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New routes towards reutericyclin analogues†

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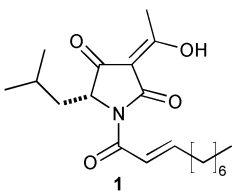
A range of *N*-acylpyrrolo[3,4-*c*]isoxazoles and derived *N*-acyltetramides has been prepared *via* a nitrile oxide dipolar cycloaddition approach, as analogues of the acyltetramic acid metabolite reutericyclin, of interest for its antibiotic potential against Gram-positive bacteria including hospital-acquired infections of resistant *Clostridium difficile*.

In order to combat the growing resistance to generally administered antibiotics, such as penicillin and methicillin, the research community is endeavouring to find new compounds that actively inhibit problematic resistant bacteria.[1] This effort has identified a number of potential candidates, one example of which is reutericyclin (**1**), isolated in 2000 by Jung *et al.* from *Lactobacillius reuteri* LTH2584.[2] Reutericyclin belongs to the 3-acyltetramic acid group of natural products (**2**), characterised by a pyrrolidine-2,4-dione unit carrying an acyl group at C-3.[3] Molecules containing this motif exhibit a range of bio-activities including antibiotic, antitumor, antiviral, antiulcerative, fungicidal and cytotoxic properties.[4] Interest in the antibiotic activity of tetramic acids has recently been stimulated by their key relationship to the inducers of bacterial quorum sensing.[5] Reutericyclin and derivatives display varying inhibition in Gram-positive bacteria.[2,6] The most interesting of these results is the inhibition of growth of resistant bacterium *Clostridium difficile*, a leading cause of antibiotic-associated diarrhoea in hospitalized patients which can lead to mortalities in persons with a compromised immune system.[7]



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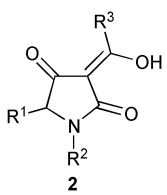

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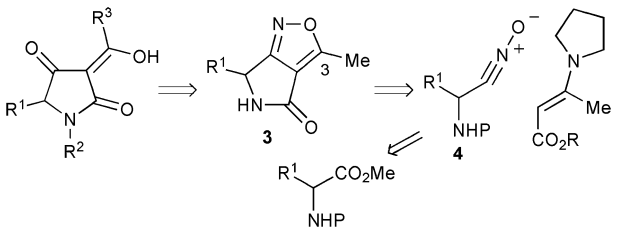
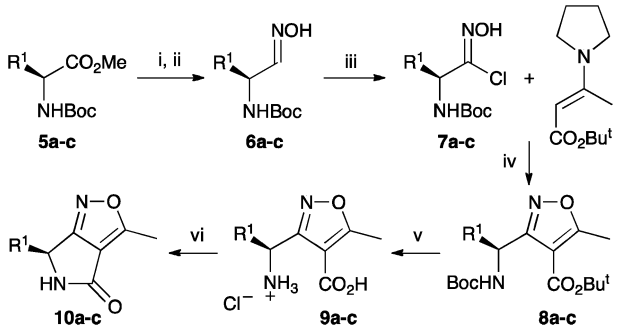


Scheme 1 The pyrrolo[3,4-*c*]isoxazole strategy (P = protecting group).

We have, over many years, explored the synthesis of the acyltetramic acid moiety[8] and other cyclic tricarbonyl systems,[9] most recently using pyrroloisoxazoles as masked acyltetramic acids and as core building blocks for peripheral elaboration.[10,11] Our 2nd generation strategy (Scheme 1)[10] uses pyrrolo[3,4-*c*]isoxazoles **3** (*cf.* pyrrolo[3,4-*d*]isoxazoles in our 1st generation approach[11]) formed by cycloaddition of nitrile oxides **4**, available in three steps from α-amino esters, with enamino ester dipolarophiles. We report here significant practical improvements in this strategy (principally in lactam closure) and its application to access novel bicyclic reutericyclin analogues. Reutericyclin has *R*-configuration at C-5, and is presumably biosynthesised from *R*-leucine,[3,12] however we have conducted our studies in the more readily available, less costly *S*-series: the chemistry should, of course, be equally applicable to the enantiomeric series.[13]

The commercially available methyl esters of *S*-valine, *S*-leucine and *S*-phenylglycine were efficiently N-protected (Boc_2O, Et_3N, CH_2Cl_2, 0–20 °C; 99, 97 and 99%, respectively). The protected amino esters (**5a–c**, respectively) were selectively reduced to the corresponding aldehydes using DIBAL-H at −78 °C (91, 93 and 87%), which were converted directly to the oximes **6a–c** ($H_2NOH{\cdot}HCl$, NaOAc, aq. EtOH, 2–8 °C; 86, 79 and 88%) to inhibit potential racemisation (Scheme 2). Treatment with NCS ($CHCl_3$ reflux) afforded C-chloro-oximes **7**, either used directly (**7a,c**) or isolated (**7b**; 75%); an extended reaction time for chlorination (18 h) led to better results when using the hydroximoyl chlorides **7** (*vide infra*) than in our previous reports.

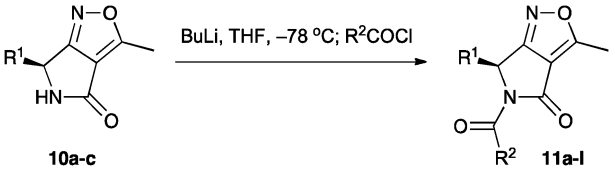


Scheme 2 Synthesis of pyrroloisoxazoles **10**. (a) R^1 = $CHMe_2$; (b) R^1 = CH_2CHMe_2; (c) R^1 = Ph. Reagents: (i) DIBAL-H, toluene, −78 °C; (ii) $H_2NOH{\cdot}HCl$, NaOAc, aq. EtOH, 2–8 °C; (iii) NCS, $CHCl_3$ reflux, 18 h; (iv) Et_3N, $CHCl_3$ reflux; (v) TFA, 20 °C; 2 M aq. HCl; (vi) T3P, EtOAc, 0–20 °C, 17 h (with **9a,b**) or PS-CDI, Et_3N, DMF–CH_2Cl_2, 20 °C, 17 h (with **9c**).

The key dipolar cycloaddition step was performed by addition of Et_3N to the chloro-oximes in the presence of the pyrrolidine enamine of *tert*-butyl acetoacetate and pyrrolidine (separately prepared; toluene reflux, Dean–Stark conditions; 99%) to form the nitrile oxide *in situ* and complete the cycloaddition ($CHCl_3$ reflux) to afford isoxazoles **8a,c** (49 and 56% from **6a,c**) and **8b** (60% from **7b**). Simultaneous deprotection of the *N*-Boc amine and *tert*-butyl ester cleavage was achieved by acid treatment (TFA, 20 °C; then 2 M aq. HCl to give hydrochloride salts of better stability for handling and on storage) to leave amino acid salts **9a–c** (99, 70 and 68%).

The final stage in assembly of the pyrroloisoxazoles, closure of the pyrrolo ring, was initially completed by our previously reported method using *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDCI) (*N*-hydroxysuccinimide, Et_3N, DMF, 0–20 °C) which required column chromatography and yielded the pyrroloisoxazoles **10a,b** in unreliable yields (ranging 8–60%).[10b] Other peptide coupling reagents were investigated: whilst PyBroP failed, HATU did produce **10a** in 40% yield.[14] The variable performance could be improved by using a polystyrene-supported carbodiimide (supplied as PS-CDI; Argonaut Technologies™) (Et_3N, DMF–CH_2Cl_2, 20 °C, 17 h) that reliably afforded **10a** (66%), still however requiring column chromatographic purification and a costly alternative. Finally the simplest and most reliable lactam closure was achieved using the recently commercialised cyclic propylphosphonic anhydride (supplied as T3P; Archemica™).[15] Thus a base (Et_3N) was added to the salts **10a,b** in EtOAc followed by T3P (0–20 °C, 17 h). The pyrroloisoxazoles **10a,b** were isolated pure without needing chromatography in good yields (59 and 68%). The phenylglycine-derived **10c** was unsuccessful with T3P but could be prepared reliably by the PS-CDI protocol (50%). We have thus revealed two improved protocols for lactam closure to pyrroloisoxazoles **10**: using T3P or PS-CDI.[16]

The last stage in the synthesis of the masked reutericyclin analogues was to perform an *N*-acylation. As base we selected to use BuLi (THF, −78 °C). Carboxylic esters were investigated as acylating agents but without success. However, acyl chlorides proved to be effective acylating agents to produce the *N*-acyl

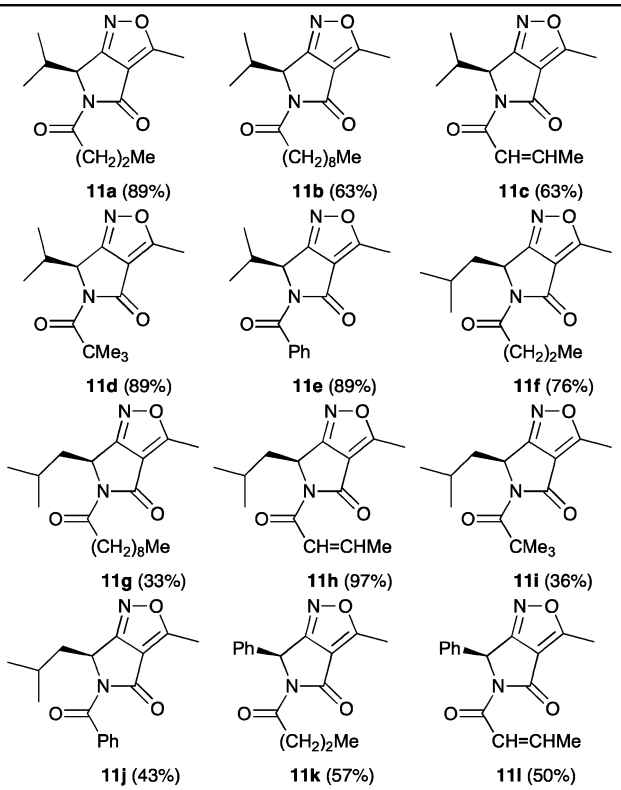


Scheme 3 *N*-Acylation of pyrroloisoxazoles **10**, see Table 1.

derivatives **11** (Scheme 3).[13] A variety of acyl chlorides were selected including long and short aliphatic chains, an α,β-unsaturated chain, a hindered branched moiety and an aromatic substituent, and all afforded *N*-acyl products **11** in yields of 33–97% (Table 1).‡ Longer chain, aromatic or more hindered acyl chlorides required a slightly longer time for complete reaction than the shorter, unhindered, examples; a standard reaction time of 3 h was eventually employed. The constitution of the *N*-(but-2-enyl)-6-(2-methylpropyl)pyrroloisoxazole **11c** was confirmed by an X-ray crystal structure (Fig. 1).§

This completed the synthesis of the reutericyclin analogues. Next we determined to create some tetramide analogues, by N–O bond cleavage of the pyrroloisoxazole nucleus. This was achieved for bicycles **11a,f** by hydrogenolysis (1 atm H_2, Pd–C) to afford the enaminoketones (tetramides) **12a,b** (49 and 52%) (Scheme 4). To demonstrate an alternative protocol, and because hydrogenation would be likely to reduce an unsaturated *N*-acyl group,[10b] N–O cleavage of **11l** was accomplished by $Mo(CO)_6$ (aq. MeCN; then 2 M aq. HCl) to give enaminoketone **12c** (60%).[17] Attempted hydrolysis of the enamine to generate acyltetramic acid

Table 1 Masked reutericyclin analogues: *N*-acyl pyrroloisoxazoles **11**



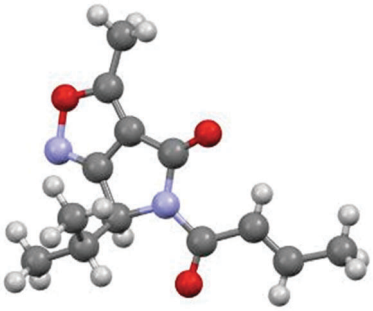
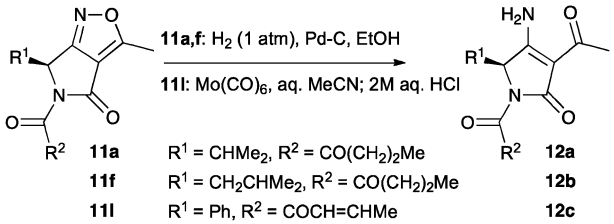


Fig. 1 X-ray crystal structure of *N*-acyl pyrroloisoxazole **11c**.



Scheme 4 Formation of *N*-acyltetramides **12** from pyrroloisoxazoles **11**.

either returned unchanged enaminoketone (*e.g.* H_2O at 20 °C or 2 M aq. HCl at reflux; $NaNO_2$, 3 M aq. H_2SO_4) or led to *N*-deacylation (aq. NaOH, 2 M at reflux or 0.1 M at 20 °C).

In conclusion, we have developed a synthetic route, based on a nitrone 1,3-dipolar cycloaddition, from amino acids to *N*-acylpyrrolo[3,4-*c*]isoxazoles **11** as reutericyclin analogues, and presented a diverse selection of 12 novel compounds. Furthermore, we have demonstrated the conversion of these heterobicycles into *N*-acyltetramides **12**. All of these new compounds are currently undergoing biological evaluation.

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Notes and references

‡ Typical procedure for *N*-acylpyrrolo[3,4-*c*]isoxazole formation: (*S*)-5-butyryl-6-isopropyl-3-methyl-5,6-dihydro-4*H*-pyrrolo[3,4-*c*]isoxazol-4-one **11a**. (*S*)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4*H*-pyrrolo[3,4-*c*]-isoxazol-4-one **10a** (50.0 mg, 0.277 mmol) was suspended in dry THF (20 mL) stirred at −78 °C under a nitrogen atmosphere. *n*-Butyl-lithium (0.201 mL, 1.41 M in hexanes, 0.283 mmol) was added and the reaction stirred for 15 min at this temperature, during which time the solution turned yellow. Butanoyl chloride (29.6 mg, 27.0 μL, 0.283 mmol) was then added in two portions over 10 min and the mixture stirred at −78 °C for a further 3 h before quenching by addition of satd. NH_4Cl solution. The mixture was tested for pH to ensure neutrality had been achieved and then separated between water (20 mL) and EtOAc (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the *title compound* **11a** as a yellow oil (62 mg, 89%); $[\alpha]_D^{20}$ +36.0 (c 5.00 × 10^{-3}, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3025, 1725 (C═O), 1689 (C═O), 1650, 1389, 1250, 1131; δ_H (400 MHz; $CDCl_3$) 0.50 (3H, d, J = 6.8, CH(CH_3)$_2$) 0.92 (3H, t, J = 7.2, CH_2CH_3), 1.17 (3H, d, J = 6.8, CH(CH_3)$_2$), 1.55–1.61 (2H, m, CH_2CH_3), 2.61 (3H, s, 3-CH_3), 2.68–2.72 (1H, m, CH(CH_3)$_2$), 2.81, 2.92 (each 1H, dt, J = 7.6, 14.8, CH_2CO), 5.13 (1H, d, J = 4, CHN); δ_C (100 MHz; $CDCl_3$) 11.8, 12.8, 13.1 (CH_3), 17.0 (CH_2CH_3), 17.9 (CH_3), 27.0 (CH(CH_3)$_2$), 38.2 (CH_2CO), 59.9 (CHN), 113.0, 159.5, 165.0 (isoxazole-C), 168.6, 173.2 (CO). HRMS (ESI): MNa^+ 273.1211; $C_{13}H_{18}N_2O_3$ requires MNa^+ 273.1210.

§ Crystal data for **11c**: $C_{13}H_{16}N_2O_3$, M = 248.28, orthorhombic, $P2_12_12_1$, a = 6.9698(12), b = 9.5108(16), c = 19.233(3) Å, V = 1274.9(4) $Å^3$, Z = 4, μ(Mo-Kα) = 0.71073 Å, 11 350 reflections measured at 150 K on a Bruker APEX 2 CCD diffractometer, 2618 unique data, R_{int} = 0.034, R[for 2390 data with $F^2 > 2\sigma(F^2)$] = 0.032, wR_2 (all data) = 0.078, 227 parameters. H atoms were freely refined. Absolute structure {x = 0.0(6)} could not be determined reliably. CCDC 959645.

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