

Synthesis of nucleoside analogues in a ball mill: fast, chemoselective and high yielding acylation without undesirable solvents

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SUPPORTING INFORMATION

All chemicals were purchased from commercial suppliers (Sigma, Aldrich or Fluka) and used without further purification.

¹H or ¹³C NMR spectra were recorded on a Bruker DPX-300, III-400 or DRX-500 at 20°C. All spectra were recorded at ambient temperature in either CDCl₃ or *d*₆-DMSO. Chemical shifts are quoted relative to TMS in CDCl₃ or DMSO in *d*₆-DMSO. HPLC grade solvents were used as supplied by Sigma-Aldrich.

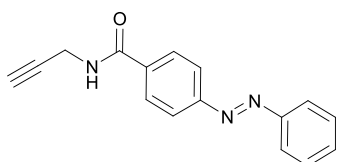
Mass spectra were recorded using a Waters GCT Premier (EI) or VG Quattro II Triple Quadrupole Mass Spectrometer (Electrospray). Mass spectrometry was performed by Analytical Services and Environmental Projects (ASEP) at Queen's University of Belfast.

Melting points were recorded using a Stuart SMP10.

Samples from ball-milled reaction mixtures to be analysed by RP-HPLC were removed from the vessel by adding the HPLC eluting solvent (see below) to effect complete dissolution of the reactions mixtures. Aliquots (5 - 20 µl) of these solutions containing equimolar quantities of the azobenzene were injected onto the HPLC (Merck Hitachi D7000 system) and chromatograms were recorded using a Merck Hitachi Diode Array Detector L-7455, following isocratic elution at 1.0 ml min⁻¹ using 100 mM triethylammonium acetate, pH 6.5 in 65:35 (v/v) acetonitrile / water (18.2 MΩ) as the mobile phase and a Phenomenex Clarity 5µm Oligo-RP (150 x 4.60 mm) as the stationary phase.

All ball-mill reactions were performed using a Retsch Mixer Mills MM 400 according to the conditions described in the main text and below. The hydration levels of DABCO, DBU and DMAP were analysed by ¹H NMR (400MHz) and found to be less than 2%.

N-(*p*-phenylazo)benzamido propyne (3a).

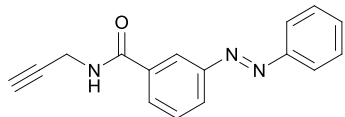


A stainless steel jar (25 ml) was charged with a mixture of *p*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**2a**)¹ (399 mg, 1.23 mmol), propargylamine (160 µl, 2.50 mmol, 2 eq.), DMAP (243 mg, 1.99 mmol, 1.6 eq.) and a 15.0 mm stainless steel ball. The vessel was vibrated at

28 Hz for 10 minutes and then was washed with ethyl acetate (2x10 ml). This solution was transferred to a separating funnel and further diluted with ethyl acetate (40 ml) and then washed with 2M HCl (aq) (3 x 25 ml) followed by saturated Na₂CO₃ (aq) (3 x 25 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and reduced *in vacuo* without further purification to yield pure **3a** (305 mg, 1.16 mmol, 94%). m.p. 173-175°C. ¹H NMR (300 MHz, CDCl₃, TMS) δ = 2.31 (1H, t, *J*_{1,2} = 2.5, CH), 4.29 (2H, dd, *J*_{2,1} = 2.5, *J* = 5.2, CH₂), 6.43 (1H, br s, NH), 7.49 – 7.57 (3H, m, *m*-, *p*-PhN₂), 7.91 – 7.99 (6H, m, *o*-PhN₂, *o*-,

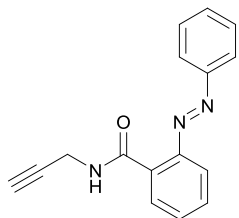
m-PhCO); ^{13}C NMR (76MHz, CDCl_3 , TMS) δ = 30.3, 72.6, 79.7, 123.4, 123.5, 128.5, 129.6, 132.1, 135.8, 152.9, 154.8, 166.8; EI + MS m/z = 263 (M), calc. 263.1059, found 263.1072.

N-(*m*-phenylazo)benzamido propyne (**3b**).



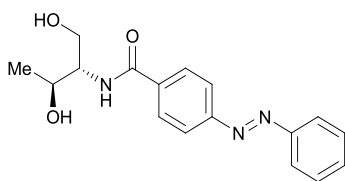
A stainless steel jar (25 ml) was charged with a mixture of *m*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**2b**)¹ (398 mg, 1.23 mmol), propargylamine (160 μl , 2.50 mmol, 2 eq.), DMAP (247 mg, 2.02 mmol, 1.6 eq.), and a 15.0 mm stainless steel ball. The vessel was vibrated at 28 Hz for 10 minutes and then was washed with ethyl acetate (2x10 ml). This solution was transferred to a separating funnel and further diluted with ethyl acetate (40 ml) and then washed with 2M HCl (aq) (3 x 25 ml) followed by saturated Na_2CO_3 (aq) (3 x 25 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and reduced *in vacuo* without further purification to yield pure **3b** (298 mg, 1.13 mmol, 92 %). m.p. 129–131 °C. ^1H NMR(300 MHz, CDCl_3 , TMS) δ = 2.31 (1H, t, $J_{1,2}$ = 2.5, CH), 4.30 (2H, dd, $J_{1,2}$ = 2.5, J = 5.2, CH_2), 6.47 (1H, br s, NH), 7.45 – 7.65 (4H, m, *p*-, *m*- PhN_2 , *m*-PhCO), 7.90 – 7.99 (3H, m, *o*- PhN_2 , *p*- N_2PhCO), 8.04 – 8.11 (1H, m, *p*-PhCO), 8.27 (1H, t, J = 1.68, *o*-PhCO); ^{13}C NMR (76MHz, CDCl_3 , TMS) δ = 29.9, 72.1, 79.3, 120.5, 123.0, 126.4, 129.2, 129.6, 129.6, 131.5, 134.8, 152.4, 152.5, 166.37; EI + MS m/z = 263 (M), calc. 263.1059, found 263.1058.

N-(*o*-phenylazo)benzamido propyne (**3c**).



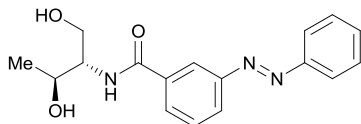
A stainless steel jar (25 ml) was charged with a mixture of *o*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**2c**)¹ (449 mg, 1.39 mmol), propargylamine (200 μl , 2.90 mmol, 2 eq.), DMAP (300 mg, 2.45 mmol, 1.6 eq.), and a 15.0 mm stainless steel ball. The vessel was vibrated at 28 Hz for 10 minutes and then was washed with ethyl acetate (2x10 ml). This solution was transferred to a separating funnel and further diluted with ethyl acetate (40 ml) and then washed with 2M HCl (aq) (3 x 25 ml) followed by saturated Na_2CO_3 (aq) (3 x 25 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and reduced *in vacuo* without further purification to yield pure **3c** (331 mg, 1.09 mmol, 90 %). m.p. 103–105 °C. ^1H NMR(300 MHz, CDCl_3 , TMS) δ = 2.31 (1H, t, $J_{1,2}$ = 2.6, CH), 4.33 (2H, dd, $J_{1,2}$ = 2.6, J = 4.7, CH_2), 7.53 – 7.65 (5H, m, *m*-, *p*- PhN_2 , *p*- N_2PhCO , *p*-COPh N_2), 7.81 – 7.88 (1H, m, *o*- N_2PhCO), 7.89 – 7.97 (2H, m, *o*-Ph N_2), 8.37 – 8.45 (1H, m, *o*-PhCO), 9.07 (1H, br s, NH); ^{13}C NMR (76MHz, CDCl_3 , TMS) δ = 29.9, 71.8, 79.8, 115.7, 123.5, 129.4, 130.5, 131.6, 131.7, 132.0, 132.3, 149.3, 152.3, 165.3; EI + MS m/z = 263 (M), calc. 263.1059, found 263.1053.

2S,3S-(2-*N*-(*p*-phenylazo)benzamido)-1,3-dihydroxybutane (**6a**).



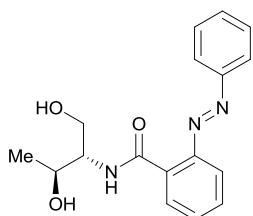
A stainless steel jar (25 ml) was charged with a mixture of *p*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**2a**) (155 mg, 0.48 mmol), D-threoinol (102 mg, 0.99 mmol, 2 eq.), and DMAP (91 mg, 0.74 mmol, 1.5 eq.), 50 μ l (0.51 mmol) of ethyl acetate and a 15.0 mm stainless steel ball. The vessel was vibrated at 28 Hz for 10 minutes, washed with ethyl acetate (2x5 ml) and the solution applied to a silica gel column eluting with ethyl acetate. Appropriate fractions were reduced *in vacuo* to yield pure **6a** (119 mg, 0.38 mmol, 79 %). ^1H NMR (300MHz, d^6 -DMSO) δ = 1.09 (3H, d, J = 6.3, CH_3), 3.58 – 3.66 (2H, m, CH_2OH), 3.87 – 3.99 (2H, m, 2 x CH), 4.58 – 4.68 (2H, m, 2xOH), 7.57 – 7.67 (3H, m, *m*-, *p*-PhN₂), 7.90 – 8.00 (5H, m, *o*-PhN₂, *m*-PhCO, NH), 8.06 – 8.13 (2H, m, *o*-PhCO); ^{13}C NMR (76MHz, d^6 -DMSO) δ = 20.5, 57.1, 60.7, 65.2, 122.6, 123.1, 129.0, 129.9, 132.4, 137.3, 152.3, 153.6, 166.2; ES - MS ($\text{C}_{17}\text{H}_{18}\text{N}_3\text{O}_3$), m/z = 312 (M - H), calc. 312.1348, found 312.1350

2S,3S-(2-*N*-(*m*-phenylazo)benzamido)-1,3-dihydroxybutane (**6b**).



A stainless steel jar (25 ml) was charged with a mixture of *m*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**2b**) (123 mg, 0.38 mmol), D-threoinol (83 mg, 0.79 mmol, 2 eq.), DMAP (53 mg, 0.43 mmol, 1.5 eq.), 50 μ l (0.51 mmol) of ethyl acetate and a 15.0 mm stainless steel ball. The vessel was vibrated at 28 Hz for 10 minutes, washed with ethyl acetate (2x5 ml) and the solution applied to a silica gel column eluting with ethyl acetate. Appropriate fractions were reduced *in vacuo* to yield pure **6b** (2 mg, 0.29 mmol, 76 %). Mp 146–148 °C. ^1H NMR (300MHz, d^6 -DMSO) δ = 1.10 (3H, d, J = 6.1, CH_3), 3.45 – 3.70 (2H, m, CH_2OH), 3.87 – 4.02 (2H, m, 2 x CH), 4.58 – 4.71 (2H, m, 2xOH), 7.52 – 7.76 (4H, m, *m*-, *p*-PhN₂, *m*-N₂PhCO), 7.87 – 7.98 (2H, m, NH, *p*-PhCO), 7.99 – 8.12 (3H, m, *p*-N₂PhCO, *o*-PhN₂), 8.36 – 8.42 (1H, m, *o*-PhCO); ^{13}C NMR (76MHz, d^6 -DMSO) δ = 20.1, 56.9, 60.3, 64.8, 121.5, 122.5, 124.5, 129.3, 129.4, 130.1, 131.7, 136.1; 151.6, 151.8, 165.7; ES + MS ($\text{C}_{17}\text{H}_{20}\text{N}_3\text{O}_3$), m/z = 314 (M + H⁺), calc. 314.1505, found 314.1511.

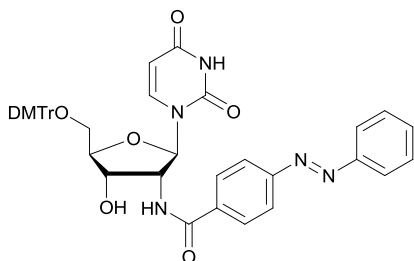
2S,3S-(2-*N*-(*o*-phenylazo)benzamido)-1,3-dihydroxybutane (**6c**).



A stainless steel jar (25 ml) was charged with a mixture of *o*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**1c**) (165 mg, 0.51 mmol), D-threoinol (116 mg, 1.10 mmol, 2 eq.), DMAP (106 mg, 0.87 mmol, 1.5 eq.), 50 μ l (0.51 mmol) of ethyl acetate and a 15.0 mm stainless steel ball. The vessel was vibrated at 28 Hz for 10 minutes, washed with ethyl acetate (2x5 ml) and the solution applied to a silica gel column eluting with ethyl acetate. Appropriate fractions were reduced *in vacuo* to yield pure **6c** (132 mg, 0.42 mmol, 83 %). Mp 136–138 °C. ^1H NMR (300MHz, d^6 -DMSO) δ = 1.07 (3H, d, J = 6.3, CH_3), 3.43 – 3.60 (2H, m, CH_2OH), 3.90 – 4.06 (2H, m, 2 x CH), 4.72 (2H, br d, 2xOH), 7.56 – 7.68 (5H, m, *m*-, *p*-PhN₂, *p*-N₂PhCO, *p*-COPhN₂), 7.71 – 7.77 (1H, m, *o*-COPhN₂), 7.92 – 8.06 (3H, m, *o*-PhN₂,

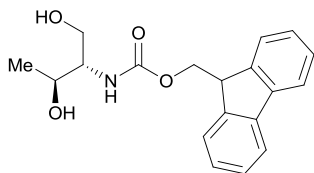
o-N₂PhCO), 8.36 (1H, d, *J* = 8.7, NH); ¹³C NMR (76MHz, d⁶-DMSO) δ = 20.3, 56.4, 60.4, 63.9, 115.6, 123.2, 129.4, 129.9, 130.8, 131.2, 132.1, 134.0, 148.8, 151.6, 165.74; EI + MS (C₁₇H₁₉N₃O₃) *m/z* = 313 (M), calc. 313.1426, found 313.1412.

5'-O-(4,4'-dimethoxytrityl)-2'-(*N*-(*p*-phenylazo)benzamido)-2'-deoxyuridine (8).



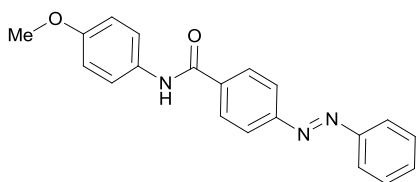
A stainless steel jar (25 ml) was charged with a mixture of 5'-O-(4,4'-dimethoxytrityl)-2'-amino-2'-deoxyuridine (139 mg, 0.25 mmol, 2 eq.), *p*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**2a**) (39 mg, 0.12 mmol), DMAP (24 mg, 0.20 mmol), 25 μ l (0.26 mmol) of ethyl acetate and a 15.0 mm stainless steel ball. The vessel was vibrated at 28Hz for 10 minutes, washed with DCM. The product was isolated following silica gel column chromatography eluting with a gradient of MeOH in DCM in presence of 0.1% v/v of TEA. The organic solvent was removed *in vacuo* to yield pure **8** (81mg, 0.10 mmol, 90 %). Characterisation by NMR and mass spectrometry according to ref 1.

2S,3S-(2-*N*-(9H-fluoren-9-yl)methylcarbamato)-1,3-dihydroxybutane (10).



A stainless steel jar (25 ml) was charged with a mixture of 9-fluorenylmethyl *N*-succinimidyl-carbonate (305 mg, 0.90 mmol), D-threoinol (193 mg, 1.84 mmol, 2 eq.), DMAP (153 mg, 1.36 mmol, 1.5 eq.), 90 μ l (0.92 mmol) of ethyl acetate and a 15.0 mm stainless steel ball. The vessel vibrated at 28Hz for 10 minutes. The vessel was vibrated at 28 Hz for 10 minutes, washed with ethyl acetate (2x10 ml) and the solution applied to a silica gel column eluting with ethyl acetate. Appropriate fractions were reduced *in vacuo* to yield pure **10** (235mg, 0.71 mmol, 79 %). ¹H NMR (300MHz, CDCl₃, TMS) δ = 1.20 (3H, d, *J* = 6.13, CH₃), 2.27 (1H, br s, CH₂OH), 2.40 (1H, br s, CHOH), 3.56 (1H, br s, CHNH), 3.77 – 3.90 (2H, m, CH₂OH), 4.11 – 4.27 (2H, m, CHOH, CH FMOC), 4.36 – 4.54 (2H, m, CH₂ FMOC), 5.37 – 5.53 (1H, m, NH), 7.32 (2H, ψ t, *J*_{9,11} = 7.4, H₃, H_{3'}), 7.41 (2H, ψ t, *J*_{10,12} = 7.3, H₄, H_{4'}), 7.61 (2H, d, *J*_{11,9} = 7.3, H₂, H_{2'}), 7.77 (2H, d, *J*_{12,10} = 7.4, H₅, H_{5'}); ¹³C NMR (76MHz, CDCl₃, TMS) δ = 20.8, 47.7, 65.4, 67.1, 69.3, 120.4, 125.4, 127.4, 128.1, 141.8, 144.4; ES + MS (C₁₉H₂₁NO₄Na) *m/z* = 350 (M + Na), calc. 350.1365, found 350.1368.

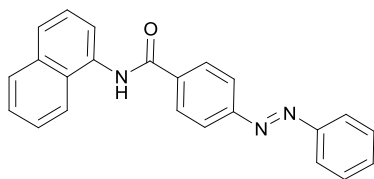
***N*-(4-methoxyphenyl)-4-(phenyldiazenyl)benzamide (12).**



A stainless steel jar (25 ml) was charged with a mixture of *p*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**2a**)¹ (86 mg, 0.27 mmol), *p*-anisidine (69 mg, 0.55 mmol, 2 eq.), DMAP (56 mg, 0.46 mmol, 1.7 eq.), and a 15.0 mm stainless steel ball. The vessel was vibrated at 28 Hz for 20 minutes and then was washed with ethyl acetate (2x10 ml). This solution was transferred to a separating funnel and further diluted with ethyl acetate (40 ml) and then washed with 2M

HCl (aq) (3 x 25 ml) followed by saturated NaHCO₃ (aq) (3 x 25 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and reduced *in vacuo* without further purification to yield pure **14** (70 mg, 0.21 mmol, 78 %). Mp 218–221 °C. ¹H NMR (300MHz, d⁶-DMSO) δ = 3.76 (3H, s, OMe), 6.96 (2H, d, $J_{2,4}$ = 8.9, *o*-PhOMe), 7.58 – 7.66 (3H, m, *m*-, *p*-PhN₂), 7.71 (2H, d, $J_{4,2}$ = 8.9, *m*-PhOMe), 7.91 – 7.98 (2H, m, *o*-PhN₂), 8.01 (2H, d, $J_{6,7}$ = 8.4, *o*-COPhN₂), 8.17 (2H, d, $J_{7,6}$ = 8.4, *m*-COPhN₂), 10.33 (1H, br s, NH); ¹³C NMR (76MHz, CDCl₃, TMS) δ = 55.56, 114.15, 122.43, 122.75, 123.14, 129.28, 129.94, 132.42, 137.54, 152.30, 153.74, 156.06, 164.61; ES + MS (C₂₀H₁₈N₃O₂) m/z = 332 (M + H⁺), calc. 332.1399, found 332.1388.

***N*-(naphthalen-1-yl)-4-(phenyldiazenyl)benzamide (14).**



A stainless steel jar (25 ml) was charged with a mixture of *p*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (**2a**)¹ (125 mg, 0.39 mmol), 1-naphthylamine (115 mg, 0.80 mmol, 2 eq.), DMAP (73 mg, 0.60 mmol, 1.5 eq.) and a 15.0 mm stainless steel ball. The vessel vibrated at 30Hz for 120 minutes, washed with ethyl acetate (2x10 ml). This solution was transferred to a separating funnel and further diluted with ethyl acetate (40 ml) and then washed with 2M HCl (aq) (3 x 25 ml) followed by saturated NaHCO₃ (aq) (3 x 25 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and reduced *in vacuo* without further purification to yield pure **12** (89 mg, 0.25 mmol, 65 %). Mp 187–190. °C ¹H NMR (400MHz, CDCl₃, TMS) δ = 7.49 – 7.63 (6 H, m, *m*-, *p*- PhN₂, H8, H7, H3), 7.77 (1 H, d, J = 8.3, H4), 7.89 – 8.02 (4 H, m, *o*-PhN₂, H9, H6), 8.05 (3 H, d, $J_{4,5}$ = 8.4, *m*-COPhN₂, H2), 8.14 (2H, d, $J_{5,4}$ = 8.2, *o*-COPhN₂), 8.28 (1H, br s, NH); ¹³C NMR (101MHz, CDCl₃, TMS) δ = 120.6, 123.2, 123.6, 125.8, 126.2, 126.4, 126.6, 128.3, 128.9, 129.2, 131.7, 134.2, 136.5, 152.6, 154.6; ES + MS (C₂₃H₁₈N₃O) m/z = 352 (M + H⁺), calc. 352.1450, found 352.1450.

References

1. S. Keiper and J. S. Vyle, *Angew. Chem. Int. Ed.*, 2006, **45**, 3306-3309.