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A Straightforward Stereoselective Synthesis of D- and L-5-Hydroxy-4-hydroxymethyl-2-cyclohexenylguanine

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A novel and facile synthesis of 5-hydroxy-4-hydroxymethyl-2-cyclohexenylguanine 1 is described. The key steps involve a Diels–Alder reaction of ethyl (2E)-3-acetoxy-2-propenoate 2 as dienophile with Danishefsky’s diene 3 to build up the six-membered ring skeleton, a Fraser-Reid reductive rearrangement of the adduct using LiAlH₄, and base-moiety introduction using a Mitsunobu reaction. Optically pure D- and L-1 were obtained via resolution of intermediate 7 with (R)-(+-)-methylmandelic acid. The synthetic procedure toward racemic 1 consists of only five steps and has proven to be highly efficient toward the synthesis of cyclohexenyl nucleosides.

Introduction

Recently, we have demonstrated¹ that both D- and L-cyclohexenylguanine (1) (Figure 1) are highly potent and selective anti-herpes virus (HSV-1, HSV-2, VZV, CMV) agents. Their activity profile is similar to that of the known antiviral drugs acyclovir and ganciclovir, and they represent the most potent antiviral nucleosides containing a six-membered carbohydrate mimic that have ever been reported. Remarkably, the antiviral activity profiles of both enantiomers are very similar. We have described an enantioselective synthesis of the D- and L-enantiomers of 1 starting from (R)-carvone.¹² However, the synthesis is long and time-consuming, and it is not suited for the preparation of large amounts of the final products that are needed for full biological evaluation.

We hereby report a short and facile synthesis of 1 and its two enantiomers (Schemes 1–3). The key step is a Diels–Alder reaction³ of ethyl (2E)-3-acetoxy-2-propenoate 2 with Danishefsky’s diene 3 to construct the six-membered ring skeleton ⁴ with the desired trans orientation of the substituents in positions 4 and 5. Simultaneous reduction and rearrangement using lithium aluminum hydride (LAH) leads to triol 6. After protection as benzylidene acetal 7, the base moiety is introduced using a Mitsunobu reaction.

Results and Discussion

The preparation of dienophile (E)-2 and diene 3 for the Diels–Alder reaction is presented in Scheme 4. Ethyl (2E)-3-acetoxy-2-propenoate 2¹ can easily be obtained on a multigram scale by formylation of ethyl acetate and acetylation of the sodium salt of the α-formyl ester using acetyl chloride. A mixture of (E)-2 and (Z)-2 was obtained when the temperature during reaction and distillation was not well controlled. However, the (Z)-2 isomer can easily be converted quantitatively into (E)-2 via treatment of the mixture of (Z)-2 and (E)-2 with thiophenol in the presence of 2,2’-azobis(2-methylpropionitrile) (AIBN). Danishefsky’s diene 3¹ was readily obtained (50 g scale) by silylation of (E)-4-methoxy-3-buten-2-one (TMSCI, ZnCl₂, Et₃N) followed by distillation.

The Diels–Alder reaction was carried out by heating a mixture of neat 2 and 3 in the presence of hydroquinone at 180 °C for 1.5 h (Scheme 1). After removal of the volatiles, the residue was distilled under high vacuum and the adduct was obtained in 71% yield as a 4:1

Figure 1. Structure of (±)-cyclohexenyl G and assignment of D- and L-nomenclature.
yield over the two steps. The $\alpha$-configuration of the 1-OH substituent of 7 was established by an NOE experiment (Figure 2): irradiation of the proton at C1 gave enhanced signals of H2/3, H3, and H6.

Introduction of the purine base moiety was performed according to our previously reported procedure, i.e., 7 was reacted with 2-amino-6-chloropurine under Mitsunobu reaction conditions to generate the chloropurine 10a with inversion of the configuration at C1 (Scheme 2). The N$_2$-isomer (14%) 10b was also isolated. Finally, acidic hydrolysis of 10a gave the racemic (±)-1 in 27% overall yield starting from 7. The spectral data of (±)-1 are identical to those of the previously reported enantiomer(s).

The synthesis of optically pure $\alpha$-1 and $\beta$-1 was achieved via resolution of intermediate 7 followed by introduction of the base moiety on the enantiopure cyclohexene precursor using a Mitsunobu reaction (Scheme 3). Several methods for resolving 7 could be envisaged, i.e., kinetic resolution using Sharpless epoxidation, enzymatic methods such as enantioselective esterification using a lipase, or formation of diastereomeric esters.

Sharpless epoxidation has the disadvantage that half of the material will be lost because conversion of the resulting epoxide back to the double bond is rather tedious. In a first experiment, esterification of 7 using vinyl acetate and Lipase PS (Amano) gave only 33% of enantiomeric excess. As both enantiomers of 7 were needed, we preferred to synthesize the diastereomeric esters of 7 using a chiral acid. The use of the cheap and commercially available pyroglutamic acid 11 for this purpose was not satisfactory due to the difficult separation of the resulting diastereomers. The use of (−)-naproxene 12 was also attempted. However, the best result with this chiral acid was 38% of diastereomeric excess because only partial separation of its esters on silica gel could be achieved. Finally, we retained (R)-(-)-O-methylmandelic acid, which has been applied earlier to resolve the diastereomeric esters of cyclohexenyl nucleosides.

Acylation of 7 was carried out with (R)-(−)-O-methylmandelic acid in the presence of DCC and DMAP in 79% yield. Careful separation of the two diastereoisomers 13a and 13b by chromatography with a gradient of hexane and EtOAc gave pure 13a (eluting first) and 13b, respectively. The diastereomeric purity of 13a and 13b was checked by HPLC and found to be 97% for 13a and 98% for 13b. Hydrolysis of the ester group of 13a or 13b with KOH/MeOH provided 7a and 7b in good yields. The enantiomeric purity of 7a and 7b was examined by chiral chromatography, and was 97% and 98%, respectively.

Conversion of 7a into $\alpha$-1 and of 7b into $\beta$-1 was accomplished according to the same procedure as for (±)-1. The enantiomeric purity of $\alpha$-1 and $\beta$-1 was 99% and

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Scheme 1. Synthesis of the Cyclohexenyl Precursor

![Scheme 1](image)

$^a$ Key: (a) 180 °C, hydroquinone, 1.5 h, 62%; (b) LiAlH$_4$, THF, 0 °C, 66%; (c) PhCH(OMe)$_2$, PTSA, 1,4-dioxane, rt, 24 h, 70%; (d) MnO$_2$, CH$_2$Cl$_2$, rt, 21 h, 83%; (e) NaBH$_4$, CeCl$_3$·7H$_2$O, MeOH, rt, 2 h, 90%.

Figure 2. Assignment of the α-configuration of 1-OH using NOE experiments.

mixture of the endo and exo adducts 4a and 4b, respectively (as determined by $^1$H NMR analysis). During the distillation, the temperature should be carefully controlled to avoid the formation of phenol derivative 5 as the major product. The highly functionalized adduct 4 is sensitive to acid and moisture and is best reduced immediately. The stereochemical ambiguity in the adduct is removed during the next reaction. Reduction of the mixture 4a/4b with excess LiAlH$_4$, according to the procedure of Fraser-Reid, gave triol 6 in 64% yield. This reaction involves the reduction of two ester groups and concomitant rearrangement of the silyloxyenol ether to the enone intermediate, which is then further reduced to 6.

Reaction of triol 6 with benzaldehyde dimethyl acetal in the presence of PTSA at room temperature for 1 day gave rise to benzylidene acetal 7 (70% yield). Prolongation of the reaction time (2 days) gave rise to a mixture of 7 and 8 (ranging from 6:1 to 3:1). As separation of 7 and 8 proved difficult, the mixture was oxidized to enone 9, which was then reduced using NaBH$_4$ in the presence of CeCl$_3$·7H$_2$O. In this way, only 7 was obtained in 74% yield over the two steps. The α-configuration of the 1-OH substituent of 7 was established by an NOE experiment (Figure 2): irradiation of the proton at C1 gave enhanced signals of H2/3, H3, and H6.

97%, respectively, according to chiral chromatographical analysis (Figure 3). The absolute configuration of \( \text{D-1} \) and \( \text{L-1} \) was established via comparison with an authentic sample of \( \text{D-1} \) that was obtained via the enantioselective synthesis starting from \((\text{R})\)-carvone.\(^1\) By analogy, the absolute configuration of the intermediates \( \text{13a/13b} \) and \( \text{7a/7b} \) were also established.

In summary, we have developed a short and facile synthesis for the preparation of racemic \( \text{1} \) and its two enantiomers. The key steps involve a Diels–Alder reaction of enone \( \text{2} \) with Danishefsky diene \( \text{3} \) and the reductive rearrangement of the adduct \( \text{4} \). The synthesis consists of only 5 steps (for \((\pm)\)-\text{1}) and it represents a highly efficient approach toward the preparation of cyclohexenyl nucleosides.

### Experimental Section

**General Methods.** All air-sensitive reactions were carried out under nitrogen. THF and \( \text{Et}_2\text{O} \) were distilled from sodium/benzophenone, 1,4-dioxane from CaH\(_2\), and \( \text{CH}_2\text{Cl}_2 \) from P\(_2\)O\(_5\).
Ethyl (2E)-3-acetoxy-2-propenoate 2 and Danishefsky’s diene 3 were prepared according to the literature procedures. HPLC analysis was performed on a Merck-Hitachi L-6200 A liquid chromatograph equipped with a Merck-Hitachi L-4000 UV detector, using a Bio-Sil D90-10 column (250 x 10 mm). Eluent A: hexanes−EtOAc 70:30; Eluent B: hexanes−EtOH 70:30; detection: 200 nm; detection: 225 nm. Chiral HPLC analysis was performed on a Waters 6000 controller liquid chromatograph equipped with a Waters 2487 UV detector, using a Chiralpak AD column (250 x 4.6 mm). Eluent A: hexanes−EtOH 95:5, flow: 1.0 mL/min, detection: 200 nm; Eluent B: hexanes−EtOH 70:30 containing 0.2% TFA, flow: 1.0 mL/min, detection: 260 nm.

Isomerization of the Z/E Mixture 2 to Ethyl (2E)-3-Acetoxy-2-propenoate (2). A mixture of (Z)-2 and (E)-2 (39100, 52.5 g, 332 mmol) was treated with thionophenol (16.3 mL, 17.5 g, 159 mmol) and 2-azobis(2-methylpropionitrile) (AIBN, 8.31 g, 50.6 mmol) at 80 °C for 2.5 h. The reaction mixture was cooled to rt and taken up in ethyl acetate (400 mL) that was washed with an 0.01 N NaOH aqueous solution (250 mL). The organic layer was dried over Na2SO4 and concentration to leave a pale yellow oil. Distillation under vacuum (53 °C, 0.5 mmHg) to afford a white solid. 

Conversion of 9 to 7. A mixture of 7/8 (3.1:1.45 g, 1.79 mmol) and activated manganese dioxide (MnO2, 1.56 g, 17.9 mmol, 10 equiv) in dry CH2Cl2 (15 mL) was stirred at rt for 24 h. The reaction mixture was treated with benzaldehyde dimethyl acetal (6.2 mL, 41.2 mmol) and sodium hydride (1.2 equiv), the mixture was stirred at rt for 0.5 h and extracted with EtOAc (3x). The combined organic layers were washed with water and brine, dried over sodium sulfate and concentrated. The residue was purified on silica gel (hexanes−EtOAc 1:1) to afford 7 (5.06 g, 70% yield) as a white crystalline solid. A total of 600 mg (13%) of 6 was recovered: mp 105−106 °C; 1H NMR (CDCl3) δ 1.78 (td, 1H, J = 12.1, 9.9 Hz), 2.17−2.19 (m, 2H), 2.44−2.66 (m, 2H), 3.61 (t, 1H, J = 10.8 Hz), 3.67 (d, 1H, J = 11.1, 9.2 Hz), 4.26 (dd, 1H, J = 10.8, 4.6 Hz), 4.49 (m, 1H), 5.41 (dt, 1H, J = 9.9, 1.6 Hz), 5.60 (s, 1H), 5.72 (d, 1H, J = 9.9 Hz), 7.36−7.57 (m, 5H); 13C NMR (CDCl3) δ 38.4 (t), 39.9 (d), 67.7 (d), 70.6 (t), 77.7 (d), 102.1 (d), 124.9 (d); HRMS m/z calcd for C14H14O3Na (M + Na+) 255.0979, found 255.0995. Anal. Calcd for C13H12O3: C, 72.39; H, 6.94. Found: C, 72.07; H, 6.82.

When the reaction time was prolonged for 2 days, a mixture of 7 and 8 (ratio ranging from 3:1 to 6:1) was obtained.

Conversion of 9 to 7. To a mixture of LiAlH4 (25 g, 658 mmol) in dry THF (110 mL). The mixture was cooled in an ice bath and carefully treated with water (25 mL) for 15 min, a 15% aqueous NaOH solution (25 mL) for 15 min, and finally water (75 mL). After being stirred at room temperature for 0.5 h, the resulting mixture was filtered and the slurry was washed with water (5 x 100 mL) and EtOAc (3 x 100 mL). The layers were separated, and the aqueous layer was washed with ethyl acetate (3 x 100 mL). The aqueous layer was evaporated to dryness to give a brown gummy residue, which was chromatographed on silica gel (EtOAc/MeOH 91:9) to give 6 (6.74 g, 66%) as a light yellow syrup: 1H NMR (DMSO-d6) δ 1.37 (td, 1H, J = 11.7, 9.9 Hz), 1.92−2.10 (m, 2H), 3.24−3.45 (m, 2H), 3.63 (dt, 1H, J = 10.2, 4.4 Hz), 4.07 (m, 1H), 4.49 (t, 1H, J = 5.3 Hz, OH), 4.63 (d, 1H, J = 5.1 Hz, OH), 4.70 (d, 1H, J = 5.9 Hz, OH), 5.52 (d, 1H, J = 11.0 Hz), 5.57 (d, 1H, J = 11.0 Hz); 13C NMR (DMSO-d6) δ 42.0 (t), 47.2 (d), 62.2 (t), 65.9 (d), 66.3 (d), 72.7 (d), 132.8 (d); HRMS m/z calcld for C9H10O (M+) 162.0783, found 162.0778.

(±)-(4R*,7S*,8aR*)-2-Phenyl-4a,7,8,8a-tetrahydro-4H-1,3-benzodioxin-7-ol (7). Compound 6 (4.49 g, 31.1 mmol) was treated with benzaldehyde dimethyl acetal (6.2 mL, 41.2 mmol) in the presence of p-toluenesulfonic acid monohydrate (PTSA, 300 mg, 1.58 mmol) in dry 1,4-dioxane (140 mL) at rt for 24 h. Ice was added, and the mixture was stirred at rt for 0.5 h and extracted with EtOAc (3x). The combined organic layers were washed with water and brine, dried over sodium sulfate and concentrated. The residue was purified on silica gel (hexanes−EtOAc 1:1) to afford 7 (5.06 g, 70% yield) as a white crystalline solid. A total of 600 mg (13%) of 6 was recovered: mp 105−106 °C; 1H NMR (CDCl3) δ 1.78 (td, 1H, J = 12.1, 9.9 Hz), 2.17−2.19 (m, 2H), 2.44−2.66 (m, 2H), 3.61 (t, 1H, J = 10.8 Hz), 3.67 (d, 1H, J = 11.1, 9.2 Hz), 4.26 (dd, 1H, J = 10.8, 4.6 Hz), 4.49 (m, 1H), 5.41 (dt, 1H, J = 9.9, 1.6 Hz), 5.60 (s, 1H), 5.72 (d, 1H, J = 9.9 Hz), 7.36−7.57 (m, 5H); 13C NMR (CDCl3) δ 38.4 (t), 39.9 (d), 67.7 (d), 70.6 (t), 77.7 (d), 102.1 (d), 124.9 (d); HRMS m/z calcld for C14H14O3Na (M + Na+) 255.0979, found 255.0995. Anal. Calcd for C13H12O3: C, 72.39; H, 6.94. Found: C, 72.07; H, 6.82.
at 0 °C was added DCC (3.42 g, 16.5 mmol) in portions. The reaction mixture was allowed to warm to rt over 1.5 h and was stirred for an additional 1.5 h at rt. CH₂Cl₂ (500 mL) was added, and the mixture was filtered. The filtrate was washed with an aqueous 1 M H₃PO₄ solution (90 mL), water (90 mL), and a saturated aqueous NaHCO₃ solution (45 mL), dried over Na₂SO₄, and concentrated. The crude product as white crystals (7.11 g) was subjected to chromatography on silica gel (35–70 nm) packed with hexane and eluting with a mixture of hexanes–EtOAc, slowly increasing the polarity. On elution with hexanes–EtOAc 71:29, 1.34 g of 13a (93% de), a mixture of 13a and 13b (1.96 g), and 1.22 g of 13b (79% de) were obtained (4.52 g, 79% yield). The diastereomeric excess was determined by HPLC.

The fractions containing 13a and 13b were again submitted to chromatographic purification yielding 589 mg (97% of diastereomeric purity) of 13a and 426 mg (99% of diastereomeric purity) of 13b.

Compound 13a: mp 67–68 °C; ¹H NMR (CDCl₃) δ 1.91 (dt, 1H, J = 12.0, 10.2 Hz), 2.55 (ddd, 1H, J = 11.1, 7.5, 3.5 Hz), 2.60 (ddd, 1H, J = 10.9, 7.0, 3.9 Hz), 3.41 (s, 3H), 3.61 (t, 1H, J = 11.4 Hz), 3.71 (ddd, 1H, J = 11.4, 9.1, 3.1 Hz), 4.24 (dd, 1H, J = 11.0, 4.4 Hz), 4.78 (s, 1H), 5.47 (s, 1H), 5.59 (s, 1H), 5.59–5.68 (m, 1H), 6.32–6.74 (m, 1H), 7.32–7.54 (m, 10H), aromatic protons); ¹³C NMR (CDCl₃) δ 34.1 (t), 39.7 (d), 57.3 (q), 70.3 (t), 70.6 (d), 77.1 (d), 82.6 (d), 101.8 (d), 126.1 (d), 127.2 (d), 127.4 (d), 128.0 (d), 128.4 (d), 128.7 (d), 129.0 (d), 136.1 (s), 138.0 (s), 170.4 (s, CO). Anal. Calcd for C₂₃H₂₄O₅: C, 72.61; H, 6.36. Found: C, 72.64; H, 6.40.

Compound 13b: ¹H NMR (CDCl₃) δ 1.91 (dt, 1H, J = 12.0, 9.9 Hz), 2.37 (ddd, 1H, J = 10.8, 7.3, 2.9 Hz), 2.56 (m, 1H), 3.39 (s, 3H), 3.57 (t, 1H, J = 11.4 Hz), 3.64 (ddd, 1H, J = 12.4, 9.2, 3.2 Hz), 4.24 (dd, 1H, J = 11.0, 4.6 Hz), 4.76 (s, 1H), 5.47 (s, 1H), 5.43–5.57 (m, 1H), 5.54–5.70 (m, 1H), 7.30–7.48 (m, 10H, aromatic protons); ¹³C NMR (CDCl₃) δ 33.6 (t), 39.5 (d), 57.1 (q), 70.1 (t), 70.4 (d), 76.8 (d), 82.3 (d), 101.8 (d), 126.0 (d), 127.0 (d), 127.4 (d), 128.0 (d), 128.1 (d), 128.7 (d), 128.5 (d), 128.8 (d), 129.3 (s), 135.9 (s), 137.9 (s), 170.2 (s, CO); HRMS m/z calc for C₂₂H₂₃O₅Na (+M Na⁺) 403, found 403. Anal. Calcd for C₂₂H₂₃O₅Na: C, 72.61; H, 6.36. Found: C, 72.44; H, 6.42.

Hydrolysis of 13a to o-7a and 13b to l-7b. A solution of 13a (500 mg, 1.31 mmol, 97% de) in 20% aqueous NaOH (27 mL, 133 mmol), THF (27 mL), and MeOH (27 mL) was heated under reflux for 2 h. The organic solvent was evaporated under vacuum, and the remaining aqueous phase was diluted with water (30 mL) and extracted with CH₂Cl₂ (4 × 60 mL). The combined organic layers were washed with a saturated NaHCO₃ solution (60 mL) and brine (60 mL), dried over Na₂SO₄, and evaporated. The residue was chromatographed on silica gel (hexanes–EtOAc 2:1) to give 7a (291 mg, 95% yield, 97% ee according to chiral HPLC analysis using eluent A) as a white solid: mp 95 °C. Anal. Calcd for C₁₄H₁₅O₄: C, 72.39; H, 6.94. Found: C, 72.33; H, 6.70.

The same procedure was followed for conversion of 13b to 7b (91% yield, 99% ee): mp 94–95 °C. Anal. Calcd for C₁₄H₁₅O₄·0.5H₂O: C, 69.69; H, 7.10. Found: C, 69.57; H, 6.94.

(±)-[1S,4R,5S]-9-(5-Hydroxy-4-hydroxymethyl-2-cyclohexen-1-yl)guanine (1). To a mixture of 7 (696 mg, 3 mmol), 2-amino-6-chloropurine (1.02 g, 6 mmol), and triphenylphosphine (PPh₃, 1.57 g, 6 mmol) in dry 1,4-dioxane (30 mL) was added slowly a solution of DEAD (945 mL, 6 mmol) in dry 1,4-dioxane (10 mL). The reaction was stirred at rt overnight and concentrated. The residue was absorbed on silica gel and chromatographed (CH₂Cl₂–MeOH 100:1 and 50:1) to afford crude 10a (2 g) and the N₂-epimer 10b (140 mg) as a white solid.

Crude 10a (2 g) was treated with TFA–H₂O (3:1, 20 mL) at rt for 2 days. The reaction mixture was concentrated and coevaporated with toluene. The residue was chromatographed on silica gel (CH₂Cl₂–MeOH 50:1 and 10:1) to give (±)-1 (233 mg, 27% overall yield starting from 7). The physicochemical properties (¹H NMR, ¹³C NMR, MS, UV) of 1 are identical to those previously reported.⁴

(15R,4S,5R)-9-(5-Hydroxy-4-hydroxymethyl-2-cyclohexen-1-yl)guanine (o-1). The same procedure as described for the preparation of (±)-1 was used. The analytical sample was obtained by crystallization with a mixture of disopropyl ether–MeOH (8:2). The enantiomeric excess (99% ee) was determined by Chiral HPLC analysis using eluent B): mp 275–280 °C dec. Anal. Calcd for C₁₂H₁₅N₅O₃·2(H₂O): C, 46.00; H, 6.11; N, 22.35. Found: C, 46.30; H, 5.81; N, 22.18.

(1R,4S,5R)-9-(5-Hydroxy-4-hydroxymethyl-2-cyclohexen-1-yl)guanine (l-1). The same procedure as described for the preparation of (±)-1 was used. The analytical sample (97% ee) was obtained by crystallization from a mixture of EtOAc–MeOH (4:3): mp 273–277 °C dec. Anal. Calcd for C₁₂H₁₅N₅O₃·2(H₂O): C, 46.00; H, 6.11; N, 22.35. Found: C, 46.21; H, 5.86; N, 21.73.

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