**Supplementary Information**

# *In vivo* delivery of transcription factors with multifunctional oligonucleotides

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Berkeley, California 94720
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## Experimental Methods Section:

## Methods

### S1. Materials

3'-azide functionalized oligonucleotides were purchased from Intergrated DNA Technologies. All chemicals for the DART synthesis were purchased from Sigma-Aldrich. 0.1 M phosphate buffered saline (PBS), syringes, needles, pipette tips, eppendorf tubes, and nuclear magnetic resonance (NMR) tubes were purchased from VWR. $^{1}$H-NMR spectra were recorded in $CDCl_3$, $CD_3OD$ or $D_2O$ on a Varian 400 spectrometer equipped with a Sun workstation at 300K. TMS ($\delta\ (ppm)_H = 0.00$) was used as the internal reference. $^{13}$C-NMR spectra were recorded in either $CDCl_3$, $CD_3OD$ and $D_2O$ at a 100MHz on a Varian 400 spectrometer, using the central resonances of $CDCl_3$ ($\delta\ (ppm)_C = 77.23$) and methanol ($\delta\ (ppm)_C = 49.15$) as the internal references. Chemical shifts are reported in ppm and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Coupling constants, *J*, are reported in hertz (Hz). High-resolution mass spectra (HRMS) were obtained on a AB SCIEX TOF/TOF 5800 system and are reported as m/z (relative intensity). Accurate masses are reported for the molecular ion ($M^+$) or a suitable fragment ion. Chemicals were purchased from Aldrich or VWR and used without further purification. All solvents were purified using standard methods. Flash chromatography was carried out using silica gel (230-400 mesh). All reactions were performed under anhydrous conditions under $N_2$ or Argon and monitored by TLC on Kieselgel 60 F254 plates (Merck). Detection was accomplished by examination under UV light (254 nm) and by charring with 10 % sulfuric acid in methanol. The Nrf2 expression plasmid was obtained from Addgene (plasmid 21553). HepG2 cells and Hepa-1C1C7 were obtained from the biosciences divisional services at UC Berkeley. Female 6-8 weeks old C57BL6/J mice were purchased from Charles River.

### S2. Synthesis of the DARTs

The synthesis of the DARTs is shown in Figures S1 and S2. The key intermediate in the DART synthesis was the compound **5**, which is composed of a bivalent galactose, conjugated to a hydrophobic C32 chain through an acid degradable acetal linkage. The compound **5** also contains a strained alkyne, which was used to conjugate to azide functionalized oligonucleotides, via copper-free click chemistry. The synthesis of **5** is shown in Figure S1. A detailed description of the synthetic protocols used to generate **5** is described below.

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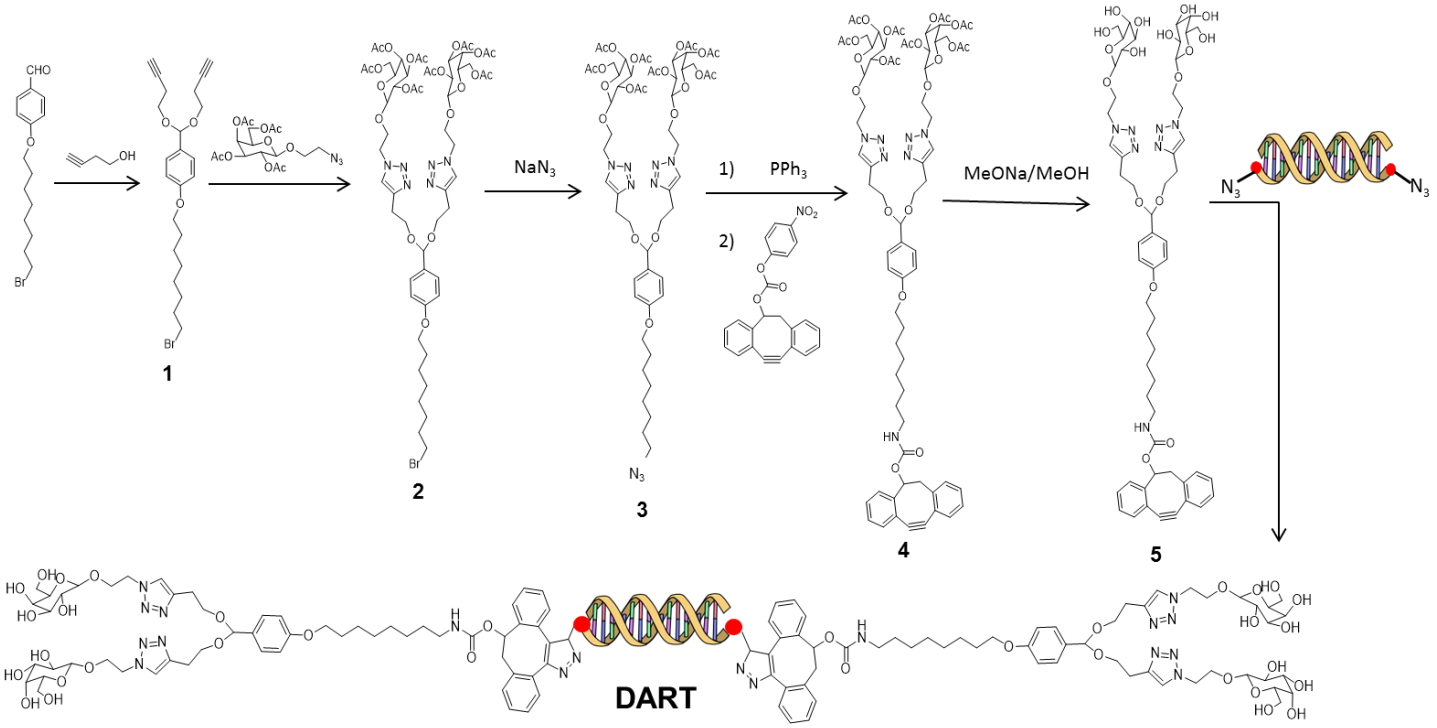
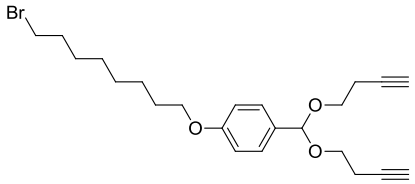
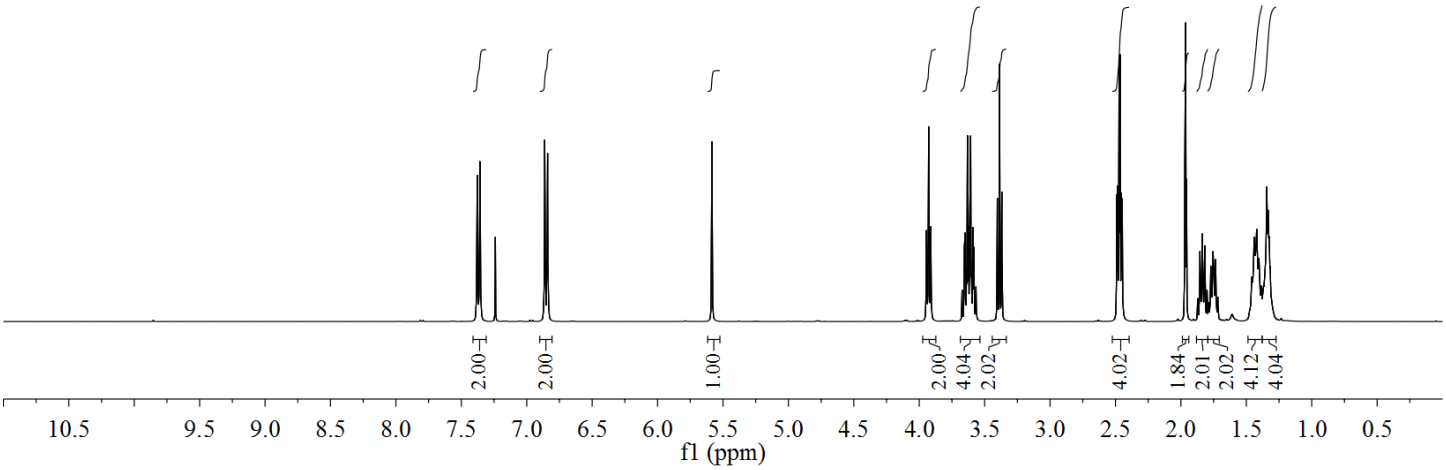



**Supplementary Figure S1. DART synthesis.**

### S2.1 Synthesis of compound 5

*Synthesis of 1-(bis(but-3-yn-1-yloxy)methyl)-4-((8-bromooctyl)oxy)benzene (**1**):* To a stirred solution of 4-((8-bromooctyl)oxy)benzaldehyde (1.88 g, 6.0 mmol) and 3-butyn-1-o1 (1.68 g, 24.0 mmol, 4.0 equiv) in dry THF (15 mL) was added 5 A sieves (4 g) and p-toluenesulfonic acid (180 mg, 1.0 mmol, 0.16 equiv). The reaction mixture was stirred overnight at room temperature. The reaction was then quenched with triethylamine (0.7 mL, 5.0 mmol). The mixture was filtrated and concentrated under reduced pressure. The resulting compound was purified by column chromatography on silica gel (EtOAc/Hexane, 1:15 v/v) to afford **1** (1.2g, 46.1%). $^{1}$H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.37 (d, 2 H), 6.85 (d, 2 H), 5.58 (s, 1 H), 3.93 (t, 2 H), 3.62 (m, 4 H), 3.39 (t, 2 H), 2.47 (td, 4 H), 1.96 (t, 2 H), 1.84 (m, 2 H), 1.75 (m, 2 H), 1.42 (m, 4 H), 1.33 (m, 4 H). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 159.4, 130.1, 128.1, 114.3, 101.4, 81.6, 69.5, 68.1, 63.3, 34.2, 33.0, 29.4, 28.9, 28.3, 26.2, 20.1.

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2.00
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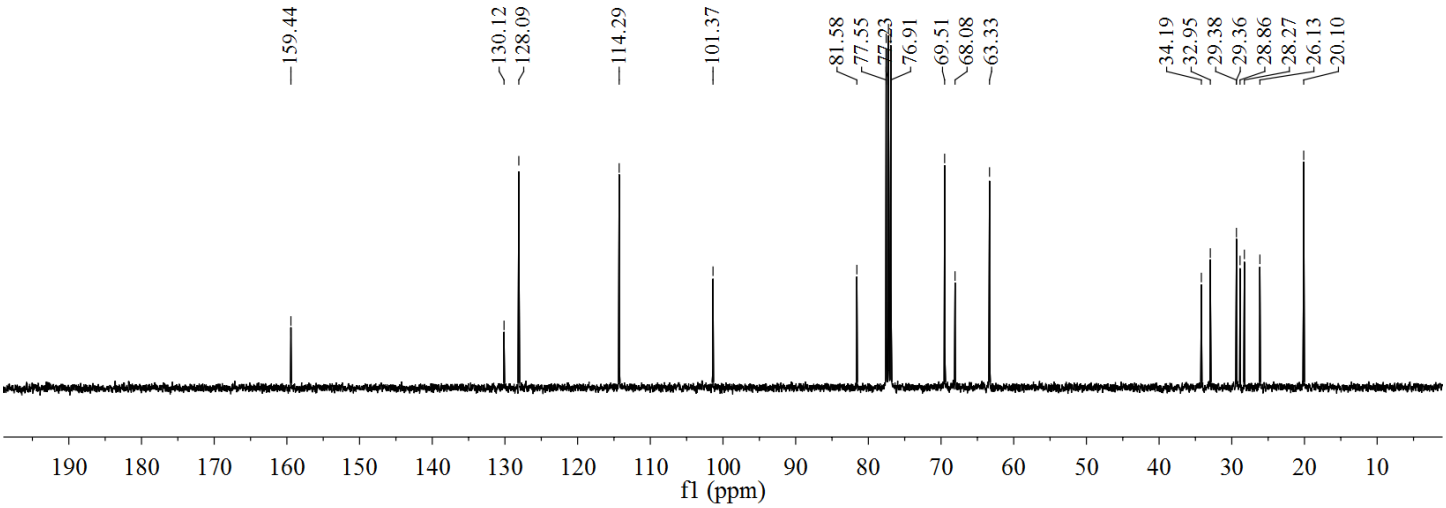
4.02
1.84
2.01
2.02
4.12
4.04
10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)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114.29
101.37
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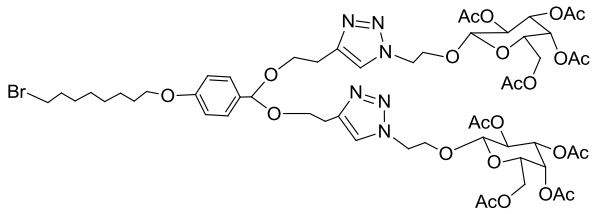
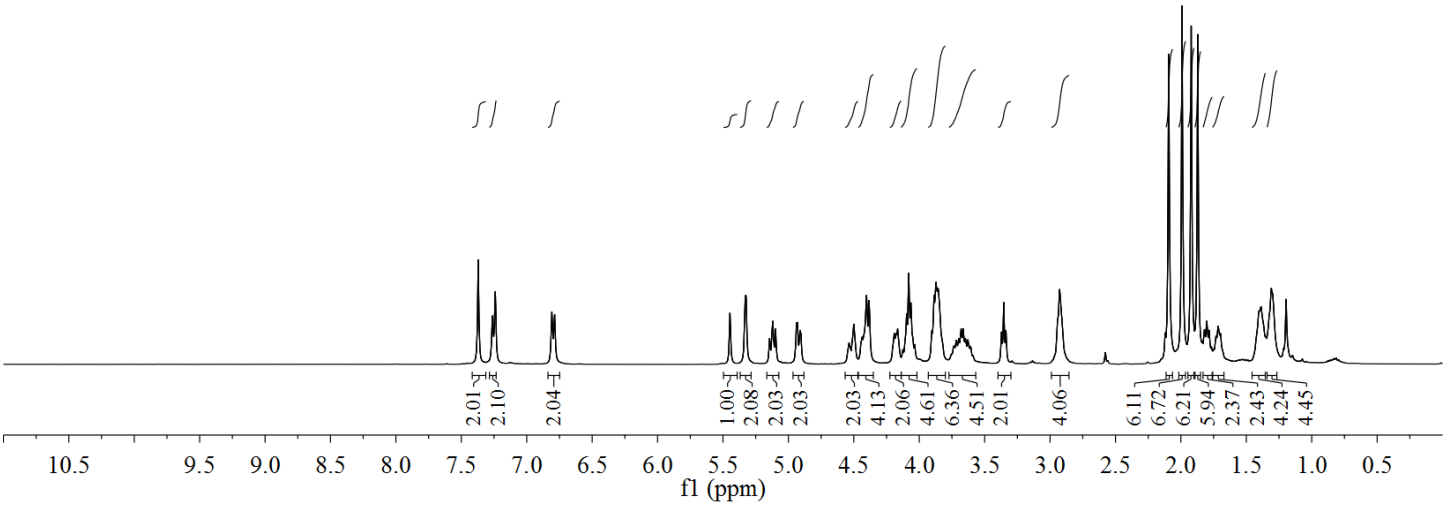
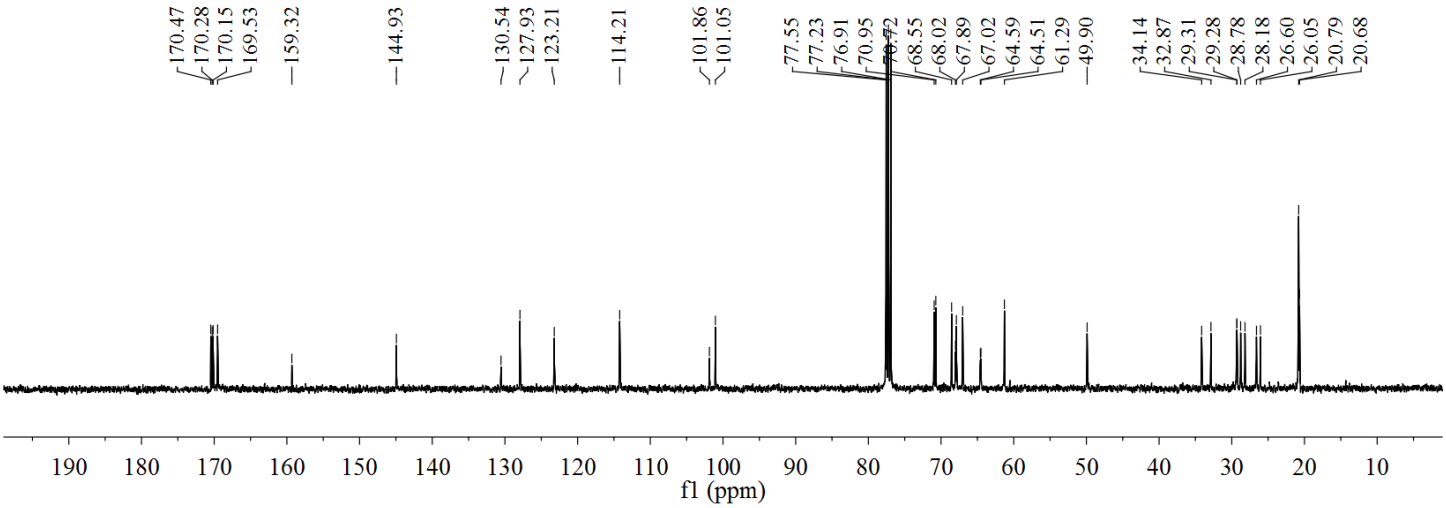
77.55
77.23
76.91
69.51
68.08
63.33
34.19
32.95
29.38
29.36
28.86
28.27
26.13
20.10
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

*Synthesis of [(2S,3R,4R,5S,6S)-6-{2-[4-(2-{[2-(1-{2-[(2S,3S,4R,5R,6S)-3,4,5-Triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-1H-1,2,3-triazol-4-yl)ethoxy][p-(8-bromooctyloxy)phenyl]methoxy}ethyl)-1H-1,2,3-triazol-1-yl]ethoxy}-3,4,5-triacetoxytetrahydro-2H-pyran-2-yl]methyl acetate (**2**):* To a stirred solution of compound **1** (22.95 mg, 0.05 mmol) and 2-azidoethyl, 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (66 mg, 0.2 mmol, 4.0 equiv) in DMF was added CuI (1.9 mg, 0.01 mmol, 0.2 equiv) and DIPEA (6.25 mg, 0.05 mmol) and the mixture was stirred for 48 h at room temparature. The solvent was removed in vacuo. The residue was purifed by silica gel column chromatography (hexane:acetone=2:1) to afford **2** (55 mg, 86.6%). $^{1}$H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.37 (s, 2 H), 7.25 (d, 2 H), 6.80 (d, 2 H), 5.45 (s, 1 H), 5.32 (s, 2 H), 5.12 (t, 2 H), 4.92 (d, 2 H), 4.52 (m, 2 H), 4.41 (m, 4 H), 4.18 (m, 2 H), 4.08, (m, 4H), 3.88 (m, 6 H), 3.67, (m, 4 H), 3.35 (t, 2 H), 2.93 (m, 4 H), 2.09 (b, 6 H), 1.99 (s, 6 H), 1.92 (s, 6 H), 1.87 (s, 6 H), 1.79 (m, 2 H), 1.72 (m, 2H), 1.40 (m, 4 H), 1.31 (m, 4 H). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 170.5, 170.3, 170.2, 169.5, 159.3, 144.9, 130.5, 127.9, 114.2, 101.9, 101.1, 71.0, 70.7, 68.6, 68.0, 67.9, 67.0, 64.6, 64.5, 61.3, 49.9, 34.1, 32.9, 29.3, 29.3, 28.8, 28.1, 26.6, 26.0, 20.8, 20.7. ESI-MS m/z Found: 1291.4150, calculated: 1291.4116 for $C_{55}H_{77}O_{23}N_6BrNa$ $[M+Na]^+$.

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114.21
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68.02
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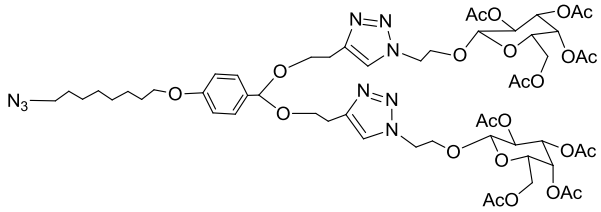
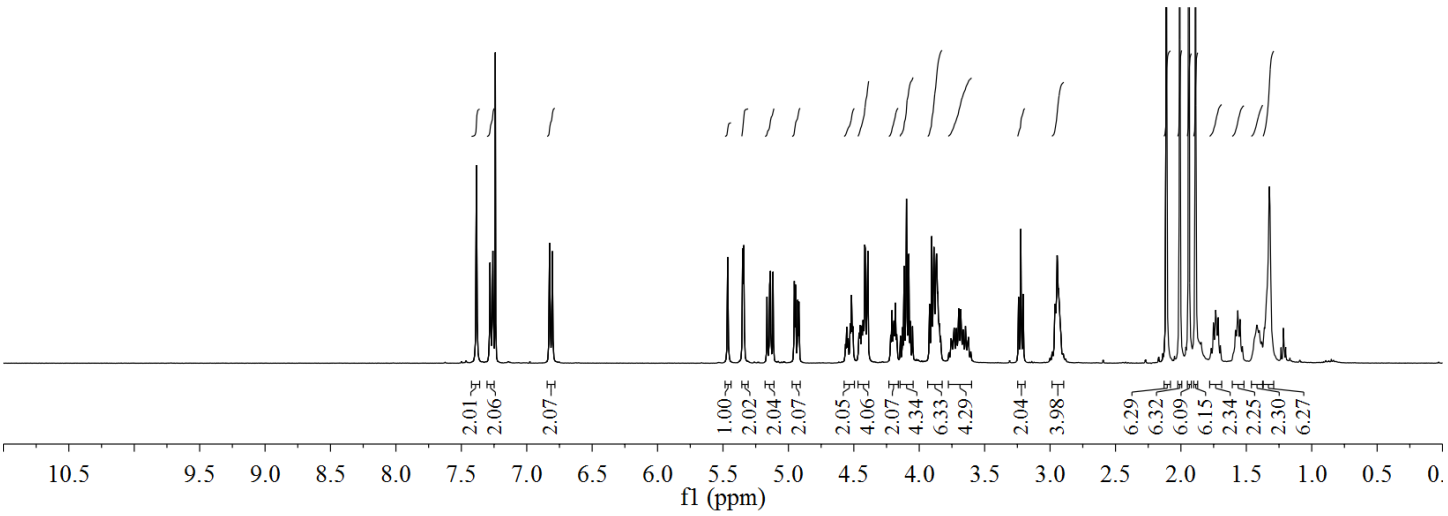
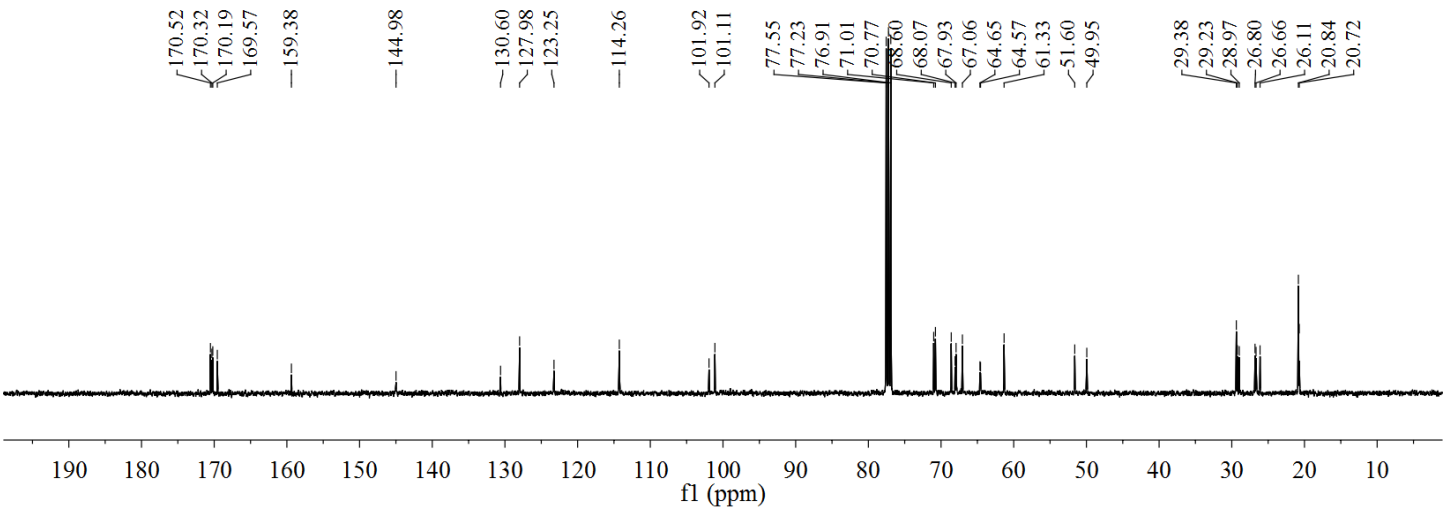
67.02
64.59
64.51
61.29
49.90
34.14
32.87
29.31
29.28
28.78
28.18
26.60
26.05
20.79
20.68
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

*Synthesis of [(2S,3R,4R,5S,6S)-6-{2-[4-(2-{[2-(1-{2-[(2S,3S,4R,5R,6S)-3,4,5-Triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-1H-1,2,3-triazol-4-yl)ethoxy][p-(8-azidooctyloxy)phenyl]methoxy}ethyl)-1H-1,2,3-triazol-1-yl]ethoxy}-3,4,5-triacetoxytetrahydro-2H-pyran-2-yl]methyl acetate (**3**):* To a stirred solution of compound **2** (500 mg, 0.4 mmol) in anhydrous DMF was added 10 fold excess $NaN_3$. The reaction mixture was stirred at 60 $^oC$ for 12 h. The mixture was filtrated and concentrated under reduced pressure. The residue was purifed by silica gel column chromatography (hexane:acetone 2:1) to give the pure product as a liquid (yield 95%). $^1H$ NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.38 (s, 2 H), 7.27 (d, 2 H), 6.81 (d, 2 H), 5.46 (s, 1 H), 5.35 (d, 2 H), 5.14 (t, 2 H), 4.94 (d, 2 H), 4.54 (m, 2 H), 4.42 (m, 4 H), 4.20 (m, 2 H), 4.10, (m, 4 H), 3.87 (m, 6 H), 3.69, (m, 4 H), 3.22 (t, 2 H), 2.95 (m, 4 H), 2.11 (s, 6 H), 2.01 (s, 6 H), 1.94 (s, 6 H), 1.89 (s, 6 H), 1.74 (m, 2 H), 1.56 (m, 2 H), 1.40 (m, 2 H), 1.31 (m, 6 H). $^{13}C$ NMR (100 MHz, $CDCl_3$): *δ (ppm)* 170.5, 170.3, 170.2, 169.5, 159.4, 145.0, 130.6, 128.0, 114.3, 101.9, 101.1, 71.1, 70.8, 68.6, 68.1, 67.9, 67.1, 64.7, 64.6, 61.3, 51.6, 50.0, 29.4, 29.2, 29.0, 26.8, 26.7, 20.8, 20.7. ESI-MS m/z Found: 1254.5044, calculated: 1254.5025 for $C_{55}H_{77}O_{23}N_9Na$ $[M+Na]^+$.

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71.01
70.77
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68.07
67.93
67.06
64.65
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61.33
51.60
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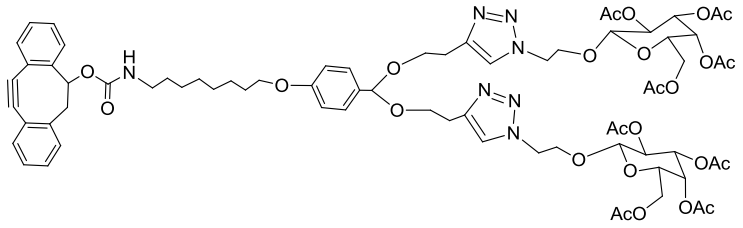
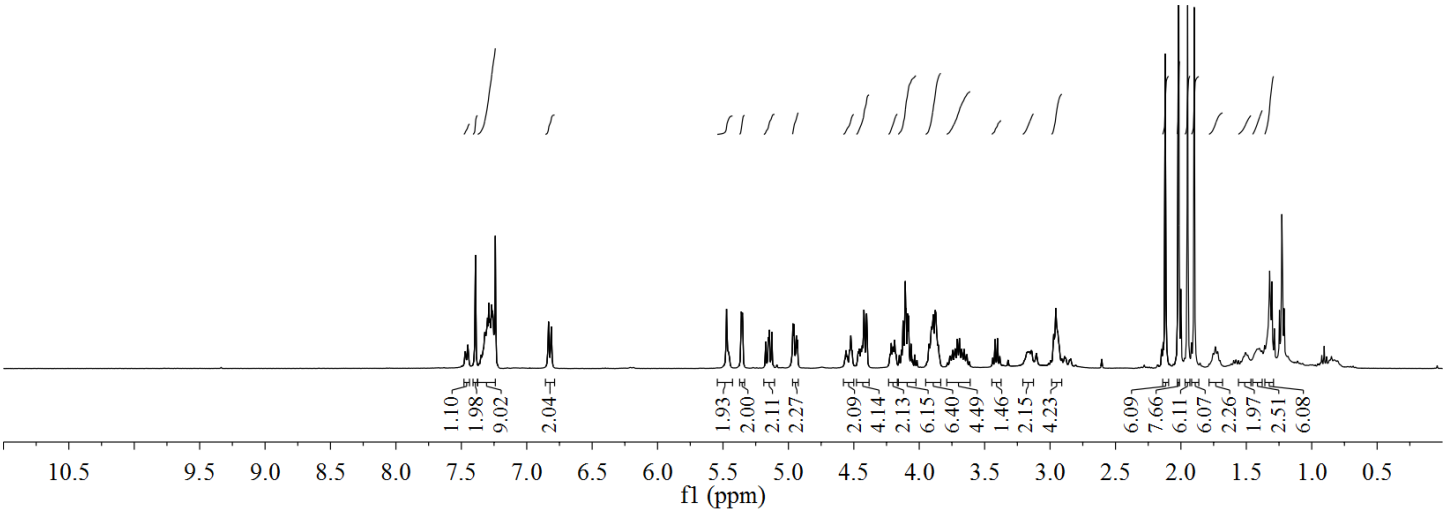
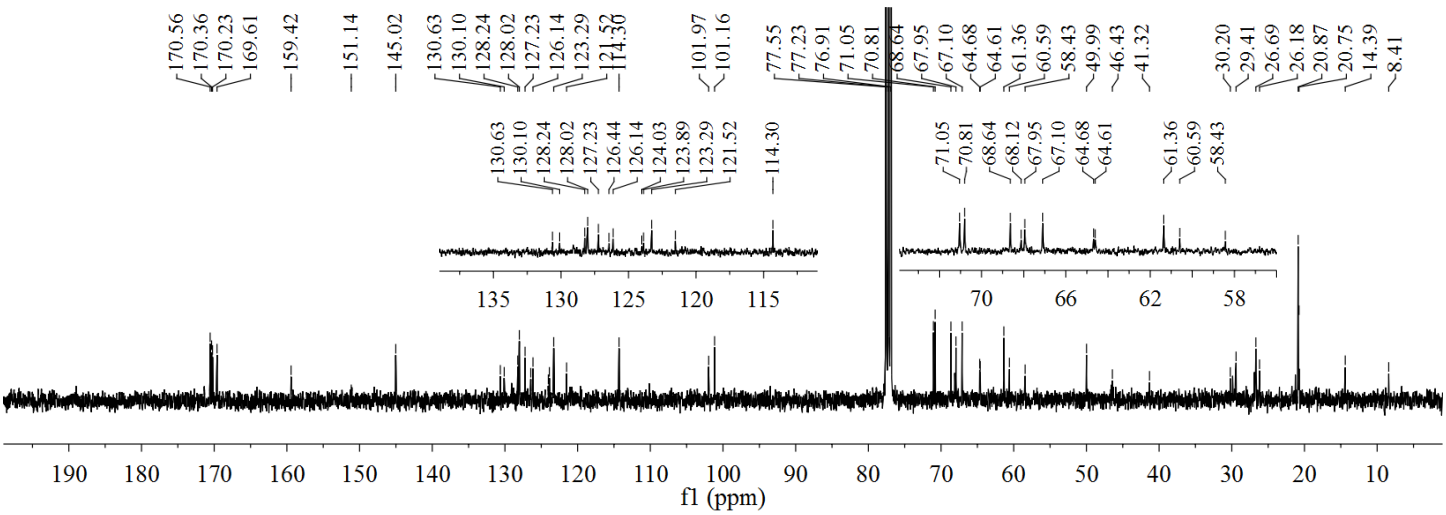
29.38
29.23
28.97
26.80
26.66
26.11
20.84
20.72
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

*Synthesis of [(2S,3R,4R,5S,6S)-6-{2-[4-(2-{[2-(1-{2-[(2S,3S,4R,5R,6S)-3,4,5-Triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-1H-1,2,3-triazol-4-yl)ethoxy][p-(8-{tricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yloxycarbonylamino}octyloxy)phenyl]methoxy}ethyl)-1H-1,2,3-triazol-1-yl]ethoxy}-3,4,5-triacetoxytetrahydro-2H-pyran-2-yl]methyl acetate (**4**):* To a stirred solution of compound **3** (250 mg, 0.203 mmol) in a mixture solvent composed of THF/$H_2O$ (8/2) was added $PPh_3$ (63.8 mg, 0.243 mmol). The reaction mixture was stirred overnight at room temperature (RT). The mixture was concentrated under reduced pressure and the residue was purifed by silica gel column chromatography, using $CH_2Cl_2$:$CH_3OH$:$Et_3N$, 10:1:0.1 as the eluent, to give the crude product, to which p-nitrophenyl modified cyclooctyne (78.15 mg, 0.203 mmol) and $Et_3N$ (45 μl, 0.323 mmol) were added. After overnight strring at RT, the reaction mixture was concentrated and the residue was purified by column chromatography (Hexane:Acetone=1:1) to give compound **4** as a liquid (112 mg, 38%). $^1$H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.46 (d, 1 H), 7.39 (s, 2 H), 7.30 (m, 9 H), 6.82 (d, 2 H), 5.47 (s, 2 H), 5.36 (d, 2 H), 5.15 (t, 2 H), 4.95 (d, 2 H), 4.54 (m, 2 H), 4.44 (m, 4 H), 4.21 (m, 2 H), 4.09 (m, 6 H), 3.90 (m, 6 H), 3.70 (m, 4 H), 3.41 (q, 2 H), 3.15 (m, 2 H), 2.96 (m, 4H), 2.13 (s, 6 H), 2.02 (s, 6 H), 1.95 (s, 6H), 1.90 (s, 6 H), 1.74 (m, 2 H), 1.52 (m, 2 H), 1.39 (m, 2 H), 1.31 (m, 6 H). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 170.6, 170.4, 170.3, 169.6, 159.4, 151.1, 145.0, 130.6, 130.0, 128.2, 128.0, 127.2, 126.4, 126.1, 124.0, 123.9, 123.3, 121.5, 114.3, 102.0, 101.2, 71.0, 70.8, 68.6, 68.1, 68.0, 67.1, 64.7, 64.6, 61.4, 58.4, 50.0, 46.4, 41.3, 30.2, 29.4, 26.7, 26.2, 20.9, 20.8, 8.4. ESI-MS m/z Found: 1474.5851, calculated: 1474.5800 for $C_{72}H_{89}O_{25}N_7Na$ $[M+Na]^+$.

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170
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100
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80
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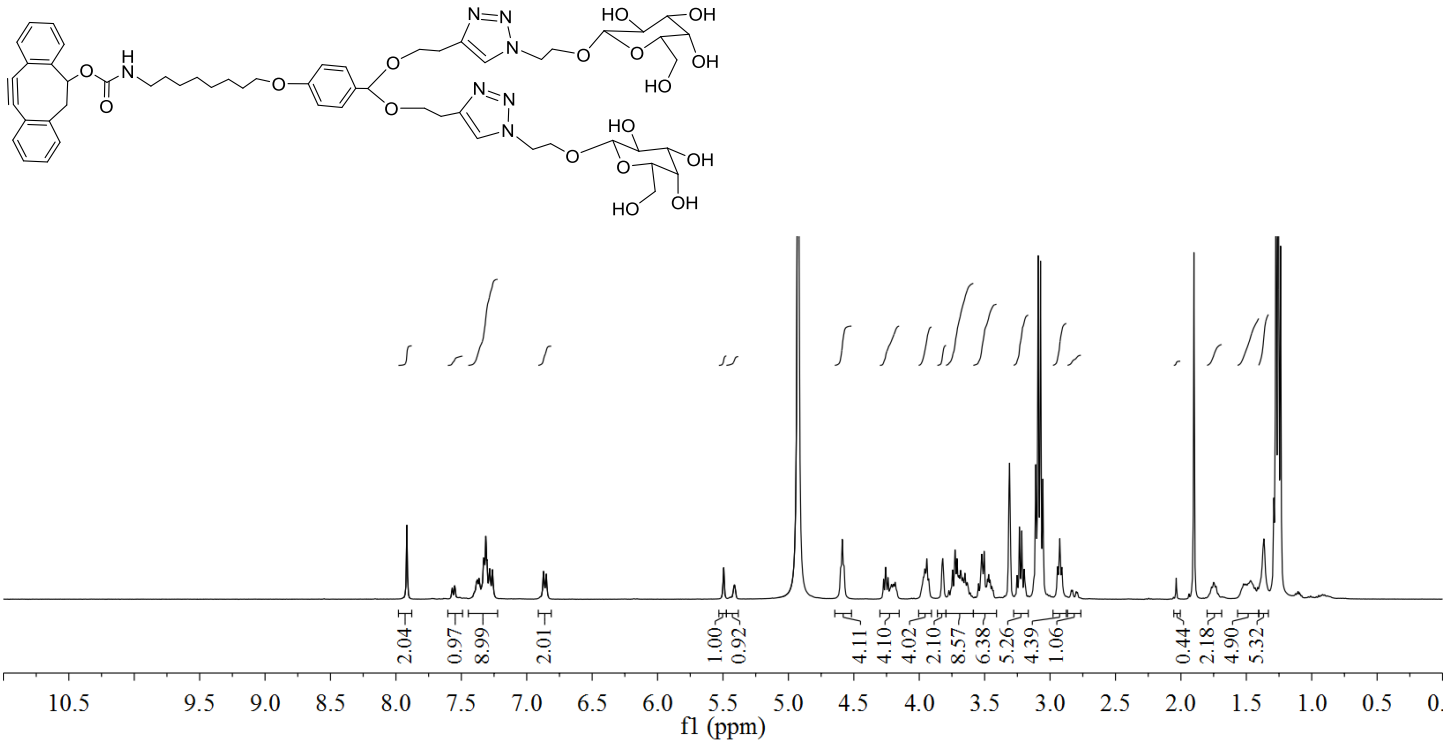
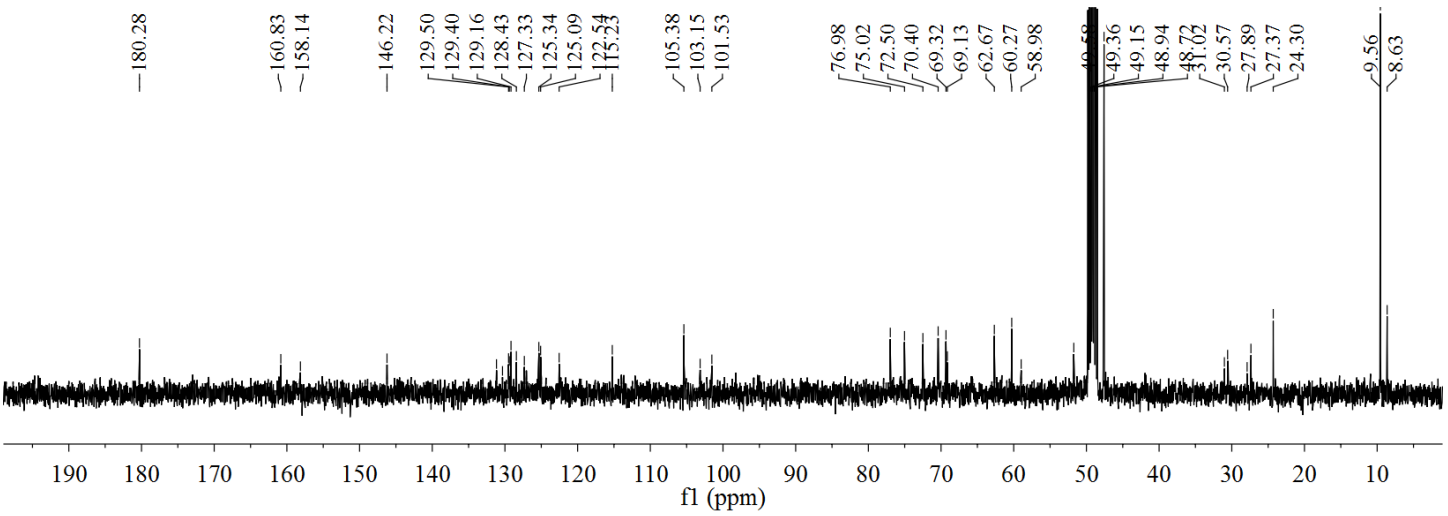
60
50
40
30
20
10
f1 (ppm)

*Synthesis of 1-{2-[(2S,3S,4R,5S,6S)-3,4,5-Trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-4-(2-{[2-(1-{2-[(2S,3S,4R,5S,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-1H-1,2,3-triazol-4-yl)ethoxy][p-(8-{tricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yloxycarbonylamino}octyloxy)phenyl]methoxy}ethyl)-1H-1,2,3-triazole (**5**):* To a stirred solution of **4** (100 mg, 0.07 mmol) in degassed MeOH was added $NaOCH_3$ (30.75 mg, 0.57 mmol) at room temperature, and the mixture was stirred for 1 h. The reaction was quenched with Amberlite IR-120 cation exchange resin and the pH of the reaction was brought to 8. The mixture was concentrated and the residue was purified by column chromatography (MeCN:$H_2O$:$Et_3N$,10:1:0.1) to give compound **5** as a liquid (62 mg, 82%). $^1$H NMR (400 MHz, MeOD): *δ (ppm)* 7.92 (s, 2 H), 7.56 (d, 1 H), 7.45 – 7.22 (m, 9H), 6.86 (d, 2 H), 5.49 (s, 1H), 5.41 (s, 1 H), 4.59 (t, 4 H), 4.30 – 4.15 (m, 4 H), 3.94 (t, 4 H), 3.82 (s, 2 H), 3.80 – 3.59 (m, 8 H), 3.59 – 3.41 (m, 6 H), 3.28 – 3.17 (m, 5 H), 2.93 (t, 4 H), 2.81 (m, 1 H), 1.75 (m, 2 H), 1.56 – 1.44 (m, 5 H), 1.39 (m, 5 H). $^{13}$C NMR (100 MHz, MeOD): *δ (ppm)* 180.3, 160.8, 158.1, 146.2, 131.2, 130.3, 129.5, 129.4, 129.2, 128.4, 127.3, 125.3, 125.1, 122.5, 115.2, 105.4, 103.2, 101.5, 77.0, 75.0, 72.5, 70.4, 69.3, 69.1, 62.7, 60.3, 59.0, 51.7, 31.0, 30.6, 27.9, 27.4, 24.3, 8.63. ESI-MS m/z Found: 1138.4947, calculated: 1138.4955 for $C_{56}H_{73}O_{17}N_7Na$ $[M+Na]^+$. The purity of **5** was further verified via analytical HPLC, performed on a Shimadzu system with a CBM-20A Prominence communication bus module, a SPD-M20A Prominence Diode Array detector, a DGU-20A5 Prominence degasser, and two LC-6AD pumps. The column used for the HPLC analysis was a XBridge C8 (5μm) 4.6x150mm column, and the elution was performed with 0.5% triethylamine in $H_2O$ (A) and 0.1% 0.5% triethylamine in acetonitrile (B). The gradient used was: 0-4 min, 30% B; 4-25 min, 30% to 80% B; 25-27 min, 80% B.

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103.15
101.53
76.98
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72.50
70.40
69.32
69.13
62.67
60.27
58.98
49.36
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48.94
48.72
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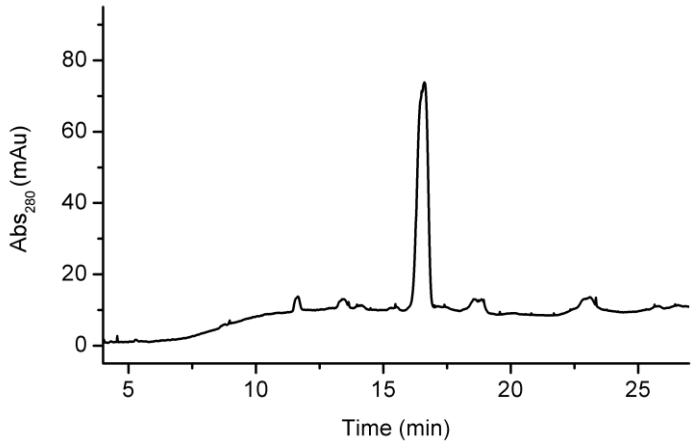
f1 (ppm)


Abs280 (mAu)
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5 10 15 20 25
Time (min)

## S2.2 Synthesis of DARTs

The DARTs were synthesized by conjugating 3'-azide functionalized oligonucleotides containing the Nrf2 ARE sequence, (forward strand 5'- GTC ACA GTG ACT CAG CAG AAT CTG TTT TT-$N_3$-3') and (reverse strand 5'- CAG ATT CTG CTG AGT CAC TGT GAC TTT TT-$N_3$-3'), with compound **5**, via copper free click chemistry. A 100 nmole of 3'-azide functionalized oligonucleotide and 600 nmole of compound **5** were mixed together in 10µL of PBS (pH 10), incubated overnight at room temperature, and purified via a Bio-Spin 6 column. The conjugation of compound **5** to the individual oligonucleotides was determined by gel electrophoresis, (see Figure S2). The double stranded DARTs were generated by hybridizing equal molar ratios of the modified oligonucleotides**.** The oligonucleotides were incubated at 80°C for 2 min in PBS (pH 10), were cooled down to room temperature for 30 min, and analyzed with gel electrophoresis.

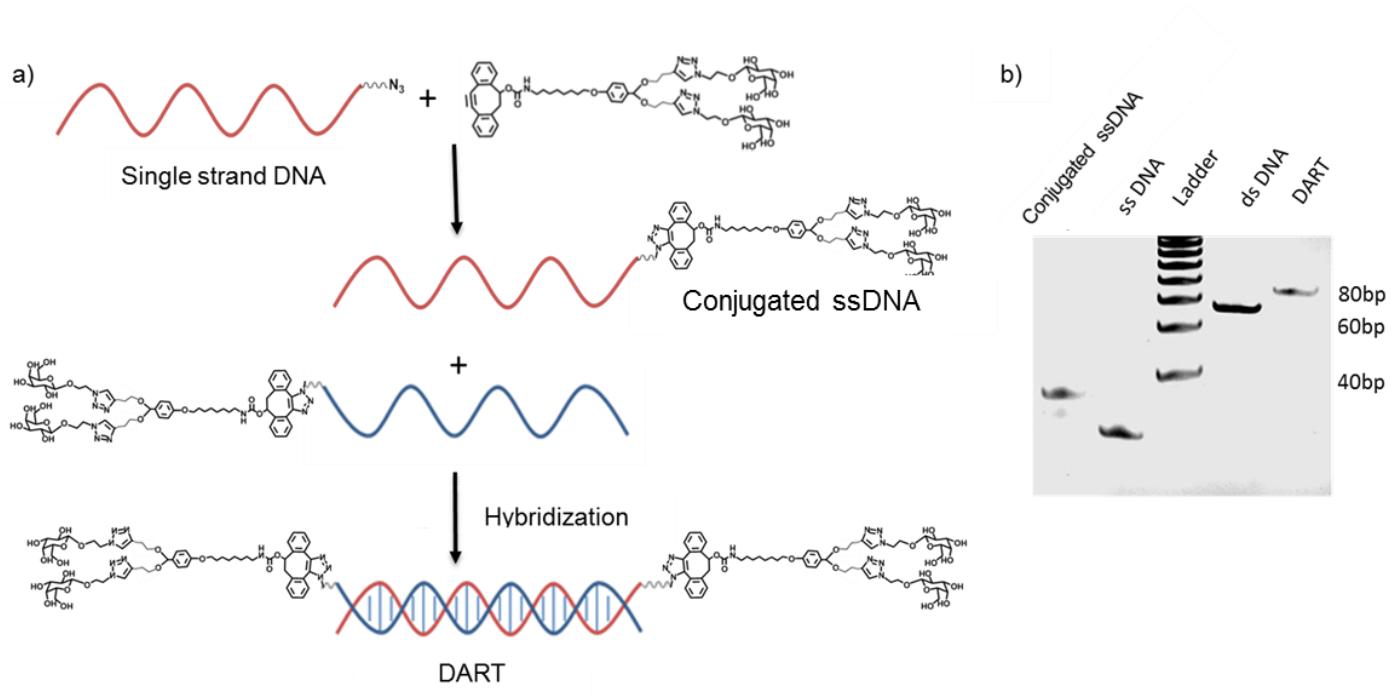


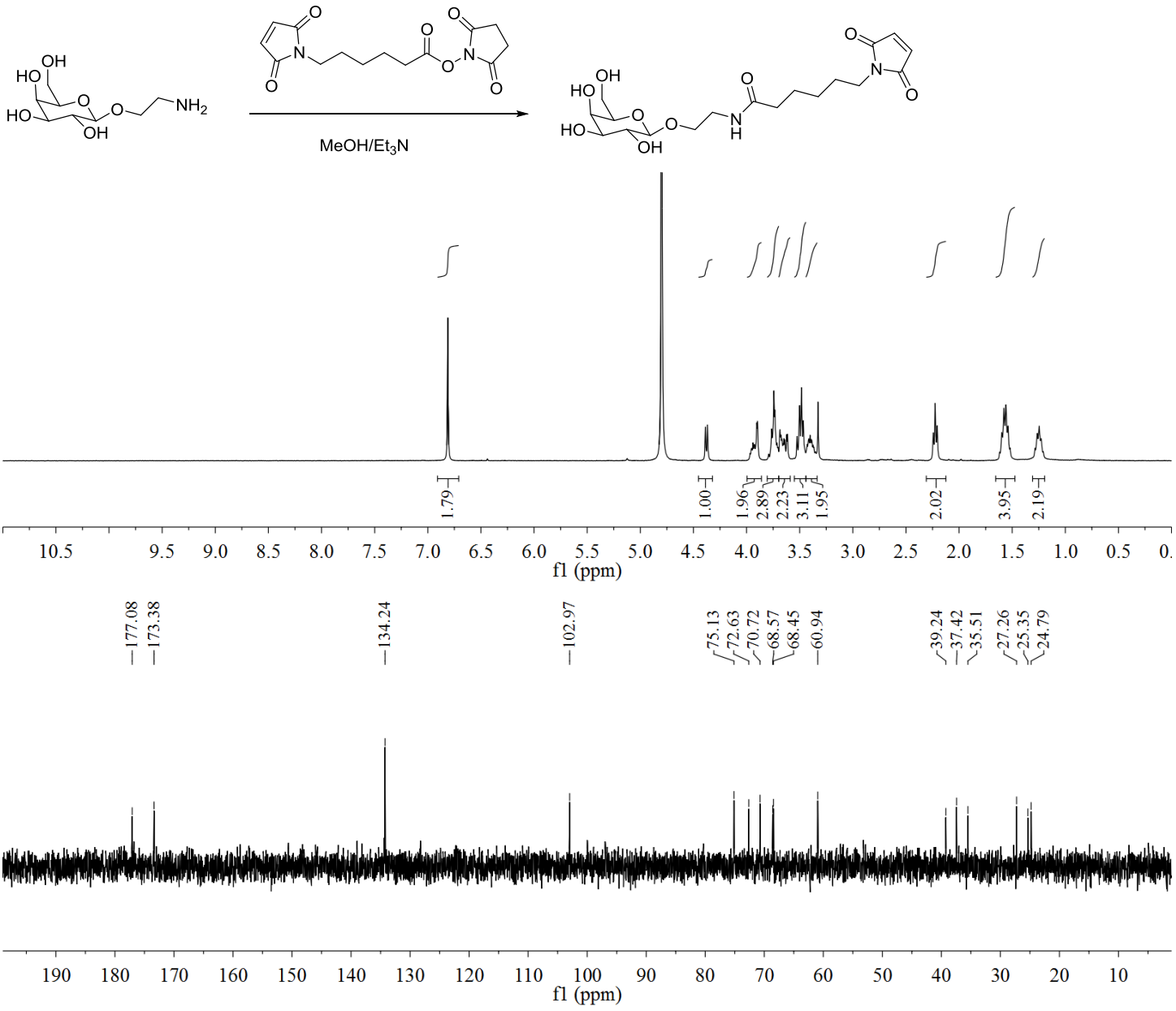

**Figure S2. Conjugation of compound 5 to azide-modified oligonucleotides.**

**a**, DARTs were synthesized by conjugating azide-modified oligonucleotides containing the Nrf2 consensus sequence with compound **5**, followed by hybridization.

**b**, Characterization of DART synthesis. Polyacrylamide gel electrophoresis confirms the conjugation of **5** to ssDNA, as evidenced by the mobility shift in the ssDNA conjugate lane. ssDNA conjugate lane migrates slower than unmodified DNA. Similar changes in mobility were seen between DART and dsDNA.

**S2.3 Synthesis of Gal-DNA**

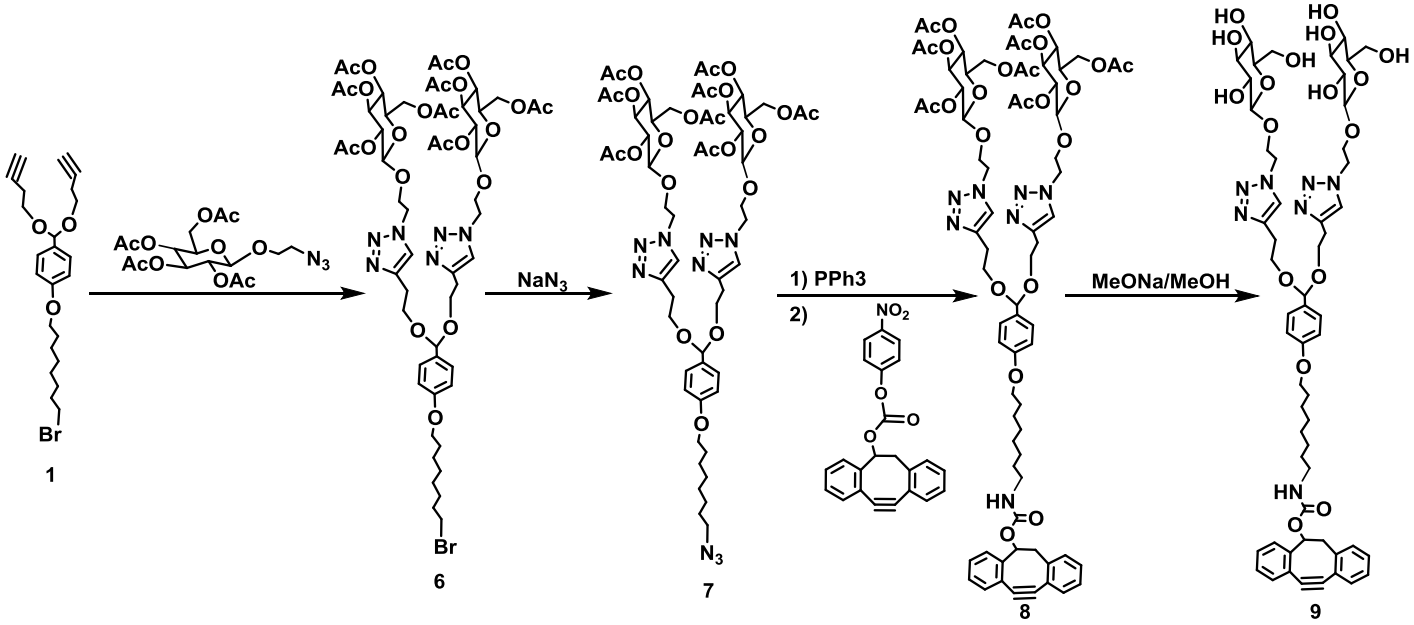
Galactose-maleimide was synthesized following published procedure.[1] Briefly, to a stirred solution of 2-aminoethyl galactoside (15 mg, 0.067 mmol) in MeOH (1 mL) was added a solution of 6-maleimidohexanoic acid *N*-hydroxysuccinimide ester (30 mg, 0.097 mmol) in MeOH (1 mL) and triethylamine (20 µL, 0.143mmol). The reaction mixture was stirred at room temperature for 2 h, and then concentrated to dryness. The residue was purified by column chromatography ($CH_2Cl_2$:MeOH=85:15) to give 6'-maleimidohexanamidoethyl β-D-Galactopyranoside (12 mg, 44%). $^1$H NMR (400 MHz, $D_2O$): *δ (ppm)* 6.81 (s, 2H), 4.38 (d, 1H), 4.00 – 3.86 (m, 2H), 3.81 – 3.70 (m, 3H), 3.65 (m, 2H), 3.49 (m, 3H), 3.38 (m, 2H), 2.22 (t, 2H), 1.66 – 1.47 (m, 4H), 1.31 – 1.19 (m, 2H). $^{13}$C NMR (100 MHz, $D_2O$): *δ (ppm)* 177.1, 173.4, 134.2, 103.0, 75.1, 72.6, 70.7, 68.6, 68.5, 60.9, 39.2, 37.4, 35.5, 27.3, 25.4, 24.8. ESI-MS m/z Found: 439.1684, calculated: 439.1687 for $C_{18}H_{28}O_9N_2Na$ $[M+Na]^+$.



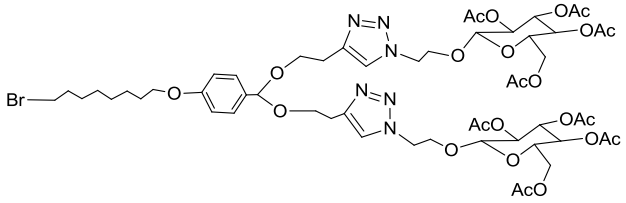
3’-Thiol modified C3 S-S functionalized oligonucleotides containing the Nrf2 consensus sequence, (forward strand 5'- GTC ACA GTG ACT CAG CAG AAT CTG TTT TT/3ThioMC3-D/-3') and (reverse strand 5'- CAG ATT CTG CTG AGT CAC TGT GAC TTT TT/3ThioMC3-D /-3') were purchased from IDT. 1mM oligonucleotide solution (5µL) was mixed with 50mM TCEP (2µL) and incubated at RT for 30 min. 1mg/mL galactose-maleimide dissolved in 20µL DI water was added and incubated at RT for 24hr. After overnight incubation at room temperature, the excess galactose-maleimide was eliminated through a Bio-Spin 6 column, and analyzed with gel electrophoresis to verify successful conjugation. Double stranded galactose DNA was generated by hybridizing equal molar ratios of the galactose modified oligonucleotides**.** The oligonucleotides were incubated at 80°C for 2 min, cooled down to room temperature for 30 min, and then analyzed by gel electrophoresis.

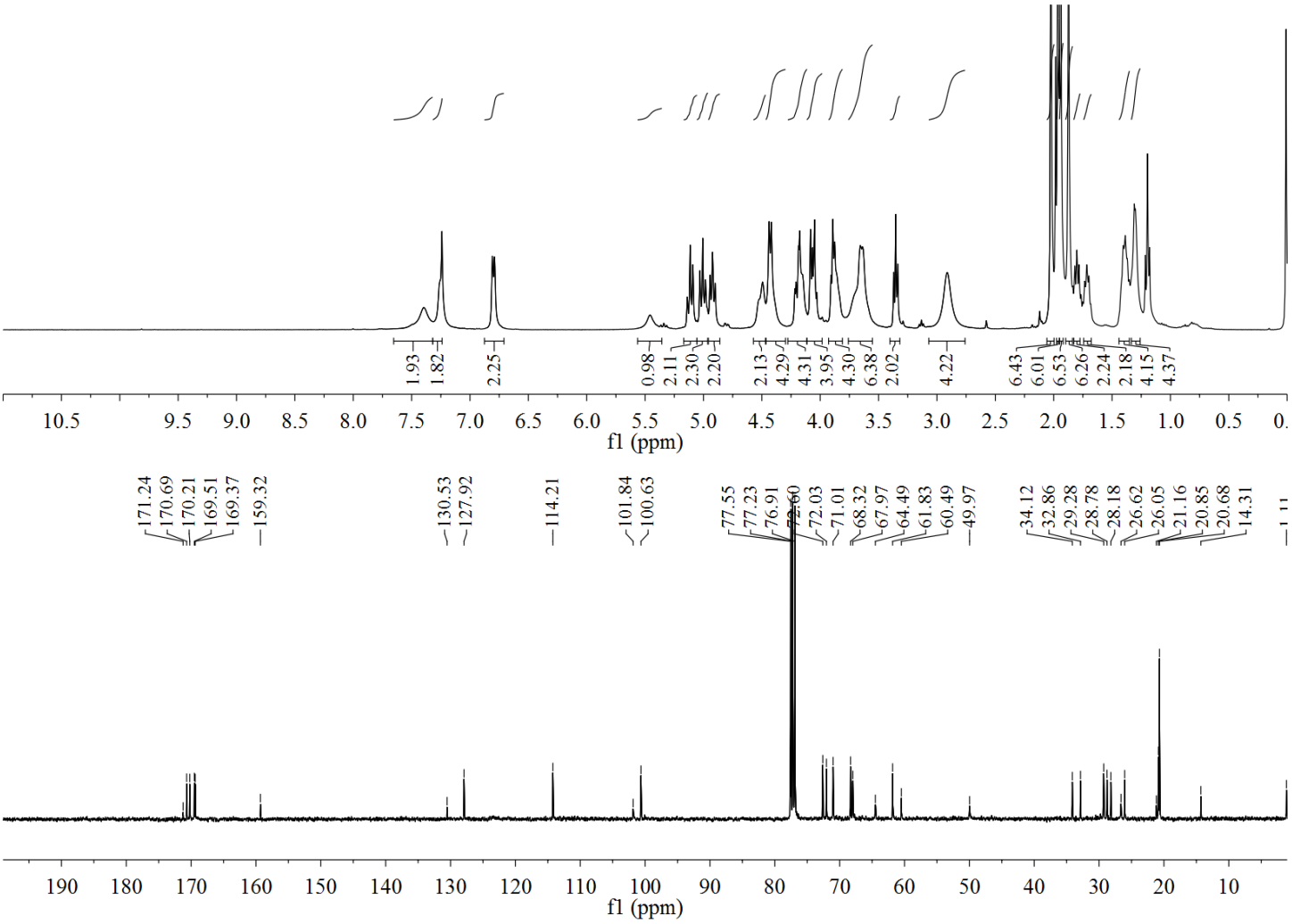
**S2.4 Synthesis of Glu-DARTs**

Glu-DARTs are identical to the DARTs, except that they contain glucose instead of galactose. The synthesis of the Glu-DARTs was accomplished via a procedure similar to that of the DARTs, as shown below.

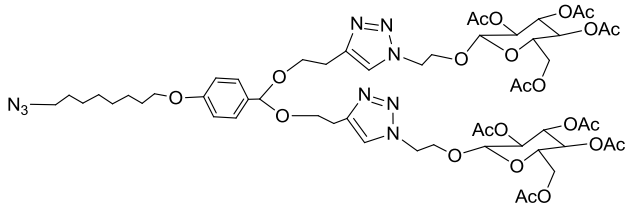


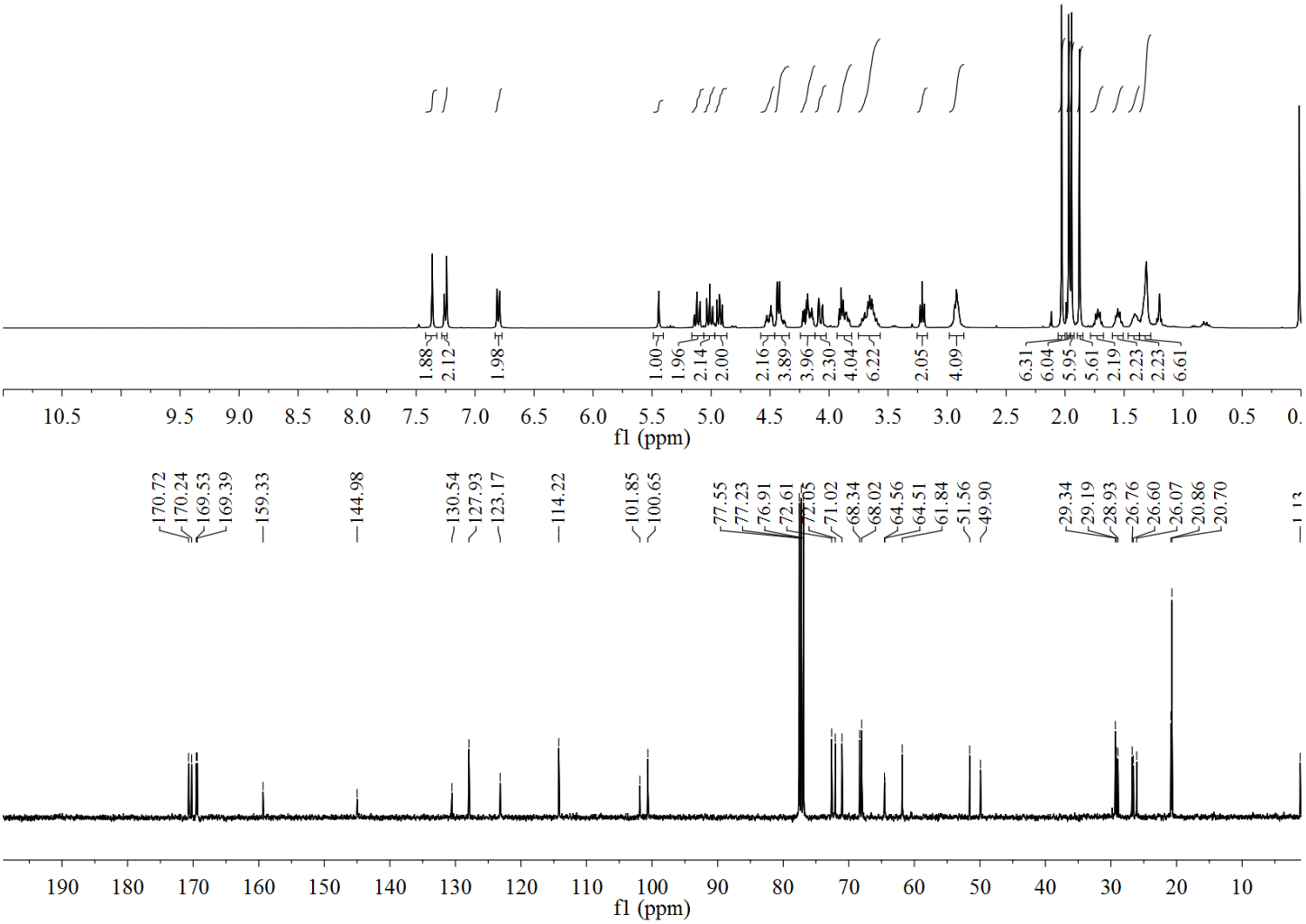

*Synthesis of [(2S,3S,4R,5S,6S)-6-{2-[4-(2-{[2-(1-{2-[(2S,3S,4R,5S,6S)-3,4,5-Triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-1H-1,2,3-triazol-4-yl)ethoxy][p-(8-bromooctyloxy)phenyl]methoxy}ethyl)-1H-1,2,3-triazol-1-yl]ethoxy}-3,4,5-triacetoxytetrahydro-2H-pyran-2-yl]methyl acetate (6):* To a stirred solution of compound **1** (47 mg, 0.11 mmol) and 2-azidoethyl, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (102 mg, 0.24 mmol, 2.2 equiv) in DMF was added CuI (2.3 mg, 0.012 mmol, 0.1 equiv) and DIPEA (14.8 mg, 0.11 mmol) and the mixture was stirred for 48 h at room temparature. The solvent was removed in vacuo. The residue was purifed by silica gel column chromatography (hexane:acetone=2:1) to afford **6** (120 mg, 87.5%). $^{1}$H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.40 (s, 2 H), 7.25 (d, 2 H), 6.80 (d, 2 H), 5.46 (s, 1 H), 5.10 (t, 2 H), 5.01 (t, 2 H), 4.92 (t, 2 H), 4.49 (m, 2 H), 4.43 (d, 4 H), 4.20 (m, 4 H), 4.05, (m, 4H), 3.89 (m, 4 H), 3.65, (m, 6 H), 3.35 (t, 2 H), 2.91 (b, 4 H), 2.02 (s, 6 H), 1.96 (s, 6 H), 1.94 (s, 6 H), 1.87 (s, 6 H), 1.80 (m, 2 H), 1.71 (m, 2H), 1.38 (m, 4 H), 1.31 (m, 4 H). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 170.7, 170.2, 169.5, 169.4, 159.3, 144.9, 130.5, 127.9, 114.2, 101.8, 100.6, 72.6, 72.0, 71.0, 68.3, 68.0, 64.5, 61.8, 50.0, 34.1, 32.9, 29.3, 28.8, 28.1, 26.6, 26.1, 20.9, 20.7.






*Synthesis of [(2S,3S,4R,5S,6S)-6-{2-[4-(2-{[2-(1-{2-[(2S,3S,4R,5S,6S)-3,4,5-Triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-1H-1,2,3-triazol-4-yl)ethoxy][p-(8-azidooctyloxy)phenyl]methoxy}ethyl)-1H-1,2,3-triazol-1-yl]ethoxy}-3,4,5-triacetoxytetrahydro-2H-pyran-2-yl]methyl acetate (**7**):* To a stirred solution of compound **6** (120 mg, 0.094 mmol) in anhydrous DMF was added 10 fold excess $NaN_3$. The reaction mixture was stirred at 60 $^oC$ for 12 h. The mixture was filtrated and concentrated under reduced pressure. The residue was purifed by silica gel column chromatography (hexane:acetone=2:1) to give **7** as foaming liquid (100 mg, 86%). $^1H$ NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.36 (s, 2 H), 7.25 (d, 2 H), 6.80 (d, 2 H), 5.44 (s, 1 H), 5.11 (t, 2 H), 5.00 (t, 2 H), 4.93 (t, 2 H), 4.51 (m, 2 H), 4.41 (m, 4 H), 4.17 (m, 4 H), 4.07, (m, 2 H), 3.87 (m, 4 H), 3.66 (m, 6 H), 3.21 (t, 2 H), 2.92 (m, 4 H), 2.03 (s, 6 H), 1.97 (s, 6 H), 1.95 (s, 6 H), 1.88 (s, 6 H), 1.72 (m, 2 H), 1.54 (m, 2 H), 1.39 (m, 2 H), 1.31 (m, 6 H). $^{13}C$ NMR (100 MHz, $CDCl_3$): *δ (ppm)* 170.7, 170.2, 169.5, 169.4, 159.3, 145.0, 130.5, 127.9, 114.2, 101.9, 100.7, 72.6, 72.1, 71.0, 68.3, 68.0, 64.6, 64.5, 61.8, 51.6, 49.9, 29.3, 29.2, 28.9, 26.8, 26.6, 26.0, 20.9, 20.7.



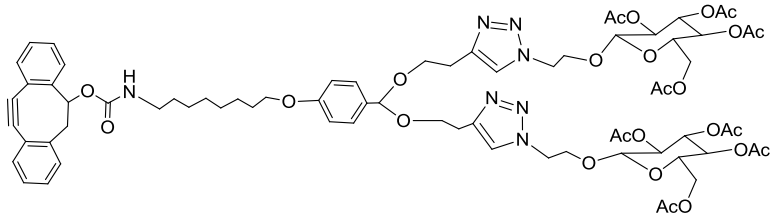



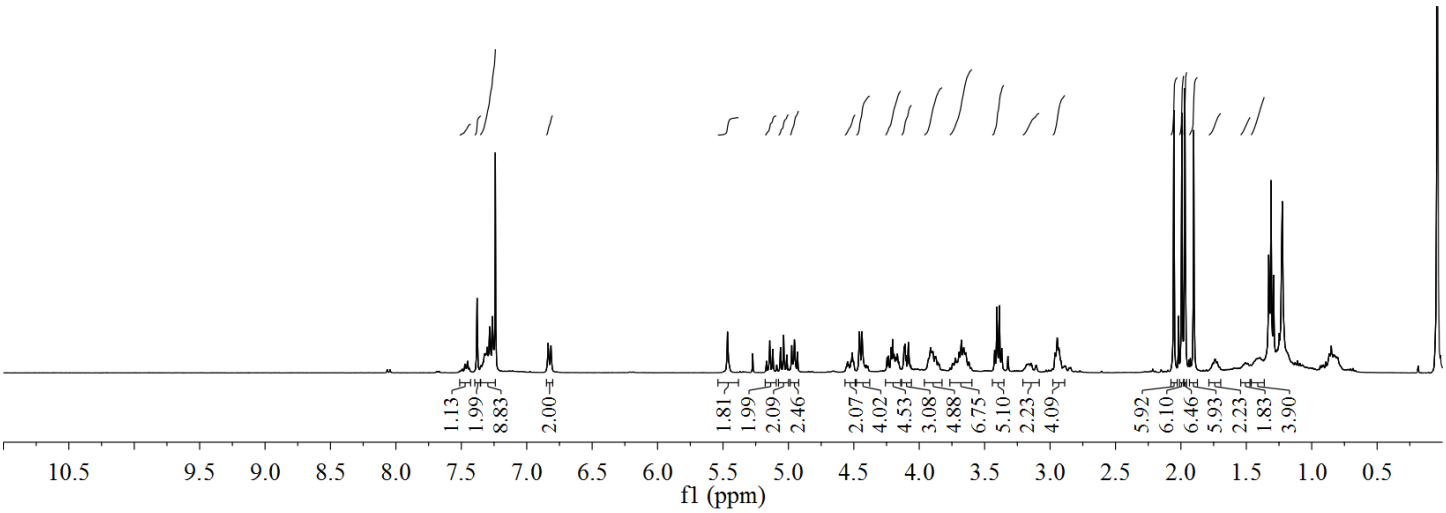


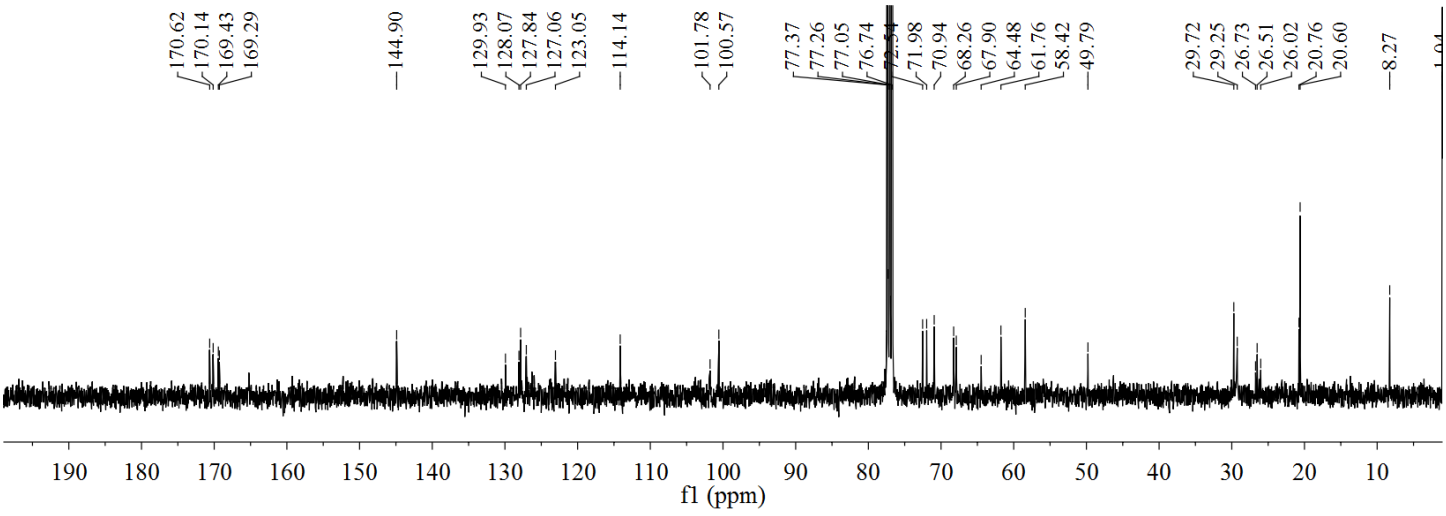


*Synthesis of [(2S,3S,4R,5S,6S)-6-{2-[4-(2-{[2-(1-{2-[(2S,3S,4R,5S,6S)-3,4,5-Triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-1H-1,2,3-triazol-4-yl)ethoxy][p-(8-{tricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yloxycarbonylamino}octyloxy)phenyl]methoxy}ethyl)-1H-1,2,3-triazol-1-yl]ethoxy}-3,4,5-triacetoxytetrahydro-2H-pyran-2-yl]methyl acetate (**8**):* To a stirred solution of compound **7** (41 mg, 0.033 mmol) in THF/$H_2O$ (8/2) was added $PPh_3$ (12.3 mg, 0.047 mmol). The reaction mixture was stirred overnight at room temperature. The mixture was concentrated under reduced pressure and the residue was purifed by silica gel column chromatography ($CH_2Cl_2$:$CH_3OH$:$Et_3N$=10:1:0.1) to give the crude product, to which p-nitrophenyl modified cyclooctyne (16 mg, 0.039 mmol) and $Et_3N$ (10 μl, 0.072 mmol) were added. After overnight strring at RT, the reaction mixture was concentrated and the residue was purified by column chromatography

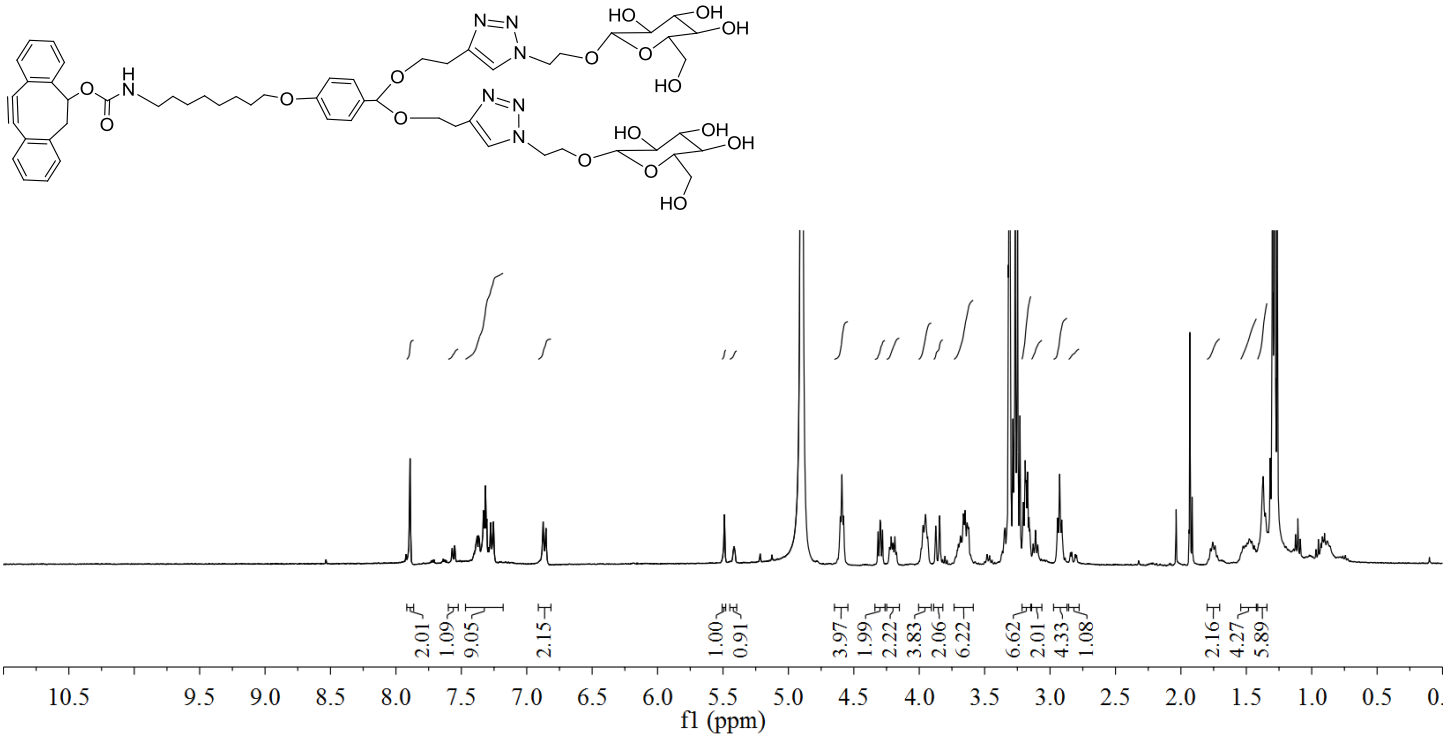
(hexane:acetone=1:1) to give compound **8** as a liquid (18 mg, 37%). $^{1}$H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.46 (d, 1 H), 7.37 (s, 2 H), 7.35 – 7.24 (m, 9 H), 6.83 (d, 2 H), 5.47 (s, 2 H), 5.13 (t, 2 H), 5.03 (t, 2 H), 4.95 (t, 2 H), 4.52 (m, 2 H), 4.42 (m, 4 H), 4.20 (m, 4 H), 4.09 (m, 3 H), 3.96 – 3.83 (m, 5 H), 3.68 (m, 7 H), 3.40 (q, 5 H), 3.14 (m, 2 H), 2.92 (m, 4H), 2.05 (s, 6 H), 1.99 (s, 6 H), 1.97 (s, 6H), 1.90 (s, 6 H), 1.73 (m, 2 H), 1.51 (m, 2 H), 1.40 (m, 4 H). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 170.6, 170.1, 169.4, 169.3, 144.9, 129.9, 128.1, 127.8, 127.1, 123.1, 114.1, 101.8, 100.6, 72.5, 72.0, 70.9, 68.3, 67.9, 64.5, 61.8, 58.4, 49.8,29.7, 29.3, 26.7, 26.5, 26.0, 20.8, 20.6, 8.3.

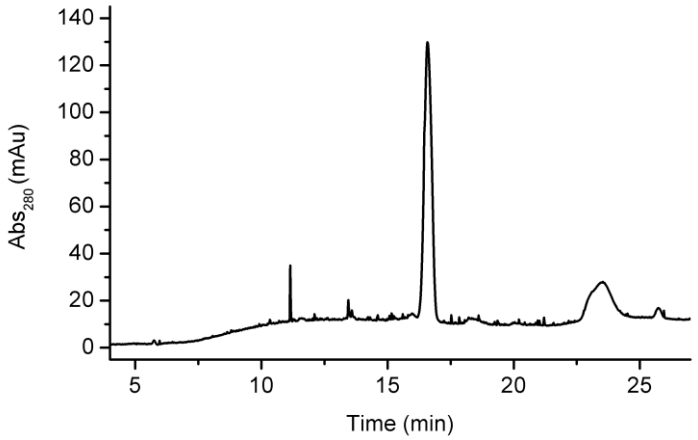


*Synthesis of 1-{2-[(2S,3S,4R,5R,6S)-3,4,5-Trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-4-(2-{[2-(1-{2-[(2S,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy]ethyl}-1H-1,2,3-triazol-4-yl)ethoxy][p-(8-{tricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yloxycarbonylamino}octyloxy)phenyl]methoxy}ethyl)-1H-1,2,3-triazole (9):* To a stirred solution of compound **8** (18 mg, 0.012 mmol) in degassed MeOH was added $NaOCH_3$ (5.5 mg, 0.102 mmol) at room temperature, and the mixture was stirred for 1 h. The reaction was then quenched with the addition of Amberlite IR-120 cation exchange resin, and the pH was brought to 8. The mixture was concentrated and the residue was purified by column chromatography ($MeCN:H_2O:Et_3N$=10:1:0.1) to give compound **9** as a liquid (10 mg, 75%). $^1$H NMR (400 MHz, MeOD): *δ (ppm)* 7.89 (s, 2 H), 7.56 (d, 1 H), 7.44 – 7.23 (m, 9H), 6.86 (d, 2 H), 5.49 (s, 1H), 5.41 (s, 1 H), 4.59 (t, 4 H), 4.30 (t, 2 H), 4.25 – 4.15 (m, 2 H), 4.00 – 3.91 (m, 4 H), 3.86 (d, 2 H), 3.73 – 3.59 (m, 6 H), 3.18 (m, 6 H), 3.10 (t,2 H), 2.92 (t, 4 H), 2.82 (m, 1 H), 1.76 (m, 2 H), 1.48 (m, 4 H), 1.36 (m, 6 H). ESI-MS m/z Found: 1138.4953, calculated: 1138.4955 for $C_{56}H_{73}O_{17}N_7Na$ $[M+Na]^+$. The purity of compound **9** was further verified via analytical HPLC, performed on a Shimadzu system with a CBM-20A Prominence communication bus module, a SPD-M20A Prominence Diode Array detector, a DGU-20A5 Prominence degasser, and two LC-6AD pumps. The column used for the HPLC analysis was a XBridge C8 (5μm) 4.6x150mm column, and the elution was performed with 0.5% triethylamine in $H_2O$ (A) and 0.1% 0.5% triethylamine in acetonitrile (B). The gradient used was: 0-4 min, 30% B; 4-25 min, 30% to 80% B; 25-27 min, 80% B.






Glu-DARTs were synthesized by conjugating 3'-azide functionalized oligonucleotides containing the Nrf2 ARE sequence, (forward strand 5'- GTC ACA GTG ACT CAG CAG AAT CTG TTT TT-$N_3$-3') and (reverse strand 5'-CAG ATT CTG CTG AGT CAC TGT GAC TTT TT-$N_3$-3'), with compound **9** via copper free click chemistry. A 100 nmole of 3'-azide functionalized oligonucleotide and 600 nmole of compound **9** were mixed together in 10µL of PBS (pH 10), incubated overnight at room temperature, and purified via a Bio-Spin 6 column. The conjugation of compound **9** to the individual oligonucleotides was determined by gel electrophoresis. Double stranded Glu-DARTs were generated by hybridizing equal molar ratios of the modified oligonucleotides**.**

**S3. Hydrolysis kinetics of DARTs**

DART hydrolysis was investigated by gel electrophoresis. 1.5 μM of DART solutions were prepared in pH 5 and in pH 7.4 PBS solutions, incubated for various time points at 37°C, and then analyzed by gel electrophoresis using a 4-20% TBE polyacrylamide gel (Biorad) stained with ethidium bromide. The percent hydrolysis was determined by quantifying the bands comprising the intact and hydrolyzed DARTs, using ChemiDoc XRS (Bio-rad), see Figure S3. Intact DARTs, DARTs with one acetal hydrolyzed, and DARTs with two acetals hydrolyzed were quantified. The percent hydrolysis of the DARTs was determined by integrating the two bands that represented DART hydrolysis products, one acetal hydrolyzed and both acetals hydrolyzed, and then dividing by the total DARTs present, which equaled the integration of the three bands corresponding to intact DART, one acetal hydrolyzed, and both acetals hydrolyzed. The one acetal hydrolyzed DART was multiplied by 0.5 to represent it as one acetal.

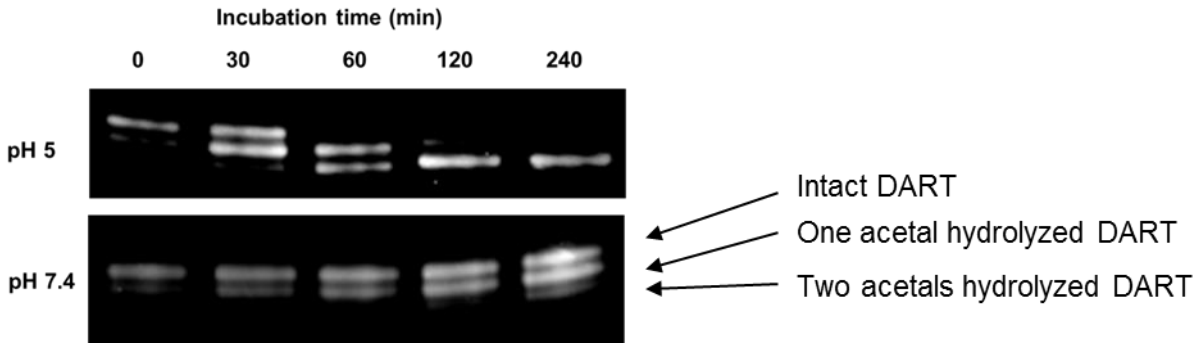



**Figure S3. The hydrolysis of DARTs is pH-dependent.** Intact and hydrolyzed DARTs have different electrophoretic mobility. The percent hydrolysis of DARTs was determined based on the intensity of the gel bands corresponding to intact and hydrolyzed DARTs. DARTs rapidly hydrolyzed at pH 5, but were stable at pH 7.4.

**S4. pH sensitive liposome leakage assay**

Liposomes containing calcein (200 mg/mL) dye were prepared with DPPC (Dipalmitoylphosphatidylcholine) as previously described by Lorin et al.[2]. Liposomes were diluted in 100 μL of either pH 5 or 7.4 PBS solutions, in a 96-well plate. 10 μL of DART stock solutions at various concentrations were added to the liposomes to generate DART concentrations between 10 to 30 μg/mL in triplicate. The plate was shaken and the fluorescence was measured after 30 min of incubation ($\lambda_{exc}$=490 nm and $\lambda_{ems}$=515 nm). The negative control was PBS added to the liposomes, and the positive control was triton-X 100 (1 mg/mL). The percentage of calcein leakage was calculated according to the following equation:

$$\% \text{ leakage} = (I_t - I_n) / (I_{max} - I_n)$$

where $I_t$ is the fluorescence intensity at 30 min after addition of DARTs, $I_n$ the fluorescence intensity from the PBS negative control, and $I_{max}$ the fluorescence intensity at 30 min after addition of triton-X 100.

**S5. Recombinant Nrf2 protein expression and purification**

Nrf2 was expressed in Rosetta2 expression *E.coli* following the procedure of Chumanov et al.[3]. The Nrf2 in the soluble fraction was purified with a nickel affinity column. A 100 mM of imidazole containing elution buffer (50 mM Phosphate Buffer, pH8, 300 mM NaCl) was used to elute the pure Nrf2. Purification steps were analyzed by SDS-PAGE gel electrophoresis. Purified Nrf2 protein was desalted using PD10 column or dialysis.

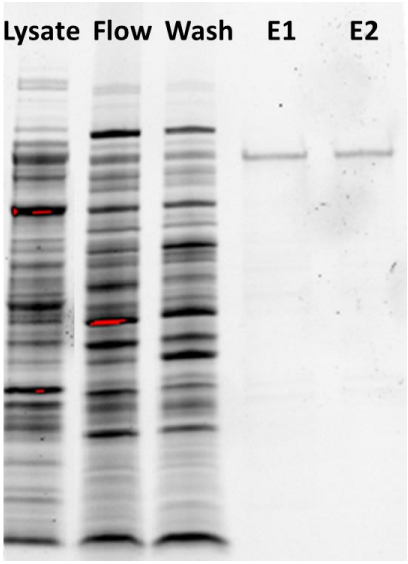



**Figure S4. Nrf2 can be purified with a nickel affinity column.** E.coli lysate has Nrf2 induction. Samples consisting of the flow through, wash, elution with 100mM imidazole (E1), and elution with 200mM imidazole (E2) were analyzed with SDS PAGE electrophoresis. Elution with 100mM or 200mM imidazole gave pure Nrf2.

**S6. Competitive ELISA between ARE DNA and DARTs for binding to Nrf2**

A competitive ELISA assay was conducted to compare the binding affinity of DARTs to unmodified ARE DNA. Nrf2 ELISA plates (TransAM Nrf2 ELISA (Active Motive)), containing ARE DNA as bait, were mixed with 75 nM Nrf2 and various amounts of ARE DNA or DART (75 nM, 150 nM, 600 nM: equivalent to 1:1, 1:2, 1:8 Nrf2 to DNA or DART molar ratio). ELISA was then performed for Nrf2 following the manufacturer's protocol, and percent inhibition was calculated. Specific inhibition values were calculated by normalization against the maximum amount of Nrf2 binding that could be inhibited with free ARE DNA.

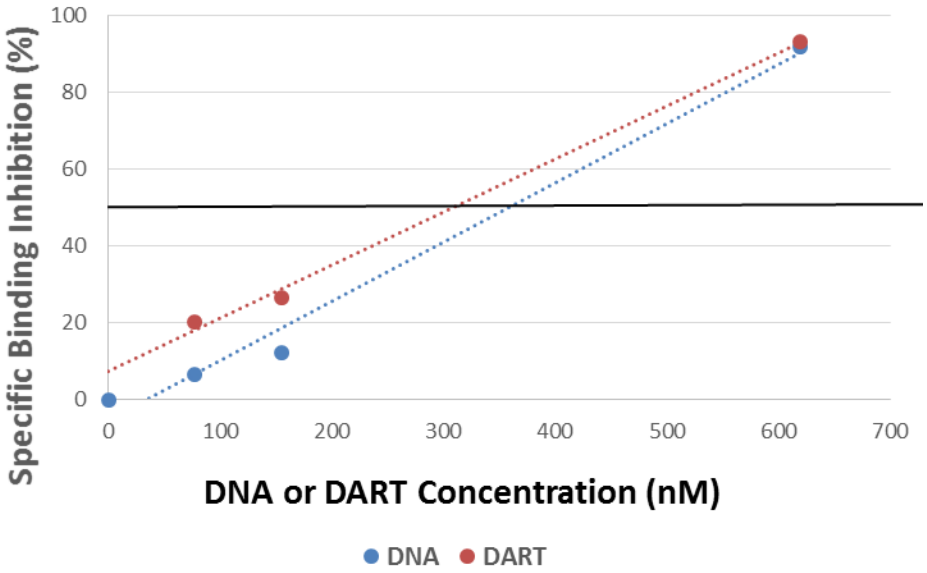



**Figure S5. The binding affinities of DARTs and ARE DNA to Nrf2 are similar.**

Binding of Nrf2 to surface immobilized DNA was competitively inhibited by ARE DNA or DARTs. Increased amounts of ARE DNA or DARTs resulted in higher binding inhibition of Nrf2. The estimated $IC_{50}$ values are similar between ARE DNA and the DARTs.

**S7. Fluorescent labeling of Nrf2**

500 µg of Nrf2 (500 µg/mL) was reacted with 5 times molar excess of Alexa555-NHS-ester, Alexa647-NHS-ester (Life Technologies), or IRDye 800CW-NHS-ester (LI-COR Biosciences) at 4°C overnight in 0.1 M phosphate buffer (pH 8.5). A PD-10 column was used to remove unreacted Alexa dyes. The concentration of Nrf2 and conjugated Alexa555, Alexa647, or IRDye 800CW were measured with a Nanodrop 2000 (Thermo).

**S8. Binding of Nrf2 to DARTs in serum**

An Electrophoretic mobility shift assay was conducted to study the binding of the DARTs with Alexa555-Nrf2. Fluorescently labeled Nrf2 was labeled with Alexa555, as described in S7. Various molar ratios of DARTs (100 pmole, 1µL) to Alexa555-Nrf2 (0, 50, 100, 200, or 400 pmole) were mixed in Nrf2 binding buffer (4mM $MgCl_2$ and 0.1 µM MafG in PBS) containing 10% fetal bovine serum without heat inactivation (total volume 10µL). After incubation for 15 min in 37°C, samples were analyzed by gel electrophoresis using a 4-20% TBE polyacrylamide gel (Biorad) stained with SYBR safe DNA stain (Life Technologies).

**S9. *In vitro* delivery of Nrf2 with DARTs**

DARTs or Gal-DNA were mixed with Nrf2 at equal molar ratios and incubated in binding buffer for 15 min before addition to HepG2 cells. For all *in vitro* studies, 0.6 µM of Nrf2 or 0.6 µM of Nrf2 complexed with 0.6 µM of DARTs were used.

**S9.1 Intracellular localization of Nrf2 delivered by DARTs or Gal-DNA**

The intracellular localization of Nrf2 delivered by the DARTs was observed by confocal microscopy (Swept Field Confocal microscope-Prairie Technology). Nrf2 was labeled with Alexa555 to visualize its intracellular localization. Alexa555-Nrf2 and DART or Gal-DNA were mixed at equal molar ratios. HepG2 cells in 35 cm culture dishes were treated with 0.6 μM of Alexa555-Nrf2, prepared with or without DART or Gal-DNA, for 30 min in OptiMEM medium with 1% FBS. After wash and additional 4hr incubation in culture medium, 100nM of LysoTracker Green DND-26 (Life Technologies) was added to the cells to visualize endosomes and lysosomes. Nuclei were stained with Hoechst (1μg/mL). HepG2 cells were washed 3 times with PBS and images were taken using confocal microscopy (see below for Gal-DNA images).

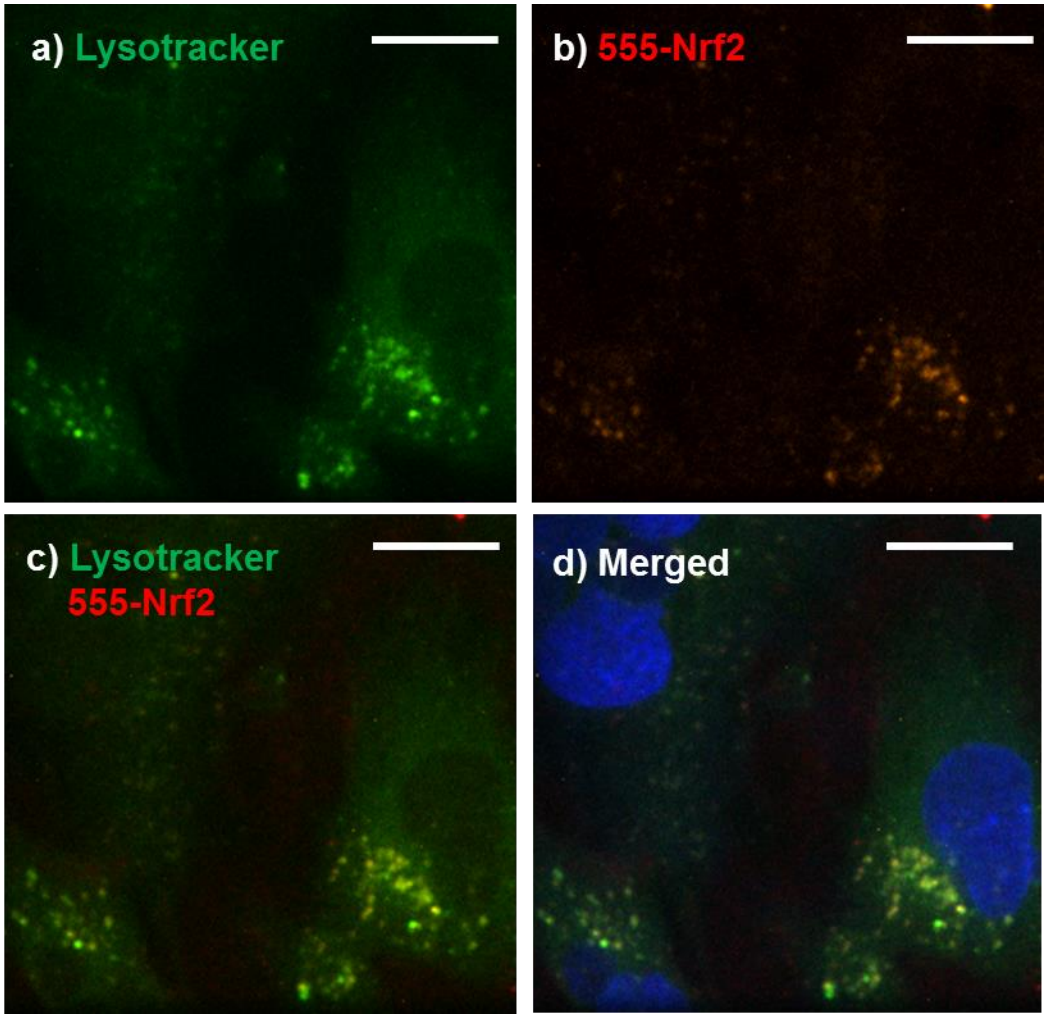



**Figure S6. Nrf2 delivered by Gal-DNA is located in lysosomes.**

Alexa555 labeled Nrf2 (red color) was complexed with Gal-DNA and incubated with HepG2 cells. Lysosomes were counterstained with Lysotracker green (green color). (a) Fluorescence of the green channel imaging Lysotracker green fluorescence, (b) Fluorescence of the red channel imaging Alexa555 labeled Nrf2 complexed with Gal-DNA, (c) Merged image of the green channel and the red channel, orange and yellow colors demonstrate that Nrf2 delivered by Gal-DNA is predominantly in lysosomes, (d) Merged image of the

red, green, and blue channel (hoechst staining of nuclei), the merged image has no accumulation of Nrf2 in the nucleus. (Scale bar: 2.5 µm).

**S9.2 Intracellular uptake analysis of Cy3-Glu-DART in HeLa cells**

3'-azide functionalized oligonucleotides containing the Nrf2 consensus sequence, (forward strand 5'-/Cy3/ GTC ACA GTG ACT CAG CAG AAT CTG TTT TT-$N_3$-3') and (reverse strand 5'- CAG ATT CTG CTG AGT CAC TGT GAC TTT TT-$N_3$-3') were purchased from Integrated DNA Technologies. Glu-DART modification was conducted as described earlier. HeLa cells were treated with either Cy3-DNA or Cy3-Glu-DARTs in OptiMEM medium with 1% FBS for 24hr. HeLa cells were washed 3 times with PBS and analyzed by flow cytometry (Guava easyCyte™).

**S9.3 Intracellular ROS analysis of HepG2 cells treated with Nrf2 delivered by DARTs**

HepG2 cells with 80% confluency in a 24-well plate were treated with 200 µM hydrogen peroxide for 4 hours in OptiMEM. After PBS washing, 0.6 µM of free Nrf2 or DART-Nrf2 were added to the cells in OptiMEM with 1% FBS and incubated overnight. Cells were washed with PBS and treated with 2 µM of 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-$H_2$DCFDA). After 30 min of incubation, cells were detached by trypsin treatment. Flow cytometry (Guava easyCyte™) was used to quantify the level of ROS, and the mean fluorescence at 488 nm excitation was calculated by Incyte™.

**S10. Cytotoxicity analysis of HepG2 cells treated with DARTs or DART-Nrf2**

HepG2 cells were plated at a cell density of $10^5$ in a 96 well plate in DMEM with 10% FBS and cultured for 24 hours with different concentrations of DARTs or DART-Nrf2. The percentage cell viability was determined using a cell counting kit (Dojindo). The cell viability was compared against untreated controls and is shown in Figures S7 and S8.

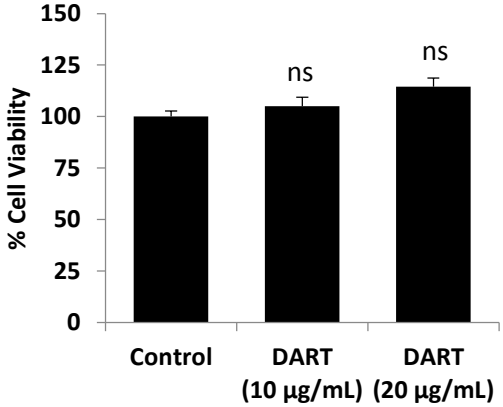



**Figure S7. DARTs have minimal toxicity to HepG2 cells.** Mean ± S.E, n=6, ns=statistically not significant with respect to control.

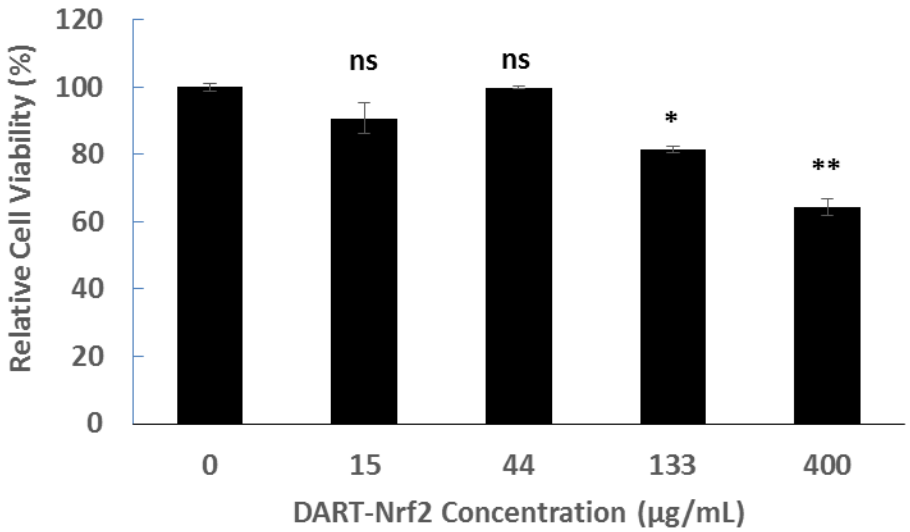



**Figure S8. DART-Nrf2 has minimal toxicity to HepG2 cells.** DART-Nrf2 has no toxicity to HepG2 cells at 44 µg/mL (0.65 µM). Mean ± S.E, n=4, ns=statistically not significant with respect to control.

**S11. Delivery of Nrf2 *in vivo* with DARTs**

For all the *in vivo* experiments, C57BL/6 female mice at 6 weeks of age were treated in accordance with the policies of the animal ethics committee of the University of California at Berkeley.

**S11.1 Biodistribution of Nrf2 delivered by DARTs**

Nrf2 was labeled with IRDye 800CW (LI-COR Biosciences) using the same procedure to conjugate the Alexa dyes as described in S7. Mice were intravenously injected with either free Nrf2 or DART-Nrf2 (1.25 mg/kg of Nrf2 and 0.423 mg/kg of DARTs in a 100 µL of PBS). Mice were euthanized by $CO_2$ asphyxiation 4 h after injection and the liver, lung, heart, kidney, spleen, and brain were harvested. Ex vivo imaging of organs was performed using an IVIS imaging system. IRDye fluorescence from the organs was quantified. Briefly, the organs were homogenized in 1 mL of PBS, and centrifuged at 13,000 g for 30 min. IRDye fluorescence intensity in 100 µL of the supernatant was quantified using a plate reader ($\lambda_{ex}/\lambda_{em}$=760/800; Tecan). To correct for background fluorescence, the fluorescence from control organs was subtracted from the fluorescence measured in the organs of Nrf2 or DART-Nrf2 treated mice.

**S11.2 Pharmacokinetics of Nrf2 delivered by DARTs**

Mice were intravenously injected with either free Alexa647-Nrf2 or DART-Alexa647-Nrf2 complexes (1.25 mg/kg of Nrf2 and 0.423 mg/kg of DARTs in a 100 µL of PBS). After various time points the mice were euthanized by $CO_2$ asphyxiation and the blood was collected by cardiac puncture. The plasma was isolated by centrifugation and the plasma fluorescence was quantified using a plate reader ($\lambda_{ex}/\lambda_{em}$=640/680; Tecan), to measure the serum Alexa647-Nrf2 concentration. A standard curve was generated with Alexa647-Nrf2 to

calibrate the fluorescence reading from the plasma. The half-lifes were calculated by plotting the logarithm of Nrf2 serum concentration versus time (see Table S1).

| | **Half-life (min)** |
|---|---|
| **Nrf2** | **82** |
| **DART-Nrf2** | **104** |

**Table S1. Half-life of Nrf2 and Nrf2/DART complexes *in vivo*.** The serum half-life of Nrf2 and DART-Nrf2 complexes were determined by measuring the fluorescence of Alexa647 labeled Nrf2. DART complexation causes a modest increase in Nrf2 serum half-life.

**S11.3 Serum TNF-α analysis of mice treated with DARTs**

Mice were administered free Nrf2, DART-Nrf2, or DARTs, at a dose of 5 mg/kg of Nrf2 and 1.69 mg/kg of DARTs. 24 h after administration of the samples, the blood was collected by cardiac puncture. Serum TNF-α levels were measured with a mouse TNF-α ELISA kit (eBioscience) following the manufacturer's instruction, and the results are shown in Figure S9. The control mice for this experiment were untreated mice.

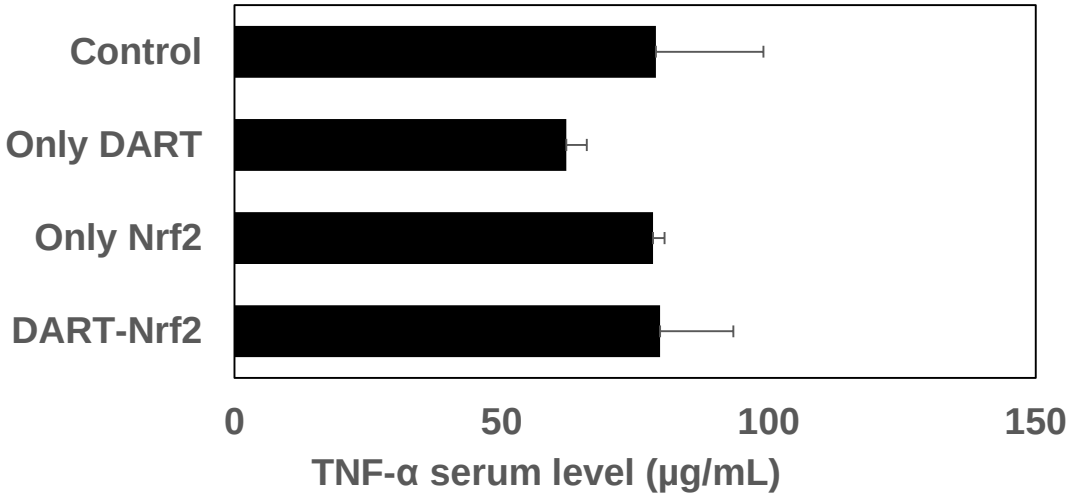



**Figure S9. DARTs cause minimal inflammation *in vivo*.** The serum levels of TNF-α were not significantly altered in mice treated with DARTs, Nrf2, or DART-Nrf2 versus untreated controls, mean ± S.E, n=3, all samples are statistically not significant to control.

**S11.4 Casp1 expression in mice treated with DART-Nrf2**

Mice were administered free Nrf2 or DART-Nrf2 at a dose of 5 mg/kg of Nrf2 and 1.69 mg/kg of DARTs. 24 h after administration of the samples, the liver was collected. The expression levels of Casp1, a gene not regulated by Nrf2, were measured with reverse transcription PCR (RT-PCR) as described in S7.1. The

expression levels of Casp1 were not significantly altered in mice treated with Nrf2, or DART-Nrf2 versus untreated controls.

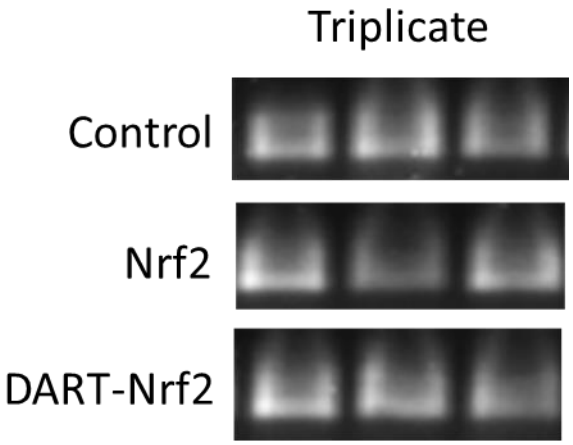



**Figure S10. DART-Nrf2 has minimal effect on Casp1 expression *in vivo*.** The expression levels of Casp1 were not significantly altered in mice treated with Nrf2, or DART-Nrf2 versus untreated controls.

**S12. Uptake of Glu-DARTs in HeLa cells**

Transcription factors have the potential to treat diseases in organs outside of the liver. Therefore we performed experiments to test the modularity of the DART delivery strategy, using glucose terminated DARTs labeled with Cy3 (Cy3-Glu-DARTs). The uptake of Cy3-Glu-DARTs in the tumorigenic HeLa cell line, which over-expresses glucose transporters, was investigated by flow cytometry. HeLa cells were treated with 0.6 μM of either Cy3-DNA or Cy3-Glu-DARTs in OptiMEM medium with 1% FBS for 24hr. HeLa cells were washed 3 times with PBS and analyzed by flow cytometry (Guava easyCyte™).

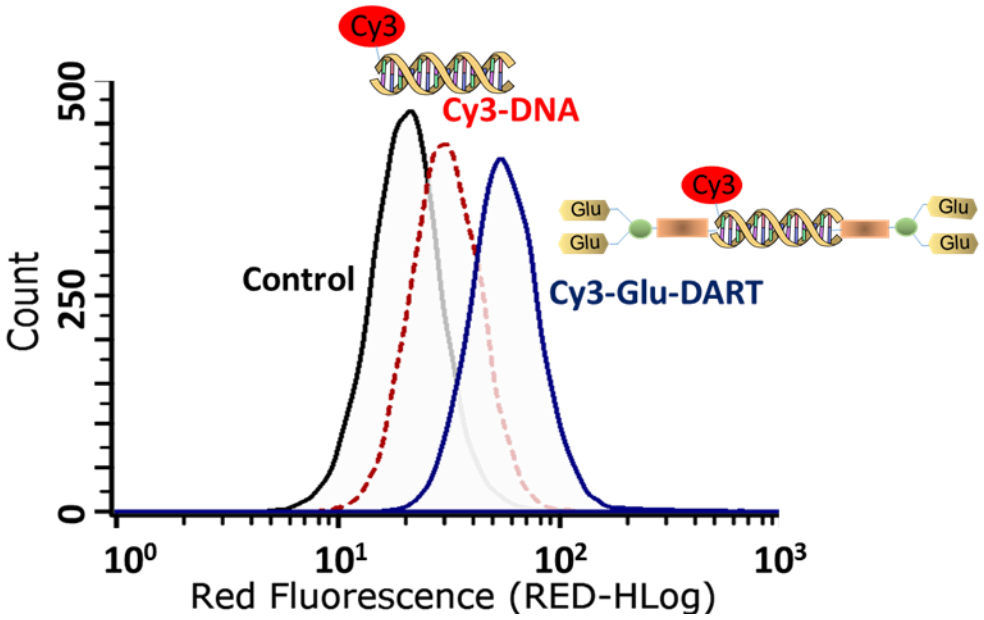


**Figure S11. Glucose targeted DARTs are internalized by HeLa cells by a factor of 4 greater than free DNA.** Red dashed line represents Cy3-DNA treated HeLa cells and blue solid line represents Cy3-Glu-DART treated HeLa cells.

### S13. Dynamic Light Scattering (DLS) measurement of DARTs

DLS measurements on DART-Nrf2 solutions were obtained using a Zetasizer Nano ZS (Malvern Instruments, UK). Individual DART (50μM) and Nrf2 (50μM) samples were prepared in PBS and filtered through a 0.22 μm centrifugal filter unit (Millipore, MA) prior to mix and complexation. Equal molar ratio of DARTs and Nrf2 were mixed in PBS for 15 minutes at room temperature. The size of DART-Nrf2 complexes were measured and plotted in Figure S12.

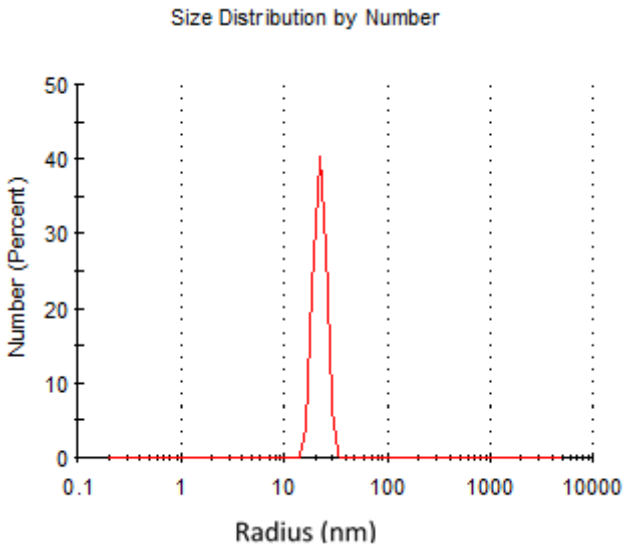



**Figure S12. DART-Nrf2 does not form large aggregates.** DLS of DART-Nrf2.

### S14. Statistics

Statistical analyses were performed using a student t-test and p-values for each experiment were determined. Statistically significant data ($p<0.05$) are depicted using the '*' symbol and $p<0.01$ data are depicted using the '**' symbol.

### References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