Stereospecific Total Synthesis of *d*-Biotin from L(+)-Cysteine[1]

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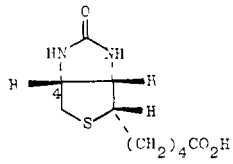
Abstract: The stereospecific total synthesis of the growth promotant *d*-biotin (**1**) is described. The preparation begins with L(+)-cysteine (**2**) and therefore requires no chemical resolution. The aldehyde **7** is synthesized in four steps from **2** and converted to the trans olefin **20** via a Grignard reaction to the vinyl alcohol **19** followed by a Claisen rearrangement. In line with pertinent observations described in model systems, the trans olefin **20** undergoes a dramatic oxidative cyclization-rearrangement to yield the key bromourethane **21**. The chemistry of the derived amino bromide **22** is elucidated and involves a further rearrangement implicating the aziridine **23**. Treatment of the bromo lactam **29** with azide under S_N2 conditions yields the desired cis azido lactam **32** which is converted to *d*-bisnorbiotin methyl ester **37**. An independent synthesis of **37** in an optically pure state is related in order to confirm the identity and optical purity of the product. The final sequence involves chain elongation of **37** employing a malonate ester alkylation of the derived thiophanium bromide **46**. The resultant *d*-biotin (**1**) is shown to be identical in all respects with the natural product.

In a series of biological investigations, Bateman[2] discovered that a diet abnormally high in egg white content generated a toxic reaction in experimental animals, causing a severe dermatitis as well as other degenerative effects. Certain nutrients such as yeast, liver, milk, and egg yolk were later found by Boas[3] to exert a curative influence, and in fact wholly prevented egg-white toxicity. He called the active principle "protective factor X". However, György and DuVigneaud,[4] working with liver concentrates, actually isolated in 1940 a pure sample of this substance, which they dubbed vitamin H. At about the same time, Kögl,[5] who was studying Bios BII, an egg yolk concentrate known to promote optimal growth in yeast, separated the active component and called the substance biotin.

The structure of vitamin H was elucidated in 1942 by DuVigneaud[6] and subsequently confirmed by synthesis.[7] Although Kögl persisted in the assignment of an isomeric structure to his substance,[8] a total microbiological profile carried out by Krueger and Peterson[9] suggested the identity of biotin and vitamin H. Recent discoveries[10] of important applications of biotin in the areas of growth promotion and nutrition have fostered a renewed interest in the total synthesis of this molecule. These efforts have culminated in the recent development of several novel preparations of biotin.[11]

We were intrigued with the possibility of using L(+)-cysteine (**2**) as starting material for our synthesis. Biosynthetic studies had confirmed the facile incorporation of L(+)-cysteine into the biotin framework.[12] In addition, a recent chemical correlation[13] of D-glucose with *d*-dethiobiotin, prepared by Raney nickel desulfurization of *d*-biotin (**1**), the biologically



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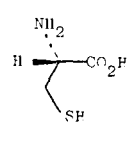

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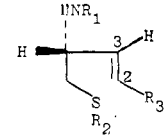
active enantiomer, provided unequivocal chemical proof that the configuration of L(+)-cysteine correlated with that of the C(4) carbon of *d*-biotin. Finally, an x-ray structure determination[14] of the bis-α-bromoanilide of carbon dioxide biotin confirmed the absolute configuration of *d*-biotin (**1**).

Our synthetic plan called for the establishment of the C(2)-C(3) bond with suitable functionality for the attachment of the two heteroatoms at these positions in a stereocontrolled fashion. An olefin of general structure **3**, for example, would fulfill these objectives.

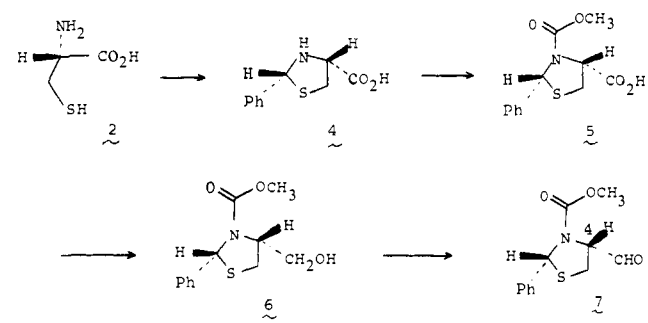


3a R_3 = cis
3b R_3 = trans

Fortunately, numerous methods exist for the stereospecific production of olefins, allowing a choice of a **3a** or **3b** substrate to be constructed. In addition, we anticipated various reactions which would afford a stereospecific cis or trans mode of electrophilic addition to this double bond, thus giving even greater versatility to our intermediate. Specifically, **3a** requires a cis addition of the ultimate heteroatoms, while **3b** necessitates a trans motif.

Toward this end, the reactive functionality present in L(+)-cysteine had to be protected and the carboxyl group reduced to the oxidation level of an aldehyde, since a Wittig reaction was anticipated for the preparation of an olefin of type **3a**. Therefore, as depicted in Scheme I, L(+)-cysteine (**2**) was

Scheme I



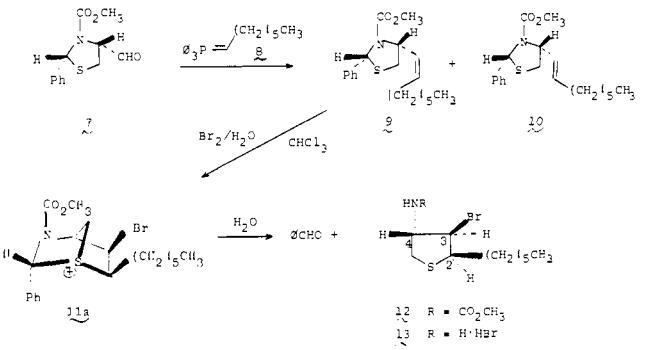
condensed with benzaldehyde to yield the desired thiazolidine **4**. Since further protection of the nitrogen atom was necessary, the methylurethane **5** was selected in the hope that this grouping might later serve as a precursor for the imidazolidone ring of biotin. This was prepared easily from **4** by reaction with methyl chloroformate. The carboxyl function was next reduced selectively in the presence of the urethane moiety by the action of diborane to yield the alcohol **6**, which was oxidized to the key intermediate aldehyde **7** by a modified Collins[15] procedure.

This entire sequence proceeded smoothly and the aldehyde **7** was thus available in an overall yield of 74% from L(+)-cysteine. An x-ray structure determination was carried out on the alcohol **6** and showed a cis relationship of the phenyl and hydroxy methylene substituents.[16]

NMR studies revealed the epimeric purity at C(4) of the aldehyde **7** to be >90%. Furthermore, the compound was easily epimerized at C(3) by either chromatography or treatment with tertiary amines. Fortunately, the crude aldehyde **7** could be used directly without any further and perhaps treacherous purification.

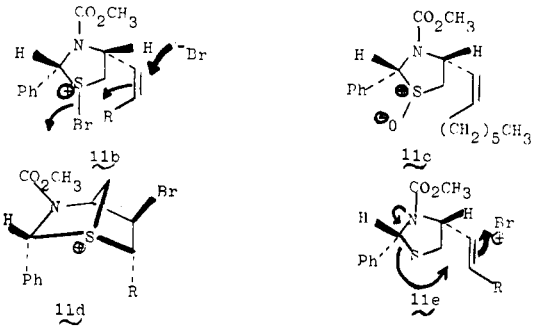
Wittig reaction of the aldehyde **7** with the hexylphosphorane **8**[17] (Scheme II) yielded the cis olefin **9,** contaminated by only

Scheme II



trace amounts of the trans isomer **10.** Upon treatment of the cis olefin **9** with bromine in chloroform to which 1 equiv of water had been added, a remarkable and totally stereospecific rearrangement was found to occur.

Rather than a straightforward addition of bromine to the double bond, the optically active tetrahydrothiophene bromide **12** was obtained as a pure diastereomer. Careful examination of the reaction mixture demonstrated the total absence of other possible stereoisomers. This unique observation may implicate anchimeric stabilization of the initial olefinic bromonium ion by the proximate sulfur atom to form a sulfonium cationic intermediate such as **11a.** Alternatively, initial attack of the bromonium species may occur at sulfur to generate a monocyclic sulfonium cation **11b.** Such a transient intermediate may



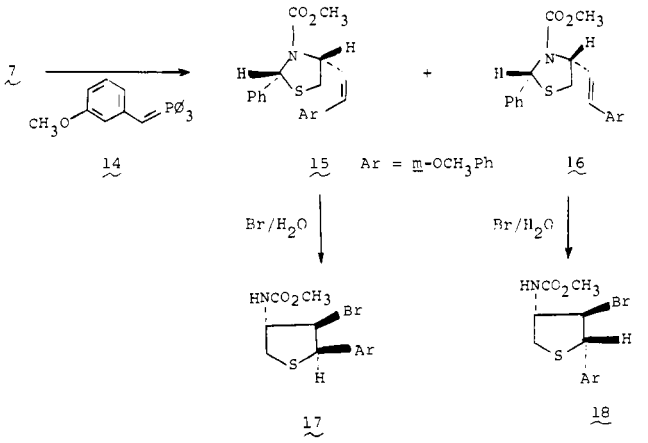
then direct a stereospecific addition to the neighboring double bond, in the manner shown.[17a] Support for this latter mechanism was obtained by the isolation of the sulfoxide **11c** in quantitative yield upon peroxidation of the olefin **9.** This pathway may not be especially favored in the case of trans olefins **3b,** since the bicyclic moiety **11d** would possess a destabilizing 1,3 interaction. In this case, a direct fragmentation of the thiazolidine system may be occurring as depicted in **11e.**

At any rate, this result not only necessitates a trans mode of addition of bromine and sulfur to the double bond, but also guarantees the attachment of the bromide substituent at C(3) to be trans to the carbomethoxyamino function at C(4) as desired. Reaction of the cation **11a** with water then presumably generates the bromide **12** and benzaldehyde, which was also isolated from the reaction. An x ray was carried out on the derived amino bromide hydrobromide **13,** prepared by treatment of **12** with HBr/acetic acid, and completely confirmed these structural designations.

At this juncture, we realized that the β orientation of bromine at C(3) was well set up for an S_N2 displacement by a nitrogen nucleophile to afford the desired cis relationship of nitrogens at C(3) and C(4). Furthermore, in view of our mechanistic rationale, the β configuration of the side chain at C(2) (which would lead to *epi*-biotin) could be precisely reversed if the bromination were carried out on a trans rather than cis olefinic precursor. This alteration should invert the stereochemistry at C(2), but still force the incoming bromine to assume the desired β orientation.

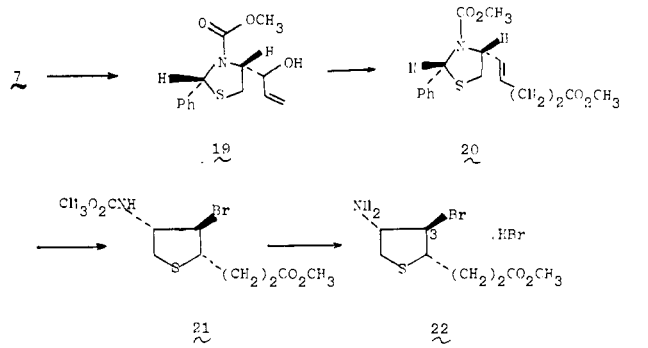
To test this theory, the aldehyde **7** was reacted with the phosphorane **14**[18] to produce an easily separable mixture of cis and trans styrenes **15** and **16** (Scheme III). A pure sample of each compound was then treated with bromine as before,

Scheme III



and a unique bromide **17** or **18** was produced in a completely stereospecific fashion. No trace of the other stereoisomeric bromide could be detected in each individual reaction. With this result in hand, our synthetic strategy was clear. Bromination of a pure trans olefin of general structure **3b** would provide an intermediate analogous to the model compound **18** whose stereochemistry is ideally suited for ultimate conversion to *d*-biotin. To this end, the aldehyde **7** was reacted with a vinyl Grignard reagent to produce the vinyl alcohol **19** (Scheme IV).

Scheme IV

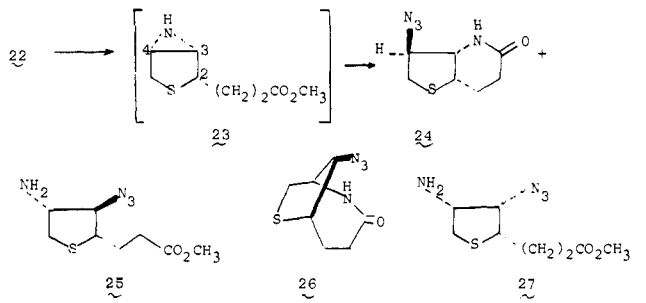


This compound underwent a Claisen rearrangement and afforded the trans olefin **20** in excellent yield. After some developmental work, the desired bromo urethane **21** was available from the olefin **20** by reaction with pyridinium hydrobromide perbromide in methanol. The product was produced stereospecifically, and no trace of any other stereoisomer could be detected. Of utmost importance was the fact that the beautifully crystalline intermediate **21** was still optically active, implying that epimerization had not occurred at C(3) in any

of the previous intermediates. The ultimate correlation of this bromo urethane **21** with *d*-biotin verified that the optical purity at this point is 100%. Interestingly, HBr/acetic acid treatment of the bromo urethane **21** cleanly hydrolyzed the carbomethoxyamino function leaving the ester group untouched. The resultant amino bromide hydrobromide **22** was assumed to have the indicated structure on the basis of mechanistic analogy. This fact was later demonstrated by an x ray on a subsequent intermediate.

Elaboration of the newly won amino bromide hydrobromide **22** in the direction of *d*-biotin required a direct S_N2 displacement of the C(3) bromine by nitrogen with inversion. Although flanked by two α substituents on the same side of the molecule from which azide should attack, the molecule smoothly incorporated the azido moiety giving a 9:1 mixture of the trans azido lactam **24** and the amino azido ester **25** (Scheme V). We

Scheme V

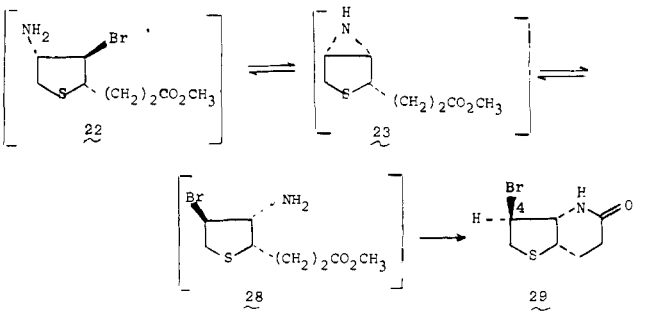


had at first optimistically assigned these products the desired structures **26** and **27**, respectively. However, the mass spectrum of the azido lactam was inconsistent with **26** but could be easily rationalized in terms of the isomeric alternative **24.** Once again, a complete x-ray structure determination was carried out on the trans azido lactam **24** and unequivocally confirmed these results. The obtention of the rearranged products **24** and **25** implicates the intermediacy of the aziridine **23.** Ring opening of **23** by azide from the now unencumbered β side leads to **24** by attack at C(4) and **25** by reaction at C(3). The marked predominance of **24** is clearly a result of the substitution at C(2) which sterically crowds approach to C(3). Finally, the irreversible nature of the aziridine ring opening by azide was demonstrated by the fact that a pure sample of the amino azido ester **25** did not afford any **24** under the reaction conditions.

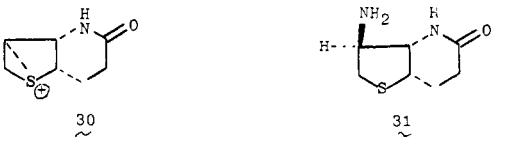
Therefore, we were confronted with the problem that our carefully constructed amino bromide **22,** whose stereochemistry seemed at first ideally suited for transformation into the elusive all-cis framework of biotin, was rearranging to a useless progenitor.

This rearrangement to aziridines was unavoidable, and its occurrence was observed in a variety of studies aimed at the direct displacement of bromine by a variety of nucleophiles, both inter- and intramolecularly.[19] Since the incoming nucleophile was incorporated at C(4) in a trans relationship to the lactam function, we reasoned that should the nucleophile itself be a leaving group, rather than the desired nitrogen, a subsequent S_N2 displacement on it would then create the stipulated all-cis stereochemistry about the tetrahydrothiophene ring.

When the amino bromide hydrobromide **22** was simply refluxed in acetic acid, an equilibrium was set up between the two isomeric amino bromides **22** and **28** via the intermediate aziridine **23.** Owing to the fortunate length of the side chain at C(2), the particular amino bromide **28** was removed from the equilibrium as it formed the beautifully crystalline, optically active trans bromo lactam **29** in quantitative yield. All that remained was to replace the bromide of **29** with azide at C(4) with inversion.

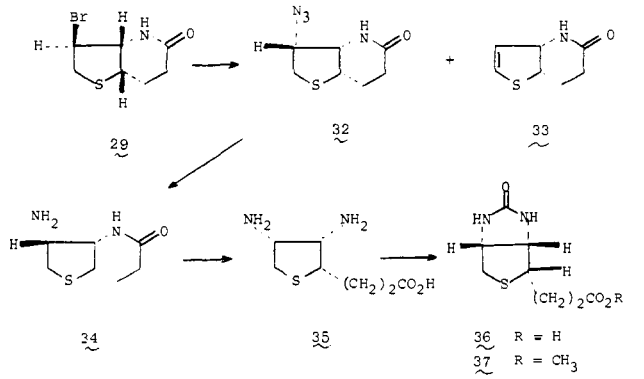


Reaction of the trans bromide **29** with sodium azide in dioxane/water quantitatively produced the previously obtained trans azido lactam **24.** The displacement had in fact occurred with 100% retention at C(4)! This presumably results from anchimeric assistance by sulfur in the solvolysis of the bromide via the intermediate sulfonium cation **30.** This identity of the trans azido lactams was further confirmed by their hydrogenation to the same trans amino lactam **31.**



The ultimate solution to the preparation of the desired cis azido lactam **32** consisted in carrying out the reaction with lithium azide in a polar aprotic solvent which favored an S_N2 mechanism (Scheme VI). Under these conditions, no trace of

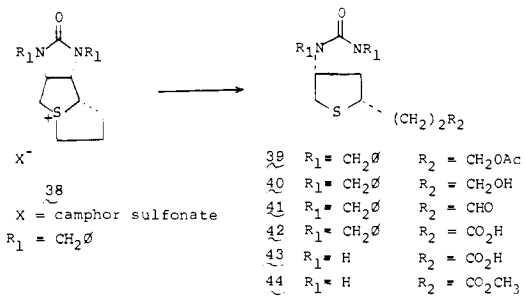
Scheme VI



the undesired trans azide **24,** a product of an S_N1 type reaction, could be detected. The major product, in addition to the cis azide **32,** was the dihydrothiophene **33,** a product of an E2 elimination. Under a wide variety of conditions, varying mostly the polarity of the solvent, either only pure **24** or a mixture of **32** and **33** was obtained.

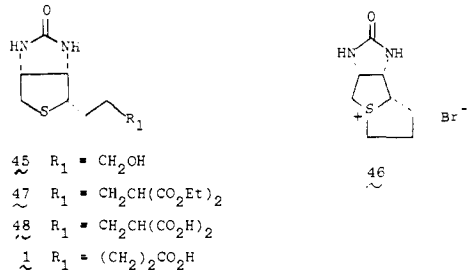
Hydrogenation of the cis azide **32** smoothly afforded the nicely crystalline cis amino lactam **34,** which was clearly isomeric at C(4) with the previously described trans analogue **31.** The sequence of barium hydroxide hydrolysis to the diamino acid **35,** followed by treatment with a phosgene solution in aqueous bicarbonate, smoothly produced *d*-bisnorbiotin **36,** isolated as its methyl ester **37.**

At this junction, an independent synthesis of authentic *d*-bisnorbiotin methyl ester was carried out in order to unequivocally prove our structural assignments and independently check the optical purity of **37.** Therefore, the dibenzylcamphor sulfonate salt **38,** an optically pure intermediate used in the commercial synthesis of biotin,[20] was reacted first with sodium acetate followed by sodium hydroxide to produce the acetate **39** and the alcohol **40,** respectively.[21] Moffatt oxidation[22] gave a respectable yield of the aldehyde **41,** which was further oxidized to the desired acid **42** by silver oxide. Debenzylation was



effected by refluxing the acid **42** in 48% HBr to yield authentic *d*-bisnorbiotin **43 = 36,** isolated as its methyl ester **44 = 37.** Our synthetic sample **37** was identical in all respects with this authentic material **44,** thus verifying the structural designations. Furthermore, a comparison of optical purities demonstrated that our synthesis had preserved 100% of the optical integrity present in L(+)-cysteine. Finally, basic hydrolysis of our material regenerated *d*-bisnorbiotin, which is itself a natural product of the microbiological degradation of *d*-biotin. The identity of our sample with the natural metabolite was easily demonstrated.[23]

The completion of our synthesis necessitated the conversion of *d*-bisnorbiotin methyl ester **37** to *d*-biotin (**1**). Reduction of **37** with lithium borohydride yielded *d*-bisnorbiotinol **45,** which was smoothly converted to the thiophanium bromide **46.** Treatment of the salt **46** with sodium diethylmalonate yielded the diester **47,** which was isolated as the diacid **48.** Decarboxylation in refluxing water cleanly produced *d*-biotin (**1**), identical in all respects with the natural product.



It is interesting to note that the amino group of L(+)-cysteine (**2**) which was ultimately to be attached to C(4) in the resulting biotin structure has in fact been secured to C(3), a result of the novel rearrangement of the amino bromide **22.** This result underscores the interplay of synthetic design and experimental fact that often renders synthetic organic chemistry so fascinating. In summary, we have accomplished a total stereospecific synthesis of *d*-biotin from L(+)-cysteine, a preparation which requires no resolution of any intermediates and proceeds without detectable racemization.

Experimental Section[24]

4-(*R*)-Carboxy-2-(*R*)-phenylthiazolidine (4). To a solution of 60.0 g (0.342 mol) of L(+)-cysteine hydrochloride hydrate in 525 mL of water was added 36 g (0.368 mol) of potassium acetate. After a solution was obtained, 525 mL of 95% ethanol was added followed immediately by 42 mL (44.2 g, 0.417 mol) of benzaldehyde, added in one portion. The product thiazolidine **4** soon began to crystallize. The reaction was kept at 25 °C for 3 h and an additional 3 h at 0 °C. The product was filtered, washed with ethanol, and dried to afford 72 g (98%) of the thiazolidine **4**: mp 159–160 °C (ethanol); $[\alpha]^{25}_D$ −135.1° (*c* 1.03, Me_2SO); IR (KBr) 2700–2400 (NH_3^+), 1600–1550 (CO_2^-), 1360 cm^{-1}; NMR (Me_2SO) δ 7.40 (m, 5 H, Ph), 6.8 (bm, 1 H, NH), 5.8 (s, 1 H, CH), 4.4–4.0 (dd, 1 H, $CHCO_2H$), 3.5–3.0 (m, 2 H, CH_2); mass spectrum *m/e* 209 (M^+), 170, 164, 137 (base).

Anal. Calcd for $C_{10}H_{11}NO_2S$ (209.27): C, 57.40; H, 5.30; N, 6.69; S, 15.32. Found: C, 56.88; H, 5.29; N, 6.81; S, 15.88.

3-Carbomethoxy-4-(*R*)-carboxy-2-(*R*)-phenylthiazolidine (5). To a mixture of 60 g of sodium carbonate in 330 mL of water and 220 mL of 10% sodium bicarbonate was added 62.7 g (0.3 mol) of the thiazolidine **4.** A solution of 23.0 mL (28.4 g, 0.3 mol) of methyl chloroformate in 75 mL of ether was added dropwise over 0.5 h. The reaction mixture was stirred at 25 °C for 1 h and acidified to pH 1 by the dropwise addition of 50 mL of 6 N hydrochloric acid. The mixture was extracted several times with methylene chloride. The organic phases were dried over sodium sulfate and evaporated to afford 77.4 g (97%) of the urethane **5** as a white, crystalline solid: mp 129–130 °C (ethyl acetate/petroleum ether); $[\alpha]^{25}_D$ +122.8° (*c* 1.04, CH_3OH); IR ($CHCl_3$) 3000–2400 (acid OH), 1720 (urethane), 1700 (acid), 1450, 1380 cm^{-1}; NMR ($CDCl_3$) δ 10.27 (s, 1 H, CO_2H), 7.8–7.3 (m, 5 H, Ph), 6.3 (s, 1 H, CH), 5.1 (dd, 1 H, $CHCO_2H$), 3.8 (s, 3 H, OCH_3), 3.6 (d, 2 H, CH_2); mass spectrum *m/e* 267 (M^+), 236, 222, 208, 135 (base), 152.

Anal. Calcd for $C_{12}H_{13}NO_4S$ (267.30): C, 54.00; H, 4.87; N, 5.25; S, 11.98. Found: C, 54.03; H, 4.94; N, 5.27; S, 11.63.

3-Carbomethoxy-4-(*R*)-hydroxymethyl-2-(*R*)-phenylthiazolidine (6). To a solution of 80.0 g (0.3 mol) of the urethane **5** in 150 mL of dry tetrahydrofuran was added dropwise at 25 °C 400 mL (0.4 mol) of a 1 M solution of diborane in tetrahydrofuran. The reaction mixture was stored at 25 °C for 0.5 h and then quenched with 800 mL of water. The mixture was treated with 300 mL of 10% sodium bicarbonate and extracted several times with methylene chloride. The organic phases were dried over sodium sulfate and evaporated to yield 75.0 g (99%) of the alcohol **6** as a white solid: mp 85–86 °C (methylene chloride/hexane); $[\alpha]^{25}_D$ +139.5° (*c* 1.08, acetone); IR (KBr) 3500–3400 (OH), 1700 (urethane), 1450, 1360 cm^{-1}; NMR ($CDCl_3$) δ 7.3 (bs, 5 H, Ph), 6.12 (s, 1 H, CH), 4.5 (dd, 1 H, $CHCH_2$), 3.9 (d, 2 H, CH_2OH), 3.6 (s, 3 H, OCH_3), 3.6–2.8 (m, 3 H, CH_2 + OH); mass spectrum *m/e* 253 (M^+), 222 (base), 195, 194, 176.

Anal. Calcd for $C_{12}H_{15}NO_3S$ (253.22): C, 56.90; H, 5.97; N, 5.53; S, 12.66. Found: C, 56.71; H, 5.79; N, 5.46; S, 12.41.

3-Carbomethoxy-4-(*R*)-formyl-2-(*R*)-phenylthiazolidine (7). To a mixture of 1200 mL of dry methylene chloride and 77.6 mL of dry pyridine was added 48 g (0.48 mol) of chromium trioxide (mechanical stirring). After 15 min at 25 °C, the reaction mixture was treated in one portion with a solution of 20.24 g (0.08 mol) of the alcohol **6** in 50 mL of dry methylene chloride. The reaction mixture was stirred for an additional 15 min and decanted. The reaction flask was further washed with three 200-mL portions of methylene chloride. The supernatant was combined with the washings and the mixture was evaporated. The residue was triturated with three 200-mL portions of ether. The ether phases were combined and washed with 1 N hydrochloric acid until pH of the aqueous layer was 1. The organic phase was washed with water, dried over magnesium sulfate, and evaporated to afford 16.0 g (80%) of the aldehyde **7** as a colorless oil, pure by TLC and used directly in the next step: $[\alpha]^{25}_D$ +151.7° (*c* 0.95, CH_3OH); IR ($CHCl_3$) 1720 (urethane), 1700 (CHO), 1440, 1380 cm^{-1}; NMR ($CDCl_3$) δ 9.4 (s, 1 H, CHO), 7.4–7.0 (bs, 5 H, Ph), 6.1 (s, 1 H, CH), 4.8 (dd, 1 H, $CHCHO$), 3.6 (s, 3 H, OCH_3), 3.2 (d, 2 H, CH_2); mass spectrum *m/e* 251 (M^+), 222 (base), 195.

***cis*-3-Carbomethoxy-4-(*R*)-(oct-2-enyl)-2-(*R*)-phenylthiazolidine (9).** A suspension of 13.86 g (0.0314 mol) of *n*-hexyltriphenylphosphonium bromide[17] in 200 mL of dry tetrahydrofuran was treated under nitrogen with 19.6 mL of *n*-butyllithium (1.6 N in hexane). The blood-red solution was allowed to stir at 25 °C for 15 min and treated dropwise with 7.9 g (0.0314 mol) of the aldehyde **7** in 50 mL of dry tetrahydrofuran. After 20 min at 25 °C and 10 min at 50 °C, the reaction mixture was cooled to room temperature and stored for 1 h.

The mixture was partitioned between water and methylene chloride. The aqueous phase was further extracted with methylene chloride. The extracts were dried over sodium sulfate and evaporated to yield 20 g of residue, which was filtered through 100 g of silica using ethyl acetate/hexane, 1:1. The cis olefin **9** was eluted first and obtained in a yield of 6.18 g (59%) as a colorless oil: $[\alpha]^{25}_D$ +223.0° (*c* 1.02, CH_3OH); IR (CH_2Cl_2) 1700 (urethane), 1440, 1380 cm^{-1}; NMR ($CDCl_3$) δ 7.4–7.2 (bs, 5 H, Ph), 6.2 (s, 1 H, CH), 5.5 (m, 2 H, CH=CH), 5.2 (m, 1 H, $CHCH_2$), 3.6 (s, 3 H, OCH_3), 3.3–2.4 (m, 2 H, CH_2), 2.2 (m, 2 H, allylic CH_2), 1.4–1.0 (bm, 8 H, $(CH_2)_4$), 1.0–0.8 (t, 3 H, CH_3); mass spectrum *m/e* 333 (M^+), 286, 195, 164, 118 (base).

Anal. Calcd for $C_{19}H_{27}NO_2S$ (333.49): C, 68.43; H, 8.16; N, 4.20; S, 9.61. Found: C, 68.31; H, 8.11; N, 4.59; S, 9.67.

3-(*R*)-Bromo-2-(*R*)-*n*-hexyltetrahydrothiophene-4-(*S*)-carbamic Acid Methyl Ester (12). A solution of 1.99 g (0.006 mol) of the cis olefin **9** in 60 mL of chloroform to which 0.11 mL of water had been added was treated with 60 mL of a 0.1 N solution of bromine in

chloroform at 25 °C. After 20 min, the reaction mixture was evaporated to dryness and chromatographed over 100 g of silica, eluting with ethyl acetate/hexane, 1:1. The desired bromo urethane **12** was collected and afforded 1.5 g (77%) of product as a white, crystalline solid: mp 85–86 °C (petroleum ether); $[\alpha]^{25}_D$ −129.2° (*c* 1.03, CH_3OH); IR (KBr) 3400 (NH), 1700 (urethane), 1550 cm^{-1}; NMR ($CDCl_3$) δ 5.42 (bd, 1 H, NH), 4.8–4.5 (m, 2 H, CHCH), 3.71 (s, 3 H, OCH_3), 3.7–3.5, 2.8–2.6 (m, 3 H, CH_2SCH), 2–1.1 (m, 10 H, $(CH_2)_5$), 0.88 (t, 3 H, CH_3); mass spectrum *m/e* 325/323 (M^+), 294, 244.

Anal. Calcd for $C_{12}H_{22}BrNO_2S$ (324.29): C, 44.45; H, 6.84; N, 4.32; S, 9.89; Br, 24.64. Found: C, 44.63; H, 6.97; N, 4.15; S, 10.07; Br, 24.69.

4-(*S*)-Amino-3-(*R*)-bromo-2-(*R*)-*n*-hexyltetrahydrothiophene Hydrobromide (13). A solution of 32 mg (0.1 mmol) of the bromo urethane **12** in 3 mL of a saturated solution of gaseous hydrogen bromide in acetic acid was stored in the dark at 25 °C for 20 h. The solution was evaporated to dryness to yield 32 mg (91%) of the amino bromide hydrobromide **13**. The product was recrystallized from methanol/ether to afford 23 mg of pure material as a white solid: mp 155–156 °C; $[\alpha]^{25}_D$ +135.0° (*c* 1.07, CH_3OH); IR (KBr) 3000–2500 (NH_3^+), 1600, 1500 cm^{-1}; NMR (D_2O) δ 5.6 (m, 1 H, CHBr), 4.8 (m, H, C*H*NH_3), 4.13 (m, 1 H, CH), 4.2–3.5 (m, 2 H, CH_2S), 2.6–1.5 (m, 10 H, $(CH_2)_5$), 1.6–1.4 (t, 3 H, CH_3); mass spectrum *m/e* 266/264 (M^+), 186 (M − Br), 169 (M − Br − NH_3), 152.

Anal. Calcd for $C_{10}H_{20}BrNS{\cdot}HBr$ (347.17): C, 34.60; H, 6.10; N, 4.03; S, 9.24; Br, 46.04. Found: C, 34.57; H, 6.07; N, 3.97; S, 9.32; Br, 45.99.

***cis*- and *trans*-3-Carbomethoxy-4-(*R*)-(3-methoxystyryl)-2-(*R*)-phenylthiazolidine (15 and 16).** To a solution of 0.06 mol of the phosphorane **14**[18] in 500 mL of dry tetrahydrofuran was added 15.0 g (0.06 mol) of the aldehyde **7** in 100 mL of dry tetrahydrofuran. The reaction mixture was stirred at 25 °C for 1.5 h. The solid was filtered off, and the filtrate was evaporated to yield 30.0 g of residue. This material was filtered through 500 g of silica, eluting with ethyl acetate/hexane (3:7) to afford 11.0 g (52%) of the olefins **15** and **16**. Both materials exist as viscous oils. Data for **15**: IR (CH_2Cl_2) 1700 (urethane), 1600, 1440, 1370 cm^{-1}; NMR ($CDCl_3$) δ 7.6–6.9 (m, 9 H, aromatic), 6.56 (d, 1 H, olefin), 6.22 (s, 1 H, CHPh), 5.79 (dd, 1 H, olefin), 5.31 (m, 1 H, CH), 3.80 (s, 3 H, OCH_3), 3.58 (s, 3 H, CO_2CH_3), 3.4–2.8 (m, 2 H, CH_2); UV max (CH_3OH) 283 (ε 2550), 241 sh (10 600), 210 infl (36 100); mass spectrum *m/e* 355 (M^+), 309, 233, 160 (base).

Anal. Calcd for $C_{20}H_{21}NO_3S$ (355.46): C, 67.58; H, 5.96; N, 3.94; S, 9.02. Found: C, 67.58; H, 6.17; N, 3.85; S, 8.90.

Data for **16**: IR ($CHCl_3$) 1700 (urethane), 1600, 1450, 1360 cm^{-1}; NMR ($CDCl_3$) δ 7.6–6.9 (m, 9 H, aromatic), 6.8–6.3 (m, 2 H, olefin), 6.24 (s, 1 H, CHPh), 5.10 (m, 1 H, CH), 3.8 (s, 3 H, OCH_3), 3.6 (s, 3 H, CO_2CH_3), 3.5–2.8 (m, 2 H, CH_2); UV max 297 nm (ε 3000), 257 (14 200), 214 (30 400); mass spectrum *m/e* 355 (M^+), 309, 233 (base), 218, 192.

Anal. Calcd for $C_{20}H_{21}NO_3S$ (355.46): C, 67.58; H, 5.96; N, 3.94. Found: C, 67.48; H, 6.17; N, 3.92.

3-(*R*)-Bromo-2-(*R*)-(3-methoxyphenyl)tetrahydrothiophene-4-(*S*)-carbamic Acid Methyl Ester (17). To a solution of 1.46 g (0.0041 mol) of the cis olefin **15** in 40 mL of dry chloroform at 25 °C were added 0.074 mL of water and 41 mL of a 0.1 N solution of bromine in chloroform. After 0.5 h at 25 °C, the reaction mixture was evaporated, and the residue chromatographed over silica, eluting with benzene/ethyl acetate, 95:5. The bromide **17** was obtained as the only isolable product in a yield of 0.923 g (65%). The product was recrystallized from methanol and was obtained as white needles: mp 110–111 °C; $[\alpha]^{25}_D$ +94.4° (*c* 1.0, acetone); IR (KBr) 3400 (NH), 1700 (urethane), 1550, 1280 cm^{-1}; NMR ($CDCl_3$) δ 7.2–6.9 (m, 4 H, aromatic), 5.5 (bd, 1 H, NH), 4.9–4.7 (m, 3 H, CHCHCH), 3.8 (s, 3 H, OCH_3), 3.7 (s, 3 H, CO_2CH_3), 3.0–2.8 (dd, 2 H, CH_2); UV max 283 nm (ε 2300), 276 (2400), 220 infl (10 400); mass spectrum *m/e* 345/347 (M^+), 266, 212, 191 (base).

Anal. Calcd for $C_{13}H_{16}BrNO_3S$ (346.25): C, 45.10; H, 4.66; N, 4.05; S, 9.26; Br, 23.08. Found: C, 45.02; H, 4.75; N, 3.89; S, 9.20; Br, 22.95.

3-(*R*)-Bromo-2-(*S*)-(3-methoxyphenyl)tetrahydrothiophene-4-(*S*)-carbamic Acid Methyl Ester (18). To a solution of 1.20 g (0.0034 mol) of the trans olefin **16** in 35 mL of dry chloroform at 25 °C was added 0.061 μL of water and 34 mL of a 0.1 N solution of bromine in chloroform. After 0.5 h at 25 °C, the reaction mixture was evaporated, and the residue was chromatographed over silica, eluting with benzene/ethyl acetate, 95:5. The bromide **18** was obtained as the only isolable product in a yield of 0.506 g (43%). The product was recrystallized from methanol and yielded glistening plates: mp 179–180 °C; IR (KBr) 3380 (NH), 1710 (urethane), 1570, 1300 cm^{-1}; NMR ($CDCl_3$) δ 7.4–6.8 (m, 4 H, aromatic), 5.2 (bd, 1 H, NH), 4.6–4.0 (m, 3 H, CHCHCH), 3.8 (s, 3 H, OCH_3), 3.6 (s, 3 H, CO_2CH_3), 3.2–3.0 (m, 2 H, CH_2); UV max 283 nm (ε 2200), 276 (2400), 225 infl (9000); mass spectrum *m/e* 345/347 (M^+), 265 (M^+ − Br), 212, 191 (base), 145.

Anal. Calcd for $C_{13}H_{16}BrNO_3S$ (346.25): C, 45.10; H, 4.66; N, 4.05; S, 9.26; Br, 23.08. Found: C, 44.86; H, 4.66; N, 3.98; S, 9.08; Br, 22.99.

3-Carbomethoxy-4-(*R*)-(1-hydroxyprop-2-en-1-yl)-2-(*R*)-phenylthiazolidine (19). To a mixture of 310 mL (0.440 mol) of 1.42 N (THF) vinylmagnesium chloride solution (Ventron Corp.) and 250 mL of dry methylene chloride was added dropwise over 10 min at −75 °C a solution of 14.2 g (0.0565 mol) of the aldehyde **7** in 50 mL of dry methylene chloride. The reaction mixture was stirred at −75 °C for 0.5 h and quenched at that temperature by the dropwise addition of 30 mL of methanol. After the addition of 150 mL of saturated ammonium chloride, the mixture was warmed to 25 °C and extracted several times with methylene chloride. The organic phases were washed with water, dried over sodium sulfate, and evaporated to afford 13.7 g (87%) of the vinyl alcohol **19** as a viscous oil. The material was used directly in the next step. For analysis, a sample was chromatographed over silica, eluting with benzene/ethyl acetate, 1:1, to provide a colorless oil: $[\alpha]^{25}_D$ +101.6° (*c* 1.0, CH_3OH); IR ($CHCl_3$) 3600–3400 (OH), 1700 (urethane), 1460, 1380 cm^{-1}; NMR ($CDCl_3$) δ 7.6–7.3 (m, 5 H, aromatic), 6.2 (s, 1 H, CHPh), 6.1–5.2 (m, 3 H, vinyl), 4.5–4.1 (m, 2 H, CHCHN), 3.7 (s, 3 H, OCH_3), 3.5–2.8 (m, 2 H, CH_2), 2.3 (bs, 1 H, OH); mass spectrum *m/e* 203 (M^+), 146 (base).

Anal. Calcd for $C_{14}H_{17}NO_3S$ (279.36): C, 60.19; H, 6.13; N, 5.01; S, 11.18. Found: C, 59.88; H, 6.25; N, 4.92; S, 11.08.

Methyl (3-Carbomethoxy-2-(*R*)-phenylthiazolidin-4-(*R*)-yl)-4-pentenoate (20). A solution of 44 g (0.158 mol) of the vinyl alcohol **19** 191.5 g (1.58 mol) of trimethyl orthoacetate, and 100 mL of a 20% solution of propionic acid/benzene in 2 L of benzene was heated for 24 h at 92 °C (Dean-Stark trap). The reaction mixture was cooled, washed with 2 N sodium carbonate and water, dried over magnesium sulfate, and evaporated to yield 50.0 g (95%) of the trans olefin **20**, pure by GC, as a colorless oil: $[\alpha]^{25}_D$ +88.3° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1730 (ester), 1700 (urethane), 1430, 1350 cm^{-1}; NMR ($CDCl_3$) δ 7.3 (m, 5 H, aromatic), 6.18 (s, 1 H, CHPh), 5.8–5.6 (m, 2 H, J_{trans} = 16 Hz, olefin), 4.84 (dd, 1 H, CH), 3.65 (s, 3 H, OCH_3), 3.4–2.6 (m, 2 H, CH_2), 2.3 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 335 (M^+), 222, 195 (base).

Anal. Calcd for $C_{17}H_{21}NO_4S$ (335.42): C, 60.88; H, 6.31; N, 4.18; S, 9.56. Found: C, 60.81; H, 6.24; N, 4.32; S, 9.15.

3-(*R*)-Bromo-2-(*S*)-carbomethoxyethyltetrahydrothiophene-4-(*S*)-carbamic Acid Methyl Ester (21). To a solution of 1.6 g (4.77 mmol) of the trans olefin **20** in 40 mL of dry methanol was added at 0 °C 1.50 g (4.77 mmol) of pyridine hydrobromide perbromide. After 5 min, the solution was refluxed for 1 h, cooled, and evaporated. The residue was partitioned between methylene chloride and 1 N hydrochloric acid. The organic phase was dried over sodium sulfate and evaporated. The residue was chromatographed over silica, eluting with benzene/ethyl acetate, 4:1. Benzaldehyde is first eluted, followed cleanly by the desired bromo urethane **21**. The product is obtained as 0.71 g (47%) of a white, crystalline solid: mp 139–140 °C (ethyl acetate/petroleum ether); $[\alpha]^{25}_D$ −17.7° (*c* 1.04, CH_3OH); IR (KBr) 3400 (NH), 1740 (ester), 1720 (urethane), 1540, 1280 cm^{-1}; NMR ($CDCl_3$) δ 5.2 (bd, 1 H, NH), 4.4–3.9 (m, 2 H, CHCH), 3.7 (s, 3 H, OCH_3), 3.7 (s, 2 H, CH_2), 3.5–3.0 (m, 1 H, CHS), 3.0–1.8 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 294/296 (M^+ − OCH_3), 262/264, 245 (M^+ − HBr), 170 (base).

Anal. Calcd for $C_{10}H_{16}BrNO_4S$ (326.21): C, 36.82; H, 4.94; N, 4.29; S, 9.83; Br, 24.50. Found: C, 37.13; H, 5.22; N, 4.41; S, 9.79; Br, 24.47.

3-[4-(*S*)-Amino-(*R*)-bromotetrahydrothiophen-2-(*S*)-yl]propionic Acid Methyl Ester Hydrobromide (22). A solution of 5.5 g (10.68 mmol) of the bromo urethane **21** in 55 mL of acetic acid, which had been previously saturated with gaseous hydrogen bromide, was stored at 25 °C in the dark for 20 h. The solution was evaporated and the residue was recrystallized from ethyl acetate to afford 4.6 g (79%) of pure amino bromide hydrobromide **22** as a white, crystalline solid: mp 160–161 °C; $[\alpha]^{25}_D$ + 18.2° (*c* 0.99, CH_3OH); IR (KBr) 3000–2700

(NH_3^+), 1740 (ester), 1240 cm^{-1}; NMR ($CDCl_3/CD_3OD$) δ 4.5–4.0 (b, 3 H, NH_3), 4.4–3.8 (m, 2 H, CHCH), 3.7 (s, 3 H, OCH_3), 3.6–3.0 (m, 3 H, CH_2SCH), 2.8–1.8 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 268/270 ($M^+ + H$), 236/238, 187 (base).

Anal. Calcd for $C_8H_{14}BrNO_2S \cdot HBr$ (349.10): C, 27.53; H, 4.33; N, 4.01; Br, 45.78; S, 9.19. Found: C, 27.59; H, 4.22; N, 4.20; Br, 45.92; S, 9.14.

3-(*S*)-Amino-4-(*S*)-azidotetrahydrothiophene-2-(*S*)-propionic Acid Lactam (24). A solution of 788 mg (2.26 mmol) of the amino bromide hydrobromide **22** in 45 mL of dry dimethylformamide was treated with 2.0 g of sodium azide and heated at 100 °C for 4 h. The mixture was evaporated, and the residue partitioned between 1 N hydrochloric acid and methylene chloride. The organic phase was dried over sodium sulfate and evaporated to yield 408 mg (91%) of the trans azido lactam **24**. Recrystallization from ethyl acetate yielded white needles: mp 174–175 °C; $[\alpha]^{25}_D$ −100.0° (*c* 1.11, Me_2SO); IR (CH_2Cl_2) 3300 (NH), 2090 (N_3), 1660 cm^{-1} (amide); NMR ($CDCl_3$) δ 7.2 (bd, 1 H, NH), 4.3–3.6 (bm, 3 H, CHCHCH), 3.4–2.4 (m, 2 H, CH_2), 2.4–1.7 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 198 (M^+), 170 ($M^+ - N_2$), 127, 97 (base).

Anal. Calcd for $C_7H_{10}N_4OS$ (198.25): C, 42.41; H, 5.08; N, 28.06; S, 16.17. Found: C, 42.39; H, 5.03; N, 28.21; S, 15.83.

Acidification of the aqueous phase, followed by extraction with methylene chloride, yielded 50 mg (9%) of the amino azido ester **25** as a colorless oil upon evaporation of the organic extracts: IR (CH_2Cl_2) 3400–3200 (NH_2), 2090 (N_3), 1740 (ester), 1435 cm^{-1}.

3-(*R*)-Amino-4-(*S*)-bromotetrahydrothiophene-2-(*S*)-propionic Acid Lactam (29). To a mixture of 1.1 g (0.134 mol) of sodium acetate in 42 mL of acetic acid was added 4.2 g (0.012 mol) of the amino bromide hydrobromide **22**. The solution was heated under reflux for 7 h, cooled, and evaporated. The residue was taken up in methylene chloride and washed with 10% sodium bicarbonate. The organic phase was dried over sodium sulfate and evaporated to yield 2.70 g (96%) of pure trans bromo lactam **29**. For analysis, a sample was recrystallized from ethyl acetate to afford white needles: mp 208–209 °C; $[\alpha]^{25}_D$ −90.7° (*c* 1.05, Me_2SO); IR (KBr) 3250 (NH), 1660 cm^{-1} (lactam); NMR ($CDCl_3$) δ 7.2 (m, 1 H, NH), 4.4–2.4 (m, 5 H), 2.4–1.8 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 235/347 (M^+), 156 ($M^+ - Br$), 129 (base).

Anal. Calcd for $C_7H_{10}BrNOS$ (236.14): C, 35.61; H, 4.27; N, 5.93; S, 13.58; Br, 33.84. Found: C, 35.75; H, 4.30; N, 5.87; S, 13.70; Br, 33.77.

Solvolysis of the Trans Bromo Lactam (29). A mixture of 400 mg (1.70 mmol) of the trans bromo lactam **29** and 520 mg (8.0 mmol) of sodium azide in 8 mL of dioxane/water (2:1) was heated under reflux for 21 h. The reaction mixture was evaporated, and the residue was partitioned between water and methylene chloride. The organic phases were dried over sodium sulfate and evaporated to yield 285 mg (85%) of pure trans azido lactam **24**, mp 174–175 °C, identical in all respects with the sample prepared from the amino bromide hydrobromide **22**.

3-(*S*),4-(*S*)-Diaminotetrahydrothiophene-2-(*S*)-propionic Acid δ-Lactam (31). A solution of 198 mg (1.0 mmol) of the trans azido lactam **24** in 100 mL of absolute ethanol was treated with 200 mg of 10% Pd/C and hydrogenated at 25 °C and 45 psi for 16 h. The autoclave was vented, and the catalyst filtered off. The filtrate was evaporated to afford 170 mg (99%) of pure trans amino lactam **31**. For analysis, a sample was recrystallized from ethyl acetate to yield **31** as a white, crystalline solid: mp 173–174 °C; $[\alpha]^{25}_D$ −22.7° (*c* 1.03, CH_3OH); IR (KBr) 3300–3100 (NH), 1650 (lactam), 1400, 1300 cm^{-1}; NMR ($CDCl_3$) δ 7.8 (b, 1 H, NH), 4.0–3.4 (m, 3 H, CHCHCH), 3.2–2.6 (m, 2 H, CH_2), 2.6–2.0 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 172 (M^+), 162, 130, 98, 75 (base).

Anal. Calcd for $C_7H_{12}N_2OS$ (172.25): C, 48.81; H, 7.02; N, 16.26; S, 18.61. Found: C, 48.61; H, 6.93; N, 16.50; S, 18.45.

3-(*S*)-Amino-4-(*R*)-azidotetrahydrothiophene-2-(*S*)-propionic Acid Lactam (32). A solution of 1.65 g (7.02 mmol) of the trans bromo lactam **29** in 33 mL of dry dimethylformamide to which 1.0 g (20.4 mmol) of lithium azide had been added was heated to 130 °C for 2.5 h. The reaction mixture was evaporated, and the residue was partitioned between methylene chloride/water. The organic phases were dried and evaporated to yield 1.21 g of product mixture. This residue was chromatographed on 12 thick layer silica plates, eluting with chloroform/methanol, 9:1. The less polar band, appearing at R_f 0.2, was isolated and afforded 0.227 g (16%) of the cis azido lactam **32** as a colorless oil: $[\alpha]^{25}_D$ −47.0° (*c* 0.99, CH_3OH); IR (CH_2Cl_2) 3200 (NH), 2090 (N_3), 1665 (lactam), 1350 cm^{-1}; NMR ($CDCl_3$) δ 6.3 (bs, 1 H, NH), 4.2–3.6 (bm, 3 H, CHCHCH), 3.1–2.4 (m, 2 H, CH_2), 2.4–1.8 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 156 ($M^+ - N_3$), 129, 127, 97 (base).

Isolation of the major band at R_f 0.1 yielded 0.771 g (71%) of *cis*-3-(*S*)-amino-2,3-dihydro-2-thiophenepropionic acid lactam (**33**) as a colorless oil: IR (CH_2Cl_2) 3300 (NH), 1650 cm^{-1} (lactam); NMR ($CDCl_3$) δ 7.5 (bs, 1 H, NH), 6.4 (d, 1 H, J = 11 Hz, olefin CHS), 5.5 (dd, 1 H, J = 11 and 4 Hz, olefin CH), 4.7 (bd, 1 H, C*H*NH), 4.2 (m, 1 H, CHS), 3.0–2.0 (m, 4 H, CH_2CH_2).

3-(*S*)-4-(*R*)-Diaminotetrahydrothiophene-2-(*S*)-propionic Acid δ-Lactam (34). A solution of 100 mg (0.51 mmol) of the cis azido lactam **32** in 100 mL of absolute ethanol was treated with 100 mg of 10% Pd/C and hydrogenated at 25 °C and 45 psi for 16 h. The autoclave was vented, and the catalyst was filtered off. The filtrate was evaporated, and the residue was chromatographed on two thick layer silica plates, eluting with chloroform/methanol/ammonium hydroxide, 89:10:1. The desired product was isolated at R_f 0.3 and weighed 60 mg (68%). For analysis, a sample was recrystallized from ethyl acetate to yield the cis amino lactam **34** as a white, crystalline solid: mp 108–109 °C; $[\alpha]^{25}_D$ −24.9° (*c* 0.49, CH_3OH); IR (CH_2Cl_2) 3300 (NH), 1660 cm^{-1} (lactam); NMR ($CDCl_3$) δ 7.2 (bs, 1 H, NH), 4.0 (s, 2 H, NH_2), 3.7 (m, 1 H, CH), 3.4–2.6 (m, 3 H), 2.5 (m, 1 H, CH), 2.4–1.7 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 173 ($M^+ + H$), 98, 75 (base).

Anal. Calcd for $C_7H_{12}N_2OS$ (172.21): C, 48.81; H, 7.02; N, 16.27; S, 18.61. Found: C, 48.77; H, 7.00; N, 16.18; S, 18.57.

***d*-Bisnorbiotin Methyl Ester (37).** To a solution of 60 mg (0.349 mmol) of the cis amino lactam **34** in 7 mL of water was added 1.5 g (7.93 mmol) of barium hydroxide monohydrate. The mixture was refluxed for 20 h and cooled, and the solid was filtered off and washed with water. The filtrate, containing the diamino acid **35**, was concentrated to 10 mL, cooled to 0 °C, and treated with gaseous phosgene until an acid pH was obtained. The mixture was evaporated to dryness and treated with 20 mL of dry methanol and 1 drop of concentrated sulfuric acid. The reaction mixture was heated under reflux for 1 h and cooled, and the suspended salts were filtered off and washed with methanol. The filtrate was concentrated and partitioned between water and methylene chloride/methanol, 4:1. The organic phases were dried over sodium sulfate and evaporated to afford 36 mg (45%) of pure *d*-bisnorbiotin methyl ester (**27**), mp 165–166 °C, after recrystallization from ethyl acetate: $[\alpha]^{25}_D$ +55.7° (*c* 0.96, Me_2SO); IR (KBr) 3300–3200 (NH), 1730 (ester), 1700 cm^{-1} (imidazolidone); NMR (Me_2SO) δ 6.4 (bd, 1 H, NH), 4.4–4.1 (m, 2 H, CHCH), 3.59 (s, 3 H, OCH_3), 3.3–2.5 (m, 3 H, CH_2SCH), 2.4 (t, 2 H, $CH_2CO_2CH_3$), 1.8 (m, 2 H, CH_2); mass spectrum *m/e* 230 (M^+), 199, 170, 97 (base), 87.

Anal. Calcd for $C_9H_{14}N_2O_3S$ (230.29): C, 46.94; H, 6.13; N, 12.16; S, 13.92. Found: C, 46.80; H, 5.98; N, 12.39; S, 14.02.

***d*-Bisnorbiotinol (45).** A solution of 230 mg (1.0 mmol) of *d*-bisnorbiotin methyl ester (**37**) in 10 mL of dry tetrahydrofuran was treated with 44 mg (2.0 mmol) of lithium borohydride and heated under reflux for 3.5 h. The reaction mixture was cooled, acidified with 1 N hydrochloric acid, and evaporated to dryness. The residue was recrystallized from water to afford a first crop of 75 mg of pure alcohol **45**. The mother liquors were found to contain an additional 101 mg of pure *d*-bisnorbiotinol (**45**). Total yield 176 mg (87%) of product: mp 189–191 °C (water); $[\alpha]^{25}_D$ +60.3° (*c* 1.02, Me_2SO); IR (KBr) 3400 (OH), 3300–3100 (NH), 1690 (imidazolidone), 1490 cm^{-1}; NMR (Me_2SO) δ 9.1 (bs, 2 H, NHNH), 6.5 (bs, 2 H, CHCH), 4.6–4.0 (m, 3 H, CH_2OH), 3.2–2.5 (m, 3 H, CH_2SCH), 1.8–1.4 (m, 4 H, CH_2CH_2); mass spectrum *m/e* 202 (M^+), 184 ($M^+ - H_2O$), 142, 57 (base).

Anal. Calcd for $C_8H_{14}N_2O_2S$ (202.28): C, 47.50; H, 6.98; N, 13.85; S, 15.85. Found: C, 47.52; H, 6.96; N, 13.76; S, 15.69.

***all-cis-d*-3,4-(2′-Ketoimidazolido)-1,2-trimethylenethiophanium Bromide (46).** A solution of 250 mg (1.24 mmol) of *d*-bisnorbiotinol (**45**) in 5 mL of acetic acid which had been saturated with gaseous hydrogen bromide was heated at 100 °C for 0.5 h. The reaction mixture was cooled and evaporated. The residue was triturated with methanol. The thiophanium bromide **46** separated and was collected. After recrystallization from water/acetone, 180 mg (68%) of pure product **46**, mp 221–222 °C, $[\alpha]^{25}_D$ +14.3° (*c* 1.01, H_2O), was obtained: IR (KBr) 3300–3200 (NH), 1710 (imidazolidone), 1410 cm^{-1}; NMR (D_2O) δ 5.5 (bs, 2 H, CHCH), 4.8–4.0 (m, 3 H, CH_2SCH), 4.2 (b, 2 H, CH_2S), 3.2–2.8 (m, 4 H, CH_2CH_2).

Anal. Calcd for $C_8H_{13}BrN_2OS$ (265.18): C, 36.24; H, 4.94; N, 10.56; S, 12.09; Br, 30.14. Found: C, 36.25; H, 4.82; N, 10.42; S, 12.33; Br, 29.91.

***d*-Biotin (1).** A solution of 0.46 g (0.03 mol) of sodium in 10 mL of diethyl malonate was treated with 2.65 g (0.01 mol) of the thiophanium bromide **46.** The mixture was heated at 120 °C for 6 h, cooled, and partitioned between water/chloroform. The organic phase was further washed with water and evaporated in vacuo. The residue, containing the diester **47,** was heated under reflux for 3 h with a mixture of 12 g of barium hydroxide and 70 mL of water/methanol, 5:2. The mixture was diluted with water and acidified with dilute sulfuric acid. After filtration of the barium sulfate the filtrate was heated under reflux, filtered hot, and concentrated. The diacid **48,** mp 190 °C dec, was deposited upon cooling. This product was smoothly decarboxylated by heating a 0.2 N aqueous solution at 180 °C for 0.5 h. The reaction mixture was concentrated to afford a sample of pure *d*-biotin (**1**), which separated on cooling, mp 230–232 °C, mmp 230–232 °C, identical in all respects with an authentic sample: IR (KBr) 3300–3250 (NH), 2700–2500 (acid), 1705 (imidazolidone), 1690 cm^{-1} (acid); NMR (Me_2SO) δ 6.7 (bs, 1 H, NH), 6.5 (bs, 1 H, NH), 4.30 (m, 2 H, CHCH), 3.15 (b, 1 H, CHS), 2.75 (m, 2 H, CH_2S), 2.22 (t, 2 H, CH_2), 1.5 (bm, 6 H, $(CH_2)_3$); mass spectrum *m/e* 244 (M^+), 184, 112, 97 (base), 85.

Anal. Calcd for $C_{10}H_{16}N_2O_3S$ (244.29): C, 49.16; H, 6.60; N, 11.47; S, 13.12. Found: C, 48.94; H, 6.43; N, 11.50; S, 13.40.

***d*-Bisnorbiotin Methyl Ester (44) from the Aldehyde 41.** A solution of 2.65 g (0.0076 mol) of the aldehyde **41** (prepared from the camphorsulfonate salt **38**[21]) in 20 mL of methylene chloride/methanol (1:1) was added at 25 °C to a suspension of 2.7 g (0.016 mol) of silver nitrate in 20 mL of an aqueous solution containing 1.24 g (0.031 mmol) of sodium hydroxide. The reaction mixture was vigorously stirred for 0.25 h and filtered. The filtrate was concentrated and partitioned between methylene chloride and 1 N sodium hydroxide. The aqueous phase was acidified and extracted several times with methylene chloride. These latter extracts were dried over sodium sulfate and evaporated to yield 1.6 g (61%) of the dibenzyl acid **42.** This product was used directly and heated under reflux in 40 mL of 48% aqueous HBr. The reaction mixture, now containing *d*-bisnorbiotin (**43**), mp 211–216 °C dec, was taken up in 30 mL of absolute methanol and a catalytic amount of concentrated sulfuric acid. The solution was heated under reflux for 1 h, cooled, and concentrated to dryness. The residue was partitioned between water/methylene chloride, and the material from the organic extracts was chromatographed over silica, eluting with chloroform/methanol, 9:1. A sample of pure *d*-bisnorbiotin methyl ester (**44**), mp 163–164 °C (ethyl acetate), was isolated, identical in all respects with the material **37**, prepared from L(+)-cysteine, mmp 163–164 °C. The optical rotation of this optically pure sample was found to be $[\alpha]^{25}_D$ +55.7° (*c* 0.96, Me_2SO) as compared to $[\alpha]^{25}_D$ +55.7° (*c* 0.96, Me_2SO) of the material **27** prepared from L(+)-cysteine. Therefore, the optical purity of synthetic *d*-bisnorbiotin methyl ester (**27**) is equal to 100%.

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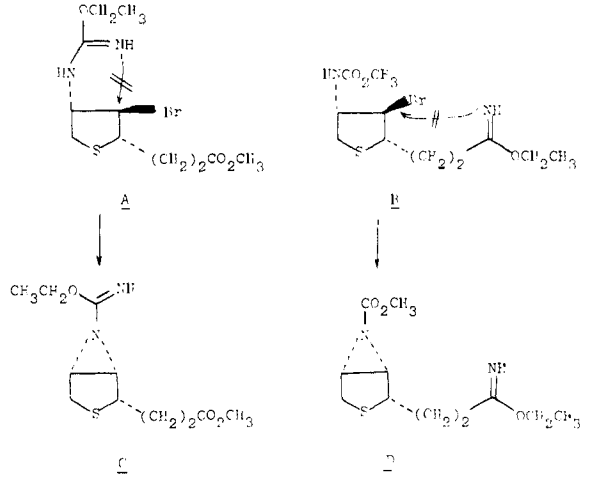
References and Notes

(1) For preliminary communication see P. N. Confalone, G. Pizzolato, E. G. Baggiolini, D. Lollar, and M. R. Uskoković, *J. Am. Chem. Soc.*, **97**, 5936 (1975). We wish to dedicate this paper to Prof. R. B. Woodward on the occasion of his 60th birthday.
(2) W. G. Bateman, *J. Biol. Chem.*, **26**, 263 (1916).
(3) M. A. Boas, *Biochem. J.*, **21**, 712 (1927).
(4) P. György, V. Du Vigneaud, et al., *Science*, **92**, 609 (1940).
(5) F. Kögl, *Ber.*, **68A**, 16 (1935).
(6) V. Du Vigneaud et al., J. Biol. Chem., **146**, 475, 487 (1942).
(7) S. A. Harris, D. E. Wolf, R. Mozingo, and K. Folkers, *Science*, **97**, 447 (1943); S. A. Harris, D. E. Wolf, R. Mozingo, G. E. Arth, R. C. Anderson, N. R. Easton, and K. Folkers, *J. Am. Chem. Soc.*, **67**, 2096 (1945); S. A. Harris, D. E. Wolf, R. Mozingo, R. C. Anderson, G. E. Arth, N. R. Easton, D. Heyl, A. N. Wilson, and K. Folkers, *ibid.*, **66**, 1756 (1944); A. Grüssner, J. P. Bourquin, and O. Schnider, *Helv. Chim. Acta*, **28**, 510, 517 (1945); B. R. Baker, M. V. Querry, S. Bernstein, S. R. Safir, and Y. Subbarow, *J. Org. Chem.*, **12**, 167 (1947); B. R. Baker, M. V. Querry, W. L. McEwen, S. Bernstein, S. R. Safir, L. Dortman, and Y. Subbarow, *ibid.*, **12**, 186 (1947); B. R. Baker, W. L. McEwen, and W. N. Kinley, *ibid.*, **12**, 1322 (1947); M. W. Goldberg and L. H. Sternbach, U. S. Patents 2 489 232, 2 489 235, and 2 489 238 (1949).
(8) F. Kögl and E. J. Ten Ham, *Hoppe-Seyler's Z. Physiol. Chem.*, **279**, 140 (1943).
(9) K. K. Krueger and W. H. Peterson, *J. Biol. Chem.*, **173**, 497 (1948).
(10) J. C. Bauernfeind, *Feedstuffs*, **41**, 32 (1969); R. Blair and C Whitehead, *ibid.*, **48**, 30 (1976); D. B. McCormick, *Nutr. Rev.*, **33**, 97 (1975).
(11) S. Bory, M. J. Luche, B. Moreau, S. Lavielle, and A. Marquet Cercoa, *Tetrahedron Lett.*, 827 (1975); H. Kuzuhara, H. Ohrui, and S. Emoto, *ibid.*, 1185 (1975); H. Ohrui and S. Emoto, *ibid.*, 2765 (1975); S. I. Zav'yalov et al., *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, **24**, 1533 (1975); P. N. Confalone, G. Pizzolato, and M. R. Uskokovic, *Helv. Chim. Acta*, **59**, 1005 (1976); *J. Org. Chem.*, **42**, 135 (1977); *ibid.*, in press; G. F. Field, Abstracts, 172nd National Meeting of the American Chemical Society, San Francisco, Calif., Aug 1976.
(12) A. Lezius, E. Ringelmann, and F. Lynen, *Biochem. Z.*, **336**, 510 (1963).
(13) H. Kuzuhara, H. Ohrui, and S. Emoto, *Tetrahedron Lett.*, 1185 (1970).
(14) J. Trotter and J. A. Hamilton, *Biochemistry*, **5**, 713 (1966).
(15) R. Ratcliffe and R. Rodehorst, *J. Org. Chem.*, **35**, 4000 (1970).
(16) This result amends the trans structures postulated in our preliminary communication. For a recent x ray on a related thiazolidine see R. Parthasarathy, B. Paul, and W. Korytnyk, *J. Am. Chem. Soc.*, **98**, 6634 (1976).
(17) C. T. Eyles and S. Trippett, *J. Chem. Soc.*, 67–71 (1966).
(17a) This mechanism was first suggested by B. M. Trost, personal communication, 1975.
(18) G. S. Grinenko, E. V. Popova, and V. I. Maksimov, *Zh. Org. Khim.*, **7**, 935 (1971).
(19) Examples of the latter approach are the imino ethers A and B, both prepared



in a straightforward fashion from the bromo urethane **21.** These intermediates yielded only the aziridines C and D under a variety of conditions.
(20) M. W. Goldberg and L. H. Sternbach, U.S. Patent 2 489 235.
(21) For experimental details see G. F. Field, U.S. Patent 3 687 967.
(22) K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, **87**, 5661, 5670 (1965).
(23) We thank Professor D. B. McCormick, Cornell University, for supplying a sample of natural *d*-bisnorbiotin. Cf. D. B. McCormick, S. Iwahara, L. D. Wright, and H. C. Li, *J. Biol. Chem.*, **244**, 1393 (1969).
(24) Melting points were determined on a Rinco Model M-50 melting point apparatus and are uncorrected. IR spectra were obtained using a Beckman IR-9 spectrophotometer. A Cary 14 recording spectrophotometer was used for UV absorption spectra. NMR spectra were determined with Varian T-60 and HA-100 spectrometers using tetramethylsilane as the internal reference. Mass spectra were recorded on a CEC 21-110B mass spectrometer at 70 eV using a direct insertion probe. Thin layer chromatography was carried out using Merck F-254 silica gel plates.