline 93 would be a useful precursor to lead compounds, and we therefore concentrated efforts on improving the enantioselectivity of this reaction by exploring conditions and catalysts.

Scheme 2.28 – Effect of pyridyl substitution on enantioselectivity of imine cyclization.

2.3.3 **Enantioselective PTC – Screening and Synthesis**

To begin with, we attempted to improve the enantioselectivity of the cyclization of imine 92 to indoline 93 by investigating the catalyst component of this reaction. The most

* These investigations were carried out together with Dr Jamie Wolstenhulme.
common core scaffold of chiral phase transfer catalysts is based on the cinchona alkaloid structure, although several alternative scaffolds based on axially-chiral BINOL derivatives and other motifs have also been developed over the years. In order to expedite screening, reactions were performed on a relatively small scale (40 mg imine 92), and only the enantioselectivity of each reaction was initially evaluated (Figure 2.11). In general, catalysts based on the quinine scaffold performed better than catalysts based on quinidine, and the Maruoka\textsuperscript{107} and Lygo\textsuperscript{108} catalysts (I and J) were largely ineffective at controlling product stereochemistry. The highest level of enantioselectivity was attained with the quininium salt F.

**Figure 2.11** – Screen of phase-transfer catalysts for the cyclization of imine 92 to indoline 93.
Having identified the best catalyst, we conducted a screen of solvents, catalyst concentrations, and temperatures. Key findings are summarized here and in Table 2.4:

1. Of all the investigated solvents, toluene afforded the most favorable balance of reactivity and selectivity.

2. Lowering the catalyst loading to 5 mol % caused a complete loss of conversion at -50 °C, but led to improved enantioselectivity at -30 °C. Raising the catalyst loading to 20 mol % resulted in a loss of enantioselectivity at -30 °C.

3. Addition of CH₂Cl₂ to the reaction mixture (10% v/v) largely recovered reactivity at -50 °C, but led to a dramatic drop in enantioselectivity.

![Catalyst Reaction Scheme](image)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Catalyst loading (eq)</th>
<th>Temperature (°C)</th>
<th>e.r. (93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhMe</td>
<td>0.1</td>
<td>-50</td>
<td>86:14</td>
</tr>
<tr>
<td>iPrO</td>
<td>0.1</td>
<td>-50</td>
<td>No reaction</td>
</tr>
<tr>
<td>PhF</td>
<td>0.1</td>
<td>-35</td>
<td>53:47</td>
</tr>
<tr>
<td>9:1 PhMe/iPrOH</td>
<td>0.1</td>
<td>-50</td>
<td>51:49</td>
</tr>
<tr>
<td>PhMe</td>
<td>0.05</td>
<td>-50</td>
<td>No reaction</td>
</tr>
<tr>
<td>PhMe</td>
<td>0.05</td>
<td>-30</td>
<td>89:11</td>
</tr>
<tr>
<td>PhMe</td>
<td>0.2</td>
<td>-30</td>
<td>84:16</td>
</tr>
<tr>
<td>9:1 PhMe/CH₂Cl₂</td>
<td>0.05</td>
<td>-50</td>
<td>64:36</td>
</tr>
</tbody>
</table>

*Table 2.4 – Screen of solvents, catalyst loadings, and temperatures.*

We applied the optimized reaction conditions to the cyclization of imine 92 in the presence of catalyst F and its pseudoenantiomer C. On a relatively small reaction scale (50 mg imine 92), enantiomeric ratios of 89:11 and 15:85 were obtained, and the diastereomeric ratio exceeded 20:1 for both catalysts. Augmenting the scale of these reactions to 1 gram of imine 92 led to a slight drop in both e.r. and d.r. (Scheme 2.29). Nevertheless, we were satisfied that the enantioenrichment would be sufficient in both
cases to enable an effective resolution either by recrystallization or semi-preparatory chiral HPLC.

Scheme 2.29 – Optimized reaction conditions for the enantioselective cyclization of imine 92.

Attempts to improve the enantiomeric excess by recrystallization proved unsuccessful. However, we succeeded in identifying conditions that enable the separation of both enantiomers of 93 on a YMC amylose-SA semi-preparatory HPLC column. This allowed us to obtain the two enantiomeric forms of indoline 93 in e.r. > 99:1. N-Acetylation of both enantiomers and hydrogenolysis of the O-linked benzyl groups afforded the common intermediates (R,R)-100 and (S,S)-100, which would serve as precursors to optically-pure KDM2A inhibitor candidates. (Scheme 2.30). The absolute stereochemistry of these common intermediates was assigned by X-ray crystallography (Figure 2.12).

Scheme 2.30 – Synthesis of optically-pure intermediates (S,S)-100 and (R,R)-100.
Figure 2.12 – X-ray crystal structure of HCl salt of indoline (S,S)-100.

The two enantiomeric forms of indoline 102 could be obtained in e.r. > 99:1 by O-alkylation of (S,S)-100 and (R,R)-100 with 1,7-dibromohexane, followed by substitution with pyrrolidine (Scheme 2.31). An evaluation of KDM2A inhibition using the RapidFire activity assay revealed that the (2S,3S)-indoline core (derived from imine cyclization catalyzed by F) is more potent than the (2R,3R)-indoline core (derived from imine cyclization catalyzed by C) (Table 2.5).

Scheme 2.31 – Synthesis of optically-pure KDM2A inhibitors (S,S)-102 and (R,R)-102.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S,S)-102</td>
<td>0.39 (6.4 ± 0.06)</td>
</tr>
<tr>
<td>(R,R)-102</td>
<td>0.84 (6.1 ± 0.07)</td>
</tr>
</tbody>
</table>

Table 2.5 – Inhibitory activities of enantiomeric indolines (S,S)-102 and (R,R)-102.

* X-ray diffraction data was collected and the structure solved by John Joliffe.
Finally, the optically-pure indoline \((S,S)-108\), previously obtained via a chiral resolution of the indoline core (subsection 2.3.1), could be synthesized from intermediate \((S,S)-100\). The hydroxyl substituent of the pyridyl ring was converted to a triflate group in good yield, and Sonogashira coupling with alkyne 105, followed by hydrogenation, afforded indoline \((S,S)-108\) in e.r. > 99:1 (Scheme 2.32). The identity of this compound could be confirmed by comparison of the HPLC retention time with that of the more potent enantiomer obtained via chiral resolution (IC\(_{50}\) = 0.16 µM). An extensive activity screen using a panel of representative KDMs confirmed that \((S,S)-108\) is a highly selective inhibitor of KDM2A (Table 2.6). In addition, \((S,S)-108\) was found to be inactive towards a representative panel of methyl-lysine binding domains.\(^*\)

### Scheme 2.32 – Synthesis of optically-pure \((S,S)-108\) from intermediate \((S,S)-100\).

### Table 2.6 – AlphaScreen™ IC\(_{50}\) values (µM) of \((S,S)-108\) against a panel of KDMs.\(^†\)

<table>
<thead>
<tr>
<th>KDM2A</th>
<th>KDM3A</th>
<th>KDM4A</th>
<th>KDM4C</th>
<th>KDM4D</th>
<th>KDM5B</th>
<th>KDM5C</th>
<th>KDM6B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>39</td>
<td>639</td>
<td>430</td>
<td>321</td>
<td>17</td>
<td>16</td>
<td>20</td>
</tr>
</tbody>
</table>

With a robust asymmetric synthetic strategy towards a promising lead compound in hand, we turned our attention to the evaluation of the potential biological activity of these KDM2A inhibitors within cells. The next chapter of this thesis will focus on attempts to modulate both on-target and off-target cellular activity by altering key molecular features in a rational manner.

\(^*\) MBD screening was conducted at the SGC-Toronto by Guillermo Sensteinra and Dr Masoud Vedadi.

\(^†\) For a statistical analysis of the data, see section 8.1 in the appendix.
3. **LEAD OPTIMIZATION**

3.1 **Cellular Activity**

3.1.1 **Inhibition of Histone Demethylation**

Cellular inhibitory activity was measured using an immunofluorescence (IF) assay to quantify H3K36me2 in HeLa cells. Experiments were performed with two cell lines: wild-type (WT) cells containing catalytically-active KDM2A, and mutant (MUT) cells containing constitutively-inactive KDM2A. Excitingly, when indolines \((S,S)-102\) (IC\(_{50}\) 0.39 µM) and \((S,S)-108\) (IC\(_{50}\) 0.16 µM) were tested in this assay, both compounds demonstrated a dose-dependent effect on cellular H3K36me2 levels in WT cells (Figure 3.1). Indoline \((S,S)-108\) was particularly promising, as it induced a significant increase in the intracellular concentration of H3K36me2 at relatively low compound concentrations (39 ± 11 % increase at approx. 1 µM). As a control, the pan-2-OG dependent deoxygenase inhibitor IOX1\(^{46}\) was tested in the same assay and was found to inhibit demethylation of H3K36me\(_2\) at considerably higher concentrations (＞15 µM). However, it is uncertain whether this is a consequence of KDM2A inhibition or the effect of general cytotoxicity.

Cytotoxicity towards HeLa cells was measured by monitoring overall cell numbers over a range of compound concentrations. Indolines \((S,S)-102\) and \((S,S)-108\) were found to be cytotoxic when dosed above 10 µM, so that their effects on cellular H3K36me2 levels at higher concentrations (＞5 µM) should be viewed with caution, as these may not entirely reflect on-target activity. In order to better understand the nature of both on-target and

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* These experiments were done by Dr Stephanie Hatch, Target Discovery Institute.
off-target contributions to cellular activity, we decided to take advantage of recent advances in the field of quantitative transcriptomics.

Figure 3.1 – Inhibition of H3K36me2 demethylation in HeLa cells: A) Structures of KDM2A inhibitors (S,S)-102 and (S,S)-108, and pan 2-OG deoxygenase inhibitor IOX1. B) IF assay and cytotoxicity of IOX1. C) IF assay and cytotoxicity of (S,S)-102. D) IF assay and cytotoxicity of (S,S)-108.
3.1.2 Transcriptomics*

Effects on gene expression levels were explored using a highly multiplexed 3’ mRNA sequencing method as previously described by Gapp and co-workers.\(^{109}\) Within a diverse panel of in-house compounds, our series of indoline-containing KDM2A inhibitors was represented by \((S,S)-108\) and 89, which we considered to be a suitable structurally-analogous “inactive” control (IC\(_{50}\) 17 µM). When dosed at a concentration of 10 µM, both molecules influenced expression levels of more than 200 genes in HAP1 cells. However, at a concentration of 1 µM, only the active analogue \((S,S)-108\) had a significant effect on expression levels (Figure 3.2-A). We postulate that this concentration dependence may be a consequence of predominantly off-target effects at high concentrations, as opposed to a more specific effect resulting from KDM2A inhibition at low concentrations. The overlap of the gene expression signatures of \((S,S)-108\) and 89 is depicted in Figure 3.2-B.

![Chemical structures](image)

**Figure 3.2** – Quantitative transcriptomics analysis of gene expression changes caused by \((S,S)-108\) and 89: A) Total number of genes with altered expression levels. B) Overlap of gene expression changes.

* Experiments and data analysis were done by Barbara Mair and Dr Thomas Konopka respectively.
We concluded that, while our lead compound \((S,S)-\textbf{108}\) is clearly able to penetrate into cells and influence H3K36me2 demethylation, it is not suitable for use as a chemical probe due to the magnitude of off-target activity at higher compound concentrations, which we felt would interfere with studies investigating the roles of KDM2A in biology. We therefore sought to develop an inhibitor with a wider on-target activity window\(^*\) by modifying the structure of \((S,S)-\textbf{108}\) so as to reduce the number and magnitude of non-specific interactions within cells.

3.2 Addressing Off-Target Activity and Cytotoxicity

3.2.1 Linker Modifications

In 1997, C. A. Lipinski published a review article analyzing various physical and chemical properties of drug candidates that had successfully advanced to phase II clinical trials.\(^{110}\) The insights provided by this landmark publication continue to impact common practices in medicinal chemistry today by influencing library design and guiding strategies for lead optimization. Specifically, Lipinski proposed a set of guidelines (commonly known as the “rule-of-5”) to help identify hits and lead compounds that would be more likely to progress through the drug discovery pipeline as a result of favorable physicochemical and pharmacokinetic properties:

1) Fewer than 5 H-bond donors (i.e. O-H and N-H bonds)

2) Molecular weight < 500 g mol\(^{-1}\).

3) \(\log P \leq 5\).

4) < 10 H-bond acceptors (i.e. O and N atoms).

\(^*\) Range of compound concentrations over which cellular effects are predominantly due to KDM2A inhibition (i.e. on-target) as opposed to non-specific interactions (i.e. off-target).
Although it was never our intention to develop a molecule that could potentially form the basis of a drug discovery program, we reasoned that the insights gained from Lipinski’s analysis could also be applied to the development of chemical probes. In particular, we recognized that most of our indoline-based KDM2A inhibitors were characterized by both a higher molecular weight and ClogP than recommended by the “rule-of-5” guidelines (Figure 3.3). This particular combination of traits did not appear to limit compound solubility; however, we attributed the observed off-target activity and cytotoxicity of indoline (S,S)-108 to its relatively high lipophilicity (ClogP = 5.88)*111. Our reasoning was largely based on the work of J. D. Hughes and co-workers, who observed a strong correlation between logP and in vivo toxicity and identified compound promiscuity as a key contributor to this trend.112 We therefore focused our efforts on identifying chemical modifications to (S,S)-108, which would increase polarity and thereby improve the ratio of on-target to off-target activity.

Figure 3.3 – The molecular weight and ClogP of indoline (S,S)-108 are both higher than recommended according to Lipinski’s guidelines.

We considered the saturated hydrocarbon chain of indoline (S,S)-108 and its analogues to be the most obvious molecular feature that we could modify in order to reduce lipophilicity. Initially, we hoped that replacing the alkyl chain of 108 with a more polar polyethylene-glycol (PEG) linker would be a viable solution. Indoline 159

*Calculated using the Molinspiration™ cheminformatics web-based tool (www.molinspiration.com).
(ClogP = 3.30) was therefore synthesized from racemic 100 and tested for inhibitory activity against KDM2A (Scheme 3.1). Unfortunately, a dramatic loss of potency was observed, suggesting that simply incorporating multiple heteroatoms into the linear alky chain was unlikely to represent a viable strategy for reducing lipophilicity. Attempts were also made to introduce an amide linker via Pd-catalyzed carbonylative coupling\textsuperscript{113} of indoline 53 and carboxylation using Knochel’s “turbo Grignard” reagent.\textsuperscript{114} However, neither of these methods yielded the desired products in our hands (Scheme 3.2). In order to accelerate the discovery of modifications that would increase polarity while maintaining potency, we turned to a rational-design approach based on computational docking.

![Scheme 3.1](image1)

\textbf{Scheme 3.1} – Synthesis of racemic PEG-linked indoline 159.

![Scheme 3.2](image2)

\textbf{Scheme 3.2} – Attempted synthesis of amide linkers via carbonylative coupling or carboxylation of indoline 53.
3.2.2 Computational Docking

Computational docking has become an increasingly important tool in drug discovery, as it enables the prediction of ligand binding conformations and important ligand-protein interactions. When combined with structural insights from X-ray crystallography, it represents a powerful strategy for both hit identification and lead optimization. This is perhaps best illustrated by the computer-aided discovery of a highly potent series of non-nucleoside HIV reverse transcriptase inhibitors by K. S. Anderson and W. L. Jorgensen using a combination of virtual screening and computational docking of inhibitor candidates.\(^{115}\)

In spite of numerous attempts, the acquisition of an X-ray co-crystal structure of indoline (S,S)-108 bound to KDM2A was not accomplished.\(^*\) In order to obtain a structural insight into the inhibitor-protein interaction, we therefore relied on computational docking using X-ray crystal structures of KDM2A in both apo\(^{116}\) and peptide-bound\(^{38}\) states available through the protein data bank (PDB).\(^{117}\) We made the assumption that (S,S)-108 would bind within the active site of the enzyme’s JmjC domain, and we initially used the open-source program AutoDock Vina\(^{118}\) to predict the most likely binding conformation within this area. AutoDock Vina is a grid-based docking program, which samples all possible ligand positions and conformations within a predefined region of the protein target and allocates a “score” based on steric repulsion, coulombic forces, and van der Waals interactions. In these scoring calculations, the protein structure is represented as a static grid, so that conformational changes of the protein upon ligand binding are not considered. Similarly, the solvent is approximated as a continuous medium, and solvent contributions to both the

\(^*\) X-ray crystallography trials with KDM2A were carried out by researchers at the SGC.
enthalpy and entropy of binding consequently do not feature in the overall scoring algorithm.

Figures 3.4 illustrates the highest-scoring docking solution using indoline \((S,S)-108\) and the peptide-bound structure of KDM2A (PDB code: 4QXC). In this proposed complex, the protonated \(N\)-alkyl pyrrolidine group extends into the peptide binding pocket, mimicking the dimethyl-lysine sidechain of the peptide substrate. The indoline core adopts a conformation that overlaps with the remainder of the peptide (Figure 3.5). Although we did not identify any specific interactions that would be consistent with our SAR studies (e.g. significance of the \(C3\) substituents and pyridyl ring), the need for a conformationally-flexible chain linking the indoline core and the pyrrolidine head group could potentially be explained by the presence of a narrow channel through which the dimethyl-lysine sidechain is threaded to access the enzyme’s catalytic site.

![Figure 3.4](image)

**Figure 3.4** – Docking solution of indoline \((S,S)-108\) in KDM2A active site using *AutoDock Vina*. 
While we recognized the limitations of computational docking as a method of obtaining accurate structural information, we envisaged using it as a tool to identify potential modifications to the inhibitor’s alkyl chain. A virtual screen of analogues of (S,S)-108 derived from commercially-available or easily accessible poised linker synthons was conducted using the docking program Molsoft-ICM. In general, modifications that constrained linker flexibility resulted in poor docking scores, and identifying synthetically-tractable alterations that would increase polarity proved challenging. Eventually, exploring possible changes in the vicinity of the pyrrolidine head-group revealed that a β-hydroxyl motif could potentially undergo a number of favorable H-bonding interactions with key residues in the KDM2A active site (Figure 3.6). We therefore identified indoline 160 as a promising synthetic target. The presence of an additional stereogenic center on the alkyl chain posed a challenge, and we decided it would be important to consider a number of possible stereoisomers of 160 in order to maximize the likelihood of discovering an improved KDM2A inhibitor.

* Work done in collaboration with Helene Pierson, under the supervision of Dr Brian Marsden, SGC.
Scheme 3.3 illustrates the synthesis of the enantiomeric alkyne fragments 163 and 164 from the chiral pool reagents (R)- and (S)-epichlorohydrin. The chemoselectivity of reactions involving various nucleophiles and epichlorohydrin has been studied extensively, as it dictates the absolute stereochemistry of the reaction product.\textsuperscript{120} Direct displacement of chloride results in overall retention of configuration, whereas epoxide-opening followed by intramolecular substitution results in inversion of configuration. K. Urata demonstrated that complete inversion of configuration could be achieved with oxygen-based nucleophiles under phase-transfer catalysis,\textsuperscript{121} and this methodology was later applied to the synthesis of analogues of the fatty-acid derived acetogenin class of natural products by R. L. Grée.\textsuperscript{122} Our choice of reagents and reaction conditions draws on the insights provided by these investigations.
Scheme 3.3 – Synthesis of enantiomeric alkynes 163 and 164 from R(-)-epichlorohydrin and S(+)-epichlorohydrin respectively.

Alkynes 163 and 164 subsequently underwent Sonogashira coupling with optically-pure indolines (R,R)-157 and (S,S)-157, to afford all possible stereoisomeric products featuring a syn-arrangement of functional groups at the indoline C2 and C3 positions. Reduction of the alkyne linker of each product by hydrogenation over a palladium on charcoal catalyst afforded the stereoisomeric alkane-linked compounds 166, 168, 170, and 172 (Scheme 3.4).

Scheme 3.4 – Synthesis of stereoisomeric alkyne- and alkane-linked KDM2A inhibitor candidates, containing linker modifications suggested by computational docking.
Unfortunately, biological testing revealed that all isomers were substantially weaker inhibitors of KDM2A than analogues with unfunctionalized hydrocarbon chains (Figure 3.7). This setback highlights the limitations of using computational docking to assist inhibitor discovery. In our case, the conclusions derived from *in silico* screening and docking were ultimately based on flawed assumptions and approximations. In particular, our hypothesis that the inhibitors occupy the peptide binding site would eventually be invalidated using a variety of experimental techniques (see Chapter 4).

**Figure 3.7** – RapidFire IC₅₀ values for isomeric inhibitor candidates incorporating linker modifications suggested by computational docking. pIC₅₀ values and errors are shown in parentheses.
### 3.2.3 Indoline Core Modifications

Given the apparent challenge of introducing polar modifications to the linker and alkyl chain without compromising inhibitory activity, we decided to focus our efforts on incorporating more heteroatoms in the indoline core itself. Previous work in the Smith group by Dr Alan Lamb explored enantioselective synthetic approaches to 4- and 6-aza-indolines, and we therefore envisaged synthesizing potential KDM2A inhibitors based on these scaffolds.

To begin with, regioisomeric aminopyridines 175 and 177 were synthesized in two steps from the relevant chloropyridine starting reagents. Benzyl cyanide and stoichiometric TDA-1 were used in both SNAr reactions, and the resulting products were reduced to the desired anilines using zinc powder and NH₄Cl (Scheme 3.5). TDA-1 is a “trident” phase-transfer catalyst initially reported by G. Soula and first applied to substitution reactions of chloropyridines by P. Ballesteros.

![Scheme 3.5 – Synthesis of aminopyridines 175 and 177.](image)

Condensation of aminopyridine 175 with 5-bromo-3-pyridinecarboxaldehyde afforded the desired imine product 178, albeit after a longer reaction time than required for the equivalent aniline 47. Conversely, condensation of aminopyridine 177 with 5-bromo-3-pyridinecarboxaldehyde was not achieved under a variety of reaction conditions.
The lack of reactivity of 4-substituted aminopyridines with aromatic aldehydes had also been encountered by Dr Alan Lamb in the course of his investigations, and we therefore decided to focus our synthetic efforts on generating aza-indolines from imine 178. Cyclization of 178 under racemic reaction conditions afforded an inseparable mixture of diastereomers, which were subsequently acetylated using acetyl chloride and pyridine. The major syn diastereomer was isolated and subjected to a Stille-Migita cross-coupling reaction with vinylstannane 57 to afford aza-indoline 181 ($\text{ClogP} = 4.95$) in moderate yield (Scheme 3.6).

**Scheme 3.6** – Synthesis of aza-indoline 181. The relative stereochemistry of intermediate 180 was assigned by analogy to indolines 52 and 53.

As an alternative approach to increasing the polarity of the indoline ring system, we envisaged incorporating a polar aromatic heterocycle as one of the substituents on the C3 position. To this end, synthesis of the thiazole-containing indoline 189 was accomplished (Scheme 3.7). Nucleophilic aromatic substitution of 2-chlorothiazole 182 by 2-nitrophenylacetonitrile 183 proved challenging, and the low yield of this transformation is attributed to a number of side reactions involving the initial product 184. Reduction of 184 by hydrogenation over a palladium on charcoal catalyst did afford the desired aniline,
but partial decomposition of this product in the course of silica gel column chromatography made complete purification of \textbf{185} difficult. Condensation of the impure aniline with 5-bromo-3-pyridinecarboxaldehyde was found to proceed very slowly (approx. 15% conversion after 48 h), and instead of the expected imine \textbf{186}, indoline \textbf{189} was obtained as a single diastereomer. This suggests that imine \textbf{186} undergoes spontaneous cyclization once formed, possibly \textit{via} a zwitterionic intermediate (\textbf{187} and \textbf{188}) as previously proposed for imine \textbf{75} (Subsection 2.2.2). A trace of aminal \textbf{190} was also observed, although the amount of isolated material was insufficient to enable complete characterization. To complete the synthesis of the final KDM2A inhibitor candidate \textbf{193} (ClogP = 4.39), \textbf{189} was acetylated to afford indoline \textbf{192}, which underwent Stille-Migita cross-coupling with vinylstannane \textbf{57} (Scheme 3.8).

\begin{center}
\textbf{Scheme 3.7} – Synthesis of thiazole-containing indoline \textbf{189}. Imine \textbf{186} was not isolated, as it underwent spontaneous cyclization to indoline \textbf{189} and aminal \textbf{190}. The stereochemistry of indoline \textbf{189} was tentatively assigned by analogy to indolines \textbf{49} and \textbf{50}.
\end{center}
Scheme 3.8 – Synthesis of thiazole-containing KDM2A inhibitor candidate 193.

Aza-indoline 181 and thiazole 193 were tested for inhibition of KDM2A using the RapiFdire assay. Unfortunately, both compounds were found to be substantially weaker inhibitors than their more lipophilic analogues (Figure 3.8). We concluded that a more methodical approach would be required to identify a successful strategy for incorporating polar functionality while maintaining inhibitory activity.

Figure 3.8 – RapidFire IC₅₀ values of aza-indoline 181 and thiazole 193, along with pIC₅₀ values and errors. Activities of the previously-discussed indolines 58 and 59 are shown for comparison.

3.2.4 N-Acyl Group Modifications

Since Lipinski’s seminal analysis highlighting the relationships between certain physical and chemical properties of pharmaceutical candidates and their likelihood of success in the drug discovery pipeline, lipophilic efficiency (LiPE) has emerged as a particularly insightful parameter with respect to lead optimization.¹²⁶ It was first defined
by P. D. Leeson and B. Springthorpe according to the equation: \( \text{LiPE} = pIC_{50}^* - \log P \). It is important to recognize that on-target biological activity depends on the precise interplay of both enthalpic and entropic contributions to the relevant ligand-protein interaction.

That said, due to the importance of the hydrophobic effect in ligand binding, increasing compound lipophilicity generally results in enhanced biological activity. As discussed previously, this enhanced activity often arises at the expense of greater promiscuity and hence toxicity. LiPE is therefore a measure of how efficiently a molecule is able to balance the desirable traits of high biological activity and moderate lipophilicity.

Figure 3.9 shows a LiPE plot of our indoline-based KDM2A inhibitors divided into 2 compound series. Series A corresponds to molecules featuring different indoline C2/C3 substituents and linker motifs, while molecules in series B contain different indoline N-acyl groups. For the compounds in series A, we observed a general correlation between biological activity and lipophilicity: Reducing ClogP led to a loss of inhibitory activity. In contrast, altering the identity of the N-acyl group appeared to have a much less pronounced effect on inhibition. We therefore concluded that the N-acyl group would offer the most promising handle for introducing polarity without compromising inhibitory activity, and we envisaged incorporating a selection of polar functional groups at the carbonyl α-position (Scheme 3.9).

Scheme 3.9 – Modular synthetic approach towards incorporating polar functionality in the indoline N-acyl motif.

* \( pIC_{50} = -\log([IC_{50} (\mu M)] \).
† Series B compounds were synthesized by Dr Jamie Wolstenhulme.
The previously-established limited reactivity of the indoline nitrogen necessitated the synthesis of α-functionalized acetyl chlorides as acylating reagents. Thus, incorporation of a cyano group was accomplished by in situ formation of 2-cyanoacetyl chloride from its carboxylic acid precursor, followed by reaction with the indoline core 50. Sonogashira coupling with alkyne 105 and hydrogenation over a palladium on charcoal catalyst afforded the saturated alkyl-linked indoline 196 (Scheme 3.10).

**Scheme 3.10** – Synthesis of α-cyano-acetyl indoline 196.
We envisioned the preparation of a hydroxylated analogue through N-acylation of indoline 50 with benzyloxyacetyl chloride 197, followed by Sonogashira coupling, alkyne reduction, and deprotection. We chose to employ a benzyl protecting group, as we anticipated that this would enable simultaneous reduction and deprotection in a single step. Initial acylation and Sonogashira coupling afforded indoline intermediate 199, albeit in modest yield. Treatment of this intermediate with hydrogen under Pd/Rh catalysis was successful in effecting alkyne double reduction; however O-debenzylation was not observed. Attempts to deprotect the hydroxyl group via high-pressure hydrogenolysis over a variety of catalysts and subsequently with BF$_3$/Na$^{129}$ proved unsuccessful (Scheme 3.11).

![Scheme 3.11 – Synthesis of alkyne-linked indoline 199 and unsuccessful benzyl-ether deprotection.](image)

We therefore decided to adopt an alternative protecting group strategy and synthesized the TBS-protected indoline 203 from glycolic acid. Sonogashira coupling of 203 with alkyne 105 afforded indoline 205 in moderate yield. However, deprotection of the silyl ether with TBAF gave rise to a mixture of inseparable products 206 and 207 (Scheme 3.12). Moreover, the amide bond of the desired indoline 206 was found to be highly susceptible to cleavage even under relatively mild conditions, such as those encountered in the course of aqueous work-up with NaHCO$_3$ and purification by silica gel column chromatography. This suggests that even if the alkyne linker of 207 could be reduced cleanly, the lack of
stability of the alkyl-linked product means that it is unlikely to be a suitable KDM2A chemical probe candidate.

Scheme 3.12 – Synthesis of alkyne-linked indoline 205. TBS-deprotection by TBAF afforded the desired product 206, which was susceptible to uncontrolled cleavage of the amide bond.

We reasoned that replacing the hydroxyl group in 206 with a less electron-withdrawing primary amine would improve stability towards uncontrolled cleavage of the amide bond. The amine would be masked in the form of a comparatively unreactive phthalimide group, which we anticipated could be removed with N₂H₄ at the end of the synthetic sequence. Thus, indoline 50 was acylated with α-(N-phthalimido)-acetyl chloride 209 to afford indoline 210, which underwent Sonogashira coupling followed by hydrogenation of the alkyne linker. The resulting product 212 was cautiously treated with aqueous N₂H₄ to afford the indoline 213 (ClogP = 4.64) in reasonably good yield (Scheme 3.13). At first glance, 213 appeared to be substantially more stable than its hydroxyl-containing analogue would likely have been, although some cleavage of the amide bond was observed after prolonged exposure to N₂H₄.
Finally, the acetyl-free indolines 207 and 214 were synthesized to provide further insight into the importance of the N-acyl group with respect to inhibition (Scheme 3.14). These compounds are predicted to be more lipophilic than their acetylated analogues and were found to undergo gradual oxidation to indolenines and other products upon exposure to air. Nonetheless, we anticipated that an evaluation of their biological activities would be instructive.

Scheme 3.13 – Synthesis of α-aminoacetyl-containing indoline 213.

Scheme 3.14 – Synthesis of acetyl-free indolines 207 and 214.

The effect of varying the indoline N-acyl group on inhibitory activities was evaluated using the RapidFire assay (Figure 3.10). Excitingly, α-aminoacetyl-indoline 213 was found
to be a substantially more potent inhibitor than acetyl-indoline 108 (213: IC$_{50}$ 0.12 μM; LiPE 2.3 vs. 108: IC$_{50}$ 0.25 μM; LiPE 0.7). We postulate that the greater inhibitory activity of 213 is a consequence of electrostatic or H-bonding interactions between the primary amine and either residues in the enzyme binding site or ambient solvent molecules. Replacing the acetyl group of 108 with an α-cyanoacetyl motif led to a drop in potency. However, the magnitude of this change was relatively small when compared to the observed losses of potency as a result of increasing the polarity of the indoline core or linker (subsections 3.2.1, 3.2.3). Finally, removing the acetyl group completely led to a marginal improvement in inhibitory activity at the expense of increased lipophilicity (214: IC$_{50}$ 0.20 μM, LiPE 0.4). Together, these observations appear to confirm our hypothesis that the indoline N-acyl group provides a promising handle for modulating polarity without compromising inhibition of KDM2A.

Figure 3.10 – RapidFire IC$_{50}$ and ClogP values of KDM2A inhibitors with different indoline N-acyl groups. pIC$_{50}$ values and errors are shown in parentheses.
Given the susceptibility of the α-hydroxyacetyl amide bond to hydrolysis under relatively mild conditions, we were concerned that a similar effect would occur with indoline 213. We therefore monitored a 20 mM aqueous solution of 213 at rt by LCMS. Unfortunately, even after a period of 1 hour, hydrolysis of the amide bond to afford indoline 214 was observed. 214, in turn, undergoes gradual oxidation to the corresponding indolenine and other products. This decomposition led to a marked drop in inhibitory activity, and retesting the aqueous stock solution of 213 gave rise to IC\textsubscript{50} values of 0.36 μM and 2.0 μM after one and two freeze-thaw cycles respectively. A possible explanation for the lability of the amide bond in 213 is the occurrence of intramolecular general base catalysis involving the terminal amino group (Scheme 3.11).

Scheme 3.11 – Facile hydrolysis of the amide bond of 213, possibly as a result of general base catalysis.

The requirement for chemical probes to demonstrate unambiguous, on-target cellular activity presented a particularly difficult challenge in this project. While we were ultimately unable to provide a convincing solution to this problem, we identified a promising strategy for modulating key physical properties of lead compounds while maintaining inhibitory activity towards KDM2A. We anticipate that future work based on this approach could eventually yield a unique KDM2A chemical probe candidate (subsection 4.3.4). The next chapter of this thesis will primarily focus on exploring the mechanism of KDM2A inhibition using a broad range of experimental techniques.
4. MECHANISM OF INHIBITION

4.1 Identifying the Inhibitor Class

4.1.1 Inhibitor Demethylation

Computational docking of (S,S)-108 in the KDM2A active site suggests that the inhibitor’s pyrrolidine head-group mimics the dimethyl-lysine sidechain of the histone substrate (Subsection 3.2.2). We therefore reasoned that a dimethylamine head-group might be susceptible to catalytic demethylation. To test this hypothesis, dimethylamine-capped indoline 216 was synthesized from alkyl bromide 101 (Scheme 4.1). As expected, indoline 216 was found to be a weaker inhibitor of KDM2A than its pyrrolidine-capped analogue (100: IC50 0.29 μM).

Scheme 4.1 – Synthesis of dimethylamine-capped indoline 216 and possible demethylation by KDM2A.

216 was incubated with up to one equivalent of KDM2A in the presence of excess 2-OG and FeII and monitored by mass spectrometry for enzyme-catalyzed demethylation; however, no conversion of 216 to 217 was observed. While we realized that this absence
of reactivity did not necessarily invalidate our hypothesis regarding inhibitor occupancy of the peptide binding site, it highlighted the need for further considerations of the potential modes of enzyme inhibition.

4.1.2 Kinetics of Enzyme Inhibition

The demethylation of H3K36me2 and its peptidic analogue by KDM2A most likely proceeds via an ordered, sequential mechanism. Crystallographic and spectroscopic investigations on closely related 2-OG deoxygenases are consistent with initial coordination of 2-OG to Fe$^{II}$ in the active site, followed by consecutive binding of the peptide substrate and O$_2$.\textsuperscript{130,131} Figure 4.1 summarizes the possible modes of inhibition based on this catalytic mechanism. In addition, work by W. W. Cleland\textsuperscript{132} and J. S. Blanchard\textsuperscript{133} on the theory of multi-substrate enzyme catalysis provides the basis for the derivation of a steady state rate law accounting for all possible KDM2A-inhibitor interactions.

\[
v = \frac{v_{\text{max}}[2OG][P][O_2]}{(k_{-1} \cdot \frac{k_{-2}}{k_1} + \frac{k_{-3}}{k_1} \cdot K_P[O_2] + K_{2OG}[P][O_2]) \left(1 + \frac{[I]}{K_{i1}}\right) + \left(\frac{k_{-2}}{k_2} \cdot K_{O_2}[2OG] + K_P[2OG][O_2] \right) \left(1 + \frac{[I]}{K_{i2}}\right) + K_{O_2}[2OG][P] \left(1 + \frac{[I]}{K_{i3}}\right) + [2OG][P][O_2] \left(1 + \frac{[I]}{K_{i4}}\right)}}
\]

Figure 4.1 – Ordered, sequential mechanism of KDM2A-catalyzed demethylation of the peptide substrate (P) and possible modes of dead-end inhibition, giving rise to a theoretical steady-state rate law. $v_{\text{max}}$ is the maximum rate of reaction and $K_X = \frac{[E][X]}{[E\cdot X]}$. 

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We envisaged using the RapidFire mass spectrometry platform to measure the initial rate of KDM2A-catalyzed peptide-demethylation over a range of 2-OG and inhibitor concentrations and hence establish whether our inhibitor binds the enzyme competitively with respect to 2-OG (i.e. $K_{i2}$, $K_{i3}$, $K_{i4} \gg 0$). This approach has previously been used to confirm the mode of inhibition of a variety of 2-OG mimics.\(^4\),\(^5\),\(^9\),\(^13\)4 To begin, the effect of varying the concentration of 2-OG on the consumption of the dimethylated peptide substrate was measured with the aim of determining a practical 2-OG concentration range for analysis (Figure 4.2). Intriguingly, the conversion of substrate to product appeared to reach a maximum when $[2\text{-OG}] = 5$ μM before diminishing at higher cofactor concentrations, indicating a degree of substrate inhibition. To our knowledge, inhibition of KDM2A by 2-OG has not been reported in the literature. However, L. M. Mirica noted that KDM4C is subject to substrate inhibition and proposed that this is a consequence of competition between 2-OG and O₂.\(^13\)5 In order to ensure that oxygen would not be the rate-limiting substrate, we focused on a range of 2-OG concentrations corresponding to the “regular”, positive relationship between substrate concentration and reaction rate – in this case, $[2\text{-OG}] \leq 3$ μM.

**Figure 4.2** – Substrate inhibition of KDM2A by 2-OG. Concentrations are: 0, 1, 5, 10, 20, 50, 100, and 200 μM.
The relationship between [2-OG] and the initial rate of enzyme-catalyzed demethylation at different concentrations of the optically-pure inhibitor (S,S)-108 (IC$_{50}$ 0.16 μM) is depicted in Figure 4.3, along with the corresponding Lineweaver-Burk plot.$^{136}$ The observation that $v_{\text{max}}$ decreases with increasing inhibitor concentration allows us to rule out a mode of inhibition that is purely competitive with respect to 2-OG.

**Figure 4.3** – A) Plot of [2-OG] vs. initial rate of peptide demethylation. The concentration of the peptide substrate is 10 μM. B) Lineweaver-Burke plot, illustrating non-competitive inhibition wrt 2-OG.$^*$

$^*$For a complete regression analysis of these results, see subsection 8.2.2 in the appendix.
We envisaged using an analogous approach to explore the mode of KDM2A inhibition with respect to the peptide substrate. As this would entail measuring the initial rate of demethylation over a range of peptide concentrations, it was important to ensure that the peptide would not saturate the mass spectrometer’s detector, thus obscuring the obtained results. No signal saturation at peptide concentrations up to 50 μM was observed (Figure 4.4).

Figure 4.4 – MS extracted ion chromatogram (EIC) integrated areas as a function of [peptide].

The relationship between [peptide] and the initial rate of enzyme-catalyzed demethylation at different concentrations of (S,S)-108 is depicted in Figure 4.5, along with the corresponding Lineweaver-Burk plot. As before, the observation that $v_{\text{max}}$ decreases with increasing inhibitor concentration suggests a mode of inhibition that is not purely competitive with respect to the peptide substrate.
Figure 4.5 – A) Plot of [peptide] vs. initial rate of peptide demethylation. The concentration of the 2-OG cosubstrate is 10 μM. B) Lineweaver-Burke plot, illustrating non-competitive inhibition kinetics of \((S,S)-108\) wrt the peptide.∗

4.2 Confirmation of Binding

4.2.1 Biolayer Interferometry

Having explored the kinetics of enzyme inhibition, we sought to obtain more information about the stoichiometry and thermodynamic parameters of the enzyme-

∗For a regression analysis of both graphs, see section 8.2 in the appendix.
inhibitor interaction. We initially envisaged employing isothermal titration calorimetry (ITC) to achieve this. In an ITC experiment, heat exchange between a sample cell and a reference cell within an adiabatic container is measured. The sample cell contains a solution of protein or ligand, and the exothermic or endothermic ligand-protein interaction is monitored during the course of titration. This provides information about the stoichiometry as well as the enthalpic and entropic contributions to binding. Unfortunately, we were unable to find precedent for the application of ITC to KDM-ligand interactions, and attempts to identify conditions that provided an adequate signal-to-noise ratio for our system proved unsuccessful. Moreover, the low sensitivity of ITC necessitates the commitment of a relatively large quantity of protein, which precluded optimization of an ITC-based assay for KDM2A binding.

To gain an insight into the strength of the inhibitor-KDM2A binding interaction and determine the corresponding $K_D$, we therefore turned to a more sensitive technique: bio-layer interferometry (BLI). In a BLI experiment, a protein or ligand is immobilized on the tip of a fiber-optic biosensor, usually by incorporating a biotin-tag on either species and exploiting the effectively irreversible biotin-streptavidin binding interaction. Immersion of the tip in a solution of the corresponding ligand or protein results in ligand-protein association, which increases the optical thickness of the biosensor. This change in optical thickness alters the interference pattern of white light that is reflected from the tip of the fiber-optic biosensor, and the consequential wavelength shift may be measured in real time (Figure 4.7).
Figure 4.7 – Principles of bio-layer interferometry (BLI): The association of immobilized protein/ligand with its binding partner increases the optical thickness of the bio-layer at the tip of a fiber-optic biosensor. The resulting wavelength shift of reflected white light may be measured in real time.

Initial work carried out within the SGC focused on engineering a biotinylated version of KDM2A to be used in BLI assays. However, the incorporation of a biotin tag at various locations in the protein sequence led to a dramatic loss of catalytic activity. It was therefore necessary to synthesize a biotinylated inhibitor instead. Previous research by Dr Katherine England in the C. Schofield research group revealed that a long linker connecting biotin and the inhibitor scaffold (ideally 23 PEG units) is essential for minimizing non-specific interactions between streptavidin and KDM2A. With this in mind, we synthesized the biotin-tagged indoline 224 (Scheme 4.2).
Scheme 4.2 – Synthesis of the biotinylated indoline 224 for BLI investigations.

A Cu-catalyzed click reaction was employed to connect the biotin tag 220 to the indoline inhibitor scaffold 223. We reasoned that functionalization at the indoline N-acyl position would be most likely to afford a compound that largely retains inhibitory activity towards KDM2A (subsection 3.2.4). However, a substantial loss of potency relative to the acetylated analogue 59 was observed (12 μM vs. 0.22 μM), and we considered that the residual, weak inhibitory activity of 224 could be primarily a consequence of non-specific interactions between the long PEG chain and KDM2A. This was supported by the observation that, while 224 bound effectively to the streptavidin-coated biosensor tips (Figure 4.8-A), there was no significant difference between binding of immobilized 224 to KDM2A and binding of the PEG-biotin control to KDM2A (Figure 4.8-B). An alternative
experimental approach was therefore required to confirm and measure the strength of inhibitor-KDM2A association.

Figure 4.8 – BLI binding curves: A) Immobilization of biotinylated compounds on streptavidin-coated biosensor tips. B) Association with KDM2A. 225 and 226 were used as positive and negative controls respectively.
4.2.2  Native Mass Spectrometry*

Native (i.e. non-denaturing) mass spectrometry has become an increasingly important method for studying biomolecular interactions, and we therefore envisaged its application to studying the association of (S,S)-108 and KDM2A. Encouragingly, we observed a clear complex of the enzyme and (S,S)-108, corresponding to a 1:1 binding stoichiometry (Figure 4.9). However, the strength of this interaction was insufficient to accurately determine the corresponding dissociation constant ($K_D$) using this method. It is possible that the 2-OG cofactor and/or peptide substrate (which had been omitted for this experiment) are required for stronger association, and studies exploring the potential cooperativity of these binding interactions by native MS are underway.

Figure 4.9 – Native MS spectra of apo-KDM2A (bottom) and a 1:10 mixture of KDM2A and (S,S)-108. The +13, +14, and +15 charge states are depicted.

* This work was carried out by Shane Chandler, under the supervision of Prof Justin Benesch, CRL.
4.3 Covalent Labelling of KDM2A

4.3.1 Synthesis of a Photoaffinity Inhibitor

Due to the difficulty of obtaining an X-ray co-crystal structure of our lead compound bound to KDM2A, an alternative approach was required to identify the inhibitor binding site. The use of photoaffinity probes in combination with modern experimental techniques in proteomics to characterize protein-ligand interactions forms the basis of a continually-growing area of research.\textsuperscript{140-142} Within the field of KDMs, C. Schofield and co-workers recently demonstrated the application of the 4-component Ugi reaction\textsuperscript{143} to the synthesis of a series of photoaffinity probes based on the 2-OG deoxygenase inhibitor IOX1.\textsuperscript{144} We therefore sought to synthesize a photoreactive indoline inhibitor of KDM2A to study the inhibitor-enzyme binding interaction.

A variety of photoreactive functional groups have been employed in the development of photoaffinity probes, including aryl azides, benzophenones, and diazirines. We anticipated that installing an aliphatic diazirine motif through $N$-acylation of the indoline core would be most likely to afford an inhibitor that is both potent and as structurally similar as possible to previously synthesized lead compounds. An examination of the recent literature in this area revealed that aliphatic diazirines have been used successfully in a number of photo-crosslinking studies. For example, Krom and Meijler incorporated an aliphatic diazirine into the alkyl chain of the fatty acid-derived signaling molecule 3-oxo-C\textsubscript{12}-HSL and demonstrated selective covalent labelling of the bacterial transcriptional activator LasR.\textsuperscript{145} Similarly, J. W. Keillor and co-workers synthesized a diazirine-containing cinnamic acid derivative, which helped reveal conformational changes of the pig liver transglutaminase binding site upon ligand association.\textsuperscript{146}
Scheme 4.3 illustrates the synthesis of the racemic diazirine-containing KDM2A inhibitor 230. The preparation of diazirine 228 from levulinic acid has been described by J. C. Jewett and co-workers.\textsuperscript{147} We subsequently converted this intermediate to the corresponding acyl chloride, which was used to effect N-acylation of indoline 50. Stille-Migita cross-coupling of the diazirine-containing product 229 with vinylstannane 57 under slightly milder reaction conditions than previously employed (60 °C vs. 85 °C) afforded the target photoreactive indoline 230 in moderate yield.

Scheme 4.3 – Synthesis of the photoreactive KDM2A inhibitor 230.

Encouragingly, 230 was found to be a reasonable potent inhibitor of KDM2A, and we therefore proceeded with our envisaged photo-crosslinking trials. To ensure homogenous and reproducible sample irradiation and minimize the impact of radiation-induced protein denaturation, solutions of KDM2A and 230 were maintained at 4 °C and exposed to light at a wavelength of 350 nm using a CaproBox (Caprotec, Berlin). Furthermore, we included an excess of NiCl\textsubscript{2} in the photo-crosslinking solution, guided by the observation of X. Wang and co-workers that KDM2A is stabilized towards aggregation by Ni\textsuperscript{II}.\textsuperscript{67} Finally, we ensured that KDM2A and 230 were incubated at 4 °C for 45 min prior
to irradiation to guarantee that a thermodynamic equilibrium of protein-ligand association would be reached before covalent labelling.

Photo-crosslinking of KDM2A and **230** was monitored using a Q-TOF mass spectrometer. Excitingly, the formation of the covalently-modified protein was found to progress as expected with increasing duration of sample irradiation, and after an irradiation time of 15 min, the proportion of labelled protein appeared to reach a maximum value of approximately 18%. A small amount of doubly-labelled KDM2A was also detected after longer irradiation times (Figure 4.10).

**Figure 4.10** – Progress of KDM2A photo-crosslinking as a function of irradiation time – KDM2A (1 μM), **230** (4 μM), 2-OG (10 μM), and NiCl₂ (50 μM) in MES buffer (pH 7.0).
4.3.2 Synthesis of a Bifunctional Photoaffinity Probe Candidate

Photoaffinity probes frequently contain functional motifs such as biotin tags or fluorescent markers, which make them useful tools for studying their protein targets in a cellular context. In particular, such functionalized photoaffinity probes may be used to validate target engagement and explore off-target activity. We therefore decided to synthesize a modified photoaffinity probe for KDM2A, containing a terminal alkyne “handle”, which would be poised to react via a Cu-catalyzed click reaction with the azide group of modified biotin after photo-crosslinking. In theory, cellular pulldown studies could subsequently be used to confirm binding to KDM2A within cells, thus complementing our previously-described approach based on transcriptomics (subsection 3.1.2).

Scheme 4.4 illustrates an efficient, two-step synthesis of the bifunctional diazirine 233. Terminal alkyne 232 could be synthesized in moderate yield from 2,3-dihydrofuran, employing synthetic methodology previously discovered within the group of D. M. Hodgson. To obtain the desired diazirine product, use of neat, condensed NH₃ instead of a concentrated NH₃/MeOH solution was found to be essential.

Scheme 4.4 – Synthesis of bifunctional diazirine 233.

Unfortunately, all attempts to acylate indoline 50 using 233 via direct amide coupling proved unsuccessful. We postulate that this is a consequence of the relatively low reactivity of the indoline nitrogen. In addition, we were unable to convert 233 into the corresponding acyl chloride 235, which appeared to be unstable, even at low temperatures (Scheme 4.5).
Scheme 4.5 – Unsuccessful direct coupling of diazirine 233 to the indoline core.

We reasoned that direct coupling of 233 to a primary amino group appended to the indoline core would potentially offer a solution to this problem. To investigate this hypothesis, we synthesized indoline 239 via acylation of 50 with phthalimide 236, followed by Stille-Migita cross-coupling and amine deprotection using \( \text{N}_2\text{H}_4 \). Encouragingly, the additional methylene unit between the terminal amino group and the amide carbonyl bond in 239 appeared to confer stability towards hydrolysis. We therefore anticipate that indoline 239 (\( \text{clogP} = 4.09 \)) might prove to be a suitable alternative to the previously-discussed hydrolytically-labile inhibitor 213 (subsection 3.2.4). Finally, amide coupling of 239 with diazirine 233 afforded the desired bifunctional photoreactive indoline 240 in good yield (Scheme 4.6).

Encouragingly, 240 was found to undergo efficient photo-crosslinking with KDM2A, although a small amount of double-labelling was also observed (Figure 4.11). Before proceeding with any cellular pulldown studies using 240, we turned our attention to identifying the inhibitor binding site. This would involve degradation of the covalently-modified protein into a series of peptide fragments, followed by peptide sequencing using state-of-the-art LC-MS/MS analysis.
Scheme 4.6 – Synthesis of 240, a potential photoaffinity probe for cellular target engagement studies.

Figure 4.11 – Photo-crosslinking of 240 and KDM2A – KDM2A (1 μM), 240 (4 μM), NiCl₂ (50 μM), and 2-OG (10 μM) in MES buffer (pH 7.0).
4.3.3 **KDM2A digest and MS/MS**

To obtain a solution of peptide fragments, which would be amenable to sequencing by LC-MS/MS, a sample containing a mixture of 230-labelled and unlabelled KDM2A was first subjected to an elastase digest. Elastase is a serine protease, and the isoform that was employed for our analysis (elastase II) is selective for peptide bonds with hydrophobic sidechains in the P1 position (N-terminal side of amide).\(^{149}\) We anticipated that the use of a protease with such broad substrate selectivity would lead to a series of overlapping KDM2A peptide fragments and therefore provide a clear indication of the principle binding site of 230.

Following chromatographic separation of the peptide fragments, the identity and position of covalently-modified amino acid residues was determined by MS/MS fragmentation analysis.\(^*\) In total, 29 amino acids were identified as being susceptible to photo-crosslinking. An examination of the distribution of photo-crosslinked residues revealed that the highest proportion of labelled peptides corresponded to the protein’s N-terminal region, and residues located within the sequence 21RYEDDGI5DDEIEGKRTFDL\(_{40}\) were by far the most susceptible to photo-crosslinking – 52% of all labelled peptides contained residues in this region. The next highest frequency region (121MTMAQWTRYYETPEEEREKL\(_{140}\)) encompassed residues present in 12% of all labelled peptides. The distribution of labelled peptides across the protein sequence is illustrated in Figure 4.12, and Figure 4.13 shows a typical peptide fragmentation spectrum used to identify the locations of modified residues.

\(^*\) Data was collected and processed by Dr Rebecca Konietzy in the research group of Prof. Benedikt Kessler, TDI. An illustration of the entire KDM2A sequence labelling distribution is shown in subsection 8.3.2 of the appendix.
Figure 4.12 – Distribution of labelled peptides, showing apparent preference for photo-crosslinking of residues near the protein N-terminus. Observed ratios of labelled to unlabelled peptides are displayed above each bar.

Figure 4.13 – MS/MS fragmentation pattern, showing labelling of residue E31. An intense peak corresponding to fragmentation of the bond formed by photo-crosslinking of 230 and KDM2A is also present (m/z = 545.33).
While the results appear to imply that the photoaffinity inhibitor 230 binds KDM2A at a site near the enzyme’s N-terminus, they also highlight a substantial degree of apparently random, non-specific labelling. In particular, we noted that the majority of covalently-modified residues are acidic (65% of labelled residues are either Asp or Glu), although some regions with high densities of acidic residues but no labelling were also observed. A possible explanation for the apparently preferred photo-crosslinking of acidic residues may be related to the unique photochemistry of aliphatic diazirines.

It has been shown both theoretically and experimentally that diazirines isomerize to diazo-compounds upon irradiation with UV-light (Scheme 4.7).\textsuperscript{150} Diazo-compounds can fragment to release N\textsubscript{2} and a highly reactive and short-lived singlet carbene species, which is expected to react instantaneously with residues in the protein binding site or with the solvent (carbene lifetime: 10\textsuperscript{-10} - 10\textsuperscript{-9} sec).\textsuperscript{151} Alternatively, diazo-compounds can undergo protonation to form diazonium ions, which may be sufficiently long-lived to dissociate from the binding site and react relatively non-selectively with particularly nucleophilic and/or negatively-charged sidechains (diazonium lifetime: approx. 10\textsuperscript{-1} sec).\textsuperscript{152} Indeed, J. B. Cohen and co-workers attributed the labelling of principally Asp, Glu, and Tyr residues on the nicotinic acetylcholine receptor by a diazirine-containing etomidate derivative to the generation of a relatively long-lived electrophilic species such as a diazonium ion or stabilized carbocation.\textsuperscript{153} Moreover, the chemoselective alkylation of protein carboxyl groups by diazo compounds has been demonstrated by R. T. Raines.\textsuperscript{154}

![Scheme 4.7 – Photolysis of diazirines via diazo-intermediate.](image-url)
To estimate the relative magnitudes of selective and unselective covalent labelling of KDM2A by 230, an excess of the non-photoreactive inhibitor 108 (IC$_{50}$ 0.25 μM) was added to the photo-crosslinking solution (Figure 4.14). 230 and 108 are predicted to compete for binding to the enzyme; however, only a minor reduction in the photolabelling efficiency was observed as a result of addition of 108 (9% vs. 12%). This suggests that the perceived distribution of covalently modified residues largely reflects non-selective reactions involving a positively-charged, long-lived electrophilic species derived from diazirine 230 and either negatively-charged or nucleophilic side chains of KDM2A.

![Chemical structures of 230 and 108](image)

**Figure 4.14** – Photocrosslinking competition involving 230 and 108 – KDM2A (1 μM), 230 (4 μM), NiCl$_2$ (50 μM), and 2-OG (10 μM) and 108 (20 μM) in MES buffer (pH 7.0) and was irradiated at 350 nm for 12 min.

We postulate that it may be possible to distinguish between selective and non-selective covalent modifications of KDM2A by monitoring changes in the distribution of photo-crosslinked residues as a function of irradiation time or due to the presence of a
competitive binder such as 108. However, a different photoreactive functional group is ultimately required to provide a clear indication of the location of the inhibitor binding site. F.M. Richards first reported the use of 3-trifluoromethyl-3-aryldiazirine (TFMD) as a photoreactive group in photo-crosslinking studies. It is reasoned that the diazo-compound derived from this functionality is less readily protonated to afford a long-lived diazonium species and is therefore more likely to bring about selective labelling of binding site residues. We therefore propose that the trifluoromethyl-diazirine 242 might be a more suitable photoaffinity probe for future work on this project (Figure 4.15). To complement these studies, H-D exchange MS could also be used to reveal conformational changes upon inhibitor binding to KDM2A and thus hopefully provide a clear picture of the dynamics of this interaction.

![Figure 4.15](image)

**Figure 4.15** – Potential TFMD-based photoaffinity probe for more selective photo-crosslinking of KDM2A.

### 4.3.4 Project Conclusion and Prospects for Future Work

The aim of this project was to develop a chemical probe for the histone lysine demethylase KDM2A that would satisfy the SGC criteria regarding potency, selectivity, and cellular activity (Subsection 2.2.1). After identifying a promising hit in a screen of known binders to methyl-lysine reading domains and HMTs, we synthesized a library of chiral indoline-based KDM2A inhibitors and explored key SARs around this scaffold. Synthetic methodology relying on enantioselective phase-transfer catalysis was subsequently applied to the asymmetric synthesis of lead compounds, and we ultimately discovered a
novel inhibitor of KDM2A that displays very promising in vitro potency and selectivity towards this target (IC\textsubscript{50} 0.16 μM, > 70-fold selectivity). A subsequent evaluation of the cellular compound activity focused on inhibition of H3K36me2 demethylation and made use of transcriptomics to explore the resulting impact on gene expression patterns. We attempted to address important limitations associated with compound cytotoxicity and promiscuity using a rational approach based on computational docking and an analysis of key physical properties. Finally, we investigated the mechanism of KDM2A inhibition using a variety of experimental techniques, including enzyme kinetics studies, native mass spectrometry, and photo-crosslinking with a photoreactive inhibitor. Although a number of questions regarding the nature of the protein-inhibitor interaction still remain unanswered, notably the actual strength of inhibitor-KDM2A association and the precise location of the inhibitor binding site, we identified potential strategies for resolving these issues.

To our knowledge, (S,S)-108 is the first KDM2A-selective inhibitor to demonstrate a significant effect on cellular H3K36me2 levels at sub-micromolar doses (Subsection 3.1.1). However, its cytotoxicity and off-target activity at higher concentrations compromise its suitability as a chemical probe. As a potential strategy to overcome this problem, we identified the indoline N-acyl group as a promising handle for modulating compound lipophilicity. Polar follow-up candidates that are possibly more stable to hydrolytic decomposition than indoline 213 (clogP 4.64, IC\textsubscript{50} 0.12 μM) are illustrated in Figure 4.15.
Figure 4.15 – Follow-up candidates to 213: A series of α-amino acid derivatives (243) might be stabilized towards amide hydrolysis due to steric hindrance around the N-acyl bond. The α-carboxyacetyl indoline 244 is expected to exist as a zwitterion at neutral pH and may therefore retain membrane permeability. The β-aminoacetyl indoline 239 has already been synthesized as an intermediate towards diazirine 240 (Scheme 4.6).

The discovery of a completely novel class of selective KDM2A inhibitors that display an apparently unique inhibitory mechanism illustrates the successful application of asymmetric organocatalysis to an exciting area of research within chemical biology – epigenetics. In this project, the availability of robust, enantioselective synthetic methodology was essential for the efficient synthesis of optically-pure lead compounds. The next chapter of this thesis therefore focuses on the development of new chiral cation-directed reactions, which provide access to three-dimensional scaffolds that could potentially form the basis of future chemical probe discovery programs.
5. **ENANTIOSELECTIVE SYNTHESIS OF POISED FRAGMENTS**

5.1 Background

5.1.1 3-D Poised Fragments

Recent years have witnessed a growing interest in the development of asymmetric fragments for drug discovery. The creation of international collaborative projects such as the 3-D fragment consortium\(^{156}\) and the European Lead Factory,\(^ {157}\) which aim to address the current need for small, chiral scaffolds, is testament to the importance of such compounds for the discovery of novel biologically-active molecules. We regard the development of new enantioselective synthetic methodology as a potentially valuable contribution to these efforts.

In the context of drug discovery, a *fragment* may be defined as a molecule with a molecular weight < 300 gmol\(^{-1}\) and log\(P < 3.\)\(^ {158}\) An alternative characterization of overall fragment size based on the total number of “heavy” (i.e. non-hydrogen) atoms is also commonly adopted. In order for a molecule to qualify as a fragment according to this definition, the total number of atoms other than H ≤ 15.\(^ {159}\) The principle advantage of screening molecules of this size as opposed to larger, more drug-like compounds is the ability of fragment libraries to statistically cover a larger proportion of the available chemical space and thus give rise to higher hit-discovery rates.\(^ {160}\) Fragments that are found to interact independently with the desired biological target may subsequently be linked to
afford potent and selective lead compounds. One of the challenges associated with this approach is the requirement for sensitive biophysical techniques to measure the relatively weak affinities of proteins for fragment-sized molecules (μM to mM levels).161

The discovery of the anti-cancer drug Navitoclax by Abbott Laboratories provides an elegant example of the promise of fragments in chemical biology. As illustrated in Figure 5.1, two small molecules that were found to bind weakly to the surface of the anti-apoptotic transmembrane protein Bcl-XL were connected and subsequently elaborated to afford a highly potent series of inhibitors of the Bcl proteins.162

![Figure 5.1](image)

**Figure 5.1** – Fragment-based discovery of the potent anti-cancer drug Navitoclax. **K**\(_D\) and **K**\(_i\) values indicate activity towards Bcl-XL.

In order to realize the full potential of fragment-based drug discovery (FBDD), it is crucial that fragment libraries maximize their coverage of available chemical space. Consequently, a combination of both sp\(^2\)-rich (“flatland”) and sp\(^3\)-rich (3-D) fragments is desirable. The generation of chiral fragments poses a particular challenge, as an effective control of their absolute stereochemistry is ultimately required for the development of enantiopure drug candidates. In addition, the ideal fragment contains multiple reactively-orthogonal functional groups that serve as “handles” for connection to other chemical entities.163 With this in mind, we sought to expand the scope of cation-directed enantioselective C-acylation reactions recently developed within the Smith group to access...
unique, enantioenriched 3-D fragments that would be “poised” to react via one or more reactive sites.

5.1.2 Enantioselective C-Acylation of Enolates

The principle challenge associated with enantioselective C-acylation of enolate anions is their ambident reactivity, which gives rise to mixtures of regioisomeric products resulting from acylation on either oxygen or carbon. In general, O-acylation is kinetically favored in both the gas phase and in solution.\textsuperscript{164-166} To overcome this intrinsic preference, strategies have been developed based on the use of preformed enolate derivatives such as silyl ketene acetals by G. C. Fu,\textsuperscript{167} A. D. Smith,\textsuperscript{168} and E. N. Jacobsen\textsuperscript{169} (Figure 5.2 – A-C). More recently, intermolecular enantioselective C-acylation of 3-substituted lactams has also been accomplished by B. M. Stoltz and co-workers via a Ni-catalyzed 3-component coupling reaction (Figure 5.2 – D).\textsuperscript{170}

![Figure 5.2 – Previous approaches to enantioselective C-acylation of enolate derivatives.](image)

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Within the Smith group, the enantioselective synthesis of a series of spirobiindanones was recently achieved using an organocatalytic approach (Figure 5.3 – A).\textsuperscript{171} This reaction proceeds through chiral cation-directed C-acylation of the indanone substrate with an intramolecular ester. We anticipated that the principles of this approach could potentially be applied to the enantioselective synthesis of a unique poised spirolactam fragment (Figure 5.3 – B).

**Figure 5.3** – A) Chiral cation-directed enantioselective synthesis of spirobiindanones. B) Proposed enantioselective synthesis of a poised spirolactam fragments and potential approaches to fragment elaboration.

### 5.2 Development of a Poised Spirolactam Fragment

#### 5.2.1 Substrate Synthesis

The synthesis of phenyl 2-(bromomethyl)benzoate 247 is illustrated in Scheme 5.1. Phenyl ester 246 was easily prepared from commercially-available o-toluic acid, and subsequent radical bromination afforded benzyl bromide 247 in good yield. We envisaged
alkylating an $N$-protected pyrrolidinone with this compound to obtain a substrate for cyclization.

**Scheme 5.1** – Synthesis of 2-(bromomethyl)benzoate 247 for alkylation of pyrrolidinone.

Initial attempts to alkylate $N$-benzylpyrrolidinone 248 with 247 proved unsuccessful, affording a complex mixture of products in addition to largely unreacted pyrrolidinone starting material (Scheme 5.2 – A). Deprotonation of 248 by lithium amide bases has been documented in the literature, and we therefore postulate that the resulting lithium-enolate irreversibly deprotonates alkyl bromide 247 at the benzylic position rather than undergoing alkylation. By contrast, the less basic lithium enolate of $N$-Boc-pyrrolidinone 249 was found to react smoothly with 247, affording the cyclization substrate 250 in moderate yield (Scheme 5.2 – B).

**Scheme 5.2** – A) Attempted alkylation of $N$-benzylpyrrolidinone 248 with bromide 247. B) Successful alkylation of $N$-Boc-pyrrolidinone 249 with benzyl bromide 247.

250 was found to undergo cyclization to the desired spirolactam 251 in the presence of KO'Bu or alternatively a combination of solid Cs$_2$CO$_3$ and TBAB (Scheme 5.3). However, under either set of reaction conditions, conversion of substrate to product appeared to
plateau at approximately 40%. To investigate whether this was a consequence of a retro-acylation process induced by the released phenoxide, we monitored a mixture of 251, phenol, and KO'Bu at rt. The reversibility of C-acylation was confirmed by the recovery of an approximately equimolar mixture of 250 and 251 after 48 h. We concluded that an alternative substrate would therefore be required to achieve a favorable cyclization yield and ultimately a high degree of enantiocontrol under asymmetric reaction conditions.

Scheme 5.3 – Cyclization of phenyl ester 250 afforded spirolactam 251, but the phenoxide leaving group was found to induce retro-acylation.

Issues associated with the reactivity of phenoxide had also been encountered in the course of the development of methodology towards enantiomerically-enriched spirobiindanones. These problems were eventually overcome by replacing the substrate’s phenyl ester with a pentafluorophenyl ester, which generates a less nucleophilic phenoxide byproduct. Thus, we focused on the synthesis of the pentafluorophenyl ester equivalent of 250. Unfortunately, alkylation of N-Boc pyrrolidinone 249 by alkyl bromide 254 afforded a complex mixture of products (Scheme 5.4), possibly as a result of competitive deprotonation of 254 by the lithium enolate of 249 or competitive reactions involving the pentafluorophenyl ester.

Scheme 5.4 – Attempted direct alkylation of N-Boc-pyrrolidine 249 by bromide 254.
We therefore envisaged an alternative route to the desired pentafluorophenyl ester substrate *via* an ester coupling of the corresponding benzoic acid. Alkylation of 249 by methyl 2-(bromomethyl)benzoate 256 afforded pyrrolidinone 257 in good yield. However, attempted deprotection of the methyl ester by LiOH or KOTMS to access benzoic acid 258 resulted in preferential hydrolysis of the lactam (Scheme 5.5).

**Scheme 5.5** – Synthesis of pyrrolidinone 257 and lack of chemoselectivity with respect to hydrolysis of its methyl ester.

Benzoic acid 258 was eventually synthesized by hydrogenolysis of the corresponding benzyl ester 261. Subsequent carbodiimide coupling of the acid with pentafluorophenol afforded pyrrolidinone 255 in good yield (Scheme 5.6).

**Scheme 5.6** – Synthesis of pentafluorophenyl ester cyclization substrate 255.
5.2.2 Attempted Enantioselective cyclization

Encouragingly, pyrrolidinone 255 was found to undergo efficient cyclization to spirolactam 251 in the presence of KO\textsuperscript{t}Bu, although purification of the reaction product proved challenging due to its lack of stability towards silica gel column chromatography (Scheme 5.7).

![Scheme 5.7 – Racemic cyclization of pyrrolidinone 255 to spirolactam 251.](image)

We estimate the pKa of 255 to be close to the empirical upper limit for interfacial phase-transfer catalysis (approx. 23).\textsuperscript{173} Nonetheless, we proceeded with a screen of asymmetric reaction conditions using N-benzyl quinidinium A as the catalyst (Table 5.1).

![Table 5.1 – (i) As determined by TLC. (ii) As determined by analysis of the crude H NMR spectrum.](image)
Unfortunately, conversion levels were found to be exceptionally poor with all bases screened. Enantiomeric ratios could be measured after cyclization with KOH(s) and the phosphazene base BTP,\(^{174}\) but an essentially racemic mixture of products was obtained in both cases. Finally, attempts to augment the basicity of KOH and thereby enhance the reaction rate by addition of a crown ether\(^{175}\) failed to improve results. We concluded that pyrrolidinone 255 is not sufficiently reactive under the conditions attainable under enantioselective phase transfer catalysis and that an alternative, more acidic cyclization substrate is required. We considered that cyclization of the corresponding oxindole would be instructive, despite the fact that the resulting spiro-oxindole product would no longer qualify as a true fragment.

### 5.3 Spiro-Oxindoles

#### 5.3.1 Substrate Synthesis

Spiro-oxindoles feature in a broad range of natural products and biologically-active molecules, and a variety of synthetic strategies towards these privileged scaffolds have therefore been developed.\(^{176,177}\) However, to the best of our knowledge, enantioselective acylation at the oxindole C3 position has not been reported. A notable recent contribution to spiro-oxindole synthetic methodology is the discovery of a reaction involving the enantioselective C-H oxidation of an achiral spirocyclic substrate by a supramolecular ruthenium complex within the group of T. Bach (Scheme 5.8).\(^{178}\) Unfortunately, racemization of the initial alcohol product of oxidation via a retro-aldol process leads to a trade-off between enantioselectivity and overall yield.
Scheme 5.8 – Enantioselective C-H oxidation to form spiro-oxindoles developed by T. Bach and co-workers.

Synthesis of the pentafluorophenyl ester cyclization substrate 263 is illustrated in Scheme 5.9. N-methyloxindole 258 was accessed via a Wolff-Kishner reduction\(^\text{179}\) of N-methylisatin and subjected to a Knoevenagel condensation\(^\text{180}\) with benzaldehyde 259 to afford oxindole 260 as a 1:3 mixture of E- and Z-olefins. Hydrogenation of this mixture with a palladium on carbon catalyst cleanly afforded benzoic acid 261. The fact that lactone 262 was not observed suggests that reduction of the alkene function proceeds more rapidly than hydrogenolysis of the benzyl ester moiety. Finally, carbodiimide coupling of acid 261 with pentafluorophenol afforded 263 in good yield.

Scheme 5.9 – Synthesis of oxindole cyclization precursor 263.
5.3.2  **Enantioselective Cyclization**

Oxindole 263 was found to cyclize cleanly to the desired racemic spiro-oxindole product 264 in the presence of aqueous $K_2CO_3$ and TBAB (Scheme 5.10). Encouraged by the markedly greater reactivity of this scaffold towards intramolecular $C$-acylation relative to the previous pyrrolidinone system, we envisaged a methodical screen of enantioselective reaction conditions. In order to expedite screening, reactions were performed on a relatively small scale (15 mg 263), and only the enantioselectivity of each reaction was initially evaluated.

Scheme 5.10 – Racemic cyclization of oxindole 263 to spiro-oxindole 264.

To begin, phase-transfer catalysts based on the four commercially-available $N$-benzyl cinchona alkaloid scaffolds were investigated together with a selection of aqueous bases. We hoped that this limited screen would provide an initial insight into the sensitivity of the reaction towards modifying these components. The cinchonidinium salt (D) performed marginally better than its stereoisomeric analogues (entries 1-4), giving rise to a very promising level of enantioenrichment (e.r. 75:25). The identity of the aqueous base was found to have only a minor effect on enantioselectivity (entries 5-10), suggesting that the base’s metallic counterion is readily displaced from the deprotonated substrate by the ammonium species and likely does not feature in the cyclization transition state (Table 5.2).
Table 5.2 – Primary screen of main cinchona alkaloid scaffolds and aqueous bases.

Next, we conducted an extensive screen of chiral phase-transfer catalysts (Figure 5.4). The presence of a free hydroxyl group at the C9 position of the cinchona catalyst scaffold was found to be essential for attaining high levels of enantioenrichment (D vs. E, K vs. O), implying a possible substrate-catalyst H-bonding interaction. In addition, polyaromatic substituents such as naphthyl and anthracenyl ring systems were found to enhance enantioselectivity (J – M). The most favorable result was ultimately achieved with quininium-based catalyst N, which gave rise to a 92:8 mixture of enantiomers. Alternative catalysts based on dual quaternary-ammonium/H-bonding functionality (Q) or a chiral biaryl scaffold (R) did not perform well.
Having identified a selective catalyst, we investigated the impact of varying substrate concentration, catalyst loading, and water content on enantioselectivity. Key findings are summarized here and in Tables 5.3 and 5.4.

**Figure 5.4** – Screen of phase-transfer catalysts for the cyclization of 263 to 264.

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1. Lowering the concentration of 263 from 0.05 M to 0.025 M led to a marginal improvement in the e.r. (entries 1 vs. 2).

2. Reducing the catalyst loading from 10 to 5 mol % did not affect enantioselectivity but resulted in a markedly slower reaction rate based on TLC analysis (entries 1 vs. 3).

3. Aqueous bases gave rise to higher e.r. values than their solid equivalents (entries 4-7). Raising the water content did not have a significant impact on enantioselectivity (entries 3 vs. 4).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Concentration of 263</th>
<th>Catalyst loading</th>
<th>e.r.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K₂CO₃ (50% aq.)</td>
<td>0.05 M</td>
<td>0.1 eq.</td>
<td>92:8</td>
</tr>
<tr>
<td>2</td>
<td>K₂CO₃ (50% aq.)</td>
<td>0.025 M</td>
<td>0.1 eq.</td>
<td>93:7</td>
</tr>
<tr>
<td>3</td>
<td>K₂CO₃ (50% aq.)</td>
<td>0.05 M</td>
<td>0.05 eq.</td>
<td>92:8</td>
</tr>
<tr>
<td>4</td>
<td>K₂CO₃ (25% aq.)</td>
<td>0.05 M</td>
<td>0.1 eq.</td>
<td>92:8</td>
</tr>
<tr>
<td>5</td>
<td>K₃PO₄ (50% aq.)</td>
<td>0.05 M</td>
<td>0.1 eq.</td>
<td>91:9</td>
</tr>
<tr>
<td>6</td>
<td>K₂CO₃ (s.)</td>
<td>0.05 M</td>
<td>0.1 eq.</td>
<td>88:12</td>
</tr>
<tr>
<td>7</td>
<td>K₃PO₄ (s.)</td>
<td>0.05 M</td>
<td>0.1 eq.</td>
<td>88:12</td>
</tr>
</tbody>
</table>

Table 5.3 – Effect of substrate concentration, catalyst loading, and use of solid/aqueous bases on reaction enantioselectivity.

The reaction medium was found to have a substantial effect on the degree of enantiocontrol, and non-polar, aromatic solvents generally gave rise to higher product e.r. values (Table 5.4). Finally, lowering the reaction temperature to 0 °C resulted in a further improvement of enantioselectivity (to 94:6), although the reaction was found to slow down considerably under these conditions based on TLC analysis.
Table 5.4 – Effect of reaction solvent on enantioselectivity.

Guided by these screening results, we were ultimately able to obtain 264 in good yield and with an e.r. of 95:5 (Scheme 5.11). Product enantioenrichment could subsequently be augmented to 98:2 by recrystallization. The high level of enantiocontrol exhibited by this reaction demonstrates the potential of cation-directed intramolecular C-acylation as an approach to the asymmetric synthesis of spirocyclic compounds.

Scheme 5.11 – Optimized enantioselective reaction conditions for cyclization of 263 to 264.
5.3.3 **Prospects for Future Work**

Derivitization of spiro-oxindole 264 proved challenging. Given the insights of T. Bach and co-workers regarding the tendency of alcohol 265 to undergo racemization via a retro-aldol reaction, we anticipated that nucleophilic addition to the ketone carbonyl would probably afford a mixture of products and ultimately lead to an erosion of enantioenrichment (Scheme 5.12). In addition, protection via formation of a ketal was not accomplished, probably as a result of the neopentylic nature of the ketone.

**Scheme 5.12** – Lability of alcohol 265 as observed by T. Bach and co-workers.$^{178}$

The chemoselective functionalization of amides using catalytic In\(^+\) complexes has been reported by H. Nagashima,$^{181}$ D. J. Dixon,$^{182}$ and P. Huang.$^{183}$ Unfortunately, attempts to apply this methodology to either reduction or reductive alkynylation of the amide carbonyl of 264 were unsuccessful (Scheme 5.13). We postulate that this is a result of electronic deactivation of the amide by the oxindole aromatic ring. We were also unable to activate the amide carbonyl with more potent electrophilic reagents such as trifluoromethanesulphonic anhydride (Tf\(_2\)O) and phosphorus oxychloride (POCl\(_3\)).

**Scheme 5.13** – Attempted functionalization of amide carbonyl using Ir\(^+\) catalysis.
Finally, we anticipated that removal of the \( N \)-methyl group to afford the secondary amide would facilitate functionalization. However, attempts to effect this transformation using \( \text{BBr}_3 \), \( \text{HBr} \), and high-pressure hydrogenation over a palladium on charcoal catalyst were unsuccessful, resulting in either recovery of the unreacted starting material or extensive decomposition.

![Scheme 5.14](image)

**Scheme 5.14** – Further attempted reactions of spiro-oxindole 264.

Despite these setbacks regarding the derivatization of spiro-oxindole 264, we anticipate that a range of alternative poised chiral fragments could potentially be synthesized via an enantioselective \( C \)-acylation approach. The lessons learned in the course of this project will hopefully provide a valuable insight for future work within the group. In particular, we confirmed the importance of employing a pentafluorophenyl ester acylating agent, and we explored the limits of base-induced interfacial phase-transfer catalysis with respect to the substrate pKa. Potential substrates for cation-directed intramolecular \( C \)-acylation to afford 3-D fragments are depicted in Figure 5.5.
Figure 5.5 – Potential substrates for cation-directed enantioselective C-acylation: A) N-Boc-4-Thiazolidinone scaffold is expected to be more acidic than its pyrrolidinone equivalent due to carbanion-stabilization by sulfur\(^\text{185}\). B) N-Boc-β-lactam is expected to be more acidic than its pyrrolidinone equivalent due to the smaller ring size\(^\text{186}\). C) Potential acyclic substrate.
6. **EXPERIMENTAL**

6.1 **General Experimental**

6.1.1 **General Synthetic Procedures I**

**Reaction Conditions**

Reactions were carried out in flame-dried glassware under an atmosphere of argon unless stated otherwise. Room temperature (rt) refers to 20-25 °C. Temperatures of 0 °C were obtained using an ice/water bath. Temperatures of -78 °C were obtained using a dry ice/acetone bath. Reflux conditions were obtained using an oil bath or a Drysyn® heating block equipped with a contact thermometer. Temperatures of 0 °C or below which had to be maintained for extended periods of time were obtained using a Julabo FT902 immersion cooler.

**Solvents**

Acetonitrile, dichloromethane, diethyl ether, methanol, toluene, and tetrahydrofuran were purified by filtration through activated alumina columns employing the method of Grubbs *et al.* Dimethylsulfoxide, dimethylformamide, and *N*-methylpyrrolidinone were purchased as anhydrous solvents in a Sure/Seal™ bottle from Sigma-Aldrich. All other solvents were used as supplied without prior purification.

**Reagents and Catalysts**

All reagents were used directly as supplied by major chemical suppliers or following purification procedures described by Perrin and Armarego.
Chromatography

Thin layer chromatography was performed on Merck Kieselgel 60 F$_{254}$ 0.25 mm pre-coated aluminium plates. Product spots were visualized under UV light ($\lambda = 254$ nm) and/or by staining with potassium permanganate solution, vanillin solution, or ninhydrin solution. Flash pressure column chromatography was performed using VWR silica gel 60 (40-63 μm particle size) using head pressure by means of a nitrogen line.

Nuclear Magnetic Resonance Spectrometry

NMR spectroscopy was carried out using Bruker Avance spectrometers in the deuterated solvent stated, using the residual non-deuterated solvent signal as an internal reference. $^{19}$F NMR spectra were referenced externally to CFCl$_3$ in CDCl$_3$. Magnetic field strengths are quoted in MHz and refer to the resonance frequency of the relevant nucleus. Chemical shifts are quoted in ppm with signal splittings recorded as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), sextet (sex), septet (sept), octet (oct), nonet (non) and multiplet (m). The abbreviation br. is to denote broad, app. to denote apparent, Ph to denote phenyl, and C=O to denote carbonyl groups. Coupling constants, $J$, are measured to the nearest 0.1 Hz for $^1$H NMR spectra and to the nearest 1 Hz for $^{13}$C NMR spectra and are presented as observed. For rotameric molecules, spectra were obtained at 348 K in deuterated benzene or deuterated dimethylsulfoxide. Assignment of spectra was assisted by the results of COSY, HSQC, and HMBC experiments. A forward slash (/) separates ambiguous assignments for a particular peak.

Infrared Spectroscopy

Infrared spectra were recorded neat or as a film on a Bruker Tensor 27 FTIR spectrometer equipped with an attenuated total reflectance attachment with internal calibration. Absorption maxima ($\lambda_{\text{max}}$) are quoted in wavenumbers (cm$^{-1}$).

* VT-NMR spectra were obtained by Dr Barbara Odell.
Mass Spectrometry

Low resolution mass spectra were recorded on a Micromass LCT Premier spectrometer under conditions of electrospray ionization (ESI). High resolution mass spectra were recorded on Bruker MicroTOF and Micromass GCT spectrometers under conditions of electrospray ionization (ESI), field ionization (FI), or chemical ionization (CI). Values are reported as a ratio of mass to charge in Daltons.

Melting Points

Melting points were determined using a Reichert melting point apparatus and are uncorrected.

Polarimetry

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm (using the sodium D line, 589 nm). Concentrations are reported in g/100 mL. Temperatures are reported in °C.

HPLC

Analytical chiral HPLC was performed on a Dionex UltiMate 3000 system comprising a Dionex LPG-3400A pump, WPS-3000SL autosampler, TCC-3000SD column compartment, DAD-3000 diode-array detector, fitted with the appropriate Daicel Chiralpak column (dimensions: 0.46 cm φ x 25 cm) and corresponding guard column (0.4 cm φ x 1 cm). Wavelengths (λ) are reported in nm, retention times (τ_R) are reported in minutes and solvent flow rates are reported in mL min⁻¹. Semi-preparatory HPLC was performed on the same system, fitted with a YMC Chiral amylose-SA S-5μm column (dimensions: 250 x 10.0 mml.D).
6.1.2  General Synthetic Procedures II

**General Procedure 1 – Racemic cyclization of imines to form indolines**

KO\'Bu (1.1-1.8 eq.) was added to a solution of imine (1 eq.) in toluene and stirred at 0 °C until completion. NH₄Cl (saturated aq., approx. 10 mL/mmol imine) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated.

**General Procedure 2 – Acylation of indolines with acyl chlorides or chloroformates**

Acyl chloride (2-5 eq.) was added portionwise/dropwise to a solution of indoline (1 eq.) and pyridine (2-5 eq.) in CH₂Cl₂. The reaction mixture was stirred at rt until completion. NaHCO₃ (saturated aq., approx. 10 mL/mmol indoline) was added, and the mixture was extracted with CH₂Cl₂. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated.

**General Procedure 3 – Hydrogenation and hydrogenolysis over Pd/C**

Pd/C (wet degussa type, 10% w/w substrate) was added to a solution of the substrate in the specified solvent. The suspension was first degassed three times with N₂ using a pump-flood procedure and then degassed three times with H₂ using a pump-flood procedure. The reaction mixture was stirred under a H₂ atmosphere until completion and flushed with N₂. The suspension was filtered through Celite™, eluted with EtOAc, and concentrated.

**General procedure 4 – Stille cross-coupling**

A solution of aryl bromide/triflate (1 eq.) and vinyl stannane (1.2 eq.) in NMP was degassed with Ar. Pd(Ph₃)₄ (0.1 eq.) was added, and the mixture was stirred at the specified temperature until completion. The reaction mixture was allowed to cool to rt, diluted with CH₂Cl₂, and washed three times with H₂O, then brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated.
General procedure 5 – Sonogashira cross-coupling in $N,N$-diisopropylamine

Indoline (1 eq.), PdCl$_2$(PPh$_3$)$_2$ (0.05 eq.), and Cul (0.05 eq.) were degassed three times in a Schlenk tube with Ar. A degassed solution of alkyne (1.2 eq.) in $N,N$-diisopropylamine was added, and the reaction mixture was stirred at the specified temperature until completion. The reaction mixture was allowed to cool to rt, filtered through Celite™ and eluted with EtOAc. The filtrate was washed 3 times with H$_2$O, then brine, dried over Na$_2$SO$_4$, filtered, and concentrated.

6.2 Experimental Procedures

6.2.1 Chemical Synthesis

2-Phenylacetonitrile nitrobenzene 46

Nitrile 46 was prepared according to a modified literature procedure.$^{66}$ NaOH (aq., 50% w/w, 12 mL, 150 mmol) was added to a suspension of 1-fluoro-2-nitrobenzene (3.2 mL, 30 mmol), benzyl cyanide (3.5 mL, 30 mmol), and tetrabutylammonium bisulfate (10.2 g, 30.0 mmol) in toluene (80 mL). The mixture was stirred at 0 °C for 60 min. HCl (aq., 1 M, 150 mL) was added, and the mixture was extracted with CH$_2$Cl$_2$. The combined organic extracts were washed with brine, dried over MgSO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (10% EtOAc/petroleum ether) afforded nitrile 46 as a yellow solid (3.34 g, 14.0 mmol, 47%).

$^1$H (400 MHz, CDCl$_3$): δ 8.08 (1H, dd, $J$ 8.3, 1.3, H6), 7.68-7.77 (2H, m, H3, H4), 7.53-7.59 (1H, ddd, $J$ 8.3, 7.4, 1.3, H5), 7.30-7.41 (5H, m, Ph), 6.18 (1H, s, H7).

$^{13}$C (101 MHz, CDCl$_3$): δ 147.6 (C1), 134.1 (C4), 134.1 (i-Ph), 130.9 (C3), 130.5 (C2), 129.7 (C5), 129.3 (Ph), 128.7 (Ph), 127.9 (Ph), 125.8 (C6), 118.6 (CN), 38.3 (C7).

HRMS (ES$^+$): C$_{14}$H$_{10}$N$_2$NaO$_2$ + ([M+Na]$^+$) requires 261.0634; found 261.0640.
IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3065, 3033, 2867, 2242, 1527, 1347, 736, 699.

MP: 47-48 °C.

2-(3-Nitropyridin-4-yl)-2-phenylacetonitrile 176

Nitrile 176 was prepared according to a modified literature procedure.$^{125}$ Benzyl cyanide (1.20 mL, 10.4 mmol) was added to a suspension of tris[2-(2-methoxyethoxy)ethyl]amine (3.0 mL, 9.4 mmol) and KOH (2.33 g, 41.6 mmol) in THF (25 mL). The mixture was stirred at 50 °C for 10 min, and a solution of 3-nitro-4-chloropyridine (1.48 g, 9.36 mmol) in THF (15 mL) was added over 1 h by syringe pump. The mixture was stirred at 50 °C for a further 30 min, then allowed to cool to rt and quenched with NH$_4$Cl (saturated aq., 50 mL). The mixture was extracted with EtOAc, and the combined organic extracts were washed with pH 6 phosphate buffer, then brine, dried over MgSO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (22% EtOAc/petroleum ether) afforded nitrile 176 as a brown solid (1.23 g, 5.14 mmol, 55%).

$^1$H (400 MHz, CDCl$_3$): $\delta$ 9.28 (1H, s, $H_2$), 8.92 (1H, d, $J = 5.1$, $H_6$), 7.72 (1H, d, $J = 5.1$, $H_5$), 7.36-7.45 (3H, m, Ph), 7.29-7.35 (2H, m, Ph), 6.22 (1H, s, $H7$).

$^{13}$C (101 MHz, CDCl$_3$): $\delta$ 154.7 (C6), 146.8 (C2), 143.3, 139.4, 132.4, 129.7 (Ph), 129.3 (Ph), 128.0 (Ph), 124.4 (C5), 117.3 (CN), 38.0 (C7).

HRMS (ES$^+$): [C$_{13}$H$_9$O$_2$N$_3$Na]$^+$ ([M+Na]$^+$) requires 262.05870; found 262.05884.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3081, 2248, 1595, 1568, 1530, 1348, 824, 762, 698.

MP: 52-54 °C.
Nitrile 174 was prepared according to a modified literature procedure. Benzyl cyanide (1.20 mL, 10.4 mmol) was added to a suspension of tris[2-(2-methoxyethoxy)ethyl]amine (3.0 mL, 9.4 mmol) and KOH (2.33 g, 41.6 mmol) in THF (25 mL). The mixture was stirred at 50 °C for 10 min, and a solution of 2-chloro-3-nitropyridine (1.48 g, 9.36 mmol) in THF (15 mL) was added over 1 h by syringe pump. The mixture was stirred at 50 °C for a further 30 min, then allowed to cool to rt and quenched with NH₄Cl (saturated aq., 50 mL). The mixture was extracted with EtOAc, and the combined organic extracts were washed with pH 6 phosphate buffer, then brine, dried over MgSO₄, filtered, and concentrated. Purification by flash pressure column chromatography (22% EtOAc/petroleum ether) afforded nitrile 174 as a yellow solid (1.02 g, 4.26 mmol, 46%).

$^1$H (400 MHz, CDCl₃): δ 8.89 (1H, dd, J 4.6, 1.5, H6), 8.32 (1H, dd, J 8.3, 1.7, H4), 7.48 (1H, dd, J 8.3, 4.6, H5), 7.38-7.44 (2H, m, Ph), 7.24-7.32 (3H, m, Ph), 6.25 (1H, s, H7).

$^{13}$C (101 MHz, CDCl₃): δ 153.7 (C6), 149.5, 143.9, 133.8 (C4), 133.2, 129.2 (Ph), 128.9 (Ph), 128.3 (Ph), 124.2 (C5), 118.0 (CN), 42.0 (C7).

HRMS (ES$^+$): [C$_{13}$H$_9$O$_3$N$_3$Na]$^+$ ([M+Na]$^+$) requires 262.0587; found 262.0588.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3109, 2250, 1597, 1528, 1350, 1207, 849, 748, 696.

MP: 96-98 °C.
Ethyl 2-cyano-2-(nitrophenyl)acetate 73

![Ethyl 2-cyano-2-(nitrophenyl)acetate](image)

Ethyl cyanoacetate (4.3 mL, 40 mmol) was added dropwise to a suspension of KOtBu (4.9 g, 44 mmol) in THF (50 mL) and stirred at 0 °C for 10 min. 1-Fluoro-2-nitrobenzene (2.1 mL, 20 mmol) was added dropwise and stirred at 60 °C for 16 h. The mixture was diluted with EtOAc, and HCl (aq., 1 M, 30 mL) was added. The solution with extracted with EtOAc. The combined organic extracts were washed with brine, dried over MgSO₄, filtered, and concentrated. Purification by flash pressure column chromatography (45% Et₂O/hexane) afforded nitrile 73 as a yellow oil (1.9 g, 8.0 mmol, 40%).

$^1$H (400 MHz, CDCl₃): δ 8.23 (1H, d, J 7.8, H6), 7.74-7.81 (2H, m, H3, H4), 7.65 (1H, m, H5), 5.66 (1H, s, H7), 4.30 (2H, q, J 7.1, CH₂CH₃), 1.32 (3H, t, J 7.1, CH₂CH₃).

$^{13}$C (101 MHz, CDCl₃): δ 163.5 (C=O), 147.3 (C1), 134.5 (C3/C4), 131.5 (C3/C4), 130.6 (C5), 126.0 (C6), 125.2 (C2), 114.4 (CN), 63.8 (C7), 41.2 (CH₂CH₃), 13.8 (CH₂CH₃).

HRMS (ES⁺): C₁₁H₁₀NaN₂O₄⁺ ([M+Na⁺]⁺ requires 257.0538; found 257.0541.

IR: $v_{max}$ (film)/cm⁻¹ 3034, 2841, 2241, 1746 (C=O), 1528, 1347, 1216, 1023, 789, 739, 699.

Methyl 2-(nitrophenyl)-2-phenylacetate 62

![Methyl 2-(nitrophenyl)-2-phenylacetate](image)

A solution of methyl phenylacetate (0.56 mL, 4.0 mmol) in THF (20 mL) was added to a solution of KHMDS (0.5 M in toluene, 16.8 mL, 8.40 mmol) in THF (20 mL) and stirred at -78 °C for 10 min. 1-Fluoro-2-nitrobenzene (0.42 mL, 4.0 mmol) was added dropwise, and the mixture was stirred at -60 °C for 30 min. The solution was allowed to warm to 0 °C, and H₂O (10 mL) was added. The

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mixture was extracted with EtOAc. Purification by flash pressure column chromatography (10% Et₂O/petroleum ether) afforded ester 62 as a pale yellow oil (335 mg, 1.24 mmol, 31%).

\(^1\)H (400 MHz, CDCl₃): δ 7.56 (1H, dd, J 7.9, 1.3, H6), 6.95-7.14 (6H, m, H3, Ph), 6.72 (1H, app td, J 7.9, 1.3, H4), 6.58 (1H, app td, J 7.9, 1.2, H5), 5.75 (1H, s, H7), 3.31 (3H, s, CH₃).

\(^1^3\)C (101 MHz, CDCl₃): δ 172.1 (CO₂Me), 149.9 (C1), 137.5 (i-Ph), 134.6 (C2), 133.0 (C4), 132.0 (C3), 130.0 (Ph), 129.6 (Ph), 128.3 (Ph), 128.2 (C5), 125.2 (C6), 54.0 (C7), 52.4 (CH₃).

HRMS (ES⁺): C₁₁H₈NaNO₄⁺ ([M+Na⁺]) requires 294.0742; found 294.0745.

IR: ν_{max} (film)/cm⁻¹ 2872, 1737 (C=O), 1526, 1350, 1204, 1165, 1009, 737, 703.

Methyl 2-(nitrophenyl)acetate 82

Ester 82 was prepared according to a literature procedure. Two drops of concentrated H₂SO₄ were added to a stirred solution of 2-nitrophenylacetic acid (6.0 g, 33 mmol) in CH₃OH. The solution was stirred at 60 °C for 16 h, then concentrated. The resulting residue was dissolved in EtOAc and washed with H₂O, then with brine. The organic layer was dried over MgSO₄, filtered, and concentrated to afford ester 82 as a colorless oil (6.6 g, 33 mmol, quant). The spectral data matched those previously reported in the literature.

\(^1\)H (400 MHz, CDCl₃): δ 8.12 (1H, dd, J 8.0, 1.3, H6), 7.61 (1H, app td, J 7.5, 1.2, H4), 7.49 (1H, app td, J 8.0, 1.3, H5), 7.37 (1H, dd, J 7.5, 1.2, H3), 4.04 (2H, s, H7), 3.72 (3H, s, CO₂CH₃).

\(^1^3\)C (101 MHz, CDCl₃): δ 170.4 (C=O), 148.7 (C1), 133.6 (C4), 133.3 (C3), 129.7 (C2), 128.6 (C5), 125.3 (C6), 52.2 (CO₂CH₃), 39.5 (C7).

LCMS (ES⁺'): 218.1 ([M+Na⁺]).

IR: ν_{max} (film)/cm⁻¹ 3004, 2955, 1737 (C=O), 1524, 1347, 1196, 1171, 713.
Ester 83 was prepared according to a literature procedure. A mixture of ester 82 (5.0 g, 26 mmol), paraformaldehyde (3.08 g, 103 mmol), tetrabutylammonium iodide (380 mg, 1.03 mmol), and K$_2$CO$_3$ (14.2 g, 103 mmol) in toluene (50 mL) was stirred at 50 °C for 5 h. The reaction was quenched with H$_2$O, and the mixture was extracted with toluene. The combined organic extracts were washed with brine, dried over MgSO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (10% EtOAc/petroleum ether) afforded ester 83 as a pale yellow oil (4.97 g, 24.1 mmol, 94%). The spectral data matched those previously reported in the literature.

$^{1}$H (400 MHz, CDCl$_3$): δ 8.12 (1H, d, J 7.9, H6), 7.66 (1H, app t, J 7.5, H4), 7.54 (1H, app t, J 7.9, H5), 7.40 (1H, d, J 7.5, H3), 6.55 (1H, app s, H8a), 5.89 (1H, app s, H8b), 3.73 (3H, s, CO$_2$C$_6$H$_3$).

$^{13}$C (101 MHz, CDCl$_3$): δ 165.2 (C=O), 147.8 (C1), 139.7 (C7), 133.7 (C4), 132.9 (C2), 132.1 (C3), 129.3 (C5), 127.5 (C8), 124.5 (C6), 52.3 (CO$_2$CH$_3$).

LRMS (ES$^+$): 208.1 ([M+H$^+$]), 230.1 ([M+Na$^+$]).

IR: $\nu_{\text{max}}$(film)/cm$^{-1}$ 2954, 1721 (C=O), 1524, 1347, 1208, 812, 788.

2-Phenylnitrile aniline 47

Aniline 47 was prepared according to a modified literature procedure. Zinc powder (8.24 g, 126 mmol) and NH$_4$Cl (10.1 g, 189 mmol) were added to a solution of nitrile 46 (3.00 g, 12.6 mmol) in 5:1 acetone/H$_2$O (240 mL). The mixture was stirred at rt for 20 min, then filtered through Celite$^\text{TM}$, eluted with EtOAc, and washed with water, then brine. The organic layer was dried over MgSO$_4$,
filtered, and concentrated. Purification by flash pressure column chromatography (13% EtOAc/petroleum ether) afforded aniline 47 as an orange solid (2.16 g, 10.4 mmol, 82%).

$^1$H (400 MHz, CDCl$_3$): δ 7.30-7.44 (6H, m, H3, Ph), 7.21 (1H, app td, J 7.8, 1.2, H5), 6.89 (1H, app td, J 7.8, 1.2, H4), 6.74 (1H, dd, J 7.8, 1.2, H6), 5.19 (1H, s, H7), 3.54 (2H, br s, NH$_2$).

$^{13}$C (101 MHz, CDCl$_3$): δ 143.8 (C1), 133.9 (i-Ph), 129.6 (C5), 129.5 (C3), 129.4 (Ph), 128.5 (Ph), 127.7 (Ph), 120.0 (C2), 119.5 (C4), 118.9 (CN), 117.5 (C6), 39.0 (C7).

HRMS (ES$^+$): C$_{14}$H$_{12}$N$_2$Na$^+$ ([M+Na]$^+$) requires 231.0893; found 231.0901.

IR: $\nu$ max (film)/cm$^{-1}$ 3482, 3374, 3076, 3014, 2259, 1628, 1496, 1455, 751, 698.

MP: 72-73 °C.

2-(3-aminopyridin-4-yl)-2-phenylacetonitrile 177

Aniline 177 was prepared according to a modified literature procedure. Zinc powder (2.73 g, 41.8 mmol) and NH$_4$Cl (3.35 g, 62.7 mmol) were added to a solution of nitrile 176 (1.00 g, 4.18 mmol) in 5:1 acetone/H$_2$O (25 mL). The mixture was stirred at rt for 20 min, then filtered through Celite$^\text{TM}$, eluted with EtOAc, and washed with water, then brine. The organic layer was dried over MgSO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (3% MeOH/CH$_2$Cl$_2$) afforded aniline 177 as a yellow solid (443 mg, 2.12 mmol, 51%).

$^1$H (400 MHz, CDCl$_3$): δ 8.09-8.16 (2H, m, H2, H6), 7.38-7.45 (3H, m, Ph), 7.31-7.36 (2H, m, Ph), 7.23 (1H, d, J 4.9, H5), 5.15 (1H, s, H7), 3.66 (2H, br s, NH$_2$).

$^{13}$C (101 MHz, CDCl$_3$): δ 140.9 (C2/C6), 139.9, 139.6 (C2/C6), 132.3, 129.7 (Ph), 129.1 (Ph), 127.7 (Ph), 127.3, 123.0 (C5), 117.7 (CN), 38.4 (C7).
HRMS (ES\textsuperscript{+}): \([C_{13}H_{12}N_3]^+ ([M+H]^+))\) requires 210.10257; found 210.10274.

IR: \(v_{\text{max}}\) (film)/cm\(^{-1}\) 3391, 3208, 2251, 1636, 1566, 1495, 1422, 1331, 1072, 733, 698.

MP: 110-112 °C.

\((E)-((S\acute{\text{r}}\text{-Bromopyridin-3\acute{\text{-}}}\text{-yl})\text{methylene})\text{amino})\text{phenyl-2-phenylacetonitrile 48}\)

5-Bromo-3-pyridinecarboxaldehyde (800 mg, 4.30 mmol) was added to a suspension of aniline 47 (597 mg, 2.87 mmol) and MgSO\textsubscript{4} (1.73 g, 14.3 mmol) in dry toluene (25 mL). The mixture was stirred at rt for 2 h, then filtered and concentrated. The crude product was triturated with ice-cooled Et\textsubscript{2}O to afford imine 48 as a yellow solid (1.02 g, 2.71 mmol, 94%).

\(^1\text{H}\) (400 MHz, CDCl\textsubscript{3}): \(\delta 8.85 (1H, d, J 1.7, H2'/H6'), 8.74 (1H, d, J 2.2, H2'/H6'), 8.33-8.39 (2H, m, H9, H4'), 7.45 (1H, dd, J 7.6, 1.5, H3), 7.35 (1H, app td, J 7.7, 1.5, H5), 7.19-7.33 (6H, m, Ph, H4), 7.05 (1H, dd, J 7.7, 1.2, H6), 5.83 (1H, s, H7).

\(^{13}\text{C}\) (101 MHz, CDCl\textsubscript{3}): \(\delta 156.1 (C9), 153.4 (C2'/C6'), 148.9 (C2'/C6'), 147.8 (C1), 137.2 (C4'), 135.8, 132.9, 131.2, 129.7 (C5), 129.0 (Ph/C4), 128.9 (C3), 128.0 (Ph/C4), 127.9 (Ph/C4), 127.7 (Ph/C4), 121.4 (C5'), 119.8 (CN), 117.8 (C6), 37.7 (C7).

HRMS (ES\textsuperscript{+}): \(C_{20}H_{14}^{79}\text{BrN}_3\text{Na}^+ ([M+Na]^+)\) requires 398.0263; found 398.0265.

IR: \(v_{\text{max}}\) (film)/cm\(^{-1}\) 3061, 2894, 2244, 1629, 1551, 1493, 1452, 764, 696.

MP: 115-118 °C.
(E)-2-(2-((3-Bromobenzylidene)amino)phenyl)-2-phenylacetonitrile 123

3-Bromobenzaldehyde (0.25 mL, 2.1 mmol) was added to a suspension of aniline 47 (370 mg, 1.78 mmol) and MgSO₄ (1.07 g, 8.89 mmol) in dry toluene (12 mL). The mixture was stirred at rt for 16 h, then filtered and concentrated. Purification by flash pressure column chromatography (8-15% Et₂O/petroleum ether) afforded imine 123 as a yellow solid (497 mg, 1.32 mmol, 74%).

1H (400 MHz, C₆D₆): δ 7.78 (1H, app t, J 1.4, H2'), 7.55 (1H, s, H9), 7.48 (1H, dd, J 7.6, 1.4, H3), 7.41 (1H, app dt, J 7.8, 1.4, H6'), 7.21-7.27 (3H, m, H4', o-Ph), 6.85-7.01 (5H, m, m-Ph, p-Ph, H4, H5), 6.74 (1H, app t, J 7.8, H5'), 6.53 (1H, dd, J 7.6, 1.4, H6), 5.66 (1H, s, H7).

13C (101 MHz, C₆D₆): δ 159.3 (C9), 149.0 (C1), 138.6 (C1'), 137.2 (i-Ph), 134.9 (C4'), 132.6 (C2'), 132.2 (C2), 130.8 (C5'), 129.8 (C5), 129.4 (p-Ph), 129.2 (C3), 128.5 (o-Ph), 128.1 (p-Ph), 127.8 (C4), 127.8 (C6'), 123.6 (C3'), 120.4 (CN), 118.5 (C6), 38.5 (C7).

HRMS (ES⁺): C₂₁H₁₆⁷⁹BrN₂⁺ ([M+H]⁺) requires 375.0497; found 375.0488.

IR: νmax (film)/cm⁻¹: 3062, 3029, 2900, 2243, 1627, 1563, 1487, 1193, 786, 698.

MP: 123-126 °C.

(E)-2-(2-(((5-Bromofuran-2-yl)methylene)amino)phenyl)-2-phenylacetonitrile 138

5-Bromo-2-furaldehyde (333 mg, 1.90 mmol) was added to a suspension of aniline 47 (330 mg, 1.58 mmol) and MgSO₄ (954 mg, 7.92 mmol) in CH₂Cl₂ (12 mL). The mixture was stirred at rt for 16 h, then filtered and concentrated. Purification by flash pressure column chromatography
(20% Et₂O/petroleum ether) afforded imine 138 as a yellow oil (398 mg, 1.09 mmol, 69%).

\(^1\)H (400 MHz, C₆D₆): δ 7.41-7.48 (2H, m, H3, H9), 7.29-7.35 (2H, m, o-Ph), 6.86-6.99 (5H, m, H4, H5, m-Ph, p-Ph), 6.47 (1H, dd, J 7.3, 1.9, H6), 6.28 (1H, d, J 3.4, H3’/H4’), 5.84-5.91 (2H, m, H7, H3’/H4’).

\(^{13}\)C (101 MHz, C₆D₆): δ 153.6 (C2’), 148.4 (C1), 147.5 (C9), 144.3 (C5’), 137.3 (i-Ph), 132.8 (C2), 129.7 (C5), 129.4 (m-Ph/p-Ph), 129.2 (C3), 128.5 (o-Ph), 128.0 (C4), 120.5 (CN), 118.0 (C6), 117.8 (C3’/C4’), 114.7 (C3’/C4’), 38.1 (C7).

HRMS (ESI): C₁₉H₁₃⁷⁹BrNaN₂O⁺ ([M+Na]⁺) requires 387.0109; found 387.0096.

IR: \(\nu_{\text{max}}\) (film)/cm⁻¹ 3029, 2914, 2244, 1628, 1466, 1018, 753, 697.

† 1 peak obscured by solvent signal.

\((E)-2-(2-(((4-Bromofuran-2-yl)methylene)amino)phenyl)-2-phenylacetonitrile 132\)

Imine 132 was prepared according to a modified literature procedure.\(^{100}\) Pyrrolidine (14 µL, 0.17 mmol) was added to a stirred slurry of aniline 47 (350 mg, 1.68 mmol), 4-bromo-2-furaldehyde (382 mg, 2.18 mmol), and 3 Å molecular sieves (1.7 g) in CH₂Cl₂ (10 mL). The mixture was stirred at 50 °C for 18 h, then filtered through Celite™, eluted with CH₂Cl₂, and concentrated. Purification by flash pressure column chromatography (10-45% Et₂O/petroleum ether) afforded imine 132 as an orange oil (359 mg, 0.98 mmol, 59%).

\(^1\)H (400 MHz, C₆D₆): δ 7.41-7.47 (2H, m, H3, H9), 7.29-7.34 (2H, m, o-Ph), 6.86-6.98 (6H, m, H4, H5, H5’, m-Ph, p-Ph), 6.54 (1H, s, H3’), 6.43 (1H, app m, H6), 5.89 (1H, s, H7).

\(^{13}\)C (101 MHz, C₆D₆): δ 153.6 (C2’), 148.4 (C1), 147.5 (C9), 144.3 (C5’), 137.3 (i-Ph), 132.8 (C2), 129.7 (C5), 129.4 (m-Ph/p-Ph), 129.2 (C3), 128.5 (o-Ph), 128.0 (C4), 120.5 (CN), 118.0 (C6), 117.8 (C3’), 38.1 (C7).
HRMS (ES\(^+\)): C\(_{19}\)H\(_{13}\)BrNaN\(_2\)O\(_2\) \([\text{M+Na}]^+\) requires 387.0109; found 387.0095.

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3141, 2922, 2244, 1626, 1493, 1213, 925, 754, 697.

† 1 peak obscured by solvent signal.

(E)-2-(((5-Bromopyridin-3-yl)methylene)amino)phenyl)-2-phenylacetonitrile 64

\[
\begin{array}{c}
\text{CO}_2\text{Me} \\
\text{Ph} \\
62 \quad \xrightarrow{\text{H}_2/\text{Pd/C, EtOAc, rt}} \quad \text{NH}_2 \\
\text{NO}_2
\end{array}
\quad \begin{array}{c}
\text{CO}_2\text{Me} \\
\text{Ph} \\
63 \quad \xrightarrow{5\text{-bromo-3-pyridinecarboxaldehyde, MgSO}_4, \text{toluene, rt}} \quad \text{Br} \\
\end{array}
\]

Pd/C (wet degussa type, 16 mg) was added to a solution of ester 62 (160 mg, 0.59 mmol) in EtOAc (6 mL). The suspension was degassed 3 times with hydrogen using a pump-flood procedure and placed under hydrogen for 2.5 h. The mixture was filtered through Celite\textsuperscript{TM}, eluted with EtOAc, and concentrated at 26 °C. The resulting residue was dissolved in toluene (6 mL), and MgSO\(_4\) (355 mg, 2.95 mmol) and 5-Bromo-3-pyridinecarboxaldehyde (120 mg, 0.65 mmol) were added. The mixture was stirred at rt for 16 h, then filtered and concentrated. Purification by flash pressure column chromatography (17% EtOAc/petroleum ether) afforded imine 64 as a yellow oil (220 mg, 0.54 mmol, 91%).

\(^1\)H (400 MHz, C\(_6\)D\(_6\)): δ 8.63 (1H, d, \(J\ 2.0,\ H2'/H6'\)), 8.59 (1H, d, \(J\ 2.0,\ H2'/H6'\)), 8.12 (1H, app t, \(J\ 2.0,\ H4'\)), 7.59 (1H, s, H9), 7.34-7.44 (3H, m, H3, o-Ph), 6.98-7.14 (5H, m, H4, H5, m-Ph, p-Ph), 6.67 (1H, dd, J 7.6, 1.5, H6), 5.74 (1H, s, H7), 3.32 (3H, s, CO\(_2\)CH\(_3\)).

\(^{13}\)C (101 MHz, C\(_6\)D\(_6\)): δ 173.3 (C=O), 155.6 (C9), 153.5 (C2'/C6'), 149.5 (C2'/C6'), 149.4 (C1), 138.7, 137.4 (C4'), 135.6, 133.9, 130.0 (C3/o-Ph), 129.8 (C3/o-Ph), 129.3 (C5), 128.6 (Ph/C4), 127.9 (Ph/C4), 127.8 (Ph/C4), 121.9 (C5'), 117.7 (C6), 53.6 (C7), 52.1 (CO\(_2\)CH\(_3\)).

HRMS (ES\(^+\)): C\(_{21}\)H\(_{17}\)BrNaN\(_2\)O\(_2\) \([\text{M+Na}]^+\) requires 431.0371; found 431.0364.

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3024, 2949, 1735 (C=O), 1628, 1199, 1153, 1018, 765, 698.
Methyl (E)-2-((5-Bromopyridin-3-yl)methylene)amino)phenyl)propanoate 85

Pd/C (wet degussa type, 120 mg) was added to a solution of ester 83 (1.2 g, 5.8 mmol) in EtOAc (35 mL). The suspension was degassed 3 times with hydrogen using a pump-flood procedure and placed under hydrogen for 2 h. The mixture was filtered through Celite™, eluted with EtOAc, and concentrated at 26 °C. The resulting residue was dissolved in toluene (40 mL), and powdered MgSO₄ (3.8 g, 32 mmol) and 5-Bromo-3-pyridinecarboxaldehyde (1.2 g, 6.4 mmol) were added. The mixture was stirred at rt for 1 h, then filtered and concentrated. Purification by flash pressure column chromatography (32% Et₂O/petroleum ether) afforded imine 85 as a pale yellow solid (1.2 g, 3.4 mmol, 58%).

¹H (400 MHz, CDCl₃): δ 8.90 (1H, d, J 2.0, H6’), 8.77 (1H, d, J 2.0, H2’), 8.46 (1H, app t, J 2.0, H4’), 8.44 (1H, s, H9), 7.35 (1H, app m, H3), 7.28-7.33 (2H, m, H4, H5), 7.06 (1H, dd, J 7.5, 1.4, H6), 4.23 (1H, q, J 7.2, H7), 3.63 (3H, s, CO₂CH₃), 1.51 (3H, d, J 7.2, H8).

¹³C (101 MHz, CDCl₃): δ 175.3 (C=O), 154.8 (C9), 153.0 (C2’), 148.9 (C6’), 148.2 (C1), 137.2 (C4’), 136.0 (C2), 133.4 (C3’), 128.1 (C4/C5), 128.0 (C3), 127.5 (C4/C5), 121.4 (C5’), 117.3 (C6), 51.9 (CO₂CH₃), 41.0 (C7), 17.8 (C8).

HRMS (ES⁺): C₁₆H₁₅BrNaN₂O₂⁺ ([M+Na]⁺) requires 369.0215; found 369.0220.

IR: νmax (film)/cm⁻¹ 2982, 2948, 1732 (C=O), 1628, 1208, 743, 696.

MP: 65-66 °C.
(E)-2-(2-(((5-(Benzyloxy)pyridin-3-yl)methylene)amino)phenyl)-2-phenylacetonitrile 92

A solution of aldehyde 91 (4.86 g, 22.8 mmol) in toluene (25 mL) was added to a slurry of aniline 47 (4.0 g, 19 mmol) and MgSO₄ (11.4 g, 94.7 mmol) in toluene (75 mL). The mixture was stirred at rt for 16 h, filtered, and concentrated. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded imine 92 as a pale yellow solid (6.05 g, 15.0 mmol, 79%).

¹H (500 MHz, C₆D₆): δ 8.59 (1H, d, J 2.9, H6'), 8.41 (1H, d, J 1.6, H2'), 7.74 (1H, dd, J 2.9, 1.6, H4'), 7.63 (1H, s, H9), 7.43 (1H, dd, J 7.5, 1.7, H6), 7.18-7.24 (4H, m, Ph), 7.13 (2H, app t, J 7.6, Ph), 7.05 (1H, app t, J 7.4, Ph), 6.84-7.00 (5H, m, H4, H5, Ph), 6.50 (1H, dd, J 7.8, 1.4, H3), 5.65 (1H, s, H7), 4.72 (1H, d, J 12.0, OCH₂H₃Phi), 4.67 (1H, d, J 12.0, OCH₂H₃Phi).

¹³C (126 MHz, C₆D₆): δ 157.6 (C9), 155.4 (C5'), 148.5 (C1), 144.7 (C2'), 142.9 (C6'), 136.8 (i-Ph), 136.5 (i-Ph), 132.4 (C3'), 131.8 (C2), 129.5 (C4/C5/Ph), 129.1 (C4/C5/Ph), 128.9 (C6), 128.6 (Ph), 128.5 (Ph), 127.7 (C4/C5/Ph), 127.5 (C4/C5/Ph), 120.0 (CN), 118.2 (C3), 117.9 (C4'), 70.3 (OCH₂Ph), 38.3 (C7).‡

‡ 2 peaks obscured.

HRMS (ES⁺): [C₃₀H₂₂O₃N₃]⁺ ([M+H]⁺) requires 404.17574; found 404.17587.

IR: νmax (film)/cm⁻¹ 3063, 2879, 2243, 1628, 1589, 1454, 1425, 1317, 1283, 1175, 737, 696.

MP: 78-80 °C.
(E)-2-(3-(((5-Bromopyridin-3-yl)methylene)amino)pyridin-2-yl)-2-phenylacetonitrile \(178\)

5-Bromopyridinecarboxaldehyde (289 mg, 1.55 mmol) was added to a suspension of aniline \(175\) (250 mg, 1.19 mmol) and MgSO\(_4\) (719 mg, 5.97 mmol) in toluene (10 mL) and stirred at rt for 24 h. The mixture was filtered and concentrated. The crude product was triturated three times with ice-cooled Et\(_2\)O to afford imine \(178\) as a yellow solid. (265 mg, 0.70 mmol, 59%, contains minor impurities).

\(^1\)H (400 MHz, CDCl\(_3\)): \(\delta\) 8.69 (1H, d, \(J\) 2.0, \(H2'/H6'\)), 8.61 (1H, d, \(J\) 2.0, \(H2'/H6'\)), 8.36 (1H, dd, \(J\) 4.8, 1.6, \(H6\)), 8.21 (1H, app t, \(J\) 2.0, \(H4'\)), 8.16 (1H, s, \(H9\)), 7.17-7.25 (3H, m, \(H4, Ph\)), 7.11-7.16 (1H, dd, \(J\) 7.8, 4.8, \(H5\)), 6.98-7.10 (3H, m, \(Ph\)), 5.74 (1H, s, \(H7\)).

\(^{13}\)C (101 MHz, CDCl\(_3\)): \(\delta\) 158.0 (C9), 153.8 (C2'/C6'), 150.1, 149.0 (C2'/C6'), 148.4 (C6), 143.4, 137.4 (C4'), 134.7, 132.4, 128.9 (Ph), 128.1 (Ph), 127.8 (C4/Ph), 125.5 (C4/Ph), 124.4 (C5), 121.5 (C5'), 118.9 (CN), 41.1 (C7).

HRMS (ES\(^+\)): [C\(_{19}\)H\(_{14}\)N\(_4\)\(^{79}\)Br\(^+\)]\(^+\) (\([M+H]^+\)) requires 377.0396; found 377.0400.

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3059, 2245, 1626, 1493, 1437, 1200, 1018, 748, 696.

MP: 116-118 °C.
(2RS, 3SR)-2-(5′-Bromopyridin-3′-yl)-3-phenylindoline-3-carbonitrile 49 and (2RS, 3RS)-2-(5′-bromopyridin-3′-yl)-3-phenylindoline-3-carbonitrile 50

Indolines 49 and 50 were prepared according to general procedure 1, using KOtBu (656 mg, 5.85 mmol), imine 48 (1.10 g, 2.92 mmol) in toluene (50 mL). The reaction mixture was stirred at 0 °C for 20 min. Purification by flash pressure column chromatography (18% EtOAc/petroleum ether) afforded separately indoline 49 as a colorless solid (338 mg, 0.90 mmol, 31%) and indoline 50 as a colorless solid (580 mg, 1.54 mmol, 53%).

N.B. Stereochemistry assigned by nOe analysis, with red arrows indicating through-space interactions.

(2RS,3SR)-2-(5′-Bromopyridin-3′-yl)-3-phenylindoline-3-carbonitrile 49

$^1$H (500 MHz, CD$_6$D): δ 8.46 (1H, d, J 2.0, H₂'), 8.36 (1H, d, J 2.0, H₆'), 7.16 (1H, app s H₄'), 7.05 (1H, app td, J 7.7, 1.2, H₆), 6.99 (1H, app d, J 7.7, H₄), 6.69-6.75 (3H, m, o-, p-Ph), 6.60-6.68 (3H, m, H₅, m-Ph), 6.41 (1H, app d, J 7.7, H₇), 6.49 (1H, d, J 3.4, H₂), 2.72 (1H, d, J 3.4, NH).

$^{13}$C (126 MHz, CD$_6$D): δ 151.4 (C6'), 150.4 (C7a), 147.8 (C2'), 137.8 (C4'), 134.7 (i-Ph), 134.4 (C3'), 130.7 (C6), 128.9 (Ph), 128.9 (Ph), 128.7 (Ph), 128.2 (C3a) 126.1 (C4), 121.8 (C5), 121.5 (CN), 120.7 (C5'), 111.0 (C7), 71.9 (C2), 56.0 (C3).

HRMS (ES⁺): C$_{20}$H$_{14}$BrN$_3$Na$^+$ ([M+Na]$^+$) requires 398.0263; found 398.0270.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3354, 3059, 2242, 1608, 1485 1262, 752, 701.

MP: 133-136 °C.

(2RS,3RS)-2-(5′-Bromopyridin-3′-yl)-3-phenylindoline-3-carbonitrile 50

$^1$H (500 MHz, CD$_6$D): δ 8.65 (1H, app s, H₆'), 8.08 (1H, app s, H₂'), 7.91 (1H, app t, J 1.9, H₄'), 7.17-
7.20 (2H, m, o-Ph), 6.95-7.04 (4H, m, H6, m-Ph, p-Ph), 6.67 (1H, app d, J 7.7, H4), 6.60 (1H, app td, J 7.7, 0.9, H5), 6.43 (1H, app d, J 7.7, H7), 4.21 (1H, d, J 3.2, H2), 2.93 (1H, d, J 3.2, NH).

\(^{13}\text{C}\ (126\text{ MHz, }\text{C}_6\text{D}_6):\ \delta 152.3 (C6'), 150.6 (C7a), 148.1 (C2'), 138.5 (C4'), 137.4 (\text{-Ph}), 134.6 (C3'), 130.7 (C6), 129.5 (Ph), 129.2 (Ph), 128.7 (Ph), 127.5 (C3a), 126.2 (C4), 121.5 (C5), 121.4 (C5') 118.8 (CN), 111.2 (C7), 75.4 (C2), 58.9 (C3).

HRMS (ES\(^{+}\)): C\(_{20}\)H\(_{14}\)\(_{79}\)BrN\(_3\)Na\(^{+}\) ([M+Na\(^{+}\)] requires 398.0263; found 398.0270.

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3343, 3058, 2242, 1607, 1484, 1470, 1257, 752, 702.

MP: 69-70 °C.

**Ethyl (2RS, 3RS)-2-(5-bromopyridin-3-yl)-3-cyanoindoline-3-carboxylate 77**

Pd/C (wet degussa type, 10% palladium by weight, 80 mg) was added to a solution of nitrile 73 (800 mg, 3.42 mmol) in EtOAc (20 mL). The suspension was degassed 3 times with hydrogen using a pump-flood procedure and placed under hydrogen for 45 min. The mixture was filtered through Celite\textsuperscript{TM}, eluted with EtOAc, and concentrated at 26 °C. The resulting residue was dissolved in toluene (24 mL), and MgSO\(_4\) (2.06 g, 17.1 mmol) and 5-Bromo-3-pyridinecarboxaldehyde (699 mg, 3.76 mmol) were added. The mixture was stirred at rt for 16 h, then filtered and concentrated. Purification by flash pressure column chromatography (28% EtOAc/petroleum ether) afforded indoline 77 as a 97:3 mixture of diastereoisomers as a pale yellow solid (889 mg, 2.38 mmol, 68%).

N.B. Stereochemistry assigned by X-ray crystallography. Major diastereoisomer (as drawn):

\(^1\text{H}\ (400\text{ MHz, }\text{C}_6\text{D}_6):\ \delta 8.69 (1H, d, J 1.9, H2'), 8.60 (1H, d, J 2.2, H6'), 7.85 (1H, app t, J 2.0, H4'), 7.09 (1H, app d, J 7.9, H4), 6.98 (1H, app td, J 7.7, 1.2, H6), 6.62 (1H, app td, J 7.7, 1.0, H5), 6.34 (1H, app
Methyl (2RS,3SR)-2-(5-bromopyridin-3-yl)-3-phenylindoline-3-carboxylate 65 and Methyl (2RS,3RS)-2-(5-bromopyridin-3-yl)-3-phenylindoline-3-carboxylate 66

Indolines 65 and 66 were prepared according to general procedure 1, using KO\textsuperscript{t}Bu (72 mg, 0.65 mmol), and imine 64 (240 mg, 0.59 mmol) in toluene (11 mL). The reaction mixture was stirred at 0 °C for 20 min. Purification by flash pressure column chromatography (18% EtOAc/petroleum ether) afforded separately indoline 65 as a colorless solid (76 mg, 0.19 mmol, 31%) and indoline 66 as a colorless solid (66 mg, 0.16 mmol, 27%).

N.B. Stereochemistry assigned by nOe analysis, with red arrows indicating through-space interactions.

Methyl (2RS,3SR)-2-(5-bromopyridin-3-yl)-3-phenylindoline-3-carboxylate 65

\(^1\text{H} (500 \text{ MHz}, \text{C}_6\text{D}_6): \delta 8.68 (1\text{H}, \text{d}, J 1.7, H2'), 8.52 (1\text{H}, \text{d}, J 2.4, H6'), 7.51 (1\text{H}, \text{app d}, J 7.6, H4), 7.40
(1H, app t, J 1.9, H4'), 7.16 (1H, obscured, H6), 6.83 (1H, app td, J 7.6, 1.0, H5), 6.69-6.79 (3H, m, m- Ph, p-Ph), 6.50 (1H, app d, J 7.7, H7), 6.44 (2H, m, o-Ph), 5.55 (1H, d, J 3.6, H2), 3.26 (3H, s, CO2CH3), 2.91 (1H, d, J 3.3, NH).

13C (126 MHz, C6D6): δ 173.7 (C=O), 151.2 (C7a), 150.4 (C6'), 149.1 (C2'), 138.8 (i-Ph), 138.8 (C4'), 137.4 (C3'), 130.0 (C6), 128.6 (o-Ph), 128.4 (m-Ph/p-Ph), 127.9 (m-Ph/p-Ph), 127.4 (C4), 120.5 (C5), 120.5 (C5'), 110.5 (C7), 67.8 (C2), 67.2 (C3), 52.6 (CH3).†

† 1 peak obscured by solvent signal.

HRMS (ES⁺): [C21H17BrN2O2]⁺ ([M+H]⁺) requires 409.0546; found 409.0538.

IR: νmax (film)/cm⁻¹ 3361, 1730 (C=O), 1605, 1483, 1237, 1041, 712, 699.

MP: 118-121 °C.

Methyl (2RS,3RS)-2-(5-bromopyridin-3-yl)-3-phenylindoline-3-carboxylate 66

1H (500 MHz, C6D6): δ 8.64 (1H, d, J 2.2, H6'), 8.34 (1H, d, J 1.7, H2'), 7.79 (1H, app t, J 2.1, H4'), 7.37 (1H, app d, J 7.4, H4), 7.32 (2H, m, o-Ph), 7.01-7.11 (4H, m, H6, m-Ph, p-Ph), 6.77 (1H, app td, J 7.5, 1.0, H5), 6.46 (1H, app d, J 7.7, H7), 4.57 (1H, d, J 2.4 Hz, H2), 3.06 (1H, app s, NH), 2.89 (3H, s, CH3).

13C (126 MHz, C6D6): δ 171.0 (C=O), 151.0 (C7a), 150.9 (C6'), 148.4 (C2'), 142.8 (i-Ph), 138.7 (C4'), 137.7 (C3'), 129.7 (C6), 129.5 (C3a), 129.1 (m-Ph/p-Ph), 128.5 (C4), 128.4 (o-Ph), 128.4 (m-Ph/p-Ph), 120.7 (C5'), 120.5 (C5), 110.6 (C7), 73.0 (C2), 67.4 (C3), 51.8 (CH3).

HRMS (ES⁺): [C22H18BrN2O2]⁺ ([M+H]⁺) requires 409.0546; found 409.0536.

IR: νmax (film)/cm⁻¹ 3354, 1735 (C=O), 1604, 1484, 1260, 886, 752, 703.

MP: 72-76 °C.
Indolines 124 and 125 were prepared according to general procedure 1, using KOtBu (278 mg, 2.48 mmol) and imine 123 (465 mg, 1.24 mmol) in toluene (20 mL). The reaction mixture was stirred at 0 °C for 15 min. Purification by flash pressure column chromatography (13% Et2O/petroleum ether) afforded separately indoline 124 as a colorless solid (118 mg, 0.31 mmol, 25%) and indoline 125 as a colorless solid (194 mg, 0.52 mmol, 42%).

N.B. Stereochemistry assigned by analogy to previously reported similar indolines.74

(2RS,3SR)-2-(3-Bromophenyl)-3-phenylindoline-3-carbonitrile 124

1H (500 MHz, C6D6): δ 7.14 (1H, app t, J 1.8, H2'), 7.02-7.09 (3H, m, H4, H6, H4'), 6.90 (1H, app d, J 7.8, H6'), 6.68-6.82 (5H, m, Ph), 6.65 (1H, app td, J 7.5, 1.0, H5), 6.57 (1H, app t, J 7.8, H5'), 6.41 (1H, app d, J 7.6, H7), 4.84 (1H, d, J 3.3, H2), 2.78 (1H, d, J 3.3, NH).

13C (126 MHz, C6D6): δ 150.7 (C7a), 139.1 (C1'), 135.1 (i-Ph), 131.8 (C4'), 131.3 (C2'), 130.5 (C6), 129.9 (C5'), 129.0 (C3a), 128.7 (o/m/p-Ph), 128.7 (o/m/p-Ph), 128.4 (o/m/p-Ph), 126.8 (C6'), 126.1 (C4), 122.8 (C3'), 122.0 (CN), 121.5 (C5), 110.8 (C7), 74.4 (C2), 55.9 (C3).

HRMS (ES+): C21H16BrN2+ ([M+H]+) requires 375.0497; found 375.0485.

IR: νmax (film)/cm⁻¹: 3360, 3060, 2854, 2241, 1608, 1484, 1469, 1204, 791, 751, 698.

MP: 140-143 °C.

(2RS,3RS)-2-(3-Bromophenyl)-3-phenylindoline-3-carbonitrile 125

1H (500 MHz, C6D6): δ 7.40 (1H, app t, J 1.8, H2'), 7.22-7.30 (3H, m, H4', o-Ph), 6.97-7.06 (5H, m, H6, H6', m-Ph, p-Ph), 6.69-6.78 (2H, m, H4, H5'), 6.60 (1H, app dt, J 7.5, 0.9, H5), 6.42 (1H, app d, J 7.9,
$^1$H (400 MHz, C$_6$D$_6$): $\delta$ 7.32-7.41 (2H, m, o-Ph), 6.94-7.06 (4H, m, H6, p-Ph, m-Ph), 6.87 (1H, d, $J$ 0.7, H5'), 6.80 (1H, app d, $J$ 7.5, H4), 6.57 (1H, app td, $J$ 7.5, 0.8, H5), 6.38 (1H, app d, $J$ 7.8, H7), 6.33 (1H, app s, H3'), 4.47 (1H, d, $J$ 2.5, H2), 3.10 (1H, d, $J$ 2.5, NH).

$^{13}$C (101 MHz, C$_6$D$_6$): $\delta$ 153.4 (C2'), 150.3 (C7a), 141.9 (C5'), 139.2 (i-Ph), 130.6 (C6), 129.0 (p-Ph), 127.8 (C3a), 127.7 (o-Ph), 126.1 (C4), 121.3 (C5), 119.0 (CN), 112.9 (C3'), 110.9 (C7), 101.1 (C4'), 71.1 (C2), 57.7 (C3).

HRMS (ES$^+$): C$_{18}$H$_{13}$BrNaN$_2$O$^+$ ([M+Na]$^+$) requires 387.0109; found 387.0097.

Indoline 133 was prepared according to general procedure 1, using KO'Bu (109 mg, 0.98 mmol) and imine 132 (325 mg, 0.89 mmol) in toluene (12 mL). The reaction mixture was stirred at 15 °C for 15 min. Purification by flash pressure column chromatography (15% Et$_2$O/petroleum ether) afforded indoline 133 as a colorless solid (240 mg, 0.65 mmol, 73%).

N.B. Stereochemistry assigned by analogy to previously reported similar indolines.$^{74}$
IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3356, 2239, 1606, 1484, 1469, 1256, 1126, 924, 745.

MP: 137-140 °C.

$(2R,3R)/(2S,3S)-2$-(5-(Benzyloxy)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 93

The catalyst (70 mg, 0.11 mmol) was added to a solution of imine 92 (880 mg, 2.18 mmol) in toluene (33 mL), and the suspension was stirred at -30 °C for 30 min. CsOH·H$_2$O (732 mg, 4.36 mmol) was added and stirred at -30 °C for 16 h. The mixture was diluted with EtOAc (40 mL), and NH$_4$Cl (sat. aq., 40 mL) was added. The mixture was allowed to warm to rt, the organic and aqueous layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (35% EtOAc/petroleum ether) afforded indoline 93 as a 85:15 mixture of enantiomers as a colorless solid (787 mg, 1.95 mmol, 89%). Resolution by semi-preparatory chiral HPLC (YMC chiral amylose SA, 5% iPrOH, 95% hexane, 5.0 mL/min) afforded indoline $(R,R)$-93 as a colorless solid (e.r. > 99:1).

$(2S,3S)-2$-(5-(Benzyloxy)pyridin-3-yl)-3-phenylindoline-3-carbonitrile $(S,S)$-93

Indoline $(S,S)$-93 was prepared in an analogous manner to indoline $(R,R)$-93 using a pseudoenantiomeric catalyst (47 mg, 0.074 mmol), imine 92 (600 mg, 1.49 mmol), and CsOH·H$_2$O (500 mg, 2.97 mmol) in toluene (19 mL). The reaction mixture was stirred at -30 °C for 16 h. Purification by flash pressure column chromatography (35% EtOAc/petroleum ether) afforded indoline 93 as a 89:11 mixture of enantiomers as a colorless solid (538 mg, 1.33 mmol, 90%). Resolution by semi-preparatory chiral HPLC (YMC chiral amylose SA, 5% iPrOH, 95% hexane, 5.0 mL/min) afforded indoline $(R,R)$-93 as a colorless solid (e.r. > 99:1).
mL/min) afforded indoline (S,S)-93 as a colorless solid (e.r. > 99:1).

N.B. Stereochemistry assigned by x-ray crystallography of indoline (S,S)-93.

\(^1\)H (400 MHz, C\(_6\)D\(_6\)): \(\delta 8.58 (1H, d, J 2.9, H2'/H6')\), 7.91 (1H, d, J 1.4, H2'/H6'), 7.63 (1H, app t, J 2.0, H4'), 7.27 (2H, m, Ph), 6.93-7.14 (7H, m, H6, Ph), 6.74 (1H, app d, J 7.6, H4), 6.61 (1H, app t, J 7.6, H5), 6.44 (1H, app d, J 8.0, H7), 4.73 (1H, d, J 11.8, OCH\(_3\)H\(_6\)Ph), 4.69 (1H, d, J 11.8, OCH\(_3\)H\(_6\)Ph), 4.40 (1H, d, J 3.2, H2), 3.06 (1H, d, J 3.0, NH).\(^\dagger\)

\(^{13}\)C (101 MHz, C\(_6\)D\(_6\)): \(\delta 155.3 (C5')\), 150.6 (C7a), 142.2 (C2'/C6'), 140.1 (C2'/C6'), 137.5, 136.8, 133.0, 130.3 (C6/Ph), 129.1 (C6/Ph), 128.7 (C6/Ph), 128.6 (C6/Ph), 127.5, 125.9 (C4), 121.0 (C5), 120.5 (C4'), 119.0 (CN), 110.8 (C7), 75.7 (C2), 70.4 (OCH\(_3\)Ph), 58.6 (C3).\(^\ddagger\)

\(^\ddagger\) Remaining 2 peaks obscured.

\(^\dagger\) Remaining 3 peaks obscured.

HRMS (ES\(^{+}\)): [C\(_{27}\)H\(_{22}\)ON\(_3\)]\(^{+}\) ([M+H]\(^{+}\)) requires 404.1757; found 404.1763.

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3355, 2925, 2248, 1606, 1470, 1435, 1322, 1171, 1026, 745, 698.

MP: 48-50 °C.

\([\alpha]_D^{25.0} (R,R) = +26 \text{ (c = 0.1, CHCl}_3\); [\alpha]_D^{25.0} (S,S) = -19 \text{ (c = 0.1, CHCl}_3\).

Chiral HPLC: (Chiralpak IA, 10% iPrOH, 90% hexane, 1.0 mL/min, \(\lambda = 254\) nm, 20 \(\mu\)L injection)

\(\tau_\alpha (R,R) = 33.2\) min, \(\tau_\beta (S,S) = 27.5\) min.
(25,35)-2-(5-Hydroxypyridin-3-yl)-3-phenyldimoline-3-carbonitrile 266

Indoline 266 was prepared according to general procedure 3 using indoline (5S,5S)-93 (195 mg, 0.49 mmol) and Pd/C (wet degussa type, 20 mg) in MeOH (14 mL). The reaction mixture was stirred under a H₂ atmosphere for 2 h. The reaction mixture was filtered through Celite and concentrated to afford indoline 266 as a colorless solid (154 mg, 0.49 mmol, quant).

¹H (500 MHz, CD₃OD): δ 8.06 (1H, d, J 2.6, H2'/H6'), 7.72 (1H, d, J 1.6, H2'/H6'), 7.37-7.52 (5H, m, Ph), 7.21-7.32 (2H, m, H4', H6), 6.92 (2H, app d, J 8.5, H4, H7), 6.84 (1H, app t, J 7.5, H5), 5.08 (1H, app s, H2), 3.35 (1H, app s, NH).¹

¹³C (126 MHz, CD₃OD): δ 155.8 (C5'), 152.7 (C7a), 140.0 (C2'/C6'), 138.8 (C2'/C6'), 138.5, 136.0, 131.5 (C6), 130.1 (Ph), 129.9 (Ph), 128.7 (Ph), 128.4, 126.1 (C4), 123.6 (C4'), 121.1 (C5), 120.0 (CN), 111.9 (C7), 76.3 (C2), 59.8 (C3).

¹ OH peak not observed due to exchange with MeOD.


IR: ν max (film)/cm⁻¹ 3348, 2922, 2241, 1608, 1428, 1299, 1214, 1136, 994, 756.

MP: 110-112 °C.

[α]D²⁵ = +12 (c = 0.1, CHCl₃).
\[ \text{5-((2S,3S)-3-Cyano-3-phenylindolin-2-yl)pyridin-3-yl trifluoromethanesulfonate 267} \]

\[
\begin{align*}
\text{\begin{tikzpicture}
\node (n1) at (0,0) {\text{CN}};
\node (n2) at (0.5,0.5) {\text{N}};
\node (n3) at (1.5,0.5) {\text{SO-}};
\node (n4) at (2,0) {\text{CF3}};
\end{tikzpicture}}
\end{align*}
\]

\[ N,N\text{-disopropylethylamine (156 \mu L, 0.89 mmol) was added to a solution of indoline 266 (140 mg, 0.45 mmol) and N-phenyl-bis(trifluoromethanesulfonimide) (176 mg, 0.49 mmol) in CH}_2\text{Cl}_2 (10 mL) at 0 \text{ °C. The reaction mixture was allowed to warm to rt and stirred for 1 h. H}_2\text{O (10 mL) was added, the layers separated, and the aqueous layer was extracted 3 times with CH}_2\text{Cl}_2. The combined organic extracts were washed with brine, dried over Na}_2\text{SO}_4, filtered, and concentrated. Purification by flash pressure column chromatography (3.5% EtOAc/petroleum ether) afforded indoline 267 as a colorless hygroscopic solid (170 mg, 0.38 mmol, 85%).} \]

\[ ^1\text{H (400 MHz, C}_6\text{D}_6): \delta \text{ 8.36 (1H, d, J 2.7, H2’/H6’), 8.11 (1H, d, J 1.7, H2’/H6’), 7.63 (1H, app t, J 2.1, H4’), 6.92-7.06 (4H, m, H6, Ph), 6.63 (1H, dd, J 7.9, 1.4, H4), 6.59 (1H, app td, J 7.5, 0.9, H5), 6.41 (1H, app d, J 8.2, H7), 4.21 (1H, d, J 3.4, H2), 2.83 (1H, d, J 3.4, NH).} \]

\[ ^{13}\text{C (101 MHz, C}_6\text{D}_6): \delta \text{ 150.0 (C5’), 149.1 (C2’/C6’), 146.9 (C7a), 143.6 (C2’/C6’), 136.8, 134.8, 130.4 (C6/Ph), 129.3 (C6/Ph), 129.0 (Ph), 128.6 (C4’), 127.0, 125.8 (C4), 121.5 (C5), 119.2 (q, J 321.2, CF3), 118.1 (CN), 111.1 (C7), 74.8 (C2), 58.5 (C3).} \]

\[ \dagger 1 \text{ peak obscured by solvent signal.} \]

\[ \ddagger 1 \text{ peak obscured by solvent signal.} \]

\[ ^{19}\text{F (377 MHz, C}_6\text{D}_6): \delta \text{ -73.1.} \]

\[ \text{HRMS (ES’): [C}_{22}\text{H}_{15}\text{O}_3\text{N}_3\text{F}_3^{19}\text{S}]^+ ([M+H]^+) \text{ requires 446.0781; found 446.0780.} \]

\[ \text{IR: } \nu_{\text{max}} \text{(film)/cm}^{-1} \text{ 3059, 2249, 1607, 1427, 1217, 1138, 837, 756, 700.} \]

\[ [\alpha]_D^{25.0} = +31 \text{ (c = 0.1, CHCl}_3). \]
Indoline 52 was prepared according to general procedure 2, using indoline 49 (250 mg, 0.66 mmol), acetyl chloride (57 µL, 0.80 mmol), and pyridine (64 µL, 0.80 mmol) in CH$_2$Cl$_2$ (13 mL). The reaction mixture was stirred at rt for 15 min. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded indoline 52 as a colorless solid (270 mg, 0.65 mmol, 98%).

$^1$H (500 MHz, C$_6$D$_6$, 348 K): δ 8.23 (1H, d, $J$ 2.2, H6'), 8.10 (1H, app br s, H7), 7.87 (1H, app s, H2'), 7.08 (1H, app t, $J$ 7.8, H6), 6.88 (2H, m, o-Ph), 6.71-6.84 (6H, m, H4, H4', H5, m-Ph, p-Ph), 5.62 (1H, s, H2), 1.64 (3H, s, CH$_3$).

$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): δ 168.2 (C=O), 151.3 (C6'), 146.9 (C2'), 144.6 (C7a), 137.4 (C4'), 134.3 (C3'), 133.7 (C3a/i-Ph), 131.6 (C6), 129.5 (m-Ph/p-Ph), 129.1 (m-Ph/p-Ph), 129.1 (o-Ph), 126.8 (C4), 125.7 (C5), 121.4 (CN), 120.8 (C5'), 117.6 (C7), 72.9 (C2), 55.8 (C3), 24.0 (CH$_3$).†

† 1 peak obscured by solvent signal.

HRMS (ES$^+$): C$_{22}$H$_{16}$BrN$_3$NaO$^+$ ([M+Na]$^+$) requires 440.0369; found 440.0371.

IR: $\nu_{\text{max}}$(film)/cm$^{-1}$ 3062, 2361, 2236, 1677 (C=O), 1478, 1385, 1020, 756, 699.

MP: 126-128 °C.
Indoline 53 was prepared according to general procedure 2, using indoline 50 (400 mg, 1.06 mmol), acetyl chloride (91 µL, 1.3 mmol), and pyridine (103 µL, 1.28 mmol) in CH₂Cl₂ (20 mL). The reaction mixture was stirred at rt for 15 min. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded indoline 53 as a colorless solid (379 mg, 0.91 mmol, 85%).

¹H (500 MHz, C₆D₆, 348 K): δ 8.56 (1H, d, J 2.2, H₆'), 8.41 (1H, d, J 1.6, H₂'), 8.04 (1H, app br s, H₇), 7.55 (1H, app t, J 1.8, H₄'), 7.17 (2H, m, o-Ph), 7.06 (1H, app t, J 7.8, H₆), 6.96-7.03 (4H, m, H₄, m-Ph, p-Ph), 6.74 (1H, app t, J 7.8, H₅), 5.18 (1H, br s, H₂), 1.49 (3H, s, CH₃).

¹³C (126 MHz, C₆D₆, 348 K): δ 167.9 (C=O), 152.9 (C₆'), 147.1 (C₂'), 143.4 (C₇a), 140.8 (i-Ph), 136.8 (C₄'), 136.0 (C₃'), 131.4 (C₆), 130.2 (m-Ph/p-Ph), 129.8 (C₃a), 129.5 (m-Ph/p-Ph), 126.5 (C₄), 126.2 (o-Ph), 126.1 (C₅), 121.8 (C₅'), 118.9 (CN), 117.5 (C₇), 74.0 (C₂), 57.9 (C₃), 23.7 (CH₃).

HRMS (ES⁺): C₂₂H₁₆⁷⁹BrN₃NaO⁺ ([M+Na]⁺) requires 440.0369; found 440.0370.

IR: ν_max (film)/cm⁻¹ 3011, 2236, 1679 (C=O), 1479, 1389, 1020, 756, 700.

MP: 90-94 °C.
A suspension of indoline 52 (120 mg, 0.29 mmol), PdCl$_2$(PPh$_3$)$_2$ (10 mg, 0.014 mmol), and Cul (3.0 mg, 0.014 mmol) in diisopropylamine (4 mL) was degassed and placed under an Ar atmosphere before ethynyltrimethylsilane (50 µL, 0.34 mmol) was added. The mixture was stirred at 65 °C for 5.5 h, then filtered through Celite and eluted with CH$_2$Cl$_2$. The filtrate was washed with water, then with brine, dried over MgSO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded alkyne 116 as a pale yellow solid (121 mg, 0.28 mmol, 96%).

$^1$H (500 MHz, C$_6$D$_6$, 348 K): δ 8.43 (1H, app s, H6’), 8.15 (1H, app br s, H7), 7.85 (1H, app s, H2’), 7.06 (1H, app td, J 7.8, 1.4, H6), 6.85-6.94 (3H, m, H4’, o-Ph), 6.67-6.83 (5H, m, H4, H5, m-Ph, p-Ph), 5.62 (1H, s, H2), 1.60 (3H, s, CH$_3$), 0.12 (9H, s, Si(CH$_3$)$_3$).

$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): δ 168.2 (C=O), 153.0 (C6’), 148.2 (C2’), 144.8 (C7a), 137.1, 133.9 (C4’), 132.2, 131.5 (C6), 129.4 (m-Ph/p-Ph), 129.2 (m-Ph/p-Ph), 129.1 (o-Ph), 126.7 (C4), 125.6 (C5), 121.5 (CN), 120.5 (C5’), 117.7 (C7), 102.0 (C8/C9), 99.5 (C8/C9), 73.3 (C2), 55.9 (C3), 24.0 (CH$_3$), 0.02 (Si(CH$_3$)$_3$).†

† 1 peak obscured by solvent signal.

HRMS (ES$^+$): C$_{27}$H$_{25}$N$_3$NaOSi$^+$ ([M+Na]$^+$) requires 458.1659; found 458.1655.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3040, 2961, 2160, 2158, 1678 (C=O), 1600, 1479, 1385, 1127, 846, 758, 698.

MP: 125-127 °C.
A suspension of indoline 53 (150 mg, 0.36 mmol), PdCl₂(PPh₃)₂ (13 mg, 0.018 mmol), and CuI (3.5 mg, 0.018 mmol) in diisopropylamine (4 mL) was degassed and placed under an Ar atmosphere before ethynyltrimethylsilane (61 µL, 0.43 mmol) was added. The mixture was stirred at 65 °C for 5.5 h, then filtered through Celite™. The filtrate was washed three times with water, then with brine, dried over MgSO₄, filtered, and concentrated. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded alkyne 117 as a colorless solid (141 mg, 0.32 mmol, 89%).

¹H (500 MHz, C₆D₆, 348 K): δ 8.78 (1H, app s, H₂'/H₆'), 8.47 (1H, app s, H₂'/H₆'), 8.10 (1H, app br s, H₇), 7.59 (1H, app s, H₄'), 7.13-7.18 (2H, obscured, o-Ph), 7.03 (1H, app t, J 7.8, H₆), 6.95-7.01 (4H, m, H₄, m-Ph, p-Ph), 6.71 (1H, app t, J 7.8, H₅), 5.16 (1H, s, H₂), 1.47 (3H, s, CH₃), 0.14 (9H, s, Si(CH₃)₃).

¹³C (126 MHz, C₆D₆, 348 K): δ 167.9 (C=O), 154.6 (C₂'/C₆'), 148.0 (C₂'/C₆'), 143.6 (C₇a), 141.1, 136.6 (C₄'), 134.0, 131.3 (C₆), 130.2 (m-Ph/p-Ph), 129.7, 129.4 (m-Ph/p-Ph), 126.4 (C₄), 126.2 (o-Ph), 126.0 (C₅), 118.9 (CN), 117.6 (C₇), 102.2 (C₈/C₉), 99.9 (C₈/C₉), 74.5 (C₂), 58.0 (C₃), 23.6 (CH₃), 0.04 (Si(CH₃)₃).

‡ 1 peak obscured by solvent signal.

HRMS (ES⁺): [C₂₇H₂₆N₃OSi]⁺ ([M+H]⁺) requires 436.1840; found 436.1850.

IR: νmax (film)/cm⁻¹ 2961, 2160, 1680 (C=O), 1479, 1385, 1250, 847, 759, 698.

MP: 155-157 °C.
TBAF (1.0 M in THF, 0.3 mL, 0.3 mmol) was added dropwise to a solution of alkyne \textbf{116} (110 mg, 0.25 mmol) in THF (5 mL) at 0 °C and stirred at rt for 15 min. The mixture was diluted with EtOAc (10 mL) and washed three times with water, then with brine, dried over MgSO\(_4\), filtered, and concentrated. Purification by flash pressure column chromatography (36% EtOAc/petroleum ether) afforded alkyne \textbf{118} as a colorless oil (90 mg, 0.25 mmol, 99%).

\(^1\)H (500 MHz, C\(_6\)D\(_6\), 348 K): \(\delta\) 8.36 (1H, app s, H\(_2'\)/H\(_6'\)), 8.17 (1H, app br s, H7), 7.86 (1H, app s, H2'/H6'), 7.07 (1H, app t, \(J\) 7.8, H6), 6.89 (2H, m, o-Ph), 6.83 (1H, app s, H4'), 6.70-6.81 (5H, m, H4, H5, m-Ph, p-Ph), 5.61 (1H, s, H2), 2.58 (1H, s, H9), 1.60 (3H, s, CH\(_3\)).

\(^{13}\)C (126 MHz, C\(_6\)D\(_6\), 348 K): \(\delta\) 168.2 (C=O), 153.0 (C\(_2'\)/C\(_6'\)), 148.5 (C\(_2'\)/C\(_6'\)), 144.8 (C7a), 137.5, 133.8 (C4'), 132.3, 131.5 (C6), 129.4 (m-Ph/p-Ph), 129.1 (m-Ph/p-Ph), 129.1 (o-Ph), 126.7 (C4), 125.6 (C5), 121.4 (CN), 119.5 (C5'), 117.7 (C7), 81.6 (C8/C9), 80.3 (C8/C9), 73.2 (C2), 55.9 (C3), 24.0 (CH\(_3\)).

\(^\dagger\) 1 peak obscured by solvent signal.

HRMS (ES\(^+\)): [C\(_{24}\)H\(_{17}\)N\(_3\)NaO\(^+\)] (\([\text{M+Na}]^+\)) requires 386.1264; found 386.1268.

IR: \(v_{\text{max}}\) (film)/\(\text{cm}^{-1}\) 3285, 3023, 2261, 1676 (C=O), 1478, 1386, 1025, 757, 700.
(2RS,3RS)-1-Acetyl-2-(5-ethynylpyridin-3-yl)-3-phenylindoline-3-carbonitrile 119

TBAF (1.0 M in THF, 0.35 mL, 0.35 mmol) was added dropwise to a solution of alkyne 117 (120 mg, 0.28 mmol) in THF (5 mL) at 0 °C and stirred at rt for 15 min. The mixture was diluted with EtOAc (10 mL) and washed three times with water, then with brine, dried over MgSO₄, filtered, and concentrated. Purification by flash pressure column chromatography (36% EtOAc/etroleum ether) afforded alkyne 119 as a colorless oil (94 mg, 0.26 mmol, 92%).

¹H (500 MHz, C₆D₆, 348 K): δ 8.71 (1H, app s, H2'/H6'), 8.46 (1H, app s, H2'/H6'), 8.12 (1H, app br s, H7), 7.53 (1H, app s, H4'), 7.14-7.20 (2H, obscured, o-Ph), 7.05 (1H, app t, J 7.9, H6), 6.95-7.03 (4H, m, H4, m-Ph, p-Ph), 6.73 (1H, app t, J 7.6, H5), 5.16 (1H, s, H2), 2.66 (1H, s, H9), 1.45 (3H, s, CH₃).

¹³C (126 MHz, C₆D₆, 348 K): δ 167.9 (C=O), 154.5 (C2'/C6'), 148.3 (C2'/C6'), 143.6 (C7a), 141.0, 137.0 (C4'), 133.9, 131.3 (C6), 130.2 (m-Ph/p-Ph), 129.7, 129.4 (m-Ph/p-Ph), 126.4 (C4), 126.2 (o-Ph), 126.0 (C5), 120.5 (C5'), 118.9 (CN), 117.5 (C7), 82.1 (C8/C9), 80.5 (C8/C9), 74.4 (C2), 58.0 (C3), 23.6 (CH₃).

HRMS (ES⁺): [C₂₄H₁₇N₃NaO⁺]⁺ ([M+Na⁺]) requires 386.1264; found 386.1265.

IR: ν max (film)/cm⁻¹ 3286, 3021, 2260, 1675 (C=O), 1480, 1385, 1025, 753, 697.
Indoline 78 was prepared according to general procedure 2, using indoline 77 (840 mg, 2.26 mmol), acetyl chloride (0.20 mL, 2.7 mmol), and pyridine (0.20 mL, 2.7 mmol) in CH₂Cl₂ (35 mL). The reaction mixture was stirred at rt for 15 min. Purification by flash pressure column chromatography (36% EtOAc/petroleum ether) afforded indoline 78 as a colorless solid (819 mg, 1.97 mmol, 87%).

$^1$H (500 MHz, C₆D₆, 348 K): δ 8.44 (1H, d, J 1.8, H6'), 8.25 (1H, d, J 1.6, H2'), 7.72 (1H, app br s, H7), 7.36 (1H, app t, J 1.8, H4'), 7.00 (1H, app t, J 7.8, H6), 6.77 (1H, app t, J 7.6, H5), 5.73 (1H, s, H2), 3.48 (2H, q, J 7.1, OCH₂CH₃), 1.55 (3H, s, CH₃), 0.63 (3H, t, J 7.1, OCH₂CH₃).¹

$^{13}$C (126 MHz, C₆D₆, 348 K): δ 167.3 (C=O), 163.9 (C=O), 152.1 (C6'), 147.2 (C2'), 143.5 (C7a), 137.2 (C4'), 133.6 (C3'), 131.4 (C6), 127.1 (C4), 125.2 (C5), 125.0 (C3a), 120.8 (C5'), 117.5 (CN), 116.7 (C7), 69.5 (C2), 63.6 (OCH₂CH₃), 55.6 (C3), 13.4 (CH₃), 13.3 (OCH₂CH₃).

¹ 1 peak obscured by solvent signal.

HRMS (ES⁺): [C₁₉H₁₆BrNa₃O₃]$^+$ ([M+Na]$^+$) requires 436.0273; found 436.0276.

IR: $\nu_{max}$ (film)/cm⁻¹ 2984, 2248, 1754, 1677 (C=O), 1481, 1385, 1253, 1021, 755, 705.

MP: 91-93 °C.
Methyl (2RS,3SR)-1-acetyl-2-(5-bromopyridin-3-yl)-3-phenylindoline-3-carboxylate 67

Indoline 67 was prepared according to general procedure 2, using indoline 65 (65 mg, 0.16 mmol), acetyl chloride (14 µL, 0.19 mmol), and pyridine (15 µL, 0.19 mmol) in CH$_2$Cl$_2$ (5 mL). The reaction mixture was stirred at rt for 15 min. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded indoline 67 as a colorless paste (72 mg, 0.16 mmol, 99%).

$^1$H (500 MHz, C$_6$D$_6$, 348 K): δ 8.41 (1H, app br s, H7), 8.24 (1H, d, J 2.1, H2'/H6'), 8.02 (1H, d, J 1.4, H2'/H6'), 7.30 (1H, app d, J 7.7, H4), 7.15 (1H, obscured, H5), 7.00 (1H, app s, H4'), 6.90 (1H, app td, J 7.7, 1.0, H6), 6.77-6.81 (3H, m, Ph), 6.72-6.77 (2H, m, Ph), 6.31 (1H, s, H2), 3.19 (3H, s, CO$_2$CH$_3$), 1.83 (3H, s, CH$_3$).

$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): δ 172.6 (C=O), 167.9 (C=O), 150.3 (C2'/C6'), 147.1 (C2'/C6'), 144.7 (C7a), 137.1 (C4'), 136.9, 136.2, 130.4 (C6), 129.5, 128.6 (Ph), 128.1 (Ph), 127.9 (Ph), 124.4 (C5), 120.4 (C5'), 117.5 (C7), 69.6 (C2), 67.9 (CO$_2$CH$_3$), 52.8 (C3), 23.7 (CH$_3$).†

† 1 peak obscured by solvent signal.


IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3044, 1731, 1673 (C=O), 1477, 1389, 1231, 939, 730, 699, 662.
(2RS,3SR)-1-Acetyl-2-(3-bromophenyl)-3-phenyldione-3-carbonitrile 126

Indoline 126 was prepared according to general procedure 2, using indoline 124 (100 mg, 0.27 mmol), acetyl chloride (23 μL, 0.32 mmol), and pyridine (26 μL, 0.32 mmol) in CH₂Cl₂ (6 mL). The reaction mixture was stirred at rt for 25 min. Purification by flash pressure column chromatography (15% EtOAc/petroleum ether) afforded indoline 126 as a colorless solid (97 mg, 0.23 mmol, 87%).

¹H (500 MHz, C₆D₆, 348 K): δ 8.48 (1H, app br s, H7), 7.13 (1H, app td, J 7.7, 1.4, H6), 6.93 (2H, app d, J 7.3, o-Ph), 6.73-6.88 (6H, m, m-Ph, p-Ph, H4, H4’, H5), 6.61 (1H, app br s, H2’), 6.40 (1H, app d, J 7.8, H6’), 6.33 (1H, app t, J 7.8, H5’), 5.52 (1H, s, H2), 1.62 (3H, s, CH₃).

¹³C (126 MHz, C₆D₆, 348 K): δ 168.6 (C=O), 145.2 (C7a), 139.2, 134.2, 131.8, 131.4, 131.3 (C6), 130.3, 129.2, 128.8, 128.8, 126.6, 125.7, 125.5 (C5), 123.1 (CN), 121.7 (C3’), 117.9 (C7), 75.1 (C2), 56.2 (C3), 24.1 (CH₃).

‡ 1 peak obscured by solvent signal.

HRMS (ES⁺): C₂₃H₁₇₇⁹BrNa₂O⁺ ([M+Na]+) requires 439.0422; found 439.0415.

IR: νmax (film)/cm⁻¹ 3064, 2236, 1677 (C=O), 1478, 1387, 1282, 757, 728, 694.

MP: 125-128 °C.
(2RS,3RS)-1-Acetyl-2-(3-bromophenyl)-3-phenylindoline-3-carbonitrile 127

Indoline 127 was prepared according to general procedure 2, using indoline 125 (170 mg, 0.45 mmol), acetyl chloride (39 μL, 0.54 mmol), and pyridine (44 μL, 0.54 mmol) in CH₂Cl₂ (10 mL). The reaction mixture was stirred at rt for 25 min. Purification by flash pressure column chromatography (16% EtOAc/petroleum ether) afforded indoline 127 as a colorless solid (179 mg, 0.43 mmol, 95%).

¹H (500 MHz, C₆D₆, 348 K): δ 8.42 (1H, app br s, H7), 7.37 (1H, app s, H2’), 7.20-7.25 (2H, m, o-Ph), 7.19 (1H, app d, J 8.2, H4’), 7.13 (1H, app t, J 7.9, H6), 7.06 (1H, app d, J 7.6, H4), 6.96-7.04 (3H, m, m-Ph, p-Ph), 6.90 (1H, app d, J 7.9, H6’), 6.77 (1H, app t, J 7.6, H5), 6.68 (1H, app t, J 7.9, H5’), 5.08 (1H, s, H2), 1.48 (3H, s, CH₃).

¹³C (126 MHz, C₆D₆, 348 K): δ 168.3 (C=O), 144.1 (C7a), 141.6 (i-Ph), 141.0 (C1’), 133.2 (C4’), 131.6 (C5’), 131.2 (C6), 130.5 (C2’), 130.2 (m-Ph/p-Ph), 130.0 (C3a), 129.3 (m-Ph/p-Ph), 126.4 (C4), 126.2 (o-Ph), 125.9 (C5), 125.3 (C6’), 124.0 (C3’), 118.8 (CN), 117.7 (C7), 76.4 (C2), 58.4 (C3), 23.8 (CH₃).


IR: ν_max (film)/cm⁻¹ 3063, 2238, 1676 (C=O), 1463, 1387, 1242, 757, 738, 696.

MP: 149-152 °C.
(2RS,3RS)-1-Acetyl-2-(5-bromofuran-2-yl)-3-phenylindoline-3-carbonitrile 139 and (2RS,3SR)-1-Acetyl-2-(5-bromofuran-2-yl)-3-phenylindoline-3-carbonitrile 140

KO’Bu (68 mg, 0.60 mmol) was added to a solution of imine 138 (200 mg, 0.55 mmol) in toluene (8 mL) and stirred at 0 °C for 10 min. NH₄Cl (saturated aq., 6 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over MgSO₄, and filtered. The resulting residue (200 mg) was dissolved in CH₂Cl₂ (10 mL) with pyridine (53 μL, 0.66 mmol). Acetyl chloride (47 μL, 0.66 mmol) was added dropwise, and the solution was stirred at rt for 10 min. NaHCO₃ (saturated aq., 5 mL) was added, and the mixture was extracted with CH₂Cl₂. The combined organic extracts were washed with brine, dried over MgSO₄, filtered, and concentrated. Purification by flash pressure column chromatography (3.5% Et₂O/toluene) afforded separately indoline 140 as a colorless oil (115 mg, 0.28 mmol, 52%) and indoline 139 as a colorless oil (71 mg, 0.17 mmol, 32%).

N.B. Stereochemistry assigned by nOe analysis, with red arrows indicating through-space interactions.

(2RS,3RS)-1-Acetyl-2-(5-bromofuran-2-yl)-3-phenylindoline-3-carbonitrile 140

¹H (500 MHz, CD6d6, 348 K): δ 8.30 (1H, app br s, H7), 7.18-7.23 (2H, m, o-Ph), 7.06-7.13 (2H, m, H4, H6), 6.93-7.01 (3H, m, m-Ph, p-Ph), 6.77 (1H, app td, J 7.6, 0.8, H5), 5.85 (1H, d, J 3.4, H3'/H4'), 5.80 (1H, d, J 3.4, H3'/H4'), 5.27 (1H, br s, H2), 1.53 (3H, s, CH₃).
$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): δ 168.0 (C=O), 153.2 (C2'), 143.4 (C7a), 140.5 (i-Ph), 131.0 (C6), 130.1 (m-Ph), 130.0 (p-Ph), 129.4 (C3a), 126.3 (o-Ph), 126.2 (C4), 125.7 (C5), 123.9 (C5'), 118.7 (CN), 118.0 (C7), 113.4 (C3'/C4'), 111.9 (C3'/C4'), 70.9 (C2), 56.9 (C3), 23.2 (CH$_3$).

HRMS (ES$^+$): [C$_{21}$H$_{16}$BrN$_2$O$_2$]$^+$ ([M+H]$^+$) requires 407.0390; found 407.0382.

IR: $\nu_{\text{max}}$(film)/cm$^{-1}$ 3128, 3064, 2241, 1677 (C=O), 1600, 1479, 1387, 1351, 1122, 735, 668.

(2RS,3SR)-1-Acetyl-2-(5-bromofuran-2-yl)-3-phenylindoline-3-carbonitrile 139

$^1$H (500 MHz, C$_6$D$_6$, 348 K): δ 8.35 (1H, app br s, H7), 7.13-7.14 (2H, m, o-Ph), 7.10 (1H, app td, J 7.7, 1.2, H6), 6.91-7.02 (3H, m, m-Ph, p-Ph), 6.82 (1H, app d, J 7.7, H4), 6.74 (1H, app td, J 7.7, 0.9, H5), 5.60 (1H, s, H2), 5.48 (1H, d, J 3.2, H4'), 5.43 (1H, d, J 3.2, H3'), 1.63 (3H, s, CH$_3$).

$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): δ 168.2 (C=O), 151.5 (C2'), 144.7 (C7a), 134.0 (i-Ph), 131.2 (C6), 130.1 (C3a), 129.4 (p-Ph), 128.9 (m-Ph), 128.9 (o-Ph), 126.4 (C4), 125.3 (C5), 123.2 (C5'), 121.0 (CN), 118.1 (C7), 112.7 (C3'), 112.5 (C4'), 70.1 (C2), 55.4 (C3), 23.5 (CH$_3$).

HRMS (ES$^+$): [C$_{21}$H$_{16}$BrN$_2$O$_2$]$^+$ ([M+H]$^+$) requires 407.0390; found 407.0382.

IR: $\nu_{\text{max}}$(film)/cm$^{-1}$ 3065, 2236, 1678 (C=O), 1600, 1478, 1386, 1351, 1125, 755, 698.

(2SR,3RS/SR)-1-Acetyl-2-(4-bromofuran-2-yl)-3-phenylindoline-3-carbonitrile 134

Indoline 134 was prepared according to general procedure 2, using indoline 133 (133 mg, 0.36 mmol), acetyl chloride (31 μL, 0.44 mmol), and pyridine (35 μL, 0.44 mmol) in CH$_2$Cl$_2$ (7 mL). The reaction mixture was stirred at rt for 10 min. Purification by flash pressure column chromatography (15% EtOAc/petroleum ether) afforded indoline 134 as a yellow oil (144 mg, 0.38 mmol, 97%).
$^{1}$H (500 MHz, C$_6$D$_6$, 348 K): $\delta$ 8.26 (1H, app br s, H7), 7.20-7.25 (2H, m, o-Ph), 7.07-7.13 (2H, m, H4, H6), 6.94-7.02 (3H, m, m-Ph, p-Ph), 6.92 (1H, s, H5'), 6.78 (1H, app t, J 7.6, H5), 6.08 (1H, s, H3'), 5.25 (1H, br s, H2), 1.52 (3H, s, CH$_3$).

$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): $\delta$ 167.8 (C=O), 152.2 (C2'), 143.3 (C7a), 142.6 (CS'), 140.5 (i-Ph), 131.0 (C6), 130.2 (m-Ph), 129.4 (p-Ph), 128.9 (C3a), 126.3 (o-Ph), 126.1 (C4), 125.8 (C5), 118.6 (CN), 118.0 (C7), 112.9 (C3'), 101.3 (C4'), 70.8 (C2), 56.7 (C3), 23.2 (CH$_3$).

HRMS (ES$^+$): [C$_{21}$H$_{16}$BrN$_2$O$_2$]$^+$ ([M+H]$^+$) requires 407.0390; found 407.038.

IR: $\nu_{\text{max}}$(film)/cm$^{-1}$ 3061, 2236, 1677 (C=O), 1479, 1387, 1352, 924, 755, 697.

(2RS,3RS)-1-acetyl-2-(5-(benzyloxy)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 95 and (2RS,3SR)-1-Acetyl-2-(5-(benzyloxy)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 94

Racemic:

`BuOK (706 mg, 6.29 mmol) was added to a solution of imine 92 (1.27 g, 3.15 mmol) in toluene (60 mL) and stirred at 0 °C for 25 min. NH$_4$Cl (saturated aq., 60 mL) was added, and the mixture was extracted 3 times with EtOAc. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded an inseparable mixture of diastereomers (853 mg,
2.11 mmol, 67%). This mixture (755 mg, 1.87 mmol) was dissolved in CH$_2$Cl$_2$ (40 mL) with pyridine (212 μL, 2.63 mmol). Acetyl chloride (181 μL, 2.53 mmol) was added dropwise, and the solution was stirred at rt for 30 min. NaHCO$_3$ (saturated aq., 40 mL) was added, and the mixture was extracted with CH$_2$Cl$_2$. The combined organic extracts were washed with brine, dried over MgSO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (15% EtOAc/petroleum ether) afforded separately diastereomers 95 as a colorless solid (376 mg, 0.84 mmol, 45%) and 94 as a colorless solid (167 mg, 0.38 mmol, 20%).

Asymmetric:

Indolines (R,R)-95 and (S,S)-95 were prepared according to general procedure 2 using indoline (R,R)-93 (157 mg, 0.39 mmol), acetyl chloride (33 μL, 0.47 mmol), and pyridine (38 μL, 0.47 mmol) in CH$_2$Cl$_2$ (8.5 mL) and indoline (S,S)-93 (216 mg, 0.54 mmol), acetyl chloride (46 μL, 0.64 mmol), and pyridine (52 μL, 0.64 mmol) in CH$_2$Cl$_2$ (10 mL). Purification by flash pressure column chromatography (50% EtOAc/petroleum ether) afforded indolines (R,R)-95 (165 mg, 0.37 mmol, 95%) and (S,S)-95 (230 mg, 0.52 mmol, 96%) respectively.

(2RS,3RS)-1-acetyl-2-(5-(benzyloxy)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 95

$^1$H (500 MHz, C$_6$D$_6$, 348 K): δ 8.45 (1H, app s, H2’/H6’), 8.33 (1H, app br s, H7), 8.26 (1H, app s, H2’/H6’), 7.22 (2H, app d, J 7.6, o-Ph), 6.97-7.13 (11H, m, H4, H4’, H6, m-Ph, p-Ph, H11, H12, H13), 6.77 (1H, app t, J 7.6, H5), 5.20 (1H, br s, H2), 4.50-4.61 (2H, d, J 12.5, H9), 1.48 (3H, br s, CH$_3$).

$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): δ 168.2 (C=O), 156.1 (CS’), 144.0 (C7a), 141.7 (C2’/C6’), 141.3, 140.9 (C2’/C6’), 136.8 (C10), 134.8, 131.2, 130.2, 129.9, 129.4, 129.1, 126.4, 126.3 (o-Ph), 125.9 (C5), 119.7 (C4’), 119.0 (CN), 117.7 (C7), 74.7 (C2), 71.2 (C9), 58.3 (C3), 23.7 (CH$_3$).†

† 2 peaks obscured.

HRMS (ES$^+$): [C$_{29}$H$_{24}$N$_3$O$_2$]$^+$ ([M+H]$^+$) requires 446.1863; found 446.1854.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2981, 2241, 1675 (C=O), 1479, 1386, 1277, 753, 669.

MP: 61-64 °C.
\([\alpha]^{25.0}_D (R,R) = +76 \ (c = 0.1, \text{CHCl}_3); \ [\alpha]^{25.0}_D (S,S) = -84 \ (c = 0.1, \text{CHCl}_3).\]

(2RS,3SR)-1-Acetyl-2-(5-(benzoxo)pyridin-3-yl)-3-phenylindoline-3-carbonitrile \(94\)

\(^1\)H (500 MHz, \(\text{CD}_3\text{OD}\), 348 K): \(\delta 8.61 (1\,\text{H}, \text{app br s}, H7), 8.45 (1\,\text{H}, \text{d}, J 2.7, H2'/H6'), 8.28 (1\,\text{H}, \text{app s}, H2'/H6'), 7.19-7.24 (2\,\text{H}, \text{m}, o-\text{Ph}), 6.87-7.12 (11\,\text{H}, \text{m}, H4, H4', H6, m-\text{Ph}, p-\text{Ph}, H11, H12, H13), 6.70 (1\,\text{H}, \text{app td}, J 7.6, 1.0, H5), 5.09 (1\,\text{H}, \text{br s}, H2), 4.42 (1\,\text{H}, \text{d}, J 11.7, H9), 4.38 (1\,\text{H}, \text{d}, J 11.7, H9'), 1.36 (3\,\text{H}, \text{br s}, \text{CH}_3).

\(^{13}\)C (126 MHz, \(\text{CD}_3\text{OD}\), 348 K): \(\delta 168.0 (\text{C}=\text{O}), 155.5 (C5'), 143.5 (C7a), 141.1 (C2'/C6'), 140.7, 140.0 (C2'/C6'), 136.1, 134.4, 130.9, 129.8, 129.0, 128.7, 127.9, 126.0, 125.9, 125.7 (C5), 118.8 (C4'/CN), 118.7 (C4'/CN), 117.3 (C7), 73.9 (C2), 70.3 (C9), 57.8 (C3), 23.3 (CH3).‡

‡ 2 peaks obscured.

HRMS (ES\(^{+}\)): \([C_{29}H_{24}N_3O_2]^+ ([\text{M+H}]^+)\) requires 446.1863; found 446.1860.

IR: \(\nu_{\text{max}}\) (film)/\(\text{cm}^{-1}\) 3033, 2239, 1675 (C=O), 1594, 1479, 1385, 1277, 738, 698.

MP: 63-68 °C.

\(2\) peaks obscured.

\(^{13}\)C (126 MHz, \(\text{CD}_3\text{OD}\), 348 K): \(\delta 168.0 (\text{C}=\text{O}), 155.5 (C5'), 143.5 (C7a), 141.1 (C2'/C6'), 140.7, 140.0 (C2'/C6'), 136.1, 134.4, 130.9, 129.8, 129.0, 128.7, 127.9, 126.0, 125.9, 125.7 (C5), 118.8 (C4'/CN), 118.7 (C4'/CN), 117.3 (C7), 73.9 (C2), 70.3 (C9), 57.8 (C3), 23.3 (CH3).‡

‡ 2 peaks obscured.

HRMS (ES\(^{+}\)): \([C_{29}H_{24}N_3O_2]^+ ([\text{M+H}]^+)\) requires 446.1863; found 446.1860.

IR: \(\nu_{\text{max}}\) (film)/\(\text{cm}^{-1}\) 3033, 2239, 1675 (C=O), 1594, 1479, 1385, 1277, 738, 698.

MP: 63-68 °C.

\(2\) peaks obscured.

HRMS (ES\(^{+}\)): \([C_{29}H_{24}N_3O_2]^+ ([\text{M+H}]^+)\) requires 446.1863; found 446.1860.

IR: \(\nu_{\text{max}}\) (film)/\(\text{cm}^{-1}\) 3033, 2239, 1675 (C=O), 1594, 1479, 1385, 1277, 738, 698.

MP: 63-68 °C.

Indoline \(96\) was prepared according to general procedure 3, using indoline \(94\) (150 mg, 0.34 mmol) and Pd/C (wet degussa type, 15 mg) in MeOH (10 mL). The reaction mixture was stirred under a \(\text{H}_2\) atmosphere for 5.5 h. The crude residue was triturated with toluene to afford indoline \(96\) as a colorless solid (75 mg, 0.21 mmol, 63%).

\(^1\)H NMR (500 MHz, \(\text{CD}_3\text{OD}\)): \(\delta 8.28 (1\,\text{H}, \text{app br s}, H7), 7.77 (1\,\text{H}, \text{d}, J 2.4, H6'), 7.59 (1\,\text{H}, \text{app td}, J 7.8, 1.1, H6), 7.38 (1\,\text{H}, \text{d}, J 1.3, H2'), 7.31 (1\,\text{H}, \text{app td}, J 7.8, 1.1, H5), 7.19-7.28 (4\,\text{H}, \text{m}, H4, Ph), 7.15 (2H,
m, Ph), 6.61 (1H, app t, J 1.9, H4'), 6.30 (1H, s, H2), 2.16 (3H, br s, CH3).1

$^1$C NMR (126 MHz, CD3OD): δ 171.5 (C=O), 155.7 (C5'), 145.3 (C7a), 140.0 (C2'), 138.5 (C6'), 134.5 (C3'), 133.7 (i-Ph), 132.4 (C6), 130.4 (Ph), 129.8 (Ph), 129.8 (Ph), 127.6 (C4), 127.0 (C5), 122.5 (C4'), 122.3 (CN), 118.8 (C7), 72.9 (C2), 56.6 (C3), 24.2 (CH3).‡

‡ OH peak not observed due to exchange with MeOD.

‡ 1 peak obscured.


IR: υmax (film)/cm⁻¹ 3065, 2236, 1671 (C=O), 1591, 1479, 1388, 1280, 700, 625.

MP: 132-136 °C.

(2RS,3SR)-1-Acetyl-2-(5-hydroxypyridin-3-yl)-3-phenyldiolined-3-carbonitrile 100

Indoline 100 was prepared according to general procedure 3, using indoline 95 (390 mg, 0.88 mmol) and Pd/C (wet degussa type, 36 mg) in MeOH (24 mL). The reaction mixture was stirred under a H2 atmosphere for 5 h. The crude residue was triturated with toluene to afford indoline 100 as a colorless solid (308 mg, 0.87 mmol, 98%). The non-racemic forms of indoline 100 were prepared from (R,R)-95 and (S,S)-95, following an identical protocol.

$^1$H NMR (500 MHz, CD3OD): δ 8.33 (1H, app br s, H7), 8.15 (1H, app br s, H6'), 8.06 (1H, app br s, H2'), 7.57 (1H, app t, J 7.7, H6), 7.36-7.46 (4H, m, H4, m-Ph, p-Ph), 7.32 (1H, app t, J 7.7, H5), 7.28 (2H, m, o-Ph), 7.05 (1H, app t, J 2.0, H4'), 5.75 (1H, s, H2), 1.99 (3H, br s, CH3).1

$^{13}$C NMR (126 MHz, CD3OD): δ 171.2 (C=O), 156.3 (C5'), 144.2 (C7a), 141.0 (i-Ph), 140.0 (C6'), 139.6
(C2'), 136.6 (C3'), 132.2 (m-Ph/p-Ph), 130.4 (m-Ph/p-Ph), 127.2 (C5), 127.2 (C4), 126.9 (o-Ph), 121.7 (C4'), 119.8 (CN), 118.5 (C7), 74.1 (C2), 58.8 (C3), 24.0 (CH3).‡

OH peak not observed due to exchange with MeOD.

† 1 peak obscured.


IR: νmax (film)/cm⁻¹ 3062, 2234, 1671 (C=O), 1591, 1480, 1387, 1278, 757, 673.

MP: 120-125 °C.

[α]D25^25.0 (R,R) = +72 (c = 0.1, CHCl₃); [α]D25^25.0 (S,S) = -73 (c = 0.1, CHCl₃).

5-((2R,3R)/(2S,3S)-1-Acetyl-3-cyano-3-phenylindolin-2-yl)pyridin-3-yl trifluoromethanesulfonate

5-((2R,3R)/(2S,3S)-1-Acetyl-3-cyano-3-phenylindolin-2-yl)pyridin-3-yl trifluoromethanesulfonate 157

Diisopropylethylamine (49 μL, 0.28 mmol) was added to a solution of indoline (R,R)-100 (50 mg, 0.14 mmol) and N-phenyl-bis(trifluoromethylsulphonimide) (100 mg, 0.28 mmol) in CH₂Cl₂ (4 mL) at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 3 h. H₂O (10 mL) was added, and the mixture was extracted with CH₂Cl₂. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded indoline (R,R)-157 as a colorless hygroscopic solid (53 mg, 0.11 mmol, 79%).

5-((2S,3S)-1-acetyl-3-cyano-3-phenylindolin-2-yl)pyridin-3-yl trifluoromethanesulfonate 157
Indoline (S,S)-157 was prepared in an analogous manner to indoline (R,R)-157, using indoline (S,S)-100 (148 mg, 0.42 mmol), diisopropylethylamine (0.15 mL, 0.83 mmol), and N-phenylbis(trifluoromethylsulfonimide) (298 mg, 0.83 mmol) in CH₂Cl₂ (12 mL). The reaction mixture was stirred at rt for 3 h. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded indoline (S,S)-157 as a colorless hygroscopic solid (189 mg, 0.39 mmol, 93%).

Alternatively, indoline (S,S)-157 was prepared according to general procedure 2, using indoline 267 (52 mg, 0.12 mmol), acetyl chloride (17 µL, 0.23 mmol), and pyridine (19 µL, 0.23 mmol) in CH₂Cl₂ (2 mL). The reaction mixture was stirred at rt for 20 min. Purification by flash pressure column chromatography (30% EtOAc/petroleum ether) afforded indoline (S,S)-157 as a colorless hygroscopic solid (52 mg, 0.11 mmol, 92%).

$^1$H (500 MHz, C₆D₆, 348 K): δ 8.44 (1H, app s, H2'/H6'), 8.34 (1H, d, J 2.1, H2'/H6'), 7.96 (1H, app br s, H7), 7.24 (1H, app s, H4'), 7.11-7.21 (2H, obscured, o-Ph), 7.06 (1H, app t, J 7.9, H6), 6.94-7.03 (4H, m, H4, m-Ph, p-Ph), 6.74 (1H, app t, J 7.6, H5), 5.23 (1H, s, H2), 1.50 (3H, s, CH₃).

$^{13}$C (126 MHz, C₆D₆, 348 K): δ 167.7 (C=O), 148.4 (C2'/C6'), 147.4, 144.2 (C2'/C6'), 143.2, 140.4, 136.2, 131.4 (C6), 130.3 (m-Ph/p-Ph), 129.7, 129.6 (m-Ph/p-Ph), 127.1 (C4'), 126.6 (C4), 126.2 (o-Ph), 126.2 (C5), 118.6 (CN), 117.2 (C7), 73.6 (C2), 57.7 (C3), 23.5 (CH₃).

‡ CF₃ signal obscured due to insufficient signal-to-noise ratio.

$^{19}$F (377 MHz, C₆D₆): δ -72.7.

HRMS (ES+): [C₂₃H₁₇O₄N₃F₃S⁺]⁺ ([M+H]+) requires 488.0886; found 488.0887.

IR: $\nu_{max}$ (film)/cm⁻¹: 2981, 2236, 1678 (C=O), 1479, 1429, 1385, 1219, 1138, 835, 752, 698.

$[\alpha]_{D}^{25.0}$ (R,R) = +51 (c = 0.1, CHCl₃), $[\alpha]_{D}^{25.0}$ (S,S) = -60 (c = 0.1, CHCl₃).
KO'Bu (65 mg, 0.58 mmol) was added to a solution of imine 178 (200 mg, 0.53 mmol) in toluene and stirred at 0 °C for 20 min. NH₄Cl (saturated aq., 20 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (50% EtOAc/petroleum ether) afforded a 1:1.2 inseparable mixture of diastereoisomers (87 mg, 0.23 mmol, 44%). 60 mg (0.16 mmol) of the residue was dissolved in CH₂Cl₂ (6 mL). Pyridine (19 µL, 0.24 mmol) was added, followed by acetyl chloride (17 µL, 0.24 mmol). The mixture was stirred at rt for 2 h. NaHCO₃ (saturated aq., 6 mL) was added, and the mixture was extracted with CH₂Cl₂. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered and concentrated. Purification by flash pressure column chromatography afforded indoline 180 as a colorless hygroscopic solid as a 19:1 mixture of diastereoisomers (38 mg, 0.09 mmol, 57%).

N.B. Stereochemistry assigned by analogy to indolines 52 and 53.

¹H (500 MHz, C₆D₆, 348 K): δ 8.57 (1H, d, J 1.9, H2'/H6'), 8.33 (1H, d, J 1.9, H2'/H6'), 8.19 (1H, app br s, H7), 7.98 (1H, dd, J 4.7, 1.3, H5), 7.48 (1H, app t, J 1.9, H4'), 7.23 (2H, app d, J 8.0, Ph), 6.93-7.03 (3H, m, Ph), 6.63 (1H, dd, J 8.2, 4.7, H6), 5.17 (1H, s, H2), 1.41 (3H, s, CH₃).

¹³C (126 MHz, C₆D₆, 348 K): δ 168.6 (C=O), 153.1 (C2'/C6'), 149.8, 147.4 (C5), 146.9 (C2'/C6'), 139.8, 137.5, 136.7 (C4'), 135.5, 130.3 (Ph), 129.5 (Ph), 126.2 (Ph), 125.0 (C6), 124.3 (C5'), 121.9 (CN), 117.5 (C7), 72.3 (C2), 59.4 (C3), 23.2 (CH₃).

HRMS (ES⁺): [C₂₁H₁₆⁺BrN₄O⁺ ([M+H]⁺) requires 419.0502; found 419.0504.

180
IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3035, 2241, 1682 (C=O), 1439, 1354, 1227, 701, 683.

1-({5’-Azidopentyl})pyrrolidine 114

Azide 113 was prepared according to a literature procedure.$^{191}$ NaN$_3$ (286 mg, 4.42 mmol) was added to a solution of 1,5-dibromopentane (1.2 mL, 8.8 mL) in DMF (30 mL) and stirred at 50 °C for 3 h. The mixture was allowed to cool to rt, diluted with EtOAc, and washed with H$_2$O, then brine. The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (3.5% Et$_2$O/hexane) afforded 113 as a colorless liquid (557 mg, 2.90 mmol, 66%). Azide 113 was not fully characterized due to concerns about its toxicity and stability.

A solution of azide 113 (200 mg, 1.04 mmol) in dry THF (2 mL) was added dropwise to a solution of pyrrolidine (0.96 mL, 12 mmol) in dry THF (3 mL) and stirred at 50 °C for 1 h. Water (5 mL) was added, and the mixture was extracted with Et$_2$O. The combined organic extracts were washed with brine, dried over MgSO$_4$, filtered, and concentrated to afford azide 114 as a yellow liquid (171 mg, 0.94 mmol, 90%).

$^1$H (400 MHz, CDCl$_3$): $\delta$ 3.27 (2H, t, $J$ 7.0, H5’), 2.37-2.55 (6H, m, H1’, H2), 1.81 (4H, m, H3), 1.49-1.68 (4H, m, H2’, H4’), 1.41 (2H, app m, H3’).

$^{13}$C (101 MHz, CDCl$_3$): $\delta$ 56.4 (C1’), 54.3 (C2), 51.5 (C5’), 28.9 (C2’/C4’), 28.5 (C2’/C4’), 24.9 (C3’), 23.5 (C3).

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3399, 2935, 2091 (N=N=N), 1459, 1266, 879.

HRMS: [C$_9$H$_{19}$Na$_4$]$^+$ ([M+H]$^+$) requires 183.1604; found 183.1605.
(2RS,3SR)-1-Acetyl-3-phenyl-2-(5-((5-(pyrrolidin-1-yl)pentyl)-1H-1,2,3-triazol-4-yl)pyridin-3-yl)indoline-3-carbonitrile 121

Indoline 121 was prepared according to a modified literature procedure.\textsuperscript{192} CuSO\textsubscript{4} (aq., 2.5 mM, 0.55 mL, 0.0014 mmol) and sodium-L-ascorbate (aq., 12.5 mM, 0.55 mL, 0.0069 mmol) were added to a solution of alkyne 118 (25 mg, 0.070 mmol) and azide 114 (15 mg, 0.083 mmol) in \textsuperscript{1}BuOH (1.1 mL). The mixture was stirred at rt for 16 h. H\textsubscript{2}O (4 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated. Purification by preparatory TLC (6% MeOH/CH\textsubscript{2}Cl\textsubscript{2} + 0.5% aq. NH\textsubscript{4}OH) afforded indoline 121 as a colorless solid (9.3 mg, 0.017 mmol, 26%).

\textsuperscript{1}H (500 MHz, C\textsubscript{6}D\textsubscript{6}, 348 K): \(\delta 8.75 (1H, d, J 2.0, H6’), 8.32 (1H, app br s, H7), 8.04 (1H, app s, H2’), 7.65 (1H, app t, J 2.0, H4’), 7.12 (1H, app td, J 7.8, 1.4, H6), 7.04-7.09 (2H, m, Ph), 6.84 (1H, dd, J 7.7, 0.9, H4), 6.72-6.82 (5H, m, H5, H9, Ph), 5.82 (1H, s, H2), 3.69 (2H, t, J 7.1, H11), 2.36 (4H, m, H17), 2.26 (2H, t, J 7.0, H15), 1.70 (3H, s, CH\textsubscript{3}), 1.61 (4H, m, H18), 1.47 (2H, app quin, J 7.2, H12), 1.31 (2H, app quin, J 7.2, H13).

\textsuperscript{13}C (126 MHz, C\textsubscript{6}D\textsubscript{6}, 348 K): \(\delta 168.5 (C=O), 148.4 (C6’), 147.6 (C9), 145.1 (C2’), 144.2 (C7a), 134.2, 132.9 (C4’), 131.7, 131.5 (C6), 129.3 (Ph), 129.3, 129.1 (Ph), 128.9 (Ph), 127.6, 126.7 (C4), 125.6 (C5), 121.7, 120.0, 117.9 (C7), 73.7 (C2), 56.3 (C15), 56.1 (C3), 54.6 (C17), 50.4 (C11), 30.5 (C12), 28.9 (C14), 24.9 (C13), 24.4 (C18), 24.1 (CH\textsubscript{3}).

HRMS (ES\textsuperscript{+}): [C\textsubscript{33}H\textsubscript{36}N\textsubscript{7}O\textsuperscript{+}] ([M+H\textsuperscript{+}]\textsuperscript{+}) requires 546.2976; found 546.2983.

IR: \textit{v_{max}} (film)/cm\textsuperscript{-1} 2937, 2793, 2261, 1674 (C=O), 1600, 1478, 1388, 1352, 1283, 733, 700.

MP: 116-123 °C.
Indoline 122 was prepared according to a modified literature procedure.\textsuperscript{192} CuSO\(_4\) (aq., 10 mM, 0.33 mL, 0.0033 mmol) and sodium-L-ascorbate (aq., 50 mM, 0.13 mL, 0.0065 mmol) were added to a solution of alkyne 119 (24 mg, 0.070 mmol) and azide 114 (12 mg, 0.066 mmol) in 3:1 \(^t\)BuOH/H\(_2\)O (1 mL). The mixture was stirred at rt for 16 h. H\(_2\)O (4 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated. Purification by preparatory TLC (6% MeOH/CH\(_2\)Cl\(_2\) + 0.5% aq. NH\(_4\)OH) afforded indoline 122 as a colorless solid (9.5 mg, 0.017 mmol, 26%).

\(^1\)H (500 MHz, C\(_6\)D\(_6\), 348 K): δ 9.06 (1H, d, J 2.0, H6'), 8.61 (1H, d, J 2.0, H2'), 8.36 (1H, app t, J 2.0, H4'), 8.32 (1H, app br s, H7), 7.25 (2H, m, Ph), 7.11 (1H, app t, J 7.6, H6), 7.08 (1H, app d, J 7.5, H4), 6.95-7.05 (3H, m, Ph), 6.88 (1H, s, H9), 6.76 (1H, app t, J 7.5, H5), 5.35 (1H, s, H2), 3.72 (2H, t, J 7.3, H11), 2.37 (4H, m, H17), 2.27 (2H, t, J 7.3, H15), 1.62 (4H, m, H18), 1.55 (3H, s, CH\(_3\)), 1.50 (2H, app quin, J 7.5, H12), 1.33 (2H, app quin, J 7.3, H14), 1.13 (2H, app quin, J 7.3, H13).

\(^{13}\)C (126 MHz, C\(_6\)D\(_6\), 348 K): δ 168.2 (C=O), 149.1 (C6'), 148.0 (C2'), 144.4, 144.0, 141.2, 134.7 (C4'), 131.3 (C6/C9), 131.2 (C6/C9), 130.2 (Ph), 129.8, 129.3 (Ph), 126.4 (C4), 126.3 (Ph), 125.9 (C5), 120.4, 119.2, 117.8 (C7), 75.0 (C2), 58.3 (C3), 56.3 (C15), 54.6 (C17), 50.5 (C11), 30.5 (C12), 28.9 (C14), 25.0 (C13), 24.4 (C18), 23.7 (CH\(_3\)).\textsuperscript{\#}

\(\# 1\) peak obscured.

HRMS (ES\(^+\)): [C\(_{33}\)H\(_{36}\)N\(_7\)O\(^+\)] \(\text{([M+H]\(^+\)})\) requires 546.2976; found 546.2980.

IR: \(\nu_{max}\) (film)/cm\(^{-1}\) 2935, 2793, 2261, 1673 (C=O), 1600, 1478, 1386, 1353, 912, 734.

MP: 109-114 °C.
Vinylstannane 55 was prepared according to a literature procedure. Pd_{2}dba_{3} (16 mg, 0.018 mmol), cyc_{3}PHBF_{4} (26 mg, 0.071 mmol), and DIPEA (25 µL, 0.14 mmol) were added to a solution of CH_{2}Cl_{2} (15 mL) and stirred at rt for 10 min. 6-Heptyn-1-ol (400 mg, 3.57 mmol) was added, and the mixture was cooled to 0 °C. A solution of Bu_{3}SnH (1.15 mL, 4.28 mmol) in CH_{2}Cl_{2} (5 mL) was added dropwise over 5 min and stirred at 0 °C for 2.5 h. The mixture was concentrated and the residue purified by flash pressure column chromatography (10% EtOAc/petroleum ether) to afford vinylstannane 55 as a colorless liquid (1.20 g, 2.98 mmol, 84%). The spectral data matched those previously reported in the literature.

^{1}H (400 MHz, CDCl_{3}): δ 5.93 (1H, app dt, J 19.0, 5.6, H6), 5.86 (1H, app d, J 19.0, H7), 3.65 (2H, app td, J 6.6, 5.4, H1), 2.15 (2H, td, J 6.9, 5.6, H5), 1.28-1.63 (20H, m), 0.74-0.98 (15H, m).

^{13}C (101 MHz, CDCl_{3}): δ 149.4 (C6), 127.3 (C7), 63.0 (C1), 37.8 (C5), 32.7, 29.1, 28.7, 27.3, 25.2, 13.7, 9.4.

LCMS (ES\(^{+}\)): 291.1 ([M(^{120}Sn)-2(C_{4}H_{9})+H\(^{+}\)], 427.2 ([M(^{120}Sn)+Na\(^{+}\)]).

(E)-1-(7-(Tributylstanny)hept-6-en-1-yl)pyrrolidine 57

PPh_{3} (972 mg, 3.71 mmol) was added portionwise to a solution of vinylstannane 55 (1.15 g, 2.85 mmol) and CBr_{4} (1.23 g, 3.71 mmol) in CH_{2}Cl_{2} (20 mL), and the mixture was stirred at 0 °C for 1 h. Ice-cooled pentane (50 mL) was added. The white precipitate was removed by filtration, and the filtrate was concentrated. The resulting residue was dissolved in THF (10 mL) and added to a solution of pyrrolidine (1.20 mL, 13.9 mmol) in THF (10 mL). The mixture was stirred at 50 °C for 4 h. H_{2}O (20 mL) was added and the mixture was extracted with EtOAc. The combined organic
extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded vinylstannane 57 as a yellow oil (413 mg, 0.90 mmol, 32%).

$^1$H (400 MHz, CDCl$_3$): δ 5.96 (1H, app dt, J 18.9, 5.8, H6), 5.86 (1H, app d, J 18.9, H7), 2.49 (4H, m, H2’), 2.42 (2H, t, J 7.1, H1), 2.14 (2H, app q, J 6.7, H5), 1.78 (4H, m, H3’), 1.39-1.55 (10H, m), 1.23-1.37 (8H, m), 0.82-0.93 (15H, m).

$^{13}$C (101 MHz, CDCl$_3$): δ 149.6 (C6), 127.1 (C7), 56.7 (C1), 54.2 (C2’), 37.8 (C5), 29.1, 29.0, 28.9, 27.3, 27.2, 23.4 (C3’), 13.7, 9.4.

HRMS (ES$^+$): [C$_{23}$H$_{48}$N$_{12}$O$_{12}$Sn]$^+$ ([M+H]$^+$) requires 458.2807; found 458.2809.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2956, 2926, 2872, 2853, 1457, 1146, 989.

(2RS,3SR)-1-Acetyl-3-phenyl-2-{5-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl}indoline-3-carbonitrile 58

Indoline 58 was prepared according to general procedure 4, using indoline 52 (60 mg, 0.14 mmol), vinylstannane 57 (78 mg, 0.17 mmol), and Pd(PPh$_3$)$_4$ (17 mg, 0.014 mmol) in NMP (1.5 mL). The reaction mixture was stirred at 85 °C for 1.5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded indoline 58 as a pale yellow oil (38 mg, 0.075 mmol, 54%).

$^1$H (500 MHz, C$_6$D$_6$, 348 K): δ 8.41 (1H, app br s, H7), 8.29 (1H, d, J 2.0, H6’), 7.91 (1H, app s, H2’), 7.13 (1H, obscured, H6), 7.00 (2H, m, o-Ph), 6.68-6.88 (6H, m, H4, H4’, H5, m-Ph, p-Ph), 5.86 (1H, d,
J 16.1, H8) 5.89 (1H, dt, J 16.1, 6.2, H9), 5.71 (1H, s, H2), 2.42 (4H, m, H16), 2.37 (2H, t, J 7.2, H14) 1.93 (2H, app q, J 6.9, H10), 1.67 (3H, s, CH3), 1.63 (4H, m, H17), 1.49 (2H, app quin, J 7.1, H13), 1.23-1.35 (4H, m, H11, H12).

13C (126 MHz, C6D6, 348 K): δ 168.4 (C=O), 148.4 (C6'), 147.2 (C2'), 145.1 (C7a), 134.5 (C9), 134.1, 133.4, 132.1, 131.4 (C6), 131.3, 129.2, 128.9, 126.5 (C8), 126.5 (C4), 125.5 (C5), 121.6 (CN), 117.8 (C7), 73.6 (C2), 56.6 (C3), 56.0 (C14), 54.5 (C16), 33.4 (C10), 29.5 (C11/C12/C13), 29.5 (C11/C12/C13), 27.6 (C11/C12), 24.3 (C17), 24.0 (CH3).‡

‡ 2 peaks obscured.

HRMS (ES'): [C33H37N4O][+1] ([M+H]+) requires 505.2962; found 505.2962.

IR: νmax (film)/cm⁻¹ 2931, 2787, 2260, 1679 (C=O), 1478, 1387, 1024, 756, 731, 696.

(2RS,3RS)-1-Acetyl-3-phenyl-2-(5-{[(E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl]pyridin-3-yl})indoline-3-carbonitrile 59

Indoline 59 was prepared according to general procedure 4, using indoline 53 (40 mg, 0.095 mmol), vinylstannane 57 (48 mg, 0.11 mmol), and Pd(PPh3)4 (11 mg, 0.0095 mmol) in NMP (1.2 mL). The reaction mixture was stirred at 85 °C for 2 h. Purification by flash pressure column chromatography (5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 59 as a pale yellow oil (19 mg, 0.038 mmol, 40%).

1H (500 MHz, C6D6, 348 K): δ 8.66 (1H, d, J 2.0, H6'), 8.45 (1H, d, J 2.0, H2'), 8.39 (1H, app br s, H7), 7.42 (1H, app t, J 2.0, H4'), 7.21-7.30 (2H, m, o-Ph), 7.12 (1H, obscured, H6), 7.07 (1H, app d, J 7.6,
H4), 6.95-7.04 (3H, m, m-Ph, p-Ph), 6.77 (1H, app t, J 7.6, H5), 5.94-6.10 (2H, m, H8, H9), † 5.24 (1H, s, H2), 2.43 (4H, m, H17), 2.38 (2H, t, J 7.1, H14), 1.93 (2H, app q, J 6.9, H10), 1.64 (4H, m, H17), 1.42-1.56 (5H, m, H13, CH3), 1.24-1.35 (4H, m, H11, H12).

† 1H NMR (500 MHz, C6D6): 5.96 (1H, dt, J 16.1, 6.7, H9), 5.86 (1H, d, J 16.1, H8).

13C (126 MHz, C6D6, 348 K): δ 168.3 (C=O), 149.9 (C6'), 147.3 (C2'), 144.1 (C7a), 141.4, 135.1 (C9), 134.8, 134.1, 131.3 (C6), 130.6, 130.2 (m-Ph/p-Ph), 129.4 (m-Ph/p-Ph), 126.8 (C8), 126.5 (C4), 126.3 (o-Ph), 126.0 (C5), 119.1 (CN), 117.7 (C7), 74.9 (C2), 58.3 (C3), 56.8 (C14), 54.7 (C16), 33.7 (C10), 29.6 (C11/C12/C13), 29.6 (C11/C12/C13), 27.9 (C11/C12), 24.5 (C17), 23.7 (CH3). ‡

‡ 1 peak obscured.

HRMS (ES†): [C33H37N4O]+ ([M+H]+) requires 505.2962; found 505.2967.

IR: νmax (film)/cm⁻¹ 3028, 2931, 2791, 2261, 1678 (C=O), 1479, 1386, 1239, 1176, 1133, 756, 680.

Ethyl (2R5,3R5)-1-acetyl-3-cyano-2-(5-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl)indoline-3-carboxylate 79

Indoline 79 was prepared according to general procedure 4, using indoline 78 (70 mg, 0.17 mmol), vinylstannane 57 (108 mg, 0.24 mmol), and Pd(PPh3)4 (20 mg, 0.017 mmol) in NMP (2.0 mL). The reaction mixture was stirred at 85 °C for 1 h. Purification by flash pressure column chromatography (5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 79 as a pale yellow oil (30 mg, 0.060 mmol, 36%, contains minor impurities).

† 1H (500 MHz, C6D6, 348 K): δ 8.51 (1H, d, J 2.2, H6'), 8.31 (1H, d, J 2.2, H2'), 8.12 (1H, app br s, H7), 7.26 (1H, app s, H4'), 7.22 (1H, app d, J 7.5, H4), 7.08 (1H, app td, J 7.7, 1.3, H6), 6.82 (1H, app td, J
7.7, 0.9, \(H5\)), 5.97 (1H, dt, \(J16.1, 6.4, H9\)), 5.91 (1H, d, \(J16.1, H8\)), 5.81 (1H, br s, \(H2\)), 3.51 (2H, q, \(J7.0, CH2CH3\)), 2.41 (4H, m, \(H16\)), 2.37 (2H, t, \(J7.1, H14\)), 1.90 (2H, app q, \(J6.4, H10\)), 1.64 (4H, m, \(H17\)), 1.59 (3H, s, COCH3), 1.46 (2H, app quin, \(J7.1, H13\)), 1.20-1.32 (4H, m, \(H11, H12\)), 0.65 (3H, t, \(J7.0, CH2CH3\)).

\[^{13}\text{C}\] (126 MHz, \(C6D6\), 348 K): \(\delta\) 168.0 (NCOCH3), 164.4 (CO2Et), 149.5 (C6'), 147.8 (C2'), 144.5 (C7a), 135.3 (C9), 134.2 (C3'/C5'), 132.0 (C3'/C5'), 131.7 (C6), 131.4 (C4'), 127.4 (C4), 126.4 (C8), 125.4 (C5), 125.4 (C3a) 118.3 (CN), 117.4 (C7), 70.6 (C2), 63.8 (CH2CH3), 56.8 (C14), 56.4 (C3), 54.7 (C16), 33.6 (C10), 29.6 (C13), 29.5 (C11/C12), 27.8 (C11/C12), 24.5 (C17), 23.9 (NCOCH3), 13.7 (CH2CH3).

HRMS (ES\(^+\)): [C\(_{30}\)H\(_{37}\)N\(_4\)O\(_3\)]\(^\text{+}\) ([M+H]\(^+\)) requires 501.2860; found 501.2847.

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 2932, 2788, 2260, 1757 (C=O), 1680 (C=O), 1481, 1463, 1387, 1252, 1024, 754.

Methyl (2RS,3SR)-1-acetyl-3-phenyl-2-(5-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl)indoline-3-carboxylate 69

Indoline 69 was prepared according to general procedure 4, using indoline 67 (50 mg, 0.11 mmol), vinylstannane 57 (71 mg, 0.16 mmol), and Pd(PPh\(_3\))\(_4\) (13 mg, 0.011 mmol) in NMP (1.5 mL). The reaction mixture was stirred at 85 °C for 1.2 h. Purification by flash pressure column chromatography (6.5% MeOH/CH\(_2\)Cl\(_2\) + 0.5% aq. \(NH_2OH\)) afforded indoline 69 as a pale yellow oil (40 mg, 0.074 mmol, 67%).

\(^1\text{H}\) (500 MHz, \(C6D6\), 348 K): \(\delta\) 8.64 (1H, app br s, \(H7\)), 8.29 (1H, app s, \(H2'/H6'\)), 8.07 (1H, app s, \(H2'/H6'\)), 7.36 (1H, app d, \(J7.7, H4\)), 7.21 (1H, app td, \(J7.7, 1.3, H5\)), 6.77-6.95 (7H, m, \(H4', H6, Ph\)), 6.45 (1H, s, \(H2\)), 5.90 (1H, d, \(J16.0, H8\)), 5.84 (1H, dt, \(J16.0\ Hz, 6.1, H9\)), 3.23 (3H, s, CO\(_2\)CH\(_3\)), 2.42
(4H, m, H16), 2.39 (2H, t, J 7.2, H14), 1.88-1.99 (5H, m, H10, CH3), 1.64 (4H, m, H17), 1.49 (2H, app quin, J 6.8, H13), 1.23-1.39 (4H, m, H11, H12).

13C (126 MHz, CD6D6, 348 K): δ 173.2 (C=O), 168.6 (C=O), 147.9 (C2′/C6′), 147.9 (C2′/C6′), 145.5 (C7a), 137.7, 134.3, 134.1, 133.4, 131.4, 130.7 (C6), 130.2, 129.0 (Ph), 128.8 (Ph), 127.0 (Ph), 124.6 (C5), 118.0 (C7), 70.6 (C2), 68.4 (CO2CH3), 56.8 (C3), 54.7 (C14/C16), 53.1 (C14/C16), 33.6 (C10), 29.7 (C11/C12/C13), 29.7 (C11/C12/C13), 27.8 (C11/C12), 24.5 (C17), 24.1 (CH3).†

‡ 2 peaks obscured.

HRMS (ES+): [C34H40N3O3]+ ([M+H]+) requires 538.3064; found 538.3058.

IR: νmax (film)/cm⁻¹ 2931, 2786, 1731 (C=O), 1673 (C=O), 1478, 1390, 1229, 729.

Methyl (2RS,3RS)-1-acetyl-3-phenyl-2-{5-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl}indoline-3-carboxylate 70

Indoline 70 was prepared according to general procedure 4, using indoline 68 (40 mg, 0.089 mmol), vinylstannane 57 (57 mg, 0.12 mmol), and Pd(PPh3)4 (10 mg, 0.0087 mmol) in NMP (2.0 mL). The reaction mixture was stirred at 85 °C for 4.5 h. Purification by flash pressure column chromatography (5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 70 as a pale yellow oil (20 mg, 0.037 mmol, 41%).

1H (500 MHz, dmsod6, 363 K): δ 8.49 (1H, d, J 1.9, H2′/H6′), 8.19 (1H, d, J 1.9, H2′/H6′), 7.99 (1H, app br s, H7), 7.63 (1H, app d, J 7.6, H4), 7.53 (1H, app s, H4′), 7.44 (2H, app d, J 7.6, o-Ph), 7.37 (3H, m, m-Ph, p-Ph), 7.30 (1H, app t, J 7.3, H5), 7.19 (1H, app t, J 7.6, H6), 6.35 (1H, d, J 16.0, H8), 6.26
(1H, dt, J 16.0, 6.7, H9), 6.18 (1H, s, H2), 3.23 (3H, s, CO2CH3), 2.42 (4H, m, H16), 2.38 (2H, t, J 7.3, H14), 2.19 (2H, app q, J 7.2, H10), 2.15 (3H, s, CH3), 1.67 (4H, m, H17), 1.40-1.51 (4H, m, H11/H12/H13), 1.33-1.39 (2H, app quin, J 6.9, H11/H12/H13).

13C (126 MHz, dmso-D6, 363 K): δ 169.3 (C=O), 167.4 (C=O), 146.9 (C2′/C6′), 146.0 (C2′/C6′), 141.6 (C7a), 141.3, 133.5, 133.3, 132.1, 130.6, 128.4, 128.3, 127.3, 127.1, 125.3, 123.5, 115.5 (C7), 69.1 (CO2CH3), 55.1 (C3), 53.0 (C16), 51.2 (C14), 31.7 (C10), 27.9 (C11/C12/C13), 27.7 (C11/C12/C13), 26.1 (C11/C12/C13), 23.2 (CH3), 22.8 (C17).

‡ 2 peaks obscured.

HRMS (ES''): [C34H40N3O3]\(+\) ([M+H]\+) requires 538.3064; found 538.3066.

IR: v\(_{\text{max}}\) (film)/cm\(^{-1}\) 2929, 1741 (C=O), 1671 (C=O), 1479, 1389, 1223, 966, 716, 697.

(2RS,3SR)-1-Acetyl-3-phenyl-2-(3-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)phenyl)indoline-3-carbonitrile 128

Indoline 128 was prepared according to general procedure 4, using indoline 126 (38 mg, 0.091 mmol), vinylstannane 57 (66 mg, 0.15 mmol), and Pd(PPh3)4 (11 mg, 0.0095 mmol) in NMP (1.6 mL). The reaction mixture was stirred at 85 °C for 2.5 h. Purification by flash pressure column chromatography (5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 128 as a pale yellow oil (27 mg, 0.054 mmol, 59%).

1H (500 MHz, C6D6, 348 K): δ 8.69 (1H, app d, J 8.2, H7), 7.12-7.20 (1H, obscured, H6), 7.03 (2H, m, o-Ph), 6.74-6.88 (6H, m, m-Ph, p-Ph, H4, H4', H5), 6.64 (1H, app t, J 7.5, H5'), 6.55 (1H, br s, H2').
6.43 (1H, app d, J 7.5, H6’), 6.00 (1H, d, J 15.8, H8), 5.84 (1H, dt, J 15.8, 6.8, H9), 5.64 (1H, s, H2), 2.35-2.49 (6H, m, H14, H16), 2.00 (2H, app q, J 6.8, H10), 1.69 (3H, s, CH3), 1.59-1.67 (4H, m, H17), 1.50 (2H, app quin, J 6.8, H13), 1.35 (4H, m, H11, H12).

13C (126 MHz, C6D6, 348 K): δ 169.2 (C=O), 145.4 (C7a), 138.8, 136.8, 134.3, 132.3 (C9), 131.4, 129.8 (C8), 129.2, 129.1, 128.9, 127.9, 126.5, 126.2, 125.5, 122.1, 117.9 (C7), 75.4 (C2), 56.8 (C14/C16), 56.1 (C3), 54.7 (C14/C16), 33.6 (C10), 29.9 (C11/C12), 29.7 (C13), 27.8 (C11/C12), 24.3 (C17), 24.3 (CH3). 1

† 3 peaks obscured.

HRMS (ESI): [C34H38N3O]+ ([M+H]+) requires 504.3009; found 504.2998.

IR: νmax (film)/cm⁻¹ 2930, 2789, 1677 (C=O), 1477, 1352, 1281, 728, 697.

(2RS,3RS)-1-Acetyl-3-phenyl-2-(3-(E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)phenylindoline-3-carbonitrile 129

Indoline 129 was prepared according to general procedure 4, using indoline 127 (50 mg, 0.12 mmol), vinylstannane 57 (77 mg, 0.17 mmol), and Pd(PPh3)4 (14 mg, 0.012 mmol) in NMP (2.0 mL). The reaction mixture was stirred at 85 °C for 1.5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 129 as a pale yellow oil (35 mg, 0.070 mmol, 58%).

1H (500 MHz, C6D6, 348 K): δ 8.62 (1H, app d, J 7.8, H7), 7.33 (2H, m, o-Ph), 7.24 (1H, app s, H2’), 7.17-7.22 (2H, m, H4’, H6), 7.11 (1H, app d, J 7.6, H4), 6.98-7.07 (4H, m, m-Ph, p-Ph, H5’), 6.95 (1H,
app d, J 7.3, H6', 6.80 (1H, app t, J 7.6, H5), 6.25 (1H, d, J 15.6, H8), 6.11 (1H, dt, J 15.6, 6.8, H9), 5.22 (1H, s, H2), 2.35-2.47 (6H, m, H14, H16), 2.05 (2H, app q, J 6.7, H10), 1.60-1.68 (4H, m, H17), 1.57 (3H, s, CH3), 1.51 (2H, app quin, J 6.9, H13), 1.37 (4H, m, H11, H12).

13C (126 MHz, C6D6, 348 K): δ 168.9 (C=O), 144.4 (C7a), 141.9, 139.9, 138.9, 132.7 (C9), 131.2 (C6), 130.2 (m-Ph/p-Ph/C'S'), 130.1 (C8), 130.1 (m-Ph/p-Ph/C'S'), 129.2 (m-Ph/p-Ph/C'S'), 128.9, 127.3 (C2'), 126.4 (C4), 126.3 (o-Ph), 125.8 (C5), 125.2 (C6'), 125.0 (C4'), 119.1 (CN), 117.7 (C7), 77.1 (C2), 58.5 (C3), 56.8 (C14), 54.7 (C16), 33.7 (C10), 29.8 (C11/C12), 29.7 (C13), 27.9 (C11/C12), 24.3 (C17), 24.0 (CH3).

HRMS (ES'): [C34H38N3O]+ ([M+H]+) requires 504.3009; found 504.2993.

IR: νmax (film)/cm⁻¹ 2930, 2790, 1678 (C=O), 1478, 1387, 1282, 729, 697.

7-Bromohept-1-yn 104

Alkyne 104 was prepared according to a literature procedure.104 PPh3 (1.22 g, 4.64 mmol) was added portionwise to a solution of 6-heptyn-1-ol (400 mg, 3.57 mmol) and CBr4 (1.54 g, 4.64 mmol) in CH2Cl2 (8 mL), and the mixture was stirred at 0 °C for 1.5 h. Ice-cooled hexane (30 mL) was added. The white precipitate was removed by filtration, and the filtrate was concentrated. Purification by flash pressure column chromatography (100% petroleum ether) afforded alkyne 104 as a colorless liquid (537 mg, 3.07 mmol, 86%). The spectral data matched those reported in the literature.

1H (400 MHz, CDCl3): δ 3.42 (2H, t, J 6.8, H7), 2.22 (2H, app m, H3), 1.96 (1H, t, J 2.7, H1), 1.89 (2H, app m, H6), 1.48-1.64 (4H, m, H4, H5).

13C (101 MHz, CDCl3): δ 84.1 (C2), 68.5 (C1), 33.5 (C7), 32.3 (C6), 27.6 (C4/C5), 27.3 (C4/C5), 18.3 (C3).
A solution of alkyne 104 (280 mg, 1.60 mmol) in THF (4 mL) was added to a solution of pyrrolidine (1.3 mL, 16 mmol) in THF (4 mL). The mixture was stirred at 50 °C for 1.5 h. H₂O was added, and the mixture was extracted with Et₂O. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded alkyne 105 as a yellow liquid (245 mg, 1.48 mmol, 41%).

¹H (400 MHz, CDCl₃): δ 2.37-2.53 (6H, m, H₇, H₉), 2.18 (2H, td, J 7.0, 2.7, H₃), 1.93 (1H, t, J 2.7, H₁), 1.76 (4H, m, H₁₀), 1.47-1.61 (4H, m, H₄, H₆), 1.43 (2H, app quin, J 7.1, H₅).

¹³C (101 MHz, CDCl₃): δ 84.6 (C₂), 68.1 (C₁), 56.5 (C₇/C₉), 54.2 (C₇/C₉), 28.6 (C₄/C₆), 28.4 (C₄/C₆), 26.8 (C₅), 23.4 (C₁₀), 18.3 (C₃).

HRMS (ES⁺): [C₁₁H₂₀N⁺] ([M+H]⁺) requires 166.1590; found 166.1592.

IR: νmax (film)/cm⁻¹ 3311 (C-H), 2936, 2787, 1461, 1147, 734, 631.

(2R,3S)-1-Acetyl-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)pyridin-3-yl)indoline-3-carbonitrile 106

Indoline 106 was prepared according to general procedure 5, using indoline 52 (70 mg, 0.17 mmol), alkyne 105 (41 mg, 0.25 mmol), PdCl₂(PPh₃)₂ (5.9 mg, 0.0084 mmol), and Cul (1.6 mg, 0.0084 mmol).
in N,N-diisopropylamine (2.5 mL). The reaction mixture was stirred at 70 °C for 1 h. Purification by flash pressure column chromatography (5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 106 as a pale yellow oil (55 mg, 0.11 mmol, 66%).

¹H (500 MHz, C₆D₆, 348 K): δ 8.45 (1H, d, J 1.9, H6'), 8.26 (1H, app br s, H7), 7.84 (1H, app br s, H2'), 7.08 (1H, app td, J 7.7, 1.3, H6), 6.89-6.99 (3H, m, H4', o-Ph), 6.76-6.86 (4H, m, H4, m-Ph, p-Ph), 6.73 (1H, app t, J 7.7, H5), 5.63 (1H, s, H2), 2.39 (4H, m, H16), 2.33 (2H, t, J 6.9, H14), 2.09 (2H, t, J 6.6, H10), 1.57-1.67 (7H, m, H17, CH₃), 1.32-1.44 (6H, m, H11, H12, H13).

¹³C (126 MHz, C₆D₆): δ 168.4 (C=O), 152.7 (C6'), 147.5 (C2'), 144.7 (C7a), 137.9, 136.8, 133.5 (C4'), 132.0, 131.5 (C6), 129.4 (Ph), 129.1 (Ph), 128.9 (Ph), 126.6, 125.7, 121.6 (C5'/CN), 121.4 (C5'/CN), 117.8 (C7), 95.4 (C8/C9), 77.7 (C8/C9), 72.8 (C2), 56.6 (C14), 55.7 (C3), 54.6 (C16), 29.1 (C11/C12/C13), 28.9 (C11/C12/C13), 27.4 (C11/C12/C13), 24.3 (C17), 24.1 (CH₃), 19.9 (C10).

HRMS (ES⁺): [C₃₃H₃₅N₄O⁺]([M+H]⁺) requires 503.2805; found 503.2792.

IR: ν_max (film)/cm⁻¹ 2935, 2790, 2232, 1680 (C=O), 1478, 1387, 1024, 757, 700.

(2RS,3RS)-1-Acetyl-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)pyridin-3-yl)indoline-3-carbonitrile 107

Indoline 107 was prepared according to general procedure 5, using indoline 53 (70 mg, 0.17 mmol), alkyne 105 (41 mg, 0.25 mmol), PdCl₂(PPh₃)₂ (5.9 mg, 0.0084 mmol), and Cul (1.6 mg, 0.0084 mmol) in N,N-diisopropylamine (2.5 mL). The reaction mixture was stirred at 70 °C for 1.25 h. Purification by flash pressure column chromatography (5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline
107 as a pale yellow oil (66 mg, 0.092 mmol, 79%). The non-racemic form of indoline 107 was prepared from (S,S)-157, following an identical protocol.

$^1$H (500 MHz, C₆D₆, 348 K): δ 8.81 (1H, d, J 1.9, H6’), 8.46 (1H, d, J 2.2, H2’), 8.21 (1H, app br s, H7), 7.60 (1H, app s, H4’), 7.19 (2H, m, o-Ph), 7.06 (1H, app t, J 7.7, H6), 6.95-7.04 (4H, m, H4, m-Ph, p-Ph), 6.73 (1H, app t, J 7.7, H5), 5.18 (1H, br s, H2), 2.40 (4H, app br s, H16), 2.33 (2H, t, J 6.9, H14), 2.13 (2H, t, J 6.8, H10), 1.62 (4H, m, H17), 1.47 (3H, s, CH₃), 1.34-1.45 (6H, m, H11, H12, H13).

$^{13}$C (126 MHz, C₆D₆): δ 168.1 (C=O), 154.4 (C6’), 147.2 (C2’), 143.6 (C7a), 140.9 (C3’/i-Ph), 136.1 (C3a), 133.8 (C3’/i-Ph), 131.3 (C3a), 130.6 (C6), 130.2 (C4/m-Ph/p-Ph), 129.4 (C4/m-Ph/p-Ph), 126.4 (C4/m-Ph/p-Ph), 126.3 (o-Ph), 126.1 (C5), 119.0 (C5’/CN), 118.3 (C5’/CN), 117.7 (C7), 96.0 (C8/C9), 77.9 (C8/C9), 74.2 (C2), 58.0 (C3), 56.5 (C14), 54.6 (C16), 29.1 (C11/C12/C13), 28.9 (C11/C12/C13), 27.4 (C11/C12/C13), 24.3 (C17), 23.7 (CH₃), 20.0 (C10).

HRMS (ES⁺): [C₃₃H₃₅N₄O]⁺ requires 503.2805; found 503.2790.

IR: ν_max (film)/cm⁻¹ 2934, 2789, 2232, 2341, 1678 (C=O), 1479, 1389, 1275, 755, 705.

\[ [\alpha]_{D}^{25.0} (S,S) = -55 (c = 0.1, CHCl₃) \]

(2SR,3RS)-1-Acetyl-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)furan-2-yl)indoline-3-carbonitrile 143

Indoline 143 was prepared according to general procedure 5, using indoline 140 (50 mg, 0.12 mmol), alkyne 105 (26 mg, 0.16 mmol), PdCl₂(PPh₃)₂ (4 mg, 0.006 mmol), and Cul (1 mg, 0.006 mmol) in N,N-diisopropylamine (5 mL). The reaction mixture was stirred at 70 °C for 2.5 h. Purification by flash pressure column chromatography (4.5 % MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 143 as a pale yellow oil (51 mg, 0.10 mmol, 83%).
\[ ^1 \text{H} \quad (500 \text{ MHz, } \text{C}_6\text{D}_6, 348 \text{ K}): \delta \quad 8.39 \quad (1\text{H, app br s, } H7), \quad 7.20-7.27 \quad (2\text{H, m, } o-\text{Ph}), \quad 7.08-7.14 \quad (2\text{H, m, } H4, H6), \quad 6.92-7.01 \quad (3\text{H, m-Ph, } p-\text{Ph}), \quad 6.77 \quad (1\text{H, app td, } J \text{ 7.6 Hz, } 0.9, H5), \quad 6.22 \quad (1\text{H, d, } J \text{ 3.4, } H3'/H4'), \quad 5.90 \quad (1\text{H, d, } J \text{ 3.4, } H3'/H4'), \quad 5.31 \quad (1\text{H, br s, } H2), \quad 2.38 \quad (4\text{H, m, } H16), \quad 2.31 \quad (2\text{H, t, } J \text{ 6.9, } H14), \quad 2.13 \quad (2\text{H, t, } J \text{ 6.8, } H10), \quad 1.62 \quad (4\text{H, m, } H17), \quad 1.55 \quad (3\text{H, s, } CH_3), \quad 1.33-1.42 \quad (6\text{H, m, } H11, H12, H13). \]

\[ ^{13} \text{C} \quad (126 \text{ MHz, } \text{C}_6\text{D}_6): \delta \quad 168.3 \quad (C=O), \quad 150.9, 143.4, 140.5, 139.7, 131.0 \quad (C_6), \quad 130.1 \quad (m-Ph/p-Ph), \quad 129.3 \quad (m-Ph/p-Ph), \quad 126.4 \quad (o-Ph), \quad 126.2 \quad (C4), \quad 125.7 \quad (C5), \quad 118.8 \quad (CN), \quad 118.1 \quad (C7), \quad 115.6 \quad (C3'), \quad 110.2 \quad (C4'), \quad 96.8 \quad (C8/C9), \quad 71.8 \quad (C8/C9), \quad 71.0 \quad (C2), \quad 57.0 \quad (C3), \quad 56.6 \quad (C14), \quad 54.6 \quad (C16), \quad 29.2 \quad (C11/C12/C13), \quad 28.8 \quad (C11/C12/C13), \quad 24.3 \quad (C17), \quad 23.3 \quad (CH_3), \quad 20.0 \quad (C10). \]

‡ 1 peak obscured.

HRMS (ES\textsuperscript{+}): [C\textsubscript{32}H\textsubscript{34}N\textsubscript{3}O\textsubscript{2}\textsuperscript{+}] \quad ([M+H]\textsuperscript{+}) \quad requires \quad 492.2645; \quad found \quad 492.2639.

IR: \nu_{\text{max}} \quad (film)/cm\textsuperscript{-1} \quad 2935, \quad 2792, \quad 2260, \quad 1680 \quad (C=O), \quad 1478, \quad 1388, \quad 1352, \quad 1022, \quad 756, \quad 698.

(2SR,3SR)-1-Acetyl-3-phenyl-2-({5-(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)furan-2-yl})indoline-3-carbonitrile 141

Indoline 141 was prepared according to general procedure 5, using indoline 139 (50 mg, 0.12 mmol), alkyne 105 (26 mg, 0.16 mmol), PdCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2} (4 mg, 0.006 mmol), and Cul (1 mg, 0.006 mmol) in N,N-diisopropylamine (5 mL). The reaction mixture was stirred at 70 °C for 1.5 h. Purification by flash pressure column chromatography (4% MeOH/CH\textsubscript{2}Cl\textsubscript{2} + 0.5% aq. NH\textsubscript{4}OH) afforded indoline 141 as a pale yellow oil (50 mg, 0.10 mmol, 82%).

\[ ^1 \text{H} \quad (500 \text{ MHz, } \text{C}_6\text{D}_6, 348 \text{ K}): \delta \quad 8.75 \quad (1\text{H, app br s, } H7), \quad 7.23 \quad (2\text{H, m, } o-\text{Ph}), \quad 7.07 \quad (1\text{H, app t, } J \text{ 7.5, } H6), \quad 6.94-7.03 \quad (3\text{H, m, } m-\text{Ph, } p-\text{Ph}), \quad 6.74 \quad (1\text{H, dd, } J \text{ 7.6, 1.2, } H4), \quad 6.69 \quad (1\text{H, app td, } J \text{ 7.6, 1.0, } H5), \quad 5.84 \quad (1\text{H,
d, J 3.4, H3'/H4'), 5.55 (1H, br s, H2), 5.39 (1H, d, J 3.4, H3'/H4'), 2.35 (4H, m, H16), 2.27 (2H, t, J 7.1, H14), 1.98 (2H, t, J 6.5, H10), 1.62 (4H, m, H17), 1.54 (3H, br s, CH3), 1.34 (2H, app quin, J 6.7, H11/H12/H13), 1.21-1.30 (4H, m, H11/H12/H13).

13C (126 MHz, C6D6): δ 168.4 (C=O), 149.2, 144.6, 139.0, 133.9, 131.2 (C6), 129.3 (m-Ph/p-Ph), 128.9 (m-Ph/p-Ph), 128.7 (o-Ph), 126.3 (C4), 125.3 (C5), 121.2 (CN), 118.2 (C7), 114.7 (C3'/C4'), 111.1 (C3'/C4'), 96.0 (C8/C9), 71.3 (C8/C9), 69.9 (C2), 56.5 (C14), 55.3 (C3), 54.6 (C16), 29.0 (C11/C12/C13), 28.7 (C11/C12/C13), 27.3 (C11/C12/C13), 24.2 (C17), 23.6 (CH3), 19.8 (C10).

‡ 1 peak obscured.


IR: νmax (film)/cm⁻¹ 2934, 2789, 2259, 1679 (C=O), 1479, 1387, 1351, 1021, 756, 697.

(2SR,3RS)-1-Acetyl-3-phenyl-2-(4-(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)furan-2-yl)indoline-3-carbonitrile 135

Indoline 135 was prepared according to general procedure 5, using indoline 134 (50 mg, 0.12 mmol), alkyne 105 (28 mg, 0.17 mmol), PdCl2(PPh3)2 (4 mg, 0.006 mmol), and Cul (1 mg, 0.006 mmol) in N,N-diisopropylamine (5 mL). The reaction mixture was stirred at 70 °C for 16 h. Purification by flash pressure column chromatography (4.5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 135 as a pale yellow oil (15 mg, 0.031 mmol, 24%).

1H (500 MHz, C6D6): δ 8.68 (1H, app br s, H7), 7.24 (2H, m, o-Ph), 7.20 (1H, s, H5'), 7.00-7.13 (2H, m, H4, H6), 6.90-6.97 (3H, m, m-Ph, p-Ph), 6.72 (1H, app td, J 7.5, 1.1, H5), 6.21 (1H, br s, H3'), 5.08 (1H, br s, H2), 2.37 (4H, m, H16), 2.31 (2H, t, J 7.0, H14), 2.19 (2H, t, J 6.7, H10), 1.61 (4H, m, H17), 1.34 (2H, app quin, J 6.7, H11/H12/H13), 1.21-1.30 (4H, m, H11/H12/H13).
1.33-1.49 (9H, m, H11, H12, H13, CH3).

13C (126 MHz, CD6): δ 168.2 (C=O), 150.8, 146.6 (C5'), 143.4, 140.4, 131.0 (C4/C6), 130.1 (m-Ph/p-Ph), 129.4 (m-Ph/p-Ph), 126.3 (o-Ph), 125.9 (C4/C6), 125.7 (C5), 118.8 (CN), 118.2 (C7), 112.6 (C3'), 110.4 (C8/C9), 93.4 (C8/C9), 70.6 (C2), 56.7 (C3), 56.6 (C14), 54.6 (C16), 29.1 (C11/C12/C13), 27.4 (C11/C12/C13), 24.2 (C17), 23.3 (CH3), 20.0 (C10).

‡ 2 peaks obscured.


IR: νmax (film)/cm⁻¹ 2948, 2248, 1676 (C=O), 1479, 1387, 1352, 1275, 756, 698.

(2R,3R)-1-Acetyl-3-phenyl-2-(5-(7-(pyrrolin-1-yl)heptyl)pyridin-3-yl)indoline-3-carbonitile 108

Indoline 108 was prepared according to general procedure 3, using indoline 107 (50 mg, 0.099 mmol) and Pd/C (wet degussa type, 5 mg) in MeOH (2.5 mL). The reaction mixture was stirred under a H₂ atmosphere for 4.5 h. Purification by flash pressure column chromatography (5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 108 as a colorless oil (37 mg, 0.073 mmol, 74%).

The non-racemic form of indoline 108 was prepared from (S,S)-107, following an identical protocol (e.r. >99:1).

1H (500 MHz, CD6): δ 8.69 (1H, app br s, H7), 8.50 (1H, d, J 2.0, H6'), 8.46 (1H, d, J 1.7, H2'), 7.20-7.28 (3H, m, H4', o-Ph), 7.10 (1H, app t, J 7.7, H6), 7.03 (1H, app d, J 7.7, H4), 6.94-7.01 (3H, m, m-Ph, p-Ph), 6.74 (1H, app td, J 7.5, 0.9, H5), 5.13 (1H, br s, H2), 2.36-2.48 (6H, m, H14, H16), 2.09 (2H, t, J 7.3, H8), 1.65 (4H, m, H17), 1.38-1.55 (5H, m, H13, CH₃), 1.22-1.33 (4H, m, H9, H12), 0.98-1.17
Indoline 144 was prepared according to general procedure 3, using indoline 143 (41 mg, 0.083 mmol) and Pd/C (wet degussa type, 4 mg) in MeOH (1.5 mL). The reaction mixture was stirred under a H₂ atmosphere for 4.5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 144 as a colorless oil (23 mg, 0.046 mmol, 56%).

(4H, m, H10, H11).

¹³C (126 MHz, C₆D₆): δ 168.5 (C=O), 152.3 (C6’), 146.6 (C2’), 144.0 (C7a), 141.1, 139.2, 133.7, 133.3 (C4’), 131.3 (C6), 130.2 (m-Ph/p-Ph), 129.4 (m-Ph/p-Ph), 126.4 (C4), 126.3 (o-Ph), 126.0 (C5), 119.1 (CN), 117.7 (C7), 74.5 (C2), 58.2 (C3), 57.0 (C14/C16), 54.8 (C14/C16), 33.1 (C8), 31.4 (C9/C12), 29.9 (C10/C11), 29.9 (C13), 28.2 (C9/C12), 24.3 (C17), 23.8 (CH₃). 1

† 1 peak obscured.

HRMS (ES⁺): [C₃₃H₃₉N₄O]⁺ ([M+H]⁺) requires 507.3118; found 507.3106.

IR: νmax (film)/cm⁻¹ 2929, 2787, 2261, 1677 (C=O), 1479, 1385, 1239, 1028, 755, 669.

[α]D²⁵ (S,S) = -87 (c = 0.1, CHCl₃).

Chiral HPLC: (Chiralpak ODH, 15% t-PrOH, 85% hexane, 1.3 mL/min, λ = 254 nm, 20 μL injection) τᵣ (R,R) = 8.7 min, τᵣ (S,S) = 11.4 min.

(2SR,3RS)-1-Acetyl-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)heptyl)furan-2-yl)indoline-3-carbonitrile 144
$^{13}$C (126 MHz, CD$_6$D$_6$): δ 168.4 (C=O), 158.7, 148.8, 143.6, 140.8, 130.9 (C4/C6), 130.0 (m-Ph/p-Ph), 129.2 (m-Ph/p-Ph), 126.3 (o-Ph), 126.0 (C4/C6), 125.6 (C5), 119.0 (CN), 118.1 (C7), 110.2 (C3'), 106.7 (C4'), 71.2 (C2), 57.0 (C14), 57.0 (C3), 54.7 (C16), 30.0 (C10/C11/C12), 29.9 (C9/C13), 29.7 (C10/C11/C12), 28.6 (C8), 28.5 (C9/C13), 28.2 (C10/C11/C12), 24.3 (C17), 23.4 (CH$_3$).†

† 1 peak obscured.

HRMS (ES$^+$): [C$_{32}$H$_{38}$N$_3$O$_2$]$^+$ ([M+H]$^+$) requires 496.2959; found 496.2940.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2933, 2789, 2263, 1673 (C=O), 1479, 1384, 1351, 1022, 756, 698.

(2SR,3SR)-1-Acetyl-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)heptyl)furan-2-yl)indoline-3-carbonitrile 142

Indoline 142 was prepared according to general procedure 3, using indoline 141 (40 mg, 0.081 mmol) and Pd/C (wet degussa type, 4 mg) in MeOH (1.5 mL). The reaction mixture was stirred under a H$_2$ atmosphere for 1 h. Purification by flash pressure column chromatography (4% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded indoline 142 as a colorless oil (26 mg, 0.052 mmol, 64%).

$^1$H (500 MHz, CD$_6$D$_6$): δ 8.54 (1H, app br s, H7), 7.24 (2H, app d, J 7.5, o-Ph), 7.14 (1H, app t, J 7.7, H6), 6.95-7.02 (3H, m, m-Ph, p-Ph), 6.86 (1H, app d, J 7.7, H4), 6.78 (1H, app t, J 7.7, H5), 5.70 (1H, s, H2), 5.60 (1H, d, J 3.0, H3'), 5.38 (1H, d, J 3.0, H4'), 2.38-2.48 (6H, m, H14, H16), 2.04 (2H, t, J 7.4, H8), 1.74 (3H, s, CH$_3$), 1.65 (4H, m, H17), 1.50 (2H, app quin, J 7.4, H13), 1.32 (2H, app quin, J 7.4, H12), 1.04-1.22 (4H, m, H9, H11), 1.00 (2H, app quin, J 7.1, H10).

$^{13}$C (126 MHz, CD$_6$D$_6$): δ 168.6 (C=O), 157.9, 147.4, 145.1, 134.7, 131.1 (C6), 129.0 (Ph), 129.0 (Ph), 129.0 (Ph), 126.3 (C4), 125.1 (C5), 121.4 (CN), 118.2 (C7), 110.9 (C3'), 106.0 (C4'), 70.6 (C2), 56.9 (C14), 55.6 (C3), 54.7 (C16), 29.9 (C9/C10/C11/C13), 29.9 (C9/C10/C11/C13), 29.7
(C9/C10/C11/C13), 28.4 (C9/C10/C11/C13), 28.2 (C8/C12), 28.2 (C8/C12), 24.5 (C17), 23.6 (CH3).†

‡ 1 peak obscured.

HRMS (ES⁺): [C_{32}H_{38}N_{3}O_{2}]⁺ ([M+H]⁺) requires 496.2959; found 496.2938.

IR: ν_{max}(film)/cm⁻¹ 2933, 2789, 1671 (C=O), 1477, 1389, 1353, 1022, 757, 699. (2SR,3RS)-1-Acetyl-3-phenyl-2-(4-(7-(pyrrolidin-1-yl)heptyl)furan-2-yl)indoline-3-carbonitrile 136

Indoline 136 was prepared according to general procedure 3, using indoline 135 (10 mg, 0.020 mmol) and Pd/C (wet degussa type, 1.5 mg) in MeOH (1.0 mL). The reaction mixture was stirred under a H₂ atmosphere for 16 h. Purification by flash pressure column chromatography (4% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 135 as a colorless oil (6.0 mg, 0.012 mmol, 60%).

¹H (500 MHz, C₆D₆): δ 8.80 (1H, app br s, H7) 7.31 (2H, o-Ph), 7.06-7.14 (2H, m, H4, H6), 6.91-6.98 (4H, m, H5', m-Ph, p-Ph), 6.74 (1H, app td, J 7.6, 0.9, H5), 6.01 (1H, br s, H3'), 5.22 (1H, br s, H2), 2.39-2.46 (6H, m, H14, H16), 2.06 (2H, t, J 7.6, H8), 1.65 (4H, m, H17), 1.48-1.59 (5H, m, H13, CH₃), 1.26-1.38 (4H, m, H9, H12), 1.14-1.25 (4H, m, H10, H11).

¹³C (126 MHz, C₆D₆): δ 168.4 (C=O), 150.8, 143.7, 140.8, 140.4 (C5'), 131.0 (C6), 130.1 (m-Ph/p-Ph), 129.3 (m-Ph/p-Ph), 127.3, 126.3 (o-Ph), 126.1 (C4), 125.7 (C5), 118.9 (CN), 118.2 (C7), 110.8 (C3'), 71.2 (C2), 57.0 (C3), 57.0 (C14/C16), 54.8 (C14/C16), 30.2 (C9/C12), 30.0 (C10/C11), 30.0 (C10/C11), 29.9 (C13), 28.3 (C9/C12), 25.2 (C8), 24.3 (C17), 23.4 (CH₃).†

‡ 1 peak obscured.

HRMS (ES⁺): [C_{32}H_{34}N_{3}O_{2}]⁺ ([M+H]⁺) requires 492.2645; found 492.2638.
K₂CO₃ (146 mg, 1.06 mmol) was added to a solution of indoline 96 (75 mg, 0.21 mmol) in acetone (15 mL), followed by 1,7-dibromoheptane (140 µL, 0.84 mmol). The mixture was stirred under reflux for 16 h, allowed to cool to rt, and concentrated. The remaining residue was dissolved in EtOAc, washed three times with H₂O, then brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (40% EtOAc/petroleum ether) afforded indoline 98 as a pale yellow oil (36 mg, 0.068 mmol, 32%, contains minor impurities).

\[ \text{IR: } \nu_{\text{max}} \text{(film)/cm}^{-1} 2933, 2789, 2248, 1673 (\text{C}=\text{O}), 1479, 1384, 1351, 1022, 756, 698. \]

\[ \text{(2RS,3SR)-1-Acetyl-2-((7-bromoheptyl)oxy)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 98} \]

\[ \begin{array}{c}
\text{O} \\
\text{N} \\
\text{C}_5 \text{H}_3 \\
\text{C}_6 \text{H}_6 \\
\text{N} \\
\text{C}_2 \text{H}_3
\end{array} \]

\[ \text{HRMS (ES\textsuperscript{+}): } [\text{C}_{29}\text{H}_{31}^{79}\text{BrN}_3\text{O}_3]^{+} ([\text{M+H}]^{+}) \text{ requires 532.1594; found 532.1592.} \]

\[ \text{IR: } \nu_{\text{max}} \text{(film)/cm}^{-1} 3028, 2929, 2857, 1676 (\text{C}=\text{O}), 1599, 1497, 1387, 1321, 1188, 757, 701. \]
(2RS,3RS)-1-Acetyl-2-(5-((7-bromoheptyloxy)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 101

K$_2$CO$_3$ (214 mg, 1.55 mmol) was added to a solution of indoline 100 (110 mg, 0.31 mmol) in acetone (20 mL), followed by 1,7-dibromohexane (0.20 mL, 1.2 mmol). The mixture was stirred under reflux for 8 h, allowed to cool to rt, and concentrated. The remaining residue was dissolved in EtOAc, washed three times with H$_2$O, then brine. The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (40% EtOAc/petroleum ether) afforded indoline 101 as a pale yellow oil (63 mg, 0.12 mmol, 38%, contains minor impurities). The non-racemic forms of indoline 101 were prepared from (R,R)-100 and (S,S)-100, following an identical protocol.

$^1$H (500 MHz, C$_6$D$_6$, 348 K): δ 8.43 (1H, d, J 2.5, H$^6$), 8.38 (1H, app br s, H$^7$), 8.28 (1H, d, J 1.9, H$^2$), 7.24 (2H, m, o-Ph), 7.10 (1H, app td, J 7.9, 1.2, H$^6$), 7.06 (1H, app d, J 7.6, H$^4$), 7.04 (1H, app t, J 2.2, H$^4$), 6.96-7.03 (3H, m, m-Ph, p-Ph), 6.76 (1H, app td, J 7.7, 0.9, H$^5$), 5.23 (1H, s, H$^2$), 3.47 (2H, app td, J 6.7, 1.6, H$^9$), 2.99 (2H, t, J 6.8, H$^{15}$), 1.44-1.57 (5H, m, H$^{14}$, CH$_3$), 1.35 (2H, app quin, J 7.4, H$^{10}$), 1.10 (2H, app quin, J 7.5, H$^{13}$), 1.04 (2H, app quin, J 7.5, H$^{11}$), 0.96 (2H, app quin, J 7.2, H$^{12}$).

$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): δ 168.4 (C=O), 156.2 (C$^5$), 143.8 (C$^7$a), 141.2 (C$^2$), 140.9 (i-Ph), 139.8 (C$^6$), 134.8 (C$^3$), 131.3 (C$^6$), 130.2 (m-Ph/p-Ph), 129.4 (m-Ph/p-Ph), 126.4 (C$^4$), 126.2 (o-Ph), 126.0 (C$^5$), 119.1 (CN), 118.6 (C$^4$), 117.6 (C$^7$), 74.2 (C$^2$), 68.5 (C$^9$), 58.1 (C$^3$), 34.0 (C$^{15}$), 33.1 (C$^{14}$), 29.3 (C$^{10}$), 28.8 (C$^{12}$), 28.4 (C$^{13}$), 26.0 (C$^{11}$), 23.7 (CH$_3$).

‡ 1 peak obscured.

HRMS (ES$^+$): [C$_{29}$H$_{31}$BrN$_3$O$_2$]$^+$ ([M+H]$^+$) requires 532.1594; found 532.1580.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3063, 2935, 2258, 1676 (C=O), 1595, 1479, 1386, 1238, 927, 756, 698.
(2RS,3SR)-1-acetyl-3-phenyl-2-(5-((7-(pyrrolidin-1-yl)heptyl)oxy)pyridin-3-yl)indole-3-carbonitrile

K₂CO₃ (29 mg, 0.21 mmol) was added to a solution of indoline 98 (22 mg, 0.04 mmol) in acetonitrile (3.0 mL), followed by pyrrolidine (14 µL, 0.17 mmol). The mixture was stirred at 60 °C for 2.5 h. H₂O was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (4.5-5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 99 as a colorless oil (14 mg, 0.027 mmol, 65%).

1H (500 MHz, C₆D₆, 348 K): δ 8.36 (1H, app br s, H7), 8.09 (1H, d, J 2.8, H6'), 7.72 (1H, app br s, H2'), 7.10 (1H, app td, J 7.7, 1.2, H6), 7.01 (2H, app d, J 7.3, o-Ph), 6.77-6.86 (4H, m, H4, m-Ph, p-Ph), 6.74 (1H, app td, J 7.7, 1.0, H5), 6.32 (1H, app br s, H4'), 6.57 (1H, br s, H2), 3.32 (2H, app m, H9), 2.93-2.46 (6H, m, H15, H17), 1.61-1.69 (7H, m, H18, CH₃), 1.50 (2H, app quin, J 7.3, H14), 1.39 (2H, app quin, J 6.8, H10), 1.32 (2H, app quin, J 6.9, H13), 1.15-1.23 (4 H, m, H11, H12).

13C (126 MHz, C₆D₆, 348 K): δ 168.6 (C=O), 155.4 (C5'), 144.9 (C7a), 141.1 (C2'), 138.7 (C6'), 133.9 (i-Ph), 132.9 (C3'), 131.5 (C6), 129.2 (Ph), 129.2 (Ph), 129.0 (Ph), 126.6 (C4), 125.6 (C5), 121.7 (CN), 119.6 (C4'), 117.7 (C7), 73.0 (C2), 68.5 (C9), 56.9 (C15/C17), 55.8 (C3), 54.8 (C15/C17), 29.9 (C11/C12), 29.8 (C14), 29.5 (C10), 28.1 (C13), 26.5 (C11/C12), 24.3 (C18), 24.1 (CH₃).†

† 1 peak obscured.
HRMS (ES\textsuperscript{+}): [C\textsubscript{33}H\textsubscript{39}N\textsubscript{4}O\textsubscript{2}]\textsuperscript{+} ([M+H]\textsuperscript{+}) requires 523.3068; found 523.3068.

IR: \(\nu_{\text{max}}\) (film)/cm\textsuperscript{-1} 2932, 2235, 1679 (C=O), 1478, 1387, 1183, 1030, 756, 699.

(2RS,3SR)-1-Acetyl-3-phenyl-2-{5-((7-{pyrrolidin-1-yl}heptyl)oxy)pyridin-3-yl}indoline-3-carbonitrile 102

K\textsubscript{2}CO\textsubscript{3} (58 mg, 0.42 mmol) was added to a solution of indoline 101 (45 mg, 0.085 mmol) in acetonitrile (6 mL), followed by pyrrolidine (28 \(\mu\)L, 0.34 mmol). The mixture was stirred at 60 °C for 3 h. H\textsubscript{2}O was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated. Purification by flash pressure column chromatography (5\% MeOH/CH\textsubscript{2}Cl\textsubscript{2} + 0.5\% aq. NH\textsubscript{4}OH) afforded indoline 102 as a colorless oil (24 mg, 0.046 mmol, 54\%). The non-racemic forms of indoline 102 were prepared from (\(R,R\))-101 and (\(S,S\))-101, following an identical protocol (e.r. > 99:1).

\textsuperscript{1}H (500 MHz, C\textsubscript{6}D\textsubscript{6}, 348 K): \(\delta\) 8.42 (1H, d, \(J\ 2.5\), H6\textsuperscript{'}), 8.39 (1H, app br s, H7), 8.28 (1H, d, \(J\ 1.2\), H2\textsuperscript{'}), 7.24 (2H, m, o-Ph), 7.05-7.13 (2H, m, H4, H6), 6.96-7.04 (4H, m, H4\textsuperscript{'}-m-Ph, p-Ph), 6.76 (1H, app td, \(J\ 7.6\), 0.9, H5), 5.22 (1H, br s, H2), 3.50 (2H, app td, \(J\ 6.4\), 1.7, H9), 2.37-2.48 (6H, m, H15, H17), 1.65 (4H, m, H18), 1.46-1.54 (5H, m, H14, CH\textsubscript{3}1), 1.43 (2H, app quin, J 6.8, H10), 1.29 (2H, app quin, J 7.1, H13), 1.18 (4H, m, H11, H12).

\textsuperscript{13}C (126 MHz, C\textsubscript{6}D\textsubscript{6}, 348 K): \(\delta\) 168.5 (C=O), 156.3 (C5\textsuperscript{'}), 143.9 (C7a), 141.1 (C2\textsuperscript{'}), 141.0 (i-Ph), 139.9 (C6\textsuperscript{'}), 134.8 (C3\textsuperscript{'}), 131.3 (C6), 130.2 (m-Ph/p-Ph), 129.4 (m-Ph/p-Ph), 128.7 (C3a), 126.4 (C4), 126.3 (o-Ph), 126.0 (C5), 119.2 (CN), 118.7 (C4\textsuperscript{'}), 117.6 (C7), 74.3 (C2), 68.7 (C9), 58.2 (C3), 56.9 (C15), 205.
54.8 (C17), 30.0 (C11/C12), 29.8 (C14), 29.6 (C10), 28.1 (C13), 26.4 (C11/C12), 24.3 (C18), 23.8 (CH₃).


IR: νmax (film)/cm⁻¹ 2932, 2789, 1677 (C=O), 1595, 1479, 1386, 1278, 1032, 873, 755, 698.

[α]D²⁵ = +57 (c = 0.1, CHCl₃); [α]D²⁵ = -48 (c = 0.1, CHCl₃).

Chiral HPLC: (Chiralpak ODH, 15% iPrOH, 85% hexane, 1.3 mL/min, λ = 254 nm, 20 μL)
τ,R (R,R) = 11.8 min, τ,S (S,S) = 16.7 min.

(2RS,3RS)-1-Acetyl-2-(5-((7-(dimethylamino)heptyl)oxy)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 216

Dimethylamine hydrochloride (20 mg, 0.23 mmol) was added to a solution of indoline 101 (30 mg, 0.056 mmol) in acetonitrile, followed by K₂CO₃ (40 mg, 0.28 mmol). The mixture was stirred 50 °C for 4.5 h. H₂O was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 216 as a colorless oil (19 mg, 0.038 mmol, 68%).

¹H (500 MHz, C₆D₆, 348 K): 6 8.42 (1H, d, J 2.2, H6'), 8.35 (1H, app br s, H7), 8.27 (1H, app s, H2'), 7.24 (2H, app d, J 7.6, Ph), 7.06-7.13 (2H, m, H4, H6), 6.95-7.05 (4H, m, H4', Ph), 6.77 (1H, app t, J 7.6, H5), 5.24 (1H, s, H2), 3.54 (2H, t, J 6.5, H9), 2.18 (2H, t, J 7.1, H15), 2.13 (6H, s, H17), 1.54 (3H, s, CH₃), 1.46 (2H, app quin, J 7.3, H10), 1.40 (2H, app quin, J 7.2, H14), 1.25 (2H, app quin, J 7.5, H13), 1.11-1.21 (4H, m, H11, H12).
\( ^{13}C \) (126 MHz, C₆D₆, 348 K): \( \delta \) 168.3 (C=O), 156.5 (C₅'), 144.1 (C7a), 141.3 (C2'), 141.3, 140.4 (C₆'), 134.8, 131.3 (C₆), 130.0, 129.4 (Ph), 126.4 (C4), 126.3 (Ph), 125.9 (C₅), 119.2 (C₄'), 119.1 (CN), 117.7 (C7), 74.8 (C2), 69.2 (C9), 60.4 (C3), 45.9 (C17), 29.9 (C11/C12), 29.6 (C10), 28.4 (C14), 28.0 (C13), 26.5 (C11/C12), 23.7 (CH₃).

HRMS (ES⁺): [C₃₁H₃₇N₄O₂]⁺ ([M+H]⁺) requires 497.2911; found 497.2909.

IR: \( \nu_{\text{max}} \) (film)/cm\(^{-1}\) 2937, 2858, 1678 (C=O), 1595, 1479, 1387, 1279, 1032, 756.

\((2RS,3RS)-1\text{-Acetyl-2-}(5-(2-(2-bromoethoxy)ethoxy)ethoxy)ethoxy)pyridin-3-yl)-3\text{-phenylindoline-3-carbonitrile 158}\)

\[\begin{align*}
\text{K}_2\text{CO}_3 \text{ (272 mg, 1.97 mmol) was added to a solution of indoline 100 (140 mg, 0.39 mmol) in acetone (25 mL), followed by 1,2-bis(2-bromoethoxy)ethane (410 mg, 1.58 mmol). The mixture was stirred at 50 °C for 2 h, allowed to cool to rt, and concentrated. The remaining residue was dissolved in EtOAc, washed three times with H₂O, then brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (3.5% MeOH/CH₂Cl₂) afforded indoline 158 as an orange paste (80 mg, 0.15 mmol, 37%, contains minor impurities).}

^{1}H \text{ (500 MHz, C₆D₆, 348 K): } \delta \text{ 8.41 (1H, d, J 2.3, H6'), 8.33 (1H, app br s, H7), 8.26 (1H, app s, H2'), 7.23 (2H, app d, J 7.7, o-Ph), 7.11 (1H, app t, J 7.8, H6), 7.04-7.08 (2H, m, H4, H4'), 6.95-7.04 (3H, m, m-Ph, p-Ph), 6.77 (1H, app t, J 7.6, H5), 5.22 (1H, s, H2), 3.65 (2H, app m, H9), 3.41 (2H, t, J 6.3, H15), 3.35 (2H, app s, H10), 3.24-3.33 (4H, m, H12, H13), 3.06 (2H, t, J 6.3, H16), 1.53 (3H, s, CH₃).

^{13}C \text{ (126 MHz, C₆D₆, 348 K): } \delta \text{ 168.4 (C=O), 156.1 (C₅'), 143.8 (C7a), 141.3 (C2'), 141.0 (i-Ph), 139.8 (C₆'), 134.7 (C3'), 131.2 (C6), 130.1 (m-Ph/p-Ph), 129.3 (m-Ph/p-Ph), 126.2 (C4), 126.2 (o-Ph), 125.9}
\end{align*}\]
(C5), 119.2 (C4'), 119.1 (CN), 117.6 (C7), 74.2 (C2), 71.6 (C15), 71.2 (C10), 70.9 (C12/C13), 69.9 (C12/C13), 68.2 (C9), 58.1 (C3), 31.0 (C16), 23.7 (CH3).‡

‡ 1 peak obscured.


IR: νmax (film)/cm⁻¹ 3026, 2923, 2249, 1674 (C=O), 1598, 1479, 1479, 1384, 1279, 1061, 735, 697.

(2RS,3RS)-1-Acetyl-3-phenyl-2-(5-((2-(2-(2-(pyrrolidin-1-yl)ethoxy)ethoxy)ethoxy)pyridin-3-yl)indoline-3-carbonitrile 159

K₂CO₃ (63 mg, 0.45 mmol) was added to a solution of indoline 158 (50 mg, 0.091 mmol) in acetonitrile (6 mL), followed by pyrrolidine (30 µL, 0.36 mmol). The mixture was stirred at 60 °C for 3 h. H₂O was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 159 as a pale yellow oil (38 mg, 0.070 mmol, 77%).

¹H (500 MHz, C₆D₆, 348 K): δ 8.41 (1H, d, J 2.8, H6'), 8.33 (1H, app br s, H7), 8.26 (1H, app s, H2'), 7.24 (2H, m, o-Ph), 7.12 (1H, app t, J 8.0, H6), 6.96-7.09 (5H, m, H4, H4', m-Ph, p-Ph), 6.78 (1H, app t, J 7.4, H5), 5.24 (1H, s, H2), 3.69 (2H, app m, H9), 3.51 (2H, t, J 6.0, H15), 3.36-3.47 (6H, m, H10, H12, H13), 2.63 (2H, t, J 6.0, H16), 2.46 (4H, m, H18), 1.56-1.64 (4H, m, H19), 1.55 (3H, s, CH₃).

¹³C (126 MHz, C₆D₆, 348 K): δ 168.3 (C=O), 156.3 (C5'), 144.0 (C7a), 141.5 (C2'), 141.3 (i-Ph), 140.5 (C6'), 134.8 (C3'), 131.3 (C6), 130.2 (m-Ph/p-Ph), 129.9 (C3a), 129.4 (m-Ph/p-Ph), 126.4 (C4), 126.3
HRMS (ES\textsuperscript{+}): [C\textsubscript{32}H\textsubscript{37}N\textsubscript{4}O\textsubscript{4}]\textsuperscript{+} ([M+H]\textsuperscript{+}) requires 541.2809; found 541.2788.

IR: \nu_{\text{max}}(\text{film})/\text{cm}^{-1} 2875, 2842, 1675 (C=O), 1595, 1479, 1387, 1279, 1124, 757.

(2RS,3RS)-1-Acetyl-2-(5-((Z)-7-hydroxyhept-1-en-1-yl)pyridin-3-yl)-3-phenylindoline-3-carbonitrile

Indoline 110 was prepared according to a modified literature procedure.\textsuperscript{97} InCl\textsubscript{3} (66 mg, 0.30 mmol) was dissolved in THF (0.9 mL) and cooled to -78 °C. Dibal-H (1.0 M in hexanes, 0.28 mL, 0.28 mmol) was added and stirred at -78 °C for 45 min. A solution of 6-heptyn-1-ol (22 mg, 0.20 mmol) in THF (0.6 mL) was added, followed by Et\textsubscript{3}B (1.0 M in hexanes, 0.19 mL, 0.19 mmol), and the mixture was stirred at -78 °C or 4 h. The reaction was allowed to warm to rt, and a solution of indoline 53 (110 mg, 0.26 mmol) in DMF (0.9 mL) was added, followed by a solution of Pd(PPh\textsubscript{3})\textsubscript{4} in THF (4.6 mM, 0.35 mL, 0.0016 mmol). The reaction mixture was stirred at 60 °C for 7 h, then diluted with EtOAc, washed with NaHCO\textsubscript{3} (saturated aq), and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated. Purification by flash pressure column chromatography (3.5% MeOH/CH\textsubscript{2}Cl\textsubscript{2}) afforded indoline 110 as a colorless oil (42 mg, 0.093 mmol, 58%, contains minor impurities).

\textsuperscript{1}H (500 MHz, C\textsubscript{6}D\textsubscript{6}, 348 K): \delta 8.59 (1H, app s, H\textsubscript{6}''), 8.46 (1H, app s, H2''), 8.34 (1H, app br s, H7), 7.42 (1H, app s, H4''), 7.24 (2H, m, Ph), 7.14 (1H, app t, J 7.8, H6), 7.08 (1H, app d, J 7.6, H4), 6.96-7.05 (3H, m, Ph), 6.80 (1H, app t, J 7.6, H5), 6.07 (1H, d, J 11.7, H8), 5.53 (1H, dt, J 11.7, 7.6, H9), 5.24
(1H, s, H2), 3.38 (2H, app q, J 6.3, H14), 2.02 (2H, app q, J 7.4, H10), 1.52 (3H, s, CH3), 1.33 (2H, app quin, J 6.7, H13), 1.13-1.24 (4H, m, H11, H12), 0.94 (1H, t, J 5.5, OH).

$^{13}$C (126 MHz, C₆D₆, 348 K): δ 168.3 (C=O), 152.2 (C6'), 147.1 (C2'), 144.0 (C7a), 141.2, 136.9 (C9), 134.7, 133.7, 133.2 (C4'), 131.3 (C6), 130.2 (Ph), 129.9, 129.4 (Ph), 126.5 (C4), 126.3 (Ph), 126.0 (C5), 125.6 (C8), 119.1 (CN), 117.7 (C7), 74.8 (C2), 63.0 (C14), 58.3 (C3), 33.3 (C13), 30.0 (C11/C12), 29.0 (C10), 26.1 (C11/C12), 23.7 (CH₃).

HRMS (ES^+): [C_{29}H_{30}N_{3}O_{2}]^+ ([M+H]^+) requires 452.2333; found 452.2326.

IR: $\nu_{\text{max}}$ (film)/cm⁻¹ 3420 (O-H), 3061, 2243, 1676 (C=O), 1479, 1385, 978, 755, 700.

(Z)-7-{5-((2RS,3RS)-1-Acetyl-3-cyano-3-phenylindolin-2-yl)pyridin-3-yl}hept-6-en-1-yl 4-methylbenzenesulfonate 111

$\rho$-Toluenesulfonyl chloride (38 mg, 0.20 mmol) was added to a solution of indoline 110 (30 mg, 0.066 mmol) and pyridine (32 µL, 0.41 mmol) in CH₂Cl₂ (5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min, allowed to warm to rt, and stirred for a further 20 h. H₂O (5 ml) was added, and the mixture was extracted 3 times with CH₂Cl₂. The combined organic extracts were washed with H₂O, then brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (55% EtOAc/petroleum ether) afforded indoline 111 as a colorless solid (41 mg, 0.068 mmol, 95%).

$^1$H (500 MHz, C₆D₆, 348 K): δ 8.56 (1H, d, J 1.6, H6'), 8.46 (1H, d, J 1.9, H2'), 8.32 (1H, app br s, H7), 7.77 (2H, app d, J 8.2, H18), 7.38 (1H, app s, H4'), 7.24 (2H, app d, J 8.2, Ph), 7.13-7.18 (1H, obscured, H6), 7.10 (1H, app d, J 7.6, H4), 6.97-7.06 (3H, m, Ph), 6.76-6.88 (3H, m, H5, H19), 6.04 (1H, d, J 11.3, 210
$^1$H (500 MHz, CD$_2$O$_6$, 348 K): $\delta$ 8.61 (1H, d, $J$ 1.5, H6'), 8.46 (1H, d, $J$ 1.9, H2'), 8.36 (1H, app br s, H7), 7.43 (1H, app s, H4'), 7.25 (2H, m, Ph), 7.13-7.18 (1H, obscured, H6), 7.10 (1H, app d, $J$ 7.6, H4), 7.08 (1H, app d, $J$ 7.3, H5), 6.99 (1H, dt, $J$ 11.7, 6.6, H9), 6.85 (1H, dt, $J$ 11.7, 7.4, H8), 5.42 (1H, dt, $J$ 11.7, 7.4, H9), 5.25 (1H, d, J 6.5, H14), 4.90 (1H, d, J 6.5, H13), 4.83 (1H, dt, $J$ 11.7, 6.6, H12), 1.92-1.99 (5H, m, H10, H21), 1.53 (3H, s, CH$_3$), 1.32 (2H, app quin, J 6.9, H13), 0.93-1.11 (4H, m, H11, H12).

$^{13}$C (126 MHz, CD$_2$O$_6$, 348 K): $\delta$ 168.2 (C=O), 152.1 (C6'), 147.2 (C2'), 144.4, 144.0 (C7a), 141.2, 136.4 (C9), 135.7, 134.5, 133.7, 133.2 (C4'), 131.3 (C6), 130.2 (Ph), 129.4 (Ph), 126.5 (C4), 126.3 (Ph), 126.0 (C5), 125.8 (C8), 119.1 (CN), 117.6 (C7), 74.7 (C2), 70.5 (C14), 58.3 (C3), 29.5 (C11/C12), 29.4 (C13), 28.8 (C10), 25.6 (C11/C12), 23.7 (CH$_3$), 21.4 (C21).

‡ 2 peaks obscured.

HRMS (ES$^+$): [C$_{36}$H$_{36}$N$_3$O$_4$S]$^+$ ([M+H]$^+$) requires 606.2421; found 606.2414.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3029, 2927, 1677 (C=O), 1479, 1387, 1189, 1033, 1011, 665.

MP: 104-106°C.

(2RS,3RS)-1-Acetyl-3-phenyl-2-(5-(Z)-(2-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl)indoline-3-carbonitrile 109

Pyrrole (23 µL, 0.28 mmol) was added to a solution of indoline 111 (17 mg, 0.028 mmol) in CH$_3$CN (2 mL). The reaction mixture was stirred at 50 °C for 1 h, then allowed to cool to rt and diluted with EtOAc. The solution was washed twice with H$_2$O, then with brine. The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) to afford indoline 109 as a pale yellow oil (9.2 mg, 0.018 mmol, 64%).
6.94-7.06 (3H, m, Ph), 6.81 (1H, app t, J 7.6, H5), 6.07 (1H, d, J 11.7, H8), 5.57 (1H, dt, J 11.6, 7.4, H9), 5.23 (1H, s, H2), 2.43 (4H, m, H16), 2.38 (2H, t, J 7.3, H14), 2.07 (2H, app q, J 7.0, H10), 1.64 (4H, m, H17), 1.52 (3H, s, CH3), 1.45 (2H, app quin, J 7.1, H13), 1.22-1.33 (4H, m, H11, H12).

13C (126 MHz, C6D6, 348 K): δ 168.2 (C=O), 152.2 (C6'), 147.1 (C2'), 144.1 (C7a), 141.3, 137.0 (C9), 134.7, 133.7, 133.2 (C4'), 131.3 (C6), 130.2 (Ph), 129.9, 129.4 (Ph), 126.4 (C4), 126.3 (Ph), 125.9 (C5), 125.5 (C8), 119.1 (CN), 117.7 (C7), 74.8 (C2), 58.3 (C3), 56.9 (C14), 54.7 (C16), 30.4 (C11/C12), 29.7 (C13), 29.3 (C10), 27.9 (C11/C12), 24.5 (C17), 23.7 (CH3).

HRMS (ES‘): [C33H37ON4]+ ([M+H]+) requires 505.2962; found 505.2961.

IR: νmax (film)/cm⁻¹ 3028, 2931, 2242, 1676 (C=O), 1479, 1387, 1026, 755, 698.

(2RS,3SR)-1-Acetyl-3-phenyl-2-(5-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl)-2,3-dihydro-1H-pyrrolo[3,2-b]pyridine-3-carbonitrile 181

Indoline 181 was prepared according to general procedure 4 using indoline 180 (30 mg, 0.072 mmol), vinylstannane 57 (42 mg, 0.093 mmol), and Pd(PPh3)4 (8 mg, 0.007 mmol) in NMP (1.5 mL). The reaction mixture was stirred at 85 °C for 1.5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 181 as a pale yellow oil (20 mg, 0.040 mmol, 55%).

1H (500 MHz, C6D6, 348 K): δ 8.66 (1H, d, J 1.8, H2'/H6'), 8.44 (1H, app br s, H7), 8.38 (1H, d, J 2.0, H2'/H6'), 8.03 (1H, dd, J 4.8 Hz, 1.3, H5), 7.35 (1H, app s, H4'), 7.31 (2H, m, Ph), 6.95-7.05 (3H, m, Ph), 6.70 (1H, dd, J 8.2, 4.8, H6), 5.98-6.08 (2H, m, H8, H9), 5.29 (1H, s, H2), 2.43 (4H, m, H16), 2.39 (2H, t, J 7.2, H14), 1.96 (2H, app br s, H10), 1.64 (4H, m, H17), 1.44-1.53 (5H, m, H13, CH3), 1.28-
1.36 (4H, m, H11, H12).

$^{13}$C (126 MHz, C$_6$D$_6$, 348 K): δ 169.0 (C=O), 150.0, 147.2 (C2'/C6'), 147.0 (C5), 140.2 (C2'/C6'), 140.0, 135.4 (C8/C9), 134.8, 133.6, 130.6 (C4'), 130.2 (Ph), 129.4 (Ph), 126.7 (C8/C9), 126.3 (Ph), 124.9 (C6), 124.3 (CN), 117.6 (C7), 73.2 (C2), 59.6 (C3), 56.7 (C14), 54.7 (C16), 33.6 (C10), 29.6 (C11/C12/C13), 29.5 (C11/C12/C13), 27.9 (C11/C12/C13), 24.5 (C17), 23.3 (CH$_3$).\(^{1}\)

‡ $^1$H NMR (400 MHz, C$_6$D$_6$): δ 5.98 (1H, dt, J 16.0, 6.5, H9), 5.88 (1H, d, J 16.0, H8).

‡ 1 peak obscured.

HRMS (ES$^+$): [C$_{23}$H$_{36}$N$_5$O]$^+$ ([M+H]$^+$) requires 506.2914; found 506.2914.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2931, 2790, 2261, 1682 (C=O), 1439, 1381, 1277, 1024, 969, 804, 698.

2-(2-Nitrophenyl)-2-(thiazol-2-yl)acetonitrile 182

Thiazole 182 was prepared according to a modified literature procedure.$^{195}$ A solution of 2-nitrophenylacetonitrile (800 mg, 4.93 mmol) in DMSO (8 mL) was added dropwise to a suspension of 2-chlorothiazole (0.42 mL, 4.9 mmol) and Cs$_2$CO$_3$ (3.54 g, 10.9 mmol) in DMSO (8 mL) and stirred at 80 °C for 16 h. The mixture was allowed to cool to rt, and NH$_4$Cl (saturated aq.) was added. The mixture was extracted with CH$_2$Cl$_2$. The combined organic extracts were washed three times with H$_2$O, then brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (20% EtOAc/petroleum ether) afforded nitrile 182 as an orange solid (344 mg, 1.40 mmol, 28%).

$^1$H (400 MHz, CDCl$_3$): δ 8.18 (1H, dd, J 7.8, 1.2, H3), 7.97 (1H, dd, J 7.8, 1.3, H6), 7.74-7.83 (2H, m, H5, H4'), 7.63 (1H, app td, J 7.8, 1.3, H4), 7.39 (1H, d, J 3.4, H5'), 6.61 (1H, s, H7).

$^{13}$C (101 MHz, CDCl$_3$): δ 161.3, 147.1, 143.6 (C5/C4'), 134.6 (C5/C4'), 131.2 (C6), 130.5 (C4), 128.2, 125.9 (C3), 121.2 (C5'), 116.7 (CN), 37.2 (C7).
HRMS (ES\textsuperscript{+}): [C\textsubscript{11}H\textsubscript{7}O\textsubscript{2}Na\textsubscript{32}S]\textsuperscript{+} ([M+Na]\textsuperscript{+}) requires 268.01512; found 268.01512.

IR: \(\nu_{\text{max}}\) (film)/cm\textsuperscript{-1} 3118, 2947, 2249, 1526, 1497, 1346, 787, 735.

MP: 40-41 °C.

\begin{align*}
(2RS,3RS)-2-(5-Bromopyridin-3-yl)-3-(thiazol-2-yl)indoline-3-carbonitrile 189
\end{align*}

Pd/C (wet Degussa type, 30 mg) was added to a solution of nitrile 182 (300 mg, 1.22 mmol) in MeOH (12 mL). The suspension was first degassed 3 times with \(N_2\) and then 3 times with \(H_2\) using a pump-flood procedure. The mixture was stirred under a \(H_2\) atmosphere for 18 h, then flushed with \(N_2\) before being filtered through Celite\textsuperscript{TM}, eluted with EtOAc, and concentrated. Purification by flash pressure column chromatography (25\% EtOAc/petroleum ether) afforded an orange solid (150 mg, impure), which was re-dissolved in toluene (6 mL). MgSO\textsubscript{4} (414 mg, 3.44 mmol) and 5-bromo-3-pyridinecarboxaldehyde (166 mg, 0.89 mmol) were added, and the mixture was stirred at rt for 48 h. The mixture was filtered and concentrated. Purification by flash pressure column chromatography (40\% EtOAc/petroleum ether) afforded indoline 189 as a yellow solid (62 mg, 0.16 mmol, 13%).

N.B. Stereochemistry was assigned by analogy to indolines 49 and 50.

\(^1\)H (500 MHz, \(C_6D_6\)): \(\delta\) 8.54 (1H, d, \(J\) 2.4, \(H_6'\)), 8.46 (1H, d, \(J\) 1.9, \(H_2'\)), 7.42 (1H, app t, \(J\) 2.1, \(H_4'\)), 7.07 (1H, d, \(J\) 3.3, \(H_{11}\)), 6.98-7.05 (2H, m, \(H_4, H_6\)), 6.62 (1H, app td, \(J\) 7.6, 0.9, \(H_5\)), 6.38 (1H, app d, \(J\) 7.9, \(H_7\)), 6.16 (1H, d, \(J\) 3.3, \(H_{10}\)), 4.72 (1H, d, \(J\) 3.5 \(H_2\)), 2.79 (1H, d, \(J\) 3.3, \(NH\)).

\(^{13}\)C (126 MHz, \(C_6D_6\)): \(\delta\) 164.5 (C8), 151.7 (C6'), 150.3 (C7a), 147.9 (C2'), 143.8 (C11), 137.6 (C4'), 133.8 (C3'), 131.5 (C4/C6), 127.4 (C3a), 126.2 (C4/C6), 121.6 (C5), 121.3 (C10), 120.8 (C5'), 119.4
(CN), 111.5 (C7), 72.0 (C2), 54.7 (C3).

HRMS (ES'):[C_{17}H_{12}N_{4}^{79}Br^{32}S]\) requires 382.9961; found 382.9963.

IR: \nu_{max} (film)/cm^{-1} 3187, 2952, 1609, 1485, 1423, 1261, 1022, 885, 748, 706.

MP: 152-155 °C.

(2RS,3RS)-1-Acetyl-2-(5-bromopyridin-3-yl)-3-(thiazol-2-yl)indoline-3-carbonitrile 192

\[
\begin{align*}
\text{Indoline 192 was prepared according to general procedure 2, using indoline 189 (52 mg, 0.14 mmol), acetyl chloride (24 µL, 0.33 mmol), and pyridine (26 µL, 0.33 mmol) in CH}_2\text{Cl}_2 (3 mL). The reaction mixture was stirred at rt for 1 h. NaHCO}_3 \text{ (saturated aq., 3 mL) was added, and the mixture was stirred at rt for a further 10 min. Purification by flash pressure column chromatography (42% EtOAc/petroleum ether) afforded indoline 192 as a pale yellow solid (55 mg, 0.13 mmol, 92%).}
\end{align*}
\]

\(^1\text{H NMR (400 MHz, CDCl}_3\): } \delta 8.43 (1H, d, J 1.3, H6'), 8.36 (1H, app br s, H7), 8.07 (1H, d, J 1.9, H2'), 7.80 (1H, d, J 3.3, H11), 7.61 (1H, app td, J 7.9, 1.2, H6), 7.49 (1H, app d, J 7.6, H4), 7.28-7.35 (2H, m, H4', H5), 7.24 (1H, d, J 3.3, H10), 6.08 (1H, br s, H2), 2.24 (3H, br s, CH}_3\).

\(^{13}\text{C NMR (100.6 MHz, CDCl}_3\): } \delta 168.0 (C=O), 162.2 (C8), 151.3 (C6'), 146.3 (C2'), 143.9 (C11), 143.3 (C7a), 136.9 (C4'), 132.4 (C6), 132.2 (C3'), 126.4 (C4), 125.8 (C5), 121.9 (C10), 120.5 (C5'), 118.6 (CN), 117.7 (C7), 71.5 (C2), 53.2 (C3), 24.2 (CH}_3\).

† 1 peak obscured.

HRMS (ES'):[C_{19}H_{14}^{79}BrN_{4}OS]\) requires 425.0066; found 425.0066.

IR: \nu_{max} (film)/cm^{-1} 3051, 2248, 1677 (C=O), 1478, 1384, 1219, 1020, 756, 704.

MP: 188-190 °C.
Indoline 193 was prepared according to general procedure 4 using indoline 192 (40 mg, 0.094 mmol), vinylstannane 57 (55 mg, 0.12 mmol), and Pd(PPh₃)₄ (14 mg, 0.012 mmol) in NMP (1.5 mL). The reaction mixture was stirred at 85 °C for 2 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 193 as a pale yellow oil (29 mg, 0.057 mmol, 60%).

¹H NMR (500 MHz, C₆D₆): δ 8.68 (1H, app br s, H7), 8.37 (1H, d, J 2.0, H6'), 8.09 (1H, app s, H2'), 7.33 (1H, d, J 3.3, H11), 7.07 (1H, app t, J 7.7, H6), 6.97 (1H, app br s, H4'), 6.93 (1H, app d, J 7.9, H4), 6.71 (1H, app td, J 7.6, 0.9, H5), 6.20 (1H, d, J 3.3, H10), 5.88 (1H, dt, J 16.0, 6.5, H14), 5.83 (1H, br s, H2), 5.78 (1H, d, J 16.0, H13), 2.32-2.44 (6H, m, H19, H21), 1.88 (2H, app q, J 6.8, H15), 1.64 (4H, m, H22), 1.38-1.55 (5H, m, H18, CH₃), 1.27 (4H, m, H16, H17).

¹³C NMR (126 MHz, C₆D₆): δ 168.5 (C=O), 163.6 (C8), 148.7 (C6'), 147.3 (C2'), 144.8 (C7a), 143.8 (C11), 134.8 (C14), 133.5 (C5'), 132.4 (C6), 131.5 (C4'), 131.1 (C3'), 126.7 (C4), 126.2 (C13), 125.6 (C5), 121.9 (C10), 119.8 (CN), 118.0 (C7), 72.7 (C2), 56.8 (C19), 54.7 (C21), 54.3 (C3), 33.7 (C15), 29.6 (C18), 29.6 (C16/C17), 27.8 (C16/C17), 24.3 (C22), 24.0 (CH₃).†

† 1 peak obscured.

HRMS (ES⁺): [C₃₀H₃₄N₅O₅]⁺ ([M+H]⁺) requires 512.2479; found 512.2478.

IR: νmax (film)/cm⁻¹ 2931, 2790, 2194, 1679 (C=O), 1478, 1386, 1351, 1024, 756.
Alkyne 164 was prepared according to a modified literature procedure.\(^{122}\) NaOH (50% w/w aq., 0.64 mL, 24 mmol) and tetrabutylammonium bromide (341 mg, 1.06 mmol) were added to a solution of 3-butyn-1-ol (0.80 mL, 11 mmol) in hexane (20 mL). \(R\)-(−)-epichlorohydrin (1.9 mL, 24 mmol) was added dropwise, and the mixture was stirred at 45 °C for 3 h. The mixture was allowed to cool to rt, diluted with Et\(_2\)O, and washed three times with H\(_2\)O, then brine. The organic layer was dried over Na\(_2\)SO\(_4\), filtered, and concentrated to afford a pale yellow liquid (1.13 g). The crude product (250 mg) was dissolved in a solution of pyrrolidine (1.65 mL, 19.8 mmol) in EtOH (10 mL), and the mixture was stirred at 100 °C for 2.5 h. The solution was allowed to cool to rt, NH\(_4\)Cl (saturated aq., 25 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed three times with H\(_2\)O, then brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated. Purification by flash pressure column chromatography afforded alkyne 164 as a yellow hygroscopic solid (165 mg, 0.84 mmol, 42%).

\(^1\)H (400 MHz, CDCl\(_3\)): \(\delta\) 3.86 (1H, app qt, \(J\) 9.6, 6.0, 3.8, \(H_7\)), 3.61 (2H, t, \(J\) 7.2, \(H_4\)), 3.52 (1H, dd, \(J\) 10.0, 3.8, \(H_6\)), 3.44 (1H, dd, \(J\) 10.0, 6.0, \(H_6'\)), 3.39 (1H, br s, \(OH\)), 2.59-2.71 (3H, m, \(H_8, H_10\)), 2.42-2.54 (4H, m, \(H_3, H_{10'}\)), 2.36 (1H, dd, \(J\) 12.1, 3.8, \(H_8'\)), 1.97 (1H, t, \(J\) 2.7, \(H_1\)), 1.70-1.82 (4H, m, \(H_{11}\)).

\(^{13}\)C (101 MHz, CDCl\(_3\)): \(\delta\) 81.2 (\(C_2\)), 73.6 (\(C_6\)), 69.4 (\(C_1\)), 69.3 (\(C_4\)), 67.9 (\(C_7\)), 58.4 (\(C_8\)), 54.1 (\(C_{10}\)), 23.5 (\(C_{11}\)), 19.7 (\(C_3\)).

HRMS (ES\(^+\)): [C\(_{11}\)H\(_{20}\)O\(_2\)N\(^+\)]\(^{+}\) ([M+H]\(^{+}\)) requires 198.1489; found 198.1490.

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3480 (br, O-H), 3300 (sharp, C-H), 2876, 2803, 1117, 1063.

\([\alpha]_D^{25.0} = -26\) (c = 0.1, CHCl\(_3\)).
Alkyne 163 was prepared according to the same procedure as 164, using NaOH (50% w/w aq., 0.64 mL, 24 mmol), tetrabutylammonium bromide (341 mg, 1.06 mmol), 3-butyne-1-ol (0.80 mL, 11 mmol), and S-(+)‐epichlorohydrin (1.9 mL, 24 mmol) in hexane (20 mL). The reaction mixture was stirred at 45 °C for 4 h. The crude product (600 mg) was dissolved in a solution of pyrrolidine (4.0 mL, 48 mmol) in EtOH (25 mL), and the mixture was stirred at 100 °C for 2 h. The solution was allowed to cool to rt, and NH₄Cl (saturated aq., 50 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed three times with H₂O, then brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography afforded alkyne 163 as a yellow hygroscopic solid (419 mg, 2.12 mmol, 45%).

The spectral and mass properties of alkyne 163 are identical to those of alkyne 164.

\[ \alpha_D^{25.0} = +19 \ (c = 0.1, \text{CHCl}_3). \]

Indoline 165 was prepared according to general procedure 5 using indoline \((R,R)-157\) (25 mg, 0.051 mmol), alkyne 163 (14 mg, 0.072 mmol), PdCl₂(PPh₃)₂ (1.8 mg, 0.0026 mmol), and Cul (< 1 mg) in diisopropylamine (1.2 mL). The reaction mixture was stirred at 70 °C for 2.5 h. Purification by
flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline **165** as a pale yellow gum (14 mg, 0.026 mmol, 51%).

**¹H NMR (500 MHz, C₆D₆, 348 K):** δ 8.79 (1H, d, J 1.7, H6'), 8.46 (1H, d, J 1.8, H2'), 8.22 (1H, app br s, H7), 7.58 (1H, app s, H4'), 7.18-7.22 (2H, m, o-Ph), 7.07 (1H, app t, J 7.9, H6), 6.95-7.05 (4H, m, H4, m-Ph, p-Ph), 6.73 (1H, app t, J 7.5, H5), 5.18 (1H, app br s, H2), 3.84 (1H, app sex, J 4.8, H14), 3.32-3.51 (4H, m, H11, H13), 2.58 (1H, dd, J 12.1, 8.7, H15), 2.45 (2H, m, H17), 2.35-2.42 (3H, m, H10, H15'), 2.31 (2H, m, H17'), 1.52 (4H, m, H18), 1.48 (3H, s, CH₃).‡

**¹³C NMR (126 MHz, C₆D₆, 348 K):** δ 168.0 (C=O), 154.4 (C6'), 147.4 (C2'), 143.7 (C7a), 141.1 (C3'/i-Ph), 136.4 (C4'), 133.9 (C3'/i-Ph), 131.3 (C6), 130.2 (C4/m-Ph/p-Ph), 129.7 (C3a), 129.4 (C4/m-Ph/p-Ph), 126.4 (C4/m-Ph/p-Ph), 126.3 (o-Ph), 126.0 (C5), 122.2 (C5'/CN), 119.0 (C5'/CN), 117.6 (C7), 92.9 (C8/C9), 78.8 (C8/C9), 74.7 (C11/C13), 74.5 (C2), 70.0 (C11/C13), 69.1 (C14), 59.7 (C15), 58.0 (C3), 54.9 (C17), 24.4 (C18), 23.7 (CH₃), 21.6 (C10).

‡ OH signal not observed.

**HRMS (ES⁺):** [C₃₃H₃₅O₃N₄⁺ ([M+H]⁺)] requires 535.2704; found 535.2706.

**IR:** ν_{max} (film)/cm⁻¹ 3412 (br, O-H), 2934, 2882, 2249, 1676 (C=O), 1479, 1387, 1119, 758, 698.

[α]_{D}^{25.0} = -74 (c = 0.1, CHCl₃).
(2R,3R)-1-Acetyl-2-(5-((R)-2-hydroxy-3-(pyrrolidin-1-yl)propoxy)-but-1-yn-1-yl)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 167

Indoline 167 was prepared according to general procedure 5 using indoline (R,R)-157 (25 mg, 0.051 mmol), alkyne 164 (14 mg, 0.072 mmol), PdCl₂(PPh₃)₂ (1.8 mg, 0.0026 mmol), and Cul (< 1 mg) in diisopropylamine (1.2 mL). The reaction mixture was stirred at 70 °C for 1.5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 167 as a pale yellow gum (17 mg, 0.032 mmol, 62%).

¹H NMR (500 MHz, C₆D₆, 348 K): δ 8.80 (1H, d, J 1.8, H₆'), 8.45 (1H, d, J 2.3, H₂'), 8.22 (1H, app br s, H₇), 7.58 (1H, app t, J 1.9, H₄'), 7.16-7.25 (2H, m, o-Ph), 7.06 (1H, app t, J 7.8, H₆), 6.94-7.04 (4H, m, H₄, m-Ph, p-Ph), 6.72 (1H, app t, J 7.6, H₅), 5.17 (1H, app br s, H₂), 3.83 (1H, app sex, J 4.6, H₁4), 3.34-3.49 (4H, m, H₁₁, H₁₃), 2.58 (1H, dd, J 12.1, 8.7, H₁₅), 2.44 (2H, m, H₁₇), 2.34-2.41 (3H, m, H₁₀, H₁₅'), 2.26-2.34 (2H, m, H₁₇'), 1.51 (4H, m, H₁₈), 1.46 (3H, s, CH₃). ‡

¹³C NMR (126 MHz, C₆D₆, 348 K): δ 168.0 (C=O), 154.3 (C₆'), 147.4 (C₂'), 143.7 (C₇a), 141.1 (C₃'/i-Ph), 136.3 (C₄'), 133.8 (C₃'/i-Ph), 131.2 (C₆), 130.2 (C₄/m-Ph/p-Ph), 129.7 (C₃a), 129.3 (C₄/m-Ph/p-Ph), 126.4 (C₄/m-Ph/p-Ph), 126.2 (o-Ph), 126.0 (C₅), 122.2 (C₅'/CN), 118.9 (C₅'/CN), 117.6 (C₇), 92.9 (C₈/C₉), 78.8 (C₈/C₉), 74.6 (C₁₁/C₁₃), 74.5 (C₂), 70.0 (C₁₁/C₁₃), 69.0 (C₁₄), 59.7 (C₁₅), 58.0 (C₃), 54.8 (C₁₇), 24.4 (C₁₈), 23.6 (CH₃), 21.6 (C₁₀). ‡ OH peak not observed.


IR: νₘₐₓ (film)/cm⁻¹ 3415 (br, O-H), 2923, 2247, 1676 (C=O), 1479, 1385, 1275, 1117, 1024, 750, 696.

[α]D²⁵ = +52 (c = 0.1, CHCl₃).
(2S,3S)-1-Acetyl-2-{5-[(4S)-2-hydroxy-3-(pyrrolidin-1-yl)propoxy]but-1-yn-1-yl}pyridin-3-yl)-3-phenylindoline-3-carbonitrile 169

Indoline 169 was prepared according to general procedure 5 using indoline (5S)-157 (60 mg, 0.12 mmol), alkyne 105 (26 mg, 0.14 mmol), PdCl₂(PPh₃)₂ (4.3 mg, 0.0060 mmol), and CuI (< 1 mg) in diisopropylamine (2.5 mL). The reaction mixture was stirred at 70 °C for 2 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 169 as a pale yellow gum (42 mg, 0.079 mmol, 64%).

The spectral and mass properties of indoline 169 are identical to those of indoline 167.

[α]₀²₅.₀ = -60 (c = 0.1, CHCl₃).

(2S,3S)-1-Acetyl-2-{5-[(4S)-2-hydroxy-3-(pyrrolidin-1-yl)propoxy]but-1-yn-1-yl}pyridin-3-yl)-3-phenylindoline-3-carbonitrile 171

Indoline 171 was prepared according to general procedure 5 using indoline (5S)-157 (60 mg, 0.12 mmol), alkyne 164 (26 mg, 0.14 mmol), PdCl₂(PPh₃)₂ (4.3 mg, 0.0060 mmol), and CuI (< 1 mg) in diisopropylamine (2.5 mL). The reaction mixture was stirred at 70 °C for 2 h. Purification by flash
pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 171 as a pale yellow gum (42 mg, 0.079 mmol, 64%).

The spectral and mass properties of indoline 171 are identical to those of indoline 165.

\[ \alpha \rmd^{25} = -85 \, (c = 0.1, \text{CHCl}_3) \].

(2R,3R)-1-Acetyl-2-(5-(4-((S)-2-hydroxy-3-(pyrrolidin-1-yl)propoxy)butyl)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 166

Indoline 166 was prepared according to general procedure 3 using indoline 165 (12 mg, 0.022 mmol) and Pd/C (wet degussa type, 2 mg) in MeOH (1.2 mL). The reaction mixture was stirred at rt under a H₂ atmosphere for 3 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 166 as a pale yellow oil (8.2 mg, 0.015 mmol, 69%).

\( ^{1}H \) NMR (500 MHz, C₆D₆, 348 K): \( \delta \) 8.49 (1H, d, \( J 1.8, H6' \)), 8.45 (1H, d, \( J 1.8, H2' \)), 8.41 (1H, app br s, \( H7 \)), 7.21-7.30 (3H, m, \( H4', o-\text{Ph} \)), 7.17 (1H, app td, \( J 7.8, 1.2, H6 \)), 7.08 (1H, app d, \( J 7.8, H4 \)), 6.96-7.05 (3H, m, \( m-\text{Ph}, o-\text{Ph} \)), 6.78 (1H, app td, \( J 7.5, 0.9, H5 \)), 5.22 (1H, s, \( H2 \)), 3.89 (1H, app sex, \( J 4.4, H14 \)), 3.42 (2H, m, \( H13, H13' \)), 3.26 (2H, app td, \( J 6.5, 2.5, H11 \)), 2.61 (1H, dd, \( J 12.2, 8.6, H15 \)), 2.45-2.52 (2H, m, \( H17 \)), 2.43 (1H, dd, \( J 12.2, 4.4, H15' \)), 2.34 (2H, m, \( H17' \)), 2.23 (2H, t, \( J 7.4, H8 \)), 1.49-1.57 (7H, m, \( H18, CH₃ \)), 1.45 (2H, app quin, \( J 7.4, H9 \)), 1.37 (2H, app quin, \( J 7.1, H10 \)).

\( ^{13}C \) NMR (126 MHz, C₆D₆, 348 K): \( \delta \) 168.3 (C=O), 152.3 (C₆′), 146.6 (C₂'), 144.0 (C₇a), 141.3, 138.8, 133.7, 133.6 (C₄'), 131.2 (C₆), 130.1 (m-Ph/p-Ph), 129.3 (m-Ph/p-Ph), 126.4 (C₄), 126.2 (o-Ph), 125.9 (C₅), 119.0 (CN), 117.7 (C₇), 74.9 (C₂), 74.6 (C₁₃), 71.6 (C₁₁), 69.1 (C₁₄), 59.9 (C₁₅), 58.3 (C₃), 54.8
(C17), 32.9 (C8), 29.6 (C10), 27.9 (C9), 24.4 (C18), 23.7 (CH₃).²

‡ OH peak not observed.

‡ 1 peak obscured.

HRMS (ES⁺): [C₃₃H₃₉O₃N₄]⁺ ([M+H]⁺) requires 539.3017; found 539.3010.

IR: νₘₐₓ (film)/cm⁻¹ 3418 (br, O-H), 2927, 2247, 1678 (C=O), 1479, 1385, 1275, 1119, 750, 706.

[α]Ｄ²⁵ = +52 (c = 0.1, CHCl₃).

(2R,3R)-1-Acetyl-2-[(5-((R)-2-hydroxy-3-(pyrrolidin-1-yl)propoxy)butyl)pyridin-3-yl]-3-phenylindoline-3-carbonitrile 168

Indoline 168 was prepared according to general procedure 3 using indoline 167 (13 mg, 0.024 mmol) and Pd/C (wet degussa type, 2 mg) in MeOH (1.2 mL). The reaction mixture was stirred at rt under a H₂ atmosphere for 2.5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 168 as a pale yellow oil (8.3 mg, 0.015 mmol, 64%).

¹H NMR (500 MHz, CD₆D₆, 348 K): δ 8.49 (1H, d, J 2.0, H6'), 8.45 (1H, d, J 2.1, H2'), 8.41 (1H, app br s, H7), 7.22-7.30 (3H, m, H4’, o-Ph), 7.14 (1H, app t, J 8.2, H6), 7.08 (1H, app d, J 7.4, H4), 6.96-7.05 (3H, m, m-Ph, p-Ph), 6.79 (1H, app td, J 7.6, 0.9, H5), 5.23 (1H, s, H2), 3.89 (1H, app sex, J 4.5, H14), 3.42 (2H, m, H13, H13’), 3.26 (2H, app t, J 6.3, H11), 2.61 (1H, dd, J 12.0, 8.6, H15), 2.39-2.52 (3H, m, H15’, H17), 2.35 (2H, m, H17’), 2.23 (2H, t, J 7.4, H8), 1.49-1.59 (7H, m, H18, CH₃), 1.45 (2H, app quin, J 7.3, H9), 1.37 (2H, app quin, J 7.3, H10).¹
$^{13}$C NMR (126 MHz, C$_6$D$_6$, 348 K): δ 168.3 (C=O), 152.3 (C6'), 146.7 (C2'), 144.1 (C7a), 141.3, 138.9, 133.8, 133.6 (C4'), 131.3 (C6), 130.2 (m-Ph, p-Ph), 129.9, 129.3 (m-Ph, p-Ph), 126.4 (C4), 126.3 (o-Ph), 125.9 (C5), 119.1 (CN), 117.7 (C7), 74.9 (C2), 74.6 (C13), 71.7 (C11), 69.2 (C14), 59.9 (C15), 58.4 (C3), 54.9 (C17), 33.0 (C8), 29.7 (C10), 28.0 (C9), 24.5 (C18), 23.8 (CH$_3$).

‡ OH peak not observed.

HRMS (ES$^+$): [C$_{33}$H$_{39}$O$_3$N$_4$]$^+$ ([M+H]$^+$) requires 539.3017; found 539.3010.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3422 (br, O-H), 2947, 2246, 1676 (C=O), 1479, 1387, 1117, 751, 698.

$[^{[\alpha}_{D}^{25.0}$ = +55 (c = 0.1, CHCl$_3$).

(2S,3S)-1-Acetyl-2-(5-((4-((S)-2-hydroxy-3-(pyrrolidin-1-yl)propoxy)butyl)pyridin-3-yl)-3-phenylindoline-3-carbonitrile 170

Indoline 170 was prepared according to general procedure 3 using indoline 169 (22 mg, 0.041 mmol) and Pd/C (wet degussa type, 2.5 mg) in MeOH (1.8 mL). The reaction mixture was stirred at rt under a H$_2$ atmosphere for 2.5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded indoline 170 as a pale yellow oil (15 mg, 0.028 mmol, 68%).

The spectral and mass properties of indoline 170 are identical to those of indoline 168.

$[^{[\alpha}_{D}^{25.0}$ = -47 (c = 0.1, CHCl$_3$).
Indoline 172 was prepared according to general procedure 3 using indoline 171 (30 mg, 0.056 mmol) and Pd/C (wet degussa, 3 mg) in MeOH (1.8 mL). The reaction mixture was stirred at rt under a hydrogen atmosphere for 2 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 172 as a pale yellow oil (22 mg, 0.041 mmol, 73%).

The spectral and mass properties of indoline 172 are identical to those of indoline 166.

[α]_D^{25.0} = -52 (c = 0.1, CHCl₃).

Indoline 198 was prepared according to general procedure 2, using indoline 50 (60 mg, 0.16 mmol), benzyloxyacetyl chloride (38 µL, 0.24 mmol), and pyridine (19 µL, 0.24 mmol) in CH₂Cl₂ (5 mL). The reaction mixture was stirred at rt for 2 h. Purification by flash pressure column chromatography (25% EtOAc/petroleum ether) afforded indoline 198 as a colourless paste (62 mg, 0.12 mmol, 74%).

¹H NMR (500 MHz, C₆D₆, 348 K): δ 8.54 (1H, d, J = 1.8, H6'), 8.39 (1H, d, J = 1.5, H₂'), 8.31 (1H, app br s, H7), 7.50 (1H, app s, H4'), 7.12-7.25 (2H, obscured, Ph), 7.00-7.10 (5H, m, H4, H6, Ph), 6.91-6.99
(3H, m, Ph), 6.83-6.91 (2H, m, Ph), 6.74 (1H, app t, J 7.5, H5), 5.68 (1H, s, H2), 4.10 (1H, d, J 11.7, H9), 3.96 (1H, d, J 11.7, H9'), 3.64 (2H, s, OCH2Ph).

13C NMR (126 MHz, C6D6, 348 K): δ 167.5 (C=O), 152.8 (C6'), 147.0 (C2'), 143.4 (C7a), 140.5, 137.4, 136.8 (C4'), 136.0, 131.4 (C6), 130.1 (Ph), 129.6, 129.4 (Ph), 129.0 (Ph), 128.9 (Ph), 128.5 (Ph), 126.7 (C5), 126.4 (C4), 126.3 (Ph), 121.7 (C5'), 118.8 (CN), 118.0 (C7), 73.7 (C9), 72.5 (C2), 71.2 (OCH2Ph), 58.2 (C3).


IR: νmax (film)/cm⁻¹: 3079, 2896, 1767 (C=O), 1599, 1479, 1393, 1099, 1020, 750, 696.

(2RS,3RS)-1-(2-(Benzyloxy)acetyl)-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)pyridin-3-yl)indoline-3-carbonitrile 199

Indoline 199 was prepared according to general procedure 5, using indoline 198 (45 mg, 0.086 mmol), alkyne 105 (16 mg, 0.094 mmol), PdCl2(PPh3)2 (3 mg, 0.004 mmol), and Cul (< 1 mg) in N,N-diisopropylamine (2 mL). The reaction mixture was stirred at 70 °C for 5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 199 as a yellow oil (15 mg, 0.025 mmol, 29%).

1H NMR (500 MHz, C6D6, 348 K): δ 8.79 (1H, d, J 1.8, H6'), 8.45 (1H, d, J 2.1, H2'), 8.39 (1H, d, J 7.8, H7), 7.57 (1H, app t, J 2.0, H4'), 7.16-7.23 (4H, m, Ph), 7.02-7.12 (4H, m, H4, H6, Ph), 6.85-6.98 (4H, m, Ph), 6.74 (1H, app td, J 7.8, 0.8, H5), 5.77 (1H, s, H2), 4.13 (1H, d, J 11.7, H9), 3.99 (1H, d, J 11.7, H9'), 3.67 (2H, d, J 13.6, OCH2Ph), 2.40 (4H, m, H20), 2.34 (2H, t, J 6.9, H18), 2.12 (2H, t, J 6.7,
$^1$H NMR (400 MHz, CDCl$_3$): δ 4.19 (2H, s, CH$_2$), 0.93 (9H, s, Si(CH$_3$)$_3$), 0.92 (9H, s, Si(CH$_3$)$_3$), 0.29 (6H, s, Si(CH$_3$)$_3$), 0.11 (6H, s, Si(CH$_3$)$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$): δ 171.9 (C=O), 62.3 (CH$_2$), 25.8 (Si(CH$_3$)$_3$), 25.5 (Si(CH$_3$)$_3$), 17.7 (Si(CH$_3$)$_3$), 3.6 (Si(CH$_3$)$_3$), -4.8 (Si(CH$_3$)$_3$), -5.5 (Si(CH$_3$)$_3$).

Tert-butyldimethylsilyl 2-((tert-butyldimethylsilyloxy)acetate 202

Ester 202 was prepared according to a literature procedure. Imidazole (3.76 g, 55.2 mmol) was added to a solution of glycolic acid (1.0 g, 13 mmol) and tert-butyldimethylchlorosilane (4.16 g, 27.6 mmol) in N,N-DMF (15 mL) and stirred at rt for 18 h. The reaction mixture was poured onto H$_2$O and extracted with petroleum ether. The combined organic extracts were washed with NaHCO$_3$ (saturated aq.), then brine, dried over Na$_2$SO$_4$, filtered, and concentrated to afford ester 202 as a colourless liquid (2.14 g, 7.04 mmol, 54%). The spectral data matched those reported in the literature.
(2RS,3RS)-2-(5-Bromopyridin-3-yl)-1-(2-((tert-butyldimethylsilyl)oxy)acetyl)-3-phenylindoline-3-carbonitrile 203

1 drop of DMF was added to a solution of ester 202 (0.50 g, 1.6 mmol) in CH$_2$Cl$_2$ (5 mL) at 0 °C. Oxalyl chloride (0.13 mL, 1.5 mmol) was added dropwise, and the mixture was stirred at 0 °C for 1 h and allowed to warm to rt. The reaction mixture was transferred to a solution of indoline 50 (80 mg, 0.21 mmol) and pyridine (0.10 mL, 1.3 mmol) in CH$_2$Cl$_2$ (5 mL) via cannula and stirred at 0 °C for 15 min. NaHCO$_3$ (saturated aq., 15 mL) was added, and the mixture was extracted with CH$_2$Cl$_2$. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (10-15% EtOAc/petroleum ether) afforded indoline 203 as a colorless paste (58 mg, 0.11 mmol, 50%).

$^1$H NMR (500 MHz, C$_6$D$_6$, 348 K): δ 8.59 (1H, d, J 2.0, H6'), 8.54 (1H, d, J 1.3, H2'), 8.28 (1H, app d, J 7.6, H7), 7.65 (1H, app t, J 2.0, H4'), 7.12-7.20 (2H, obscured, Ph), 7.07 (1H, app t, J 7.9, H6), 6.94-7.05 (4H, m, H4, Ph), 6.74 (1H, app t, J 7.5, H5), 5.77 (1H, s, H2), 3.93 (1H, d, J 13.9, H9), 3.83 (1H, d, J 13.9, H9'), 0.73 (9H, s, SiC(CH$_3$)$_3$), -0.14 (3H, s, Si(CH$_3$)$_3$), -0.15 (3H, s, Si(CH$_3$)$_3$).

$^{13}$C NMR (126 MHz, C$_6$D$_6$, 348 K): δ 168.8 (C=O), 152.9 (C6'), 147.2 (C2'), 143.5, 140.7, 136.8 (C4'), 136.1, 131.4 (C6), 130.2 (C4/Ph), 129.5, 129.4 (C4/Ph), 126.5 (C5), 126.5 (C4/Ph), 126.3 (Ph), 121.8 (C5/CN), 118.9 (C5/CN), 117.8 (C7), 72.3 (C2), 65.8 (C9), 58.2 (C3), 26.2 (SiC(CH$_3$)$_3$), 18.7 (SiC(CH$_3$)$_3$), -5.1 (SiCH$_3$), -5.2 (SiCH$_3$').

HRMS (ES+): [C$_{28}$H$_{31}$O$_2$N$_3$-79Br-28Si] + ([M+H]+) requires 548.1363; found 548.1364.

IR: $\nu_{\text{max}}$(film)/cm$^{-1}$ 2953, 2857, 1676 (C=O), 1479, 1391, 1256, 1099, 837, 781.
(2RS,3RS)-1-(2-((Tert-butyldimethylsilyl)oxy)acetyl)-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)pyridin-3-yl)indoline-3-carbonitrile 205

Indoline 203 (48 mg, 0.088 mmol), PdCl$_2$(PPh$_3$)$_2$ (6 mg, 0.009 mmol), and Cul (< 1 mg) were degassed 3 times in a Schlenk tube with Ar. A degassed solution of alkyne 105 (22 mg, 0.13 mmol) and triethylamine (30 µL, 0.22 mmol) in toluene (2 mL) was added, and the reaction mixture was stirred at 70 °C for 3.5 h. The reaction mixture was allowed to cool to rt, filtered through Celite™ and eluted with EtOAc. The filtrate was washed 3 times with H$_2$O, then brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (4.5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded indoline 205 as a pale yellow paste (21 mg, 0.033 mmol, 38%).

$^1$H NMR (500 MHz, C$_6$D$_6$): $\delta$ 8.88 (1H, d, $J$ 2.0, H$6'$), 8.63 (1H, d, $J$ 2.2, H$2'$), 8.47 (1H, app br s, H$7$), 7.73 (1H, app t, $J$ 2.1, H$4'$), 7.17-7.20 (2H, m, Ph), 7.03 (1H, app td, $J$ 7.9, 1.4, H$6$), 6.97 (1H, app dd, $J$ 7.6, 0.7, H$4$), 6.89-6.96 (3H, m, Ph), 6.67 (1H, app td, $J$ 7.6, 1.0, H$5$), 5.83 (1H, s, H$2$), 3.87 (1H, d, $J$ 14.0, H$9$), 3.75 (1H, d, $J$ 14.0, H$9'$), 2.34-2.42 (4H, m, H18), 2.30 (2H, t, $J$ 7.1, H16), 2.06 (2H, t, $J$ 6.7, H12), 1.63 (4H, m, H19), 1.26-1.43 (6H, m, H13, H14, H15), 0.72 (9H, s, Si(CH$_3$)$_3$), -0.15 (3H, s, Si(CH$_3$)$_2$), -0.16 (3H, s, Si(CH$_3$)$_2$).

$^{13}$C NMR (126 MHz, C$_6$D$_6$): $\delta$ 168.9 (C=O), 154.3 (C6’), 147.3 (C2’), 143.7, 140.7, 136.1 (C4’), 134.0, 131.3 (C6), 130.1 (Ph), 129.3 (Ph), 128.9, 126.5 (C5), 126.4 (C4), 126.3 (Ph), 122.6 (C5’), 119.1 (CN), 117.8 (C7), 96.0 (C10/C11), 77.9 (C10/C11), 72.2 (C2), 65.7 (C9), 58.2 (C3), 56.6 (C16), 54.6 (C18), 29.1 (C13/C14/C15), 28.9 (C13/C14/C15), 27.4 (C13/C14/C15), 26.2 (Si(CH$_3$)$_3$), 24.3 (C19), 20.0 (C12), 18.6 (Si(CH$_3$)$_3$), -5.11 (Si(CH$_3$)$_2$), -5.34 (Si(CH$_3$)$^+$).

HRMS (ES$^+$): [C$_{39}$H$_{49}$N$_4$O$_2$Si$^{28}$]$^+$ ([M+H]$^+$) requires 633.3625; found 633.2627.
IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3059, 2920, 2263, 2268, 1684 (C=O), 1482, 1389, 1281, 1226, 1215, 758.

(2RS,3RS)-2-(5-Bromopyridin-3-yl)-1-(2-cyanoacetyl)-3-phenylindoline-3-carbonitrile 194

1 drop of DMF was added to a solution of cyanoacetic acid (200 mg, 2.35 mmol) in CH$_2$Cl$_2$ (8 mL), and the mixture was cooled to 0 °C. Oxalyl chloride (0.18 mL, 2.1 mmol) was added dropwise and the mixture was stirred at 0 °C for 1 h. 4 mL of the reaction mixture was transferred to a solution of indoline 50 (60 mg, 0.16 mmol) in CH$_2$Cl$_2$ (4 mL) at 0 °C, and the mixture was stirred at 0 °C for 25 min. NaHCO$_3$ (sat aq., 10 mL) was added, and the mixture was extracted with DCM. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (30% EtOAc/Petroleum ether) afforded indoline 194 as a colorless solid (56 mg, 0.13 mmol, 79%).

$^1$H NMR (500 MHz, C$_6$D$_6$, 348 K): $\delta$ 8.55 (1H, d, $J$ 1.9, H6'), 8.28 (1H, d, $J$ 1.6, H2'), 7.84 (1H, app br s, H7), 7.42 (1H, app s, H4'), 7.08 (2H, m, Ph), 6.95-7.04 (4H, m, H6, Ph), 6.93 (1H, app d, $J$ 7.5, H4), 6.70 (1H, app t, $J$ 7.6, H5), 5.01 (1H, s, H2), 2.30 (1H, d, $J$ 18.5, H9), 2.24 (1H, d, $J$ 18.5, H9').

$^{13}$C NMR (125.75 MHz, C$_6$D$_6$, 348 K): $\delta$ 160.0 (C=O), 153.5 (C6'), 146.7 (C2'), 142.2, 139.9, 136.6 (C4'), 134.5, 131.5 (C6/Ph), 130.4 (C6/Ph), 129.9 (C6/Ph), 127.3 (C5), 126.7 (C4), 126.1 (Ph), 122.0 (C5'), 118.4 (CN), 117.5 (C7), 112.5 (CN), 73.6 (C2), 58.2 (C3), 26.6 (C9).

† 1 peak obscured.

HRMS (ES$^+$/): [C$_{23}$H$_{16}$BrN$_4$O]$^+$ ([M+H]$^+$) requires 443.0502; found 443.0502.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3059, 2920, 2263, 2268, 1684 (C=O), 1482, 1389, 1281, 1226, 1215, 758.

MP: 93-95 °C.
(2RS,3RS)-1-(2-Cyanoacetyl)-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)pyridin-3-yl)indoline-3-carbonitrile 195

Indoline 195 was prepared according to general procedure 5 using indoline 194 (40 mg, 0.090 mmol), alkyne 105 (16 mg, 0.099 mmol), PdCl₂(PPh₃)₂ (3.2 mg, 0.0045 mmol), and CuI (< 1 mg) in diisopropylamine (2 mL). The reaction mixture was stirred at 70 °C for 4.5 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 195 as a colorless oil (19 mg, 0.036 mmol, 40%, contains impurities).

¹H NMR (500 MHz, C₆D₆, 348 K): δ 8.80 (1H, d, J 1.5, H6′), 8.34 (1H, d, J 1.8, H2′), 8.01 (1H, app br s, H7), 7.47 (1H, app s, H4′), 7.12 (2H, m, Ph), 6.93-7.08 (5H, m, H4, H6, Ph), 6.72 (1H, app t, J 7.5, H5), 5.06 (1H, br s, H2), 2.40 (4H, m, H18), 2.28-2.37 (4H, m, H9, H16), 2.14 (2H, t, J 6.6, H12), 1.64 (4H, m, H19), 1.34-1.50 (6H, m, H13, H14, H15).

¹³C NMR (126 MHz, C₆D₆, 348 K): δ 160.2 (C=O), 154.9 (C6′), 146.8 (C2′), 142.6, 140.3, 136.0 (C4′), 132.5, 131.5 (C4/C6/Ph), 130.4 (C4/C6/Ph), 129.7 (C4/C6/Ph), 127.2 (C5), 126.6 (C4/C6/Ph), 126.2 (Ph), 122.9 (C5′), 118.5 (CN), 117.7 (C7), 112.7 (CN), 96.6 (C10/C11), 77.8 (C10/C11), 74.1 (C2), 58.4 (C3), 56.6 (C9/C16), 54.7 (C18), 29.2 (C13/C14/C15), 29.0 (C13/C14/C15), 27.6 (C9/C16), 26.7 (C13/C14/C15), 24.5 (C19), 20.1 (C12). ‡

‡ 1 peak obscured.

HRMS (ES⁺): [C₃₄H₃₄N₅O⁺]⁺ ([M+H]⁺) requires 528.2763; found 528.2762.

IR: v max (film)/cm⁻¹ 3026, 2934, 2261, 1677 (C=O), 1480, 1394, 756, 698.
(2RS,3RS)-1-(2-Cyanoacetyl)-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)heptyl)pyridin-3-yl)indoline-3-carbonitrile 196

Indoline 196 was prepared according to general procedure 3 using indoline 195 (16 mg, 0.030 mmol) and Pd/C (wet degussa type, 2 mg) in MeOH (2 mL). The reaction mixture was stirred at rt under a H2 atmosphere for 4 h. Purification by flash pressure column chromatography (4.5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 196 as a colorless oil (14 mg, 0.026 mmol, 87%, contains minor impurities).

1H NMR (500 MHz, C6D6, 348 K): δ 8.49 (1H, app s, H6'), 8.35 (1H, app s, H2'), 8.23 (1H, app br s, H7), 7.09 (1H, app t, J 7.9, H6), 6.97-7.06 (4H, m, H4, Ph), 6.78 (1H, app t, J 7.6, H5), 5.12 (1H, br s, H2), 2.37-2.49 (8H, m, H9, H16, H18), 2.21 (2H, t, J 7.6, H10), 1.65 (4H, m, H19), 1.50 (2H, app quin, J 7.4, H15), 1.27-1.41 (4H, m, H11, H14), 1.19 (2H, app quin, J 7.5, H13), 1.12 (2H, app quin, J 7.5, H12).†

13C NMR (126 MHz, C6D6, 348 K): δ 160.4 (C=O), 153.0 (C6'), 146.3 (C2'), 143.0, 140.5, 139.5, 133.3 (C4'), 132.5, 131.5 (C6), 130.4 (C4/Ph), 129.7 (C4/Ph), 127.1 (C5), 126.6 (C4/Ph), 126.3 (Ph), 118.6 (CN), 117.8 (C7), 112.8 (CN), 74.5 (C2), 58.7 (C3), 56.9 (C16), 54.7 (C18), 33.2 (C10), 31.1 (C11/C14), 29.9 (C13), 29.8 (C15), 29.5 (C12), 28.2 (C11/C14), 26.8 (C9), 24.5 (C19).‡

† 3 peaks obscured.
‡ 1 peak obscured.

HRMS (ES+): [C34H38N5O]+ ([M+H]+) requires 532.3071; found 532.3073.

IR: vmax (film)/cm⁻¹ 3028, 2935, 2262, 1677 (C=O), 1480, 1461, 1393, 755, 698.
2-(1,3-Dioxoisooindolin-2-yl)acetyl chloride 209

Acyl chloride 209 was prepared according to a literature procedure.\(^{197}\) DMF (1 drop) was added to a suspension of N-phthaloylglycine (1.00 g, 4.87 mmol) in CH\(_2\)Cl\(_2\) (35 mL) and cooled to 0 °C. Oxalyl chloride (0.82 mL, 9.8 mmol) was added dropwise, and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was concentrated at rt to afford acyl chloride 209 as a yellow solid (1.12 g, 5.01 mmol, quant). The spectral data matched those reported in the literature.

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 7.88 \) (2H, m, Ar), 7.75 (2H, m, Ar), 4.79 (2H, s, CH\(_2\)).

\(^{13}\)C NMR (101 MHz, CDCl\(_3\)): \(\delta 169.2 \) (C=O), 166.7 (C=O), 134.8 (Ar), 131.7 (Ar), 124.1 (Ar), 47.7 (CH\(_2\)).

\((2R,3R)-2\)-(5-Bromopyridin-3-yl)-1-(2-(1,3-dioxoisooindolin-2-yl)acetyl)-3-phenylindoline-3-carbonitrile 210

Indoline 210 was prepared according to general procedure 2, using indoline 50 (80 mg, 0.21 mmol), acyl chloride 209 (119 mg, 0.53 mmol), and pyridine (43 \(\mu\)L, 0.53 mmol) in CH\(_2\)Cl\(_2\) (6.5 mL). The reaction mixture was stirred at rt for 16 h. Purification by flash pressure column chromatography (30-35% EtOAc/petroleum ether) afforded indoline 210 as a colorless solid (75 mg, 0.13 mmol, 63%).

\(^1\)H NMR (400 MHz, dmso-D\(_6\)): \(\delta 8.79 \) (1H, d, \(J 1.9\), H\('6\)'), 8.57 (1H, d, \(J 1.6\), H\('2\)'), 8.11 (1H, app s, H\('7\)'), 7.82-7.93 (4H, m, H13, H14), 7.60 (1H, app t, \(J 7.8\), H6), 7.50-7.57 (2H, m,
$^1$H NMR (400 MHz, C$_6$D$_6$, 348 K): $\delta$ 8.81 (1H, app s, H6'), 8.52 (1H, app s, H2'), 7.99 (1H, app br s, Ph), 7.43-7.50 (2H, m, H4, Ph), 7.32-7.42 (3H, m, H5, Ph), 6.22 (1H, s, H2), 4.92 (1H, d, J 17.2, H9), 4.14 (1H, d, J 17.2, H9').

$^{13}$C NMR (101 MHz, dms-DM): $\delta$ 166.5 (C=O), 164.3 (C=O), 150.9 (C6'), 145.8 (C2'), 140.9, 138.1, 136.4 (C4'), 134.4, 134.2 (C13/C14), 131.1, 130.6 (C6), 129.1 (Ph), 128.6 (C4/Ph), 128.4, 127.7, 125.8 (C5/Ph), 125.3 (C4/Ph), 125.1 (C5/Ph), 122.8 (C13/C14), 119.8 (C5'/CN), 117.8 (C5'/CN), 116.4 (C7), 69.5 (C2), 56.4 (C3).

HRMS (ES$^+$): [C$_{30}$H$_{20}$O$_3$N$_4$Br]$^+$ ([M+H]$^+$) requires 563.0713; found 563.0714.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2980, 1720, 1688, 1482, 1424, 1389, 733, 715.

MP: 250-252 °C.

(2RS,3RS)-1-(2,1,3-Dioxoisindolin-2-yl)acetyl)-3-phenyl-2-(5-(pyrrolidin-1-yl)hept-1-yn-1-yl)pyridin-3-yl)indoline-3-carbonitrile 211

Indoline 211 was prepared according to general procedure 5 using indoline 210 (62 mg, 0.11 mmol), alkyne 105 (23 mg, 0.14 mmol), Pd(Ph$_3$)$_2$Cl$_2$ (4 mg, 0.006 mmol), and Cul (approx. 1 mg) in N,N-diisopropylamine (2 mL). The reaction mixture was stirred at 70 °C for 3 h. Purification by flash pressure column chromatography (4.5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded indoline 211 as a pale yellow oil (34 mg, 0.053 mmol, 48%).
$7, 7.37-7.50 (2H, m, H13/H14), 7.29 (2H, app d, J 7.4, Ph), 2.33 (2H, t, J 7.0, H21), 2.13 (2H, t, J 6.7, H17), 1.62 (4H, m, H24), 1.32-1.51 (6H, m, H18, H19, H20).

$13$C NMR (101 MHz, C$_6$D$_6$, 348 K): δ 167.5 (C=O), 164.9 (C=O), 154.6 (C6'), 147.2 (C2'), 142.9, 140.7, 136.4 (C4'), 134.0 (C13/C14), 133.1, 131.2 (C4/C6/Ph), 130.3 (Ph), 129.5 (C4/C6/Ph), 126.6 (C5), 126.3 (C4/C6/Ph), 126.1 (Ph), 123.7 (C13/C14), 122.7 (C5'/CN), 118.7 (C5'/CN), 117.9 (C7), 95.9 (C15/C16), 78.2 (C15/C16), 73.7 (C2), 58.6 (C3), 56.6 (C21), 54.6 (C23), 41.1 (C9), 29.0 (C18/C19/C20), 29.0 (C18/C19/C20), 27.5 (C18/C19/C20), 24.4 (C24), 20.1 (C17).‡

‡ 2 peaks obscured.

HRMS (ES$^+$): [C$_{41}$H$_{38}$N$_5$O$_3$]$^+$ ([M+H]$^+$) requires 648.2969; found 648.2963.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2938, 2245, 1721, 1686, 1595, 1481, 1422, 1389, 954, 715.

(2RS,3RS)-1-(2-(1,3-Dioxoisindolin-2-yl)acetyl)-3-phenyl-2-(5-(7-(pyrrolidin-1-yl)heptyl)pyridin-3-yllindoline-3-carbonitrile **212**

Indoline **212** was prepared according to general procedure 3, using indoline **211** (27 mg, 0.042 mmol) and Pd/C (wet degussa type, 3 mg) in MeOH (2.5 mL). The reaction mixture was stirred at rt under a H$_2$ atmosphere for 24 h. Purification by flash pressure column chromatography (4.5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded indoline **212** as a colorless oil (17 mg, 0.026 mmol,
62%).

$^1$H NMR (400 MHz, CD$_6$D$_6$, 348 K): δ 8.47-8.58 (2H, m, $H2'$, $H6'$), 8.27 (1H, app br s, $H7$), 7.36-7.45 (3H, m, $H4'$, $H13$/$H14$), 7.32 (2H, app d, $J$ 7.6, Ph), 6.98-7.12 (5H, m, $H4$, $H6$, Ph), 6.88-6.96 (2H, m, $H13$/$H14$), 6.76 (1H, app t, $J$ 6.9, $H5$), 2.36-2.49 (6H, m, $H21$, $H23$), 2.28 (2H, t, $J$ 6.9, $H15$), 1.64 (4H, m, $H16$, $H20$), 1.28-1.39 (2H, app quin, $J$ 7.3, $H19$), 1.13-1.28 (4H, m, $H17$, $H18$).

$^{13}$C NMR (101 MHz, CD$_6$D$_6$, 348 K): δ 167.5 ($C=O$), 165.1 ($C=O$), 152.6 ($C2'$/$C6'$), 146.8 ($C2'/C6'$), 143.4, 140.8, 139.4, 133.9 ($C13$/$C14$), 133.5 ($C4'$), 133.1, 132.9, 131.2 ($C4/C6/Ph$), 130.4, 130.3 ($C4/C6/Ph$), 129.4 ($C4/C6/Ph$), 126.5 (C5), 126.3 ($C4/C6/Ph$), 126.2 (Ph), 123.6 ($C13$/$C14$), 118.7 (CN), 117.9 (C7), 73.9 (C2), 59.0 (C3), 56.9 (C21), 54.7 (C23), 41.2 (C9), 33.3 (C15), 31.2 ($C16$/$C20$), 30.0 ($C17$/$C18$), 29.8 ($C16$/$C20$), 29.6 ($C17$/$C18$), 28.2 (C19), 24.4 (C24).

HRMS (ES$^+$): [C$_{41}$H$_{42}$N$_5$O$_3$]$^+$ ([M+H]$^+$) requires 652.3288; found 652.3281.

IR: $\nu_{\text{max}}$(film)/cm$^{-1}$ 2932, 2241, 1721, 1687, 1481, 1423, 1389, 1110, 734, 715.

(2RS,3RS)-1-Glycyl-3-phenyl-2-(5-(7-pyrrolidin-1-yl)heptyl)pyridin-3-yl)indoline-3-carbonitrile 213

N$_2$H$_4$·H$_2$O (7.0 µL, 0.13 mmol) was added to a solution of indoline 212 (17 mg, 0.026 mmol) in EtOH (2 mL) and stirred at 60 °C for 1 h. The mixture was filtered and concentrated. CH$_2$Cl$_2$ was added to the remaining residue, the resulting precipitate was removed by filtration, and the filtrate was concentrated. This process was repeated 4 times. Purification by flash pressure column chromatography (5.5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) to afford indoline 213 as a pale yellow oil (9.4 mg, 0.018 mmol, 69%, contains minor impurities).
$^1$H NMR (400 MHz, C$_6$D$_6$): $\delta$ 8.53 (1H, app br s, H7), 8.50 (1H, d, J 2.0, H6$'$), 8.46 (1H, d, J 1.8, H2$'$), 7.19-7.24 (3H, m, H4', Ph), 7.10 (1H, app t, J 7.7, H6), 7.03 (1H, dd, J 7.7, 0.8, H4), 6.92-6.99 (3H, m, H4), 6.80-6.88 (3H, m, Ph), 6.73 (1H, app td, J 7.7, 1.0, H5), 5.20 (1H, br s, H2), 2.72 (2H, br s, H9), 2.36-2.48 (6H, m, H16, H18), 2.10 (2H, t, J 7.5, H10), 1.65 (4H, m, H19), 1.50 (2H, app quin, J 7.6, H15), 1.28 (4H, m, H11, H14), 1.12 (2H, app quin, J 7.2, H12/H13), 1.05 (2H, app quin, J 7.2, H12/H13), 0.77 (3H, br s, NH$_2$, includes H$_2$O).

$^{13}$C NMR (101 MHz, C$_6$D$_6$): $\delta$ 172.1 (C=O), 152.4 (C6$'$), 146.5 (C2$'$), 143.9, 141.0, 139.2, 133.5 (C4$'$), 133.3, 131.4 (C6), 130.2 (Ph), 129.4 (Ph), 126.5 (C4), 126.3 (Ph), 126.2 (C5), 119.1 (CN), 117.6 (C7), 73.1 (C2), 58.4 (C3), 57.0 (C16), 54.8 (C18), 46.2 (C9), 33.1 (C10), 31.3 (C11/C14), 30.0 (C12/C13), 29.8 (C15), 29.5 (C12/C13), 28.2 (C11/C14), 24.3 (C19).

† 1 peak obscured.

HRMS (ES$^+$): [C$_{33}$H$_{40}$N$_5$O]$^+$ ([M+H]$^+$) requires 522.3227; found 522.3227.

IR: $\nu_{max}$ (film)/cm$^{-1}$ 3378 (broad, N-H), 2241, 2930, 1681 (C=O), 1598, 1480, 1393, 1222, 772.

5-((2S,3S)-3-Cyano-1-(2-(1,3-dioxoisindolin-2-yl)acetyl)-3-phenylindolin-2-yl)pyridin-3-yl trifluoromethanesulfonate 268

Indoline 268 was prepared according to general procedure 2, using indoline 267 (80 mg, 0.18 mmol), Acyl chloride 209 (240 mg, 1.07 mmol), and pyridine (87 µL, 1.1 mmol) in CH$_2$Cl$_2$ (3 mL).

The reaction mixture was stirred at rt for 1 h. Purification by flash pressure column chromatography (8% EtOAc/CHCl$_3$) afforded indoline 268 as a colourless gum (108 mg, 0.17 mmol, 95%).

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Indoline 269 was prepared according to general procedure 4, using indoline 268 (80 mg, 0.13 mmol), vinylstannane 57 (76 mg, 0.16 mmol), and Pd(PPh3)4 (15 mg, 0.013 mmol) in NMP (3 mL). The reaction mixture was stirred at 80 °C for 2.5 h. Purification by flash pressure column chromatography (5% MeOH/CH2Cl2 + 0.5% aq. NH4OH) afforded indoline 269 as a colourless gum.
(70 mg, 0.11 mmol, 85%).

$^1$H NMR (500 MHz, C$_6$D$_6$, 348 K): $\delta$ 8.68 (1H, app s, H6'), 8.52 (1H, app s, H2'), 8.21 (1H, app br s, H7), 7.55 (1H, app s, H4'), 7.40 (2H, m, H13), 7.34 (2H, app d, J 7.7, o-Ph), 6.96-7.10 (5H, m, H4, H6, m-Ph, p-Ph), 6.90 (2H, m, H14), 6.74 (1H, app t, J 7.6, H5), 6.01-6.15 (2H, m, H15, H16), $^*$ 5.54 (1H, s, H2), 4.21 (1H, d, J 16.2, H9), 4.02 (1H, d, J 16.2, H9'), 2.43 (4H, m, H23), 2.37 (2H, t, J 7.2, H21), 1.96 (2H, app q, J 6.4, H17), 1.62 (4H, m, H24), 1.48 (2H, app quin, J 6.9, H20), 1.26-1.39 (4H, m, H18, H19).

$^{13}$C NMR (126 MHz, C$_6$D$_6$, 348 K): $\delta$ 167.6 (C=O), 165.2 (C=O), 150.2 (C6'), 147.3 (C2'), 143.3, 140.8, 135.2 (C15/C16), 135.0, 134.0 (C14), 133.2, 133.1, 131.2 (C6), 130.8 (C4'), 130.4 (m-Ph), 129.5 (p-Ph), 126.9 (C15/C16), 126.7 (C5), 126.4 (o-Ph), 126.2 (C4), 123.7 (C13), 118.8 (CN), 118.0 (C7), 74.0 (C2), 58.9 (C3), 56.7 (C21), 54.6 (C23), 41.3 (C9), 33.6 (C17), 29.5 (C18/C19), 29.3 (C20), 27.8 (C18/C19), 24.4 (C24).$^*$

$^*$ $^1$H NMR (400 MHz, C$_6$D$_6$): $\delta$ 6.05 (1H, dt, J 15.9, 6.6, H16), 5.94 (1H, d, J 15.9, H15).

‡ 1 peak obscured.

HRMS (ES$^+$): [C$_{41}$H$_{40}$O$_3$N$_5$]$^+$ ([M+H]$^+$) requires 650.3126; found 650.3120.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2932, 2261, 1721 (C=O), 1688 (C=O), 1481, 1423, 1389, 715.

$[\alpha]_D^{25.0} = +17$ (c = 0.1, CHCl$_3$).
N₂H₄·H₂O (22 µL, 0.46 mmol) was added dropwise to a solution of indoline 269 (60 mg, 0.093 mmol) in EtOH (4.8 mL) and stirred at 60 °C for 1.5 h. The mixture was filtered and concentrated. CH₂Cl₂ was added to the remaining residue, the resulting precipitate was removed by filtration, and the filtrate was concentrated. This process was repeated 4 times. Purification by flash pressure column chromatography (7.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) to afford indoline 270 as a pale yellow oil (29 mg, 0.056 mmol, 59%).

¹H NMR (500 MHz, C₆D₆, 348 K): δ 8.65 (1H, d, J 2.1, H₆'), 8.44 (1H, d, J 2.1, H₂'), 8.36 (1H, app br s, H₇), 7.39 (1H, app t, J 1.9, H₄'), 7.23 (2H, m, o-Ph), 7.10-7.19 (1H, obscured, H₆), 7.07 (1H, app d, J 7.7, H₄), 6.94-7.05 (3H, m, m-Ph, p-Ph), 6.77 (1H, app td, J 7.6, 1.0, H₅), 5.94-6.10 (2H, m, H₁₅, H₁₆), 5.34 (1H, s, H₂), 2.88 (1H, d, J 16.8, H₉), 2.82 (1H, d, J 16.8, H₉'), 2.42 (4H, m, H₂₃), 2.38 (2H, t, J 7.10, H₂₁), 1.94 (2H, app q, J 6.6, H₁₇), 1.64 (4H, m, H₂₄), 1.47 (2H, app quin, J 7.0, H₂₀), 1.22-1.39 (4H, m, H₁₈, H₁₉), 0.95 (2H, br s, NH₂).

¹³C NMR (126 MHz, C₆D₆, 348 K): δ 171.7 (C=O), 149.7 (C₆'), 146.8 (C₂'), 140.9, 134.8, 134.4 (C₁₅/C₁₆), 133.5, 131.0, 130.3 (C₆), 129.9 (C₄'), 129.6 (m-Ph/p-Ph), 129.0 (m-Ph/p-Ph), 126.5 (C₁₅/C₁₆), 126.2 (C₄), 126.0 (o-Ph), 125.8 (C₅), 118.7 (CN), 117.3 (C₇), 73.1 (C₂), 58.2 (C₃), 56.5 (C₂₁), 54.4 (C₂₃), 46.1 (C₉), 33.3 (C₁₇), 29.3 (C₁₈/C₁₉/C₂₀), 29.2 (C₁₈/C₁₉/C₂₀), 27.6 (C₁₈/C₁₉), 24.2 (C₂₄).†

† ¹H NMR (400 MHz, C₆D₆): δ 5.96 (1H, dt, J 16.0, 6.6, H₁₆), 5.86 (1H, d, J 16.0, H₁₅).

‡ 1 peak obscured.
HRMS: [C_{33}H_{33}ON_{3}]^{+} ([M+H])^+ requires 520.3071; found 520.3069.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3380 (br, N-H), 2931, 1681 (C=O), 1599, 1480, 1393, 1221, 773.

$[\alpha]_{D}^{25.0} = -63$ (c = 0.1, CHCl$_3$).

(2RS,3RS)-3-Phenyl-2-(5-{(7-(pyrrolidin-1-yl)hept-1-yn-1-yl)pyridin-3-yl})indoline-3-carbonitrile 207

Indoline 207 was prepared according to general procedure 5, using indoline 50 (215 mg, 0.57 mmol), alkyne 105 (104 mg, 0.63 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (20 mg, 0.03 mmol), and CuI (5 mg, 0.03 mmol) in N,N-diisopropylamine (5 mL). The reaction mixture was stirred at 60 °C for 3 h. Purification by flash pressure column chromatography (4.5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded indoline 207 as a pale yellow oil (80 mg, 0.17 mmol, 31%).

$^1$H NMR (500 MHz, C$_6$D$_6$): $\delta$ 8.96 (1H, d, $J = 2.0$, H6'), 8.18 (1H, d, $J = 2.0$, H2'), 8.00 (1H, app t, J 2.0, H4'), 7.22 (2H, m, Ph), 6.93-7.06 (4H, m, H6, Ph), 6.73 (1H, app d, J 7.6, H4), 6.59 (1H, app td, J 7.6, 0.9, H5), 6.42 (1H, app d, J 7.6, H7), 4.29 (1H, d, J 3.2, H2), 2.99 (1H, d, J 3.2, NH), 2.38 (4H, m, H16), 2.33 (2H, t, J 6.9, H14), 2.15 (2H, t, J 6.6, H10), 1.63 (4H, m, H17), 1.33-1.50 (6H, m, H11, H12, H13).

$^{13}$C NMR (125.75 MHz, C$_6$D$_6$): $\delta$ 153.8 (C6'), 150.8 (C7a), 148.4 (C2'), 138.3 (C4'), 137.8, 132.3, 130.6 (C6/Ph), 129.5 (C6/Ph), 129.1 (C6/Ph), 127.7, 126.2 (C4), 121.8 (C5'), 121.3 (C5), 118.9 (CN), 111.1 (C7), 95.1 (C8/C9), 78.4 (C8/C9), 75.8 (C2), 58.9 (C3), 56.7 (C14), 54.7 (C16), 29.3 (C11/C12/C13), 29.1 (C11/C12/C13), 27.5 (C11/C12/C13), 24.3 (C17), 20.0 (C10).†

† 1 peak obscured.

HRMS (ES$^+$): [C$_{33}$H$_{33}$N$_4$]^{+} ([M+H]$^+$) requires 461.2700; found 461.2695.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2936, 1726, 1607, 1470, 1450, 1263, 752.
Indoline 214 was prepared according to general procedure 3 using indoline 207 (20 mg, 0.04 mmol) and Pd/C (degussa type, 2 mg) in MeOH (1.5 mL). The reaction mixture was stirred at rt under a H₂ atmosphere for 16 h. Purification by flash pressure column chromatography (5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 214 as a colorless oil (14 mg, 0.029 mmol, 72%).

¹H NMR (500 MHz, C₆D₆): δ 8.57 (1H, d, J 2.0, H6'), 8.11 (1H, d, J 2.0, H2'), 7.83 (1H, app t, J 2.0, H4'), 7.28 (2H, m, Ph), 6.97-7.06 (4H, m, H6, Ph), 6.75 (1H, app d, J 7.6, H4), 6.61 (1H, app td, J 7.6, 0.9, H5), 6.47 (1H, app d, J 8.1, H7), 4.45 (1H, d, J 3.0, H2), 3.31 (1H, d, J 3.0, NH), 2.31-2.49 (8H, m, H8, H14, H16), 1.63 (4H, m, H17), 1.46-1.57 (4H, m, H9, H13), 1.35 (2H, app quin, J 7.5, H10/H11/H12), 1.17-1.29 (4H, m, H10/H11/H12).

¹³C NMR (126 MHz, C₆D₆): δ 151.8 (C6'), 151.2 (C7a), 147.8 (C2'), 138.3, 138.0, 135.7 (C4'), 132.1, 130.6 (C6/Ph), 129.4 (C6/Ph), 129.0 (C6/Ph), 127.9, 126.3 (C4), 121.3 (C5), 119.3 (CN), 111.1 (C7), 76.4 (C2), 59.1 (C3), 57.0 (C14), 54.8 (C16), 33.5 (C8), 31.7 (C9/C13), 30.0 (C10/C11/C12), 29.8 (C9/C13), 29.7 (C10/C11/C12), 28.1 (C10/C11/C12), 24.3 (C17).†

† 1 peak obscured.


IR: νmax (film)/cm⁻¹ 3027, 2930, 2797, 1608, 1471, 1259, 1220, 749, 700.
Indoline 271 was prepared according to general procedure 4, using indoline 50 (60 mg, 0.16 mmol), vinylstannane 57 (87 mg, 0.19 mmol), and Pd(PPh₃)₄ (18 mg, 0.016 mmol) in NMP (3.2 mL). The reaction mixture was stirred at 85 °C for 3 h. Purification by flash pressure column chromatography (4.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 271 as a colorless paste (42 mg, 0.091 mmol, 57%).

¹H (500 MHz, C₆D₆): δ 8.72 (1H, d, J 2.1, H6'), 8.11 (1H, d, J 2.1, H2'), 8.02 (1H, app t, J 2.1, H4'), 7.26 (2H, m, o-Ph), 6.96-7.06 (4H, m, H6, m-Ph, p-Ph), 6.75 (1H, app d, J 7.4, H4), 6.61 (1H, app td, J 7.5, 0.9, H5), 6.47 (1H, app d, J 7.9, H7), 6.17-6.23 (2H, m, H8, H9), 4.41 (1H, d, J 3.1, H2), 3.15 (1H, d, J 3.1, NH), 2.34-2.47 (6H, m, H14, H16), 2.00 (2H, app q, J 6.8, H10), 1.64 (4H, m, H17), 1.51 (2H, app quin, J 7.1, H13), 1.27-1.38 (4H, m, H11, H12).

¹³C (126 MHz, C₆D₆): δ 151.0 (C7a), 149.7 (C6'), 148.4 (C2'), 137.8 (i-Ph), 134.6 (C9), 133.9 (C5'), 132.3 (C4'), 132.3 (C3'), 130.6 (C6), 129.4 (m-Ph), 129.0 (p-Ph), 128.4 (o-Ph), 127.9 (C3a), 126.9 (C8), 126.3 (C4), 121.3 (C5), 119.2 (CN), 111.2 (C7), 76.3 (C2), 59.0 (C3), 56.8 (C14), 54.7 (C16), 33.8 (C10), 29.7 (C13), 29.7 (C11/C12), 27.8 (C11/C12), 24.3 (C17).

HRMS: [C₃₁H₃₅N₄]⁺ ([M+H]⁺) requires 463.2856; found 463.2853.

IR: ν_max (film)/cm⁻¹ 2933, 2798, 1608, 1472, 1260, 1220, 1026, 773, 645.
Ester 146 was prepared following a literature procedure. Two drops of concentrated H₂SO₄ were added to a stirred solution of L-(+)-mandelic acid (1 g, 6.57 mmol) in iPrOH. The solution was stirred at 65 °C for 16 h, then concentrated. The resulting residue was dissolved in EtOAc and washed with H₂O, then with brine. The organic layer was dried over MgSO₄, filtered, and concentrated to afford ester 146 as a colorless solid (1.2 g, 5.57 mmol, 85%). The spectral data matched those reported in the literature.

**I'H (400 MHz, CDCl₃):** δ 7.29-7.46 (5H, m, Ph), 5.13 (1H, s, PhCH), 5.08 (1H, hept, J 6.3, OCH(CH₃)₂), 1.29 (3H, d, J 6.1, CH₃a), 1.12 (4 H, d, J 6.1, CH₃b).

**I''C (101 MHz, CDCl₃):** δ 173.2 (CO₂iPr), 138.5 (i-Ph), 128.5 (Ph), 128.3 (Ph), 126.4 (Ph), 72.9 (PhCHOH), 70.2 (OCH(CH₃)₂), 21.7 (CH₃a), 21.4 (CH₃b).

**LCMS (ES⁺):** 217.1 ([M+Na⁺]).

**IR:** νmax (film)/cm⁻¹: 3484 (br, O-H), 2983, 2361, 1725 (C=O), 1265, 1183, 1105, 1067, 733, 700.

**MP:** 39-41 °C (lit 43 °C).

[α]D²⁰ = +109.1 ° (c = 1.0, CHCl₃), lit [α]D²² = +103.6 ° (c = 1.0, CHCl₃).

Chloroformate 147 was prepared following a literature procedure. Triphosgene (1.15 g, 3.86 mmol) was added to a solution of ester 146 (1.00 g, 5.15 mmol) in toluene (5 mL) at 0 °C.
Quinoline (0.67 mL, 5.7 mmol) was added dropwise and stirred at rt for 2 h. The mixture was cooled to 0 °C, and HCl (aq. 1 M, 10 mL) was added. The mixture was extracted with Et₂O, and the combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated to afford chloroformate 147 as a brown, hygroscopic solid (1.32 g, 5.14 mmol, quant). The spectral data matched those reported in the literature.

¹H (400 MHz, CDCl₃): δ 7.39-7.50 (5H, m, Ph), 5.92 (1H, s, PHCH), 5.10 (1H, hept, J 6.3, OCH(CH₃)₂), 1.29 (3H, d, J 6.1, CH₃a), 1.16 (3 H, d, J 6.1, CH₃b).

¹³C (101 MHz, CDCl₃): δ 166.4 (C'O₂iPr), 150.6 (OC(O)Cl), 132.0 (i-Ph), 129.9 (Ph), 128.9 (Ph), 127.7 (Ph), 80.3 (PHCH), 70.4 (OCH(CH₃)₂), 21.6 (CH₃a), 21.3 (CH₃b).

LCMS (ES⁺): 279.0 ([M+35Cl]+Na⁺), 281.0 ([M+37Cl]+Na⁺).

ν_max (film): 2984, 1776 (C=O), 1746 (C=O), 1139, 1101, 1008, 820, 734, 695.

[α]_D^20 = +106.3 ° (c = 1.0, CHCl₃), lit [α]_D^19.7 = +93.1 ° (c = 1.73, CHCl₃).

(S)-2-Isopropoxy-2-oxo-1-phenylethyl (2R,3R)-2-(5-bromopyridin-3-yl)-3-cyano-3-phenylindoline-1-carboxylate 148 and (S)-2-Isopropoxy-2-oxo-1-phenylethyl (2S,3S)-2-(5-bromopyridin-3-yl)-3-cyano-3-phenylindoline-1-carboxylate 149

Indolines 148 and 149 were prepared according to general procedure 3 using indoline 50 (220 mg, 0.59 mmol), chloroformate 147 (330 mg, 1.29 mmol), and pyridine (0.12 mL, 1.5 mmol) in CH₂Cl₂ (12 mL). The reaction mixture was stirred at rt for 8 h. Purification by flash pressure column chromatography (5% EtOAc/petroleum ether) afforded separately indoline 148 as a colorless solid.
(91 mg, 0.15 mmol, 25%) and indoline 149 as a colorless solid (99 mg, 0.17 mmol, 29%).

(S)-2-Isoproxy-2-oxo-1-phenylethyl (2R,3R)-2-(5-bromopyridin-3-yl)-3-cyano-3-phenylindoline-1-carboxylate 148

1H NMR (500 MHz, C6D6): δ 8.69 (1H, app s, H6'), 8.53 (1H, app s, H2'), 8.29 (1H, app br s, H7), 7.74 (1H, app s, H4'), 7.12-7.18 (2H, obscured, Ph), 6.85-7.07 (10H, app br s, H4, H6, Ph), 6.65 (1H, app td, J 7.6, 1.0, H5), 5.81 (1H, s, H10), 5.32 (1H, br s, H2), 4.87 (1H, sept, J 6.3, H13), 0.92 (3H, d, J 6.3, H14), 0.77 (3H, d, J 6.3, H14').

‡ 2 peaks obscured.

HRMS (ES⁺): [C32H27O4N3Br]⁺ ([M+H]⁺) requires 596.1180; found 596.1179.

IR: v max (film)/cm⁻¹ 2972, 1722, 1485, 1393, 1217, 1103, 760, 696.

MP: 40-42 °C.

[α]D²⁵ = +102 (c = 0.1, CHCl₃).

(S)-2-Isoproxy-2-oxo-1-phenylethyl (2S,3S)-2-(5-bromopyridin-3-yl)-3-cyano-3-phenylindoline-1-carboxylate 149

1H NMR (500 MHz, C6D6): δ 8.49 (1H, app s, H6'), 8.46 (1H, app br s, H2'), 8.27 (1H, app br d, J 7.4, H7), 7.54 (1H, app br s, H4'), 7.24 (2H, m, Ph), 6.91-7.07 (9H, m, H6, Ph), 6.88 (1H, dd, J 7.7, 0.8, H4), 6.64 (1H, app td, J 7.6, 1.0, H5), 6.01 (1H, s, H10), 5.62 (1H, s, H2), 4.87 (1H, sept, J 6.2, H13), 0.89 (3H, d, J 6.2, H14), 0.74 (3H, d, J 6.2, H14').

13C NMR (126 MHz, C6D6): δ 168.4 (C=O), 152.3 (C6'), 151.8 (C=O), 147.0 (C2'), 142.4 (C7a), 140.4 (i-Ph), 136.9 (C4'), 136.1 (C3'), 134.1 (i-Ph), 131.4 (C6), 130.2 (Ph), 129.7 (C3a), 129.7 (Ph), 129.4
(Ph), 129.3 (Ph), 127.9 (Ph), 126.4 (Ph), 126.3 (C4), 125.8 (C5), 121.5 (C5'), 118.8 (CN), 116.4 (C7), 76.8 (C10), 73.8 (C2), 70.1 (C13), 57.7 (C3), 21.8 (C14), 21.5 (C14').

HRMS (ES⁺): [C₃₂H₂₇O₄N₃Br]⁺ ([M+H]⁺) requires 596.11780; found 596.1179.

IR: ν_max (film)/cm⁻¹ 2978, 1722, 1485, 1391, 1221, 1103, 756, 696.

MP: 126-128 °C.

[α]_D^25.0 = +3.0 (c =0.1, CHCl₃).

(2RS,3RS)-2-(5-Bromopyridin-3-yl)-1-(3-(methyl-3H-diazirin-3-yl)propanoyl)-3-phenylindoline-3-carbonitrile 229

Diazirine 228 was prepared according to a modified literature procedure.¹⁴⁷ A solution of NH₃ in MeOH (7 N, 5.0 mL, 35 mmol) was added dropwise to a solution of levulinic acid (0.44 mL, 4.3 mmol) in MeOH (1 mL) at 0 °C and stirred for 3 h. A solution of hydroxylamine O-sulfonic acid (510 mg, 4.95 mmol) in MeOH (1.5 mL) was added and stirred at rt for 16 h. The excess NH₃ was removed by blowing the mixture down with N₂. The white precipitate was filtered off and the filtrate concentrated. The remaining residue was dissolved in MeOH (0.5 mL) and cooled to 0 °C. NEt₃ (0.90 mL, 6.5 mmol) was added and stirred at 0 °C for 5 min. I₂ was added portionwise until the yellow colour of the solution remained (approx. 1 g). The solution was diluted with EtOAc, washed with HCl (aq., 1 M), Na₂S₂O₃ (aq., 10% w/w), and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated.

The crude residue (210 mg) was dissolved in CH₂Cl₂ (12 mL) and 1 drop DMF was added. The solution
was cooled to 0 °C, and oxalyl chloride (0.30 mL, 3.3 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 1.5 h and concentrated.

The crude residue (78 mg) was dissolved in CH2Cl2 (1.5 mL) and added dropwise to a solution of indoline 50 (50 mg, 0.13 mmol) and pyridine (43 µL, 0.53 mmol) in CH2Cl2 (2 mL) and stirred at rt for 30 min. NaHCO3 (saturated aq., 5 mL) was added, and the mixture was extracted with CH2Cl2. The combined organic extracts were washed with brine, dried over Na2SO4, filtered, and concentrated. Purification by flash pressure column chromatography (25% EtOAc/petroleum ether) afforded indoline 229 as a colourless gum (40 mg, 0.082 mmol, 62%).

1H NMR (500 MHz, C6D6, 348 K): δ 8.56 (1H, d, J 2.1, H6'), 8.42 (1H, d, J 1.9, H2'), 8.06 (1H, app br s, H7), 7.56 (1H, app t, J 2.1, H4'), 7.20 (2H, m, o-Ph), 6.94-7.10 (5H, m, H4, H6, m-Ph, p-Ph), 6.73 (1H, app td, J 7.6, 0.8, H5), 5.25 (1H, s, H2), 1.57 (2H, app m, H9), 1.42 (2H, t, J 6.7, H10), 0.56 (3H, s, CH3).

13C NMR (126 MHz, C6D6, 348 K): δ 169.0 (C=O), 152.6 (C6'), 146.6 (C2'), 142.7, 140.3, 136.4 (C4'), 135.4, 130.9 (C6), 129.8 (m-Ph/p-Ph), 129.1 (m-Ph/p-Ph), 126.1 (C4), 125.9 (C5), 125.8 (o-Ph), 121.4 (C5'), 118.3 (CN), 117.2 (C7), 73.2 (C2), 57.5 (C3), 29.7 (C9/C10), 29.0 (C9/C10), 24.7 (C11), 19.7 (C12).†

† 1 peak obscured.


IR: νmax (film)/cm⁻¹ 3069, 2933, 1672 (C=O), 1477, 1261, 1020, 737, 700.
(2RS,3RS)-1-(3-(Methyl-3H-diazirin-3-yl)propanoyl)-3-phenyl-2-(5-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl)indoline-3-carbonitrile 230

Indoline 230 was prepared according to general procedure 4 using indoline 229 (35 mg, 0.072 mmol), vinylstannane 57 (39 mg, 0.086 mmol), and Pd(PPh₃)₄ (8 mg, 0.007 mmol) in NMP (1.5 mL). The reaction mixture was stirred at 60 °C for 3.5 h. Purification by flash pressure column chromatography (6.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 230 as a pale yellow paste (14 mg, 0.024 mmol, 34%).

¹H NMR (500 MHz, C₆D₆, 348 K): δ 8.65 (1H, app s, H6'), 8.48 (1H, app s, H2'), 8.36 (1H, app br s, H7), 7.45 (1H, app s, H4'), 7.28 (2H, app d, J 7.5, o-Ph), 7.12 (1H, app t, J 7.9, H6), 7.08 (1H, app d, J 7.8, H4), 6.96-7.06 (3H, m, m-Ph, p-Ph), 5.98-6.10 (2H, m, H13, H14), 5.35 (1H, s, H2), 2.41 (4H, m, H22), 2.36 (2H, t, J 7.3, H19), 1.93 (2H, app q, J 6.4, H15), 1.56-1.73 (6H, m, H9, H23), 1.34-1.51 (4H, m, H10, H18), 1.22-1.34 (4H, m, H16, H17), 0.53 (3H, s, CH₃).

¹³C NMR (126 MHz, C₆D₆, 348 K): δ 169.8 (C=O), 150.0 (C6'), 147.2 (C2'), 143.8, 141.2, 135.2 (C13/C14), 134.8, 133.9, 131.2 (C6), 130.8 (C4'), 130.2 (m-Ph/p-Ph), 129.4 (m-Ph/p-Ph), 126.8 (C13/C14), 126.5 (C4), 126.3 (C5), 126.1 (o-Ph), 118.9 (CN), 117.8 (C7), 74.5 (C2), 58.3 (C3), 56.7 (C19), 54.6 (C22), 33.6 (C15), 30.3 (C9), 29.6 (C10/C17/C18/C19), 29.6 (C10/C17/C18/C19), 29.5 (C10/C17/C18/C19), 27.8 (C17/C18), 25.1 (C11), 24.5 (C23), 20.0 (CH₃). ‡¹

† ¹H NMR (500 MHz, C₆D₆): δ 6.00 (1H, dt, J 15.9, 6.7, H14), 5.94 (1H, d, J 15.9, H13).

‡ 1 peak obscured.

HRMS (ES⁺): [C₃₆H₄₁ON₆]⁺ ([M+H]+) requires 573.3336; found 573.3339.
IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2930, 2788, 2360, 1678 (C=O), 1479, 1350, 756, 669.

$(2RS,3RS)$-2-(5-Bromopyridin-3-yl)-1-(hex-5-ynoyl)-3-phenylindoline-3-carbonitrile 222

Oxalyl chloride (0.30 mL, 3.6 mmol) was added dropwise to a solution of 5-hexynoic acid (0.20 mL, 1.8 mmol) and DMF (1 drop) in CH$_2$Cl$_2$ (20 mL) at 0 °C and stirred for 1.5 h. The reaction mixture was allowed to warm to rt and concentrated to afford acyl chloride 221 as a yellow oil (224 mg, 1.72 mmol, 96%).

A solution of acyl chloride 221 (56 mg, 0.43 mmol) in CH$_2$Cl$_2$ (1 mL) was added to a solution of indoline 50 (80 mg, 0.21 mmol) and pyridine (34 µL, 0.43 mmol) in CH$_2$Cl$_2$ (3 mL) and stirred at rt for 1 h. NaHCO$_3$ (saturated aq., 3 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (25% EtOAc/petroleum ether) afforded indoline 222 as a pale yellow oil (94 mg, 0.20 mmol, 95%).

$^1$H NMR (500 MHz, C$_6$D$_6$, 348 K): $\delta$ 8.57 (1H, app s, H$^6'$), 8.47 (1H, app s, H$^2'$), 8.18 (1H, app br s, H7), 7.59 (1H, app s, H4'), 7.14-7.21 (2H, obscured, o-Ph), 7.07 (1H, app t, J 7.7, H6), 6.94-7.04 (4H, m, H4, m-Ph, p-Ph), 6.74 (1H, app t, J 7.7, H5), 5.33 (1H, s, H2), 1.99 (2H, app m, H9), 1.79-1.90 (2H, td, J 6.8, 2.5, H11), 1.56 (1H, t, J 2.6, H13), 1.52 (2H, app quin, 6.8, H10).

$^{13}$C NMR (126 MHz, C$_6$D$_6$, 348 K): $\delta$ 170.4 (C=O), 152.9 (C6'), 147.2 (C2'), 143.4 (C7a), 140.8 (i-Ph), 136.8 (C4'), 136.0 (C3'), 131.3 (C6), 130.2 (m-Ph/p-Ph), 129.5 (m-Ph/p-Ph), 126.5 (C4), 126.2 (o-Ph), 126.2 (C5), 121.8 (C5'), 118.9 (CN), 117.8 (C7), 83.5 (C12), 73.6 (C2), 69.9 (C13), 57.9 (C3), 34.2 (C9), 23.7 (C10), 17.9 (C11).
‡ 1 peak obscured.

HRMS (ES’): [C_{26}H_{21}ON_{3}^{79}Br]^+ ([M+H]^+) requires 470.08625; found 470.08636.

IR: \nu_{\text{max}} (film)/cm\(^{-1}\) 3288 (sharp, C-H), 3068, 2977, 1674 (C=O), 1479, 1391, 1271, 1020, 756, 698.

(2RS,3RS)-1-(Hex-5-ynoyl)-3-phenyl-2-{5-[(E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl]pyridin-3-yl}indoline-3-carbonitrile 223

Indoline 223 was prepared according to general procedure 4, using indoline 222 (60 mg, 0.13 mmol), vinylstannane 57 (77 mg, 0.17 mmol), and Pd(PPh\(_3\))\(_4\) (15 mg, 0.013 mmol) in NMP (3 mL). The reaction mixture was stirred at 85 °C for 2.5 h. Purification by flash pressure column chromatography (5% MeOH/CH\(_2\)Cl\(_2\) + 0.5% aq. NH\(_4\)OH) afforded indoline 223 as a pale yellow oil (32 mg, 0.057 mmol, 44%, contains minor impurities).

\(^1\)H NMR (500 MHz, C\(_6\)D\(_6\), 348 K): \delta 8.67 (1H, d, J 1.4, H6'), 8.54 (1H, d, J 1.7, H2'), 8.45 (1H, app br s, H7), 7.49 (1H, app s, H4'), 7.28 (2H, app d, J 7.4, o-Ph), 7.14 (1H, app t, J 8.0, H6), 7.10 (1H, app d, J 7.7, H4), 6.95-7.06 (3H, m, m-Ph, p-Ph), 6.79 (1H, app t, J 6.8, H5), 5.99-6.10 (2H, m, H14, H15), 5.45 (1H, s, H2), 2.43 (4H, m, H22), 2.37 (2H, t, J 7.2, H20), 2.06 (2H, app m, H9), 1.89-1.97 (2H, app br s, H16), 1.85 (2H, td, J 6.8, 2.5, H11), 1.64 (4H, m, H23), 1.51-1.60 (3H, m, H10, H13), 1.48 (2H, app quin, J 7.0, H19), 1.22-1.35 (4H, m, H17, H18).

\(^{13}\)C NMR (126 MHz, C\(_6\)D\(_6\), 348 K): \delta 170.7 (C=O), 149.9 (C6'), 147.4 (C2'), 144.1, 141.3, 135.1 (C14/C15), 134.8, 134.1, 131.2 (C6), 130.7 (C4'), 130.2 (m-Ph/p-Ph), 129.4 (m-Ph/p-Ph), 126.9 (C14/C15), 126.5 (C4), 126.4 (o-Ph), 126.1 (C5), 119.1 (CN), 118.0 (C7), 83.6 (C12/C13), 74.5 (C2), 251
69.9 (C12/C13), 58.3 (C3), 56.7 (C20), 54.7 (C22), 34.4 (C9), 33.6 (C16), 29.5 (C17/C18/C19), 29.5 (C17/C18/C19), 27.8 (C17/C18/C19), 24.5 (C23), 23.9 (C10), 17.9 (C11).†

† 1H NMR (400 MHz, C₆D₆): δ 5.97 (1H, dt, J 16.1, 6.6, H15), 5.87 (1H, d, J 16.1, H14).

‡ 1 peak obscured.

HRMS (ES⁺): [C₃₇H₄₁ON₄]^⁺ ([M+H]^⁺) requires 557.32749; found 557.32733.

IR: νmax (film)/cm⁻¹ 2930, 2252, 1674 (C=O), 1599, 1479, 1391, 1273, 754.

N-(71-azido-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69-
Tricosaoxahenheptacontyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-
yl)pentanamide 220

Azide 220 was prepared according to a modified literature procedure.¹⁹⁸ N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (7.7 mg, 0.040 mmol) and 1-hydroxy-7-azabenzotriazole (5.4 mg, 0.039 mmol) were added to a solution of amino-(PEG)₂₃-azide (40 mg, 0.036 mmol) and D-biotin (8.9 mg, 0.036 mmol) in N,N-DMF (0.5 mL) and stirred at rt for 18 h. The reaction mixture was diluted with CH₂Cl₂ and washed 3 times with H₂O, then brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (8% MeOH/CH₂Cl₂ + 1% aq. NH₄OH) afforded azide 220 as a colorless oil (37 mg, 0.028 mmol, 78%).

¹H NMR (500 MHz, CDCl₃): δ 6.69 (1H, s, NH), 5.77 (1H, app s, NH), 4.98 (1H, app s, NH), 4.51 (1H, dd, J 7.6, 5.0, H3), 4.33 (1H, dd, J 7.6, 4.8, H4), 3.60-3.71 (96H, m), 3.57 (2H, t, J 5.2), 3.42-3.47 (2H, t, J 5.5), 3.40 (2H, t, J 5.2, CH₂N₃), 3.16 (1H, ddd, J 8.0, 7.5, 4.6, H5), 2.92 (1H, dd, J 12.8, 5.0, H2), 2.74 (1H, d, J 12.8, H2'), 2.23 (2H, app m, H9), 1.62-1.80 (4H, m, H6, H8), 1.46 (2H, app quin, J 7.4, H7).
\(^{13}\text{C}\) NMR (126 MHz, CDCl\(_3\)): \(\delta \) 173.1 (C=O), 163.2 (C=O), 70.7, 70.7, 70.6, 70.5, 70.4, 70.4, 70.1, 70.0, 69.9, 61.7 (C4), 60.1 (C3), 55.3 (C5), 50.7 (CH\(_2\)N\(_3\)), 40.5 (C2), 39.1 (CONHCH\(_2\)), 35.8 (C9), 28.0 (C6), 28.0 (C7), 25.5 (C8).

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 2869, 2108 (N=N=N), 1702, 1457, 1349, 1251, 1104, 949.

\(N\)-(71-(4-(4-((2RS,3RS)-3-Cyano-3-phenyl-2-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl)indolyl-1-yl)-4-oxobutyl)-1H-1,2,3-triazol-1-yl)-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69-tricosaoxahenheptacontyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide 224

Indoline 224 was prepared according to a modified literature procedure.\(^{192}\) CuSO\(_4\) (5 mM aq., 175 \(\mu\)L, 0.88 \(\mu\)mol) and sodium-L-ascorbate (25 mM aq., 85 \(\mu\)L, 0.0021 mmol) were added to a solution of indoline 223 (12 mg, 0.018 mmol) and azide 220 (25 mg, 0.015 mmol) in 1:1 \(^t\)BuOH/H\(_2\)O (1 mL) and stirred at 70 °C for 14 h. The reaction mixture was allowed to cool to rt, diluted with H\(_2\)O, and extracted with CH\(_2\)Cl\(_2\). The combined organic extracts were washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated. Purification by flash pressure column chromatography (9% MeOH/CH\(_2\)Cl\(_2\) + 1% aq. NH\(_4\)OH) afforded indoline 224 as a colorless oil (19 mg, 0.010 mmol, 56%, contains minor impurities).

\(^1\)H NMR (500 MHz, C\(_6\)D\(_6\)): \(\delta \) 8.78 (1H, app br s, H7), 8.71 (1H, d, \(J\) 1.9, H6'), 8.63 (1H, d, \(J\) 1.9, H2'), 7.62 (1H, app s, H4'), 7.39 (2H, app d, \(J\) 7.6, Ph), 7.14-7.20 (1H, obscured, H6), 7.11 (1H, br t, NH), 253
7.05-7.10 (3H, m, H4, Ph), 6.97-7.04 (3H, m, H13, Ph), 6.76 (1H, app td, J 7.5, 0.8, H5), 6.09 (1H, dt, J 15.9, 6.8, H17), 5.99 (1H, d, J 15.9, H16), 5.79 (1H, br s, H2), 5.71 (1H, app s, NH), 4.06 (2H, t, J 5.2, H15), 3.95 (1H, dd, J 7.6, 4.8, H4''), 3.85 (1H, dd, J 7.6, 5.0, H3''), 3.30-3.58 (9H, m), 3.23-3.28 (2H, m), 2.77 (1H, app ddd, J 8.2, 7.2, 4.7, H2''), 2.46-2.59 (4H, m), 2.44 (4H, m, H23), 2.38 (2H, t, J 7.40, H22), 2.26 (2H, m), 2.00 (1H, app oct, J 6.8), 1.92 (2H, app q, J 6.9, H18), 1.71-1.87 (3H, m, H6'', H21), 1.65 (5H, m, H6'', H24), 1.43-1.55 (2H, m), 1.23-1.42 (6H, m).

13C NMR (126 MHz, CDCl3): δ 173.4 (C=O), 171.6, 164.7 (C=O), 149.7 (C6'), 147.3 (C2'), 147.0, 140.9, 134.9 (C17), 134.6, 134.4, 131.3 (C6), 130.9 (C4'), 130.2 (Ph), 129.4 (Ph), 126.7 (C16), 126.6 (Ph), 126.5 (C4), 126.0 (C5), 122.5 (C13), 119.4 (CN), 117.9 (C7), 73.7 (C2), 71.4, 71.3, 71.3, 71.1, 71.1, 71.0, 70.9, 70.7, 70.6, 70.0, 62.1 (C3''), 60.8 (C4''), 56.8 (C22), 56.3 (C2''), 54.7 (C23), 50.3 (C15), 40.9 (C5''), 40.0, 36.4, 35.3, 33.8 (C18), 29.5, 29.5, 28.9, 28.9 (C6''), 27.9, 26.5, 25.2, 25.0, 24.3 (C24).

HRMS (ES+): [C95H155O26N10S3]3+ ([M+3H]3+) requires 628.3617; found 628.3614.

IR: νmax (film)/cm⁻¹ 2871, 1701, 1460, 1349, 1257, 1105, 949.

[α]D25 = +8.6 (c = 0.1, CHCl₃).

4-Oxonon-8-ynoic acid 232

Acid 232 was prepared according to a literature procedure.¹⁴⁸ tBuLi (1.7 M in pentane, 8.5 mL, 14 mmol) was added dropwise to a solution of 2,3-dihydrofuran (1.3 mL, 17 mmol) in THF (16 mL) at -78 °C. The solution was allowed to warm to -5 °C over 1 h, then re-cooled to -78 °C. (5-Iodopent-1-yn-1-yl)trimethylsilane (3.2 g, 12.0 mmol) was added dropwise, and the reaction mixture was allowed to warm to rt and stirred for 16 h. The mixture was cooled to 0 °C, quenched with NH₄Cl (saturated aq.), and extracted with Et₂O. The combined organic extracts were washed...
with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. The resulting residue was dissolved in THF (32 mL), and Jones reagent (2 M, 18 mL, 36 mmol) was added dropwise with vigorous stirring. The mixture was stirred at rt for 4 h, diluted with Et$_2$O (35 mL) and H$_2$O (35 mL), and stirred for a further 30 min. The aqueous phase was extracted with Et$_2$O, and the combined organic extracts were washed with H$_2$O and extracted with NaOH (10% aq.). The basic extract was cooled to 0 °C, acidified to pH 2 with HCl (3M, aq.), and extracted with CH$_2$Cl$_2$. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated to afford acid 232 as a colorless solid (1.16 g, 6.90 mmol, 58%). The spectral data matched those reported in the literature.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 2.74 (2H, t, $J$ 6.0, H$_2$/H$_3$), 2.57-2.68 (4H, m, H$_2$/H$_3$, H$_5$), 2.23 (2H, td, $J$ 7.0, 2.7, H$_7$), 1.97 (1H, t, $J$ 2.7, H$_9$), 1.81 (2H, app quin, $J$ 7.0, H$_6$).\(^{1}\)

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 208.1 (C=O), 178.7 (C=O), 83.4 (C$_8$), 69.1 (C$_9$), 40.9 (C$_2$/C$_3$/C$_5$), 36.8 (C$_2$/C$_3$), 27.7 (C$_2$/C$_3$/C$_5$), 22.1 (C$_6$), 17.6 (C$_7$).

$^{1}$ CO$_2$H peak was not observed.


IR: $\nu_{\max}$ (film)/cm$^{-1}$ 3289 (sharp, C-H), 1707, 1404, 1184, 1101, 929, 643.

MP: 42-44 °C (lit 45 °C).

3-[(3-[(Pent-4-yn-1-yl)-3H-diazirin-3-yl]propanoic acid 233

Diazirine 233 was prepared according to a modified literature procedure.\(^{146}\) Anhydrous ammonia (approx. 4 mL) was condensed in a flask at -40 °C, and a solution of acid 232 (410 mg, 2.44 mmol) in MeOH (1.5 mL) was added dropwise. The mixture was stirred at -40 °C for 3.5 h. A solution of
hydroxylamine-O-sulphonic acid (317 mg, 2.80 mmol) in MeOH (2.5 mL) was added dropwise over 30 min. The reaction mixture was stirred at -35 °C for 1 h, then allowed to warm to rt and stirred for 14 h. The remaining ammonia was allowed to evaporate, and the crude residue was filtered and washed with MeOH. The filtrate was concentrated and dissolved in CH₂Cl₂ (1.5 mL). NEt₃ (0.40 mL, 2.9 mmol) was added, followed by a solution of I₂ in CH₂Cl₂ (0.5 g in 3 mL) until the yellow color persisted. The reaction mixture was diluted with EtOAc, washed with HCl (1 M aq.), Na₂S₂O₃ (aq., 10% w/w), and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (30% EtOAc/CH₂Cl₂) afforded diazirine 233 as a pale yellow oil (85 mg, 0.47 mmol, 19%).

¹H NMR (400 MHz, CDCl₃): δ 2.14-2.21 (4H, m, H₂, H₇), 1.96 (1H, t, J 2.7, H₉), 1.77 (2H, t, J 7.7, H₃), 1.56 (2H, t, J 7.4, H₅), 1.34 (2H, app quin, 7.4, H₆).

¹³C NMR (101 MHz, CDCl₃): δ 177.5 (C=O), 83.2 (C₈), 69.0 (C₉), 31.5 (C₅), 28.1 (C₂), 27.9 (C₃), 27.6 (C₄), 22.6 (C₆), 17.8 (C₇).

HRMS (ES⁻): [C₉H₁₁N₂O₂]⁻ (M⁻H⁻) requires 179.0826; found 179.0823.

IR: νₑₓₘₓ (film)/cm⁻¹ 3299 (sharp, C-H), 3044, 1712 (C=O), 1585, 1435, 1289, 927, 646.

(2RS,3RS)-2-(5-Bromopyridin-3-yl)-1-(3-(1,3-dioxoisoindolin-2-yl)propanoyl)-3-phenylindoline-3-carbonitrile 237

Acyl chloride 236 was prepared according to a modified literature procedure.²⁹⁷ Oxalyl chloride (0.39 mL, 4.6 mmol) was added dropwise to a solution of 3-phthalimidopropionic acid (0.50 g,
2.3 mmol) and DMF (2 drops) in CH$_2$Cl$_2$ (20 mL) at 0 °C and stirred for 1 h. The reaction mixture was allowed to warm to rt and concentrated to afford acyl chloride 236 as a yellow solid (509 mg, 2.14 mmol, 94%).

Acyl chloride 236 (114 mg, 0.48 mmol) was added portionwise to a solution of indoline 50 (120 mg, 0.32 mmol) and pyridine (40 µL, 0.48 mmol) in CH$_2$Cl$_2$ (10 mL) and stirred at rt for 4 h. NaHCO$_3$ (saturated aq., 10 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (12% EtOAc/CHCl$_3$) afforded indoline 237 as a colorless solid (142 mg, 0.23 mmol, 72%).

$^1$H NMR (500 MHz, dmso-D$_6$, 363 K): δ 8.68 (1H, d, J 1.9, H6'), 8.48 (1H, d, J 1.5, H2'), 8.10 (1H, app br s, H7), 7.92 (1H, app s, H4'), 7.76-7.85 (4H, m, H14, H15), 7.57 (1H, app t, J 7.8, H6), 7.37-7.48 (4H, m, H4, Ph), 7.26-7.35 (3H, m, H5, Ph), 5.98 (1H, s, H2), 3.79 (2H, app m, H10), 3.01 (1H, br s, H9), 2.72 (1H, br s, H9').

$^{13}$C NMR (126 MHz, dmso-D$_6$, 363 K): δ 169.2 (C=O), 167.9 (C=O), 151.6 (C6'), 146.7 (C2'), 142.2 (C7a), 139.2 (C3a), 137.2 (C4'), 135.8 (C3'), 134.7 (C14/C15), 132.1 (C13), 131.4 (C6), 129.9 (Ph), 129.5 (Ph), 126.2 (C4/C5/Ph), 126.1 (C4/C5/Ph), 126.0 (C4/C5/Ph), 123.4 (C14/C15), 120.7 (C5'), 118.9 (CN), 117.4 (C7), 71.0 (C2), 56.9 (C3), 33.9 (C10), 33.6 (C9).

‡ 1 peak obscured.

HRMS: [C$_{31}$H$_{22}$O$_3$N$_4$]$^+$ ([M+H]$^+$) requires 577.0870; found 577.0869.

IR: v$_{max}$ (film)/cm$^{-1}$ 3049, 2248, 1704, 1675, 1389, 1221, 1172, 1110, 871, 732.

MP: 194-196 °C.
Indoline 238 was prepared according to general procedure 4, using indoline 237 (60 mg, 0.10 mmol), vinylstannane 57 (57 mg, 0.13 mmol), Pd(PPh₃)₄ (12 mg, 0.01 mmol) in NMP (2 mL). The reaction mixture was stirred at 85 °C for 2 h. Purification by flash pressure column chromatography (5.5% MeOH/CH₂Cl₂ + aq. NH₄OH) afforded indoline 238 was a colorless oil (54 mg, 0.08 mmol, 78%).

**¹H NMR (500 MHz, C₆D₆, 348 K):** δ 8.61 (1H, app s, H6'), 8.55 (1H, app s, H2'), 8.40 (1H, app br s, H7), 7.50 (1H, app s, H4'), 7.34-7.43 (2H, m, H14), 7.29 (2H, app d, J 7.7, o-Ph), 7.05-7.13 (2H, m, H4, H6), 6.99-7.05 (2H, m, m-Ph), 6.90-6.99 (3H, m, H15, p-Ph), 6.76 (1H, app t, J 7.7, H5), 3.74 (1H, app t, J 7.7, H5), 3.65 (1H, app t, J 7.7, H5), 3.32-3.53 (8H, m, H22, H24, H9, H9'), 1.94 (1H, app q, J 6.7, H18), 1.64 (4H, m, H25), 1.48 (2H, m, H21), 1.24-1.39 (4H, m, H19, H20).

**¹³C NMR (126 MHz, C₆D₆, 348 K):** δ 168.7 (C=O), 167.7 (C=O), 149.9 (C6'), 147.2 (C2'), 143.7 (C7a), 141.0 (i-Ph), 134.9 (C17), 134.7 (C3'/C5'), 133.8 (C3'/C5'), 133.7 (C15), 132.9 (C13), 131.2 (C6), 130.7 (C4'), 130.1 (m-Ph), 129.3 (p-Ph), 126.8 (C16), 126.3 (C4), 126.3 (o-Ph), 126.2 (C5), 123.3 (C14), 118.9 (CN), 118.0 (C7), 74.4 (C2), 58.3 (C3), 56.7 (C22), 54.7 (C24), 34.5 (C9/C10), 34.4 (C9/C10), 33.6 (C18), 29.6 (C19/C20/C21), 29.5 (C19/C20/C21), 27.8 (C19/C20/C21), 24.4 (C25).‡

‡ 1 peak obscured.

HRMS: [C₄₂H₄₂O₃N₅]⁺ ([M+H]⁺) requires 664.3282; found 664.3279.

IR: ν<sub>max</sub> (film)/cm⁻¹ 2933, 1709, 2436, 1642, 1381, 1282, 1198, 1122, 829, 722.
(2RS,3RS)-1-(3-Aminopropanoyl)-3-phenyl-2-(5-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl)indoline-3-carbonitrile 239

Hydrazine monohydrate (18 µL, 0.35 mmol) was added to a solution of indoline 238 (46 mg, 0.069 mmol) in MeOH (2mL) and stirred at 50 °C for 4 h. The mixture was filtered and concentrated. CH₂Cl₂ was added to the remaining residue, the resulting precipitate was removed by filtration, and the filtrate concentrated. Purification by flash pressure column chromatography (5.5% MeOH/CH₂Cl₂ + 0.5% aq. NH₄OH) afforded indoline 239 as a pale yellow oil (29 mg, 0.054 mmol, 78%).

¹H NMR (500 MHz, C₆D₆, 348 K): δ 8.66 (1H, app s, H6'), 8.51 (1H, app s, H2'), 8.44 (1H, app br s, H7), 7.45 (1H, app s, H4'), 7.27 (2H, app d, J 6.8, o-Ph), 7.14 (1H, app t, J 7.8, H6), 7.08 (1H, app d, J 7.8, H4), 6.94-7.05 (3H, m, m-Ph, p-Ph), 6.78 (1H, app t, J 7.6 H5), 5.95-6.10 (2H, m, H12, H13), 5.45 (1H, s, H2), 2.65 (1H, app sex, J 6.8, H10), 2.58 (1H, app sex, J 6.8, H10'), 2.43 (4H, m, H20), 2.38 (2H, t, J 7.2, H18), 1.83-2.05 (4H, m, H9, H9', H14), 1.65 (4H, m, H21), 1.48 (2H, app quin, J 6.8, H17), 1.23-1.39 (4H, m, H15, H16), 0.63 (3H, br s, NH₂, includes H₂O signal).

¹³C NMR (126 MHz, C₆D₆, 348 K): δ 171.0 (C=O), 149.9 (C6'), 147.3 (C2'), 144.0 (C7a), 141.3, 135.1 (C13), 134.7, 134.1, 131.2 (C6), 130.7 (C4'), 130.2 (m-Ph/p-Ph), 129.3 (m-Ph/p-Ph), 126.8 (C12), 126.5 (C4), 126.3 (o-Ph), 126.0 (C5), 119.1 (CN), 118.0 (C7), 74.3 (C2), 58.3 (C3), 56.8 (C18), 54.7 (C20), 40.1 (C10), 38.4 (C9), 33.7 (C14), 29.6 (C15/C16/C17), 29.6 (C15/C16/C17), 27.9 (C15/C16/C17), 24.5 (C21).‡

‡ 1 peak obscured.

HRMS: [C₃₄H₄₀ON₅]⁺ ([M+H]⁺) requires 534.3227; found 534.3228.
IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3345 (br, N-H), 3027, 2929, 1672 (C=O), 1598, 1479, 1391, 1276, 755.

$N$-{3-[(2RS,3RS)-3-Cyano-3-phenyl-2-(5-((E)-7-(pyrrolidin-1-yl)hept-1-en-1-yl)pyridin-3-yl)indolin-1-yl]-3-oxopropyl]-3-(3-pent-4-yn-1-yl)-3H-diazirin-3-yl}propanamide 240

Indoline 240 was prepared according to a modified literature procedure.$^{145}$ 1-Hydroxybenzotriazole hydrate (8.5 mg, 0.047 mmol), $N$-{3-Dimethylaminopropyl}-$N'$-ethylcarbodiimide hydrochloride (9 mg, 0.05 mmol), and $N,N$-diisopropylethylamine (13 µL, 0.075 mmol) were added to a solution of indoline 239 (20 mg, 0.037 mmol) in DMF (0.6 mL). A solution of diazirine 233 (6.8 mg, 0.037 mmol) in DMF (0.4 mL) was added, and the reaction mixture was stirred at rt for 9 h. H$_2$O was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (5% MeOH/CH$_2$Cl$_2$ + 0.5% aq. NH$_4$OH) afforded indoline 240 as a pale yellow oil (17 mg, 0.024 mmol, 66%).

$^1$H NMR (500 MHz, C$_6$D$_6$, 348 K): $\delta$ 8.66 (1H, d, $J$ 1.7, H6'), 8.52 (1H, d, $J$ 1.8, H2'), 8.37 (1H, app br s, H7), 7.47 (1H, app s, H4'), 7.26 (2H, m, o-Ph), 7.15 (1H, obscured, H6), 7.09 (1H, app d, $J$ 7.4, H4), 6.97-7.07 (3H, m, m-Ph, p-Ph), 6.79 (1H, app t, $J$ 7.4, H5), 5.99-6.17 (2H, m, H21, H22), 5.47 (1H, s, H2), 5.26 (1H, s, NH), 3.18 (2H, app m, H10), 2.43 (4H, m, H29), 2.39 (2H, t, $J$ 7.14, H27), 2.27 (1H, app m, H9), 2.02 (1H, app br s, H9'), 1.97 (2H, app br s, H23), 1.83 (2H, td, $J$ 6.8, 2.6, H18), 1.74 (1H, t, $J$ 2.6, H20), 1.65 (4H, m, H30), 1.41-1.54 (4H, m, H25, H26), 1.26-1.38 (6H, m, H13, H14, H24), 1.19 (2H, app quin, $J$ 7.0, H16), 1.08 (2H, quin, $J$ 7.2, H17).
$^{13}$C NMR (126 MHz, C$_6$D$_6$, 348 K): $\delta$ 170.8 (C=O), 170.6 (C=O), 149.9 (C6'), 147.1 (C2'), 143.6 (C7a), 141.0 (i-Ph), 135.2 (C22), 134.6 (C5'), 133.8 (C3'), 131.2 (C6), 130.9 (C4'), 130.2 (m-Ph), 129.4 (p-Ph), 126.7 (C21), 126.6 (C4), 126.3 (o-Ph), 126.3 (C5), 118.9 (CN), 117.7 (C7), 83.8 (C19), 74.2 (C2), 69.6 (C20), 58.1 (C3), 56.8 (C27), 54.7 (C29), 36.3 (C9), 35.5 (C10), 33.6 (C23), 32.5 (C16), 30.5, 29.6, 29.5, 29.0, 28.2 (C15), 27.9, 24.4 (C30), 23.4 (C17), 18.4 (C18).‡

‡ 1 peak obscured.

HRMS: [C$_{43}$H$_{50}$O$_2$N$_7$]$^+$ ([M+H]$^+$) requires 696.4021; found 696.4019.

IR: $\nu$$_{\text{max}}$(film)/cm$^{-1}$ 3298 (sharp, C-H), 2932, 2790, 1670, 1542, 1479, 1395, 756, 698.

$^{1}$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.74 (2H, t, $J$ 7.2, H5), 2.51 (2H, t, $J$ 8.1, H3), 1.99 (2H, app quin, $J$ 7.6, H4), 1.52 (9H, C(CH$_3$)$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 173.3 (C=O), 150.1 (C=O), 82.6 (C(CH$_3$)$_3$), 46.4 (C5), 32.8 (C3), 27.9

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$^{T}$ert-buty1 2-oxopyrrolidine-1-carboxylate 249

Pyrrolidinone 249 was prepared according to a literature procedure.$^{199}$ 2-Pyrrolidinone (4.0 g, 47 mmol) was dissolved in acetonitrile (38 mL) and cooled to 0 °C. A solution of di-$^{t}$ert-butyl dicarbonate (11.3 g, 51.7 mmol) in CH$_3$CN (16 mL) was added dropwise. N,N-dimethylaminopyridine (574 mg, 4.7 mmol) was added, and the mixture was allowed to warm to rt and stirred at rt for 1 h. The mixture was concentrated and the remaining residue was dissolved in EtOAc and washed with H$_2$O. The pH of the aqueous wash was adjusted to approx. 7 using HCl (aq., 1 M) and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (25% EtOAc/petroleum ether) afforded pyrrolidinone 249 as a pale yellow hygroscopic solid (7.96 g, 43 mmol, 91%). The spectral data matched those reported in the literature.

$^{1}$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.74 (2H, t, $J$ 7.2, H5), 2.51 (2H, t, $J$ 8.1, H3), 1.99 (2H, app quin, $J$ 7.6, H4), 1.52 (9H, C(CH$_3$)$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 173.3 (C=O), 150.1 (C=O), 82.6 (C(CH$_3$)$_3$), 46.4 (C5), 32.8 (C3), 27.9
(C(H\textsubscript{3}))\textsubscript{3}, 17.3 (C4).

LCMS (ES\textsuperscript{+}): 208.2 ([M+Na\textsuperscript{+}]).

IR: \(\nu_{\text{max}}\) (film)/cm\textsuperscript{-1} 2979, 1782, 1750, 1712, 1366, 1308, 1252, 1150, 1018, 778.

**Phenyl 2-methylbenzoate 246**

Ester 246 was prepared according to a literature procedure.\textsuperscript{200} o-Toluic acid (5.0 g, 37 mmol) and DMF (3 drops) were dissolved in CH\textsubscript{2}Cl\textsubscript{2} (75 mL) and cooled to 0 °C, and oxalyl chloride (6.2 mL, 73.4 mmol) was added dropwise. The solution was allowed to warm to rt, stirred for 4 h, and concentrated to afford acyl chloride 245 as a pale yellow oil (5.69 g, 36.8 mmol, quant.). Sodium hydride (60% dispersion in mineral oil, 700 mg, 17.5 mmol) was added to a solution of phenol (913 mg, 9.71 mmol) in THF (20 mL) at 0 °C and stirred for 30 min. A solution of acyl chloride 245 (1.5 g, 9.7 mmol) in THF (20 mL) was added dropwise, the mixture was allowed to warm to rt, and stirred for 30 min. H\textsubscript{2}O (30 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated. Purification by flash pressure column chromatography (4% Et\textsubscript{2}O/petroleum ether) afforded ester 246 as a colorless oil (1.97 g, 9.30 mmol, 96%). The spectral data matched those reported in the literature.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 8.20 (1H, app d, \(J\) 7.95, H6), 7.40-7.56 (3H, m, H4, Ph), 7.18-7.40 (5H, m, H3, H5, Ph), 2.71 (3H, s, C\textsubscript{6}H\textsubscript{5}).

\textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}): \(\delta\) 165.8 (C=O), 150.9 (i-Ph), 141.3 (C1/C2), 132.7 (C4/Ph), 131.9 (C3/C5/Ph), 131.1 (C6), 129.5 (C4/Ph), 128.5 (C1/C2), 125.9 (C3/C5/Ph), 125.8 (C3/C5/Ph), 121.8 (C3/C5/Ph), 21.9 (CH\textsubscript{3}).

IR: \(\nu_{\text{max}}\) (film)/cm\textsuperscript{-1} 3042, 1736 (C=O), 1488, 1290, 1242, 1190, 1043, 734, 688.
Perfluorophenyl 2-methylbenzoate 253

Pentafluorophenol (1.79 g, 9.70 mmol) was added to a solution of acyl chloride 245 (1.5 g, 9.7 mmol) and triethylamine (1.62 mL, 11.6 mmol) in CH$_2$Cl$_2$ (100 mL) and stirred at rt for 20 min. NH$_4$Cl (saturated aq., 100 mL) was added, and the mixture was extracted with CH$_2$Cl$_2$. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated. Purification by flash pressure column chromatography (0.5% Et$_2$O/petroleum ether) afforded ester 253 as a colorless solid (2.73 g, 9.03 mmol, 93%).

$^1$H NMR (400 MHz, CDCl$_3$): δ 8.20 (1H, dd, $J$ 8.2, 1.3, H6), 7.56 (1H, app td, $J$ 7.6, 1.4, H4), 7.30-7.44 (2H, m, H3, H5), 2.68 (3H, s, CH$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$): δ 162.8 (C=O), 142.3, 133.9 (C4), 132.2 (C3/C5), 131.7 (C6), 126.2 (C3/C5), 125.9, 21.8 (CH$_3$).

$^{19}$F NMR (377 MHz, CDCl$_3$): δ -152.5 (m), -158.2 (app t, $J$ 21.9), -162.5 (m).

‡ C$_6$F$_5$ not observed due to insufficient signal-to-noise ratio.

IR: $\nu_{\text{max}}$(film)/cm$^{-1}$ 1760 (C=O), 1518, 1228, 1028, 995, 733.

MP: 31-32 °C.

Phenyl 2-(bromomethyl)benzoate 247

Benzyl bromide 247 was prepared according to a literature procedure. N-bromosuccinimide (1.51 g, 8.48 mmol) and benzoyl peroxide (41 mg, 0.17 mmol) were added to a solution of ester 246 (1.8 g, 8.5 mmol) in CCl$_4$ (25 mL) at 0 °C. The mixture was allowed to warm to rt and stirred under
reflux for 3.5 h. The mixture was allowed to cool to rt over 14 h and filtered. The filtrate was washed with H$_2$O, then brine. The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (3% Et$_2$O/petroleum ether) afforded benzyl bromide 247 as a colorless solid (1.70 g, 5.84 mmol, 69%). The spectral data matched those reported in the literature.

$^1$H NMR (400 MHz, CDCl$_3$): δ 8.22 (1H, dd, J 7.8, 1.2, H6), 7.51-7.63 (2H, m, H3, H4), 7.42-7.51 (3H, m, H5, Ph), 7.23-7.35 (3H, m, Ph), 5.02 (2H, s, ArCH$_2$Br).

$^{13}$C NMR (101 MHz, CDCl$_3$): δ 165.0 (C=O), 150.6 (i-Ph), 139.9 (C1/C2), 133.1 (C3/C4), 131.8 (C6), 131.8 (C3/C4), 129.5 (CS/Ph), 128.7 (CS/Ph), 128.4 (C1/C2), 126.1 (Ph), 121.7 (Ph), 31.3 (ArCH$_2$Br).

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 1733 (C=O), 1486, 1250, 1189, 1161, 1054, 1043, 689.

MP: 42-44 °C (lit 50-51 °C).

Perfluorophenyl 2-(bromomethyl)benzoate 254

Benzyl bromide 254 was prepared according to a modified literature procedure.$^{201}$ N-bromosuccinimide (1.12 g, 6.30 mmol) and benzoyl peroxide (31 mg, 0.13 mmol) were added to a solution of ester 253 (2.4 g, 6.3 mmol) in CCl$_4$ (20 mL) at 0 °C. The mixture was allowed to warm to rt and stirred under reflux for 24 h. The mixture was allowed to cool to rt and filtered. The filtrate was washed with H$_2$O, then brine. The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by flash pressure column chromatography (0.5% Et$_2$O/petroleum ether) afforded benzyl bromide 254 as a colorless solid (1.47 g, 3.86 mmol, 61%).

$^1$H NMR (400 MHz, CDCl$_3$): δ 8.26 (1H, dd, J 8.0, 1.4, H6), 7.67 (1H, app td, J 7.8, 1.4, H4), 7.59 (1H, dd, J 7.8, 1.4, H3), 7.52 (1H, app td, J 7.6, 1.4, H5), 4.95 (2H, s, ArCH$_2$Br).
$^{13}$C NMR (101 MHz, CDCl$_3$): δ 161.9 (C=O), 141.0 (C1/C2), 134.4 (C4), 132.3 (C6), 132.2 (C3), 128.9 (C5), 125.4 (C1/C2), 30.5 (ArCH$_2$Br).

$^{19}$F NMR (377 MHz, CDCl$_3$): δ -152.1 (m), -157.6 (t, $J$ 22.3), -162.1 (m).

‡ C$_4$F$_5$ peaks not observed due to insufficient signal-to-noise ratio.

HRMS (Cl$^+$): [C$_{14}$H$_{10}$BrF$_5$O$_2$N$^+$] requires 397.9815; found 397.9809.

IR: $\nu_{\text{max}}$ (film) cm$^{-1}$ 1759 (C=O), 1516, 1233, 1030, 995, 760, 700.

MP: 82-84 °C.

Methyl 2-(bromomethyl)benzoate 256

Benzyl bromide 256 was prepared according to a literature procedure.$^{201}$ N-bromosuccinimide (2.37 g, 13.3 mmol) and benzoyl peroxide (65 mg, 0.27 mmol) were added to a solution of methyl o-toluate (1.9 mL, 13 mmol) in CCl$_4$ (105 mL) at 0 °C. The mixture was allowed to warm to rt and stirred under reflux for 2.5 h. The mixture was allowed to cool to rt and filtered. The filtrate was washed with H$_2$O, then brine. The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated.

Purification by flash pressure column chromatography (3% Et$_2$O/petroleum ether) afforded benzyl bromide 256 as a colorless solid (2.09 g, 9.12 mmol, 69%). The spectral data matched those reported in the literature.

$^1$H NMR (400 MHz, CDCl$_3$): δ 7.98 (1H, dd, $J$ 7.7, 1.2, H6), 7.44-7.54 (2H, m, H3, H4), 7.38 (1H, app td, $J$ 7.6, 1.9, H5), 4.97 (2H, s, ArCH$_2$Br), 3.95 (3H, s, CO$_2$CH$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$): δ 167.0 (C=O), 139.2 (C1/C2), 132.5 (C3/C4), 131.6 (C3/C4), 131.3 (C6), 129.0 (C1/C2), 128.5 (C5), 52.3 (CO$_2$CH$_3$), 31.5 (ArCH$_2$Br).

IR: $\nu_{\text{max}}$ (film) cm$^{-1}$ 1749 (C=O), 1434, 1294, 1076, 1046, 798, 761.

265
Pyrrolidinone 250 was prepared according to a modified literature procedure.\textsuperscript{202} A solution of \textit{N,N}-diisopropylamine (0.30 mL, 2.1 mmol) in THF (5 mL) was cooled to -78 °C, and \textit{t}BuLi (2.4 M in hexane, 0.81 mL, 1.9 mmol) was added dropwise. The mixture was allowed to warm to 0 °C and stirred for 10 min before being re-cooled to -78 °C. A solution of pyrrolidinone 249 (300 mg, 1.62 mmol) in THF (5 mL) was added dropwise and stirred for 30 min. A solution of benzyl bromide 247 (519 mg, 1.78 mmol) in THF (5 mL) was added dropwise over 5 min and stirred at -70 °C to -75 °C for 2 h. The reaction mixture was diluted with Et\textsubscript{2}O (20 mL), allowed to warm to 0 °C, and quenched with NH\textsubscript{4}Cl (saturated aq., 30 mL). The mixture was extracted with Et\textsubscript{2}O, and the combined organic extracts were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated. Purification by flash pressure column chromatography (20% EtOAc/petroleum ether) afforded pyrrolidinone 250 as a colorless paste (287 mg, 0.73 mmol, 45%).

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 8.16 (1H, dd, \(J\) 7.9, 1.4, \(H9\)), 7.53 (1H, app td, \(J\) 7.5, 1.4, \(H11\)), 7.34-7.48 (4H, m, \(H10, H12, Ph\)), 7.28 (1H, m, \(Ph\)), 7.19-7.24 (2H, m, \(Ph\)), 3.72 (1H, dddd, \(J\) 11.5, 8.2, 2.7, \(H5\)), 3.66 (1H, dd, \(J\) 13.3, 5.5, \(H6\)), 3.49 (1H, dddd, \(J\) 11.2, 10.4, 2.0, \(H5'\)), 3.14 (1H, dd, \(J\) 13.3, 8.5, \(H6'\)), 2.96 (1H, app m, \(H3\)), 2.01 (1H, app m, \(H4\)), 1.75 (1H, app m, \(H4'\)), 1.51 (9H, s, C(CH\textsubscript{3})\textsubscript{3}).

\textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}): \(\delta\) 175.1 (C=O), 165.7 (C=O), 150.7 (C=O/i-Ph), 150.3 (C=O/i-Ph), 141.8 (C7/C8), 132.8 (C11), 132.1 (C10/C12/Ph), 131.3 (C9), 129.5 (C10/C12/Ph), 128.8 (C7/C8), 126.7 (C10/C12/Ph), 125.9 (Ph), 121.7 (Ph), 82.7 (C(CH\textsubscript{3})\textsubscript{3}), 45.3 (C3), 44.4 (C5), 34.1 (C6), 28.0 (C(CH\textsubscript{3})\textsubscript{3}),
HRMS (ES\textsuperscript{+}): C\textsubscript{23}H\textsubscript{25}O\textsubscript{5}N\textsubscript{2}Na ([M+Na\textsuperscript{+}]) requires 418.1630; found 418.1633.

IR: \(\nu_{\text{max}}\) (film) cm\textsuperscript{-1}: 1782, 1755, 1714, 1601, 1518, 1313, 1231, 1148, 1028, 994.

\textit{Tert}-butyl 3-(2-(methoxycarbonyl)benzyl)-2-oxopyrrolidine-1-carboxylate 257

Pyrrolidinone 257 was prepared according to a modified literature procedure.\textsuperscript{202} A solution of \(N,N\)-diisopropylamine (0.45 mL, 3.2 mmol) in THF (10 mL) was cooled to -78 °C, and \(^9\)BuLi (2.4 M in hexane, 1.24 mL, 2.97 mmol) was added dropwise. The mixture was allowed to warm to 0 °C and stirred for 10 min before being re-cooled to -78 °C. A solution of pyrrolidinone 249 (500 mg, 2.70 mmol) in THF (10 mL) was added dropwise and stirred for 30 min. A solution of benzyl bromide 256 (618 mg, 2.70 mmol) in THF (10 mL) was added dropwise over 5 min and stirred at -70 °C to -75 °C for 1 h. The reaction mixture was diluted with Et\textsubscript{2}O (40 mL), allowed to warm to 0 °C, and quenched with NH\textsubscript{4}Cl (saturated aq., 50 mL). The mixture was extracted with Et\textsubscript{2}O, and the combined organic extracts were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated. Purification by flash pressure column chromatography (20% EtOAc/petroleum ether) afforded pyrrolidinone 257 as a colorless paste (636 mg, 1.91 mmol, 71%).

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 7.90 (1H, dd, \(J\) 7.8, 1.3, \(H9\)), 7.44 (1H, app td, \(J\) 7.5, 1.4, \(H11\)), 7.28-7.34 (2H, \(H10\), \(H12\)), 3.89 (3H, s, CO\textsubscript{2}CH\textsubscript{3}), 3.73 (1H, ddd, \(J\) 11.0, 8.4, 2.6, \(H5\)), 3.66 (1H, dd, \(J\) 12.7, 4.2, \(H6\)), 3.50 (1H, ddd, \(J\) 11.5, 10.2, 2.4, \(H5'\)), 2.90 (1H, dd, \(J\) 12.7, 9.2, \(H6'\)), 2.85 (1H, app m, \(H3\)), 1.97 (1H, app m, \(H4\)), 1.72 (1H, app m, \(H4'\)), 1.53 (9H, s, C(CH\textsubscript{3})\textsubscript{3}).

\textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}): \(\delta\) 175.1 (C=O), 167.9 (C=O), 150.4 (C=O), 140.8 (C7/C8), 132.1 (C11), 267
131.6 (C10/C12), 130.8 (C9), 129.8 (C7/C8), 126.5 (C10/C12), 82.7 (C(CH3)3), 52.1 (CO2CH3), 45.4 (C3), 44.4 (C5), 34.3 (C6), 28.0 (C(CH3)3), 24.0 (C4).


IR: \( \nu_{\text{max}} \) (film)/cm\(^{-1}\) 2973, 1780, 1748, 1713, 1296, 1256, 1150, 1086, 945.

**Benzyl 2-(hydroxymethyl)benzoate 259**

Ester 259 was prepared according to a literature procedure.\(^{203}\) A suspension of phthalide (4.00 g, 29.8 mmol) was stirred in NaOH (aq., 1 M, 30 mL) at 100 °C for 1 h. The mixture was allowed to cool to rt and concentrated by creating an azeotropic mixture with toluene. The remaining solid residue was dissolved in DMF (20 mL), and benzyl bromide (3.55 mL, 29.8 mmol) was added. The mixture was stirred at rt for 2 h. H\(_2\)O (40 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated. Purification by flash pressure column chromatography (20% EtOAc/petroleum ether) afforded ester 259 as a colorless solid (5.33 g, 22.0 mmol, 74%, contains minor impurities). The spectral data matched those reported in the literature.

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.07 (1H, dd, \( J \) 7.9, 1.2, H6), 7.55, (1H, app td, \( J \) 7.5, 1.4, H4), 7.45-7.50 (3H, m, H3, Ph), 7.34-7.45 (4H, m, H5, Ph), 5.39 (2H, s, ArCO2CH\(_2\)Ph), 4.81 (2H, s, ArCH\(_2\)OH), 3.77 (1H, br s, OH).

\(^{13}\)C NMR (101 MHz, CDCl\(_3\)): \( \delta \) 167.7 (C=O), 143.1, 135.5, 133.1 (C4), 131.2 (C6), 130.3 (C3/Ph), 128.7, 128.6 (C5/Ph), 128.4 (C3/Ph), 128.2 (C5/Ph), 127.8 (C5/Ph), 67.1 (ArCO2CH\(_2\)Ph), 64.7 (ArCH\(_2\)OH).

IR: \( \nu_{\text{max}} \) (film)/cm\(^{-1}\) 3264 (br, O-H), 1764 (C=O), 1258, 1024, 733, 697.

MP: 52-54 °C.
Benzyl 2-(bromomethyl)benzoate 260

![Chemical Structure](image)

Triphenylphosphine (3.1 g, 12 mmol) was added to a solution of ester 259 (2.4 g, 9.9 mmol) and carbon tetrabromide (3.9 g, 12 mmol) in CH₂Cl₂ (100 mL) at 0 °C and stirred for 1 h. Ice-cooled hexane was added, the resulting suspension filtered, and the filtrate concentrated. Purification by flash pressure column chromatography (5% Et₂O/petroleum ether) afforded ester 260 as a yellow oil (2.66 g, 8.72 mmol, 88%).

¹H NMR (400 MHz, CDCl₃): δ 8.02 (1H, dd, J 7.8, 1.2, H₆), 7.45-7.55 (4H, m, H₄, Ph), 7.37-7.45 (4H, m, H₃, H₅, Ph), 5.41 (2H, s, ArCO₂CH₂Ph), 4.98 (2H, s, ArCH₂Br).

¹³C NMR (101 MHz, CDCl₃): δ 166.3 (C=O), 139.3, 135.6, 132.6 (C₄), 131.7 (Ph), 131.4 (C₆), 129.0, 128.6 (C₃/C₅/Ph), 128.5 (C₃/C₅/Ph), 128.4 (Ph), 128.4 (C₃/C₅/Ph), 67.1 (ArCO₂CH₂Ph), 31.5 (ArCH₂Br).

IR: νmax (film)/cm⁻¹ 3033, 1765 (C=O), 1254, 1151, 1111, 756, 697.

*Tert*-butyl 3-(2-((benzyloxy)carbonyl)benzyl)-2-oxopyrrolidine-1-carboxylate 261

![Chemical Structure](image)

Pyrrolidinone 261 was prepared according to a modified literature procedure.²⁰² A solution of N,N-diisopropylamine (0.54 mL, 3.9 mmol) in THF (12 mL) was cooled to -78 °C, and ΒuLi (2.4 M in hexane, 1.5 mL, 3.6 mmol) was added dropwise. The mixture was allowed to warm to 0 °C and stirred for 10 min before being re-cooled to -78 °C. A solution of pyrrolidinone 249 (600 mg,
3.2 mmol) in THF (12 mL) was added dropwise and stirred for 30 min. A solution of benzyl bromide 260 (990 mg, 3.2 mmol) in THF (12 mL) was added dropwise over 5 min and stirred at -70 °C to -75 °C for 1.5 h. The reaction mixture was diluted with Et₂O (40 mL), allowed to warm to 0 °C, and quenched with NH₄Cl (saturated aq., 50 mL). The mixture was extracted with Et₂O, and the combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (17% EtOAc/petroleum ether) afforded pyrrolidinone 261 as a colorless solid (557 mg, 1.36 mmol, 42%).

1H NMR (400 MHz, CDCl₃): δ 7.94 (1H, dd, J 7.8, 1.3, H9), 7.42-7.49 (3H, m, H11, Ph), 7.28-7.41 (5H, m, H10, H12, Ph), 5.35 (2H, s, OCH₂Ph), 3.61-3.70 (2H, m, H5, H6), 3.43 (1H, ddd, J 10.8, 7.5, 2.1, H5’), 3.02 (1H, dd, J 13.4, 9.2, H6’), 2.87 (1H, app m, H3), 1.88 (1H, app m, H4), 1.66 (1H, app m, H4’), 1.55 (9H, s, C(CH₃)₃).

13C NMR (101 MHz, CDCl₃): δ 175.1 (C=O), 167.2 (C=O), 150.4 (C=O), 140.9 (C7), 135.8 (i-Ph), 132.1 (C11), 131.7 (C12), 130.9 (C9), 129.9 (C8), 128.6 (Ph), 128.5 (Ph), 128.3 (Ph), 126.6 (C10), 82.7 (OC(CH₃)₃), 66.8 (OCH₂Ph), 45.4 (C3), 44.4 (C5), 34.1 (C6), 28.0 (C(CH₃)₃), 23.9 (C4).

MP: 87-88 °C.

HRMS (ES‘): [C₂₅H₂₇O₅N₂Na]⁺ ([M+Na]⁺) requires 432.1781; found 432.1778.

IR: νmax (film)/cm⁻¹ 2982, 1782, 1748, 1713, 1315, 1250, 1152, 752.

2-((1-(Tert-butoxycarbonyl)-2-oxopyrrolidin-3-yl)methyl)benzoic acid 258

Pyrrolidinone 258 was prepared according to general procedure 3 using pyrrolidinone 261 (500 mg, 1.22 mmol) and Pd/C (degussa type, 50 mg) in THF (15 mL). The reaction mixture was stirred at rt
under a H₂ atmosphere for 5.5 h to afford pyrrolidinone 258 as a colorless solid (382 mg, 1.20 mmol, quant).

¹H NMR (500 MHz, CDCl₃): δ 8.07 (1H, dd, J 7.9, 1.3, H9), 7.50 (1H, app td, J 7.6, 1.4, H11), 7.31-7.39 (2H, m, H10, H12), 3.70-3.79 (2H, m, H5, H6), 3.52 (1H, ddd, J 10.9, 9.9, 2.8, H5'), 3.09 (1H, dd, J 13.4, 9.0, H6'), 2.99 (1H, app m, H3), 1.99 (1H, app m, H4), 1.75 (1H, app m, H4'), 1.54 (9H, s, C(CH₃)₃).

¹³C NMR (126 MHz, CDCl₃): δ 175.4 (C=O), 171.9 (C=O), 150.4 (C=O), 141.8 (C7/C8), 133.0 (C11), 132.0 (C9), 131.8 (C10/C12), 128.6 (C7/C8), 126.7 (C10/C12), 82.8 (OC(CH₃)₃), 45.3 (C3), 44.4 (C5), 34.2 (C6), 28.0 (C(CH₃)₃), 24.1 (C4).

† CO₂H peak is not observed.


IR: ν max (film)/cm⁻¹ 3289, 1776, 1716, 1369, 1307, 1257, 947, 752.

MP: 105-108 °C.

**Tert-butyl 2-oxo-3-(2-((perfluorophenoxy)carbonyl)benzyl)pyrrolidine-1-carboxylate 255**

Pyrrolidinone 255 was prepared according to a modified literature procedure.²⁰⁴

N,N-dicyclohexylcarbodiimide (155 mg, 0.75 mmol) was added to a solution of pyrrolidinone 258 (200 mg, 0.63 mmol) and pentafluorophenol (127 mg, 0.69 mmol) in THF (10 mL) and stirred at rt for 1 h. The reaction mixture was filtered and concentrated. Purification by flash pressure column chromatography (40% Et₂O/petroleum ether) afforded pyrrolidinone 255 as a colorless solid.
(262 mg, 0.54 mmol, 86%).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.22 (1H, dd, $J$ 7.9, 1.3, $H_9$), 7.60 (1H, app td, $J$ 7.6, 1.3, $H_{11}$), 7.47 (1H, app d, $H_{12}$), 7.42 (1H, app td, $J$ 7.9, 1.3, $H_{10}$), 3.74 (1H, ddd, $J$ 10.8, 8.7, 2.4, $H_5$), 3.66 (1H, dd, $J$ 13.6, 5.4, $H_6$), 3.51 (1H, ddd, $J$ 11.1, 7.8, 2.1, $H_5'$), 3.11 (1H, dd, $J$ 13.5, 8.5, $H_6'$), 2.90 (1H, app m, $H_3$), 2.02 (1H, app m, $H_4$), 1.72 (1H, app m, $H_4'$), 1.52 (9H, s, C(CH$_3$)$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 174.9 (C=O), 162.7 (C=O), 150.3 (C=O), 143.1 (C7/C8), 134.1 (C11), 132.5 (C12), 131.9 (C9), 127.1 (C10), 125.9 (C7/C8), 82.8 (OC(CH$_3$)$_3$), 45.2 (C3), 44.3 (C5), 34.2 (C6), 28.0 (OC(CH$_3$)$_3$), 24.1 (C4).†

$^{19}$F NMR (377 MHz, CDCl$_3$): $\delta$ -152.5 (m), -157.8 (t, $J$ 21.8), -162.1 (m).

† Peaks corresponding to C$_6$F$_5$ system obscured.

HRMS (ES$^+$): [C$_{23}$H$_{20}$O$_5$NF$_5$Na]$^+$ ([M+Na]$^+$) requires 508.1154; found 508.1151.

IR: $\nu_{max}$ (film)/cm$^{-1}$ 2983, 1769, 1757, 1715, 1520, 1316, 1232, 1151, 1029, 995.

MP: 126-130 °C.

*Tert-butyl 1,2'-dioxo-1,3-dihydrospiro[indene-2,3':pyrrolidine]-1'-carboxylate 251*

KO'Bu (18 mg, 0.16 mmol) was added to a solution of pyrrolidinone 255 (40 mg, 0.082 mmol) in toluene (2 mL) and stirred at -10 °C for 2.5 h. NH$_4$Cl (saturated aq.) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated. Purification by preparatory TLC (40% Et$_2$O/petroleum ether) afforded spiro-pyrrolidinone 251 as a colorless solid (12 mg, 0.040 mmol, 48%).
\(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta 7.74\) (1H, app d, \(J 7.6, H6\)), \(7.64\) (1H, app t, \(J 7.6, H8\)), \(7.50\) (1H, app d, \(J 7.6, H9\)), \(7.40\) (1H, app t, \(J 7.6, H7\)), \(4.15\) (1H, app m, \(H5_A\)), \(3.86\) (1H, app td, \(J 9.8, 2.2, H5_B\)), \(3.77\) (1H, d, \(J 17.0, H5' A\)), \(3.01\) (1H, d, \(J 17.0, H5' B\)), \(2.50\) (1H, ddd, \(J 12.8, 7.7, 2.2, H4_A\)), \(1.54\) (9H, s, C(CH\(_3\))\(_3\)).

\(^13\)C NMR (126 MHz, CDCl\(_3\)): \(\delta 203.0\) (C\(_2'\)), \(171.8\) (C2), \(153.3\) (NCO\(_2\)Bu), \(150.1\) (C3'/C4'), \(135.6\) (C8), \(134.4\) (C3'/C4'), \(127.9\) (C7), \(126.3\) (C9), \(124.8\) (C6), \(83.3\) (OC(CH\(_3\))\(_3\)), \(60.5\) (C3), \(44.0\) (C5), \(37.6\) (C5'), \(28.7\) (C4), \(28.0\) (OC(CH\(_3\))\(_3\)).

HRMS (ES\(^+\)): [C\(_{17}\)H\(_{19}\)O\(_4\)N\(_2\)Na]\(^+\) (\([M+Na]\)^+) requires 324.1206; found 324.1206.

IR: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 1781, 1746, 1714, 1368, 1312, 1278, 1153, 960, 749.

MP: 74-76 °C.

1-Methylindolin-2-one 258

Oxindole 258 was prepared according to a literature procedure.\(^{205}\) 1-Methylisatin (5.0 g, 31 mmol) was dissolved in hydrazine-hydrate (78-82%, 34 mL) and stirred at 95 °C for 2.5 h. The reaction mixture was allowed to cool to rt, ice-cooled H\(_2\)O (40 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated. Recrystallization from EtOAc/hexane afforded oxindole 258 as an orange solid (2.45 g, 16.6 mmol, 54%). The spectral data matched those reported in the literature.

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 7.21-7.33\) (2H, m), \(7.04\) (1H, app t, \(J 7.5\)), \(6.82\) (1H, app d, \(J 7.8\)), \(3.52\) (2H, s, ArCH\(_2\)), \(3.21\) (3H, s, CH\(_3\)).

\(^13\)C NMR (101 MHz, CDCl\(_3\)): \(\delta 175.0\) (C=O), 145.1, 127.8, 124.4, 124.2, 122.3, 108.0, 35.7 (CH\(_3\)), 26.1 (ArCH\(_2\)).
IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 1703 (C=O), 1614, 1470, 1347, 1124, 750.

Benzyl 2-formylbenzoate 259

![Benzyl 2-formylbenzoate](image)

Ester 259 was prepared according to a literature procedure.$^{206}$ $K_2CO_3$ (1.1 g, 8.0 mmol) and benzyl bromide (0.80 mL, 6.7 mmol) were added to a solution of 2-formylbenzoic acid (1.0 g, 6.7 mmol) in $N,N$-DMF (6 mL). The reaction mixture was stirred at rt for 2.5 h, diluted with EtOAc, and washed three times with H$_2$O, then brine. The organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated to afford ester 259 as a colorless liquid (1.6 g, 6.7 mmol, quant). The spectral data matched those reported in the literature.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 10.64 (1H, s CHO), 8.01 (1H, app m), 7.95 (1H, app m), 7.60-7.70 (2H, m), 7.45-7.50 (2H, m, Ph), 7.34-7.45 (3H, m, Ph), 5.43 (2H, s, OCH$_2$Ph).

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 192.0 (C=O), 166.0 (C=O), 137.1, 135.2, 132.9, 132.4, 131.9, 130.4, 128.7 (Ph), 128.6, 128.4 (Ph), 128.4 (Ph), 67.2 (OCH$_2$Ph).

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3041, 1712, 1695, 1256, 1193, 1073, 1029, 747, 696.

2-((1-Methyl-2-oxindolin-3-yl)methyl)benzoic acid 261

Oxindole 260 was prepared according to a modified literature procedure.$^{207}$ A solution of oxindole 258 (1.20 g, 8.15 mmol), ester 259 (2.15 g, 8.97 mmol), and piperidine (81 µL, 0.82 mmol) in EtOH
was stirred at reflux for 17 h. The mixture was allowed to cool to rt and concentrated. The remaining residue was dissolved in EtOAc and washed 3 times with NaHCO₃ (saturated aq.) and twice with HCl (aq., 1 M). The organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (7% EtOAc/toluene) afforded oxindole 260 as an orange oil and as a 1:3 mixture of E/Z alkene isomers (1.70 g, 4.60 mmol, 56%).

Oxindole 261 was prepared according to general procedure 3, using oxindole 260 (1.6 g, 4.3 mmol) and Pd/C (degussa, 160 mg) in THF (45 mL). The reaction mixture was stirred at rt under a H₂ atmosphere for 3.5 h to afford oxindole 261 as a pale yellow oil (1.22 g, 4.33 mmol, quant).

¹H NMR (400 MHz, CDCl₃): δ 9.62 (1H, br s, CO₂H), 8.09 (1H, dd, J 7.8, 1.2, H11), 7.47 (1H, app td, J 7.5, 1.3, H13), 7.36 (1H, app td, J 7.5, 1.0, H12), 7.19-7.30 (2H, m, H5/H6, H14), 6.93 (1H, app t, J 7.5, H5/H6), 6.80 (1H, app d, J 7.8, H4/H7), 6.77 (1H, app d, J 7.4, H4/H7), 3.97 (1H, t, J 7.3, H3), 3.84 (1H, dd, J 13.3, 6.4, H8), 3.38 (1H, dd, J 13.3, 8.5, H8'), 3.20 (3H, s, CH₃).

¹³C NMR (101 MHz, CDCl₃): δ 177.8 (C=O), 171.7 (C=O), 143.9, 140.4, 132.6 (C14), 132.4 (C13), 131.8 (C11), 129.3, 128.6, 127.9 (C5/C6), 127.0 (C12), 124.6 (C4/C7), 122.2 (C5/C6), 107.9 (C4/C7), 46.2 (C3), 35.4 (C8), 26.2 (CH₃).

HRMS (ES⁺): [C₁₇H₁₅O₃N⁺Na⁺]⁺ ([M+Na⁺]⁺) requires 304.0944; found 304.0941.

IR: νmax (film)/cm⁻¹ 3060, 1706, 1638, 1469, 1254, 1124, 1042, 746.

Perfluorophenyl 2-((1-methyl-2-oxoindolin-3-yl)methyl)benzoate 263

Oxindole 263 was prepared according to a modified literature procedure.²⁰⁴ N,N-dicyclohexylcarbodiimide (244 mg, 1.19 mmol) was added to a solution of oxindole 261
(278 mg, 0.99 mmol) and pentafluorophenol (183 mg, 0.99 mmol) in THF (12 mL) and stirred at rt for 45 min. The reaction mixture was filtered and concentrated. Purification by flash pressure column chromatography (15% EtOAc/petroleum ether) afforded oxindole 263 as a yellow paste (352 mg, 0.79 mmol, 80%).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.29 (1H, dd, J 8.0, 1.3, H11), 7.63 (1H, app td, J 7.6, 1.4, H13), 7.48 (1H, app td, J 7.8, 1.2, H12), 7.43 (1H, app d, J 7.7, H14), 7.26 (1H, app t, J 7.7, H5/H6), 6.94 (1H, app td, J 7.6, 0.9, H5/H6), 6.76-6.85 (2H, m, H4, H7), 3.69-3.85 (2H, m, H3, H8), 3.41 (1H, dd, J 13.0, 7.7, H8' ), 3.21 (3H, s, CH$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 177.1 (C=O), 162.5 (C=O), 143.9, 142.5, 133.8 (C13), 133.1 (C14), 132.2 (C11), 128.3, 128.0 (C5/C6), 127.4 (C12), 126.0, 124.4 (C4/C7), 122.2 (C5/C6), 108.0 (C4/C7), 46.0 (C3), 35.5 (C8), 26.1 (CH$_3$).

$^{19}$F NMR (377 MHz, CDCl$_3$): $\delta$ -152.3 (m), -158. (t, J 22.0), -162.3 (m).

‡ C$_6$F$_5$ peaks not observed due to insufficient signal-to-noise ratio.

HRMS (ES$^+$): [C$_{23}$H$_{15}$O$_3$NF$_5$]$^+$ ([M+H]$^+$) requires 448.0967; found 448.0966.

IR: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3081, 2947, 1709 (C=O), 1682 (C=O), 1611, 1470, 1377, 750.

$^1$'-Methylspiro[indene-2,3'-indoline]-1,2'(3H)-dione 264

Racemic: K$_2$CO$_3$ (aq., 50% w/w, 56 µL, 0.40 mmol) was added to a suspension of oxindole 263 (36 mg, 0.080 mmol) and tetrabutylammonium bromide (2.6 mg, 0.88 mmol) in toluene (1.6 mL) and stirred at rt for 5 h. NaHCO$_3$ (saturated aq., 3 mL) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$, filtered,
and concentrated. Purification by flash pressure column chromatography (40% Et₂O/petroleum ether) afforded oxindole 264 as a colorless solid (14 mg, 0.053 mmol, 66%).

**Asymmetric:** A suspension of oxindole 263 (250 mg, 0.56 mmol) and the catalyst (31 mg, 0.056 mmol) in m-xylene (22 mL) was stirred at 0 °C for 10 min. K₂CO₃ (aq., 50% w/w, 383 µL, 2.79 mmol) was added, and the reaction mixture was stirred at 0 °C for 48 h. NH₄Cl (saturated aq.) was added, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated. Purification by flash pressure column chromatography (40% Et₂O/petroleum ether) afforded oxindole 264 as a colorless solid (110 mg, 0.42 mmol, 76%, e.r. 95:5). The enantioenrichment could be augmented by recrystallization from 1:1 Et₂O/petroleum (e.r. > 98:2).

¹H NMR (400 MHz, CDCl₃): δ 7.82 (1H, app d, J 7.7, H10), 7.71 (1H, app td, J 7.7, 1.2, H12), 7.62 (1H, app d, J 7.7, H13), 7.47 (1H, app t, J 7.5, H11), 7.33 (1H, app td, J 7.8, 1.3, H5/H6), 7.01 (1H, app td, J 7.5, 1.0, H5/H6), 6.88-6.96 (2H, m, H4, H7), 3.83 (1H, d, J 17.2, H15), 3.45 (1H, d, J 17.2, H15'), 3.30 (3H, s, CH₃).

¹³C NMR (101 MHz, CDCl₃): δ 199.8 (C=O), 174.9 (C=O), 153.8, 144.8, 135.6 (C12), 135.1, 130.2, 128.9 (C5/C6), 128.1 (C11), 126.5 (C13), 125.5 (C10), 122.9 (C5/C6), 122.0 (C4/C7), 108.6 (C4/C7), 63.0 (C3), 37.6 (C15), 26.8 (CH₃).

HRMS (ES⁺): [C₁₇H₁₃O₂N⁺Na⁺] requires 286.0839; found 286.0839.

IR: vₘₐₓ (film)/cm⁻¹ 3045, 2891, 1722, 1697, 1609, 1468, 1346, 1275, 1088, 750.

MP: 178-180 °C.

[α]₂⁰° = -94 (c = 0.1, CHCl₃, e.r. 95:5).

Chiral HPLC: (Chiralpak ADH, 10% iPrOH, 90% hexane, 1.0 mL/min, λ = 254 nm, 20 µL injection) τ₀ (minor) = 17.1 min, τ₀ (major) = 22.9 min.
6.2.2 Biochemical Experimental Procedures

2-OG Competition

Solutions containing H3K36me2 peptide (20 μM), Fe\textsuperscript{II}(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (20 μM), sodium-L-ascorbate (200 μM), and 2-OG (0.4 μM, 0.8 μM, 1.2 μM, 2.0 μM, 4.0 μM, 6.0 μM) in MES buffer (pH 7.0) were prepared in a deep 96-well plate (total volume per well = 600 μL). A solution of KDM2A in MES buffer (50 mM MES, 100 mM NaCl [pH 7.0]) was prepared (total volume = 12 mL) and divided equally into 3 falcon tubes, to which was added (S,S)-\textsuperscript{108} (tube A: 0 nM, tube B: 500 nM, tube C: 1000 nM). After incubation at 0 °C for 45 min, 600 μL of solution A was added to each well, and the progress of demethylation was monitored via automated sampling using the RapidFire mass spectrometry platform (Agilent Technologies, Wakefield MA). The relative concentration of H3K36me1 was determined by integration of the extracted ion chromatogram corresponding to the H3K36me1 peptide using MassHunter software (Agilent Technologies). This procedure was repeated with solutions B and C using freshly-prepared 2-OG wells. Initial rates were determined by calculating the slope of the linear region of the reaction (see section 8.2 in the appendix for raw data and regression analysis).

Peptide Competition

Solutions containing 2-OG (20 μM), Fe\textsuperscript{II}(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (20 μM), sodium-L-ascorbate (200 μM), and H3K36me2 peptide (2 μM, 4 μM, 10 μM, 20 μM, 32 μM, 80 μM) in MES buffer (pH 7.0) were prepared in a deep 96-well plate (total volume per well = 600 μL). A solution of KDM2A in MES buffer (50 mM MES, 100 mM NaCl [pH 7.0]) was prepared (total volume = 12 mL) and divided equally into 3 falcon tubes, to which was added (S,S)-\textsuperscript{108} (tube A: 0 nM, tube B: 500 nM, tube C: 1000 nM). After incubation at 0 °C for 45 min, 600 μL of solution A was added to each well, and the progress of demethylation was monitored via automated sampling using the RapidFire mass spectrometry platform (Agilent Technologies, Wakefield MA). The relative concentration of H3K36me1 was determined by integration of the extracted ion chromatogram corresponding to
the H3K36me1 peptide using MassHunter software (Agilent Technologies). This procedure was repeated with solutions B and C using freshly-prepared peptide wells. Initial rates were determined by calculating the slope of the linear region of the reaction (see section 8.2 in the appendix for raw data and regression analysis).

**Bio-layer Interferometry (BLI)**

The following stock solutions were prepared using a freshly prepared HEPES buffer (25 mM HEPES, 50 mM NaCl, 0.5% glycerol [pH 7.5]).

1. Blank solution (1000 μL)
   - [(NH₄)₂Fe³⁺(SO₄)₂·6H₂O] – 10 μM
2. Biotin control (500 μL)
   - D-Biotin – 10 μM
   - [(NH₄)₂Fe³⁺(SO₄)₂·6H₂O] – 10 μM
3. **225** positive control (500 μL)
   - **225** – 10 μM
   - [(NH₄)₂Fe³⁺(SO₄)₂·6H₂O] – 10 μM
4. **224** (500 μL)
   - **224** – 10 μM
   - [(NH₄)₂Fe³⁺(SO₄)₂·6H₂O] – 10 μM
5. KDM2a solution (200 μL)
   - KDM2a – 5 μM
   - [(NH₄)₂Fe³⁺(SO₄)₂·6H₂O] – 10 μM
   - 2-oxoglutarate (2-OG) – 10 μM

Binding experiments were carried out using a FortéBio Octet Red96 instrument. The strepavidin-coated tips (FortéBio 18-5019) were initially left in the blank solution for 120 s to obtain a baseline measurement. The tips were subsequently transferred to the solutions containing the biotinylated
compounds and loaded for 300 s and then returned to the blank solution for a further 300 s. The tips were incubated in the KDM2a solution for 450 s and then returned to the blank solution.

**Photoaffinity Labelling of KDM2A with 230 or 240**

A solution containing the following components was prepared using MES buffer (50 mM MES, 100 mM NaCl [pH 7.0]) and incubated at 0 °C for 45 min.

- KDM2a – 1 µM
- **230** or **240** – 4 µM
- Ni²⁺Cl₂ – 50 µM
- 2-OG – 10 µM

The solution was irradiated in 60 µL aliquots with 350 nm uv-light at 1-4 °C using a CaproBox™ (Caprotec, Berlin). Irradiation times longer than 5 min were carried out in 5 min pulses, separated by 2 min intervals to prevent sample warming. Samples were analyzed by liquid-chromatography/mass-spectrometry (see subsection 8.3.1 in the appendix for mass spectra corresponding to UV-activation of 230).
7. References


Mix, K. A.; Raines, R. T., Optimized Diazot Scaffold for Protein Esterification. Org. Lett. 2015, 17 (10), 2358-2361.


Cox, O. B.; Spencer, J.; Brennan, P. In Design and utilisation of a poised fragment library in the search for inhibitors of PHIP(2), an atypical bromodomain, American Chemical Society: 2016; pp MEDI-399.


8. **APPENDIX**

8.1 **Biochemical Assay Data**

8.1.1 **AlphaScreen Data**

**KDM2A**

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295
**JMJD2D**

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**JMJD3A**

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**8.1.2 RapidFire Data**

**KDM2A**

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<th>pIC50 SD</th>
<th>pIC50 SEM</th>
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8.2 2-OG and Peptide Competition

8.2.1 Raw Data Graphs

2-OG Competition

![Graphs showing 2-OG competition with different inhibitor concentrations.](image-url)
Peptide Competition
### 8.2.2 Regression Analysis

#### 2-OG Competition Michaelis-Menten Plot

<table>
<thead>
<tr>
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<tr>
<td><strong>Best-fit values</strong></td>
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<tr>
<td>$V_{\text{max}}$</td>
<td>20743</td>
<td>13233</td>
<td>9020</td>
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<td>$K_{m}$</td>
<td>0.8909</td>
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<td>0.06468</td>
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<td><strong>95% CI (profile likelihood)</strong></td>
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<tr>
<td>$V_{\text{max}}$</td>
<td>18318 to 23805</td>
<td>12618 to 13899</td>
<td>8183 to 10004</td>
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<tr>
<td>$K_{m}$</td>
<td>0.6455 to 1.237</td>
<td>0.578 to 0.756</td>
<td>0.48 to 0.8431</td>
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<td><strong>Goodness of Fit</strong></td>
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<td>Degrees of Freedom</td>
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<td>$R$ square</td>
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<td>$Sy.x$</td>
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<td><strong>Constraints</strong></td>
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<td>$K_{m}$</td>
<td>$K_{m} &gt; 0$</td>
<td>$K_{m} &gt; 0$</td>
<td>$K_{m} &gt; 0$</td>
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<td># of X values</td>
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<td># Y values analyzed</td>
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2-OG Competition Lineweaver-Burk Plot

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<td><strong>Best-fit values ± SE</strong></td>
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<td>Slope</td>
<td>3.895e-005 ± 3.466e-006</td>
<td>4.713e-005 ± 9.89e-007</td>
<td>7.454e-005 ± 2.244e-006</td>
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<tr>
<td>Y-intercept</td>
<td>5.421e-005 ± 8.417e-006</td>
<td>7.887e-005 ± 2.402e-006</td>
<td>0.0001064 ± 5.451e-006</td>
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<tr>
<td>X-intercept</td>
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<td>-1.673</td>
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<td>1/slope</td>
<td>25673</td>
<td>21216</td>
<td>13416</td>
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| **95% Confidence Intervals** |                     |                      |                      |
| Slope                | 2.933e-005 to 4.857e-005 | 4.439e-005 to 4.988e-005 | 6.831e-005 to 8.077e-005 |
| Y-intercept          | 3.085e-005 to 7.758e-005 | 7.22e-005 to 8.554e-005 | 9.125e-005 to 0.0001215 |
| X-intercept          | -2.542 to -0.6608     | -1.91 to -1.46       | -1.755 to -1.145     |

| **Goodness of Fit** |                     |                      |                      |
| R square            | 0.9693               | 0.9982               | 0.9964               |
| Sy.x                | 0.00001352           | 0.00003859           | 0.000008757          |

| **Is slope significantly non-zero?** |                     |                      |                      |
| F                                 | 126.3                | 2271                 | 1103                 |
| DFn, DFd                          | 1, 4                 | 1, 4                 | 1, 4                 |
| P value                           | 0.0004               | <0.0001              | <0.0001              |
| Deviation from zero?              | Significant          | Significant          | Significant          |

| **Equation** |                     |                      |                      |
| Y = 3.895e-005*X + 5.421e-005     | Y = 4.713e-005*X + 7.887e-005 | Y = 7.454e-005*X + 0.0001064 |

| **Data** |                     |                      |                      |
| Number of X values | 6                      | 6                      | 6                      |
| Maximum number of Y Replicates | 1                      | 1                      | 1                      |
| Total number of values | 6                      | 6                      | 6                      |
| Number of missing values | 0                      | 0                      | 0                      |
# Peptide Competition Michaelis-Menten Plot

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<th>([S,S\text{-108}] = 250) nM</th>
<th>([S,S\text{-108}] = 500) nM</th>
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<td><strong>Best-fit values</strong></td>
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<td>(V_{\text{max}})</td>
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<td>26670</td>
<td>16719</td>
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<td>(K_{\text{m}})</td>
<td>4.98</td>
<td>5.537</td>
<td>4.784</td>
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<td><strong>Std. Error</strong></td>
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<tr>
<td>(V_{\text{max}})</td>
<td>1840</td>
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<td>920.8</td>
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<tr>
<td>(K_{\text{m}})</td>
<td>0.5982</td>
<td>0.4497</td>
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<td><strong>95% CI (profile likelihood)</strong></td>
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<td></td>
</tr>
<tr>
<td>(V_{\text{max}})</td>
<td>44045 to 54121</td>
<td>24806 to 28744</td>
<td>14295 to 19842</td>
</tr>
<tr>
<td>(K_{\text{m}})</td>
<td>3.589 to 6.868</td>
<td>4.408 to 6.936</td>
<td>2.777 to 8.14</td>
</tr>
<tr>
<td><strong>Goodness of Fit</strong></td>
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<tr>
<td>Degrees of Freedom</td>
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<tr>
<td>(R^{2})</td>
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<td>(S_{y.x})</td>
<td>1440</td>
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<td>736.3</td>
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<td><strong>Constraints</strong></td>
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<tr>
<td>(K_{\text{m}})</td>
<td>(K_{\text{m}} &gt; 0)</td>
<td>(K_{\text{m}} &gt; 0)</td>
<td>(K_{\text{m}} &gt; 0)</td>
</tr>
<tr>
<td><strong>Number of points</strong></td>
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<td></td>
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<tr>
<td># of X values</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td># Y values analyzed</td>
<td>6</td>
<td>6</td>
<td>6</td>
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# Peptide Competition Linewaver-Burk Plot

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<tr>
<th></th>
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<tbody>
<tr>
<td><strong>Best-fit values ± SE</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.0001034 ± 2.457e-006</td>
<td>0.0001778 ± 7.781e-006</td>
<td>0.0002195 ± 8.41e-006</td>
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<tr>
<td>X-intercept</td>
<td>-0.2018</td>
<td>-0.2374</td>
<td>-0.312</td>
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<tr>
<td>1/slope</td>
<td>9667</td>
<td>5623</td>
<td>4555</td>
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<table>
<thead>
<tr>
<th>95% Confidence Intervals</th>
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<th></th>
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<tbody>
<tr>
<td>Slope</td>
<td>9.662e-005 to 0.0001103</td>
<td>0.0001562 to 0.0001994</td>
<td>0.0001962 to 0.0002429</td>
</tr>
<tr>
<td>Y-intercept</td>
<td>1.769e-005 to 2.406e-005</td>
<td>3.215e-005 to 5.23e-005</td>
<td>5.761e-005 to 7.939e-005</td>
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<tr>
<td>X-intercept</td>
<td>-0.2451 to -0.163</td>
<td>-0.3257 to -0.1657</td>
<td>-0.396 to -0.2424</td>
</tr>
</tbody>
</table>

| **Goodness of Fit**     |                     |                       |                       |
| R square                | 0.9977               | 0.9924                | 0.9942                |
| Sy.x                    | 0.000002071          | 0.00000656            | 0.00000709            |

| **Is slope significantly non-zero?** |                     |                       |                       |
| F          | 1773                | 522.4                 | 681.5                 |
| DFn, DFd   | 1, 4                | 1, 4                  | 1, 4                  |
| P value    | <0.0001             | <0.0001               | <0.0001               |
| Deviation from zero?   | Significant          | Significant            | Significant            |

| **Equation**            |                     |                       |                       |
| Y = 0.0001034*X + 2.087e-005 | Y = 0.0001778*X + 4.222e-005 | Y = 0.0002195*X + 6.85e-005 |

| **Data**                |                     |                       |                       |
| Number of X values      | 6                   | 6                      | 6                      |
| Maximum number of Y replicates | 1               | 1                      | 1                      |
| Total number of values  | 6                   | 6                      | 6                      |
| Number of missing values| 0                   | 0                      | 0                      |
8.3  Photoaffinity Labelling

8.3.1  Mass Spectra of UV-Activation of 230

Progress of photoactivation of 230 (mw 572.33) by irradiation with UV-light (250 nm) for 1 min, 2 min, 3 min, 5 min, 10 min, 15 min.
8.3.2 Distribution of Photo-Crosslinked Residues on KDM2A

KDM2A sequence (residues 1-519): Blue bars indicate relative proportion of peptides that contain covalently-modified residue.
## 8.4 HPLC Traces and X-ray Crystallography

### 8.4.1 HPLC Traces

![HPLC trace diagram](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time</th>
<th>Area mAU*min</th>
<th>Height mAU</th>
<th>Relative Area %</th>
<th>Relative Height %</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.a.</td>
<td>Peak 1</td>
<td>n.a. 27.457</td>
<td>n.a. 23.060</td>
<td>n.a. 24.426</td>
<td>n.a. 50.37</td>
<td>n.a. 56.36</td>
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<tr>
<td>1</td>
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<td>34.633</td>
<td>22.723</td>
<td>18.910</td>
<td>49.63</td>
<td>43.64</td>
<td>n.a.</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
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<td>100.00</td>
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<td>Peak Name</td>
<td>Retention Time (min)</td>
<td>Area (mAU*min)</td>
<td>Height (mAU)</td>
<td>Relative Area (%)</td>
<td>Relative Height (%)</td>
<td>Amount</td>
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<td>--------------</td>
<td>-------------------</td>
<td>---------------------</td>
<td>--------</td>
</tr>
<tr>
<td>1</td>
<td>Peak 1</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>27.787</td>
<td>5.507</td>
<td>5.390</td>
<td>87.68</td>
<td>89.06</td>
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<tr>
<td></td>
<td></td>
<td>35.893</td>
<td>0.774</td>
<td>0.662</td>
<td>12.32</td>
<td>10.94</td>
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<tr>
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<td>6.281</td>
<td>6.053</td>
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</table>

**Integration Results**

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time (min)</th>
<th>Area (mAU*min)</th>
<th>Height (mAU)</th>
<th>Relative Area (%)</th>
<th>Relative Height (%)</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Peak 1</td>
<td>27.480</td>
<td>474.103</td>
<td>423.118</td>
<td>99.61</td>
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<td>33.530</td>
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<td>475.979</td>
<td>425.167</td>
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<td>100.00</td>
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</tbody>
</table>

**Diagram:**

- Chart showing the response in mAU and retention time in minutes.
- Peaks 1 and 2 are identified with retention times 27.787 and 35.893 minutes, respectively.
<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time (min)</th>
<th>Area (mAU*min)</th>
<th>Height (mAU)</th>
<th>Relative Area (%)</th>
<th>Relative Height (%)</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.a.</td>
<td>Peak 1</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
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<td>6.418</td>
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<td>32.503</td>
<td>41.297</td>
<td>30.945</td>
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<td>82.82</td>
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</table>

**Integration Results**

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Retention Time (min)</th>
<th>Area (mAU*min)</th>
<th>Height (mAU)</th>
<th>Relative Area (%)</th>
<th>Relative Height (%)</th>
<th>Amount</th>
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<tbody>
<tr>
<td>n.a.</td>
<td>Peak 1</td>
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<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
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<td>2.924</td>
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<td>Area</td>
<td>Height</td>
<td>Relative Area</td>
<td>Relative Height</td>
<td>Amount</td>
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<tr>
<td>-----</td>
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<td>--------</td>
<td>---------------</td>
<td>-----------------</td>
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<tr>
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<td>n.a.</td>
<td>n.a.</td>
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### Integration Results

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<th>Peak Name</th>
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<th>Height mAU</th>
<th>Relative Area %</th>
<th>Relative Height %</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
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<td>299.556</td>
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<td>100.00</td>
<td>n.a.</td>
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<tr>
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<td>16.723</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>Relative Area (%)</td>
<td>Relative Height (%)</td>
<td>Amount</td>
</tr>
<tr>
<td>-----</td>
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<td>-------------</td>
<td>------------------</td>
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### Integration Results

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<th>No.</th>
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<th>Area</th>
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<th>Relative Area</th>
<th>Relative Height</th>
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<td>n.a.</td>
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Integration Results

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<th>Area (mAU*min)</th>
<th>Height (mAU)</th>
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<td>1</td>
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<td>15.997</td>
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<td>Relative Area (%)</td>
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### X-ray Crystallography Data

X-ray Crystallographic Data and Structure Refinement for 77, 007JDJ14

<table>
<thead>
<tr>
<th>Identification code</th>
<th>007JDJ14</th>
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<tbody>
<tr>
<td>Empirical formula</td>
<td>C_{17}H_{15}BrClN_{3}O_{2}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>408.68</td>
</tr>
<tr>
<td>Temperature</td>
<td>150 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>C 2/c</td>
</tr>
</tbody>
</table>
| Unit cell dimensions| a = 41.1184 Å, α = 90°  
                            b = 8.77720 Å, β = 113.8427°  
                            c = 22.7766 Å, γ = 90° |
| Volume              | 7518.66 Å³ |
| Z,Z'                | Z: 16 Z': 0 |
| Density (calculated)| 1.44 Mg m⁻³ |
| Absorption coefficient| 2.342 mm⁻¹ |
| F(000)              | 3296.0 |
| Crystal size        | 0.04 x 0.18 x 0.40 mm³ |
| Theta range for data collection | 5.122° to 27.496° |
| Reflections collected| 15307 |
| Independent reflections | 8581 |
| Absorption correction| Multi-scan |
| Refinement method   | Full-matrix least squares on F² |
| Goodness-of-fit on F²| 0.974 |
| Final R indices [I>2σ(I)] | R¹ = 0.0656, wR² = 0.1703 |
| R indices (all data) | R¹ = 0.0888, wR² = 0.1852 |
X-ray Crystallographic Data and Structure Refinement for 100, 030JDJ15

Identification code: 030JDJ15
Empirical formula: C_{23}H_{21}ClN_{3}O_{2.5}
Formula weight: 414.89
Temperature: 150 K
Wavelength: 1.54180 Å
Crystal system: Triclinic
Space group: P 1
Unit cell dimensions:
- a = 8.6005 Å
- b = 8.7390 Å
- c = 15.6102 Å
- α = 103.198°
- β = 92.916°
- γ = 98.395°
Volume: 1125.70 Å^{3}
Z, Z’: Z: 2 Z’: 0
Density (calculated): 1.22 Mg m^{-3}
Absorption coefficient: 1.706 mm^{-1}
F(000): 434.0
Crystal size: 0.01 x 0.03 x 0.20 mm^{3}
Theta range for data collection: 5.218° to 75.980°
Reflections collected: 22875
Independent reflections: 8722
Absorption correction: Multi-scan
Refinement method: Full-matrix least squares on F^2
Goodness-of-fit on F^2: 0.996
Final R indices [I>2σ(I)]: R^1 = 0.0753, wR^2 = 0.2132
R indices (all data): R^1 = 0.0800, wR^2 = 0.2208

Crystallographic Information files
The crystallographic information files (.cif) for all X-ray structures in this thesis can be found in the attached CD.