Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

A FRET-based Fluorogenic Phosphine for Live Cell Imaging with the Staudinger Ligation

Matthew J. Hangauer and Carolyn R. Bertozzi

Supporting Information

1. General methods

All chemical reagents obtained from commercial suppliers were used without further purification unless noted. All reactions were performed in flame-dried glassware with the exception of reactions performed in aqueous media. Degassed solvents were obtained from at least three freeze-pump-thaw cycles, followed by placement under an $Ar_{(g)}$ atmosphere. Water obtained from a Milli-Q purification system was used in all manipulations. Dichloromethane and acetonitrile were distilled under a nitrogen atmosphere and dried over CaH_2 prior to use. Methanol, *N*,*N*-dimethylformamide, and *N*,*N*-diisopropylethylamine were used as received from commercial sources without further purification.

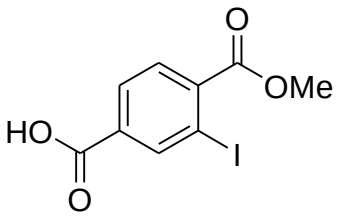
Flash chromatography was performed using Merck 60 Å 230-400 mesh silica gel. Analytical thin layer chromatography was performed using glass-backed Analtech Uniplate silica gel plates. Reversed-phase HPLC was performed on a Varian Pro Star system with a Varian UV-Vis detector model 345 on a Microsorb C-18 preparative column (21.4 x 250 mm) at a flow rate of 20 mL/min.

NMR spectra were obtained on Bruker DRX-500 and AVQ-400 MHz spectrometers at the UC-Berkeley College of Chemistry NMR Facility. ^{1}H and ^{13}C NMR spectra were obtained without ^{31}P-filtering; ^{13}C resonances are unassigned and reported as observed. High resolution mass spectrometry was performed at the UC-Berkeley Mass Spectrometry Laboratory (FAB) or the Howard Hughes Medical Institute Mass Spectrometry Laboratory at UC-Berkeley (MALDI). Uncorrected melting points were determined using a Thomas Hoover capillary melting point apparatus.

Kinetics studies were performed in black polystyrene 96-well microtiter plates (Thermo Electron Corp.) and fluorescence emission readings were measured using a 96-well plate fluorescence reader (Molecular Devices). Absorption spectra were recorded using 1 cm quartz cells on a Cary-100 UV-Vis spectrophotometer. Fluorescence spectra were recorded using 1 cm quartz cells on a Photon Technology International Quanta Master 4 L-format scanning spectrophotometer equipped with an LPS-220B 75-W xenon lamp and power supply, A-1010B lamp housing with integrated igniter, switchable 814 photon counting/analog photomultiplier detection unit, and MD5020 motor driver.

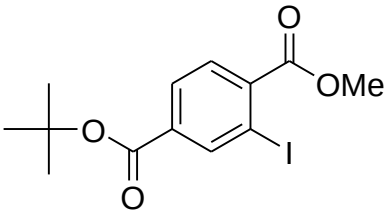
Microscopy was performed on a Zeiss Axiovert 200M inverted microscope equipped with a 63 × 1.4 numerical aperture Plan-Apochromat oil immersion lens. A 175-W xenon lamp housed in a Sutter DG4 illuminator linked to the microscope by an optical fiber assured shuttering and illumination. SLIDEBOOK software (Intelligent Imaging Solutions) was used to control the microscope and the camera. Image stacks spaced 0.5 μm apart were acquired by using a CoolSNAP HQ charged-coupled device camera (Roper Scientific). The image stacks were digitally deconvolved using the nearest-neighbor algorithm of SLIDEBOOK. All image acquisition and processing was performed under identical conditions for test and control samples.

2. Synthetic procedures



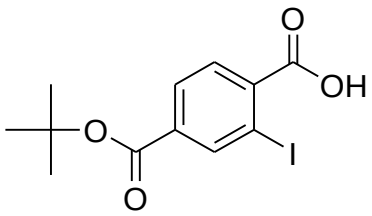
2

2-Iodo-terephthalic acid 1-methyl ester (2): **2** was prepared as reported previously.[1]



3

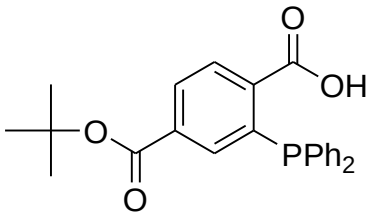
2-Iodo-terephthalic acid 4-*tert*-butyl ester 1-methyl ester (3): **2** (15.0 g, 49.2 mmol) was dissolved in 150 mL of dichloromethane in a 500 mL round-bottomed flask. 4-Dimethylaminopyridine (3.01 g, 24.6 mmol) and anhydrous *tert*-butanol (7.06 mL, 73.9 mmol) were added. The solution was cooled to 0 °C and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (11.3 g, 59.1 mmol) was added. The resulting solution was stirred for 10 min at 0 °C, then allowed to warm to RT and was stirred for 2 d. The crude product mixture was washed with 150 mL of H_2O (2x), 150 mL of saturated $NaHCO_{3(aq)}$ (1x), and the aqueous layer was back extracted with an additional 150 mL of dichloromethane. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was then recrystallized from hot methanol and H_2O to yield pale yellow crystals (15.0 g, 84%). m.p. 73-74 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.56 (s, 9H), 3.91 (s, 3H), 7.75 (d, 1H, J = 8.0 Hz), 7.94 (dd, 1 H, J = 8.0, 1.6 Hz), 8.50 (d, 1H, J = 1.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 28.04, 52.68, 82.22, 93.30, 128.66, 130.36, 135.34, 138.48, 141.81, 163.26, 166.51. HRMS (FAB): Calcd for $C_{13}H_{16}O_4I$ $[M+H]^+$ 363.0093, found 363.0089.



4

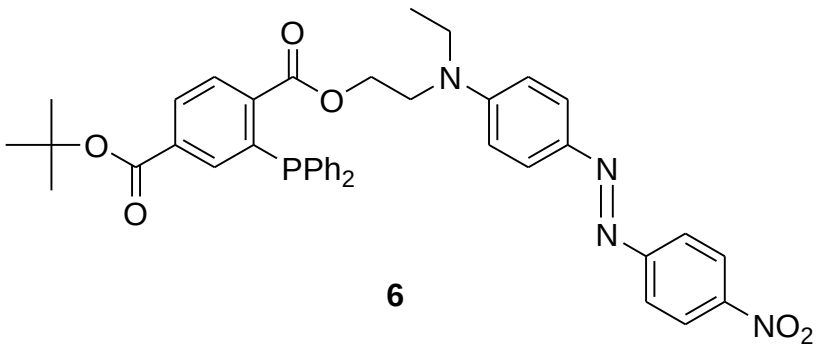
2-Iodo-terephthalic acid 4-*tert*-butyl ester (4): **3** (1.28 g, 3.53 mmol) was dissolved in 40 mL of 3:1 methanol:H_2O in a 100 mL round-bottomed flask. Lithium hydroxide (0.127 g, 5.30 mmol) was added and the solution was stirred for 28 h at RT. The methanol solvent was removed in vacuo and 100 mL of dichloromethane was added to the remaining aqueous solution. The aqueous layer was brought to pH 10 by addition of saturated $NaHCO_{3(aq)}$, the organic layer was removed and discarded, and 200 mL of additional dichloromethane was added. The aqueous layer was acidified to pH 1 by addition of 1 M $HCl_{(aq)}$, and the organic layer was isolated and washed with brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was recrystallized from hot methanol and H_2O

to yield yellow crystals (1.15 g, 94%). m.p. 146-147 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.59 (s, 9H), 7.98-8.02 (m, 2H), 8.58 (app s, 1H), 11.91 (br s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 28.07, 82.56, 93.98, 128.78, 131.53, 136.03, 136.62, 142.46, 163.34, 171.00. HRMS (FAB): Calcd for $C_{12}H_{14}O_4I$ $[M+H]^+$ 348.9937, found 348.9937.



5

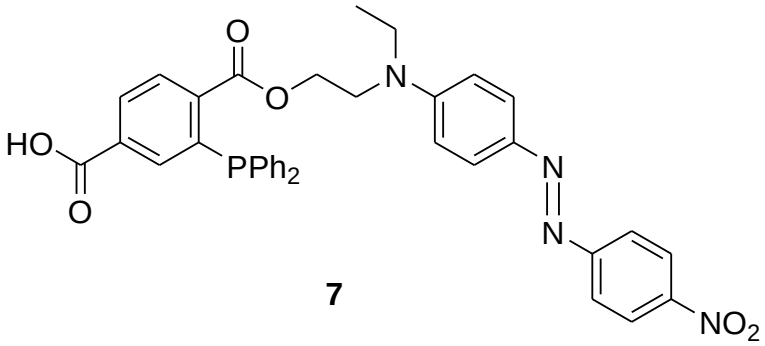
2-Diphenylphosphanyl-terephthalic acid 4-*tert*-butyl ester (5): **4** (5.16 g, 14.8 mmol), K_2CO_3 (4.10 g, 29.6 mmol), and $Pd(OAc)_2$ (10.0 mg, 0.0445 mmol) were dissolved in 200 mL of acetonitrile in a 500 mL round-bottomed flask fitted with a condenser. The solution was degassed in vacuo, placed under $Ar_{(g)}$, and diphenylphosphine (2.58 mL, 14.8 mmol) was added. The solution was heated to reflux and became dark red. After 42 h, the crude product mixture was concentrated in vacuo, redissolved in 250 mL of dichloromethane and added to 150 mL of H_2O. 1 M $HCl_{(aq)}$ was added to adjust the aqueous layer to pH 3, then the organic layer was isolated, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was recrystallized from methanol and H_2O to yield a yellow solid (4.32 g, 72%). m.p. 175-177 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.44 (s, 9H), 7.31-7.36 (m, 9H), 7.51-7.56 (m, 2H), 7.99-8.01 (d, 1H, J = 7.2 Hz), 8.17-8.19 (dd, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 27.92, 81.63, 128.47, 128.55, 128.85, 128.93, 131.42, 133.86, 134.07, 135.11, 135.94, 136.11, 137.21, 137.31, 142.04, 142.33, 164.55, 169.99. ^{31}P NMR ($CDCl_3$, 162 MHz): δ_P -3.60. HRMS (FAB): Calcd for $C_{24}H_{24}O_4P$ $[M+H]^+$ 407.1412, found 407.1412.



6

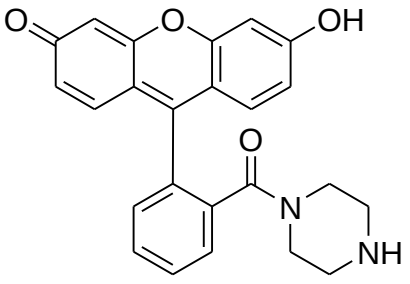
2-Diphenylphosphanyl-terephthalic acid 4-*tert*-butyl ester 1-(2-{ethyl-[4-(4-nitro-phenylazo)-phenyl]-amino}-ethyl) ester (6): Disperse red 1 (2.03 g, 6.46 mmol) and 4-dimethylaminopyridine (28.4 mg, 0.232 mmol) were dissolved in 30 mL of acetonitrile in a 100 mL three neck round-bottomed flask. The solution was degassed in vacuo and placed under $Ar_{(g)}$. Separately, **5** (0.944 g, 2.32 mmol) was dissolved in 10 mL of degassed acetonitrile. The solution of **5** in acetonitrile was added to the three neck flask, and the resulting solution was stirred for 10 min at RT. *N,N'*-Dicyclohexylcarbodiimide (0.479 g, 2.32 mmol) was added to the reaction under $Ar_{(g)}$ and the reaction was stirred for 16 h at RT in the dark. The crude product mixture was concentrated in vacuo and purified by flash chromatography on silica gel (10:1 cyclohexane:EtOAc) to yield a dark red solid (1.35 g, 83%). m.p. 68-69 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.17-1.20 (t, 3H, J = 6.8 Hz), 1.43 (s, 9H), 3.42-3.47 (q, 2H, J = 7.2 Hz), 3.54-3.57 (t, 2H, J =

6.4 Hz), 4.33-4.37 (t, 2H, *J* = 6.4 Hz), 6.72-6.75 (d, 2H, *J* = 9.2 Hz), 7.27-7.35 (m, 10H), 7.53-7.54 (m, 1H), 7.86-7.92 (app t, 4H, *J* = 10.0 Hz), 7.98-8.00 (m, 1H), 8.02-8.07 (m, 1H), 8.27-8.29 (d, 2H, *J* = 8.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 12.30, 27.91, 45.60, 48.37, 61.95, 81.60, 111.46, 122.63, 124.64, 126.25, 128.61, 128.68, 129.01, 130.58, 131.73, 131.83, 132.03, 133.84, 134.04, 134.91, 135.16, 136.70, 136.89, 137.10, 137.21, 140.86, 141.14, 143.79, 147.34, 151.12, 156.69, 164.38, 166.23. ^{31}P NMR ($CDCl_3$, 162 MHz): δ_P -3.62. HRMS (FAB): Calcd for $C_{40}H_{40}N_4O_6P$ $[M+H]^+$ 703.2685, found 703.2671.



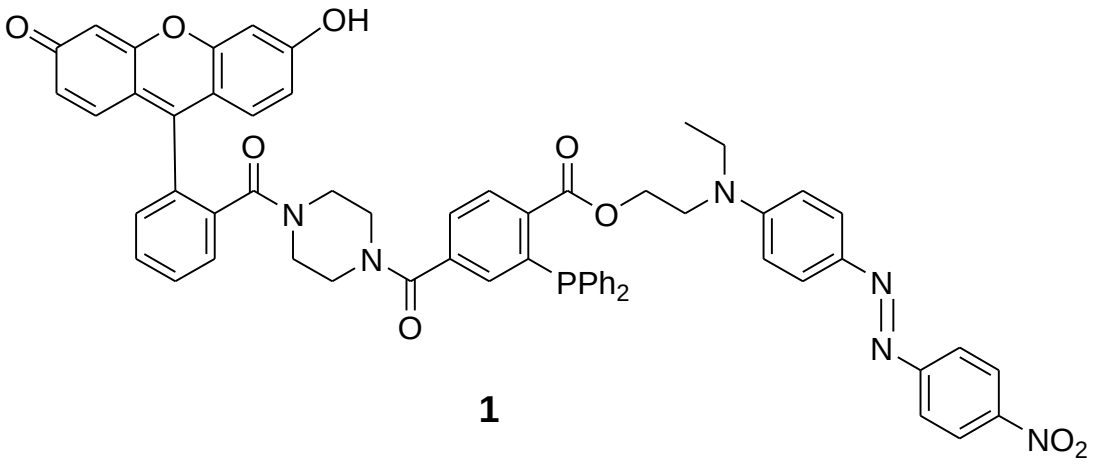
7

2-Diphenylphosphanyl-terephthalic acid 1-(2-{ethyl-[4-(4-nitro-phenylazo)-phenyl]-amino}-ethyl) ester (7): 6 (1.08 g, 1.54 mmol) was dissolved in 3.2 mL of degassed dichloromethane in a 10 mL round-bottomed flask under $Ar_{(g)}$. Trifluoroacetic acid (1.48 mL, 19.9 mmol) and triethylsilane (0.61 mL, 3.8 mmol) were added. The solution was stirred for 5.5 h at RT in the dark. The crude product mixture was diluted with dichloromethane and washed with H_2O. The aqueous layer was separated, acidified to pH 4 with 1 M $HCl_{(aq)}$, and back extracted with dichloromethane. The organic layers were combined, concentrated in vacuo, and purified by flash chromatography on silica gel (40:1 dichloromethane:methanol) to yield a dark red solid (0.66 g, 66%). m.p. 158-160 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.19-1.21 (t, 3H, *J* = 6.8 Hz), 3.45-3.46 (q, 2H, *J* = 6.8 Hz), 3.57-3.59 (t, 2H, *J* = 6.0 Hz), 4.35-4.38 (t, 2H, *J* = 5.6 Hz), 6.73-6.76 (d, 2H, *J* = 8.8 Hz), 7.32-7.37 (m, 10H), 7.69 (s, 1H), 7.87-7.93 (m, 4H), 8.01-8.06 (m, 2H), 8.29-8.31 (m, 2H), 11.79 (br s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 12.32, 45.58, 48.34, 62.12, 111.51, 122.66, 124.65, 126.26, 128.71, 128.78, 129.14, 129.76, 130.57, 132.64, 133.79, 133.99, 135.57, 136.79, 136.89, 138.14, 138.33, 141.12, 141.41, 143.80, 147.32, 151.16, 156.70, 166.29, 170.15. ^{31}P NMR ($CDCl_3$, 162 MHz): δ_P -4.32. HRMS (MALDI): Calcd for $C_{36}H_{32}N_4O_6P$ $[M+H]^+$ 647.2059, found 647.2066.



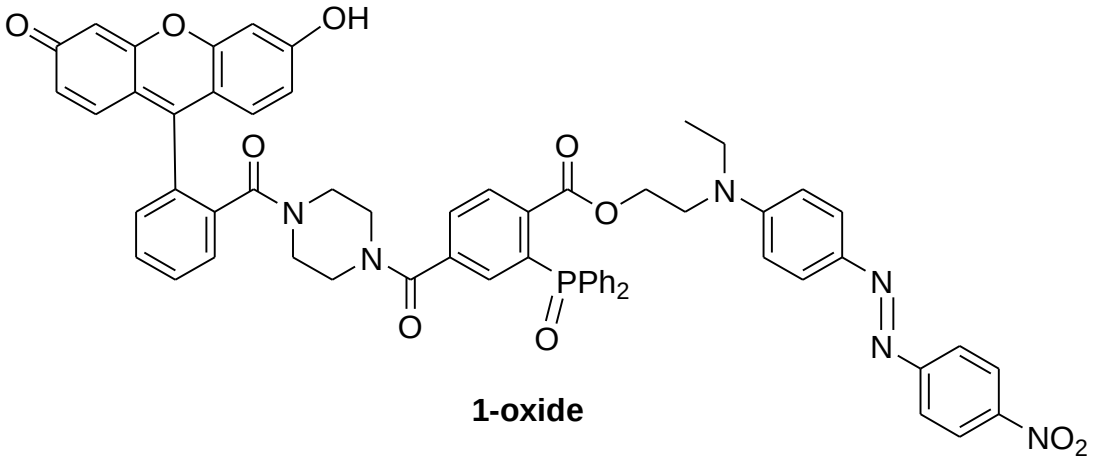
8

6-Hydroxy-9-[2-(piperazine-1-carbonyl)-phenyl]xanthen-3-one (8). 8 was prepared as reported previously.[2]



1

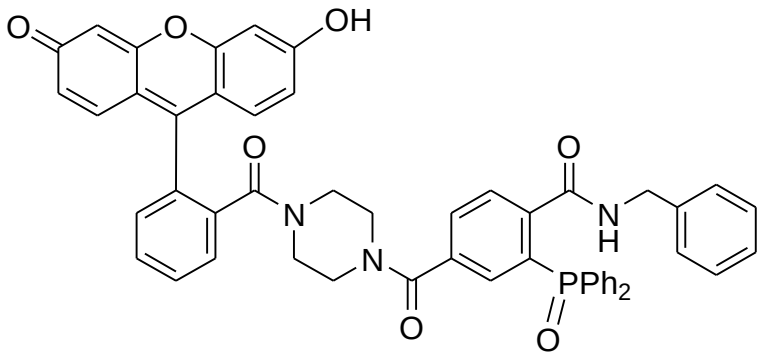
2-Diphenylphosphanyl-4-{4-[2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-benzoyl]-piperazine-1-carbonyl}-benzoic acid 2-{ethyl-[4-)4-nitro-phenylazo)-phenyl]-amino}-ethyl ester (1). **7** (259 mg, 0.401 mmol) and *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (228 mg, 0.601 mmol) were dissolved in 5 mL of degassed *N*,*N*-dimethylformamide under $Ar_{(g)}$. *N*,*N*-Diisopropylethylamine (0.14 mL, 0.80 mmol) was added and the solution was stirred for 20 min at RT. **8** (176 mg, 0.441 mmol) was added under $Ar_{(g)}$ and the solution was stirred for 16 h at RT in the dark. The crude product mixture was concentrated in vacuo and purified by flash chromatography on silica gel (100% dichloromethane to 10:1 dichloromethane:methanol) to yield a dark red solid (268 mg, 65%). **1** was further purified by reversed-phase HPLC (40% acetonitrile/60% water to 100 % acetonitrile over 40 min, maximum peak elution at 35.4 min) prior to use in all assays. m.p. 161-162 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.15-1.83 (t, 3H, J = 6.8 Hz), 2.92-3.00 (m, 4H), 3.30-3.54 (m, 8H), 4.33 (t, 2H, J = 6.4 Hz), 6.70-6.81 (m, 7H), 7.01 (s, 2H), 7.22-7.29 (m, 10H), 7.39 (s, 1H), 7.46-7.48 (d, 2H, J = 6.8 Hz), 7.65 (s, 2H), 7.84-7.91 (dd, 4H, J = 8.8, 19.2 Hz), 8.03 (s, 1H), 8.28-8.30 (d, 2H, J = 8.8 Hz), 8.91 (br s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 12.28, 41.82, 45.57, 47.12, 48.34, 62.04, 103.92, 111.43, 114.99, 122.00, 122.63, 124.64, 126.20, 127.30, 127.93, 128.76, 128.83, 129.12, 129.57, 130.12, 130.74, 131.35, 131.82, 132.43, 133.71, 133.91, 134.74, 135.34, 135.53, 136.92, 137.69, 140.94, 141.24, 143.77, 147.34, 151.05, 151.47, 156.67, 157.42, 165.98, 167.73, 168.92. ^{31}P NMR ($CDCl_3$, 162 MHz): δ_P -4.37. HRMS (MALDI): Calcd for $C_{60}H_{50}N_6O_9P$ $[M+H]^+$ 1029.3377, found 1029.3345.



1-oxide

2-(Diphenyl-phospinoyl)-4-{4-[2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-benzoyl]-piperazine-1-carbonyl}-benzoic acid 2-{ethyl-[4-(4-nitro-phenylazo)-phenyl]-amino}-ethyl ester (1-oxide). **1-oxide** was isolated as a byproduct during flash chromatography of **1** and is a dark red solid (10.5 mg). **1-oxide** was further purified by reversed-phase HPLC (5% acetonitrile/95% water to 60% acetonitrile over

40 min, hold at 60% acetonitrile from 40 to 60 min, maximum peak elution at 52.0 min) prior to use in all assays. m.p. 195-196 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.14-1.15 (m, 3H), 3.20-3.22 (m, 8H), 3.37-3.42 (m, 4H), 3.93 (br s, 2H), 6.65-6.74 (m, 5H), 6.83-7.05 (m, 2H), 7.40-7.64 (m, 16H), 7.86-7.94 (m, 5H), 7.99-8.01 (d, 1H, J = 9.2 Hz), 8.33-8.39 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz): δ_C 12.41, 40.00, 42.45, 46.35, 50.11, 63.83, 104.49, 112.58, 112.77, 115.73, 123.64, 125.75, 127.21, 127.30, 127.69, 128.77, 130.09, 130.18, 130.95, 131.23, 131.83, 132.17, 132.59, 132.70, 132.84, 132.94, 133.67, 133.72, 136.38, 138.59, 138.72, 138.86, 144.98, 148.85, 152.87, 153.04, 153.76, 158.13, 159.22, 167.80, 169.70, 171.1. ^{31}P NMR (CD_3OD, 162 MHz): δ_P 33.74. HRMS (MALDI): Calcd for $C_{60}H_{50}N_6O_{10}P$ $[M+H]^+$ 1045.3326, found 1045.3297.



9

***N*-Benzyl-2-(diphenyl-phosphinoyl)-4-{4-[2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-benzoyl]-piperazine-1-carbonyl}-benzamide (9).** **1** (50 mg, 0.049 mmol) was dissolved in 0.6 mL of degassed 3:1 acetonitrile:H_2O in a 2.5 mL round-bottomed flask under $Ar_{(g)}$. Benzyl azide (0.031 mL, 0.25 mmol) was added and the solution was stirred for 4 h at RT in the dark. The crude product mixture was concentrated in vacuo and purified by flash chromatography on silica gel (50:1 to 10:1 dichloromethane:methanol) to yield a bright orange solid (35 mg, 86%). **9** was further purified by reversed-phase HPLC (5% acetonitrile/95% water to 60% acetonitrile over 40 min, maximum peak elution at 32.3 min) prior to use in all assays. m.p. 273-274 °C (decomp). ^{1}H NMR (CD_3OD, 400 MHz): δ_H 3.12-3.18 (m, 4H), 3.47-3.51 (m, 4H), 4.04 (s, 2H), 6.54 (s, 4H), 7.02-7.04 (m, 2H), 7.19-7.29 (m, 6H), 7.37-7.40 (m, 3H), 7.46-7.53 (m, 4H), 7.59-7.72 (m, 9H). ^{13}C NMR (CD_3OD, 125 MHz): δ_C 29.02, 41.38, 43.19, 102.98, 115.10, 121.17, 126.90, 127.37, 128.13, 128.29, 128.39, 129.18, 129.40, 129.60, 129.82, 130.01, 130.37, 130.54, 130.79, 131.19, 131.40, 131.77, 131.85, 132.26, 132.27, 132.47, 132.56, 134.95, 136.16, 136.23, 137.67, 141.94, 142.00, 152.17, 157.52, 167.88, 168.23, 168.85. ^{31}P NMR (CD_3OD, 162 MHz): δ_P 33.50. HRMS (FAB): Calcd for $C_{51}H_{41}N_3O_7P$ $[M+H]^+$ 838.2682, found 838.2675.

3. Kinetics of the Staudinger ligation between phosphine 1 and benzyl azide

A stock solution of phosphine **1** (1.10 mM in ethanol) was diluted 1:10 in aq. KH_2PO_4 (10 mM) buffer (pH 7.0). Three serial five-fold dilutions of this 110 μM buffered solution were prepared (22, 4.4, and 0.88 μM). 100 μL aliquots of each dilution of phosphine **1** were mixed with 100 μL aliquots of benzyl azide (10, 5, 2.5, 1.25, 0.625, and 0 mM in acetonitrile) to give final solvent mixtures of 1:1 aq. KH_2PO_4 (10 mM) : acetonitrile with phosphine **1** concentrations of 11, 2.2, and 0.44 μM and benzyl azide concentrations of 5, 2.5, 1.25, 0.625, 0.3125, and 0 mM. Fluorescence intensities (λ_{ex} = 495 nm, λ_{em} = 515 nm) were recorded at 0, 2, 4, 6, 8, 10, 14, 20, and 30 min after mixing. The concentration of

Staudinger ligation product **9** was determined by interpolation of a standard curve which related fluorescence of **9** to its concentration (0-3 μM) in 1:1 aq. KH_2PO_4 (10 mM) : acetonitrile. It was independently verified that no intermolecular quenching from released quencher after ligation occurs at the concentrations of the assay (data not shown). Data analysis under pseudo first-order conditions was performed as previously reported.[3]

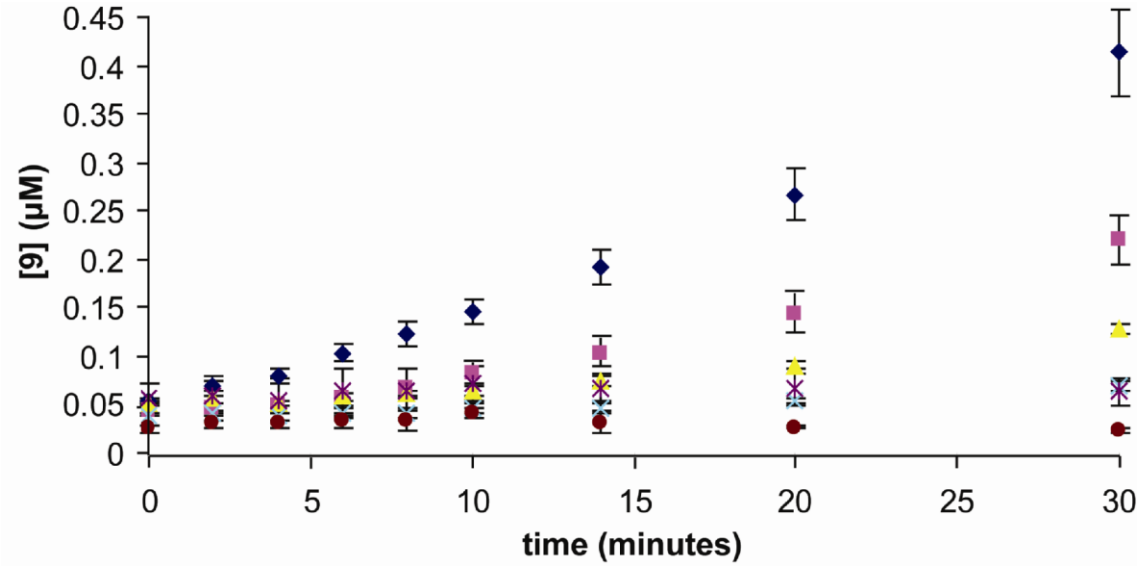


Figure S1. Timecourse of reaction between **1** and benzyl azide producing the fluorescent product **9** with an initial concentration of **1** = 11 μM. Data are shown for varying initial benzyl azide concentrations (mM): 5 (◆), 2.5 (■), 1.25 (▲), 0.625 (✕), 0.3125 (✳), 0 (●).

4. Photophysical characterization

Extinction coefficients were determined by plotting a standard Beer-Lambert plot. Relative fluorescence quantum yields were determined according to the method of Fery-Forgues et al.[4] Specifically, **1**, **1-oxide**, and **9** were dissolved in Dulbecco's phosphate-buffered saline (PBS (Gibco)), pH 7.4, and their concentrations were adjusted to yield absorbance values between 0.1-0.5 at the excitation wavelength. Each sample was then diluted tenfold using additional PBS and emission spectra were recorded with a fluorimeter. All solutions were excited at 470 nm, and emission spectra were integrated. Quantum yields were determined using fluorescein (NIST-traceable standard in 0.1 N NaOH, Molecular Probes), Φ_F = 0.95 +/- 0.03, as the standard.[5]

5. Labeling of azido-murine dihydrofolate reductase (mDHFR)

mDHFR was metabolically labeled with azidohomoalanine in place of methionine as reported previously.[1] Azido-DHFR and native DHFR (400 ng) were incubated for 20 h at RT with 12.5 μM phosphine **1** in 20 μL PBS under denaturing conditions with 4 M urea in the dark. The crude product mixture was then diluted with SDS-PAGE loading buffer (4x), heated for 3 min at 95 °C, and loaded onto a Criterion XT Precast Gel (12% Bis-Tris). After electrophoresis, the gel was imaged directly for fluorescence using a Typhoon 9410 Variable Mode Imager (Amersham Biosciences) using fluorescein settings (λ_{ex} = 532 nm, 526 SP emission filter). The gel was then Zn-stained for total protein content

using the GelCode E-Zinc Reversible Stain Kit (Pierce). Protein concentrations were determined using the BioRad DC protein assay kit with BSA as a standard.

6. Cell culture conditions

HeLa cells (human cervical cancer) and CHO cells (Chinese hamster ovary) were grown in Dulbecco's Modified Eagle Medium (DMEM (Gibco)), and F-12 Nutrient Mixture [HAM] (Gibco), respectively, and supplemented with 10% FCS, penicillin (100 units/mL) and streptomycin (0.1 mg/mL). Cells were maintained in a 5% CO_2, water-saturated atmosphere at 37 °C. Except where noted, all rinsing and handling of live cells for flow cytometry and microscopy experiments was performed in the appropriate media with 10% FCS.

7. Flow cytometry

Live cell labeling with **1**:

CHO cells were seeded at a density of 60,000 cells per well (determined by Coulter cell counter) in 24-well polystyrene tissue culture plates in 400 μL F-12 media with 10% FCS. Peracetylated *N*-α-azidoacetylmannosamine (Ac_4ManNAz) was administered to cells by adding 4 μL (for a final concentration of 100 μM Ac_4ManNAz) of 10 mM Ac_4ManNAz in ethanol stock solution to each well and allowing evaporation of the ethanol prior to addition of cells and media. Cells were incubated with Ac_4ManNAz in three independent wells, or in the case of controls without any added Ac_4ManNAz in six independent wells, for 3 d at 37 °C. The cells were rinsed with media (1x) and then incubated with 25 μM phosphine **1** (diluted from a 10 mM DMSO stock solution) in media, or media alone for control cells, for 8 h at 37 °C. Next, the cells were rinsed with media (3x) and with PBS (1x). The cells were lifted with 200 μL 1 mM EDTA in PBS, transferred to wells in a 96-well v-bottom plate and pelleted by centrifugation (3500 *g* for 3 min) at 4 °C. Finally, the cells were rinsed with 200 μL PBS with 1% FCS (3x), resuspended in 400 μL PBS with 1% FCS and analyzed using a FACScalibur flow cytometer (Becton Dickinson) equipped with a 488 nm argon laser. Gating was performed based on untreated live control cells and a minimum of 20,000 live cells were gated per sample. Measurements were performed on three independent samples for each of three tested conditions (+azide/+**1**, -azide/+**1**, -azide/-**1**), with error bars representing the standard deviation of the mean. Data were analyzed using FloJo (Tree Star) software.

Annexin V-PE staining of live cells treated with **1**:

CHO cells prepared and treated with **1** as above were further stained with Annexin V-PE (BD Biosciences). Pelleted cells were resuspended at a concentration of 10^6 cells/mL in Binding Buffer (BD Biosciences), and 5 μL of Annexin V-PE solution was added per 2 x 10^5 cells. The cells were incubated for 15 min at RT with gentle vortexing in the dark, diluted 5x with Binding Buffer and analyzed by flow cytometry as above. Single color (+azide/+**1**/-Annexin V-PE and +azide/-**1**/+Annexin V-PE) control samples were used to aid in compensation for two color flow cytometry (data not shown). A minimum of 10,000 cells were analyzed per sample and measurements were performed on three independent samples for each of three tested conditions (+azide/+**1**, -azide/+**1**, -azide/-**1**), with error bars representing the standard deviation of the mean. Data were analyzed using FloJo (Tree Star) software.

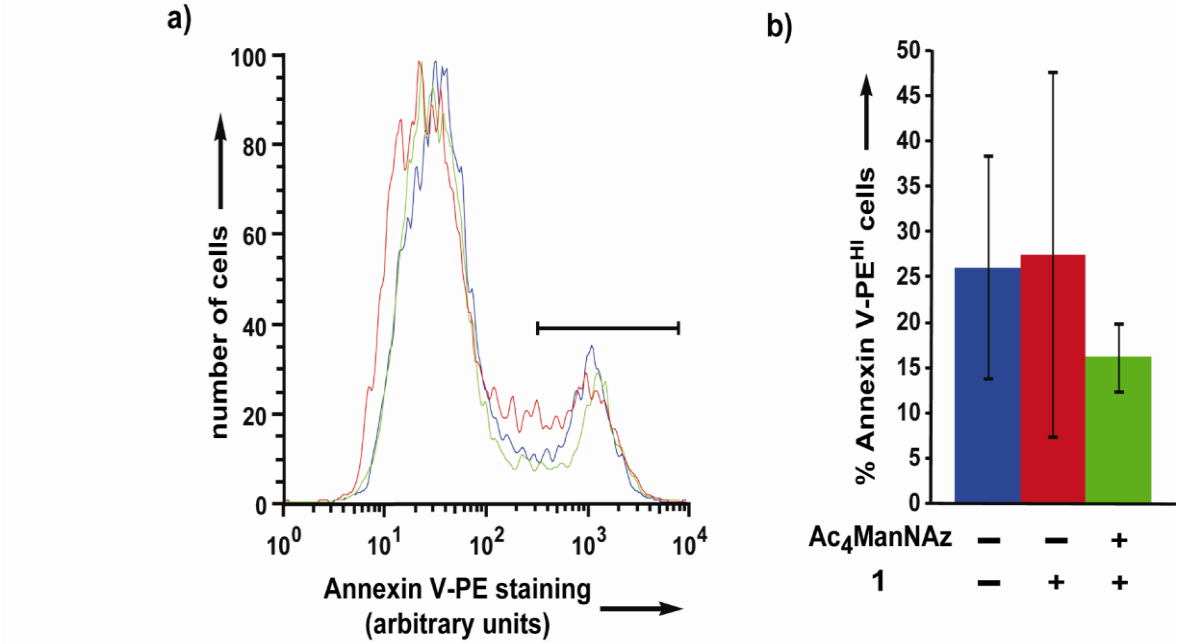


Figure S2. Flow cytometry analysis of Ac_4ManNAz-treated CHO cells labeled by phosphine **1** followed by early apoptotic marker Annexin V-PE. Cells were treated with Ac_4ManNAz for 3 days and phosphine **1** for 8 hours. Control cells were either untreated, or treated with phosphine **1** alone. a) Representative histogram of all ungated cells with the blue, red and green data representing -azide/-**1**, -azide/+**1**, and +azide/+**1** respectively; the populations under the horizontal bar are quantified in b). b) Bar graph illustrating % of cells in the Annexin V-PE^{HI} stained population; error bars represent the standard deviation of the mean for triplicate measurements.

8. Live cell fluorescence microscopy

Live cell labeling with **1** *and localization with a live cell Golgi marker:*

HeLa cells were seeded at a density of 25,000 cells per well (determined by Coulter cell counter) in 250 µL DMEM with 10% FCS on 8-well Nunc Lab-Tek Chambered Cover Glass microscopy slides (Fisher). Ac_4ManNAz was administered to the cells by adding 1.25 µL (for a final concentration of 50 µM Ac_4ManNAz) of 10 mM Ac_4ManNAz in ethanol stock solution to each well and allowing evaporation of the ethanol prior to addition of cells and media. Cells were incubated with Ac_4ManNAz for 40 h at 37 °C. The cells were rinsed with media (3x) and each well was filled with 50 µM phosphine **1** (diluted from a 10 mM DMSO stock solution) in media, sealed with tape, inverted, and incubated for 8 h at 37 °C. Slide inversion during labeling prevented non-fluorescent debris from accumulating on the slide surface, but had no effect on the fluorescence images. For localization with a live cell Golgi marker, the cells were rinsed with media (3x) and a 500 µM BODIPY® TR C_5-ceramide complexed to BSA (Molecular Probes) stock solution, diluted 100x with media, was added to the cells. After incubating for 30 min at 4 °C, the cells were rinsed with cold media (2x) and incubated with media at 37 °C for 30 min. The cells were then rinsed with media (2x) and treated with 15 µg/mL Hoechst 33342 (Molecular Probes) in PBS with 1% FCS for 2 min. After rinsing with media (2x), the cells were treated with 500 nM propidium iodide (Aldrich) in PBS with 1% FCS for 2 min. Finally, the live cells were rinsed (2x) and imaged in media.

Localization with a fixed cell Golgi marker:

Live HeLa cells were labeled with Ac_4ManNAz, **1**, Hoechst 33342, and propidium iodide as described above. The cells were then rinsed with media (2x) and fixed with cold methanol for 5 min. The cells were blocked with 1% BSA in PBS for 15 min at RT and then incubated with 0.5 µg/mL anti-

Golgin 97 mouse mAb (Molecular Probes) in PBS with 1% BSA for 1.5 h at RT. The cells were rinsed with PBS (3 x 8 min) and blocked with 1% BSA in PBS for 10 min at RT. Then the cells were incubated with 5 μg/mL goat anti-mouse IgG-Alexa Fluor 647 (Molecular Probes) in PBS with 1% BSA for 1 h at RT. The cells were then rinsed with PBS (3 x 8 min) and imaged in PBS.

Supporting Information references

[1] K. L. Kiick, E. Saxon, D. A. Tirrell, C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 19.
[2] P. V. Chang, J. A. Prescher, M. J. Hangauer, C. R. Bertozzi, *J. Am. Chem. Soc.* **2007**, *129*, 8400.
[3] G. A. Lemieux, C. L. De Graffenried, C. R. Bertozzi, *J. Am. Chem. Soc.* **2003**, *125*, 4708.
[4] S. Fery-Forgues, D. Lavabre, *J. Chem. Educ.* **1999**, *76*, 1260.
[5] J. H. Brannon, D. J. Magde, *J. Phys. Chem.* **1978**, *82*, 705.

Full references with 10 or more authors from the printed version

[5] a) S. T. Laughlin, N. J. Agard, J. M. Baskin, I. S. Carrico, P. V. Chang, A. S. Ganguli, M. J. Hangauer, A. Lo, J. A. Prescher, C. R. Bertozzi, *Methods Enzymol.* **2006**, *415*, 230.
[6] a) Y. Kho, S. C. Kim, C. Jiang, D. Barma, S. W. Kwon, J. Cheng, J. Jaunbergs, C. Weinbaum, F. Tamanoi, J. Falck, Y. Zhao, *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 12479; c) M. A. Kostiuk, M. M. Corvi, B. O. Keller, G. Plummer, J. A. Prescher, M. J. Hangauer, C. R. Bertozzi, G. Rajaiah, J. R. Falck, L. G. Berthiaume, *FASEB J.*, in press.
[12] c) T. Hosoya, T. Hiramatsu, T. Ikemoto, M. Nakanishi, H. Aoyama, A. Hosoya, T. Iwata, K. Maruyama, M. Endo, M. Suzuki, *Org. Biomol. Chem.* **2004**, *2*, 637.