Pergamon

PII: S0957-4166(97)00019-0

Tetrahedron: Asymmetry, Vol. 8, No. 5, pp. 677–681, 1997

0957-4166/97 $17.00 + 0.00

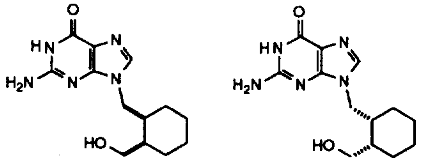
Resolution and stereoselective synthesis of the herpes thymidine kinase inhibitor L-653180

Derek J. Von Langen * and R. L. Tolman
Merck Research Laboratories, Rahway, New Jersey 07065, USA

Abstract: Resolution of L-653180, (±)-9-{[(Z)-2-(hydroxymethyl)cyclohexyl]-methyl}guanine, a racemic, nonsubstrate inhibitor of HSV-TK, was achieved and the absolute stereochemistry of the enantiomers was determined. Enzyme inhibition was shown to reside in the enantiomer with [1*S*,2*R*] stereochemistry in the cyclohexyl sidechain. An enantioselective synthesis of the more active L-653180 stereoisomer was devised. © 1997 Elsevier Science Ltd. All rights reserved.

During investigations on identifying novel inhibitors of herpes simplex virus thymidine kinase (HSV-TK), L-653180, prepared as the racemate **1a** and **1b**, (Figure 1), was found to be a potent, nonsubstrate inhibitor of HSV-TK.[1] Separation and independent testing of the enantiomers was undertaken to determine if they possessed differential activity towards HSV-TK.

The most expedient way to separate the enantiomers was to utilize chiral HPLC. It was found that baseline separation of the enantiomers could be achieved on a ChiralPak AD column (Figure 2). Although L-653180 was sparingly soluble in the mobile phase (hexane:ethanol 95:5), several milligrams of each enantiomer were isolated for biological assay. The faster isomer, identified as **1a**, (T_r=13.4 min) had a K_i=0.14 μM whereas the slower isomer, **1b**, (T_r=16.7 min) had a K_i=17 μM against the HSV-TK as calculated by the Dixon plot of 1/V against inhibitor concentration at various thymidine concentrations.



1a **1b**

Figure 1. Enantiomers of L-653180

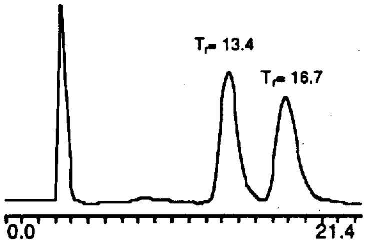
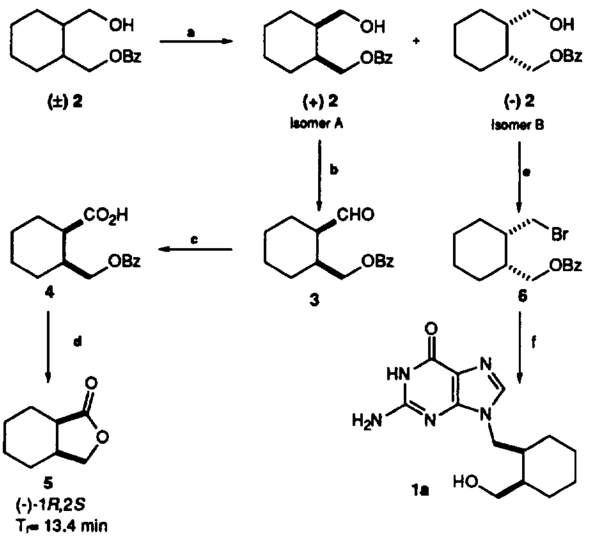


Figure 2. Chiral separation of enantiomers **1a** and **1b**

---

* Corresponding author.

Once we had determined the enantiomers possessed differential activity, we turned our attention towards establishing the absolute stereochemistry of each enantiomer. The racemic hydroxybenzoate **2**, an intermediate in the synthesis of L-653180,[2] could easily be separated by preparative chiral chromatography[3] yielding enantiomers (+)**2** and (−)**2** each of >98% *ee*. The absolute stereochemistry of (+)**2** was determined to be 1*R*, 2*S* by conversion of (+)**2** to the known lactone **5**[4] (Scheme 1). Thus the alcohol (+)**2** was oxidized to the corresponding carboxylic acid **4**, which was then deprotected and cyclized under acidic conditions. Lactone **5** was identical to a sample of known absolute configuration by chiral HPLC and optical rotation. The other enantiomer, (−)**2**, must then possess the opposite absolute stereochemistry, 1*S*, 2*R*.



Scheme 1. Optical trace to determine the absolute stereochemistry for the individual enantiomers of L-653,180. a) ChiralCel OD, hexanes:isopropanol 98:2; b)TPAP, NMMO, DCM, sieves; c)$NaClO_2$, NH_3SO_3H, aq THF; d) triethylamine, MeOH then dil HCl; e) CBr_4, MeOH; f).[2]

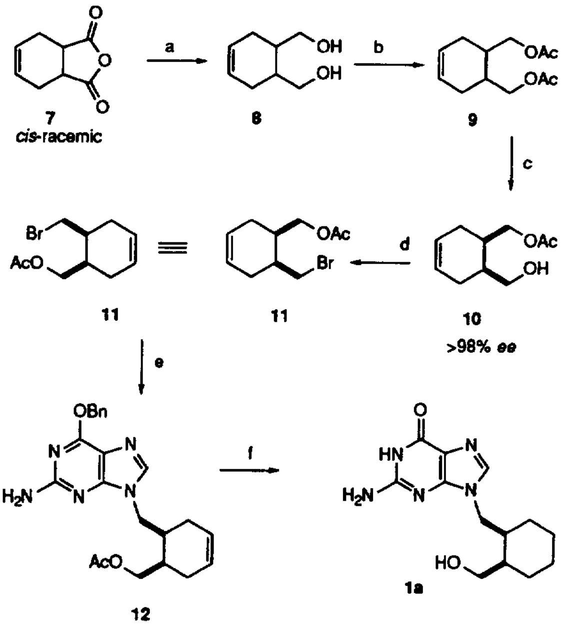
To determine the stereochemistry of the final compounds, (−)**2** was converted to its corresponding 9-alkyl guanine derivative for comparison with **1a** and **1b**. Following the literature procedure,[2] (−)**2**, was converted to its bromide **6** and then alkylated and deprotected to afford a compound which was identified as the faster enantiomer, **1a**, by chiral HPLC. Thus the absolute stereochemistry of **1a**, the more active enantiomer of L-653180, is determined to be 1*S*, 2*R*.

With the chirality of the synthetic target now determined we wished to develop an inexpensive, efficient route to **1a**. Scheme 2 outlines the synthesis in which the enantioselectivity is derived from an enzymatic hydrolysis of a prochiral intermediate. The inexpensive phthlate ($15/kg), **7**, was reduced to the racemic diol **8**. Treatment with acetyl chloride gave the meso-diacetate **9** which was selectively hydrolyzed with porcine pancreatic lipase[5] to the chiral monoacetate **10** in greater than 99% *ee*. Conversion of **10** to its corresponding bromide **11**, and coupling with the protected purine gave acetate **12** which was deprotected to afford **1a** in >98% *ee* and good overall yield.

In summary, the resolution of a novel HSV-TK inhibitor, L-653180 has been achieved. The stereochemistry of the enantiomers has been determined. A short, efficient, enantioselective synthesis of the more active enantiomer, **1a**, has been devised.

Experimental section

^{1}H NMR (500 MHz) and ^{13}C NMR (125.8 MHz) were measured on a Varian Unity 500 spectrometer. Chemical shifts are reported in units of δ relative to internal chloroform at 7.24 ppm for ^{1}H NMR



Scheme 2. Enantioselective synthesis of 1a. a) LAH, THF; b) AcCl, pyr.; c) PPL, pH 7 buffer; d) CBr_4, Ph_3P, CH_2Cl_2; e) NaH, DMF, 2-amino-6-benzyloxypurine; f) K_2CO_3 then 10% Pd/C, H_2.

and 77.00 ppm for ^{13}C NMR. Spectra were recorded in $CDCl_3$ unless noted otherwise. Analytical thin-layer chromatography (TLC) was performed on precoated silica plates (60F-254, 0.2mm thick E. Merck). Visualization of the plates was accomplished with UV light, ethanolic phosphomolybdic acid or ammonium molybdate/ceric sulfate/sulfuric acid charring procedures. Chromatography refers to vacuum filtration chromatography using Kieselgel 60G for thin layer chromatography as the adsorbent. HPLC analyses were performed on a Rainin Dynamax system using a PDA-1 photodiode array detector or single channel UV-1A detector under the following methods: A: ChiralCel OD (4.6×250 mm), Hexane:isopropanol (98:2), 1mL/min, λ=220 nm, rt; B: YMC Basic (4.6×150 mm) MeOH:H_2O (0.1% TFA) 50:50, 0.5 mL/min, rt; C: ChiralPak AD (4.6×250 mm), Hexane:EtOH 80:20 1 mL/min, λ=252 nm, 40°C.

Preparation of 1(3H)-(3S,4R)-hexahydroisobenzofuranone 5

A solution of (+)-(Z)-2-(benzyloxymethyl)cyclohexanemethanol (0.065 g, 0.26 mmol) in dichloromethane (2 mL) was treated with crushed 4A molecular sieves (0.130 g), N-methylmorpholine-N-oxide (0.046 g, 0.39 mmol) and TPAP (0.005 g, 2 mole %) and stirred at room temperature for 30 min. The reaction mixture was then transferred to a pad of silica gel and eluted with hexane:EtOAc (4:1) to yield **3** as a colorless oil (51 mg) and was used without purification. A solution of aldehyde **3**, THF (2 mL), 1 M $NaClO_2$ (0.3 mL), 1 M NH_3SO_3H (0.3 mL) and 2.7 M NaH_2PO_4 (0.12 mL) was stirred at 0°C for 45 min. The bright yellow solution was diluted with dichloromethane and water. The phases were separated and the organic washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give the carboxylic acid **4** as a colorless solid (0.056 g). This material was taken into 1 mL of triethylamine/methanol/water (4/4/1) and warmed to reflux overnight. After cooling to room temperature the reaction was acidified with 1 M HCl, extracted with ether, dried and concentrated to afford lactone **5** as an oil. ^1H NMR δ 4.19 (dd, 1H, J=5,9 Hz), 3.95 (d, 1H, J= 9Hz), 2.6–2.69 (m, 1H), 2.40–2.50 (m, 1H), 2.15 (d, 1H, J=5Hz), 1.75–1.85 (m, 1H), 1.55–1.70 (m, 3H), 1.18–1.30 (m, 3H). ^{13}C NMR δ 178.4, 71.6, 39.3, 35.2, 27.0, 23.3, 22.7, 22.4. HPLC Method A: T_r=13.1 min [compound **5** coelutes with a reference[4] sample of the (−)-1*R*,2*S* isomer of the lactones, the racemic lactones have T_r=13.1 and T_r=14.9 min for the (−)-1*R*,2*S* and (+)-1*S*,2*R* isomers, respectively]; $[\alpha]_D^{25}$=−46.3 (c=1.0, $CHCl_3$) lit. $[\alpha]_D^{25}$=−45.9 (c=1.1, $CHCl_3$).

Preparation of cis-*1,2-dihydroxymethylcyclohex-4-ene 8*

To a 0°C suspension of lithium aluminum hydride (10 g, 4 eq) in dry THF (250 mL) was added portionwise phthalic anhydride (15 g, 98.6 mmol) over 20 min. The mixture was brought to room temperature, warmed to reflux for 6 h then cooled to 0°C. The reaction was quenched with saturated sodium sulfate solution, filtered and concentrated. The residue was taken into ethyl acetate washed with water, brine, dried and concentrated to yield **8** as a colorless viscous oil (12.3 g, 88%). ^1H NMR δ 5.62 (s, 2H), 3.76–3.70 (m, 2H), 3.72–3.55 (m, 4H), 2.20–1.98 (m, 6H), ^{13}C NMR δ 125.6, 63.7, 37.8, 26.9. MS: (M+H)=143 m/z.

Preparation of cis-*1,2-diacetoxymethylcyclohex-4-ene 9*

To a solution of *cis*-1,2-dihydroxymethylcyclohex-4-ene (4.0 g, 28.2 mmol), pyridine (6.8 mL, 3 eq) and dichloromethane (60 mL) was added acetyl chloride (4.2 mL, 2.1 eq). A colorless precipitate forms and the mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane and washed with water, 1 N hydrochloric acid (×2), brine, dried and concentrated to give **9** as a slightly orange oil (6.3 g, 98%). ^1H NMR δ 5.64 ppm (s, 2H), 4.12–4.07 (m, 2H), 4.04–3.98 (m, 2H), 2.29–2.12 (m, 4H), 2.03 (s, 6H), 1.96–1.88 (m, 2H), ^{13}C NMR δ 172.6, 125.4, 63.5, 37.6, 26.9, 20.8. MS: (M+H)=227 m/z.

*Preparation of 1(*R*)-acetoxymethyl-2(*S*)-hydroxymethylcyclohex-4-ene 10*

A suspension of *cis*-1,2-diacetoxymethylcyclohex-4-ene (2.3 g, 10 mmol), pH 7 phosphate buffer (25 mL) and porcine pancreatic lipase (0.200 g, Sigma Type II) was vigorously stirred. The reaction was kept constant by addition of 1 M sodium hydroxide from a pH controller and syringe pump. After 16 h addition of 1 eq hydroxide was complete and the mixture was filtered through a pad of Celite. The Celite was washed with ether and the filtrates were washed with ether (×3). The combined ether washes were washed with brine, dried and concentrated to yield **10** as a slightly yellow oil (1.6 g, 89%). ^1H NMR δ 5.68–5.60 ppm (m, 2H), 4.21 (dd, 1H, J=7,11 Hz), 3.95 (dd, 1H, J=8,11 Hz), 3.68 (dd, 1H, J=7,11 Hz), 3.57 (dd, 1H, J=7,11 Hz), 2.29–2.12 (m, 4H), 2.05 (s, 3H), 2.01–1.85 (m, 3H), ^{13}C NMR δ 171.4, 125.6, 125.0, 64.9, 63.8, 37.1, 33.1, 26.8, 25.9, 20.9. $[\alpha]_D^{25}$=−17 (c=0.42, chloroform), MS: (M+H) 185 m/z.

*Preparation of 1(*R*)-acetoxymethyl-2(*S*)-bromomethylcyclohex-4-ene 11*

To a solution of dichloromethane (20 mL), tetrabromomethane (3.37 g) and 1(*R*)-acetoxymethyl-2(*S*)-hydroxymethylcyclohex-4-ene (1.25 g, 6.8 mmol) was added a solution of triphenylphosphine (2.13 g) in dichloromethane (5 mL) and the reaction stirred in the dark for four days. The reaction was concentrated to a dark oil and triturated with ether. The solid was filtered and the filtrate concentrated and purified by silica gel chromatography to yield **11** as a nearly colorless oil (1.25 g, 75%): $[\alpha]_D^{25}$=−11.3 (c=1.1, $CHCl_3$); ^1H NMR δ 5.64 ppm (dd, 2H, J=8,10 Hz), 4.09–4.0 (m, 2H), 3.48 (dd, 1H, J=7,10 Hz), 3.38 (dd, 1H, J=8,10 Hz), 2.40–2.33 (m, 1H), 2.31–2.13 (m, 3H), 2.08 (s, 3H), 2.10–1.94 (m, 2H), ^{13}C NMR δ 173.5, 129.9, 129.0, 50.6, 49.9, 37.9, 36.5, 24.0, 22.9, 20.8.

*Preparation of 9-{[1(*R*)-acetoxymethyl-2(*S*)-cyclohex-4-enyl]methyl}2-amino-6-benzyloxypurine 12*

To a suspension of 2-amino-6-benzyloxypurine (0.22 g, 1 mmol) in DMF (2 mL) was added sodium hydride (0.037 g) and the mixture stirred at room temperature for 30 min. A solution of 1(*R*)-acetoxymethyl-2(*S*)-bromomethylcyclohex-4-ene (0.272 g) in DMF (0.75 mL) and the reaction stirred at 60°C for 16 h. The reaction was neutralized with acetic acid and concentrated. The desired compound **12** was purified by silica gel chromatography (chloroform:methanol 97:3) as a colorless foam (0.16 g, 40%): ^1H NMR δ 8.02 ppm (s, 1H), 7.65 (s, 1H), 7.49 (d, 2H, J=8Hz), 7.38–7.26 (m, 3H), 5.65 (dd, 2H, J=7,10 Hz), 5.56 (s, 2H), 4.33 (dd, 1H, J=7,10 Hz), 4.12 (dd, 1H, J=7,10 Hz), 4.06–3.97 (m, 2H), 2.40 (bs, 1H), 2.23–1.75 (m, 6H), 2.20 (s, 3H), ^{13}C NMR δ 172.3, 159.1, 152.7, 150.0, 141.5, 139.7, 128.3, 128.2, 127.9, 125.4, 124.8, 114.9, 67.9, 64.3, 44.4, 35.3, 33.6, 26.8, 21.0.

*Preparation of 9-{[1(*R*)-hydroxymethyl-2(*S*)-cyclohexyl]methyl}guanine 1a*

To a solution of 9-{[1(*R*)-acetoxymethyl-2(*S*)-cyclohex-4-enyl]methyl}2-amino-6-benzyloxypurine (0.078 g), and aqueous methanol (1 mL) was added potassium carbonate (0.052 g) and the reaction stirred for 90 min at room temperature. The reaction was concentrated to remove the methanol and the residue partitioned between chloroform and water. The chloroform fraction was separated, dried and concentrated. The residue was taken into methanol (2 mL) and a catalytic amount of 10% Pd/C added. The mixture was stirred under a hydrogen balloon for 5 h filtered and concentrated to a colorless solid. The desired product was purified by silica gel chromatography (chloroform:methanol:water 90:10:1), to give **1a** (0.029 g, 70%): This material was identical in all respects with **1a** obtained by separation from the racemate by chiral chromatography: HPLC Method C, T_r=13.4 min, HPLC Method B T_r=6.9 min, 99.2% area; $[\alpha]_D^{25}$=+10.7 (c=0.1, MeOH); ^{1}H NMR (d_6-DMSO) δ 10.44 ppm (bs, 1H), 7.66 (s,1H), 6.39 (bs, 2H), 4.46 (t, 1H, J=5Hz), 3.90 (m, 2H), 3.55–3.30 (m, 2H, partially obscured by water), 2.18 (m, 1H), 1.8–1.1 (m, 9H), ^{13}C NMR δ 156.8, 153.4, 151.4, 137.8, 116.4, 60.9, 43.2, 37.4, 25.8, 22.9, 22.8.

References

1. Ashton, W. T., Meurer, L. C., Tolman, R. L., Karkas, J. D., Liou, R., Perry, H. C., Czelusniak, S. M., and Klein, R. J. *Nucleosides Nucleotides* **1989**, *8*, 1157.
2. Bourne, N., Bravo, F. J., Ashton, W. T., Meurer, L. C., Tolman, R. L., Karkas, J. D., and Stanberry, L. R. *Antimicrob. Agents Chemother.* **1992**, *36*, 2020.
3. The racemate (±)**2** was separated on a ChiralcelOD column (20×250mm) eluting with 2% isopropanol in hexanes, 8 mL/min. The faster enantiomer was identified as (+)**2** ($[\alpha]_D^{25}$=+22.6 (c=1.1, $CHCl_3$), T_r=43.7 min) and the slower as (−)**2** ($[\alpha]_D^{25}$=−23 (c=1.0, $CHCl_3$), T_r=46.8 min).
4. Hamilton, G. S., Huang, Z., Yang, X.-J., Patch, R. J., Narayanan, B. A., and Ferkany, J. W. *J. Org. Chem.* **1993**, *58*, 7263.
5. Laumen, K., and Schneider, M. *Tetrahedron Lett.* **1985**, *26*, 2073.

(Received in USA 6 September 1996; accepted 30 December 1996)