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13. ABSTRACT (Maximum 200 Words) Organosulfur compounds have demonstrated protective activity against sulfur mustard (HD), bis(2-chloroethyl) sulfide. Twenty three compounds, many representing new compositions of matter, have been prepared in the first half of this program. These include derivatives of thiavitamin E, glutathione, thiacholesterol, pyridine and benzothiazole. The program has expanded the ability to prepare unsymmetric disulfides of interest and the utility of 2-mercaptobenzothiazole and alkoxycarbonylsulphenyl chlorides in this program. These compounds, largely based upon monothiols, form the platform upon which to develop targets related to BAL and related systems. We currently are awaiting details of bioassay studies.					
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Principal Investigator's Signature

5/01/02
Date

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5. Introduction/Background

The purpose of this program is to develop chemical substances that can serve as medical counter-measures to mustard gas (HD) exposure. Our previous work demonstrated that some types of organosulfur compounds have the ability to protect cells against mustard gas, the extent of protection being a function of several things, including the structural details of the target compound.

Those organosulfur compounds which have demonstrated value in this program all contain divalent sulfur or possess the ability to produce divalent sulfur-containing species under relatively mild conditions. Second, they all are able to function as good nucleophiles or be converted into good nucleophiles under mild conditions. Third, they all appear to be able to act as good anti-oxidants (free-radical inhibitors) by either electron-transfer or hydrogen atom-transfer processes. Many of these compounds are able to function as ligands (or to create them *in vivo*) and a number appear to be able to function as bidentate (or higher) ligands. This ability may influence one-electron transfer processes involving metallic cations as well as the ability of metals to catalyze enzymic reactions, cross cell membranes, and so forth. The functional groups which are most often associated with the desired biological activity include disulfides, thiols and potential/latent thiols.

Our earlier research demonstrated that derivatives of the anti-oxidants glutathione and thiavitamin E (the thiol analog of vitamin E) are among the organosulfur compounds that provide some protection against HD exposure.

This present program has expanded the number of derivatives of glutathione and thiavitamin E available for testing against HD. It also has created a number of new compositions of matter which, because of their molecular fragments, may also exhibit activity. For example, this program has led to the creation of several new sulfur-containing pyridine *N*-oxides. Finally, during this program period we created a number of lipid-like materials possessing potentially efficacious functional groups. These compounds, whose syntheses began by converting cholesterol to its sulfur analog (thiacholesterol), include several disulfides. During this period we also have laid the foundations for synthesizing derivatives of a second important type of lipid, the acylglyceride. These targets are not only of interest because of their potential value in protecting cell membranes from HD. They also are the lead-in compounds

for the preparation of derivatives of BAL, a thiol containing compound known to provide some protection against HD.

A total of twenty-three compounds were submitted during the first half of this contract period. (This exceeds the number suggested in the original SOW.) Their structures are shown in the following Table. We await clear/complete bioassay results.

Table 1.1 Compounds Submitted
Thiacholesterol Derivatives

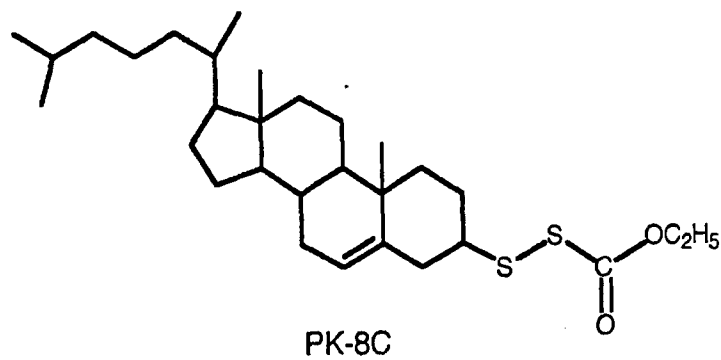
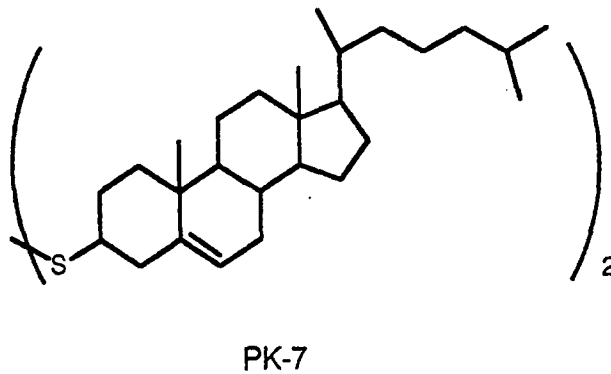
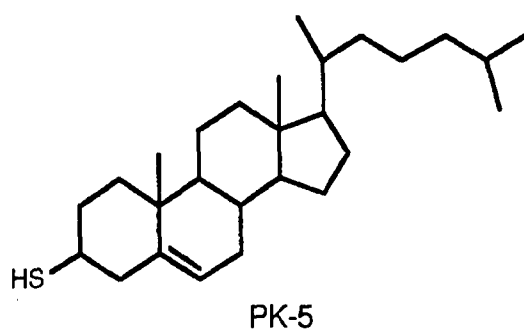
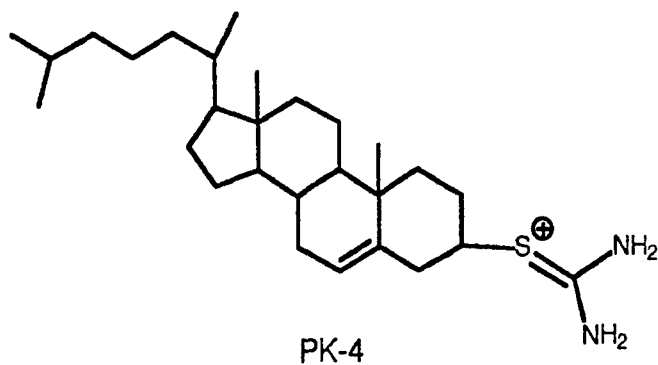
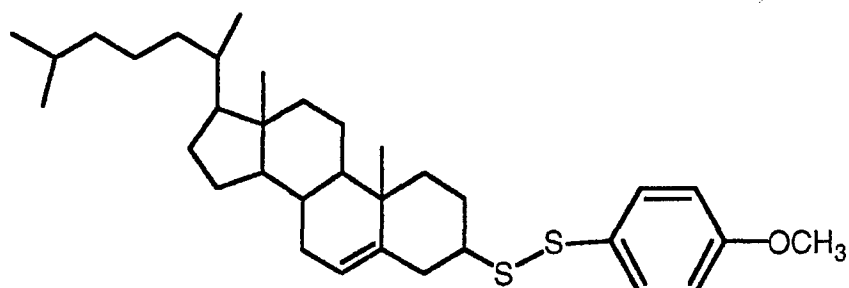
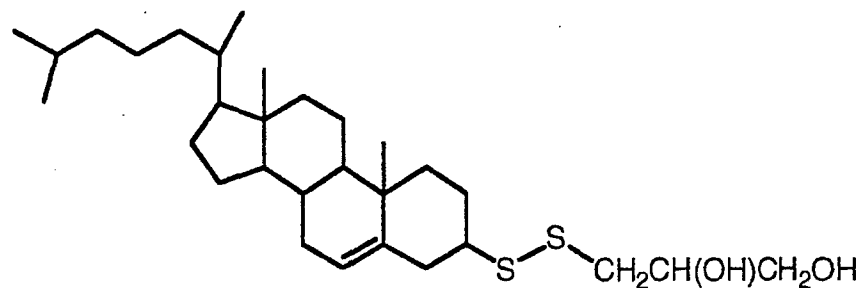


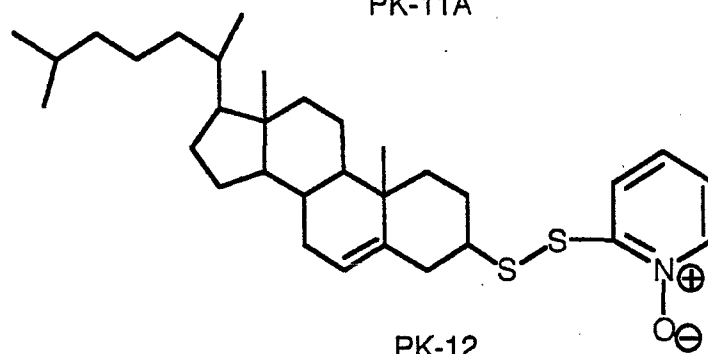
Table 1.2 Compounds Submitted
Thiacholesterol Derivatives (con't)



PK-9

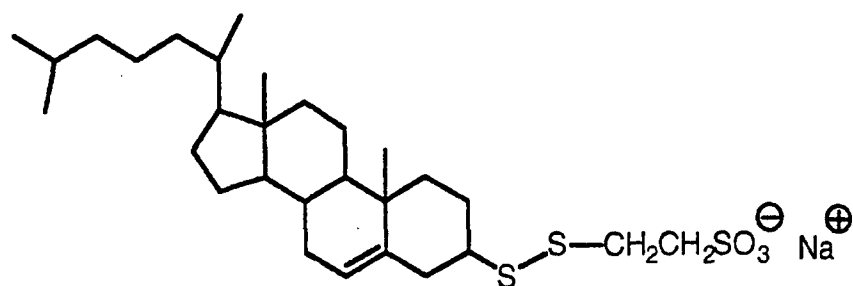


PK-11A

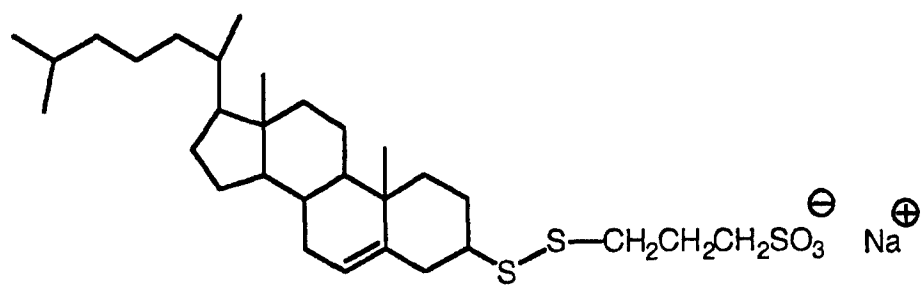


PK-12

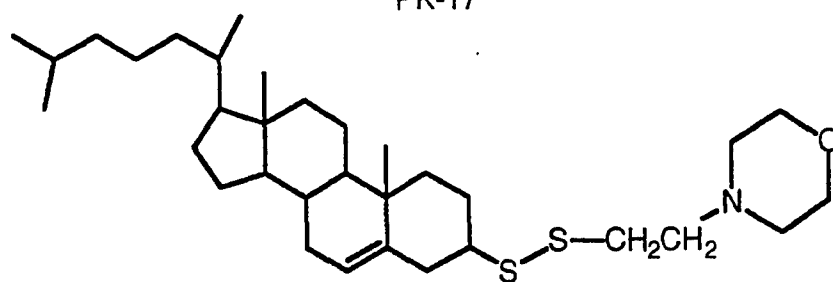
Table 1.3 Compounds Submitted
Thiacholesterol Derivatives (con't)



PK-14A

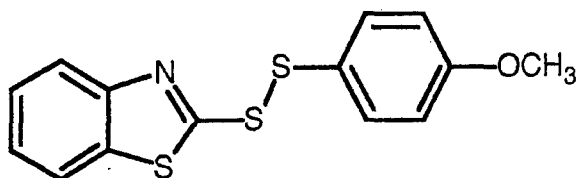


PK-17

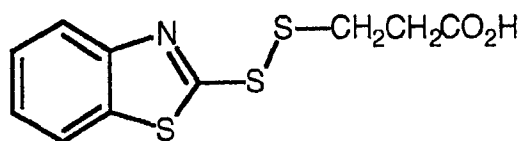


PK-18

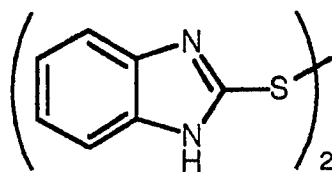
Table 1.4 Compounds Submitted
Heterocyclic Systems



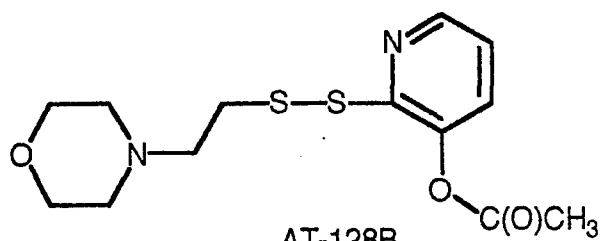
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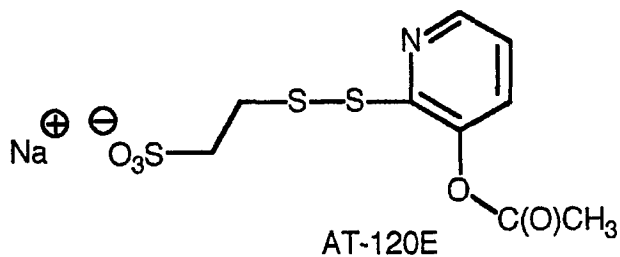
AK-1



AT-134

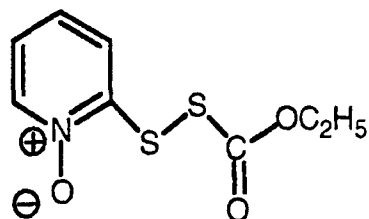


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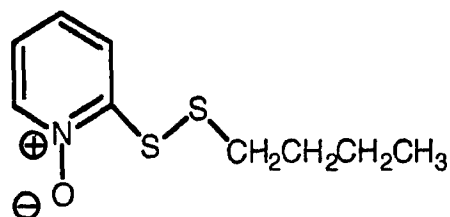


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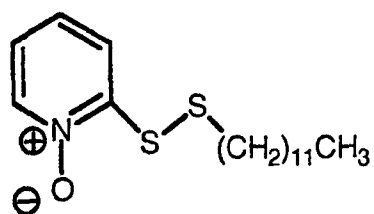
Table 1.5 Compounds Submitted
Heterocyclic Systems (con't)



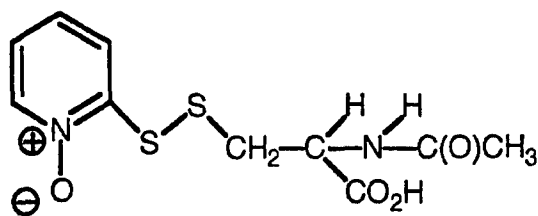
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TYLII-202/3

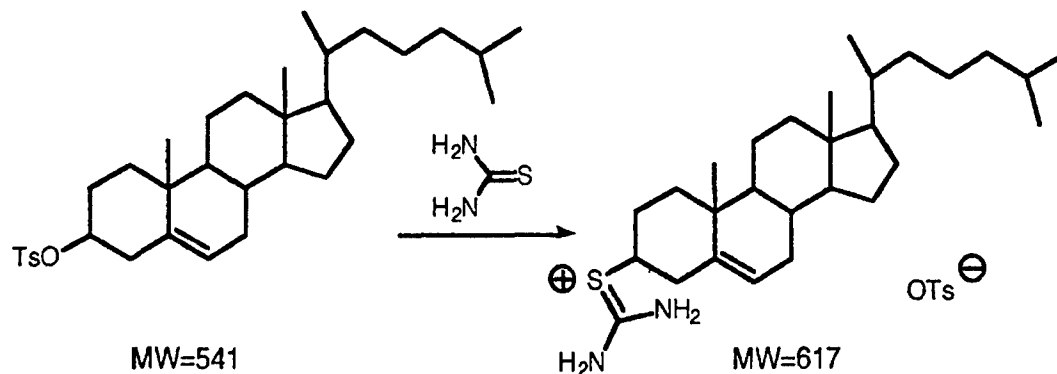
6. Body

Syntheses

All of the target compounds gave satisfactory elemental analyses and molecular spectra (infrared and proton and carbon nmr). Many have been prepared several times and a typical (not yield-maximized) preparation is presented in this section. Equations depict the final step of the syntheses of each target compound submitted during the first portion of this contract period. Melting points which are shown are uncorrected.

Target Compounds - Thiacholesterol Derivatives

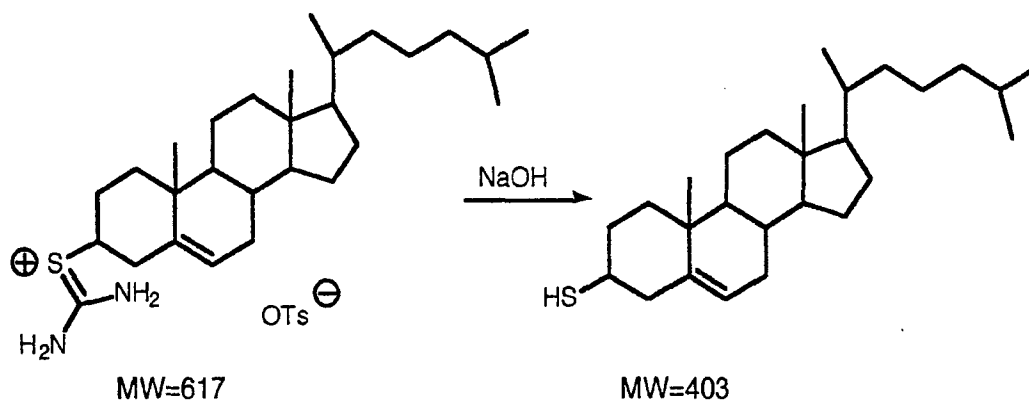
Cholesterylisothiuronium Tosylate (PK-4)



A mixture of 5.4 g (0.010 mol) cholesteryl tosylate, thiourea (9.0 g, 0.12 mol), dry (KOH) pyridine (5 mL) and 50 mL of absolute ethanol was refluxed for 3 h. The resulting *hot* solution was treated with sufficient water to bring the mixture to the cloud point. The reaction mixture then was cooled to room temperature and the resulting solid removed by vacuum filtration. The filtered solid was suspended in 240 mL of acetone and the mixture heated to reflux. The insoluble salt was separated from this mixture by filtration to afford 3.6 g of crude product, mp 236 - 238 °C. This crude salt was recrystallized from ethanol to afford 2.9 g (0.0046 mol, 46% yield) of salt, mp 237 - 239 °C.

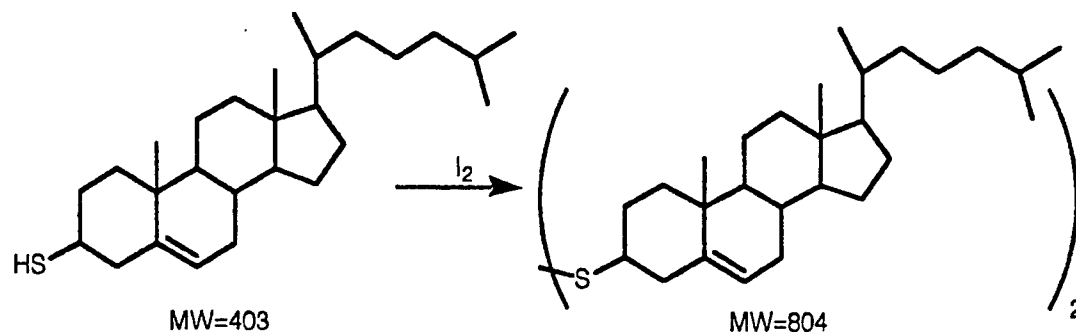
ANAL. Calcd. for C ₃₅ H ₅₆ N ₂ O ₃ S ₂ :	C, 68.14 ; H, 9.15 ; N, 4.54.
Found:	C, 68.18 ; H, 9.37 ; N, 4.67.

This experiment was repeated several times with similar results.

Thiacholesterol (PK-5)

A mixture of cholesterylisothiuronium tosylate (2.5 g, 0.0040 mol), sodium hydroxide (0.068 g, 0.012 mol) and absolute ethanol (55 mL) was refluxed until the system became homogeneous (approx. 0.5 h). After this, 5 mL of water was added and the mixture heated for an additional 2 h. The reaction mixture then was poured on to 100 mL of ice water and treated with ≈ 1 mL of glacial acetic acid. The resulting, slightly acidic mixture was filtered and the isolated solid recrystallized from 5:1 acetone:methanol mixture to afford 1.2 g (0.0030 mol, 75% yield) of white crystals of thiacholesterol, mp 95 - 96 °C. The TLC (silica gel, chloroform eluent, iodine visualization) of this solid exhibited a single spot.

ANAL. Calcd. for $\text{C}_{27}\text{H}_{46}\text{S}$:	C, 80.53; H, 11.51.
Found:	C, 79.91; H, 12.06.

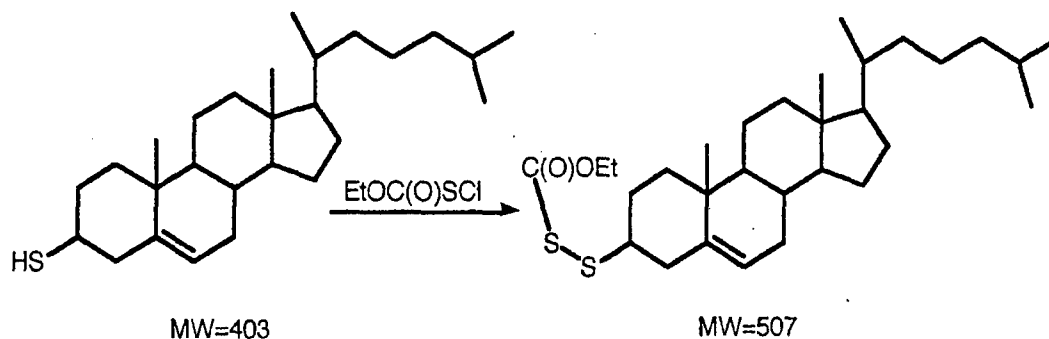
Dicholesteryl Disulfide (PK-7)

To a solution of 2.00 g (0.00496 mol) of thiacholesterol in a mixture 15 mL of hexane and 50 mL of absolute ethanol there was added a solution of iodine (0.63 g, 0.0025 mol) in 10 mL of ethanol. Addition was dropwise and with stirring, required 10 min, and was conducted at rt. After addition was complete the resulting precipitate was removed by filtration and washed with 315 mL of ethanol. This crude product was recrystallized from a benzene-acetone (1:1 v/v) mixture to yield 1.6 g (0.002 mol; 80 %) of the desired of disulfide, mp 141-143 °C.

ANAL. Calcd. for $C_{54}H_{90}S_2$: C, 80.73; H, 11.29.
 Found: C, 79.93; H, 11.74.

This experiment was repeated twice with similar results.

Cholesteryl Ethoxycarbonyl Disulfide (PK-8C)



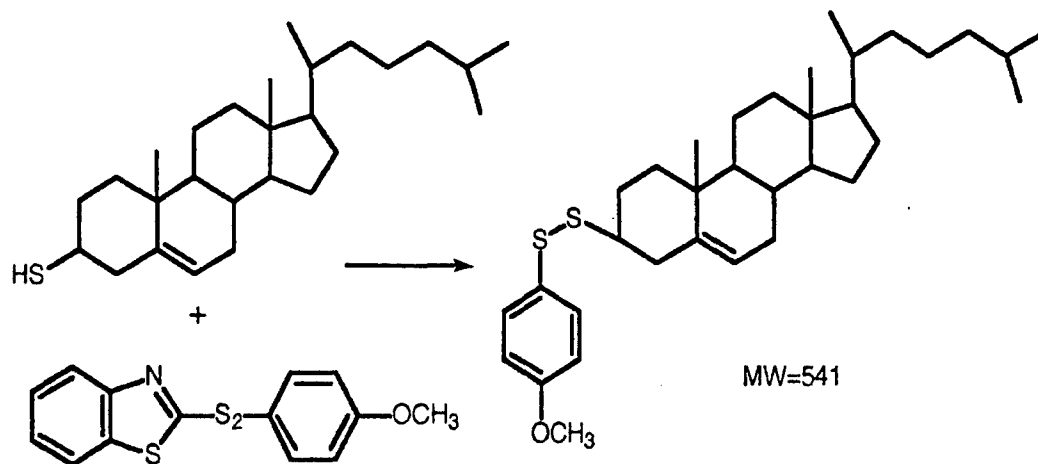
A solution of 4 g, (0.01 mol) of thiacholesterol in a mixture of 15 mL of chloroform and 15 mL of absolute ethanol was added, dropwise and with stirring, to a solution of ethoxycarbonylsulfonyl chloride (1.39 g, 0.0100 mol) in 10 mL of absolute ethanol. The reaction was conducted in a nitrogen atmosphere and at 0 - 5 °C. After 2 h the ice bath was removed and the reaction mixture stirred for 3 days at rt. Then the solvent was removed (rotary evaporator) and the orange-yellowish oil column chromatographed (eluent chloroform; iodine visualization; silica gel, 230 - 400 mesh, 2 mL/min).

The TLC of this substance indicated three spots (aluminum oxide, eluent hexane:chloroform $\approx$ 8:1, iodine visualization). The attempted purification of this compound by column chromatography (neutral alumina, 80-200 mesh, Brockman activity 1; l = 30 cm, d = 4 cm, eluent hexane:chloroform = 8:1; 2 mL/min) was unsuccessful. (Column chromatography monitored by TLC.) The desired ethoxycarbonyl cholesteryl disulfide decomposed during chromatography.

The synthesis was repeated several times, each yielding a product mixture which contained substantial amounts of the desired product. Ultimately purification of the desired product was achieved by column chromatography of several partially purified products using silica gel/hexane-benzene (1:1). Isolated yields ranged from 25 - 40 %. After standing in a refrigerator for several days, in the presence of a few drops of hexane, the isolated oil solidified to form white crystals, mp 58 - 60 °C.

ANAL. Calcd. for $\text{C}_{30}\text{H}_{50}\text{O}_2\text{S}_2$: C, 71.09; H, 9.94.

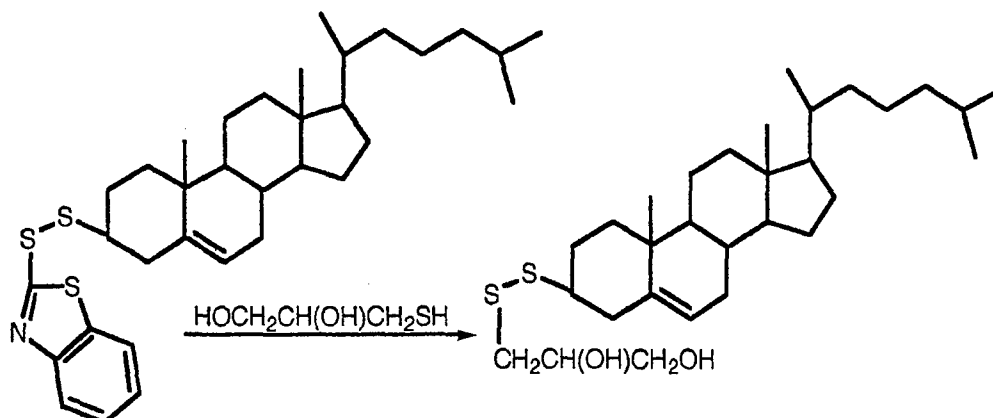
Found: C, 71.14; H, 10.58.

Cholesteryl 4-Methoxyphenyl Disulfide (PK-9)

A solution of thiacholesterol (2.64 g; 0.00656 mol) in 13 mL of chloroform was added, dropwise and with stirring, to a solution of 2.00 g (0.00656 mol) of 2-benzothiazolyl 4-methoxyphenyl disulfide in 100 mL of chloroform. The reaction mixture was maintained at rt and the reaction progress monitored by TLC. As soon as the reaction was completed (≈ 48 h), the chloroform solution was washed first with 5 % aqueous sodium hydroxide (2 x 110 mL) and then with water (2 x 110 mL). The chloroform layer was dried (MgSO_4) and the drying agent removed by filtration. The solvent was removed (rotary evaporator) to yield 2.8 g of the crude product. Recrystallization from acetone yielded 2.0 g (0.0037 mol; 56 %) of pure disulfide as white crystals, mp 70 - 72 °C.

ANAL. Calcd. for $\text{C}_{34}\text{H}_{52}\text{OS}_2$: C, 75.50; H, 9.69.
 Found: C, 75.55; H, 9.92.

Cholesteryl 2,3-Dihydroxypropyl Disulfide (PK-11A)



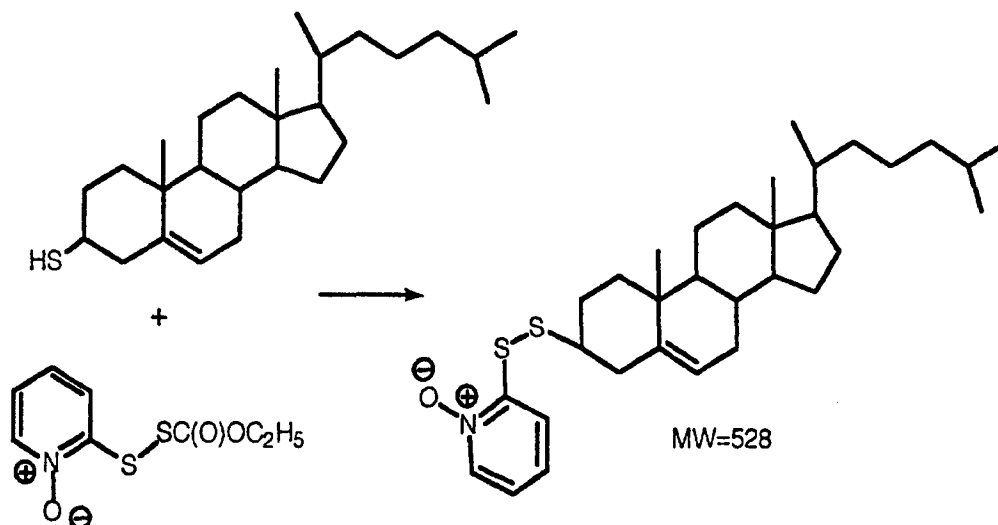
A solution of 1.265 g (0.01054 mol) of 3-mercapto-1,2-propanediol (90 % wt aq. solution) in 55 mL of water was added, dropwise, to a well-stirred solution 5.99 g (0.01054 mol) of 2-benzothiazolyl cholesteryl disulfide in a mixture 264 mL of chloroform and 105 mL of ethanol. The reaction mixture then was stirred at rt for 96 h. The chloroform-rich phase was separated from aqueous layer using a separatory funnel and the organic phase then washed successively with 5 % aq. sodium hydroxide (2 x 110 mL) and then with water (2 x 110 mL). The washed solution then was dried (MgSO_4). The drying agent then was removed by filtration and the solvent removed (rotary evaporator) to afford the crude product.

The crude product was column chromatographed (silica gel [200-400 mesh], eluent: CHCl_3 followed by a mixture of chloroform and methanol (5:1 v/v), and the fractions monitored by TLC, iodine visualization). The fractions with $R_f = 0.7$ were combined and the solvent removed by rotary evaporation to yield 3 g of crude product. Recrystallization from ethanol gave 2.5 g (0.0049 mol, 47 %) of pure cholesteryl 2,3-dihydroxypropyl disulfide as white crystals, mp 98 - 100 °C.

ANAL. Calcd. for $\text{C}_{30}\text{H}_{52}\text{O}_2\text{S}_2$: C, 70.81; H, 10.30.

Found: C, 71.06; H, 10.23.

Cholesteryl 2-Pyridyl Disulfide *N*-oxide (PK-12)



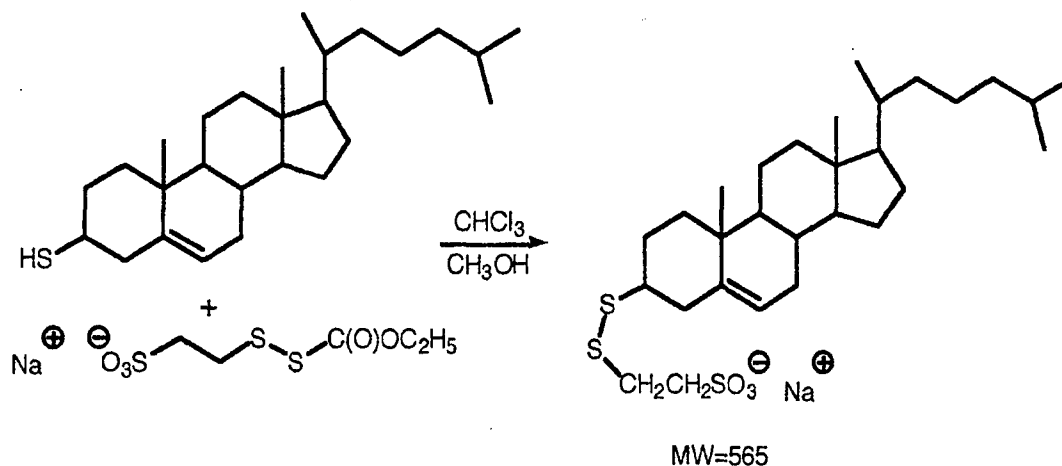
Thiacholesterol 2.00 g (0.00500 mol) was added, dropwise and with stirring, to a solution of 1.15 g (0.0050 mol) of ethoxycarbonyl 2-pyridyl disulfide *N*-oxide in methanol (35 mL). The reaction was conducted at room temperature and under nitrogen. After 30 min, 40 mL of chloroform was added. The mixture then was stirred for 48 h at room temperature and under nitrogen. After this the volatiles were removed using a rotary evaporator.

The residue was column chromatographed (silica gel; flow rate $\approx$ 5 mL/min; dimensions $l = 30$ cm, $d = 4$ cm; eluent chloroform:methanol (4:1 v/v)). The fractions were monitored by TLC (silica gel; similar eluent). Upon concentration (rotary evaporator) and drying (in vacuum; P_2O_5) overnight, the fractions with $R_f = 0.75$ gave 0.70 g (0.0013 mol, 27 %) of cholesteryl 2-pyridyl disulfide *N*-oxide, mp 167 - 169 °C.

ANAL. Calcd. for $C_{32}H_{49}NOS_2$: C, 72.81; H, 9.36.

Found: C, 72.83; H, 9.52.

Sodium Cholesteryl 2-(Sulfonatoethyl) Disulfide (PK-14A)



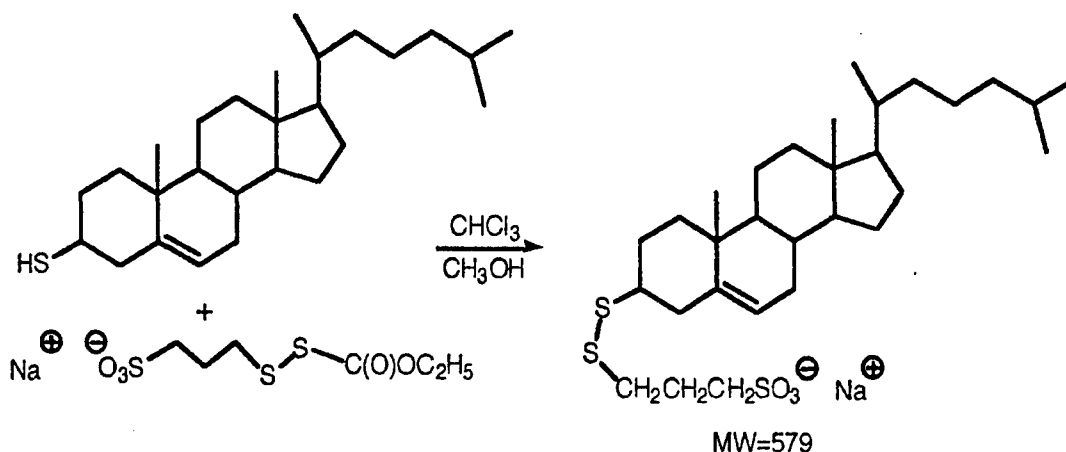
A solution of 1.00 g (0.00248 mol) of thiacholesterol in 15 mL of chloroform was added, dropwise and with stirring, to a solution of 0.598 g (0.00223 mol) of sodium ethoxycarbonyl 2-(sulfonatoethyl) disulfide in 15 mL of methanol at rt. The addition and subsequent reaction were conducted under nitrogen. Stirring was continued at rt for 48 h and the progress of the reaction monitored by TLC (silica gel, chloroform eluent, iodine visualization). Then the solvent was removed (rotary evaporator) and the residue purified by column chromatography (substrate: silica gel 230-400 mesh; l=30 cm, d=2 cm, eluent: (1) chloroform [1 L] and then (2) methanol (1.2 L); flow rate 5 mL/min. The purity of the fractions was monitored by TLC (silica gel plate, eluent chloroform, iodine visualization).

Upon concentration (rotary evaporator) and drying in vacuum (overnight; P_2O_5) the fractions with $R_f = 0,0$ gave 0.91g of the desired, crude disulfide. This product was washed with methanol (2 x 15 mL), removed by filtration and dried in vacuum (overnight; P_2O_5) to yield 0.870 g (0.00154 mol, 69.1%) of the final disulfide as white crystals, dec. 220 - 250 °C.

ANAL. Calcd. for $C_{29}H_{49}NaO_3S_2$:	C, 61.66; H, 8.74.
Found:	C, 61.00; H, 9.09.

This compound appears to form an emulsion in water, that is, it acts as a surfactant.

Sodium Cholesteryl 3-(Sulfonatopropyl) Disulfide (PK-17)

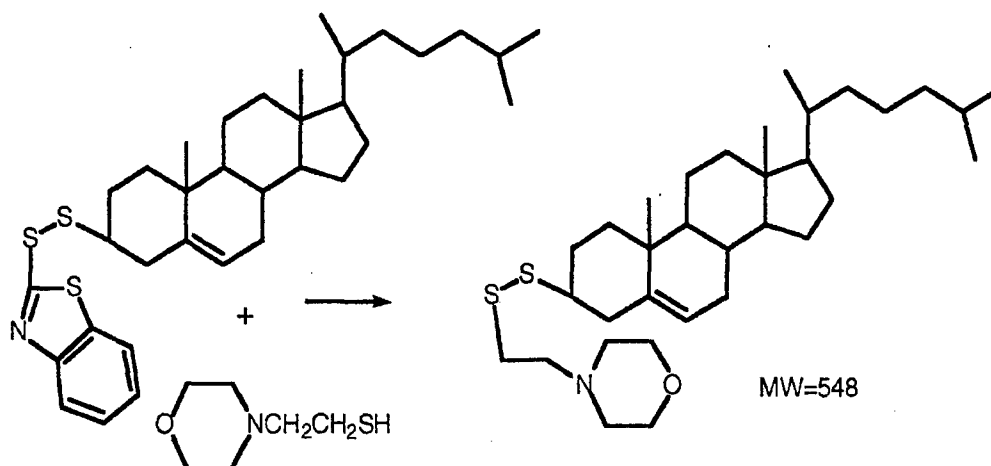


A solution of 1.00 g (0.00248 mol) of thiacholesterol in 15 mL of CHCl_3 was added, dropwise and with stirring, to a solution of 0.598 g (0.00212 mol) of sodium ethoxycarbonyl 3-(sulfonatopropyl) disulfide in 15 mL of methanol. The addition was conducted at 20 °C and under nitrogen. Stirring was continued at rt for 72 h and the progress of the reaction monitored by TLC (silica gel plate; chloroform eluent; iodine visualization). After the reaction was complete the solvent was removed (rotary evaporator) and the residue purified by column chromatography (substrate:silica gel [230 – 400 mesh]; elution rate $\approx$ 5mL/min; $l=30$ cm; $d=2$ cm, eluent: (1) chloroform (0.7 L) and then (2) methanol (0.9 L)). The composition of the fractions was monitored using TLC (silica gel plate; eluent chloroform; iodine visualization).

After removing the solvent and drying the residue overnight (in vacuum; P_2O_5) there resulted 1.10 g of crude disulfide. This product was washed with methanol (2 x 7 mL), and the washed solid isolated by filtration. This was then dried (in vacuum; P_2O_5) overnight to yield 0.950 g (0.00164 mol; 77.4%) of the desired disulfide as white crystals, dec. 200 - 230 °C.

ANAL. Calcd. for $\text{C}_{30}\text{H}_{51}\text{NaO}_3\text{S}_3$:	C, 62.24; H, 8.88.
Found:	C, 61.52; H, 9.17.

Cholesteryl 2-(*N*-Morpholino)ethyl Disulfide (PK-18)



A solution of 0.778 g (0.00532 mol) of 2-(*N*-morpholino)ethanethiol in 11 mL of chloroform was added, dropwise and with stirring, to a suspension of 3.00 g (0.00528 mol) of cholesteryl 2-benzothiazolyl disulfide in 80 mL of chloroform. Addition was conducted at rt and under nitrogen. Addition required about 30 min and during this time the reaction mixture became homogeneous. The solution was maintained at rt and the reaction's progress monitored by TLC (silica gel plate, eluent chloroform, iodine visualization).

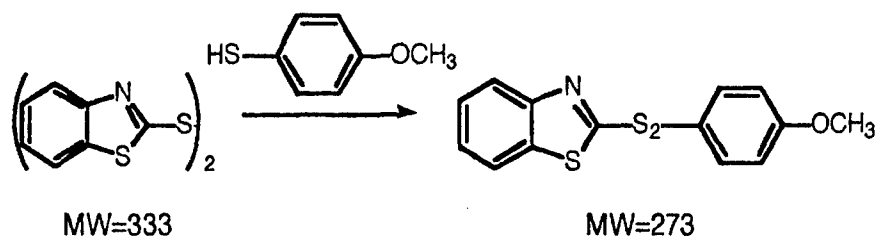
As soon as the reaction was completed (≈ 48 h), the chloroform solution was washed initially with 5 % aqueous sodium hydroxide (2 x 11 mL) and then with water (2 x 11 mL) and dried (MgSO_4). The drying agent was removed by vacuum filtration and the solvent then removed (rotary evaporator) to yield 3.10 g of crude product. Recrystallization from methanol yielded 2.20 g (0.00401 mol, 75.9 %) of disulfide as white crystals, mp 59 - 60 °C.

ANAL. Calcd. for $\text{C}_{33}\text{H}_{57}\text{NOS}_2$: C, 72.34; H, 10.49.
 Found: C, 72.19; H, 10.76.

This reaction was repeated twice with similar results.

Target Compounds - Heterocyclic Systems

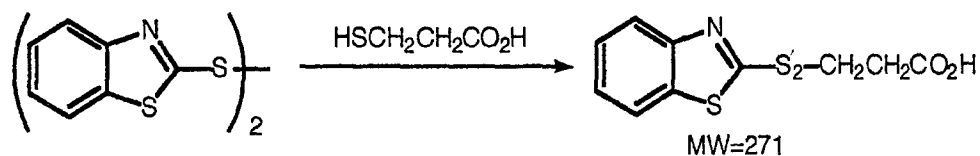
2-Benzothiazolyl 4-Methoxyphenyl Disulfide (PK-16)



A slurry of 2,2'-dithiobis(benzothiazole) (0.0200 mol) in 50 mL of chloroform was treated with 2.80 g (0.0200 mol) of 4-methoxybenzenethiol. After about 45 min. of stirring at 25 °C the reaction mixture became homogeneous. The resulting chloroform solution was washed successively with 5 % aqueous sodium hydroxide (2 x 50 mL), water (2 x 50 mL) and then dried (MgSO_4). The drying agent was removed by filtration and the solvent removed on a rotary evaporator (reduced pressure) to afford an oily residue (5.95 g) which solidified while drying overnight at 0.01 torr. Recrystallization (ethanol solvent) afforded 4.8 g (0.018 mol, 90 %) of the desired 2-benzothiazolyl 4-methoxyphenyl disulfide as off-white crystals, mp 59 - 60 °C.

ANAL. Calcd. for $\text{C}_{14}\text{H}_{11}\text{NOS}_3$: C, 55.06; H, 3.63; N, 4.59.

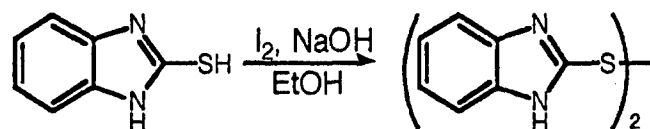
Found: C, 54.60; H, 3.58; N, 4.66.

2-Benzothiazolyl 2-Carboxyethyl Disulfide (AK-1)

A solution of 3-mercaptopropanoic acid (1.06 g, 0.0100 mole) in 25 mL of chloroform was added, dropwise and with stirring, to a suspension of 2,2'-dithiobisbenzothiazole (3.32 g, 0.0100 mole) in 150 mL of chloroform. Addition required 15 min. The reaction mixture became homogeneous after 30 min. The reaction mixture then was stirred for an additional 1.5 h, during which time a solid was deposited. TLC (silica gel, chloroform eluent, iodine visualization) revealed the absence of any starting material. The resulting solid (2.10 g) was separated from the mixture by filtration (vacuum) and the chloroform solution allowed to stand in the refrigerator overnight. This afforded an additional 0.500 g of solid which was separated by filtration (vacuum). The two solids that were collected were identical and combined to yield, after recrystallization from methanol, 2.40 g (0.00885 mol; 88.5 %) of the desired product, mp 160 - 161 °C.

ANAL. Calcd. for $\text{C}_{10}\text{H}_9\text{NO}_2\text{S}_3$: C, 44.26; H, 3.34; N, 5.16.

Found: C, 44.16; H, 3.49; N, 4.99.

2-Benzimidazolyl Disulfide (AT-134)

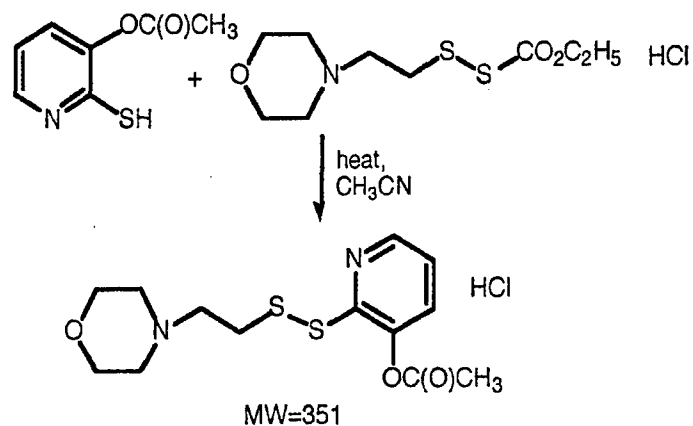
MW=298

A sample of 10 g (0.067 mol) of 2-mercaptobenzimidazole was suspended in 100 mL of absolute ethanol and the mixture stirred magnetically. To this solution there was added, dropwise and with stirring, a solution of one equivalent of iodine and sodium hydroxide dissolved in 150 mL of ethanol. The resulting mixture was stirred until the iodine color disappeared (≈ 2 h). Approximately one-half of the solvent was removed under reduced pressure and the residue then treated with 100 mL of water. The resulting suspension was extracted with 3 x 100 mL of methylene chloride. The isolated methylene chloride solution was dried (MgSO_4) and the drying agent removed by filtration. The resulting dried solution was concentrated (rotary evaporator) to afford 9.1 g of the crude desired disulfide. This solid was recrystallized from ethanol to afford 7.3 g (0.025 mol; 73 %) of the desired product which melted with decomposition at $\approx 206^\circ\text{C}$.

ANAL. Calcd. for $\text{C}_{14}\text{H}_{10}\text{N}_4\text{S}_2$: C, 56.36; H, 3.38; N, 18.78; S, 21.49.

Found: C, 56.41; H, 3.01; N, 18.99; S, 21.66.

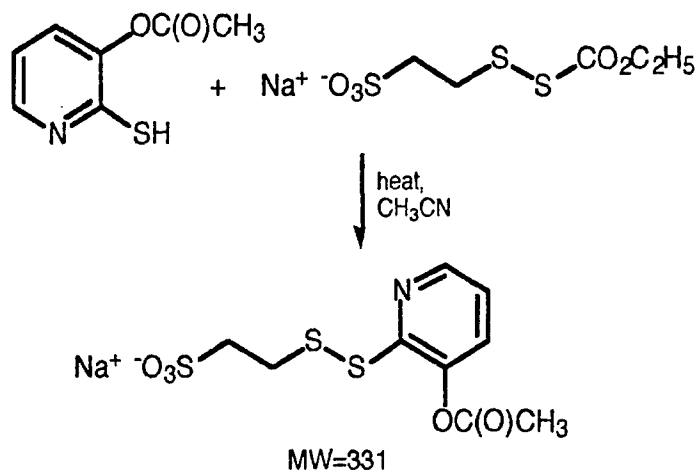
2-Acetylpyridyl 2-(*N*-Morpholino)ethyl Disulfide Hydrochloride (AT-128B)



A sample of 4.0 g (0.024 mol) of 3-acetoxy-2-mercaptopyridine was suspended in 100 mL of acetonitrile with vigorous magnetic stirring. To this there was added an equivalent amount of the required unsymmetric disulfide pictured in the equation as its hydrochloride salt. The mixture then was warmed to 50 °C and maintained at this temperature for 3 h with stirring. The resulting mixture then was cooled to room temperature and the majority of the solvent removed using a rotary evaporator. The solid which was formed was removed by vacuum filtration and then recrystallized twice from ethanol. There resulted 2.8 g (0.0080 mol, 33 %) of the desired product, mp 161-163 °C.

ANAL. Calcd. for $C_{13}H_{19}ClN_2O_3S_2$: C, 44.50; H, 5.46; N, 7.98; S, 18.27.
 Found: C, 44.24; H, 5.25; N, 7.75; S, 18.54.

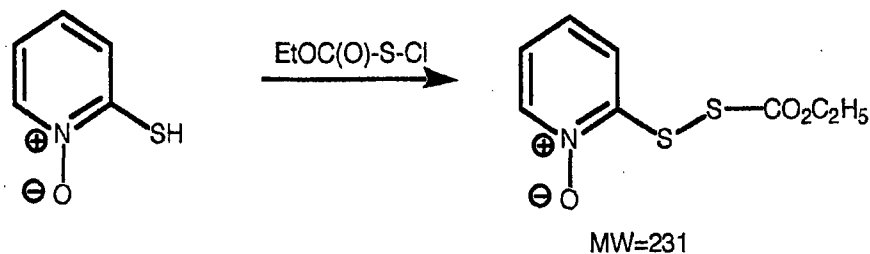
Sodium 2-Acetylpyridyl 2-sulfonatoethyl Disulfide (AT-120E)



A sample of 4.2 g (0.025 mol) of 3-acetoxy-2-mercaptopyridine was suspended in 110 mL of acetonitrile with overhead stirring. To this there was added an equivalent amount of the required unsymmetric disulfide pictured in the equation above as the sodium salt. The reacting system was warmed to 50 - 55 °C and maintained at this temperature for $\approx$ 4 h with stirring. The resulting mixture then was cooled to room temperature and the majority of the solvent removed using a rotary evaporator. The solid which was formed was removed by vacuum filtration and then recrystallized twice from aq. methanol (9:1 methanol:water). There resulted 2.6 g (0.0079 mol, 32 %) of the desired product, mp 144 - 147 °C, as white crystals.

ANAL. Calcd. for $\text{C}_9\text{H}_{10}\text{NNaO}_5\text{S}_3$: C, 32.62; H, 3.04; N, 4.23.
 Found: C, 31.71; H, 3.60; N, 4.02.

Ethoxycarbonyl 2-Pyridyl Disulfide *N*-oxide (TYLII 182/22)

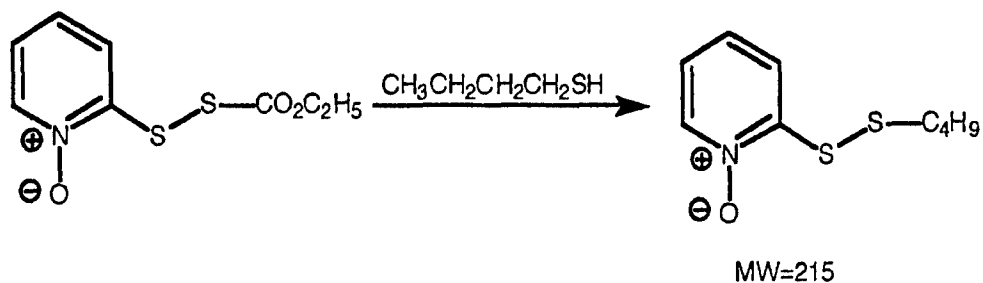


A solution of 2-mercaptopyridine *N*-oxide (1.4 g, 0.011 mol) in 6 mL of trifluoroacetic acid was added to a solution of ethoxycarbonylsulfonyl chloride (1.7 g, 0.012 mol) in trifluoroacetic acid (9 mL). The addition, which was conducted at -15 °C, with stirring and under a nitrogen atmosphere, required 15 min. The cold bath then was removed and the reaction mixture stirred for an additional 30 min at room temperature. The volatiles then were removed (rotary evaporator) at 30 °C and the residue dissolved in 20 mL of acetic acid. The acetic acid then was evaporated under reduced pressure and at 30 °C.

This procedure was repeated ten times (10 x 20 mL = 200 mL of acetic acid *in toto*). The resulting mass was suspended in 20 mL of heptane, mixture stirred for 5 min and the volatiles removed in vacuo at 30 °C. This procedure was repeated twenty times (*in toto* 20 x 20 mL = 400 mL of heptane). The solid residue was dried for overnight (P₂O₅) at room temperature to yield 2.29 g (0.0099 mol, 90 %) of the desired disulfide as a white powder, mp 87 - 88 °C.

ANAL. Calcd. for C₈H₉NO₃S₂: C, 41.54; H, 3.92; N, 6.06; S, 27.73.
 Found: C, 41.29; H, 3.87; N, 5.83; S, 26.15.

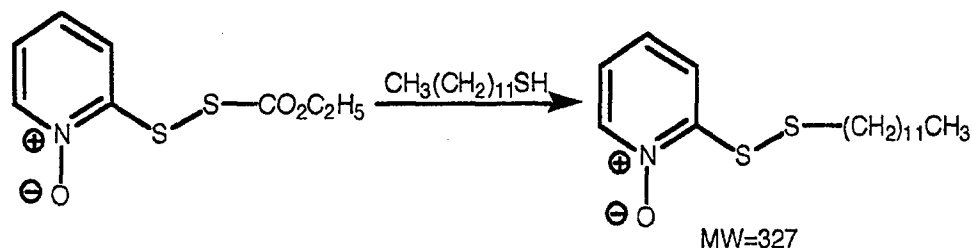
Butyl 2-Pyridyl Disulfide *N*-oxide (TYLII-218/1)



A sample of 5.3 g (0.024 mol) of ethoxycarbonyl 2-pyridyl disulfide *N*-oxide was mixed with 60 mL of acetonitrile. To this there was added an equivalent amount of butanethiol (syringe) and the resulting mixture stirred at 20 °C for 1 h. It then was warmed to 50 °C for 1 h. After this the reaction mixture was concentrated using a rotary evaporator to afford a viscous straw-colored oil. This was purified using low pressure column chromatography (silica gel/chloroform). Ultimately, there resulted 5.1 g (0.023 mol, 99 %) of the desired unsymmetric disulfide as an oil, n_D^{20} 1.6226. Attempted distillation led to decomposition at approx. 185 - 195 °C.

ANAL. Calcd. for $C_9H_{13}NOS_2$: C, 50.20; H, 6.08; N, 6.50.

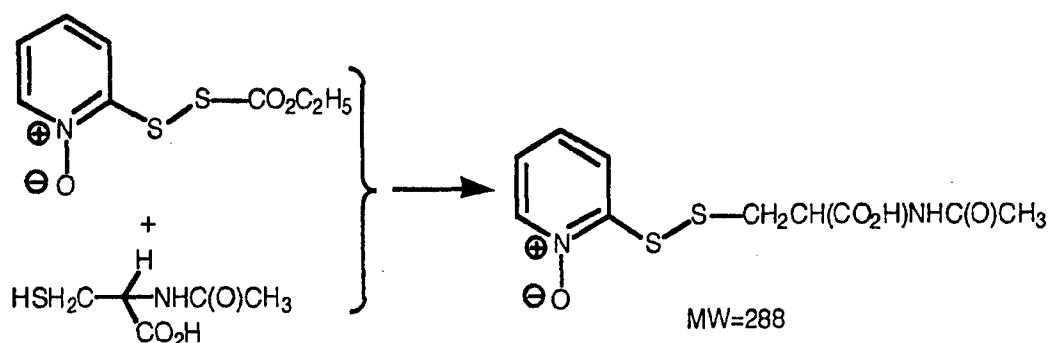
Found: C, 49.14; H, 6.05; N, 6.53.

Dodecyl 2-Pyridyl Disulfide *N*-oxide (TYLII-230/1)

A sample of 5.0 g (0.022 mol) of ethoxycarbonyl 2-pyridyl disulfide *N*-oxide was mixed with 65 mL of acetonitrile. To this there was added an equivalent amount of dodecanethiol (syringe) and the resulting mixture stirred at 20 °C for 1 h. It then was warmed to 40 °C for 2 h. After this the reaction mixture was concentrated using a rotary evaporator to afford a viscous, straw-colored oil. This oil was washed with $\approx$ 5 mL of cold ethanol. After standing for approx. a week in a refrigerator, the resulting oil solidified to form 2.8 g (0.0083 mol, 38 % yield) of the desired product, mp 38 °C.

Based upon the elemental analysis the compound is believed to exist as a hydrate.

ANAL. Calcd. for $C_{17}H_{29}NOS_2 \cdot 1/2 H_2O$:	C, 60.67; H, 8.98; N, 4.16.
Found:	C, 60.50; H, 8.92; N, 4.07.

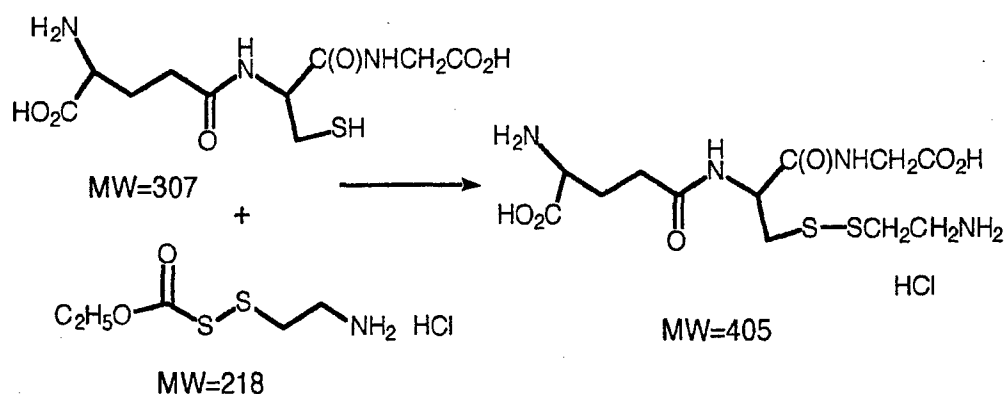
***N*-Acetyl-L-cysteinyl 2-Pyridyl Disulfide *N*-oxide (TYLII-202/3)**

A sample of 3.0 g (0.013 mol) of ethoxycarbonyl 2-pyridyl disulfide *N*-oxide was mixed with 100 mL of acetonitrile. To this there was added, with stirring, an equivalent amount of *N*-acetylcysteine and the resulting mixture stirred at 20 °C for 2 h. It then was warmed to 50 °C for 1 h. After this the reaction mixture was concentrated using a rotary evaporator to afford an off-white solid. This solid was then recrystallized twice from ethanol to afford 2.5 g (0.0087 mol, 67 %) of the desired product, mp 180 - 182 °C. The sample, like other pyridine oxides, appears to be slightly hygroscopic.

ANAL. Calcd. for C ₁₀ H ₁₂ N ₂ O ₄ S ₂ :	C, 41.65; H, 4.19; N, 9.71; S, 22.24.
Found:	C, 41.87; H, 4.15; N, 9.64; S, 22.78.

**Target Compounds -
Miscellaneous**

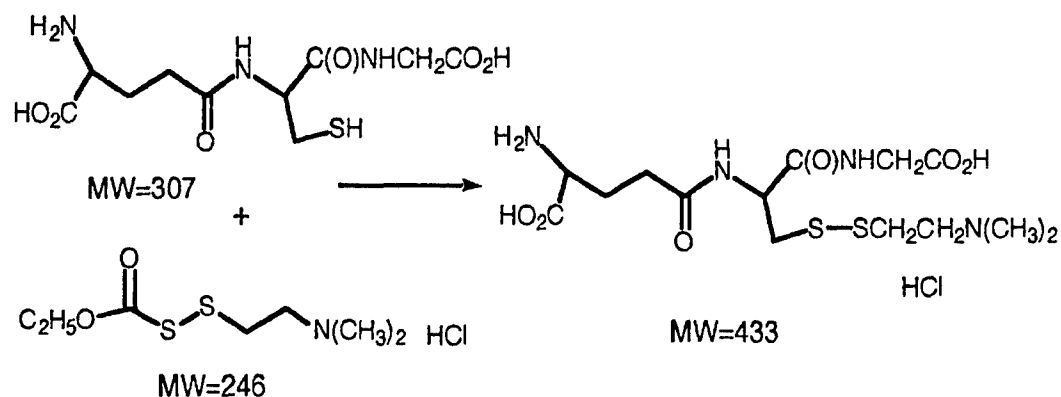
2-Aminoethyl Glutathionyl Disulfide Hydrochloride (TYLII 72)



A solution of 2-aminoethyl ethoxycarbonyl disulfide hydrochloride (1.1 g, 5.0 mmol) in methanol (25 mL) was added, in one portion and with a stirring, to a suspension of glutathione (1.5 g, 5.0 mmol) in methanol (65 mL). The resulting mixture then was stirred vigorously for 23 h to afford a suspension. This suspension was filtered and the collected solid washed with ether (2 x 50 mL) and then dried in vacuum (P_2O_5 ; 10 h; 70 °C). There resulted 1.5 g (73 %) of 2-aminoethyl glutathionyl disulfide hydrochloride, mp ≈ 138 °C (dec ≈ 178 °C).

ANAL. Calcd. for $\text{C}_{12}\text{H}_{23}\text{ClN}_4\text{O}_6\text{S}_2$: C, 34.41; H, 5.35; N, 13.37; S, 15.31.
Found: C, 35.16; H, 5.68; N, 13.11; S, 15.02.

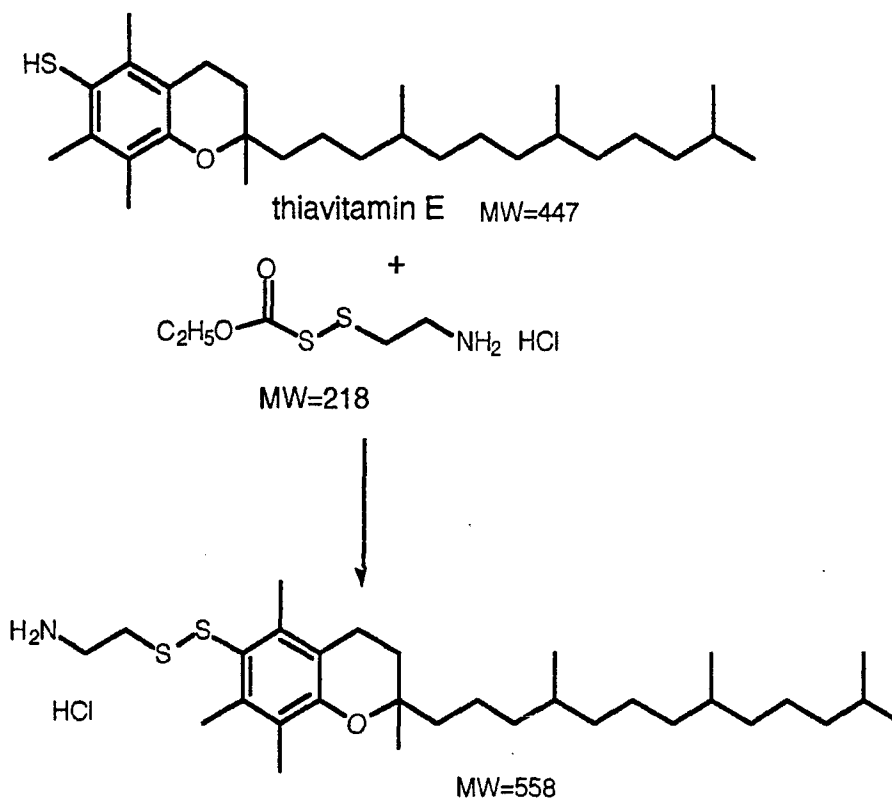
2-*N,N*-Dimethylaminoethyl Glutathionyl Disulfide Hydrochloride (TYLII-166/3)



A solution of 2-*N,N*-dimethylaminoethyl ethoxycarbonyl disulfide hydrochloride (1.3 g, 5.2 mmol) in methanol (30 mL) was added, in one portion and with a stirring, to a suspension of glutathione (1.5 g, 4.9 mmol) in methanol (75 mL). The resulting mixture then was stirred vigorously for 24 h to afford a suspension. This suspension was filtered and the collected solid washed with ether (2 x 50 mL) and then dried in vacuum (P_2O_5 ; 12 h; 70 °C). There resulted 1.4 g (3.33 mmol; 68 %) of 2-*N,N*-dimethylaminoethyl glutathionyl disulfide hydrochloride, mp ≈ 108 °C (dec ≈ 142 °C).

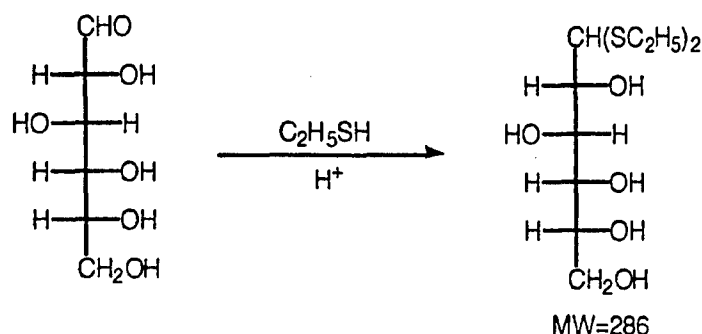
ANAL. Calcd for $C_{14}H_{27}ClN_4O_6S_2 + H_2O$: C, 36.16; H, 6.29; N, 13.37; S, 15.31.
Found: C, 35.16; H, 5.68; N, 13.11; S, 15.02.

2-Aminoethyl Thiavitamin E Disulfide Hydrochloride (ALT-63A)



To a solution of thiavitamin E (2.6 g, 5.8 mmol) dissolved in absolute ether (10 mL) there was added, in one portion and with stirring, a solution of 2-aminoethyl ethoxycarbonyl disulfide hydrochloride (1.27 g, 5.82 mmol) in anhydrous acetonitrile (100 mL) (nitrogen atmosphere). The mixture was stirred under nitrogen for 0.5 h at 20 °C. Then the reaction mixture was refluxed for 2 h (until a negative reaction was demonstrated with Ellman's reagent). The reaction mixture then was stirred overnight at room temperature. A slightly yellow, oily solid obtained was collected by vacuum filtration. This was washed first with a small amount of water and then with a small portion of *cold* hexane (moderately soluble). Ultimately it was dried in a vacuum dessicator (P_2O_5) overnight to afford 2.3 g of crude product. This was triturated with cold methanol to afford 2.0 g (3.6 mmol; 62 %) of the desired product as a slightly orange-colored amorphous powder. This sample decomposed upon heating.

ANAL. Calcd. for $C_{31}H_{56}ClNOS_2$:	C, 66.68; H, 10.11; N, 2.51; S, 11.48.
Found:	C, 66.92; H, 10.17; N, 2.51; S, 11.71.

1,1-Di-(1',1'-dithiapropryl) D-Glucose (AT-172A)


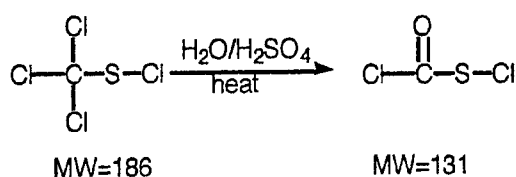
A sample of D-glucose (18 g; 0.10 mol) was dissolved in a mixture of dioxane:water ($\approx 2/1$:v/v; approx. 100 mL). To this there was added 5 mL of trifluoroacetic acid and 14.0 g (0.23 mol) of ethanethiol (via syringe). The reaction mixture was warmed to $\approx 30^\circ\text{C}$ and stirred at this temperature for 2 h. At this time an additional 7 mL of thiol was added. After stirring overnight the reaction mixture was combined with 300 mL of benzene and the water removed by azeotropic distillation (Dean-Stark trap). The volatiles were then removed under reduced pressure (rotary evaporator) and the residue washed with a small amount of cold water. The resulting solid was washed once with cold methanol and the volatiles removed under vacuum. There resulted 6.2 g (0.022 mol; 22 %) of the desired compound as white crystals, $116\text{--}118^\circ\text{C}$.

ANAL. Calcd. for $\text{C}_{10}\text{H}_{22}\text{O}_5\text{S}$: C, 41.94; H, 7.74.
 Found: C, 42.02; H, 7.86.

Non-Target Compounds

The following describe our synthesis of selected compounds germane to this program.

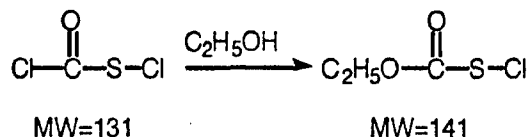
Chlorocarbonylsulfenyl Chloride –



Into a three-necked rb flask (N₂ atmosphere) there was placed a mixture of 18 mL of H₂O and 210 mL of conc. sulfuric acid. To this there then was added, with vigorous stirring and in one portion, 186 g (1.00 mol) of perchloromethylmercaptan. The resulting mixture, maintained at 45-50 °C, became yellow and was stirred for a total of 2 h. It then was transferred to a separatory funnel and the upper, yellow layer isolated. This crude ethoxycarbonylsulfenyl chloride was dried (MgSO₄) and the product used without further purification. The yield was 107 g, ≈ 82 %.

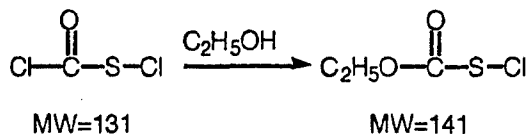
The procedure was repeated a number of times with minor variations (e.g., varying drying agent) and comparable results.

Ethoxycarbonylsulfonyl Chloride – Procedure A



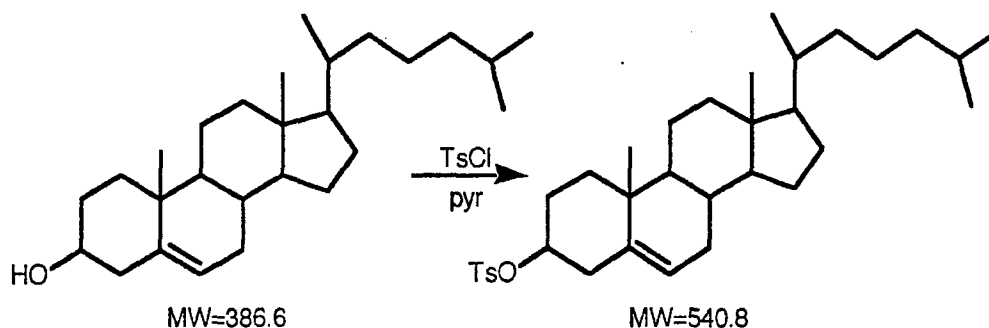
Into a three-necked, rb flask there was placed 105 g (0.744 mol) of chlorocarbonylsulfonyl chloride. The system, under a nitrogen blanket, then was stirred magnetically and maintained at $\approx 25^\circ\text{C}$ while 37 g (0.80 mol) of absolute ethanol was added dropwise and with stirring; addition required approx. 2 h. The resulting mixture then was stirred at 25°C for about 6 h after which the desired ethoxycarbonylsulfonyl chloride was obtained by vacuum distillation (bp $56 - 61^\circ\text{C}/21$ torr; n_D^{20} 1.4777-1.4740).

Procedure B



Using a three-necked, rb flask (nitrogen atmosphere), a sample of 38 g (0.29 mol) of crude chlorocarbonylsulfonyl chloride was warmed to 30°C . To this there was added, dropwise and with stirring, 14 g (0.30 mol) of ethanol and the reaction temperature then maintained at $30 - 35^\circ\text{C}$ for 6 h. It then was allowed to remain at room temperature overnight. The crude mixture was purified by vacuum distillation to yield 11.7 g (0.083 mol) of ethoxycarbonylsulfonyl chloride (bp $45 - 53^\circ\text{C}/20$ torr). A second fraction, 9.7 g (0.074 mol), was isolated with bp $53 - 56^\circ\text{C}/20$ torr. The combined yield was 55 %.

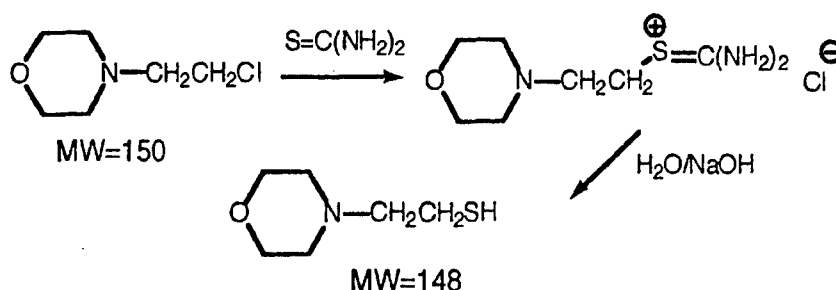
This procedure has been conducted a number of times with minor variations but with generally similar results.

Cholesteryl Tosylate

A mixture of cholesterol (19.3 g, 0.0500 mol), tosyl chloride (16.3 g, 0.0855 mol) and 75 mL of dried (KOH) pyridine was stirred for 26 h at room temperature in a three-necked, rb flask which was equipped with a drying tube (CaCl_2). The reaction mixture then was poured into ≈ 110 mL of ice water. The resulting precipitate was washed with 4 x 100 mL of ice water and then air dried. There resulted 27.1 g (approx. 100 %) of crude product, melting range 121-126 $^\circ\text{C}$. TLC (silica gel, chloroform eluent, iodine visualization) indicated the presence of only one substance. This dried solid was dissolved in ≈ 20 mL of refluxing benzene and then ≈ 40 mL of pentane added. A solid precipitated. After cooling the mixture, this solid was removed by filtration and dried to yield 20.9 g (0.0387 mol, 77% yield) of the desired product, mp 127-130 $^\circ\text{C}$.

Other runs afforded the product in yields ranging from ≈ 60 to ≈ 80 % yield.

2-(*N*-morpholino)ethanethiol



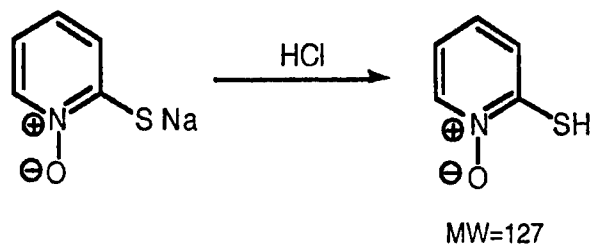
Procedure A

N-(2-Chloroethyl)morpholine hydrochloride (19 g, 0.13 mol) was added to a solution of thiourea (10 g, 0.13 mol) in 200 mL of water and the mixture then refluxed for 2 h. After the reaction mixture was cooled to room temperature, it was added to 400 mL of cold acetone and the resulting precipitate collected by vacuum filtration. This filtered solid then was washed with ether and dried in a vacuum desiccator (P_2O_5) to afford 19 g of white crystals corresponding to the intermediate isothiuronium salt (see equation above).

All of the following steps were performed under an atmosphere of nitrogen in order to ensure minimal thiol oxidation after the conversion of the isothiuronium salt to corresponding mercaptan. The isothiuronium salt (19 g, 0.071 mol), prepared as described above, was dissolved in 100 mL of degassed water and a solution of sodium hydroxide (9.0 g, 0.23 mol) in 50 mL of water added in one portion. The reaction mixture then was heated for 1.5 h at 60 °C. After the mixture was cooled to room temperature 80 mL of ethyl ether was added and the resulting mixture stirred for 10 min. The ether layer then was removed and the pH of the aqueous layer adjusted to 7.0 using 50 % aq. sulfuric acid. The acidified aqueous phase then was reextracted with 3 x 100 mL of ethyl ether. The combined ether extracts were dried (MgSO_4), the drying agent removed by filtration, and volatiles removed by rotary evaporation. Approximately 6 g (60 %) of a colorless liquid was obtained. This product, used without further purification, was stored under nitrogen in a refrigerator until needed.

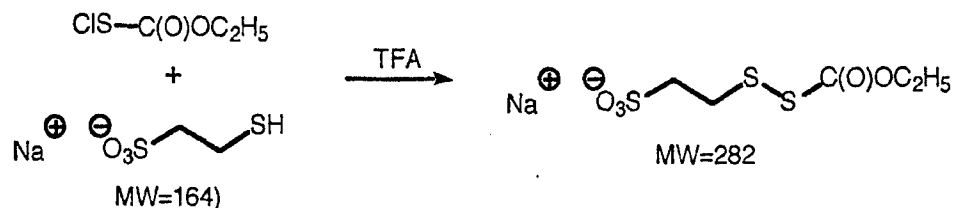
Procedure B

The above experiment was repeated using 37 g of 4-(2-chloroethyl)morpholine hydrochloride to give 11.7 g (66 %) of crude mercaptan. Combined material from these two runs (17 g) was vacuum distilled to afford 15 g of pure 2-(*N*-morpholino)ethanethiol, bp 147-150 °C ($\approx$ 90 torr).

2-Mercaptopyridine-*N*-oxide

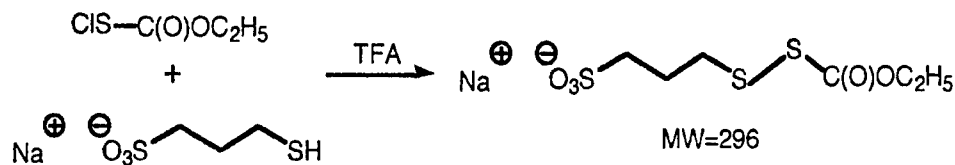
The sodium salt of 2-mercaptopyridine-*N*-oxide monohydrate (8.0 g, 0.048 mol) was dissolved in water (20 mL). This solution was cooled to 0-5 °C and 6 mL of cold, concentrated hydrochloric acid was added, dropwise and with stirring. The resulting white precipitate was removed by filtration and then washed with 140 mL of cold water. After washing, the solid was dried (20 °C) under vacuum (P₂O₅) overnight to afford 4.85 g (0.0382 mol, 80%) of 2-mercaptopyridine-*N*-oxide.

Sodium Ethoxycarbonyl 2-Sulfonatoethyl Disulfide



To a solution of 2.0 g (0.014 mol) of ethoxycarbonylsulfonyl chloride in 5 mL of trifluoroacetic acid at $\approx -7^\circ\text{C}$ there was added, dropwise and with stirring, a solution of 2.1 g (0.013 mol) of sodium 2-mercaptoethane sulfonate (MESNA) in 10 mL of trifluoroacetic acid. Addition required 25 min and was conducted under a nitrogen blanket. The reaction mixture then was allowed to warm to 10°C (30 min), the cold bath removed and the mixture stirred for 30 min at room temperature. The solvent was removed in vacuum at temperatures below 35°C . The residue was maintained at 0.1 torr for 3 h to yield 4.71 g of solid crude product. This product was dissolved in 27 mL of methanol and insoluble materials removed by filtration. The filtrate was poured into 1800 mL of ether and the resulting precipitate removed by filtration (20 nm filter). This solid was dried (P_2O_5) at 70°C overnight to afford 2.87 g (0.010 mol; 71%) of the desired product.

Sodium Ethoxycarbonyl 3-Sulfonatopropyl Disulfide



A solution of 2.0 g (0.014 mol) of ethoxycarbonylsulfonyl chloride in 5 mL of trifluoroacetic acid was cooled to $-7\text{ }^{\circ}\text{C}$. To this there was added, with stirring and under a nitrogen blanket, a solution of 2.28 g (0.0128 mol) of 3-(sodium sulfonate)propyl mercaptan in 10 mL of trifluoroacetic acid; addition required 30 min. The mixture was allowed to warm to $10\text{ }^{\circ}\text{C}$ for 30 min. The cold bath then was removed and the mixture stirred for 30 min at room temperature. The volatiles then were removed in a vacuum at $< 30\text{ }^{\circ}\text{C}$. The residue was maintained at 0.1 torr for 7 h and 4.99 g of solid crude product were obtained. This product was dissolved in 25 mL of methanol, filtered, and the filtrate poured into 1.8 L of ether. The resulting precipitate was separated by filtration using a 20 nm filter. It then was dried (P_2O_5) at $70\text{ }^{\circ}\text{C}$ overnight to afford 3.02 g (0.010 mol; 78%) of the desired product.

7. Key Research Accomplishments

1. Organosulfur analogs of several natural products, including both thiacholesterol and thiavitamin E, have been prepared in good yield, and shown to be stable under a variety of conditions.
2. A wide range of target molecules containing the S-S linkage have been prepared in good yield and shown to be stable towards disproportionation. These targets include fragments derived from glutathione, thiacholesterol, pyridine, pyrimidine, thiavitamin E, thioglycerol and morpholine
3. The use of 2-benzothiazolyl disulfide and perchloromethyl mercaptan in the synthesis of molecules containing the S-S bond has been greatly expanded.

8. Reportable Outcomes

- Glutathione and other amino acid containing thiols are converted to unsymmetric disulfides in high yields. These are stable under ordinary conditions.
- Cholesterol and other sterols, can be converted to the corresponding thiols in excellent yield and these, in turn, can be converted to a wide range of derivatives possessing an S-S bond.
- Thiacholesterol, thiavitamin E, and other lipid soluble systems can be converted to derivatives which can be "dispersed" in water by converting them to surfactants. These surfactants may have either cationic or anionic water-soluble fragments.

9. Conclusions

No biological data was provided to our group prior to the completion of the first half of this program. Therefore, we can reach no conclusions about the biological activity of these targets. However, we have firmly established that virtually any potential target containing a sulfur-to-sulfur bond can be prepared using techniques discovered/advanced in our labs. Further we have demonstrated the ability to convert sulfur-free starting materials into sulfur-containing analogs in good yields (e.g., cholesterol $\rightarrow$ thiacholesterol). We also have shown that physical properties (e.g., water dispersibility) can be varied according to the nature of fragments attached to one or both of the sulfur atoms in molecules containing an S-S bond.