Supplementary Material for Chemical Communications

Electronic Supplementary Information

A NADPH substitute for selective photo-initiation of reductive bioprocesses via two photon induced electron transfer

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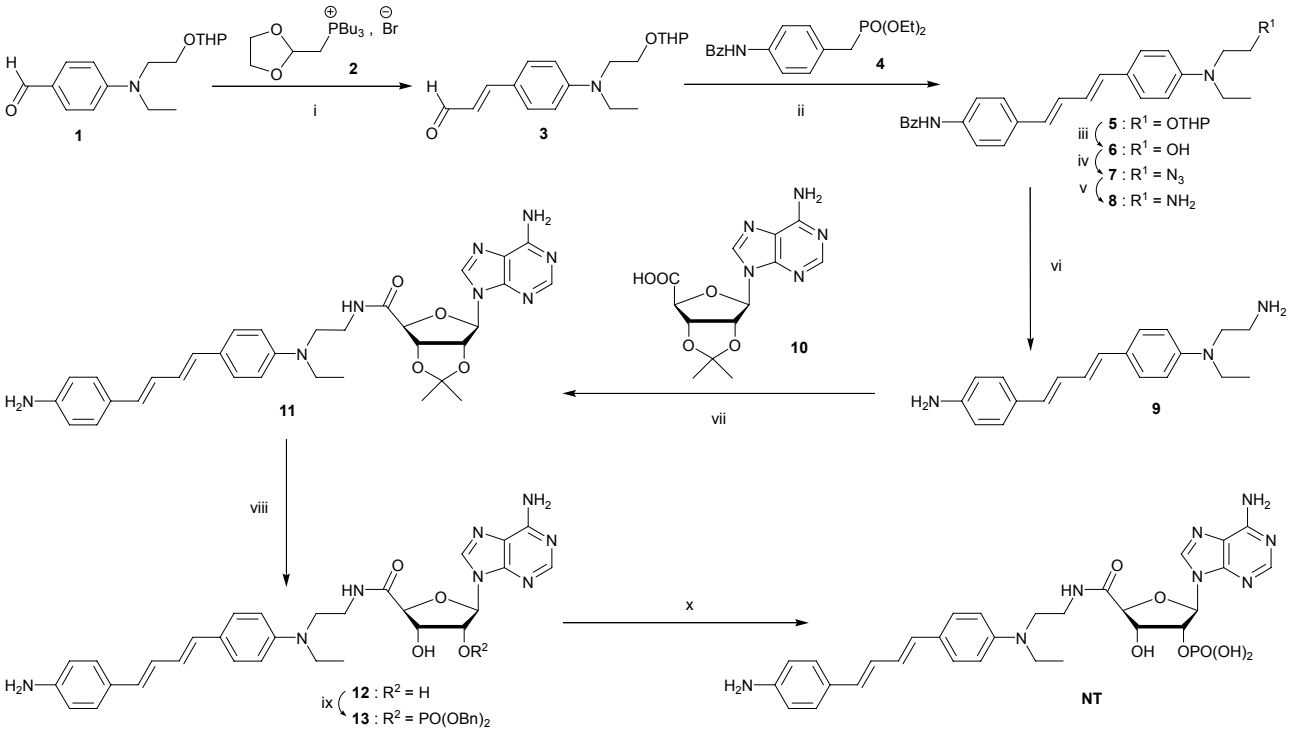
1. Synthesis of the nanotrigger NT.



Scheme S1 i: a, **2**, THF, NaH; b, AcOH, CH_2Cl_2 (92%); ii: **4**, NaH, THF (63%); iii: HCl, CH_2Cl_2 (95%); iv: a, TsCl, pyridine; b, NaN_3, *N*,*N*-dimethylacetamide (69%); v: a, Ph_3P, toluene; b, THF, H_2O (100%); vi: NaOH, EtOH (75%); vii: **10**, HBTU, DMF, Et_3N (57%); viii: TFA, H_2O, THF (96%); ix: a, $(iPr)_2N$-$P(OBn)_2$, tetrazole; b, O_2 (69%); x, Me_3SiBr, CH_2Cl_2 (88%).

(2*E*)-3-[4-[Ethyl[2-[(tetrahydro-2*H*-pyran-2-yl)oxy]ethyl]amino]phenyl]-2-propenal (3). To a solution of **1**[S1] (4 g, 14.9 mmol) in 40 mL of anhydrous THF, were added **2**[S2] (7.13 g, 19.37 mmol) and NaH (60% dispersion in mineral oil, 1.70 g, 44.7 mmol). The mixture was stirred at rt for 19 h. After addition of water at 0°C, THF was evaporated. After extraction with CH_2Cl_2 and drying (Na_2SO_4), the solvent was removed under reduced pressure. The residue was diluted in 70 mL of CH_2Cl_2. Silica gel (30 g), water (2.5 mL) and acetic acid (200 µL) were added and the mixture was stirred at rt for 2.5 days. The mixture was filtered through silica gel (CH_2Cl_2/ethyl acetate 50:50). The resulting solution was neutralized with Na_2CO_3, washed with water, dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography (CH_2Cl_2/ethyl acetate 98:2 then 95:5) to yield 4.06 g (92%) of **3**; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 9.58 (d, J = 7.9 Hz, 1H), 7.43 (d, J = 9.0 Hz, 2H), 7.36 (d, J = 15.8 Hz, 1H), 6.71 (d, J = 9.0 Hz, 2H), 6.52 (dd, J = 15.8 Hz, J = 7.9 Hz, 1H), 4.6 (m, 1H), 3.96-3.75 (m, 2H), 3.65-3.56 (m, 4H), 3.49 (q, J = 7.1 Hz, 2H), 1.85-1.50 (m, 6H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 193.48, 153.74, 150.12, 130.55, 123.21, 121.16, 111.31, 98.93, 64.66, 62.06, 49.90, 45.38, 30.37, 25.16, 19.22, 11.94; HRMS (EI) calcld for $C_{18}H_{25}NO_3$ (M^+) *m/z* 303.1834, found 303.1815.

***N*-[4-[4-(4-[Ethyl-[(2-tetrahydropyran-2-yloxy)ethyl]amino]phenyl)buta-1,3-dienyl]phenyl]benzamide (5).** To a solution of **3** (1 g, 3.3 mmol) in 40 mL of anhydrous THF under argon, were added NaH (60% dispersion in mineral oil, 3.8 g, 9.5 mmol) and phosphonate **4**[S3] (1.2 g, 3.43 mmol). The mixture was stirred at rt for 24 h. After addition of ice, THF was evaporated. After extraction with CH_2Cl_2 (2 x 75 mL) and drying (Na_2SO_4), the solvent was removed under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/ethyl acetate 99:1 then 97:3) to yield 0.83 g (63%) of **5**; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 7.91 (dd, J = 7.9 Hz, J = 1.6 Hz, 2H), 7.88 (s, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.61-7.44 (m, 4H), 7.35 (d, J = 8.8 Hz, 2H), 6.88 (dd, J = 9.9 Hz, J = 15 Hz, 1H), 6.74-6.62 (m, 4H), 6.57 (d, J = 8.8 Hz, 1H), 4.64 (m, 1H), 3.99-3.84 (m, 2H), 3.67-3.6 (m, 4H), 3.48 (q, J = 7.1 Hz, 2H), 1.88-1.58 (m, 8H), 1.24 (t, J = 7.0 Hz, 2H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 165.69, 147.28, 136.67, 134.77, 134.18, 132.00, 131.58, 129.43, 129.22, 128.52, 127.62, 126.98, 127.10, 124.96, 124.63, 120.38, 111.58, 99.02, 64.99, 62.15, 49.98, 45.27, 30.50, 25.27, 19.35, 12.16; HRMS (ES^+) calcd

for $C_{32}H_{36}N_2O_3$ ($M^{+\cdot}$) *m/z* 496.2726, found 496.2741. Anal. Calcd for $C_{32}H_{36}N_2O_3$ (496.65): C, 77.15; H, 7.10; N, 5.80. Found: C, 76.81; H, 7.34; N, 5.75.

***N*-[4-[4-[4-[Ethyl-(2-hydroxyethyl)amino]phenyl]buta-1,3-dienyl)phenyl]benzamide (6).** To a solution of **5** (0.83 g, 1.67 mmol) in 120 mL of CH_2Cl_2, was added 6 N HCl (6 mL). The mixture was refluxed for 12 h. The organic layer was discarded, and water was added. The mixture was made basic with 6 N NaOH and extracted with CH_2Cl_2. After drying (Na_2SO_4), the solvent was evaporated to yield 0.65 g (95 %) of **6**; ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 8.3 Hz, *J* = 1.5 Hz, 2H), 7.83 (s, 1H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.61-7.52 (m, 3H), 7.45 (d, *J* = 8.6 Hz, 2H), 7.34 (d, *J* = 8.8 Hz, 2H), 6.88-6.73 (m, 4H), 6.62 (d, *J* = 9.6 Hz, 1H), 6.57 (d, *J* = 9.7 Hz, 1H), 3.83 (t, *J* = 5.8 Hz, 2H), 3.52 (t, *J* = 5.8 Hz, 2H), 3.46 (q, *J* = 6.9 Hz, 2H), 1.6 (br. s, 1H), 1.19 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 165.49, 147.74, 136.73, 134.96, 134.32, 132.85, 131.86, 129.63, 129.48, 128.82, 127.69, 126.96, 126.75, 126.00, 125.28, 120.17, 112.61, 60.26, 52.44, 45.65, 11.95; HRMS (ES^+) calcd for $C_{27}H_{28}N_2O_2$ ($M^{+\cdot}$) *m/z* 412.2151, found 412.2154.

***N*-[4-[4-[4-[(2-Azidoethyl)ethylamino]phenyl]buta-1,3-dienyl]phenyl]benzamide (7).** Alcohol **6** (1.05 g, 2.5 mmol), pyridine (12 mL) and tosyl chloride (1.5 g, 7.87 mmol) were stirred under argon at 0°C for 3 h. Dichloromethane (200 mL) and water (50 mL) were added. The organic layer was dried (Na_2SO_4), and the solution was concentrated to 100 mL. To this solution were added NaN_3 (1.2 g, 18.5 mmol) and 50 mL of *N*,*N*-dimethylacetamide. The mixture was heated under reflux for 12 h. Dichloromethane (50 mL) and water (50 mL) were added. The organic layer was dried (Na_2SO_4), and the solvents were evaporated. The residue was purified by column chromatography (CH_2Cl_2) to yield 0.759 g (69%) of **7**; ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7 Hz, 2H), 7.81 (s, 1H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.59-7.52 (m, 3H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 6.89-6.64 (m, 4H), 6.66 (d, *J* = 15.0 Hz, 1H), 6.59 (d, *J* = 6.6 Hz, 1H), 3.54-3.46 (m, 6H), 1.21 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75.32 MHz, $CDCl_3$) δ 165.49, 146.70, 136.78, 134.97, 134.30, 132.79, 131.80, 129.71, 129.44, 128.78, 127.79, 126.97, 126.74, 126.09, 125.39, 120.21, 112.16, 49.53, 48.99, 45.56, 12.25; HRMS (ES^+) calcd for $C_{27}H_{27}N_5ONa$ ($[M+Na]^+$) *m/z* 460.2113, found 460.2117.

***N*-[4-[4-[4-[(2-Aminoethyl)ethylamino]phenyl]buta-1,3-dienyl]phenyl]benzamide (8).** To a solution of **7** (0.50 g, 1.14 mmol) in toluene (50 mL), was added Ph_3P (0.33 g, 1.27 mmol) in toluene (50 mL). The mixture was heated under reflux for 7 h. After cooling, 130 mL of THF and 0.5 mL of H_2O were added and the mixture was refluxed for 5 days. The solvents were evaporated under reduced pressure. The residue was crystallized in ether to yield 0.47 g (100%) of **8**; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 7.9 (d, J = 7.2 Hz, 2H), 7.86 (s, 1H), 7.63 (d, J = 8.5 Hz, 2H), 7.58-7.49 (m, 5H), 7.45 (d, J = 8.5 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H), 6.91-6.78 (m, 2H), 6.71 (d, J = 8.8 Hz, 2H), 6.59 (dd, J = 15.5 Hz, J = 4.8 Hz, 2H), 3.46-3.39 (m, 4H), 2.96 (t, J = 6.8 Hz, 2H), 1.19 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75.48 MHz, $CDCl_3$) δ 168.02, 150.12, 138.02, 134.88, 133.35, 132.5, 130.92, 129.97, 129.82, 128.62, 128.49, 128.02, 127.77, 126.55, 125.10, 119.14, 113.27, 53.28, 42.75, 37.70, 13.15; HRMS (EI) calcd for $C_{27}H_{29}N_3O$ (M^+) *m/z* 411.2310, found 411.2336.

***N*-[4-[4-[4-Aminophenyl)buta-1,3-dienyl]phenyl]*N*-ethylethan-1,2-diamine (9).** To a solution of **8** (0.300 g, 0.729 mmol) in ethanol (70 mL), was added 6 N NaOH (20 mL). The mixture was heated under reflux for 12 h. The solvents were evaporated. The organic layer was washed with H_2O, dried and evaporated. The crude product was acidified with 1 N HCl (2 mL) and the resulting salt was purified by reversed phase (C18) MPLC (H_2O/MeOH, gradient from 100:0 to 0:100). To the purified salt were added CH_2Cl_2 and aqueous K_2CO_3. The organic layer was dried (Na_2SO_4) and the solvent was evaporated to yield 0.225 g (75%) of **9**; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 7.9 (d, J = 8.0 Hz, 2H), 7.7-7.4 (m, 4H), 7.2 (d, J = 3.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 6.65 (d, J = 8.0 Hz, 2H), 3.35 (m, 4H), 2.85 (t, J = 4.0 Hz, 2H), 1.9-1.6 (m, 4H), 1.1 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 151.91, 148.72, 141.11, 134.08, 130.54, 129.31, 128.07, 126.89, 126.01, 125.49, 114.97, 11.41, 53.29, 43.73, 39.91, 12.72; HRMS (EI) calcd for $C_{20}H_{25}N_3$ (M^+) *m/z* 307,2048, found 307.2057.

***N*-[2-[[4-[4-(4-Aminophenyl)buta-1,3-dienyl]phenyl]ethylamino]ethyl]-1-(6-amino-9*H*-purin-9-yl)-1-deoxy-2,3-*O*-(1-methylethylidene)-β-*D*-ribofuranuronamide (11).** To a solution of **9** (0.09 g, 0.29 mmol) in 10 mL of distilled DMF under argon, were added **10**[S4] (0.093 g, 0.029

mmol), Et_3N (0.043 g, 0.435 mmol) and HBTU (0.164 g, 0.435 mmol). The mixture was stirred for 3 days. The DMF was evaporated under reduced pressure, and the residue was purified by reversed phase (C18) MPLC (H_2O/MeOH, gradient from 50:50 to 0:100, then MeOH/CH_2Cl_2, gradient from 100:0 to 75:25), to yield 0.100 g (57%) of **11**; ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.01 (s, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 6.88-7.72 (m, 3H), 6.72-6.52 (m, 6H), 6.3 (s, 1H), 6.04 (m, 4H), 5.6 (m, 1H), 5.02 (m, 1H), 4.54 (m, 1H), 3.43-3.24 (m, 6H), 1.63 (s, 3H), 1.42 (s, 3H), 1.1 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75.48 MHz, $CDCl_3$) δ 167.29, 157.22, 152.97, 150.71, 147.06, 140.38, 140.36, 133.72, 130.01, 128.03, 127.11, 126.87, 126.09, 117.08, 115.14, 113.68, 112.41, 87.67, 84.01, 81.88, 49.68, 44.51, 34.37, 26.96, 25.62, 12.44; HRMS (ES^+) calcd for $C_{33}H_{38}N_8O_4Na$ $[M+Na]^+$ *m/z* 633.2914, found 633.2908.

***N*-[2-[[4-[4-(4-Aminophenyl)buta-1,3-dienyl]phenyl]ethylamino]ethyl]-1-(6-amino-9*H*-purin-9-yl)-1-deoxy-β-*D*-ribofuranuronamide (12).** To a solution of 11 (0.120 g, 0.197 mmol) in THF / H_2O (1 mL / 0.5 mL), was added trifluoroacetic acid (3 mL). The mixture was stirred for 13 h at rt. The solvents were evaporated. Dichloromethane and aqueous K_2CO_3 were added to the residue. The organic layer was dried (Na_2SO_4) and the solvent was removed to yield 0.108 g (96%) of 12; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.33 (s, 1H), 8.04 (d, 2H, J = 8.0 Hz), 7.57 (d, 2H, J = 8.0 Hz), 7.12-6.88 (m, 4H), 6.82-6.47 (m, 7H), 6.08 (m, 5H), 4.95 (m, 2H), 4.15 (m, 1H), 3.48-3.33 (m, 6H), 1.08 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75.48 MHz, $CDCl_3$) δ 166.78, 157.40, 134.43, 134.10, 131.80, 131.75, 130.99, 129.89, 126.51, 123.90, 123.79, 123.15, 122.31, 120.94, 120.83, 118.79, 117.49, 114.53, 85.14, 79,01, 78.74, 64.92, 50.52, 42.72, 34.89, 12.52; HRMS (ES^+) calcd for $C_{30}H_{34}N_8O_4Na$ $[M+Na]^+$ *m/z* 593.2595, found 593.2524.

Phosphoric acid mono[5-[2-[[4-[4-(4-aminophenyl)buta-1,3-dienyl]phenyl]ethylamino]ethyl-carbamoyl]-2-[6-aminopurin-9-yl]-4-hydroxytetrahydrofuran-3-yl] ester (NT). To a solution of dibenzyl diidopropylphosphoramidite (0.100 g, 0.29 mmol) and 1-*H*-tetrazole (0.307 g, 0.44 mmol) in 3 mL of CH_2Cl_2 under argon, was added **12** (0.165 g, 0.29 mmol). The mixture was stirred at rt for 2 h (the reaction was monitored by LCMS). The mixture was stirred for 24 h at 25 °C under air. Water (20 mL) and CH_2Cl_2 (60 mL) were added. The organic layer was separated and washed with 15 mL of an aqueous saturated solution of $NaHCO_3$, dried (Na_2SO_4) and

evaporated, giving **13** (69%) as an equimolar mixture of two regioisomers (4-OH and 5-OH) which could be separated by HPLC. Air was removed from a solution of **13** in anhydrous CH_2Cl_2 by blowing argon. Then Me_3SiBr (0.132 g, 0.87 mmol) was added and the mixture was stirred at 0 °C for 12 h. Water was added and the mixture was stirred again for 1 h. The organic layer was separated, dried (Na_2SO_4) and evaporated to yield 0.114 g (88%) of **NT**; ^{1}H NMR (300.13 MHz, $CDCl_3$) : δ 8.6 (s, 1H), 8.2 (s, 1H), 7.61 (d, 2H, J = 8Hz), 7.41 (d, 2H, J = 8Hz), 7.71-6.88 (m, 3H), 6.52-6.73 (m, 6H), 6.3 (m, 1H), 5.3-5.36 (m, 2H), 4.32-4.36 (m, 1H), 3.5 (m, 7H), 3.5-3.3 (m, 6H), 1.1 (t, 3H, J = 7.2Hz); ^{13}C NMR (75.48 MHz, $CDCl_3$) δ 167.31, 158.03, 134.43, 134.11, 131.83, 132.05, 131.11, 129.67, 126.92, 123.90, 123.71, 123.19, 122.35, 121.08, 120.97, 118.15, 117.59, 114.83, 85.25, 81.23, 79.01, 72.74, 67.14, 37.70, 33.73, 13.04; ^{31}P NMR: (121.49 MHz, $CDCl_3$) δ 2.28. ESMS *m/z* 651.2 $[M+H]^+$, $C_{30}H_{36}N_8O_7P$ requires 651.2; HRMS (ES^+) calcd for $C_{30}H_{33}N_8O_7PNa_3$ $[M-2H+3Na]^+$ *m/z* 717.1903, found 717.1907.

2. Optical spectroscopy

General methods

UV/Vis spectra were recorded on a Jasco V-570 double beam spectrophotometer.

Steady-state fluorescence measurements were performed at room temperature using an Edinburgh Instruments (FLS 920) spectrometer working in photon-counting mode. Corrected emission spectra were obtained for each compound at $\lambda_{ex} = \lambda_{max}$(abs) with $A_{\lambda ex} < 0.1$ to minimize internal absorption. Fluorescence quantum yields were measured using standard methods on air equilibrated samples at room temperature. Fluorescein in 0.1 M NaOH (ϕ = 0.90) was used as a reference.[S5]

TPA (two-photon absorption) measurements were conducted by investigating the TPEF (two-photon excited fluorescence) of nanotrigger **NT** in Tris buffer (pH 7.4) using a Ti-sapphire laser delivering 150 fs excitation pulses, according to the experimental protocol established by Xu and Webb.[S6] This experimental protocol allows avoiding contributions from excited-state absorption that are known to result in largely overestimated TPA cross-sections. Fluorescein in 0.01 M NaOH, whose TPEF action cross-sections are well-known,[S6] served as the reference, taking into account the necessary corrections for the refractive index of the solvents.[S7] More details about the

experimental setup have been previously published.[S7] Quenching experiments were performed in 50 mM Tris buffer (pH 7.4) from Aldrich, using FAD from Sigma.

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