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Synthesis, Resolution, and Determination of the Absolute Configuration of the Enantiomers of *cis*-4,5-dihydroxy-1,2-dithiane 1,1-dioxide, an HIV-1 NCp7 Inhibitor

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Abstract—The anti-HIV activity of (±)-*cis*-4,5-dihydroxy-1,2-dithiane 1,1-dioxide [(±)-*cis*-1,1-dioxo-[1,2]-dithiane-4,5-diol, NSC-624151] and its attack on the zinc finger domain of the HIV-1 nucleocapsid p7 (NCp7) protein has been established [Rice, W. G.; Baker, D. C.; Schaeffer, C. A.; Graham, L.; Bu, M.; Terpening, S.; Clanton, D.; Schultz, R.; Bader, J. P.; Buckheit, R. W.; Field, L.; Singh, P. K. Turpin, J. A. *Antimicrob. Agents Chemother*. **1997**, *41*, 419]. In order to determine which enantiomer of NSC-624151 is the more active component, the compound was resolved via its bis-'Mosher ester', which was prepared via its reaction with two equiv of (−)-(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. The diastereoisomeric esters were separated, and each ester was hydrolyzed to yield enantiomers with $[\alpha]_D^{21}$ +151° (*c* 0.5, MeOH) and $[\alpha]_D^{21}$ −146° (*c* 0.5, MeOH). Single-crystal X-ray analysis of the (−)-bis-'Mosher ester' showed that the (−)-enantiomer is the (4*S*, 5*R*)-compound. The (−)-enantiomer (NSC 693195) was ca. twice as active (EC_{50} 8.8±0.2 μM) as its (+)-counterpart (NSC 693194) (EC_{50} 16.2±2.4 μM) in the XTT assay against HIV-1. All three compounds were found to be approximately equally effective in promoting Zn ejection from the NCp7 zinc finger. As the more anti-HIV active enantiomer is only slightly more active than the racemic form, it appears to offer no advantages over the racemic form.

Introduction

It is well established that the human immunodeficiency virus type 1 (HIV-1) has an extreme tendency to mutate to forms that are resistant to existing antiviral therapies.[1] The most common approach to combating the rapid emergence of drug-resistant HIV is to treat infected individuals with a cocktail of drugs that simultaneously disrupt virus replication through multiple modalities.[2–4] Such cocktails include the combination of drugs that target the viral reverse transcriptase or protease,[5–7] and drugs are under development that target the viral integrase[8–11] or the viral gp120 glycoprotein.[12] Because these drugs ultimately suffer from cross-resistance within drug classes and the development of resistance to new drug entities,[4,13] it is highly desirable to create new drug classes that target atypical viral structures, especially those that are highly conserved, and, hence, resistant to mutation.

For some time it has been recognized that in all lenti- and oncoretroviruses, the nucleocapsid (NC) proteins contain one or two highly conserved Cys-Xaa_2-Cys-Xaa_4-His-Xaa_4-Cys (CCHC, where Xaa is any amino acid) peptide segments[14,15] that are coordinated to zinc and are known as retroviral zinc fingers.[16,17] The spacing and the zinc-chelating residues (three Cys and one His) of the zinc finger motif are absolutely conserved, and mutations in the metal-chelating residues lead to a noninfectious virus.[18–20] The zinc finger domains within the $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ precursor polyproteins are

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involved in a number of essential viral processes including reverse transcription,[21,22] integration,[23] protease processing,[24] and packaging of viral RNA.[18] The mature NCp7 protein results from the processing of these precursor polyproteins,[25] and the zinc fingers of this protein are needed to initiate further infection.[21,26–28] Hence, compounds that attack the retroviral zinc finger may function as inhibitors in more than one step of the HIV-1 replication cycle and may provide an improved approach to anti-HIV chemotherapy.[29–31]

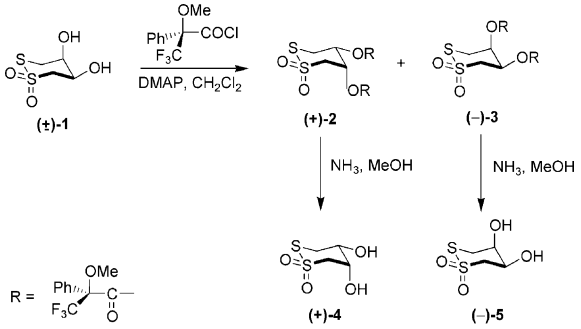
It has been shown that within the HIV-1 nucleocapsid p7 protein, the sulfur atoms of the Cys residues that are coordinated to zinc can be covalently modified through electrophilic attack by various functional groups.[32] Among active compounds are aromatic C-nitroso compounds,[33,34] 2,2′-dithiobisbenzamides and derivatives,[35,36] cyclic 2,2′-dithiobisbenzamides,[37,38] pyridinioalkanoyl esters and thioesters,[39–41] and thiolcarbamates.[42]

The NCI's XTT-based cytoprotection assay for anti-HIV compounds[43] had identified *cis*- and *trans*-4,5-dihydroxy-1,2-dithiane 1,1-dioxide[44] as inhibitors of HIV-1 replication. Our extension of this research was to determine which enantiomer of the more potent cis compound (±**1**) was responsible for the anti-HIV activity through interaction with NCp7.

Results and Discussion

Resolution of (±)-*cis*-4,5-dihydroxy-1,2-dithiane 1,1-dioxide [(±)-1] (Scheme 1)

Previous studies using the XTT-based cytoprotection assay[45] had identified (±)-*cis*-4,5-dihydroxy-1,2-dithiane 1,1-dioxide (±-**1**)[44] as the more potent of the two (*cis–trans*) stereoisomers. In order to determine which enantiomer of the *cis* isomer is the more active anti-HIV agent, resolution was attempted by a number of methods, including the use of lipases and hydrolases on appropriate ester derivatives, as well as separation of the bis-benzoyl derivative of (±)-**1** on a chiral D-phenylglycine HPLC column (data not shown). Neither method proved successful.[46] Conversion of (±)-**1** to a set of diesters with an optically active acyating agent and separation of the resulting diastereomers was the next option. Thus (±)-**1** was converted by reaction with (−)-(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride and DMAP in dry CH_2Cl_2 into its 'Mosher esters' **2** and **3**.[47,48] Diastereomers (+)-**2** and (−)-**3** were then separated on a silica gel column and subsequently hydrolyzed to the diols (+)-**4** and (−)-**5**, respectively, with 40% NH_3 in MeOH. Then, in order to determine the absolute stereochemistry at C-4 and C-5, a single-crystal X-ray structure of compound (−)-**3** was obtained, which indicated the (−)-enantiomer (−)-**5** to be the (4*S*, 5*R*) compound, based on apparent relative configurations of the stereogenic centers with the known chirality of the ester ligands. Details of the X-ray structural determination are in Table 1, and the ORTEP structure of (−)-**3** is shown in Figure 1.



Scheme. 1. Resolution of (±)-**1** into its enantiomers **4** and **5**.

Anti-HIV-1 activity

Evaluation of the enantiomers (+)-**4** and (−)-**5** in the XTT-based cytoprotection assay revealed slightly more

Table 1. Crystal structure data for compound (−)-**3**

Empirical formula	$C_{24}H_{22}F_6O_8S_2$	
Formula weight	616.54	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$ (no. 19)	
Unit cell dimensions	$a = 7.536(3)$ Å	$\alpha = 90°$
	$b = 12.886(5)$ Å	$\beta = 90°$
	$c = 27.470(11)$ Å	$\gamma = 90°$
Volume, Z	2668(2) Å^3, 4	
Density (calculated)	1.535 mg/m^3	
Absorption coefficient	0.288 mm^{-1}	
$F(000)$	1264	
Crystal size	0.30 × 0.22 × 0.20 mm	
Θ range for data collection	2.17 to 22.58°	
Limiting indices	$0 \le h \le 8$, $0 \le k \le 13$, $-29 \le l \le 29$	
Reflections collected	4158	
Independent reflections	3443 ($R_{int} = 0.1354$)	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	3440/0/361	
Goodness-of-fit on F^2	1.108	
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0857$, $wR2 = 0.1324$	
R indices (all data)	$R1 = 0.2059$, $wR2 = 0.1865$	
Absolute structure parameter	0.0(3)	
Largest diff. peak and hole	0.308 and −0.289 eÅ^{-3}	

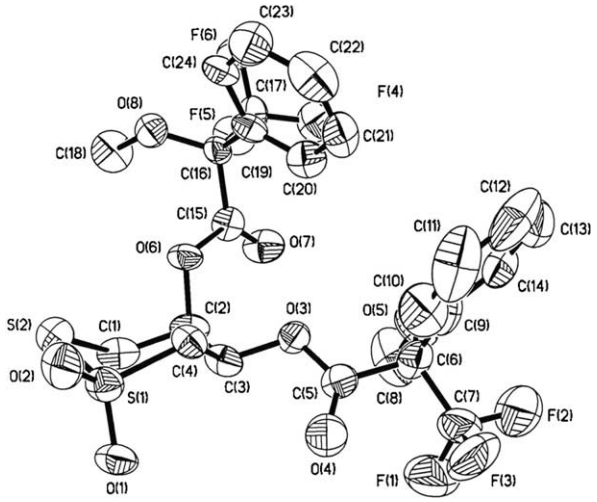


Figure 1. ORTEP view of bis-Mosher ester (−)-**3** shown as 50% thermal ellipsoids.

potent antiviral activity by the (−)-enantiomer **5** (NSC-693195), which showed an EC_{50} of 8.8 μM and an CC_{50} of 132 μM, compared with an EC_{50} of 16.2 μM and an CC_{50} of 134 μM for the (+)-enantiomer **(+)-4** (Table 2). Thus the (−)-enantiomer **(−)-5** is almost twice as active as its (+)-enantiomer, and the CC_{50} values are equivalent.

Using an assay that measures the ejection of zinc from the HIV-1 p7 nucleocapsid protein (NCp7) protein,[45] these compounds were found to target the highly conserved retroviral zinc fingers. The assay measures the decrease in relative fluorescence (reported in relative fluorescence units, RFU) following the loss of zinc from the C-terminal zinc finger. The loss of fluorescence occurs as the Trp^{37} residue folds away from the aqueous environment. The greater the percent decrease of RFU, which is measured at time intervals of 0, 3, 10 and 30 min, the better is the ability of the compound to eject zinc from the zinc finger. The results (Table 2) show that all three compounds, the **(±)-1**, **(+)-4**, and **(−)-5**, show approximately equivalent rates of zinc ejection in the assay.

While no studies to determine the precise mode of action for these compounds have been undertaken, their behavior in the NCp7 zinc ejection assay indicates that they are likely substrates for a nucleophilic reaction with specific Cys residues of the zinc fingers in a stepwise fashion similar to that established for pyridinioalkanoyl esters.[40] The chemical reaction would be similar to that established for the decomposition in base of these cyclic 1,2-dithiane 1,1-dioxides as reported by Field and co-workers[44] or that accepted for the hydrolysis of cyclic thiosulfonates in general.[49] It is interesting to note that, while OH substitution at C-4 and C-5, as well as the *cis* or *trans* configuration of the –OH substituents, are important in determining anti-HIV activity as determined in the XTT-based cytoprotection assay (–OAc or –H substitution renders the compounds essentially inactive),[45] neither the presence of, nor the relative configurations of, these substituents has much effect on the rate of ejection of zinc in the Trp^{37} assay. (See data in Table 2 as well as that reported for the –OH and –OAc analogues in the previous paper.[45]) These observations, together with the fact that the anti-HIV activities of the two enantiomers [**(+)-4** and **(−)-5**] differ only by a factor of two, would seem to indicate that the presence of substituents, as well as their stereochemistry, play a more important role in some other phenomena that are important in determining anti-HIV activity—drug delivery, compound stability, host-cell toxicity (as indicated by CC_{50} values), or possibly other properties—and that, at least with these compounds, the interaction with the zinc finger protein per se is itself less selective and is a process that has far lower demands on the finer aspects of the structure of the interacting species.

Table 2. XTT Cytoprotection assay and NCp7 zinc ejection assay for compounds **(±)-1**, **(+)-4**, and **(−)-5**

Compd[a]	XTT Assay[b]		NCp7 Trp37Assay (RFU)[c]			
	EC_{50} (μM)	CC_{50} (μM)	0	3	10	30 min
(±)-1	12.2±2.7	122±5	295	136	75	42
(+)-4	16.2±2.4	134±1	280	146	75	41
(−)-5	8.8±0.2	132±3	279	117	69	45

[a]The National Cancer Institute's NSC numbers are as follows: **(±)-1**, NSC-624151; **(+)-4**, −693194; **(−)-5**, −693195.
[b]The assay was run according to the protocols previously established using an XTT-based cytoprotection study with CEM-SS cells and HIV-1.[34,45] EC_{50}, effective concentration for 50% cytoprotection; CC_{50}, cytotoxic concentration, 50%; that is, compound concentration to reduce cell viability 50%. The mean values of duplicate runs are reported.
[c]The NCp7 activity assay was conducted as previously described using NCp7 protein and monitoring of the ejection of Zn^{2+} by the fluorescence of Newport Green following the chelation of Zn^{2+}.[40]

Single-crystal X-ray structure of (−)-3

The X-ray crystal structure of **(−)-3** was determined at 20 °C on a Siemens R3m/V diffractometer. A suitable crystal was mounted using epoxy on a glass fiber. Unit cell dimensions were calculated from the angular setting of 35 well-centered reflections having 2θ values in the range of 15–25°. Compound **(−)-3** was found to crystallize in the orthorhombic system. Axial dimensions were confirmed by use of axial photographs. Two unique octants of data were collected for a total of 4158 (3443 unique) reflections. Corrections for Lorentz and polarization effects were applied during data reduction; absorption effects were ignored ($m = 0.288\ mm^{-1}$). The $|E^*E - 1|$ statistics, as well as observed systematic absences, were uniquely consistent with the space group $P2_12_12_1$. The structure was solved by direct methods using the Siemens SHELXTL-93 (Version 5.0) proprietary software package, and refined by Full-Matrix Least Squares on F^2 until the structure converged to R (R_wF^2) = 8.57% (13.24%). All non-hydrogen atoms were anisotropically refined. Hydrogen atoms were placed at calculated positions and introduced into the refinement as fixed contributors with an isotropic U value of 0.08 $Å^2$. The ORTEP view of bis-Mosher ester **(−)-3** as 50% thermal ellipsoids is shown in Figure 1 and its crystal structure data are given in Table 1.[50]

Experimental

General procedures (chemistry)

Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Analytical TLC was performed on aluminum-backed plates coated with E. Merck Silica Gel-60 F-254. The developed plates were air-dried and visualized by irradiation with UV light and/or by dipping in 10 wt% phosphomolybdic acid solution in ethanol, followed by heating at 120–140 °C. Flash column chromatography was performed on Silica Gel-60 (230–400 mesh). The HPLC analysis was carried out on a Beckman System Gold instrument using a Regis no. 731221 Pirkle D-phenylglycine (25 cm × 1 cm i.d.) column. Optical rotations were measured with a Perkin–Elmer Model 243 automatic polarimeter for solutions in a 1-dm cell at the indicated temperature. ^1H and ^{13}C NMR spectra

were recorded at 250 and 62.9 MHz, respectively, on a Bruker AC 250 instrument using the indicated solvent. ^{1}H NMR shifts are reported as δ (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard; multiplicities are first-order values in Hz: s, singlet; bs, broad singlet; d, doublet; t, triplet; dd, double of doublets; db, broad doublet; q, quartet; m, multiplet. ^{13}C NMR chemical shifts are reported as δ (ppm) relative to the chemical shift of the solvent used ($CDCl_3$: 77.0 ppm; CD_3OD: 49.0 ppm). Elemental analyses were performed by Atlantic Microlabs, Inc. of Atlanta, GA, USA. Chemicals were of reagent grade and were used directly. Solvents were distilled and dried by literature procedures before use. All evaporations were carried out with a rotary evaporator under vacuum and below 40 °C.

(2*S*,4*R*,5*S*)-(+)-3,3,3-Trifluoro-2-methoxy-2-phenylpropionic acid 1,1-dioxo-4-[(2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyloxy][1,2]dithiane-5-yl ester [(+)-2] and (2*S*,4*S*,5*R*)-(−)-3,3,3-trifluoro-2-methoxy-2-phenylpropionic acid 1,1-dioxo-4-[(2*S*)-2-methoxy-2-phenyl-3,3,3-trifluoropropionyloxy][1,2]dithiane-5-yl ester [(−)-3]. To **(±)-1** (0.225 g, 1.22 mmol) and *N*,*N*-dimethylaminopyridine (0.597 g, 4.89 mmol) in dry CH_2Cl_2 (5 mL) was added (−)-(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.750 g, 2.97 mmol) over 30 min at 0 °C under N_2. The reaction mixture was allowed to gradually rise to room temperature and stir at that temperature for 20 h. Then, the reaction mixture was cooled in an ice bath, washed with N HCl, followed by satd aq $NaHCO_3$, and finally with dist H_2O. The CH_2Cl_2 layer was dried with anhyd Na_2SO_4 and concentrated, and the crude product was purified on a column of silica gel with 85:15 hexanes–EtOAc to obtain **(+)-2** (0.252 g, 34%) as the 1st fraction; mp: 162–163 °C: $[\alpha]_D^{21}$ +81° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.39 (m, 10H), 5.79 (m, 1H), 5.59 (m, 1H), 3.67 (m, 3H), 3.49 (s, 3H), 3.40 (dd, 1H, *J* = 15.5 Hz, *J* = 5.1 Hz), 3.25 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.35, 165.19, 131.29, 131.14, 130.10, 129.95, 128.77, 128.61, 126.91, 125.16, 120.59, 116.02, 85.40, 84.94, 84.44, 84.01, 70.78, 65.73, 58.47, 55.52, 55.38, 34.15. Anal. calcd for $C_{24}H_{22}F_6O_8S_2$: C, 46.75; H, 3.60; S, 10.40. Found: C, 46.79; H, 3.58; S, 10.29. The 2nd fraction yielded diastereomer **(−)-3** (0.250 g, 33%) as white crystals: mp: 184–185 °C (from hexanes–EtOAc); $[\alpha]_D^{21}$ −120° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.38 (m, 10H), 5.76 (m, 2H), 3.73 (d, 1H, *J* = 15.4 Hz), 3.54 (m, 3H), 3.45 (s, 3H), 3.40 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.41, 165.31, 131.17, 131.07, 130.07,130.01, 128.73, 128.65, 127.31, 126.96, 125.27, 125.18, 120.67, 120.58, 116.09, 115.97, 85.38, 84.93, 84.48, 84.03, 70.84, 66.28, 58.36, 55.58, 55.32, 34.47. Anal. calcd for $C_{24}H_{22}F_6O_8S_2$: C, 46.75; H, 3.60; S, 10.40. Found: C, 46.68; H, 3.66; S, 10.32. For single-crystal X-ray data, see Table 1 and Figure 1.

(+)-*cis*-4,5-Dihydroxy-1,2-dithiane 1,1-dioxide [(+)-4]. To **(+)-2** (0.167 g, 0.271 mmol) was added 40% NH_3 in MeOH (4 mL) over 5 min under N_2 at room temperature. The solid went into solution as 40% NH_3 in MeOH was added, and the solution turned pale yellow in color. After 1.5 h, TLC showed complete disappearance of the diester. The reaction mixture was then concentrated under vacuum, and the crude oil was purified by silica gel chromatography using 95:5 CH_2Cl_2–MeOH as the eluent to give **(+)-4** (0.0212 g, 43%) as a white solid: mp 133–134 °C; $[\alpha]_D^{21}$ +151° (*c* 0.5, CD_3OD); ^{1}H NMR (CD_3OD): δ 4.20 (m, 1H), 4.13 (m, 1H), 3.66 (dd, 1H, *J* = 12.6 Hz, *J* = 11.0 Hz), 3.47 (dd, 1H, *J* = 14.7 Hz, *J* = 1.4 Hz), 3.37 (m, 2H); ^{13}C NMR (CD_3OD) δ 71.44, 65.92, 61.62, 38.63. Anal. calcd for $C_4H_8O_4S_2$: C, 27.74; H, 4.66; S, 32.77. Found: C, 27.75; H, 4.58; S, 32.57.

(−)-*cis*-4,5-Dihydroxy-1,2-dithiane 1,1-dioxide [(−)-5]. As for **(+)-2**, compound **(−)-3** (0.159 g, 0.258 mmol) was deacylated and purified to give **(−)-5** (0.019 g, 40%) as a white solid: mp 131–132 °C; $[\alpha]_D^{21}$ −146° (*c* 0.5, CD_3OD); ^{1}H NMR (CD_3OD): δ 4.19 (m, 1H), 4.13 (m, 1H), 3.66 (dd, 1H, *J* = 12.8 Hz, *J* = 10.9 Hz), 3.47 (dd, 1H, *J* = 14.7 Hz, *J* = 1.5 Hz), 3.37 (m, 2H); ^{13}C NMR (CD_3OD) δ 71.42, 65.91, 61.62, 38.63. Anal. calcd for $C_4H_8O_4S_2 \cdot 0.14C_4H_8O_2$: C, 27.86; H, 4.68; S, 32.62. Found: C, 27.85; H, 4.59; S, 32.44.

Antiviral activity determinations

The antiviral activities were determined in an XTT [2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)-carbonyl]-2*H*-tetrazolium hydroxide]-based cytoprotection assay using CEM-SS cells and HIV-1 as previously described.[34,45] Details of the results are in Table 2.

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