The synthesis and conformational aspects of a novel dioxopiperazine—a possible β-turn constraint

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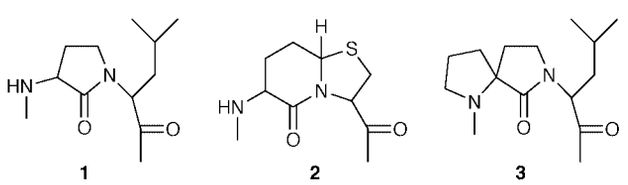
A synthetic route to a novel α,α-diamino β-keto ethoxycarbonyl-containing dioxopiperazine, capable of mimicking a β-turn, is reported. The *cis* configuration of the dioxopiperazine has been rationalised by NMR spectroscopy, while computational energy calculations have been used to explain the reluctance to cyclise of N-terminal partially protected dipeptides containing α,α-amino groups.

Introduction

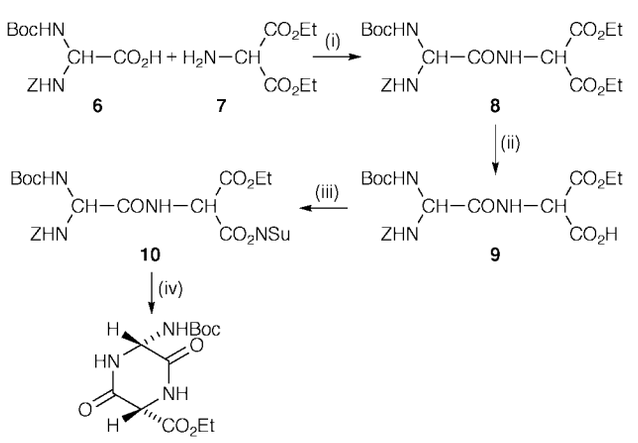
The biological activity of peptides and proteins is often enshrined in a small loop of amino acid sequence which has been generally designated a β-turn.[1] This β-turn motif has received a great deal of attention as the conformational basis for designing pharmaceutically desirable peptidomimetics for application as peptide receptor agonists and antagonists.[2] The constraining of peptides by cyclisation into cyclic penta-[3] and hexapeptides[4] offers a means of maximising the β-turn conformations, although even at this level of constraint there are many energy minimised conformations present. An alternative means of constraining peptides for the development of peptidomimetics is to mimic a β-turn using a rigid building block, which can influence the conformation of biologically active motifs.[5] Amongst the best known examples, (*S*)- and (*R*)-Gly[ANC-2] **1**,[6] BTD **2**,[7] and (*S*,*S*)-spiro-Pro-Leu **3**[8] have been



known to stabilise β-turns in linear or cyclic peptides. However when these are incorporated[5] into cyclic pentapeptides, only in the case of spiro-insert **3** was there evidence that the insert appeared at the β-turn. In cyclohexapeptides the BTD insert **2** provided a stable and typical βII′/βII arrangement. Our interest in cyclopentapeptide analogues,[9] led us to the molecular modelling (A. Slater using ICI Viking programme) of the minimum constraint requirement for a β-turn, which would fit tightly at the turn without causing steric hindrance, and preserve the polar backbone at the β-turn. The result turned out to be a reverse *cis*-amide link in the form of a novel dioxopiperazine ring as portrayed in structure **4**. This we saw as a prototype for β-turn stabilisation, and as the nucleus of a template to induce β-sheet structures, a topic which has seen a great deal of activity of late.[10] In order to produce a unit compatible with contemporary protocols for peptide synthesis the synthon **5** became our target.

Results and discussion

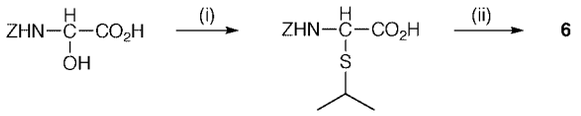
In attempting the synthesis of dioxopiperazine **5**, it was discovered very quickly that we had focused on a rather demanding combination of circumstances in that it contains β-keto and α,α-diamino components. Precursors of the former would be liable to facile decarboxylation while the α,α-diamino unit would have to be orthogonally protected. The eventual route that proved successful is outlined in Scheme 1. Whilst diester **7**



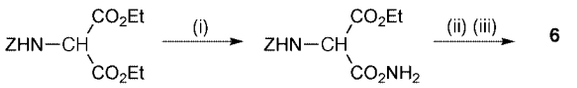
Scheme 1 *Reagents and conditions*: (i) DCC–HOBT; (ii) 1 eq. base; (iii) DCC–HONSu; (iv) H_2/Pd/C.

was commercially available, the diprotected diamino acid **6** proved more difficult to obtain.

gem-Diamino alkyl units have been explored for many years in the synthesis of *retro inverso* peptide analogues,[11] but only two of the published methods proved fruitful in our hands. The synthetic route based on the work of Bock *et. al.*[12] (Scheme 2) and synthesis *via* our adaptation of a procedure by Waki *et al.*[13] (Scheme 3) were the only synthetic sequences to give significant



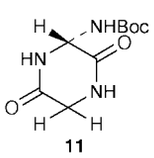
Scheme 2 *Reagents and conditions*: (i) Me_2CHSH–AcOH–H_2SO_4; (ii) Boc-NH_2–$HgCl_2$.



Scheme 3 *Reagents and conditions*: (i) DCC–HONSu–NH_3; (ii) TIB, pyridine, t-BuOH; (iii) base.

yields of **6**. The coupling of **6** and **7** was routine and best accomplished by DCC–HOBT to give dipeptide **8**, which was then subjected to various conversions in order to synthesise dioxopiperazine **5**. The literature is full of facile syntheses of dioxopiperazines from dipeptide esters, many being formed far too readily[14] as side products. Yet, when the Z-amino protecting group was removed from **8** by catalytic hydrogenation over palladium/charcoal, and the free amino diester was subjected to typical conditions for cyclisation to dioxopiperazine (refluxing in ethanol for periods up to 96 hours, or hydrogenation at elevated pressures), there was no evidence that **5** had been formed. For a similar amino dipeptide diethyl ester, but without an α,α-diamino combination these conditions sufficed.[15] To test that the cyclisation was not impeded by the bulkiness of the Boc-amino group, the alternative route was also tested, by removing the Boc group in **8** with trifluoroacetic acid. After neutralisation with DIEA (diisopropylethylamine), stirring at room temperature for 24 h, followed by refluxing in ethanol overnight, no tendency to cyclise was detected.

Eventually, a successful synthetic route was achieved by carefully hydrolysing **8** to a mono ester **9**, followed by the activation of the carboxy group as its *N*-hydroxysuccinimide ester **10**. Removal of the Z-group protection in **10**, using H_2/Pd/C in the presence of base, led to the spontaneous cyclisation to the required synthon **5**. A side reaction product (in 13% yield) during a cyclisation attempt was identified as the decarboxylated analogue **11**. So, although the tendency for the dioxopiperazine



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and some of the linear mono ester precursors to decarboxylate was troublesome, the biggest surprise was the lack of cyclisation between the released α-amino group with the esters at the C-terminal positions. This could be due either to the lack of nucleophilicity of the amino group or the lack of reactivity of β-keto esters. The latter seemed unlikely as there was precedence in the literature,[15] supporting their adequacy as dioxopiperazine precursors. We therefore initiated empirical energy calculations as described later in this paper to try and clarify the position.

It was very important to the applicability of the proposed insert **5** as a constraining unit, that the side chain protected amino and carboxy groups were pointing in the same direction—*i.e.* in a *cis* disubstituted ring. The synthesis of **5** had been carried out from a dipeptide precursor **10** which was racemic at both chiral centres. So four diastereoisomeric dioxopiperazines [*RR* and *SS* (*cis*-forms) or the *RS* and *SR* (*trans*-forms)] could emanate from such a system. All the dioxopiperazine material isolated from the synthesis of **5** was therefore analysed by NMR spectroscopy, and only one form which we believe is the *cis*-form (we cannot differentiate

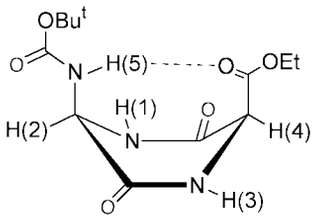


Fig. 1

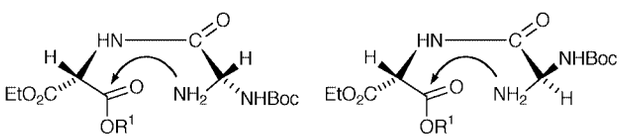


Fig. 2

between the *RR* or *SS* forms) was characterised as depicted in Fig. 1. The characterisation was made on the basis of the following NMR data and arguments. The dioxopiperazine **5** showed the following signals in its ^{1}H NMR spectrum. The numbering refers to atoms listed in Fig. 1: δ_H (250 MHz; d_6-DMSO; Me_4Si) 1.2 (3H, t, CH_2CH_3), 1.40 [9H, s, $C(CH_3)_3$], 4.2 (2H, q, CH_2CH_3), 4.65 [1H, s, C*H*(4)], 5.22 [1H, d, *J* 11, C*H*(2)], 7.25 [1H, d, *J* 11, N*H*(5)], 8.5 [1H, s, N*H*(1)] and 8.80 [1H, s, N*H*(3)]. The boat form was assumed as very few chair forms of dioxopiperazines have been found to exist in the solution phase.[16] In $CDCl_3$ solution at room temperature, two separate signals for both H(2) and H(4) were seen, giving evidence of two conformers, since on heating to 60 °C, the separate signals for both H(2) and H(4) almost coalesced. Both signals underwent simultaneous upfield shifts, indicative of increased shielding, due to ring inversion. This increased shielding can occur in two situations: (a) if the ring is in the chair conformation and the substituents are *trans*; (b) the conformation is in the boat form and the substituents are *cis*. However, further evidence for the boat conformation comes from the H-coupling constants in $CDCl_3$ solution: δ H(4) 4.70, $J_{H(3)-H(4)} = 4$ Hz, H(2) 5.52, $J_{H(1)-H(2)} = 5.6$ Hz, H(5) 6.08, $J_{H(5)-H(2)} = 8$ Hz.

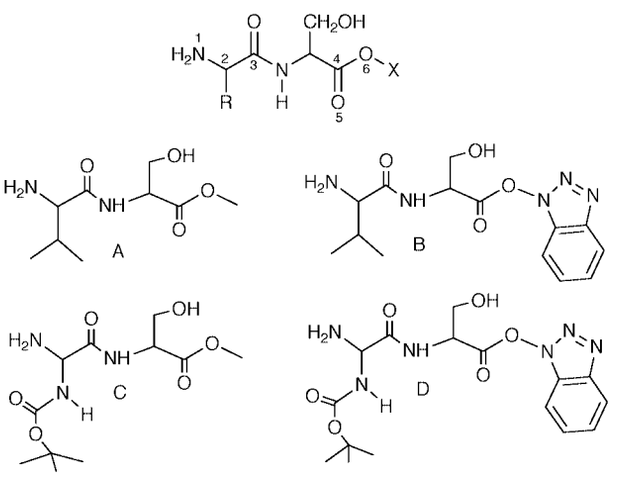
If our configurational deductions for the *cis*-dioxopiperazine **5** are correct, then this *cis*-form has preferentially formed from a dipeptide which contains four diastereoisomers. There is precedence in the literature[17] where self-condensation of DL-amino acid esters preferentially formed *cis*-dioxopiperazines. This was attributed to the rates of cyclisation of two kinds of precursor diastereoisomeric dipeptide esters, and showed that pre-*cis*-peptide esters cyclised faster than pre-*trans* in methanol solution. Rationalising our own results in this way we would have to argue that in the representations in Fig. 2 the pre-*cis* is more energetically favoured than the pre-*trans* form. So this selectivity, and why the α,α-diamino precursor dipeptide was so reluctant to cyclise, led us to our computational energy calculations using a model system designed to compare the N-terminal α,α-diamino system with a conventional sterically hindered N-terminal valyl unit. A C-terminal seryl residue was used, as this could be later used in an alternative synthesis of analogues of **5**. The HOBT active ester was used for comparison with a C-terminal alkyl ester.

Computational energy calculations

Molecular orbital calculations were carried out on empirical structures for molecules, using the AM1[18] method of the MOPAC 93 program.[19] The preliminary calculations of heat of formation and formulae for the representative molecules calculated, appear in Fig. 3. All calculations were carried out assuming the gas phase, and these heat of formation figures were inserted into eqn. (1) to calculate the nett energy change due to

Table 1 Nett energy change due to cyclisation

	Chirality	Conformation after cyclisation	Energy/ kcal mol^{-1}	Calculated atomic charges					
				1	2	3	4	5	6
A	LD	*trans*	−0.980	−0.343	−0.030	0.259	0.289	−0.347	−0.276
	LL	*cis*	0.270	−0.344	−0.031	0.262	0.272	−0.347	−0.286
B	LD	*trans*	−8.890	−0.338	−0.033	0.274	0.291	−0.292	−0.174
	LL	*cis*	−7.500	−0.345	−0.030	0.264	0.288	−0.295	−0.171
C	LL	*trans*	3.780	−0.361	0.107	0.310	0.281	−0.366	−0.254
	DL	*cis*	4.170	−0.363	0.105	0.307	0.277	−0.365	−0.253
D	LL	*trans*	−7.110	−0.362	0.109	0.308	0.292	−0.297	−0.166
	DL	*cis*	−6.570	−0.367	0.104	0.311	0.290	−0.294	−0.179



	Chirality of dipeptide	Heat of formation of dipeptides/ kcal mol^{-1}
A	LD	−192.66
	LL	−193.54
B	LD	−21.69
	LL	−22.71
C	LL	−266.82
	DL	−266.77
D	LL	92.87
	DL	92.97

	Heat of formation of DKP's *etc.*/kcal mol^{-1}
CH_3OH	−57.05
HOBt	106.01
DKP *trans* (isopropyl)	−136.59
DKP *cis* (isopropyl)	−136.22
DKP *trans* (Boc-amino)	−205.99
DKP *cis* (Boc-amino)	−205.55

Fig. 3

Nett energy (kcal mol^{-1}) =

$$(H_{\text{dioxopiperazine}} + H_{\text{X–OH}}) - (H_{\text{dipeptide}}) \quad (1)$$

cyclisation, where H = Heat of formation, X = CH_3 or benzotriazole. The results appear in Table 1. If the energy value is positive, the reaction is deemed to be thermodynamically impossible.

The results show that the use of an active ester (reactions A and C compared with B and D) is very important in nett energy changes, although it is not manifested in a great increase in the electrophilicity of the ester carbonyl carbon (4), but electron densities at oxygens (5) and (6) do show change. The result confirms the difficulties we have had in cyclisation without the use of an active ester. The effect of the Boc-amino group compared to the isopropyl group at carbon (2) has increased the electrophilicity of carbons (2) and (3), so the increase in the nucleophilicity on the N(1) α-amino group is therefore a little surprising. However, if the difference in charge between atoms (1) and (2) are calculated the average difference in the atoms bearing the isopropyl group is −0.373, but is changed to an average value of −0.257 for the two examples (C and D) bearing the Boc-amino substituent. This difference could well explain the reduced reactivity of the α-amino group. At the present time the model is not refined enough to distinguish for us the configurational selectivity which was experienced in the synthesis, but this might be more of a kinetic rather than a thermodynamic effect.

To further check whether the difficulties in cyclisation were due to the Boc-amino group substituent, we attempted to synthesise *cyclo*-(Val-Ser)[20] and *cyclo*-(Boc-amino α-Gly-Ser), under similar conditions. Under conditions[20] of refluxing in methanol over 5 days only the former dioxopiperazine could be produced.

Experimental

^{1}H NMR spectra were recorded, either at 250 MHz on a Bruker WM 250 spectrometer, or at 400 MHz on a Bruker AC-400 FT spectrometer, using tetramethylsilane as internal standard. Chemical shifts are given in ppm; J values are given in Hz. Abbreviations used, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were determined both at the EPSRC Centre, Swansea, and at Zeneca Pharmaceuticals, on a VG 12 - 253 for low resolution EI/CI, and for high resolution (or FAB) a VG Autospec mass spectrometer using Cs^+ ions for bombardment on samples dissolved either in a thioglycerol or NOBA (3-nitrobenzyl alcohol). The numbers in brackets after *m/z* values represent the intensity of the peak relative to the base peak. CHN Analyses were carried out on either a Carlo Erba 1106 or a Perkin Elmer 2400 CHN instrument. Mp's were determined on a Gallenkamp hot stage apparatus and are uncorrected.

Column chromatography was routinely carried out on silica gel 60 (Merck) (in a few cases neutral silica gel was used) which was prepared as follows: silica gel 60 (Merck) (355 g) was stirred in 0.1 M sodium hydroxide solution for 30 min. After removal of base the silica was re-dispersed in buffer solution (pH 7, 1 L), followed by filtration and washing with water (3 L), and finally with pure methanol (1 L). After filtration, the silica was partially air dried, before being dried in an air oven at 120 °C for 16 h.

Synthesis of 2-(*N*-benzyloxycarbonylamino)-2-(*N*-*tert*-butoxycarbonylamino)ethanoic acid 6

Method A (based on the method of Bock *et al.*[12]). A suspension of *N*-benzyloxycarbonyl-2-hydroxyglycine[21] (29.0 g, 0.129 mol) and propane-2-thiol (39.3 g, 0.516 mol) in glacial acetic acid (100 mL) was cooled in an ice bath under stirring, followed by the addition of conc. sulfuric acid (15 mL). After stirring the mixture at ambient temperature for 2 days, it was poured into ice and the aqueous solution was extracted with ethyl acetate. Extraction of the organic phase with 5% sodium hydrogen carbonate, followed by acidification of the aqueous layer and re-extraction of the product into ethyl acetate, gave on work up and trituration with petroleum ether (40–60 °C), *N*-benzyloxycarbonyl-2-isopropylthioglycine (28.5 g, 78%), as a white crystalline solid, mp 82–84 °C (lit.[12] 82–84 °C); δ_H (250 MHz;

d_6-DMSO) 1.19 and 1.23 [6 H, 2d, *J* 7.0, $(CH_3)_2CH$], 3.09 [1 H, m, $(CH_3)_2CH$], 5.05 [1 H, d, NHC*H*, (partially obscured by a singlet at 5.05)], 5.05 (2 H, s, $C_6H_5CH_2$), 7.35 (5 H, s, $C_6H_5CH_2$), 7.35 (1 H, d, *J* 8.5, N*H*CH); *m/z* CI (NH_3): 284 (7%) $(M + H)^+$, 210 (4) $[M + H - (CH_3)_2CS]^+$, 193(2) $(M - C_7H_7)^+$, 152(46) $(NH_3COOCH_2Ph)^+$.

N-Benzyloxycarbonyl-2-isopropylthioglycine (4.1 g, 14.5 mmol), *tert*-butylcarbamate (7.3 g, 62.3 mmol) and mercury(II) chloride (5.9 g, 21.7 mmol) in tetrahydrofuran (75 mL) were heated at 60 °C under nitrogen for 20 h. The cooled mixture was diluted with diethyl ether (150 mL) and filtered. The filtrate was further diluted with diethyl ether (200 mL), and the organic phase extracted with 10% sodium hydroxide solution. Work up after acidification of the alkaline aqueous layer, gave a colourless gum which on trituration with diethyl ether, and recrystallisation from the same solvent gave 2-Boc-amino-2-Z-aminoethanoic acid as a white crystalline compound (1.17 g, 25%), mp 158–159 °C (decomp.) (lit.[12] 158–159 °C decomp.) (Found: C, 55.7; H, 6.2; N, 8.6. $C_{15}H_{20}N_2O_6$ requires: C, 55.55; H, 6.2; N, 8.6%); δ_H (100 MHz; d_6-DMSO) 1.43 [9H, s, $(CH_3)_3C$], 5.08 (2H, s, $C_6H_5CH_2$), 5.35 (1H, t, *J* 8, NHC*H*), 7.3 (1H, br d, partially obscured, N*H*CH), 7.38 (5H, s, $C_6H_5CH_2$), 7.8 (1H, br d, *J* 8, N*H*CH); *m/z* (FAB) 347 (16.5%) $(M + Na)^+$, 325 (7.6) $(M + H)^+$, 269 (35) $(M + H - C_4H_8)^+$, 152 (9.1), 91 (100) $(C_7H_7)^+$. A major by-product in the mother liquor was identified as ZNHCH(OH)COOH.

Method B (adapted from the work of M. Waki *et al.*[13]). *N*-Benzyloxycarbonyl-2-amino-monoethyl malonate[22] (10.0 g, 35 mmol), *N*-hydroxysuccinimide (6.13 g, 53.2 mmol) in dry tetrahydrofuran was cooled to 0 °C. DCC (8.06 g, 39.05 mmol) was added, and the reaction mixture was stirred vigorously at 0 °C for 1 h, when dry tetrahydrofuran (100 mL) saturated with gaseous ammonia was added *via* a syringe needle through a septum. After one more hour of stirring at 0 °C, the precipitated dicyclohexylurea and hydroxysuccinimide were removed by filtration and the filtrate, after rotary evaporation, was re-dissolved in hot ethyl acetate (100 mL). After extraction of the ethyl acetate layer with 10% citric acid followed by sodium bicarbonate solution and water, the dried solvent layer on rotary evaporation began to precipitate out *N-benzyloxycarbonyl-2-carboxamidoglycine* (9.45 g, 95%), mp 112–114 °C (from hot isopropanol) (Found: C, 55.8; H, 5.7; N, 9.85. $C_{13}H_{16}N_2O_5$ requires: C, 55.7; H, 5.75; N, 10.0%); δ_H (60 MHz; d_6-DMSO) 1.07 (3 H, t, *J* 7, CH_3CH_2), 3.95 (2 H, q, *J* 7, CH_3CH_2), 4.63 (1 H, d, *J* 8, NHC*H*), 4.87 (2 H, s, $C_6H_5CH_2$), 7.13 (5H, s, $C_6H_5CH_2$), 7.20–7.70 (3 H, m, N*H*CH and CONH_2); *m/z* (EI) 280 (2.4%) M^+, 263 (0.5) $(M - NH_3)^+$, 237 (2.4) $(M - CONH)^+$, 91 (100) $(C_7H_7)^+$.

N-Benzyloxycarbonyl-2-carboxamidoglycine (5.0 g, 17.8 mmol) and TIB [*I,I*-bis(trifluoro-acetoxy)iodo]benzene (11.48 g, 26.7 mmol), in dry 2-methylpropan-2-ol (150 mL) were refluxed for 15 min, and then pyridine (4.22 g, 53.4 mmol) was added, the reaction being complete in 1 h. After removal of solvent by rotary evaporation, the residue was re-dissolved in chloroform, and the insoluble pyridinium trifluoroacetate was removed by filtration. Work up of the organic layer using the usual acid–base extractions described before, yielded on rotary evaporation, a colourless oil (5.21 g, 83%), purified by column chromatography with chloroform as eluent, to give *ethyl 2-N-benzyloxycarbonylamino-2-tert-butyloxycarbonylaminoethanoate*, mp 122 °C (chloroform–light petroleum) (Found: C, 58.1; H, 7.0; N, 7.9. $C_{17}H_{24}N_2O_6$ requires: C, 57.9; H, 6.9; N, 7.95%); δ_H (250 MHz; d_6-DMSO) 1.17 (3 H, t, *J* 7, CH_3CH_2), 1.39 [9 H, s, $(CH_3)_3C$], 4.11 (2 H, q, CH_3CH_2), 5.06 (2 H, s, $C_6H_5CH_2$), 5.32 [1 H, t, *J* 7.5, $(NH)_2CH$], 7.36 (5 H, s, $C_6H_5CH_2$), 7.60 (1 H, d, *J* 7, N*H*CH), 8.02 (1 H, d, *J* 7.7, CHN*H*); *m/z* (CI) 353 (10%) $(M + H)^+$; 297 (57) $(M + H - C_4H_8)^+$, 253 (61) $(297 - CO_2)^+$, 236 (10) $(253 - NH_3)^+$, 102 (100) $(NH_2{=}CHCO_2Et)^+$. This ethyl ester (4.65 g, 13.2 mmol) in a 7:3 (*v*:*v*) mixture of ethanol–water, homogenised by vigorous stirring or using an ultrasonic bath, was hydrolysed by adding an equimolar amount of 1 M potassium hydroxide, over 30 min–1 h, and by stirring at ambient temperature for 3 h. Work up procedures as described under method A gave 4.2 g (95%) of acid **6**, with analytical data identical to the product of method A.

Diethyl [2-(benzyloxycarbonylamino)-2-(*tert*-butoxycarbonylamino)ethanoylamino]malonate, 8

Acid **6** (4.2 g, 13.0 mmol) and diethyl aminomalonate **7** (4.1 g, 19.4 mmol) were dissolved in a minimum volume of dry DMF, and cooled in an ice-bath. HOBT (2.56 g, 19 mmol) was added followed by dropwise addition of triethylamine until the pH of the mixture reached 7–7.5 (monitored with moist pH paper). DCC (2.81 g, 13.65 mmol) was added, and soon precipitation of dicyclohexylurea occurred. After 12 h stirring at approx 0 °C, the precipitate was removed by filtration, and the filtrate rotary evaporated. The gummy residue was dissolved in a minimum volume of hot ethyl acetate, and left to cool to precipitate more of the urea. After further filtration, the filtrate was diluted with more ethyl acetate and washed with 10% citric acid solution and sodium bicarbonate as described earlier. Two neutral components detected by TLC, in the dried ethyl acetate layer, were purified by silica gel chromatography. Fraction eluted in 96:4 (*v*:*v*) chloroform–methanol was crystallised from ethyl acetate–light petroleum as *dipeptide diester* **8** (9.39 g, 75%) mp 114 °C, δ_H (100 MHz; d_6-DMSO) 1.21 (6 H, t, *J* 7, CH_3CH_2), 1.40 [9 H, s, $(CH_3)_3C$], 4.19 (4 H, q, *J* 7, CH_3CH_2), 5.03 (1 H, d, *J* 7, NHC*H*), 5.09 (2 H, s, $C_6H_5CH_2$), 5.48 [1 H, t, *J* 8.4, $(NH)_2CH$], 7.16 and 7.60 [2 H, br d, *J* 8.4, $(NH)_2CH$], 8.65 (1 H, d, *J* 7, N*H*CH); *m/z* (FAB) 504 (1.5%) $(M + Na)^+$, 482 (2.5) $(M + H)^+$, 426 (6) $(482 - C_4H_8)^+$, 365 (7) $(482 - BocNH_2)^+$, 321 (14) $(365 - CO_2)^+$, 231 (37) $(321 + H - C_7H_7)^+$, 91 (100) $(C_7H_7)^+$. A by-product (0.5 g, 10%) eluted from the column with 92:8 (*v*:*v*) chloroform–methanol was identified as ZNHCH(OH)CONHCH$(CO_2Et)_2$.

Monoethyl 2-[2-(benzyloxycarbonylamino)-2-(*tert*-butoxycarbonylamino)ethanoylamino]malonate, 9

Dipeptide diester **8** (4.6 g, 9.55 mmol) was hydrolysed with 1 M potassium hydroxide under the general conditions described for making **6** in method B above, giving *monoethyl dipeptide ester* **9** (4.24 g, 98%), as a white crystalline compound, mp 99 °C (from ethanol–light petroleum) (Found: C, 51.1; H, 6.0; N, 8.8. $C_{20}H_{27}N_3O_9{\cdot}H_2O$ requires: C, 51.1; H, 6.2; N, 8.9%); δ_H (250 MHz; d_6-DMSO) 1.19 (3 H, t, *J* 7, CH_3CH_2), 1.39 [9 H, s, $(CH_3)_3C$], 4.15 (2 H, q, *J* 7, CH_3CH_2), 4.88 (1 H, d, *J* 6.7, NHC*H*), 5.06 (2 H, s, $C_6H_5CH_2$), 5.46 [1 H, t, *J* 8.1, $(NH)_2CH$], 7.32 and 7.70 [2 H, 2br s, $(NH)_2CH$], 8.40 (1 H, br s, N*H*CH); *m/z* (FAB): 929 (1.2%) $(2 \times M + Na)^+$, 476 (12.4) $(M + Na)^+$, 454 (8.6) $(M + H)^+$, 398 (18.1) $(454 - C_4H_8)^+$, 337 (7.7) $(454 - BocNH_2)^+$, 203 (38.5) $(337 + H - C_7H_7OCO)^+$, 91 (100) $(C_7H_7)^+$.

Ethyl 5-(*N-tert*-butoxycarbonylamino)-3,6-dioxopiperazine-2-carboxylate 5, *via* ethyl succinimido-2-[2-(benzyloxycarbonylamino)-2-(*tert*-butoxycarbonylamino)ethanoylamino]malonate 10

Monoethyl dipeptide ester **9** (1.0 g, 2.2 mmol) and *N*-hydroxysuccinimide (0.38 g, 3.3 mmol) (previously dried over KOH at 80 °C for 10 h), were dissolved in dry DMF (20 mL) and cooled in an ice bath with stirring. DCC (0.477 g, 2.31 mmol) was added and within 5 min dicyclohexylurea started precipitating, and the reaction was deemed complete within 30 min. The precipitate was removed by filtration, the DMF removed by rotary evaporation. (In trial runs the reaction was worked up at this stage by re-dissolution of the residue in ice-cold ethyl acetate. Any further precipitated urea was removed before subjecting a

concentrated solution to chromatography on silica gel. The main fractions containing *N-hydroxysuccinimido ester* ***10*** (0.72 g, 60%), were eluted with a solvent mixture of chloroform–(4–10%) methanol. However over 30% of the yield was lost by reaction of the active ester with methanol at the chromatography stage, so in subsequent reactions the active ester was not isolated.) In the 'non-isolated' cases the DMF solution of the active ester **10**, was diluted to 200 mL, and 20% Pd/C (100 mg) added. Hydrogen gas was purged through under stirring for 24 h, and the catalyst removed by repeated filtration through thick filter paper. The filtrate was rotary evaporated at <35 °C, and the gummy residue re-dissolved in ethyl acetate for subsequent extraction of acid and base material. The neutral fraction remaining appeared to contain a number of components (by TLC), which were separated by column chromatography on silica gel. *Dioxopiperazine* **5** was eluted in the later fractions (97 : 3, *v* : *v* chloroform–methanol), which after evaporation gave a colourless oil (0.26 g, 40%), which crystallised on trituration with ether. Mp 188–190 °C (from chloroform) (Found: C, 46.4; H, 6.1; N, 13.0. $C_{12}H_{19}N_3O_6{\cdot}\frac{1}{2}\ H_2O$ requires: C, 46.45; H, 6.5; N, 13.54%); δ_H (400 MHz; d_6-DMSO) details appear in the discussion; δ_H (400 MHz, $CDCl_3$; ambient temp; atoms as numbered in Fig. 1) 1.3 (3 H, m, CH_3CH_2), 1.48 [9 H, s, $(CH_3)_3C$], 4.38 (2 H, m CH_3CH_2), 4.69 and 4.70 (1H, 2d, $J_{3,4}$ 3.5, $J_{3,4}$ 4.0, *H*(4) conformers), 5.52 and 5.55 [1H, d and dd, $J_{1,2}$ or $J_{2,5}$ 5.6, $J_{5,2}$ 8, $J_{1,2}$ 3 *H*(2) conformers], 5.77 and 6.08 [1 H, br s and d, $J_{5,2}$ 8, *H*(5)], 6.49 and 6.52 [1 H, br s, *H*(3)], 6.58 and 5.68 [1 H, br s, *H*(1)]. At 60 °C in $CDCl_3$, *H*(4) gave a broad singlet at 4.66, and *H*(2) a broad singlet at 5.52 and doublet at 5.50.

A by-product (13% yield) of some of the attempts to synthesise **5** was identified as *3-tert-butyloxycarbonylamino-piperazin-2,5-dione* (**11**), a white crystalline solid, mp 93–101 °C (lowered by traces of *N*-hydroxysuccinimide); δ_H (400 MHz; d_6-DMSO) 1.39 [9 H, s, $(CH_3)_3C$], 3.6 (1 H, dd, C*H*-H), 3.89 (1 H, d, CH-*H*), 4.92 (1 H, dd, C*H*-NHBoc), 7.96 (1 H, d, N*H*), 8.12 (1 H, s, N*H*), 8.53 (1 H, s, N*H*); 2D COSY spectrum showed C*H* at 4.92 coupled to both N*H*'s at δ 7.96 and 8.53, the C*H* at 3.60 coupled to both N*H* at 8.12 and C*H* at δ 3.89. ^{13}C DEPT 135 confirmed a CH_2 signal at δ 44.26. *m*/*z* (CI): 246 $(M + NH_4)^+$.

Cyclo(Val-L-Ser)[20] and Cyclo(Val-D-Ser)[20]

Comparison of the effect of isopropyl *vis à vis* Boc-amino side chains on the cyclisation yields in dioxopiperazine formation was checked under the known conditions[20] that had proved successful for the isopropyl case: Z-Val-Ser-OMe[22] or Z-Val-D-Ser-OMe[20] (0.103 g, 0.3 mmol) were independently suspended in absolute methanol (10 mL) with 10% Pd/C (10 mg), and stirred under hydrogen for 15 h. After removal of catalyst and evaporation of solvent, the crude residues were suspended in absolute methanol (10 mL) and refluxed for 110 h. Removal of solvent and work up gave the cyclo(Val-Ser), mp 246–247 °C (lit.,[20] 245–249 °C), and cyclo(Val-D-Ser), mp 224–225 °C (lit.,[20] 230–232 °C), with NMR data identical to published data. For the analogue with the Boc-amino side chain:

(2-*N*-Benzyloxycarbonylamino-2-*N*-*tert*-butoxycarbonyl)ethanoyl-L-serine methyl ester. 2-Boc-amino-2-Z-aminoethanoic acid **6** (1.04 g, 3.22 mmol) and HOBT (0.493 g, 3.22 mmol) in DMF (20 mL) were cooled in an ice bath, before being treated with L-serine methyl ester hydrochloride (0.499 g, 3.22 mmol), triethylamine (0.9 mL, 6.44 mmol) and DCC (0.663 g, 3.22 mmol), in that order, and the total mixture was stirred at room temperature for 24 h. After evaporation of DMF, dicyclohexylurea and HOBT precipitated out of cold acetone and ethyl acetate respectively. Combined filtrates in ethyl acetate were washed successively with 10% citric acid, 0.1 M $NaHCO_3$ and water, and after drying, the solvent was evaporated to a crude residue. Chromatography on silica gel (with gradient of ethyl acetate–light petroleum) gave a white product, mp 149–151 °C (62% yield); δ_H (400 MHz d_6-DMSO) 1.4 [9 H, s, $(CH_3)_3C$], 3.6–3.8 (5 H, s and d, OCH_3 and CH_2OH), 4.3–4.4 (1 H, br s, $CHCH_2OH$), 5.05 (2 H, s, CH_2O), 5.3–5.4 (1 H, t, NHC*H*NH), 7.0 (1 H, br s, CHCON*H*), 7.3–7.4 (5 H, m, C_6H_5), 7.5–7.6 (1 H, br s, OCON*H*), 7.9 (1 H, br s, OCON*H*); δ_C (d_6-DMSO) 28.1 [$C(CH_3)$], 51.8 and 54.9 (2 × *C*H, and OCH_3), 61.1 (CH_2OH), 65.7 (CH_2O), [$(CH_3)_3C$], 128 and 136.6 (C_6H_5), 167.68 and 170.3 (4 × *C*O). Product of hydrolysis of this ester had *m*/*z* (*electrospray*): 434 $(M + Na)^+$. $C_{18}H_{25}N_3O_8{\cdot}$Na requires 434.

Attempted cyclisation. When the above dipeptide ester was refluxed after hydrogenation under exactly the same conditions as for the valyl analogues, no dioxopiperazine formed.

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