Improved Synthesis of the Bifunctional Chelator p-SCN-Bn-HOPO

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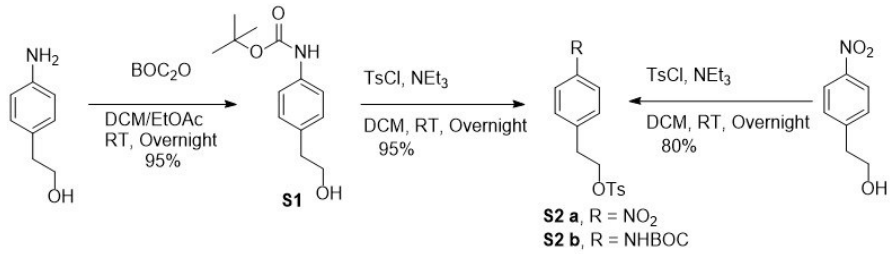


Scheme S1. Synthesis of tosylated reagents used for Schemes 1 and 2.

Synthesis of 2-(4-teritiarybutyl-aminophenyl)ethan-1-ol (S1) :

To 2-(4-aminophenyl)ethan-1-ol (13.7 g, 0.1 mol) in 750 mL dichloromethane was added di-tert-butyl decarbonate (43.6 g, 0.2 mol) in 750 mL dichloromethane dropwise using dropping funnel at 0̊ C. Then the reaction mixture was stirred at room temperature overnight. Then the solvent was evaporated under vacuum. The crude mixture is dissolved in 200 mL dichloromethane and extracted with brine using a separatory funnel. The organic layer was dried over Na_2SO_4, and evaporated to dryness to obtain crude product, which was purified using silica column chromatography eluting with 95:5 (DCM:EtOAc) resulted **S1** in 95% yield (22.6 g, 0.095 mol).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, *J*=7.6 Hz, 2H), 7.10 (d, *J*=8.36 Hz, 2H), 6.57 (s, 2H), 3.77 (t, *J*=6.56 Hz, 2H), 2.77 (t, J=6.56 Hz, 2H), 1.74 (s, 1H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ; 153.06, 136.85, 133.16, 129.67, 119.02, 80.62, 63.85, 36.56, 28.47;. HRMS (ESI) *m/z* calcd for $C_{13}H_{19}NO_3$ ([M + H]$^+$), 238.1443, found 238.1434; $C_{13}H_{19}NO_3$ ([M + Na]$^+$), 260.1263, found 260.1263.

4-nitrophenethyl 4-methylbenzenesulfonate (S2a):

To a solution of 2-(4-nitrophenyl)ethanol (2.07 g, 12.4 mmol) and triethyl amine (5.85 g, 57.8 mmol) in dichloromethane (80 mL) was added p-toulnesulfonylchloride (2.36 g, 12.4 mmol) in THF (60 mL) drop wise at 0^{o}C under N_2. The resulting solution was stirred at room temperature for overnight. Then the solvent was evaporated under reduced pressure. The resulting residue was dissolved in methylene chloride, washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude compound was purified by silica column chromatography using hexane:DCM 30:70 as eluent to obtain white yellowish solid. Yield: 80%. (3.18 g, 9.91 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J*=10.9 Hz, 2H), 7.67 (d, 7.10 *J*=10.35 Hz, 2H), 7.27-7.30 (m, 4H), 4.29 (t, *J*=8 Hz, 2H), 3.08 (t, J=8 Hz, 2H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ; 147.22, 145.26, 144.37, 132.87, 130.02, 129.98, 127.98,127.23, 123.92, 69.61, 42.19, 35.30, 21.79, 14.33;.
HRMS (ESI) *m/z* calcd for $C_{15}H_{15}NO_5S$ ([M + H]$^+$), 322.07, found 322.076; $C_{15}H_{15}NO_5S$ ([M + Na]$^+$), 344.04, found 344.056.

Synthesis of Synthesis of 2-(4-teritiarybutyl aminophenyl)ethyl 4-methylbenzenesulfonate (S2b):

To compound **S1** (2.37 g, 10 mmol) in 75 mL dichloromethane and triethyl amine (3.3 g, 33 mmol) was added p-toulenesulfonylchloride (2 g, 10.5 mmol) in 50 mL THF dropwise using dropping funnel at 0̊ C. Then the reaction mixture is stirred at room temperature for overnight. Then the solvent was evaporated under vacuum. The obtained product **S2b** (3.7 g) 95% was used in next step without further purification. There is about 5% of unreacted compound **S1** (monitored via TLC using DCM:Hexanes 3:1). Column purification results in degradation of compound **S2b** to compound **S1** so it is not ideal to run the mixture through a silica column.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (d, *J*=8.34 Hz, 2H), 7.23 (d, , *J*=8.04 Hz, 2H), 7.19 (d, *J*=7.98, 2H), 6.96 (d, *J*=8.58, 2H), 6.49 (s, 1H), 4.11 (t, J=7.14 Hz, 2H), 2.84 (t, J=7.08 Hz, 2H), 2.38 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ; 152.75,

144.73, 137.20, 132.80, 129.81, 129.43, 127.83, 127.04, 118.65, 80.53, 70.77, 42.01, 34.65, 28.35, 21.65, 14.17;. HRMS (ESI) *m/z* calcd for $C_{20}H_{25}NO_5S$ ([M + H]$^+$), 392.1532, found 392.1534; $C_{20}H_{25}NO_5S$ ([M + Na]$^+$), 414.1351, found 414.135.

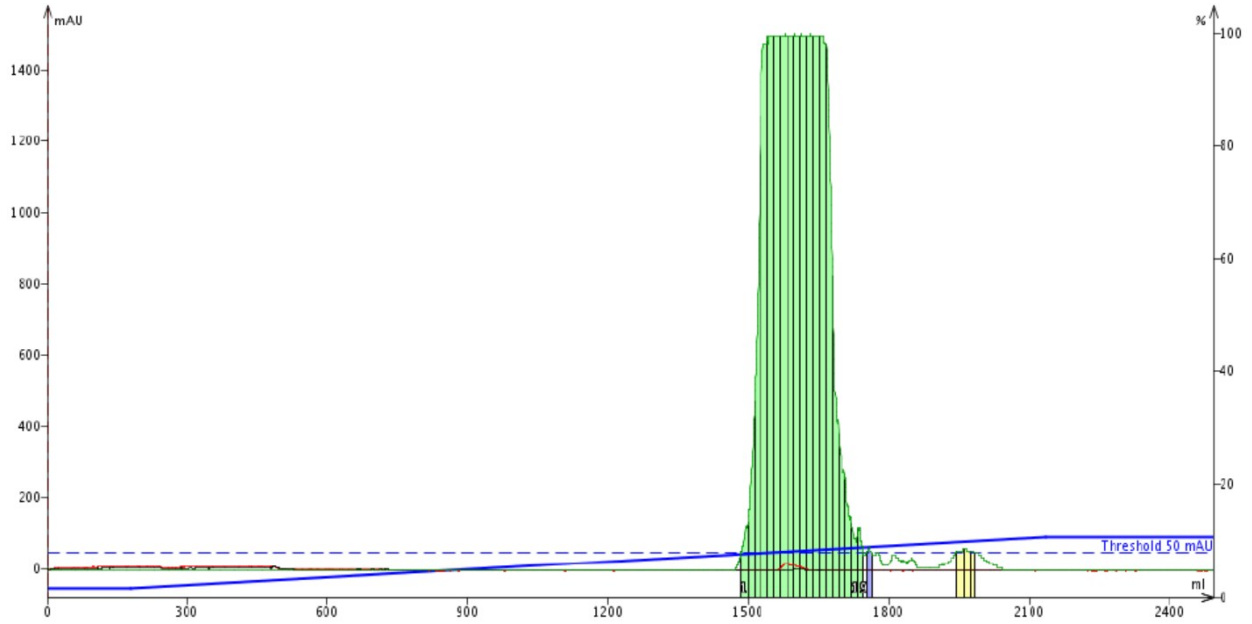


Figure S1. Flash chromatography purification of compound **4** using an eluent of DCM: MeOH over a SNP-silica gel column.

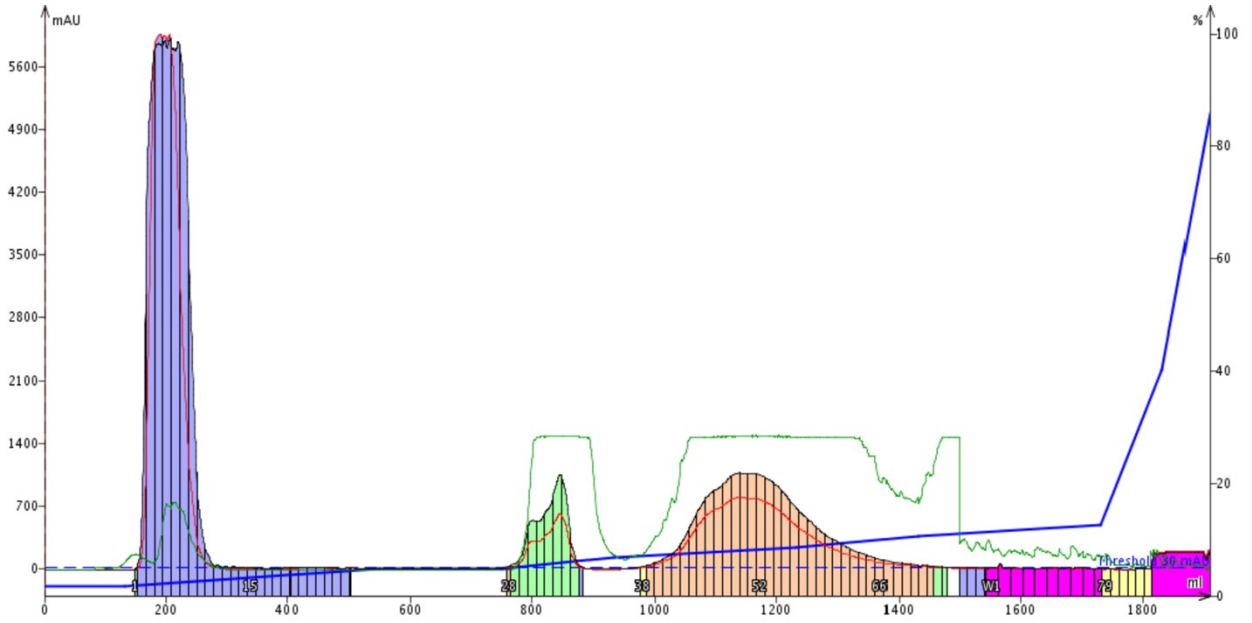


Figure S2: Flash purification of compound **5** using an eluent of DCM: MeOH over a SNP-silica gel column.

Trial	4-nitrophenylethyl-bromide equiv.	K_2CO_3 equiv.	compound 3 (g)	Time	Power	Temp °C	% yield
A3S4-O1	1	5	0.2	5min -	50 W	25-> 40 °C	3.1
				1h	25 W	40 °C	
				10min	25 W	40->25 °C	
A3S4-O2	2	5	0.2	5min	50 W	25-> 40 °C	3.5
				1h	25 W	40 °C	
				10min	25 W	40->25 °C	
A3S4-O3	3	5	0.3	5min	50 W	25-> 40 °C	12.3
				1h	25 W	40 °C	
				10min	25 W	40->25 °C	
A3S4-O4	4	5	0.3	5min	50 W	25-> 40 °C	2.3
				1h	25 W	40 °C	
				10min	25 W	40->25 °C	

Table S1. Microwave reaction for the synthesis of tert-butyl (4-((tert-butoxycarbonyl)(3-((4-nitrophenethyl)amino)propyl)amino)butyl)(3-((tert-butoxycarbonyl)amino)propyl)carbamate (**4**, Scheme 2)

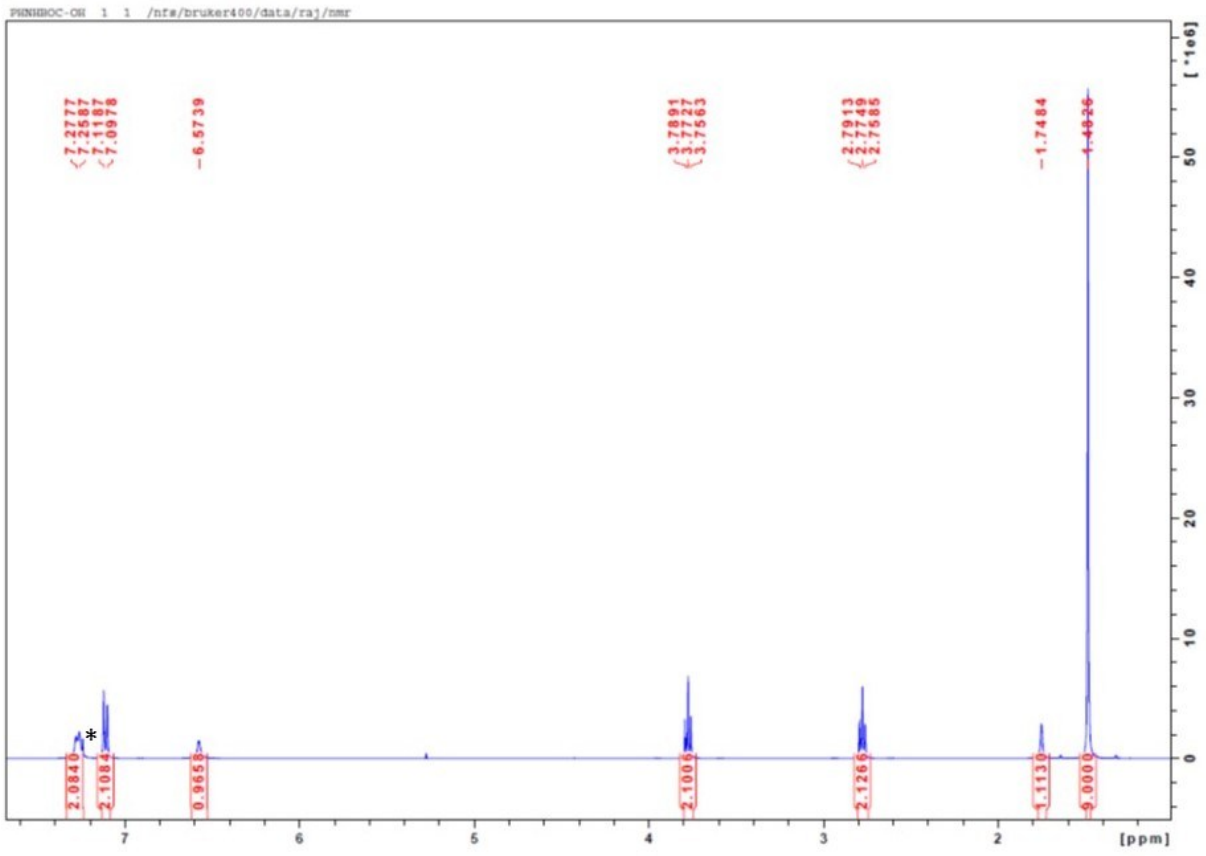


Figure S3. ^{1}H NMR of compound **S1** in *$CDCl_3$.

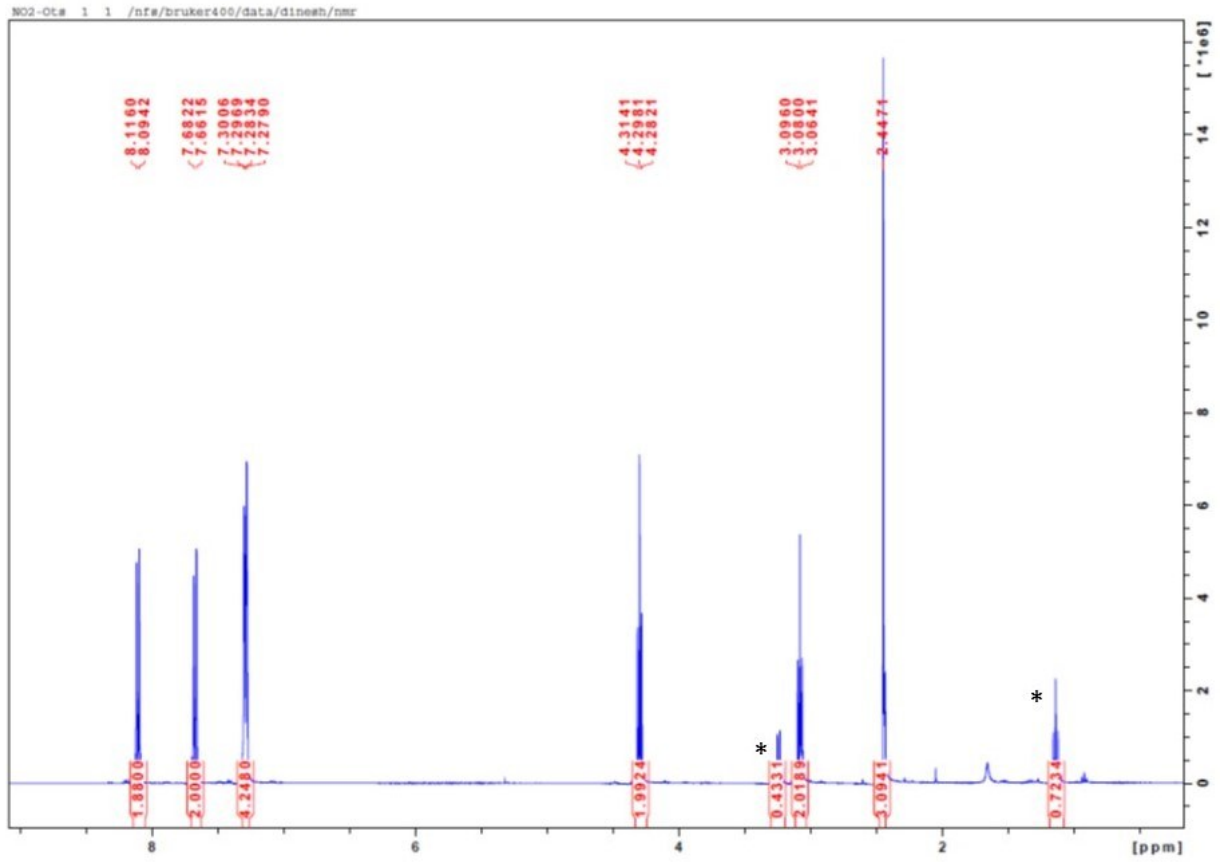


Figure S4. ^{1}H NMR of compound **S2a** in $CDCl_3$, * ethylacetate.

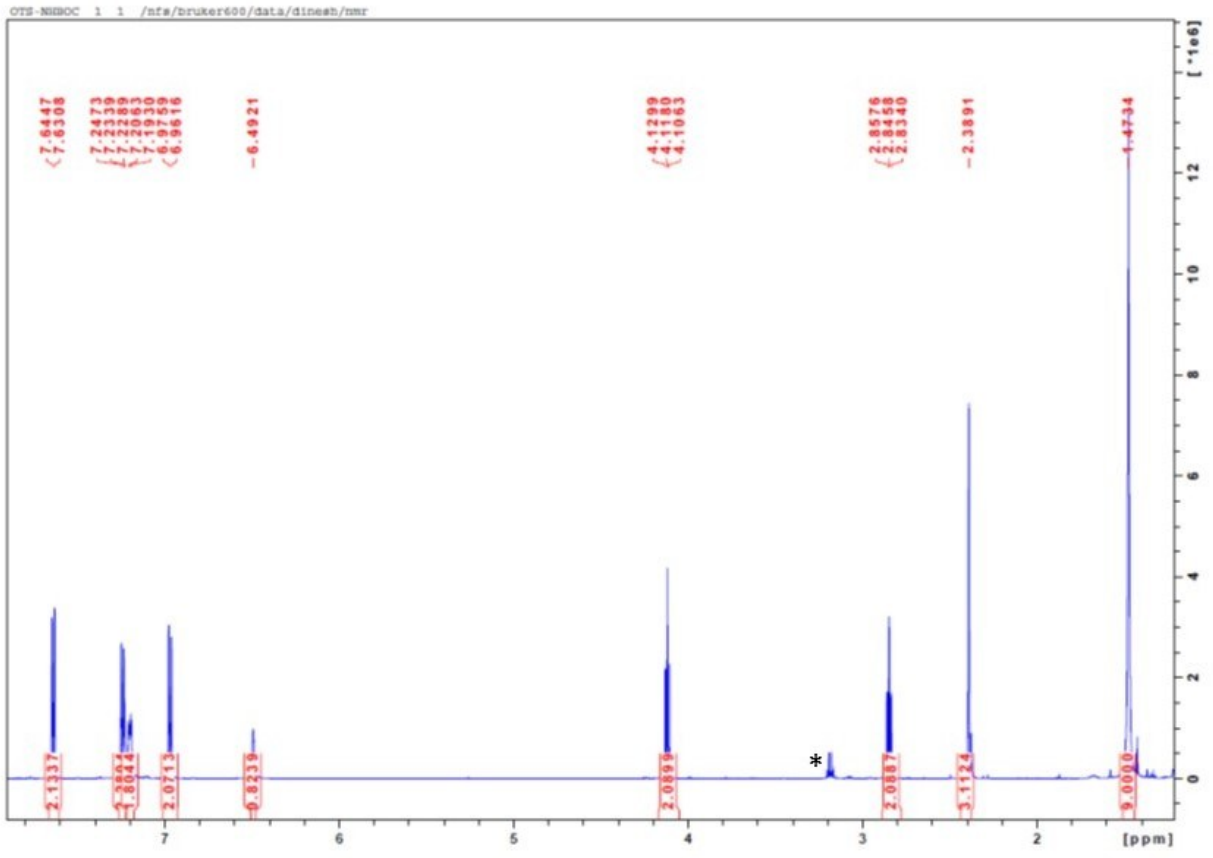


Figure S5. ^{1}H NMR of compound **S2b** in $CDCl_3$. *ethylacetate

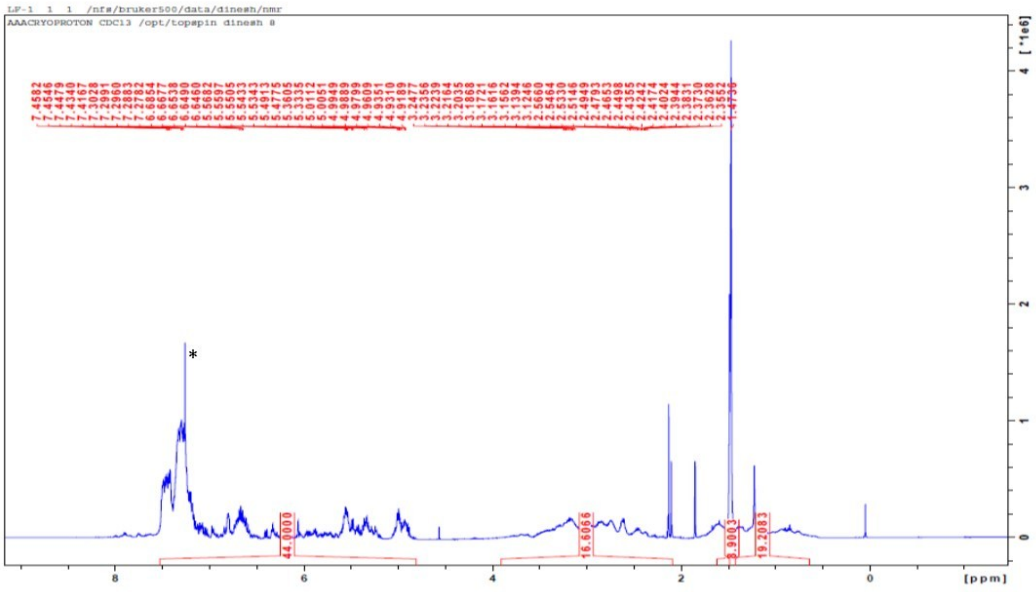


Figure S6. ^{1}H NMR of compound **1** in *$CDCl_3$.

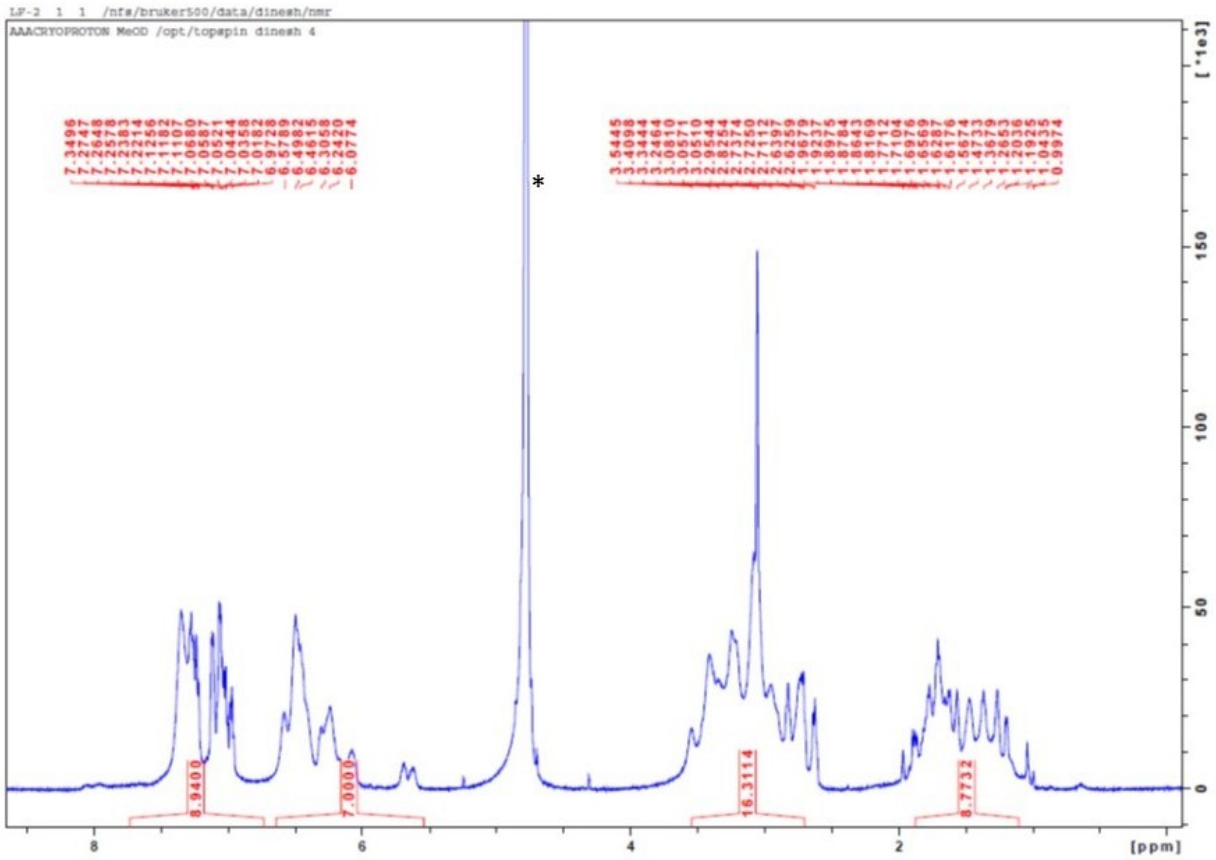


Figure S7. ^{1}H NMR of compound **2** in CD_3OD*

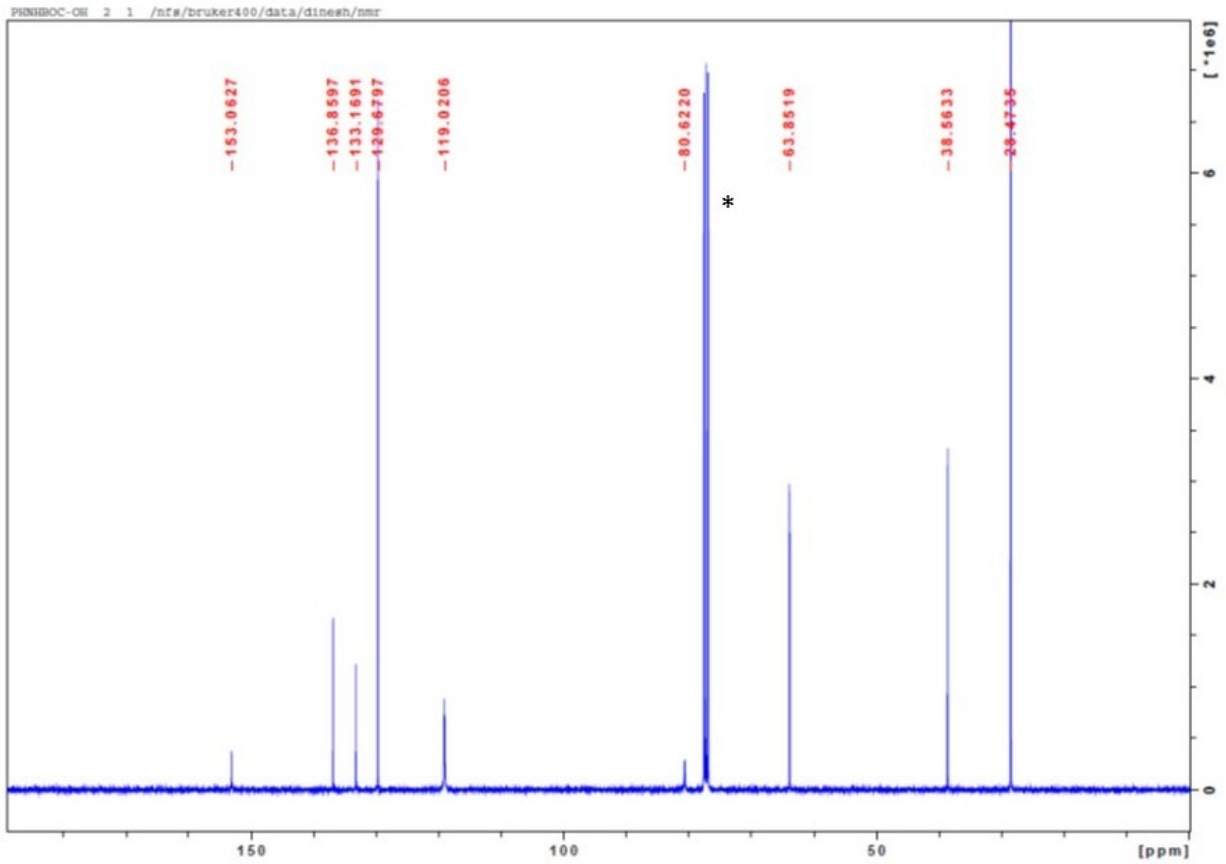


Figure S8. ^{13}C NMR of compound **S1** in *$CDCl_3$.

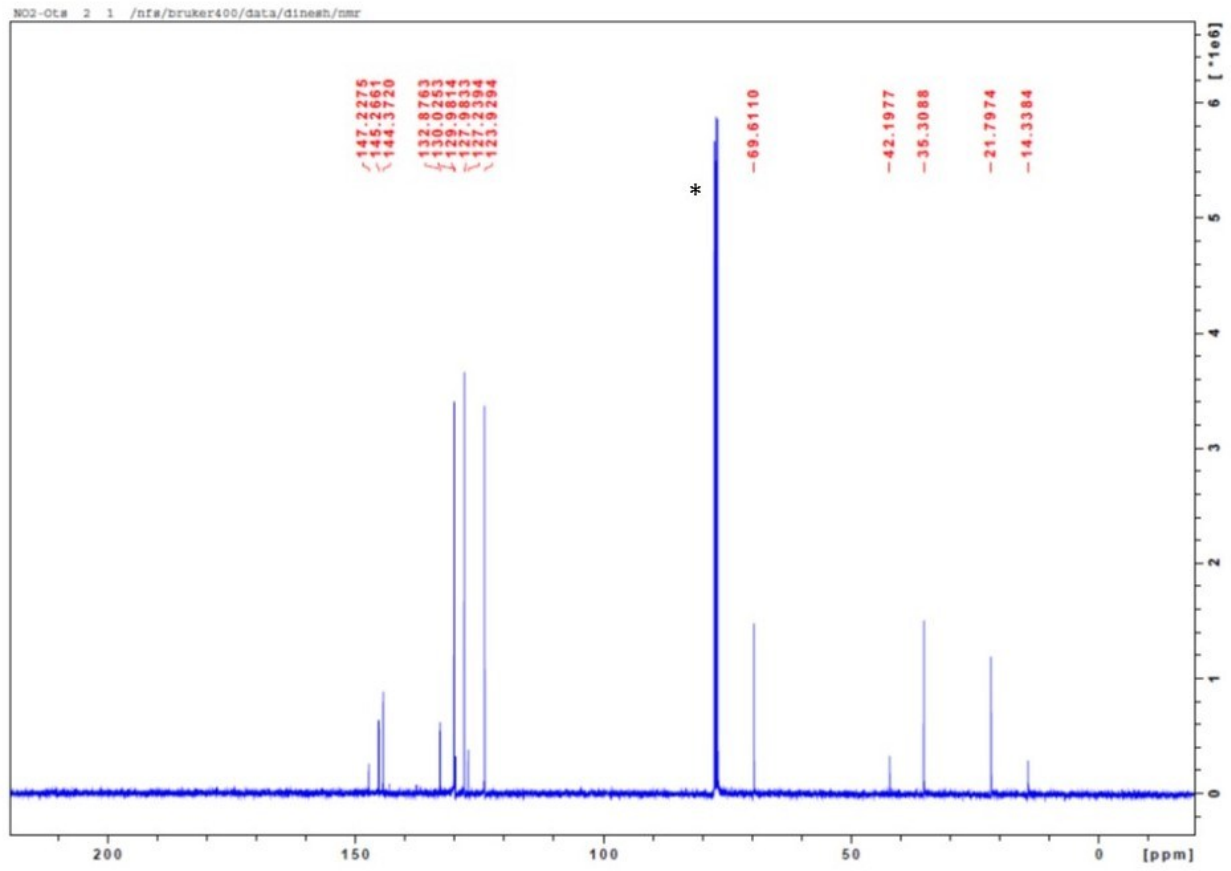


Figure S9. ^{13}C NMR of compound **S2a** in *$CDCl_3$.

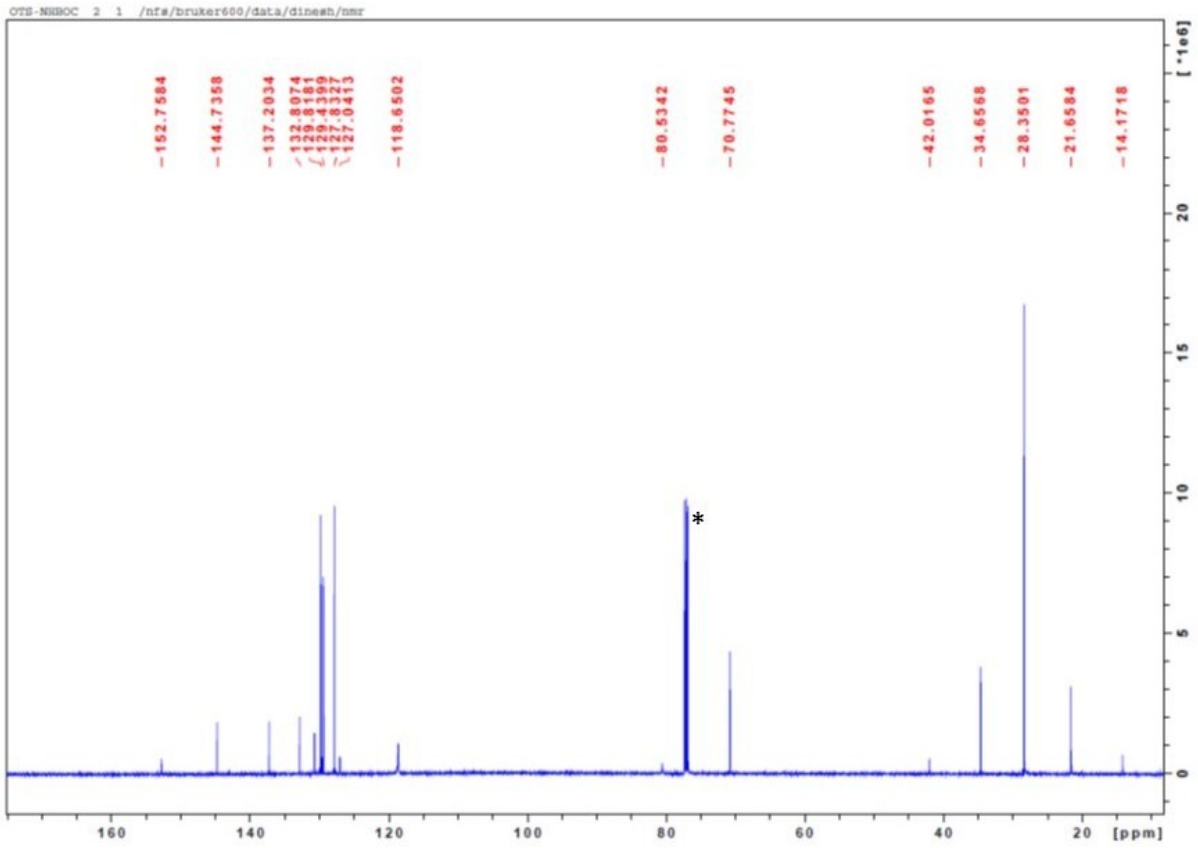


Figure S10. ^{13}C NMR of compound **S2b** in $*CDCl_3$.

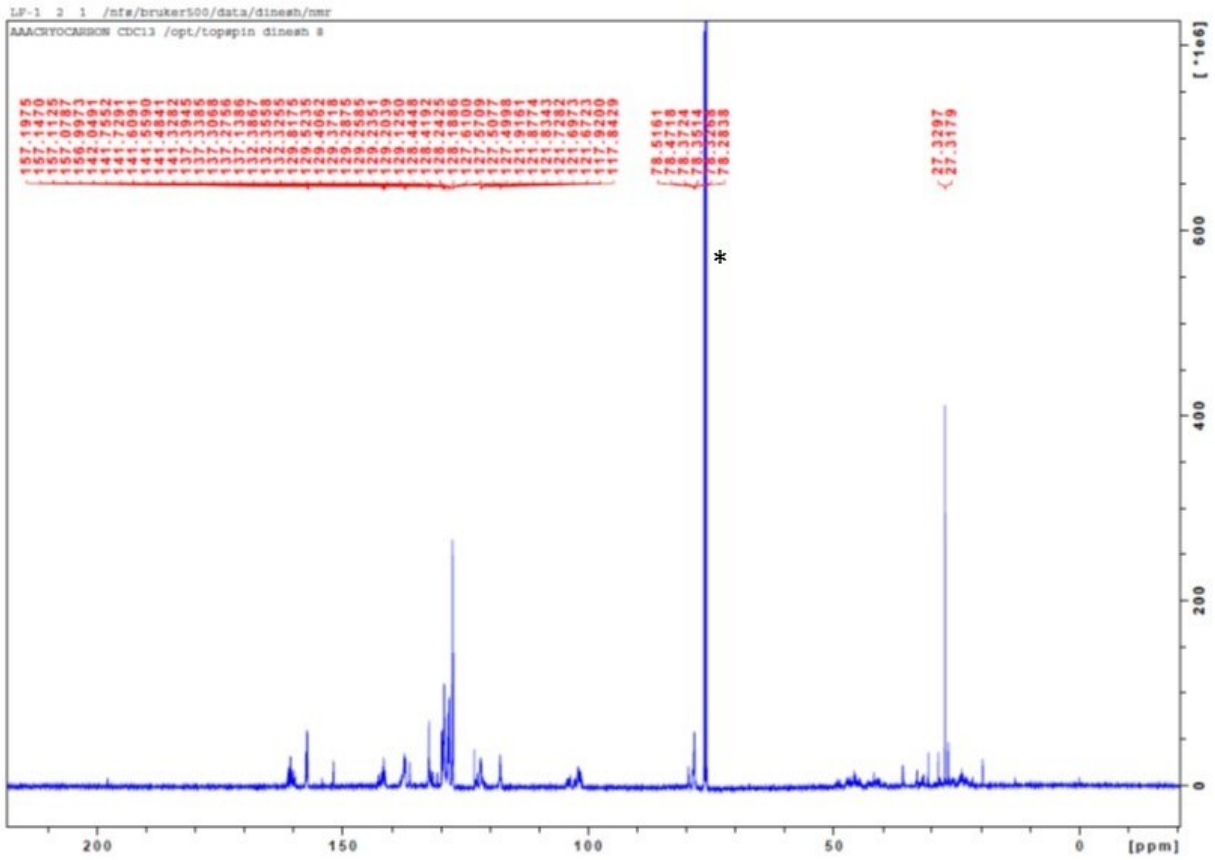


Figure S11. ^{13}C NMR of Compound **1** in *$CDCl_3$.

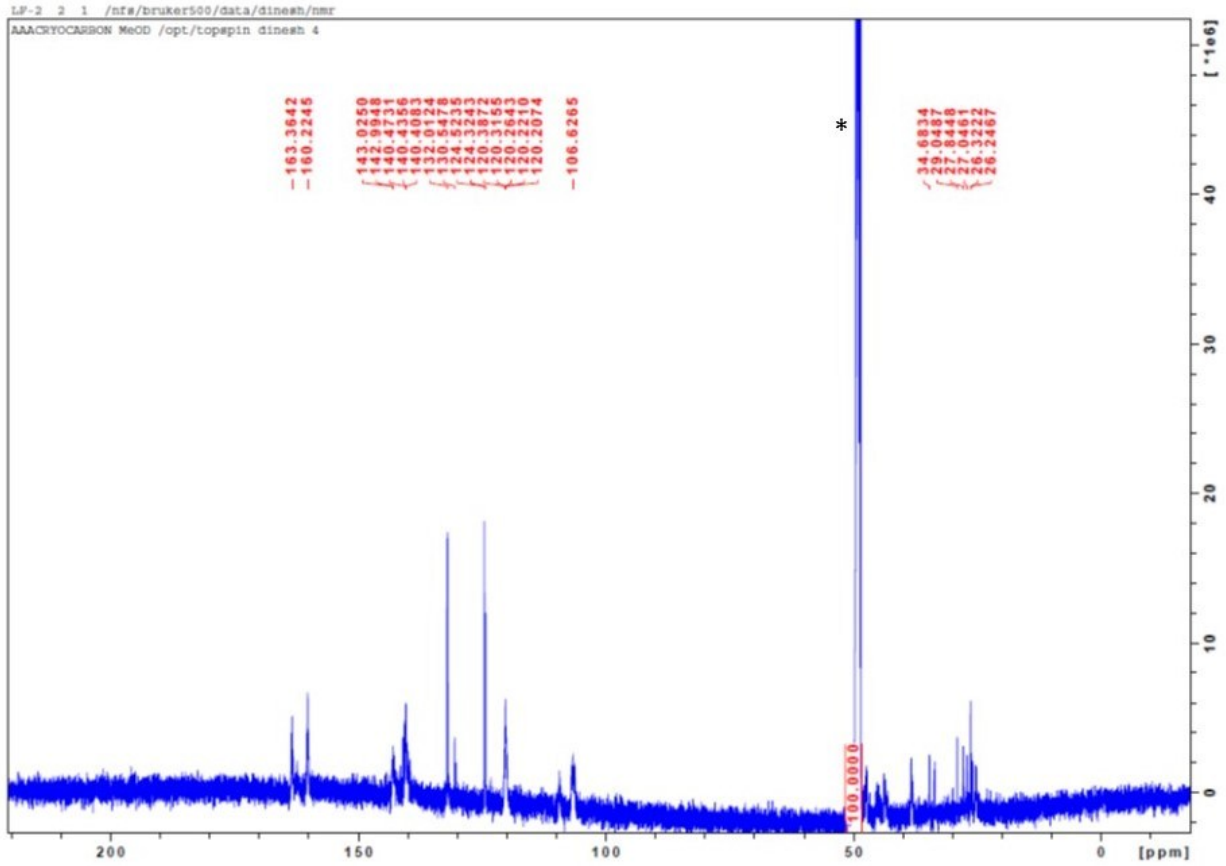
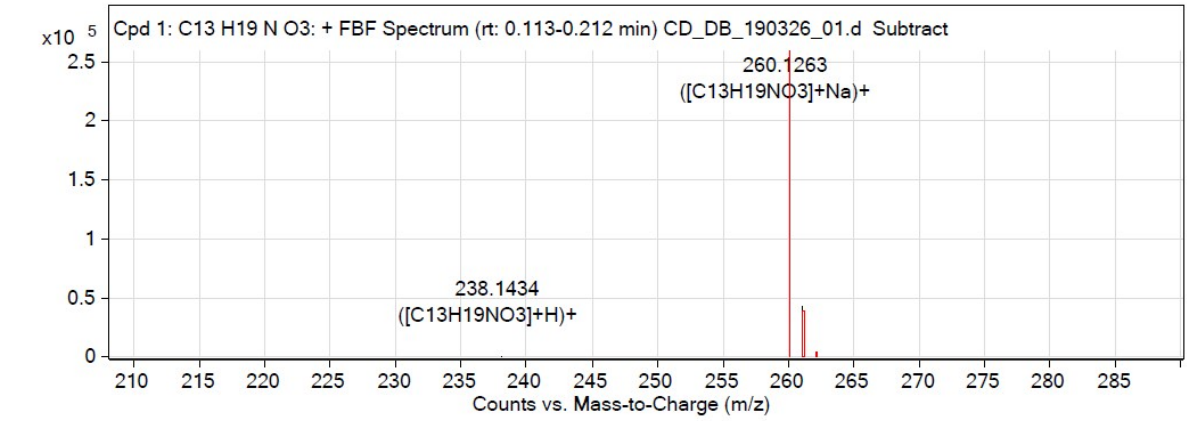


Figure S12. ^{13}C NMR of compound **2** in $*CD_3OD$

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C13 H19 N O3	0.137	237.1371	259480	C13 H19 N O3	237.1365	2.56

MS Zoomed Spectrum



MS Spectrum Peak List

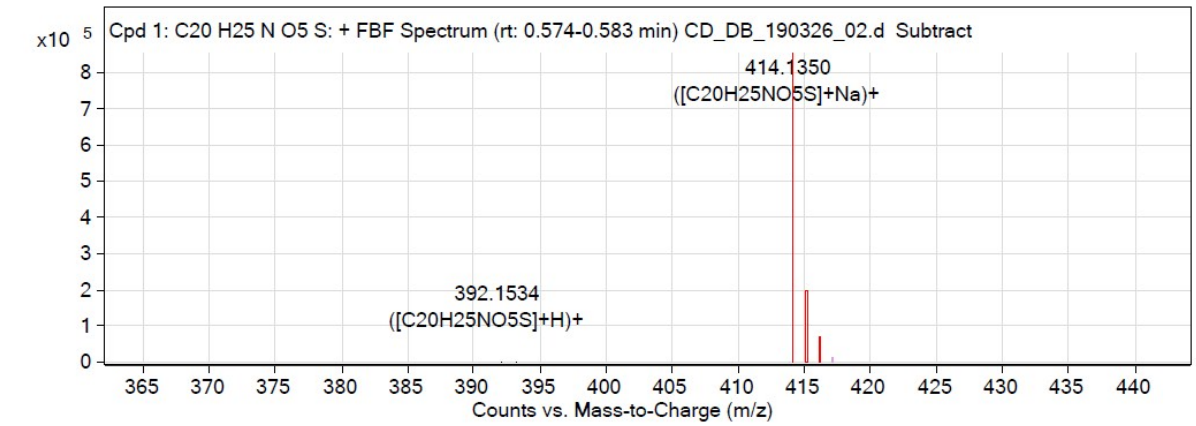
m/z	z	Abund	Formula	Ion
238.1434	1	363.05	C13H19NO3	(M+H)+
260.1263	1	259480.03	C13H19NO3	(M+Na)+
261.1298	1	42344.59	C13H19NO3	(M+Na)+
262.1323	1	4623.75	C13H19NO3	(M+Na)+

Figure S13. HRMS of Compound **S1**

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C20 H25 N O5 S	0.118	391.1458	854402	C20 H25 N O5 S	391.1453	1.13

MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
392.1534	1	1202.18	C20H25NO5S	(M+H)+
393.1643	1	156	C20H25NO5S	(M+H)+
414.135	1	854401.63	C20H25NO5S	(M+Na)+
415.1381	1	175544.03	C20H25NO5S	(M+Na)+
416.1357	1	49103.63	C20H25NO5S	(M+Na)+

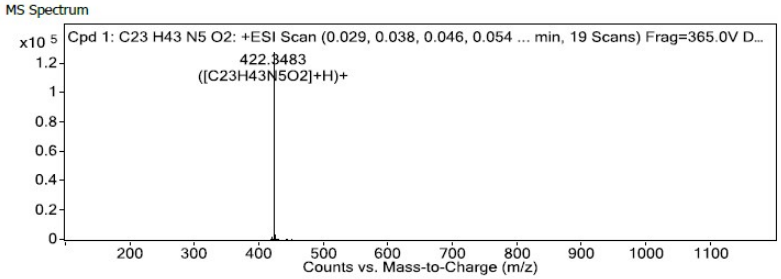
Figure S14. HRMS of Compound **S2b**

Compound Table

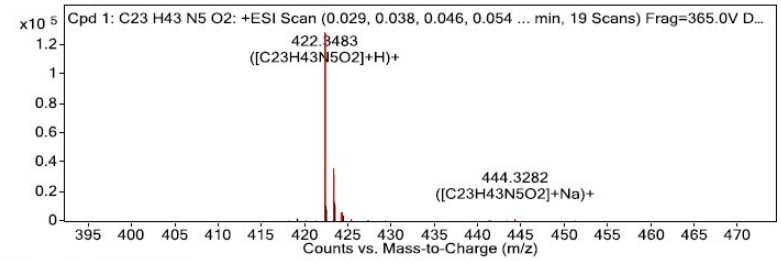
Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C23 H43 N5 O2	0.038	421.341	127973	C23 H43 N5 O2	421.3417	-1.68	C23 H43 N5 O2	C23 H43 N5 O2

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C23 H43 N5 O2	422.3483	0.038	Find By Formula	421.341

MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

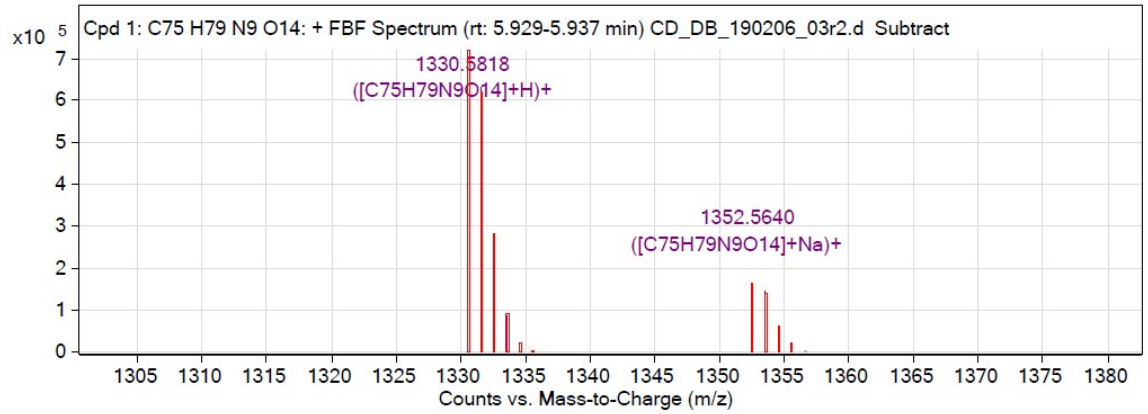
m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
422.3483	422.349	1.44	1	127973.05	C23H43N5O2	(M+H)+
423.351	423.3519	2.31	1	32352.66	C23H43N5O2	(M+H)+
424.3538	424.3547	2.28	1	4210.75	C23H43N5O2	(M+H)+
425.3568	425.3574	1.53	1	439.63	C23H43N5O2	(M+H)+
444.3282	444.3309	6.13	1	555.23	C23H43N5O2	(M+Na)+
445.3313	445.3339	5.7	1	122.12	C23H43N5O2	(M+Na)+
446.3169	446.3367	44.37	1	69.03	C23H43N5O2	(M+Na)+

Figure S15. HRMS of Spermine mono Ph-NHBOC (Intermediate for compound **1**)

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C75 H79 N9 O14	5.821	1329.5747	720544	C75 H79 N9 O14	1329.5746	0.07

MS Zoomed Spectrum



MS Spectrum Peak List

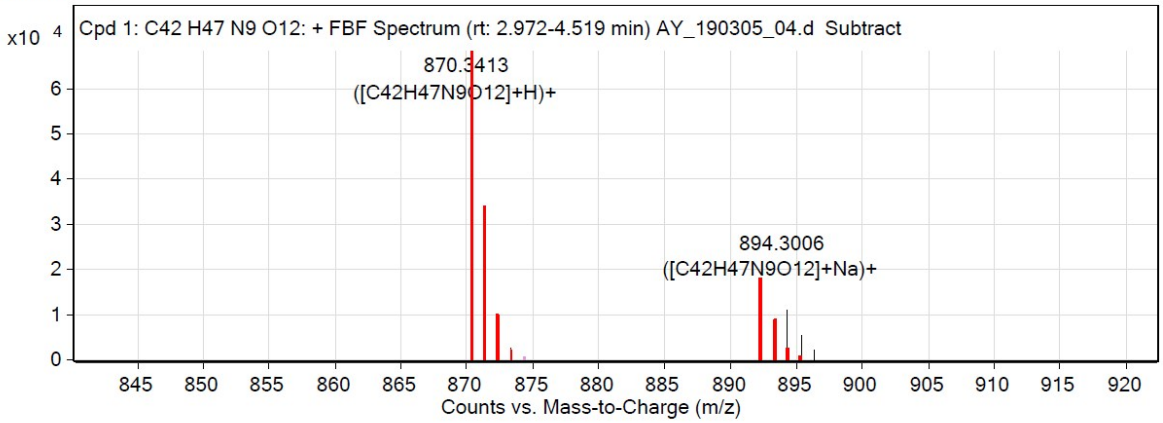
m/z	z	Abund	Formula	Ion
1330.5818	1	720544.25	C75H79N9O14	(M+H)+
1331.5852	1	631059.25	C75H79N9O14	(M+H)+
1332.589	1	273066.38	C75H79N9O14	(M+H)+
1333.5906	1	85510.94	C75H79N9O14	(M+H)+
1334.5927	1	20738.16	C75H79N9O14	(M+H)+
1335.5927	1	4785.6	C75H79N9O14	(M+H)+
1352.564	1	162156	C75H79N9O14	(M+Na)+
1353.5673	1	145619.59	C75H79N9O14	(M+Na)+
1354.5696	1	64523.8	C75H79N9O14	(M+Na)+
1355.5724	1	20166.56	C75H79N9O14	(M+Na)+

Figure S16. HRMS of compound **1**

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C42 H47 N9 O12	3.33	869.3298	68297	C42 H47 N9 O12	869.3344	-5.36

MS Zoomed Spectrum



MS Spectrum Peak List

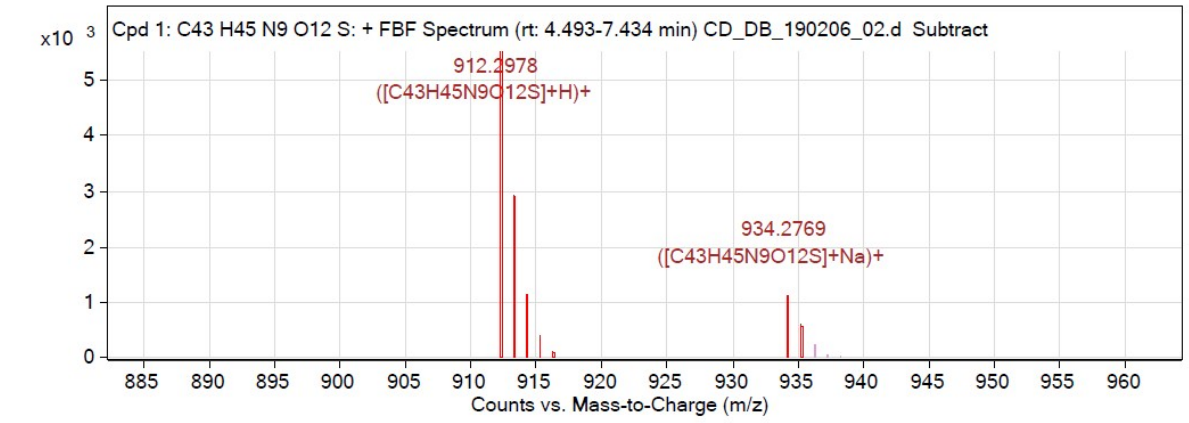
m/z	z	Abund	Formula	Ion
870.3413	1	68297.38	C42H47N9O12	(M+H)+
871.344	1	33455.82	C42H47N9O12	(M+H)+
872.3469	1	9982.67	C42H47N9O12	(M+H)+
873.3492	1	2310.85	C42H47N9O12	(M+H)+
892.3181	1	7168.24	C42H47N9O12	(M+Na)+
893.3166	1	4341.65	C42H47N9O12	(M+Na)+
894.3006	1	10859.19	C42H47N9O12	(M+Na)+
895.3028	1	5284.43	C42H47N9O12	(M+Na)+
896.304	1	2237.01	C42H47N9O12	(M+Na)+

Figure S17. HRMS of Compound **2**

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C43 H45 N9 O12 S	5.972	911.2892	5519	C43 H45 N9 O12 S	911.2908	-1.74

MS Zoomed Spectrum



MS Spectrum Peak List

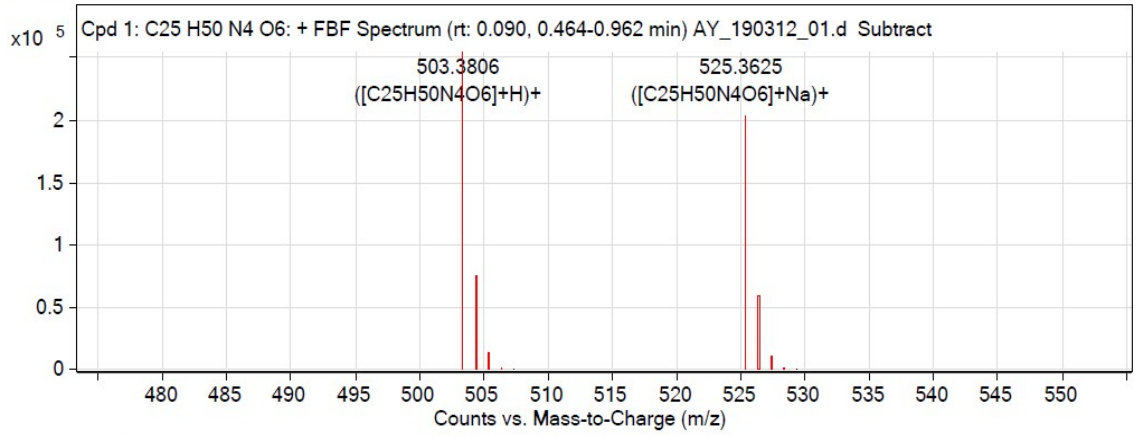
m/z	z	Abund	Formula	Ion
912.2978	1	5518.52	C43H45N9O12S	(M+H)+
913.3007	1	2926.74	C43H45N9O12S	(M+H)+
914.2999	1	1135.67	C43H45N9O12S	(M+H)+
915.2907	1	395.78	C43H45N9O12S	(M+H)+
916.279	1	107.02	C43H45N9O12S	(M+H)+
934.2769	1	1094.94	C43H45N9O12S	(M+Na)+
935.2777	1	593.23	C43H45N9O12S	(M+Na)+

Figure S18. HRMS of Compound **3**

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C25 H50 N4 O6	0.115	502.3732	202787	C25 H50 N4 O6	502.373	0.41

MS Zoomed Spectrum



MS Spectrum Peak List

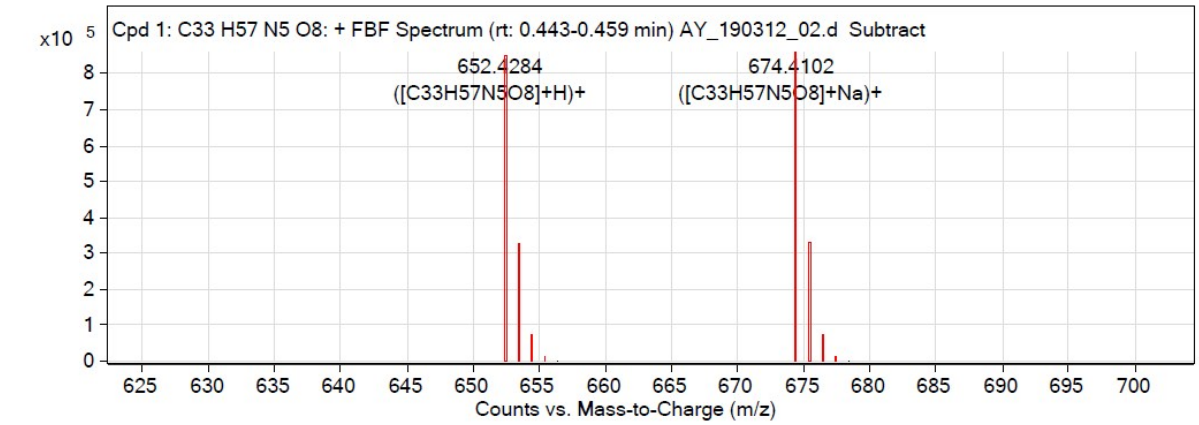
m/z	z	Abund	Formula	Ion
503.3806	1	254455.78	C25H50N4O6	(M+H)+
504.3836	1	65923.58	C25H50N4O6	(M+H)+
505.3861	1	12195.28	C25H50N4O6	(M+H)+
506.3882	1	1613.74	C25H50N4O6	(M+H)+
507.389	1	169.6	C25H50N4O6	(M+H)+
525.3625	1	202787.02	C25H50N4O6	(M+Na)+
526.3653	1	56712.75	C25H50N4O6	(M+Na)+
527.3678	1	10332.87	C25H50N4O6	(M+Na)+
528.3703	1	1396.42	C25H50N4O6	(M+Na)+
529.3719	1	162.67	C25H50N4O6	(M+Na)+

Figure S19. HRMS of Compound **4**

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C33 H57 N5 O8	0.135	651.4211	859526	C33 H57 N5 O8	651.4207	0.63

MS Zoomed Spectrum



MS Spectrum Peak List

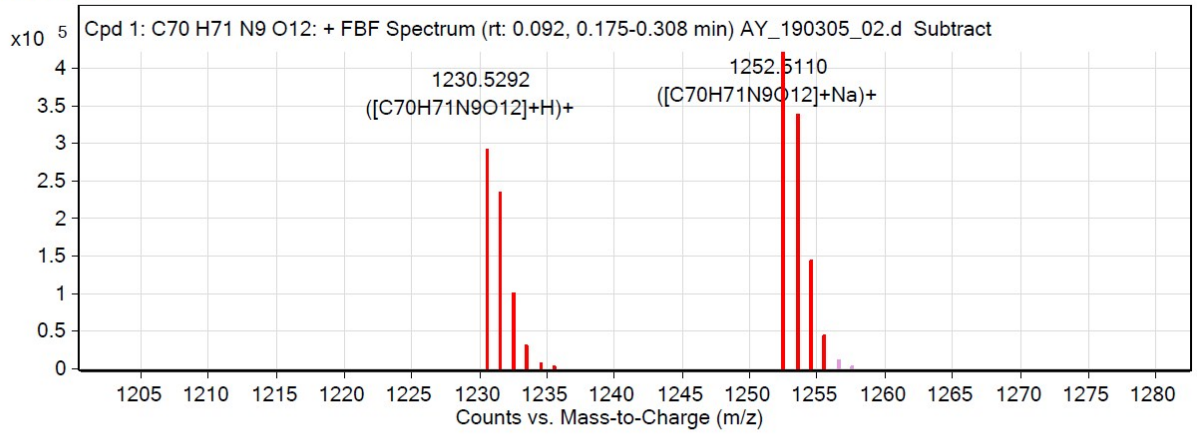
m/z	z	Abund	Formula	Ion
652.4284	1	849852.94	C33H57N5O8	(M+H)+
653.4319	1	297926.72	C33H57N5O8	(M+H)+
654.4342	1	63774.71	C33H57N5O8	(M+H)+
655.4366	1	10315.5	C33H57N5O8	(M+H)+
656.4387	1	1462.34	C33H57N5O8	(M+H)+
674.4102	1	859525.94	C33H57N5O8	(M+Na)+
675.4136	1	306508.88	C33H57N5O8	(M+Na)+
676.4159	1	66397.7	C33H57N5O8	(M+Na)+
677.4181	1	10765.14	C33H57N5O8	(M+Na)+
678.4211	1	1767.19	C33H57N5O8	(M+Na)+

Figure S20. HRMS of Compound **5**

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C70 H71 N9 O12	0.109	1229.522	291642	C70 H71 N9 O12	1229.5222	-0.2

MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
1230.5292	1	291642.47	C70H71N9O12	(M+H)+
1231.5322	1	234697.89	C70H71N9O12	(M+H)+
1232.5354	1	96760.02	C70H71N9O12	(M+H)+
1233.5379	1	27606.99	C70H71N9O12	(M+H)+
1234.5437	1	7242.72	C70H71N9O12	(M+H)+
1235.5522	1	2086.66	C70H71N9O12	(M+H)+
1252.511	1	421751.31	C70H71N9O12	(M+Na)+
1253.5144	1	322793.06	C70H71N9O12	(M+Na)+
1254.5179	1	143499.13	C70H71N9O12	(M+Na)+
1255.5205	1	42572.86	C70H71N9O12	(M+Na)+

Figure S21. HRMS of Compound **6**

LCMS for HOPO-NH2

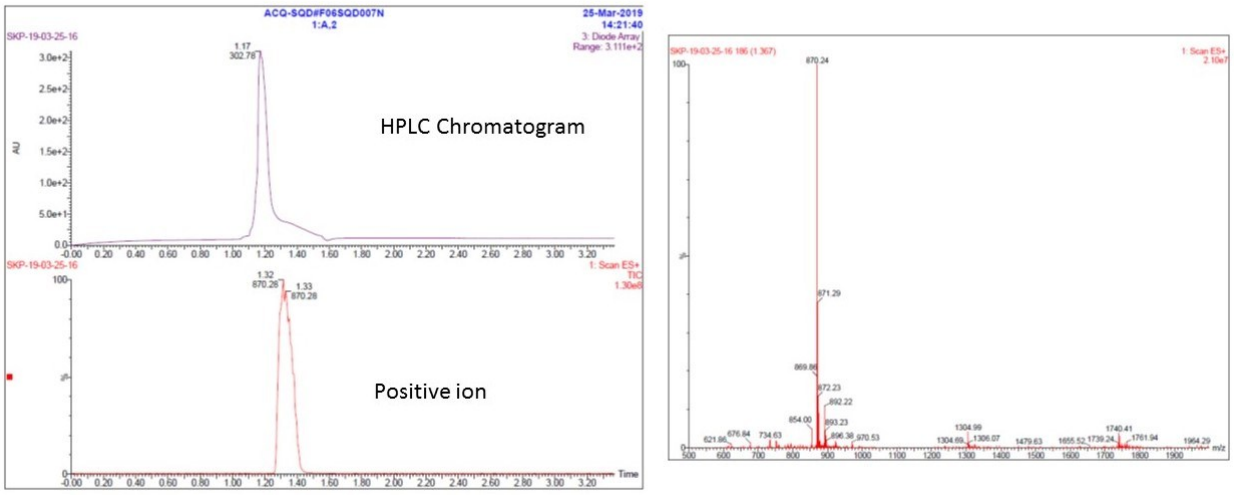


Figure S22. LCMS of compound **2**

LCMS for HOPO-NCS

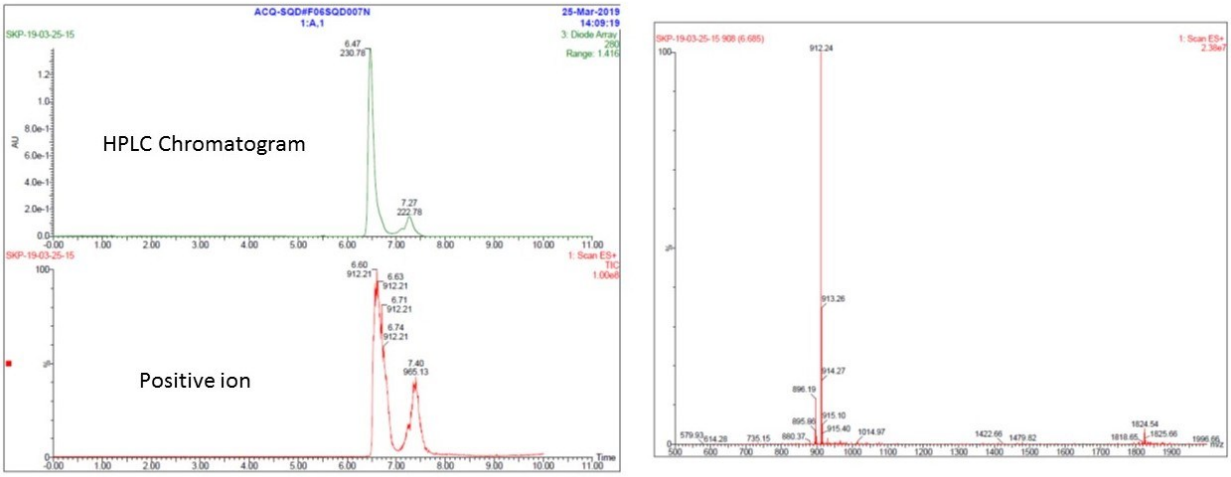


Figure S23. LCMS of compound **3**. Peak at 7.30 min is the Fe(III) HOPO-Bn-pNCS. Fe(III) is picked up in the HPLC assay.

Author Contributions:

N. V. S. D. K. B is the principal investigator, lead the study, designed, conducted experiments on the new four step scheme and helped improve yields on 9-step synthesis

A. Y : conducted experiments to improve yields on 9-step synthesis; supervised undergraduates who also performed experiments to improve yields on 9-step synthesis

M. C: performed experiments represented in Tables 1,2 and Table S1 to improve yields on 9-step synthesis under guidance of A.Y.

J. A: performed experiments represented in Tables 1,2 and Table S1 to improve yields on 9-step synthesis under guidance of A.Y.

H. T. C: performed experiments on new four step synthesis under guidance of N.V.S.D.K.B.

K. M. T: performed experiments represented in Tables 1,2 and Table S1 to improve yields on 9-step synthesis.

M. A. D: provided leadership, direction and insight towards improvement on 9-step synthesis that made possible the experiments represented in Tables 1,2. Contributed to preparation of manuscript.

S. P: developed and implemented HPLC purification of final product.

J. W. B: provided guidance and direction on the purification of final product.

J. S. L: provided overall leadership, direction and focus of the entire project.

L. C. F: initiated entire project; provided day-to-day management; contributed to writing of manuscript.

C. M. D: provided leadership and hands-on direction of project, specifically the new four step synthesis and also improvements on the nine-step synthesis, provided guidance on outlining manuscript and writing of manuscript.

The manuscript was written by N. V. S. D. K. B; A. Y; L. C. F; and C.M.D.; and commented by all authors.