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SYNTHESIS OF D-BIOTIN FROM CYSTEINE*

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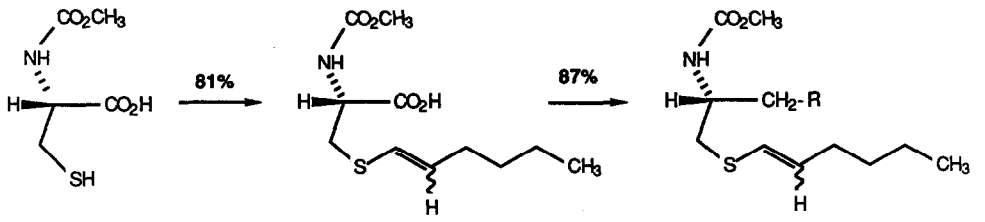
ABSTRACT: A NOVEL APPROACH TO D-BIOTIN FROM CYSTEINE, WHICH UTILIZES THE NITRILE OXIDE TO ENOLTHIOETHER DOUBLE BOND CYCLIZATION FOR THE FORMATION OF C_2-C_3 OF THE THIOPHANE RING AND THE INCORPORATION OF THE 3-N IN THE BIOTIN MOLECULE, IS DESCRIBED. THE CIS RELATION OF THE N-BUTYL SIDE CHAIN AND THE SULFUR IN RESPECT TO ISOXAZOLIDINE RING IN THE BICYCLIC DIASTEREOMERS 9 AND 12 IS CHARACTERIZED BY AN NMR SIGNAL FOR THE α-METHYLENE PROTONS OF THE SIDE CHAIN AS A MULTIPLET AT δ1.82 AND 1.80, RESPECTIVELY, DOWNFIELD FROM THE MULTIPLET AT 1.33 AND 1.32, RESPECTIVELY, FOR THE OTHER TWO METHYLENES OF THE SIDE CHAIN.

WE HAVE RECENTLY DESCRIBED[1] A SYNTHESIS OF D-BIOTIN IN WHICH C_2-C_3 OF THE THIOPHANE RING AND THE CORRESPONDING TWO CHIRAL CENTERS HAVE BEEN FORMED BY A STEREOSELECTIVE [3+2] NITRONE TO ENOLTHIOETHER DOUBLE BOND CYCLIZATION. COROLLARY TO THIS APPROACH, WE HAVE INVESTIGATED THE CORRESPONDING NITRILE OXIDE ENOLTHIOETHER DOUBLE BOND CYCLIZATION, WHICH LED TO A SYNTHESIS OF DEOXYBIOTIN (19), THE MICROBIOLOGICAL CONVERSION OF WHICH TO D-BIOTIN IS A KNOWN PROCESS.

PREPARATION OF THE CYCLIZATION SUBSTRATE, A MIXTURE OF THE CIS AND TRANS NITRO-ENOLTHIOETHER 6 IS SKETCHED IN SCHEME 1. N-METHOXYCARBONYL-L-CYSTEINE (1), PREPARED FROM L-CYSTINE, WAS TREATED IN DIOXANE WITH AN EXCESS OF N-HEXYNE IN THE PRESENCE OF AIBN AS RADICAL INITIATOR TO GIVE A MIXTURE OF THE CORRESPONDING CIS AND TRANS ENOLTHIOETHERS 2 IN 81% OVERALL YIELD. THE CORRESPONDING ALCOHOL 3 WAS OBTAINED BY FIRST FORMING A MIXED ANHYDRIDE WITH METHYLCHLOROFORMATE FOLLOWED BY TREATMENT WITH SODIUM BOROHYDRIDE. THE MOST EFFICIENT SEQUENCE FOR THE CONVERSION OF 3 TO THE NITRO COMPOUND 6 WAS FOUND TO BE OVER THE MESYLATE 4 AND IODIDE 5, BOTH PREPARED BY STANDARD METHODS. CONVERSION OF 5 TO 6 WAS ACHIEVED WITH SODIUM NITRITE IN DIMETHYLFORMAMIDE IN THE PRESENCE OF UREA AND PHLOROGLUCINOL[2] IN ORDER TO MINIMIZE THE FORMATION OF THE CORRESPONDING NITRITE ESTER. THE NITRO COMPOUND 6 (AS A 1:1 MIXTURE OF GEOMETRICAL ISOMERS) CRYSTALLIZES FROM HEXANE-METHYLENE CHLORIDE AS RELATIVELY SHARP MELTING WHITE NEEDLES.

*WE DEDICATE THIS PAPER TO PROF. H. WYNBERG ON THE OCCASION OF HIS 65TH BIRTHDAY.

Scheme 1



1 2

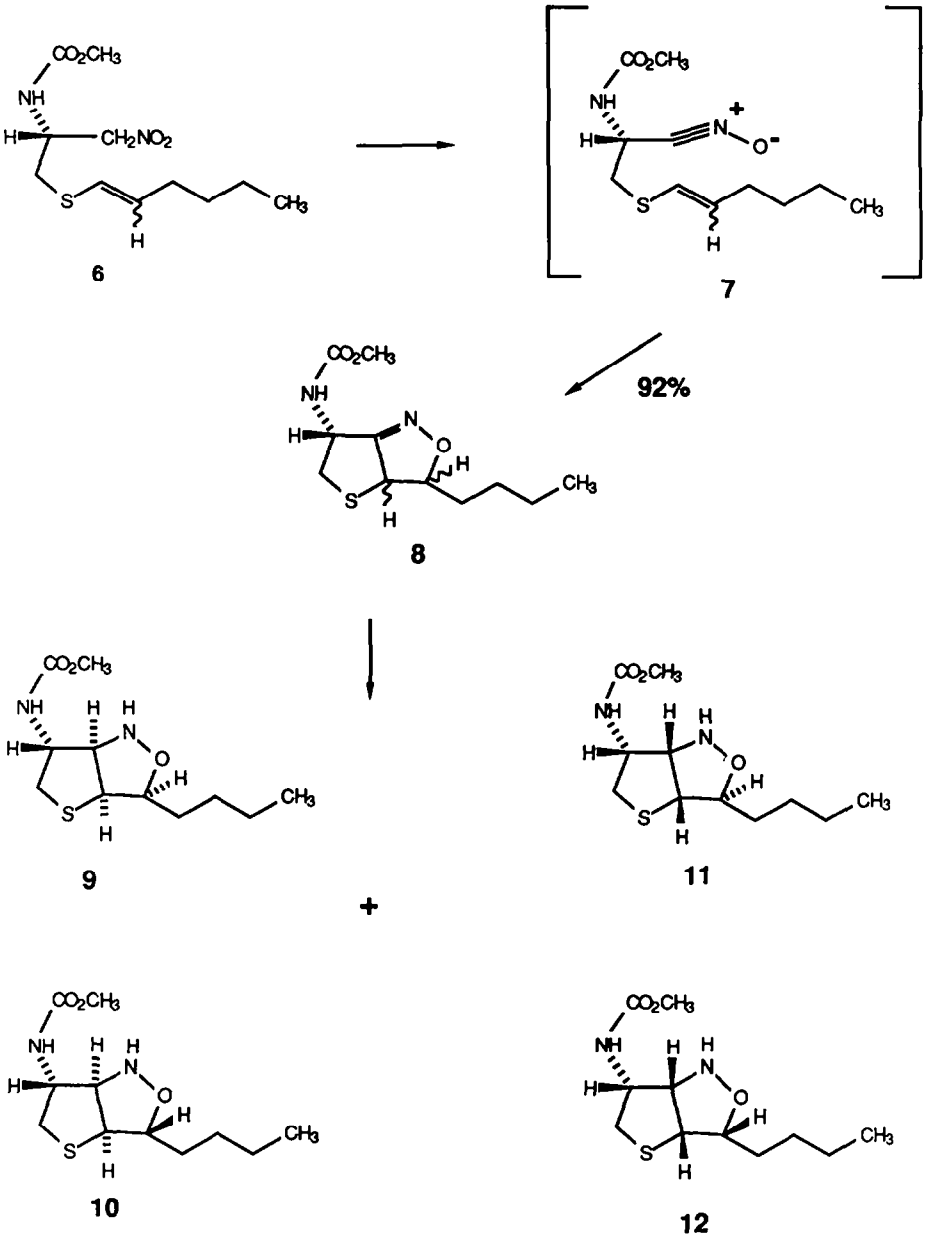
3 R = OH
4 R = OMs (98%)
5 R = I (90%)
6 R = NO_2 (60%)

REFLUXING OF 6 IN BENZENE IN THE PRESENCE OF AN EXCESS OF PHENYLISOCYANATE GENERATES A MIXTURE OF ISOMERIC ISOXAZOLINES 8. HIGH PRESSURE LIQUID CHROMATOGRAPHY HAD TO BE USED TO SEPARATE EACH OF THESE ISOMERS, WHICH WERE FORMED IN APPROXIMATELY EQUAL AMOUNTS. VERY EASILY SEPARABLE BY COLUMN CHROMATOGRAPHY WERE, ON THE OTHER HAND, THE ISOXAZOLIDINES 9, 10, 11, AND 12, WHICH WERE GENERATED WHEN THE ABOVE ISOXAZOLINES MIXTURE WAS TREATED WITH DIISOBUTYLALUMINUM HYDRIDE IN TOLUENE AT -78°.

THE STRUCTURES OF THE ISOXAZOLIDINES 9-12 WERE IDENTIFIED BY CONVERSION OF 11 AND 12 TO DEOXYBIOTIN 19 AS ILLUSTRATED BELOW, BY X-RAY SINGLE CRYSTAL ANALYSES FOR THE DIASTEREOMERS 10 AND 12, AND BY THE FOLLOWING ^{1}H NMR COMPARISON. THE ^{1}H NMR OF 12, WHICH HAS THE N-BUTYL SIDE CHAIN AND THE THIOPHANE SULFUR ON THE SAME SIDE OF THE ISOXAZOLIDINE RING EXHIBITS THE SIGNAL FOR THE α-METHYLENE PROTONS OF THE SIDE CHAIN AS A MULTIPLET AT δ1.82, DOWNFIELD FROM THE MULTIPLET AT 1.33 FOR THE OTHER TWO METHYLENES OF THE SIDE CHAIN. THIS DOWNFIELD SHIFT (δ1.80) WAS OBSERVED ALSO, AND AS EXPECTED, FOR THE TRANS-ISOMER 9. THE ISOMERS 10 AND 11, WITH SIDE CHAINS TRANS TO THE SULFUR IN RESPECT TO THE ISOXAZOLIDINE RING, EXHIBIT ALL THREE SIDE CHAIN METHYLENES UPFIELD, THE MULTIPLETS AT δ1.38 AND 1.35, RESPECTIVELY.

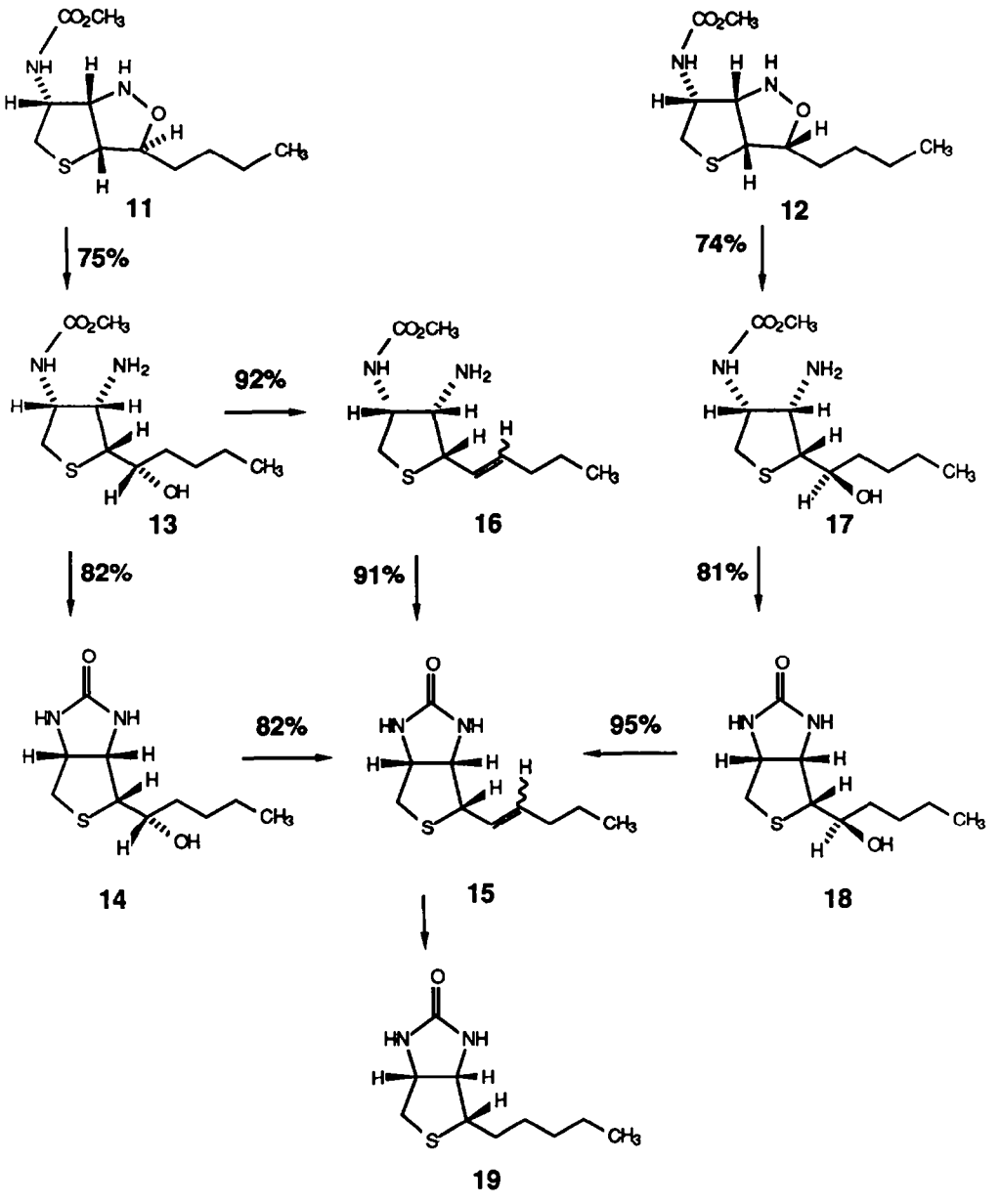
HYDROGENATION OF 11 AND 12 (AS SINGLE COMPONENTS OR AS A MIXTURE) WITH PALLADIUM-ON-CHARCOAL IN 1:1 MIXTURE OF ACETIC ACID/WATER GAVE THE AMINO ALCOHOLS 13 AND 17 IN GOOD YIELDS. THESE PRODUCTS WERE CYCLIZED TO THE IMIDAZOLIDINONES 14 AND 18 BY REFLUXING IN A MIXTURE OF DIOXANE-WATER IN THE PRESENCE OF BARIUM HYDROXIDE. DEHYDRATION TO THE MIXTURE OF DISUBSTITUTED OLEFINS 15 WAS EFFECTED WITH CATALYTIC AMOUNTS OF p-TOLUENESULFONIC ACID IN BOILING TOLUENE. NONE OF THE EXOCYCLIC ISOMERS WERE OBSERVED. ALTERNATIVELY, 13 CAN BE FIRST DEHYDRATED TO 16 AND THE LATTER CYCLIZED TO 15. INTERESTINGLY, THIS ALTERNATIVE ROUTE GENERATES SEVERAL BY-PRODUCTS IF APPLIED ON 17. CATALYTIC HYDROGENATION OF 15 WITH PALLADIUM-ON-CHARCOAL USING ACETIC ACID-WATER

Scheme 2



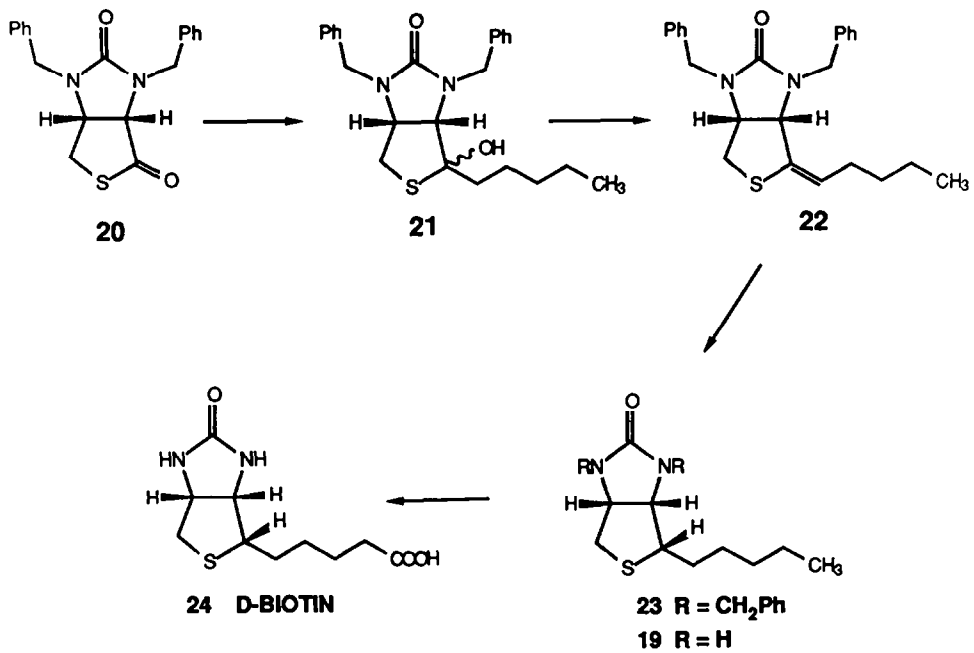
AS SOLVENT GIVES DEOXYBIOTIN 19. ALL THE PHYSICAL AND SPECTROSCOPICAL DATA OF THE SO PREPARED DEOXYBIOTIN (INCLUDING OPTICAL ROTATION AND MIXED MELTING POINT) ARE IDENTICAL WITH THOSE OF AN AUTHENTIC SAMPLE, PREPARED FROM A WELL KNOWN INTERMEDIATE 20 (SCHEME 4) OF THE STERNBACH D-BIOTIN SYNTHESIS[3].

Scheme 3



THE STERNBACH INTERMEDIATE 20 WAS REACTED WITH N-PENTYLMAGNESIUM BROMIDE AND THE RESULTING CARBINOL 21 WAS DEHYDRATED IN BOILING ACETIC ACID. HYDROGENATION OF 22 OVER RANEY NICKEL LED TO THE ALL-CIS INTERMEDIATE 23, WHICH WAS DEBENZYLATED TO THE DEOXYBIOTIN 19 WITH SODIUM IN LIQUID AMMONIA.

Scheme 4



THE CONVERSION OF 19 TO D-BIOTIN IS A KNOWN MICROBIOLOGICAL OXIDATION PROCESS[4].

REFERENCES

1) E.G. BAGGIOLINI, H.L. LEE, G. PIZZOLATO, M.R. USKOKOVIĆ, J.AMER.CHEM.SOC., 1982, 104, 6460.

2) N. KORNBLUM, H.O. LARSON, R.K. BLACKWOOD, D.D. MOOBERRY, E.P. OLIVETO, G.E. GRAHAM, J.AMER.CHEM.SOC., 1956, 78 1497.

3) (A) M.W. GOLDBERG; L.H. STERNBACH, U.S. PATENTS 2,489,232; 2,489,235; AND 2,489,238 (1949); (B) L.H. STERNBACH, COMPR. BIOCHEM., 1963, *11*, 66.

4) S. OGIWO, S. FUJIMOTO, H. WADA, Y. TANIGAWA, U.S. PATENT 3,859,167.

Experimental Section

General Methods. 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 HA-100 spectrometer using tetramethylsilane as the internal reference. Mass spectra were recorded on a CEC 21-110B mass spectrometer at 70eV using direct insertion probe. Thin layer chromatography was carried out using Merck F-254 silica gel plates.

N-Methoxycarbonyl-L-cysteine (1). A solution of 13.8 g (0.13 mol) of anhydrous sodium carbonate, dissolved in 180 mL of water was mixed with 120 mL of a solution of 10% sodium bicarbonate in water, to which 14.4 g (0.06 mol) of L-cystine were added. The resulting suspension was cooled at 0°C and treated dropwise with 12.3 g (10.1 mL, 0.13 mol) of methylchloroformate. After addition, the reaction mixture was allowed to come to room temperature and vigorously stirred for 4 hrs. It was then cooled again at 0°C and adjusted to pH 2 with 5N hydrochloric acid, allowed to come to room temperature, saturated with sodium chloride and extracted with 3 x 150 mL of ethyl acetate. The combined organic layers were dried and evaporated *in vacuo* to give 19.16 g of N-carboxymethyl-L-cystine. This was dissolved in 200 mL of dry liquid ammonia and treated at -60°C to -70°C with metallic sodium added portionwise in small pieces and waiting, after each addition, that the initially blue solution turned colorless again. Altogether, 4.8 g (.209 mol) of sodium were used. When the blue color persisted, a few crystals of ammonium chloride were added until the color was discharged and then the ammonia was allowed to evaporate. The residue was treated with 100 mL of saturated ammonium chloride solution, the pH adjusted to 2 with 5N hydrochloric acid, and extracted with 3 x 150 mL of ethyl acetate. The organic layers were combined, dried and evaporated *in vacuo* to give 18.0 g (91% yield) of N-methoxycarbonyl-L-cysteine (1) as a thick, pale yellow oil. ^{1}NMR ($CDCl_3$) δ 1.50 (t, J =9.0 Hz, 1H), 3.03 (dd, J = 9.0, 5.0 Hz, 2H), 3.74 (s, 3H), 4.67 (m, 1H), 5.76 (bs, 1H).

(Z and E)(-S-(Hexen-1-yl)-N-(methoxycarbonyl)-cysteine (2). A solution of 10.74 g (0.06 mol) of N-(methoxycarbonyl)-cysteine (1), 7.15 g (10 mL, 0.087 mol) of 1-hexyne and 500 mg of 2,2'-bisazo-(2-methylpropionitrile) in 20 mL of dioxane was heated at 85°C for 10 hrs, cooled at room temperature and diluted with 200 mL of ether and extracted with 3 x 100 mL of a 2N solution of sodium hydroxide. The combined alkaline solutions were washed with 3 x 100 mL of ether and adjusted to pH 2 with 3N hydrochloric acid (at 0°C). The resulting mixture was extracted with 3 x 150 mL of ethyl acetate. The combined organic extracts were washed with 3 x 50 mL of brine, dried and evaporated to give 14.0 g (89% yield) of (Z and E)-2-(hexen-1-yl)-N-(methoxycarbonyl)-cysteine (2), as a pale, yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (bt, J = 6.0 Hz, 3H), 1.33 (m, 4H), 2.06 (m, 2H), 3.10 (m, 2H), 3.70 (s, 3H), 4.60 (m, 1H), 5.33 to6.00 (m, 2H), 9.76 (bs, 1H).

[2R(5Z and 5E)]-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]propanol (3). To a solution of 32.0 g (0.123 mol) of (Z and E)-S-(hexen-1-yl)-N-(methoxycarbonyl)cysteine (2) in 250 mL of freshly distilled dry tetrahydrofuran cooled at 0°C, 13.6 g (0.134 mol) of triethylamine and 12.8 g (0.135 mol) of methylchloroformate were subsequently added dropwise. After addition, the reaction mixture was stirred for 2 1/2 hrs, filtered and slowly added to a suspension of 22.7 g (0.600 mol) of sodium borohydride in 100 mL of water at0°C. The resulting mixture was allowed to come to room temperature, stirred for 3 hrs, cooled again at 0°C and treated with 65 mL of 5N hydrochloric acid, added dropwise. Finally, it was extracted with 3 x 150 mL of ethyl acetate and the combined organic layers were washed with 3 x 50 mL of a 2N potassium bicarbonate solution, followed by 3 x 50 mL of brine, dried and evaporated *in vacuo* to give 25.8 g (87% crude yield) of **3** as an almost colorless thick oil. For analytical purposes, a small amount of the product (consisting of an approximately 1:1 mixture of Z and E geometrical isomers mixture) was separated into its components, using a Waters Associates High Pressure liquid chromatograph Model 244 and an 8' x 3/8" PORASIL A® column, eluted with a mixture of hexane-ethyl acetate (1:1).

Z-Isomer: $[\alpha]^{25}D$ -26.2° (c 0.8, EtOH); ^1H NMR ($CDCl_3$) 0.91 (bt, J = 6.0 Hz, 3H),1.32 (m, 4H),2.16 (m, 2H), 2.87 (d, J = 6.0 Hz, 2H), 3.68 (s, 3H), 3.80 (m, 2H), 5.70 (m, 1H), 5.60 (dt, J = 9.2, 7.0 Hz, 1H), 5.92 (d, J= 9.2 Hz, 1H); IR ($CHCl_3$) 3650, 3450, 1725, 1625,1523 cm^{-1}; mass spectrum, m/e(%) 247(24), 172(39), 118(90), 86(100).

Anal. Calcd for $C_{11}H_{21}NO_3S$: C, 53.41; H, 8.56; N, 5.66. Found: C, 53.53; H, 8.37; N, 5.75.

E-Isomer: $[\alpha]^{25}D$ =33.24° (c 0.8, EtOH); ^1H NMR ($CDCl_3$) 0.90 (bt, J = 6.0 Hz, 3H), 1.32 (m, 4H), 2.07 (m, 2H), 2.86 (d, J = 6.0 Hz, 2H), 3.68 (s, 3H), 3.80 (m, 2H), 5.70 (m, 1H), 5.70 (dd, J= 14.3, 6.0 Hz, 1H), 5.94 (d, J = 14.3 Hz, 1H); IR ($CHCl_3$) 3625, 3450, 1700, 1615, 1513, 950 cm^{-1}; mass spectrum, m/e(5) 247(18), 172(32), 118(84), 86(100).

Anal. Calcd for $C_{11}H_{21}NO_3S$: C, 53.41; H, 8.56; N, 5.66. Found: C, 53.71; H, 8.77; N, 5.71.

[2R-(5Z and 5E)]-3-(1-Hexen-1-yl-thio)-2-[(methoxycarbonyl)-amino]-1-[(methylsulfonyl)oxy]propane (4). A solution of 13.1 g (0.050 mol) of [2R(5Z and 5E]-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]propanol (**3**) in 60 mL of anhydrous pyridine was treated dropwise at 0°C with 8.6 g (0.075 mol) of methanesulfonyl chloride. After addition, the reaction mixture was further stirred at 0°C for 4 hrs. Then it was diluted with 10 mL of water, stirred for 30 min, adjusted to pH 2 with 2N hydrochloric acid, extracted with 3 x 100 mL of ethyl acetate. The combined organic phases were washed with water, 2N potassium bicarbonate solution, brine, dried and evaporated *in vacuo* to give 16.2 g (99% yield) of **4** as a thick, pale yellow oil. ^1H NMR ($CDCl_3$) 0.90 (bt, J = 6.0 Hz, 3H), 1.33 (m, 4H), 2.10 (m, 2H), 2.86 (d with free splittings, J = 8.0 Hz, 2H), 3.03 (s, 3H), 3.67 (s, 3H), 4.00 (m, 1H), 4.33 (m, 2H), 5.13 (bd, J = 8.0 Hz, 1H), 5.36 to 6.03 (m, 2H); IR ($CHCl_3$) 3380, 1720, 1610, 1500, 950 cm^{-1}.

[2S(5Z and 5E)]-3-(1-Hexen-1-yl-thio)-1-iodo-2-[(methoxycarbonyl)amino]-propane (5). A mixture of 16.2 g (0.050 mol) of[2R(5Z and 5E)]-3-(1-hexen-1-yl-thio)-2[(methoxycarbonyl)amino]-1-[(methoxysulfonyl)oxy]propane (**4**), 22.5 g (0.150 mol) of sodium iodide and 350 mL of acetone was refluxed for 5 hrs. After cooling, the solvent was evaporated *in vacuo*, the residue treated with 100 mL of

water and extracted with 3 x 100 mL of ethyl acetate. The combined organic phases were washed with 2N sodium thiosulfate solution, water, brine and evaporated *in vacuo* to give 16.1 g (90.7% yield) of **5** as a thick, yellow-bronw oil. ^{1}H NMR ($CDCl_3$) 0.90 (bt, J = 6.0 Hz, 3H), 1.36 (m, 4H), 2.10 (m, 2H), 2.90 (m, 2H), 3.50 (m, 2H), 3.67 (s, 3H),d 5.33 to 6.06 (m, 2H); IR ($CHCl_3$) 3320, 1420, 1610, 1500, 950 cm^{-1}.

[2R(5Z and 5E)]-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]-1-nitropropane (6). A solution of 6.8 g (0.019 mol) of [2S(5Z and 5E)]-3-(1-hexen-1-yl-thio)-1-iodo-2-[(methoxycarbonyl)amino]propane (**5**), 2.5 g (0.042 mol) of urea, 2.4 g (0.019 mol) of phloroglucinol and 2.9 g (0.042 mol) of sodium nitrite in 100 mL of dimethylformamide was stirred at room temperature under nitrogen, for 30 hrs. It was then treated with 100 mL of water, extracted with 3 x 100 mL of ether and the combined organic layers washed subsequently with water, 2N sodium thiosulfate solution, water, 2N potassium bicarbonate and brine. Evaporation *in vacuo* gave 5.39 g of a yellow residue which was purified on a silica gel column (100 g), eluted with an ethyl acetate-hexane mixture (2:3) to give 3.1 g (59% yield) of pure (**6**) as a light yellow powder. Crystallization from methylene chloride-hexane gave 2.9 g (55%) of crystalline product (mp 75-76°C). ^{1}H NMR ($CDCl_3$) 0.90 (bt, J = 6.0 Hz, 3H), 1.38 (m, 4H), 2.10 (m, 2H), 2.44 (m, 2H), 3.68 (s, 3H), 4.33 (m, 1H), 4.50 to 5.00 (m, 2H), 5.22 (bs, 1H), 5.60 to 6.00 (m, 2H); IR (KBr) 3335, 1694, 1660, 1553, 1536 cm^{-1}; mass spectrum, m/e(%) 276, 150, 129, 87.

Anal. Calcd for $C_{11}H_{20}N_2O_4S$: C, 47.81; H, 7.30; N, 10.14. Found: C, 47.61; H, 7.17; N, 10.23.

Cyclization of[2R(5Z and 5E)]-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)-amino-1-nitropropane (6). A mixture of 100 g (0.0036 mol) of [2R(5Z and 5E)]-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]-1-nitropropane (**6**), and 1.29 g (0.0109 mol) of phenylisocyanate in 30 mL of anhydrous benzene to which a few drops of triethylamine were added, was stirred at room temperature, under argon, for 40 hrs. The reaction mixture was then treated with 5 mL of water, stirred at room temperature for one hour, diluted with 100 mL of benzene and the organic phase washed subsequently with water and brine, dried and evaporated to give 0.980 g of a light brown residue. This was applied on a 200 g silica gel

column, eluted with a 1:2 mixture of ethyl acetate-hexane to give 0.859 g (92.4% yield) of a mixture of isoxazolines **8**. The components of the mixture were separated by high pressure liquid chromatography using a Waters Associates Chromatograph Model244 and an 8' x 3/8" PORASIL A® column, eluted with a mixture of hexane-ethyl acetate (5:1) to give the following isomers (in order of elution):

0.111 g of [3S-(3β,3aβ,6α)]-3-Butyl-3,3a,5,6-tetrahydrothieno[3,2-c]-isoxazol-6-yl carbamic acid methyl ester. Crystallization from hexane-methylene chloride gave white crystals: mp 83-85°C. $[\alpha]^{25}D$ -262.2° (c 0.23, EtOH); ^1H NMR ($CDCl_3$) 0.92 (br t, J = 6.0 Hz, 3H), 1.38 (m, 4H), 1.86 (m, 2H), 2.96 (dd, J =11.0, 9.3 Hz), 3.50 (dd, J = 11.0, 9.0 Hz, 1H), 3.70 (3H, s), 4.56 (m, 2H), 4.89 (m, 1H),a 5.41 (br d, 1H); mass spectrum, m/e(%) 258(7), 201(12), 183(8), 172(11), 155(126), 101(100). IR (KBr): 3345, 1698, 1647, 1525 cm^{-1}

Anal. Calcd for $C_{11}H_{16}N_2O_3S$: C, 51.14; H, 7.02; N, 10.84. Found: C, 51.22; H, 7.07; N, 10.78.

0.149 g of [3R-(3α,3aβ,6α)]-3-Butyl-3,3a,5,6-tetrahydrothieno[3,2-c]-isoxazol-6-yl carbamic acid methyl ester. Crystallization from hexane-methylene chloride gave white crystals, mp 110-113°C: $[\alpha]^{25}D$ -164.6° (c 0.2, EtOH); ^1H NMR ($CDCl_3$) 0.92 (br t, J = 6.0 Hz, 3H), 1.40 (m, 4H), 1.56 (m, 2H), 2.80 (dd, J = 11.0, 9.0 Hz, 1H), 3.48 (dd, J = 11.0, 8.0 Hz, 1H), 3.70 (s, 3H), 4.66 to 5.16 (m, 3H), 5.46 (br s, 1H); mass spectrum, m/e(%) 258(47), 201(69), 183(35), 155(50), 101(100).

0.119 g of[3R-(3α,3aα,6α)-3-Butyl-3,3a,5,6-tetrahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid methyl ester. Crystallization from methylene chloride afforded white crystals, mp 92-94°C: $[\alpha]^{25}D$ +310.7° (c 0.3, EtOH); ^1H NMR ($CDCl_3$) (bt, J = 6.0 Hz, 3H), 1.40 (m, 4H), 1.84 (m, 2H), 3.06 (dd, J = 12.0, 3.0 Hz, 1H), 3.46 (dd, J = 12.0, 6.0 Hz, 1H), 3.69 (s, 3H), 4.60 (m, 2H), 4.87 (td, J = 6.0, 3.0 Hz, 1H), 5.16 (bs, 1H); IR (KBr) 3295, 1728, 1715, 1634, 1540 cm^{-1}; mass spectrum, m/e(%) 258(12), 201(14), 166(29), 101(100).

Anal. Calcd for $C_{11}H_{18}N_2O_3S$: C, 51.14; H, 7.02; N, 10.84. Found: C, 51.36; H, 6.98; N, 10.78.

0.122 g of [3S-(3β,3aα,6α)]-3-Butyl-3,3a,5,6-tetrahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid methyl ester. Crystallization from methylene

chloride-hexane gave white crystals, mp 102-104°C: $[\alpha]^{25}D$ +185.8 (c 0.3, EtOH); ^1H NMR 0.91 (bt, J = 6.0 Hz, 3H), 1.40 (m, 4H), 1.60 (m, 2H), 3.04 (dd, J = 12.0, 3.0, 1H), 3.32 (dd, J = 17.0, 6.0 Hz, 1H), 3.69 (s, 3H), 4.80 (m, 1H), 5.00 (m, 1H), 5.05 (d, J = 10.0 Hz, 1H), 5.20 (bs, 1H); IR (KBr) 3360, 1705, 1625, 1540 cm^{-1}; mass spectrum, m/e(%) 258(79), 201(32), 166(70), 101(100).

Anal. Calcd for $C_{11}H_{18}N_2O_3S$: C, 51.14; H, 7.02; N, 10.84. Found: C, 51.27; H, 7.03; N, 10.84.

[3S-(3β,3aβ,6α,6aβ)-3-Butylhexahydrothieno[3,2-c]-isoxazol-6-yl carbamic acid methyl ester (11). To a solution of 0.774 g (3.0 mmol) of[3S-(3β,3aβ,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester in 30 mL of anhydrous toluene, kept under argon at -78°C, 10 mL (15.0 mmol) of a 1.5 molar solution of diisobutylaluminum hydride solution in toluene was added dropwise. After addition, the reaction mixture was stirred at -78°C for 1 hr. It was then treated at -78°C with 2 mL of methanol, allowed to come to room temperature, combinedwith a 2N solution of aqueous sodium potassium tartrate solution and extracted with ethyl acetate. The combined organic phases were washed with brine, dried and evaporated to give 0.763 g of crude product. Purification by chromatography on silica using hexane-ethyl acetate (1:1) as eluent gave 0.598 g of pure **11**. Crystallization from methylene chloride-hexane gave white crystals, mp 121-122°C. $[\alpha]^{25}D$ -222.8° (c 0.3, EtOH); ^1H NMR ($CDCl_3$) δ 0.87 (br m, 3H), 1.35 (m, 6H), 2.74 (t, J = 10.4, 1H), 2.97 (dd, J = 10.4, 6.0 Hz, 1H), 3.69 (s, 3H), 3.72 (m, 1H), 3.90 to 4.30 (br m, 3H),d 5.64 (br s, 2H); IR3420, 3280, 1720, 1705, 1510 cm^{-1}; mass spectrum, m/e(%) 260(10, M^+), 159(9), 102(100),

Anal. Calcd for $C_{11}H_{20}N_2O_3S$: C, 50.75; H, 7.74; N, 10.76. Found: C, 50.42; H, 7.89; N, 10.41.

[3R-(3α,3aβ,6α,6aβ)-3-Butylhexahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid methyl ester (12). Following the procedure described for the preparation of **11**, [3R-(3α,3aβ,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid methyl ester was converted to **12**, which was obtained as white crystals from hexane-methylene chloride, mp 105-106°C. $[\alpha]^{25}D$ -232.5° (c 0.3, EtOH); ^1H NMR ($CDCl_3$) δ 0.91 (br t, J = 6.0 Hz, 3H), 1.33 (m, 4H), 1.82 (m, 2H),

2.57 (dd, J = 10.4, 9.2, Hz, 1H), 2.88 (dd, J = 10.4, 6.0 Hz, 1H), 3.67 (s, 3H), 3.70 (m, 1H), 3.90 to 4.40 (m, 3H), 5.37 (br s, 1H), 5.70 (br d, J = 8.0 Hz,1H): IR 3350, 3200, 1705, 1530 cm^{-1}; mass spectrum, m/e(%) 260(12, M^+), 159(10), 153(11), 126(43), 102(100). Anal. Calcd for $C_{11}H_{20}N_2O_3S$: C, 50.75; H, 7.74; N, 10.76. Found: C, 50.44; H, 7.76; N, 10.53.

[3R-(3α,3aα,6α,6aα)]-3-Butylhexahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid methyl ester (10). Following the procedure described for the preparation of **11**, [3R-(3α,3aα,6α)-3-butyl-3,3a,5,6-tetrahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid methyl ester was converted to **10**; white crystals from hexane-methylene chloride, mp 143-144°C. $[\alpha]^{25}D$ +176.8° (c 0.3, EtOH): ^1H NMR ($CDCl_3$) δ 0.91 (br t, J = 6.0 Hz, 3H), 1.38 (m, 6H), 2.73 (dd, J = 12.0, 2.4 Hz, 1H), 3.76 (dd, J = 12.0, 4.4 Hz, 1H), 3.67 (s, 3H), 3.70 (m, 1H),s 3.90 to 4.20 (m, 2H), 4.35 (m, 1H), 5.20 (br d, J = 8.0 Hz, 1H),d 5.65 (br s, 1H) ppm: IR (KBr) 3325, 3210, 1695, 1540 cm^{-1}; mass spectrum, m/e(%) 260(9, M^+), 159(9), 102(100).

Anal. Calcd for $C_{11}H_{20}N_2O_3S$: C, 50.75; H, 7.74; N, 10.76. Found: C, 51.07; H, 7.95; N, 10.63.

[3S-(3β,3aα,6α,6aα)]-3-Butylhexahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid methyl ester (9). Following the procedure described for the preparation of **11**, [3s-(3β,3aα,6α)-3-butyl-3,3a,5,6-tetrahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid methyl ester was converted to **9**, which was obtained as white crystals from hexane-methylene chloride, mp 147-149°C. $[\alpha]^{25}D$ +185.5° (c 0.3, EtOH): ^1H NMR ($CDCl_3$) δ 0.91 (br t, J = 6.0 Hz, 3H), 1.32 (m, 4H), 1.80 (m, 2H), 2.58 (br d, J = 11.2 Hz, 1H), 3.14 (dd, J = 11.2, 4.0 Hz, 1H), 3.67 (s, 3H), 3.70 (m, 1H), 4.0 (dd, J = 6.0, 4.0 Hz, 1H),d 4.19 (br d, J = 6.0 Hz, 1H), 4.40 (m, 1H), 5.20 (br s, 1H)d, 5.60 (br s, 1H) ppm; IR (KBr) 3320, 3210, 1695, 1550 cm^{-1}; mass spectrum, m/e(%) 200(13, M^+), 159(10), 126(44), 102(100).

Anal. Calcd for $C_{11}H_{20}N_2O_3S$: C, 50.75; H, 7.74; N, 10.76. Found: C, 50.71; H, 7.84; N, 10.62.

[2R-[2α(S*),3α,4α]]-α-Butyltetrahydro-4-[(methoxycarbonyl)amino]-3-amino-2-thiophenemethanol (13). A mixture of 0.175 g (0.672 mmol) of [3S-(3β,3aβ,6α,6aβ)]-3-butylhexahydrothieno-[3,2-c]-isoxazol-6-yl carbamic acid

methyl ester (**11**) and 0.050 g of 10% palladium on charcoal in 15 mL of a 1:1 mixture of acetic acid and water was hydrogenated at room temperature and under atmospheric pressure for 24 hrs. It was then neutralized by careful addition of 1N potassium bicarbonate solution and, after addition of 50 mL of ethyl acetate, filtered through Celite® and washed with 5 x 50 mL of ethyl acetate. The separated and combined organic layers were washed with 3 x 20 mL of brine, dried and evaporated *in vacuo* to give 0.164 g (93% yield) of crude (**13**). This was further purified by column chromatography on 30 g of silica gel, using ethyl acetate-methanol (95:5) as eluent to give 0.111 g (63% yield) of pure **16**. Crystallization from methylene chloride-methanol gave white crystals, mp 134-135°C; $[\alpha]^{25}D$ -108.6 (c 0.3, EtOH). ^1H NMR ($CDCl_3$) δ 0.92 (br t, J = 6.0 Hz, 3H), 1.44 (br s, 6H), 1.75 (br s, 3H), 2.58 (t, J = 10.0 Hz, 1H), 3.10 (dd, J = 10.0, 3.4 Hz, 1H), 3.28 to 3.50 (br m, 1H), 3.50 to3.90 (br m, 2H), 3.67 (s, 3H), 4.20 (br m, 1H),s 5.48 (br d, J = 7.0 Hz, 1H) ppm; IR (KBr) 3390, 3335, 1690, 1540 cm^{-1}; mass spectrum, m/e(%) 102(22), 75(100).

Anal. Calcd for $C_{11}H_{22}N_2O_3S$: C, 50.36; H, 8.45; N, 10.68. Found: C, 50.20; H, 8.34; N, 10.52.

[2R-[2α(R*),3α,4α]]-α-Butyltetrahydro-4-[(methoxycarbonyl)amino]-3-amino-2-thiophenemethanol (17). A mixture of 0.120 g (0.461 mmol) of [3R-(3α,3aβ,6α,6aβ)]-3-butylhexahydrothieno-[3.2-c]-isoxazol-6-yl carbamic acid methyl ester (**12**) and 0.050 g of 10% palladium on charcoal in 15 mL of a 1:1 mixture of acetic acid and water was hydrogenated at room temperature and under atmospheric pressure for 24 hrs. It was then neutralized by careful addition of 1N potassium bicarbonate solution and, after addition of 50 mL of ethyl acetate, filtered through Celite® and washed with 5 x 10 mL of ethyl acetate. The separated and combined organic layers were washed with 3 x 20 mL of brine, dried and evaporated *in vacuo* to give 0.113 g (93% yield) of crude (**17**). Purification by column chromatography gave 0.086 g (71%) of pure **17**. Crystallization from hexane-methylene chloride gave white crystals, mp 104-105°C. $[\alpha]^{25}D$ -53.5° (c 0.2, EtOH). ^1H NMR ($CDCl_3$) 0.91 (br s, 3H), 1.40 (br s, 4H), 1.76 (br s, 3H)d, 2.62 (dd, J = 11.6, 5.0 Hz, 1H), 3.07 (dd, J = 11.6, 4.8 Hz, 1H), 3.54 (br s, 2H), 3.66 (s, 3H)sd, 3.93 (br m, 1H), 4.21 (br m, 1H), 6.07 (br m, 1H) ppm; IR (KBr) 3335, 1685, 1538 cm^{-1}; mass spectrum, m/e(%) 102(23), 75(100).

Anal. Calcd for $C_{11}H_{22}N_2O_3S$: C, 50.36; H, 8.45; N, 10.08. Found: C, 50.60; H, 8.71; N, 10.58.

[3aS-[3aβ,4α(S*),6aβ-4-(1-hydroxypentyl)-1H-tetrahydrothieno[3,4-d]imidazol-2(3H)-one (14). A mixture of 0.014 g (0.053 mmol) of [2R-[2α(S*),3α,4α]]-α-butyltetrahydro-4-[(methoxycarbonyl)amino]-3-amino-2-thiophenemethanol (**13**), 0.2 g of barium hydroxide, 1 mL of dioxane and 2 mL of water was refluxed under argon for 1 hr. It was then acidified with 1N hydrochloric acid and extracfted with 4 x 20 mL of ethyl acetate. The combined organic layers were washed with 2N potassium bicarbonate solution and brine, dried and evaporated *in vacuo* to give 0.0115 g (93%) of **14**. Crystallization from methylene chloride-hexane gave white crystals, mp 222-224°C. ^{1}H NMR (CD_3OD): δ 0.93 (br t, J = 6.0 Hz, 3H), 1.50 (br, s, 6H), 2.70 (d, J = 13.0 Hz, 1H), 3.00 (dd, J = 13.0, 4.0, 1H), 3.64 (br m, 1H), 4.50 (m, 2H) ppm; mass spectrum, m/e(%) 145(26), 86(100).

[3aS-[3aβ,4α(R*),6aβ]-4-(1-Hydroxypentyl)-1H-tetrahydrothieno-[3,4-d]imidazol-2(3H)-one (18). A mixture of 0.030 g (0.114 mmol) of [2R-[2α(R*),3α,4α]]-α-butyltetrahydro-4-[(methoxycarbonyl)amino]-3-amino-2-thiophenemethanol (**17**) and 0.2 g of barium hydroxide, 1.5 mL of dioxane and 2 mL of water was refluxed under argon for 1 hr. It was then acidified with 1N hydrochloric acid and extracted with 4 x 20 mL of ethyl acetate. The combined organic layers were washed with 2N potassium bicarbonate solution and brine, dried and evaporated *in vacuo* to give 0.0215 g (82% yield) of **18**. Crystallization from methyl chloride-hexane gave white crystals, mp 196-198°C. ^{1}H NMR (CD_3OD) δ 0.94 (br t, J = 6.0 Hz, 3H), 1.44 (br s, 6H),2.69 (d, J= 13.0 Hz, 1H), 2.88 (dd, J = 13.0, 4.0 Hz, 1H), 3.70 (br m, 1H), 4.40 (m, 2H); mass spectrum, m/e (%) 144(48), 97(30), 84(100).

[3aS-(3aβ,4α,6aβ)-4-pentyltetrahydro-1H-thieno[3,4-d]imidazol-2(3H)-one (19). A solution of 20.0 mg (0.087 mmol) of [3aS-[3aβ,4α(S*),6aβ]-4-(1-hydroxypentyl)-1H-tetrahydrothieno-[3,4-d]imidazol-2-(3H)-one (**14**) and 20.0 mg of p-toluenesulfonic acid monohydrate in 2 mL of dry toluene was refluxed under argon for 1 hr. After cooling, 5 mL of a 2N sodium bicarbonate solution was added and the mixture extracted with ethyl acetate. The combined organic phases were washed with brine, dried and evaporated to dryness to give 18.0 mg of **15** (mixture of isomers). This was dissolved in a 1:1 mixture of acetic acid and water and hydrogenated at room temperature over 50 mg of palladium on charcoal (15 h). After filtration of the

catalyst and removal of the solvent *in vacuo*, the residue was diluted with ethyl acetate, washed with 2N aqueous potassium bicarbonate solution and brine, dried and evaporated to dryness to give 16.6 mg of **19**. Crystallization from ethanol-water gave white crystals, mp 182-183°C. $[\alpha]^{25}D$ +80.2° (c 0.3, EtOH). ^1H NMR (D_6-DMSO) δ 0.87 (t, J = 6.0 Hz, 3H), 1.32 (br ;m, 2H), 2.58 (d, J = 12.1 Hz, 1H), 2.81 (dd, J = 12.2, 4.4 Hz, 1H), 3.10 (br m, 1H), 4.11 (m, 1H), 4.29 (m, 1H), 6.33 (br s, 1H), 6.39 (br s, 1H); IR (KBr) 3255, 1705, 1470 cm^{-1}; mass spectrum, m/e(%) 214 (14, M^+), 171(13), 154(129), 115(26), 97(100).

Using the same procedure, [3aS-[3aβ,4α(R*),6aβ]-4-(1-hydroxypentyl)-1H-tetrahydrothieno-[3,4-d]imidazol-2(3H)-one (**18**) was also converted to **19**.

(3aS)-1,3-Dibenzyl-4-pentylidene-3aβ,6aβ-1H-hexahydrothieno[3,4-d]imidazol-2-one (22). In a reaction flask with reflux condenser, dropping funnel and magnetic stirrer, was added 1.24 g (51 mmol) of magnesium turning and 5 mL of dry tetrahydrofuran under a nitrogen atmosphere. 7.55 g (50 mmol) of n-pentyl-bromide in 50 mL of dry tetrahydrofuran was placed into the dropping funnel. Five mL of this solution and small crystal of iodine were added to magnesium in the flask and the mixture was gently warmed with a heat gun until the reaction started. The solution from dropping funnel was added slowly to keep reaction going. When addition was complete, the reaction mixture was refluxed for an additional hour. After it was cooled with an ice bath, 5 g (14.8 mmol) of the thiolactone **20** in 50 mL of dry tetrahydrofuran was added over a period of 30 min, which was followed by ice bath removal and heating to reflux for 4 hr. Seventy mL of an ice-water mixture was added and the pH was brought to 1 with 2N HCl. Tetrahydrofuran was removed *in vacuo* and the residue was extracted with 3 x 100 mL of ethyl acetate. The combined extracts were washed with brine until neutral, dried over sodium sulfate anhydrous and evaporated to give 6.4 g of crude tertiary alcohol **21**.

Dehydration to **22** was carried out on the crude product **21** or after a partial purification by a silica gel column chromatography (70-230 Mesh, 1:1 benzene-ethyl acetate), using half of the material each way.

A solution of 3.2 g of crude **21** in 32 mL of glacial acetic acid was refluxed two hours and then evaporated. 100 mL of methylene chloride was added

to the residue, and this solution was washed with 3 x 30 mL of 2N Na_2CO_3 and then with brine until neutral, dried over sodium sulfate anhydrous and evaporated to give 3.05 g of the crude **22**. This material was chromatographed on 300 g of Silica gel (70-230 Mesh) column with 1:1 benzene-ethyl acetate, to give 2.255 g (77.75%) of amorphous (3aS)-1,3-dibenzyl-4-pentylidene-3aβ,6aβ-1H-hexahydro-thieno[3,4-d]imidazol-2-one (**22**) as pale yellow oil, $[\alpha]^{25}D$ +233.53° (c 0.99, EtOH). [The analytical sample of **22** was prepared from produce of the experiment using the partially purified intermediate **21**].

^1H NMR ($CDCl_3$) δ0.88 (brt, J = 5.6 Hz, 3H), 1.2-1.5 (m, 4H), 2.04 (brt, J = 7.0 Hz, 2H), 2.92 (d, J = 4.4 Hz, 2H), 4.00 (d, J = 14.8 Hz, 1H), 4.02 (d, J = 15.8 Hz,1H), 4.77 (d, J = 14.8 Hz, 1H), 4.91 (d, J = 15.8 Hz, 1H), 4.00-4.30 (2m, 2H)s, 5.42 (t, J = 7.2 Hz, 1H), 7.24 (brs, 5H), 7.25 (brs, 5H).

Anal. Calcd for $C_{24}H_{28}N_2OS$: C, 73.48; H, 7.19; N, 7.13; S, 8.16. Found: C, 73.23; H, 7.15; N, 7.25; S, 8.24.

(3aS)-1,3-Dibenzyl-4-n-pentyl-3aβ,4α,6aβ-1H-tetrahydrothieno[3,4-d]imidazol-2-one (23). To the solution of 500 mg (1.227 mmol) of (3aS)-1,3-dibenzyl-4-pentylidene-3aβ,6aβ-1H-hexahydrothieno[3,4-d]imidazol-2-one (**22**) in 50 mL of methanol was added 500 mg of Ra-Ni catalyst and this reaction mixture was hydrogenated at 40-50°C and 300 psi pressure in a glass liner. After filtration and evaporation to dryness, the residue was chromatographed on a 100 g Silical gel (70-230 Mesh) column with 2:1 hexane-ethyl acetate to give 305 mg (61%) of crystalline (3aS)-1,3-dibenzyl-4-n-pentyl-3aβ,4α,6aβ-1H-tetrahydrothieno[3,4-d]-imidazol-2-one (**23**), mp 62-64°C, $[\alpha]^{25}D$ -7.87° (c 1.0, CH_3OH).

Anal. Calcd for $C_{24}H_{30}N_2OS$: C, 73.06; H, 7.66; N, 7.10; S, 8.13. Found: C, 73.08; H, 7.90; N, 7.03; S, 7.92.

(3aS)-4-n-Pentyl-3aβ,4α,6aβ-1H-tetrahydrothieno[3,4-a]imidazol-2(3H)-one (19). To the solution of 500mg (1.26 mmol) of (3aS)-1,3-dibenzyl-4-n-pentyl-3aβ,4α,6aβ-1H-tetrahydrothieno[3,4-d]imidazol-2-one (**23**) in 10 mL of anhydrous tetrahydrofuran and 50 mL of anhydrous liquid ammonia, cooled with dry ice-propanol bath, was added in portions, 600 mg of sodium metal and stirred overnight. An excess of ammonium chloride was added until blue color

disappeared. After all ammonia has evaporated, the reaction mixture was made acidic with 1N HCl and extracted with 4 x 100 mL of ethyl acetate. The ethyl acetate extracts were washed with brine, dried over anhydrous sodium sulfate and evaporated. It gave crude, crystalline (3aS)-4-n-pentyl-3aβ,4α,6aβ-1H-tetrahydrothieno[3,4-d]imidazol-2-one (**19**) in quantitative yield. Recrystallization from ethanol-water, mp 178-180°C. $[\alpha]^{25}D$ +85.36° (c 0.8786, EtOH). ^1H NMR (d_6-DMSO) δ0.87 (t, J = 6.0 Hz, 3H), 1.32 (bm, 2H), 2.58 (d, J = 12.1 Hz, 1H), 2.81 (dd, J= 12.2, 4.4 Hz, 1H), 3.10 (bm, 1H),4.11 (m, 1H), 4.29 (m, 1H), 6.33 (bs, 1H), 6.39 (bs, 1H). Mass spectrum, m/e: 214 (M^+).

Anal. Calcd for $C_{10}H_{18}N_2OS$: C, 56.04; H,d 8.47; N, 13.07; S, 14.96. Found: C, 55.90; H, 8.45; N, 12.82; S, 14.69.

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