Supplementary Information

Unbiased identification of the liposome protein corona using photoaffinity based chemoproteomics

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Supplementary Figures and Tables

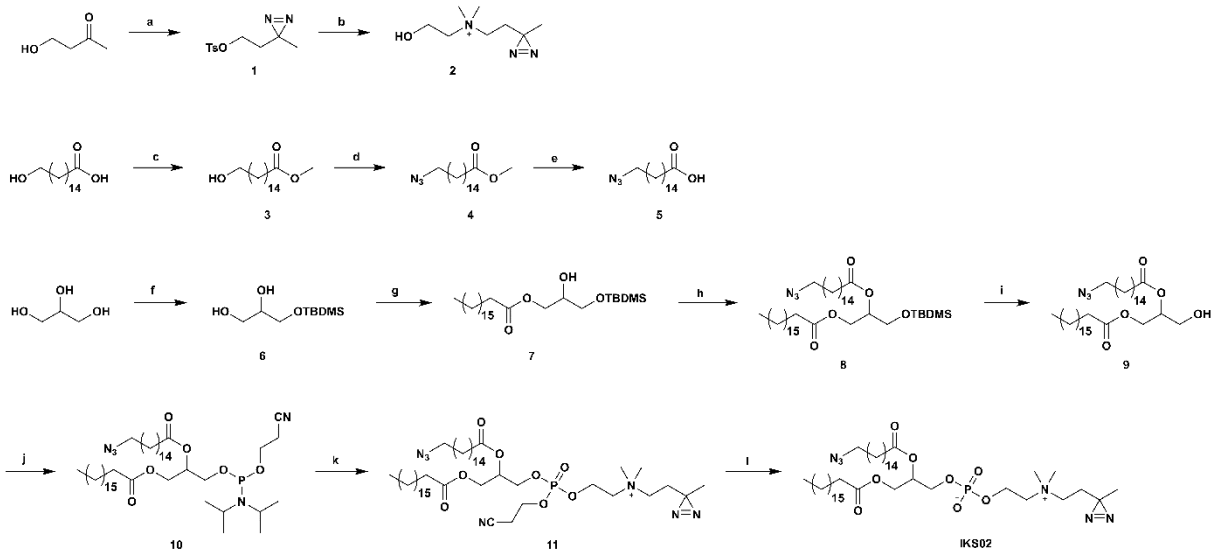

Figure S1. Synthesis scheme of the PAL probe IKS02. Reagents and conditions: (a) 1. 7N NH_3 in methanol; 2. NH_2OSO_3H; 3. I_2, Et_3N; 4. TsCl, pyridine, 20% over 4 steps; (b) DMEA, acetone, quant.; (c) AcCl, MeOH, 92%; (d) 1. MsCl, Et_3N, DCM; 2. NaN_3, DMF, 70 °C, 91% over 2 steps; (e) 4M NaOH, dioxane, 95%; (f) ^{t}BDMSCl, imidazole, DCM:DMF (1:1), -18 °C, 34%; (g) Stearic acid, DCC, DMAP, DCM, 76%; (h) **5**, DCC, DMAP, DCM, 64%; (i) $Et_3N{\cdot}3HF$, THF, quant.; (j) $PCl(OCH_2CH_2CN)(N^{i}Pr_2)$, Et_3N, DCM, 74%; (k) 1. **2**, imidazole, DCM; 2. ^{t}BuOOH, DCM, 10% over 2 steps; (l) $^{t}BuNH_2$, DCM, 48%. Detailed procedures are described in the Chemical Synthesis section of the Supplementary Information.

Formulation name	Lipid composition (mol%)	Size avg. (nm)	PDI	Zeta Potential (mV)
Myocet	55% POPC: 45% Cholesterol	105.3	0.034	-7.4 ± 2.1
AmBisome	53% DSPC : 21% DSPG : 26% Cholesterol	107.2	0.087	-24.5 ± 3.2
EndoTAG-1	51.5% DOTAP : 48.5% DOPC	101.4	0.044	+41.6 ± 4.6
Myocet + IKS02	50% POPC : 45% Cholesterol : 5% IKS02	102.1	0.042	-6.5 ± 1.8
AmBisome + IKS02	48% DSPC : 21% DSPG : 26% Cholesterol : 5% IKS02	104.8	0.079	-23.3 ± 2.7
EndoTAG-1 + IKS02	51.5% DOTAP : 43.5% DOPC : 5% IKS02	108.6	0.053	+43.2 ± 3.9
Myocet + DOPE-LR	54% POPC : 45% Cholesterol : 1% DOPE-LR	99.1	0.089	-5.2 ± 3.0
AmBisome + DOPE-LR	52% DSPC : 21% DSPG : 26% Cholesterol : 1% DOPE-LR	101.7	0.092	-28.9 ± 5.0
EndoTAG-1 + DOPE-LR	51.5% DOTAP : 47.5% DOPC : 1% DOPE-LR	104.5	0.063	+46.6 ± 5.9

Formulations in clinic/trials

Formulation name	Lipid composition (mol%)	Size avg. (nm)	Surface charge	Encapsulated drug
Myocet	54% POPC : 45% Cholesterol : 1% DOPE-LR	150-200 nm	zwitterionic neutral	Doxorubicin
AmBisome	53% DSPC : 21% DSPG : 26% Cholesterol	78 nm	anionic	Amphotericin B
EndoTAG-1	51.5% DOTAP : 48.5% DOPC	200 nm	cationic	Paclitaxel

Table S1. Dynamic Light Scattering and zeta potential measurements for the formulations used in this study. All formulations were made through thin film hydration and extrusion described in the Biological Methods & Proteomics section of the Supplementary Information. Liposome composition and size of formulations used in clinic or clinical trials obtained from Ref. 1.

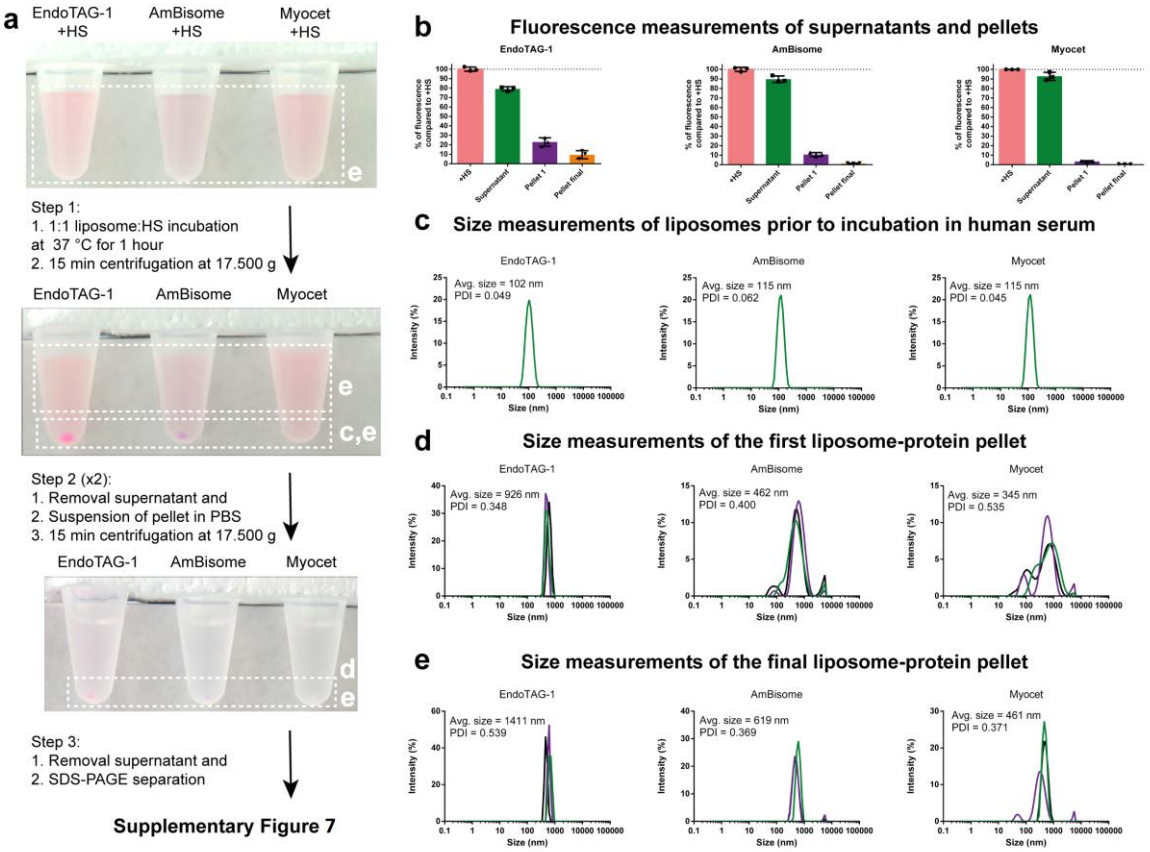

Figure S2. Sedimentation of liposome-protein complexes. (a) Liposomes containing 1 mol% fluorescent lipid (DOPE-LR) were incubated in human serum at a 1:1 ratio, followed by centrifugation for 15 minutes at 17,500 g. The supernatant was removed, the pellet was resuspended in PBS and the centrifugation and wash step repeated twice. After the last removal of the supernatant, the liposome-protein complexes were resolved on SDS-PAGE as shown in Figure S7. (b) Fluorescence measurements of supernatant and pellet from the steps described in **a**. Pellets were resuspended in PBS. All samples were performed in triplicate. Fluorescence of DOPE-LR (560 ex./583 em.) was determined using a fluorescence plate reader (Tecan M200, Tecan Life Sciences). (c) Dynamic Light Scattering (DLS) size measurements of the extruded liposomes prior to incubation in human serum. (d) DLS size measurements of the liposome-protein pellet after the first centrifugation step. (e) DLS size measurements of the liposome-protein pellet after the final centrifugation step. Pellets were re-suspended in PBS (100 µL) for DLS measurements.

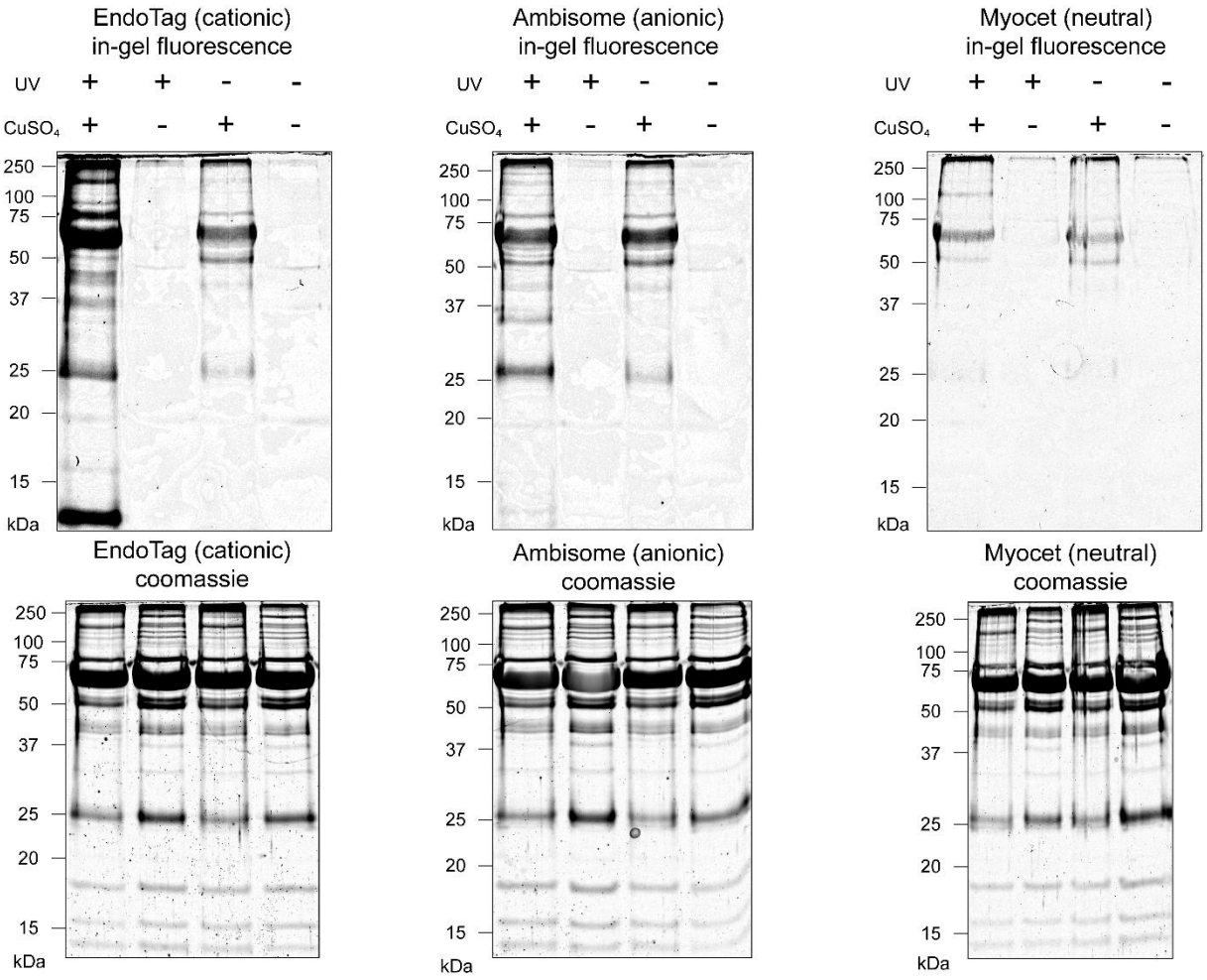

Figure S3. In-gel fluorescence and Coomassie stained SDS-PAGE gels for the photoaffinity method, displaying the fluorescently labelled protein corona (in-gel fluorescence) and the total protein content (Coomassie Blue). 10 µg total protein was loaded in each lane, as determined by BCA assay. Gels were run on a 10% polyacrylamide gel as described in the Biological Methods & Proteomics section.

ISOQuant Configuration

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Table S2. ISOQuant label-free quantification (LFQ) configuration based on the TOP3 approach.

Abundance	Protein name	Protein entry	IEP	MW (kDa)	average ppm -UV	average ppm +UV
1	Prothrombin	THRB	5.54	72	3101	49658
2	Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2	6.43	107	18647	50201
3	Apolipoprotein A-I	APOA1	5.44	31	7831	27202
4	Inter-alpha-trypsin inhibitor heavy chain H3	ITIH3	5.39	100	6056	22664
5	Clusterin	CLUS	5.87	53	1023	14509
6	C4b-binding protein alpha chain	C4BPA	7.02	69	6198	19139
7	Hyaluronan-binding protein 2	HABP2	6.11	65	1187	14124
8	Kininogen-1	KNG1	6.38	73	1639	14413
9	Protein AMBP	AMBP	5.9	40	6520	18286
10	Apolipoprotein A-IV	APOA4	5.12	45	929	10798
11	Apolipoprotein C-III	APOC3	5.06	11	659	8709
12	Antithrombin-III	ANT3	6.33	53	1398	7563
13	Complement C1r subcomponent	C1R	5.8	82	1766	6873
14	Serum paraoxonase/arylesterase 1	PON1	4.93	40	2906	7862
15	Apolipoprotein E	APOE	5.49	36	902	4623
16	Coagulation factor X	FA10	5.59	56	718	2562
17	Coagulation factor IX	FA9	5.19	53	1199	2735
18	Apolipoprotein A-II	APOA2	6.64	11	71	1159
19	Apolipoprotein C-II	APOC2	4.44	11	0	1064
20	Apolipoprotein C-I	APOC1	9.43	9	314	717

Abundance	Protein name	Protein entry	IEP	MW (kDa)	average ppm -UV	average ppm +UV
1	Apolipoprotein E	APOE	5.49	36	326	22878
2	Apolipoprotein A-I	APOA1	5.44	31	2469	11527
3	Serum amyloid P-component	SAMP	6.13	26	284	3038
4	Beta-2-glycoprotein 1	APOH	7.87	40	438	2682
5	Apolipoprotein C-I	APOC1	9.43	9	1024	2730
6	Apolipoprotein A-IV	APOA4	5.12	45	0	1589
7	Apolipoprotein C-III	APOC3	5.06	11	245	1130
8	Apolipoprotein A-II	APOA2	6.64	11	170	686
9	Apolipoprotein D	APOD	4.87	22	0	435
10	Apolipoprotein C-II	APOC2	4.44	11	119	390

Abundance	Protein name	Protein entry	IEP	MW (kDa)	average ppm -UV	average ppm +UV
1	Lysozyme C	LYSC	9.34	17	587	1438
2	Apolipoprotein C-III	APOC3	5.06	11	414	1170

Figure S4. Abundance profiles for proteins meeting the selection criteria. Displayed as plots, showing the protein entry and abundancy in both +UV and -UV samples, as well as in table format.

Human serum

Abundance	Protein name	Protein entry	IEP	MW (kDa)	average ppm
1	Serum albumin	ALBU	5.89	71.4	26.08
2	Complement C3	CO3	5.99	188.7	65.56
3	Serotransferrin	TRFE	6.78	79.3	29.71
4	Alpha-2-macroglobulin	A2MG	6.04	164.7	58.25
5	Immunoglobulin heavy constant gamma 1	IGHG1	8.20	36.6	16.61
6	Alpha-1-antitrypsin	A1AT	5.25	46.9	19.39
7	Immunoglobulin heavy constant mu	IGHM	6.37	50.1	21.16
8	Apolipoprotein A-I	APOA1	5.44	30.8	14.74
9	Immunoglobulin heavy constant gamma 2	IGHG2	7.45	36.5	17.66
10	Vitamin D-binding protein	VTDB	5.16	54.5	23.22
11	Immunoglobulin heavy constant alpha 1	IGHA1	6.09	38.5	18.53
12	Immunoglobulin kappa constant	IGKC	6.13	11.9	10.02
13	Complement factor H	CFAH	6.21	143.8	54.33
14	Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2	6.43	106.9	42.45
15	Apolipoprotein A-IV	APOA4	5.12	45.4	21.84
16	Complement factor B	CFAB	6.69	86.9	36.53
17	Haptoglobin	HPT	6.15	45.9	23.01
18	Immunoglobulin heavy constant gamma 3	IGHG3	7.79	42.3	22.70
19	Plasminogen	PLMN	6.93	93.3	39.75
20	C4b-binding protein alpha chain	C4BPA	7.02	69.1	32.04
21	Complement C4-B	CO4B	6.90	194.3	74.06
22	Plasma protease C1 inhibitor	IC1	6.11	55.4	27.83
23	Antithrombin-III	ANT3	6.33	53.1	27.46
24	Beta-2-glycoprotein 1	APOH	7.87	39.6	23.83
25	Hemopexin	HEMO	6.60	52.4	28.01
26	Enolase 1	ENO1	6.19	46.9	26.35
27	Immunoglobulin heavy constant gamma 4	IGHG4	7.13	36.5	23.53
28	Inter-alpha-trypsin inhibitor heavy chain H4	ITIH4	6.56	103.6	46.05
29	Complement C5	CO5	6.11	190.0	75.04
30	Gelsolin	GELS	5.87	86.1	40.66
31	Alpha-1-antichymotrypsin	AACT	5.19	47.8	28.00
32	Transthyretin	TTHY	5.42	16.0	17.81
33	Apolipoprotein A-II	APOA2	6.64	11.3	16.98
34	Inter-alpha-trypsin inhibitor heavy chain H1	ITIH1	6.35	101.8	47.40
35	Angiotensinogen	ANGT	5.88	53.4	31.44
36	Complement C1s subcomponent	C1S	4.66	78.2	39.63
37	Prothrombin	THRB	5.54	71.5	38.02
38	Ceruloplasmin	CERU	5.35	123.1	55.47
39	Apolipoprotein E	APOE	5.49	36.3	26.92
40	Heparin cofactor 2	HEP2	6.47	57.2	34.57
41	Alpha-2-HS-glycoprotein	FETUA	5.35	40.1	28.83
42	Hemoglobin subunit alpha	HBA	9.20	15.3	22.17
43	Apolipoprotein C-III	APOC3	5.06	10.9	19.64
44	Hemoglobin subunit beta	HBB	6.91	16.1	22.34
45	Complement C1r subcomponent	C1R	5.80	81.7	44.15
46	Complement C1q subcomponent subunit B	C1QB	8.87	26.9	27.27
47	N-acetylmuramoyl-L-alanine amidase	PGRP2	7.29	62.8	39.03
48	Alpha-2-antiplasmin	A2AP	5.87	54.9	36.26
49	Complement C1q subcomponent subunit C	C1QC	8.54	26.0	27.85
50	Complement C4-A	CO4A	6.68	194.4	83.69

Table S3. Abundancy of proteins in human serum determined with LFQ based on the TOP3 approach, analysed with the ISOQuant software.

51	Histidine-rich glycoprotein	HRG	7.13	60.5	39.56
52	Clusterin	CLUS	5.87	53.1	36.98
53	Alpha-1B-glycoprotein	A1BG	5.50	54.8	37.77
54	Leucine-rich alpha-2-glycoprotein	A2GL	6.53	38.4	32.98
55	Serum amyloid P-component	SAMP	6.13	25.5	28.88
56	Kininogen-1	KNG1	6.38	73.0	45.14
57	Immunoglobulin heavy constant alpha 2	IGHA2	5.85	37.4	33.41
58	Haptoglobin-related protein	HPTR	6.71	39.5	34.75
59	Coagulation factor XII	FA12	7.55	70.1	45.54
60	Apolipoprotein C-I	APOC1	9.43	9.3	26.25
61	Corticosteroid-binding globulin	CBG	5.61	45.3	37.31
62	Apolipoprotein C-II	APOC2	4.44	11.3	25.91
63	Protein AMBP	AMBP	5.90	39.9	36.27
64	CD5 antigen-like	CD5L	5.15	39.6	36.26
65	Serum paraoxonase/arylesterase 1	PON1	4.93	39.9	36.61
66	Complement component C9	CO9	5.28	64.7	45.31
67	Apolipoprotein L1	APOL1	5.49	44.0	38.84
68	Vitronectin	VTNC	5.45	55.1	42.85
69	Vitamin K-dependent protein S	PROS	5.35	77.2	50.51
70	Afamin	AFAM	5.55	71.0	48.85
71	Apolipoprotein D	APOD	4.87	21.6	32.48
72	Immunoglobulin J chain	IGJ	4.91	18.6	31.82
73	Pigment epithelium-derived factor	PEDF	5.97	46.5	41.82
74	Carboxypeptidase B2	CBPB2	7.54	49.0	43.51
75	Kallistatin	KAIN	7.58	48.7	43.76
76	Plasma kallikrein	KLKB1	8.10	73.5	52.53
77	Properdin	PROP	7.75	53.8	46.18
78	Complement factor I	CFAI	7.31	68.1	51.15
79	Insulin-like growth factor-binding protein complex acid labile subunit	ALS	6.37	66.8	50.72
80	Carboxypeptidase N subunit 2	CPN2	5.59	61.4	49.00
81	Retinol-binding protein 4	RET4	5.68	23.4	36.68
82	Thyroxine-binding globulin	THBG	5.88	46.7	44.85
83	Apolipoprotein M	APOM	5.63	21.6	36.74
84	Serum amyloid A-4 protein	SAA4	9.41	14.9	36.09
85	Alpha-1-acid glycoprotein 1	A1AG1	4.74	23.7	37.83
86	Alpha-1-acid glycoprotein 2	A1AG2	4.85	23.9	38.25
87	Lumican	LUM	6.19	38.8	43.99
88	Immunoglobulin heavy constant delta	IGHD	8.10	42.8	46.30
89	Zinc-alpha-2-glycoprotein	ZA2G	5.66	34.5	43.05
90	Immunoglobulin lambda variable 1-51	LV151	6.86	12.5	36.45
91	Apolipoprotein C-IV	APOC4	9.13	14.9	38.34
92	Tetranectin	TETN	5.38	22.9	40.11
93	Hemoglobin subunit delta	HBD	8.23	16.2	39.13

Table S3. Continued.

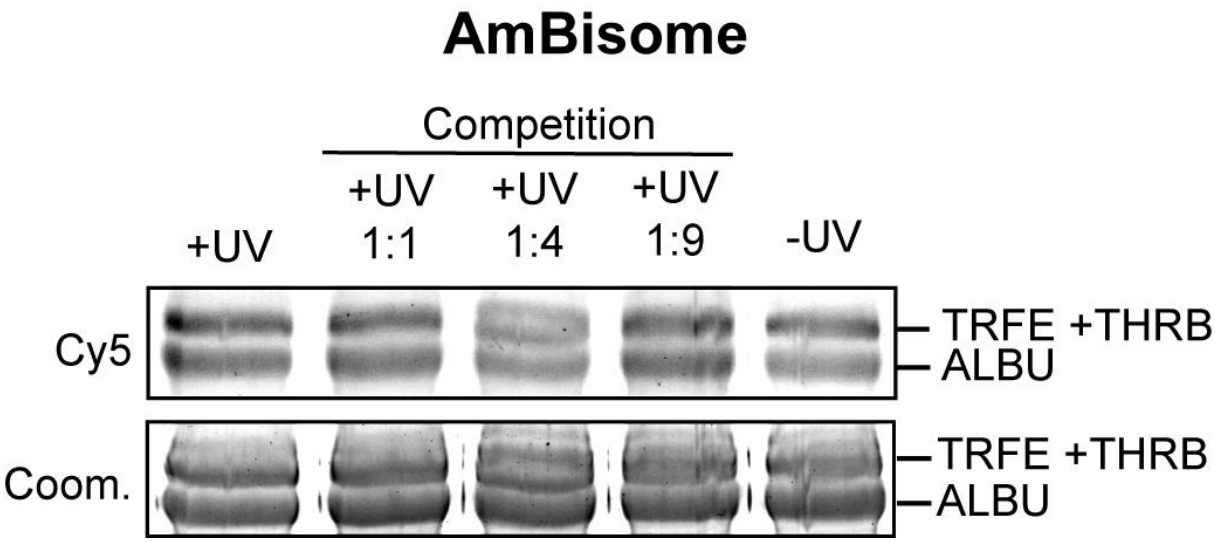


Figure S5. Competitive binding of human serum albumin (ALBU), transferrin (TRFE) and prothrombin (THRB). Increasing concentrations (1:1 to 1:9 molar ratios) of unlabeled AmBisome liposomes were incubated, together with AmBisome liposomes containing IKS02 (5 mol%), in a predefined mixture of purified human serum proteins (see Figure 4). Captured proteins were separated by SDS-PAGE and visualized by in-gel fluorescence (Cy5). Protein loading determined by Coomassie Blue (coom.).

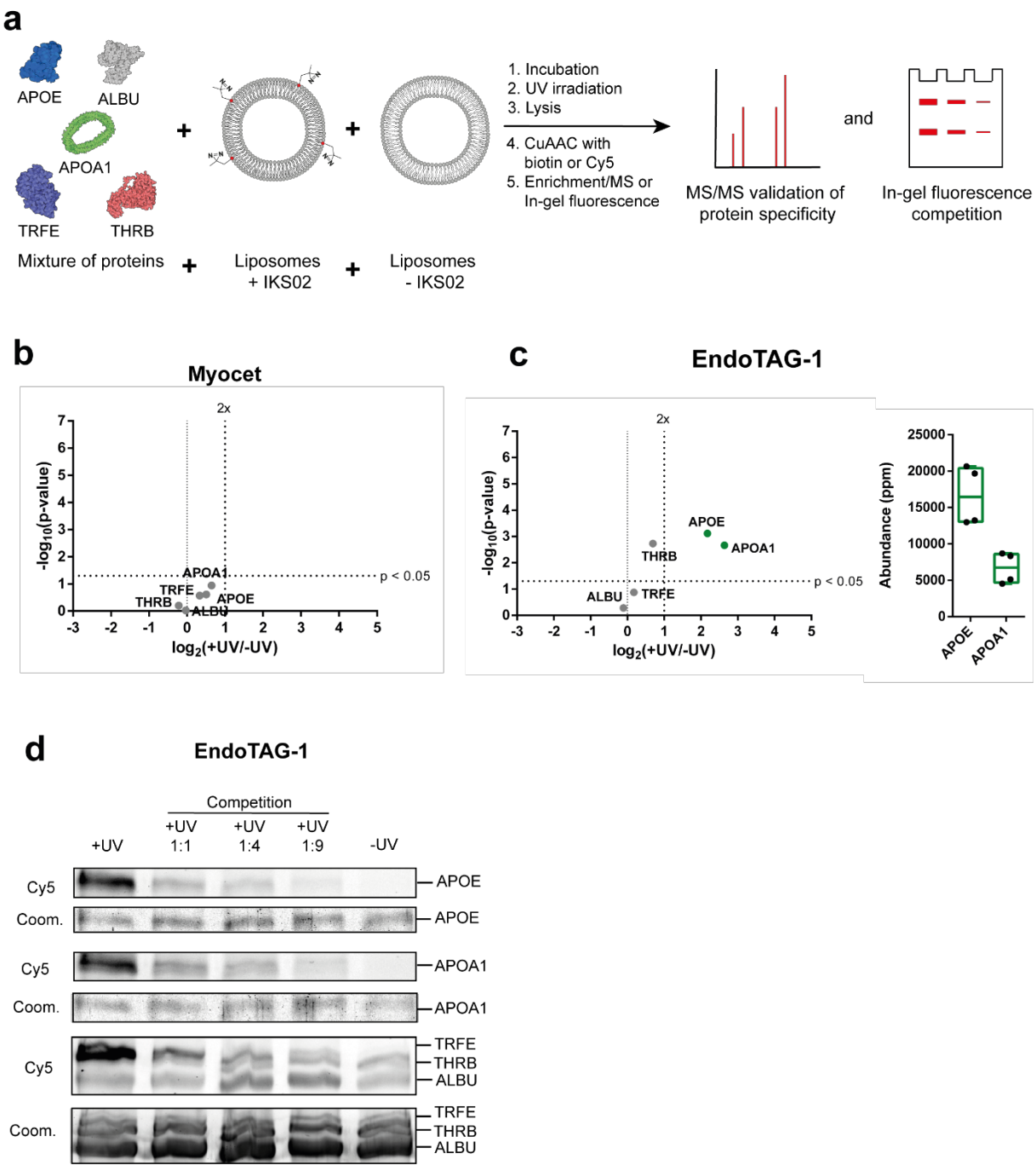


Figure S6. Validation of apolipoprotein E and A1 binding to Myocet and EndoTAG-1 liposomes. (a) Liposomes, containing 5 mol% IKS02, were incubated in a mixture of purified human serum proteins consisting of apolipoprotein E (APOE, 2 µgmL-1), serum albumin (ALBU, 25 µgmL-1), apolipoprotein A-I (APOA1, 2 µgmL-1), transferrin (TRFE, 10 µgmL-1) and prothrombin (THRB, 2 µgmL-1). (b,c) Volcano plot of protein enrichment over background (log2(+UV/-UV)) plotted against the statistical significance of this comparison (-log10(p-value)). Proteins meeting all selection criteria labelled in green. For EndoTAG-1, abundance plot of apoE and apoA1 within the +UV samples. (d) Competition assay of apolipoprotein E and A1 binding. Increasing concentrations (1:1 to 1:9 molar ratios) of unlabelled EndoTAG-1 liposomes were incubated, together with EndoTAG-1 liposomes containing IKS02 (5 mol%), in the above predefined mixture of purified human serum proteins. Captured apoE and apoA1 on the

surface of IKS02-labeled EndoTAG-1 liposomes were separated by SDS-PAGE and visualized by in-gel fluorescence (Cy5). Protein loading determined by Coomassie Blue (coom.). Protein structures were obtained from the protein data bank (PDB): (APOE: 2L7B, APOA1: 1AV1, ALBU: 1E78, THRB: 6C2W, TRFE: 1D3K). Illustrations were generated using Illustrate.

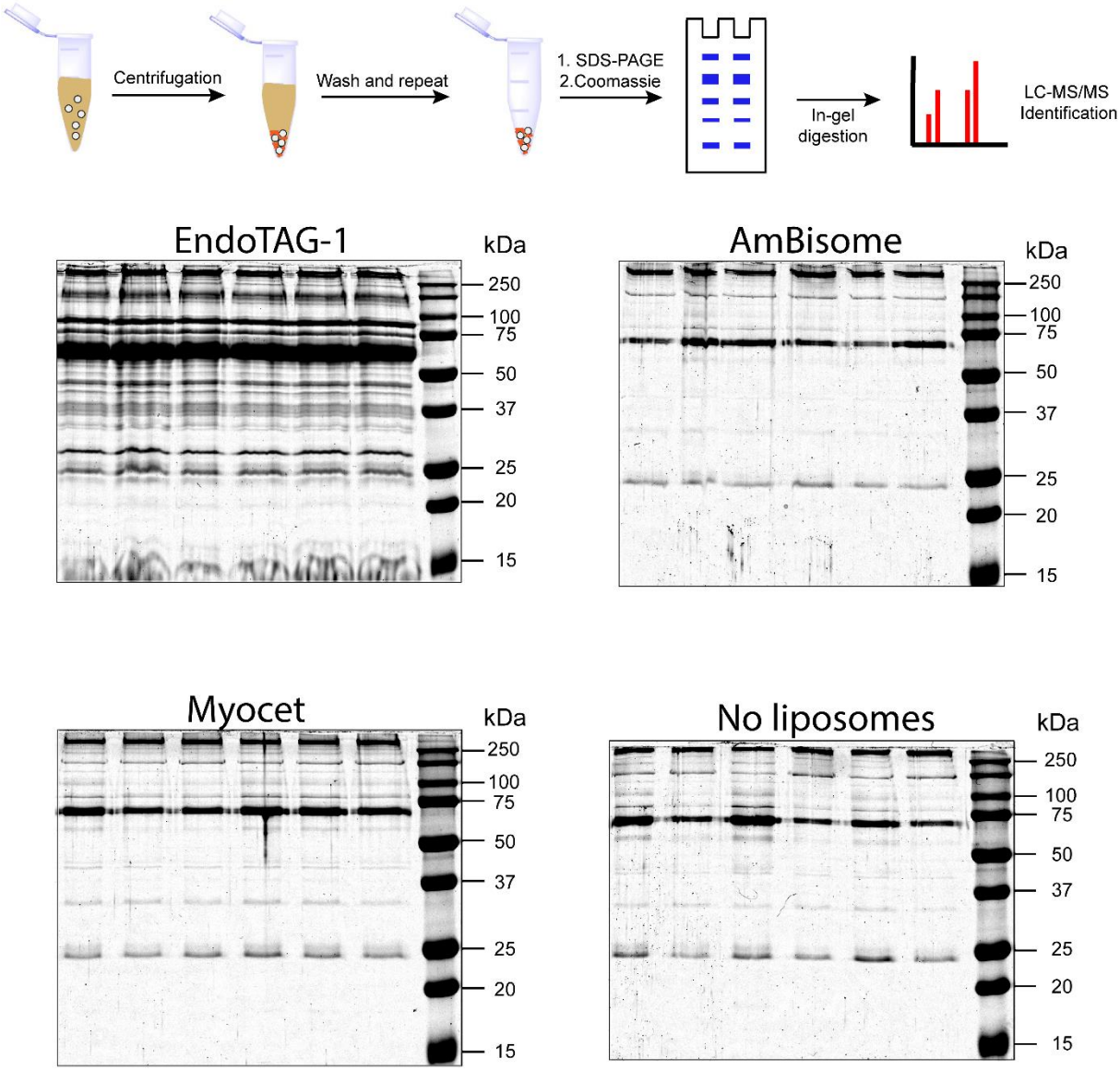


Figure S7. Gel electrophoresis (SDS-PAGE) of protein coronas isolated *via* centrifugation, displaying Coomassie Blue stained replicates (n=6) used for in-gel digestion. The total amount of liposome-protein complexes isolated by centrifugation were loaded in each lane without correction. Gels were run on a 10% polyacrylamide gel as described in the Biological Methods & Proteomics section.

Abundance	Protein name	Protein entry	IEP	MW (kDa)	average ppm
1	Serum albumin	ALBU	5,89	71	166624
2	Complement C3	CO3	5,99	189	125283
3	Immunoglobulin heavy constant mu	IGHM	6,37	50	42156
4	Apolipoprotein E	APOE	5,49	36	38054
5	Serotransferrin	TRFE	6,78	79	20767
6	Alpha-2-macroglobulin	A2MG	6,04	165	18900
7	Immunoglobulin heavy constant alpha 1	IGHA1	6,09	39	11451
8	Apolipoprotein A-IV	APOA4	5,12	45	10705
9	Apolipoprotein A-I	APOA1	5,44	31	9582
10	Clusterin	CLUS	5,87	53	6738
11	Haptoglobin	HPT	6,15	46	6114
12	Apolipoprotein D	APOD	4,87	22	2933

Table S4. Protein abundancy for the AmBisome protein corona from the centrifugation method determined with LFQ based on the TOP3 approach, analysed with the ISOQuant software.

Myocet					
Abundance	**Protein name**	**Protein entry**	**IEP**	**MW (kDa)**	**average ppm**
1	Serum albumin	ALBU	5.89	71	132524
2	Complement C3	CO3	5.99	189	61074
3	Pyruvate carboxylase_ mitochondrial	PYC	6.41	130	51995
4	Vitronectin	VTNC	5.45	55	51013
5	Apolipoprotein E	APOE	5.49	36	47754
6	Propionyl-CoA carboxylase alpha chain_ mitochondrial	PCCA	7.27	81	45110
7	Immunoglobulin heavy constant mu	IGHM	6.37	50	43059
8	Inter-alpha-trypsin inhibitor heavy chain H1	ITIH1	6.35	102	31785
9	Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2	6.43	107	29841
10	Prothrombin	THRB	5.54	72	28366
11	Alpha-2-macroglobulin	A2MG	6.04	165	17305
12	Serotransferrin	TRFE	6.78	79	16843
13	Apolipoprotein A-IV	APOA4	5.12	45	14697
14	Clusterin	CLUS	5.87	53	11392
15	Immunoglobulin heavy constant alpha 1	IGHA1	6.09	39	10089
16	Alpha-1-antitrypsin	A1AT	5.25	47	9162
17	Haptoglobin	HPT	6.15	46	7963
18	Serum paraoxonase/arylesterase 1	PON1	4.93	40	7590
19	Apolipoprotein A-I	APOA1	5.44	31	7056
20	Hyaluronan-binding protein 2	HABP2	6.11	65	5028
21	Apolipoprotein C-II	APOC2	4.44	11	4953
22	Heparin cofactor 2	HEP2	6.47	57	3657
23	Apolipoprotein D	APOD	4.87	22	1762
24	Dermcidin	DCD	6.14	11	1472

Table S5. Protein abundancy for the Myocet protein corona from the centrifugation method determined with LFQ based on the TOP3 approach, analysed with the ISOQuant software.

No liposomes

Abundance	Protein name	Protein entry	IEP	MW (kDa)	average ppm
1	Serum albumin	ALBU	5.89	71	111535
2	Complement C3	CO3	5.99	189	104869
3	Apolipoprotein E	APOE	5.49	36	35444
4	Serotransferrin	TRFE	6.78	79	28106
5	Alpha-2-macroglobulin	A2MG	6.04	165	21894
6	Immunoglobulin heavy constant alpha 1	IGHA1	6.09	39	21604
7	Alpha-1-antitrypsin	A1AT	5.25	47	20143
8	Immunoglobulin heavy constant mu	IGHM	6.37	50	19366
9	Apolipoprotein A-I	APOA1	5.44	31	18041
10	Immunoglobulin heavy constant gamma 1	IGHG1	8.2	37	17181
11	Apolipoprotein A-IV	APOA4	5.12	45	14567
12	Haptoglobin	HPT	6.15	46	12884
13	Clusterin	CLUS	5.87	53	12679
14	Complement C4-B	CO4B	6.9	194	8851
15	Complement C4-A	CO4A	6.68	194	8448
16	Vitronectin	VTNC	5.45	55	7579
17	Complement component C9	CO9	5.28	65	6327
18	Lactotransferrin	TRFL	8.02	80	5262
19	Apolipoprotein C-III	APOC3	5.06	11	5039
20	Lysozyme C	LYSC	9.34	17	4995
21	Gelsolin	GELS	5.87	86	4868
22	Apolipoprotein D	APOD	4.87	22	4548
23	Apolipoprotein L1	APOL1	5.49	44	4278
24	Alpha-1-antichymotrypsin	AACT	5.19	48	4244
25	Inter-alpha-trypsin inhibitor heavy chain H1	ITIH1	6.35	102	3949
26	Prothrombin	THRB	5.54	72	3850
27	Inter-alpha-trypsin inhibitor heavy chain H4	ITIH4	6.56	104	3623
28	Immunoglobulin kappa constant	IGKC	6.13	12	3404
29	Complement factor B	CFAB	6.69	87	3360
30	Serum paraoxonase/arylesterase 1	PON1	4.93	40	3319
31	Zinc-alpha-2-glycoprotein	ZA2G	5.66	34	2927
32	Dermcidin	DCD	6.14	11	2892
33	Apolipoprotein C-II	APOC2	4.44	11	2849
34	Kininogen-1	KNG1	6.38	73	2766
35	Polymeric immunoglobulin receptor	PIGR	5.44	84	2617
36	Desmoglein-1	DSG1	4.72	115	2596
37	Serum amyloid A-4 protein	SAA4	9.41	15	2372
38	Beta-2-glycoprotein 1	APOH	7.87	40	2063
39	Immunoglobulin heavy constant gamma 3	IGHG3	7.79	42	1923
40	Immunoglobulin J chain	IGJ	4.91	19	1624
41	Prolactin-inducible protein	PIP	8.1	17	1374
42	Cystatin-A	CYTA	5.22	11	1330
43	Annexin A1	ANXA1	6.67	39	1125
44	Zymogen granule protein 16 homolog B	ZG16B	7.56	23	1070
45	Hemoglobin subunit beta	HBB	6.91	16	1020
46	Apolipoprotein A-II	APOA2	6.64	11	993
47	Protein S100-A8	S10A8	6.65	11	726
48	Alpha-1-acid glycoprotein 1	A1AG1	4.74	24	657
49	Apolipoprotein C-IV	APOC4	9.13	15	653
50	Apolipoprotein C-I	APOC1	9.43	9	369

Table S6. Background protein abundancy of the centrifugation method, determined with LFQ based on the TOP3 approach, analysed with the ISOQuant software.

EndoTag

Abundance	Protein name	Protein entry	IEP	MW (kDa)	average ppm
1	Complement C3	CO3	5.99	189	107751
2	Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2	6.43	107	56604
3	Inter-alpha-trypsin inhibitor heavy chain H1	ITIH1	6.35	102	52182
4	Kininogen-1	KNG1	6.38	73	41911
5	Inter-alpha-trypsin inhibitor heavy chain H3	ITIH3	5.39	100	36208
6	Serum albumin	ALBU	5.89	71	36107
7	Prothrombin	THRB	5.54	72	36037
8	Clusterin	CLUS	5.87	53	30526
9	Complement C4-B	CO4B	6.9	194	30066
10	Serum paraoxonase/arylesterase 1	PON1	4.93	40	24734
11	Alpha-2-macroglobulin	A2MG	6.04	165	23372
12	Ceruloplasmin	CERU	5.35	123	20035
13	Alpha-1-antitrypsin	A1AT	5.25	47	19392
14	Antithrombin-III	ANT3	6.33	53	19378
15	Gelsolin	GELS	5.87	86	16063
16	Hyaluronan-binding protein 2	HABP2	6.11	65	14756
17	Apolipoprotein E	APOE	5.49	36	14519
18	Vitronectin	VTNC	5.45	55	14243
19	Immunoglobulin heavy constant mu	IGHM	6.37	50	13398
20	Complement C1s subcomponent	C1S	4.66	78	12534
21	Complement C4-A	CO4A	6.68	194	12532
22	Plasma protease C1 inhibitor	IC1	6.11	55	12190
23	Immunoglobulin heavy constant alpha 1	IGHA1	6.09	39	12126
24	Inter-alpha-trypsin inhibitor heavy chain H4	ITIH4	6.56	55	11681
25	Heparin cofactor 2	HEP2	6.47	57	11613
26	Immunoglobulin heavy constant gamma 1	IGHG1	8.2	37	11328
27	Apolipoprotein A-I	APOA1	5.44	31	10414
28	Complement C1r subcomponent	C1R	5.8	82	7903
29	Complement C5	CO5	6.11	190	7690
30	Histidine-rich glycoprotein	HRG	7.13	61	7685
31	Protein AMBP	AMBP	5.9	40	7498
32	Apolipoprotein A-IV	APOA4	5.12	45	7359
33	Apolipoprotein F	APOF	5.31	36	6835
34	Vitamin K-dependent protein Z	PROZ	5.59	46	6812
35	Lumican	LUM	6.19	39	6654
36	C-reactive protein	CRP	5.32	25	6423
37	Vitamin K-dependent protein C	PROC	5.85	53	6359
38	Coagulation factor IX	FA9	5.19	53	6183
39	Complement component C9	CO9	5.28	65	5813
40	Beta-Ala-His dipeptidase	CNDP1	4.98	57	5729
41	Vitamin K-dependent protein S	PROS	5.35	77	5542
42	Alpha-2-antiplasmin	A2AP	5.87	55	5530
43	Apolipoprotein M	APOM	5.63	22	5496
44	Serotransferrin	TRFE	6.78	79	4806
45	Haptoglobin	HPT	6.15	46	4769
46	Thrombospondin-1	TSP1	4.53	133	4165
47	Coagulation factor X	FA10	5.59	56	3970
48	Alpha-1-antichymotrypsin	AACT	5.19	48	3725
49	Immunoglobulin kappa constant	IGKC	6.13	12	3478
50	Phosphatidylcholine-sterol acyltransferase	LCAT	5.69	50	3298

Table S7. Protein abundancy for the EndoTAG-1 protein corona from the centrifugation method determined with LFQ based on the TOP3 approach, analysed with the ISOQuant software.

51	Complement factor H	CFAH	6.21	144	3160
52	Ficolin-3	FCN3	6.25	33	3065
53	Apolipoprotein D	APOD	4.87	22	2992
54	Angiotensinogen	ANGT	5.88	53	2944
55	Cartilage oligomeric matrix protein	COMP	4.16	85	2936
56	Protein Z-dependent protease inhibitor	ZPI	8.6	51	2681
57	Apolipoprotein A-II	APOA2	6.64	11	2512
58	Galectin-3-binding protein	LG3BP	4.95	66	2411
59	Lipopolysaccharide-binding protein	LBP	6.27	54	2373
60	Endoplasmin	ENPL	4.56	93	2312
61	C4b-binding protein alpha chain	C4BPA	7.02	69	2268
62	Secreted phosphoprotein 24	SPP24	8.39	25	2140
63	Apolipoprotein C-II	APOC2	4.44	11	2136
64	Thrombospondin-4	TSP4	4.25	109	2072
65	Haptoglobin-related protein	HPTR	6.71	40	1926
66	Plasma serine protease inhibitor	IPSP	9.75	46	1885
67	Apolipoprotein C-III	APOC3	5.06	11	1831
68	Platelet glycoprotein Ib alpha chain	GP1BA	5.87	72	1663
69	Selenoprotein P	SEPP1	7.72	43	1613
70	Mannan-binding lectin serine protease 1	MASP1	5.16	81	1608
71	CD5 antigen-like	CD5L	5.15	40	1454
72	Apolipoprotein L1	APOL1	5.49	44	1426
73	Complement C1q subcomponent subunit C	C1QC	8.54	26	1420
74	SPARC-like protein 1	SPRL1	4.53	76	1310
75	Hemopexin	HEMO	6.6	52	1299
76	Insulin-like growth factor-binding protein complex acid labile subunit	ALS	6.37	67	1292
77	Immunoglobulin heavy constant gamma 3	IGHG3	7.79	42	1283
78	Serum paraoxonase/lactonase 3	PON3	5.11	40	1244
79	Transthyretin	TTHY	5.42	16	1191
80	Alpha-1B-glycoprotein	A1BG	5.5	55	1184
81	Phosphatidylinositol-glycan-specific phospholipase D	PHLD	5.92	93	1124
82	Immunoglobulin heavy constant alpha 2	IGHA2	5.85	37	1085
83	Immunoglobulin heavy constant gamma 2	IGHG2	7.45	37	1085
84	Vitamin D-binding protein	VTDB	5.16	55	1066
85	Alpha-2-HS-glycoprotein	FETUA	5.35	40	1002
86	Complement C1q subcomponent subunit B	C1QB	8.87	27	996
87	Fermitin family homolog 3	URP2	6.57	77	955
88	Complement component C6	CO6	6.37	108	954
89	Complement factor B	CFAB	6.69	87	864
90	Carboxypeptidase N subunit 2	CPN2	5.59	61	854
91	Prenylcysteine oxidase 1	PCYOX	5.78	57	841
92	C4b-binding protein beta chain	C4BPB	4.87	29	772
93	14-3-3 protein zeta/delta	1433Z	4.53	28	561
94	Complement C1q subcomponent subunit A	C1QA	9.45	26	543
95	Pregnancy zone protein	PZP	5.96	165	530
96	Serum amyloid P-component	SAMP	6.13	26	527
97	Lysozyme C	LYSC	9.34	17	517
98	Kallistatin	KAIN	7.58	49	514
99	Immunoglobulin heavy constant gamma 4	IGHG4	7.13	36	455
100	Extracellular superoxide dismutase [Cu-Zn]	SODE	6.17	26	442
101	Immunoglobulin J chain	IGJ	4.91	19	402
102	N-acetylmuramoyl-L-alanine amidase	PGRP2	7.29	63	359
103	Beta-2-glycoprotein 1	APOH	7.87	40	342
104	Retinol-binding protein 4	RET4	5.68	23	200
105	Zinc-alpha-2-glycoprotein	ZA2G	5.66	34	200
106	Hemoglobin subunit beta	HBB	6.91	16	174
107	Prolactin-inducible protein	PIP	8.1	17	168
108	Alpha-1-acid glycoprotein 2	A1AG2	4.85	24	159
109	Dermcidin	DCD	6.14	11	130
110	Apolipoprotein C-IV	APOC4	9.13	15	110
111	Protein S100-A8	S10A8	6.65	11	90
112	Apolipoprotein C-I	APOC1	9.43	9	57

Table S7. Continued.

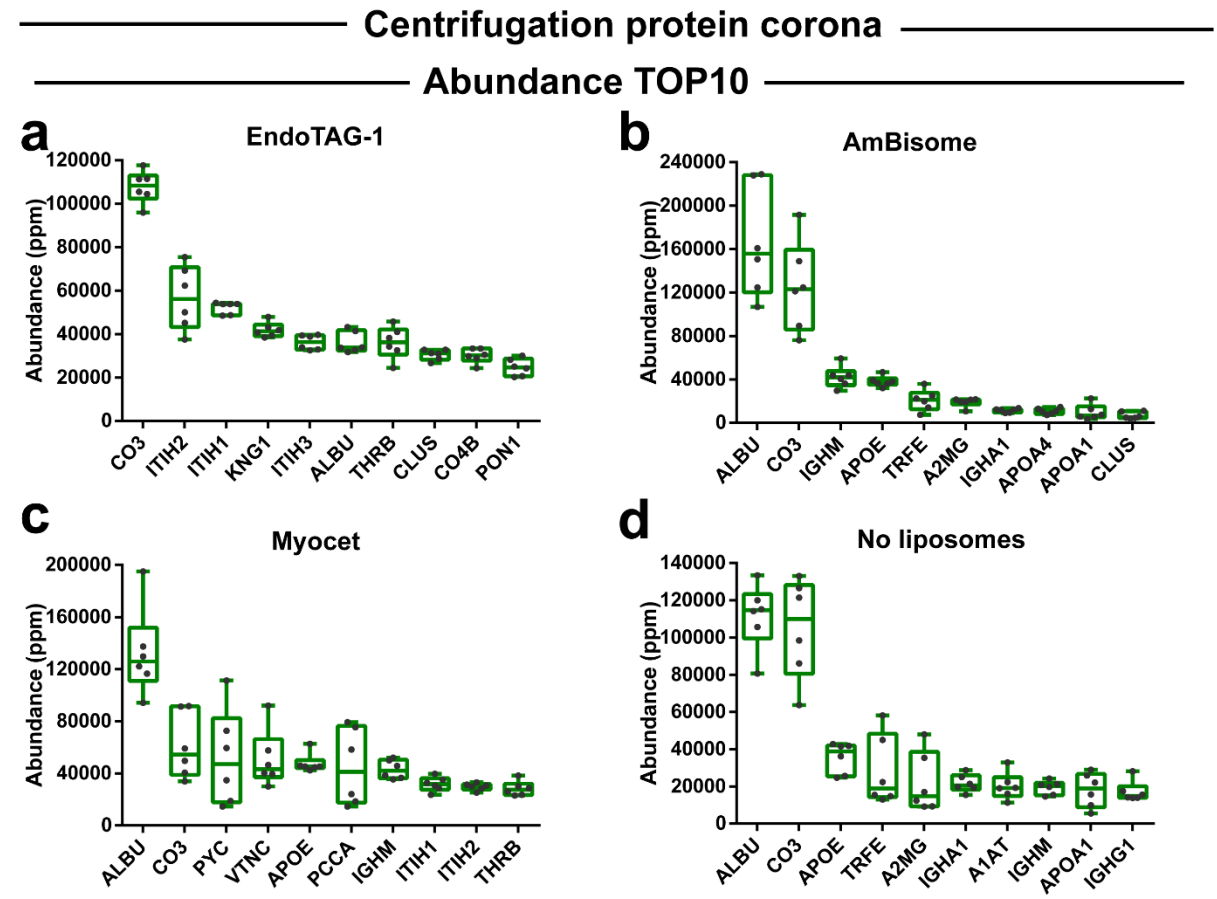


Figure S8. Top 10 most abundant proteins in the corona determined by the centrifugation method for each formulation as well as the negative control in which buffer without liposomes was added to the serum. Complete abundancy lists can be found as table format in Tables S4-7.

Chemical Synthesis

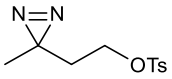
General

All solvents and reagents were obtained from common commercial sources (Sigma Aldrich, Acros Organics, Alfa Aesar, Fluka, Merck) and used as received without further purification, unless stated otherwise. All reactions were performed under a nitrogen atmosphere, unless stated otherwise. Column chromatography was performed using silica gel (40-63 µm, 60 Å, Screening Devices, The Netherlands) or high purity silica gel (40-63 µm, 60 Å, Sigma-Aldrich). TLC analysis was performed on Merck silica gel 60/Kieselguhr F_{254}, 0.25 mm TLC plates. Compounds were visualized by UV adsorption or $KMnO_4$ stain (K_2CO_3 (15 g), $KMnO_4$ (2 g), and H_2O (200 mL)). ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AV 400 MHz or 850 MHz spectrometer. Chemical shifts are reported in ppm (δ), relative to the deuterated solvent as internal standard. Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, br s= broad singlet, m = multiplet), coupling constants (J) reported in Hz. High resolution mass spectra were recorded by direct injection (2 µL of a 1 mM solution in methanol) using a mass spectrometer (Thermo Finnigan LTQ Orbitrap) with an electrospray ion source run in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C), and with a resolution R = 60,000 at m/z 400 (mass range m/z = 150-2,000) and dioctylphthalate (m/z = 391.28428) as a “lock mass”. The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). During the chemical synthesis of IKS02, no unexpected or unusually high safety hazards were encountered.

Chemical Procedures

Synthesis of diazirine and diacylglycerol building blocks

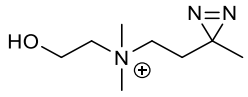
2-(3-methyl-3H-diazirin-3-yl)ethyl 4-methylbenzenesulfonate (1)[2]



To 7N methanolic ammonia (11.2 mL, 79 mmol, 7 eq.) was added 4-hydroxybutan-2-one (0.98 mL, 11.35 mmol, 1 eq.) at 0 °C under nitrogen atmosphere. After stirring at 0 °C for 2.5 hours, the solution turned dark yellow. To the solution was added hydroxylamine-O-sulfonic acid (1.48 g, 13.05 mmol, 1.1 eq.) in methanol (9.7 mL) dropwise. The solution turned light yellow and was stirred overnight at room temperature until a white suspension was formed. The solid was filtered off and the ammonia was evaporated by gently blowing nitrogen through the solution. The solution was cooled down to 0 °C and to the solution was added triethylamine (1.6 mL, 11.35 mmol, 1 eq.), then was added in portions molecular iodine (±2 g) until the brown colour persisted. After 2 hours, the solution was quenched by the addition of brine (40 ml) and extracted with diethyl ether (3x). The organic layers were combined, washed with sodium thiosulfate (1x) and brine (1x). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. To the crude was added pyridine (8 mL) and *p*-toluenesulfonylchloride (2.30 g, 12 mmol, 1.1 eq.). After stirring overnight at room temperature, the solution was poured onto ice (120 g). The solution was quenched with concentrated hydrogen chloride (10 mL), which was added dropwise. The mixture was extracted with diethyl ether (3x). The organic layers were combined and washed with saturated sodium bicarbonate (1x) and brine (1x). The collected organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. Flash column chromatography (silica gel, 8% ethyl acetate in petroleum ether) yielded **1** (640 mg, 2.50 mmol, 20%).

TLC: R_f = 0.4 (dichloromethane/methanol, 80:20 v/v). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 3.94 (t, J = 6.4 Hz, 2H), 2.45 (s, 3H), 1.66 (t, J = 6.4 Hz, 2H), 0.99 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 145.17, 130.04, 128.08, 125.37, 65.23, 34.27, 23.50, 21.78, 19.88.

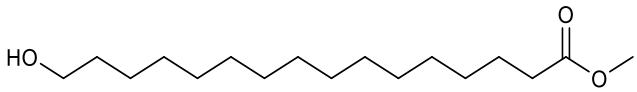
2-hydroxy-N,N-dimethyl-N-(2-(3-methyl-3H-diazirin-3-yl)ethyl)ethan-1-aminium (2)[2]



To a solution of **1** (200 mg, 0.78 mmol, 1.1 eq.) in acetonitrile (700 µl) was added 2-dimethylaminoethanol (72 µl, 0.71 mmol, 1 eq.). After stirring overnight at 80 °C, additional **13** (10 mg, 0.039 mmol, 0.55 eq.) was added. After stirring overnight at 80 °C, the mixture was concentrated under reduced pressure to give the yellow/brown solid **2** (134 mg, 0.78 mmol, quant).

^{1}H NMR (400 MHz, MeOD) δ 7.71 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 4.02 – 3.89 (m, 2H), 3.50 – 3.44 (m, 2H), 3.43 – 3.40 (m, 2H), 3.13 – 3.09 (m, 4H), 2.38 (s, 3H), 1.88 – 1.80 (m, 2H), 1.07 (s, 2H). **^{13}C NMR** (100 MHz, MeOD) δ 143.62, 141.73, 129.87, 126.93, 66.43, 61.33, 56.81, 52.33, 52.29, 52.25, 29.28, 21.31, 19.35, 0.81.

Methyl 16-hydroxyhexadecanoate (3)

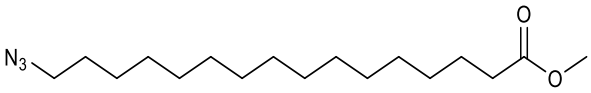


To a solution of 16-hydroxyhexadecanoic acid (1.70 g, 6.6 mmol, 1 eq.) in methanol (100 mL) was added acetyl chloride (3.24 mL, 44.7 mmol, 8 eq.) at 0 °C. After stirring overnight, additional acetyl chloride (3 mL, 41.4 mmol) was added. Monitoring by TLC showed complete conversion after 2 hours. The mixture was then concentrated under reduced pressure and dissolved in dichloromethane. The solution was washed with a saturated sodium bicarbonate solution (2x), water (2x) and brine (1x). Every aqueous phase was extracted with dichloromethane (1x). The organic layers were combined,

dried over anhydrous sodium sulfate and evaporated under reduced pressure, yielding **3** as a white solid (1.73 g, 6.06 mmol, 92%).

$\mathbf{R_f}$ = 0.8 (Pentanes/Ethyl acetate, 75:25 v/v). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.63 (t, J = 6.6 Hz, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.66 – 1.50 (m, 4H), 1.38 – 1.19 (m, 22H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 63.24, 51.60, 34.26, 32.94, 29.77, 29.76, 29.74, 29.72, 29.57, 29.39, 29.29, 25.87, 25.17, 25.10

Methyl 16-azidohexadecanoate (4)[3]

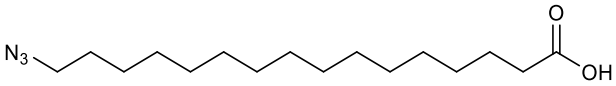


To a solution of **3** (1.70 g, 6.06 mmol) and triethylamine (5.07 mL, 36.4 mmol, 6 eq.).in methanol (100 mL) was added methanesulfonyl chloride (1.88 mL, 24.2 mmol, 4 eq.) dropwise at 0 °C. After addition, the mixture was allowed to warm to room temperature and monitoring by TLC showed complete conversion of the starting materials after 3 hours. The mixture was concentrated under reduced pressure, dissolved in dichloromethane and the solution was washed with a saturated sodium bicarbonate solution (1x), water (1x) and brine (1x). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure, yielding the mesylate intermediate which was taken to the next step without further purification.

To a solution of mesylate intermediate in N,N-dimethylformamide (40 mL) was added sodium azide (2.15 g, 33 mmol) and the solution was stirred at 70 °C for 2 hours. The mixture was concentrated under reduced pressure, dissolved in DCM and washed with water (3x), a saturated sodium bicarbonate solution (1x) and brine (1x). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. Flash column chromatography (silica gel,5-10% ethyl acetate in pentane) yielded **4** as a white solid (1.71 g, 5.51 mmol, 91%)

R_f = 0.8 (Pentanes/Ethyl acetate, 75:15 v/v). **^{1}H NMR** (400 MHz, CDCl3) δ 3.66 (s, 3H), 3.25 (t, *J* = 8 Hz, 2H), 2.29 (t, *J* = 8 Hz, 2H), 1.66 - 1.59 (m, 4H), 1.42- 1.25 (m, 22H). **^{13}C NMR** (100 MHz, CDCl3) δ 174.43, 51.61, 51.56, 34.24, 29.75, 29.71, 29.66, 29.61, 29.57, 29.38, 29.38, 28.96

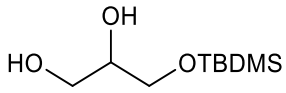
16-Azidohexadecanoic acid (5)[3]



To a solution of **4** (1.70 g, 5.49 mmol) in tetrahydrofuran and dioxane (1:1, 15 mL) was added a 4M NaOH solution (15 mL) and the reaction was stirred at room temperature overnight. The mixture was diluted with ethyl acetate (200 mL) and washed with a 1M HCl solution (2x), water (1x) and brine (1x). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure yielding **5** as a white solid (1.62 g, 5.21 mmol, 95%)

R_f = 0.2 (Pentanes/Ethyl acetate, 75:25 v/v). **^{1}H** NMR (400 MHz, CDCl3) δ 3.25 (t, *J* = 8 Hz, 2H), 2.34 (t, *J* = 8 Hz, 2H), 1.66 - 1.59 (m, 4H), 1.35- 1.25 (m, 22H). **^{13}C** NMR (100 MHz, CDCl3) δ 180.53, 51.60, 29.74, 29.70, 29.66, 29.60, 29.55, 29.36, 29.28, 29.17, 28.95, 26.84, 24.78

3-((*tert*-Butyldimethylsilyl)oxy)propane-1,2-diol (6)

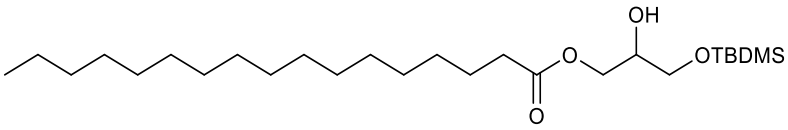


To a solution of *tert*-Butyldimethylsilyl chloride (1.0 gram, 6.6 mmol, 1 eq.) in dichloromethane (25 mL) was added dropwise a solution of glycerol (17.8 gram, 198.6 mmol, 30 eq.) and imidazole (1.35 gram, 19.8 mmol, 3 eq.) in dichloromethane (30 mL) and DMF (12 mL) at -18 °C. After stirring the solution for one hour at -18 °C, water (50 mL) was added. The resulting mixture was extracted with dichloromethane (3x). The organic layers were combined and washed with water (1x) and brine (1x), dried over anhydrous sodium sulfate and evaporated under reduced pressure. Flash column

chromatography (silica gel, 40% ethyl acetate in pentane) yielded **6** as a transparent oil (478 mg, 2.27 mmol, 34%).

R_f = 0.54 (Pentane/Ethyl acetate, 50:50 v/v). **^{1}H NMR** (400 MHz, CDCl3) δ 3.81 – 3.55 (m, 5H), 0.90 (s, 9H), 0.08 (s, 6H). **^{13}C NMR** (100 MHz, CDCl3) δ 71.71, 64.89, 64.35, 64.19, 25.98, 18.39, -5.34.

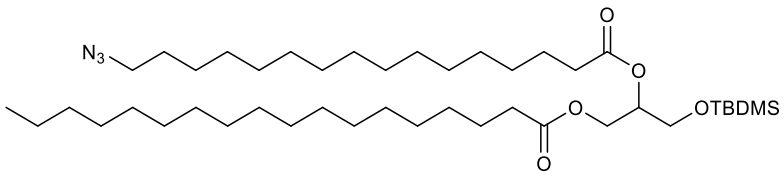
3-((*tert*-Butyldimethylsilyl)oxy)-2-hydroxypropyl propionate (7)



To a solution of stearic acid (295 mg, 1.04 mmol, 0.7 eq.) in dichloromethane (8 mL) were added N,N'-dicyclohexylcarbodiimide (257 mg, 1.04 mmol, 0.7 eq.) and 4-dimethylaminopyridine (90 mg, 0.74 mmol, 0.5 eq.). After stirring for 30 minutes at room temperature, the solution was cooled down to 0 °C. To the cooled solution was added 5 (310 mg, 1.48 mmol, 1 eq.). The solution was stirred at 0 °C for 30 min, allowed to warm up to room temperature and stirred overnight. The formed suspension was filtered and the filtrate was washed with saturated sodium bicarbonate solution (2x), water (2x) and brine (1x). The separate aqueous layers were extracted with dichloromethane (1x). The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. Flash column chromatography (silica gel, 6% ethyl acetate in pentane), yielded 6 as a mixture of regioisomers (2°:1° = 7:43, determined by 1H-NMR) (374 mg, 0.790 mmol, 76%).

R_f = 0.65 & 0.7 (Pentane/Ethyl acetate, 80:20 v/v). **^{1}H NMR** (400 MHz, CDCl3) δ 4.19 – 4.05 (m, 1H), 3.92 – 3.84 (m, 1H), 3.84 – 3.72 (m, 1H), 3.67 (dd, J = 10.1, 4.6 Hz, 1H), 3.60 (dd, J = 10.1, 5.6 Hz, 1H), 2.33 (t, J = 7.6 Hz, 2H), 1.70 – 1.55 (m, 2H), 1.25 (s, 28H), 0.94 – 0.77 (m, 12H), 0.07 (d, J = 2.6 Hz, 6H). **^{13}C NMR** (100 MHz, CDCl3) δ 174.13, 74.38, 65.12, 63.81, 63.02, 62.70, 34.55, 34.34, 32.07, 29.84, 29.82, 29.80, 29.75, 29.61, 29.51, 29.42, 29.29, 25.97, 25.09, 22.84, 14.27, -5.33.

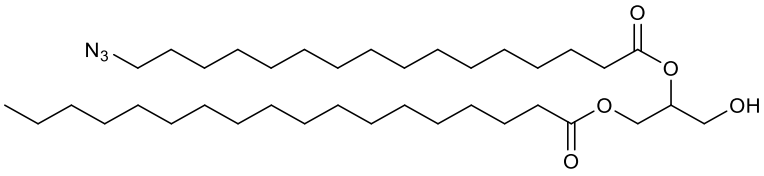
2-((16-Azidohexadecanoyl)oxy)-3-((tert-butyldimethylsilyl)oxy)propyl stearate (8)



To a solution of **5** (221 mg, 0.746 mmol, 1.05 eq.) in dichloromethane (8 mL), were added N,N'-dicyclohexylcarbodiimide (185 mg, 0.746 mmol, 1.05 eq.) and 4-dimethylaminopyridine (65 mg, 0.530 mmol, 0.75 eq.). After stirring for 30 minutes at room temperature, **7** was added (336 mg, 0.751 mmol, 1 eq.) and the solution was stirred overnight. The formed suspension was filtered and the organic phase was washed with saturated sodium bicarbonate solution (2x), water (2x) and brine (1x). The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. Flash column chromatography (silica gel, 1.5% ethyl acetate in pentane) yielded **12** (340 mg, 0.452 mmol, 64%) as a mixture of regioisomers.

$\mathbf{R_f}$ = 0.3 (Pentane/Ethyl acetate, 95:5 v/v). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.17 – 4.95 (m, 1H), 4.33 (dd, J = 11.8, 3.7 Hz, 1H), 4.15 (dd, J = 11.8, 6.3 Hz, 1H), 3.79 – 3.54 (m, 2H), 3.25 (t, J = 7.0 Hz, 2H), 2.29 (dd, J = 7.9, 7.1, 2.1 Hz, 4H), 1.68 – 1.51 (m, 6H), 1.25 (s, 50H), 0.96 – 0.76 (m, 12H), 0.07 (s, 6H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.61, 173.26, 71.80, 62.58, 61.58, 51.62, 34.49, 34.30, 32.07, 29.84, 29.81, 29.79, 29.77, 29.69, 29.63, 29.51, 29.44, 29.30, 29.27, 29.25, 28.98, 26.86, 25.08, 25.05, 22.83, 14.26, -5.36 (d, J = 4.2 Hz).

2-((16-Azidohexadecanoyl)oxy)-3-hydroxypropyl stearate (9)



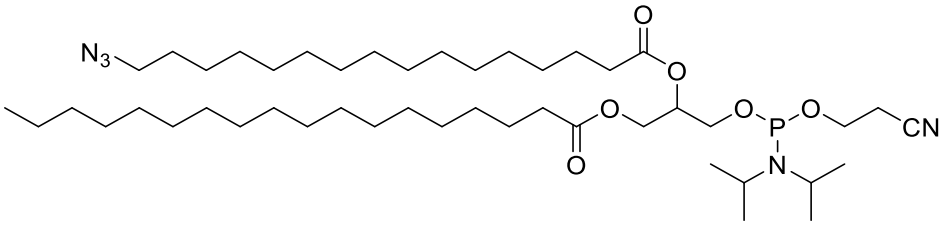
To a solution of **8** (340 mg, 0.452 mmol, 1 eq.) in acetonitrile: tetrahydrofuran (1:1, 8 mL) was added triethylamine trihydrofluoride (0.74 mL, 4.52 mmol, 10 eq.). After stirring overnight at room temperature, the solution was quenched on ice with a saturated sodium bicarbonate solution. After

extraction with dichloromethane (4x), the organic layers were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Flash column chromatography (silica gel, 20% ethyl acetate in pentane) yielded **9** (289 mg, 0.452 mmol, quant) as a mixture of regioisomers.

R_f = 0.8 (Pentane/Ethyl acetate, 80:20 v/v). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.08 (p, J = 5.0 Hz, 1H), 4.32 (dd, J = 11.9, 4.5 Hz, 1H), 4.24 (dd, J = 11.9, 5.6 Hz, 1H), 4.21 – 4.13 (m, 1H), 3.75 – 3.71 (m, 1H), 3.25 (t, J = 7.0 Hz, 2H), 2.38 – 2.29 (m, 4H), 1.66 – 1.55 (m, 8H), 1.35 – 1.21 (m, 48H), 0.88 (t, J = 6.4 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 72.24, 62.11, 61.71, 51.64, 34.44, 34.26, 32.08, 29.85, 29.81, 29.79, 29.77, 29.69, 29.63, 29.52, 29.42, 29.40, 29.31, 29.27, 29.24, 28.99, 26.87, 25.09, 25.04, 22.85, 14.28.

Phosphoramidite synthesis of IKS

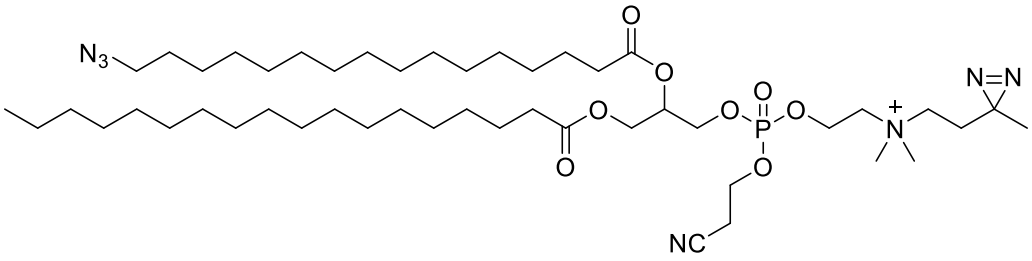
2-((16-Azidohexadecanoyl)oxy)-3-(((2-cyanoethoxy)(diisopropylamino) phosphanyl)oxy)propyl stearate (10)



A solution of **13** (200 mg, 0.313 mmol, 1 eq.) and diisopropylethylamine (329 µl, 1.88 mmol, 6 eq.) in dry dichloromethane (5 mL) was dried over freshly oven-dried 3Å molecular sieves and stored under nitrogen atmosphere. The mixture was transferred to a dry flask under nitrogen atmosphere and to the solution was added 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (250 mg, 1.056 mmol, 3 eq.). After stirring for 1.5 hours the solution was concentrated under reduced pressure until 600 mbar. Flash column chromatography, (high purity silica gel pre-treated with 5% triethylamine in pentane, 3% ethyl acetate and 3% Et_3N in pentane) yielded **16** (194 mg, 0.231 mmol, 74%)**.** The product was stored in 20% triethylamine in dichloromethane (2 mL) under nitrogen atmosphere overnight. For the next reaction, the product was concentrated under reduced pressure until 60 mbar for maximum 10 minutes.

R_f = 0.6 (Pentane/Ethyl acetate/Et_3N, 90:7:3 v/v/v)). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.29 – 4.06 (m, 2H), 3.92 – 3.72 (m, 2H), 3.67 – 3.54 (m, 1H),3.25 (t, J = 7.0 Hz, 1H), 2.63 (td, J = 6.5, 2.3 Hz, 2H), 2.54 (q, J = 7.2 Hz, 4H), 2.30 (tt, J = 7.0, 3.5 Hz, 4H), 1.68 – 1.54 (m, 6H), 1.32 – 1.18 (m, 50H), 1.17 (q, J = 2.9 Hz, 12H), 0.87 (t, J = 6.8 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.61, 69.68, 69.52, 64.03, 58.64, 58.46, 51.63, 46.38, 43.43, 43.31, 34.30 (d, J = 4.3 Hz), 32.07, 29.82 (d, J = 4.6 Hz), 29.66 (d, J = 5.9 Hz), 29.51, 29.45, 29.30, 28.98, 26.86, 25.03, 24.75, 24.65, 24.57, 22.83, 14.27, 11.69. ^{31}P NMR (162 MHz, $CDCl_3$) δ 150.08, 149.50.

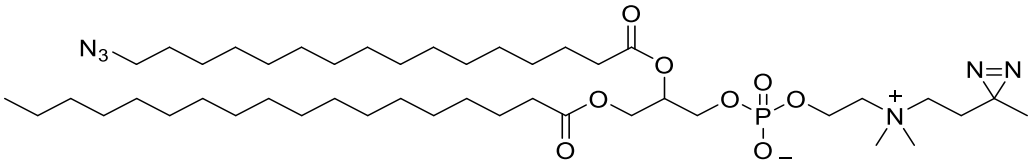
2-(((2-((16-Azidohexadecanoyl)oxy)-3-(stearoyloxy)propoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N-dimethyl-N-(2-(3-methyl-3H-diazirin-3-yl)ethyl)ethan-1-aminium (11)



A solution of **10** (194 mg, 0.231 mmol, 1 eq.) in dry dichloromethane (5 mL) was dried over freshly oven-dried 3Å molecular sieves under nitrogen atmosphere. To the solution were added **2** (79 mg, 0.231 mmol, 1 eq.) and tetrazole (1.03 mL, 0.462 mmol, 2 eq.). After stirring for 45 minutes, additional tetrazole (0.51 mL, 0.231 mmol, 1eq.) and **15** (20 mg, 0.058 mmol, 0.25 eq.) were added. After 1 hour, ^{31}P-NMR indicated complete conversion of the starting material (main peak shifted from 150 ppm to 140 ppm) to the solution was added *tert*-Butyl hydroperoxide (66 µL, 0.347 mmol, 1.5 eq.). After 45 minutes, ^{31}P-NMR showed oxidation was complete (peak shifted from 140 ppm to -3 ppm), the solution was diluted with dichloromethane and washed with saturated sodium bicarbonate (1x) and brine (1x). The collected organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. Flash column chromatography (high purity silica gel, 10% methanol in dichloromethane) yielded **11** (20.5 mg, 5.54 µmol, 10%).

R_f = 0.3 (dichloromethane/methanol, 80:20 v/v). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.88 – 4.63 (m, 2H), 4.50 – 4.31 (m, 3H), 4.27 – 4.12 (m, 4H), 3.65 (d, J = 5.4 Hz, 2H), 3.40 (s, 6H), 3.25 (t, J = 7.0 Hz, 2H), 3.11 (qd, J = 7.3, 4.8 Hz, 4H), 2.90 (s, 2H), 2.43 – 2.26 (m, 4H), 1.90 (s, 2H), 1.65 – 1.52 (m, 6H), 1.25 (s, 50H), 1.17 (s, 3H), 0.87 (t, J = 6.7 Hz, 3H). **^{31}P NMR** (162 MHz, $CDCl_3$) δ -2.68, -3.33. **ESI-HRMS** (*m/z*) $C_{48}H_{91}N_7O_8P^+$: $[M]^+$ calculated: 925.27, found: 923.98.

2-((16-Azidohexadecanoyl)oxy)-3-(stearoyloxy)propyl (2-(dimethyl(2-(3-methyl-3H-diazirin-3-yl)ethyl)ammonio)ethyl) phosphate (IKS02)



To a solution of **17** (10.26 mg, 11.08 µmol) in dry dichloromethane (3 mL) was added a mixture of *tert*-butylamine and dichloromethane (1:1, 100 µL) and the mixture was stirred at room temperature for 3 hours. The solution was concentrated under reduced pressure. The product was purified with column chromatography (high purity silica gel, 17% methanol in dichloromethane) and yielded **IKS02** as a white solid (5.66 mg, 5.32 µmol, 48%).

$\mathbf{R_f}$ = 0.3 (dichloromethane/methanol, 70:30 v/v). **^{1}H NMR** (850 MHz, $CDCl_3$) δ 4.55 (s, 1H), 4.40 (s, 3H), 4.27 (dd, J = 11.7, 5.3 Hz, 2H), 4.24 (dd, J = 11.5, 4.6 Hz, 2H), 4.01 (s, 1H), 3.86 (s, 2H), 3.58 (t, J = 8.4 Hz, 2H), 3.30 (s, 6H), 3.25 (t, J = 7.0 Hz, 2H), 2.31 (t, J = 7.7 Hz, 6H), 1.87 (q, J = 8.8 Hz, 2H), 1.59 (h, J = 7.1 Hz, 6H), 1.33 – 1.18 (m, 48H), 1.15 (s, 3H), 0.88 (t, J = 7.1 Hz, 3H). **^{31}P NMR** (162 MHz, $CDCl_3$) δ -1.82. **ESI-HRMS** (*m/z*) $C_{45}H_{87}N_6O_8P$: $[M]^+$ calculated: 871.1780, found: 871.63958.

Biological Methods & Proteomics

General

All solvents and reagents were obtained from common commercial sources (Sigma Aldrich, Acros Organics, Alfa Aesar, Fluka, Merck) and used without further purification, unless stated otherwise. Dynamic light scattering and zeta potential measurements were performed on a Malvern Zetasizer Nano ZS. For light irradiation, a CaproBox™ (Caprotec Bioanalytics GmbH) was used with a wavelength of 350 nm and applying a 300 nm light filter. Human serum was purchased from Sigma-Aldrich (Non Heat Inactivated, Human Male AB plasma, USA origin, sterile-filtered, product code: H4522) with a protein concentration of 60.2 µg/µl determined by a Pierce BCA Protein Assay Kit (Thermo Scientific). The serum was aliquoted, snap-frozen with liquid nitrogen and stored for a maximum of 6 months at -80 °C. Albumin from human serum (SRP6182), Human transferrin (T3309) and recombinant human apolipoprotein E3 (SRP4696) were purchased from Sigma-Aldrich. Human prothrombin (RP-43087) was purchased from Thermo-Fisher Scientific. Recombinant human Apolipoprotein A1 (ab50239) was purchased from Abcam B.V. (Amsterdam, The Netherlands).

Evaporation of solvents with a vacuum centrifuge was performed using an Eppendorf speedvac (Eppendorf Concentrator Plus 5301). Sequencing grade modified trypsin was purchased from Promega (product code = V5111). Acetonitrile (LC-MS grade) and methanol (LC-MS grade) were purchased from Biosolve. Formic acid (LC-MS grade) was purchased from Actu-All Chemicals. BioSpin columns were purchased from Bo-Rad. The Empore C18 47-mm extraction disks (model 2215) were purchased from 3M™ Purification. Enolase digest standard was purchased from Waters MassPREP™.

Throughout the biological and proteomic methods, no unexpected or unusually high safety hazards were encountered.

Biological and Proteomic Procedures

Liposome preparation

Lipids were combined from stock solutions (10 mM in $CHCl_3$:MeOH 1:1 v/v) at the desired molar ratios. The solvents were evaporated under a nitrogen flow and traces of solvents were removed *in vacuo* for at least 30 minutes. Lipid films were hydrated with the desired volume of 20 mM HEPES (pH 7.4), vortexed and warmed to 65 °C for 5 minutes. The mixture was extruded thirteen times through two stacked 100 nm polycarbonate membranes (Nucleopore Track-Etch, Whatman) using an Avanti Mini Extruder (Avanti Polar Lipids). Size and surface charge were measured by Dynamic Light Scattering (DLS) and Zeta Potential measurement and liposomes were stored in the dark at 4 °C for no longer than two weeks.

Photoaffinity method

Incubation, crosslinking and click chemistry

Liposomes containing the photoaffinity probe (25 µL, 5 mM) were added to pre-warmed human serum (37 °C, 25 µL, 60.26 mg/ml protein) and incubated in the dark at 37 °C for 1 hour. For every liposome formulation, twelve replicates were prepared. Half of the replicates were irradiated with 350 nm light for 15 minutes, while cooling. The other replicates were kept at room temperature in the dark for 15 minutes. Afterwards, the liposomes were solubilized by addition of 10 µL 0.2% Triton X-100 in ultrapure water and incubation for 30 minutes. The samples were diluted by adding 140 µL of 0.1% SDS in ultrapure water. Aliquots of 100 µL were taken and protein precipitation was performed according to Wessel and Flügge.[4] Briefly, ultrapure water (400 µL), methanol (650 µL), chloroform (200 µL) and ultrapure water (150 µL) were added sequentially, followed by vigorous vortexing and centrifugation (3000 *g,* 10 min, rt). The liquid layers were removed, the pellet resuspended with methanol (600 µL) and centrifuged (14,000 *g*, 5 min, rt). The supernatant was discarded and the pellet was dissolved in HEPES buffer containing 0.5% SDS (200 µL, 100 mM, pH 8.0).

A BCA assay was performed to determine the protein concentration and the samples were diluted to a volume of 450 µL with HEPES buffer with 0.5% SDS (100 mM, pH 8.0) with a protein concentration of 0.5-1.0 mg/mL. For each protein sample, click reagent mixture (50 µL) was added from a 10x concentrated stock to give a final concentration of 100 µM $CuSO_4$, 1000 µM sodium ascorbate, 500 µM THPTA, 5000 µM aminoguanidine and 20 µM Cy5-alkyne or Biotin-alkyne, followed by incubation at room temperature for 1 hour. Methanol (650 µL), chloroform (150 µL) and ultrapure water (150 µL) were added sequentially, the mixture vortexed and centrifuged (3000 *g*, 10 min, rt). The liquid layers were removed, resuspended with methanol (600 µL) and centrifuged (14,000 *g*, 5 min, rt). The pellet was air-dried at room temperature for 5-10 minutes and resuspended in freshly prepared denaturing buffer (250 µL, 6 M urea, 25 mM NH_4HCO_3) and used for in-gel fluorescence imaging or enrichment. Alternatively, samples were snap-frozen with liquid nitrogen and stored for no longer than 2 weeks at -80 °C.

SDS-Page and in-gel fluorescence imaging

Protein concentration was determined by BCA assay prior to loading samples for in-gel fluorescence. To a volume corresponding to 10 µg of protein was added Laemmli buffer (4x stock) and the proteins were resolved on a 12.5% PA gel at 180 V. The subset of fluorescent proteins was imaged on a Typhoon FLA 9500 (GE Healthcare), followed by staining of all the proteins with Coomassie Brilliant Blue R-250 staining solution (Bio-Rad) and imaging on a ChemiDoc MP system (Bio-Rad).

Reduction and alkylation

To lipid-protein samples conjugated to biotin, 5 µL (1 M DTT; 20 mM final concentration) was added. Samples were vortexed, centrifuged and incubated at 56 °C while shaking (600 rpm) for 30 minutes. The samples were allowed to cool down to room temperature, after which 40 µl 0.5 M iodoacetamide (80 mM final concentration) was added and the samples incubated at room temperature in the dark for 30 minutes. Afterwards, 20 µL 1 M DTT (100 mM final concentration) was added and the samples

were vortexed and incubated at 56 °C for 5 minutes. Reduced and alkylated proteins were used directly for avidin bead enrichment.

Enrichment and on-bead digestion

Avidin agarose beads (50% slurry, 100 µL per sample, Thermo Fisher Scientific) were washed three times with PBS (10 mL PBS per 400 µL slurry), centrifuging at 2500 *g* for 3 minutes. The beads were resuspended in PBS (1 mL PBS per 100 µL slurry) and divided over 15 mL tubes in 1 mL fractions. An additional 2 mL PBS was added to each tube, after which the denatured and alkylated protein samples were added and the samples were shaken gently in an overhead shaker at RT for at least 3 hours. Beads were pelleted (2,500 g, 5 min) and the supernatant discarded. The beads were washed twice with SDS in PBS (0.5% w/v, 10 mL), three times with PBS (10 mL) and twice with ultrapure water (10 mL). In between each washing step, the samples were vortexed, centrifuged (2,500 g, 5 min) and the supernatants were discarded. The washed beads were resuspended in 250 µL on-bead digestion buffer (100 mM TRIS pH 8.0, 100 mM NaCl, 1 mM $CaCl_2$ and 2% v/v acetonitrile (LC-MS grade)) and transferred to 1.5 mL low-binding Eppendorf tubes, after which 10 µL 0.1 µg/µL trypsin was added and the samples were incubated at 37 °C while shaking (950 rpm) overnight. To the samples was added 12.5 µL formic acid, after which they were loaded onto Bio-Spin columns (Bio-Rad) and the flow-through was collected by centrifugation (2,500 g, 2 min) in low-binding Eppendorf tubes. The samples were desalted using the StageTips procedure described below.

Protein binding validation experiment

Human serum albumin (ALBU, 25 µg), transferrin (TRFE, 10 µg), apolipoprotein A1 (APOA1, 2 µg), apolipoprotein E3 (APOE, 2 µg) and prothrombin (THRB, 2 µg) were mixed in a total volume of 17.5 µL (PBS) for each replicate. To each replicate was added liposomes containing IKS02 (7.5 µL, 5 mM). For competition experiments, liposomes without IKS02 added were according to the competitive ratio (5 mM, 1:1 = 7.5 µL, 1:4 = 30 µL, 1:9 = 67.5 µL). The mixture was incubated at 37 °C for 1 hour followed

by liposome solubilisation with 1% Triton X-100 (5 µL). Proteins were precipitated by addition of ultrapure water, up to a volume of 100 µL, methanol (100 µL) and chloroform (50 µL), followed by vigorous vortexing and centrifugation (3000 g, 10 min, rt). The liquid layers were removed, the pellet resuspended with methanol (200 µL) and centrifuged (14,000 g, 5 min, rt). The supernatant was discarded and the pellet was dissolved in HEPES buffer (45 µL, 100 mM, pH 8.0). For each protein sample, click reagent mixture (5 µL) was added from a 10x concentrated stock to give a final concentration of 100 µM CuSO4, 1000 µM sodium ascorbate, 500 µM THPTA, 5000 µM aminoguanidine and 20 µM Cy5-alkyne or Biotin-alkyne, followed by incubation at room temperature for 1 hour. Protein precipitation was repeated as prior to the click reaction and the pellet was dissolved in PBS (50 µL) from which an aliquot was taken to perform a BCA assay. For in-gel fluorescence measurement, aliquots containing 10 µg protein were analysed by SDS-PAGE and in-gel fluorescence as described before. For MS/MS experiments, aliquots containing 20 µg protein were taken for reduction and alkylation and further steps as described before.

Centrifugation method

Centrifugation, washing and SDS-PAGE

The centrifugation method for protein corona determination was performed as previously described.[5–7] Briefly, human serum (100 µL, 60.26 µg/µL protein) was thawed on ice and warmed to 37 °C prior to incubation with liposomes (100 µL, 1 mg/mL) at 37 °C in low-binding Eppendorf tubes for one hour. The samples were centrifuged (17,500 g, 15 min) and the supernatant was discarded. The pellets were washed by dissolving in PBS (100 µL, pH 7.4) and centrifugation (17,500 g, 15 min). This washing step was performed two more times, after which the pellets were dissolved in 1% SDS containing Laemmli buffer (20 µL), denatured at 95 °C for 5 minutes and resolved on a 12.5% poly acrylamide gel. The gel was fixed and stained using Coomassie Brilliant Blue R-250 staining solution,

imaged on a ChemiDoc MP system (Bio-Rad) followed by in-gel reduction, alkylation and digestion as described below.

In-gel reduction, alkylation and digestion

The SDS-PAGE gel lanes were cut in in pieces of approximately 3 mm and transferred to 1.5 mL low-binding Eppendorf tubes. The gel pieces were washed with 25 mM NH_4HCO_3/acetonitrile (95:5, v/v) (400 µL) for 30 minutes and twice with 50 mM NH_4HCO_3/acetonitrile (50:50 v/v, 400 µL) for 30 minutes. The gel pieces were dehydrated by the addition of acetonitrile (300 µL, 10 min), after which the liquids were removed and the gel pieces were dried with a vacuum centrifuge. The gel pieces were hydrated with a 10 mM DTT in 100 mM NH_4HCO_3 solution (200 µL) and incubated at 56 °C for 1 hr. The excess liquid was removed, 55 mM IAA in 100 mM NH_4HCO_3 (200 µL) added and the solution incubated at room temperature in the dark for 45 minutes. The gel pieces were subsequently washed with 100 mM NH_4HCO_3 (200 µL) for 10 minutes and acetonitrile (200 µL) for 10 minutes. These washing steps were repeated two more times and the pieces were dried with a vacuum centrifuge.

The gel pieces were hydrated with digestion buffer (200 µL, 5 ng/µL trypsin in 50 mM NH_4HCO_3/acetonitrile 90:10 v/v) and incubated at 37 °C overnight. Formic acid in 50 mM NH_4HCO_3 (100 µL, 5:95 v/v) was added and the supernatants of the corresponding gel lanes were combined. To the gel pieces was added a solution of acetonitrile/50 mM NH_4HCO_3/formic acid (50:45:5 v/v, 100 µL) followed by incubation at room temperature for 45 minutes. The gel pieces were sonicated for 5 minutes and the supernatants were combined with the previous supernatants of the corresponding gel lanes. This last extraction step was performed one more time. Finally, a solution of acetonitrile/50 mM NH_4HCO_3/formic acid (90:5:5 v/v, 100 µL) was added and incubated at room temperature for 5 minutes. The supernatants were combined and dried using a vacuum centrifuge. The protein digests were dissolved in 100 µL StageTip solution A (0.5% (v/v) formic acid in ultrapure water) and desalted, using the StageTip procedure described below, before analysis by UPLC MS/MS.

In-solution reduction, alkylation and digestion

Six aliquots of human serum (20 µL, 60.26 mg/ml protein) were precipitated according to Wessel and Flügge[4]. Briefly, ultrapure water (480 µL), methanol (650 µL), chloroform (200 µL) and ultrapure water (150 µL) were added sequentially, followed by vigorous vortexing and centrifugation (3000 *g,* 10 min, RT). The liquid layers were removed, the pellet resuspended with methanol (600 µL) and centrifuged (14,000 *g*, 5 min, RT). The supernatant was discarded and the pellet was dissolved in freshly prepared denaturing buffer (250 µL, 6M Urea and 25 mM $NaHCO_3$). A BCA assay was performed to determine the protein concentration and aliquots were taken corresponding to 100 µg of protein, followed by dilution to 100 µL with denaturing buffer. To the sample was added 5 µL 0.2 M DTT and the sample was incubated at 56 °C for 30 minutes, followed by the addition of 25 µL 0.2 M Iodoacetamide and incubation at room temperature for 30 minutes. An additional 20 µL 0.2 M DTT was added and the sample was incubated at 56 °C for 5 minutes. Aliquots of 22.5 µL (15 µg of protein) were transferred to low-binding Eppendorf tubes and diluted to 200 µL with digestion buffer (100 mM TRIS pH 8.0, 100 mM NaCl, 1 mM $CaCl_2$ and 2% v/v acetonitrile (LC-MS grade)), to reduce the urea concentration to ~0.6 M, after which 3 µL, 0.1 µg/µL trypsin (1:50 w/w trypsin:protein) was added and the samples were incubated at 37 °C while shaking (950 rpm) overnight. After digestion, 10 µL formic acid was added and the samples were desalted using the StageTip procedure described below.

StageTip desalting

The protein digest desalting procedure was conducted as previously described.[8] Briefly, C_{18} extraction disks (47 mm) were placed in 200 µL pipette tips. These StageTips were conditioned, loaded, washed and eluted, following the scheme below. The eluted fractions were collected into low-binding Eppendorf tubes, dried using a vacuum centrifuge and stored at -20 °C or immediately prepared for UPLC-MS/MS measurements.

STAGE	BUFFER
Conditioning 1	50 µL MeOH (LC-MS grade)
Conditioning 2	50 µL StageTip solution B: 0.5% (v/v) formic acid, 80% (v/v) acetonitrile and 19.5% ultrapure water
Conditioning 3	50 µL StageTip solution A: 0.5% (v/v) formic acid in ultrapure water
Loading	Sample
Washing	100 µL StageTip solution A
Elution	100 µL StageTip solution B

NanoUPLC-MS/MS analysis

LC-MS was performed as described previously.[9] Peptide samples were dissolved in 50 µL LC-MS sample solution (ultrapure water:acetonitrile:formic acid 97:3:0.1) containing 10 fmol/µL enolase digest as an internal standard for label-free quantification. DMSO was not added to the LC solvents. Instead, a lower source temperature (80 °C instead of 100 °C) was used. A trap–elute protocol was used, in which a digest is loaded on a trap column and eluted and separated on the analytical column. The samples were brought on this trap column at a flow rate of 10 µl/min with 99.5% solvent A for 2 min, after which the column was switched to the analytical column. The peptide separation was achieved using a multistep concave gradient based on the gradients described elsewhere.[10] After washing with 90% solvent B, the column was re-equilibrated to initial conditions.

The detailed protocol is specified below:

TIME (MIN)	GRADIENT COMPOSITION (%B)	FLOW RATE (NL/MIN)
0	1.0	300
2.4	1.0	300
4.2	5.0	300
10.2	7.6	300

15.6	10.3	300
21	13.1	300
25.8	16.1	300
30.6	19.2	300
35.4	22.4	300
40.2	25.7	300
45	29.1	300
49.8	32.6	300
54	36.2	300
58.2	40.0	300
58.8	90.0	300
60.3	90.0	300
61.2	90.0	300
61.5	1.0	300
70.8	1.0	300

The rear seals of the pump were flushed every 30 min with 10% (v/v) ACN. [Glu1]-fibrinopeptide B (GluFib) was used as a lock mass compound. The auxiliary pump of the LC system was used to deliver this peptide to the reference sprayer (0.2 µl/min). As MS acquisition method, UDMS^{e} method was set up as described previously.[10] Briefly, these settings include that the mass range was set from 50 to 2,000 Da, with a scan time of 0.6 s in positive resolution mode. To be able to use the low-energy MS mode, the collision energy was set to 4 V in the trap cell. Besides, the transfer cell collision energy was ramped using drift-time-specific collision energies for the elevated energy scan[11]. The lock mass was sampled every 30 s.

MS acquisition method

The Synapt G2Si mass spectrometer (Waters) operating with Masslynx for acquisition and PLGS for peptide identification was used for analysis. The following settings in positive resolution mode were

used: source temperature of 80°C, capillary voltage 3.0 kV, nano flow gas of 0.25 Bar, purge gas 250 L/h, trap gas flow 2.0 ml/min, cone gas 100 L/h, sampling cone 25V, source offset 25, lock mass acquiring was done with a mixture of Leu Enk (556.2771) and Glu Fib (785.84265), lock spray voltage 2.5 kV, Glufib fragmentation was used as calibrant. An UDMS^{e} data-independent acquisition method was used for analysis. Briefly, the mass range is set from 50 to 2,000 Da with a scan time of 0.6 seconds in positive, resolution mode. The collision energy is set to 4 V in the trap cell for low-energy MS mode. For the elevated energy scan, the transfer cell collision energy is ramped to higher collision energies and data is recorded. The lock mass was sampled every 30 seconds and used for accurate determination of parent ions mass after peak picking. The PLGS search engine was used for peptide identification against the Uniprot human database to which the streptavidin, avidin, yeast enolase and trypsin sequences were manually added. The ISOQuant software[10] was used for label free quantification of proteins using 50 fmol of yeast enolase digest as benchmark.

Proteomic analysis

Configuration parameters for label-free quantification (LFQ) in the ISOQuant software are listed in Supplementary Table 2. For quantification, +UV and –UV replicates for all samples were compared in separate groups. The protein lists were filtered by excluding proteins that are considered as contamination (e.g. keratins), non-endogenous (e.g. trypsin, avidin) or non-reproducible (not present in six out of six +UV or centrifugation samples). For the volcano plots, the ratio of average ppm for each protein was calculated and is displayed as a logarithmic value (^{2}log). Furthermore, the p-value was determined by multiple t-tests comparing the replicates of each group using the GraphPad Prism software. In addition, a Benjamini-Hochberg correction was applied to adjust the p-value for multiple comparisons. The final adjusted p-value is displayed as a logarithmic value (^{10}log). Proteins that were exclusive for +UV samples or did not occur more than once in the –UV samples, making a t-test impossible, were labelled as ‘exclusive’ and are listed next to the volcano plot. Abundance plots were generated by plotting the ppm values of all six replicates. Similar statistical analysis was performed for

validation experiments, with slight modifications: (1) the number of replicates here was four, but proteins still had to be present in four out of four replicates. (2) A Benjamini-Hochberg correction was no longer performed as the processing did not require a high amount of comparisons for the t-test. Instead, p-values were directly taken from t-tests and displayed as the logarithmic value (^{10}log). Absolute quantification was achieved from the same LFQ in ISOQuant, based on a comparison to the internal standard (ENLS digest, 50 fmol). The proteins that passed the criteria for the volcano plots were selected. The average absolute amount of these proteins in the +UV samples was corrected for the average in the –UV samples. For heat map construction, the sum of ppm values for all 'accepted' proteins within the sample was taken and the relative abundance of every protein was calculated as a ratio expressed in percentages of this value. For the photoaffinity method, the proteins within the enrichment and p-value boundaries of the volcano plot were considered as 'accepted'. For the centrifugation method, all besides the initially filtered proteins were considered as 'accepted'. Fully processed proteomic data for all samples is provided in two separate excel spreadsheets.

The mass spectrometry proteomics data have also been deposited to the ProteomeXchange Consortium via the PRIDE[12] partner repository with the dataset identifier PXD016229.

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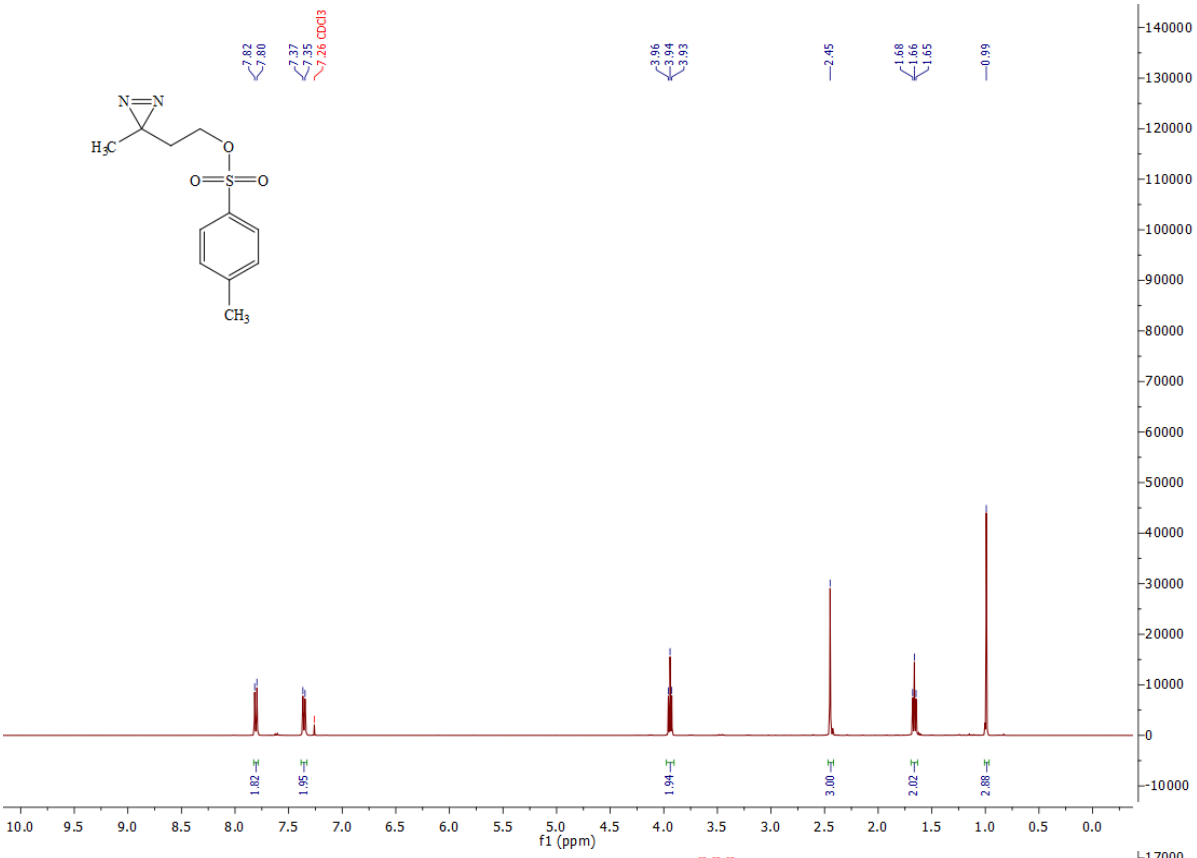
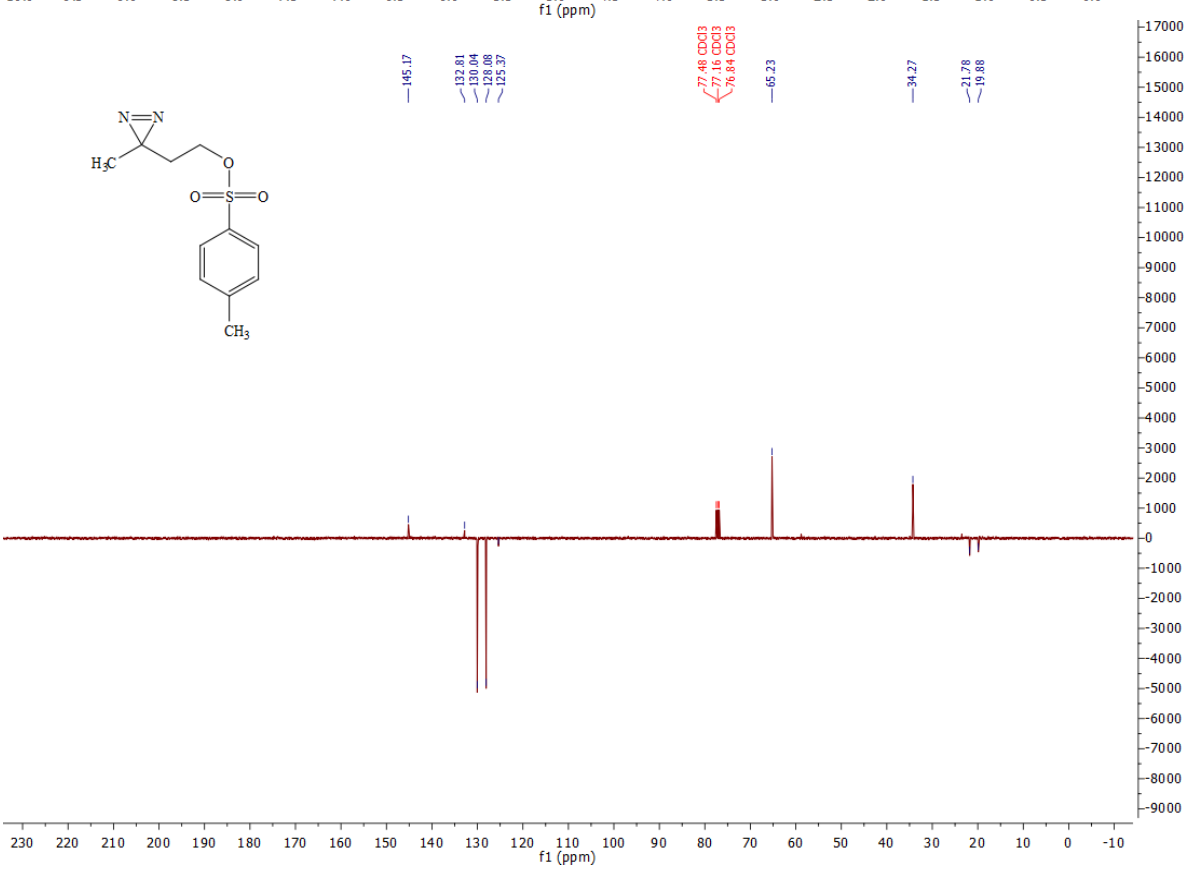
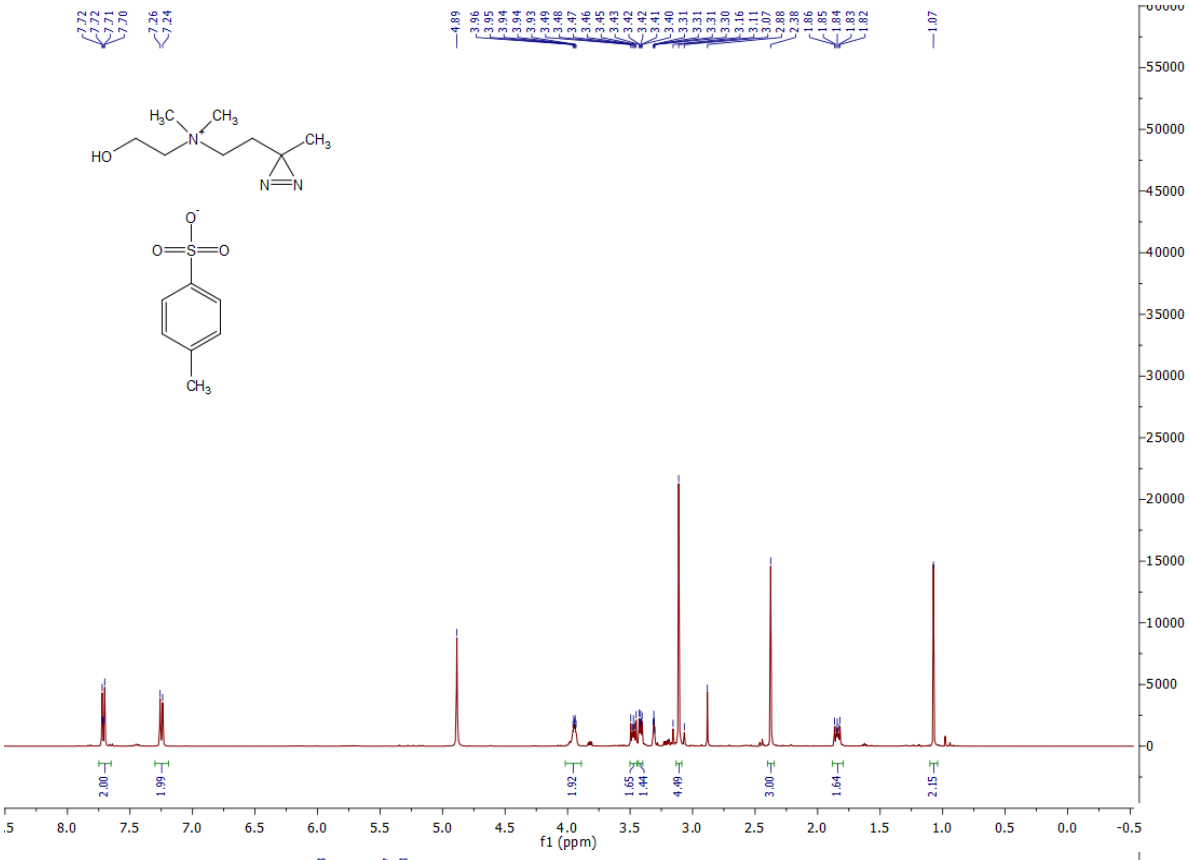
NMR and MS spectra



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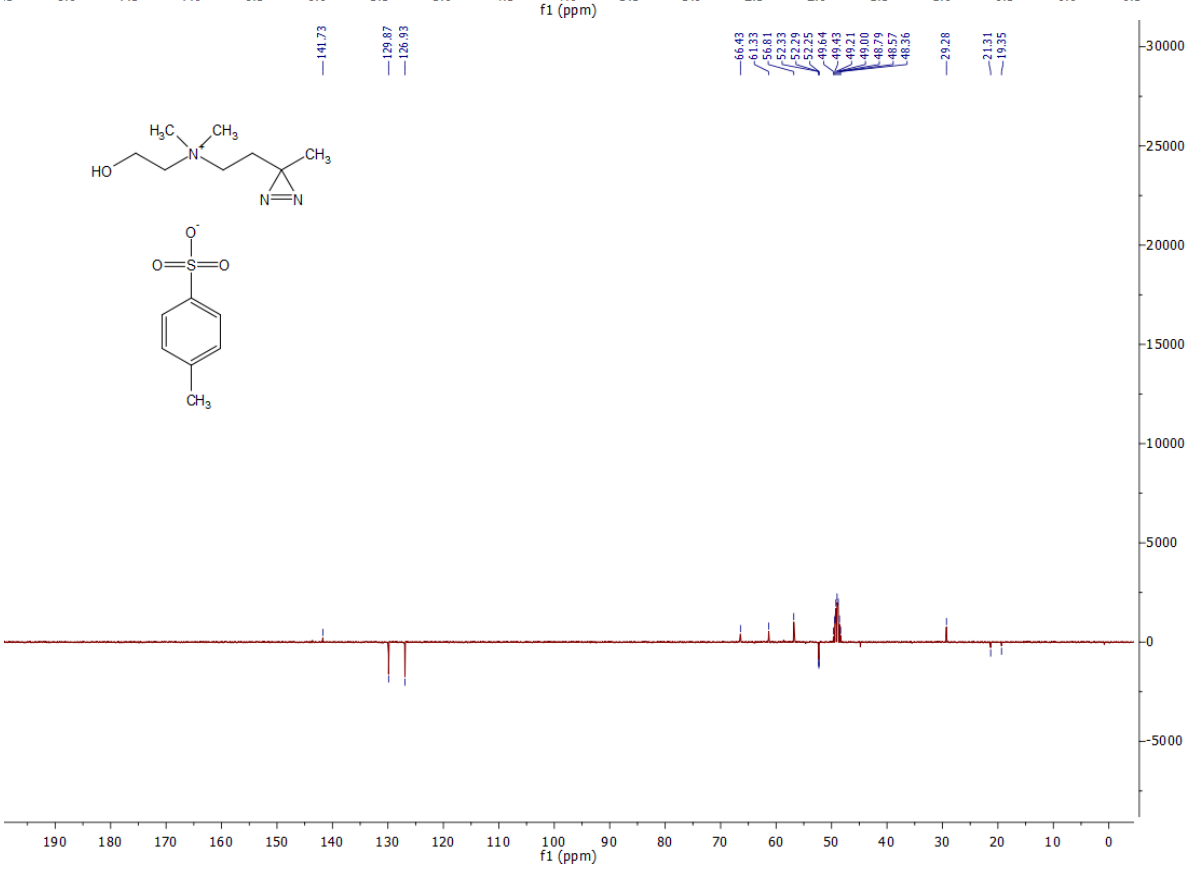
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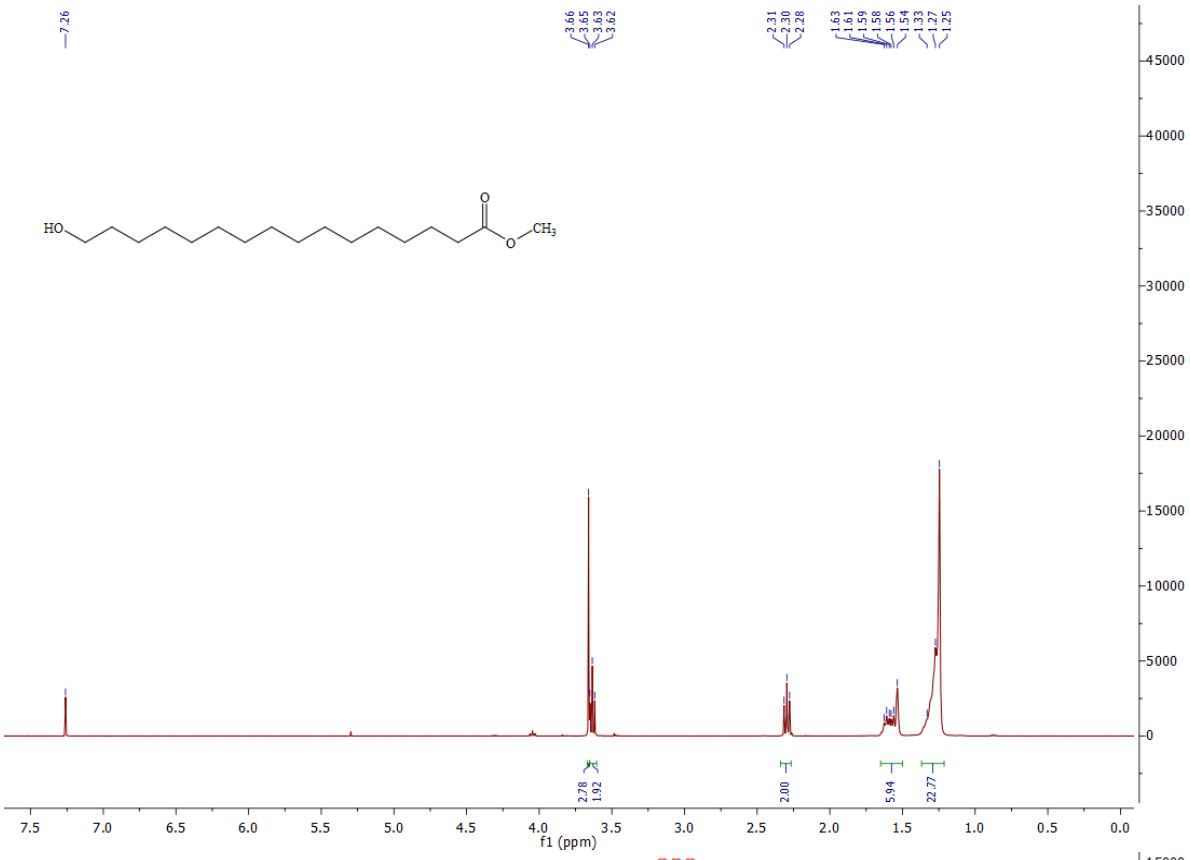
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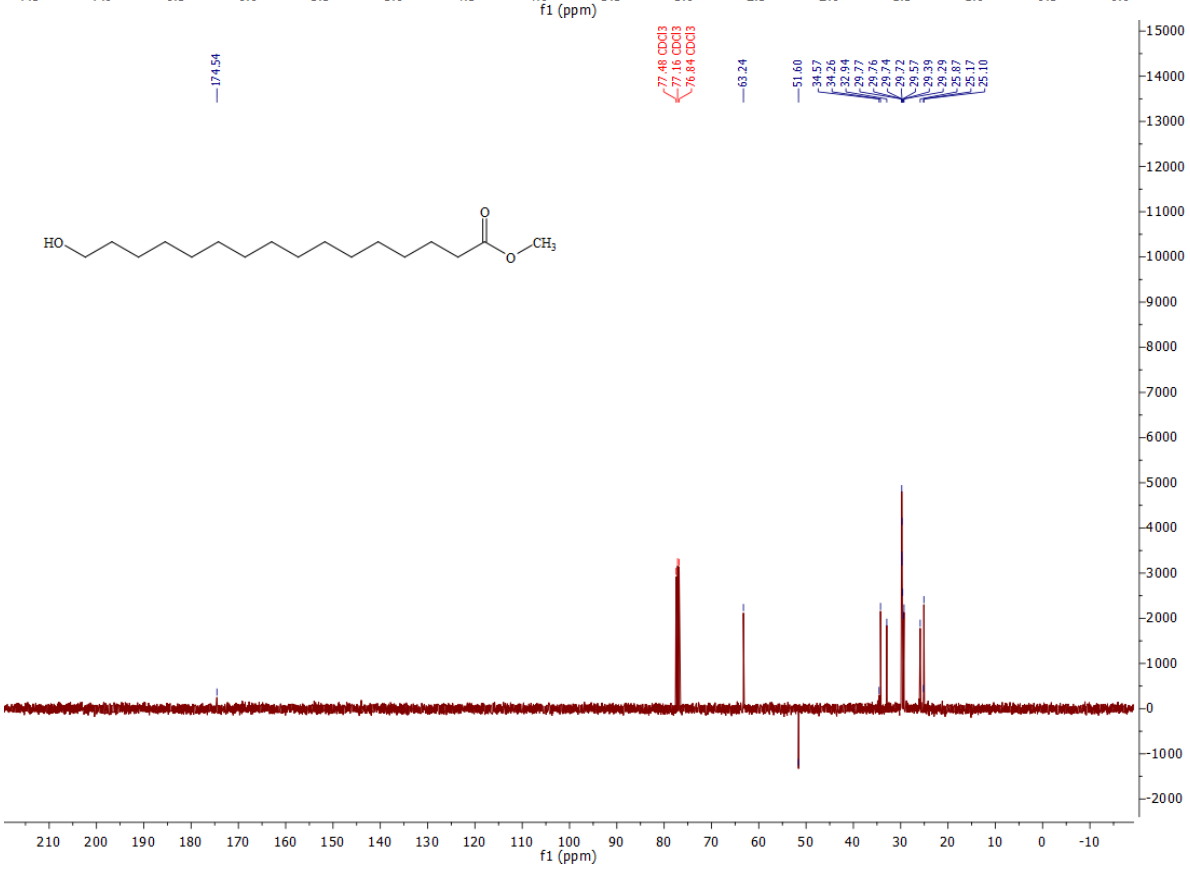
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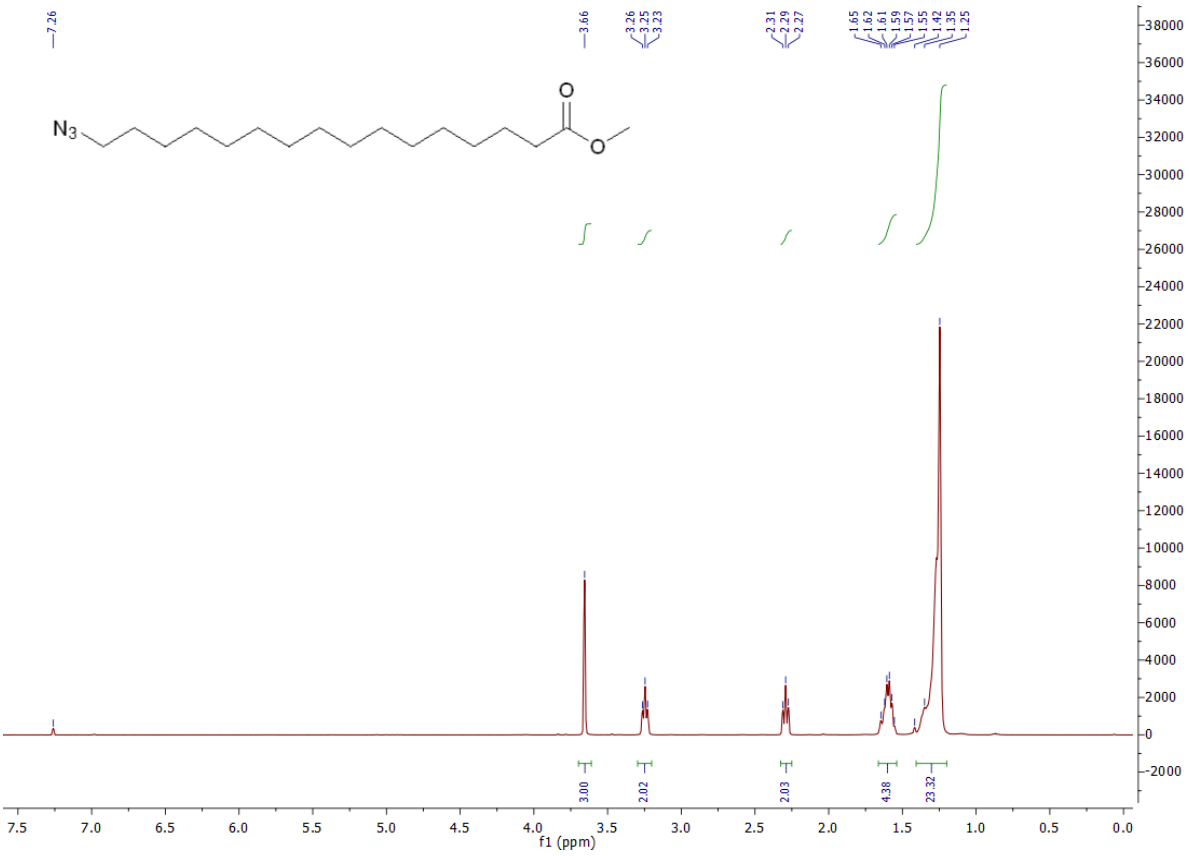
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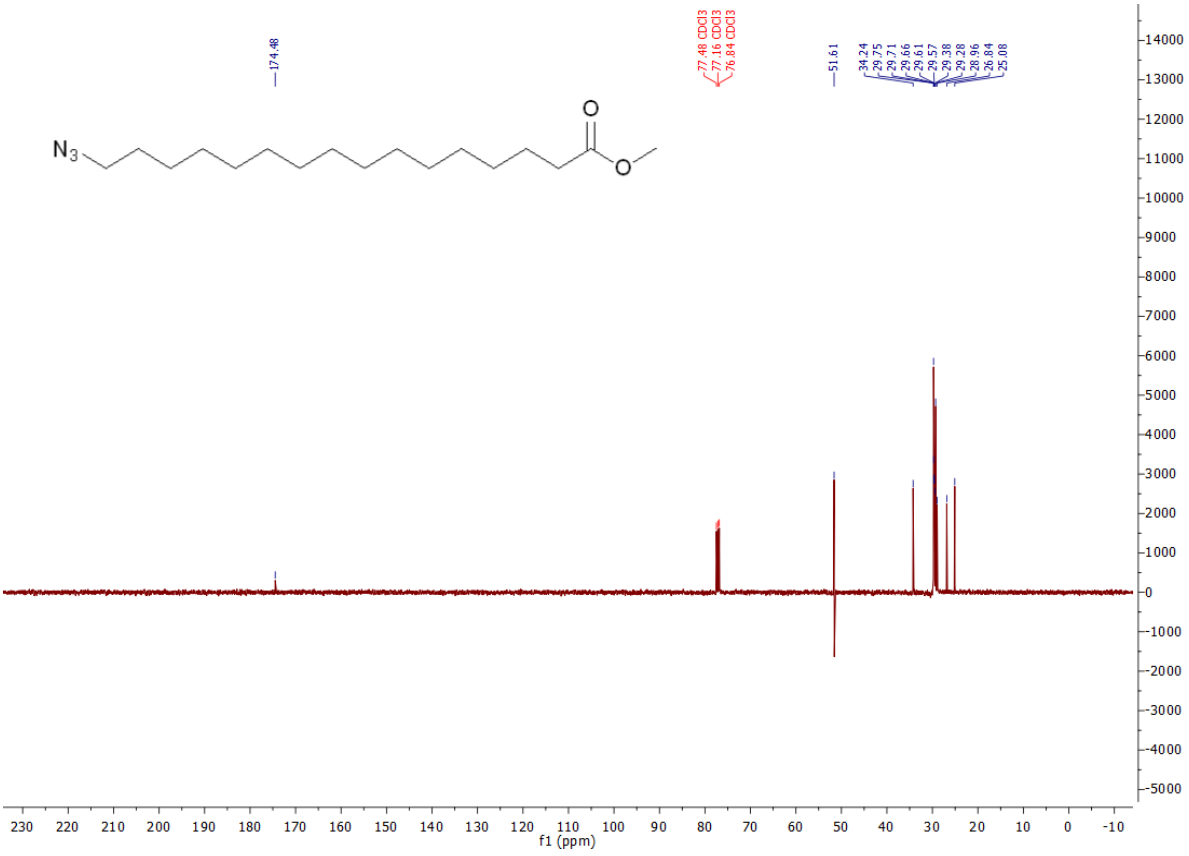
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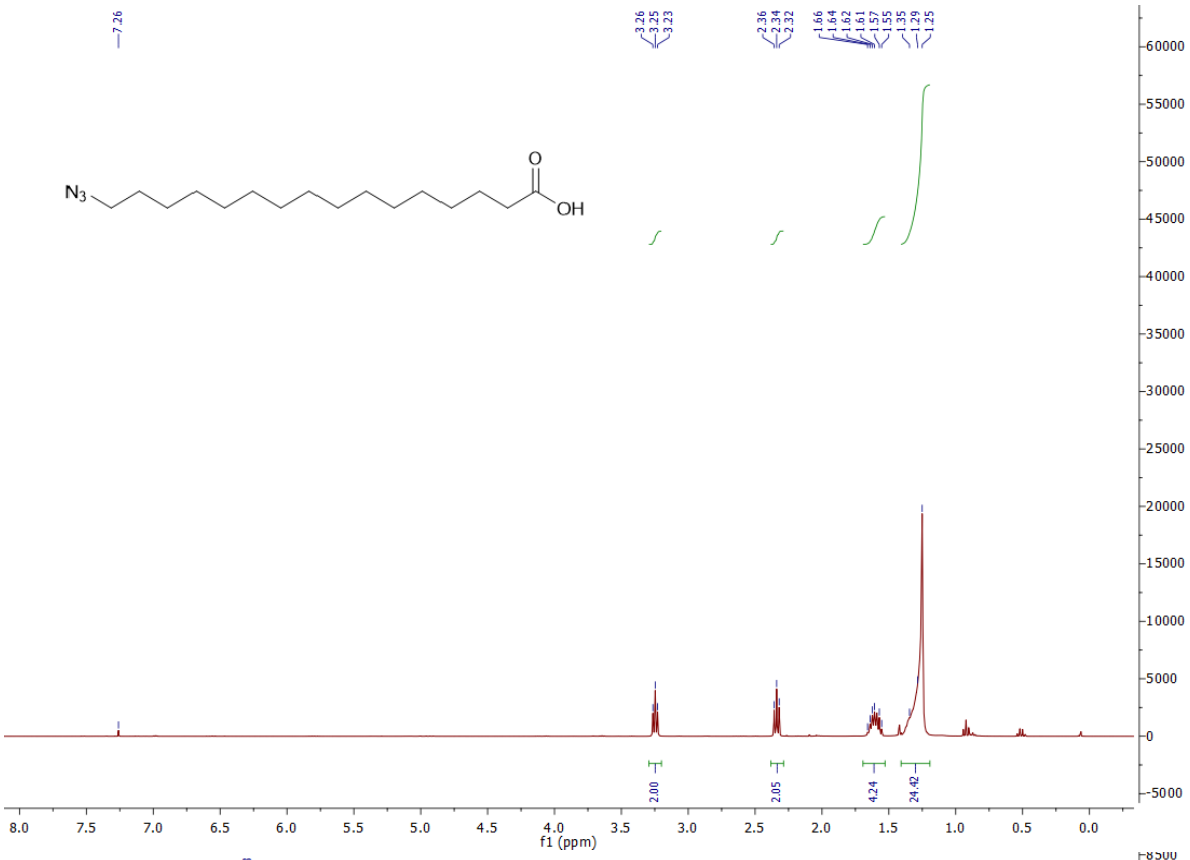
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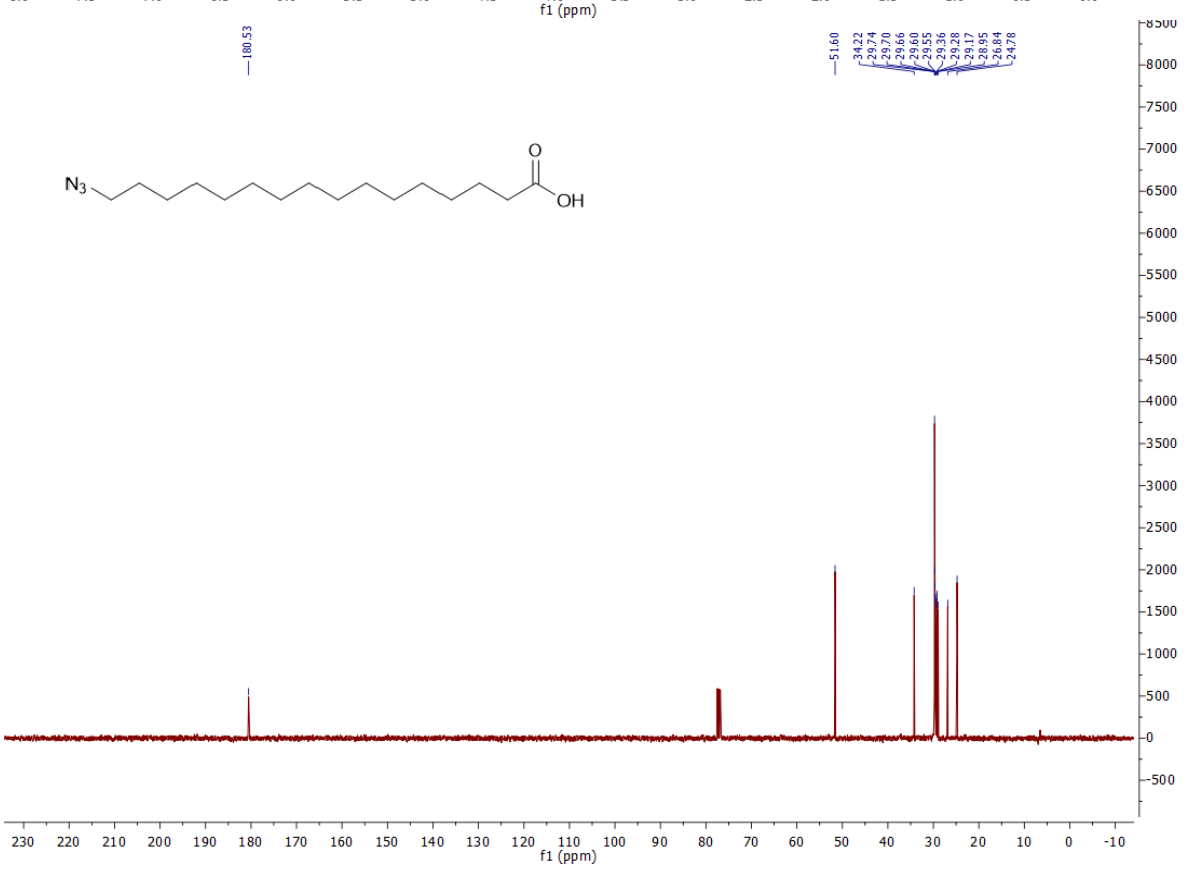
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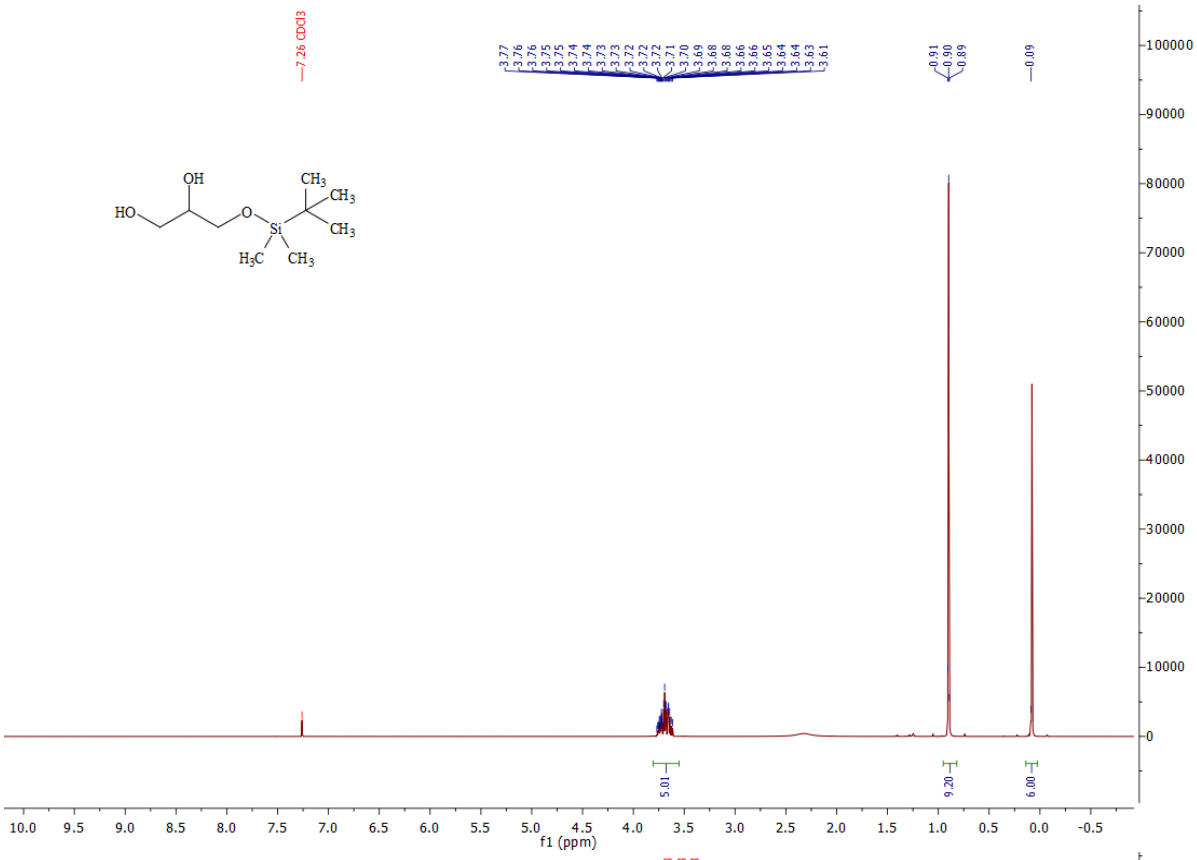
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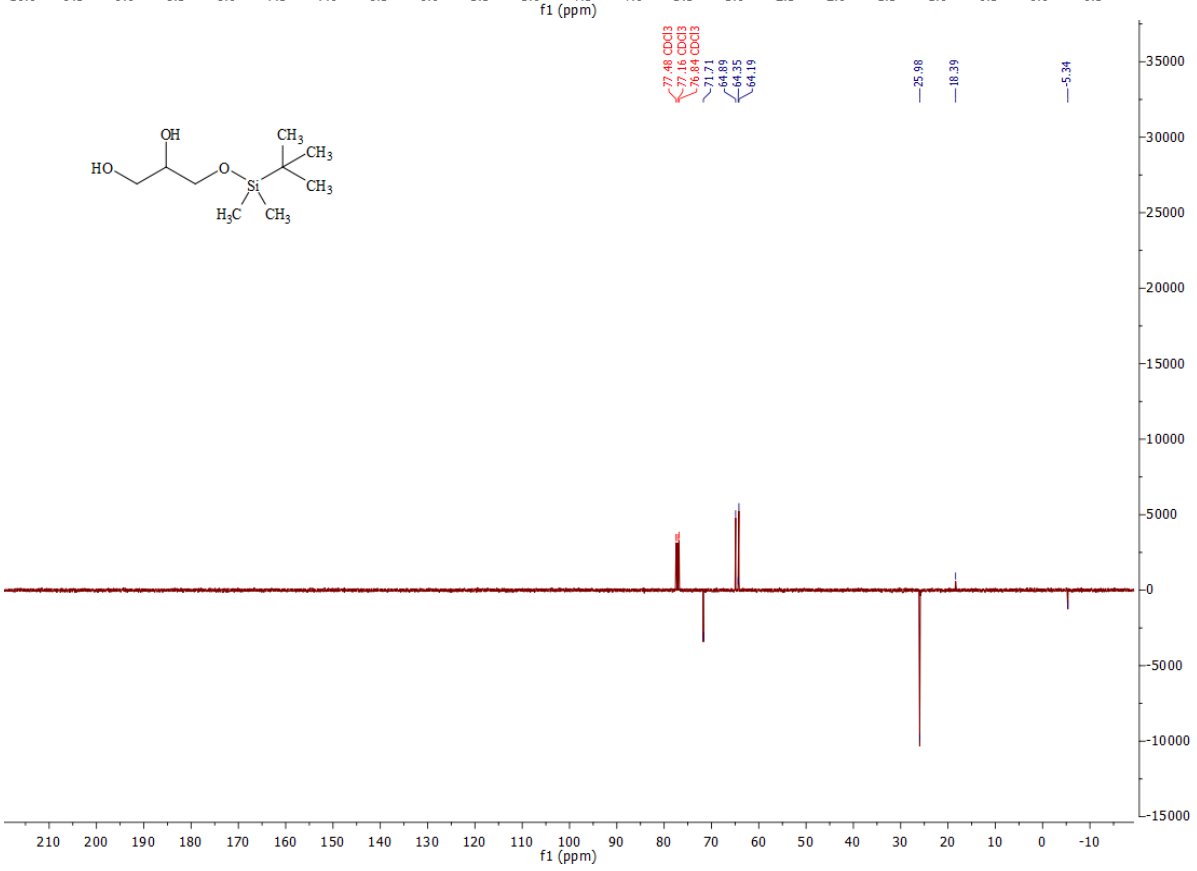
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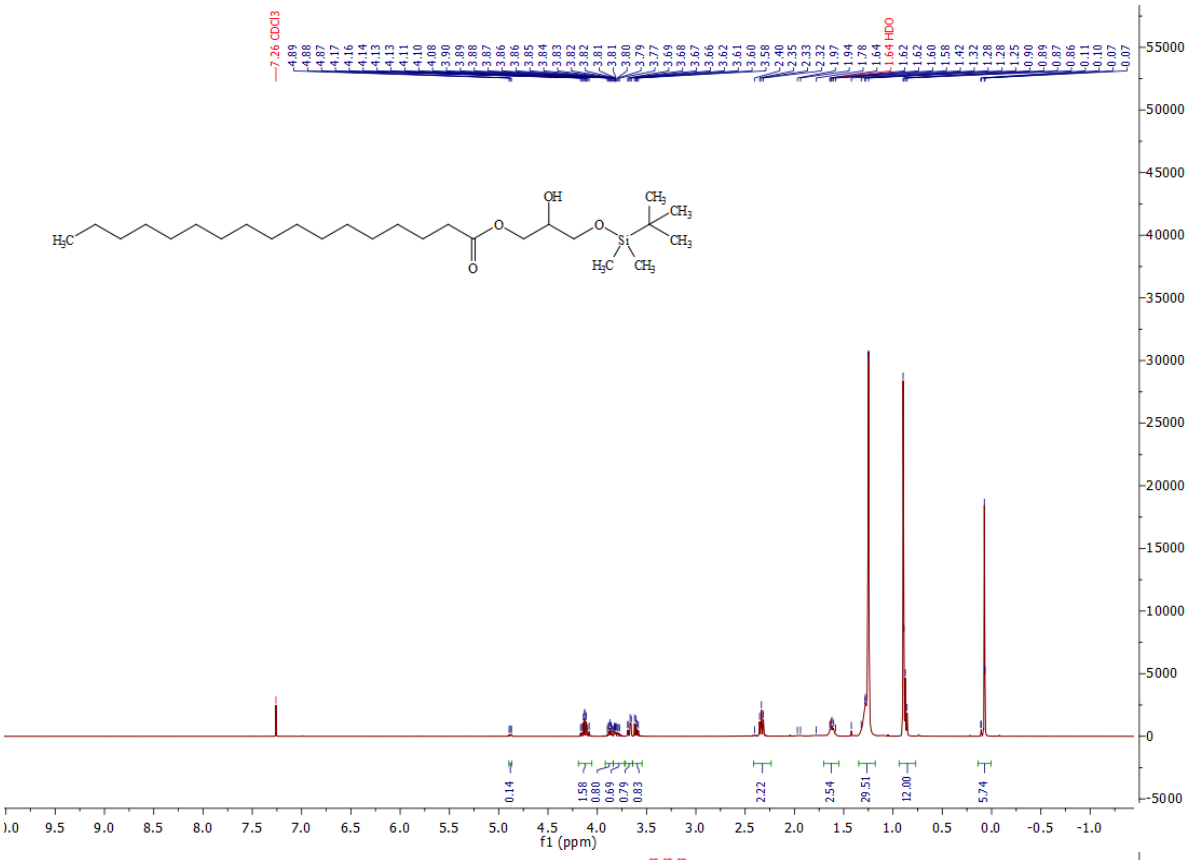
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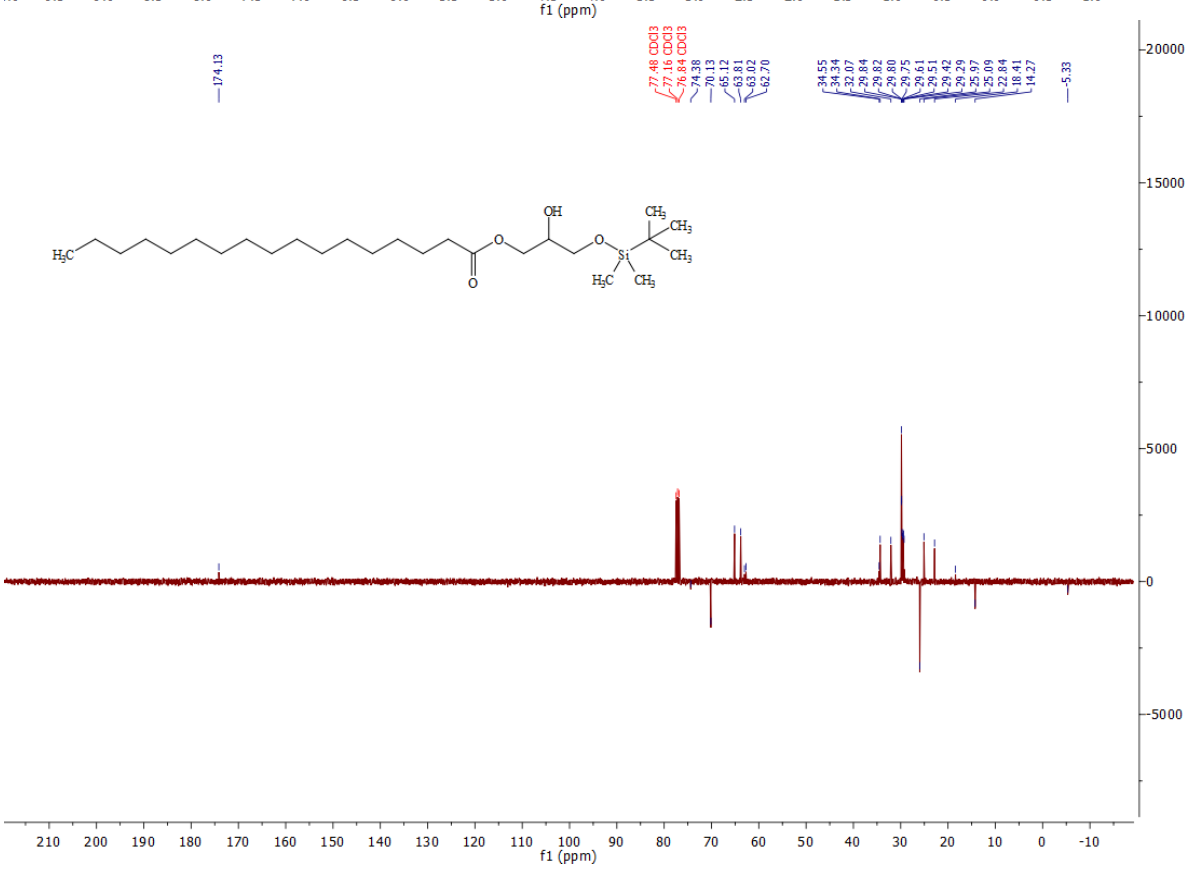
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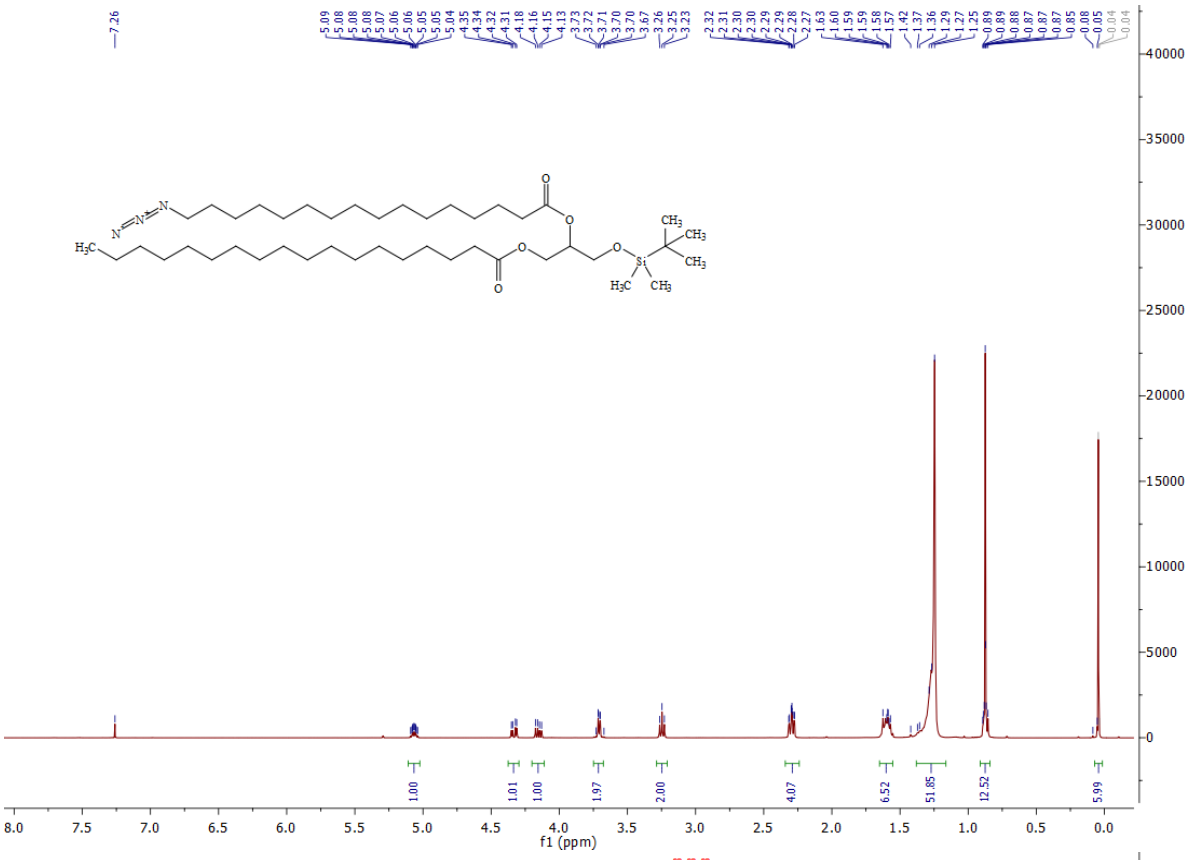
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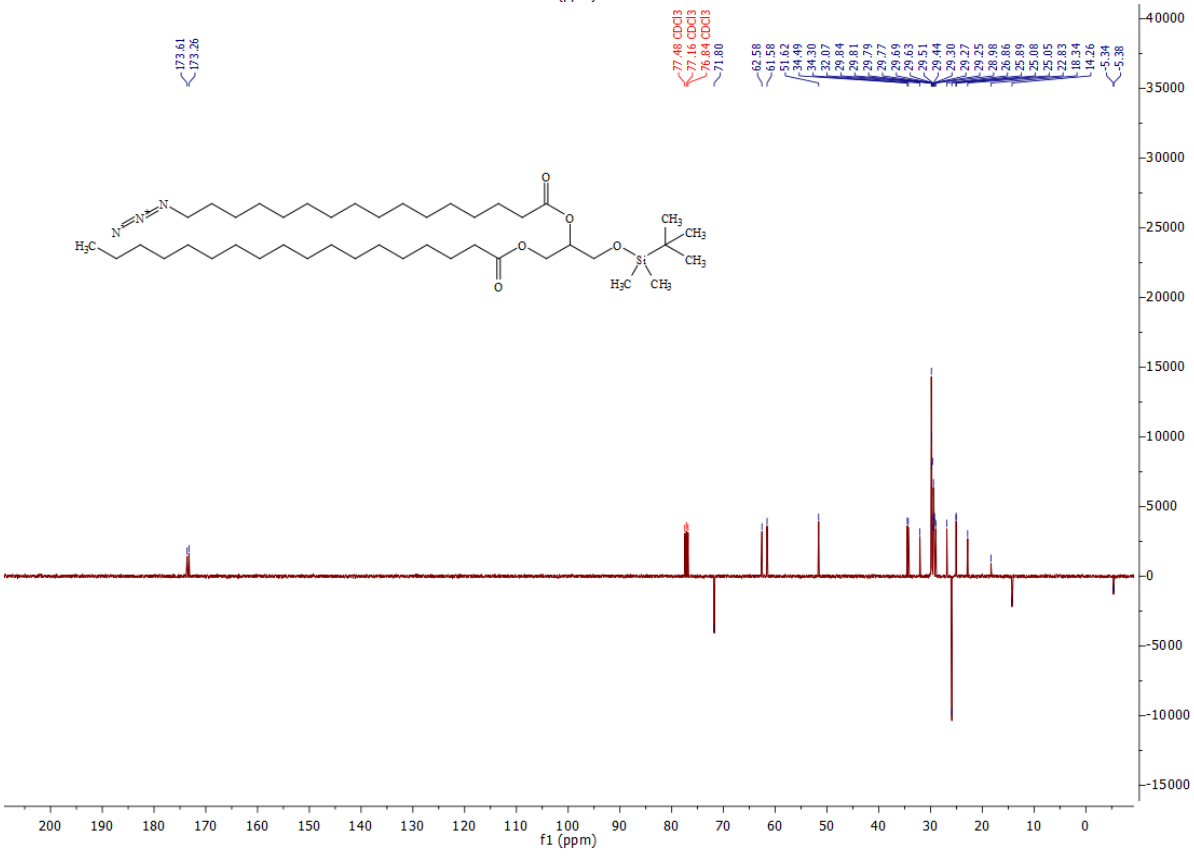
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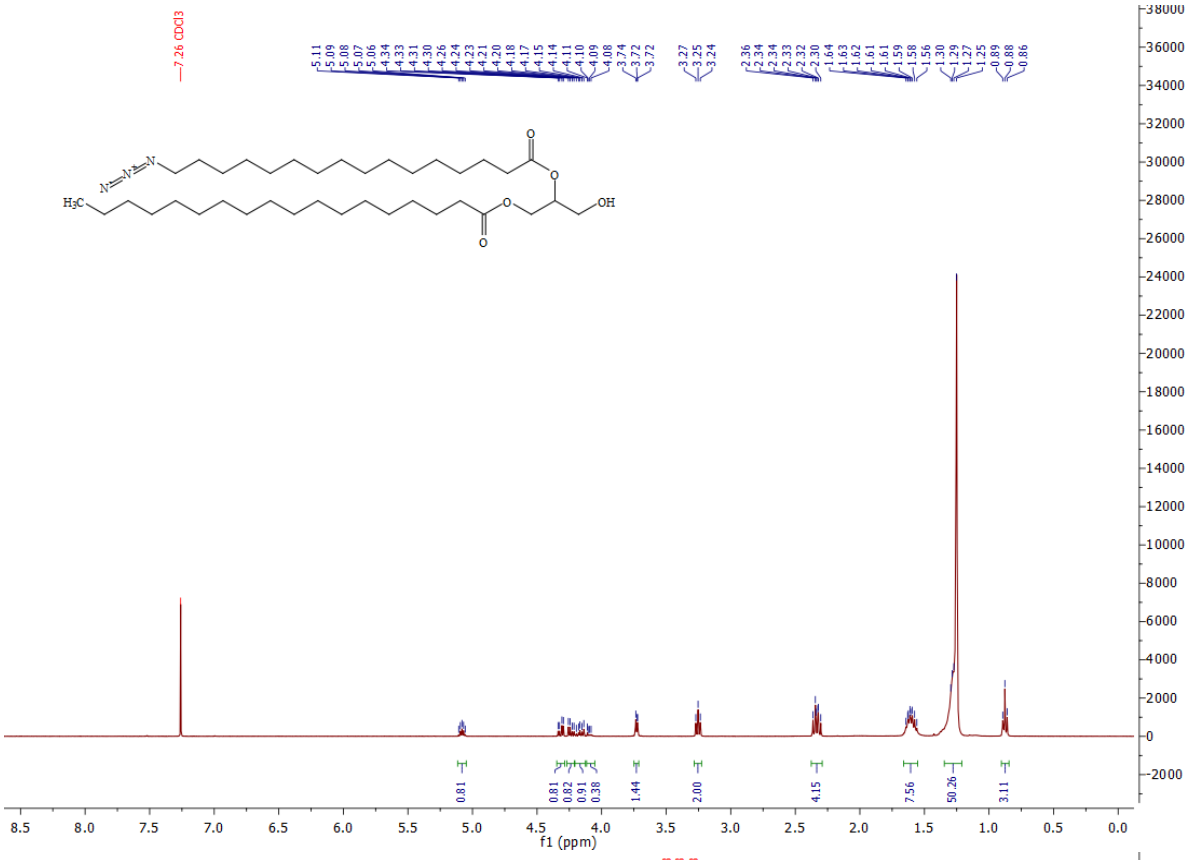
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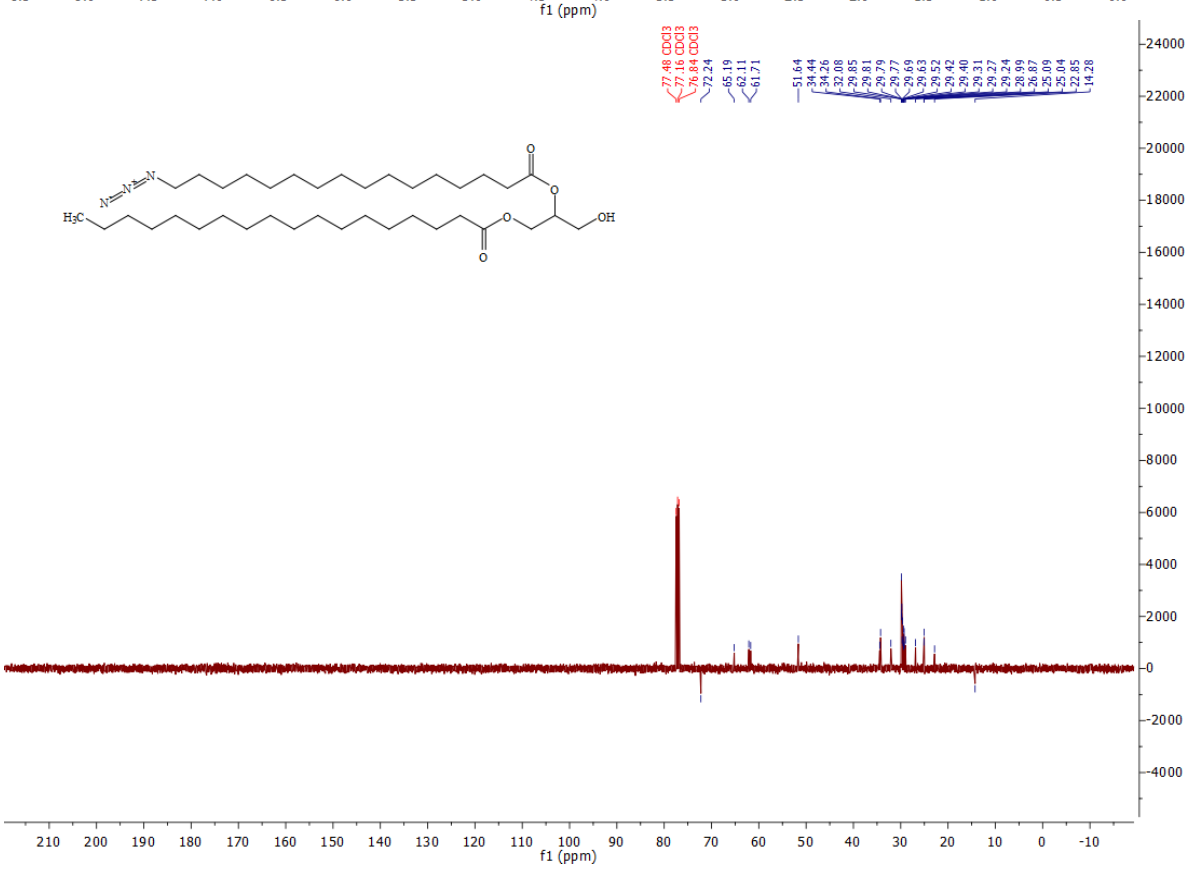
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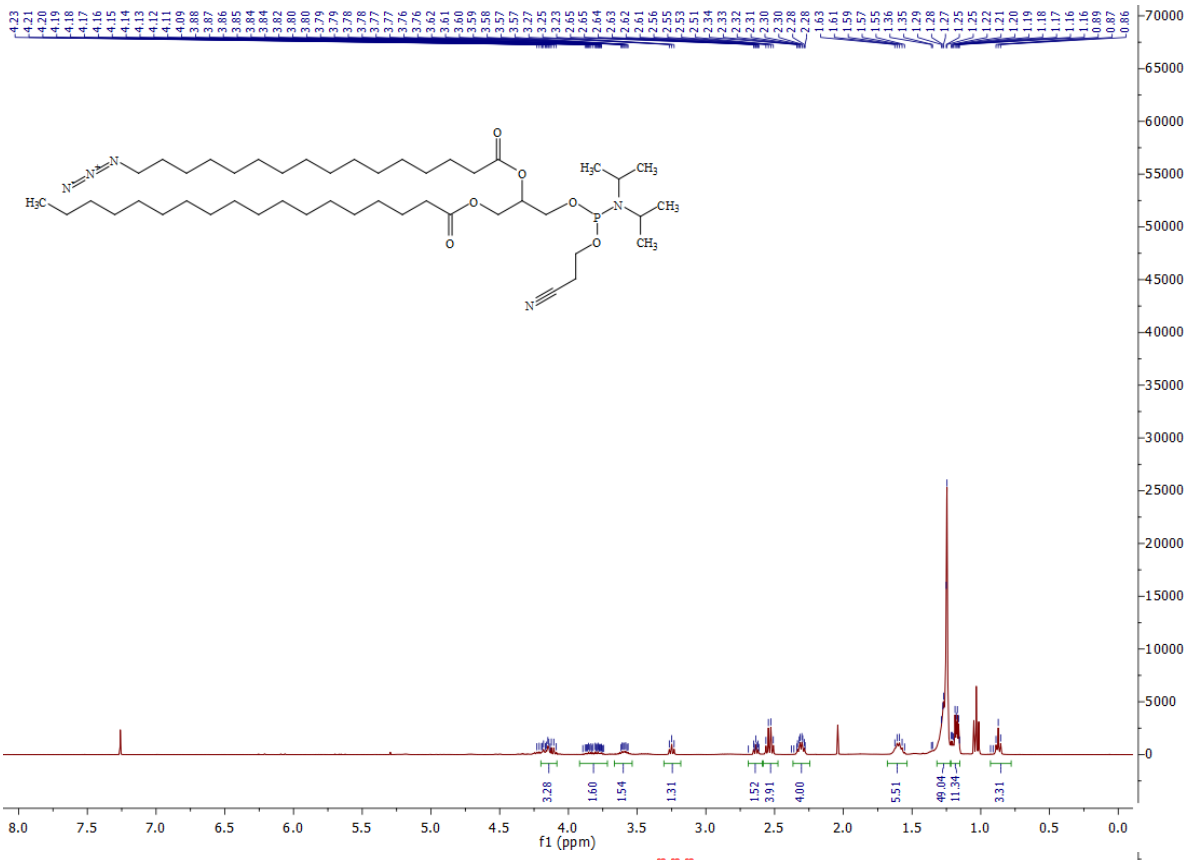
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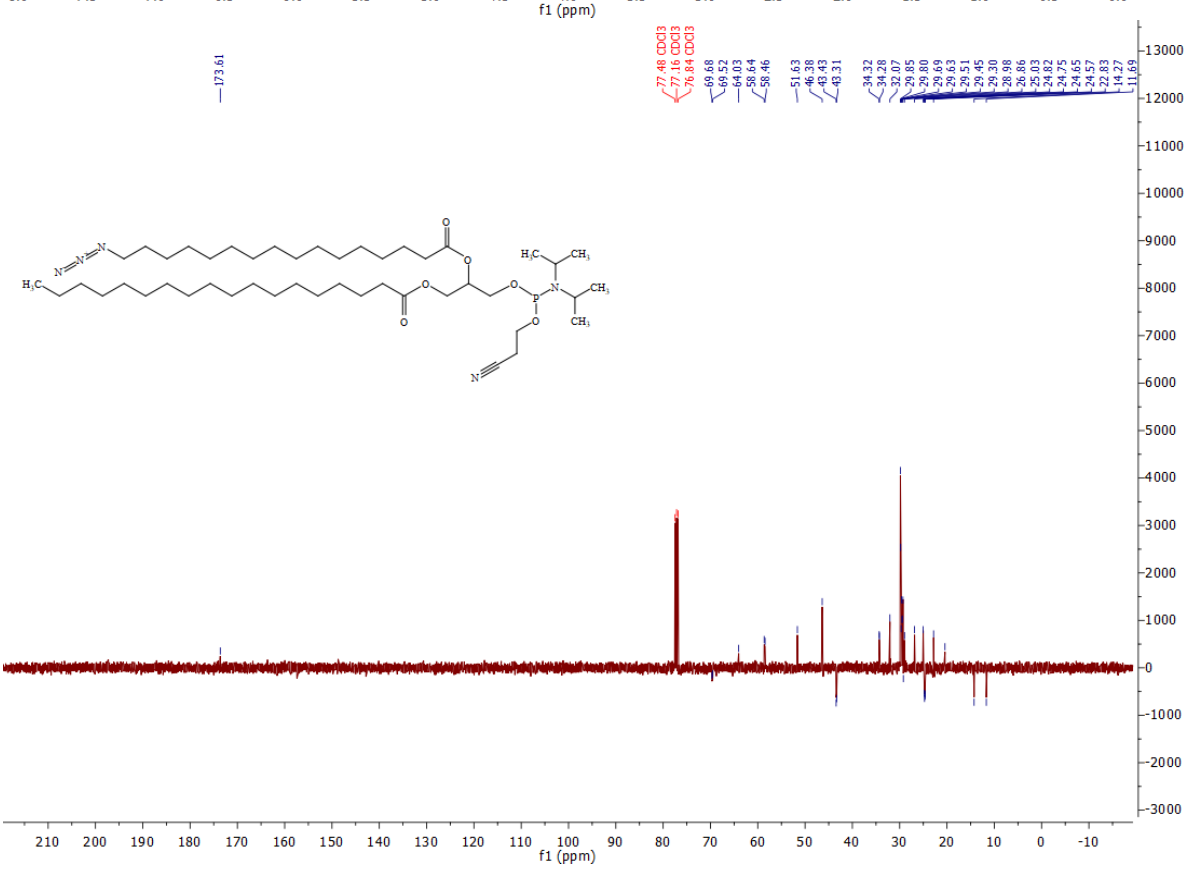
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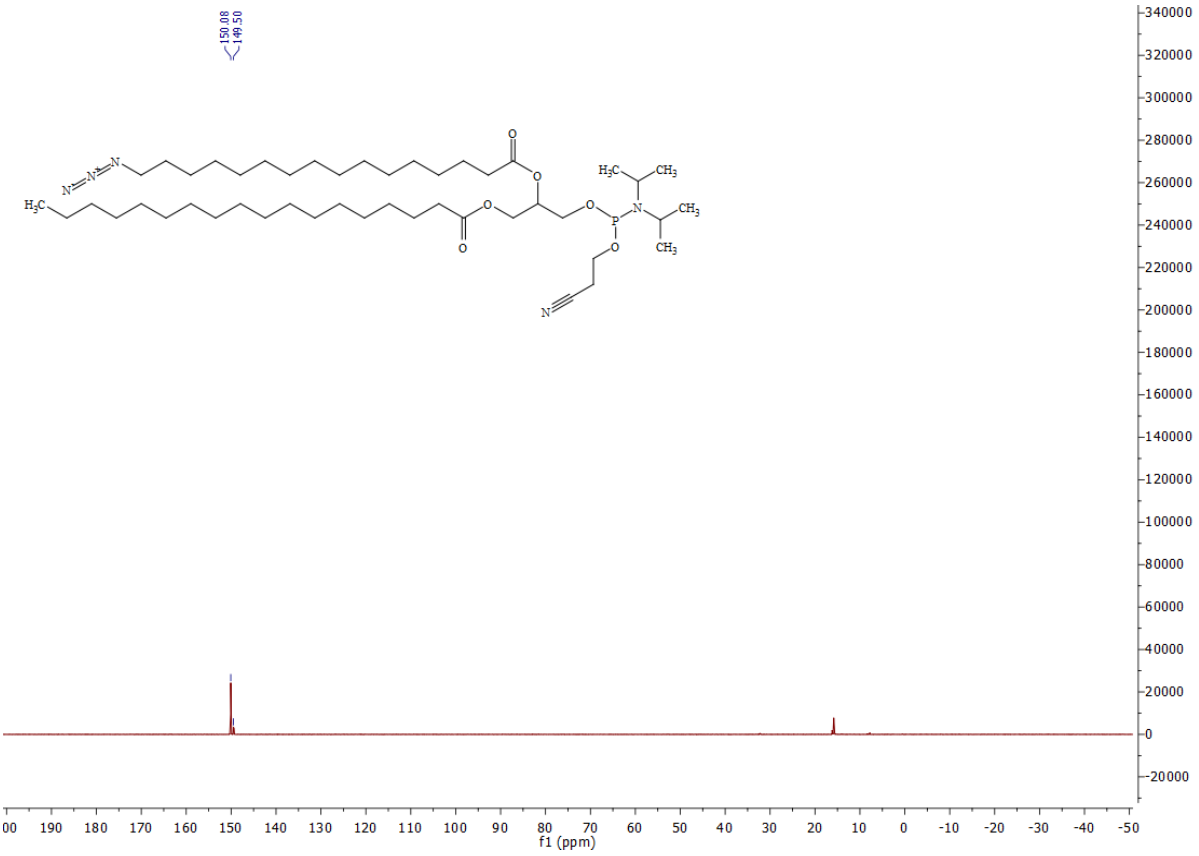
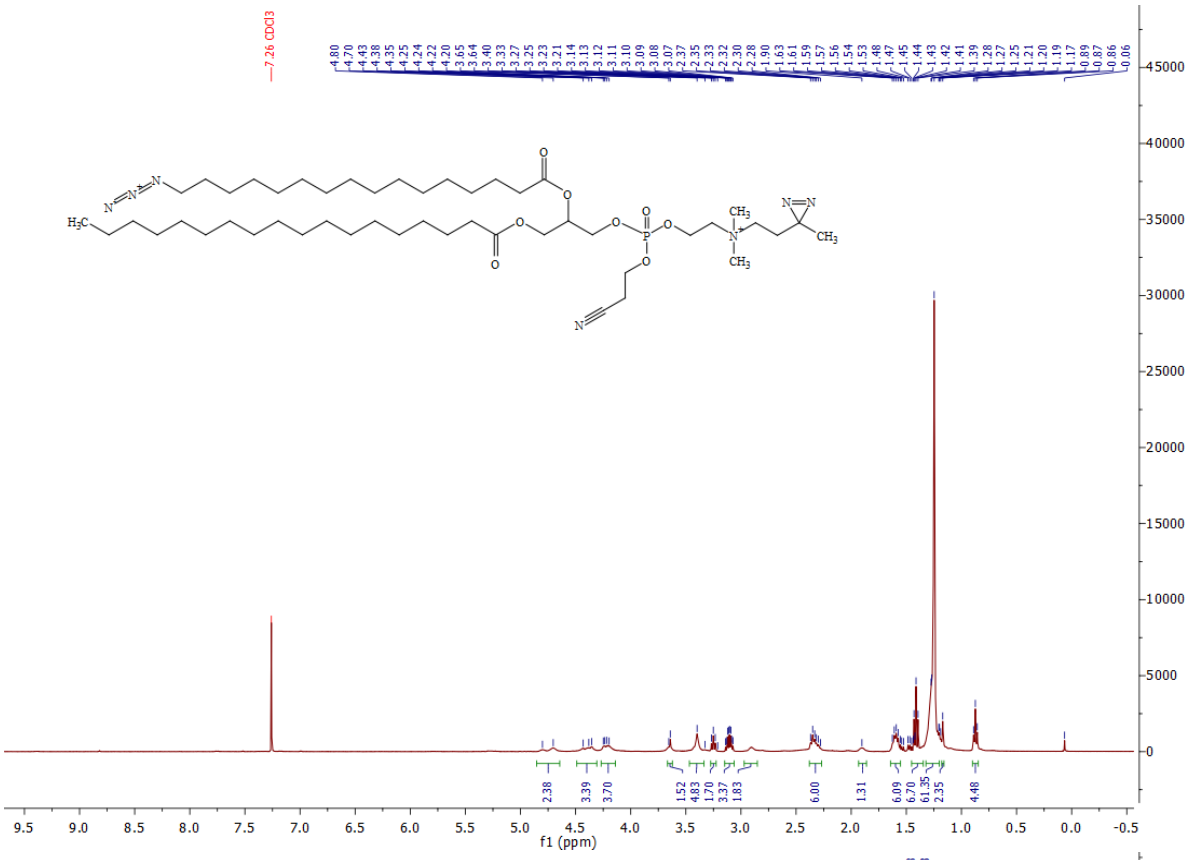
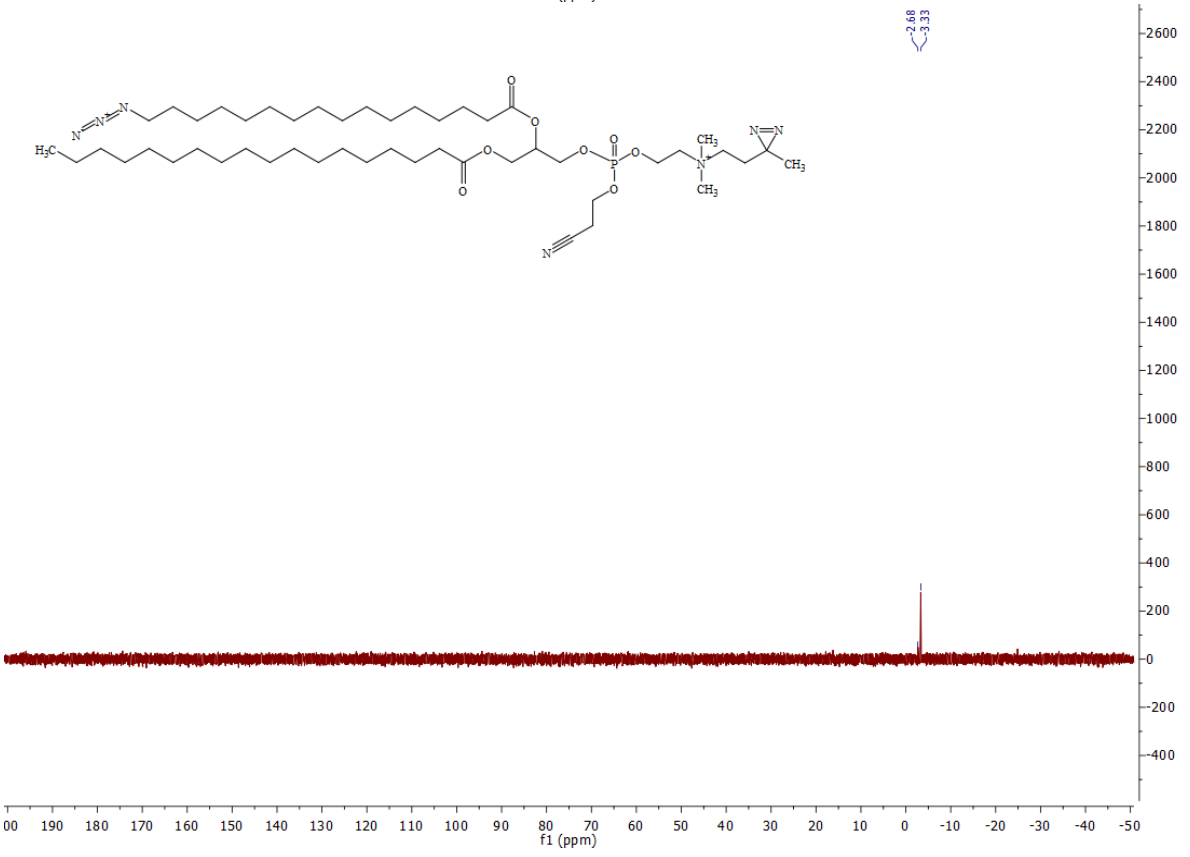
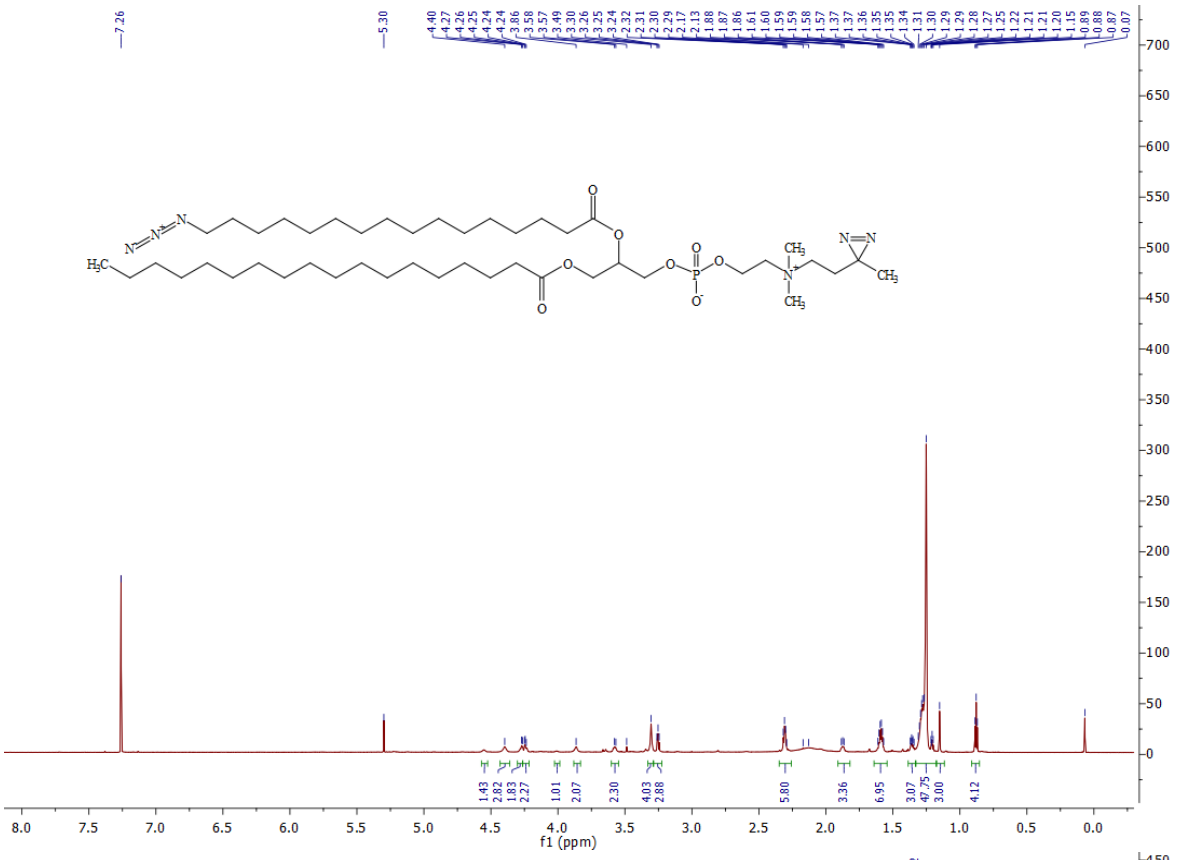
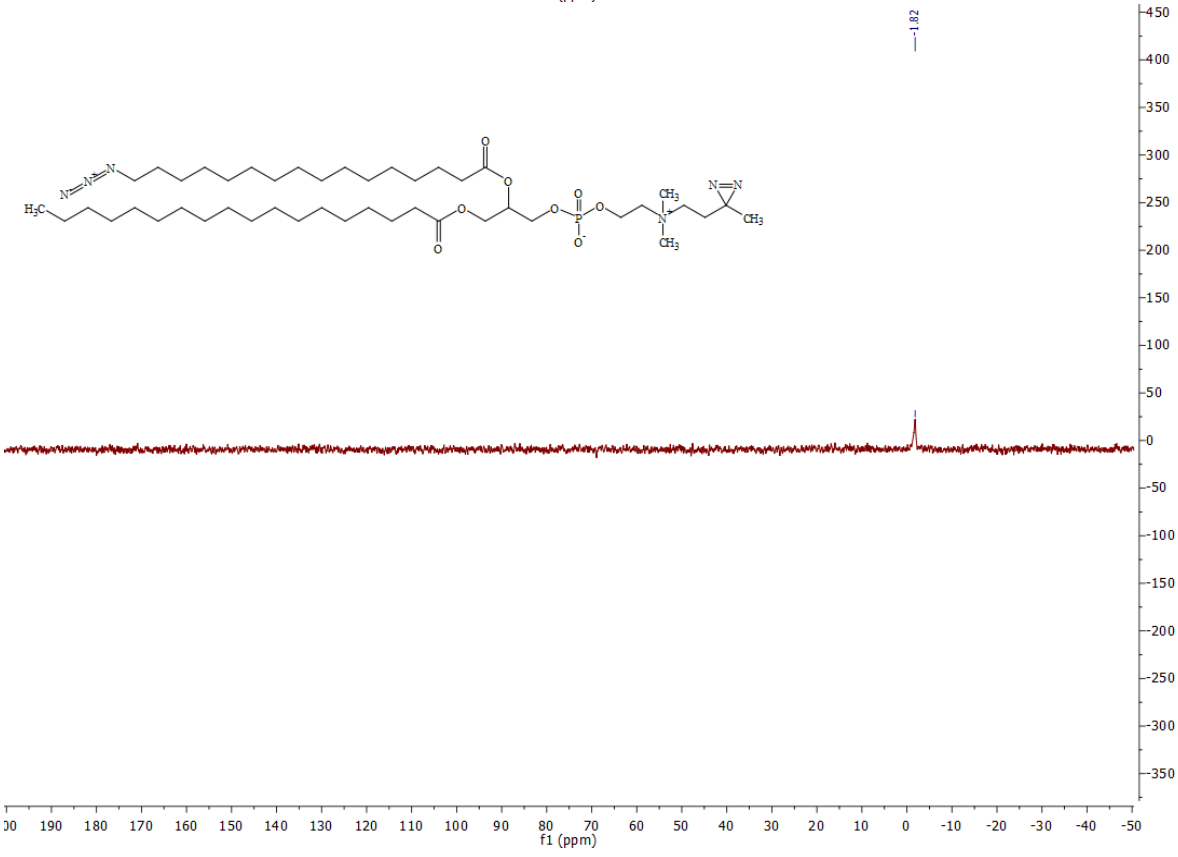
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
69.68
69.52
64.03
58.64
58.46
51.63
46.38
43.43
43.31
34.32
34.28
32.07
29.85
29.80
29.69
29.63
29.51
29.45
29.30
28.98
26.86
25.03
24.82
24.75
24.65
24.57
22.83
14.27
11.69
f1 (ppm)

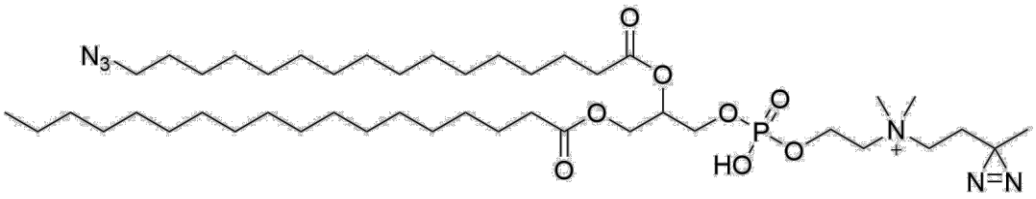

150.08
149.50
340000
320000
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
-20000
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50
f1 (ppm)


7.26 CDCl3
f1 (ppm)


2.68
3.33
f1 (ppm)


7.26
5.30
1.43
2.82
1.83
2.27
1.01
2.07
2.30
4.03
2.88
5.80
3.36
6.95
3.07
47.75
3.00
4.12
f1 (ppm)


1.82
f1 (ppm)



Exact Mass: 871,64