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Synthesis of 2′-β-*C*-methyl-neplanocin derivatives as anti-HCV agents

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ABSTRACT

The synthesis of 2′-β-*C*-methyl-neplanocin derivatives is described. The key intermediate cyclopentenyl alcohol **12** is prepared from sugar **5** in 12 steps. Coupling of **12** with appropriately protected purine, 7-deaza pyrimidine, uracil and pyrimidine bases via the Mitsunobu reaction followed by deprotection afforded the target cyclopentenyl nucleosides (**18**–**23**, **27**). The synthesized compounds were evaluated as potential inhibitors of the hepatitis C virus (HCV) in vitro. Unfortunately, none of them show anti-HCV activity below EC_{50} 100 μM.

Hepatitis C virus (HCV) is the pathogen associated with the majority of sporadic and transfusion related non-A and non-B hepatitis infections. Although HCV is often asymptomatic, it can progress to chronic hepatitis, leading to liver cirrhosis and hepatocellular carcinoma in up to 80% of patients.[1,2] Since there is no vaccine available, efficacious therapies are urgently needed to combat this important viral disease.

Ourselves and others have previously demonstrated that some C_2'-methyl modified non-natural nucleosides are potent inhibitors of HCV replication. It has been demonstrated that the 2′-β-*C*-methyl derivatives are efficient chain terminators of HCV genome replication without significant toxicity.[3–5]

Carbocyclic nucleosides are biologically interesting molecules that can display important antitumor and antiviral activities. Such nucleosides are chemically more stable and are not subject to the action of the enzymes that cleave the glycosyl linkage in conventional nucleosides. Often, introduction of unsaturation can increase the biological potency and specificity when compared to the corresponding saturated carbocyclic analogues.[6] (−)-Neplanocin A (NPA, **2**), originally isolated from the culture filtrate of the soil fungus *Ampullariella regularis*, is a carbocyclic nucleoside with potent antiviral and antitumor activity and is a powerful inhibitor of *S*-adenosylhomocysteine hydrolase.[7]

In recent years, attention has been increasingly focused on structural modifications of carbocyclic nucleosides. It has been discovered that many synthetic analogues of NPA, including abacavir[8] and carbovir,[9] exhibit potent anti-viral activity. Combining the structural features of both compounds **1** and **2**, we speculated that a 2′-methyl modification on NPA such as **3** and **4** (Fig. 1) would reduce cytotoxicity and introduce anti-HCV activity.

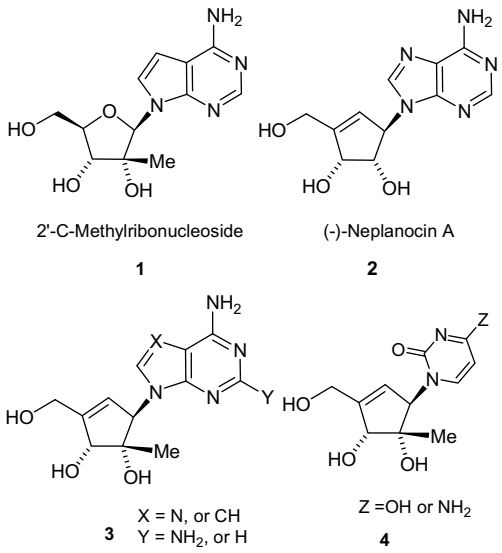

Figure 1. C_2'-Methyl adenosines and (−)-neplanocin A.

Several synthetic methodologies have been reported toward NPA. Ohira and co-workers reported the so far shortest method using a C–H insertion reaction of a methylidene carbene as a key step.[10] Based on this strategy, herein we describe the synthesis of 2-β-*C*-methyl-neplanocin A derivatives **3** and **4**, and their antiviral properties.

The intermediate **6** can be conveniently synthesized from 3′,5′-*O*-(4-methylbenzoyl)-2′-*C*-methyl-ribose[11] **5** according to the reported procedure[12] (Scheme 1). Protection of the 5′-primary hydroxyl of **6** with a *tert*-butyldiphenylsilyl (TBDPS) group,

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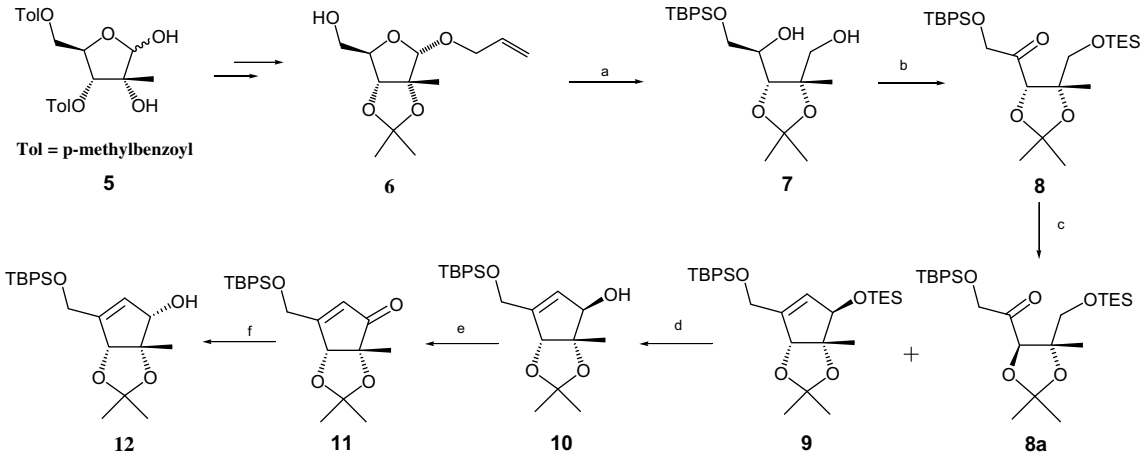
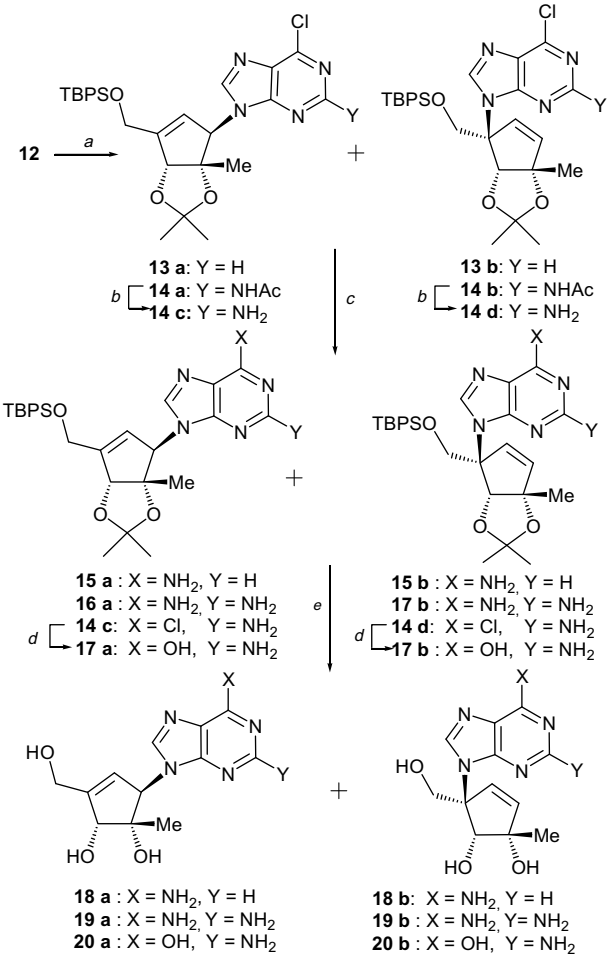


Scheme 1. Reagents and conditions: (a) (i) TBDPSCl, imidazole, DMF; (ii) [(dppp)$NiCl_2$], DIBAL-H, toluene; (iii) $NaBH_4$, MeOH, 82% for three steps; (b) (i) TESCl, imidazole, DMF; (ii) Dess–Martin periodinane, DCM; (c) TMSC(Li)N_2, THF, 0 °C (40–55% for **9** and 5–10% for **8a**); (d) PPTS, MeOH; (e) $PySO_3$, Et_3N, DCM, DMSO; (f) $NaBH_4$, $CeCl_3$, MeOH.

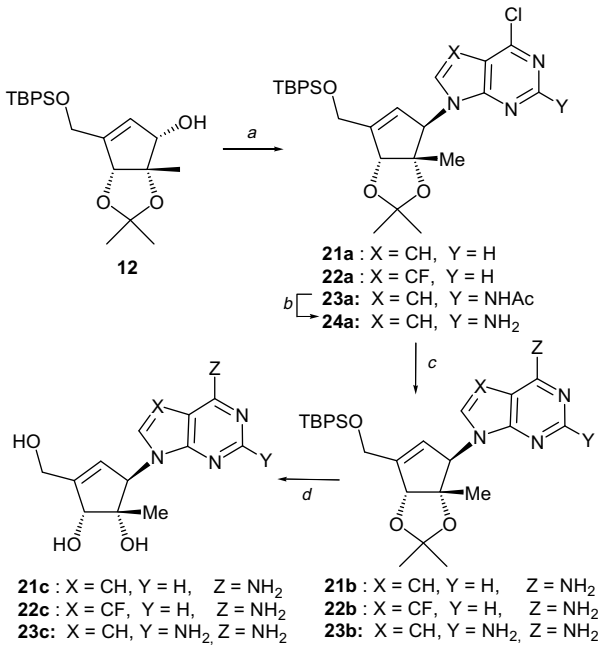
followed by selective cleavage of the allyl ether using a slight excess of diisobutylaluminum hydride in the presence of a catalytic amount of [$NiCl_2$(dppp)][13] afforded the hemiketal intermediate in excellent yield. With the addition of an excess amount of DIBAL-H, the hemiketal could be reduced further to give the corresponding diol **7** in one pot with good yield.[13] Selective protection of the newly formed primary alcohol as a triethylsilyl (TES) ether, followed by a Dess–Martin periodinane oxidation of the secondary alcohol gave ketone **8**. Cyclization *via* intramolecular C–H insertion of the alkylidenecarbene derived from ketone **8** by exposure to lithiotrimethylsilyl-diazomethane provided the desired product **9** with 40–54% yield, while epimerization at the 3′ position gave rise to byproduct **8a**.[14] In contrast to the previous report of Ohira who obtained a 2.7:1 epimeric mixture of the cyclopentene derivative during the synthesis of NPA,[10] we only got a single cyclopentene product **9**. Selective TES deprotection of compound **9** using PPTS/MeOH, followed by oxidation of alcohol **10** with trioxidesulfur-pyridine complex provided ketone **11**. Its selective reduction with $NaBH_4$ in the presence of cerium(III) chloride furnished key intermediate **12** and its epimer **10** in a 4:1 ratio with 90% total yield.[15]

With the key intermediate **12** in hand, the base components of the carbocyclic nucleosides were coupled with **12** using the Mitsunobu reaction[16] to obtain the desired nucleosides **13a**, **14a**, **21a**–**23a**, and **25a** (Schemes 2–4). To synthesize the purine analogues (Scheme 2), the cyclopentenyl alcohol **12** was treated with 6-chloropurine and N^2-acetylamino-6-chloropurine under standard Mitsunobu conditions to give **13a** and **14a**. Surprisingly we also obtained the minor allylic migration byproducts **13b** and **14b**.[17] When compared to reported values, the reaction rate as well as the yield was decreased, which we attribute to steric hinderance caused by the 2′-*C*-methyl group. In addition, we observed the effect of the 2′-*C*-methyl group in previous steps when we reduced **11** to **12** and found a mixture of diastereomers **12** and **10** rather than a single isomer **12**. We also investigated this Mitsunobu reaction under microwave-assisted conditions at 80 °C and noted that the reaction was completed in only 1 minute (Scheme 3), compared to thermal conditions, which usually required about 16 hours or longer. The mixture of **13a**–**b** and **14a**–**b** was converted to the desired 4-amino derivatives **18a**–**b** and **19a**–**b**[18] by ammonolysis at elevated temperature followed by deprotection of the *tert*-butyl diphenyl silyl and isopropylidene groups with 4 N HCl in MeOH. Compounds **14a**–**b** were converted to the corresponding guanine nucleosides **20a**–**b** by the previously reported method.[19]

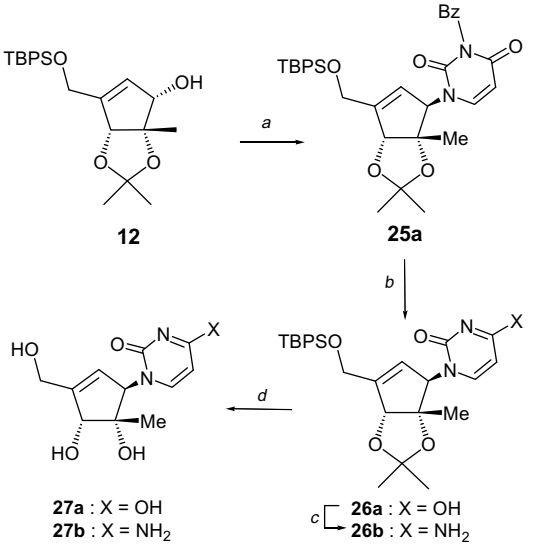
The 7-deaza and 7-F-7-deaza purine analogues were prepared using a similar procedure (Scheme 3). Treatment of **12** with 7-deaza-6-chloropurine, 7-F-deaza-6-chloropurine, and N^2-acetylamino-7-deaza-6-chloropurine, as described above, gave **21a**, **22a**, and **23a**, respectively. The N^2-acetyl group in **23a** was removed using aqueous NaOH to provide compound **24a**. Compounds **21a**, **22a**, and **24a** were treated with methanolic ammonia at 80 °C (sealed



Scheme 2. Reagents and conditions: (a) Ph_3P, DIAD, THF, rt, 30–75%; (b) 1 N NaOH, MeOH; (c) methanolic ammonia, 18 h, 80 °C; (d) 1 N NaOH, reflux; (e) 4 N HCl, MeOH, rt.



Scheme 3. Reagents and conditions: (a) Ph_3P, DIAD, THF, rt, 20–30%; or microwave 80 °C, 1 min, 40–50%; (b) 1 N NaOH, MeOH; (c) methanolic ammonia, 18 h, 80 °C; (d) 4 N HCl, MeOH, rt.



Scheme 4. Reagents and conditions: (a) Ph_3P, DIAD, THF, rt, 28%; (b) 1 N NaOH, MeOH; (c) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, MeCN, 0 °C–rt, 24 h then 30% NH_4OH, rt, 5 h; (d) 4 N HCl, MeOH, rt.

tube, 16–36 h) to afford **21b**, **22b**, and **23b**; deprotection of these derivatives with 4 N HCl in methanol at room temperature provided the free carbocyclic nucleosides **21c**, **22c**, and **23c** in high yields ranging from 95% to 99%.

The synthesis of the cytosine and uracil derivatives of 2′-β-*C*-methyl-neplanocin A was carried out using a similar strategy, (Scheme 4). Briefly, the cyclopentenol **12** was coupled with *N*-Bz uracil followed by deprotection of the benzoyl group, *tert*-butyl diphenyl silyl group, and isopropylidene groups to yield compound **27a**. Compound **26a** was converted to the corresponding cytosine analogue **27b** by the reported method.[20]

All the synthesized nucleosides were evaluated as potential inhibitors of HCV using the cell-based bicistronic replicon assay, modified for RNA quantitation by RNase protection.[3] While none of the analogs showed cytotoxicity below 100 μM, unfortunately they were inactive in the inhibition of HCV replication when tested up to 100 μM. A successful inhibitory action requires the nucleosides to be converted to their corresponding nucleoside triphosphates; therefore, one possible reason for the inactivity of the cyclopentenyl nucleoside is its inability to be phosphorylated by the appropriate kinases.[4]

In summary, a series of novel 2′-β-*C*-methyl-neplanocin analogues were successfully synthesized and their biological activity was evaluated. Microwave irradiation improved a key Mitsunobu coupling reaction. To the best of our knowledge, this represents the first documented case of the synthesis of neplanocin analogues under these conditions. Further structure–activity relationship and other biological evaluations of these analogues are currently underway, and will be reported in due course.

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(d, J = 10.5 Hz, 1H), 1.41 (s, 3H); Compound **19a**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.56 (s, 1H), 5.82 (s, 1H), 5.23 (s, 1H), 4.40 (s, 1H), 4.25 (dd, J = 7.5 Hz, 2H), 0.84 (s, 3H); Compound **19b**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (s, 1H), 6.50 (d, J = 6.0 Hz, 1H), 6.14 (d, J = 6.0 Hz, 1H), 4.12 (s, 1H), 3.98 (d, J = 11 Hz, 1H), 3.71 (d, J = 11 Hz, 1H), 1.36 (s, 3H); Compound **20a**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (s, 1H), 5.92 (m, 1H), 5.24 (m, 1H), 4.45 (s, 1H), 4.28 (dd, J = 14, 14 Hz, 2H), 0.93 (s, 3H); Compound **20b**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.82 (s, 1H), 6.60 (d, J = 5.5 Hz, 1H), 6.20 (d, J = 5.5 Hz, 1H), 4.21 (s, 1H), 4.02 (d, J = 10.5 Hz, 1H), 3.84 (d, J = 10.5 Hz, 1H), 1.42 (s, 3H); Compound **21c**: ^{1}H NMR (500 MHz, CD_3OD) δ 8.31 (s, 1H), 7.26 (s, 1H), 6.95 (s, 1H), 5.87 (s, 1H), 5.73 (s, 1H), 4.52 (s, 1H), 4.35 (br s, 2H), 0.84 (s, 3H); Compound **22c**: ^{1}H NMR (500 MHz, CD_3OD) δ 8.24 (s, 1H), 7.12 (s, 1H), 5.80 (s, 1H), 5.71 (s, 1H), 4.46 (s, 1H), 4.32 (dd, J = 17.0, 17.0 Hz, 2H), 0.85 (s, 3H); Compound **23c**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.64 (d, J = 5.0 Hz, 1H), 6.42 (d, J = 5.0 Hz, 1H), 5.87 (s, 1H), 5.41 (s, 1H), 4.46 (s, 1H), 4.36 (dd, J = 16.0, 16.0 Hz, 2H), 0.86 (s, 3H); Compound **27a**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.32 (d, J = 4.5 Hz, 1H), 5.67 (s, 1H), 5.66 (d, J = 4.5 Hz, 1H), 5.40 (s, 1H), 4.34 (s, 1H), 4.28 (dd, J = 13.0, 13.0 Hz, 2H), 1.10 (s, 3H); Compound **27b**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.64 (br s, 1H), 6.08 (br s, 1H), 5.68 (s, 1H), 5.42 (s, 1H), 4.38 (s, 1H), 4.25 (dd, J = 13.5, 13.5 Hz, 2H), 1.13 (s, 3H).

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