Supporting Information for

Original article

Building bioorthogonal click-release capable artificial receptors on cancer cell surface for imaging, drug targeting and delivery

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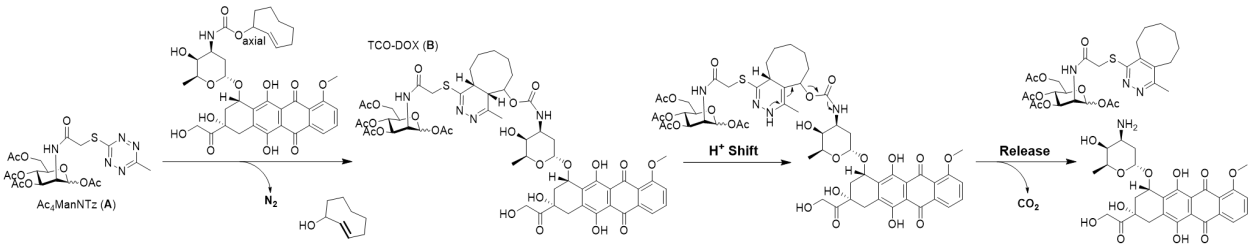
Table of contents:

1 Click release of TCO-caged agents 3
1.1 Click and release of TCO-caged Doxorubicin: 3
1.2 Click and release of TCO-caged ARV771: 3
2 Statistical analysis. 4
3. Chemical synthesis. 4
3.1 Synthesis of Ac_4ManNTz (**1**) 4
3.1.1 3-methyl-6-(methylthio)-1,2,4,5-tetrazine: 4
3.1.2 3-bromo-6-methyl-1,2,4,5-tetrazine (**4**): 5
3.1.3 2-((6-methyl-1,2,4,5-tetrazin-3-yl)thio)acetic acid (**5**): 5
3.1.4 Ac_4ManNTz (**1**): 6
3.2 Synthesis of Ac_4ManNPhTz (**2**) 7
3.2.1 4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzoic acid (**6**): 7
3.2.2 Ac_4ManNPhTz (**2**): 8
3.3 Synthesis of Ac_4ManNPh (**3**) 9
3.4 Synthesis of TCO-caged prodrugs 10
3.4.1 Synthesis of TCO-4-Nitrophenyl Carbonate 10
3.4.2 Synthesis of TCO-DOX 10
3.4.3 Synthesis of TCO-ARV771 11
4. Figures 12
4.1 Cytotoxicity of Ac_4ManNTz, Ac_4ManNPhTz, Ac_4ManNPh. 12
4.2 Metabolically engineered cell-surface glycoconjugates on A549 cells 13
4.3 Metabolically engineered cell-surface glycoconjugates on HeLa cells 13
4.4 Metabolic glycoengineering dependent on concentration of Ac_4ManNTz 14
4.5 TCO-Biotin labeling 14
4.6 Time course for tetrazine-TCO labeling 15
4.7 Metabolism of Ac_4ManNTz in various cell lines 16
4.8 Simultaneous labelling of two cell surface reporters 16
5. Tables 17
5.1 **Table S1**. Antibodies used for Western blotting analysis 17
6. Spectrum 18
7. Reference 26

1 Click release of TCO-caged agents

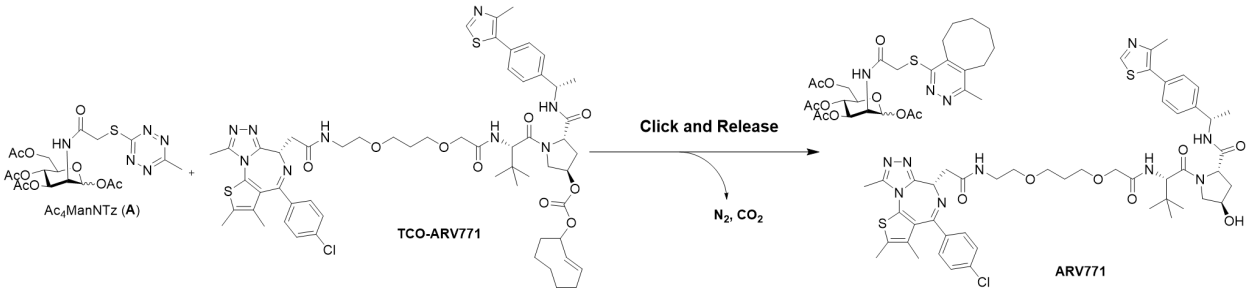
1.1 Click and release of TCO-caged Doxorubicin:



Procedure for click and release:

TCO-caged Doxorubicin (TCO-Dox, 20 μM) was dissolved in PBS (containing 5 % DMSO). After the solution was equilibrated at 37 ºC, Ac_4ManNTz (40 μM) was added, and the solution was thoroughly mixed and incubated at 37 ºC in the dark. The progress of the reaction was monitored by HPLC/PDA analysis, revealing the formation of the doxorubicin. The released doxorubicin was quantified by using a reference solution of doxorubicin hydrochloride in PBS.

1.2 Click and release of TCO-caged ARV771:



The click and release experiments of TCO-caged ARV771 were conducted in the same manner. With TCO-ARV771 (20 μM) dissolved in PBS (containing 5% DMSO), Ac_4ManNTz (40 μM) was added, and the solution was thoroughly mixed and incubated at 37 ºC in the dark. The progress of the reaction was monitored by HPLC/PDA analysis. The formation and quantification of the ARV771 were evaluated by using a reference solution of ARV771 in 5 % DMSO in PBS.

2 Statistical analysis

Data were analyzed and visualized using GraphPad Prism 7 software. All statistical tests were made and compared by two-tailed Student's t-test between two experimental groups. $P < 0.05$ was considered to be significant.

3. Chemical synthesis

3.1 Synthesis of Ac_4ManNTz (1)

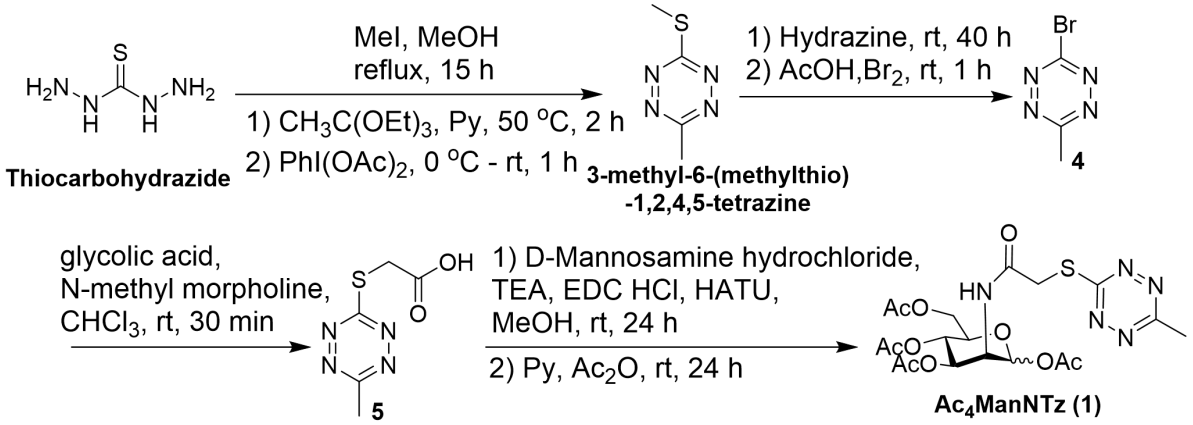
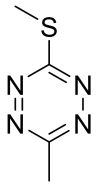


Figure S1: Synthesis of Ac_4ManNTz (**1**).

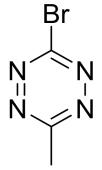
3.1.1 3-methyl-6-(methylthio)-1,2,4,5-tetrazine:



This compound was prepared according to the method described in the literature.[1-2] Thiocarbohydrazide (10 g, 94.20 mmol) was dissolved in ethanol (283 mL) and refluxed. To this mixture was added the solution of Iodomethane (6.5 mL, 103.62 mmol) in ethanol (42 mL) slowly with vigorous stirring and further refluxed for 1 hour. The reaction mixture was allowed to cool to room temperature. This mixture was left in freeze overnight to let the product precipitate out, which was decanted/filtered, and vacuum dried to get white crystalline solid (10.68 g, 43.07 mmol). The intermediate was taken further. To 5.6 g of this ethanol (140 mL) was added and heated to 55°C and triethyl orthoacetate (4.55 mL, 24.83 mmol) and stirred for 5 minutes, the solution turns straw red. To this mixture triethylamine (3.46 mL, 24.83 mmol) was

added and refluxed for 30 minutes. The reaction mixture was removed from the hot bath and added $NaNO_2$ (3.27 g, 47.41 mmol) followed by the slow addition of TFA (1.90 mL, 24.83 mmol). After the complete addition, the reaction mixture was refluxed for 1 hour and then brought to room temperature. Then 150 mL of hexane was added and diluted with 300 mL of water and extracted with diethyl ether (3×). The solvent was dried, and the crude product was purified by flash chromatography (SiO_2, 10-20% EtOAc in Hexane) to get red oil in 30 % yield (953 mg, 6.70 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.95 (s, 3H), 2.71 (s, 3H).

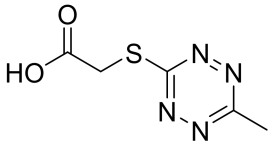
3.1.2 3-bromo-6-methyl-1,2,4,5-tetrazine (4):



This compound was prepared according to the method described in the literature.[3] To a stirred solution of 3-methyl-6-(methylthio)-1,2,4,5-tetrazine (766 mg, 5.39 mmol) in ethanol (34 mL) was added 1 M hydrazine in THF (6.46 mL, 6.46 mmol) and stirred at room temperature for 40 hours. The reaction mixture was then concentrated and purified by flash chromatography (10-20% MeOH in EtOAc) to get a red-colored solid in 47% yield (322 mg, 2.55 mmol) and was directly taken to the next step.

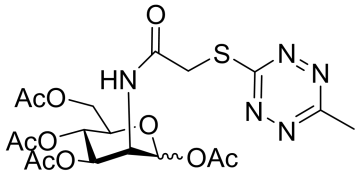
This intermediate 3-hydrazineyl-6-methyl-1,2,4,5-tetrazine (314 mg, 2.5 mmol) was dissolved in acetic acid (6.5 mL) to which was added Br_2 (166 µl, 3.23 mmol) and stirred for 1 hour at room temperature. The reaction mixture was then diluted with water and extracted DCM, dried over anhydrous $MgSO_4$. The crude mixture was concentrated and purified by flash chromatography (20% EtOAc in Hexane) to get red crystals of the pure product in 74% yield (322 mg, 1.85 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.4, 161.4, 20.9.

3.1.3 2-((6-methyl-1,2,4,5-tetrazin-3-yl)thio)acetic acid (5):



This compound was synthesized based on a reported method.[4] To the solution of thioglycolic acid (96 µl, 1.37 mmol) and N-methyl morpholine (278 µl, 2.53 mmol) in chloroform (8 mL) at room temperature was added the solution of 6-methyl bromotetrazine (200 mg, 1.15 mmol) in chloroform (3.5 mL) slowly under nitrogen atmosphere. The reaction was allowed to stir for 30 minutes followed by the addition of 2.5 mL of 15% HCl and extracted with DCM. The organic phase was washed with water and solvent was dried to get the crude product, which was purified by flash chromatography (5% MeOH in DCM) to get the red solid (76 mg, 0.42 mmol). ^{1}H NMR (500 MHz, CD_3OD) δ 4.18 (s, 2H), 2.93 (s, 3H); ^{3}C NMR (126 MHz, CD_3OD) δ 175.3, 171.5, 167.2, 33.4, 20.7.

3.1.4 Ac_4ManNTz (1):



This compound was synthesized based on a reported method.[5] D-mannosamine hydrochloride (95 mg, 0.438 mmol) was added to a solution of tetrazine derivative (68 mg, 0.365 mmol) in dry methanol (10 mL). Triethylamine (61 µL, 0.438 mmol) was added, and the reaction mixture was stirred for 5 min at room temperature. The solution was cooled to 0 °C, and HATU (347 mg, 0.71 mmol) was added, followed by EDC·HCl (180 mg, 0.91 mmol). The reaction mixture was allowed to warm to room temperature overnight, at which TCL monitor the reaction was complete. The reaction mixture was concentrated and the crude ManNTz was purified by silica gel chromatography, eluting with methanol-dichloromethane (1:5, v/v). The crude product was directly used for the acetylation without further purification.

Acetic anhydride (345 µL, 3.65 mmol) and DMAP (5 mg, 0.0365 mmol) were added to a solution of ManNTz in pyridine (5 mL), and the reaction mixture was stirred overnight at room temperature. The solution was concentrated, resuspended in CH_2Cl_2, and washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by silica gel chromatography, eluting with hexanes-ethyl acetate (6:4, v/v). The fractions containing product were concentrated to get a red crystalline solid Ac_4ManNTz (α/β isomers: ~1/3; 59 mg, 25% yield). ^{1}H NMR (500 MHz, $CDCl_3$)

δ 7.03 (d, *J* = 10.0 Hz, 1H), 5.94 (d, *J* = 1.8 Hz, 1H), 5.12 (dd, *J* = 10.2, 4.3 Hz, 1H), 4.59 (ddd, *J* = 10.1, 4.4, 1.9 Hz, 1H), 4.54 (t, *J* = 10.2 Hz, 1H), 4.13 (dd, *J* = 12.4, 4.7 Hz, 1H), 4.00 – 3.80 (m, 4H), 2.95 (s, 3H), 2.14 – 1.86 (m, 12H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 169.7, 169.2, 168.0, 167.0, 166.4, 165.8, 90.4, 69.0, 67.9, 63.9, 60.8, 48.3, 32.3, 19.8, 19.8, 19.6, 19.4; HRMS (ESI) calculated for $C_{19}H_{26}N_5O_{10}S$ $[M+H]^+$ *m/z* 516.1400, found 516.1395.

3.2 Synthesis of Ac_4ManNPhTz (2)

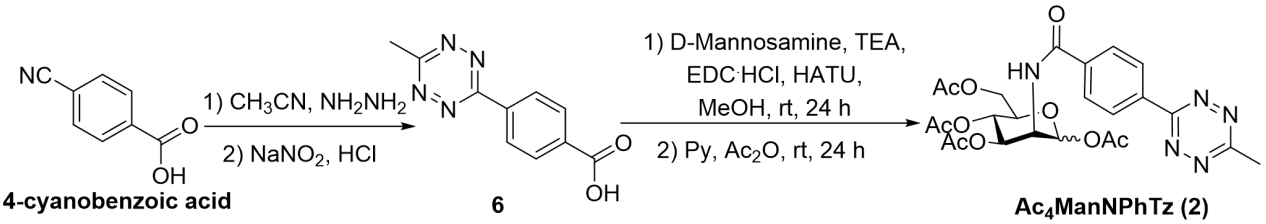
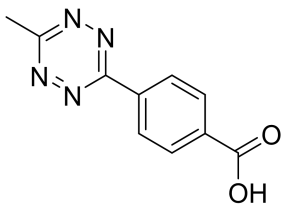


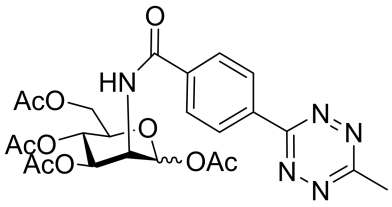
Figure S2: Synthesis of Ac_4ManNPhTz (**2**).

3.2.1 4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzoic acid (6):



This compound was synthesized based on a reported method.[6] To the ice-cooled mixture of p-Cyanobenzoic acid (1.0 g, 6.79 mmol), acetonitrile (4 mL, 67.96 mmol), and 3-mercaptopropionic acid (592 µL, 6.79 mmol) was added hydrazine hydrate (16.5 mL, 339.5 mmol) dropwise under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 18 hours until completion. The reaction mixture was then cooled to 0 °C in ice-bath condition and the solution of $NaNO_2$ (9.37 g, 135.8 mmol) was added. Then 1 N HCl was added until the evolution of gas ceased and extracted with ethyl acetate three times. The organic phase was combined and washed with brine dried over anhydrous Na_2SO_4, and concentrated to get the crude product, which was purified by flash chromatography (60-80% EtOAc in Hexane) to get purple-colored crystalline solid in 56% yield (822 mg, 3.8 mmol).

3.2.2 Ac_4ManNPhTz (2):



D-mannosamine hydrochloride (120 mg, 0.55 mmol) was added to a solution of tetrazine derivative (100 mg, 0.46 mmol) in dry methanol (10 mL). Triethylamine (129 µl, 0.92 mmol) was added, and the reaction mixture was stirred for 5 min at room temperature. The solution was cooled to 0 ºC, and HATU (589 mg, 1.15 mmol) was added, followed by EDC·HCl (228 mg, 1.15 mmol). The reaction mixture was allowed to warm to room temperature overnight, at which TCL monitor the reaction was complete. The reaction mixture was concentrated and the crude ManNPhAz was purified by silica gel chromatography, eluting with methanol-dichloromethane (1:5, v/v). The crude product was directly used for the acetylation without further purification.

Acetic anhydride (436 µl, 4.625 mmol) and DMAP (6 mg, 0.046 mmol) were added to a solution of ManNPhAz in pyridine (5 mL), and the reaction mixture was stirred overnight at room temperature. The solution was concentrated, resuspended in CH_2Cl_2, and washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by silica gel chromatography, eluting with hexanes-ethyl acetate (1:1, v/v). The fractions containing product were concentrated to get a red crystalline solid Ac_4ManNPhAz (61 mg, 24% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (t, J = 1.8 Hz, 1H), 8.81 (dt, J = 7.8, 1.5 Hz, 1H), 8.11 (dt, J = 7.7, 1.5 Hz, 1H), 7.75 (t, J = 7.8 Hz, 1H), 6.55 (d, J = 9.1 Hz, 1H), 6.23 (d, J = 1.9 Hz, 1H), 5.48 (dd, J = 10.1, 4.3 Hz, 1H), 5.37 (t, J = 9.9 Hz, 1H), 4.95 (ddd, J = 9.1, 4.3, 1.9 Hz, 1H), 4.37 – 4.28 (m, 1H), 4.20 – 4.10 (m, 2H), 3.15 (s, 3H), 2.31 – 2.02 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.2, 169.6, 168.2, 167.8, 166.5, 163.4, 134.8, 132.5, 131.4, 131.3, 129.9, 125.9, 91.7, 77.3, 77.0, 76.7, 70.3, 69.1, 65.3, 61.9, 49.9, 21.2, 20.9, 20.8, 20.7, 20.6; HRMS (ESI) calculated for $C_{24}H_{28}N_5O_{10}S$ $[M+H]^+$ *m/z* 546.1836, found 546.1831.

3.3 Synthesis of Ac_4ManNPh (3)

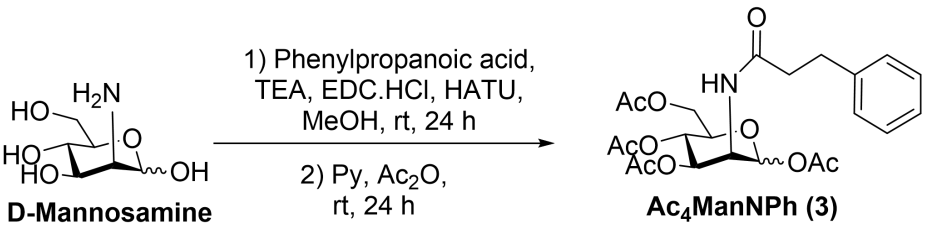


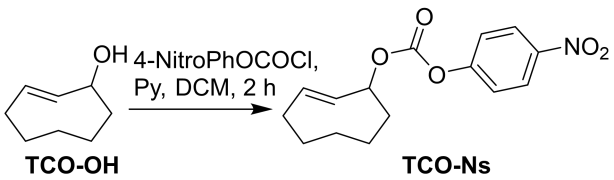
Figure S3: Synthesis of Ac_4ManNPh (**3**).

D-mannosamine hydrochloride (50 mg, 0.23 mmol) was added to a solution of phenylpropanoic acid (35 mg, 0.23 mmol) in dry methanol (5 mL). Triethylamine (62 µl, 0.46 mmol) was added, and the reaction mixture was stirred for 5 min at room temperature. The solution was cooled to 0 ^{o}C, and HOBt (78 mg, 0.58 mmol) was added, followed by EDC·HCl (112 mg, 0.58 mmol). The reaction mixture was allowed to warm to room temperature overnight, at which TCL monitor the reaction was complete. The reaction mixture was concentrated and the crude ManNPh was purified by silica gel chromatography, eluting with methanol-dichloromethane (1:10, v/v). The crude product was directly used for the acetylation without further purification.

Acetic anhydride (230 µL, 2.32 mmol) was added to a solution of ManNPh in pyridine (5 mL), and the reaction mixture was stirred overnight at room temperature. The solution was concentrated, resuspended in CH_2Cl_2, and washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by silica gel chromatography, eluting with hexanes-ethyl acetate (2:1, v/v). The fractions containing product were concentrated to yield Ac_4ManNPh (25 mg, 23 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.29 (t, *J* = 1.8 Hz, 2H), 7.24 – 7.22 (t, *J* = 7.8, 1.5 Hz, 1H), 7.22 – 7.21 (d, *J* = 7.7, 1.5 Hz, 2H), 5.89 – 5.76 (m, 2H), 5.31 -5.04 (m, 2H), 4.79 – 4.60 (m, 1H), 4.37 – 4.28 (m, 1H), 4.27 – 4.21 (m, 1H), 4.09 – 3.99 (m, 1H), 3.01 – 2.94 (m, 2H), 2.66 – 2.56 (m, 2H), 2.17 – 2.04 (s, 9H), 1.96 – 1.94 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6, 172.9, 170.6, 170.1, 170.0, 169.7, 169.6, 168.3, 168.1, 140.2, 140.1, 128.7, 128.6, 128.3, 128.2, 126.5, 126.4, 91.5, 90.5, 73.5, 71.3, 70.1, 68.8, 65.4, 65.2, 62.1, 61.9, 49.6, 49.3, 38.0, 37.9, 31.4, 31.4, 20.8, 20.7, 20.6, 20.6, 20.5.

3.4 Synthesis of TCO-caged prodrugs

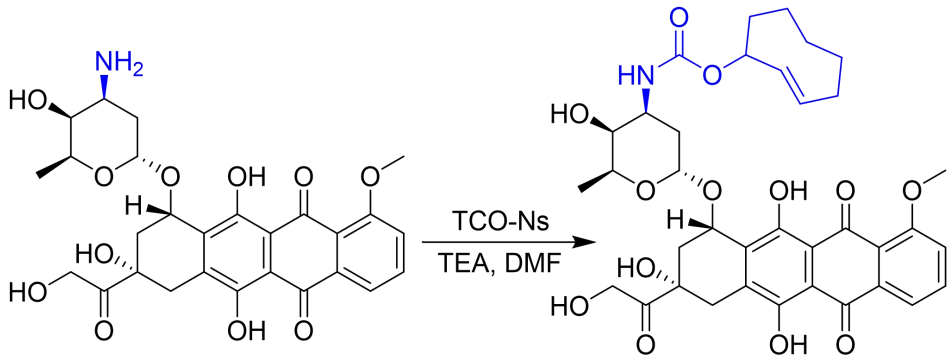
3.4.1 Synthesis of TCO-4-Nitrophenyl Carbonate



TCO-OH **TCO-Ns**

Compound **TCO-Ns** was synthesized according to reference.[7] To a solution of compound **TCO-OH** (500 mg, 3.7 mmol) in CH_2Cl_2 (10 mL) was added pyridine (0.75 mL, 9.3 mmol) under N_2. A solution of 4-nitrophenyl chloroformate (0.85 g, 4.1 mmol) in CH_2Cl_2 (10 mL) was added and the reaction mixture was stirred at rt for 2 h. A sat. aq. Solution of NH_4Cl (25 ml) was used to stop the reaction. After phase separation, the aqueous layer was extracted with CH_2Cl_2 twice. The combined organic layers were washed with a sat. aq. NaCl solution, dried over Na_2SO_4, and concentrated. The crude produce was purified by flash column (5% EtOAc in Hexane) to yield compound **TCO-Ns** (530 g, mmol, 49.2 % yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 – 8.27 (d, *J* = 9.1 Hz, 2H), 7.41 – 7.39 (d, *J* = 9.1 Hz, 2H), 6.01 – 5.95 (ddd, *J* = 16.5, 11.3, 3.8 Hz, 1H), 5.58 – 5.54 (dd, *J* = 16.5, 2.2 Hz, 1H), 5.44 (m, 1H), 2.54 – 2.52 (m, 1H), 2.24 – 2.20 (m, 1H), 2.11 – 2.00 (m, 2H), 1.96 – 1.89 (m, 1H), 1.83 – 1.70 (m, 2H), 1.57 – 1.53 (m, 1H), 1.21 – 1.14 (m, 1H), 0.88 – 0.81 (m, 1H).

3.4.2 Synthesis of TCO-DOX

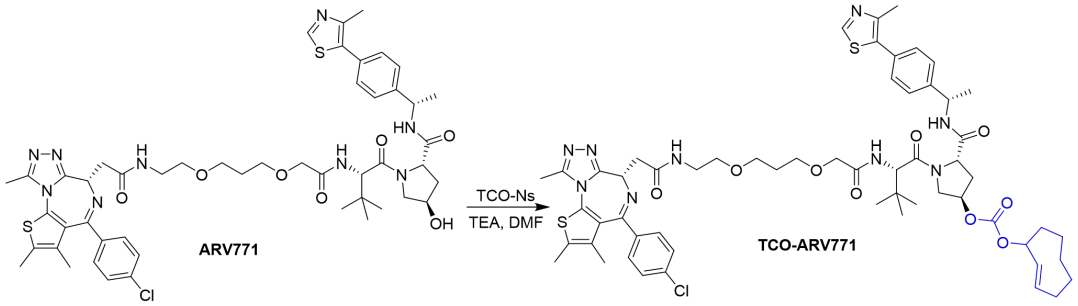


DOX **TCO_DOX**

The TCO-DOX was synthesized according to the reference.[8] The TCO-Ns (75 mg, 0.26 mmol) was dissolved in DMF (5 mL). Triethylamine (36 μL, 0.26 mmol) was added, followed by doxorubicin hydrochloride (71 mg, 0.13 mmol). The mixture was stirred in the dark at room

temperature for 3 days. The solvent was removed under high vacuum and the residue was purified by Prep-RP-HPLC using methanol and water as the eluent. The product fraction was lyophilized to yield TCO-Dox (52 mg, 57 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.97 (s, 1H), 13.25 (s, 1H), 8.03 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.77 (t, *J* = 8.1 Hz, 1H), 7.39 – 7.36 (m, 1H), 5.82 – 5.66 (m, 1H), 5.58 – 5.40 (m, 2H), 5.34 – 5.17 (m, 2H), 5.10 – 5.01 (m, 1H), 4.74 (s, 2H), 4.51 (s, 1H), 4.17 – 4.09 (m, 1H), 4.07 (s, 3H), 3.86 (bs, 1H), 3.67 (s, 1H), 3.27 (d, *J* = 18.8 Hz, 1H), 3.08 – 2.93 (m, 2H), 2.47 – 2.36 (m, 1H), 2.34 – 2.27 (m, 1H), 2.22 – 2.13 (m, 1H), 2.08 – 1.62 (m, 11H), 1.31 – 1.25 (m, 4H), 1.07 – 0.94 (m, 1H), 0.83 – 0.78 (m, 1H).

3.4.3 Synthesis of TCO-ARV771



The TCO-ARV771 was synthesized in the same manner. The TCO-Ns (12 mg, 0.04 mmol) was dissolved in DMF (5 mL). Triethylamine (5.6 µL, 0.04 mmol) was added, followed by doxorubicin hydrochloride (20 mg, 0.02 mmol). The mixture was stirred in the dark at room temperature for 3 days. The solvent was removed under high vacuum and the residue was by Prep-RP-HPLC using methanol and water as the eluent. The product fraction was lyophilized to yield TCO-ARV771 (4.5 mg, 19 % yield). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.98 (s, 1H), 7.49 – 7.36 (m, 8H), 5.82 – 5.70 (m, 1H), 5.57 – 5.46 (m, 1H), 5.27 – 5.19 (m, 2H), 5.03 – 4.95 (m, 1H), 4.69 – 4.54 (m, 3H), 4.16 (t, *J* = 12.6 Hz, 1H), 4.03 – 3.84 (m, 3H), 3.68 – 3.55 (m, 6H), 3.50 – 3.41 (m, 3H), 2.71 (s, 3H), 2.49 – 2.39 (m, 8H), 2.17 – 1.78 (m, 7H), 1.76 – 1.66 (m, 4H), 1.65 – 1.54 (m, 2H), 1.53 – 1.42 (m, 4H), 1.03 (s, 9H), 0.91 – 0.81 (m, 2H).

4. Figures

4.1 Cytotoxicity of Ac_4ManNTz, Ac_4ManNPhTz, Ac_4ManNPh.

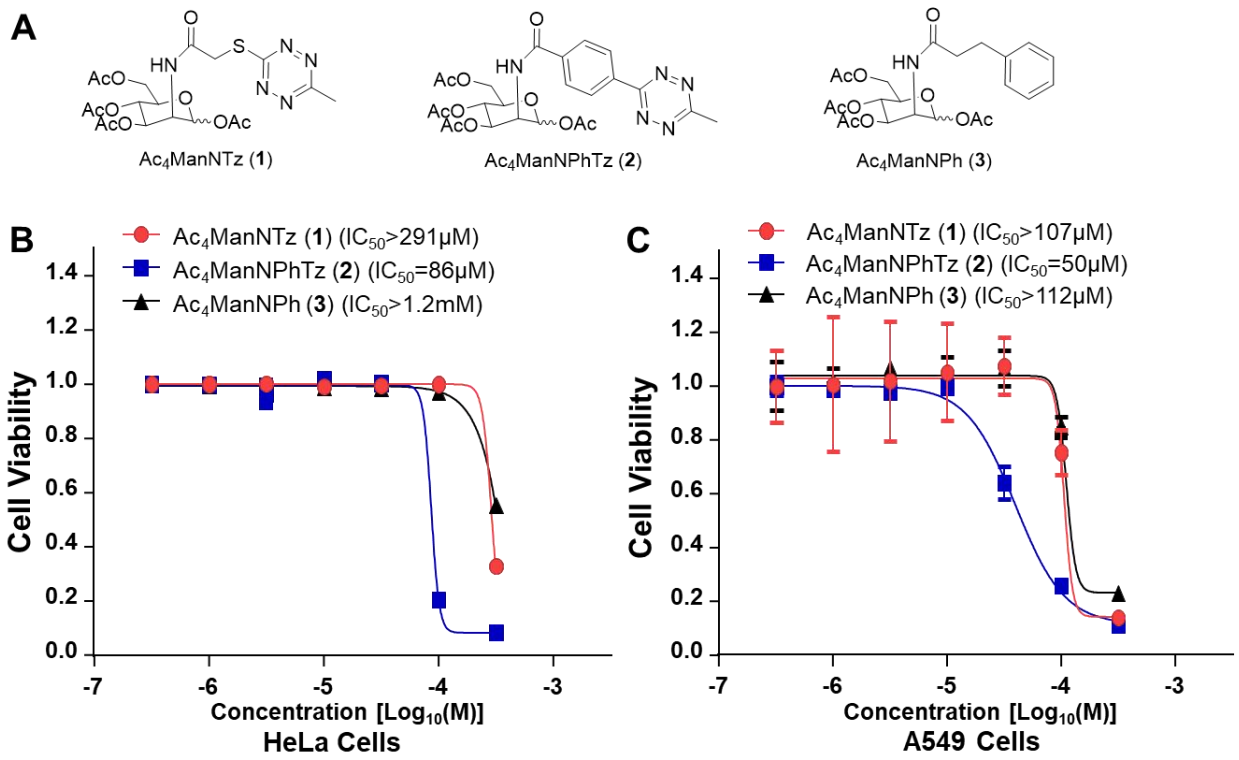


Figure S4: Cytotoxicity of tetrazine tagged sugars. A) Structure of tagged sugars, Ac_4ManNTz (**1**), Ac_4ManNPhTz (**2**) and Ac_4ManNPh (**3**). **B, C**) Cytotoxicity of tagged sugars on HeLa and A549 cancer cells.

4.2 Metabolically engineered cell-surface glycoconjugates on A549 cells

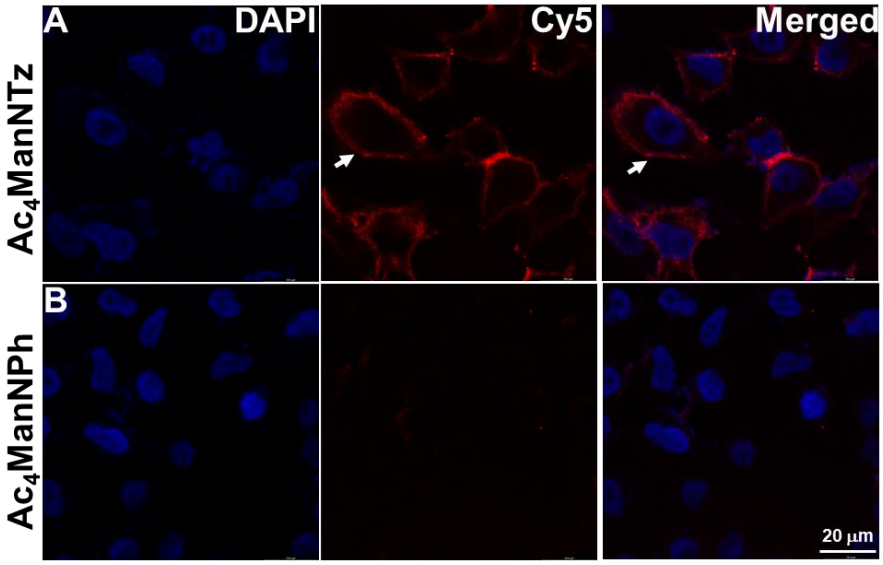


Figure S5: A, B) Confocal images showing the A549 cells pretreated by the Ac_4ManNTz (**1**) could be specifically labeled on the cell surface (arrow) while the control group pretreated by the Ac_4ManNPh (**3**) displayed barely visible fluorescent signal.

4.3 Metabolically engineered cell-surface glycoconjugates on HeLa cells

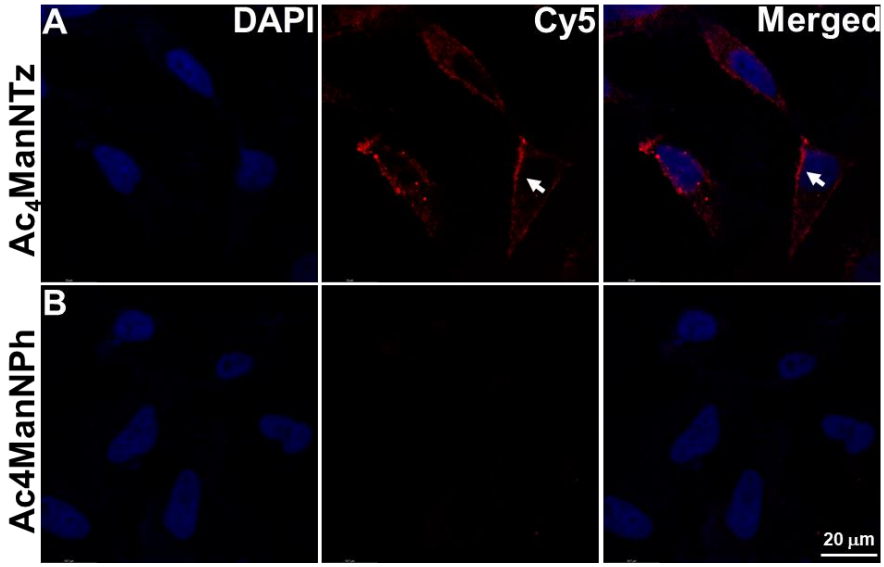


Figure S6: A, B) Confocal images showing the HeLa cells pretreated by the Ac_4ManNTz (**1**) could be specifically labeled on the cell surface (arrow) while the control group pretreated by the Ac_4ManNPh (**3**) displayed barely visible fluorescent signal.

4.4 Metabolic glycoengineering dependent on concentration of $Ac_4ManNTz$

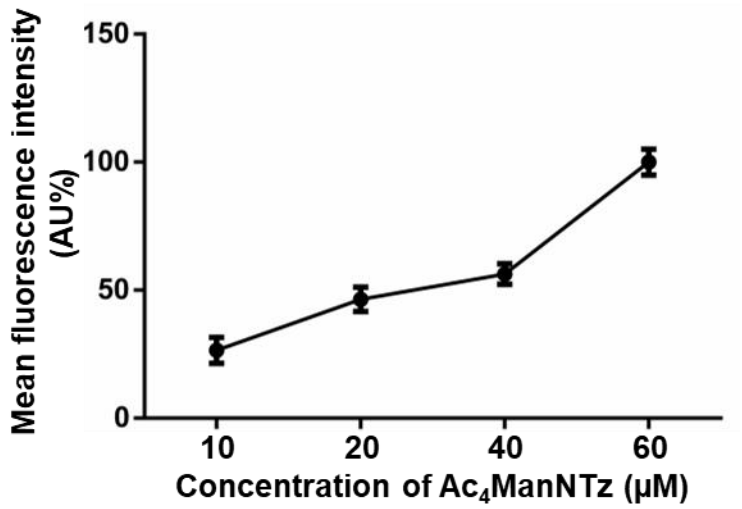
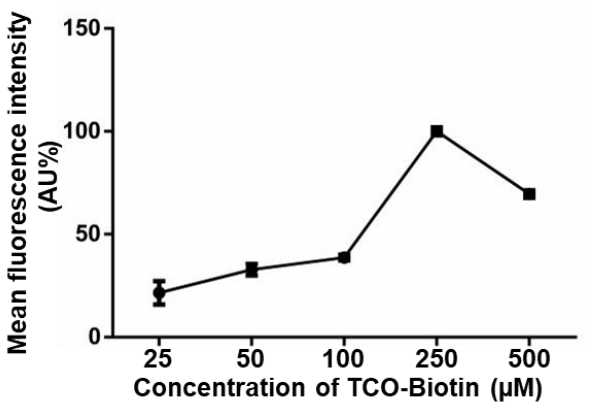


Figure S7: Cell lines were incubated in the presence of $Ac_4ManNTz$ at various concentration (0, 10µM, 20µM, 40µM, 60µM) for 3days, washed, and treated with TCO-Biotin 1mM for 1h. The cells were washed again and stained with streptavidin-AF647 (20 µg/mL). The fluorescence signal measured by flow cytometry is reported in mean fluorescence intensity with arbitrary units.

4.5 TCO-Biotin labeling



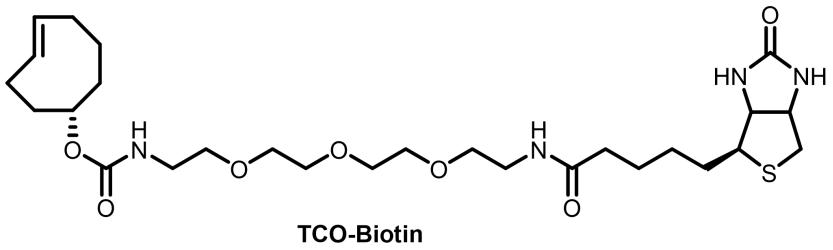


Figure S8: Cell lines were incubated in the presence of Ac_4ManNTz (50 μM) for 3days, washed, and treated with TCO-Biotin at various concentration for 1h. The cells were washed again and stained with streptavidin-AF647 (20 μg/mL). The fluorescence signal measured by flow cytometry is reported in mean fluorescence intensity with arbitrary units.

4.6 Time course for tetrazine-TCO labeling

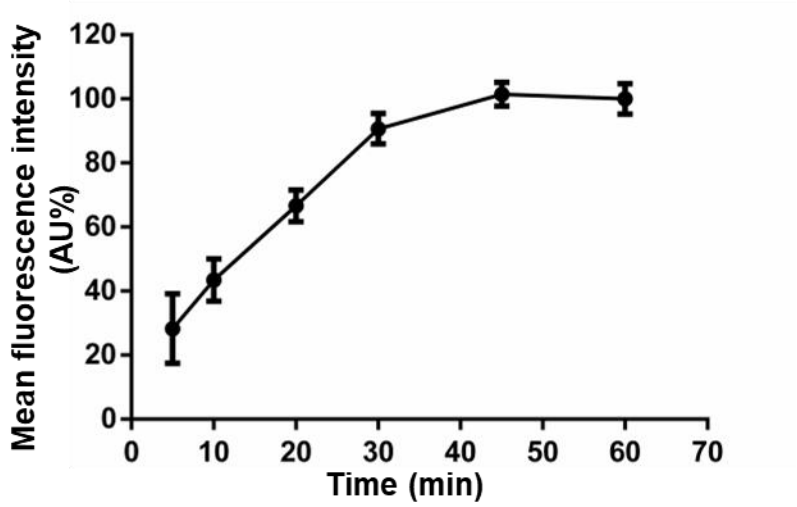


Figure S9: Time course of the cell-surface labeling reaction, as monitored by flow cytometry. Cell lines were incubated in the presence of Ac_4ManNTz (50 μM) for 3 days, washed, and treated with 0.25 mM of TCO-Biotin by varying duration of reaction time. The cells were washed again and stained with streptavidin-AF647 (20 μg/mL). The fluorescence signal measured by flow cytometry is reported in mean fluorescence intensity with arbitrary units.

4.7 Metabolism of $Ac_4ManNTz$ in various cell lines

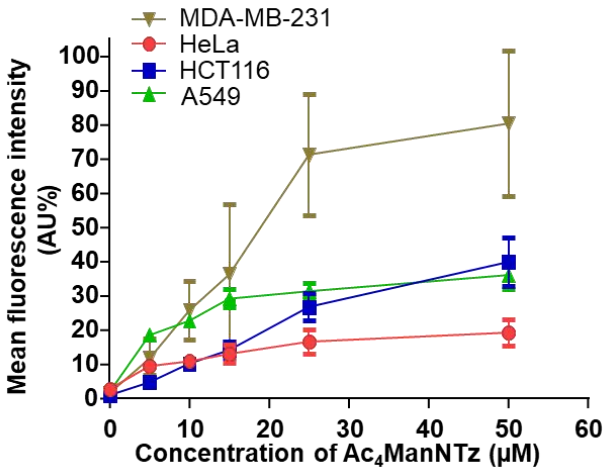


Figure S10: Cell lines were incubated in the presence of or absence of 25 μM of $Ac_4ManNTz$ for 3 days, washed, and treated with 250 μM of TCO-Biotin for 30 min. The cells were washed again and stained with streptavidin-AF647 (20 μg/mL). The fluorescence signal measured by flow cytometry is reported in arbitrary units, and error bars represent the standard deviation for three replicates.

4.8 Simultaneous labelling of two cell surface reporters

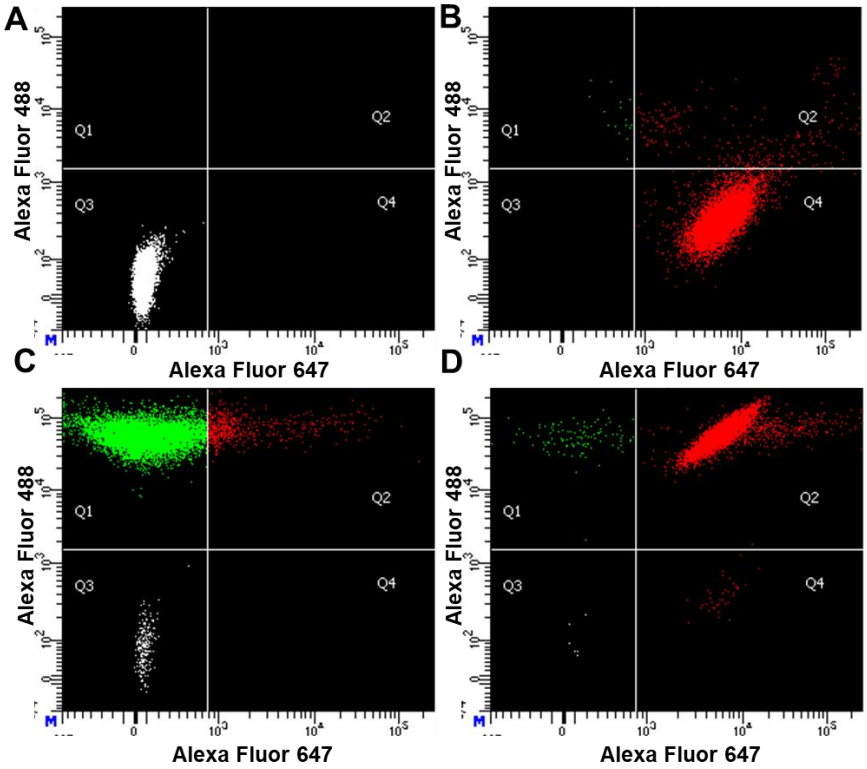


Figure S11: **Simultaneous labelling of two cell surface reporters.** MDA-MB-231 cells were cultured in the presence of both Ac_4ManNTz (25 μM) and Ac_4ManNAz (25 μM) for 72h, followed by concurrent treatment with DBCO-FITC (25 μM, 30 min at 37 °C) to covalently tag cell surface azides and TCO-Biotin (0.25 mM, 30 min at 37 °C), streptavidin-AF647 to tag tetrazines, respectively. Flow cytometry histograms of MDA-MB-231 cells incubated with **A**) neither Ac_4ManNTz nor Ac_4ManNAz, **B**) Ac_4ManNTz only, **C**) Ac_4ManNAz only, and **D**) both Ac_4ManNTz and Ac_4ManNAz, followed by the DBCO-FITC and TCO-Biotin and Streptavidin-AF647.

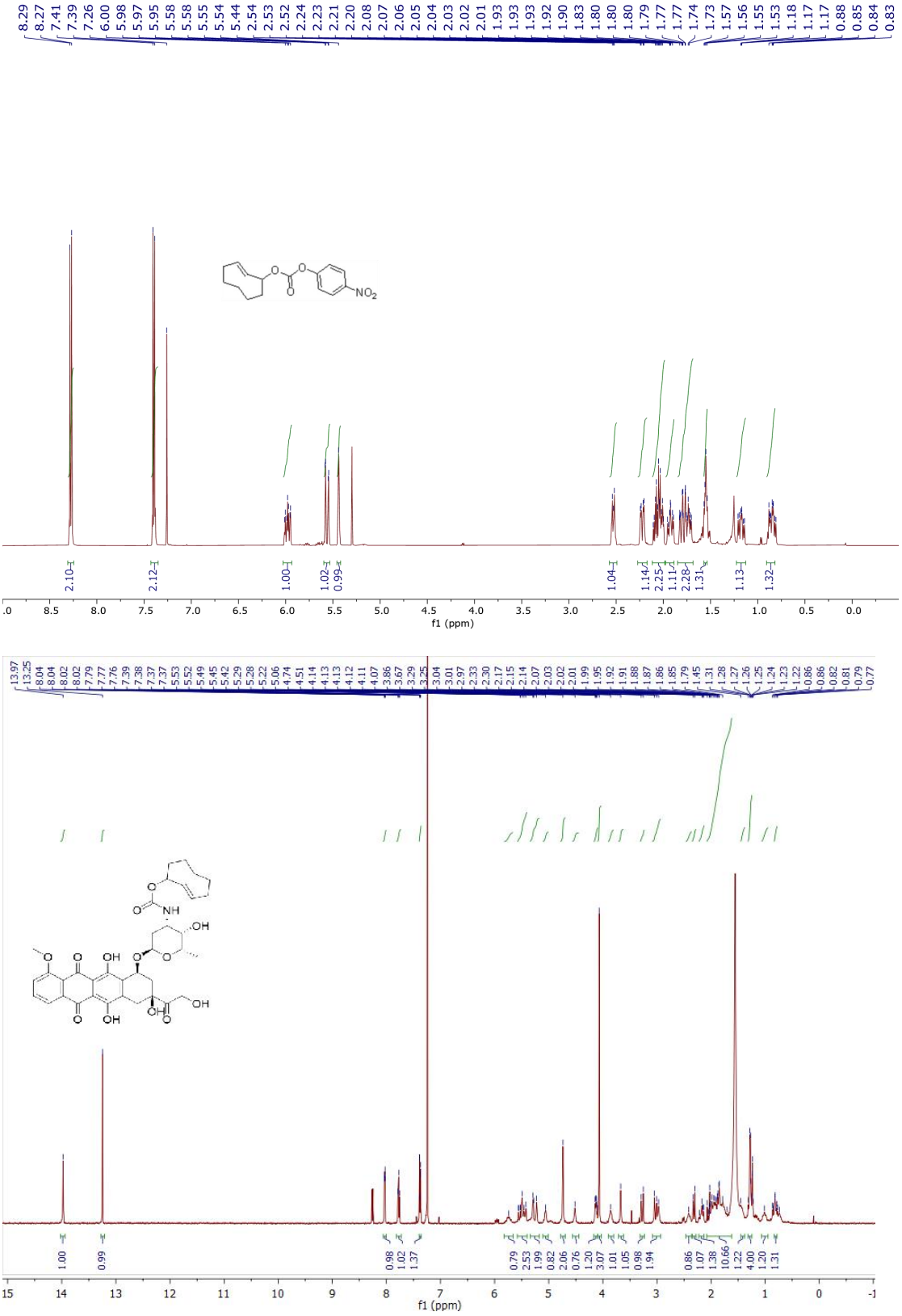
5. Tables

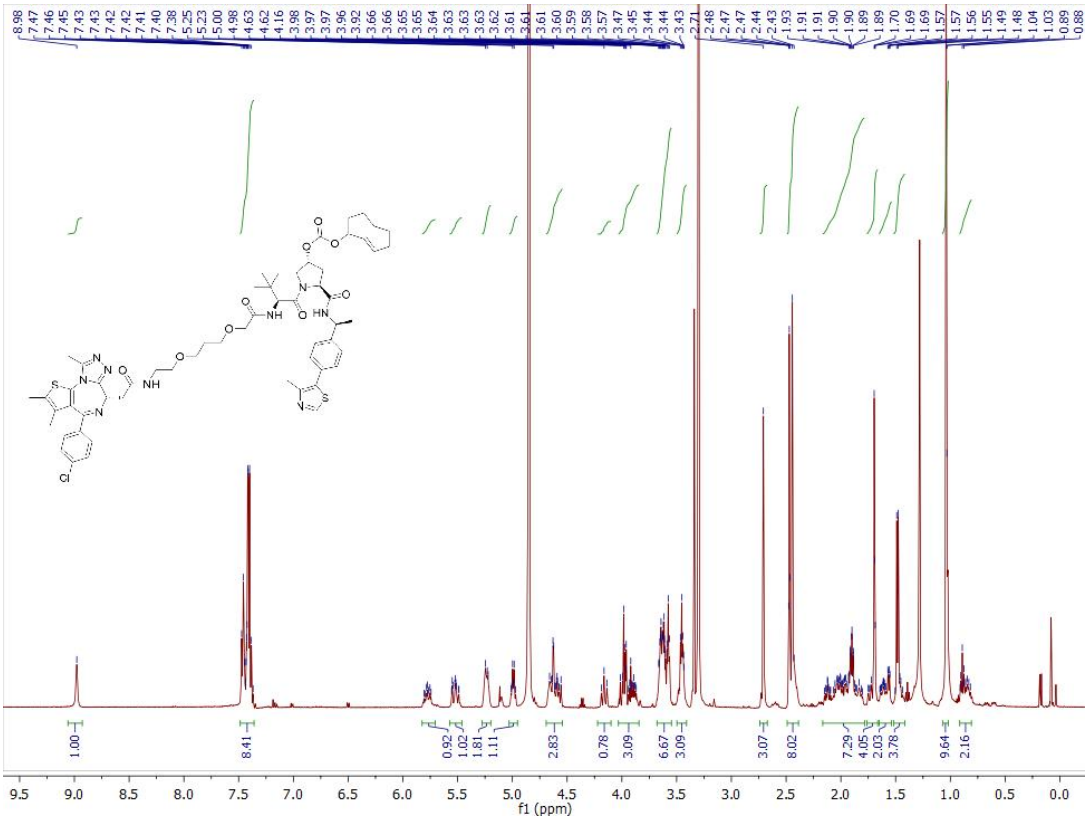
5.1 Table S1 Antibodies used for Western blotting analysis.

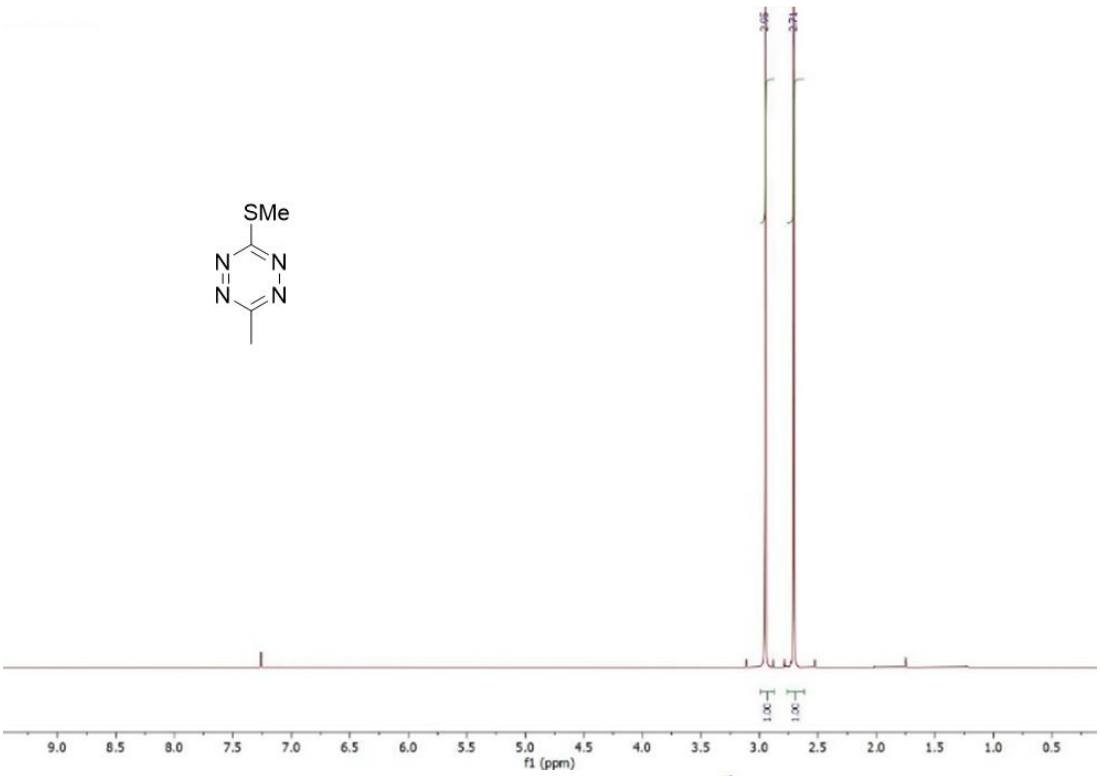
Antibody	**Antibody isotype**	**Catalog #**	**Concentration**
BRD4[1]	Rabbit IgG Monoclonal	13440S	1:1000
c-Myc[2]	Mouse IgG Monoclonal	M4439	1:1000
GAPDH[2]	Mouse IgG Monoclonal	G8795	1:1000
Secondary antibody[2]	Anti-rabbit IgG	A6154	1:3000
Secondary antibody[2]	Anti-mouse IgG	A28177	1:4000

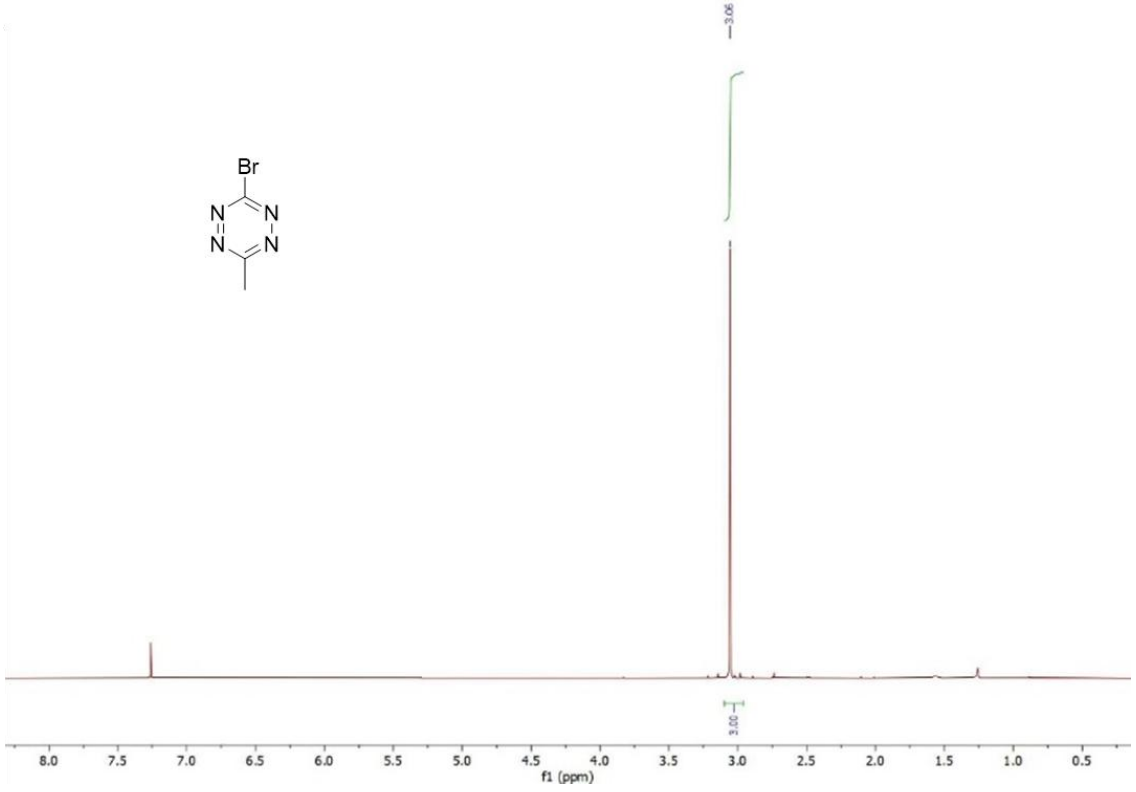
Footnotes: [1] Cell signaling, Danvers, MA, USA; [2]Sigma, St. Louis, MO, USA.

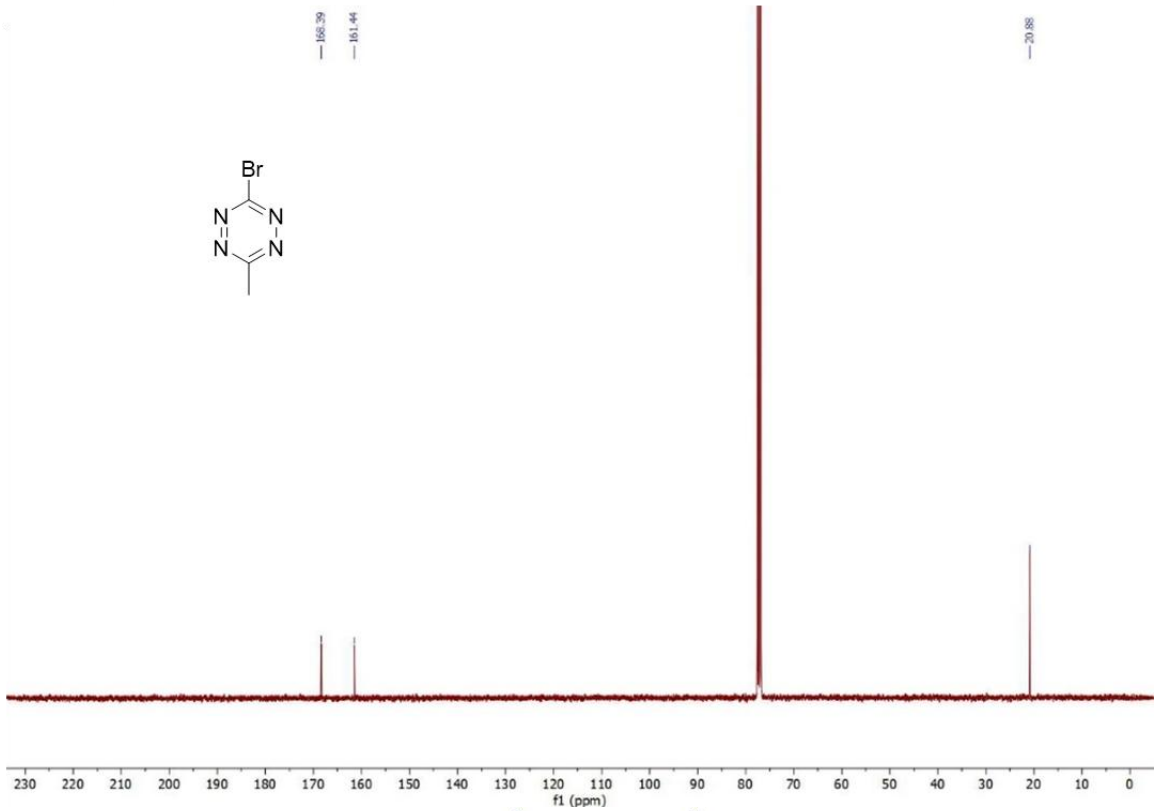
6. Spectrum

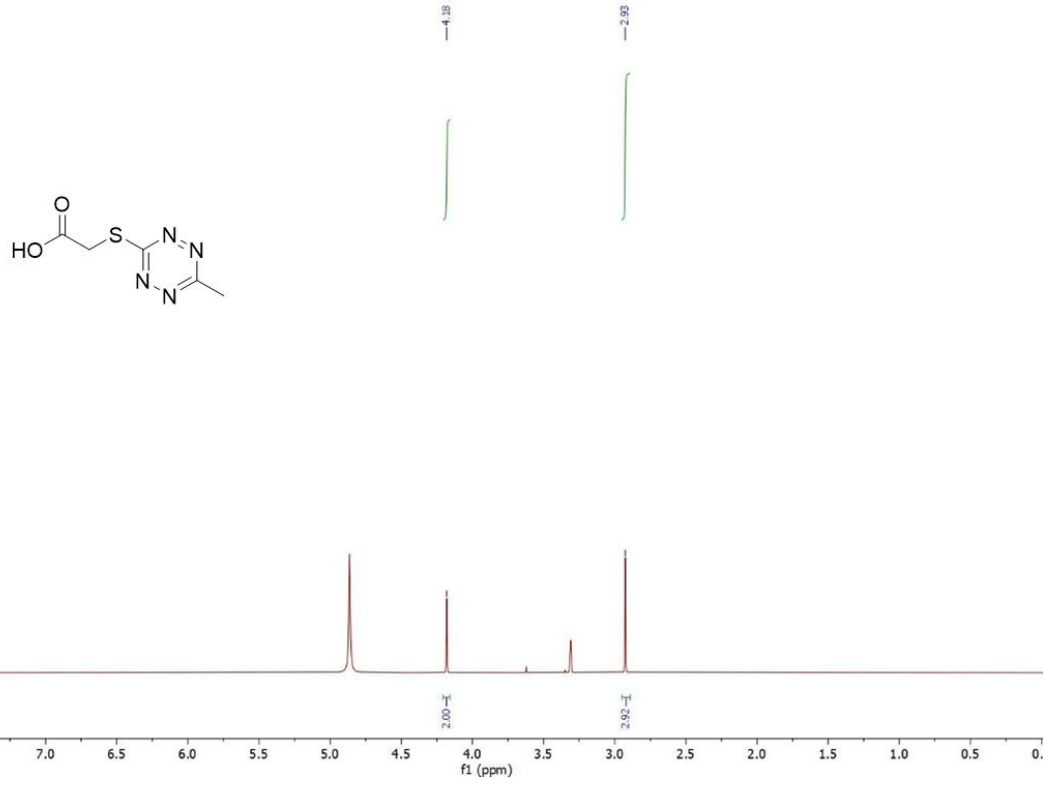


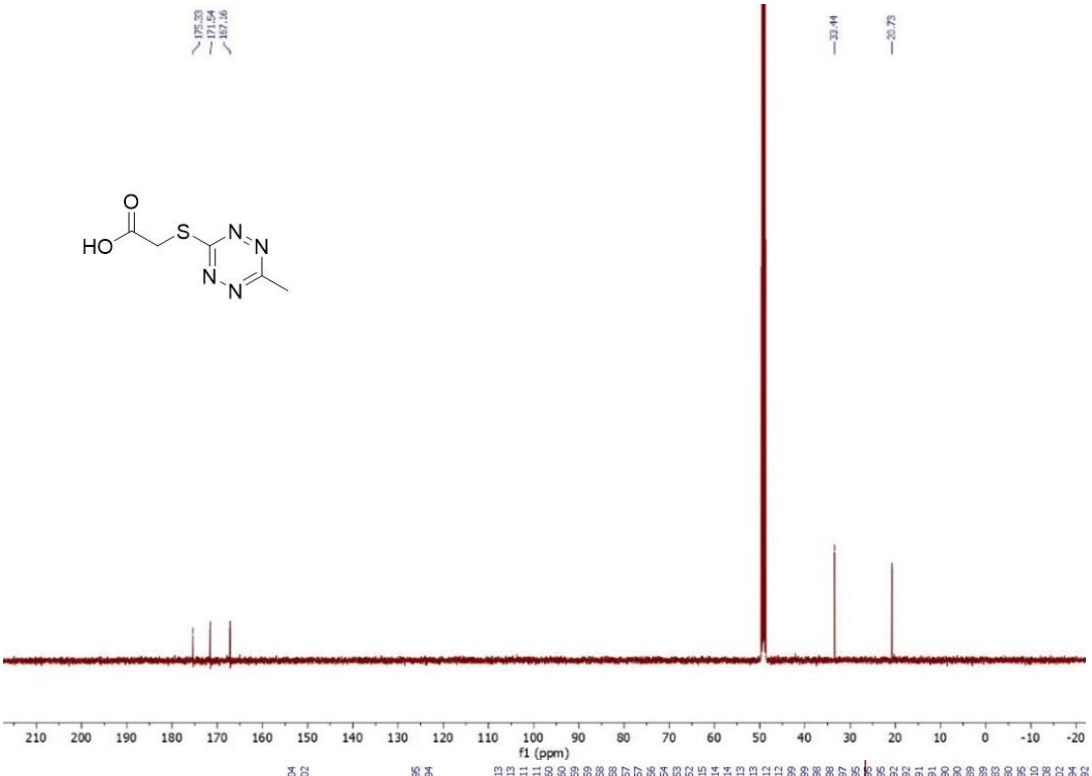

f1 (ppm)

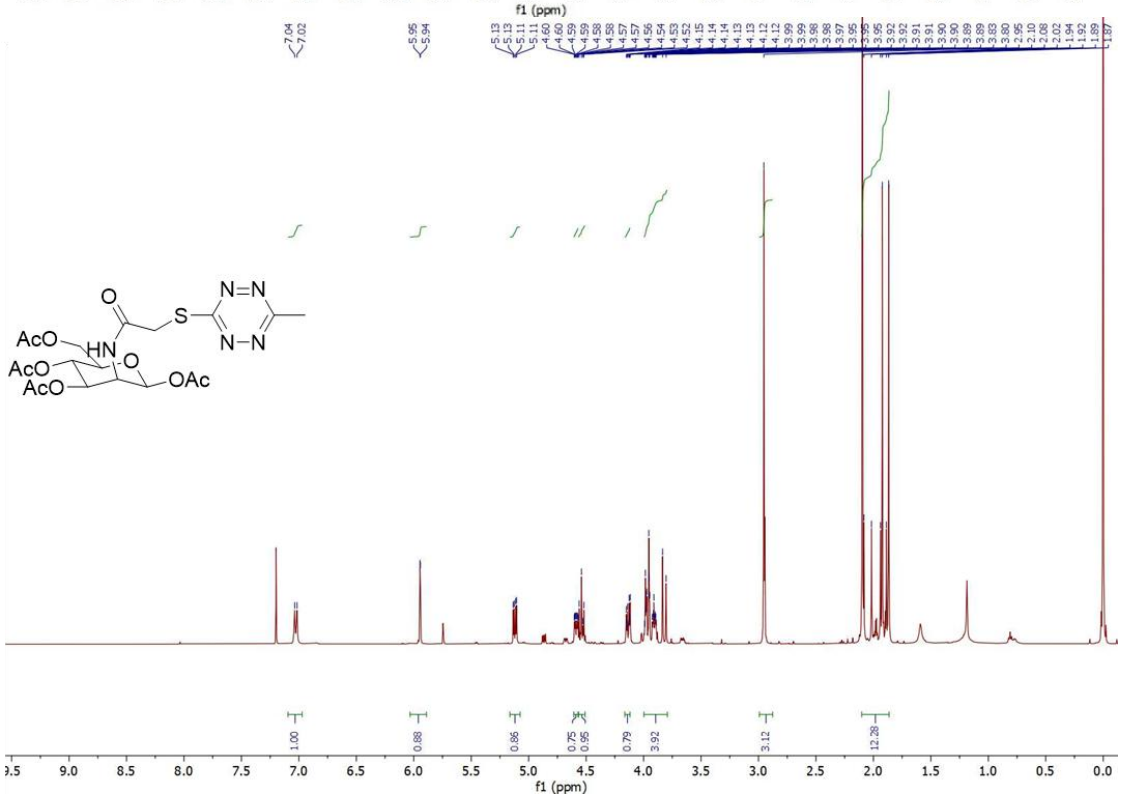

SMe
N
N
N
N
2.66
2.71
1.00
1.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

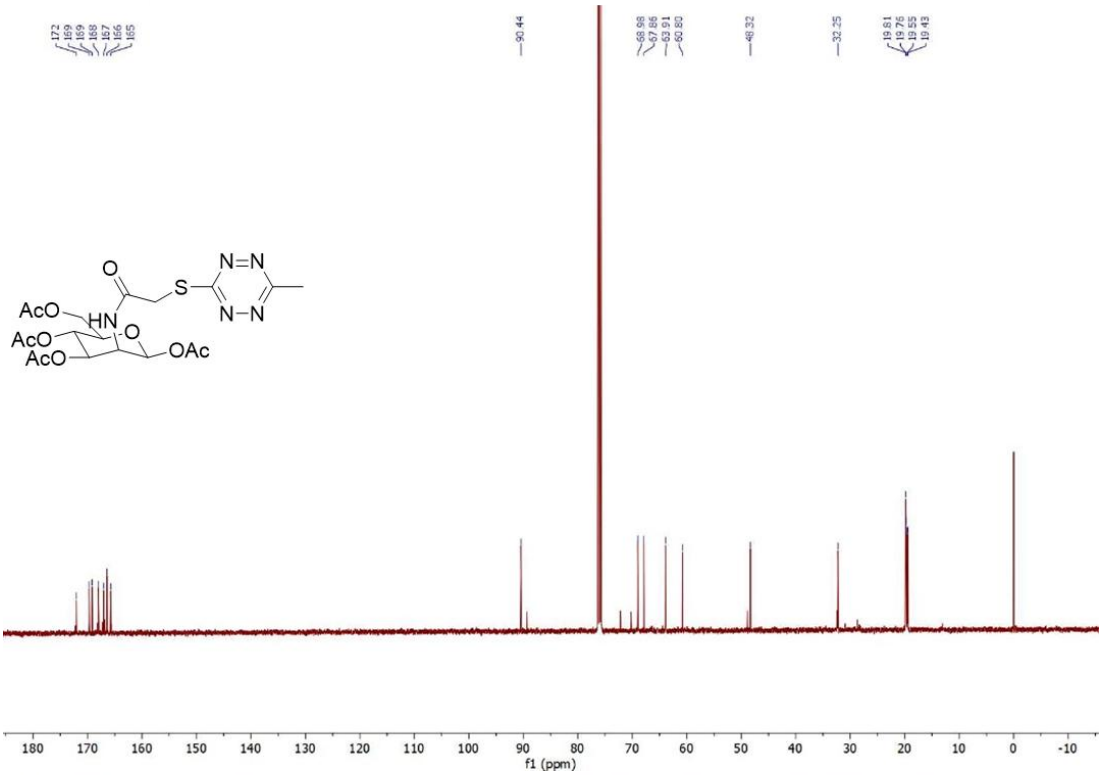

Br
N
N
N
N
3.06
3.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

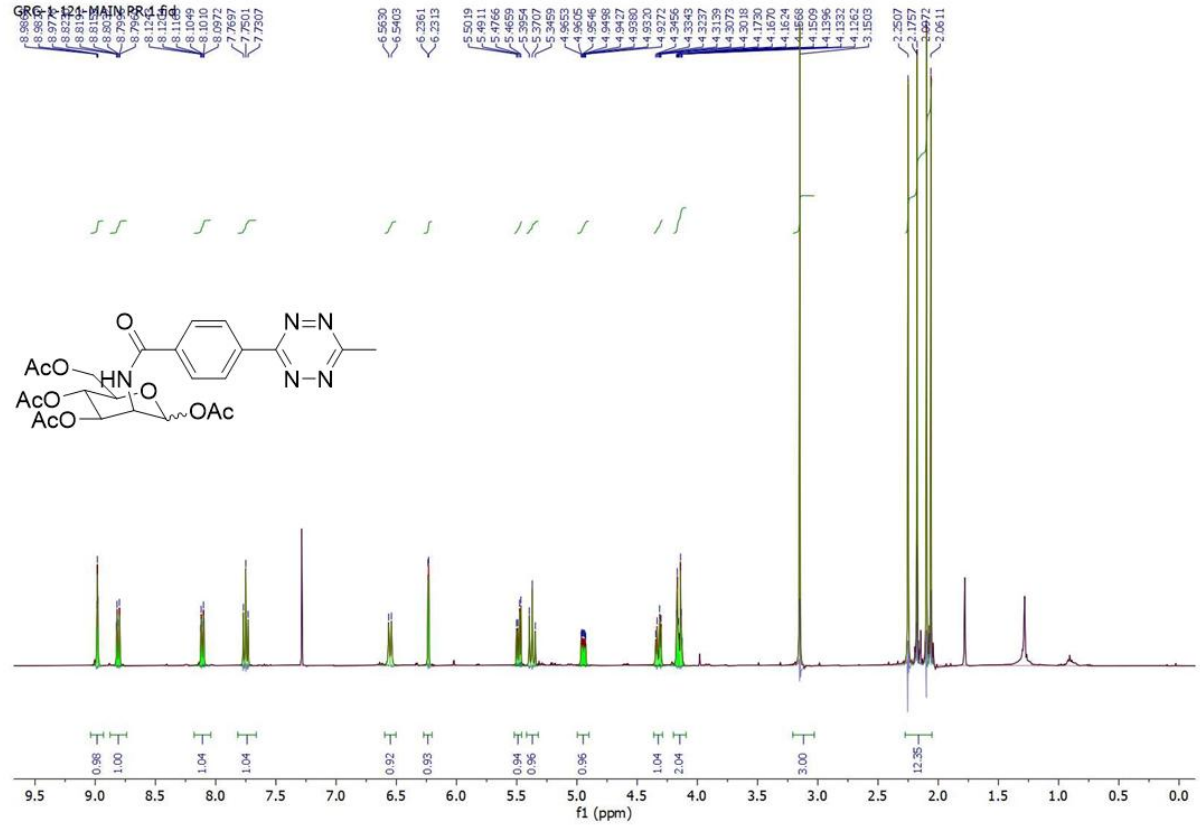

168.39
161.44
20.88
Br
N
N
N
N
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

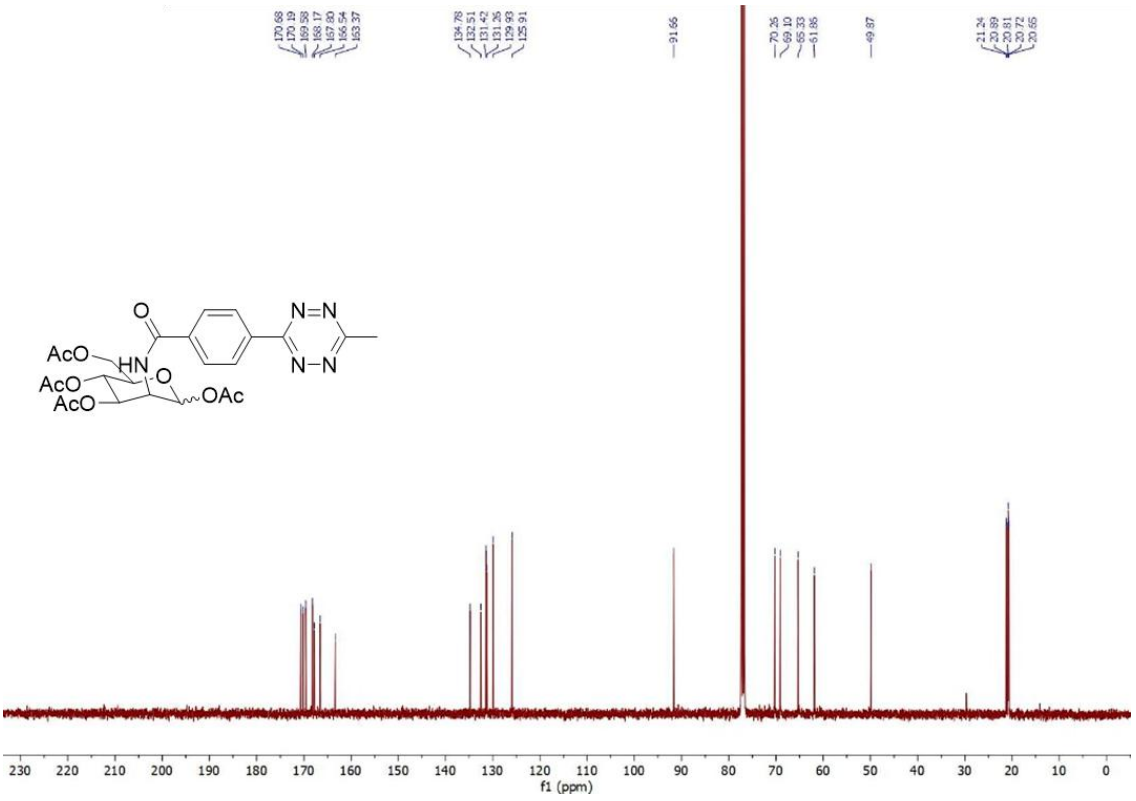

4.18
2.93
O
HO
S
N
N
N
N
2.00
2.92
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

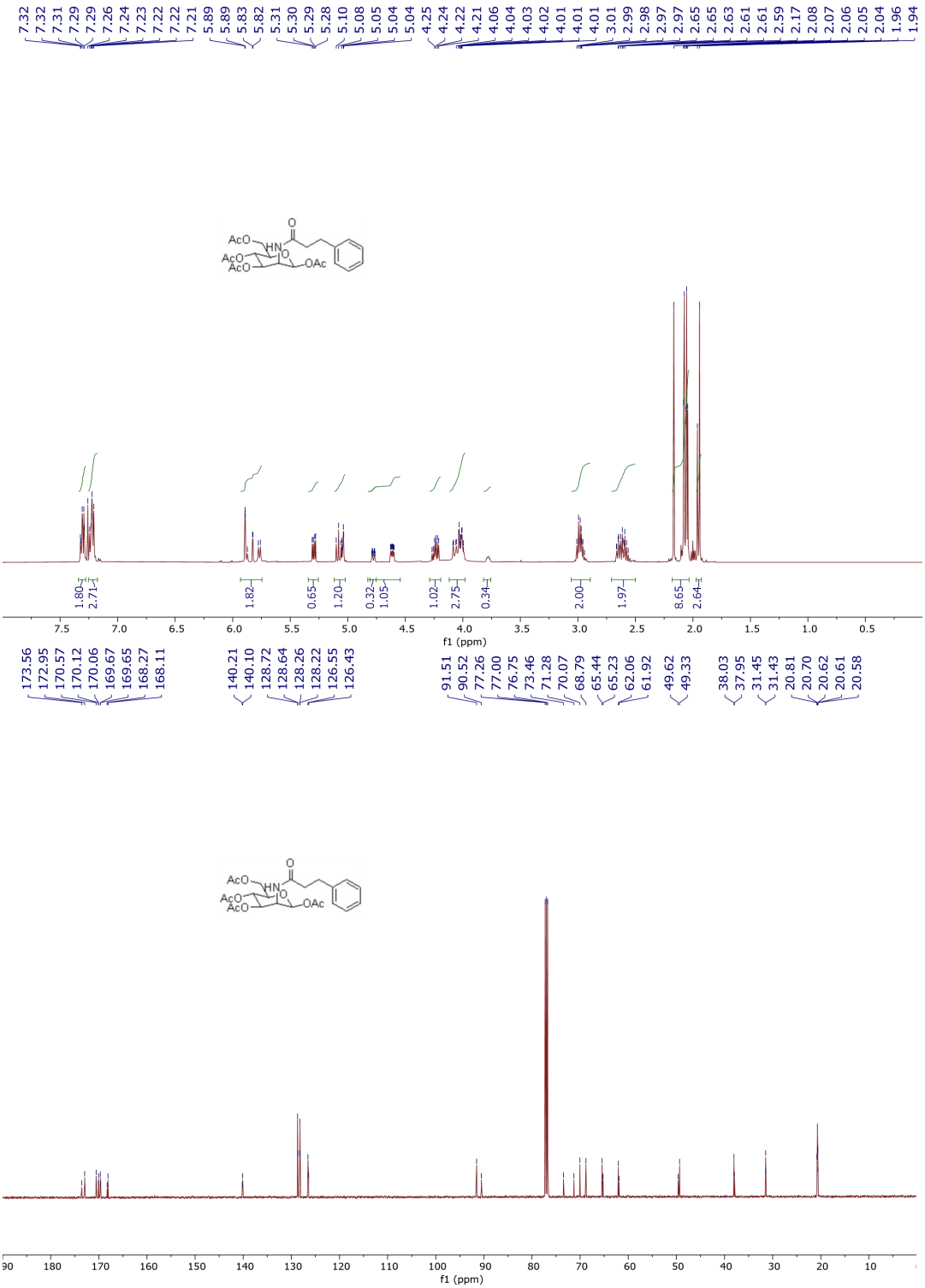

175.33
171.54
167.16
33.44
20.73
HO
O
S
N
N
N
N
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


7.04
7.02
5.95
5.94
5.13
5.13
5.12
5.11
5.11
4.60
4.60
4.59
4.59
4.59
4.58
4.58
4.57
4.57
4.56
4.54
4.53
4.52
4.15
4.14
4.14
4.13
4.12
4.12
3.99
3.99
3.98
3.98
3.97
3.95
3.95
3.95
3.92
3.92
3.91
3.91
3.90
3.90
3.89
3.89
3.83
3.80
2.95
2.10
2.08
2.02
1.94
1.93
1.89
1.87
O
S
N=N
N–N
AcO
HN
AcO
O
AcO
OAc
1.00
0.88
0.86
0.75
0.95
0.79
3.92
3.12
12.28
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


AcO
AcO
AcO
HN
O
S
N=N
N–N
OAc
f1 (ppm)


GRG-1-121-MAIN-PR-1.fid
AcO
AcO
AcO
HN
O
N=N
N–N
OAc
f1 (ppm)


170.68
170.19
169.58
168.17
167.80
166.54
163.37
134.78
132.51
131.42
131.36
129.93
125.91
91.66
70.25
69.10
65.33
61.86
49.97
21.24
20.99
20.81
20.72
20.65
O
N=N
N−N
AcO
HN
O
AcO
AcO
OAc
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.32 7.32 7.31 7.29 7.29 7.26 7.24 7.23 7.22 7.22 7.21
5.89 5.89 5.83 5.82 5.31 5.30 5.29 5.28 5.10 5.08 5.05 5.04 5.04
4.25 4.24 4.22 4.21 4.06 4.04 4.03 4.02 4.01 4.01 4.01 3.01 2.99 2.98 2.97 2.97 2.65 2.65 2.63 2.61 2.61 2.59 2.17 2.08 2.07 2.06 2.05 2.04 1.96 1.94
AcO
HN
O
AcO
AcO
OAc
1.80 2.71 1.82 0.65 1.20 0.32 1.05 1.02 2.75 0.34 2.00 1.97 8.65 2.64
f1 (ppm)
173.56 172.95 170.57 170.12 170.06 169.67 169.65 168.27 168.11
140.21 140.10 128.72 128.64 128.26 128.22 126.55 126.43
91.51 90.52 77.26 77.00 76.75 73.46 71.28 70.07 68.79 65.44 65.23 62.06 61.92 49.62 49.33
38.03 37.95 31.45 31.43 20.81 20.70 20.62 20.61 20.58
f1 (ppm)

7. Reference

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