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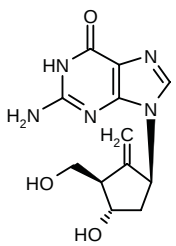
CCC: 0377-8282/99

BMS-200475

Anti-HBV

Entecavir
SQ-34676

(1*S*,3*R*,4*S*)-9-[4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine



$C_{12}H_{15}N_5O_3$ Mol wt: 277.2850

CAS: 142217-69-4
CAS: 209216-23-9 (as monohydrate)

EN: 182634

Synthesis

The regioselective reaction of cyclopentadiene (I) and sodium (1) or commercial sodium cyclopentadienide (II) (2, 3) with benzyl chloromethyl ether (III) by means of the chiral catalyst (–)-diisopinocampheylborane in THF, followed by hydroxylation with H_2O_2/NaOH, gives (1*S*-*trans*)-2-(benzyloxymethyl)-3-cyclopenten-1-ol (IV), which is regioselectively epoxidized with *tert*-butyl hydroperoxide and vanadyl acetylacetonate in 2,2,4-trimethylpentane, yielding [1*S*-(1α,2α,3β,5α)-2-(benzyloxymethyl)-6-oxabicyclo[3.1.0]hexan-3-ol (V). The protection of (V) with benzyl bromide and NaH affords the corresponding ether (VI), which is condensed with 6-*O*-benzylguanine (VII) by means of LiH in DMF to give the guanine derivative (VIII). The protection of the amino group of (VIII) with 4-methoxyphenyl(diphenyl)chloromethane (IX), TEA and DMAP in dichloromethane gives intermediate (X), which is oxidized at the free hydroxyl group with methylphosphonic acid, DCC and oxalic acid in DMSO (1) or Dess Martin periodinane in dichloromethane (2, 3), yielding the cyclopentanone derivative (XI). The reaction of (XI) with (i) $Zn/TiCl_4/CH_2Br_2$ complex in THF/CH_2Cl_2 (1), (ii) activated $Zn/PbCl_2/CH_2I_2/TiCl_4$ in THF/CH_2Cl_2 (2), (iii) Nysted reagent/$TiCl_4$ in THF/CH_2Cl_2 (2, 3) or (iv) Tebbe reagent in toluene (2) affords the corresponding methylene derivative (XII), which is partially deprotected with 3N HCl in hot THF, providing the dibenzylated compound (XI). Finally, this compound is treated with BCl_3 in dichloromethane (1-3). Scheme 1.

Description

Hydrate, m.p. >220 °C, $[\alpha]_D^{22}$ +34° (c 0.3, water) (1); monohydrate, white crystalline solid, m.p. 234-6 °C (decomp.) for the bulk sample and m.p. 255 °C (decomp.) for an analytical sample recrystallized from water, $[\alpha]_D$ +33.2° (c 0.3, water) (2); $[\alpha]_D$ +35.0° (c 0.38, water) (3).

Introduction

Infection with hepatitis B virus (HBV), the prototype member of a small family of related hepadnaviruses, is a major cause of morbidity and mortality in all regions of the world. There are an estimated 350 million carriers of the virus (4). According to the World Health Organization, hepatitis B results in 1-2 million deaths every year worldwide. In Europe alone, it has been estimated that 1-2 million people are infected with HBV every year, and of these, about 90,000 will be infected persistently with HBV and some 22,000 will eventually die from cirrhosis or primary liver cancer (5). Up to 80% of cases of primary liver cancer are attributed to hepatitis B, which is second only to tobacco among the known human carcinogens (6).

The development of vaccines for active immunization against hepatitis B virus has been one of the great successes in preventive medicine. First-generation hepatitis B vaccines, derived from the plasma of carriers, were first introduced in 1982 and are still used in many parts of the world. Second-generation vaccines based on recombinant DNA vaccines, most of which are yeast-derived, have replaced plasma-derived vaccines in many countries (7).

The primary goal of treatment of patients with chronic HBV is to suppress HBV replication before there is

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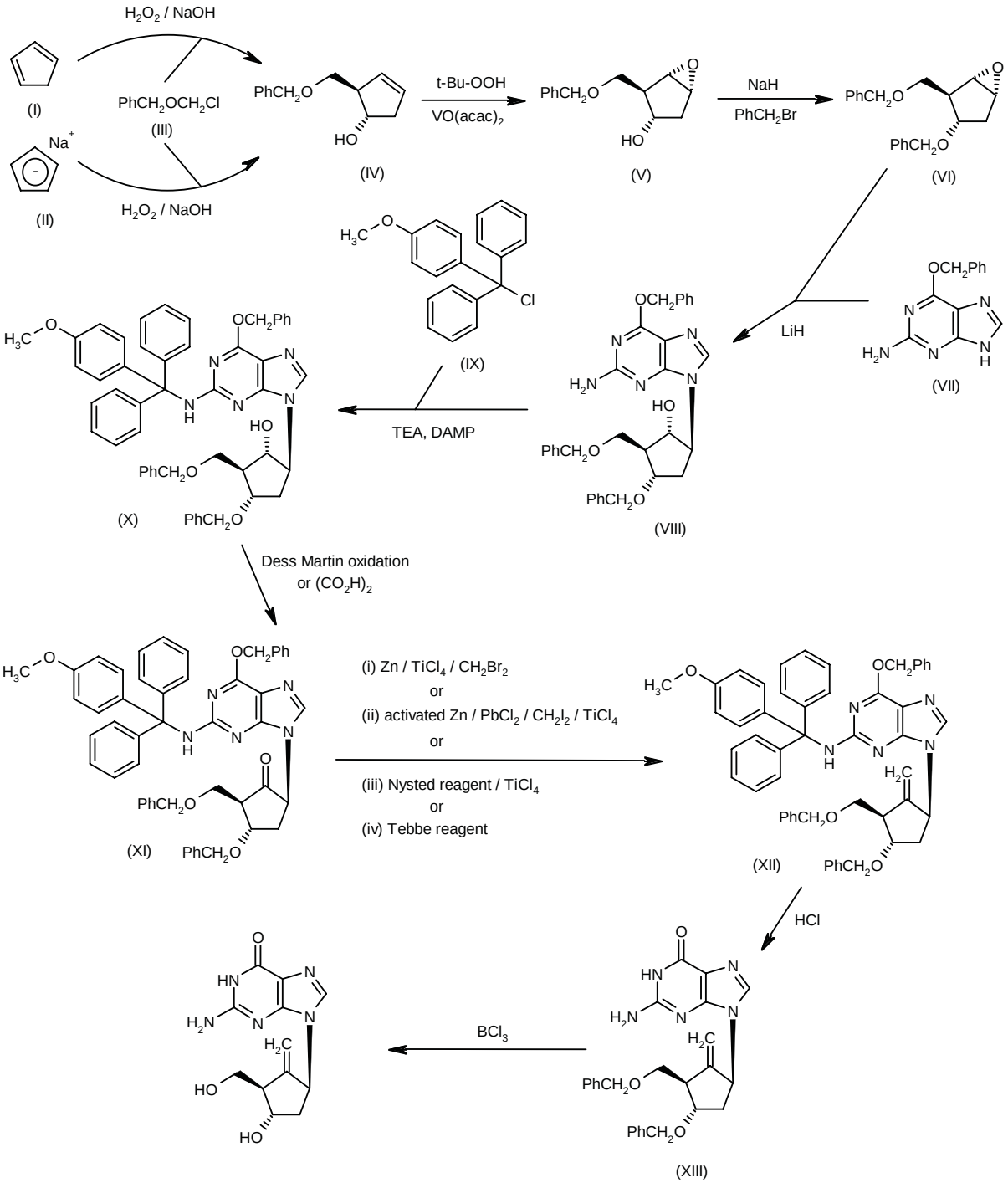
Scheme 1: Synthesis of BMS-200475

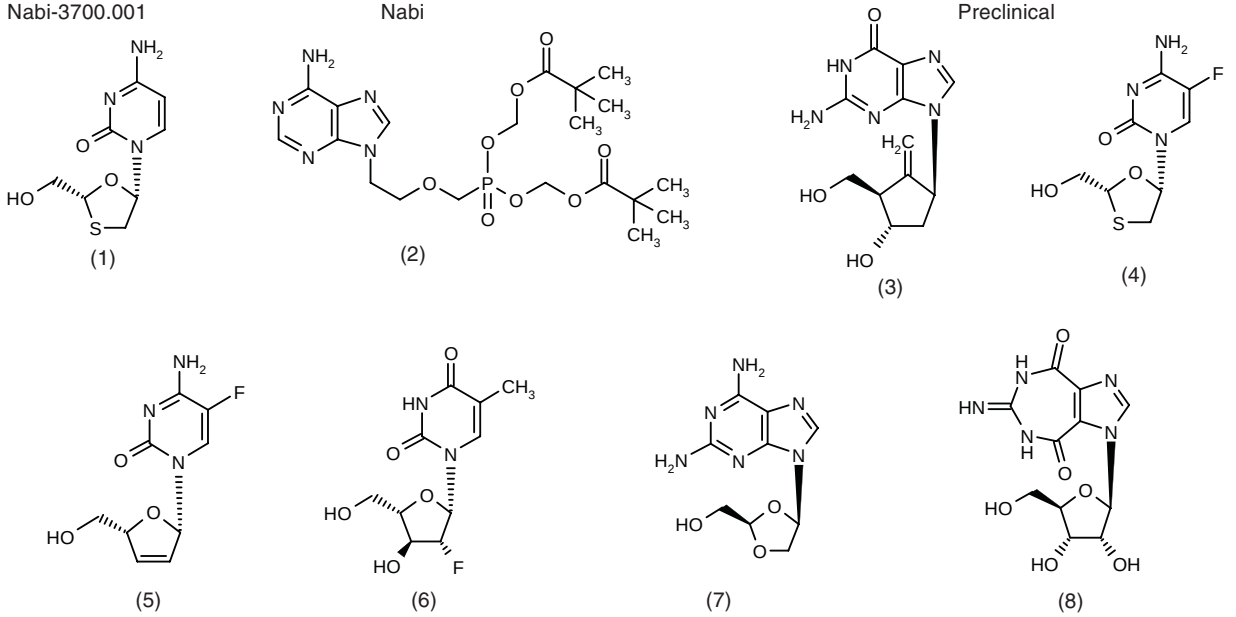


irreversible liver damage. Recombinant α interferon (IFN-α), introduced in 1985, was the first compound to be approved in most countries for the treatment of chronic hepatitis B; however IFN-α induces long-term remissions in only 25-40% of patients, is expensive and is often poorly tolerated.

During the last decade there has been a great deal of interest in developing nucleoside analogs which inhibit reverse transcriptase and hepatitis B DNA polymerase. These new agents are more potent inhibitors of hepatitis B replication than IFN-α. Lamivudine (3TC, Heptodin®), introduced in 1995 for HIV disease, was launched this

Table I: Chemical structures of nucleoside analogs launched and under development for treatment of HBV infection (Prous Science Ensemble database).

Compound	Company	Status
1. Lamivudine	BioChem Pharma/Glaxo Wellcome	L-1999
2. Adefovir dipivoxil	Gilead	Phase III
3. BMS-200475	Bristol-Myers Squibb	Phase II
4. Emtricitabine	Triangle	Phase I/II
5. β-L-Fd4C	Vion	Preclinical
6. Clevudine	Triangle	Preclinical
7. DAPD	Triangle	Preclinical
8. Nabi-3700.001	Nabi	Preclinical



year by BioChem Pharma and Glaxo Wellcome as the first oral antiviral treatment for chronic hepatitis B. Three nucleoside analogs, adefovir dipivoxil (Gilead), BMS-200475 (Bristol-Myers Squibb) and emtricitabine (Coviracil®; Triangle) are undergoing phase III, II and I/II clinical development, respectively, while others are under preclinical evaluation. The structures of lead nucleoside analogs in development for HBV are shown in Table I.

A major problem with lamivudine treatment is the development of drug resistance. Lamivudine-resistant hepatitis B is characterized by amino acid site mutations in the YMDD locus of the catalytic domain of the HBV polymerase. Ideally, therapeutic agents used in combination therapy should have additive or synergistic activity against HBV infection and delay or prevent the development of drug resistance. The efficacy of strategies combining lamivudine with interferon or other antiviral agents with different mechanisms of action, or of combining nucleoside analogs with immunomodulatory therapy, remains to be determined in clinical trials.

In the search for new antiviral agents, scientists at Bristol-Myers Squibb synthesized a series of 4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl purines and pyrimidines and identified SQ-34676 (BMS-200475) as being worthy of further evaluation. The compound was originally targeted as an antiherpesvirus agent (8), although later studies proved its highly superior anti-HBV activity.

Pharmacological Actions

BMS-200475 was shown in early studies to be a potent inhibitor of hepatitis B virus replication *in vitro* in HepG2.2.15 cells (EC_{50} = 3.75 nM), while inducing cytotoxicity only at concentrations fully 8000 times lower (CC_{50} = 30 μM) (9-11). Treatment with BMS-200475 had no apparent inhibitory effects on mitochondrial DNA content (11). BMS-200475 was found to have selective in vitro activity against HBV as compared to HIV, influenza virus, human cytomegalovirus, varicella zoster virus and herpes simplex virus type 1. Asymmetrical synthesis of the compound provided a good overall yield with high optical purity. Poor HBV activity was shown by the enantiomer of BMS-200475 and also the adenine, thymine and 5-iodouracil nucleoside base analogs of the compound in comparison to BMS-200475 (3, 11).

In a separate study in human hepatoma cells, BMS-200475 was found to be specifically taken up and phosphorylated to its mono-, di- and triphosphate esters. The

uptake of BMS-200475 by HepG2 cells was linear between 1 and 25 μM, but intracellular triphosphate accumulated most efficiently in the low μM range. The half-life was approximately 15 h. BMS-200475 was shown to be more efficiently phosphorylated to its triphosphate form than lamivudine, penciclovir or lobucavir, and this phosphorylation of BMS-200475, especially at low concentrations, was indicated as being one reason for its high potency against HBV (12).

The multifunctional viral polymerase (Pol) is the key enzyme in the unique hepadnavirus replication scheme and is an important target for viral inhibition. The triphosphate forms of BMS-200475 and lobucavir inhibited HBV, woodchuck hepatitis virus (WHV) and duck hepatitis B virus (DHBV) *in vitro* to a similar extent; both were significantly more active than other nucleosides tested (ganciclovir, lamivudine, acyclovir, ddG and SQ-32829). Furthermore, BMS-200475 and lobucavir blocked the three distinct phases of hepadnavirus replication: priming, reverse transcription and DNA-dependent DNA synthesis (13).

The woodchuck is a commonly used animal model for hepatitis B infection. In one *in vivo* study, daily treatment of chronically infected animals with BMS-200475 (0.02-0.5 mg/kg p.o.) for periods of 1-3 months led to effective suppression of WHV, as manifested by decreased levels of WHV DNA and reduced endogenous hepadnaviral polymerase activity. Viral DNA was nondetectable using a dot blot hybridization technique in animals treated for 3 months with BMS-200475; analysis using a more sensitive PCR assay showed that mean WHV titers decreased significantly as a result of the treatment. Upon discontinuation of the drug, hepatitis viremia gradually returned to pretreatment levels (14).

In another woodchuck study, BMS-200475 was administered once daily (0.02 or 0.1 mg/kg) to chronically infected WHV carriers for 84 days. WHV viremia was reduced by 10- to 1000-fold after just 1 week of treatment with the title compound at both doses. All carriers treated at the higher dose and 4 of 6 treated at the lower dose had reductions of >1000-fold in WHV viremia by the third week of therapy; this level of suppression was maintained for 6-8 weeks after the drug was discontinued. Serum WHV DNA returned to pretreatment or detectable levels 8-12 weeks after discontinuing treatment (15).

A subsequent study evaluated the effects of maintenance therapy of chronically infected WHV carriers with BMS-200475. Nineteen woodchucks were treated once daily for 8 weeks with the agent (0.5 mg/kg p.o.), and serum WHV DNA dropped below limits of detection after 1-5 weeks of treatment. Six woodchucks were then withdrawn from drug therapy, causing viral DNA to rebound to pretreatment levels within 1-8 weeks, while the remaining 13 continued treatment with BMS-200475 using a once-weekly dosing regimen (0.5 mg/kg p.o.). Viral DNA serum levels remained fully undetectable in 12 of 13 animals 16 weeks after discontinuation of daily drug dosing. These results indicate that once viral suppression is successfully achieved, maintenance therapy using a much less frequent dosing schedule is feasible (16, 17).

The ability of BMS-200475 to inhibit DHBV infection in primary duck hepatocytes (EC_{50} = 0.13 nM) and in live ducklings has also been demonstrated. *In vivo* in infected ducks, BMS-200475 decreased viral DNA levels in the liver by 96, 83 and 45% at doses of 1.0, 0.1 and 0.01 mg/kg/day by oral gavage. Its activity was slightly superior to that of lobucavir and highly superior to that of lamivudine *in vivo* (18).

Pharmacokinetics and Metabolism

The results obtained *in vitro* in HepG2 and hepatitis B virus-transfected 2.2.15 human hepatoma cell lines demonstrated that the metabolism of BMS-200475 is similar. The di- and triphosphate products were the primary metabolites obtained. Accumulation of the triphosphate was rapid and was detectable down to a concentration of 5 nM of added drug. Maximum triphosphate accumulation at 25 μM was 29 pmol/10(6) cells. Accumulation was slow and kinetics of the triphosphate were linear for 3 days before beginning to decline. The intracellular half-life of BMS-200475 triphosphate was approximately 15 h in both cell lines (12, 19, 20).

Clinical Studies

In the first clinical trial conducted with the compound, BMS-200475 was administered to healthy volunteers as single oral doses of 1, 2.5, 5, 10, 20 or 40 mg p.o. according to a randomized, double-blind, placebo-controlled design. Pharmacokinetics were evaluated using blood and urine samples collected for 14 days postdosing. Safety was evaluated by physical examination and laboratory testing before escalation to each subsequent dose level. BMS-200475 was well tolerated, with an incidence of treatment-related adverse events similar to that for placebo (31% *vs.* 33% for placebo). Side effects of the study drug, all of which were mild and reversible, included drowsiness/fatigue, headache and lightheadedness/dizziness. Pharmacokinetic assessment revealed that the drug is well absorbed after oral dosing, with dose-dependent increases in peak plasma concentrations and AUC values. Plasma drug concentrations declined in a biexponential fashion, with a mean terminal $t_{1/2}$ of 55 h. More than 50% of the administered dose was eliminated in the urine as unchanged drug. Renal tubular secretion appeared to play an important role, with renal clearance values ranging from 300-600 ml/min (21).

BMS-200475 is currently in phase II trials in the U.S. Development of the compound is also being conducted outside the U.S. (22).

Manufacturer

Bristol-Myers Squibb Co. (US).

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