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# Synthesis of a Cyclic Peptide Polyamine: A Comparative Study of Routes to *Cyclo*-(Lys-Lys-Gly)$_2$

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***Abstract.*** Several methods for the preparation of *cyclo*-(Lys-Lys-Gly)$_2$ were compared. Cyclisation of linear hexapeptides of the type H-[Lys(Z)-Lys(Z)-Gly]$_2$-X was most efficient using excess DPPA with the free amino acid (i.e. X = OH), but purification and scale-up were problematical. Much more efficient was cyclo-dimerisation of H-Lys(Z)-Lys(Z)-Gly-OPFP.TFA, using $Cs_2CO_3$ as the neutralising base; purification from this procedure was easy, and scale-up involved no loss of yield. The cyclo-dimerisation route proceeded in 38% overall yield from the tripeptide Boc-Lys(Z)-Lys(Z)-Gly-OMe, whereas the best yield *via* the linear pathway was 20.5% from the same tripeptide starting material.

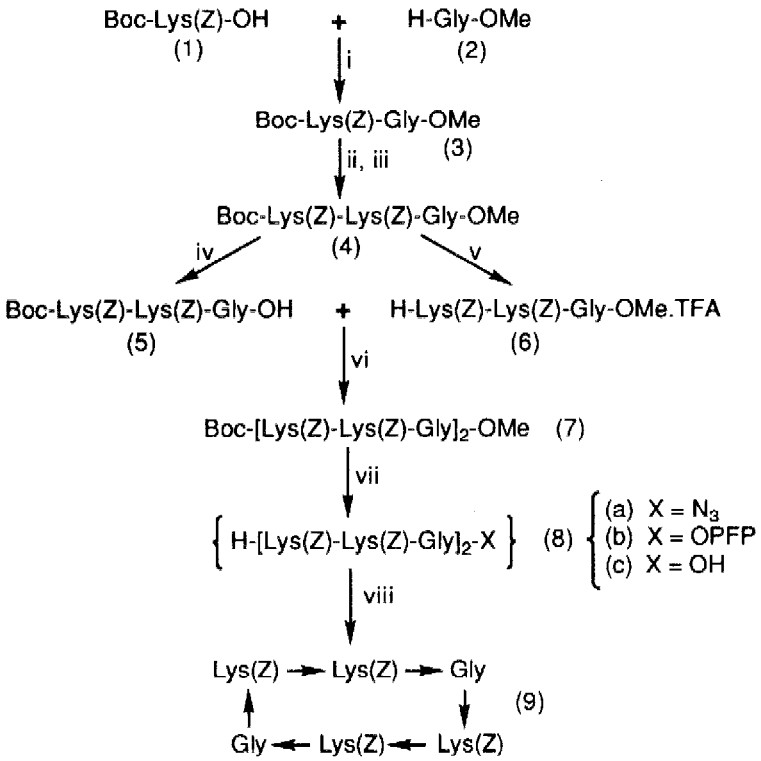
Linear peptides are typified by the large amount of conformational freedom that they possess, in contrast to the well defined secondary and tertiary structure that is usually seen in proteins. The presence of one or more rings within a peptide substantially reduces the number of conformations that are accessible, and cyclic peptides are particularly important in medicinal chemistry.[1] Nevertheless, a single ring rarely generates peptides for which a pronounced conformational minimum is apparent. We wished to prepare peptides with additional constraints (in particular, possessing multiple rings) in order to study conformational effects, and ultimately with the intention of designing peptidic enzyme mimics.

As a precursor to such molecules, we envisaged a small cyclic peptide containing several lysine residues, in which the ε-nitrogens could be cross-linked in order to generate polycyclic peptides. We selected *cyclo*-(Lys-Lys-Gly)$_2$ because its twofold symmetry would allow a convergent synthetic strategy, and because the glycine residues might aid β-turn formation whilst simultaneously guaranteeing racemisation-free fragment couplings. In this paper, we describe our findings on the synthesis of *cyclo*-(Lys-Lys-Gly)$_2$, and these results should offer a useful model for the preparation of peptides of general structure *cyclo*-(X-Y-Gly)$_2$.

For all of our studies, we were able to use Boc-Lys(Z)-Lys(Z)-Gly-OMe (4) as a common building block. This was prepared by standard solution phase peptide synthesis, as outlined in Scheme 1, giving access to multi-gram quantities of the protected tripeptide (4). Selective deprotections liberated the free acid (5) and free α-amine (6), which were then coupled using DCC/ HOBt[2] to give the linear hexapeptide Boc-[Lys(Z)-Lys(Z)-Gly]$_2$-OMe (7) in 51% yield.

Four methods of cyclisation were explored: *via* the azide[3], *via* the pentafluorophenyl ester[4], or by direct cyclisation of the free amino acid using BOP[5] or DPPA[6]. The results are summarised in Table 1, and

show that the azide (with or without HOBt catalysis)[7] and pentafluorophenyl ester routes gave no indication of the desired cyclic peptide (9) by FAB mass spectrometry. The parent ion for (9) was observed in the crude product from cyclisation using BOP, but many by-products were present, and isolation of the pure cyclic peptide proved impossible. Much cleaner was the reaction using DPPA/ $NaHCO_3$ over $3^1/_2$ days, from which pure *cyclo*-[Lys(Z)-Lys(Z)-Gly]$_2$ (9) was obtained in 20% yield after gel filtration (LH20/ DMF), dry packed flash chromatography (silica, $CHCl_3$/ MeOH/ AcOH), and reprecipitation from hot methanol. This awkward purification procedure was necessary because of the low solubility of the hexapeptides, and similar problems had been encountered during isolation of the linear hexapeptide precursors.



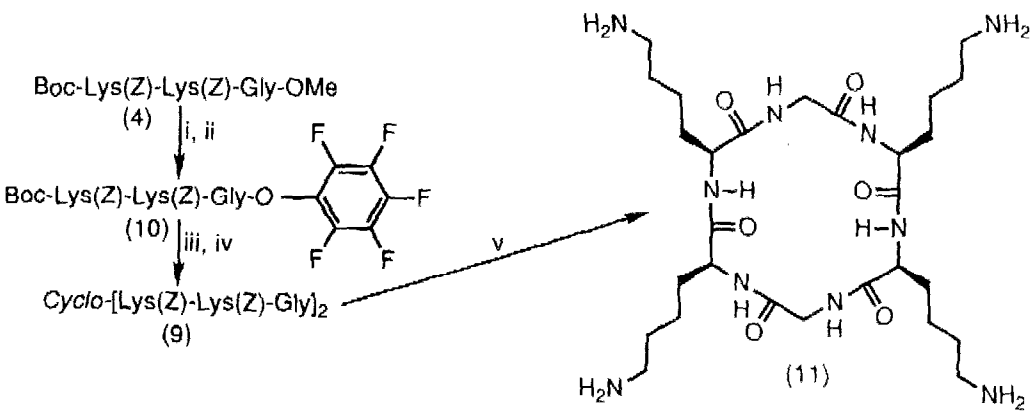

**Scheme 1.** Reagents: i, DCC/ HOBt (98%); ii, 90% TFA (aq); iii, (1)/ DCC/ HOBt/ DIPEA (84% for ii+iii); iv, KOH/ $H_2O$/ MeOH (91%); v, 90% TFA (aq); vi, DCC/ HOBt/ DIPEA [51% of (7a) for v+vi]; vii, see notes 3,4 & 5 for the formation of (8a), (8b) and (8c) respectively (as their TFA or HCl salts); viii, see Table 1 for conditions used for the final cyclisation.

To summarise the results, we found that the cyclisation of H-[Lys(Z)-Lys(Z)-Gly]$_2$-X (8) (X = $N_3$, OPFP, OH) was accomplished most cleanly using DPPA with the free acid (8c). A large excess of the coupling reagent, and longer reaction times, led to significantly improved yields [up to 46% for the cyclisation step, although only 20.5% overall from the tripeptide (4)], but the addition of HOBt was not beneficial,[7] and isolation of the pure product was problematical. On larger scales, the cyclisations were less efficient and, given these limitations, an alternative approach was explored.

| Cyclisation | [Peptide] (Wt.) | [DPPA] | [HOBt] | Temp. | Time | Yield |
|---|---|---|---|---|---|---|
| Azide[3] | 1.1 mM | - | 0 or cat. | 0°C | 65 h | 0% |
| PFP ester[4] | 1.2 mM | - | - | 90°C | 18.5 h | 0% |
| BOP[5] | 0.95 mM | - | - | RT | 84 h | * |
| DPPA[6] | 0.57 mM (145 mg) | 2 eq. | - | RT | 3.5 d | 20% |
| DPPA[6] | 0.92 mM (100 mg) | 2 eq. | - | RT | 7 d | 35% |
| DPPA[6] | 0.92 mM (100 mg) | 2 eq. | 10 eq. | RT | 7 d | 30% |
| DPPA[6] | 0.92 mM (100 mg) | 2 eq. | 30 eq. | RT | 7 d | 10% |
| DPPA[6] | 0.92 mM (100 mg) | 10 eq. | - | RT | 7 d | 46% |
| DPPA[6] | 0.92 mM (500 mg) | 10 eq. | - | RT | 7 d | 20% |

**Table 1.** Results from the cyclisation of H-[Lys(Z)-Lys(Z)-Gly]$_2$-X under a variety of conditions. The cyclisation procedures are described in the notes[3-6]; * detected by FAB-MS, but not isolated.

Cyclo-dimerisation has been used for the synthesis of several cyclic peptides possessing $C_2$ symmetry,[8] and our target molecule was a potential candidate for this tactic. Of crucial importance regarding (9) are the two glycine residues, which allow racemisation-free activation of the tripeptide precursor. The cyclo-dimerisation of tripeptide pentafluorophenyl esters had the best literature precedent, although the failure of H-[Lys(Z)-Lys(Z)-Gly]$_2$-OPFP to undergo cyclisation did not bode well. But these misgivings proved unfounded, for neutralisation of the TFA salt of H-Lys(Z)-Lys(Z)-Gly-OPFP (0.02M in DMF) with $NaHCO_3$ allowed isolation of the desired cyclic hexapeptide (9) in 26% yield. Even using these unoptimised conditions, this route already bettered the linear approach in overall yield. Moreover, the isolation of the product turned out to be straightforward, as simple reprecipitation from hot methanol gave the pure cyclic hexapeptide (9).




**Scheme 2.** Reagents: i, KOH/ $H_2O$/ MeOH (91%); ii, PFP-OH/ DCC (100%); iii, TFA/ $CH_2Cl_2$ (1:1) (100%); iv, see Table 2; v, $H_2$/ $Pd(OH)_2$-C/ TFE (100%)

| Base (5 eq) | [Peptide] | Scale | Time/ Temp. | Yield |
|---|---|---|---|---|
| $NaHCO_3$ | 0.02 M | 480 mg | 3 d/ RT | 26% |
| $NaHCO_3$ | 0.10 M | 300 mg | 3 d/ RT | 26% |
| $NaHCO_3$ | 0.005 M | 480 mg | 3 d/ RT | 23% |
| $KHCO_3$ | 0.02 M | 260 mg | 3 d/ RT | 26% |
| DIPEA | 0.02 M | 400 mg | 3 d/ RT | 22% |
| $Cs_2CO_3$[9] | 0.02 M | 400 mg | 3 d/ RT | 36% |
| $Cs_2CO_3$[9] | 0.02 M | 1.78 g | 3 d/ RT | 42% |

**Table 2.** Results from the cyclo-dimerisation of H-[Lys(Z)-Lys(Z)-Gly]$_2$-OPFP.TFA.

We expected the cyclo-dimerisation to be accutely concentration dependant, as inter- and intra-molecular steps are involved in the reaction. We were therefore astonished to find that a four- to five-fold increase or decrease in concentration had virtually no effect on the yield (Table 2). Because of the ionophoric nature of many cyclic peptides, we decided to see whether the choice of base (with its associated cation) might affect the yield. As shown in Table 2, the use of caesium carbonate[9] led to a dramatic increase in yield; FAB mass spectra of the cyclic product (9) in the presence of equimolar amounts of Group I metal ions failed to show a strong preference for chelation to $Cs^+$ instead of $Na^+$ or $K^+$, so the caesium effect[10] is probably operating here. To our delight, increasing the scale of the reaction led to improved yields; overall, the cyclo-dimerisation strategy gave up to 38% yield of (8) from the fully protected tripeptide (4).

Finally, removal of the benzyloxycarbonyl protection from the ε-nitrogens of (9) was accomplished by hydrogenolysis over Pearlman's catalyst in trifluoroethanol (TFE) as solvent; other work in our laboratory had already shown that palladium on charcoal was a poor catalyst for such $N^{\varepsilon}$-deprotections of lysyl peptides.

The synthesis of *cyclo*-[Lys(Z)-Lys(Z)-Gly]$_2$ (9) has given valuable insight into the best route to such $C_2$ symmetric cyclic hexapeptides. Of particular note is that the cyclo-dimerisation strategy proceeded in good overall yield from the tripeptide (4), allowed easy isolation of the desired product, and could be carried out on larger scales (and in quite high concentration) without any loss of yield. We believe that this methodology should work well with most cyclic hexapeptides of general structure *cyclo*-(X-Y-Gly)$_2$, and its application to the cyclo-dimerisation of cross-linked tripeptides is discussed in the following paper.

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1) For the use of peptides as targets for drug research, see Dutta, A.S.; *Chem. Br.*, **1989**, *25*, 159
2) Konig, W; Geiger, R.; *Chem. Ber.*, **1970**, *103*, 788, 2024 and 2034.
3) The methyl ester (7) was converted into the hydrazide (96% yield), and the Boc protection was removed using 90% TFA (aq). Cyclisation *via* the azide was attempted by the addition of t-butyl nitrite/ HCl in DMF at ca. -16°C (for 1.5 h), followed by further stirring over DIPEA and HOBt at 0°C for 12 h.. For a leading reference, see Kessler, H.; Gemmecker, G.; Haupt, A.; Klein, M.; Wagner, K; Will, M.; *Tetrahedron*, **1988**, *44*, 745.
4) Hydrolysis of methyl ester (7) (KOH/ $H_2O$/ DMF; 80% yield) gave the free acid, which was reacted with PFP-OH/ DCC in DMF to give the PFP ester of (7) (61%). After removal of the Boc protection (TFA/ $CH_2Cl_2$, 1:1), the product was added dropwise over 2.5 h to pyridine at 90°C, and stirred for a further 16 h. For a leading reference, see Schmidt, U.; Kroner, M.; Griesser, H.; *Synthesis*, **1991**, 294.
5) After hydrolysis of the methyl ester of (7),[4] followed by removal of the Boc-protection using 90% TFA (aq), the resulting free hexapeptide (as the TFA salt) was stirred with BOP in DMF over excess base. For a leading reference, see Jouin, P.; Poncet, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B.; *J. Org. Chem.*, **1989**, *54*, 617.
6) The procedure was the same as note 5, replacing BOP with DPPA. For a leading reference, see Boger, D.L.; Yohannes, D.; *J. Org. Chem.*, **1990**, *55*, 6000.
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9) These reactions involved the use of 2.5 equivalents of $Cs_2CO_3$, corresponding to 5 equivalents of available base, and 5 equivalents of $Cs^+$ ions.
10) Buter, J.; Kellogg, R.M.; F. van Bolhuis, F.; *J. Chem. Soc., Chem. Commun.*, **1990**, 282.

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