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BMS-200475, A NOVEL CARBOCYCLIC 2'-DEOXYGUANOSINE ANALOG WITH POTENT AND SELECTIVE ANTI-HEPATITIS B VIRUS ACTIVITY IN VITRO

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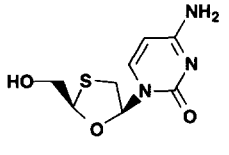
Abstract: BMS-200475, a novel carbocyclic analog of 2'-deoxyguanosine, is a potent inhibitor of hepatitis B virus in vitro (ED_{50} = 3 nM) with relatively low cytotoxicity (CC_{50} = 21-120 μM). A practical 10-step asymmetric synthesis was developed affording BMS-200475 in 18% overall chemical yield and >99% optical purity. The enantiomer of BMS-200475 as well as the adenine, thymine, and iodouracil analogs are much less active.

Hepatitis B is one of the most prevalent viral diseases in the world and is known to be a major cause of chronic liver disease, leading to cirrhosis/hepatocellular carcinoma.[1] Three hundred million individuals worldwide are chronically infected with the hepatitis B virus (HBV) and there is a continuing need for new therapies for individuals infected with HBV. In the United States, alpha interferon is the only approved therapy for chronically infected individuals. Lamivudine (**1**) and famciclovir (**2**), a prodrug of penciclovir (**3**), are currently in clinical trials for chronic HBV infection, and other nucleoside analogs with anti-HBV activity have recently been reported.[2] In this communication we report the synthesis and in vitro anti-HBV activity of BMS-200475 (**4**), a carbocyclic analog of 2'-deoxyguanosine in which the furanose oxygen is replaced with an exocyclic double bond.[3,4] Additionally, we report the synthesis and anti-HBV activities of the adenine (**5**), thymine (**6**), and 5-iodouracil (**7**) base analogs of BMS-200475, and the enantiomer of BMS-200475 (**8**).



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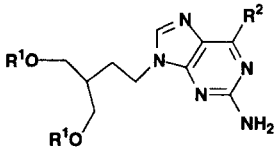
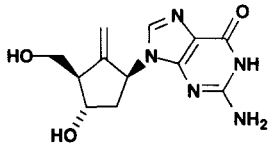


2 R^1 = Ac, R^2 = H
3 R^1 = H, R^2 = OH



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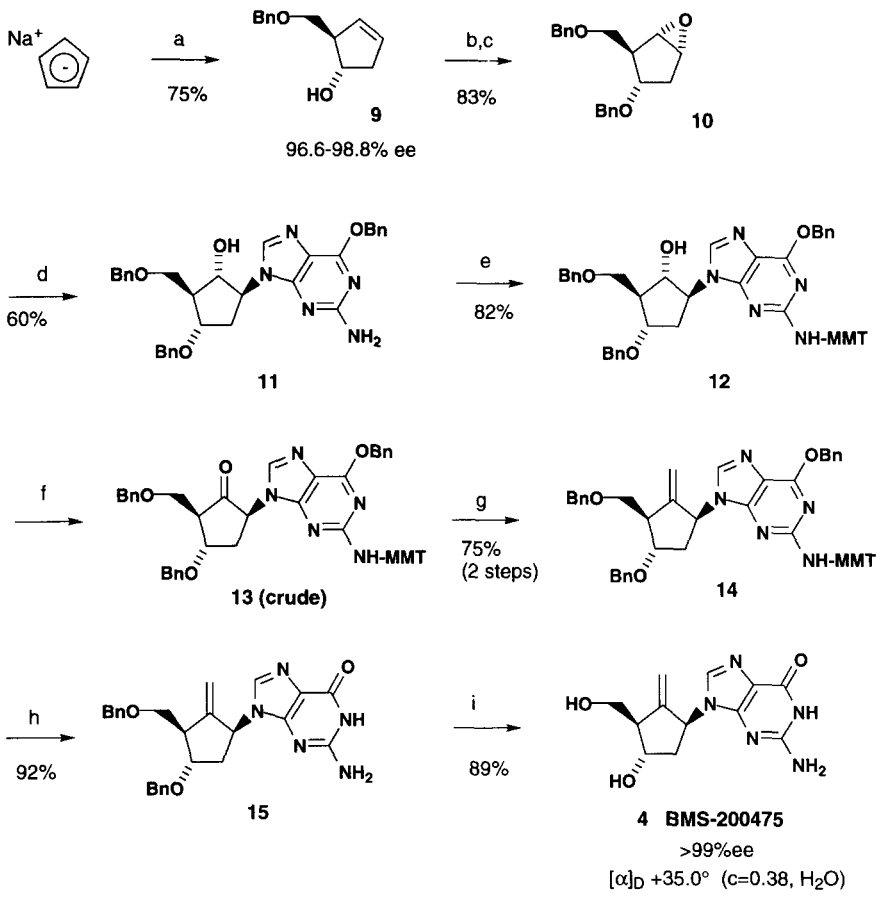
The synthesis of BMS-200475 (**4**) is shown in Scheme 1. The known[5] chiral cyclopentyl epoxide **10** proved to be a useful synthon for the preparation of **4** as well as its base analogs **5-7**. However, the preparation of **10**, especially on a large scale, was severely compromised by low yields (ca. 25%) in the

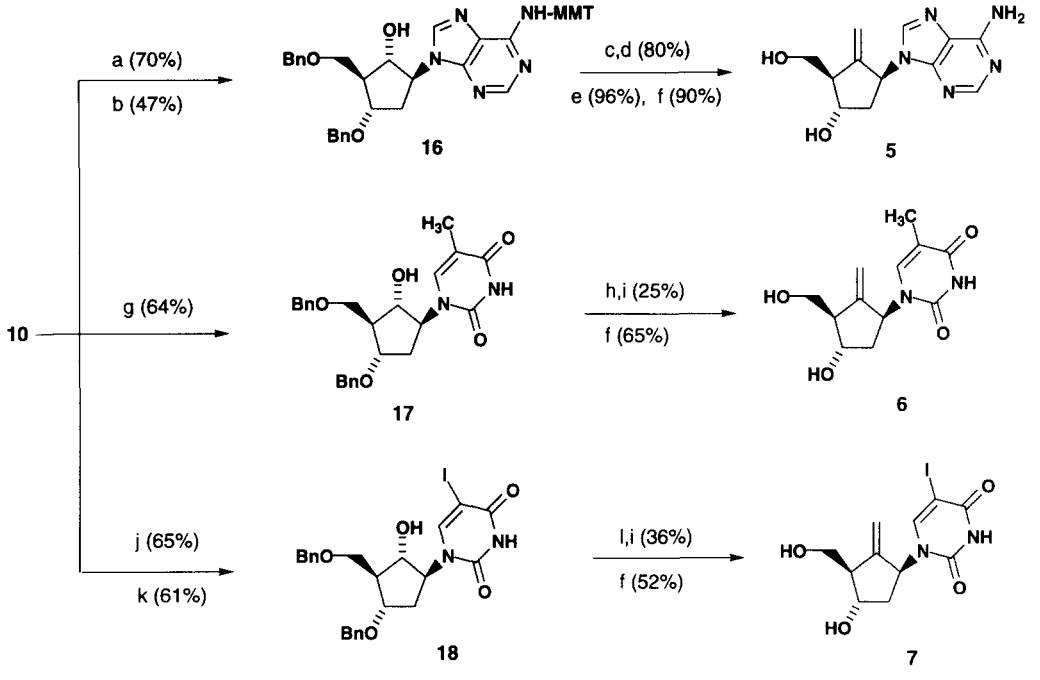
Scheme 1.



(a) (i) $BnOCH_2Cl$, THF, -65 to -78 °C, (ii) diisopinylcampheylborane (prepared from (+)-α-pinene), THF, -65 to -78 °C, (iii) aq. NaOH, H_2O_2 ; (b) $VO(acac)_2$, *t*-BuOOH, CH_2Cl_2; (c) BnBr, NaH, Bu_4NI, DMF; (d) 6-benzyloxy-2-aminopurine, LiH, DMF, 125 °C; (e) 4'-monomethoxytrityl chloride, TEA, DMAP, CH_2Cl_2; (f) Dess-Martin reagent, *t*-BuOH, CH_2Cl_2; (g) Nysted reagent, $TiCl_4$, THF; (h) aq. HCl, THF, MeOH, 55°C; (i) BCl_3, CH_2Cl_2, -78 °C

generation of intermediate **9** when using sodium cyclopentadienide prepared in situ from cyclopentadiene and sodium.[6] We were gratified to find that the use of commercial sodium cyclopentadienide[7] improved the yield of **9** 3-fold to 75% (96.6-98.8% ee). Reaction of **10** with 6-benzyloxy-2-aminopurine (2 equiv) and LiH (0.5 equiv) in DMF at 125 °C for 2 h afforded the N-9 adduct **11** in 60% yield following chromatography.[8] Also isolated were small amounts of the corresponding N-7 adduct and the N-9 regiomer resulting from attack at the other epoxide site. Protection of the purine amino group was found to be required for the subsequent oxidation of the cyclopentyl alcohol. Thus, **11** was treated with 4'-monomethoxytrityl chloride in CH_2Cl_2 in the presence of triethylamine and DMAP to afford **12** in 82%

Scheme 2.



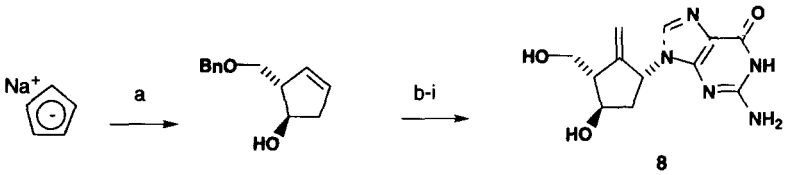
(a) adenine, LiH, DMF, 120 °C; (b) 4'-monomethoxytrityl chloride, pyr, DMAP, CH_2Cl_2; (c) Dess-Martin reagent, *t*-BuOH, CH_2Cl_2; (d) Nysted reagent, $TiCl_4$, THF; (e) 1 N HCl, MeOH, THF; (f) BCl_3, CH_2Cl_2, -78 °C; (g) thymine, LiH, DMF, 140 °C; (h) DMSO, DCC, $CH_3PO_3H_2$; (i) Zn, $TiCl_4$, CH_2Br_2, THF, CH_2Cl_2; (j) uracil, NaH, DMF, 130 °C; (k) I_2, aq. HNO_3, dioxane, 90 °C; (l) pyridinium dichromate, molecular sieves, CH_2Cl_2;

yield. Initially, Moffatt oxidation (DCC, DMSO, and methylphosphonic acid)[9] followed by Lombardo methylenation (Zn, $TiCl_4$, CH_2Br_2, THF, CH_2Cl_2)[10] was employed to afford crude **14**, which was deprotected (aq. HCl, THF, MeOH, 55 °C) on the purine ring to afford penultimate intermediate **15** in only 23% overall yield (3 steps). It was clear from analysis of the crude intermediates that both the oxidation and methylenation steps were responsible for the low overall yield. For example, a major side-product formed during the Moffatt oxidation was an internal cyclopentenone resulting from β-elimination of the 3'-benzyloxy group from the initially formed cyclopentanone **13**. A number of different oxidation and methylenation reagents were investigated in an attempt to improve the yield of **12** → **14**. Attempted TPAP-NMMO[11] oxidation of **12** provided only a mixture of starting material and undesired cyclopentenone product. However, Dess-Martin[12] reagent cleanly provided the desired crude cyclopentanone **13**. For the methylenation of **13**, the Tebbe,[13] Nysted,[14] and Pb-modified Lombardo[15] procedures were all superior in terms of yield and purity compared to the unmodified Lombardo conditions. In terms of convenience and amenability to scale-up, the Nysted procedure was employed for this sequence. Thus, Dess-Martin oxidation of **12** followed by Nysted methylenation afforded **14** in 75% overall yield. Deprotection of the purine employing the conditions described above provided **15** in 92% yield. Finally, debenzylation of the

carbocycle (excess BCl_3, CH_2Cl_2, -78 °C) afforded **4** in 89% yield (>99% ee) following crystallization from water.[16] The overall yield of **4** starting from commercial sodium cyclopentadienide is 18%. This chemistry has been employed to prepare >20g of **4**.

The synthesis of the adenine, thymine, and iodouracil analogs (**5-7**) is shown in Scheme 2; analogs **6** and **7** were prepared prior to the development of the optimized oxidation/methylenation sequence described above.[17-19] Compound **8**, the enantiomer of **4**, was prepared as shown in Scheme 3.[20]

Scheme 3.



93.6% ee

8

>99%ee

$[\alpha]_D$ -34.6° (c=0.3, H_2O)

(a) (i) $BnOCH_2Cl$, THF, -65 to -78 °C, (ii) diisopinylcampheylborane (prepared from (-)-α-pinene), THF, -65 to -78 °C, (iii) aq. NaOH, H_2O_2; (b)- (i) see Scheme 1

The anti-HBV activity[21] of analogs **4-8** and several other nucleoside analogs is shown in Table 1. Compound **4** with an EC_{50} of 0.003 μM emerged as the most active analog tested in cell culture. The adenine analog (**5**) was 43-fold less potent, while the thymine (**6**) and 5-iodouracil (**7**) analogs were much less potent.

Table 1. Activity of Nucleoside Analogs Against HBV in HepG2.2.15 Cells.

Compound	EC_{50} (μM)
4 (BMS-200475)	0.003
5	0.128
6	>100
7	10.5
8	100
1 (3TC)	0.2
3 (penciclovir)	≥100
carbocyclic 2'-dG	0.05

Compound **8**, the enantiomer of **4**, was 30,000-fold less potent, demonstrating that the anti-HBV activity resides solely in the enantiomer whose absolute configuration resembles that of a natural nucleoside. As shown in Table 1, **4** shows greater anti-HBV potency than 3TC (**1**), penciclovir (**3**) and carbocyclic 2'-deoxyguanosine (carbocyclic 2'-dG)[22] in our cell culture assay .

Table 2 displays the high degree of selectivity of **4** as an anti-HBV agent. The potencies of **4** against HIV (human immunodeficiency virus), influenza, HCMV (human cytomegalovirus), HSV-1 (herpes simplex virus type 1), and VZV (varicella zoster virus) are at least 3000-fold weaker than the potency against HBV. The cytotoxicity of **4** varies depending on the cell line tested, ranging from 21 to 120 μM (Table 2).

Table 2. Activity of **4** (BMS-200475) Against Other Viruses.

Virus	Cell Line	Antiviral Activity (EC_{50}, μM)	Cytotoxicity[a] (CC_{50}, μM)
HBV	HepG2.2.15	0.003	30
HIV	CEM-SS	>10	21
Influenza	MDBK	>80	78
HCMV	HFF	15	ND
HSV-1	WI-38	≥32	>90
VZV	WI-38	30-60	120

[a]Determined by either MTT or XTT assays; ND = not determined directly. Visual loss of HFF cells was noted at 90 and 150μM after 10 days.

Thus **4** (BMS-200475) is shown to be a highly potent and selective anti-HBV agent with relatively low cytotoxicity in a variety of cell lines. BMS-200475 is currently undergoing further evaluation, both in vitro and in vivo.

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6. The yields reported by other laboratories for this conversion fall within the range of 33-44% (References 5a and 5b).

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16. Compound **4** (BMS-200475): ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.54 (bs, 1H), 7.66 (s, 1H), 6.42 (s, 2H), 5.36 (m, 1H), 5.10 (m, 1H), 4.87 (d, J = 3.4 Hz, 1H), 4.84 (m, 1H), 4.56 (m, 1H), 4.23 (m, 1H), 3.53 (m, 2H), 2.52 (m, 1H, partially overlaps with solvent), 2.22 (m, 1H), 2.04 (m, 1H). Anal. calcd for $C_{12}H_{15}N_5O_3$•1.0 H_2O: C, 48.81; H, 5.80; N, 23.72. Found: C, 48.81; H, 5.70; N, 23.86.

17. Compound **5**: $[\alpha]_D^{25}$ +51.5° (c = 0.28, 1 N HCl). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (s, 1H), 8.10 (s, 1H), 7.23 (bs, 2H), 5.56 (m, 1H), 5.11 (m, 1H), 4.93 (m, 2H), 4.52 (m, 1H), 4.28 (m, 1H), 3.60 (m, 2H), 2.57 (m, 1H), 2.45 (m, 1H), 2.09 (m, 1H). Anal. calcd for $C_{12}H_{15}N_5O_2$: C, 55.16; H, 5.79; N, 26.80. Found: C, 55.00; H, 5.43; N, 26.89.

18. Compound **6**: $[\alpha]_D^{25}$ +59.0° (c = 0.30, H_2O). ^{1}H NMR (270 MHz, DMSO-d_6): δ 11.22 (bs, 1H), 7.28 (d, J = 1.1 Hz, 1H), 5.51 (m, 1H), 5.14 (s, 1H), 4.78 (s, 1H), 4.75 (s, 2H), 4.16 (bs, 1H), 3.54 (bs, 2H), 2.50 (m, 1H, partially overlaps with solvent), 1.93 (m, 2H), 1.74 (s, 3H). Anal. calcd for $C_{12}H_{16}N_2O_4$•0.4 H_2O: C, 55.53; H, 6.53; N, 10.80. Found: C, 55.49; H, 6.29; N, 10.84.

19. Compound **7**: $[\alpha]_D^{25}$ +63.0° (c = 0.30, MeOH). ^{1}H NMR (270 MHz, DMSO-d_6): δ 7.92 (s, 1H), 5.47 (m, 1H), 5.19 (m, 1H), 4.87 (m, 1H), 4.82 (m, 2H), 4.14 (m, 1H), 3.59 (m, 2H), 2.45 (m, 1H, partially overlaps with solvent), 1.99 (m, 2H). Anal. calcd for $C_{11}H_{13}N_2O_4I$•0.32 H_2O: C, 35.72; H, 3.72; N, 7.58. Found: C, 35.97; H, 3.55; N, 7.32.

20. Compound **8**: ^{1}H NMR (400 MHz, DMSO-d_6): spectrum identical to that of compound **4**. Anal. calcd for $C_{12}H_{15}N_5O_3$•1.5 H_2O: C, 47.36; H, 5.96; N, 23.02. Found: C, 47.33; H, 5.68; N, 23.01.

21. HepG2.2.15 human liver cells, which harbor integrated HBV genomes, secrete substantial amounts of infectious HBV virus particles bearing viral DNA genomes into the medium. Antiviral effects are scored as reductions in the amount of HBV DNA present in the media after treatment of the cells with the drug for 9 days. HBV DNA is released from secreted virus particles by alkali treatment, immobilized onto membranes and quantitated by hybridization with a radiolabeled HBV DNA probe.

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