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Synthesis of 4-Amino Substituted and 4-Unsubstituted 1,10-Phenanthroline-3-carboxylic Acid Derivatives as Potential DNA Cleavage Reagents

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Abstract: The synthesis of several new 4-amino substituted and 4-unsubstituted 1,10-phenanthroline-3-carboxylic acid derivatives are reported. The 4-amino derivatives (**5,6,7**) were prepared from 1,4-dihydro-4-oxo-1,10-phenanthroline-3-carboxylic acid (**2**) via aromatization and 4-chlorination, followed by nucleophilic substitution at C4 by the appropiate alkyl amine. The 4-unsubstituted derivatives (**10,11,12**) were prepared by aromatization and 4-chlorination of ethyl 1,4-dihydro-4-oxo-1,10-phenanthroline-3-carboxylate. The substituent at C4 was removed by conversion to the 4-tosyl hydrazide, followed by base-catalysed elimination of the hydrazide group. These phenanthroline derivatives were designed as metal-ion-complex mediated DNA cleavage reagents. In both series the 3-carboxylic acid side chain was coupled to β-alanine to facilitate its conjugation with DNA binding molecules.

Abbreviations

BOP: benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate; DCM: dichloromethane; EDTA: ethylenediamine tetraacetic acid; HOBt: 1-hydroxybenzotriazole; TEA: triethylamine.

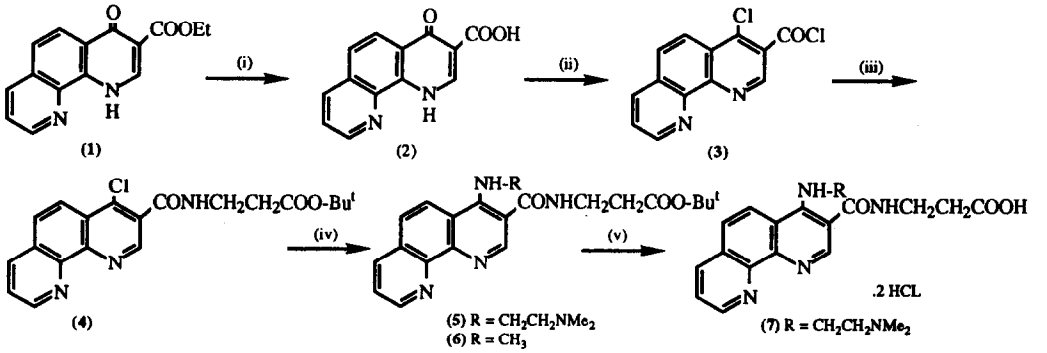
Introduction

Stable complexes formed between transitional elements and organic ligands that facilitate the oxidation-reduction cycle of the metal ions, such as EDTA-iron[1-3] and 1,10-phenanthroline-copper,[4-6] can generate reactive hydroxy radicals in the presence of a suitable redox system such as molecular oxygen and sodium ascorbate.[5] Utilising the hydroxy radicals generated to cleave the DNA sugar-phosphate backbone, these metal complexes have been employed in the study of DNA-ligand interactions, either in the free form, or covalently attached to the DNA binding molecule.[3-6]

The use of a tethered 1,10-phenanthroline system as DNA cleavage reagent has been reported in some detail by Sigman *et al.*[4-6] However, the method described in general requires the presence of a cysteinyl residue for the post-synthetic incorporation of the 5-iodoacetamido-1,10-phenanthroline intermediate. For our purpose, a 1,10-phenanthroline carboxylic acid derivative is more suitable for the study of peptide DNA interactions, as the DNA cleavage phenanthroline moiety can be coupled directly to the amino terminal of a DNA binding peptide of interest, which is prepared using standard solid phase peptide synthesis, thus avoiding post-synthesis derivatization.

In the literature, there are few reports of 1,10-phenanthroline carboxylic acids. 1,4-Dihydro-4-oxo-1,10-phenanthroline-3-carboxylic acid (**2**) has been used as a common synthetic intermediate for 3-unsubstituted derivatives after 3-decarboxylation.[7] The 3-carboxylic acid group has not been used as a synthetic functional handle. Furthermore, 4-unsubstituted 1,10-phenanthroline-3-carboxylic acid derivatives have not been reported. Since the substitution at C3 would not interfere sterically with the chelation property of the 1,10-phenanthroline system, it would provide an excellent synthetic handle for its conjugation with DNA binding ligands.

In this report, the synthesis of several new 1,10-phenanthroline-3-carboxylic acid derivatives with 4-amino-substitution is reported. Also, the pathway for the removal of the 4-substituent to give the heretofore unknown 1,10-phenanthroline-3-carboxylic acid is reported for the first time.



(i) NaOH, reflux;
(ii) $POCl_3$, cat. DMF, reflux;
(iii) β-Ala-O-Bu^t, TEA, DCM, RT;
(iv) RNH_2, DMF, 60-70°;
(v) HCl in Chloroform, RT.

Scheme 1.

Results and Discussion

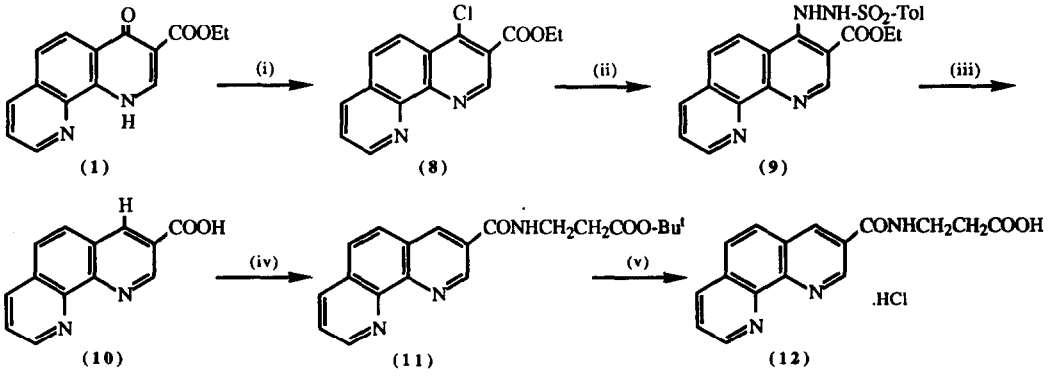
The synthesis of both series of the title 1,10-phenanthroline-3-carboxylic acid derivatives, the 4-amino substituted (**5, 6, 7**) and the 4-unsubstituted derivatives (**10, 11, 12**), are illustrated in Scheme 1 and 2 respectively. Both series were prepared from the common precursor, ethyl 1,4-dihydro-4-oxo-1,10-phenanthroline-3-carboxylate (**1**). Compound (**1**) was obtained in good yield from the facile condensation of 8-aminoquinoline and diethyl ethoxymethylenemalonate, followed by cyclisation in boiling phenyl ether.[7]

4-amino derivatives

The 4-amino substituted derivatives were synthesized according to the common pathway (Scheme 1). The ethyl ester of the precursor (**1**) was hydrolysed, and the resulting carboxylic acid (**2**) was aromatized and chlorinated to the acid chloride (**3**) with phosphoryl chloride. Thus, the 1,4-dihydro-4-oxo-pyridine system of (**2**) was converted to the 4-chloropyridine system in (**3**). The 3-carboxyl chloride derivative (**3**) was treated with β-alanine *t*-butyl ester in the presence of triethylamine to give (**4**), the precursor possessing the penultimate synthetic handle for coupling to a peptide chain. The *t*-butyl ester of the side chain was removed by acidolysis after the 4-substitution has been elaborated.

At this stage, the 4-chloropyridine system of the 4-chloro-1,10-phenanthroline-3-carboxy-β-alanine *t*-butyl ester (**4**) is an important intermediate for a whole host of 4-substituted derivatives, which can be prepared via the facile nucleophilic displacement of the 4-chloro atom. The preparation of the 4-amino derivatives in general was achieved by treating the 4-chloro intermediate (**4**) with the desired primary amino component in excess in DMF at 60-70 °C for 2-4 h. Pure products were obtained after simple work up procedures.

In this work, the preparation of two amino substituents was illustrated. A simple 4-aminopyridine system, represented by the 4-(N-methylamino) derivative (**5**), was choosen to increase the electron density at N1, and consequently the complexation affinity for metal ions. The N,N-dimethylethylenediamine substituent, which is cationic at physiological pH, was choosen for its ability to enhance DNA binding affinity.[8,9] Introduction of this 4-substituent gave the *t*-butyl ester (**6**), which was deprotected using HCl in chloroform to give the free acid (**7**). Compound (**7**) was conjugated with DNA intercalator molecules to further enhance the DNA binding affinity. Compound (**7**) and its intercalator conjugates were found to have remarkable DNA cleavage activity and specificity, the result of which will be reported elsewhere.



(i) $POCl_3$, cat.DMF, reflux;
(ii) Tos-hydrazide, chloroform, RT;
(iii) $NaHCO_3$ in aq./ethylene glycol, reflux;
(iv) β-Ala-O-Bu^t, BOP, HOBt, DMF, RT;
(v) HCl in chloroform.

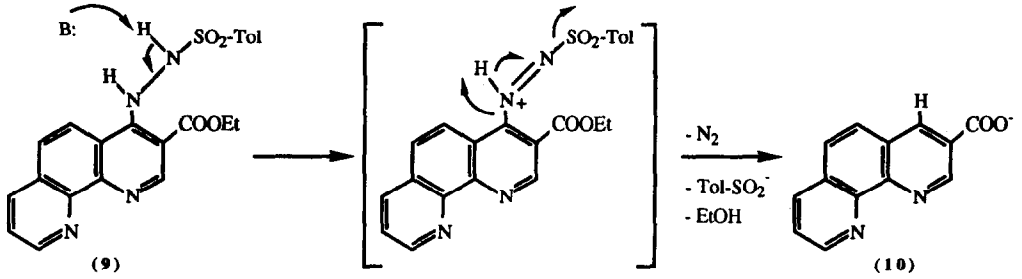
Scheme 2.

4-unsubstituted derivative

In the synthesis of 1,10-phenanthrolines from the precursor (**1**), the products always retain a functional group at C4. The replacement of the 4-substitution with a proton is formally a reductive step. Direct reduction of the 4-oxo or 4-chloro derivatives would probably lead to the reduction of the ring system, thus extra re-oxidation steps would be required to regain aromaticity. An indirect, non-reductive pathway has been reported for the elimination of the 4-substituent in an analogous acridine system.[10] It required the conversion of the 4-functionality to the 4-tosyl hydrazide in a pyridinyl system. The subsequent base catalysed E2 elimination of the tosyl hydrazide is initiated by the abstraction of the proton from the sulphonated N atom (N1), leading to the heterolytic cleavage of the C4-N2' bond, presumably via the diazo intermediate (Scheme 3).

In this work, the ethyl 4-oxo-3-carboxylate (**1**) was chlorinated and aromatized with phosphoryl chloride to give the ethyl 4-chloro-3-carboxylate (**8**). The 4-chloro atom was displaced by toluenesulphonyl hydrazide to

give the 4-hydrazido derivative (**9**). The 4-hydrazide group was removed with concomitant hydrolysis of the 3-ethyl ester in boiling aqueous sodium bicarbonate-ethylene glycol to give 1,10-phenanthroline-3-carboxylic acid (**10**). The 3-carboxylic acid (**10**) was coupled with the linker β-alanine *t*-butyl ester using BOP-HOBt to give (**11**). The *t*-butyl ester was removed by acidolysis in HCl saturated chloroform to give the final product, 1,10-phenanthroline-3-carboxy-β-alanine (**12**), which is suitable for N-terminal coupling in peptide synthesis (Scheme 2).



Scheme 3.

Summary

The syntheses of a series of 1,10-phenanthroline-3-carboxylic acid derivatives are reported. The 4-amino derivatives were prepared via the displacement of the 4-chloro atom produced as a consequence of aromatization using phosphoryl chloride. For the 4-unsubstituted derivatives, the 4-chloro atom can be removed non-reductively by conversion to the 4-tosyl hydrazide, which was then eliminated via a base-catalysed mechanism. These derivatives can be conjugated to potential DNA binding ligands via the 3-carboxy-β-alanine side chain.

Experimental

Melting points were measured using a Buchi melting point apparatus. Analytical thin layer chromatography was performed on Merck (Darmstadt, Germany) precoated silica gel 60 F254 plates. Nmr spectroscopy were performed on a Bruker AM300 spectrometer. ^1H chemical shifts were referenced to tetramethyl silane (0.0 ppm) in $CDCl_3$ solvents, or residual methanol solvent peak (3.30 ppm) in CD_3OD or D_2O with methanol as internal standard. ^{13}C chemical shifts were referenced to the solvent peaks, $CDCl_3$ (77.0 ppm) in $CDCl_3$ solvent mix, or methanol (49.0 ppm) in CD_3OD or D_2O. The coupling constants are reported in Hertz. Molecular formula determinations were carried out using high resolution FAB mass spectrometry, performed at the Victorian College of Pharmacy, Parkville, Victoria, Australia. Cation exchange resin Macro-prep 50S was purchased from Bio-Rad Laboratories, USA. Phosphoryl chloride, toluenesulphonyl hydrazide, N,N-dimethylethylenediamine, methylammonium chloride and HOBt were purchased from Aldrich Chemical Co., USA. β-Alanine *t*-butyl ester hydrochloride, and BOP were purchased from Novabiochem, Switzerland. The intermediates (**1**) and (**2**) were prepared according to a literature method.[7]

Preparation of 4-chloro-1,10-phenanthroline-3-carboxy-β-alanine t-butyl ester (4)

Compound (**2**) (1 g) was refluxed in phosphoryl chloride (5 ml) with a catalytic amount of DMF (0.05 ml) for 1 h. The mixture was concentrated under vacuum, and residual phosphoryl chloride removed by azeotropic distillation with benzene under vacuum. The resulting 4-chloro-3-carboxyl chloride (**3**) was not isolated and was used in the subsequent step without further purification. The crude product (**3**) was dissolved in DMF (10 ml), cooled in ice, and treated with β-alanine *t*-butyl ester hydrochloride (1.1 equiv., 820 mg) and TEA (4 equiv., 2 ml). The mixture was stirred for 2 h at 0°, and at room temperature for a further 16 h. The reaction mixture was diluted with 1 M $NaHCO_3$, and extracted with DCM (3x100 ml). The combined extracts were dried ($MgSO_4$) and concentrated under vacuum to a thick oil. The product (**4**) was purified via column chromatography (Alumina activity AII, eluting with 1% methanol in DCM), and crystallised from petroleum ether and DCM as pale yellow prisms, mp. 158°, yield 1.22 g (76%). HR-MS $[MH]^+$ $C_{20}H_{21}ClN_3O_3$ calc. 386.1291, obs. 386.1217. ^1H-nmr δ ($CDCl_3$) 1.422 (s, CH_3 Bu^t), 2.635 (t, J 6.0, αCH_2 β-Ala), 3.756 (q, J 6.0, βCH_2 β-Ala), 7.187 (bt, NH), 7.622 (dd, J4.3, 8.0, H8), 7.794 (d, J 9.2, H6), 8.096

(d, J 9.2, H5), 8.191 (dd, J 1.5, 8.0, H7), 9.113 (s, H2), 9.143 (dd, J 1.5, 4.3 H9). ^{13}C-nmr δ ($CDCl_3$) 28.07 (CH_3 Bu^t), 34.87 (αCH_2 β-Ala), 35.77 (βCH_2 β-Ala), 81.29 (q-C Bu^t), 122.10 (C5), 123.89 (C8), 125.98 (C3), 128.26 (C6), 128.82 (C6a), 130.10 (C4a), 135.99 (C7), 139.18 (C4), 145.32 (C6b), 147.23 (C4b), 148.84 (C2), 150.97 (C9), 164.61 (3-CO), 171.73 (CO β-Ala).

Preparation of 4-[N-methylamino]-1,10-phenanthroline-3-carboxy-β-alanine t-butyl ester (5)

Compound **(4)** (200 mg) was treated with excess methylammonium chloride (500 mg) in DMF (6 ml) and TEA (4 ml) at 60° for 3 h. The reaction mixture was concentrated to a thick oil under vacuum, diluted with water (50 ml), and extracted with ethyl acetate (2x50 ml). The desired product was recrystallised from DMF-water as a white powder, mp. 207°, yield 150 mg (88%). HR-MS $[MH]^+$ $C_{21}H_{25}N_4O_3$ calc. 381.1927, obs. 381.1897. ^{1}H-nmr δ (CD_3OD) 1.481 (s, CH_3 Bu^t), 2.630 (t, J 6.7, αCH_2 β-Ala), 3.119 (s, CH_3 NMe), 3.648 (t, J6.7, βCH_2 β-Ala), 7.669 (dd, J 4.4, 8.1, H8), 7.756 (d, J 9.3, H6), 8.137 (d, J 9.2, H5), 8.334 (dd, J 1.4, 8.1, H7), 8.597 (s, H2), 9.012 (dd, 1.5, 4.2, H9). ^{13}C-nmr δ (CD_3OD) 28.39 (CH_3 Bu^t), 33.16 (NMe), 35.90 (αCH_2 β-Ala), 37.23 (βCH_2 β-Ala), 82.02 (q-C Bu^t), 113.56 (C4a), 119.75 (C3), 121.65 (C5), 124.51 (C8), 125.31 (C6), 130.12 (C6a), 137.31 (C7), 146.58 (C6b), 147.16 (C4b), 150.36 (C2), 150.84 (C9), 152.49 (C4), 171.67 (3-CO), 172.77 (CO β-Ala).

Preparation of 4-[N-[2-(N',N'-dimethylamino)ethyl]amino]-1,10-phenanthroline-3-carboxy-β-alanine t-butyl ester (6)

Compound **(4)** (5.5 g) was treated with excess N,N-dimethylethylenediamine (8 ml) in DMF (40 ml) at 60° for 3 h. The reaction mixture was concentrated under vacuum to about 5 ml and the product precipitated by dilution with water to 100 ml. The desired compound was recrystallised from methanol-water to give pale yellow prisms, mp. 176°, yield 4.75 g (79%). HR-MS $[MH]^+$ $C_{24}H_{32}N_5O_3$ calc. 438.2505, obs. 438.2489. ^{1}H-nmr δ ($CDCl_3$) 1.478 (s, CH_3 Bu^t), 2.309 (s, CH_3 NMe), 2.57-2.65 (m, αCH_2 β-Ala and CH_2NMe_2), 3.64-3.77 (m, βCH_2 β-Ala and 4-$NHCH_2$), 7.246 (m, NH β-Ala), 7.616 (dd, J4.3, 8.1, H8), 7.686 (d, J 9.2, H6), 7.811 (m, 4-NH), 8.090 (d, J 9.2, H5), 8.217 (dd, J 1.6, 8.1, H7), 8.927 (s, H2), 9.165 (dd, J 1.6, 4.3, H9). ^{13}C-nmr δ ($CDCl_3$) 28.12 (CH_3 Bu^t), 34.97 (αCH_2 β-Ala), 35.50 (βCH_2 β-Ala), 45.22 (NMe_2), 46.03 (CH_2NMe_2), 59.02 (4-$NHCH_2$), 81.33 (q-C Bu^t), 113.05 (C4a), 119.28 (C3), 122.08 (C5), 123.16 (C8), 123.71 (C6), 128.69 (C6a), 135.53 (C7), 146.24 (C6b), 148.16 (C4b), 148.65 (C2), 150.20 (C9), 153.21 (C4), 168.31 (3-CO), 171.88 (CO β-Ala).

Preparation of 4-[N-[2-(N',N'-dimethylamino)ethyl]amino]-1,10-phenanthroline-3-carboxy-β-alanine dihydrochloride (7)

Compound **(6)** (3.4 g) was dissolved in chloroform (10 ml) and treated with HCl saturated chloroform (100 ml) at RT for 1 h. The resulting dihydrochloride precipitate was collected and washed with chloroform to give a hygroscopic solid, mp. 187°, yield 3.66 g (85%). HR-MS $[MH]^+$ $C_{20}H_{24}N_5O_3$ calc. 382.1879, obs. 382.1855. ^{1}H-nmr δ (CD_3OD) 2.743 (t, J 6.2, αCH_2 β-Ala), 3.68-3.76 (m, βCH_2 β-Ala & CH_2NMe_2), 4.115 (m, 4-$NHCH_2$), 7.911 (dd, J 4.3, 8.2, H8), 8.163 (d, J 9.3, H6), 8.56-8.65 (H5 and H7), 8.652 (s, H2), 9.164 (m, H9). ^{13}C-nmr δ (CD_3OD) 34.08 (αCH_2 β-Ala), 37.41 (βCH_2 β-Ala), 42.41 (CH_2NMe_2), 44.32 (NMe_2), 57.41 (4-$NHCH_2$), 113.64 (C4a), 118.94 (C3), 121.28 (C5), 127.06 (C8), 128.13 (C6), 130.61 (C6a), 137.10 (C6b), 137.77 (C7), 139.50 (C4b), 142.79 (C2), 152.48 (C9), 155.45 (C4), 167.51 (3-CO), 175.31 (CO β-Ala).

Preparation of ethyl 4-chloro-1,10-phenanthroline-3-carboxylate (8)

Compound **(1)** (1 g) was refluxed in phosphoryl chloride (5 ml) and DMF (0.05 ml) for 1 h. The mixture was concentrated under vacuum, and purified by column chromatography (Alumina activity AII, eluting with DCM). The desired product was crystallised from ethyl acetate-petroleum ether as fine white needles, mp. 121°, yield 780 mg (73%). HR-MS $[MH]^+$ $C_{15}H_{12}ClN_2O_2$ calc. 287.0587, obs. 287.0608. ^{1}H-nmr δ ($CDCl_3$) 1.464 (t, J 7.1, CH_3 Et), 4.515 (q, J 7.1, CH_2 Et), 7.689 (dd, J 4.3, 8.1, H8), 7.929 (d, J 9.2, H6), 8.277 (dd, J 1.7, 8.1, H7), 8.374 (d, J 9.2, H5), 9.221 (dd, J 1.7, 4.3, H9), 9.421 (s, H2). ^{13}C-nmr δ ($CDCl_3$) 14.10 (CH_3 Et), 62.11 (CH_2 Et), 122.40 (C5), 123.99 (C8), 125.08 (C4a), 126.49 (C3), 128.15 (C6), 129.08 (C6a), 135.95 (C7), 142.59 (C4), 145.17 (C6b), 147.84 (C4b), 149.96 (C2), 150.91 (C9), 164.30 (3-CO).

Preparation of ethyl 4-[N^2-(p-toluenesulphonyl hydrazido)]-1,10-phenanthroline-3-carboxylate (9)

Compound **(8)** (6 g) was dissolved in chloroform (150 ml) and a solution of tosyl hydrazide (1 equiv., 3.9 g) in chloroform (150 ml) was added. The mixture was allowed to react at RT for 16 h. The resulting white precipitate was collected, washed with DCM-petroleum ether (1:1 mixture), mp. 185-190° (dec.), yield 8.52 g (93%). HR-MS $[MH]^+$ $C_{22}H_{21}N_4O_4S$ calc. 437.1283 obs. 437.1268. ^{1}H-nmr δ (1:1 $CD_3OD/CDCl_3$) 1.485 (t, J 7.1, CH_3 Et), 2.413 (s, CH_3 Tos), 4.492 (q, J 7.1, CH_2 Et), 7.364 (d, J 8.2, H3,3' Tos), 7.785 (d, J 8.2, H2,2' Tos), 7.963 (dd, J 4.4, 8.3, H8), 8.098 (d, J 9.6, H6), 8.571 (dd, J 1.5, 8.3, H7), 9.198 (dd, J 1.5, 4.3, H9), 9.238 (s, H2), 9.488 (bd, J 9.4, H5). ^{13}C-nmr δ (1:1 $CD_3OD/CDCl_3$) 12.83 (CH_3 Et), 20.29 (CH_3 Tos), 62.63 (CH_2 Et), 104.94 (C4a), 116.36 (C3), 123.38 (C5), 125.90 (C6), 125.97 (C8), 127.64 (C2,2' Tos), 129.42 (C3,3' Tos), 129.57 (C6a), 132.15 (C1 Tos), 136.13 (C7), 137.35 (C6b), 137.66 (C4-Tos), 143.07 (C2), 145.25 (C4b), 150.50 (C9), 157.16 (C4), 164.94 (3-CO).

Preparation of 1,10-phenanthroline-3-carboxylic acid (10)

Compound **(9)** (5 g) was finely powdered and added slowly to a boiling mixture of sodium bicarbonate (1 M, 50 ml) and ethlene glycol (100 ml), such that the evolution of gas was not too rigorous. The mixture was refluxed for a further 1 h after the addition has been completed, cooled and diluted to 400 ml with water. The mixture was filtered, the filtrate was adjusted to pH 2.5 with HCl (5 M), and applied to a bed of strongly acidic cation exchange resin (TEA form). The resin was washed with distilled water and the product was eluted with TEA in water (2.5%). The eluant was evaporated to dryness and the product was recrystallised from ethanol-ethyl acetate as an off white powder, mp. >300°, yield 1.86 g (70%). HR-MS $[MH]^+$ $C_{13}H_9N_2O_2$ calc. 225.0664, obs. 225.0692. ^{1}H-nmr δ (CD_3OD) 7.741 (dd, J 4.4, 8.2, H8), 7.913, 7.966 (AB-Quartet, J 8.9, H5 & H6), 8.431 (dd, J 1.6, 8.2, H7), 8.849 (d, J 1.9, H4), 9.048 (dd, J 1.6, 4.4, H9), 9.572 (d, J 1.9, H2). ^{13}C-nmr δ (CD_3OD) 124.80 (C8), 127.91 (C5), 128.39 (C6),

129.55 (C4a), 130.75 (C6a), 134.22 (C3), 137.97 (C4), 138.37 (C7), 146.55 (C6b), 147.17 (C4b), 151.07 (C9), 152.20 (C2), 172.44 (3-CO).

Preparation of 1,10-phenanthroline-3-carboxy-β-alanine t-butyl ester (11)

Compound (**10**) (900 mg), β-alanine *t*-butyl ester (1.1 equiv., 800 mg), BOP (1.1 equiv., 1.95 g), HOBt (1.1 equiv., 540 mg) and di-isopropylethylamine (2.2 equiv., 2.02 ml) were stirred in DMF (20 ml) for 16 h at RT. The mixture was concentrated under vacuum, and diluted with water to 300 ml. The resulting precipitate was collected and recrystallised from ethanol-water as fine plates, mp. 241°, yield 1 g (71%). HR-MS $[MH]^+$ $C_{20}H_{22}N_3O_3$ calc. 352.1661, obs. 352.1687. ^{1}H-nmr δ ($CDCl_3$) 1.439 (s, CH_3 Bu^t), 2.608 (t, J 6.0, αCH_2 β-Ala), 3.765 (q, J 6.0, βCH_2 β-Ala), 7.309 (bt, NH), 7.635 (dd, J 4.3, 8.1, H8), 7.808 (s, H5 & H6), 8.230 (dd, J 1.7, 8.1, H7), 8.673 (d, J 2.2, H4), 9.165 (dd, J 1.7, 4.3, H9), 9.438 (d, J 2.2, H2). ^{13}C-nmr δ ($CDCl_3$) 28.10 (CH_3 Bu^t), 34.94 (αCH_2 β-Ala), 35.69 (βCH_2 β-Ala), 81.51 (q-C Bu^t), 123.66 (C8), 126.66 (C5), 127.51 (C6), 127.73 (C4a), 128.97 (C3), 129.40 (C6a), 135.67 (C4), 136.08 (C7), 145.78 (C6b), 147.60 (C4b), 147.66 (C2), 150.66 (C9), 165.22 (3-CO), 172.13 (CO β-Ala).

Preparation of 1,10-phenanthroline-3-carboxy-β-alanine (12)

Compound (**11**) (1 g) was dissolved in DCM (10 ml) and treated with HCl saturated chloroform (100 ml) for 1 h. The mixture was evaporated to dryness and the product recrystallised from methanol-ethyl acetate as a white powder, mp. 266° (dec.), yield 900 mg (95%). HR-MS $[MH]^+$ $C_{16}H_{14}N_3O_3$ calc. 296.1035, obs. 296.1047. ^{1}H-nmr δ (D_2O) 2.727 (t, J 6.5, αCH_2 β-Ala), 3.610 (t, J 6.5, βCH_2 β-Ala), 7.603, 7.696 (AB-quartet, J 9.0, H5 & H6), 8.091 (dd, J 5.4, 8.2, H8), 8.164 (d, J 1.7, H4), 8.700 (d, J 1.7, H2), 8.810 (d, J 8.1, H7), 8.934 (d, J 5.0, H9). ^{13}C-nmr δ (D_2O) 33.45 (αCH_2 β-Ala), 36.07 (βCH_2 β-Ala), 125.44 (C8), 126.15 (C6), 127.79 (C4a), 129.28 (C5), 129.72 (C6a), 129.95 (C3), 135.21 (C6b), 135.73 (C4), 138.59 (C4b), 143.91 (C9), 145.87 (C7), 148.43 (C2), 165.74 (3-CO), 176.07 (CO β-Ala).

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References

1. Van Dyke, M.W.; Hertzberg, R.P.; Dervan, P.B.; *Proc. Natl. Acad. Sci. USA*, **1982**, 79, 5470-5474.
2. Hertzberg, R.P.; Dervan, P.B.; *J. Am. Chem. Soc.*, **1982**, 104, 313-315.
3. Sluka, J.P., Horvath, S.J., Bruist, M.F., Simon, M.I., Dervan, P.B.; *Science*, **1987**, 238, 1129-1132.
4. Chen, C.-H.B.; Sigman, D.S.; *Science*, **1987**, 237, 1197-1201.
5. Sigman, D.S.; Kuwabara, M.D.; Chen, C.-H.B.; Bruice, T.W.; *Methods in Enzymology*, **1991**, 208, 414-433.
6. Chen, C.-H.B.; Mazumder, A.; Constant, J.-F.; Sigman, D.S.; *Bioconjagate Chem.*, **1993**, 4, 69-77.
7. Snyder, H.R.; Freier, H.F.; *J. Am. Chem. Soc.*, **1946**, 68, 1320-1322.
8. Atwell, G.J.; Cain, B.F.; Baguley, B.C.; Finlay, G.J.; Denny, W.A.; *J. Med. Chem.*, **1984**, 27, 1481-1485.
9. Wakelin, L.P.G.; Atwell, G.J.; Rewcastle, G.W.; Denny, W.A.; *J. Med. Chem.*, **1987**, 30, 855-861.
10. Albert, A.; *"The Acridines, their preparation, physical, chemical and biological properties and uses"*, Edward Arnold Ltd., London, 1966.

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