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GLY/LYS- CONTAINING PEPTIDE MACROCYCLES: SYNTHESIS AND CYCLIZATION STUDIES

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Abstract: Symmetric and lipophilic side-chain protected cyclic peptides have been prepared by solid-phase synthesis using an Fmoc-protocol on a TFA-sensitive resin to prepare the linear precursors, which have undergone cyclization with several *in situ* activating and coupling reagents. The cyclic peptides have been obtained in high yields and purity.

Most naturally occurring ionophoric molecules form a hydrophilic cavity with a hydrophobic exterior when they bind a suitable ion[1]. Such conformational characteristics can be reproduced by using synthetic cyclic peptides as model compounds[2] in which high symmetry and some degree of molecular flexibility are forced upon the peptide skeleton[3]. These structural criteria can be met, in a first approach, by cyclic octa-[4] and nonapeptides of the sequence cyclo[Gly_n-Lys(Z)]$_m$ (n=3, m=2, **1**; n=2, m=3, **2**; Fig. 1) whose key features include: i) lipophilic protected lysine side chains; ii) high C_2 or C_3 symmetry; iii) a tridimensional cavity defined by the cycle and the side chains, and iv) the possibility of generating an anion-binding site by deprotection of the lysine ε-amino groups[5].

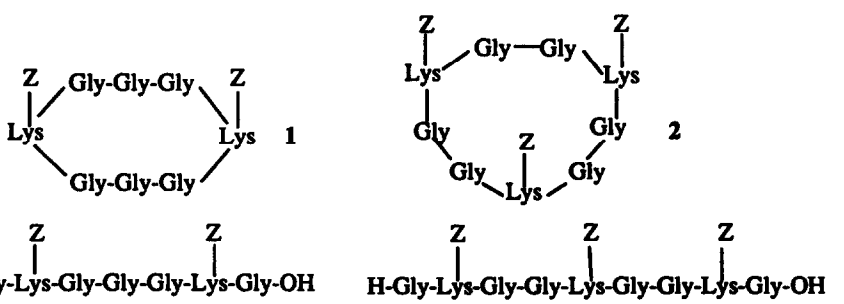

Figure 1[6]

Structures **1** and **2** have been synthesized from their linear precursors, **3** and **4**, by a combination of solid phase and solution methods. The assembly of **3** and **4** was done on a TFA-labile *p*-alkoxymethyl-type solid support[7] which allows the use of mild Fmoc-based chemistry during peptide chain assembly (Fig. 2). The first amino acid (C-terminal glycine) was anchored as the active ester of **5** upon an amino-functionalized resin as described[7].

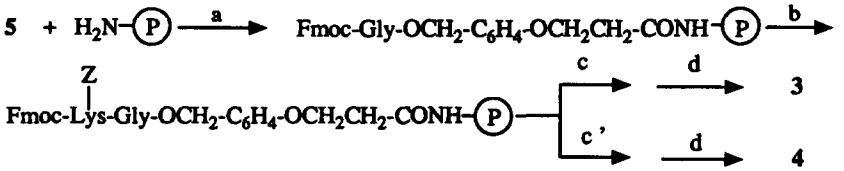

Figure 2. Solid phase synthesis of linear precursors. Reagents and conditions: a. HOBt in DMF /CH_2Cl_2; 14 h, rt. b. i) 1:1 piperidine / DMF, 30 min, rt; ii) Fmoc-Lys(Z)-OH / DCC in CH_2Cl_2; 90 min, rt. c and c'. Six and seven coupling cycles, respectively, according to sequence. d. i)1:1 piperidine /DMF, 30 min, rt; ii) 45 % (v/v) TFA / CH_2Cl_2.

After removal of N-terminal Fmoc groups and acidolysis with TFA, the crude, side-chain protected linear peptides **3** and **4** were homogeneous by HPLC and were used without further purification in the next reaction step. Both products were satisfactorily characterized by amino acid analysis, ^{1}H-NMR (500 MHz) and FAB-MS (**3**, m/z= 885 $(M+H)^+$; **4**, m/z= 1147 $(M+H)^+$). Overall (synthesis and acidolysis) yields for both peptides were in the 90-95% range.

Table 1. Coupling agent-promoted cyclizations of peptide **3** [a]

Reagent	Yield[b] (%)	Recovery[c] (%)
DEPC	> 99	65
DPPA	> 99	70
BOP	> 99	68
WOODWARD K	30	15
HOBt / DCC	65	20
HONSu / DCC	65	25

a. General cyclization protocol was as follows: i) Removal of HOAc from **3** by repeated lyophilization from water and drying over P_2O_5 and KOH at high vacuum; ii) Dissolving in DMF to 10^{-4}M and neutralization with 10 equivalents of N,N-diisopropylethylamine; iii) Addition of 3 equivalents of coupling reagent. The reaction was kept at 4 ° C under slow stirring. Analytical HPLC samples were taken at 6, 12, 24, 48 and 96 h.
b. Determined by HPLC. Reactions were considered finished when no change in peak area was observed in two consecutive determinations.
c. Based on amount of cyclic peptide isolated after precipitation with water, centrifugation and lyophilization from HOAc.

For the preparation of cyclopeptides from linear precursors two different approaches are generally available . In one of them, activation and coupling (*i.e.*, ring closure) steps are separated by an intermediate purification of the C-terminal carboxyl-activated peptide derivative. Alternatively, both processes can be combined in a single operation with the aid of *in situ* activating-coupling reagents[8,9]. In our case we opted for this second alternative. In a preliminary study, the effect of several coupling agents on the extent and recovery of the cyclization reaction has been investigated using 3 as model substrate (Figure 3 and Table 1).

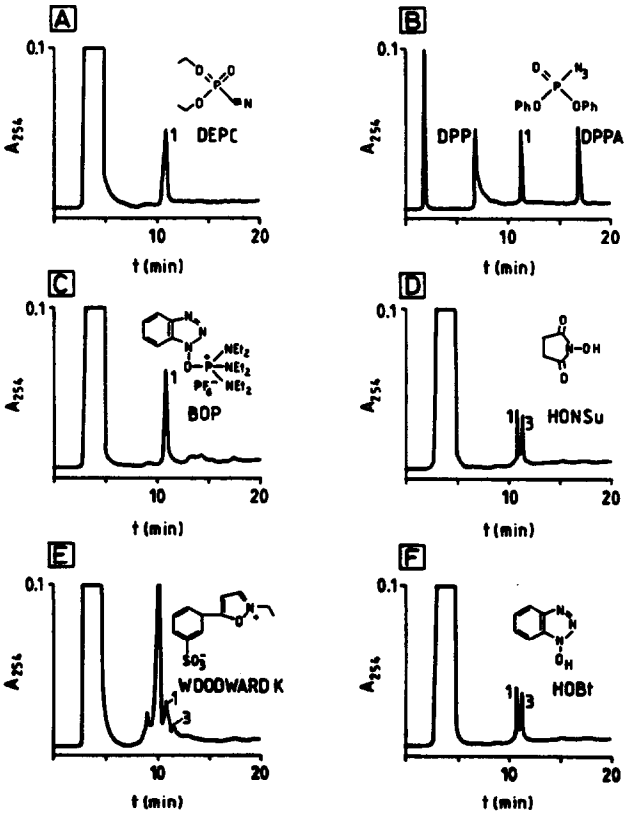

Figure 3. HPLC analyses of the cyclization of 3 with several coupling agents. Conditions: Val-U-Pak C_{18}-silica column (25 cm x 46 mm; 5 μm); flow 1.0 ml/min; 10-95% linear acetonitrile (0.036% TFA) gradient in water (0.045%) over 20 min.

Of all the coupling reagents investigated, DEPC, DPPA and BOP were the most effective ones, giving in all cases near-quantitative yields (less than 1% starting product detectable by HPLC) after 6 h. Recoveries of 65-70% cyclization product were obtained in all three cases after purification. However, as DEPC is highly toxic and requires careful handling in a well-ventilated hood, the latter two reagents are preferable. The other three coupling reagents (Woodward K, HONSu/DCC and HOBt/DCC) did not perform as well, even at long reaction times. Based on these results, DPPA- and BOP-mediated cyclizations of **4** to **2** were successfully carried out in 99 % yield (65 % recovered product). Both **1** and **2** were characterized by FAB-MS (m/z= 867 $(M+H)^+$, m/z= 1129 $(M+H)^+$, respectively) and by their NMR spectra, in which the α-NH signals clearly showed the existence of the desired C_2 and C_3 symmetries, respectively.

Binding properties of both cyclic peptides **1** and **2** and their deprotected analogues are presently under study.

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6.- The abbreviations used to denote amino acid derivatives and peptides are those recomended by the IUPAC-IUB Commission on Biochemical Nomenclature. Other abbreviations used: BOP= benzotriazol-1-yl-tris(dimethylamino)phosphonium hexafluorophosphate, DCC= N, N'-dicyclohexylcarbodiimide, DEPC= diethylphosphorocyanidate, DMF= N, N-dimethyl-formamide, DPPA= diphenylphosphorylazide, FAB-MS= fast atom bombardment mass spectrometry, Fmoc= 9-fluorenylmethyloxycarbonyl, HOBT= 1-hydroxybenzotriazole, HONSu= N-hydroxysuccinimide, Tcp= 2,3,5-trichlorophenyl, TFA= trifluoroacetic acid, Z= benzyloxycarbonyl.

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