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Chemoenzymatic arabinosylation of 2-aminopurines bearing the chiral fragment of 7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazines

Barbara Z. Eletskaya, Irina D. Konstantinova, Alexander S. Paramonov, Roman S. Esipov, Dmitry A. Gruzdev, Alexey Yu. Vigorov, Galina L. Levit, Anatoly I. Miroshnikov, Victor P. Krasnov and Valery N. Charushin

Experimental

(3*S*)-7,8-Difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine ((*S*)-**1**),[S1] (3*R*)-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine ((*R*)-**1**),[S2] (3*S*)-4-(2-aminopurin-6-yl)aminohexanoyl-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine ((*S*)-**3**),[S3] (3*R*)-4-(2-aminopurin-6-yl)aminohexanoyl-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine ((*R*)-**3**)[S3] were obtained as described previously. Other reagents are commercially available. Preparation of *E. coli* purine nucleoside phosphorylase (PNP; the product of the *deoD*gene; EC 2.4.2.1; 52 units/mg; 15 mg/mL) and uridine phosphorylase (UP) was accomplished according to the known procedure[S4]. Solutions of recombinant *E. coli* UP and PNP in 5 mM potassium phosphate buffer (pH 7.0) with activities 900 and 918 units per mL, respectively, were used.[S4]

The solvents were purified according to traditional methods and used freshly distilled. Optical rotations were measured on a Perkin Elmer 341 polarimeter. The ^{1}H, ^{19}F, and ^{13}C NMR spectra of compounds (*R*)-**3** and (*S*)-**3** were recorded on a Bruker Avance 500 spectrometer (500, 470, and 125 MHz, respectively) with TMS and hexafluorobenzene as internal references in DMSO-d_6 at 100 °C. The ^{1}H and ^{13}C NMR spectra of arabinosides **5** and **6** were recorded on Bruker DRX-700 spectrometer (700 and 176 MHz, respectively) in DMSO-d_6 at 30 °C. Microanalyses were performed using Perkin Elmer 2400 II analyzer. Analytical TLC was performed using Sorbfil plates (Imid, Russia). Flash-column chromatography was performed using Silica gel 40 (230–400 mesh) (Alfa Aesar, UK). Analytical HPLC of compounds (*R*)-**3** and (*S*)-**3** was performed on an Agilent 1200 instrument using a Kromasil Cellucoat column (150×4.6 mm, 5 μm), detection at 316 nm, 0.8 mL/min flow rate, MeCN–H_2O 25:75 mixture as an eluent. The HRMS spectra of compounds (*R*)-**3** and (*S*)-**3** were registered on a Bruker maXis Impact HD instrument operating in positive ion mode with ESI probe installed at N_2 flow rate 4 L/min, nebulizer pressure 0.4 bar. The probe voltage was set to 4.5 kV.

HPLC monitoring of arabinosylation progress was performed on Waters system (Waters 1525, Waters 2487, Breeze 2; USA) with Ascentis® Express C18 column (2.7 μm, 7.5×3.0 mm) using eluent A (0.1% aqueous TFA) and eluent B (0.1% aqueous TFA–70% aqueous MeCN); linear gradient elution: A → A–B 1:1, 20 min; flow rate 1 mL/min, UV detection at 254 and 280 nm. The HRMS spectra of arabinosides (*R*)-**5**, (*S*)-**5** and (*R*)-**6** were registered on an Agilent 6224 instrument, ESI-TOF LC/MS in positive ion mode, electrospray ionization.

4-(2-Aminopurin-6-yl)-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazines (3). General procedure: 2-amino-6-chloropurine **2** (0.21 g, 1.24 mmol) and TFA (0.47 mL, 6.20 mmol) were added to a solution of amine (*R*)-**1** or (*S*)-**1** (0.46 g, 2.48 mmol) in CF_3CH_2OH (8 mL). The reaction mixture was refluxed for 17 h, and then evaporated to dryness. The residue was treated with MeOH (10 mL), filtered through a bed of basic alumina and eluted with MeOH (10×10 mL). The methanolic solution was evaporated to dryness; the residue was purified by flash column chromatography (SiO_2, from $CHCl_3$ to 10% MeOH in $CHCl_3$).

(3*R*)-4-(2-Aminopurin-6-yl)-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine [(*R*)-3]: yield 0.100 g (26%), amorphous solid, $[\alpha]_D^{20}$ +17.6 (*c* 0.8, MeOH), *ee*>99.5%, HPLC (Kromasil Cellucoat, MeCN–H_2O 25:75): τ 14.8 min. ^{1}H NMR (500 MHz, DMSO-d_6, 100 °C) δ: 1.33 (d, *J* 6.7 Hz, 3 H, Me), 4.17 (dd, *J* 10.9, 2.5 Hz, 1 H, H-2B), 4.39 (dd, *J* 10.9, 1.3 Hz, 1 H, H-2A), 5.55 (br. s, 2 H, NH_2), 5.73 (m, 1 H, H-3), 6.77 (m, 1 H, H-6), 7.60 (ddd, *J* 9.4, 5.5, 2.5 Hz, 1 H, H-5), 7.75 (s, 1 H, purine H-8) ppm. ^{19}F NMR (475 MHz, DMSO-d_6, 100 °C) δ: 0.89 (ddd, *J* 20.9, 8.2, 2.5 Hz, 1 F, F-8), 17.50 (ddd, *J* = 21.0, 10.2, 5.5 Hz, 1 F, F-7). ^{13}C NMR (125 MHz, DMSO-d_6, 100 °C) δ: 15.01, 47.43, 69.30, 105.77 (d, *J* 18.0 Hz), 114.28, 118.51 (dd, $J_{C–F}$ 7.5, 3.5 Hz), 122.83, 135.35 (dd, $J_{C–F}$ 10.2, 3.2 Hz), 136.75, 138.66 (dd, $J_{C–F}$ 242.5, 5.6 Hz), 145.23 (dd, $J_{C–F}$ 239.9, 10.2 Hz), 151.63, 155.01, 158.82. HRMS, *m/z*: 319.1112 $[M+H]^+$, (calc. for $C_{14}H_{13}F_2N_6O$, *m/z*: 319.1113).

(3*S*)-4-(2-Aminopurin-6-yl)-7,8-difluoro-3-methyl-2,3-dihydro-4*H*-[1,4]benzoxazine [(*S*)-3]: yield 0.103 g (26%), amorphous solid, $[\alpha]_D^{20}$ −17.7 (*c* 1.0, MeOH), *ee*>99.5%, HPLC (Kromasil Cellucoat, MeCN–H_2O 25:75): τ 16.3 min. NMR spectra were identical to those of (*R*)-enantiomer **(*R*)-3**. HRMS, *m/z*: 319.1114 $[M+H]^+$, (calc. for $C_{14}H_{13}F_2N_6O$, *m/z*: 319.1113).

Enzymatic 1-*β*-D-arabinosylation of nucleobases (*R*)-4 and (*S*)-4. General procedure: UP (145 units) and PNP (145 units for (*R*)-**4** or 115 units for (*S*)-**4**) were added to a solution of 4-[6-(2-aminopurin-6-yl)aminohexanoyl]-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-

[1,4]benzoxazine [(*S*)-**4** (25 mg, 58 μmol) or (*R*)-**4** (20 mg, 46 μmol)] and Ara-U (21 mg, 87 μmol, 1.5 equiv.) in 5 mM KH_2PO_4 buffer solution (pH 7.0) (23 ml). The reaction mixture was gently stirred at 55 °C. The reaction progress was monitored by HPLC. After 7 days, warm reaction mixture was extracted by $CHCl_3$ (3×30 mL). The extract was evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (SiO_2, from $CHCl_3$ to 7% EtOH in $CHCl_3$).

(3*R*)-4-[6-(2-Amino-9-(β-D-arabinofuranosyl)purin-6-yl)aminohexanoyl]-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine [(*R*)-5]: yield 17 mg (65 %), amorphous solid, $[\alpha]_D^{20}$ +6 (*c* 0.2, DMF). HPLC: τ 16.2 min (96.6%) [(*R*)-**5**], τ 17.1 min (3.4%) [(*R*)-**4**]. ^{1}H NMR (700 MHz, DMSO-d_6, 30 °C) δ: 1.12 (m, 3 H, Me), 1.36 (m, 2 H, hexanoyl 2×H-4), 1.56-1.65 (m, 4 H, hexanoyl 2×H-3 and 2×H-5), 2.48 (m, 1 H, hexanoyl H-2b, partially overlapped by DMSO signal), 2.67 (m, 1 H, hexanoyl H-2a), 3.41 (m, 2 H, hexanoyl 2×H-6, overlapped by H_2O signal), 3.61 (dd, *J* 11.7, 5.1 Hz, 1 H, Ara H-5b), 3.66 (dd, *J* 11.7, 4.4 Hz, 1 H, Ara H-5a), 3.75 (m, 1 H, Ara H-4), 4.03 (m, 1 H, Ara H-2), 4.08 (m, 1 H, Ara H-3), 4.16 (br. d, *J* 10.6 Hz, 1 H, benzoxazine H-2b), 4.36 (br. d, *J* 10.6 Hz, 1 H, benzoxazine H-2a), 4.71 (m, 1 H, benzoxazine H-3), 5.08 (br. s. 1 H, Ara OH-5), 5.47 (br. s. 1 H, Ara OH-3), 5.61 (br. s. 1 H, Ara OH-2), 5.80 (br. s, 2 H, NH_2), 6.08 (d, *J* 4.6 Hz, 1 H, Ara H-1), 6.93 (ddd, *J* 9.2, 9.2, 8.8 Hz, 1 H, benzoxazine H-6), 7.18 (br. s, 1 H, hexanoyl NH-6), 7.71 (m, 1 H, benzoxazine H-5), 7.77 (s, 1 H, purine H-8). ^{13}C NMR (176 MHz, DMSO-d_6, 30 °C) δ: 15.07 (CH_3), 24.39 (hexanoyl C-3), 25.90 (hexanoyl C-4), 28.86 (hexanoyl C-5), 33.40 (hexanoyl C-2), 39.63 (hexanoyl C-6, overlapped by DMSO signal), 40.93 (benzoxazine C-3), 61.03 (C-5'), 69.79 (benzoxazine C-2), 75.42 and 75.50 (Ara C-2 and C-3), 83.10 (Ara C-1), 84.04 (Ara C-4), 106.73 (d, $J_{C–F}$ 18.3 Hz, benzoxazine C-6), 112.53 (purine C-5), 119.23 (benzoxazine C-5a), 121.74 (benzoxazine C-5), 135.65 (benzoxazine C-8a), 136.11 (m, benzoxazine C-8), 136.55 (purine C-8), 139.37 (dd, $J_{C–F}$ 243.2, 15.0 Hz, benzoxazine C-7), 150.9 (purine C-4), 154.75 and 160.10 (purine C-2 and C-6), 170.95 (hexanoyl CO). HRMS, *m/z*: 564.2297 $[M+H]^+$, (calc. for $C_{25}H_{32}O_5N_7F_2$, *m/z*: 564.2382).

(3*S*)-4-[6-(2-Amino-9-(β-D-arabinofuranosyl)purin-6-yl)aminohexanoyl]-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine [(*S*)-5]: UP (115 units) and PNP (145 units) were added to a solution of compound (*S*)-**4** (25 mg, 58 μmol) and Ara-U (21 mg, 87 μmol, 1.5 equiv.) in 5 mM KH_2PO_4 buffer solution (pH 7.0, 23 mL). A mixture was gently stirred at 55 °C for 7 days. Then the warm reaction mixture was extracted by $CHCl_3$ (3×30 mL). The extract was evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (SiO_2, from $CHCl_3$ to 7 % EtOH in $CHCl_3$). Yield 15 mg (46%), amorphous solid, $[\alpha]_D^{20}$ +50 (*c* 0.2, DMF). ^{1}H NMR (700 MHz, DMSO-d_6, 30 °C) δ: 1.11 (m, 3 H, Me), 1.35 (m, 2 H, hexanoyl 2×H-4), 1.56-1.65 (m, 4 H, hexanoyl 2×H-3 and 2×H-5), 2.48 (m, 1 H, hexanoyl H-2b, partially overlapped by DMSO signal), 2.66 (m, 1 H, hexanoyl H-2a), 3.40 (m, 2 H, hexanoyl 2×H-6, overlapped by H_2O signal), 3.60 (m, 1 H, Ara H-5b), 3.65 (m, 1 H, Ara H-5a), 3.74 (m, 1 H, Ara H-4), 4.02 (m, 1 H, Ara H-2), 4.08 (m, 1 H, Ara H-3), 4.15 (d, *J* 11.0 Hz, 1 H, benzoxazine H-2b), 4.36 (d, *J* 11.0 Hz, 1 H, benzoxazine H-2a), 4.70 (m, 1 H, benzoxazine H-3), 5.07 (m, 1 H, OH-5 Ara), 5.46 (d, *J* 3.9 Hz, 1 H, Ara OH-3), 5.60 (d, *J* 4.0 Hz, 1 H, Ara OH-2), 5.79 (br. s, 2 H, NH_2), 6.08 (d, *J* 4.6 Hz, 1 H, Ara H-1), 6.93 (ddd, *J* 9.8, 9.8, 8.8 Hz, 1 H, benzoxazine H-6), 7.17 (br. s, 1 H, hexanoyl NH-6), 7.63 (m, 2 H, benzoxazine H-5), 7.76 (s, 1 H, purine H-8). ^{13}C NMR (176 MHz, DMSO-d_6, 30 °C) δ: 15.07 (CH_3), 24.40 (hexanoyl C-3), 25.90 (hexanoyl C-4), 28.89 (hexanoyl C-5), 33.40 (hexanoyl C-2), 39.13 (hexanoyl C-6, overlapped by DMSO signal), 39.23 (benzoxazine C-3, overlapped by DMSO signal), 61.03 (Ara C-5), 69.78 (benzoxazine C-2), 75.42 and 75.51 (Ara C-2 and C-3), 83.09 (Ara C-1), 84.03 (Ara C-4), 106.72 (d, $J_{C\text{–}F}$ 17.7 Hz, benzoxazine C-6), 112.49 (purine C-5), 119.21 (benzoxazine C-5a), 121.74 (benzoxazine C-5), 135.63 (d, $J_{C\text{–}F}$ 12 Hz, benzoxazine C-8a), 136.42 (purine C-8), 136.63 (m, benzoxazine C-8), 138.80 (dd, $J_{C\text{–}F}$ 243.0, 14.0 Hz, benzoxazine C-7), 150.64 (purine C-4), 154.75 and 160.00 (purine C-2 and C-6), 170.94 (hexanoyl CO). HRMS, *m/z*: 564.2300 $[M+H]^+$, (calc. for $C_{25}H_{32}O_5N_7F_2$, *m/z*: 564.2382).

(3*R*)-4-[2-Amino-9-(β-D-arabinofuranosyl)purin-6-yl]-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine [(*R*)-6]: UP (180 units) and PNP (290 units) were added to a solution of compound (*R*)-**3** (25 mg, 80 μmol) and Ara-U (29 mg, 120 μmol) in 5 mM KH_2PO_4 buffer solution (pH 7.0) (40 mL). The reaction mixture was gently stirred at 55 °C for 15 days, and then concentrated under reduced pressure to a volume of 2 mL. Resulting solution was subjected to column chromatography (Octadecyl–Si 100 polyol, 0.03 mm, 30×120 mm) using a linear gradient 10 → 40% EtOH−H_2O as an eluent. Yield 15 mg (43%), amorphous solid, $[\alpha]_D^{20}$ +50 (*c* 0.3, DMF). HPLC: τ 15.6 min (96.9%) [(*S*)-**5**]. ^1H NMR (700 MHz, DMSO-d_6, 30 °C) δ: 1.34 (d, *J* 6.6 Hz, 3 H, Me), 3.61 (m, 1 H, Ara H-5B), 3.66 (m, 1 H, Ara H-5A), 3.76 (m, 1 H, Ara H-4), 4.08 (m, 1 H, Ara H-2), 4.10 (m, 1 H, Ara H-3), 4.16 (d, *J* ~ 10.5 Hz, 1 H, benzoxazine H-2B), 4.44 (d, *J* 10.9 Hz, 1 H, benzoxazine H-2A), 5.04 (br. t, *J* 4.8 Hz, 1 H, OH-5 Ara), 5.49 (d, *J* 3.7 Hz, 1 H, OH-3 Ara), 5.62 (d, *J* 5.0 Hz, 1 H, OH-2 Ara), 5.64 (m, 1 H, benzoxazine H-3), 6.15 (br. s, 2 H, NH_2), 6.16 (d, *J* 4.5 Hz, 1 H, Ara H-1), 6.87 (m, 1 H, benzoxazine H-6), 7.60 (m, 2 H, benzoxazine H-5), 7.98 (s, 1 H, purine H-8) ^{13}C NMR (176 MHz, DMSO-d_6, 30 °C) δ: 16.02 (CH_3), 48.45 (benzoxazine C-3), 61.46 (Ara C-5), 70.06 (benzoxazine C-2), 75.83 and 75.97 (Ara C-2 and C-3), 83.68 (Ara C-1), 84.71 (Ara C-4), 107.0 (d, $J_{C\text{–}F}$ 17.8 Hz, benzoxazine C-6), 114.49 (purine C-5), 119.78

(dd, $J_{C–F}$ 9.4, 3.7 Hz, benzoxazine C-5), 123.11 (benzoxazine C-5a), 136.07 (dd, $J_{C–F}$ 10.6, 2.4 Hz benzoxazine C-8a), 139.8 (purine C-8), 139.43 (dd, $J_{C–F}$ = 256.3, 15.0 Hz, benzoxazine C-7), 146.410 (dd, $J_{C–F}$ 239.6, 10.6 Hz, benzoxazine C-8), 152.58, 154.52 and 159.8 (purine C2, C4 and C6).). HRMS, *m/z*: 451.1474 $[M+H]^+$, (calc. for $C_{19}H_{20}O_5N_6F_2$, *m/z*: 451.1546).

References for Supplementary Materials

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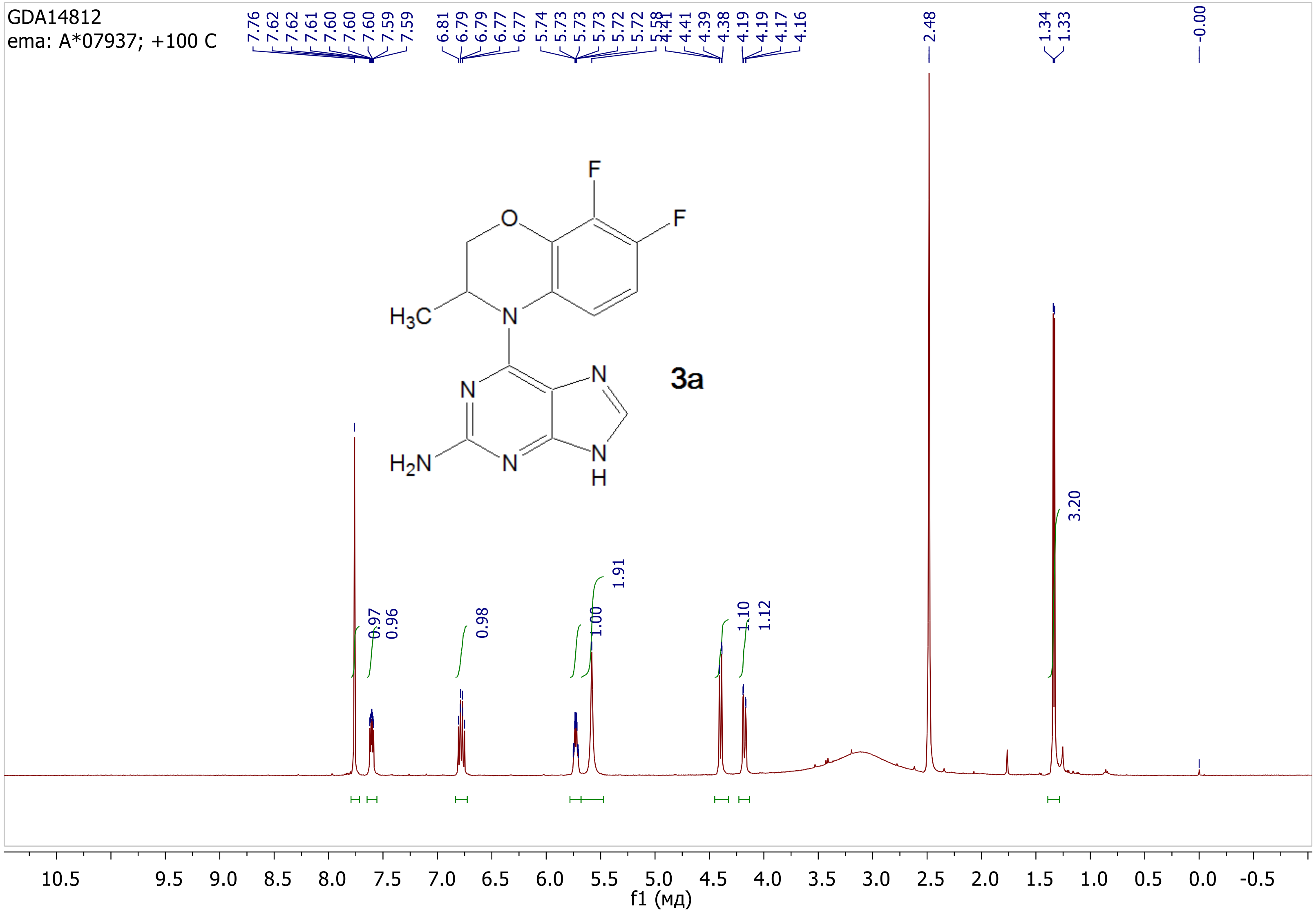

Figure S1 ^{1}H NMR spectrum of compound **3** (500 MHz, DMSO-d_6, 100 °C).

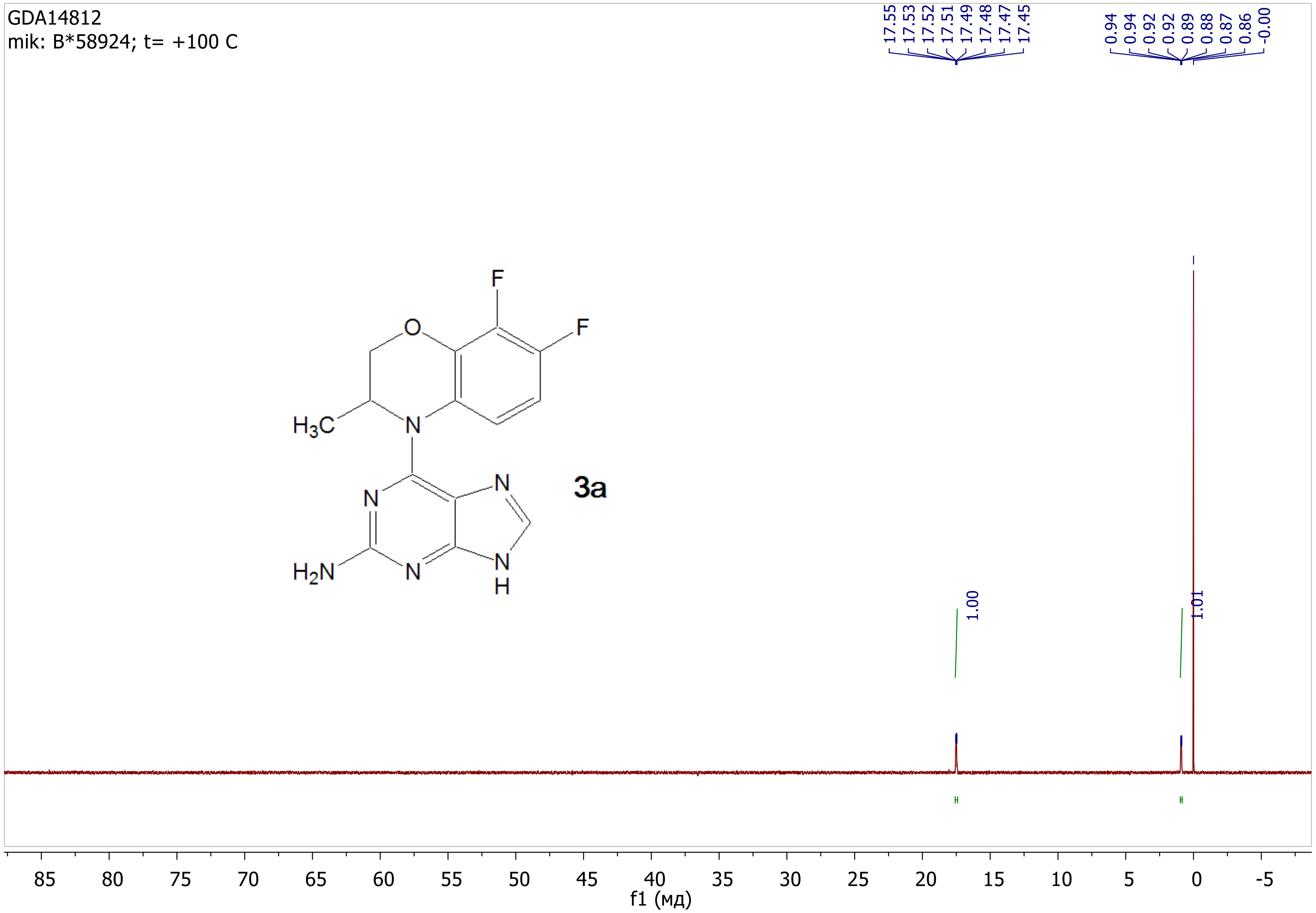

Figure S2 ^{19}F NMR spectrum of compound **3** (470 MHz, DMSO-d_6, 100 °C).

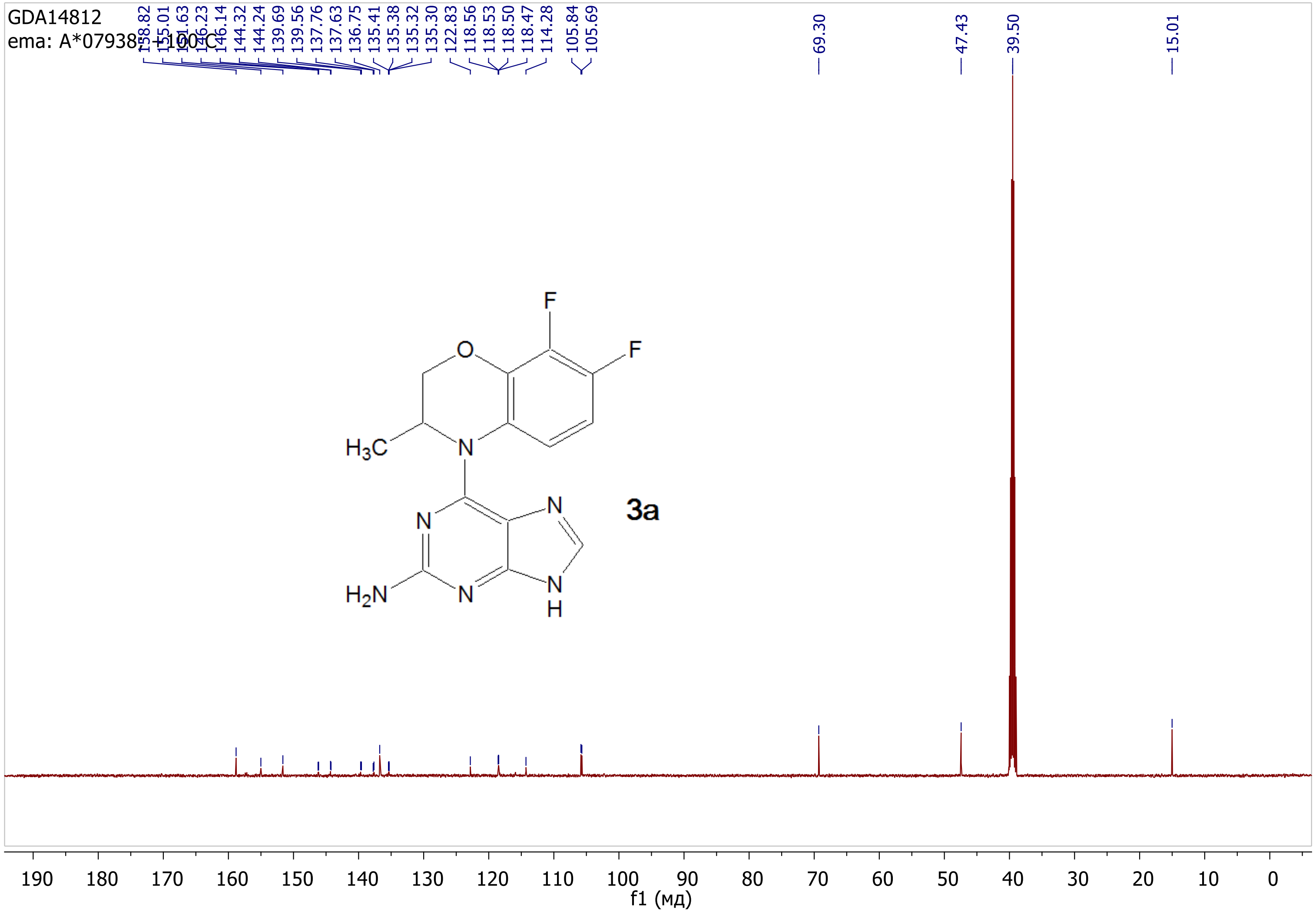

Figure S3 ^{13}C NMR spectrum of compound **3** (125 MHz, DMSO-d_6, 100 °C).

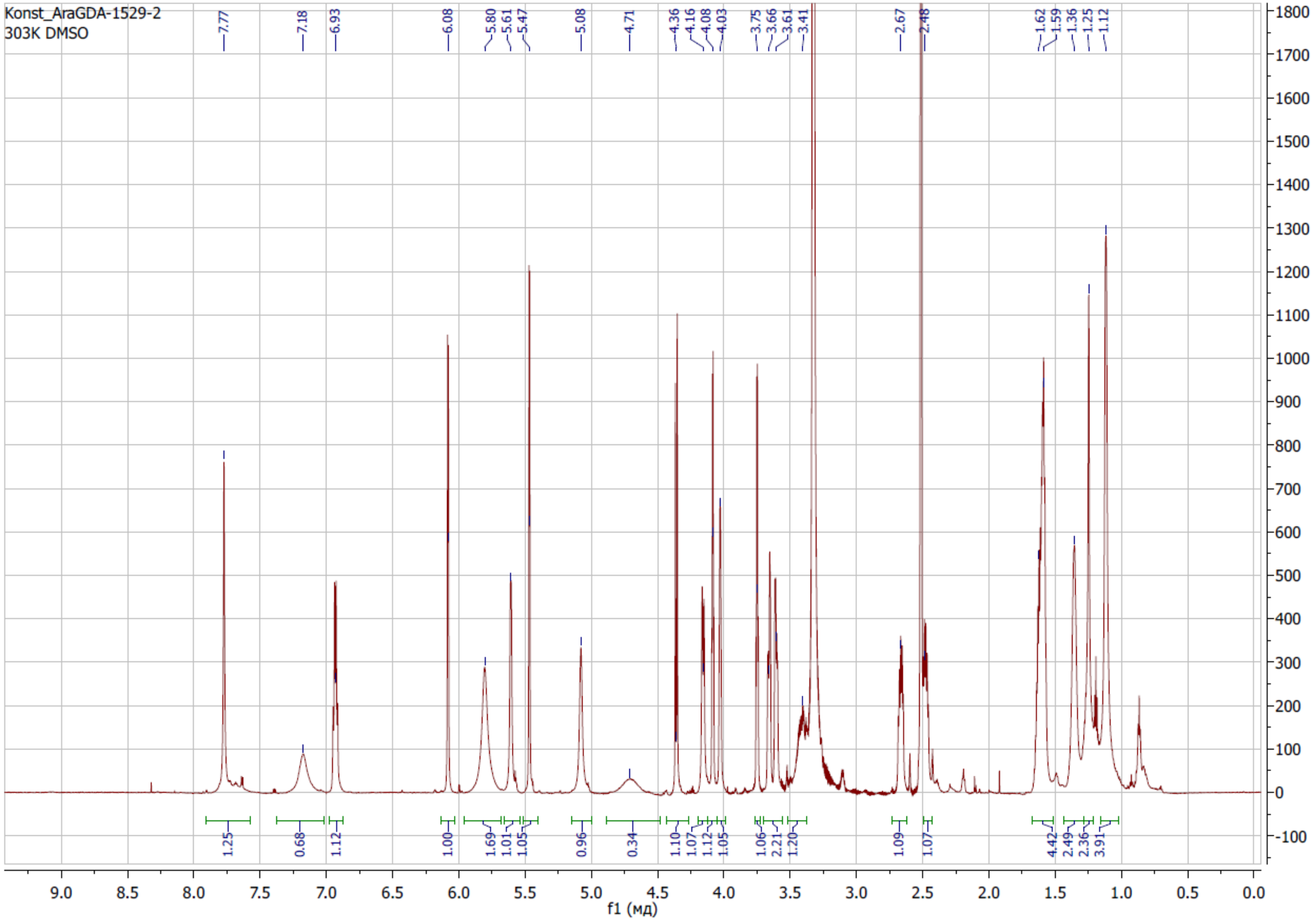

Figure S4 ^{1}H NMR spectrum of compound (*R*)-**5** (700 MHz, DMSO-d_6, 30 °C).

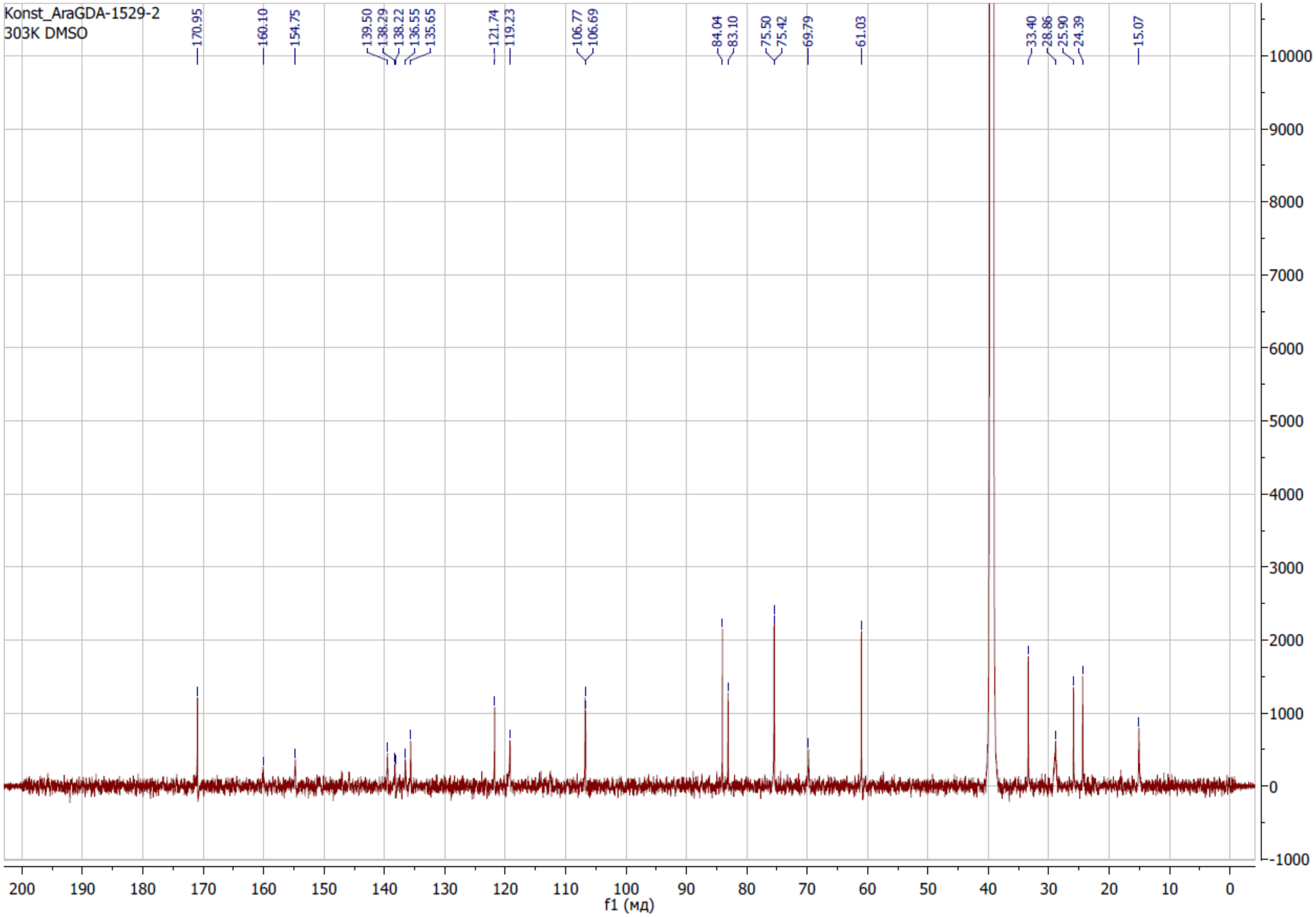

Figure S5 ^{13}C NMR spectrum of compound (*R*)-**5** (176 MHz, DMSO-d_6, 30 °C).

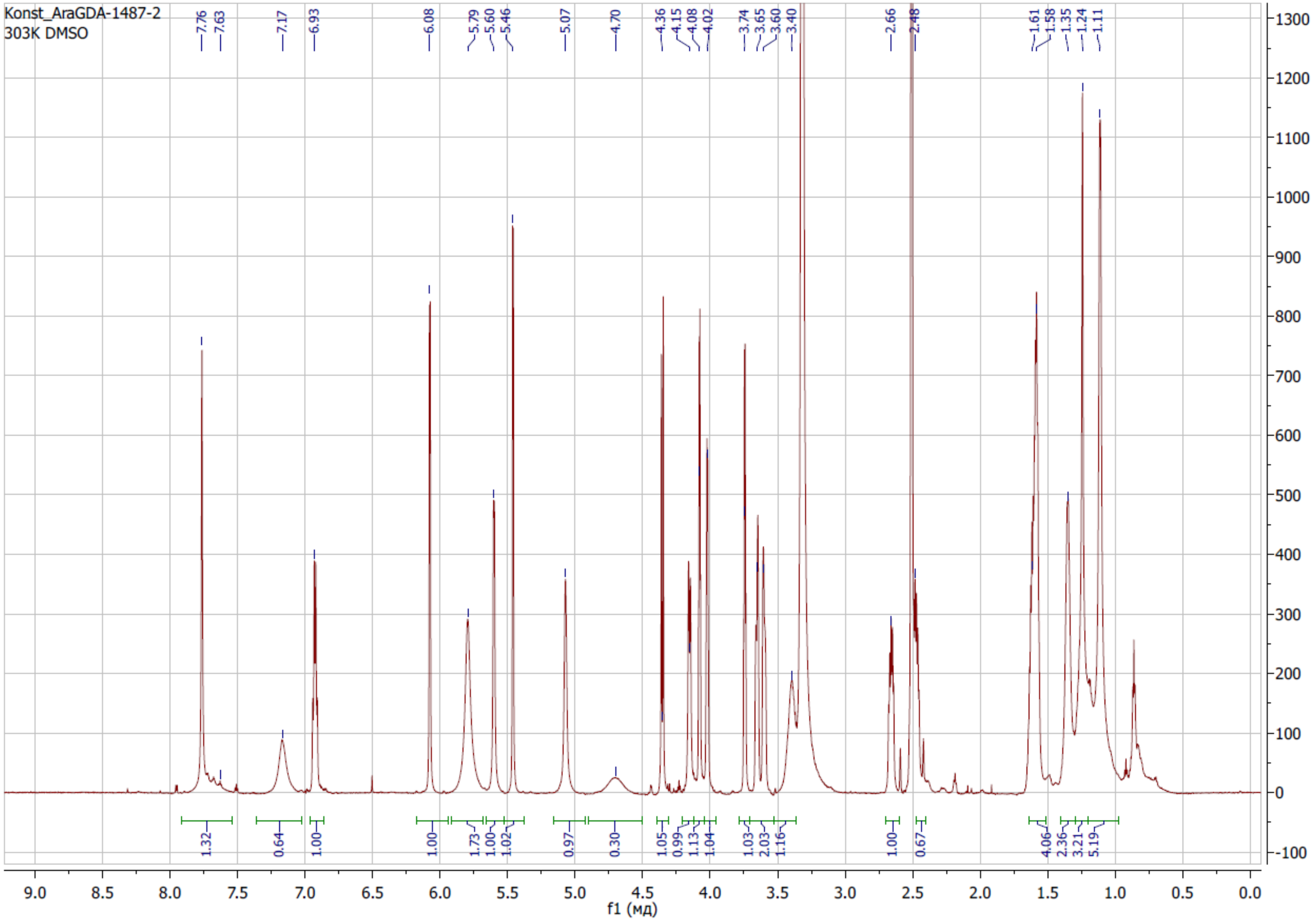

Figure S6 ^{1}H NMR spectrum of compound (*S*)-**5** (700 MHz, DMSO-d_6, 30 °C).

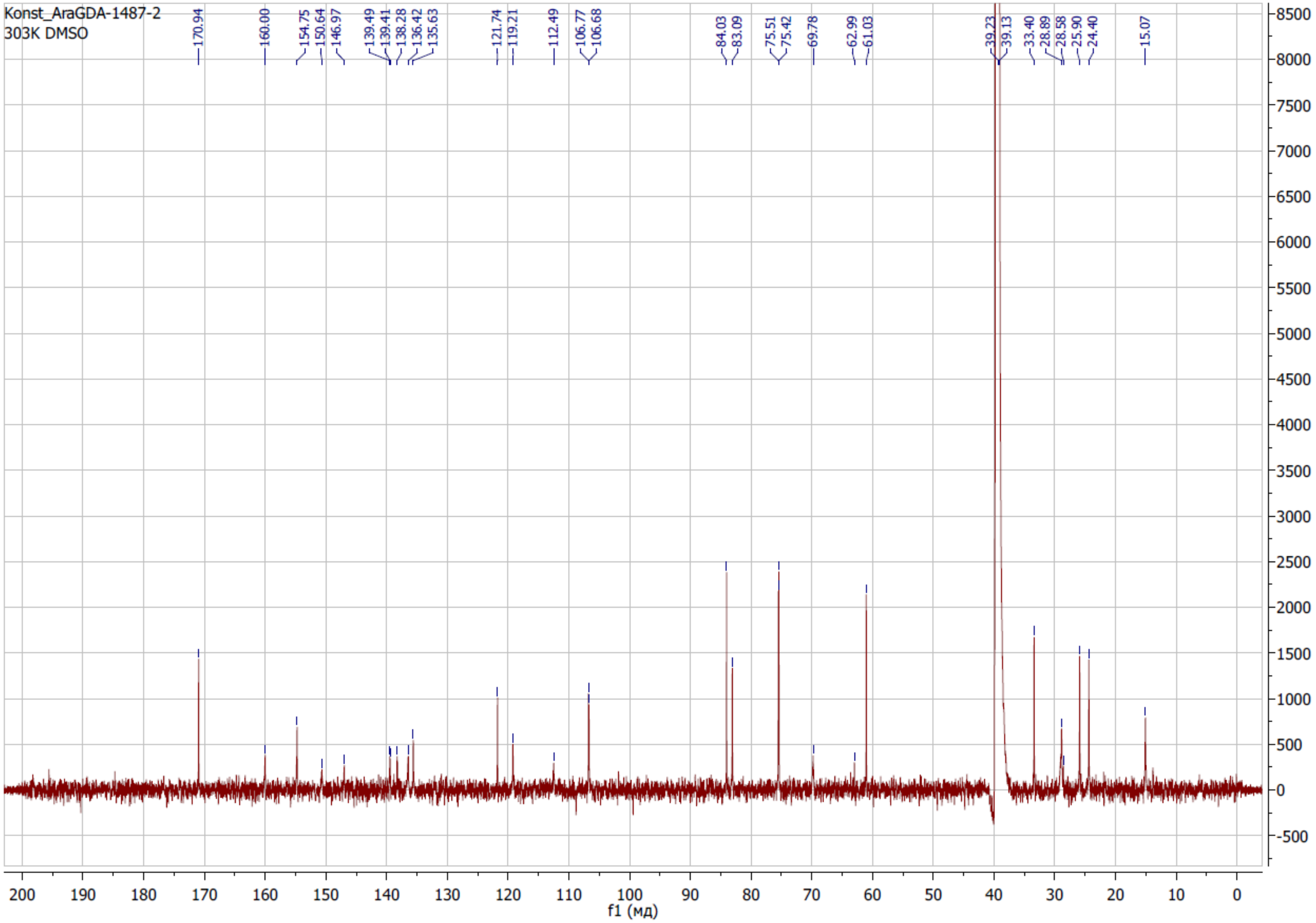

Figure S7 ^{13}C NMR spectrum of compound (*S*)-**5** (176 MHz, DMSO-d_6, 30 °C).

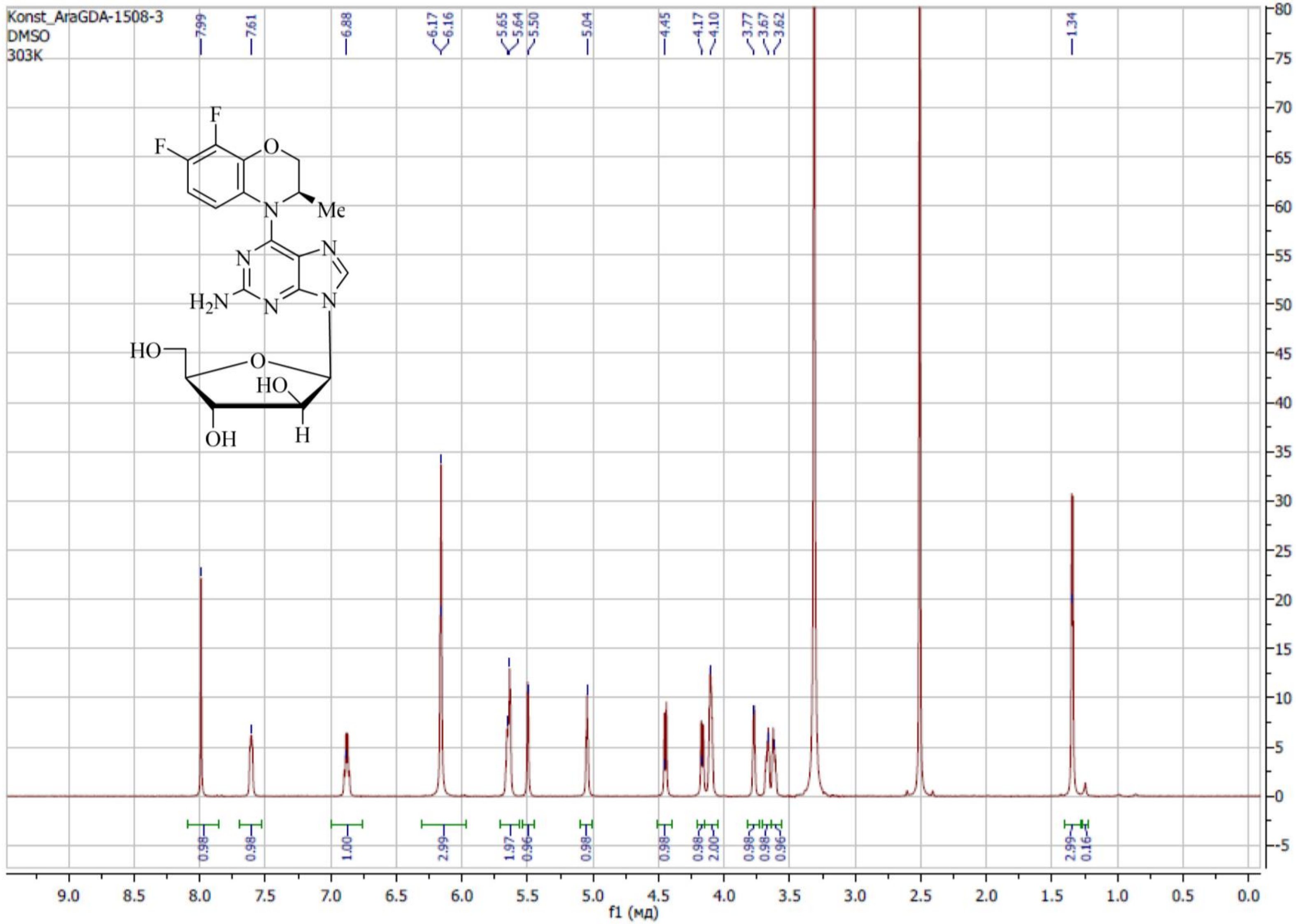

Figure S8 ^{1}H NMR spectrum of compound (*R*)-**6** (700 MHz, DMSO-d_6, 30 °C).

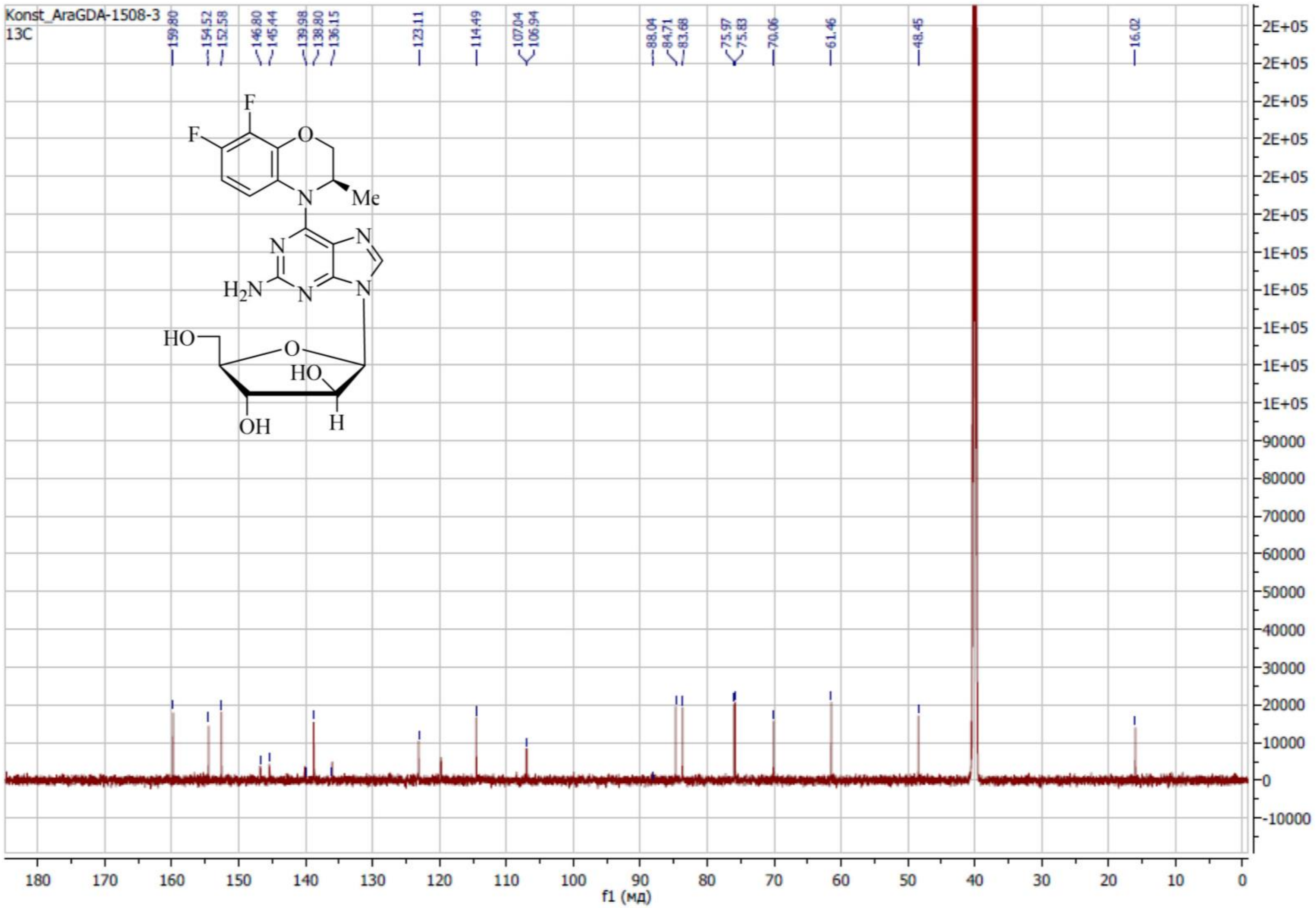

Figure S9 ^{13}C NMR spectrum of compound (*R*)-**6** (176 MHz, DMSO-d_6, 30 °C).

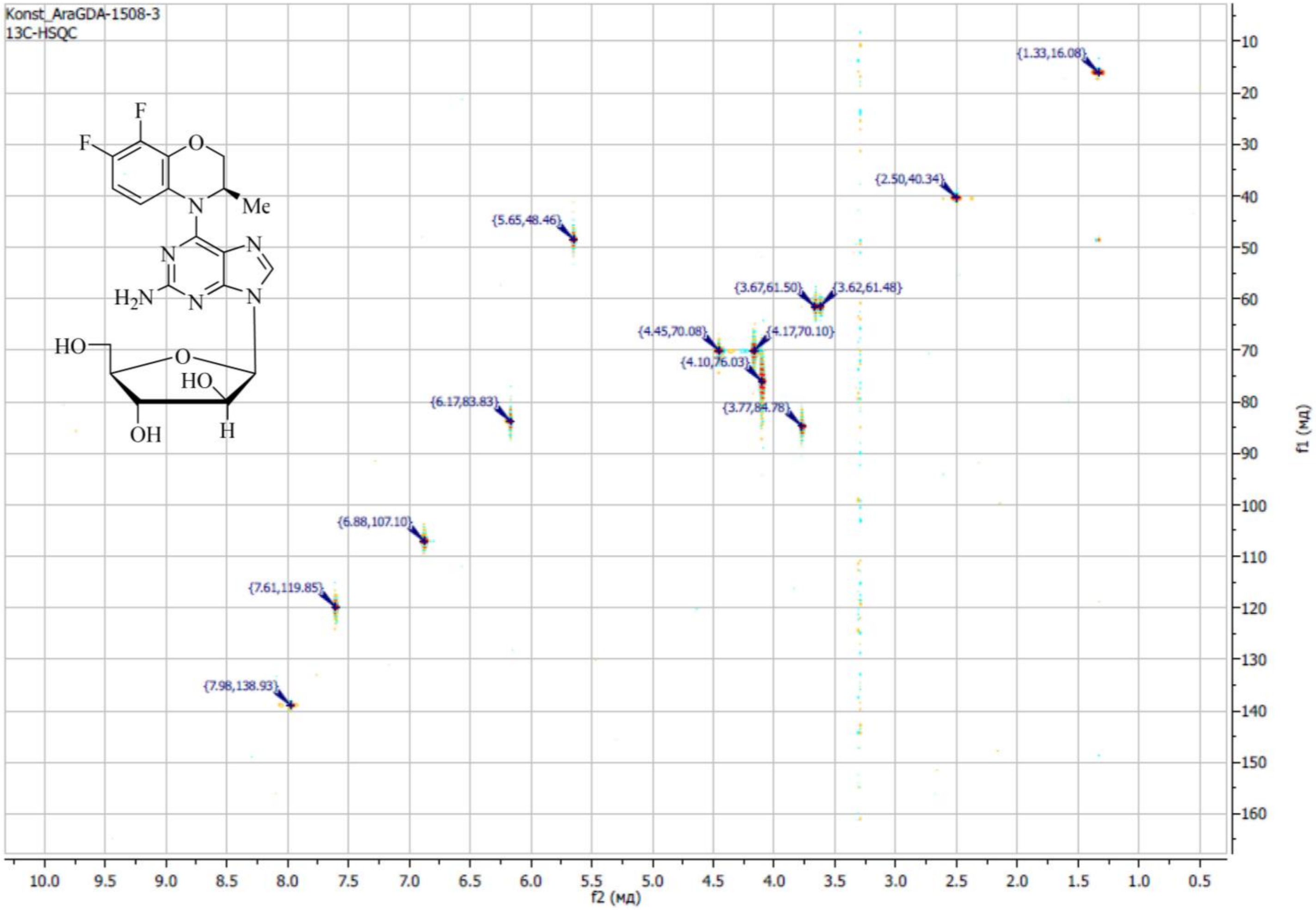

Figure S10 ^{1}H–^{13}C HSQC NMR spectrum of compound (*R*)-**6** (DMSO-d_6, 30 °C).

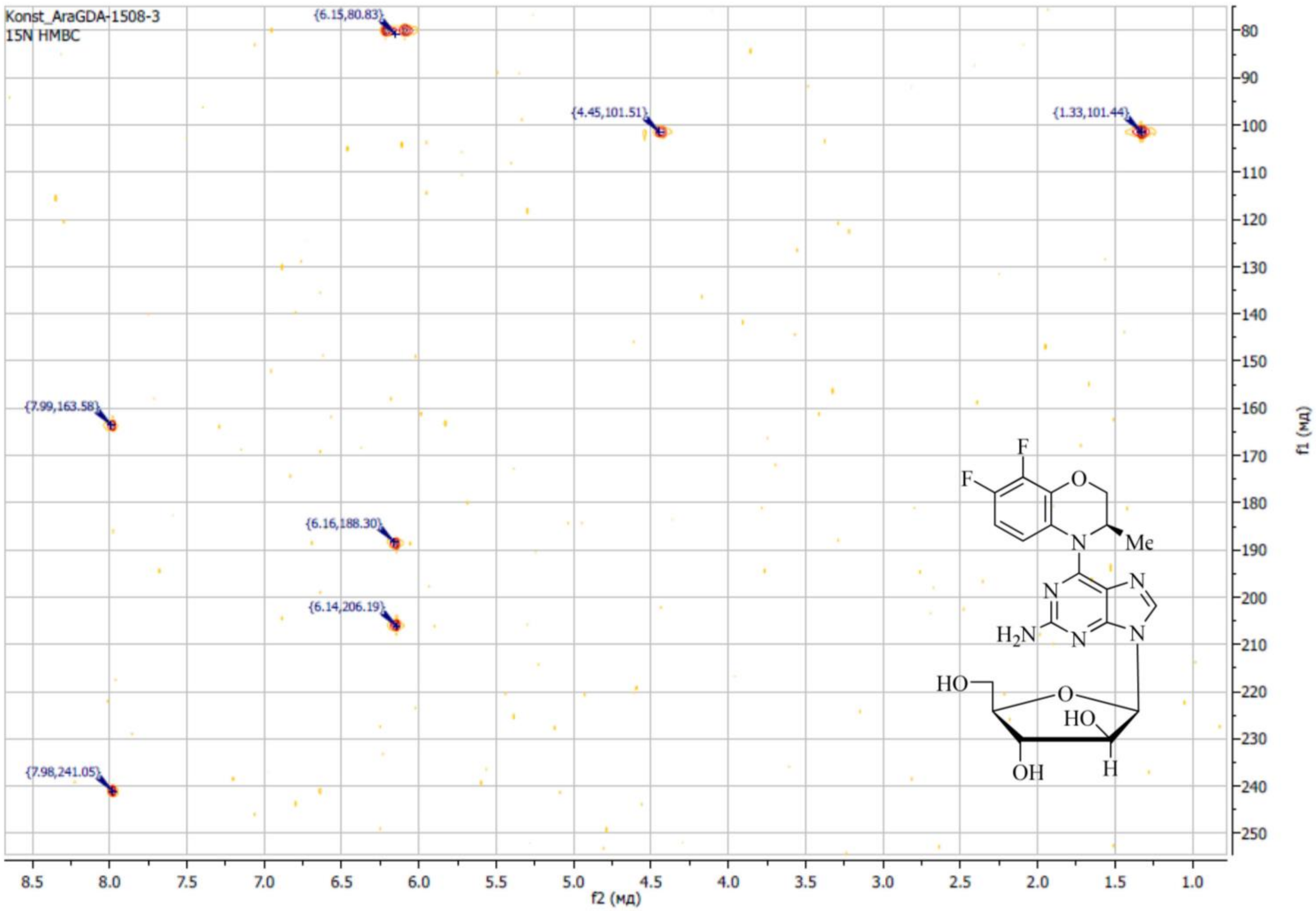

Figure S11 ^{1}H–^{15}N HMBC NMR spectrum of compound (*R*)-**6** (DMSO-d_6, 30 °C).