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Synthesis of a Ring-Oxygenated Variant of the 2-Carboxy-6-hydroxyoctahydroindole Core of Aeruginosin 298-A from Glucose

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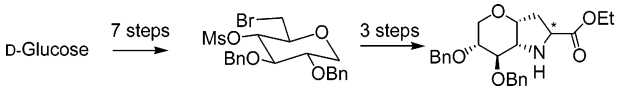
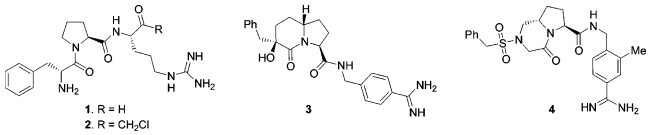


The design and synthesis of a new core structure, a ring-oxygenated variant of 2-carboxy-6-hydroxyoctahydroindole (Choi) from D-glucose, is reported. Choi, a rigid bicyclic unnatural amino acid, is the core structure of about 15 aeruginosins natural compounds. These compounds are thrombin, trypsin, and factor VIIa inhibitors and Choi is important for their biological activity. The ring-oxygenated variant of 2-carboxy-6-hydroxyoctahydroindole can potentially be used as a surrogate of Choi in the design and synthesis of aeruginosin-based thrombin inhibitors.

Introduction

Thrombin (factor IIa) is a key trypsin-like serine protease of the blood coagulation cascade, it is also the most potent activator of platelet aggregation. Thrombin inhibitors are important agents in treating or preventing thrombosis disorders. Many thrombin inhibitors have been designed and synthesized in recent years.[1−4] Substrate analogue strategy has been used in the design of reversible thrombin inhibitor D-Phe-Pro-Argininal (**1**)[5] and the irreversible potent inhibitor chromethyl ketone D-Phe-Pro-ArgCH_2Cl (**2**) (PPACK).[6] These compounds and the crystal structures of thrombin complexes with inhibitors including PPACK were often used as the starting point for structure optimizations of novel thrombin inhibitors.[7,8] Conformational restricted inhibitors that can preorganize into optimal conformations for the binding site are potentially more selective and could have better therapeutic profiles. Flexible inhibitors can adopt multiple conformations but they need to rearrange and fit into a protease binding site. Conformational strained thrombin inhibitor with an indolizidinone motif **3** and fused piperazinone system **4** showed good inhibitory activity.



Despite much research effort, it is still very challenging to find an orally active, safe, and effective agent for treating or preventing thrombosis disorders. The major anticoagulants heparin and warfrin were introduced about 50 years ago. Currently recombinant hirudin and argatroban are approved for the treatment of thrombosis associated with heparin induced thrombocytopenia (HIT). Hirudin (66 amino acids) and its analogue hirulog (20 amino acids) are polypetides isolated from medicinal leeches.[9] Argatroban is a synthetic small molecule thrombin inhibitor, it was recently approved for use as an anticoagulant for treating prophylaxis, HIT, and associated thrombotic complications.[10] Like heparin, both hirudin and argatroban are administered parenterally. Ximelagatran is an orally active thrombin inhibitor and

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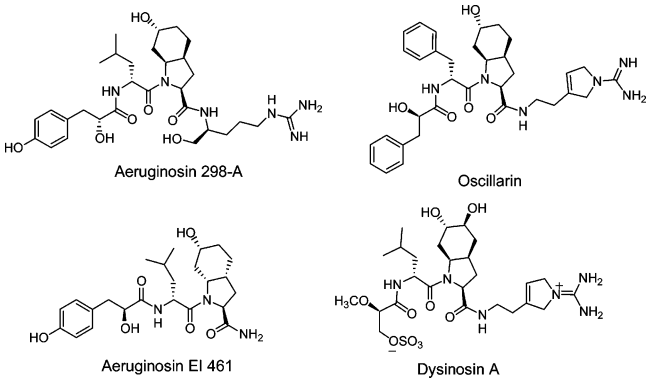
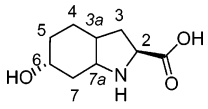


FIGURE 1. Structures of aeruginosian 298-A, occillarin, dysinosin A, and aeruginosin EI 461.

is in a phase III clinical trial as an antithrombotic drug.[11]

In recent years, a new class of marine nature products, the aeruginosins, were isolated from cyanobacterial blooms.[12−20] They are small linear peptides containing unnatural amino acids and they are found to be serine protease inhibitors. The approximately 16 compounds in this family share a common new bicyclic amino acid core structure 2-carboxy-6-hydroxyoctahydroindole (Choi) (**5**).



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Aeruginosins are thrombin, trypsin, and factor VIIa inhibitors and this new amino acid is the critical determinant of their biological activity. The 14 initially reported members mostly contain the configuration of 2*S*,3a*S*,6*R*,7a*S* in their azabicyclic core. The structures of several aeruginosins are shown in Figure 1. Aeruginosin 298-A exhibits thrombin and trypsin inhibition at IC_{50} of 0.5 and 1.7 μM but does not significantly affect papain, chymotrypsin, and plasmin.[15,16] Oscillarin is a relatively newer member of the family and it is a potent thrombin inhibitor with $IC_{50} = 28$ nM.[22] It has the same Choi structure as in aeruginosin 298-A, but with different amino acids. The crystal structure of thrombin inhibited by oscillarin was also published. Other than the typical

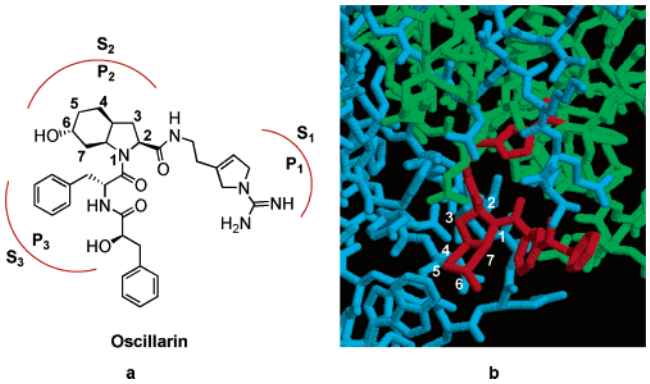

FIGURE 2. X-ray crystal structures of thrombin inhibited by oscillarin. This is obtained from protein data bank code 1R1W.[22] The Choi ring is number labeled in the molecular structure (a) and the crystal structure (b). The substrate binding sites are also labeled in the oscillarin structure.

stereochemistry observed in aeruginosin 298-A, the opposite configuration of the Choi bicycle has also been reported. Aeruginosin EI461[23] contains one less amino acid unit and the Choi has opposite stereochemistry to that of aeruginosin 298-A. Dysinosin A contains a new Choi structure with 5,6-dihydroxyl substituents, and it is a factor VIIa inhibitor (Ki = 0.11 μM) and thrombin inhibitor (Ki = 0.45 μM).[25,26]

Structure Design

The X-ray crystal structures of thrombin inhibiting by several aeruginosins have been obtained through the inhibitor and thrombin−hirugen complexes.[21,22,25] The binding mode of aeruginosin 298-A resembles closely that of D-Phe-Pro-Arg chloromethyl ketone[6] and other serine protease inhibitors.[1−4] Aeruginosin 298-A binds to the active site of thrombin in a noncovalent way forming an antiparallel strand with thrombin.[21] The five-membered ring of the Choi residue occupies the hydrophobic binding site, while its six-membered ring projects out and loosely interacts with Try 60 and Tyr 60 from thrombin. The active site where the Choi group interacts has many degrees of freedom. The crystal structures of oscillarin and dysinosin-A thrombin complexes revealed similar binding patterns as aeruginosin 298-A. Dysinosin-A forms a hydrogen bonding network with thrombin complex; however, the 5,6-dihydroxyoctahydroindole group appears not to have hydrogen bonding interactions with the P_2 binding pocket even though there are polar amino acid residues in the pocket.[25] The crystal structure of oscillarin with thrombin complex is shown in Figure 2. The amide NH from the octahydroindole carboxamide forms a hydrogen bond with Ser 256; however, no interactions were observed with the 6-hydroxyl group of

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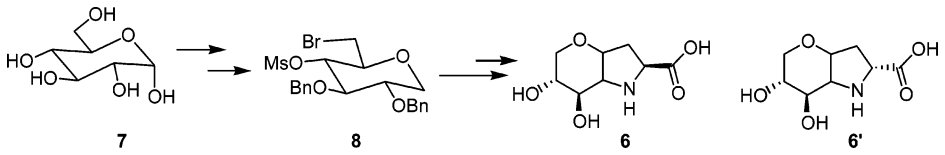
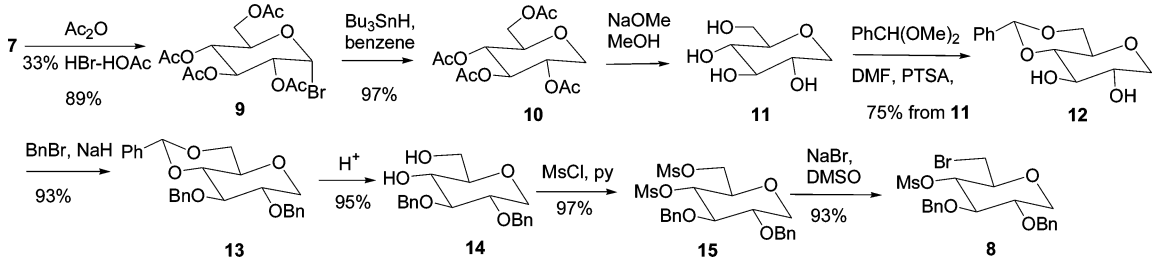
SCHEME 1. Synthetic Plan To Obtain the Choi Analogues from Methyl α-D-Glucopyranose

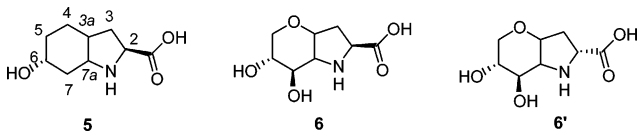


SCHEME 2. Synthesis of the Mesylate Bromide Intermediate 8 from D-Glucose



the Choi moiety. The terminal phenyl group has no interaction with the enzyme.[22]

On the basis of the crystal structures and the binding sites interactions, we can design novel inhibitors with similar core structure motifs but with different substituents and functionalization to enhance binding, activity, and pharmacokinetic properties. In all the crystal structures of aeruginosin−thrombin complexes so far, Choi moieties of the aeruginosins which occupy the S_2 binding site generally have a good degree of freedom. Thrombin generally can tolerate imprecise binding from different molecules. The rigid bicyclic amino acid structure is very important in defining the conformation of the molecule and it is essential in their antithrombin activity. The 6-hydroxyl group projects out and the Choi core is not interacting strongly with residues at the active site. Thus modifications on the core structure in this area should not impact negatively on binding to the protein while some changes will increase contact and enhance binding. Such changes include introducing an oxygen at the C-4 position and/or adding an additional hydroxyl group to the C-5 or C-7 position, these lead to structures **6** and **6′** as surrogates for **5**. Because pharmacokinetics are very



unpredictable, these changes will provide other possibilities that might provide scope for improving the pharmacokinetic profile of this compound class if required. The availability of different stereoisomers is also important in elucidating structure−activity relationships. Studies to rationalize electronic and steric influence of different inhibitors have not given good correlations so far. Modification from the natural analogues can allow us to understand the stereoelectronic factors that determine the activity of these inhibitors. These proposed Choi analogues are novel structures and will have great potential in discovering better thrombosis inhibitors.

Results and Discussion

Because of the important biological activity of aeruginosins, the total syntheses of these natural products have been carried out.[23−24,26−30] The Choi core structure is crucial for their antithrombotic activity. Several methods have been developed for the synthesis of Choi.[26−31] The majority of strategies involve the use of amino acids as starting materials. These include tyrosine oxidation−rearrangement[27] and Birch reduction of *O*-methyltyrosine followed by benzylation and cyclization.[28−30] A third method involves glutamic acid as the starting material through intramolecular *N*-acyloxyiminium ion carbocyclization reactions.[22] Other methods include using a pyrrolidine as starting material[32] and a synthesis that utilized catalytic phase transfer alkylation strategy.[30,33] To optimize thrombin inhibitor structure and discover novel antithrombosis drugs, we designed and synthesized novel analogues of the unnatural amino acid Choi for the preparation of aeruginosin-based thrombin inhibitors. The Choi analogues **6** and **6′** can be prepared from readily available D-glucose **7**, through an intermediate mesylate bromide **8** (Scheme 1). The mesylate **8** can be used to synthesize the products by extending the molecule with a two carbon unit that can be converted to the cyclic amino acid later.

The synthesis of intermediate mesyalte bromide **8** is shown in Scheme 2. The D-glucose **7** is converted to the bromosugar **9**. Reduction of **9** with tributyltin hydride gave the deoxygenated tetraacetate **10**. Deacetylating with ethanol and sodium ethoxide afforded tetraol **11** in quantitative yield. The selective protection of **11** to the acetal **12** was carried out smoothly with dimethoxyl benzylidene acetal in DMF. Dibenzylation and deprotection to free the 4,6-dihydroxyl groups followed by dimesylation gave dimesylate **15** with excellent yields. Monodisplace-

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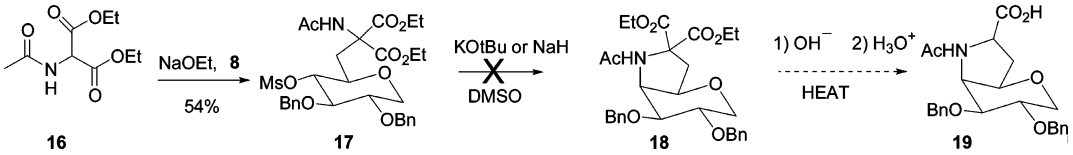
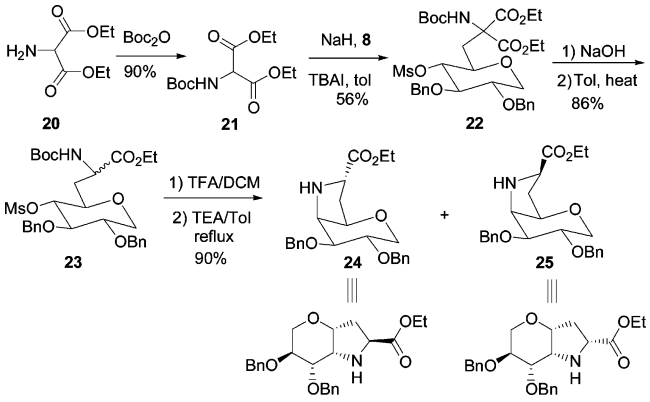
SCHEME 3. Alkylation of Intermediate 8 by Diethyl *N*-Acetylmalonate



SCHEME 4. Alkylation of Intermediate 8 by Diethyl *N*-Boc Malonate to Choi Analogues



ment of the primary mesylate was achieved with sodium bromide in DMSO at 60 °C to furnish intermediate **8**.

To extend intermediate **8** with the two-carbon glycine unit, several experiments were carried out. These include the alkylation using the anion from diethyl malonate. Diethyl acetamidomalonate **16** was first used as the two-carbon unit and the alkylation of the anion from **16** with bromide **8** led to the formation of intermediate **17** (Scheme 3). Bromide **8** reacted with diethyl acetamidomalonate sluggishly with low yield under typical reaction conditions (e.g., NaOEt/EtOH, reflux; NaH, THF or toluene reflux). Successful alkylation was achieved in 54% yield by adding TBAI as a catalyst presumably through a more reactive iodide intermediate and the nucleophilicity of the anion is enhanced by interacting with TBAI. However, the acetamide was not acidic enough to be deprotonated with a relatively mild base and afford the S_N2 displacement of the mesylate to give intermediate **18**. Various conditions (NaH or KO-*t*-Bu in THF, toluene, DMF, or DMSO) were tested—in the best case only about 10% yield of product was formed, possibly due to partial ester hydrolysis and/or mesylate decomposition.

With the promising result of diethyl malonate, we anticipated that the cyclization problem could be avoided by using free amine to displace the mesylate. In this route (Scheme 4), *N*-Boc protected diethyl aminomalonate **21** was used as the coupling reagent, and the anion generated from **21** was alkylated with **8** under slightly modified reaction conditions (NaH, with TBAI as a catalyst, reflux overnight in toluene). The alkylation product **22** was obtained in 56% yield. The monodecarboxylation of **22** was carried out with a sodium hydroxide in water and ethanol mixture to hydrolyze one ester group followed by refluxing to remove the carboxylate to give an inseparable diastereomer mixture of intermediate **23** in 86% yield. The deprotection of **23** with TFA and cyclization of the resulting amine under refluxing condition with TEA as catalyst furnished the cyclized Choi analogues **24** and **25** in very good yield. The two diasteromers **24** and **25** can be isolated with flash chromatography. Intermediates **24** and **25** are the protected versions of **6** and **6′**. The fact that they can be separated easily allows us to study the stereochemical influences of the Choi core structures to the aeruginosin analogues' properties.

The stereochemistry of compounds **24** and **25** is confirmed by their ^{1}H NMR and 2D-NOESY spectra. Figure 3 shows the assigned structures and the expected NOE

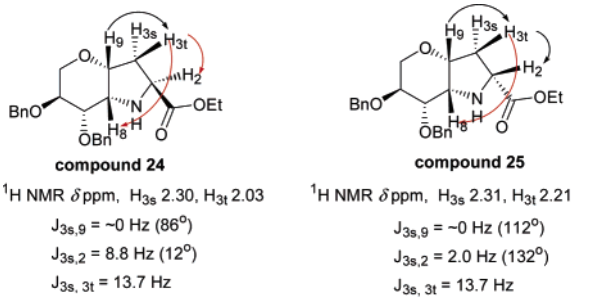


FIGURE 3. Structures of compounds **24** and **25** and the NOE among different protons.

among hydrogens from the five-membered ring. The 2D-NOESY spectra of the two diastereomers' aliphatic regions are shown in the Supporting Information.

The signals corresponding to methylene protons 3s and 3t can be assigned unambiguously from 2D-NOESY spectra. There should be more NOE interactions for proton 3t than 3s (Figure 3). In both isomers, there is an additional NOE cross-peak for H_{3t} and H_8. H_{3s} will not have NOE interactions with H_8. With this assignment confirmed, we can further assign the stereochemistry at C-2 base on the coupling constant differences of the two ^{1}H NMR spectra. For compound **24**, proton 3t is a ddd centered at 2.03 ppm, 3s is a dd centered at 2.30 ppm, the coupling constants of H_{3s} are 13.7 Hz for the geminal coupling and 8.8 Hz for the H−C−C−H J_3 coupling with H_2. Molecular modeling of **24** indicated that the dihedral angle of $H_2-C_2-C_3-H_{3s}$ is about 12° and that of $H_9-C_9-C_3-H_{3s}$ is about 86°; these explain the splitting pattern and that the J_3 of H_9 and H_{3s} is very small, therefore the NMR signals degenerate to a dd. For diastereomer **25**, proton 3t is a multiplet with pattern ddd centered at 2.21 ppm, and proton 3s is a dd centered at 2.31 ppm. The coupling constants of H_{3s} are 13.7 Hz for the geminal coupling and 1.9 Hz for the H−C−C−H coupling with H_2 and/or H_9. The molecular model of compound **25** indicated that the H_9-C_9 bond is almost perpendicular to the C_3-H_{3s} bond, and the J_3 for $H_{3s}-H_9$ is therefore very small. The dihedral angle for $H_2-C_2-C_3-H_{3s}$ is about 131°, which explains the observed splitting pattern of dd and a smaller coupling constant J_3 for $H_{3s}-H_2$.

Compounds **24** and **25** can be used in the synthesis of aeruginosin analogues directly by coupling at the amino

group. They can also be converted to the unprotected Choi analogues **6** and **6′**. We are currently synthesizing aeruginosin 298-A and oscillarin analogues containing the novel Choi core structures.

Conclusions

We have designed and synthesized ring-oxygenated variants of Choi analogues from D-glucose in short sequences efficiently. The two protected ring oxygenated variants of Choi synthesized can be used in the synthesis of aeruginosin 298-A analogues. The synthetic strategies are versatile in constructing inhibitor core structures with different stereochemistry. Because of the abundance of chiral hydroxyl groups in carbohydrate raw materials, different stereoisomers can be synthesized readily from different sugar molecules by similar methods. During the ring cyclization step to form the bicyclic structure, both configurations of the amino acids can be obtained in one step. These isomers are diastereomers of each other, thus they can be readily separated by chromatography. This allows quick access to different stereoisomers at a very late stage of the synthesis. The availability of novel Choi core structures with different stereochemistries will be useful in helping us to understand the stereochemical influences of substituents to the inhibitor properties. The biochemical information obtained from these isomers can be used to establish structure−activity relationships. Our future efforts will be the design and synthesis of derivatives from these novel core structures and the study of their antithrombotic activities. This will potentially lead to the discovery of novel thrombin inhibitor drugs that are useful in the treatment of blood coagulation.

Experimental Section

1,5-Anhydro-2,3-di-*O*-benzyl-4,6-di-*O*-methylsulfonyl-D-glucitol (15). Compound **14** (2 g, 5.8 mmol) was dissolved in 10 mL of DCM and 8 mL of pyridine. Methanesulfonyl chloride (1.8 mL, 23.3 mmol) was then added to the reaction mixture, which was left stirring under anhydrous condition until completion (usually 12 h). The reaction mixture was then poured into a beaker containing 15 g of crushed ice and 15 mL of saturated aq $NaHCO_3$ and was left stirring in an ice bath for 1 h. The organic layer was extracted with ethyl acetate three times and the combined organic layer was washed with water and brine,and then dried over Na_2SO_4. Concentration and purification by flash chromatography gave **15** as colorless crystals (2.8 g, 97% yield). Mp 104−105 °C, $[\alpha]_D$ +26.0 (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 7.34−7.26 (m, 10H), 5.06 (d, 1H, J = 11.1 Hz), 4.70 (d, 1H, J = 11.1 Hz), 4.64 (d, 1H, J = 11.5 Hz), 4.64 (d, 1H, J = 11.5 Hz), 4.49−4.43 (m, 2H), 4.29 (dd, 1H, J = 11.3, 5.26 Hz), 4.08−4.04 (m, 1H), 3.72−3.65 (m, 2H), 3.59 (ddd, 1H, J = 9.8, 5.2, 2.3 Hz), 3.24−3.20 (m, 1H), 3.02 (s, 3H), 2.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.5, 137.3, 128.6, 128.5, 128.1, 127.9, 127.8, 127.7, 82.4, 78.4, 77.1, 76.1, 75.3, 73.2, 67.8, 67.7, 38.5, 37.5; HRMS calcd for $C_{22}H_{28}O_9S_2$ + Na 523.1072, found 523.1083.

1,5-Anhydro-2,3-di-*O*-benzyl-6-bromo-4-*O*-methylsulfonyl-D-glucitol (8). The mixture of the dimesylate **15** (1.34 g, 2.7 mmol), NaBr (1.65 g, 16.0 mmol), TBAB (0.26 g, 0.80 mmol), and DMSO (10 mL) was stirred at 60 °C for 12 h; usually by then the synthesis was completed. The reaction mixture was cooled to room temperature then poured into 20 mL of water, the water phase was extracted with ethyl acetate 4 times, and the combined organic phase was dried over sodium sulfate. Concentration and purification on a silica gel column afford the pure product (1.26 g, 93% yield) as a white crystal. Mp 64−67 °C, $[\alpha]_D$ +27.7 (*c* 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 7.34−7.27 (m, 10H), 5.07 (d, 1H, J = 11.1 Hz), 4.68 (d, 1H, J = 11.1 Hz), 4.65 (d, 1H, J = 11.5 Hz), 4.58 (d, 1H, J = 11.5 Hz), 4.36 (t, 1H, J = 9.1 Hz), 4.10−4.06 (m, 1H), 3.75−3.64 (m, 2H), 3.51 (dt, 1H, J = 7.2, 2.2 Hz), 3.41 (dd, 1H, J = 11.3, 7.3 Hz), 3.26−3.21 (m, 1H), 2.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.6, 137.4, 128.6, 128.5, 128.2, 127.9, 127.8, 127.7, 82.4, 79.8, 78.7, 77.8, 75.3, 73.2, 67.8, 38.6, 31.8. HRMS calcd for $C_{21}H_{25}BrO_6S$ + Na 507.0453, found 507.0453.

Preparation of Compound 17. Diethyl acetamidomalonate **16** (214 mg, 0.99 mmol) was dissolved in 5 mL of anhydrous THF, then NaH (40 mg, 0.99 mmol, 60% oil immersion) was added to the solution. The reaction mixture was stirred at room temperature for half an hour. Then compound **8** (160 mg, 0.33 mmol) in 5 mL of anhydrous THF was injected and the reaction mixture was stirred at room temperature for 1 h. TBAI (37 mg, 0.1 mmol) was added and the reaction mixture was stirred at room temperature for another hour before the reaction mixture was heated to reflux for 2 days. The reaction mixture was cooled to 0 °C and 5 mL of 0.5 N HCl was added. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and the crude was purified by flash chromatography to give the product as a white solid (110 mg, 54%). Mp 107−109 °C, $[\alpha]_D$ +13.3 (*c* 0.7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.37−7.24 (m, 10 H), 6.66 (s, 1H), 4.84 (ABq, 2H, J = 9.8 Hz), 4.64 (d, 1H, J = 11.7 Hz), 4.55 (d, 1H, J = 11.7 Hz), 4.36−4.31 (m, 1H), 4.25−4.11 (m, 4H), 3.81−3.77 (m, 1H), 3.61−3.54 (m, 2H), 3.30 (dd, 1H, J = 10.7, 9.8 Hz), 3.06−2.95 (m, 2H), 2.98 (s, 3H), 2.37 (dd, 1H, J = 14.7, 9.8 Hz), 2.02 (s, 3H), 1.23−1.18 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 168.4, 167.3, 137.8, 137.7, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 82.7, 80.1, 78.5, 75.3, 74.6, 73.3, 67.7 64.1, 62.7, 62.3, 39.2, 34.6, 23.0, 13.9, 13.7.

Preparation of Compound 22. Diethyl 2-[(*tert*-butyloxycarbonyl)amino]malonate (**21**) was prepared according to literature procedure.[34] Compound **21** (1.4 g, 5.1 mmol) was dissolved in 15 mL of dry toluene under N_2. NaH (204 mg, 5.1 mmol) was then added to the solution. The reaction mixture was left stirring at room temperature for half an hour. Then compound **8** (820 mg, 1.7 mmol) was added and the reaction mixture was stirred at room temperature for an hour. TBAI (188 mg, 0.51 mmol) was added and the reaction mixture was heated to reflux for 17 h. The reaction mixture was cooled to 0 °C and 10 mL of 0.5 N HCl was added. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 75 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and the crude was purified by flash chromatography to give the product as a viscous colorless oil (630 mg, 56%). $[\alpha]_D$ +8.3 (*c* 0.93, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) mixture of conformers δ 7.42−7.20 (m, 10 H), 6.03 (br s, 0.3H), 5.87 (br s, 0.7H), 5.13−4.96 (m, 0.3H), 4.86 (br s, 1.4H), 4.70−4.54 (m, 2.3H), 4.35−4.09 (complex, 5H), 3.78 (d, 1H, J = 9.8 Hz), 3.68−3.52 (m, 2H), 3.39 (t, 1H, J = 9.8 Hz), 3.08−2.78 (complex, 5H), 2.37 (dd, 1H, J = 14.7, 10.7 Hz), 1.42 (s, 9H), 1.24−1.18 (complex, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 167.7, 153.9, 137.8, 128.4, 128.3, 128.2, 127.9, 127.7, 127.4, 82.7, 81.8, 80.5, 80.3, 80.1, 79.0, 78.6, 75.4, 75.1, 74.4, 73.2, 73.0, 67.0, 64.0, 62.6, 62.1, 39.2, 38.6, 35.3, 35.0, 28.1, 27.8, 13.9, 13.7. HRMS calcd for $C_{33}H_{45}NO_{12}S$ + Na 702.2560, found 702.2568.

Preparation of Compound 23. NaOH (1 N, 2 mL) in 10 mL of ethanol was added dropwise at room temperature to a stirred solution of compound **22** (360 mg, 0.53 mmol). After the reaction was complete (TLC analysis, usually 5 h), the reaction mixture was acidified with 1 N HCl to pH 2−3 and extracted with chloroform five times. The combined organic

(34) Schneider, H.; Sigmund, G.; Schricker, B.; Thirring, K.; Berner, H. *J. Org. Chem.* **1993**, *58*, 683−689.

layer was dried over Na_2SO_4. Solvent was removed under reduced pressure to afford crude product, which was subsequently decarboxylated by heating in 15 mL of toluene at 75 °C for 2 h. Solvent was removed under reduced pressure and the crude was purified by flash chromatography. Compound **23** (322 mg, 86%) was obtained as viscous oil (a mixture of inseparable diasteromers): ^{1}H NMR (400 MHz, $CDCl_3$) (the NMR spectrum is complicated due to the presence of two diasteromers) δ 7.36−7.19 (m, 10 H), 5.24−5.19 (m, 1H), 5.07 (d, 0.6H, J = 11.7 Hz), 5.02 (d, 0.4H, J = 11.7 Hz), 4.73−4.37 (complex, 4H), 4.25−3.91 (complex, 4H), 3.70−3.58 (m, 2H), 3.43 (dt, 0.5H, J = 9.8, 2.0 Hz), 3.32 (t, 0.5H, J = 9.8 Hz), 3.18−3.10 (m, 1H), 2.81 (s, 1.2H), 2.77 (s, 1.8H), 2.30 (dd, 0.5H J = 14.7, 6.8 Hz), 2.09 (m, 0.5H), 1.97−1.84 (m, 1H), 1.43 (singlet with shoulder, 9H), 1.24 (t, 1.2H, J = 6.84 Hz), 1.23 (t, 1.8H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 171.9, 155.5, 154.9, 137.7, 137.6, 137.5, 137.4, 128.55, 128.47, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 82.6, 81.1, 80.9, 79.8, 78.9, 77.2, 75.6, 75.4, 75.2, 73.1, 67.8, 67.7, 61.4, 61.3, 38.67, 38.58, 34.7, 34.2, 28.2, 14.1, 14.0. HRMS calcd for $C_{30}H_{41}NO_{10}S$ + Na 630.2349, found 630.2359.

Preparation of Compounds 24 and 25. Compound **23** (270 mg, 0.44 mmol), 12 mL of DCM, and 3 mL of TFA were mixed at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 h before the solvent was evaporated under reduced pressure. Saturated $NaHCO_3$ (15 mL) and 25 mL of EtOAc were added to the residue. The aqueous layer was extracted with EtOAc five times. The combined organic layer was dried over Na_2SO_4. Solvent was removed under reduced pressure to afford crude product, which was dissolved in 10 mL of toluene. TEA (70 μL, 0.50 mmol) was added to the reaction mixture which was then heated to reflux for 2 h. After this period of time, solvent was removed under reduced pressure and the crude was purified by flash chromatography to give pure diasteoromers **24** (80 mg, 44%) and **25** (84 mg, 46%). Compound **24** was obtained as a colorless syrup. $[\alpha]_D$ +11.0 (c 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.24 (m, 10 H), 4.78 (q, 2H, J = 2.0 Hz), 4.69 (q, 2H, J = 11.7 Hz), 4.17 (q, 2H, J = 6.84 Hz,), 4.01−3.91 (m, 4H), 3.65 (dd, 1H, J = 8.8, 4.9 Hz), 3.60 (dd, 1H, J = 4.9, 2.0 Hz), 3.13−3.07 (m, 1H), 2.39 (br, 1H), 2.30 (dd, 1H, J = 13.7, 8.8 Hz), 2.03 (ddd, 1H, J = 13.7, 6.8, 4.9 Hz), 1.26(t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 138.6, 138.5, 128.3, 127.6, 127.55, 127.50, 80.3, 78.2, 74.6, 73.3, 71.7, 67.5, 61.0, 60.3, 56.9, 36.9, 14.2. HRMS calcd for $C_{24}H_{29}NO_5$ + H 412.2124, found 412.2130.

Compound **25** was obtained as a colorless syrup. $[\alpha]_D$ +94.8 (c 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.24 (m, 10 H), 4.84 (q, 2H, J = 2.0 Hz), 4.65 (q, 2H, J = 5.9 Hz), 4.16(q, 2H, J = 6.8 Hz), 3.91−3.74 (m, 5H), 3.58 (dd, 1H, J = 4.9, 2.0 Hz), 3.02 (t, 1H, J = 10.7 Hz), 2.55 (br, 1H), 2.31 (dd, 1H, J = 13.7, 2.0 Hz,), 2.21 (ddd, 1H, J = 13.7, 9.8, 3.9 Hz), 1.23 (t, 3H, J = 6.8 Hz,); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 138.6, 138.4, 128.4, 127.7, 127.6, 79.6, 77.4, 74.1, 73.7, 70.8, 67.2, 61.1, 58.21, 37.5, 14.2. HRMS calcd for $C_{24}H_{29}NO_5$ + H 412.2124, found 412.2133.

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Note Added after ASAP Publication. References 24, 25, 26, and 31 were incorrectly cited in the text in the version published ASAP September 27, 2005; the corrected version was published ASAP September 29, 2005.

Supporting Information Available: The procedures for preparing compounds **9**−**14**, the ^{1}H and ^{13}C NMR spectra of compounds **8**−**15**, **17**, and **22**−**25**, and the 2D NOESY, 2D-COSY spectra for compounds **24** and **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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