COMMUNICATION

Synthesis of the C50 Diastereomers of the C33-C51 Fragment of Stambomycin D

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Yongchen Wang,[a‡] Venkaiah Chintalapudi,[a‡] Haraldur G. Gudmundsson,[a] Gregory L. Challis[bc] and Edward A. Anderson*[a]

As products of genome mining, the stereochemical assignment of the macrolide antibiotics stambomycins A–D has been made on the basis of sequence analysis of the associated polyketide synthase, aside from two stereocentres at C28 and C50. Here we describe syntheses of two C50 diastereomers of the C33–C51 region of the stambomycins, which support the PKS-based configurational assignment, and establish a strategy suitable for access to the extended stambomycin framework.

The stambomycins are a family of macrolide natural products discovered and isolated from *Streptomyces ambofaciens* by Challis, Aigle and co-workers using a genome mining approach (Figure 1a).[1-4] Differing only in the nature of their C26 alkyl sidechains, these molecules exhibit potent anti-tumour and anti-bacterial activities. The connectivity, and the identity of the majority of the stereocentres of the natural product framework, could be predicted by sequence analysis of the polyketide synthases responsible for its assembly,[5-8] with the exception of the stereocentres at C28 and C50 which are installed by cytochrome P450-catalyzed oxidations.[9] While an unequivocal validation of stereochemistry remains to be achieved, confirmation of the sequence analysis predicted stereostructure would underline the value of this method,[10-14] compared to other structural assignment techniques such as NMR spectroscopy[15-18] (including associated computational analysis),[19-24] which can be ambiguous for molecules of such complexity.[25-28]

We recently described a synthesis of the C1–C27 region of stambomycin D, in which the spectroscopic data of the synthetic truncate showed a good match with that of the natural product.[29] Continuing from that work, we report here the synthesis of two C50 diastereomers of the C33–C51 segment of the stambomycins, and comparison of the associated spectroscopic data with the natural product, which further supports the sequence analysis based stereochemical prediction. This study also identifies suitable conditions for deprotection of the target fragments, bearing in mind the potential sensitivities in these molecules (specifically the C50 allylic oxygen functionality) and, for future work, compatibility

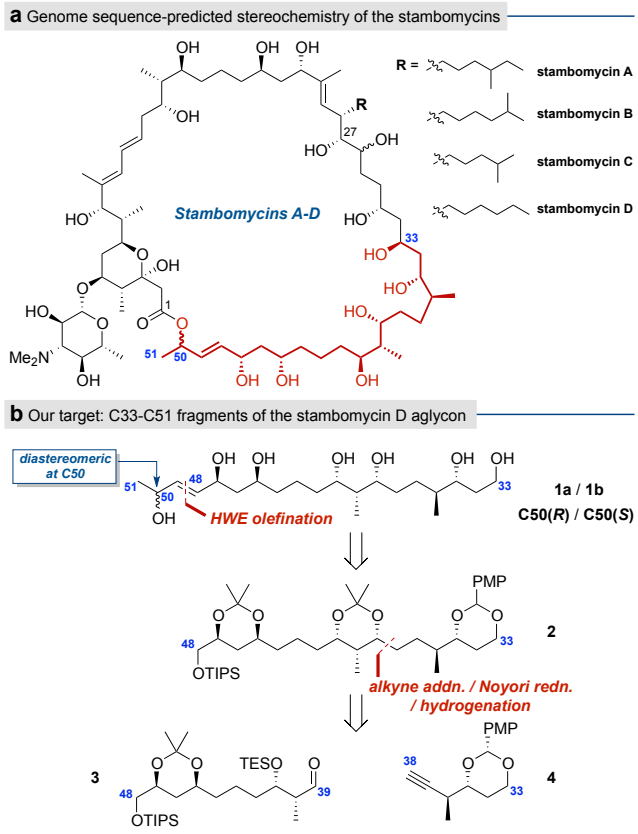


Figure 1. (a) Stambomycins A–D; (b) the C50 diastereomeric C33–C51 fragments **1a** and **1b**, and planned retrosynthesis.

[a] Chemistry Research Laboratory, University of Oxford, 12 Mansfield Road, Oxford, OX1 3TA, U.K.
[b] Department of Chemistry and Warwick Integrative Synthetic Biology Centre, University of Warwick, Coventry, CV4 7AL, U.K.
[c] Department of Biochemistry and Molecular Biology and ARC Centre of Excellence for Innovations in Peptide and Protein Science, Biomedicine Discovery Institute, Monash University, Clayton, Victoria 3800, Australia

† Electronic supplementary information (ESI) available: Experimental details and data. See DOI:

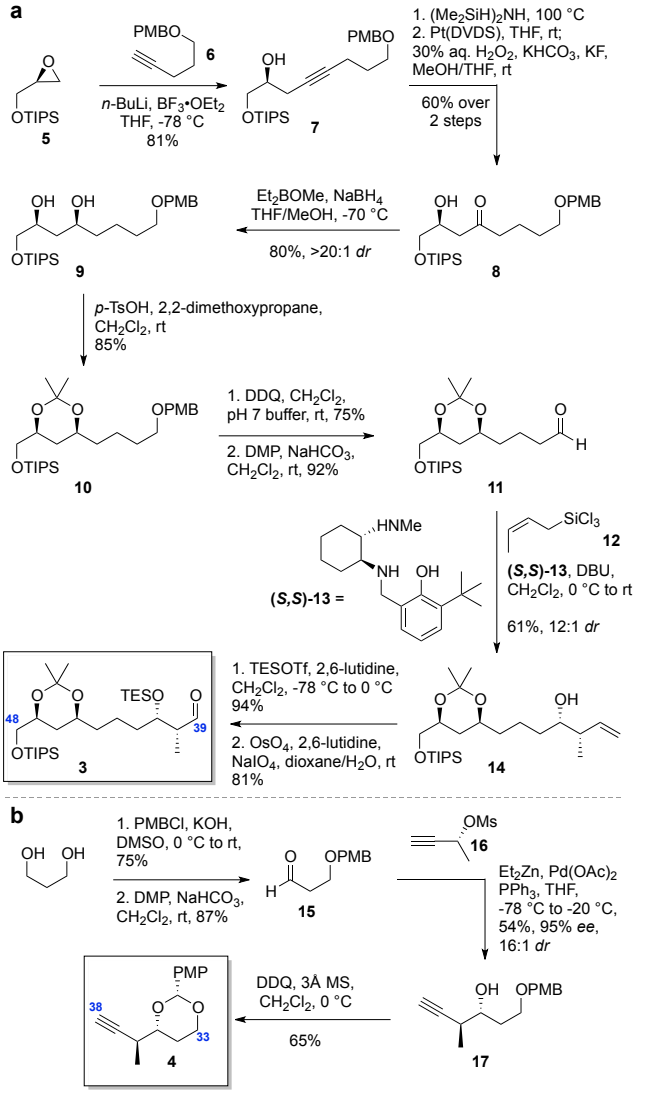
with the previously synthesized C10–C13 diene. In doing so, we demonstrate the ability to install either stereochemistry at C50 at a late synthetic stage, thus laying the groundwork for further studies on the full-length natural product.

Our retrosynthetic analysis of the C33–C51 fragments **1a** and **1b** is shown in Figure 1b. This commences with a late-stage Horner-Wadsworth-Emmons (HWE) olefination / asymmetric reduction at C48 on the C33–C48 motif **2**. Further disconnection at the centre of this unit reveals the C39–C48 aldehyde **3** and C33–C38 alkyne **4**; the union of these two fragments could potentially be achieved by addition of an acetylide derived from **4** to aldehyde **3**, followed by an oxidation / asymmetric reduction / hydrogenation sequence to install the required C39 stereochemistry and C37–C38 saturation.

Synthesis of the C39–C48 aldehyde **3** (Scheme 1a) commenced with addition of alkyne **6** to TIPS-protected (*R*)-glycidol **5**, which afforded homopropargylic alcohol **7** in 81% yield. **7** was treated with 1,1,3,3-tetramethyldisilazane as a prelude to intramolecular hydrosilylation[30, 31] catalyzed by platinum(0) 1,3-divinyl-1,1,3,3-tetramethyldisiloxane,[32] with Tamao oxidation of the intermediate cyclic siloxane furnishing hydroxyketone **8** in good yield (60%). Subsequent Narasaka-Prasad diastereoselective reduction[33] of **8** with diethylmethoxyborane and sodium borohydride gave 1,3-*syn* diol **9** in excellent yield and stereoselectivity (80%, >20:1 *dr*). Treatment of this diol with 2,2-dimethoxypropane and *p*-TsOH afforded acetonide **10** (85%), which enabled confirmation of the relative stereochemistry of the *syn* diol through the Rychnovsky method.[34,35] Deprotection of the PMB ether in **10** (DDQ, 75%) followed by Dess-Martin oxidation afforded aldehyde **11** (92%). To install the next two adjacent stereocentres, a Leighton crotylation[36] was performed on **11**, which proceeded with excellent diastereoselectivity; the stereochemistry of the newly-introduced hydroxyl-bearing stereocentre was confirmed by Mosher ester analysis.[37, 38] Alcohol **14** was then treated with TES triflate and 2,6-lutidine to generate the corresponding TES ether in high yield (94%). Finally, oxidative cleavage of the terminal alkene[39] afforded the C39–C48 aldehyde **3** (81%).

Preparation of the C33–C38 alkyne **4** (Scheme 1b) began with mono-PMB protection of propane-1,3-diol (75%), followed by Dess-Martin oxidation to aldehyde **15** (87%). Enantio- and diastereoselective allenylzinc addition[40-42] of propargylic mesylate **16** to aldehyde **15** gave propargylic alcohol **17** in 54% yield (95% *ee*, 16:1 *dr*), with the stereochemistry of the newly-installed alcohol confirmed by Mosher ester analysis. Treatment of **17** with DDQ in dry CH_2Cl_2[43] effected oxidation to the PMP acetal **4** (65%).

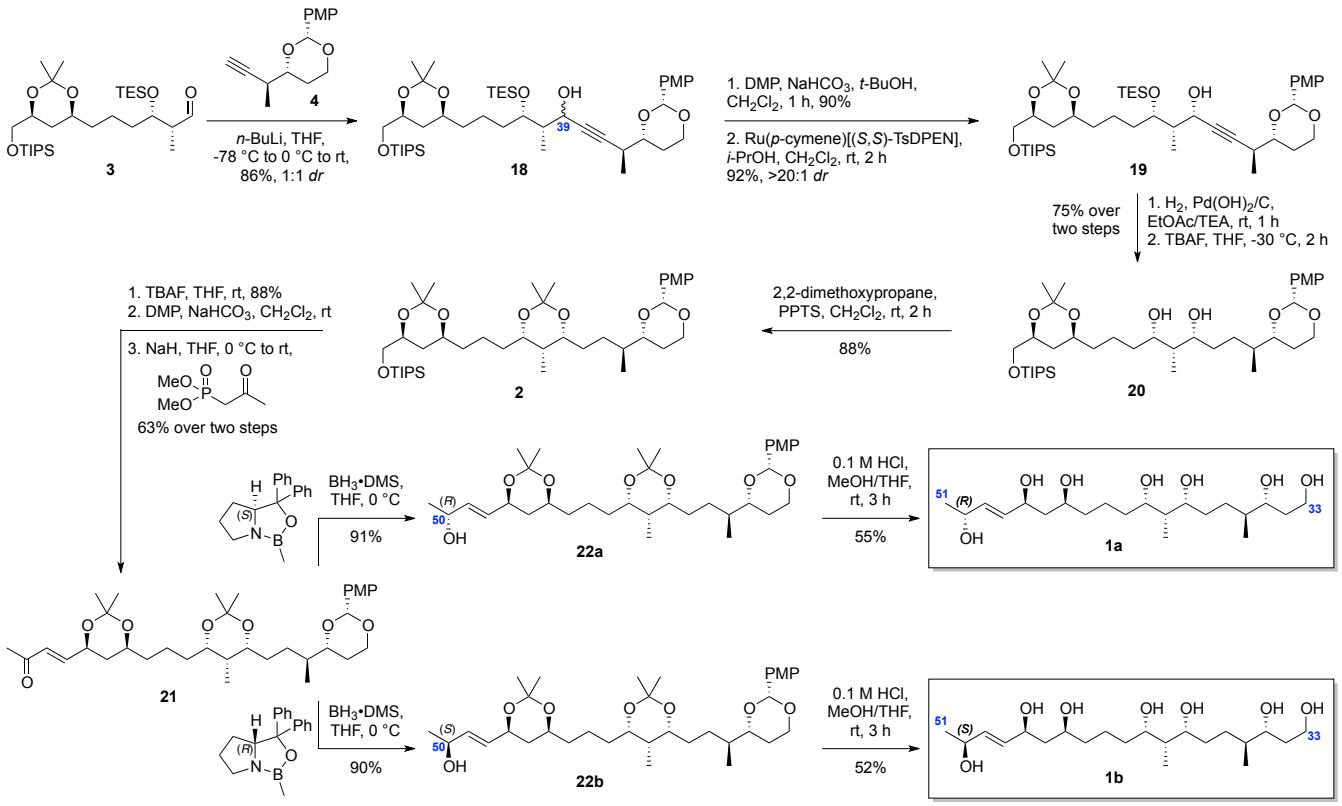
With fragments **3** and **4** in hands, attention turned to construction of the C33-C48 skeleton (Scheme 2). The union of **3** and **4** was achieved using *n*-BuLi, which gave a 1:1 mixture of the two diastereomeric propargylic alcohols **18** in 86% yield. To install the required stereocentre at C39, these diastereomers were oxidized (Dess-Martin periodinane, 90%), then reduced using Noyori asymmetric transfer hydrogenation,[44] which gave propargylic alcohol **19** in excellent yield and diastereoselectivity (92%, >20:1 *dr*). Hydrogenation of the triple bond in **19**



Scheme 1. (a) Synthesis of C39–C48 aldehyde **3**; and (b) synthesis of C33–C38 alkyne **4**.

proceeded smoothly in the presence of triethylamine, which suppressed unwanted deprotection of the TES ether and PMP acetal. The crude residue was directly reacted with TBAF to obtain diol **20** in 75% yield over two steps. Protection of **20** as an acetonide gave the C33–C48 fragment **2** in 87% yield; at this point the stereochemistry at C39 was confirmed by the Rychnovsky-Evans method.[45,35]

Completion of the C33–C51 fragments began with deprotection of the TIPS ether in **2** (88%), followed by oxidation and HWE olefination to form enone **21** in 63% yield over two steps. Due to the stereochemical uncertainty at C50, **21** was treated with both (*S*)- and (*R*)-CBS reagents to afford the C50-(*R*)-enol **22a** (91%) and the C50-(*S*)-enol **22b** (90%) respectively, ready for the final deprotection. This proved challenging, as under acid conditions typically required to deprotect 1,3-*syn*-acetonides (e.g. 1 M HCl, 80% AcOH, or cat. TsOH), the C50 allylic alcohol proved susceptible to degradation (for example by ionization / elimination or ionization / cyclization), while milder conditions led to only partial deprotection and degradation on extended reaction times. After significant



Scheme 2. Synthesis of the C33–C51 Fragments **1a** (C50(*R*)) and **1b** (C50(*S*))

exploration, we found that treatment of **22a** with 0.1 M HCl in 2:1 MeOH/THF for 3 h at ambient temperature afforded **1a** in a pleasing 55% yield. These conditions are particularly appealing, as they were also those optimized for deprotection of the C1–C27 fragment in our previous work.[29] Application of these conditions to the epimer **22b** gave **1b** in 52% yield. Purification of **1a** and **1b** proved non-trivial due to their extremely high polarity: normal phase chromatography was unsuitable, but high purity samples could be obtained using reversed phase HPLC.

With heptaols **1a** and **1b** in hands, we set about comparing their NMR spectroscopic data with that of stambomycin D (Figure 2). Although the synthesized and natural materials are cyclic and acyclic respectively, the conformational flexibility of the stambomycin D macrocycle likely confers the ability to adopt 'acyclic-like' conformations in local regions of structure, rendering such comparison informative if not definitive.[46] In the event, the ^{1}H and ^{13}C NMR spectra of the synthetic and natural materials showed a good match, with the expected exceptions of C50 which is incorporated into the macrolactone in the natural product but is a free alcohol in **1a** and **1b**, and at the point of truncation (C33–C35). It is worth noting that the most significant chemical shift difference of the synthetic fragments lay at C49 ($\Delta\delta(^{13}C)$ = 0.04 ppm); this suggests that with authentic and synthetic full-length materials in hand, the identification of the C50 stereocentre is likely to be achievable, and underlines the sensitivity of NMR spectroscopy in the discrimination of subtle stereochemical differences. A further discrepancy was noted at C37, where the synthetic fragments showed distinct environments for the diastereotopic protons (as observed by HSQC). This suggested that a minor reassignment of the ^{1}H NMR data for the natural product may be required, which was confirmed by re-examination of the original HSQC data for the stambomycins. In general, our synthetic C33–C51 fragments show good agreement with stambomycin D, particularly in the central region (C36–C45), further validating the predicted stereochemistry of the natural product.

In conclusion, we have synthesized two diastereomers of the 'southern' C33–C51 region of stambomycin D, which

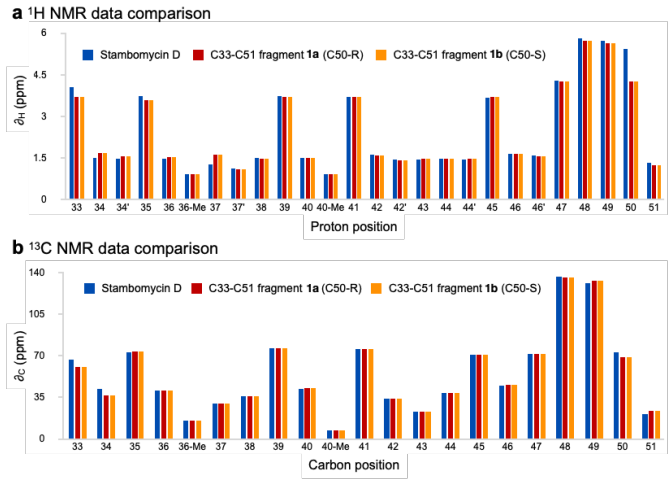


Figure 2. Comparison of ^{1}H and ^{13}C NMR Data of **1a** and **1b** with Stambomycin D.

displayed a good match of spectroscopic data with the natural product, particularly in the central region (C36–C45) of the fragments. This work further supports the sequence-predicted stereochemical assignment of the stambomycins. Importantly, the deprotection conditions applied to these fragments correlate with those employed for the C1–C27 segment, laying the groundwork for global deprotection of the entire C1–C51 framework. Efforts towards this goal will be reported in due course.

We thank the Marie Skłodowska-Curie actions for an Individual Fellowship (V.C., GA No 702385), and the Leverhulme Trust for funding (H.G.G., RPG-2015-003). E.A.A. thanks the EPSRC for additional support (EP/S013172/1). Dr Lijiang Song (University of Warwick) is thanked for re-examining the original stambomycin HSQC data.

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡These authors contributed equally to this work.

1 L. Laureti, L. Song, S. Huang, C. Corre, P. Leblond, G. L. Challis and B. Aigle, Identification of a Bioactive 51-membered Macrolide Complex by Activation of a Silent Polyketide Synthase in Streptomyces Ambofaciens, *Proc. Nat. Acad. Sci. USA*, 2011, **108**, 6258.

2 N. Ziemert, M. Alanjary and T. Weber, The Evolution of Genome Mining in Microbes – a Review, *Nat. Prod. Rep.*, 2016, **33**, 988.

3 P. J. Rutledge and G. L. Challis, Discovery of Microbial Natural Products by Activation of Silent Biosynthetic Gene Clusters, *Nat. Rev. Microbiol.*, 2015, **13**, 509.

4 E. J. N. Helfrich, S. Reiter and J. Piel, Recent Advances in Genome-based Polyketide Discovery, *Curr. Opin. Biotech.*, 2014, **29**, 107.

5 B. Aigle, G. Challis, L. Laureti, L. Song and P. Leblond; Stambomycin and Derivatives, Their Production and Their Use as Drugs.; US20120142622 A1, 2012.

6 A. T. Keatinge-Clay, Stereocontrol within Polyketide Assembly Lines, *Nat. Prod. Rep.*, 2016, **33**, 141.

7 D. H. Kwan, Y. Sun, F. Schulz, H. Hong, B. Popovic, J. C. C. Sim-Stark, S. F. Haydock and P. F. Leadlay, Prediction and Manipulation of the Stereochemistry of Enoylreduction in Modular Polyketide Synthases, *Chem. Biol.*, 2008, **15**, 1231.

8 A. T. Keatinge-Clay, A Tylosin Ketoreductase Reveals How Chirality is Determined in Polyketides, *Chem. Biol.*, 2007, **14**, 898.

9 L. J. Song, L. Laureti, C. Corre, P. Leblond, B. Aigle and G. L. Challis, Cytochrome P450-mediated Hydroxylation is Required for Polyketide Macrolactonization in Stambomycin Biosynthesis, *J. Antibiot.*, 2014, **67**, 71.

10 Z. J. Low, J. Xiong, Y. Xie, G.-L. Ma, H. Saw, H. Thi Tran, S. L. Wong, L. M. Pang, J. Fong, P. Lu, J.-F. Hu, Y. Liang, Y. Miao and Z.-X. Liang, Discovery, Biosynthesis and Antifungal Mechanism of the Polyene-polyol Meijiemycin, *Chem. Commun.*, 2020, **56**, 822.

11 Y. Hu, M. Wang, C. Wu, Y. Tan, J. Li, X. Hao, Y. Duan, Y. Guan, X. Shang, Y. Wang, C. Xiao and M. Gan, Identification and Proposed Relative and Absolute Configurations of Niphimycins C–E from the Marine-Derived Streptomyces sp. IMB7-145 by Genomic Analysis, *J. Nat. Prod.*, 2018, **81**, 178.

12 M. C. Kim, H. Machado, K. H. Jang, L. Trzoss, P. R. Jensen and W. Fenical, Integration of Genomic Data with NMR Analysis Enables Assignment of the Full Stereostructure of Neaumycin B, a Potent Inhibitor of Glioblastoma from a Marine-Derived Micromonospora, *J. Am. Chem. Soc.*, 2018, **140**, 10775.

13 I. Pérez-Victoria, D. Oves-Costales, R. Lacret, J. Martín, M. Sánchez-Hidalgo, C. Díaz, B. Cautain, F. Vicente, O. Genilloud and F. Reyes, Structure Elucidation and Biosynthetic Gene Cluster Analysis of Caniferolides A–D, New Bioactive 36-membered Macrolides from the Marine-derived Streptomyces Caniferus CA-271066, *Org. Biomol. Chem.*, 2019, **17**, 2954.

14 M. C. Kim, J. M. Winter, R. Cullum, Z. Li and W. Fenical, Complementary Genomic, Bioinformatics, and Chemical Approaches Facilitate the Absolute Structure Assignment of Lonostatin, a Linear Polyketide from a Rare Marine-Derived Actinomycete, *ACS Chem. Biol.*, 2020, **15**, 2507.

15 N. Matsumori, D. Kaneno, M. Murata, H. Nakamura and K. Tachibana, Stereochemical Determination of Acyclic Structures Based on Carbon−Proton Spin-Coupling Constants. A Method of Configuration Analysis for Natural Products, *J. Org. Chem.*, 1999, **64**, 866.

16 Y. Kobayashi, J. Lee, K. Tezuka and Y. Kishi, Toward Creation of a Universal NMR Database for the Stereochemical Assignment of Acyclic Compounds: The Case of Two Contiguous Propionate Units, *Org. Lett.*, 1999, **1**, 2177.

17 J. Lee, Y. Kobayashi, K. Tezuka and Y. Kishi, Toward Creation of a Universal NMR Database for the Stereochemical Assignment of Acyclic Compounds: Proof of Concept, *Org. Lett.*, 1999, **1**, 2181.

18 E. Fleury, M.-I. Lannou, O. Bistri, F. Sautel, G. Massiot, A. Pancrazi and J. Ardisson, Advances in the Universal NMR Database: Toward the Determination of the Relative Configurations of Large Polypropionates, *Eur. J. Org. Chem.*, 2009, **2009**, 4992.

19 K. Ermanis, K. E. B. Parkes, T. Agback and J. M. Goodman, The Optimal DFT Approach in DP4 NMR Structure Analysis – Pushing the Limits of Relative Configuration Elucidation, *Org. Biomol. Chem.*, 2019, **17**, 5886.

20 S. G. Smith and J. M. Goodman, Assigning the Stereochemistry of Pairs of Diastereoisomers Using GIAO NMR Shift Calculation, *J. Org. Chem.*, 2009, **74**, 4597.

21 S. G. Smith and J. M. Goodman, Assigning Stereochemistry to Single Diastereoisomers by GIAO NMR Calculation: The DP4 Probability, *J. Am. Chem. Soc.*, 2010, **132**, 12946.

22 M. M. Zanardi and A. M. Sarotti, GIAO C–H COSY Simulations Merged with Artificial Neural Networks Pattern Recognition Analysis. Pushing the Structural Validation a Step Forward, *J. Org. Chem.*, 2015, **80**, 9371.

23 M. O. Marcarino, M. a. M. Zanardi, S. Cicetti and A. M. Sarotti, NMR Calculations with Quantum Methods: Development of New Tools for Structural Elucidation and Beyond, *Acc. Chem. Res.*, 2020, **53**, 1922.

24 M. O. Marcarino, S. Cicetti, M. M. Zanardi and A. M. Sarotti, A Critical Review on the Use of DP4+ in the Structural Elucidation of Natural Products: the Good, the Bad and the Ugly. A Practical Guide, *Nat. Prod. Rep.*, 2021, DOI: 10.1039/D1NP00030F.

25 H. Fuwa, Structure Determination, Correction, and Disproof of Marine Macrolide Natural Products by Chemical Synthesis, *Org. Chem. Front.*, 2021, **8**, 3990.

26 M. Heinrich, J. J. Murphy, M. K. Ilg, A. Letort, J. Flasz, P. Philipps and A. Fürstner, Total Synthesis of Putative Chagosensine, *Angew. Chem. Int. Ed.*, 2018, **57**, 13575.

27 B. Y. Han, N. Y. S. Lam, C. I. MacGregor, J. M. Goodman and I. Paterson, A Synthesis-enabled Relative Stereochemical

Assignment of the C1–C28 Region of Hemicalide, *Chem. Commun.*, 2018, **54**, 3247.
28 D. Paul, A. Kundu, S. Saha and R. K. Goswami, Total Synthesis: the Structural Confirmation of Natural Products, *Chem. Commun.*, 2021, **57**, 3307.
29 J. Lim, V. Chintalapudi, H. G. Gudmundsson, M. Tran, A. Bernasconi, A. Blanco, L. Song, G. L. Challis and E. A. Anderson, Synthesis of the C1–C27 Fragment of Stambomycin D Validates Modular Polyketide Synthase-Based Stereochemical Assignments, *Org. Lett.*, 2021, **23**, 7439.
30 K. Tamao, K. Maeda, T. Tanaka and Y. Ito, Intramolecular Hydrosilation of Acetylenes: Regioselective Functionalization of Homopropargyl Alcohols, *Tetrahedron Lett.*, 1988, **29**, 6955.
31 J. A. Marshall and M. M. Yanik, Synthesis of β-Hydroxy Ketones and Vinylsilanes from Homopropargylic Alcohols by Intramolecular Hydrosilation, *Org. Lett.*, 2000, **2**, 2173.
32 S. E. Denmark and W. Pan, Intramolecular Hydrosilylation and Silicon-Assisted Cross-Coupling: An Efficient Route to Trisubstituted Homoallylic Alcohols, *Org. Lett.*, 2001, **3**, 61.
33 K. Narasaka and F.-C. Pai, Stereoselective Reduction of β Hydroxyketones to 1,3-diols Highly Selective 1,3-asymmetric Induction via Boron Chelates, *Tetrahedron*, 1984, **40**, 2233.
34 S. D. Rychnovsky and D. J. Skalitzky, Stereochemistry of Alternating Polyol Chains: ^{13}C NMR Analysis of 1,3-diol Acetonides, *Tetrahedron Lett.*, 1990, **31**, 945.
35 See the Supporting Information for details.
36 L. M. Suen, M. L. Steigerwald and J. L. Leighton, A new and More Powerfully Activating Diamine for Practical and Scalable Enantioselective Aldehyde Crotylsilylation Reactions, *Chem. Sci.*, 2013, **4**, 2413.
37 J. A. Dale and H. S. Mosher, Nuclear Magnetic Resonance Enantiomer Regents. Configurational Correlations via Nuclear Magnetic Resonance Chemical Shifts of Diastereomeric Mandelate, O-methylmandelate, and .alpha.-methoxy-.alpha.-trifluoromethylphenylacetate (MTPA) esters, *J. Am. Chem. Soc.*, 1973, **95**, 512.
38 T. R. Hoye, C. S. Jeffrey and F. Shao, Mosher Ester Analysis for the Determination of Absolute Configuration of Stereogenic (chiral) Carbinol Carbons, *Nat. Protoc.*, 2007, **2**, 2451.
39 W. Yu, Y. Mei, Y. Kang, Z. Hua and Z. Jin, Improved Procedure for the Oxidative Cleavage of Olefins by OsO_4–$NaIO_4$, *Org. Lett.*, 2004, **6**, 3217.
40 J. A. Marshall and N. D. Adams, Synthesis of Chiral Enantioenriched Homopropargylic Alcohols from Propargylic Mesylates via Chiral Allenylzinc Intermediates, *J. Org. Chem.*, 1998, **63**, 3812.
41 J. A. Marshall and N. D. Adams, Addition of Allenylzinc Reagents, Prepared in situ from Nonracemic Propargylic Mesylates, to Aldehydes. A New Synthesis of Highly Enantioenriched Homopropargylic Alcohols, *J. Org. Chem.*, 1999, **64**, 5201.
42 C. Botuha, F. Chemla, F. Ferreira, A. Pérez-Luna and B. Roy, Allenylzinc Reagents: New Trends and Synthetic Applications, *New J. Chem.*, 2007, **31**, 1552.
43 Y. Oikaw, T. Yoshioka and O. Yonemitsu, Protection of Hydroxy Groups by Intramolecular Oxidative Formation of Methoxybenzylidene Acetals with DDQ, *Tetrahedron Lett.*, 1982, **23**, 889.
44 K. Matsumura, S. Hashiguchi, T. Ikariya and R. Noyori, Asymmetric Transfer Hydrogenation of Alpha,Beta-Acetylenic Ketones, *J. Am. Chem. Soc.*, 1997, **119**, 8738.
45 D. A. Evans, D. L. Rieger and J. R. Gage, ^{13}C NMR Chemical Shift Correlations in 1,3-diol Acetonides. Implications for the Stereochemical Assignment of Propionate-derived Polyols, *Tetrahedron Lett.*, 1990, **31**, 7099.
46 I. Paterson, R. Britton, K. Ashton, H. Knust and J. Stafford, Synthesis of Antimicrofilament Marine Macrolides: Synthesis and Configurational Assignment of a C_5–C_{16} Degradation Fragment of Reidispongiolide A, *Proc. Nat. Acad. Sci. USA*, 2004, **101**, 11986.