Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1707–1730

BIOORGANIC & MEDICINAL CHEMISTRY

2,2′-Dithiobisbenzamides Derived from α-, β- and γ-Amino Acids Possessing Anti-HIV Activities: Synthesis and Structure–Activity Relationship

J. V. N. Vara Prasad,[a,*] Joseph A. Loo,[a] Frederick E. Boyer,[a] Michael A. Stier,[a] Rocco D. Gogliotti,[a] William J. Turner,[a] Patricia J. Harvey,[a] Melissa R. Kramer,[a] David P. Mack,[a] Jefferey D. Scholten,[b] Stephen J. Gracheck[c] and John M. Domagala[a]

[a]*Department of Chemistry, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105, USA*
[b]*Department of Biochemistry, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105, USA*
[c]*Department of Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105, USA*

Received 14 January 1998; accepted 20 April 1998

Abstract—Nucleocapsid protein (NCp7), which contains highly conserved retroviral zinc fingers, is essential in the early as well as the late phase of human immunodeficiency virus (HIV) life cycle and constitutes a novel target for AIDS therapy. HIV-1 NCp7 is a basic 55 amino acid protein containing two $C(X)_2C(X)_4H(X)_4C$ motif zinc fingers flanked by basic amino acids on each side. 2,2′-dithiobisbenzamides have previously been reported to release zinc from these NCp7 zinc fingers and also to inhibit HIV replication. Specifically, 2,2′-dithiobisbenzamides derived from simple amino acids showed good antiviral activities. The benzisothiazolone **3**, the cyclic derivative of **2**, was selected for clinical trials as an agent for AIDS therapy. Herein we report the syntheses and antiviral activities, including therapeutic indices, of 2,2′-dithiobisbenzamides derived from α-, β- and γ-amino acids. Electrospray ionization mass spectrometry was used to study the zinc-ejection activity of these compounds. Among the α-amino acid derived 2,2′-dithiobisbenzamides, analogues containing alkyl side chains were found to be antivirally active with good therapeutic indices. 2,2′-Dithiobisbenzamides, derived from β- and γ-amino acids, were found to possess better antiviral and therapeutic efficacies than the α-amino acid analogues. Thus compound **59** was found to possess an EC_{50} of 1.9 μM with a therapeutic index of > 50. Interestingly, 2,2′-dithiobisbenzamides derived from α-amino acids containing a protected acid function and polar side chains also exhibited very good antiviral activity.

Introduction

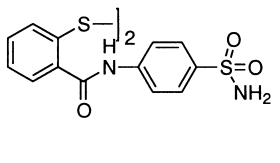
Targets of antiretroviral therapy for patients infected with human immunodeficiency virus (HIV), the putative agent responsible for AIDS, include reverse transcriptase[1] and protease[2] enzymes. Due to the recent encouraging results obtained with combination therapies[3,4] relative to monotherapies, there is an increased interest in anti-HIV compounds targeted against novel viral targets.[5,6]

Screening of selected compounds, from the Parke-Davis chemical collection by the National Cancer Institute against HIV-1 in cell culture resulted in the identification of 2,2′-dithiobisbenzamides as anti-HIV compounds (i.e. **1** and **2**). These compounds displayed low micromolar activity against laboratory and clinical isolates of HIV, including the strains resistant to both nucleoside and non-nucleoside reverse transcriptase inhibitor. These compounds also showed synergistic activity with reverse transcriptase inhibitors (AZT, ddC) and the protease inhibitor (KNI-272). Thus, these 2,2′-dithiobisbenzamides could potentially be used in combination with other known anti-HIV drugs. Due to its novel mechanism of action (vide infra) and its potential for combination HIV therapy, compound **2** (**CI-1013**, **DIBA-4**) was selected for preclinical evaluation. Recently we reported a structure-activity relationship on

*Corresponding author.

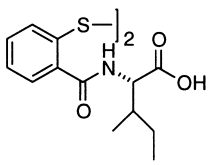
0968-0896/98/$—see front matter
PII: S0968-0896(98)00118-7

the basic benzamide structure (**1** (**DIBA-1**) and **2**) and showed that certain (α-amino acid analogues were among the most active.[7] Benzisothiazolone (**3**, **CI-1012**), a cyclic compound derived directly from **2**, was selected for clinical trials as an agent for AIDS therapy. Rice and co-workers also reported a wide variety of disulfides, for example **4**, as anti-HIV agents.[8] Another disulfide containing macrolide (**5**, SRR-SB3) was also reported to be an anti-HIV agent[9] and is likely to act by a similar mechanism to these 2,2-dithiobisbenzamides. Azodicarbonamide (**6**, ADA),[10] introduced into Phase I/II clinical trials in Europe for advanced AIDS, was also shown to inhibit HIV-1 replication by targeting the nucleocapsid protein.[11] We wished to expand on the observation that α-amino acids conferred good activity to the 2,2′-dithiobisbenzamides. Herein we report the syntheses and a detailed structure–activity relationship of various 2,2-dithiobisbenzamides derived from α-, β-, and γ-amino acids, including anti-HIV activities and therapeutic indices.



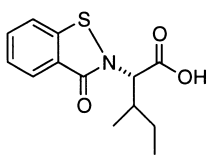
1 (DIBA-1)

EC_{50}: 3.4 μM
TC_{50}: 120 μM



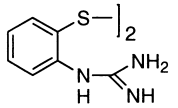
2 (CI-1013, DIBA-4)

EC_{50}: 7.0 μM
TC_{50}: 140 μM



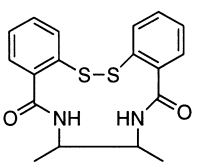
3 (CI-1012)

EC_{50}: 11 μM
TC_{50}: >100 μM



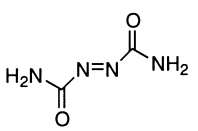
4

EC_{50}: 1.2 μM
TC_{50}: 38.4 μM



5 (SRR-SB3)

EC_{50}: 1.8 μg/mL
TC_{50}: 15.9 μg/mL



6 (ADA)

EC_{50}: 38 μM
TC_{50}: >200 μM

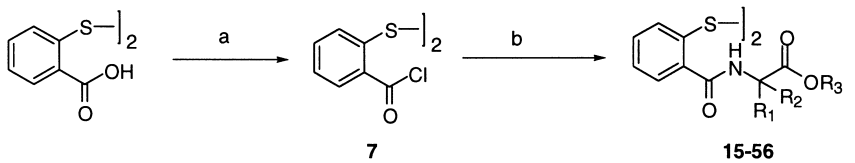
Chemistry

The compounds described in this paper were synthesized via coupling of 2,2-dithiobisbenzoyl chloride **7**, prepared from the commercially available 2,2′-dithiobisbenzoic acid and thionyl chloride,[12] with the corresponding amino acids as shown in Scheme 1. Couplings with **7** were performed under a variety of conditions. The amino acid was converted to the corresponding silyl amine by treatment with *N*-methyl-*N*-(trimethylsilyl)acetamide followed by reaction with **7**. Alternately, the amine was converted to its potassium salt by treatment with potassium *t*-butoxide in ethanol and this was coupled with **7**. Finally, the amino acid esters were also coupled with dithiobisbenzoyl chloride **7** in the presence of a base like *N*-methylmorpholine or triethylamine.

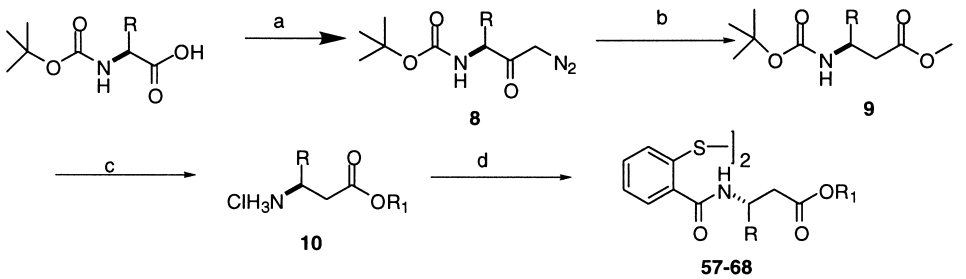
β-Amino acids, used for the preparation of compounds **57**–**68**, were synthesized from the corresponding protected α-amino acids via the diazoketone derivatives **8**. Homologation into methyl ester, **9** was accomplished via treatment with silver benzoate.[13] The methyl esters thus obtained were saponified using potassium carbonate in methanol/water and the Boc group was removed with a 4 N hydrochloric acid solution in dioxane to obtain the fully deprotected β-amino acids **10** (Scheme 2). The γ-amino acids,[14] used for the synthesis of compounds **69**–**76**, were prepared from the corresponding N-protected γ-amino aldehydes **11**[15] via Wittig reaction to obtain the α- and β-unsaturated esters **12**. Hydrogenation of **12** gave the saturated N-protected γ-amino esters **13**. Carboxyl and amino protective groups were removed by saponification and acid treatment, respectively, to give the fully deprotected γ-amino acids **14**, which were further used to couple to **7** (Scheme 3).

Biological Assays

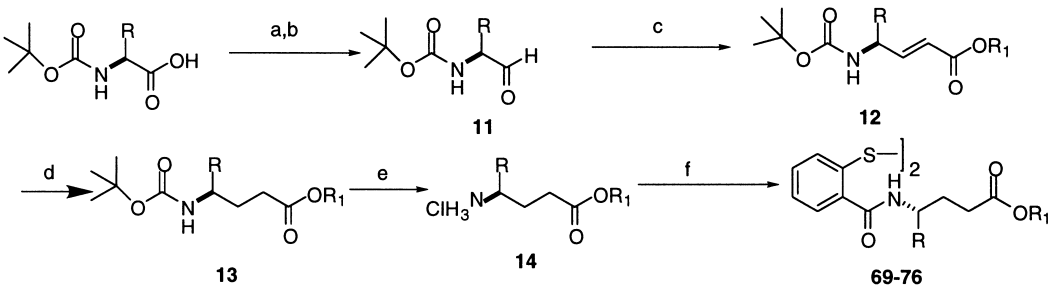
All the compounds reported in this paper were tested for anti-HIV activity in an in vitro culture assay with HIV-IIIB infected human lymphocyte derived CEM cells using the XTT cytopathic method at Southern Research Institute.[16] The assay measures the protection conferred by the drug to the lymphocyte cells against the cytopathic effects of HIV. The EC_{50} indicates the concentration of the drug that provides 50% protection against HIV. The TC_{50} is the concentration of drug that elicits drug induced cytotoxicity in 50% of uninfected cells. Some compounds were tested two to three times and the averages of the runs were reported. Gel shift assay was performed to measure the ability of selected compounds to inhibit the binding of NCp7 to its target ψRNA. Selected compounds were also tested for their ability to extrude zinc from NCp7 using electrospray mass spectroscopy as described earlier.[17] The physical properties and biological data of the compounds are shown in Tables 1–6.



Scheme 1. Reactions conditions: (a) thionyl chloride reflux, overnight; (b) i. 1 equiv of amino acid or its ester, 4.0 equiv of N-methyl-N-(trimethylsilyl)acetamide, *N*-methylmorpholine dichloromethane, rt, 30 min, followed by addition of bisacid chloride, rt, 4–12 h; or ii. Dichloromethane, triethylamine or *N*-methylmorpholine, rt, overnight, or iii. 1 equiv of amino acid or its ester, potassium *t*-butoxide, ethanol, 0 °C, 1 h, followed by addition of bisacid chloride, rt, 4–12 h.



Scheme 2. Reactions conditions: (a) 1 equiv of amino acid, isobutyl chloroformate, triethylamine, −78 °C, 30 min, THF, 1.1 equiv of diazomethane in ether, rt; (b) silver benzoate, triethylamine, methanol; (c) i. $R_1 = H$; potassium carbonate, $MeOH,H_2O$; ii. 4 N HCl in dioxane, rt, 45 min; (d) bisacid chloride, **6**, coupling as in Scheme 1.



Scheme 3. Reaction conditions: (a) EDCl, HOBT, *N*-methylmorpholine, HCl·HN(OMe)Me, 0 °C to rt; (b) LAH, 0 °C; (c) Wittig reagent, rt; (d) hydrogenation; (e) i. R = H, 3 N NaOH, rt; ii. 4 N HCl in dioxane, rt; (f) bisacid chloride, **7**, coupling as in Scheme 1.

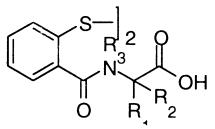
Results and Discussion

2,2′-Dithiobisbenzamides derived from α-amino acids

As reported earlier,[7] 2,2′-dithiobisbenzamides derived from DL-isoleucine (**2**), L-isoleucine (**15**), or D-isoleucine (**16**) exhibited similar antiviral activities as well as therapeutic indices, a result which suggests that chirality is not a factor (Table 1). Hence 2,2′-dithiobisbenzamides derived from either DL- or L-amino acids were used in this current study. The side chain of the amino acid portion in 2,2′-dithiobisbenzamides derived from various α-amino acids was varied and their antiviral activities are shown in Table 1. Among 2,2′-dithiobisbenzamides possessing straight chain alkyl groups **18**–**23**, compounds possessing *n*-butyl and *n*-pentyl side chains on the α-amino acid portion (**21** and **22**, respectively) showed better antiviral activities. 2,2′-Dithiobisbenzamides **2**, **24**–**30** derived from α-amino acids containing branching at the β-or γ-position of the amino acid showed good antiviral activities with good therapeutic indices. However, 2,2′-dithiobisbenzamide analogues **31**–**34**, which are disubstituted at the α-position of the amino acids, either did not exhibit good antiviral activity or had poor therapeutic indices. Thus aminoisobutyric acid analogue **31** and α-methylmethionine analogue **34** showed EC_{50}s of 25 μM and 46 μM, respectively, whereas cyclopentyl and cyclohexyl analogues **32** and **33** showed increased cellular toxicities. Among 2,2′-dithiobisbenzamides possessing a phenyl ring (**35**–**37**), compound **37**, with a phenyl ring two

atoms away from the α-position, exhibited better antiviral activity (EC_{50}: 6 μM) relative to their lower homologs **35** and **36** (EC_{50}s: 25 and >100 μM, respectively). Reduction of the phenyl ring of **37** to give the cyclohexyl analogue **38** did not affect the antiviral activity. Similarly, placement of the hydroxy or amino substituents at the *para* position of the phenyl ring of compound **36** to give **39** and **41** showed no improvement in antiviral activities over **36**. 2,2′-Dithiobisbenzamides derived from α-amino acids possessing polar functional groups on the side chain, that is, carboxyl, hydroxyl, ether moieties (compounds **42**–**47**), displayed negligible antiviral activities. However, asparagine and glutamine derived analogues **48** and **49** exhibited EC_{50}s of 51 and 18.3 μM, respectively. Interestingly, the benzamide with a tertiary amide moiety (compound **50**, the *N*-methyl

Table 1. Dithiobisbenzamides varying amino acid side chains and their physical and antiviral activities tested against HIV in cell culture



Entry	R_1	R_2	R_3	Chirality	Mp(°C)	Molecular formula	Elements analyzed	EC_{50} (μM)	TC_{50} (μM)	TI[c]
2	$CH(CH_3)CH_2CH_3$	H	H	DL	225–229	$C_{26}H_{32}N_2O_6S_2{\cdot}0.26H_2O$	CHN	9.0	>120	>13.3[a]
15	$CH(CH_3)CH_2CH_3$	H	H	L	195–197	$C_{26}H_{32}N_2O_6S_2{\cdot}0.7H_2O$	CHN	7.0	140	20[a]
16	$CH(CH_3)CH_2CH_3$	H	H	D	194–196	$C_{26}H_{32}N_2O_6S_2{\cdot}0.6H_2O$	CHN	6.3	113	17.9[a]
17	H	H	H	-	210–214	$C_{18}H_{16}N_2O_6S_2$	CH	51.3	>100	>1.9[a]
18	CH_3	H	H	DL	226–227	$C_{20}H_{20}N_2O_6S_2$	CHN	35.3	>100	>2.8[a]
19	CH_2CH_3	H	H	DL	238–240	$C_{22}H_{24}N_2O_6S_2{\cdot}0.1H_2O$	CHN	25	>100	>4.0
20	$CH_2CH_2CH_3$	H	H	L	218–220	$C_{24}H_{28}N_2O_6S_2{\cdot}1.4H_2O$	CHN	28.5	>80	>2.8
21	$CH_2CH_2CH_2CH_3$	H	H	L	218–220	$C_{26}H_{32}N_2O_6S_2{\cdot}0.5H_2O$	CHN	7.4	51	6.9
22	$CH_2CH_2CH_2CH_2CH_3$	H	H	DL	160–163	$C_{28}H_{36}N_2O_6S_4$	CHN	6.9	62.7	9.1
23	$CH_2CH_2SCH_3$	H	H	L	201–202	$C_{24}H_{28}N_2O_6S_4{\cdot}0.6H_2O$	CHN	43	>100	>2.3
24	$CH(CH_3)_2$	H	H	DL	231–233	$C_{24}H_{28}N_2O_6S_2$	CHN	12	>100	>8.3[a]
25	$C(CH_3)_3$	H	H	L	132–135	$C_{26}H_{32}N_2O_6S_2$	CHN	18	>100	>5.5
26	$CH_2CH(CH_3)_2$	H	H	L	204–206	$C_{26}H_{32}N_2O_6S_2$	CHN	8.9	130	>14.6[a]
27	$CH_2C(CH_3)_3$	H	H	L	264–265	$C_{28}H_{36}N_2O_6S_2{\cdot}0.25H_2O$	CHN	5.7	92	16.1
28	$CH_2CH(CH_3)=CH_2$	H	H	L	159–161	$C_{26}H_{28}N_2O_6S_2{\cdot}0.61H_2O$	CHN	12	>100	>8.3
29	CH_2(cyclopentyl)	H	H	DL	164–166	$C_{30}H_{36}N_2O_6S_2$	-[b]	8.3	47	5.7
30	CH_2(cyclohexyl)	H	H	L	230–232	$C_{32}H_{40}N_2O_6S_2{\cdot}0.8H_2O$	CHN	2.45	23	9.4
31	CH_3	CH_3	H	-	244–245	$C_{24}H_{24}N_2O_6S_2{\cdot}0.7H_2O$	CHN	25	>100	>4.0
32	$CH_2CH_2CH_2CH_2$		H	-	227–229	$C_{26}H_{28}N_2O_6S_2{\cdot}1.9H_2O$	CHN	>16	16	1
33	$CH_2CH_2CH_2CH_2CH_2$		H	-	254–255	$C_{28}H_{32}N_2O_6S_2{\cdot}1.3H_2O$	CHN	6.9	25.6	3.7
34	$CH_2CH_2SCH_3$	CH_3	H	L	215–217	$C_{26}H_{32}N_2O_6S_4{\cdot}1.0H_2O$	CHN	46	>100	>2.2
35	C_6H_5	H	H	L	231–232	$C_{30}H_{24}N_2O_6S_2{\cdot}1.1H_2O$	CHN	25	>100	>4.0
36	$CH_2C_6H_5$	H	H	L	143–147 (d)	$C_{32}H_{28}N_2O_6S_2{\cdot}0.4H_2O$	CHN	>100	>100	1.0
37	$CH_2CH_2C_6H_5$	H	H	L	227–229	$C_{34}H_{32}N_2O_6S_2{\cdot}0.83H_2O$	CHN	6.0	68	11.3
38	$CH_2CH_2C_6H_{11}$	H	H	L	242–244	$C_{34}H_{44}N_2O_6S_2{\cdot}0.81H_2O$	CHN	5.7	75	13.2
39	$CH_2C_6H_5$(*para*-OH)	H	H	L	196–201	$C_{32}H_{28}N_2O_8S_2$	CHN	65	>165	1.0
40	$CH_2C_6H_5$(*para*-NHCbz)	H	H	L	228–230	$C_{48}H_{42}N_4O_{10}S_2{\cdot}1.58H_2O$	CHN	13	>100	7.7
41	$CH_2C_6H_5$(*para*-NH_2)	H	H	L	164–165	$C_{26}H_{32}N_2O_6S_2{\cdot}HBr$	-[b]	>15	15	<1.0
42	CH_2COOH	H	H	L	177–178	$C_{22}H_{20}N_2O_{10}S_2$	CHN	>100	>100	1.0
43	CH_2CH_2COOH	H	H	L	205–206	$C_{24}H_{24}N_2O_{10}S_2{\cdot}1.8H_2O$	CHN	>100	>100	1.0
44	$CH_2CH_2CH_2COOH$	H	H	L	259–260	$C_{26}H_{28}N_2O_{10}S_2$	CHN	>100	>100	1.0
45	$CH_2CH_2CH_2CH_2NH_2$	H	H	L	195–197	$C_{26}H_{34}N_4O_6S_2{\cdot}2.0HCl{\cdot}3.5H_2O$	CHNSCl	>100	>100	1.0
46	CH_2OH	H	H	L	205–206	$C_{20}H_{20}N_2O_8S_2{\cdot}0.3H_2O$	CHN	>100	>100	1.0
47	CH_2O^tBu	H	H	L	206–207	$C_{28}H_{36}N_2O_8S_2$	CHN	>100	>100	1.0
48	CH_2CONH_2	H	H	L	208–211	$C_{22}H_{22}N_4O_8S_2$	CHNS	51	>100	2.0
49	$CH_2CH_2CONH_2$	H	H	L	192–194 (d)	$C_{24}H_{26}N_4O_8S_2{\cdot}1.7H_2O$	CHNS	18.3	>100	5.5
50	$CH_2CH(CH_3)_2$	H	CH_3	L	120 (d)	$C_{28}H_{36}N_2O_6S_2{\cdot}0.3H_2O$	CHN	>100	>100	1.0
51	$CH_2CH_2CH_2CH_2$ (R_1 and R_3)	H		L	88–90	$C_{24}H_{24}N_2O_6S_2{\cdot}0.85H_2O$	CHN	>100	>100	1.0

[a]Taken from reference[7].

[b]Purities were checked by HPLC.

[c]$TI = TC_{50}/EC_{50}$.

derivative of **26**) did not show any antiviral activity, indicating the importance of the NH moiety in the molecule. For this reason, the proline analogue **51** did not show any antiviral activity. This observation is consistent with our previous results.[7]

Variation of spacer in the amino acids

Next, the spacer between the amine and carboxylic acid was varied in these 2,2′-dithiobisbenzamides to give **17**, **52**–**56** and are shown in Table 2. Increases in antiviral activities were observed with the increase in spacer from $n=1$ to $n=4$. Thus increasing the spacer length from $n=1$ (**17**) to $n=2$ (**52**) resulted in ~sevenfold increase in antiviral activity. A further twofold increase in anti-HIV activity was engendered by increasing the spacer from $n=2$ (**52**) to $n=3$ (**53**), and another twofold increase in potency was observed from $n=3$ (**53**) to $n=4$ (**54**) or 5 (**55**). Though the cellular toxicities also increased modestly, compounds **54** and **55** exhibited a therapeutic indices of 38 and 44, respectively. However, the analogue containing a 6-carbon atom spacer **56** showed antiviral activity similar to compound **53** ($n=3$), and a further increase in toxicity. This is very interesting observation because among the 2,2′-dithiobisbenamides **54** and **55** represent the most potent of the compounds described to date with EC_{50}s of 1.7 and 1.8 μM, respectively.

2,2′-Dithiobisbenzamides derived from β- and γ-amino acids

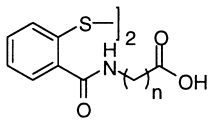
Since 2,2′-dithiobisbenzamides showed improved potencies with increased methylene spacing between the acid and the amide nitrogen, various 2,2′-dithiobisbenzamides containing β- and γ-amino acids were synthesized (**57**–**76**) and are shown in Table 3. Generally, 2,2′-dithiobisbenzamides derived from β-amino acids (**57**–**68**) showed an increase in anti-HIV activities relative to 2,2′-dithiobisbenzamides derived from α-amino acids (Table 3). Since the cellular toxicities are similar, 2,2′-dithiobisbenzamides derived from β-amino acids showed better overall therapeutic indices. Among these derivatives, compound **59** containing a *n*-butyl side chain was the most potent, with a higher therapeutic index relative to the lead compound **2**. Although, 2,2′-dithiobisbenzamides derived from γ-amino acids (**69**–**79**) with or without unsaturation exhibited good anti-HIV activities, their cellular toxicities were also increased relative to the 2,2′-dithiobisbenzamides derived from α- and β-amino acids leading to lower therapeutic indices. Interestingly, analogues **67** and **75** containing the benzyl group in the side chain and derived from β- and γ-amino acids, respectively, show much better antiviral activity and therapeutic index compared to the α-amino acid analogue **36**, which was inactive.

2,2′-Dithiobisbenzamides derived from amino derivatives

To explore the importance of the carboxylic group on the antiviral activities, various analogues derived from amino acid esters, amides, or amino alcohols were synthesized and are shown in Table 5.

The α-amino acid ester analogues (**80**–**86**) containing simple alkyl side chains exhibited little antiviral activity, in direct contrast to their acid analogues. Moreover, 2,2′-dithiobisbenzamides derived from amino alcohols (containing alkyl or phenyl side chains) **87**–**89** were toxic. However, α-amino acid ester analogues (**90**–**94**), which contain the polar functional groups in the side chain, amino (**90** and **91**), hydroxy (**92** and **93**) and cyano (**94**), exhibited better anti-HIV activities relative

Table 2. Dithiobisbenzamides varying spacer between amine and carboxylic groups and their physical and antiviral activities tested against HIV in cell culture

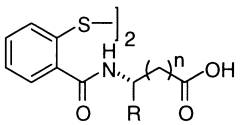


Entry	*n*	mp (°C)	Molecular formula	Elements analyzed	EC_{50} (μM)	TC_{50} (μM)	TI[b]
17	1	210–214	$C_{18}H_{16}N_2O_6S_2$	CH	51.3	>100	>1.9[a]
52	2	201–203	$C_{20}H_{20}N_2O_6S_2$	CHN	7.8	120	15.4[a]
53	3	165–167	$C_{22}H_{24}N_2O_6S_2$	CHN	3.7	>100	27.0
54	4	172–173	$C_{24}H_{28}N_2O_6S_2$	CHN	1.7	64	37.7
55	5	137–139	$C_{26}H_{32}N_2O_6S_2$	CHN	1.8	80	44.4
56	6	190–192	$C_{28}H_{36}N_2O_6S_2 \cdot 0.4H_2O$	CHN	3.4	44	12.9

[a]Taken from ref[7].
[b]$TI = TC_{50}/EC_{50}$.

to the free acid analogues (**45** and **46**) shown in Table 1. When a side chain amine group of **90** was protected using Boc- or Cbz-group (**95** and **96**) the antiviral activity is reduced or the toxicity is increased. Interestingly, the analogues containing amide functionalities in either the backbone or side chain (**97**–**100**) exhibited very good anti-HIV activities usually with good therapeutic efficacies. Thus, compound **100** showed EC_{50}: 3.3 μM with a therapeutic index of 27.5. It appears that hydrogens on the amide nitrogen are important for antiviral activity, since **101**, which is an *N*-methoxy-*N*-methyl amide showed no viral activity. 2,2′-Dithiobisbenzamide analogues containing two amide groups (**102** and **103**) also did not exhibit any antiviral activities.

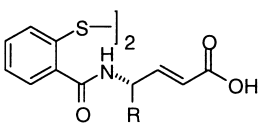
Table 3. Dithiobisbenzamides derived from β-and γ-amino acids possessing various side chains and their physical and antiviral activities tested against HIV in cell culture



Entry	R	*n*	Chirality	Mp (°C)	Molecular formula	Elements analyzed	EC_{50} (μM)	TC_{50} (μM)	TI[a]
57	CH_3	1	DL	137–138	$C_{22}H_{24}N_2O_6S_2{\cdot}0.6H_2O$	CHN	29.9	59.5	2.0
58	$CH_2CH_2CH_3$	1	L	219–224	$C_{28}H_{36}N_2O_6S_2$	CHN	3.2	> 100	> 31.55
59	$CH_2CH_2CH_2CH_3$	1	L	231–232	$C_{30}H_{40}N_2O_6S_2$	CHN	1.9	> 100	> 52.6
60	$CH(CH_3)_2$	1	L	228–229	$C_{26}H_{32}N_2O_6S_2$	CHNS	5.1	> 100	> 19.6
61	$CH(CH_3)CH_2CH_3$	1	L	220–224	$C_{28}H_{36}N_2O_6S_2{\cdot}0.4H_2O$	CHNS	3.2	> 100	> 31.2
62	Cyclohexyl	1	L	271–272	$C_{32}H_{40}N_2O_6S_2{\cdot}0.3H_2O$	CHNS	4.3	76	17.7
63	$CH_2CH(CH_3)_2$	1	L	231–232	$C_{28}H_{36}N_2O_6S_2{\cdot}0.5H_2O$	CHNS	3.4	92	27
64	$CH_2C(CH_3)_3$	1	L	208–210	$C_{30}H_{40}N_2O_6S_2{\cdot}0.4H_2O$	CHNS	5.2	> 100	19.2
65	CH_2(cyclohexyl)	1	L	197–200	$C_{34}H_{44}N_2O_6S_2{\cdot}1.2H_2O$	CHNS	6.4	42	6.6
66	C_6H_5	1	DL	236–237	$C_{32}H_{28}N_2O_6S_2{\cdot}0.6H_2O$	CHN	6.9	> 100	14.5
67	$CH_2C_6H_5$	1	L	211–214	$C_{34}H_{32}N_2O_6S_2{\cdot}0.53H_2O$	CHN	2.1	67	31.9
68	$CH_2CH_2CH_2CH_2NH$(Cbz)	1	L	146–157	$C_{44}H_{50}N_4O_{10}S_2{\cdot}0.38H_2O$	CHN	7.1	97	13.7
69	$CH_2CH_2CH_3$	2	L	256–258	$C_{28}H_{36}N_2O_6S_2$	CHN	2.5	50	20
70	$CH_2CH_2CH_2CH_3$	2	L	225–226	$C_{30}H_{40}N_2O_6S_2{\cdot}0.3H_2O$	CHN	4.1	25	6.1
71	$CH(CH_3)_2$	2	L	254–255	$C_{28}H_{36}N_2O_6S_2$	CHN	4.3	74	17.2
72	$CH(CH_3)CH_2CH_3$	2	L	253–256	$C_{30}H_{40}N_2O_6S_2$	CHN	4.9	60	12.2
73	Cyclohexyl	2	L	284–286(d)	$C_{34}H_{44}N_2O_6S_2{\cdot}1.1H_2O$	CHN	7.8	24	3.1
74	CH_2(cyclohexyl)	2	L	231–233	$C_{36}H_{48}N_2O_6S_2$	CHN	> 8.5	8.5	1.0
75	$CH_2C_6H_5$	2	L	223–226	$C_{36}H_{36}N_2O_6S_2{\cdot}0.42H_2O$	CHN	2.1	67	31.7
76	$CH_2CH(CH_3)_2$	2	L	251–252	$C_{30}H_{40}N_2O_6S_2{\cdot}0.9H_2O$	CHN	4.0	> 100	> 25

[a]$TI = TC_{50}/EC_{50}$.

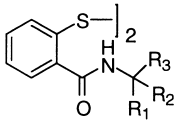
Table 4. Dithiobisbenzamides derived from α,β-unsaturated amino acids possessing various side chains and their physical and antiviral activities tested against HIV in cell culture



Entry	R	Mp (°C)	Molecular formula	Elements analyzed	EC_{50} (μM)	TC_{50} (μM)	TI[a]
77	$CH(CH_3)_2$	223–225	$C_{28}H_{32}N_2O_6S_2{\cdot}0.7H_2O$	CHN	4.8	74	15.4
78	$CH(CH_3)CH_2CH_3$	238–241	$C_{30}H_{36}N_2O_6S_2{\cdot}0.77H_2O$	CHN	5.0	70	14
79	$CH_2CH(CH_3)_3$	238–240	$C_{30}H_{36}N_2O_6S_2$	—[b]	3.7	53	14.3

[a]$TI = TC_{50}/EC_{50}$.
[b]Purity was checked by HPLC.

Table 5. Dithiobisbenzamides derived from protected amino acids possessing various side chains and their physical and antiviral activities tested against HIV in cell culture



Entry	R_1	R_2	R_3	Chirality	Mp (°C)	Molecular formula	Elements analyzed	EC_{50} (μM)	TC_{50} (μM)	TI[a]
80	$CH_2CH(CH_3)_3$	H	$COOCH_3$	L	205–206	$C_{28}H_{36}N_2O_6S_2{\cdot}0.7H_2O$	CHN	> 100	> 100	> 1.0
81	$CH_2CH(CH_3)_3$	H	COO^tBu	L	214–215	$C_{34}H_{48}N_2O_6S_2$	CHN	69	> 100	1.0
82	$CH_2CH(CH_3)_3$	H	$COOCH_2CH=CH_2$	L	185–188	$C_{32}H_{38}N_2O_6S_2{\cdot}0.34H_2O$	CHN	25	> 100	> 4.0
83	$CH_2CH(CH_3)_3$		$HCOOCH_2C_6H_5$	L	147–148	$C_{40}H_{44}N_2O_6S_2{\cdot}0.36H_2O$	CHN	> 73	73	1.0
84	$CH(CH_3)CH_2CH_3$	H	COO^tBu	L	170–171	$C_{34}H_{48}N_2O_6S_2$	CHN	> 95	95	–
85	$COOCH_3$	H	$COOCH_3$	-	163–164	$C_{24}H_{24}N_2O_{10}S_2$	CHN	> 100	> 100	1.0
86	$CH_2CH_2CH_2CH_2$		COO^tBu	L	60–64	$C_{32}H_{40}N_2O_6S_2{\cdot}0.4CHCl_3$	CHN	> 16	16	1
87	$CH_2CH(CH_3)_3$	H	CH_2OH	L	195–196	$C_{26}H_{36}N_2O_4S_2$	CHN	> 12.4	12.4	1.0
88	C_6H_5	H	CH_2OH	L	235–236	$C_{30}H_{28}N_2O_4S_2{\cdot}0.2H_2O$	CHN	> 21	21	1.0
89	$CH(CH_3)_2$	H	$CH=CHCH_2OH$	L	173–175	$C_{30}H_{40}N_2O_4S_2$	-[b]	> 9.0	9.0	1.0
90	$CH_2CH_2CH_2CH_2NH_2$	H	$COOCH_3$	L	160 (d)	$C_{28}H_{38}N_4O_6S_2{\cdot}2.0\ HCl{\cdot}0.75H_2O$	CHNSCl	14.9	> 100	6.7
91	$CH_2CH_2CH_2CH_2NH_2$[c]	H	CH_2COOCH_3	L	> 143	$C_{30}H_{42}N_4O_6S_2{\cdot}2.0HBr$	CHN	10.4	> 100	> 9.6
92	$CH(OH)CH_3$	H	$COOCH_3$	L	169–172	$C_{24}H_{28}N_2O_8S_2{\cdot}0.5H_2O$	CHNS	4.4	67	15.2
93	CH_2OH	H	$COOCH_3$	L	167–169	$C_{22}H_{24}N_2O_8S_2{\cdot}0.15H_2O$	CHNS	2.34	60	25.6
94	CH_2CN	H	$CH_2CH_2COOCH_3$	L	188–196	$C_{28}H_{30}N_4O_6S_2{\cdot}0.4H_2O$	CHN	5.2	69	13.3
95	$CH_2CH_2CH_2CH_2NHCbz$	H	CH_2COOCH_3	L	143–147	$C_{46}H_{54}N_4O_{10}S_2$	CHN	> 16	16	1.0
96	$CH_2CH_2CH_2CH_2NHBoc$	H	COO^tBu	L	81–83	$C_{44}H_{66}N_4O_{10}S_2{\cdot}0.75CHCl_3$	CHNS	23	23	1.0
97	$CH_2CH(CH_3)_3$	H	$CONH_2$	L	244–246	$C_{26}H_{34}N_4O_4S_2{\cdot}1.9H_2O$	CHN	6.5	21	3.2
98	H	H	CH_2CONH_2	-	205–207	$C_{20}H_{22}N_4O_4S_2{\cdot}0.75H_2O$	CHNS	1.4	22	15.7
99	CH_2CONH_2	H	COO^tBu	L	215–217	$C_{30}H_{38}N_4O_8S_2$	-[b]	5.2	> 100	19.2
100	$CH_2CH_2CONH_2$	H	COO^tBu	L	197–198	$C_{32}H_{42}N_4O_8S_2{\cdot}0.5H_2O$	CHN	3.3	91	27.5
101	$CH_2CH(CH_3)_3$	H	$CON(OCH_3)CH_3$	L	124–126	$C_{30}H_{42}N_4O_6S_2{\cdot}0.47H_2O$	CHN	> 20	20	1.0
102	$CONH_2$	H	$CONH_2$	-	247–249	$C_{20}H_{20}N_6O_6S_2$	-[b]	> 100	> 100	1.0
103	CH_2CONH_2	H	$CONH_2$	-	254–255	$C_{22}H_{24}N_6O_6S_2$	-[b]	> 21	21	1.0

[a]$TI = TC_{50}/EC_{50}$.
[b]Purity was checked by HPLC.
[C]HBr salt.

Conclusions from SAR

In summary, SAR of these 2,2′-dithiobisbenzamides shows several important features: (1) Analogues containing straight-chain or α- or β-branched alkyl groups in the side chain of amino acid portion, were preferred as they demonstrate excellent antiviral activities; (2) Variation of spacer between the amine and carboxylic acid is fruitful, yielding 2,2′-dithiobisbenzamides possessing excellent antiviral activities; (3) Analogues containing either a longer straight alkyl side chain (**21** and **22**) in the α-amino acid analogues or a longer spacer between amine and acid portion (**54**–**56**) gave more cytotoxicity, which may be due to less specificity or other cellular effects; (4) 2,2′-Dithiobisbenzamides containing acid functionality exhibited better antiviral activities than other polar functional group analogues; (5) It is also apparent that the presence of one polar group (preferably acid functionality) is necessary for the anti-HIV activity of 2,2′-dithiobisbenzamides, whereas two polar groups are detrimental for anti-HIV activity; (6) 2,2′-Dithiobisbenzamides containing a tertiary amide did not exhibit good antiviral activities.

It is not surprising to note that analogues containing carboxylic functionality are preferred compounds considering the fact that the zinc finger domains and linker region (Fig. 1) of NCp7 contains basic amino acid residues.[18,19] Molecular modeling studies also showed that these aromatic disulfides could be docked efficiently into the zinc finger domains.[8] Our SAR also supports the overall conclusion from molecular modeling studies performed by Rice and co-workers.[8] In conclusion, an effective ligand should possess a hydrophobic (preferably alkyl side chains) and hydrophilic (preferably carboxyl functionality) components for interaction with NCp7 protein.

Mechanism of action

Rice et al.[6] demonstrated in their initial report that HIV nucleocapsid protein (NCp7) was the target for these 2,2′-dithiobisbenzamides effecting antiviral activity. The

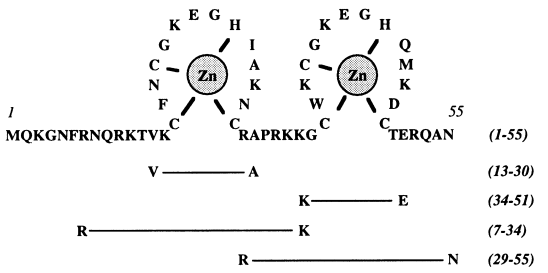


Figure 1. Amino acid sequence of nucleocapsid protein, NCp7, and the peptide fragments used for this study.

biological roles of the nucleocapsid zinc fingers include RNA packaging, dimerization, assembly of viral structure and reverse transcription of viral RNA genome.[20] These highly conserved nucleocapsid proteins are essential in multiple phases of the retroviral replication cycle.[21–23] The HIV-1 NCp7 (Fig. 1) is a basic 55 amino acid protein containing two $C(X)_2C(X)_4H(X)_4C$ motif zinc fingers flanked by basic amino acids on each side.[18,19] These motifs are known to bind Zn^{+2} with subpicomolar affinities.[24] The NMR solution structure of NCp7 as well as NCp7 bound to ψRNA have been reported.[25]

Rice et al. and later Tummino et al. demonstrated that the 2,2-dithiobisbenzamides cause extrusion of zinc from the zinc fingers of NCp7 resulting in a functionally ineffective form of NCp7.[5,26] Turpin et al.[27] have observed the direct consequences of NCp7 zinc extrusion in HIV infected cells in cell culture with similar aromatic disulfides. Thus they have shown that 2,2′-dithiobisbenzamides chemically modify the mutationally intolerant retroviral zinc fingers in infected cells, interrupting protease-mediated maturation of virions leading ultimately to the production of compromised virions. It was also found that the relative rates of HIV inactivation by various dithiobenzamides correlate with their relative kinetic rates of NCp7 zinc ejection.[28] Turpin et al. also showed that this class of disulfides (example **1**) impairs the ability of HIV-1 virions to initiate reverse transcription through their action on the retroviral zinc finger, thereby blocking further rounds of replication.[29] Spectrometric studies of 2,2-dithiobisbenzamides using electrospray ionization mass spectrometry (ESI-MS), 500 MHz one- and two-dimensional nuclear magnetic resonance spectroscopy and circular dichroism spectroscopy demonstrated that Zn ejection is accompanied by formation of covalent complexes between 2,2′-dithiobisbenzamide monomers and Cys residues of Zn-depleted NCp7 protein.[17] Zinc is ejected sequentially from the less stable C-terminal zinc finger, and then the more stable N-terminal zinc finger.[17] Characterization of various intermediates during the formation of final products in the reaction of NCp7 protein with 2,2′-dithiodipyridine and disulfiram was also reported using electrospray-mass spectrometry studies.[30] It has also been shown that similar dithiobenzamides with the amide substituent *para* instead of *ortho* do not extrude zinc or exhibit antiviral activity.[6,8,18b]

Characterization of zinc ejection by electrospray ionization mass spectrometry (ESI-MS)

The time course study of zinc ejection from NCp7 protein, as measured by changes in mass of Zn-loaded NCp7 using ESI mass spectrometry, was performed with selected 2,2′-dithiobisbenzamides (**2**, **33**, **51**, **59**, **72**,

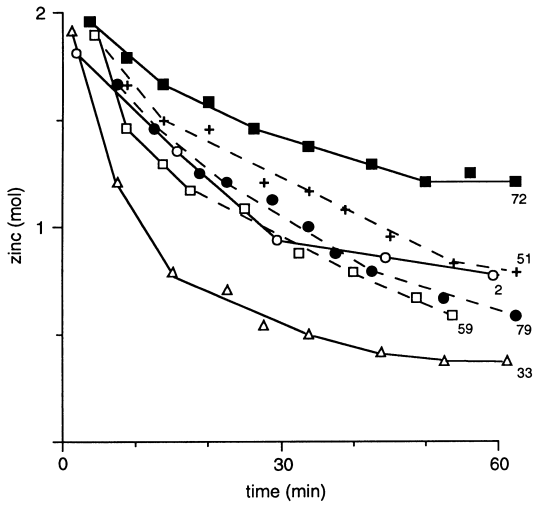


Figure 2. Time course study of total zinc ejection from NCp7 measured by ESI-MS. The absolute abundances (peak intensity) for each protein–zinc species were summed in each mass spectrum acquired at each time point and normalized to the total protein ion signal. For example, all of the ion signals for the NCp7-Zn_1 species were counted together (i.e. [NCp7 + Zn] + [NCp7 + Zn + drug] + [NCp7 + Zn + 2drug] + etc). The following symbols were used to represent the data for the various compounds studied: ■, 72; +, 51; O, 2; ●, 79; □, 59; Δ, 33.

and **79**) derived from various amino acids (Fig. 2). After addition of five molar equivalents of the compound to the protein, the major components, after 1 h, were the starting material (NCp7 + 2Zn) and mono-modified (NCp7 + X + Zn) molecules, where X represents half of the disulfide drug molecule. At later time periods, the apo-protein and its various covalently-modified forms (e.g. NCp7 + 2X) were the major products. The data shows that up to two zinc atoms are being ejected upon the covalent addition of these compounds to NCp7-Zn_2 protein during a 1 h reaction time, similar to that previously observed with compound **2**. Figure 3 shows mass spectra from two time periods after addition of the compounds to NCp7-Zn_2. Although all of the compounds studied by ESI-MS showed some zinc-ejection activity, the nature with which they acted was somewhat variable. For example, compounds **33** and **51** appeared to form more abundant covalent adducts upon zinc-ejection relative to **59** (Fig. 3). However, a direct correlation between the zinc ejection data and the antiviral activities of the present set of compound, was not observed. More surprising is that compound **51** (proline amide) also ejects zinc, though it did not show any antiviral activity. This result confirm that disulfides themselves eject zinc and that they need not be first converted to their cyclic analogues, benzisothiazolones,

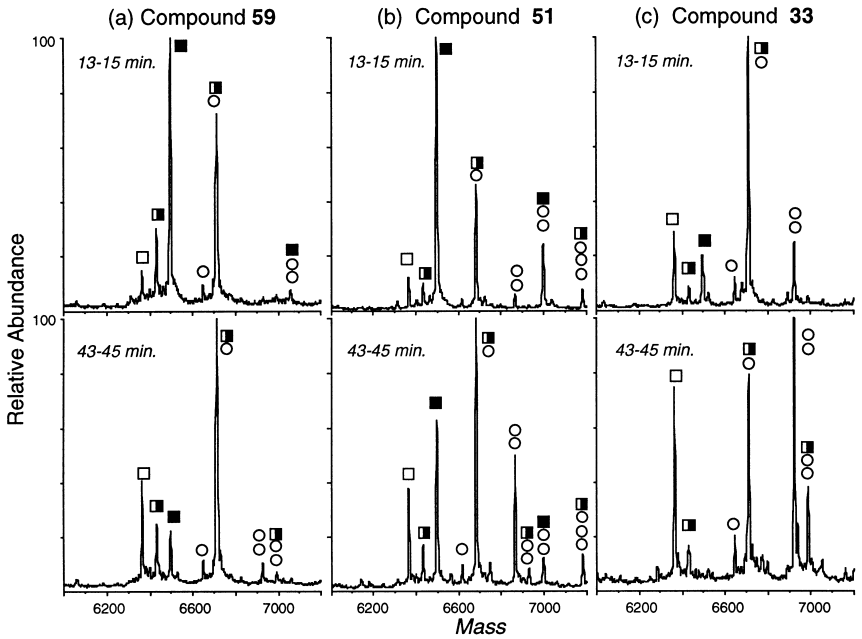


Figure 3. Time course study of zinc ejection from NCp7 by compounds (a) **59**, (b) **51**, and (c) **33** as monitored by ESI-MS. Deconvoluted mass spectra acquired 13–15 > min (top) and 43–45 min (bottom) after 150 μM of the compound was added to a solution containing 30 μM NCp7, 60 μM $ZnCl_2$, and 10 μM ammonium acetate pH 6.9. The square symbol represents the amount of zinc bound to NCp7: a completely filled square (■) designates 2 bound-zinc, a half-filled square (l) designates 1 bound-zinc, and an open square (□) designates zero bound-zinc (apo-protein). The circle (○) symbol represents covalently-bound compound to NCp7, with the number of circles equal to the number of molecules binding.

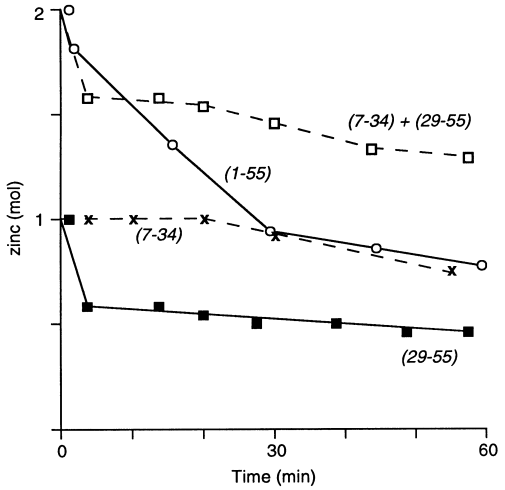


Figure 4. Time course study of total zinc ejection by **2** from zinc-bound peptides derived from the NCp7 amino acid sequence (see Fig. 1) measured by ESI-MS. The following symbols were used to represent the data for the various peptides studied: ■, peptide 29-55; x, peptides 7–34; O, 1–55 (NCp7). Peptides 13–30 and 34–51 showed no ejection of zinc in the presence of **2** over a 1 h period. The open square (□) symbol designate points resulting from the addition of data from peptides 7–34 (x) and 29–55 (■).

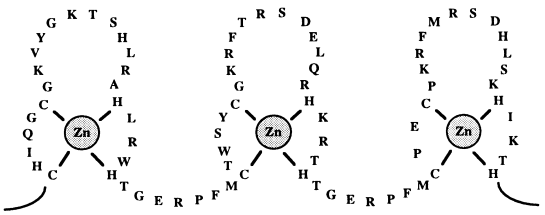


Figure 5. Amino acid sequence of SP1 protein.

in situ. Since these experiments were conducted on naked NCp7 in the absence of ψRNA, it is possible that recognition could be a factor in the NCp7–ψRNA–drug ternary complex.

The mechanism of action of **2** was further investigated by ESI-MS using peptide fragments derived from the NCp7 structure. Peptides composed of the amino acid sequence for the N-terminal zinc finger motif (residues 13–30) and the C-terminal zinc finger structure (residues 34–51) (see Fig. 1) showed very little zinc-ejection activity or covalent drug-binding over a 1 h period. However, peptides containing either the N- or C-terminal zinc fingers with the highly basic central linker region attached (residues 7–34 and 29–55, respectively) showed moderate zinc-ejection and drug-binding activity over the same time period, with the C-terminal finger/linker peptide exhibiting much more reactivity (Fig. 4). The rate of zinc ejection for either of the finger/linker peptides or the combined sum is not as fast as full length 1–55 NCp7, indicating the importance of the total conformation of NCp7 on its reactivity and stability. The Arg29-Gly35 region is highly conserved in human and simian immunodeficiency virus strains. The linker region appears to affect the reactivity of disulfide compounds towards zinc-bound NCp7 in a still unknown manner.

Table 6. Binding and antiviral data of selected 2,2′-bisdithiobenzamides as measured by gel shift assay

Entry	Gel shift IC_{50} (μM)	HIV EC_{50} (μM)
36	66.2	>100
59	19.1	1.9
76	12.9	4.0
79	5.89	3.7

Gel shift assay of selected 2,2′-dithiobisbenzamides

To further address the mechanism of action of these compounds, a representative group of 2,2′-dithiobisbenzamides was chosen to test their ability to inhibit ψRNA–NCp7 complex formation as measured using a gel shift assay. This represents a functional assay for NCp7 and the results are shown in Table 6. Antivirally inactive compound **36** has the highest IC_{50} value in this assay, while the active compounds versus HIV showed 5–19 μM inhibition of ψRNA–NCp7 binding. Although the correlation is not tight, it seems reasonable in qualitative terms given the error in these biological measurements.

Conclusions

Through a systematic variation of the amino acid portion of these 2,2′-dithiobisbenzamide analogues a compound **59**, with improved antiviral activity (fourfold) and improved therapeutic index relative to the lead compound **2**, was obtained. Structural features that improve antiviral activities were studied and found to be quite consistent. More importantly, structural features that increase cellular toxicities were identified, which may shed some light on the selectivity towards viral zinc fingers relative to mammalian zinc fingers. Gel shift studies in our laboratories using SP1 zinc finger protein (a mammalian zinc finger containing the CCHH motif, Figure 5)[31] have shown no binding with disulfide **2** (>100 μM at 4 h and >50 μM at 24 h), indicating selectivity towards NCp7 protein. Moreover, the fact that these compounds exert in vitro antiviral activity without excessive cellular toxicity on either primary cell

lines indicates that there exists a degree of specificity toward the retroviral zinc fingers.[18b]

The exact mechanism of action of these compounds with the NCp7 and any cellular targets is uncertain, as these highly reactive disulfides are capable of multiple reaction in vivo, to form many potentially active species (e.g. free thiols, mixed disulfides with other cellular proteins, etc). Nevertheless, these structurally simple and easily synthesized compounds appear to possess good anti-HIV activity, a reasonable therapeutic index, and importantly have a different mode of action from known anti-HIV agents, which are approved or in clinical trials.

Experimental

Biological assays. The expression and isolation of NCp7 protein were reported previously.[26,27] Peptide fragments of NCp7 were synthesized by the University of Michigan Protein and Carbohydrate Structure Facility (Ann Arbor, MI, USA).

NCp7 gel shift assay. The RNA binding experiments were carried out at room temperature in a binding buffer containing 50 mM Tris–HCl pH 7.5, 100 mM KCl, 20 mM $MgCl_2$, 10% glycerol. The NCp7 at 0.5 μM was incubated with each compound at six concentrations (200–0 μM). At 24 h, a 50 μL aliquot from each was added to an equal volume of ^{32}P-labeled ψRNA (< 100 pM) containing 0.01 μg/mL calf thymus DNA. After mixing, the solutions were incubated for 10 min at room temperature. Separation of RNA and RNA–protein complexes was accomplished by gel electrophoresis on a 8% nondenaturing polyacrylamide gel (39/1 crosslinking) using a running buffer of 50 mM Tris, 45 mM boric acid, 0.5 mM EDTA, pH 8.4. The gels were pre-run at 4 °C after loading with voltage on. The gels were dried and then imaged using a Molecular Dynamics Phosphor Imager. After quantitation of the images, IC_{50} values were calculated based on the concentration of compound incubated with protein prior to the addition of RNA.

SP1 Gel shift assay. The DNA corresponding to the 99 amino acid DNA binding domain of SP1 was obtained by PCR from a plasmid containing the human coding sequence for SP1. The primers utilized in the PCR reaction added a Nde I and Hind III restriction site to the sequence for cloning purposes. The PCR reaction was carried out using Vent Polymerase followed by A-tailing and was ligated into the Invitogen TA cloning kit plasmid. Colonies were initially chosen by restriction digest and verified by sequencing. The Nde I/Hind III fragment was then cloned into pET21a(+) which had been digested with the same restriction enzymes and dephosphorylated. The ligation product was transformed into the INVF' cells from the TA cloning kit, colonies again chosen by restriction digest, and verified by sequencing. Isolated plasmid containing the correct DNA was then transformed into BL21(DE3) cells for expression. The cells were then grown to an OD600 of 0.6 in 300 mL of LB media supplemented with 100 μM zinc chloride and 100 mg/mL Ampicillin. Isopropyl-galactosidase (1 mM) was added and after 2 h of further growth, the cells were harvested. The cells were aliquotted and centrifuged for 30 min, 4 °C, 2500 rpm. The supernatant was then discarded and the pellets were stored at −80 °C. Partial purification of the peptide was achieved via a scaled-up version of Desjarlais and Berg.[32] The pellet weight was multiplied by a factor of 0.0177 to give the amount of buffer to be added (25 mM Tris–HCl pH 8.0, 100 μM zinc chloride, 5 mM DTT). The pellets were liquefied and mixed until uniform. The cell paste was then aliquotted into 1.5 mL sterile Eppendorf tubes. The tubes were then placed in a boiling water bath for 15 min. 1 M KCl was added to a concentration of 150 mM/tube. The tubes were then vortexed and spun in an Eppendorf centrifuge at 14,000 rpm for 30 min at 4 °C. The supernatant was then removed to a fresh tube. After pooling the protein, it was aliquotted and stored at −80 °C. The protein obtained was then pooled together, aliquotted, and stored at −80 °C. Protein sequence was verified by amino acid analysis and the protein concentration was estimated to be 580 nM. The DNA binding experiments were carried out at room temperature in a binding buffer consisting of 50 mM Tris–HCl, pH 7.5, 100 mM KCl, 20 mM $MgCl_2$, and 10% glycerol. Partially purified SP1 zinc finger protein (37.7 ng) was incubated with each compound at two concentrations (50 and 5 μM). At 1, 4, and 24 h, a 19 mL aliquot from each was added to 6 μL of ^{32}P labeled SP1 DNA consensus sequence (< 100 pM). After mixing, the solutions were incubated at room temperature for 15 min. Separation of DNA and the DNA–protein complex was achieved by gel electrophoresis on an 8% nondenaturing polyacrylamide gel (39/1 crosslinking) using a running buffer of 50 mM Tris, 45 mM boric acid, 0.5 mM EDTA, pH 8.4. The gels were pre-run at 4 °C for 30 min at 150V and continued for 60 min at 250V at 4 °C after loading the entire reaction with the voltage on. The gels were dried and then imaged using a Molecular Dynamics Phosphorimager. After quantitation of the images using the ImageQuant software package, percentage shift values were calculated based on the volume of the DNA–protein complex band compared to the total volume of all bands present.

Chemical synthesis. Melting points were determined in open capillary tubes on a Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra were

determined in KBr pellet on a Nicolet FT IR SX-20 spectrophotometer. Proton magnetic resonance (NMR) spectra were recorded on a 300 or 400 MHz Varian Unity spectrometer. Chemical shifts are reported in δ values relative to tetramethylsilane. Mass spectra were recorded on a Finnigan TSQ-70 spectrometer. Elemental analyses were performed on a Perkin–Elmer 240 elemental analyzer or at Robertson Microlit Laboratories (Madison, NJ, USA). Flash or medium pressure chromatography was performed using silica gel 230–400 mesh. All starting materials were commercially available unless otherwise noted.

General procedures for the synthesis of 2,2′-dithiobisbenzamides. The general procedures used in synthesis of the compounds described above are very much similar to the ones described previously.[7] The synthesis and spectral data of compounds reported earlier were not given.

General method A. Initially amino acid hydrochloride (2–2.5 equiv) was taken in 10 mL of dichloromethane and treated with *N*-methylmorpholine (2–2.5 equiv) to liberate free base. To it *N*-methyl-*N*-(trimethylsilyl)acetamide (2–4.5 equiv) was added. The reaction mixture was stirred at room temperature for 1.5 h. To it bisacid chloride (1 equiv) was added. The reaction mixture was stirred at room temperature 4–16 h. Solvents were evaporated. The residue was stirred with 3 N hydrochloric acid for 1 h, diluted with ethyl acetate and washed with 3 N hydrochloric acid. The product was extracted into saturated sodium bicarbonate solution and the free acid was liberated by added 6 N hydrochloric acid to get the final product. The final product was purified by washing with either ether or 10% ethanol in ether, filtered and dried.

General method B. To the amino acid ester (1 equiv) taken in dichloromethane was added *N*-methylmorpholine or triethylamine (1.2–10 equiv). To it bisacid chloride (0.5 equiv) in dichloromethane was added dropwise at 0–10 °C. The reaction mixture was stirred at room temperature for 18 h. The mixture was concentrated, suspended in water and filtered. The solids were dissolved in chloroform, washed with water, dried and concentrated. The residue was triturated with ether/hexanes (1/1) to obtain the final product, which was purified by flash silica gel chromatography, if needed.

General method C. Potassium *t*-butoxide (0.6 equiv) was taken in 20 mL of ethanol and stirred at room temperature for 5 min. To it amino acid (1 equiv) was added and kept under stirring for 15–20 min until it is homogeneous. To it bisacid chloride (0.25 equiv) in dichloromethane (5 mL) was added and kept under stirring for 3 h. After 3 h, ethanol was evaporated and the residue was taken in water and ethyl acetate. Aqueous layer was acidified with 3 N hydrochloric acid to obtain precipitate. Then, it was extracted into saturated sodium bicarbonate solution and the free acid was liberated by added 6 N hydrochloric acid to get the final product. The final product was purified by washing with either ether or 10% ethanol in ether, filtered and dried.

General method D. Amino acid (1 equiv) was taken in absolute ethanol and treated with a solution of sodium (1 equiv) in absolute ethanol, keeping the temperature between −30 to −50 °C. After 2 h, 2,2′-dithiobisbenzoyl chloride was added portionwise over 1 h. After stirring overnight at room temperature, the mixture was concentrated and the residue was suspended in water. After filtering the insoluble material, the filtrate was adjusted to pH 3.0 with 1 N hydrochloric acid and the solid obtained was collected. The product was again taken in aqueous sodium bicarbonate solution and acidified with hydrochloric acid to obtain pure material, which was dried.

Examples

[*S*-(*R,*R**)-2-{2-[2-(1-Carboxy-2-methyl-butylcarbamoyl)-phenyldisulfanyl]-benzoyl-amino}-3-methyl-pentanoic acid (15).** This compound was synthesized according to the general procedure B using isoleucine *t*-butyl ester (5.70 g, 30.4 mmol), pyridine (50 mL), 2,2′-dithiobisbenzoyl chloride (4.80 g,14.0 mmol) and 70 mL of CH_2Cl_2. Isolated yield: 38%; This material was dissolved in 50 mL CH_2Cl_2 and reacted with 50 mL trifluoroacetic acid at 35 °C. After 2 h, the mixture was concentrated and the residue triturated with ether. The solids were suspended in EtOAc/$CHCl_3$ and filtered. Isolated yield: 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (bs, 2H), 8.73 (d, 2H), 7.65 (d, 4H), 7.45 (m, 2H), 7.31 (t , 2H), 4.35 (t, 2H), 1.96 (m, 2H), 1.53 (m, 2H), 1.33 (m, 2H), 0.96 (d, 6H), 0.89 (t, 6H); IR (KBr) 1722, 1641 cm^{-1}; MS (ESI) *m*/*z* 266.

(+,−)-2-{2-[2-(1-Carboxy-propylcarbamoyl)-phenyldisulfanyl]benzoylamino}-butyric acid (19). This compound was synthesized according to the general procedure B using bisacid chloride (1.64 g, 4.77 mmol), potassium *t*-butoxide (1.29 g, 11.45 mmol) and corresponding DL-amino acid hydrochloride (2.64 g, 19.08 mmol). Isolated yield: 62%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (t, 6H), 1.66 (m, 2H), 1.86 (m, 2H), 4.31 (m, 2H), 7.33 (t, 2H), 7.47 (t, 2H), 7.66 (d, 2H), 7.72 (d, 2H), 8.83 (d, 2H); IR (KBr) 3330, 2973, 1716, 1639, 1529, 1288, 1249, 1233, 748 cm^{-1}; MS (ESI) *m*/*z* 238, 323, 463, 475.

[*S*-(*R,*R**)-2-{2-[2-(1-Carboxy-butylcarbamoyl)-phenyldisulfanyl]benzoylamino}-pentanoic acid (20).** This compound was synthesized according to the general method

A using bisacid chloride (2.0 g, 5.85 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (6.81 g, 46.84 mmol), corresponding amino acid hydrochloride (1.8 g, 11.71 mmol), *N*-methylmorpholine (4.74 g, 46.84 mmol) and 40 mL of dichloromethane. Isolated yield: 70%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (t, 6H), 1.47 (m, 4H), 1.81 (m, 4H), 4.4 (m, 2H), 7.33 (t, 2H), 7.47 (t, 2H), 7.66 (t, 2H), 7.72 (d, 2H), 8.83 (d, 2H); IR (KBr) 3279, 2961, 2873, 1723, 1640, 1534, 1260, 746 cm^{-1}; MS (ESI) *m*/*z* 252, 286, 503.

[*S*-(*R,*R**)-2-{2-[2-(1-Carboxy-pentylcarbamoyl)-phenyldisulfanyl]benzoylamino}-hexanoic acid (21).** This compound was synthesized according to the general procedure C using bisacid chloride (1.64 g, 4.77 mmol), potassium *t*-butoxide (1.29 g, 11.45 mmol) and corresponding DL-amino acid hydrochloride (2.50 g, 19.08 mmol). Isolated yield: 68%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (t, 6H), 1.28 (m, 8H), 1.75 (m, 4H), 4.31 (m, 2H), 7.28 (t, 2H), 7.42 (t, 2H), 7.61 (d, 2H), 7.66 (d, 2H), 8.77 (d, 2H); IR (KBr) 3282, 2957, 2870, 1718, 1643, 1538, 1458, 1251, 755 cm^{-1}; MS (ESI) *m*/*z* 266.

[*S*-(*R,*R**)-2-{2-[2-(1-Carboxy-hexylcarbamoyl)-phenyldisulfanyl]benzoylamino}-heptanoic acid (22).** This compound was synthesized according to the general procedure C using bisacid chloride (1.64 g, 4.77 mmol), potassium *t*-butoxide (1.29 g, 11.45 mmol) and corresponding DL-amino acid (2.76 g, 19.08 mmol). Isolated yield: 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (t, 6H), 1.31 (m, 8H), 1.44 (m, 4H), 1.77 (m,4H), 4.36 (m, 2H), 7.31 (t, 2H), 7.45 (t, 2H), 7.64 (d, 2H), 7.67 (d, 2H), 8.83 (d, 2H); IR (KBr) 3286, 2955, 2860, 1729, 1634, 1532, 1461, 1247, 745 cm^{-1}; MS (ESI) *m*/*z* 280, 559.

[*S*-(*R,*R**)-2-{2-[2-(1-Carboxy-3-methylsulfanyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-4-methylsulfanyl-butyric acid (23).** This compound was synthesized according to the general procedure C using bisacid chloride (1.64 g, 4.77 mmol), potassium *t*-butoxide (1.29 g, 11.45 mmol) and corresponding DL-amino acid hydrochloride (2.84 g, 19.08 mmol). Isolated yield: 52%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (s, 6H), 2.61 (m, 8H), 4.53 (m, 2H), 7.31 (t, 2H), 7.47 (t, 2H), 7.64 (d, 2H), 7.72 (t, 2H), 8.89 (d, 2H); IR (KBr) 3337, 2917, 1727, 1639, 1530, 1433, 1224, 748 cm^{-1}; MS (ESI) *m*/*z* 284, 567.

[*S*-(*R,*R**)]-2-{2-[2-(1-Carboxy-2,2-dimethyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-3,3-dimethylbutyric acid (25).** This compound was synthesized according to the general method A using bisacid chloride (1.57 g, 4.5 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (11.1 mL, 36 mmol), corresponding amino acid (1.50 g, 11 mmol) and 120 mL of dichloromethane. Isolated yield: 15%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03 (s, 18H), 4.29 (m, 2H), 7.25 (t, 2H), 7.39 (t, 2H), 7.54 (d, 2H), 7.59 (d, 2H), 8.56 (d, 2H); MS (ESI) *m*/*z* 532.

[*S*-(*R,*R**)]-2-{2-[2-(1-Carboxy-3,3-dimethyl-butylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-4,4-dimethylpentanoic acid (27).** This compound was synthesized according to the general method A using bisacid chloride (0.59 g, 1.72 mmol), *N*-methyl-*N*-(trimethylsilyl)-acetamide (8.6 g, 59.17 mmol), corresponding amino acid (1.0 g, 6.88 mmol), *N*-methylmorpholine (1.39 g, 13.76 mmol) and 20 mL of dichloromethane. Isolated yield: 82%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (s, 18H), 1.61 (m, 4H), 4.36 (m, 2H), 7.29 (t, 2H), 7.42 (t, 2H), 7.62 (t, 2H), 7.67 (t, 2H), 8.86 (d, 2H); IR (KBr) 3313, 2956, 1711, 1637, 1544, 1434, 1251, 867, 747 cm^{-1}; MS (ESI) *m*/*z* 280, 561.

[*S*-(*R,*R**)-2-{2-[2-(1-Carboxy-3-methyl-but-3-enylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-4-methyl-pent-4-enoic acid (28).** This compound was synthesized according to the general method A using bisacid chloride (1.32 g, 3.87 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (2.25 g, 15.48 mmol), corresponding amino acid hydrochloride (1.0 g, 7.75 mmol), *N*-methylmorpholine (1.56 g, 15.48 mmol) and 40 mL of dichloromethane. Isolated yield: 49%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.75 (s, 6H), 2.55 (m, 4H), 4.61 (m, 2H), 4.83 (brs, 4H), 7.31 (t, 2H), 7.44 (t, 2H), 7.64 (t, 2H), 7.66 (t, 2H), 8.83 (d, 2H); IR (KBr) 3386, 3293, 2923, 1729, 1639, 1527, 1434, 1258, 898, 747 cm^{-1}; MS (ESI) *m*/*z* 264.

(+,−)-2-{2-[2-(1-Carboxy-2-cyclopentyl-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-3-cyclopentyl-propionic acid (29). This compound was synthesized according to the general method A using bisacid chloride (0.54 g, 1.59 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.85 g, 12.72 mmol), DL-cyclopentylalanine (0.5 g, 3.18 mmol), *N*-methylmorpholine (1.29 g, 12.72 mmol) and 20 mL of dichloromethane. Isolated yield: 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (m, 4H), 1.31–2.03 (m, 18H), 4.36 (m, 1H), 5.06 (dd, 1H), 7.39 (t, 2H), 7.62 (t, 2H), 7.83 (d, 2H), 7.92 (d, 2H), 8.8 (d, 2H) IR (KBr) 3411, 2950, 2864, 1737, 1599, 1530, 1448, 1222, 747 cm^{-1}; MS (ESI) *m*/*z* 290, 292.

[*S*-(*R,*R**)]-2-{2-[2-(1-Carboxy-2-cyclohexyl-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-3-cyclohexylpropionic acid (30).** This compound was synthesized according to the general method A using bisacid chloride (1.0 g, 2.92 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.7 g, 11.68 mmol), L-cyclohexylalanine hydrochloride (1.33 g, 6.41 mmol), triethylamine (1.3 g, 12.82 mmol) and 20 mL of dichloromethane. Isolated yield: 51%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.77–1.25 (m, 11H), 1.39–1.86 (m, 15H), 4.36 (m, 2H), 7.33 (t, 2H), 7.44

(t, 2H), 7.66 (d, 4H), 8.83 (d, 2H), 12.61 (brs, 2H); IR (KBr) 3287, 2923, 1727, 1631 cm^{-1}; MS (ESI) *m*/*z* 306.

2-{2-[2-(1-Carboxy-1-methyl-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-2-methyl-propionic acid (31). The title compound was synthesized according to the general method A using bisacid chloride (1.0 g, 2.92 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.70 g, 11.68 mmol), corresponding amino acid (1.5 g, 7.4 mmol), *N*-methylmorpholine (1.20 g, 11.68 mmol) and 20 mL of dichloromethane. Isolated yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47 (s, 12H), 7.17 (t, 2H), 7.44 (t, 2H), 7.66 (d, 4H), 8.72 (s, 2H), 12.31 (brs, 2H); IR (KBr) 3329, 2988, 1712, 1633, 1528, 748 cm^{-1}; MS (ESI) *m*/*z* 238.

Cyclopentanecarboxylic acid, 1,1′-[dithiobis[(2,1-phenylenecarbonyl)imino]bis[-, (32). The title compound was synthesized according to the general method A using bisacid chloride (1.0 g, 2.92 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.70 g, 11.68 mmol), corresponding amino acid (0.82 g, 6.41 mmol), *N*-methylmorpholine (1.20 g, 11.68 mmol) and 20 mL of dichloromethane. Isolated yield: 52%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.69 (m, 8H), 2.09 (m, 8H), 7.3 (t, 2H), 7.43 (t, 2H), 7.61 (d, 2H), 7.64 (d, 2H), 8.65 (s, 2H), 12.28 (brs, 2H); IR (KBr) 3297, 2959, 1710, 1630, 1530, 1323, 748 cm^{-1}; MS (ESI) *m*/*z* 264.

Cyclohexanecarboxylic acid, 1,1′-[dithiobis[(2,1-phenylenecarbonyl)imino]bis[-, (33). The title compound was synthesized according to the general method A using bisacid chloride (1.0 g, 2.92 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.7 g, 11.68 mmol), corresponding amino acid (0.92 g, 6.41 mmol), *N*-methylmorpholine (1.2 g, 11.68 mmol) and 20 mL of dichloromethane. Isolated yield: 60%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.28 (brm, 2H), 1.55 (brs, 10H), 1.73 (brm, 4H), 2.15 (brd, 4H), 7.31 (t, 2H), 7.44 (t, 2H), 7.61 (d, 2H), 7.66 (d, 2H), 8.53 (s, 2H), 12.28 (brs, 2H); IR (KBr) 3288, 2935, 1711, 1632, 1529, 748 cm^{-1}; MS (ESI) *m*/*z* 278.

(+,−)-2-{2-[2-(1-Carboxy-1-methyl-3-methylsulfanyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-2-methyl-4-methylsfulfanyl-butyric acid (34). This compound was synthesized according to the general procedure C using bisacid chloride (1.64 g, 4.77 mmol), potassium *t*-butoxide (1.29 g, 11.45 mmol) and corresponding DL-α-methylmethionine (3.11 g, 19.08 mmol). Isolated yield: 76%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (s, 6H), 2.03 (s and m, 8H), 2.47 (m, 4H, partially obscured by dmso peak), 2.33 (m, 2H), 7.31 (t, 2H), 7.44 (t, 2H), 7.61 (d, 2H), 7.66 (d, 2H), 8.66 (d, 2H); IR (KBr) 3325, 2918, 1709, 1629, 1520, 1448, 751 cm^{-1}; MS (ESI) *m*/*z* 298.

[*R*-(*R,*R**)] (2-{2-[Carboxy-phenyl-methyl)-carbamoyl]-phenyldisulfanyl}-benzoylamino)-phenyl-acetic acid (35).** *t*-Butyl ester of the title compound was prepared according to the general method B using bisacid chloride (0.43 g, 1.25 mmol), corresponding amino acid *t*-butyl ester hydrochloride (0.60 g, 2.89 mmol), triethyl amine (1.2 g) in 30 mL of dichloromethane. Resulting compound was taken in 10 mL of dichloromethane and stirred with 0.73 g of trifluoroacetic acid at room temperature to afford the product. Isolated yield: 10%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.08 (d, 2H), 7.43 (m, 14H), 7.60 (t, 2H), 7.82 (m, 2H); MS (ESI) *m*/*z* 286.

[*S*-(*R,*R**)]-2-{2-[2-(1-Carboxy-2-phenyl-ethylcarabamoyl)-phenyldisulfanyl]-benzoylamino}-3-phenyl-propionic acid (36).** (a) *t*-Butyl ester of the title compound was prepared according to the general method B using bisacid chloride (0.87 g, 2.9 mmol), corresponding amino acid *t*-butyl ester hydrochloride (1.30 g, 5.05 mmol), triethyl amine (1.2 g) in 20 mL of dichloromethane. Isolated yield: 54%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (s, 18H), 3.03 (dd, 2H), 3.11 (dd, 2H), 4.50 (m, 2H), 7.17 (m, 2H), 7.25 (m, 5H), 7.47 (t, 2H), 7.50 (d, 2H), 7.53 (d, 2H), 8.94 (d, 2H). (b) The above compound was dissolved in 10 mL of dichloromethane. To this solution was added 1.5 mL of trifluoroacetic acid and the mixture was stirred at room temperature for 48 h. The solvents were evaporated and the residue was triturated with hexanes to obtain the title compound which was dried in vacuo. Isolated yield: 99%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.03 (dd, 2H), 3.22 (dd, 2H), 4.64 (m, 2H), 7.19 (t, 2H), 7.31 (m, 10H), 7.42 (t, 2H), 7.55 (m, 4H), 8.92 (d, 2H); IR (KBr) 3306, 3030, 1721, 1641, 1528, 1260, 749 cm^{-1}; MS (ESI) *m*/*z* 300, 601.

[*R*-(*R,*R**)]-2-{2-[2-(1-Carboxy-3-phenyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-4-phenyl-butyric acid (37).** The title compound was synthesized according to the general method A using bisacid chloride (1.53 g, 4.46 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (3.24 g, 11.68 mmol), corresponding amino acid (2.0 g, 11.16 mmol), *N*-methylmorpholine (2.26 g, 22.32 mmol) and 40 mL of dichloromethane. Isolated yield: 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.11 (m, 4H), 2.64–2.83 (m, 4H), 4.33 (m, 2H), 7.17–7.4 (m, 12H), 7.5 (t, 2H), 7.69 (d, 2H), 7.75 (d, 2H), 8.95 (d, 2H), 12.69 (brs, 2H); IR (KBr) 3284, 2926, 1717, 1639, 1532, 747, 699 cm^{-1}; MS (ESI) *m*/*z* 314.

[*S*-(*R,*R**)]-2-{2-[2-(1-Carboxy-3-cyclohexyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-4-cyclohexyl-butyric acid (38).** The title compound was synthesized according to the general method A using bisacid chloride (1.48 g, 4.34 mmol), *N*-methyl-*N*-(trimethylsilyl)-acetamide (4.27 g, 43.24 mmol), corresponding amino acid (2.0 g, 10.81 mmol), *N*-methylmorpholine (4.37 g,

43.24 mmol) and 40 mL of dichloromethane. Isolated yield: 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (m, 4H), 1.03–1.26 (m, 12H), 2.55–2.92 (m, 14H), 7.31 (t, 2H), 7.44 (t, 2H), 7.64 (d, 2H), 7.69 (d, 2H), 8.83 (d, 2H), 12.64 (brs, 2H); IR (KBr) 3290, 2922, 2851, 1715, 1642, 1532, 742 cm^{-1}; MS (ESI) *m*/*z* 320.

[*S*-(*R,*R**)]-2-(2-{2-[1-Carboxy-2-(4-hydroxy-phenyl)-ethylcarbamoyl]-phenyldisulfanyl}-benzoylamino)-3-(4-hydroxy-phenyl)-propionic acid (39).** (a) To the bisacid chloride (0.57 g, 0.167 mmol) dissolved in 20 mL of dichloromethane was added tyrosine (*t*-butyl) *t*-butyl ester hydrochloride (1.10 g, 3.34 mmol) and triethylamine (0.7 g) as described in the general method B, isolated yield: 52%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (s, 18H), 1.37 (s, 18H), 3.08 (m, 4H), 4.5 (m, 2H), 6.89 (d, 4H), 7.22 (d, 4H), 7.28 (t, 2H), 7.42 (t, 2H), 7.53 (d, 2H), 7.58 (d, 2H), 8.94 (d, 2H). (b) The above compound (0.57 g) was dissolved in 10 mL of dichloromethane. To this, 1.0 mL of trifluoroacetic acid was added and stirred at room temperature for 24 h. The solvents were evaporated and the residue on triturating with hexanes afforded the title compound. Isolated yield: 50%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.92 (dd, 2H), 3.08 (dd, 2H), 4.53 (m, 2H), 6.66 (d, 4H), 7.14 (d, 4H), 7.28, (t, 2H), 7.42 (t, 2H), 7.58 (m, 4H), 8.86 (d, 2H), 9.22 (s, 2H); MS (ESI) *m*/*z* 316.

[*S*-(*R,*R**)]-3-(4-Benzyloxycarbonylamino-phenyl)-2-(2-{2-[2-(4-benzyloxycarbonylamino-phenyl)-1-carboxy-ethylcarbamoyl]-phenyldisulfanyl}-benzoylamino)-propionic acid (40).** This compound was synthesized according to the general procedure C using bisacid chloride (1.0 g, 2.41 mmol), potassium *t*-butoxide (0.43 g, 1.21 mmol) and corresponding amino acid hydrochloride (obtained via deprotection of the corresponding amino acid (1.0 g), where α-amine was protected as Boc derivative (2.41 mmol)]. Isolated yield: 48%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.14 (dd, 4H), 4.58 (m, 2H), 5.11 (s, 4H), 7.22 (m, 6H), 7.39 (m, 16H), 7.58 (d, 2H), 7.55 (d, 2H), 8.89 (d, 2H), 9.7 (s, 2H), 12.82 (brs, 2H); IR (KBr) 3310, 1716, 1528, 1223, 745 cm^{-1}; MS (ESI) *m*/*z* 451.

[*S*-(*R,*R**)]-3-4-(Amino-phenyl)-2-(2-{2-[2-(4-amino-phenyl)-1-carboxy-ethylcarbamoyl]-phenyldisulfanyl}-benzoylamino)-propionic acid (41).** The title compound was synthesized by treating **40** (0.15 g) with 31% HBr in acetic acid solution (4 mL) at room temperature for 20 min. The solvents were evaporated and the residue on triturating with ether afforded solid, which is hygroscopic. Isolated yield: 92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.28 (dd, 2H), 3.36 (dd, 2H), 5.40 (m, 2H), 7.20 (d, 4H), 7.28 (d, 2H), 7.20–7.6 (m, 10H), 7.36 (d, 2H), 7.66 (t, 2H), 7.79 (d, 2H), 7.94 (d, 1H), 8.31 (brs, 1H), 8.97 (d, 2H), 9.8 (brs, 4H); IR (KBr) 3422, 2920, 1732, 1630, 1511, 741 cm^{-1}; MS (ESI) *m*/*z* 315, 329.

[*S*-(*R,*R**)]-2-{2-[2-(1,2-Dicarboxy-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-succinic acid (42).** This compound was synthesized according to the general procedure C using bisacid chloride (1.1 g, 3.2 mmol), corresponding amino acid *t*-butyl ester hydrochloride (2.0 g), *N*-methylmorpholine (1.06 g) in 45 mL of dichloromethane; The crude compound obtained was dissolved in 10 mL of dichloromethane. To it 0.41 g of trifluoroacetic acid was added and stirred at room temperature for 24 h. The solvents were evaporated and the residue was triturated with hexanes to obtain the title compound, which was dried in vacuo. Isolated yield: 25%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.88 (m, 4H), 4.78 (m, 2H), 7.36 (m, 2H), 7.52 (m, 2H), 7.71 (m, 4H), 8.98 (d, 2H); MS (ESI) *m*/*z* 536.

[*S*-(*R,*R**)]-2-{2-[2-(1,3-Dicarboxy-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-pentanedioic acid (43).** *t*-Butyl ester of the title compound was prepared according to general method B using bisacid chloride (1.05 g, 3.0 mmol), corresponding amino acid *t*-butyl ester hydrochloride (2.0 g, 6.7 mmol), triethylamine (1.2 g) in 45 mL of dichloromethane; The crude compound obtained was dissolved in 10 mL of dichloromethane. To this, 2.86 g of trifluoroacetic acid was added and stirred at room temperature for 24 h. The solvents were evaporated and the residue was triturated with hexanes to obtain the title compound which was dried in vacuo. Isolated yield: 16%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.03 (m, 2H), 2.50 (m, 4H), 4.42 (m, 2H), 7.32 (t, 2H), 7.45 (t, 2H), 7.67 (d, 2H), 7.72 (d, 2H), 8.85 (d, 2H).

[*S*-(*R,*R**)]-2-{2-[2-((1,4-Dicarboxy-butylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-hexanedioic acid (44).** *t*-Butyl ester of the title compound was prepared according to the general method B using bisacid chloride (1.12 g, 3.2 mmol), corresponding amino acid *t*-butyl ester hydrochloride (2.0 g, 7.3 mmol), triethylamine (1.2 g) in 55 mL of dichloromethane. The crude compound obtained was dissolved in 10 mL of dichloromethane. To it trifluoroacetic acid was added and stirred at room temperature for 24 h. The solvents were evaporated and the residue was triturated with hexanes to obtain the title compound which was dried in vacuo. Isolated yield: 30%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.64 (m, 8H), 2.54 (t, 4H), 4.36 (m, 2H), 7.30 (t, 2H), 7.45 (t, 2H), 7.67 (m, 4H), 8.84 (d, 2H); MS (ESI) *m*/*z* 296.

6-Amino-2-{2-[2-(5-amino-1-carboxy-pentylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-hexanoic acid (45). A solution of **90** (0.51 g, 0.86 mmol) in 100 mL of 1 N HCl was heated to 50 °C for 7 h. The solution was cooled and concentrated to give after drying 0.41 g of compound **45** as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (brs,

2H), 8,86 (d, 2H), 7.88 (brs, 6H), 7.76 (d, 2H), 7.65 (d, 2H), 7.48 (t, 2H), 7.33 (t, 2H), 4.37 (m, 2H), 2.78 (m, 4H), 1.83 (m, 4H), 1.60 (m, 2H), 1.48 (m, 2H), IR (KBr) 3426, 2926, 1630 cm^{-1}; MS (ESI) *m*/*z* 563.

(*S*-*R,*R**)]-2-{2-[2-(2-Carbamoyl-1-carboxy-ethylcarbamoyl)-phenyldidsulfanyl]-benzoylamino}-succinamic acid (48).** This compound was prepared from **99** (1.31 g, 2.0 mmol) using 15 mL of dichloromethane and 15 mL of trifluoroacetic acid. The sample was concentrated and the residue was triturated with ethanol and 1 N HCl to yield 0.74 g of final product as a solid, mp 208–211 °C. ^{1}H NMR (DMSO-d_6) δ 12.74 (brs, 2H), 8.85 (d, 2H), 7.67 (dd, 4H), 7.48 (t, 2H), 7.43 (brs, 2H), 7.31 (t, 2H), 6.98 (brs, 2H), 4.72 (q, 2H), 2.72 (dd, 2H), 2.60 (dd, 2H); IR (KBr) 3270, 1724, 1645, 748 cm^{-1}; MS (ESI) *m*/*z* 533.

(*S*-*R,*R**)]-4-Carbamoyl-2-{2-[2-(3-carbamoyl-1-carboxy-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-butyric acid (49).** This compound was prepared from **100** (1.05 g, 1.6 mmol) using 15 mL of dichloromethane and 15 mL of trifluoroacetic acid. The residue was triturated with ethanol and 1 N HCl to yield 0.74 g of final product as a solid. ^{1}H NMR (DMSO-d_6) δ 12.65 (bs, 2H), 8.88 (d, 2H), 7.69 (d, 2H), 7.60 (d, 2H), 7.42 (t, 2H), 7.31 (bs, 2H), 7.26 (t, 2H), 6.80 (brs, 2H), 4.31 (m, 2H), 2.20 (t, 4H), 2.02 (m, 2H), 1.88 (m, 2H); IR (KBr) 3264, 1718, 1643, 1538, 747 cm^{-1}; MS (APCI) *m*/*z* 281.

[*S*-(*R,*R**)]-2-{2-[2-(1-Carboxy-3-methyl-butyl)-methyl-carbamoyl]-phenyldisulfanyl}-benzoyl)-methyl-amino]-4-methyl-pentanoic acid (50).** *t*-Butyl ester of the title compound was prepared according to the general method B using bisacid chloride (1.02 g, 2.98 mmol), corresponding amino acid *t*-butyl ester hydrochloride (1.5 g, 7.5 mmol), triethylamine (1.15 mL, 8.25 mmol) in 40 mL of dichloromethane. The crude compound obtained was dissolved in 10 mL of dichloromethane. To it trifluoroacetic acid was added and stirred at room temperature for 24 h. The solvents were evaporated and the residue was triturated with hexanes to obtain the title compound, which was dried in vacuo. Isolated yield: 23%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.8 (m, 12H), 1.48 (m, 4H), 1.73 (m, 2H), 2.36 (s, 6H), 7.45 (t, 2H), 5.02 (m, 2H), 7.20 (m, 6H), 7.75 (m, 2H); MS (ESI) *m*/*z* 282.

[*S*-(*R,*R**)]-(1H-Pyrrolidine-2-carboxylic acid, 1,1′-[dithio-bis(2,1-phenylenecarbonyl)]bis-, (51).** The title compound was synthesized from **83** (1.36 g, 2.2 mmol) using 15 mL trifluoroacetic acid in 15 mL dichloromethane to afford 0.78 g of the title compound as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, 2H), 7.43–7.20 (m, 6H), 4.76 (m, 2H), 3.28 (m, 4H), 2.39 (m, 2H), 2.18 (m, 2H), 2.10–1.85 (m, 4H); IR (KBr) 2976, 1740, 1601, 1426, 1179, 751 cm^{-1}; MS (ESI) *m*/*z* 499.

4-{2-(3-Carboxy-propylcarbamoyl)-phenyldilsulfanyl]-benzoylamino}-butyric acid (53). *t*-Butyl ester of the title compound was prepared according to the general method B using bisacid chloride (1.96 g, 5.7 mmol), corresponding amino acid *t*-butyl ester hydrochloride (2.0 g, 12.5 mmol), triethylamine (1.25 mL, 12.0 mmol) in 85 mL of dichloromethane. The crude compound obtained was dissolved in 10 mL of dichloromethane. To it trifluoroacetic acid was added and stirred at room temperature for 24 h. The solvents were evaporated and the residue was triturated with hexanes to obtain the title compound which was dried in vacuo. Isolated yield: 23%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.75 (m, 4H), 2.30 (m, 4H), 3.26 (m, 4H), 7.27 (t, 2H), 7.42 (t, 2H), 7.59 (m, 4H), 8.61 (m, 2H); MS (ESI) *m*/*z* 238.

6-{2-[2-(5-Carboxy-pentylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-hexanoic acid (56). This compound was synthesized according to the general procedure C using bisacid chloride (1.10 g, 3.0 mmol), sodium ethoxide (prepared from 0.30 g of sodium and 40 mL of ethanol) and corresponding amino acid (3.0 g, 20 mmol). Isolated yield: 43%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (m, 8H), 1.48 (m, 8H), 2.18 (t, 4H), 3.24 (m, 4H), 7.27 (t, 2H), 7.39 (t, 2H), 7.60 (m, 4H), 8.57 (m, 2H); MS (ESI) *m*/*z* 280.

(±)3-{2-(2-Carboxy-1-methyl-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-butyric acid (57). This compound was synthesized according to the general procedure C using bisacid chloride (1.1 g, 3.0 mmol), potassium *t*-butoxide (1.35 g, 1.2 mmol) and corresponding amino acid (2.0 g, 19.3 mmol) and 20 mL of ethanol. Isolated yield: 37%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (d, 6H), 2.43 (m, 4H), 4.29 (m, 2H), 7.26 (m, 2H), 7.35 (m, 2H), 7.59 (m, 4H), 8.52 (m, 2H); MS (ESI) *m*/*z* 487.

[*S*-(*R,*R**)]-(3-{2-[2-(1-Carboxymethyl-butylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-hexanoic acid) (58).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.79 g, 2.75 mmol), *N*-methylmorpholine (1.0 g (1.08 mL), 9.85 mmol), bisacid chloride (0.70 g, 2.04 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.43 g (1.58 mL), 9.85 mmol), isolated yield: 46%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, 6H), 1.39 (m, 4H), 1.51 (m, 4H), 2.49 (m, 4H), 4.29 (m, 2H), 7.30 (t, 2H), 7.43 (t, 2H), 7.56 (d, 2H), 7.64 (d, 2H), 8.46 (d, 2H), 11.4 (br s, 2H); IR (KBr) 3280, 3060, 2958, 2932, 2872, 1700, 1637, 1587, 1538, 1462, 1432, 1321, 1227, 1185, 1037, 915, 873, 744, 696. 652 cm^{-1}; MS (ESI) *m*/*z* 266.3.

[*S*-(*R,*R**)]-(3-{2-[2-(1-Carboxymethyl-pentylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-heptanoic acid) (59).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.95 g, 5.23 mmol), *N*-methylmorpholine (1.11 g (1.21 mL), 10.98 mmol), bisacid chloride (0.78 g, 2.27 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.60 g (1.76 mL), 10.98 mmol). Isolated yield: 67%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (t, 6H), 1.34 (m, 8H), 1.55 (m, 4H), 2.47 (m, 4H), 4.28 (m, 2H), 7.30 (t, 2H), 7.43 (t, 2H), 7.56 (d, 2H), 7.64 (d, 2H), 8.45 (d, 2H), 12.22 (s, 2H); IR (KBr) 3278, 3060, 3028, 2957, 2930, 2860, 1701, 1636, 1587, 1537, 1459, 1433, 1413, 1321, 1288, 1251, 1227, 1183, 1138, 1037, 913, 872, 747, 697, 652 cm^{-1}; MS (ESI) *m*/*z* 280.3.

[*S*-(*R,*R**)]-(3-{2-[2-(1-Carboxymethyl-2-methyl-propyl-carbamoyl)-phenyldisulfanyl]-benzoylamino}-4-methyl-pentanoic acid) (60).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.72 g, 4.29 mmol), *N*-methylmorpholine (0.91 g (0.99 mL), 9.02 mmol), bisacid chloride (0.64 g, 1.87 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.31 g (1.45 mL), 9.02 mmol). Isolated yield: 27%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (m, 12H), 1.85 (m, 2H), 2.41–2.56 (m, 4H), 4.20 (m, 2H), 7.30 (t, 2H), 7.43 (t, 2H), 7.55 (d, 2H), 7.64 (d, 2H), 8.42 (d, 2H), 12.19 (s, 2H); IR (KBr) 3286, 3062, 2963, 2935, 2874, 1705, 1635, 1587, 1536, 1462, 1432, 1409, 1322, 1284, 1184, 745 cm^{-1}; MS (ESI) *m*/*z* 266.4.

[*S*-(*R,*R**)]-(3-{2-[2-(1-Carboxymethyl-2-methyl-butyl-carbamoyl)-phenyldisulfanyl]-benzoylamino}-4-methyl-hexanoic acid) (61).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.71 g, 3.91 mmol), *N*-methylmorpholine (0.83 g (0.9 mL), 8.19 mmol), bisacid chloride (0.58 g, 1.70 mmol) and *N*-methyl-*N*-(trimethylsilyl)acetamide (1.19 g (1.31 mL), 8.19 mmol). Isolated yield: 98%; 1H NMR (300 MHz, DMSO-d_6) δ 0.89 (m, 12H), 1.18 (m, 2H), 1.48 (m, 2H), 1.64 (m, 2H), 2.46 (m, 4H), 4.26 (m, 2H), 7.29 (t, 2H), 7.42 (t, 2H), 7.54 (d, 2H), 7.64 (d, 2H), 8.46 (d, 2H), 12.13 (br s, 2H); IR (KBr) 3286, 2965, 1702, 1635, 1587, 1538, 1432 cm^{-1}; MS (APCI) *m*/*z* 280.5.

[*S*-(*R,*R**)]-(3-{2-[2-(2-Carboxy-2-cyclohexyl-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-3-cyclohexyl-propionic acid) (62).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.55 g, 2.65 mmol), *N*-methylmorpholine (0.56 g (0.61 mL), 5.56 mmol), bisacid chloride (0.45 g, 1.15 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (0.81 g (0.89 mL), 5.56 mmol). Isolated yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6) (0.99–1.16 (m, 10H), 1.49 (m, 2H), 1.61 (m, 2H), 1.73 (m, 8H), 2.41–2.58 (m, 4H), 4.19 (m, 2H), 7.29 (t, 2H), 7.41 (t, 2H), 7.55 (d, 2H), 7.64 (d, 2H), 8.41 (d, 2H), 12.17 (brs, 2H); IR (KBr) 3281, 3061, 2927, 2852, 1670, 1635, 1587, 1538, 1447, 1432, 1416, 1323, 1284, 743 cm^{-1}; MS (ESI) *m*/*z* 306.5.

[*S*-(*R,*R**)]-(3-{2-[2-(1-Carboxymethyl-3-methyl-butyl-carbamoyl)-phenyldisulfanyl]-benzoylamino}-5-methyl-hexanoic acid) (63).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.50 g, 2.75 mmol), *N*-methylmorpholine (0.58 g (0.64 mL), 5.78 mmol) bisacid chloride (0.45 g, 1.31 mmol), *N*-methyl-*N*-trimethylsilyl)acetamide (0.84 g (0.93 mL), 5.78 mmol). Isolated yield: 29.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (m, 12H), 1.29 (m, 2H), 1.55 (m, 2H), 1.70 (m, 2H), 2.48 (m, 4H), 4.37 (m, 2H), 7.30 (t, 2H), 7.42 (t, 2H), 7.55 (d, 2H), 7.64 (d, 2H), 8.43 (d, 2H), 12.21 (brs, 2H); IR (KBr) 3283, 3060, 2956, 2932, 2870, 1712, 1633, 1536, 1463, 1434, 1313, 1283 cm^{-1}; MS (ESI) *m*/*z* 280.4.

[*S*-(*R,*R**)]-(3-{2-[2-(1-Carboxymethyl-3,3-dimethyl-butyl-carbamoyl)phenyldisulfanyl]-benzoylamino}-5,5-dimethyl-hexanoic acid) (64).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.88 g, 4.50 mmol), *N*-methylmorpholine (0.96 g (1.04 mL), 9.44 mmol), bisacid chloride (0.67 g, 1.96 mmol), *N*-methyl-(*N*-trimethylsilyl) acetamide (1.37 g (1.52 mL), 9.44 mmol) to yield 0.95 g of beige solid. Isolated yield: 82%; ^{1}H NMR (400 MHz, DMSO-d_6) γ 0.95 (s, 18H), 1.35 (d, 2H), 1.66 (dd, 2H), 2.37–2.52 (m, 4H), 4.43 (m, 2H), 7.29 (t, 2H), 7.40 (t, 2H), 7.54 (d, 2H), 7.62 (d, 2H), 8.48 (d, 2H), 12.22 (s, 2H); IR (KBr) 3299, 3062, 2955, 2867, 1712, 1632, 1530, 1465, 1433, 1366, 1322, 1285, 1252, 1202, 747 cm^{-1}; MS (ESI) *m*/*z* 294.5.

[*S*-(*R,*R**)]-(3-{2-[2-(1-Carboxymethyl-2-cyclohexyl-ethyl-carbamoyl)-phenyldisulfanyl]-benzoylamino}-4-cyclohexyl-butyric acid) (65).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.87 g, 3.92 mmol), *N*-methylmorpholine (0.83 g (0.91 mL), 8.24 mmol) bisacid chloride (0.59 g, 1.71 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.20 g (1.32 mL), 8.24 mmol). Isolated yield 40%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (m, 2H), 0.95 (m, 2H), 1.14 (m, 6H), 1.41 (m, 6H), 1.60 (m, 8H), 1.95 (br d, 2H), 2.46 (m, 4H), 4.42 (m, 2H), 7.30 (t, 2H), 7.42 (t, 2H), 7.52 (d, 2H), 7.63 (d, 2H), 8.42 (d, 2H), 12.21 (br s, 2H); IR (KBr) 3422, 3368, 3339, 3312, 2922, 2850, 1712, 1631, 1587, 1529, 1447, 1434, 1319, 1283 cm^{-1}; MS (ESI) *m*/*z* 320.5.

(±)-3-{2-[2-(2-Carboxy-1-phenyl-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-3-phenyl-propionic acid (66). This compound was synthesized according to the general

procedure C using bisacid chloride (0.7 g, 2.0 mmol), potassium *t*-butoxide (0.7 g, 8.5 mmol) and corresponding amino acid (2.0 g, 12.1 mmol) and 20 mL of ethanol. Isolated yield: 37%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.77 (m, 4H), 5.39 (m, 2H), 7.16–7.93 (m, 18H), 9.10 (d, 2H), 12.32 (s, 2H); MS (ESI) *m*/*z* 599.

[*S*-(*R,*R**)]-(3-{2-[2-(1-Carboxymethyl-2-phenyl-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-4-phenylbutyric acid) (67).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (1.00 g, 4.64 mmol), *N*-methylmorpholine (0.99 g (1.07 mL), 9.74 mmol) bisacid chloride (0.70 g, 2.02 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.42 g (1.56 mL), 9.74 mmol). Isolated yield: 59%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (m, 4H), 2.89 (d, 2H), 4.47 (m, 2H), 7.19 (m, 2H), 7.27 (m, 10H), 7.40 (t, 2H), 7.47 (d, 2H), 7.55 (d, 2H), 8.57 (d, 2H), 12.29 (s, 2H); IR (KBr) 3293, 3091, 3028, 2923, 1710, 1637, 1586, 1528, 1433, 1404, 1286, 1258, 1204, 1162, 1082, 1035, 907, 869, 747, 701 cm^{-1}; MS (ESI) *m*/*z* 314.4.

[*S*-(*R,*R**)]-(7-Benzyloxycarbonylamino-3-{2-[2-(5-benzyloxycarbonylamino-1-carboxymethyl-pentylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-heptanoic acid) (68).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (1.31 g, 3.96 mmol), *N*-methylmorpholine (0.84 g (0.91 mL), 8.32 mmol) bisacid chloride (0.59 g, 1.72 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.21 g (1.34 mL), 8.32 mmol). Isolated yield: 74%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (m, 10H), 1.54 (m, 2H), 2.46 (m, 4H), 2.98 (m, 2H), 4.25 (m, 2H), 4.99 (s, 4H), 7.24–7.37 (m, 12H), 7.43 (t, 2H), 7.57 (d, 2H), 7.64 (d, 2H), 8.44 (d, 2H), 12.24 (s, 2H); IR (KBr) 3332, 3063, 2931, 2861, 1705, 1637, 1534, 1455, 1434, 1311, 1258, 1140, 1029, 746, 698 cm^{-1}; MS (ESI) *m*/*z* 429.4.

[*S*-(*R,*R**)]-(4-(2-{2-[1-(2-Carboxy-ethyl)-butylcarbamoyl]-phenyldisulfanyl}-benzoylamino)-heptanoic acid) (69).** The title compound was synthesized according to the general method A using corresponding γ-amino acid hydrochloride (0.89 g, 4.90 mmol), *N*-methylmorpholine (1.04 g (1.14 mL), 10.29 mmol), bisacid chloride (0.73 g, 2.13 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.49 g (1.65 mL), 10.29 mmol). Isolated yield: 59%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, 6H), 1.38 (m, 4H), 1.48 (m, 4H), 1.67 (m, 2H), 1.81 (m, 2H), 2.31 (t, 4H), 3.96 (m,2H), 7.31 (t, 2H), 7.42 (t, 2H), 7.57 (d, 2H), 7.67 (d, 2H), 8.31 (d, 2H), 12.05 (s, 2H); IR (KBr) 3277, 3172, 2957, 2932, 2871, 1708, 1632, 1536, 1450, 1433, 1317, 1259, 1181, 1092, 1037, 744, 699 cm^{-1}; MS (ESI) *m*/*z* 280.3.

[*S*-(*R,*R**)]-(4-(2-{2-[1-(2-Carboxy-ethyl)-pentylcarbamoyl]-phenyldisulfanyl}-benzoylamino)-octanoic acid) (70).** The title compound was synthesized according to the general method A using corresponding γ-amino acid hydrochloride (0.96 g, 4.90 mmol), *N*-methylmorpholine (1.04 g (1.14 mL), 10.29 mmol) bisacid chloride (0.73 g, 2.13 mmol), *N*-methyl-*N*-trimethylsilyl)acetamide (1.49 g (1.65 mL), 10.29 mmol). Isolated yield: 32%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (t, 6H), 1.33 (m, 8H), 1.50 (m, 4H), 1.67 (m, 2H), 1.79 (m, 2H), 2.31 (t, 4H), 3.94 (m, 2H), 7.30 (t, 2H), 7.42 (t, 2H), 7.57 (d, 2H), 7.65 (d, 2H), 8.31 (d, 2H), 12.05 (s, 2H); IR (KBr) 3281, 3064, 2956, 2930, 2839, 1706, 1631, 1587, 1537, 1450, 1434, 1316, 1288, 1258, 1173, 1091, 745, 698, 652 cm^{-1}; MS (ESI) *m*/*z* 294.4.

[*S*-(*R,*R**)]-(4-(2-{2-[1-(2-Carboxy-ethyl)-2-methyl-propylcarbamoyl]-phenyldisulfanyl}-benzoylamino)-5-methylhexanoic acid) (71).** The title compound was synthesized according to the general method A using corresponding γ-amino acid hydrochloride (1.68 g, 9.25 mmol), *N*-methylmorpholine (1.96 g (2.14 mL), 19.42 mmol), bisacid chloride (1.38 g, 4.02 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (2.82 g (3.12 mL), 19.42 mmol). Isolated yield: 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (t, 12H), 1.65 (m, 2H), 1.81 (m, 4H), 2.32 (m, 4H), 3.78 (m, 2H), 7.30 (t, 2H), 7.42 (t, 2H), 7.57 (d, 2H), 7.67 (d, 2H), 8.28 (d, 2H), 12.08 (s, 2H); IR (KBr) 3285, 3062, 2963, 2932, 2874, 1702, 1632, 1587, 1537, 1446, 1434, 1318, 1304, 1262, 742, 696 cm^{-1}; MS (ESI) *m*/*z* 280.4.

[*S*-(*R,*R**)]-(4-(2-{2-[1-(2-Carboxy-ethyl)-2-methyl-butylcarbamoyl]-phenyldisulfanyl}-benzoylamino)-5-methylheptanoic acid) (72).** The title compound was synthesized using general method A using corresponding γ-amino acid hydrochloride (1.40 g, 7.15 mmol), *N*-methylmorpholine (1.52 g (1.65 mL), 15.02 mmol), bisacid chloride (1.07 g, 3.11 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (2.18 g (2.42 mL), 15.02 mmol). Isolated yield: 77%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (s, 12H), 1.18 (m, 2H), 1.49 (m, 2H), 1.61 (m, 4H), 1.81 (m, 2H), 2.29 (m, 4H), 3.84 (m, 2H), 7.30 (t, 2H), 7.42 (t, 2H), 7.56 (d, 2H), 7.66 (d, 2H), 8.32 (d, 2H), 12.06 (s, 2H); IR (KBr) 3277, 3062, 2964, 2932, 2876, 1702, 1632, 1538, 1457, 1434, 1302, 746, 697 cm^{-1}; MS (ESI) *m*/*z* 294.4.

[*R*-(*R,*R**)]-(4-{2-[2-(3-Carboxy-1-cyclohexyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-4-cyclohexylbutyric acid) (73).** The title compound was synthesized according to the general method A using corresponding γ-amino acid hydrochloride (0.38 g, 1.71 mmol), *N*-methylmorpholine (0.36 g (0.40 mL), 3.60 mmol), bisacid chloride (0.26 g, 0.75 mmol), *N*-methyl-*N*-(trimethylsilyl)-acetamide (0.52 g (0.58 mL), 3.60 mmol). Isolated yield: 23%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.00–1.28 (m, 10H), 1.44 (m, 2H), 1.60–1.89 (m, 14H), 2.28 (m, 4H), 3.79 (m, 2H), 7.29 (t, 2H), 7.41 (t, 2H), 7.56 (d, 2H),

7.66 (d, 2H), 8.27 (d, 2H), 12.05 (s, 2H); IR (KBr) 3306, 3060, 2924, 2851, 1706, 1631, 1587, 1536, 1449, 1433, 1326, 1303, 1288, 1260, 955, 889, 744, 697 cm^{-1}; MS (CI) *m/z* 320.

[*R*-(*R,*R**)]-(4-{2-[2-(3-Carboxy-1-cyclohexylmethyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-5-cyclohexyl-pentanoic acid) (74).** The title compound was synthesized according to the general method A using corresponding γ-amino acid hydrochloride (1.42 g, 6.02 mmol), *N*-methylmorpholine (1.27 g (1.4 mL), 12.65 mmol), bisacid chloride (0.90 g, 2.62 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.84 g (2.03 mL), 12.65 mmol). Isolated yield: 83%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84 (m, 2H), 0.95 (m, 2H), 1.14 (m, 6H), 1.30 (m, 2H), 1.42 (m, 4H), 1.63 (m, 10H), 1.75 (m, 2H), 1.91 (m, 2H), 2.30 (t, 4H), 4.08 (m, 2H), 7.30 (t, 2H), 7.41 (t, 2H), 7.54 (d, 2H), 7.65 (d, 2H), 8.30 (d, 2H), 12.04 (s, 2H); IR (KBr) 3306, 3060, 2924, 2851, 1706, 1631, 1587, 1536, 1449, 1433, 1326, 1303, 1288, 1260, 955, 889, 744, 697 cm^{-1}; MS (ESI) *m/z* 334.6.

[*R*-(*R,*R**)]-(4-{2-[2-(1-Benzyl-3-carboxy-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-5-phenyl-pentanoic acid) (75).** The title compound was synthesized according to the general method A using corresponding γ-amino acid hydrochloride (0.60 g, 2.61 mmol), *N*-methylmorpholine (0.55 g (.60 mL), 5.49 mmol) bisacid chloride (0.39 g, 1.14 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (0.80 g (0.88 mL), 5.49 mmol). Isolated yield: 33%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.74 (m, 2H), 1.84 (m, 2H), 2.35 (m, 4H), 2.85 (d, 4H), 4.19 (m, 2H), 7.19 (m, 2H), 7.27 (m, 10H), 7.39 (t, 2H), 7.44 (d, 2H), 7.56 (d, 2H), 8.43 (d, 2H), 12.06 (s, 2H); IR (KBr) 3300, 3062, 3028, 2924, 1710, 1633, 1586, 1534, 1453, 1446, 1434, 1323, 1308, 1299, 1257, 1160, 1086, 1036, 944, 747, 699 cm^{-1}; MS (CI) *m/z* 328.4.

[*S*-(*R,*R**)]-4-(2-{2-[1-(2-Carboxy-ethyl)-3-methyl-butyl-carbamoyl]-phenyldisulfanyl}-benzoylamino)-6-methyl-heptanoic acid (76).** The title compound was synthesized according to the general method A using bisacid chloride (0.22 g, 0.64 mmol), *N*-methyl-*N*-(trimethylsilyl) acetamide (1.5 g, 10.24 mmol), corresponding amino acid (0.5 g, 2.561 mmol), *N*-methylmorpholine (1.04 g, 10.24 mmol) and 10 mL of dichloromethane. Isolated yield: 48%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (d, 6H), 0.92 (d, 6H), 1.26 (m, 2H), 1.5 (m, 2H), 1.58–1.86 (m, 8H), 2.31 (t, 4H), 4.06 (m, 2H), 7.29 (d, 2H), 7.42 (d, 2H), 7.55 (d, 2H), 7.66 (d, 2H), 8.32 (d, 2H), 12.07 (s, 2H); IR (KBr) 3276, 2956, 1711, 1629, 1538, 744 cm^{-1}; MS (ESI) *m/z* 282.

[*S*-(*R,*R**)]-(EE)-(4-{2-[2-(3-Carboxy-1-isopropyl-allyl-carbamoyl)-phenyldisulfanyl]benzoylamino}-5-methyl-hex-2-enoic acid) (77).** The title compound was synthesized according to the general method A using corresponding unsaturated γ-amino acid hydrochloride (0.58 g, 3.12 mmol), *N*-methylmorpholine (0.66 g (0.72 mL), 6.55 mmol), bisacid chloride (0.47 g, 1.36 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (0.95 g (1.05 mL), 6.55 mmol). Isolated yield: 28%; ^{1}H NMR (400 MHz, DMSO-d_6) (0.95 (m, 12H), 1.95 (m, 2H), 4.50 (m, 2H), 5.95 (d, 2H), 6.86 (dd, 2H), 7.33 (t, 2H), 7.46 (t, 2H), 7.62 (d, 2H), 7.65 (d, 2H), 8.73 (d, 2H), 12.36 (s, 2H); IR (KBr) 3415, 3384, 3314, 3298, 3060, 2963, 2928, 1706, 1640, 1587, 1529, 1461, 1316, 1257, 1178, 983, 747, 698 cm^{-1}; MS (ESI) *m/z* 278.4.

[*S*-(*R,*R**)]-(*EE*)-(4-{2-[2-(1-sec-Butyl-3-carboxy-allyl-carbamoyl)-phenyldisulfanyl]-benzoylamino}-5-methyl-hept-2-enoic acid) (78).** The title compound was synthesized according to the general method A using corresponding unsaturated γ-amino acid hydrochloride (0.96 g, 4.96 mmol), *N*-methylmorpholine (1.05 g (1.14 mL), 10.41 mmol), bisacid chloride (0.74 g, 2.16 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.51 g (1.67 mL), 10.41 mmol) to yield 0.22 g of beige solid. Isolated yield: 18%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (m, 12H), 1.21 (m, 2H), 1.51 (m, 2H), 1.75 (m, 2H), 4.57 (m, 2H), 5.95 (d, 2H), 6.85 (dd, 2H), 7.31 (t, 2H), 7.46 (t, 2H), 7.62 (d, 2H), 7.66 (d, 2H), 8.75 (d, 2H), 12.34 (s, 2H); IR (KBr) 3426, 3343, 3314, 3300, 3060, 3026, 2964, 2928, 2877, 1704, 1638, 1587, 1530, 1454, 1383, 1307, 1285, 1259, 1166, 982, 748, 698 cm^{-1}; MS (ESI) *m/z* 306.5.

[*S*-(*R,*R**)]-(*EE*)-(4-{2-[2-(3-Carboxy-1-isobutyl-allyl-carbamoyl)-phenyldisulfanyl]-benzoylamino}-6-methyl-hept-2-enoic acid (79).** The title compound was synthesized according to the general method A using corresponding β-amino acid hydrochloride (0.72 g, 4.29 mmol), *N*-methylmorpholine (0.91 g (0.99 mL), 9.02 mmol), bisacid chloride (0.64 g, 1.87 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.31 g (1.45 mL), 9.02 mmol). Isolated yield : 27%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (m, 12H), 1.44 (m, 2H) 1.58 (m, 2H), 1.69 (m, 2H), 4.72 (m, 2H), 5.89 (d, 2H), 6.80 (dd, 2H), 7.31 (t, 2H), 7.44 (t, 2H), 7.64 (d, 2H), 7.66 (d, 2H), 8.75 (d, 2H), 12.19 (s, 2H); IR (KBr) 3274, 2957, 1702, 1632, 1534, 1284, 745 cm^{-1}; MS (ESI) *m/z* 292, 324.

[*S*-(*R,*R**)]-2-(2-{2-[1-(1-Methoxycarbonyl-3-methyl-butyl-carbamoyl]-phenyldisulfanyl}-benzoylamino)-4-methyl-pentanoic acid methyl ester (80).** The title compound was synthesized according to the general method A using bisacid chloride (2.07 g, 6.04 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (7.0 g, 48.34 mmol), corresponding amino acid methyl ester hydrochloride (2.0 g, 12.08 mmol), *N*-methylmorpholine (4.89 g, 48.34 mmol) and 10 mL of dichloromethane. Isolated yield: 53%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (d, 6H), 0.94 (d, 6H), 1.58 (m, 2H), 1.76 (m, 4H), 3.66 (s, 6H), 4.54 (m, 2H),

7.33 (d, 2H), 7.47 (d, 2H), 7.66 (d, 2H), 7.69 (d, 2H), 8.97 (d, 2H); IR (KBr) 3320, 2956, 1744, 1637, 1532, 747 cm^{-1}; MS (ESI) *m*/*z* 280.

[*S*-(*R,*R**)]-2-(2-{2-[1-(1-*t*-Butoxycarbonyl-3-methyl-butyl-carbamoyl]-phenyldisulfanyl}-benzoylamino)-4-methyl-pentanoic acid *t*-butyl ester (81).** The title compound was synthesized according to the general method A using bisacid chloride (1.23 g, 3.59 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (2.6 g, 17.94 mmol), corresponding amino acid methyl ester hydrochloride (2.0 g, 8.97 mmol), *N*-methylmorpholine (1.81 g, 17.94 mmol) and 40 mL of dichloromethane. Isolated yield: 70%; ^1H NMR (400 MHz, DMSO-d_6) δ 0.92 (d, 6H), 0.94 (d, 6H), 1.44 (s, 18H), 1.55 (m, 2H), 1.75 (m, 4H), 4.36 (m, 2H), 7.33 (d, 2H), 7.47 (d, 2H), 7.64 (s, 2H), 7.65 (s, 2H), 8.87 (d, 2H); IR (KBr) 3314, 2958, 1729, 1636, 1530, 1156, 745 cm^{-1}; MS (ESI) *m*/*z* 266, 645.

[*S*-(*R,*R**)]-2-(2-{2-[1-(1-Allyloxycarbonyl-3-methyl-butyl-carbamoyl]-phenylsidusulfanyl}-benzoylamino)-4-methyl-pentanoic acid allyl ester (82).** The title compound was synthesized according to the general method A using bisacid chloride (0.8 g, 2.33 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.7 g, 11.66 mmol), corresponding amino acid allyl ester p-toluenesulfonate (2.0 g, 5.83 mmol), *N*-methylmorpholine (1.18 g, 11.66 mmol) and 40 mL of dichloromethane. Isolated yield: 70%; ^1H NMR (400 MHz, DMSO-d_6) δ 0.89 (d, 6H), 0.94 (d, 6H), 1.61 (m, 2H), 1.76 (m, 4H), 4.37 (m, 2H), 4.61 (m, 4H), 5.22 (dd, 2H), 5.33 (dd, 2H), 5.94 (m, 2H), 7.33 (t, 2H), 7.47 (t, 2H), 7.64 (d, 2H), 7.68 (d, 2H), 9.0 (d, 2H); IR (KBr) 3309, 2957, 1742, 1635, 1531, 745 cm^{-1}; MS (ESI) *m*/*z* 306, 613.

[*S*-(*R,*R**)]-2-(2-{2-[1-(1-Benzloxycarbonyl-3-methyl-butyl-carbamoyl]-phenyldisulfanyl}-benzoylamino)-4-methyl-pentanoic acid benzyl ester (83).** The title compound was synthesized according to the general method A using bisacid chloride (0.696 g, 2.03 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.46 g, 10.16 mmol), corresponding amino acid benzyl ester *p*-toluenesulfonate (2.0 g, 5.08 mmol), *N*-methylmorpholine (1.03 g, 10.16 mmol) and 40 mL of dichloromethane. Isolated yield: 60%; ^1H NMR (400 MHz, DMSO-d_6) δ 0.84 (d, 6H), 0.89 (d, 6H), 1.54 (m, 2H), 1.72 (m, 4H), 4.5 (m, 2H), 5.12 (s, 4H), 7.31 (m, 12H), 7.39 (t, 2H), 7.58 (d, 2H), 7.61 (d, 2H), 8.97 (d, 2H); IR (KBr) 3298, 2956, 1741, 1634, 1531, 745 cm^{-1} MS (ESI) *m*/*z* 356, 713.

[*S*-(*R,*R**)]-(2-{2-[2-(1-*t*-Butoxycarbonyl-2-methyl-butyl-carbamoyl)-phenyldisulfanyl]-benzoylamino}-3-methyl-pentanoic acid *t*-butyl ester) (84).** This compound was prepared according to the general method B using isoleucine *t*-butyl ester (48.0 g, 256.37 mmol), bisacid chloride (40.0 g, 116.53 mmol), *N*-methylmorpholine (27.23 g (29.6 mL), 269.19 mmol) to yield 62.9 g (83.7%) of white solid (diastereomeric mixture). ^1H NMR (400 MHz, DMSO-d_6) δ 0.89 (t, 6H), 0.96 (m, 6H), 1.21–1.38 (m, 2H), 1.44 (s, 18H), 1.40–1.61 (m, 2H), 1.85–2.03 (m, 2H), 4.24 and 4.42 (t and dd, 2H), 7.32 (t, 2H), 7.45 (t, 2H), 7.64 (m, 4H), 8.70 and 8.77 (d and d, 2H); IR (KBr) 3322, 3060, 2969, 2933, 2876, 1729, 1647, 1587, 1523, 1431, 1368, 1351, 1301, 1282, 1155, 1038, 847, 745 cm^{-1}; MS (APCI) *m*/*z* 266.3.

(+,−)2-(2-{2-[(Bismethoxycarbopnyl-methyl)-carbamoyl]-phenyldisulfanyl}-benzoylamino)malonic acid dimethyl ester (85). The title compound was synthesized according to the general method A using bisacid chloride (1.03 g, 3.09 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.8 g, 12.39 mmol), corresponding amine (2.25 g, 12.39 mmol), *N*-methylmorpholine (1.25 g, 12.39 mmol) and 25 mL of dichloromethane. Isolated yield: 45%; ^1H NMR (400 MHz, DMSO-d_6) δ 3.75 (s, 12H), 7.36 (t, 2H), 7.42 (d, 2H), 7.5 (t, 2H), 7.97 (d, 2H), 9.31 (s, 2H); IR (KBr) 3164, 3061, 2890, 1749, 1683, 1445, 1278, 743 cm^{-1}; MS (ESI) *m*/*z* 282.3, 563.4.

[*S*-(*R,*R**)])(1*H*-Pyrrolidine-2-carboxylic acid, 1,1(-[dithiobis(2,1-phenylenecarbonyl)]bis,bis(1,1-dimethylethyl) ester (86).** This compound was prepared according to the general method B using bisacid chloride (1.0 g, 3 mmol) in 15 mL of dichloromethane and proline-*t*-butyl ester (1.08 g, 6 mmol), *N*-methylmorpholine (0.99 mL, 9 mmol) in 10 mL of dichloromethane. The compound was purified by chromatography (SiO_2, $CHCl_3$/MeOH, 98/2) to afford 1.59 g of the title compound as a solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.70 (m, 2H), 7.33 (m, 4H), 7.24 (m, 2H), 4.57 (m, 2H), 3.83–3.31 (m, 4H), 2.99 (m, 2H), 1.99 (m, 6H), 1.51 (s, 18H); IR (KBr) 2976, 1736, 1637, 1414, 1152 cm^{-1}; MS (APCI) *m*/*z* 613.

[*S*-(*R,*R**)]-Benzamide, 2,2(-dithiobis[*N*-[1-hydroxymethyl)-3-methylbutyl] (87).** The title compound was synthesized according to the general method A using corresponding amino alcohol (1.17 g, 10.8 mmol), *N*-methylmorpholine (0.83 g (0.91 mL), 8.24 mmol) bisacid chloride (1.59 g, 4.3 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (3.2 mL, 19.9 mmol). Isolated yield 29%; ^1H NMR (300 MHz, DMSO-d_6) δ 0.91 (d, 12H), 1.41 (m, 4H), 1.71 (m, 2H), 3.40 (m, 4H), 4.73 (t, 2H), 7.29 (d, 2H), 7.39 (t, 2H), 7.62 (m, 4H), 8.23 (d, 2H); MS (ESI) *m*/*z* 252.

[*R*-(*R,*R**)]-Benzamide, 2,2′-dithiobis[*N*-(2-hydroxy-1-phenyl-ethyl) (88).** The title compound was synthesized using general method A using bisacid chloride (1.0 g, 2.94 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (3.4 mL, 11.76 mmol), corresponding amino alcohol (1.0 g, 4.0 mmol) and 70 mL of dichloromethane. Isolated yield: 74%; ^1H NMR (300 MHz, DMSO-d_6) δ 3.33

(m, 2H), 5.05 (m, 2H), 7.36 (m, 14H), 7.62 (d, 2H), 7.79 (d, 2H), 8.94 (d, 2H); MS (ESI) *m/z* 274.

[*S*-(*R,*R**)]-Benzamide, 2,2′-dithiobis[*N*-hydroxy-1-(1-methylethyl)-3-pentenyl] (89).** The title compound was synthesized according to the general method A using bisacid chloride (2.74 g, 8.0 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (4.65 g, 32 mmol), corresponding amino alcohol (0.78 g, 4.0 mmol), *N*-methylmorpholine (3.20 g, 32 mmol) and 40 mL of dichloromethane. Isolated yield: 41%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (d, 6H), 0.93 (d, 6H), 1.36 (m, 2H), 1.54 (m, 2H), 1.69 (m, 2H), 3.92 (m, 4H), 4.58 (m, 2H), 4.69 (t, 2H), 5.66 (m, 4H), 7.31 (t, 2H), 7.44 (t, 2H), 7.61 (d, 2H), 7.66 (d, 2H), 8.55 (d, 2H); IR (KBr) 3314, 2955, 1634, 1532, 746 cm^{-1}; MS (ESI) *m/z* 278, 557.

6-Amino-2-{2-[2-(5-amino-1-carboxy-pentylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-hexanoic acid methyl ester (90). A solution of **96** (1.54 g, 1.75 mmol) in 30 mL of dichloromethane was treated with HCl gas at 0 °C. After 18 h the solution was concentrated to a solid and dissolved in methanol. HCl gas was bubbled in at 0 °C and it was stirred for 3 h. The solution was concentrated to afford 1.38 g of **90** as a foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.02 (d, 2H), 7.91 (bs, 4H), 7.76 (dd, 2H), 7.64 (d, 2H), 7.49 (t, 2H), 7.34 (t, 2H), 4.42 (m, 2H), 3.68 (s, 6H), 2.77 (m, 4H), 1.81 (m, 2H), 1.61 (m, 4H), 1.45 (m, 4H); IR (KBr) 2950, 1739, 1638, 1529, 1163, 745 cm^{-1}; MS (ESI) *m/z* 591.

[*S*-(*R,*R**)]-7-Amino-3-{2-[2-(5-amino-1-methoxycarbonyl-methyl-pentylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-heptanoic acid methyl ester (91).** Synthesized from **95** (1.0 g, 1.13 mmol) by stirring in 33% HBr/AcOH (20 mL) for 1 h at room temperature. Diethyl ether was then added to cause a solid to precipitate. The precipitate was filtered off and washed several times with diethyl ether followed by several washes with ethyl acetate to yield a beige solid. Isolated yield: 76.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (m, 4H), 1.56 (m, 8H), 2.59 (d, 4H), 2.78 (m, 4H), 3.60 (s, 6H), 4.30 (m, 2H), 7.32 (t, 2H), 7.45 (t, 2H), 7.62 (d, 2H), 7.69 (brs, 6H), 8.52 (d, 2H); IR (KBr) 3229, 2947, 2867, 1733, 1634, 1539, 1457, 1436, 1314, 1161, 1036, 989, 867, 747 cm^{-1}; MS (ESI) *m/z* 619.4.

[*S*-(*R,*R**)]-(*N*,*N*′-[Dithiobis(2,1-phenylenecarbonyl)bis-L-threonine-bis-dimethyl ester) (92).** This compound was prepared according to the general method B using bisacid chloride (2.01 g, 5.9 mmol) in 35 mL of dichloromethane and threonine methyl ester hydrochloride (2.0 g, 11.8 mmol), *N*-methylmorpholine (2.72 mL, 24.8 mmol) in 40 mL of dichloromethane. The organic layer was washed with 1 N HCl and **92** precipitated from the organic layer. The solid was dried to give 2.15 g. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (d, 2H), 7.70 (dd, 2H), 7.61 (dd, 2H), 7.44 (t, 2H), 7.29 (t, 2H), 4.90 (d, 2H), 4.48 (m, 2H), 4.16 (m, 2H), 3.63 (s, 6H), 1.13 (d, 6H); IR (KBr) 3315, 1742, 1625, 1539, 1086 cm^{-1}; MS (APCI) *m/z* 537.

[*S*-(*R,*R**)]-(*N*,*N*′-[Dithiobis(2,1-phenylenecarbonyl)bis-L-serine-bis-dimethyl ester) (93).** This compound was prepared according to the general method B using bisacid chloride (1.50 g, 4.4 mmol) in 30 mL of dichloromethane and serine methyl ester hydrochloride (1.37 g, 8.8 mmol), *N*-methylmorpholine (1.93 mL, 17.6 mmol) in 40 mL of dichloromethane. The organic layer was washed with 1 N HCl, saturated bicarbonate and brine. The solution was concentrated to give 1.5 g of a foam. The compound was purified by chromatography (SiO_2, $CHCl_3$/MeOH, 95/5) to afford 0.98 g of a solid. The sample was recrystallized from hot $CHCl_3$ to give 0.87 g of **93** as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (d, 2H), 7.71 (d, 2H), 7.60 (d, 2H), 7.44 (t, 2H), 7.28 (t, 2H), 5.03 (t, 2H), 4.48 (m, 2H), 3.75 (t, 4H), 3.62 (s, 6H); IR (KBr) 3434, 3331, 1750, 1738, 1644, 1544, 748 cm^{-1}; MS (APCI) *m/z* 509.

[*S*-(*R,*R**)]-(5-Cyano-4-{2-[2-(1-cyanomethyl-3-methoxy-carbonyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-pentanoic acid methyl ester) (94).** This compound was prepared according to the general method A using bisacid chloride (0.18 g, .52 mmol) in 35 mL of dichloromethane and threonine methyl ester hydrochloride (2.0 g, 11.8 mmol), *N*-methylmorpholine (0.13 g (0.14 mL), 1.30 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (0.36 g (0.40 mL), 2.49 mmol) in 40 mL of dichloromethane. Isolated yield: 19.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.88 (m, 4H), 2.46 (m, 4H), 2.83 (m, 4H), 3.58 (s, 6H), 4.21 (m, 2H), 7.33 (t, 2H), 7.43 (t, 2H), 7.62 (d, 2H), 7.68 (d, 2H), 8.78 (d, 2H); IR (KBr) 3326, 3316, 3059, 2952, 2851, 2250, 1734, 1641, 1586, 1532, 1436, 1355, 1307, 1261, 1206, 1172, 1091, 1038, 985, 872, 789, 749, 699 cm^{-1}; MS (APCI) *m/z* 291.

[*S*-(*R,*R**)]-(7-Benzyloxycarbonylamino-3-{2-[2-(5-benzyl-oxycarbonylamino-1-methoxycarbonylmethyl-pentylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-heptanoic acid methyl ester) (95).** The title compound was synthesized according to the general method B using β-amino acid (3.33 g, 9.66 mmol), bisacid chloride (1.44 g, 4.20 mmol), *N*-methylmorpholine (2.05 g (2.23 mL), 20.28 mmol) to yield 2.75 g of beige solid. Isolated yield: 74%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23–1.50 (m, 8H), 1.54 (m, 2H), 2.56 (d, 4H), 2.98 (m, 4H), 3.56 (s, 6H), 4.27 (m, 2H), 4.99 (s, 4H), 7.24–7.37 (m, 12H), 7.43 (t, 2H), 7.56 (d, 2H), 7.64 (d, 2H), 8.48 (d, 2H); IR (KBr) 3325, 3279, 3062, 2944, 2860, 1733, 1690, 1633, 1586, 1537, 1456, 1435, 1313, 1262, 1202, 1158, 1137, 1034, 993, 876, 742, 697, 652 cm^{-1}; MS (ESI) *m/z* 443.5.

[*S*-(*R,*R**)]-6-*t*-Butoxycarbonylamino-2-{2-[2-(1-*t*-butoxycarbonyl)-5-*t*-butoxycarbonylamino-pentylcarbamoyl)-phenylcarbamoyl)phenydisulfanyl]-benzoylamino}-hexanoic acid *t*-butyl ester (96).** This compound was prepared according to the general method B using bisacid chloride (1.0 g, 3.0 mmol) in 15 mL of dichloromethane and γ-*N*-Boc-lysine-*t*-butyl ester hydrochloride (2.24 g, 6.6 mmol), *N*-methylmorpholine (1.65 mL, 15 mmol) in 20 mL of dichloromethane. The compound was purified by chromatography (SiO_2, $CHCl_3$/MeOH, 95/5) to afford 2.25 g of **96** as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, 2H), 7.52 (d, 2H), 7.32 (t, 2H), 7.18 (t, 2H), 6.73 (bs, 2H), 4.63 (m, 4H), 3.07 (m, 4H), 1.93 (m, 2H), 1.76 (m, 2H), 1.45 (s, 18H), 1.55–1.30 (m, 8H), 1.34 (s, 18H); IR (KBr) 3351, 2976, 1714, 1694, 1648, 1525, 1159 cm^{-1}, MS (APCI) *m*/*z* 876.

[*S*-(*R,*R**)]Benzamide, 2,2′-dithiobis[*N*-[1-(aminocabonyl)-3-methylbutyl] (97).** The title compound was synthesized according to the general method A using bisacid chloride (2.5 g, 7.3 mmol), *N*-methyl-*N*-(trimethylsilyl)-acetamide (8.49 g, 58.44 mmol), L-leucine amide (2.2 g, 14.61 mmol), *N*-methylmorpholine (5.91 g, 58.44 mmol) and 40 mL of dichloromethane. Isolated yield: 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (d, 6H), 0.94 (d, 6H), 1.72 (m, 2H), 1.55 (m, 4H), 4.47 (m, 2H), 7.06 (brs, 2H), 7.31 (t, 2H), 7.44 (t, 2H), 7.47 (brs, 2H), 7.64 (d, 2H), 7.75 (d, 2H), 8.64 (d, 2H); IR (KBr) 3373, 3281, 2956, 1670, 1633, 1530, 743 cm^{-1}; MS (ESI) *m*/*z* 531.

Benzamide, 2,2′-dithiobis[*N*-(3-amino-3-oxopropyl) (98). This compound was prepared according to the general method A using bisacid chloride (2.74 g, 8 mmol) in 40 mL of dichloromethane and β-alanine amide (2.0 g, 16 mmol) and *N*-methyl-*N*-(trimethylsilyl)acetamide (7.7 mL, 48 mmol) in 40 mL of dichloromethane. After 18 h the mixture was concentrated and triturated with 1 N HCl. Acetonitrile was added and the precipitate was filtered and washed with diethyl ether. The solid was dried to afford 0.83 g of **98**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.67 (t, 2H), 7.60 (d, 4H), 7.43 (m, 4H), 7.27 (t, 2H), 6.86 (brs, 2H), 2.48 (m, 4H), 2.36 (m, 4H); IR (KBr) 3268, 1663, 1633, 737 cm^{-1}; MS (APCI) *m*/*z* 447.

[*S*-(*R,*R**)]-2-{2-[2-(1-*t*-Butoxycarbamoyl-carbamoyl)-phenyldisulfanyl]benzoylamino}-succinamic acid *t*-butyl ester (99).** This compound was prepared according to the general method B using bisacid chloride (1.0 g, 3.0 mmol) in 15 mL of dichloromethane and arginine-*t*-butyl ester hydrochloride (1.48 g, 6.6 mmol), *N*-methylmorpholine (1.65 mL, 15 mmol) in 20 mL of dichloromethane. A precipitate formed which was collected and washed with ether. The solid was treated with boiling isopropanol and filtered hot to yield 1.15 g of the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.91 (d, 2H), 7.63 (d, 4H), 7.46 (m, 4H), 7.31 (t, 2H), 6.98 (bs, 2H), 4.60 (m, 2H), 2.70 (m, 2H), 2.57 (m, 2H), 1.41 (s, 18H); IR (KBr) 3327, 1731, 1669, 1639, 1539, 1154 cm^{-1}; MS (APCI) *m*/*z* 646.

[*S*-(*R,*R**)]-2-{2-[2-(1-*t*-Butoxycarboyl-3-carbamoyl-propylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-4-carbamoyl-butyric acid *t*-butyl ester (100).** This compound was prepared according to the general method B using bisacid chloride (1.5 g, 4.5 mmol) in 25 mL of dichloromethane and glutamine-*t*-butyl ester hydrochloride (2.36 g, 9.9 mmol), *N*-methylmorpholine (2.47 mL, 22.5 mmol) in 30 mL of dichloromethane. A precipitate formed which was collected and washed with ether. The solid was recrystallized from ethanol to afford 1.21 g of **100** as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.95 (d, 2H), 7.71 (d, 2H), 7.64 (d, 2H), 7.47 (t, 2H), 7.35 (bs, 2H), 7.33 (t, 2H), 6.85 (brs, 2H), 4.25 (m, 2H), 2.25 (t, 4H), 2.03 (m, 2H), 1.93 (m, 2H), 1.43 (s, 18H); IR (KBr) 3398, 1731, 1651, 1156, 744 cm^{-1}; MS (APCI) *m*/*z* 675.

[*S*-(*R,*R**)]2,2-Dithiobis[*N*-[1-(methoxymethylamino)-carbonyl]-3-methylbutyl]benzamide] (101).** The title compound was synthesized according to the general method A using bisacid chloride (1.59 g, 4.64 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (2.7 g, 18.56 mmol), corresponding amino acid amide hydrochloric acid salt (0.49 g, 2.32 mmol), *N*-methylmorpholine (1.88 g, 18.56 mmol) and 40 mL of dichloromethane. Isolated yield: 30%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (d, 6H), 0.94 (d, 6H), 1.42 (m, 2H), 1.75 (m, 4H), 3.14 (s, 6H) 3.81 (s, 6H), 4.97 (m, 2H), 7.31 (t, 2H), 7.44 (t, 2H), 7.64 (d, 2H), 7.70 (d, 2H), 8.81 (d, 2H); IR (KBr) 3324, 2957, 1638, 1531, 746 cm^{-1}; MS (ESI) *m*/*z* 309, 619.

2-{2-[2-(Dicarbamoylmethyl-carbamoyl)-phenyldisulfanyl]-benzoylamino}-malonamide (102). The title compound was synthesized using general method A using bisacid chloride (2.93 g, 8.55 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (4.96 g, 34.2 mmol), corresponding amine (2.0 g, 17.09 mmol), *N*-methylmorpholine (3.46 g, 34.2 mmol) and 25 mL of dichloromethane. Isolated yield: 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33 (t, 2H), 7.39 (d, 2H), 7.5 (t, 2H), 7.72 (s, 4H), 7.89 (s, 2H), 7.94 (d, 2H), 8.17 (s, 2H); IR (KBr) 3342, 3177, 1715, 1644, 1448, 743 cm^{-1}; MS (ESI) *m*/*z* 208, 252.

[*S*-(*R,*R**)] 2-{2-[2-(1,2-Dicarbamoyl-ethylcarbamoyl)-phenyldisulfanyl]-benzoylamino}-succinamide (103).** This compound was prepared according to the general method B using bisacid chloride (0.82 g, 2.39 mmol), hydrochloric acid salt of asparagine amide (1.0 g, 5.97 mmol), *N*-methylmorpholine (1.21 g, 11.94 mmol), 1.30 mmol), *N*-methyl-*N*-(trimethylsilyl)acetamide (1.74 g, 11.94 mmol) in 40 mL of dichloromethane. Isolated yield: 42%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.58

(m, 4H), 4.69 (m, 2H), 6.94 (brs, 2H), 7.14 (brs, 2H), 7.31 (t, 2H), 7.39 (s, 4H), 7.48 (t, 2H), 7.66 (d, 2H), 7.77 (d, 2H), 8.69 (d, 2H); IR (KBr) 3395, 3312, 3203, 1663, 1530, 1432, 1332, 745 cm^{-1}; MS (APCI) m/z 266.4.

Zinc ejection by electrospray ionization mass spectrometry (ESI-MS). Positive ion ESI-MS analyses were performed with a Finnigan MAT 900Q forward geometry hybrid mass spectrometer (Bremen, Germany) equipped with a position-and-time-resolved-ion-counting (PATRIC) focal-plane array detector after the magnet and before quadrupole analyzer.[33] Protein solutions for ESI-MS measurements were prepared at a concentration of 10–30 μM in 10 mM ammonium acetate (pH 6.9) with two times the protein molar concentration of zinc ($ZnCl_2$) in water. Stock solutions of the disulfide compounds were prepared at a concentration of ca. 5–10 mM in acetonitrile/methanol/THF mixtures. Sample solutions were infused through the ESI source at a flow rate of 1 μL min^{-1}.

Acknowledgements

All the EC_{50} and TC_{50} measurements were performed at Southern Research Institute, Birmingham, AL. All spectra reported were recorded at the Parke-Davis Analytical Research Section facility. The authors thank all those who participated in anti-HIV activity testing and measurement of the analytical data. We also thank Ms Susan Hagen, Dr. James Kaltenbronn and Dr. Alex Bridges for proof reading as well their suggestions.

References

1. Romero, D. L. *Ann. Rep. Med. Chem.* **1994**, *29*, 123; and references therein.

2. (a) Moyle, G.; Gazzard, B. *Drugs* **1996**, *51*, 701; and references therein. (b) Kempf, D. J.; Sham, H. L. *Curr. Pharmaceut. Design* **1996**, *2*, 225.

3. Connolly, K. J.; Hammer, S. M. *Antimicrob. Agents Chemother.* **1995**, *38*, 2491.

4. Mascolini, M. *J. Int. Assoc. Physicians AIDS Care* **1996**, 22.

5. DeClercq, E. *J. Med. Chem.* **1995**, *38*, 2491.

6. Rice, W. G.; Supko, J. G.; Malspeis, L.; Buckheit, R. W.; Clanton, D.; Bu, M.; Graham, L.; Schaeffer, C. A.; Turpin, J. A.; Domagala, J. M.; Gogliotti, R.; Bader, J. P.; Halliday, S. M.; Coren, L.; Sowder, R. C.; Author, L. O.; Henderson, L. E. *Science* **1995**, *270*, 1194.

7. (a) Domagala, J. M.; Bader, J. P.; Gogliotti, R. D.; Sanchez, J. P.; Stier, M. A.; Song, Y.; Vara Prasad, J. V. N.; Tummino, P. J.; Scholten, J.; Harvey, P.; Holler, T.; Gracheck, S.; Hupe, D.; Rice, W. G.; Schultz, R. *Bioorg. Med. Chem.* **1997**, *5*, 569. (b) Domagala, J. M.; Gogliotti, R.; Sanchez, J. P.; Stier, M. A.; Musa, K.; Song, Y.; Loo, J.; Reily, M.; Tummino, P.; Harvey, P.; Hupe, D.; Sharmeen, L.; Mack, D.; Scholten, J.; Saunders, J.; McQuade, T. *Drug Design Discov.* **1997**, *15*, 49.

8. Rice, W. G.; Turpin, J. A.; Schaeffer, C. A.; Graham, L.; Clanon, D.; Buckheit, Jr., R. W.; Zaharevitz, D.; Summers, M. F.; Wallqvist, A.; Covell, D. G. *J. Med. Chem.* **1996**, *39*, 3606.

9. Witvrouw, M.; Balzarini, J.; Pannecouque, C.; Jhaumeer-Laulloo, S.; Este, J. A.; Schols, D.; Chereanov, P.; Schmit, J.-C.; Debyser, Z.; Vandamme, A.-M.; Desmyter, J.; Ramadas, S. R.; De Clercq, E. *Antimicrob. Agents Chemother.* **1997**, *41*, 262.

10. Vandevelde, M.; Wityrouw, M.; Schmit, J. C.; Sprecher, S.; De Clercq, E.; Tassignon, J.-P. *AIDS Res. Hum. Retro.* **1996**, *12*, 567.

11. Rice, W. G.; Turpin, J. A.; Huang, M.; Clanton, D.; Buckheit, R. W.; Covell, D. G.; Wallqvist, A.; McDonnell, N. B.; DeGuzman, R. N.; Summers, M. F.; Zalkow, L.; Bader, J. P.; Haugwitz, R. D.; Sausville, E. A. *Nature Medicine* **1997**, *3*, 341.

12. Okachi, R.; Niino, H.; Kitaura, K.; Mineura, K.; Nakamizo, Y.; Murayama, Y.; Ono, T.; Nakamizo, A. *J. Med. Chem.* **1985**, *28*, 1772.

13. Pluncinska, K.; Liberek, B. *Tetrahedron* **1987**, *43*, 3509.

14. Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6568; and references therein.

15. Fehrentz, J.-A.; Castro, B. *Synthesis* **1983**, 676.

16. Buckheit, Jr., R. W.; Hollingshead, M. G.; Germany Decker, J.; White, E. L.; McMahon, J. B.; Allen, L. B.; Ross, L. R.; Decker, D.; Westbrook, L.; Shannon, W. M.; Weislow, O.; Bader, J. P.; Boyd, M. R. *Antiviral Res.* **1993**, *21*, 247.

17. Loo, J. A.; Holler, T. P.; Sanchez, J.; Gogliotti, R. ; Maloney, L.; Reily, M. D. *J. Med. Chem.* **1996**, *39*, 4313.

18. Henderson, L. E.; Copeland, T. D.; Sowder, R. C.; Smythers, G. W.; Oroszlan, S. *J. Biol. Chem.* **1981**, *256*, 8400.

19. South, T. L.; Blake, P. R.; Sowder, R. C.; Arthur, L. O.; Henderson, L. E.; Summers, M. F. *Biochemistry* **1990**, *29*, 7786.

20. (a) Darlix, J.-L.; Lapadat-Tapolsky, M.; De Rocquigny, H.; Roques, B. P. *J. Mol. Biol.* **1995**, *254*, 523. (b) Rice, W. G.; Turpin, J. A. *Med. Virol.* **1996**, *6*, 187. (c) Poon, D. T. K.; Aldovini, A. *J. Virol.* **1996**, *70*, 6607.

21. Aldovini, A.; Young, R. A. *J. Virol.* **1990**, *64*, 1920–1926.

22. Gorelick, R. J.; Henderson, L. E.; Hanser, J. P.; Rein, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *85*, 8420.

23. Darlix, J.-L.; Vincent, A.; Gabus, C.; De Rocquigny, H.; Roques, B. P. *C. R. Acad. Sci. III* **1993**, *316*, 763.

24. Mely, Y.; Cornille, F.; Foueniw-Zaluski, M. C.; Darlix, J. L.; Roques, B. P.; Gerard, D. *Biopolymers* **1991**, *31*, 899.

25. (a) Summers, M. F.; Henderson, L. E.; Chance, M. R.; Bess, J. W. Jr.; South, T. L.; Blake, P. R.; Sagi, I.; Perez-Alvarado, G.; Sowder, R. C., III; Hare, D. R.; Arthur, L. O. *Pro. Sci.* **1992**, *8*, 586. (b) De Guzman, R. N.; Wu, Z. R.; Stalling, C. C.; Pappalardo, L.; Borer, P. N.; Summers, M. F. *Science* **1998**, *279*, 384.

26. Tummino, P. J.; Scholten, J. D.; Harvey, P. J.; Holler, T. P.; Maloney, L.; Gogliotti, R.; Domagala, J. M.; Hupe, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 969.

27. Turpin, J. A.; Terpening, S. J.; Schaeffer, C. A.; Yu, G.; Glover, C. J.; Felsted, R. L.; Sausville, E. A.; Rice, W. G. *J. Virol.* **1996**, *70*, 6180.

28. Tummino, P. J.; Harvey, P. J.; McQuade, T.; Domagala, J.; Gogliotti, R.; Sanchez, J.; Song, Y.; Hupe, D. *Antimicrob. Agents Chemother.* **1997**, *41*, 394.

29. Turpin, J. A.; Schaeffer, C. A.; Terpening, S. J.; Graham, L.; Bu, M.; Rice, W. G. *Antiviral Chem. Chemother.* **1997**, *8*, 60.

30. Hathout, Y.; Fabris, D.; Han, M. S.; Sowder, R. C.; Henderson, L. E.; Fenselau, C. *Drug Metab. Dispos.* **1996**, *24*, 1395.

31. Narayan, V. A.; Kriwack, R. W.; Caradonna, J. P. *J. Biol. Chem.* **1997**, *272*, 7801.

32. Desjarlais, J. R.; Berg, J. M. *Proteins: Struct., Funct. Gene.* **1992**, *12*, 101.

33. Loo, J. A.; Pesch, R. *Anal. Chem.* **1994**, *66*, 3659.