Tetrahedron Letters, Vol.31, No.3,pp399-402, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

THE USE OF CROWN ETHERS IN PEPTIDE CHEMISTRY: PART 3 SYNTHESIS OF AN ENKEPHALIN DERIVATIVE USING 18-CROWN-6 AS A NON-COVALENT AMINO PROTECTING GROUP

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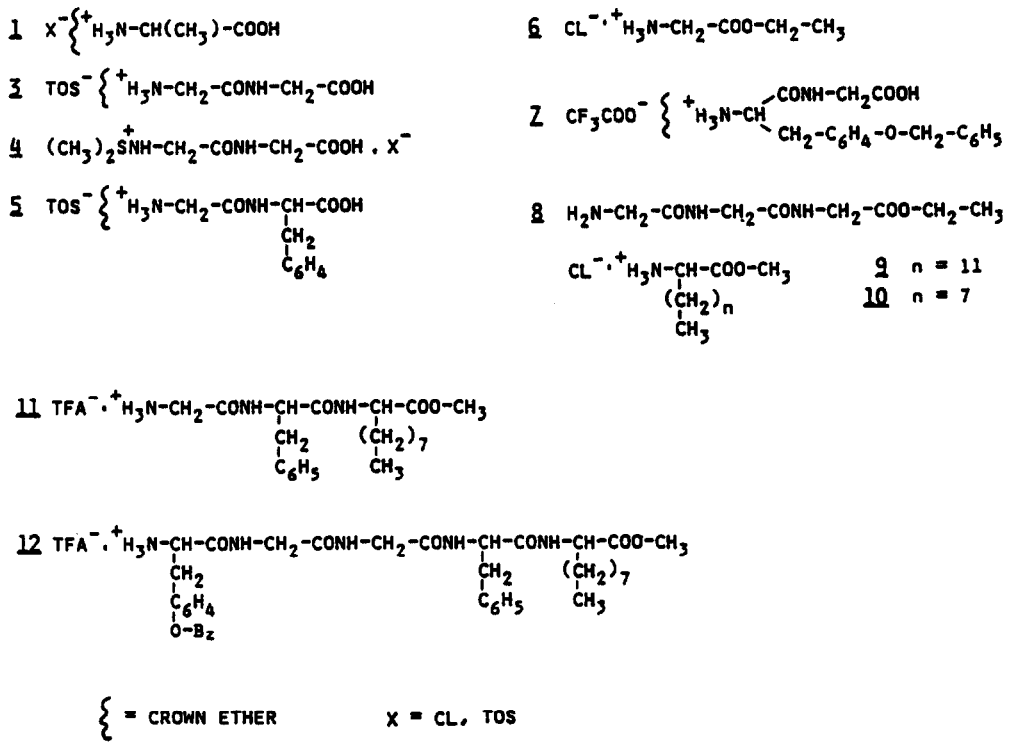
Summary: The efficacy of amino acid protection with crown ethers is demonstrated by the solution synthesis of an enkephalin pentapeptide derivative. Extraction of the organic phase with a KCl solution after each coupling steps is used for the deprotection of the peptide intermediate.

The use of crown compounds for the protection of the amino group of amino acids offers, in principle, some advantages over the more commonly used groups such as t-boc and fmoc. Thus the non-covalent nature of the interaction between the crown ether and the ammonium ion and their large affinity for inorganic ions[1] can provide the basis for a rapid but mild protection and deprotection scheme.

In order to test this hypothesis we have studied the behaviour of amino acid and peptide complexes with 18-crown-6 in various organic solvents and in the conditions usually employed for peptide synthesis[2,3].

When the alanine complex **1** was reacted with equivalent amounts of 1,3-dicyclohexyl-carbodiimide (DCC) in either chloroform of acetonitrile, it was found that the hydrogen bond in the O-acyl derivative **2** induced, first deprotection of the amino group and subsequently oligomerisation of the amino acid (scheme 1). To favour solute-solvent interactions and thus avoid the amino group deprotection, DMSO was hence used as the reaction solvent. Furthermore the alanine complex was replaced with the dipeptide complex **3** in an attempt to separate the amino group from the DCC nitrogen in the O-acyl derivative **2**. Under these new conditions the oligomerisation process resulted inhibited; however complex reacted with the solvent to form the DMSO-peptide dehydration product **4**.

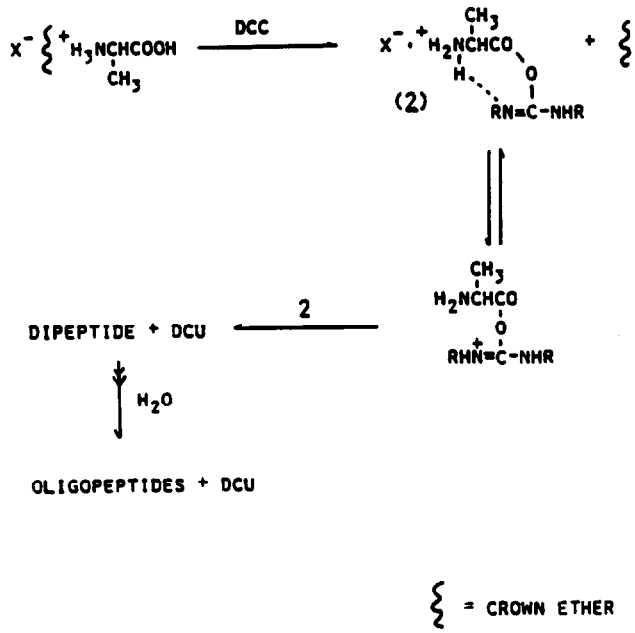
Further progress toward the initial aim of this study was made possible by the elucidation of the mechanism leading to **4** and in this communication we show that selective coupling is achieved in good yields by using dipeptide complexes and dimethylformamide (DMF) as reaction solvent. The synthesis of the [α-aminodecanoyl]-5-enkephalin derivative **12** is described as an example of the successful application of this scheme.



The dipeptide complexes 3,5 and 7 were prepared according to procedures described in the earlier work [1,2]. Before use they were re-crystallised and shown to be homogeneous by elemental analysis and ^{1}H-NMR. To optimise the reaction conditions the synthesis of the glycine tripeptide 8 was performed and it was found that the use of one-to-two fold excess complex improved the yields to about 85%. Reactions were typically carried out as follows: Gly-OEt 6 (140 mg, 1mmol) and triethylamine (0.14 ml, 1 mmol) were dissolved in DMF (2 ml) and mixed with a DMF solution (3 ml) containing one equivalent each of complex 3 and DCC. After 24 hrs dicyclohexylurea (DCU) and solvent were removed; the resulting oily material dissolved in chloroform and washed with dil. HCl, H_2O and dil. NaOH. To remove the crown protective group a final extraction with a saturated KCl solution (pH 8) was performed. Evaporation of the solvent afforded the glycine tripeptide 8 whose degree of purity and chemical composition were determined by ^{1}H-NMR and FAB mass spectrometry. Similar results were obtained when the fatty-amino acid esters 9 and 10 were used in place of the glycine ester 6.

The synthesis of the enkephalin derivative 12 was performed as follows. Tripeptide 11 was prepared in 83% yield from the Gly-Phe complex 5 and the ester 10, using an HOBT-DCC mediated coupling. After the extraction and deprotection procedures described above, 11 was purified by semi-preparative reverse phase HPLC and its chemical composition

SCHEME I



ascertained by NMR and FAB mass spectrometry[4]. Purified tripeptide ester (27 mg, 0.05 mmoles) and triethylamine (7.1 ul, 1 eq) were then dissolved in chilled DMF (2 ml) and added to a second chilled DMF solution containing two equivalents each of HOBT (15.6 mg), DCC (21 mg) and the Tyr-Gly complex **7** (50.8 mg). The reaction was allowed to proceed at r.t. for 24 hrs before DCU and solvent were removed. Deprotection with a saturated KCl solution and purification by reverse phase HPLC afforded the O-benzyl pentapeptide **12** in about 50% yield[5].

References and notes

1. M.Hiraoka, "Crown compounds: their characteristics and applications", Elsevier Publ.s, Amsterdam, 1982.
2. P.Mascagni, C.B.Hyde, M.Charalambous and K.J.Welham, J.Chem.Soc., Perkin trans.II, 323-327, (1987)
3. C.B.Hyde, K.J.Welham and P.Mascagni, J.Chem.Soc., Perkin trans. II, in press.
4. FAB mass spectra were run on a VGZAB-SE spectrometer:
   M^+ (less counter-ion), expected for 11, 406; M^+ found, 406.
   ^{1}H-NMR spectra were run on a Bruker AM-500 spectrometer; using $CDCl_3$ as solvent and TMS as internal standard 11 gave the following δ values:

7.95 (d,1H, NH-Phe); 7.67 (d,1H, NH-aminodecanoyl); 7.74 (br,1-2H, NH_3^+-Gly); 7.18 (m,5H, Ar-Phe); 4.85 (m,1H, Hα-aminodecanoyl); 4.33 (m,1H, Hα-Phe); 3.98 (d,1H, Hα-Gly); 3.65 (d,1H, Hα-Gly); 3.63 (s,3H, COOMe); 2.85 (m,2H, Hβ-Phe); 1.55 (br,14H, CH_2's-aminodecanoyl); 0.88 (t,3H, CH_3-aminodecanoyl).

5. M^+ calculated for 12, 716; M^+ found, 716.
^{1}H-NMR (DMSO-D_6; 8.73 (br,1H, NH-Phe); 8.48 (d,1H, NH-aminodecanoyl); 8.18 (s,2H, NH_3^+-Tyr); 7.77-6.96 (Ar-Phe, Tyr, O-Bz plus NH-Gly); 5.51 (t,1H, NH-Gly); 5.07 (s,2H, CH_2-OBz); 4.63 (m,1.3H, Hα-aminodecanoyl); 4.22 (m,1H, Hα-Phe); 3.99 (m,1H, Hα-Tyr); 3.86-3.65 (dd's,4H, Hα-Gly's); 3.62 (s,3H, COOMe); 3.04-2.77 (m,4H, Hβ-Tyr, Phe); 1.58-1.51 (m,4H, Hβ,γ-aminodecanoyl); 1.24 (br,10H, CH_2's-aminodecanoyl); 0.84 (t,3H CH_3-aminodecanoyl).

(Received in UK 23 November 1989)