Supporting Information

Design of S-S Bond Containing Maleimide-Conjugated *closo*-Dodecaborate (SSMID): Identification of Its Unique Modification Sites on Albumin and Investigation of Intracellular Uptake

Satomu Ishii,[a,b] Shinichi Sato,[a] Hiroya Asami,[c] Tomoko Hasegawa,[c] Jun-ya Kohno[c] and Hiroyuki Nakamura[a,*]

[a] *Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, 4259, Nagatsuta-cho, Midori-ku, Yokohama, 226-8503, Japan.*

[b] *School of Life Science and Technology, Tokyo Institute of Technology, 4259, Nagatsuta-cho, Midori-ku, Yokohama, 226-8503, Japan.*

[c] *Department of Chemistry, Facility of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588, Japan.*

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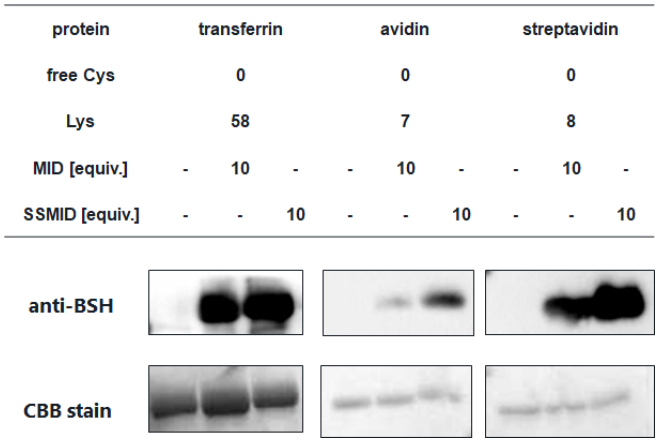
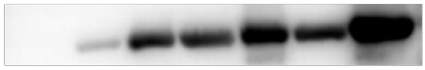
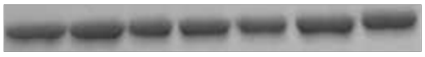


Figure S1. Modification of transferrin, avidin and streptavidin by MID or SSMID.

(A)

Lane	1	2	3	4	5	6	7
MID [mM]	-	1	10	-	-	-	-
SSMID [mM]	-	-	-	1	10	1	10
Reaction time [h]	-	2	2	2	2	12	12
anti-BSH antibody							
CBB stain							
Relative intensity (reference MID = 1)	-	0.18	1.0	1.21	1.79	1.39	3.98

(B)

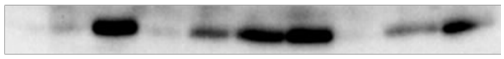
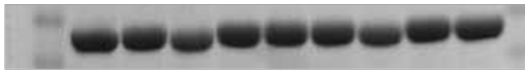
Lane	1	2	3	4	5	6	7	8	9
MID [mM]	-	1	1	1	1	-	-	-	-
SSMID [mM]	-	-	-	-	-	1	1	1	1
NAS [mM]	-	-	10	-	-	-	10	-	-
NEM [mM]	-	-	-	10	-	-	-	10	-
IAA [mM]	-	-	-	-	10	-	-	-	10
anti-BSH antibody									
CBB stain									
Relative intensity (reference MID = 1)	-	1	0	0.22	0.54	0.84	0	0.10	0.69

Figure S2. Western blot analysis of MID (**1**) and SSMID (**2**) conjugation to BSA. (A) Concentration- and reaction time-dependent conjugation efficacy; (B) Conjugation efficacy of MID and SSMID to BSA pretreated with capping agents: *N*-acetyl succinimide (NAS), *N*-ethylmaleimide (NEM), and 2-iodoacetamide (IAA). BSA (1 mM) was used for all experiments.

MASCOT Search Results

Protein View: ALBU_BOVIN

Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4

Database:	SwissProt
Score:	63
Expect:	0.0063
Nominal mass (M_r):	69248
Calculated pI:	5.82
Taxonomy:	Bos taurus

Sequence similarity is available as an NCBI BLAST search of ALBU_BOVIN against nr.

Search parameters

Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications:	Carbamidomethyl (C), Oxidation (HW), Oxidation (M), NakamuraLab_SSMIDIAA(C) (C), NakamuraLab_SSMIDIAA(K) (K)
Mass values searched:	100
Mass values matched:	62

Protein sequence coverage: 79%

Matched peptides shown in ***bold red***.

```
  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
601 STQTALA
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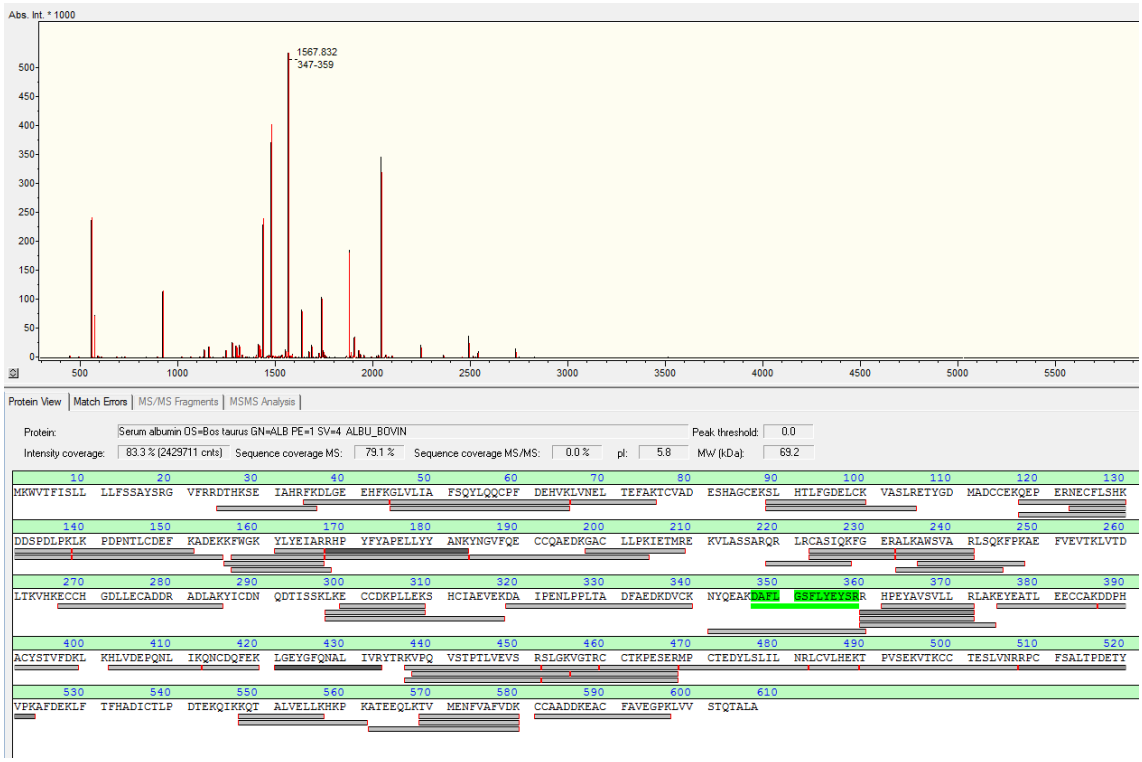
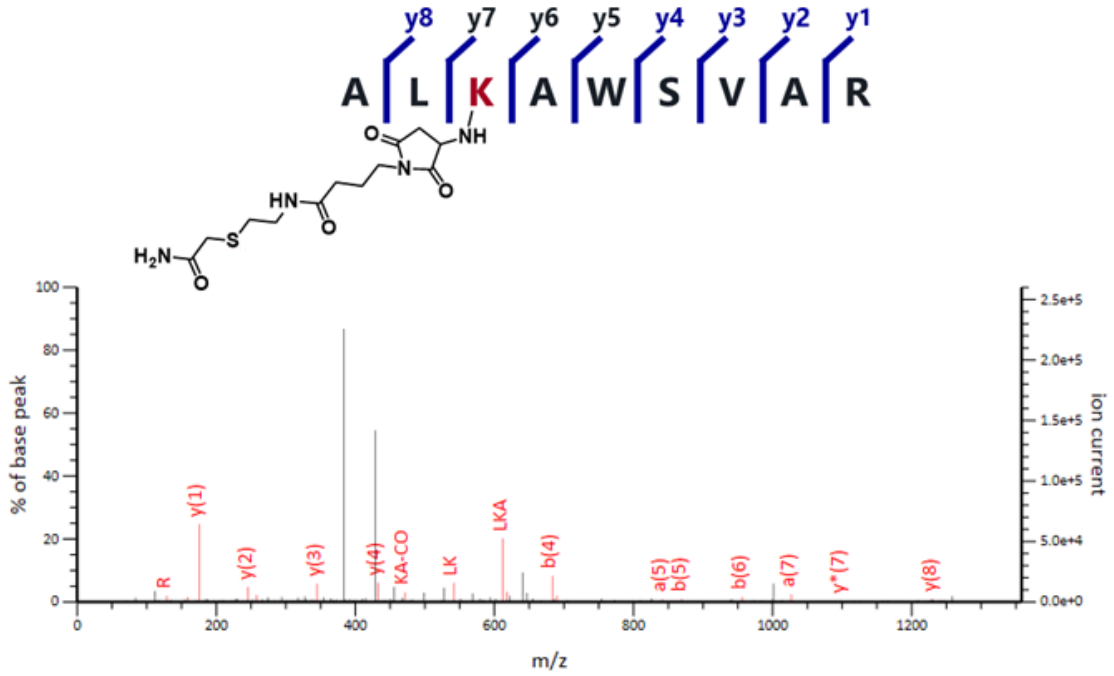
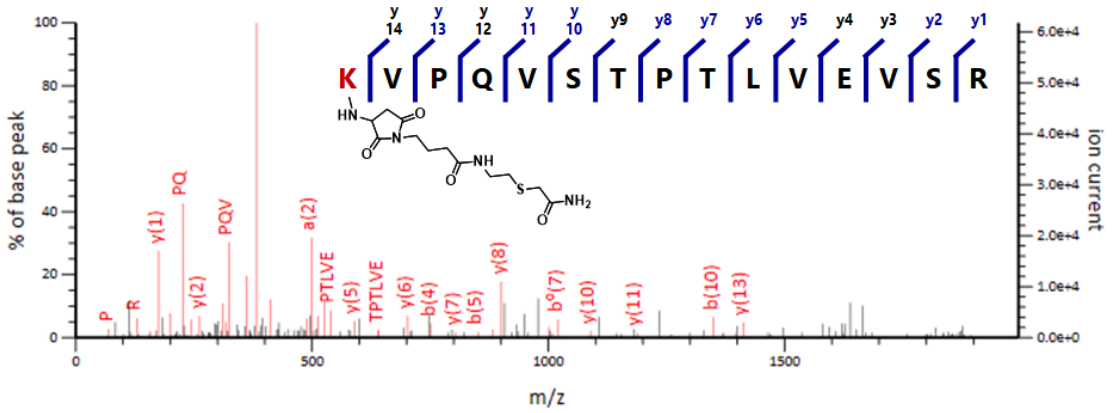


Figure S3. Protein sequence coverage of BSA obtained by LC-MALDI analysis



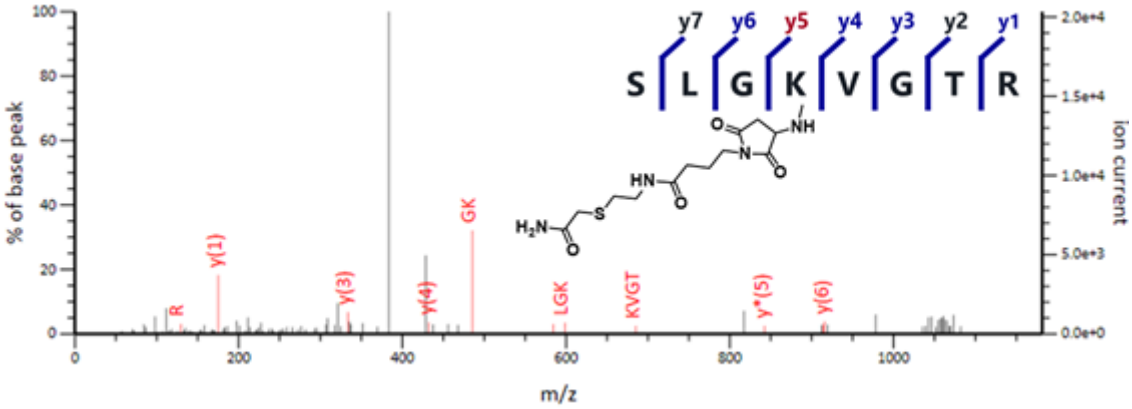
#	Immon.	a	a*	a^0	b	b*	b^0	d	Seq.	v	w	y	y*	y^0	#
1	44.0495	44.0495			72.0444			44.0495	A						9
2	86.0964	157.1335			185.1285			115.0866	L	1171.5677	1170.5724	*1229.6459*	1212.6194	1211.6354	8
3	400.2013	584.3225	567.2959		**612.3174**	595.2908		228.1707	K	744.3787	743.3835	*1116.5619*	1099.5353	1098.5513	7
4	44.0495	655.3596	638.3330		**683.3545**	666.3280			A	673.3416		*689.3729*	672.3464	671.3624	6
5	159.0917	**841.4389**	824.4124		**869.4338**	852.4073			W	487.2623		*618.3358*	601.3093	600.3253	5
6	60.0444	928.4709	911.4444	910.4604	**956.4658**	939.4393	938.4553	912.4760	S	400.2303	399.2350	*432.2565*	415.2300	414.2459	4
7	72.0808	**1027.5393**	1010.5128	1009.5288	1055.5343	1038.5077	1037.5237	1013.5237	V	301.1619	314.1823	*345.2245*	328.1979		3
8	44.0495	**1098.5765**	1081.5499	1080.5659	1126.5714	1109.5448	1108.5608		A	230.1248		*246.1561*	229.1295		2
9	129.1135								R	74.0237	73.0284	*175.1190*	158.0924		1

Figure S4. MS/MS analysis of modified peptide fragment containing Lys211.



#	Immon.	a	a*	a^0	b	b*	b^0	d	d'	Seq.	v	w	w'	y	y*	y^0	#
1	400.2013	400.2013	383.1748		428.1962	411.1697		44.0495		K							15
2	72.0808	499.2697	482.2432		**527.2646**	510.2381		485.2541		V	1467.7802	1480.8006		1511.8428	1494.8162	1493.8322	14
3	70.0651	596.3225	579.2959		624.3174	607.2908		570.3068		P	1370.7274	1369.7322		***1412.7744***	1395.7478	1394.7638	13
4	101.0709	724.3811	707.3545		**752.3760**	735.3494		667.3596		Q	1242.6688	1241.6736		1315.7216	1298.6951	1297.7110	12
5	72.0808	823.4495	806.4229		**851.4444**	834.4178		809.4338		V	1143.6004	1156.6208		***1187.6630***	1170.6365	1169.6525	11
6	60.0444	910.4815	893.4549	892.4709	938.4764	921.4499	920.4658	894.4866		S	1056.5684	1055.5732		***1088.5946***	1071.5681	1070.5841	10
7	74.0600	1011.5292	994.5026	993.5186	1039.5241	1022.4975	1021.5135	995.5343	997.5135	T	955.5207	968.5411	970.5204	***1001.5626***	984.5360	983.5520	9
8	70.0651	1108.5819	1091.5554	1090.5714	1136.5769	1119.5503	1118.5663	1082.5663		P	858.4680	857.4727		***900.5149***	883.4884	882.5043	8
9	74.0600	1209.6296	1192.6031	1191.6191	1237.6245	1220.5980	1219.6140	1193.6347	1195.6140	T	757.4203	770.4407	772.4199	***803.4621***	786.4356	785.4516	7
10	86.0964	1322.7137	1305.6871	1304.7031	**1350.7086**	1333.6821	1332.6980	1280.6667		L	644.3362	643.3410		***702.4145***	685.3879	684.4039	6
11	72.0808	1421.7821	1404.7556	1403.7715	1449.7770	1432.7505	1431.7664	1407.7664		V	545.2678	558.2882		***589.3304***	572.3039	571.3198	5
12	102.0550	1550.8247	1533.7981	1532.8141	1578.8196	1561.7931	1560.8090	1492.8192		E	416.2252	415.2300		***490.2620***	473.2354	472.2514	4
13	72.0808	1649.8931	1632.8666	1631.8825	1677.8880	1660.8615	1659.8775	1635.8775		V	317.1568	330.1772		***361.2194***	344.1928	343.2088	3
14	60.0444	1736.9251	1719.8986	1718.9146	1764.9200	1747.8935	1746.9095	1720.9302		S	230.1248	229.1295		***262.1510***	245.1244	244.1404	2
15	129.1135									R	74.0237	73.0284		***175.1190***	158.0924		1

Figure S5. MS/MS analysis of modified peptide fragment containing Lys413.



#	Immon.	a	a*	a^0	b	b*	b^0	d	d'	Seq.	v	w	w'	y	y*	y^0	#
1	60.0444	60.0444		42.0338	88.0393		70.0287	44.0495		S							8
2	86.0964	173.1285		155.1179	201.1234		183.1128	131.0815		L	971.4727	970.4775		1029.5510	1012.5244	1011.5404	7
3	30.0338	230.1499		212.1394	258.1448		240.1343			G				***916.4669***	899.4404	898.4563	6
4	400.2013	657.3389	640.3123	639.3283	685.3338	668.3072	667.3232	301.1870		K	487.2623	486.2671		859.4454	842.4189	841.4349	5
5	72.0808	756.4073	739.3807	738.3967	784.4022	767.3756	766.3916	742.3916		V	388.1939	401.2143		***432.2565***	415.2300	414.2459	4
6	30.0338	813.4287	796.4022	795.4182	841.4236	824.3971	823.4131			G				***333.1881***	316.1615	315.1775	3
7	74.0600	914.4764	897.4499	896.4658	942.4713	925.4448	924.4608	898.4815	900.4608	T	230.1248	243.1452	245.1244	276.1666	259.1401	258.1561	2
8	129.1135									R	74.0237	73.0284		***175.1190***	158.0924		1

Figure S6. MS/MS analysis of modified peptide fragment containing Lys431.

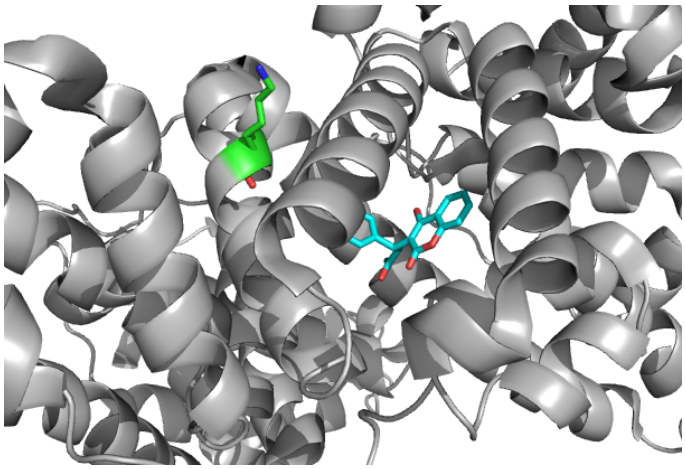


Figure S7. Lysine residue corresponding BSA Lys211 in warfarin binding site of HSA. Lys211 of BSA is conserved in the structure of HSA (HSA Lys212). Green: lysine residue. Blue: warfarin. PDB file: 2BXD.

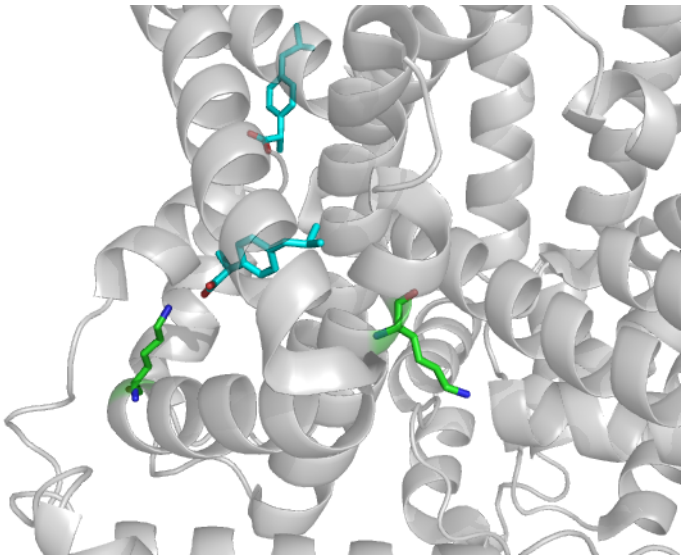


Figure S8. Lysine residues corresponding to BSA Lys413 and Lys431 in ibuprofen binding site of HSA. Lys413 and Lys431 of BSA are conserved in the structure of HSA (HSA Lys414 and Lys432). Green: lysine residues corresponding to BSA Lys413 (left) and Lys431 (right) residue. Blue: ibuprofen. PDB file: 2BXG.

1. General procedure for the synthesis of SSMID

NMR spectra were recorded with a Bruker AVANCE-400 (400 MHz for ^{1}H) or ASCEND-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C, 160 MHz for ^{11}B) instrument in the indicated solvent. ^{11}B NMR spectra were obtained by the $^{11}B–^{1}H$ decoupling method. Chemical shifts are reported in units of parts per million (ppm) relative to the signal (δ =0.00 ppm) for internal tetramethylsilane for solutions in $CDCl_3$ (δ = 7.26 ppm for ^{1}H, δ = 77.0 ppm for ^{13}C) or CD_3CN (δ = 1.94 ppm for ^{1}H, δ = 118.26 ppm for ^{13}C). IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Only the strongest and/or structurally important peaks are reported as IR data given in cm^{-1}. High-resolution mass spectrometry (HRMS) was performed with a Bruker ESI-TOF-MS (micrOTOF II). All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light (254 nm), and were visualized using an aqueous alkaline $KMnO_4$ solution. Silica gel (Fuji Silysia, CHROMATOREX PSQ60B, 50–200 μm) was used for column chromatography. All chemicals and purified proteins for biological experiments were obtained from commercial sources and were used without further purification.

Synthesis of 1,4-dioxane-*closo*-dodecaborate complex (1)

This compound was synthesized according to the literature procedure.[1] Briefly, *closo*-dodecaborate tetrabutylammonium form (1.3 g, 2.0 mmol) converted from the commercially available *closo*-dodecaborate triethylammonium form, $(Et_3NH)_2[B_{12}H_{12}]$, by ion-exchange was dissolved in 1,4-dioxane (70 mL) and $NaBF_4$ (1.1 g, 10 mmol) and HCl (4 M, 1 mL) were added. The mixture was heated to 100 °C and stirred for 12 h. After cooling to ambient temperature, the precipitates were removed by filtration and the organic layer was concentrated under reduced pressure. Recrystallization from ethanol gave compound **1** as a white solid (430 mg, 87% yield). ^{1}H NMR (400 MHz, CD_3CN) δ 4.50 (m, 4H), 3.85 (m, 4H), 3.07 (m, 8H), 1.60 (m, 8H), 1.35 (m, 8H), 0.97 (t, J = 7.2 Hz, 12H).

Synthesis of *N-tert*-butoxycarbonylcystamine (2)

This compound was synthesized according to the literature procedure.[2] Briefly, cystamine dihydrochloride (1.00 g, 4.44 mmol) was dissolved in methanol (70 mL) and triethylamine (1.85 mL, 13.33 mmol) was added at 0°C. The mixture was stirred for 30 min and then di-*tert*-butyldicarbonate (1.01 mL, 4.44 mmol) was added dropwise at 0°C. The reaction mixture was allowed to r.t. and stirred for 5 h. The solvent was evaporated, and 1 N NaOH solution was added (5 mL). The mixture was extracted with CH_2Cl_2 (2 x 5 mL) and the combined organic phases

were washed with H_2O (2 x 5 mL). The organic layer was then dried over $MgSO_4$ and concentrated in vacuo to yield compound **2** as a white solid (448 mg, 46% yield). ^{1}H NMR ($CDCl_3$) δ 3.45 (m, 2H), 3.00 (t, *J* = 6.16 Hz, 2H), 2.77 (q, *J* = 6.19 Hz, 4H), 1.45 (s, 9H).

Synthesis of *N-tert*-butoxycarbonylcystamine-*closo*-dodecaborate conjugate (3)

Compound **1** (471 mg, 1 mmol), *N-tert*-butoxycarbonylcystamine **2** (261mg, 1 mmol) and $NaHCO_3$ (270 mg, 3.2 mmol) were dissolved in dry MeCN (5 mL) and the mixture was stirred under refluxed conditions for 10 h. Inorganic precipitates were filtered and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH =10:1) to give compound **3** as a white solid (571 mg, 79% yield). ^{1}H NMR (400 MHz; CD_3CN) δ 3.85 (t, *J* = 5.36 Hz, 2H), 3.67 (m, 4H), 3.58 (s, 1H), 3.44 (t, *J* = 7.16 Hz, 2H), 3.37 (q, *J* = 6.48 Hz, 2H), 3.24 (t, *J* = 5.3 Hz, 2H), 3.12 (m, 8H), 2.88 (m, 2H), 2.81 (t, *J* = 6.62 Hz, 1H), 1.63 (m, 8H), 1.44 (s, 9H),1.38 (m, 8H), 0.99 (t, *J* = 7.34 Hz, 12H). ^{13}C NMR (125 MHz; CD_3CN) δ 71.34, 69.05, 68.97, 64.92, 64.78, 58.36, 46.79, 45.73, 39.49, 37.91, 33.07, 27.64, 23.33, 19.34, 12.81. ^{11}B NMR (160 MHz; CD_3CN) δ 5.87, −16.77, −17.58, −22.08. IR (ATR, cm^{-1}): 2959, 2866, 2463, 1698, 1457, 1380, 1249, 1161, 1047, 1014, 862, 719, 420, 403; HRMS (ESI, negative) m/z calcd. for $C_{29}H_{74}B_{12}N_3O_4S_2$ $[M]^-$: 722.6334, found: 722.6235. m.p. 96-97 °C.

Synthesis of tetrabutylammonium form of maleimide-cystamine-*closo*-dodecaborate conjugate (6)

Compound **3** (346 mg, 0.55 mmol) was dissolved in CH_3CN (18 mL) and 4N HCl dioxane (6 mL) was added dropwise at 0 ℃. The mixture was stirred at room temperature for 4 h and then TEA was added dropwise. After evaporation, the residue was dissolved in CH_2Cl_2 and wash with 1 N NaOH. The organic solvents were removed under reduced pressure to give compound crude **4** as a yellow oil (300 mg).Crude **4** (150 mg, 0.24 mmol), 4-maleimidobutyric acid **5** (53 mg, 0.29 mmol), (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP;106 mg, 0.24 mmol), $NaHCO_3$ (65 mg, 0.77 mmol) were dissolved in CH_2Cl_2 and stirred at room temperature for overnight. The reaction mixture was washed 3 times with 1N HCl solution followed by 3 times with brine and then concentrated under the reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 20:1 to 10:1) to give

compound **6** as a white amorphous (115 mg, 2 steps 60% yield). ^{1}H NMR (400 MHz; CD_3CN) δ 6.77 (s, 2H), 3.85 (t, *J* = 5.38 Hz, 3H), 3.68- 3.64 (m, 6H), 3.51-3.42 (m, 9H), 3.24 (t, *J* = 5.32 Hz, 3H), 3.11-3.05 (m, 8H), 2.89 (t, *J* = 6.60 Hz, 2H), 1.84 (m, 4H), 1.63 (m, 8H), 1.44 (m, 8H), 0.99 (t, *J* = 7.34 Hz, 12H). ^{13}C NMR (125 MHz; D_2O) δ 173.24, 172.26, 172.20, 172.10, 135.16, 73.4, 73.09, 72.19, 71.90, 69.76, 69.69, 69.13, 68.46, 67.87, 59.27, 59.25, 59.22, 53.51, 49.81, 49.04, 48.44, 47.63, 47.32, 46.76, 46.65, 39.28, 39.05, 38.95, 37.96, 37.90, 36.62, 35.84, 33.75, 33.71, 33.50, 30.86, 30.68, 25.16, 25.14, 24.67, 24.55, 24.25, 20.25, 20.24, 13.75. ^{11}B NMR (160 MHz; D_2O) δ 6.19, -16.75, −17.71, −22.36. IR (ATR, cm^{-1}): 2959, 2929, 2872, 2465, 1702, 1635, 1457, 1407, 1378, 1281, 1140, 1106, 1046, 1015, 880, 829, 718, 694; HRMS (ESI, negative) m/z calcd. for $C_{16}H_{37}B_{12}N_3O_5S_2Na$ $[M]^-$: 568.3268, found: 568.3274.

Synthesis of tetramethylammonium form of maleimide-cystamine-*closo*-dodecaborate conjugate (SSMID)

Compound **6** (115 mg, 0.14 mmol) was dissolved in methanol/ethanol (1/1; 5 mL) and a solution of tetramethylammonium (TMA) chloride (77 mg, 0.7 mmol) in 2 mL (methanol/ethanol = 1/1) was added. SSMID was precipitated as a white solid (42 mg, 42%): ^{1}H NMR (500 MHz; CD_3CN): δ 7.31 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 3.75-3.70 (m, 1H), 3.54-3.52 (m, 2H), 3.51-3.42 (m, 4H), 3.30-3.24 (m, 2H), 3.23 (s, 12H), 2.46 (d, *J* = 7.2 Hz, 2H), 1.88-1.82 (m, 1H), 1.38 (d, *J* = 6.8 Hz, 4H), 0.90 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (125 MHz; D_2O) δ 172.04, 170.93, 169.59, 163.26, 135.21, 59.31, 59.29, 59.26, 37.27, 36.50, 31.24, 28.84, 26.31, 24.27, 24.25, 20.27, 13.75; ^{11}B NMR (160 MHz; D_2O) δ 6.74, -16.77, −17.58, −22.14. IR (ATR, cm^{-1}): 2925, 2468, 1700, 1635, 1485, 1445, 1118, 1049, 1017, 948, 822, 719, 694, 455, 418; HRMS (ESI, negative) m/z calcd. for $C_{16}H_{38}B_{12}N_3O_5S_2$ $[M]^-$: 546.3459, found: 546.3394. m.p. 244-246 °C.

2. Protein modification with MID or SSMID

For the modification with MID or SSMID, a solution of BSA (final concentration 100 μM) in PBS buffer was added MID (final concentration 10 mM). The solution was briefly vortexed and incubated at room temperature for 12 h. The mixture was added 5×SDS-PAGE sample buffer, boiled at 95 °C for 5 min and subjected to SDS-polyacrylamide gel (10% acrylamide) electrophoresis (PAGE). The gel was stained by CBB and obtained image with a Molecular Imager ChemiDoc XRS (Bio Rad).

3. Western blot detection of boron conjugated albumin

Acrylamide gel which obtained by SDS-PAGE was transferred to polyvinylidene difluoride (PVDF) membrane (GE Healthcare), blocked with Immuno Block (DS Pharma) for 1 h. The membrane was washed by TTBS (Tween-Tris buffered saline) for 10 min in 3 times. The resulting membrane was treated with anti-B_{12} cluster ($B_{12}H_{11}SH$, BSH) antibody (1000 times diluted) at the condition of 4 °C for 12 h. HRP-conjugated secondary antibody (anti-mouse-HRP, Santa Cruz, 3000 times diluted) was treated at room temperature for 1 h, then washed by TTBS buffer for 10 min in 3 times. The chemiluminescence images were obtained by Fusion Solo 4S (Vilber Lourmat) after added detection reagent Luminata™ Forte (Millipore) or ImmunoStar LD (Wako Pure Chemical Industries, Ltd.).

4. Blocking experiment of albumin

To a solution of BSA (final concentration 100 μM) or BSA, which was capped with 10 mM NEM or NAS on ahead, in 10 mM PBS buffer for 1h was added MID or SSMID (final concentration 10 mM). The solution was briefly vortexed and incubated at room temperature for 12 h. The mixture was boiled for 5 min and subjected to SDS-polyacrylamide gel (10% acrylamide) electrophoresis (PAGE).

5. In-gel digestion for mass spectrometric characterization of SSMID-protein conjugates

The entire acrylamide gel was rinsed with ultrapure water for a few hours, and bands (spots) of interest were excised with a clean scalpel. The gel pieces cut into a cube size was transferred to a tube and they were washed with ultrapure water for 10 minutes three times. The gels pieces were treated with 100 μL of 100 mM ammonium bicarbonate/acetonitrile (1:1, vol/vol) solution and incubate tubes for 10 min at 37 °C. The supernatant was removed and the gels were treated with 100 μL of 100 mM ammonium bicarbonate solution and incubate tubes for 10 min. The supernatant was removed and the gels were treated with 100 μL of 100 mM ammonium bicarbonate/acetonitrile (1:1, vol/vol) solution and incubate tubes for 10 min at 37 °C. The supernatant was removed and the gels were treated with 50 μL of acetonitrile and incubate tubes at 37℃ until gel pieces become white and shrink. The shrunk gels pieces were treated with 50 μL of 100 mM DTT / 100 mM ammonium bicarbonate solution, vortex briefly and incubate tubes for 30 min at 37 °C. The tubes were cooled down to room temperature and the supernatant was

removed completely. For capping free SH groups, 50 μL of 100 mM iodoacetamide / 100 mM ammonium bicarbonate solution was added and incubated for 30 min at room temperature in the dark. The gels pieces were washed with 100 μL of 100 mM ammonium bicarbonate solution and 100 μL of 100 mM ammonium bicarbonate/acetonitrile (1:1, vol/vol) solution. The gel pieces were shrunk with acetonitrile. A solution of trypsin in Tris buffer (pH 8.0) were added to the dry gel pieces. The samples were incubated overnight at 37℃. The digestion was quenched with adding TFA (final 0.1 %), and the samples were desalinated through Cleanup C18 Pipette Tips (Agilent). The solution (2 μL) were mixed on MALDI plate mix with 1 μL of CHCA.

6. Immunocytochemical study (ICC) of boron conjugated albumin

Cells were seeded at a density of 3×10^5 cells/ml with media 0.1 mL on the cover glasses in 6 well plate dishes, and incubated for 12h. Solution of MID-conjugated albumin (100 ppm boron concentration) was added and the cells were incubated for 6 hours. The cells were washed three times in the dish with 1 mL of PBS buffer, fixed with 4% paraformaldehyde (PFA) for 10 min, and treated with 0.4 % TritonX for 5 min. Added the anti-MID antibody (Rabbit polyclonal antibody against MID was produced by Scrum Inc.) 500 times diluted by Can get signal solution B (TOYOBO Inc.) at the condition of 4 °C for overnight, the cells were washed three times in the dish with 1 mL of PBS buffer. Added the FITC-conjugated secondary antibody (anti-Rabbit-FITC) 500 times diluted at the condition of 4 °C for 2 h. The cells were washed with PBS buffer three times. Nuclei were counterstained with DAPI. Fluorescence signals were observed using a confocal laser microscope (LMS780 spectral confocal system, Zeiss).

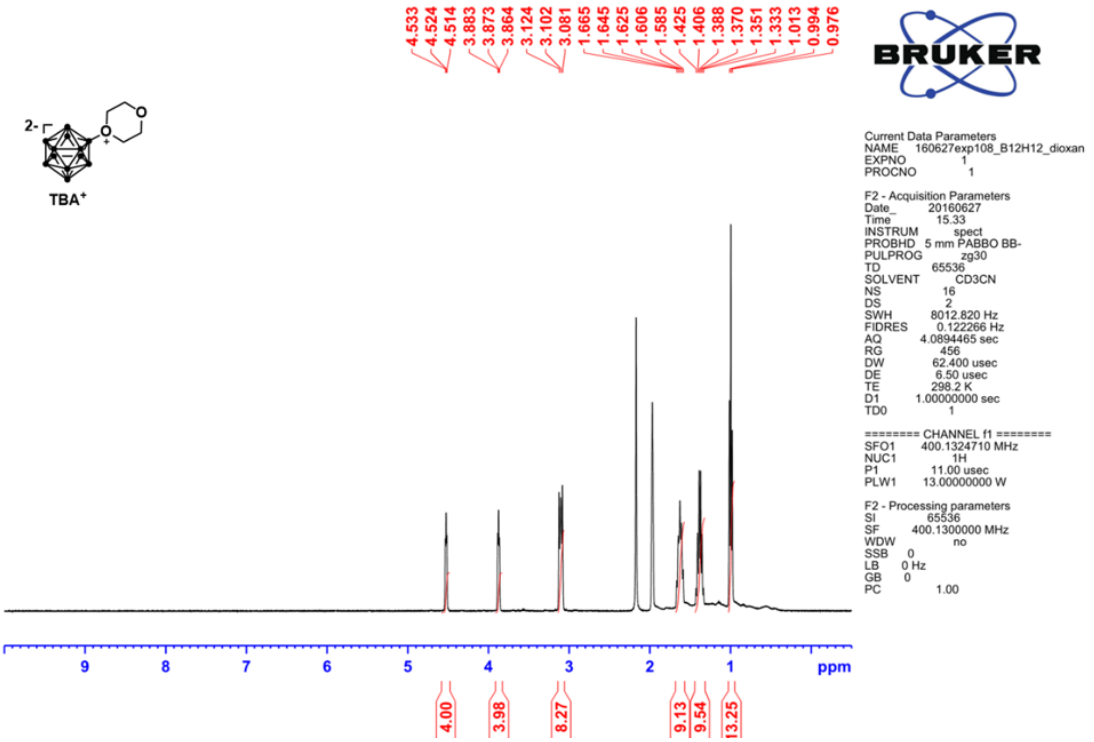
7. References

[1] I. B. Sivaev, N. Y. Kulikova, E. A. Nizhnik, M. V. Vichuzhanin, Z. A. Starikova, A. A. Semioshkin and V. I. Bregadze, *J. Organomet. Chem.*, 2008, **693**, 519-525.

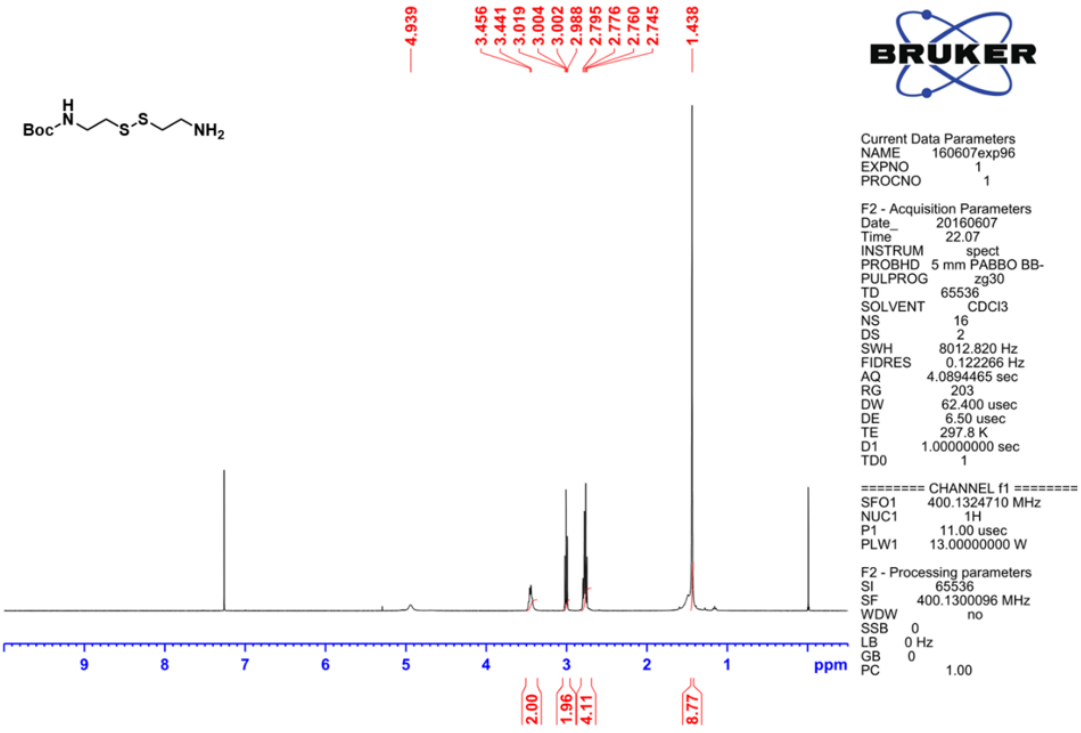
[2] R. R. Castillo, D. Lozano and M. Vallet-Regi, *Bioconjug. Chem.*, 2018, **29**, 3677-3685.

8. NMR Spectral data

1,4-Dioxane-*closo*-dodecaborate complex: ^{1}H-NMR (500 MHz, CD_3CN)



***N-tert*-butoxycarbonylcystamine (2):** ^{1}H-NMR (500 MHz, CD_3CN)



***N-tert*-butoxycarbonylcystamine-*closo*-dodecaborate conjugate (3)**

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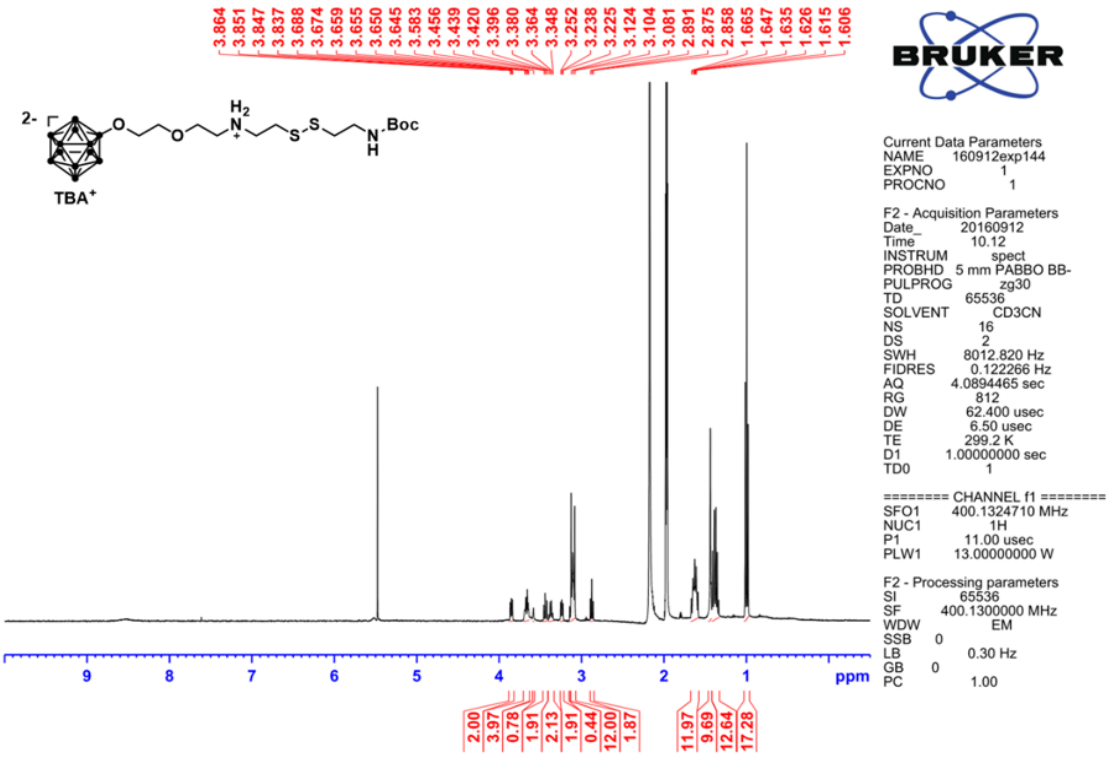
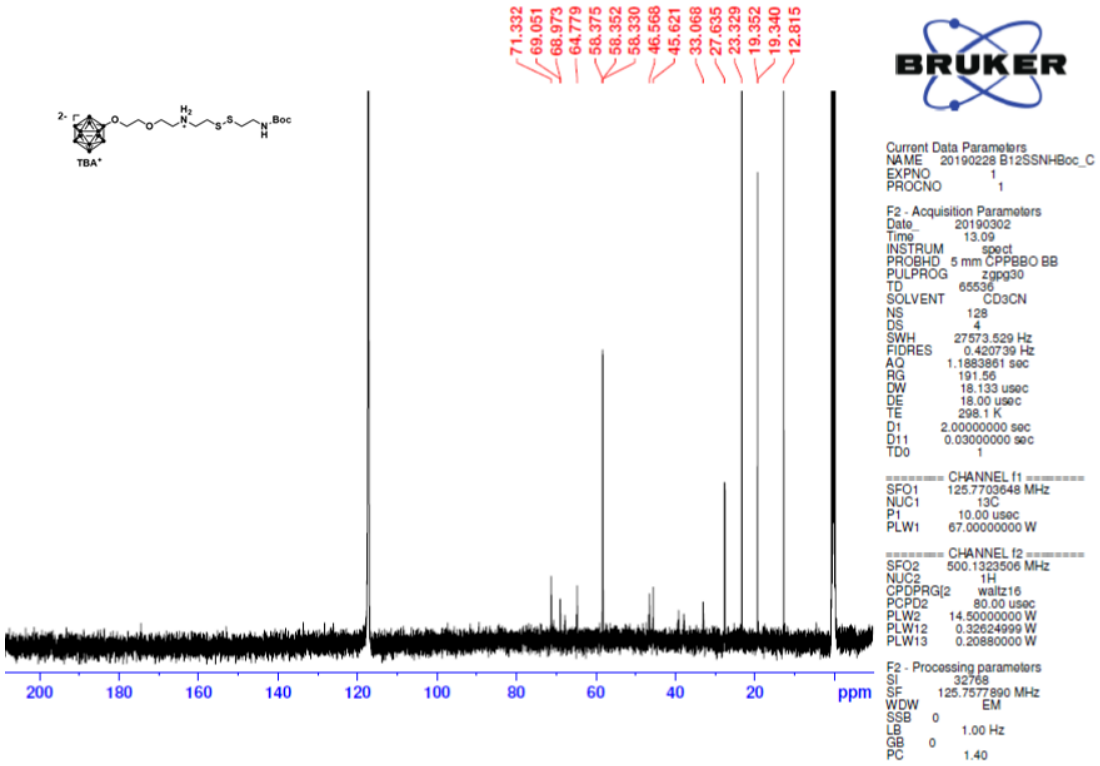
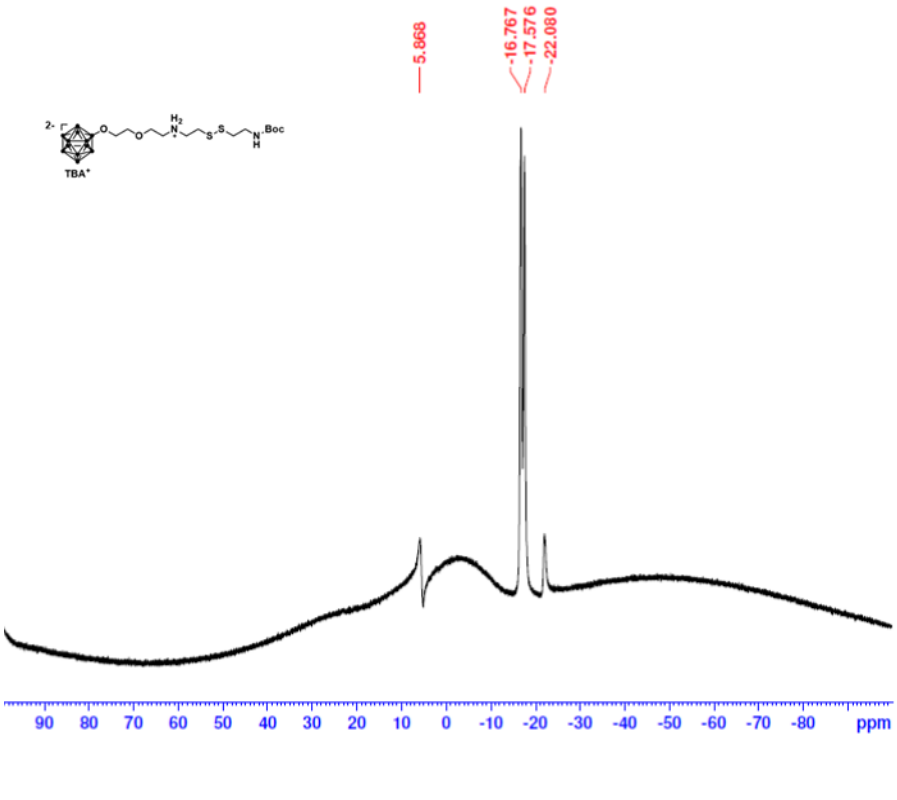


^{13}C-NMR (125 MHz, CD_3CN)



^{11}B-NMR (160 MHz, CD_3CN)



BRUKER

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PROCNO 1

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TD0 1

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NUC1 11B
P1 11.93 usec
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F2 - Processing parameters
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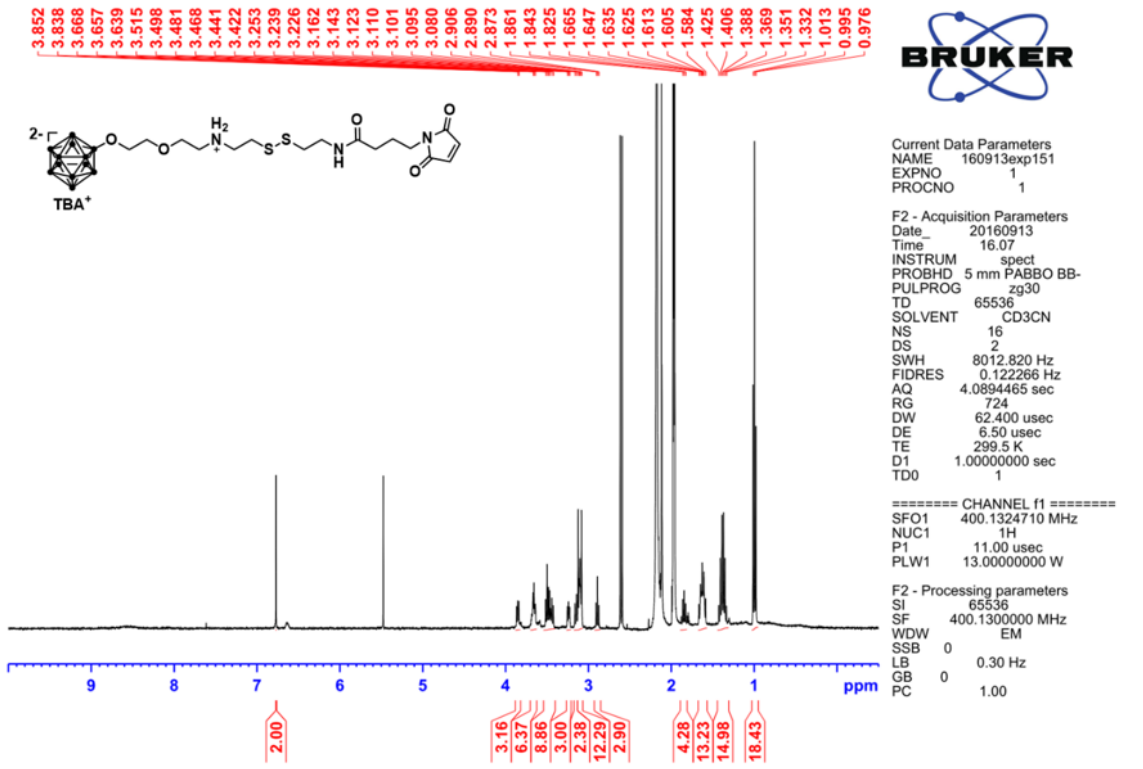
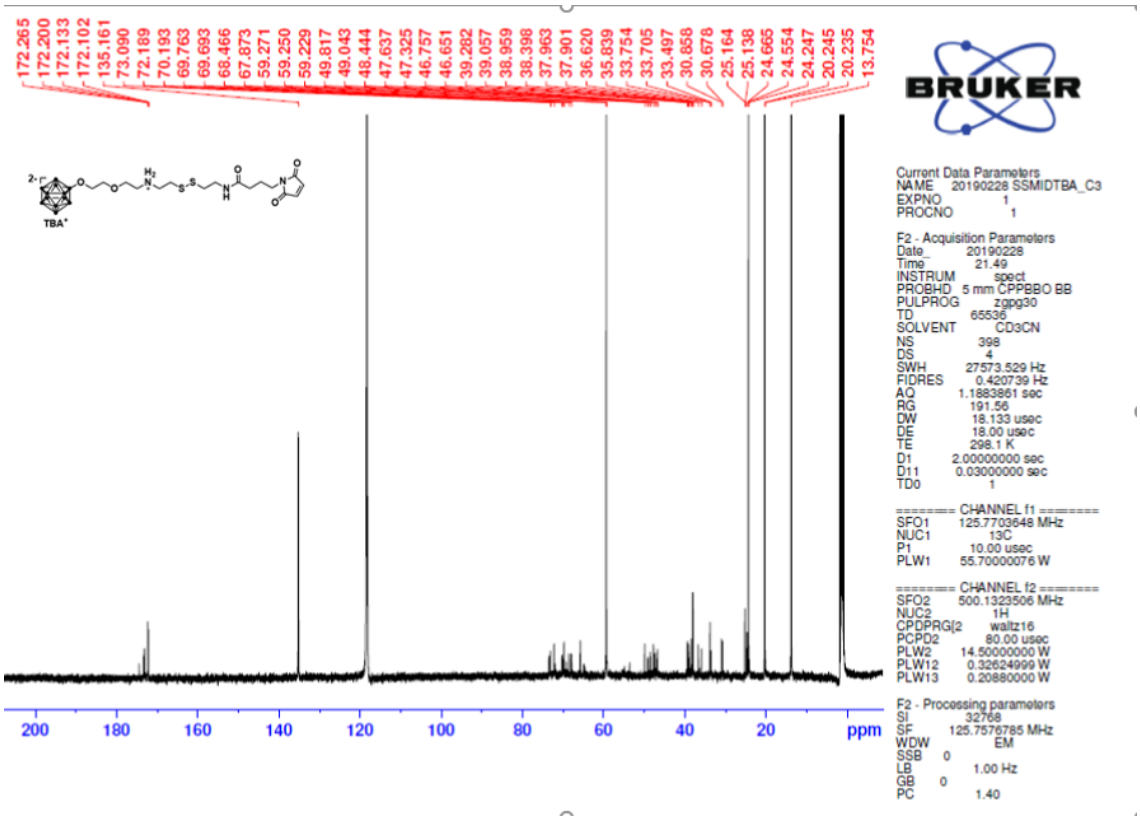
Tetrabutylammonium form of maleimide-cystamine-*closo*-dodecaborate conjugate (6)

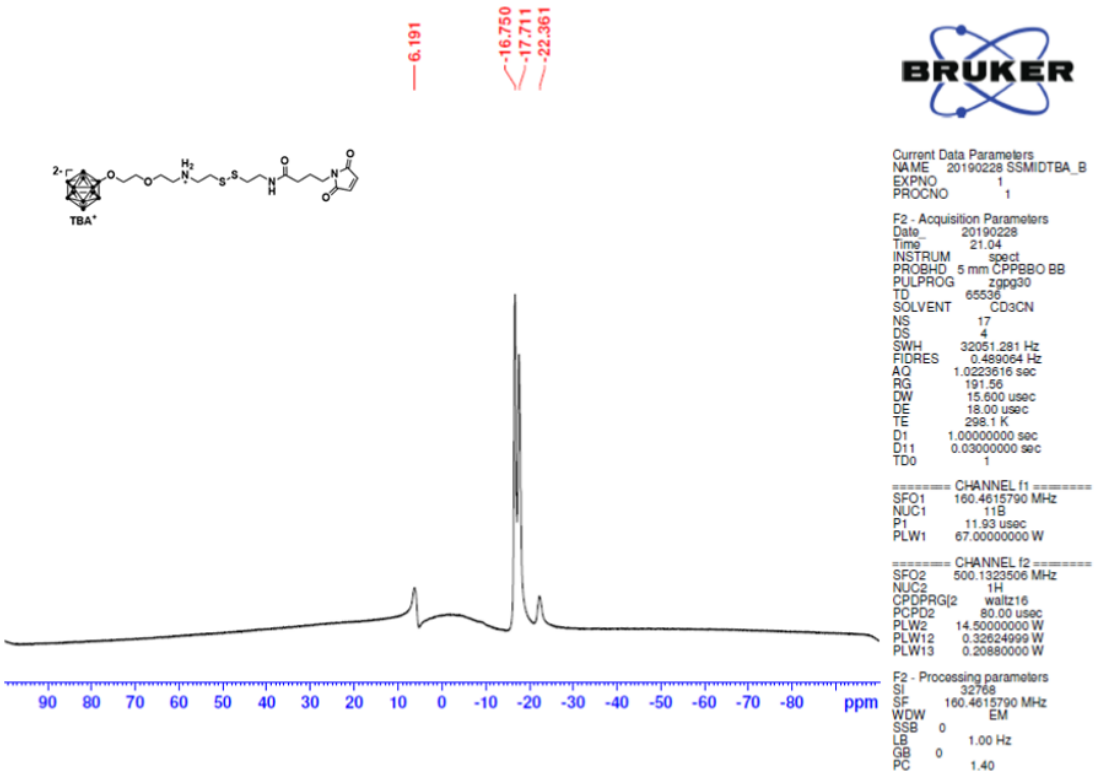
^{1}H-NMR (500 MHz, CD_3CN)



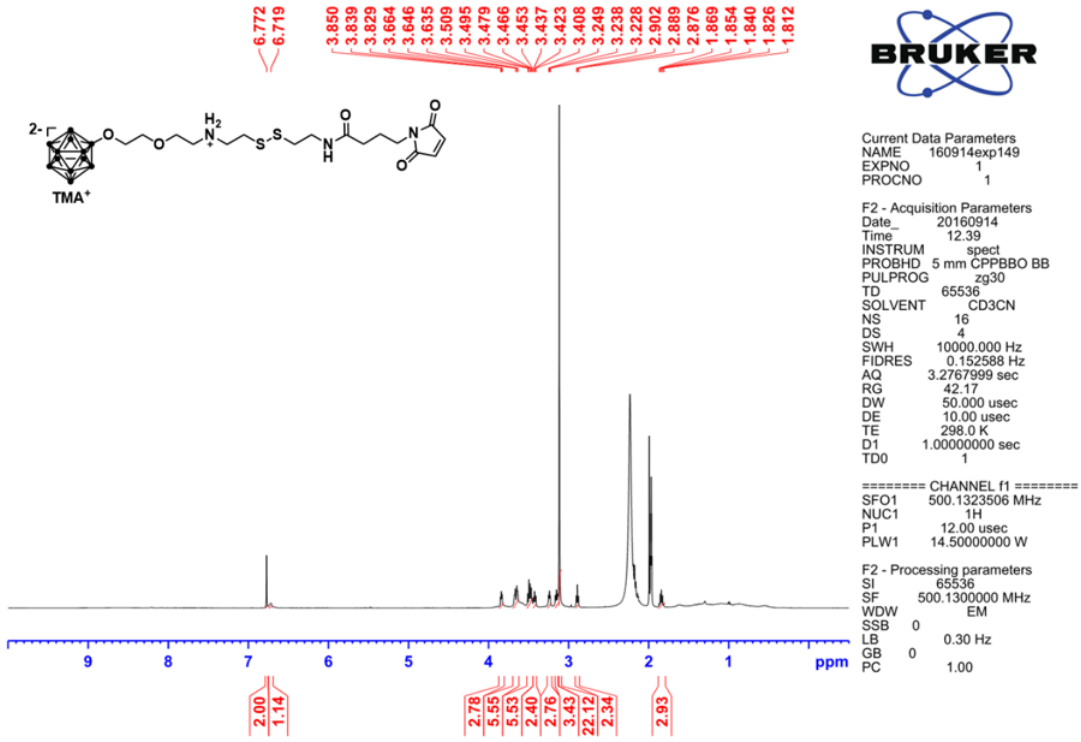
^{13}C-NMR (125 MHz, CD_3CN)



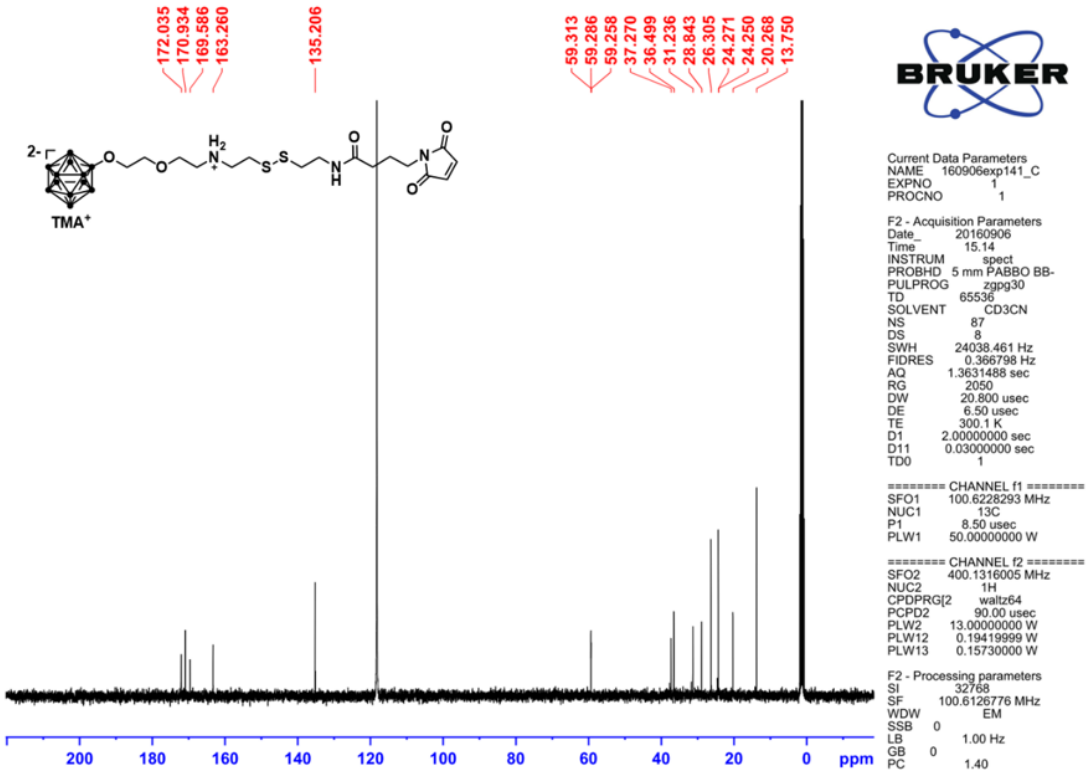
^{11}B-NMR (160 MHz, CD_3CN)



Tetramethylammonium form of maleimide-cystamine-*closo*-dodecaborate conjugate (SSMID): ^{1}H-NMR (500 MHz, CD_3CN)



^{13}C-NMR (125 MHz, CD_3CN)



^{11}B-NMR (160 MHz, CD_3CN)