Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 4455–4458

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Evaluation of Optically Pure Dioxolanes as Inhibitors of Hepatitis C Virus RNA Replication

Sanjib Bera,[a] Leila Malik,[a] Balkrishen Bhat,[a] Steven S. Carroll,[b] Malcolm MacCoss,[c] David B. Olsen,[b] Joanne E. Tomassini[b] and Anne B. Eldrup[a,*]

[a]*Department of Medicinal Chemistry, Isis Pharmaceuticals, Carlsbad, CA 92008, USA*
[b]*Department of Biological Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[c]*Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*

Received 19 June 2003; revised 3 September 2003; accepted 4 September 2003

Abstract—A series of optically pure 1,3-dioxolane nucleoside mimics was synthesized by a synthetic route that allowed incorporation of a 5*R*-methyl substituent from commercially available starting materials. The pyrrolo[2,3-*d*]pyrimidine heterocycle was chosen as a substitute for the purine derivative. Coupling of the pyrrolo[2,3-*d*]pyrimidine and the dioxolane was performed under solid–liquid phase transfer conditions. The ability to inhibit HCV RNA replication was assessed in a cell based subgenomic replicon assay. None of the described compounds displayed significant anti-HCV activity.

Hepatitis C virus (HCV) is the pathogen associated with the majority of chronic hepatitis infections world wide and is a major cause of liver disease and transplantations. The currently recommended antiviral therapy consists of interferon alpha and ribavirin, a treatment that leads to only moderate sustained response rates. HCV encodes a series of viral proteins: the NS2/3 autoprotease, the NS3 serine protease and NTPase/helicase, and NS5B, the RNA dependent RNA polymerase (RdRp).[1] The polymerase is essential to viral replication and proliferation, and hence represents a valid drug discovery target. Non-nucleoside inhibitors (NNIs) and nucleoside inhibitors (NIs) of virally encoded polymerases have been validated for other targets, for example, HIV. While a range of NNI inhibitors of HCV RNA replication have been reported,[2–5] few NIs have been identified.[6–10]

Some of the NS5B NIs described have encompassed alteration of the 2′ position, either in the form of a 2′-*O*-methyl[6] or a 2′-*C*-methyl modification[6–8] (Fig. 1). Other reports[9,10] have indicated dioxolane based nucleoside triphosphates to be inhibitors of HCV RdRp mediated RNA synthesis. Hence, it remains unclear if the presence of a 2′-OH/2′-*O*-methyl is strictly necessary for activity in a cell based assay.

Nucleosides where the 3′ carbon has been replaced by a heteroatom such as oxygen or sulfur, have been demonstrated to be effective in cancer and viral chemotherapy.[11–18] For example, (−)-L-β-1,3-oxathionyl cytosine (3TC, Lamivudine) **4** is an effective anti-HIV and anti-HBV agent while (−)-L-β-1,3-dioxanyl cytosine **5** (O-ddC) has demonstrated anti-tumor,[19] anti-HIV and anti-HBV activity[15] (Fig. 2). In the purine series, 1,3-dioxanylguanine **6**[17,20] has demonstrated significant anti-HIV activity. Furthermore, dideoxynucleosides containing a 4-aminopyrrolo[2,3-*d*]pyrimidine moiety have been demonstrated to display increased chemical and enzymatic stability[21] relative to the corresponding purine derivatives. Based on the above findings, we decided to

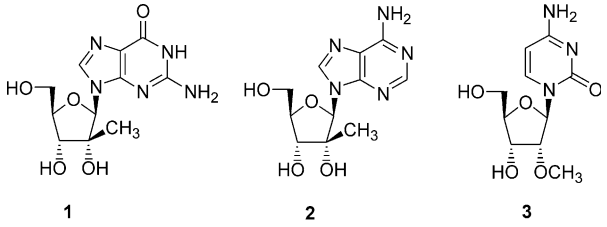


Figure 1. Examples of known HCV polymerase inhibitors, modified at the C2 or O2 position.

*Corresponding author. Tel.: +1-760-603-3852; fax: +1-760-603-4654; e-mail: aeldrup@isisph.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.09.008

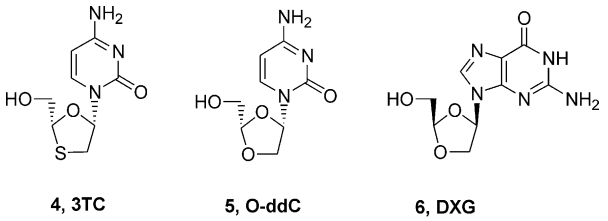


Figure 2. Examples of dioxolane and oxathiolane based antiviral and anti-neoplastic nucleosides.

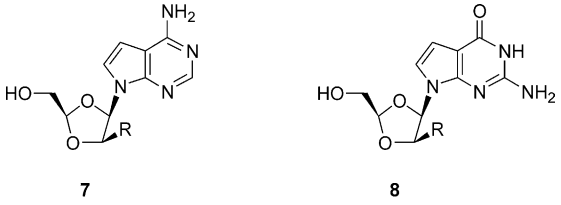
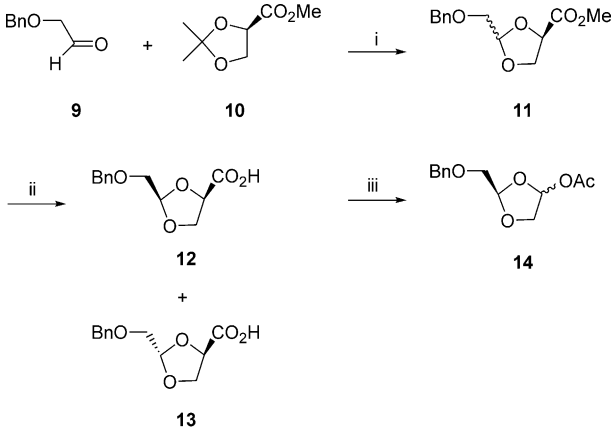


Figure 3. Target compounds. R can be H or methyl.

synthesize and evaluate the dioxolane nucleosides of general structure **7** and **8** (Fig. 3).

Chemistry

The derivatives **7** were synthesized via transketalization of the known 2-benzyloxy acetaldehyde **9** and commercially available methyl-(*R*)-2,2-dimethyl-1,3-dioxalane-4-carboxylate **10** in the presence of *p*-TSA to give the 1,3-dioxolane derivative **11** as an inseparable 1:1 mixture of diastereomers. Saponification of the methyl esters **11** with LiOH in THF, followed by acidification with aqueous H_2SO_4, gave a mixture of dioxolane carboxylic acids **12** and **13,** which were separated by silica gel column chromatography (dichloromethane/acetic acid, 98:2). The stereochemistry of the carboxylic acid derivatives **12** was confirmed by NOE. Oxidative decarboxylation of the *cis* carboxylic acid derivative **12** with $Pb(OAc)_4$ in acetonitrile in the presence of pyridine gave the key acetate intermediate **14**, as a diastereoisomeric mixture (Scheme 1).

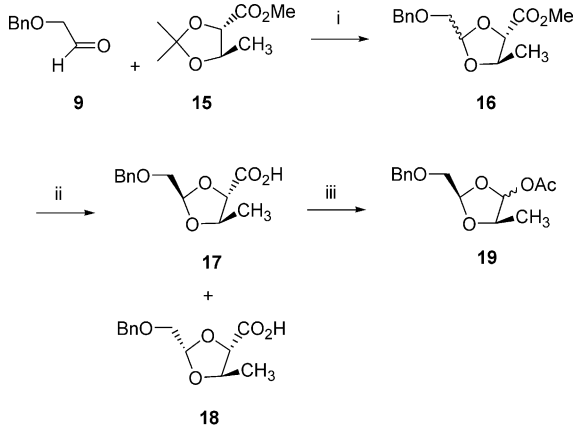


Scheme 1. Reagents and conditions: (i) *p*-TsOH, toluene, 80 °C, 1 h; (ii) (a) 1 M aqueous LiOH/THF (1:1), (b) 1 N HCl or H_2SO_4 (pH 2–3); (iii) $Pb(OAc)_4$, acetonitrile, pyridine.

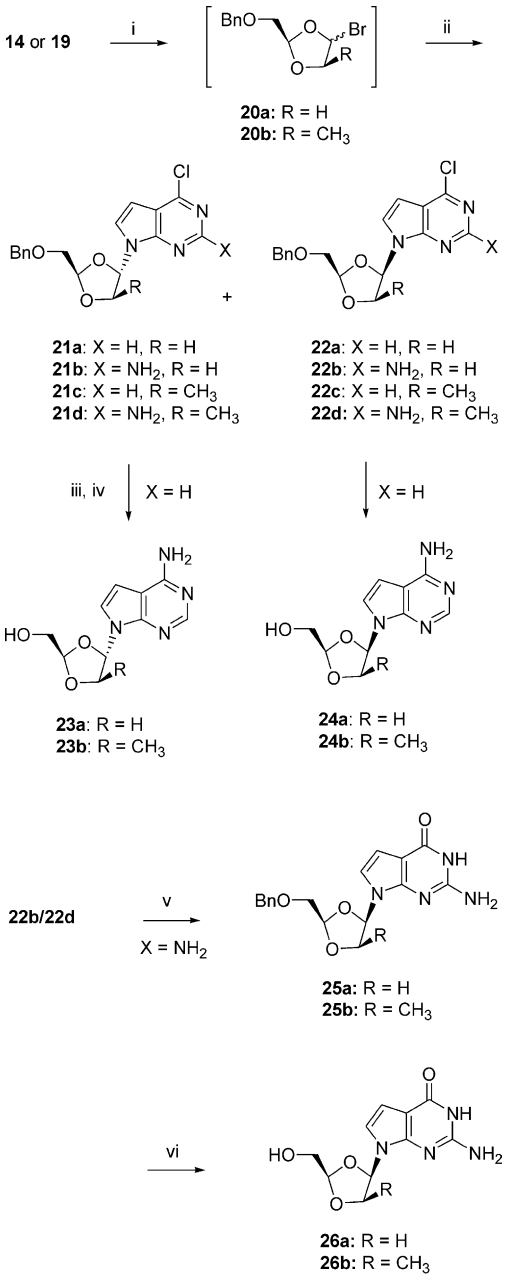
For the synthesis of the 5-methyl-1,3-dioxolane nucleoside analogues, the methyl (4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylate **15** was used as the starting material. Treatment of **15** with aldehyde **9** resulted in a 2*R*,*S*-diastereoisomeric mixture of the carboxy methyl-ester derivatives **16** in favor of the 2*R*-isomer. Saponification of the methyl ester with LiOH, followed by acidification, afforded the carboxylic acid derivatives **17** and **18**, which were easily separable by silica gel column chromatography. The structure of the major isomer **17** was determined by NOE. Treatment of **17** with $Pb(OAc)_4$ and pyridine in acetonitrile gave an anomeric mixture of the acetates **19** (Scheme 2).

Attempted Vorbrüggen coupling[22] of the acetate **14** with in situ silylated 4-chloropyrrolo[2,3-*d*]pyrimidine in the presence of trimethylsilyl trifluoromethane-sulfonate was ineffective and resulted in poor yield. However, in a more general approach, in situ formation of the dioxolanyl bromide **20a** was accomplished from the acetate **14** using bromotrimethylsilane (TMSBr). The thus formed bromide was reacted with 4-chloropyrrolo[2,3-*d*]pyrimidine under solid–liquid phase transfer conditions, in the presence of tris-[2-(2-methoxyethoxy)ethyl]amine (TDA-1) and KOH[21,23] in acetonitrile to afford a separable mixture (2:3) of α- and β-nucleoside derivatives **21a** and **22a** in 38% overall yield.[24] Under similar conditions, coupling of the 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine derivative gave a mixture of the nucleosides **21b** and **22b** as a 1:1.4 mixture in 39% overall yield (Scheme 3). Amination of the nucleoside derivatives **21a** and **22a** separately, with saturated methanolic ammonia, was followed by deprotection of the benzyl group with Pd/C and ammonium formate in refluxing methanol to furnish the desired nucleosides **23a** and **24a**.[25]

For the synthesis of the 5-methyl dioxolane derivatives, acetate **19** was treated with TMSBr in dichloromethane to give the bromo derivative **20b**, which on condensation with 4-chloropyrrolo[2,3-*d*]pyrimidine in the presence of TDA-1 and KOH in acetonitrile afforded a mixture of **21c** and **22c** (1:1.5) in 32% overall yield. Treatment of **21c** and **22c** separately with methanolic ammonia followed by hydrogenolysis with $Pd(OH)_2$ on



Scheme 2. Reagents and conditions: (i) *p*-TsOH, toluene, 80 °C, 1 h; (ii) (a) 1 M aqueous LiOH/THF (1:1), (b) 1 N HCl or H_2SO_4 (pH 2–3); (iii) $Pb(OAc)_4$, acetonitrile, pyridine.



Scheme 3. Reagents and conditions: (i) TMSBr, dichloromethane, 0 °C to room temperature; (ii) 4-chloropyrrolo[2,3-*d*]pyrimidine or 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine, TDA-1, KOH, acetonitrile; (iii) methanolic ammonia, 80 °C; (iv) ammonium formate, methanol, reflux; (v) 2 M NaOH/dioxane (1:1), 2-mercaptoethanol, 100 °C; (vi) $Pd(OH)_2$, cyclohexene/ethanol (1:2), 80 °C, 2 h (**26a**) or Pd/C, ammonium formate, methanol, reflux (**26b**).

carbon in methanol gave the target nucleosides **23b** and **24b**.[26] Similarly, coupling of 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine gave a mixture of **21d** and **22d**. Treatment of **22b** with mercaptoethanol in the presence of NaOH gave the 2-aminopyrrolo[2,3-*d*]pyrimidin-4-one derivative **25a**, which on reduction with $Pd(OH)_2$ in ethanol/cyclohexene (2:1) afforded the target dioxolane nucleoside **26a**.[27] Similar treatment of **22d** with mercaptoethanol in the presence of NaOH, followed by transfer hydrogenation in the presence of Pd/C and ammonium formate in methanol, gave **26b**.

Biological Evaluation

Compounds **23**, **24**, **26** were evaluated as inhibitors of HCV replication in a cell-based, subgenomic replicon assay as previously described.[6] None of the described compounds displayed significant inhibition of HCV RNA replication ($EC_{50} > 50$ μM). Based on previous art, these compounds are likely inhibitors of HBV polymerase and HIV reverse transcriptase. Work is in progress to evaluate anti-HBV/anti-HIV activity.

Results and Discussion

Synthetic routes to a series of pyrrolo[2,3-*d*]pyrimidin-7-yl)-1,3-dioxolanes were devised using phase transfer conditions to effect coupling of either (2*R*)-2-benzyloxymethyl-4-acetoxy-1,3-dioxolane or (2*R*)-2-benzyloxymethyl-4-acetoxy-(5*R*)-methyl-1,3-dioxolane to the appropriate 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine. Compounds were evaluated as inhibitors of HCV RNA replication in a cell based assay and found to be inactive. In principle, the inactivity of these compounds in the subgenomic replicon assay could reflect lack of uptake and/or metabolism to the corresponding triphosphate. However, compounds of similar structure are known inhibitors of viral and human polymerases indicating a level of uptake and metabolism sufficient to elicit activity. Hence it appears more likely that the basis for the observed inactivity is inability to elicit recognition from the HCV RdRp. If so, this would point to the 2′-hydroxy or 2′-methoxy as essential pharmacophores for recognition by the HCV RdRp. Given the fact that HCV polymerase utilizes ribonucleoside triphosphates as substrates for the incorporation of ribonucleoside monophosphates against an RNA template, recognition of the 2′ position is likely to be highly refined. Work is in progress to determine if the corresponding triphosphates are substrates and/or inhibitors of the HCV RdRp.

References and Notes

1. De Francesco, R.; Tomei, L.; Altamura, S.; Summa, V.; Migliaccio, G. *Antiviral Res.* **2003**, *58*, 1.
2. Chan, L.; Reddy, J.; Proulx, M.; Das, S.; Pereira, O.; Wang, W.; Siddiqui, A.; Yannopoloulos, C. G.; Poisson, C.; Turcotte, N.; Drouin, A.; Hicham Alaoui-Ismaili, M. H.; L'Heureux, L.; Bilimoria, D.; Nguyen-Ba, N. *J. Med. Chem.* **2003**, *46*, 1283.
3. Wang, M.; Ng, K. K.-S.; Cherney, M. M.; Chan, L.; Yannopoulos, J. B.; Morin, N.; Nguyen-Ba, N.; Alaoui-Ismaili, M. H.; Bethell, R. C.; James, M. N. G. *J. Biol. Chem.* **2003**, *278*, 11 9489.
4. Dhanak, D.; Duffy, K. J.; Johnston, V. K.; Lin-Goerke, J.; Darcy, M.; Shaw, A. N.; Gu, B.; Silverman, C.; Gates, A. T.; Nonnemacher, M. R.; Earnshaw, D. L.; Casper, D. J.; Kaura, A.; Baker, A.; Greenwood, C.; Gutshall, L. L.; Maley, D.; DelVecchio, A.; Macarron, R.; Hofmann, G. A.; Alnoah, Z.; Cheng, H. Y.; Chan, G.; Khandekar, S.; Keenan, R. M.; Sarisky, R. T. *J. Biol. Chem.* **2002**, *277*, 38322.
5. Gu, B.; Johnston, V. K.; Gutshall, L. L.; Ngeyun, T. T.; Gontarek, R. R.; Darcy, M. G.; Tedesco, R.; Dhanak, D.;

Duffy, K. J.; Kao, C. C.; Sarisky, R. T. *J. Biol. Chem.* **2003**, *278*, 16602.
6. Carroll, S. S.; Tomassini, J.; Bosserman, M.; Krista, K.; Stahlhut, M. W.; Eldrup, A. B.; Bhat, B.; Hall, D.; Simcoe, A. L.; LaFemina, R.; Rutkowski, C. A.; Shim, S.; Wolanski, B.; Yang, Z.; Migliaccio, G.; De Francesco, R.; Kuo, L. C.; MacCoss, M.; Olsen, D. B. *J. Biol. Chem.* **2003**, *278*, 11979.
7. Migliaccio, G.; Tomassini, J.; Carroll, S. S.; Tomei, L.; Altamura, S.; Bhat, B.; Bartholomew, L.; Bosserman, M.; Ceccacci, A.; Colwell, L. F.; Cortese, R.; De Francesco R.; Eldrup, A. B.; Getty, K.; Hou, X. S.; LaFemina, R.; Ludmerer, S.; MacCoss, M.; McMasters, D.; Stahlhut, M. W.; Olsen, D. B.; Hazuda, D.; Flores, O. *J. Biol. Chem.*, in press.
8. Sommadossi, J. P.; Lacolla, P., Int. Patent Appl. (2001) WO 01/190121.
9. Ismaili, H. M. A.; Moulay, A.; Cheng, Y.-X.; Lavallee, J.-F.; Siddiqi, A.; Storer, R. Int. Patent Appl. WO 01/60315, 2002.
10. Storer, R. US Patent 6,566,365, 2003.
11. Belleau, B.; Dixit, D.; Nguyen-Ba, N.; Kraus, J. L. *Abstr. TOC1*, Abst. 5th International Conference on AIDS, Montreal, Quebec, Canada, 1989; p 515.
12. Soudeyns, H.; Yao, X. J.; Gao, Q.; Belleau, B.; Kraus, J. L.; Nguyen-Ba, N.; Spira, B.; Wainberg, M. A. *Antimicrob. Agents Chemother.* **1991**, *35*, 1386.
13. Norbeck, D. W.; Spanton, S.; Broder, S.; Mitsuya, H. *Tetrahedron Lett.* **1989**, *30*, 6263.
14. Chu, C. K.; Beach, J. W.; Jeong, L. S.; Choi, B. G.; Comer, F. I.; Alves, A. J.; Schinazi, R. F. *J. Org. Chem.* **1991**, *56*, 6503.
15. Kim, H. O.; Shanmuganathan, K.; Alves, A. J.; Jeong, L. S.; Beach, J. W.; Schinazi, R. F.; Chang, C. N.; Cheng, Y. C.; Chu, C. K. *Tetrahedron Lett.* **1992**, *33*, 6899.
16. Siddique, M. A.; Brown, W. L.; Nguyen-Ba, N.; Dixit, D. M.; Mansour, T. S.; Hooker, E.; Viner, K. C.; Cameron, J. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1543.
17. Belleas, B. R.; Evans, C. A.; Tae, H. L. A.; Jin, H.; Dixit, D. M.; Mansour, T. S. *Tetrahedron Lett.* **1992**, *33*, 6949.
18. Jeong, L. S.; Schinazi, R. F.; Beach, J. W.; Kim, H. O.; Shanmuganathan, K.; Nampalli, S.; Chun, M. W.; Chung, W. K.; Choi, B. G.; Chu, C. K. *J. Med. Chem.* **1993**, *36*, 2627.
19. Grove, K. L.; Guo, X.; Liu, S. H.; Gao, Z.; Chu, C. K.; Cheng, Y. C. *Cancer Res.* **1995**, *55*, 3008.
20. Kim, H. O.; Schinazi, R. F.; Satyanarayana, N.; Shanmuganathan, K.; Cannon, D. L.; Alves, A. J.; Jeong, L. S.; Beach, J. W.; Chang, C. N.; Chu, C. K. *J. Med. Chem.* **1993**, *36*, 30.
21. Seela, F.; Muth, H. P.; Bindig, U. *Synthesis* **1988**, 670.
22. Gunic, E.; Girardet, J. L.; Pietrzkowski, Z.; Esler, C.; Wang, G. *Bioorg. Med. Chem.* **2001**, *9*, 163.
23. Ramzaeva, N.; Mittelbach, C.; Seela, F. *Helv. Chim. Acta* **1999**, *82*, 12.
24. **General glycosidation procedure**: To a solution of acetate **14** or **19** (1 mmol) in dichloromethane (6 mL) was added TMSBr (2 mmol) dropwise at 0 °C. The solution was stirred at 0 °C for 15 min, then at room temperature for 30 min. The solvent was evaporated under reduced pressure, and co-evaporated with acetonitrile. The bromo derivative **20** was dissolved in acetonitrile (5 mL) and added to a vigorously stirred suspension of the appropriate 4-chloropyrrolo[2,3-*d*]pyrimidine (1 mmol), tris-[2-(2-methoxyethoxy)ethyl]amine (0.3 mmol) and powdered KOH (3 mmol) in acetonitrile (6 mL). After 30 min, the solution was poured into a mixture of diethylether (100 mL) and saturated aqueous $NaHCO_3$ (100 mL). The organic phase was separated, dried over Na_2SO_4, filtered, evaporated and subsequently purified over silica gel to give the various derivatives **21** (13–16%) and **22** (19–23%).
25. ^{1}H NMR (200 MHz, methanol-d_4) of **23a**: δ 8.09 (s, 1H), 7.27 (d, J = 3.8 Hz, 1H), 6.64 (m, 2H), 5.46 (t, J = 3.4 Hz), 4.44 (dd, J = 5.6, 9.2 Hz, 1H), 4.32 (dd, J = 3.2, 9.2 Hz, 1H), 3.61 (d, J = 3.2 Hz, 2H); ^{1}H NMR (MeOH-d_4) of **24a**: δ 8.08 (s, 1H), 7.40 (d, J = 3.6 Hz, 1H), 6.59 (m, 2H), 5.09 (t, J = 3.2 Hz), 4.36 (dd, J = 2, 9.6 Hz, 1H), 4.24 (dd, J = 5.6, 9.6 Hz, 1H), 3.73 (d, J = 3.2 Hz, 2H).
26. ^{1}H NMR (200 MHz, methanol-d_4) of **23b**: δ 8.27 (s, 1H), 7.65 (d, J = 3.8 Hz, 1H), 6.95 (d, J = 3.8 Hz, 1H), 6.12 (d, J = 5.4 Hz, 1H), 5.54 (t, J = 3.2 Hz, 1H), 4.58 (m, 1H), 3.67 (d, J = 3.2 Hz, 2H), 1.44 (d, J = 6.4 Hz, 3H); ^{1}H NMR (200 MHz, methanol-d_4) of **24b**: δ 8.26 (s, 1H), 7.92 (d, J = 3.7 Hz, 1H), 6.89 (d, J = 3.7 Hz, 1H), 6.64 (d, J = 4.8 Hz, 1H), 5.10 (t, J = 2.6 Hz, 1H), 4.36 (m, 1H), 3.73 (d, J = 2.6 Hz, 2H), 0.92 (d, J = 6.2 Hz, 3H).
27. ^{1}H NMR (D_2O + methanol-d_4) of **26a**: δ 6.82 (d, J = 3.8 Hz, 1H), 6.28 (d, J = 3.8 Hz, 1H), 6.20 (m, 1H), 4.94 (t, J = 3.1 Hz, 1H), 4.23 (dd, J = 2, 9.8 Hz, 1H), 4.07 (dd, J = 5.8, 9.8 Hz, 1H), 3.55 (d, J = 2.2 Hz, 2H); ^{1}H NMR (CD_3CN) of **26b**: δ 6.97 (d, J = 3.8 Hz, 1H), 6.34 (d, J = 3.8 Hz, 1H), 6.27 (d, J = 5.2 Hz, 1H), 5.16 (bs, 2H), 5.02 (t, J = 3.2 Hz, 1H), 4.35 (m, 1H), 3.79 (m, 2H), 0.97 (d, J = 6.2 Hz, 3H).