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SYNTHESIS AND BIOLOGICAL EVALUATION OF INOSINE PHOSPHONATES.

Mikhail Abramov and Piet Herdewijn*

Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, K.U. Leuven,

Minderbroedersstraat 10, BE-3000 Leuven

*Address correspondence to piet.herdewijn@rega.kuleuven.be

EXPERIMENTAL

General. For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware under N_2 . Reagents and solvents were provided by Acros, Fluka, or Pharma Waldhof. TLC: Precoated aluminum sheets (Fluka silica gel/TLC cards, 254 nm); the spots were visualized with UV light. Column chromatography (CC): ICN silica gel 63–200 60 P. ^{1}H-, ^{13}C-and ^{31}P-NMR spectra: a Bruker Avance 300-MHz, or a Bruker Avance 500-MHz spectrometer. For clarity's sake, NMR signals of nucleoside-sugar-moiety H- and C-atoms are indicated with primes. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface; samples were infused in i-PrOH/H_2O 1 : 1 at 3 cm^3/min.

2-(Carboxy(hypoxanthine-9-yl)methoxy)-3-hydroxypropanoic acid 4. To a suspension of 2'-deoxyinosine **3** (1.08 g, 4 mmol) in water (200 cm^3) was added a solution of potassium permanganate (2.52 g, 16 mmol) in water (200 cm^3) and 1N KOH (12 cm^3). After stirring for 2 days the excess of permanganate was destroyed with methanol (2 cm^3) and the reaction mixture was filtered through celite and evaporated to ca 20 cm^3. The solution was acidified to pH 4.5 with 1N HCl and white fine precipitate was filtered off and dried. Yield of **4:** 500 mg (42%), m.p 180-3 ^{o}C (dec). ^{1}H NMR (300 MHz, DMSO-d_6, 25 ^{o}C): δ = 3.60 (d, 2H, *J* 4.3 Hz, H5'), 3.98 (t, 1H, *J* 4.3

Hz, H4'), 6.26 (s, 1H, H1'), 8.08 (s, 1H, H2), 8.12 (s, 1H, H8), 12.44 (br s, 1H, H1). ^{13}C NMR (75 MHz, $CDCl_3$, 25 ^{o}C): δ = 62.1 (C2'), 78.3 (C3'), 79.8 (C1'), 124.5 (C5), 139.4 (C8), 148.9 (C4), 146.8 (C2), 156.9 (C6), 167.0 and 171.0 (COOH)). HRMS calcd for $C_{10}H_{10}N_4O_7$, [MH^-] 297.0471, found 297.0474.

3'-*O*-Acetyl-1-benzyloxymethyl-2'-deoxyinosine 5. To a suspension of 2'-deoxyinosine **3** (7.5 g, 29.7 mmol) in pyridine (150 cm^3) MMTrCl (11.02 g, 35.7 mmol) was added in one portion at RT. The reaction mixture was stirred for 36 h and turn to yellow-orange color. The reaction was monitored by TLC (10% MeOH/DCM). When the starting material has disappeared the reaction mixture was cooled in an ice bath and acetic anhydride (10 cm^3, 106 mmol) was added drop wise using additional funnel. The reaction mixture was stirred overnight at RT and methanol (10 cm^3) was added carefully to decompose excess of Ac_2O. Upon completion the reaction mixture was concentrated and coevaporated twice with toluene. The residue was dissolved in DCM, washed with H_2O, dried over Na_2SO_4, and purified by column chromatography on silica gel with 1:2:98 TEA/MeOH/DCM to 1:4:95 TEA/MeOH/DCM. Yield of 3'-O-acetyl-2'-deoxy-5'-O-monomethoxytrityldeoxyinosine: 15g (90%).

The separated compound was dissolved in DCM (200 cm^3) and cooled in an ice-bath. DIPEA (23.0 cm^3, 132 mmol) and benzyl chloromethyl ether (6.0 cm^3, 32 mmol) were added. The reaction solution was stirred for 2 h at RT. The progress of the reaction was followed by TLC (5:95 MeOH/DCM). Upon completion the reaction mixture was concentrated and coevaporated twice with toluene. The crude material was purified by column chromatography on silica gel with 1.5:100 MeOH/DCM) to yield 3'-*O*-acetyl-1-benzyloxymethyl-2'-deoxy-5'-*O*-monomethoxy-tritylinosine 16g (70%). The separated compound was dissolved in DCM (70 cm^3) and MeOH (30 cm^3) and TsOH (4.81 g, 28.0 mmol) was added at 0 ^{o}C. The reaction solution turns to yellow and was stirred for 30 min under argon at 0 ^{o}C. The reaction was monitored by TLC (5:95 MeOH/DCM). Upon completion, the reaction mixture was neutralized with triethylamine (5.2 cm^3,

37 mmol), washed with water, dried and purified by column chromatography on silica gel using 3:97 MeOH/DCM. Yield of compound **5**: 8.0 g (83%), off white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ^{o}C): δ = 2.14 (s, 3H, Ac), 2.48 (dd, $J_{H,H}$=5.4 Hz, $J_{H,H}$=14.0 Hz, 1H, H2'), 3.00 (m, 1H, H2'), 3.92 (m, 2H, H5'), 4.24 (br s, 1H, OH5'), 4.68 (s, 2H, Bn), 5.06 (dd, $J_{H,H}$=3.5 Hz, $J_{H,H}$=10.0 Hz, 1H, H4'), 5.53 (m, 1H, H3'), 5.58 (s, 2H, CH_2O), 6.25 (dd, $J_{H,H}$=5.4 Hz, $J_{H,H}$=9.8 Hz, 1H, H1'), 7.32 (m, 5H, Ph), 7.91 (s, 1H, H2), 8.13 (s, 1H, H8). ^{13}C NMR (75 MHz, $CDCl_3$, 25 ^{o}C): δ = 21.0 (Ac), 38.4 (C2'), 63.0 (C5'), 72.1 and 74.5 (CH_2OCH_2), 76.0 (C3'), 87.1 (C1'), 87.2 (C4'), 126.5 (C5), 127.9, 128.1, 128.5, and 136.5 (all Ph), 139.7 (C8), 145.2 (C4), 147.3 (C2), 156.3 (C6), 170.3 (Ac). HRMS calcd for $C_{20}H_{22}N_4O_6$, [MH^+] 415.1618, found 415.1601.

1-Benzyloxymethyl-2'-deoxyinosine 5'-carboxylic acid 6. To a colorless solution of compound **5** (3.0 g, 7.2 mmol) in MeCN (30 cm^3) and water (10 cm^3) iodobenzene diacetate (5.1 g, 15.9 mmol) and TEMPO (0.25 g, 1.6 mmol) were added at RT. The yellow reaction solution was stirred for 8 h. The reaction was monitored by TLC (10:90 MeOH/$CHCl_3$). Upon completion, the reaction mixture was concentrated, coevaporated twice with toluene and purified by column chromatography on silica gel with 5-20% MeOH/DCM to yield 3'-acetyl-1-benzyloxymethyl-2'--deoxyinosine 5'-carboxylic acid 3.0 g (97%). The separated compound was dissolved in saturated NH_3 in MeOH (50 cm^3) and kept overnight. The reaction mixture was concentrated, the solid was suspended in DCM (5 cm^3), washed with ether and dry. Yield of compound **6:** 2.5 g (92%), tan solid, m.p.> 250 (dec). ^{1}H NMR (300 MHz, DMSO-d_6, 25 ^{o}C): δ = 2.23 (m, 2H, H2'), 4.15 (m, 1H, H3'), 4.41 (m, 1H, H4'), 4.60 (s 2H, CH_2O), 5.51 (s 2H, CH_2O), 6.39 (m, 1H, H1'), 7.29 (m, 5H, Ph), 8.46 (s, 1H, H2), 9.17 (s, 1H, H8). ^{13}C NMR (75 MHz, $CDCl_3$, 25 ^{o}C): δ = 41.7 (C2'), 66.8 (C3'), 71.0 (C1'), 74.9 and 75.2 (CH_2OCH_2), 84.8 (C4'), 123.3 (C5), 128.0, 128.7, and 137.9 (all Ph), 141.1 (C8), 147.6 (C4), 14149.1 (C2), 156.5 (C6), 174.0 (COOH)). HRMS calcd for $C_{18}H_{18}N_4O_6$, [MH^+] 387.1307, found 387.1292.

1- Benzyloxymethyl -9-(2',3'-dideoxy-3',4'-didehydro-β-D-erythrofuranosyl)hypoxanthine 7. To a tan solution of carboxylic acid **6** (2.3 g, 6.0 mmol) in DMF (20 cm^3) N,N-dimethylformamide dineopentyl acetal (12. 0 cm^3, 43 mmol) was added using a syringe. The reaction solution was stirred for 6 h under argon at 130 °C. The progress of the reaction was followed by TLC (20:80 MeOH/$CHCl_3$). Upon completion the reaction mixture was cooled and concentrated. The crude product was purified by column chromatography on silica gel using 3:97 MeOH/DCM. Yield of compound **7:** 1.6 g (76%), light-brown glass. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ^{o}C): δ = 2.96 (m, 1H, H2'), 3.84 (m, 1H, H2'), 4.68 (s, 2H, Bn), 5.26 (dd, $J_{H,H}$=2.7 Hz, $J_{H,H}$=5.0 Hz, 1H, H3'), 5.59 (s, 2H, CH_2O), 6.50 (dd, $J_{H,H}$=2.7 Hz, $J_{H,H}$=5.0 Hz, 1H, H4'), 6.70 (dd, $J_{H,H}$=3.5 Hz, $J_{H,H}$=9.4 Hz, 1H, H1'), 7.32 (m, 5H, Ph), 7.91 (s, 1H, H2), 8.13 (s, 1H, H8). ^{13}C NMR (75 MHz, $CDCl_3$, 25 ^{o}C): δ = 36.1 (C2'), 71.8 and 74.2 (CH_2OCH_2), 83.3 (C1'), 99.5 (C3'), 124.0 (C5), 127.9, 128.1, 128.5, and 136.7 (all Ph), 137.3 (C8), 144.7 (C2), 146.9 (C4), 147.6 (C4'), 156.3 (C6). HRMS calcd for $C_{17}H_{16}N_4O_3$, [MH^+] 325.1301, found 325.1290.

1- Benzyloxymethyl -9-(4'-β-(dibenzylphosphono)methoxy -2',3'-dideoxy-2',3'-didehydro-β-D-erythrofuranosyl)hypoxanthine 8. To a light yellow solution of 1- benzyloxymethyl -9-(2',3'-dideoxy-3',4'-didehydro-β-D-erythrofuranosyl)hypoxanthine **7** (290 mg, 0.89 mmol) in DCM (5 cm^3) PhSeCl (188 mg, 0.98 mmol) was added at -70 ^{o}C. The reaction suspension was stirred for 1 h under argon at -50 ^{o}C . Dibenzyl (hydroxymethyl)phosphonate [11] (523 mg, 1.8 mmol) was added in one portion and reaction mixture was kept overnight at -20 ^{o}C. Lithium perchlorate (114 mg, 1.1 mmol) was added and the mixture was allowed to warm to 0 ^{o}C. The reaction mixture was diluted with DCM (40 cm^3), washed with 5% $NaHCO_3$ and dried. The crude product was purified by column chromatography with 2:98 MeOH/DCM. To the collected compound (290 mg, 0.38 mmol) 30% H_2O_2 (2 eq) was added and the reaction mixture was stirred for 1 h. The reaction was monitored by TLC (5:95 MeOH/DCM). Upon completion the reaction mixture was concentrated

and the crude product was purified by column chromatography on silica gel using 3:97 MeOH/DCM. Yield of compound **8:** 104 mg (45%), white foam.

Alternatively, to a brown solution of **7** (350 mg, 1.08 mmol) and dibenzyl (hydroxymethyl)phosphonate (630 mg, 2.16 mmol) in DCM (20 cm^3) 1M iodine monobromide DCM (1.62 cm^3, 1.62 mmol) was added at -25 ^oC. The reaction suspension was stirred with a magnetic stir bar for 45 min under argon at -35 ^oC and turned to orange color. The reaction mixture was diluted with DCM (20 cm^3) washed with bisulfite and sat $NaHCO_3$. The crude product was purified by column chromatography on silica gel with 2:98 MeOH/DCM. A tan solution of this compound (300 mg, 0.40 mmol, 37 % yield) in dioxane (10 cm^3) and DBU (0.18 cm^3, 1.2 mmol) was stirred for 1.5 h under argon at 65 ^oC. Upon completion the reaction mixture was cooled and concentrated. The residue was dissolved in DCM and washed with 5% AcOH (20 cm^3). The crude product was purified by column chromatography on silica gel using 3:97 MeOH/DCM. Yield of **8:** 200 mg (80%), tan foam.

^1H NMR (300 MHz, $CDCl_3$, 25 ^oC): δ = 3.82 (dd, $J_{H,H}$=8.0 Hz, $J_{H,H}$=14.0 Hz, 1H, H5'), 3.97 (dd, $J_{H,H}$=9.2 Hz, $J_{H,H}$=14.0 Hz, 1H, H5'), 4.67 (s, 2H, CH_2O), 5.08 (m, 4H, Bn), 5.56 (s, 2H, CH_2O), 5.83 (s, 1H, H4'), 6.27 (m, 2H, H2', H3'), 6.84 (s, 1H H1'), 7.32 (m, 15H, Ph), 7.83 (s, 1H, H2), 8.12 (s, 1H, H8). ^{31}P NMR (121 MHz, $CDCl_3$, 25 ^oC): δ = 21.3. HRMS, calcd for $C_{32}H_{31}N_4O_7P$, [MH^-] 615.2009, found 615.1998.

9-(4'-β-phosphonomethoxy -β-D-erythrofuranosyl)hypoxanthine di(triethylammonium) salt 1. To a colorless solution of 1-benzyloxymethyl -9-(4'-β-(dibenzylphosphono)methoxy -2',3'-dideoxy-2',3'-didehydro-β-D-erythrofuranosyl)hypoxanthine **8** (60 mg, 0.1 mmol) and N-methylmorpholine N-oxide (29.7 mg, 0.25 mmol) in acetone (2 cm^3) and water (0.5 cm^3) OsO_4 in t-butanol (0.122 cm^3, 9.76 μmol) was added. The reaction solution was stirred for 8 h under argon at RT. The reaction was monitored by TLC (10/90 MeOH/$CHCl_3$). Upon completion the reaction mixture was concentrated and the crude product was purified by column chromatography on silica

gel with 7:93 MeOH/DCM. Yield of protected hypoxantine phosphonate: (45 mg, 71%). A suspension of protected hypoxantine phosphonate (20 mg) and 5% Pd/C (10 mg) in methanol (2 cm^3) and 1 M TEAB (200 uL) was hydrogenated overnight at normal pressure. The reaction was monitored by TLC. 10:90 MeOH/$CHCl_3$. Upon completion, the reaction mixture was filtered through Celite and solvent was removed under reduced pressure. The yield of the obtained compound **1** as a white lyophilized powder was quantitive. Additional purification could be performed on a Source 15Q ion exchange column (Amersham Biosciences) using a TEAB gradient from 0 to 0.5 M in 30 minutes. ^{1}H NMR (300 MHz, D_2O, 25 ^{o}C): δ = 3.51 (dd, $J_{H,H}$=9.0 Hz, $J_{H,H}$=13.0 Hz, 1H, H5'), 3.70 (dd, $J_{H,H}$=9.0 Hz, $J_{H,H}$=13.0 Hz, 1H, H5'), 4.30 (d, $J_{H,H}$=4.6 Hz, 1H, H3'), 4.92 (dd, $J_{H,H}$=4.6 Hz, $J_{H,H}$=6.2 Hz, 1H, H2'), 5.17 (s, 1H, H4'), 6.14 (d, $J_{H,H}$=6.3 Hz, 1H, H1'), 8.11 (s, 1H, H2), 8.31 (s, 1H, H8). ^{13}C NMR (75 MHz, D_2O, 25 ^{o}C): δ = 64.2 (d, J=157 Hz, C5'), 73.5(C2'), 74.5(C3'), 86.9(C1'), 109.1(C4'), 123.2(C5), 139.7(C8)145.9(C2), 148.8(C4), 158.2(C6). ^{31}P NMR (121 MHz, D_2O, 25 ^{o}C): δ = 14.2. HRMS, calcd for $C_{10}H_{13}N_4O_8P$, [MH^-] 347.0394, found 347.0392.

9-(2', 3'-dideoxy-4'-β-phosphonomethoxy -β-D-erythrofuranosyl)hypoxanthine di(triethylammonium) salt 2. A suspension of protected hypoxantine phosphonate **8** (25 mg) and 5% Pd/C (10 mg) in methanol (2 cm^3) and 1 M TEAB (200 uL) was hydrogenated overnight at normal pressure. The reaction was monitored by TLC (10:90 MeOH/$CHCl_3$). Upon completion the reaction mixture was filtered through Celite and solvent was removed under reduced pressure. The yield of the obtained compound **2** as a tan lyophilized powder was quantitive. Additional purification could be performed on a Source 15Q ion exchange column (Amersham Biosciences) using a TEAB gradient from 0 to 0.5 M in 30 minutes. ^{1}H NMR (300 MHz, D_2O, 25 ^{o}C): δ = 2.20 (m, 2H, H3'), 2.56 (m, 2H, H2'), 3.42-3.61 (m, 2H, H5'), 5.35 (m, 1H, H4'), 6.40 (m, 2H, H1'), 8.12 (s, 1H, H2), 8.38 (s, 1H, H8). ^{31}P NMR (121 MHz, D_2O, 25 ^{o}C): δ = 15.5. HRMS, calcd for $C_{10}H_{15}N_4O_8P$, [MH^-] 315.0494, found 315.0494.