Synthesis of β-Amino Acid Peptides. Part 4.[1] Cyclo-peptides of β-Amino Acids

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Cyclo-peptides of substituted β-amino acids have been prepared by direct and indirect methods. These include achiral (**1a**, **1b**), chiral (**1c**), and racemic examples (**1c**)

We have investigated the synthesis of linear and tri- and hexa-peptides derived from β-amino acids. Initial aims included the assessment of selective protection, coupling methods, and identification of side reactions. In addition, it was of interest to establish whether cyclo-tripeptides could be isolated, and to investigate their ease and method of preparation and a comparison of some of their properties.

Here we report the synthesis of cyclo-tri-β-aminoisovaleryl (**1a**), cyclo-tri and hexa-β-aminopivaloyl (**1b**), and an (±)- and (+)-cyclo-tri-β-aminobutyryl (**1c**). The synthesis of cyclo-tri-β-alanyl has been described in full[2] and in part.[3,4]

$$\ulcorner[NHCR^1R^2.CR^3_2.CO]_n\urcorner$$

(1)

a; $R^1 = R^2 = Me$, $R^3 = H$, $n = 3$
b; $R^1 = R^2 = H$, $R^3 = Me$, $n = 3, 6$
c; $R^1 = Me$, $R^2 = R^3 = H$, $n = 3$

$$H[NHCR^1R^2.CR^3_2.CO]_3OR^4$$

(2)

a; $R^1 = R^2 = Me$, $R^3 = R^4 = H$
b; $R^1 = R^2 = R^4 = H$, $R^3 = Me$
c; $R^1 = Me$, $R^2 = R^3 = R^4 = H$
d; $R^1 = Me$, $R^2 = R^3 = H$, $R^4 = TCP$

As reported, the only coupling procedures which worked effectively with β-aminoisovaleric acid were the oxazinone and pivaloyl mixed anhydride methods,[5] restricting approaches to indirect routes. Reaction of the tripeptide (**2a**) with pivaloyl chloride in pyridine provided the cyclo-tripeptide (**1a**) in 38.6% yield. Neither t.l.c. nor mass spectral evidence supported the presence of cyclo-dimers.

Coupling reactions with β-aminopivalic acid were equivocal when using DCCI, CMA, and active esters.[6] In considering cyclisation of the tripeptide (**2b**) we were influenced by the foregoing and chose the anhydride method using *o*-phenylene-phosphochloridite (OPPC).[7] The reaction afforded two neutral, ninhydrin-negative substances in an overall yield of 97%. Separation was effected by washing, from which the water-soluble cyclo-tripeptide (**1b**; $n = 3$) could be isolated in a yield of 52%. Recrystallisation of the water-insoluble fraction provided the cyclo-hexapeptide (**1b**; $n = 6$) in 45% yield.‡

Cyclisation under similar conditions as used for (**1b**) of the racemic β-aminobutyroyl-tripeptide (**2c**) provided the (±)-cyclo-peptide (**1c**) in a crude yield of 40%. Initial attempts to cyclise the (+)-tripeptide (**2c**) under the same conditions were unsuccessful. By using an excess of diethyl phosphite a homogeneous solution could be obtained from which the (+)-cyclo-tripeptide (**1c**) crystallised in 40% yield. The optically active cyclo-peptide (**1c**) proved to be insoluble in a variety of covalent and more ionic solvents. A second rate utilised the (+)-tripeptide active ester (**2d**) which provided 24% of the identical (+)-cyclo-peptide (**1c**).

Reviewing the experimental results, it was unfortunate that conditions for cyclisation reactions could not be standardised because of differing solubilities of linear and cyclic compounds.

Examination of crude cyclic peptides by t.l.c. and mass spectroscopy demonstrated that only in the cyclisation reaction of (**2b**) could any trace of cyclo-dimeric material be found.

In general it may also be concluded from the ready isolation of β-cyclo-tripeptides that prohibitive conformational, angular, and transannular strain is relatively unimportant in the transition state leading to the formation of the 12-membered cyclo-peptides.[4] Conformational mobility of the cyclo-tripeptides should be severely restricted apart from cyclo-(tri-β-alanyl)[2] and the interesting possibility arises of conformational diastereoisomerism in cyclo-tri-β-peptides containing both *cis*- and *trans*-amide bonds.

Experimental

M.p.s were determined on Gallenkamp or Electrothermal Apparatus. Mass spectra were obtained using the PCMU Service. Optical rotations were measured on a Bellingham and Stanley Polarimeter. Solvents were purified and dried before use according to published methods.[8] Diethyl phosphite was distilled under reduced pressure and the fraction used had b.p. 50 °C, 2 mmHg.[9] Neutral products, excepting cyclo-peptides, were isolated by washing in ethyl acetate successively with hydrochloric acid (0.5M), sodium hydroxide (0.5M), and water; this was followed by drying over anhydrous magnesium sulphate and evaporation under reduced pressure using a rotary evaporator. T.l.c. on Merck Kieselgel (0.25 mm) employed the following solvent systems (v/v): (A) ethyl acetate, (B) butanol–acetic acid–water (4:1:1), (C) ethanol–water–ammonia (*d* 0.880) (8:1:1), (D) methanol, (E) propanol–water (1:1), and (F) butan-2-ol–3% ammonia (100:44). Light petroleum refers to the fraction of b.p. 40—60 °C.

The Synthesis of Cyclo-peptides of β-*Aminoisovaleric Acid* (**1a**), β-*Aminopivalic Acid* (**1b**), *and of* (+)- and (±)-β-*Aminobutyric Acid* (**1c**).—*Cyclo-(tri-*β*-aminosovaleryl)* (**1a**). The tripeptide (**2a**)[5] hydrochloride (351.5 mg, 1 mol) was dissolved in pyridine (300 ml) and pivaloyl chloride (1.21 g, 10 mmol) added with stirring. The solution was heated to 100 °C for 4 h and evaporated to dryness. The residue was dissolved in water (10 ml) and the aqueous solution extracted four times with ethyl acetate–butanol (3:1, aliquot 10 ml). The combined extracts

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‡ Cyclisation of the corresponding hexapeptide was achieved using OPPC in 19% yield.

were evaporated and the residue, dissolved in methanol (10 ml), shaken with Dowex 50-X8 cation exchange resin (H^+ form, 50 g). The resin was filtered off and washed with methanol. Evaporation of the methanol left a gum which crystallized under light petroleum and was recrystallized from ethyl acetate–light petroleum yielding the *product* (**1a**) (114 mg, 38.5%), m.p. 254—256 °C, raised to 257—258 °C on further recrystallization, R_{FA} 0.40, R_{FB} 0.85 [Found: C, 60.5; H, 9.1; N, 14.1%; $M^{+\cdot}$ 297. $(C_5H_9NO)_n$ requires C, 60.6; H, 9.2; N, 14.1%; $M^{+\cdot}$ 297].

Preparation of Cyclo-(tri-β-aminopivaloyl) (**1b**, n = 3) *and Cyclo-(hexa-β-aminopivaloyl)* (**1b**; n = 6).—A solution of the corresponding free tripeptide (**2b**)[6] (315 mg, 1 mmol) in diethyl phosphite (10 ml) was cooled to 0 °C and OPPC (192 mg, 1.1 mmol) added gradually with stirring. After 5 min, the solution was diluted with diethyl phosphite (200 ml) and triethylamine (202 mg, 2 mmol) added. The mixture was stirred for 1 h at room temperature, heated under reflux for 15 min, and then evaporated. Water was added to the residue and insoluble material (120 mg) filtered off. Evaporation of the filtrate and further addition of water to the residue gave a further crop of insoluble material (14 mg). The combined crude product (134 mg, 45%) was recrystallised from methanol to furnish the pure *cyclo-hexapeptide* (**1b**; $n = 6$), m.p. 302—305.5 °C, R_{FB} 0.65, R_{FC} 0.91, R_{FD} 0.66 [Found: C, 60.4; H, 8.8; N, 14.3%; $M^{+\cdot}$ 594. $(C_5H_9NO)_n$ requires C, 60.6; H, 9.15; N, 14.1%; $M^{+\cdot}$ 594]. The aqueous solution was desalted with Dowex 50-X8 (H^+ form, 50 g) and De-acidite FF-IP (OH-form, 50 g) ion exchange resins. The eluate was evaporated yielding the crude tripeptide derivative (155 mg, 52%). A sample was sublimed (140 °C, 0.03 mmHg) to yield the crystalline *cyclo-tripeptide* (**1b**; $n = 3$), m.p. 255—259.5 °C, R_{FC} 0.78 [Found: C, 60.6; H, 9.3; N, 14.4%; $M^{+\cdot}$ 297. $(C_5H_9NO)_n$ requires C, 60.6; H, 9.2; N, 14.1%; $M^{+\cdot}$ 297]. Inverse addition of reagents afforded 19% of the cyclo-hexapeptide (**1b**; $n = 6$) and no cyclo-tripeptide (**1b**; $n = 3$).

Preparation of the Cyclo-tripeptides of (RS)- *and* (R)-3-*Aminobutyric Acid: Preparation of* (±)-*Cyclo-(tri-β-aminobutyroyl)* (**1c**).—A solution of the (±)-free tripeptide (**2c**)[1] (100 mg, 0.35 mmol) in diethyl phosphite (5 ml) was cooled to 0 °C and OPPC (67.2 mg, 0.385 mmol) added gradually with stirring. After 5 min the solution was diluted with diethyl phosphite (75 ml) and triethylamine (70.8 mg, 0.1 ml, 0.70 mmol) added. The mixture was stirred overnight at room temperature and then heated under reflux for 15 min. The crude product (40 mg, 40%), m.p. 260 °C, precipitated from the cooled solution and was filtered off. The product was examined by t.l.c., R_{FE} 0.67; R_{FB} 0.45 (ninhydrin negative). Sublimation (220—230 °C, 0.25 mmHg) of the crude product yielded the crystalline *cyclo-tripeptide* (**1c**) (20 mg, 20%) m.p. 300 °C [Found: C, 56.5; H, 8.2; N, 16.3%; $M^{+\cdot}$ 255. $(C_4H_7NO)_n$ requires C, 56.45; H, 8.3; N, 16.5%; $M^{+\cdot}$ 255].

The diethyl phosphite filtrate was evaporated and the residue desalted in the usual way to yield an additional quantity of cyclo-peptide (30 mg).

Preparation of (+)-*Cyclo-(tri-β-aminobutyroyl)* (**1c**).—A suspension of (−)-free tripeptide (**2c**)[1] (100 mg, 0.37 mmol) in diethyl phosphite (80 ml) was cooled to 0 °C and OPPC (71 mg, 0.407 mmol) added with stirring. After 5 min triethylamine (74.8 mg, 0.1 ml, 0.74 mmol) was added and the heterogeneous mixture stirred at room temperature for 1 h. The reaction mixture was gently heated under reflux until homogeneous (36 min) and heating continued for the next 15 min. The reaction was cooled to room temperature, when a solid material separated from solution. The precipitate (40 mg, 40%), m.p. 340 °C was filtered off and purified by sublimation (240—245 °C, 2×10^{-3} mm Hg) to yield a fine crystalline *product* (**1c**) (33 mg, 33%), m.p. 340 °C, R_{FB} 0.24, R_{FF} 0.42, $[\alpha]^{20}$ + 15.0° (*c.* 1 in glacial acetic acid) [Found: C, 56.55; H, 8.4; N, 16.5%; $M^{+\cdot}$ 255. $(C_4H_7NO)_n$ requires C, 56.45; H, 8.3; N, 16.5%; $M^{+\cdot}$ 255].

Evaporation of the filtrate yielded a residue which gave 8 mg of authentic cyclic peptide (**1c**) on recrystallisation from glacial acetic acid and ether thus raising the overall yield to 48%.

Preparation of (+)-*Cyclo-(tri-β-aminobutyroyl)* (**1c**): *Preparation of* 2,4,5-*Trichlorophenyl-β-benzyloxycarbonyl-*L-*aminobutyroyl-*L-*β-aminobutyroyl-*L-*β-aminobutyrate.*—The *N*-protected tripeptide acid[1] (0.4 g, 0.98 mmol) 2,4,5-trichlorophenol (0.21 g, 1.1 mmol), and DCCI (0.23 g, 1.1 mmol) in pyridine (40 ml) were mixed together and worked up in the usual manner. The *N*-protected *tripeptide active ester* was isolated (0.32 g, 56%), m.p. 192—194 °C, raised to 196—198 °C on recrystallization from methanol–ether (Found: C, 53.1; H, 5.3; Cl, 18.3; N, 7.0. $C_{26}H_{31}Cl_3N_3O_6$ requires C, 53.1; H, 5.3; Cl, 18.1; N, 7.15%). A solution of the foregoing tripeptide active ester (250 mg, 0.43 mmol) in methanol (50 ml) and concentrated hydrochloric acid (0.1 ml) was purged with nitrogen gas and 10% palladium on charcoal (50 mg) added. Hydrogen was passed through the mixture until evolution of carbon dioxide ceased. The solution was filtered through a HiFlo-filter bed and the filtrate evaporated to afford a white solid residue. The foregoing crude ester (**2d**) hydrochloride in dimethylformamide (20 ml) was added dropwise with vigorous stirring to pyridine (80 ml) at 115 °C over 2 h, and stirred for a further 1 h. The solution was evaporated, and the residue triturated with water, then with 50% aqueous methanol, and finally with methanol. The solid product (23 mg, 23.7%), m.p. 340 °C, R_{FB} 0.24; R_{FF} 0.42, was recrystallized from glacial acetic acid–ether and had characteristics identical with those of the previously isolated (+)-cyclo-(tri-β-aminobutyroyl) (**1c**).

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