Table 2. Comparison of the Antiviral Activity of **35a** and Other PIs against Multidrug Resistant Clinical Isolates in PHA-PBMs Cells[a]

virus	EC_{50} (μM)			
	35a	ATV	LPV	DRV (**1a**)
HIV-$1_{ERS104pre}$ (X4)	0.0019 ± 0.0015	0.0027 ± 0.0006	0.031 ± 0.004	0.004 ± 0.001
HIV-$1_{MDR/B}$ (X4)	0.0145 ± 0.0001 (8)	0.470 ± 0.007 (174)	> 1 (> 32)	0.034 ± 0.008 (9)
HIV-$1_{MDR/C}$ (X4)	0.0037 ± 0.0018 (2)	0.039 ± 0.003 (14)	0.437 ± 0.004 (14)	0.009 ± 0.005 (2)
HIV-$1_{MDR/G}$ (X4)	0.0026 ± 0.0004 (1)	0.019 ± 0.008 (7)	0.181 ± 0.023 (6)	0.026 ± 0.009 (7)
HIV-$1_{MDR/TM}$ (X4)	0.0275 ± 0.0055 (14)	0.075 ± 0.003 (28)	0.423 ± 0.082 (14)	0.022 ± 0.015 (6)
HIV-$1_{MDR/MM}$ (R5)	0.0050 ± 0.0023 (3)	0.205 ± 0.024 (76)	0.762 ± 0.115 (25)	0.017 ± 0.005 (4)
HIV-$1_{MDR/JSL}$ (R5)	0.0275 ± 0.0009 (14)	0.293 ± 0.099 (109)	> 1 (> 32)	0.023 ± 0.005 (6)

[a] The amino acid substitutions identified in the protease-encoding region of HIV-$1_{ERS104pre}$, HIV-1_B, HIV-1_C, HIV-1_G, HIV-1_{TM}, HIV-1_{MM}, HIV-1_{JSL} compared to the consensus type B sequence cited from the Los Alamos database include L63P; L10I, K14R, L33I, M36I, M46I, F53I, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L; L10I, I15V, K20R, L24I, M36I, M46L, I54V, I62V, L63P, K70Q,V82A, L89M; L10I, V11I, T12E, I15V, L19I, R41K, M46L, L63P, A71T, V82A, L90M; L10I, K14R, R41K, M46L, I54V, L63P, A71V, V82A, L90M; I93L, L10I, K43T, M46L, I54V, L63P, A71V, V82A, L90M, Q92K; and L10I, L24I, I33F, E35D, M36I, N37S, M46L, I54V, R57K, I62V, L63P, A71V, G73S, V82A, respectively. HIV-$1_{ERS104pre}$ served as a source of wild-type HIV-1. The EC_{50} values were determined by using PHA-PBMs as target cells, and the inhibition of p24 Gag protein production by each drug was used as an end point. The numbers in parentheses represent the fold changes of EC_{50} values for each isolate compared to the EC_{50} values for wild-type HIV-$1_{ERS104pre}$. All assays were conducted in duplicate, and the data shown represent mean values (±1 standard deviations) derived from the results of two or three independent experiments.

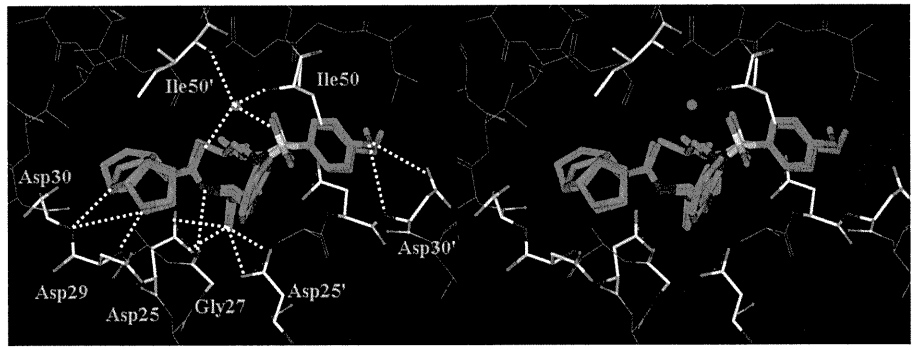

Figure 2. Stereoview of inhibitor **35a** modeled into the active site of HIV-1 protease and superimposed on the X-ray crystal structure of **1b** (PDB code 3I7E).

the series comparable to inhibitor **2**. However, the 4-amino derivative **36** exhibited very comparable enzyme inhibitory and antiviral potency similar to **1a**.

We have examined inhibitor **35a** for its activity against a panel of multidrug-resistant HIV-1 variants and compared it with that of other clinically available PIs including **1a**. The results are shown in Table 2. All inhibitors showed high antiviral activity against an HIV-1 clinical strain isolated from a drug-naive patient (wild-type).[19] Compound **35a** displayed the most potent activity with an EC_{50} of 1.9 nM. When tested against multidrug-resistant HIV-1 variants, compound **35a** retained impressively high activity to all variants with EC_{50} values ranging from 2.6 to 27.5 nM. In contrast, other inhibitors, except **1a**, exhibited substantial loss of activity. Interestingly, **1a** and **35a** showed similar fold-change of EC_{50} against most multidrug-resistant HIV strains. The results indicated that **35a** is highly active against multidrug-resistant HIV-1 variants. This inhibitor outperformed the clinically available PIs with exceedingly high antiviral activity and compared well with **1a**, which currently stands as the leading PI for the treatment of drug-resistant HIV infection.

In order to obtain molecular insights into the enzyme–inhibitor interactions of **35a** in the protease active site, an active model of **35a** was created. A stereoview of the overlaid structure of **35a** with the X-ray structure of inhibitor **1b**-bound HIV-1 protease is shown in Figure 2. Inhibitor **35a** was modeled starting from the X-ray crystal structure of **1b**. The conformation of **35a** was optimized using the MMFF94 force field,[37] as implemented in Molecular Operating Environment (version 2009.10, Chemical Computing Group, Montreal, Canada). The modeled structure maintains the important binding interactions (hydroxyl group with Asp25 and Asp25′ carboxylates; cyclic ether oxygens with Asp29 and Asp30 backbone NH bonds; methoxy oxygen with the Asp30′ backbone NH bond; carbonyl oxygen and sulfonamide oxygen with a water molecule binding to Ile50 and Ile50′) that are observed in the crystal structure of **1b**-bound HIV-1 protease.

Conclusions

We have reported the structure-based design of novel HIV-1 protease inhibitors incorporating a stereochemically defined 4-hexahydrofuropyranol-derived urethanes as the P2-ligand. The inhibitors were designed to make extensive interactions including hydrogen bonding with the protein backbone of the HIV-1 protease active site. The synthesis of (3a*S*,4*S*,7a*R*)-hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol [(−)-**7**, Tp-THF] was carried out in optically active form using (3a*R*,6a*S*)-3,3a,6,6a-tetrahydro-2*H*-cyclopenta[*b*]furan-2-one as the starting material. Inhibitor **35a** has shown excellent enzyme inhibitory activity and antiviral potency comparable to that of approved PI **1a**. Furthermore, it has shown excellent activity against multi-PI-resistant variants, superior to other FDA approved inhibitors examined. The data are comparable to those of **1a**. We have carried out detailed structure–activity studies that indicated that the stereochemistry of the Tp-THF ligand and position of its oxygens are critical to the ligand's high enzyme affinity.

An active model of **35a** was created based upon the X-ray crystal structure of **1b**-bound HIV-1 protease. The overlaid structures revealed that both oxygens of the Tp-THF ligand can interact with the Asp29 and Asp30 backbone NHs, similar to the bis-THF ligand oxygens. Furthermore, the extra methylene unit in the Tp-THF ligand appears to fill in the hydrophobic pocket in the S2-site more effectively compared to the bis-THF in **1a**. The design of an inhibitor targeting the protein backbone may serve as an important guide to combat drug resistance. Further design and chemical modifications are currently underway.

Experimental Section

General Experimental Methods. All anhydrous solvents were obtained according to the following procedures: diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone under argon; toluene, methanol, acetonitrile, and dichloromethane were distilled from calcium hydride; benzene was distilled from sodium. Other solvents were used without purification. All moisture-sensitive reactions were carried out in flame-dried flasks under argon atmosphere. Reactions were monitored by thin layer chromatography (TLC) using Silicycle 60A-F254 silica gel precoated plates. Flash column chromatography was performed using Silicycle 230−400 mesh silica gel. Yields refer to chromatographically and spectroscopically pure compounds. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova-300 (300 and 75 MHz), Bruker Avance ARX- 400 (400 and 100 MHz), or DRX-500 (500 and 125 MHz). High and low resolution mass spectra were carried out by the Mass Spectroscopy Center at Purdue University. The purity of all test compounds was determined by HRMS and HPLC analysis in the different solvent systems. All test compounds showed ≥95% purity.

(1*S*,2*R*)-2-[1-(*tert*-Butyldimethylsilyloxy)cyclopent-3-en-2-yl]-ethyl Acetate (5). To a stirred suspension of lithium aluminum hydride (93 mg, 2.45 mmol) in dry Et_2O (6 mL) was added dropwise a solution of (−)-(1*S*,5*R*)-2-oxabicyclo[3.3.0]oct-6-en-3-one (**4**) (150 mg, 1.19 mmol) in Et_2O (4 mL + 1 mL rinse) at 0 °C under argon. The reaction mixture was vigorously stirred at this temperature for 1.5 h. Water (0.1 mL) was then carefully added followed by addition of 3 M NaOH (0.1 mL) and then water (0.3 mL). The solution was stirred until formation of a white precipitate was complete. EtOAc (3 mL) and then Na_2SO_4 were added, and the resulting suspension was filtered out. The amorphous solid was washed several times with EtOAc (5 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude oil was purified by flash chromatography on silica gel using hexanes/EtOAc (1:1) as the eluent to give the resulting diol (145 mg, 95%) as a colorless oil. TLC: R_f = 0.28 (hexanes/EtOAc = 1:2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.74 (m, 1H), 5.56 (m, 1H), 4.48 (dt, J = 2.4, 6.6 Hz, 1H), 3.84 (m, 1H), 3.71 (ddd, J = 3.6, 8.7, 10.0 Hz, 1H), 2.75 (m, 1H), 2.67 (m, 1H), 2.36 (d, J = 17.1 Hz, 1H), 1.98−1.75 (m, 1H).

To a stirred solution of the diol (76 mg, 0.59 mmol) in CH_2Cl_2 (3 mL) was added 2,4,6-collidine (1.2 mmol, 155 μL) followed by acetyl chloride (50 μL, 0.71 mmol) at −78 °C under argon. The resulting solution was stirred at this temperature for 5 h at which point additional acetyl chloride (0.25 μL, 0.24 mmol) was added. The solution was stirred for 2 h, and then saturated aqueous $NaHCO_3$ solution was added. The two layers were separated, and the aqueous layer was washed with CH_2Cl_2 (3 × 5 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude oil was purified by flash chromatography on silica gel using hexanes/EtOAc (6:1, then 4:1) as the eluent to give the monoacetate (88 mg, 87%) as a colorless oil. TLC: R_f = 0.26 (hexanes/EtOAc = 2:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.80−5.72 (m, 1H), 5.64−5.58 (m, 1H), 4.40 (dt, J = 2.4, 5.6 Hz, 1H), 4.20 (t, J = 7.2 Hz, 2H), 2.74−2.56 (m, 2H), 2.33 (d, J = 17.1 Hz, 1H), 2.06 (s, 3H), 2.04−1.88 (m, 1H), 1.87−1.73 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1, 132.4, 128.4, 72.7, 63.9, 47.2, 42.1, 26.8, 21.0. HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_9H_{15}O_3$ 171.1021; found 171.1020.

To a stirred solution of the above acetate (54 mg, 0.32 mmol) and 2,6-lutidine (74 μL, 0.63 mmol) in CH_2Cl_2 (1 mL) was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (125 mg, 108 μL) at −78 °C under argon. The mixture was stirred for 10 min, at which point reaction completion was observed. Saturated aqueous $NaHCO_3$ solution (1 mL) and additional CH_2Cl_2 (2 mL) were added. The two layers were separated, and the aqueous layer was further extracted with CH_2Cl_2 (2 × 2 mL). The combined organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude oil was purified by column chromatography on silica gel using hexanes/EtOAc (20:1) as the eluent to afford silylated product **5** (90 mg, > 99%) as a colorless oil. TLC: R_f = 0.68 (hexanes/EtOAc = 3:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.68 (s, 2H), 4.45 (dt, J = 5.1, 6.3 Hz, 1H), 4.14 (t, J = 6.9 Hz, 2H), 2.67−2.55 (m, 1H), 2.47 (dd, J = 6.9, 15.4 Hz, 1H), 2.23 (dd, J = 4.8, 15.4 Hz, 1H), 2.04 (s, 3H), 2.01−1.85 (m, 1H), 1.72−1.56 (m, 1H), 0.88 (s, 9H), 0.06 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.2, 132.7, 128.4, 73.6, 63.8, 45.9, 41.0, 27.4, 25.8, 21.0, 18.1, −4.6, −5.0.

(4*S*,4a*S*,7a*R*)-4-(*tert*-Butyldimethylsilyloxy)hexahydrofuro-[2,3-*b*]pyrane (6). To a stirred solution of **5** (76 mg, 0.27 mmol) in MeOH (2 mL) was added K_2CO_3 (37 mg, 0.27 mmol). The solution was stirred at 23 °C for 2 h. Then saturated aqueous NH_4Cl solution (2 mL) was added to the mixture. EtOAc was added, and the two layers were separated. The aqueous layer was extracted with EtOAc (4 × 3 mL). The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The resulting oil was purified by flash chromatography on silica gel using hexanes/EtOAc (7:1) as the eluent to give the corresponding alcohol (64 mg, 98%) as a colorless oil. This intermediate was used immediately for the subsequent reaction. TLC: R_f = 0.29 (hexanes/EtOAc = 5:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.72.5.62 (m, 2H), 4.52 (dt, J = 6.0, 6.9 Hz, 1H), 3.74−3.60 (m, 2H), 2.80−2.68 (m, 1H), 2.49 (ddt, J = 1.8, 7.2, 16.3 Hz, 1H), 2.34−2.29 (m, 1H), 2.06 (br s, 1H), 1.90−1.62 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 132.9, 128.3, 74.0, 61.1, 46.5, 40.6, 31.2, 25.8, 18.2, −4.7, −5.0.

A stream of ozonized oxygen was bubbled through a solution of the above alcohol (63.8 mg, 0.26 mmol) in CH_2Cl_2 (15 mL) at −78 °C until the blue color persisted (5 min). After the solution was flushed with nitrogen, Me_2S (0.5 mL) was added. The solution was warmed to 0 °C and stirred over a 2 h period following which anhydrous Na_2SO_4 was added. The solution was left at room temperature overnight and then filtered and concentrated in vacuo. The resulting solid was quickly passed through a short column of silica gel using hexanes/EtOAc (3:1) as the eluent to afford the hemiacetal (99 mg) as a white-solid mixture of isomers which was submitted directly to the next step. TLC: R_f = 0.26 (hexanes/EtOAc = 3:1). To an ice-cold solution of the crude diacetal (~0.25 mmol) and Et_3SiH (0.16 mL, 1.0 mmol) in CH_2Cl_2 (3 mL) under argon, was slowly added BF_3−Et_2O (60 μL, 0.5 mmol). The mixture was stirred at 0 °C for 10 min. Saturated aqueous $NaHCO_3$ solution (2 mL) and additional CH_2Cl_2 were added. The two phases were separated and the aqueous layer was further extracted with CH_2Cl_2 (3 × 2 mL). The combined organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude oil was purified by column chromatography on silica gel using hexanes/EtOAc (7:1) as the eluent to give bicyclic acetal **6** (38 mg, 55% 3 steps) as an amorphous solid. TLC: R_f = 0.50 (hexanes/EtOAc = 3:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.95 (d, J = 3.4 Hz, 1H), 4.24−4.08 (m, 2H), 3.92 (dt, J = 8.1, 9.1 Hz, 1H), 3.85 (ddd, J = 2.0, 4.5, 12.2 Hz, 1H), 3.30 (dt, J = 2.0, 12.3 Hz, 1H), 2.39 (m, 1H), 2.07 (tt, J = 9.4, 12.0 Hz, 1H), 1.91−1.66 (m, 2H), 1.58−1.48 (m, 1H), 0.89 (s, 9H), 0.07 (s, 3H), 0.067 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 101.2, 68.4, 67.8, 61.1, 47.2, 30.3, 25.7, 22.4, 18.2, −4.6, −4.8.

(3a*S*,4*S*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4ol [(−)-7]. Bicyclic compound **6** (36 mg, 0.139 mmol) was dissolved in

THF (1 mL), and tetrabutylammonium fluoride (1 M solution THF, 0.21 mL, 0.21 mmol) was added to the solution. The mixture was stirred for 2 h at 23 °C. Saturated aqueous NH_4Cl solution was added (2 mL), followed by EtOAc (2 mL). The two phases were separated, and the aqueous layer was further extracted with EtOAc (4 × 3 mL). The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The resulting compound was purified by flash chromatography on silica gel using hexanes/EtOAc (1:2 then 1:3) as the eluent to afford pure alcohol (−)-**7** (19 mg, 94%) as an amorphous solid. TLC: R_f = 0.15 (hexanes/EtOAc = 1:3). $[\alpha]_D^{23}$ −29.6 (*c* 1.06, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ 4.99 (d, J = 2.7 Hz, 1H), 4.25−4.16 (m, 2H), 3.96 (q, J = 7.5 Hz, 1H), 3.90 (ddd, J = 2.4, 4.8, 12.3 Hz, 1H), 3.34 (td, J = 3.0, 11.7 Hz, 1H), 2.58−2.45 (m, 1H), 2.14−1.98 (m, 1H), 1.96−1.82 (m, 1H), 1.80−1.62 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 101.4, 68.4, 67.5, 61.0, 46.3, 29.4, 21.8. HRMS-CI (*m*/*z*): $[M + H]^+$ calcd for $C_9H_{15}O_3$ 127.0759; found 127.0757.

(3a*R*,4*R*,7a*S*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol [(+)-7]. Cyclopentenediol **8** was prepared as described previously.[27b] The same synthetic sequence was the applied on the diol as for the synthesis of (−)-**7**. Ligand (+)-**7** was obtained in high enantiomeric purity [99% ee, $[\alpha]_D^{23}$ +22.3 (*c* 0.22, $CHCl_3$)].

2-Ethoxy-2,3,6,7-tetrahydrobenzofuran-4(5*H*)-one (10). To a stirred solution of 2-diazo-1,3-cyclohexanedione (300 mg, 2.17 mmol) in freshly distilled ethyl vinyl ether (5 mL) was added $[Rh_2(OAc)_4]$ (10 mg, 0.02 mmol). The mixture was stirred at room temperature for 5 h, after which the reaction was diluted with Et_2O and a few drops of pyridine were added. A red precipitate formed. The solution was filtered on a short pad of silica, flushing with Et_2O/THF (4:1) as eluent. After evaporation, the residue was purified by column chromatography on silica gel using hexanes/CH_2Cl_2/THF (8:1:1) as the eluent to furnish benzofuranone derivative **17** (347 mg, 88%). TLC: R_f = 0.29 (hexanes/EtOAc = 1:1). ^1H NMR ($CDCl_3$, 400 MHz) δ 5.72 (dd, J = 3.3, 7.4 Hz, 1H), 3.88 (m, 1H), 3.62 (m, 1H), 2.92 (ddt, J = 2.2, 7.4, 15.8 Hz, 1H), 2.70−2.62 (m, 1H), 2.52−2.37 (m, 2H), 2.33 (t, J = 6.5 Hz, 2H), 2.12−1.95 (m, 2H), 1.24 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 Hz) δ 195.2, 175.7, 112.3, 108.5, 65.0, 36.3, 32.7, 23.8, 21.5, 14.9.

2-Ethoxyhexahydrobenzofuran-4(2*H*)-one (11). To a solution of the ketone **10** (140 mg, 0.77 mmol) in EtOAc (9 mL) was added 5% Pd/C (128 mg, 60 μmol), and the mixture was stirred under H_2 (1 atm) for 1.5 h at room temperature. The mixture was then filtered on Celite and the pad washed with EtOAc. Evaporation of the solvent furnished the corresponding crude ketone **11** as an essentially pure mixture of diastereoisomers (130 mg, dr = 9:1). The ketone was directly submitted to the next step without purification. TLC major isomer: R_f = 0.35 (hexanes/EtOAc = 2:1).

***cis*-Octahydrobenzofuran-4-ol [(±)-12].** A solution of ketone **11** (130 mg, ca. 0.7 mmol) in CH_2Cl_2 (10 mL) was cooled to −78 °C under Ar. L-Selectride (1 M solution, 0.9 mL, 0.9 mmol) was slowly added to the solution over 5 min and the reaction mixture was stirred for 1.5 h at −78 °C. Upon complete conversion, Et_3SiH (0.6 mL, 437 mg, 3.7 mmol) was added followed by dropwise addition of TMSOTf (380 μL, 466 mg, 2.1 mmol). The solution was stirred for 2.5 h while slowly warming to 0 °C. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution (5 mL). The two phases were separated, and the aqueous phase was extracted with Et_2O (5×). The combined organic layer was washed with brine, dried ($MgSO_4$), and evaporated under vacuum. The residue was purified by column chromatography on silica gel using hexanes/EtOAc (3:1 to 2:1) as the eluent to yield the desired alcohol (±)-**12** (78 mg, 71% over two steps) as a colorless oil. TLC: R_f = 0.25 (hexanes/EtOAc = 1:2). ^1H NMR ($CDCl_3$, 400 MHz) δ 4.01 (dt, J = 4.6, 8.8 Hz, 1H), 3.88−3.82 (m, 2H), 3.78 (dt, J = 7.1, 8.7 Hz, 1H), 2.31 (m, 1H), 2.12−1.90 (m, 2H), 1.74−1.50 (m, 5H), 1.32−1.22 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 Hz) δ 77.6, 69.1, 66.7, 43.2, 30.2, 26.9, 25.9, 16.2.

(3a*S*,4*S*,7a*R*)-Octahydrobenzofuran-4-ol [(−)-12]. Racemic alcohol **12** (70 mg, 0.5 mmol) was dissolved in THF (5 mL), and vinyl acetate (120 μL, 1.25 mmol) was added. Amano lipase PS-30 (30 mg) was added, and the resulting suspension was stirred at 15−17 °C. After 48 h, 30 mg of additional enzyme was added and the mixture was left for additional 48 h until ~54% conversion was reached (NMR and GC). The resulting suspension was diluted with Et_2O and filtered on Celite and the filter cake rinsed with Et_2O. After evaporation of the remaining solvent, the residue was purified by column chromatography using hexanes/EtOAc (5:1, 3:1, then 2:1) as the eluent to yield acetyl furanol **13** (38 mg, 41%) and the desired enantioenriched (−)-hexahydrobenzofuranol (−)-**12** (24 mg, 35%). The enantiomeric excess of the 2,4-dinitrobenzoate derivative of (−)-**12** was determined to be 98.8% ee by chiral HPLC: column ChiralPak IA, hexane/isopropanol (90/10 to 50/50, 40 min), 1 mL/min, 35 °C, λ = 254 nm, t_R major = 16.54 min, t_R minor = 37.1 min.

2-[3-(*tert*-Butyldimethylsilyl)oxy)-1-hydroxypropyl]cyclopentanone (15). A solution of lithium diisopropylamide (14 mmol), freshly prepared by adding *n*-BuLi (1.6 M solution in hexanes, 8.75 mL, 14 mmol) to diisopropylamine (1.97 mL, 1.42 g, 14 mmol) in THF (30 mL) at 0 °C under argon followed by stirring for 30 min, was cooled to −78 °C, and cyclopentanone **14** (1.12 mL, 1.07 g, 12.7 mmol) in THF (5 mL) was added dropwise over 10 min. After being stirred at −78 °C for 1.5 h, 3-*tert*-butyldimethylsilyloxypropionaldehyde (1.55 g, 8.2 mmol) in THF (20 mL) was added dropwise over 5 min. The mixture was stirred for an additional 2 h, and the reaction was quenched by addition of saturated aqueous NH_4Cl solution (10 mL). Following dilution with Et_2O, the two phases were separated, and the aqueous phase was extracted with Et_2O (2×). The combined organic phase was washed with brine, dried ($MgSO_4$), filtered, and evaporated. The residue was quickly purified by column chromatography on silica gel using hexanes/EtOAc (20:1 to 10:1) as the eluent to give **15** as a 3:1 mixture of diastereoisomers (2.13 g, 95%). Light yellow oil. TLC: R_f = 0.37 and 0.23 (hexanes/EtOAc = 5:1). ^1H NMR ($CDCl_3$, 400 MHz) δ 4.27 (dt, J = 3.1, 9.3 Hz, 0.3H), 4.10 (s, 1H), 3.91 (m, 1H), 3.87 (m, 0.3H), 3.85−3.75 (m, 2.6H), 2.38−2.30 (m, 6.5H), 1.80−1.56 (m, 5.2H), 0.88 (brs, 12H), 0.06 (s, 2H), 0.05 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 222.8, 220.4, 70.4, 70.2, 62.6, 60.5, 54.5, 53.9, 39.1, 38.7, 37.0, 36.6, 26.4, 25.9, 25.8, 23.5, 20.7, 20.5, 18.2, −5.5, −5.6. HRMS-CI (*m*/*z*): $[M - OH]^+$ calcd for $C_{14}H_{27}O_2Si$ 255.1780; found 255.1785.

2,3,6,7-Tetrahydrocyclopenta[*b*]pyran-4(5*H*)-one (16). To a solution of DMSO (425 μL, 468 mg, 6 mmol) in CH_2Cl_2 (3 mL) was added $(CF_3CO)_2O$ (406 μL, 609 mg, 2.9 mmol) dropwise at −78 °C under argon. The resulting mixture was stirred at that temperature for 45 min. Then a precooled solution of ketone **15** (272 mg, 1 mmol) in CH_2Cl_2 (3 mL) was added. The reaction mixture was stirred at −78 °C for 30 min, then at −15 °C for 15 min and cooled back to −78 °C. Et_3N (1.25 mL, 911 mg, 9 mmol) was added, and the mixture was stirred at −78 °C for 45 min. The reaction was quenched by addition of saturated aqueous NH_4Cl solution and the mixture warmed to room temperature. The two phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3×) and then EtOAc (1×). The combined organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography using hexanes/EtOAc (20:1, then 15:1 with a few drops of acetic acid) as the eluent to give the corresponding diketone (221 mg, 82%) as a light orange oil. TLC: R_f = 0.37 (hexanes/EtOAc = 10:1). ^1H NMR ($CDCl_3$, 400 MHz) δ 12.7 (br s, 1H), 3.90 (t, J = 6.2 Hz, 0.66H), 3.89 (t, J = 6.5 Hz, 2H), 3.46 (t, J = 7.8 Hz, 0.33H), 2.86 (dt, J = 3.0, 6.2 Hz, 0.66H), 2.58 (t, J = 7.2 Hz, 2H), 2.45 (t, J = 6.5 hz, 2H), 2.40 (t, J = 7.9 Hz, 2H), 2.31−2.19 (m, 0.66H), 2.10−1.97 (m, 0.66H), 1.95−1.82 (m, 2H), 0.86 (s, 9H), 0.86 (s, 3H), 0.04 (s, 1H), 0.03 (s, 1H), 0.03 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 212.9, 206.1, 203.6, 175.4, 110.9, 62.4, 59.6, 58.5, 45.6, 38.7, 37.8, 37.0, 25.7, 25.6, 25.0, 20.6, 20.3, 18.1, −5.6. HRMS-CI (*m*/*z*): $[M + H]^+$ calcd for $C_{14}H_{26}O_3Si$ 271.1729; found 271.1733.

Diketone (54 mg, 0.2 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C under argon. Trifluoroacetic acid (90 μL, 134 mg, 1.2 mmol) was then added dropwise. The mixture was stirred at 0 °C for 30 min and then warmed to room temperature and stirred for 4 h. As completion was reached, solid $NaHCO_3$ (~150 mg) was then added and the mixture diluted with EtOAc. After being stirred for 10 min, the suspension was filtered on a small Celite pad. The solvent was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using hexanes/EtOAc (4:1) as the eluent to furnish α,β-unsaturated ketone **16** (26 mg, 94%) as a colorless oil. TLC: R_f = 0.23 (hexanes/EtOAc = 3:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.49 (t, J = 6.9 Hz, 2H), 2.59−2.45 (m, 6H), 1.89 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 189.6, 178.5, 114.5, 69.5, 35.4, 32.6, 25.6, 19.0.

Octahydrocyclopenta[*b*]pyran-4-ol [(±)-18]. A solution of α,β-unsaturated ketone **16** (109 mg, 0.79 mmol) in EtOAc (6 mL) was loaded with 10% Pd/C (50 mg, 0.047 mmol) and carefully placed under H_2 (1 atm). The mixture was stirred at room temperature for 12 h. The suspension was then filtered over a Celite pad, the pad washed with EtOAc, and the resulting solution evaporated under reduced pressure. The essentially pure ketone (81 mg) was directly carried out to the next step without further purification. TLC: R_f = 0.37 (hexanes/EtOAc = 3:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.22−4.15 (m, 2H), 3.69 (td, J = 2.8, 12.0 Hz, 1H), 2.71 (ddd, J = 7.2, 12.3, 15.7 Hz, 1H), 2.48 (dt, J = 4.0, 9.0 Hz, 1H), 2.23 (ddt, J = 1.4, 2.8, 15.7 Hz, 1H), 2.00−1.80 (m, 5H), 1.71−1.63 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.2, 82.8, 65.9, 55.1, 38.5, 33.3, 28.4, 22.8.

The ketone was diluted in CH_2Cl_2 (5 mL) under argon and cooled to −78 °C. L-Selectride (1 M solution in THF, 0.80 mL, 0.8 mmol) was added dropwise, and the resulting mixture was stirred at this temperature for 2 h. Hydrogen peroxide (30% aqueous solution, 3 mL) and 3 N NaOH aqueous solution were added, and the mixture was warmed to 23 °C and stirred for 5 h. The phases were separated and the aqueous phase extracted with CH_2Cl_2 (4×). The combined organic phase was washed with brine, dried (Mg_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using hexanes/EtOAc (4:1, then 1.5:1) as the eluent to yield *cis*-bicyclic alcohol (±)-**18** (77 mg, 68% two steps) as a colorless oil. TLC: R_f = 0.13 (hexanes/EtOAc = 2:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.11 (dt, J = 5.6, 11.1 Hz, 1H), 3.91 (ddd, J = 2.0, 4.5, 11.7 Hz, 1H), 3.84−3.81 (m, 1H), 3.33 (dt, J = 2.3, 11.9 Hz, 1H), 2.17−2.08 (m, 1H), 1.92−1.81 (m, 1H), 1.79−1.55 (m, 7H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 80.5, 68.3, 65.4, 47.0, 32.6, 29.7, 21.6, 21.3.

(4*S*,4a*S*,7a*S*)-Octahydrocyclopenta[*b*]pyran-4-ol [(−)-18]. Racemic alcohol (±)-**18** (68 mg, 0.48 mmol) was dissolved in THF (5 mL), and vinyl acetate (225 μL, 2.4 mmol) was added. Amano lipase PS-30 (30 mg) was added, and the resulting suspension was stirred at 15−20 °C. The mixture was left stirring for >48 h until around 50% conversion was reached (as seen by NMR). The resulting suspension was diluted with Et_2O and filtered on Celite, and the filter cake was rinsed with Et_2O. After evaporation of the remaining solvent, the residue was purified by column chromatography using hexanes/EtOAc (5:1, 3:1, then 1.5:1) to yield the desired enantio enriched pyranol (−)-**18** (25 mg, 37%). $[\alpha]_D^{20}$ −47.5 (*c* 1.32, $CHCl_3$). An enantiopurity of 94.1% ee for the alcohol was measured by chiral HPLC analysis of the corresponding activated carbonate **31d**: column ChiralPak IA, 0.7 mL/min, hexanes/IPA (98:2 to 85:15, from 0 to 45 min), λ = 210 nm, T = 30 °C, t_R minor = 22.4 min, t_R major = 23.3 min.

(±)-*endo*,*cis*-Bicyclo[4.3.0]nonan-2-ol [(±)-19]. Enone **17**[33] (106 mg, 0.77 mol) was dissolved in THF (10 mL), and the flask was purged with argon. Pd/C 10% (60 mg, 0.06 mmol) was added to the solution, and the resulting suspension was stirred under hydrogen (1 atm). TLC monitoring first shows isomerization of the enone, through migration of the olefin to the internal position, followed by slow formation of the reduced cis-product. After 12 h, the solution was filtered on a pad of Celite and the solvent removed in vacuo. The residue was purifed by flash column chromatography on silica gel using hexanes/EtOAc (30:1 to 10:1) to give the reduced ketone (98 mg, 92%). TLC: R_f = 0.65 (hexanes/EtOAc = 5:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.62−2.54 (m, 1H), 2.48−2.38 (m, 1H), 2.38−2.23 (m, 2H), 2.08−1.98 (m, 1H), 1.94−1.30 (m, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.6, 53.1, 42.9, 39.6, 31.0, 27.2, 26.6, 23.8, 23.0. A solution of the ketone (135 mg, 0.98 mmol) in CH_2Cl_2 (3 mL) was cooled to −78 °C under argon. L-Selectride (1 M solution THF, 1.2 mL) was added dropwise to the solution, and the reaction mixture was stirred at −78 °C for 1 h. Hydrogen peroxide solution (30% solution, 1.5 mL) and then NaOH (3 M solution, 1.5 mL) were added, and the mixture was warmed to 23 °C and stirred for 1 h. After dilution with water (2 mL) and then addition of Na_2SO_3 saturated aqueous solution (3 mL), the aqueous phase was successively extracted with CH_2Cl_2 (4×). The combined organic phase was dried (Na_2SO_4), filtered, and evaporated in vacuo. The residue was purified by column chromatography on silica gel using hexanes/EtOAc (6:1) to yield racemic alcohol (±)-**19** (92 mg, 66%) as a colorless oil. TLC: R_f = 0.25 (hexanes/EtOAc = 5:1). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.96 (m, 1H), 2.26−2.17 (m, 1H), 1.93 (m, 1H), 1.79−1.53 (m, 7H), 1.47−1.15 (5 H), 0.96 (dq, J = 3.3, 13.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 71.6, 46.4, 40.1, 31.5, 29.5, 27.0, 23.9, 21.4, 21.2. HRMS-EI (*m*/*z*): $[M - OH]^+$ calcd for C_9H_{15} 122.1096, found 122.1097.

(1*R*,2*S*,6*R*)-Bicyclo[4.3.0]nonan-2-ol [(−)-19]. Racemic **19** (86 mg, 0.62 mmol) was dissolved in THF (5 mL), and vinyl acetate (0.5 mL) was added. Amano lipase PS-30 (60 mg) was added, and the resulting suspension was stirred at 23 °C until 50% conversion was reached (NMR) in ~6 h. The resulting suspension was diluted with Et_2O and filtered on Celite, and the filter cake was rinsed with Et_2O. After evaporation of the remaining solvent, the residue was purified by column chromatography using hexanes/EtOAc (8:1, 6:1, then 4:1) to yield acetate **21** and the desired enantioenriched (−)-indanol (−)-**19** (38.5 mg, 45% yield). $[\alpha]_D^{20}$ −28.3° (*c* 1.02, $CHCl_3$), ($[\alpha]_D^{20}$ lit. −27.2° (*c* 1.0, $CHCl_3$)).[38] The enantiomeric excess of the 2,4-dinitrobenzoate derivative was determined to be 89.9% ee by chiral HPLC, column ChiralPak IA, hexane/isopropanol (100/0 to 90/10, 15 min; 90/10 to 80/20, 15 min), 1 mL/min, t_R minor = 16.58 min, t_R major = 19.5 min.

3-[(4-Iodotetrahydrofuran-3-yl)oxy]propan-1-ol (23). To a solution of freshly distilled 2,5-dihydrofuran (700 mg, 0.740 mL, 10 mmol), in a mixture of dry 1,3-propanediol/dimethoxyethane (1:1, 5 mL) at 0 °C under argon was successively added NH_4OAc (77 mg, 1 mmol), followed by *N*-iodosuccinimide (11 mmol, 2.47 g). The mixture was warmed to 23 °C and stirred for 12 h protected from light. The reaction was quenched by addition of saturated aqueous Na_2SO_3 and then diluted with water. The mixture was extracted with Et_2O/EtOAc (1:1). The combined organic phase was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using hexanes/EtOAc (4:1, 3:1, then 2.5:1) to give iodo alcohol **23** (1.2 g, 45%) as a pale yellow oil. TLC: R_f = 0.3 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.33 (m, 1H), 4.29−4.19 (m, 3H), 4.04 (dd, J = 2.2, 9.8 Hz, 1H), 3.79 (dd, J = 1.5, 9.8 Hz, 1H), 3.76−3.69 (m, 3H), 3.60 (m, 1H), 1.81 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 88.2, 76.1, 71.8, 67.9, 60.6, 32.3, 23.4.

3-[(4-Iodotetrahydrofuran-3-yl)oxy]propanal (24). Oxalyl chloride (580 mg, 392 μL, 4.6 mmol) was diluted in CH_2Cl_2 (12 mL) under argon, and the solution was cooled to −78 °C. Dry DMSO (715 mg, 650 μL, 9.15 mmol) in CH_2Cl_2 (3 mL) was added to the cold solution dropwise, and the mixture was stirred for 30 min. A solution of alcohol **23** (500 mg, 1.83 mmol) in CH_2Cl_2 (4 mL) was then added slowly, and the mixture was kept stirring for an additional hour at −78 °C. Et_3N (1.3 g, 1.8 mL,

12.8 mmol) was then introduced. The white suspension was stirred at −78 °C for 20 min and slowly warmed to room temperature. A 0.5 M phosphate buffer solution pH 5.5 (20 mL) was added. The two phases were separated, and the resulting aqueous phase was extracted with Et_2O (4×). The combined organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash column chromatography using hexanes/EtOAc (6:1 to 4:1) to yield the desired aldehyde **24** (433 mg, 86%) as a light yellow oil. TLC: R_f = 0.76 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.77 (t, J = 1.3 Hz, 1H), 4.35 (m, 1H), 4.30−4.19 (m, 3H), 4.04 (dd, J = 2.3, 9.8 Hz, 1H), 3.92 (ddd, J = 5.3, 6.7, 9.5 Hz, 1H), 3.77 (dd, J = 1.7, 10.1 Hz, 1H), 3.75 (ddd, J = 5.2, 6.2, 9.5 Hz, 1H), 2.69 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.1, 88.3, 76.1, 71.8, 63.1, 43.7, 23.3.

Hexahydro-2*H*-furo[3,4-*b*]pyran-4-ol [(±)-25]. To a solution of aldehyde **24** (100 mg, 0.37 mmol) in DME (10 mL) was successively added indium (60 mg, 0.55 mmol), CuI (48 mg, 0.25 mmol), and a catalytic amount of iodine (10 mg, 0.037 mmol). After the suspension was stirred for 5 min, water (4 mL) was added and the mixture was stirred at room temperature for 4 h. The suspension was filtered on a Celite pad, washing the pad with THF. The solvent was reduced under vacuum and the resulting aqueous phase acidified with 1 M HCl and saturated with NaCl. The aqueous phase was extracted with EtOAc, and the combined organic phase was dried over $MgSO_4$. After filtration and evaporation, the crude was purified by flash column chromatography on silica gel using hexanes/EtOAc (1:1 to 1:5) to provide the bicyclic alcohol (±)-**25** (25 mg, 47%) as a mixture of diastereoisomers. TLC: R_f = 0.28 (EtOAc 100%). Pyridinium chlorochromate (74 mg, 0.346 mmol) was added to a suspension of flame-dried 4 Å molecular sieves in CH_2Cl_2 (2 mL) at room temperature under argon. A solution of the above alcohol (25 mg, 0.173 mmol) in CH_2Cl_2 (1.5 mL) was transferred to the suspension at 0 °C, and the solution was stirred for 1 h at 0 °C. The reaction was quenched by addition of isopropanol, and the mixture was filtered on a silica pad, flushing with Et_2O. After evaporation of the solvent, the corresponding ketone thus obtained was used directly in the next step. TLC: R_f = 0.45 (hexanes/EtOAc = 1:1). The ketone was redissolved in EtOH (1.5 mL). The solution was cooled to −20 °C, and $NaBH_4$ (25 mg, 0.66 mmol) was added at once. After being stirred at this temperature for 30 min, the reaction was quenched by addition of saturated aqueous NH_4Cl solution (1.5 mL). The solution was extracted with EtOAc and the combined organic phase dried (Na_2SO_4), filtered, and evaporated. The corresponding racemic alcohol (±)-**25** was purified by flash column chromatography using hexanes/EtOAc (1:1 to 1:5) as the eluent. Colorless oil (12 mg, 50% two steps). TLC: R_f = 0.25 (100% EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.26 (m, 1H), 4.05 (t, J = 3.0 Hz, 1H), 4.04−3.95 (m, 3H), 3.94−3.85 (m, 2H), 3.40 (dt, J = 2.5, 11.8 Hz, 1H), 2.60 (m, 1H), 1.94 (d, J = 4.0 Hz, 1H), 1.80 (ddt, J = 4.6, 11.5, 12.5 Hz, 1H), 1.74 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 78.3, 74.5, 67.1, 66.4, 65.0, 45.5, 30.0.

To a solution of racemic (±)-**25** (10 mg, 0.07 mmol) in dry THF (1 mL) under an argon atmosphere was added vinyl acetate (60 mg, 65 μL, 0.7 mmol) followed by addition of immobilized Amano Lipase PS-30 (10 mg) on Celite-545. The mixture was stirred at 15−20 °C for 2 days until > 50% conversion could be observed by NMR of aliquots. The resulting suspension was diluted in Et_2O and filtered on a small Celite pad. The solvents were evaporated and the residue was purified by flash chromatography using hexanes/EtOAc (1:1 to 1:5) as the eluent to give enantiomeric alcohol **25** (4.6 mg, 46%) as a colorless oil. An enantiopurity of > 99.5% ee for the alcohol was measured by analysis of the corresponding activated carbonate **31f** on chiral HPLC (column ChiralPak IC, hexane/isopropanol 52:48, 1 mL/min, λ = 215 nm, T = 24 °C, t_R minor = 14.4 min, t_R major = 15.5 min).

(3a*R*,6a*R*)-Tetrahydrofuro[2,3-*b*]furan-3(2*H*)-one (28). Enantiomerically pure (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-ol (bis-THF) **27** (85 mg, 0.65 mmol) was diluted in dry CH_2Cl_2 (6 mL) under argon. The solution was cooled to 0 °C, and anhydrous Na_2HPO_4 (52 mg, 0.36 mol) was added. Dess−Martin periodinane (360 mg, 0.85 mmol) was added at once at 0 °C and the resulting suspension warmed to 23 °C and stirred for 3 h. The reaction was then quenched by successive addition of saturated aqueous $NaHCO_3$ and saturated aqueous Na_2SO_3 solutions (1.5 + 1.5 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 and then EtOAc. The combined organic phases were dried (Na_2SO_4), filtered on a small pad of silica gel, and evaporated to dryness. The residue was purified by column chromatography on silica gel using hexanes/EtOAc (3:1) to furnish ketone **28** (73 mg, 87%) as a white crystalline solid. TLC: R_f = 0.57 (hexanes/EtOAc = 1:1). Spectral data corresponded to those previously reported in the literature.[35]

(3a*S*,7a*R*)-Tetrahydro-2*H*-furo[2,3-*b*]pyran-5(3*H*)-one (29). $AlMe_3$ (25% w/w hexanes, 250 μL, 0.6 mmol) was diluted in dry CH_2Cl_2 (5 mL) under argon, and the solution was cooled to −78 °C. A solution of ketone **28** (64 mg, 0.5 mmol) in dry CH_2Cl_2 (5 mL) was slowly added dropwise. After 10 min, $TMSCHN_2$ (2 M solution in Et_2O, 275 μL, 0.55 mmol) was added. The mixture was stirred for 2 h while warming to −30 °C. Saturated Rochelle's salt solution (5 mL) was added, and the mixture was stirred for 1 h. The phases were separated. The aqueous phase was extracted with CH_2Cl_2, and the combined organic phase was dried ($MgSO_4$). The solution was filtered on a small silica gel pad, flushing with Et_2O, and the collected organic phase was evaporated. A crude mixture of the desired ketone along with α-silylated derivatives and isomers was then obtained. The mixture was redissolved in THF (5 mL). AcOH (6 drops) and TBAF (0.5 mL, 0.5 mmol) were successively added. The resulting mixture was stirred at 23 °C for 3 h and evaporated to dryness. The residue was purified by flash column chromatography on silica gel using hexanes/EtOAc (5:1) as the eluent to give ketone **29** (45 mg, 63%). TLC: R_f = 0.35 (hexanes/EtOAc = 2:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.49 (d, J = 6.8 Hz, 1H), 4.11 (d, J = 18.2 Hz, 1H), 4.10 (m, 1H), 3.92 (d, J = 18.2 Hz, 1H), 3.74 (dt, J = 6.5, 8.9 Hz, 1H), 2.85 (m, 1H), 2.71 (d, J = 6.3, 15.6 Hz, 1H), 2.48 (d, J = 3.9, 15.6 Hz, 1H), 2.15 (m, 1H), 1.55 (ddt, J = 7.7, 8.9, 12.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.7, 100.9, 67.5, 67.1, 39.2, 36.2, 31.3.

(3a*S*,5*R*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-5-ol (30). A solution of ketone **29** (25 mg, 0.173 mmol) dissolved in CH_2Cl_2 (5 mL) was cooled to −78 °C under argon. L-Selectride (1 M in THF, 200 μL, 0.2 mmol) was added dropwise. The solution was stirred at this temperature for 3 h and quenched by addition of saturated aqueous NH_4Cl solution. The aqueous phase was extracted with EtOAc. The combined organic extract was dried (Na_2SO_4), filtered, and evaporated. The crude was purified by column chromatography on silica gel using hexanes/EtOAc (2:1, 1:1, then 1:2) to yield alcohol **30** as a 5:1 mixture of diastereoisomers (18 mg, cis major). The stereoisomers were separated in the subsequent synthesis of the mixed activated carbonate **31g**. TLC: R_f = 0.25 (hexanes/EtOAc = 1:2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.08 (d, J = 3.8 Hz, 0.2H), 5.05 (d, J = 3.3 Hz, 1H), 4.16−4.11 (m, 1.2H), 3.95−3.84 (m, 1.6H), 3.81−3.70 (m, 2H), 3.63 (m, 1H), 3.27 (dd, J = 7.9, 11.2 Hz, 0.2H), 2.35−1.70 (m, 6H).

(3a*S*,4*S*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (4-Nitrophenyl) Carbonate (31a). Furopyranol ligand (−)-**7** (9 mg, 0.063 mmol) was diluted in CH_2Cl_2 (0.5 mL) under argon. The solution was cooled to 0 °C, and dry pyridine (17 μL, ~0.21 mmol) was added. 4-Nitrophenyl chloroformate (24 mg, 0.12 mmol) was added at once to the solution, upon which a white precipitate formed. The mixture was stirred for 2 h while warming to room temperature. Upon completion, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel using hexanes/EtOAc (6:1, then 3:1) as the eluent to give the corresponding

activated carbonate **31a** (18 mg, >99%). TLC: R_f = 0.25 (hexanes/EtOAc = 3:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.29 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.7 Hz, 2H), 5.30−5.19 (m, 1 H), 5.07 (d, J = 2.7 Hz, 1H), 4.28 (dt, J = 3 Hz, 1H), 4.04−3.95 (m, 2H), 3.47−3.37 (m, 1H), 2.80−2.68 (m, 1H), 2.30−2.10 (m, 1H), 2.05−1.90 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.3, 151.7, 145.4, 125.3, 121.7, 101.1, 75.4, 68.5, 60.5, 43.2, 25.8, 22.5.

(3a*R*,4*R*,7a*S*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (4-Nitrophenyl) Carbonate (31b). The title compound was obtained from (+)-**7** as described for (−)-**7** in 86% yield after purification by column chromatography on silica gel using hexanes/EtOAc (6:1, then 3:1). Spectral data were consistent with those recorded for **31a**.

(3a*R*,4*S*,7a*R*)-Octahydrobenzofuran-4-yl (4-Nitrophenyl) Carbonate (31c). The title compound was obtained from (−)-**12** as described for (−)-**7** in 83% yield after purification by column chromatography on silica gel using hexanes/EtOAc (8:1 to 6:1). TLC: R_f = 0.7 (hexanes/EtOAc = 3:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (d, J = 9.2 Hz, 2H), 7.39 (d, J = 9.2 Hz, 2H), 5.07 (m, 1H), 4.13−4.05 (m, 2H), 3.90 (q, J = 8.2 Hz, 1H), 2.72 (m, 1H), 2.10−2.00 (m, 2H), 1.90−1.68 (m, 4H), 1.55−1.45 (m, 1H), 1.34−1.23 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.4, 151.9, 145.2, 125.2, 121.7, 77.7, 77.1, 66.5, 41.2, 27.0, 26.2, 25.4, 18.0.

((4*S*,4a*R*,7a*S*)-Octahydrocyclopenta[*b*]pyran-4-yl) (4-Nitrophenyl) Carbonate (31d). The title compound was obtained from (−)-**18** as described for (−)-**7** in 85% yield after purification by column chromatography on silica gel using hexanes/CH_2Cl_2/THF (4:1:0 then 4:1:0.1) as the eluent. TLC: R_f = 0.31 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (d, J = 9.1 Hz, 2H), 7.38 (d, J = 9.1 Hz, 2H), 5.21 (m, 1H), 4.00 (ddd, J = 1.8, 4.7, 12.0 Hz, 1H), 3.93 (dt, J = 2.5, 2.7 Hz, 1H), 3.43 (dt, J = 2.1, 12.0 Hz, 1H), 2.36 (m, 1H), 2.04−1.82 (m, 4H), 1.82−1.62 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5, 151.9, 145.3, 125.3, 121.8, 80.7, 77.3, 65.0, 43.7, 32.6, 26.3, 22.3, 21.7.

(3a*R*,4*S*,7a*R*)-Octahydro-1*H*-inden-4-yl (4-Nitrophenyl) Carbonate (31e). The title compound was obtained from (−)-**19** as described for (−)-**7** in 90% yield after purification by column chromatography on silica gel using hexanes/EtOAc (20:1 to 10:1) as the eluent. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (d, J = 9.1 Hz, 2H), 7.38 (d, J = 9.1 Hz, 2H), 5.05 (m, 1H), 2.41 (m, 1H), 2.05 (m, 1H), 1.98−1.24 (m, 11H), 1.05 (dq, J = 3.4, 12.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.7, 151.9, 145.2, 125.2, 121.8, 80.7, 42.8, 40.2, 31.3, 26.6, 25.7, 23.4, 22.4, 21.3.

(4*S*,4a*S*,7a*R*)-Hexahydro-2*H*-furo[3,4-*b*]pyran-4-yl (4-Nitrophenyl) Carbonate (31f). The title was obtained from (−)-**25** as described for (−)-**7** in >99% yield following column chromatography purification on silica gel using hexanes/EtOAc (3:1, then 2:1) as the eluent. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (d, J = 9.1 Hz, 2H), 7.38 (d, J = 9.1 Hz, 2H), 5.32 (m, 1H), 4.20−3.88 (m, 6H), 3.50 (m, 1H), 2.81 (m, 1H), 2.10−1.90 (m, 2H).

[(3a*S*,5*R*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-5-yl] (4-Nitrophenyl) Carbonate (31g). The title compound was obtained from **30** as described for (−)-**7** in 70% yield. Purification and separation from the 5-*epi* diastereoisomer were performed following flash column chromatography on silica gel using hexanes/EtOAc (3:1, 2:1, then 1:1) as the eluent. Amorphous solid (70% from a 5:1 mixture of diastereoisomers). TLC: R_f = 0.16 (hexanes/EtOAc = 2:1). ^{1}H NMR (C_6D_6, 800 MHz) δ 7.64 (d, J = 9.0 Hz, 2H), 6.69 (d, J = 9.0 Hz, 2H), 4.76 d, J = 3.6 Hz, 1H), 4.35 (m, 1H), 4.02 (dt, J = 3.8, 8.6 Hz, 1H), 3.94 (dt, J = 2.8, 13.0 Hz, 1H), 3.60 (q, J = 8.0 Hz, 1H), 3.12 (dd, J = 2.0, 13.0 Hz), 2.04 (m, 1H), 1.67 (dq, J = 3.1, 15.1 Hz, 1H), 1.50 (m, 1H), 1.46−1.38 (m, 2H). ^{13}C NMR (C_6D_6, 200 MHz) δ 154.9, 151.9, 145.2, 124.9, 121.2, 100.7, 72.0, 67.4, 63.8, 35.9, 27.9, 27.3.

(3a*S*,4*S*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl-(2*S*,3*R*)-4-(*N*-isobutyl-4-methoxyphenyl sulfonamido)-3-hydroxy-1-phenylbutan-2-yl Carbamate (35a). . Sulfonamide isostere **32** (42 mg, 0.08 mmol) was dissolved in a 30% TFA solution in CH_2Cl_2 (3 mL), the solution was stirred at 23 °C for 2 h after which the solvent was evaporated under reduced pressure. The corresponding Boc-deprotected intermediate (0.08 mmol) was then diluted in dry acetonitrile (0.8 mL) at 0 °C under argon and Et_3N (0.3 mL, 0.2 mmol) was added. A solution of activated carbonate **31a** (18.6 mg, 0.06 mmol) in acetonitrile or THF (0.5 mL) was then added to the mixture. The reaction was stirred at 23 °C until completion was reached (2−3 days). The solution was then evaporated *in vacuo* and the resulting residue purified by flash chromatography on silica gel using hexanes/EtOAc (2:1 then 1:1) as the eluent to afford the inhibitor **35a** as a amorphous solid (19.8 mg, 55%). TLC R_f = 0.35 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.71 (d, J = 8.9 Hz, 2H), 7.33−7.17 (m, 5H), 6.97 (d, J = 8.9 Hz, 2H), 5.05−4.90 (m, 1H), 4.93 (d, J = 3.6 Hz, 1H), 4.84 (d, J = 8.4 Hz, 1H), 4.15 (dt, J = 2.4, 9.0 Hz, 1H), 3.87 (s, 3H), 3.98−3.76 (m, 4H), 3.31 (t, J = 11.7 Hz, 1H), 3.22−2.90 (m, 4H), 2.90−2.78 (m, 2H), 2.48−2.32 (m, 1H), 1.96−1.25 (m, 5H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.1, 155.5, 137.6, 129.8, 129.4, 128.4, 126.5, 114.3, 101.1, 72.9, 70.2, 68.5, 60.9, 58.9, 55.7, 54.9, 53.8, 43.5, 35.6, 27.3, 26.2, 22.3, 20.2, 19.9. HRMS-ESI (m/z): [M + Na]$^+$ calcd for $C_{29}H_{40}N_2O_8NaS$ 599.2403, found 599.2406.

(3a*S*,4*S*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-4-(4-Amino-*N*-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl Carbamate (36). The title compound was obtained from **31a** and sulfonamide isostere **33** as described for inhibitor **35a**, in 64% yield following purification by flash chromatography using $CHCl_3$/2% MeOH as the eluent. TLC: R_f = 0.45 (hexanes/EtOAc = 1:3). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55 (d, J = 8.7 Hz, 2H), 7.32−7.16 (m, 5H), 6.67 (d, J = 8.7 Hz, 2H), 4.97 (m, 1H), 4.93 (d, J = 3.4 Hz, 1H), 4.85 (d, J = 8.7 Hz, 1H), 4.20−4.11 (m, 3H), 3.92−3.80 (m, 5H), 3.31 (dt, J = 2.2, 11.9 Hz, 1H), 3.15 (dd, J = 8.1, 15.2 Hz, 1H), 3.05 (dd, J = 4.2, 14.1 Hz, 1H), 3.01−2.80 (m, 3H), 2.75 (dd, J = 6.6, 13.4 Hz, 1H), 2.40 (m, 1H), 1.97−1.60 (m, 4H), 1.46 (m, 1H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5, 150.7, 137.7, 129.5, 129.5, 128.4, 126.5, 126.2, 114.1, 101.1, 72.8, 70.1, 68.5, 60.8, 58.9, 54.8, 53.8, 43.4, 35.5, 27.3, 26.2, 22.2, 20.2, 19.9. HRMS-ESI (m/z): [M + Na]$^+$ calcd for $C_{28}H_{39}N_3O_7NaS$ 584.2406; found 584.2402.

(3a*S*,4*S*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-Hydroxy-4-(4-(hydroxymethyl)-*N*-isobutylphenylsulfonamido)-1-phenylbutan-2-yl Carbamate (37). The title compound was obtained from **31a** and sulfonamide isostere **34** as described for inhibitor **35a** in 72% yield following purification by flash chromatography on silica gel using $CHCl_3$/2% MeOH as the eluent. Amorphous solid. TLC: R_f = 0.23 (hexanes/EtOAc = 1:2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 8.1 Hz, 2H), 7.32−7.17 (m, 5H), 4.96 (m, 1H), 4.93 (d, J = 3.2 Hz, 1H), 4.85 (d, J = 8.5 Hz, 1H), 4.80 (s, 2H), 4.15 (t, J = 8.5 Hz, 1H), 3.92−3.80 (m, 4H), 3.70 (s, 1H), 3.31 (t, J = 11.6 Hz, 1H), 3.16 (dd, J = 8.0, 15.0 Hz, 1H), 3.10−2.95 (m, 3H), 2.88−2.76 (m, 2H), 2.41 (m, 1H), 2.04 (m, 1H), 1.95−1.78 (m, 2H), 1.76−1.56 (m, 2H), 1.47 (m, 1H), 0.93 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.6, 146.2, 137.6, 137.1, 129.4 128.5, 127.6, 127.1, 126.5, 101.1, 72.8, 70.2, 68.4, 64.2, 60.8, 58.8, 54.9, 53.7, 43.4, 35.5, 27.3, 26.2, 22.2, 20.1, 19.9. HRMS-ESI (m/z): [M + Na]$^+$ calcd for $C_{29}H_{40}N_2O_8NaS$ 599.2403, found 599.2414.

(3a*R*,4*R*,7a*S*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl ((2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (35b). The title compound was obtained from **31b** and sulfonamide isostere **32** in 65% yield as described for inhibitor **35a**, following purification by column chromatography on silica gel using hexanes/EtOAc (3:1, then 1.5:1) as the eluent. White amorphous solid. TLC: R_f = 0.44 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, J = 8.9 Hz, 2H), 7.31−7.26 (m, 2H), 7.25−7.20 (m, 3H), 6.98 (d, J = 8.9 Hz, 2H), 5.00 (m, 1H), 4.97 (d, J = 2.7 Hz, 1H), 4.88 (d, J = 8.0 Hz, 1H), 4.17 (t, J = 7.7 Hz, 1H), 3.99−3.72 (m, 6H), 3.87 (s, 3H), 3.31 (dt, J = 1.9, 12.0 Hz, 1H), 3.13 (dd, J = 8.4, 15.0 Hz, 1H), 3.08−2.84 (m, 4H), 2.79 (dd, J = 6.7, 13.4 Hz, 1H), 2.53 (m, 1H), 2.00 (m, 1H), 1.83 (m, 1H), 1.73

(m, 1H), 1.68−1.54 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 155.7, 137.7, 129.8, 129.5, 128.5, 126.5, 114.3, 101.2, 72.6, 70.2, 68.4, 60.8, 58.7, 55.6, 55.1, 53.7, 43.6, 35.3, 27.3, 26.2, 22.5, 20.1, 19.9. HRMS-ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{29}H_{40}N_2O_8NaS$ 599.2403, found 599.2407.

(3a*R*,4*S*,7a*R*)-Octahydrobenzofuran-4-yl (2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl Carbamate (35c). The title compound was obtained from **31c** and sulfonamide isostere **32** in 75% yield as described for inhibitor **35a**, following purification by column chromatography on silica gel using hexanes/EtOAc (3:1, then 2.5:1) as the eluent. TLC: R_f = 0.39 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (d, J = 8.9 Hz, 2H), 7.311−7.16 (m, 5H), 6.98 (d, J = 8.9 Hz, 2H), 4.83 (m, 2H), 3.95−3.75 (m, 5H), 3.87 (s, 3H), 3.68 (q, J = 8.1 Hz, 1H), 3.14 (dd, J = 8.4, 15.2 Hz, 1H), 3.08 (dd, J = 4.1, 14.1 Hz, 1H), 3.05−2.99 (m, 1H), 2.96 (dd, J = 8.4, 13.4 Hz, 1H), 2.87−2.75 (m, 2H), 2.35 (m, 1H), 1.83 (m, 1H), 1.70−1.40 (m, 7H), 1.20 (m, 1H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.0, 156.1, 137.7, 129.7, 129.5, 129.4, 128.4, 126.4, 114.3, 73.0, 71.8, 66.6, 58.8, 55.6, 54.7, 53.7, 41.2, 35.6, 27.3, 27.2, 27.0, 25.7, 20.1, 19.9, 17.7. HRMS-ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{30}H_{42}N_2O_7NaS$ 597.2610, found 597.2621.

(4*S*,4a*R*,7a*S*)-Octahydrocyclopenta[*b*]pyran-4-yl ((2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (35d). The title compound was obtained from **31d** and sulfonamide isostere **32** in 81% yield as described for inhibitor **35a**, following purification by column chromatography on silica gel using hexanes/EtOAc (3:1, then 2.5:1) as the eluent. TLC: R_f = 0.58 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, J = 8.9 Hz, 2H), 7.30−7.17 (m, 5H), 6.96 (d, J = 8.9 Hz, 2H), 4.94 (m, 1H), 4.81 (d, J = 8.1 Hz, 1H), 3.86 (s, 3H), 3.90−3.76 (m, 4H), 3.33 (t, J = 11.9 Hz, 1H), 3.13 (dd, AB, J = 8.3, 15.0 Hz, 1H), 3.08−2.91 (m, 3H), 2.85 (m, 1H), 2.79 (dd, J = 6.8, 13.5 Hz, 1H), 2.04 (m, 1H), 1.81 (m, 2H), 1.76−1.64 (m, 3H), 1.64−1.49 (m, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.0, 156.0, 137.7, 129.8, 129.4, 128.4, 126.4, 114.3, 80.5, 72.7, 71.7, 65.2, 58.7, 55.6, 54.8, 53.7, 44.1, 35.6, 32.5, 27.2, 26.6, 22.0, 21.6, 20.1, 19.8. HRMS-ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{30}H_{42}N_2O_7S$ 597.2610, found 597.2612.

(3a*R*,4*S*,7a*R*)-Octahydro-1*H*-inden-4-yl-(2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl Carbamate (35e). The title compound was obtained from **31e** and sulfonamide isostere **32** as described for inhibitor **35a**. Following preliminary purification by flash chromatography using hexanes/CH_2Cl_2/THF (8:1:1) as the eluent, the inhibitor was obtained as a mixture of unseparable isomeric compounds. Compound **35e** was derivatized into the corresponding *N*,*O*-isopropylidene compound by treatment of **35e** (20 mg) with 2,2-dimethoxypropane (0.1 mL) and a catalytic amount of *p*TSA (1.5 mg) in dry CH_2Cl_2 (1 mL) for 8 h at 23 °C. After neutralization with Et_3N, the organic phase was evaporated to dryness. Following a quick silica gel column (hexanes/EtOAc = 8:1), the resulting inhibitor was purified by HPLC: preparative HPLC column Sunfire Prep C18 OBD, 30 mm × 100 mm, eluent MeOH/H_2O 85:15 (30 min) and then 90:10 (15 min), flow rate 15 mL·min^{-1}, t_R = 42 min. The isopropylidene derivative was then obtained as a colorless oil (24 mg). The product was then taken into MeOH (2 mL). *p*TSA·H_2O (36 μmol, 1.5 mg) was added, and the resulting solution was refluxed for 6 h. After neutralization with a few drops of Et_3N, the solution was evaporated and the residue purified by column chromatography on silica gel using hexanes/CH_2Cl_2/THF (8:1:1) to give inhibitor **35e** (15 mg, 43% from **31e**). TLC: R_f = 0.35 (hexanes//EtOAc = 5:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (d, J = 8.9 Hz, 2H), 7.32−7.18 (m, 5H), 6.97 (d, J = 8.9 Hz, 2H), 4.79 (m, 1H), 4.70 (d. J = 8.1 Hz, 1H), 3.90 (m, 1H), 3.87 (s, 3H), 3.81 (m, 1H), 3.18−3.02 (m, 3H), 2.98−2.82 (m, 2H), 2.78 (dd, J = 6.6, 13.2 Hz, 1H), 2.10 (m, 1H), 1.90 (m, 1H), 1.82 (m, 1H), 1.74−1.19 (m, 11H), 0.95 (m, 1H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.0, 156.4, 137.7, 129.9, 129.5, 129.4, 128.5, 126.4, 114.3, 74.9, 72.8, 58.8, 55.6, 54.8, 53.8, 43.1, 39.9, 35.7, 31.3, 27.2, 26.9, 26.1, 23.5, 22.2, 21.3, 20.1, 19.9. HRMS-ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{31}H_{44}N_2O_6NaS$ 595.2818, found 595.2816.

(4*S*,4a*S*,7a*R*)-Hexahydro-2*H*-furo[3,4-*b*]pyran-4-yl ((2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (35f). The title compound was obtained from **31f** and sulfonamide isostere **32** in 75% yield as described for inhibitor **35a**, following purification by column chromatography using hexanes/EtOAc (3:1, then 2.5:1) as the eluent. TLC: R_f = 0.24 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 800 MHz) δ 7.70 (d, J = 8.8 Hz, 2H), 7.30 (m, 2H), 7.24−7.20 (m, 3H), 6.97 (d, J = 8.8 Hz, 2H), 5.05 (m, 1H), 4.83 (d, J = 8.5 Hz, 1H), 4.03 (t, J = 3.2 Hz, 1H), 3.96 (m, 1H), 3.87 (s, 3H), 3.87 (s, 3H), 3.88−3.81 (m, 5H), 3.62 (t, J = 8.3 Hz, 1H), 3.39 (t, J = 11.5 Hz, 1H), 3.14 (dd, J = 8.4, 15.0 Hz, 1H), 3.02 (dd, J = 4.0, 14.1 Hz, 1H), 2.99−2.94 (m, 2H), 2.84 (dd, J = 8.7, 14.1 Hz, 1H), 2.77 (dd, J = 6.6, 13.4 Hz, 1H), 2.51 (m, 1H), 1.81 (m, 1H), 1.78 (dq, J = 4.5, 12.4 Hz, 1H), 1.71 (dd, J = 5.4, 12.4 Hz, 1H), 0.91 (d J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 200 MHz) δ 163.0, 155.5, 137.5, 129.6, 129.45, 129.38, 128.5, 126.6, 114.3, 78.4, 74.4, 72.6, 70.0, 66.1, 64.9, 58.8, 55.6, 54.9, 53.7, 42.7, 35.4, 27.2, 26.9, 20.1, 19.8. HRMS-ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{29}H_{40}N_2O_8S$ 599.2403, found 599.2397.

(3a*S*,5*R*,7a*R*)-Hexahydro-2*H*-furo[2,3-*b*]pyran-5-yl ((2*S*,3*R*)-3-Hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate (35g). The title compound was obtained from **31g** and sulfonamide isostere **32** in 86% yield as described for inhibitor **35a**, following purification by column chromatography on silica gel using hexanes/EtOAc (gradient 3:1 to 1.5:1) as the eluent. TLC: R_f = 0.33 (hexanes/EtOAc = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (d, J = 8.9 Hz, 2H), 7.32−7.26 (m, 2H), 7.25−7.17 (m, 3H), 6.98 (d, J = 8.9 Hz, 2H), 4.98 (d, J = 3.5 Hz, 1H), 4.89 (d, J = 8.7 Hz, 1H), 4.54 (m, 1H), 4.11 (dt, J = 3.5, 8.3 Hz, 1H), 3.87 (s, 3H), 3.90−3.77 (m, 4H), 3.74 (m, 1H), 3.56 (d, J = 12.7 Hz, 1H), 3.12 (dd, J = 8.5, 15.1 Hz, 1H), 3.09−2.91 (m, 3H), 2.84 (dd, J = 8.5, 14.1 Hz, 1H), 2.79 (dd, J = 6.8, 13.4 Hz, 1H), 2.08 (m, 1H), 2.04−1.93 (m, 2H), 1.90−1.76 (m, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.4, 155.7, 137.6, 129.7, 129.5, 128.5, 126.5, 114.4, 101.0, 72.5, 68.0, 67.1, 65.4, 58.8, 55.6, 54.9, 53.8, 36.2, 35.8, 28.3, 27.8, 27.2, 20.1, 19.9. HRMS-ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{29}H_{40}N_2O_8NaS$ 599.2403, found 599.2397.

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Supporting Information Available: HPLC and HRMS data of inhibitors **35a**−**g**, **36**, and **37**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Conway, B. HAART in Treatment-Experienced Patients in the 21st Century: The Audacity of Hope. *Future Virol.* **2009**, *4*, 39–41.
(2) Bartlett, J. A.; DeMasi, R.; Quinn, J.; Moxham, C.; Rousseau, F. Overview on the Effectiveness of Triple Combination Therapy in

Antiretroviral-Naive HIV-1 Infected Adults. *AIDS* **2001**, *15*, 1369–1377.
(3) Walensky, R. P.; Paltiel, A. D.; Losina, E.; Mercincavage, L. M.; Schackman, B. R.; Sax, P. E.; Weinstein, M. C.; Freedberg, K. A. The Survival Benefits of AIDS Treatment in the United States. *J. Infect. Dis.* **2006**, *194*, 11–19.
(4) Sepkowitz, K. A. AIDS: The First 20 Years. *N. Engl. J. Med.* **2001**, *344*, 1764–1772.
(5) Palella, F. J., Jr.; Delaney, K. M.; Moorman, A. C.; Loveless, M. O.; Fuhrer, J.; Satten, G. A.; Aschman, D. J.; Holmberg, S. D. Declining Morbidity and Mortality among Patients with Advanced Human Immunodeficiency Virus Infection. *N. Engl. J. Med.* **1998**, *338*, 853–860.
(6) De Clercq, E. Anti-HIV Drugs: 25 Compounds Approved within 25 Years after the Discovery of HIV. *Int. J. Antimicrob. Agents* **2009**, 307–320.
(7) Pillay, D.; Bhaskaran, K.; Jurriaans, S.; Prins, M.; Masquelier, B.; Dabis, F.; Gifford, R.; Nielsen, C.; Pedersen, C.; Balotta, C.; Rezza, G.; Ortiz, M.; de Mendoza, C.; Kucherer, C.; Poggensee, G.; Gill, J.; Porter, K. The Impact of Transmitted Drug Resistance on the Natural History of HIV Infection and Response to First-Line Therapy. *AIDS* **2006**, *20*, 21–28.
(8) Grabar, S.; Pradier, C.; Le Corfec, E.; Lancar, R.; Allavena, C.; Bentata, M.; Berlureau, P.; Dupont, C.; Fabbro-Peray, P.; Poizot-Martin, I.; Costagliola, D. Factors Associated with Clinical and Virological Failure in Patients Receiving a Triple Therapy Including a Protease Inhibitor. *AIDS* **2000**, *14*, 141–149.
(9) Little, S. J.; Holte, S.; Routy, J. P.; Daar, E. S.; Markowitz, M.; Collier, A. C.; Koup, R. A.; Mellors, J. W.; Connick, E.; Conway, B.; Kilby, M.; Wang, L.; Whitcomb, J. M.; Hellmann, N. S.; Richman, D. D. Antiretroviral-Drug Resistance among Patients Recently Infected with HIV. *N. Engl. J. Med.* **2002**, *347*, 385–394.
(10) Grant, R. M.; Hecht, F. M.; Warmerdam, M.; Liu, L.; Liegler, T.; Petropoulos, C. J.; Hellmann, N. S.; Chesney, M.; Busch, M. P.; Kahn, J. O. Time Trends in Primary HIV-1 Drug Resistance among Recently Infected Persons. *JAMA, J. Am. Med. Assoc.* **2002**, *288*, 181–188.
(11) Hue, S.; Gifford, R. J.; Dunn, D.; Fernhill, E.; Pillay, D. On behalf of the UK Collaborative Group on HIV Drug Resistance. Demonstration of Sustained Drug-Resistant Human Immunodeficiency Virus Type 1 Lineages Circulating among Treatment-Naive Individuals. *J. Virol.* **2009**, *83*, 2645–2654.
(12) Lucas, G. M. Antiretroviral Adherence, Drug Resistance, Viral Fitness and HIV Disease Progression: A Tangled Web Is Woven. *J. Antimicrob. Chemother.* **2005**, *55*, 413–416.
(13) Spaltenstein, A.; Kazmierski, W. M.; Miller, J. F.; Samano, V. Discovery of Next Generation Inhibitors of HIV Protease. *Curr. Top. Med. Chem.* **2005**, *5*, 1589–1607.
(14) Ghosh, A. K.; Sridhar, P. R.; Kumaragurubaran, N.; Koh, Y.; Weber, I. T.; Mitsuya, H. Bis-tetrahydrofuran: A Privileged Ligand for Darunavir and a New Generation of HIV-Protease Inhibitors That Combat Drug-Resistance. *ChemMedChem* **2006**, *1*, 939–950.
(15) Ghosh, A. K.; Sridhar, P. R.; Leshchenko, S.; Hussain, A. K.; Li, J.; Kovalevsky, A. Yu.; Walters, D. E.; Wedekind, J. E.; Grum-Tokars, V.; Das, D.; Koh, Y.; Maeda, K.; Gatanaga, H.; Weber, I. T.; Mitsuya, H. Structure-Based Design of Novel HIV-1 Protease Inhibitors To Combat Drug Resistance. *J. Med. Chem.* **2006**, *49*, 5252–5261.
(16) Ghosh, A. K.; Xu, C.-X.; Rao, K. V.; Baldridge, A.; Agniswamy, J.; Wang, Y.-F.; Weber, I. T.; Aoki, M.; Miguel, S. G. P.; Amano, M.; Mitsuya, H. Probing Multidrug-Resistance/Protein−Ligand Interaction with New Oxatricyclic Designed Ligands in HIV-1 Protease Inhibitors. *ChemMedChem* **2010**, *5*, 1850–1854.
(17) FDA News Release, June 23, 2006: FDA Approves New HIV Treatment for Patients Who Do Not Respond to Existing Drugs. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2006/ucm108676.htm.
(18) On October 21, 2008, FDA expanded approval to Prezista (darunavir), coadministered with ritonavir and with other antiretroviral agents, for the treatment of HIV-1 infection in treatment-naive adult patients. In addition to the traditional approval, a new dosing regimen for treatment-naive adult patients was approved.
(19) Koh, Y.; Nakata, H.; Maeda, K.; Ogata, H.; Bilcer, G.; Devasamudram, T.; Kincaid, J. F.; Boross, P.; Wang, Y.-F.; Tie, Y.; Volarath, P.; Gaddis, L.; Harrison, R. W.; Weber, I. T.; Ghosh, A. K.; Mitsuya, H. Novel Bis-tetrahydrofuranylurethane-Containing Nonpeptidic Protease Inhibitor (PI) UIC-94017 (TMC114) with Potent Activity against Multi-PI-Resistant Human Immunodeficiency Virus in Vitro. *Antimicrob. Agents Chemother.* **2003**, *47*, 3123–3129.
(20) De Meyer, S.; Azijn, H.; Surleraux, D.; Jochmans, D.; Tahri, A.; Pauwels, R.; Wigerinck, P.; de Bethune, M.-P. TMC114, a Novel Human Immunodeficiency Virus Type 1 Protease Inhibitor Active against Protease Inhibitor-Resistant Viruses, Including a Broad Range of Clinical Isolates. *Antimicrob. Agents Chemother.* **2005**, *49*, 2314–2321.
(21) Lefebvre, E.; Schiffer, C. A. Resilience to Resistance of HIV-1 Protease Inhibitors: Profile of Darunavir. *AIDS Rev.* **2008**, *10*, 131–142.
(22) Ghosh, A. K.; Chapsal, B. D.; Weber, I. T.; Mitsuya, H. Design of HIV Protease Inhibitors Targeting Protein Backbone: An Effective Strategy for Combating Drug Resistance. *Acc. Chem. Res.* **2008**, *41*, 78–86.
(23) Tie, Y.; Boross, P. I.; Wang, Y.-F.; Gaddis, L.; Hussain, A. K.; Leshchenko, S.; Ghosh, A. K.; Louis, J. M.; Harrison, R. W.; Weber, I. T. High Resolution Crystal Structures of HIV-1 Protease with a Potent Non-Peptide Inhibitor (UIC-94017) Active against Multi-Drug-Resistant Clinical Strains. *J. Mol. Biol.* **2004**, *338*, 341–352.
(24) King, N. M.; Prabu-Jeyabalan, M.; Nalivaika, E. A.; Wigerinck, P.; de Bethune, M.-P.; Schiffer, C. A. Structural and Thermodynamic Basis for the Binding of TMC114 a Next-Generation Human Immunodeficiency Virus Type 1 Protease Inhibitor. *J. Virol.* **2004**, *78*, 12012–12021.
(25) Kovalevsky, A. Y.; Liu, F.; Leshchenko, S.; Ghosh, A. K.; Louis, J. M.; Harrison, R. W.; Weber, I. T. Ultra-High Resolution Crystal Structure of HIV-1 Protease Mutant Reveals Two Binding Sites for Clinical Inhibitor TMC114. *J. Mol. Biol.* **2006**, *363*, 161–173.
(26) Amano, M.; Koh, Y.; Das, D.; Li, J.; Leschenko, S.; Wang, Y.-F.; Boross, P. I.; Weber, I. T.; Ghosh, A. K.; Mitsuya, H. A Novel Bis-tetrahydrofuranylurethane-Containing Nonpeptidic Protease Inhibitor (PI), GRL-98065, Is Potent against Multi-PI-Resistant Human Immunodeficiency Virus in Vitro. *Antimicrob. Agents Chemother.* **2007**, *51*, 2143–2155.
(27) (a) Nakashima, H.; Masayuki, S.; Taniguchi, T.; Ogasawara, K. Chiral Preparation of Polyoxygenated Cyclopentanoids. *Synthesis* **2000**, *6*, 817–823. (b) Laumen, K.; Schneider, M. P. A Facile Chemoenzymatic Route to Optically Pure Building Blocks for Cyclopentanoid Natural Products. *J. Chem. Soc., Chem. Commun.* **1986**, 1298–1299.
(28) Ishihara, K.; Kurihara, H.; Yamamoto, H. An Extremely Simple, Convenient, and Selective Method for Acylating Primary Alcohols in the Presence of Secondary Alcohols. *J. Org. Chem.* **1993**, *58*, 3791–3793.
(29) Nara, M.; Terashima, S.; Yamada, S. Stereochemical Studies—LVII Synthesis of Optically Active Compounds by the Novel Use of Meso-Compounds—1. Efficient Synthesis of Two Structural Types of Optically Pure Prostaglandin Intermediates. *Tetrahedron* **1980**, *36*, 3161–3170.
(30) Moriarty, R. M.; Bailey, B. R., III.; Prakash, O.; Prakash, I. Capture of Electron-Deficient Species with Aryl Halides. New Syntheses of Hypervalent Iodonium Ylides. *J. Am. Chem. Soc.* **1985**, *107*, 1375–1378.
(31) Müller, P.; Allenbach, Y. F.; Bernardinelli, G. On the Enantioselectivity of Transition Metal-Catalyzed 1,3-Cycloadditions of 2-Diazocyclohexane-1,3-diones. *Helv. Chim. Acta* **2003**, *86*, 3164–3178.
(32) Ferrie, L.; Reymond, S.; Capdevielle, P.; Cossy, J. Formal Chemoselective Synthesis of Leucascandrolide A. *Org. Lett.* **2007**, *9*, 2461–2464.
(33) Tsantali, G. G.; Takakis, I. M. Expeditious Copper-Catalyzed Conjugate 1,4-Addition of Bromo[2-(1,3-dioxolan-2-yl)ethyl]magnesium to α,β-Cycloalkenones and Subsequent Tranformations. *J. Org. Chem.* **2003**, *68*, 6455–6458.
(34) Shen, Z.-L.; Geo, G.-L.; Loh, T.-P. Indium-Copper and Indium-Silver Mediated Barbier Grignard Type Alkylation Reaction of Aldehydes Using Unactivated Alkyl Halides in Water. *J. Org. Chem.* **2008**, *73*, 3922–3924.
(35) Ghosh, A. K.; Leschenko, S.; Noetzel, M. Stereoselective Photochemical 1,3-Dioxolane Addition to 5-Alkoxymethyl-2(5*H*)-furanone: Synthesis of Bis-tetrahydrofuranyl Ligand for HIV Protease Inhibitor UIC-94017 (TMC-114). *J. Org. Chem.* **2004**, *69*, 7822–7829.
(36) Toth, M. V.; Marshall, G. R. A Simple Continuous Fluorometric Assay for HIV Protease. *Int. J. Pept. Protein Res.* **1990**, *36*, 544–550.
(37) Halgren, T. A. MMFF VII. Characterization of MMFF94, MMFF94s, and Other Widely Available Force Fields for Conformational Energies and for Intermolecular-Interaction Energies and Geometries. *J. Comput. Chem.* **1999**, *20*, 730–748.
(38) Hodgson, D. M.; Lee, G. P.; Marriot, R. E.; Thompson, A. J.; Wisedale, R.; Witherington, J. Isomerisations of Cycloalkene- and Bicycloalkene-Derived Achiral Epoxides by Enantioselective α-Deprotonation. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2151–2161.

Synthesis and Anti-human Immunodeficiency Virus Activity of the Skeleton Isomers of 3′,4′-Di-(*O*)-(−)-camphanoyl-(+)-khellactone

Hiromi NISHIOKA,[a] Koji UESUGI,[a] Norihiro UEDA,[a] Yuka KONDO,[a] Megumi TSUJI,[a] Hitoshi ABE,[b] Takashi HARAYAMA,[a] Takayuki HAMASAKI,[c] Masanori BABA,[c] and Yasuo TAKEUCHI*,[a]

[a] *Division of Pharmaceutical Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University; 1–1–1 Tsushima-Naka, Kita-ku, Okayama 700–8530, Japan:* [b] *Faculty of Engineering, Toyama University; 3190 Gofuku, Toyama 930–8555, Japan: and* [c] *Division of Antiviral Chemotherapy Center for Chronic Viral Diseases Graduate School of Medical and Dental Sciences, Kagoshima University; 8–35–1 Sakuragaoka, Kagoshima 890–8544, Japan.*

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Optically active structural isomers (1b—f and *dst*-1b—f) of 3′,4′-di-(*O*)-(−)-camphanoyl-(+)-khellactone (DCK) were synthesized and their anti-human immunodeficiency virus (HIV) activity was investigated. The value of the sensitivity index (SI) of 1b was greater than that of DCK.

Key words anti-human immunodeficiency virus; coumarin; asymmetric dihydroxylation; new Mosher's method; 3′,4′-di-(*O*)-(−)-camphanoyl-(+)-khellactone

3′,4′-Di-(*O*)-(−)-camphanoyl-(+)-khellactone (DCK) is well known as an anti-human immunodeficiency virus (HIV) compound with remarkably high activity. Lee and his coworkers reported the structure–activity relationship (SAR) of the analogue of DCK (**1a**); in particular, they discussed the optimization of substituents on the angular benzo[2,1-*b*:4,3-*b*′]dipyran skeleton.[1—6] We believe that one of the most important studies on the SAR of DCK must involve the arrangement of an angular benzodipyran skeleton. In this paper, we describe the synthesis and anti-HIV activity of the optically active structural isomers (**1b**—**f** and *dst*-**1b**—**f**) of DCK (Fig. 1).

Our desired compounds (**1b**—**d** and *dst*-**1b**—**d**) can be obtained from hydroxycoumarin by the method used to synthesize DCK. 6-, 5-, or 8-hydroxycoumarin (**2b**—**d**) and benzodipyran derivatives (**3b**—**d**) were successfully prepared by mainly using our reported method.[7] The Sharpless asymmetric dihydroxylation of **3b**—**d** with dihydroquinine-2,5-diphenyl-4,6-pyrimidinediyl diether ((DHQ)$_2$PYR) afforded the optically active diols (**4b**—**d**), which exhibited an *R,R* configuration. On the other hand, dihydroxylation of **3b**—**d** with dihydroquinidine-2,5-diphenyl-4,6-pyrimidinediyl diether ((DHQD)$_2$PYR) yielded *S,S* diols (*ent*-**4b**—**d**). Camphanoylation of **4b**—**d** afforded the final products (**1b**[8]—**d**). Other isomers (**1e**, **f** and *dst*-**1e**, **f**) were prepared in the similar manner (Chart 1). In the case of asymmetric dihydroxylation, even though the values of yield and enantiomeric excess (*ee*) were never high, we could still obtain **4b**—**f** and those enantiomer (*ent*-**4b**—**f**) (Table 1).

The absolute structure of **4b**—**f** was determined by using the modified Mosher's method (Fig. 2).[9] The reaction of **4b**—**f** and *ent*-**4b**—**f** with (*R*)-α-methoxy-α-trifluoromethylaphenylacetic acid ((*R*)-MTPA) afforded monoester (**5b**—**f** and *dst*-**5b**—**f**), where the hydroxy group of the benzylic position was selectively esterified. The difference ($\Delta\delta$) between the chemical shift (ppm) of each peak in the ^{1}H-NMR of **5b**—**f** and *dst*-**5b**—**f** is shown in Fig. 2.

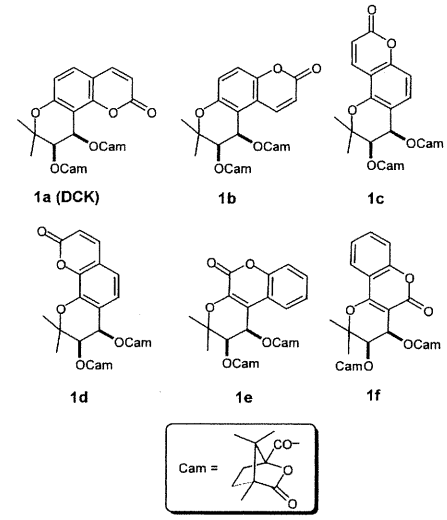


Fig. 1. Structure of DCK and Its Skeleton Isomers

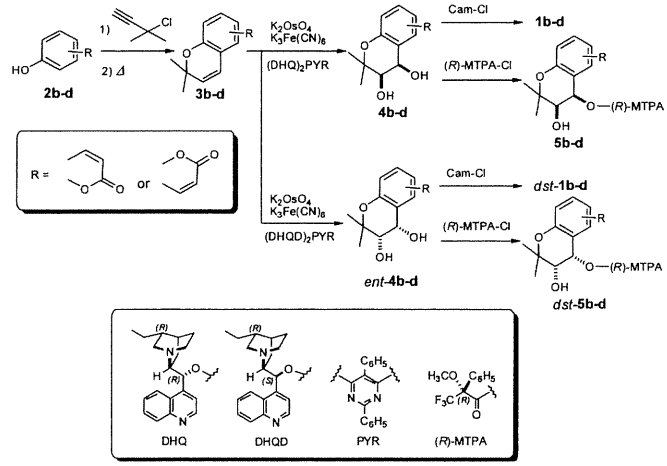


Chart 1

Table 1. Dihydroxylation of **3b**—**f** with (DHQ)$_2$PYR or (DHQD)$_2$PYR Ligand *via* Chart 1

Starting material (**3**)	Ligand	Product (**4**)	Yield %[a]	*ee* %[b]
3b	(DHQ)$_2$PYR	**4b**	23	57
3b	(DHQD)$_2$PYR	*ent*-**4b**	43	75
3c	(DHQ)$_2$PYR	**4c**	58	57
3c	(DHQD)$_2$PYR	*ent*-**4c**	66	68
3d	(DHQ)$_2$PYR	**4d**	24	67
3d	(DHQD)$_2$PYR	*ent*-**4d**	54	68
3e	(DHQ)$_2$PYR	**4e**	28	53
3e	(DHQD)$_2$PYR	*ent*-**4e**	61	58
3f	(DHQ)$_2$PYR	**4f**	72	90
3f	(DHQD)$_2$PYR	*ent*-**4f**	41	76

a) After recrystallization. *b*) Determined by chiral HPLC analysis.

* To whom correspondence should be addressed. e-mail: take@pharm.okayama-u.ac.jp

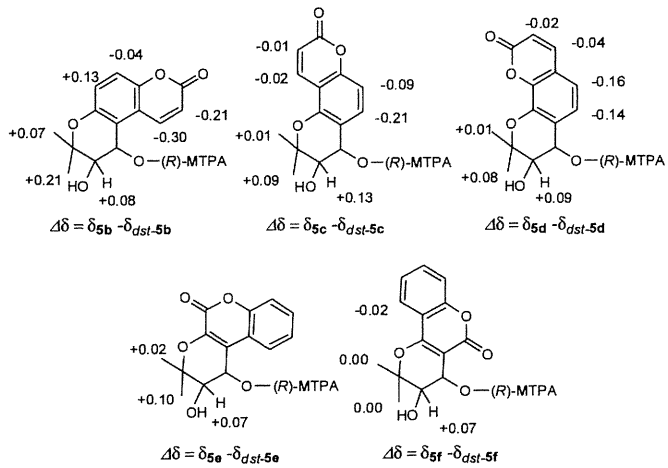


Fig. 2. Analysis Using New Mosher's Method for the Absolute Structure of **5**

Table 2. Anti-HIV Activity of **1**

Conpound (**1**)	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI (CC_{50}/EC_{50})[c]
1a (DCK)	0.17	>4.0	>23
1b	0.23	52	226
dst-**1b**	>100	>100	—
1c	4.3	>100	>23
dst-**1c**	>100	>100	—
1d	>100	>100	—
dst-**1d**	>100	>100	—
1e	>100	>100	—
dst-**1e**	>100	>100	—
1f	>100	>100	—
dst-**1f**	1.79	30.3	16
AZT[d]	0.076	>20	>263

a) EC_{50}: 50% effective concentration based on the inhibition of HIV-1-induced cell death in virus-infected MT-4 cells. *b*) CC_{50}: 50% cytotoxic concentration based on the reduction of viability in mock-infected MT-4 cells. *c*) Selectivity index: ratio CC_{50}/EC_{50}. *d*) AZT (zidovudine) was used as positive control.

The activity of compound (**1**) against HIV-1 replication was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells. Briefly, the cells (1×10^5 cells/ml) were infected with HIV-1 at a multiplicity of infection (MOI) of 0.1 and were cultured in the presence of various concentrations of the test compounds. After the cells were incubated for 4 d at 37 °C, the number of viable cells was monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The cytotoxicity of the compounds was simultaneously evaluated with their antiviral activity, on the basis of the viability of mock-infected cells. All experiments were repeated twice.

Among **1b**—**f** and those diastereomers (*dst*-**1b**—**f**), we could not find any derivative whose anti-HIV activity was more potent than that of DCK. However, the selective index (SI) of **1b**, whose anti-HIV activity (EC_{50}=0.23 μM) was almost the same as that of DCK, was greater than that of DCK (Table 2). We expect the DCK analogues to also exhibit anti-HIV activity.

Acknowledgment We are grateful to the SC-NMR Laboratory of Okayama University for the use of the facilities.

References and Notes

1) Huang L., Kashiwada Y., Cosentino L. M., Fan S., Lee K.-H., *Bioorg. Med. Chem. Lett.*, **4**, 593—598 (1994).
2) Huang L., Kashiwada Y., Cosentino L. M., Fan S., Chen C. H., McPhail A. T., Fujioka T., Mihashi K., Lee K.-H., *J. Med. Chem.*, **37**, 3947—3955 (1994).
3) Xie L., Crimmins M. T., Lee K.-H., *Tetrahedron Lett.*, **36**, 4529—4532 (1995).
4) Xie L., Takeuchi Y., Cosentino L. M., McPhail A. T., Lee K.-H., *J. Med. Chem.*, **44**, 664—671 (2001).
5) Xie L., Takeuchi Y., Cosentino L. M., Lee K.-H., *J. Med. Chem.*, **42**, 2662—2672 (1999).
6) Tang J., Qian K., Zhang B.-N., Chen Y., Xia P., Yu D., Xia Y., Yang Z.-Y., Chen C.-H., Morris-Natschke S. L., Lee K.-H., *Bioorg. Med. Chem.*, **18**, 4363—4373 (2010).
7) Nishita Y., Uesugi K., Satoh Y., Harayama T., *Yakugaku Zasshi*, **118**, 599—608 (1998).
8) **1b**: Colorless needles. mp 210—212 °C [*de*=90.5%] (AcOEt–hexane). IR (KBr) cm^{-1}: 1790, 1730. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, s, 3″-CH_3), 0.98 (3H, s, 3′-CH_3), 1.10, 1.11 (each 3H, each s, 7″-CH_3), 1.13 (6H, s, 7′-CH_3), 1.45, 1.48 (each 3H, each s, 8-CH_3), 1.67—2.54 (8H, m, CH_2), 5.40 (1H, d, J=4.5 Hz, 9-H), 6.46 (1H, d, J=9.9 Hz, 2-H), 6.67 (1H, d, J=4.5 Hz, 10-H), 7.11 (1H, d, J=9.3 Hz, 6-H), 7.34 (1H, d, J=9.3 Hz, 5-H), 7.50 (1H, d, J=9.9 Hz, 1-H). FAB-MS (positive ion mode) *m/z*: 623. *Anal.* Calcd for $C_{34}H_{38}O_{11}$: C, 65.58, H, 6.15. Found: C, 65.50, H, 6.18. $[\alpha]_D^{23.1}$ −60.36 (c=0.76, $CHCl_3$). Diastereomer excess was determined by chiral HPLC analysis.
9) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Org. Chem.*, **56**, 1296—1298 (1991).

Original article

Synthesis of 1-benzyl-3-(3,5-dimethylbenzyl)uracil derivatives with potential anti-HIV activity

*Yohei Isono[1], Norikazu Sakakibara[1], Paula Ordonez[2], Takayuki Hamasaki[2], Masanori Baba[2], Masahiro Ikejiri[3], Tokumi Maruyama[1]**

[1]Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, Sanuki City, Japan
[2]Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan
[3]Faculty of Pharmacy, Osaka Ohtani University, Tondabayashi, Japan

*Corresponding author e-mail: maruyama@kph.bunri-u.ac.jp

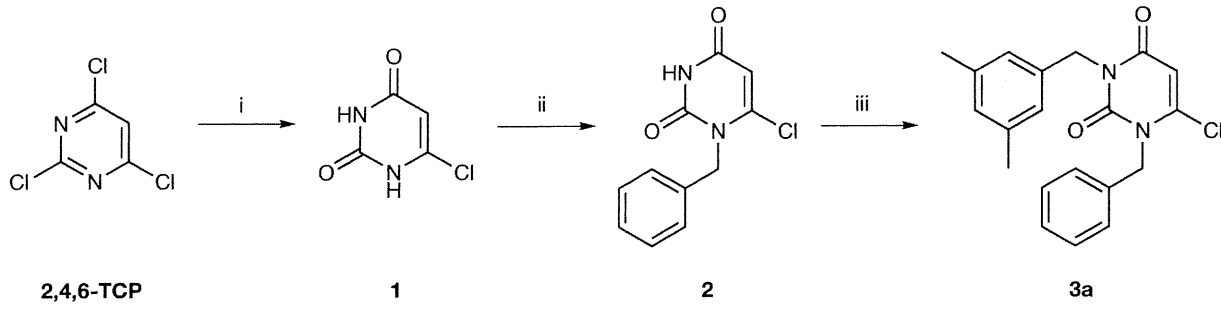
Background: Nine novel uracil analogues were synthesized and evaluated as inhibitors of HIV-1.
Methods: Key structural modifications included replacement of the 6-chloro group of 1-benzyl-6-chloro-3-(3,5-dimethylbenzyl)uracil by other functional groups or N^1-alkylation of 3-(3,5-dimethylbenzyl)-5-fluorouracil.
Results: These compounds showed only micromolar potency against HIV-1 in MT-4, though two of them; 6-azido-1-benzyl-3-(3,5-dimethylbenzyl) uracil and 6-amino-1-benzyl-3-(3,5-dimethylbenzyl) uracil were highly potent (half maximal effective concentration =0.067 and 0.069 μM) and selective (selectivity index =685 and 661), respectively. Structure-activity relationships among the newly synthesized uracil analogues suggest the importance of the H-bond formed between 6-amino group of 6-amino-1-benzyl-3-(3,5-dimethylbenzyl) uracil and amide group of HIV-1 reverse transcriptase.
Conclusions: We discovered two 6-substituted 1-benzyl-3-(3,5-dimethylbenzyl) uracils, (6-azido-1-benzyl-3-(3,5-dimethylbenzyl) uracil and 6-amino-1-benzyl-3-(3,5-dimethylbenzyl) uracil) as novel anti-HIV agents. These compounds should be further pursued for their toxicity and pharmacokinetics *in vivo* as well as antiviral activity against non-nucleoside reverse transcriptase inhibitor-resistant strains.

Introduction

Non-nucleoside reverse transcriptase inhibitor (NNRTI), which is not the basic structure of nucleoside, binds at an allosteric binding site that is present in the reverse transcriptase (RT) [1–3]. In 1989, Baba *et al.* [4] first discovered that 1-[(2-hydroxyethoxy) methyl]-6-phenylthiothymine (HEPT) had a strong anti-HIV-1 activity, though its 5′-triphosphate derivative (HEPT-TP) showed no inhibitory effect on HIV-1 transcriptase. At present, there are a variety of pharmaceutical agents that can be classified as NNRTIs, such as nevirapine (NVP), efavirenz (EFV), delavirdine (DLV) and etravirine (ETR). NNRTIs have a high level of drug safety and good adherence, however, they have a disadvantage which is a tendency to evolve drug-resistant viruses caused by mutations of their binding site in HIV-1 RT. NVP, EFV and DLV bind to similar sites of HIV RT, thus HIV-1 resistant to one of these compounds shows cross-resistance to others; by contrast, ETR appears to bind the multiple sites of RT. As a result, ETR retains the activity against NVP, EFV or DLV-resistant mutants.

Since the discovery of HEPT, related uracil derivatives have been subsequently synthesized. Interestingly, one of them, SJ-3366, has potent antiviral activity against HIV-2 as well as HIV-1 [5]. All of these compounds are 6-substituted uracil derivatives, which are comparatively difficult to synthesize in large quantities; therefore, 1,3-disubstituted uracils have been synthesized and examined for their anti-HIV-1 activity [6]. The introduction of 3-methylbenzyl group at N3-position of uracil was highly effective in inhibiting HIV-1 replication *in vitro*. Moreover, 3-(3,5-dimethybenzyl) uracil derivatives exhibited an excellent anti-HIV-1 activity. As for the substituent of N^1 position, cyanomethyl group and benzyl group have brought about good results. Based on the estimation that the hydrogen bond accepting nitrogen of the cyanomethyl group might be related to the strong anti-HIV-1 activity, the

Figure 1. Synthesis of 6-chloro-1-benzyl-3-(3,5-dimethylbenzyl)uracil (3a)



Reagents and conditions: i, 2.5 M NaOH aq, 120°C, 1 h; ii, BnBr, K_2CO_3, DMF, 70°C, 1h; iii, 2.6-dimethylbenzylalcohol, DIAD, PPh_3, THF, 50°C, 16 h.

introduction of 2-picolyl group or 4-picolyl group at N^1-position of the uracil analogue has been investigated and resulted in further elevation of anti-HIV-1 activity. These results were confirmed by a computing science method, in which the docking energy of a substrate on HIV-1 RT has been calculated [7].

An attempt at introducing a methyl or iodo group at C5-position of 1-benzyl-3-(3,5-dimethybenzyl)uracil resulted in significant decrease of anti-HIV-1 activity, suggesting that the bulky group at C5-position of uracil disturbs the binding to HIV-1 RT. However, a fluorine atom is about the same van der Waals radius as a hydrogen atom, and it makes the compound metabolically and chemically stable, thus many pharmaceutical agents possess fluorine atom(s) [8]. These backgrounds prompted us to prepare 1-substituted 3-(3,5-dimethybenzyl)-5-fluorouracils and examine their anti-HIV-1 activity. In addition, we attempt, in this report, to introduce a small group at 6-position of 1-benzyl-3-(3,5-dimethybenzyl)uracil to explore the effect of 6-substitution on the uracil ring.

Methods

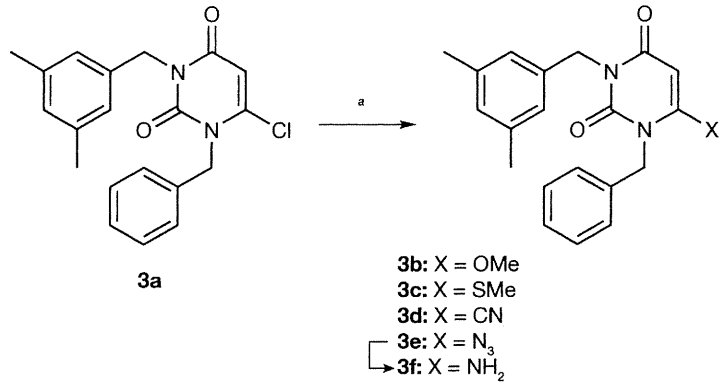
Preparation of 6-chloro-1-benzyl-3-(3,5-dimethylbenzyl)uracil (3a)

Synthesis of 6-substituted analogues of 1-benzyl-3-(3,5-dimethylbenzyl)uracil were accomplished from the key compound 6-chloro-1-benzyl-3-(3,5-dimethylbenzyl) uracil (**3a**) that could be synthesized from commercially available 2,4,6-trichloropyrimidine (2,4,6-TCP; Figure 1). Thus, 2,4,6-TCP was treated with 2.5 M NaOH to give 6-chlorouracil (**1**) in 74% yield [9]. Then, compound **1** was treated with benzyl bromide to give the corresponding 1-benzylated congeners (**2**) in 55% yield as previously reported by Ichikawa *et al.* [10]. This result indicates that the electron-withdrawing chlorine atom facilitates the dissociation of N^1-H to produce an intermediary uracil-1-anion [7]. Compound **2** was condensed with 3,5-dimethylbenzyl alcohol using the Mitsunobu reaction [11] to afford 3-(3,5-dimethylbenzyl) congener (**3a**).

Synthesis of 6-substituted-1-benzyl-3-(3,5-dimethylbenzyl)uracil (3b–3f)

Briefly, displacement of the 6-chloro group of **3a** was carried out by nucleophilic substitution in suitable solvents to give the 6-substituent derivatives (Figure 2). First of all, Gogoi *et al.* [12] and Ohkura *et al.* [13] have independently reported that 6-chloro-1,3-dimethyluracil underwent nucleophilic substitution to give the 6-methoxy- and 6-thiomethoxy-1,3-dimethyluracil under special reactive condition such as phase transfer or photoreaction condition. We examined displacement of **3a** with NaOMe in MeOH of 6-chloro derivative (**3a**) under normal conditions to give 1-benzyl-6-methoxy-3-(3,5-dimethylbenzyl)uracil (**3b**) in 63% yield. A similar reaction of **3a** with sodium thiomethoxide produced the corresponding 6-thiomethoxyl derivative (**3c**) in 60% yield. These reactions proceed rapidly even at room temperature and the 6-chloro group of **3a** has been proved to be facile for nucleophilic substitution. Synthesis of 6-cyano derivative (**3d**) was carried out in a similar way reported by Nagata and Yoshioka [14]. Thus, compound **3a** was treated with potassium cyanide at 50°C overnight to give **3d** in 59% yield. As for the azido substitution of 6-chloro function, no report except for the reaction of 6-chloro-1,3-dimethyluracil with sodium azide in MeOH has been published [15]. In a similar way, compound **3a** was treated with NaN_3 in DMF to give 6-azido derivative (**3e**) in moderate yield. The structures of **3d** and **3e** were confirmed by

Figure 2. Synthesis of 6-substituted-1-benzyl-3-(3,5-dimethylbenzyl)uracil (**3b–3f**)



C6-substitution of 6-chloro congener (**3a**)

Entry	Product	Conditions
1	**3b**	MeONa, MeOH, rt, 30 min, 63%
2	**3c**	MeSNa, DMF, rt, 30 min, 60%
3	**3d**	KCN, DMF, 50°C, 16 h, 59%
4	**3e**	NaN_3, DMF, rt, 2 h, 90%
5	**3f**	$LiAlH_4$, THF, 0°C, 30 min, 79%

[a]See tabulated data.

IR spectra in which characteristic absorptions due to 6-cyano or 6-azido group was observed at 2,231 or 2,133 cm^{-1}, respectively. Then, selective reduction of 6-azido analogue (**3e**) to 6-amino congener was explored. Hirota *et al.* [16] reported that reduction of 6-azido-1,3-dimethyluracil to 6-amino derivative using tetralin under thermal condition (150°C, 5 min) in 90% yield. To avoid the decomposition at high-temperature, reduction of **3e** was carried out with lithium aluminum hydride to give 6-amino compound **3f** in 79% yield. As described above, synthesis of 6-substituted analogues of 1-benzyl-3-(3,5-dimethylbenzyl)uracil (**3b–3f**) was accomplished from 6-chloro derivative (**3a**) without any special device or reagents.

Preparation of 3-(3,5-dimethylbenzyl)-5-fluorouracil (9)

Next, synthesis of 1-substituted analogues of 3-(3,5-dimethylbenzyl)-5-fluorouracil (**10a–10d**) from 5-fluorouracil was attempted. It is reasonable to prepare these compounds from a key compound, 3-(3,5-dimethylbenzyl)-5-fluorouracil (**9**). However, it is not easy to introduce an alkyl group at N3 of 5-fluorouracil. Baker *et al.* [17] reported that alkylation with allylic-type halides, such as allyl bromide and benzyl chloride, occurred at the N^1-position. Our group reported the method to obtain N3-alkylated 5-fluorouracil, in which N3-alkylation of the 5,6-adduct was involved as a key step [18]. We employed this method for the synthesis of 3-(3,5-dimethylbenzyl)-5-fluorouracil (**9**; Figure 3). Thus, 5-fluorouracil was reacted with *N*-chlorosuccinimide to give the 5,6-adduct (**6**) in 58% yield. Compound **6** is supposed to cause steric hindrance at N^1 due to sp^3 hybrid orbital of C6 and the bulky methoxy group so that alkylation is preferable at N3-position. Condensation of **6** with 3,5-dimethylbenzyl alcohol using triphenylphosphine and diisopropylazodicarboxylate (DIAD) resulted in the formation of 3-(3,5-dimethylbenzyl) derivative (**7**; Figure 3). In this reaction, it was difficult to remove impurities by column chromatography or recrystallization process from the product. Therefore, without purification, 3-(3,5-dimethylbenzyl)uracil derivative (**7**) was reacted with 5% palladium on activated carbon in MeOH under the hydrogen atmosphere. However, only 5-fluorouracil was obtained instead of **8**, suggesting reduction of both chloro and 3,5-dimethylbenzyl group and subsequent elimination of the resulting product. Next, reduction of **7** was accomplished by zinc-catalyzed hydrogenation to give the dehalogenated product (**8**) successfully. However, **8** was so unstable that it gradually transformed into **9** even at room temperature. Accordingly, the crude **8** was subjected to elimination reaction using K_2CO_3 in MeOH to give 3-(3,5-dimethylbenzyl)-5-fluorouracil (**9**) as white crystals in 74% overall yield from **6** in three steps. The structure of **9** was confirmed as N-3 substituted product by bathochromic shift of UV spectra in which absorption maximum was observed in MeOH at 268 nm, while in 0.1 M NaOH *aq*, shifts to 299 nm.

N^1-alkylation of key compound 9

N^1-Alkylation of the key compound **9** (Figure 3) was achieved by the conventional manner. Thus, compound **9** was alkylated with bromoacetonitrile in the presence of K_2CO_3 in DMF to give the 1-cyanomethyl congener (**10a**) in 89% yield. In a similar way, **9** was treated with benzyl bromide in DMF to give **10b** in 92% yield. Also, alkylation of **9** with 2-(chloromethyl)pyridene or

4-(chloromethyl)pyridene furnished **10c** or **10d** in 78% or 88% yield, respectively.

Anti-HIV assay

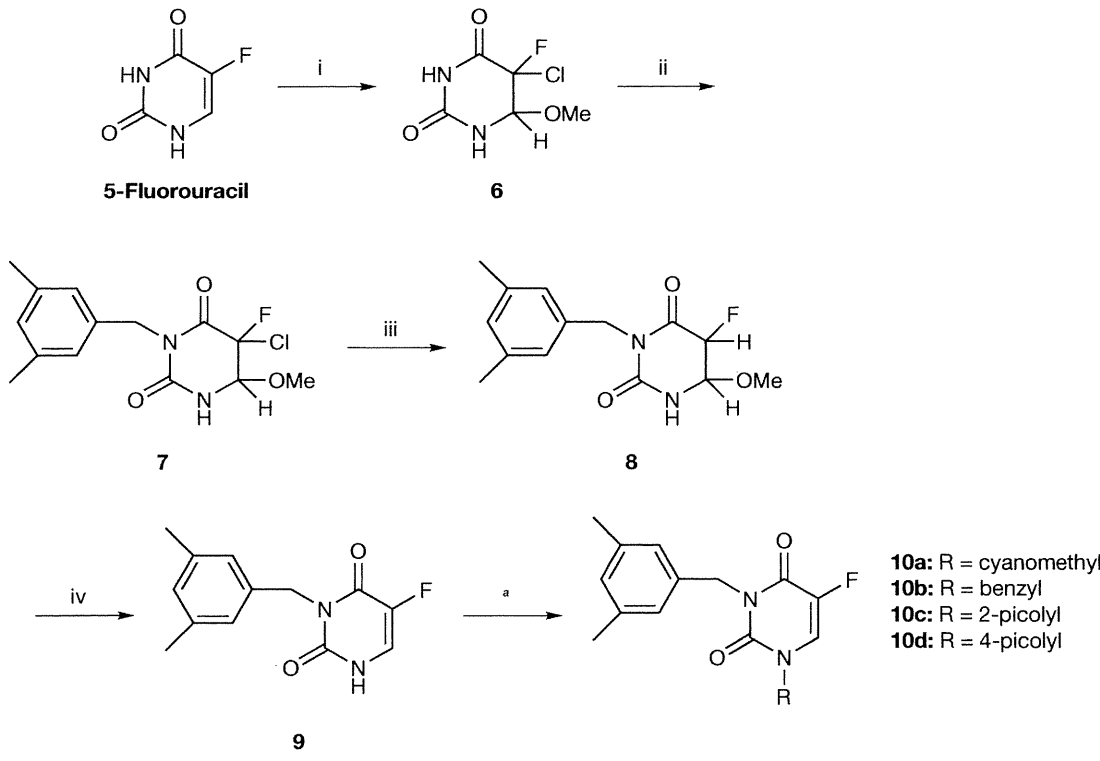
MT-4 cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml of penicillin G, and 0.10 g/ml of streptomycin. The III_B strain of HIV-1 was used throughout the experiment. The virus was propagated and titrated in MT-4 cells. Virus stocks were stored at -80°C until use. The anti-HIV-1 activity of the test compounds was determined by the inhibition of virus-induced cytopathogenicity in MT-4 cells [19]. Briefly, MT-4 cells (1×10^5 cells/ml) were infected with HIV-1 at a multiplicity of infection (MOI) of 0.1 and were cultured in the presence of various concentrations of the test compounds. After a 4-day incubation at 37°C in 5% CO_2, the number of viable cells was monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method [20]. The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity, based on the viability of mock-infected cells, as determined by the MTT method.

Materials

Instrumentation

^1H NMR and ^{13}C NMR spectra were taken with a Ultrashield™ 400 Plus FT NMR System (BRUKER, Yokohama, Japan). Chemical shifts and coupling constants (*J*) were given in δ and Hz, respectively. Melting points were determined on a Yanaco MP-500D (Yanaco, Tokyo, Japan). Elementary analyses were

Figure 3. Synthesis of 3-(3,5-dimethylbenzyl)-5-fluorouracil (**9**)



N1-alkylation of 5-flourouracil congener (**7**)

Entry	Product	Conditions
1	**10a**	$BrCH_2CN$, K_2CO_3, DMF, rt, 2 h, 89%
2	**10b**	BnBr, K_2CO_3, DMF, rt, 3 h, 92%
3	**10c**	2-picolyl chloride, NaI, K_2CO_3, DMF, rt, 16 h, 78%
4	**10d**	4-picolyl chloride, NaI, K_2CO_3, DMF, rt, 16 h, 88%

Reagents and conditions: i, NCS MeOH, 50°C, 16 h; ii, 3,5-dimethylbenzylalcohol, DIAD, PPh_3, THF, rt, 16 h; iii, Zn, NH_4Cl, MeOH, rt, 6 h; iv, K_2CO_3, MeOH, rt, 16 h. [a]See tabulated data.

determined by a Perkin Elmer Series II CHNS/O Analyzer 2400 (Perkin Elmer, Yokohama, Japan). High-resolution mass spectrometry was performed on a APEX IV mass spectrometer (BRUKER) with electrospray ionization mass spectroscopy (ESI-MS).

Compounds

6-Chlorouracil (1)

A mixture of 2,4,6-Trichloropyrimidine (TCI, 13 ml, 113 mmol) and NaOH (18.1 g,0.45 mol, 4 *eq*) in water (185 ml) was refluxed for 1 h, then added concentrated HCl (ca. 40 ml) over a pH range 2–3, and recrystallized at 4°C. The residue was filtered off, and recrystallized from MeOH to give white crystals (12.26 g, 83.67 mmol, 74%).

Mp>300 °C. ^{1}H NMR (DMSO-d_6): δ 11.20, 12.05 (1 H, br s, NH), 5.72 (1 H, d, H5, *J* 1.6).

1-Benzyl-6-chlorouracil (2)

6-Chlorouracil (**1**; 6.79 g, 46.3 mmol) was dissolved in DMF (150 ml) and K_2CO_3 (3.2 g, 23.2 mmol) and benzyl bromide (7.32 ml, 61.6 mmol) were added to the solution, then kept at 70°C for 1 h. Water was added to the mixture and the aqueous mixture was extracted with ethyl acetate. The organic extracts were washed with saturated sodium chloride solution, and dried with sodium sulfate. After removal of the organic solvent, the residual syrup was chromatographed over the column of silica gel (ethyl acetate: hexane =1:1) to give **2** as white crystals (5.97 g, 55%).

HRMS (ESI) Calcd for $C_{11}H_9ClN_2NaO_2$ $[M+Na]^+$: 259.0245. Found 259.0249. Mp 164.6-164.9 °C. ^{1}H-NMR (DMSO-d_6): δ 11.75 (1 H, br s, NH), 7.26-7.40 (5 H, m, -$CH_2C_6\underline{H}_5$), 5.99 (1 H, s, H5), 5.16 (2 H, s, -$C\underline{H}_2C_6H_5$).

1-Benzyl-6-chloro-3-(3,5-dimethylbenzyl) uracil (3a)

A solution of compound **3** (0.70 g, 2.97 mmol), triphenylphosphine (1.56 g, 5.94 mmol), 3,5-Dimethylbenzylalcohol (787.0 µl, 5.94 mmol) and TMAD (*N,N,N',N'*-tetramethylazodicarboxamide, 1.02 g, 5.94 mmol) in THF (25.0 ml) was stirred overnight at 50°C. After 12 h stirring, the solution was concentrated to a small volume. The residual solution was purified by silica gel column chromatography (20%→25% ethyl acetate in hexane) to give as a syrup **3a** (0.91 g, 2.57 mmol, 86%).

HRMS (ESI) Calcd for $C_{20}H_{19}ClN_2NaO_2$ $[M+Na]^+$: 377.1027. Found 377.1037. ^{1}H NMR ($CDCl_3$): δ 6.79-7.37 (8 H, m, -$CH_2C_6\underline{H}_3$, -$CH_2C_6\underline{H}_5$),5.96 (1 H, s, H5 of uracil), 5.27 (2 H, s, -CH_2), 5.05 (2 H, s, -CH_2), 2.28 (6 H, s, -CH_3×2).

1- Benzyl-3-(3,5-dimethylbenzyl)-6-methoxyuracil (3b)

A mixture of compound **3a** (0.52 g, 1.46 mmol) and 28% sodium methoxide in methanol solution (0.48 ml, 2.48 mmol) in MeOH (25.0 ml) was stirred for 0.5 h at room temperature. The mixture was acidified with an acetic acid, and water was added to the mixture and the aqueous mixture was extracted with ethyl acetate. The organic extracts were washed with water, 5% sodium bicarbonate solution and saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was recrystallized from 40% AcOEt in hexane to give white crystals of **3b** (0.32 g, 0.92 mmol, 63%).

HRMS (ESI) Calcd for $C_{21}H_{22}N_2NaO_3$ $[M+Na]^+$: 373.1523. Found 373.1535. Mp 93.3-95.7 °C. ^{1}H NMR ($CDCl_3$): δ 6.84-7.37 (8 H, m, -$CH_2C_6\underline{H}_3$, -$CH_2C_6\underline{H}_5$), 5.14 (1 H, s, H5 of uracil), 5.07 (2 H, s, -CH_2), 5.05 (2 H, s, -CH_2), 3.85 (3 H, s, -OCH_3), 2.27 (3 H, s, -CH_3), 2.27 (3 H, s, -CH_3).

1-Benzyl-3-(3,5-dimethylbenzyl)-6-thiomethoxyuracil (3c)

A compound **3a** (0.50 g, 1.41 mmol) and sodium thiomethoxide (0.13 g, 1.83 mmol) in DMF (10.0 ml) was stirred for 0.5 h at room temperature. The reaction was quenched with AcOH (2.0 ml), and the mixture was evaporated *in vacuo* and the residue was extracted with benzene. The organic extracts were washed with water, 5% sodium bicarbonate solution and saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residual solution was purified by silica gel column chromatography (33%→50% ethyl acetate in hexane) to white crystals of **3c** (0.31 g, 0.85 mmol, 60%).

HRMS (ESI) Calcd for $C_{20}H_{22}N_2NaO_2S$ $[M+Na]^+$: 389.1294. Found 389.1305. Mp 119.0-120.4 °C. ^{1}H NMR ($CDCl_3$): δ 6.89-7.38 (8 H, m, -$CH_2C_6\underline{H}_3$, -$CH_2C_6\underline{H}_5$), 5.52 (1 H, s, H5 of uracil), 5.20 (2 H, s, -CH_2), 5.08 (2 H, s, -CH_2), 2.40 (3 H, s, -SCH_3), 2.28 (3 H, s, -CH_3), 2.28 (3 H, s, -CH_3).

1- Benzyl-6-cyano-3-(3,5-dimethylbenzyl) uracil (3d)

Compound **3a** (0.31 g, 0.86 mmol) was dissolved in DMF (10.0 ml) and potassium cyanide (0.17 g, 2.58 mmol) was added to the solution, then kept at 50°C overnight. After condensation of the solution, the residual syrup was recrystallized from 40% AcOEt in hexane to give white crystals **3d** (0.17 g, 0.50 mmol, 59%).

HRMS (ESI) Calcd for $C_{21}H_{19}N_3NaO_2$ $[M+Na]^+$: 368.1370. Found 386.1384. IR (Nujol) cm^{-1}: 2231. Mp 158.1-158.6 °C. ^{1}H NMR ($CDCl_3$): δ 7.08-7.68 (8 H, m, -$CH_2C_6\underline{H}_3$, -$CH_2C_6\underline{H}_5$), 6.88 (1 H, s, H5 of uracil), 5.07 (2 H, s, -CH_2), 4.97 (2 H, s, -CH_2), 2.29 (3H, s, -CH_3), 2.29 (3 H, s, -CH_3).

6-Azido-1-benzyl-3-(3,5-dimethylbenzyl) uracil (3e)

A mixture of compound **3a** (0.34 g, 0.94 mmol) and sodium azide (0.07 g, 1.03 mmol) in DMF (5.0 ml) was stirred for 0.5 h at room temperature and then water

was added to the mixture, and the aqueous mixture was extracted with ethyl acetate. The organic extracts were washed with water, saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (25%→33% ethyl acetate in hexane) to give a caramel **3e** (0.31 g, 0.85 mmol, 90%).

HRMS (ESI) Calcd for $C_{20}H_{19}N_5NaO_2$ $[M+Na]^+$: 384.1431. Found 386.1439. IR (Nujol) cm^{-1}: 2133. ^1H NMR ($CDCl_3$): δ 6.89-7.37 (8 H, m, $-CH_2C_6\underline{H}_3$, $-CH_2C_6\underline{H}_5$), 5.56 (1 H, s, H5 of uracil), 5.06 (2 H, s, $-CH_2$), 5.04 (2 H, s, $-CH_2$), 2.27 (3 H, s, $-CH_3$), 2.27 (3 H, s, $-CH_3$).

6-Amino-1-benzyl-3-(3,5-dimethylbenzyl) uracil (**3f**)
Compound **3e** (0.92 g, 2.53 mmol) was dissolved in dry THF (15 ml), under nitrogen atmosphere. To this stirred solution was carefully added $LiAlH_4$ (0.10 g, 2.53 mmol) at 0°C. Stirring was continued at 0°C for 0.5 h and quenched by the addition of MeOH (3.0 ml), until no effervescence was observed. Water (20 ml) was then added and the product was extracted with EtOAc. The combined organic extracts were washed with water, saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (6.3% MeOH in $CHCl_3$) to give a caramel **3f** (0.67 g, 2.01 mmol, 79%).

HRMS (ESI) Calcd for $C_{20}H_{21}N_3NaO_2$ $[M+Na]^+$: 358.1526. Found 358.1526. ^1H NMR ($CDCl_3$): δ 6.86-7.43 (8 H, m, $-CH_2C_6\underline{H}_3$, $-CH_2C_6\underline{H}_5$), 5.17 (2 H, s, $-CH_2$), 5.09 (2 H, s, $-CH_2$), 5.00 (1 H, s, H5 of uracil), 4.16 (2 H, br s, $-NH_2$), 2.29 (6 H, s, $-CH_3$×2).

5-Chloro-5,6-dihydro-5-fluoro-6-methoxyuracil (**6**)
5-Fluorouracil (5FU, Sigma–Aldrich, Tokyo, Japan; 6.50 g, 50.0 mmol) was dissolved in MeOH (350.0 ml) and N-Chlorosuccinimide (Sigma–Aldrich, 13.30 g, 100.0 mmol) was added to the solution, and then stirred for overnight at 50°C. The mixture was evaporated, and was recrystallized from 50% EtOH in H_2O to give white crystals of **4** (5.73 g, 29.2 mmol, 58%).

Mp 213.5-215.3 °C. ^1H NMR (DMSO-d_6): δ 11.19 (1 H, br s, 3-NH), 9.21 (1 H, br s, 1-NH), 5.00 (1 H, d, H6, *J* 4.8), 3.38 (3 H, d, -OMe, *J* 1.2).

3-(3,5-Dimethylbenzyl)-5-fluorouracil (**9**)
A solution of compound **6** (3.51 g, 17.86 mmol), triphenylphosphine (9.36 g, 35.72 mmol), 3,5-dimethylbenzylalcohol (3.93 ml, 26.79 mmol) and DIAD (diisopropyl azodicarboxylate, WAKO, Osaka, Japan, 3.58 ml, 18.22 mmol) in THF (270.0 ml) was stirred overnight at room temperature. After 12 h stirring, the solution was concentrated to a small volume. The residual solution was purified by silica gel column chromatography (25%→33% ethyl acetate in hexane) to give as a syrup **7** (7.92 g, impurity-containing yellow syrup). Next, Compound **7** (7.92 g) was dissolved in MeOH (200.0 ml) and ammonium chloride (5.39 g, 71.44 mmol) and zinc powder (6.59 g, 71.44 mmol) were added to the solution, and then stirred for 6 h at room temperature. The mixture was extracted with ethyl acetate. The organic extracts were washed with water, saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated to give **8** (7.44 g, impurity-containing yellow syrup). Then, **8** was dissolved in MeOH (300.0 ml) and K_2CO_3 (11.02 g, 79.5 mmol) was added to the solution, and then stirred overnight at room temperature. The mixture was filtrated, and was extracted with ethyl acetate. The organic extracts were washed with water, saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (40%→50% ethyl acetate in hexane) to give white crystals **9** (3.29 g, 13.27 mmol, 74%).

HRMS (ESI) Calcd for $C_{13}H_{13}FN_2NaO_2$ $[M+Na]^+$: 271.0853. Found 271.0844. Mp 163.0-167.1 °C. UV: max 268.0nm (MeOH) max 298.7nm (NaOH) ^1H NMR ($CDCl_3$): δ 9.27 (1 H, br s, 1-NH), 7.17 (1 H, s, H6 of uracil), 7.04 (2 H, s, H2+H6 of $-C_6H_3$), 6.92 (1 H, s, H4 of $-C_6H_3$), 5.04 (2 H, s, $-C\underline{H}_2C_6H_3$), 2.28 (6 H, s, $-CH_3$×2).

1-Cyanomethyl-3-(3,5-dimethylbenzyl)-5-fluorouracil (**10a**)
Compound **9** (0.25 g, 1.00 mmol) was dissolved in DMF (10.0 ml), and K_2CO_3 (0.42 g, 3.00 mmol) and bromoacetonitrile (0.28 ml, 4.00 mmol) was added to the solution, and then stirred for 2 h at room temperature. The mixture was quenched by the addition of 1.0 M HCl *aq* (3.0 ml), and then extracted with ethyl acetate. The organic extracts were washed with 5% sodium bicarbonate solution, saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (33% AcOEt in hexane) to give white crystals of **10a** (0.26 g, 0.89 mmol, 89%).

HRMS (ESI) Calcd for $C_{15}H_{14}FN_3NaO_2$ $[M+Na]^+$: 310.0962. Found 310.0950. Mp 177.1-178.6 °C. ^1H NMR ($CDCl_3$): δ 7.30 (1 H, d, H6 of uracil, *J* 6.4), 7.08 (2 H, s, H2+H6 of $-C_6H_3$), 6.93 (1 H, s, H4 of $-C_6H_3$), 5.07, (2 H, s, $-CH_2$), 4.66 (2 H, s, $-CH_2$), 2.29 (3 H, s, $-CH_3$), 2.29 (3 H, s, $-CH_3$).

1-Benzyl-3-(3,5-dimethylbenzyl)-5-fluorouracil (**10b**)
Compound **9** (0.10 g, 0.40 mmol) was dissolved in DMF (4.0 ml), and benzyl bromide (0.19 ml, 1.60 mmol) and K_2CO_3 (0.17 g, 1.20 mmol) was added to the solution, and then stirred for 3 h at room temperature. The mixture was quenched by the addition of 1.0 M HCl *aq*, and then extracted with ethyl acetate. The organic extracts were

washed with 5% sodium bicarbonate solution, saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (20% AcOEt in hexane) to give white crystals of **10b** (0.13 g, 0.37 mmol, 92%).

HRMS (ESI) Calcd for $C_{20}H_{19}FN_2NaO_2$ $[M+Na]^+$: 361.1323. Found 361.1320. Mp 103.3-104.8 °C. ^1H NMR (DMSO-d_6): δ 8.36 (1 H, d, H6 of uracil, *J* 6.4), 7.30-7.38 (5 H, m, $-C_6H_4$), 6.86 (1 H, s, H4 of $-C_6H_3$), 6.83 (2 H, s, H2+H6 of $-C_6H_3$), 4.92, (2 H, s, $-CH_2$), 4.91 (2 H, s, $-CH_2$), 2.20 (6 H, s, $-CH_3$×2).

*3-(3,5-Dimethylbenzyl)-1-(2-picolyl)-5-fluorouracil (***10c***)*
Compound **9** (0.25 g, 1.00 mmol) was dissolved in DMF (10.0 ml), and K_2CO_3 (1.11 g, 8.00 mmol), NaI (0.03 g, 0.2 mmol), and 2-picolylchloride hydrochloride (0.66 g, 1.60 mmol) was added to the solution, and then stirred for overnight at room temperature. The mixture was quenched by the addition of 1.0 M HCl *aq*, and then extracted with ethyl acetate. The organic extracts were washed with saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (50% AcOEt in hexane) to give white crystals of **10c** (0.26 g, 0.78 mmol, 78%).

HRMS (ESI) Calcd for $C_{19}H_{18}FN_3NaO_2$ $[M+Na]^+$: 362.1275. Found 362.1265. Mp 114.3-115.5 °C. ^1H NMR (DMSO-d_6): δ 8.52 (1 H, ddd, H6 of 2-picolyl, *J* 4.8, 1.6 and 0.8), 8.34 (1 H, d, H6 of uracil, *J* 6.4), 7.80 (1 H, ddd, H4 of 2-picolyl, *J* 7.6, 7.6 and 1.6),7.37 (1H, d, H3 of 2-picolyl, *J* 7.6) 7.32 (1H, ddd, H5 of 2-picolyl, *J* 7.6, 4.8 and 0.8) 6.87 (1 H, s, H4 of $-C_6H_3$), 6.82 (2 H, s, H2+H6 of $-C_6H_3$), 5.04, (2 H, s, $-CH_2$), 4.92 (2 H, s, $-CH_2$), 2.21 (6 H, s, $-CH_3$×2).

*3-(3,5-Dimethylbenzyl)-1-(4-picolyl)-5-fluorouracil (***10d***)*
Compound **9** (0.25 g, 1.00 mmol) was dissolved in DMF (10.0 ml), and K_2CO_3 (1.11 g, 8.00 mmol), NaI (0.03 g, 0.2 mmol), and 4-picolylchloride hydrochloride (0.66 g, 1.60 mmol) was added to the solution, and then stirred overnight at room temperature. The mixture was quenched by the addition of 1.0 M HCl *aq*, and then extracted with ethyl acetate. The organic extracts were washed with saturated sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (33% AcOEt in hexane) to give a caramel **10d** (0.30 g, 0.88 mmol, 88%).

HRMS (ESI) Calcd for $C_{19}H_{18}FN_3NaO_2$ $[M+Na]^+$: 362.1275. Found 362.1277. ^1H NMR (DMSO-d_6): δ 8.60 (1 H, dd, H2+H6 of 4-picolyl, *J* 4.4 and 1.6), 8.43 (1 H, d, H6 of uracil, *J* 6.4), 7.36 (2 H, dd, H3+H5 of 4-picolyl, *J* 4.4 and 1.6), 6.93 (1 H, s, H4 of $-C_6H_3$), 6.91 (2 H, s, H2+H6 of $-C_6H_3$), 5.02, (2 H, s, $-CH_2$), 4.98 (2 H, s, $-CH_2$), 2.27 (6 H, s, $-CH_3$×2).

Results

Structure-activity relationship

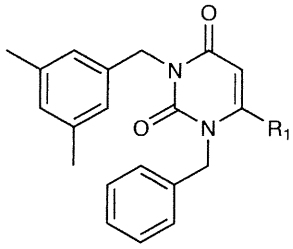
Antiviral activity of the 6-substituted 1-benzyl-3-(3,5-dimethylbenzyl)uracils (**3b–3f**) and 1-substituted 3-(3,5-dimethybenzyl)-5-fluorouracils (**10a–10d**) were examined for their inhibitory effects on HIV-1-induced cytopathogenicity and cell viability in MT-4 cells. As comparison, MKC-442 (emivirine), NVP and 4′-ethynylstavudine (4′-Ed4T) were also tested [19–21]. In a series of 6-substituted analogues (**3b–3f**), 6-methoxy derivative (**3b**) provided a comparable result to BBF-29, while in 6-thiomethoxy analog (**3c**), anti-HIV-1 activity was reduced as compared to BBF-29 (Figure 4), suggesting that smaller electronegativity in the chalcogen atom at the 6-position in the uracil skeleton caused reduced anti-HIV-1 activity. Next, introduction of linear group, such as cyano and azido groups at 6-carbon, was investigated for the structure–activity relationship. It is interesting that the 6-azido derivative (**3e**) showed excellent anti-HIV-1 activity and emerged as the most potent inhibitor in this series with 50% effective concentration (EC_{50}) of 0.067 ±0.011 μM (50% cytotoxic concentration [CC_{50}] 45.9 ±0.7 μM and selectivity index [SI] 685). By contrast, the 6-cyano compound (**3d**) showed only moderate anti-HIV-1 activity. These wildly divergent results could be explained by the following. Since the steric effect is similar for both groups, the difference presumably comes from the anti-HIV-1 activity of their metabolites. To investigate the possibility that 6-azido derivative (**3e**) showed strong antiviral activity after converting metabolically to the 6-amino congener, the anti-HIV-1 activity of compound **3f** was evaluated and found that it had comparable potency to that of 6-azido derivative (**3e**) with EC_{50} of 0.069 ±0.006 μM (CC_{50} 45.6 ±0.9 μM and SI 661). This result supports our assumption that **3e** is reduced to **3f** in cell cultures, and the metabolite (**3f**) inhibits HIV-1 RT. To prove this hypothesis, it is necessary to perform the anti-HIV-1 assay for **3e** and **3f** in a cell-free system.

5-Fluorouracil analogues (**10a–10d**) had reduced anti-HIV-1 activity and cytotoxicity in MT-4 cell (Figure 4), suggesting that the introduction of fluorine into C5-position of uracil ring results in reduced anti-HIV-1 activity, presumably because the strong electron-withdrawing effect of fluorine causes electron deficiency of uracil ring and reduced affinity to the HIV-1 RT.

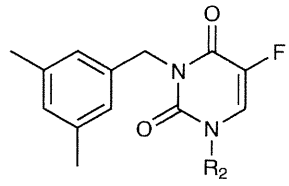
Discussion

We have previously demonstrated that some novel 1,3-disubstituted uracils selectively inhibit HIV-1 replication in cell cultures [6,7]. In this report, hydrogen bonding interaction (H-bond) between ligand and amide group of Lys 101 residue as well as the hydrophobic interaction is important for the binding of uracil derivatives to HIV-1 RT. Strong anti-HIV-1 activity of the 6-amino

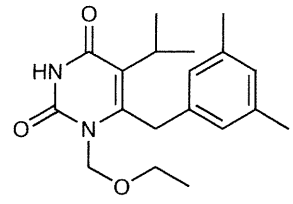
Figure 4. Antiviral activity of 1,3-disubstituted uracils against HIV-1



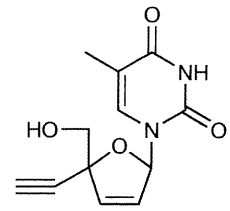
BBF-29, 3b–3f



10a–10d



MCK-442 (emivirine)



4′-Ed4T

Compound	R_1	R_2	EC_{50}, μM	CC_{50}, μM	SI
BBF-29	H	–	0.28	44.7	160
3b	OMe	–	0.76 ±0.19	45.7 ±0.2	60
3c	SMe	–	7.2 ±2.5	46.8 ±1.9	7
3d	CN	–	2.1 ±0.7	>100	>48
3e	N_3	–	0.067 ±0.011	45.9 ±0.7	685
3f	NH_2	–	0.069 ±0.006	45.6 ±0.9	661
10a	–	Cyanomethyl	15.3 ±3.2	>100	>7
10b	–	Benzyl	1.1 ±0.4	51.8 ±1.2	47
10c	–	2-picolyl	1.4 ±0.4	>100	>71
10d	–	4-picolyl	2.0 ±0.3	>100	>50
MKC-442			0.010 ±0.004	>100	>10,000
Nevirapine			0.061 ±0.007	>100	>1,639
4′-Ed4T			0.028 ±0.002	>100	>3,571

CC_{50}, concentration of compound that reduces the normal uninfected MT-4 cell viability by 50%. EC_{50}, concentration of compound required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells. SI, selectivity index (CC_{50}/EC_{50}).

derivative (**3f**) can be explainable by the H-bond formed between 6-amino group of **3f** and amide group of HIV-1 RT. Accordingly, the discrepancy of anti-HIV-1 activity between the 6-azido (**3e**) and 6-cyano analogues (**3d**) is caused by the antiviral activity of their metabolite. The 6-azido analogue (**3e**) may be converted to 6-amino congener (**3f**) and exert the anti-HIV-1 activity. To develop these compounds for the treatment of HIV-1, the antiviral activity against drug-resistant mutants is important. Therefore, it is interesting to know weather **3e** and **3f** inhibit the replication of NNRTI-resistant strains.

In conclusion, we discovered two 6-substituted 1-benzyl-3-(3,5-dimethylbenzyl) uracils as novel anti-HIV agents. These compounds should be further pursued for their toxicity and pharmacokinetics *in vivo* as well as antiviral activity against NNRTI-resistant strains.

Disclosure statement

All authors are inventors of the patent currently submitted to Japan Patent Office.

References

1. De Clercq E. Strategies in the design of antiviral drugs. *Nat Rev Drug Discov* 2002; **1**:13–25.
2. De Clercq E. New Approaches toward anti-HIV Chemotherapy. *J Med Chem* 2005; **48**:1297–1313.
3. De Clercq E. The design of drugs for HIV and HCV. *Nat Rev Drug Discov* 2007; **6**:1001–1018.
4. Baba M, Tanaka H, De Clercq E, *et al.* Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. *Biochem Biophys Res Commun* 1989; **165**:1375–1381.
5. Buckheit RW, Jr., Watson K, Fliakas-Boltz V, *et al.* SJ-3366, a unique and highly potent nonnucleoside reverse transcriptase inhibitor of human immunodeficiency virus type 1 (HIV-1) that also inhibits HIV-2. *Antimicrob Agents Chemother* 2001; **45**:393–400
6. Maruyama T, Kozai S, Yamasaki T, *et al.* Synthesis and antiviral activity of 1,3-disubstituted uracils against HIV-1 and HCMV. *Antivir Chem Chemother* 2003; **14**:271–279.
7. Maruyama T, Kozai S, Demizu Y, *et al.* Synthesis and anti-HIV-1 and anti-HCMV activity of 1-substituted 3-(3,5-dimethylbenzyl) uracil derivatives. *Chem Pharm Bull (Tokyo)* 2006; **54**:325–333.
8. Maruyama T, Ikejiri M, Izawa K, Onishi T. Synthesis and biological activity of fluorinated nucleosides. In Ojima I (Editor). *Fluorine in Medicinal Chemistry and Chemical Biology*. Chichester: John Wiley & Sons 2009; pp165–198.

9. Cresswell RM, Wood HCS. The biosynthesis of pteridines. I. The synthesis of riboflavine. *J Chem Soc* 1960; 4768–4775.
10. Ishikawa I, Itoh T, Melik-Ohanjanian RG, *et al.* Synthesis and X-Ray Analysis of 1-Benzyl-6-chlorouracil. *Heterocycles* 1990; **31**:1641–1646.
11. Mitsunobu O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* 1981; **1**:1–28.
12. Gogoi M, Sandhu JS, Baruah JN. Phase-transfer catalyzed preparation of 6-alkoxy and 6-thioalkoxy-1,3-dimethyluracils. *Indian Journal of Chemistry, Section B* 1984; **23B**:851–852.
13. Ohkura K, Uchiyama S, Aizawa K, Nishijima K, Seki K. Photochemical synthesis of naphthocyclobutapyrimidines *via* 1,2-cycloaddition of 6-chloro-1,3-dimethyluracil with naphthalenes. *Heterocycles* 2002; **57**:1403–1408.
14. Nagata W, Yoshioka M. Organic Reactions. Vol. 25. New York: John Wiley & Sons, Inc. 1977; pp. 255–476.
15. Bhuyan PJ, Borah HN, Sandhu JS. Studies on uracils: an efficient method for the synthesis of novel 1-allyl-6-(1,2,3-triazolyl) analogs of HEPT. *Journal of the Chemical Society, Perkin Transactions 1* 1999; **21**:3083–3084.
16. Hirota K, Maruhashi K, Asao T, Senda S. Pyrimidine derivatives and related compounds. XLIV. Thermolysis of 6-azido-1,3-dimethyluracil to a pyrimido [5,4-g] pteridine derivative. *Chem Pharm Bull (Tokyo)* 1982; **30**:3377–3379.
17. Baker BR, Jackson GDF. Nonclassical antimetabolites XXIV. Alkylation of 5-fluorouracil. *J Pharm Sci* 1965; **54**:1758–1762.
18. Kozai S, Fukagawa T, Maruyama T. A new method for the synthesis of N3-alkylated analogs of 5-fluorouracil. *Journal of the Chemical Society, Perkin Transactions 1* 1998; **19**:3145–3146.
19. Baba M, De Clercq E, Tanaka H, *et al.* Potent and selective inhibition of human immunodeficiency virus type 1 (HIV-1) by 5-ethyl-6-phenylthiouracil derivatives through their interaction with the HIV-1 reverse transcriptase. *Proc Natl Acad Sci U S A* 1991; **88**:2356–2360.
20. Pauwels R, Balzarini J, Baba M, *et al.* Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J Virol Methods* 1988; **20**:309–321.
21. De Clercq E, Descamps J, Verhelst G, *et al.* Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. *J Infect Dis* 1980; **141**:563–574.

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LETTER

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Synthesis of 4′-Ethynyl-2′-deoxy-4′-thioribonucleosides and Discovery of a Highly Potent and Less Toxic NRTI

Kazuhiro Haraguchi,*[,†] Hisashi Shimada,[†] Keigo Kimura,[†] Genta Akutsu,[†] Hiromichi Tanaka,[†] Hiroshi Abe,[‡] Takayuki Hamasaki,[§] Masanori Baba,[§] Elizabeth A. Gullen,[∥] Ginger E. Dutschman,[∥] Yung-Chi Cheng,[∥] and Jan Balzarini[⊥]

[†]School of Pharmacy, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

[‡]RIKEN Advanced Science Institute, Nanomedical Engineering Laboratory, 2-1, Hirosawa, Wako-shi, Saitama 351-0198, Japan

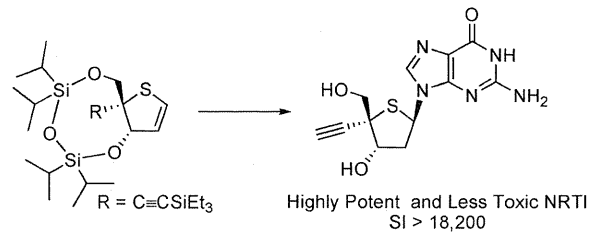
[§]Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 89-8544, Japan

[∥]Department of Pharmacology, School of Medicine, Yale University, New Haven, Connecticut 06520, United States

[⊥]Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Supporting Information

ABSTRACT: The synthesis of 4′-ethynyl-2′-deoxy-4′-thioribonucleosides was carried out utilizing an electrophilic glycosidation in which 4-ethynyl-4-thiofuranoid glycal **16** served as a glycosyl donor. Electrophilic glycosidation between **16** and the silylated nucleobases (N^4-acetylcytosine, N^6-benzoyladenine, and N^2-acetyl-O^6-diphenylcarbamoylguanine) was carried out in the presence of *N*-iodosuccinimide (NIS), leading to the exclusive formation of the desired β-anomers **29**, **33**, and **36**. Anti-HIV studies demonstrated that these 4′-thio nucleosides were less cytotoxic to T-lymphocyte (i.e., MT-4 cells) than the corresponding 4′-ethynyl derivatives of 2′-deoxycytidine (**44**), 2′-deoxyadenosine (**45**), and 2′-deoxyguanosine (**46**). Comparison of the selectivity indices (SI) was made between 4′-thionucleosides (**32**, **41**, and **43**) and the corresponding 4′-oxygen analogues **44**-**46** by using the reported CC_{50} and EC_{50} values. In the case of cytosine and adenine nucleosides, comparable SI values were obtained as follows: **32** (545) and **44** (458); **41** (>230) and **45** (1630). In contrast, 4′-ethynyl-2′-deoxy-4′-thioguanosine **43** was found to possess a SI value of >18200, which is 20 times better than that of **46** (933).



KEYWORDS: 4′-Thionucleosides, glycal, electrophilic glycosidation, anti-HIV-1 activity, nucleoside reverse transcriptase inhibitors

Nucleoside analogues are recognized as an important class of biologically active compounds, especially as antiviral and antitumor agents.[1−3] Among their sugar-modified analogues, 4′-thionucleosides, in which the oxygen atom in the furanose ring is replaced with a sulfur atom, have attracted much attention since the discovery of the antiviral and antitumor activities of 4′-thiothymidine (**1**) and 2′-deoxy-4′-thiocytidine (**2**) (Figure 1).[4] Also, it has been reported that 4′-substituted thymidines such as the 4′-azido (**3**), 4′-methoxy (**4**), 4′-cyano (**5**), and 4′-ethynyl (**6**) derivatives exhibit potent anti-HIV activity.[5]

Having been stimulated by the above findings, we synthesized the 4′-substituted analogues **7**−**12** of 4′-thiothymidine (Figure 2) and found promising anti-HIV activity in the 4′-azido (**8**), the 4′-cyano (**11**), and the 4′-ethynyl (**12**) derivatives.[6] This finding led us to investigate the present study where synthesis of the 4′-ethynyl analogues having other nucleobases (cytosine, adenine, and guanine) was carried out.

In our previous study,[6] the synthesis of **7**−**12** was accomplished through nucleophilic substitution of the 4′-acetoxy derivative **13** (Figure 3). The 4′-acetoxy leaving group of **13** was introduced by diacetoxylation of the 4′,5′-anhydro derivative **14** with $Pb(OAc)_4$. Compound **14** was prepared by a series of reactions initiated with NIS-mediated electrophilic glycosidation between silylated thymine and TIPDS (1,1,3,3- tetraisopropyldisiloxane-1,3-diyl)-protected 4-thiofuranoid glycal **15**.[7] In the present study, to enable a diverse set of nucleobases to be introduced, the 4-thiofuranoid glycal **16** already substituted at the 4-position with the triethylsilylethynyl group was employed as a glycosyl donor.

Our plan to introduce an ethynyl group in a tetrahydrothiophene ring is visualized in Scheme 1. Aldol reaction between **A** and formaldehyde gives **B**, which is then converted to the *O*-silyl-protected **C**. The formyl group of **C** is reacted with dimethyl

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