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CHEMISTRY

trans-Dihydroxypiperidines: Synthesis, Stereochemistry, and Anti-HIV Activity

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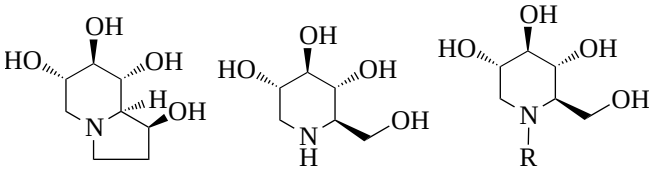
The search for medicines for HIV and AIDS treatment is among the most acute problems of modern science all over the world. However, the experience of using the vast majority of anti-HIV preparations shows that this virus soon develops resistance to the drugs (actually, within several months after the beginning of the therapy). Therefore, a continuous search for new classes of organic compounds exhibiting anti-HIV activities is required [1, 2].

Data on the structure of the main HIV-1 enzymes, namely, reverse transcriptase and protease, are widely used in the search for appropriate inhibitors. Several nucleoside (azidothymidine, zalcitabine, stavudine) and nonnucleoside (nevirapine, delavirdine, efavirenz) preparations are now widely used for inhibiting HIV replication at the reverse transcriptase step. Virus protease is inhibited by sakvinavir, ritanovir, indinavir, and other drugs [3].

In the late 1990s, data concerning the discovery of an absolutely new class of HIV replication inhibitors, polyhydroxylated alkaloids isolated from the fruits of the Australian chestnut *Castanospermus australe*, were published. These include castanospermin **1**, 2-deoxynojuerymycin **2** and its N-alkylated analogues **3**–**5**, swansonine, and other compounds [2–4], which are imino- or azasugars in which the oxygen atom of the pyranose ring is replaced by nitrogen. The report on this alkaloid family suppressing the development of HIV infection triggered a stream of studies dealing with the synthesis of their homologues and analogues, most often, on the basis of natural sugars (see for example, [5]), and evaluation of their ability to inhibit enzyme systems.



1 **2** **3**: R = Me, **4**: R = Et, **5**: R = Bu.

The biological activities of castanospermin **1**, one of the 32 possible optically active stereoisomers, are quite diverse, but inhibition of HIV replication and replication of other viruses is most significant [2]. The HIV inhibition is thought to take place upon glycosylation of the viral shell protein in which the cell glucosidase uses an iminosugar molecule instead of *D*-glucose. This substitution results in incompleteness of the HIV protein shell and in the inhibition of replication of the immunodeficiency virus.

In this study, while working on the quest for and preparation of new anti-HIV compounds using a biomimetic approach, we isolated a common structural fragment, *trans*-3,4-dihydroxypiperidine, from the family of polyhydroxylated alkaloids **1**–**5** containing several chiral centers and suggested that this fragment may prove to be an anti-HIV pharmacophore. To verify this assumption, we chose target compounds, namely, racemic and chiral C- and N-substituted *trans*-3,4-dihydroxypiperidines **1**–**12**, and carried out a stereoselective synthesis and conformational study of these compounds. The compounds were tested in vitro for anti-HIV activity and toxicity in cell lines of human origin infected with different strains of HIV-1. The results were highly successful and promising. The simplest azasugars, *trans*-dihydroxypiperidines **1**–**12**, containing only two hydroxy groups, were found to be relatively nontoxic and to suppress the development of HIV infection to different extents. Within this group, we chose the most promising leading compounds that can be recommended for in-depth study [6]. The new group of anti-HIV substances we found is especially promising for further studies because the anti-HIV activity of enantiomers of the leading compounds can be increased manyfold and because their synthesis is rather simple and economical.

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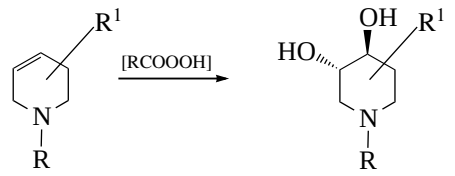
Synthesis of target compounds 1–12. All C- and N-substituted *trans*-3,4-dihydroxypiperidines **1**–**12** were synthesized by *trans*-hydroxylation of two series of tetrafluoroborates or trifluoroacetates of C- and N-substituted 1,2,5,6-tetrahydropyridines (THPs) **13**–**24** by treatment with trifluoroperacetic acid or free THP bases by treatment with performic acid. Under these conditions, the nitrogen atom is blocked, which prevents the formation of N oxides deactivating the double bond.



13–**24** **1**–**12**

1, **13**. R = R^1 = H;
2, **14**. R = Me, R^1 = H;
3, **15**. R = Et, R^1 = H;
4, **16**. R = *n*-Pr, R^1 = H;
5, **17**. R = *n*-Bu, R^1 = H;
6, **18**. R = Bn, R^1 = H;
7, **19**. R = Bn, R^1 = 6-Me;
8, **20**. R = Bn, R^1 = 3-Me;
9, **21**. R = Bn, R^1 = 4-Me;
10, **22**. R = Bn, R^1 = 4-Ph;
11, **23**. R = $(CH_2)_2OH$, R^1 = H;
12, **24**. R = MeCHPh, R^1 = H.

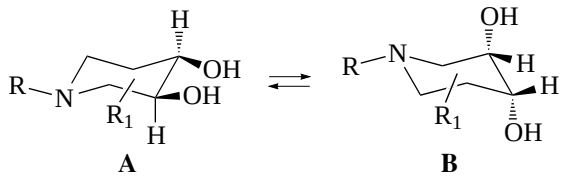
The first series of THPs **14**–**21** was prepared by reduction of 1-benzylpyridinium or 1-benzyl-2-, -3-, and -4-picolinium chlorides with $NaBH_4$ in ethanol at 0°C. The second series of THPs **25** and **26** was synthesized by transamination of 1-(2,4-dinitrophenyl)pyridinium chloride (Zincke salt) by ethanolamine and (*S*)-phenylethylamine with subsequent reduction of the resulting new pyridinium salts (THPs **23, 24**) with $NaBH_4$ in ethanol at 0°C. THP **13**, unsubstituted at nitrogen, was prepared by removing the *N*-benzyl group from THP **18** by hydrogenolysis over palladium black. THP **22** was produced upon dehydration of 1-benzyl-4-hydroxy-4-phenylpiperidine, which was, in turn, synthesized by the addition of phenyllithium to 1-benzylpiperidin-4-one. The individual *trans* isomers of 3,4-dihydroxy derivatives **1**–**12** were isolated as free bases by column chromatography on silica gel or by salt crystallization. The chemical purity of the target compounds obtained in each step was checked by GC/MS and elemental analyses; the spatial structure and the diastereomeric composition were determined using high-resolution ^1H and ^{13}C NMR spectra, and the presence of an intramolecular hydrogen bond was established from the data of IR spectroscopy [6].

Conformational analysis. The *trans* geometry of 3,4-dihydroxypiperidines **1**–**6** and **11**, without substituents in the piperidine ring, was confirmed by ^1H and ^{13}C NMR data. The ^1H NMR spectral patterns and the magnitudes of vicinal spin–spin coupling constants are similar for different diols (Table 1). A typical example is the spectrum of 1-benzyl-3,4-dihydroxypiperidine **6**. The great magnitudes of vicinal spin–spin coupling constants ($^3J_{2H_a,3H}$, $^3J_{5H_a,4H}$, and $^3J_{3H_a,4H}$) are indicative of the diaxial orientation of the protons at C-3 and C-4, which implies the diequatorial orientation of the

Table 1. Vicinal spin–spin coupling constants of protons at C-2, C-3, C-4, and C-5 in diols

^3J, Hz	**2**	**3**	**4**	**5**	**6**	**7**	**10**	**11**
$^3J_{2H_a,3H_a}$	11.64	11.44	10.88	11.23	9.60	7.86	4.17	9.29
$^3J_{2H_e,3H_a}$	4.27	4.35	4.19	4.22	4.40		1.94	4.64
$^3J_{3H_e,4H_e}$						4.8		
$^3J_{3H_a,4H_a}$	9.03	8.93	9.14	8.87	8.40	8.7	–	9.29
$^3J_{4H_a,5H_a}$	12.02	11.81	11.70	11.87	10.6	8.97	–	10.7
$^3J_{4H_a,5H_e}$	4.84	4.89	4.98	–	4.80		–	–
$^3J_{5H_e,6H_e}$						5.26		
$^3J_{5H_a,6H_e}$					4.2	3.91		
$^3J_{6H_e,6\text{-}CH_3}$						6.60		

3,4-hydroxy groups. Thus, it follows that the conformation equilibrium **A** ⇔ **B** for diols **1**–**6** and **11** is shifted almost completely toward the diequatorial conformer **A**.



A **B**

The predominant 3e,4e,6a-conformation for 6-methyl-3,4-dihydroxypiperidine **7** was established in line with the diequatorial orientation of the hydroxy groups at C-3 and C-4 (large $^3J_{3H_a,4H}$, $^3J_{4H_a,5H}$, and $^3J_{4H,5H}$ spin–spin coupling constants, equal to 8.7, 8.07, and 8.15 Hz, and small $^3J_{3H_e,4H}$ and $^3J_{6H_e,5H}$ constants, equal to 4.81 and 4.05 Hz, respectively) and preferentially axial orientation of the 6-methyl group (the small $^3J_{5H_a,6H}$, $^3J_{5H_e,6H}$ constants correspond to the equatorial orientation of the proton at C-6) (Table 1).

The introduction of substituents into the 3(Me) and 4(Me, Ph) positions of the piperidine ring in diols **8**–**10** shifts the conformational equilibrium **A** ⇔ **B** toward the diaxial conformer **B**. For instance, the diaxial orientation of the 3,4-dihydroxy groups in diol **9** was established in double resonance experiments on the basis of the $^3J_{2H_a,3H}$ (3.7 Hz) and $^3J_{2H_e,3H}$ (2.1 Hz) values, which correspond to the equatorial orientation of the proton at C-3 and, hence, the axial orientation of the 3-hydroxy group. The orientation of the 4-hydroxy group should also be axial, as stipulated by the *trans*-hydroxylation conditions. The *trans*-diaxial orientations of the 3,4-hydroxy groups in diols **8** and **10**, containing quaternary centers at C-3 and C-4, were established in a similar way. For diol **8**, the $^3J_{2H_a,3H}$ (4.17 Hz) and $^3J_{2H_e,3H}$ (1.94 Hz) values point to the equatorial orientation of the proton at C-3 and, hence, the axial orientation of the hydroxy group at C-3. A similar situation is observed for diol **10** ($^3J_{2H_a,3H}$, 4.10 Hz; $^3J_{2H_e,3H}$, 1.9 Hz). In view of the *trans*-hydroxylation conditions, the second hydroxy group at C-4 should also be axial in both diol **8** and diol **10**. This pattern of conformations is supplemented by the data of IR spectra (Table 2).

The IR spectra of diols **2**–**6** exhibit intense bands at about 3600 cm^{-1} (stretching vibrations of the free 4-OH group) and 3625–3630 cm^{-1} (vibrations of the free 3-OH group) and a low-intensity band at 3510–3525 cm^{-1}. In the spectra of dilute solutions, we assigned this band to vibrations of the hydroxy group involved in an intramolecular bond with nitrogen [7].

Table 2. Stretching vibrations of the hydroxy group in 3,4-dihydroxypiperidines **2**–**6** and **8**–**10**, ν_{OH}, cm^{-1}

Compound	Vibrations of the hydroxyl		
	involved in an intermolecular bond	involved in an intramolecular bond	free
1-Me-3-hydroxypiperidine	3050–3580	3540	3625
1-Me-4-hydroxypiperidine	3020–3580	–	3618
2	3010–3570	3510 (vw)	3600; 3627
3	3025–3570	3515 (vw)	3598; 3628
4	3020–3580	3520 (vw)	3600; 3628
5	3030–3570	3515 (vw)	3600; 3627
6	3040–3560	3525 (vw)	3600; 3625
8	3110–3570	3510	3600, 3633
9	3110–3470	3520	3615
10	3130–3580	3510	3605

The low intensity of this band may be indicative of essential predominance of conformer **A** in the **A** ⇔ **B** equilibrium. This is in line with ^{1}H NMR data for diols **2**–**6**. The situation changes sharply for compounds **9** and **10**: in particular, in dilute solutions, a clearly defined band appears at 3510–3520 cm^{-1}, which is due to stretching vibrations of the hydroxy group involved in an intramolecular bond. Simultaneously, the long-wavelength absorption band for the free hydroxyl either disappears (in the spectra of **9** and **10**) or becomes less intense (in the spectrum of **8**). According to the above ^{1}H NMR data, the hydroxy groups in diols **8**–**10** are diaxial. In this case, the orientation of the axial 3-OH group is convenient for the formation of a nonstrained six-membered ring due to an intramolecular hydrogen bond.

Anti-HIV activity of the hydroxylated piperidines. The antiviral and cytotoxic properties of *trans*-hydroxylated piperidines **1**–**12** were studied. The cytotoxic properties were determined with respect to the transplantable T-lymphoblastoid CEM SS cell line. The CEM SS cell line is a biologically cloned variant capable of virus-induced syncytium formation. It is widely used to study HIV and its inhibitors. The antiviral properties were studied with respect to the acute infection model CEM SS/HIV-1-BRU. The reference strain HIV-1_{BRU} is actively replicated in T-lymphoblastoid cell lines. To assay the antiviral activity of the piperidine derivatives, the CEM SS cells were incubated at 37°C with different concentrations of the preparations (100, 10, 1, 0.1, and 0.01 μg/ml) and infected with a virus with a multiplicity of 10^3 $TCID_{50}$ (Tissue Culture Infectious Dose-50). After 24-hour incubation, the unbound virus was removed by low-velocity centrifugation (1000 rpm, 7 min), and the cell precipitate was re-sus-

Table 3. Anti-HIV activity, toxicity, and chemotherapeutical index for some piperidine derivatives

Compound	% of infection for the HIV-1_{BRU} cell line at the concentration (μg/ml)					ED_{50}, μg/ml	CD_{50}, μg/ml[a]	Therapeutical index
	100	10	1	0.1	0.01			
1	17.0	18.0	73.0	115.1	–	2.7	310	115
2	93.9	94.4	95.8	101.2	–	>100	195	<2
3	87.7	80.9	87.5	91.3	–	>100	190	<2
4	89.9	89.7	94.4	98.3	–	>100	210	<2
5	87.6	84.7	80.0	110.6	–	>100	750	<7.5
6	97.7	98.1	73.2	71.0	–	>100	1600	<16
6/1[b]	15.0	16.0	76.0	93.0	–	2.8	1300	460
6/2[c]	93.2	97.1	77.3	98.0	–	>100	1030	<10
7	14.0	48.0	37.0	102.0	–	0.6	650	1080
7/1[b]	–	45.0	108.7	109.0	100	8.6	725	85
8	93.8	95.7	72.8	92.6	–	>100	410	<4
9	95.7	100.3	89.8	99.1	–	>100	740	<7
10	57.7	95.2	94.9	95.8	–	160	450	3
11	21.5	52.0	100.0	102.0	–	12	1100	90
12	94.8	95.1	84.3	101.8	–	>100	1670	<16
Stavudine	[d]							400
Azidothymidine (AZT)	[d]							1000

[a] For Sup T1 cells; [b] as the hydrochloride; [c] as the 3,4-diacetate; [d] published data.

pended in a fresh growth medium with the corresponding concentrations of the compounds under study. The infection development was monitored for 5 days with estimation of the cytopathogenic effect (cytolysis, syncytium formation), and the concentration of the p24 viral antigen in the culture broth was determined using enzyme immunoassay (EIA) (Innogenetics, Belgium). The results of testing of the anti-HIV activity and toxicity and the chemotherapeutical index (selectivity index) for some of the compounds are listed in Table 3. The greatest chemotherapeutical indices were noted for the leading compounds **6/1** and **7**. Judging by published data, these values are quite comparable with the values for anti-HIV preparations that are currently in use.

The most important outcome of this study is identification of a new, promising and relatively nontoxic group of anti-HIV agents, namely, (±)-*trans*-3,4-dihydroxypiperidines. In the series of these compounds, a certain relationship was found between the structure, the preferred conformation, and the anti-HIV activity (toxicity). Indeed, the variation of the anti-HIV activity in the "free base–hydrochloride" pairs for diols **6** and **7** was found to be substantially dissimilar (Table 3); the hydrochloride of diol **6** was nearly 30 times more active than the free base, whereas the activity of the free base of **7** was 13 times as high as the activity of its hydrochloride. A sharp increase in the toxicity in the case of compounds that occur predominantly in a conformation with diaxial orientation of both hydroxy groups should also be noted. These data should be taken into account in considering the conformation–activity relationships.

EXPERIMENTAL

The ^1H and ^{13}C spectra were recorded on a Varian VXR-400 spectrometer operating at 400 MHz using $CDCl_3$ and as the solvent and TMS as the internal standard. The chemical shifts are given in the δ scale. The GC/MS spectra were measured on an HP-5990 instrument with an HP-5972 mass selective detector (a 30 m × 0.2 mm quartz capillary column with the HP-5MS stationary phase and temperature programming from 70 to 250°C with a heating rate of 30 K/min). Thin-layer chromatography was carried out on DC-Alufolien-Alumina oxide plates (Merck, Germany). Column chromatography was carried out on columns packed with silica gel 60 (Merck, Germany). IR spectra were recorded for saturated solutions in CCl_4. Then, the solutions were diluted, usually tenfold, until the spectra recorded on a UR-20 spectrometer stopped changing. Due to the very low solubility of hydroxylated pyridines in CCl_4, the concentration of the solute was not determined. To study the cytotoxic properties, CEM SS cells were cultivated in the presence of different doses of the preparation for 5–7 days. After completion of the specified period, the cell viability was determined by the MTT method. This method is based on measuring the ability

of the cells under study to transform the readily soluble yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) bromide into insoluble intracellular crystals of MTT formazan. The efficiency of this transformation reflects the general level of dehydrogenase activity of the cells under study and, within certain limits, is directly proportional to the living cell concentration. The amount of formazan that can be optically determined (540 nm) is directly proportional to the amount of living cells. The cell control containing no preparation was taken as 100%.

1,2,5,6-TRPs **14**–**18** and **20**–**22** were synthesized by the reduction of the corresponding pyridinium salts (25 mmol) with sodium tetrahydroborate (60 mmol) in ethanol (60 mmol) at 0°C. THPs **23** and **24** were prepared according to published procedures [14, 15]. The structures of the resulting compounds **14**–**18** and **20**–**22** were confirmed by spectroscopy resorting to published data [8–14]. Diols **1**–**5** and **10** were obtained by oxidation of THPs **13–17** and **22** by a 25% hydrogen peroxide solution in 98% formic acid for seven days at 30°C. Diols **6, 7**, and **12** were prepared by the oxidation of the corresponding THPs **18, 19**, and **24** with trifluoroacetic acid in dichloromethane as described previously [15].

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