SNAP-tagging Live Cells via Chelation-Assisted Copper-Catalyzed Azide-Alkyne Cycloaddition

Daniel J. Stone,[1] Miguel Macias-Contreras,[1] Shaun M. Crist,[1] Christelle F. T. Bucag,[1] Gwimoon Seo,[2] and Lei Zhu[1,*]

1. Department of Chemistry and Biochemistry, Florida State University, 95 Chieftan Way, Tallahassee, FL 32306-4390, USA

2. Institute of Molecular Biophysics, Florida State University, 91 Chieftan Way, Tallahassee, FL 32306-4380, USA

lzhu@fsu.edu

Supporting Information

Table of Contents

1. Additional structure

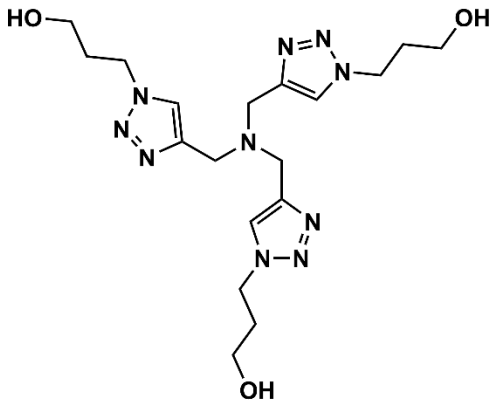

Fig. S1 Chemical structure of THPTA (tris(3-hydroxypropyltriazolylmethyl)amine), the copper-binding ligand used in this work.

2. ESI-MS-TOF spectra

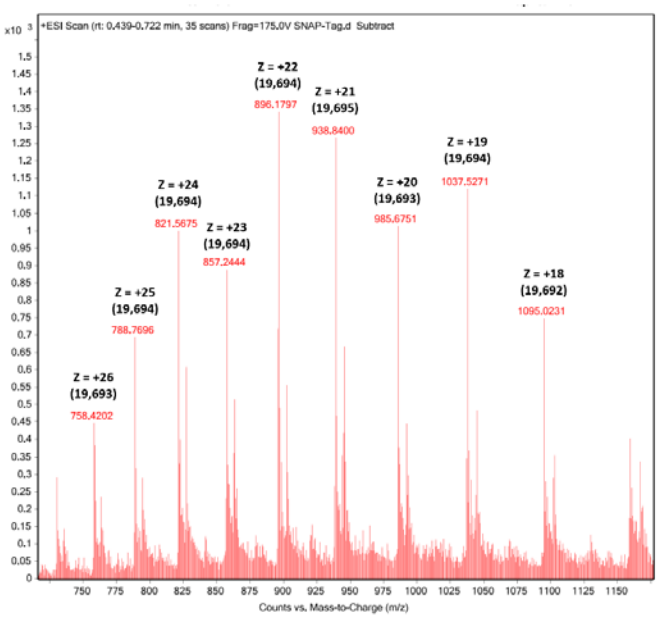

Fig. S2 ESI-TOF-MS spectrum of SNAP-tag protein (purchased from NEB). The charge (Z) is listed above each m/z peak, while the m value is listed in the parenthesis. The calculated MW of the intact SNAP-tag protein is 19,694, while the calculated MW of the mono-DTT adduct is 19,829.

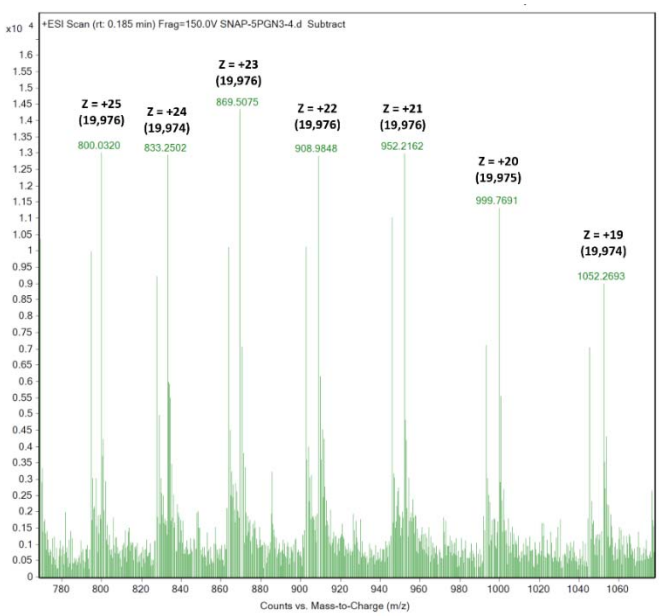

Fig. S3 ESI-TOF-MS spectrum of the reaction mixture of SNAP-tag protein (purchased from NEB) and **5PG-AZIDE**. The charge (Z) is listed above each m/z peak, while the m value is listed in the parenthesis. The calculated MW of the conjugate of the intact SNAP-tag protein and **5PG-AZIDE** is 19,840, while the calculated MW of the conjugate of the mono-DTT adduct and **5PG-AZIDE** is 19,975.

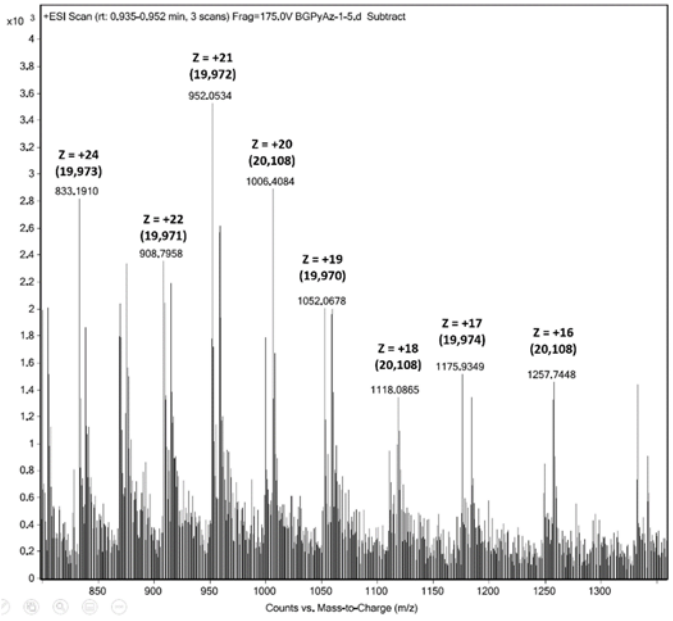

Fig. S4 ESI-TOF-MS spectrum of the reaction mixture of SNAP-tag protein (purchased from NEB) and **BG-PyAz-1**. The charge (Z) is listed above each m/z peak, while the m value is listed in the parenthesis. The calculated MW of the conjugate of the intact SNAP-tag protein and **BG-PyAz-1** is 19,973, while the calculated MW of the conjugate of the mono-DTT adduct and **BG-PyAz-1** is 20,108.

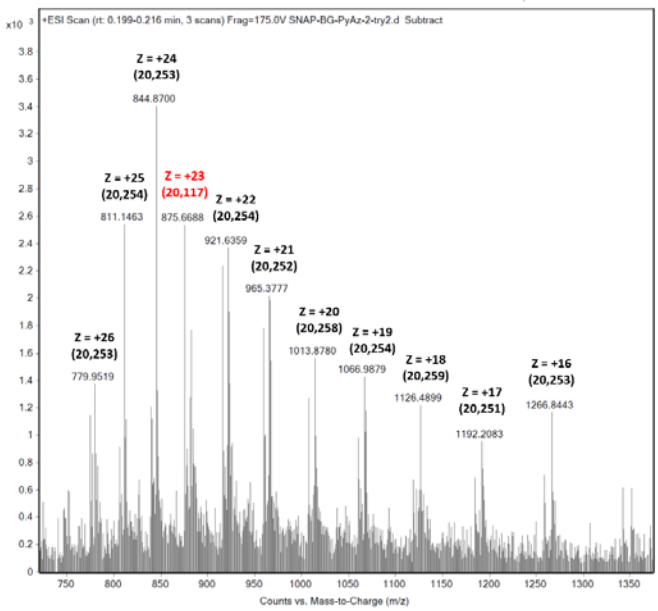

Fig. S5 ESI-TOF-MS spectrum of the reaction mixture of SNAP-tag protein (purchased from NEB) and **BG-PyAz-2**. The charge (Z) is listed above each m/z peak, while the m value is listed in the parenthesis. The calculated MW of the conjugate of the intact SNAP-tag protein and **BG-PyAz-2** is 20,118 (marked in red), while the calculated MW of the conjugate of the mono-DTT adduct and **BG-PyAz-2** is 20,253.

3. SDS-PAGE

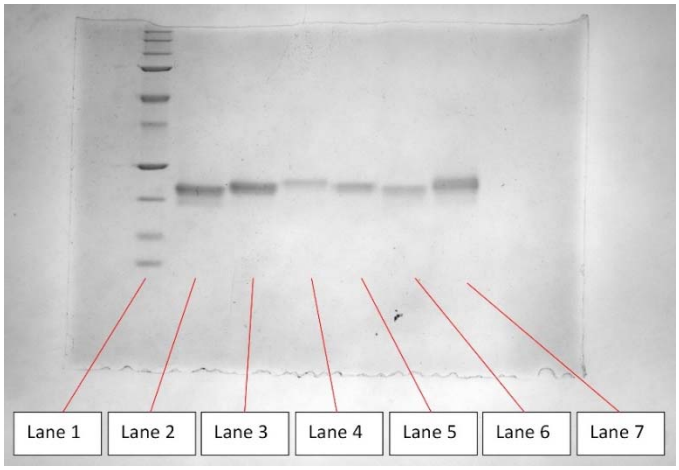

Fig. S6 SDS-PAGE gel stained by Coomassie Blue G250. Lane 1 – ladder; Lane 2 – SNAP-tag only; Lane 3 – SNAP-tag and **BG-PyAz-2**, after SEC; Lane 4 – **BG-PyAz-2**-attached SNAP-tag and Sulfo-Cy5-alkyne treated with the CuAAC cocktail, after SEC; Lane 5 – **BG-PyAz-2**-attached SNAP-tag and Sulfo-Cy5-alkyne without the CuAAC cocktail, after SEC; Lane 6 – SNAP-tag and Sulfo-Cy5-alkyne treated with the CuAAC cocktail without **BG-PyAz-2**, after SEC; and Lane 7 - **BG-PyAz-2**-attached SNAP-tag and Sulfo-Cy5-alkyne treated with the CuAAC cocktail, without SEC separation.

4. Additional images

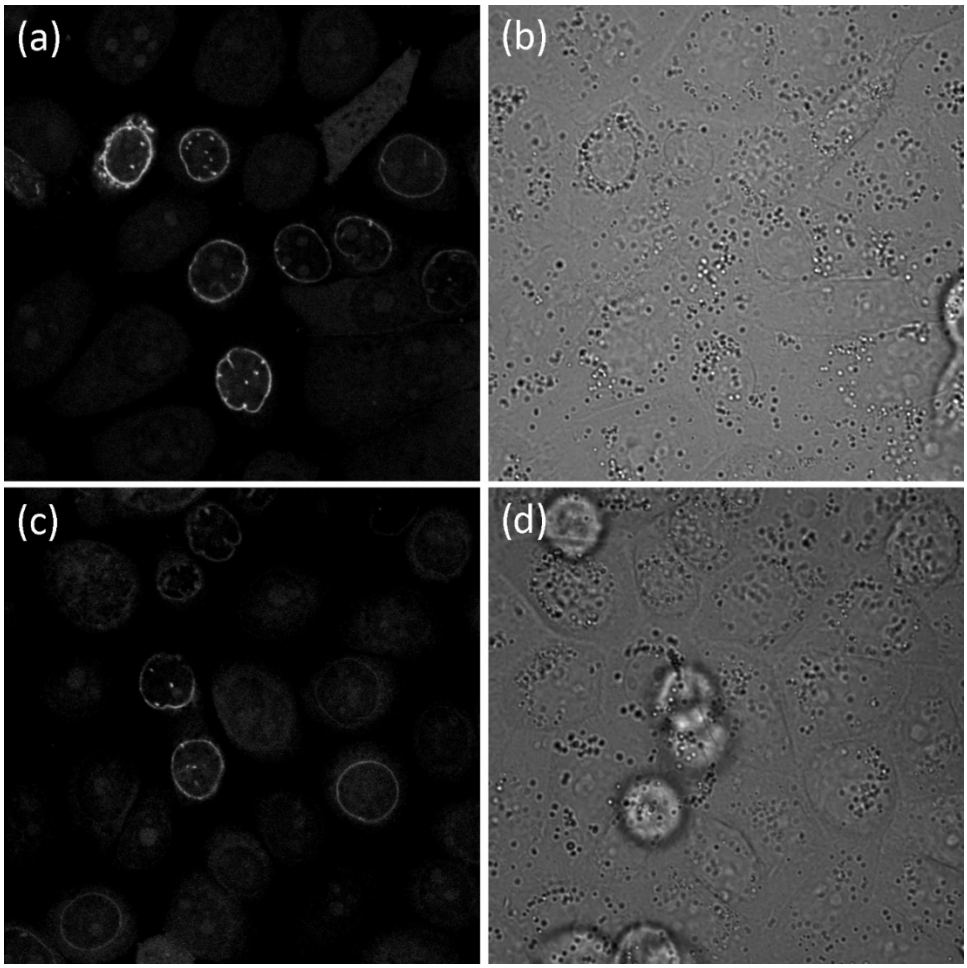

Fig. S7 Fluorescence (a, c) and bright field (b, d) images of SNAP-LaminA-expressing HeLa cells labeled by **BG-PyAz-2** (a and b, 3 μM) or **BG-AZIDE** (c and d, 3 μM) for 1 h, followed by fixation (4% PFA) and permeabilization (0.2% Triton 100-X), and subsequent treatment with $CuSO_4$ (40 μM) and 5/6-TAMRA-PEG_4-alkyne (6 μM) as parts of the Click-iT reagent for 30 minutes at rt. λ_{ex} = 543 nm, emission window 560−620 nm.

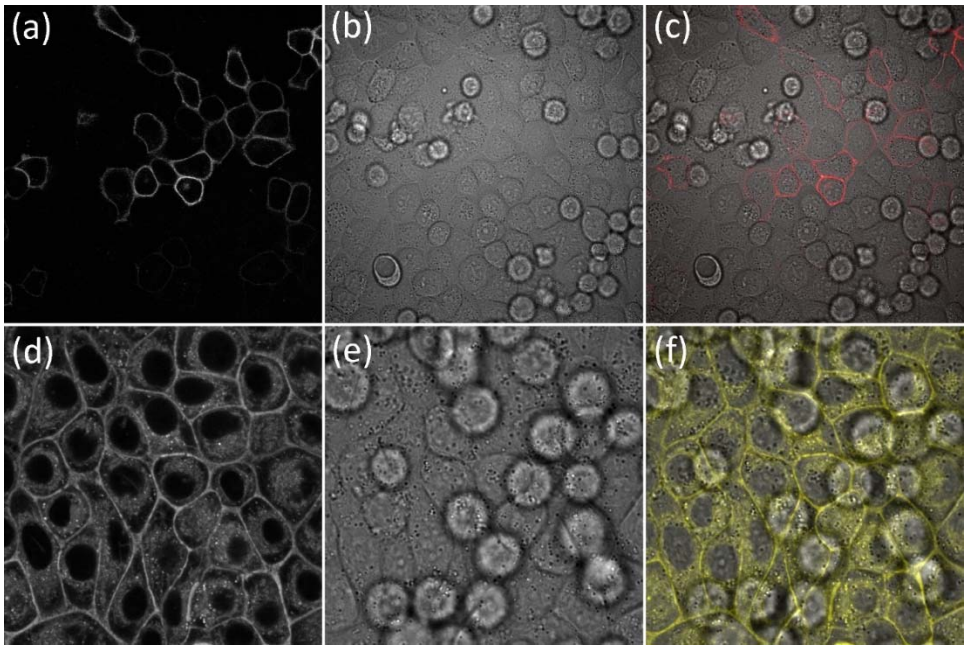

Fig. S8 Fluorescence (left), bright field (middle), and overlay (right) images of pSNAP$_f$-ADRβ2-expressing HeLa cells labeled by the membrane impermeable SNAP-Surface-647 (top row, 1 μM, λ_{ex} = 633 nm, λ_{em} > 650 nm) or the membrane permeable SNAP-Cell-TMR-Star (bottom row, 1 μM, λ_{ex} = 543 nm, emission window 560-620 nm) for 30 minutes at 37 °C. SNAP-Cell-TMR-Star shows non-specific staining on cell membranes and inside cells.

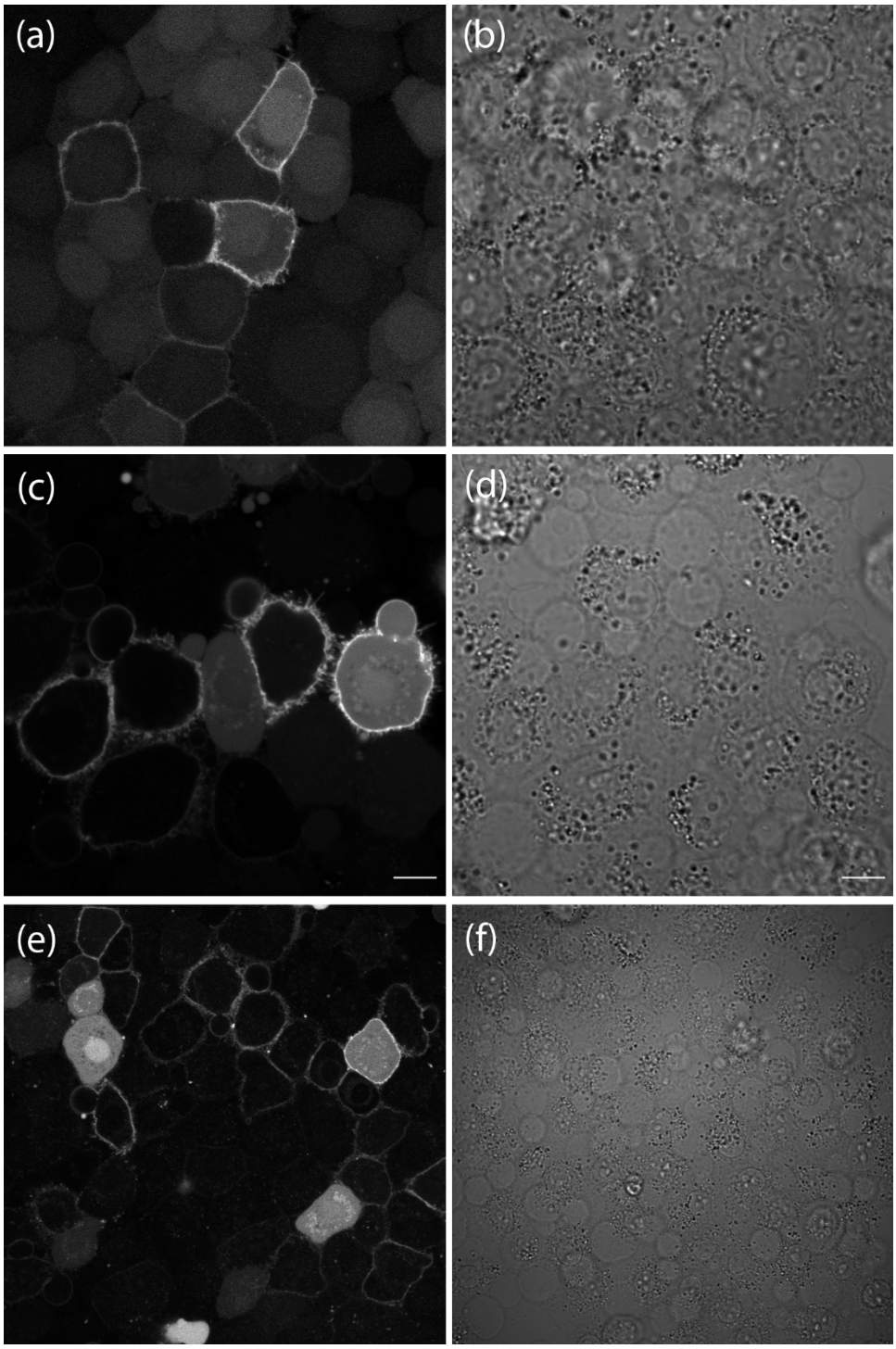

Fig. S9 Fluorescence (a, c, e) and bright field (b, d, f) images of pSNAP$_f$-ADRβ2-expressing live HeLa S3 cells labeled by **5PG-AZIDE** (a and b, 3 μM), **BG-PyAz-1** (c and d, 3 μM), and **BG-AZIDE** (e and f, 3 μM) with an incubation time of 1 h, followed by labeling with sulfo-Cy5-alkyne (6 μM, 30 minutes at rt) using 40 μM $CuSO_4$ as part of the Click-iT reagent. λ_{ex} = 633 nm, λ_{em} > 650 nm.

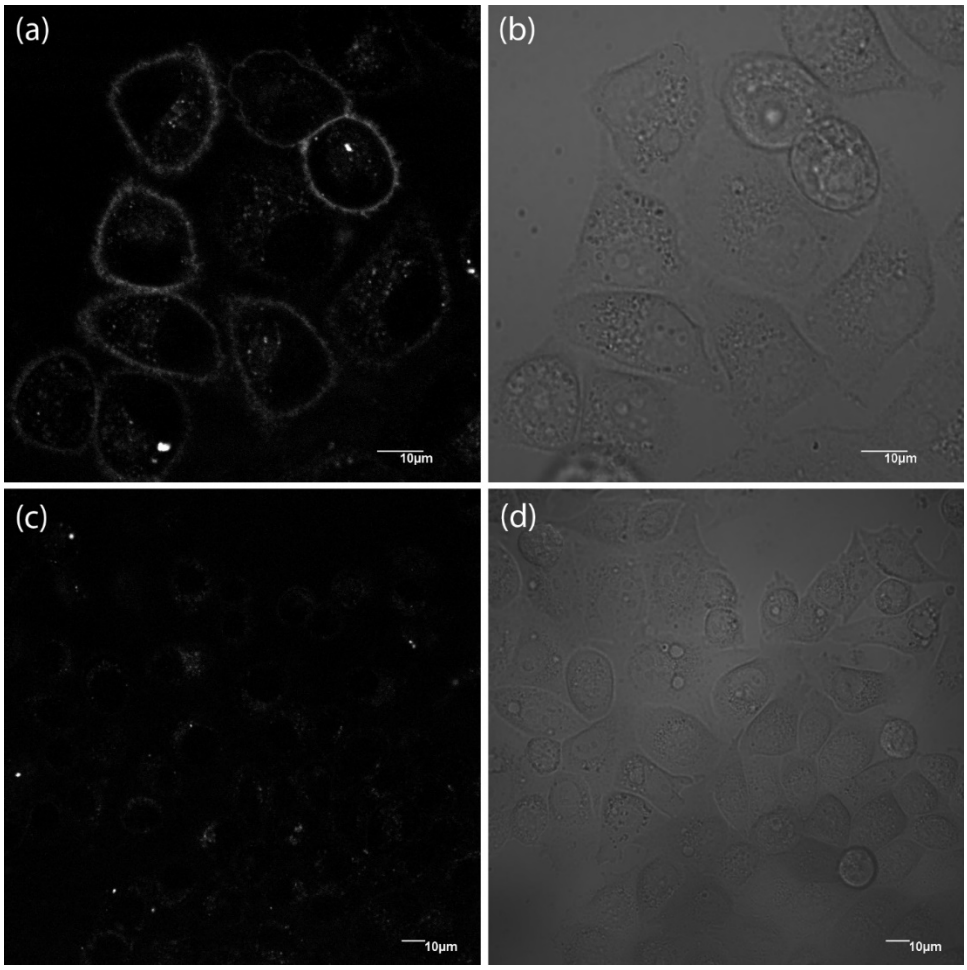

Fig. S10 Fluorescence (a, c) and bright field (b, d) images of pSNAP$_f$-ADRβ2-expressing HeLa cells labeled by **5PG-AZIDE** (a and b, 3 µM) or **BG-AZIDE** (c and d, 3 µM) for 30 min at 37 °C, followed by sulfo-Cy5-alkyne (6 µM) using a homemade CuAAC cocktail for 10 minutes at rt. [$Cu(OAc)_2$] = 20 µM, [sodium ascorbate] = 0.5 mM, [THPTA] = 0.1 mM. λ_{ex} = 633 nm, λ_{em} > 650 nm.

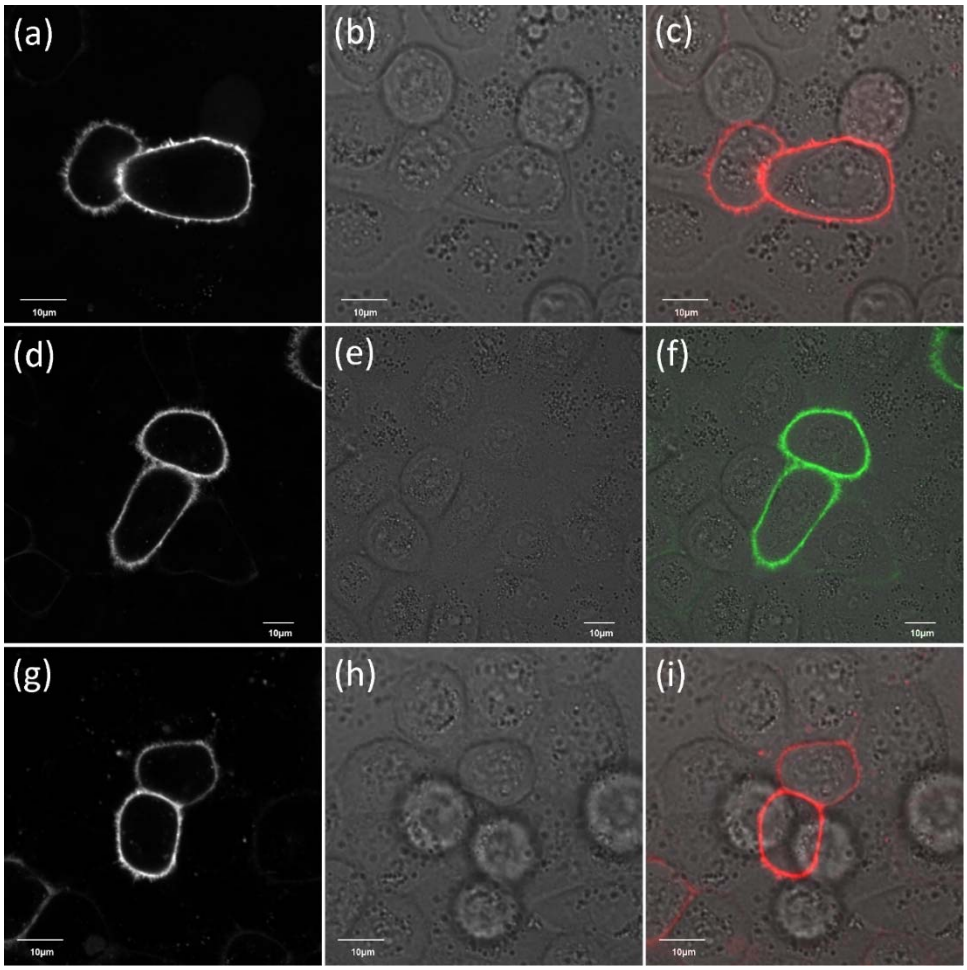

Fig. S11 Fluorescence (left), DIC (middle), and overlay (right) images of pSNAP$_f$-ADRβ2-expressing <u>live</u> HeLa cells (selected clusters from the view field) labeled by **BG-PyAz-2** (a-f) at 3 μM each for 30 min at 37 °C, followed by sulfo-Cy5-alkyne (a-c, 6 μM) or 6-FAM-alkyne (d-f, 6 μM) for 10 min at 37 °C using a homemade CuAAC cocktail in PBSA: [$Cu(OAc)_2$] = 20 μM, [SA] = 0.5 mM, [THPTA] = 0.1 mM. Frames g-I were acquired from the experiment in which **BG-PyAz-2** (3 μM) was mixed with the CuAAC cocktail for 30 minutes to produce the labeling media. The pseudo one-step labeling occurred over 10 mins at 37 °C. For Cy5, λ_{ex} = 633 nm, λ_{em} > 650 nm. For FAM, λ_{ex} = 488 nm, λ_{em} =500-600 nm.

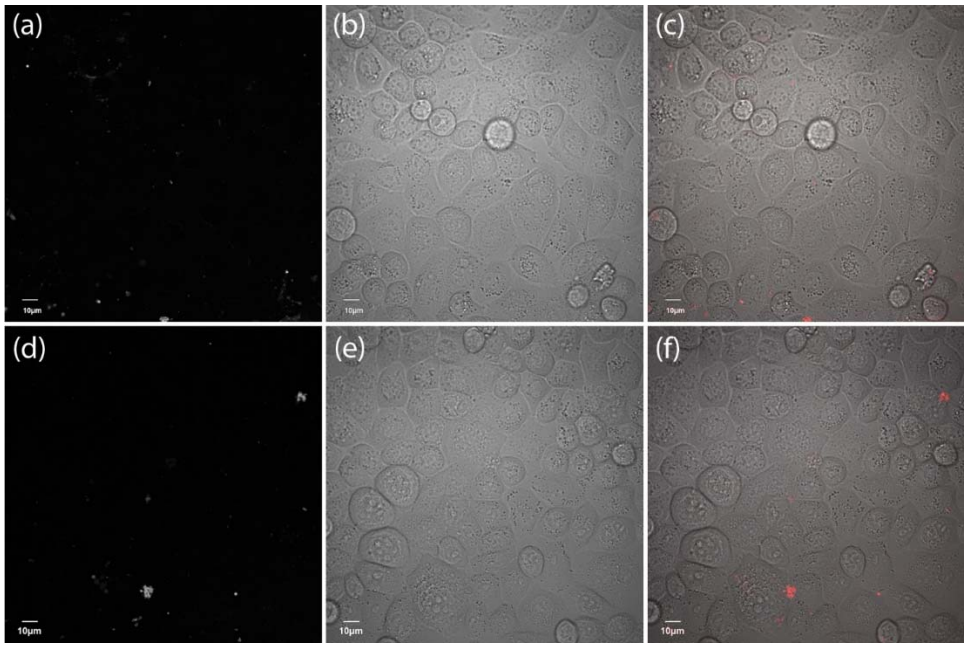

Fig. S12 Fluorescence (left), DIC (middle), and overlay (right) images of pSNAP$_f$-ADRβ2-expressing live HeLa cells incubated with DBCO-sulfo-Cy5 (a-c, 6 μM in PBSA) or sulfo-Cy5-alkyne (d-f, 6 μM in the homemade CuAAC cocktail) for 5 minutes at 37 °C. For d-f, [$Cu(OAc)_2$] = 20 μM, [SA] = 0.5 mM, [THPTA] = 0.1 mM. λ_{ex} = 633 nm, λ_{em} > 650 nm.

5. Copies of ^{1}H and ^{13}C NMR spectra of new compounds

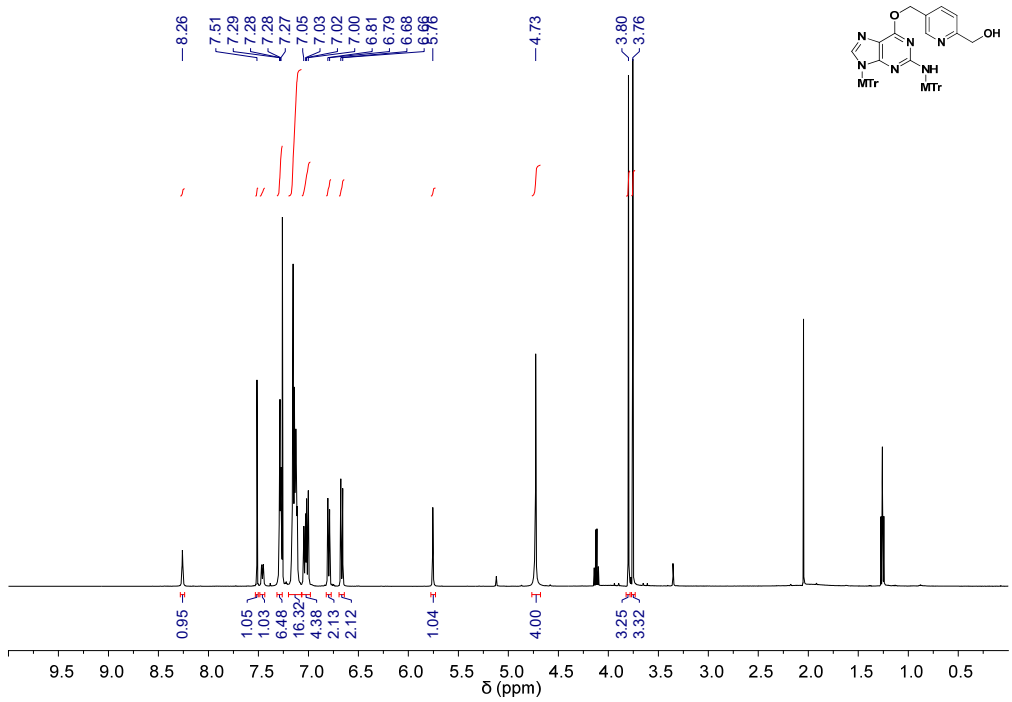

Fig. S13 ^{1}H NMR (500 MHz, $CDCl_3$) of compound **2**.

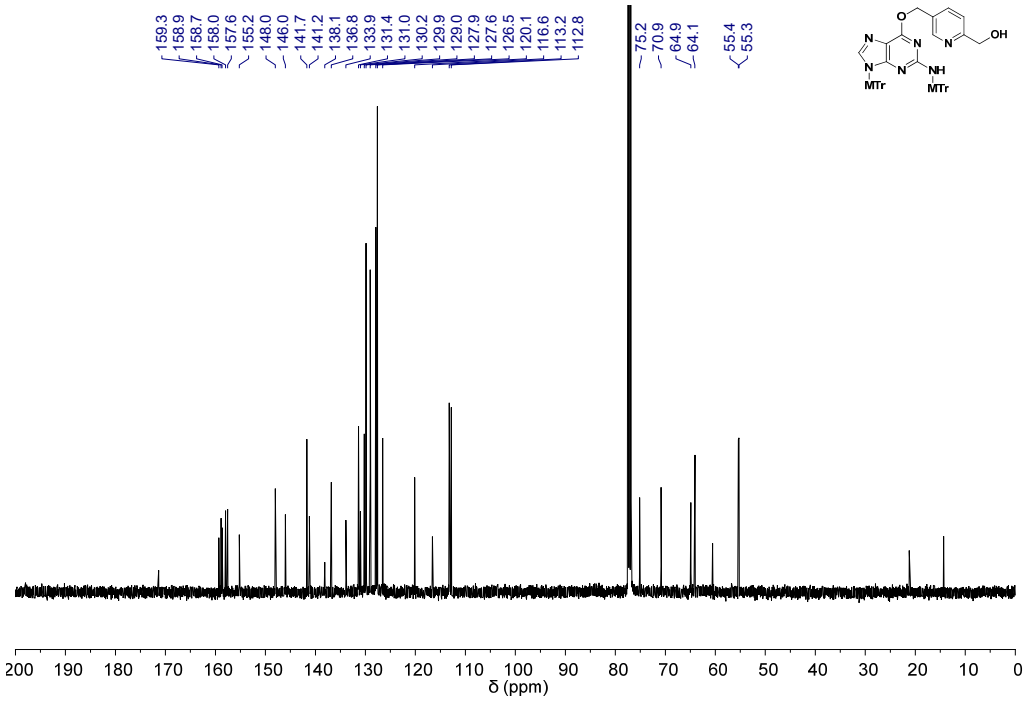

Fig. S14 ^{13}C NMR (125 MHz, $CDCl_3$) of compound **2**. The peaks at 171, 60, 21, 14 ppm are attributed to residual ethyl acetate in the sample.

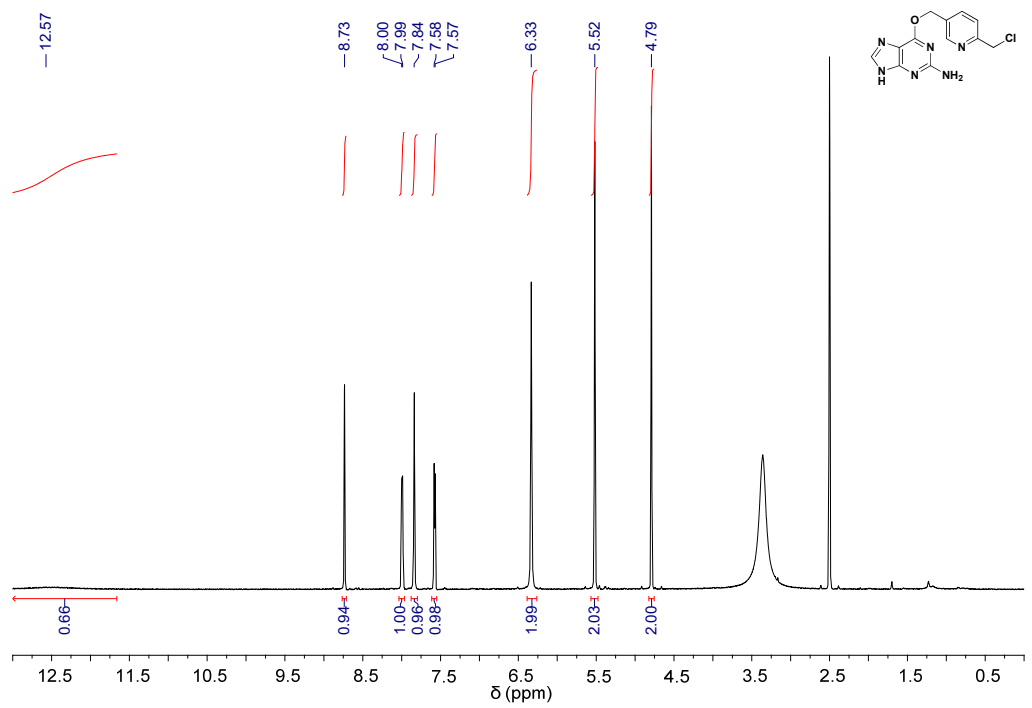

Fig. S15 ^{1}H NMR (600 MHz, DMSO-d_6) of compound **3**.

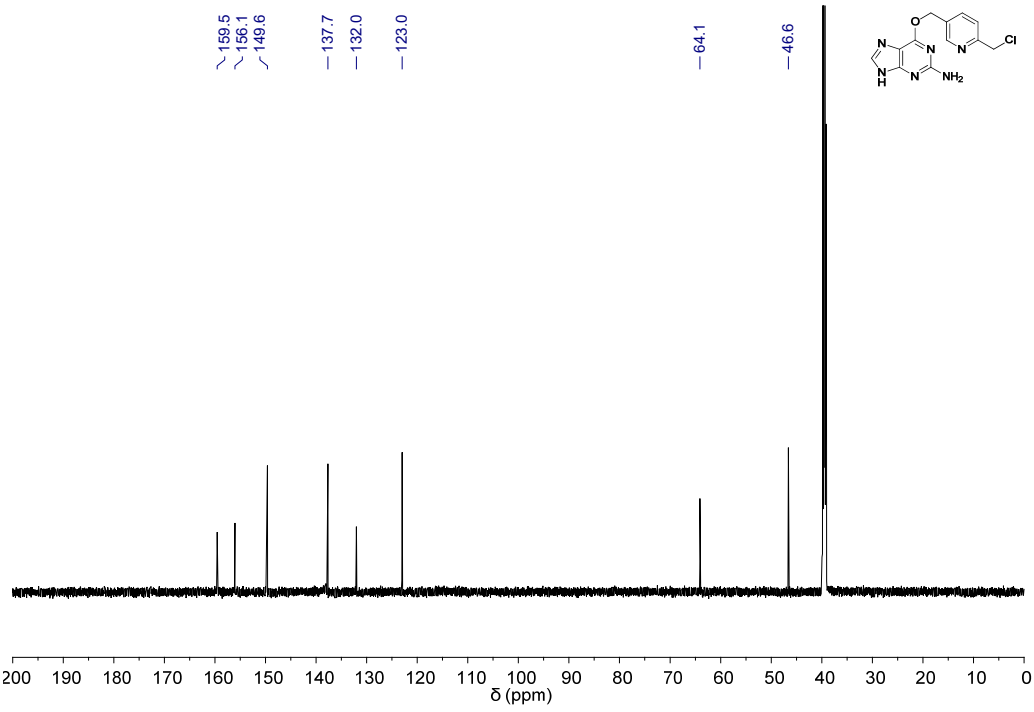

Fig. S16 ^{13}C NMR (150 MHz, DMSO-d_6) of compound **3**.

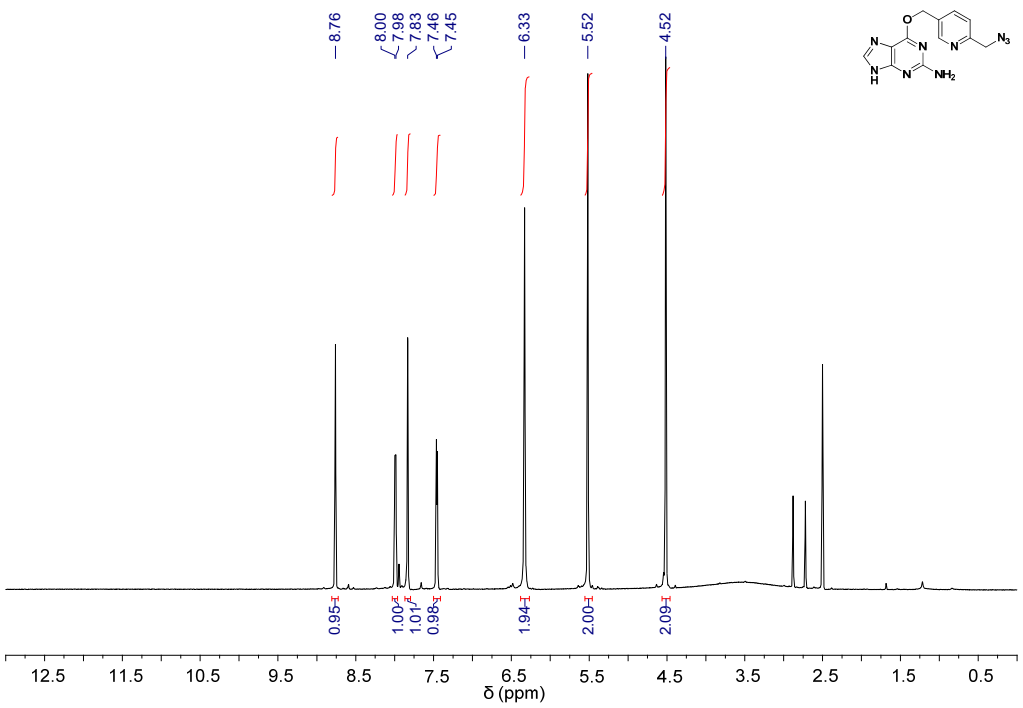

Fig. S17 ^{1}H NMR (600 MHz, DMSO-d_6) of **5PG-AZIDE**.

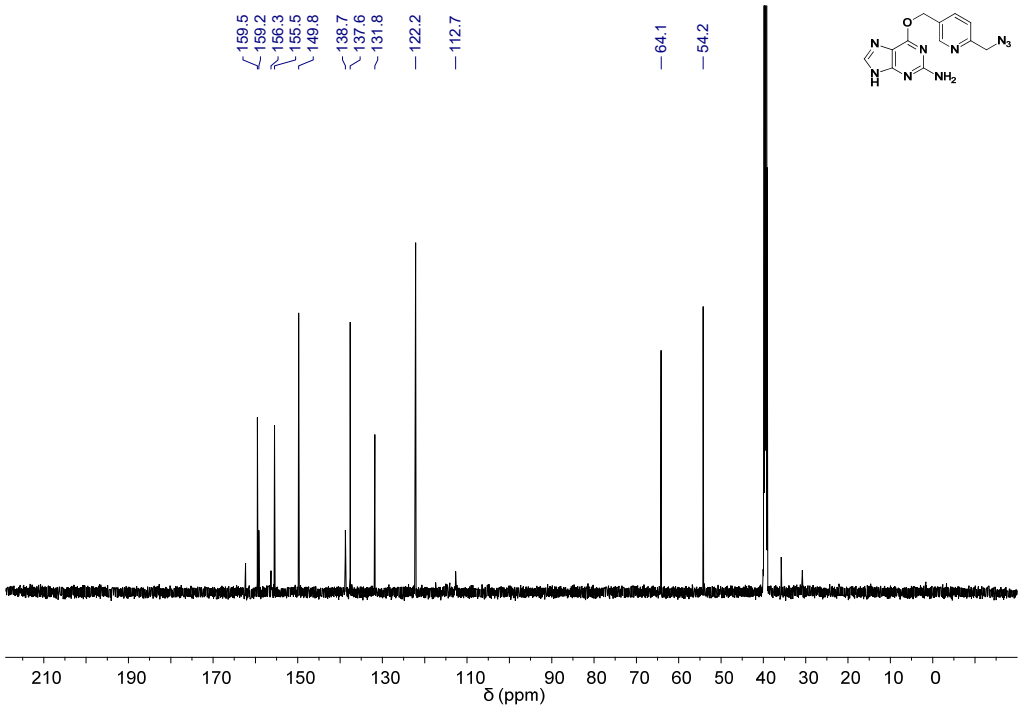

Fig. S18 ^{13}C NMR (150 MHz, DMSO-d_6) of **5PG-AZIDE**.

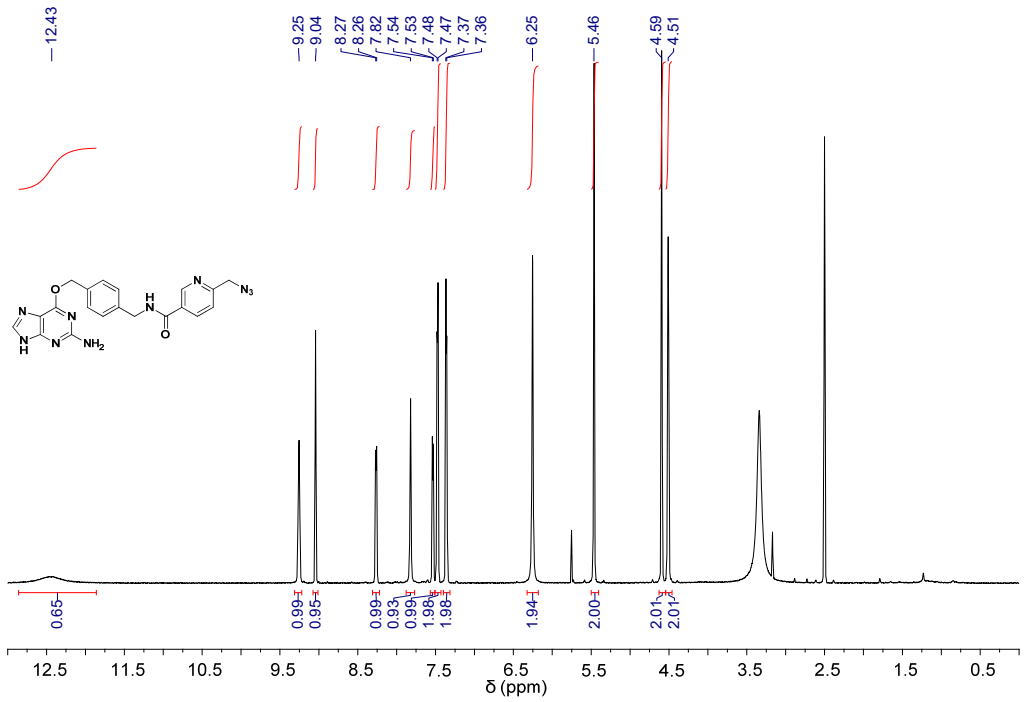

Fig. S19 ^{1}H NMR (600 MHz, DMSO-d_6) of **BG-PyAz-1**.

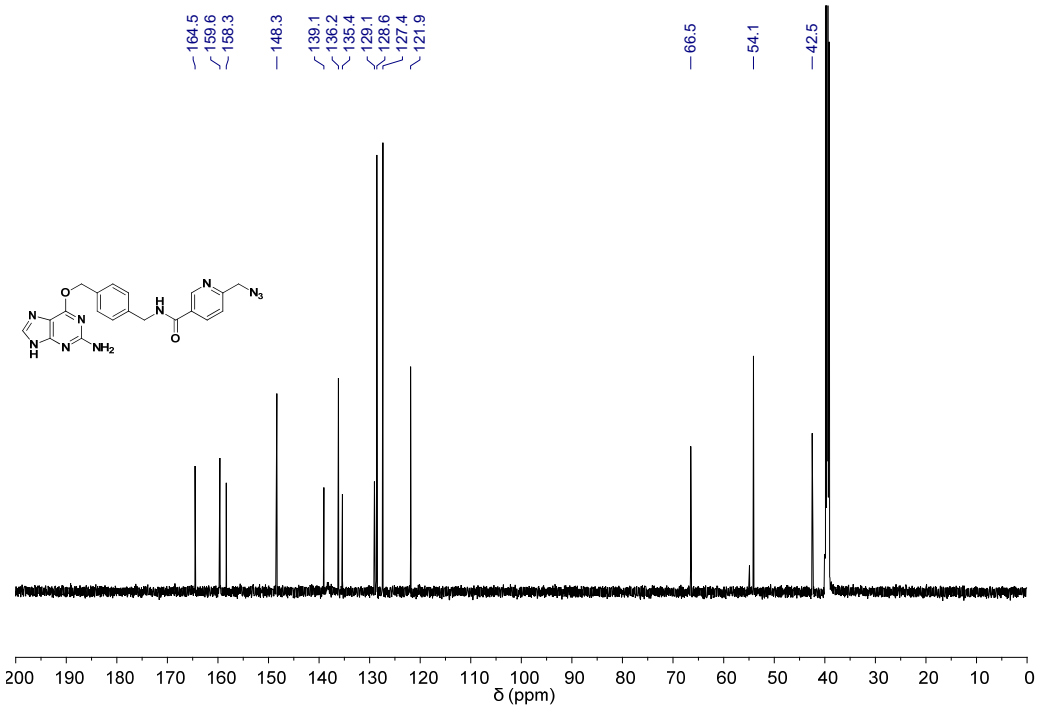

Fig. S20 ^{13}C NMR (150 MHz, DMSO-d_6) of **BG-PyAz-1**.

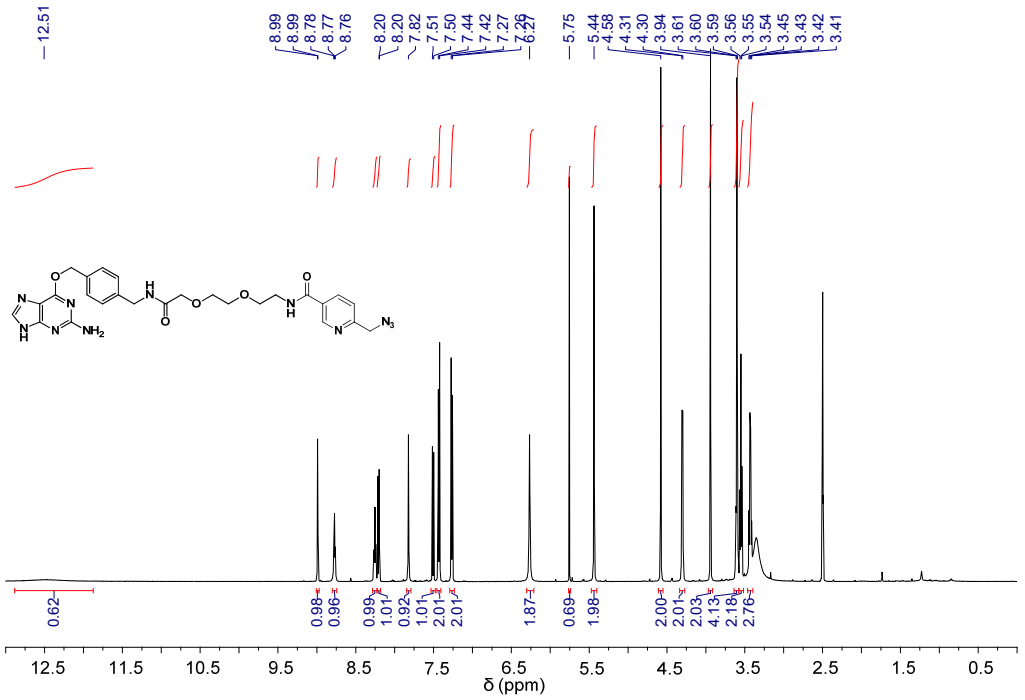

Fig. S21 ^{1}H NMR (500 MHz, DMSO-d_6) of **BG-PyAz-2**.

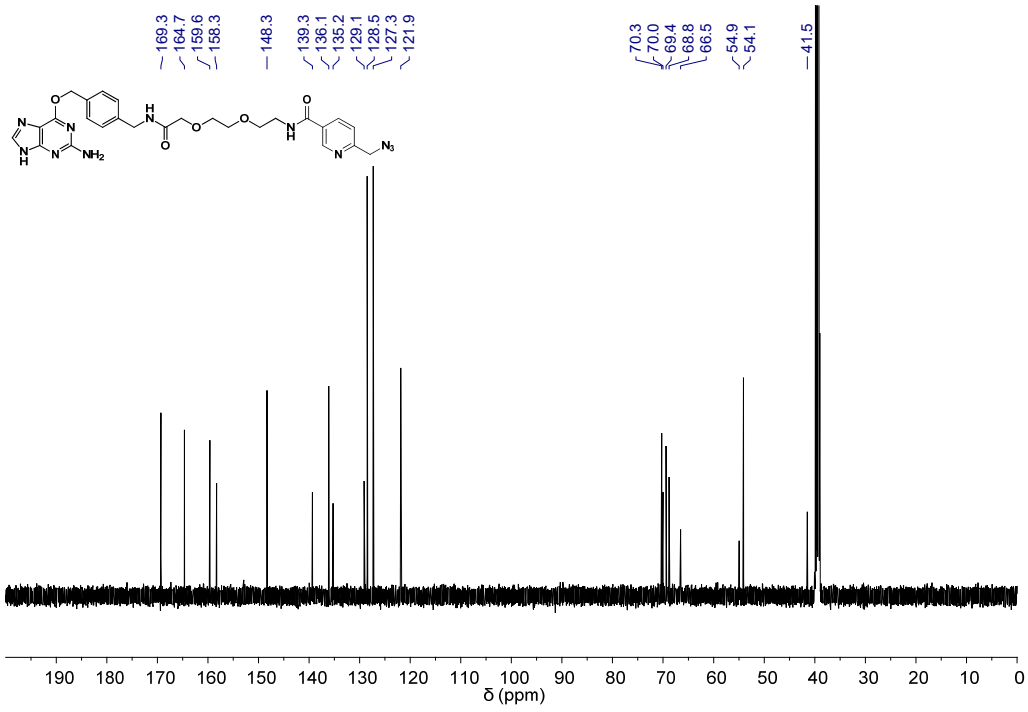

Fig. S22 ^{13}C NMR (125 MHz, DMSO-d_6) of **BG-PyAz-2**.