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Synthesis of Carbapyochelins via Diastereoselective Azidation of 5-(Ethoxycarbonyl)methylproline Derivatives

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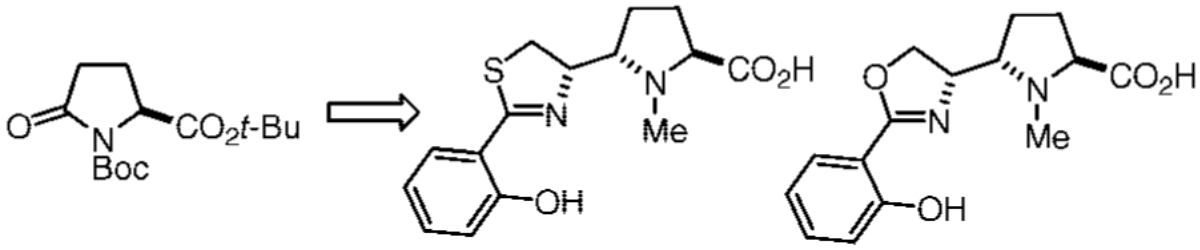
Abstract

Two configurationally stable carbon-based analogues of pyochelin have been prepared from Boc-pyroglutamic acid-*tert*-butyl ester in 11 and 13 steps. Introduction of the amino group was achieved by a highly diastereoselective electrophilic azidation reaction to afford novel bis-α-amino acid proline derivatives.



Pyochelin (**1**) is a phenolate siderophore isolated from *Pseudomonas aeruginosa* that enhances microbial growth by solublizing ferric iron and accelerating iron transport.[1] Studies on *P. aeruginosa* iron uptake systems have focused largely on the recognition of pyochelin by bacterial outer membrane receptors.[2] These efforts have benefited from the synthesis and evaluation of various pyochelin analogues, many of which chelate and transport iron(III) with similar efficiency compared to the natural product.[3]

Recently, it has been shown that the endogenous protein siderocalin (also lipocalin 2, NGAL) is able to bind and sequester iron as complexes with siderophores, thus starving pathogens of this required nutrient.[4] While siderocalin tightly binds a number of small-molecule phenolate siderophores such as carboxymycobactins, enterochelin, and parabactin, it does not bind to pyochelin. We have proposed that the presence of a sterically demanding sulfur-containing thiazoline ring, which replaces the oxazoline ring present in the other phenolate siderophores, interferes with a conserved water-mediated hydrogen bond between these siderophores and

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Supporting Information Available: Detailed experimental procedures for compounds **5** and **12**–**16**, and spectral data for all new compounds, as well as crystal structure data (CIF) for **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

siderocalin.4c,d In contrast, pyochelin does bind to chicken Ex-FABP, a protein with high sequence homology to human siderocalin in the binding calyx region.[5] The fact that chicken Ex-FABP also shows affinity for a variable spectrum of microbial siderophores raises the possibility that these proteins are part of an antibacterial defense strategy employed across the phylum.

To parse the ligand recognition parameters of both siderocalin and chicken Ex-FABP, we sought to design and synthesize analogues of pyochelin that would demonstrate both protein and iron binding capabilities. Because pyochelin undergoes epimerization at the 2″ position through the general mechanism shown in Figure 1, efforts to synthesize and isolate **1** in diastereomerically pure form have met with limited success.[6] We envisioned this problem could be circumvented by incorporating a methylene unit in place of the ring A sulfur, a position with no established role in iron or siderocalin binding. In addition, we sought to target the oxazoline variant **3** to test our steric clash hypothesis through the design of an oxo analogue. These analogues would potentially provide useful information on the discreet stereochemical requirements for siderophore specific protein–ligand interactions. Here, we describe our efforts toward novel proline-based siderophores, resulting in a diastereoselective synthesis of amino acids **2** and **3**.

Our initial synthesis plan relied on intramolecular nitrene insertion as the key step for introduction of the ring B nitrogen. Toward this end, we prepared known proline diester **5**,[7] as an inseparable 16:1 trans:cis mixture via lithium triethylborohydride reduction and Horner–Wadsworth–Emmons olefination of (*N*-Boc)-*tert*-butyl pyroglutamate (**4**).[8] Chemoselective ester reduction was followed by carbamate formation to give **6** in 64% yield after purification (Scheme 1). Unfortunately, rhodium(II)-catalyzed C–H insertion[9] resulted in poor yields of the desired 5-membered cyclic carbamate. Attempts to employ silver(I)-catalyzed conditions, which have shown enhanced conversion in the case of some secondary C–H bond insertions, 10 resulted in slightly improved albeit unsatisfactory yields.

Given these results, we decided to explore potentially regio- and diastereoselective electrophilic azidation[11] as an alternative strategy (Scheme 2). When **5** was treated with LHMDS and trisyl azide at −78 °C, followed by AcOH quench, we obtained a mixture of starting material, ring-opened starting material (**9**), and low yields of desired product **8**, along with unidentified byproducts. We reasoned that the electron-withdrawing and sterically demanding Boc -group prevents reaction of the desired carbanion with the azide source. Anticipating the necessary introduction of an *N*-methyl group, we opted to replace the *N*-Boc group prior to azide transfer. Thus, acidic cleavage of the Boc group followed by treatment with anion exchange resin in MeOH and filtration gave the crude amine, which was then subjected to reductive alkylation to afford **10**.

When *N*-methyl proline **10** was subjected to the same azide transfer condition used for substrate **5**, we observed a marked increase in conversion and yield of the desired azidoester. Optimal conditions utilized 2.2 equiv of LHMDS and 2.0 equiv of trisyl azide in THF with AcOH quench (entry 1). Reducing the amount of LHMDS to 1.1 equiv (entry 2) resulted in exclusive formation of the α-diazoester, as did the use of 4-nitrophenylsulfonyl azide as an electrophile (entry 5). The potassium enolate of **10** reacted to give only the desired azide product, irrespective of the amount of base used (entries 3 and 4).

Analysis of the ^{1}H NMR of **11** indicated the presence of only one diastereomer (under optimized azidation conditions). Although the configuration of the newly formed stereocenter could not be determined at this stage, we considered the two possible transition states shown in Figure 2. Path A depicts the minimization of 1,3-allylic strain, followed by approach of trisyl azide from the Re-face of the enolate. Alternatively, a sixmembered chelation transition state

may lead to axial approach of trisyl azide onto a low-energy half-chair conformer (Fürst–Plattner principle). Both of these models result in the formation of the *anti* product. On the basis of this, we anticipated that the newly formed α-amino acid center would possess an *S* configuration to match that of the 4′ position in pyochelin.[12] This tentative assignment was later confirmed by X-ray diffraction of an amide derivative (vide infra).

Completion of the synthesis of pyochelin analogues **2** and **3** is depicted in Scheme 3. Thus, azide reduction of **11** and EDC-mediated coupling of the crude amine to 2-benzyloxybenzoic acid gave amide **12** in 93% yield. Reduction of the ethyl ester was followed by hydrogenolysis of the benzyl ether and dehydrative cyclization with the Burgess reagent[13] to give oxazoline **15**. The thiazoline ring was formed over two steps by thiolysis of the oxazoline ring with hydrogen sulfide in MeOH/NEt_3, and subsequent dehydration again with the Burgess reagent.[14]

Our efforts to carry out TFA-mediated deprotection of *tert*-butyl esters **14** and **15** (with anisole scavenger) were initially met with difficulties. The apparent sensitivity of the oxazoline and thiazoline rings in the presence of water and excess TFA resulted in appreciable amounts (>20%) of hydrated ring-opened products. This side reaction could be minimized by a careful workup procedure involving dilution of the reaction mixture with ethyl acetate, concentration in vacuo, and addition of a precooled diethyl ether/water mixture. Separation of the water layer and lyophilization then gave **2** and **3** in high yields without appreciable hydrolysis. RP-HPLC (C_{18}) analysis of the crude amino acids showed each to have >95% purity following deprotection (see the Supporting Information).

To determine the configuration of the newly formed stereocenter of **11** we relied primarily on the synthesis of crystalline derivatives, since detailed 2D NMR studies gave ambiguous results. While some intermediates in Scheme 3 were solids, none of these compounds provided crystals of sufficient quality for X-ray diffraction. We were pleased to find that reduction of azide **11** followed by coupling to 2-methoxymethyloxybenzoic acid[15] gave 84% yield of amide **16**, which readily crystallized out of CH_2Cl_2/hexane. Single-crystal X-ray diffraction revealed an *S* configuration at the 1′ center (IUPAC numbering), thus confirming our earlier prediction.

In summary, we have described a route toward two configurationally stable analogues of pyochelin. We are currently investigating the ability of ferric complexes of **2** and **3** to bind siderocalin and chicken Ex-FABP. Studies are also underway to explore the scope of the highly diastereoselective electrophilic azidation for the synthesis of other functionalized proline chimeras with orthogonally protected *N*- and *C*-termini. To the best of our knowledge, this work represents the first stereoselective synthesis of bis-proline structures related to **11** (Scheme 4). This class of residues should find utility in a variety of peptidomimetic applications.

Experimental Section

(2*S*,5*S*)-5-Ethoxycarbonylmethyl-1-methylpyrrolidine-2-carboxylic Acid *tert*-Butyl Ester (10)

A solution of **5** (9.20 g, 25.7 mmol) in 50 mL of 25% TFA/DCM was stirred at rt for 30 min, diluted with 100 mL of EtOAc, then evaporated under reduced pressure and dried under high vacuum to remove excess trifluoroacetic acid. The crude amine trifluoroacetate salt was taken up in 100 mL of EtOH and treated with 38 g of Dowex-X2 anion exchange resin (OH^- form). The mixture was stirred gently until the pH of the solution reached 8–9. The resin was then removed by filtration and washed repeatedly with EtOH. Evaporation of filtrate gave the crude amine as a yellow oil (attempts to isolate the free amine by aqueous extraction were hampered by its water solubility). The amine was treated with formaldehyde (30% solution in EtOH, 41.8 mL, 515 mmol) and 1 M aq. HCl until a pH of 2 was obtained. A catalytic amount of 10% Pd/

C was added and the flask was then purged and filled with an H_2 atmosphere (balloon). The reaction mixture was stirred 18 h at rt and purged with a stream of Ar, then the catalyst was removed by filtration over celite with MeOH rinsing. The filtrate was concentrated, diluted with sat. aq. $NaHCO_3$, and extracted with EtOAc. Drying over Na_2SO_4, filtration, and concentration under reduced pressure gave a crude 16:1 diastereomeric mixture of *N*-methylated amines which were separated by chromatography over silica gel (33% Et_2O/hexanes as eluant). The trans isomer **10** was obtained as a pale yellow oil (3.63 g, 52% from **5**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (q, J = 7.0 Hz, 2H), 3.53 (dd, J = 8.2, 2.3 Hz, 1H), 3.42 (septet, J = 4.4 Hz, 1H), 2.59 (dd, J = 14.4, 4.1 Hz, 1H), 2.43 (s, 3H), 2.30–2.04 (m, 3H), 1.86–1.76 (m, 1H), 1.70–1.60 (m, 1H), 1.47 (s, 9H), 1.22 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 172.2, 80.7, 67.0, 60.2, 59.7, 38.8, 35.3, 29.9, 28.2, 27.3, 14.2. HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{14}H_{25}NO_4$ 272.1856, found 272.1859.

(2*S*,5*S*,1′*S*)-5-(Azidoethoxycarbonylmethyl)-1-methylpyrrolidine-2-carboxylic Acid *tert*-Butyl Ester (11)

A solution of **10** (900 mg, 3.32 mmol) in dry THF (7 mL) was cooled to −78 °C and treated with LHMDS (1 M solution in THF, 7.30 mL, 7.30 mmol) dropwise. The enolate was allowed to form over 1 h at the same temperature and treated with a precooled (−78 °C) solution of 2,4,6-triisopropylbenzenesulfonyl azide (2.05 g, 6.63 mmol) in THF (12 mL). After 2 min the reaction was quenched by adding glacial AcOH (750 μL, 13.3 mmol) and then allowed to stir 16 h at rt. The reaction was concentrated, taken up in 10% aq. Na_2CO_3, and extracted with EtOAc, then the organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was purified by chromatography over silica gel (10–20% EtOAc/hexanes gradient as eluant). The desired azide **11** was obtained as a yellow oil (735 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27 (d, J = 2.6 Hz, 1H), 4.25 (q, J = 7.0 Hz, 2H), 3.71 (d, J = 7.6 Hz, 1H), 3.58 (dt, J = 8.8, 3.5 Hz, 1H), 2.53 (s, 3H), 2.10 (m, 2H), 1.87 (m, 2H), 1.47 (s, 9H), 1.29 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 168.9, 80.9, 67.3, 64.7, 63.0, 61.7, 34.8, 28.2, 28.1, 25.1, 14.1; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{14}H_{24}N_4O_4$ 313.1876, found 313.1884.

Data for the α-diazoester byproduct: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (q, J = 6.9 Hz, 2H), 4.05 (dd, J = 8.4, 4.9 Hz, 1H), 3.56 (d, J = 8.2 Hz, 1H), 2.43 (s, 3H), 2.14–2.03 (m, 1H), 1.90–1.72 (m, 3H), 1.26 (t, J = 7.1 Hz, 3H), 1.48 (s, 9H); ESI-MS (*m*/*z*) $[MH]^+$ calcd for $C_{14}H_{23}N_3O_4$ 298.17, found 298.26.

(2*S*,5*S*,4′*S*)-5-[2-(2-Hydroxyphenyl)-4,5-dihydrooxazol-4-yl]-1-methylpyrrolidine-2-carboxylic Acid (3)

Oxazoline ester **14** (40 mg, 116 μmol) was treated with anisole (126 μL, 1.16 mmol) followed by 1.5 mL of a 90% TFA/DCM solution. The reaction was stirred at rt for 6 h, then diluted with 5 mL of EtOAc, and concentrated in vacuo (this step is necessary to remove excess TFA and avoid hydrolytic opening of the oxazoline ring). The crude residue was then partitioned between water and Et_2O (precooled to 0 °C) and separated, then the aqueous layer was washed with an additional portion of Et_2O. The aqueous layer was then frozen and lyophilized to yield pure **3 TFA** as a hygroscopic white solid (38 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (dd, J = 7.6, 1.6 Hz, 1H), 7.45 (ddd, J = 8.6, 7.4, 2.0 Hz, 1H), 6.99 (dd, J = 8.6, 0.8 Hz, 1H), 6.92 (dt, J = 7.4, 1.2 Hz, 1H), 4.91 (ddd, J = 10.2, 7.8, 3.9 Hz, 1H), 4.61 (t, J = 9.4 Hz, 1H), 4.27 (t, J = 8.4 Hz, 1H), 3.92 (m, 1H), 2.93 (s, 3H), 2.16 (m, 1H), 2.01 (m, 1H), 1.72 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.1, 166.3, 159.1, 158.0 (q, TFA), 134.2, 128.2, 119.1, 116.7, 109.6, 69.3, 66.7, 63.3, 51.9, 36.4, 26.7, 23.1; HPLC C_{18} retention time (0–90% MeCN in H_2O with 0.1% formic acid, linear gradient, 20 min) = 9.78 min; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{15}H_{18}N_2O_4$ 291.1339, found 291.1349.

(2*S*,5*S*,4'*S*)-5-[2-(2-Hydroxyphenyl)-4,5-dihydrothiazol-4-yl]-1-methylpyrrolidine-2-carboxylic Acid (2)

Thiazoline ester **15** (35 mg, 110 μmol) was deprotected by using the same procedure described for **14**. Lyophilization of the final aqueous extracts yielded pure **2 ·TFA** as a white solid (40 mg, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (m, 2H), 6.99 (m, 2H), 5.17 (dt, J = 9.3, 3.1 Hz, 1H), 4.18 (m, 1H), 3.89 (m, 1H), 3.61 (dd, J = 11.7, 9.4 Hz, 1H), 3.22 (dd, J = 10.9, 9.4 Hz, 1H), 2.85 (s, 3H), 2.15 (m, 2H), 2.03 (m, 1H), 1.81 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 168.1, 158.0 (q, TFA), 156.0, 132.3, 129.2, 118.4, 115.7, 114.9, 73.8, 67.1, 52.0, 46.0, 36.9, 34.8, 27.2; HPLC C_{18} retention time (0–90% MeCN in H_2O with 0.1% formic acid, linear gradient, 20 min) = 10.38 min; mp 68–70 °C; HRMS (ESI-TOF) (m/z) $[M + H]^+$ calcd for $C_{15}H_{18}N_2O_3S$ 307.1111, found 307.1115.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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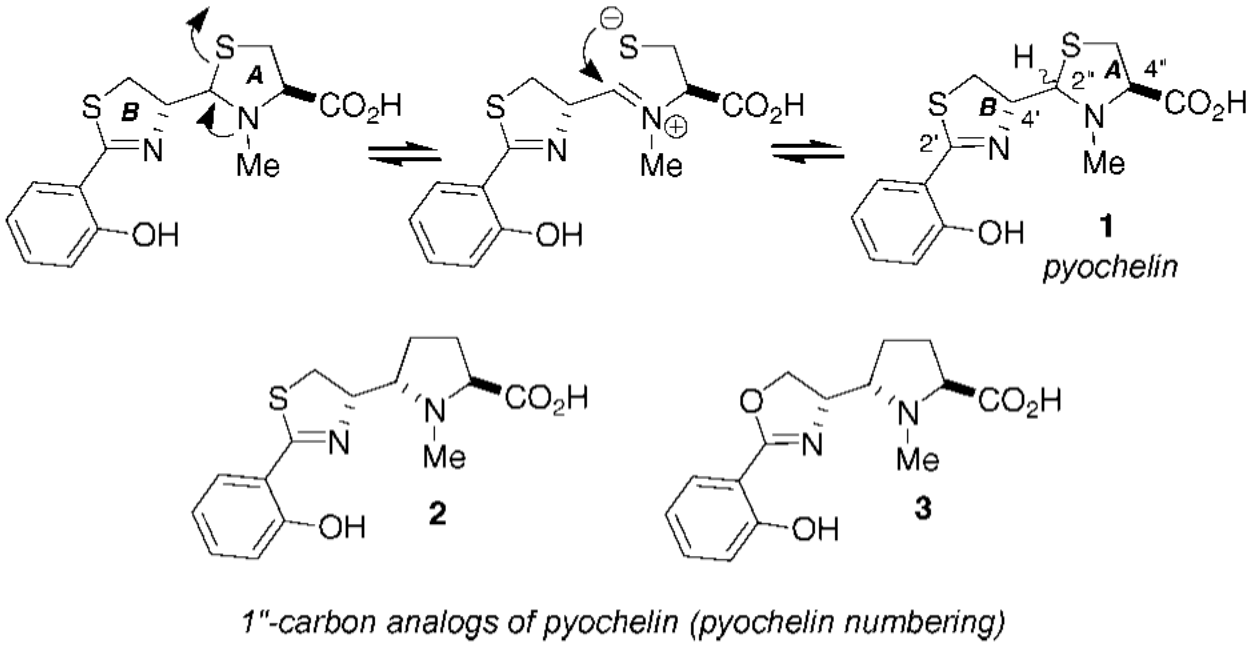
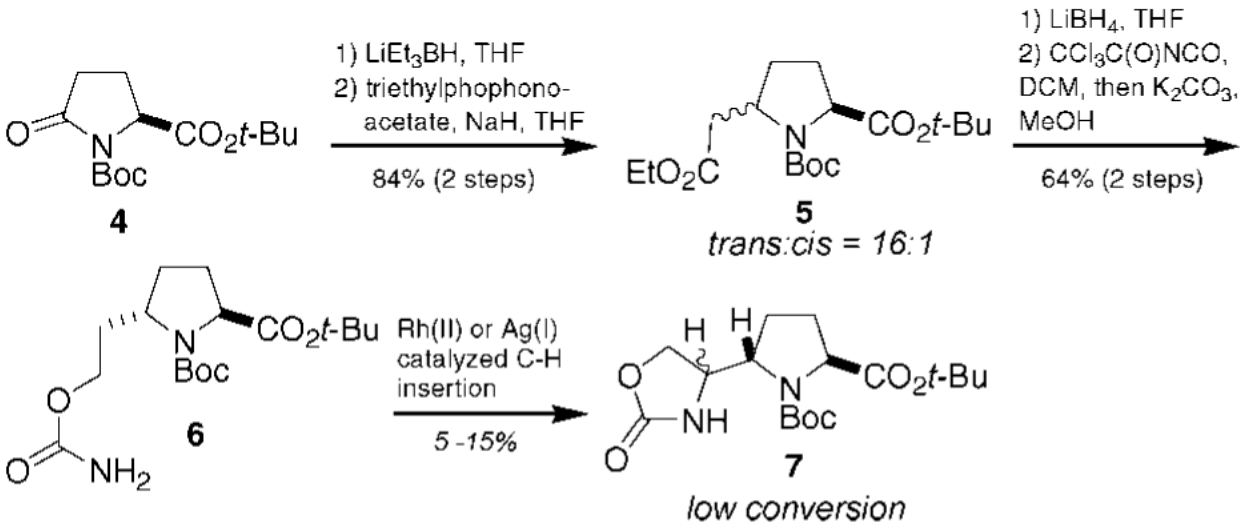
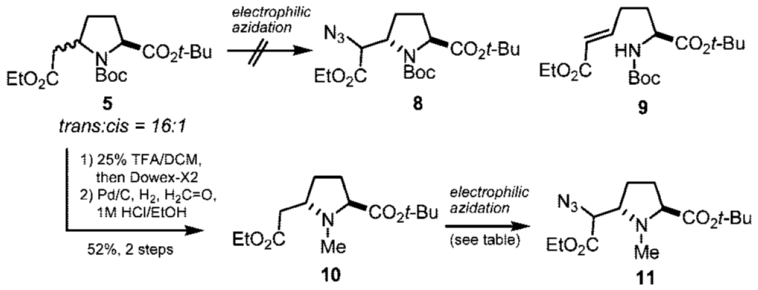


Figure 1.
Pyochelin and configurationally stable proline-based analogues.



Scheme 1.
Attempted C–H Insertion Route



entry	base	electrophile (2 eq.)	isolated % yield 11	diazo
1	2.2 eq LHMDS	Trisyl-N_3	71	2
2	1.1 eq LHMDS	Trisyl-N_3	-	53
3	2.2 eq KHMDS	Trisyl-N_3	68	-
4	1.1 eq KHMDS	Trisyl-N_3	68	-
5	2.2 eq LHMDS	4-$NO_2PhSO_2N_3$	-	70
6	2.2 eq LHMDS	*p*-ABSA	36	-

Scheme 2.
Electrophillic Azidation Reactions

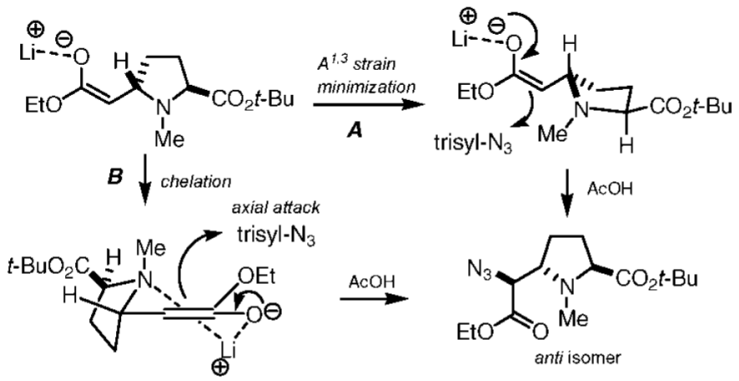
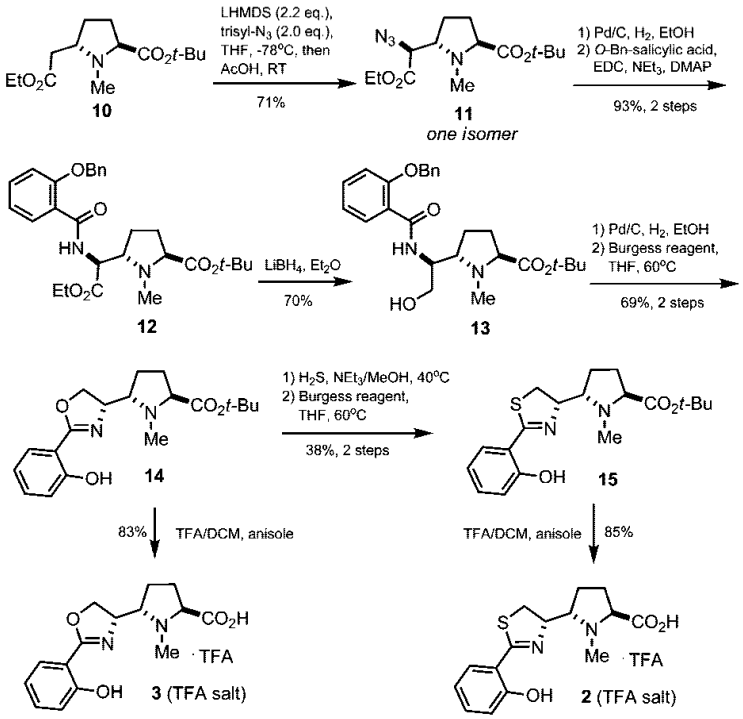
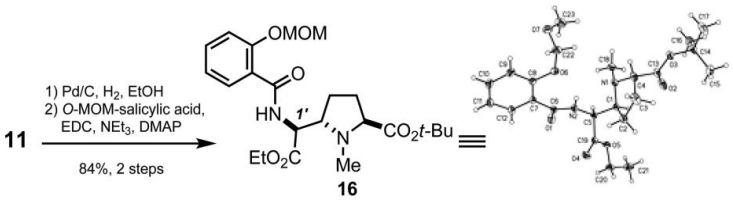


Figure 2.
Proposed transition state models for the electrophilic azidation of 10.



Scheme 3.
Synthesis of Carbapyochelins 2 and 3



Scheme 4.
Synthesis and X-ray Structure of 16