Stereospecific Synthesis of Peptide Analogs with *allo*-Threonine and D-*allo*-Threonine Residues

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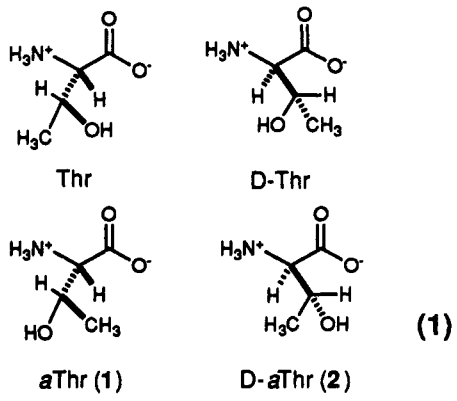
The nonproteinogenic β-hydroxy α-amino acids *allo*-threonine (*a*Thr, **1**) and D-*allo*-threonine (D-*a*Thr, **2**) are not part of the common arsenal of protein building blocks. However, they are found as constituents of an increasing group of biologically active peptides, such as the phytotoxic syringopeptins,[1] the antiviral agent viscosin,[2] the antibiotic cyclodepsipeptide complex CDPC 3510 isolated from the genus *Fusarium*,[3] and glycopeptidolipid antigens from mycobacteria responsible for pulmonary and disseminated infectious diseases particularly in AIDS patients.[4,5] Interestingly, the variable oligosaccharide moiety of these cell wall glycopeptides is attached to the hydroxyl group of D-*allo*-threonine, which serves therefore as a focal point of SAR investigations.[6] Substitution of threonine residues in peptide or protein backbones in the region of scissile peptide bonds with analogs **1** or **2** is also expected to render these linkages more stable to proteolysis. Specifically, incorporation of D-*allo*-threonine for threonine conserves the natural (*R*)-stereochemistry at the β-carbon while inversion of the α-configuration inhibits enzymatic attack.[7]



(1)

In spite of their biological relevance, the use of amino acids **1** and **2** has been considerably hampered by their high cost. Protocols for a de novo asymmetric synthesis or preparation from L-threonine are available but require multistep procedures and careful purification.[8,9] A direct interconversion of peptide sequences with threonine, *allo*-threonine, and D-*allo*-threonine residues would greatly facilitate the study and application of these attractive structural variants. We have recently developed a mild and highly specific procedure for the preparation of peptide oxazolines,[10] and we now report an application of this methodology for the desired direct transformation of threonine and D-threonine peptide sequences into the corresponding *allo*-threonine and D-*allo*-threonine analogs.

The peptide *cis*-oxazoline **5** is readily prepared by treatment of Cbz-Val-Thr-OMe (**3**) with ((methoxycarbonyl)sulfamoyl)triethylammonium hydroxide, inner salt (**4**, Burgess Reagent).[10] The intramolecular cyclization proceeds with inversion of the configuration at the threonine β-carbon (Figure 1). Acid hydrolysis of **5** at room temperature results in an intermediate *O*-acyl amine **i**,[9a,11] which smoothly undergoes an intramolecular *O*→*N*-acyl shift[12] to **iii** upon adjustment of the pH of the reaction mixture to 9.5 with K_2CO_3. Cbz-Val-*a*Thr-OMe (**6**) is isolated in >98% diastereomeric purity according to ^1H and ^{13}C NMR and 62% overall chemical yield.

As illustrated in Figure 1, this reaction sequence can be repeated with analog **6**, which is thus converted back to the threonine peptide **3** via *trans*-oxazoline **7**. Cbz-Val-Thr-OMe obtained from this double inversion cycle was identical in all regards to the starting peptide **3**, adding further proof for the complete stereoselectivity of this process.

The intramolecular *O*→*N*-acyl shift that converts the initially generated threonine ester to the peptide during the oxazoline hydrolysis sequence occurs readily even with sterically hindered acyl groups and longer peptide sequences (Figure 2). In dipeptide Cbz-Aib-Thr-OMe (**8**), the *allo*-threonine is generated next to a sterically highly demanding 2-methylalanine (Aib) residue. The isolation or purification of the intermediate heterocycle **9** is not necessary; analog Cbz-Aib-*a*Thr-OMe (**10**) is also obtained directly after addition of aqueous acid, followed by base, to the Burgess reaction mixture. In an analogous fashion, tetrapeptide ester **12** is isolated as a single isomer in 65% yield by this residue-selective β-inversion process.

The glycopeptide antibiotic ramoplanose (UK-71,903) contains a macrocycle composed of 16 amino acids, of which

(1) Ballio, A.; Barra, D.; Bossa, F.; Collina, A.; Grgurina, I.; Marino, G.; Moneti, G.; Paci, M.; Pucci, P.; Segre, A.; Simmaco, M. *FEBS Lett.* **1991**, *291*, 109.

(2) Burke, T. R.; Knight, M.; Chandrasekhar, B. *Tetrahedron Lett.* **1989**, *30*, 519.

(3) Flippin, L. A.; Jalali-Araghi, K.; Brown, P. A. *J. Org. Chem.* **1989**, *54*, 3006.

(4) (a) Riviere, M.; Puzo, G. *J. Biol. Chem.* **1991**, *266*, 9057. (b) Riviere, M.; Puzo, G. *Biochemistry* **1992**, *31*, 3575. (c) Gurjar, M. K.; Saha, U. K. *Tetrahedron Lett.* **1991**, *32*, 6621.

(5) For other bioactive compounds with *a*Thr and D-*a*Thr residues, see: (a) Sheehan, J. C.; Drummond, P. E.; Gardner, J. N.; Maeda, K.; Mania, D.; Nakamura, S.; Sen, A. K.; Stock, J. A. *J. Am. Chem. Soc.* **1963**, *85*, 2867. (b) Bodanszky, M.; Izdebski, J.; Muramatsu, I. *J. Am. Chem. Soc.* **1969**, *91*, 2351. (c) Hori, M.; Iwasaki, H.; Horii, S.; Yoshida, I.; Hongo, T. *Chem. Pharm. Bull.* **1973**, *21*, 1175. (d) König, W. A.; Loeffler, W.; Meyer, W. H.; Uhmann, R. *Chem. Ber.* **1973**, *106*, 816. (e) Aydin, M.; Lucht, N.; König, W. A.; Lupp, R.; Lung, G.; Winkelmann, G. *Liebigs Ann. Chem.* **1985**, 2285.

(6) Brennan, P. J. In *Microbial Lipids*; Academic Press: London, 1988; Vol. 1; pp 203-298.

(7) Allen, M. C.; Wade, R. *Int. J. Peptide Protein Res.* **1988**, *32*, 89.

(8) Leading references: (a) Schöllkopf, U. *Tetrahedron* **1983**, *39*, 2085. (b) Seebach, D.; Juaristi, E.; Miller, D. D.; Schickli, C.; Weber, T. *Helv. Chim. Acta* **1987**, *70*, 237. (c) Belekon, Y. N.; Bulychev, A. G.; Vitt, S. V.; Struchkov, Y. T.; Batsanov, A. S.; Timofeeva, T. V.; Tsyryapkin, V. A.; Ryzhov, M. G.; Lysova, L. A.; Bakhmutov, V. I.; Belikov, V. M. *J. Am. Chem. Soc.* **1985**, *107*, 4252. (d) Kuzuhara, H.; Watanabe, N.; Ando, M. *J. Chem. Soc., Chem. Commun.* **1987**, 95. (d) Ito, Y.; Sawamura, M.; Shirakawa, E.; Hayashizaki, K.; Hayashi, T. *Tetrahedron* **1988**, *44*, 5253. (e) Reno, D. S.; Lotz, B. T.; Miller, M. J. *Tetrahedron Lett.* **1990**, *31*, 827. (f) Genet, J. P.; Pinel, C.; Mallart, S.; Juge, S.; Thorimbert, S.; Laffitte, J. A. *Tetrahedron: Asymm.* **1991**, *2*, 555. (g) Beaulieu, P. L. *Tetrahedron Lett.* **1991**, *32*, 1031.

(9) (a) Elliott, D. F. *J. Chem. Soc.* **1949**, 589; **1950**, 62. (b) Pfister, K.; Robinson, C. A.; Shabica, A. C.; Tishler, M. *J. Am. Chem. Soc.* **1949**, *71*, 1101.

(10) Wipf, P.; Miller, C. P. *Tetrahedron Lett.* **1992**, *33*, 907.

(11) (a) Bergmann, M.; Miekeley, A. *Z. Physiol. Chem.* **1924**, *140*, 128. (b) Frump, J. A. *Chem. Rev.* **1971**, *71*, 483.

(12) (a) Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Ed.; Academic Press: New York, 1979; Vol. 2; pp 210. (b) Meyers, A. I.; Knaus, G.; Kamata, K. *J. Am. Chem. Soc.* **1974**, *95*, 268. (c) Wakamiya, T.; Tarumi, Y.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 2686.

0022-3263/93/1958-1575$04.00/0

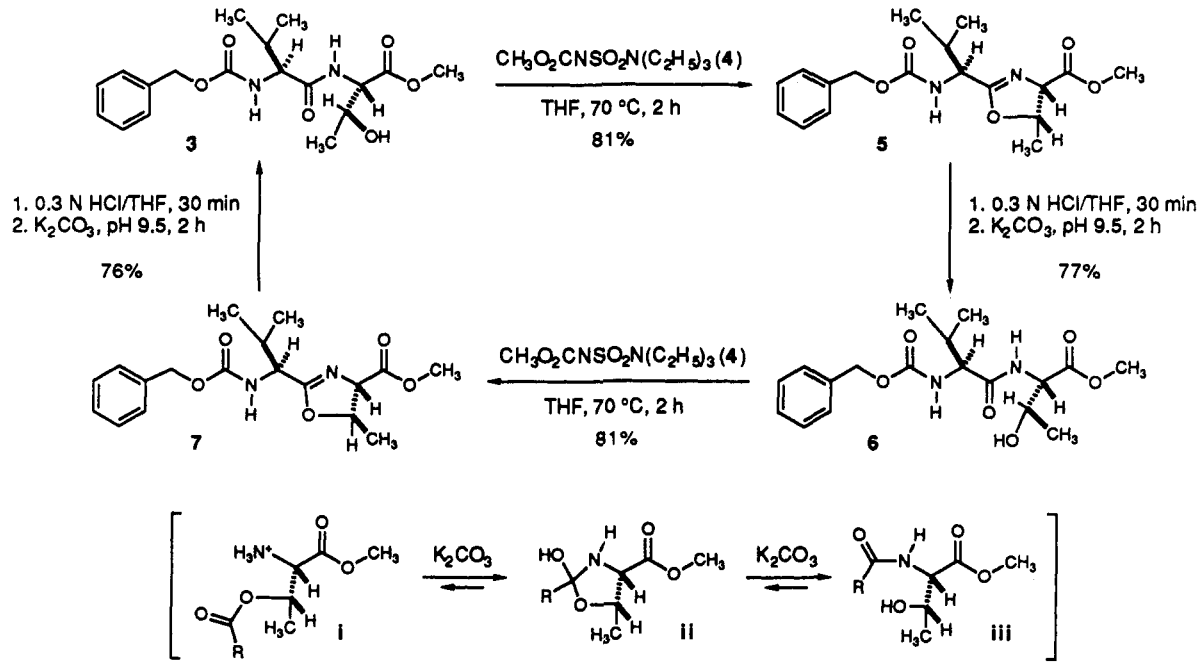


Figure 1. Interconversion of Thr and *a*Thr residues in a dipeptide segment via hydrolysis of the intermediate oxazolines **5** and **7**. An *O*→*N*-acyl shift leads to the regeneration of the Val-*a*Thr amide bond from the amino ester **i**.

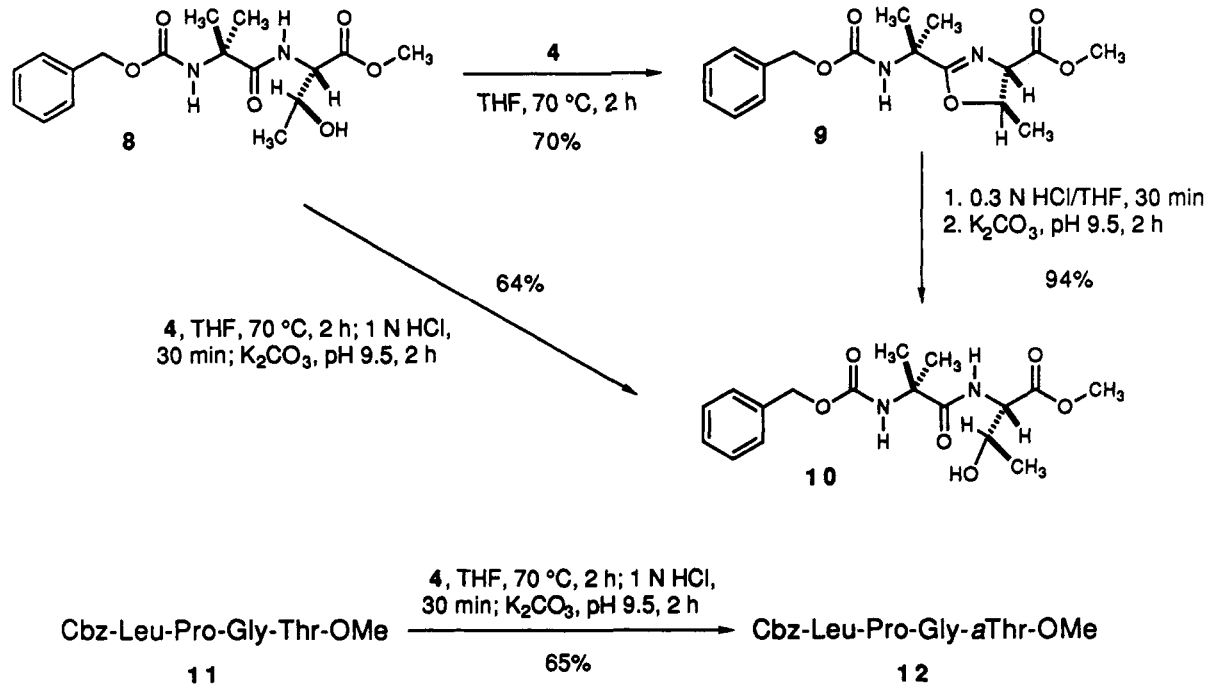


Figure 2. Preparation of Cbz-Aib-*a*Thr-OMe and Cbz-Leu-Pro-Gly-*a*Thr-OMe from Cbz-Aib-Thr-OMe and Cbz-Leu-Pro-Gly-Thr-OMe, respectively.

two are D-*allo*-threonines and one is a L-*allo*-threonine.[13] To further probe the application of our methodology for the rapid synthesis of peptide sequences with these unusual building blocks, we decided to prepare the ^{11}Tyr-^{12}D-*a*Thr-^{13}Tyr segment of ramoplanose. Acylation of Tyr(OEt)-OMe (**13**) with Boc-Tyr(OBn)-D-Thr-OH, readily obtained from commercially available D-threonine and tyrosine, via the mixed anhydride,[14] provides the tripeptide **14** in 85% yield (Figure 3). Subsequent β-carbon inversion on D-Thr occurs selectively and without any cleavage of the acid labile N-terminal protective group. The desired Tyr-D-*a*Thr-Tyr sequence **15** is thus isolated in 60% yield and could easily be combined with further ramoplanose segments.

In conclusion, we have demonstrated a novel interconversion of Thr, D-Thr, *a*Thr, and D-*a*Thr residues by direct manipulation of peptide segment structures. Key features include the highly specific cyclization of β-hydroxy-α-amino acid residues with Burgess reagent and a mild acid/base hydrolysis of the intermediate heterocycle. An

(13) Skelton, N. J.; Harding, M. M.; Mortishire-Smith, R. J.; Rahman, S. K.; Williams, D. H.; Rance, M. J.; Ruddock, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7522.

(14) Anderson, G. W.; Zimmermann, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.

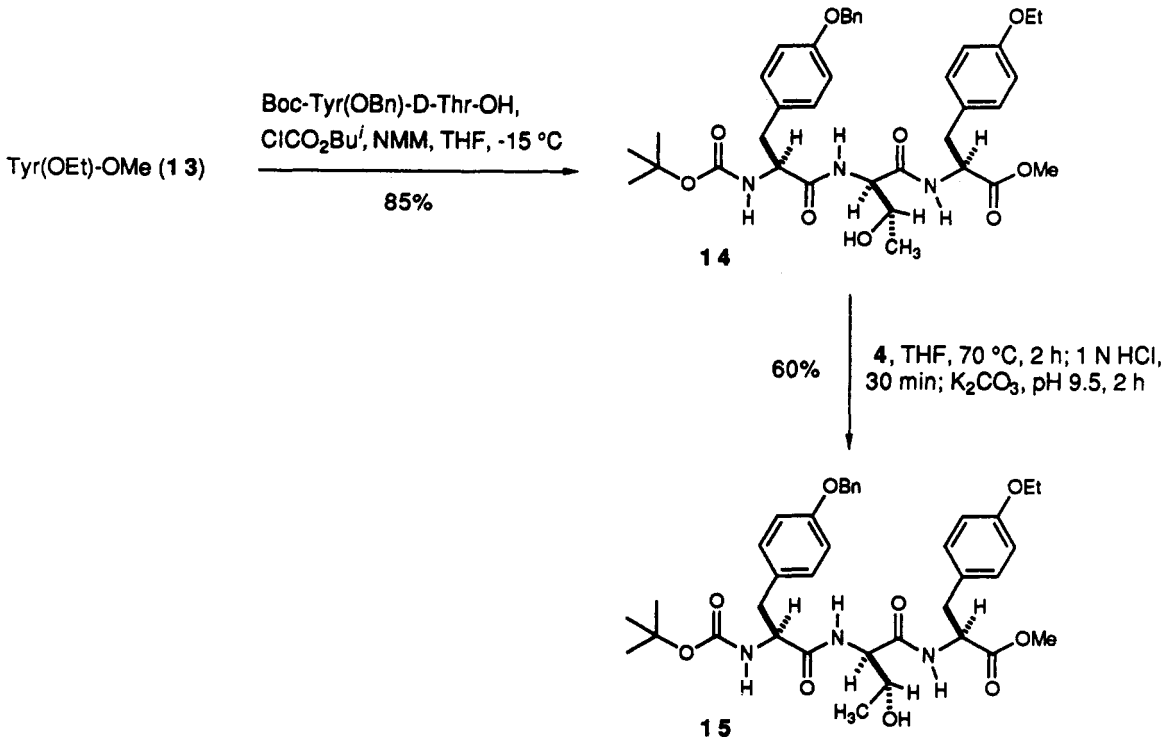


Figure 3. The tripeptide Boc-Tyr-D-Thr-Tyr-OMe is converted into the ^{11}Tyr-^{12}D-αThr-^{13}Tyr segment of ramoplanose (UK-71,903).

intramolecular *O*→*N*-acyl shift regenerates the amide backbone. Application of this straightforward technique greatly facilitates the preparation of the growing number of αThr- and D-αThr-containing peptides of nonribosomal origin. More importantly even, the specific inversion of configuration at the functionalized β-carbon of threonine, which is involved in many biological molecular recognition processes, will allow the study of the enzyme-inhibitory and conformational effects of peptide analogs with these unusual point mutations. Ultimately, as the hydrolysis of the intermediately formed oxazoline generates a peptide where an X-Thr amide bond is replaced by an ester bond, this strategy might also be of interest for the site-selective cleavage of polypeptides[15] at β-hydroxy-α-amino acid residues.

Experimental Section

General. Ether and THF were distilled from either sodium or potassium/benzophenone ketyl under Ar or N_2 immediately prior to use. NMR spectra were recorded at 300 MHz for ^{1}H and at 75 MHz for ^{13}C in $CDCl_3$ unless noted otherwise. High-resolution mass spectra were obtained by peak matching with reference peaks of known *m/z*. Chromatography was performed on silica gel according to the Still protocol.[16] Aib, 2-methylalanine; Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; Gly, glycine; Leu, L-leucine; Pro, L-proline; Thr, L-threonine; Tyr, L-tyrosine; Val, L-valine.

General Procedure. Preparation of Cbz-Val-αThr-OMe (6) from Cbz-Val-Thr-OMe (3). A solution of Cbz-Val-Thr-OMe (3,[10] 100 mg, 0.27 mmol) in 2 mL of THF in a Pyrex tube was flushed with Ar and treated with Burgess reagent (4, 72 mg, 0.31 mmol). The mixture was heated at 70–75 °C for 2 h and diluted to 10 mL with THF. This solution was treated with 10 mL of an aqueous 0.5 M HCl solution and stirred for 15 min at 22 °C. The pH of the solution was adjusted to 9.5 by addition of a saturated K_2CO_3 solution, and the reaction was monitored by TLC. After 30 min, the rearrangement was complete. The solution was neutralized to pH = 7 with an aqueous 1 M HCl solution, and the THF was evaporated. The aqueous layer was extracted with EtOAc (2 × 60 mL), and the combined organic extracts were washed with brine (1 × 20 mL) and dried (Na_2SO_4). Chromatography on SiO_2 (EtOAc) yielded Cbz-Val-αThr-OMe (6, 71 mg, 71%) as a white foam: IR (neat) 3090, 3050, 2960, 1750, 1710, 1680, 1535, 1455, 1445, 1380, 1360, 1290, 1240, 1150, 1090, 1050, 770, 735, 700 cm^{-1}; ^{1}H NMR δ 7.33–7.26 (m, 5 H), 7.16 (d, 1 H, J = 7.3 Hz), 5.55 (d, 1 H, J = 8.4 Hz), 5.11, 5.06 (AB, 2 H, J = 11.7 Hz), 4.67–4.65 (m, 1 H), 4.13–4.08 (m, 2 H), 3.77 (s, 3 H), 2.11–2.04 (m, 1 H), 1.18 (d, 3 H, J = 6.0 Hz), 0.98–0.92 (m, 6 H); ^{13}C NMR δ 172.2, 170.5, 156.7, 136.1, 128.6, 128.3, 128.1, 68.7, 67.2, 60.5, 58.2, 52.6, 31.1, 19.2, 18.9, 18.0; MS (Cl) *m/z* (relative intensity) 367 ([M + H]$^+$, 20), 323 (16), 259 (12), 215 (10), 206 (20), 162 (25), 108 (10), 91 (100), 72 (20); $[\alpha]_D$ = −22° (*c* = 0.8, EtOH, 23 °C). Isolation of the intermediate oxazoline 5[10] in 81% yield and subsequent hydrolysis gave 6 in 77% yield from 5.

Preparation of Cbz-Val-Thr-OMe (3) from Cbz-Val-αThr-OMe (6). According to the general procedure, Cbz-Val-αThr-OMe (6) was converted in 62% overall yield into Cbz-Val-αThr-OMe[10] (3).

Preparation of Cbz-Aib-αThr-OMe (10) from Cbz-Aib-Thr-OMe (8). According to the general procedure, Cbz-Aib-Thr-OMe (8) was converted in 64% overall yield into Cbz-Aib-αThr-OMe (10): IR (neat) 3320, 2980, 1730, 1669, 1522, 1456, 1387, 1333, 1263, 1213, 1177, 1136, 1090, 1020, 741, 698 cm^{-1}; ^{1}H NMR δ 7.40–7.27 (m, 5 H), 7.10 (d, 1 H, J = 9.0 Hz), 5.45 (s, 1 H), 5.07 (s, 2 H), 4.70–4.62 (m, 1 H), 4.25–4.13 (m, 1 H), 3.75 (s, 3 H), 1.52 (s, 3 H), 1.50 (s, 3 H), 1.11 (d, 3 H, J = 6.48 Hz); ^{13}C NMR δ 174.5, 170.5, 155.5, 135.9, 128.6, 128.3, 128.2, 68.2, 67.1, 58.3, 57.1, 52.5, 26.0, 24.9, 18.5; MS (FAB) *m/z* 353 ([M + H]$^+$); $[\alpha]_D$ = +31.7 (*c* = 1.1, CH_2Cl_2, 24 °C). Isolation of the intermediate oxazoline 9[10] in 70% yield and subsequent hydrolysis gave 10 in 94% yield from 9.

Preparation of Cbz-Leu-Pro-Gly-αThr-OMe (12) from Cbz-Leu-Pro-Gly-Thr-OMe (11). According to the general procedure, Cbz-Leu-Pro-Gly-Thr-OMe (11) was converted in 65% overall yield into Cbz-Leu-Pro-Gly-αThr-OMe (12): IR (neat) 3312, 3065, 2957, 2872, 1661, 1530, 1451, 1381, 1329, 1256, 1123, 1045, 912, 733, 698 cm^{-1}; ^{1}H NMR δ 7.60 (d, 1 H, J = 7.8 Hz), 7.51 (t, 1 H, J = 6.0 Hz), 7.35–7.25 (m, 5 H), 5.64 (d, 1 H, J = 8.8 Hz), 5.20 (m, 1 H), 5.08, 5.01 (AB, 2 H, J = 12.2 Hz), 4.60–4.50 (m, 1 H), 4.48 (dd, 1 H, J = 8.1, 1.9 Hz), 4.35–4.25 (m, 2 H), 4.10–4.00

(15) (a) Schepartz, A.; Cuenoud, B. *J. Am. Chem. Soc.* **1990**, *112*, 3247. (b) Hoyer, D.; Cho, H.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 3249.
(16) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(m, 1 H), 4.00–3.85 (m, 1 H), 3.72–3.69 (m, 1 H), 3.67 (s, 3 H), 3.65–3.55 (m, 1 H), 2.14–2.06 (m, 2 H), 2.05–1.97 (m, 2 H), 1.74–1.64 (m, 1 H), 1.47–1.43 (m, 2 H), 1.26 (d, 3 H, J = 6.5 Hz), 0.95–0.90 (m, 6 H); ^{13}C NMR δ 172.8, 172.1, 170.6, 169.2, 156.3, 136.2, 128.5, 128.2, 128.0, 68.4, 67.0, 61.0, 58.5, 52.2, 50.9, 47.8, 43.0, 40.9, 28.9, 25.3, 24.5, 23.2, 21.8, 19.6; MS (FAB) m/z 535 ([M + H]$^+$); $[\alpha]_D$ = −1.1 (c = 0.9, CH_2Cl_2).

Preparation of Boc-Tyr(OBn)-D-*a*Thr-Tyr(OEt)-OMe (15) from Boc-Tyr(OBn)-D-Thr-Tyr(OEt)-OMe (14). According to the general procedure, Boc-Tyr(OBn)-D-Thr-Tyr-(OEt)-OMe (14, prepared by standard[14] mixed anhydride coupling of Boc-Tyr(OBn)-D-Thr-OH and Tyr(OEt)-OMe) was converted in 60% overall yield into Boc-Tyr(OBn)-D-*a*Thr-Tyr(OEt)-OMe (15): IR (neat) 3295, 2978, 2928, 1740, 1696, 1684, 1615, 1647, 1539, 1512, 1456, 1368, 1246, 1177, 1117, 1047, 918, 824, 735 cm^{-1}; ^{1}H NMR δ 7.48–7.27 (m, 6 H), 7.09 (d, 2 H, J = 8.5 Hz), 7.03 (d, 2 H, J = 8.6 Hz), 7.05–7.00 (m, 1 H), 6.89 (d, 2 H, J = 8.5 Hz), 6.79 (d, 2 H, J = 8.5 Hz), 5.17 (d, 1 H, J = 7.0 Hz), 4.76 (s, 2 H), 4.85–4.75 (m, 1 H), 4.45 (dd, 1 H, J = 7.9, 4.2 Hz), 4.38–4.28 (m, 1 H), 4.12 (q, 2 H, J = 7.1 Hz), 3.85–3.75 (m, 1 H), 3.69 (s, 3 H), 3.11–2.91 (m, 4 H), 2.00 (br s, 1 H), 1.38 (s, 9 H), 1.36 (t, 3 H, J = 6.9 Hz), 0.97 (d, 3 H, J = 6.0 Hz); ^{13}C NMR δ 172.3, 172.0, 169.9, 158.1, 157.9, 155.5, 137.0, 130.3, 130.2, 128.6, 128.0, 127.6, 127.5, 115.1, 114.6, 80.4, 70.0, 68.8, 63.4, 57.5, 56.3, 53.5, 52.4, 37.4, 37.1, 28.3, 18.9, 14.8; MS(FAB) m/z 678 ([M + H]$^+$), $[\alpha]_D$= +8.0 (c = 0.8, CH_2Cl_2, 24 °C).

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Supplementary Material Available: ^{1}H and ^{13}C NMR spectra for compounds **3**, **6**, **8**, **10–12**, **14**, and **15** (15 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.