Synthesis of Nucleosides and Related Compounds. XXXI.[1] Resolution of 9-[*c*-4, *t*-5-Bis(hydroxymethyl)cyclopent-2-en-*r*-1-yl]-9*H*-adenine [(±)-BCA] by Means of High-Pressure-Mediated Deamination with Adenosine Deaminase

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Deamination of an anti-human immunodeficiency virus (anti-HIV) carbocyclic adenine nucleoside, 9-[*c*-4, *t*-5-bis(hydroxymethyl)cyclopent-2-en-*r*-1-yl]-9*H*-adenine (BCA) by adenosine deaminase under a variety of pressures (1 bar—6 kbar) was investigated. It was found that, while (+)-BCA was practically unaffected, the deamination of (−)-BCA was remarkably enhanced under 4 kbar to give the corresponding (−)-hypoxanthine derivative [(−)-BCH)] in nearly 100% ee.

Keywords adenosine deaminase; high-pressure; enzymatic resolution; anti-HIV activity; carbocyclic adenine nucleoside; 9-cyclopentenylhypoxanthine

Previously, we reported that 9-[*c*-4,*t*-5-bis(hydroxymethyl)cyclopent-2-en-*r*-1-yl]-9*H*-adenine (BCA) showed significant anti-human immunodeficiency virus (HIV) activity.[2] The racemic BCA corresponds to the hybrid nucleoside of carbovir[3,4] and carbocyclic oxetanocin,[5,6] both of which are potent anti-HIV reagents (Chart 1). Chemical resolution of racemic BCA and biological evaluation of each enantiomer revealed that only (−)-BCA showed anti-HIV activity.[7] The absolute structure of (−)-BCA was determined to be (1*R*,4*S*,5*R*) by its total synthesis from (−)-Corey lactone.[8,9]

In order to provide an economical preparation of (−)-BCA, we have investigated the deamination of (±)-BCA by adenosine deaminase to the corresponding hypoxanthine derivative and found that, though the reaction did not proceed at standard atmospheric pressure, the reaction carried out under high-pressure results in the formation of (−)-BCH {(1*R*,4*S*,5*R*)-9-[4,5-bis(hydroxymethyl)cyclopent-2-en-1-yl]-1*H*,9*H*-hypoxanthine} in nearly 100% ee. The findings that the optimum pressure for the reaction was *ca.* 4 kbar (which can be achieved in a large-scale apparatus), and that BCH can be chemically converted to BCA, provide the basis for an extremely economical route to (−)-BCA. In this paper, we report these studies in detail.

Results and Discussion

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), which catalyzes the hydrolysis of adenosine to inosine and ammonia, is important physiologically in purine metabolism. In recent years, this enzyme has also been utilized for the preparation or resolution of pharmacologically active purine nucleoside analogues.[3,10]

Before investigating the deamination, we examined the inhibition of adenosine deaminase by racemic BCA and each enantiomer. As a result, it was found that, while the enantiomers did not act as substrates, they inhibited the deamination of adenosine by this enzyme. Among the two enantiomers, the binding ability of (+)-BCA to the enzyme was much stronger than that of (−)-BCA (Table I).

Next, we investigated the deamination of BCA by adenosine deaminase under a variety of high-pressures, for the following two reasons. 1) A well-known advantage of the high-pressure technique over reactions carried out under atmospheric pressure in the field of synthetic organic chemistry[11–13] is an increase of the reaction rate when the corresponding reaction has a large negative activation volume ($\Delta V^{\neq}$).[14] 2) Though high-pressure investigation in the area of enzymatic organic synthesis might be useful not only for the preparation of pharmacologically active substances or their intermediates but also for elucidation of the catalytic mechanism of enzymes, there has been no report so far of a remarkable pressure effect.

Since (−)-BCA is readily available from (−)-Corey lactone, we first investigated the deamination of (−)-BCA to give (−)-BCH under various pressures at 22 °C for 12 h. As expected, the rate of deamination increased with

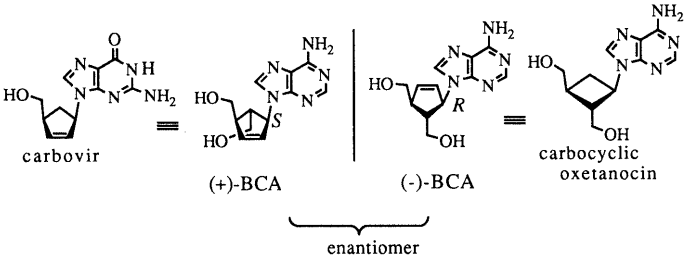

Chart 1

TABLE I. Inhibition of the Deamination of Adenosine with Adenosine Deaminase by BCA[a]

	Substrate	Inhibitor	K_i (μM)
(−)-BCA	−	+	260
(+)-BCA	−	+	68
(±)-BCA	−	+	100

a) +: acted as; −: did not act as.

increasing pressure. It reached a maximum at *ca.* 4 kbar, and then decreased dramatically at 6 kbar. This means that inactivation of adenosine deaminase occurs between 5 and 6 kbar (Table II). The inactivation was irreversible, because the enzyme pressurized at 6 kbar no longer catalyzed the deamination of BCA after the pressure was decreased again.

On the basis of these observations, we conducted the deamination of (±)-BCA and both of its enantiomers at 4 kbar at 22 °C in order not only to resolve the racemic BCA into each enantiomer with high enantiomeric excess (ee), but also to clarify the difference between the deamination rate of (−)-BCA and that of (+)-BCA. Thus, (−)-BCA was converted to (−)-BCH in quantitative yield within 40 h, whereas the conversion yield of (+)-BCA to (+)-BCH was only 5% even at 80 h. Moreover, the deamination of (±)-BCA for 80 h provided (−)-BCH in 31% conversion yield. For determination of the ee, the (−)-BCH thus obtained was derivatized to its (*S*)-(−)-2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) diester, whose 500 MHz ^{1}H-NMR examination revealed that the ee of (−)-BCH was more than 99%. The results of the above study are summarized in Table III.

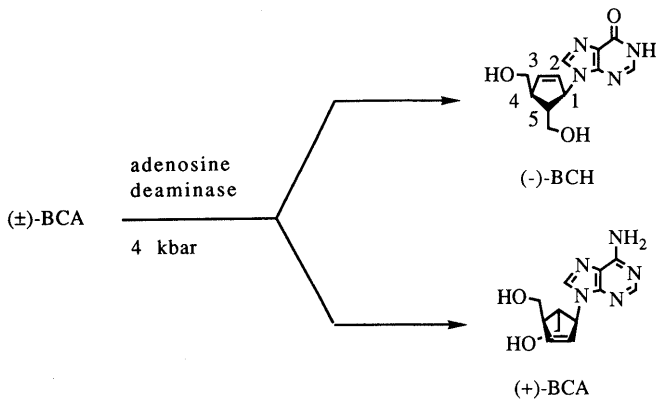

Chart 2

Table II. Deamination of (−)-BCA with Adenosine Deaminase under Various Pressures at 22 °C for 12 h

Reaction pressure	Conversion to BCH (%)[a]
1 bar (control)	0
2.0 kbar	8
3.0 kbar	21
4.0 kbar	40
5.0 kbar	30
6.0 kbar	4

a) Conversion (%) represents BCH formed/total BCA initially used and was estimated by high-performance liquid chromatography (HPLC) (Waters; column, μ-Poracil C_{18}; solvent, dioxane : water = 1 : 7; detection, UV 254 nm; retention times, BCH = 3 min and BCA = 5.5 min).

Table III. Deamination of (−)-BCA, (+)-BCA, and (±)-BCA with Adenosine Deaminase under 4 kbar at 22 °C

Reaction time (h)	Conversion to BCH (%)[a]		
	(−)-BCA	(+)-BCA	(±)-BCA
20	62	2	12
40	100	3	18
60	—	4	25
80	—	5	31

a) See the corresponding footnote in Table II.

Finally, the conversion of (−)-BCH to (−)-BCA was carried out essentially in the same manner as reported previously for the conversion of hypoxanthine to adenine.[15] Thus, as shown in Chart 3, (−)-BCH was converted to the diacetate (**1**), whose chlorination by thionyl chloride–*N*,*N*-dimethylformamide afforded the 6-chloropurine derivative (**2**). Amination of the latter gave the desired (−)-BCA. All reactions proceeded in nearly quantitative yields. We have therefore achieved the enzymatic resolution of (±)-BCA into (−)-BCA with a high enantiomeric excess. Moreover, it is noteworthy that compound **2** would be a versatile intermediate for the synthesis of (−)-BCA analogues because the chlorine atom of **2** can be substituted by various groups such as alkylamino, alkoxyl, and mercapto groups.

In summary, we have found that (−)-BCA binds less strongly to the adenosine deaminase but is much more susceptible to deamination under high-pressure than (+)-BCA. Though the mechanism of the acceleration of deamination under high-pressure remains obscure, it could involve conformational change of the enzyme under high-pressure. The irreversible inactivation of the adenosine deaminase at rather higher pressure (6 kbar), as mentioned above, tends to support this mechanism.

This high-pressure technique should be applicable widely to deamination by adenosine deaminase of substrates which are rather inert either under atmospheric pressure or at higher temperature, and may also be useful for elucidation of the deamination mechanism.[16–18]

Further studies on the deamination of adenosine itself as well as other adenine nucleoside analogues by adenosine deaminase under high-pressure are in progress.

Experimental

All melting points were determined on a micro-hot stage (Yanagimoto) and are uncorrected. ^{1}H-NMR spectra at 60 MHz and 500 MHz were recorded with JEOL JNM-PMX 60 and JEOL JNM-FX 500 spectrometers, respectively, using tetramethylsilane (TMS) as an internal standard. The abbreviations of signal patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; br, broad; brs, broad singlet. Low- and high-resolution mass spectra (MS) were obtained on JEOL JMS-DX303

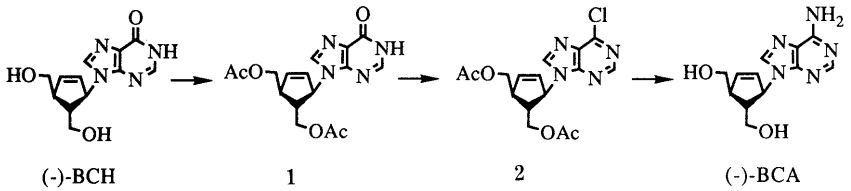

Chart 3

and JEOL JMS-AX500 mass spectrometers, respectively. Wakogel (C-200) and Merck Kiesel-gel 60 F254 were employed for silica gel column and thin layer chromatography (TLC), respectively. The ratios of mixtures of solvents for chromatography are shown as volume/volume.

(±)-BCA, (−)-BCA, and (+)-BCA were synthesized as previously reported.[2,7-9] Adenosine deaminase type VI from calf intestinal mucosa was purchased from Sigma Chemical Co.

Inhibition of Adenosine Deaminase by BCA The initial velocities were measured by the method of Kalckar[19,20] in 3 ml of 0.05 M phosphate buffer, pH 7.0, with two adenosine concentrations (20 and 40 μM) in the presence of different fixed concentrations of BCA (0, 40, 80, 120 μM) and 0.0075 U enzyme at 25 °C. (+)-BCA behaved as a competitive inhibitor, whereas (−)-BCA acted as a noncompetitive one. The K_i values were estimated from a Dixon plot. Details will be reported elsewhere.

Deamination of (±)-BCA, (−)-BCA, and (+)-BCA by Adenosine Deaminase under High-Pressure High-pressure reactions were carried out by using a piston-cylinder apparatus equipped with a KP.15.B pump (Hikari Kouatsu Kiki Ltd., Co., Japan). A solution of (−)-BCA (5 mg, 19 μM) and adenosine deaminase (19 units) in phosphate buffer (pH 7.0, 4.7 ml) was placed in a Teflon tube (4.7 ml) with a Teflon stopper. The tube was placed in a high-pressure reactor and pressurized to 2—6 kbar at 22 °C. The pressure was measured by a manganin coil and the accuracy was ±0.1 kbar. The pressure was released and the reaction mixture was concentrated under reduced pressure to give a residue, which was submitted to silica gel column chromatography. Elution with $CHCl_3$–MeOH (5 : 1) gave BCH, which was recrystallized from MeOH. Due to the low yield, (+)-BCH was not isolated.

(−)-BCH {(1*R*,4*S*,5*R*)-9-[4,5-bis(hydroxymethyl)cyclopent-2-en-1-yl]-1*H*,9*H*-hypoxanthine}: mp 225—227 °C (MeOH), $[\alpha]_D^{22}$ −37.0° (*c* = 0.15, MeOH). High-resolution MS *m/z* Calcd for $C_{12}H_{14}N_4O_3$ (M^+): 262.1065. Found. 262.1066. ^{1}H-NMR (CD_3OD, 500 MHz) δ: 2.35 (quint, 1H, *J* = 7 Hz, 5-H), 2.81 (m, 1H, 4-H), 3.66 (dd, 1H, *J* = 11, 7 Hz, CHH′OH), 3.73 (dd, 1H, *J* = 11, 7 Hz, CHH′OH), 3.79 (d, 2H, *J* = 7 Hz, CH_2OH), 5.56 (dt, 1H, *J* = 7, 2 Hz, 1-H), 5.85 (dt, 1H, *J* = 7, 2 Hz, 3-H), 6.15 (dt, 1H, *J* = 7, 2 Hz, 2-H), 8.09, 8.03 (s, each 1H, hypoxanthine-H).

The (−)-MTPA diester of (−)-BCH was obtained in a usual manner (*N*,*N*′-dicyclohexylcarbodiimide-*N*,*N*′-dimethylaminopyridine in CH_2Cl_2 for 3 h at room temperature): mp 205—207 °C (hexane–ethyl acetate).

The ee of (−)-BCH obtained from (±)-BCA at 4 kbar was determined by comparison of the 500 MHz ^{1}H-NMR spectrum with that of (−)-MTPA diester obtained from racemic BCH (mp 170—172 °C), which was prepared by treatment of a racemic BCA precursor, 9-(*t*-5-benzyloxymethyl-*c*-4-hydroxymethylcyclopent-2-en-*r*-1-yl)-6-chloro-9*H*-purine[2] with boron trichloride in dichloromethane at −78 °C.

Synthesis of (−)-BCA from the Corresponding (−)-BCH a) Acetylation of (−)-BCH Acetic anhydride (1 ml) was added to a solution of (−)-BCH (83 mg, 0.32 mmol) in pyridine (2 ml) and the solution was kept stirring at room temperature for 12 h. The residue obtained after evaporation of the solvent was chromatographed over silica gel. Elution with $CHCl_3$–MeOH (20 : 1) afforded (1*R*,4*S*,5*R*)-9-[4,5-bis(acetoxymethyl)cyclopent-2-en-1-yl]-1*H*,9*H*-hypoxanthine (**1**: 96%).

1: mp 134—136 °C (AcOEt), $[\alpha]_D^{21}$ +3.7° (*c* = 1.0, $CHCl_3$). High-resolution MS *m/z* Calcd for $C_{16}H_{18}N_4O_5$ (M^+): 346.1276. Found. 346.1289. ^{1}H-NMR ($CDCl_3$, 60 MHz) δ: 2.00 (3H, s, 5-CH_2OCOCH_3), 2.07 (3H, s, 4-CH_2OCOCH_3), 2.30—3.17 (2H, m, 4-H, 5-H), 4.03—4.50 (4H, m, 4-CH_2OAc, 5-CH_2OAc), 5.57 (1H, dd, *J* = 6, 2 Hz, 1-H), 5.87 (1H, dd, *J* = 3, 3 Hz, 3-H), 6.15 (1H, dd, *J* = 3, 3 Hz, 2-H), 7.90 (1H, s, purine-H), 8.30 (1H, s, purine-H).

b) Chlorination of the Diacetate (1) of (−)-BCH with Thionyl Chloride *N*,*N*-Dimethylformamide (0.1 ml, 1.3 mmol) and thionyl chloride (0.2 ml, 2.6 mmol) were added to a solution of the diacetate (91 mg, 0.26 mmol) in $CHCl_3$ (5 ml) and the solution was stirred at 65 °C for 4 h. After addition of ice-water to the reaction mixture, followed by basification by the addition of $NaHCO_3$, the product was extracted with $CHCl_3$. The residue obtained after evaporation of the solvent was chromatographed over silica gel. Elution with AcOEt–hexane (1 : 1) afforded 87 mg (92%) of (1*R*,4*S*,5*R*)-9-[4,5-bis(acetoxymethyl)cyclopent-2-en-1-yl]-6-chloro-9*H*-purine (**2**) as an oil.

2: Oil, $[\alpha]_D^{23}$ +33.3° (*c* = 3.1, $CHCl_3$). High-resolution MS *m/z* Calcd for $C_{15}H_{17}ClN_4O_4$ (M^+), $C_{16}H_{17}Cl^*N_4O_4$ ($M^+ + 2$): 364.0937, 366.0908. Found. 364.0913, 366.0887. ^{1}H-NMR ($CDCl_3$, 60 MHz) δ: 2.00 (3H, s, 5-CH_2OCOCH_3), 2.07 (3H, s, 4-CH_2OCOCH_3), 2.26—3.23 (2H, m, 4-H, 5-H), 4.17—4.56 (4H, m, 4-CH_2OAc, 5-CH_2OAc), 5.69 (1H, dd, *J* = 6, 2 Hz, 1-H), 5.92 (1H, dd, *J* = 3, 3 Hz, 3-H), 6.23 (1H, dd, *J* = 3, 3 Hz, 2-H), 8.23 (1H, s, purine-H), 8.76 (1H, s, purine-H).

c) Preparation of (−)-BCA from the 6-Chloropurine Derivative (2) Ammonia gas was passed under ice-cooling into a solution of the chloropurine (**2**: 244 mg) in absolute methanol (25 ml) for 1 h, and the whole mixture was heated in a sealed tube at 90 °C for 20 h. The residue obtained after evaporation of the solvent was chromatographed on silica gel. Elution with AcOEt–MeOH (8 : 1) gave 180 mg (77%) of the adenine derivative [(−)-BCA]. The sample was identical with an authentic sample[8] in all respects examined.

(−)-BCA: Colorless needles, mp 206—207 °C (MeOH), $[\alpha]_D^{23}$ −32.1° (*c* = 0.28).

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References

1) Part XXX in this series, A. Toyota, N. Katagiri, and C. Kaneko, *Synthetic Commun.*, in press.
2) N. Katagiri, M. Nomura, H. Sato, C. Kaneko, K. Yusa, and T. Tsuruo, *J. Med. Chem.*, **35**, 1882 (1992).
3) R. Vince and M. Hua, *J. Med. Chem.*, **33**, 17 (1990).
4) R. Vince and J. Brownell, *Biochem. Biophys. Res. Commun.*, **168**, 912 (1990).
5) D. W. Norbeck, E. Kern, S. Hayashi, W. Rosenbrook, H. Shama, T. Herrin, J. J. Plattner, R. Clement, R. Swanson, N. Shipkowitz, D. Hardry, K. Marsh, G. Arnett, W. Shannon, S. Broder, and H. Mitsuya, *J. Med. Chem.*, **33**, 1281 (1990).
6) G. S. Bisacchi, A. Braitman, C. W. Cianci, J. M. Clark, A. K. Field, M. E. Hagen, D. R. Hockstein, M. F. Malley, T. Mitt, W. A. Slusarchyk, J. E. Sundeen, B. J. Terry, A. V. Tuomari, E. R. Weaver, M. G. Young, and R. Zahler, *J. Med. Chem.*, **34**, 1415 (1991).
7) N. Katagiri, T. Shiraishi, H. Sato, A. Toyota, C. Kaneko, K. Yusa, T. Ohhara, and T. Tsuruo, *Biochem. Biophys. Res. Commun.*, **184**, 154 (1992).
8) N. Katagiri, A. Toyota, T. Shiraishi, H. Sato, and C. Kaneko, *Tetrahedron Lett.*, **33**, 3507 (1992).
9) N. Katagiri, H. Sato, S. Arai, A. Toyota, and C. Kaneko, *Heterocycles*, **34**, 1097 (1992).
10) N. Shimada, S. Hasegawa, S. Saito, T. Nishikiori, A. Fujii, and T. Takita, *J. Antibiotics*, **40**, 1788 (1987).
11) K. Matsumoto, A. Sera, and T. Uchida, *Synthesis*, **1985**, 1.
12) K. Matsumoto and A. Sera, *Synthesis*, **1985**, 999.
13) N. S. Isaacs, *Tetrahedron*, **47**, 8463 (1991).
14) T. Asano and W. J. Le Noble, *Chem. Rev.*, **78**, 407 (1978).
15) S. Shuto, T. Obara, M. Toriya, M. Hosoya, R. Snoeck, G. Andrei, J. Balzarini, and E. De Clercq, *J. Med. Chem.*, **35**, 324 (1992).
16) W. Jones, L. C. Kurz, and R. Wolfenden, *Biochemistry*, **28**, 1242 (1989).
17) W. M. Kati and R. Wolfenden, *Biochemistry*, **28**, 7919 (1989).
18) M. Orozco, E. I. Canela, and R. Franco, *J. Org. Chem.*, **55**, 2630 (1990).
19) H. M. Kalckar, *J. Biol. Chem.*, **167**, 445 (1947).
20) H. M. Kalckar, *J. Biol. Chem.*, **167**, 461 (1947).