

-1-

METHOD OF MAKING (S-R\*,R\*)-3-METHYL-2-(3-OXO-3H-BENZO(D)ISOTHIAZOL-2-YL)PENTANOIC ACID AND (S-(R\*,R\*)), L-2-(2-(2-(1-CARBOXY-2-METHYLBUTYLCARBAMOYL)PHENYLDISULFONYL)BENZOYL-AMINO)-3-METHYLPENTANOIC ACID

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## FIELD OF THE INVENTION

The present invention provides a synthesis of [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid and [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyle)phenyldisulfonyl]-benzoylamino]-3-methylpentanoic acid.

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## BACKGROUND OF THE INVENTION

The compound [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid is useful as an antiviral agent and can be used to treat patients infected with the HIV virus. The compound [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyle)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid is an intermediate in the synthesis of [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid and can be used to treat bacterial and viral infections, including patients infected with the HIV virus. See, for example, United States Patent Number 5,463,122, which is hereby incorporated by reference. Thus, it would be useful to have a simple, high yield method for synthesizing [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid and [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyle)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid.

-2-

## SUMMARY OF THE INVENTION

The present invention provides a method of making  
[S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-  
5 2-yl)pentanoic acid that comprises reacting 2,2'-  
dithiosalicylic acid with a halogenating agent to make  
2,2'-dithiobisbenzoyl halide; reacting the 2,2'-  
dithiobisbenzoyl halide with L-isoleucine in  
tetrahydrofuran or tetrahydrofuran and a base to make  
10 [S-(R\*,R\*)],L-2-[2-[2-(1-carboxy-2-methylbutyl-  
carbamoyl)phenyldisulfonyl]benzoylamino]-3-  
methylpentanoic acid; and reacting the [S-(R\*,R\*)],  
L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)-  
phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid  
15 with a halogen oxidizing agent to make [S-(R\*,R\*)]-  
3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic  
acid.

In a preferred embodiment, the halogenating agent  
is thionyl chloride.

20 In another preferred embodiment, the 2,2'-  
dithiobisbenzoyl halide is 2,2'-dithiobisbenzoyl  
chloride.

In another preferred embodiment, the base is  
sodium bicarbonate.

25 In another preferred embodiment, the halogen  
oxidizing agent is chlorine, bromine, or iodine.

In more preferred embodiment, the halogen  
oxidizing agent is bromine.

Also provided by the present invention is a method  
30 of making [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]-  
isothiazol-2-yl)pentanoic acid that comprises reacting  
2,2'-dithiosalicylic acid with thionyl chloride to make  
2,2'-dithiobisbenzoyl chloride; reacting the 2,2'-  
dithiobisbenzoyl chloride with at least two equivalents  
35 of L-isoleucine in tetrahydrofuran or tetrahydrofuran  
and sodium bicarbonate to make [S-(R\*,R\*)],L-2-[2-[2-

-3-

(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]-benzoylamino]-3-methylpentanoic acid; and reacting the [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutyl-carbamoyl)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid with bromine and acetic acid to make [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]-isothiazol-2-yl)pentanoic acid.

Also provided is a method of making [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)-phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid that comprises reacting 2,2'-dithiosalicylic acid with a halogenating agent to make 2,2'-dithiobisbenzoyl halide; and reacting the 2,2'-dithiobisbenzoyl halide with L-isoleucine in tetrahydrofuran or tetrahydrofuran and a base to make [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid.

In another preferred embodiment of the method, the halogenating agent is thionyl chloride.

In another preferred embodiment of the method, the 2,2'-dithiobisbenzoyl halide is 2,2'-dithiobisbenzoyl chloride.

In another preferred embodiment, the base is sodium bicarbonate.

Also provided is a method of making [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutyl-carbamoyl)-phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid that comprises reacting 2,2'-dithiosalicylic acid with thionyl chloride to make 2,2'-dithiobisbenzoyl chloride; and reacting the 2,2'-dithiobisbenzoyl chloride with L-isoleucine in tetrahydrofuran or tetrahydrofuran and a base to make [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyl-disulfonyl]benzoylamino]-3-methylpentanoic acid.

- 4 -

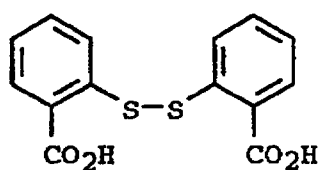
## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of making  
[S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-  
5 2-yl)pentanoic acid and [S-(R\*,R\*)],L-2-{2-[2-(1-  
carboxyl-2-methylbutylcarbamoyl)phenyldisulfonyl]-  
benzoylamino}-3-methylpentanoic acid in accordance with  
Scheme I below.

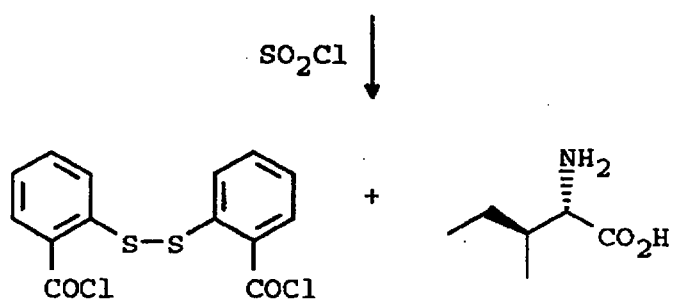
-5-

## Scheme I

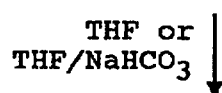
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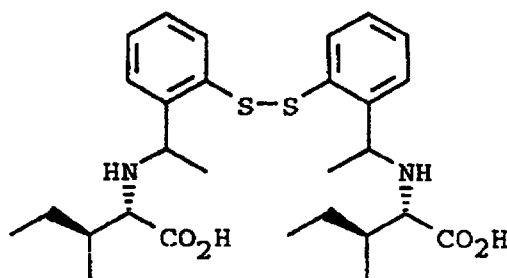
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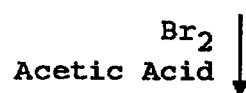
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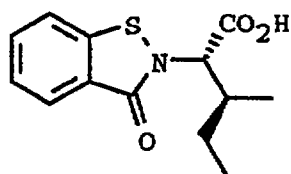
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-6-

In the first step of the synthesis, 2,2'-dithiosalicylic acid, which can be purchased from Aldrich, Milwaukee, Wisconsin, is converted to its corresponding acid halide, 2,2'-dithiobisbenzoyl halide, by reacting the 2,2'-dithiosalicylic acid with a halogenating agent. Those skilled in the art are familiar with the conversion of an acid to an acid halide. Examples of suitable halogenating agents include, but are not limited to, thionyl chloride, phosgene, phosphorus trichloride, phosphorus pentachloride, and phosphorus tribromide.

In a preferred embodiment, the halogenating agent is thionyl chloride, and the acid halide is an acid chloride. The term "halogen" includes chlorine, bromine, fluorine, and iodine.

In general, the reaction is carried out under an inert atmosphere in an aprotic solvent. Examples of suitable aprotic solvents include, but are not limited to, toluene, heptane, hexane, and acetonitrile. In a preferred embodiment, the solvent is toluene. The 2,2'-dithiobisbenzoyl halide can be used in the next step as obtained, i.e. in crude form, or can be purified by methods well-known to those skilled in the art.

In the second step of the synthesis, 2,2'-dithiobisbenzoyl halide is reacted with L-isoleucine in tetrahydrofuran or a mixture of tetrahydrofuran and a base to form [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)-phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid. Use of tetrahydrofuran (THF) or a solution of THF and a base provides for higher yields and higher purities and is less toxic and carcinogenic than other solvents used in the formation of amides from chlorides and  $\alpha$ -amino acids. Moreover, the use of THF as a solvent is not obvious, as L-isoleucine has only limited solubility in

-7-

THF, and THF is generally decomposed by hydrogen chloride, which is generated in the reaction. Moreover, the present synthesis does not require any protection/deprotection steps for the  $\alpha$ -amino acid and is less prone to racemization, resulting in products having superior optical activity. The [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)-phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid can be used in the next step as obtained or can be further purified by methods well-known to those skilled in the art. Examples of suitable bases include, but are not limited to, sodium bicarbonate, sodium carbonate, potassium carbonate, and potassium bicarbonate. Preferably, the base is sodium bicarbonate.

In the third step of the synthesis, [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)-phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid is reacted with a halogen oxidizing agent to make [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid. In this step, the disulfide bond of the [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid is oxidized and the intermediate sulphenyl bromide cyclized to form [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid. Suitable halogen oxidizing agents include, but are not limited to bromine, chlorine, and iodine. In a preferred embodiment, the halogen oxidizing agent is bromine.

In general, in this step of the synthesis, a slurry of [S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid in acetic acid is stirred with the addition of a halogen oxidizing agent. After stirring, the precipitate formed is filtered. The

-8-

product precipitates directly from the reaction mixture in a high state of purity.

Next, the precipitate, which is crude [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid is purified by extraction with an ether, preferably methyl-t-butyl ether, and water. The ether extracts are combined and concentrated to afford an oil. The oil is dissolved in an ether and then heptane is added. The resulting precipitate is filtered, washed and dried to yield [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid.

The following examples are intended to illustrate specific embodiments of the present invention and are not intended to limit the specification, including the claims, in any way.

#### EXAMPLE 1

##### 2,2'-Dithiobisbenzoyl chloride

To a 2 L three-necked round-bottomed flask fitted with a mechanical stirrer, a thermometer, and a reflux condenser with a nitrogen inlet are charged 2,2'-dithiosalicylic acid (120 g), toluene (600 mL), dimethylformamide (1 mL), and thionyl chloride (128 g). With stirring, the mixture is heated at 75°C for 21 hours under nitrogen to give a clear yellow solution. The solution is heated to 80°C and filtered through diatomaceous earth on a Buchner funnel. The filter cake is washed with toluene (100 mL), and the combined filtrates are transferred to a 2 L three-necked round-bottomed flask fitted with a mechanical stirrer, a thermometer, and a distillation head. The hot solution is concentrated to a total volume of 300 mL under vacuum (5 mm Hg) and to a final batch temperature of 65°C. Fresh toluene (500 mL) is added to the mixture which is reheated to 80°C. The hot

-9-

solution is concentrated to a total volume of 300 mL under vacuum (5 mm Hg), and a further charge of fresh toluene (500 mL) is made. The mixture is reheated to 80°C and the solution concentrated to a final volume of 400 mL under vacuum (5 mm Hg) and to a final batch temperature of 65°C to give a thick yellow slurry. The slurry is cooled to 10°C, stirred for 2 hours, and filtered on a Buchner filter. The filter cake is washed with cold toluene (100 mL) and dried in a vacuum oven at 40°C to -45°C to give 120 g (89.3%) of the title compound as pale yellow crystals; mp 156.2-157.9°C.

<sup>1</sup>H NMRS (δ, CDCl<sub>3</sub>, 200 MHz): 8.41-8.37 (m, 2H, aromatic H), 7.78-7.74 (m, 2H, aromatic H), 7.60-7.51 (m, 2H, aromatic H), 7.43-7.34 (m, 2H, aromatic H).

## EXAMPLE 2

[S-(R\*,R\*)]1,L-2-[2-[2-(1-carboxy-2-methylbutyl-carbamoyl)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid

To a 1 L three-necked round-bottomed flask fitted with a mechanical stirrer, a thermometer, and a reflux condenser with a nitrogen inlet are charged 2,2'-dithiobisbenzoyl chloride (75.0 g), L-isoleucine (62.8 g), sodium bicarbonate (55.3 g) and THF (750 mL). With stirring, the slurry is heated at 54°C to 58°C under nitrogen for 20 minutes and then at 60°C to 65°C for 8 hours. The reaction mixture is cooled to room temperature and is slowly poured into a 3 L three-necked round-bottomed flask fitted with a mechanical stirrer containing a rapidly stirred mixture of concentrated hydrochloric acid (60 g), water (550 mL), and methyl t-butyl ether (800 mL). The 1 L flask is rinsed successively with THF (150 mL), methyl t-butyl ether (150 mL), and water (150 mL), and the rinses are added to the 3 L flask. The two-phase

-10-

mixture is stirred at room temperature for 30 minutes and then allowed to settle. The lower aqueous layer is separated, and the upper organic layer is washed with three portions of water (2 x 375 mL, 180 mL). To the rapidly stirred organic layer is added hexane (1060 mL) to give a thick white slurry which is stirred at room temperature for 2 hours. The solids are filtered on a Buchner filter, washed with hexane (150 mL), and dried in a vacuum oven at 65°C to give 105.9 g (91.0%) of the crude title compound.

To a 2 L three-necked round-bottomed flask fitted with a mechanical stirrer, a distillation head, and a thermometer are charged the crude title compound (95.2 g) and THF (1620 mL). The pale yellow solution is heated under reflux, and THF (1140 mL) is distilled out of the flask. The THF solution in the flask is cooled to room temperature and to this, with rapid agitation, is added hexane (760 mL) to give a thick white slurry which is stirred at room temperature for 2 hours. The solids are filtered on a Buchner filter, washed with hexane (375 mL) and dried in a vacuum oven at 67°C to give 93.8 g (98.5%) of the title compound as a white solid; HPLC 99.1% (by area); mp 207-210°C; <sup>1</sup>H NMRS (δ, DMSO, 200 MHz): 12.8-12.4 (br s, 2H, CO<sub>2</sub>H), 8.72 (d, J = 8.3 Hz, 2H, NH), 7.68-7.64 (m, 4H, aromatic H), 7.50-7.41 (m, 2H, aromatic H), 7.35-7.27 (m, 2H, aromatic H), 4.39-4.32 (m, 2H, NCH), 1.95 (m, 2H, CH<sub>3</sub>CH), 1.53 (m, 2H, CH<sub>2</sub>), 1.31 (m, 2H, CH<sub>2</sub>), 0.97 (d, J = 6.9 Hz, 6H, CH<sub>3</sub>CH), 0.89 (m, 6H, CH<sub>3</sub>CH<sub>2</sub>).

## EXAMPLE 3

[S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid

To a 500 mL three-necked round-bottomed flask fitted with a mechanical stirrer, a thermometer, and an addition funnel with a nitrogen inlet are charged

-11-

[S-(R\*,R\*)], L-2-[2-[2-(1-carboxy-2-methylbutyl-carbamoyl)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid (25 g) and acetic acid (163 mL).

To the stirred slurry under nitrogen is added dropwise  
5 over 15 minutes a solution of bromine (7.9 g) in acetic acid (12 mL). The orange slurry is stirred at room temperature for 4 hours under nitrogen, filtered on a Buchner funnel, and washed with heptane (2 x 50 mL) to give 31.7 g of the crude solvent-wet title compound.

10 To a 500 mL separatory funnel are charged the crude title compound (31.7 g), methyl *t*-butyl ether (69 mL) and water (66 mL). After extraction, the lower water layer is separated and extracted with methyl *t*-butyl ether (19 mL). The methyl *t*-butyl ether layers  
15 are combined, washed with water (50 mL), and concentrated to an oil using a rotary evaporator under vacuum (25 mm Hg) and to a final batch temperature of 50°C. The oil is held under vacuum at 50°C for 1 hour, dissolved in methyl *t*-butyl ether (80 mL), and filtered  
20 on a Buchner filter. The filtrates are transferred to a 250 mL three-necked round-bottomed flask fitted with a mechanical stirrer, a thermometer, and an addition funnel with a nitrogen inlet. To the warm (45°C) stirred methyl *t*-butyl ether solution is added heptane  
25 (40 mL). The turbid solution is held at 45°C for 1 hour and a further charge of heptane (19 mL) is made. The slurry is cooled to room temperature, then to 10°C and stirred for 1 hour. The solids are filtered on a Buchner funnel, washed with heptane (40 mL), and dried  
30 in a vacuum oven at 45°C for 16 hours to give 19.3 g (77.5%) of the title compound as white crystals;

mp 122-123°C;

<sup>1</sup>H NMRS (δ, CDCl<sub>3</sub>, 200 MHz): 8.81 (br s, 1H, CO<sub>2</sub>H),  
8.07 (d, J = 7.8 Hz, 1H, aromatic H), 7.67-7.54 (m, 2H,  
35 aromatic H), 7.44-7.36 (m, 1H, aromatic H), 5.28 (d, J = 9.4 Hz, 1H, CHN), 2.28-2.23 (m, 1H, CHCH<sub>3</sub>),

-12-

1.42-1.38 (m, 1H, CH<sub>2</sub>), 1.29-1.20 (m, 1H, CH<sub>2</sub>), 1.11 (d, J = 6.8 Hz, 3H, CHCH<sub>3</sub>), 0.91 (t, J = 7.3 Hz, 3H, CH<sub>2</sub>CH<sub>3</sub>).

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## EXAMPLE 4

Antiviral Activity of [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid

[S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid (Example 3) causes the  
10 extrusion of zinc from the nucleocapsid protein (NCp7) of HIV-1. The NC protein is highly conserved among all retroviruses (South T., Blake P., et al., Biochemistry, 1990;29:7786) and is essential for viral infectivity (Aldovini A. and Young R., J. Virology, 1990;64:1920  
15 and Gorelick R., Nigida S., et al., J. Virology, 1990;64:3207). The zinc is normally held in NC proteins by 1 or 2 zinc fingers. In the case of HIV-1, 2 zinc fingers are present (Summers M., South T., et al., Biochemistry, 1990;29:329) and are involved  
20 specifically with the PSI site on viral RNA which controls the packaging of viral RNA. Interference of this packaging causes the formation of non-infectious virions (Dannull J., Surovoy A., et al., EMBO, 1994;13:1525). It has previously been shown that  
25 compounds that cause zinc extrusion have potent anti-HIV activity in multiple cell lines and against all retroviruses (Rice W., Schaeffer C., et al., Nature, 1993;361:473).

A fluorescence-based assay has been developed to  
30 monitor the ejection of zinc from purified HIV-1 NCp7. The fluorophore, N-(6-methoxy-8-quinoly)-p-toluenesulfonamide (TSQ), has an increased fluorescent signal upon bonding zinc ion in solution. The NCp7 protein containing 2 Zn-fingers and 2 Zn ions is  
35 incubated with drug causing the extrusion of Zn ions. The released Zn is then sequestered by the TSQ and the

-13-

increased fluorescence monitored relative to control. The assay was performed as follows: 10  $\mu$ M compound was added to 2.8  $\mu$ M NCp7 and 47  $\mu$ M TSQ in 20  $\mu$ L of pH 7.4 buffer at 26°C for 90 minutes. Fluorescence  
5 (excitation 355 nM emission 400 nM) was monitored versus time. Controls were the NCp7 under assay conditions without drug, and apo NCp7 (no Zn) with drug. The % Zn extrusion was calculated based on the actual fluorescence measured divided by the  
10 fluorescence of all theoretical Zn extruded (5.6  $\mu$ M)  $\times$  100.

The test systems utilized to establish the cellular antiviral activity of [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid are  
15 well recognized in the art and are routinely employed for such purpose. For example, the assay utilized to evaluate the compound's activity against the HIV virus is that employed by the U.S. National Cancer Institute as described by Weislow O.S., et al., J. Natl. Cancer  
20 Inst., 1989;81:577-586, incorporated herein by reference.

The procedure is designed to detect agents acting at any stage of the virus reproductive cycle. The assay basically involves the killing of T4 lymphocytes  
25 by HIV. Small amounts of HIV are added to cells, and at least two complete cycles of virus reproduction are necessary to obtain the required cell killing. Agents which interact with virions, cells, or virus gene-products to interfere with viral activities will  
30 protect cells from cytolysis. The system is automated in several features to accommodate large numbers of candidate agents, and is generally designed to detect anti-HIV activity. However, compounds which degenerate or are rapidly metabolized in the culture conditions  
35 may not show activity in this screen.

-14-

Another test system utilized to evaluate the invention compounds is called HIV H9 assay. The HIV H9 cell assay measures the inhibitor concentration required to suppress HIV-1 virus replication. In this system, viral growth occurs through multiple rounds of the life-cycle. Any suppression of the replication kinetics results in a geometric decrease in virus production. As a result, this assay is a sensitive means of measuring the ability of a compound to inhibit HIV-1 viral replication.

The H9 T-cell line is batch infected with HIV virus at a multiple of infection (MOI) of 0.01. After 2 hours absorption, the cells are washed, resuspended in RPMI-1640 (a readily available medium well-known to those skilled in the art)/10% fetal calf serum, and seeded at  $5 \times 10^3$  cells/well of a 96-well plate. A duplicate plate of uninfected H9 cells is prepared for the cytotoxicity assay. Drugs are serially diluted 1/3.16 in dimethylsulfoxide (DMSO), transferred to media at an 8X concentration, and then added to the cultures in triplicate. The final DMSO concentration of 0.002 (0.2%).

Viral production is measured by room temperature assay and cytotoxicity is measured by XTT assay at 7 days post-infection. The XTT assay is well-known to those skilled in the art. See, for example, J. Natl. Cancer Inst., 1989;81:577-586. The room temperature assay is performed as a modification of Borroto-Esoda and Boone, J. Virol., 1991;65:1952-1959 and quantitated using a Molecular Dynamics Phosphoimager with Imagequant software. The XTT assay is performed as a modification of Roehm, et al., J. Immuno. Methods., 1991;142:257-265 and quantitated using a molecular Devices Thermomax plate reader with Softmax software.

Data is electronically transferred to a Microsoft Excel spreadsheet for analysis. The room temperature

-15-

assay values equivalent to 50% and 90% inhibition of virus production are calculated from the untreated controls. The concentrations of inhibitor required to produce these values ( $IC_{50}$  and  $IC_{90}$ ) are interpolated from data points flanking these room temperature activities. The XTT assay values equivalent to 50% cytotoxicity are calculated from the untreated controls. The concentrations of inhibitor required to produce this value are interpolated from data points flanking these XTT values.

Yet another test system employed to determine antiviral activity is called the CEM cell assay.

T4 lymphocytes (CEM cell line) are exposed to HIV at a virus to cell ratio approximately 0.05, and plated along with noninfected control cells in 96-well microliter plates.

Candidate agent is dissolved in dimethylsulfoxide (unless otherwise noted), then diluted 1:200 in cell culture medium. Further dilutions (half- $\log_{10}$ ) are prepared before adding to an equal volume of medium containing either infected or noninfected cells.

Cultures are incubated at 37°C in a 5% carbon dioxide atmosphere for 6 or 7 days. The tetrazolium salt, XTT, is added to all wells, and cultures are incubated to allow formazan color development by viable cells, J. National Cancer Institute, 1989;81:577-586. Individual wells are analyzed spectrophotometrically to quantitate formazan production, and in addition are viewed microscopically for detection of viable cells confirmation of protective activity.

Drug-tested virus-infected cells are compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, etc.) on the same plate. Data are reviewed in

-16-

comparison with other tests done at the same time and a determination about activity is made.

Table 1 below presents the results of the compound in the zinc extrusion assay described above. The compound was evaluated for its ability to cause the extrusion of zinc from nucleocapsid protein NCp7 (expressed as % relative to control).

TABLE 1. Zn Extrusion From the Zn Fingers of HIV-1 Nucleocapsid Protein (NCp7)

| Compound of Example | % Zn Extrusion Relative to Control |
|---------------------|------------------------------------|
| 3                   | 30                                 |

Table 2 below presents data for the compound of Example 3 when evaluated in the H9 and the CEM cell assays.

TABLE 2. Anti-HIV Activity CEM Cell Assay

| Compound of Example | EC <sub>50</sub> (μM) <sup>a</sup> | TC <sub>50</sub> (μM) <sup>b</sup> |
|---------------------|------------------------------------|------------------------------------|
| 3                   | 14                                 | >100                               |

<sup>a</sup> Effective concentration that protects cells from viral cytopathic effects

<sup>b</sup> Toxic concentration that inhibits the growth of cells 50% relative to control

-17-

## CLAIMS

1. A method of making [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid, the  
5 method comprising:
  - a. reacting 2,2'-dithiosalicylic acid with a  
halogenating agent to make 2,2'-  
dithiobisbenzoyl halide;
  - b. reacting the 2,2'-dithiobisbenzoyl halide  
10 with L-isoluecine in tetrahydrofuran or  
tetrahydrofuran and a base to make  
[S-(R\*,R\*)],L-2-{2-[2-(1-carboxy-2-methyl-  
butylcarbamoyl)phenyldisulfonyl]benzoyl-  
amino}-3-methylpentanoic acid; and
  - c. reacting the  
15 [S-(R\*,R\*)],L-2-{2-[2-(1-carboxy-  
2-methylbutylcarbamoyl)phenyldisulfonyl]-  
benzoylamino}-3-methylpentanoic acid with a  
halogen oxidizing agent to make [S-(R\*,R\*)]-  
20 3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-  
yl)pentanoic acid.
2. The method of Claim 1 wherein the halogenating  
agent is thionyl chloride.
3. The method of Claim 1 wherein the 2,2'-  
dithiobisbenzoyl halide is 2,2'-dithiobisbenzoyl  
chloride.
4. The method of Claim 1 wherein the base is sodium  
bicarbonate.
5. The method of Claim 1 wherein the halogen  
oxidizing agent is chlorine, bromine or iodine.



-18-

6. The method of Claim 5 wherein the halogen oxidizing agent is bromine.
7. A method of making [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid, the method comprising:
- a. reacting 2,2'-dithiosalicylic acid with thionyl chloride to make 2,2'-dithiobisbenzoyl chloride;
  - b. reacting the 2,2'-dithiobisbenzoyl chloride with at least two equivalents of L-isoluecine in tetrahydrofuran or tetrahydrofuran and a base to make [S-(R\*,R\*)],L-2-{2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]-benzoylamino}-3-methylpentanoic acid; and
  - c. reacting the [S-(R\*,R\*)],L-2-{2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]-benzoylamino}-3-methylpentanoic acid with bromine and acetic acid to make [S-(R\*,R\*)]-3-methyl-2-(3-oxo-3H-benzo[d]isothiazol-2-yl)pentanoic acid.
8. A method of making [S-(R\*,R\*)],L-2-{2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]-benzoylamino}-3-methylpentanoic acid, the method comprising:
- a. reacting 2,2'-dithiosalicylic acid with a halogenating agent to make 2,2'-dithiobisbenzoyl halide; and
  - b. reacting the 2,2'-dithiobisbenzoyl halide with L-isoluecine in tetrahydrofuran or tetrahydrofuran and a base to make [S-(R\*,R\*)],L-2-{2-[2-(1-carboxy-2-methyl-



-19-

butylcarbamoyl)phenyldisulfonyl]benzoyl-amino]-3-methylpentanoic acid.

9. The method of Claim 8 wherein the halogenating agent is thionyl chloride.
10. The method of Claim 8 wherein the 2,2'-dithiobisbenzoyl halide is 2,2'-dithiobisbenzoyl chloride.
11. The method of Claim 8 wherein the base is sodium bicarbonate.
12. A method of making [S-(R\*,R\*)],L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]-benzoylamino]-3-methylpentanoic acid, the method comprising:
  - 5 a. reacting 2,2'-dithiosalicylic acid with thionyl chloride to make 2,2'-dithiobisbenzoyl chloride; and
  - b. reacting the 2,2'-dithiobisbenzoyl chloride with L-isoluecine in tetrahydrofuran or  
10 tetrahydrofuran and a base to make [S-(R\*,R\*)],L-2-[2-[2-(1-carboxy-2-methylbutylcarbamoyl)phenyldisulfonyl]benzoylamino]-3-methylpentanoic acid.

