Synthesis of a Chiral Serine Aldehyde Equivalent and Its Conversion to Chiral α-Amino Acid Derivatives

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Abstract: We report a new methodology for the synthesis of chiral nonproteinaceous α-amino acids, which involves protection of the carboxyl group of serine as a cyclic ortho ester. This reduces the acidity of the α-proton, allowing for oxidation of the side chain of serine to an aldehyde without racemization. A variety of carbonyl addition reactions, such as Grignard, Reformatsky, and Wittig additions, can then be carried out, leading to a wide range of amino acids. Very good stereocontrol is achieved, allowing for the selective synthesis of all four diastereomers of β-hydroxy-α-amino acids. The method readily allows for stereospecific incorporation of both C and H isotopes in amino acid side chains.

Introduction

Nonproteinaceous α-amino acids possess enormous structural diversity and a broad range of biological activity.[1] In the past decade, there has been a surge of interest in the development of new methods for their synthesis and for their use as synthons in more elaborate syntheses.[2] This synthetic effort has been an integral part of the growth of asymmetric organic synthesis.[3] A number of elegant approaches have recently been described for the asymmetric synthesis of various α-amino acids in their optically pure forms.[4] Most of these methods involve the derivatization of glycine equivalents attached to a chiral template.[5] Other strategies have relied on the indirect homologation of the serine side chain.[6] These methodologies generally suffer from a lack of flexibility in terms of stereochemical control at the β-carbon. Several indirect routes from D-serine have been described for obtaining a chiral L-serine aldehyde equivalent, in which the aldehyde originates from the acid group of the amino acid.[7] These chiral serine aldehydes have found many synthetic applications,[8] but their utility is limited by the oxidation of the alcohol moiety to the corresponding carboxylic acid that is required late in the synthetic scheme. In this preliminary account, we wish to report a simple and direct approach for the synthesis of a novel chiral L-serine aldehyde equivalent and illustrate the versatility of this trifunctional synthon for the synthesis of polyfunctional α-amino acids.

Our approach was based on the reasoning that the acidity of the α-proton of L-serine could be substantially reduced by masking the carboxylic acid as a base-stable cyclic ortho ester.[9] The decreased acidity of the α-proton would diminish serine's tendency

(1) (a) *Chemistry and Biochemistry of the Amino Acids*; Barrett, G. C., Ed.; Chapman and Hall: London, 1985. (b) Wagner, I.; Musso, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 816–828.

(2) For some reviews of the use of amino acids in asymmetric syntheses, see: (a) Martens, J. *Top. Curr. Chem.* **1984**, *125*, 165–246. (b) Ottenheijm, H. C. J. *Chimia* **1985**, *39*, 89–98. (c) Coppola, G. M.; Schuster, H. F. *Asymmetric Synthesis. Construction of Chiral Molecules Using Amino Acids*; John Wiley & Sons: Toronto, 1987.

(3) For some reviews of early developments in asymmetric synthesis and the importance of amino acids as targets and reagents, see: (a) Kagan, H. B.; Fiaud, J. C. *Top. Stereochem.* **1978**, *10*, 175–285. (b) ApSimon, J. W.; Seguin, R. P. *Tetrahedron* **1979**, *35*, 2797–2842. (c) Halpern, J. *Science* **1982**, *217*, 401–407. (d) Mosher, H. S.; Morrison, J. D. *Science* **1983**, *221*, 1013–1019.

(4) (a) Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon: Toronto, 1989. See also references contained within: (b) Arnold, L. D.; Drover, J. C. G.; Vederas, J. C. *J. Am. Chem. Soc.* **1987**, *109*, 4649–4659. (c) Arnold, L. D.; May, R. G.; Vederas, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 2237–2241. (d) Jung, M. E.; Jung, Y. H. *Tetrahedron Lett.* **1989**, *30*, 6637–6640. (e) Blaser, D.; Seebach, D. *Liebigs Ann. Chem.* **1991**, 1067–1078. (f) Reetz, M. T. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1531–1546.

(5) A variety of approaches have been used. Most commonly, the bond between the α-carbon and the side chain is formed using an anion at the α-center, with the stereochemistry at the α-center controlled by a chiral ring system containing the α-carbon. Variations occur in the type of ring system used. For oxazolidinine, see: (a) Seebach, D.; Müller, S. G.; Gysel, V.; Zimmermann, J. *Helv. Chim. Acta* **1988**, *71*, 1303–1318. For imidazolidinone, see: (b) Fitzi, R.; Seebach, D. *Tetrahedron* **1988**, *44*, 5277–5292. (c) Seebach, D; Dziadulewicz, E.; Behrendt, L.; Cantoreggi, S.; Fitzi, R. *Liebigs Ann. Chem.* **1989**, 1215–1232. For bis-lactim ether, see: (d) Schöllkopf, U. *Top. Curr. Chem.* **1983**, *109*, 65–84. For glycine Schiff base Ni(II) complex, see: (e) Belokon, 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*, 4252–4259. The polarity of the bond formation can be reversed by using an electrophilic glycine template, see: (f) Sinclair, P. J.; Zhai, D.; Reibenspies, J.; Williams, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 1103–1104. (g) Williams, R. M. *Aldrichimica Acta* **1992**, *25*, 11–25. The α-center can be located outside of the ring; see: (h) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757–6761. (i) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1987**, *109*, 7151–7157. Alternatively, the α-carbon to nitrogen bond can be formed by aminating an *N*-acyloxazolidone enolate; see: (j) Evans, D. A.; Britton, T. C.; Dorow, R. L.; Dellaria, J. F. *J. Am. Chem. Soc.* **1986**, *108*, 6395–6397. (k) Evans, D. A.; Britton, T. C. *J. Am. Chem. Soc.* **1987**, *109*, 6881–6883.

(6) Several methods rely on nucleophilic opening of a cyclic serine derivative. For an aziridine, see: (a) Baldwin, J. E.; Adlington, R. M.; Robinson, N. G. *J. Chem. Soc., Chem. Commun.* **1987**, 153–155. For a β-lactone, see refs 4b,c. Other approaches convert the side chain to a nucleophile and use it in displacements (Sasaki, N. A.; Hashimoto, C.; Potier, P. *Tetrahedron Lett.* **1987**, *28*, 6069–6072) or to form a Wittig ylide (Sibi, M. P.; Renhowe, P. A. *Tetrahedron Lett.* **1990**, *31*, 7407–7410).

(7) Garner developed a cyclic oxazolidine aldehyde; see: (a) Garner, P. *Tetrahedron Lett.* **1984**, *25*, 5855–5858. (b) Garner, P.; Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361–2364. (c) Garner, P.; Park, J. M.; *Org. Synth.* **1991**, *70*, 18–26. Rappoport produced both an acyclic *N*-phenylsulfonyl-protected aldehyde, see: (d) Maurer, P. J.; Takahata, H.; Rapoport, H. *J. Am. Chem. Soc.* **1984**, *106*, 1095–1098. And an *N*-9-phenylfluoren-9-yl cyclic carbamate, see: (e) Lubell, W.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 3824–3831. Both compounds have the aldehyde originating fron the acid moiety. He also reported an acyclic *N*-(9-phenylfluoren-9-yl)serine derivative (ref 7e) with the aldehyde originating from the serine alcohol and the acid protected as a dimethyl acetal. However, no amino acids were reported to be synthesized with this aldehyde. The use of α-amino aldehydes for amino acid synthesis is reviewed in ref 4f and in (f) Jurczak, J.; Golebiowski, A. *Chem. Rev.* **1989**, *89*, 149–164. In ref 4f, Reetz reports a di-*N*-benzyl-protected serinal with the original serine side chain protected as a *t*-BDMS or benzyl ether, based on unpublished data. Recently, a stable Boc-protected serinal has been prepared from D-glucosamine hydrochloride by Giannis and Henk. It can be reacted with unstabilized ylides to give allylamino alcohols; see: (g) Giannis, A.; Henk, T. *Tetrahedron Lett.* **1990**, *31*, 1253–1256.

(8) For a few examples of syntheses with the aldehydes reported in ref 7, see: (a) Garner, P.; Park, J. M.; Malecki, E. *J. Org. Chem.* **1988**, *53*, 4395–4398. (b) Herold, P. *Helv. Chim. Acta* **1988**, *71*, 354–362. (c) Beaulieu, P. L.; Duceppe, J.-S.; Johnson, C. *J. Org. Chem.* **1991**, *56*, 4196–4204. (d) Casiraghi, G.; Colombo, L.; Rassu, G.; Spanu, P. *J. Chem. Soc., Chem. Commun.* **1991**, 603–604. (e) Roemmele, R. C.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1866–1875. (f) Coleman, R. S.; Carpenter, A. J. *Tetrahedron Lett.* **1992**, *33*, 1697–1700. (g) Reetz, M. *Pure Appl. Chem.* **1992**, *64*, 351–359. (h) Reetz, M. T.; Drewes, M. W.; Schmitz, A. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1141–1143.

(9) For a review of the synthesis and use of ortho esters, see: DeWolfe, R. H. *Synthesis* **1974**, *3*, 153–172.

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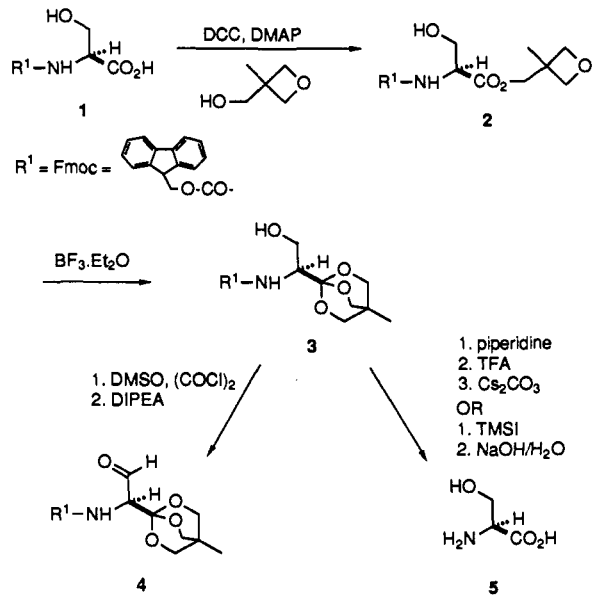
Scheme I

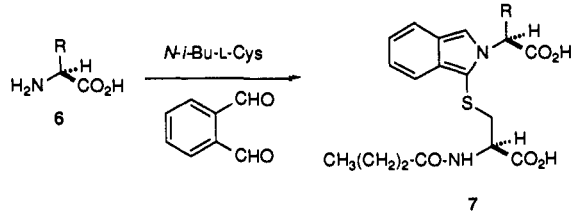


to enolize and epimerize, allowing for the direct oxidation of the hydroxyl side chain to the corresponding aldehyde without loss of chirality. To our knowledge, ortho esters of α-amino acids have never been reported.

Results

Synthesis of Protected Serine Aldehyde. In 1982, Corey[10] reported the facile preparation of 4-methyl-2,6,7-trioxabicyclo[2.2.2] ortho esters (OBO) from the boron trifluoride catalyzed rearrangement of the corresponding 3-methyl-3-(hydroxymethyl)oxetane ester. In applying this method for the protection of serine, we initially chose acid-stable N$^{\alpha}$-fluoren-9-ylmethoxycarbonyl (Fmoc) for amine protection because of its stability to the Lewis acid conditions required for the rearrangement, and also because of the known crystallinity of Fmoc amino acid derivatives. Oxetane ester **2** is prepared in high yield (80–85%) from the addition of Fmoc-L-serine (**1**) to a mixture of DCC (1.2 equiv), 3-methyl-3-(hydroxymethyl)oxetane (20 equiv) and DMAP (0.05 equiv) (Scheme I). The large excess of oxetane alcohol can be recovered and is required to minimize esterification of the serine side chain (the resultant dimer has been isolated and characterized). The oxetane ester is then converted to the OBO ester. In order to isolate high yields of the OBO ester, it is essential to maintain the concentration of boron trifluoride at less than 0.1 equiv. With these conditions, the rearrangement is complete after 12 h at room temperature. The protected derivative is a crystalline solid which is stable at room temperature and to silica gel.[11] Removal of the protecting groups from **3** (and from other β-hydroxy analogs described below) can be accomplished by at least two methods. The first method (procedure A) is a three-step "one-pot" procedure in which the protected amino acid is first treated with piperidine (20% in CH_2Cl_2) followed by evaporation. The free amine derivative is then reacted with aqueous trifluoroacetic acid in CH_2Cl_2 to give the corresponding dihydroxy ester, and the reaction is followed by evaporation. Finally, hydrolysis of the dihydroxy ester with Cs_2CO_3 (5 equiv, MeOH:H_2O, 1:1.5)[12] gives free amino acid L-serine, **5**. It is critical to remove the Fmoc group while the ortho ester is intact: if removal is attempted with either piperidine or with Cs_2CO_3 after the ortho ester has been ring-opened, considerable racemization occurs. For the second method (procedure B), the protected amino acid is treated with iodotrimethylsilane (TMSI) for 12 h at 80 °C and then extracted from Et_2O into 0.5 N NaOH. In both procedures, the deprotected L-serine is purified by cation exchange chromatography. The chiral purity of the amino acid is determined by HPLC after derivatization of the free amine with *o*-phthaladehyde (OPA) and *N*-isobutyryl-L-cysteine (*N*-*i*-Bu-L-Cys)[13] (Scheme II). The piperidine/TFA/Cs_2CO_3 procedure results in 1–2% racemization of serine, while the TMSI procedure causes <0.2% racemization during the deprotection procedure (limit of detection approximately 0.2% D). Less racemization was observed when procedure A was used to deprotect other derivatized amino acids.

Scheme II



Fmoc-L-serine-OBO ester (**3**) is oxidized under Swern conditions to give aldehyde **4** in quantitative yield, with 97–99% ee. This aldehyde is chemically and chirally stable at room temperature for at least several weeks (indefinitely at −20 °C) and is used without further purification. In fact, chromatographic purification of **4** on silica gel results in complete racemization, although the ortho ester remains intact. It is stable in solution (no change in optical rotation after 190 h in EtOAc at room temperature) but slowly racemizes in the presence of a base (0.7% racemization per hour with 0.2 M *N*,*N*-diisopropylethylamine (DIPEA) in EtOAc; 1.3%/h if 0.5 M H_2O is also present). The chiral purity of **4** was directly assessed by ^{1}H NMR analysis in the presence of chiral shift reagents (Eu(hfc)$_3$) with comparison to analogous intermediates obtained from D,L-serine (Figure 1). Racemization was also assessed by HPLC analysis after reduction of the aldehyde ($NaBH_4$), deprotection (procedure A or B), and derivatization (OPA and *N*-*i*-Bu-L-Cys). Comparison with the protected starting alcohol **3** that was deprotected and derivatized under identical conditions allowed the determination of any racemization occurring during the oxidation step.

The protected serine aldehyde **4** is very reactive toward a variety of classical reagents used for addition to aldehydes; those described below constitute some of our initial attempts and are illustrative of the many possible transformations.

Grignard Additions. Grignard addition with MeMgBr (2.5 equiv, −78 °C) results in protected L-threonine **10** in 57% yield with a threo (2*S*,3*R*):erythro (2*S*,3*S*) ratio of 94:6, with >98% ee. (Scheme III). The primary byproduct is the recovered racemic starting aldehyde (25%). Racemization of the unreacted aldehyde may be due to enolization during the reaction, but this is difficult to ascertain as the aldehyde is known to racemize during the column purification needed to isolate it. Higher yields of product (77%) are obtained if the reaction is carried out at room temperature, but lower diastereoselectivity is observed (84:16). Similarly, reaction of **4** with PhMgBr (4 equiv, 25 °C) provides protected L-phenylserine **11** (85% yield) with a 83:17 threo (2*S*,3*R*):erythro (2*S*,3*S*) ratio and >98% ee (57% yield and 86:14 threo:erythro at −78 °C). The resulting β-hydroxy intermediates can be reoxidized using Swern conditions to give the corresponding ketones **8** and **9** in excellent yields (85–99%). The protected ketones are more chirally stable than **4** and can be purified by chromatography on silica gel without racemization. The structures and chiral purity of **8** and **10** and the diastereomeric

(10) Corey, E. J.; Raju, N. *Tetrahedron Lett.* **1983**, *24*, 5571–5574.
(11) Traces of acid in $CDCl_3$ will cause the ortho ester to ring-open; this can be prevented by prefiltering the $CDCl_3$ through basic alumina.
(12) Kaestle, K. L.; Anwer, M. K.; Audhya, T. K.; Goldstein, G. *Tetrahedron Lett.* **1991**, *32*, 327–330.
(13) Brückner, H.; Wittner, R.; Godel, H. *J. Chromatogr.* **1989**, *476*, 73–82.

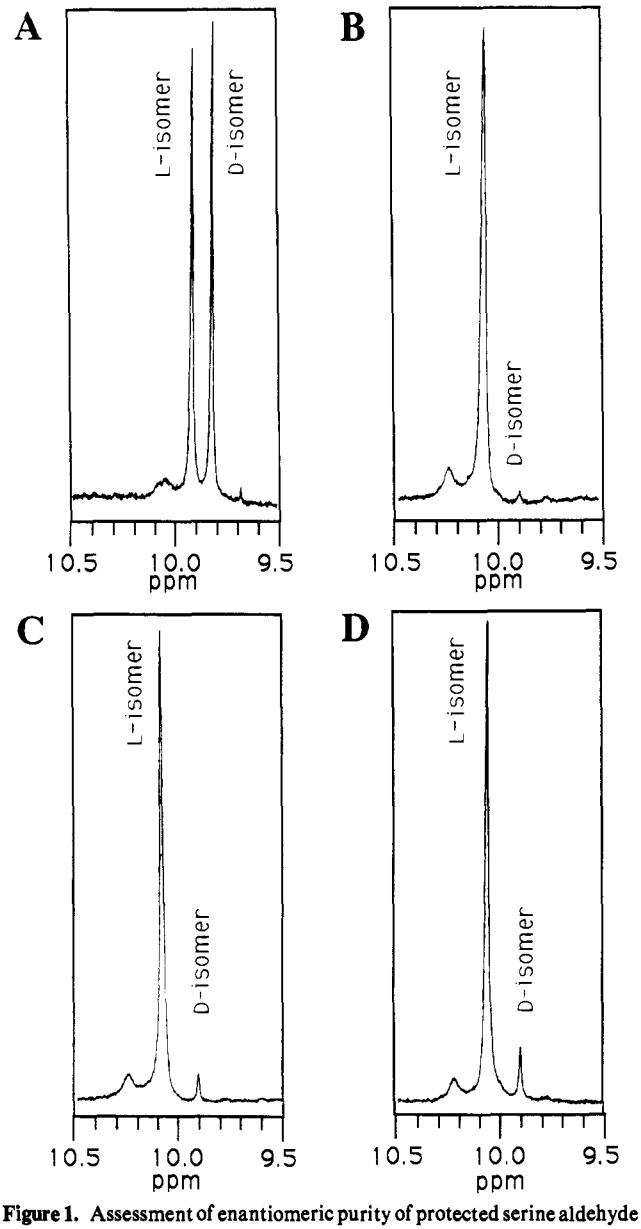


Figure 1. Assessment of enantiomeric purity of protected serine aldehyde **4** by chiral shift ^{1}H NMR studies. Spectra were obtained at 200 MHz using 10.0 mg of **4** in 500 μL of benzene-d_6. (A) D,L-Fmoc-Ser(ald)-OBO ester (**4**) + 100 μL of 50 mg/mL Eu(hfc)$_3$ in benzene-d_6 (0.18 equiv); % D expected: 50.0, measured (by peak integration): 51.0. (B) L-Fmoc-Ser(ald)-OBO ester (**4**) + 100 μL of 50 mg/mL Eu(hfc)$_3$ in benzene-d_6 (0.18 equiv); % D expected 0.0, measured 0.9. (C) As for B, spiked with 40 μL of the D,L-Fmoc-Ser(ald)-OBO ester/Eu(hfc)$_3$ solution used in A; % D expected 3.0, measured 3.8. (D) As for B, spiked with 90 μL of the D,L-Fmoc-Ser(ald)-OBO ester/Eu(hfc)$_3$ solution used in A; % D expected 6.4, measured 7.6.

configuration of **10** were confirmed by an independent synthesis from L-threonine using the Fmoc/OBO ester protection scheme. In addition, the crystal structure of **8** has been obtained. Both ketones **8** and **9** can subsequently be reduced ($LiBH_4$, quantitative) to regenerate the corresponding alcohol but with the opposite configuration at the β-carbon. A diastereoselectivity of 91:9 erythro (2*S*,3*S*):threo (2*S*,3*R*) is observed for threonine derivative **12** and a >98:<2 erythro(2*S*,3*S*):threo(2*S*,3*R*) ratio for L-phenylserine derivative **13**. The selectivity can be reversed if $Zn(BH_4)_2$ is used as the reducing agent, giving 32:68 erythro (2*S*,3*S*):threo(2*S*,3*R*) when **8** is reduced. Deprotection of β-hydroxy derivatives by procedure A or B gives the corresponding α-amino acids. HPLC analysis after derivatization allows for both diastereomeric and enantiomeric ratios to be determined, as the four possible isomers are well resolved (Figure 2). The

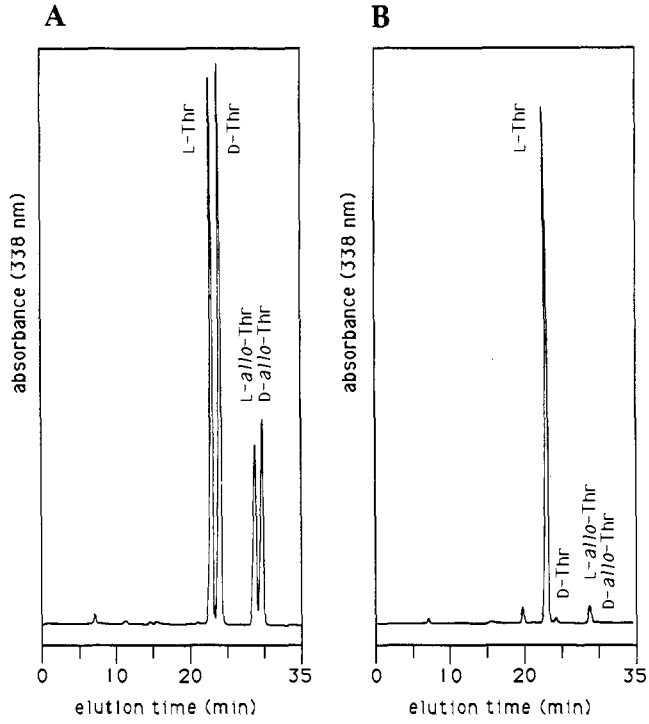
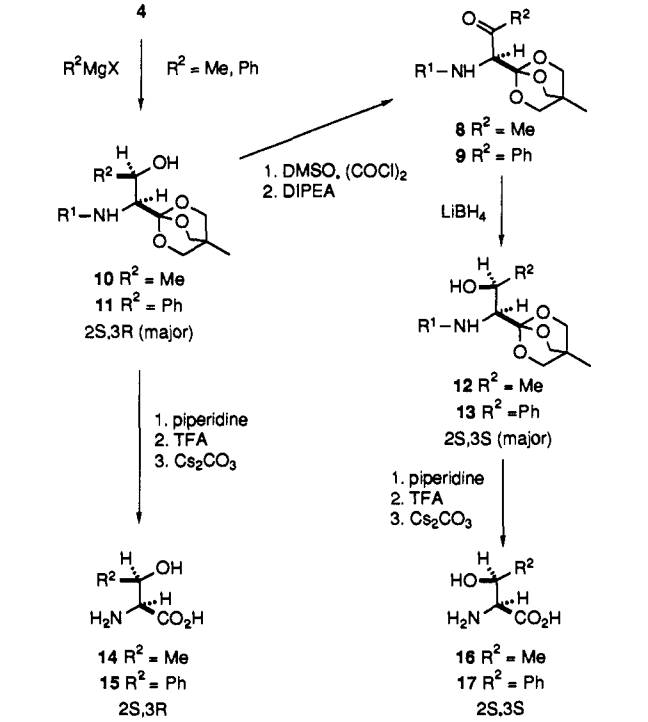


Figure 2. Assessment by HPLC of enantiomeric and diastereomeric purity of deprotected derivatized threonine analogs. Amino acid samples (approximately 1 mg/mL in 0.01 N HCl, 40 μL) were mixed with borate buffer (0.133 M, pH 10.4, 80 μL), OPA (5 mg/mL in borate buffer, 40 μL), and *N*-*i*-Bu-L-Cys (20 mg/mL in borate buffer, 40 μL). After 1 min, 25 μL was injected on a Waters Radial-Pak C-18 cartridge column and eluted at 2 mL/min with a gradient of 100% sodium acetate buffer solution (30 mM, pH 6.5) to 55% methanol in 35 min with detection at 338 nm: (A) 70:30 mixture of D,L-Thr:D,L-*allo*-Thr standards; (B) deprotected **10**, the crude MeMgBr adduct of Fmoc-Ser(ald)-ortho **4**, showing 94:6 Thr:*allo*-Thr with 98.0% ee.

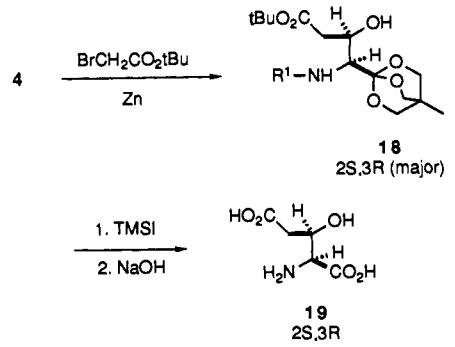
Scheme III



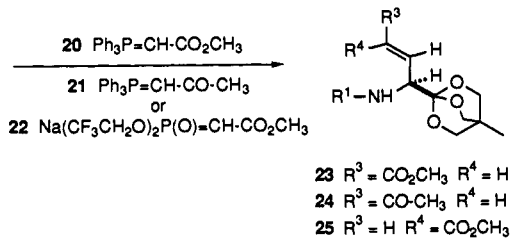
diastereomeric ratios can also be determined by ^{1}H NMR by integration of the amide protons of the protected amino acids or the α-protons of the deprotected products.

Reformatsky Addition. Reformatsky reaction of aldehyde **4**

Scheme IV



Scheme V



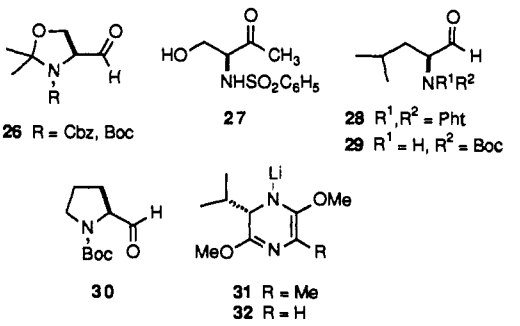
with the organozinc derived from *tert*-butyl bromoacetate gives a 92:8 mixture of threo (2*S*,3*R*):erythro (2*S*,3*S*) β-hydroxyglutamic acid derivative **18** in 73% yield (Scheme IV). Deprotection with TMSI removes all protecting groups to give (2*S*,3*R*)-β-hydroxyglutamic acid (**19**) in good yield, with >98% ee.

Wittig Reaction. Olefination of **4** with stabilized Wittig reagents **20** or **21** (Scheme V) produces β,γ-didehydro derivatives **23** or **24** in excellent yields, with high *E* selectivity (>95:<5 *E*:*Z*). *Z* isomer **25** can be obtained preferentially (70% yield, 90:10 *Z*:*E*) by using anion **22** derived from $(CF_3CH_2O)_2P(O)CH_2CO_2CH_3$ and NaH (–78 °C, THF). The *E* and *Z* isomers can easily be separated by flash chromatography on silica gel. The enantiomeric excess (ee) was 99% for **23**, 90% for **24**, and 93% for **25**, as determined by ^1H NMR in the presence of $Pr(hfc)_3$. Attempts at acylation with (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (Mosher's acid) and BOP/NMM after removal of the Fmoc group with piperidine were unsuccessful with ketone **24** and resulted in small amounts of partially racemized derivatives (approximately 70% ee) with esters **23** and **25**. Deprotection attempts using procedure A or B were unsuccessful. However, preliminary results of deprotection when alternate N-protecting groups are used appear promising, as the Cbz- and Boc-protected aldehydes can be reacted with unstabilized ylides and successfully deprotected. These protected unsaturated intermediates should lend themselves to further synthetic transformations.[4f]

Discussion

The carbonyl addition reactions demonstrate a striking diastereoselectivity for an acyclic system. For example, the Grignard addition of MeMgBr at –78 °C gives a 94:6 ratio (threo:erythro) of diastereomers, in sharp contrast to the 1:1 mixture of diastereomers obtained by Beaulieu et al.[14] when MeMgBr was added to Garner's Cbz-protected cyclic oxazolidone aldehyde **26**. The ratios obtained are similar to those observed for Grignard addition to *N*,*N*-dibenzyl-protected α-amino aldehydes, though with opposite selectivity.[8h] The ketone reduction selectivities ($LiBH_4$, –78 °C, 91:9 erythro:threo for Me ketone **8**, 98:2 for Ph ketone **9**) can be compared to the $NaBH_4$ reduction of a *N*-phenylsulfonyl-protected methyl ketone derivative of serine **27**, which gave a diastereoselectivity of 70:30.[7d]

The high diastereoselectivity of Reformatsky addition of *tert*-butyl bromoacetate to aldehyde **4** (92:8 threo(2*S*,3*R*):erythro (2*S*,3*S*)) is in sharp contrast to the only other reported Reformatsky reaction with an *N*-protected α-amino aldehyde. When the organozinc derivative of isopropyl acetate was added to *N*-phthalyl-protected leucinal **28**, a 55:45 syn:anti ratio of adducts was obtained.[15] Condensation of lithiated ethyl acetate with Boc-L-leucinal (**29**) also gave minimal diastereoselectivity (60:40 syn:anti);[16] cyclic Boc-L-prolinal (**30**) gave somewhat better results (80:20 syn:anti).[17] Reetz et al.[8h] observed high selectivity that was oppposite (95:5 anti:syn) to our results with the addition of lithiated methyl acetate to *N*,*N*-dibenzyl-protected α-amino aldehydes.



The stereochemical outcome observed in the carbonyl additions is consistent with a nonchelation-controlled Felkin–Anh attack on the aldehyde from the face opposite to the OBO ester blocking group (*re* face attack, Figure 3).[18] The results would also be consistent with a chelation-controlled model,[19] with the nitrogen as the chelating heteroatom, except that a reversal in reduction diastereoselectivity is observed when the chelating reagent $Zn(BH_4)_2$[20] is used. This reversal suggests that nonchelation control is normally in effect. The increase in reduction diastereoselectivity with increasing size of the ketone substituent agrees with the Felkin–Anh model predictions.[18a]

The addition results are also supported by the X-ray crystal structure of the protected threonine ketone derivative **8** (Figure 4), which clearly shows that one side of the carbonyl is much more accessible for attack. This is the *re* face from which nonchelation-controlled reduction occurs with Thr **8** and phenylserine **9** ketones. It corresponds to the side of nucleophilic attack on Ser aldehyde **4**, assuming this compound adopts a similar conformation. In the crystal structure, the carbonyl is slightly twisted from the Felkin–Anh configuration so that the carbonyl and the urethane nitrogen are almost eclipsed. The ketone oxygen is not within hydrogen-bonding distance of the urethane NH, but an oxygen of the ortho ester group of a neighboring molecule is. Thus, the structure observed may be somewhat distorted from the actual structure in solution, but it still supports the proposed Felkin–Anh mode of attack. These ground-state arguments of nonchelation diastereoselective control agree with those made by Reetz based on the X-ray crystal structure of *N*,*N*-dibenzylphenylalaninal.[8g]

Additions on the Garner aldehyde derived from D-serine (**26**), which result in L-β-hydroxy amino acids, occur preferentially as a nonchelation-controlled Felkin–Anh attack on the *si* face (as do additions to α-amino aldehydes derived from D-amino acids, such as leucinal (**29**) and *allo*-threoninal).[7f] The net result is the opposite (erythro, or anti) diastereomer to that in our method. A recent report[8f] uses vinylzinc chloride to obtain a reversal of addition stereochemistry (syn:anti 6:1), in which a coordinated

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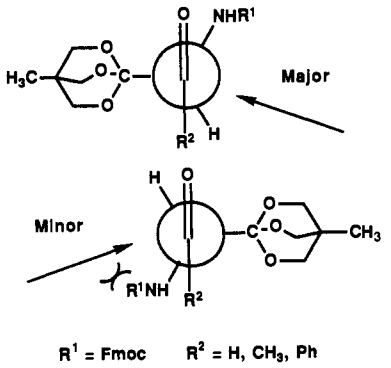

Figure 3. Felkin–Anh model predicting direction of attack on the side-chain carbonyl.

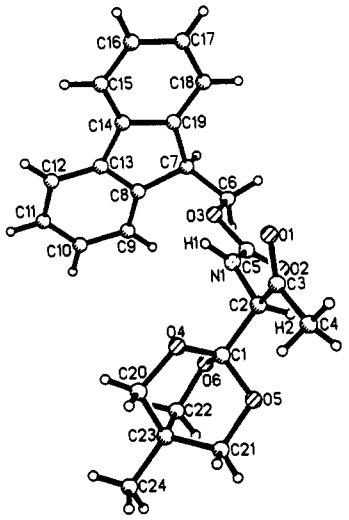

Figure 4. X-ray diffraction structure of Fmoc-Thr(ket)-OBO ester (**8**), the protected threonine ketone derivative.

delivery of a nucleophile is proposed. It should be noted that the Garner aldehyde cannot be isolated enantiomerically pure (ee = 93–95%).[7b] Rapoport synthesis of β-hydroxy α-amino acids from *N*-phenylsulfonyl-protected amino ketones **27** also produces the erythro diastereomer.[7d,8e]

The instability observed when deprotection of β,γ-didehydro-δ-keto derivatives was attempted is not without literature precedent. Beaulieu et al.[8c] prepared a number of vinylglycine derivatives by Wittig reactions with Garner's aldehyde **26**. Derivatives in which the alkene was conjugated with a methyl ester or aromatic ring could not be oxidized to give the amino acid. This result is surprising, as one would expect the conjugation to help stabilize the alkene. Similar results were obtained with chiral Boc-protected serinal, which has been prepared and reacted with stabilized ylides to give allylamino alcohols.[7g] The side-chain alcohol could not be oxidized to give the amino acid unless the alkene was first reduced. When Schöllkopf's bis-lactim ether enolate (**31**, derived from L-Val-L-Ala) was used in a "Michael-type" addition to methyl acrylates containing a leaving group in the β-position, the desired α-methyl-β,γ-didehydro-δ-keto products were obtained.[21] However, if the α-methyl was replaced with a proton by using the L-Val-L-Gly-derived enolate **32**, only the isomerized α,β-didehydro derivatives were obtained. The rapid isomerization of β,γ-didehydroglutamic acid to the α,β-didehydro derivative has been reported by Bory et al.,[22] who found that isomerization was complete after 1 h in a pyridine/CH_2Cl_2 solution. They also determined a half-life of $t_{1/2}$ = 2 min in a pH 8.2 methanol/phosphate buffer solution, with $t_{1/2}$ = 6 min at pH 7.

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(22) Bory, S.; Gaudry, M.; Marquet, A. *New J. Chem.* **1986**, *10*, 709–713.

The derivatization and HPLC analysis procedure used for analysis of the deprotected amino acids provides a rapid and accurate quantitation of both enantiomeric and diastereomeric purity in a single assay and is thus much more useful than Mosher acid derivatives. Standard retention times can be determined by using *N*-*i*-Bu-L-Cys and *N*-*i*-Bu-D-Cys, avoiding the need to prepare the D-amino acid analogs. The method is very sensitive as it can detect as little as 0.1–0.2% of the enantiomer or diastereomer. Its accuracy is limited by the enantiomeric purity of the *N*-*i*-Bu-L-Cys used to form the diastereomeric derivative. An improved synthesis[23] of this compound provides it in >99.6% ee.[24] By using this assay, it became apparent that the commercially available L-Ser used as a starting material was contaminated by 1–2% D-Ser, as has been noted in the literature.[7b,25] This purity cannot be improved by recrystallization. However, enantiomeric purity increases during preparation of the protected intermediates, so that when Fmoc-Ser-OBO ester is deprotected with TMSI, it is found to contain only 0.8% D-Ser. Deprotection with the Cs_2CO_3 procedure (procedure A) causes 1–2% racemization with serine, which could likely be minimized if the cleavage time in basic solution was optimized. Since serine is known to be the common amino acid most prone to racemization,[26] other amino acids should show an even lower decrease in enantiomeric purity. This is demonstrated by the minimal racemization found when threonine, phenylserine, and β-hydroxyglutamic acid were deprotected by procedure A. The increased chiral stability of compounds with an additional β-alkyl substituent is also supported by the stability of the protected threonine and phenylserine ketone derivatives to chromatography on silica gel.

Conclusions

In summary, we describe a new strategy for the synthesis of a chiral serine aldehyde equivalent by masking the carboxylic functional group of serine as an ortho ester. Both L- and D-amino acid derivatives can be prepared starting directly from the commercially available L- or D-serine, respectively. We are also investigating other amine protecting groups, other addition reactions to the OBO ester protected chiral aldehyde, and OBO ester protection of other amino acids. The large degree of stereocontrol exhibited during additions to this protected aldehyde should permit the stereoselective synthesis of a variety of highly functionalized chiral α-amino acids. The methodology also allows for incorporation of carbon and hydrogen isotopes late in the synthetic scheme, thereby minimizing their loss.

Experimental Section

General Methods. Fmoc-succinimide was purchased from Raylo Chemicals, BF_3·Et_2O and DMAP from Fluka, L-serine and DCC from Chemical Dynamics, L-threonine from Schweizerhall, and D-serine, D,L-serine, L-cysteine, D,L-cysteine, 3-(hydroxymethyl)-3-methyloxetane, isobutyryl chloride, oxalyl chloride, and most other reagents from Aldrich Chemical Company. CH_2Cl_2, DMSO, and DIPEA were distilled from CaH_2, THF and Et_2O from Na/benzophenone. Most reactions were carried out under N_2 in glassware dried overnight at 120 °C or flame-dried before use.

(23) Obtaining good yields of *N*-*i*-Bu-L-Cys without racemization was difficult when the procedure reported by Brückner, Wittner, and Godel[13] was used (4–5% racemization observed). Reaction of L-Cys_2 in 2:1 H_2O:dioxane with 10 eq of NaOH and 5 eq of isobutyryl chloride for 10 min was found to give much better yields of (*N*-*i*-Bu-L-Cys)$_2$ with minimal racemization (<0.2%). Reduction with Zn/2 N HCl gave the desired *N*-*i*-Bu-L-Cys.
(24) Racemization of *N*-*i*-Bu-L-Cys was determined by derivatization with L-alanine and analysis by HPLC, detection limit approximately 0.2% of one diasteromer (Waters 125-Å, 8- × 100-mm μ-Bondapak C_{18} Radial-Pak cartridge column, 2 mL/min; 100% 30 mM sodium acetate buffer, pH 6.5; linear gradient over 35 min to 60:40 buffer:MeOH; detection at 338 nm); L-Ala-*N*-*i*-Bu-L-Cys elutes at 29.9 min, D-Ala-*N*-*i*-Bu-L-Cys at 32.8 min.
(25) Commercial L-Ser is reported as commonly containing 0.5–1.5% D-Ser: Peptide Institute, Inc., Osaka, Japan, 1992–1993 catalog, pp II and 116.
(26) Smith, G. G.; Reddy, G. V. *J. Org. Chem.* **1989**, *54*, 4529–4535.

NMR spectra were recorded in $CDCl_3$ (referenced to TMS at 0.00 ppm for ^1H NMR, to $CDCl_3$ at 77.00 ppm for ^{13}C NMR), acetone-d_6 (referenced to 2.04 ppm for ^1H NMR, 29.80 ppm for ^{13}C NMR), benzene-d_6 (referenced to 7.15 ppm for ^1H NMR), or D_2O (referenced to 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid at 0.00 ppm for both ^1H NMR and ^{13}C NMR) on a Bruker AC-200 or AM-250 spectrometer. $CDCl_3$ used for NMR samples containing an ortho ester was prefiltered through basic alumina to remove traces of acid. IR spectra were recorded on a Bomem MB-100 FT-IR spectrophotometer. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. Melting points were determined on a Mel-Temp apparatus in an open capillary tube and are uncorrected. Low- and high-resolution mass spectral analyses were carried out by Gaston Boulay at the Université de Sherbrooke. Elemental analyses were determined by M-H-W Laboratories in Phoenix, AZ. HPLC analyses were performed using a Waters 600E system controller with Waters 600 multisolvent delivery system, Model 481, or 486 variable wavelength UV/vis detector, and Waters 745 data module. TLC was carried out on Merck aluminum backed silica gel 60 F_{254}, with visualization by UV, ninhydrin solution (2% in EtOH), or I_2. TLC solvent systems commonly used are as follows: A, 1:1 EtOAc:hexane; B, 3:1 EtOAc:hexane; C, 1:1:1:1 H_2O:EtOAc:*n*-BuOH:MeOH.

***N*-(9-Fluorenylmethyloxycarbonyl)-L-serine, Fmoc-L-Ser, 1.** L-Ser (25.5 g, 0.243 mol) and $Na_2CO_3 \cdot H_2O$ (33.2 g, 0.268 mol, 1.1 equiv) were dissolved in H_2O (300 mL) and added dropwise to a stirred solution of Fmoc-succinimide (85.9 g, 0.255 mol, 0.95 equiv) in dioxane (550 mL) cooled in ice to 8 °C. The solution was allowed to warm to room temperature overnight and was poured into H_2O (500 mL) after 24 h. The solution was extracted with CH_2Cl_2 (3 x 500 mL), and the organic fractions were combined, washed (1 x 500 mL 1 N HCl), dried ($MgSO_4$), and evaporated to dryness. The white solid obtained could be recrystallized from EtOAc/hexane, Et_2O/hexane, or $CHCl_3$; in all cases, extensive drying in vacuo was required to remove all the solvent; EtOAc and Et_2O were particularly difficult to remove. White crystals (78.6 g, 94% yield) were collected by filtration: mp 74–86 °C; TLC (65:25:4:3 $CHCl_3$:MeOH:H_2O:AcOH) R_f 0.42; IR (Nujol mull) 3313 (m), 3400–2400 (br), 1741 (s), 1673 (s), 1534 (s), 1224 (m), 1087 (m), 1054 (m), 758 (s), 737 (s) cm^{-1}; ^1H NMR (acetone-d_6, 250 MHz) δ 7.86–7.28 (m, 8H, Fmoc =C*H*), 6.54 (d, J = 8.0 Hz, 1H, N*H*), 4.40–4.21 (m, 4H, Fmoc C*H*C*H*$_2$O, α-C*H*), 3.98 (dd, J = 11.0, 4.6 Hz, 1H, β-CH*H*), 3.89 (dd, J = 11.1, 3.9 Hz, 1H, β-C*H*H); ^{13}C NMR (acetone-d_6, 62.9 MHz) δ 172.19 ($\underline{C}O_2H$), 157.02 ($\underline{C}ONH$), 145.05, 142.09 (Fmoc =$\underline{C}$=), 128.50, 127.93, 126.16, 120.77 (Fmoc =$\underline{C}$H), 67.37 (Fmoc $\underline{C}H_2O$), 63.06 (β-$\underline{C}H_2$), 57.23 (α-$\underline{C}H$), 47.99 (Fmoc $\underline{C}HCH_2$).

***N*-(9-Fluorenylmethyloxycarbonyl)-L-serine 3-Methyl-3-(hydroxymethyl)oxetane Ester, Fmoc-L-Ser-oxetane Ester, 2.** Fmoc-L-Ser (**1**) (1.97 g, 4.74 mmol) was dissolved in CH_2Cl_2 (50 mL) and added dropwise over 1 h to a stirred solution of DCC (1.47 g, 7.11 mmol, 1.5 equiv), DMAP (29.0 mg, 0.237 mmol, 0.05 equiv), and 3-methyl-3-(hydroxymethyl)-oxetane (9.68 g, 94.8 mmol, 20 equiv) cooled to 0 °C. After 3 h following completion of addition, the solution was filtered to remove DCU. It was then washed with 1% NH_4Cl (2 x 125 mL) and 5% $NaHCO_3$ (1 x 125 mL), dried ($MgSO_4$), and evaporated to dryness, yielding a white foam. The aqueous fractions were saved for recovery of oxetane alcohol. The product was purified by flash chromatography (silica gel, 40:1 $CHCl_3$:IPA or 2:1 EtOAc:hexane, loaded in CH_2Cl_2), yielding 1.76 g (90%) of a white foam. This was crystallized from EtOAc/hexane to give 1.42 g (73%) of colorless crystals. The main byproducts of the reaction were the dimer resulting from acylation of one serine side chain, Fmoc-Ser-*O*-(Fmoc-Ser-oxetane) (TLC, solvent B: R_f 0.44), and Fmoc-Ser-*N*-acylurea (TLC, solvent B: R_f 0.63).

Oxetane alcohol was recovered by evaporating the aqueous fractions to near dryness and then extracting them with EtOAc (3 x 50 mL). The organic fractions were combined, dried ($MgSO_4$), and evaporated to a viscous liquid. The liquid was distilled under vacuum to yield 6.16 g (67% of theoretical recovery) of 3-methyl-3-(hydroxymethyl)oxetane (^1H NMR and bp identical to authentic sample). **2**: mp 106–107 °C; $[\alpha]^{25}_D$ –6.4° (c = 1.01, EtOAc); TLC (solvent B) R_f 0.35; IR (Nujol mull) 3453 (w), 3382 (w), 3305 (w), 1748 (s), 1701 (s), 1609 (vw), 1541 (m), 1340 (w), 1212 (w), 1191 (m), 1083 (s), 1051 (m), 966 (m), 763 (m), 738 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.78–7.28 (m, 8H, Fmoc =C*H*), 5.76 (br d, J = 6.9 Hz, 1H, N*H*), 4.59–4.42 (m, 8H, Fmoc C*H*$_2$O, 3 oxetane C*H*$_2$O), 4.24 (t, J = 6.9 Hz, 1H, Fmoc C*H*CH$_2$), 4.18–4.08 (m, 2H, α-C*H*, β-C*H*H), 3.96–3.87 (m, 1H, β-CH*H*), 2.88 (t, J = 6.3 Hz, 1H, O*H*), 1.30 (s, 3H, oxetane C*H*$_3$); ^{13}C NMR (gated and decoupled, gated couplings indicated before assignments, $CDCl_3$, 50.3 MHz) δ 170.67 (s, $\underline{C}OO$), 156.21 (s, $\underline{C}ONH$), 143.77, 143.65, 141.28 (s, Fmoc =$\underline{C}$=), 127.70, 127.05, 125.05, 119.97 (d, Fmoc =$\underline{C}$H), 79.45 (t, oxetane $\underline{C}H_2O$), 68.97 (t, oxetane $\underline{C}H_2OCO$), 67.17 (t, Fmoc $\underline{C}H_2O$), 63.29 (t, β-$\underline{C}H_2$), 56.35 (d, α-$\underline{C}H$), 47.09 (d, Fmoc $\underline{C}HCH_2$), 39.65 (s, oxetane $\underline{C}CH_3$), 20.69 (q, oxetane C$\underline{C}H_3$); MS (CI, CH_4) m/z 412 (MH^+, 100), 394 (MH^+ – 18, 95), 381 (MH^+ – 31, 26); HRMS (CI, CH_4) calcd for $C_{23}H_{26}O_6N$ 412.1760, found 412.1767 ±0.0011 (MH^+). Anal. Calcd for $C_{23}H_{25}O_6N$: C, 67.14; H, 6.12; N, 3.40. Found: C, 66.94; H, 6.01; N, 3.37.

1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*)-1-amino-2-hydroxyethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-Ser-OBO Ester, 3. Fmoc-L-Ser-oxetane ester (**2**) (1.00 g, 2.43 mmol) was dissolved in freshly distilled CH_2Cl_2 (15 mL) and cooled to 0 °C under N_2. A solution of $BF_3 \cdot Et_2O$ (40 μL of a 20% (v/v) solution in CH_2Cl_2, 0.065 mmol, 0.027 equiv) was added, and the solution was stirred and allowed to warm to room temperature. After 8 h, Et_3N (100 μL, 0.72 mmol, 0.29 equiv) was added and the solution evaporated to dryness. The residue was purified by flash column chromatography (silica gel, 3:1 EtOAc:hexane, loaded in CH_2Cl_2), giving 0.855 g (85%) of a white foam. Recrystallization from EtOAc/hexane gave 0.757 g (76%) of colorless crystals: mp 146–147 °C; $[\alpha]^{25}_D$ –21.1° (c = 1.01, EtOAc); TLC (solvent A) R_f 0.18, (solvent B) R_f 0.43; IR (Nujol mull) 3456 (w), 3391 (w), 1699 (s), 1529 (m), 1244 (w), 1044 (s), 1013 (m), 991 (m), 739 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.78–7.26 (m, 8H, Fmoc =C*H*), 5.36 (br d, J = 9.3 Hz, 1H, N*H*), 4.39 (d, J = 7.0 Hz, 2H, Fmoc C*H*$_2$O), 4.25 (t, J = 7.0 Hz, 1H, Fmoc C*H*CH$_2$), 4.00–3.87 (m, 2H, α-C*H*, β-C*H*H), 3.94 (s, 6H, 3 ortho C*H*$_2$O), 3.75–3.64 (m, 1H, β-CH*H*), 2.55 (br dd, J = 8.4, 3.9 Hz, 1H, O*H*), 0.82 (s, 3H, ortho C*H*$_3$); ^{13}C NMR (gated and decoupled, gated couplings indicated before assignments, $CDCl_3$, 50.3 MHz) δ 154.21 (s, $\underline{C}ONH$), 144.07, 143.97, 141.25 (s, Fmoc =$\underline{C}$=), 127.60, 126.99, 125.18, 119.99 (s, Fmoc =$\underline{C}$H), 108.54 (s, ortho $\underline{C}O$), 72.71 (t, ortho $\underline{C}H_2O$), 66.99 (t, Fmoc $\underline{C}H_2O$), 61.94 (t, β-$\underline{C}H_2$), 55.19 (d, α-$\underline{C}H$), 47.17 (d, Fmoc $\underline{C}HCH_2$), 30.58 (s, ortho $\underline{C}CH_3$), 14.26 (q, ortho $\underline{C}CH_3$); MS (CI, CH_4) m/z 412 (MH^+, 80), 394 (MH^+ – 18, 53), 367 ($M\underline{H}^+$ – 45, 100); HRMS (CI, CH_4) calcd for $C_{23}H_{26}O_6N$ 412.1760, found 412.1767 ± 0.0011 (MH^+). Anal. Calcd for $C_{23}H_{25}O_6N$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.14; H, 5.91; N, 3.43.

General Procedure for Removal of Protecting Groups: L-Serine, 5. Procedure A. Fmoc-L-Ser-OBO ester (**3**) (0.517 g, 1.26 mmol) was stirred with 15 mL of 20% piperidine in CH_2Cl_2 for 40 min at room temperature. The solvent was removed under vacuum, and CH_2Cl_2 (15 mL), TFA (350 μL), and H_2O (250 μL) were added to the white residue. After the solution was stirred for 15 min at room temperature, the solvent was again removed under vacuum. The oily residue was dissolved in 15 mL of MeOH, and 4 mL of H_2O and 21 mL of a 10% (wt/vol) Cs_2CO_3 solution (6.4 mmol, 5.1 equiv) were added. After being stirred for 18 h at room temperature, the solution was filtered through Celite and acidified with 2 N HCl (approximately 4 mL) to pH <3. The solution was loaded on a cation exchange column (Bio-Rad AG 50W-X8 100–200 mesh, hydrogen form, 1 x 12 cm), washed with 0.01 N HCl and H_2O, and then eluted with 5% Et_3N in H_2O (alternately, a 1 M NH_4OH solution could be used for elution). The eluate was evaporated to dryness under vacuum to give 0.120 g (91%) of a white solid. Recrystallization gave 0.109 g (82%) of white needles: mp 215.5-216.5 °C dec (lit.[27] mp 228 °C dec); spectral data identical to authentic serine; enantiomeric purity 96.8% ee (Ser starting material 97.8% ee), as determined by HPLC after derivatization (see below for conditions).

Procedure B. Fmoc-L-Ser-OBO ester (**3**) (0.106 g, 0.258 mmol) was stirred with TMSI (500 μL, 3.5 mmol, 14 equiv) at 75 °C for 24 h. After the solution was cooled, Et_2O (3 mL) was carefully added followed by the dropwise addition of 0.5 N NaOH (5 mL). The organic layer was removed and washed with 0.5 N NaOH (2 x 4 mL). The aqueous fractions were combined, washed (2 x 5 mL of Et_2O), and then acidified to pH <3 with 2 N HCl. The sample was purified on a cation exchange column as in procedure A, giving 0.0249 g (92%) of a white solid after evaporation. Recrystallization (H_2O/acetone) gave 0.0183 g (67%) of fine needles: mp 213–214 °C dec; spectral data identical to authentic serine; enantiomeric purity 98.4% ee, as determined by HPLC.

1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*)-1-amino-2-oxoethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-Ser(ald)-OBO Ester, 4. Fmoc-L-Ser-OBO ester (**3**) (0.203 g, 0.494 mmol) was dissolved in 1.5 mL of freshly distilled CH_2Cl_2 under N_2 and cooled to –78 °C (dry ice/acetone). Freshly distilled oxalyl chloride (70 μL, 0.80 mmol, 1.6 equiv) was added to 8 mL of CH_2Cl_2 in a separate flask under N_2 and

(27) *The Merck Index, ninth edition*; Windholz, M., Ed.; Merck & Co., Inc.: Rahway, NJ, 1976.

the solution cooled to −78 °C. Dry DMSO (115 μL, 1.63 mmol, 3.3 equiv) was added to the oxalyl chloride solution and the mixture stirred at −78 °C for 10 min. The alcohol solution was then transferred by cannula and rinsed with 1 mL of CH_2Cl_2. The resulting cloudy, white solution was stirred for 80 min at −78 °C, after which DIPEA (430 μL, 2.47 mmol, 5 equiv) was added. The solution was warmed to 0 °C and stirred for an additional 40 min. CH_2Cl_2 (50 mL) was added; the solution was washed with ice-cold 3% NH_4Cl (2 x 75 mL) and saturated NaCl (1 x 75 mL), dried ($MgSO_4$), and evaporated to dryness, yielding 0.207 g (102%) of a white solid foam. The aldehyde was reasonably pure by TLC and ^1H NMR and had in excess of 97% ee (as determined below). It was used without further purification as attempts at chromatography (silica gel or alumina) resulted in racemization and/or decomposition, and recrystallization was difficult, although possible (EtOAc/hexane). The aldehyde was moderately stable at room temperature and could be stored indefinitely at −20 °C with no noticeable loss in chemical or chiral purity. It was also stable in solution, with no change in optical rotation after 190 h in EtOAc at room temperature. **4**: mp 163–164.5 °C; $[\alpha]^{25}_D$ −82.2° (c = 1.02, EtOAc); TLC (solvent A) R_f 0.43, (solvent B) R_f 0.67; IR (cast from CH_2Cl_2) 3356 (m), 3063 (w), 2949 (w), 2884 (w), 1723 (s br), 1552 (m), 1450 (w), 1334 (w), 1248 (w), 1075 (s), 1047 (s), 1014 (m), 760 (m), 740 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 9.72 (s, 1H, C*H*O), 7.77–7.28 (m, 8H, Fmoc =C*H*), 5.41 (d, J = 8.9 Hz, 1H, N*H*), 4.65 (d, J = 9.0 Hz, 1H, α-C*H*), 4.45–4.22 (m, 3H, Fmoc C*H*C*H*$_2$O), 3.96 (s, 6H, 3 ortho C*H*$_2$O), 0.84 (s, 3H, ortho C*H*$_3$); ^1H NMR (benzene-d_6, 250 MHz) δ 9.70 (s, 1H, C*H*O), 7.55–7.10 (m, 8H, Fmoc =C*H*), 5.53 (d, J = 9.3 Hz, 1H, N*H*), 4.97 (d, J = 9.3 Hz, 1H, α-C*H*), 4.34 (dd, J = 10.5, 7.5 Hz, 1H, Fmoc CHC*H*HO), 4.25 (dd, J = 10.5, 7.1 Hz, 1H, Fmoc CHCH*H*O) 4.05 (t, J = 7.3 Hz, 1H, Fmoc C*H*CH$_2$O) 3.30 (s, 6H, 3 ortho C*H*$_2$O), −0.17 (s, 3H, ortho C*H*$_3$); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 195.53 (CHO), 156.18 (CONH), 143.88, 141.26 (Fmoc =C=), 127.64, 127.04, 125.22, 119.91 (Fmoc =CH), 107.24 (ortho CO), 72.95 (ortho CH$_2$O), 67.46 (Fmoc CH$_2$O), 63.32 (α-CH), 47.25 (Fmoc CHCH$_2$), 30.93 (ortho CCH$_3$), 14.29 (ortho CCH$_3$); MS (CI, CH_4) m/z 410 (MH^+, 100), 381 (MH^+ − 29, 6), 279 (MH^+ − 13, 16); HRMS (CI, CH_4) calcd for $C_{23}H_{24}O_6N$: 410.1603, found: 410.1615 ± 0.0011 (MH^+). Anal. Calcd for $C_{23}H_{23}O_6N$: C, 67.47; H, 5.66; N, 3.42. Found: C, 67.21; H, 5.82; N, 3.45.

Determination of Enantiomeric Purity. Method A. NMR Chiral Shift Analysis. Solutions of 50 mg/mL Eu(hfc)$_3$ or Pr(hfc)$_3$ and 20 mg/mL aldehyde were prepared in benzene-d_6. Aliquots of the chiral shift reagent solution were added until optimum peak separation was obtained. A base-line separation of the aldehyde CHO peak was achieved when 500 μL of aldehyde solution and 100 μL of Eu(hfc)$_3$ solution (0.18 equiv Eu(hfc)$_3$) were used, with peaks at 9.92 ppm (L-serine derivative) and 9.83 ppm (D-serine derivative) (Figure 1A). The amount of Eu(hfc)$_3$ was not critical; 0.33 equiv gave peaks at 10.25 and 10.00 ppm, but there was more line broadening and interference from Eu(hfc)$_3$ signals when greater amounts were used. Samples of crude aldehyde derivative were then examined by standard addition, using 0.18 eq of Eu(hfc)$_3$ and adding aliquots of Fmoc-D-Ser(ald)-OBO ester premixed with 0.18 equiv of Eu(hfc)$_3$ (Figures 1B–D).

Method B. Reduction, Deprotection, Derivatization, and HPLC Analysis. Crude Fmoc-Ser(ald)-ortho **4** (0.0341 g, 0.0833 mmol) was dissolved in 2 mL of CH_2Cl_2 and 2 mL of MeOH, and $NaBH_4$ (10 mg, 0.26 mmol) was added. The reaction was quenched with 25 mL of 5% NH_4Cl after 10 min; the solution was extracted into CH_2Cl_2 (2 x 20 mL), dried ($MgSO_4$), and evaporated to give 0.0282 g of a white foam (82%). The protecting groups were removed with TMSI (see deprotection procedure B), and the crude cleaved product was derivatized as follows. A solution of the amino acid (10–40 μL of approximately 1 mg/mL) was mixed with borate buffer (80 μL of a 0.133 M solution, pH 10.4), o-phthaladehyde (40 μL of a 5 mg/mL solution in borate buffer), and N-isobutyryl-L-cysteine (40 μL of a 20 mg/mL solution in borate buffer).[13,23,24] After 5 min, 25 μL of this solution was injected onto a Waters 125-Å 8- x 100-mm μ-Bondapak C_{18} Radial-Pak cartridge column (2 mL/min; 100% 30 mM sodium acetate buffer, pH 6.5; linear gradient over 25 min to 60:40 buffer:MeOH; detection at 338 nm). Retention times were determined by using various combinations of L-serine, D-serine, N-isobutyryl-L-cysteine, and N-isobutyryl-D-cysteine. The L-Ser-L-*i*-Bu-Cys diastereomer (and any D-Ser-D-*i*-Bu-Cys) eluted at 19.1 min, with the D-Ser-L-*i*-Bu-Cys (and L-Ser-D-*i*-Bu-Cys) derivative at 20.5 min. The extent of racemization caused by oxidation of Fmoc-Ser-OBO ester was determined by comparing the deprotected, reduced aldehyde sample with a sample of deprotected Fmoc-Ser-OBO ester starting material. In one set of experiments carried out at the same time, samples of Fmoc-Ser(ald)-OBO ester were reduced, then deprotected by procedure A or B, and found to contain 2.2% or 1.4% D-Ser, respectively. Fmoc-Ser-OBO ester deprotected by procedure A gave product with 1.6% D-Ser, while procedure B resulted in 0.8% D-Ser. The consistent difference indicated that the oxidation was causing less than 0.6% racemization, while the Cs_2CO_3 deprotection was causing 0.8% racemization. Commercial L-Ser starting material contained 1.1% D-Ser when analyzed under identical conditions.[25] Enantiomeric purities of >97% ee were routinely obtained for the reduced, deprotected, aldehyde. This HPLC analysis method was also used to assess both diastereomeric and enantiomeric ratios for a variety of other amino acids after synthesis. Its sensitivity was around 0.1%, with its accuracy limited by the enantiomeric purity of *N*-*i*-Bu-L-Cys.[23,24]

Grignard Addition of MeMgBr to Fmoc-L-Ser(ald)-OBO Ester, 4: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*,2*R*)-1-amino-2-hydroxypropyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-Thr-OBO Ester, 10. Crude Fmoc-L-Ser(ald)-OBO ester (**4**) (0.201 g, 0.468 mmol, assuming 100% yield of the aldehyde from oxidation) was dissolved in dry CH_2Cl_2 (2 mL), diluted with dry Et_2O (15 mL), and cooled to −78 °C (dry ice/acetone) under N_2. A solution of MeMgBr in Et_2O (Aldrich, 3.0 M, 900 μL, 2.7 mmol, 5.8 equiv) was added quickly by syringe, and the solution was stirred vigorously. After 7 min, the reaction was quenched by pouring the solution into 100 mL of 5% NH_4Cl. CH_2Cl_2 (100mL) was added, and the organic layer was separated, washed with 5% NH_4Cl (1 x 100 mL) and saturated NaCl (1 x 100 mL), dried ($MgSO_4$), and evaporated to dryness, yielding 0.222 g of a white foam. The crude product was purified by flash column chromatography (silica gel, 2:1 EtOAc:hexane, loaded in CH_2Cl_2) to give 0.113 g of a white solid (57% yield for two steps, oxidation and Grignard reaction) and 0.484 g (25%) of recovered aldehyde (racemic). ^1H NMR integration of the amide protons indicated a 94:6 threo (2*S*,3*R*):erythro (2*S*,3*S*) ratio; deprotection with TMSI (procedure B), derivatization with o-phthaladehyde and N-isobutyryl-L-cysteine, and analysis by HPLC also indicated a 94:6 ratio of L-Thr:L-*allo*-Thr, with 96% ee. Retention times were identical to those of standards prepared from L-Thr (25.3 min), D-Thr (26.3 min), L-*allo*-Thr (30.3 min), and D-*allo*-Thr (31.0 min) (Waters 125-Å 8- x 100-mm μ-Bondapak C_{18} Radial-Pak cartridge column, 2 mL/min; 100% 30 mM sodium acetate buffer, pH 6.5; linear gradient over 35 min to 45:55 buffer:MeOH; detection at 338 nm). Two recrystallizations of the protected derivative (EtOAc/hexane) gave the threo isomer in >98% de. **10**: mp 182–183.5 °C; $[\alpha]^{25}_D$ −12.3° (c = 1.01, EtOAc); TLC (solvent A) R_f 0.26 (threo), 0.24 (erythro), (solvent B) R_f 0.50, (20:1 $CHCl_3$:IPA) R_f 0.46 (threo), 0.42 (erythro); IR (Nujol mull) 3518 (m), 3454 (m), 1735 (s), 1509 (s), 1285 (m), 1214 (m), 1055 (s), 1046 (s), 977 (m), 972 (m), 750 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) threo (2*S*,3*R*) isomer δ 7.78–7.26 (m, 8H, Fmoc =C*H*), 5.36 (br d, J = 10.3 Hz, 1H, N*H*), 4.44–4.35 (m, 3H, Fmoc C*H*$_2$O, β-C*H*), 4.30–4.25 (m, 1H, Fmoc C*H*CH$_2$), 3.95 (s, 6H, 3 ortho C*H*$_2$O), 3.76 (d, J = 10.3 Hz, 1H, α-C*H*), 2.94 (s, 1H, O*H*), 1.13 (d, J = 6.4 Hz, 3H, Thr C*H*$_3$), 0.83 (s, 3H, ortho C*H*$_3$); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 156.88 (CONH), 144.05, 143.85, 141.24 (Fmoc =C=), 127.54, 126.95, 125.14, 119.87 (Fmoc =CH), 108.78 (ortho CO), 72.83 (ortho CH$_2$O), 66.93 (Fmoc CH$_2$O), 65.15 (β-CH), 57.73 (α-CH), 47.26 (Fmoc CHCH$_2$), 30.63 (ortho CCH$_3$), 18.99 (Thr CH$_3$), 14.29 (ortho CCH$_3$); MS (CI, CH_4) m/z 426 (MH^+, 67), 408 (MH^+ − 18, 100), 381 (MH^+ − 45, 74); HRMS (CI, CH_4) calcd for $C_{24}H_{28}O_6N$ 426.1916, found 426.1919 ± 0.0012 (MH^+). Anal. Calcd for $C_{24}H_{27}O_6N$: C, 67.75; H, 6.40; N, 3.29. Found: C, 67.53; H, 6.42; N, 3.26.

Deprotection of 10: L-Thr, 14. Fmoc-L-Thr-OBO ester (**10**) (0.376 g, 0.884 mmol) was deprotected according to procedure A, with Et_3N elution from the cation exchange column giving 0.101 g (96%) of a colorless solid. Recrystallization (H_2O/acetone) gave 0.0890 g (85%) of crystals: mp 251–252 °C dec (lit.[27] mp 255–257 °C dec); spectral data identical to authentic Thr; enantiomeric purity 99.0% ee, diastereomeric purity >99.8% de, as determined by HPLC (Thr standard 99.0% ee).

Grignard Addition of PhMgBr to Fmoc-L-Ser(ald)-OBO Ester, 4: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*,2*R*)-1-amino-2-phenyl-2-hydroxyethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-(2*S*,3*R*)-phenylserine-OBO Ester, 11. Crude Fmoc-L-Ser(ald)-OBO ester (**4**) (0.446 g, 1.03 mmol, assuming 100% yield in the oxidation) was dissolved in 3 mL of dry CH_2Cl_2 and diluted with 40 mL of dry Et_2O under N_2. A solution of PhMgBr in Et_2O (Aldrich, 3.0 M, 1.5 mL, 4.5 mmol, 4.4 equiv) was added quickly by syringe at room temperature and the resulting solution stirred vigorously. After 3 min, the reaction was worked up as for the addition product of MeMgBr, **10**, yielding 0.625 g of a white foam. Flash column chromatography (silica gel, 1:1 EtOAc:hexane,

loaded in CH_2Cl_2) gave 0.427 g (85% yield for two steps, oxidation and Grignard reaction) of a white solid foam. ^1H NMR integration of the amide and β-CH protons indicated a 83:17 threo (2S,3R):erythro (2S,3S) ratio; deprotection with TMSI, derivatization with *o*-phthaladehyde and *N*-isobutyryl-L-cysteine, and analysis by HPLC also indicated a 85:15 ratio, with 97.0% ee. Retention times of the threo isomers were identical to those of standards prepared from D,L-*threo*-phenylserine (113.6 min for L, 118.2 min for D); the other peaks were assumed to be the erythro isomers (131.7 min for L, 135.4 min for D), and they agreed with the retention times of the erythro isomers prepared later (Waters 125-Å 8-x 100-mm μ-Bondapak C_{18} Radial-Pak cartridge column, 2 mL/min; 100% 30 mM sodium acetate buffer, pH 6.5; linear gradient over 150 min to 63:37 buffer:MeOH; detection at 338 nm). Attempts at recrystallization (EtOAc/hexane, Et_2O/hexane) tended to produce a solid coating, with an identical diastereomeric ratio. If the addition was done at −78 °C, the yield decreased to 57% and the diastereoselectivity only increased slightly (to 86:14 threo:erythro by NMR analysis of the crude protected derivative; 90:10 by HPLC analysis of the deprotected product). **11**: mp 92–98 °C; TLC (solvent A) R_f 0.26 (threo), 0.23 (erythro); IR (cast from CH_2Cl_2) 3510 (br m), 3443 (br m), 3056 (w), 2949 (m), 2882 (m), 1722 (s), 1517 (m), 1450 (m), 1399 (w), 1234 (m), 1196 (m), 1049 (s), 1022 (m), 993 (w), 760 (m), 738 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) major isomer = threo (83%), minor = erythro (17%) δ 7.75–7.14 (m, 13H, Fmoc =C*H* + Ph =C*H*), 5.50 (d, *J* = 10.3 Hz, 0.8H, threo N*H*), 5.37–5.27 (m, 0.8H, threo β-C*H*), 4.90-4.87 (m, 0.4H, erythro N*H*, β-C*H*), 4.33–4.08 (m, 4H, Fmoc C*HCH*$_2$, α-C*H*), 4.00 (s, 5.0H, 3 threo ortho CH_2O), 3.97 (s, 1.0H, 3 erythro ortho CH_2O), 3.44 (s, 0.8H, threo O*H*), 3.34 (s, 0.2H, erythro O*H*), 0.84 (s, 3H, ortho CH_3); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 156.22 (CONH), 144.13, 144.00, 141.20 (Fmoc =C=), 140.01 (Ph =C=), 128.15 (Ph =CH), 127.52, 126.94 (Fmoc =CH), 125.85, 125.24 (Ph =CH), 125.16, 119.84 (Fmoc =CH), 108.86 (ortho CO), 72.88 (ortho CH_2O), 70.80 (β-CH), 66.93 (Fmoc CH_2O), 58.63 (α-CH), 47.08 (Fmoc CHCH$_2$), 30.74 (ortho CCH$_3$), 14.31 (ortho CCH$_3$); MS (CI, CH_4) *m/z* 488 (MH^+, 41), 470 (MH^+ − 18, 37), 381 (MH^+ − 107, 100); HRMS (CI, CH_4) calcd for $C_{29}H_{30}O_6N$ 488.2073, found 488.2068 ± 0.0014 (MH^+). Anal. Calcd for $C_{29}H_{29}O_6N$: C, 71.44; H, 5.99; N, 2.87. Found: C, 71.49; H, 6.06; N, 2.79.

Deprotection of 11: L-(2S,3R)-Phenylserine, 15. Fmoc-L-phenylserine-OBO ester (**11**) (0.351 g, 0.719 mmol) was deprotected according to procedure A, with NH_4OH elution from the cation exchange column giving 0.118 g (91%) of a white solid: 76% de by ^1H NMR; 80% de and 97% ee by HPLC. Recrystallization (H_2O/EtOH) gave 0.072 g (55%) of colorless crystals: mp 180–185 °C dec (lit.[29] mp 194–195 °C dec); enantiomeric purity 99.0% ee, diastereomeric purity 80% de, as determined by HPLC; TLC (solvent C) R_f 0.63 (both threo and erythro); ^1H NMR (D_2O, 250 MHz) major isomer = threo (90%), minor = erythro (10%) δ 7.47 (br s, 5H, Ph =C*H*), 5.37 (d, *J* = 4.0 Hz, 0.1H, erythro β-C*H*), 5.32 (d, *J* = 4.2 Hz, 0.9H, threo β-C*H*), 4.10 (d, *J* = 4.0 Hz, 0.1H, erythro α-C*H*), 3.93 (d, *J* = 4.1 Hz, 0.9H, threo α-C*H*); ^{13}C NMR (D_2O, 50.3 MHz) threo isomer δ 174.91 (CO_2H), 142.02 (Ph =C=), 131.43, 131.82, 128.75 (Ph =CH), 74.20 (β-CH), 63.76 (α-CH), weak peaks (erythro isomer) also observed at δ 129.19 (Ph =CH), 74.01 (β-CH), 63.33 (α-CH). Anal. Calcd for $C_{29}H_{29}O_6N$ C, 59.66; H, 6.12; N, 7.73. Found: C, 59.57; H, 6.23; N, 7.84.

Oxidation of Me Grignard Adduct 10: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1S)-1-amino-2-oxopropyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-Thr(ket)-OBO Ester, 8. Fmoc-L-Thr-OBO ester (**10**) (0.556 g, 1.31 mmol) was oxidized under Swern oxidation conditions identical to those used for obtaining Fmoc-L-Ser(ald)-OBO ester (**4**). After workup, 0.550 (99%) g of a white foam was obtained, essentially pure by NMR. Recrystallization yielded colorless needles (0.417 g, 75%) suitable for X-ray analysis. The ketone could be purified by column chromatography over silica gel without racemization. **8**: mp 134–135 °C; $[\alpha]^{25}_D$ −71.6° (*c* = 1.00, EtOAc); TLC (solvent A) R_f 0.46, (solvent B) R_f 0.65; IR (Nujol mull) 3352 (w), 1725 (s), 1709 (s), 1543 (m), 1357 (m), 1240 (s), 1067 (m), 1045 (s), 999 (s), 771 (w), 753 (m), 744 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.77–7.25 (m, 8H, Fmoc =C*H*), 5.69 (br d, *J* = 8.9 Hz, 1H, N*H*), 4.64 (d, *J* = 8.8 Hz, 1H, α-C*H*), 4.42–4.20 (m, 3H, Fmoc C*HCH*$_2$), 3.94 (s, 6H, 3 ortho CH_2O), 2.33 (s, 3H, COCH_3), 0.82 (s, 3H, ortho CH_3); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 202.57 (COCH$_3$), 155.98 (CONH), 143.92, 143.84, 141.23 (Fmoc =C=), 127.61, 127.01, 125.19, 119.88 (Fmoc =CH), 106.96 (ortho CO), 72.97 (ortho CH_2O), 67.26 (Fmoc CH_2O), 63.08 (α-CH), 47.13 (Fmoc CHCH$_2$), 30.70 (ortho CCH$_3$), 29.79 (COCH$_3$), 14.23 (ortho CCH$_3$); MS (CI, CH_4) *m/z* 424 (MH^+, 100), 379 (MH^+ − 45, 46); HRMS (CI, CH_4) calcd for $C_{24}H_{26}O_6N$ 424.1760, found 424.1767 ± 0.0012 (MH^+). Anal. Calcd for $C_{24}H_{25}O_6N$: C, 68.07; H, 5.95; N, 3.31. Found: C, 67.81; H, 6.02; N, 3.28.

X-Ray Experiments. The monoclinic crystals (fw 423.47), space group $P2_1$, had *a* = 10.133(2) Å, *b* = 9.086(1) Å, *c* = 11.423(1) Å, β = 95.73(1)°, V = 1046.6(2) Å^3, Z = 2, d_c = 1.344 g cm^{-3}. Data were obtained on a Siemens R3m/V diffractometer at 175 K (Mo Kα radiation, 2560 reflections collected, 2213 reflections observed with $F > 6.0\sigma(F)$). The structure was solved on a Siemens SHELXTL PLUS (VMS) by direct methods, with refinement by full-matrix least-squares, and hydrogens were determined by a Riding model, with refinement by isotropic *U*. The final residuals were *R* = 0.0387 and R_w = 0.0434 based on 305 parameters, with maximum Δ/σ = 0.01, and maximum and minimum peak heights in the final difference map were 0.22 and −0.20 eÅ^{-3}.

Oxidation of Ph Grignard Adduct 11: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1S)-1-amino-2-phenyl-2-oxoethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-phenylserine(ket)-OBO Ester, 9. Fmoc-L-phenylserine-OBO ester (**11**) (0.121 g, 0.248 mmol) was oxidized under conditions similar to those used for obtaining Fmoc-L-Ser(ald)-OBO ester (**4**), except that 2 eq of oxalyl chloride and 4 equiv of DMSO were used and the reaction was stirred at −78 °C for 130 min. The reaction was quenched with 6 equiv of DIPEA and the solution stirred at 0 °C for 70 min. Workup gave 0.117 g of a white foam solid. Purification by flash column chromatography (silica gel, 1:1 EtOAc:hexane, loaded in CH_2Cl_2) produced 0.102 g (85%) of a colorless solid, which resisted attempts at recrystallization: mp 85–92 °C; $[\alpha]^{25}_D$ −28.7° (*c* = 0.99, EtOAc); TLC (solvent A) R_f 0.59; IR (cast from $CDCl_3$) 3403 (w), 3063 (w), 2947 (w), 2882 (m), 1724 (s), 1693 (s), 1512 (m), 1449 (m), 1218 (s), 1048 (s), 1007 (m), 761 (m), 738 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.09–7.24 (m, 13H, Fmoc =C*H* + Ph =C*H*), 6.01 (d, *J* = 9.4 Hz, 1H, N*H*), 5.63 (d, *J* = 9.4 Hz, 1H, α-C*H*), 4.35 (d, *J* = 7.2 Hz, 2H, Fmoc CHC*H*$_2$), 4.23 (t, *J* = 7.0 Hz, 1H, Fmoc C*H*CH$_2$), 3.87 (s, 6H, 3 ortho CH_2O), 0.75 (s, 3H, ortho CH_3); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 195.08 (COPh), 155.91 (CONH), 143.87, 141.21 (Fmoc =C=), 136.43 (Ph =C=), 133.28, 129.37, 128.21 (Ph =CH), 127.56, 126.98, 125.19, 119.80 (Fmoc =CH), 107.30 (ortho CO), 72.93 (ortho CH_2O), 67.32 (Fmoc CH_2O), 57.41 (α-CH), 47.08 (Fmoc CHCH$_2$), 30.67 (ortho CCH$_3$), 14.19 (ortho CCH$_3$); MS (EI, 70 eV) *m/z* 485 (M^+, 63), 263 (M^+ − 222, 100); HRMS (EI, 70 eV) calcd for $C_{29}H_{27}O_6N$ 485.1838, found 485.1849 ± 0.0014 (M^+). Anal. Calcd for $C_{29}H_{27}O_6N$: C, 71.74; H, 5.67; N, 2.89. Found: C, 71.71; H, 5.67; N, 2.81.

Reduction of Oxidized Me Grignard Adduct 8: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1S,2S)-1-amino-2-hydroxypropyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-*allo*-Thr-OBO Ester, 12. Crude Fmoc-L-Thr(ket)-OBO ester (**8**) (0.269 g, 0.642 mmol) and $LiBH_4$ (0.058 g, 2.7 mmol, 4.2 equiv) were cooled to −78 °C in a 50-mL flask under N_2. A 1:1 solution of CH_2Cl_2:CH_3OH (30 mL, cooled to −78 °C) was added and the solution stirred at −78 °C for 10 h. After being warmed to room temperature, the solution was poured into 5% NH_4Cl (100 mL) and CH_2Cl_2 (50 mL) was added. The organic layer was separated, washed with 5% NH_4Cl (1 x 100 mL) and saturated NaCl (1 x 100 mL), dried ($MgSO_4$), and evaporated to dryness, yielding 0.2537 g of a white foam solid (93% yield for two steps, oxidation and reduction). NMR analysis (NH integration) indicated a 92:8 allo:threo diastereomer ratio, whereas deprotection, derivatization, and analysis by HPLC showed a 93:7 ratio, with <0.2% D-*allo*-Thr (see synthesis of **10** for HPLC conditions). Crystallization of the crude product (Et_2O/hexane) produced 0.189 g (69% yield for two steps) of a white solid as a first crop (96:4 erythro (2S,3S):threo (2S,3R) by NMR analysis of protected derivative, 96:4 by HPLC of deprotected, derivatized amino acid). A second recrystallization improved the diastereoselectivity to 98:2 (by NMR). The diastereoselectivity could also be improved by recrystallizing the deprotected amino acid. **12**: mp 143–144.5 °C; $[\alpha]^{25}_D$ −36.8° (*c* = 1.00, EtOAc); TLC (solvent A) R_f 0.24; IR (Nujol mull) 3518 (w), 3446 (w), 3413 (w), 3303 (w), 1733 (s), 1519 (m), 1283 (m), 1243 (m), 1043 (s), 995 (s), 758 (m), 739 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.78–7.26 (m, 8H, Fmoc =C*H*), 5.04 (d, *J* = 9.7 Hz, 1H, N*H*), 4.51–4.22 (m, 3H, Fmoc C*HCH*$_2$), 4.05–3.75 (m, 1H, β-C*H*), 3.94 (s, 6H, 3 ortho CH_2O), 3.83 (dd, *J* = 9.7, 7.3 Hz, 1H, α-C*H*), 3.48 (d, *J* = 3.1 Hz, 1H, O*H*), 1.18 (d, *J* = 6.2 Hz, 3H, Thr CH_3), 0.83 (s, 3H, ortho CH_3); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 156.60 (CONH), 144.06, 143.78, 141.25 (Fmoc =C=), 127.56, 126.94, 125.04, 119.87 (Fmoc =CH), 108.63 (ortho CO), 72.67 (ortho CH_2O), 67.49 (β-CH), 66.79 (Fmoc CH_2O), 59.26 (α-CH), 47.27 (Fmoc

(28) *The Aldrich Handbook of Fine Chemicals*; Aldrich Chemical Co.: Milwaukee, WI, 1992.

(29) Ozaki, Y.; Maeda, S.; Miyoshi, M.; Matsumoto, K. *Synthesis* **1979**, 216–217.

CHCH$_2$), 30.57 (ortho CCH$_3$), 19.18 (Thr CH$_3$), 14.23 (ortho CCH$_3$); MS (CI, CH$_4$) m/z 426 (MH$^+$, 100), 408 (MH$^+$ - 18, 85), 381 (MH$^+$ - 45, 89); HRMS (CI, CH$_4$) calcd for $C_{24}H_{28}O_6N$ 426.1916, found 426.1911 ± 0.0012 (MH$^+$). Anal. Calcd for $C_{24}H_{27}O_6N$: C, 67.75; H, 6.40; N, 3.29. Found: C, 67.70; H, 6.38; N, 3.30.

Deprotection of 12: L-*allo*-Thr, 16. Fmoc-L-*allo*-Thr-OBO ester (**12**) (0.097 g, 0.23 mmol, 92% de) was deprotected according to procedure A, with Et_3N elution from the cation exchange column giving 0.024 g (89%) of a white solid: 99.0% ee, 92% de by HPLC. Recrystallization (H_2O/acetone) gave 0.017 g (61%) of white crystals: mp 259–260 °C dec (lit.[28] mp 272 °C dec); spectral data identical to authentic *allo*-Thr; enantiomeric purity 99.0% ee, diastereomeric purity 96% de, as determined by HPLC (Thr standard 99.0% ee).

Reduction of Oxidized Phe Grignard Adduct 9: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*,2*S*)-1-amino-2-phenyl-2-hydroxyethyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-(2*S*,3*S*)-phenylserine-OBO Ester, 13. Crude Fmoc-L-β-ket-Phe-OBO ester **9** (0.070 g, 0.145 mmol) and $LiBH_4$ (0.011 mg, 0.52 mmol, 3.6 equiv) were cooled to −78 °C in a 10-mL flask under N_2. A 5:2 solution of CH_2Cl_2:CH_3OH (7 mL) was cooled to −78 °C and added. After being stirred for 7 h, the solution was allowed to warm to room temperature and then poured into 5% NH_4Cl (20 mL). CH_2Cl_2 (20 mL) was added, and the organic layer was separated, washed with 5% NH_4Cl (1 x 20 mL) and saturated NaCl (1 x 20 mL), dried ($MgSO_4$), and evaporated to dryness, yielding 0.067 g of a white foam solid (94% yield). NMR analysis (NH/β-H integration) indicated a >98:<2 erythro(2*S*,3*S*):threo(2*S*,3*R*) diastereomer ratio, whereas deprotection, derivatization, and analysis by HPLC (see synthesis of **11** for conditions) showed a 98:2 ratio, with <0.2% D-β-hydroxy-Phe. Crystallization (CH_2Cl_2/Et_2O/hexane) produced 0.047 g (66% yield for two steps) of translucent crystals as a first crop (>98:<2 erythro:threo by NMR analysis of protected derivative): mp 150-151 °C; TLC (solvent A) R_f 0.23; IR (cast from CH_2Cl_2) 3494 (m), 3448 (m), 3063 (w), 2948 (m), 2883 (m), 1718 (s), 1519 (m), 1450 (m), 1246 (m), 1048 (s), 1005 (s), 761 (m), 739 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.74–7.13 (m, 13H, Fmoc =C*H* + Ph =C*H*), 4.89–4.70 (m, 2H, N*H*, β-C*H*), 4.37–3.90 (m, 4H, Fmoc C*HCH*$_2$, α-C*H*), 4.08 (d, J = 1.6 Hz, 1H, O*H*), 3.97 (s, 6H, 3 ortho C*H*$_2$O), 0.85 (s, 3H, ortho C*H*$_3$); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 155.72 (CONH), 144.13, 143.76, 141.23 (Fmoc =C=), 140.10 (Ph =C=), 127.94, 127.86, 127.64 (Ph =CH), 127.54, 126.95 (Fmoc =CH), 126.85, 125.31 (Ph =CH), 125.03, 119.84 (Fmoc =CH), 108.75 (ortho CO), 74.25 (β-CH), 72.83 (ortho CH$_2$O), 66.89 (Fmoc CH$_2$O), 58.65 (α-CH), 47.02 (Fmoc CHCH$_2$), 30.73 (ortho CCH$_3$), 14.29 (ortho CCH$_3$); MS (EI, 70 eV) m/z 487 (M$^+$, 11), 469 (M$^+$ − 18, 15), 381 (M$^+$ − 106, 100); HRMS (EI, 70 eV) calcd for $C_{29}H_{29}O_6N$ 487.1995, found 487.1999 ± 0.0018 (M$^+$). Anal. Calcd for $C_{29}H_{29}O_6N$: C, 71.44; H, 5.99; N, 2.87. Found: C, 71.17; H, 6.13; N, 2.90.

Deprotection of 13: L-(2*S*,3*S*)-Phenylserine, 17. Fmoc-L-phenylserine-OBO ester (**13**) (0.061 g, 0.12 mmol) was deprotected according to procedure B, with NH_4OH elution from the cation exchange column giving 0.020 g (91%) of a white solid: 96% de, 99.6% ee by HPLC. Recrystallization (H_2O/EtOH) gave 0.011 g (52%) of colorless crystals: mp 175 °C dec; enantiomeric purity 99.2% ee, diastereomeric purity 99.2% de, as determined by HPLC; TLC (solvent C) R_f 0.63 (threo and erythro); ^{1}H NMR (D_2O, 250 MHz) δ 7.49–7.42 (m, 5H, Ph =C*H*), 5.37 (d, J = 4.1 Hz, 1H, erythro β-C*H*), 4.10 (d, J = 4.1 Hz, 1H, erythro α-C*H*); ^{13}C NMR (D_2O, 62.9 MHz) δ 174.01 (CO$_2$H), 139.72 (Ph =C=), 131.71, 129.20 (Ph =CH), 74.00 (β-CH), 63.35 (α-CH). Anal. Calcd for $C_{29}H_{29}O_6N$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.40; H, 5.98; N, 7.90.

Reformatsky Addition to Fmoc-L-Ser(ald)-OBO Ester (4): 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*,2*R*)-1-amino-3-(*tert*-butyloxycarbonyl)-2-hydroxypropyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-(2*S*,3*R*)-β-hydroxy-Glu(*O*-*t*-Bu)-OBO Ester, 18. Zinc powder (100 mesh, 0.251 g, 3.85 mmol, 6 equiv), iodine (1 small crystal), and dry THF (15 mL) were refluxed for 20 min in a dry 25-mL flask under N_2. A solution of crude Fmoc-L-Ser(ald)-OBO ester (**4**) (0.282 g, 0.642 mmol, 1 equiv, assuming 100% yield of oxidation) and *tert*-butyl bromoacetate (520 μL, 3.21 mmol, 5 equiv) in THF (5 mL) was added quickly to the refluxing solution and the solution rinsed with 3 mL of THF. The solution was refluxed for 20 min, allowed to cool to room temperature, and then poured into 100 mL of 5% NH_4Cl. CH_2Cl_2 (75 mL) was added, and the organic layer was removed, washed with 5% NH_4Cl (1 x 100 mL) and saturated NaCl (1 x 100 mL), dried ($MgSO_4$), and evaporated to dryness, yielding 0.490 g of a clear oil. Purification by flash column chromatography (silica gel, 1:1 EtOAc:hexane) gave 0.246 g (73% yield for two steps, oxidation and Reformatsky reaction) of a white solid. If present, unreacted aldehyde was first reduced with $NaBH_4$, as separation was difficult. Deprotection with TMSI, derivatization with *o*-phthaladehyde and *N*-isobutyryl-L-cysteine, and analysis by HPLC indicated a 92:8 ratio of threo:erythro L-β-hydroxyglutamic acid, with 99.0% ee. Retention times were as follows: threo-L-β-hydroxyglutamic acid (21.0 min), *threo*-D-β-hydroxyglutamic acid (22.4 min), *erythro*-L-β-hydroxyglutamic acid (26.2 min), and *erythro*-D-β-hydroxyglutamic acid (34.0 min) (Waters 100-Å 8- x 100-mm μ-Bondapak C_{18} Radial-Pak cartridge column, 2 mL/min; 100% 30 mM sodium acetate buffer, pH 6.5; linear gradient over 42 min to 93:7 buffer:MeOH; detection at 338 nm). Deprotection, derivatization, and HPLC analysis showed a 92:8 threo (2*S*,3*R*):erythro (2*S*,3*S*) diastereomer ratio, with 99.0% ee. Recrystallization (EtOAc/hexane) gave 0.155 g (46%) of colorless crystals: mp 90–93 °C; TLC (solvent A) R_f 0.34; IR (Nujol mull) 3515 (w), 3387 (w), 1721 (s), 1705 (s, sh), 1524 (m), 1232 (m), 1155 (s), 1045 (s), 1012 (m), 759 (m), 740 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.77–7.26 (m, 8H, Fmoc =C*H*), 5.36 (d, J = 10.5 Hz, 1H, N*H*), 4.62 (dd, J = 8.3, 4.6 Hz, 1H, β-C*H*), 4.40 (d, J = 7.5 Hz, 1H, Fmoc C*H*HO), 4.39 (d, J = 6.7 Hz, 1H, Fmoc CH*H*O), 4.25 (br t, J = 6.8 Hz, 1H, Fmoc C*H*CH$_2$), 3.94 (s, 6H, 3 ortho C*H*$_2$O), 3.85 (d, J = 10.3 Hz, 1H, α-C*H*), 3.10 (s, 1H, O*H*), 2.42 (dd, J = 15.8, 8.4 Hz, 1H, γ-C*H*H), 2.31 (dd, J = 15.9, 4.6 Hz, 1H, γ-CH*H*), 1.46 (s, 9H, *t*-Bu C*H*$_3$), 0.83 (s, 3H, ortho C*H*$_3$); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 170.72 (CO$_2$-*t*-Bu), 156.59 (CONH), 144.15, 143.80, 141.26 (Fmoc =C=), 127.58, 126.98, 125.20, 119.90 (Fmoc =CH), 108.59 (ortho CO), 80.79 (*t*-Bu C), 72.73 (ortho CH$_2$O), 66.94 (Fmoc CH$_2$O), 66.23 (β-CH), 56.62 (α-CH), 47.22 (Fmoc CHCH$_2$), 39.46 (γ-CH$_2$), 30.62 (ortho CCH$_3$), 28.06 (*t*-Bu CH$_3$), 14.26 (ortho CCH$_3$); MS (EI, 70 eV) m/z 525 (M$^+$, 1), 507 (M$^+$ − 18, 0.5), 481 (M$^+$ − 44, 8), 454 (M$^+$ − 73, 22), 381 (M$^+$ − 144, 30), 273 (M$^+$-252, 100); HRMS (EI, 70 eV) calcd for $C_{29}H_{35}O_8N$ 525.2362, found 525.2354 ± 0.0015 (M$^+$). Anal. Calcd for $C_{29}H_{35}O_8N$: C, 66.27; H, 6.73; N, 2.67. Found: C, 66.01; H, 6.73; N, 2.58.

Deprotection of 18: L-(2*R*,3*S*)-β-Hydroxy-Glu·NH$_4$, 19. Fmoc-β-hydroxy-Glu-OBO ester **18** (0.114 g, 0.218 mmol) was deprotected according to procedure B, with NH_4OH elution from the cation exchange column giving 0.036 g (91%) of the monoammonium salt, 92:8 threo:erythro, 99.0% ee. Recrystallization (H_2O/EtOH) gave 0.021 g (55%) of the monoammonium salt: mp 205–206 °C dec (lit.[30] mp 190 °C dec); enantiomeric purity 99.6% ee, diastereomeric purity 94:6 threo:erythro, as determined by HPLC; TLC (solvent C) R_f 0.16 (threo); ^{1}H NMR (D_2O, 250 MHz) δ 4.51–4.44 (m, 1H, β-C*H*), 3.89 (d, J = 3.4 Hz, 0.06H, erythro α-C*H*), 3.72 (d, J = 3.3 Hz, 0.94H, threo α-C*H*), 2.61 (dd, J = 15.4, 4.7 Hz, 1H, γ-C*H*H), 2.49 (dd, J = 15.2, 8.6 Hz, 1H, γ-CH*H*); ^{13}C NMR (D_2O, 62.9 MHz) δ 181.43, 175.52 (CO$_2$H), 70.11 (β-CH), 61.66 (α-CH), 44.69 (γ-CH$_2$). Anal. Calcd for $C_5H_9O_5N$·NH$_3$: C, 33.33; H, 6.73; N, 15.55. Found: C, 33.45; H, 6.57; N, 15.37.

Attempts to isolate the HCl salt (after purification on a cation exchange column, the sample was evaporated, dissolved in 2 N HCl, then evaporated, and recrystallized from EtOH/H_2O) gave lower yields (0.018 mg, 41%) but increased diastereoselectivity (>98:<2 threo:erythro, 99.0% ee): ^{1}H NMR (D_2O, 200 MHz) δ 4.60–4.51 (m, 1H, β-C*H*), 3.93 (d, J = 4.8 Hz, 1H, threo α-C*H*), 2.88 (dd, J = 16.4, 4.4 Hz, 1H, γ-C*H*H), 2.69 (dd, J = 16.4, 8.6 Hz, 1H, γ-CH*H*); ^{13}C NMR (D_2O, 50.3 MHz) δ 177.38, 174.26 (s, CO$_2$H), 68.91 (d, β-CH), 61.06 (d, α-CH), 41.79 (t, γ-CH$_2$).

Wittig Addition of Ph$_3$P=CHCO$_2$CH$_3$ to Fmoc-L-Ser(ald)-OBO ester, 4: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*)-1-amino-3-(methyloxycarbonyl)-(*E*)-2-propenyl]-4-methyl-2,6,7-trioxabicyclo-[2.2.2]octane, Fmoc-L-*trans*-β,γ-dehydro-Glu(OCH$_3$)-OBO Ester, 23. Crude Fmoc-L-Ser(ald)-OBO ester (**4**) (1.107 g, 2.64 mmol, assuming 100% yield in oxidation), Ph$_3$P=CHCO$_2$CH$_3$ (0.957 g, 2.90 mmol, 1.1 equiv), and dry CH_2Cl_2 (150 mL) were added to a flask, and the solution was stirred at room temperature for 2 h. The reaction was evaporated to dryness and the resulting solution was then purified by flash column chromatography (silica gel, 1:1 EtOAc:hexane), yielding 1.136 g of a white solid (94% for two steps, oxidation and Wittig reaction) with a >95:<5 *E*:*Z* ratio (as determined by NMR integration of alkene protons). It was possible to resolve the two isomers by chromatography, but the same *E*:*Z* ratio was seen in the crude product. Attempts at recrystallization were unsuccessful, with some isomerization occurring to give the α,β-unsaturated isomer. Enantiomeric purity was assessed by chiral shift ^{1}H NMR by the method of standard addition. Near base-line resolution of the ortho ester CH$_3$ peak was obtained using 0.29 equiv of Pr(hfc)$_3$ with a 20 mg/mL solution of alkene in benzene-d_6. Integration or peak height measurement (L-

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isomer at δ –0.76 ppm, D-isomer at δ –0.65 ppm, as determined with D,L standard) showed 99.2% ee (detection limit approximately 0.2% D). **23**: mp 140–141 °C; $[\alpha]^{25}_D$ –22.8° (*c* = 0.98, EtOAc); TLC (solvent A) R_f 0.51 (*E*), 0.39 (*Z*); IR (Nujol mull) 3322 (w), 1722 (s), 1706 (s), 1657 (w), 1533 (m), 1311 (m), 1283 (m), 1232 (m), 1051 (s), 1012 (m), 987 (m), 739 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.78–7.25 (m, 8H, Fmoc =C*H*), 7.02 (dd, *J* = 15.8, 4.9 Hz, 1H, CHC*H*=), 5.99 (dd, *J* = 15.8, 1.8 Hz, 1H, =C*H*CO_2), 5.17 (d, *J* = 9.3 Hz, 1H, N*H*), 4.59 (ddd, *J* = 9.2, 4.9, 1.4 Hz, 1H, α-C*H*), 4.42 (d, *J* = 7.7 Hz, 2H, Fmoc CH_2O), 4.24 (t, *J* = 7.0 Hz, 1H, Fmoc C*H*CH_2), 3.92 (s, 6H, 3 ortho CH_2O), 3.74 (s, 3H, CO_2CH_3), 0.81 (s, 3H, ortho CH_3); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 166.47 (CO_2CH_3), 155.94 (CONH), 144.00, 143.71 (Fmoc =C=), 143.19 (CHCH=), 141.18 (Fmoc=C=), 127.64, 127.00, 125.08 (Fmoc=CH), 122.35 (=CHCO$_2$), 119.92 (Fmoc=CH), 107.72 (ortho CO), 72.89 (ortho CH_2O), 67.00 (Fmoc CH_2O), 56.00 (α-CH), 51.55 (CO_2CH_3), 47.16 (Fmoc CHCH$_2$), 30.76 (ortho CCH_3), 14.22 (ortho CCH_3); MS (CI, CH_4) *m*/*z* 494 ($MC_2H_5^+$, 7), 480 (MCH_3^+, 8), 466 (MH^+, 100), 421 (MH^+ – 45, 13); HRMS (CI, CH_4) calcd for $C_{26}H_{28}O_7N$ 466.1866, found 466.1861 ± 0.0014 (MH^+). Anal. Calcd for $C_{26}H_{27}O_7N$: C, 67.09; H, 5.85; N, 3.00. Found: C, 67.37; H, 5.83; N, 2.99.

Wittig Addition of Ph_3P=CHCOCH$_3$ to Fmoc-L-Ser(ald)-OBO Ester, 4: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*)-1-amino-4-oxo-(*E*)-2-pentenyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-*trans*-γ-methylketone-vinyl-Gly-OBO Ester, 24. Crude Fmoc-L-Ser(ald)-OBO ester (**4**) (0.055 g, 0.12 mmol, assuming 100% yield in oxidation), Ph_3P=CHCOCH$_3$ (0.053 g, 0.17 mmol, 1.4 equiv), and dry CH_2Cl_2 (3 mL) were added to a flask, and the solution was stirred at room temperature for 25 h under N_2. The solution was evaporated to dryness and then purified by flash column chromatography (silica gel, 1:1 EtOAc:hexane), yielding 0.0494 g of a white solid (89% for two steps, oxidation and Wittig reaction) with a >95:<5 *E*:*Z* ratio (as determined by NMR integration of alkene protons). Attempts at recrystallization were unsuccessful, resulting in decomposition. Enantiomeric purity was assessed by chiral shift ^1H NMR using 0.22 equiv of Pr(hfc)$_3$ with a 20 mg/mL solution of alkene in benzene-d_6. The ortho ester CH_3 proton peak heights were measured (L-isomer at δ –0.47 ppm, D-isomer at δ –0.40 ppm, as determined with a D,L standard) and indicated 90% ee. **24**: mp 80–87 °C; $[\alpha]^{25}_D$ –25.6° (*c* = 1.04, EtOAc); TLC (solvent A) R_f 0.34 (*E*); IR (cast from CH_2Cl_2) 3334, (br w), 3056 (br w), 2946 (w), 2883 (m), 1719 (s), 1679 (m), 1636 (w), 1522 (m), 1246 (s), 1049 (s), 1018 (m), 999 (m), 761 (w), 741 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.79–7.28 (m, 8H, Fmoc=C*H*), 6.83 (dd, *J* = 16.1, 4.8 Hz, 1H, CHC*H*=), 6.21 (dd, *J* = 16.1, 1.6 Hz, 1H, =C*H*CO), 5.14 (d, *J* = 9.2 Hz, 1H, N*H*), 4.61 (ddd, *J* = 8.6, 4.1, 1.4 Hz, 1H, α-C*H*), 4.43 (d, *J* = 7.6 Hz, 2H, Fmoc CH_2O), 4.24 (t, *J* = 6.9 Hz, 1H, Fmoc C*H*CH_2), 3.94 (s, 6H, 3 ortho CH_2O), 2.28 (s, 3H, COCH_3), 0.83 (s, 3H, ortho CH_3); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 198.02 ($COCH_3$), 155.98 (CONH), 144.00, 143.74 (Fmoc =C=), 141.62 (CHCH=), 141.30 (Fmoc =C=), 131.35 (=CHCO), 127.68, 127.03, 125.05, 119.96 (Fmoc=CH), 107.79 (ortho CO), 72.93 (ortho CH_2O), 67.06 (Fmoc CH_2O), 56.10 (α-CH), 47.29 (Fmoc CHCH$_2$), 30.80 (ortho CCH_3), 27.59 ($COCH_3$), 14.24 (ortho CCH_3); MS (CI, CH_4) *m*/*z* 450 (MH^+, 100), 405 (MH^+ – 45, 15); HRMS (CI, CH_4) calcd for $C_{26}H_{28}O_6N$ 450.1916, found 450.1931 ± 0.0012 (MH^+). Anal. Calcd for $C_{26}H_{27}O_6N$: C, 69.47; H, 6.05; N, 3.12. Found: C, 69.42; H, 6.07; N, 3.01.

Wittig Addition of Na(CF$_3$CH$_2$O)$_2$P(O)=CHCO$_2$CH$_3$ to Fmoc-L-Ser(ald)-OBO Ester, 4: 1-[*N*-(9-Fluorenylmethyloxycarbonyl)-(1*S*)-1-amino-3-(methyloxycarbonyl)-(*Z*)-2-propenyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane, Fmoc-L-cis-β,γ-dehydro-Glu(OCH$_3$)-OBO Ester, 25. Crude Fmoc-L-Ser(ald)-OBO ester (**4**) (0.367 g, 0.850 mmol, assuming 100% yield in oxidation) was dissolved in 10 mL of dry THF under N_2 and cooled to –78 °C. NaH (0.050 g, 2.1 mmol, 2.5 equiv) was suspended in 150 mL of dry THF under N_2 and cooled to 0 °C. $(CF_3CH_2O)_2P(O)CH_2CO_2CH_3$ (Aldrich, 467 μL, 2.21 mmol, 2.6 equiv) was added dropwise over 5 min, and the solution was cooled to –78 °C. The aldehyde solution was transferred by cannula and rinsed with 5 mL of THF. After 35 min, the solution was poured into 200 mL of 5% NH_4Cl and 200 mL of CH_2Cl_2 was added. The organic layer was removed, washed with 5% NH_4Cl (1 x 200 mL) and saturated NaCl (1 x 200 mL), dried ($MgSO_4$), and evaporated to dryness. The crude product was purified by flash column chromatography (silica gel, 1:1 EtOAc:hexane) to give 0.248 g (63%) of the *Z* isomer as a white foam solid. The *E* isomer was also isolated (0.027 g, 7%), giving a yield of 70% and a *Z*:*E* selectivity of 90:10 for the reaction. Some unreacted aldehyde (0.053 g, 15%) was also recovered. Chiral shift ^1H NMR analysis (0.24 equiv of Pr(hfc)$_3$ with a 20 mg/mL solution of alkene in benzene-d_6) showed an ee of >93% (ortho ester CH_3 peak height measurement: L-isomer at δ –0.17 ppm, D-isomer at δ –0.27 ppm). **25**: mp 60–66 °C; $[\alpha]^{25}_D$ –34.5° (*c* = 0.97, EtOAc); TLC (solvent A) R_f 0.39 (*Z*), 0.51 (*E*); IR (Nujol mull) 3355 (br w), 1723 (br s), 1660 (w), 1514 (m), 1319 (m), 1198 (s), 1049 (s), 1016 (m), 761 (w), 741 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.76–7.25 (m, 8H, Fmoc=C*H*), 6.05 (dd, *J* = 11.4, 9.2 Hz, 1H, CHC*H*=), 5.94 (d, *J* = 11.5 Hz, 1H, =C*H*CO_2), 5.74 (br t, *J* = 8.4 Hz, 1H, α-C*H*), 5.29 (br d, *J* = 7.5 Hz, 1H, N*H*), 4.36 (d, *J* = 7.5 Hz, 2H, Fmoc CH_2O), 4.24 (t, *J* = 7.1 Hz, 1H, Fmoc C*H*CH_2), 3.93 (s, 6H, 3 ortho CH_2O), 3.74 (br s, 3H, CO_2CH_3), 0.80 (s, 3H, ortho CH_3); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 165.80 (CO_2CH_3), 155.55 (CONH), 144.11, (Fmoc =C=), 143.99, 141.22 (CHCH= and Fmoc =C=), 127.53, 126.95, 125.18 (Fmoc=CH), 122.51 (=CHCO$_2$), 119.82 (Fmoc=CH), 108.00 (ortho CO), 72.91 (ortho CH_2-O), 67.41 (br, Fmoc CH_2O), 51.94 (α-CH or CO_2CH_3), 51.41 (α-CH or CO_2CH_3), 47.16 (Fmoc CHCH$_2$), 30.72 (ortho CCH_3), 14.24 (ortho CCH_3); MS (EI, 70 eV) *m*/*z* 465 (M^+, 45), 421 (M^+ – 44, 24), 385 (M^+ – 80, 70), 270 (M^+ – 195, 100), 243 (M^+ – 222, 85), 238 (M^+ – 227, 92); HRMS (EI, 70 eV) calcd for $C_{26}H_{27}O_7N$ 465.1787, found 465.1792 ± 0.0014 (M^+). Anal. Calcd for $C_{26}H_{27}O_7N$: C, 67.09; H, 5.85; N, 3.01. Found: C, 67.20; H, 6.03; N, 2.84.

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Supplementary Material Available: A listing of tables of positional and thermal parameters of the X-ray crystallographic determination of Fmoc-Thr(ket)-OBO ester (**8**) (8 pages); a table of observed and calculated structure factors (10 pages). Ordering information is given on any current masthead page.