Thiopeptide Synthesis. α-Amino Thionoacid Derivatives of Nitrobenzotriazole as Thioacylating Agents

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Introduction

There has been considerable interest recently in the synthesis and properties of thiopeptides in which the −CSNH− group replaces one or more peptide bonds.[1] These modified peptides have demonstrated increased activity *in vivo* as biological response modifiers, neuroeffectors, and immunomodulators due to the stability of their thioamide bonds toward enzymatic degradation as compared to that of their oxygenated counterpart.[2] Synthetic routes employed to prepare these thiopeptides included replacement of oxygen by sulfur using P_4S_{10} or Lawesson's phosphetane disulfide reagent,[3] and thioesters[4] or dithioesters[5] of *N*-protected amino acids. Several procedures also have been reported for monothionation of peptides using *N*-protected amino monothioacids and benzotriazolyloxytris(pyrrolidino) phosphonium hexafluorophosphate (PYBOP) and some of its derivatives.[6] Unfortunately, these methods displayed lack of reaction site specificity, low yields and purity because of side reactions, and loss of enantiomeric integrity in the final product, apparently because of racemization induced by the thioacylating agents.

Recently, a major improvement was described[7] for the site specific incorporation of thioamide linkages into a growing peptide under mild conditions using thioacylbenzimidazolinones of amino acid derivatives as thioacylating agents (Scheme 1). In our hands, this method proceeds with about 2% loss of enantiomeric purity, as demonstrated by HPLC analysis of the reaction product **10a**, formed in reaction with α-methylbenzylamine. This procedure, although superior to previous methods, still suffers from the formation of benzimidazole **2** as a significant byproduct, and the overall yield for the four-step process was only about 20%. Furthermore, our recent attempt[8] to use this procedure failed due to the limited reactivity of the benzimidazolinone **4** as a thioacylating agent. To overcome these limitations, we have developed a new method for thiopeptide synthesis.

Results and Discussion

Our present work describes the use of α-amino thionoacid derivatives of nitrobenzotriazole as thioacyl transfer reagents in thiopeptide synthesis. The synthetic strategy is similar to that applied in the previous work[7] for the preparation of benzimidazolinone thioacylating agents. However, improvement was sought in two areas. First, an efficient thioacylating agent was needed that possessed a better leaving group than the benzimidazolinone group and that could be readily cleaved under mild conditions, thus allowing shorter reaction time for peptide segment coupling to take place without racemization.[9] Thioacyl halides are not candidates because they are unstable even at low temperature and only a few are known.[10] Similarly, thioacyl anhydrides are not easily accessible and are unstable.[10] The benzotriazole group was chosen because the benzotriazole anion is an excellent leaving group[11] and has the advantage that its derivatives are frequently quite stable. Second, it was important to avoid the formation of the benzimidazole byproduct **2** with the concomitant purification requirement and loss of yield. This avoidance was achieved by using 4-nitro-1,2-phenylenediamine as a latent triazole source. The introduction of an electron-withdrawing group para to the unacylated amino group reduces its nucleophilicity and its participation for the formation of any benzimidazole.

The procedure for the preparation of benzotriazole thioacylating agents (**9a**−**d**,**f**) is illustrated in Scheme 2. Coupling was effected between 4-nitro-1,2-phenylenediamine and the *N*-BOC amino acid in THF at 0 °C using mixed carbonic anhydride methodology for peptide synthesis.[12] After isolation, this process, in all examples, gave the crystalline anilide **6** in 90−92% yield. Direct thionation of **6** was achieved with a mixture of P_4S_{10} and anhydrous Na_2CO_3 in THF. The reaction proceeded smoothly for a total of 3 h at 0 °C and rt to afford thioamides **7** in excellent yield (86−88%) with the exception of **7e** which was obtained in 16% yield. In the latter case, the major product was acylthioimidate **8** (64% yield), formed in competitive cyclization between the nucleophilic sulfur of the thioamide group and the carbonyl oxygen of the β-methylester of aspartic acid. In general a better yield of **7** was obtained when thionation

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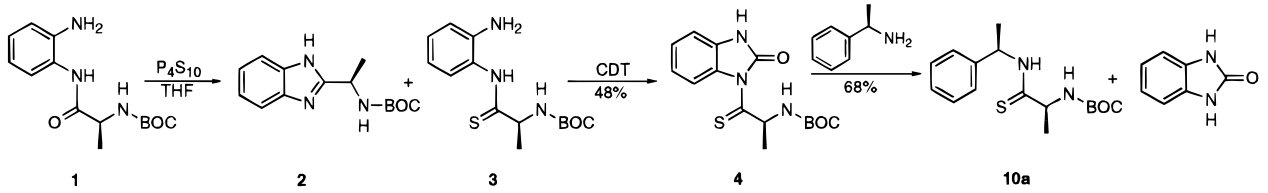
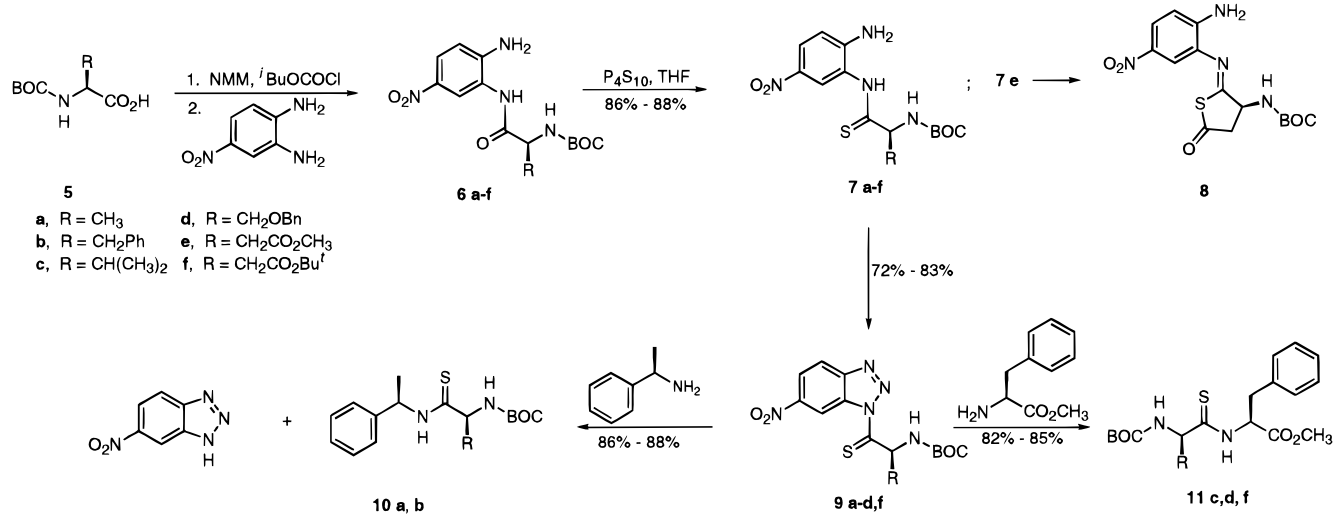
Scheme 1. Benzimidazolinone Route



Scheme 2. Benzotriazole Route



of **6** was carried out using limited P_4S_{10} (40−50 mol %); this reaction gave only the corresponding thioamide.

The structure of **7** was established by combustion analysis and ^{13}C NMR spectroscopy which showed a characteristic signal for the thioamide carbon at lower field (δ 200−213 ppm) which was absent in the starting compound. Intramolecular diazonium cyclization of **7** using nitrous acid generated *in situ* with $NaNO_2$ in AcOH gave benzotriazoles **9** in 72−83% yield. These compounds are stable orange solids and can be stored for months at 0 °C without decomposition. However, the aspartic acid benzotriazole derivative **9f** is unstable and should be prepared immediately prior to use.

Having established a suitable procedure for the synthesis of the desired benzotriazoles of the α-amino thionoacids, we next examined their efficiency and reactivity as thioacylating reagents. Initial experiments were carried out with α-methylbenzylamine as the amine component, coupling with **9a** and **9b** to optimize the reaction conditions and to test for enantiomeric integrity. The best results were obtained when α-methylbenzylamine was added slowly over a 40 min period to a solution of **9a** or **9b** in THF at 0 °C. Reaction took place almost instantly as evidenced by the change of color of the reaction medium from orange to colorless. After the addition was completed, the product was easily separated from the reaction mixture by simple chromatography through a plug of silica gel, eluting with hexane/EtOAc, 2/1. Compounds **10a** and **10b** were obtained in 88% and 86% yield, respectively. Their enantiomeric purity was evaluated by HPLC using racemic samples of **9a** and **9b** as controls, from which the corresponding **10a** and **10b** were prepared. The HPLC analysis indicated the enantomeric ratio (er) of the products to be in excess of 99/1.

Couplings were then conducted with an amino acid residue known to be somewhat sensitive to racemization. Thus, thiobenzotriazole derivatives **9c**,**d**,**f** were coupled with phenylalanine methyl ester in THF under the exact conditions used for coupling α-methylbenzylamine with **9a** and **9b**. The product thiopeptides **11c**,**d**,**f** were obtained in 82−85% yield and were determined by HPLC analysis to be enantiomerically pure. The amino acids utilized in this study were selected to provide some scope to the coupling, for example, aliphatic and aromatic side chain (Ala and Phe), hindered amino acids (Val), and functionality (Ser and Asp-β-methyl and β-*tert*-butyl ester).

In summary we provide a mild, convenient, efficient, and racemization-free route for incorporation of a thioamide linkage into peptides employing inexpensive and easily prepared reagents.

Experimental Section

General. Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded in solution in the indicated deuterated solvents at 400 and 100.6 MHz, respectively, and chemical shifts (δ) are reported relative to either a tetramethylsilane internal standard or the signals due to the solvent; coupling constants, *J*, are reported in hertz. TLC was carried out using precoated sheets (Merck silica gel 60-F_{250}, 0.2 mm) which, after development, were visualized at 254 nm, and/or by spraying with 0.3% ninhydrin solution in *tert*-BuOH/AcOH (93/3) and heating. Merck silica gel 60 (70−230 mesh) was used for column chromatography. The enantiomeric purities of the thiopeptide products were determined by analytical HPLC on a 300 × 3.9 mm normal phase microsorb column utilizing detection at 254 nm. All reactions were conducted under a nitrogen or argon atmosphere. Solvents were distilled prior to use: THF from Na/benzophenone; *N*-methylmorpholine and triethylamine from CaH and then stored over 4 Å molecular sieves. Organic extracts were dried over anhydrous Na_2SO_4 and filtered, and the filtrate was evaporated under reduced pressure at 30−40 °C unless otherwise indicated. Microanalysis was performed by the College of Chemistry Micro Analytical Laboratory, University of California, Berkeley.

General Procedure for the Preparation of Compounds 6a−f. Coupling of α-*N*-BOC L-Amino Acids with 4-Nitro-1,2-phenylenediamine. *N*-Methylmorpholine (2.2 mL, 20 mmol) was added to a solution of the α-*N*-BOC L-amino acid

(**5a**−**f**) in THF (100 mL) at −20 °C, followed by dropwise addition of isobutyl chloroformate (1.3 mL, 10 mmol). The mixture was stirred for 10 min, 4-nitro-1,2-phenylenediamine (1.53 g, 10 mmol) was added, and the resulting slurry was stirred at −15 °C for 2 h and at rt overnight. The precipitate was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (250 mL), and the solution washed successively with 1 M $NaHPO_4$, brine, 5% $NaHCO_3$, and brine, and then dried and evaporated to dryness. Crystallization of the residue from EtOAc/hexane afforded pure **6a**−**f** as yellow solids in 90−92% yield.

α-*N*-BOC-L-alanine 2-amino-5-nitroanilide (6a): mp 187 °C; $[\alpha]^{22}_D = -46.5°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 9.33 (s, 1H), 8.08 (d, 1H, J = 1.9), 7.86 (dd, 1H, J = 9.0, 2.5), 7.21 (d, 1H, J = 6.1), 6.74 (d, 1H, J = 9.1), 6.44 (s, 2H), 4.09 (m, 1H), 1.38 (s, 9H), 1.23 (d, 3H, J = 7.2); ^{13}C NMR δ 172.4, 155.5, 149.8, 135.4, 123.3, 122.2, 121.2, 113.5, 78.3, 50.3, 28.2, 17.4. Anal. Calcd for $C_{14}H_{20}N_4O_5$: C, 51.9; H, 6.2; N, 17.3. Found: C, 51.9; H, 6.4; N, 17.4.

α-*N*-BOC-L-phenylalanine 2-amino-5-nitroanilide (6b): mp 186 °C; $[\alpha]^{22}_D = +16.8°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 9.41 (br s, 1H), 7.96 (d, 1H, J = 1.16), 7.87 (dd, 1H, J = 6.6, 2.4), 7.31 (m, 1H), 6.74 (d, 1H, J = 9.0), 6.38 (s, 2H), 4.31 (m, 1H), 3.03 (m, 1H), 2.9 (m, 1H), 1.34 (s, 9H); ^{13}C NMR δ 171.3, 155.7, 149.9, 137.8, 135.4, 129.3, 129.3, 128.1, 126.4, 123.4, 122.4, 121.0, 113.5, 78.4, 56.4, 37.0, 28.1. Anal. Calcd for $C_{20}H_{24}N_4O_5$: C, 60.0; H, 6.0; N, 14.0. Found: C, 60.1; H, 6.2; N, 13.8.

α-*N*-BOC-L-valine 2-amino-5-nitroanilide (6c): mp 139−140 °C; $[\alpha]^{22}_D = -63.8°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 9.37 (s, 1H), 8.47 (s, 1H), 7.8 (d, 1H, J = 9.0), 7.07 (d, 1H, J = 7.4), 6.7 (d, 1H, J = 9.0), 6.4 (s, 2H), 3.9 (m, 1H), 1.36 (s, 9H), 0.9 (dd, 6H, J = 11.5, 5.9); ^{13}C NMR δ 171.0, 156.5, 150.1, 135.9, 123.7, 122.4, 121.6, 114.0, 78.8, 61.2, 60.2, 30.1, 28.6, 19.1, 14.5. Anal. Calcd for $C_{16}H_{24}N_4O_5$: C, 54.5; H, 6.8; N, 15.9. Found: C, 54.7; H, 7.0; N, 16.0.

α-*N*-BOC-*O*-benzyl-L-serine 2-amino-5-nitroanilide (6d): mp 149−150 °C; $[\alpha]^{22}_D = -21.7°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 9.5 (s, 1H), 7.8 (d, 1H, J = 8.8), 7.2 (m, 5H), 7.15 (d, 1H, J = 6.2), 6.7 (d, 1H, J = 8.9), 6.4 (s, 2H), 4.5 (s, 2H), 4.3 (d, 1H, J = 5.7), 3.6 (br s, 2H), 1.36 (s, 9H); ^{13}C NMR δ 170.2, 156.0, 150.4, 138.6, 135.9, 128.7, 127.9, 124.0, 122.9, 121.5, 114.0, 79.0, 72.6, 70.0, 55.2, 28.6, 23.6. Anal. Calcd for $C_{21}H_{26}N_4O_6$: C, 58.6; H, 6.1; N, 13.0. Found: C, 58.5; H, 6.0; N, 13.0.

α-*N*-BOC-L-aspartic acid *β*-methyl ester α-2-amino-5-nitroanilide (6e): mp 103 °C; $[\alpha]^{22}_D = -39.3°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 9.5 (s, 1H), 8.0 (s, 1H), 7.8 (dd, 1H, J = 9.0, 2.5), 7.3 (d, 1H, J = 7.0), 6.7 (d, 1H, J = 9.0), 6.4 (s, 2H), 4.4 (m, 1H), 3.5 (s, 3H), 2.8 (dd, 1H, J = 16.0, 5.9), 2.6 (dd, 1H, J = 16.0, 8.0), 1.36 (s, 9H); ^{13}C NMR δ 171.3, 170.7, 155.9, 150.6, 135.8, 124.0, 123.2, 121.4, 113.9, 79.1, 52.0, 51.8, 36.3, 28.6, 25.9. Anal. Calcd for $C_{16}H_{22}N_4O_7$: C, 50.2; H, 5.8; N, 14.7. Found: C, 50.4; H, 5.9; N, 14.6.

α-*N*-BOC-L-aspartic acid *β*-*tert*-butyl ester α-2-amino-5-nitroanilide (6f): mp 187 °C; $[\alpha]^{22}_D = -39.9°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 9.38 (s, 1H), 8.0 (s, 1H), 7.8 (dd, 1H, J = 9.0, 2.5), 7.2 (d, 1H, J = 7.2), 6.7 (d, 1H, J = 9.0), 6.4 (s, 2H), 4.3 (m, 1H), 2.7 (dd, 1H, J = 16.0, 6.3), 2.5 (dd, 1H, J = 16.0, 7.7), 1.36 (s, 18H); ^{13}C NMR δ 170.7, 169.9, 155.8, 150.5, 135.8, 123.9, 123.1, 121.5, 114.0, 80.7, 79.0, 60.2, 51.9, 28.6, 28.2, 21.2, 14.5. Anal. Calcd for $C_{19}H_{28}N_4O_7$: C, 53.8; H, 6.7; N, 13.2. Found: C, 53.9; H, 6.7; N, 12.9.

General Procedure for the Preparation of Thioanilides 7a−**f.** Under a flow of argon, P_4S_{10} (1.1 g, 2.5 mmol)[13] was mixed with Na_2CO_3 (0.27 g, 2.5 mmol) in THF (100 mL). The mixture was stirred for 1 h at 25 °C and then cooled to 0 °C. To this clear solution was added anilide **6** (5 mmol), and the reaction mixture was stirred at 0 °C for 30 min and at rt for 2.5 h. The mixture was filtered through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc/heptane (2/1, 75 mL) and washed with 5% $NaHCO_3$ (2 × 30 mL), and the aqueous layers were back-extracted with EtOAc/heptane (75 mL). The combined organic layers were washed with brine, dried, and evaporated to an oil. Upon addition of Et_2O (∼50 mL) and 1 h of cooling, the precipitated yellow solid **7** was collected, washed with Et_2O (∼15 mL), and dried at rt.

α-*N*-BOC-L-alanine 2-amino-5-nitrothioanilide (7a): yield 88%; mp 184 °C; $[\alpha]^{22}_D = -78.3°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 10.2 (s, 1H), 7.95 (dd, 1H, J = 9.1, 2.5), 7.84 (s, 1H), 7.44 (d, 1H, J = 5.0), 6.76 (d, 1H, J = 9.1), 6.46 (s, 1H), 4.42 (m, 1H), 1.38 (s, 9H), 1.37 (d, 3H, J = 6.7); ^{13}C NMR δ 208.6, 155.7, 150.8, 135.2, 124.9, 124.7, 122.4, 113.7, 78.6, 56.9, 28.2, 20.1. Anal. Calcd for $C_{14}H_{20}N_4O_4S$: C, 49.4; H, 5.9; N, 16.5. Found: C, 49.5; H, 5.9; N, 16.2.

α-*N*-BOC-L-phenylalanine 2-amino-5-nitrothioanilide (7b): yield 87%; mp 175 °C; $[\alpha]^{22}_D = +48.8°$ (*c* 1.95, DMSO); ^{1}H NMR (DMSO) δ 10.21 (s, 1H), 7.92 (dd, 1H, J = 6.4, 2.7), 7.5 (m, 1H), 7.33 (m, 5H), 6.73 (d, 1H, J = 9.1), 6.4 (s, 2H), 4.56 (m, 1H), 3.06 (m, 2H), 1.35 (s, 9H); ^{13}C NMR δ 207.1, 155.9, 150.7, 137.3, 135.2, 129.5, 128.0, 126.5, 124.4, 122.2, 113.6, 78.6, 28.2. Anal. Calcd for $C_{20}H_{24}N_4O_4S$: C, 57.7; H, 5.8; N, 13.4. Found: C, 57.7; H, 6.0; N, 13.2.

α-*N*-BOC-L-valine 2-amino-5-nitrothioanilide (7c): yield 87%; mp 104−105 °C (crystallized with one molecule of ether); $[\alpha]^{22}_D = -87°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 10.2 (s, 1H), 7.9 (d, 1H, J = 9.1), 7.7 (s, 1H), 7.4 (d, 1H, J = 6.4), 6.4 (s, 2H), 3.9−3.5 (m, 1H), 2.05 (m, 1H), 1.4 (s, 9H), 0.97−0.92 (dd, 6H, J = 14.0, 6.5); ^{13}C NMR δ 208.4, 157.0, 151.4, 135.7, 125.7, 125.5, 125.0, 122.9, 114.2, 79.1, 68.0, 65.4, 31.6, 28.7, 19.7, 15.6. Anal. Calcd for $C_{16}H_{24}N_4O_4S$ $(C_2H_5)_2O$: C, 54.3; H, 7.8; N, 12.7. Found: C, 54.5; H, 8.0; N, 12.8.

α-*N*-BOC-*O*-benzyl-L-serine 2-amino-5-nitrothioanilide (7d): yield 84%; mp 161−162 °C; $[\alpha]^{22}_D = -23.6°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 10.16 (s, 1H), 7.9 (dd, 1H, J = 9.0, 2.6), 7.7 (s, 1H), 7.3 (s, 1H), 7.3 (m, 5H), 6.3 (s, 2H), 4.6 (dd, 1H, J = 12.4, 6.0), 4.5 (dd, 2H, J = 19.0, 11.0), 3.7 (m, 2H), 1.37 (s, 9H); ^{13}C NMR δ 205.3, 156.1, 151.1, 138.8, 128.7, 128.0, 125.5, 125.1, 122.9, 114.2, 79.3, 72.8, 71.9, 61.2, 28.6, 23.6. Anal. Calcd for $C_{21}H_{26}N_4O_5S$: C, 56.5; H, 5.9; N, 12.5. Found: C, 56.4; H, 5.9; N, 12.5.

α-*N*-BOC-L-aspartic acid *β*-methyl ester α-2-amino-5-nitrothioanilide (7e) and acylthioimidate (8) were obtained as a mixture (1.6 g) from **6e** following the general procedure described above. Separation by column chromatography, eluting with hexane/EtOAc (2/1), afforded first pure acylthioimidate **8** (1.2 g, 3.27 mmol, 64%) as a yellow solid: mp 200 °C dec; R_f = 0.29 (hexane/EtOAc, 1/1); $[\alpha]^{22}_D = -33.5°$ (*c* 0.89, DMSO); ^{1}H NMR (DMSO) δ 8.0 (dd, 1H, J = 9.0, 2.5), 7.9 (d, 1H, J = 2.4), 7.8 (d, 1H, J = 8.0), 6.7 (d, 1H, J = 9.1), 6.5 (s, 1H), 4.4 (m, 1H), 3.2 (dd, 1H, J = 18.0, 9.0), 2.7 (dd, 1H, J = 18.0, 3.7), 1.35 (s, 1H); ^{13}C NMR δ 212.6, 177.2, 156.2, 151.7, 136.0, 127.0, 126.7, 118.4, 114.7, 80.0, 57.2, 36.7, 28.6. Anal. Calcd for $C_{15}H_{18}N_4O_5S$: C, 49.2; H, 4.9; N, 15.3. Found: C, 49.3; H, 4.9; N, 14.9.

Further elution with hexane/EtOAc (2/1) gave thioanilide **7e** (300 mg, 0.76 mmol, 16% yield): mp 146−147 °C after two crystallizations from THF; R_f = 0.20 (hexane/EtOAc, 1/1); $[\alpha]^{22}_D = -87.5°$ (*c* 0.88, DMSO); ^{1}H NMR (DMSO) δ 10.17 (s, 1H), 7.9 (dd, 1H, J = 9.0, 2.5), 7.8 (s, 1H), 7.5 (d, 1H, J = 5.9), 6.7 (d, 1H, J = 9.0), 6.4 (s, 2H), 4.7 (dd, 1H, J = 13.8, 6.2), 3.5 (s, 3H), 3.0 (dd, 1H, J = 16.6, 5.8), 2.8 (dd, 1H, J = 16.6, 8.0), 1.3 (s, 9H); ^{13}C NMR δ 205.9, 171.0, 156.1, 151.2, 135.7, 125.5, 125.2, 122.9, 114.2, 79.3, 57.7, 52.0, 28.6. Anal. Calcd for $C_{16}H_{22}N_4O_6S$: C, 48.2; H, 5.6; N, 14.1. Found: C, 48.3; H, 5.5; N, 14.1.

α-*N*-BOC-L-aspartic acid *β*-*tert*-butyl ester α-2-amino-5-nitrothioanilide (7f): yield 85%; mp 204 °C; $[\alpha]^{22}_D = -89.4°$ (*c* 1.0, DMSO); ^{1}H NMR (DMSO) δ 10.21 (s, 1H), 7.9 (dd, 1H, J = 9.1, 6.5), 7.7 (s, 1H), 7.4 (d, 1H, J = 6.1), 6.7 (d, 1H, J = 9.0), 6.4 (s, 2H), 4.6 (m, 1H), 2.9 (dd, 1H, J = 16.0, 7.4), 2.7 (dd, 1H, J = 16.0, 7.4), 1.38 (s, 9H); ^{13}C NMR δ 210.8, 174.6, 160.7, 156.0, 140.5, 130.2, 130.0, 127.7, 119.0, 85.6, 84.0, 62.5, 33.0, 26.0, 19.3. Anal. Calcd for $C_{19}H_{28}N_4O_6S$: C, 51.8; H, 6.4; N, 12.7. Found: C, 51.8; H, 6.5; N, 12.8.

General Procedure for the Preparation of Benzotriazoles 9a−**d,f.** To a solution of thioanilide **7** (2 mmol) dissolved by gentle warming at 40 °C and then cooled to 0 °C in glacial acetic acid (diluted with 5% water, 15 mL) was added $NaNO_2$ (0.21 g, 3 mmol) in portions over 5 min with stirring. After 30 min, ice water (∼100 mL) was added, and the precipitated product was filtered and washed with water. The orange solid was dried *in vacuo* at rt overnight and then at 50 °C for 4 h to afford benzotriazoles **9a**−**d** and **9f** as amorphous solids of sufficient purity for use in the next step.

(13) Commercial P_4S_{10} was purified by Soxhlet extraction with carbon disulfide. The crystalline P_4S_{10} which formed in the boiler flask was used in these procedures.

1-(*N*-BOC-L-thionoalaninyl)-6-nitrobenzotriazole (9a): yield 79%; mp 124 °C (ether); $[\alpha]^{22}_D = +20.7°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.6 (s, 1H), 8.44 (d, 1H, *J* = 9.0), 8.30 (d, 1H, *J* = 9.0), 6.19 (m, 1H), 5.4 (d, 1H, 8.6), 1.63 (d, 3H, *J* = 6.8), 1.4 (s, 9H); ^{13}C NMR δ 212.0, 149.5, 148.7, 131.4, 122.2, 121.5, 112.9, 80.1, 56.7, 28.4, 22.5. Anal. Calcd for $C_{14}H_{17}N_5O_4S$: C, 47.9; H, 4.9; N, 19.9. Found: C, 48.0; H, 5.0; N, 19.9.

1-(*N*-BOC-L-thionophenylalaninyl)-6-nitrobenzotriazole (9b): yield 81%; mp 127−128 °C (ether); $[\alpha]^{22}_D = +223.2°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.6 (s, 1H), 8.4 (d, 1H, *J* = 8.6), 8.2 (d, 1H, *J* = 8.4), 7.2 (s, 5H), 6.5 (s, 1H), 5.4 (m, 1H), 3.3 (m, 1H), 3.0 (m, 1H), 1.39 (s, 9H); ^{13}C NMR δ 209.0, 155.1, 149.6, 149.0, 153.3, 131.7, 129.4, 128.5, 127.3, 122.2, 121.5, 112.7, 80.6, 62.1, 42.8, 28.3. Anal. Calcd for $C_{20}H_{21}N_5O_4S$: C, 56.2; H, 5.0; N, 16.4. Found: C, 56.3; H, 5.0; N, 16.4.

1-(*N*-BOC-L-thionovalinyl)-6-nitrobenzotriazole (9c): yield 77%; amorphous; $[\alpha]^{22}_D = +43.9°$ (*c* 1.0, $CHCl_3$) ^1H NMR ($CHCl_3$) δ 9.7 (s, 1H), 8.4 (d, 1H, *J* = 8.8), 8.3 (d, 1H, *J* = 8.9), 6.1 (m, 1H), 5.4 (d, 1H, *J* = 9.6), 2.3 (m, 1H), 1.4 (s, 9H), 1.08 (d, 3H, *J* = 6.6), 0.97 (d, 3H, *J* = 6.8); ^{13}C NMR δ 210.0, 155.6, 149.5, 149.1, 131.8, 122.2, 121.5, 112.8, 80.4, 65.7, 34.3, 28.3, 20.2, 17.0. Anal. Calcd for $C_{16}H_{21}N_5O_4S$: C, 50.7; H, 5.6; N, 18.5. Found: C, 50.8; H, 5.6; N. 18.4.

1-(*N*-BOC-*O*-benzyl-L-thionoserinyl)-6-nitrobenzotriazole (9d): yield 83%; amorphous; $[\alpha]^{22}_D = -6.2°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.6 (s, 1H), 8.4 (d, 1H, *J* = 7.6), 8.2 (d, 1H, *J* = 8.9), 7.2 (s, 1H), 7.1 (m, 5H), 6.3 (m, 1H), 5.8 (d, 1H, *J* = 8.7), 4.5 (dd, 2H, *J* = 31.7, 12.0), 3.9 (m, 2H), 1.6 (s, 9H); ^{13}C NMR δ 205.7, 155.3, 149.6, 148.9, 137.0, 131.9, 128.3, 127.8, 127.6, 122.1, 121.4, 112.8, 80.6, 73.2, 71.8, 61.0, 28.4. Anal. Calcd for $C_{21}H_{23}N_5O_4S$: C, 55.1; H, 5.1; N, 15.3. Found: C, 55.3; H, 5.0; N, 15.1.

1-(*N*-BOC-*O*-*β*-*tert*-butyl-L-α-thionoaspartyl)-6-nitrobenzotriazole (9f). To a solution of thioanilide **7f** (330 mg, 0.75 mmol) dissolved in THF/AcOH (1/1, 5 mL) at rt and then cooled to 0 °C was added $NaNO_2$ (77 mg, 1.1 mmol) with stirring. After 30 min, EtOAc/heptane (3/1, 60 mL) was added, and the solution was washed successively with H_2O (3 × 30 mL), $NaHCO_3$ (3 × 30 mL), and brine, was dried, and then was evaporated to dryness, to give a pale yellow foam that was immediately used in the next step: yield 72%; ^1H NMR ($CDCl_3$) δ 9.6 (s, 1H), 8.4 (d, 1H, *J* = 7.2), 8.3 (d, 1H, *J* = 9.0), 6.3 (m, 1H), 5.9 (br s, 1H), 3.0 (dd, 1H, *J* = 15.0, 5.3), 2.8 (dd, 1H, *J* = 14.5, 6.8), 1.37 (s, 9H), 1.36 (s, 9H); ^{13}C NMR δ 210.0, 168.5, 146.6, 146.3, 126.3, 122.3, 121.6, 117.2, 116.6, 112.7, 82.3, 58.1, 41.1, 28.3, 28.2, 28.0, 27.9.

Procedure for Coupling Thioacylating Reagents 9a and 9b with (*R*)-α-Methylbenzylamine and HPLC Analysis. To a cooled solution (0 °C) of thioacylating reagent **9a** or **9b** (2 mmol) in 30 mL of THF was added dropwise a solution of α-methylbenzylamine (0.26 mL, 2 mmol) in 10 mL of THF over a period of 40 min. After the addition was completed, insoluble material was removed by filtration, and the filtrate was evaporated and the residue chromatographed (hexane/EtOAc, 2/1) to give **10a** or **10b** in 88% and 86% yield, respectively. To determine the enantiomeric purities of the products, crude samples of **10a** and **10b** were analyzed by HPLC, and the results were compared with those obtained from samples prepared from treatment of racemic **9a** and **9b** with (*R*)-α-methylbenzylamine. The HPLC analysis revealed that the er's of **10a** and **10b** were >99/1. For *N*-BOC-DL-alanine (*R*)-α-methylbenzylthioamide: mobile phase hexane/EtOAc (8/1); flow rate 1 mL/min; for *S*,*R* diastereomer, t_R 17.4 min; for *R*,*R* diastereomer, t_R 20.6 min. For *N*-BOC-DL-phenylalanine (*R*)-α-methylbenzylthioamide: mobile phase hexane/EtOAc (8/1); flow rate 1 mL/min; for *S*,*R* diastereomer, t_R 11.4 min; for *R*,*R* diastereomer, t_R 12.2 min.

***N*-BOC-L-alanine α-methylbenzylthioamide (10a):** oil; R_f = 0.54 (hexane/EtOAc, 1/1); $[\alpha]^{22}_D = +84.8°$ (*c* 1.3, $CHCl_3$); ^1H NMR ($CHCl_3$) δ 8.7 (s, 1H), 7.2 (s, 5H), 5.6 (m, 1H), 5.3 (s, 1H), 1.5 (d, 3H, *J* = 6.9), 1.4 (d, 3H, *J* = 6.8), 1.3 (s, 9H); ^{13}C NMR δ 204.0, 155.7, 141.3, 128.7, 127.5, 126.4, 80.2, 54.1, 28.3, 21.4, 20.5. Anal. Calcd for $C_{16}H_{24}N_2O_2S$: C, 62.3; H, 7.8; N, 9.1. Found: C, 62.2; H, 8.0; N, 9.1.

***N*-BOC-L-phenylalanine α-methylbenzylthioamide (10b):** mp 112 °C; R_f = 0.6 (hexane/EtOAc, 1/1); $[\alpha]^{22}_D = +115°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$ δ 7.5 (s, 1H), 7.29−7.1 (m, 10H), 5.5 (s, 1H), 4.5 (dd, 1H, *J* = 14.0, 6.2), 3.2 (dd, 1H, *J* = 12.9, 5.8), 3.1 (m, 1H), 1.7 (m, 1H), 1.49 (s, 9H), ^{13}C NMR δ 201.4, 155.2, 141.0, 136.9, 129.3, 128.7, 127.7, 126.4, 80.3, 63.0, 54.2, 41.9, 28.3, 19.9. Anal. Calcd for $C_{22}H_{28}N_2O_2S$: C, 68.7; H, 7.4; N, 7.3. Found: C, 68.9; H, 7.5; N, 7.2.

General Procedure for Coupling Thioacylating Reagents 9c,d,f with L-Phenylalanine Methyl Ester. Formation of Thiopeptides 11c,d,f. To a cooled solution (0 °C) of thioacylating reagent **9c**,**d**,**f** (2 mmol) in 30 mL of THF was added dropwise a solution of L-phenylalanine methyl ester (2 mmol, generated by treating L-phenylalanine methyl ester hydrochloride with 100 mol % of triethylamine at 0 °C) in 10 mL of THF over a period of 40 min. After the addition was completed, evaporation of the solvent and purification of the residue by chromatography (hexane/EtOAc, 2/1) afforded thiopeptides **11c**,**d**,**f** in 82−85% yield.

***N*-BOC-L-thionovalyl-L-phenylalanine methyl ester (11c):** oil; R_f = 0.8 (hexane/EtOAc, 1/1); $[\alpha]^{22}_D = +44.4°$ (*c* 0.8, $CHCl_3$); ^1H NMR ($CHCl_3$) δ 8.2 (d, 1H, *J* = 6.6), 7.25 (m, 3H), 7.1 (d, 2H, *J* = 6.5), 5.4 (m, 1H), 5.2 (s, 1H), 4.0 (m, 1H), 3.7 (s, 3H), 3.3 (dd, 1H, *J* = 14.0, 6.1), 3.1 (dd, 1H, *J* = 14.0, 5.1), 1.43 (s, 9H), 0.9 (d, 6H, *J* = 6.4); ^{13}C NMR δ 205.6, 170.9, 155.6, 135.3, 129.3, 128.3, 127.3, 58.3, 52.4, 36.5, 33.1, 28.3, 19.6, 17.9. Anal. Calcd for $C_{20}H_{30}N_2O_4S$: C, 60.9; H, 7.7; N, 7.1. Found: C, 60.6; H, 7.5; N, 7.4.

HPLC analysis established the er of the product to be >99/1 by comparison with a sample prepared from **9c** with racemic DL-phenylalanine methyl ester. For *N*-BOC-L-thionovalyl-DL-phenylalanine methyl ester: mobile phase hexane/EtOAc, 8/1; flow rate 1 mL/min; for *S*,*S* diastereomer, t_R 11.4 min; for *S*,*R* diastereomer, t_R 16.2 min.

***N*-BOC-*O*-benzyl-L-thionoserinyl-L-phenylalanine methyl ester (11d):** mp 79−80 °C (hexane); R_f = 0.79 (hexane/EtOAc, 1/1); $[\alpha]^{22}_D = +116.8°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.6 (s, 1H), 7.3 (m, 5H), 7.2 (m, 3H), 7.1 (d, 2H, *J* = 2.9), 5.3 (m, 1H), 4.5 (d, 2H, *J* = 4.7), 4.0 (m, 1H), 3.6 (s, 3H), 3.3 (dd, 1H, *J* = 14.0, 60), 3.1 (dd, 1H, *J* = 13.8, 5.0), 1.4 (s, 9H); ^{13}C NMR δ 200.0, 170.6, 155.0, 137.3, 135.4, 129.3, 128.5, 128.0, 127.2, 80.6, 58.6, 52.4, 36.3, 28.2, 28.0. Anal. Calcd for $C_{25}H_{32}N_2O_5S$: C, 63.5; H, 5.9; N, 5.9. Found: C, 63.7; H, 6.7; N, 5.9.

(*N*-BOC-*O*-*β*-*tert*-butyl-L-α-thionoaspartyl)-L-phenylalanine methyl ester (11f): oil; R_f = 0.57 (hexane/EtOAc, 3/1); $[\alpha]^{22}_D = +71.4°$ (*c* 0.77, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.79 (br s, 1H), 7.32−7.25 (m, 3H), 7.15 (d, 2H, *J* = 6.7), 5.8 (br s, 1H), 5.29 (m, 1H), 4.7 (br s, 1H), 3.7 (d, 3H, *J* = 4.5), 3.29 (dd, 1H, *J* = 13.8, 6.2), 3.18 (dd, 1H, *J* = 13.9, 5.4), 3.05 (d, 1H, *J* = 12.9), 2.7 (m, 1H), 1.43 (s, 9H), 1.42 (s, 9H); ^{13}C NMR δ 202.3, 171.0, 170.6, 155.1, 135.4, 129.3, 129.2, 128.6, 127.3, 81.8, 80.5, 58.8, 58.6, 56.7, 52.4, 40.4, 36.5, 28.3, 28.0. Anal. Calcd for $C_{23}H_{34}N_2O_6S$: C, 59.2; H, 7.4; N, 6.0. Found: C, 58.7; H, 7.4; N, 6.3.

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