2-Propanephosphonic acid anhydride (T3P)-mediated segment coupling and head-to-tail cyclization of sterically hindered peptides

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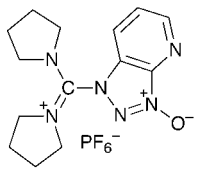
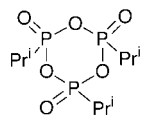
In the course of comparing the effectiveness of an HOAt-derived coupling reagent (HAPyU) and a phosphonic acid-based condensation agent (T3P) by cyclization of model sequences, we found that T3P was a superior reagent with regard to conversion of the linear peptide into the stereochemically intact cyclic monomer when sterically hindered amino acids are found at the cyclization site.

Although the stepwise coupling of urethane-protected amino acids in peptide chemistry normally causes no problems with regard to loss of configuration at the reactive carboxy residue, head-to-tail cyclizations of short peptide chains having the all-L-configuration (*e.g.* penta- and hexa-peptides) or the corresponding segment condensation often remain problematic.[1c,2] For this reason, such systems represent good models for evaluating the usefulness of newly developed coupling methods. HOBt- and, more importantly, HOAt-derived coupling reagents,[3] have been evaluated and recommended as efficient tools for solving some of the most intractable problems such as reaction rate, degree of epimerization, and extent of oligomerization.[4]† Various authors have compared the efficiency of condensation reagents derived from HOBt (*e.g.* TBTU, HBTU), HOAt (*e.g.* HATU, HAPyU) and phosphoric and phosphonic acids (*e.g.* DPPA, T3P[5]) in cyclization reactions and have confirmed the superiority of the HOBt-[6] and, especially, HOAt-based reagents[4] with regard to these problems.



HAPyU


T3P

For peptide cyclization involving sterically hindered α,α-dialkyl- or *N*-methyl-amino acids at the *N*-terminal position there has been little systematic effort to uncover the important factors which influence the efficiency of the cyclization process. For loss of configuration during ordinary segment coupling it has long been known that increasing steric hindrance in the amino component correlates with increasing loss of configuration.[7] In the case of cyclization of the four possible linear precursors which lead to cyclo(Aib-Phe-D-Pro-Ala), only the isomer bearing N-terminal Ala gave the cyclopeptide in significant amount (44%).[8] The other three isomers cyclized in only trace amounts (2–3%), suggesting that factors other than steric effects at the N-terminal position are important. Although some investigators have deliberately avoided attempting cyclization at an N-Me amino acid, for example in the case of cyclosporin,[9] others have reported effective depsipeptide cyclization at N-terminal sites occupied by MeLeu (oxime ester, > 48%;[10] BOP-Cl, 87%[11]) or MeVal (acid chloride, 25%[12]). Since none of these previously reported systems involved the difficult all-L-configuration, the present study was initiated.

In the course of segment condensation studies leading to the ACP decapeptide (acyl carrier protein 74-65) one of us (P. H.) showed the effectiveness of 2-propanephosphonic acid anhydride (T3P) as a condensation reagent. This result, along with Wenger's demonstration[9] that T3P and the BOP reagent were equally effective in the cyclosporin case (65% cyclization), led to a reexamination of the T3P reagent in segment condensations and head-to-tail cyclizations of all-L-pentapeptides, especially those bearing sterically hindered amino acids at the cyclization site. Whereas comparison of HAPyU, a reagent which we had previously shown to be one of the most effective coupling reagents for peptide cyclization,[4] and T3P in ordinary [2 + 1] segment condensations in the presence of Pr^i_2NEt for the model tripeptide Z-Phe-Val-Pro-NH_2 showed far more epimerization for the T3P case (29.7 *vs.* 10.8%) along with a lower yield (58.6 *vs.* 88.8%), upon substitution of the more hindered H-Aib-NH_2 for H-Pro-NH_2 the loss of configuration for T3P, while significant, was substantially less than for HAPyU (14.6 *vs.* 41.1%) (Table 3).‡ However, under the normal conditions for HAPyU reactions, the T3P reaction was extremely slow.

In order to evaluate the cyclization utility of T3P, we studied the ring closure of several linear pentapeptides (model peptides **1**, **4**–**7** and thymopoietin-derived sequences **2** and **3**) by analytical HPLC using T3P and HAPyU as condensation reagents. For pentapeptide **1** optimization studies were carried out with T3P under various reaction conditions (temperature,

1 AANMeAAA
2 R(Ac)KAVY [SP5]
3 R(Ac)K(Hmb)AVY [(Hmb)Ala[3]-SP5]
4 AibAAAA
5 (*N*-Me)AAAAA
6 (*N*-Me)FAAAA
7 (Tmob)AAAAA

Table 1 AA(*N*-Me)AAA cyclization in DMF (after 24 h reaction)

Entry	Coupling reagent	P:B:CR[a]	Yield (%) all-L-CM[b]	AA(*N*-Me)-AAa CM[b]	all-L-CD[c]
1	HAPyU	1:3:1.1	55	1.6	33
2	T3P	1:6:5	70	2.9	5
3[d]	T3P	1:6:5	65	2.2	10
4	T3P	1:6:1.5	73	9.3	7
5	T3P	1:3:1.5	66	10	8
6[e]	T3P	1:6:5	52	5.9	16

[a] P = linear peptide, B = base (Pr^i_2NEt), CR = coupling reagent; 10^{-3} M. [b] Cyclic monomer. [c] Cyclic dimer. [d] Temperature at the beginning of the reaction = 0 °C. [e] 10^{-2} M.

Table 2 Cyclization of N-terminally sterically hindered peptides in DMF

	Yield (%) after 80 h			
	HAPyU cyclization[a]		T3P cyclization[b]	
Sequence	all-L-CM[c]	epimerized CM[c,d]	all-L-CM[c]	epimerised CM[c,d,e]
AibAAAA	22	64	38	2
(*N*-Me)AAAAA	4	69	24	5
(*N*-Me)FAAAA	1	87	15	6
(Tmob)AAAAA	13	59	6	22

[a] For conditions, see Table 1, entry 1. [b] For conditions, see Table 1, entry 2. [c] Cyclic monomer. [d] Due to C-terminal epimerisation of the all-L precursor during reaction. [e] After 80 h, 40–60% linear peptide (both epimerized and unepimerized) was still detectable.

concentration, excess of reagent and base) and the results compared with the best results previously obtained with HAPyU. In the case of all peptides which lack a sterically demanding amino acid in the N-terminal position (Table 1), differences between T3P- and HAPyU-mediated reactions are not significant, although for the cyclization of AA(*N*-Me)AAA at 10^{-3} M concentration, regardless of reaction conditions, T3P gives a higher yield of the all-L-cyclic monomer than the corresponding HAPyU reagent (Table 1). Under the best conditions T3P led to a level of epimerization (2.2%) which is only slightly higher than that observed with HAPyU (1.6%). For the T3P case, the degree of epimerization appears to depend more on the excess of condensation reagent and concentration rather than an excess of base (Table 1). The rate of T3P-mediated cyclization is comparable to that previously observed for HAPyU; after approximately 2 min the reaction is about 90% complete in all cases. In accordance with previous results for HAPyU (J. Klose, unpublished) T3P-induced cyclization at lower temperatures leads to significantly enhanced cyclodimerization (Table 1). The cases of SP5 and (Hmb)Ala[3]-SP5 are very similar for the two activators in that comparable amounts of all-L-cyclic monomer are formed with T3P leading to somewhat less epimerization (data not shown). In all three cases significantly larger amounts of the undesired all-L-cyclodimer is formed for the HAPyU system.

Whereas for these cyclization reactions involving relatively unhindered amino acids no clear superiority of T3P can be shown, the situation is drastically different in the case of cyclization *via* peptide bond formation at a sterically hindered amino acid (Table 2). Here, HAPyU-mediated cyclization yields almost exclusively the C-terminally epimerized cyclic monomer (59–87%).§ In contrast, T3P-promoted reactions yield the desired all-L-cyclic monomer as the major product, even though the overall yield of cyclic product is not high due to the sluggishness of the reaction, which was allowed to proceed for 80 h. Even at that point cyclization was incomplete. As expected, the tendency to give the all-L-cyclic monomer is lower the more hindered is the N-terminal substituent (increasing from **4** to **7**) and formation of all-L-cyclic product fails almost completely for the sterically most demanding Tmob-residue. The differences observed with regard to C-terminal stereomutation are reminiscent of the differences seen in comparison of HBTU and HAPyU or HATU, where the former leads to extensive C-terminal epimerization.[2,4]

In summary, the data presented show that T3P is at least as effective as HAPyU in the relatively demanding cyclization of all-L-pentapeptides, and remarkably more efficient for systems having a sterically hindered amino acid, such as Aib, (*N*-Me)Ala and (*N*-Me)Phe, at the cyclization site. Whereas the HAPyU-promoted cyclization yields the C-terminally epimerized product almost exclusively, the use of T3P gives the stereo-retained cyclic monomer as the main product, albeit in rather low yield.

Table 3 [2 + 1] Segment condensation of Z-Phe-Val-Aib-NH_2[a]

Coupling reagent (equiv.)	Base (equiv.)	Residue yield[b] (%)	LD (%)[c]	Yield of LL-, LD-tripeptide[d] (%)
HATU (1)	Pri_2NEt (2)	82.9	40.3	100
HATU (1)	TMP (2)	74.6	31.0	100
HATU (1)[e]	Pri_2NEt (2)	95.2	41.5 (LL)	100
HAPyU (1.1)	Pri_2NEt (3)	82.2	41.1	100
HAPyU (1.1)	TMP (3)	74.6	34.0	100
T3P (5)[f]	Pri_2NEt (6)	34.8	14.6	5.3
T3P (5)[f]	TMP (6)	33.3	13.1	3.1
T3P (5)[g]	Pri_2NEt (6)	48.1	22.3	3.2
T3P (5)[g]	TMP (6)	43.1	16.6	7.0
T3P (5)[f]	DMAP (6)	54.7	11.6	4.9

[a] Test couplings were carried out by adding the base to a stirred and ice-cooled solution of Z-Phe-Val-OH, H-Aib-NH_2 and the coupling reagent (each 1 equiv., 0.125 M in DMF). The mixture was stirred at 0 °C for 1 h at room temperature overnight. Work-up was carried out as described in L. A. Carpino, D. Ionescu and A. El-Faham, *J. Org. Chem.*, 1996, **61**, 2463, Table 1, footnote *a*. [b] Calculated based on the weight of the crude product. [c] Pure LL or LD peptides were co-injected onto HPLC to determine epimerization by comparison of retention times. [d] Calculated based on integration of the LL- and LD-tripeptide peaks relative to all other HPLC peaks. [e] Z-Phe-D-Val-OH was used in this case. [f] 24 h reaction time. [g] 90 h reaction time.

Notes and references

† Abbreviations: Aib = aminoisobutyric acid; DPPA = diphenylphosphoryl azide; HAPyU = 1-[3-oxido-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ium-1-yl(pyrrolidin-1-yl)methylene]pyrrolidinium hexafluorophosphate; HATU = *N*-[3-oxido-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ium-1-yl(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate; HBTU = *N*-[3-oxido-1*H*-benzotriazol-3-ium-1-yl(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate; Hmb = 2-hydroxy-4-methoxybenzyl; HOAt = 1-hydroxy-7-azabenzotriazole; HOBt = 1-hydroxybenzotriazole; TBTU = *N*-[3-oxido-1*H*-benzotriazol-3-ium-1-yl-(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate; Tmob = 2,4,6-trimethoxybenzyl; TMP = 2,4,6-trimethylpyridine; T3P = PPA = 2-propanephosphonic acid anhydride.

‡ Segment condensations were performed as outlined in Table 3.

§ Generally, the amount of C-terminal epimerization was determined by analytical HPLC (cyclization of the authentic C-terminally D-Ala-containing analogues was carried out to give the authentic D-Ala cyclomonomers for establishment of the HPLC retention times) and examination of the products by ES-MS.

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