JOC Article

Stereoselective Synthesis of a Potent Thrombin Inhibitor by a Novel P2−P3 Lactone Ring Opening†

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The concise synthesis of a potent thrombin inhibitor was accomplished by a mild lactone aminolysis between an orthogonally protected bis-benzylic amine and a diastereomerically pure lactone. The lactone was synthesized by the condensation of L-proline methyl ester with an enantiomerically pure hydroxy acid, which in turn was synthesized by a highly stereoselective (>500:1 er) and productive (100000:1, S/C) enzymatic reduction of an α-ketoester. In addition, a second route to the enantiomerically pure lactone was accomplished by a diastereoselective ketoamide reduction.

Introduction

Thrombin is a trypsin-like protease enzyme that plays a critical role in intrinsic and extrinsic blood coagulation. As a result of the enzymatic activation of numerous coagulation factors, thrombin is activated to cleave fibrinogen, producing fibrin, which is directly responsible for blood clotting. An imbalance between these factors and their endogenous activators and inhibitors can give rise to a number of disease states such as myocardial infarction, unstable angina, stroke, ischemia, restenosis following angioplasty, pulmonary embolism, deep vein thrombosis, and arterial thrombosis. Thrombin is involved in many of these processes and is the most potent stimulator of platelet aggregation known. Thus, molecules that could selectively inhibit the formation of thrombin or that could modulate the activity of thrombin would have the potential to regulate many of the above-mentioned disease states.[1] Members of the class of thromboembolitic agents (e.g., warfarin, hirudin, coumadin, and bivalirudin[2]) are plagued by numerous limitations. Consequently, the aggressive search for a potent, selective, and bioavailable thrombin inhibitor is widespread.[3] An intensive effort by Merck has led to the identification of thrombin inhibitor **1**. This molecule contains the common P1, P2, P3 structural motif found in many potential thrombin inhibitors (Scheme 1).[4]

The existing route to **1** from the corresponding synthons (P1, P2, and P3) resulted in a linear synthesis of the target molecule and incorporated standard peptide couplings and multiple protection/deprotection manipulations.[5] Accordingly, a more concise assembly of **1** was desired, which was envisioned as arising via an aminolysis of lactone P2−P3 with an appropriate P1 benzylic amine. Herein, we describe the total synthesis of the target thrombin inhibitor **1** utilizing this convergent strategy.

Results and Discussion

P1 Synthesis. The cornerstone of our strategy was predicated on an efficient synthesis of the P2−P3 lactone and its subsequent aminolysis with an appropriate P1 amine. This event would unmask the correct skeletal framework of the target molecule. From the outset we realized that one of the synthetic challenges would be the differentiation between two benzylic amine positions in P1. The existing synthetic approach to this orthogonally protected, bis-benzylic amine counterpart utilized a cyano ester, but the route was operationally cumbersome.[5] Thus, we initially chose to improve upon this approach.

† Dedicated to Professor Gary H. Posner on the occasion of his 60th birthday.

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⊥ Department of Molecular Systems.

(1) (a) Vacca, J. P. *Curr. Opin. Chem. Bio.* **2000**, *4*, 394. (b) Raghavan, S. A. V.; Dikshit, M. *Drugs Future* **2002**, *27*, 669.

(2) Gladwell, T. D. *Clinical Therap.* **2002**, *24*, 38.

(3) Fevig, J. M.; Wexler, R. R. *Ann. Rep. Med. Chem.* **1999**, *34*, 81.

(4) This nomenclature refers to the sequence D-Phe-Pro-Arg, which mimics fibrinogen (thrombin's natural substrate).

(5) Morrissette, M. M.; Stauffer, K. J.; Williams, P. D.; Lyle, T. A.; Vacca, J. P.; Krueger, J. A.; Lewis, S. D.; Lucas, B. J.; Wong, B. K.; White, R. B.; Miller-Stein, C.; Lyle, E. A.; Wallace, A. A.; Leonard, Y. M.; Welsh, D. C.; Lynch, J. J.; McMasters, D. R. *Bioorg. Med. Chem. Lett.* Manuscript submitted.

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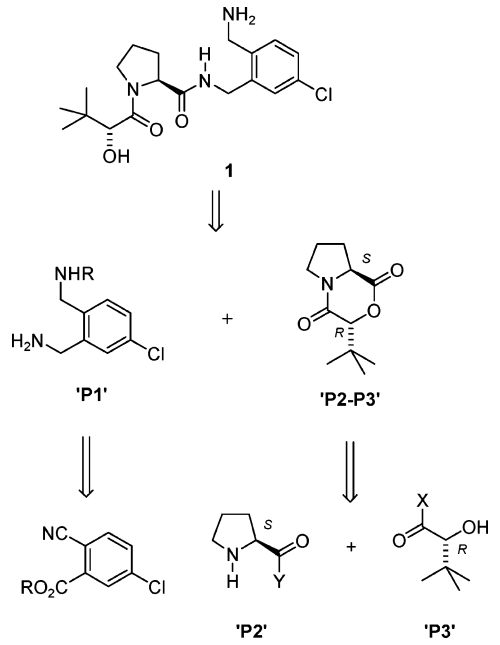
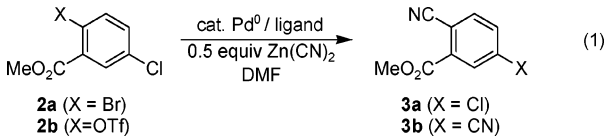
SCHEME 1



The existing methodology to prepare cyano ester **3a** hinged on the palladium-catalyzed cyanation[6] of methyl 2-bromo-5-chlorobenzoate (**2a**) (eq 1). However, in our hands, this was a capricious and tedious reaction that resulted in significant amounts of over cyanated arene **3b**. Although slight modifications offered initial improvements and resulted in high yields of the corresponding nitrile **3a** (>92%), the final product was still contaminated with 3−5% of bis-nitrile **3b** regardless of the source of palladium used.[7] In retrospect, this was not surprising since the desired product **3a** is a highly activated aryl chloride itself, which is readily cyanated at the temperatures necessary for cyanation of the starting bromide (80 °C).[8]

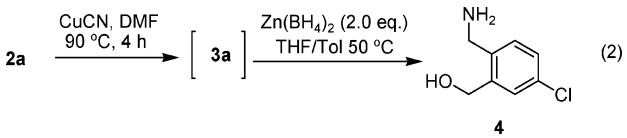


(1)

In an attempt to circumvent this problem, we envisioned that palladium-catalyzed cyanation of the corresponding aryl triflate **2b** would occur at a much lower temperature and allow for selective monocyanation.[9] Indeed, the cyanation of **2b** proceeded well at 50 °C; however, we were again plagued by over cyanation (1−2%).

Realizing that palladium-based cyanations were problematic, we turned our attention to the stoichiometric copper(I) cyanation (Rosenmund−von Braun reaction)[10] of **2a**. When **2a** was heated with 1.1 equiv of CuCN in DMF at 90 °C for 4 h, a clean transformation (>90% assay yield) to **3a** occurred with minimal over cyantion to **3b** (<0.4% by HPLC) on >200 g scale.[11]

Initial attempts to globally reduce a toluene stream of cyano ester **3a** to the corresponding amino alcohol **4** involved using excess lithium aluminum hydride in THF at various reaction temperatures. In every case, these reductions produced dark reaction mixtures with a complex array of products including dechlorination of the aromatic ring. Other reducing agents (i.e., BH_3−DMS, $NaBH_4$, DIBAL-H) also provided unsatisfactory results. Upon further investigation, we found that reductions that utilized freshly prepared $Zn(BH_4)_2$[12] provided smooth reduction of **3a** to **4** with minimal byproduct formation (eq 2).



(2)

In this case, it was clear by ^{1}H NMR and LC/MS data of reaction mixtures that the ester was rapidly reduced followed by slow reduction of the nitrile.[13] Thus, addition of a toluene stream of **3a** to a THF solution of $Zn(BH_4)_2$ (2.0 equiv.) at 50 °C for 12−18 h gave >98% HPLC assay yield of **4**. Typical isolated yields starting were 60−65% (from **2a**).[14]

Amino alcohol **4** was protected with Boc_2O to afford *N*-Boc arene **5** in a 93% yield (Scheme 2). Treatment of

(6) (a) Tschaen, D. M.; Desmond, R.; King, A. O.; Fortin, M. C.; Pipik, B.; King, S.; Verhoeven, T. R. *Synth. Commun.* **1994**, *24*, 887. (b) Maligres, P. E.; Waters, M. S.; Fleitz, F.; Askin, D. *Tetrahedron Lett.* **1999**, *40*, 8193.

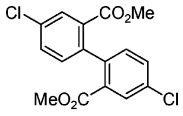
(7) Due to the fact that we were unable to reject the bis-nitrile **3b** impurity during the latter stages of the synthesis, a route that curbed its formation was necessary.

(8) (a) Sundermeier, M.; Zapf, A.; Beller, M.; Sans, J. *Tetrahedron Lett.* **2001**, *42*, 6707. (b) Jin, F.; Confalone, P. N. *Tetrahedron Lett.* **2000**, *41*, 3271. (c) Akita, Y.; Shimazaki, M.; Ohta, A. *Synthesis* **1981**, 974. (d) Anderson, Y.; Langstrom, B. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1395.

(9) For related procedures involving the Pd-catalyzed cyanation of aryl triflates, see: (a) Drechsler, U.; Hanack, M. *Synlett* **1998**, 1207. (b) Anderson, B. A.; Bell, E. C.; Ginah, F. O.; Harn, N. K.; Pagh, L. M.; Wepsiec, J. P. *J. Org. Chem.* **1998**, *63*, 8224.

(10) (a) Rosenmund, K. W.; Struck, E. *Chem. Ber.* **1919**, *52*, 1749. (b) Von Braun, J.; Manz, G. *Liebigs Ann. Chem.* **1931**, *448*, 111. For reviews see: (c) Ellis, G. P.; Romney-Alexander, T. M. *Chem. Rev.* **1987**, *87*, 779. (d) Lindley, J. *Tetrahedron* **1984**, *40*, 1433. (e) Ito, T.; Watanabe, K. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 419.

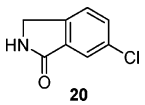
(11) Two other impurities were identified as byproducts arising from this process. One (0.4 LCAP) was identified as methyl 5-bromo-2-cyanobenzoate. We attribute this impurity as a result of halogen exchange of the desired product **4** with the CuBr formed during the reaction. The other (1.2% LCAP) is the corresponding homocoupled product **19**.



19

(12) For leading references on the synthesis and use of $Zn(BH_4)_2$, see: (a) Narasimhan, S.; Balakumar, R. *Aldrichim. Acta* **1998**, *31*, 19. (b) Narasimhan, S.; Madhavan, S.; Ganeshwar Prasad, K. *J. Org. Chem.* **1995**, *60*, 5314.

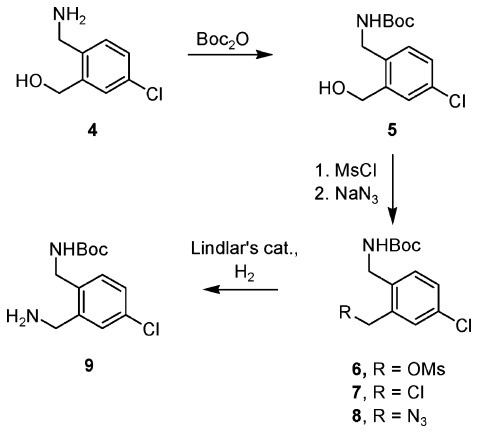
(13) A significant byproduct in other reduction systems (especially BH_3-DMS) was the corresponding lactam **20**, which we believe forms from rapid nitrile reduction followed by ring closure on the unreduced ester. Identifying a reducing agent that preferentially reduced the ester functionality prior to the nitrile was vital in limiting formation of **20**.



20

(14) Crystallizing the amino alcohol at this point allowed us to remove minor impurities that we were unable to reject downstream.

SCHEME 2



an isopropyl acetate (IPAc) solution of **5** with TEA and MsCl provided a 97:3 ratio of **6**/**7** after warming to room temperature. The reaction was diluted with DMF, treated with NaN_3, and aged at ambient temperature for 24−48 h (as determined by the conversion of **6** and **7**). Reaction mixtures that contained elevated levels of **7** routinely required longer reaction times (or higher DMF ratios) for complete conversion. Washing the IPAc with water afforded a clean stream of azide **8**. The above one-vessel protocol for transformation of alcohol **5** to azide **8** was demonstrated on >200 g scale, affording a 93% overall assay yield from **5** (**8** in IPAc).

Azide **8** was next reduced to amine **9** with Lindlar's catalyst/H_2.[15] Initial hydrogenolysis screens were performed in alcoholic solvents. Reductive dechlorination was a significant competing side reaction under these conditions. Reaction variables of solvent, temperature, pressure, azide concentration, and catalyst loading were screened for efficiency of transformation and suppression of the des-Cl impurity. Optimal conditions were achieved utilizing Lindlar's catalyst (2−5 mol %) in IPAc, at 30−40 °C, and 20−80 psi H_2. The addition of TEA improved the rate of reaction [1 equiv (no benefit observed at > 1 equiv)] as did the periodic purging of the reaction atmosphere with fresh hydrogen (N_2 removal). After catalyst filtration, the direct isolation of **9** was accomplished by solvent removal and crystallization from tri-*n*-propylamine.

P2−P3 Synthesis. We have discovered two methods for the stereoselective assembly of diastereomerically pure lactone (*S*,*R*)-**16**. The first approach to lactone (*S*,*R*)-**16** centered on the synthesis of (*R*)-3,3-dimethyl-2-hydroxybutyric acid [(*R*)-**14**]. Although this is a relatively simple chiral hydroxy acid, and these types of molecules have been commonly used as synthetic building blocks,[16] only sparse reports on the synthesis of either antipode of this molecule existed.[17−21] To develop an efficient approach to this compound, catalytic, asymmetric reductions of the corresponding α-ketoester **12** were investigated.[22] Ethyl 3,3-dimethyl-2-oxobutanoate (**12**) was synthesized either by the addition of *t*-BuMgCl to diethyl oxalate (**10**)[23,24] or by the alkylation of 3,3-dimethyl-2-oxobutanoic acid (**11**) with EtBr/DBU.[25,26] Both methods rapidly afforded ketoester **12**[27,28] in high yields (Scheme 3).

The asymmetric reduction of **12** was accomplished with the isolated enzyme KRED1001 (Scheme 4). KRED1001

(15) Corey, E. J.; Nicolaou, K. C.; Balanson, R. D.; Machida, Y. *Synthesis* **1975**, 590.

(16) Coppola, G. M.; Schuster, H. F. α-*Hydroxy Acids in Enantioselective Syntheses*; VCH: Weinheim 1997.

(17) The classical resolution with cinchonidine has been reported, but is low yielding and requires multiple crystallizations: Tanabe, T.; Yajima, S.; Imaida, M. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 2178.

(18) The conversion of 3,3-dimethyl-2-oxobutanoic acid (**11**) to (*R*)-2-hydroxy-3,3-dimethylbutanoic acid has been reported to proceed with a high level of enantioselectivity using the cell line *Proteus vulgaris*, H_2 gas, and benzyl viologen: (a) Schummer, A.; Yu, H.; Simon, H. *Tetrahedron* **1991**, *47*, 9019. (b) Simon, H.; Bader, J.; Günther, H.; Neumann, S.; Thanos, J. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 539.

(19) The synthesis of (*S*)-2-hydroxy-3,3-dimethylbutanoic acid has been accomplished by diazotization of L-*tert*-leucine: (a) Quast, H.; Leybach, H. *Chem. Ber.* **1991**, *124*, 849. (b) Van Draanen, N. A., Arseniyadis, S.; Crimmins, M. T.; Heathcock, C. H. *J. Org. Chem.* **1991**, *56*, 2499. (c) Hartwig, W.; Schoellkopf, U. *Liebigs Ann. Chem.* **1982**, 1952. This material can also be obtained from the racemate by the classical resolution with either brucine (ref 10) or (*S*)-phenylethylamine: (d) Zhang, W.-Y.; Jakiela, D. J.; Maul, A.; Knors, C.; Lauher, J. W.; Helquist, P.; Enders, D. *J. Am. Chem. Soc.* **1988**, *110*, 4652. (e) Masamune, S.; Reed, L. A., III; Davis, J. T.; Choy, W. *J. Org. Chem.* **1983**, *48*, 4441. For other syntheses of racemic 2-hydroxy-3,3-dimethylbutanoic acid, see: (f) Enholm, E. J. Lavieri, S.; Cordóva, T.; Ghiviriga, I. *Tetrahedron Lett.* **2003**, *44*, 531.

(20) Similarly, the asymmetric reduction of methyl 3,3-dimethyl-2-oxobutanoate using stoichiometric amounts of borohydrides has been used to form the corresponding (*S*)-carbinol: (a) Brown, H. C.; Cho, B. T.; Park, W. S. *J. Org. Chem.* **1986**, *51*, 1. 3396. (b) Brown, H..; Pai, G. G. *J. Org. Chem.* **1985**, *50*, 1384. This material has also been prepared by a multistep synthesis: Ko, K.-Y.; Frazee, W. J.; Eliel, E. L. *Tetrahedron* **1984**, *40*, 1333.

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(23) Rabjohn, N.; Harbert, C. A. *J. Org. Chem.* **1970**, *35*, 3726.

(24) Jaeger, D. A.; Broadhurst, M. D.; Cram, D. J. *J. Am. Chem. Soc* **1979**, *101*, 717.

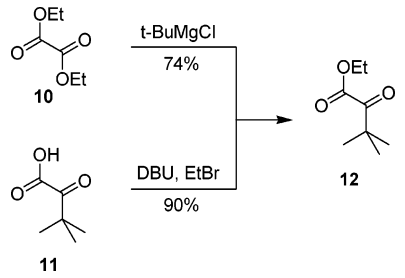
(25) Rambaud, M.; Bakasse, M.; Duguay, G.; Villieras, J. *Synthesis* **1988**, 566.

(26) Ono, N.; Yamada, T.; Saito, T.; Tanada, K.; Kaji, A. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2401

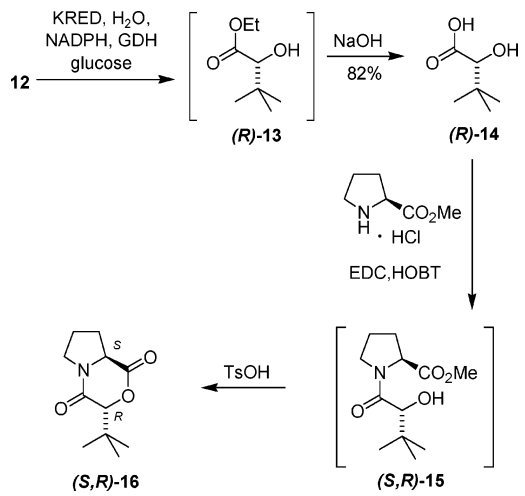
(27) For some alternative syntheses of keto ester **12**, see: (a) Reetz, M. T.; Heimbach, Schwellnus, K. *Tetrahedron Lett.* **1984**, *25*, 511. (b) Seyferth, D.; Weinstein, R. M.; Hui, R. C.; Wang, W.-L.; Archer, C. M. *J. Org. Chem.* **1991**, *56*, 5768. (c) de las Heras, M. A.; Vaquero, J. J.; García-Navio, J. L.; Alvarez-Builla *J. Org. Chem.* **1996**, *61*, 9009. (d) Wright, J. B. *J. Org. Chem.* **1955**, *77*, 4883.

(28) For review articles on the synthesis of α-keto acids, see: (a) Kovács, L. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 471. (b) Cooper, A. J. L.; Ginos, J. Z.; Meister, A. *Chem. Rev.* **1983**, *83*, 321.

SCHEME 3



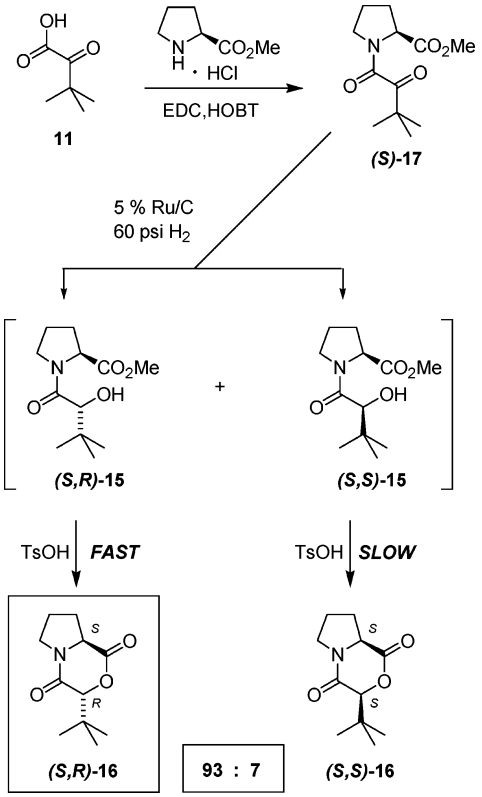
SCHEME 4



is a commercially available ketoreductase that has been used for the asymmetric reduction of β-ketoesters but, to the best of our knowledge, has not been applied to the asymmetric reduction of α-ketoesters. The reduction is cofactor-dependent. Typical operating parameters utilized ketoester **12** (45 mg/mL), NADPH (0.1−0.5 mg/mL), glucose dehydrogenase (0.5−2 mg/mL), KRED1001 (0.1−2 mg/mL), and glucose (70 mg/mL) in an appropriate buffer solution (e.g., phosphate, MOPS, etc.) at 25−40 °C with the pH being maintained between 6 and 8. The source of hydride for this ketone reduction was glucose. As monitored by HPLC, the conversion is quantitative and occurs with an extremely high degree of stereoselectivity (>500: 1; *R*/*S*). The hydroxy ester (*R*)-**13** could be isolated as an oil and then saponified to the corresponding enantiomerically pure hydroxy acid (*R*)-**14** without epimerization. But in a more direct process, a simple pH adjustment (pH >13) of the crude enzymatic reaction mixture (post-reduction) immediately saponified the ethyl ester (*R*)-**13**. After pH adjustment (ca. pH = 2), the desired product (*R*)-**14** was extracted into ethyl acetate and the KRED, GDH, NADPH, and glucose remained in the aqueous phase. Removal of the EtOAc and crystallization from heptane afforded the enantiomerically pure (*R*)-3,3-dimethyl-2-hydroxybutyric acid (*R*)-**14** in an 82% isolated yield (>99.5% ee) and was successfully demonstrated on a 200 g scale.

Amide formation between the enantiomerically pure hydroxyacid and L-proline methyl ester (EDC, HOBT, CH_3CN) afforded a mixture of the hydroxy ester (*S*,*R*)-**15** and the lactone (*S*,*R*)-**16** after aqueous workup. Treatment of the mixture with catalytic TsOH in toluene with the concomitant removal of MeOH from the system afforded exclusively the lactone (*S*,*R*)-**16**, which was

SCHEME 5



crystallized from heptane. This procedure was demonstrated on > 200 g scale to provide lactone (*S*,*R*)-**16** as a white crystalline solid (73% isolated yield, >99 LCWP, >99.5% de).

Lactone (*S*,*R*)-**16** could also be accessed by a complementary method in which the carbinol stereochemistry was installed by the substrate-controlled asymmetric reduction of ketoamide (*S*)-**17** (Scheme 5). Similar asymmetric reductions of α-keto proline amides[29] and α,β-unsaturated proline amides[30] have been reported to occur with low levels of stereochemical induction.

Ketoamide (*S*)-**17** was prepared in a single step (84%) by coupling ketoacid **11** with L-proline methyl ester (EDC, HOBT). An initial screen of catalytic hydrogenation reducing agents indicated that Ru/C was superior to Pt/C, Pd/C, or Rh/C for the substrate-controlled reduction of the carbonyl group. The latter hydrogenation catalysts resulted in decreased rate of carbonyl reduction.[31] The resulting mixture of diastereomeric hydroxy esters was lactonized with catalytic TsOH to afford a 93:7 ratio of lactones [(*S*,*R*)-**16**/(*S*,*S*)-**16**]. Moreover, it was observed that the cyclization of hydroxy ester (*S*,*R*)-**15** to the

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(30) Mallat, T.; Baiker, A. *Appl. Catal. A: General* **2000**, *200*, 3. Prolinol-based keto amides (hemiacetals) have been reduced with good stereoselectivity: Pansare, S. V.; Ravi, R. G. *Tetrahedron* **1998**, *54*, 14549.

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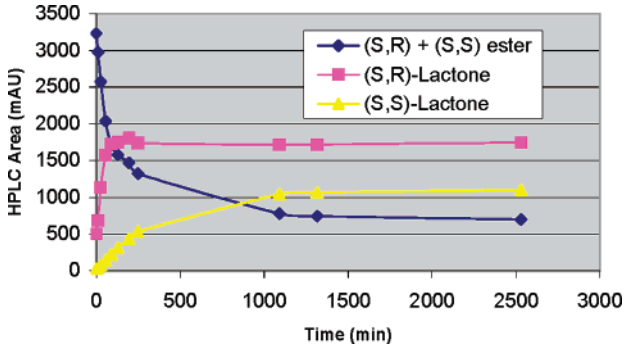


FIGURE 1. Ester (*S*,*R*)-**15** and (*S*,*S*)-**15**[34] and lactone (*S*,*R*)-**16** and (*S*,*S*)-**16** component profiles [HPLC area (mAU) vs time].

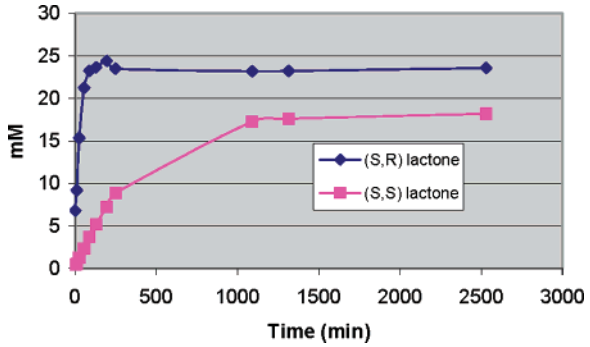


FIGURE 2. Formation of lactones (*S*,*R*)-**16** and (*S*,*S*)-**16** [concentration (mM) vs time].

desired lactone (*S*,*R*)-**16** occurred more rapidly than the cyclization of the diastereomeric hydroxy ester (*S*,*S*)-**15** to the lactone (*S*,*S*)-**16**.

To further probe this phenomenon, an equimolar mixture of diastereomeric esters (*S*,*R*)-**15** and (*S*,*S*)-**15** was prepared by the EDC/HOBT coupling of *rac*-hydroxy acid **14** and L-proline methyl ester HCl. These esters were lactonized in toluene at 24 °C, with 10% TsOH. The steady-state formation of lactone (*S*,*R*)-**16** occurred in less than 2 h.[32] The molar rate of (*S*,*R*)-**16** formation was more rapid than the diastereomeric lactonization of hydroxy ester (*S*,*R*)-**15** to lactone (*S*,*S*)-**16** [$k_{rel} = 17$]. Component profiles are provided in Figures 1 and 2. A similar bicyclic lactam has been prepared by cyclization of an amino group to a proline methyl ester tether. [33]

A conformational search using molecular mechanics (MMFFs, 4r distance-dependent dielectric) revealed two conformers for each of the diastereomeric lactones; the two conformers differed only in the five-membered ring pucker. The lower-energy conformer of each stereoisomer was optimized at the 6-31G** level of theory, and the resulting structures are shown in Figure 3. The energy of (*S*,*R*)-**16** was found to be higher than that of (*S*,*S*)-**16**, $\Delta E = +0.85$ kcal/mol, which is probably due to the steric repulsion caused by the axial *t*-Bu group.

The more rapid lactonization of the *S*,*R* isomer is therefore likely to be due not to the relative stabilities of the products, which in fact favors the *S*,*S* isomer, but to a lower energy transition state or intermediate along the lactonization pathway.[35]

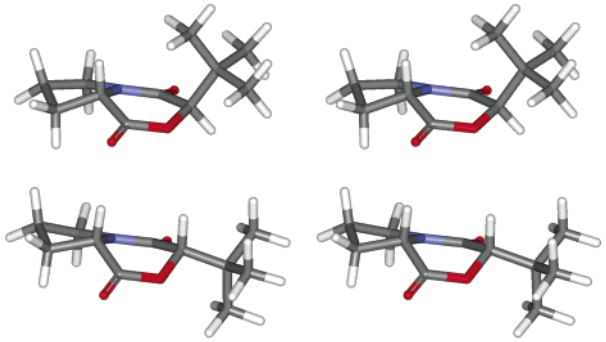
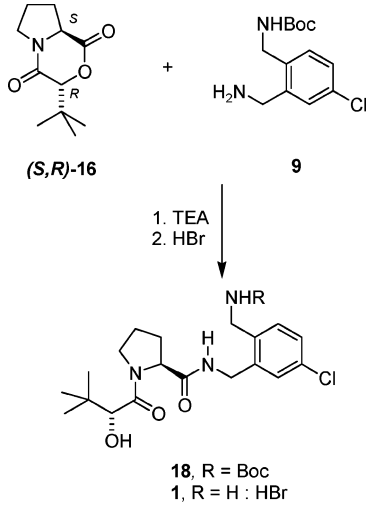


FIGURE 3. Stereoscopic views of the structures of (*S*,*R*)-**16** (top) and (*S*,*S*)-**16** (bottom) optimized at the 6-31G** level.

SCHEME 6



Lactone Amination and Completion of the Synthesis. The coupling of the two components [**9** and (*S*,*R*)-**16**] was accomplished in either triethylamine or 2-propanol and occurred without the need for a catalyst or base additive (Scheme 6). While many lactone aminolysis reactions require more rigorous conditions or are facilitated by the addition of catalysts,[36] the facile nature of this amidation can be attributed to the inherent strain in lactone (*S*,*R*)-**16**. Indeed, initial results indicate that the rate of aminolysis with amine **9** is faster with lactone (*S*,*R*)-**16** than with the more stable diastereomeric lactone (*S*,*S*)-**16** [$k_{rel} = 2$].

Other polar solvents screened (NMP, DMF, DIPEA, CH_3CN, THF) resulted in slightly slower coupling rates or incompatibility with the lactone (MeOH, EtOH). Alternatively, the lactone opening in THF could be accelerated by performing the reaction at 40 °C in the presence of HOAc. Regardless of the method for coupling the two fragments, the subsequent workup included a wash with 2 M citric acid to remove amine **9** followed by

(32) In this experiment, MeOH was not removed from the system; therefore, the lactonization was not driven entirely to completion.
(33) Zhang, N.; Nubbemeyer, U. *Synthesis* **2002**, 242.
(34) Ester (*S*,*R*)-**15** and (*S*,*S*)-**15** were not resolvable by HPLC.
(35) Consistent with this hypothesis is the finding that the lactone hydrate, a model for a tetrahedral intermediate, is calculated at the 6-31G** level to be 2.2 kcal/mol lower in energy for the *S*,*R* isomer than for the *S*,*S* isomer.
(36) Liu, W.; Xu, D. D.; Repic, O.; Blacklock, T. J. *Tetrahedron Lett.* **2001**, *42*, 2439.

a wash with 0.2 N NaOH (or saturated Na_2CO_3) to hydrolyze and remove unreacted lactone (*S*,*R*)-**16**. This process occurred without epimerization of either stereocenter. Penultimate (*S*,*R*)-**18** could be isolated as an amorphous solid following solvent removal, but this compound was typically utilized in the deprotection without isolation. Numerous conditions were screened for the unmasking of the benzylamine by *N*-Boc deprotection. Problems ranged from sluggish reactivity to product decomposition (spontaneous lactonization with extrusion of the corresponding diamine). The optimized conditions for *N*-Boc removal incorporated the addition of a 6 wt % HBr (3 eq) solution to an anhydrous isopropyl acetate (IPAc) solution of the substrate. This afforded the target molecule HBr salt **1** as a white amorphous solid in an overall 80% isolated yield from lactone (*S*,*R*)-**16**.

Conclusions

In summary, the ring opening of a diastereomerically pure bicyclic lactone with an orthogonally protected bisbenzylic amine set the desired framework for a potent thrombin inhibitor. The synthesis is highlighted by two efficient syntheses of the diasteromerically pure lactone (*S*,*R*)-**16**. The first synthesis relied on a highly stereoselective enzymatic reduction of an α-ketoester (>500:1 er) while a complementary approach utilized a substrate-controlled diastereomeric reduction of a proline ketoamide.

Experimental Section

Amino Alcohol 4.[37] To a mechanically stirred heterogeneous mixture of CuCN (220 g, 2.4 mol) in DMF (2.5 L) under an atmosphere of nitrogen was added methyl-2-bromo-5-chlorobenzoate[7] (550 g, 2.2 mol). The reaction was heated to 90 °C for 4 h and then cooled to 10 °C to give a bright yellow heterogeneous mixture. Toluene (2.5 L) was added followed by the slow addition of a 9:1 aqueous solution (2.5 L) of 10% (w/v) NH_4Cl/30% NH_4OH to maintain the internal temperature <25 °C. The flask was then exposed to air and the resulting biphasic mixture stirred at ambient temperature for 12 h. The deep blue aqueous layer was removed and an additional 2.5 L of the 9:1 aqueous ammonia solution added and stirred for 2 h. This process was repeated until the aqueous layer was no longer blue (typically three washes were needed). The clear and colorless toluene layer was washed with brine (1.0 L) and azeotropically dried (K_f < 100 ppm). HPLC assay of the final toluene solution determined the yield to be 91% (390 g).

To a stirred slurry of $ZnCl_2$ (260 g, 1.9 mol) in THF (2.0 L) at ambient temperature was added a 2.0 M solution of $LiBH_4$ in THF (2.0 L, 4.0 mol) under an atmosphere of nitrogen. A mild exotherm was noticed during this addition. The resulting mixture was then heated to 50 °C for 90 min followed by the slow addition of the aforementioned toluene (2.0 L) solution of methyl-5-bromo-2-cyanobenzoate (390 g, 2.0 mol) at a rate to maintain the internal temperature <65 °C. Once addition was complete, the reaction was allowed to proceed for 12 h at 60 °C. The resulting heterogeneous mixture was cooled to 10 °C at which point 3 N HCl (3.0 L) was added slowly to maintain the internal temperature < 25 °C. This biphasic mixture was then heated at 40 °C for 30 min and separated at that temperature. Fresh toluene (2.5 L) was added to the acidic aqueous layer and then cooled to 10 °C. Next, addition of 10 N NaOH was added slowly until the bottom aqueous layer was at pH 12. The biphasic mixture was warmed to 40 °C, and the layers were separated at this temperature. The toluene layer was then concentrated in vacuo until a concentration of ca. 125 g/L was achieved and cooled to 5 °C, and heptane was added as antisolvent. Amino alcohol **4** was isolated by vacuum filtration as an off-white crystalline solid (235 g, 70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 2.0 Hz, 1H), 7.25−7.17 (m, 2H), 4.59 (s, 2H), 3.96 (s, 2H) 4.10−2.60 (bs, NH_2 and OH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 143.3, 138.4, 133.7, 131.1, 130.2, 127.8, 64.2, 44.9. Anal. Calcd for $C_8H_{10}ClNO$: C, 55.99; H, 5.87; N, 8.16; Cl, 20.66. Found: C, 55.95; H, 5.83; N, 8.18; Cl, 20.60.

***N*-Boc Alcohol 5.** To a stirred cooled (10 °C) slurry of amino alcohol **4** (230 g, 1.4 mol) in toluene (2.5 L) was slowly added a solution of Boc-anhydride (300 g, 1.4 mol) in toluene (500 mL). After complete addition, the reaction was allowed to warm to ambient temperature and stirred until complete consumption of the starting material was evident by HPLC (ca. 6 h). The reaction was then concentrated to approximately $^1/_2$ of its initial volume, at which point the product began to precipitate. Heptane was added as antisolvent and the product collected and dried by vacuum filtration as a crystalline white solid (342 g, 93%): mp 92.1 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (bs, 1H), 7.33−7.20 (m, 2H), 5.50−4.80 (bs, 1H), 4.70 (s, 2H), 4.34 (s, 2H), 2.80−2.20 (bs, 1H), 1.44 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.1, 140.6, 135.5, 133.5, 130.7, 129.1, 128.3, 80.1, 62.7, 41.5, 28.4 (3C); IR (Nujol mull) 3387, 3316, 2925, 2854, 1682, cm^{-1}. Anal. Calcd for $C_{13}H_{18}ClNO_3$: C, 57.46; H, 6.68; N, 5.15; Cl, 13.05. Found: C, 57.20; H, 6.71; N, 4.96; Cl, 13.19.

***N*-Boc Amine 9.** To a solution of alcohol **5** (322.9 g, 1.19 mol) in isopropyl acetate (1292 mL) and triethylamine (241 g, 2.38 mol) at −15 °C was slowly added mesyl chloride (163.5 g, 1.43 mol) at such a rate as to maintain the internal temperature below 0 °C. The resulting slurry was then warmed to room temperature (HPLC assay of the slurry determined that the reaction achieved a >99% conversion to **6** and **7**). This slurry was diluted with 1400 mL of DMF, and then sodium azide (177.7 g, 2.73 mol) was added. After the mixture was stirred at room temperature for 3 days, H_2O (1000 mL) was added and the resulting two-phase solution separated. The organic layer was washed three additional times with water and then dried. This solution of azide **8** was diluted to 2300 mL with isopropyl acetate followed by the addition of triethylamine (111 g, 1.10 mol) and Lindlar's catalyst (70.0 g) and then hydrogenation (20−30 psig hydrogen, 35 °C, 23 h). The catalyst was filtered through a plug of Solka Floc, and then solvent was removed under vacuum (80−70 mmHg, 19−30 °C) to about 0.5 the original volume. Tri-*n*-propylamine (350 mL) was added and the concentration continued (50−59 mmHg, 38 °C) until distillation stopped. During the final distillation, solids precipitated and the crystallization continued as the IPAc was removed. Filtration afforded 242.1 g (75%) of amine **9** as a white solid: mp 77.7 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31−7.20 (m, 3H), 5.94 (bs, 1NH), 4.31 (bd, J = 5.4 Hz, 2H), 3.89 (s, 2H), 1.58 (s, 2H), 1.44 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 155.8, 142.2, 135.9, 133.4, 130.8, 128.8, 127.5, 79.4, 43.9, 42.0, 28.5 (3C); IR (Nujol mull) 3358, 3291, 3169, 2925, 2854, 1696 cm^{-1}. Anal. Calcd for $C_{13}H_{19}ClN_2O_2$: C, 57.67; H, 7.07; N, 10.35; Cl, 13.09. Found: C, 57.61; H, 7.19; N, 10.27; Cl, 13.06.

Ethyl 3,3-Dimethyl-2-oxobutanoate (12) (Method A). To a mixture of 3,3-dimethyl-2-oxobutanoic acid (**11**) (35 g, 83 wt % by HPLC) and 260 mL of MTBE was slowly added DBU (46.5 g). The addition was slightly exothermic. The reaction was cooled to 37 °C, and bromoethane (57 g) was added and stirred for 24 h at 38 °C. The reaction mixture was washed with 1 N HCl and 10 wt % aq NaCl, dried over Na_2SO_4, and concentrated in vacuo to afford 37.0 g of ketoester **12** as a light yellow oil [97% corrected yield (93 wt % by ^{1}H NMR, 92.5 LCWP), 89 LCAP]: bp 90 °C (40 mmHg); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.30 (q, J = 7.2 Hz, 2H), 1.34 (t, J = 7.2 Hz, 3H), 1.24 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 202.1, 163.9, 61.7, 42.6, 25.7 (3C), 14.1; IR (thin film) 2976, 1738 cm^{-1}. Anal. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.79; H, 8.79.

Ethyl 3,3-Dimethyl-2-oxobutanoate (12) (Method B). To a solution of 498 g of diethyl oxalate in 2 L of toluene at

−78 °C was added *tert*-butylmagnesium chloride (1 M in THF) (3.85 L) over 1 h while the internal temperature was maintained below −60 °C. After 1 h, the reaction mixture was quenched with 3 N HCL (1 L) and water (1 L) and allowed to warm to room temperature. The organic phase was separated and washed with water. The solvent was removed in vacuo to yield 598.6 g of crude ketoester, which was purified by vacuum distillation (90 °C, 40 mm Hg) to produce 443.3 g (74% yield) of ketoester (90 LCWP, 73 LCAP, 95 wt % by ^{1}H NMR).

(*R*)-2-Hydroxy-3,3-dimethylbutanoic Acid [(*R*)-14]. The following components were rapidly stirred at room temperature: 4.6 L of 400 mM phosphate buffer stock solution (see the general Experimental Section (within the Supporting Information) for the preparation of all stock solutions), 12 L of the glucose stock solution, 320 mL of the GDH stock solution, 73.6 mL of the NADP stock solution, 12.8 mL of the KRED1001 stock solution, and 295.2 g of ketoester **12**. The pH was maintained at 7.0 by the addition of 5% NaOH during the course of the reaction. [The enantiomerically pure ethyl ester (*R*)-**13** could be isolated at this stage, although the saponification reaction was most frequently performed on the crude reaction mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.33−4.21 (m, 2H), 3.80 (d, J = 7.6 Hz, 1H), 2.83 (dd, J = 7.6, 1.4 Hz, 1H), 1.32 (t, J = 7.2 Hz, 3H), 0.981 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.3, 78.4, 61.2, 35.2, 25.8, 14.2; IR (neat) 3515, 2960, 1727 cm^{-1}; $[\alpha]^{25}_{365}$ −84 (*c* 1, MeOH); >95% ee] After complete reduction (8 h, HPLC analysis), 720 mL of NaOH (50% v/v) was added and stirred for 75 min to effect complete saponification. The final hydrolyzed solution was neutralized to pH = 2 with concentrated H_2SO_4 and then extracted with EtOAc. The solvent was removed in vacuo and the residue crystallized from heptane to afford 212.7 g of (*R*)-2-hydroxy-3,3-dimethylbutanoic acid (*R*)-**14** as a white solid (86% yield, >99.5% ee): mp 49.8 °C (lit.[17] mp 51 °C); $[\alpha]^{25}_{365}$ −46 (*c* 1, MeOH);[38] ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (s, 1H), 1.04 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.7, 78.3, 35.2, 25.8 (3C); IR (Nujol mull) 3356, 2918, 2853, 1733 cm^{-1}. Anal. Calcd for $C_6H_{12}O_3$: C, 54.53; H, 9.15. Found: C, 54.16; H, 9.09.

Lactone (*S*,*R*)-16 (Method A). A solution of L-proline methyl ester hydrochloride (272.1 g, 1.64 mol) in 3 L of acetonitrile was cooled to 0 °C, and diisopropylethylamine (215.6 g, 1.67 equiv) was added. After 15 min, HOBT (61.5 g, 0.45 mol), hydroxy acid (*R*)-**14** (200.1 g, 1.51 mol), and EDC (350.9 g, 1.83 mole) were added sequentially, and the resulting mixture was stirred at 0 °C for 5 h (90% HPLC assay yield). The mixture was quenched with 1 L of 3 N HCl and diluted with dichloromethane (3 L). The organic portion was separated and washed with 3 N HCl, saturated $NaHCO_3$, and then 10 wt % aqueous NaCl. The solvent was removed in vacuo to yield a crude mixture of ester (*S*,*R*)-**15** and lactone (*S*,*R*)-**16** (376.5 g). This solid was dissolved in toluene (2 L) and placed in a 5 L three-neck round-bottomed flask that was equipped with a short-path distillation head. To this was added PTSA (56.0 g, 0.30 mol) and the mixture heated to 45 °C at 100 mmHg. After 6 h at 45 °C, the conversion to lactone (*S*,*R*)-**16** was complete (100% by HPLC). During the distillation, approximately 500 mL of toluene was removed. The mixture was cooled to ambient temperature, washed twice with saturated $NaHCO_3$ and saturated NaCl, and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was recrystallized from heptane to afford 213.2 g of lactone (*S*,*R*)-**16** as a white solid [67% isolated yield, 99.5 LCAP, 100 LCWP, 0.17 LCAP lactone (*S*,*S*)-**16**]: mp 111.6 °C; $[\alpha]^{25}_{365}$ −214 (*c* 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.51 (s, 1H), 4.22 (dd, J = 9.6, 6.4 Hz, 1 H), 3.80−3.68 (m, 1H), 3.59−3.52 (m, 1H), 2.54−2.42 (m, 1H), 2.11−1.86 (m, 3H), 1.12 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 167.8, 163.1, 89.6, 57.2, 45.8, 37.4, 30.5, 26.2 (3C), 21.9; IR (Nujol mull) 2922, 2853, 1743, 1673 cm^{-1}. Anal. Calcd for $C_{11}H_{17}NO_3$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.57; H, 8.27; N, 6.51.

Lactone (*S*,*S*)-16. An authentic sample of this lactone was prepared in an identical fashion to the preparation of the diastereomeric lactone [(*S*,*R*)-**16**]: mp 162.5 °C; $[\alpha]^{25}_{365}$ −444 (*c* 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.35 (s, 1H), 4.26 (t, J = 7.7 Hz, 1 H), 3.64−3.53 (m, 2H), 2.41−2.32 (m, 2H), 2.01−1.96 (m, 2H), 1.28 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.8, 164.2, 84.6, 57.7, 45.1, 34.0, 28.3, 26.0 (3C), 23.3; IR (Nujol mull) 2923, 2853, 1739, 1691 cm^{-1}. Anal. Calcd for $C_{11}H_{17}NO_3$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.45; H, 8.25; N, 6.75.

Ketoamide (*S*)-17. To a solution of D-proline methyl ester hydrochloride (1.42 g, 8.5 mmol) in 26 mL of methylene chloride at 0 °C was slowly added diisopropylethylamine (1.50 mL) over a 5 min while the internal temperature was maintained at <5 °C. To the reaction mixture were added hydroxybenzotriazole hydrate (210 mg, 1.5 mmol), 3,3-dimethyl-2-oxobutanoic acid (1.00 g, 7.7 mmol), and EDC hydrochloride (1.63 g, 8.5 mmol), and this was stirred overnight at room temperature and then quenched with 3 N HCl. The layers were separated, and the organic portion was washed with 3 N HCl and saturated sodium bicarbonate, dried over sodium sulfate, concentrated in vacuo, and purified by silica gel chromatography to afford 1.56 g (84%) of ketoamide (*S*)-**17** as a white solid: mp 53.0 °C; $[\alpha]^{25}_{365}$ −382 (*c* 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) 3:1 rotameric mixture; major rotamer δ 4.58−4.50 (m, 1H), 3.77 (s, 3H), 3.54−2.45 (m, 2H), 2.17−1.90 (m, 4H), 1.30 (s, 9H); minor rotomer 4.68 (dd, J = 8.4, 4.0 Hz, 1H), 3.74 (s, 3H), 3.70−3.63 (m, 2H), 2.30−2.17 (m, 4H), 1.30 (s, 9H); ^{13}C NMR (75.5 MHz, C_6D_6) mixture of rotamers δ 207.5, 207.2, 173.2, 172.2, 165.8, 163.9, 59.8, 58.6, 52.3, 52.1, 47.4, 47.1, 43.7, 43.6, 31.6, 29.1, 27.4, 26.7, 26.5, 25.0, 22.5; IR 2923, 2852, 1747, 1706, 1630 cm^{-1}. Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.81. Found: C, 59.76; H, 8.07; N, 5.80.

Lactone (*S*,*R*)-16 (Method B). Ketoamide (0.5 g, 2.07 mmol), 5% Ru/C (0.25 g), and methanol (50 mL) were combined and degassed by three alternating nitrogen/vacuum cycles. The mixture was heated to 50 °C and pressurized to 60 psig of hydrogen pressure. After 72 h, no ketoamide remained (GC). The mixture was cooled to room temperature, depressurized and filtered through a Celite packed sintered glass funnel. The methanol was removed in vacuo to yield the crude hydroxy ester. The hydroxy ester was dissolved in 15 mL of toluene, and PTSA (60 mg, 0.15 equivalents) was added. The mixture was stirred at room temperature and vacuum applied (40 mmHg) to remove the methanol, which was formed as a byproduct. After 3 h, HPLC analysis indicated that the cyclization was complete. The ratio of (*S*,*R*)-lactone **16** to (*S*,*S*)-lactone **16** was 10:1. The toluene was removed in vacuo, and the (*S*,*R*)-lactone **16** was purified by silica gel chromatography (4:1 dichloromethane/EtOAc) to yield 293 mg of lactone (*S*,*R*)-**16** [1.39 mmol, 67% yield from ketoamide (*S*)-**9**].

Amide 18. A slurry of the lactone (*S*,*R*)-**16** (19.14 g, 90.60 mmol) and amine **9** in triethylamine (100 mL) was stirred overnight. The initial slurry became homogeneous overnight. The reaction mixture was diluted with isopropyl acetate and cooled to 7 °C, and 1 M citric acid (250 mL) was added while the internal temperature was maintained at less than 16 °C. The mixture was separated, and the organic portion was washed with 1 M citric acid (100 mL), water (100 mL), 1 M Na_2CO_3 (150 mL), and water (20 mL). The solvent was removed in vacuo to provide **Boc 18** (45.42 g that contained 6.6 wt % (^{1}H NMR) isopropyl acetate (3.02 g), corrected weight = 42.4 g, 97% yield) as a glass. This material was used in the subsequent step without further purification.

(37) Corral, C.; Madroñero, S.; Vega, S. *Anal. Quim.* **1972**, 851.

(38) The literature value[17] for the specific rotation of enantiomerically pure (*R*)-2-hydroxy-3,3-dimethylbutanoic acid is: $[\alpha]^{20}_D$ +65 [*c* 1, aqueous ammonium molybdate (50 mg/10 mL)] and $[\alpha]^{20}_D$ −4.3 (*c* 4, H_2O). Correspondingly, the specific rotation for (*S*)-2-hydroxy-3,3-dimethylbutanoic acid is $[\alpha]^{20}_D$ −63 [*c* 1, aqueous ammonium molybdate (48.5 mg/10 mL)]. The rotation of this (*S*)-antipode (purchased from Aldrich Chemical Co., Milwaukee, WI), under our conditions, was $[\alpha]^{20}_{365}$ +46.3 (*c* 1, MeOH).

HBr Salt of 1. A stock solution of 6.0 wt % HBr was generated by sparging HBr gas through isopropyl acetate. In a separate vessel, an ambient temperature solution of Boc amine **18** (9.62 g, 20.0 mmol) in isopropyl acetate (100 mL) was treated with the stock solution of HBr in isopropyl acetate (80.6 g solution, 4.85 g HBr, 60.0 mmol) via addition funnel over 30 min. The resultant slurry was aged for 1 h at ambient temperature. The slurry was filtered under N_2, sequentially washed with isopropyl acetate, methyl acetate and dried under vacuum to afford 8.12 g of an amorphous white solid (85% isolated yield corrected for purity of 97 LCAP): ^{1}H NMR (300 MHz, CD_3OD) δ 7.55−7.30 (m, 3H), 4.62 (d, AB, 1H, J = 5.1 Hz), 4.33−4.12 (m, 4H), 4.09 (s, 1H), 3.86−3.61 (m, 2H), 2.28−1.82 (m, 4H) 0.93 (s, 9H); HRMS *m*/*z* calcd for $C_{19}H_{29}BrClN_3O_3$ (M^+) 382.1892, found 382.1891. Compound **1** that was prepared by this method was identical to an authentic sample of **1**.[5]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectral data for compounds **9**, **12**, (*R*)-**13**, (*R*)-**14**, (*S*,*R*)-**16**, (*S*,*S*)-**16**, (*R*)-**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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