Supplementary Material for

Clickable fluorophores for biological labeling – with or without copper

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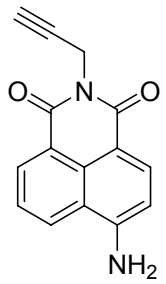
1. General Remarks

Unless otherwise indicated, all starting materials were obtained from commercial suppliers (Sigma-Aldrich, Fluka) and used without further purification. Analytical thin-layer chromatography (TLC) was performed on Polygram SIL G/UV 254 pre-coated plastic TLC plates with 0.25 mm silica gel from Macherey-Nagel + Co. Silica gel column chromatography was carried out with Flash silica gel (0.040–0.063 mm) from Merck. The NMR spectra were recorded on a Bruker DRX-250, Bruker DRX-300 or Varian Inova 600 MHz spectrometer. Chemical shifts (δ) are given in parts per million (ppm) using solvent signals as the reference. Coupling constants (J) are reported in Hertz (Hz). Splitting patterns are designated as s (singlet), d (doublet), t (triplet), quint (quintuplet), m (multiplet), dd (doublet of a doublet).

2. Synthesis of "clickable" dyes

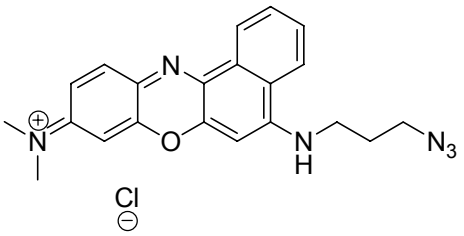


2b: A mixture of 4-amino-1,8-naphthalic anhydride (0.908 g, 4 mmol) and propargylamine (2 eq.) were heated at 80 ºC overnight in DMF. After cooling, the solution was poured into 200 ml of ice-cold water. The precipitate was filtered, washed with water and acetone and dried *in vacuo*. Compound **2b** was obtained as a yellowish solid. Yield: 778 mg, 73 %. m.p. 298-300 ^{o}C. ^{1}H ($CDCl_3$): δ = 3.05 (1H, s); 4.73(2H, s); 6.85 (1H, d, J = 8.4 Hz); 7.65 (1H, t, J = 7.7 Hz); 8.19 (1H, d, J = 8.4 Hz); 8.42 (1H, d, J = 7.3 Hz); 8.62 (1H, d, J = 8.4 Hz). Anal. Calcd. for $C_{15}H_{10}N_2O_2$: C, 71.06; H, 4.52; N, 10.86. Found: C, 71.08; H, 4.41; N, 10.87.

3a: 4-(2-azidoethoxy)benzaldehyde was synthesized by reacting 4-(2-hydroxyethoxy)benzaldehyde (1.4 g, 9.3 mmol) with carbon tetrabromide (3.5 g, 10.23 mmol), triphenylphosphine (4.93 g, 18.6 mmol) and sodium azide (1.24 g, 18.6 mmol) in DMF at room temperature for 24 h. The solvent was removed *in vacuo*. The collected material was dissolved in dichloromethane, washed with H_2O, Et_2O and dried with $MgSO_4$, and was purified by flash column (EtOAc / Hexane = 1 : 1) to give a colorless gum (90%). ^{1}H NMR ($CDCl_3$): δ = 3.55 (2H, t, J = 4.7 Hz); 4.13 (2H, t, J = 4.7 Hz); 6.93 (2H, d, J = 8.8 Hz); 7.59 (2H, d, J = 8.8 Hz); 9.68 (1H, s,). ^{13}C NMR ($CDCl_3$) δ = 49.76, 67.19, 114.50, 130.24, 131.75, 161.83, 190.22.). Anal. Calcd for $C_9H_9N_3O_2$: C, 56.54; H, 4.74; N, 21.98; Found: C, 56.45; H, 4.82; N, 21.90.

Compound **3a** was synthesized following the literature: [Xiaolin Zhang, Yi Xiao, and Xuhong Qian. Organic Letters (2008), 10(1), 29-32.] Yield: 364 mg, 30%. m.p. 141-143. ^{1}H NMR (CD_3Cl): δ = 0.94 (6H, t, J = 7.5 Hz); 1.33 (6H, s); 2.30 (4H, q, J = 7.5 Hz); 2.52 (6H, s); 3.67 (2H, t, J = 4.9); 4.21 (2H, t, J = 5.1 Hz); 7.03 (2H, d, J = 8.5 Hz); 7.18 (2H, d, J = 8.8 Hz). ^{13}C NMR (CDCl3) δ = 24.9; 26.2; 28.1; 53.0; 55.4; 74.3; 113.0; 116.1; 116.5; 117.1; 120.4; 122.0; 131.6; 147.0; 151.2; 153.4; 158.5. HRMS (PI-EI) calcd for $C_{25}H_{30}BF_2N_2O$ $[M]^+$: 465.2511; found: 465.2503.

3b: 4-(2-propyn-1-yloxy)-benzaldehyde was synthesized following the literature method.(F. Lu, S. Xiao, Y. Li, *Macromolecules* **2004,** *37,* 7444). A similar procedure for **3a** was used to prepare the expected **3b**. Yield: 0.25 g, 44 %. m.p. 145-147 ^{o}C. ^{1}H NMR ($CDCl_3$): δ = 7.15 (d, J = 8.8 Hz, 2H), 7.03 (d, J = 8.8 Hz, 2H), 4.72 (d, J = 2.2 Hz, 2H), 2.48(s, 6H), 2.45 (s, 1H), 2.30 (q, J=7.57 Hz, 4H), 1.28 (s, 6H), 0.94 (t, J=7.5 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ = 158.6, 153.1, 139.7, 137.9, 132.2, 130.7, 129.1, 127.7, 114.5, 64.2, 47.8, 28.3, 16.6, 14.1, 12.0, 11.3. HRMS (PI-EI) calcd for $C_{26}H_{29}BF_2N_2O$ [M+]: 434.2341; found: 434.2340.



6a: The reactant 5-dimethylamino-2-nitrosophenol was synthesized following the literature procedure (V. H. J. Frade, P. J. G. Coutinho, J. C. V. P. Moura, M. Sameiro T. Goncalves. Tetrahedron 63 (2007) 1654–1663].

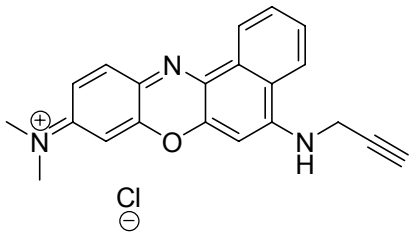
Synthesis of (3-azidopropyl)-1-naphthylamine: To a solution of 1-naphthylamine (2 g; 14.0 mmol) in ethanol (10 mL), 3-bromo-1-propanol (1.33 mL; 14.7 mmol) was added and the resulting mixture was refluxed for 10 h and monitored by TLC (silica: chloroform–methanol, 5.8:0.2). The solvent was removed under reduced pressure and the crude mixture was purified by dry chromatography (silica: chloroform and chloroform–methanol, 5.8:0.2). The obtained product (10 mmol) was reacted with carbon tetrabromide (3.77 g, 11 mmol), triphenylphosphine (5.3 g, 20 mmol) and sodium azide (1.24 g, 20 mmol) in DMF at room temperature for 24 h. DMF was removed *in vacuo*, and the collected material was dissolved in dichloromethane, washed with H_2O, Et_2O and dried with $MgSO_4$. The collected solution was concentrated and purified by flash column (EtOAc:Hexane = 1:1) to give a colorless gum. (1.47 g, 90%).The Yield: 1.15g, 74%. m.p. 173–175 ^oC. IR (KBr): ν = 3450, 3102, 2960, 2915, 2120, 1623, 1581, 1523, 1490, 1458, 1390, 1326, 1151 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.08 - 2.25 (2H, m); 3.55 (2H, t, J = 6.8 Hz); 3.84 (2H, t, J = 6.0 Hz); 6.68 (1H, d, J = 6.0 Hz); 7.27 (1H, d, J = 7.0 Hz); 7.38 (1H, t, J = 7.5 Hz); 7.42 – 7.50 (2H, m); 7.77 – 7.85 (2H, m). ^{13}C NMR ($CDCl_3$) δ = 31.0; 40.5; 48.3; 104.5; 117.5; 119.8; 123.4; 124.3; 126.1; 126.9; 128.8; 134.5; 143.2. HRMS (FAB) calcd. for $C_{13}H_{14}N_4$ $[M+H]^+$: 226.1216; found: 226.1212.

6a was obtained by condensation of 5-dimethylamino-2-nitrosophenol hydrochloride with (3-azidopropyl)-1-naphthylamine in 10 mL acidic ethanol. Specifically, to a cold solution (ice bath) of 5-dimethylamino-2-nitrosophenol hydrochloride (610 mg, 3 mmol) in ethanol (10 mL), (3-azidopropyl)-1-naphthylamine (658 mg, 2.91 mmol) and concentrated hydrochloride acid (0.25 mL) were added. The mixture was refluxed for about 9 hours and monitored by TLC (silica: chloroform and chloroform–methanol, 6:1). The solvent was removed under reduced pressure and the crude mixture was purified by dry chromatography (silica: chloroform–methanol, 5.5:0.5). N-[5-(3-Azidopropylamino)-9H-benzo[a]-phenoxazin-9-ylidene]-N-methyl-methanaminium chloride (**6a**) was obtained as a blue solid.

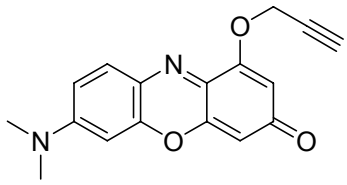
Yield: 710 mg, 65%. m.p. 184-186 ^oC. ^1H NMR (CD_3Cl): δ = 2.03-2.18 (m, 2H,); 3.20 (s, 6H); 3.54 (t, J = 6.9 Hz, 2H); 3.84 (t, J = 6.9 Hz, 2H); 6.61 (d, J = 2.5 Hz, 1H), 6.72 (s, 1H); 6.97

(dd, J = 2.2 Hz, J = 2.2 Hz, 1H); 7.76 (s, 1H); 7.79 (s, 1H); 7.85 (m, 1H); 8.80 (t, J = 4.6 Hz, 1H); 9.40 (d, J = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ = 27.1; 30.2; 42.6; 45.8; 99.0; 118.1; 120.6; 120.7; 121.3; 122.2; 122.6; 126.5; 126.9; 127.1; 129.3; 132.9; 147.0; 147.8; 148.6; 164.4. HRMS (PI-LSI) calcd. for $C_{21}H_{21}N_6O$ [M^+]: 373.1777; found: 373.1770.

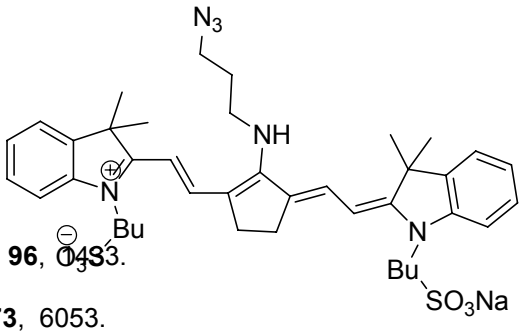
6b: The compound was obtained by using a similar method as **6a**. The alkylation of 1-naphthylamine (0,5 g, 3,49 mmol) with propargyl bromide (1.04 g, 6.98 mmol) afforded propargyl-1-naphthylamine (0,46 g, 73 %) (Helv. Chim. Acta, 56 (1), 1973. 478-489.), which was then used in the coupling reaction to give the desired compound **6b**. Yield: 0.57g, 68 %. m.p. 133-135 °C. ^{1}H NMR (CD_3OD): δ = 1.25 (s, 2H), 2.52 (s, 1H), 3.30 (s, 6H), 7.00 (s, 1H), 7.36 (s, 1H), 7.60 (m, 1H), 8.05 (m, 1H), 8.47 (d, J = 8.4 Hz, 1H), 8.70 (d, J = 8.4 Hz, 1H), 9.37 (d, J = 5.5 Hz, 1H), 10.03 (d, J = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ = 31.1; 42.6; 70.5; 78.4; 99.0; 118.0; 120.5; 120.7; 121.2; 122.3; 122.6; 126.6; 126.9; 127.1; 129.3; 133.0; 147.0; 147.8; 148.7; 164.4. HRMS (PI-LSI) calcd. for $C_{21}H_{18}N_3O$ [M+]: 328.1450; found: 328.1453.



7: 147 mg (0.5 mmol) of 7-dimethylamino-1-hydroxy-phenoxazone[1] was refluxed with (82 mg, 0.55 mmol) of propargyl bromide, 207 mg of K_2CO_3 in acetone for 24 h. After cooling, the reaction was filtered, concentrated on a rotavapor, and then purified by flash column. Yield: 76.5 mg, 52%. m.p. > 300 °C. ^{1}H NMR ($CDCl_3$): 7.70 (d, J= 7.5, 1H), 6.71 (m, 1H), 6.44 (s, 1H), 6.44 (s, 1H), 6.26 (s, 1H), 6.15 (s, 1H), 4,84 (d, 2H, J = 2.2 Hz), 3.15 (s, 6H), 2.59 (t, 1H, J = 2.2 Hz). HRMS (PI-EI) calcd. for $C_{17}H_{14}N_2O_3$ [M'^+]: 294.1004; found: 294.1002.



9a: IR-806 (Sigma-Aldrich) (0.1 g, 0.136 mmol) and 3-azidopropylamine[2] (0.054 g, 0.54 mmol) in DMF (5



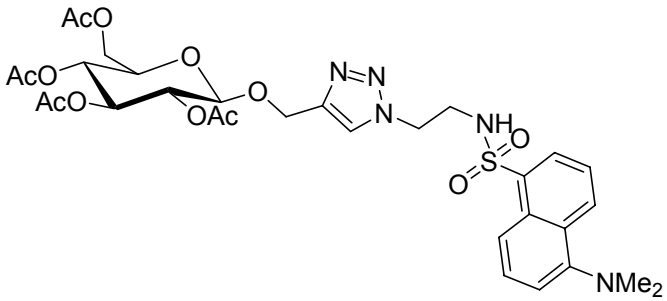
[1] Kotoucek, M.; Martinek, M.; Ruzicka, E. *Monatsh. Chem.* , 1965, **96**, 1433.

[2] A. J. Lampkins; E. J. O'Neil; B. D. Smith. *J. Org. Chem.,* 2008, **73**, 6053.

mL) was stirred in the dark for 24 hours. The solution was poured into MTBE (50 mL) and the precipitated product was filtered and washed with several portions of MTBE (0.095 g, 93%). M.p.: 200-201 °C. IR (neat) ν = 1416, 1503, 1570, 1712, 2097, 2978, 3069 cm-1. ^{1}H NMR (DMSO): δ = 1.59 (12H, s); 1.67 – 1.76 (8H, m); 1.78 (2H, quint., J = 6.4 Hz); 2.03 (2H, quint., J = 6.4 Hz); 2.46 – 2.51 (3H, m); 2.80 (2H, t, J = 7.2 Hz); 3.43 (2H, t, J = 6.8 Hz); 3.54 (2H, t, J = 6.4 Hz); 3.78 - 3.85 (2H, m); 3.88 - 3.94 (4H, m); 5.62 (2H, d, J = 12.8 Hz); 7.02 (2H, t, J = 7.5 Hz); 7.13 (2H, d, J = 7.9 Hz); 7.25 (2H, t, J = 7.9 Hz); 7.41 (2H, d, J = 7.2 Hz); 7.79 (2H, d, J = 12.8 Hz). ^{13}C NMR: δ = 22.5; 25.2; 25.9; 27.7; 28.4; 39.9; 42.2; 44.0; 47.0; 47.7; 48.5; 50.8; 64.8; 78.9; 79.1; 96.3; 109.1; 121.1; 121.9; 122.3; 126.1; 128.0; 139.7; 142.8; 164.5; 166.1. HRMS (ESI) calcd. for $C_{40}H_{51}N_6O_6S_2^-$ [M]$^-$: 775.3317, found: 775.3312.

3. Conjugates

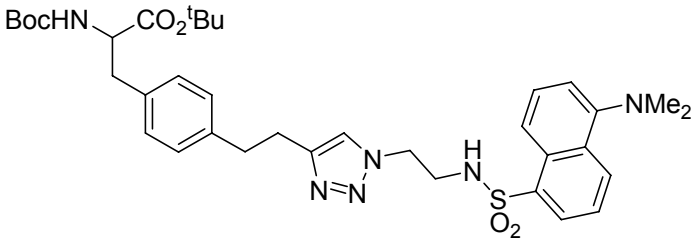
3.1. General procedure for the synthesis of conjugates: Azido- or alkyne bearing fluorophores **1**-**11** (1 eq.) and modified building blocks **A**-**F**[3,4,5,6,7,8] (1.1 eq.) were stirred in acetonitrile-water (50 v/v%) mixture at r.t. in the presence of 10% CuI and triethylamine 20 % for 16 hours.



1A: (72%). ^{1}H NMR (DMSO-d_6) δ = 1.99 (3H, s); 2.00 (3H, s); 2.01 (3H, s); 2.08 (3H, s); 2.88 (6H, s); 3.33 – 3.44 (2H, m); 3.74 (1H, ddd, J = 2.5 Hz, J = 4.6 Hz, J = 9.9 Hz); 4.17 (1H, dd, J = 2.5 Hz, J = 12.4 Hz); 4.24 (1H, dd, J = 4.6 Hz, J = 12.4 Hz); 4.41 (2H, dd, J = 5.0 Hz, J = 10.3 Hz); 4.69 (1H, d, J = 8.1 Hz); 4.76 (1H, d, J = 12.7 Hz); 4.85 (1H, d, J = 12.7 Hz); 4.94 (1H, dd, J = 7.8 Hz, J = 9.2 Hz); 5.10 (1H, dd, J = 9.6 Hz; J = 9.6 Hz); 5.22 (1H, dd, J = 9.6 Hz, J = 9.6 Hz); 5.86 (1H, t, J = 6.0 Hz); 7.18 (1H, d, J = 7.5 Hz);

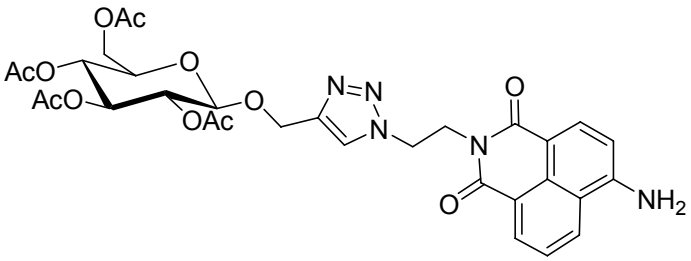
[3] T. Maki; K. Ishida. J. Org. Chem., 2007, **72**, 6427.
[4] U. Sirion; H. J. Kim; J. H. Lee; J. W. Seo; B. S. Lee; S. J. Lee; S. J. Oh; D. Y. Chi *Tetrahedron Lett.*, 2007, **48**, 3953.
[5] A. Deiters; T. A. Cropp; M. Mukherji; J. W. Chin; J. C. Anderson; P. G. Schultz J. Am. Chem. Soc. 2003, **125**, 11782.
[6] W. S. Aldridge; B. J. Hornstein; S. Serron; D. M. Dattelbaum; J. R. Schoonover; T. J. Meyer *J. Org. Chem.*, 2006, **71**, 5186.
[7] A. Nagy; A. Kotschy *Tetrahedron Lett.*, 2008, **49**, 3782.
[8] A. K. Pathak; V. Pathak; L. E. Seitz; K. N. Tiwari; M. S. Akhtar; R. C. Reynolds *Tetrahedron Lett.*, 2001, **42**, 7755.

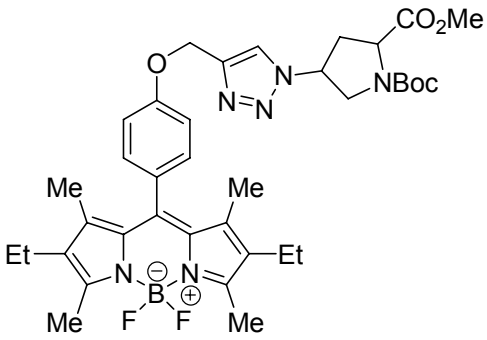
7.5 – 7.55 (3H, m); 8.18 (1H, d, J = 8.5 Hz); 8.23 (1H, dd, J = 1.0 Hz, J = 7.5 Hz), 8.55 (1H, d, J = 8.5 Hz). ^{13}C NMR δ = 20.5; 20.6; 20.7; 20.8; 42.7; 45.4; 50.3; 61.7; 62.7; 68.3; 71.5; 71.9; 72.6; 99.7; 115.4; 118.5; 123.1; 124.4; 128.6; 129.4; 129.5; 129.9; 130.8; 134.2; 148.8; 152.0; 169.4; 169.6; 170.1; 170.7. HRMS (ESI) calcd. for $C_{31}H_{40}N_5O_{12}S$ $[M+H]^+$: 706.2394, found: 706.2392.



1C: (99%). ^{1}H NMR (DMSO-d_6) δ = 1.40 (18H, s); 2.87 (6H, s); 2.98 (2H, dd, J = 6.0 Hz, J = 14.2 Hz); 3.40 – 3.45 (2H, m); 4.36 – 4.38 (2H, m); 5.01 (1H, d, J = 8.1 Hz); 5.07 (2H, s); 5.83 (1H, t, J = 5.7 Hz); 6.87 (2H, d, J = 8.5 Hz); 7.08 (2H, d, J = 8.5 Hz); 7.15 (1H, d, J = 8.5 Hz); 7.47 (1H, s); 7.49 – 7.53 (2H, m); 8.19 (1H, d, J = 8.5 Hz); 8.23 (1H, dd, J = 1.0 Hz, J = 7.1 Hz), 8.55 (1H, d, J = 8.5 Hz). ^{13}C NMR δ = 27.9; 28.3; 37.6; 42.7; 45.4; 50.1; 54.9; 61.7; 79.6; 81.9; 114.6; 115.3; 118.5; 123.0; 124.1; 128.6; 129.1; 129.4; 129.5; 129.9; 130.5; 130.8; 134.3; 143.9; 152.0; 155.0; 157.0; 170.1. HRMS (ESI) calcd. for $C_{35}H_{47}N_6O_7S$ $[M+H]^+$: 695.3226, found: 695.3218.

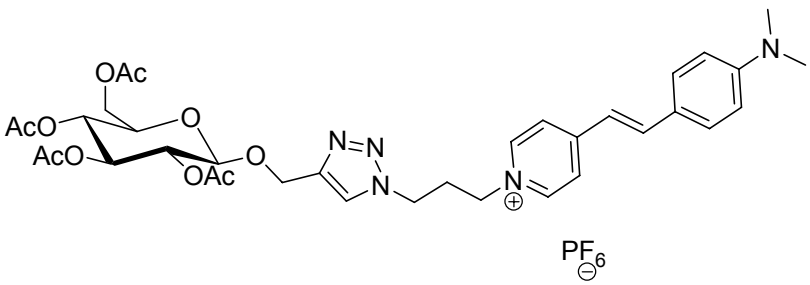
2aA: (75%). ^{1}H NMR (DMSO-d_6) δ = 1.98 (3H, s); 2.00 (3H, s); 2.02 (3H, s); 2.09 (3H, s); 3.77 (1H, ddd, J = 2.6 Hz, J = 4.7 Hz, J = 10.2 Hz); 4.16 (1H, dd, J = 2.1 Hz, J = 12.4 Hz); 4.23 (1H, dd, J = 4.7 Hz, J = 12.4 Hz); 4.55 – 4.66 (4H, m); 4.70 – 4.77 (1H, m); 4.80 – 4.90 (4H, m); 5.00 (1H, dd, J = 8.1 Hz, J = 9.8 Hz); 5.08 (1H, t, J = 9.8 Hz); 5.22 (1H, t, J = 9.4 Hz); 5.33 (2H, bs); 6.69 (1H, d, J = 8.1 Hz); 7.45 (1H, t, J = 8.1 Hz); 7.7 (1H, s); 8.03 (1H, d, J = 8.5 Hz); 8.20 (1H, d, J = 8.5 Hz), 8.41 (1H, d, J = 7.2 Hz). ^{13}C NMR δ = 20.5; 20.6; 20.7; 20.8; 39.5; 42.6; 48.3; 61.9; 62.0; 68.5; 71.2; 71.7; 72.8; 98.8; 109.2; 110.2; 119.6; 121.9; 123.9; 124.5; 127.7; 129.8; 131.6; 134.1; 150.2; 163.6; 164.4; 169.4; 169.5; 170.2; 170.7. HRMS (ESI) calcd. for $C_{31}H_{34}N_5O_{12}$ $[M+H]^+$: 668.2204, found: 668.2193.



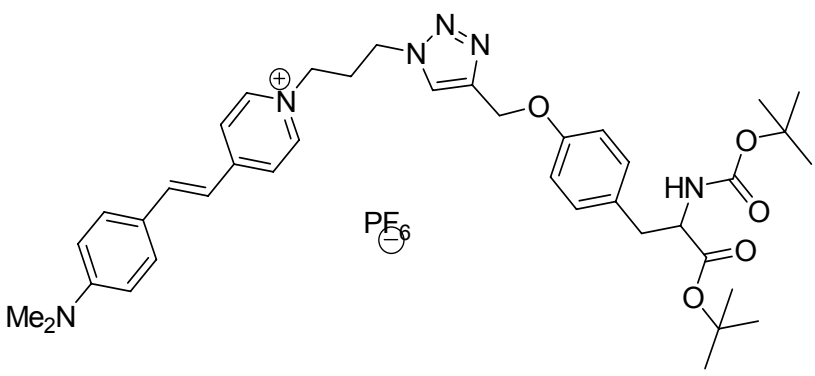


3bD: (64%). ^{1}H NMR (DMSO-d_6) δ = 0.97 (6H, t, J = 7.2 Hz); 1.30 (6H, s); 1.42, 1.47 (9H, 2S); 2.29 (4H, q, J = 7.2 Hz); 2.51 (6H, s); 2.53 – 2.68 (1H, m); 2.91 – 3.00 (1H, m); 3.68 – 3.75 (3H, m); 3.82 – 3.94 (1H, m); 4.14 – 4.21 (1H, m); 4.41 – 4.52 (1H, m); 5.18 – 5.19 (1H, m); 5.22 (2H, s); 7.08 (2H, d, J = 8.6 Hz); 7.17 (2H, d, J = 8.6 Hz); 7.85 (1H, bs). ^{13}C NMR δ = 11.8; 12.4; 14.1; 14.6; 17.0; 22.6; 28.2; 29.3; 29.6; 31.9; 60.3; 61.9; 81.1; 115.2; 121.6; 121.7; 128.5; 129.6; 131.1; 132.7; 138.3; 139.9; 144.0; 153.5; 158.6; 172.1. HRMS (ESI) calcd. for $C_{37}H_{48}BF_2N_6O_5$ $[M+H]^+$: 705.3747, found: 705.3744.

4A: (98%). ^{1}H NMR (DMSO-d_6) δ = 1.88 (3H, s); 1.93 (3H, s); 1.97 (3H, s); 2.01 (3H, s); 2.30 – 2.40 (1H, m); 3.10 (6H, s); 3.90 – 4.10 (3H, m); 4.10 – 4.25 (2H, m); 4.40 – 4.50 (3H, m); 4.55 – 4.68 (2H, m); 4.72 – 4.94 (3H, m); 5.2 – 5.27 (1H, m); 6.76 (1H, dd, J = 5.2 Hz, J = 8.6 Hz); 6.78 (1H, d, J = 8.6 Hz); 6.95 – 7.19 (2H, m); 7.55 (1H, dd, J = 4.9 Hz, J = 8.6 Hz); 7.6 (1H, d, J = 8.6 Hz), 7.85 – 7.95 (1H, m); 7.99 – 8.08 (2H, m); 8.65 – 8.75 (2H, m). ^{13}C NMR δ = 20.0; 20.1; 20.2; 20.3; 30.4; 39.5; 46.2; 56.2; 56.5; 61.5; 61.8; 67.9; 70.5; 70.7; 71.9; 98.5; 111.8; 116.9; 122.2; 124.2; 130.0; 142.2; 143.2; 143.4; 151.8; 153.7; 168.9; 169.1; 169.4; 169.9. HRMS (ESI) calcd. for $C_{35}H_{44}N_5O_{10}$ $[M]^+$: 694.3088, found: 694.3086.

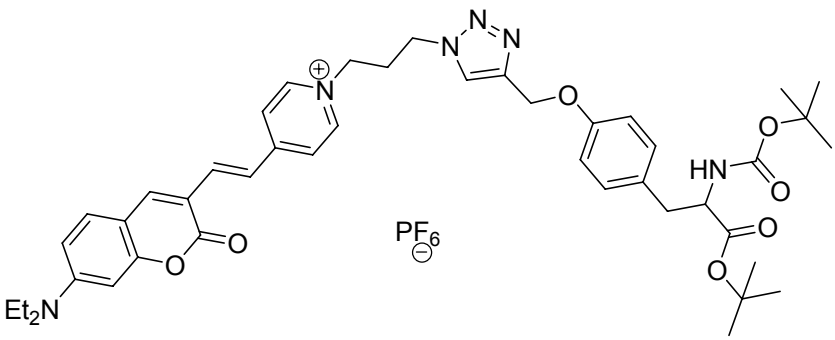


4C: (83%). ^{1}H NMR (DMSO-d_6) δ = 1.29, 1.30, 1.31, 1.32 (18H, s); 2.30 – 2.36 (2H, m); 2.65 – 2.85 (2H, m); 3.00 (6H, s); 3.85 – 3.94 (1H, m); 4.15 – 4.23 (1H, m); 4.26 – 4.34 (1H, m); 4.39 –

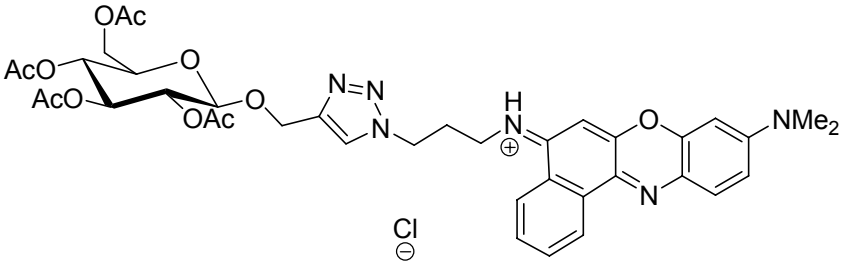


4.49 (2H, m); 4.87 – 4.94 (1H, m); 5.00 – 5.05 (1H, m); 6.69 (2H, d, J = 8.1 Hz); 6.73 (1H, d, J = 8.5 Hz); 6.75 (1H, dd, J = 8.5 Hz, J = 19.2 Hz); 6.90 (1H, m); 7.04 – 7.14 (4H, m); 7.55 (2H, d, J = 8.9 Hz); 7.86 (1H, d, J = 16.2 Hz); 7.93 – 8.01 (2H, m); 8.60 – 8.70 (2H, m). ^{13}C NMR δ = 30.7; 30.9; 31.2; 33.4; 38.7; 43.2; 48.4; 59.4; 63.6; 81.2; 83.4; 115.0; 117.1; 120.0; 125.4; 125.5; 133.2; 133.3, 133.4, 145.5; 146.5; 146.7; 147.4; 155.1; 157.0, 158.5; 159.3; 174.3. HRMS (ESI) calcd. for $C_{39}H_{51}N_6O_5$ $[M]^+$: 683.3920, found: 683.3905.

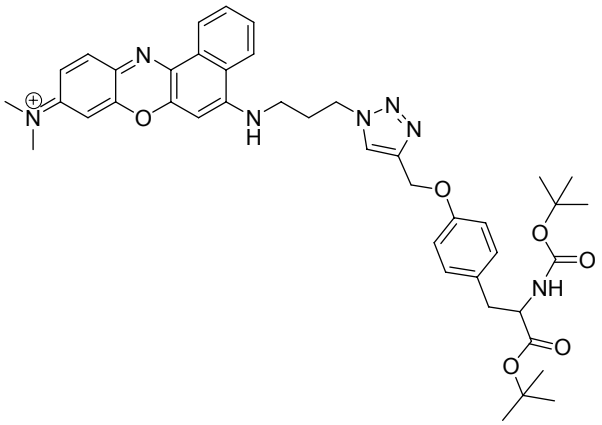
5C: (60%). ^{1}H NMR (DMSO-d_6) δ = 1.17 (6H, t, J = 7.0 Hz); 1.34, 1.36, (18H, s); 2.39 – 2.60 (2H, m); 2.78 – 2.85 (2H, m); 3.51 (4H, q, J = 7.0 Hz); 3.59 – 3.63 (1H, m); 3.80 – 3.90 (1H, m); 4.20 – 4.30 (1H, m); 4.44 – 4.57 (3H, m); 5.04 – 5.11 (1H, m); 6.45 – 6.80 (3H, m); 6.85 – 6.90 (1H, m); 7.01 – 7.20 (3H, m); 7.47 – 7.86 (2H, m); 8.03 – 8.24 (3H, m); 8.71 – 8.85 (1H, m). HRMS (ESI) calcd. for $C_{44}H_{55}N_6O_7$ $[M]^+$: 779.4127, found: 779.4138.



6aA: (99%). ^{1}H NMR (DMSO-d_6) δ = 1.90 (3H, s); 1.92 (3H, s); 1.96 (3H, s); 2.02 (3H, s); 2.34 (1H, t, J = 6.5 Hz); 3.24 (6H, s); 3.74 (2H, t, J = 6.5 Hz); 3.98 – 4.02 (1H, m); 4.05 (1H, dd, J = 1.8 Hz, J = 12.3 Hz); 4.20 (1H, dd, J =5.3 Hz, J = 12.9 Hz); 4.58 (2H, t, J = 7.0 Hz); 4.63 (1H, d, J = 12.3 Hz); 4.73 – 4.8 (2H, m); 4.88 – 4.93 (2H, m); 5.24 (1H, t, J = 9.5 Hz); 6.74 (1H, s); 6.97 (1H, s); 7.16 – 7.23 (1H, m); 7.78 (1H, d, J = 8.8 Hz); 7.84 (1H, t, J = 7.6 Hz), 7.93 (1H, t, J = 7.6 Hz); 8.13 (1H, s); 8.40 (1H, d, J = 8.2 Hz); 8.72 (1H, d, J = 7.6 Hz); 9.90 (1H, s). ^{13}C NMR δ = 20.1; 20.2; 20.3; 20.4; 28.6; 40.5; 45.7; 47.0; 54.7; 61.5; 61.9; 68.0; 70.5; 70.7; 71.9; 93.4; 95.6; 98.6; 115.4; 123.3; 123.8; 124.4; 129.5; 130.4; 131.8; 132.0; 133.3; 143.0; 147.3; 151.3; 155.1; 157.4; 168.9; 169.1; 169.4; 169.9. HRMS (ESI) calcd. for $C_{38}H_{43}N_6O_{11}$ $[M]^+$: 759.2989, found: 759.2991.

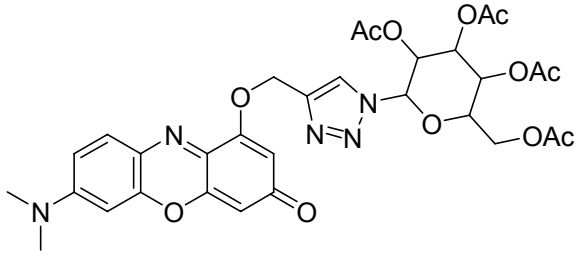


6aC: (42%). ^{1}H NMR (DMSO-d_6) δ = 1.38 (9H, s); 1.40 (9H, s); 2.65 – 2.7 (2H, m); 2.92 – 2.99 (2H, m); 3.25 (6H, s); 3.90 – 3.98 (2H, m); 4.37 (1H, dd, J = 6.1 Hz, J = 13.1 Hz); 4.70 (2H, t, J = 6.1 Hz); 4.95 (1H, d, J = 7.8 Hz); 5.02 (2H, s); 6.52 (1H, s), 6.59 (1H, s); 6.84 – 6.87 (2H, m); 6.93 – 6-97 (1H, m); 7.00 – 7.06 (2H, m); 7.69 (1H, d, J = 8.7 Hz); 7.75 – 7.79 (2H, m); 8.13 (1H, s); 8.71 (1H, d, J = 7.8 Hz); 9.28 (1H bs).

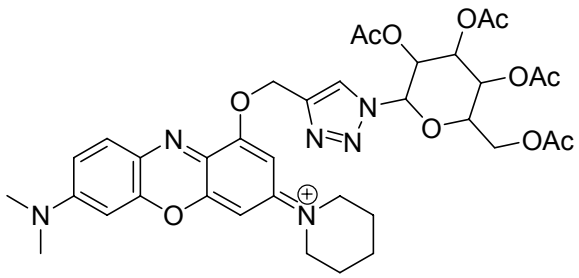


^{13}C NMR δ = 27.9; 28.3; 28.8; 37.5; 40.9, 45.8; 47.9; 54.9; 61.7; 79.6; 81.9; 93.6; 96.0; 114.6; 124.2; 124.5; 126.1; 128.7; 128.9; 130.5; 130.6; 131.9; 132.1; 143.7; 146.1; 147.0; 148.7; 151.4; 154.6; 155.0; 157.2; 158.8; 162.7; 166.4; 170.9. HRMS (ESI) calcd. for $C_{42}H_{50}N_7O_6$ $[M]^+$: 748.3822, found: 748.3824.

7B: (45%). ^{1}H NMR (DMSO-d_6) δ = 1.79 (3H, s); 1.96 (3H, s); 2.00 (3H, s); 2.02 (3H, s); 3.09 (6H, s); 4.11 (2H, m); 4.38 (1H, m); 5.19 (1H, t, J = 9.9 Hz); 5.23 (2H, s); 5.56 (1H, t, J = 9.3 Hz); 5.71 (1H, t, J = 9.3 Hz); 5.92 (1H, s); 6.19 (1H, s); 6.41 (1H, d, J = 9.3 Hz); 6.58 (1H, s); 6.78 (1H, d, J = 8.8 Hz); 7.57 (1H, d, J = 8.8 Hz); 8.62 (1H, s). ^{13}C NMR (HMQC) δ = 19.4; 19.7; 19.9; 20.0; 39.5; 61.1; 61.2; 67.0; 69.5; 71.7; 83.3; 95.6; 101.7; 106.7; 110.2; 123.9; 131.0. HRMS (ESI) calcd. for $C_{31}H_{34}N_5O_{12}$ $[M+H]^+$: 668.2204, found: 668.2197.

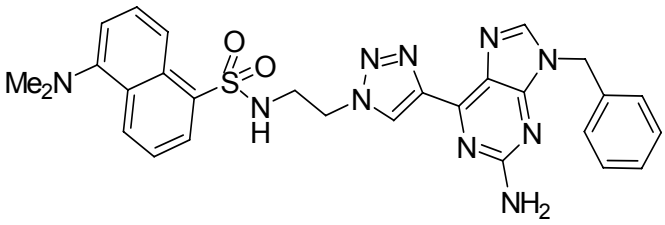


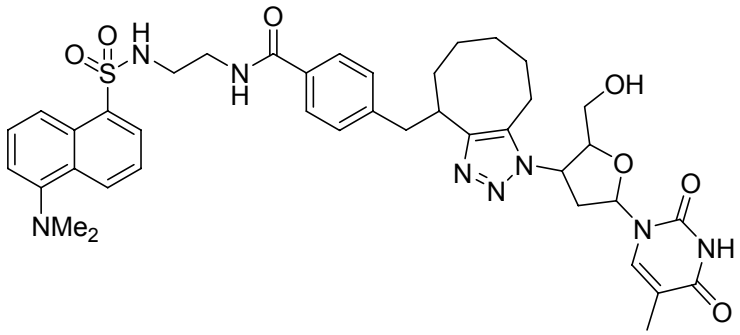
8B: (53%). ^{1}H NMR (DMSO-d_6) δ = 1,70, 1.73 (6H, s); 1.78 (3H, s); 1.96 (3H, s); 2.00 (3H, s); 2.02 (3H, s); 3.28 (6H, s); 3.91 (4H, s); 4.11 (2H, m); 4.39 (1H, m); 5.19 (1H, t, J = 9.3 Hz); 5.49 (2H, s); 5.57 (1H, t, J = 9.3 Hz); 5.68 (1H, t, J = 9.3 Hz); 6.41 (1H, d, J = 8.8 Hz); 6.74 (1H, s); 6.88 (1H, s); 7.01 (1H, s); 7.19 (1H, d, J = 8.2 Hz); 7.74 (1H, d, J 8.8 Hz); 8.67 (1H, s). ^{13}C NMR (HMQC) δ = 19.2; 19.6; 19.7; 19.8; 23.0; 25.8;



40.3; 49.1; 61.1; 61.6; 66.9; 69.5; 71.4; 72.7; 83.3; 91.6; 95.3; 96.5; 115.1; 123.9; 132.9. HRMS (ESI) calcd. for $C_{36}H_{43}N_6O_{11}^+$ $[M]^+$: 735.2984, found: 735.2981.

1E: (80%). ^{1}H NMR (DMSO-d_6) δ = 2.76 (6H, s); 3.26 – 3.30 (2H, m); 4.49 - 4.60 (2H, m); 5.29 – 5.40 (2H, m); 6.60 – 6.70 (2H, m); 7.08 (1H, s); 7.30 – 7.38 (2H, m); 7.40 – 7.60 (2H, m); 8.00 – 8.18 (2H, m); 8.20 – 8.40 (3H, m); 8.87 (1H, s). HRMS (ESI) calcd. for $C_{28}H_{29}N_{10}O_2S^+$ $[M+H]^+$: 569.2190, found: 569.2179.





11F: (83%). Mixture of two regioisomers 77-33%. ^{1}H NMR (DMSO-d_6) δ = 1.00 – 1.11 (1H, m); 1.30 – 1.55 (5H, m); 1.66 – 1.74 (1H, m); 1.81, 1.83 (3H, s, regioisomers); 2.55 – 2.77 (3H, m); 2.81 (6H, s); 2.83 – 2.86 (1H, m); 2.90 – 2.94 (2H, m); 2.98, 3.08 (1H, dd, J = 7.6 Hz, J = 13.5 Hz, regioisomers); 3.23 – 3.28 (3H, m); 3.36 -3.40 (1H, m); 3.49 -3.62 (1H, m); 3.64 – 3.72 (1H, m); 4.18 (1H, dq, J = 3.5 Hz, J = 12.3 Hz); 5.03 – 5.11 (1H, m); 5.30 (1H, dt, J = 4.7 Hz, J = 17.6 Hz); 6.42 – 6.54 (1H, m); 7.12 – 7.23 (2H, m); 7.32 (1H, d, J = 7.0 Hz); 7.53 – 7.65 (4H, m); 7.71, 7.78 (1H, s, regioisomers); 8.03 – 8.07 (1H, m); 8.09 – 8.11 (1H, m); 8.25 – 8.33 (2H, m); 8.43 – 8.45 (1H, m); 11.33, 11.35 (1H, s, regioisomers). HRMS (ESI) calcd. for $C_{37}H_{49}N_8O_7S^+$ $[M+H]^+$: 785.3445, found: 785.3419.

4. *In-vitro* Cytostatic Activity of compounds 9b and 10

Adherent, epithelial-like **C**hinese **H**amster **O**vary cells (CHO-K1, ATCC® number: CCL-61) were cultured at 37°C in 5% CO_2 atmosphere and grown in Ham's F12 medium containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 160 μg/mL gentamycin and 1.176 g/L sodium bicarbonate (Sigma).[9,10,11]

[9] Sigma, Ham's Nutrient Mixtures **N6760** medium powder, **N4388** medium powder + 25 mM HEPES

Cells were seeded into 96-well plates at a density of 5 x 10^3 cells per well. After 24-hour incubation, cells were treated with compounds **9b** and **10** at a concentration range from 5 x10^{-2} to 10^2 μM in 200 μL serum free Ham's F12 medium (c_{DMSO} = 2.5 v/v %) for 3 hrs. As a control we used serum free medium or DMSO treatment. After treatment cells were washed twice with serum free medium and further cultivated for 72 hrs in serum-containing medium.

After 3 days viability of the cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay.[12,13] The respiratory chain and other electron transport systems[14] reduce MTT that results in the formation of non-water-soluble violet formazan crystals within the cell.[15] The amount of these crystals can be determined spectrophotometrically and serves as an estimate for the number of mitochondria and hence the number of living cells in the well.[16] The following protocol was applied: 45 µl MTT-solution (2 mg/ml) was added to each well. After 3.5 hrs of incubation cells were centrifuged for 5 min (900 g) and the supernatant was removed. The obtained formazan crystals were dissolved in DMSO and the optical density (OD) of the samples was measured at λ = 540 and 620 nm using an ELISA Reader (iEMS Reader, Labsystems, Finland). OD_{620} values were substracted from OD_{540} values. The percent of cytostasis was calculated using the following equation:

$$\text{inhibition of cell proliferation (cytostasis)} = [1 - (OD_{treated}/OD_{control})] \times 100;$$

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[10] Puck TT, et al. Genetics of somatic mammalian cells III. Long-term cultivation of euploid cells from human and animal subjects. J. Exp. Med. 108: 945-956, 1958.

[11] Kao FT, Puck TT. Genetics of somatic mammalian cells, VII. Induction and isolation of nutritional mutants in Chinese hamster cells. Proc. Natl. Acad. Sci. USA 60: 1275-1281, 1968.

[12] Slater, T. F., Sawyer, B., and Sträuli, U. (1963) Studies on succinate-tetrazolium reductase systems : III. Points of coupling of four different tetrazolium salts III. Points of coupling of four different tetrazolium salts. Biochimica et Biophysica Acta 77, 383-393.

[13] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55–63.

[14] Liu, Y B; Peterson, D A; Kimura, H; Schubert, D. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. J Neurochem. 1997;69:581–593.

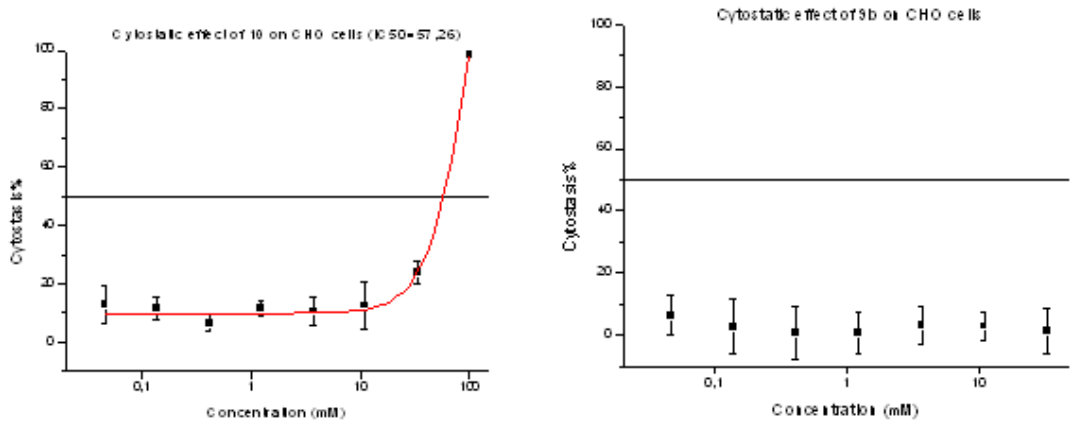
[15] Altman, F P. Tetrazolium salts and formazans. Prog Histochem Cytochem. 1976;9(**3**):1–56.

[16] Denizot, F; Lang, R. Rapid colorimetric assay for cell growth and survival. J Immunol Methods. 1986;89:271–277.

where $OD_{treated}$ and $OD_{control}$ correspond to the optical densities of the treated and the control cells, respectively. In each case two independent experiments were carried out with 4–8 parallel measurements. The 50% inhibitory concentration (IC_{50}) values were determined from the dose-response curves. The curves were defined using MicrocalTM Origin1 (version 6.0) software.

We studied the cytostatic activity of the compounds **9b** and **10** *in vitro*. Therefore CHO cells were treated with **9b** and **10** at 5 $x10^{-2}$ to 10^2 μM concentration range and the viability of the cells was determined by using MTT-assay.

Data summarized in the figure below show that **9b** did not have a toxic effect inhibiting proliferation, whereas **10** exhibited a modest, but not negligible antiproliferative effect on CHO cells (20 uM<IC_{50}< 100 uM > 20 μM < 100 μM).



5. Fluorescence Labeling and Cell Imaging

Culture conditions for CHO cells were the same as described above.[5,6] Cells were seeded into eight-well Lab-Tek Borosilicate chamber slide (Lab-Tek Chambered Borosilicate Coverglass System, 155411, Nunc, Rochester, NY, USA) at 5 x 10^3 cells per well density. For biolabeling experiments, cells were incubated for 2 days in either culture medium or in medium supplemented with 100 μM ManNAz (Invitrogen, tetraacetylated N-azidoacetyl-D-mannosamine; C33366; stock solution was prepared with ethanol and water: 5.52 mg was dissolved in 1 ml ethanol and 4 ml distilled water was added). After incubation the medium was gently aspirated, and the cells were fixed in methanol at -20 °C for 10 minutes followed by acetone for 1 min. The cells then were washed with 200 μL of PBS (pH = 7.0) three times and treated with a reaction solution containing ***(i)*** 20 μM compound **9b**, 50 μM $CuSO_4$/50 μM Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) and 1 mM sodium ascorbate in PBS (pH 7.0) or ***(ii)*** 20 μM compound **10** in PBS (pH = 7.0) at 37°C for 20 min. After

incubation cells were washed with 200 μL of serum free RPNI 1640 medium three times. In case of live-cell labeling the cells were washed with serum free RPNI 1640 medium and reaction time was 1 h.

Confocal luminescence imaging was performed on an Olympus Fluoview500 (Olympus). Excitation sources were: argon ion laser (488 nm); Melles Griot and He-Ne laser (632 nm). The objective was an 10x /0.40 oil-immersion lens.