Synthesis of Pyrimidine 1′,3′-Anhydro-β-D-*psico*- and -*sorbo*-furanosyl Nucleosides

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Abstract: A novel approach for the synthesis of pyrimidine 1′,3′-anhydro-β-D-*psico*- and -*sorbo*-furanosyl nucleosides **2** and **3**, respectively, has been developed. The approach described here employs a readily available O^2,3′-anhydro-β-D-fructofuranosyluracil (**1**) as a key starting compound.

Key words: pyrimidine nucleosides, hexofuranosides, anhydro derivatives

Oligonucleotides containing rigid nucleosides display a number of exciting biological properties, which are of importance for possible biomedical applications.[1] The furanose ring of natural nucleosides is flexible and exists in an equilibrium of the N- and S-type conformations.[2] The introduction of a bridge between diverse carbon atoms of the pentofuranose moieties results in the fixation of the furanose ring in one of the possible conformations. Among such rigid nucleosides, the 1′,3′-anhydro nucleosides of D-psicofuranose have been recently independently synthesized by Chattopadhyaya and co-workers[3] and by us[4,5] employing a convergent approach.[6–8] In the present communication, we report on the synthesis of pyrimidine 1′,3′-anhydro-β-D-*psico*- and -*sorbo*-furanosyl nucleosides **2**–**4** (Figure 1) employing O^2,3′-anhydro-β-D-fructofuranosyluracil (**1**)[9,10] as a key starting compound.

2, B = U (uracil-1-yl)
3, B = C (cytosin-1-yl)
4

Figure 1

Treatment of the anhydro nucleoside **1** with (4-monomethoxy)trityl (MMTr) chloride in pyridine afforded, after silica gel column chromatography, mainly 1′,6′-di-*O*-MMTr (**5**; 42%) and 6′-*O*-MMTr (**6**; 22%) derivatives, along with the isomeric 4′,6′-di-*O*-MMTr nucleoside **7** (7%). Benzylation of the former followed by chromatographic separation gave the desired compound **8** (48%), its derivative **9** (25%), as well as two minor products, the epoxide **10** (6%) and fructoside **11** (10%). The formation of the epoxide **10** likely results from an intramolecular nucleophilic attack of a 4′-alkoxide at the 3′-carbon atom leading to the O^2,3′-anhydro ring-opening. The transient existence of similar pyrimidine *ribo*-epoxides under alkaline conditions has been previously postulated[11] and 2′,3′-anhydrouridine was recently synthesized from O^2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil on treatment with NaH in anhydrous DMSO.[12] It was shown that 2′,3′-anhydrouridine is readily converted back into O^2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil by heating or by treating it with Et_3N–MeOH at room temperature.[12] Note that standard detritylation of the epoxide **10** afforded the anhydro nucleoside **1** in high yield pointing to the 3′,4′-*psico*-epoxide structure of compound **10**. The formation of the fructosides **9** and **11** may be accounted for an acidic neutralization of the reaction mixture before chromatography (Scheme 1).

Cleavage of the anhydro ring of **8** was achieved under alkaline hydrolysis to afford the 1′,4′,6′-O-protected fructoside **9** in 88% yield. Its conventional mesylation followed by detritylation gave the fructoside **12** in 77% combined yield. Treatment of the latter with MeONa–MeOH under reflux resulted in the 1′,3′-anhydro ring-closure (**13**; 46%). Catalytic debenzylation of the psicoside **13** gave the desired 1-(1,3-anhydro-β-D-psicofuranosyl)uracil (**2**; 67%).

Compound **7** was alternatively transformed into the anhydro psicoside **2** in five steps: (i) cleavage of the O^2,3′-anhydro ring (0.5 M NaOH in a mixture of EtOH–dioxane–H_2O, 2:1:1; 95%); (ii) selective 1′-O-benzoylation (BzCl/anhyd pyridine; 0 °C, 16 h) followed by (iii) conventional mesylation (59%, combined); (iv) the 1′,3′-anhydro ring-closure on treatment with 0.5 M MeONa/MeOH under reflux (46%), and (v) detritylation (75%).

Uracil nucleoside **13** was transformed to 1-(1,3-anhydro-β-D-psicofuranosyl)cytosine (**3**; 68%)[13,3b] through intermediary formation of cytosine derivative **14**[14] by consecutive treatments with (i) Ac_2O/DMAP/pyridine, (ii) tris(triazolyl)-phosphate in MeCN, (iii) aqueous ammonia, (iv) NH_3/MeOH, and (v) H_2/Pd/C in MeOH.

Treatment of compound **5** with 0.5 M NaOH in dioxane–H_2O (1:1, v/v) gave the 1,6-di-*O*-MMTr *fructo*-nucleoside

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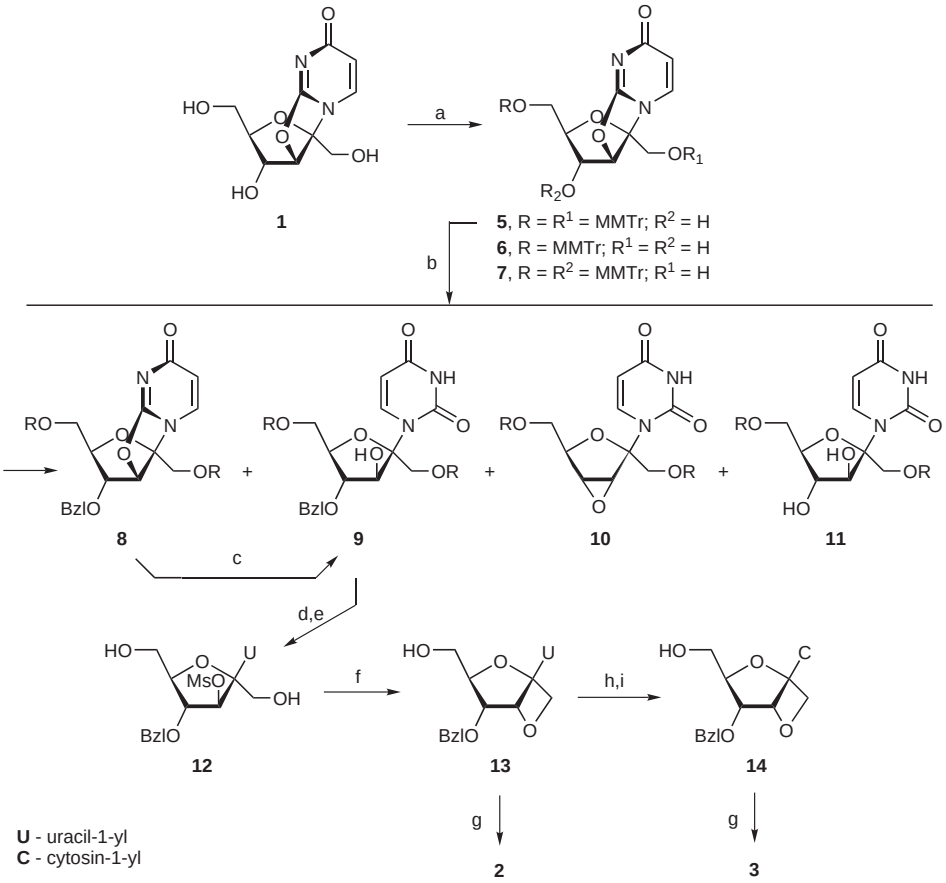
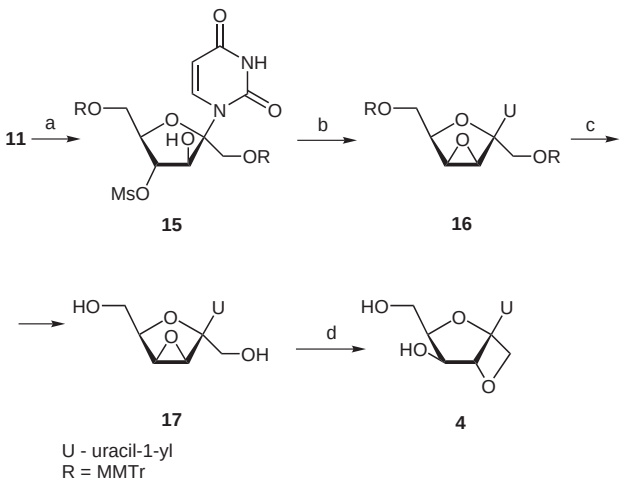


Scheme 1 *Reagents and conditions*: (a) (1) MMTrCl/anhyd pyridine, r.t., 20 h; (2) SiO_2, elution with a MeOH gradient (0–10%, v/v) in $CHCl_3$ **5** (42%), **6** (22%), **7** (7%); (b) (1) NaH/THF, 0–4 °C, 5 min; + BzlBr, 4 °C to 20 °C, 20 h; (2) SiO_2, elution with a MeOH gradient (0–1%, v/v) in $CHCl_3$: **8** (48%), **9** (25%), **10** (6%), **11** (10%); (c) 1 M NaOH/dioxane–H_2O, 1:1 (v/v), r.t., 1 h (88%); (d) MsCl/anhyd pyridine, 0 °C to 20 °C 20 h (87%); (e) 2% *p*-TsOH/$CHCl_3$–MeOH (7:3, v/v), r.t. 10 min (89%); (f) 1 M MeONa/MeOH, reflux, 5 h (46%); (g) H_2/5% Pd/C/MeOH r.t., 24 h: **2** (67%); **3** (62%); (h) Ac_2O/DMAP/pyridine, r.t., 30 min (96%); (i) (1) tris(triazolyl)phosphate/MeCN; (2) 25% aq ammonia/dioxane 1:5 (v/v), r.t., 16 h; (3) NH_3/MeOH, r.t., 6 h (70%).

(**11**) in 97% yield. Its careful mesylation in anhydrous pyridine gave the mesylate **15** (89%), treatment of which with MeONa/MeOH led to the 3′,4′-anhydro derivative **16** (80%). Detritylation of the latter gave 1-(3,4-anhydro-β-D-tagatofuranosyl)uracil (**17**; 84%). Finally, the tagatoside **17** was isomerized into 1-(1,3-anhydro-β-D-sorbofuranosyl)uracil (**4**) upon treatment either with NaH in anhydrous DMF at 75–80 °C for seven minutes [48%; uracil (20%) and the starting **17** (28%) have been also isolated] or with MeONa/MeOH at reflux for 40 minutes [61%; uracil: 14%; **17**: 20%; Scheme 2].

No formation of isomeric 1-(1,4-anhydro-β-D-fructofuranosyl)uracil was detected. In the case of a rather rigid furanose ring of the epoxide **17**, such a regioselectivity of an intramolecular attack of the O1′-oxygen at C3′-carbon atom maybe explained by a greater spatial proximity of these atoms vs. the O1′–C4′. Indeed, a geometry optimization of compound **17** using the HyperChem® program (Hypercube, Inc., 2002; release 7.1; AMBER force field) furnished the 2.9 Å and 3.3 Å distances between the O1′–C3′ and O1′–C4′ atoms, respectively (Figure 2).

The structure of the synthesized compounds was proved by ^{1}H NMR and ^{13}C NMR data. The assignment of all the ^{1}H and ^{13}C resonances was made by 2D [^{1}H,^{1}H] and [^{1}H,^{13}C] correlation spectra and in some cases by homo-decoupling experiments. The values of the H3′/H4′ and



Scheme 2 *Reagents and conditions*: (a) MsCl/anhyd pyridine, 0–20 °C, 16 h (89%); (b) 0.5 M MeONa/MeOH, r.t., 3 h (80%); (c) 60% aq CF_3COOH, 45–50 °C, 4 h (84%); (d) (1) NaH/anhyd DMF, 75–80 °C, 7 min (48%); (2) MeONa/MeOH, reflux, 40 min (61%).

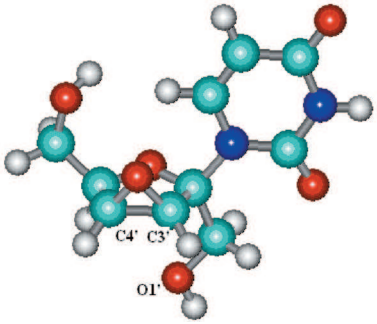


Figure 2 Computer-generated (molecular mechanics; AMBER) representation of 1-(3,4-anhydro-β-D-tagatofuranosyl)uracil (**17**).

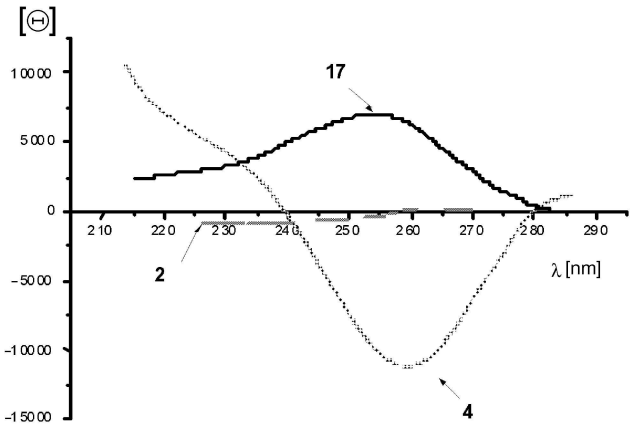


Figure 3 The CD spectra of 1-(1,3-anhydro-β-D-psicofuranosyl)-uracil (**2**, ···), its *sorbo*-isomer **4** (---), and 1-(3,4-anhydro-β-D-tagatofuranosyl)uracil (**17**, ——) in MeOH.

H4′/H5′ proton–proton coupling constants for the psicosides **2** and **3** are similar to those for the relevant thymine and cytosine nucleosides[3] as well as adenine[4] counterpart. Moreover, the aforementioned couplings are in a fair correspondence with the respective torsion angles of 45° and 168° deduced from the energy minimized structures of 1,3-anhydro-β-D-*psico* nucleosides.

By going from the psicoside **2**[15] to the sorboside **4**,[16] rather remarkable changes in the coupling constants and chemical shifts have been observed. Thus, the vicinal $J_{3',4'} < 0.5$ Hz and $J_{4',5'} = 3.35$ Hz couplings are again in a fair correspondence with the respective torsion angles of 85° and 38°, which are found in the energy-minimized structure of the sorboside **4**. As might be expected, an inversion of a hydroxyl group configuration at C4′ resulted in displacements of the H5′ resonance to a lower field ($\Delta\delta = -0.35$ ppm). The most striking observation consists in the considerable displacement to a lower field of the C3′ resonance ($\Delta\delta = -4.56$ ppm) on passing from the psicoside **2** to the sorboside **4**. The most probable explanation may be the anisotropic effect of the C^2=O carbonyl group of the aglycon on the C3′ atom resulting from the formation of an intramolecular hydrogen bond between the 4′-OH group and the oxygen of the C^2=O carbonyl. A downfield shift of the 4′-OH resonance by 0.39 ppm in the ^{1}H NMR spectrum of the sorboside **4** vs. the psicoside **2** agrees with the C4′-OH···O=C^2 hydrogen bond.

The NMR data for the psicoside **10**[17] correlate well with those published for 2′,3′-anhydrouridine;[12] the most characteristic are the high-field resonances at $\delta = 57.37$ ppm (C3′), 56.12 ppm (C4′).

The formation of the C4′-OH···O=C^2 hydrogen bond leads to a *syn*-conformation about the glycosyl bond. In this respect, the CD spectra of nucleosides **2** and **4** feature interesting changes in the Cotton effects (Figure 3). No clear CD maxima are observed in the spectrum of the psicoside **2**. On the contrary, the CD curve of the sorboside **4** exhibits a large negative CD band in the B_{2u} spectral region, and a positive B_{1u} and E_{1ua} CD bands. Thus, the sorboside **4** displays approximately mirror-image CD curve vs. uridine and closely related uracil nucleosides[18,19] and essentially identical CD spectrum with that of 6,1′-propanouridine,[20] which is the pure *syn*-conformer about the glycosyl bond. These data gave further support to the C4′-OH···O=C^2 hydrogen bond. It is noteworthy that the CD curve of the tagatoside **17** exhibits, like that of uridine, a positive B_{2u} CD band, whereas is anomalous in the B_{1u} and E_{1ua} spectral regions.

In conclusion, a novel approach for the synthesis of pyrimidine 1′,3′-anhydro-β-D-*psico*- and -*sorbo*-furanosyl nucleosides has been developed. The approach described here employs a readily available O^2,3′-anhydro-β-D-fructofuranosyluracil (**1**) as a key starting compound that may be transformed into a number of pyrimidine anhydro hexofuranosyl nucleosides.

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References

(1) (a) Jepsen, J. S.; Sorensen, M. D.; Wengel, J. *Oligonucleotides* **2004**, *14*, 130. (b) Petersen, M.; Wengel, J. *Trends Biotechnol.* **2003**, *21*, 74. (c) Imanishi, T.; Obika, S. *Chem. Commun.* **2002**, 1653. (d) Leumann, C. J. *Bioorg. Med. Chem.* **2002**, *10*, 841.

(2) Thibaudeau, C.; Chattopadhyaya, J. *Stereoelectronic Effects in Nucleosides and Nucleotides and their Structural Implications*; Uppsala University Press: Sweden, **1999**, jyoti@boc.uu.se; ISBN 91-506-1351-0.

(3) (a) Pradeepkumar, P. I.; Zamaratski, E.; Földesi, A.; Chattopadhyaya, J. *J. Chem. Soc., Perkin Trans. 2* **2001**, 402. (b) Pradeepkumar, P. I.; Amirkhanov, N. V.; Chattopadhyaya, J. *Org. Biomol. Chem.* **2003**, *1*, 81. (c) Pradeepkumar, P. I.; Cheruku, P.; Plashkevych, O.; Acharya, P.; Gohil, S.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **2004**, *126*, 11484.

(4) Roivainen, J.; Vepsäläinen, J.; Azhayev, A.; Mikhailopulo, I. A. *Tetrahedron Lett.* **2002**, *43*, 6553.

(5) Note that the structure of compound **16** in ref. 4 was erroneously described to 1-(1,3-anhydro-β-D-psicofuranosyl)thymine. Single-crystal X-ray analysis showed that compound **16** actually is methyl 1-deoxy-1-(N^1-thyminyl)-β-D-psicofuranoside: (Roivainen, J.; Reuter, H.;

Mikhailopulo, I. A., unpublished). The structures of the anhydro nucleosides **9**, **12**, and **13** have been proved by X-ray analyses (Roivainen, J. A.; Reuter, H.; Mikhailopulo, I. A., unpublished).

(6) (a) Hrebabecky, H.; Farkas, J.; Sorm, F. *Coll. Czech. Chem. Commun.* **1972**, *37*, 2059. (b) Hrebabecky, H.; Farkas, J.; Sorm, F. *Coll. Czech. Chem. Commun.* **1974**, *39*, 1098.

(7) Elliott, R. D.; Niwas, S.; Riordan, J. M.; Montgomery, J. A.; Secrist, J. A. III *Nucleosides Nucleotides* **1992**, *11*, 97.

(8) Sarma, M. S. P.; Megati, S.; Klein, R. S.; Otter, B. A. *Nucleosides Nucleotides* **1995**, *14*, 393.

(9) (a) Sanchez, R. A.; Orgel, L. E. *J. Mol. Biol.* **1970**, *47*, 531. (b) Shannahoff, D. H.; Sanchez, R. A. *J. Org. Chem.* **1973**, *38*, 593.

(10) Holy, A. *Nucleic Acids Res.* **1974**, *1*, 289.

(11) Codington, J. F.; Doerr, I. L.; Fox, J. J. *J. Org. Chem.* **1964**, *29*, 564; and references therein..

(12) Miah, A.; Reese, C. B.; Song, Q.; Sturdy, Z.; Neidle, S.; Simpson, I. J.; Read, M.; Rayner, E. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3277.

(13) The ^{1}H NMR and ^{13}C MNR data for the psicoside **3** are in agreement with those previously published.[3b]

(14) Divakar, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171.

(15) 1-(1,3-Anhydro-β-D-psicofuranosyl)uracil (**2**): amorphous powder; UV (MeOH): λ_{max} = 257 nm (ε 9500), λ_{min} = 227 (ε 2700) nm. ^{1}H NMR (500.13 MHz, DMSO-d_6): δ_{TMS} = 7.44 (d, 1 H, $J_{5,6}$ = 7.99 Hz, H6), 5.63 (d, 1 H, H5), 5.25 (d, 1 H, $J_{3',4'}$ = 3.91 Hz, H3′), 5.21 (br s, 1 H, 4′-OH), 5.00 (d, 1 H, $J_{1',1''}$ = 8.13 Hz, H1′), 4.82 (br t, 1 H, *J* = 4.10 Hz, 6′-OH), 4.57 (d, 1 H, H1′′), 4.07 (ddd, 1 H, $J_{4',5'}$ = 8.59 Hz, $J_{5',6'}$ = 2.0 Hz, $J_{5',6''}$ = 5.68 Hz, H5′), 4.03 (br m, 1 H, H4′), 3.75 (br d, 1 H, $J_{6',6''}$ = 12.9 Hz, H6′), 3.50 (br m, 1 H, H6′′) ppm. ^{13}C NMR (125.77 MHz, DMSO-d_6): δ_{TMS} = 163.22 (C4), 149.24 (C2), 141.60 (C6), 101.96 (C5), 90.85 (C2′), 86.84 (C3′), 83.30 (C5′), 77.83 (C1′), 69.65 (C4′), 60.57 (C6′) ppm. Anal. Calcd for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93. Found: C, 46.77; H, 4.63; N, 10.90.

(16) 1-(1,3-Anhydro-β-D-sorbofuranosyl)uracil (**4**): amorphous powder; UV (MeOH): λ_{max} = 256 nm (ε 9900), λ_{min} = 227 nm (ε 2800). ^{1}H NMR (500.13 MHz, (DMSO-d_6): δ_{TMS} = 11.39 (br s, 1 H, N^3H), 7.40 (d, 1 H, $J_{5,6}$ = 8.05 Hz, H6), 5.73 (d, 1 H, H5), 5.60 (d, 1 H, *J* = 4.42 Hz, 4′-OH), 5.17 (s, 1 H, $J_{3',4'}$ < 0.5 Hz, H3′), 5.04 (d, 1 H, $J_{1',1''}$ = 8.28 Hz, H1′), 4.80 (t, 1 H, *J* = 5.70 Hz, 6′-OH), 4.57 (d, 1 H, H1′′), 4.42 (m, 1 H, $J_{4',5'}$ = 3.35 Hz, $J_{5',6'}$ = 5.54 Hz, $J_{5',6''}$ = 5.88 Hz, H5′), 4.10 (br t, 1 H, H4′), 3.74 (ddd, 1 H, $J_{6',6''}$ = 11.60 Hz, H6′), 3.63 (ddd, 1 H, H6′′) ppm. ^{13}C NMR (125.77 MHz, DMSO-d_6): δ_{TMS} = 163.23 (C4), 149.65 (C2), 141.68 (C6), 102.45 (C5), 92.96 (C2′), 91.40 (C3′), 84.92 (C5′), 78.61 (C1′), 70.91 (C4′), 58.79 (C6′). Anal. Calcd for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93. Found: C, 46.75; H, 4.61; N, 10.88.

(17) 1-[3,4-Anhydro-1,6-di-*O*-(4-monomethoxy)-trityl-β-D-psicofuranosyl]uracil (**10**): amorphous powder; UV (MeOH): λ_{max} = 231.5 nm (ε 26900), λ_{max} = 263 nm (ε 9000), λ_{min} = 225 nm (ε 23500), λ_{min} = 255 nm (ε 8800). ^{1}H NMR (500.13 MHz, DMSO-d_6): δ_{TMS} = 11.35 (s, 1 H, N^3H), 7.71 (d, 1 H, $J_{5,6}$ = 8.14 Hz, H6), 5.47 (d, 1 H, H5), 4.92 (d, 1 H, $J_{3',4'}$ = 2.54 Hz, H3′), 4.56 (t, 1 H, $J_{5',6'}$ = $J_{5',6''}$ = 4.26 Hz, H5′), 3.92 (d, 1 H, $J_{4',5'}$ < 0.5 Hz, H4′), 3.61 (d, 1 H, $J_{1',1''}$ = 9.29 Hz, H1′), 3.16 (d, 1 H, H-1′′), 3.19 (d, H6′ and H6′′) ppm. ^{13}C NMR (125.77 MHz, DMSO-d_6): δ_{TMS} = 163.14 (C4), 150.47 (C2), 141.64 (C6), 100.63 (C5), 63.21 (C1′), 95.92 (C2′), 57.37 (C3′), 56.12 (C4′), 79.83 (C5′), 62.65 (C6′) ppm. Anal. Calcd for $C_{50}H_{44}N_2O_8$: C, 74.98; H, 5.54; N, 3.50. Found: C, 74.83; H, 5.50; N, 3.42.

(18) Miles, D. W.; Robins, R. K.; Eyring, H. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 1138.

(19) (a) Miles, D. W.; Robins, M. J.; Robins, R. K.; Winkley, M. W.; Eyring, H. *J. Am. Chem. Soc.* **1969**, *91*, 824. (b) Miles, D. W.; Robins, M. J.; Robins, R. K.; Winkley, M. W.; Eyring, H. *J. Am. Chem. Soc.* **1969**, *91*, 831. (c) Miles, D. W.; Inskeep, W. H.; Robins, M. J.; Winkley, M. W.; Robins, R. K.; Eyring, H. *J. Am. Chem. Soc.* **1970**, *92*, 3872.

(20) Yoshimura, Y.; Ueda, T.; Matsuda, A. *Tetrahedron Lett.* **1991**, *32*, 4549.