A Second-Generation Cycloaddition Route to 5-Substituted 3-Acyltetramic Acids

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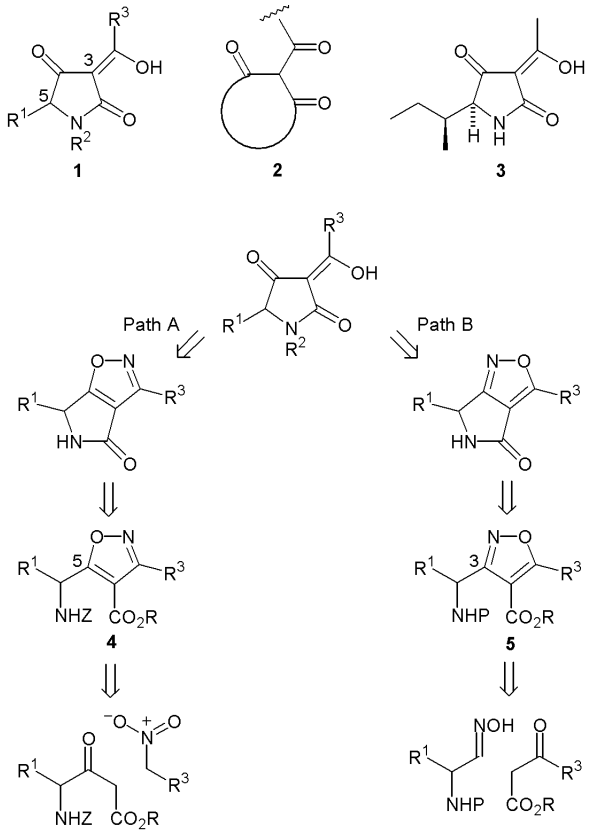
Abstract: The 1,3-dipolar cycloaddition of α-aminonitrile oxides, formed from α-amino-acids, to enamines of β-ketoesters affords 3-(1-aminoalkyl)isoxazole-4-carboxylic esters that are converted *via* pyrrolo[3,4-*c*]isoxazol-4-ones into 5-substituted 3-acetyltetramic acids.

Key words: acyltetramic acid, isoxazole, nitrile oxide, dipole, cycloaddition

During our synthetic studies towards the 3-acyltetramic acids **1**, a group of biologically active metabolites containing the (enolised) tricarbonyl motif **2**[1] and exemplified by the structurally simplest example, tenuazonic acid **3**,[2] we have developed the strategy summarized in Scheme 1, path A.[3] This uses *5-(1-aminoalkyl)*isoxazole-4-carboxylic esters **4** as latent tricarbonyl units, and the highly polar enolic functionality is masked until late in the sequence.



Scheme 1 (P = Protecting group)

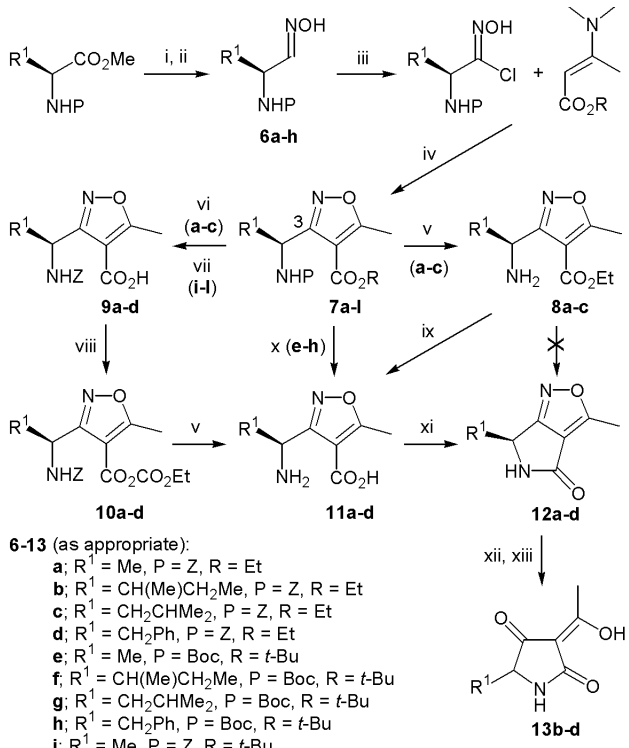
The path A approach is successful for 5-(1-aminomethyl)-isoxazoles **4** (R^1 = H) but we have so far been unable to extend it to more substituted examples, **4** ($R^1 \neq$ H),[4] and thence to 5-substituted 3-acyltetramic acids. We report now on a second-generation isoxazole strategy that overcomes this difficulty.

N–O Bond cleavage and hydrolysis of isoxazoles, to reveal a 1,3-dicarbonyl functionality, renders the N–O regiochemistry irrelevant. Our new strategy is thus based on *3-(1-aminoalkyl)*isoxazole-4-carboxylic esters **5**, Scheme 1, path B. The 4-carboxyisoxazoles of path A are prepared by 1,3-dipolar cycloaddition of nitrile oxides to enamines of γ-amino-β-ketoesters derived from α-amino-acids; path B involves reversing the origins of dipole and dipolarophile components, i.e. α-aminonitrile oxides derived from α-amino-acids, with enamines of β-ketoesters as dipolarophiles.

Table 1: Yields of Oximes **6a-h** and of Isoxazole cycloadducts **7a-l**

R^1	P	**6**, % from protd. amino-ester	R	**7**, % from oxime **6**
Me	Z	**6a**, 63	Et	**7a**, 65
$CH(Me)CH_2Me$	Z	**6b**, 65	Et	**7b**, 63
CH_2CHMe_2	Z	**6c**, 67	Et	**7c**, 71
CH_2Ph	Z	**6d**, 66	Et	**7d**, 42
Me	Boc	**6e**, 67	*t*-Bu	**7e**, 59
$CH(Me)CH_2Me$	Boc	**6f**, 54	*t*-Bu	**7f**, 52
CH_2CHMe_2	Boc	**6g**, 55	*t*-Bu	**7g**, 54
CH_2Ph	Boc	**6h**, 78	*t*-Bu	**7h**, 48
Me	Z		*t*-Bu	**7i**, 54
$CH(Me)CH_2Me$	Z		*t*-Bu	**7j**, 54
CH_2CHMe_2	Z		*t*-Bu	**7k**, 75
CH_2Ph	Z		*t*-Bu	**7l**, 64

Initially methyl esters of the N-benzyloxycarbonyl-(*S*)-α-amino-acids alanine, isoleucine, leucine and phenylalanine were reduced (DIBAL-H, toluene, –78 °C) to the corresponding aldehydes, which were not stored but converted directly ($NH_2OH \cdot HCl$, NaOAc, EtOH aq., 70 °C) to the corresponding stable, solid oximes **6a-d** as *syn/anti* mixtures, without epimerisation. Subsequently this was repeated with the N-*tert*-butoxycarbonylamino-esters to afford oximes **6e-h** (Scheme 2, Table 1).[5] Treatment of the oximes **6** with N-chlorosuccinimide ($CHCl_3$, reflux) resulted in C-chlorination. When these crude hydroxi-



6-13 (as appropriate):
a; R^1 = Me, P = Z, R = Et
b; R^1 = CH(Me)CH_2Me, P = Z, R = Et
c; R^1 = CH_2CHMe_2, P = Z, R = Et
d; R^1 = CH_2Ph, P = Z, R = Et
e; R^1 = Me, P = Boc, R = *t*-Bu
f; R^1 = CH(Me)CH_2Me, P = Boc, R = *t*-Bu
g; R^1 = CH_2CHMe_2, P = Boc, R = *t*-Bu
h; R^1 = CH_2Ph, P = Boc, R = *t*-Bu
i; R^1 = Me, P = Z, R = *t*-Bu
j; R^1 = CH(Me)CH_2Me, P = Z, R = *t*-Bu
k; R^1 = CH_2CHMe_2, P = Z, R = *t*-Bu
l; R^1 = CH_2Ph, P = Z, R = *t*-Bu

Reagents: i, DIBAL-H, toluene, –78ºC; ii, $NH_2OH.HCl$, NaOAc, EtOH aq., 70ºC iii, N-chlorosuccinimide, $CHCl_3$, reflux; iv, Et_3N; v, HBr-AcOH, 25ºC; vi, NaOH aq., reflux; vii, TFA, CH_2Cl_2, 0ºC; viii, EtOCOCl, Et_3N, THF, 0→25ºC; ix, NH_3 aq., reflux; x, TFA, 25ºC; xi, EDCI, N-hydroxysuccinimide, DMF, 0→25ºC; xii, H_2, Pd-C, MeOH, 25ºC; xiii, 2M NaOH aq., 90ºC

Scheme 2

moyl chlorides were treated with Et_3N in the presence of an enamine formed from ethyl or *tert*-butyl acetoacetate and pyrrolidine (toluene, reflux),[6] the nitrile oxides formed *in situ* underwent regiospecific cycloaddition with spontaneous elimination of pyrrolidine[7] to afford the appropriate protected 3-(1-aminoalkyl)isoxazole-4-carboxylates **7a-l** in good yields (Table 1).[8]

The next stage was closure to a pyrroloisoxazolone. Treatment of the 3-(N-benzyloxycarbonylalkyl)isoxazoles **7a-c** with HBr-AcOH (33% w/v; 20 °C, 16h) afforded, on basification, amino-esters **8a-c** (89, 79 and 96%, respectively), which distilled unchanged and could not be cyclised by a range of techniques. This is in accord with our earlier findings for ethyl 5-aminomethyl-3-methylisoxazole-4-carboxylate.[3] In order to further activate the C-4 carboxy group, the N-benzyloxycarbonyl-4-carboxylic acids **9a-d** were prepared. Acids **9a** (88%), **9b** (62%) and **9c** (66%) were obtained by saponification (NaOH aq., reflux, 4 h) of ethyl esters **7a-c**, respectively; alternatively and more efficiently, the acids **9a-d** were prepared by acidolysis (TFA, CH_2Cl_2, 0 °C, 3 h) of the *tert*-butyl esters **7i-l**, respectively, in high yield (Table 2). Conversion into the mixed anhydrides **10a-d** (EtOCOCl, Et_3N, THF, 0→20 °C) was complete after 16 h, as judged by ^1H NMR spectroscopy. However, on brief treatment of these anhydrides with HBr-HOAc (33% w/v; 20 °C, 1 h), instead of the cyclisation expected on the basis of our earlier report on the path A strategy,[3] the major products isolated were the amino-acids **11a-d** as their HBr salts (Table 2).[9]

Table 2: Yields of Acids **9**, Amino-acids **11**, Pyrroloisoxazolones **12** and 3-Acetyltetramic acids **13**.

R^1	**9**, % from **7i-l**	**11**, % from **9** or from **7e-h**	**12**, % from **11**	**13**, % from **12**
Me	**9a**, 96	**11a**, 52 or 92	**12a**, 45	
CH(Me)CH_2Me	**9b**, 82	**11b**, 49 or 89	**12b**, 86	**13b**, 74
CH_2CHMe_2	**9c**, 98	**11c**, 47 or 87	**12c**, 85	**13c**, 63
CH_2Ph	**9d**, 95	**11d**, 75 or 88	**12d**, 75	**13d**, 47

We decided therefore to complete lactam closure to the desired 5,6-dihydro-4*H*-pyrrolo[3,4-*c*]isoxazol-4-ones from the amino acids **11**. In addition to the results above, amino-acids **11a** (86%) and **11b** (94%) were also obtained by hydrolysis (1M NH_3 aq., reflux, 3 h) of the amino-esters **8a** and **8b**, respectively. The method of choice for preparation of amino-acids **11** was, however, direct acidolysis (TFA, 20 °C, 4 h) of the N-*tert*-butoxycarbonylisoxazole *tert*-butyl esters **7e-h**, to afford amino-acids **11a-d**, respectively, isolated as their HCl salts (2M HCl, 20 °C, 0.5 h) (Table 2). After evaluating a number of C-activation protocols, we opted for lactam formation using the water-soluble carbodiimide 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), to permit extractive removal of the urea by-product. Thus the HCl salts of **11a-d** in DMF were treated with N-hydroxysuccinimide and EDCI (0→20 °C, 12 h) to afford pyrroloisoxazolones **12a-d** in good yield (Table 2).[10] The identity of lactams **12b** and **12c** was confirmed by X-ray crystal structure determinations.[11]

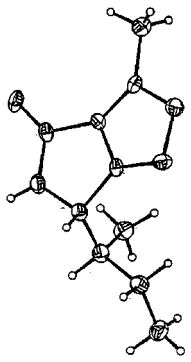


Figure 1 X-ray crystal structure of pyrroloisoxazolone **12b**

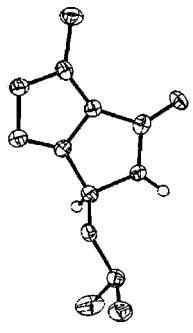


Figure 2 X-ray crystal structure of pyrroloisoxolone **12c**

Unmasking of the tricarbonyl functionality was accomplished *via* hydrogenolysis of the bicyclic lactams **12a-d** (1 atm. H_2, 10% Pd-C, MeOH, 20 °C, 12 h) and hydrolysis of the intermediate enamino-ketones (2M NaOH aq., 90 °C, 16 h) to afford the 3-acetyltetramic acids **13b-d** (Table 2).[12] 5-(1-Methylpropyl)-3-acetyltetramic acid **13b** has the structure of the antitumour[13] fungal metabolite tenuazonic acid, and examination by ^{1}H NMR spectroscopy[14] revealed that this hydrolysis step had generated tenuazonic acid **3** and its C-5 epimer as a 1:2 mixture of diastereoisomers.[15,16] When we employed the minimum conditions found to give complete hydrolysis (0.05M NaOH aq., 50 °C, 20 h), the mixture was improved to 4:1 in favour of natural tenuazonic acid **3**.[17]

We have thus demonstrated that the isoxazole strategy for preparation of 5-substituted 3-acyltetramic acids is successful using 3-aminoalkylisoxazole-4-carboxylates; the shortest route is **6**→**7** →**11**→**12**→**13**. Elaboration studies on the pyrroloisoxazolones **12** as building blocks for more complex 3-acyltetramic acids are underway.

Acknowledgement

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References and Notes

(1) For a recent review and leading references, see: Royles, B. J. L. *Chem. Rev.* **1995**, *95*, 1981; Jones, R. C. F.; Begley, M. J.; Peterson, G. E.; Sumaria, S. *J. Chem. Soc. Perkin Trans. 1* **1990**, 1959.

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(4) We have to date been unable to form the required pyrrolidine enamines of γ-amino-β-ketoesters other than 4-amino-3-oxobutanoates.

(5) *Cf*. Chung, Y. J.; Ryu, E. J.; Keum G.; Kim, B. H. *Bioorg. Chem*. **1996**, *4*, 209; Kim, B. H.; Chung, Y. J.; Ahn, H. J.; Ha, T.-K. *Bull. Korean Chem. Soc*. **1996**, *17*, 401.

(6) The enamine geometry is undetermined; Scheme 2 shows the *E*-isomer for convenience.

(7) Stork, G.; McMurry, J.E. *J. Am. Chem. Soc.* **1967**, *89*, 5461.

(8) *(*S,S*)-3-(1-tert-Butyloxycarbonylamino-2-methylbutyl)-4-tert-butyloxycarbonyl-5-methylisoxazole* **7f**: To (2*S*,3*S*)-2-*tert*-butyloxycarbonylamino-3-methylpentanaldoxime **6f** (6.77 g, 27.63 mmol) in chloroform (600 ml) at 0 °C was added N-chlorosuccinimide (4.06 g, 30.40 mmol) in portions over 20 min and the mixture heated under reflux for 1.5 h, by which time no oxime remained by tlc. *tert*-Butyl 3-pyrrolidino-2-butenoate (11.66 g, 55.26 mmol) was added in one portion followed by triethylamine (3.07 g, 30.39 mmol) dropwise to the refluxing mixture over a period of 3 h *via* syringe pump. The mixture was heated under reflux for a further 2 h, cooled and poured into deionised water (600 ml). The organic phase was separated, washed with citric acid solution (2M, 2 x 600 ml), sodium hydroxide solution (5% w/v, 500 ml) and saturated brine (500 ml), dried ($MgSO_4$) and concentrated under reduced pressure to yield a dark oil purified by chromatography on silica gel, eluting with hexane:ethyl acetate (8:1 v/v) to yield the *title compound* (5.24 g, 52%) as a yellow oil (Found: C, 61.73; H, 8.66; N, 7.85%; MH^+, 369.2389. $C_{19}H_{32}N_2O_5$ requires C, 61.93; H, 8.75; N, 7.60%; MH, 369.2389); $[\alpha]_D^{20}$ –40 (*c* 1.8 in $CHCl_3$); λ_{max}(EtOH)/nm 217 (ε/dm^3 mol^{-1} cm^{-1} 11,500); ν_{max}($CHCl_3$)/cm^{-1} 3448, 2978, 1709, 1600, 1505, 1442, 1370, 1314 1170, 1121 and 1018; δ_H(400 MHz; $CDCl_3$) 0.75 (3H, d, *J* 6.8, C*H*$_3$CH), 0.80 (3H, t, *J* 7.3, C*H*$_3$CH$_2$), 1.06 (1H, m, CH$_3$CH*H*), 1.33 and 1.50 (each 9H, s, (C*H*$_3$)$_3$), 1.53 (1H, m, CH$_3$C*H*H), 1.77 (1H, m, CH$_3$C*H*), 2.53 (3H, s, 5-C*H*$_3$), 4.92 (1H, m, C*H*NH) and 5.73 (1H, br d, *J* 10.3, N*H*); δ_C(100 MHz; $CDCl_3$) 11.1 (CH_3), 13.3 (5-CH_3), 15.8 (CH_3), 24.0 (CH_3CH_2), 28.0 (C(*C*H$_3$)$_3$), 28.1 (C(*C*H$_3$)$_3$), 38.1 (CH_3*C*H), 52.0 (CHNH), 78.9 (*C*(CH$_3$)$_3$), 82.3 (*C*(CH$_3$)$_3$), 108.7 (C-4), 155.2 (OCONH), 161.4 (C-3), 162.7 (C-5) and 175.2 (*C*O$_2$^{t}Bu); *m/z*(EI) 369 (MH^+, 11%), 311, 255, 239, 211, 155, 137 and 57 (100).

(9) A low yield (≤ 18%) of pyrroloisoxazolone **12b** was obtained in some attempts using mixed anhydride **10b**.

(10) *(6S)-3-Methyl-6-[(1S)-methylpropyl]-5,6-dihydro-4H-pyrrolo-[3,4-c]isoxazol-4-one* **12b**: To (*S*,*S*)-3-(1-amino-2-methylbutyl)-5-methylisoxazole-4-carboxylic acid **11b**, as the HCl salt (0.489 g, 1.98 mmol), and N-hydroxysuccinimide (0.251 g, 2.18 mmol) in dry DMF (30 ml) at 0 °C was added EDCI (0.456 g, 2.38 mmol) in portions over 0.5 h and the mixture left to stir at 20 °C for 12 h. Triethylamine (0.602 g, 5.95 mmol) was added dropwise over 3 h *via* syringe pump and the mixture left to stir for a further 5 h at 20 °C before concentration under reduced pressure. The residue was dissolved in ethyl acetate (25 ml), washed successively with water (25 ml), hydrochloric acid (2M, 2 x 25 ml), saturated sodium hydrogen carbonate solution (2 x 25 ml) and saturated brine (25 ml), dried ($MgSO_4$) and concentrated under reduced pressure to afford the *title compound* (0.33 g, 86%) as an off-white solid; a portion was recrystallised from hexane:ethyl acetate to yield colourless crystals, m.p. 110 °C (Found: C, 61.70; H, 7.24; N, 14.31%; M^+, 194.1055. $C_{10}H_{14}N_2O_2$ requires C, 61.84; H, 7.26; N, 14.42%; M, 194.1055); $[\alpha]_D^{26}$ +20 (*c* 1.9 in $CHCl_3$); λ_{max}(EtOH)/nm 228 (ε/dm^3 mol^{-1} cm^{-1} 8,600); ν_{max}($CHCl_3$)/cm^{-1} 3443, 3035, 2968, 1710, 1655, 1529, 1382, 1326 and 1126; δ_H(300 MHz; $CDCl_3$) 0.92 (3H, d, *J* 6.7, C*H*$_3$CH), 0.98 (3H, t, *J* 7.4, C*H*$_3$CH$_2$), 1.41 and 1.57 (each 1H, m, CH$_3$CH*H*), 1.84 (1H, m, CH$_3$C*H*), 2.61 (3H, s, 3-C*H*$_3$), 4.55 (1H, d, *J* 5.7, C*H*NH) and 6.74 (1H, br s, N*H*); δ_C(75 MHz; $CDCl_3$) 11.4 (*C*H$_3$CH$_2$), 12.0 (3-CH_3), 13.8 (*C*H$_3$CH), 25.6 (CH$_3$*C*H$_2$), 37.8 (CH$_3$*C*H), 57.6 (CHNH), 114.2 (C-3a), 163.7 (C-6a), 166.0 (C-3) and 171.3 (CONH); *m/z*(EI) 194 (M^+, 8%), 179, 165, 151, 137 (100), 123, 110, 95, 70 and 57. The structure was confirmed by an X-ray crystal structure determination, ref 11.

(11) Crystal data for **12b** and **12c** (m.p. 170-171 °C from hexane: ethyl acetate) is deposited at the Cambridge Crystallographic Database.

(12) UV spectroscopy indicated hydrolysis of the enaminoketone derived from **12a** was complete, but acyltetramic acid **13a** proved too polar to be extracted from aqueous solution.

(13) For leading references to the toxicity spectrum of tenuazonic acid, see: Lebrun, M. H.; Nicolas, L.; Boutar, M.; Gaudemer, F.; Ranomenjanahary, S.; Gaudemer, A. *Phytochemistry* **1988**, *27*, 77.

(14) *Cf*. Poncet, J.; Jouin, P.; Castro, B.; Nicolas, L.; Boutar, M.; Gaudemer, A. *J. Chem. Soc., Perkin Trans. 1* **1990**, 611.

(15) Precursor **12b** was found to be a single diastereoisomer.

(16) Although the 3-acetyltetramic acids **13c,d** are consistent with samples reported to retain optical activity [**13c**: m.p. 110 °C (lit. 114-114.5 °C; Yuki, H.; Tohira, Y.; Aoki, B.; Kano, T.; Takama, S.; Yamazaki, T. *Chem. Pharm. Bull*. **1967**, *15*, 1107). **13d**: m.p. 132-135 °C (lit. 133-134 °C; Schmidlin, T.; Tamm, C. *Helv. Chim. Acta* **1980**, *63*, 121)], we assume epimerisation comparable to **13b**, *cf*. ref. 13.

(17) Attempted acidic hydrolysis of the enaminoketone formed from **12b** was unsuccessful.

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