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RESOLUTION OF 9-(*c*-4,*t*-5-BISHYDROXYMETHYLCYCLOPENT-2-EN-*r*-1-YL)-9*H*-ADENINE AND SELECTIVE INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS BY THE (-) ENANTIOMER

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Summary: Two enantiomers of 9-(*c*-4,*t*-5-bishydroxymethylcyclopent-2-en-*r*-1-yl)-9*H*-adenine (BCA) which showed a potent and selective anti-HIV effects have been synthesized and evaluated against human immunodeficiency virus type 1. The result demonstrated that the potent-HIV activity of racemic BCA is expressed solely by the (-) isomer.

9-(*c*-4,*t*-5-Bishydroxymethylcyclopent-2-en-*r*-1-yl)-9*H*-adenine (BCA) has been found recently to show significant protection of MT-4 cells from the cytopathic effects of HIV-1 (1,2). Significant antiviral effects are observed at BCA concentrations that are approximately 200-fold below cytotoxic concentrations for the host cells (2), making BCA a high-priority candidate for further development as a potentially useful new antiviral drug for the treatment and prophylaxis of AIDS.

The general synthetic route recently reported for the preparation of BCA and related analogues could give the racemic form of a variety of carbocyclic nucleosides (2-7). Taking the synthesis of racemic BCA as a typical example, our route gave the intermediate (*c*-4,*t*-5-bishydroxymethylcyclopent-2-enylamine: **5**) in 3 steps from the *syn*-adduct (**2**) derived from hetero Diels-Alder reaction of 5-benzyloxymethyl-1,3-cyclopentadiene (**1**) and tosyl cyanide. Condensation of **5** with 5-amino-4,6-

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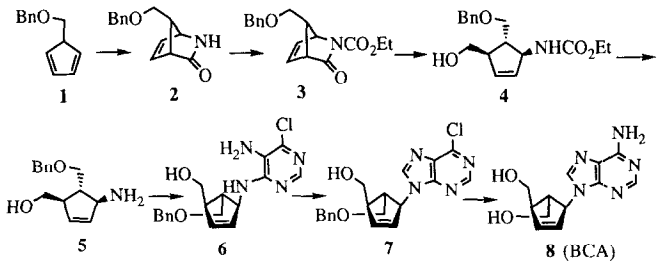


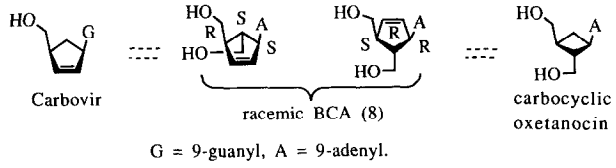
Fig. 1. Synthetic scheme for (±)-BCA.

dichloropyrimidine afforded **6**, which on cyclization gave **7**. Replacement of the chloro group of **7** by ammonia followed by deblocking of benzyl group afforded racemic BCA (**8**) (Fig. 1).

The biological evaluations of previously synthesized carbocyclic nucleoside analogues have been carried out with mixtures of (+) and (-) enantiomers and, so far, only the enantiomers that are analogous to the naturally occurring β-D-nucleosides have been found to exert the activity (7).

Racemic BCA (**8**) has a hybrid structure between Carbovir (7) and carbocyclic oxetanocin (8) [the absolute structures are defined for the Carbovir analogue (**A**) relative to the cyclopentenyl skeleton, while for the carbocyclic oxetanocin analogue (**B**) relative to bishydroxymethyl groups on cyclopentenyl ring and hence, there is possibility that, on this typical racemic compound (**8**), each and/or both enantiomers could exert the anti-HIV activity (Fig. 2).

Thus, we have been interested in separation of the optical isomers of racemic BCA (**8**) in order to evaluate both of the enantiomers for the anti-viral activities. After finding out that the ester (a mixture of **10** and **11**) obtained by treatment of the 6-chloropurine (**7**) with (1*S*)-(-)-camphanic acid chloride (**9**) could be separated readily into each diastereomer (**10** and **11**) by HPLC, we have prepared both enantiomers of BCA as enantiomerically pure compounds and found that (-)-BCA is a potent inhibitor of HIV-1 while the corresponding (+)-BCA is inactive (Fig. 3).



G = 9-guanyl, A = 9-adenyl.

Fig. 2. Structural relationship of each enantiomer of (±)-BCA with Carbovir and carbocyclic oxetanocin.

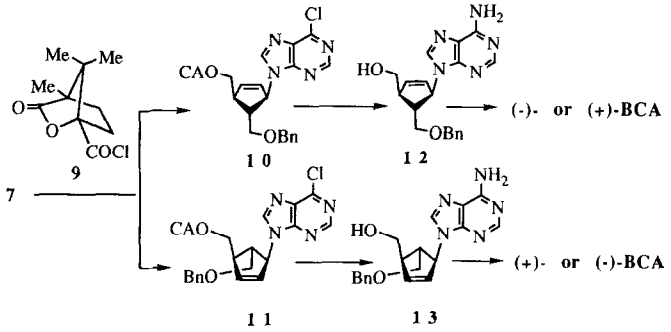


Fig. 3. Separation of (1*S*)-(-)-camphanyl esters of 6-chloro derivative of (±)-BCA.

MATERIALS AND METHODS

The 6-chloropurine derivative (**7**), the synthetic precursor of 9-(*c*-4,*t*-5-bishydroxymethylcyclopent-2-en-*r*-1-yl)-9*H*-adenine (racemic BCA, Scheme 1) was synthesized as previously reported (2). TLC was performed on Merck silica gel 60F-254 and silica gel column chromatography on Wako-gel C-200.

*Preparation of (1S)-(-)-camphanic acid esters (**10** and **11**) of 9-(t-5-benzyloxymethyl-c-4-hydroxymethylcyclopent-2-en-r-1-yl)-6-chloro-9H-purine (**7**).* To a solution of **7** (210 mg, 0.57 mmol) in anhydrous THF (5 ml) were added (1*S*)-(-)-camphanic acid chloride (240 mg, 1.22 mmol) and triethylamine (230 mg, 2.28 mmol), successively. After being stirred for 16 h at room temperature, the mixture was poured into ice-water, and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue, which was subjected to silica gel column chromatography. Elution with hexane-ethyl acetate (1:1) gave a mixture of **10** and **11** (244 mg, 78%). The mixture was subjected to HPLC for separation.

*Separation of the diastereomeric mixture of camphanate into **10** and **11** by means of HPLC.* HPLC was performed with Waters Associates instrument (M 6000 pump: U6K injector) under the following conditions: packing; μ poracil, column; 1/4" x 1', solvent; hexane-EtOH (25: 1), flow rate; 3 ml/min^{-1}, detector; UV absorption at 254 nm. Retention time: **10** (less polar); 24 min, **11** (more polar); 25.5 min.

*Preparation of (-)- and (+)-BCA from the corresponding camphanates (**10** and **11**) via the adenines (**12** and **13**).* The (-)-enantiomer of BCA as a typical procedure. NH_3 gas was passed over a solution of **10** (80 mg, 0.145 mmol) in MeOH (10 ml) with ice-salt cooling for 5 min. The solution was heated in a sealed tube at 70-80 °C for 16 h. The residue obtained after evaporation of the solvent *in vacuo* was chromatographed on silica gel column. Elution with ethyl acetate-MeOH (8:1) gave **12** (40 mg, 78%), mp 186-187 °C (colorless needles from ethyl acetate-MeOH), $[\alpha]_D^{26}$ -45.0 (*c* 0.8, MeOH). All spectral data were identical with those of the corresponding racemic compound (2).

The benzyl group of **12** was removed by the method previously reported (3) to give (-)-BCA, mp 206-207 °C, $[\alpha]_D^{28}$ -24.7 (*c* 1.4, MeOH).

Anal. Calc'd for $C_{12}H_{15}N_5O_2$: C, 55.16; H, 5.79; N, 26.81. Found: C, 55.06; H, 5.80; N, 26.75. All spectral data of (-)-BCA were identical with those of the racemic compound (**8**) (2).

In the same manner, (+) BCA was obtained from **11**. (+)-BCA, mp 206-207 °C, $[\alpha]_D^{28}$ +27.62 (*c* 1.6, MeOH).

Antiviral Assay.

HIV-1 (MN) (9) was provided by AIDS Research and Reference Reagent Program of the National Institute of Allergy and Infectious Diseases. Antiviral activity of the compounds was assessed by measurement of their inhibitory effects on HIV-1(IIIB or MN)-induced cytopathogenicity in MT-4 cells, as described in Weislow *et al.* (10). The cells were exposed to HIV-1 (IIIB) at m.o.i. of 0.002 or infected by cocultivation of chronically HIV-1 (MN) infected H9 cells, and cultured for 6 days in the presence of various concentration of the drug. The viable cells were measured by the XTT (2,3-bis[2-methoxy-4-nitro-5-sulfopheny]-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide). The cytotoxicity of the compounds was also determined.

RESULTS AND DISCUSSION

The compounds were compared for their inhibitory effects on the cytopathogenicity of HIV-1 (IIIB or MN) in MT-4 cells (Table 1). As a reference compound, 2', 3'-dideoxycytidine (DDC) was also included. The

Table 1. In Vitro Antiviral Activity of 9-(*c*-4,*t*-5-Dihydroxymethylcyclopent-2-en-*r*-1-yl)-9*H*-adenine (BCA) against HIV-1 in MT-4 Cells

Compound	Virus	ED_{50}[a], μg/ml	ID_{50}[b], μg/ml	TI[c]
(±)-BCA	IIIB	1.8 ± 0.3	>100	>56
(-)-BCA	IIIB	0.71 ± 0.37	>100	>141
(+)-BCA	IIIB	>100	>100	----
DDC	IIIB	0.069 ± 0.020	48 ± 7	695
(±)-BCA	MN	4.6 ± 1.4	>100	>22
(-)-BCA	MN	1.9 ± 1.1	>100	>53
(+)-BCA	MN	>100	>100	----
DDC	MN	0.12 ± 0.02	29 ± 7	242

[a] The effective dose, 50% (ED_{50}), represents the concentration of compound that increases formazan production in infected cultures to 50% of untreated, uninfected cell controls.
[b] The inhibitory dose, 50% (ID_{50}), represents the toxic concentration of drug that reduces formazan production in uninfected cultures to 50% determined by simple linear interpolation from the data.
[c] The therapeutic index (TI) was determined by dividing the ID_{50} by the ED_{50}.

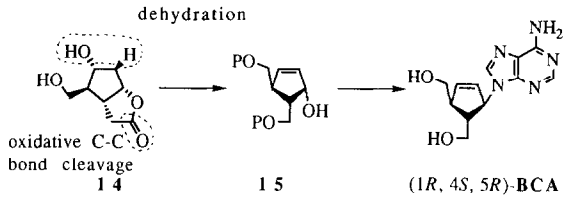


Fig. 4. Synthetic methodology for (1*R*,4*S*,5*R*)-BCA from (-)-Corey lactone.

racemic mixture of BCA showed anti-HIV-1 activity, with its ED_{50} values being 1.8 ± 0.3 and 4.6 ± 1.4 μg/ml evaluated by IIIB and MN, respectively. The (-) isomer of BCA was active, but (+) isomer of BCA did not inhibit the replication of HIV-1 at concentrations of up to 100 μg/ml. This result indicates that the anti-HIV-1 activity of racemic BCA is expressed solely by the (-) isomer. ED_{50} of (-)-BCA was 0.71 ± 0.37 and 4.6 ± 1.4 μg/ml evaluated by IIIB and MN, respectively. These values are 2.2-2.5-fold more higher than the ED_{50} of the racemic BCA.-(-) BCA did not markedly reduced the viability of mock-infected MT-4 cells at concentrations up to 100 μg/ml. Although (-)-BCA required a higher concentration than DDC to inhibit HIV-1 replication, it is an interesting candidate for further study because of its low toxicity.

Knowing that Mitsunobu reaction of cyclopentanols and cyclopentenols with 6-chloropurine afforded the 9-substituted purines with inversion of configuration (11), we are currently investigating the synthesis of (1*R*,4*S*,5*R*)-BCA through the cyclopentenol (**15**) from (-)-Corey lactone (**14**) (Fig. 4). Such work would provide the means to determine unambiguously the absolute configuration of the (-)-BCA.

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