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Synthesis of Cyclic Peptides via Efficient New Coupling Reagents

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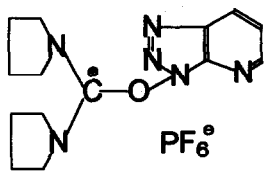
ABSTRACT:
The efficiency of various coupling reagents in promoting the cyclization of linear peptides has been compared. Newly developed reagents based on 1-hydroxy-7-azabenzotriazole were found to be highly effective and led to remarkably diminished racemization.

Due to their restricted conformational flexibility, cyclic peptides are of great interest in connection with structure-activity relationships, especially the elucidation of bioactive conformations.
In the synthesis of homodetic cyclic peptides, the readiness of an open chain precursor to cyclize depends on the size of the ring to be closed, and usually no difficulties arise for the cyclization of peptides containing seven or more amino acid residues. Although ring closure with hexa- and pentapeptides is more hampered, the cyclization is enhanced by the presence of turn structure-inducing amino acids such as glycine, proline or a D-amino acid[1,2]. For linear peptides which do not contain amino acid residues which stabilize turn structures the cyclization reaction may be an inherently improbable or slow process, and side reactions, such as cyclodimerization, may dominate even at high dilutions (10^{-3}- 10^{-4}M)[3]. Moreover, the prolonged existence of the activated carboxyl group increases the likelihood of racemization of the C-terminal residue. The extent of racemization may be diminished by application of the azide method[4] or modified azide methods using DPPA[5,6]. However, these cyclization methods are extremely slow, usually requiring many hours or even several days[4,7]. In comparison with DPPA, TBTU[8] and BOP[9] provide fast cyclizations[10,11], but may also lead to racemization levels which are comparable to those observed with DCC/HOBt[12].

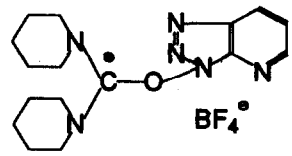
The aim of the present study was to compare the utility of various coupling reagents for peptide cyclization, including some newly developed reagents based on 1-hydroxy-7-azabenzotriazole (HOAt)[13] which has recently been recommended as a substitute for HOBt in solution-based peptide synthesis. Replacement of HOBt by HOAt led to significantly improved coupling efficiencies and racemization suppression[14].

Using the linear GnRH-derived decapeptide 3 as a model, it was found that the newly developed uronium salts 1 (HAPyU)[14,15] and 2 (TAPipU)[14,15], derived from HOAt, were highly effective for the cyclization (Fig 1A)[16]. These reagents led to complete cyclization within less than

30 min at a peptide concentration of 1.5 mM, whereas TBTU, TOPPipU[17] and DPPA gave only 60%, 10% and 12% cyclization, respectively. A 10 %-excess of 1 or 2 was found to be sufficient for quantitative ring closure[16]. As previously noted for the corresponding pyrrolidine and piperidine analogs of the BOP reagent[19] the pyrrolidine salt 1 proved to be more active than the piperidine analog 2.



HAPyU, 1



TAPipU, 2

H-Nal-D-Cpa-D-Pal-Gln-Tyr-D-Arg-Leu-Arg-Pro-Lys(Ac)-OH 3

H-Val-Arg-Lys(Ac)-Ala-Val-Tyr-OH 4

H-Arg-Lys(Ac)-Ala-Val-Tyr-OH 5

In addition, compounds 1 and 2 were tested for side-chain cyclizations of peptides containing D-amino acids and were found to be more effective than TBTU, BOP, TOPPipU and DPPA for the formation of rings incorporating 3, 4, 5 and 7 amino acid residues (data not shown).
The cyclization was considerably accelerated by increasing the peptide concentration (Fig 1B). Thus, the linear decapeptide was cyclized within 2 min at a peptide concentration of 0.1 M when reagents 1 and 2 were used. Surprisingly, even at high peptide concentrations (0.1 - 0.2 M) no intermolecular reactions were observed, indicating that at least for the head-to-tail and side-chain cyclizations studied, application of the principle of dilution[3] is not required.

Linear hexapeptides, constructed exclusively from L-amino acids and not containing glycine or proline, are known to cause problems during attempted ring closure reactions[2,20]. Indeed, our attempts to cyclize the hexapeptide 4, (10^{-2}M) failed, when BOP and TBPipU[18] were used (Fig 1C). Only 5 - 10 % of the desired cyclopeptide was formed, whereas a moderate yield (25 %) was obtained by use of compound 2. The cyclization is however accompanied by extensive racemization (BOP 24%, TBPipU 7%, TAPipU 8%) of the C-terminal tyrosine residue, as demonstrated by HPLC analysis of the reaction mixture[21]. In remarkable contrast to these results, with HAPyU, 1, the all L-cyclohexapeptide, 4, was formed in 55 % yield within 30 min and less than 0.5 % of the D-Tyr-isomer was detected in the reaction mixture.

These results demonstrate the superiority of coupling reagents derived from 1-hydroxy-7-azabenzotriazole, especially HAPyU, 1, for promoting peptide cyclization quickly and with a minimum of racemization.

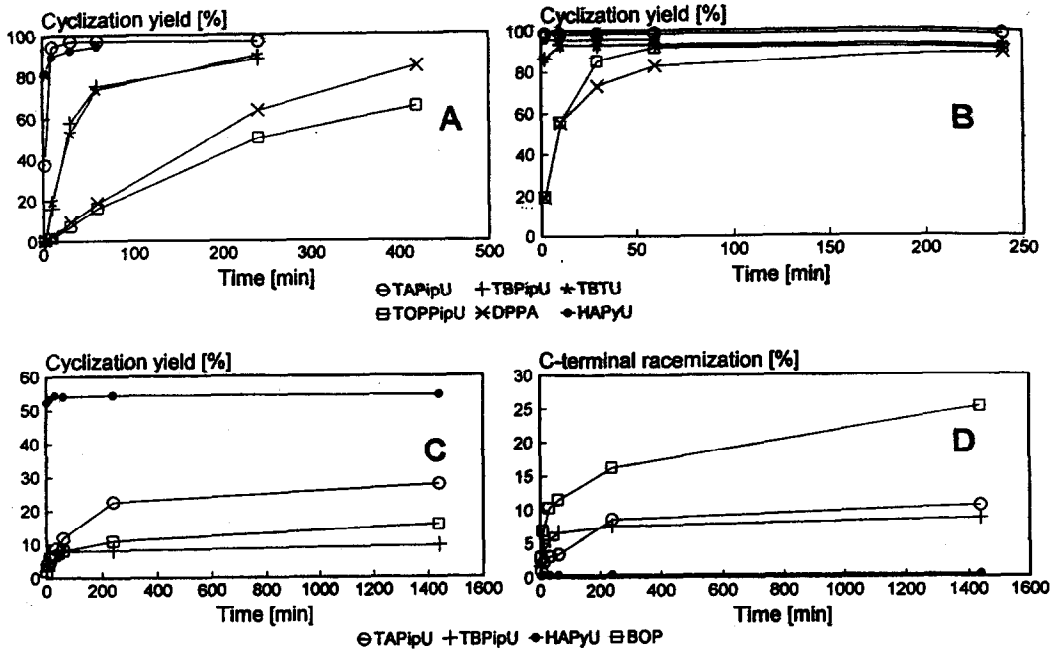


Fig 1. Comparison of different coupling reagents for peptide cyclization. A: Cyclization of 3 c=0.0015 M); B: Cyclization of 3 (c=0.1 M); C: Cyclization of 4 (c=0.01 M); D: Racemization of C-terminal Tyr during the cyclization of 4.

Naturally, even these new activating agents cannot overcome difficulties arising from unfavourable conformations of linear pentapeptides devoid of glycine and proline and containing only L-amino acids[20]. Thus, our initial attempts to cyclize the human splenin[22]-derived pentapeptide H-Arg-Lys(Ac)-Ala-Val-Tyr-OH, 5, at peptide concentrations of 0.01 and 0.001 M using HAPyU 1 resulted predominantly in the formation of the corresponding dimer and cyclodimer, in contrast to the results obtained with hexapeptide 4. Formation of the corresponding cyclopentapeptide appears to require initial racemization of the C-terminal tyrosine residue, as observed earlier using similar sequences[20] (see however ref. 23). Thus, using reagent 1 only negligible amounts of cyclo(Arg-Lys(Ac)-Ala-Val-D-Tyr) were formed, whereas with TBPipU, TAPipU and TOPPipU up to 12 % of this cyclopeptide was obtained due to the higher racemization potency of the latter reagents.

Acknowledgments

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6. *Abbreviations:* BOP: benzotriazolyloxytris-(dimethylamino)-phosphoniumhexafluorophosphate; DCC: dicyclohexylcarbodiimide; DIEA: diisopropylethylamine; DMF: dimethylformamide; DPPA: diphenylphosphorylazide; HOBt: 1-hydroxybenzotriazole; HOAt: 1-hydroxy-7-azabenzotriazole; HAPyU: O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethylenuronium hexafluorophosphate; TAPipU: O-(7-azabenzotriazol-1-yl)-1,1,3,3-pentamethylenuronium tetrafluoroborate; TBPipU: O-(benzotriazol-1-yl)-1,1,3,3-pentamethylenuronium tetrafluoroborate;TBTU: O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TOPPipU: 2-(2-oxo-pyrid-1-yl)-1,1,3,3-pentamethylenuronium tetrafluoroborate
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15. The synthesis of HOAt was carried out by methylation of commercially - available 2-nitro-3-pyridinol followed by treatment of the methyl ether with excess hydrazine[14]. The coupling reagents were prepared following the procedure described by Knorr[18].
16. Conditions of the cyclization reaction:
    0.1 mMol of peptide dissolved in DMF was treated under stirring with 0.11 mMol of coupling reagent (in case of activation by DPPA 0.2 mmol) and 0.3 mmol of DIEA. The reaction mixture was stirred at room temperature, and when cyclization was complete, as judged by reversed phase HPLC, the solvent was evaporated in vacuo. The residue was dissolved in methanol and isolated by precipitation from ethyl acetate/ether.
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