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Synthesis of Novel Spiro[2.3]hexane Carbocyclic Nucleosides via Enzymatic Resolution

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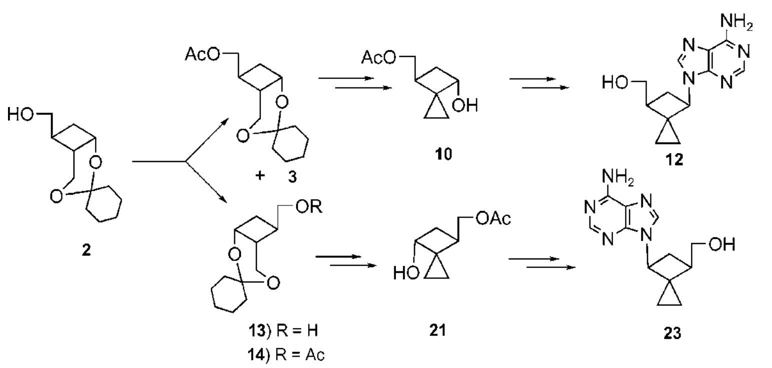
ABSTRACT



Novel *R*- and *S*-spiro[2.3]hexane nucleosides have been synthesized. The key step involved the *Pseudomonas cepacia* lipase catalyzed resolution of racemic compound 2, synthesized in seven steps starting from diethoxyketene and diethyl fumarate, to give (+)-acetate 3 and (–)-alcohol 13. (+)-Acetate 3 and (–)-acetate 14 were converted to *R*- and *S*-9-(6-hydroxymethylspiro[2.3]hexane)-4-adenine, respectively.

During the last two decades, treatment of viral infections has advanced remarkably, driven particularly by the search for effective agents for the treatment of herpes, AIDS, and viral hepatitis. In recent years, new and emerging viruses, such as new strains of hepatitis and herpes viruses, Ebola, West Nile, and SARS, have shown their lethal potential. Furthermore, the threat that viruses and other microorganisms could be used as biological weapons in warfare or bioterrorism has brought antiviral research to the forefront. Although vaccination is a valuable preventative tool for certain viral infections, new and effective antiviral agents are needed to prevent acute and chronic viral infections.

Nucleosides are the most frequently used effective class of antiviral agents, with over 20 drugs currently approved for the treatment of viral diseases and a number of candidates in the clinical trials.[1]

Since oxetanocin-A[2] was isolated and its antiviral activity described,[2,3] continuous studies have been made to explore the chemistry and biological activity of four-membered-ring-containing nucleosides (Figure 1). Since Honjo et al.[4] first

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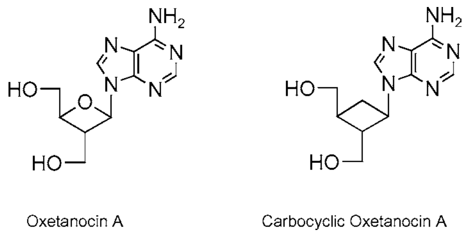

Figure 1. Analogues of oxetanocin.

reported the synthesis of cyclobutyladenine, the carbocyclic analogue of oxetanocin with its interesting biological activity[5] has prompted numerous subsequent syntheses of both parent and related compounds.[6]

Zemlicka and co-workers[7] described a new class of nucleoside analogues, the spiro[3.3]heptane and spiro[2.2]-pentane nucleosides. They found that adenosine analogue displayed some activity against human cytomegalovirus in vitro.[8] However, only limited examples of spiro nucleosides have been reported.[7,8] Herein, we wish to describe a facile method for the synthesis of novel *R*- and *S*-spiro[2.3]hexane nucleosides.

The observation of different pharmacological as well as toxicological properties of opposite enantiomers highlights the need for asymmetric synthesis.[9] In early studies, chiral resolutions were performed to obtain nonracemic intermediates.[10] Ichikawa et al.[11] employed a chiral titanium complex as the catalyst in an asymmetric [2 + 2] cyclization to provide a functionalized cyclobutyl intermediate. Jung and Sledeski[12] utilized an enzymatic desymmetrization of a *meso* cyclobutane as the enantioselective step in their formation of nonracemic cyclobutyl adenine. However, the large number of steps involved as well as the moderate enantioselectivity of enzyme-catalyzed reactions resulted in relatively low overall yield. Consequently, the need exists for more efficient and simple methods for obtaining chiral forms with high enantiomeric excess.

Table 1. Different Enzymes Tested for Maximum Optical Purity

lipases	**3** $[\alpha]_D$	**13** $[\alpha]_D$	time (h)
Candida antarctica	1.40	−3.6	13−14
Lipozyme (*Mucor mechei*)	2.40	−4.2	13−14
Porcine pancreatic	11.90	−19.0	13−14
Pseudomonas cepacia	42.00	−48.10	2

In this paper, we describe the use of *Pseudomonas cepacia* lipase promoted enzymatic resolution of intermediate **2** for the synthesis of spiro[2.3]hexane nucleosides. The synthesis of cyclobutyl precursor **2** was achieved as described in the literature.[13] Compound **2** was subjected to enzymatic resolution by using different lipases (Table 1). Among the various lipases studied, *P. cepacia* (PS) gave the highest optical and chemical yield on a multigram scale. The reaction progress was monitored by ^{1}H NMR. After 2 h, a 1:1 ratio for the H-3 proton was observed and (+)-(1*S*,2*S*,3*S*)-3-*O*-acetyl-1,2-*O*-cyclohexylidene-2,3-bis(hydroxymethyl)-1-cyclobutanol **3** and (−)-(1*R*,2*R*,3*R*)-1,2-*O*-cyclohexylidene-3-hydroxy-2,3-bis(hydroxymethyl)-1-cyclobutanol **13** were obtained. Vinyl benzoate and vinyl acetate were studied as an acylating agents; however, the later one was found to give better enantioselectivity.

To synthesize *R*-spiro[2.3]hexane carbocyclic nucleoside **12**, compound **3** was hydrolyzed with Amberlite IR 120 (H^+) in methanol, followed by the tritylation of primary alcohol to give compound **4** (Scheme 1). Subsequent silylation of the secondary alcohol gave compound **5**. Deprotection of the trityl group was effected using $BF_3 \cdot Et_2O$ to furnish **6**. Iodination of compound **6** with $CH_3P(OPh)_3I$ in THF gave iodide **7**. DBU-mediated elimination of **7** in THF under reflux conditions gave compound **8**. Attempts for cyclopropanation on compound **8** were unsuccessful; hence, the TBDPS protecting group was first deprotected using TBAF in THF to give compound **9**. Cyclopropanation of compound **9** using $(C_2H_5)_2Zn$ and CH_2I_2 under reflux conditions in ether gave (*R*)-6-acetoxymethylspiro[2.3]hexane-4-ol **10**[14] in 53% yield. The alcohol **10** was condensed with 6-chloropurine under Mitsunobu conditions to give compound **11** in 63% yield. The 6-chloro derivative was converted to compound **12** by treatment with a saturated solution of ammonia in methanol in a steel bomb at 100 °C for 24 h. Deprotection of the primary alcohol as well as ammonolysis took place under the same conditions to give (*R*)-9-(6-hydroxymethylspiro[2.3]hexane)-4-adenine **12**.[15] Following a similar procedure, the (−)-acetate **14** was converted

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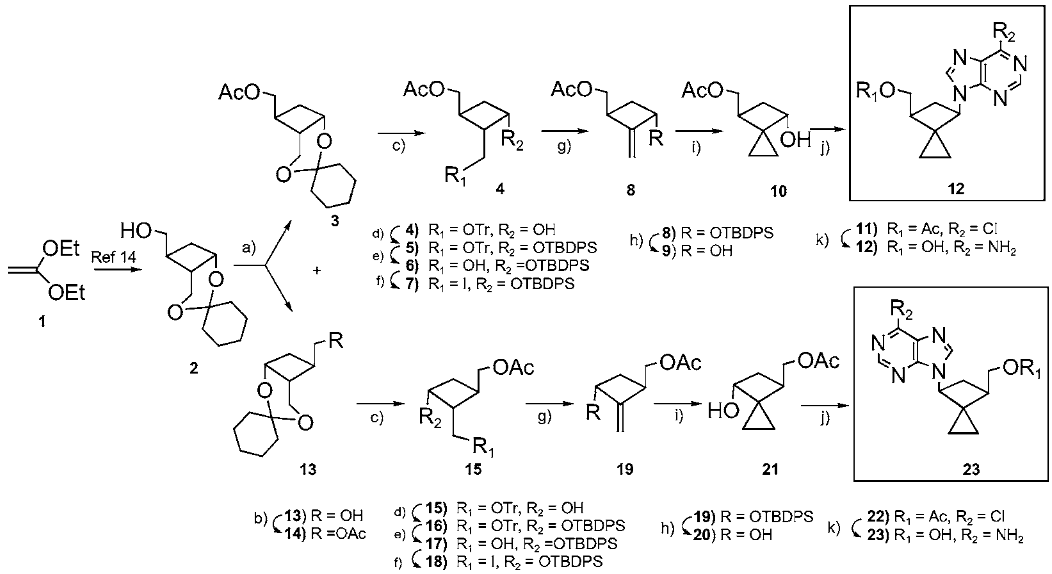
Scheme 1. Synthesis of (*R*)-D- and (*S*)-L-Spiro[2.3]hexane Carbocyclic Nucleoside[a]



[a] Reagents and conditions: (a) *P. cepacia* lipase, AcOCH=CH$_2$, 28 °C, 2 h; (b) Ac$_2$O, py, CH$_2$Cl$_2$; (c) (i) Amberlite IR-120 (H$^+$), MeOH, rt, 1 h, (ii) Ph$_3$CCl, Py, rt, overnight; (d) TBDPS-Cl, imidazole, CH$_2$Cl$_2$, rt, 1 h; (e) BF$_3$·OEt$_2$, MeOH, CH$_2$Cl$_2$, rt, 1 h; (f) Me$_3$P(OPh$_3$)I, DMF, 1 h, rt; (g) DBU, THF reflux, 24 h; (h) TBAF, THF, RT, 1 h; (i) (C$_2$H$_5$)$_2$Zn, CH$_2$I$_2$, ether, 0−45 °C; (j) 6-chloropurine, PPh$_3$, DIAD, THF, 0 °C to rt, overnight; (k) NH$_3$, MeOH, 100 °C, 24 h.

to (*S*)-9-(6-hydroxymethylspiro[2.3]hexane)-4-adenine **23**[16] in 10 steps. The optical purity was determined by chiral HPLC on a CHIRALPAK AD-H column using 2-propanol−hexane (1:1) as an eluent and found to be 98.2% ee for compound **12** and >99.9% ee for compound **23** (Figure 2).

The antiviral activity of the synthesized spiro nucleosides **12** and **23** was evaluated against HIV-1 in human PBM cells[17] The (*R*)-adenine analogue **12** exhibited moderately potent anti HIV activity (EC$_{50}$ 22.4 μM) without cytotoxicity up to 42.4 μM in PBM, 100 μM in CEM, and Vero cells. The *S*-enantiomer **23** also showed some anti-HIV activity (EC$_{50}$ 48.6 μM) (Table 2).

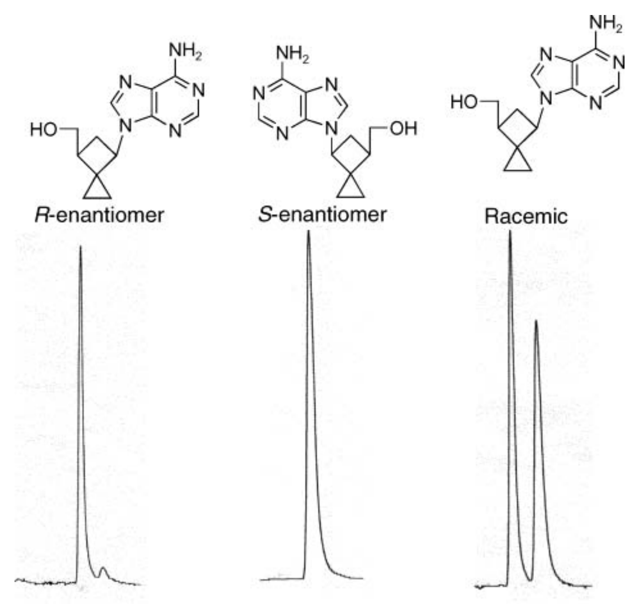


Figure 2. Chiral HPLC of enantiomers **1**, **2**, and **1** + **2**. CHIRALPAK AD-H column (250 × 4.6 mm, 2-propanol/hexane (1:1) as eluent, flow rate 0.2 mL/min, detection at 260 nm, concentrated = 1 mg/mL, volume injected = 5 μL. RT = retention time.

(14) **Compound 10:** colorless oil; $[\alpha]_D$ +119.05 (*c* 0.15, CHCl$_3$); ^{1}H NMR (CDCl$_3$); δ 0.34−0.44 (m, 2H), 0.73 (m, 1H), 0.86 (m, 1H), 2.08 (s, 3H), 2.13 (m, 1H), 2.33 (m, 1H), 2.46 (m, 1H), 4.08 (d, J = 6.83 Hz, 2H), 4.31 (t, J = 6.83 Hz, 1H); ^{13}C NMR (CDCl$_3$) δ 6.7, 7.1, 21.0, 33.9, 34.2, 66.8, 70.3, 171.9. Anal. Calcd for C$_{16}$H$_{17}$NO$_6$ (*p*-nitrobenzoyl derivative): C, 60.18; H, 5.37; N. 4.39. Found: C. 60.40; H. 5.59; N. 4.24. **Compound 21:** colorless oil; $[\alpha]_D$ −127.82 (*c* 0.4, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 0.35−0.46 (m, 2H), 0.72 (m, 1H), 0.84 (m, 1H), 2.07 (s, 3H), 2.14 (m, 1H), 2.32 (m, 1H), 2.44 (m, 1H), 4.06 (d, J = 6.73 Hz, 2H), 4.29 (t, J = 6.73 Hz, 1H); ^{13}C NMR (CDCl$_3$) δ 6.8, 7.1, 21.0, 29.9, 33.6, 34.1, 66.9, 70.3, 172.0. Anal. Calcd for C$_{16}$H$_{17}$NO$_6$ (*p*-nitrobenzoyl derivative): C, 60.28; H, 5.37; N, 4.39. Found: C, 60.36; H, 5.68; N, 4.19.

(15) **Compound 12:** white solid; mp 197−198 °C; $[\alpha]_D$ −39.09 (*c* 0.13, MeOH); UV (MeOH) λ_{max} 261.0 (ϵ 11 097, pH 2), 261.0 (ϵ 12 030, pH 7), 261.0 (ϵ 8277.5, pH 11); ^{1}H NMR [(CD$_3$)$_2$SO] δ 0.02 (m, 1H), 0.85 (m, 2H), 1.06 (m, 1H), 2.88 (m, 2H), 3.80 (t, J = 4.88 Hz, 2H), 4.84 (t, J = 4.88 Hz, 1H), 5.42 (t, J = 8.30 Hz, 1H), 8.28 (s, 1H), 8.64 (s, 1H); ^{13}C NMR [(CD$_3$)$_2$SO] δ 4.9, 10.4, 28.6, 30.3, 36.3, 50.9, 63.2, 119.2, 140.3, 150.1, 152.8, 156.4. Anal. Calcd for C$_{12}$H$_{15}$N$_5$O: C, 58.76; H, 6.16; N, 28.55. Found: C, 58.62; H, 6.20; N, 28.30.

(16) **Compound 23:** white solid; mp 197−199 °C; $[\alpha]_D$ +38.44 (*c* 0.12, MeOH); UV (MeOH) λ_{max} 261.0 (ϵ 11 640, pH 2), 261.0 (ϵ 13 767, pH 7), 261.0 (ϵ 16 727.5, pH 11); ^{1}H NMR [(CD$_3$)$_2$SO] δ 0.01 (m, 1H), 0.90 (m, 2H), 1.05 (m, 1H), 2.85 (m, 2H), 3.8 (t, J = 5.37 Hz, 2H), 4.85 (t, J = 5.37 Hz, 1H), 5.24 (t, J = 8.30 Hz, 1H), (s, 2H), 8.40 (s, 1H), 8.65 (s, 1H); ^{13}C NMR [(CD$_3$)$_2$SO] δ 5.1, 10.4, 28.6, 30.3, 36.3, 51.1, 63.0, 119.2, 140.4, 150.1, 153.0, 156.5. Anal. Calcd for C$_{12}$H$_{15}$N$_5$O: C, 58.76; H, 6.16; N, 28.55. Found: C, 58.34; H, 6.14; N, 28.01.

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Table 2. Anti-HIV-1 Activity and Cytotoxicity of Compounds **12** and **23** in Different Cells

		IC_{50} (mM)		
compd	EC_{50} (mM)	PBM	CEM	Vero
12	22.4	42.4	>100	>100
23	48.6	>100	>100	>100
AZT	0.004	>100	14.3	29.0

In summary, we have described the preparative scale enzymatic resolution of compound **2** and the synthesis of two new versatile intermediates **10** and **21**. Condensation of these intermediates with 6-chloropurine, followed by ammonolysis readily provided novel spiro[2.3]hexane nucleosides **12** and **23** in high optical purity. Furthermore, intermediates **10** and **21** are being utilized for the synthesis of other nucleosides with natural as well as unnatural heterocyclic moieties.

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Supporting Information Available: Complete Experimental Section with full characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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