Practical Synthesis of BILA 2157 BS, a Potent and Orally Active Renin Inhibitor: Use of an Enzyme-Catalyzed Hydrolysis for the Preparation of Homochiral Succinic Acid Derivatives

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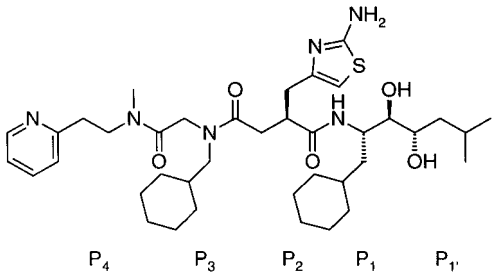
We have developed a highly convergent and stereoselective synthesis of BILA 2157 BS, a potent and orally active renin inhibitor. The synthesis proceeds in 15 distinct chemical steps (with several integrated, multistep operations) from aminodiol **4**. The key step in the synthesis involves the use of an enantiospecific, enzyme-catalyzed hydrolysis of a substituted succinate diester to provide a homochiral succinic acid derivative in 98% enantiomeric excess (≥2.5 kg scale). Recycling of the unwanted enantiomer is accomplished through base-catalyzed racemization, leading to an efficient deracemization of the starting racemic diester. The entire sequence proceeds without chromatographic purifications and delivers the product with >97% homogeneity. In addition, compared to the previously reported syntheses of BILA 2157 BS, this approach avoids the use of expensive chiral auxiliaries and cryogenics and, thus, should be amenable to the preparation of large quantities of this peptidomimetic inhibitor.

Introduction

The renin−angiotensin system (RAS) plays a central role in the regulation of blood pressure and, over the years, has been fruitful ground for the search for new therapies for the treatment of hypertension and congestive heart failure.[1,2] Following the advent and initial success of angiotensin converting enzyme (ACE) inhibitors, the occurrence of side effects associated with their use[3] has stimulated further research to identify other sites for therapeutic intervention along the RAS.[4] Incentive for the development of novel therapies based on inhibition of the aspartyl protease renin,[5] the first implicated enzyme along this pathway, is derived from the fact that renin, unlike ACE, acts on a single known substrate (angiotensinogen). Interference with this event is thus less likely to result in unwanted side effects.

Using the sequence of angiotensinogen as a starting point for rational design, replacement of the cleavable Leu-Val amide bond of this substrate by nonhydrolyzable isosteres quickly led to the discovery of potent and specific inhibitors of renin.[1,2] However, despite extensive efforts directed at the search for small, orally active inhibitors of the enzyme, lead compounds have generally remained large (MW > 600), peptidomimetic-like molecules, lacking adequate physicochemical properties for oral absorption.



BILA 2157 BS (**1**)

Enzyme	IC_{50} (nM)
renin (pH 6.0)	1.4
renin (pH 7.4)	2.5
cathepsin D	540
pepsin	11,000
gastricsin	23% inhibition at 1000

Figure 1.

Our own efforts in this field culminated in the discovery of BILA 2157 BS (**1**), a potent and specific inhibitor of human renin (Figure 1).[6] Despite a high molecular weight (MW 727) and significant amide character, BILA 2157 BS exhibited an encouraging pharmacological profile. Oral bioavailability of **1** in cynomolgus monkey was 40%, and the compound elicited a statistically significant lowering of blood pressure in sodium-depleted animals at a dose of 3 mg/kg.[6] These encouraging results led to

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(1) Greenlee, W. J. *Med. Res. Rev.* **1990**, *10*, 173.

(2) Rosenberg, S. H. Renin Inhibitors. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier: New York, 1995; Vol. 32, p 32.

(3) Side effects encountered with the use of ACE inhibitors include cough (6−14% of patients), skin rash, and angioneurotic oedema.[1,2]

(4) Most notable are nonpeptide AII receptor antagonists such as losartan; see, for example: (a) Buchholz, R. A.; Lefker, B. A.; Ravi Kiron, M. A. *Ann. Rep. Med. Chem.* **1993**, *28*, 69. (b) Greenlee, W. J.; Siegl, P. K. S. *Ann. Rep. Med. Chem.* **1992**, *27*, 59.

(5) Boger, J. *Ann. Rep. Med. Chem.* **1985**, *20*, 257.

(6) Simoneau, B.; Lavallée, P.; Anderson, P. C.; Bailey, M.; Bantle, G.; Berthiaume, S.; Chabot, C.; Fazal, G.; Halmos, T.; Ogilvie, W.; Poupart, M.-A.; Thavonekham, B.; Xin, Z.; Thibeault, D.; Bolger, G.; Panzenbeck, M.; Winquist, R.; Jung, G. L. *Bioorg. Med. Chem.* **1999**, *7*, 489.

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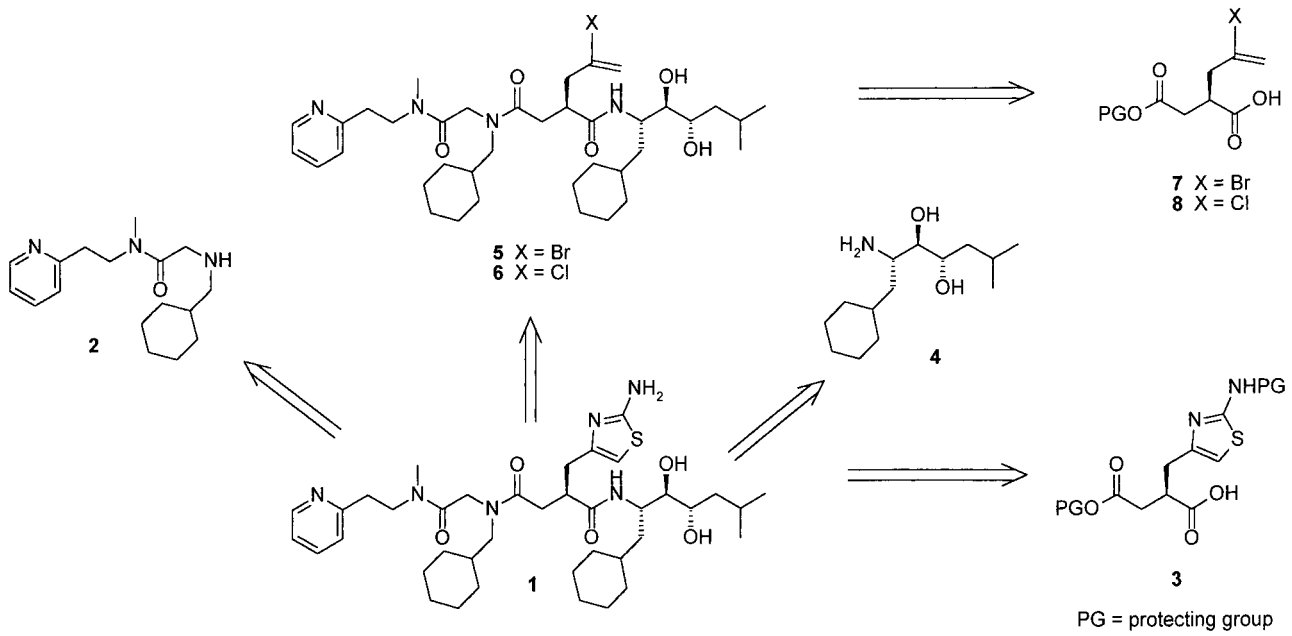
Scheme 1



the selection of BILA 2157 BS as a candidate for further preclinical evaluation. For these studies, large quantities of the inhibitor became necessary, requiring the development of a practical synthesis. In this account, we describe a stereoselective and convergent synthesis of **1**, which makes use of an environmentally sound and economical enzymatic hydrolysis for the preparation of a key homochiral succinic derivative.

Results and Discussion

The large-scale synthesis of peptidomimetic inhibitors, in general, represents a major synthetic challenge to the organic chemist.[7] In the case of renin inhibitors, the high cost usually associated with synthetic complexity must be minimized to allow possible competition with ACE inhibitors (now entering the generic market) and the more recently introduced nonpeptide angiotensin II antagonists.[4] Furthermore, due to the chronic nature and widespread occurrence of high blood pressure related conditions, lifetime treatment costs must be maintained at acceptable levels. These factors are important constraints that must be taken into consideration in the planning of a synthetic strategy.

BILA 2157 BS has four asymmetric centers, three of which are part of its dihydroxyethylene transition-state mimic ($P_1-P_{1'}$).[8] The left-hand side (P_4-P_3) is a structurally simple, achiral tertiary amide derivative. The remaining chiral center is contained within the central succinyl core of the molecule and carries a basic aminothiazolyl P_2 side chain. This heterocycle plays a critical role in the pharmacokinetic profile of **1**.[6]

First-Generation Synthesis. The "medicinal chemistry" route to BILA 2157 BS, used during SAR studies leading to its discovery, is based on a convergent assembly depicted in Scheme 1. Logical disconnections are provided by the amide linkages on both sides of the succinyl core, leading to three key intermediates **2**−**4**.[6] This scheme was subsequently modified to use vinyl bromide **7** (the vinyl bromide being a precursor to the aminothiazolyl heterocycle),[9] instead of **3**, to avoid partial epimerization of the P_2 position previously encountered during coupling with the aminodiol **4**.[10] Contamination of the final product, resulting from epimers at P_2, was found to be exceedingly difficult to eliminate in the final stages of the synthesis. The preparation of **1** thus proceeded via crystalline bromide **5**, followed by construction of the heterocycle onto the complete inhibitor backbone.[10]

Because of the amorphous nature of BILA 2157 BS,[11] and despite numerous attempts to prepare crystalline addition salts, final purification of **1** was accomplished by flash chromatography.

Second-Generation Synthesis. Following our first scale-up campaign of BILA 2157 BS,[10] we envisaged requirements for multikilogram quantities of the inhibitor, in support of full toxicology studies. With this goal in mind, the original route to **1** suffered from several limitations that severely restricted its application to large-scale preparations. Most problematic were the syntheses of succinyl fragment **7** and aminodiol **4**. The former required the use of an expensive chiral auxiliary, three cryogenic steps, and the use of pyrophoric and costly alkyllithium reagents. The reported cyanohydrin route to aminodiol **4**[12] was found to be capricious and subject to low overall yields, in addition to requiring the use of large amounts of expensive and highly toxic

(7) Beaulieu, P. L.; Lavallée, P.; Abraham, A.; Anderson, P. C.; Boucher, C.; Bousquet, Y.; Duceppe, J.-S.; Gillard, J.; Gorys, V.; Grand-Maître, C.; Grenier, L.; Guindon, Y.; Guse, I.; Plamondon, L.; Soucy, F.; Valois, S.; Wernic, D.; Yoakim, C. *J. Org. Chem.* **1997**, *62*, 3440.

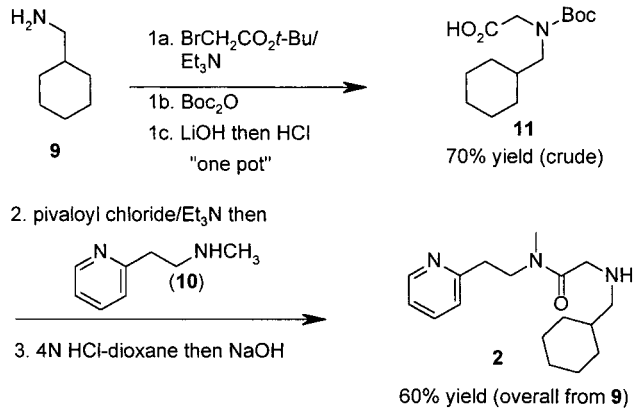
(8) Luly, J. R.; BaMaung, N.; Soderquist, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B.; Merits, I.; Bolis, G.; Greer, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1988**, *31*, 2264.

(9) (a) Morton, H. E.; Leanna, M. R. *Tetrahedron Lett.* **1993**, *34*, 4481. (b) Leanna, M. R.; Morton, H. E. *Tetrahedron Lett.* **1993**, *34*, 4485.

(10) Simoneau, B.; Lavallée, P.; Bailey, M.; Duceppe, J.-S.; Grand-Maître, C.; Grenier, L.; Ogilvie, W. W.; Poupart, M.-A.; Thavonekham, B. *Can. J. Chem.*, in press.

(11) BILA 2157 BS exists in solution as a mixture of several rotamers, due to the presence of two tertiary amide functionalities. Coupled to a high peptidic content, this phenomena confers amorphous physical properties to the molecules which have not allowed purification of the inhibitor by crystallization.

Scheme 2



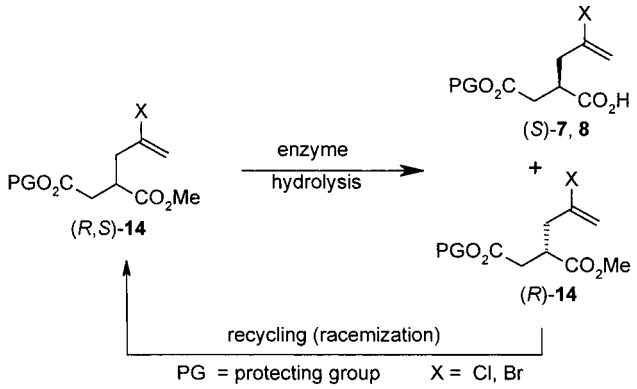
trimethylsilylcyanide. The dihydroxylation route to **4**[13] appeared more practical on a large scale, but suffered from modest control of diastereoselectivity during introduction of the hydroxyl groups. Finally, an alternative to chromatographic purification of the final product was required for large-scale synthetic purposes.

The synthesis of the left-hand side (**2**, Scheme 2) of BILA 2157 BS, used in our initial scale-up, was judged suitable for large-scale preparations. No attempts were made to further improve this procedure, which delivers this fragment in 60% overall yield (kg scale) from commercially available cyclohexylmethylamine **9** and 2-(2-methylaminoethyl)pyridine **10**, without need for purifications. The synthesis of **2** has been described elsewhere.[10]

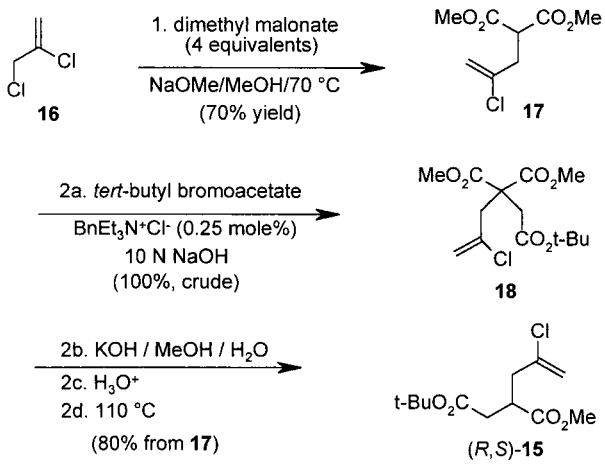
Preparation of Racemic Succinyl Fragments. After consideration of several possibilities, we settled on preparing the succinyl core (**14**) in racemic form, followed by separation of the two enantiomers by resolution. This strategy avoids the use of an expensive chiral auxiliary and seemed a particularly attractive methodology since it had the obvious possibility to be coupled with recycling of the unwanted antipode through racemization (a process often referred to as deracemization).[14]

While classical resolution processes employing diastereomeric salt formation are widely used on a large scale, and often provide the most economical way of preparing enantiomerically pure compounds,[7,14] we opted to investigate the use of enzymes for the stereospecific hydrolysis protocol illustrated in Scheme 3. Factors influencing our decision included the following: lower cost associated with such a catalytic process, the use of water as solvent, minimal waste management, operational simplicity, and availability/low cost of reagents. The use of an esterase enzyme to carry out the stereospecific hydrolysis of

Scheme 3



Scheme 4



racemate (*R*,*S*)-**14**, coupled to recycling of (*R*)-**14** through racemization, seemed most efficient and suited to our application.[15] The racemic diester (*R*,*S*)-**15** (Scheme 4) was selected as the substrate for this enzymatic resolution protocol. The *β*-*tert*-butyl ester functionality, we believed, would remain unaffected throughout the process and ensure a good control of chemoselectivity between the two carboxylic acid functions during the hydrolysis step. We also chose the more readily available and less costly vinylic chloride derivative (X = Cl in Scheme 3), rather then the previously used bromide, as precursor to the aminothiazolyl heterocycle.[10] The preparation of racemic (*R*,*S*)-**15** shown in Scheme 4 is based on classical malonate alkylation chemistry. Dimethylmalonate was first alkylated with 2,3-dichloropropene **16** using sodium methoxide as a base. A large excess (4 equiv) of the malonate was used to minimize formation of dialkylated products, but unreacted material was recovered by simple distillation and continuously recycled (small amounts of the desired monoalkylated product **17** co-distill with dimethylmalonate and recycling of the distillate allows partial recovery and an increase in yield of the product). In this way, alkylated malonate **17** was isolated in 70% yield after purification by vacuum distillation (three alkylation cycles) and was contaminated with <3% of dialkylated material. A second alkylation was then

(12) (a) Luly, J. R.; Hsiao, C.-N.; BaMaung, N.; Plattner, J. J. *J. Org. Chem.* **1988**, *53*, 6109. (b) Rosenberg, S. H.; Spina, K. P.; Woods, K. W.; Polakowski, J.; Martin, D. L.; Yao, Z.; Stein, H. H.; Cohen, J.; Barlow, J. L.; Egan, D. A.; Tricarico, A.; Baker, W. R.; Kleinert, H. D. *J. Med. Chem.* **1993**, *36*, 449. For other approaches to the synthesis of **4**, including scale-up of a modified cyanohydrin route, see: (c) Schwindt, M. A.; Belmont, D. T.; Carlson, M.; Franklin, L. C.; Hendrickson, V. S.; Karrick, G. L.; Poe, R. W.; Sobieray, D. M.; Van De Vusse, J. *J. Org. Chem.* **1996**, *61*, 9564 and references therein.

(13) Krysan, D. J.; Rockway, T. W.; Haight, A. R. *Tetrahedron: Asymmetry* **1994**, *5*, 625.

(14) (a) Collins, A. N.; Sheldrake, G. N.; Crosby, J. *Chirality in industry. The commercial manufacture and applications of optically active compounds*; John Wiley: New York, 1992. (b) Collins, A. N.; Sheldrake, G. N.; Crosby, J. *Chirality in industry II. Developments in the manufacture and applications of optically active compounds*; John Wiley: New York, 1997.

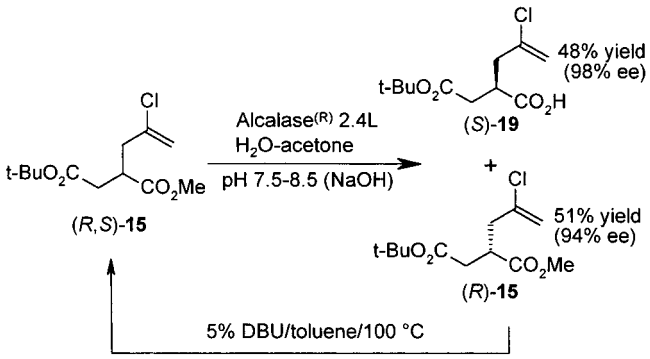
(15) (a) Bailey, M. D. U.S. Patent 5 808 085, 1998. (b) Bailey, M.; Adamson, D.; Halmos, T.; Grand-Maître, C. *Tetrahedron: Asymmetry* **1999**, in press. Similar enzymatic hydrolysis protocols for succinate derivatives have been reported by other groups: (c) Wirz, B.; Soukup, M. *Tetrahedron: Asymmetry* **1997**, *8*, 187. (d) Ozaki, E.; Sakashita, K. *Chem. Lett.* **1997**, 741. (e) Guibé-Jampel, E.; Rousseau, G.; Salaün, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1080.

performed on **17** using *tert*-butyl bromoacetate to provide crude triester **18** in quantitative yield. This alkylation, originally carried out using strong base (KO-*t*-Bu or LiHMDS) and anhydrous conditions, was modified and ultimately performed using solvent-free phase-transfer conditions.[16] The reaction took place with 10 N NaOH in the presence of a catalytic amount of benzyltriethylammonium chloride, both starting materials, and the generated product serving as the organic medium. It is noteworthy that, despite the use of 10 N NaOH, no hydrolysis of the methyl esters occurred, and crude **18** was obtained in quantitative yield by simple phase separation. The resistance of either **17** or **18** to saponification under the strongly basic reaction conditions is rationalized in terms of the high rate of alkylation of the anion derived from **17** and the negligible solubility of **18** in the aqueous phase. In an attempt to use less expensive *tert*-butyl chloroacetate as the electrophile, the rate of alkylation was reduced sufficiently for hydrolysis of the methyl esters to take place, and none of **18** was isolated. The absence of solvents in this reaction minimizes the generation of wastes and impact on the environment. Finally, decarboxylation of **18** to produce racemic (*R*,*S*)-**15** was accomplished by initial monosaponification of one methyl ester using KOH in a homogeneous methanolic solution, followed by acidification, extraction of the monoacid into toluene, and refluxing of the extract to induce decarboxylation. (*R*,*S*)-**15** was isolated by vacuum distillation in 80% yield overall from **17** (≥2.5 kg scale).

Enantiospecific, Enzymatic Hydrolysis of Racemic Succinates. Having secured access to large quantities of racemic (*R*,*S*)-**15**, we then addressed the key enzyme-catalyzed hydrolysis step. The use of enzymes for the stereospecific hydrolysis of esters is a well-established, highly practical process for the large-scale synthesis of optically active substances.[15,17]

After several esterase enzymes were screened for their effectiveness in the enantiospecific hydrolysis described in Scheme 3, a crude, readily available, and inexpensive serine protease (Subtilisin Carlsberg) preparation, Alcalase,[18a] was identified as the most promising candidate for our purpose. Following optimization of several reaction parameters (solvents, temperature, time, pH),[15a,b] it was found that hydrolysis of the (*S*)-enantiomer of (*R*,*S*)-**15** using a catalytic amount of a crude (food grade) Alcalase 2.4L preparation[18a] proceeded smoothly at pH 7.5−8.5 to give (*S*)-**19** in 48% yield and 98% ee as determined by HPLC analysis on a chiral support (see figure in the Supporting Information and Experimental Section for details). The use of purified Subtilisin Carlsberg did not offer any advantages over the crude preparation. Unreacted ester (*R*)-**15** (51% yield, 94% ee) and acid (*S*)-**19** were conveniently separated using a simple acid−base extraction protocol. The hydrolysis was carried out on ≥2.5 kg of racemate, and the crude products could be used without need of purification. The absolute stereochemistry of isolated acid (*S*)-**19** was unambiguously determined with the help of an X-ray crystal structure of a more advanced intermediate **31** (vide infra). Recycling of the unwanted (*R*)-**15** ester through racemization was accomplished by heating neat (80 °C) with a catalytic amount of DBU (previously carried out using stoichiometric amounts of DBU in refluxing toluene).[15b] Following three resolution/racemization cycles, the overall yield of (*S*)-**19** (98% ee) was increased to 82%, resulting in efficient deracemization of (*R*,*S*)-**15**.

Scheme 5



We wondered if the enzymatic protocol described above could be carried out on a more advanced intermediate encompassing the P_4-P_2 portion of the inhibitor. This strategy appears less efficient at first glance: large-scale chemistry dictates that the most economical route is usually one that carries the least amount of material through the sequence; therefore, resolution processes should be implemented as early as possible in a synthetic scheme, unless the unwanted isomer is recyclable. This approach however, would advantageously delay introduction of aminodiol **4** to a latter stage of the synthesis, thus minimizing requirements of this more valuable fragment. We therefore examined the enzymatic hydrolysis of succinate derivative (*R*,*S*)-**21** as illustrated in Scheme 6.

Cleavage of the *tert*-butyl ester of (*R*,*S*)-**15** with 4 N HCl in dioxane gave crystalline acid (*R*,*S*)-**20** (85% yield), which was coupled to P_4-P_3 (**2**) using a pivaloyl mixed anhydride. Crude racemate (*R*,*S*)-**21** was obtained as an oil in quantitative yield. Without purification, this crude ester was submitted to the enzymatic hydrolysis protocol under conditions identical to those used for (*R*,*S*)-**15**. Crude ester (*R*)-**21** was isolated in 52% yield and 97.6% ee as shown by HPLC analysis on a chiral support (see the Experimental Section for details). Acid (*S*)-**22** (94% ee), on the other hand, was isolated in lower yield (37−38%), presumably a consequence of its increased water solubility. Both compounds were obtained as oils but were of sufficient purity to be used without purification. Ester (*R*)-**21** was easily recycled by refluxing with catalytic NaOMe in methanol, again demonstrating potential for deracemization (Scheme 6). The absolute stereochemical assignment for (*S*)-**22** is based on its convergence to a more advanced intermediate for which stereocenters had been assigned with the help of a crystal structure (vide infra).

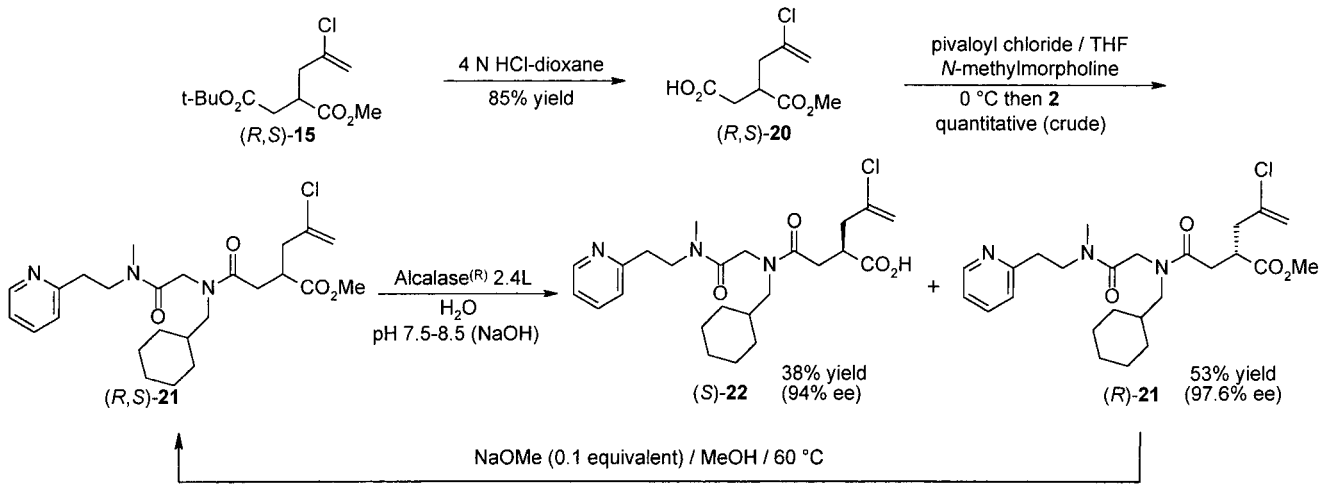
Both resolution schemes (Schemes 5 and 6) attest to the versatility and scope of the Alcalase-mediated hydrolysis. The enzyme indiscriminately carries out an almost stereospecific hydrolysis of a simple, lipophilic

(16) Singh, R. K. *Synthesis* **1985**, 54.

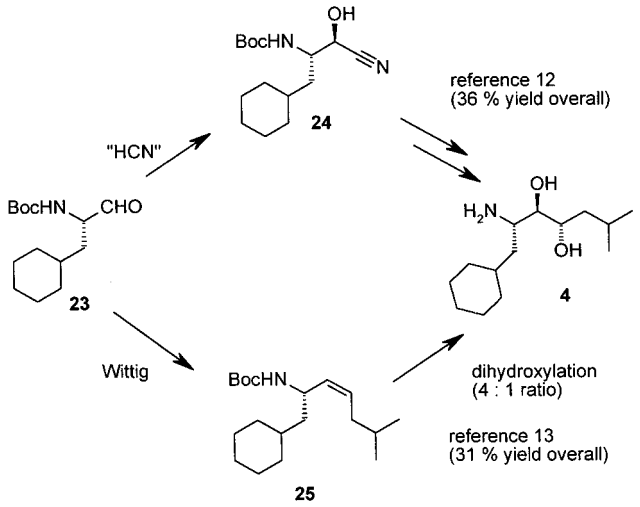
(17) (a) *Enzymes in synthetic organic chemistry*; Wong, C. H., Whitesides, G. M., Eds.; Elsevier Science, Inc.: New York, 1994. (b) *Enzyme catalysis in organic synthesis. A comprehensive handbook*; Drauz, K., Waldmann, H., Eds.; VCH Publishers: New York, 1995; Vol. 1.

(18) (a) Alcalase is a crude enzyme preparation whose main component (Subtilisin A, Subtilisin Carlsberg) is a serine endopeptidase. The crude Alcalase 2.4 L enzyme preparation is available in bulk from Novo Nordisk Biochem North America, Inc., Franklinton, NC 27525. (b) Since the extent of conversion for these reactions was not accurately measured, only approximate *E* values can be determined for these two substrates; see: Chen, C.-H.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

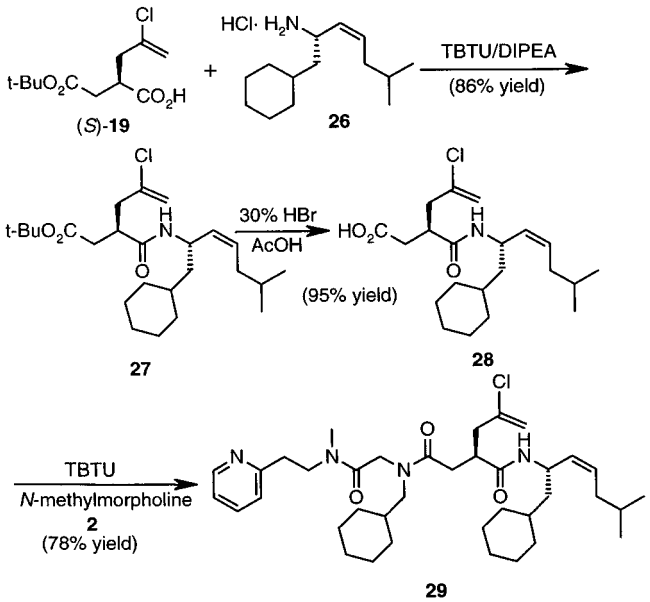
Scheme 6



Scheme 7



Scheme 8



diester **15** ($E > 200$)[18b] or a more complex amide derivative **21** ($E > 100$).[18b] This observation is consistent with reports of high levels of tolerance at the P_2 and P_3 positions (and beyond) of substrates in protease-catalyzed reactions (including subtilisin).[17a] This procedure for the preparation of homochiral succinic acid derivatives should find wide application due to its simplicity, versatility, efficiency, availability of the enzyme, and practicality for large scale production.

Elaboration of the Aminodiol Functionality. Several reports have appeared in the literature that describe syntheses of transition-state isostere **4**.[8,12,13] Two routes are particularly noteworthy as they have been shown to be amenable to scale-up. Both make use of protected (*S*)-cyclohexylalaninal **23** as chiral starting material (Scheme 7). The first route[12] proceeds via cyanohydrin **24**, which is then elaborated in a series of steps into aminodiol **4**. The second strategy converts aldehyde **23** into alkene **25** via a Wittig condensation.[13] Dihydroxylation of the alkene then gives a 4:1 mixture of diols in favor of the desired relative stereochemistry present in **4**. While both routes proceed with comparable efficiencies (31−36% yield overall), the dihydroxylation approach requires less chemical operations and thus seemed more attractive to us.

Hoping to improve on the overall yield of the latter process, we wondered if replacement of the *tert*-butyl carbamate protecting group of **25** by a more complex and chiral entity, such as the P_2 portion of our inhibitor, would favorably influence facial diastereoselectivity in the dihydroxylation of the double bond. To this end, three olefinic substrates were prepared as depicted in Scheme 8. Starting with resolved acid (*S*)-**19**, coupling with readily available allylic amine hydrochloride **26**[13] gave ester **27**. Deprotection of the carboxyl group under strong acid conditions gave **28**, which was coupled to P_4-P_3 (**2**) to give **29**, incorporating the complete backbone of BILA 2157 BS.

Dihydroxylation of compounds **27**−**29**, unlike carbamate **25**, is potentially complicated by the presence of the vinylic chloride functionality at P_2, which in principle is also susceptible to electrophilic attack by oxidizing agents. Using the oxidation conditions previously reported in the literature for alkene **25** (2.5 mol % OsO_4/2.5 equiv of *N*-methylmorpholine *N*-oxide/2-propanol)[13] we found that substantial oxidation of the vinylic chloride was taking place, leading to complex mixtures of products. Careful study of dihydroxylation parameters (solvent, water content, co-oxidant, temperature, additives) led to the optimized conditions shown in Table 1, which were then applied to the three substrates (**27**−**29**),

Scheme 9

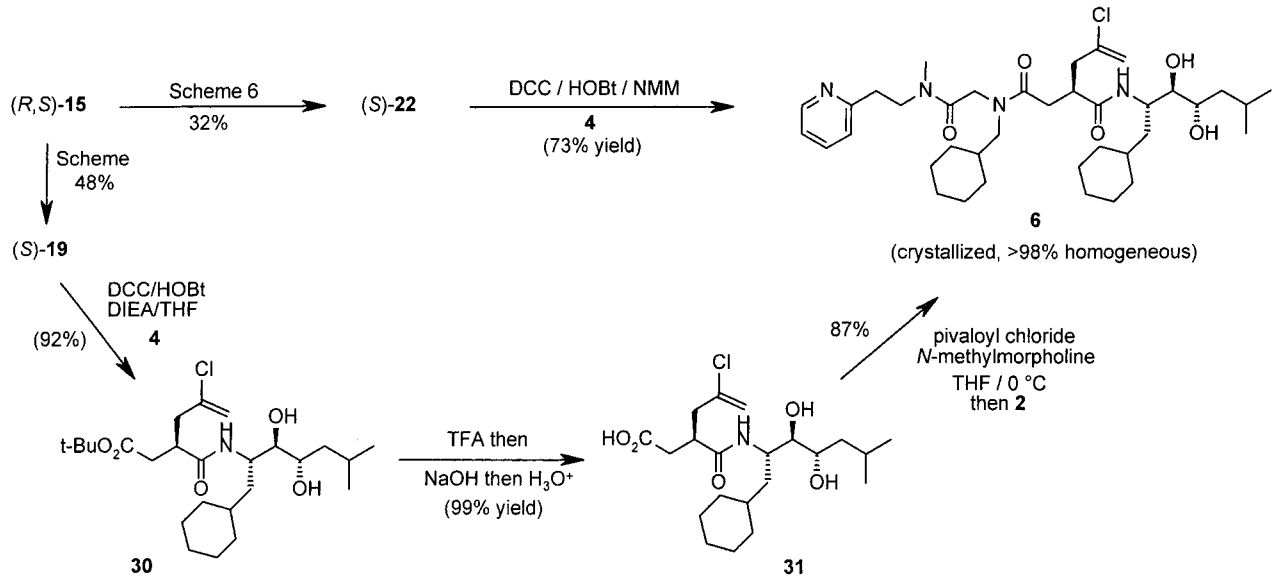
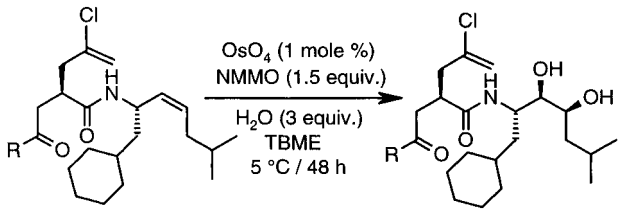


Table 1



substrate	R	ratio[a]	product	yield[b] (%)
27	O-*t*-Bu	6.5:1	30	60
28	OH	5.4:1	31	56
29	P_4-P_3	6.1:1	6	56

[a] Ratios determined by HPLC analysis of crude reaction mixture. [b] Isolated yield of isomerically homogeneous material.

resulting in modest yields of diols (55−60%) and acceptable diastereoselectivities (5.5−6.5:1 vs 4:1 for **25**). The use of additives (methylsulfonamide, quinoline, pyridine)[19,20] or performing the oxidation under asymmetric conditions (AD-mix-α or -*β*)[13,20] had no beneficial effect on the outcome of the reaction. In all cases, the desired products (**6**, **30**, **31**) precipitated out of the reaction mixture and were conveniently isolated in isomerically pure form after a simple filtration followed by recrystallization (see the Supporting Information for details on the preparation of these analytical standards). The structural identity and integrity of the diols was confirmed by comparison (NMR, HPLC, mp, rotation) to material prepared by coupling of the appropriate (*S*)-acids (**19** or **22**) with authentic aminodiol **4**[12,13] as shown in Scheme 9 (see below).

Since the slight improvements in diastereoselection observed in the dihydroxylation of substrates **29**−**31** were not sufficient to overcome problems of chemoselectivity between double bonds, this approach was abandoned in favor of our initial and more convergent assembly protocols, using aminodiol **4** as a presynthesized fragment.[21]

Inhibitor Backbone Assembly. Two alternative routes were evaluated for assembly of the inhibitor backbone and are depicted in Scheme 9. The first one uses resolved acid (*S*)-**19**, which was coupled to aminodiol **4**[12,13] using DCC-HOBt.[22] The resulting diol **30** was obtained in 92% yield as a pure (>99% homogeneous) solid. Subsequent cleavage of the *tert*-butyl ester with TFA was accompanied by the formation of small amounts of trifluoroacetylated products. Consequently, the crude acid was submitted to alkaline treatment prior to isolation, and acid **31** was then isolated in near-quantitative yield as a white crystalline solid. Completion of the backbone assembly using this route involved coupling with P_4-P_3 (**2**), conveniently carried out using pivaloyl chloride for activation of the carboxyl group of **31**. No side reactions implicating the free hydroxyl groups were noticed, and compound **6** was obtained in 87% yield after crystallization (>98.5% homogeneity by HPLC). The overall yield of **6** starting from (*R*,*S*)-**15** was 38%.

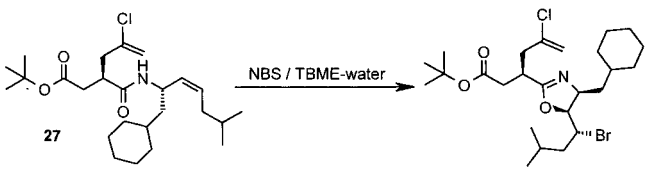
The second assembly route shown in Scheme 9 uses resolved acid (*S*)-**22** obtained as shown in Scheme 6. Thus, coupling of the crude acid to aminodiol **4**,[12,13] using again DCC-HOBt,[22] gave **6** in 73% yield, identical in all respects to material produced by the first route. The overall yield of **6** (>98.5% homogeneous) starting from (*R*,*S*)-**15** was 23%.

While the second route saves introduction of the most valuable fragment (**4**) to latter stages in the assembly

(19) (a) Schröder, M. *Chem. Rev.* **1980**, *80*, 187.

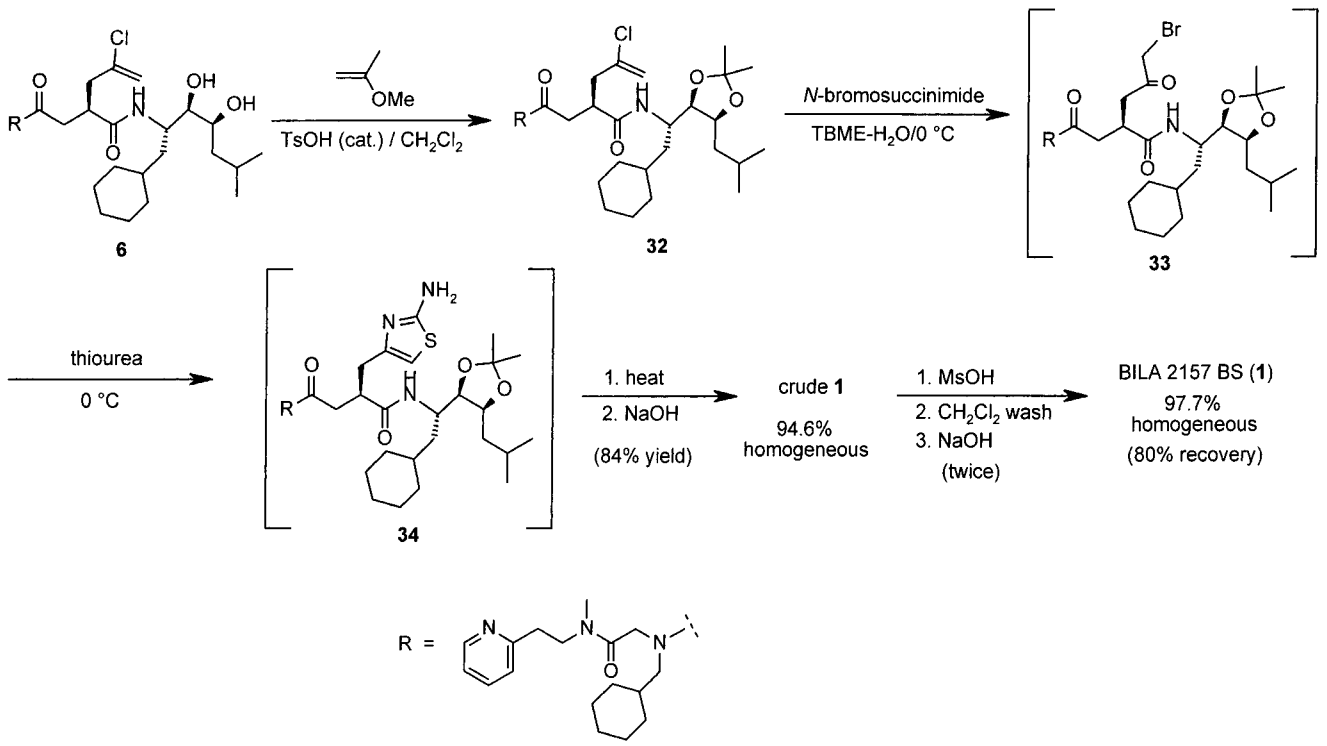
(20) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768.

(21) To circumvent side product formation associated with oxidation of the vinylic chloride functionality of compound **27**, we attempted its conversion into the aminothiazolyl heterocycle prior to double bond dihydroxylation. However, under previously used conditions,[10] upon treatment of **27** with 1 equiv of NBS, the more nucleophilic P_1 double bond underwent attack (presumably from the least hindered side) and a single isomer of the bromo derivative, with the relative stereochemistries shown below, was isolated (tentative structure based on MS and NMR).



(22) Bodansky, M. In *Principles of Peptide Synthesis*; Springler-Verlag: New York, 1984.

Scheme 10



sequence, the first process delivers **6** with greater overall efficiency. In addition, many intermediates along that sequence exhibited favorable physical properties, which allowed purification by crystallization at several steps along the sequence. In contrast, the use of the second route required the processing of crude oily materials from (*R*,*S*)-**20** until compound **6**, which could then be purified by crystallization. Nevertheless, material obtained from either routes was shown by NMR and HPLC to be free from contamination by P_2 epimers (see the Supporting Information for details on the preparation of analytical standards).

Determination of the Absolute Configuration of Stereogenic Centers. We were successful in obtaining crystals of **31** suitable for X-ray structure determination (Figure 2).[23] This allowed unambiguous assignment of the absolute configurations of all stereogenic centers in the molecule (and therefore BILA 2157 BS), including the enzymatically generated P_2 center. By convergence of several of the described routes to compound **6**, it also confirmed the relative stereochemistry of the two carbinol centers that are introduced through the dihydroxylation protocol (Table 1), as well as establishing the (*S*)-configuration of the asymmetric carbon atom of **22**, derived from enzymatic hydrolysis of (*R*,*S*)-**21**.

Construction of the Aminothiazole Heterocycle. Previously, purification of BILA 2157 BS to satisfactory levels could only be achieved using chromatographic techniques, due to the poor physical properties (amorphous solid) of **1**, and our lack of success in forming crystalline addition salts with achiral/chiral, organic or mineral acids.[10] It was therefore of outmost importance

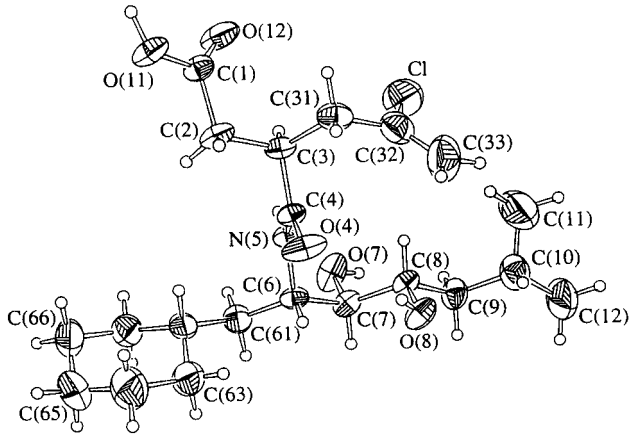


Figure 2.

that the final steps of the synthesis be carried out with maximum efficiency and control of side-product formation.

Conversion of the vinyl chloride **6** to BILA 2157 BS requires four chemical transformations as depicted in Scheme 10.[10] First, protection of the 1,2-diol in the form of an acetonide (**32**) is required to prevent side reactions in the following step. The acetonide is not isolated *per say*, but volatiles removed under reduced pressure and the residue taken up in a biphasic *tert*-butyl methyl ether (TBME)/water system. Addition of *N*-bromosuccinimide at 0 °C then converts the vinyl chloride to bromomethyl ketone **33**, which is treated in situ with thiourea to produce aminothiazole **34** as a water-soluble salt.[9,10] The cooled aqueous phase containing **34** is separated, washed with TBME in the cold (to avoid premature cleavage of the acetonide), and then heated to 60 °C to affect cleavage of the acetonide and elimination of residual volatile organic solvents. Subsequent neutralization with NaOH

(23) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

then induces precipitation of BILA 2157 BS free base in 84% yield from **6** and 94.5% homogeneity (HPLC).

Several factors are critical to the success of this integrated four-step, one-pot sequence that delivers crude **1** with ca. 5% loss in homogeneity with respect to the starting material **6**. The biphasic system used in the NBS reaction is superior to the originally reported homogeneous conditions (aqueous acetonitrile)[10] because contact of the sensitive bromoketone **33** with water is minimized. In addition, washing of the aqueous hydrobromide solution of **34** prior to cleavage of the acetonide also serves to improve the purity of the final product.

Further purification of BILA 2157 BS could be carried out by conversion to its water-soluble dimesylate salt, followed by washing with dichloromethane and reprecipitation of the free base with aqueous NaOH. After two such treatments, homogeneity was increased by ca. 3% to ~97.5%, with an 80% recovery of the material. BILA 2157 BS obtained by this route was comparable to material obtained previously[10] and was contaminated by less than 0.01% of its epimer at P_2 (HPLC).

In preliminary toxicology studies, chronic administration of BILA 2157 BS in rats and dogs resulted in toxicological findings, which precluded further development.

Conclusion

BILA 2157 BS (**1**), a potent and orally active renin inhibitor, was prepared by a highly convergent 15-step synthesis from aminodiol **4**. Several chemical steps were combined through implementation of multistep, integrated sequences and one-pot operations amenable to large scale. Key features of this synthesis include a solvent-free malonate alkylation under phase-transfer conditions and the use of an enzyme for the large-scale preparation of homochiral succinic acid derivatives (with recycling of the antipode through racemization). In addition, previously required cryogenic conditions and the use of a chiral auxiliary have been eliminated, significantly reducing the overall cost for the process. Finally, the end product was purified to ca. 97% homogeneity without chromatography.

Experimental Section

General Methods. All reagents, solvents, and starting materials were obtained from commercial sources and used as received. Benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[24] was purchased from Richelieu Biotechnologies Inc. (Montréal, Canada). Alcalase 2.4 L (food grade) was obtained from Novo Nordisk Biochem.[18a] ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR at 100 MHz. Flash chromatography[25] was performed on Merck silica gel 60 (0.040−0.063 mm). Analytical thin-layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates.

All reactions requiring anhydrous conditions were conducted under a positive pressure of argon or nitrogen gas, in oven-dried glassware.

Dimethyl (2-Chloro-2-propenyl)malonate 17. A 10 L vessel, equipped with a heating mantle, mechanical stirrer, thermometer, reflux condenser, and addition funnel, was purged with dry nitrogen gas and charged with dimethylmalonate (2642 g, 20.00 mol, 4 equiv) and anhydrous MeOH (550 mL). Sodium methoxide (1143 mL of a 25% w/w solution in MeOH, 5.00 mol, 1 equiv) was added in portions, followed by a MeOH rinse (550 mL). The reaction mixture was warmed to 62 °C, and 2,3-dichloropropene **16** (461 mL, 5.00 mol, 1 equiv) in MeOH (550 mL) was added dropwise over a period of 24−48 h while the internal temperature of the reaction was maintained above 65 °C. Additional sodium methoxide solution (57 mL) was added and the mixture heated to 70 °C for 3 h to complete the reaction. After the mixture was cooled to ambient temperature, the white slurry was filtered to remove NaCl, using MeOH for rinses, and the solvent removed under reduced pressure. Water (2 L) was added to the solid residue, and the aqueous phase was separated and extracted with ether. The organic phases were combined, washed with 1 N HCl (3 × 500 mL), water, and brine, and dried over $MgSO_4$. The ether was removed under reduced pressure and the residue fractionally distilled under vacuum to give recovered dimethylmalonate (1560 mL, bp 80 °C/1.5−2.0 Torr) containing some monoalkylated material and monoalkylated malonate **17** (620.3 g, 60% yield, Bp 79−86 °C/0.6 Torr) containing <3% dialkylated material and <5% dimethylmalonate. The distilled product was used without further purification in the next step, but a small sample was purified by flash chromatography for characterization: R_f 0.49 (4:1 hexane/ether); IR (film on NaCl) ν 1737, 1637 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.25 (broad m, 1H), 5.23 (broad d, J = 1.5 Hz, 1H), 3.81 (t, J = 7.5 Hz, 1H), 3.76 (s, 6H), 2.95 (dd, J = 7.5, 1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 168.6, 138.2, 115.2, 52.7, 49.8, 38.3; MS (ES^+) m/z 207 (MH^+), 229 ($M + Na^+$).

Recovered dimethylmalonate (1433 mL) was combined with fresh material (2000 mL) and alkylated under the conditions described above. Following three such alkylation cycles, the combined yield of distilled **17** was 70%.

Dialkylated Malonate 18. A 22 L vessel was equipped with a mechanical stirrer, thermometer, and dropping funnel and immersed in an ice−water bath. The flask was charged with 10 N NaOH (13 L), the solution was cooled to 10 °C, and benzyltriethylammonium chloride (11.58 g, 51 mmol, 0.004 equiv) was added. A mixture of malonate **17** (2627 g, 12.72 mol) and *tert*-butyl bromoacetate (2480 g, 12.72 mol) was added dropwise, maintaining the internal reaction temperature below 15 °C. The reaction mixture was then left stirring overnight at room temperature. The organic layer was separated and the aqueous phase extracted with hexane. This extract was evaporated under reduced pressure, the residue combined with the main organic layer, and the mixture used directly in the next step. A small sample was purified as follows for characterization: the crude material was diluted with ether and the solution washed with 1 N NaOH, 1 N HCl, and brine. After the solution was dried over $MgSO_4$, the solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel using 5% EtOAc in hexane as eluent. Compound **18** was obtained as a colorless oil: R_f 0.31 (10% EtOAc/hexane); IR (film on NaCl) ν 1741, 1734, 1632 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.32 (broad d, J = 1.5 Hz, 1H), 5.21 (broad s, 1H), 3.75 (s, 6H), 3.24 (s, 2H), 3.06 (s, 2H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 169.9, 169.5, 137.1, 117.6, 81.3, 54.4, 52.9, 41.8, 37.4, 27.9; MS (ES^+) m/z 321 (MH^+), 343 ($M + Na^+$). Anal. Calcd for $C_{14}H_{21}ClO_6$: C, 52.42; H, 6.60. Found: C, 52.19; H, 6.73.

Racemic Methylsuccinate (*R,S*)-15. Crude malonate derivative **18** from above (assume 12.72 mol) was diluted with MeOH (7 L) in a 22 L vessel equipped with a thermometer, mechanical stirrer, and addition funnel. The solution was cooled to 10 °C in an ice−water bath, and a solution of KOH (1000 g, 17.80 mol) in water (4 L) was added at such a rate to maintain the reaction temperature below 15 °C. After completion, the mixture was stirred overnight at room temperature. MeOH was then removed under reduced pressure and the aqueous phase washed with hexane (4 × 1 L). The aqueous layer was acidified to pH 1 with concentrated HCl (1.4 L) and saturated with NaCl (500 g). The product was extracted with toluene (3 × 2 L), and the extracts were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was then refluxed for 20 h to affect decarboxylation. The toluene was then

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

(25) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

evaporated under reduced pressure, the residue suspended in 2 N HCl, and the product extracted into hexane. After being washed with water and brine, the solution was dried (Na_2SO_4), filtered, and evaporated. The residue was distilled under vacuum to give pure succinate (*R,S*)-**15** as an oil (2664 g, 79% yield from **17**): bp 130−135 °C/0.75 Torr; R_f 0.34 (10% EtOAc/hexane); IR (film on NaCl) ν 1733, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.24 (broad d, J = 1 Hz, 1H), 5.19 (broad m, 1H), 3.71 (s, 3H), 3.14 (m, 1H), 2.76 (ddd, J = 14.5, 6.5, 1 Hz, 1H), 2.59 (dd, J = 16.5, 8.5 Hz, 1H, part of ABX), 2.54 (ddd, J = 14.5, 8, 0.5 Hz, 1H), 2.48 (dd, J = 16.5, 5.5 Hz, 1H, part of ABX), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 174.1, 170.5, 139.2, 115.0, 81.0, 51.9, 40.7, 39.2, 36.0, 28.0; MS (FAB) *m/z* 263 (MH^+). Anal. Calcd for $C_{12}H_{19}ClO_4$: C, 54.86; H, 7.29. Found: C, 54.64; H, 7.42. HPLC (ChiralPak AS 4.6 × 250 mm, 0.25% EtOH/hexane, 0.5 mL/min flow rate): (*R*)-**15** t_R 9.6 min (50%); (*S*)-**15** t_R 10.6 min (50%).

Enzymatic Hydrolysis of (*R,S*)-15. Isolation of Acid (*S*)-19 and Recovery of Ester (*R*)-15. A 12 L vessel equipped with a mechanical stirrer and a glass pH electrode was charged with water (4.25 L), acetone (260 mL), and (*R,S*)-**15** (2660 g, 10.12 mol). Alcalase 2.4 L (54 g, Novo Nordisk food grade enzyme)[18a] was added, and the pH of the mixture was adjusted to 7.2−7.5 by slow addition of 2 N NaOH (∼65 mL). The reaction mixture was then stirred vigorously, and the pH of the mixture maintained at 7.2−7.5 by addition of 2 N NaOH using an automatic titration device (pH meter coupled to a peristaltic addition pump). After 26 h, the pH of the mixture was raised and maintained at 7.5−7.8 for an additional 22 h, using 2 N NaOH. The pH was then readjusted once more to 7.8−8.1 and maintained by addition of 2 N NaOH for another 23 h. The total amount of added 2 N NaOH, after this point, was 2.38 L (4.76 mol, 94% of theoretical amount).

The reaction mixture was extracted with ether, and the combined organic layers were extracted with pH 9 aqueous NaOH (1 L, save extract), washed with brine, dried (Na_2SO_4), and concentrated to provide (*R*)-**15** as a pure colorless oil (1349 g, 51% yield): R_f, IR, ^1H NMR, ^{13}C NMR, MS (FAB) identical to that of (*R,S*)-**15**: $[\alpha]^{25}_D$ +6.7° (*c* 1.05, MeOH); $[\alpha]^{25}_{365}$ +14.4° (*c* 1.05, MeOH). Anal. Calcd for $C_{12}H_{19}ClO_4$: C, 54.86; H, 7.29. Found: C, 54.92; H, 7.33. HPLC (ChiralPak AS 4.6 × 250 mm, 0.25% EtOH/hexane, 0.5 mL/min flow rate): (*R*)-**15** t_R 9.8 min (97.2%, 94.4% ee); (*S*)-**15** t_R 11.0 min (2.8%).

The aqueous phase from the enzymatic hydrolysis was combined with the pH 9 NaOH wash from the isolation of the (*R*)-ester (above) and acidified to pH 1 with concentrated HCl (500 mL). The mixture was then extracted with ether and the extract washed with brine and dried (Na_2SO_4). Removal of volatiles under reduced pressure gave pure acid (*S*)-**19** as a clear yellowish oil (1213 g, 48% yield): R_f 0.69 (60% EtOAc/hexane); $[\alpha]^{25}_D$ −4.7° (*c* 1.02, MeOH); $[\alpha]^{25}_{365}$ −6.7° (*c* 1.02, MeOH); IR (film on NaCl) ν 3336−2617, 1732, 1716, 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.26 (d, J = 1 Hz, 1H), 5.22 (broad s, 1H), 3.17 (m, 1H), 2.81 (broad dd, J = 14.5, 6 Hz, 1H), 2.63−2.49 (m, 3H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ179.7, 170.4, 138.9, 115.3, 81.3, 40.3, 39.1, 35.5, 28.0; MS (FAB) *m/z* 249 (MH^+). Anal. Calcd for $C_{11}H_{17}ClO_4$: C, 53.12; H, 6.89. Found: C, 52.86; H, 7.01. HPLC of methyl ester prepared with diazomethane in ether (ChiralPak AS 4.6 × 250 mm, 0.25% EtOH/hexane, 0.5 mL/min flow rate): (*R*)-**15** t_R 9.9 min (1%); (*S*)-**15** t_R 10.9 min (99%, 98% ee).

Racemization of (*R*)-15. Methyl ester (*R*)-**15** (300.70 g, 1.144 mol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 8.7 g, 57 mmol, 0.05 equiv) were stirred at 80 °C under a nitrogen atmosphere for 24 h. HPLC analysis of an aliquot (partitioned between EtOAc/1 N HCl) on a chiral support showed almost complete racemization (ChiralPak AS 4.6 × 250 mm, 0.25% EtOH/hexane, 0.5 mL/min flow rate): (*R*)-**15** t_R 10.6 min (53%); (*S*)-**15** t_R 12.1 min (47%). The reaction mixture was then cooled to room temperature and diluted with EtOAc (500 mL) and the solution washed with 1 N HCl (3 × 300 mL). The aqueous phase was extracted with EtOAc, and the organic extracts were combined and washed with brine. After drying (Na_2SO_4) and removal of solvent under reduced pressure, the residue was distilled as previously described to give (*R,S*)-**15** (293.47 g, 97% yield). This material was recycled through enzymatic hydrolysis.

Preparation of Monomethylsuccinate (*R,S*)-20. Racemic diester (*R,S*)-**15** (607.76 g, 2.31 mol) was added in portions to cold (5 °C) 4 N HCl in dioxane (1.6 L) and the mixture stirred for 40 h, allowing the temperature to raise to ambient. Volatiles were then removed in a vacuum at 40 °C to give a yellow oily residue. Ether (250 mL) was added followed by hexane until slightly turbid and the solution allowed to crystallize, first at room temperature and then at 5 °C overnight. The product was collected by filtration, washed with 10% ether/hexane, followed by hexane, and dried under vacuum to constant weight, to give (*R,S*)-**20** (407.46 g, 85% yield): mp 61−64 °C; IR (NaCl film) ν 3600−2500, 1736, 1713, 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.45 (broad s, 1H), 5.27 (broad d, J = 1 Hz, 1H), 5.22 (broad s, 1H), 3.72 (s, 3H), 3.20 (m, 1H), 2.80 (ddd, J = 14.5, 6, 1 Hz, 1H, part of ABX), 2.74 (dd, J = 17, 8.5 Hz, 1H, part of ABX), 2.62 (dd, J = 17.5, 5 Hz, 1H, part of ABX), 2.58 (dd, J = 14.5, 8.5 Hz, 1H, part of ABX); ^{13}C NMR ($CDCl_3$) δ 177.3, 173.7, 138.6, 115.2, 52.0, 40.3, 38.5, 33.9; MS (FAB) *m/z* 207 (MH^+). Anal. Calcd for $C_8H_{11}ClO_4$: C, 46.50; H, 5.37. Found: C, 46.12; H, 5.35.

Coupling of (*R,S*)-20 with Amine 2. Preparation of (*R,S*)-21. Acid (*R,S*)-**20** (138.00 g, 667.9 mmol) and *N*-methylmorpholine (147 mL, 1.34 mol) were dissolved in dry THF (500 mL), and the solution was cooled in an ice−water bath under an argon atmosphere. Pivaloyl chloride (81.5 mL, 663 mmol) was added dropwise over a 30 min period and stirring continued for an additional 1 h at 5 °C. Amine **2**[10] (191.0 g, 0.660 mmol) in THF (75 mL + 25 mL rinse) was added over 30 min and the mixture stirred for 1.5 h at 5 °C. The reaction mixture was then quenched with water (100 mL) and THF evaporated under reduced pressure. The product was extracted with EtOAc, washed with water and brine, dried ($MgSO_4$/charcoal), and concentrated to give crude (*R,S*)-**21** (323 g, 102% yield) as a thick orange oil, which gave the following analytical data: R_f 0.24 (9:1 EtOAc/MeOH); IR (film on NaCl) ν 1730, 1660, 1640 cm^{-1}; ^1H NMR ($CDCl_3$, mixture of rotamers) δ 8.62 (broad m, 0.1 H), 8.58 (m, 0.4H), 8.54 (broad d, J = 5 Hz, 0.6H), 7.62 (m, 1H), 7.26−7.13 (m, 2H), 5.26−5.15 (m, 2H), 4.20 (d, J = 16 Hz, 0.5H), 4.10 (d, J = 16 Hz, 0.4H), 4.15−4.06 (m, 0.1H), 4.01−3.89 (m, 0.1H), 3.96 (d, J = 16 Hz, 0.4H), 3.84 (d, J = 16 Hz, 0.5H), 3.80−3.65 (m, 2H), 3.70 (s, 1.5H), 3.69 (s, 1.5H), 3.27 (m, 1H), 3.17 (m, 1H), 3.11−2.94 (m, 3H), 2.92 (s, 1.8H), 2.90 (s, 1.2H), 2.82−2.71 (m, 1.9H), 2.71−2.59 (m, 1.9H), 2.38 (m, 0.1H), 2.24 (m, 0.1H), 1.80−1.35 (m, 6H), 1.20 (m, 2H), 0.90 (m, 2H); ^{13} C NMR ($CDCl_3$, mixture of rotamers) δ 182.4, 174.8, 174.7, 171.3, 171.2, 167.8, 167.7, 167.4, 167.2, 158.8, 158.6, 158.1, 158.0, 149.6, 149.3, 148.9, 148.8, 139.6, 139.5, 137.0, 136.9, 136.88, 136.85, 124.0, 123.9, 123.8, 123.7, 122.2, 121.9, 121.7, 121.6, 114.9, 114.7, 114.6, 77.2, 55.1, 54.9, 53.7, 53.6, 51.9, 51.8, 51.7, 50.0, 49.6, 48.75, 48.7, 48.1, 47.4, 47.1, 40.7, 40.65, 40.6, 39.6, 39.4, 38.4, 37.1, 37.0, 36.5, 36.4, 36.2, 35.6, 35.5, 35.0, 34.9, 33.5, 33.4, 33.35, 33.3, 33.2, 33.1, 31.0, 30.93, 30.90, 30.85, 30.8, 30.75, 30.7, 30.6, 27.1, 26.4, 26.35, 26.3, 25.8; MS (FAB) *m/z* 478 (MH^+). Anal. Calcd for $C_{25}H_{36}ClN_3O_4$: C, 62.82; H, 7.59; N, 8.79. Found: C, 62.46; H, 7.71; N, 8.50.

Enzymatic Hydrolysis of (*R,S*)-21. Isolation of Acid (*S*)-22 and Recovery of Ester (*R*)-21. Racemic succinate (*R,S*)-**21** (196.2 g, 0.41 mol) was dissolved in acetone (150 mL) and the solution diluted with water (350 mL). The pH of the solution was adjusted to 7 by addition of 1 M $KHSO_4$, and 5 g of Alcalase 2.4 L[18a] was added. The solution was stirred vigorously and its pH maintained between 7.5 and 7.8 for 15 h by addition of 1 N NaOH (∼130 mL added over this time period, ∼75% of theoretical amount) using an automatic titration device (pH meter coupled to a peristaltic addition pump). The pH of the solution was then raised to 8 and maintained for another 24 h (∼30 mL of 1 N NaOH, 92% of theoretical amount). After a total of 160 mL of 1 N NaOH had been added, the reaction mixture was concentrated under reduced pressure to remove acetone and the aqueous phase

extracted with EtOAc. The aqueous phase was saved for recovery of (*S*)-**22** (see below). The organic extract was washed with water (2 × 100 mL) and brine and dried ($MgSO_4$/charcoal). The solution was then filtered through a small pad of silica gel using EtOAc (1 L) for washings. Removal of solvent under reduced pressure followed by drying under vacuum to constant weight gave crude (*R*)-**21** as a thick brown oil (103.69 g, 53% yield): $[\alpha]^{25}_D$ −2.5° (*c* 1.27, MeOH); $[\alpha]^{25}_{365}$ −15.0° (*c* 1.27, MeOH). HPLC analysis (two end to end Chiralcel OD-H columns of 15 and 25 cm, 2% EtOH/hexane isocratic, 0.5 mL/min flow rate, 260 nm UV detection): (*R*)-**21** t_R 154 min (98.8%, 97.6% ee); (*S*)-**21** t_R 171 min (1.2%). An analytical sample of this material was obtained by flash chromatography on silica gel using 2% MeOH/$CHCl_3$ then 3% MeOH/$CHCl_3$. (*R*)-**21**, was obtained as a colorless oil: R_f 0.34 (5% MeOH/$CHCl_3$); $[\alpha]^{25}_D$ −2.9° (*c* 1.24, MeOH); $[\alpha]^{25}_{365}$ −18.1° (*c* 1.24, MeOH); IR (NaCl film) ν 2924, 2851, 1736, 1644 cm^{-1}; ^1H NMR ($CDCl_3$, mixture of rotamers) δ 8.59 (broad d, J = 5 Hz, 0.1H), 8.55 (dt, J = 4, 1.5 Hz, 0.4H), 8.50 (broad d, J = 3 Hz, 0.5H), 7.66−7.56 (m, 1H), 7.23−7.09 (m, 2H), 5.26−5.13 (m, 2H), 4.19 (d, J = 16 Hz, 0.5H, part of ABX), 4.10 (d, J = 16 Hz, 0.4H, part of ABX), 4.08 (d, J = 17 Hz, 0.1H, part of ABX), 3.99 (d, J = 17 Hz, 0.1H, part of ABX), 3.95 (d, J = 16 Hz, 0.5H, part of ABX), 3.83 (d, J = 16 Hz, 0.4H, part of ABX), 3.79−3.66 (m, 2H), 3.69 (s, 1.5H), 3.67 (s, 1.5H), 3.31−2.95 (m, 5H), 2.90 (broad s, 3H), 2.86−2.50 (m, 3.5H), 2.39 (d, J = 6 Hz, 0.1H), 2.35 (d, J = 6.5 Hz, 0.1H), 2.24 (m, 0.5H), 1.80−1.36 (m, 6H), 1.30−1.08 (m, 3H), 0.98−0.80 (m, 2H); ^{13}C NMR ($CDCl_3$, mixture of rotamers) δ 174.8, 174.7, 171.3, 171.1, 167.7, 167.6, 167.3, 167.1, 159.1, 158.8, 158.2, 157.7, 148.7, 149.5, 149.2, 149.1, 139.61, 139.58, 139.55, 139.5, 136.8, 136.6, 136.5, 136.4, 123.8, 123.7, 123.6, 123.5, 122.1, 121.7, 121.5, 121.4, 114.8, 114.6, 114.5, 55.0, 54.8, 53.7, 53.6, 51.82, 51.80, 51.7, 50.0, 49.61, 48.7, 48.6, 48.5, 47.9, 47.3, 47.0, 40.7, 40.65, 40.60, 39.5, 39.3, 37.1, 37.0, 36.6, 36.4, 36.3, 36.2, 35.9, 35.8, 35.0, 34.9, 33.5, 33.3, 33.25, 33.2, 33.1, 31.0, 30.9, 30.85, 30.8, 30.75, 30.70, 30.6, 26.37, 26.35, 26.27, 26.26, 25.8; MS (ES^+) m/z 478 (MH^+). Anal. Calcd for $C_{25}H_{36}ClN_3O_4$: C, 62.82; H, 7.59; N, 8.79. Found: C, 62.51; H, 7.89; N, 8.75.

The aqueous layer from the first EtOAc extraction (see above) was acidified to pH 4 by addition of 20% aqueous $KHSO_4$ and extracted with EtOAc. The extract was filtered to remove precipitated enzyme residues and washed with water and brine. After drying ($MgSO_4$), concentration, and drying to constant weight under vacuum, crude acid (*S*)-**22** (67.35 g, 75% yield) was obtained as a brown foam: $[\alpha]^{25}_D$ +5.1° (*c* 1.30, MeOH); $[\alpha]^{25}_{365}$ +23.8° (*c* 1.30, MeOH). A small sample of this crude acid was converted to the corresponding methyl ester (*S*)-**21** using diazomethane and analyzed by HPLC on a chiral support (two end to end Chiralcel OD-H columns of 15 and 25 cm, 2% EtOH/hexane isocratic, 0.5 mL/min flow rate, 260 nm UV detection): (*R*)-**21** t_R 157 min (3%); (*S*)-**21** t_R 167 min (97%, 94% ee). An analytical sample of acid (*S*)-**22** was obtained by flash chromatography on silica gel using 5% MeOH/$CHCl_3$ as eluent: R_f 0.19 (5% MeOH/$CHCl_3$); $[\alpha]^{25}_D$ +5.2° (*c* 1.33, MeOH); $[\alpha]^{25}_{365}$ +25.9° (*c* 1.33, MeOH); IR (NaCl film) ν 3700−2300, 1726, 1657, 1642 cm^{-1}; ^1H NMR ($CDCl_3$, mixture of rotamers) δ 10.25 (broad s, 1H), 8.61 (broad d, J = 5 Hz, 0.2H), 8.59 (broad d, J = 4.5 Hz, 0.3H), 8.55 (broad d, J = 5 Hz, 0.5 H), 7.66 (dt, J = 7.5, 2 Hz, 1H), 7.28−7.15 (m, 2H), 5.31−5.18 (m, 2H), 4.12−3.84 (m, 2H), 3.78−3.62 (m, 2H), 3.28−3.00 (m, 4.5H), 2.97 (d, J = 5 Hz, 0.1H), 2.93 (s, 1.6H), 2.90 (s, 1.4H), 2.90−2.77 (m, 0.5H), 2.76−2.70 (m, 1.3H), 2.68−2.43 (m, 0.8H), 2.43−2.25 (m, 0.2H), 1.80−1.37 (m, 6H), 1.30−1.10 (m, 3H), 0.98−0.80 (m, 2H); ^{13}C NMR ($CDCl_3$, mixture of rotamers) δ 176.1, 176.0, 175.95, 172.7, 172.4, 172.2, 167.62, 167.61, 167.1, 167.0, 158.5, 158.3, 157.8, 157.4, 149.3, 149.0, 148.2, 148.1, 139.9, 139.8, 137.5, 137.4, 137.3, 124.15, 124.10, 124.0, 122.3, 122.0, 121.9, 121.7, 115.0, 114.9, 114.6, 114.5, 55.4, 55.1, 54.0, 53.9, 50.2, 49.9, 48.7, 48.6, 48.1, 47.6, 47.5, 40.5, 40.4, 40.35, 40.3, 39.6, 39.4, 39.3, 37.0, 36.9, 36.3, 36.15, 36.10, 35.9, 35.2, 35.1, 34.8, 33.5, 33.3, 33.15, 33.10, 33.0, 32.8, 30.9, 30.7, 30.6, 26.35, 26.30, 26.2, 25.7; MS (ES^+) m/z 464 (MH^+). Anal. Calcd for $C_{24}H_{34}ClN_3O_4$: C, 62.13; H, 7.39; N, 9.06. Found: C, 62.12; H, 7.65; N, 8.98.

Racemization of (*R*)-21. NaOMe (25% w/w solution in MeOH, 152 μL, 0.66 mmol, 0.1 equiv) was added to a solution of (*R*)-**21** (3.19 g, 6.67 mmol) in MeOH (10 mL). The dark red solution was stirred under reflux (60 °C) for 18 h, at which point HPLC analysis on a chiral support as described above for the pure enantiomers indicated complete racemization.

Coupling of Acid (*S*)-22 with Amine 4. Preparation of Compound 6. Aminodiol **4**[12,13] (2.00 g, 8.2 mmol), acid (*S*)-**22** (4.22 g, 9.1 mmol), hydroxybenzotriazole (1.50 g, 9.8 mmol), and *N*-methylmorpholine (5.00 g, 49.5 mmol) were dissolved in dry DMF (20 mL). Dicyclohexylcarbodiimide (1.90 g, 9.2 mmol) was added and the mixture stirred overnight at room temperature. The reaction mixture was then concentrated to half volume under vacuum and subsequently diluted with EtOAc (500 mL). Precipitated DCU was removed by filtration and the filtrate washed with water and brine. After drying ($MgSO_4$) and removal of solvent under reduced pressure a yellow solid was obtained. The crude material was dissolved in boiling EtOAc (20 mL), hexane (100 mL) was added, and the product was allowed to crystallize at room temperature. After cooling (5 °C), the material was collected by filtration and washed with 10% EtOAc/hexane and then with hexane. After drying in a vacuum, compound **6** was obtained as a white solid (4.16 g, 73% yield): R_f, IR, ^1H NMR, ^{13}C NMR, and MS data are identical to that of **6** obtained by coupling of acid **31** with amine **4**: mp 139−141 °C; $[\alpha]^{24}_D$ −32.2° (*c* 1.00, MeOH). Anal. Calcd for $C_{38}H_{61}ClN_4O_5$: C, 66.21; H, 8.92; N, 8.13. Found: C, 65.99; H, 9.02; N, 8.09. Reversed-phase HPLC (Supelcosil LC-ABZ, 4.6 × 150 mm, 5 μm particle size, 0−100% 0.1% TFA−CH_3CN in 0.1% TFA in 25 min then 100% 0.1% TFA in CH_3CN for 5 min, 1 mL/min flow rate, UV detection at 215 nm): t_R 17.0 min (99.04%, P_2 epimer of **6** not detected).

Preparation of Allylic Amine Hydrochloride 26. A 4:1 v/v mixture of AcOH/12 N HCl (200 mL) was slowly added (caution: gas evolution) to carbamate **25**[13] (154.22 g, 0.498 mol), and the mixture was stirred for 1.5 h at room temperature. An additional 300 mL of the acid mixture was added and the solution stirred for another 2 h, after which point TLC (20% EtOAc/hexane) showed complete disappearance of starting material. The reaction mixture was brought to pH 14 by slow addition of 10 N NaOH (850 mL) and extracted with ether. The combined extracts were washed with 1:1 water/brine, dried (Na_2SO_4), and concentrated to a viscous black oil. This residue was dissolved in ether (100 mL), and a 1 N solution of hydrogen chloride in ether (500 mL) was added dropwise over 1 h. The resulting slurry was concentrated by removal of ether (300 mL) under reduced pressure, hexane (200 mL) was added in portions, and the mixture was stirred for 30 min. The precipitated hydrochloride salt was collected by filtration and washed with cold 25% hexane in ether (2 × 250 mL) and then 50% hexane in ether (2 × 250 mL). After drying in air, hydrochloride **26** was obtained as a tan-colored solid (104.59 g, 85% yield): mp 220−227 °C dec; $[\alpha]^{25}_D$ +22.6° (*c* 1.04, MeOH); IR (NaCl film) ν 1601 cm^{-1}; ^1H NMR ($CDCl_3$, contains ~5% of *E*-isomer) δ 8.37 (broad s, 3H), 5.83 (m, 0.05H, *E*-isomer), 5.72 (m, 0.95H), 5.45 (m, 1H), 4.09 (broad s, 0.95H), 3.75 (broad s, 0.05H, *E*-isomer), 2.13−2.03 (m, 1H), 2.02−1.92 (m, 1H), 1.82−1.52 (m, 8H), 1.42−1.06 (m, 4H), 1.00−0.86 (m, 2H), 0.94 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 135.9, 126.0, 47.0, 41.2, 36.8, 33.8, 33.5, 32.7, 28.4, 26.3, 26.0, 25.9, 22.5, 22.2; MS (ES^+) m/z 210 (MH^+). Anal. Calcd for $C_{14}H_{28}ClN$: C, 68.40; H, 11.48; N, 5.70. Found: C, 68.23; H, 11.70; N, 5.73.

Coupling of Acid (*S*)-19 with Amine Hydrochloride 26. Preparation of Alkene 27. To a slurry of hydrochloride salt **26** (10.00 g, 40.7 mmol) in acetonitrile (50 mL) were added diisopropylethylamine (28.3 mL, 163 mmol) and a solution of acid (*S*)-**19** (10.12 g, 40.7 mmol) in acetonitrile (25 mL). TBTU (14.36 g, 44.8 mmol) was added and the mixture stirred 18 h at room temperature. Volatiles were then removed under reduced pressure and the residue dissolved in ether (250 mL). The organic solution was washed with 2.5 N NaOH (2 × 100 mL), 1 N HCl (100 mL), and brine. After drying ($MgSO_4$) and evaporation of solvents, the residue was purified by flash chromatography on silica gel using 5% EtOAc in hexane as

eluent to provide alkene **27** as a light yellow, waxy solid (15.48 g, 86% yield): mp 60.0−61.5 °C; R_f 0.47 (15% EtOAc/hexane); $[\alpha]^{25}_D$ +21.8° (*c* 1.02, MeOH); IR (film on NaCl) ν 3285, 1726, 1639 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.66 (d, J = 8 Hz, 1H), 5.43 (ddt, J = 11, 7.5, 0.5 Hz, 1H), 5.22−5.16 (m, 3H), 4.73 (m, J = 8 Hz, 1H), 2.91−2.82 (m, 1H), 2.65 (dd, J = 14, 8 Hz, 1H, part of ABX), 2.62 (dd, J = 17, 10 Hz, 1H, part of ABX), 2.38 (dd, J = 14, 7 Hz, 1H, part of ABX), 2.33 (dd, J = 17, 4 Hz, 1H, part of ABX), 2.02 (m, 2H), 1.78−1.55 (m, 7H), 1.44 (s, 9H), 1.31−1.10 (m, 5H), 0.97−0.87 (m, 2H), 0.90 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H); ^{13}C NMR δ 171.6, 171.4, 139.3, 131.3, 130.9, 115.1, 80.9, 44.5, 43.6, 41.6, 40.4, 37.0, 36.7, 34.0, 33.4, 33.3, 28.5, 28.1, 26.5, 26.2, 26.1, 22.4, 22.3. MS (ES^+) m/z 440 (MH^+), 462 (M + Na^+), 485 (M + 2Na^+). Anal. Calcd for $C_{25}H_{42}ClNO_3$: C, 68.23; H, 9.62; N, 3.18. Found: C, 68.43; H, 9.88; N, 3.36.

Preparation of Acid 28. A 30% solution of HBr in AcOH (50 mL) was cooled to 5 °C, and ester **27** (8.00 g, 8.2 mmol) was added. The mixture was stirred overnight at 5 °C. Volatiles were then removed under vacuum, and the residue was coevaporated with toluene (2 × 100 mL) under reduced pressure. The resulting oil was dissolved in ether (300 mL) and washed with water. The aqueous phase was basified to pH 7 with 1 N NaOH, and this solution was used again to wash the original ether layer. This extraction/neutralization operation was repeated until the aqueous phase remained at pH 6−7. The ether layer was then dried ($MgSO_4$) and concentrated and the residue purified by flash chromatography on silica gel using 5% MeOH/$CHCl_3$ as eluent. Acid **28** was obtained as a beige syrup that was dried at 75 °C under vacuum and solidified on standing (6.16 g, 91% yield): mp 87.2−89.4 °C; R_f 0.31 (5% MeOH/$CHCl_3$); $[\alpha]^{25}_D$ + 26.7° (*c* 1.06, MeOH); IR (film on NaCl) ν 3288, 3072−2720, 1712, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.65 (d, J = 8.5 Hz, 1H), 5.45 (dt, J = 10.5, 7.5 Hz, 1H), 5.21 (broad s, 2H), 5.21−5.14 (m, 1H), 4.76 (m, J = 8 Hz, 1H), 2.88 (m, 1H), 2.79 (dd, J = 17, 9 Hz, 1H, part of ABX), 2.65 (dd, J = 14, 8 Hz, 1H, part of ABX), 2.52 (dd, J = 17, 3.5 Hz, 1H, part of ABX), 2.46 (dd, J = 14, 7 Hz, 1H, part of ABX), 2.01 (tt, J = 7, 2 Hz, 2H), 1.78−1.56 (m, 6H), 1.44 (broad m, 1H), 1.30−1.10 (m, 5H), 0.98−0.82 (m, 2H), 0.90 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); ^{13}C NMR δ 175.8, 172.1, 138.5, 131.6, 130.4, 115.9, 44.9, 43.4, 41.6, 40.1, 36.7, 35.7, 34.1, 33.4, 33.3, 28.5, 26.5, 26.1, 22.4, 22.3; MS (ES-) m/z 382 (M − H). Anal. Calcd for $C_{21}H_{34}ClNO_3$: C, 65.69; H, 8.93; N, 3.65. Found: C, 65.48; H, 8.97; N, 3.77.

Coupling of Acid 28 to Amine 2. Preparation of 29. To a solution of acid **28** (2.250 g, 5.86 mmol) in dry THF (25 mL) were added *N*-methylmorpholine (1.93 mL, 17.6 mmol), a solution of amine **2**[10] (1.866 g, 6.45 mmol) in dry THF (10 mL), and TBTU (2.069 g, 6.45 mmol). The reaction mixture was then stirred overnight at room temperature. The mixture was diluted with ether (200 mL), washed with 2.5 N NaOH (2 × 25 mL), and concentrated under reduced pressure and the residue purified by flash chromatography on silica gel using 3% MeOH/$CHCl_3$ as eluent. Compound **29** was obtained as a yellow glass (3.021 g, 78% yield): R_f 0.23 (5% MeOH/$CHCl_3$); $[\alpha]^{25}_D$ +12.6° (*c* 1.0, MeOH); $[\alpha]^{25}_{365}$ +48.3° (*c* 1.0, MeOH); IR (NaCl film) ν 3315, 1652, 1637 cm^{-1}; ^1H NMR ($CDCl_3$, mixture of rotamers) δ 8.61 (broad d, J = 5 Hz, 0.1H), 8.57 (broad d, J = 5 Hz, 0.4H), 8.52 (broad d, J = 5 Hz, 0.5H), 7.67−7.58 (m, 1H), 7.24−7.11 (m, 2H), 6.22−6.09 (broad m, 1H), 5.47−5.35 (m, 1H), 5.24−5.10 (m, 3H), 4.75−4.63 (m, 1H), 4.35 (dd, J = 16, 13.5 Hz, 0.8H), 4.15 (d, J = 18 Hz, 0.1H), 4.07 (d, J = 17.5 Hz, 0.1H), 3.88−3.51 (m, 3H), 3.42 (dd, J = 13.5, 7.5 Hz, 0.1H, part of ABX), 3.32 (dd, J = 13.5, 7.5 Hz, 0.1H, part of ABX), 3.22 (dd, J = 15, 7.5 Hz, 0.4H, part of ABX), 3.14 (dd, J = 15, 8 Hz, 0.4H, part of ABX), 3.13−2.98 (m, 3H), 2.97−2.85 (m, 3.5H), 2.81−2−61 (m, 2H), 2.58−2.25 (m, 2.3H), 2.23 (dd, J = 16, 4 Hz, 0.1H, part of ABX), 2.11 (dd, J = 16, 4 Hz, 0.1H, part of ABX), 2.07−1.96 (m, 2H), 1.80−1.48 (m, 11H), 1.48−1.32 (m, 2H), 1.32−1.02 (m, 8H), 1.00−0.75 (m, 4H), 0.89 (broad d, J = 6.5 Hz, 3H), 0.88 (broad d, J = 6.5 Hz, 3H); ^{13}C NMR δ 172.2, 172.1, 172.0, 171.9, 171.8, 171.7, 167.8, 167.6, 167.3, 167.2, 159.0, 158.7, 158.1, 157.6, 149.8, 149.6, 149.2, 139.9, 139.8, 139.7, 136.8, 136.6, 136.5, 131.2, 131.1, 131.0, 130.9, 130.8, 130.7, 130.6, 123.8, 123.7, 123.6, 123.5, 122.1, 121.8, 121.6, 121.5, 114.8, 114.7, 114.5, 114.3, 55.1, 54.9, 53.9, 53.8, 50.0, 49.7, 48.7, 48.5, 47.9, 47.5, 47.1, 44.6, 44.5, 44.4, 44.3, 43.4, 43.3, 41.5, 41.4, 40.6, 40.5, 40.4, 40.3, 37.1, 37.0, 36.7, 36.6, 36.5, 36.3, 36.0, 35.8, 35.3, 35.2, 35.1, 35.0, 34.9, 34.0, 33.9, 33.5, 33.4, 33.3, 33.2, 33.2, 33.1, 33.0, 31.0, 30.9, 30.8, 30.7, 30.6, 30.5, 28.5, 26.5, 26.3, 26.2, 26.1, 25.8, 25.7, 22.3, 22.2; MS (ES^+) m/z 655 (MH^+), 677 (M + Na^+). Anal. Calcd for $C_{38}H_{59}ClN_4O_3$: C, 69.64; H, 9.07; N, 8.55. Found: C, 69.46; H, 9.47; N, 8.48.

General Procedure for Dihydroxylation of Alkenes 27−29. To the alkene substrate (∼10 mmol, 1 equiv) in *tert*-butylmethyl ether (20 mL) were added water (3 equiv) and *N*-methylmorpholine *N*-oxide (1.5 equiv). The solution was cooled to 5 °C, and OsO_4 (0.01 equiv of a 2.5% w/w solution in *tert*-butyl alcohol, 1.25 mL/10 mmol of substrate) was added. The mixture was stirred 48 h at 5 °C, after which volatiles were removed under reduced pressure. Warm acetonitrile (20 mL for a 10 mmol scale) was added to dissolve solids, followed by dropwise addition of water (10 mL) to precipitate diol product. After the mixture was stirred for 0.5 h at room temperature, precipitated solids were collected by filtration, washed with 2:1 CH_3CN/water (20 mL), and dried. Crude materials obtained in this way were comparable with products prepared from authentic aminodiol **4**.[12,13]

Diol 30 was obtained as an isomerically pure (NMR) white solid in 60% yield on a 9 mmol scale. IR, NMR, and MS data were identical to material prepared by coupling of acid (*S*)-**19** to authentic aminodiol **4**[12,13] (see below): mp 147−148 °C; R_f 0.57 (3:1 hexane/EtOAc); $[\alpha]^{25}_D$ −43.4° (*c* 1.01, MeOH). Anal. Calcd for $C_{25}H_{44}ClNO_5$: C, 63.34; H, 9.35; N, 2.95. Found: C, 63.42; H, 9.74; N, 3.03.

Diol 31 was obtained as a white solid in 56% yield on a 9.4 mmol scale (contaminated with 3.2% of the epimer at P_2 as determined by ^1H NMR comparison with an authentic standard in DMSO-d_6). IR, NMR, and MS data were identical to material prepared by deprotection of ester **30** (see below): mp 188−189 °C;. $[\alpha]^{25}_D$ −48.5° (*c* 1.02, MeOH). Anal. Calcd for $C_{21}H_{36}ClNO_5$: C, 60.35; H, 8.68; N, 3.35. Found: C, 60.57; H, 8.87; N, 3.52.

Diol 6 was obtained as an isomerically pure (NMR) solid in 56% yield on a 4 mmol scale. IR, NMR, and MS data were identical to material prepared by coupling of acid **31** with amine **2** or coupling of acid (*S*)-**22** with aminodiol **4**[12,13] (see below): mp 138.5−140 °C; R_f 0.47 (5% MeOH/EtOAc); $[\alpha]^{25}_D$ −30.6° (*c* 1.05, MeOH). Anal. Calcd for $C_{38}H_{61}ClN_4O_5$: C, 66.21; H, 8.92; N, 8.13. Found: C, 65.96; H, 9.21; N, 8.12.

Coupling of (*S*)-19 to Aminodiol 4. Preparation of Compound 30. A 5 L vessel fitted with a mechanical stirrer and thermometer was charged with acid (*S*)-**19** (200.0 g, 804 mmol) and dry THF (500 mL). The solution was cooled to 5 °C in an ice−water bath, and diisopropylethylamine (154 mL, 885 mmol) was added slowly. Aminodiol **4**[12,13] (195.7 g, 804 mmol), 1-hydroxybenzotriazole (114.1 g, 844 mmol), and additional THF (500 mL) were then added. A solution of 1,3-dicyclohexylcarbodiimide (170.1 g, 824 mmol) in dry THF (300 mL) was added dropwise to the cooled reaction mixture over a 1 h period. The white slurry was then stirred overnight at room temperature. The reaction mixture was diluted with EtOAc (1 L) and the precipitated dicyclohexylurea (DCU) separated by filtration (used EtOAc for rinses). The filtrate was then concentrated under reduced pressure to a white paste that was transferred to a 5 L three-necked flask and suspended in acetonitrile (1.9 L). To the vigorously stirred (mechanical stirrer) suspension was added 2 N HCl (1.6 L) and stirring continued for 1 h. The precipitated product was collected by filtration, washed with 1:1 CH_3CN/2 N HCl (2 × 500 mL) and water (3 × 500 mL), and dried in air to constant weight. Diol **30** was obtained as a white solid (353.4 g, 92% yield), containing 0.6% w/w of DCU and none of the P_2 epimer (^1H NMR). An analytical sample of this material was obtained by filtration through a pad of silica gel using 25% EtOAc/hexane as eluent: mp 148.5−150 °C; R_f 0.52 (25% EtOAc/hexane); $[\alpha]^{25}_D$ −44.6° (*c* 1.09, MeOH); IR (KBr) ν 3445, 3251, 1700, 1632 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.84 (d, J = 9 Hz, 1H), 5.36 (broad

d, J = 1 Hz, 1H), 5.22 (d, J = 1.5 Hz, 1H), 4.71 (d, J = 6.5 Hz, 1H), 4.49 (broad d, J = 4 Hz, 1H), 4.08 (broad dt, J = 9, 5 Hz, 1H), 3.09−2.99 (m, 2H), 2.92 (broad t, J = 7.5 Hz, 1H), 2.62 (dd, J = 14.5, 7 Hz, 1H, part of ABX), 2.44 (dd, J = 16, 9 Hz, 1H, part of ABX), 2.39 (dd, J = 14.5, 7 Hz, 1H, part of ABX), 2.23 (dd, J = 16, 5.5 Hz, 1H, part of ABX), 1.81−1.52 (m, 7H), 1.44 (broad m, 1H), 1.39 (s, 9H), 1.33 (broad m, 1H), 1.25−1.03 (m, 5H), 0.95−0.71 (broad m, 2H), 0.87 (d, J = 6.5 Hz, 3H), 0.79 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 174.8, 171.2, 139.3, 115.5, 81.3, 77.6, 69.6, 47.3, 42.6, 41.5, 40.6, 39.5, 37.1, 33.9, 33.7, 32.7, 28.1, 26.5, 26.2, 26.0, 24.6, 23.9, 21.8; MS (ES^+) m/z 474 (MH^+), 496 ($M + Na^+$). Anal. Calcd for $C_{25}H_{44}ClNO_5$: C, 63.34; H, 9.35; N, 2.95. Found: C, 63.44; H, 9.50; N, 3.05.

Deprotection of Ester 30. Preparation of Acid 31. Ester **30** from above (346.2 g, 726 mmol based on DCU content) was added to ice-cold trifluoroacetic acid (725 mL) and the mixture stirred overnight under nitrogen, allowing the temperature to rise back to ambient (caution, gas evolution!). Trifluoroacetic acid was evaporated under reduced pressure and the residue cooled in an ice bath. MeOH (1 L) was added carefully and the mixture stirred until homogeneous. The solution was then transferred to a 5 L three-necked flask fitted with a mechanical stirrer, thermometer, and addition funnel and immersed in an ice−water bath. NaOH (2.5 N) was added dropwise, keeping the temperature of the mixture below 25 °C, until pH 11 was reached (∼1 L). The reaction was stirred and the pH adjusted to 11−12 by periodical addition of more 2.5 N NaOH, until it remained constant for 1 h. Water (2.2 L) was added, and the slightly turbid solution filtered to remove insoluble impurities (mostly DCU). The filtrate was then diluted with water to a volume of 5 L, and concentrated HCl was added dropwise under vigorous stirring until a pH of 1 was reached (∼70 mL). The resulting suspension was stirred for an additional hour at room temperature and filtered, using water for rinses and washings (4 × 2 L). The white solid was dried in air to constant weight (299.8 g, 99% yield): mp 187−188.5 °C; $[\alpha]^{25}_D$ −51.0° (c 1.05, MeOH); IR (KBr) ν 3500−2300, 3254, 1730 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.82 (d, J = 9 Hz, 1H), 5.35 (broad s, 1H), 5.21 (broad d, J = 1 Hz, 1H), 4.67 (broad d, J = 2 Hz, 1H), 4.49 (broad d, J = 2.5 Hz, 1H), 4.09 (broad dt, J = 9, 4 Hz, 1H), 3.05 (m, 2H), 2.91 (broad d, J = 7.5 Hz, 1H), 2.63 (dd J = 15, 7.5 Hz, 1H, part of ABX), 2.46 (dd, J = 16.5, 8 Hz, 1H, part of ABX), 2.42 (dd, J = 14, 7 Hz, 1H, part of ABX), 2.28 (dd, J = 16.5, 6 Hz, 1H, part of ABX), 1.76 (m, 1H), 1.70−1.53 (m, 6H), 1.48−1.38 (m, 1H), 1.37−1.27 (broad m, 1H), 1.24−1.04 (m, 5H), 0.94−0.72 (m, 2H), 0.87 (d, J = 6.5 Hz, 3H), 0.79 (d, J = 6.5 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 173.9, 172.4, 139.2, 114.7, 76.5, 68.7, 46.7, 42.4, 40.8, 39.4, 38.6, 35.5, 33.5, 33.3, 32.0, 26.2, 25.8, 25.6, 24.1 (2 C), 21.4; MS (ES^-) m/z 416 ($M -$ H). Anal. Calcd for $C_{21}H_{36}ClNO_5$: C, 60.35; H, 8.68; N, 3.35. Found: C, 60.46; H, 8.71; N, 3.37.

Coupling of Acid 31 with Amine 2. Preparation of Compound 6. Acid **31** (241.8 g, 578 mmol) was suspended in dry THF (1.5 L) and the slurry cooled in an ice−water bath. *N*-Methylmorpholine (127.2 mL, 1.160 mol) was added slowly and the mixture stirred in ice for 30 min, until all solids dissolved. Pivaloyl chloride (70.45 g, 584 mmol) in THF (100 mL + 25 mL rinse) was added dropwise (40 min), keeping the temperature of the reaction mixture below 7 °C. After the mixture was stirred for an additional 2 h at 0−5 °C, a mixture of amine **2**[10] (175.8 g, 607 mmol) and *N*-methylmorpholine (76.3 mL, 691 mmol) in THF (100 mL + 25 mL rinse) was added dropwise (30 min). After the resulting mixture was stirred at 5 °C for 0.5 h, the ice bath was removed and the mixture stirred overnight at room temperature. Water (2 L) was then added and THF removed under reduced pressure. The residue was partitioned with EtOAc (3 L) and 5 N NaOH (250 mL). The organic layer was separated and the aqueous phase extracted with additional EtOAc. The combined organic phases were washed with 0.5 N NaOH and brine and dried over $MgSO_4$/activated charcoal. After filtration, EtOAc was removed under reduced pressure, and the thick yellow residue was redissolved in hot (60 °C) *tert*-butylmethyl ether (TBME, 2.4 L). The solution was allowed to cool slowly to room temperature, under rapid stirring, causing compound **6** to crystallize. Hexane (1.2 L) was added and the slurry stirred overnight at room temperature. The white solid was collected by filtration, washed with 2:1 TBME/hexane and hexane, and dried in air to constant weight (347.6 g, 87% yield). The material was 98.78% homogeneous (t_R 15.12 min) as determined by reversed-phase HPLC (Supelcosil LC-ABZ, 4.6 × 150 mm, 5 μm particle size, 0−100% 0.1% TFA−CH_3CN in 0.1% TFA in 20 min then 100% 0.1% TFA in CH_3CN for 5 min, 1 mL/min flow rate, UV detection at 215 nm), and contained none of the P_2 epimer as determined by co-injection with analytical standards (see the Supporting Information for preparation of epimeric P_2 standards): mp 139.5−140.5 °C; R_f 0.52 (5% MeOH/EtOAc); $[\alpha]^{25}_D$ − 30.8° (c 1.01, MeOH); IR (film on NaCl) ν 3364, 2924, 1651, 1636 cm^{-1}; ^{1}H NMR ($CDCl_3$, mixture of rotamers) δ 8.60 (dq, J = 5, 1 Hz, 0.1H), 8.56 (dq, J = 5, 1 Hz, 0.4H), 8.52 (dq, J = 5, 1 Hz, 0.5H), 7.68−7.58 (m, 1H), 7.25−7.11 (m, 2H), 6.78 (d, J = 8 Hz, 0.8H), 6.65 (d, J = 8.5 Hz, 0.1H), 6.60 (d, J = 8 Hz, 0.1H), 5.29−5.20 (m, 2H), 4.41−4.22 (m, 2H), 4.11 (broad m, 0.7H), 4.01 (dd, J = 17.5, 11 Hz, 0.1H, part of ABX), 3.92 (dd, J = 17.5, 14 Hz, 0.1H, part of ABX), 3.83−3.59 (m, 3H), 3.29−2.72 (m, 10H), 2.93 (s, 3H), 2.67−2.18 (m, 3H), 1.88 (m, 1H), 1.80−1.46 (m, 12H), 1.45−1.03 (m, 10H), 0.99−0.70 (m, 4H), 0.94, 0.93 (two d, J = 6.5 Hz, 3H total), 0.88, 0.87 (two d, J = 6.5 Hz, 3H total); ^{13}C NMR ($CDCl_3$, mixture of rotamers) δ 175.0, 174.7 (2 C), 172.0, 171.9, 171.8, 171.7, 167.7, 167.6, 167.5, 158.5, 158.2, 157.9, 157.4, 149.7, 149.5, 148.4 (broad), 139.8, 139.7, 137.3 (broad), 136.9, 136.8, 124.0, 123.9, 123.8, 122.2, 122.0, 121.9, 121.8, 115.2, 114.9, 114.7, 77.8, 77.5, 77.4, 77.2, 69.7, 69.6, 55.4, 55.3, 53.9, 53.8, 50.0, 49.7, 49.4, 49.0, 48.8, 49.5, 48.4, 47.9, 47.8, 47.5, 47.4, 42.7, 42.6, 41.4, 41.3, 41.2, 40.8, 40.6, 38.9, 38.8, 38.7, 37.2, 37.0, 36.4, 36.3, 36.3, 36.2, 35.5, 35.4, 35.2, 35.0, 34.8, 34.6, 33.9, 33.8, 33.7 (2 C), 33.6, 33.5, 33.2, 32.7, 32.6, 31.0, 30.9, 30.8, 30.7, 26.9, 26.5, 26.3, 26.2 (2 C), 26.1, 25.8, 25.7, 24.6, 24.0, 21.8, 21.7; MS (ES^-) m/z 687 ($M -$ H). Anal. Calcd for $C_{38}H_{61}ClN_4O_5$: C, 66.21; H, 8.92; N, 8.13. Found: C, 66.17; H, 9.20; N, 8.10.

Conversion of Vinyl Chloride 6 to Crude BILA 2157 BS (1). Vinyl chloride **6** (340.4 g, 0.494 mol) was dissolved in CH_2Cl_2 (2.00 L), and 2-methoxypropene (189 mL, 1.976 mol, 4 equiv) was added, followed by a catalytic amount of *para*-toluenesulfonic acid monohydrate (2.35 g, 12.35 mmol). The mixture was stirred for 64 h at room temperature, after which time TLC showed complete conversion to acetonide **32** (R_f from 0.73 to 0.83 in 7.5% MeOH/$CHCl_3$). Volatiles were removed under reduced pressure and the residue coevaporated with TBME (2 × 1 L) to yield **32** as a clear yellow oil.

The crude product from above was dissolved in TBME (1.7 L) and the solution cooled in an ice−water bath. Ice-cold water (1.7 L) was added, followed by *N*-bromosuccinimide (92.33 g, 518.7 mmol, 1.05 equiv) in one portion. After the mixture was stirred for 2.5 h at 0 °C, TLC indicated complete disappearance of starting material (R_f from 0.83 to 0.73 in 7.5% MeOH/$CHCl_3$). Thiourea (45.12 g, 593 mmol, 1.2 equiv) was added at 0 °C in one portion and the mixture stirred for 2 h in the ice−water bath. The reaction mixture was then diluted with cold (0 °C) 0.2 M NaCl (5 L) and additional TBME (1 L). After stirring, the organic layer was separated and the aqueous phase washed again with TBME (4 × 2L). The aqueous phase containing salts of compound **34** was then warmed to 65−70 °C and stirred for 2 h under a stream of nitrogen to affect deprotection of the acetonide and removal of all traces of organic solvents. After being cooled to room temperature, the aqueous solution of crude BILA 2157 BS hydrochloride was filtered through a 0.45 μm membrane. The filtrate (∼7 L) was then added dropwise (20 mL/min) to vigorously stirred 0.5 M NaOH (3 L, 1.5 mol, 3 equiv), causing precipitation of the free base of BILA 2157 BS (final pH of aqueous phase was 5.8−6.0). The precipitated product was collected by filtration, washed with water, and dried in a stream of air to give **1** (302.8 g, 84% yield) as a light tan-colored solid. Analysis by reversed-phase HPLC (Symmetry C_8 column, 0.2 × 15 cm, 0.4 mL/min flow rate, UV detection at 260 nM, 30−55% CH_3CN in 50 mM NaH_2PO_4 (pH 4.4) in 25 min gradient, then 55−80% in 10 min then linear): t_R 23.11 min (94.6% homogeneity).

Purification of BILA 2157 BS (1). Crude **1** from above (299.6 g, 412 mmol) was suspended in water (2 L), and methanesulfonic acid (73.27 g, 762 mmol, 1.85 equiv) in water (1 L) was added. NaCl (35.10 g) was added and the solution washed with CH_2Cl_2 (3 × 1.5 L). The aqueous layer was then stirred for 3 h under a stream of nitrogen to remove any last traces of solvent and the solution filtered through a 0.45 μm membrane. The solution was then added dropwise (3.5 h, 15 mL/min) to vigorously stirred 1 N NaOH (770 mL) diluted with water to a volume of 1.5 L (0.513 N final concentration). After the suspension was stirred overnight at room temperature, the precipitated product was collected by filtration, washed with water, and dried in air to constant weight (273.6 g, 91% recovery, 96.2% homogeneity by HPLC; see below for conditions).

The procedure was repeated starting with **1** from above (261 g, 0.359 mol) using 1.95 equiv of methanesulfonic acid (67.27 g, 0.700 mol) for salt formation. Neutralization was carried out using NaOH (30.54 g, 0.763 mol) in water (1.5 L). Following filtration of the precipitated free base, the product was collected and washed twice by stirring (30 min) with water (4 L). Finally, BILA 2157 BS was dried in air to give an off-white solid: mp 85−93 °C (lit.[10] mp 92−97 °C); R_f 0.25 (5% MeOH/$CHCl_3$); $[\alpha]^{25}_D$ −26.8° (*c* 1.08, MeOH); $[\alpha]^{25}_{365}$ −80.6° (*c* 1.08, MeOH) (lit.[10] $[\alpha]^{25}_D$ −26.0° (*c* 1.02, MeOH)); IR (NaCl film) ν 3700−2800, 1640 cm^{-1}; ^1H NMR (DMSO-d_6 and $CDCl_3$, mixture of rotamers) identical to that previously reported;[6,10] ^{13}C NMR ($CDCl_3$, mixture of rotamers) δ 176.1, 176.0, 175.9, 172.2, 169.0, 168.95, 168.9, 168.8, 168.0, 167.8, 167.7, 167.6, 158.9, 158.7, 158.0, 157.6, 149.75, 149.5, 149.3, 149.2, 149.15, 136.9, 136.7, 136.65, 136.6, 123.8, 123.7, 123.6, 123.5, 122.2, 121.9, 121.7, 121.5, 103.8, 103.7, 103.6, 78.3, 78.2, 78.1, 77.2, 69.8, 55.4, 55.2, 53.8, 53.7, 50.0, 49.7, 49.0, 48.9, 48.6, 48.0, 47.7, 47.4, 47.2, 42.7, 42.65, 42.55, 42.50, 42.45, 39.15, 39.1, 39.0, 37.2, 37.0, 36.5, 36.45, 36.25, 36.2, 36.0, 35.8, 35.0, 34.9, 34.8, 34.3, 34.2, 33.9, 33.8, 33.7, 33.65, 33.55, 33.2, 32.6, 30.9, 30.85, 30.8, 30.7, 26.5, 26.4, 26.35, 26.2, 26.15, 26.1, 25.85, 25.8, 25.75, 24.7, 24.0, 21.9; MS (ES^+) *m*/*z* 727 (MH^+). Anal. Calcd for $C_{39}H_{62}N_6O_5S$ (corrected for 2.85% w/w water content as determined by Karl Fisher analysis): C, 62.58; H, 8.67; N, 11.23. Found: C, 62.24; H, 8.73; N, 11.01. HPLC homogeneity (Symmetry C_8, 0.2 × 15 cm, 30−55% CH_3CN in 50 mM NaH_2PO_4 at pH 4.4 in 25 min then 55−80% CH_3CN in 50 mM NaH_2PO_4 at pH 4.4 in 10 min, 0.4 mL/min flow rate, UV detection at 260 nm): t_R 22.8 min (97.74%). P_2 epimer of BILA 2157 BS: t_R 24.05 min (<0.01%).

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Supporting Information Available: HPLC chromatograms for compounds (*R*)- and (*S*)-**15**. Experimental procedures for the preparation and characterization of isomeric analytical standards: (*R*)-**19**, P_2 epimers of compounds **1**, **6**, **27**, **30**, and **31** and diol isomers of compounds **6**, **30**, and **31**. This material is available free of charge via the Internet at http://pubs.acs.org.

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