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A NEW 3-(ETHOXYCARBONYLMETHYL)ISOXAZOLOPYRIDONE AS A PRECURSOR TO ACYLPYRIDONES

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Abstract – A new 3-(ethoxycarbonylmethyl)isoxazolo[4,3-*c*]pyridin-4-one has been prepared as a potential precursor to 3-acyl-4-hydroxypyridin-2-ones, by a route involving dipolar cycloaddition of nitrile oxides derived from β-alanine with an enamino-ester.

During a programme of synthesis towards metabolites containing the enolised heterocyclic tricarbonyl motif **1** (Figure 1),[1] we have reported dipolar cycloaddition strategies to access dihydroisoxazolo[4,5-*c*]pyridin-4-one **2** (1st generation)[2] and the [4,3-*c*] isomer **3a** (2nd generation)[3] as masked non-polar scaffolds for the 3-acyl-4-hydroxypyridin-2-one nucleus **4**. This unit is found in a group of metabolites with a range of biological activities, exemplified by pigments tenellin **5a** and bassianin **5b** from insect pathogenic fungi,[4] pyridovericin[5] (a tyrosine kinase inhibitor) and the elfamycin antibiotics.[6] In order to exploit the 2nd generation approach to provide molecules analogous to the natural products, it was essential to elaborate the bicycles **3** at the C-3 substituent. We proposed to take advantage of the acidity of the protons at this position to generate nucleophilic reactivity for chain extension. We initially felt that this would require activation of the C-3 substituent, and now wish to communicate our studies towards two new building blocks using that principle, the 3-ethoxycarbonylmethyl analogue **3b,** as an alternative to direct deprotonation of **3a** at C-3(Me).[3,7]

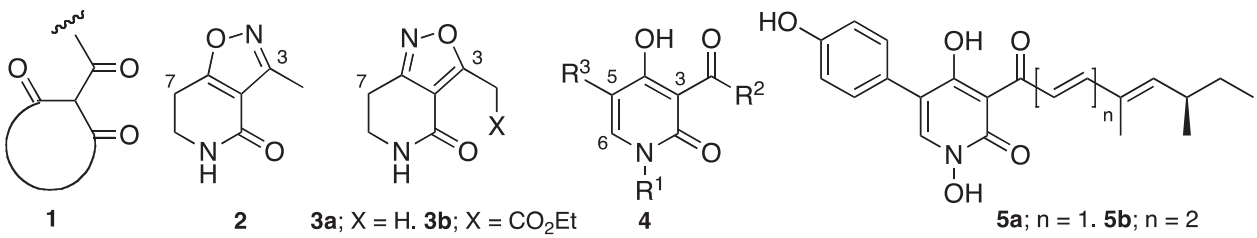
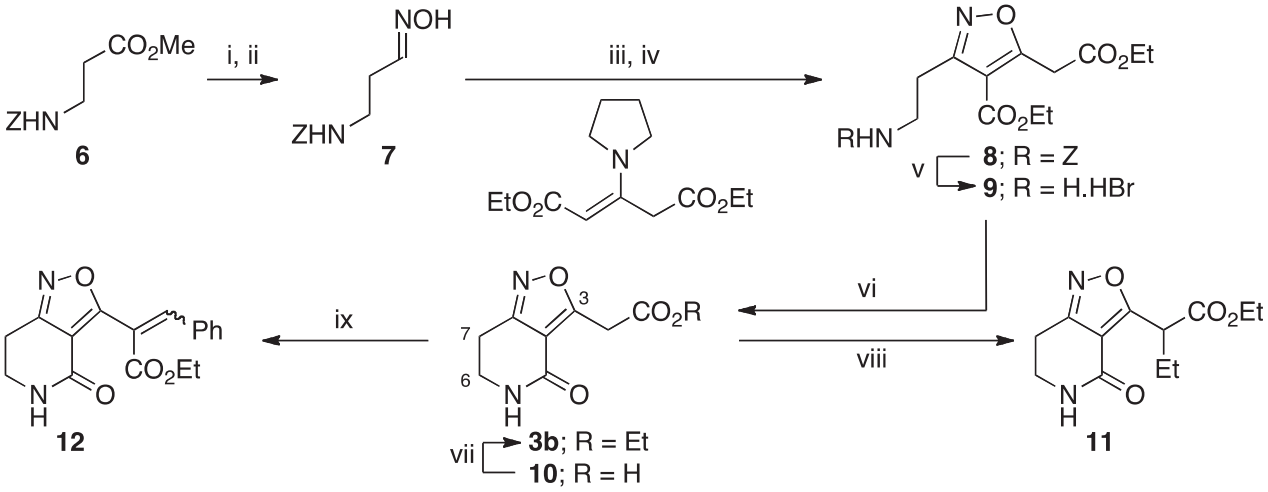


Figure 1. The heterocyclic trione motif, scaffolds and examples

The nitrile oxide dipole precursors were prepared using a sequence based on our published route from β-alanine (Scheme 1).[3] Thus N-benzyloxycarbonyl-β-alanine methyl ester **6** was reduced by DIBALH (toluene, –78 °C) and the aldehyde immediately converted into its oxime **7** (H_2NOH•HCl, NaOAc, EtOH aq., 70 °C, 10-15 min; 58% from ester **6**). Oxime **7** was C-chlorinated (1 mol eq. NCS, $CHCl_3$ reflux, 2 h; then 1 mol eq. NCS, $CHCl_3$ reflux, 18 h) and then treated directly with diethyl 3-pyrrolidino-2-pentenedioate (formed separately from pyrrolidine and diethyl 3-oxopentanedioate; toluene, reflux; 99%) in $CHCl_3$ followed by dropwise addition of triethylamine (reflux, 12 h) to complete the cycloaddition of the enamine with the nitrile oxide formed *in situ*, and the subsequent spontaneous pyrrolidine elimination.[8] Ethyl 3-(2-benzyloxycarbonylaminoethyl)-5-(ethoxycarbonylmethyl)isoxazole-4-carboxylate **8** was isolated in 52% yield. The benzyloxycarbonyl protecting group was removed (HBr-AcOH, 20 °C) to provide the corresponding amine hydrobromide **9** (96%), which on basification (Na_2CO_3 aq., 20 °C) underwent cyclization to afford the target 3-ethoxycarbonylmethyl-4,5,6,7-tetrahydroisoxazolo[4,3-*c*]pyridin-4-one **3b** (52%), along with some of the hydrolysis product, 3-carboxymethylisoxazolopyridone **10** (21%). The acid **10** could be converted into the target ester **3b** by treatment with acidic ethanol (AcCl, EtOH, 0→20 °C, 3 days, then reflux, 7 h; 91%), increasing the overall yield of isoxazolopyridone **3b** from amine salt **9** to 71%.

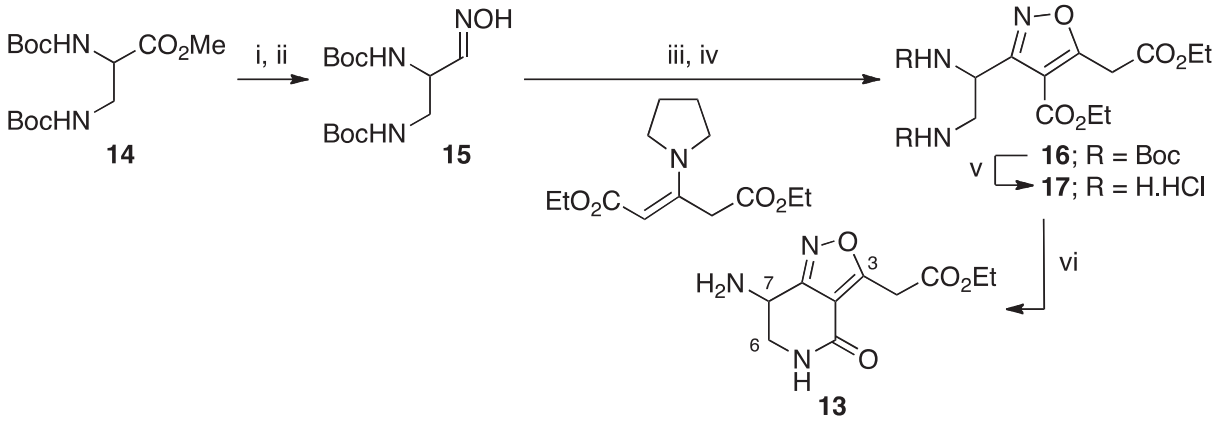


Scheme 1. Reagents: (Z = benzyloxycarbonyl) i, DIBALH, toluene, –78 °C; ii, NH_2OH.HCl, NaOAc, EtOH aq., 70 °C; iii, NCS, $CHCl_3$, reflux; iv, Et_3N and enamine; v, HBr-AcOH, 20 °C; vi, Na_2CO_3 aq.; vii, AcCl, EtOH, 0→20 °C→reflux; viii, LDA, THF, EtI, 0→20 °C→reflux; ix, LDA, THF-TMEDA, PhCHO, 0→20 °C→reflux

In unoptimised preliminary experiments we have validated the nucleophilic potential of the C-3 methylene substituent, provided by the combined activating effect of the benzyl-type position and the ethoxycarbonyl substituent in **3b**. Thus treatment of ester **3b** with base (1 mol eq. LDA, THF, 0 °C) followed by excess iodoethane in two portions (2 mol eq. at 0 °C, then 20 °C, 16 h; a further 2 mol eq., 20 °C, 4 h and reflux, 3.5 h) afforded the C-alkylation product **11** in moderate isolated yield (38%). This

result indicated the potential of the combined activating effects to overrule any N-alkylation of the piperidone ring. In a trial aldol-type reaction with an aldehyde, where any reaction at the pyridine N-atom should be reversible on workup, treatment of ester **3b** with LDA (1 mol eq., THF-TMEDA, 0 °C) followed by benzaldehyde (3 mol eq. at 0 °C, then 20 °C, 11 h and reflux, 3.5 h) led to the condensation product **12** in low yield (23%) with unknown geometry. Such condensations are relevant to the pyridone natural products where the 3-acyl substituent is often an alkenoyl group.[4-6]

Another requirement of the isoxazolopyridone scaffold is the potential for 6,7-desaturation. As a precursor for such a step, we have prepared the C-7 amino derivative **13** of 3-(ethoxycarbonylmethyl)isoxazolopyridone **3b**. We have reported elsewhere on the conversion of such 7-aminotetrahydroisoxazolopyridones into the corresponding 4,5-dihydroisoxazolopyridones.[3b] Amino compound **13** was prepared from methyl 2,3-bis(*tert*-butyloxycarbonylamino)propanoate **14** by a parallel sequence to that used to prepare **3b**. The report below reveals some low-yielding steps and was not optimized, as the requirement for 7-amino precursor **13** was superseded by the C-3 methyl analogue. Thus the methyl ester **14** was reduced to the aldehyde (DIBALH, toluene, –78 °C) and converted directly into the oxime **15** (H_2NOH•HCl, NaOAc, EtOH aq., 70 °C, 10-15 min; 86% from ester **14**) (Scheme 2).



Scheme 2. Reagents (Boc = *tert*-butyloxycarbonyl) i, DIBALH, toluene, –78 °C; ii, NH_2OH.HCl, NaOAc, EtOH aq., 70 °C; iii, NCS, $CHCl_3$, reflux; iv, Et_3N, enamine; v, TFA, 20 °C, then HCl aq.; vi, Na_2CO_3 aq.

The C-chlorination and dipolar cycloaddition sequence with diethyl 3-pyrrolidino-2-pentenedioate was carried out as reported above for oxime **7**, to afford ethyl 3-[1,2-bis(*tert*-butyloxycarbonylamino)ethyl]-5-(ethoxycarbonylmethyl)isoxazole-4-carboxylate **16** in 20% yield. Removal of the protecting groups (TFA, 20 °C; then 2M HCl aq.) afforded a hygroscopic bis-hydrochloride **17** that was basified (Na_2CO_3 aq., 20 °C) and underwent cyclization to afford the 7-amino-3-ethoxycarbonylmethylisoxazolopyridone **13** that was partially characterized, in a low 10% yield. It is very likely that, as observed above with ester **3b** and acid **10**, some ester hydrolysis took place, and that the amino acid so produced was lost in aqueous extracts. No further studies were undertaken with compound **13**.

We have thus demonstrated the synthesis of new activated isoxazolo[4,3-*c*]pyridines with potential for elaboration at the C-3 methylene substituent using nucleophilic reactivity.

EXPERIMENTAL

General: Melting points were determined using a Kofler hot stage apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1710 FT spectrometer. ^{1}H and ^{13}C NMR spectra were recorded using a Jeol JNM-LA300 spectrometer at 300 and 75MHz respectively. NMR spectra were determined in $CDCl_3$ solution (except where indicated) and chemical shifts are quoted in parts per million (ppm) from Me_4Si as internal standard. ^{1}H Coupling constants *J*, where appropriate, are quoted in Hz with multiplicities: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The prefix br (broad) is used where applicable. Mass spectra were carried out by the EPSRC National Mass Spectrometry Service Centre (Swansea) and microanalytical data by Medac Ltd analytical and chemical consultancy services. Column chromatography was carried out at medium pressure using Merck Kieselgel 60 (Art. 9385). Thin layer chromatography was carried out on silica plates (Kieselgel 60, F254, Merck Art. 554).

Diethyl 3-pyrrolidino-2-pentenedioate

Pyrrolidine (1.50 g, 21.09 mmol) was added to a solution of diethyl 3-oxopentanedioate (4.00 g, 19.78 mmol) in toluene (70 mL) and the reaction mixture was heated at reflux under a Dean-Stark water separator for 5 h. The reaction mixture was then cooled and concentrated under reduced pressure to yield the *title compound* (5.01 g, 99%) as a yellow oil, which was used without further purification; δ_H (300MHz; $CDCl_3$) 1.23, 1.27 (each 3H, t, *J* = 7.1 Hz, OCH_2CH_3), 1.94 (4H, m, $CH_2CH_2NCH_2CH_2$), 3.30 (4H, br s, CH_2NCH_2), 4.07 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.15 (2H, s, CH_2CO_2Et), 4.19 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.59 (1H, s, NC=CH).

3-(Benzyloxycarbonylamino)propanaldoxime 7

DIBALH (1.5M in toluene, 59.0 cm^3, 88.5 mmol) was added dropwise to the methyl ester **6** (10.00 g, 42.15 mmol) in dry toluene (200 mL) at –78 ºC and under nitrogen. After stirring the reaction mixture at -78 ºC for 1.5 h, hydrochloric acid (2M, 160 cm^3) was added and the mixture was allowed to warm to 0 ºC. The aqueous phase was separated and extracted with EtOAc (3 x 160 mL). The organic phases were combined, washed with water (2 x 380 mL), dried ($MgSO_4$) and concentrated under reduced pressure to yield 3-benzyloxycarbonylaminopropanal, which was used immediately to form the oxime.

To the crude aldehyde in EtOH (20 mL) was added hydroxylamine hydrochloride (5.86 g, 84.33 mmol), sodium acetate (13.84 g, 168.72 mmol) in water (40 mL). The reaction mixture was warmed to 70 ºC for 10-15 min, cooled and stored in a refrigerator overnight. The white precipitate formed was filtered, washed with water (50 mL) and recrystallised from $CHCl_3$: hexane to yield the *title compound* **7** (5.44 g, 58%) as a white solid, mp 112-114 ºC (lit.,[3] 101-104 ºC); δ_H (300MHz; $(CD_3)_2SO$) 2.38 (2H, dt, *J* = 5.1,

6.8 Hz, NCH_2CH_2), 3.14 (2H, q, *J* = 6.8 Hz, NCH_2CH_2), 5.02 (2H, s, $PhCH_2O$), 6.67 (1H, t, *J* = 5.1 Hz, CH_2CH=NOH), 7.35 (6H, m, Ar-H and NH), 10.95 (1H, s, CH=NO*H*); δ_C (75MHz; $(CD_3)_2SO$) 25.7 (NCH_2CH_2), 37.15 (NCH_2CH_2), 65.1 ($PhCH_2O$), 127.6, 127.7, 128.25 (3 x Ar-CH), 137.1 (Ar-C), 147.8 (CH=NOH), 156.0 (CONH).

Ethyl 3-(2-benzyloxycarbonylaminoethyl)-5-(ethoxycarbonylmethyl)isoxazole-4-carboxylate 8

A mixture of the oxime **8** (2.00 g, 9.00 mmol) and NCS (1.32 g, 9.89 mmol) in $CHCl_3$ (200 mL) was heated at reflux for 2 h. More NCS (2.00 g, 9.00 mmol) was then added and the reaction mixture was heated at reflux overnight, by which time none of the oxime was present by tlc. The reaction mixture was cooled and a solution of the enamine diethyl 3-pyrrolidino-2-pentenedioate (5.00 g, 19.58 mmol) in $CHCl_3$ (20 mL) was added in one portion. Et_3N (1.49 g, 14.72 mmol) was then added dropwise over 12 h, *via* a syringe pump, while heating the reaction mixture at reflux. The reaction mixture was then heated at reflux overnight, cooled and poured into water (220 mL). The organic layer was separated, washed with hydrochloric acid (2M, 2 x 220 mL), saturated aqueous $NaHCO_3$ solution (220 mL), saturated brine (220 mL), dried ($MgSO_4$) and concentrated under reduced pressure to yield a dark oil. The crude product was purified by chromatography on silica gel, eluting with EtOAc : hexane (1:4 v/v), to yield the *title compound* **8** (1.90 g, 52%) as a yellow viscous oil; (Anal. Calcd for $C_{20}H_{24}N_2O_7$: C, 59.40; H, 5.98; N, 6.92%; MH, 405.1656. Found: C, 59.54; H, 6.04; N, 7.06%; MH^+, 405.1658); ν_{max}(film)/cm^{-1} 3378, 3034, 2983, 1723, 1614, 1526, 1456, 1371, 1296, 1248, 1198, 1105, 1026; δ_H (300MHz; $CDCl_3$) 1.24, 1.32 (each 3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.09 (2H, t, *J* = 6.0 Hz, NCH_2CH_2), 3.60 (2H, q, *J* = 6.0 Hz, NCH_2CH_2), 4.08 (2H, s, CH_2CO_2Et), 4.17, 4.28 (each 2H, q, *J* = 7.1 Hz, $CO_2CH_2CH_3$), 5.06 (2H, s, $PhCH_2O$), 5.52 (1H, br t, *J* = 6.0 Hz, CONHCH_2), 7.30 (5H, s, Ar-H); δ_C (75MHz; $CDCl_3$) 14.05, 14.1 (2 x OCH_2CH_3), 27.0 (NCH_2CH_2), 33.4 (CH_2CO_2Et), 38.4 (NCH_2CH_2), 61.05, 61.8 (2 x OCH_2CH_3), 66.5 (OCH_2Ph), 110.1 (C-4), 128.0, 128.2, 128.4 (3 x Ar-CH), 136.7 (Ar-C), 156.36 (OCONH), 161.2 (C-3), 161.5 (C-5), 166.8, 170.8 (2 x CO_2Et); *m/z* (CI) 405 (MH^+, 75%), 314 (12), 271 (20), 255 (30), 222 (24), 197 (58), 126 (30), 108 (100), 106 (92).

Ethyl 3-(2-aminoethyl)-5-(ethoxycarbonylmethyl)isoxazole-4-carboxylate hydrobromide 9

HBr in glacial acetic acid (45% w/v HBr, 2.03 g, 25.09 mmol) was added to the isoxazole **8** (2.73 g, 6.75 mmol) and the reaction mixture was stirred at 20 ºC under nitrogen for 1 h. Dry Et_2O (10 mL) was added and the resultant precipitate was filtered and washed with dry Et_2O (20 mL) to give the *title compound* **9** (2.27 g, 96%) as a white solid, mp 156-157 ºC; (Found: M^+-Br, 271.1297. $C_{12}H_{19}N_2O_5Br$ requires M-Br, 271.1288); δ_H (300 MHz; $(CD_3)_2SO$) 1.20, 1.28 (each 3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.22 (4H, m, NCH_2CH_2), 4.14, 4.27 (each 2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.27 (2H, s, CH_2CO_2Et), 8.04 (3H, s, NH_3); δ_C (75 MHz; $(CD_3)_2SO$) 13.8, 13.9 (2 x OCH_2CH_3), 24.0 (NCH_2CH_2), 33.0 (CH_2CO_2Et), 36.1 (NCH_2CH_2), 60.8, 61.2 (2 x OCH_2CH_3), 109.4 (C-4), 159.6 (C-3), 160.75 (C-5), 166.9, 171.32 (2 x CO_2Et); *m/z* (EI)

271 (M^+-Br, 12%), 241 (54), 179 (22), 155 (61), 137 (46), 128 (32), 109 (45), 97 (54), 69 (31), 43 (100).

3-Ethoxycarbonylmethyl-4,5,6,7-tetrahydroisoxazolo[4,3-*c*]pyridin-4-one 3b

The hydrobromide **9** (2.45 g, 6.98 mmol) and Na_2CO_3 (1.10 g, 10.38 mmol) in water (75 mL) was stirred at room for 11 h. The reaction mixture was extracted with EtOAc (6 x 80 mL) and the organic extracts were combined and concentrated under reduced pressure to yield an off-white solid, which was recrystallised from EtOAc to yield the *title compound* **3b** as a white crystalline solid (0.81 g, 52%), mp 122-123 ºC; (Found: MH^+, 225.0873. $C_{10}H_{12}N_2O_4$ requires MH, 225.0870); ν_{max}(Nujol)/cm^{-1} 3203, 1735, 1686, 1645, 1340, 1192; δ_H (300 MHz; $CDCl_3$) 1.28 (3H, t, J = 7.1 Hz, OCH_2CH_3), 3.04 (2H, t, J = 6.5 Hz, NCH_2CH_2), 3.63 (2H, dt, J = 2.6, 6.5 Hz, NCH_2CH_2), 4.17 (2H, s, CH_2CO_2Et), 4.21 (2H, q, J = 7.1 Hz, OCH_2CH_3), 6.45 (1H, br s, CONH); δ_C (75 MHz; $CDCl_3$) 14.1 (OCH_2CH_3), 21.3 (NCH_2CH_2), 32.3 (CH_2CO_2Et), 40.7 (NCH_2CH_2), 61.9 (OCH_2CH_3), 109.5 (C-3a), 160.6 (C-7a), 162.65 (C-3), 166.8 (CONH), 167.1 (CO_2Et); *m/z* (EI) 224 (M^+, 38%), 179 (55), 152 (100), 123 (83), 94 (38), 81 (66), 69 (43), 52 (48), 43 (56).

The aqueous phase was acidified with hydrochloric acid and extracted with EtOAc (6 x 80 mL). The organic extracts were combined, washed with brine (250 mL), dried ($MgSO_4$) and concentrated under reduced pressure to yield *3-carboxymethyl-4,5,6,7-tetrahydroisoxazolo[4,3-c]pyridin-4-one* **10** as an off-white solid (0.29 g; 21%), mp 190 ºC; (Found: MH^+, 197.0556. $C_8H_8N_2O_4$ requires MH, 197.0557); ν_{max}(Nujol)/cm^{-1} 3281, 2485 (v br), 1714, 1626, 1486, 1349, 1287, 1262, 1020; δ_H (300 MHz; $(CD_3)_2SO$) 2.93 (2H, t, J = 6.5 Hz, NCH_2CH_2), 3.43 (2H, dt, J = 2.8, 6.5 Hz, NCH_2CH_2), 4.11 (2H, s, CH_2CO_2Et), 7.91 (1H, br s, CONH), 12.95 (1H, br s, COOH); δ_C (75 MHz; $(CD_3)_2SO$) 20.4 (NCH_2CH_2), 31.8 (CH_2CO_2Et), 39.6 (NCH_2CH_2), 109.1 (C-3a), 160.9 (C-7a), 161.3 (C-3), 166.8 (CONH), 168.3 (COOH); *m/z* (EI) 152 (M^+-CO_2, 52%), 123 (22), 81 (39), 43 (100).

Acetyl chloride (2.21 g; 28.15 mmol) was added dropwise to a stirred suspension of the acid **10** (30 mg; 0.153 mmol) in dry EtOH (30 mL) at 0 ºC. The reaction mixture was allowed to warm to room temperature, stirred for 3 days and then heated at reflux for 7 h. After cooling, the solvent was removed under reduced pressure to yield the *title compound* **3b** as an off-white solid (31 mg, 91%), mp 122-123 ºC; data identical to the sample described above.

3-(1-Ethoxycarbonylpropyl)-4,5,6,7-tetrahydroisoxazolo[4,3-*c*]pyridin-4-one 11

LDA, prepared by adding BuLi (2.5M in hexanes, 0.13 mL, 0.33 mmol) to diisopropylamine (36 mg, 0.36 mmol) in THF (5 mL) at 0 ºC and stirring for 10 min, was added to a solution of the isoxazolopyridone **3b** (70 mg, 0.31 mmol) in THF (10 mL), under nitrogen, at 0 ºC and the reaction mixture was stirred at this temperature for 20 min. Iodoethane (73 mg, 0.47 mmol) was then added at 0 ºC and the reaction mixture was stirred for 16 h at 20 ºC. More iodoethane (0.15 g, 0.96 mmol) was added, the reaction mixture stirred for 4 h at 20 ºC and then heated at reflux for 3.5 h. The reaction mixture was cooled, water (5 mL)

was added and the mixture extracted with EtOAc (6 x 10 mL). The organic phases were combined, washed with saturated brine (80 mL), dried ($MgSO_4$) and concentrated under reduced pressure to yield the *title compound* **11** (30 mg, 38%) as an off-white solid, mp 95 ºC; (Found: MH^+, 253.1187. $C_{12}H_{16}N_2O_4$ requires MH, 253.1183); δ_H (300 MHz; $CDCl_3$) 0.95 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 1.24 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 2.08, 2.22 (each 1H, m, CH_2CH_3), 3.05 (2H, t, *J* = 6.5 Hz, CH_2CH_2NH), 3.62 (2H, dt, *J* = 2.5, 6.5 Hz, CH_2CH_2NH), 4.19 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.48 (1H, dd, *J* = 6.4, 8.8 Hz, $CHCH_2$), 6.30 (1H, br s, CH_2CH_2NH); δ_C (75MHz; $CDCl_3$) 11.8 (CH_2CH_3), 14.1 (OCH_2CH_3), 21.3 (CH_2CH_2NH), 23.6 (CH_2CH_3), 40.6 (CH_2CH_2NH), 45.1 ($CHCO_2Et$), 61.6 (OCH_2CH_3), 108.9 (C-3a), 160.4 (C-7a), 162.7 (C-3), 169.4 (CONH), 171.3 (CO_2Et); *m/z* (EI) 252 (M^+, 6%), 224 (12), 206 (30), 179 (84), 165 (69), 137 (19), 111 (41), 94 (55), 69 (100), 55 (57), 41 (66).

3-(1-Ethoxycarbonyl-2-phenylethenyl)-4,5,6,7-tetrahydroisoxazolo[4,3-*c*]pyridin-4-one 12

LDA, prepared by adding BuLi (1.6M in hexanes, 0.20 mL, 0.32 mmol) to diisopropylamine (33.2 mg, 0.328 mmol) in THF (5 mL) at 0 ºC and stirring for 10 min, was added to a solution of the isoxazolopyridone **3b** (67.1 mg, 0.299 mmol) and TMEDA (36.2 mg, 0.312 mmol) in THF (15 mL), under nitrogen, at 0 ºC and the reaction mixture was stirred at this temperature for 20 min. Benzaldehyde (0.11 g, 1.04 mmol) was then added at 0 ºC and the reaction mixture was stirred for 11 h at 20 ºC and then heated at reflux for 2 h. The reaction mixture was cooled, hydrochloric acid (1M, 20 mL) was added and the mixture extracted with EtOAc (6 x 20 mL). The organic phases were combined, washed with saturated aqueous $NaHCO_3$ solution (80 mL), saturated brine (80 mL), dried ($MgSO_4$) and concentrated under reduced pressure to yield the crude product which was purified by chromatography on silica gel, eluting with EtOAc : hexane (4:1 v/v), to yield the *title compound* **12** (21.5 mg, 23%) as an off-white solid, mp 149 ºC; (Found: MH^+, 313.1187. $C_{17}H_{16}N_2O_4$ requires MH, 313.1182); ν_{max}(Nujol)/cm^{-1} 3198, 1713, 1668, 1643, 1596, 1323, 1257, 1089, 1061, 1042, 1026; δ_H (300 MHz; $CDCl_3$) 1.29 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.10 (2H, t, *J* = 6.5 Hz, CH_2CH_2NH), 3.59 (2H, dt, *J* = 2.8, 6.5 Hz, CH_2CH_2NH), 4.29 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 5.71 (1H, br s, CH_2CH_2NH), 7.10-7.34 (5H, m, Ar-H), 8.15 (1H, s, C=CHPh); δ_C (75MHz; $CDCl_3$) 14.15 (OCH_2CH_3), 21.6 (CH_2CH_2NH), 40.6 (CH_2CH_2NH), 61.8 (OCH_2CH_3), 110.6 (C-3a), 118.3 (C=CHPh), 128.7, 130.1, 130.6 (3 x Ar-CH), 133.1 (Ar-C), 148.2 (C=CHPh), 161.0 (C-7a), 161.2 (C-3), 164.3 (CONH), 166.9 (CO_2Et); *m/z* (EI) 312 (M^+, 11%), 294 (27), 268 (33), 239 (50), 211 (28), 138 (33), 129 (87), 115 (39), 102 (100), 94 (59), 77 (87), 51 (53), 44 (89).

2,3-bis(*tert*-Butyloxycarbonylamino)propanaldoxime 15

To methyl ester **14** (14.04 g, 44.10 mmol) in dry toluene (340 mL), at –78 ºC under nitrogen, was added DIBALH (1.5M in toluene, 111 mL, 167 mmol) dropwise over 35 min. The reaction mixture was stirred at –78 ºC for 65 min and MeOH (55 mL) was then added. The mixture was immediately poured into a solution of potassium sodium tartrate (470 g) in water (950 mL) and stirred vigorously for 1.5 h. The

aqueous phase was separated and extracted with EtOAc (2 x 500 mL). The organic phases were combined, washed with saturated brine (2 x 600 mL), dried ($MgSO_4$) and concentrated under reduced pressure to yield 2,3-bis(*tert*-butyloxycarbonylamino)propanal which was used immediately to form the oxime.

To the crude aldehyde in EtOH (35 mL) was added hydroxylamine hydrochloride (6.06 g, 87.21 mmol) and sodium acetate (14.49 g, 176.64 mmol) in water (95 mL). The reaction mixture was warmed to 70 ºC for 10-15 min, cooled and stored in a refrigerator overnight. The white precipitate was filtered, washed with water (100 mL) and dried under reduced pressure to yield the *title compound* **15** (11.55 g, 86%) as a mixture of *syn*- and *anti*-oximes which was used without further purification; mp 127-128 ºC; (Found: MH^+, 304.1870. $C_{13}H_{25}N_3O_5$ requires MH, 304.1867); δ_H (300MHz; $(CD_3)_2SO$) 1.35 (9H, s, $OC(CH_3)_3$), 1.36 (9H, s, $OC(CH_3)_3$), 3.05 (1.4H, m, NCH_2CH), 3.28 (0.6H, m, NCH_2CH), 4.10 (0.3H, m, NCH_2CH), 4.68 (0.7H, m, NCH_2CH), 6.50 (0.7H, d, J = 6.3 Hz, C*H*=NOH), 6.84 (2H, br m, 2 x NHCO), 7.10 (0.3H, d, J = 6.3 Hz, C*H*=NOH), 10.76 (0.3H, br s, CH=NO*H*), 11.06 (0.7H, br s, CH=NO*H*); δ_c (75MHz; $(CD_3)_2SO$) 28.2 ($(CH_3)_3C$), 40.8 (NCH_2CH), 42.1 (NCH_2CH), 46.4 (NCH_2CH), 50.2 (NCH_2CH), 77.7, 78.0 (2 x $OC(CH_3)_3$), 148.3, 149.38 (CH=NOH), 155.0, 155.65, 155.79 (CONH); *m*/*z* (CI) 304 (MH^+, 56%), 303 (68), 247 (58), 204 (48), 171 (100), 135 (37), 115 (82), 103 (38), 59 (41).

Ethyl 3-[1,2-bis(*tert*-butyloxycarbonylamino)ethyl]-5-(ethoxycarbonylmethyl)isoxazole-4-carboxylate 16

The procedure described above for the preparation of isoxazole **8** was utilized incorporating the following quantities: oxime **15** (0.52 g, 1.71 mmol), 2 x NCS (each 0.26 g, 1.95 mmol), $CHCl_3$ (50 mL), diethyl 3-pyrrolidino-2-pentenedioate (1.31 g, 5.13 mmol) in $CHCl_3$ (15 mL) and Et_3N (0.28 g, 2.77 mmol) over 4 h. The crude product was purified by chromatography on silica gel, eluting with EtOAc : hexane (1:4 v/v), to yield the *title compound* **16** (0.17 g, 20%) as an off-white solid, mp 84-85 ºC; (Found: MNH_4^+, 503.2713. $C_{22}H_{35}O_9N_3$ requires MNH_4, 503.2712); ν_{max}(Nujol)/cm^{-1} 3387, 3370, 1750, 1724, 1692, 1610, 1526, 1310, 1286, 1253, 1200, 1168, 1123, 1107, 1025; δ_H (300MHz; $CDCl_3$) 1.27 (3H, t, J = 7.1 Hz, OCH_2CH_3), 1.37 (3H, t, J = 7.0 Hz, OCH_2CH_3), 1.39, 1.44 (each 9H, s, $C(CH_3)_3$), 3.57 (2H, t, J = 6.0 Hz, NCH_2CH), 4.11 (2H, s, CH_2CO_2Et), 4.20 (2H, q, J = 7.1 Hz, $CO_2CH_2CH_3$), 4.35 (2H, m, $CO_2CH_2CH_3$), 5.03 (1H, br s, $HNCH_2CH$), 5.35 (1H, apparent br s, NCH_2CH), 5.91 (1H, br d, J = 8.4 Hz, CH_2CHNH); δ_C (75MHz; $CDCl_3$) 14.05, 14.08 (2 x OCH_2CH_3), 28.25, 28.3 (2 x $C(CH_3)_3$), 33.6 (CH_2CO_2Et), 43.5 (NCH_2CH), 48.6 (NCH_2CH), 61.5, 61.9 (2 x OCH_2CH_3), 79.4, 79.9 (2 x $OC(CH_3)_3$), 109.6 (C-4), 155.35, 156.1 (OCONH), 161.4 (C-3), 161.5 (C-5), 166.6, 171.2 (2 x CO_2Et); *m*/*z* (CI: NH_3) 486 (MH^+, 14%), 386 (17), 245 (59), 220 (100), 203 (21), 173 (24), 161 (35), 135 (47).

Ethyl 3-(1,2-diaminoethyl)-5-(ethoxycarbonylmethyl)isoxazole-4-carboxylate dihydrochloride 17

TFA (2 mL) was added to the isoxazole **16** (85.8 mg, 0.177 mmol) and the reaction mixture was stirred at room temperature for 4.5 h and then concentrated under reduced pressure. Hydrochloric acid (2M, 4 mL)

was then added, the reaction mixture stirred at room temperature then concentrated under reduced pressure. The residue was dissolved in water (10 mL), washed with EtOAc (2 x 10 mL) and the aqueous layer evaporated to dryness under reduced pressure to yield the *title compound* **17** as an off-white solid (61.8 mg, 98%) which was hygroscopic and used without further purification; δ_H (300 MHz; D_2O) 1.12, 1.20 (each 3H, t, J = 7.1 Hz, OCH_2CH_3), 3.58-3.77 (2H, m, NCH_2CH), 4.10 (2H, q, J = 7.1 Hz, OCH_2CH_3), 4.23 (2H, s, CH_2CO_2Et), 4.25 (2H, q, J = 7.1 Hz, OCH_2CH_3), 5.25 (1H, t, J = 6.3 Hz, NCH_2CH); δ_C (75 MHz; D_2O) 16.05, 16.1 (2 x OCH_2CH_3), 36.3 (CH_2CO_2Et), 42.2 (NCH_2CH), 47.8 (NCH_2CH), 65.9, 66.1 (2 x OCH_2CH_3), 113.3 (C-4), 159.7 (C-3), 164.85 (C-5), 172.25, 176.0 (2 x CO_2Et).

7-Amino-3-ethoxycarbonylmethyl-4,5,6,7-tetrahydroisoxazolo[4,3-*c*]pyridin-4-one 13

The procedure described above for the preparation of isoxazolopyridone **3b** was utilized incorporating the following quantities: dihydrochloride salt **17** (61.8 mg, 0.173 mmol) and Na_2CO_3 (46.0 mg, 0.434 mmol) in water (5 mL) stirred for 14 h, to yield the *title compound* **13** as an off-white solid (4.2 mg, 10%) that was partially characterized; δ_H (300 MHz; $CDCl_3$) 1.2 (3H, t, J = 7.1 Hz, OCH_2CH_3), 1.78 (2H, br s, NH_2), 3.39, 3.62 (each 1H, m, NCH_2CH), 4.11 (2H, s, CH_2CO_2Et), 4.15 (2H, q, J = 7.1 Hz, OCH_2CH_3), 4.32 (1H, dd, J = 5.2, 7.6 Hz, NCH_2CH), 5.91 (1H, br s, CONH); δ_C (75 MHz; $CDCl_3$) 14.1 (OCH_2CH_3), 32.2 (CH_2CO_2Et), 44.2 (NCH_2CH), 48.8 (NCH_2CH), 61.9 (OCH_2CH_3), 108.1 (C-3a), 162.0 (C-7a), 164.3 (C-3), 166.6 (CONH), 167.7 (CO_2Et).

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