

## SUPPLEMENTARY INFORMATION

### **Boronate Affinity-based Photoactivatable Magnetic Nanoparticles for Oriented and Irreversible Conjugation of Fc-Fused Lectin and Antibody**

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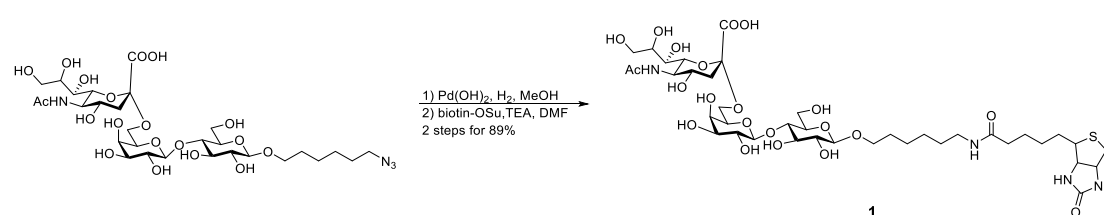
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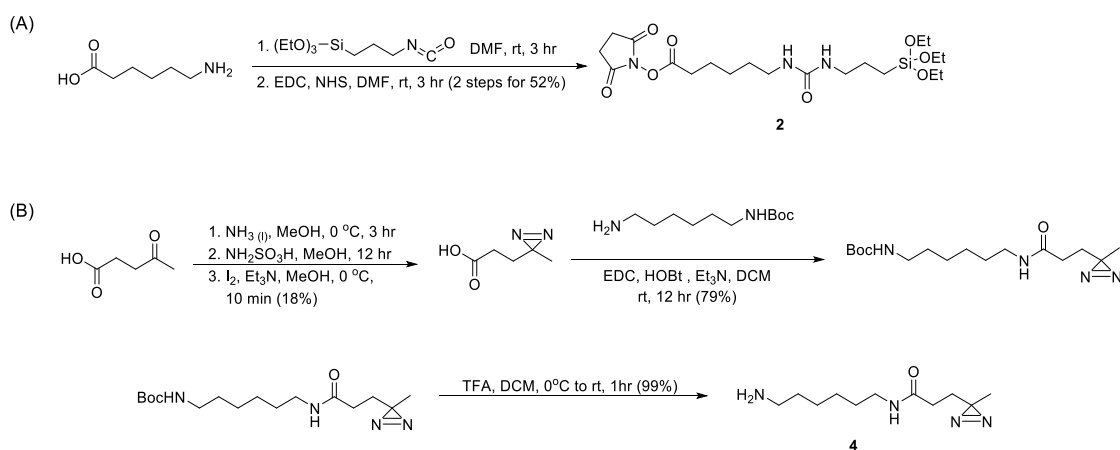
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## Materials and Methods

For chemical synthesis, all starting materials and commercially obtained reagents were purchased from Sigma-Aldrich and used as received unless otherwise noted. All reactions were performed in oven-dried glassware under nitrogen atmosphere using dry solvents.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on Bruker AV-400 spectrometer operating at 400 and 100 MHz, respectively, and were referenced to the solvent used ( $\text{CDCl}_3$  at  $\delta$  7.24 and 77.23, and  $\text{CD}_3\text{OD}$  at  $\delta$  3.31 and 49.2 for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively). Chemical shifts ( $\delta$ ) are reported in ppm using the following convention: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, and coupling constants ( $J$ ), with  $J$  reported in Hz. High-resolution mass spectra were recorded under ESI-TOF mass spectroscopy conditions. Analytical thin-layer chromatography (TLC) was performed on pre-coated plates (silica gel 60). Silica gel (E, Merck) was used for flash chromatography. Transmission electron microscopy (TEM) images were obtained by a JEM-2100 electron microscope with an operating voltage of 200 kV.



**Scheme S1.** Synthesis of biotinylated sialoside **1**.



**Scheme S2.** (A) Synthesis of *N*-hydroxysuccinimide ester triethoxysilane **2**. (B) Synthesis of photo-reactive alkyl diazirine **4**.

**Compound 1.** To a solution of NeuA $\alpha$ (2,6)Gal $\beta$ (1,4)Glc<sup>2</sup> (0.026 mmol) in MeOH (0.6 mL) was added palladium hydroxide (Pd(OH)<sub>2</sub>, 20% Pd content, 4 mg). The mixture was stirred at rt under atmosphere of hydrogen gas for 6 h. The solution was filtered through a pad of Celite. The residue was concentrated to dryness in *vacuo* and the resulting residue was dissolved in DMF (0.6 mL). *N*-Succinimide-biotin (0.039 mmol) was added to the mixture and then stirred at rt for 6 h. The solution was concentrated to dryness in *vacuo* and the resulting residue was purified by C18 reverse phase silica column (Sep-Pak Vac C18 cartridge 20cc/5g 37-55  $\mu$ m) to give **1** (20 mg, 80% for 2 steps). <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  4.62 (dd, *J* = 4.8, 12.8 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.44 (d, *J* = 8.0 Hz, 2H), 3.99 (d, *J* = 4.4 Hz, 1H), 3.94-3.54 (m, 18H), 3.33 (q, *J* = 8.8 Hz, 2H), 3.18 (q, *J* = 6.4 Hz, 2H), 3.01 (dd, *J* = 4.8, 12.8 Hz, 1H), 2.79 (d, *J* =

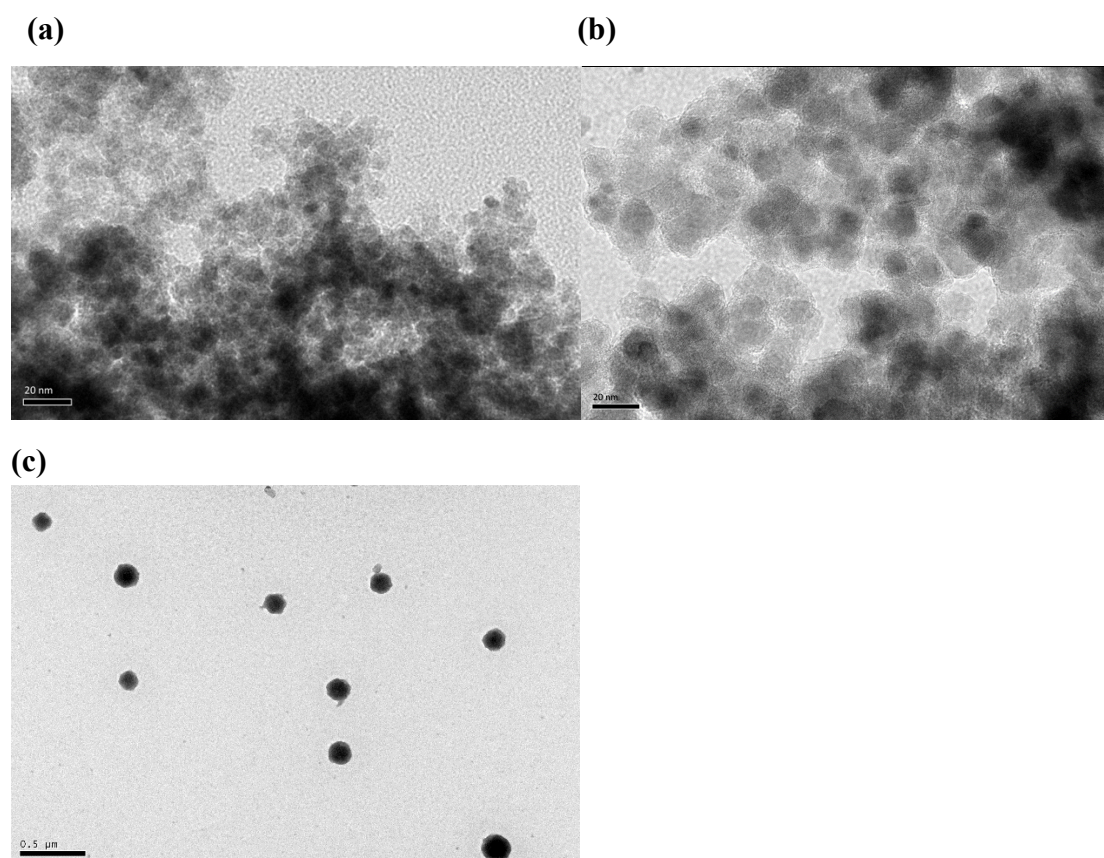
12.8 Hz, 1H), 2.72 (dd,  $J = 5.2, 12.8$  Hz, 1H), 2.26 (t,  $J = 7.2$  Hz, 2H), 2.04 (s, 3H), 1.75 (t,  $J = 12.8$  Hz, 1H), 1.67-1.61 (m, 4H), 1.54 (t,  $J = 6.8$  Hz, 2H), 1.42-1.38 (m, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  176.58, 174.93, 173.48, 165.32, 103.24, 101.94, 100.31, 79.70, 74.74, 74.65, 73.71, 72.78, 72.55, 72.39, 71.80, 70.81, 70.54, 68.53, 68.40, 63.55, 62.67, 62.10, 62.02, 60.34, 60.26, 59.35, 55.42, 51.82, 40.12, 39.73, 39.24, 35.92, 35.55, 28.67, 28.24, 28.12, 27.86, 27.70, 25.83. HRMS (ESI) calcd for  $\text{C}_{39}\text{H}_{65}\text{N}_4\text{O}_{21}\text{S}$   $[\text{M}-\text{H}]^-$   $m/z$  957.3867; found: 957.3862.

**Compound 2.** A solution of aminocaproic acid (20 mmol) in DMF (30 mL) was added 3-(triethoxysilyl)propyl isocyanate (10 mmol) dropwise under nitrogen. The solution was stirred at rt for 3 h. The mixture was concentrated to dryness *in vacuo* and then diluted with ethyl acetate. The organic layer was washed by  $\text{H}_2\text{O}$  for three times and was dried over  $\text{MgSO}_4$ , filtered, and concentrated. The crude product was then dissolved in DMF (30 mL) followed by adding EDC (15 mmol) and *N*-hydroxysuccinimide (15 mmol). The solution was stirred at rt for 3 h. The mixture was concentrated to dryness *in vacuo* and then diluted with ethyl acetate. The organic layer was washed by  $\text{H}_2\text{O}$  for three times. The organic layer was dried over  $\text{MgSO}_4$ , filtered, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/DCM 1:5) to give **2** (2.53 g, 52% for 2 steps).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.79 (q,  $J = 7.2$  Hz, 6H), 3.17 (t,  $J = 6.4$  Hz, 2H), 3.12 (t,  $J = 6.4$  Hz, 2H), 2.83 (s,

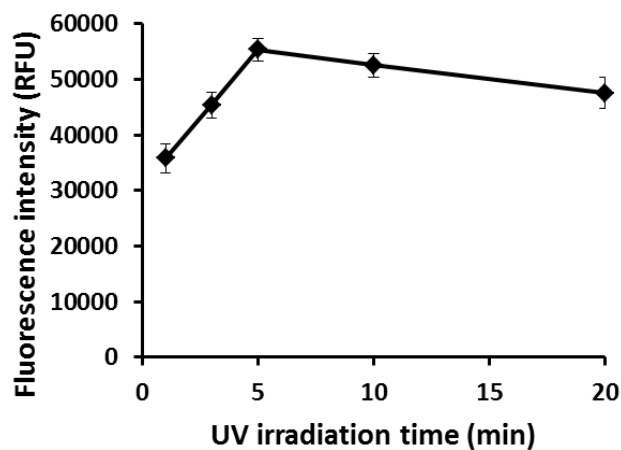
4H), 2.60 (t,  $J = 6.4$  Hz, 2H), 1.75 (p,  $J = 7.2$  Hz, 2H), 1.60 (q,  $J = 7.2$  Hz, 2H), 1.50 (q,  $J = 7.2$  Hz, 2H), 1.44 (m, 2H), 1.19 (t,  $J = 7.2$  Hz, 9H), 0.60 (dd,  $J = 8.0, 8.0$  Hz, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.32 (x2), 168.28, 158.58, 58.07 (x3), 42.51, 39.39, 30.53, 29.24, 25.47, 25.31 (x2), 23.91, 23.38, 17.98 (x3), 7.30. HRMS (ESI) calcd for  $\text{C}_{20}\text{H}_{37}\text{N}_3\text{O}_8\text{Na}$   $[\text{M}+\text{Na}]^+$   $m/z$  498.2254; found: 498.2248.

**Compound 4.** A solution of 3-(3-methyl-3H-diaziren-3-yl)propanoic acid<sup>1</sup> (0.5 mmol) in DCM (3 mL) was preactivated with EDC (0.75 mmol), HOBt (0.75 mmol), and trimethylamine (1 mmol) under nitrogen for 30 min. *N*-Boc-1,6-diaminohexane (0.6 mmol) in DCM (3 mL) was then added to the above solution and the resulting solution was stirred at rt for 12 h. The mixture was concentrated to dryness *in vacuo* and then diluted with ethyl acetate. The organic layer was washed with  $\text{H}_2\text{O}$  for three times and was dried over  $\text{MgSO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (MeOH/DCM 1:5). TFA (0.2 mL) was added to a solution of above compound (0.2 mmol) in DCM (1.8 mL) at 0 °C under nitrogen and the resulting solution was stirred at rt for 1 h. The solution was removed and dried *in vacuo* to yield **4** (33 mg, 79% for 2 steps).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  3.17 (t,  $J = 6.8$  Hz, 2H), 2.91 (t,  $J = 8.0$  Hz, 2H) 2.07 (t,  $J = 8.0$  Hz, 2H), 1.67 (m, 4H), 1.53 (t,  $J = 6.8$  Hz, 2H), 1.40 (t,  $J = 4.0$  Hz, 4H), 1.00 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  174.68, 40.85, 40.32, 31.70, 31.54, 30.25, 28.65, 27.48, 27.18, 26.51, 19.90. HRMS

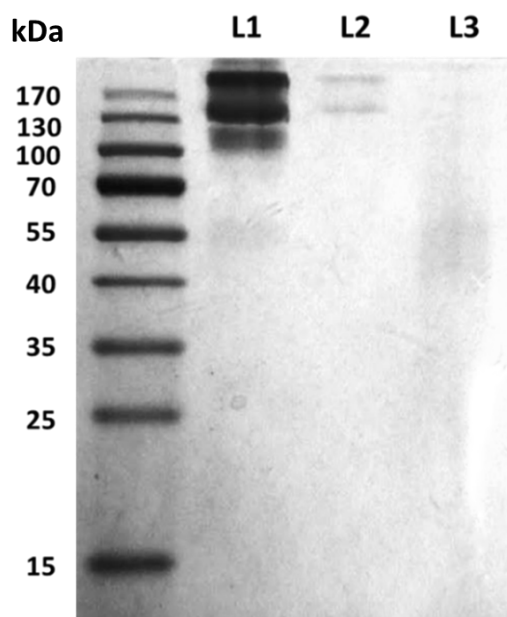
(ESI) calcd for  $C_{11}H_{23}N_4O$   $[M+H]^+$   $m/z$  227.1872; found: 227.1870.



**Figure S1.** Characterization of MNPs. TEM images of (a) magnetic core  $Fe_3O_4$ , (b) BA-Diaz@MNPs C, and (c) Siglec-2-BA-Diaz@MNP C. The scale bars in TEM images of (a), (b), and (c) are 20, 20, and 500 nm, respectively.

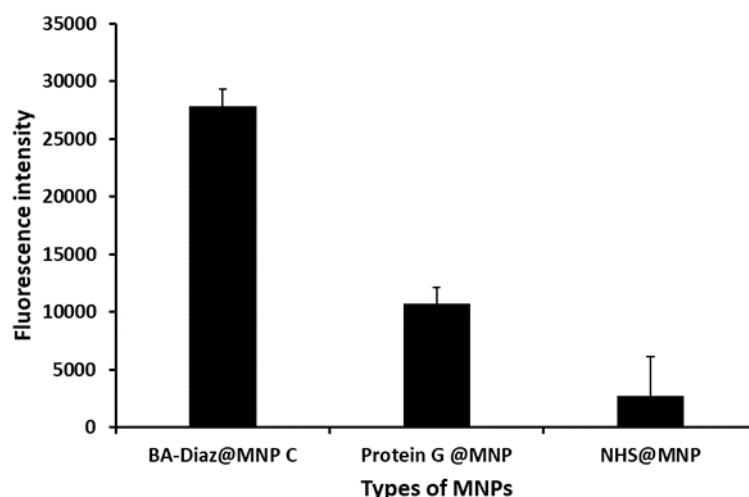


**Figure S2.** Effect of UV irradiation time on the immobilization of Siglec-2-Fc on BA-Diaz@MNPs. C. Siglec-2-Fc (50  $\mu\text{g/mL}$ ) was incubated on BA-Diaz@MNP C followed by UV irradiation for 1, 3, 5, 10 and 20 min, respectively. The fluorescence signals were measured after incubated with biotinylated sialoside **1** and Streptavidin-Cy3 followed by treatment with GdnHCl.

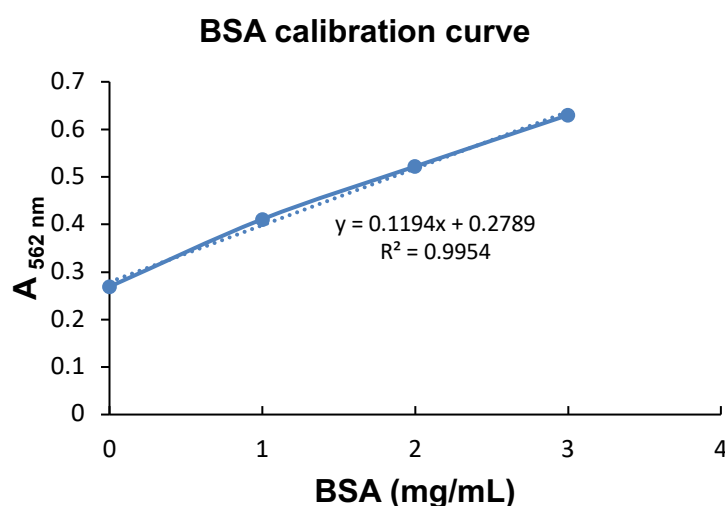


**Figure S3.** Non-reducing SDS-PAGE gel analysis of the Siglec-2-Fc@MNPs prepared by BA-Diaz-based fabrication strategy showing either traces or no detectable noncovalently attached Siglec-2-Fc on the BA-Diaz@MNP C. L1: Siglec-2-Fc (5  $\mu\text{g}$ ); L2: Siglec-2-Fc (2  $\mu\text{g}$ ) immobilized on BA@MNPs A shows partially release of Siglec-2-Fc from MNPs; L3: Siglec-2-Fc (2  $\mu\text{g}$ ) immobilized on BA-Diaz@MNP C. Protein ladder is shown in left.





**Figure S4.** Comparison of relative binding activity of Siglec-2-Fc immobilized on MNPs through oriented and irreversible conjugation utilizing BA-Diaz@MNP C, Protein G@MNP, and NHS@MNP. In each case, the same amount of Siglec-2-Fc (50  $\mu\text{g/mL}$ ) was used to be immobilized on the same weight of MNPs. Siglec-2-Fc@MNP complexes obtained from different methods were incubated first with biotinylated sialoside **1** and then Streptavidin-Cy3 followed by treatment with GdnHCl. The fluorescence signal of the supernatants were measured.



**Figure S5.** Standard curve of BSA quantified by BCA assay. The linear calibration curve of protein was observed at 562 nm. To quantify the protein amount on MNP, BSA

standard protein and the anti-SAA mAb@MNP samples were prepared and reacted with BCA reagents at 60°C for 30 min. The quantification curve was obtained by various amount of BSA (0-3 µg/µL). The MNPs were separated by a magnet and the supernatant was used for measurement of absorbance at 562 nm.

### **Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Analysis**

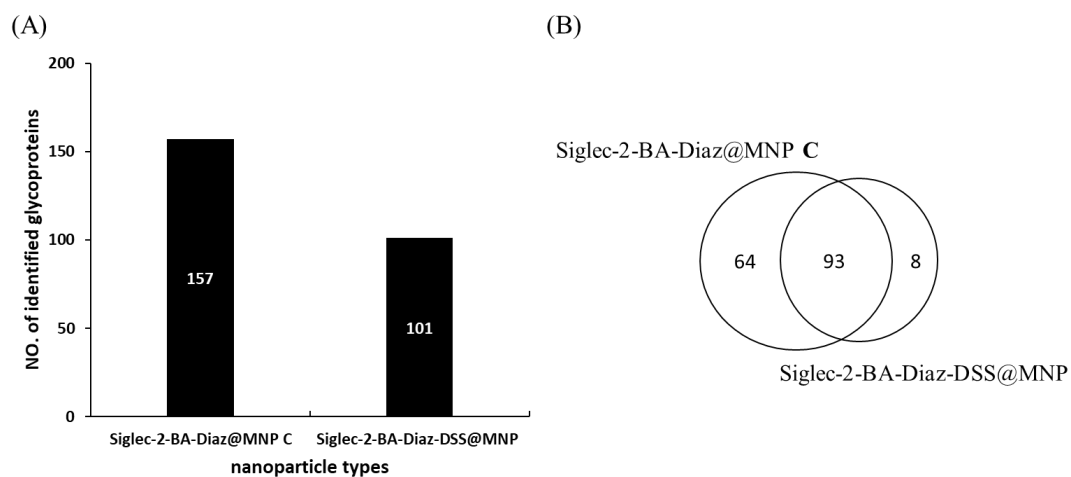
**LC-MS/MS Method.** Enriched glycopeptides from BA-Diaz@MNPs and randomly conjugated Siglec-2 MNPs (R@MNPs and R120A@MNPs) were analyzed on a nanoACQUITY UPLC system (Waters, Milford, MA) coupled with a Synapt G1 Q-ToF mass spectrometer (Waters) with a nanospray ion source. The LC mobile phase system consists of water with 0.1% formic acid (buffer A) and acetonitrile with 0.1% formic acid (buffer B). The peptides were eluted with a gradient of 0-80% buffer B (0.1% FA in ACN) for 120 min, operated in ESI-positive V mode. Data acquisition was done by data-dependent acquisition mode to automatically switch between a full MS scan (400-1600 m/z, 0.6 s.) and six MS/MS scans (100-1990 m/z, 0.6 s. for each scan) on the six most intense ions present. For comparison of enrichment efficiency of Siglec-2-BA-Diaz@MNP C and Siglec-2-BA-Diaz-DSS@MNP, LC-MS/MS analysis of MNP-enriched glycopeptides was performed on linear trap quadrupole (LTQ)-Orbitrap Velos (Thermo Fisher Scientific, Bremen, Germany) coupled to a nanoACQUITY UPLC System (Waters) through a nanospray interface (Proxeon, Odense, Denmark). The deglycosylated peptides were separated using a segmented gradient for 150-min segmented gradient with the following mobile phases: water with 0.1% formic acid (buffer A) and 2% to 80% acetonitrile with 0.1% formic acid (buffer B) at 500 nL/min flow rate. The electrospray voltage was 1.8 kV, and the ion transfer tube temperature was 300°C. Collision-induced dissociation (CID) mode using a top20 method was used,

and full MS scans were acquired in the Orbitrap mass analyzer over the  $m/z$  350–1600 range with resolution 60,000 ( $m/z$  400). The target value was 5.00E+05. The twenty-most intense peaks with charge state 2 to 4 were selected for sequencing and fragmented in the ion trap with normalized collision energy of 35%, activation  $q = 0.25$ , activation time of 10 ms, and one microscan. The exclusion duration is 60 sec. The target value was 2000 and the isolation width is 2 Da. The maximum allowed ion accumulation times was 500 ms for full scans and 100 ms for CID.

**Protein Identification.** The raw data were processed using Proteome Discoverer 2.1 (PD2.1; ThermoFisher Scientific) and peptide identification was performed by Mascot search engine (version 2.3.2) and SEQUEST search engine against the Swiss-Prot database (v2015\_12, total 20193 sequences from human). Percolator (strict false discovery rate (FDR) of 0.01 and a relaxed FDR of 0.05) was set and the mass tolerance for precursor ion mass was 10 ppm with the fragment ion tolerance as 0.1 Da. The following parameters were used: protease specified as trypsin, maximum missed cleavage set to two, variable modifications selected were methionine oxidation, cysteine alkylation by iodoacetamide (carbamidomethyl), and deamidation at asparagine or glutamine. Peptides were considered identified if their individual ion score was higher than the identity score ( $p < 0.05$ ). A decoy database search against a randomized decoy database created by PD2.1 was performed to evaluate the FDR ( $< 1\%$ ) in protein identification. Peptide-spectrum matches (PSMs) with at least high confidence and a strict maximum parsimony principle (target FDR  $< 0.01$ ) were applied for the protein level. Precursor Ions Area Detector node was used to extract the ion chromatogram for label-free quantitation. The peak area (i.e., area under the curve) of each precursor ion was obtained and the abundance of identified protein was calculated from the top three of all unique and razor peptides in Peptide and Protein Quantifier

node.

**Identified glycoproteins enriched by Siglec-2-BA-Diaz@MNP C and Siglec-2-BA-Diaz-DSS@MNP.** The enriched proteins, obtained from single biological replicate, were analyzed by the above methods. Using label-free quantification to compare the enriched glycoproteins, 36 proteins showed higher than 5-fold abundance in Siglec-2-BA-Diaz@MNP C in comparison with Siglec-2-BA-Diaz-DSS@MNP. The specificities of the materials, calculated as the ratio of glycoproteins over total proteins identified, are 20% and 24% for BA-Diaz@MNP C and BA-Diaz-DSS@MNP, respectively. The proteins commonly found from both MNP enrichments were then further analyzed by STRING database to create the CD-22 (Siglec-2) interacting network. For the protein signaling network analysis, the glycoproteins were analyzed using STRING version 11 (<http://string-db.org/>). Multiple protein names were inputted into String, the organism was set as Homo sapiens, and prediction methods included the following: neighborhood, gene fusion, cooccurrence, coexpression, experiments, databases, textmining and homology. Median confidence (0.4) was used, shown interactors was set as “custom limit=0”, and the additional (white) nodes was set as 0. A signaling network composed of predicted or known protein interactions was then obtained and Cytoscape was used to redraw the protein network to show only CD-22 interacting proteins and secondary interactions (interactions with CD-22 interacting proteins).



**Figure S6.** Glycoproteins and peptides identification were analyzed by Thermo LTQ Orbitrap Velos. (a) Identified glycoproteins from Siglec-2-BA-Diaz@MNP C and Siglec-2-BA-Diaz-DSS@MNP. (b) Overlap of identified glycoproteins from the two MNPs.

**Table S1.** Proteins identified with BA-Diaz@MNP enrichment on BJAB cells.

Acc. No.	Gene	Description	Score	#	emP	Iden-
			Mascot	Unique	AI	tified
				Peptides		in Ref
P20273	CD22 *	B-cell receptor CD22	1778	29	15.24	v
P01903	HLA-DRA*	HLA class II histocompatibility antigen, DR alpha chain	645	11	25.83	v
P04233	CD74	HLA class II histocompatibility antigen gamma chain	589	4	0.97	
Q95IE3	HLA-DRB1*	HLA class II histocompatibility antigen, DRB1-12 beta chain	513	5	12.34	v
Q30134	HLA-DRB1	HLA class II histocompatibility antigen, DRB1-8 beta chain	480	1	5.49	
Q5Y7A7	HLA-DRB1	HLA class II histocompatibility antigen, DRB1-13 beta chain	466	3	14.40	
P01860	IGHG3	Immunoglobulin heavy constant gamma 3	437	1	4.28	
P25705	ATP5A1	ATP synthase subunit alpha, mitochondrial	433	11	1.07	
P30461	HLA-B*	HLA class I histocompatibility antigen, B- 13 alpha chain	346	3	1.24	v
P04843	RPN1	Dolichyl-diphosphooligosaccharide-- protein glycosyltransferase subunit 1	325	11	1.08	
P79483	HLA-DRB3	HLA class II histocompatibility antigen, DR beta 3 chain	323	2	1.96	
P08195	SLC3A2*	4F2 cell-surface antigen heavy chain	305	11	1.41	v
P04440	HLA-DPB1*	HLA class II histocompatibility antigen, DP beta 1 chain	274	2	0.93	v
Q9GIY3	HLA-DRB1*	HLA class II histocompatibility antigen, DRB1-14 beta chain	258	1	9.00	v
P35613	BSG	Basigin OS=Homo sapiens	229	4	0.59	
P05107	ITGB2*	Integrin beta-2	209	13	1.02	v
P08575	PTPRC*	Receptor-type tyrosine-protein phosphatase C	195	9	0.33	v
P17693	HLA-G	HLA class I histocompatibility antigen, alpha chain G	167	1	0.39	
P04844	RPN2	Dolichyl-diphosphooligosaccharide-- protein glycosyltransferase subunit 2	157	4	0.35	
O00754	MAN2B1	Lysosomal alpha-mannosidase	133	3	0.12	
P02748	C9	Complement component C9	131	2	0.16	
P14625	HSP90B1	Endoplasmic OS=Homo sapiens	130	4	0.25	
P01920	HLA- DQB1*	HLA class II histocompatibility antigen, DQ beta 1 chain	123	7	2.16	v
P49755	TMED10	Transmembrane emp24 domain-containing protein 10	108	3	0.70	

P08238	HSP90AB1	Heat shock protein HSP 90-beta	108	6	0.46	
P02042	HBD	Hemoglobin subunit delta	106	1	0.23	
Q12907	LMAN2	Vesicular integral-membrane protein VIP36	94	7	1.68	
Q13724	MOGS	Mannosyl-oligosaccharide glucosidase	77	3	0.23	
P01909	HLA-DQA1	HLA class II histocompatibility antigen, DQ alpha 1 chain	73	4	1.31	
P13473	LAMP2	Lysosome-associated membrane glycoprotein 2	70	2	0.23	
Q9NR96	TLR9	Toll-like receptor 9	66	2	0.08	
P23284	PPIB	Peptidyl-prolyl cis-trans isomerase B	63	1	0.16	
Q14165	MLEC	Malectin	61	1	0.12	
P02786	TFRC*	Transferrin receptor protein 1	58	6	0.31	v
P13987	CD59	CD59 glycoprotein	55	1	0.39	
P54709	ATP1B3	Sodium/potassium-transporting ATPase subunit beta-3	54	3	1.15	
P15153	RAC2	Ras-related C3 botulinum toxin substrate 2	48	3	1.00	
P69905	HBA1	Hemoglobin subunit alpha	45	3	2.16	
Q6NUQ4	TMEM214	Transmembrane protein 214	42	2	0.13	
P11166	SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	37	1	0.15	
P43307	SSR1	Translocon-associated protein subunit alpha	37	2	0.47	
Q08380	LGALS3BP	Galectin-3-binding protein	37	4	0.32	
P28288	ABCD3	ATP-binding cassette sub-family D member 3	35	2	0.11	
Q13586	STIM1	Stromal interaction molecule 1	35	3	0.20	
P42081	CD86	T-lymphocyte activation antigen CD86	34	1	0.15	
P43308	SSR2	Translocon-associated protein subunit beta	33	2	1.15	
Q96KA5	CLPTM1L	Cleft lip and palate transmembrane protein 1-like protein	32	2	0.39	
P27797	CALR	Calreticulin	32	3	0.30	
P40926	MDH2	Malate dehydrogenase, mitochondrial	32	4	0.45	
Q92896	GLG1	Golgi apparatus protein 1	27	2	0.07	
P08134	RHOC	Rho-related GTP-binding protein RhoC	27	2	0.52	
P20645	M6PR	Cation-dependent mannose-6-phosphate receptor	17	1	0.13	
P62937	PPIA	Peptidyl-prolyl cis-trans isomerase A	0	4	0.93	

P06576	ATP5B	ATP synthase subunit beta, mitochondrial	0	9	0.95	
P60953	CDC42	Cell division control protein 42 homolog	0	2	1.00	
P32970	CD70	CD70 antigen	0	3	0.70	
P11049	CD37*	Leukocyte antigen CD37	0	1	0.17	v
P11717	IGF2R	Cation-independent mannose-6-phosphate receptor	0	3	0.05	
Q15758	SLC1A5*	Neutral amino acid transporter B(0)	0	1	0.11	v

**Table S2.** Glycoproteins identified in BJAB cells from both Siglec-2-BA-Diaz@MNP C and Siglec-2-BA-Diaz-DSS@MNP enrichment.

Accession	Gene	Description	Mascot Score	# unique peptide	emPAI	MW [kDa]
P01857	IGHG1	Immunoglobulin heavy constant gamma 1	12304	8	397.11	36.08
P20273	CD22	B-cell receptor CD22	11589	24	20.98	95.29
P01903	HLA-DRA	HLA class II histocompatibility antigen, DR alpha chain	10884	9	116.88	28.59
Q5Y7A7	HLA-DRB1	HLA class II histocompatibility antigen, DRB1-13 beta chain	6528	2	63.94	29.99
Q95IE3	HLA-DRB1	HLA class II histocompatibility antigen, DRB1-12 beta chain	6371	2	55.23	29.86
P20039	HLA-DRB1	HLA class II histocompatibility antigen, DRB1-11 beta chain	5652	1	41.17	30.14
Q30134	HLA-DRB1	HLA class II histocompatibility antigen, DRB1-8 beta chain	5509	1	26.38	29.99
Q9GIY3	HLA-DRB1	HLA class II histocompatibility antigen, DRB1-14 beta chain	4729	1	14.85	30.12
P79483	HLA-DRB3	HLA class II histocompatibility antigen, DR beta 3 chain	4632	4	28.55	29.94



P04233	CD74	HLA class II histocompatibility antigen gamma chain	4420	10	86.33	33.49
P35613	BSG	Basigin	4287	10	6.94	42.17
P25705	ATP5A1	ATP synthase subunit alpha, mitochondrial	4119	17	2.79	59.71
P05107	ITGB2	Integrin beta-2	4088	29	4.18	84.73
P08195	SLC3A2	4F2 cell-surface antigen heavy chain	3147	18	3.15	67.95
P10316	HLA-A	HLA class I histocompatibility antigen, A-69 alpha chain	3081	7	11.22	40.95
P06576	ATP5B P	ATP synthase subunit beta, mitochondrial	3079	21	10.60	56.53
Q08380	LGALS3BP	Galectin-3-binding protein	2932	17	3.98	65.29
P01920	HLA-DQB1	HLA class II histocompatibility antigen, DQ beta 1 chain	2441	10	12.90	29.97
P10319	HLA-B	HLA class I histocompatibility antigen, B-58 alpha chain	2415	1	3.47	40.31
P30443	HLA-A	HLA class I histocompatibility antigen, A-1 alpha chain	2097	4	5.70	40.82
Q13740	ALCAM	CD166 antigen	1842	12	2.16	65.06
P30504	HLA-C	HLA class I histocompatibility antigen, Cw-4 alpha chain	1829	1	2.79	40.97
P15391	CD19	B-lymphocyte antigen CD19	1567	8	1.22	61.09
P20036	HLA-DPA1	HLA class II histocompatibility antigen, DP alpha 1 chain	1414	5	10.94	29.36
P07602	PSAP	Prosaposin	1347	8	1.11	58.07
Q13586	STIM1	Stromal interaction molecule 1	1322	15	2.57	77.38
P05556	ITGB1	Integrin beta-1	1305	12	1.75	88.36
P13473	LAMP2	Lysosome-associated membrane glycoprotein 2	1239	6	1.31	44.93
P04440	HLA-DPB1	HLA class II histocompatibility antigen, DP beta 1 chain	1195	5	2.73	29.14
Q9BVK6	TMED9	Transmembrane emp24 domain-containing protein 9	1171	7	4.62	27.26

P08575	PTPRC	Receptor-type tyrosine-protein phosphatase C	1134	18	0.94	147.16
P25942	CD40	Tumor necrosis factor receptor superfamily member 5	1093	8	7.48	30.60
P43307	SSR1	Translocon-associated protein subunit alpha	1077	4	3.64	32.22
P08238	HSP90AB1	Heat shock protein HSP 90-beta	1071	10	2.08	83.21
P02786	TFRC	Transferrin receptor protein 1	1021	9	0.50	84.82
P49755	TMED10	Transmembrane emp24 domain-containing protein 10	1014	4	1.89	24.96
P04222	HLA-C	HLA class I histocompatibility antigen, Cw-3 alpha chain	948	1	2.36	40.84
P60953	CDC42	Cell division control protein 42 homolog	890	5	2.98	21.25
Q92542	NCSTN	Nicastrin	833	5	1.02	78.36
P32942	ICAM3	Intercellular adhesion molecule 3	809	6	0.61	59.50
O00461	GOLIM4	Golgi integral membrane protein 4	780	12	1.15	81.83
P04843	RPN1	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	769	15	1.46	68.53
P13987	CD59	CD59 glycoprotein	689	5	12.90	14.17
Q86UP2	KTN1	Kinectin	685	18	0.66	156.18
P13598	ICAM2	Intercellular adhesion molecule 2	675	4	0.90	30.64
P05362	ICAM1	Intercellular adhesion molecule 1	673	7	0.71	57.79
P14625	HSP90B1	Endoplasmin	659	7	0.50	92.41
P23284	PPIB	Peptidyl-prolyl cis-trans isomerase B	623	6	1.74	23.73
Q92896	GLG1	Golgi apparatus protein 1	595	9	0.38	134.46
P02748	C9	Complement component C9	592	2	0.25	63.13
P54709	ATP1B3	Sodium/potassium-transporting ATPase subunit beta-3	554	6	1.93	31.49

P09326	CD48	CD48 antigen	540	5	1.42	27.67
P62937	PPIA	Peptidyl-prolyl cis-trans isomerase A	523	5	1.28	18.00
P11279	LAMP1	Lysosome-associated membrane glycoprotein 1	519	4	0.65	44.85
O43493	TGOLN2	Trans-Golgi network integral membrane protein 2	514	5	0.47	51.08
P19440	GGT1	Gamma- glutamyltranspeptidase 1	509	3	0.33	61.37
P20138	CD33	Myeloid cell surface antigen CD33	506	3	0.47	39.80
Q12907	LMAN2	Vesicular integral-membrane protein VIP36	504	9	3.16	40.20
P05538	HLA-DQB2	HLA class II histocompatibility antigen, DQ beta 2 chain	502	1	0.85	30.37
Q70UQ0	IKBIP	Inhibitor of nuclear factor kappa-B kinase-interacting protein	491	5	0.87	39.29
P61586	RHOA	Transforming protein RhoA	458	7	7.38	21.75
Q9BY67	CADM1	Cell adhesion molecule 1	448	3	0.47	48.48
P27797	CALR	Calreticulin	430	9	2.46	48.11
Q9BUR5	APOO	MICOS complex subunit MIC26	414	3	0.70	22.27
Q96A33	CCDC47	Coiled-coil domain-containing protein 47	412	6	1.02	55.84
Q14165	MLEC	Malectin	410	3	0.59	32.21
Q7Z7H5	TMED4	Transmembrane emp24 domain-containing protein 4	397	4	1.51	25.93
Q09328	MGAT5	Alpha-1,6- mannosylglycoprotein 6-beta- N- acetylglucosaminyltransferase A	386	1	0.06	84.49
Q96EE4	CCDC126	Coiled-coil domain-containing protein 126	385	1	0.29	15.66
Q9Y3P8	SIT1	Signaling threshold-regulating transmembrane adapter 1	360	1	0.23	21.11

P15153	RAC2	Ras-related C3 botulinum toxin substrate 2	347	3	2.16	21.42
P02042	HBD	Hemoglobin subunit delta	324	2	0.87	16.05
P43121	MCAM	Cell surface glycoprotein MUC18	312	3	0.28	71.56
P69905	HBA1	Hemoglobin subunit alpha	294	4	9.00	15.25
P25445	FAS	Tumor necrosis factor receptor superfamily member 6	280	3	0.41	37.71
P61009	SPCS3	Signal peptidase complex subunit 3	268	3	1.37	20.30
Q13291	SLAMF1	Signaling lymphocytic activation molecule	239	3	0.47	37.21
P12259	F5	Coagulation factor V	186	3	0.07	251.55
Q9HDC9	APMAP	Adipocyte plasma membrane-associated protein	182	5	0.59	46.45
P02649	APOE	Apolipoprotein E	162	2	0.20	36.13
P35443	THBS4	Thrombospondin-4	124	1	0.05	105.80
P28068	HLA-DMB	HLA class II histocompatibility antigen, DM beta chain	119	3	0.93	28.92
Q96PC5	MIA2	Melanoma inhibitory activity protein 2	95	3	0.08	159.74
O75063	FAM20B	Glycosaminoglycan xylosylkinase	84	1	0.30	46.40
Q5UCC4	EMC10	ER membrane protein complex subunit 10	63	2	0.43	27.33
Q9Y639	NPTN	Neuroplastin	49	2	0.21	44.36
Q96RQ9	IL4I1	L-amino-acid oxidase	36	2	0.22	62.84
Q96DU3	SLAMF6	SLAM family member 6	23	1	0.12	37.32
Q8WUT4	LRRN4	Leucine-rich repeat neuronal protein 4	17	1	0.07	78.79
Q96LC7	SIGLEC10	Sialic acid-binding Ig-like lectin 10	0	1	0.14	76.54

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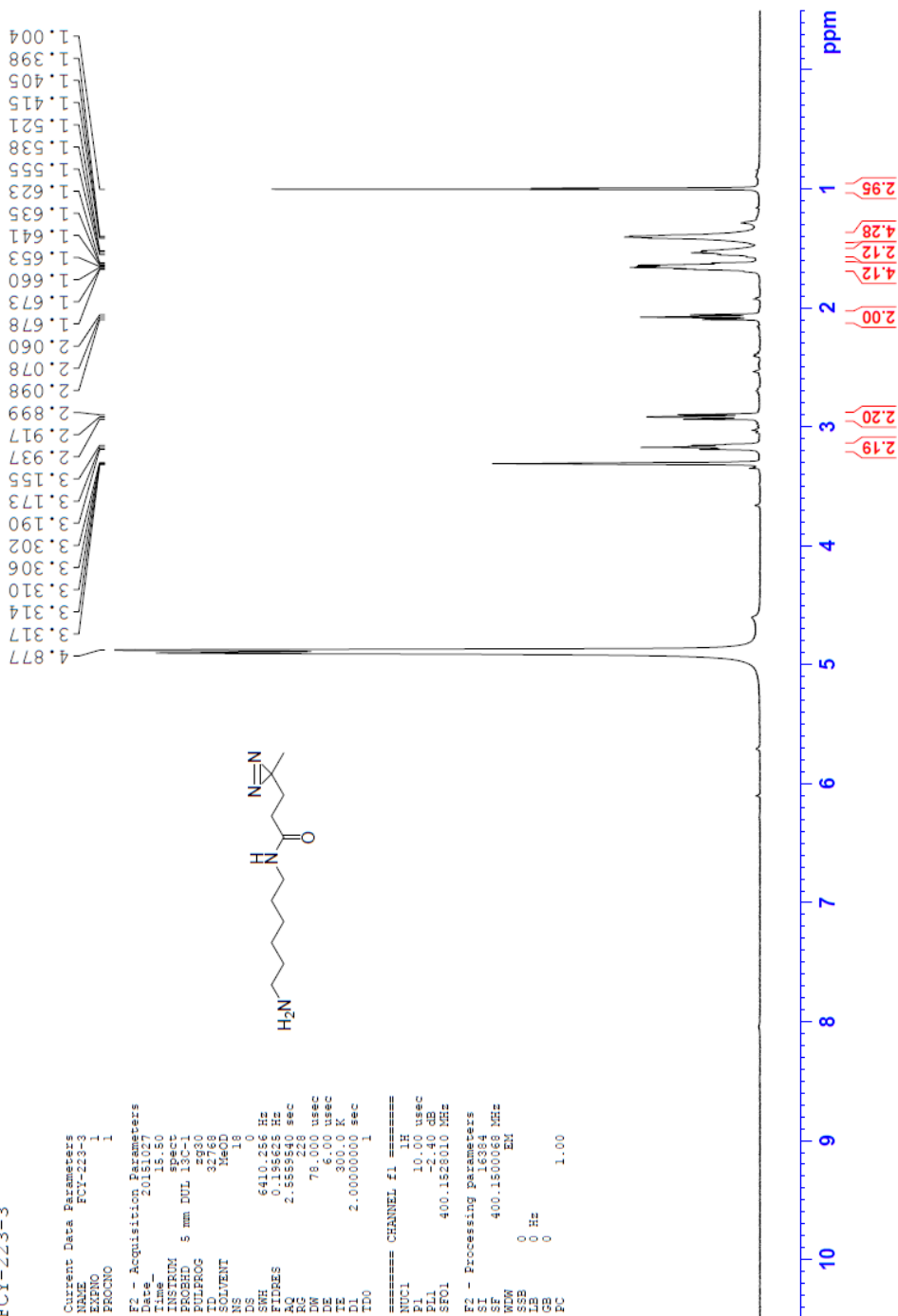
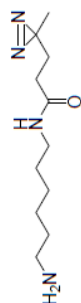
**Table S3.** Glycoproteins identified in BJAB cells with  $\geq 5$  abundance in Siglec-2-BA-Diaz@MNP C than Siglec-2-BA-Diaz-DSS@MNP

Acc. No.	Gene	Description	Mascot Score	Unique Pep-tides	emPAI	Ratio: Siglec-2- BA-Diaz@MNP C / Siglec-2-BA- Diaz- DSS@MNP
P20273	CD22	B-cell receptor CD22	11589	24	20.98	6.25
						6.64
P01903	HLA-DRA	HLA class II histocompatibility antigen	10884	9	116.88	6.31
P20039	HLA-DRB1	HLA class II histocompatibility antigen	5652	1	41.17	7.37
P08195	SLC3A2	4F2 cell-surface antigen heavy chain	3147	18	3.15	22.86
P06576	ATP5B	ATP synthase subunit beta, mitochondrial	3079	21	10.60	6.55
		HLA class I histocompatibility antigen, A-1 alpha				
P30443	HLA-A	chain	2097	4	5.70	
P15391	CD19	B-lymphocyte antigen CD19	1567	8	1.22	5.00
		HLA class II histocompatibility antigen, DP alpha 1				6.00
P20036	HLA-DPA1	chain	1414	5	10.94	
Q13586	STIM1	Stromal interaction molecule 1	1322	15	2.57	10.77
						5.74
P13473	LAMP2	Lysosome-associated membrane glycoprotein 2	1239	6	1.31	8.25
		HLA class II histocompatibility antigen, DP beta 1				
P04440	HLA-DPB1	chain	1195	5	2.73	10.89
P08575	PTPRC	Receptor-type tyrosine-protein phosphatase C	1134	18	0.94	12.94
P08238	HSP90AB1	Heat shock protein HSP 90-beta	1071	10	2.08	
P02786	TFRC	Transferrin receptor protein 1	1021	9	0.50	5.57
		HLA class I histocompatibility antigen, Cw-3 alpha				6.86
P04222	HLA-C	chain	948	1	2.36	
Q92542	NCSTN	Nicastrin	833	5	1.02	8.04
						9.00
		Dolichyl-diphosphooligosaccharide--protein				
P04843	RPN1	glycosyltransferase subunit 1	769	15	1.46	
Q86UP2	KTN1	Kinectin	685	18	0.66	9.11

P14625	HSP90B1	Endoplasmin	659	7	0.50	7.74
P02748	C9	Complement component C9	592	2	0.25	13.00
		Sodium/potassium-transporting ATPase subunit				6.38
P54709	ATP1B3	beta-3	554	6	1.93	
P09326	CD48	CD48 antigen	540	5	1.42	8.70
						5.29
P62937	PPIA	Peptidyl-prolyl cis-trans isomerase A	523	5	1.28	
P19440	GGT1	Gamma-glutamyltranspeptidase 1	509	3	0.33	5.64
		HLA class II histocompatibility antigen, DQ beta 2				5.11
P05538	HLA-DQB2	chain	502	1	0.85	
P61586	RHOA	Transforming protein RhoA	458	7	7.38	229.63
Q9BY67	CADM1	Cell adhesion molecule 1	448	3	0.47	7.06
P27797	CALR	Calreticulin	430	9	2.46	20.00
Q14165	MLEC	Malectin	410	3	0.59	17.14
P69905	HBA1	Hemoglobin subunit alpha	294	4	9.00	6.09
						6.59
Q13291	SLAMF1	Signaling lymphocytic activation molecule	239	3	0.47	
P02649	APOE	Apolipoprotein E	162	2	0.20	5.67
		HLA class II histocompatibility antigen, DM beta				5.50
P28068	HLA-DMB	chain	119	3	0.93	
						5.00
Q96PC5	MIA2	Melanoma inhibitory activity protein 2	95	3	0.08	
Q96DU3	SLAMF6	SLAM family member 6	23	1	0.12	5.60
Q96LC7	SIGLEC10	Sialic acid-binding Ig-like lectin 10	0	1	0.14	5.42

FCY-223-3

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 FWHM 12.256 Hz  
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FCY-223-C

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SFO70 400.146000 MHz  
===== CHANNEL f71 =====  
NUC71 1H  
P71 1.00 usec  
PL71 0.00 dB  
SFO71 400.146000 MHz  
===== CHANNEL f72 =====  
NUC72 1H  
P72 1.00 usec  
PL72 0.00 dB  
SFO72 400.146000 MHz  
===== CHANNEL f73 =====  
NUC73 1H  
P73 1.00 usec  
PL73 0.00 dB  
SFO73 400.146000 MHz  
===== CHANNEL f74 =====  
NUC74 1H  
P74 1.00 usec  
PL74 0.00 dB  
SFO74 400.146000 MHz  
===== CHANNEL f75 =====  
NUC75 1H  
P75 1.00 usec  
PL75 0.00 dB  
SFO75 400.146000 MHz  
===== CHANNEL f76 =====  
NUC76 1H  
P76 1.00 usec  
PL76 0.00 dB  
SFO76 400.146000 MHz  
===== CHANNEL f77 =====  
NUC77 1H  
P77 1.00 usec  
PL77 0.00 dB  
SFO77 400.146000 MHz  
===== CHANNEL f78 =====  
NUC78 1H  
P78 1.00 usec  
PL78 0.00 dB  
SFO78 400.146000 MHz  
===== CHANNEL f79 =====  
NUC79 1H  
P79 1.00 usec  
PL79 0.00 dB  
SFO79 400.146000 MHz  
===== CHANNEL f80 =====  
NUC80 1H  
P80 1.00 usec  
PL80 0.00 dB  
SFO80 400.146000 MHz  
===== CHANNEL f81 =====  
NUC81 1H  
P81 1.00 usec  
PL81 0.00 dB  
SFO81 400.146000 MHz  
===== CHANNEL f82 =====  
NUC82 1H  
P82 1.00 usec  
PL82 0.00 dB  
SFO82 400.146000 MHz  
===== CHANNEL f83 =====  
NUC83 1H  
P83 1.00 usec  
PL83 0.00 dB  
SFO83 400.146000 MHz  
===== CHANNEL f84 =====  
NUC84 1H  
P84 1.00 usec  
PL84 0.00 dB  
SFO84 400.146000 MHz  
===== CHANNEL f85 =====  
NUC85 1H  
P85 1.00 usec  
PL85 0.00 dB  
SFO85 400.146000 MHz  
===== CHANNEL f86 =====  
NUC86 1H  
P86 1.00 usec  
PL86 0.00 dB  
SFO86 400.146000 MHz  
===== CHANNEL f87 =====  
NUC87 1H  
P87 1.00 usec  
PL87 0.00 dB  
SFO87 400.146000 MHz  
===== CHANNEL f88 =====  
NUC88 1H  
P88 1.00 usec  
PL88 0.00 dB  
SFO88 400.146000 MHz  
===== CHANNEL f89 =====  
NUC89 1H  
P89 1.00 usec  
PL89 0.00 dB  
SFO89 400.146000 MHz  
===== CHANNEL f90 =====  
NUC90 1H  
P90 1.00 usec  
PL90 0.00 dB  
SFO90 400.146000 MHz  
===== CHANNEL f91 =====  
NUC91 1H  
P91 1.00 usec  
PL91 0.00 dB  
SFO91 400.146000 MHz  
===== CHANNEL f92 =====  
NUC92 1H  
P92 1.00 usec  
PL92 0.00 dB  
SFO92 400.146000 MHz  
===== CHANNEL f93 =====  
NUC93 1H  
P93 1.00 usec  
PL93 0.00 dB  
SFO93 400.146000 MHz  
===== CHANNEL f94 =====  
NUC94 1H  
P94 1.00 usec  
PL94 0.00 dB  
SFO94 400.146000 MHz  
===== CHANNEL f95 =====  
NUC95 1H  
P95 1.00 usec  
PL95 0.00 dB  
SFO95 400.146000 MHz  
===== CHANNEL f96 =====  
NUC96 1H  
P96 1.00 usec  
PL96 0.00 dB  
SFO96 400.146000 MHz  
===== CHANNEL f97 =====  
NUC97 1H  
P97 1.00 usec  
PL97 0.00 dB  
SFO97 400.146000 MHz  
===== CHANNEL f98 =====  
NUC98 1H  
P98 1.00 usec  
PL98 0.00 dB  
SFO98 400.146000 MHz  
===== CHANNEL f99 =====  
NUC99 1H  
P99 1.00 usec  
PL99 0.00 dB  
SFO99 400.146000 MHz  
===== CHANNEL f100 =====  
NUC100 1H  
P100 1.00 usec  
PL100 0.00 dB  
SFO100 400.146000 MHz



190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

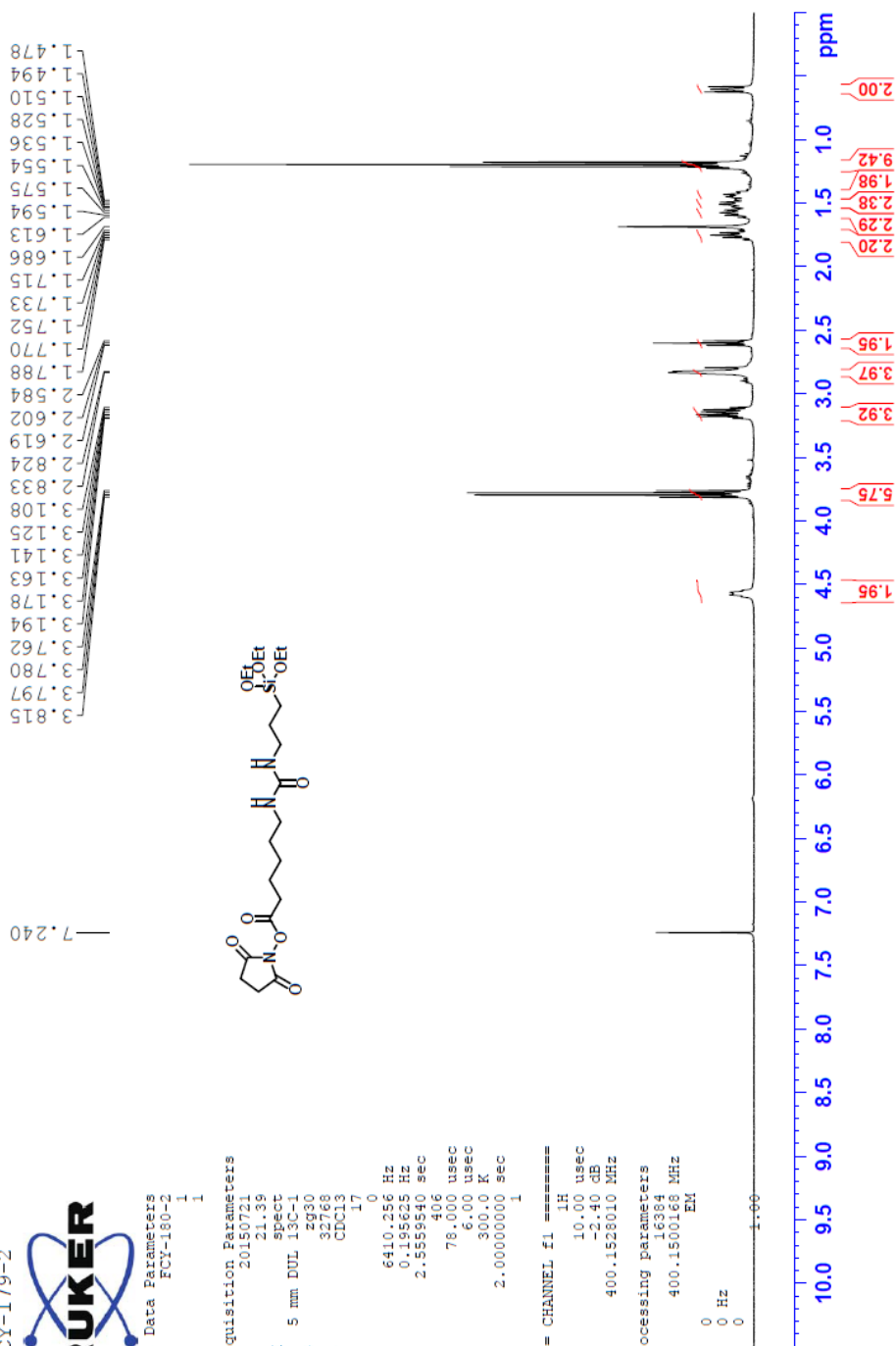
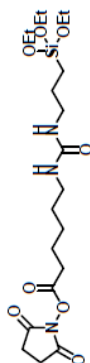


F2 - Acquisition Parameters	
Date_	2050721
Time	21.39
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
ID	32168
SOLVENT	CDCl3
NS	17
DS	0
RG	640.356 Hz
RGH	0.95625 Hz
FIDRES	0.1559540 sec
AQ	RG 406
RG	406
RGH	78.000 usec
RDW	6.00 usec
DE	300.0 K
TE	2.00000000 sec
DD1	
ID0	

```

===== CHANNEL f1 =====
NUC1
P1      10.00 usec
C1      -3.474
SF01    400.152810 MHz
=====
E2 - Processing parameters
SI      1.3984
SF      400.150168 MHz
EM
NDW
SSB     0
LB      0 Hz
GB      0
PC      1.00
=====

```

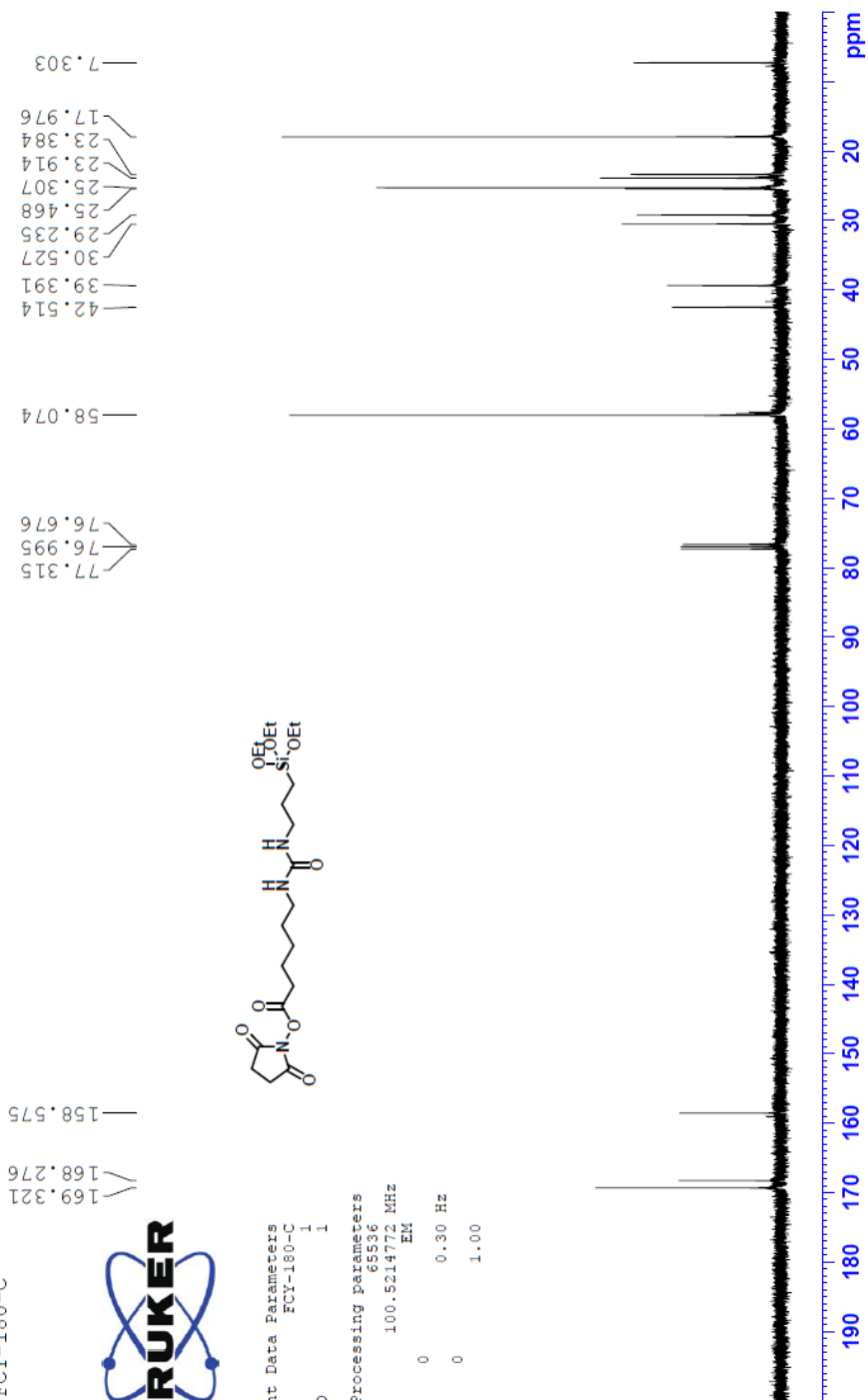
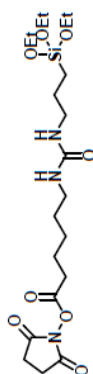


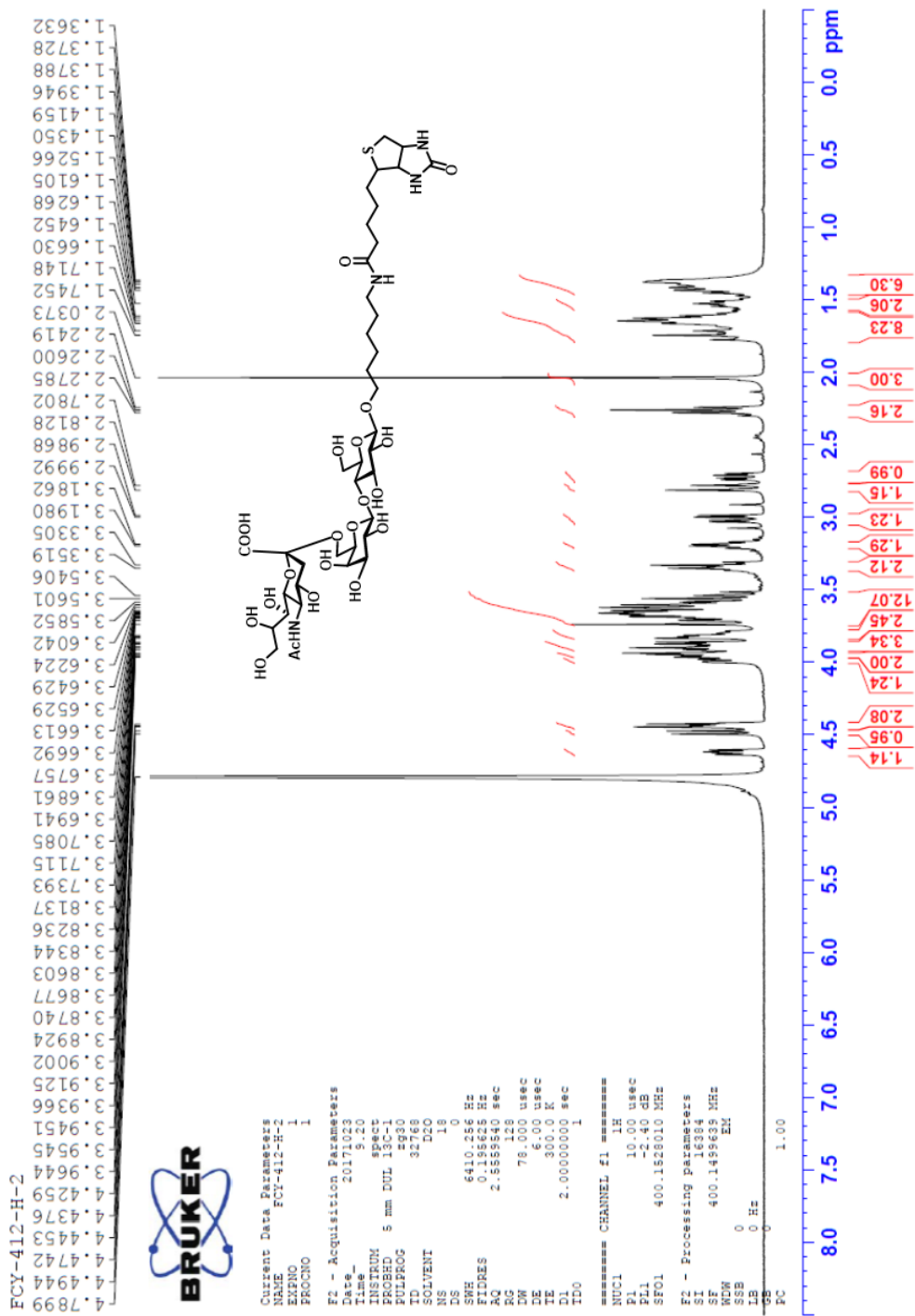
FCY-180-C

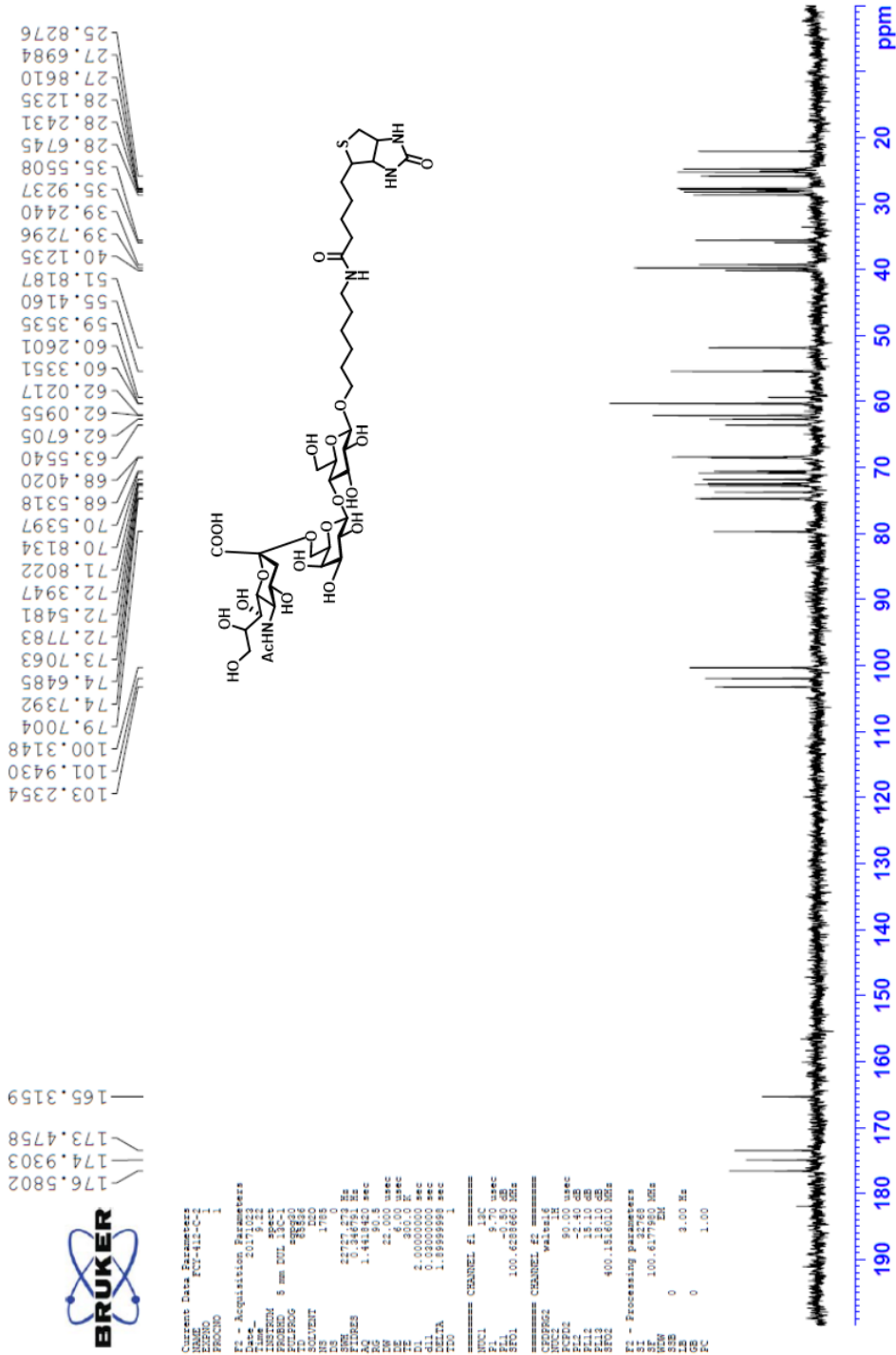


Current Data Parameters  
NAME FCY-180-C  
EXPNO 1  
PROCNO 1

F2 - Processing Parameters  
SI 65836  
SF 100.521472 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00







Current Data Parameters  
 EXPNO 1  
 PROCNO 1  
 F2 - Acquisition Parameters  
 Date\_ 20171023  
 Time 8:12  
 INSTRUM spect  
 PRGNAME 8 mm BBO 1D-1  
 F2 - Processing Parameters  
 SI 32768  
 SF 400.1416010 MHz  
 D1 2.0000000 sec  
 d11 0.0000000 sec  
 DELTA 1.8999999 sec  
 DEC 1  
 ===== CHANNEL f1 =====  
 NUCL1 13C  
 P1 9.70 usec  
 PL1 0.00 dB  
 SFO1 100.625000 MHz  
 ===== CHANNEL f2 =====  
 NUC2 1H  
 P2 9.00 usec  
 PL2 0.00 dB  
 SFO2 400.1416010 MHz  
 F2 - Processing Parameters  
 SI 32768  
 SF 400.1416010 MHz  
 D1 2.0000000 sec  
 d11 0.0000000 sec  
 DELTA 1.8999999 sec  
 DEC 1

Ref:

1. Tohru Kambe, Bruno E. Correia, Micah J. Niphakis, Benjamin F. Cravatt *J. Am. Chem. Soc.* **2014**, *136*, 10777–10782.
2. Hai Yu, Shengshu Huang, Harshal Chokhawala, Mingchi Sun, Haojie Zheng, Xi Chen *Angew. Chem. Int. Ed.* **2006**, *45*, 3938 –3944.