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Synthesis of Doubly Cross-Linked Cyclic Hexapeptides

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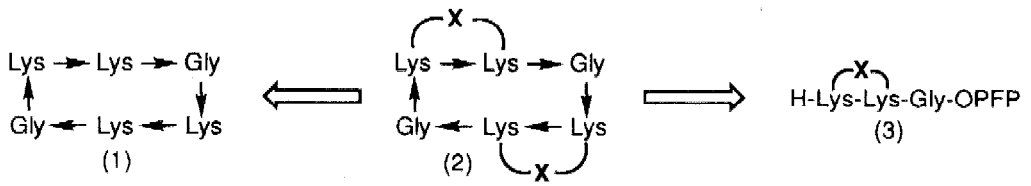
Abstract. Cyclic hexapeptides in which the adjacent N^{ε}-nitrogens of *cyclo*-(Lys-Lys-Gly)$_2$ are cross-linked by p-xylyl or $(CH_2)_6$ units have been prepared. The key steps in the syntheses of these novel types of peptide were the use of tosyl (or, less efficiently, trifluoroacetyl) as N^{ε}-protection that allowed intramolecular cross-linking of the tripeptides Boc-Lys(R)-Lys(R)-Gly-OMe (R = Ts or CF_3CO) using α,ω-dibromo-alkanes/ Cs_2CO_3, and the efficient cyclo-dimerisation of the pentafluoro-phenyl esters of the resultant cross-linked tripeptides.

One way of reducing the number of conformations that are available to a peptide is by the introduction of one or more rings. In the previous paper, we compared various methods for obtaining a monocyclic hexapeptide, *cyclo*-(Lys-Lys-Gly)$_2$ (1).[1] In this paper, we wish to describe the syntheses of peptides possessing further conformational constraints, in which both pairs of ε-nitrogens in (1) have been cross-linked, to create molecules whose general structure is depicted by (2). We were keen to use a synthetic approach that would allow the nature of the cross-linker (X) to be varied considerably,[2] so that the influence of this unit on the structure and properties of the resultant peptides could be explored.



In order to prepare such molecules, we initially considered attempting to carry out cross-linking reactions on (1). But we envisaged that there would have been severe limitations on the types of cross-linker that could have been used,[2] and there might also have been uncertainty about their location within the peptides (i.e. would

adjacent or skipped residues be connected?). Instead, we chose to discover whether tripeptides that already possessed cross-links between adjacent residues [e.g. (3)] could be induced to undergo cyclo-dimerisation, using the conditions developed by us in the previous paper. This would ensure that the location of the cross-links in the final products could be guaranteed. Moreover, we hoped that the cross-links might help to stabilise β-turns, with a concommitant increase in the yield of cyclo-dimerisation.

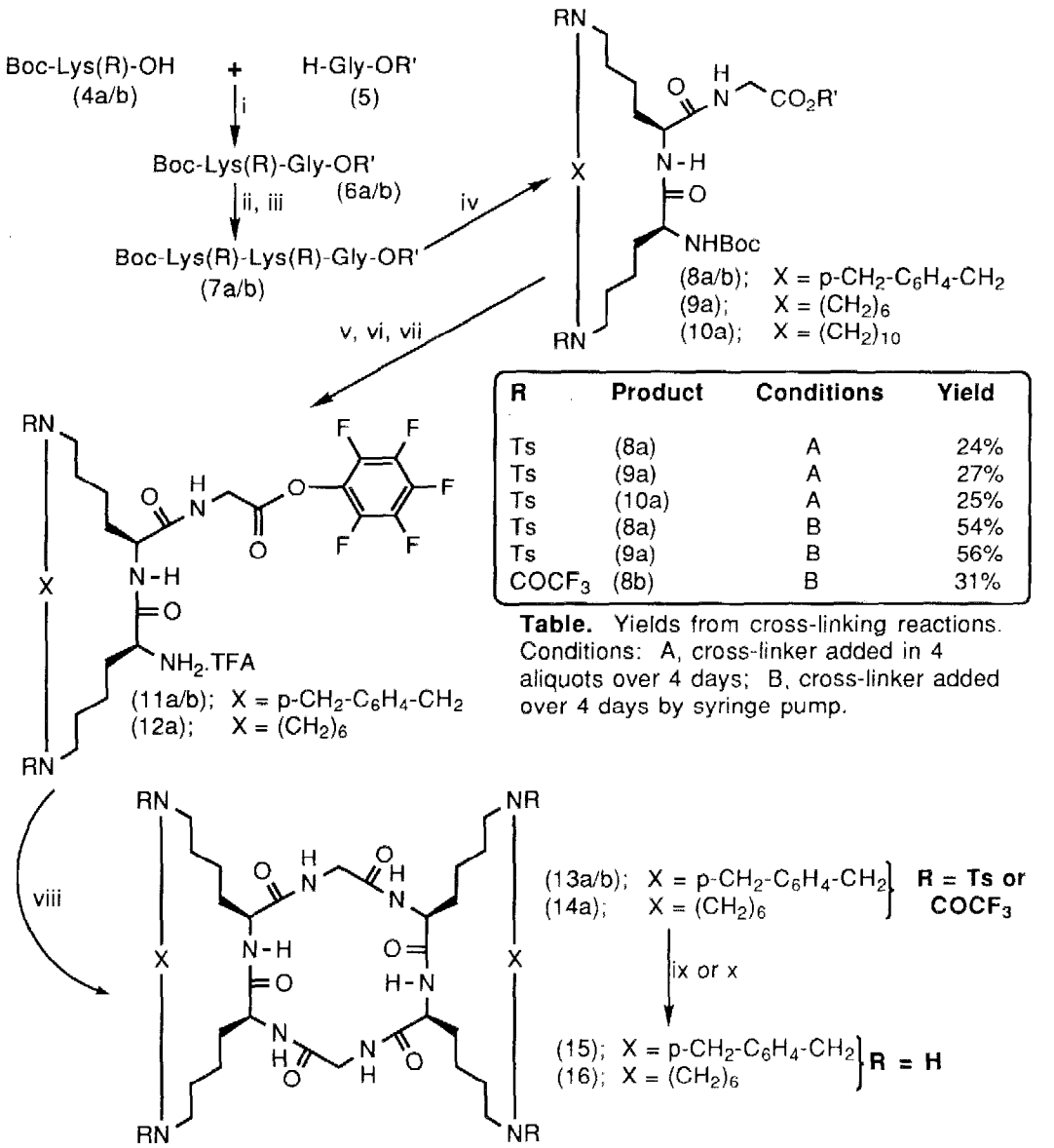
After several unsuccessful attempts to cross-link the free ε-amino groups of Boc-Lys-Lys-Gly-OMe,[3] we chose the tactic of introducing ε-nitrogen protection that was powerfully electron withdrawing; we hoped that the resultant tripeptide might readily form the dianion on both ε-nitrogens, quenching of which with an α,ω-dihaloalkane might then introduce the desired cross-link.[4] To this end, we considered the use of tosyl[5] or trifluoroacetyl[6] protection (see Scheme).

Using the di(N^{ε}-tosyl) tripeptide (7a), cross-linking was attempted using three α,ω-dibromoalkanes. The addition of the cross-linker in four portions over four days, with caesium carbonate as base, gave 24-27% yields of the p-$(CH_2)_2C_6H_4$, $(CH_2)_6$, and $(CH_2)_{10}$ cross-linked tripeptides [(8a), (9a) and (10a)]. With the viability of this method demonstrated, the p-xylyl and C_6 cross-linked derivatives were studied further. Repeating the four day addition using a syringe pump boosted the yields up to 54-56%, and this would appear to be an efficient method of preparing compounds of this type. As far as we are aware, this is the first example in peptide chemistry of a strongly electron withdrawing group being used both to protect nitrogen during synthesis and to enable an intramolecular cross-link to be introduced.

With the cross-linked tripeptides in hand, we were ready to attempt the cyclo-dimerisations using the conditions we had recently developed.[1] Thus, conversion of (8a) and (9a) into their pentafluorophenyl esters, and removal of the Boc-protection yielded the TFA salts (11a) and (12a) in overall yields of 75-85%; cyclo-dimerisation was effected by neutralisation of 0.02M solutions of these salts in DMF using solid caesium carbonate as the base, and the doubly cross-linked cyclic hexapeptides (13a) and (14a) were initially obtained in yields of about 50% (ca. 0.1 mmol scale). As observed before,[1] larger scale reactions were more efficient, with 590mg of (11a) giving a 55% yield of (13a), and 1.8g of (12a) giving (14a) in 66% yield.

We were interested in the conformational features and physical properties of the tetra-tosyl derivatives (13a/14a), but we also wanted the free tetra-amine for further synthetic work.[4] Removal of tosyl protection is well known to be a capricious transformation, and model studies on N^{ε}-tosyl protected lysine derivatives had warned us that deprotection might be tricky. In the event, standard treament with sodium in liquid ammonia[7] afforded (15) and (16), both isolated in 30% yield as their TFA salts after purification by reverse phase HPLC.

The modest yield for the final deprotection was, in fact, the only serious problem encountered in the use of tosyl both for protection and to facilitate introduction of the cross-linker. Where the free tetra-amines [e.g. (15) and (16)] were required, we thought that replacing the N^{ε}-tosyl protection by trifluoroacetyl might be beneficial; in particular, cleavage of the xylyl group might have been a significant side reaction in the reductive deprotection of (13a). Consequently, we prepared the bis(trifluoroacetyl) tripeptide (7b), and introduced the xylyl cross-linker as before, using syringe pump conditions. The cross-linked tripeptide (8b) was successfully obtained, but partial hydrolysis of the trifluoroacetyl groups during the 5 day reaction probably contributed to the disappointing 31% yield. Conversion to the pentafluorophenyl ester proceeded smoothly [(11b) in 84% yield from (8b)], but cyclo-dimerisation gave only 13% of (13b) plus 12% of the tris(trifluoroacetyl) derivative (25% total yield). Finally, deprotection of this mixture with MeOH/ NH_3(aq) gave only 40% of (15) after purification by reverse phase HPLC. Although this demonstrates that trifluoroacetyl can indeed be used in place of tosyl in



R	Product	Conditions	Yield
Ts	(8a)	A	24%
Ts	(9a)	A	27%
Ts	(10a)	A	25%
Ts	(8a)	B	54%
Ts	(9a)	B	56%
$COCF_3$	(8b)	B	31%

Table. Yields from cross-linking reactions. Conditions: A, cross-linker added in 4 aliquots over 4 days; B, cross-linker added over 4 days by syringe pump.

Scheme. For all compounds (4-14), R = Ts and R' = Me if number is suffixed by "a", and R = $COCF_3$ and R' = CH_2Ph if number is suffixed by "b". Reagents: i, DCC/ HOBt/ DMF [91% for (6a); 82% for (6b)]; ii, 90% TFA (aq); iii, (4a/b)/ DIPEA/ DCC/ HOBt/ DMF [93% for (7a); 85% for (7b) (yields for ii + iii)]; iv, Br-X-Br/ Cs_2CO_3/ DMF (see Table for X and yields); v, 40% 1M-KOH (aq)/ DMF [for (11a) and (12a)], or H_2/ Pd-C/ MeOH [for (11b)]; vi, 90% TFA (aq); vii, PFP-OH/ DCC/ DMF [78% for (11a); 66% for (12a); 84% for (11b) (yields for v + vi + vii)]; viii, Cs_2CO_3/ DMF [55% for (13a); 66% for (14a); 13% for (13b) plus 12% lacking one $COCF_3$ group]; ix, Na/ NH_3(l) [30% for (13a) to(15); 30% for (14a) to (16)]; x, sat. NH_3 (aq)/ MeOH 2:5 [40%, from (13b) + the tris(trifluoroacetyl) derivative].

this synthetic strategy, our experience is that the more robust nature of tosyl is an important advantage for the earlier synthetic steps, whilst deprotection of the sulphonamides (if required) proceeds satisfactorily.

As far as we are aware, these are the first syntheses of cyclic peptides possessing multiple intramolecular cross-links, and the strategy of using cross-linked peptide building blocks is also very unusual. But why were the cyclo-dimerisations so efficient? Our belief is that the cross-linking of the adjacent lysine residues favoured β-turns involving them;[8] this is because the lysyl side-chains would have been held in close proximity by the cross-linkers, and so there would have been no energy penalty for the unfavourable steric interaction normally associated with such β-turns. The structural features of these polycyclic peptides should therefore be unusual and interesting, and studies of such features are currently under way; in addition, we are exploring the potential host-guest properties of these molecules, in which the "wings" could fold around suitable substrates.

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REFERENCES AND NOTES

1) Bailey, P.D.; Crofts, G.A.; *Tetrahedron Lett.*, **1992**, *33*, preceding paper.

2) We wished to avoid using di-acyl cross-linkers, as further derivatisation of the ε-nitrogens would then be virtually impossible (c.f. note 4). The use of di-alkyl cross-linkers with peptides possessing free ε-NH_2 groups would run the risk of polyalkylation, and of formation of simple N-heterocycles in which both ends of the cross-linker were attached to the same nitrogen; the latter problem could be overcome by using cyclic cross-linkers for which this would be sterically impossible (e.g. p-xylyl).

3) *In situ* ($NaBH_3CN$) or stepwise ($NaBH_4$) reduction of the (di)imines derived from the tripeptide Boc-Lys-Lys-Gly-OMe and dialdehydes (OHC-X-CHO) gave complex mixtures of products, whilst careful treatment of the same tripeptide with p-$(BrCH_2)_2C_6H_4$ also failed to generate the desired product possessing a single cross-link.

4) We were particularly interested to see how the length of the cross-linkers might influence the conformations available to the final cyclic hexapeptides. We chose spacers of about 8 carbons for these model studies, with the p-xylyl derivative also selected because of the additional conformational constraints it imposed. One further objective was the synthesis of poly-lysyl peptides in which all the ε-nitrogens were interconnected by cross-links; see Bailey, P.D.; Carter, S.R.; Clarke, D.G.W.; Crofts, G.A.; Smith, P.W.; Ward, P.; *Tetrahedron Lett.*, **1992**, *33*, following paper.

5) Tosyl has been widely utilised in the formation of aza-crowns, for which its ability to stabilise negative charge and act as a protecting group is exploited. For example, see Cox, J.P.L.; Craig, A.S.; Helps, I.M.; Jankowski, K.J.; Parker, D.; Eaton, A.W.E.; Millican, A.T.; Millar, K.; Beeley, N.R.A.; Boyce, B.A.; *J. Chem. Soc., Perkin Trans 1*, **1990**, 2567.

6) Trifluoroacetamide can be used as a Gabriel equivalent, in which base induced alkylation of the nitrogen is followed by hydrolysis of the CF_3CO moiety: Harland, P.A.; Hodge, P.; *Synthesis*, **1984**, *84*, 941.

7) See Greene, T.W. *Protective Groups in Organic Synthesis* ; John Wiley and Sons, Inc.: New York, 1981; pp. 285-6, and references therein.

8) Cyclic hexapeptides usually exhibit two β-turns. Precursors that stabilise such turns would therefore be expected to undergo cyclisation more readily than those that do not.

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