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Bioorganic & Medicinal Chemistry Letters 16 (2006) 4190–4194
Bioorganic & Medicinal Chemistry Letters

Synthesis of 2-*C*-hydroxymethylribofuranosylpurines as potent anti-hepatitis C virus (HCV) agents

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Received 14 April 2006; revised 13 May 2006; accepted 29 May 2006
Available online 14 June 2006

Abstract—On the basis of potent anti-HCV activity of 2′-*C*-methyladenosine, novel 2′-*C*-hydroxymethyladenosine analogues **2a**–**c** were synthesized from D-ribose in order to lead to favorable interaction with HCV polymerase. Among compounds tested, adenosine derivative **2a** exhibited potent anti-HCV activity, indicating that the hydroxyl group of 2′-*C*-hydroxymethyl substituent led to favorable interaction with HCV polymerase.

Hepatitis C is a viral disease which afflicts an estimated 170 million people worldwide.[1] Hepatitis C virus (HCV) was identified in 1989 as the major pathogen responsible for post-transfusion non-A and non-B hepatitis.[2] Chronic infection with HCV can be developed as lethal liver diseases such as liver cirrhosis and hepatocellular carcinoma.[3]

However, effective therapeutics are still not available for the treatment of HCV-infected individuals although immunotherapy using recombinant interferon-α in combination with ribavirin is being clinically used.[4]

HCV belongs to RNA virus with a single strand RNA genome encoding a 3000 amino acid polyprotein which is processed into several viral proteins,[5,6] including the NS5b RNA dependent RNA polymerase.[7] This enzyme is essential for viral replication and has been a valid antiviral target for the development of new anti-HCV agents.

A number of nucleoside and non-nucleoside derivatives have been synthesized and tested for anti-HCV activity.[8] Among these, 2′-*C*-methyladenosine (**1**) emerged as one of the most potent inhibitors (EC_{50} = 0.3 μM) in a cell-based HCV replicon assay (Fig. 1).[9] Its guanosine[9] and cytidine[10] analogues also exhibited potent anti-HCV activity in the same assay system. These nucleosides are incorporated into proviral RNA like a substrate chain after being converted to their corresponding triphosphates and act as chain-terminators because 2′-methyl group prevents subsequent incorporation of incoming nucleoside triphosphate (NTP).[11]

On the basis of these findings, we designed the 2′-*C*-hydroxymethyl analogues **2**, which put the additional hydroxyl group to 2′-*C*-methyladenosine **1**, to expect the favorable electronic effect, in addition to the desired steric effect by 2′-*C*-hydroxymethyl group on the interaction with HCV polymerase (Fig. 1). While synthesizing the target nucleosides, we encountered that the electronic effect was more dominant factor than the steric effect in the glycosylation to obtain β-nucleosides. Herein, we report the synthesis of 2′-*C*-hydroxymethyladenosine derivatives (**2a–c**) as anti-HCV agents and their related interesting sugar chemistry.

Our initial synthetic approach was to synthesize the glycosyl donor **5** and then to condense with nucleobase, as shown in Scheme 1.

D-Ribose was treated with acetone and catalytic amount of sulfuric acid to give the acetonide **3**. Compound **3** was subjected to the aldol condensation to give the

Keywords: Hepatitis C virus; RNA dependent RNA polymerase; 2′-*C*-Hydroxymethyladenosine; Anti-HCV agent.
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doi:10.1016/j.bmcl.2006.05.089

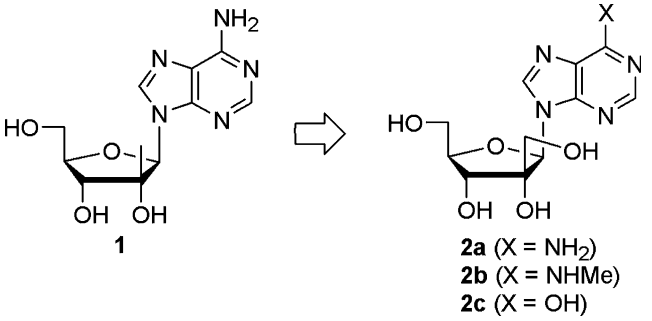
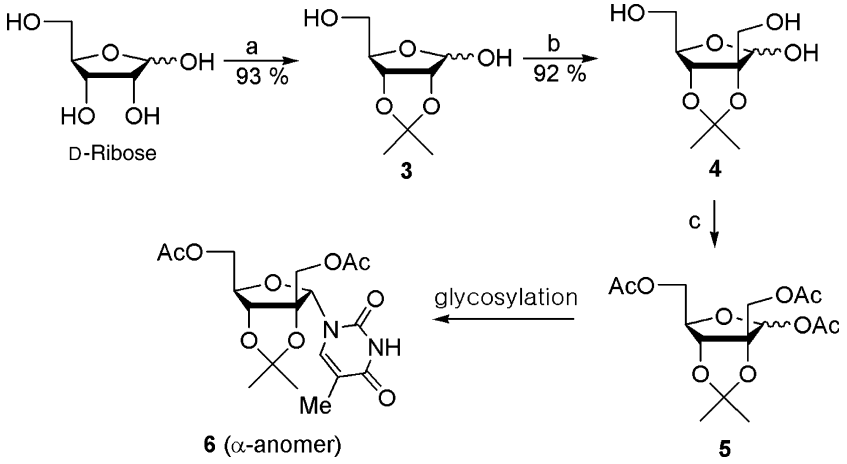


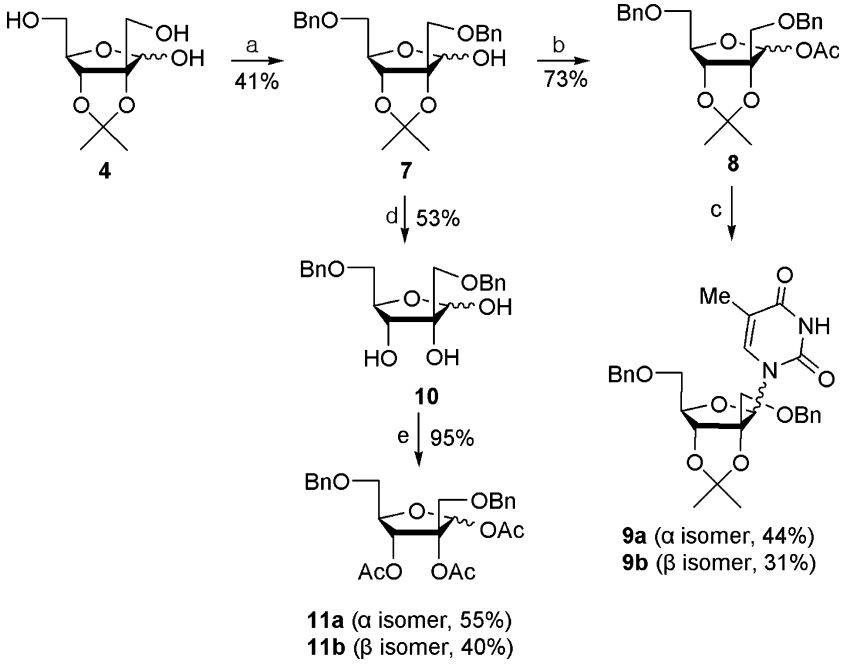
Figure 1. The rationale for the design of the target nucleosides.

2-hydroxymethyl derivative **4** as a single stereoisomer,[12] which was peracetylated to give the glycosyl donor **5**. As a model study, the glycosyl donor **5** was condensed with silylated thymine in the presence of trimethylsilyl trifluoromethane sulfonate (TMSOTf) as a Lewis acid to give the α-anomer **6** with trace amounts of its β-anomer. In this glycosylation, the neighboring group effect by the C2 acetoxymethyl group was found to be more dominant than the steric effect by 2,3-acetonide.

In order to avoid the exclusive formation of α-anomer through the neighboring group effect by 2-*C*-acetoxymethyl group, 2-*C*-hydroxymethyl group was converted to the 2-*C*-benzyloxymethyl group, as shown in Scheme 2. The benzyl group was selectively introduced at the primary hydroxyl group of **4** using organotin chemistry[13] to give the dibenzyl ether **7** as an anomeric mixture. Compound **7** was acetylated to give another glycosyl donor **8**. However, condensation of **8** with silylated thymine also afforded the α-anomer **9a** as a major product, indicating that 2-*C*-benzyloxymethyl



Scheme 1. Reagents and conditions: (a) acetone, concd H_2SO_4, rt, 2.5 h; (b) CH_2O, K_2CO_3, MeOH, reflux, 4 d; (c) Ac_2O, DMAP, NEt_3, rt, 5 h.

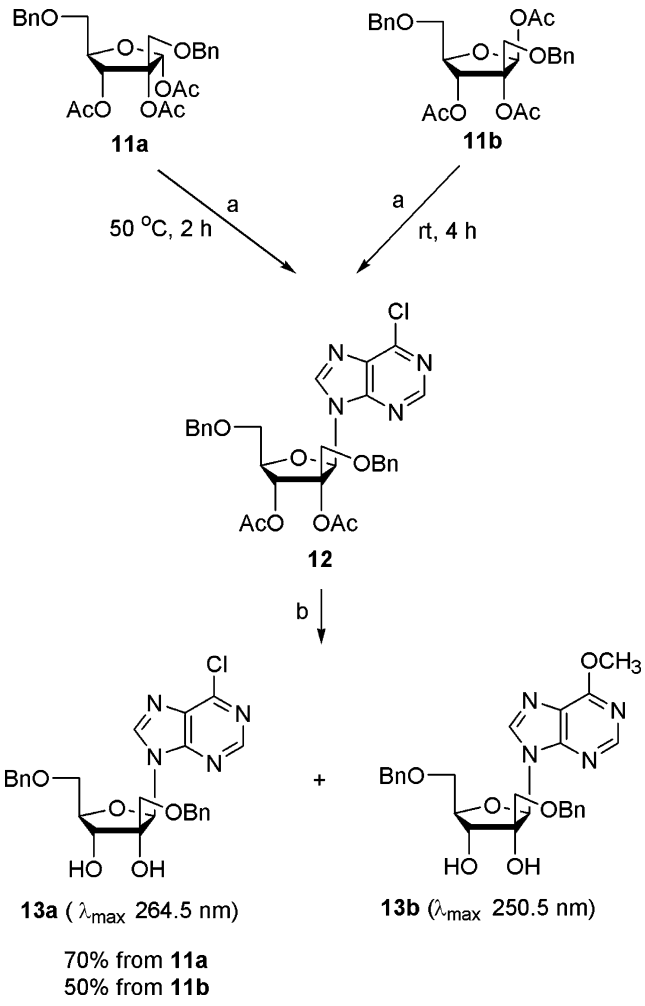


Scheme 2. Reagents and conditions: (a) i. *n*-Bu_2SnO, toluene, reflux, 15 h; ii. *n*-Bu_4NI, BnBr, 100 °C, 15 h; (b) Ac_2O, pyridine, rt, 8 h; (c) i. thymine, $(NH_4)_2SO_4$, HMDS; ii. TMSOTf, $ClCH_2CH_2Cl$, overnight; (d) 3 *N* HCl/THF (1:1), rt, 1 d; (e) Ac_2O, Et_3N DMAP, CH_2Cl_2, rt, 3 h.

group is more dominant than 2,3-acetonide from the viewpoint of stereoelectronic effect. Thus, we decided to synthesize the glycosyl donor **11** whose C2 hydroxyl group was protected with acetyl group, for the exclusive formation of the β-anomer by neighboring group participation of the C2 acyl group.

Treatment of **7** with 3 *N* HCl in THF under reflux for 1 d afforded inseparable anomeric mixture of **10**, which was decomposed when the reaction mixture was evaporated without complete neutralization. Treatment of compound **10** with acetic anhydride in the presence of DMAP and triethylamine gave **11a** (α-anomer, 55%) and **11b** (β-anomer, 40%), after purification by silica gel column chromatography. Anomeric configuration of **11a** and **11b** were readily assigned by ^{1}H NOE. The NOE effect (2.94%) was observed between the anomeric proton of **11a** and its methylene protons of 2-CH_2OBn, indicating α-anomer, while no NOE was observed on the same experiment in the case of **11b**, resulting in β-anomer.

Condensation of the acetate, **11a** or **11b** with persilylated 6-chloropurine under Vorbruggen conditions using TMSOTf yielded the same β-anomer **12** as a single isomer, through the neighboring group effect, as expected (Scheme 3). Interestingly, compound **11a** was rather unreactive toward condensation with 6-chloropurine, when compared with compound **11b**. In case of **11b**, condensation reaction was completed in 4 h at room temperature, whereas compound **11a** required higher temperature (50 °C). Treatment of **12** with sodium methoxide gave the desired product **13a** and small amounts of 6-methoxy compound **13b**. The stereochemistry of compounds **12** was established by ^{1}H NOE experiments. Irradiation on anomeric proton in **12** gave a NOE (1.26%) on the 4′-proton, indicating the β-anomer.



Scheme 3. Reagents and conditions: (a) i. 6-chloropurine, $(NH_4)_2SO_4$, HMDS, 160 °C, overnight; ii. TMSOTf, $ClCH_2CH_2Cl$; (b) NaOMe, MeOH, 1 h.

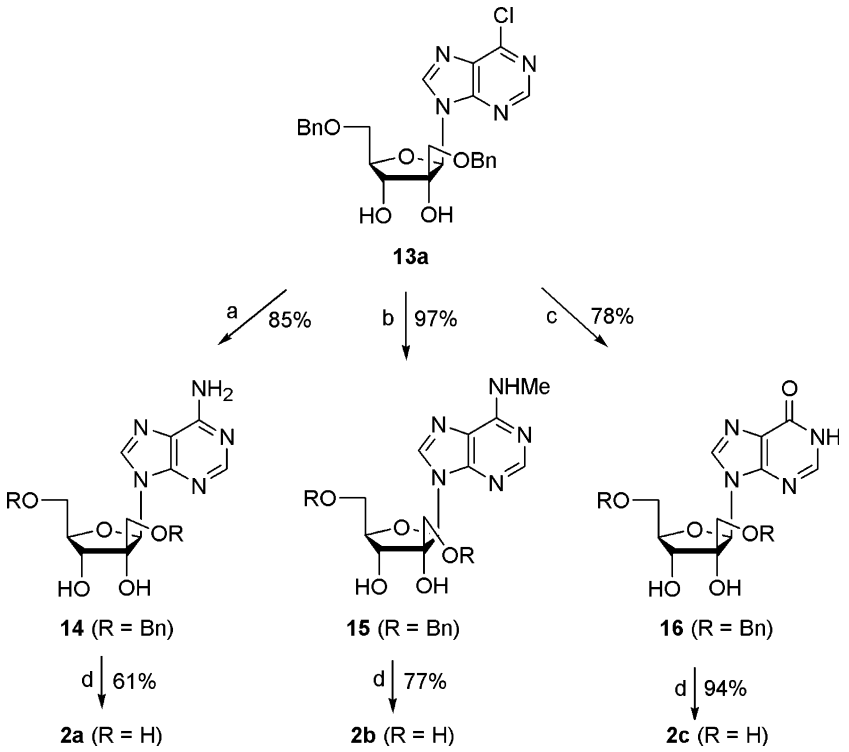
The final nucleosides **2a**–**c** were synthesized from the key intermediate **13a** (Scheme 4).

A solution of **13a** in methanolic ammonia was heated at 80 °C in a glass bomb for 1 d to give the adenine derivative **14** in 85% yield. Compound **13a** was converted to N^6-methyladenine derivatives **15** (**97**%) by heating with 40% aqueous methylamine in methanol. Compound **13a** was also converted to the hypoxanthine analogue **16** by treatment with 2-mercaptoethanol and sodium methoxide in methanol under reflux in 78% yield. It is known that compound **13a** was generally converted to **16** via the unstable intermediate **17a**, but thioketal **17b** was also formed as a stable minor adduct, as shown in Scheme 5.

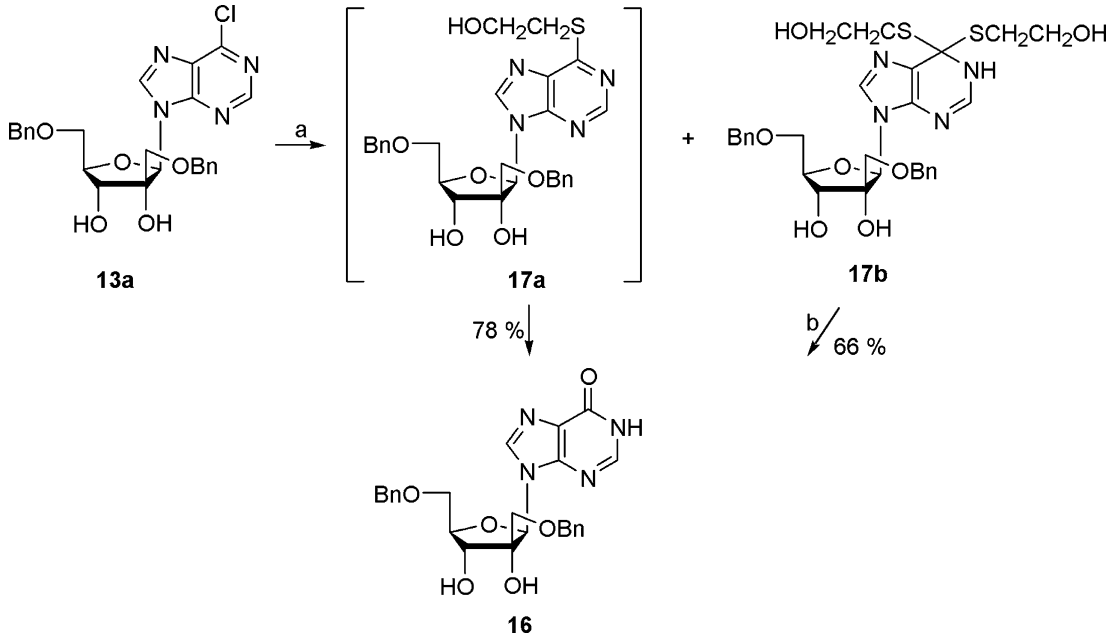
The thioketal **17b** was desulfurized to give the same compound **16** by treating with mercuric chloride and potassium acetate in acetic acid.[14] Treatment of compounds **14**–**16** with palladium black in 50% formic acid in methanol afforded the final nucleosides **2a**–**c**,[15] respectively.

All synthesized compounds were tested for anti-HCV activity using an in vitro assay system that is suitable for monitoring anti-HCV activities of compounds. This system is composed of a human hepatocarcinoma cell line (Huh-7) supporting multiplication of a HCV replicon named NK-R2AN. This replicon consists of the 5′ nontranslated region (5′NTR) and 3′NTR, which are required for replication of viral RNA, a selection marker gene neomycin phosphotransferase II and a reporter Renilla luciferase under the control of the HCV internal ribosomal entry site (IRES), and the HCV sequence encoding nonstructural proteins (NS3, 4a, 4b, 5a, and 5b) under the control of the encephalomyocarditis virus (EMCV) IRES. Antiviral activities of compounds are reflected by the reduction of *Renilla* luciferase activity in the Huh-7 cells. Among compounds tested, compound **2a** exhibited potent anti-HCV activity. Compound **2a** inhibited the replication of the replicon NK-R2AN in Huh-7 cells by 50% at 10 μM concentration. The replication of the replicon was inhibited by 2% at 1 μM concentration in the same cell line. However, other nucleosides did not show any significant antiviral activity.

In summary, on the basis of potent anti-HCV activity of 2′-*C*-methyladenosine (**1**), we have accomplished the stereoselective synthesis of β-2′-*C*-hydroxymethyladeno-



Scheme 4. Reagents and conditions: (a) NH_3/MeOH, 80 °C, 1 d; (b) 40% CH_3NH_2 in H_2O, MeOH, 80 °C, 1 h; (c) 2-mercaptoethanol, NaOMe, MeOH, 20 h; (d) Pd/black, 50% HCOOH in MeOH, 50 °C, 5 h.



Scheme 5. Reagents and conditions: (a) 2-mercaptoethanol, NaOMe, MeOH; (b) $HgCl_2$, AcOH, KOAc, reflux, overnight.

sine derivatives from D-ribose, utilizing a neighboring group effect. During the glycosylation, the electronic effect by acetyl group proved to be more dominant factor than the steric effect by CH_2OBn or isopropylidene group and CH_2OBn side chain had more impact than isopropylidene group in view of steric effect. Among compounds synthesized, adenosine derivative **2a** exhibited potent anti-HCV activity, indicating that the hydroxyl group of 2′-*C*-hydroxymethyl side chain might have favorable interaction with HCV polymerase.

Acknowledgment

This research was supported by a grant from Korea Research Foundation (KRF-2005-005-J01502).

References and notes

1. Szabo, E.; Lotz, G.; Paska, C.; Kiss, A.; Schaff, Z. *Pathol. Oncol. Res.* **2003**, *9*, 215.
2. Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.
3. Hoofnagle, J. H. *Hepatology* **1997**, *26*, 15S.
4. Lake-Bakaar, G. *Curr. Drug Targets Infect. Disord.* **2003**, *3*, 247.
5. Rosenberg, S. *J. Mol. Biol.* **2000**, *74*, 10563.
6. Takamizawa, A.; Mori, C.; Fuke, I.; Manabe, S.; Murakami, S.; Fujita, J.; Onishi, E.; Andoh, T.; Yoshida, I.; Okayama, H. *J. Virol.* **1991**, *65*, 1105.
7. Behrens, S.-E.; Tomei, L.; De Francesco, R. *EMBO J.* **1996**, *15*, 12.
8. Gordon, C. P.; Keller, P. A. *J. Med. Chem.* **2005**, *48*, 1, and references cited therein.
9. Eldrup, A. B.; Allerson, C. R.; Bennett, C. F.; Bera, S.; Bhat, B.; Bhat, N.; Bosserman, M. R.; Brooks, J.; Burlein, C.; Carroll, S. S.; Cook, P. D.; Getty, K. L.; MacCoss, M.; McMasters, D. R.; Olsen, D. B.; Prakash, T. P.; Prhavc, M.; Song, Q.; Tomassini, J. E.; Xia, J. *J. Med. Chem.* **2004**, *47*, 2283.
10. (a) Sommadossi, J.-P. PCT Patent Appl. WO 2004002422 A2, **2004**; (b) Sommadossi, J.-P. PCT Patent Appl. WO 2004003000 A2, **2004**.
11. Migliaccio, G.; Tomassini, J. E.; Carroll, S. S.; Tomei, L.; Altamura, S.; Bhat, B.; Bartholomew, L.; Bosserman, M. R.; Ceccacci, A.; Colwell, L. F.; Cortese, R.; De Francesco, R.; Eldrup, A. B.; Getty, K. L.; Hou, X. S.; LaFemina, R. L.; Ludmerer, S. W.; MacCoss, M.; McMasters, D. R.; Stahlhut, M. W.; Olsen, D. B.; Hazuda, D. J.; Flores, O. A. *J. Biol. Chem.* **2003**, *278*, 49164.
12. Ho, P.-T. *Tetrahedron Lett.* **1978**, *19*, 1623.
13. David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.
14. To a solution of compound **13a** (147 mg, 0.30 mmol) in methanol (10 mL) were added 2-mercaptoethanol (0.116 mL, 16.5 mmol), and NaOMe (89.1 mg, 16.5 mmol) and the reaction mixture was heated at reflux for 20 h. After cooling, the reaction mixture was neutralized with glacial acetic acid and evaporated. The resulting residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH 100:1 → 30:1) to give **16** (111 mg, 78%) as a colorless solid with minor formation of thioketal **17b** (40.9 mg, 22%) as a white solid. Compound **16**: UV (MeOH) λ_{max} 250.5 nm; $[\alpha]_D^{25}$ +3.90° (*c* 3.84, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 3.17 (d, 1H, *J* = 10.0 Hz, 2′-hydroxymethyl CH_a), 3.39 (d, 1H, *J* = 10.0 Hz, 2′-hydroxymethyl CH_b), 3.79 (dd, 1H, *J* = 11.2 Hz and 3.2 Hz, 5′-CH_a), 3.90 (dd, *J* = 11.2 Hz and 2.0 Hz, 5′-CH_b), 3.98 (s, 2H, benzylic CH_2), 4.13–4.16 (m, 1H, 4′-H), 4.59 (d, 1H, *J* = 9.6 Hz, 3′-H), 4.61 (s, 2H, benzylic CH_2), 6.08 (s, 1H, anomeric H), 6.81–7.34 (m, 10H, 2× Ph), 7.78 (s, 1H, H-2), 8.32 (s, 1H, H-8); ^{13}C NMR (CD_3OD, 100 MHz) δ 69.51, 69.86, 70.84, 74.64, 74.79, 81.60, 82.81, 91.99, 125.34, 128.85, 128.95, 129.03, 129.12, 129.32, 129.69, 138.45, 139.46, 146.45, 159.08. **Compound 17b**: UV (MeOH) λ_{max} 290.0 nm (sh); $[\alpha]_D^{25}$ +9.74° (*c* 0.72, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 2.84 (t, 2H, *J* = 6.4 Hz, –SCH_2–), 3.21 (d, 1H, *J* = 10.4 Hz, 2′-hydroxymethyl CH_a), 3.42 (d, 1H, *J* = 10.4 Hz, 2′-hydroxymethyl CH_b), 3.56 (td, 2H, *J* = 6.4 Hz and 1.6 Hz, –SCH_2–), 3.79 (t, 2H, *J* = 6.8 Hz, $HOCH_2CH_2S$–), 3.83–3.89 (m, 4H, benzylic CH_a, $HOCH_2CH_2S$– and 5′-CH_a), 3.95 (d, 1H, *J* = 12.0 Hz, benzylic CH_b) 3.96 (dd, 1H, *J* = 10.8 Hz and 1.6 Hz, 5′-CH_b), 4.19–4.23 (m, 1H, 4′-H), 4.66 (s, 2H, benzylic CH_2), 4.69 (d, 1H, *J* = 9.2 Hz, 3′-H), 6.21 (s, 1H, anomeric H), 6.67–7.38 (m, 10H, 2× Ph), 8.53 (s, 1H, H-2), 8.61 (s, 1H, H-8); ^{13}C NMR (CD_3OD, 100 MHz) δ 32.07, 42.31, 61.46,62.34, 69.50, 69.66, 70.57, 74.64, 74.70, 81.51, 82.86, 91.92, 128.77, 128.87, 128.97, 129.00, 129.30, 129.70, 132.19, 138.26, 139.44, 144.64, 149.41, 152.84, 161.74; To a solution of **17b** (40.0 mg, 0.065 mmol) in glacial acetic acid (2.5 mL) was added quickly a warm solution of mercuric chloride (21.6 mg, 0.08 mmol) in AcOH (1.3 mL) followed by potassium acetate in acetic acid (1.4 mL). After heating overnight, the hot solution was filtered and the precipitate was washed with diethyl ether. The reaction mixture was evaporated under reduced pressure and the residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 100:1 → 30:1) to give **16** (20.5 mg, 66%) as a colorless solid.
15. Compound **2a**: a white solid; mp 201.8–203.3 °C; MS (FAB) *m*/*z* 298.0 ($M+H^+$); UV (MeOH) λ_{max} 259.5 nm; $[\alpha]_D^{25}$ −23.81° (*c* 0.67, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 3.24 (d, 1H, *J* = 11.6 Hz, 2′-hydroxymethyl CH_a), 3.47 (d, 1H, *J* = 11.6 Hz, 2′-hydroxymethyl CH_b), 3.90 (dd, 1H, *J* = 12.4 Hz and 2.8 Hz, 5′-CH_a), 4.05 (dd, 1H, *J* = 12.4 Hz and 2.0 Hz, 5′-CH_b), 4.09–4.12 (m, 1H, 4′-H), 4.51 (d, 1H, *J* = 9.2 Hz, 3′-H), 6.15 (s, 1H, 1′-H), 8.18 (s, 1H, H-2), 8.46 (s, 1H, H-8); ^{13}C NMR (CD_3OD, 100 MHz) δ 61.35, 63.63, 69.94, 82.20, 84.40, 92.74, 120.29, 142.38, 150.35, 153.45, 157.31. Anal. Calcd for $C_{11}H_{15}N_5O_5$: C, 44.44; H, 5.09; N, 23.56. Found: C, 44.69; H, 5.48; N, 23.74. Compound **2b**: a white solid; mp 147.0–164.5 °C; MS (FAB) *m*/*z* 312.2 (M^++1); UV (MeOH) λ_{max} 265.0 nm; $[\alpha]_D^{25}$ −28.18° (*c* 2.02, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 3.07 (br s, 3H, N-CH_3), 3.17 (d, 1H, *J* = 12.0 Hz, 2′-hydroxymethyl CH_a), 3.42 (d, 1H, *J* = 12.0 Hz, 2′-hydroxymethyl CH_b), 3.85 (dd, 1H, *J* = 12.8 Hz and 3.6 Hz, 5′-CH_a), 4.00 (dd, 1H, *J* = 12.8 Hz and 1.6 Hz, 5′-CH_b), 4.04–4.08 (m, 1H, 4′-H), 4.46 (d, 1H, *J* = 8.8 Hz, 3′-H), 6.09 (s, 1H, 1′-H), 8.19 (s, 1H, H-2), 8.36 (s, 1H, H-8); ^{13}C NMR (CD_3OD, 100 MHz) 49.01, 61.37, 63.73, 70.02, 82.20, 84.42, 92.82, 120.51, 141.72, 150.55, 153.72, 157.56. Anal. Calcd for $C_{12}H_{17}N_5O_5$: C, 46.30; H, 5.50; N, 22.50. Found: C, 46.07; H, 5.74; N, 22.20. Compound **2c**: a white solid; mp 173.5–199.7 °C; MS (FAB) *m*/*z* 298.8 ($M+H^+$); UV (MeOH) λ_{max} 249.5 nm; $[\alpha]_D^{25}$ −18.79° (*c* 2.29, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 3.25 (d, 1H, *J* = 11.6 Hz, 2′-hydroxymethyl CH_a), 3.44 (d, 1H, *J* = 11.6 Hz, 2′-hydroxymethyl CH_b), 3.84 (dd, 1H, *J* = 12.4 Hz and 3.2 Hz,5′-CH_a), 3.98 (dd, 1H, *J* = 12.4 Hz and 2.4 Hz, 5′-CH_b), 4.02–4.06 (m, 1H, 4′-H), 4.45 (d, 1H, *J* = 9.2 Hz, 3′-H), 6.12 (s, 1H, 1′-H), 8.00 (s, 1H, H-2), 8.42 (s, 1H, H-8); ^{13}C NMR (CD_3OD, 100 MHz) δ 61.30, 63.48, 69.84, 82.11, 84.37, 92.31, 125.36, 141.56, 146.81, 149.89, 159.22. Anal. Calcd for $C_{11}H_{14}N_4O_6$: C, 44.30; H, 4.73; N, 18.79. Found: C, 44.14; H, 5.13; N, 18.72.