Cyclization of *all*-L-Pentapeptides by Means of 1-Hydroxy-7-azabenzotriazole-Derived Uronium and Phosphonium Reagents

Angelika Ehrlich,* Hans-Ulrich Heyne, Rüdiger Winter, Michael Beyermann, Hanka Haber, Louis A. Carpino,† and Michael Bienert

Research Institute of Molecular Pharmacology, Alfred-Kowalke-Strasse 4, D-10315 Berlin, Germany, and Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

Received June 20, 1995 (Revised Manuscript Received September 18, 1996[⊗]*)*

Due to their restricted conformational flexibility, cyclic peptides are of great interest in connection with structure−activity relationships, especially the elucidation of bioactive conformations. For linear peptides that do not contain turn structure-inducing amino acid residues, the cyclization reaction may be an inherently improbable or slow process, and side reactions, such as cyclodimerization and epimerization at the C-terminal residue, may dominate. A number of 1-hydroxy-7-azabenzotriazole-based onium salts were examined for cyclization of thymopentin-derived pentapeptides and the results compared with data from more conventional coupling reagents. The azabenzotriazol-derived coupling reagents stood out as being the most effective by far. The cyclizations proceed extremely rapidly, and in contrast to other coupling reagents, C-terminal epimerization was generally less than 10%. C-terminal D-amino acid residues favor the formation of monocyclic pentapeptide rings. A similar effect was observed for cyclization of linear *N*-methylamino acid-containing peptides, suggesting that reversible amide bond alkylation such as Hmb-modification should be useful in promoting the cyclization of pepitdes devoid of turn-inducing amino acid residues.

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 ease of cyclization is often enhanced by the presence of turn structure-inducing amino acids such as glycine, proline, or a D-amino acid.[1−3] For linear peptides that do not contain amino acid residues that 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).[4] For such slow cyclizations the increased lifetime of the intermediate activated linear peptide provides an opportunity for increased epimerization at the C-terminal residue. The extent of epimerization may be diminished by application of the azide method[5] or its modification using DPPA.[6,7] However, these methods are extremely slow, usually requiring many hours or even several days.[5,8,9] In comparison with DPPA, TBTU[10] and BOP[11] provide for fast cyclization[12] but may also lead to C-terminal epimerization levels that are comparable to those observed with DCC/HOBt.[13]

Recently, a number of new peptide coupling reagents (Figure 1) derived from HOAt[14,16] have shown advantages over the corresponding HOBt-based reagents for solution- and solid-phase peptide synthesis with regard to both speed and maintenance of chiral integrity.[17] Application of these onium reagents to the cyclization of a linear GnRH-derived decapeptide showed that they were also highly effective for head-to-tail and side chain cyclization.[18] These reagents led to complete cyclization within less than 30 min, whereas standard reagents such as TBTU, TOPPipU,[19] and DPPA caused cyclization under the same conditions to the extent of only 60, 10, and 12%, respectively.[18]

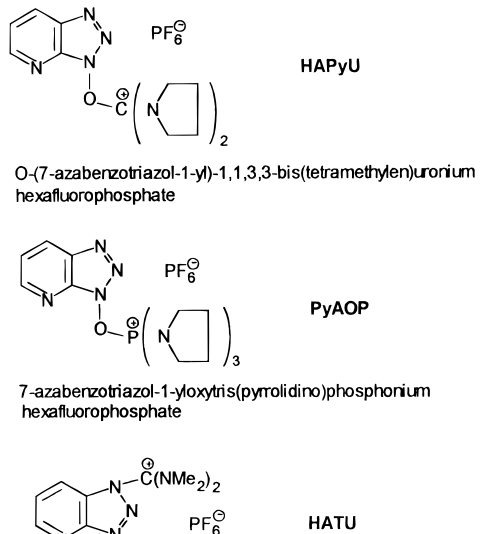


Figure 1. HOAt-derived coupling reagents used in this study.

Although azabenzotriazole-based coupling reagents are not expected to overcome difficulties arising from unfavored conformations of short, linear peptides, the observed high coupling efficiency prompted a reexamination of the cyclization of *all*-L-penta- and -hexapeptides.

† University of Massachusetts.

⊗ Abstract published in *Advance ACS Abstracts,* November 1, 1996.

(1) Dale, J. *Angew. Chem.* **1966**, *78*, 1070.
(2) Kessler, H.; Kutscher, B. *Liebigs Ann. Chem.* **1986**, 869.
(3) Kessler, H.; Kutscher, B. *Liebigs Ann. Chem.* **1986**, 914.
(4) Kopple, K. D. *J. Pharm. Sci.* **1972**, *61*, 1345.
(5) Izumiya, N.; Kato, T.; Waki, M. *Biopolymers* **1981**, *20*,1785.
(6) Brady, S. F.; Paleveda, W. J.; Arison, B. H.; Freidinger, R. M.; Nutt, R. F.; Veber, D. F. In *Peptides. Structure and Function*. Proceedings of the 8th American Peptide Symposium; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Company: Rockford, 1983; p 127.

S0022-3263(95)01108-X CCC: $12.00

As models the thymopentin-derived peptides **1**, **2**, and **3** were chosen since their cyclization is not easily achieved.

H-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-OH (1)

H-Arg(Tos)-Lys(Z)-Asp(OBn)-Val-Tyr(Bn)-OH (2)

H-Val-Arg(H^+)-Lys(Ac)-Ala-Val-Tyr-OH (3)

Peptides **1** and **3** gave by the DCC/DMAP technique[2,3] the corresponding cyclic peptides, although in both cases the C-terminal tyrosine unit had been completely epimerized to the D-configuration. In the case of peptide **2** the desired cyclic peptide was obtained but the azide coupling process was very slow (1 week) and the yield was low.[9]

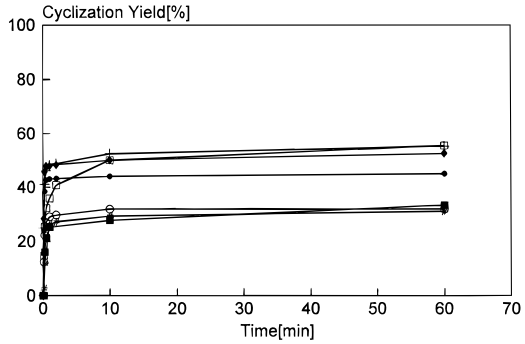


Figure 2. Cyclization of thymopentin analogs via HAPyU: ◆, H-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-OH; +, H-Arg-Lys-(Ac)-Asp(OBn)-Val-Tyr-OH; ●, H-Arg-Lys(Z)-Ala-Val-Tyr-OH; □, H-Arg-Lys(Ac)-Asp(OBn)-Val-Tyr-OH; #, H-Arg-Lys(Ac)-Ala-Val-Tyr-OH; ■, H-Arg-Lys(Ac)-Asn-Val-Tyr-OH; ○, H-Arg-Lys(Z)-Ala-Val-Tyr-OH.

Results and Discussion

Initially, a number of HOAt-based onium salts were examined for cyclization of pentapeptides **1** and **2**, and the results compared with data from more conventional coupling reagents. The HOAt-derived coupling reagents stood out as being the most effective by far, causing conversion of **1** or **2** to the corresponding cyclopeptides within 10 min in yields of 53−55% (Figure 2, Tables 1 and 2). The monocyclic structures of the isolated peptides were confirmed by ES-MS and NMR 2D sequential cross peaks.

Investigation of the chiral integrity of the cyclic products by means of GC−MS using a Chirasil-L-Val column revealed that cyclization was not accompanied by significant inversion of configuration of the activated tyrosine residue. In contrast to the DCC/DMAP case,[2,3] generally less than 10% of the D-Tyr isomer was detected in the crude products (Tables 1 and 2). In the case of *all*-L-pentapeptides **1** and **6** the less active reagents TBTU and BOP induce significantly higher degrees of epimerization (Tables 1 and 2; Figures 3 and 4).[20] The HAPyU-mediated cyclization proceeds extremely rapidly, with about 90% of the cyclization products being formed within 1 min after addition of the coupling reagent. Similarly rapid cyclizations were observed with PyAOP and HATU. Since the onium moieties in TBTU and PyBOP on the one hand, and those of HATU and PyAOP on the other, are identical, the differences in reactivity of the two series points again[18] to the favorable influence of the OAt residue on the cyclization process.[22] On the

(7) *Abbreviations:* AcOH, acetic acid; AcHmb, 2-acetoxy-4-methoxybenzyl; BOP, (1-benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate; DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DIEA, diisopropylethylamine; DMF, dimethylformamide; DPPA, diphenylphosphoryl azide; Hmb, 2-hydroxy-4-methoxybenzyl; HOBt, 1-hydroxybenzotriazole; HOAt, 1-hydroxy-7-azabenzotriazole; HAPyU, O-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate; HATU, *N*-[(dimethylamino)-*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate; PyAOP, (7-azabenzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate; PyBOP, (benzotriazol-1-yloxy)trispyrrolidinophosphonium 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; TFA, trifluoracetic acid; TFE, trifluoroethanol; TOPPipU, 2-(2-oxopyrid-1-yl)-1,1,3,3-pentamethylenuronium tetrafluoroborate.

(8) Schmidt, R.; Neubert, K. *Int. J. Peptide Protein Res.* **1991**, *37*, 502.

(9) Heavner, G. A.; Audhya, T.; Doyle, D.; Tjoeng, F. S.; Goldstein, G. *Int. J. Peptide Protein Res.* **1991**, *37*,198.

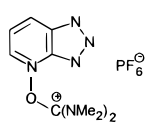
(10) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.

(11) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *14*, 1219.

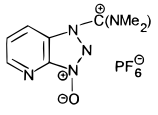
(12) (a) Felix, A. M.; Wang, Ch.-T.; Heimer E. P.; Fournier, A. *Int. J. Peptide Protein Res.* **1988**, *31*, 231−238. (b) Zimmer, S.; Hoffmann, E.; Jung, G.; Kessler, H. In *Peptides 1992*. Proceedings of the 22nd European Peptide Symposium; Schneider, C. H., Eberle A. N., Eds.; ESCOM: Leiden, 1993; p 393.

(13) Benoiton, N. L.; Lee, Y. C.; Steinauer, R.; Chen, F. M. F. *Int. J. Peptide Protein Res.* **1992**, *40*, 559.

(14) Recently, X-ray analysis[15] has shown that HATU crystallizes as the guanidinium salt shown in Figure 1 rather than as of the isomeric O-substituted form i previously assigned to this compound. HBTU ii was also shown to crystallize in guanidinium rather than the uronium form. For analogous coupling reagents (HAPyU, TAPipU, TBPipU, TBTU) for which X-ray data have not yet been obtained the traditional structural representations and nomenclature have arbitrarily been retained in the present communication. To date there is no evidence concerning the possibility of equilibration in solution between the O- and N-forms of these compounds.



i



ii

(15) Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. *Lett. Pept. Sci.* **1994**, *1*, 57.

(16) (a) Azev, Y.; Mokrushina, G. A.; Postovoskii, I. Ya.; Sheinker, Yu. N.; Anisimova, O. S. *Chem. Heterocycl. Compd.* **1976**, 1172. (b) Mokrushina, G. A.; Azev, Y.; Postovoskii, I. Ya. *Chem. Heterocycl. Compd.* **1975**, 880. (c) Azev, Y.; Mokrushina, G. A.; Postovoskii, I. Ya. *Chem. Heterocycl. Compd.* **1974**, 687. (d) Sacher, R. M.; Alt, G. H.; Darlington, W. A. *J. Agric. Food Chem.* **1973**, *21*, 132.

(17) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397. (b) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.

(18) Ehrlich, A.; Rothemund, S.; Brudel, M.; Beyermann, M.; Carpino, L. A.; Bienert, M. *Tetrahedron Lett.* **1993**, *34*, 4781.

(19) Henklein, P.; Beyermann, M.; Bienert, M.; Knorr, R. In *Peptides 1990*, Proceedings of the 21st European Peptide Symposium; Giralt, E., Andreu, D., Eds.; ESCOM: Leiden, 1991; p 67.

(20) The cyclization of various penta- and hexapeptides via HBTU was recently described (ref 21) as yielding 2−21% of the cyclopeptides after reaction times of 2−16 h. The stereochemical integrity of the activated amino acid residues was not discussed. Considering our results with TBTU (Figures 5 and 6; Table 1), it would be expected that considerable C-terminal epimerization would also occur in the case of HBTU.

(21) Sowemimo, V.; Scanlon, D.; Jones, P.; Craik, D. J. *J. Protein Chem.* **1994**, *13*, 339.

(22) It is expected that the use of HOAt-derived coupling reagents would also improve the cyclization of resin-bound peptides. In fact, HATU was found to give better results than BOP in the final head-to-tail cyclization of a resin-bound pentapeptide library constructed in four variable positions from glycine, proline, arginine, and three nonspecified amino acids.[23] Similarly, in side-chain to side-chain cyclizations HAPyU and HATU gave far better yields of the desired cyclic peptides than HBTU. However, in contrast to peptide cyclization in solution as described in the present communication, HATU-, HAPyU-, and HBTU-mediated cyclizations of resin-bound peptides are reported to be accompanied by significant amounts of alkylguanidinium peptides formed by reaction of the uronium reagent with the free amino group.[24]

Table 1. Cyclization of H-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-OH, 1. Influence of Coupling Reagents, Base, and Peptide Concentration on (a) Formation of Cyclomonomer vs Cyclodimer and (b) Epimerization of the Cyclomonomer C-Terminal Tyrosine Residue As Determined after a Reaction Time of 1 h by GC−MS Analysis

cyclomonomer[a] (%)	D-Tyr-isomer (%)	cyclodimer[a] (%)	reagent	base	concn of linear peptide (mM)
25	8.0[a]	40	HAPyU	3 equiv of DIEA	10
55	8.8	25	HAPyU	3 equiv of DIEA	1
82	nd	2	HAPyU	3 equiv of DIEA	0.1
13[b]	nd	nd	HAPyU	3 equiv of coll.	1
60	8.7	16	HAPyU	1 equiv of DIEA 2 equiv of coll.	1
56	10.9	20	PyAOP	3 equiv of DIEA	1
53	8.3	22	HATU	3 equiv of DIEA	1
50	15.1	22	TBTU	3 equiv of DIEA	1
38	20.2	13	BOP	3 equiv of DIEA	1
52	13.0	17	PyBOP	3 equiv of DIEA	1
4.3[c]	nd	nd	DPPA	2 equiv of DIEA	1

[a] Area percentages as determined by analytical HPLC analysis. [b] After 24 h 39% cyclomonomer, 22.5% D-Tyr-isomer, and 9.6% cyclodimer were formed. [c] After 24 h 23% cyclomonomer, 19.8% D-Tyr-isomer, and 7% cyclodimer were formed.

Table 2. HAPyU-Mediated Cyclization of Penta- and Hexapeptides. Influence of Sequence, Side-Chain Protection, and Reversible Backbone Alkylation on (a) Formation of Cyclomonomer vs Cyclodimer and (b) Epimerization of the C-Terminal Tyrosine Residue[a]

	peptides	cyclomonomer[b] (%)	cyclodimer[b] (%)	D-Tyr-isomer[b] (%)	concn of linear peptide (mM)
2	H-Arg(Tos)-Lys(Z)-Asp(OBn)-Val-Tyr(Bn)-OH	53	25	6.2[c]	1
3	H-Val-Arg(H^+)-Lys(Ac)-Ala-Val-Tyr-OH	25	42	7.0	1
4	H-Arg(H^+)-Lys(Z)-Asp(OBn)-Val-Tyr-OH	43	33	7.0	1
5	H-Arg(H^+)-Lys(Ac)-Asp(OBn)-Val-Tyr-OH	55	17	14.0	1
6	H-Arg(H^+)-Lys(Ac)-Ala-Val-Tyr-OH	0	35	7.0	10
		33	38	8.4[c]	1
7	H-Arg(H^+)-Lys(Z)-Ala-Val-Tyr-OH	32	56	nd	1
8	H-Arg(H^+)-Lys(Ac)-Asn-Val-Tyr-OH	33	43	7.0	1
		70	16	7.0	0.1
9	H-Arg(H^+)-Lys(Ac)-Ala-Val-D-Tyr-OH	80	nd	8.3[c]	1
10	H-Arg(H^+)-Lys(Ac)-MeGly-Val-Tyr-OH	76	11	nd	1
11	H-Arg(H^+)-Lys(Ac)-MeAla-Val-Tyr-OH	60	13	5.2[c]	1
12	H-Arg(H^+)-Lys(Ac)-(Hmb)Ala-Val-Tyr-OH	64	nd	8.6[c]	1

[a] All cyclizations were carried out in the presence of 3 equiv of DIEA for 1 h. [b] Determined by HPLC analysis except where indicated otherwise. [c] Determined by GC−MS.

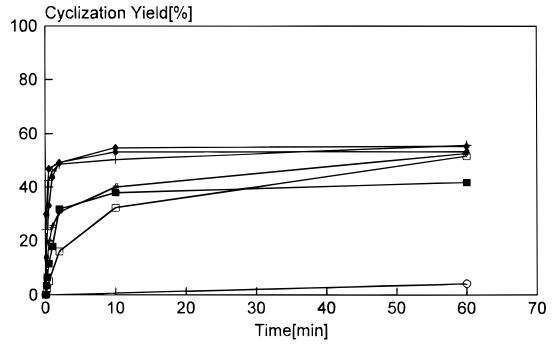


Figure 3. Time course for cyclization of H-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-OH, **1**. Comparison of different coupling reagents. Data in parentheses refer to C-terminal epimerization, as determined by GC−MS: ◆, HAPyU (8.7%); +, PyAOP (10.9%); ●, HATU (8.3%); □, TBTU (15.1%), #, PyBOP (13.0%), ■, BOP (20.2%); ○, DPPA (19.8% after 24 h).

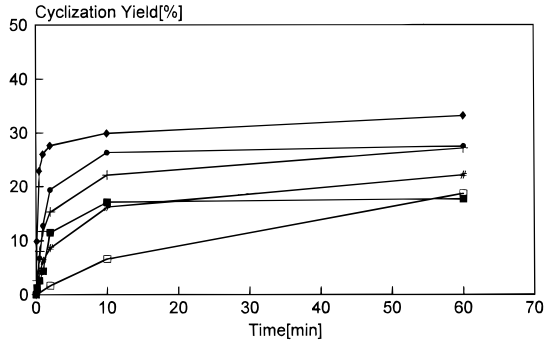


Figure 4. Time course for cyclization of H-Arg(H^+)-Lys(Ac)-Ala-Val-Tyr-OH, **6**. Comparison of different coupling reagents. Data in parentheses refer to C-terminal epimerization, as determined by GC−MS: ◆, HAPyU (8.4%); +, PyAOP (8.7%); ●, HATU (8.6%); □, TBTU (16.1%); #, PyBOP (16.8%); ■, BOP (20.7%).

other hand, for some peptides, e.g., (Figure 4), the differences among HAPyU, HATU, and PyAOP, with HAPyU providing a significantly faster cyclization, suggest that other factors are also involved. Whether some intermediate formed prior to the OAt ester is responsible for the exceptional speed of the reaction and the reduced loss of chirality remains to be elucidated. Since TBTU leads to the formation of only 10−20% of the cyclopeptide within 1 min (Figure 3), the intermediacy of less reactive species (OBt ester, oxazolone) may account for the enhanced stereomutation at the C-terminal amino acid residue.

The superiority of coupling reagents derived from HOAt for promoting peptide cyclization quickly and with a minimum of stereomutation was also demonstrated in

(23) Darlak, K.; Romanovskis, P.; Spatola, A. F. In *Peptides, Chemistry, Structure and Biology*, Proceedings of the 13th American Peptide Symposium 1993 Hodges, R. S., Smith, J. A., Eds.; ESCOM: Leiden, 1994; p 981.

(24) Story, S. C.; Aldrich, J. V. *Int. J. Peptide Protein Res.* **1994**, *43*, 292. Arttamanykul, S.; Leelasvatanakij, L.; Aldrich, J. V. 23rd Eur. Pept. Symp., Braga, Portugal, 1994, poster P76.

the cyclization of an *all*-L-hexapeptide. Cyclization of **3** with the two HOBt-based reagents BOP and TBPipU[25] gave only 5−10% of the desired cyclopeptide, whereas with the HOAt-based reagents HAPyU and TAPipU a moderate yield (25%) was obtained.[18] In addition, the extensive inversion at the C-terminal tyrosine residue observed in the case of BOP (24%)[18] is significantly diminished with HAPyU (7%) (Table 2) as demonstrated by HPLC analysis of the reaction mixture.

In an attempt to suppress further the racemization observed in the HAPyU-mediated formation of cyclic pentapeptides, DIEA was replaced by the less basic tertiary amine collidine, which has been shown to diminish racemization in both solution- and solid-phase synthesis of peptide segments.[26] Unexpectedly, in the presence of collidine, the HAPyU-mediated cyclization of peptide **1** was very slow, allowing the activated linear intermediate to undergo C-terminal inversion thereby leading to the formation of up to 25% of the D-Tyr-isomer. On the other hand, use of 1 equiv of DIEA and 2 equiv of collidine permit efficient cyclization of **1** within 1 h (Table 1). Although this technique reduced the amount of cyclodimer formed relative to the case involving the use of 3 equiv of DIEA, the amount of C-terminal epimerization (about 8%) was not significantly affected.

Cyclization of **1** is accompanied by formation of 20−25% of the cyclodimer when the reaction is performed at a peptide concentration of 1 mM, whereas dimer formation is reduced to only 2% (80% cyclomonomer) at 0.1 mM. These results contrast with those obtained in the HAPyU-assisted head-to-tail cyclizations of GnRH-decapeptides (80−90 % cyclomonomer), which proceeded smoothly without cyclodimer formation even at high peptide concentration[18] (100 and 10 mM).

Although the use of HAPyU allows for the first time efficient ring closure of thymopentin sequences **1** and **2**, the results presented are not inconsistent with the view that peptide conformation strongly influences the probability of cyclization. Initial attempts to cyclize the human splenin-derived pentapeptide **6**[18] using HAPyU at a peptide concentration of 10 mM resulted predominantly in the formation of the corresponding cyclodimer with only 7% of the monocyclic D-Tyr-isomer being formed. Even at peptide concentrations of 1 mM the formation of the monocyclic ring is less effective (33%) than for the pentapeptides **1** and **2** (>50%). These differences might be caused at least in part by variations in the side-chain protecting groups. Structural differences in the side chains of the arginine, lysine, and tyrosine residues of peptides **1**, **2**, **4**, and **5** do not significantly influence either the cyclization rate or the cyclomonomer/cyclodimer ratio, whereas in the case of peptides **6**−**8**, which lack a side chain benzyl group at position 3, the yields of the corresponding cyclomonomers are strikingly diminished. However, on the basis of the present results it is not possible to decide whether this effect is caused by the lack of space-filling protecting groups or by changes in the amino acid sequence (Asp → Ala or Asn) (see Figure 2 and Tables 1 and 2). As noted earlier,[2−4] the incorporation of turn-inducing amino acids favors the formation of monocyclic pentapeptide rings.

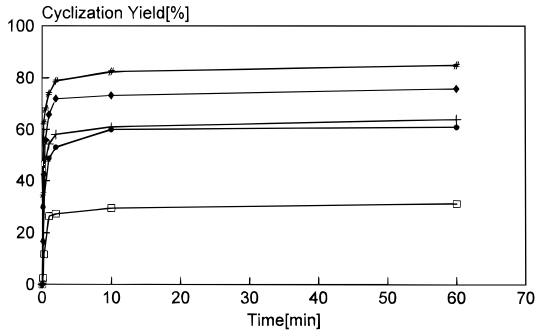


Figure 5. Influence of *N*-methylamino acid substitution and reversible backbone alkylation (Hmb) in position 3 on the HAPyU-assisted cyclization of H-Arg(H^+)-Lys(Ac)-Ala-Val-Tyr-OH, **6**, 1 mM in DMF: ◆, MeGly[3]; +, HmbAla[3]; ●, MeAla[3]; □, Ala[3]; #, Ala[3], D-Tyr[5].

Scheme 1

13 (Hmb) **14** (AcHmb)

H-Ala(Hmb)-Val-Tyr(t-Bu)-O-Trt(Cl)Resin

15 — Fmoc-Lys(Ac)-OH/ EDCI/HOBt/DIEA overnight

Fmoc-Lys(Ac)-Ala(Hmb)-Val-Tyr(t-Bu)-O-Trt(Cl)Resin

16 — standard peptide assembly

Fmoc-Arg(Pmc)-Lys(Ac)-Ala(Hmb)-Val-Tyr(t-Bu)-OTrt(Cl)Resin

17 — 1) Ac_2O (10 eq)/DIEA (10 eq)/16 h 2)TFA/C_6H_5SMe/m-cresol/3 h

Fmoc-Arg(H^+)-Lys(Ac)-Ala(AcHmb)-Val-Tyr-OH

18 — Piperidine/DMF/15 min

H-Arg-(H^+)-Lys(Ac)-Ala(Hmb)-Val-Tyr-OH

12

Thus, changing the C-terminal configuration of peptide **6** (to peptide **9**) speeds up the cyclization significantly (Table 2, Figure 5), thus supporting the data of Kessler et al.[3,27] A similar effect was observed for cyclization of the linear *N*-methylglycine- or *N*-methylalanine-containing peptides **10** and **11** (Table 2), suggesting that reversible amide bond alkylation[29−31] should be useful in promoting the cyclization of peptides devoid of turn-inducing amino acid residues. Indeed, for (Hmb)Ala-substituted-

(25) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Peptides 1990*, Proceedings of the 21st European Peptide Symposium; Girald, E., Andrew, D., Eds.; ESCOM: Leiden, 1991; p 62.

(26) (a) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695. (b) Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279.

(27) Naturally, the effect of changing the C-terminal amino acid from the L- to the D-configuration is negligible or at least less pronounced when, for whatever reasons, the L-isomer itself undergoes rapid cyclization. Thus, Feiertag et al.[28] found no influence of the C-terminal amino acid configuration on the cyclization of hexapeptides RGDSPX, which gave the cyclized peptides in high yield (80%) even for the *all*-L-peptides.

(28) Feiertag, S.; Wiesmüller, K.-H.; Nicholson, G. J.; Jung, G. In *Peptides: Chemistry, Structure and Biology*, Proceedings of the 14th American Peptide Symposium 1995 Kaumaya, P. T. P., Hodges, R. S., Eds.; ESCOM: Leiden, 1996, in press.

(29) Blaakmeer, J.; Tijsse-Klasen, T.; Tesser, G. I. *Int. J. Peptide Protein Res.* **1991**, *37*, 556.

(30) Johnson, T.; Quibell, M. *Tetrahedron Lett.* **1994**, *35*, 463.

(31) Quibell, M.; Turnell, W. G.; Johnson, T. *Tetrahedron Lett.* **1994**, *35*, 2237.

Table 3. HPLC-Retention Times and ES-MS Data of Linear and Cyclized Peptides

	linear peptide			cyclomonomer			cyclodimer		
	ES-MS		HPLC t_R	ES-MS		HPLC t_R	ES-MS		HPLC t_R
peptides	calcd	found	(min)	calcd	found	(min)	calcd	found	(min)
1	949.04	949.5	15.90	931.02	931.5	20.90	1862.04	1863.0	27.05
2	1148.40	1148.6	25.62	1130.30	1130.3	30.03	2260.60	2261.0	36.70
3	776.94	778.0	14.12	758.93	759.8	20.98	1517.86	1519.5	25.73
4	904.00	904.8	14.02	886.00	886.8	18.70	1772.00	1772.5	24.45
5	811.90	812.0	25.87	793.90	794.3	32.13	1587.80	1689.0	35.30
6	677.80	678.7	11.27	659.80	660.6	19.78	1319.60	1320.4	24.77
7	769.90	770.6	6.27	751.90	752.7	11.55	1503.80	1505.0	18.62
8	720.80	721.0	9.88	702.80	703.3	16.17	1405.60	1406.0	22.68
9	677.80	678.7	13.78	659.80	660.6	15.18	1319.60	nd	nd
10	677.80	678.7	11.42	659.80	660.2	16.63	1319.60	1320.0	25.80
11	691.83	692.7	13.67	673.82	674.6	19.50	1347.64	1348.5	23.40
12	813.96	814.8	23.75	795.94	796.8	29.45	1591.88	nd	nd

Table 4. Amino Acid Analysis of Cyclized Pentapeptides

		amino acid analysis				
		Arg	Lys	Xxx	Val	Tyr
	cyclopeptides	found				
1	cyclo[-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-]	0.82	0.98	1.01	1.0	0.56
2	cyclo[-Arg(Tos)-Lys(Z)-Asp(OBn)-Val-Tyr(Bn)-]	0.94	0.98	0.99	1.0	0.67
3	cyclo[-Val-Arg(H^+)-Lys(Ac)-Ala-Val-Tyr-]	0.83	1.0	1.08	1.78	0.9
4	cyclo[-Arg(H^+)-Lys(Z)-Asp(OBn)-Val-Tyr-]	0.99	0.99	1.18	1.0	0.42
5	cyclo[-Arg(H^+)-Lys(Ac)-Asp(OBn)-Val-Tyr-]	0.98	1.0	1.01	1.0	0.78
6	cyclo[-Arg(H^+)-Lys(Ac)-Ala-Val-Tyr-]	0.97	1.06	0.98	1.0	0.53
7	cyclo[-Arg(H^+)-Lys(Z)-Ala-Val-Tyr-]	0.97	1.01	0.93	1.0	0.34
8	cyclo[-Arg(H^+)-Lys(Ac)-Asn-Val-Tyr-]	0.96	1.04	1.21	1.0	0.39
9	cyclo[-Arg(H^+)-Lys(Ac)-Ala-Val-D-Tyr-]	0.94	1.0	0.9	0.95	0.98
10	cyclo[-Arg(H^+)-Lys(Ac)-MeGly-Val-Tyr-]	0.96	1.02	1.01	1.0	0.45
11	cyclo[-Arg(H^+)-Lys(Ac)-MeAla-Val-Tyr-]	1.02	1.04	nd	1.0	0.53
12	cyclo[-Arg(H^+)-Lys(Ac)-(Hmb)Ala-Val-Tyr-]	0.99	1.09	1.0	1.0	0.28

peptide **12,** the synthesis of which is outlined in Scheme 1, the yield of cyclomonomer is double that obtained from the unmodified sequence **6** (Table 1, Figure 5).

Standard methods were used to assemble the tripeptide sequence **15** on a chlorotrityl resin using the 2-hydroxy-4-methoxybenzyl group **13** attached to the α-amino residue of alanine. Because of hindrance toward coupling to a N-substituted amino acid, the next amino acid (Lys) was added by overnight coupling. The final amino acid (Arg) was added normally. Acetylation to stabilize the Hmb residue during removal of the peptide from the resin followed by TFA treatment gave protected pentapeptide **18** that was then deblocked by piperidine at both Fmoc and AcHmb groups to give the desired backbone-protected pentapeptide **12** (Scheme 1). An alternate method of inducing cyclization-prone conformations by reversible N-acylation of the peptide bond via $(Boc)_2O$ was recently reported.[32]

In conclusion, it has been demonstrated that the recently developed HOAt-derived coupling reagents significantly improve the head-to-tail cyclization of *all*-L-penta- and -hexapeptides devoid of turn-inducing amino acids. Although highly effective, these reagents could not fully prevent the formation of cyclodimers or the occurrence of some C-terminal epimerization. The probability of obtaining the cyclomonomer cleanly can be enhanced by application of reversible backbone alkylation. Similar methodology may also be helpful in the synthesis of cyclic peptide libraries.

Experimental Section

General Procedures. Analytical HPLC characterization of all synthesized peptides was carried out on a 5-μm Polyencap A 300 column, 250 × 4 mm. Preparative isolation of peptides was carried out by HPLC on a 10-μm Polyencap A 300 250 × 20 mm column. Peptides were eluted using linear gradients formed as follows: (a) system 1, eluant A, 0.1% TFA in water, and eluant B_1, 0.1% TFA in 50% ACN/50% water (v/v), or (b) system 2, eluant A and eluant B_2, 0.1% TFA in 80% ACN/20% water (v/v).

Amino acid analyses were performed following gas-phase hydrolysis for 20 h at 110 °C. ES-MS was performed by sample flow 1 μL/min methanol/water (1:1), 3−5 kV, 80 °C. ^{1}H-NMR spectra (1 D, 2 D Cosy,TOCSY with spin-lock time 75−80 ms and ROESY with mixing time 150 ms) were recorded at 300 K.

Racemization tests by the GC−MS technique[33] were performed on a Chirasil-Val column, 50 m, 0.25 μm. Hydrolysis was carried out in 6 N DCl/D_2O for 24 h (110 °C), and the hydrolysate was converted to the *N*-(trifluoracetyl)amino acid isopropyl ester.

Reagents. The Fmoc amino acid derivatives, 2-chlorotrityl resin, *p*-alkoxybenzyl resin, and HOBt-derived coupling reagents were purchased from NovaBiochem, Bad Soden/Ts, Germany. The synthesis of HOAt was carried out by methylation of commercially available 2-nitro-3-pyridinol followed by treatment of the resulting methyl ether with excess hydrazine.[17a] HOAt-derived coupling reagents were prepared according to the procedure described by Knorr.[10]

Syntheses of Linear Peptide Sequences. (a) Syntheses of Unprotected Linear Peptides. The syntheses of linear unprotected peptides were carried out manually using a *p*-alkoxybenzyl resin (0.6 mmol/g) by means of Fmoc amino acids and TBPipU[18] as activating agent. All amino acids were coupled using 2 equiv of Fmoc amino acid (0.3 M in DMF), 2.2 equiv of TBPipU, and 4 equiv of DIEA. Coupling times were 30 min. Couplings were monitored by the Kaiser ninhydrin test.[34] The Fmoc group was cleaved by 20% piperidine/DMF for 15 min. After completion of the syntheses the resins were

(32) Cavelier-Frontin, F.; Achmad, S.; Verducci, J.; Jacquier, R. *J. Mol. Struct.* **1993**, 286, 125.

(33) Kusomoto,S.; Matsukura, M.; Shiba, T. *Biopolymers* **1981**, *20*, 1869.

(34) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970** *34*, 595.

Table 5. ^{1}H NMR Signal Assignments and H^N-H^α Coupling Constants (DMSO-d_6, 300 K) of Cyclomonomers, Cyclodimers, and Their D-Tyr-Isomers

		HN	Ha	Hb	others	$^3J_{HN-Ha}$
	(a) Cyclo[-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-], **1a**, Cyclo[-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-D-Tyr-], **1b**, and Cyclo[-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-], **1c**					
1a	Arg[1]	8.26	3.87	1.76	Hγ 1.44; Hδ 3.13; HNε 8.49	8.0
	Lys[2]	7.95	4.01	1.65	Hγ 1.20; Hδ 1.38; Hε 2.95; HNζ 7.21	8.3
	Asp[3]	8.10	4.41	2.91		7.9
	Val[4]	8.05	3.52	2.08	Hγ 0.74/0.61	7.9
	Tyr[5]	8.04	4.21	2.91/2.87	Hδ 6.97; Hε 6.64; HO 9.17	6.0
	Z, OBn				H_2C 4.99, H_2C 5.07 } aromatic 7.25−7.39	
1b	Arg[1]	8.46	3.97	1.77	Hγ 1.44/1.30; Hδ 3.05; HNε 8.36	8.1
	Lys[2]	7.17	4.15	3.08/2.94	Hγ 1.13; Hδ 1.37; Hε 2.94; HNζ 7.20	8.7
	Asp[3]	8.50	4.11	3.08/2.94		7.1
	Val[4]	7.54	4.06	1.70	Hγ 0.70/0.65	8.9
	D-Tyr[5]	8.85	4.29	2.73	Hδ 7.00; Hε 6.62; HO 9.12	7.0
	Z, OBn				H_2C 4.98, H_2C 5.08 } aromatic 7.25−7.38	
1c	Arg[1,6]	7.56	4.23	1.69	Hγ 1.43; Hδ 3.09; HNε 8.50	
	Lys[2,7]	7.93	4.06	1.54/1.47	Hγ 1.24/1.20; Hδ 1.34; Hε 2.94; HNζ 7.18	
	Asp[3,8]	8.49	4.36	3.01/2.79		
	Val[4,9]	7.22	4.14	1.89	Hγ 0.72/0.66	
	Tyr[5,10]	8.27	4.36	3.01/2.79	Hδ 6.96; Hε 6.59; HO 9.11	
	Z, OBn				H_2C 4.98, H_2C 5.06 } aromatic 7.25−7.38	
	(b) Cyclo[-Arg(Tos)-Lys(Z)-Asp(OBn)-Val-Tyr(Bn)-], **2a**, Cyclo[-Arg(Tos)-Lys(Z)-Asp(OBn)-Val-D-Tyr(Bn)-], **2b**, and Cyclo[-Arg(Tos)-Lys(Z)-Asp(OBn)-Val-Tyr(Bn)-Arg(Tos)-Lys(Z)-Asp(OBn)-Val-Tyr(Bn)-], **2c**					
2a	Arg[1]	8.25	3.86	1.72	Hγ 1.34/1.31; Hδ 3.03; HNε 6.80	7.7
	Lys[2]	7.92	4.02	1.64	Hγ 1.20; Hδ 1.38; Hε 2.95; HNζ 7.21	8.4
	Asp[3]	8.10	4.42	2.91		8.0
	Val[4]	8.03	3.55	2.04	Hγ 0.73/0.57	8.3
	Tyr[5]	8.06	4.26	2.97/2.92	Hδ 7.10; Hε 6.90	8.6
	Tos				aromatic 7.62/7.24; H_3C 2.29	
	Z, OBn, Bn				H_2C 4.99, H_2C 5.07, H_2C 5.04 } aromatic 7.26−7.44	
2b	Arg[1]	8.43	3.93	1.72	Hγ 1.37/1.15; Hδ 2.93; HNε 6.73	8.2
	Lys[2]	7.16	4.15	1.59/1.54	Hγ 1.13; Hδ 1.37; Hε 2.93; HNζ 7.20	8.8
	Asp[3]	8.51	4.12	3.09/2.94		7.1
	Val[4]	7.55	4.05	1.70	Hγ 0.70/0.64	8.8
	D-Tyr[5]	8.87	4.32	2.78	Hδ 7.11; Hε 6.86	7.0
	Tos				aromatic 7.62/7.24; H_3C 2.30	
	Z, OBn, Bn				H_2C 4.98, H_2C 5.08, H_2C 5.01 } aromatic 7.26−7.41	
2c	Arg[1,6]	7.46	4.23	1.64	Hγ 1.36; Hδ 3.00; HNε 6.69	
	Lys[2,7]	7.94	4.00	1.53/1.47	Hγ 1.25/1.19; Hδ 1.34; Hε 2.93; HNζ 7.18	
	Asp[3,8]	8.52	4.32	3.03/2.79		
	Val[4,9]	7.15	4.16	1.88	Hγ 0.72/0.66	
	Tyr[5,10]	8.36	4.50	2.99/2.70	Hδ 7.08; Hε 6.82	
	Tos				aromatic 7.62/7.24; H_3C 2.29	
	Z, OBn, Bn				H_2C 4.98, H_2C 5.04, H_2C 4.98 } aromatic 7.25−7.41	
	(c) Cyclo[-Val-Arg-Lys(Ac)-Ala-Val-Tyr-], **3a**, and Cyclo[-Val-Arg-Lys(Ac)-Ala-Val-D-Tyr-], **3b**					
3a	Val[1]	7.21	4.24	2.16	Hγ 0.89/0.80	6.3
	Arg[2]	8.26	3.80	1.66	Hγ 1.52; Hδ 3.14; HNε 7.47	3.7
	Lys[3]	8.31	3.62	1.95	Hγ 1.22; Hδ 1.37; Hε 3.00; HNζ 7.82	7.0
	Ala[4]	8.05	4.29	1.26		8.1
	Val[5]	7.61	3.78	1.82	Hγ 0.77/0.46	8.9
	Tyr[6]	7.99	4.33	3.14/2.69	Hδ 7.02; Hε 6.60; HO 9.07	8.7
	Ac				H_3C 1.79	
3b	Val[1]	8.05	4.02	2.09	Hγ 0.75/0.69	8.9
	Arg[2]	7.92	4.11	1.70	Hγ 1.50/1.43; Hδ 3.09; HNε 7.46	7.3
	Lys[3]	7.83	3.81	1.65	Hγ 1.24; Hδ 1.38; Hε 3.01; HNζ 7.81	5.7
	Ala[4]	8.06	3.93	1.30		6.3
	Val[5]	7.04	4.07	1.88	Hγ 0.71/0.61	8.1
	D-Tyr[6]	8.33	4.46	2.83/2.68	Hδ 7.02; Hε 6.60; HO 9.07	6.8
	Ac				H_3C 1.78	
	(d) Cyclo[-Arg-Lys(Ac)-Ala-Val-Tyr-], **6a**, cyclo[-Arg-Lys(Ac)-Ala-Val-D-Tyr-], **6b**, and Cyclo[-Arg-Lys(Ac)-Ala-Val-Tyr-Arg-Lys(Ac)-Ala-Val-Tyr-], **6c**					
6a	Arg[1]	8.13	4.03	1.79/1.70	Hγ 1.45/1.38; Hδ 3.09; HNε 7.50	8.3
	Lys[2]	7.84	4.07	1.74/1.68	Hγ 1.23; Hδ 1.39; Hε 2.30; HNζ 7.83	7.5
	Ala[3]	7.93	4.08	1.32		7.7
	Val[4]	7.75	3.69	1.92	Hγ 0.76/0.59	9.0
	Tyr[5]	8.09	4.20	2.95/2.89	Hδ 6.98; Hε 6.65; HO 9.19	8.6
	Ac				H_3C 1.78	

Table 5 (Continued)

		HN	Ha	Hb	others	$^3J_{HN-Ha}$
	(d) Cyclo[-Arg-Lys(Ac)-Ala-Val-Tyr-], **6a**, cyclo[-Arg-Lys(Ac)-Ala-Val-D-Tyr-], **6b**, and Cyclo[-Arg-Lys(Ac)-Ala-Val-Tyr-Arg-Lys(Ac)-Ala-Val-Tyr-], **6c** *					
6b	Arg[1]	8.54	4.01	1.81/1.42	Hγ 1.29; Hδ 3.01; HN$^\epsilon$ 7.43	8.3
	Lys[2]	7.17	4.21	1.79/1.60	Hγ 1.19; Hδ 1.39; Hε 2.99; HN$^\zeta$ 7.81	9.0
	Ala[3]	8.25	3.79	1.37		6.5
	Val[4]	7.32	4.06	1.69	Hγ 0.72/0.66	8.8
	D-Tyr[5]	8.91	4.28	2.74	Hδ 7.00; Hε 6.62; HO 9.15	6.8
	Ac				H_3C 1.77	
6c	Arg[1,6]	8.12	3.96	1.73/1.64	Hγ 1.47; Hδ 3.11; HN$^\epsilon$ 7.50	
	Lys[2,7]	7.79	4.16	1.75/1.53	Hγ 1.20; Hδ 1.33; Hε 2.96; HN$^\zeta$ 7.81	
	Ala[3,8]	7.60	4.34	1.16		
	Val[4,9]	8.01	4.10	1.84	Hγ 0.76/0.74	
	Tyr[5,10]	8.02	4.48	3.14/2.74	Hδ 7.01; Hε 6.58; HO 9.11	
	Ac				H_3C 1.77	
	(e) Cyclo[-Arg-Lys(Ac)-MeAla-Val-Tyr-], **11a**, and Cyclo[-Arg-Lys(Ac)-MeAla-Val-D-Tyr-], **11b**					
11a	Arg[1]	7.68	4.06	1.70/1.38	Hγ 0.79; Hδ 3.10; HNε 7.49	8.4
	Lys[2]	8.07	4.38	1.80	Hγ 1.32/1.17; Hδ 1.53; Hε 2.97; HN$^\zeta$ 7.80	7.7
	MeAla[3]		3.58	1.55	H_3C 2.67	
	Val[4]	7.45	3.85	1.83	Hγ 0.82/0.59	10.0
	Tyr[5]	7.77	4.32	2.91/2.72	Hδ 6.96; Hε 6.65; HO 9.20	9.5
	Ac				H_3C 1.77	
11b	Arg[1]	8.57	3.86	1.77	Hγ 1.42; Hδ 2.99; HNε 7.39	7.5
	Lys[2]	7.20	4.56	1.66	Hγ 1.13; Hδ 1.37; Hε 2.96; HN$^\zeta$ 7.77	9.1
	MeAla[3]		3.54	1.62	H_3C 2.78	
	Val[4]	7.87	4.11	1.70	Hγ 0.81/0.73	9.8
	D-Tyr[5]	8.79	4.23	2.72	Hδ 6.99; Hε 6.62; HO 9.14	6.1
	Ac				H_3C 1.76	
	(f) Cyclo[-Arg-Lys(Ac)-(Hmb)Ala-Val-Tyr-], **12a**, and Cyclo[-Arg-Lys(Ac)-(Hmb)Ala-Val-D-Tyr-], **12b**					
12a	Arg[1]	8.31	3.77	1.87/1.83	Hγ1.37; Hδ 3.06; HN$^\epsilon$ 7.44	7.3
	Lys[2]	7.87	4.82	1.74/1.49	Hγ 1.22; Hδ 1.38; Hε 2.99; HN$^\zeta$ 7.80	9.1
	Ala[3]		3.47	1.45		
	Val[4]	7.70	3.91	1.90	Hγ 0.83/0.76	9.1
	Tyr[5]	8.30	4.03	2.98	Hδ 6.99; Hε 6.65; HO 9.17	7.5
	Ac				H_3C 1.78	
	Hmb				H_2C 4.47; H_3CO 3.95; HO 9.81; aromatic 6.40, 6.99	
12b	Arg[1]	8.57	3.90	1.75/1.42	Hγ 1.24; Hδ 2.99; HN$^\epsilon$ 7.39	7.5
	Lys[2]	7.26	4.83	1.72	Hγ 1.14; Hδ 1.41/1.37; Hε 2.97; HN$^\zeta$ 7.79	9.1
	Ala[3]		3.43	1.48		
	Val[4]	8.12	4.14	1.70	Hγ 0.81/0.73	9.7
	D-Tyr[5]	8.88	4.24	2.75	Hδ 7.01; Hε 6.63; HO 9.14	6.3
	Ac				H_3C 1.77	
	Hmb				H_2C 4.36; H_3CO 3.69; HO 9.82; aromatic 6.41, 6.90	

treated with TFA/thioanisol/*m*-cresol (9:0.5:0.5; v/v/v) for 3 h to give the crude peptide trifluoracetates that were characterized by HPLC (system 1) and ES-MS (Table 3).

(b) Syntheses of Protected Linear Sequences. The peptides were obtained via manual Fmoc solid phase synthesis on a trityl resin using the side chain-protected amino acid derivatives Tyr(Bn), Arg(Tos), Lys(Z), Lys(Ac), and Arg(NO_2). Fmoc amino acid derivatives (2 equiv) were coupled in DMF for 30 min following activation with 2 equiv of TBPipU and 4 equiv of DIEA. Couplings were monitored by the Kaiser ninhydrin test. Deprotection of the Fmoc group was achieved by treatment with 20% piperidine/DMF for 15 min. Cleavage from the resin was carried out with AcOH/DCM/TFE (2:6:2; v/v/v) for 1 h to give side chain-protected linear peptides, which were used for cyclization studies without further purification. The crude peptides were characterized by HPLC (system 2) and ES-MS (Table 3). All peptides were found to be sufficiently pure (>85% according to HPLC analysis) for use in cyclization studies without further purification.

(c) Synthesis of H-Arg(H^+)-Lys(Ac)-(Hmb)Ala-Val-Tyr-OH, 12. This sequence was obtained via the manual Fmoc strategy on the trityl resin using the following amino acid derivatives: Arg(Pmc), Lys(Ac), Tyr(tBu), and (Hmb)Ala[31] (see Scheme 1). All Fmoc amino acids (2 equiv, 0.3 M in DMF) were coupled using 2 equiv of TBPipU and 4 equiv of DIEA for 30 min except in the case of Fmoc-Lys(Ac)-OH, which was coupled via EDCI/HOBt in DCM overnight. The Fmoc-group was cleaved by 20% piperidine/DMF for 15 min. After the last coupling step the acid-labile Hmb group was temporarily stabilized through acetylation of the 2-hydroxy moiety.[30] Cleavage from the resin and deprotection of Pmc and tBu residues via TFA/thioanisol/*m*-cresol (9:0.5:0.5; v/v/v) for 3 h yielded the N-terminally Fmoc- and AcHmb-protected linear peptide **18**. Deprotection of the Fmoc group and the acetyl residue was achieved by treatment with 20% piperidine/DMF for 15 min. The crude linear Hmb-protected sequence was purified by preparative HPLC using mobile phase A and B_1. A linear gradient was formed from 1% B_1 to 70% B_1 over 70 min with a flow rate of 7 mL/min. Pure fractions were pooled and lyophilized, yielding 130 mg (26%) of pure backbone-protected peptide **12**.

Cyclization Experiments. (a) Cyclization with Uronium and Phosphonium Reagents. The linear pentapeptides (0.1 mmol) were cyclized by treatment with the corresponding coupling reagent (0.11 mmol) and 0.3 mmol of a tertiary amine (see Tables 1 and 2) in DMF. The cyclization reaction was monitored via analytical HPLC as described below using system 1 for unprotected sequences and system 2 for side chain protected peptides. After 1 h the reaction mixture was evaporated to dryness and the resulting residue dissolved in methanol and precipitated with ethyl acetate/ether. The crude peptides were examined for the desired cyclopeptides and any byproducts by HPLC analysis. Appropriate HPLC fractions were collected and examined by ES-MS to distinguish and identify the cyclomonomers and cyclodimers (Table 3). The corresponding D-Tyr-containing diastereoisomers were synthesized and used as standards in the HPLC analysis of crude cyclization products.

(b) Cyclization with DPPA. The crude linear pentapeptide **1** (0.1mmol) was dissolved in DMF, the solution adjusted to pH 8 with 0.2 mmol of DIEA, and 0.2 mmol DPPA added at room temperature. The course of the reaction was monitored

via analytical HPLC as described below using system 2. After 24 h solvent was evaporated and the residue dissolved in methanol and precipitated with ethyl acetate/ether. The crude peptides were examined by HPLC and ES-MS.

(c) HPLC Monitoring. To examine the time course of the cyclization reactions an aliquot (500 μL) of the reaction mixture was withdrawn at definite time intervals. Reaction was stopped by dilution with 500 μL of 0.1% TFA, and 50 μL of the resulting solution was injected onto the reversed-phase HPLC column with separation being performed by linear gradient elution using system 1 or 2. The cyclization yields were determined by integration of the appropriate peak areas.

(d) Isolation of Cyclomonomers and Cyclodimers by Preparative HPLC. The crude cyclopeptides (100 mg) were purified by preparative HPLC using system 1 for unprotected peptides and system 2 for protected peptides. A linear gradient was formed from 1% B_1 to 80% B_1 over 70 min with a flow rate of 7 mL/min for unprotected sequences and from 30% B_2 to 70% B_2 over 90 min with a flow rate of 10 mL/min for side chain protected peptides. Fractions were collected at 0.5 min intervals (monitoring by analytical HPLC) pooled and lyophilized, resulting in 15−30% yields of the purified peptides which were analyzed by ES-MS, amino acid analysis (Tables 3 and 4), and ^{1}H-NMR analysis (Table 5a−f).

Acknowledgment. We are grateful for financial support from the Deutsche Forschungsgemeinschaft. We thank H. Hans for technical assistance, B. Pisarz for performing amino acid analysis, and D. Runald for ES-MS data. Access for one of us (R.W.) to high-field NMR facilities in the laboratory of Prof. H. Kessler, Institute of Organic Chemistry, Technical University Munich, is gratefully acknowledged.

JO951108D