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Derivatized Amino Acids Relevant to Native Peptide Synthesis by Chemical Ligation and Acyl Transfer

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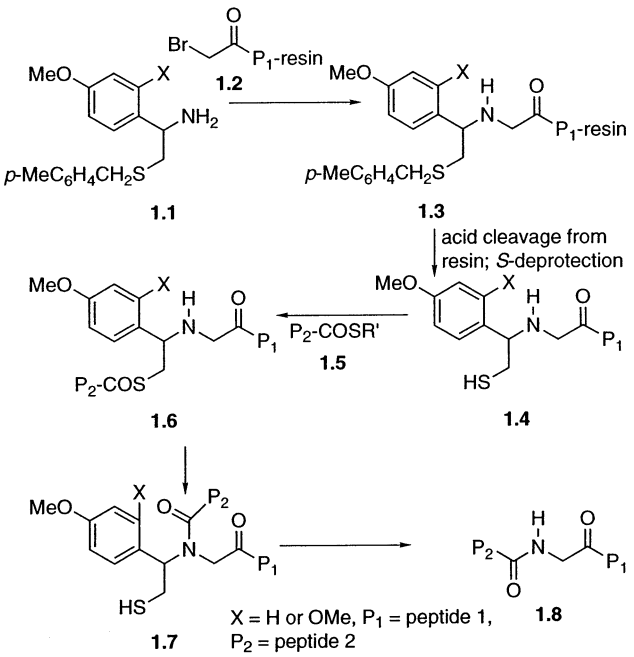
Received June 13, 2003

Three amino acids were converted into the derivatives **5.2** (from glycine), **6.4a** and **6.4b** (from alanine), and **8.3a** and **8.3b** (from *O*-benzyl serine). These N-alkylated amino acids, which can be deprotected after conversion of the carboxyl into an amide, correspond to the general structure **2.1**, a compound class of use in the study of peptide segment coupling by the ligation−acyl transfer method.

Introduction

The synthesis of large peptides and proteins by native chemical ligation[1] has attracted considerable interest, and many impressive applications have been reported.[1,2] Although the method constitutes a dramatic advance in the area of peptide and protein synthesis, the requirement for a cysteine residue at the ligation site can be an awkward restriction because cysteine is not a common amino acid, making up only 1.7%[3,4] of the residues in proteins. Accordingly, much effort has been devoted to modifying the original native chemical ligation method so that it can afford peptides with other amino acids at the ligation site.[5,6] The most ambitious development has been pioneered by Kent et al.[6] and is based on the design

SCHEME 1



of an auxiliary that is initially attached to nitrogen.[5q,r,t,6] The auxiliary must allow ligation and acyl transfer and should then be removable. In principle, the auxiliary could be attached to the eventual nitrogen terminus of one complete peptide segment; alternatively, it could be attached to each of the common amino acids, and then the appropriate derivatized amino acid, already carrying the auxiliary, would be used in the last step of assembling the peptide segment. The first of these methods has been investigated in much greater detail than the second.

Kent's method to allow ligation by means of an auxiliary at a noncysteinyl residue is based on the amines **1.1** (X = H or OMe) (Scheme 1).[6] The auxiliaries have

(1) (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776−779. (b) Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem*. **2000**, *69*, 923−960. (c) Coltart, D. M. *Tetrahedron* **2000**, *56*, 3449−3491.

(2) Wilken, J.; Kent, S. B. H. *Curr. Opin. Biotechnol*. **1998**, *9*, 412−426.

(3) (a) McCaldon, P.; Argos, P. *Proteins* **1988**, *4*, 99−122. (b) Low, D. W.; Hill, M. G.; Carrasco, M. R.; Kent, S. B. H.; Botti, P. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 6554−6559.

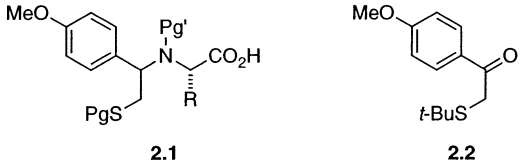
(4) Voet, D.; Voet, J. G. *Biochemistry*, 2nd ed.; Wiley: New York, 1995; p 58.

(5) For leading references, see: (a) Liu, C.-F.; Rao, C.; Tam, J. P. *Tetrahedron Lett*. **1996**, *37*, 933−936. (b) Huang, H.; Carey, R. I. *J. Peptide Res*. **1998**, *51*, 290−296. (c) Gieselman, M. D.; Xie, L.; van der Donk, W. A. *Org. Lett*. **2001**, *3*, 1331−1334. (d) Quaderer, R.; Sewing, A.; Hilvert, D. *Helv. Chim. Acta* **2001**, *84*, 1197−1206. (e) Hondal, R. J.; Nilsson, B. L.; Raines, R. T. *J. Am. Chem. Soc*. **2001**, *123*, 5140−5141. (f) Yan, L. Z.; Dawson, P. E. *J. Am. Chem. Soc*. **2001**, *123*, 526−533. (g) Tam, J. P.; Yu, Q. *Biopolymers* **1998**, *46*, 319−327. (h) Miao, Z.; Tam, J. P. *Org. Lett*. **2000**, *2*, 3711−3713. (i) Zhang, L.; Tam, J. P. *Tetrahedron Lett*. **1997**, *38*, 3−6. (j) Muir, T. W.; Dawson, P. E.; Kent, S. B. H. *Methods Enzymol*. **1997**, *289*, 266−298. (k) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett*. **2001**, *3*, 9−12. (l) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett*. **2000**, *2*, 1939−1941. (m) Soellner, M. B.; Nilsson, B. L.; Raines, R. T. *J. Org. Chem*. **2002**, *67*, 4993−4996. (n) Nilsson, B. L.; Hondal, R. J.; Soellner, M. B.; Raines, R. T. *J. Am. Chem. Soc*. **2003**, *125*, 5268−5269. (o) Canne, L. E.; Bark, S. J.; Kent, S. B. H. *J. Am. Chem. Soc*. **1996**, *118*, 5891−5896. (p) Shao, Y.; Lu, W.; Kent, S. B. H. *Tetrahedron Lett*. **1998**, *39*, 3911−3914. (q) Offer, J.; Boddy, C. N. C.; Dawson, P. E. *J Am. Chem. Soc*. **2002**, *124*, 4642−4646. (r) Offer, J.; Dawson, P. E. *Org. Lett*. **2000**, *2*, 23−26. (s) Hackeng, T. M.; Griffin, J. H.; Dawson, P. E. *Proc. Natl. Acad. Sci. U. S. A*. **1999**, *96*, 10068−10073. (t) Kawakami, T.; Akaji, K.; Aimoto, S. *Org. Lett*. **2001**, *3*, 1403−1405. (u) Marinzi, C.; Bark, S. J.; Offer, J.; Dawson, P. E. *Bioorg. Med. Chem*. **2001**, *9*, 2323−2328.

(6) Botti, P.; Carrasco, M. R.; Kent, S. B. H. *Tetrahedron Lett*. **2001**, *42*, 1831−1833.

10.1021/jo030192r CCC: $25.00
Published on Web 10/28/2003

SCHEME 2

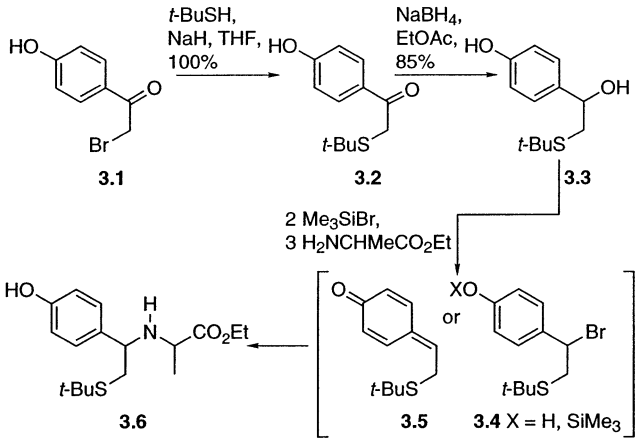


the essential property of resisting cleavage under the acidic conditions used in peptide synthesis as long as they are linked to an *amine* nitrogen; however, after ligation and acyl transfer, the attachment is to an *amide* nitrogen (see **1.7**) and each auxiliary is now easily cleaved by acid. The auxiliaries are attached to a resin-bound peptide via bromide displacement (Scheme 1, **1.1** + **1.2** → **1.3**).[6] After deprotection and resin cleavage (**1.3** → **1.4**), reaction with a thioester segment (**1.5**) results in ligation and acyl transfer (**1.4** → **1.6** → **1.7**). Finally, the auxiliary is removed under acidic conditions, giving the native peptide **1.8**.[3b,6]

Discussion

Our aim was to prepare specially derivatized amino acids that could be incorporated as the N-terminus of a peptide, the nature of the derivatization being such that sequential reaction with a thioester segment, acyl transfer, and detachment of the auxiliary could be achieved. The compounds we have made for this purpose correspond to structure **2.1** (Pg, Pg′ = protecting groups),[7] in which the role of the methoxy group is to ensure acid lability of the auxiliary *after* acyl transfer. If an amino acid corresponding to **2.1** is incorporated as the N-terminus of a peptide segment, then removal of the nitrogen and sulfur protecting groups and reaction with another peptide segment having a C-terminal thioester should result in thioester exchange, followed by S → N acyl transfer. Removal of the auxiliary would then give a native peptide. The availability of a range of derivatized amino acids **2.1**, preferably with values of R corresponding to all the common amino acids, would allow a detailed study to be made of the permissible values of R for each of the steps ligation, acyl transfer, and deprotection to work efficiently.Of a number of potential routes to **2.1**, our preference[7] was to make the compounds from protected amino acids and so we sought methods in which the C−N bond linking the amino acid unit to the auxiliary is the key step. We generally protected the sulfur as a *tert*-butyl thioether (**2.1**, Pg = Bu-*t*) but have also used a 4-methylbenzyl thioether (**2.1**, Pg = $CH_2C_6H_4$-Me-4); these protecting groups behaved well in the synthesis of **2.1** and can be removed easily in order to prime the auxiliary for ligation.

SCHEME 3

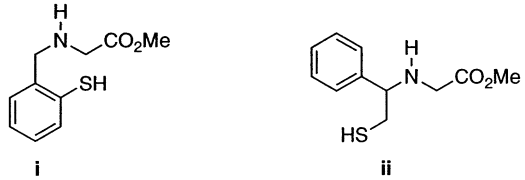


We examined a number of potential methods for nitrogen protection and, after considerable exploratory work, found that the nitrogen was best protected as its Troc carbamate (**2.1**, Pg′ = $C(O)OCH_2CCl_3$).

Our initial studies[7] were based on the use of ketone **2.2** for reductive amination with $H_2NCH_2CO_2Me$. These experiments were unsuccessful,[8] and after a great deal of further effort, we were led to consider the possibility of using quinone methides[9] as precursors to the auxiliary segment.[8] To this end, phenolic alcohol **3.3** was prepared from the known[10] bromide **3.1**, as shown in Scheme 3, and treated with 2 equiv of Me_3SiBr and 3 equiv of freshly distilled (±)-alanine ethyl ester. The expected product, **3.6**, was obtained as a 1:1 mixture of two diastereoisomers in 58% yield. Optimization of the conditions showed that use of 1 equiv of Me_3SiBr and 2 equiv of (±)-alanine ethyl ester was just as effective. Although formation of a quinone methide (**3.5**) does account for the observed products, we have no evidence to exclude the possibility of direct nucleophilic displacement from bromide **3.4** (X = H or $SiMe_3$). At this point, our plan to make compounds of type **2.1** would require that the phenolic hydroxyl of **3.6** be methylated selectively, but instead, we examined a corresponding series of reactions in which a methoxy substituent was actually in place from the beginning.

In these experiments, bromide **4.1**[11] was converted into the sulfide **2.2** (Scheme 4) and reduction then gave alcohol **4.2**. When this compound was treated with Me_3SiBr and glycine ethyl ester, using conditions optimized for the phenolic series of Scheme 3, we obtained a good yield of the desired N-alkylated product **4.3**. We had appreciated the possibility that a reactive entity generated at C(α) (see **4.2**) might be captured by neighboring

(7) Our preliminary studies (see: Coltart, D. M., Ph.D. Thesis, University of Alberta, 2000) were based on thiophenolic and 1-phenyl-2-mercaptoethyl systems exemplified by **i** and **ii**. Work on the former was stopped when similar studies by Dawson and Offer (ref 5r) were published. A noteworthy feature of **ii** (and **2.1**) is that the mercapto group ultimately released is an *alkyl* thiol and, as such, is more nucleophilic than an *aryl* thiol (cf. **i**).



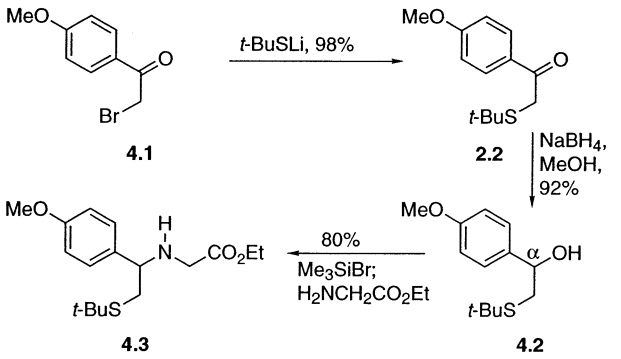
(8) The possibility of preparing compounds of type **2.1** by reductive amination or by halide displacement (cf. the present method) is shown in a generic scheme in two patents: (a) Botti, P.; Bradburne, J. A.; Kent, S. B. H.; Low, D. W. PCT Int. Appl. WO 2002/20557. (b) Kent, S. B. H.; Botti, P.; Low, D. W.; Bradburne, J. A.; Hunter, C. L., Chen, S.-Y.; Cressman, S.; Kochendoerfer, G. PCT Int. Appl. WO 2002/20034.

(9) Shevchenko, S. M.; Apushkinskii, A. G.; Gindin, V. A.; Zarubin, M. Ya. *Russ. J. Org. Chem*. **1990**, *26*, 921−925.

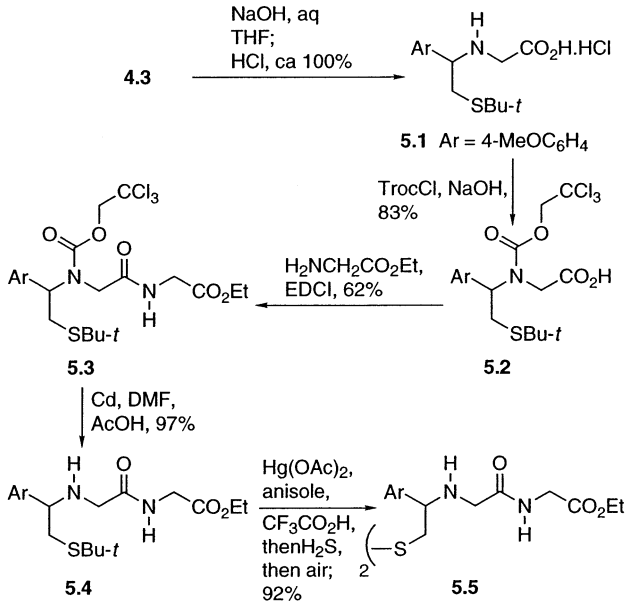
(10) Park, C.-H.; Givens, R. S. *J. Am. Chem. Soc*. **1997**, *119*, 2453−2463.

(11) Nieuwenhuis, S. A. M.; Vertegaal, L. B. J.; de Zoete, M. C.; van der Gen, A. *Tetrahedron* **1994**, *50*, 13207−13230.

SCHEME 4



SCHEME 5



group participation of the sulfur, followed by loss of the *tert*-butyl group; evidently, this is not a significant side reaction, if it occurs at all.

When alcohol **4.2** was treated with Me_3SiBr, the expected bromide was formed, but could not be purified, and so the procedure summarized in Scheme 4, in which the bromide is generated in situ, was followed.[12]

With a method for making **4.3** now available, the next tasks were to deprotect the carboxyl and protect the nitrogen to arrive at a structure that corresponds to **2.1**. In the present case (**4.3**), hydrolysis of the ethyl ester was easily effected (Scheme 5) with aqueous NaOH or LiOH and we used the resulting amino acid (**5.1**) to evaluate a number of protecting groups (Fmoc, Boc, Troc) for nitrogen, but only Troc proved satisfactory as it alone could be removed in good yield.

Derivatized Glycine. The nitrogen of **5.1** could be protected as its Troc carbamate under standard conditions (Scheme 5).[13] Coupling with glycine ethyl ester gave **5.3**, and the Troc group was removed (**5.3** → **5.4**) by the action of cadmium in DMF−AcOH, according to a known procedure.[14] The sulfur protecting group was removed by treatment with $Hg(OAc)_2$[15] in the presence of anisole (as a cation scavenger) and CF_3CO_2H. The required thiol was then released by treatment with H_2S, and oxidation, which occurred during exposure to air, afforded disulfide **5.5**. An attempt to effect oxidation with I_2−MeOH was unsuccessful and did not afford the expected disulfide. It should be noted that a disulfide is suitable for ligation chemistry when that is done in the presence of a reducing agent.[16]

The yields in the sequence of Scheme 5 were acceptable, and the route represents our optimized version of making a specially derivatized glycine corresponding to the general structure **2.1**. Coupling of **5.2** with $H_2NCH_2CO_2Et$ was, of course, done to demonstrate in a simple way that deprotection of both nitrogen and sulfur could be accomplished in a situation similar to the one that would be present when the derivatized amino acid **5.2** is the N-terminus of a peptide.

Derivatized Alanine and Serine. At this point we examined two other amino acids, L-alanine and L-serine, and for both of these it was convenient to protect the carboxyl group as its *tert*-butyl ester; the ethyl ester is suitable only for glycine, where the absence of an asymmetric center allows base hydrolysis to be used. We hoped that nitrogen protection as a Troc carbamate would again prove suitable, and in the event, this was the case.

Alcohol **4.2** was coupled with L-alanine *tert*-butyl ester (**6.1**)[17] under our optimized conditions to afford a separable mixture of the less polar (**6.2a**, 31%) and more polar (**6.2b**, 30%) adducts, respectively. The former was converted into the protected acid **6.4a**, and again we carried out a model sequence: coupling with $H_2NCH_2CONHBn$[25] (**6.4a** → **6.5a**), removal of the Troc group (Cd, DMF, AcOH, 74%; **6.5a** → **6.6a**), and deprotection of the sulfur ($Hg(OAc)_2$, CF_3CO_2H, anisole, H_2S) to obtain **6.7a**, largely

(12) We also investigated briefly the conversion of **4.2** into the corresponding chloride (Cl instead of OH in **4.2**). This was achieved by treatment with $SOCl_2$ (see Supporting Information). The chloride could not be purified by chromatography and was used crude; reaction with glycine ethyl ester was slow (CH_2Cl_2, rt, 6 h) and less efficient (64% of **4.3**) than the route of Scheme 4.

(13) Carson, J. F. *Synthesis* **1981**, 268−270.

(14) Di Giorgio, C.; Pairot, S.; Schwergold, C.; Patino, N.; Condom, R.; Farese-Di Giorgio, A.; Guedj, R. *Tetrahedron* **1999**, *55*, 1937−1958.

(15) Nishimura, O.; Kitada, C.; Fujino, M. *Chem. Pharm. Bull.* **1978**, *26*, 1576−1585.

(16) (a) Burns, J. A.; Butler, J. C.; Moran, J.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 2648−2650. (b) Tam, J. P.; Lu, Y.-A.; Liu, C.-F.; Shao, J. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 12485−12489.

(17) L-Alanine was protected as its Cbz carbamate (CbzCl, aqueous NaOH, 93%) (ref 18) and the *tert*-butyl ester was prepared using *tert*-butyl trichloroacetimidate [$Cl_3C(=NH)OBu$-*t*, $BF_3{\cdot}Et_2O$, 87%] (refs 19, 20). Hydrogenolysis then gave L-alanine *tert*-butyl ester (**6.1**) (ref 24). The Mosher amide was prepared and found to give a single peak in the ^{19}F NMR spectrum; the Mosher amides of racemic material give separated signals (^{19}F δ −69.6 and −69.4 ppm).

(18) (a) Bergmann, M.; Zervas, L. *Ber. Dtsch. Chem. Ges.* **1932**, *65*, 1192−1205. (b) Cf. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*, 2nd ed.; Springer: Berlin, 1944; pp 11−13.

(19) Armstrong, A.; Brackenridge, I.; Jackson, R. F. W.; Kirk, J. M. *Tetrahedron Lett.* **1988**, *29*, 2483−2486.

(20) (a) Other reagents, such as DCC-DMAP-*t*-BuOH (ref 21); Boc_2O-DMAP-*t*-BuOH (ref 22) gave partially racemized material. (b) Transesterification with *tert*-butyl acetate (ref 23) was very slow and proceeded in poor yield. (c) For other preparations of *tert*-butyl esters, see: Anderson, G. W.; Callahan, F. M. *J. Am. Chem. Soc.* **1960**, *82*, 3359−3363.

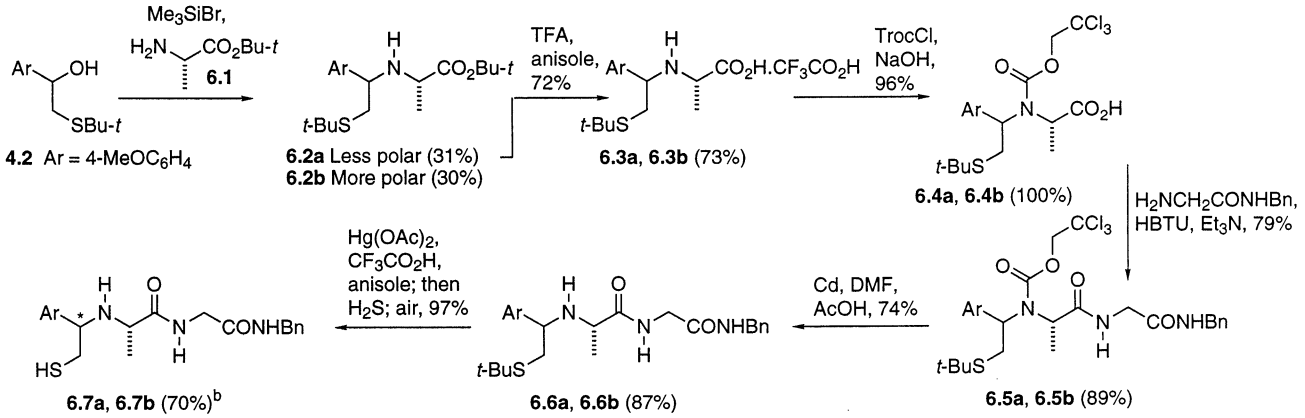
(21) Wiener, H.; Gilon, C. *J. Mol. Catal.* **1986**, *37*, 45−52.

(22) Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. *Synthesis* **1994**, 1063−1066.

(23) Taschner, E.; Chimiak, A.; Bator, B.; Sokolowska, T. *Liebigs Ann. Chem.* **1961**, *646*, 134−136.

(24) (a) Strazzolini, P.; Melloni, T.; Giumanini, A. G. *Tetrahedron* **2001**, *57*, 9033−9044. (b) L-Alanine *tert*-butyl ester is commercially available.

SCHEME 6[a,b]



[a] Yields beside the arrows refer to the "**a**" series, those in parentheses to the "**b**" series. [b]The product **6.7b** was isolated as the disulfide.

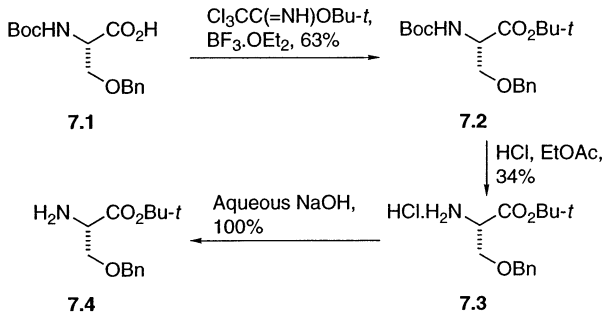
in the form of the thiol. The intermediate **6.6a** had an ee of 99.5%.The same reactions were repeated with the more polar isomer **6.2b** to give a final product (**6.7b**) that differs stereochemically from **6.7a** at the starred atom. The intermediate **6.6b** had an ee of 99.5%.

For comparison purposes, racemic amides corresponding to **6.6a** and **6.6b** were prepared from racemic alanine. Again a more polar and a less polar isomer were obtained, differing in stereochemistry at the benzylic carbon of the auxiliary.[28]

Finally, we decided to study an example of an amino acid with a functionalized side chain and chose L-serine as a suitable representative of this class. The required starting material was *O*-benzyl L-serine *tert*-butyl ester (**7.4**). Although this is a known compound,[24a] several of the literature methods we examined for making it caused extensive epimerization in the step in which the carboxyl group was converted[20] into its *tert*-butyl ester. We eventually found that the route shown in Scheme 7 is satisfactory.

N-Boc *O*-benzyl L-serine (Aldrich) was converted into its *tert*-butyl ester **7.2**[29] by the action of *tert*-butyl trichloroacetimidate.[19] Removal of the *N*-Boc group with HCl in EtOAc[30,31] took place without disturbing the ester, and the free amine (**7.4**) was obtained from the hydrochloride salt by treatment with NaOH. However, the acid treatment for the N-deprotection should not be extended beyond 14 h; hydrochloride obtained at that time (34% yield) has an ee of ≥94%, as judged by ^{19}F NMR measurements on the derived Mosher amides and was used in further work. An additional crop, obtained after 36 h (23%), had an ee of 57%.

SCHEME 7



The serine derivative **7.4** was subjected to the series of reactions shown in Scheme 8; the reactions are identical to those used for L-alanine, except for the fact that the *tert*-butyl ester unit was deprotected with $Me_3SiOSO_2CF_3$, since CF_3CO_2H caused cleavage of the benzylic C−N bond. The final products were obtained as mixtures of the disulfides shown (**8.6a** and **8.6b**) and the corresponding thiols. We did not examine **8.5a** or **8.5b** by chiral HPLC; no epimerization was found in the corresponding alanine series, as mentioned above.

Derivatized Glycine with *p*-Methylbenzyl Protection of Sulfur. To show additional generality of our method, we decided to make compound **10.5** (see Scheme 10), which has the same sulfur protecting group that was used by the Kent[6] and Dawson[5u] groups in their bromide displacement method.[6] *p*-Methylbenzyl chloride (**9.1**) was converted into thioacetate **9.2**, and treatment with BuLi gave the thiolate **9.3**. This was alkylated with bromide **4.1**,[6,11] and finally, reduction gave alcohol **9.5**. The alcohol was treated with $H_2NCH_2CO_2Et$ and Me_3SiBr, according to our general procedure, and the product **10.1** (Scheme 10) was hydrolyzed and protected on nitrogen with TrocCl. Coupling in the usual way with H_2NCH_2-CONHBn and removal of the Troc group gave **10.5**. The

(25) The compound was prepared as follows: CF_3CO_2H (5 mL) was added slowly to a stirred and cooled (0 °C) solution of $BocHNCH_2$-CONHBn (ref 26) (2.65 g, 10.0 mmol) in dry CH_2Cl_2 (5 mL). Stirring was continued at 0 °C for 3.5 h, and the solvent was evaporated. The residue was mixed with Et_2O (50 mL), and the resulting precipitate was filtered, washed thoroughly with Et_2O, and left under oil pump vacuum to give CF_3CO_2H. $H_2NCH_2CONHBn$ (ref 27) (2.79 g, 100%) as a white solid: mp 154−157 °C; FTIR (microscope) 1672 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 3.70 (s, 2 H), 4.42 (s, 2 H), 7.24−7.32 (m, 5 H) (three protons not observed); ^{13}C NMR (CD_3OD, 100 MHz) δ 41.5 (t′), 44.3 (t′), 128.5 (d′), 128.7 (d′), 129.6 (d′), 139.4 (s′), 167.1 (s′).

(26) Dinsmore, C. J.; Bergman, J. M. *J. Org. Chem.* **1998**, *63*, 4131−4134.

(27) Fournié-Zaluski, M.-C.; Coulaud, A.; Bouboutou, R.; Chaillet, P.; Devin, J.; Waksman, G.; Costentin, J.; Roques, B. P. *J. Med. Chem.* **1985**, *28*, 1158−1169.

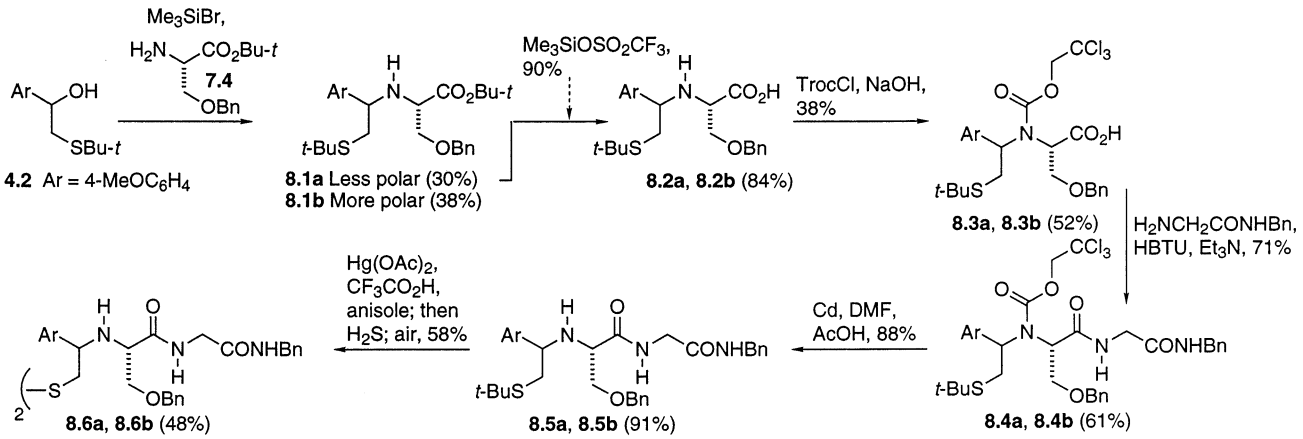
(28) In this sequence, the nitrogen was not protected as a carbamate before coupling with $H_2NCH_2CONHBn$, i.e., compounds corresponding to **6.3a** and **6.3b** were coupled directly with $H_2NCH_2CONHBn$ (EDCI, *i*-Pr_2NEt).

(29) Winterfeld, G. A.; Ito, Y.; Ogawa, T.; Schmidt, R. R. *Eur. J. Org. Chem.* **1999**, 1167−1171.

(30) Footnote 10 in: Cavelier, F.; Enjalbal, C. *Tetrahedron Lett.* **1996**, *37*, 5131−5134.

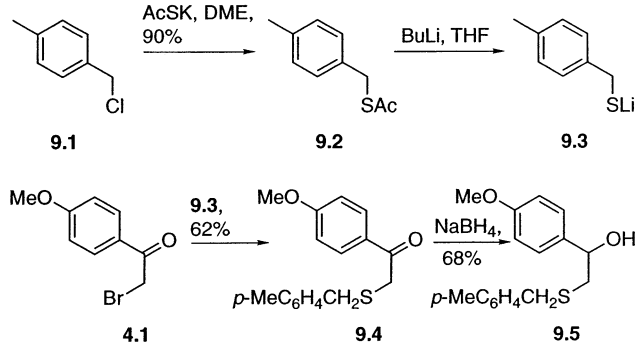
(31) Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216−3218.

SCHEME 8[a]

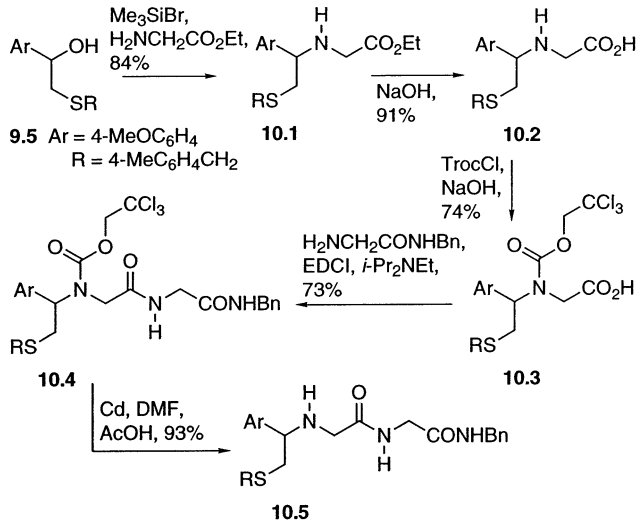


[a] Yields beside the arrows refer to the "**a**" series, those in parentheses to the "**b**" series.

SCHEME 9



SCHEME 10



protecting group in compounds of this type has been removed with HF,[6] and so our procedure clearly tolerates at least minor changes in the sulfur protecting group.

Conclusion

Our method for making derivatized amino acids of type **2.1** (Pg = Bu-*t* or *p*-$MeC_6H_4CH_2$) provides an alternative to the bromide displacement route developed by Kent et al.[6] and has been shown to work with an amino acid having a functionalized side chain. The availability of such derivatized amino acids should be helpful in studies on conformational or steric factors in the derivatizing unit that control the rate of the ligation and acyl transfer steps for different amino acids.

Experimental Section

2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethanone (2.2). Br_2 (300 μL, 5.77 mmol) was added dropwise over 10 min to a stirred and warmed (40 °C) solution of 4-methoxyacetophenone (867 mg, 5.77 mmol) in bench $CHCl_3$ (10 mL). At the end of the addition, the mixture was diluted with Et_2O (100 mL), washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated to give bromide **4.1** (1.24 g, 93%) as a white solid, which was used for the next step without purification.

A solution of the above bromide (8.82 g, 38.5 mmol) in dry THF (40 mL) was added dropwise over 10 min to a stirred and cooled (0 °C) solution of *t*-BuSLi made by slow addition of BuLi (2.5 M in hexanes, 17.7 mL, 44.3 mmol) to a stirred and cooled (0 °C) solution of *t*-BuSH (5.21 mL, 46.2 mmol) in dry THF (100 mL), the solution being stirred for 2.5 h before use. When addition was complete, the cold bath was removed and stirring was continued overnight. The mixture was diluted with Et_2O (200 mL), washed thoroughly with water (3 × 100 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel, using 1:6 EtOAc−hexanes, gave **2.2** (9.05 g, 98%) as a colorless oil: FTIR ($CDCl_3$, cast) 1671 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.34 (s, 9 H), 3.81 (s, 2 H), 3.85 (s, 3 H), 6.88−6.90 (m, 2 H), 7.89−7.91 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 30.7 (q′), 35.5 (t′), 43.6 (s′), 55.4 (q′), 113.8 (d′), 128.7 (s′), 131.1 (d′), 163.6 (s′), 194.9 (s′); exact mass *m*/*z* calcd for $C_{13}H_{18}O_2S$ 238.1027, found 238.1027.

[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]-acetic Acid Ethyl Ester (4.3). Me_3SiBr (162 μL, 1.23 mmol) was added dropwise to a stirred and cooled (0 °C) solution of alcohol **4.2** (295 mg, 1.23 mmol) in dry CH_2Cl_2 (4.0 mL). After 30 min, freshly distilled (distilled under water pump vacuum) $H_2NCH_2CO_2Et$ (253 mg, 2.46 mmol) was added in one portion. The cold bath was removed, and stirring was continued for 1 h. Evaporation of the solvent and flash chromatography of the residue over silica gel (2 × 15 cm), using 1:6 EtOAc−hexanes, gave **4.3** (323 mg, 80%) as a colorless oil: FTIR ($CDCl_3$ cast) 1739 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.22 (t, J = 7.1 Hz, 3 H), 1.31 (s, 9 H), 2.60 (br s, 1 H), 2.73 (dd, J_{AB} = 12.3 Hz, J_{AX} = 9.1 Hz, 1 H), 2.81 (dd, J_{AB} = 12.3 Hz, J_{BX} = 4.7 Hz, 1 H), 3.21 (ABq, $\Delta\nu_{AB}$ = 48.5 Hz, J_{AB} = 17 Hz, 2 H), 3.73 (dd, J_{AX} = 9.1 Hz, J_{BX} = 4.6 Hz, 1 H), 3.78 (s, 3 H), 4.14 (q, J = 7.1 Hz, 2 H), 6.86 (d, J = 8.7 Hz, 2 H), 7.24 (d, J = 8.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.3 (q′), 31.1 (q′), 37.0 (t′), 42.5

(s′), 48.8 (t′), 55.3 (q′), 60.7 (t′), 61.6 (d′), 113.9 (d′), 128.2 (d′), 134.2 (s′), 159.0 (s′), 172.1 (s′); exact mass *m*/*z* calcd for $C_{17}H_{27}NNaO_3S$ (M + Na) 348.1609, found 348.1610.

[[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethyl](2,2,2-trichloroethoxycarbonyl)amino]acetic Acid (5.2). Neat Cl_3CCH_2OCOCl (400 μL, 2.90 mmol) and 1 N NaOH (380 μL) were added alternately in 10 equal portions by syringe over 1.5 h to a stirred and cooled (0 °C) suspension of **5.1** (430 mg, 1.29 mmol) in 1 N NaOH (1.70 mL). When addition was complete, the cold bath was removed and stirring was continued for 11 h, by which time all of **5.1** had reacted. The mixture was cooled (0 °C), acidified to pH 2 with concentrated hydrochloric acid, and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 × 20 cm), using 2:100 and then 4:96 MeOH−CH_2Cl_2, gave **5.2** (510 mg, 83%) as a thick oil: FTIR ($CDCl_3$ cast) 1717, 1612 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (mixture of rotamers) δ 1.31 (s, 4 H), 1.33 (s, 5 H), 3.01−3.15 (m, 2 H), 3.67−3.83 (m containing a singlet at δ 3.77, 5 H in all), 4.73−4.81 (m, 1 H), 4.87 (ABq, $\Delta\nu_{AB}$ = 92.0 Hz, J_{AB} = 11.9 Hz, 2 H), 5.43−5.50 (m, 1 H), 6.84−6.87 (m, 2 H), 7.21−7.24 (m, 2 H) (one proton not observed in this spectrum); ^{13}C NMR ($CDCl_3$, 100 MHz) (mixture of rotamers) δ 29.1 (s′), 29.6 (s′), 30.9 (q′), 43.0 (t′), 43.2 (t′), 44.9 (t′), 45.2 (t′), 55.3 (q′), 59.6 (d′), 60.0 (d′), 75.4 (t′), 75.6 (t′), 95.1 (s′), 95.2 (s′), 114.2 (d′), 114.3 (d′), 128.4 (s′), 128.5 (d′), 129.4 (d′), 129.6 (d′), 129.7 (d′), 129.8 (d′), 154.2 (s′), 154.4 (s′), 159.6 (s′), 173.5 (s′), 174.1 (s′); exact mass *m*/*z* calcd. for $C_{18}H_{24}Cl_3NNaO_5S$ (M + Na) 494.0338, found 494.0338.

[2-[[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethyl]-(2,2,2-trichloroethoxycarbonyl)amino]acetylamino]-acetic Acid Ethyl Ester (5.3). Ethyl glycinate hydrochloride (5.2 g, 38.6 mmol) was mixed with solid K_2CO_3 (10 g, 72.4 mmol) and a few drops of saturated aqueous NaCl, and the mixture was ground with a pestle and mortar to form a thick paste. This was extracted with Et_2O, and the combined extracts were dried ($MgSO_4$) and evaporated. The resulting crude $H_2NCH_2CO_2Et$ was distilled (60 °C, water pump) to afford pure (^1H NMR) $H_2NCH_2CO_2Et$ (2.3 g, 62%).

N-(3-Dimethylamino)propyl-*N*-ethylcarbodiimide (207 mg, 1.08 mmol) was added to a stirred and cooled (0 °C) solution of acid **5.2** (510 mg, 1.08 mmol) and $H_2NCH_2CO_2Et$ (110 μL, 1.08 mmol) in dry CH_2Cl_2 (12 mL). After 15 min the cold bath was removed and stirring was continued for 2 h. The mixture was diluted with Et_2O (100 mL), washed with water (2 × 25 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 × 20 cm), using 1:99, 2:98, and then 4:96 MeOH−CH_2Cl_2, gave **5.3** (374 mg, 62%) as a gum: FTIR ($CDCl_3$ cast) 3323 (br), 1748, 1716, 1611 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) (mixture of rotamers) δ 1.24 (t, *J* = 7.1 Hz, 3 H), 1.32 (br s, 9 H), 3.04−3.29 (br m, 2 H), 3.64−3.76 (br, 2 H), 3.76 (s, 3 H), 3.81−3.96 (m, 2 H), 4.17 (q, *J* = 7.1 Hz, 2 H), 4.74−4.83 (br m, 1.6 H), 5.00−5.10 (br m, 0.4 H), 5.32−5.56 (br m, 1 H), 6.70−6.80 (br s, 1 H), 6.80−6.94 (m, 2 H), 7.21−7.30 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) (mixture of rotamers) δ 14.1 (q′), 29.8 (t′), 30.9 (q′), 41.2 (t′), 42.9 (t′), 47.7 (t′), 55.2 (q′), 59.8 (d′), 60.4 (d′), 61.4 (t′), 75.2 (s′), 75.5 (s′), 95.2 (s′), 114.2 (d′), 129.1 (d′), 129.6 (s′), 154.6 (s′), 159.6 (s′), 168.8 (s′), 169.2 (s′) (one carbon not observed in this spectrum); exact mass *m*/*z* calcd for $C_{22}H_{31}Cl_3N_2NaO_6S$ (M + Na) 579.0866, found 579.0868.

[2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]acetylamino]acetic Acid Ethyl Ester (5.4). Cd powder (1.20 g, 10.7 mmol) was added in one portion to a stirred solution of **5.3** (179 mg, 0.322 mmol) in 1:1 DMF−AcOH (8 mL). Stirring was continued for 45 min at room temperature, and the mixture was filtered through a Celite pad (2 × 4 cm), using EtOAc (3 × 20 mL). The combined filtrates and washings were washed with saturated aqueous $NaHCO_3$ (2 × 15 mL) and water (15 mL), dried ($MgSO_4$), and evaporated. The residue was kept under oil pump vacuum to remove residual DMF, and amine **5.4** (118 mg, 97%) was obtained as a pale-yellow oil: FTIR ($CDCl_3$ cast) 3307 (br), 1748, 1672, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.27 (t, *J* = 7.2, Hz, 3 H); 1.30 (s, 9 H), 1.60 (br s, 1 H), 2.73 (dd, J_{AB} = 12.8 Hz, J_{AX} = 9.5 Hz, 1 H), 2.79 (dd, J_{AB} =12.8 Hz, J_{BX} = 4.4 Hz, 1 H), 3.17 (ABq, $\Delta\nu_{AB}$ = 38.2 Hz, J_{AB} = 17.1 Hz, 2 H), 3.68 (dd, *J* = 9.5, 4.4 Hz, 1 H), 3.77 (s, 3 H), 3.98 (dd, *J* = 5.6, 3.1 Hz, 2 H), 4.19 (q, *J* = 7.2 Hz, 2 H), 6.81−6.83 (m, 2 H), 7.17−7.19 (m, 2 H), 7.70−7.72 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.3 (q′), 31.1 (q′), 36.5 (t′), 40.9 (t′), 42.7 (s′), 49.9 (t′), 55.3 (q′), 61.4 (t′), 62.4 (d′), 114.1 (d′), 128.0 (d′), 133.6 (s′), 159.2 (s′), 169.6 (s′), 171.6 (s′); exact mass *m*/*z* calcd for $C_{19}H_{31}N_2O_4S$ (M + H) 383.2004, found 383.2002.

[2-[2-[2-[[(Ethoxycarbonylmethylcarbamoyl)methyl]-amino]-2-(4-methoxyphenyl)ethyldisulfanyl]-1-(4-methoxyphenyl)ethylamino]acetylamino]acetic Acid Ethyl Ester (5.5). In this experiment the initial thiol product was not protected from air.

CF_3CO_2H (2 mL) was added to thioether **5.4** (118 mg, 0.311 mmol) contained in a flask immersed in an ice bath. The mixture was stirred, and PhOMe (50 μL), followed by $Hg(OAc)_2$ (99.3 mg, 0.311 mmol), was added. Stirring was continued for 25 min, and the solvent was evaporated. The residue was dissolved in MeCN (15 mL), and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 × 4 cm), and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings and flash chromatography of the residue over silica gel (2 × 18 cm), using 4:100 MeOH−CH_2Cl_2, gave disulfide **5.5** (92.4 mg, 92%) as a colorless oil: FTIR ($CDCl_3$ cast) 1745, 1669 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.26 (t, *J* = 7.1 Hz, 6 H), 2.74 (dd, J_{AB} = 13.7 Hz, J_{AX} = 8.0 Hz, 2 H), 3.23 (ABq, $\Delta\nu_{AB}$ = 15.4 Hz, J_{AB} = 17.0 Hz, 4 H), 2.85 (dd, J_{AB} = 13.7 Hz, J_{BX} = 5.3 Hz, 2 H), 3.71 (dd, J_{AX} = 7.8 Hz, J_{BX} = 5.4 Hz, 2 H), 3.76 (s, 6 H), 3.96 (dd, J_{AB} = 18.3 Hz, J_{AX} = 5.4 Hz, 2 H), 4.02 (dd, J_{AB} = 18.3 Hz, J_{BX} = 5.6 Hz, 2 H), 4.19 (q, *J* = 7.1 Hz, 4 H), 6.82−6.85 (m, 4 H), 7.14−7.17 (m, 4 H), 7.65 (br t, *J* = 4.5 Hz, 2 H) (two protons not observed in this spectrum); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2 (q′), 31.6 (t′), 41.0 (t′), 49.5 (t′), 55.3 (q′), 61.5 (t′), 64.3 (d′), 114.2 (d′), 128.3 (d′), 131.8 (s′), 159.4 (s′), 169.7 (s′), 170.9 (s′); exact mass *m*/*z* calcd for $C_{30}H_{43}N_4O_8S$ (M + H) 651.2522, found 651.2524.

(2*S*)-2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionic Acid *tert*-Butyl Ester (Less Polar Isomer) (6.2a) and (More Polar Isomer) (6.2b). Me_3SiBr (295 μL, 2.23 mmol) was added dropwise to a stirred and cooled (0 °C) solution of alcohol **4.2** (537 mg, 2.23 mmol) in dry CH_2Cl_2 (11 mL). After 20 min, freshly prepared amine **6.1** (see preparation of **6.1** in Supporting Information) (653 mg, 4.47 mmol) was added in one portion. The cold bath was left in place but was not recharged, stirring was continued for 2 days. Evaporation of the solvent and flash chromatography of the residue over silica gel (3 × 20 cm), using 1:7 EtOAc−hexanes, gave a fast-eluting diastereoisomer **6.2a** (262 mg, 31%) as a pale-yellow oil and a slow-eluting diastereoisomer **6.2b** (251 mg, 30%) as a colorless oil. Isomer **6.2a**: $[\alpha]^{20}_D$ −89.8° (*c* 1.0, $CHCl_3$); FTIR ($CHCl_3$ cast) 1728 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.17 (d, *J* = 7.0 Hz, 3 H), 1.30 (s, 9 H), 1.45 (s, 9 H), 2.46−2.54 (br s, 1 H), 2.66 (dd, J_{AB} = 11.7 Hz, J_{AX} = 9.5 Hz, 1 H), 2.76 (dd, J_{AB} = 12.1 Hz, J_{BX} = 4.2 Hz, 1 H), 2.93 (q, *J* = 7.1 Hz, 1 H), 3.67 (dd, J_{AX} = 9.5 Hz, J_{BX} = 4.2 Hz, 1 H), 3.78 (s, 3 H), 6.83−6.86 (m, 2 H), 7.22−7.25 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.7 (q′), 28.2 (q′), 31.0 (q′), 37.4 (t′), 42.4 (s′), 54.7 (d′), 55.3 (q′), 60.2 (d′), 80.6 (s′), 113.9 (d′), 128.3 (d′), 134.9 (s′), 158.9 (s′), 174.9 (s′); exact mass *m*/*z* calcd for $C_{20}H_{33}NNaO_3S$ (M + Na) 390.2079, found 390.2074. Isomer **6.2b**: $[\alpha]^{20}_D$ + 6.3° (*c* 1.0, $CHCl_3$); FTIR ($CHCl_3$ cast) 1729 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.22 (d, *J* = 6.9 Hz, 3 H), 1.27 (s, 9 H), 1.37 (s, 9 H), 1.9−2.5 (very br, 1 H), 2.80 (br d, *J* = 5.5 Hz, 2 H), 3.20 (q, *J* = 6.8 Hz, 1 H), 3.72−3.76 (overlapping signals containing a multiplet and a singlet at δ 3.74, 4 H in all), 6.82 (d, *J* = 8.6 Hz, 2 H), 7.21 (d, *J* = 8.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$,

125 MHz) δ 18.4 (q′), 28.1 (q′), 31.1 (q′), 35.9 (t′), 42.4 (s′), 54.7 (d′), 55.3 (q′), 60.1 (d′), 80.9 (s′), 113.9 (d′) 128.4 (d′), 158.9 (s′) (two carbons not observed in this spectrum s′); exact mass *m*/*z* calcd for $C_{20}H_{33}NNaO_3S$ 390.2079 (M + Na), found 390.2080.

(2*S*)-2-[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionic Acid Trifluoroacetate (Less Polar Isomer) (6.3a). CF_3CO_2H (2 mL) was added to a stirred and cooled (0 °C) mixture of **6.2a** (315 mg, 0.856 mmol) and PhOMe (150 μL), and stirring was continued for 5 h at 0 °C. Evaporation of the solvents and flash chromatography of the residue over silica gel (2 × 15 cm), using 8:92 and then 1:1 MeOH−CH_2Cl_2, gave **6.3a** (264 mg, 72%) as a white solid: $[\alpha]^{20}_D$ −18.1° (*c* 1.0, MeOH); FTIR (MeOH cast) 1678 cm^{-1}; ^{1}H NMR (CD_3-OD, 500 MHz) δ 1.25−1.28 (overlapping signals containing a singlet at δ 1.26, 12 H in all), 1.90 (br s, 1 H), 3.01 (dd, J_{AB} = 13.1 Hz, J_{AX} = 8.2 Hz, 1 H), 3.09−3.16 (m, 2 H), 3.73 (s, 3 H), 4.25−4.31 (m, 1 H), 4.80 (br s, 1 H), 6.89 (d, J = 8.8 Hz, 2 H), 7.21 (d, J = 8.8 Hz, 2 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 17.0 (q′), 31.3 (q′), 32.7 (s′), 44.2 (t′), 55.9 (q′), 57.1 (d′), 63.2 (d′), 115.8 (d′), 126.7 (s′), 131.0 (d′), 162.3 (s′), 173.9 (s′); ^{19}F NMR (CD_3OD, 376.5 MHz) δ −77.5; exact mass *m*/*z* calcd for $C_{16}H_{25}$-$NNaO_3S$ (M + Na) 334.1453, found 334.1450. Anal. Calcd for $C_{18}H_{26}F_3NO_5S$: C, 50.81; H, 6.16; N, 3.29; S, 7.54. Found: C, 50.55; H, 6.26; N, 3.36; S, 7.89.

(2*S*)-2-[[2-*tert*-Butylsulfanyl-1-(4-methoxyphenyl)ethyl]-(2,2,2-trichloroethoxycarbonyl)amino]propionic Acid (6.4a). Neat Cl_3CCH_2OCOCl (280 μL, 2.03 mmol) and 1 N NaOH (263 μL) were added simultaneously by syringe over 4 h to a stirred and cooled (0 °C) suspension of **6.3a** (316 mg, 0.74 mmol) in 1 N NaOH (1.18 mL). When addition was complete, the cold bath was removed and stirring was continued for 11 h, by which time all **6.3a** had reacted. The mixture was cooled (0 °C), acidified to pH 2 with concentrated hydrochloric acid, and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 × 15 cm), using 1:99, 2:99, and then 4:96 MeOH−CH_2Cl_2, gave **6.4a** (349 mg, 96%) as a white foam: $[\alpha]^{20}_D$ −61.3° (*c* 1.0, MeOH); mp 58−61 °C; FTIR (MeOH cast) 1714 cm^{-1}; ^{1}H NMR (CD_3-OD, 500 MHz) (mixture of rotamers) δ 1.34 (s, 9 H), 1.56 (d, J = 6.6 Hz, 1 H), 1.63 (d, J = 6.9 Hz, 1 H), 3.13−3.19 (m, 2 H), 3.76 (s, 3 H), 3.82−3.88 (m, 1 H), 4.61 (d, J = 11.8 Hz, 0.35 H), 4.77 (d, J = 12.9 Hz, 0.33 H), 4.91−4.98 (m, 1 H), 5.45−5.49 (m, 1 H), 6.82−6.86 (m, 2 H), 7.30−7.36 (m, 2 H) (one proton not observed in this spectrum); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.9 (q′), 17.2 (q′), 29.8 (t′), 30.1 (t′), 30.9 (q′), 42.9 (s′), 43.1 (s′), 51.6 (d′), 52.2 (d′), 55.2 (q′), 60.0 (d′), 60.8 (d′), 75.1 (t′), 75.5 (t′), 94.7 (s′), 95.3 (s′), 113.8 (d′), 114.1 (d′), 128.4 (s′), 129.9 (d′), 130.0 (d′), 152.9 (s′), 153.9 (s′), 159.4 (s′), 175.9 (s′), 176.4 (s′); exact mass *m*/*z* calcd for $C_{19}H_{26}Cl_3NNaO_5S$ (M + Na) 508.0495, found 508.0496.

[(1*S*)-1-[(Benzylcarbamoylmethyl)carbamoyl]ethyl][2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethyl]carbamic Acid 2,2,2-Trichloroethyl Ester (6.5a). *O*-Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (230 mg, 0.713 mmol) was added to a stirred mixture of acid **6.4a** (334 mg, 0.686 mmol), Et_3N (288 μL, 2.06 mmol), and the amine salt $CF_3CO_2H{\cdot}H_2NCH_2CONHB$[25] (198 mg, 0.713 mmol) in MeCN (3 mL). The mixture was stirred for 4 h, diluted with EtOAc (25 mL), and washed successively with 1 N hydrochloric acid (2 × 15 mL) and saturated aqueous $NaHCO_3$ (2 × 15 mL). The organic phase was dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (2 × 15 cm), using 4:96 MeOH−CH_2Cl_2, gave **6.5a** (345 mg, 79%) as a white crystalline solid: $[\alpha]^{20}_D$ −51.4° (*c* 1.0, $CHCl_3$); mp 61−63 °C; FTIR ($CHCl_3$ cast) 1695, 1666 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34 (s, 9 H), 1.55 (d, J = 6.9 Hz, 3 H), 3.01−3.08 (m, 1 H), 3.15−3.32 (m, 2 H), 3.66−3.79 (overlapping signals containing a singlet at δ 3.78, 4 H in all), 4.05 (dd, J = 17.7, 6.8 Hz, 1 H), 4.25 (dd, J = 15.3, 4.5 Hz, 1 H), 4.36−4.55 (m, 2 H), 5.24−540 (m, 1 H), 6.03 (br s, 0.7 H), 6.4 (br s, 0.3 H), 6.90 (d, J = 7.6 Hz, 2 H), 7.00 (br s, 1 H), 7.22−7.32 (overlapping signals, 7 H in all) (one proton not observed in this spectrum); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.1 (q′), 30.2 (s′), 31.0 (q′), 43.3 (t′), 54.8 (t′), 55.4 (t′), 61.9 (q′), 74.9 (t′), 95.0 (s′), 114.5 (d′), 127.3 (d′), 127.8 (d′), 128.6 (d′), 128.9 (d′), 129.4 (s′), 138.0 (s′), 153.0 (s′), 159.9 (s′), 168.5 (s′), 170.5 (s′); exact mass *m*/*z* calcd for $C_{28}H_{36}Cl_3N_3NaO_5S$ (M + Na) 654.1339, found 654.1337.

(2*S*)-*N*-(Benzylcarbamoylmethyl)-2-[2-*tert*-butylsulfanyl-1-(4-methoxyphenyl)ethylamino]propionamide (6.6a). Cd powder (880 mg, 7.87 mmol) was added in one portion to a stirred solution of **6.5a** (173 mg, 0.275 mmol) in 1:1 DMF−AcOH (6 mL). Stirring was continued for 3 h at room temperature, and the mixture was filtered through a Celite pad (2 × 4 cm), using EtOAc (50 mL). The combined filtrates and washings were evaporated, and flash chromatography of the residue over silica gel (2 × 20 cm), using 5:100 MeOH−CH_2Cl_2, gave **6.6a** (114 mg, 74%) as a white solid: $[\alpha]^{20}_D$ −49.8° (*c* 1.0, $CHCl_3$); FTIR ($CHCl_3$ cast) 3304, 1656 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.13 (d, J = 7.0 Hz, 3 H), 1.26 (s, 9 H), 2.62−2.75 (m, 2 H), 3.00 (q, J = 7.0 Hz, 1 H), 3.49 (dd, J = 9.2, 5.1 Hz, 1 H), 3.76 (s, 3 H), 3.88−4.04 (m, 2 H), 4.35−4.48 (m, 2 H), 4.59 (br s, 1 H), 6.81−6.85 (m, 2 H), 6.91 (br t, J = 5.0 Hz, 1 H), 7.03−7.07 (m, 2 H), 7.19−7.30 (m, 5 H), 8.19 (br t, J = 5.9 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.0 (q′), 30.9 (q′), 36.5 (t′), 42.6 (s′), 43.4 (t′), 43.5 (t′), 55.2 (q′), 61.8 (d′), 114.1 (d′), 127.3 (d′), 127.4 (d′), 127.5 (d′), 128.5 (d′), 133.9 (s′), 137.9 (s′), 159.0 (s′), 169.2 (s′), 176.2 (s′); exact mass *m*/*z* calcd for $C_{25}H_{36}N_3O_3S$ (M + H) 458.2477, found 458.2481.

The compound had ee = 99.5% (HPLC, Chirex 3020 (*S*-Leu *R*-NEA) column, 7.5% EtOH-hexane).

(2*S*)-*N*-(Benzylcarbamoylmethyl)-2-[2-mercapto-1-(4-methoxyphenyl)ethylamino]propionamide (6.7a). In this experiment, the initial thiol product was not protected from air.

CF_3CO_2H (1.0 mL) was added to thioether **6.6a** (94.4 mg, 0.207 mmol) contained in a flask immersed in an ice bath. The mixture was stirred, and anisole (40 μL) followed by $Hg(OAc)_2$ (66.0 mg, 0.207 mmol) was added. Stirring was continued for 25 min, and the solvent was evaporated. The residue was dissolved in MeCN (15 mL), and H_2S gas was bubbled through the solution for 2 min. The resulting black suspension was filtered through a tightly packed Celite column (2 × 4 cm), and the solid was washed with several portions of MeCN. Evaporation of the combined filtrate and washings and flash chromatography of the residue over silica gel (2 × 18 cm), using 4:100 MeOH−CH_2Cl_2, gave **6.7a** and the corresponding disulfide (80.8 mg, 97%) as a pale-brown oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.17 (d, J = 7.0 Hz, 3 H), 1.43 (br s, 1 H), 1.80 (br s, 1 H), 2.59−2.73 (m, 2 H), 2.97−3.05 (m, 1 H), 3.49 (dd, J = 5.7 Hz, 1 H), 3.76 (s, 3 H), 3.91 (dd, J_{AB} = 16.1 Hz, J_{AX} = 5.5 Hz, 1 H), 3.98 (dd, J_{AB} = 16.1 Hz, J_{BX} = 6.1 Hz, 1 H), 4.42 (d, J = 5.7 Hz, 2 H), 6.70 (br s, 1 H), 6.78−6.83 (m, 2 H), 7.01−7.03 (m, 2 H), 7.18−7.28 (m, 5 H), 7.92 (t, J = 5.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.2 (q′), 32.3 (t′), 43.3 (t′), 43.6 (t′), 55.3 (q′), 55.4 (q′), 63.2 (d′), 114.1 (d′), 127.5 (d′), 127.7 (d′), 127.8 (d′), 128.7 (d′), 133.0 (s′), 137.8 (s′), 159.1 (s′), 168.8 (s′), 175.8 (s′); exact mass *m*/*z* calcd for disulfide $C_{42}H_{53}N_6O_6S_2$ (M + H) 801.3462, found 801.3467. We did not establish if the compound is the trifluoroacetate salt or the free base; the percent yield quoted is based on the assumption that the compound is the free base.

Thioacetic Acid *S*-(4-Methylbenzyl) Ester (9.2). NaI (50 mg) was added to a stirred mixture of 4-methylbenzyl chloride (**9.1**) (1.27 mL, 9.59 mmol) and AcSK (1.21 g, 10.6 mmol) in dry DME (30 mL) (N_2 atmosphere). Stirring was continued for 11 h, and the mixture was diluted with Et_2O (200 mL), washed with water (3 × 75 mL), dried ($MgSO_4$), and evaporated. Flash chromatography of the residue over silica gel (2 × 20 cm), using 1:10 EtOAc−hexanes, gave **9.2** (1.56 g, 90%) as a pale-yellow oil: FTIR ($CDCl_3$ cast) 1691 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.29 (s, 3 H), 2.32 (s, 3 H), 4.10 (s, 2 H), 7.11 (ABq, $\Delta\nu_{AB}$ = 21.5, J_{AB} = 8.0 Hz, 4 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.0 (q′), 30.3 (q′), 33.2 (t′), 128.7 (d′), 129.3 (d′),

134.5 (s′), 137.0 (s′), 195.2 (s′); exact mass *m*/*z* calcd for $C_{10}H_{12}$-OS 180.0609, found 180.0610.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada and the Merck Frosst Therapeutic Research Centre for financial support. D.M.C. held an Alberta Heritage Foundation for Medical Research Fellowship. We also thank C. Boucher (Boehringer Ingelheim Canada) for ee measurements.

Supporting Information Available: NMR spectra of compounds **2.2**, **4.2**, **4.3**, chloride derived from **4.2**, **5.1**−**5.5**, *N*-Cbz-alanine *tert*-butyl ester, **6.1**, **6.2a**, **6.2b**, **6.3b**, **6.4a**, **6.4b**, **6.5a**, **6.5b**, **6.6a**, **6.6b**, **6.7a**, **6.7b**, **7.2**, **7.3**, Mosher amide of **7.3**, **8.1a**, **8.1b**, **8.2a**, **8.2b**, **8.3a**, **8.3b**, **8.4a**, **8.4b**, **8.5a**, **8.5b**, **8.6a**, **8.6b**, **9.2**, **9.5**, **10.1**−**10.5**, and experimental general techniques and procedures for chloride derived from **4.2**, **4.3** (from chloride derived from **4.2**), **5.1**, *N*-Cbz-alanine *tert*-butyl ester, **6.1**, **6.3b**, **6.4b**, **6.5b**, **6.6b**, **6.7b**, **7.2**, **7.3**, Mosher amide of **7.3**, **8.1a**, **8.1b**, **8.2a**, **8.2b**, **8.3a**, **8.3b**, **8.4a**, **8.4b**, **8.5a**, **8.5b**, **8.6a**, **8.6b**, **9.4**, **9.5**, **10.1**−**10.5**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO030192R