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Chemoselective formation and reaction of densely functionalised bicyclic tetramic acids and their biological activity

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Abstract: Weinreb amide derivatives of tetramates may be effectively accessed by chemoselective Dieckmann reaction, and further react with Grignards in a fully chemoselective reaction, giving rise to unsaturated acyl derivatives not easily available by other routes. These systems are strong chelators of calcium, and some show potent activity against Gram positive bacteria, and one is a first-in-class proteasome inhibitor.

The tetramic acid (pyrrolidine-2,4-dione) is the common structural unit in a class of natural products that possesses a wide range of biological activities including antibiotic, antifungal, antitumour and antiviral activity.^[1] Of particular interest are 3-acyl tetramic acids **1**, found in natural products with potent antibacterial activities (Figure 1).^[2]

Figure 1. Representative examples of 3-acyltetramic acid-containing natural products.

Using the self-regeneration of stereocentre (SRS) principle,^[3] we have shown that rapid access the enantiopure tetramate cores **4a-c** for three different systems, derived from serine (a: X=O, Y=H), cysteine (b: X=S, Y=H) and threonine (c: X=O, Y=Me), is possible (Scheme 1). This synthesis relies on the Dieckmann cyclisation of *N*-acylated oxazolidines **3a-c** to give tetramates **4a-c** as the major product.^[4] Although the tetramate core has no or little intrinsic biological activity,^[5] it can be functionalised to mimic features of tetramic acid-containing natural products, and some of these analogues have shown potent antibacterial activities against *E. coli* and *S. aureus*.^[6]

We recently reported the preparation of acyltetramates from **4a** using the corresponding carboxylic acid and stoichiometric amounts of DCC-DMAP, which proceeded via *O*-acylation followed by rearrangement to *C*-acylated products **6a**, but which was found to be limited in substrate scope, and not suitable for vinylic substrates in particular.^[7] In order to enable the introduction of a wider range of functionalities to the tetramate core, we decided to prepare Weinreb amides **7a-c**, for the introduction of the desired acyl group at C(6) by Grignard reaction.

Scheme 1. Previous and novel approaches to acyl tetramic acids **6a**.

Our initial attempt (Approach 1, Scheme 2), was to directly acylate tetramate **4a** using methoxy(methyl)carbamic chloride,^[8] but *O*-acylated carbamate **8** was obtained as the only product, and attempted acyl migration with DMAP^[9] or acetone cyanohydrin^[10] only delivered recovered tetramate **4a**, manifesting the good leaving group ability and low nucleophilicity of the tetramate system. In an alternative strategy (Approach 2, Scheme 2), acylation of oxazolidine **3a** gave the desired tricarbonyl **9a**, and this could be followed by Dieckman cyclisation under our previously reported conditions (with KO^tBu) or with piperidine to furnish a 1:1 mixture of Weinreb amide **7a** and ethyl ester **10a** (Table 1). However, DBU gave a better outcome, leading to exclusive formation of the desired tetramate **7a** in high yields and on a gram scale, and these conditions were also successful for **7b,c**. This remarkable process provided access to the desired densely functionalised Weinreb derivatives **7a-b** with near-complete chemoselectivity.

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Scheme 2. Preparation of Weinreb amides **7a-c**.Table 1. Base screen for the cyclisation of tricarbonyl **9a** to **7a/10a**.

Entry	Base	pK _a	Temp	Yield [%]	Ratio 7a : 10a
1	Pyridine	5.2	rt	0	-
2	Et ₃ N	10.8	rt	0	-
3	Piperidine	11.0	rt	quant.	1:1 ^[a]
4	DBU	12	rt	84	110:1 ^[b]
5	KO ^t Bu	17	Reflux	76	1:1 ^[a]

[a] Determined by NMR. [b] Determined by LC/MS

With Weinreb amides **7a-c** in hand, treatment with 2 equivalents of the desired organomagnesium bromide gave acylated compounds **11-15a-c** in yields of up to 84% (Table 2), allowing the introduction of alkyl, aryl, alkenyl and alkynyl functionalities to the tetramate core. This remarkable reaction proceeds with full chemoselectivity on the extensively conjugated and cross-conjugated substrates **7a-c**, and with no reaction at the ketone or the methyl ester or over-addition products detected by NMR or MS analysis on the reaction product mixture.

Table 2. Grignard reaction of Weinreb amides **7a-c** to give products **11-15**.

Compound	R	Yield [%]		
		a (X=O, Y=H)	b (X=S, Y=H)	c (X=O, Y=Me)
11	Me	82	63	71
12	Ph	84	76	74
13		83	75	60
14		79	72	71
15		81	66	81

Weinreb amides **7a-c** and the alkynyl analogues were found not to be fully stable, and after standing in CDCl₃ for several days, enamine **16** and tricyclic compounds **17a-c** were formed respectively (Scheme 3). The latter was confirmed from the crystal structure of **17a**.^[11]

Scheme 3. Degradation products of **7a** (A) and **15a-c** (B) in CDCl₃ and crystal structure of tricyclic **17a** shown with displacement ellipsoids drawn at 50% probability; one molecule is shown for clarity (C).

¹H and ¹³C NMR analysis of acylated compounds **11-15a-c** in CDCl₃ revealed that they exist as one major tautomer (along with a minor form observed in alkyl and alkenyl analogues **11a-c** and **14a-c**). The major form corresponds to the *Z*-*exo*-enol, while the minor one is the *E*-*exo*-enol (Figure S1); the ratio of each was solvent dependent (Figure S2). This assignment was confirmed from a ¹H – ¹³C NOE experiment of compound **11a** in C₆D₆ (Supporting Information). In contrast, carboxamide- and alkoxy-carbonyl tetramic acids exist as the *endo*-enol form, reflecting their differing capacity for stabilising resonance.^[12]

Figure 2. ^1H NMR (400 MHz, CDCl_3) and XPS calcium region of **11a** after silica purification (A), **14a** after silica purification (B) and **14a** after washing with 2 M HCl (C).

Purification of acyl-tetramic acids by column chromatography has been reported to be difficult, and this is believed to be due to the pronounced tendency of these compounds for chelation with metals.^[1c, 13] In the work reported here-in, all acylated analogues gave broad NMR signals after silica gel purification, and needed to be washed with 2 M HCl to obtain metal-free samples with well-resolved NMR spectra (Figure 2). X-ray photoelectron spectroscopic (XPS) analysis of the chelated **11a** and **14a** clearly identified the presence of calcium in both cases, but no other metals (Mg, Fe, Co...) (Table S2). After washing **14a** with 2 M HCl and analysing the solid metal-free sample again by XPS, no detectable amounts of calcium were found. Furthermore, when the metal-free sample, showing sharp NMR peaks, was washed with 2 M aqueous $\text{Ca}(\text{NO}_3)_2$, broadening of the NMR signals was observed. These results indicate that the difficult purification of acyl tetramic acids by column chromatography may well be due to chelation of trace amounts of calcium found in the silica gel. In fact, some naturally occurring tetramic acids have been isolated as the metal complexes, and this chelation capacity could play a role in their potent biological properties.^[14]

Removal of the *tert*-butyl group and the methyl ester protecting groups was of interest, not least because this would provide access to closer analogues of some natural products (Figure 1). However, *N,O*-acetal deprotection of acetylated compound **11a** was unsuccessful. All the tested conditions, including treatment with 1,3-propanedithiol,^[15] used for the deprotection of similar compounds,^[4a, 16] and other acidic conditions: THF/ H_2O /formic acid 3:1:1,^[17] TFA (2 eq) in THF/ H_2O 1:1,^[18] TFA (20 eq) in DCM,

and BiBr_3 (cat.) in MeCN^[19] resulted in quantitative recovery of starting material. This lack of reactivity of the acylated products towards hydrolysis could be due to the presence of the exocyclic enol moiety, which stabilises the lactam by hydrogen bonding.^[7] This hypothesis was supported by the successful deprotection of Weinreb amide **7a** (Scheme 4A), which has a free lactam carbonyl. To circumvent this problem, protection of the enol group was clearly necessary, but this also proved to be challenging due to the high stability - and low reactivity - of the derived enolate as a result of extensive resonance. Attempted silylation of **11a**, or treatment with MeI in the presence of CsF , conditions reported for the successful *O*-alkylation of tetrone acids,^[20] gave unreacted starting material. Finally, treatment of **11a** with methanol under Mitsunobu reaction conditions^[21] gave the methylated product, which was found to be a separable 3:1 mixture of enolates **19** and **19'** (Scheme 4B), as indicated by HMBC correlations. For this protected enol, *N,O*-acetal deprotection was successful and alcohol **21** was obtained in 74% yield. However, hydrolysis of alkene **20** proved to be more challenging and purification by flash column chromatography was unsuccessful. This could be explained by a combination of factors, including its high polarity, metal chelation and a possible retro-aldol reaction.^[22] Indeed, purification of protected alcohol **23**, prepared by direct acetylation of the crude **22**, was successful.

Acylated analogues **11a** and **14a-c** were hydrolysed with an excess of LiOH, and reflux of the crude acids in MeCN gave the decarboxylated products **24** and **25a-c** as single diastereomers. The *exo*-located *tert*-butyl group probably plays an important role in the selectivity observed in these processes.

The biological activity of acylated compounds **11a-c** - **14a-c** and Weinreb amides **7a-c** against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* was initially assessed using the hole-plate method, which allows a direct qualitative assessment of antibacterial activity (Table S3).^[2b] We found a high dependence of activity on the nature of the acyl substituent, with Weinreb amides, alkyl and aryl groups showing no or little inhibition; however, high *S. aureus* activity of the alkenyl analogues **14a-c**, and particularly **14b** was found. Similar selectivity for Gram-positive bacteria had been previously observed for related substituted tetramates.^[6-7, 23] For **17a-c**, some inhibition of both *S. aureus* and Gram-negative *E. coli* was observed. However, deprotected compounds **21** and **23** were fully inactive. Conversely, decarboxylation led to an improvement of Gram-positive activity: acetyl **24** showed some activity at 4 mg/mL, while its analogous methoxycarbonyl **11a** had been inactive, and the alkenyl analogues also displayed enhanced activities. The cysteine analogues were again superior, and the most active analogue **25b** was 27-fold more potent than the standard CephC. MIC analysis (Table S4) confirmed this analysis, with good activity against Gram-positive *S. aureus* and *S. pneumoniae*, but no Gram-negative activity. The most active analogue **25b** has MIC of 0.98 $\mu\text{g/mL}$. These results validate the effectiveness of the hole-plate method, which despite not providing accurate MIC values, represents a valuable rapid initial assay to rank analogue activity.

Scheme 4. N,O-Acetal deprotection and hydrolysis of **11a** and **14a-c**.

SAR analysis using predicted physicochemical properties was conducted (Table S5). The most active compounds are the ones with the highest clogP values and are clustered in a very tight zone of chemical space (Figure 3), a phenomenon which has been observed in other related systems.^[6] A previous study on related C(6)-acyltetramates^[6] found that those with clogP > 3 and MSA > 620 tended to be more easily transported by the efflux pump in Gram-negative *H. influenzae*. That the most active tetramates synthesised herein with selective Gram-positive activity behaved similarly and have clogP > 3 and MSA > 450 suggests that the observed lack of activity of these systems against *E. coli* could be ascribed to efflux pump (and/or lack of cell permeability) rather than a lack of intrinsic bioactivity.

Figure 3. Correlation between the Gram-positive activity of the synthesised analogues and their MSA and clogP values.

The synthesised analogues were also studied for anticancer activity; an initial high-throughput screen gave alkenyl analogues **25a** and **25b** as the only hits, of interest since these were also the ones with the highest antibacterial activity. IC₅₀ values against the five cancer cell lines (HeLa, H460, MCF7, SKOV-3 and KMS-12BM) were found to be in the 10-20 μM range (Table S6). The mechanism of action of these compounds was investigated for apoptosis, caspase activation and proteasome inhibition. The most active compound, **25b**, was found to be a reversible proteasome inhibitor (IC₅₀ 25 μM) in H460 cells (Table S7, Figure S3). The natural tetramic acid JBIR-22 has also been shown to inhibit the PPI of PAC3 homodimer, which plays a role in proteasome formation, and it could be that **25b** is acting via the same mechanism. Although JBIR-22 was also shown to be cytotoxic to HeLa cells with IC₅₀ 68 μM,^[2f] its proteasome activity has not been assessed. This work represents the first reported evidence of a tetramic acid acting as a proteasome inhibitor.

In conclusion, a fully chemoselective Grignard displacement of Weinreb amides **7a-c** has enabled the preparation of tetramic acids substituted with a range of side chain acyl groups; this alternative strategy to the classical acylation approach^[7, 12] offers a solution to the recently highlighted difficulty of the introduction of unsaturated side chains in such systems,^[24] and neatly complements the work of Schobert which has demonstrated the suitability of Lacey-Dieckman cyclisation in highly sensitive substrates.^[24] Some of these analogues had potent and selective activity against Gram-positive bacteria, and one is a first-in-class proteasome inhibitor. This outcome supports the hypothesis that preparation of simple, and more easily accessible, mimics of tetramate-containing natural products can lead to active compounds suitable for drug discovery and development.

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