Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Supporting Information

Synthesis of *gem*-difluorinated nucleoside analogues of the liposidomycins and evaluation as MraY inhibitors

Xiu-Hua Xu, [a] Amy E Trunkfield, [b] Timothy D. H. Bugg [b] and Feng-Ling Qing [a,*]

[a] *Key Laboratory of Organofluorine Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Science, 354 Fenglin Lu, Shanghai 200032, China*

[b] *Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK*

Synthesis and characterization data of compounds **5**, **10**, **11**, 3, **14**, **15**, **7, 16-19 and 4.**

COSY NMR and NOESY NMR spectra of compounds **4** and **11**

Tetrahydrofuran (THF) was distilled from sodium metal. Dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN), nitromethane (CH_3NO_2), toluene and pyridine were distilled from CaH_2. ^1H NMR spectra were recorded on a Bruker AM300 spectrometer. ^{19}F NMR spectra were recorded on a Bruker AM300 spectrometer ($FCCl_3$ as outside standard and low field is positive). All the melting points and optical rotations are uncorrected. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

2,3-Di-*O*-acetyl-5-azido-5-deoxy-D-robofuranose (5). To a cooled solution (0 ^oC) of 1,2,3-Tri-*O*-acetyl-5-azido-5-deoxy-D-ribofuranose **9** (960 mg, 3.19 mmol) in dry CH_2Cl_2 (190 mL) was slowly added HBr-HOAc (30%, 1.69 mL). After the mixture was stirred at the same temperature for 2 h, saturated aq. $NaHCO_3$ was added to quench the reaction. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether: ethyl acetate = 4: 3) to afford the compound **5** (422 mg, 51% yield): clear oil, ^1H NMR (300 MHz, $CDCl_3$) δ 5.60-5.09 (m, 3H, H-1, H-2 and H-3), 4.37-4.22 (m, 1H, H-4), 3.65-3.59 (m, 1H, H-5a), 3.50-3.43 (m, 1H, H-5b), 3.17 (s, 1H, OH), 2.16-2.07 (m, 6H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ, major 169.8 (*C*OCH_3), 169.7 (*C*OCH_3), 100.2 (C-1), 79.8 (C-4), 75.5 (C-2), 71.5 (C-3), 52.9 (C-5), 20.5 (CO*C*H_3), 20.4 (CO*C*H_3), minor 170.0 (*C*OCH_3), 169.6 (*C*OCH_3), 95.6 (C-1), 80.6 (C-4), [71.1, 71.0 (C-2，C-3)], 52.1 (C-5), 20.7 (CO*C*H_3), 20.6 (CO*C*H_3); MS (ESI) *m/z* 277.1 ($M+NH_4^+$); IR (thin film) 3457 (OH), 2940 (CH), 2104 (N_3), 1741 (C=O), 1438, 1375, 1239, 965 cm^{-1}; HRMS (ESI) calcd for $C_9H_{13}N_3O_6Na$ 282.0697, found 282.0704.

5-*O*-[5′′-Azido-5′′-deoxy-2′′,3′′-*O*-diacetyl-*β*-*D*-ribofuranosyl]-3-deoxy-3,3-*O*-difluoro-1-(uracil-1′-yl)-*β*-*D*-arabinofuranose (10). A solution of compound **5** (406 mg, 1.57 mmol), CCl_3CN (0.79 mL, 7.85 mmol) and DBU (1 drop) in CH_2Cl_2 (16 mL)

was stirred at room temperature for 1.5 h. Then solvent was removed, and the residue was purified by flash chromatography on a silica gel column (petroleum ether: ethyl acetate = 3: 1, with 1% Et_3N) to give the corresponding trichloroacetimidate derivative (480 mg, 76% yield), which was used in the next step without further characterization. To a suspension of the trichloroacetimidate derivative (480 mg, 1.19 mmol), compound **6** (256 mg, 0.97 mmol) and 4 Å MS (970 mg) in dry CH_3CN (10 mL) at -20 ^oC, was slowly added TMSOTf (0.07 mL, 0.30 mmol) in dry CH_3CN (2 mL). After being stirred at the same temperature for 1 h, the reaction was quenched with Et_3N (2 mL), filtered, and concentrated. The residue was purified by silica gel column chromatography (dichloromethane: methanol = 60 : 1) to give the compound **10** (333 mg, 68% yield) as a white solid: mp 59-61 ℃; $[\alpha]^{21}_D$ = +1.4 (*c* 0.95, CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 7.86 (d, $J_{5,6}$ 8.1 Hz, 1H, H-6), 6.03 (dd, $J_{1',2'}$ 7.2 Hz, $J_{1',F}$ 1.8 Hz, 1H, H-1′), 5.94 (d, $J_{5,6}$ 7.8 Hz, 1H, H-5), 5.54-5.31 (m, 2H, H-2″ and H-3″), 5.18 (s, 1H, H-1″), 4.50-4.32 (m, 3H, H-5′a, H-2′ and H-4″), 4.20 (dd, $J_{5'a,5'b}$ 11.7 Hz, $J_{4',5'b}$ 3.3 Hz, 1H, H-5′b), 3.80 (dd, $J_{4',5'a}$ 11.1 Hz, $J_{4',5'b}$ 2.7 Hz, 1H, H-4′), 3.66 (dd, $J_{5''a,5''b}$ 13.2 Hz, $J_{4'',5''a}$ 3.3 Hz, 1H, H-5″a), 3.48 (dd, $J_{5''a,5''b}$ 13.2 Hz, $J_{4'',5''b}$ 5.1 Hz, 1H, H-5″b), 2.15 (s, 3H, CH_3), 2.09 (s, 3H, CH_3); ^{19}F NMR (282 MHz, CD_3OD) δ –117.6 (ddd, *J* 239.7 Hz, 14.1 Hz, 12.1 Hz, 1F), –123.0 (ddd, *J* 241.4 Hz, 10.2 Hz, 5.9 Hz, 1F); ^{13}C NMR (75.5 MHz, CD_3OD) δ 171.6 (*C*OCH_3), 171.3 (*C*OCH_3), 165.8 (C-4), 152.3 (C-2), 141.4 (C-6), 124.0 (t, $J_{C3',F}$ 257.2 Hz, C-3′), 107.0 (C-1″), 104.0 (C-5), 87.1 (d, $J_{C1',F}$ 17.7 Hz, C-1′), 80.9 (C-4″), 80.2 (dd, $J_{C4',F}$ 33.5 Hz, 27.6 Hz, C-4′), 75.7 (C-2″), 75.0 (dd, $J_{C2',F}$ 25.0 Hz, 17.1 Hz, C-2′), 72.4 (C-3″), 66.4 (dd, $J_{C5',F}$ 8.4 Hz, 7.4 Hz, C-5′), 53.6 (C-5″), 20.4 (CO*C*H_3), 20.3 (CO*C*H_3); MS (ESI) *m/z* 506.1 ($M+H^+$), 523.2 ($M+NH_4^+$), 528.2 ($M+Na^+$); IR (thin film) 3406 (OH), 2938 (CH), 2107 (N_3), 1755 (C=O), 1684 (C=O), 1464, 1247, 1054 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{21}F_2N_5O_{10}Na$ 528.1149, found 528.1149.

5-*O*-[5″-Azido-5″-deoxy-*β*-*D*-ribofuranosyl]-3-deoxy-3,3-*O*-difluoro-1-(uracil-1′-yl)-*β*-*D*-arabinofuranose (11). Compound **10** (110 mg, 0.20 mmol) was dissolved

in saturated methanolic ammonia (40 mL) and methanol (20 mL). The resulting reaction mixture was stirred for 12 h. After removal of the volatile materials, the residue was purified by silica gel chromatography (dichloromethane: methanol = 10: 1) to give compound **11** (80 mg, 95 % yield) as a white solid: mp 65-68 ℃; $[\alpha]^{21}_{D}$ = –4.4 (*c* 0.66, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.80 (d, $J_{5,6}$ 8.1 Hz, 1H, H-6), 5.95 (d, $J_{1',2'}$ 6.9 Hz, 1H, H-1′), 5.79 (d, $J_{5,6}$ 8.1 Hz, 1H, H-5), 4.93 (s, 1H, H-1″), 4.37-4.27 (m, 2H, H-2′ and H-5′a), 4.10-4.08 (m, 3H, H-5′b, H-3″ and H-4″), 3.95 (d, $J_{2'',3''}$ 3.6 Hz, 1H, H-2″), 3.68 (dd, $J_{4',5'a}$ 11.1 Hz, $J_{4',5'b}$ 3.6 Hz, 1H, H-4′), 3.55 (dd, $J_{5''a,5''b}$ 13.5 Hz, $J_{4'',5''a}$ 2.7 Hz, 1H, H-5″a), 3.34 (dd, $J_{5''a,5''b}$ 13.5 Hz, $J_{4'',5''b}$ 5.1 Hz, 1H, H-5″b); ^{19}F NMR (282 MHz, CD_3OD) δ –118.4 (dt, *J* 241.7 Hz, 11.6 Hz, 1F), –123.2 (dt, *J* 239.7 Hz, 9.6 Hz, 1F); ^{13}C NMR (75.5 MHz, CD_3OD) δ 165.7 (C-4), 152.2 (C-2), 141.6 (C-6), 124.0 (dd, $J_{C3',F}$ 257.3 Hz, 254.4 Hz, C-3′), 109.5 (C-1″), 103.6 (C-5), 87.4 (d, $J_{C1',F}$ 10.0 Hz, C-1′), 82.9 (C-4″), 80.4 (dd, $J_{C4',F}$ 29.6 Hz, 24.5 Hz, C-4′), 76.1 (C-2″), 75.2 (dd, $J_{C2',F}$ 31.1 Hz, 18.3 Hz, C-2′), 72.7 (C-3″), 66.2 (t, $J_{C5',F}$ 3.6 Hz, C-5′), 54.1 (C-5″); MS (ESI) *m/z* 422.0 ($M+H^+$), 439.0 ($M+NH_4^+$), 443.9 ($M+Na^+$); IR (thin film) 3343 (OH), 2936 (CH), 2106 (N_3), 1700 (C=O), 1466, 1284, 1137, 1045 cm^{-1}; HRMS (ESI) calcd for $C_{14}H_{17}F_2N_5O_8Na$ 444.0937, found 444.0929.

5-*O*-[5″-Amino-5″-deoxy-*β*-*D*-ribofuranosyl]-3-deoxy-3,3-*O*-difluoro-1-(uracil-1′-yl)-*β*-*D*-arabinofuranose (3). A suspension of Pd/C (52 mg) and **11** (52 mg, 0.12 mmol) in MeOH (8 mL) was stirred under a hydrogen atmosphere for 30 min. Filtration and removal of the solvent gave the crude product, which was purified by column chromatography on silica gel (dichloromethane: methanol = 1: 1) to give compound **3** (40 mg, 82 % yield) as a white solid: mp 124-127 ℃; $[\alpha]^{23}_{D}$ = –16.9 (*c* 0.15, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.77 (d, $J_{5,6}$ 7.8 Hz, 1H, H-6), 5.98 (dd, $J_{1',2'}$ 6.3 Hz, $J_{1',F}$ 1.2 Hz, 1H, H-1′), 5.83 (d, $J_{5,6}$ 7.8 Hz, 1H, H-5), 4.98 (s, 1H, H-1″), 4.43-4.36 (m, 2H, H-2′ and H-5′a), 4.12-3.99 (m, 4H, H-5′b, H-2″, H-3″ and H-4″), 3.76 (dd, $J_{4',5'a}$ 11.4 Hz, $J_{4',5'b}$ 4.5 Hz, 1H, H-4′), 2.98 (dd, $J_{5''a,5''b}$ 13.2 Hz, $J_{4'',5''a}$ 3.0 Hz, 1H, H-5″a), 2.78 (dd, $J_{5''a,5''b}$ 13.2 Hz, $J_{4'',5''b}$ 7.2 Hz, 1H, H-5″b); ^{19}F NMR

(282 MHz, CD_3OD) δ –118.8 (ddd, *J* 238.9 Hz, 13.3 Hz, 11.3 Hz, 1F), –122.6 (dt, *J* 241.1 Hz, 11.0 Hz, 1F); ^{13}C NMR (75.5 MHz, CD_3OD) δ 166.0 (C-4), 152.4 (C-2), 141.5 (C-6), 124.0 (dd, $J_{C3',F}$ 258.2 Hz, 255.9 Hz, C-3′), 109.3(C-1″), 103.7 (C-5), 87.8 (d, $J_{C1',F}$ 9.8 Hz, C-1′), 84.6 (C-4″), 80.2 (dd, $J_{C4',F}$ 28.9 Hz, 24.1 Hz, C-4′), 76.3 (C-2″), 75.2 (dd, $J_{C2',F}$ 25.7 Hz, 15.9 Hz, C-2′), 73.8 (C-3″), 65.8 (t, $J_{C5',F}$ 4.8 Hz, C-5′), 45.9 (C-5″); MS (ESI) *m/z* 396.0 ($M+H^+$); IR (thin film) 3308 (OH, NH_2), 2926 (CH), 1699 (C=O), 1488, 1455, 1290, 1116, 1033 cm^{-1}; HRMS (ESI) calcd for $C_{14}H_{20}F_2N_3O_8$ 396.1213, found 396.1221.

(2*R*,3*R*)-3-*O*-Benzyl-4,4-difluoro-5-hexene-1,2,3-triol (14). To a solution of compound **13** (3.600 g, 12.06 mmol) in MeOH (36 mL) was added *p*-toluenesulfonic acid hydrate (2.200 g, 11.56 mmol). The reaction mixture was stirred overnight at room temperature, then was quenched with solid K_2CO_3 (1.700 g, 12.32 mmol). The reaction mixture was diluted with 150 mL of EtOAc and washed three times with 150 mL of brine, dried over anhydrous Na_2SO_4. After filtration and removal of the solvent in vacuo, the residue was purified by flash chromatography on silica gel (petroleum ether: ethyl acetate = 1:1) to afford compound **14** (2.832 g, 91% yield) as a clear oil: ^1H NMR (300 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5H, H_{aryl}-Bn), 6.15-5.97 (m, 1H, H-5), 5.76 (d, $J_{5,6a}$ 17.4 Hz, 1H, H-6a), 5.55 (d, $J_{5,6b}$ 11.1 Hz, 1H, H-6b), 4.92-4.82 (m, 1H, H_{alkyl}-Bn), 4.68-4.60 (m, 1H, H_{alkyl}-Bn), 3.86-3.56 (m, 4H, H-1a, H-1b, H-2, H-3), 2.37 (s, 2H, OH); ^{19}F NMR (282 MHz, $CDCl_3$) δ –101.2 (dt, *J* 254.1 Hz, 14.1 Hz, 0.04F), –102.3 (dt, *J* 254.9 Hz, 11.0 Hz, 0.96F), –105.7 (ddd, *J* 259.4 Hz, 11.0 Hz, 5.9 Hz, 0.04F), –107.1 (dt, *J* 254.9 Hz, 10.7 Hz, 0.96F); MS (ESI) *m/z* 259.1 ($M+H^+$), 276.1 ($M+NH_4^+$), 281.1 ($M+Na^+$); IR (thin film) 3394 (OH), 3035 (=CH), 1716, 1498, 1421, 1212, 1102, 989 cm^{-1}; Anal. Calcd for $C_{13}H_{16}F_2O_3$: C, 60.46; H, 6.24; Found: C, 60.29; H, 6.20.

(2*R*,3*R*)-1-*O*-Benzoyl-3-*O*-benzyl-4,4-difluoro-5-hexene-1,2,3-triol (15). To a solution of compound **14** (2.648 g, 10.26 mmol) in anhydrous CH_2Cl_2 (50 mL) and

pyridine (25 mL) was slowly added a solution of BzCl (1.24 mL, 10.26 mmol) in CH_2Cl_2 (9 mL) at -78 ^oC. After the mixture was stirred at the same temperature for 2 h, MeOH (10 mL) was added and the mixture was stirred for 30 min. Then water was added to quench the reaction. The layers were separated and the aqueous layer was extracted with ether. The combined organic layers were washed with 1 N HCl, saturated aqueous $NaHCO_3$ and brine. After the resultant solution was dried over anhydrous Na_2SO_4, and filtered, the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (petroleum ether: ethyl acetate = 4: 1) to give compound **15** (3.195 g, 86% yield) as a clear oil: ^1H NMR (300 MHz, $CDCl_3$) δ 8.04-7.95 (m, 2H, H_{aryl}-Bz), 7.58 (t, *J* 7.5 Hz, 1H, H_{aryl}-Bz), 7.44 (t, *J* 7.5 Hz, 2H, H_{aryl}-Bz), 7.35-7.29 (m, 5H, H_{aryl}-Bn), 6.20-6.03 (m, 1H, H-5), 5.79 (d, $J_{5,6a}$ 17.4 Hz, 1H, H-6a), 5.56 (d, $J_{5,6b}$ 11.1 Hz, 1H, H-6b), 4.95-4.85 (m, 1H, H_{alkyl}-Bn), 4.69 (m, 1H, H_{alkyl}-Bn), 4.60 (dd, $J_{1a,1b}$ 11.7 Hz, $J_{1a,2}$ 2.4 Hz, 1H, H-1a), 4.48 (dd, $J_{1a,1b}$ 11.7 Hz, $J_{1b,2}$ 6.3 Hz, 1H, H-1b), 4.20-4.10 (m, 1H, H-2), 3.92-3.84 (m, 1H, H-3), 2.44 (s, 1H, OH); ^{19}F NMR (282 MHz, $CDCl_3$) δ –101.3 (dt, *J* 255.8 Hz, 11.3 Hz, 0.06F), –101.7 (dt, *J* 256.9 Hz, 9.0 Hz, 0.94F), –104.2 (dt, *J* 257.2 Hz, 8.2 Hz, 0.06F), –106.6 (dt, *J* 258.3 Hz, 9.0 Hz, 0.94F); MS (ESI) *m/z* 363.1 ($M+H^+$), 380.2 ($M+NH_4^+$), 385.0 ($M+Na^+$); IR (thin film) 3474 (OH), 3035 (=CH), 1721 (C=O), 1498, 1421, 1276, 1114, 990 cm^{-1}; Anal. Calcd for $C_{20}H_{20}F_2O_4$: C, 66.29; H, 5.56; Found: C, 66.44; H, 5.64.

5-*O*-Benzoyl-3-*O*-benzyl-2-deoxy-2,2-difluoro-*D*-arabinofuranose (7). Ozone was bubbled through a solution of compound **15** (2.864 g, 7.91 mmol) in dichloromethane (60 mL) at -78 ^oC. The reaction was monitored by TLC, then the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (chloroform: methanol = 60: 1) to give compound **7** (2.477 g, 86% yield) as a clear oil: ^1H NMR (300 MHz, $CDCl_3$) δ 7.98-7.94 (m, 2H, H_{aryl}-Bz), 7.61-7.55 (m, 1H, H_{aryl}-Bz), 7.46-7.40 (m, 2H, H_{aryl}-Bz), 7.33-7.27 (m, 5H, H_{aryl}-Bn), 5.32 (d, $J_{1,F}$ 6.6 Hz, 0.55H, H-1), 5.24 (d, $J_{1,F}$ 6.9 Hz, 0.45H, H-1), 4.89 (d, *J* 11.7 Hz, 1H, H_{alkyl}-Bn), 4.62 (d, *J* 11.4 Hz, 1H, H_{alkyl}-Bn), 4.60-4.24 (m, 4H, H-3, H-4, H-5a

and H-5b), 4.03-3.96 (m, 1H, OH); ^{19}F NMR (282 MHz, $CDCl_3$) δ –109.5 (ddd, *J* 247.9 Hz, 14.4 Hz, 9.3 Hz, 0.56F), –121.9 (dd, *J* 238.6 Hz, 5.6 Hz, 0.44F), –127.1 (ddd, *J* 240.3 Hz, 14.9 Hz, 6.8 Hz, 0.44F), –128.6 (d, *J* 247.0 Hz, 0.56F); MS (ESI) *m/z* 365.1 ($M+H^+$), 382.2 ($M+NH_4^+$), 387.0 ($M+Na^+$), 403.0 ($M+K^+$); IR (thin film) 3437 (OH), 2951 (CH), 1724 (C=O), 1497, 1454, 1275, 1097, 1028 cm^{-1}; Anal. Calcd for $C_{19}H_{18}F_2O_5$: C, 62.63; H, 4.98; Found: C, 62.24; H, 5.02.

Ethanimidic acid, *N*-(5″-benzoyl-3″-*O*-benzyl-2″-deoxy-2″,2″-difluoro-*D*-ribofuranosyl)-2,3-*O*-isopropylidene1-(uracil-1′-yl)-*β*-*D*-arabinofuranosyl ester (16). A solution of compound **7** (730 mg, 2.00 mmol), CCl_3CN (1.00 mL, 10.00 mmol) and DBU (1 drop) in CH_2Cl_2 (20 mL) was stirred at room temperature for 1.5 h. Then solvent was removed, and the residue was purified by flash chromatography on a silica gel column (petroleum ether: ethyl acetate = 5: 1, with 1% Et_3N) to give the corresponding trichloroacetimidate derivative (880 mg, 87% yield), which was used in the next step without further characterization. To a suspension of the trichloroacetimidate derivative (880 mg, 1.73 mmol), compound **8** (580 mg, 2.08 mmol) and 4 Å MS (1380 mg) in dry CH_3CN (20 mL) at -20 ^oC, was slowly added TMSOTf (0.43 mL, 1.90 mmol). After being stirred at the same temperature for 6 h, the reaction was quenched with Et_3N (3 mL), filtered, and concentrated. The residue was purified by silica gel column chromatography (petroleum ether: ethyl acetate = 1: 2) to give the compound **16** (720 mg, 62% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (s, 0.45H, NH), 8.49 (s, 0.55H, NH), 8.02-7.97 (m, 2H, H_{aryl}-Bz), 7.60-7.55 (m, 1H, H_{aryl}-Bz), 7.45-7.41 (m, 2H, H_{aryl}-Bz), 7.33-7.30 (m, 6H, H_{aryl}-Bn and H-6), 5.75-5.63 (m, 2H, H-1′ and H-5), 5.29 (d, $J_{1'',F}$ 10.2 Hz, 0.55H, H-1″), 5.16 (d, $J_{1'',F}$ 10.8 Hz, 0.45H, H-1″), 4.94-3.97 (m, 11H, H-2′, H-3′, H-4′, H-5′a, H-5′b, H-3″, H_{alkyl}-Bn, H-4″, H-5″a and H-5″b), 1.97 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ –101.1 (ddd, *J* 237.7 Hz, 17.2 Hz, 10.4 Hz, 0.55F), –119.3 (dd, *J* 231.8 Hz, 9.3 Hz, 0.45F), –121.9 (dd, *J* 236.6 Hz, 4.5 Hz, 0.55F),

–122.3 (dt, *J* 231.5 Hz, 10.7 Hz, 0.45F); MS (ESI) *m/z* 672.2 ($M+H^+$), 694.3 ($M+Na^+$); IR (thin film) 2937 (CH), 1720 (C=O), 1695 (C=O), 1456, 1382, 1274, 1070, 714 cm^{-1}; Anal. Calcd for $C_{33}H_{35}F_2N_3O_{10}$: C, 59.01; H, 5.25; N, 6.26; Found: C, 58.58; H, 5.46; N, 5.94.

5-*O*-[5′′-Benzoyl-3′′-*O*-benzyl-2′′-deoxy-2′′,2′′-difluoro-*β*-*D*-ribofuranosyl]-2,3-*O*-isopropylidene1-(uracil-1′-yl)-*β*-*D*-arabinofuranose (17). Conversion of Compound **7** (728 mg, 2.00 mmol) to Compound **17** was accomplished using the similar condition as described for compound **16** with CH_3NO_2 as the solvent instead of CH_3CN. Compound **17** (403 mg, 32% in two steps) was obtained as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (s, 0.28H, NH), 8.37 (s, 0.72H, NH), 8.00-7.93 (m, 2H, H_{aryl}-Bz), 7.64-7.47 (m, 1H, H_{aryl}-Bz), 7.45-7.37 (m, 2H, H_{aryl}-Bz), 7.34-7.28 (m, 6H, H_{aryl}-Bn and H-6), 6.07 (d, $J_{1',2'}$ 3.0 Hz, 0.72H, H-1′), 5.75-5.56 (m, 1H, H-1′ and H-5), 5.57 (d, $J_{5,6}$ 8.1 Hz, 0.28H, H-5), 5.08 (d, $J_{1'',F}$ 1.5 Hz, 0.72H, H-1′′), 4.98 (d, $J_{1'',F}$ 3.0 Hz, 0.28H, H-1′′), 4.89-3.96 (m, 10H, H-2′, H-3′, H-4′，H-5′a, H-5′b, H-3′′, H_{alkyl}-Bn, H-4′′ and H-5′′a), 3.83-3.70 (m, 1H, H-5′′b), 1.58 (s, 2.16H, CH_3), 1.50 (s, 0.84H, CH_3), 1.31 (s, 0.85H, CH_3), 1.30 (s, 2.15H, CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ –105.7 (ddd, *J* 249.3 Hz, 17.2 Hz, 8.5 Hz, 0.72F), –121.2 (dd, *J* 239.4 Hz, 7.9 Hz, 0.28F), –125.3 (ddd, *J* 239.7 Hz, 16.1 Hz, 8.5 Hz, 0.28F), –127.4 (d, *J* 251.5 Hz, 0.72F); MS (ESI) *m/z* 631.3 ($M+H^+$), 653.2 ($M+Na^+$); IR (thin film) 2937 (CH), 1720 (C=O), 1695 (C=O), 1455, 1384, 1275, 1112, 713 cm^{-1}; Anal. Calcd for $C_{31}H_{32}F_2N_2O_{10}$: C, 59.04; H, 5.11; N, 4.44; Found: C, 58.90; H, 5.20; N, 4.37.

5-*O*-[3′′-*O*-Benzyl-2′′-deoxy-2′′,2′′-difluoro-*β*-*D*-ribofuranosyl]-2,3-*O*-isopropylidene-1-(uracil-1′-yl)-*β*-*D*-arabinofuranose (18). Compound **17** (277 mg, 0.44 mmol) was dissolved in saturated methanolic ammonia (33 mL) and methanol (11 mL). The resulting reaction mixture was stirred for 36 h. After removal of the volatile materials, the residue was purified by silica gel chromatography (petroleum ether: ethyl acetate = 1: 1) to give compound **18** (204 mg, 88 % yield) as a white solid: ^{1}H

NMR (300 MHz, $CDCl_3$) δ 8.99 (s, 0.28H, NH), 8.66 (s, 0.72H, NH), 7.62 (d, $J_{5,6}$ 7.8 Hz, 0.72H, H-6), 7.36-7.28 (m, 5.28H, H_{aryl}-Bn and H-6), 6.06 (d, $J_{1',2'}$ 2.7 Hz, 0.72H, H-1′), 5.73-5.66 (m, 1.28H, H-1′ and H-5), 5.08-3.76 (m, 11H, H-2′, H-3′, H-4′, H-5′a, H-5′b, H-1″, H-3″, H_{alkyl}-Bn, H-4″ and H-5″a), 3.67 (dd, $J_{5''a,\ 5''b}$ 12.3 Hz, $J_{4'',5''b}$ 4.5 Hz, 0.72H, H-5″b), 3.55 (dd, $J_{5''a,\ 5''b}$ 12.9 Hz, $J_{4'',5''b}$ 3.0 Hz, 0.28H, H-5″b), 2.08 (s, 1H, OH), 1.57 (s, 3H, CH_3), 1.36 (s, 0.85H, CH_3), 1.30 (s, 2.15H, CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ –105.9 (ddd, *J* 250.1 Hz, 17.8 Hz, 8.2 Hz, 0.72F), –120.6 (dd, *J* 239.7 Hz, 8.5 Hz, 0.28F), –124.9 (ddd, *J* 237.2 Hz, 16.6 Hz, 9.9 Hz, 0.28F), –127.0 (d, *J* 249.6 Hz, 0.72F); MS (ESI) *m/z* 527.2 ($M+H^+$), 549.2 ($M+Na^+$); IR (thin film) 3231 (OH), 2937 (CH), 1693 (C=O), 1458, 1384, 1274, 1084, 701 cm^{-1}; Anal. Calcd for $C_{24}H_{28}F_2N_2O_9$: C, 54.75; H, 5.36; N, 5.32; Found: C, 54.86; H, 5.04; N, 5.36.

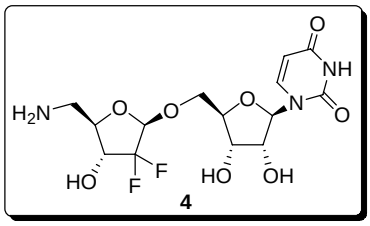
5-*O*-[5″-Azido-3″-*O*-benzyl-2″,5″-dideoxy-2″,2″-difluoro-*β*-*D*-ribofuranosyl]-2,3-*O*-isopropylidene-1-(uracil-1′-yl)-*β*-*D*-arabinofuranose (19). Compound **18** (126 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (1 mL). Then, fresh distilled pyridine (0.042 mL, 0.48 mmol) was added. After the resulting mixture was cooled to –55 ºC, a solution of trifluoromethanesulfonic anhydride (0.065 mL, 0.40 mmol) in dry CH_2Cl_2 (0.4 mL) was added dropwise to the mixture with stirring. Then, the reaction mixture was stirred for about 2 h at about –30 ºC. Water and $NaHCO_3$ solution were successively added after the mixture was warmed to the room temperature. The mixture was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4 and purified by silica gel column chromatograpy (petroleum ether: ethyl acetate = 2: 1) to afford a clear oil (135 mg). A solution of the product in acetone (30 mL) was cooled to 0 ^oC by ice bathe. Then, sodium azide (78 mg, 1.2 mmol) was added carefully with stirring. The reaction mixture was stirred at room temperature for 1h. Water was added to quench the reaction. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was quickly purified by silica gel column chromatography (petroleum ether: ethyl acetate = 2: 1) to afford the compound **19** (77 mg, 58% yield in two steps) as a white solid: ^1H NMR (300

MHz, $CDCl_3$) δ 8.58 (s, 0.28H, NH), 8.46 (s, 0.72H, NH), 7.59 (d, $J_{5,6}$ 8.1 Hz, 0.72H, H-6), 7.42-7.30 (m, 5.28H, H_{aryl}-Bn and H-6), 6.06 (d, $J_{1',2'}$ 3.0 Hz, 0.72H, H-1′), 5.82 (d, $J_{1',2'}$ 2.1 Hz, 0.28H, H-1′), 5.72 (d, $J_{5,6}$ 7.8 Hz, 0.72H, H-5), 5.64 (d, $J_{5,6}$ 9.6 Hz, 0.28H, H-5), 5.07 (d, $J_{1'',F}$ 7.8 Hz, 0.72H, H-1″), 4.97 (d, $J_{1'',F}$ 8.1 Hz, 0.28H, H-1″), 4.88-3.74 (m, 9H, H-2′, H-3′, H-4′, H-5′a, H-5′b, H-3″, H_{alkyl}-Bn and H-4″), 3.50-3.32 (m, 1H, H-5″a), 3.31-3.25 (m, 1H, H-5″b), 1.58 (s, 3H, CH_3), 1.36 (s, 0.85H, CH_3), 1.31 (s, 2.15H, CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ –105.7 (ddd, *J* 251.8 Hz, 15.2 Hz, 7.9 Hz, 0.72F), –120.5 (dd, *J* 238.8 Hz, 6.5 Hz, 0.28F), –124.6 (ddd, *J* 239.4 Hz, 16.1 Hz, 7.3 Hz, 0.28F), –126.9 (d, *J* 250.4 Hz, 0.72F); MS (ESI) *m/z* 552.2 ($M+H^+$), 574.3 ($M+Na^+$); IR (thin film) 2938 (CH), 2107 (N_3), 1695 (C=O), 1458, 1384, 1277, 1087, 700 cm^{-1}; Anal. Calcd for $C_{24}H_{27}F_2N_5O_8$: C, 52.27; H, 4.93; N, 12.70; Found: C, 52.27; H, 4.69; N, 12.76.

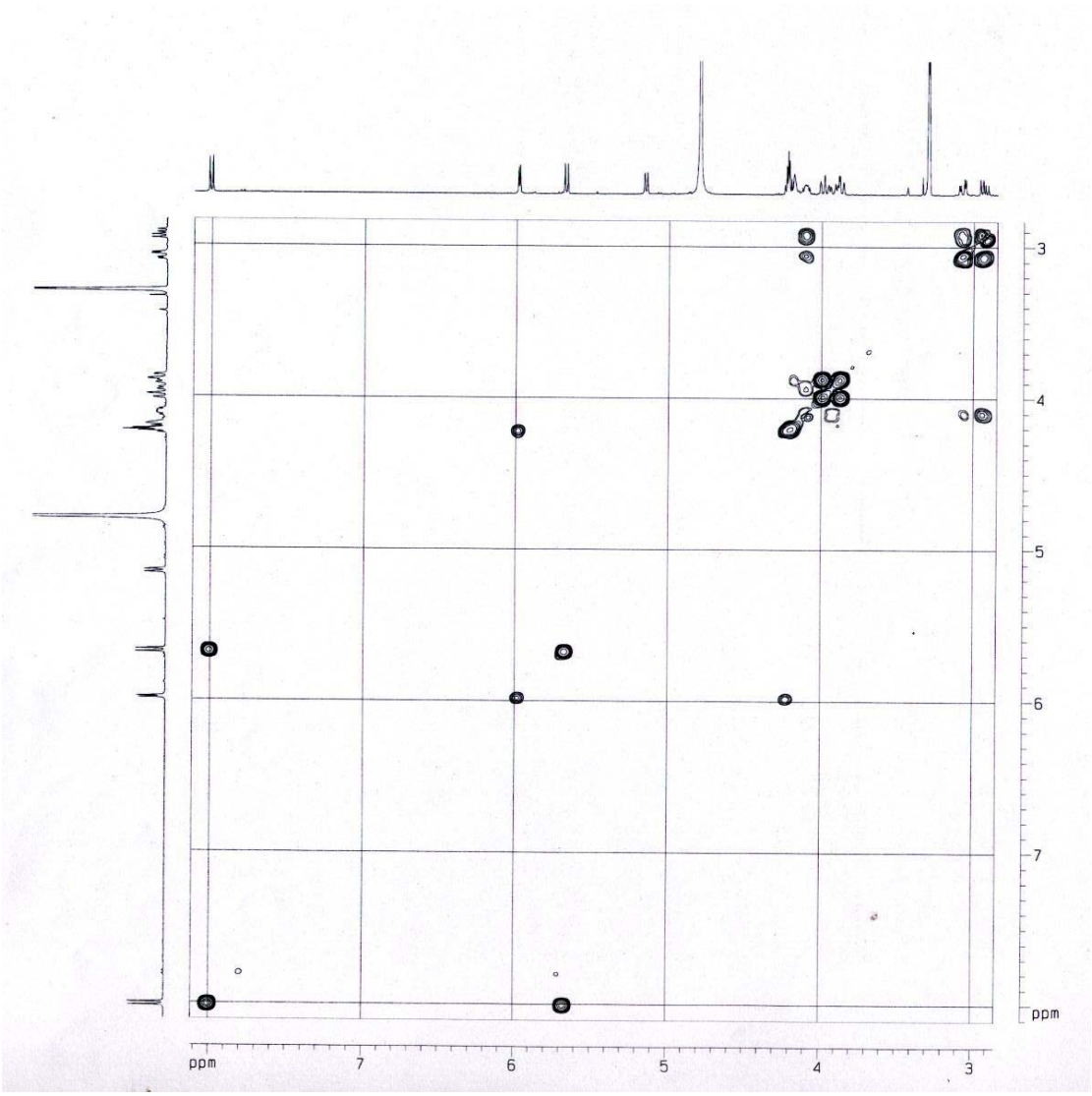
5-*O*-[5″-Amino-2″,5″-dideoxy-2″,2″-difluoro-*β*-*D*-ribofuranosyl]-1-(uracil-1′-yl) -*β*-*D*-arabinofuranose (4). Compound **19** (64 mg, 0.12 mmol) was dissolved in dry CH_2Cl_2 (6 mL). After the resulting mixture was cooled to –60 ºC, BCl_3 (1.20 mL, 1.0 M in heptane, 1.20 mmol) was added dropwise to the mixture with stirring. The mixture was then warmed to the room temperature and stirred overnight. The reaction was quenched with MeOH (3 mL), and concentrated. The residue was purified by silica gel column chromatography (dichloromethane: methanol = 5: 1) to give the compound **4** (21 mg, 45% yield) and the *α* isomer (8 mg, 18% yield) as white solids: compound **4**, mp 248-250 ℃; $[\alpha]^{27}_D$ = +22.0 (*c* 0.12, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD) δ 8.01 (d, $J_{5,6}$ 8.1 Hz, 1H, H-6), 5.98 (d, $J_{1',2'}$ 4.2 Hz, 1H, H-1′), 5.68 (d, $J_{5,6}$ 8.1 Hz, 1H, H-5), 5.15 (d, $J_{1'',F}$ 7.5 Hz, 1H, H-1″), 4.23-4.18 (m, 3H, H-2′, H-3′ and H-4′), 4.13-4.08 (m, 1H, H-4″), 4.00 (d, $J_{5'a,5'b}$ 9.8 Hz, 1H, H-5′a), 3.93 (dd, $J_{3'',F}$ 18.3 Hz, $J_{3'',4''}$ 5.7 Hz, 1H, H-3″), 3.89 (dd, $J_{5'a,5'b}$ 21.0 Hz, $J_{4',5'b}$ 2.1 Hz, 1H, H-5′b), 3.07 (dd, $J_{5''a,5''b}$ 13.8 Hz, $J_{4'',5''a}$ 3.9 Hz, 1H, H-5″a), 2.93 (dd, $J_{5''a,5''b}$ 13.5 Hz, $J_{4'',5''b}$ 7.5 Hz, 1H, H-5″b); ^{19}F NMR (282 MHz, CD_3OD) δ –109.7 (ddd, *J* 247.0 Hz, 17.8 Hz, 8.5 Hz, 1F), –128.4 (d, *J* 247.0 Hz, 1F); ^{13}C NMR (75.5 MHz, CD_3OD) δ 167.1 (C-4),

153.1 (C-2), 143.0 (C-6), 124.0 (dd, $J_{C2'',F}$ 202.2 Hz, 184.8 Hz, C-2″), 103.1 (C-5), 102.3 (dd, $J_{C1'',F}$ 32.5 Hz, 16.3 Hz, C-1″), 90.5 (C-1′), [84.9, 84.8 (C-4′, C-4″)], 76.0 (C-2′), 73.8 (dd, $J_{C3'',F}$ 24.0 Hz, 14.7 Hz, C-3″), 72.0 (C-3′), 67.7 (C-5′), 44.1 (C-5″); MS (ESI) *m/z* 396.2 ($M+H^+$), 418.0 ($M+Na^+$); IR (thin film) 3312 (OH, NH_2), 2926 (CH), 1684 (C=O), 1508, 1465, 1266, 1221, 1033 cm^{-1}; HRMS (ESI) calcd for $C_{14}H_{20}F_2N_3O_8$ 396.1213, found 396.1217.

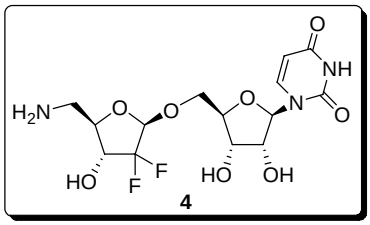
COSY NMR spectrum of compound **4**



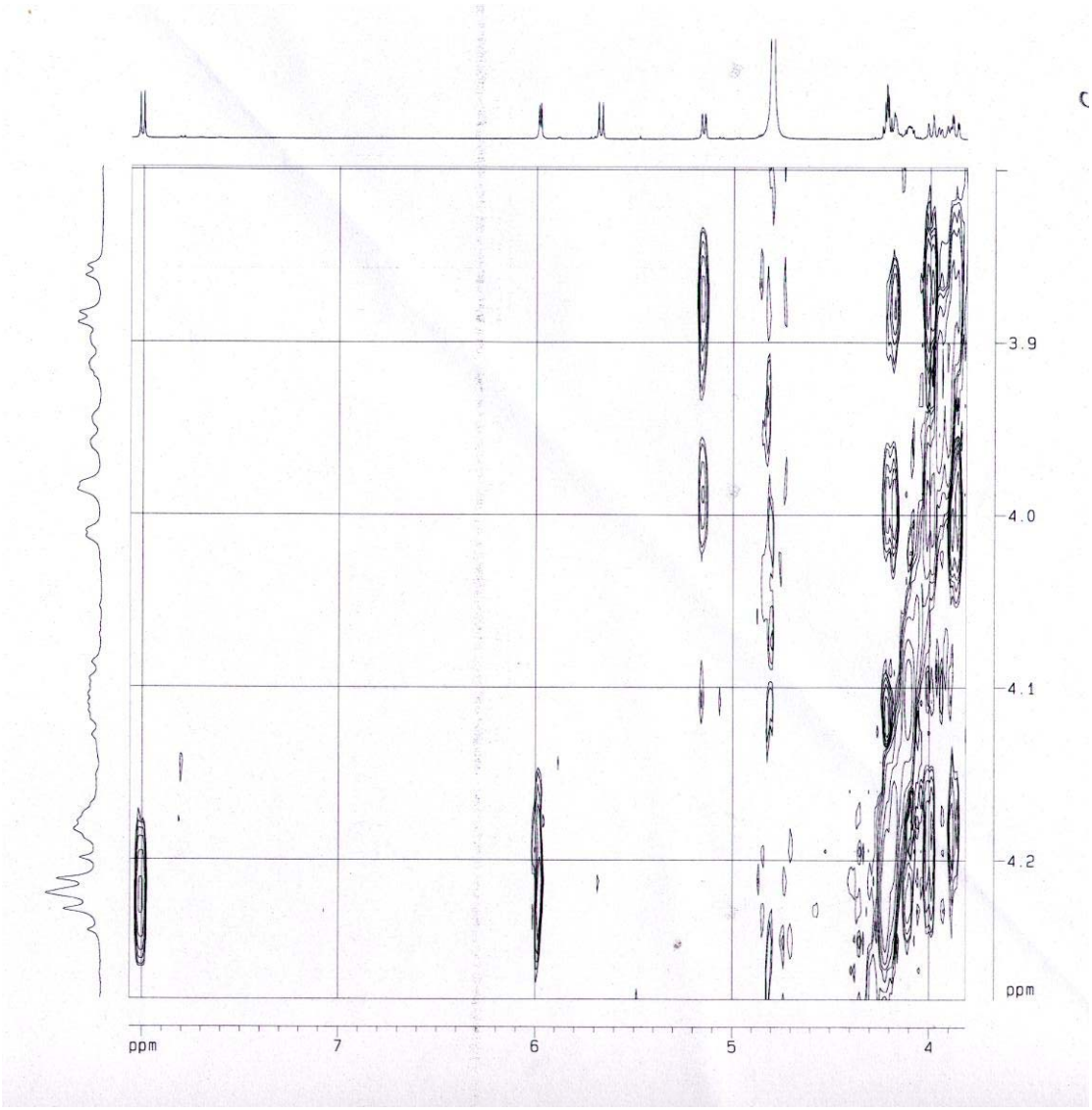
4



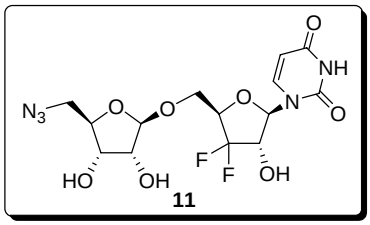
NOESY NMR spectrum of compound **4**



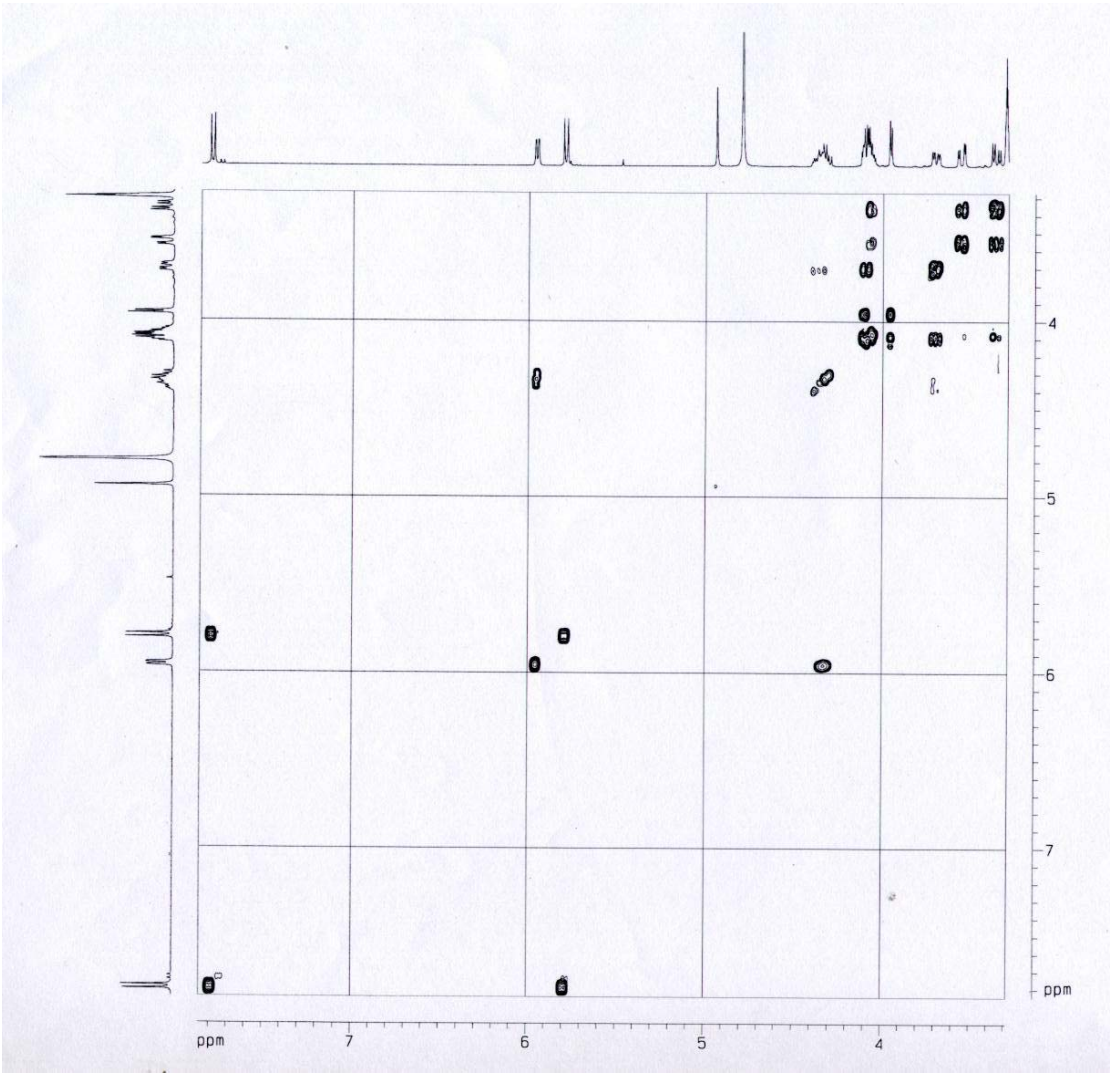
4



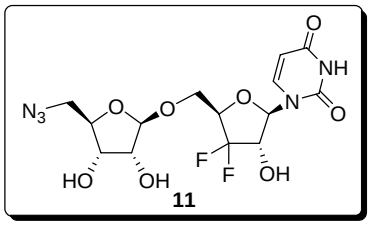
COSY NMR spectrum of compound **11**



11



NOESY NMR spectrum of compound **11**



11