Thiol and Disulfide Containing Vancomycin Derivatives Against Bacterial Resistance and Biofilm Formation

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Abstract

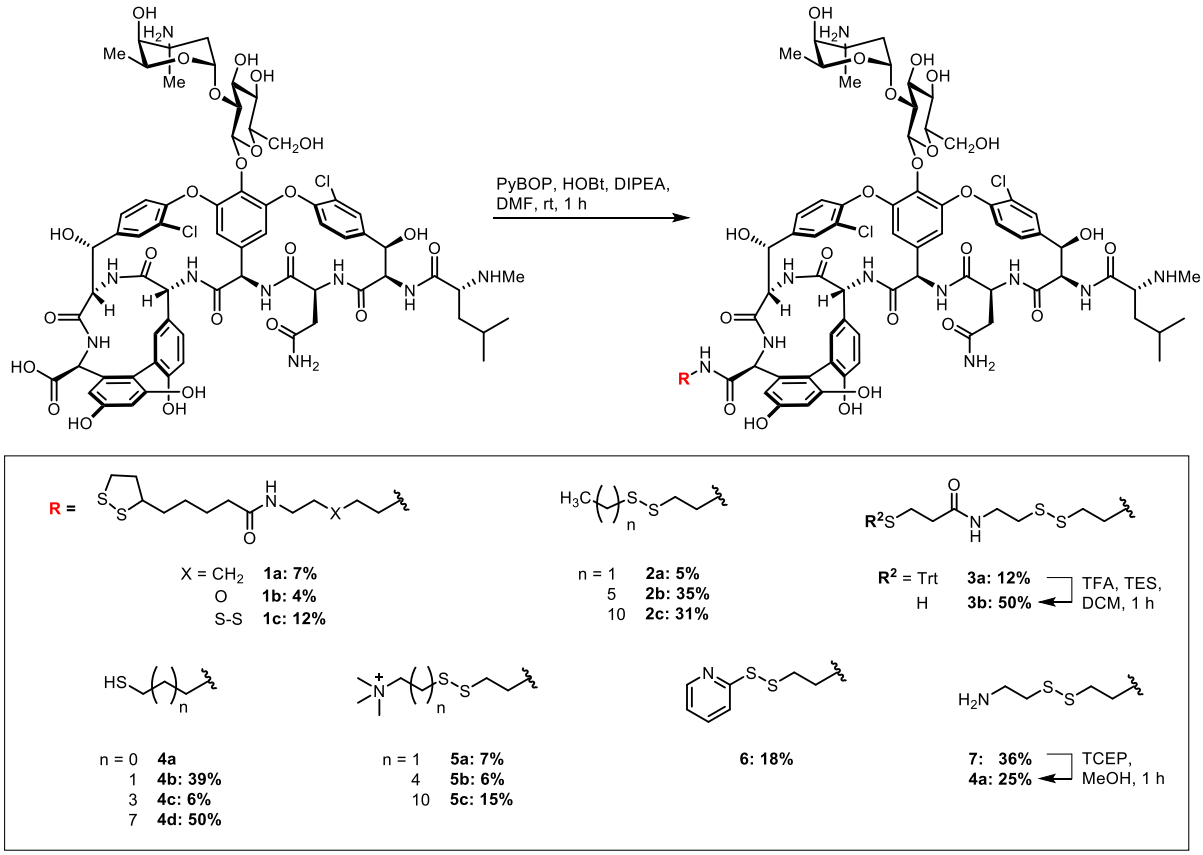
Antibiotic-resistant and biofilm-associated infections constitute a rapidly growing issue. The last resort antibiotic vancomycin is under threat, due to the increasing appearance of vancomycin resistant bacteria as well as the formation of biofilms. Herein, we report a series of novel vancomycin derivatives carrying thiol- and disulfide-containing moieties. The new compounds exhibited enhanced antibacterial activity against a broad range of bacterial strains, including vancomycin resistant microbes and Gram-negative bacteria. Moreover, all obtained derivatives demonstrated improved antibiofilm formation activity against VanB resistant *Enterococcus* compared to vancomycin. This work established a promising strategy for combating drug-resistant bacterial infections or disrupting biofilm formation and advances the knowledge on structural optimization of antibiotics with sulfur-containing modifications.

Growing multidrug bacterial resistance has become a life-threatening problem.[1] A fraction of antibiotic-resistant bacteria dubbed "the ESCAPE pathogens" including *Enterococcus faecium* and *Staphylococcus aureus* are listed as high priority targets for new treatments.[2,3] Several glycopeptide antibiotics have contributed to their sustained effectiveness in the clinic including "last resort" antibiotic vancomycin broadly used against methicillin-resistant *S. aureus* (MRSA).[4–7] However, after over 60-years of clinical use, vancomycin-resistant *S. aureus* (VRSA) and *Enterococci* (VRE) have brought new challenges to antibacterial treatment.[8–10] Consequently, there is a pressing need for renewed antibiotic discovery which overcomes the forces of evolution and selection responsible for bacterial resistance. By avoiding the molecular basis of the prevalent mechanism of resistance and providing additional mechanisms of action, drug potency against the development of resistance can be improved. Vancomycin binds to the C-terminus D-alanyl-D-alanine moiety of bacterial cell wall precursors and inhibits cell wall biosynthesis.[11,12] Various modification strategies have already been applied to increase vancomycin activity, such as ligand-binding enhancement,[13–15] binding to the pyrophosphate group of lipid II peptidoglycan,[16,17] or attachment of bacterial membrane disrupting fragments.[15,18–22] These approaches involve increasing of the residence time of antibiotics at the bacterial cell therefore improving their performance. In this respect, disulfide units might be considered as an alternative method for retaining the antibiotic on the surface of bacteria or facilitating its uptake. A number of antibiotics contain disulfide units, which have been postulated to be involved in structural rigidification (e.g., in peptide antibiotics)[23–27] or part of the pharmacophore,[28–39] show a broad range in activity against both Gram-positive and Gram-negative strains as well as biofilm formation. However, utilizing thiol-mediated uptake in bacteria was rarely investigated, and the results obtained have been mixed so far.[40]

Additionally, to growing resistance, about 80% of all chronic infections are associated with an increased survive ability of a causative pathogen in a formed biofilm.[41–43] Among them, Gram-positive *cocci* are well established bacteria related to biofilm infections. *Staphylococci* are the leaders of prosthetic related infections[44,45] followed by *Enterococci*.[46] Treatment of these infections is not always successful due to bacterial tolerance to conventional antimicrobial agents. The combined effects of the emergence of antibiotic-resistant strains and their ability to form biofilms represent a serious threat nowadays. Therefore, the development of drugs capable of combating both resistant strains and biofilm formation is particularly attractive.

In this work, we investigated the influence of sulfur-modified vancomycin derivatives on antibacterial activity against a broad range of bacterial strains., thereby rationally probing different substitution effects on the sulfur unit. Firstly, a series of vancomycin derivatives were synthesized by C-terminal modification of the glycopeptide with corresponding amines in the presence of PyBOP and HOBt as coupling reagents (Scheme 1). Lipoic acid derivatives (**1a-c**) have been chosen along the line of successful eukaryotic, thiol-mediated uptake systems, which have earlier also been evaluated with mixes success against bacteria.[40] Endolipidic disulfides (**2a-c**) were selected to increase lipophilicity, which should also positively influence residence time and potentially involve other mechanisms of actions, such as disruption of cell-wall integrity and inhibition of transglucosylase.[47] Terminal thiols were selected, both with internal disulfides (**3a**/**3b**) and without (**4a-d**), which would be most accessible to any bacterial counterpart. Finally, disulfides with polar end groups such as ammonium salts (**5a-c**), pyridine (**6**) and amine (**7**) were chosen to increase interaction with negatively charged compounds on the bacterial cell wall and improve bacteria membrane permeability.

Scheme 1. Functionalization of vancomycin at carboxylic position with sulfur-containing linkers.



The antibacterial activities of vancomycin thiol- and disulfide-containing derivatives against a selection of pathogenic clinically isolated Gram-positive strains including resistant MRSA, VISA, and VRE were determined by standard broth microdilution susceptibility tests.[48,49] The data is summarized in Table 1 (more details in Table S2 in the Supporting Information). The results reveal that the presence of the lipophilic chains is important for antibacterial activity against vancomycin intermediate, vancomycin resistant strains and *S. pneumonia*. The C6 and C11 carbon chains connected to the disulfide derivatives **2b-2c** displayed 16 to 128-fold enhanced activity for VISA, VanA and VanB strains compared to vancomycin. The derivatives with trimethyl ammonium moiety **5a-5c** were especially active against VISA and *B. subtilis* (Table S2 in the Supporting Information) and demonstrated 8-fold improvement against VRE compared to vancomycin. Moreover, C2-C5 carbon chain derivatives **5a-5b** also showed high increase in activity for *C. difficile* strain. The enhanced VRE antimicrobial activities of the vancomycin derivatives possessing lipophilic chains (**2b-2c**), or quaternary ammonium group (**5c**) might be explained by feasible contribution of the additional mechanism of action associated with disrupting bacterial cell membrane integrity. A similar effect has been reported earlier for the C-terminus modifications of vancomycin with aliphatic chains and trimethylammonium salt.[20,21,50] The lipophilic C5 chain derivative with a free thiol group **4c** was the most effective antibacterial agent against vancomycin resistant strains and also demonstrated over 16-fold improvement in activity for *S. pneumonia* and *C. difficile*. One reason for these observations might be possible compound dimerization during the *in vitro* experiments. A few examples of disulfide-containing dimers were earlier investigated by Nicolaou et al.[51] and Sundram et al.[52] demonstrating an improved activity against resistant *Enterococcus*. The vancomycin derivatives with a lipoic acid (**1a-1c**) substitutions did not alter the antibacterial profile of parent vancomycin against sensitive or resistant strains, however 4 to 8-fold increase in activity was observed against VISA, *S. pneumoniae* and *C. difficile* for the compound **1a**. The derivatives containing both disulfide bond and terminal thiol group (**3a**) revealed good potential against vancomycin intermediate and resistant strains with 8 to 64-fold enhancement in activity compared to vancomycin. Interestingly, the Trt protected analog (**3b**) of the compound **3a** did not show the loss in activity compared to its deprotected version and furthermore demonstrated the slight improvement (4-fold) against certain *C. difficile* strains and *B. subtilis* (Table S2 in the Supporting Information).

The lipo-ammonium complex[53] or sulfonium modification[21] in vancomycin derivatives were reported to have antibacterial activity against Gram-negative strains. Therefore, we sought to investigate whether the introduction of sulfur-containing linkers could achieve a similar effect. The derivatives were tested against several Gram-negative bacteria (Table S2 in the Supporting Information). Unfortunately, the modifications made did not have any effect on antibacterial activity against *E. coli*, *A. baumannii* or *P. aeruginosa*. However, we observed high activity (MIC = 1-2 μg/mL) against *M. catarrhalis* strains for the compounds possessing lipophilic chains (**2b-2c**) or Trt group (**3b**) as well as derivatives with quaternary ammonium salt (**5a-5c**) compared to vancomycin, which is resistant to these strains (Table 1). Collectively, these data provide clear and strong evidence that the presence of sulfur-containing linkers enhance the antibacterial profile of vancomycin improving the activity against a broader spectrum of bacterial strains.

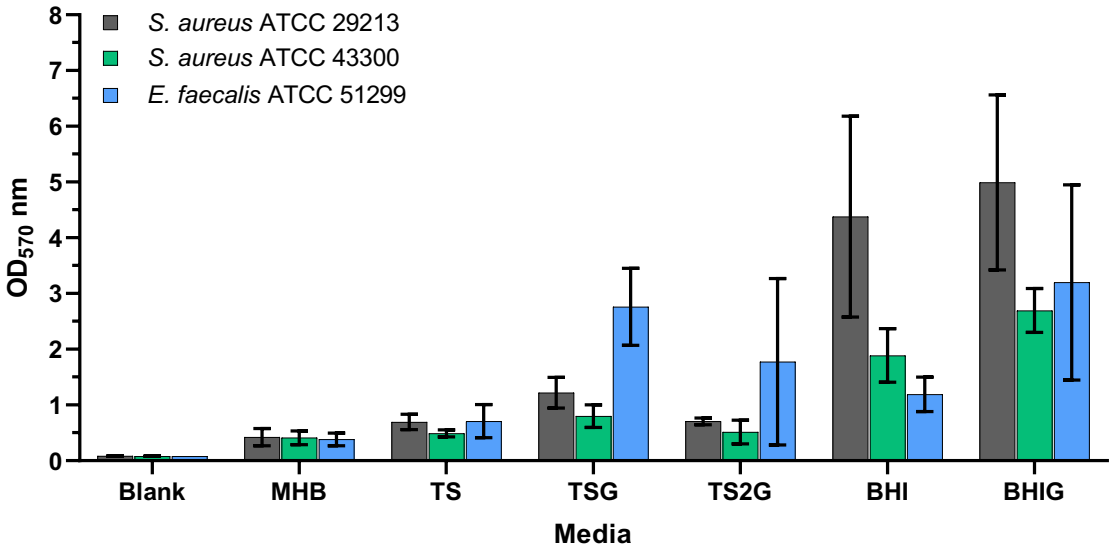
Table 1. Minimum Inhibitory Concentrations (MIC) of vancomycin derivatives against selected strains.

	MIC (µg/mL)									
	Sensitive	MRSA	VISA	hVISA	VanA	VanB	VanC			
	S. aureus[a]	*S. aureus*[b]	*S. aureus*[c]	*S. aureus*[d]	*E. faecium*[e]	*E. faecalis*[f]	*E. gallinarum*[g]	*S. pneumoniae*[h]	*C. difficile*[i]	*M. catarrhalis*[j]
VAN	1-2	1-2	8	16-32	>128	16	8-16	0.12-0.5	0.12-0.5	>32
1a	2	2	1	32	>32	4	8	0.06	0.06	4
1b	4	2	n.d.	n.d.	n.d.	4	8	n.d.	n.d.	n.d.
1c	2	2	2	2	>32	4	8	0.25	0.5	8
2a	1	1	n.d.	n.d.	n.d.	4-8	8	n.d.	n.d.	n.d.
2b	0.12-0.5	0.12-0.25	0.5	0.5	16	0.5	4-8	0.06	0.5	2
2c	1	1-2	0.5	1	2	2	4	0.25	0.12	0.5
3a	8	4	n.d.	n.d.	n.d.	2	1-2	n.d.	n.d.	n.d.
3b	0.5	0.5-1	1	1	8	2-4	4	0.25	1	2
4a	4	4	n.d.	n.d.	n.d.	2-4	2	n.d.	n.d.	n.d.
4b	4	4	8	>32	1	4	1-2	≤0.03	0.12	16
4c	8	8	2	>32	1	1-2	0.5-1	≤0.03	0.06	4
4d	4	4	2	2	2	8	8	0.5	4	8
5a	1	0.5	0.5	>32	32	16	4	0.25	≤0.015	2
5b	1	0.5-1	1	>32	16	8-16	4	0.06	≤0.015	2
5c	1	0.5	0.25	8	16	2	2	0.12	1	1
6	4	2-4	4	4	8	16	8-16	0.5	0.5	>32
7	1-2	1-2	1	1	8	8	8	≤0.03	0.5	>32

VAN: vancomycin, MRSA: methicillin resistant; VISA: vancomycin-intermidiate; hVISA: heterogenous vancomycin-intermediate; VanA, VanB, VanC: vancomycin resistant. [a]ATCC 25923, [b]ATCC 43300, [c]ATCC NRS1, [d]ATCC NRS24, [e]MMX0485, [f]ATCC 51299, [g]ATCC 700221, [h]ATCC 29212, [i]ATCC 43255 (rt 087), [j]ATCC 8193. n.d.: not determined.

Before investigating the inhibitory activity of our compounds on biofilm formation, the optimization of conditions for biofilm growth and the assessment of metabolic activity were firstly carried out. The biofilm-forming activity of six Gram-positive clinically isolated bacterial strains was investigated in six different media: MHB, TS, TSG, TS2G, BHI, and BHIG, after 24 h incubation for *Staphylococci* and *Bacillus,* and 48 h for *Enterococci*.[54] The assessment of biofilm production carried out by crystal violet (CV) staining and analysis. From the obtained results, the biomass as measured by the absorbance of CV at 570 nm revealed low values for *Enterococci* (lower 0.3) in all tested mediums (Figure S1 in the Supporting Information). Exceptionally, *E. faecalis* strain had the higher biofilm mass formation, displaying absorbance values ranging from 1.5 to 4.9 in BHI medium supplemented with 1% glucose. *B. subtilis* showed relatively high absorption values in TSG broth, however the biofilm was not uniformly formed therefore was not used for the further experiments. *Staphylococcus* spp. exhibited the highest biofilm-forming profile in BHI broth with values over 3 and over 2 for vancomycin-sensitive and MRSA strains, respectively. Moreover, it was observed that the supplementation of BHI medium with 1% glucose improved the biofilm formation for *Staphylococcus* by 20%. The results of biofilm assessment for the best performers are summarized in Figure 1.

Figure. 1 Assessment of biofilm biomass by crystal violet staining of three bacterial strains: *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 (MRSA), *E. faecalis* ATCC 51299. Strains were incubated in six different media. Error bars represent standard deviation of two independent experiments in triplicate.



Composition of the medium is likely the most important factor influencing the ability of bacteria to produce biofilm under *in vitro* conditions. Earlier studies have shown that liquid media (TS or BHI) supplemented with glucose (0.25-4%) initiate greater biofilm formation for staphylococcal and enterococcal species.[54–58] In our findings, the BHI media supplemented with 1% glucose had the best performance for the *Staphylococci* and *E. faecalis*. Moreover, Mohamed et al. reported that independent of the media *E. faecalis* produces biofilm stronger than *E. faecium*, which is in agreement with our results.[46] These results demonstrate the important role of media in biofilm production amongst Gram-positive strains.

Next, we moved to the investigation of metabolic activity of formed biofilms. The previous studies have shown the comparison characteristics between several assays evaluating viable cells and concluded that resazurin is a good alternative to other assessments of metabolic activity of Gram-positive strains.[59] Therefore, for the assessment of metabolic activity of biofilm cells, the resazurin viability assay was chosen for the Gram-positive strains.

Biofilms from the selected strains were formed over 24 h for *S. aureus* and 48 h for *E. faecalis* before the resazurin assay. The formed biofilms were treated with resazurin solutions at concentrations of 2, 4, and 8 µg/mL, respectively, and the plates were incubated at 37 °C. The relative fluorescence units (RFU) were taken every 2 min for 2 h. For all tested strains, the RFU values were increasing during 80 min of measure and then remain constant till the maximum incubation time (Figure S2 in the Supporting Information). The selection of optimal assay conditions was based on calculated statistical quality parameters (Table S5 in the Supporting Info). The conditions chosen for the resazurin assay mostly yielded an excellent Z' value (≥ 0.5). According to the obtained results the conditions for the resazurin assay selected for further experiments were set as follows: 8 µg/mL with incubation for 60 min for *S. aureus* ATCC 29213, and 4 µg/mL with incubation for 40 min for *S. aureus* ATCC 43300 and *E. faecalis* ATCC 51299. Using the previously optimized assay, novel synthesized vancomycin derivatives were tested for their ability to repress the growth and biofilm formation by three bacterial strains. The biofilms were grown in optimized earlier conditions in the presence of antibiotics at different concentrations. After incubation, MBIC was determined by resazurin assay with the chosen conditions. Colony forming units (CFU) were also defined in order to corroborate the results obtained with the resazurin-based assay.

Within vancomycin derivatives, the evaluated compounds presented MBIC values similar to their MIC data when tested against *Staphylococcus* strains. No significant improvement was observed after the vancomycin modifications compared to vancomycin itself (Table S6 in the Supporting Information). However, all new synthesized compounds completely suppressed biofilm formation of vancomycin-resistant *E. faecalis* at concentrations lower than vancomycin itself (Table 2). In particular, vancomycin derivatives with C6 and C11 lipophilic chains (**2b** and **2c**) as well as containing both a disulfide bond and free thiol group (**3a**) displayed a 32-fold increase in MBIC values compared to vancomycin. Moreover, 16-fold enhancement in inhibition activity was observed for the compounds with shorter aliphatic chains and free thiol group at the end (**4a**, **4b**) or compound with longer chain but containing trimethyl ammonium salt (**5c**). Furthermore, compared to CFU results the obtained data, revealed the quality and accurateness of these anti-biofilm assays for MBIC determination.

Table 3. Minimal biofilm inhibitory concentration (MBIC) determined by resazurin and cell counting methods

Bacterial strain	Antibiotic	MIC (µg/mL)	MBIC (µg/mL)	
			Resazurin (decrease in RFU)	CFU/mL (growth inhibition)
E. faecalis ATCC 51299	**VAN**	16	64 (98%)	64 (100%)
	1a	4	8 (100%)	8 (100%)
	1b	4	8 (97%)	8 (100%)
	1c	4	16 (100%)	16 (100%)
	2a	1	32 (100%)	32 (100%)
	2b	0.25	2 (100%)	2 (100%)
	2c	0.25	2 (92%)	2 (99%)
	3a	2-4	2 (97%)	2 (100%)
	3b	2	8 (97%)	8 (100%)
	4a	2-4	4 (99%)	4 (100%)
	4b	4	4 (95%)	4 (100%)
	4c	1-2	32 (99%)	32 (100%)
	4d	8	8 (98%)	8 (100%)
	5a	16	16 (91%)	16 (100%)
	5b	8-16	16 (87%)	16(97%)
	5c	2	4 (84%)	4 (98%)
	6	16	16 (99%)	16 (100%)
	7	8	16 (86%)	16 (98%)

In earlier work by the group of Matile, it was demonstrated that the introduction of cyclic disulfides or selenides into vancomycin structure leads to either no improvement in activity or its slight loss when tested against Gram-positive *B. subtilis*.[40] Moreover, the Gram-negative strain did not show any sensitivity to the antibiotic after its modification. However, in our work, we showed significant increase of the antibiotic activity against several sensitive and resistant strains including Gram-negative *M. catarrhalis* after the incorporation of either cyclic disulfides or other sulfur-containing linkers into vancomycin structure. The obtained results revealed the improved delivery of the modified antibiotic to several Gram-positive and Gram-negative bacteria, which prompts the further need to study the effect of thiol groups on antibiotics activity or potential thiol-mediated uptake. The high improvement in activity for the vancomycin derivatives with quaternary ammonium salts bearing a terminal tetradecyl substituents was demonstrated by the Boger group when tested against VanA resistant *Enteroccocus*.[20] Our study revealed that the introduction of a disulfide bond instead of ammonium salt with a long aliphatic chain not only has a similar increase in activity against VanA resistant strains but also appeared to be active against Gram-negative bacteria. Moreover, the combination of both disulfide bond and ammonium salt moieties for the vancomycin modification added activity against *C. difficile*. The nature of high activity and possible dimerization for vancomycin derivatives with free thiol groups should be further investigated. Nevertheless, the introduction of free thiols and disulfide moieties are expected to potentially improve the ADME profile of new antibiotics as a result of metabolization into more hydrophilic compounds for better excretion as was shown in the studies of the Judice group.[60] Moreover, the modification of the C-terminal position on vancomycin giving the prospect for the further modification of glucosamine part by the introduction of a CBP-group in order to further increase the activity of the antibiotic against vancomycin-resistant strains.[20,61]

In conclusion, we present the potential to improve the antibacterial and antibiofilm formation activity of vancomycin by its structure optimization, with sulfur-containing linkers. This strategy paves the way to new semisynthetic vancomycin analogs for the treatment of vancomycin-resistant bacterial infections and Gram-negative bacteria as well as inhibition of biofilm formation for VanB resistant enterococcus. Overall, this work provides prospective options for further investigation of disulfide and thiol - containing compounds previously underestimated in medicinal chemistry.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website. Supplementary Figures S1-S2, Tables S2, S5, S6, experimental procedures and methods, characterization data, ^{1}H and ^{13}C NMR spectra, supplementary MIC tables.

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Author Contributions

I. S. S. and K. G. designed the study, I. S. S. performed all experiments, I. S. S. and K. G. discussed the data, I. S. S. and K. G. wrote the manuscript.

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ABBREVIATIONS

MIC, minimum inhibitory concentration; MBIC, minimum biofilm inhibitory concentration; CFU, colony forming unit; RFU, relative fluorescent unit.

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Supporting information

Thiol and Disulfide Containing Vancomycin Derivatives Against Bacterial Resistance and Biofilm Formation

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I. Materials and instrumentation

The reactions were carried out under inert gas (N_2 or Ar) in oven-dried (120 °C) glass equipment and monitored for completion by TLC or UHPLC−MS (ESI). Solvents for reactions and analyses were of analytical grade. Vancomycin was purchased from Apolo Scientific. **Analytical thin-layer chromatography** (TLC) was run on Merck TLC plates silica gel 60 F254 on glass plates with the indicated solvent system; the spots were visualized by UV light (365 nm), and stained by anisaldehyde, ninhydrin, or $KMnO_4$ stain. Silica gel **column chromatography** was performed using silica gel 60 (230−400 Mesh) purchased from Sigma-Aldrich with the solvent mixture indicated. The SPE columns used were DSC-18 (Supelco, Sigma). **High performance liquid chromatography** (HPLC) were performed on a Shimadzu HPLC system (LC-20AP dual pump, CBM-20A Communication Bus Module, SPP-20, A UV/vis Detector, FRC-10A Fraction collector) using reverse-phase (RP) columns analytical: Gemini-NX C18 (150 mm x 4.6 mm; 3 µm, 10 Å), Synergy Hydro-RP (150 mm x 4.6 mm; 4 µm, 80 Å),) with a flow of 0.4 mL/min; preparative: Gemini-NX C18 (250 mm × 21.2 mm; 10 µm, 110 Å) or Synergy Hydro-RP (250 mm × 21.2 mm; 10 µm, 80 Å)) with a flow of 20 mL/min (unless otherwise stated). A solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1% HCO_2H). **Ultrahigh-performance liquid chromatography** (UHPLC) coupled to mass spectrometer (MS) experiments were performed on an Ultimate 3000 LC system (HPG-3400 RS pump, WPS-3000 TRS autosampler, TCC-3000 RS column oven, Vanquish DAD detector from Thermo Scientific) coupled to a triple quadrupole (TSQ Quantum Ultra from Thermo Scientific). The separation was performed using a RP column (Kinetex EVO C18; 50 × 2.1 mm; 1.7 µm; 100 Å, Phenomenex), a flow of 0.4 mL/min, a solvent system composed of A (H_2O + 0.1% HCO_2H) and B (MeCN + 0.1% HCO_2H) and an elution gradient starting with 5% B, increasing from 5−95% B in 3.5 min, from 95−100% B in 0.05 min, and washing the column with 100% B for 1.25 min. The filtration before UHPLC analysis were performed using Syringe Filters, Nylon 66, 0.22 µm. **IR-spectroscopy** was performed on a Varian 800 FT-IR ATR Spectrometer. **Lyophilization** was performed on a Christ Freeze-dryer ALPHA 1−4 LD plus. **High-resolution electrospray mass spectra** (HRMS (ESI)) were recorded on a timsTOF Pro TIMS-QTOF-MS instrument (Bruker Daltonics GmbH, Bremen, Germany). The samples were Experimental Section I-53 dissolved in (e.g., MeOH) at a concentration of ca. 50 µg/mL and analyzed via continuous flow injection (2 µL/ min). The mass spectrometer was operated in the positive (or negative) electrospray ionization mode at 4000 V (−4000 V) capillary voltage and −500 V (500 V) end plate offset with a N_2 nebulizer pressure of 0.4 bar and a dry gas flow of 4 mL/min at 180 °C. Mass spectra were acquired in a mass range from m/ z 50 to 2000 at ca. 20 000 resolution (m/z 622) and at 1.0 Hz rate. The mass analyzer was calibrated between m/z 118 and 2721 using an Agilent ESI-L low concentration tuning mix solution (Agilent, USA) at a resolution of 20 000 giving a mass accuracy below 2 ppm. All solvents used were purchased in best LC-MS quality. **Nuclear magnetic resonance spectra** (NMR): 1H and 13C were recorded on Avance II or III-500 (500 MHz with Cryo-BBO, TXI, BBI or BBO probes) in $CDCl_3$, CD_3OD, D_2O, CD_3CN, DMSO-d_6. Chemical shifts are given in parts per million (ppm) on the delta (δ) scale and coupling constants (J) were reported in Hz (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet or not resolved signal; br, broad signal). Chemical shifts were calibrated according to the used solvents.[1]

II. Synthesis of the compounds

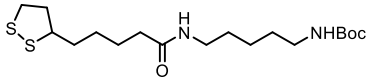
a. Synthesis of the linkers for vancomycin derivatives

Synthesis of lipoic acid derivatives with Boc-protected amine

General procedure:

Lipoic acid (1 equiv.) dissolved in DMF (0.1 M solution) was treated with DIPEA (3 equiv.). The reaction mixture was cooled down to 0 °C with an ice/water bath and HATU (2 equiv.) in DMF (0.075 M solution) was added. The mixture was stirred for 15 min followed by the addition of linkers **1.1a**, **1.1b**, or **1.1c** (1.2 equiv.) in DMF (0.05 M solution). The stirring was continued at 0 °C for 2 h. The reaction mixture was poured into water and extracted with DCM (3x5 mL). The organic phase was washed with brine (2x5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The purification methods, yields and analytical data are described below.

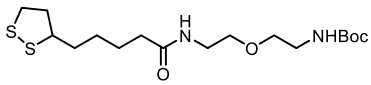
Tert-butyl (5-(5-(1,2-dithiolan-3-yl)pentanamido)pentyl)carbamate



The purification was carried out by column chromatography (EtOAc/pentane 1:1). The desired product was obtained as a yellowish solid (302 mg, 0.80 mmol, 80%).

Rf = 0.42 (EtOAc/pentane = 2:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.55 (s, 1H), 3.57 (dp, *J* = 6.4, 1.7 Hz, 1H), 3.24 (q, *J* = 6.9, 2H), 3.21 – 3.14 (m, 1H), 3.10 – 3.07 (m, 2H), 2.49 – 2.41 (m, 1H), 2.17 (t, *J* = 7.6, 2H), 1.94 – 1.86 (m 1H), 1.74 – 1.59 (m, 5H), 1.55 – 1.46 (m, 5H), 1.44 (s, 9H), 1.38 – 1.30 (m, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.84, 156.27, 56.59, 40.39, 39.42, 38.60, 36.66, 34.77, 29.91, 29.32, 29.05, 28.58, 25.58, 24.06. **IR (film)** ν_{max} = 3318, 2932, 1690, 1656, 1529, 1454, 1366, 1251, 1169, 846, 558. **HRMS (ESI)**: calcd for $C_{18}H_{34}O_3N_2NaS_2$ $[M+Na]^+$, *m/z* = 413.19065, found 413.19031.

Tert-butyl (2-(2-(5-(1,2-dithiolan-3-yl)pentanamido)ethoxy)ethyl)carbamate

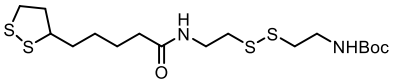


The purification was carried out by column chromatography (EtOAc/pentane = 1:1). The desired product was obtained as a yellow amorphous solid (218 mg, 0.969 mmol, 57%).

Rf = 0.4 (EtOAc/pentane = 2:1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.03 (s, 1H), 4.86 (s, 1H), 3.60 – 3.56 (m, 1H), 3.55 – 3.49 (m, 4H), 3.45 (m, 2H), 3.31 (t, *J* = 4.9 Hz, 2H), 3.21 – 3.15 (m, 1H), 3.14 – 3.08 (m, 1H), 2.82 (d, *J* = 0.7 Hz, 2H), 2.49 – 2.42 (m, 1H), 2.26 – 2.20 (t, *J* = 7.2, 2H), 1.95 – 1.87 (m, 2H), 1.76 – 1.63 (m, 5H), 1.53 – 1.47 (m, 2H), 1.46 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.12, 156.29, 69.85, 56.60, 40.40, 39.38, 38.83, 38.61, 36.47, 34.78, 29.04, 28.57, 25.54. **IR (film)** ν_{max} = 3314, 2979, 2937, 1692, 1657, 1530, 1503, 1451, 1392,

1366, 1282, 1233, 1166, 1126, 1049, 967, 845, 781, 558. **HR-ESI-MS:** calcd for $C_{17}H_{32}O_4N_2NaS_2$ $[M+Na]^+$, *m/z* = 415.16957, found 415.16943.

Tert-butyl (2-((2-(5-(1,2-dithiolan-3-yl)pentanamido)ethyl)disulfaneyl)ethyl)carbamate



The purification was carried out by column chromatography (EtOAc/pentane = 3:1). The desired product was obtained as a slightly yellow solid (57 mg, 0.165 mmol, 43%).

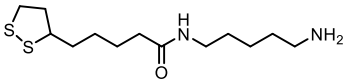
Rf = 0.38 (EtOAc/pentane = 4:1) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.39 (s, 1H), 5.00 (s, 1H), 3.60 (m, 3H), 3.48 (m, 2H), 3.24 – 3.10 (m, 2H), 2.86 (m, 2H), 2.81 (m, 2H), 2.48 (tt, *J* = 12.6, 6.5 Hz, 1H), 2.27 (t, *J* = 7.2 Hz, 2H), 1.98 – 1.89 (m, 1H), 1.72 (m, 4H), 1.51 (m, 2H), 1.47 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.45, 156.10, 79.95, 56.60, 40.43, 40.39, 38.66, 38.63, 38.14, 37.97, 36.42, 34.80, 29.05, 28.55, 25.55, 25.52. **IR (film)** ν_{max} = 3318, 2974, 2927, 1693, 1649, 1526, 1452, 1391, 1365, 1252, 1168, 1042, 949, 866, 643 **HRMS (ESI)**: calcd for $C_{17}H_{33}O_3N_2S_4$ $[M+H]^+$, *m/z* = 441.13685, found 441.13750. ***m.p.*** = 49.7-51.5 °C.

Synthesis of lipoic acid derivatives with Boc-deprotected amine

General procedure:

To a solution of Boc-protected linker (1 equiv.) in DCM (0.2 M solution), TFA (15% v/v) was added. The reaction mixture was stirred for 2 h at rt. The solvent was evaporated, and the crude was washed with ether (2x5 mL) to afford the desired products. Analytical data and yields for obtained compounds are described below.

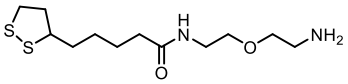
N-(5-aminopentyl)-5-(1,2-dithiolan-3-yl)pentanamide



The product was obtained as an amorphous white solid (224 mg, 0.794 mmol, 93%).

^{1}H NMR (500 MHz, MeOD) δ 3.18 (m, 2H), 2.93 (m, 2H), 2.84 (m, 2H), 2.26 – 2.16 (m, 2H), 2.03 (m 1H), 1.75 – 1.60 (m, 6H), 1.54 (m, 2H), 1.46 – 1.37 (m, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 176.07, 57.60, 41.32, 40.58, 39.87, 39.33, 36.87, 35.71, 29.93, 29.86, 28.18, 27.71, 26.73, 24.67. **IR (film)** ν_{max} = 2933, 2864, 1676, 1632, 1537, 1367, 1291, 1200, 1175, 839, 722, 706. **HRMS (ESI)**: calcd for $C_{13}H_{27}ON_2S_2$ $[M+H]^+$, *m/z* = 291.15593, found 291.15582.

N-(2-(2-aminoethoxy)ethyl)-5-(1,2-dithiolan-3-yl)pentanamide

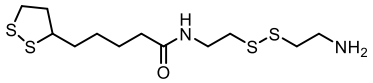


The product was obtained as an amorphous white solid (120 mg, 0.509 mmol, 81%).

^{1}H NMR (400 MHz, MeOD) δ 3.72 – 3.66 (m, 2H), 3.57 (m, 2H), 3.43 – 3.38 (m, 2H), 3.16 – 3.10 (m, 2H), 2.85 (m, 2H), 2.29 – 2.18 (m, 2H), 2.01 (m, 1H), 1.75 – 1.58 (m, 4H), 1.54 – 1.41

(m, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 175.42, 70.13, 66.79, 56.80, 40.53, 39.82, 39.14, 38.52, 36.04, 34.93, 29.06, 25.86. **IR (film)** ν_{max} = 3287, 2931, 1677, 1640, 1547, 1430, 1352, 1293, 1202, 1179, 1134, 1058, 840, 800, 722, 558, 516. **HRMS (ESI)**: calcd for $C_{12}H_{25}O_2N_2S_2$ $[M+H]^+$, *m/z* = 293.13520, found 293.13520.

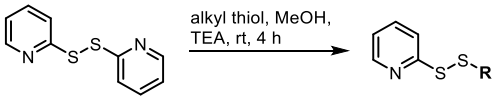
N-(2-((2-aminoethyl)disulfaneyl)ethyl)-5-(1,2-dithiolan-3-yl)pentanamide



The product was obtained as an amorphous white solid (21 mg, 0.066 mmol, 94%).

^{1}H NMR (400 MHz, MeOD) δ 3.53 – 3.47 (m, 2H), 3.30 – 3.26 (m, 3H), 3.22 – 3.04 (m, 1H), 2.30 – 2.95 (m, 2H), 2.91 – 2.76 (m, 4H), 2.29 – 2.17 (m, 2H), 2.04 – 2.0 (m, 1H), 1.75 – 1.59 (m, 4H), 1.55 – 1.40 (m, 2H). **^{13}C NMR** (101 MHz, MeOD) δ 57.56, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 41.30, 39.38, 39.33, 38.26, 36.79, 35.70, 35.41, 29.82, 26.64. **IR (film)** ν_{max} = 2932, 1675, 1650, 1430, 1356, 1292, 1204, 1176, 1145, 840, 806, 722, 558. **HRMS (ESI)**: calcd for $C_{12}H_{25}ON_2S_4$ $[M+H]^+$, *m/z* = 341.08442, found 341.08424.

Synthesis of pyridinyldisulfanyl derivatives

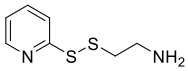


General procedure:

The synthesis was followed to the procedure described before.[2]

To a solution containing corresponding alkyl thiol (1 equiv.) in dry MeOH (0.2 M solution) purged with nitrogen, a solution containing 1,2-di(pyridin-2-yl)disulfane (1.1 equiv.) in dry MeOH (0.2 M solution) was added at room temperature. Then TEA (1.1 equiv.) was added into the reaction mixture and the resulting solution was stirred at rt for 4-6 h under an inert atmosphere. The reaction mixture was concentrated under reduced pressure and purified by column chromatography. The purification methods, yields and analytical data are described below.

2-(pyridin-2-yldisulfanyl)ethan-1-amine[3]

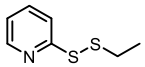


The purification was carried out by column chromatography (DCM/MeOH=10:1). The desired product was obtained as a yellow solid (31 mg, 0.176 mmol, 95%).

R*f* = 0.35 (DCM/MeOH 9:1). **^{1}H NMR** (400 MHz, MeOD) δ 8.51 (ddd, *J* = 6.3, 2.1, 0.9 Hz, 1H), 7.83 – 7.76 (td, *J* = 10.1, 2.3 Hz, 1H), 7.68 (dt, *J* = 10.1, 1.2 Hz, 1H), 7.30 (ddd, *J* = 9.3, 6.3, 1.2 Hz, 1H), 3.23 (t, *J* = 7.9 Hz, 2H), 3.10 (t, *J* = 7.9 Hz, 2H). **^{13}C NMR** (101 MHz, MeOD) δ 159.74, 150.83, 139.14, 123.18, 122.64, 39.08, 37.75. **IR (film)** ν_{max} = 2920, 1573, 1560,

1446, 1417, 1276, 1114, 1043, 760, 717 **HRMS (ESI)**: calcd for $C_7H_{11}N_2S_2$ $[M+H]^+$, *m/z* =187.03582, found 187.03587.

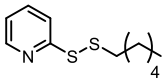
2-(ethyldisulfaneyl)pyridine[4]



The purification was carried out by column chromatography (EtOAc/pentane 1:6). The desired product was obtained as a colorless oil (103 mg, 0.97 mmol, 62%).

R*f* = 0.5 (EtOAc/pentane 1:5). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.45 (ddd, *J* = 4.8, 1.9, 1 Hz, 1H), 7.72 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.63 (ddd, *J* = 8.1, 7.4, 1.9 Hz, 1H), 7.06 (ddd, *J* = 7.4, 4.8, 1 Hz, 1H), 2.81 (q, *J* = 7.3 Hz, 2H), 1.33 (t, *J* = 7.3 Hz, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 160.81, 149.69, 137.05, 120.60, 119.66, 32.96, 14.34; **IR (film)** ν_{max} = 3045, 2970, 2925, 2868, 1572, 1560, 1445, 1416, 1373, 1275, 1253, 1144, 1116, 1082, 1043, 985, 968, 881, 758, 717; **HRMS (ESI)**: calcd for $C_7H_{10}NS_2$ $[M+H]^+$, *m/z* =172.02492, found 172.02500.

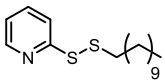
1-hexyl 2-pyridyl disulfide[5]



The purification was carried out by column chromatography (EtOAc/pentane 1:20). The desired product was obtained as a colorless oil (118 mg, 0.71 mmol, 73%).

Rf = 0.35 (EtOAc/pentane 1:20). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.46 (ddd, *J* = 4.8, 1.8, 1 Hz, 1H), 7.73 (dt, *J* = 8.2, 1 Hz, 1H), 7.64 (td, *J* = 8.2, 1.8 Hz, 1H), 7.07 (ddd, *J* = 8.2, 4.8, 1 Hz, 1H), 2.79 (t, *J* = 7.2 Hz, 2H), 1.72 – 1.65 (m, 2H), 1.42 – 1.35 (m, 2H), 1.33 – 1.20 (m, 5H), 0.87 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.44, 160.88, 149.70, 137.06, 120.58, 119.68, 39.20, 31.51, 29.05, 28.31, 22.65, 14.15. **IR (film)** ν_{max} = 3045, 2954, 2925, 2855, 1573, 1561, 1445, 1416, 1377, 1278, 1257, 1144, 1116, 1082, 1043, 985, 757, 733, 717. **HRMS (ESI)**: calcd for $C_{11}H_{18}NS_2$ $[M+H]^+$, *m/z* =228.08752, found 228.08756.

2-undecyldisulfanyl-pyridine[6]

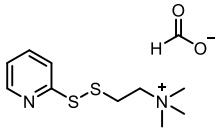


The purification was carried out by column chromatography (EtOAc/pentane 1:20). The desired product was obtained as a colorless liquid (62 mg, 0.532 mmol, 39%).

***R*$_f$** = 0.35 (EtOAc/pentane 1:20). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.46 (ddd, *J* = 4.8, 1.8, 0.9 Hz, 1H), 7.73 (dt, *J* = 8.1, 1.1 Hz, 1H), 7.63 (tdd, *J* = 7.5, 1.8, 7.4 Hz, 1H), 7.07 (ddd, *J* = 7.4, 4.8, 1.1 Hz, 1H), 2.78 (t, *J* = 7.3 Hz, 2H), 1.72 – 1.64 (m, 2H), 1.34 – 1.25 (m, 2H), 1.24 (m, 14H), 0.92 – 0.84 (t, *J* = 6.7 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 160.91, 149.72, 137.06, 120.59, 119.68, 39.21, 32.05, 29.73, 29.71, 29.62, 29.47, 29.32, 29.08, 28.64, 22.83, 14.27. **IR (film)**

ν_{max} = 2923, 2853, 1575, 1446, 1417, 1118, 1082, 1043, 986, 758, 718. **HRMS (ESI)**: calcd for $C_{16}H_{28}NS_2$ $[M+H]^+$, *m/z* =298.16577, found 298.16553

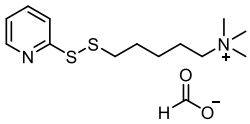
N,N,N-trimethyl-2-(pyridin-2-yldisulfaneyl)ethan-1-aminium formate



The purification was carried out by RP-HPLC (Hydro): Gradient 5% B for 14 min; 5% - 100% B for 21 min; wash. The desired product, eluting at 5.2-6.8 min, was collected, and lyophilized to afford the product as a yellow oil (36 mg, 0.5 mmol, 32%).

^{1}H NMR (500 MHz, MeOD) δ 8.51 – 8.39 (m, 1H), 7.80 – 7.65 (m, 2H), 7.46 (s, 2H), 6.85 (s, 1H), 3.80 – 3.70 (m, 2H), 3.31 – 3.25 (m, 2H), 3.20 (d, *J* = 25.4 Hz, 9H). **^{13}C NMR** (126 MHz, MeOD) δ 169.27, 149.57, 138.12, 121.93, 120.23, 65.64, 52.86, 52.75, 31.08, 30.46. **IR (film)** ν_{max} = 3368, 1572, 1559, 1445, 1417, 1342, 1146, 1113, 1084, 1043, 986, 907, 753, 717, 617 **HRMS (ESI)**: calcd for $C_{10}H_{17}N_2S_2$ $[M]^+$, *m/z* =229.08277, found 229.08286.

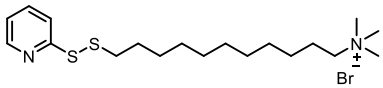
N,N,N-trimethyl-5-(pyridin-2-yldisulfaneyl)pentan-1-aminium formate



The purification was carried out by RP-HPLC (Gemini-NX): Gradient 5% B for 10 min; 5% - 100% B for 30 min; wash. The desired product, eluting at 15-19 min, was collected, and lyophilized to afford the product as a white amorphous solid (30 mg, 0.617 mmol, 18%).

^{1}H NMR (500 MHz, MeOD) δ 8.41 – 8.37 (m, 1H), 7.90 – 7.77 (m, 2H), 7.26 – 7.20 (m, 1H), 3.34 – 3.28 (m, 2H), 3.11 (s, 9H), 2.86 (t, *J* = 7.2 Hz, 2H), 1.85 – 1.74 (m, 4H), 1.49 (p, *J* = 7.7 Hz, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 161.57, 150.44, 139.30, 122.47, 121.30, 67.65, 53.65, 39.24, 29.40, 26.07, 23.58. **IR (film)** ν_{max} = 3386, 2930, 1686, 1595, 1574, 1481, 1447, 1417, 1343, 1276, 1199, 1169, 1117, 1083, 1044, 909, 823, 800, 762, 717 **HRMS (ESI)**: calcd for $C_{13}H_{23}N_2S_2$ $[M]^+$, *m/z* =271.12972, found 271.12977.

N,N,N-trimethyl-11-(pyridin-2-yldisulfaneyl)undecan-1-aminium bromide

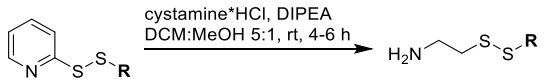


The purification was carried out by column chromatography (AlO_3, DCM/MeOH 50:1) to afford the desired product as a white amorphous solid (22.3 mg, 0.092 mmol, 56%).

R*f* = 0.34 (DCM:MeOH 50:1). **^{1}H NMR** (400 MHz, MeOD) δ 8.39 (dq, *J* = 4.9, 1.0 Hz, 1H), 7.86 (dt, *J* = 7.2, 1.0 Hz, 1H), 7.81 (dd, *J* = 9.1, 1.8 Hz, 1H), 7.81 (dd, *J* = 7.2, 1.8 Hz, 1H), 7.22 (ddd, *J* = 7.2, 4.9, 1.0 Hz, 1H), 3.36 – 3.32 (m, 2H), 3.12 (s, 9H), 2.81 (t, *J* = 7.2 Hz, 2H), 1.84 – 1.74 (m, 2H), 1.73 – 1.64 (m, 2H), 1.45 – 1.35 (m, 8H), 1.34 – 1.26 (m, 10H). **^{13}C NMR** (101 MHz, MeOD) δ 166.83, 150.27, 139.10, 122.24, 121.14, 53.49, 39.85, 30.46, 30.45, 30.43,

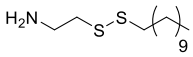
30.18, 29.85, 29.33, 27.35, 23.93. **IR (film)** ν_{max} = 3391, 2925, 2853, 1574, 1447, 1418, 1118, 764. **HRMS (ESI)**: calcd for $C_{19}H_{35}N_2S_2$ $[M]^+$, *m/z* =355.22362, found 355.22387.

Synthesis of aminoethyl disulfide derivatives



To a solution of 2-(alkyldisulfaneyl)pyridine (1.1 equiv.) in DCM cystamine hydrochloride (1 equiv.) dissolved in MeOH was added dropwise (the ratio DCM/MeOH used for the reaction is 5:1 to get 0.1M solution). Then DIPEA (2 equiv.), was added into the reaction at rt. The mixture was stirred under inert atmosphere for 4-6 h at rt. The solvents were removed under reduced pressure. Ether was added into the crude, and formed precipitation was filtered and washed a few times with Et_2O. The products were used for the next step without purification (unless otherwise stated).

2-(undecyldisulfaneyl)ethan-1-amine

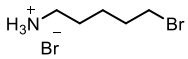


The purification wasis carried out by column chromatography (DCM/MeOH 12:1). The desired product was obtained as a colorless oil (15 mg, 0.084 mmol, 68%).

R_f = 0.30 (EtOAc:pentane 1:20). **^{1}H NMR** (400 MHz, MeOD) δ 3.28 (t, *J* = 6.7 Hz, 2H), 2.92 (t, *J* = 6.7 Hz, 2H), 2.75 (t, *J* = 7.2 Hz, 2H), 1.71 (p, *J* = 7.2 Hz, 2H), 1.46 – 1.23 (m, 18H), 0.90 (t, *J* = 6.7 Hz, 3H). **^{13}C NMR** (101 MHz, MeOD) δ 39.42, 39.31, 35.42, 33.16, 30.83, 30.80, 30.73, 30.56, 30.41, 30.22, 29.58, 23.83, 14.53. **IR (film)** ν_{max} = 3399, 2954, 2919, 2848, 2227, 1470, 721. **HRMS (ESI)**: calcd for $C_{13}H_{30}NS_2$ $[M+H]^+$, *m/z* =264.18142, found 264.18142.

Synthesis of *5-bromopentan-1-aminium bromide*

The synthesis was carried out according to the procedure.[7]



5-amino-1-pentanol (2.0 g, 0.0194 mol) was dissolved in an aqueous solution of 48% hydrobromic acid (20 mL). The mixture was stirred under reflux. After three hours the mixture was evaporated to obtain a crystalline solid. The crystals were washed with cold EtOAc (3x15 mL) to afford the desired product as a white solid. (2.37 g, 0.019 mmol, 50%).

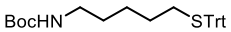
^{1}H NMR (500 MHz, D_2O) δ 3.52 (t, *J* = 6.6 Hz, 2H), 3.01 (t, *J* = 7.5 Hz, 2H), 1.90 (p, *J* = 6.8 Hz, 2H), 1.70 (p, *J* = 7.6 Hz, 2H), 1.53 (p, *J* = 8.0 Hz, 2H). **^{13}C NMR** (126 MHz, D_2O) δ 39.33, 34.36, 31.35, 25.88, 24.27. **HRMS (ESI)**: calcd for $C_5H_{13}NBr$ $[M]^+$, *m/z* = 166.02259, found 166.02226. ***m.p*** = 145.5 - 145.7 °C.

Synthesis of *tert-butyl (5-bromopentyl)carbamate*

6-bromopentylamine hydrobromide (500 mg, 2.02 mmol) was dissolved in DCM (10 mL). Di-tert-butyl dicarbonate (682 mg, 3.03 mmol) was added. Then, 1-hydroxybenzotriazole and triethylamine solution (2 mL) was added dropwise into the mixture. The reaction was stirred at rt for 40 min. The mixture was washed with sulfuric acid solution (0.5 mol/L), a saturated solution of Na_2CO_3 and brine. The organic phase was concentrated by reduced pressure. The purification was carried out by column chromatography (DCM/MeOH=9:1). The desired product was obtained as a colorless oil (410 mg, 2.02 mmol, 76%).

R*f* = 0.7 (DCM/MeOH = 9:1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.40 (t, *J* = 6.8 Hz, 2H), 3.12 - 3.08 (m, 2H), 1.87 (p, *J* = 6.9 Hz, 2H), 1.54 – 1.46 (m, 4H), 1.44 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 156.11, 40.48, 33.77, 32.46, 29.42, 28.56, 27.63, 25.49. **IR (film)** ν_{max} = 3357, 2977, 2934, 2865, 1693, 1516, 1455, 1391, 1366, 1249, 1172, 1040, 867, 781, 643. **HRMS (ESI)**: calcd for $C_{10}H_{20}O_2NBrNa$ $[M+Na]^+$, *m/z* = 288.05696, found 288.05692.

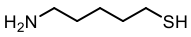
Synthesis of *tert-butyl (5-(tritylthio)pentyl)carbamate*



Triphenylmethane thiol (100 mg, 0.358 mmol) was suspended in DMSO (0.5 mL) and DBU (80 µL, 0.537 mmol) was added. The reaction turned dark red and then precipitation was observed. After 5 min of stirring, tert-butyl (5-bromopentyl)carbamate (100 mg, 0.376 mmol) in DMSO (0.2 mL) was added, and the reaction mixture was stirred for 20 min at rt. The precipitate dissolved and the reaction remained slightly pink. The reaction mixture was diluted with ethyl acetate (30 mL), and HCl (0.1 M, 30 mL) was added. The mixture was extracted with EtOAc (3x20 mL). The organic phase was collected, dried over Na_2SO_4, and concentrated by reduced pressure. The crude product was purified by column chromatography (DCM/MeOH = 10:1) to afford the desired product as a colorless oil (146 mg, 0.358 mmol, 88%).

R*f* = 0.53 (DCM/MeOH = 10:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.43 – 7.38 (m, 6H), 7.31 – 7.26 (m, 6H), 7.23 – 7.18 (m, 3H), 3.49 (d, *J* = 6.9 Hz, 1H), 3.05 - 2.98 (m, 2H), 2.14 (t, *J* = 9.1 Hz, 2H), 1.43 (s, 9H), 1.40 – 1.31 (m, 4H), 1.30 – 1.21 (m, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.14, 129.74, 127.97, 126.69, 66.60, 31.97, 29.74, 28.57, 28.43, 27.65, 26.30. **IR (film)** ν_{max} = 3362, 3057, 2975, 2858, 1701, 1595, 1492, 1444, 1391, 1249, 1170, 1083, 1035, 1001, 857, 743, 700, 676, 621. **HRMS (ESI)**: calcd for $C_{29}H_{35}O_2NNaS$ $[M+Na]^+$, *m/z* = 484.22807, found 484.22803.

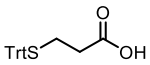
Synthesis of *5-aminopentane-1-thiol*



Tert-butyl (5-(tritylthio)pentyl)carbamate (30 mg, 0.065 mmol) was dissolved in DCM (0.325 mL) and TFA (0.325 mL, 50% v/v) was added. The color of the reaction changed to yellow. Triethylsilane (0.084 mL, 0.65 mmol) was added dropwise at rt. The color of the reaction changed to transparent. The reaction was stirred under inert atmosphere at rt for 30 min. The solvents were evaporated, and the crude was washed with pentane (5x5 mL). The product was used for the next step without additional purification.

Synthesis of *3-(tritylthio)propanoic acid*

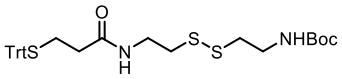
The synthesis was carried out according to the procedure.[8]



3-Mercaptopropanoic acid (6 g, 56.6 mmol) was dissolved in DCM (50 mL). Triphenyl methyl chloride (17.34 g, 62.3 mmol) previously dissolved in DCM (30 mL) was added dropwise and the mixture was stirred for 16 h at 25 °C under argon. The precipitate was immediately formed after the flask had been opened. The precipitate was filtered off and washed with diethyl ether. Pure compound was isolated as a white powder (18.2 g, 56.5 mmol, 92%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 – 7.31 (m, 12H), 7.27 – 7.23 (m, 3H), 2.28 (t, J = 7.7 Hz, 2H), 2.16 (t, J = 7.7 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO) δ 172.69, 144.36, 129.08, 128.04, 126.74, 66.16, 32.88, 26.67. **HRMS (ESI)**: calcd for $C_{22}H_{20}O_2SNa$ $[M+Na]^+$, m/z = 371.10762, found 371.10726. ***m.p.*** = 203–204 °C.

Synthesis of *tert-butyl (2-((2-(3-(tritylthio)propanamido)ethyl)disulfaneyl)ethyl)carbamate.*

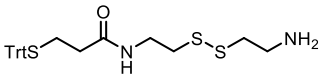


3-(tritylthio)propanoic acid **1.16** (70 mg, 0.201 mmol) was dissolved in dry DMF (4 mL), DIPEA (distilled, 105 µL, 0.603 mmol) was added. The reaction mixture was cooled down to 0 °C followed by the addition of HATU (153 mg, 0.402 mmol) and Boc-cystamine (56 mg, 0.22 mmol). The reaction mixture was stirred at rt for 3 h. The purification was carried out by column chromatography (pentane/EtOAc = 2:1) to afford the desired product as a slightly yellow amorphous solid (93 mg, 0.201 mmol, 80%).

R*f* = 0.31 (pentane:EtOAc = 2:1). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.00 (t, J = 5.5 Hz, 1H), 7.36 – 7.29 (m, 12H), 7.27 – 7.22 (m, 3H), 6.96 (t, J = 5.6 Hz, 1H), 3.30 – 3.25 (m, 2H), 3.18 (q, J = 6.4 Hz, 2H), 2.72 (dt, J = 7.8, 5.6 Hz, 4H), 2.24 (t, J = 6.9 Hz, 2H), 2.15 (t, J = 6.9 Hz, 2H), 1.37 (s, 9H). **^{13}C NMR** (126 MHz, DMSO) δ 170.15, 155.50, 144.42, 129.06, 128.01, 126.69, 77.77, 65.96, 37.94, 37.51, 37.19, 33.93, 28.21, 27.38. **IR (film)** ν_{max} = 3313, 3058, 2975, 2929, 1696, 1656, 1524, 1444, 1391, 1365, 1252, 1168, 1083, 1032, 949, 863, 744, 700,

676, 620. **HRMS (ESI)**: calcd for $C_{31}H_{38}O_3N_2S_3Na$ $[M+Na]^+$, *m/z* = 605.19368, found 605.19385.

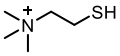
Synthesis of *N-(2-((2-aminoethyl)disulfaneyl)ethyl)-3-(tritylthio)propenamide*



The Boc-protected product **1.17a** (90 mg, 0.154 mmol) was dissolved in DCM (3 mL) and TFA (0.100 mL, 1.3 mmol, 20% v/v) was added. The reaction mixture was stirred at rt for 2 h. The solvent was evaporated, and the crude was washed with pentane. The desired product was obtained as an amorphous solid (80 mg, 0.154 mmol, 87%).

^{1}H NMR (500 MHz, MeOD) δ 7.41 – 7.37 (m, 6H), 7.32 – 7.26 (m, 6H), 7.24 – 7.20 (m, 3H), 3.47 (t, *J* = 6.7, 2H), 3.25 (t, *J* = 6.7 Hz, 2H), 2.95 (t, *J* = 6.7 Hz, 2H), 2.83 (t, *J* = 6.7 Hz, 2H), 2.41 (t, *J* = 7.2 Hz, 2H), 2.21 (t, *J* = 7.2 Hz, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 174.12, 146.17, 130.74, 128.93, 127.83, 67.83, 39.54, 39.33, 38.16, 35.84, 35.57, 28.90. **IR (film)** ν_{max} = 3058, 1675, 1547, 1444, 1202, 1136, 1034, 837, 799, 744, 722, 700, 617. **HRMS (ESI)**: calcd for $C_{26}H_{31}ON_2S_3$ $[M+H]^+$, *m/z* = 483.15930, found 483.15956.

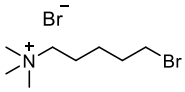
Synthesis of *2-mercapto-N,N,N-trimethylethan-1-aminium*



The synthesis was carried out according to the earlier published procedure[9] and the product was used for the next step without additional purification.

Synthesis of *5-bromo-N,N,N-trimethylpentan-1-aminium bromide*

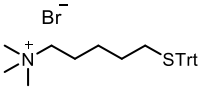
The synthesis was followed by the published procedure with a few changes.[10]



1,5 – dibromopentane (1 mL, 7.31 mmol) was dissolved in dry Et_2O (7 mL). Methylamine (2.88 mL, 36.5 mmol) was added dropwise during 5 min at 0 °C. The reaction was stirred for 2 days. The formed precipitation was filtered off, washed with dry Et_2O, and recrystallized from iPrOH/Et_2O 1:2. The product was obtained as a white solid (1.090 g, 7.3 mmol, 71%).

^{1}H NMR (400 MHz, MeOD) δ 3.50 (t, *J* = 6.6 Hz, 2H), 3.40 – 3.34 (m, 2H), 3.15 (s, 9H), 2.00 – 1.92 (m, 2H), 1.85 – 1.82 (m, 2H), 1.59 – 1.50 (m, 2H). **^{13}C NMR** (101 MHz, MeOD) δ 67.54, 53.62, 53.58, 53.54, 33.79, 33.21, 25.90, 23.13. **IR (film)** ν_{max} = 3411, 3012, 2949, 1626, 1480, 1300, 1258, 1240, 970, 911, 736, 641. **HRMS (ESI)**: calcd for $C_8H_{19}NBr$ $[M]^+$, *m/z* =208.06954, found 208.06934; ***m.p.*** = 140.1-141.3 °C.

Synthesis of *N,N,N-trimethyl-5-(tritylthio)pentan-1-aminium bromide*

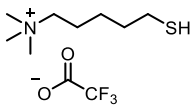


Triphenylmethane thiol (65 mg, 0.234 mmol) was suspended in DMSO (0.3 mL) and DBU (52 µL, 0.537 mmol) was added. The reaction turned dark red

and then strong precipitate was observed. After stirring at rt for 5 min, 5-bromo-N,N,N-trimethylpentan-1-aminium bromide (49 mg, 0.234 mmol) in DMSO (0.2 mL) was added, and the reaction mixture was stirred for 20 min. The solvent was evaporated and the crude product was purified by column chromatography (DCM/MeOH 100:1, 95:5, 90:5, 80:20) to afford the desired product as a white solid (51 mg, 0.234 mmol, 56%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.45 – 7.39 (m, 6H), 7.35 – 7.27 (m, 6H), 7.26 – 7.21 (m, 3H), 3.32 – 3.26 (m, 2H), 3.23 (s, 9H), 2.19 (t, J = 7.1 Hz, 2H), 1.64 – 1.54 (m, 2H), 1.55 – 1.41 (m, 2H), 1.36 – 1.30 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 144.91, 129.67, 128.04, 126.81, 66.75, 53.47, 31.42, 28.07, 25.35, 22.72. **IR (film)** ν_{max} = 3402, 1595, 1489, 1444, 1343, 1033, 969, 744, 700, 622. **HRMS (ESI)**: calcd for $C_{27}H_{34}NS$ $[M]^+$, *m/z* = 404.24065, found 404.24110. ***m.p.*** = 91.2-91.4 °C.

Synthesis of *5-mercapto-N,N,N-trimethylpentan-1-aminium*



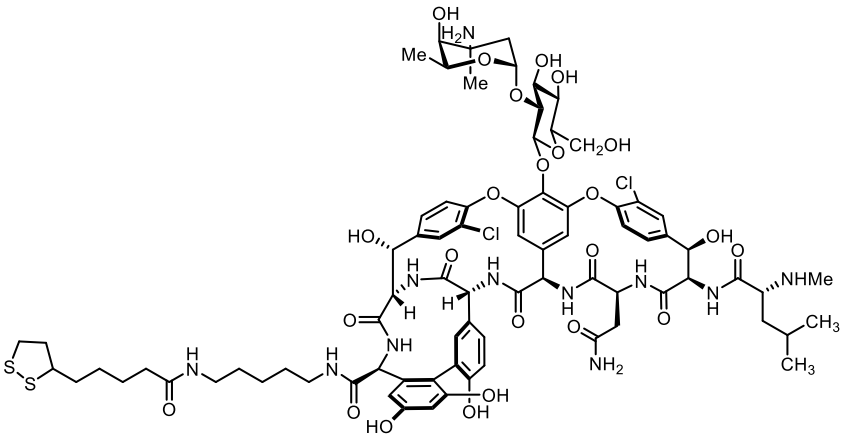
N,N,N-trimethyl-5-(tritylthio)pentan-1-aminium bromide (50 mg, 0.122 mmol) was dissolved in DCM (0.610 mL) and TFA (0.610 mL, 50% v/v) was added. The reaction turned yellow. Triethylsilane (0.200 mL, 1.22 mmol) was added, and the reaction mixture was stirred at rt for 1 h. The solvents were evaporated, and the crude was washed with pentane (5x20 mL). The resulting product was used for the next step without further purification.

b. Synthesis of vancomycin derivatives

General procedure:

Vancomycin hydrochloride (1 equiv.), PyBOP (3 equiv.), and HOBt (1 equiv.) were dissolved in dry DMF (0.05 mM, based on vancomycin). To the reaction mixture freshly distilled DIPEA (3 equiv.) was added followed by the addition of a corresponding linker (1.1 equiv.). The reaction mixture was stirred for 1 h at rt and the solvent was evaporated. The mixture was dissolved in MeCN/H_2O (1:1, with 0.1% HCO_2H) and filtered through a SPE column. The solvents were evaporated, and the compounds were purified by preparative RP-HPLC. The purification methods, analytical data, and yields for obtained compounds are described below.

Vancomycin derivative ***1a***

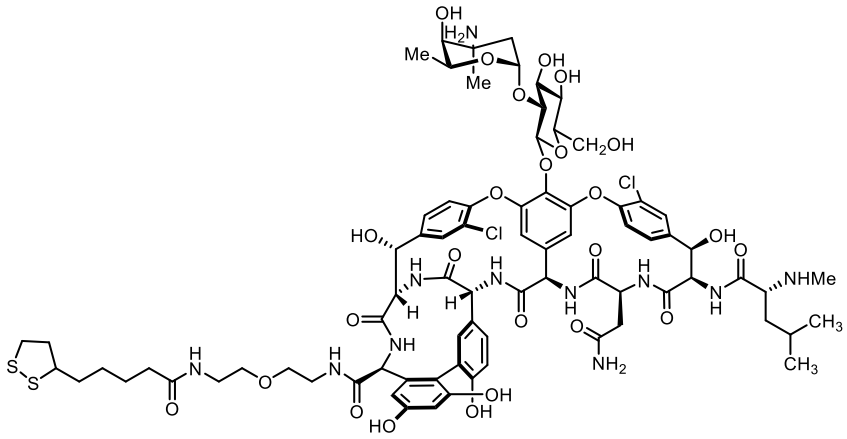


RP-HPLC (Synergy Hydro): Gradient 5% B for 12 min; 5% - 30% B for 46 min; 30% - 100% B for 4 min, wash. The desired product, eluting at 37 min, was collected, and lyophilized to afford product (4 mg, 0.0023 mmol, 7%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.60 (d, J = 2.1 Hz, 1H), 8.40 (d, J = 4.7 Hz, 1H), 8.29 (s, 1H), 7.90 -7.82 (m, 2H), 7.76 (t, J = 5.6 Hz, 1H), 7.47 (ddd, J = 23.1, 8.6, 0.6 Hz, 2H), 7.39 – 7.33 (s, 1H), 7.28 (dd, J = 25.5, 8.3 Hz,, 2H), 7.24 – 7.19 (m, 1H), 6.92 – 6.85 (m, 1H), 6.75 (dd, J = 8.4, 1.6 Hz, 1H), 6.71 – 6.65 (m, 2H), 6.36 (d, J = 2.2 Hz, 1H), 6.25 (d, J = 2.2 Hz, 1H), 5.74 (d, J = 7.9 Hz, 1H), 5.55 – 5.52 (m, 1H), 5.28 (d, J = 7.7 Hz, 1H), 5.25 – 5.19 (m, 3H), 5.14 (d, J = 3.4 Hz, 1H), 4.90 - 4.84 (m, 1H), 4.67 (q, J = 6.5 Hz, 1H), 4.43 (d, J = 4.8 Hz, 1H), 4.38 (d, J = 5.7 Hz, 1H), 4.22 (d, J = 11.0 Hz, 1H), 3.68 (d, J = 11.0 Hz, 1H), 3.64 – 3.52 (m, 4H), 3.45 (t, J = 8.8 Hz, 2H), 3.22 – 3.16 (m, 3H), 3.15 – 3.08 (m, 5H), 3.05 – 3.00 (m, 3H), 2.44 – 2.37 (m, 1H), 2.30 (s, 3H), 2.14 (dd, J = 15.9, 5.2 Hz, 1H), 2.08 – 2.03 (t, J = 7.4 Hz, 2H), 1.91 – 1.82 (m, 2H), 1.77 – 1.62 (m, 3H), 1.58 – 1.43 (m, 6H), 1.43 – 1.36 (m, 3H), 1.36 – 1.31 (m, 2H), 1.30 – 1.23 (m, 5H), 1.06 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H). **HRMS (ESI)**: calcd for $C_{79}H_{101}O_{24}N_{11}Cl_2S_2$ $[M+2H]^{2+}$, m/z = 860.79142, found 860.79230.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 0% B for 4 min, 0% - 100% B for 11 min, wash. The product was eluted at 14.9 min and was detected at λ = 280 nm.

*Vancomycin derivative **1b***

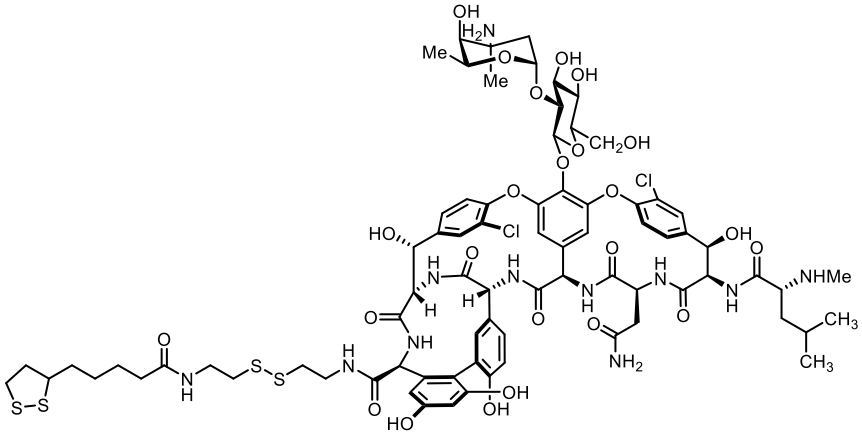


RP-HPLC (Synergy Hydro): Gradient 5% B for 12 min; 5% - 30% B for 46 min; 30% - 100% B for 4 min, wash. The desired product, eluting at 32 min, was collected, and lyophilized to afford product (2.5 mg, 0.0015 mmol, 4%) as a white solid

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.64 – 8.58 (m, 1H), 8.42 (d, J = 4.6 Hz, 1H), 8.31 (s, 2H), 7.91 (t, J = 3.4 Hz, 1H), 7.86 - 7.83 (m, 1H), 7.84 (t, J = 5.6 Hz, 1H), 7.74 (s, 1H), 7.50 (dd, J = 8.3, 1.8 Hz, 1H), 7.45 (dd, J = 8.3, 1.8 Hz, 1H), 7.36 (s, 1H), 7.32 (d, J = 8.3 Hz, 2H), 7.26 (d, J = 8.3 Hz, 1H), 7.23 – 7.19 (m, 1H), 6.88 (s, 1H), 6.76 (dd, J = 8.5, 2.0 Hz, 1H), 6.70 (d, J = 8.5 Hz, 1H), 6.67 (d, J = 12.0 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H), 6.25 (d, J = 2.0 Hz, 1H), 5.74 (d, J = 7.8 Hz, 1H), 5.54 (s, 1H), 5.29 (d, J = 7.7 Hz, 1H), 5.25 – 5.19 (m, 3H), 5.14 (d, J = 3.4 Hz, 1H), 4.90 – 4.83 (m, 1H), 4.67 (q, J = 6.5 Hz, 1H), 4.43 (d, J = 5.0 Hz, 1H), 4.39 (d, J = 5.7 Hz, 1H), 4.37 -4.33 (m, 1H), 4.22 (d, J = 10.3 Hz, 1H), 3.68 (d, J = 10.4 Hz, 1H), 3.63 – 3.56 (m, 2H), 3.56 – 3.50 (m, 2H), 3.49 – 3.45 (m, 2H), 3.43 (t, J = 5.9 Hz, 2H), 3.24 – 3.19 (m, 2H), 3.19 – 3.16 (m, 1H), 3.15 – 3.08 (m, 2H), 3.03 (t, J = 7.3 Hz, 1H), 2.44 – 2.38 (m, 2H), 2.30 (s, 3H), 2.14 (dd, J = 15.3, 5.8 Hz, 1H), 2.07 (t, J = 7.4 Hz, 2H), 1.91 – 1.81 (m, 2H), 1.76 – 1.70 (m, 1H), 1.69 – 1.62 (m, 2H), 1.58 – 1.45 (m, 4H), 1.43 – 1.37 (m, 1H), 1.37 – 1.29 (m, 2H), 1.26 (m, 2H), 1.23 (s, 1H), 1.06 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H). **HRMS (ESI)**: calcd for $C_{78}H_{99}O_{25}N_{11}Cl_2S_2$ $[M+2H]^{2+}$, m/z = 861.78105, found 861.78077.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 0% B for 4 min, 0% - 100% B for 11 min, wash. The product was eluted at 13.8 min and was detected at λ = 280 nm.

Vancomycin derivative ***1c***

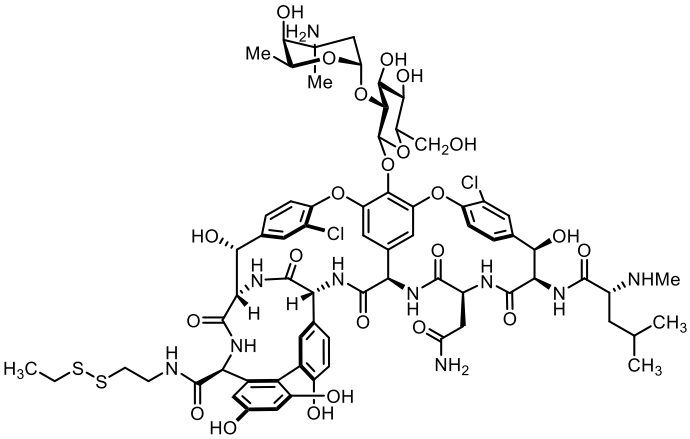


RP-HPLC (Synergy Hydro): Gradient 0% B for 14 min; 0% - 20% B for 16 min; 20% - 100% B for 2 min, wash. The desired product, eluting at 23-25 min, was collected, and lyophilized to afford product (8.5 mg, 0.04 mmol, 12%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 – 8.59 (m, 1H), 8.44 – 8.40 (m, 1H), 8.29 (s, 3H), 8.16 – 8.10 (m, 1H), 8.03 (t, J = 5.6 Hz, 1H), 7.86 (s, 1H), 7.47 (dd, J = 21.5, 8.1, 0.7 Hz, 2H), 7.38 – 7.34 (m, 1H), 7.29 (dd, J = 25.5, 8.4 Hz, 2H), 7.23 – 7.20 (m, 1H), 6.91 – 6.85 (m, 1H), 6.76 (dd, J = 8.9, 0.9 Hz, 1H), 6.70 (d, J = 8.6 Hz, 1H), 6.67 – 6.65 (m, 1H), 6.36 (d, J = 1.8 Hz, 1H), 6.24 (d, J = 1.8 Hz, 1H), 5.74 (d, J = 8.1 Hz, 1H), 5.55 – 5.52 (m, 1H), 5.29 (d, J = 7.6 Hz, 1H), 5.25 – 5.20 (m, 2H), 5.17 – 5.14 (m, 1H), 4.38 (d, J = 5.5 Hz, 1H), 4.22 (d, J = 12.4 Hz, 1H), 3.71 – 3.66 (m, 1H), 3.65 – 3.48 (m, 4H), 3.44 (q, J = 9.0, 7.0 Hz, 2H), 3.21 – 3.15 (m, 2H), 3.15 – 3.08 (m, 3H), 3.05 -3.00 (m, 1H), 2.90 – 2.82 (m, 3H), 2.80 (t, J = 6.9 Hz, 3H), 2.76 (t, J = 6.6 Hz, 1H), 2.45 – 2.39 (m, 2H), 2.30 (s, 3H), 2.17 – 2.11 (m, 1H), 2.10 – 2.04 (m, 3H), 1.91 – 1.82 (m, 3H), 1.77 – 1.61 (m, 4H), 1.58 – 1.44 (m, 7H), 1.44 – 1.39 (m, 1H), 1.38 – 1.31 (m, 4H), 1.27 (s, 3H), 1.23 (s, 2H), 1.06 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H). **HRMS (ESI)**: calcd for $C_{78}H_{99}O_{24}N_{11}Cl_2S_4$ $[M+2H]^{2+}$, m/z = 885.75567, found 885.75529.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 0% B for 4 min, 0% - 100% B for 11 min, wash. The product was eluted at 12.4 min and was detected at λ = 280 nm.

Vancomycin derivative ***2a***

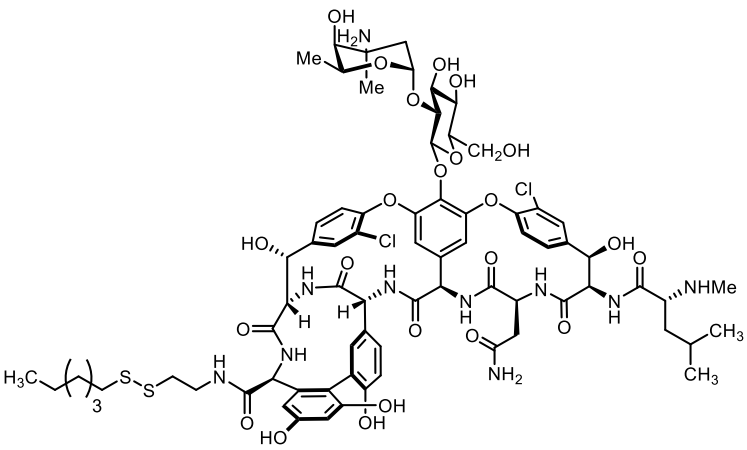


RP-HPLC (Synergy Hydro): Gradient 5% B for 12 min; 5% - 20% B for 44 min; 20% - 100% B for 4 min, wash. The desired product, eluting at 32.7-36 min, was collected, and lyophilized to afford product (1.45 mg, 0.02 mmol, 5%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 9.04 (s, 1H), 8.62 (m, 1H), 8.42 (m, 1H), 8.33 (s, 1H), 8.13 (m, 1H), 7.86 (d, J = 1.8 Hz, 1H), 7.73 (m, 1H), 7.51 – 7.43 (m, 2H), 7.39 – 7.34 (m, 1H), 7.30 (dd, J = 24.6, 8.3 Hz, 2H), 7.21 (s, 1H), 6.90 (m, 1H), 6.78 – 6.73 (dd, J = 1.6, 8.3 Hz, 1H), 6.70 (d, J = 8.5 Hz, 1H), 6.64 (m, 1H) 6.35 (d, J = 1.9 Hz, 1H), 6.24 (d, J = 2.0 Hz, 1H), 5.88 (m, 1H), 5.75 (d, J = 7.6 Hz, 1H), 5.53 (s, 1H), 5.28 (d, J = 7.8 Hz, 1H), 5.21 (m, 2H), 5.14 (m, 1H), 4.43 (m, 1H), 4.37 (d, J = 5.6 Hz, 1H), 4.22 (d, J = 11.4 Hz, 1H), 4.04 (m, 1H), 3.68 (d, J = 10.6 Hz, 1H), 3.54 (m, 2H), 3.43 (m, 2H), 3.02 (t, J = 6.8 Hz, 1H), 2.93 (m, 1H), 2.83 (m, 2H), 2.76 (q, J = 7.3 Hz, 2H), 2.54 (s, 1H), 2.52 (m, 1H), 2.30 (s, 2H), 2.14 (m, 1H), 1.84 (m, 1H), 1.73 (p, J = 6.8, 1H), 1.66 (m, 1H), 1.61 – 1.52 (m, 1H), 1.47 (m, 1H), 1.40 (m, 1H), 1.28 (t, J = 7.3 Hz, 3H), 1.24 (m, 3H), 1.06 (d, J = 6.4 Hz, 2H), 0.90 - 0.85 (dd, J = 6.6, 20.6 Hz, 6H). **HRMS (ESI)**: calcd for $C_{70}H_{86}Cl_2N_{10}O_{23}$ $[M+2H]^{2+}$, *m/z* =784.23444, found 784.23374.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 0% B for 4 min, 0% - 100% B for 11 min, wash. The product was eluted at 12.2 min and was detected at λ = 280 nm.

Vancomycin derivative ***2b***

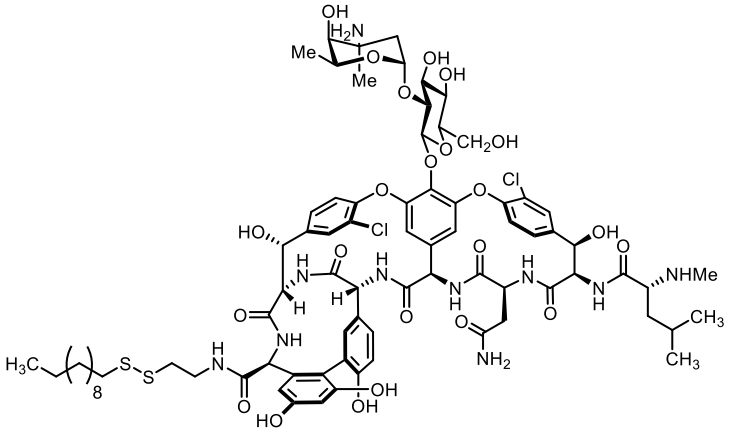


RP-HPLC (Gemini-NX): Gradient 10% B for 14 min; 10% - 30% B for 46 min; 30% - 100% B for 2 min, wash. The desired product, eluting at 28.5-30.1 min, was collected, and lyophilized to afford product (7.9 mg, 0.014 mmol, 35%) as a white solid.

^{1}H NMR (500 MHz, MeOD) δ 8.38 (s, 2H), 7.66 (d, *J* = 28.0 Hz, 2H), 7.61 (s, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 7.00 - 6.86 (m, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.43 (d, *J* = 2.3 Hz, 1H), 6.39 (d, *J* = 2.3 Hz, 1H), 5.93 – 5.78 (m, 1H), 5.52 – 5.40 (m, 2H), 5.39 – 5.29 (m, 2H), 3.90 – 3.80 (m, 2H), 3.80 – 3.71 (m, 1H), 3.67 – 3.51 (m, 3H), 2.90 – 2.81 (m, 3H), 2.74 (t, *J* = 7.2 Hz, 2H), 2.66 (s, 3H), 2.36 – 2.18 (m, 1H), 2.10 – 2.01 (m, 1H), 1.98 – 1.89 (m, 1H), 1.85 – 1.76 (m, 1H), 1.70 (p, *J* = 7.4 Hz, 3H), 1.65 – 1.56 (m, 1H), 1.50 (s, 2H), 1.45 – 1.38 (m, 2H), 1.37 – 1.25 (m, 7H), 1.19 (d, *J* = 6.1 Hz), 0.98 (d, *J* = 6.0 Hz, 3H), 0.94 (d, *J* = 6.0 Hz, 3H), 0.88 – 0.82 (t, *J* = 6.6 Hz, 3H). **HRMS (ESI):** calcd for $C_{74}H_{94}Cl_2N_{10}O_{23}S_2$ $[M+2H]^{2+}$, *m/z* =812.26520, found 812.26504.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 11 min, wash. The product was eluted at 13.9 min and was detected at λ = 280 nm.

*Vancomycin derivative **2c***

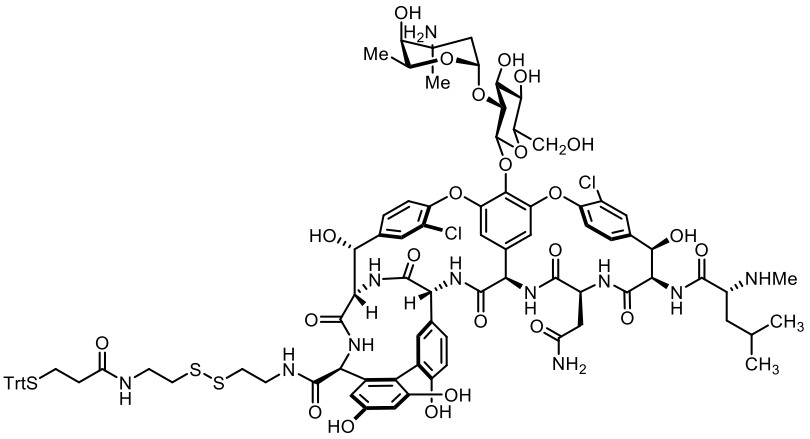


RP-HPLC (Gemini-NX): Gradient 10% B for 14 min; 10% - 50% B for 41 min; 50% - 100% B for 3 min, wash. The desired product, eluting at 33.8-36.4 min, was collected, and lyophilized to afford product (14 mg, 0.027 mmol, 31%) as a white solid.

^{1}H NMR (500 MHz, MeOD) δ 8.45 (s, 2H), 7.68 – 7.56 (m, 2H), 7.28 – 7.16 (m, 1H), 7.06 (s, 1H), 6.91 – 6.75 (m, 1H), 6.43 (d, *J* = 2.2 Hz, 1H), 6.39 (d, *J* = 2.2 Hz, 1H), 6.18 – 5.79 (m, 1H), 5.55 – 5.48 (m, 1H), 5.46 – 5.40 (m, 1H), 5.40 – 5.30 (m, 1H), 3.94 – 3.81 (m, 2H), 3.80 – 3.74 (m, 1H), 3.69 – 3.54 (m, 3H), 3.20 – 3.11 (m, 1H), 2.90 – 2.78 (m, 2H), 2.74 (t, *J* = 7.3 Hz, 2H), 2.66 (s, 3H), 2.38 – 2.15 (m, 1H), 2.10 – 2.02 (m, 1H), 2.00 – 1.92 (m, 1H), 1.88 – 1.84 (m, 1H), 1.81 – 1.76 (m, 1H), 1.70 (p, *J* = 7.2 Hz, 3H), 1.65 – 1.56 (m, 1H), 1.48 (s, 3H), 1.45 – 1.39 (m, 3H), 1.37 – 1.25 (m, 18H), 1.20 (d, *J* = 6.3 Hz, 3H), 0.96 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 6.3 Hz, 3H), 0.90 (t, *J* = 6.8 Hz, 3H). **HRMS (ESI):** calcd for $C_{79}H_{104}Cl_2N_{10}O_{23}S_2$ $[M+2H]^{+}$, *m/z* =847.30416, found 847.30358.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 11 min, wash. The product was eluted at 13.8 min and was detected at λ = 280 nm.

Vancomycin derivative ***3a***

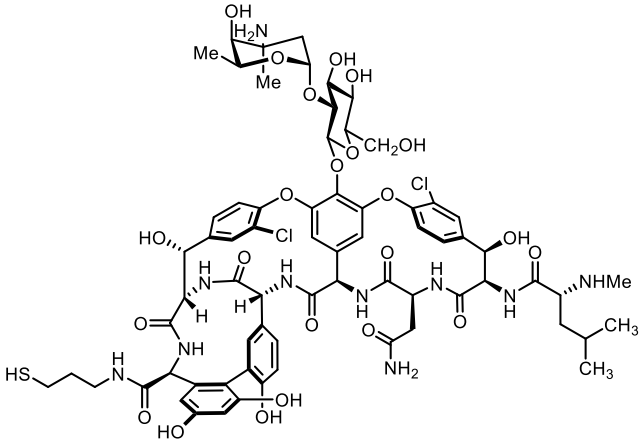


RP-HPLC (Gemini-NX): Gradient 5% B for 12 min; 5% - 40% B for 46 min; 40% - 100% B for 2 min, wash. The desired product, eluting at 43.4-46 min, was collected, and lyophilized to afford product (53.3 mg, 0.067 mmol, 50%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 -8.90 (m, 1H), 8.61 (d, J = 4.3 Hz, 1H), 8.42 (d, J = 4.3 Hz, 1H), 8.34 (s, 2H), 8.18 – 8.15 (m, 1H), 8.07 (t, J = 5.6 Hz, 1H), 7.86 (s, 1H), 7.79 – 7.71 (br, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.45 (d, J = 8.5 Hz, 1H), 7.38 – 7.28 (m, 18H), 7.27 – 7.22 (m, 5H), 7.22 – 7.19 (m, 1H), 6.88 (s, 1H), 6.76 (d, J = 8.5 Hz , 1H), 6.78 (d, J = 8.5 Hz , 1H), 6.67 – 6.61 (m, 1H), 6.36 (d, J = 1.6 Hz, 1H), 6.23 (d, J = 1.6 Hz, 1H), 5.74 (d, J = 7.9 Hz, 1H), 5.53 (s, 1H), 5.27 (J = 7.7 Hz, 1H), 5.26 – 5.19 (m, 3H), 5.14 (d, J = 3.0 Hz, 1H), 4.88 – 4.85 (m, 1H), 4.67 (q, J = 5.8 Hz, 1H), 4.43 (d, J = 4.0 Hz, 1H), 4.37 (d, J = 5.5 Hz, 2H), 4.21 (d, J = 11.4 Hz, 1H), 3.68 (d, J = 10.3 Hz, 2H), 3.59 – 3.49 (m, 2H), 3.13 (s, 1H), 3.03 (t, J = 7.2 Hz, 1H), 2.86 – 2.79 (m, 2H), 2.76 (t, J = 6.8 Hz, 2H), 2.30 (s, 3H), 2.24 (t, J = 7.6 Hz, 2H), 2.15 (t, J = 7.6 Hz, 2H), 2.13 – 2.10 (m, 1H), 1.90 – 1.83 (m, 1H), 1.78 – 1.69 (m, 1H), 1.69 -1.65 (m, 1H), 1.51 – 1.44 (m, 1H), 1.43 – 1.36 (m, 1H), 1.26 (s, 3H), 1.06 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H). **HRMS (ESI):** calcd for $C_{92}H_{105}O_{24}N_{11}Cl_2S_3$ $[M+H]^+$, m/z = 956.79311, found 956.79362.

The purity of the compound was analyzed by analytical RP- HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 11 min, wash. The product was eluted at 14.9 min and was detected at λ = 280 nm.

Vancomycin derivative ***4b***

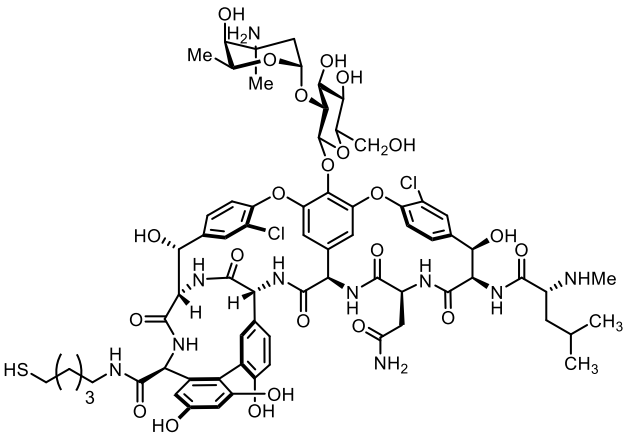


RP-HPLC (Gemini-NX): Gradient 5% B for 12 min; 5% - 30% B for 38 min; 30% - 100% B for 2 min, wash. The desired product, eluting at 23.1 -27.3 min, was collected, and lyophilized to afford product (40 mg, 0.067 mmol, 39%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.41 (s, 1H), 8.35 (s, 2H), 8.06 -8.00 (m, 1H), 7.88 – 7.83 (m, 1H), 7.80 – 7.70 (m, 1H), 7.52 – 7.43 (dd, J = 8.6, 22.3 Hz, 2H), 7.38 – 7.29 (m, 2H), 7.28 – 7.20 (m, 2H), 6.90 – 6.83 (m, 1H), 6.76 (d, J = 8.3 Hz 1H), 6.69 (d, J = 8.3 Hz 1H),6.69 – 6.60 (m, 1H), 6.36 (d, J = 2.0 Hz, 1H), 6.23 (d, J = 2.0 Hz, 1H), 5.74 (d, J = 7.4 Hz, 1H), 5.53 (s, 1H), 5.30 – 5.23 (m, 2H), 5.20 (s, 2H), 5.16 -5.10 (m, 1H), 4.89 – 4.82 (m, 1H), 4.67 (q, J = 6.2 Hz, 1H), 4.45 – 4.41 (m, 1H), 4.36 (d, J = 5.5 Hz, 1H), 4.22 (d, J = 11.8 Hz, 1H), 3.68 (d, J = 10.2 Hz, 2H), 3.54 (m, 2H), 3.09 (s, 1H), 3.03 (t, J = 7.2 Hz, 2H), 2.72 (t, J = 7.2 Hz, 2H), 2.30 (s, 3H), 2.18 – 2.11 (m, 1H), 1.89 – 1.79 (m, 3H), 1.78 – 1.69 (m, 2H), 1.65 (d, J = 13.2 Hz, 1H), 1.52 – 1.43 (m, 1H), 1.42 – 1.35 (m, 1H), 1.24 (s, 3H), 1.05 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H). **HRMS (ESI):** calcd for $C_{69}H_{84}Cl_2N_{10}O_{23}S$ $[M+2H]^{2+}$, m/z =760.73597, found 760.73639.

The purity of the compound was analyzed by analytical RP- HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 11 min, wash. The product was eluted at 12.2 min and was detected at λ = 280 nm.

Vancomycin derivative ***4c***



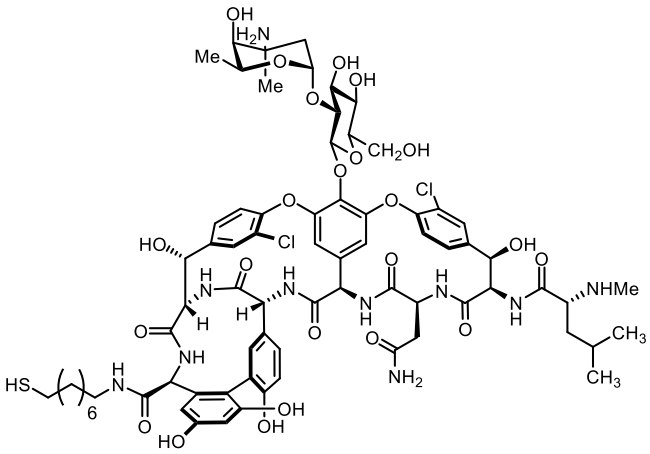
RP-HPLC (Gemini-NX): Gradient 5% B for 14 min; 5% - 25% B for 46 min; 25% - 100% B for 4 min, wash. The desired product, eluting at 28.2-29.2min, was collected and lyophilized to afford product (5.06 mg, 0.055 mmol, 6%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.63 – 8.56 (m, 1H), 8.43 – 8.38 (m, 1H), 8.31 (s, 1H), 7.92 -7.84 (m, 1H), 7.47 (dd, J = 22.8,

7.4 Hz, 1H), 7.39 – 7.32 (m, 1H), 7.29 (dd, *J* = 26.5, 8.4 Hz, 2H), 6.94 – 6.84 (m, 1H), 6.73 (dd, *J* = 27.2, 8.9 Hz, 1H), 6.36 (d, 2.2 Hz, 1H), 6.26 (d, 2.2 Hz, 1H), 5.74 (d, *J* = 8.5 Hz, 1H), 5.29 (d, *J* = 7.6 Hz, 1H), 5.26 – 5.12 (m, 2H), 4.37 (d, *J* = 5.6 Hz, 1H), 4.23 – 3.97 (m, 5H), 3.68 (d, *J* = 10.0 Hz, 1H), 3.59 – 3.50 (m, 2H), 3.48 – 3.42 (m, 2H), 3.05 – 2.99 (m, 1H), 2.71 (t, *J* = 7.4 Hz, 2H), 2.30 (s, 2H), 2.18 - 2.12 (m, 1H), 1.87 - 1.80 (m, 1H), 1.77 – 1.69 (m, 1H), 1.69 – 1.61 (m, 2H), 1.52 – 1.46 (m, 3H), 1.44 – 1.38 (m, 3H), 1.28 - 1.22 (m, 3H), 1.17 (t, *J* = 7.1 Hz, 1H), 1.06 (d, *J* = 6.3 Hz, 2H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H). **HRMS (ESI):** calcd for $C_{71}H_{87}Cl_4N_{10}O_{23}S$ $[M+2H]^{2+}$, *m/z* = 774.75162, found 774.75151.

The purity of the compound was analyzed by analytical RP- HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 11 min, wash. The product was eluted at 12.7 min and was detected at λ = 280 nm.

Vancomycin derivative ***4d***

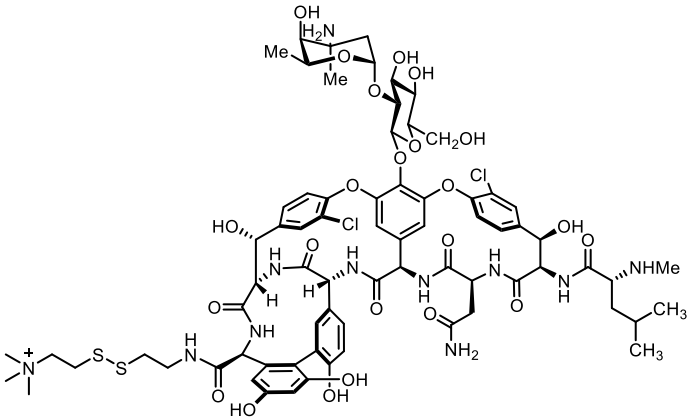


RP-HPLC (Gemini-NX): Gradient 5% B for 12 min; 5% - 35% B for 38 min; 35% - 100% B for 2 min, wash. The desired product, eluting at 31.3-36 min, was collected, and lyophilized to afford product (53.3 mg, 0.067 mmol, 50%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.29 (s, 1H), 8.97 (s, 1H), 8.62 (s, 1H), 8.44 – 8.36 (m, 1H), 8.26 – 8.16 (m, 1H), 7.86 (s, 2H), 7.78 – 7.70 (m, 1H), 7.48 (d, *J* = 9.0 Hz, 2H), 7.45 (d, *J* = 9.0 Hz, 2H), 7.39 – 7.24 (m, 3H), 7.21 (s, 1H), 6.95 – 6.83 (m, 1H), 6.75 (d, *J* = 9.2 Hz, 1H), 6.70 (d, *J* = 8.5 Hz, 2H), 6.35 (d, *J* = 2.2 Hz, 1H), 6.25 (d, *J* = 2.2 Hz, 1H), 5.88 – 5.80 (m, 1H), 5.74 (d, *J* = 7.1 Hz, 1H), 5.54 (s, 1H), 5.37 – 5.30 (br, 1H), 5.28 (d, *J* = 7.9 Hz, 1H), 5.22 (d, *J* = 7.9 Hz, 2H), 5.18 – 5.04 (m, 1H), 4.49 – 4.33 (m, 1H), 4.21 (d, *J* = 14.1 Hz, 1H), 4.05 – 3.98 (m, 1H), 3.73 – 3.65 (m, 1H), 3.59 – 3.48 (m, 2H), 3.48 – 3.41 (br, 1H), 3.19 – 3.06 (m, 4H), 3.05 – 2.98 (m, 1H), 2.70 (t, *J* = 6.7 Hz, 1H), 2.30 (s, 3H), 2.27 – 2.19 (m, 1H), 2.18 - 2.10 (m, 1H), 1.89 – 1.81 (m, 1H), 1.79 – 1.66 (m, 2H), 1.65 – 1.58 (m, 1H), 1.53 (p, *J* = 7.3 Hz, 2H), 1.50 – 1.39 (m, 5H), 1.38 – 1.22 (m, 15H), 1.07 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 4H), 0.86 (d, *J* = 6.6 Hz, 4H). **HRMS (ESI):** calcd for $C_{74}H_{94}Cl_2N_{10}O_{23}S$ $[M+H]^+$, *m/z* = 796.27900, found 796.27897.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 11 min, wash. The product was eluted at 12.5 min and was detected at λ = 280 nm.

Vancomycin derivative ***5a***

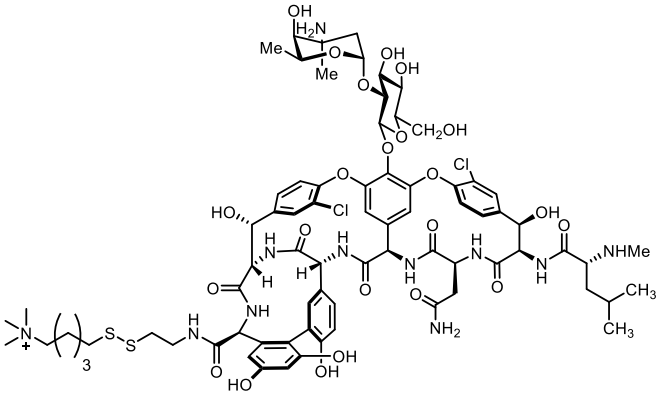


RP-HPLC (Synergy Hydro): Gradient 0% B for 14 min; 0% - 20% B for 16 min; 20% - 100% B for 2 min, wash. The desired product, eluting at 23 min, was collected, and lyophilized to afford product (3.6 mg, 0.032 mmol, 7%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.37 (s, 2H), 8.31 – 8.26 (m, 1H), 7.87 – 7.83 (m, 1H), 7.52 – 7.43 (m, 2H), 7.39 – 7.29 (m, 3H), 7.26 (d, *J* = 8.1 Hz, 1H), 7.22 – 7.18 (m, 1H), 6.90 – 6.83 (s, 1H), 6.73 (*J* = 8.4, 23.3 Hz, 2H), 6.68 (d, *J* = 10.1 Hz, 1H), 6.39 – 6.31 (m, 1H), 6.27 – 6.24 (m, 1H), 5.74 (d, *J* = 7.8 Hz, 1H), 5.53 (s, 1H), 5.31 – 5.24 (m, 2H), 5.21 (s, 2H), 5.14 (d, *J* = 4.5 Hz, 1H), 4.90 – 4.84 (m, 1H), 4.67 (q, *J* = 5.9 Hz, 1H), 4.45 – 4.40 (m, 1H), 4.38 (d, *J* = 5.3 Hz, 1H), 4.25 – 4.18 (m, 1H), 3.68 (d, *J* = 10.5 Hz, 1H), 3.63 – 3.58 (m, 4H), 3.07 (s, 6H), 3.03 (t, *J* = 7.3 Hz, 2H), 2.95 (t, *J* = 6.4 Hz, 2H), 2.30 (s, 3H), 2.18 – 2.10 (m, 1H), 1.86 – 1.80 (m, 1H), 1.72 (p, *J* = 9.7 Hz, 1H), 1.64 (d, *J* = 13.4 Hz, 1H), 1.48 (dt, *J* = 13.8, 6.8 Hz, 1H), 1.40 (dt, *J* = 13.8, 6.8 Hz, 1H), 1.23 (s, 3H), 1.05 (d, *J* = 6.1 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 3H). **HRMS (ESI)**: calcd for $C_{73}H_{93}O_{23}N_{11}Cl_2S_2$ $[M+2H]^{2+}$, *m/z* = 812.76266, found 812.76277.

The purity of the compound was analyzed by analytical RP-HPLC (Synergy Hydro): Gradient 0% B for 4 min, 0% - 100% B for 16 min, wash. The product was eluted at 2.07 min and was detected at λ = 280 nm.

Vancomycin derivative ***5b***

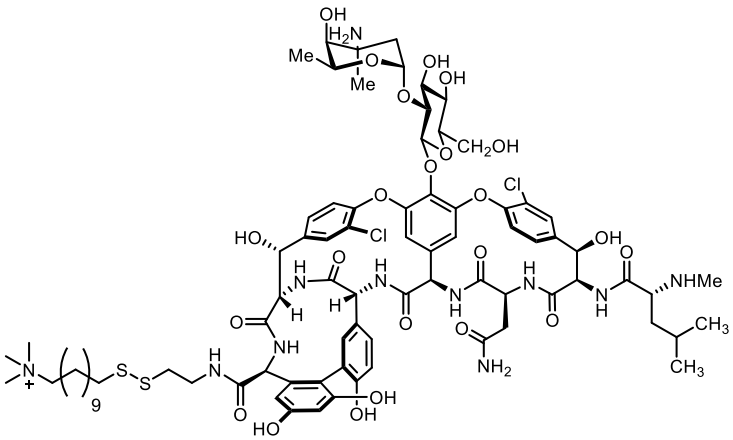


RP-HPLC (Synergy Hydro): Gradient 5% B for 14 min; 5% - 25% B for 36 min; 25% - 100% B for 3 min, wash. The desired product, eluting at 20 min, was collected, and lyophilized to afford product (10.4 mg, 0.1 mmol, 6%) as a white solid.

^{1}H NMR (500 MHz, MeOD) δ 8.36 (s, 1H), 7.72 – 7.67 (m, 1H), 7.63 (d, *J* = 6.6 Hz, 2H), 7.59 – 7.54 (m, 1H), 7.37 – 7.28 (m, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.06 (d, *J* = 2.1 Hz, 1H), 6.99 – 6.89 (m, 1H), 6.82 (d, *J* = 8.2 Hz, 1H), 6.44 (d, *J* = 2.3 Hz, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 5.90 – 5.76 (m, 1H), 5.47 (d, *J* = 6.7 Hz, 1H), 5.43 (d, *J* = 3.5 Hz, 1H), 5.39 -5.35 (m, 1H), 5.31 (d, *J* = 3.5 Hz, 1H), 4.21 (s, 1H), 3.90 – 3.79 (m, 2H), 3.79 – 3.73 (m, 1H), 3.73 – 3.60 (m, 2H), 3.59 – 3.49 (m, 2H), 2.89 (t, *J* = 7.0 Hz, 2H), 2.80 (t, *J* = 7.0 Hz, 2H), 2.66 (s, 3H), 2.34 – 2.20 (m, 1H), 2.06 (dd, *J* = 13.6, 4.4 Hz, 1H), 1.97 – 1.92 (m, 1H), 1.86 – 1.72 (m, 5H), 1.65 – 1.59 (m, 1H), 1.54 – 1.44 (m, 4H), 1.41 – 1.36 (m, 1H), 1.20 (d, *J* = 6.4 Hz, 3H), 0.99 (d, *J* = 6.0 Hz, 3H), 0.96 (d, *J* = 6.0 Hz, 3H). **HRMS (ESI):** calcd for $C_{76}H_{100}O_{23}N_{11}Cl_2S_2$ $[M+3H]^{3+}$, *m/z* =556.19318, found 556.19279.

The purity of the compound was analyzed by analytical RP-HPLC (Synergy Hydro): Gradient 0% B for 4 min, 0% - 100% B for 16 min, wash. The product was eluted at 13.1 min and was detected at λ = 280 nm.

Vancomycin derivative ***5c***



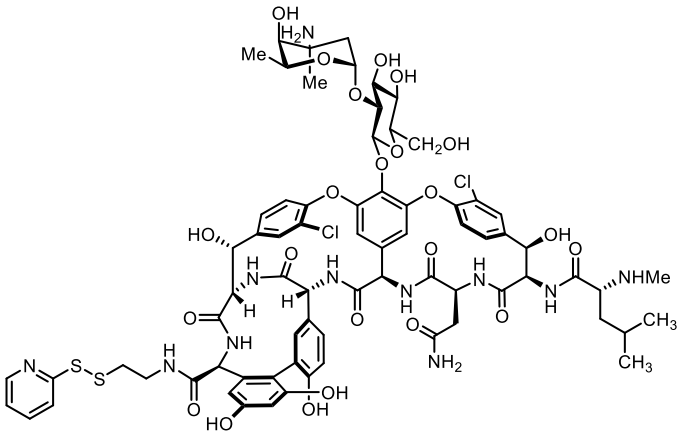
RP-HPLC (Synergy Hydro): Gradient 5% B for 14 min; 5% - 40% B for 46 min; 40% - 100% B for 2 min, wash. The desired product, eluting at 30.2 min, was collected, and lyophilized to afford product (7.23 mg, 0.028 mmol, 15%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.54 (d, *J* = 2.6 Hz, 1H), 8.41 (d, *J* = 4.8 Hz, 1H), 8.34 (s, 2H), 8.26 – 8.19 (m, 1H), 8.12 (t, *J* = 5.4 Hz, 1H), 7.86 (s, 1H), 7.81 – 7.73 (br, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.37 (s, 1H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.24 (d, *J* = 8.2 Hz, 1H), 7.21 (s, 1H), 6.83 (s, 1H), 6.76 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.67 – 6.61 (br, 1H), 6.57 (d, *J* = 11.6 Hz, 1H), 6.37 (d, *J* = 1.9

Hz, 1H), 6.23 (d, J = 1.9 Hz, 1H), 5.72 (d, J = 8.0 Hz, 1H), 5.56 (s, 1H), 5.32 (d, J = 7.6 Hz, 1H), 5.27 (s, 1H), 5.25 – 5.19 (m, 2H), 5.15 (d, J = 3.8 Hz, 1H), 4.89 – 4.82 (br, 1H), 4.67 (q, J = 6.1 Hz, 1H), 4.44 (d, J = 4.6 Hz, 1H), 4.37 (d, J = 5.4 Hz, 2H), 4.22 (d, J = 11.7 Hz, 1H), 3.71 – 3.65 (m, 2H), 3.29 – 3.21 (m, 4H), 2.86 – 2.80 (m, 2H), 2.76 (t, J = 7.2 Hz, 3H), 2.31 (s, 3H), 2.14 (dd, J = 16.0, 5.8 Hz, 1H), 1.91 – 1.81 (m, 2H), 1.76 – 1.70 (m, 2H), 1.70 – 1.61 (m, 6H), 1.52 – 1.45 (m, 1H), 1.44 – 1.33 (m, 3H), 1.35 – 1.22 (m, 18H), 1.06 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H). **HRMS (ESI):** calcd for $C_{82}H_{112}O_{23}N_{11}Cl_2S_2$ $[M+3H]^{3+}$, *m/z* =584.22448, found 584.22440.

The purity of the compound was analyzed by analytical RP-HPLC (Synergy Hydro): Gradient 0% B for 4 min, 0% - 100% B for 16 min, wash. The product was eluted at 13.1 min and was detected at λ = 280 nm.

Vancomycin derivative ***6***

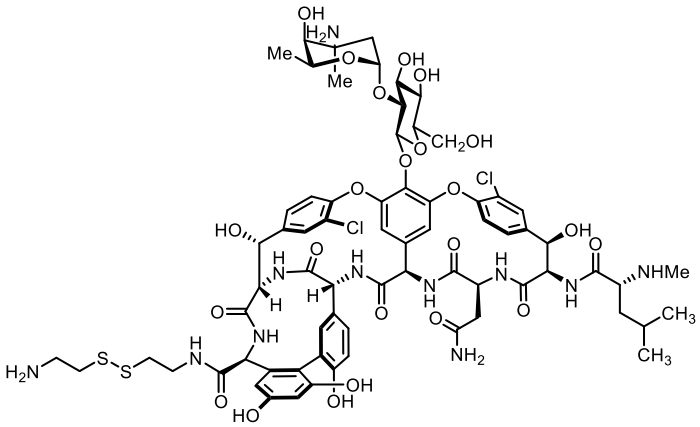


RP-HPLC (Synergy Hydro): Gradient 5% B for 10 min; 5% - 30% B for 33 min; 30% - 100% B for 2 min, wash. The desired product, eluting at 23–24.3min, was collected and lyophilized to afford product (16 mg, 0.054 mmol, 18%) as a white solid.

^{1}H NMR (500 MHz, MeOD) δ 8.44 (s, 2H), 8.38 – 8.35 (m, 1H), 7.84 – 7.77 (m, 2H), 7.70 – 7.66 (m, 1H), 7.64 – 7.60 (m, 2H), 7.59 – 7.55 (m, 1H), 7.25 – 7.19 (m, 2H), 7.05 (d, J = 2.4 Hz, 1H), 6.98 – 6.91 (m, 1H), 6.81 (d, J = 8.5 Hz, 1H), 6.43 (d, J = 2.3 Hz, 1H), 6.39 (d, J = 2.3 Hz, 1H), 5.84 – 5.76 (m, 1H), 5.48 – 5.44 (m, 1H), 5.43 (d, J = 4.2 Hz, 1H), 5.38 – 5.34 (m, 1H), 5.30 (d, J = 3.5 Hz, 1H), 4.20 – 4.17 (m, 1H), 3.90 – 3.80 (m, 2H), 3.75 (dd, J = 11.6, 4.5 Hz, 1H), 3.68 – 3.60 (m, 2H), 3.60 – 3.50 (m, 2H), 3.42 – 3.36 (m, 1H), 3.00 (t, J = 6.8 Hz, 2H), 2.84 (dd, J = 1.5 Hz, J = 15.1 Hz, 1H), 2.58 (s, 3H), 2.33 – 2.24 (m, 1H), 2.05 (dd, J = 13.6, 4.6 Hz, 1H), 1.96 – 1.90 (m, 1H), 1.80 – 1.69 (m, 2H), 1.63 – 1.55 (m, 2H), 1.49 (s, 2H), 1.20 (d, J = 6.5 Hz, 3H), 0.97 (dd, J = 14.3, 6.3 Hz, 6H); **HRMS (ESI):** calcd for $C_{73}H_{85}Cl_2N_{11}O_{23}S_2$ $[M+2H]^{2+}$, *m/z* = 808.73136, found 808.73176.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 16 min, wash. The product was eluted at 11.6 min and was detected at λ = 280 nm.

Vancomycin derivative 7

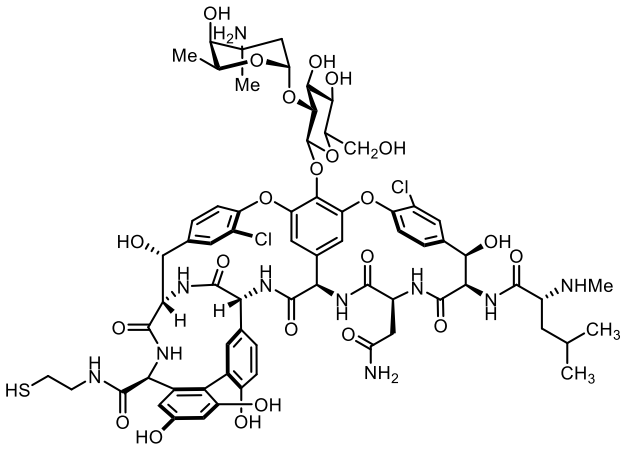


RP-HPLC (Synergy Hydro): Gradient 0% B for 12 min; 0% - 30% B for 33 min; 30% - 100% B for 10 min, wash. The desired product, eluting at 23–24.7 min, was collected and lyophilized to afford product (38 mg, 0.067 mmol, 36%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 – 8.59 (br, 1H), 8.44 (d, J = 6.3, 1H), 8.29 (s, 3H), 8.17 – 8.12 (m, 1H), 7.85 (s, 1H), 7.79 – 7.70 (m, 1H), 7.50 (d, J = 8.5, 2H), 7.45 (d, J = 8.5, 2H), 7.38 – 7.34 (br, 1H), 7.32 (d, J = 8.3 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 7.24 – 7.21 (br, 1H), 6.90 – 6.86 (m. 1H), 6.76 (dd, J = 8.4, 1.8 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.68 – 6.57 (m, 1H), 6.36 (d, J = 2.1 Hz, 1H), 6.25 (d, J = 2.1 Hz, 1H), 5.74 (d, J = 7.8 Hz, 1H), 5.54 (s, 1H), 5.29 (d, J = 7.8 Hz, 1H), 5.24 – 5.21 (m, 3H), 5.14 (d, J = 3.7 Hz, 1H), 4.90 – 4.83 (br, 1H), 4.67 (q, J = 6.4 Hz, 1H), 4.44 (d, J = 4.8 Hz, 1H), 4.38 (d, J = 5.8 Hz, 1H), 4.37 – 4.32 (m, 1H), 4.23 – 4.17 (m, 1H), 3.68 (d, J = 10.0 Hz, 1H), 3.56 – 3.43 (m, 3H), 3.11 (s, 1H), 3.03 (t, J = 7.0 Hz, 1H), 2.90 -2.81 (m, 4H), 2.80 – 2.74 (m, 1H), 2.30 (s, 3H), 2.14 (dd, J = 5.6, 16.1 Hz, 1H), 1.88 – 1.81 (m, 1H), 1.76 – 1.69 (m, 1H), 1.67 (d, J = 12.8 Hz, 1H), 1.51 – 1.45 (m, 1H), 1.44 - 1.36 (m, 1H), 1.25 (s, 3H), 1.06 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H). **HRMS (ESI):** calcd for $C_{70}H_{88}Cl_2N_{11}O_{23}S_2$ $[M+2H]^{2+}$, *m/z* =528.16188, found 528.16217.

The purity of the compound was analyzed by analytical RP-HPLC (Gemini-NX): Gradient 5% B for 4 min, 5%–100% B for 16 min, wash. The product was eluted at 2.7 min and was detected at λ = 280 nm.

Synthesis of *vancomycin derivative 4a*



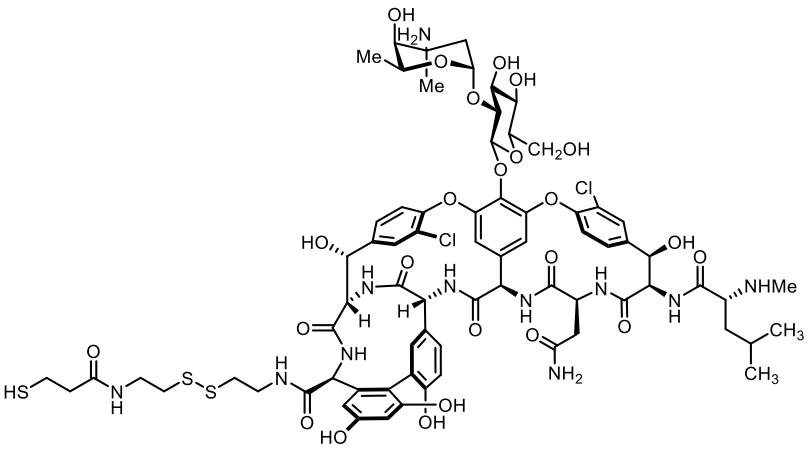
Vancomycin derivative **7** was dissolved in MeOH (0.1 mL) and H_2O (0.1 mL). TCEP (3.26 mg, 0.011 mmol) was added into the reaction. The mixture was stirred under argon for 30 min till full conversion was observed. After that, the reaction mixture was filtered through 13 mm Syringe filter, Nylon 66, 1.0 µm, and purified by semi-prep

RP-HPLC (Gemini-NX): Gradient 5% B for 7 min; 5% - 50% B for 23 min; 50% - 100% B for 2 min, wash. The desired product, eluting at 10.2-12.0 min, was collected, and lyophilized to afford product (2.1 mg, 0.0056 mmol, 25%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.2 – 8.82 (m, 1H), 8.65 – 8.57 (m, 1H), 8.46 – 8.40 (d, J = 4.2 Hz, 1H), 8.34 (s, 1H), 8.23 – 8.16 (m, 1H), 8.13 (t, J = 5.8 Hz, 1H), 7.85 (m, 1H), 7.78 – 7.70 (m, 1H), 7.49 (d, J = 8.6, 1H), 7.45 (dd, J = 0.35, 8.5), 7.37 – 7.33 (m, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 0.6 Hz, 1H), 6.92 – 6.85 (m, 1H), 6.75 (dd, J = 8.5, 1.7 Hz, 1H), 6.70 (d, J = 8.5 Hz, 2H), 6.67 – 6.59 (m, 1H), 6.35 (d, J = 2.0 Hz, 1H), 6.24 (d, J = 2.0 Hz, 1H), 5.74 (d, J = 8.0 Hz, 2H), 5.53 (s, 1H), 5.30 – 5.24 (m, 2H), 5.23 – 5.19 (m, 2H), 5.14 (d, J = 3.4 Hz, 1H), 4.90 – 4.84 (br, 1H), 4.67 (q, J = 6.2 Hz, 1H), 4.43 (d, J = 4.9 Hz, 1H), 4.36 (d, J = 5.6 Hz, 1H), 4.22 (d, J = 12.0 Hz, 1H), 3.69 -3.65 (m, 2H), 3.58 – 3.51 (m, 2H), 3.11 (s, 1H), 3.03 (t, J = 7.2 Hz, 1H), 2.59 (t, J = 7.2 Hz, 2H), 2.30 (s, 3H), 2.18 – 2.11 (m, 1H), 1.88 – 1.82 (m, 1H), 1.77 – 1.70 (m, 1H), 1.68 – 1.63 (m, 1H), 1.52 – 1.44 (m, 1H), 1.43 – 1.36 (m, 1H), 1.25 (s, 3H), 1.06 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H). **HRMS (ESI):** calcd for $C_{68}H_{82}O_{23}N_{10}Cl_2S$ $[M+2H]^{2+}$, m/z = 754.23205, found 754.22945.

The purity of the compound was analyzed by analytical RP- HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 11 min, wash. The product was eluted at 12.3 min and was detected at λ = 280 nm.

Synthesis of *vancomycin derivative 3b*

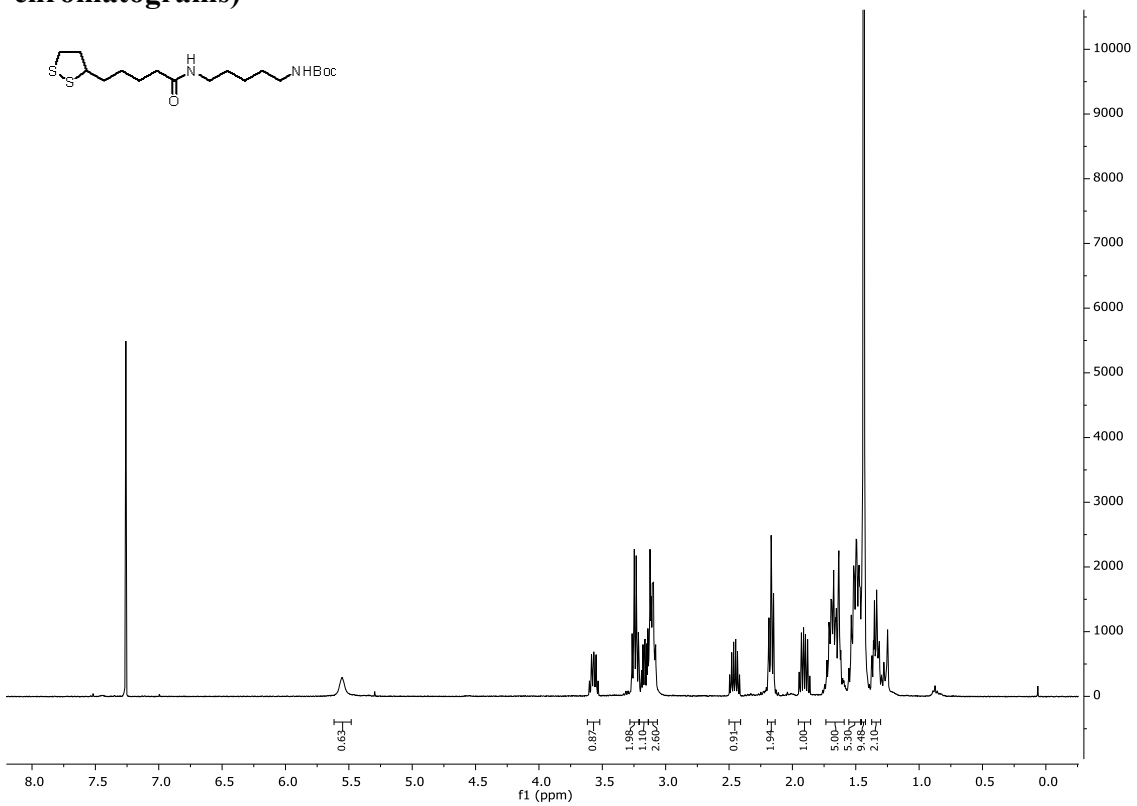


Vancomycin derivative **3a** (6 mg, 0.003 mmol) was dissolved in dry DCM (0.080 mL). TFA (0.02 mL, 0.27 mmol) and triethylsilane (0.002 mL, 0.009 mmol) were added and the reaction was stirred at rt for 1 h. The solvent was evaporated, and the crude was washed with pentane (1x2 mL). The purification carried out by semi-prep RP-HPLC (Synergy Hydro): Gradient 5% B for 7 min; 5% - 50% B for 23 min; 50% - 100% B for 2 min, wash. The desired product, eluting at 13-15 min, was collected, and lyophilized to afford product (1.23 mg, 0.003 mmol, 24%) as a white solid.

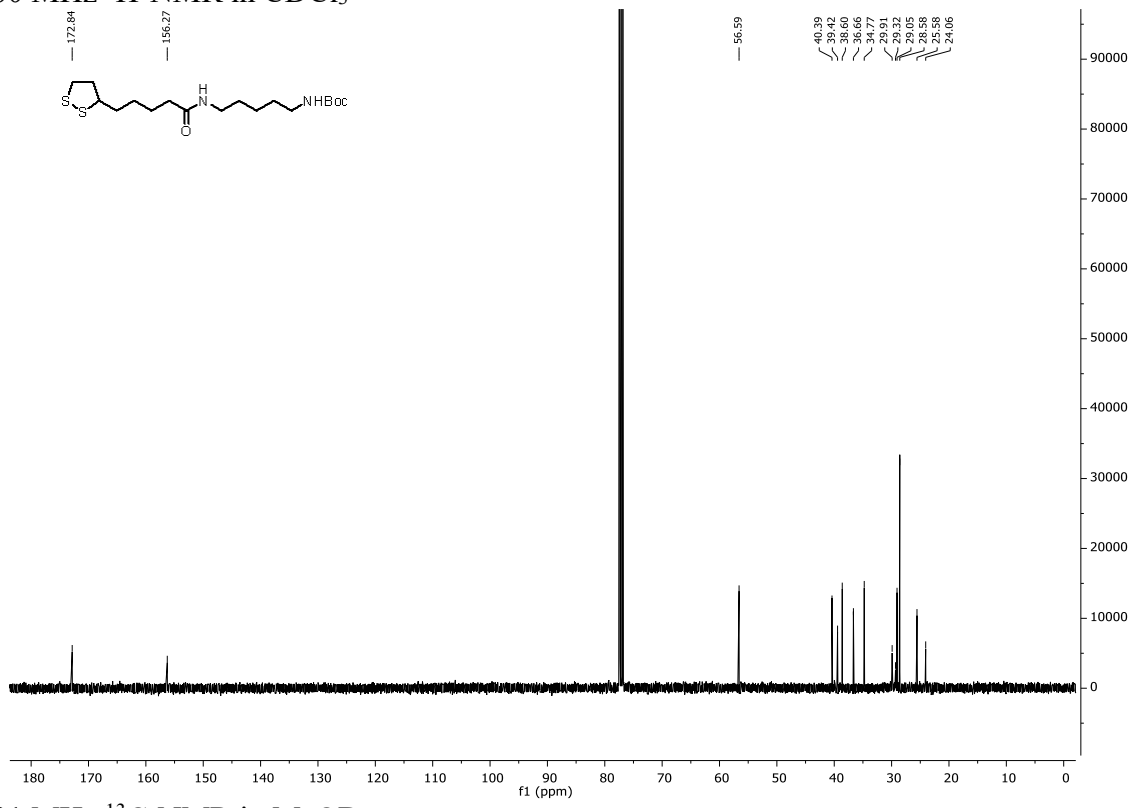
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 – 8.56 (br, 1H), 8.48 – 8.41 (br, 1H), 8.40 (s, 2H), 8.24 – 8.09 (m, 1H), 7.86 (s, 1H), 7.78 – 7.67 (br, 1H), 7.48 (dd, *J* = 21.4, 8.3 Hz, 2H), 7.36 – 7.28 (m, 3H), 7.28 – 7.20 (m, 2H), 6.91 – 6.82 (br, 1H), 6.76 (d, *J* = 7.8 Hz, 1H), 6.70 (d, *J* = 7.8 Hz, 1H), 6.68 – 6.62 (br, 1H), 6.39 – 6.34 (br, 1H), 6.26 – 6.22 (br, 1H), 5.74 (d, *J* = 8.0 Hz, 1H), 5.60 – 5.50 (m, 1H), 5.39 – 5.27 (m, 2H), 5.25 (br, 1H), 5.20 (br, 2H), 5.14 (br, 1H), 4.90 – 4.83 (br, 1H), 4.67 (q, *J* = 7.4 Hz, 1H), 4.45 – 4.41 (br, 1H), 4.39 – 4.34 (br, 1H), 4.21 (d, *J* = 11.6 Hz, 1H), 4.1 – 3.95 (br, 1H), 3.68 (d, *J* = 11.2 Hz, 1H), 3.60 – 3.50 (m, 2H), 3.05 – 3.0 (m, 3H), 2.93 – 2.78 (m, 4H), 2.61 – 2.56 (m, 1H), 2.30 (d, *J* = 2.0 Hz, 3H), 2.20 – 2.11 (m, 1H), 1.89 (d, *J* = 2.0 Hz, 2H), 1.83 – 1.68 (m, 2H), 1.64 – 1.56 (m, 1H), 1.52 – 1.44 (m, 1H), 1.43 – 1.35 (m, 1H), 1.24 (s, 3H), 1.20 (s, 2H), 1.15 – 1.08 (m, 1H), 1.07 – 1.01 (m, 3H), 0.90 (d, *J* = 6.6, 3H), 0.86 (d, *J* = 6.6 Hz, 3H). **HRMS (ESI)**: calcd for $C_{73}H_{91}O_{24}N_{11}Cl_2S_3$ $[M+2H]^{2+}$, *m/z* = 835.73833, found 835.73723.

The purity of the compound was analyzed by analytical RP- HPLC (Gemini-NX): Gradient 5% B for 4 min, 5% - 100% B for 11 min, wash. The product was eluted at 11.2 min and was detected at λ = 280 nm.

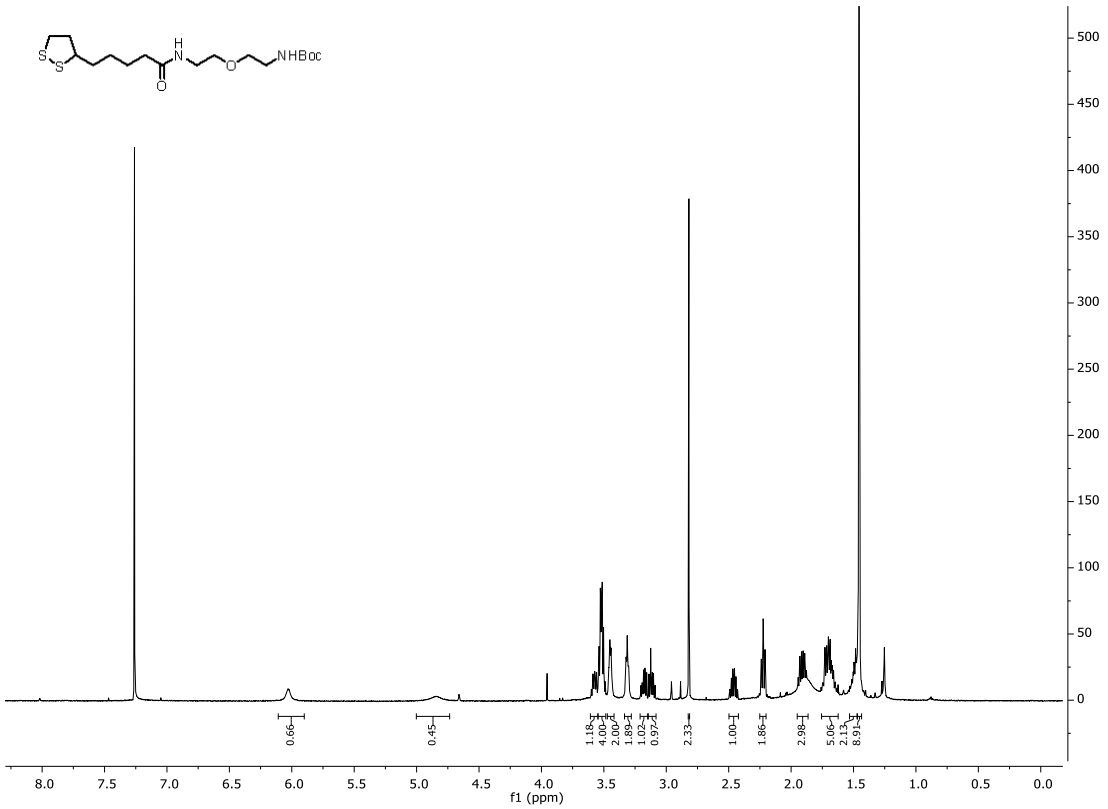
III. Characterization of all the novel compounds (NMR spectra and HPLC chromatograms)



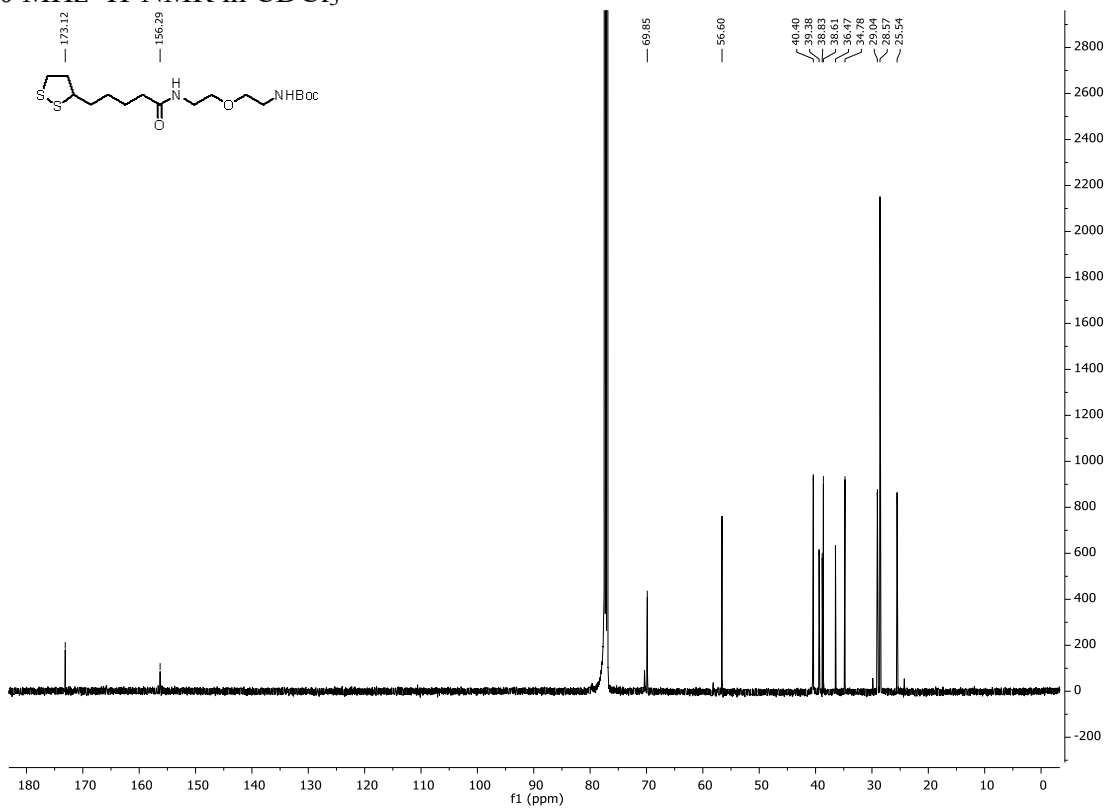
400 MHz ^{1}H-NMR in $CDCl_3$



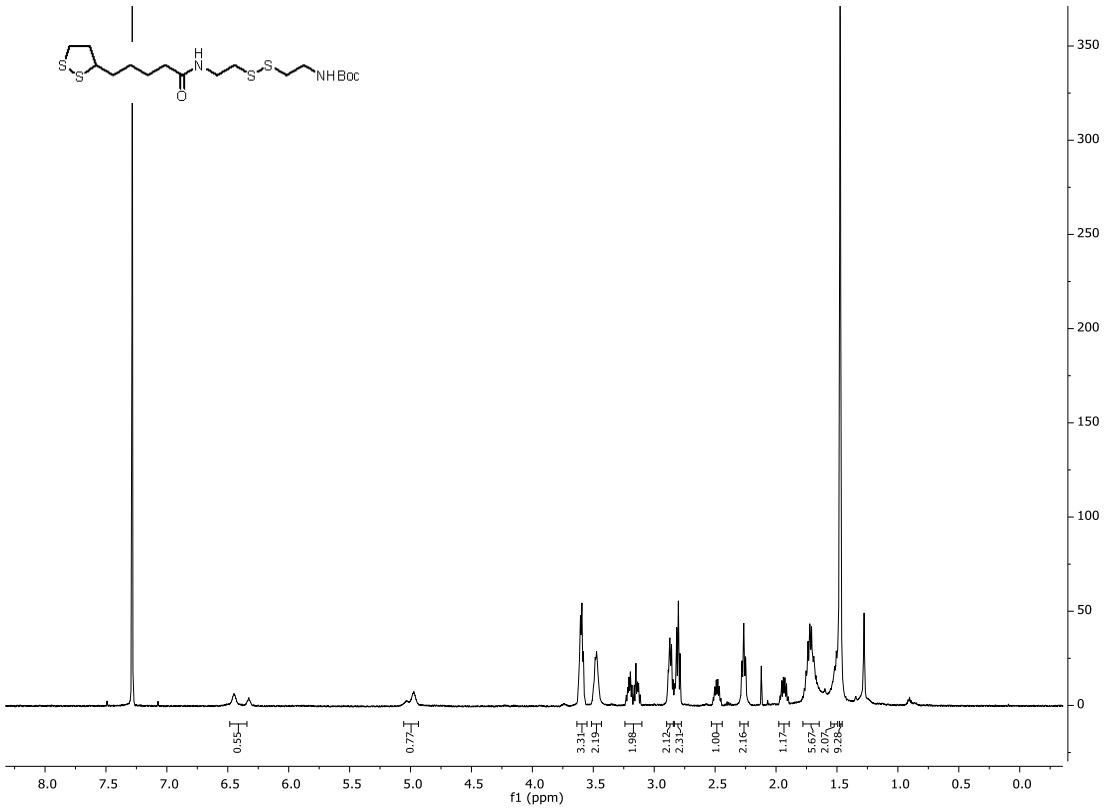
101 MHz ^{13}C-NMR in MeOD



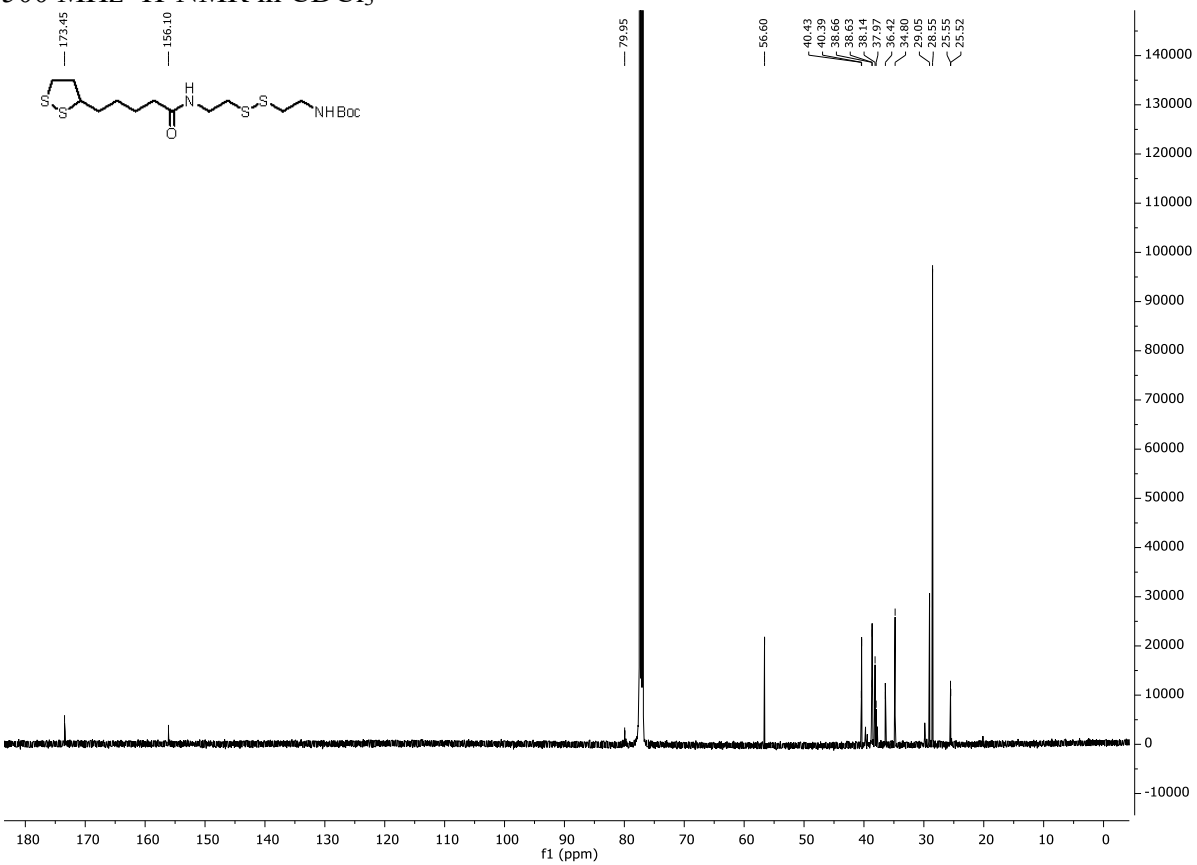
400 MHz ^{1}H-NMR in $CDCl_3$



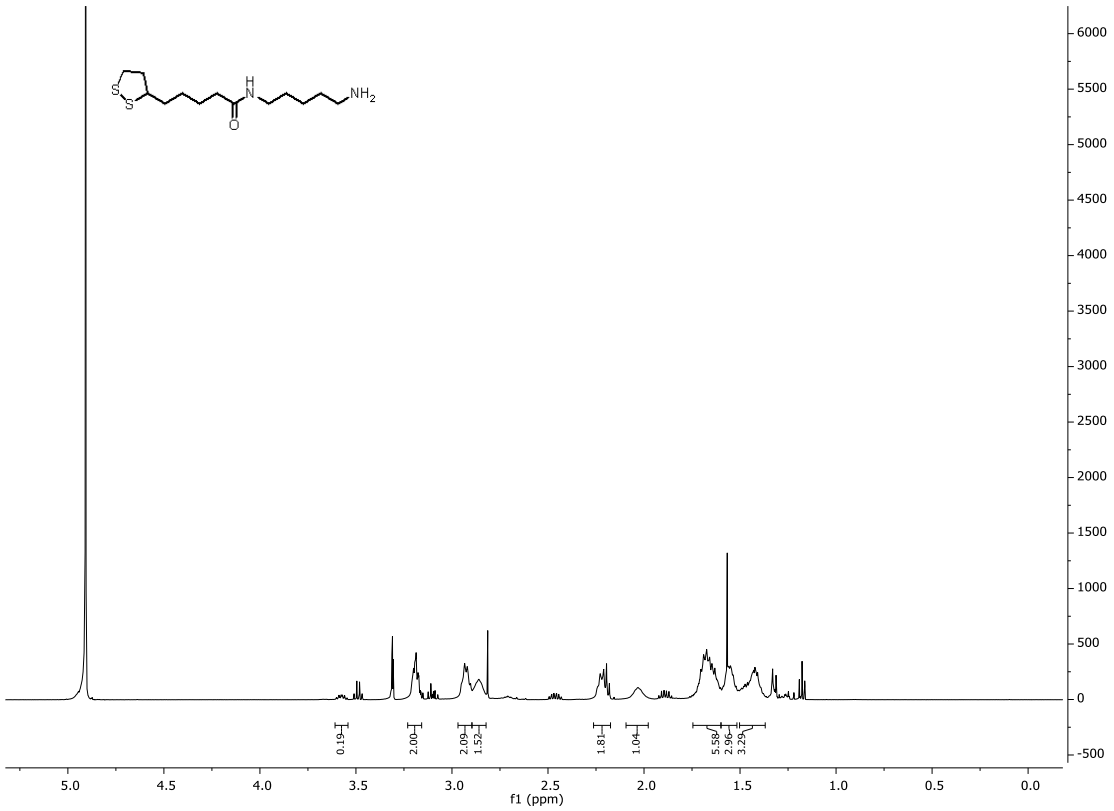
126 MHz ^{13}C-NMR in $CDCl_3$



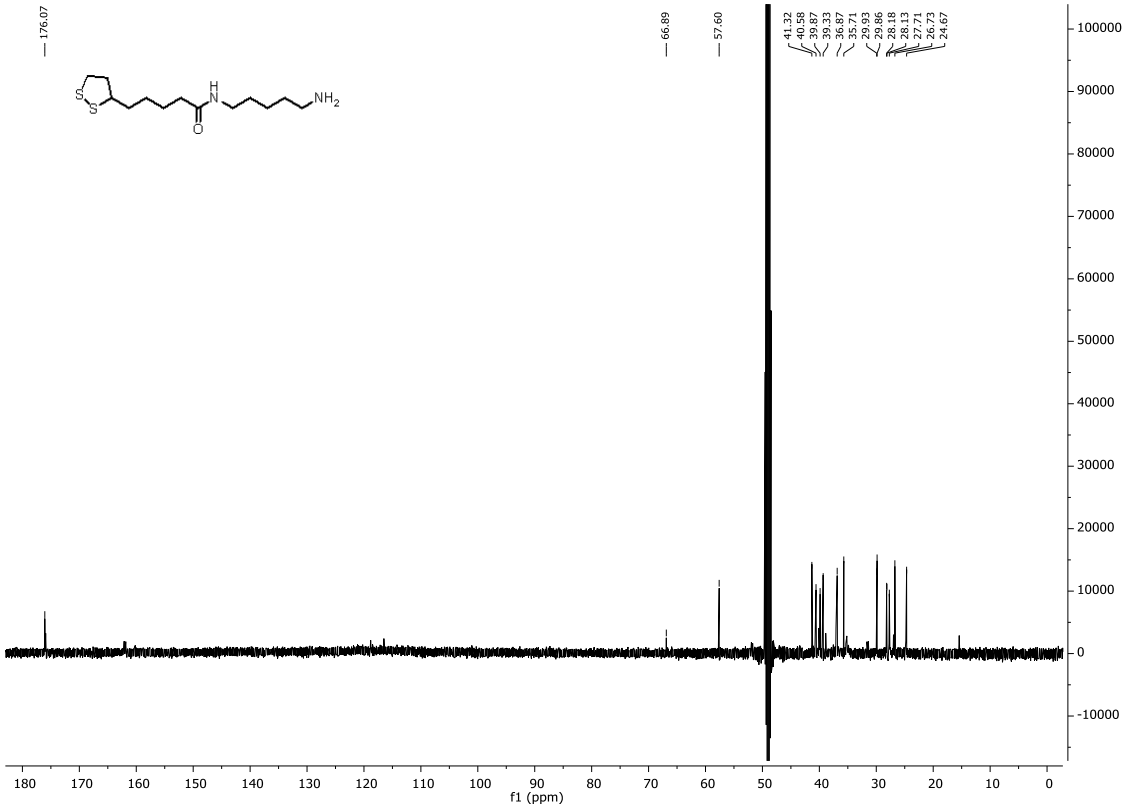
500 MHz ^{1}H-NMR in $CDCl_3$



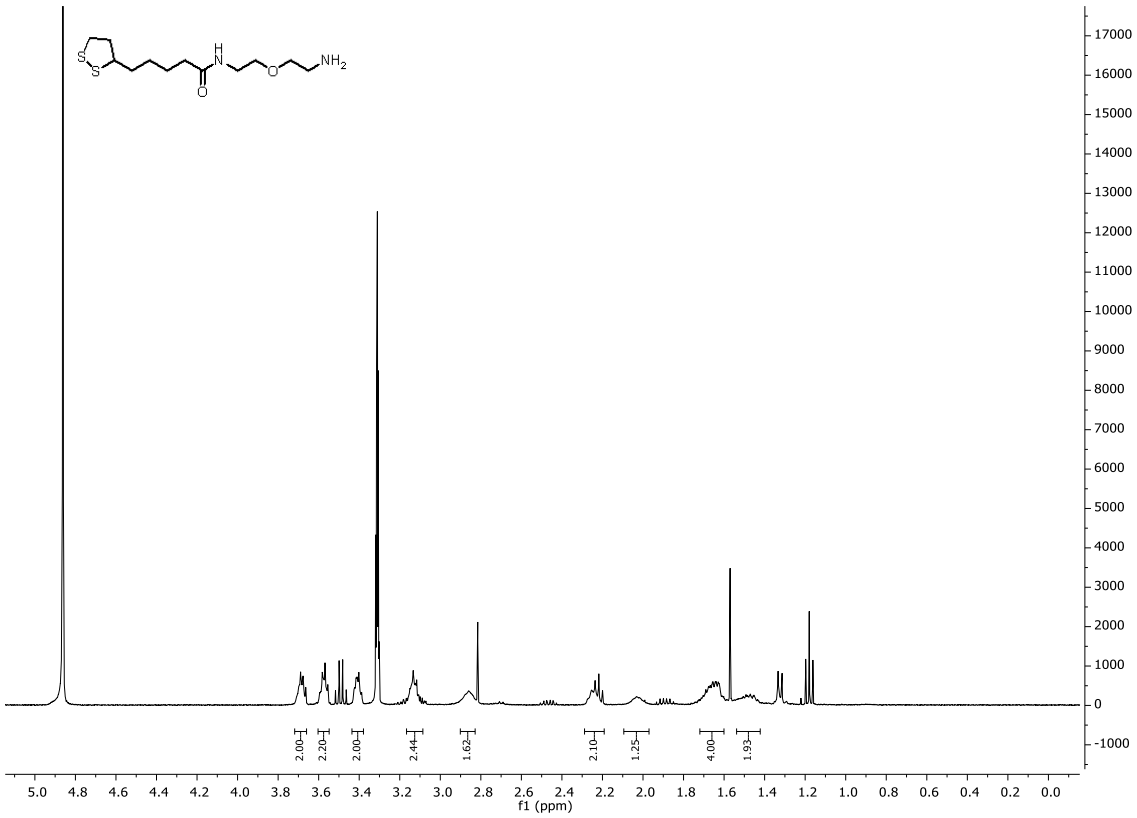
126 MHz ^{13}C-NMR in $CDCl_3$



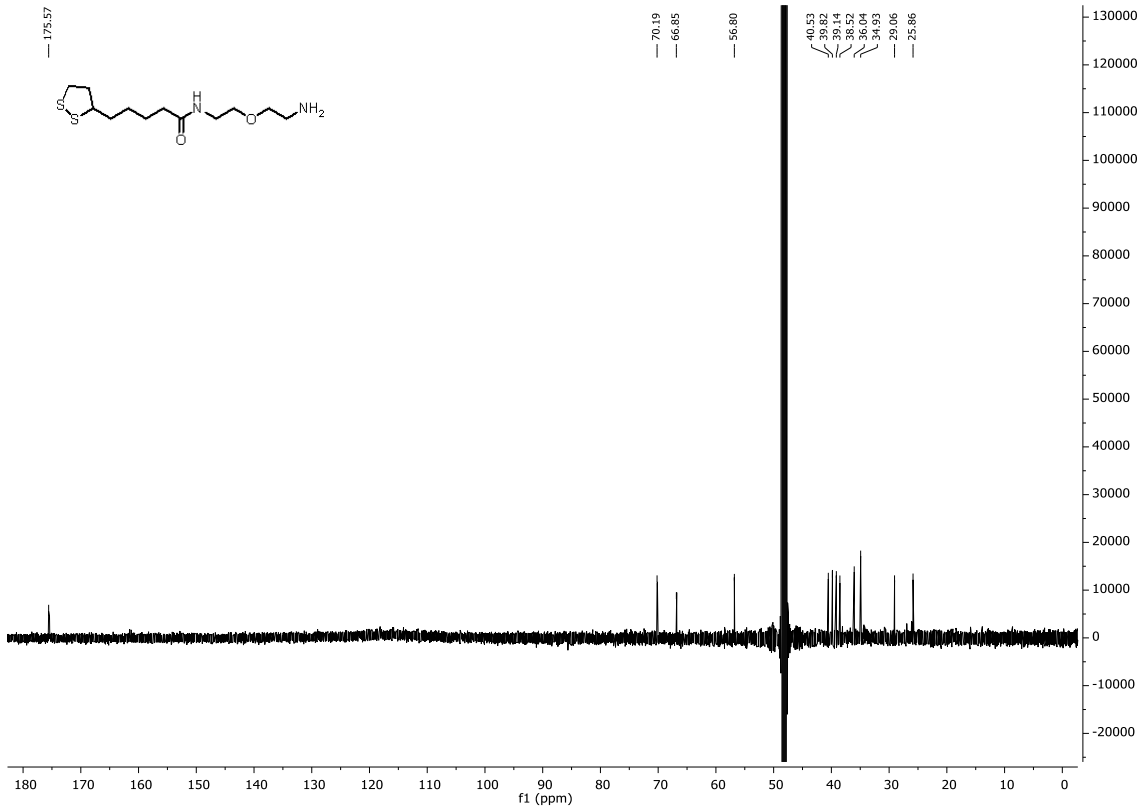
500 MHz ^{1}H-NMR in MeOD



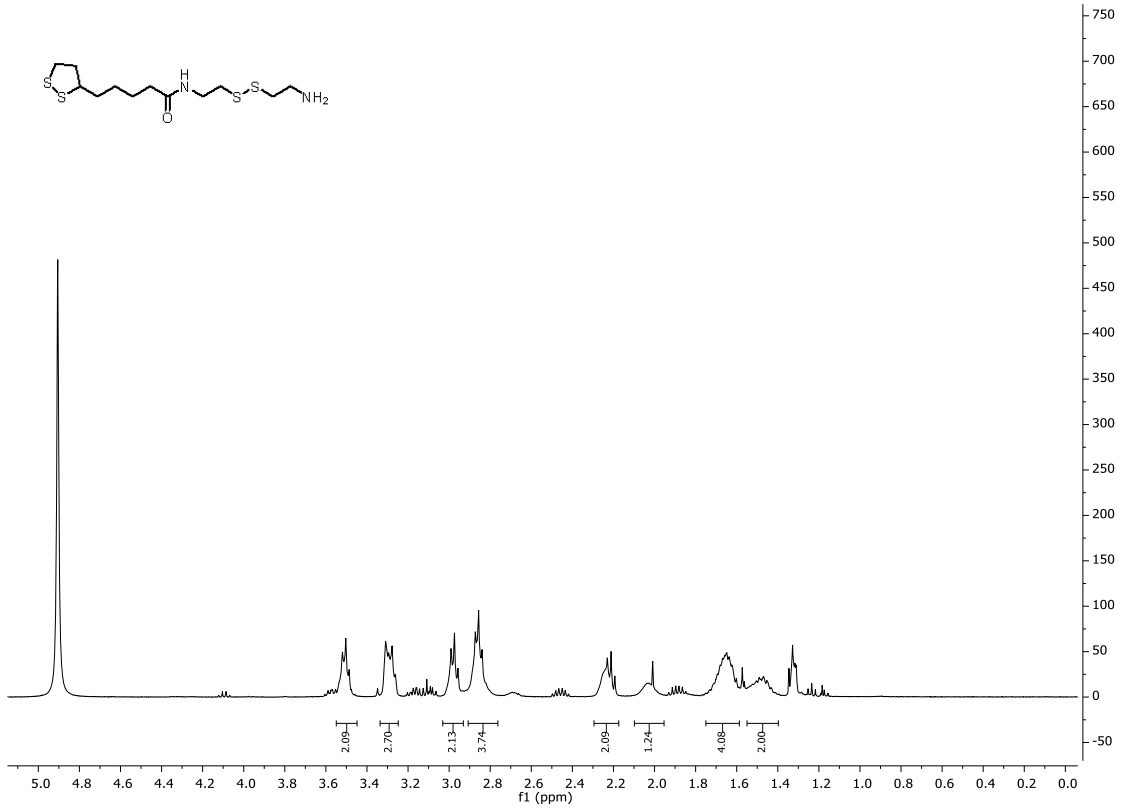
126 MHz ^{13}C-NMR in MeOD



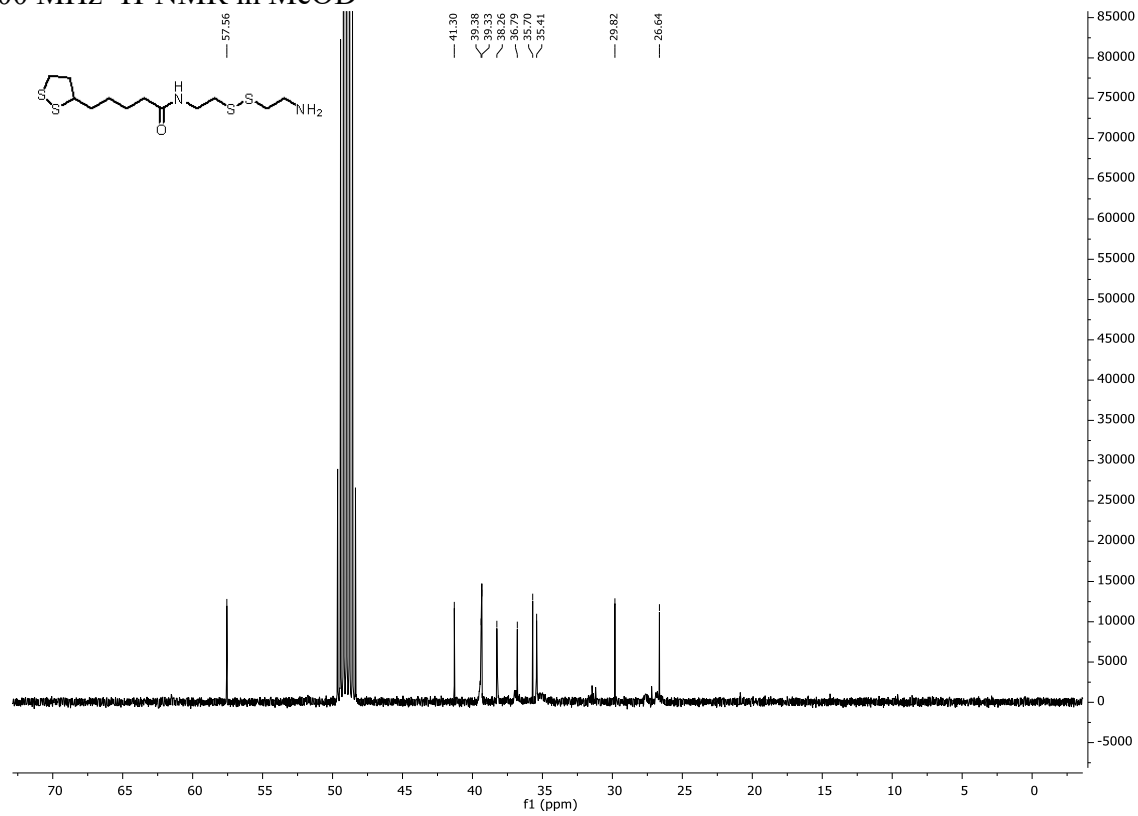
500 MHz ^{1}H-NMR in MeOD



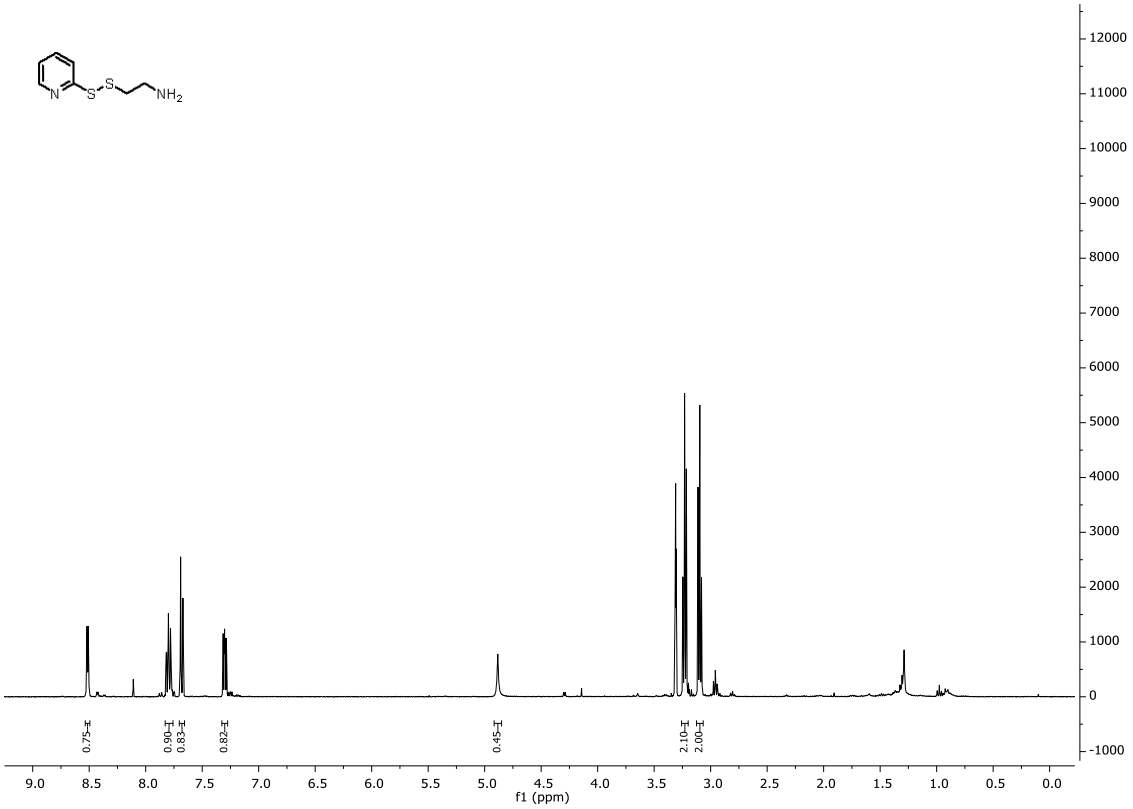
126 MHz ^{13}C-NMR in MeOD



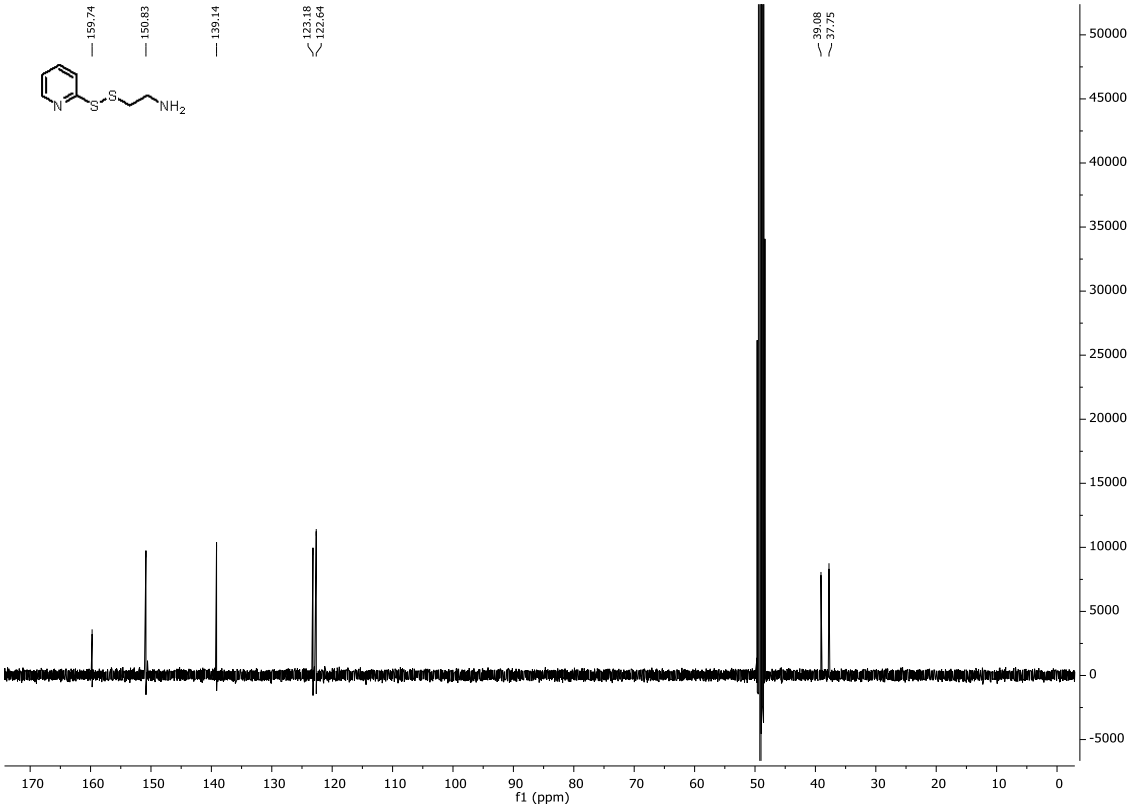
500 MHz ^{1}H-NMR in MeOD



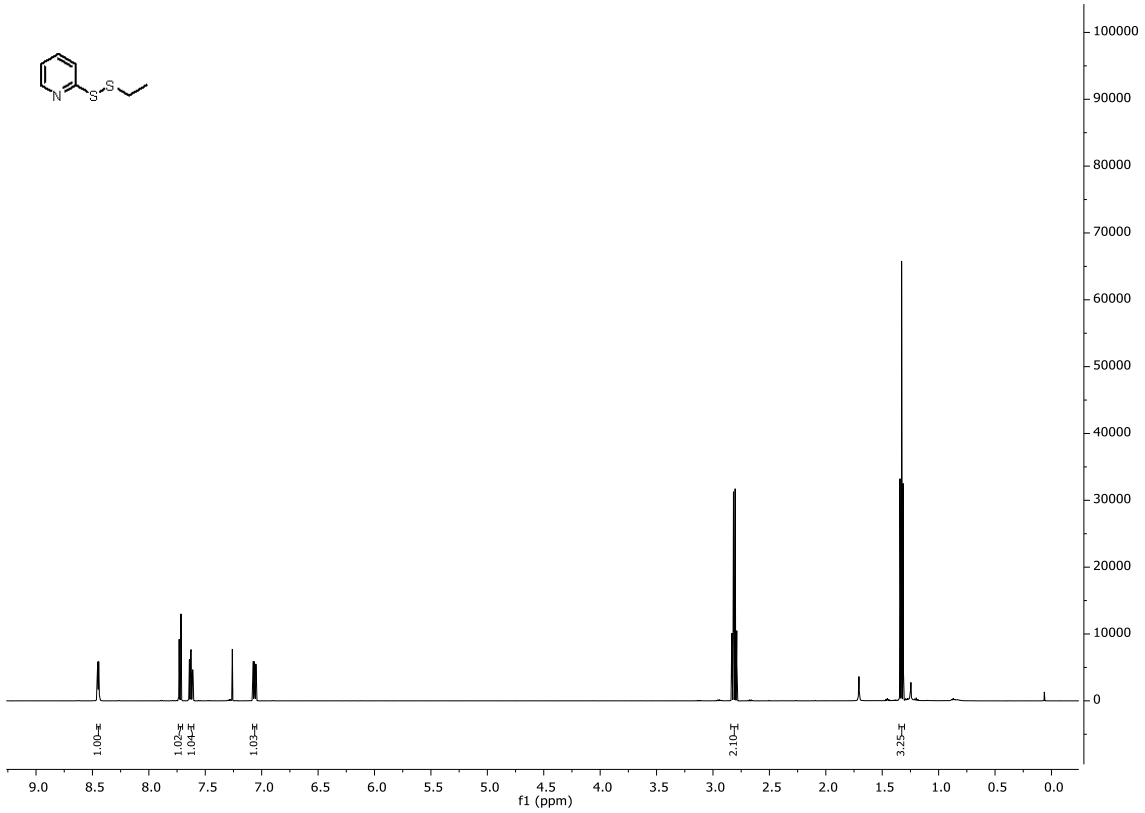
126 MHz ^{13}C-NMR in MeOD



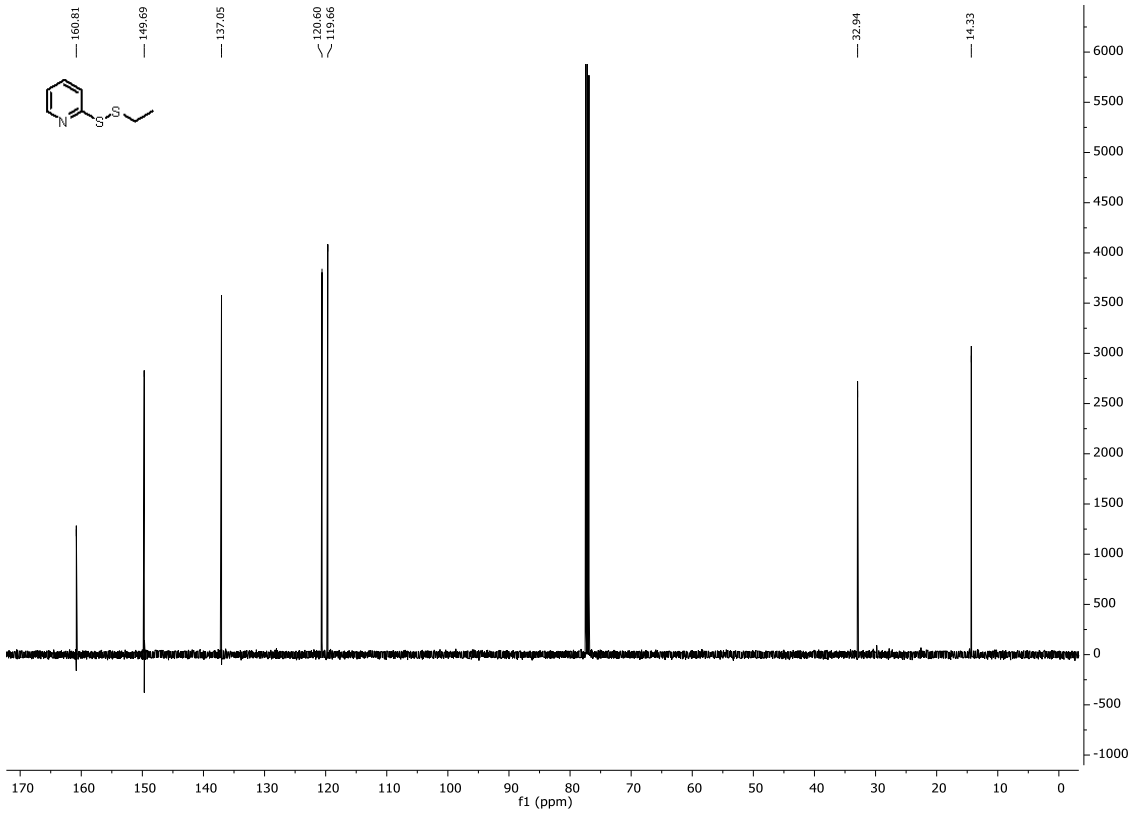
400 MHz ^{1}H-NMR in MeOD



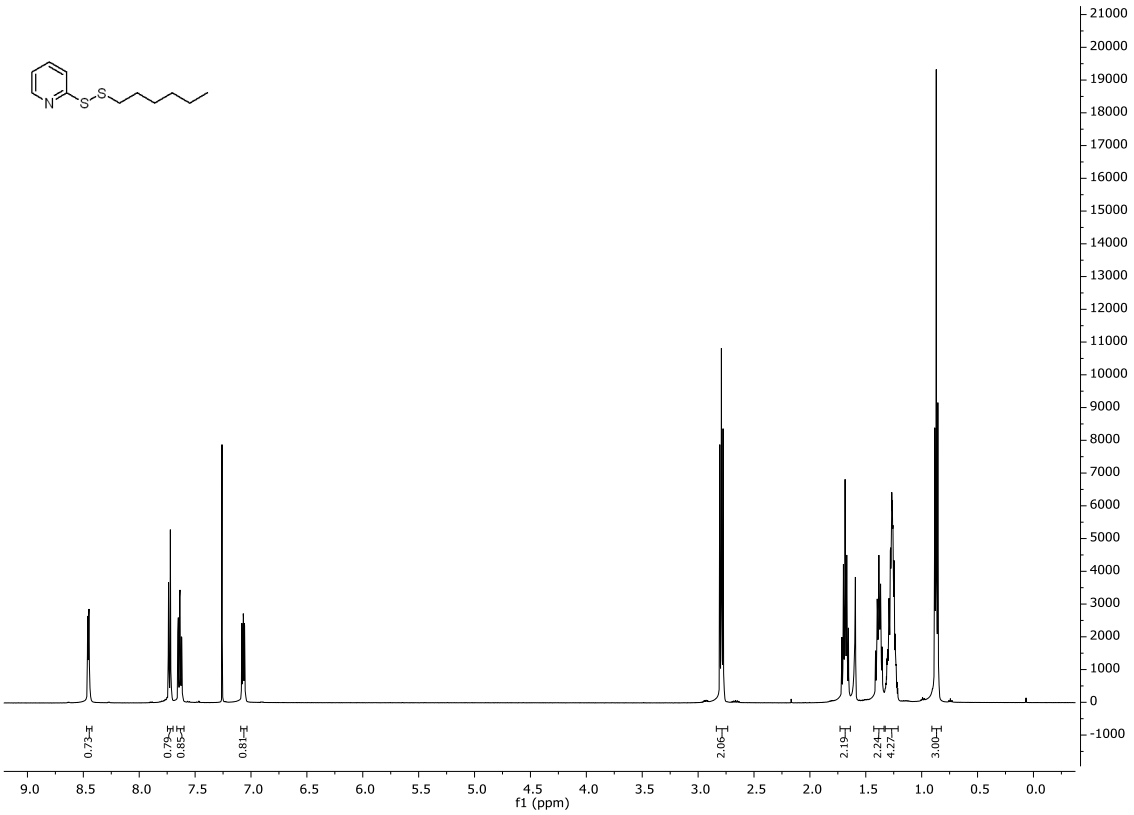
101 MHz ^{13}C-NMR in MeOD



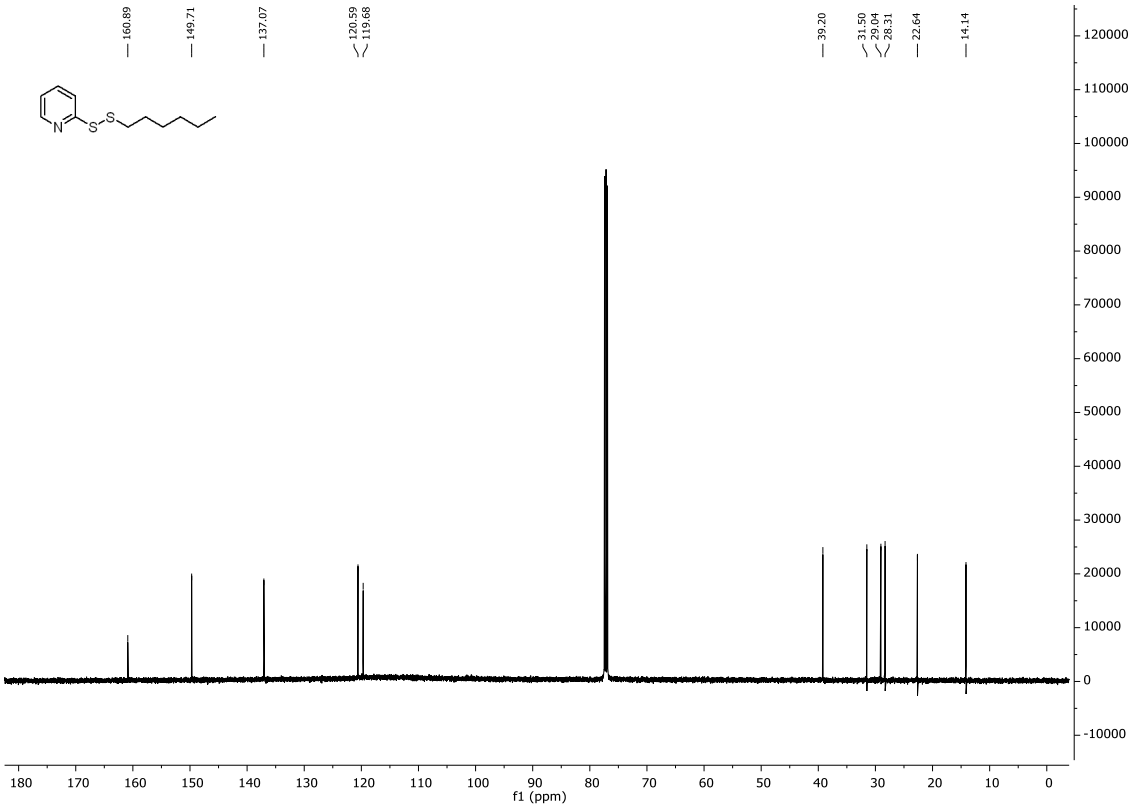
500 MHz ^{1}H-NMR in $CDCl_3$



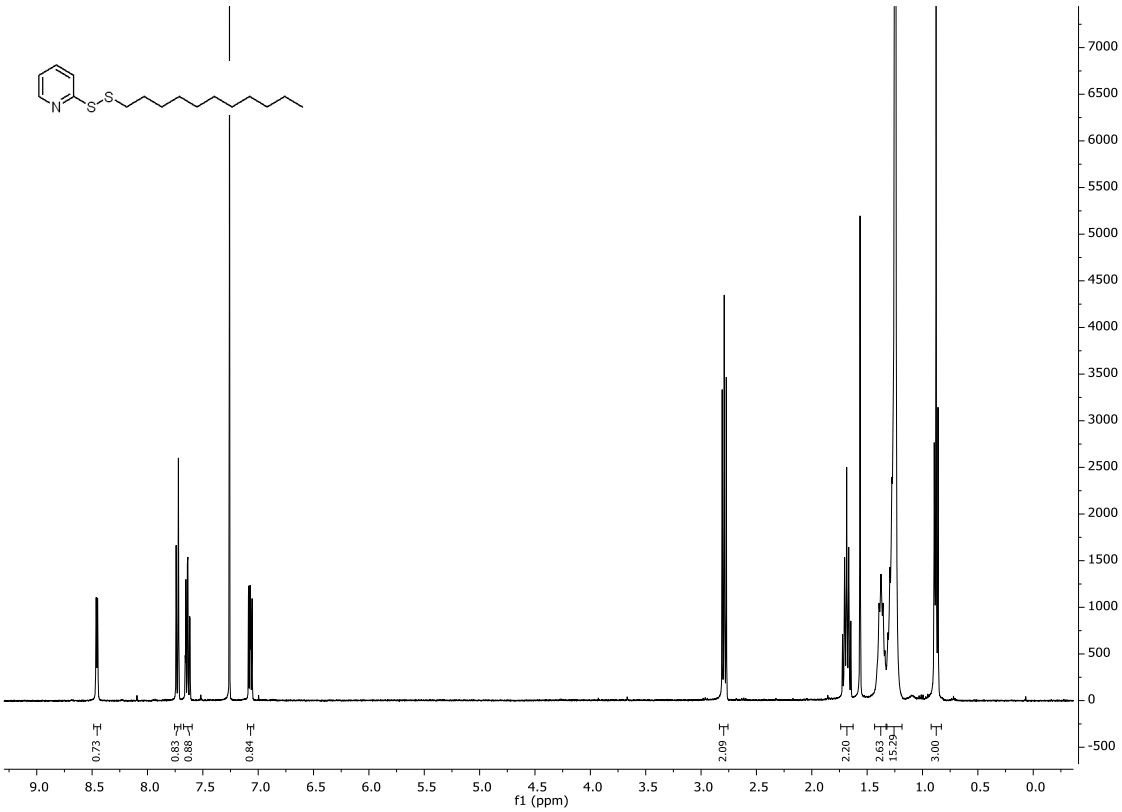
126 MHz ^{13}C-NMR in $CDCl_3$



500 MHz ^{1}H-NMR in $CDCl_3$



126 MHz ^{13}C-NMR in $CDCl_3$



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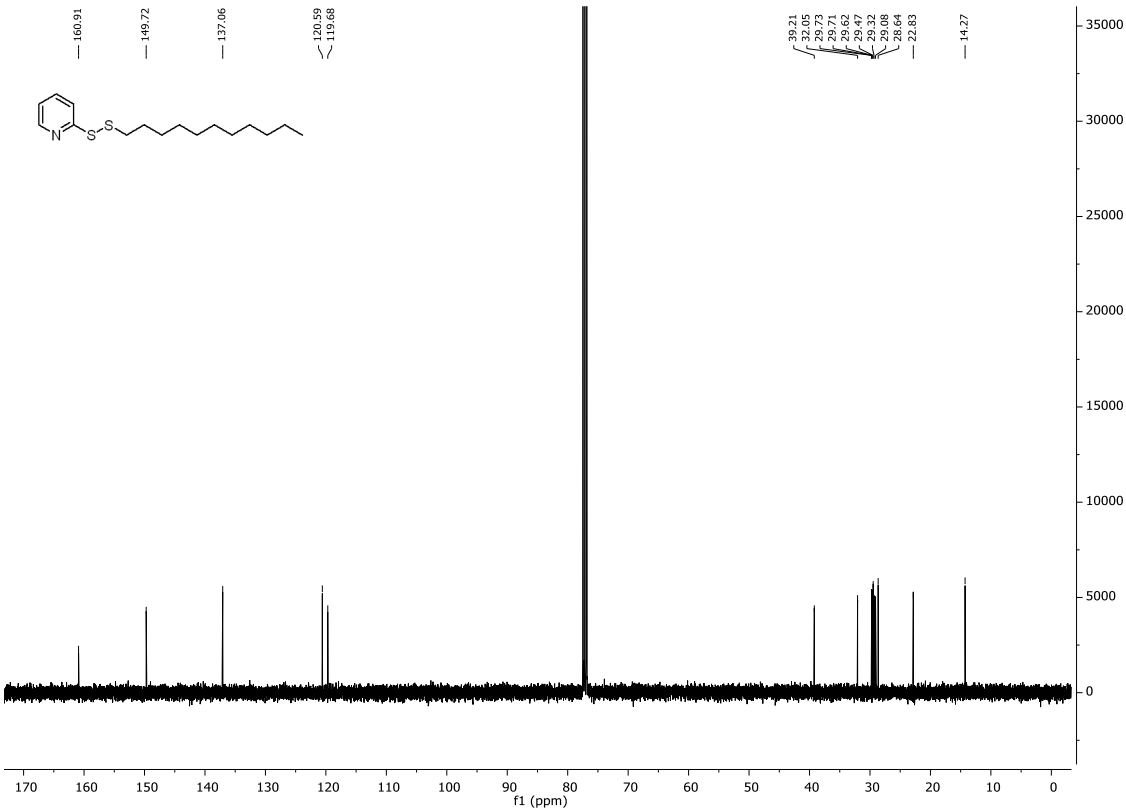

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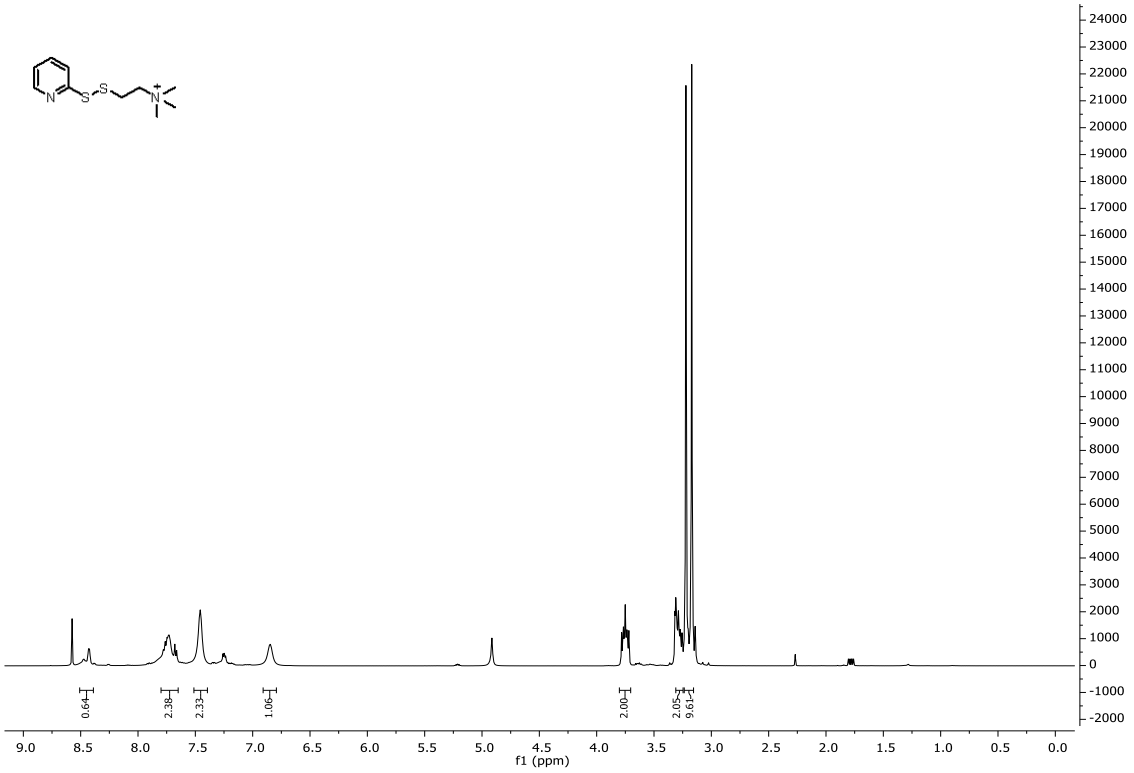
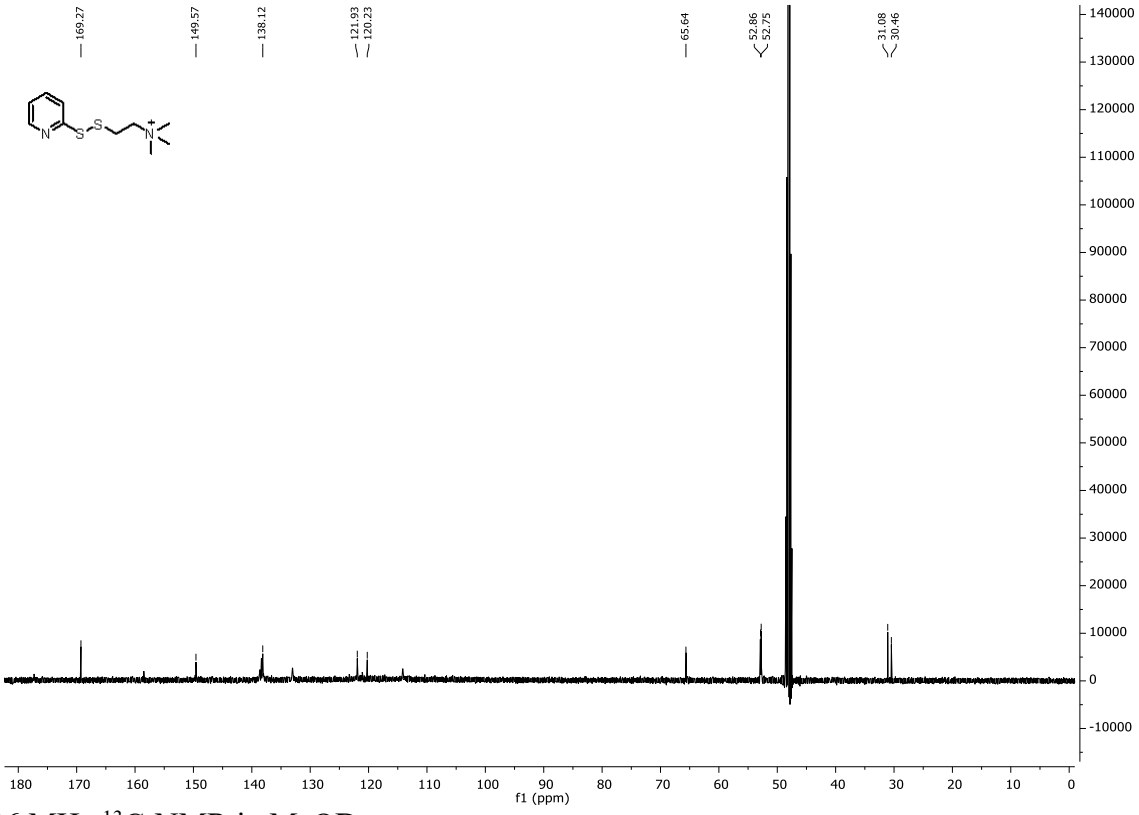


500 MHz ^{1}H-NMR in MeOD



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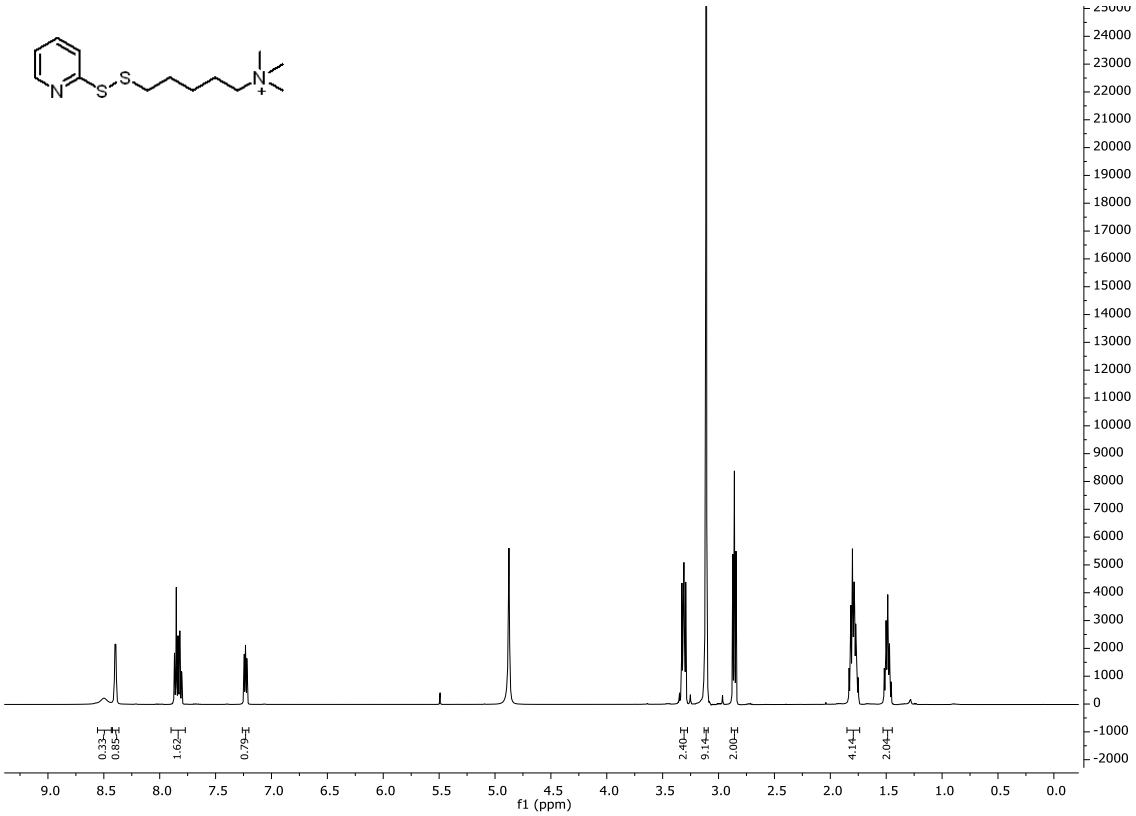
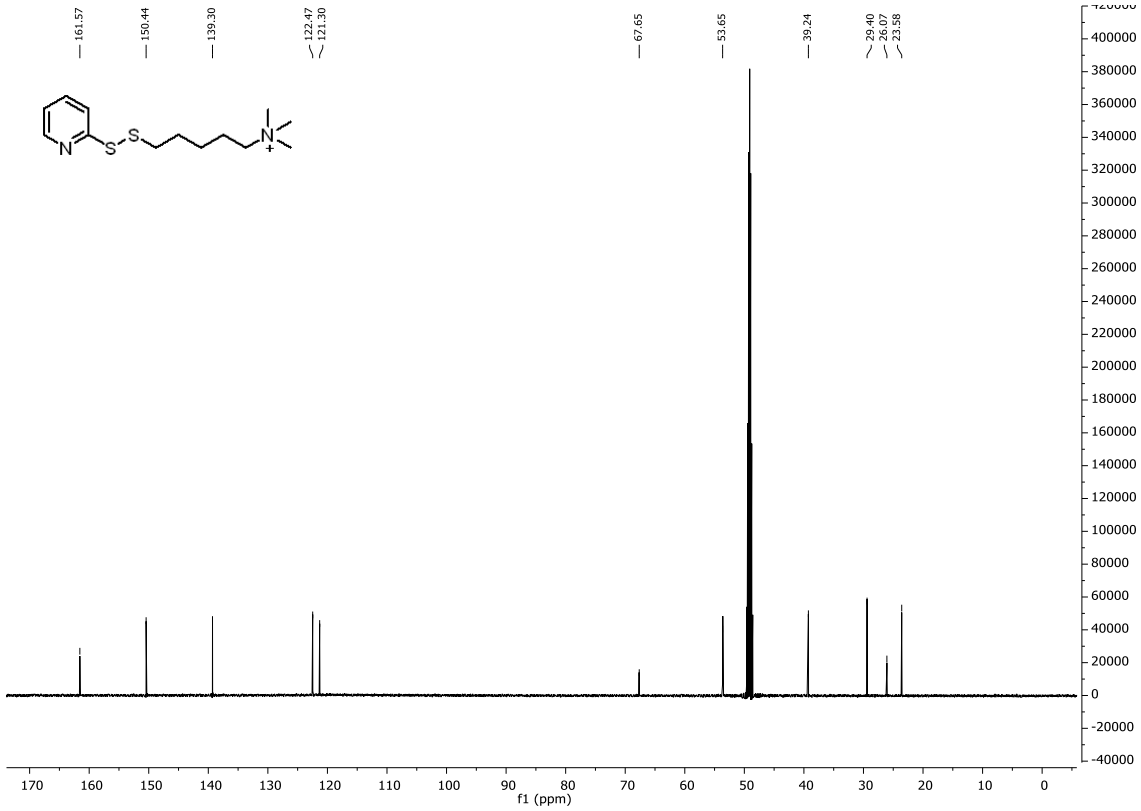
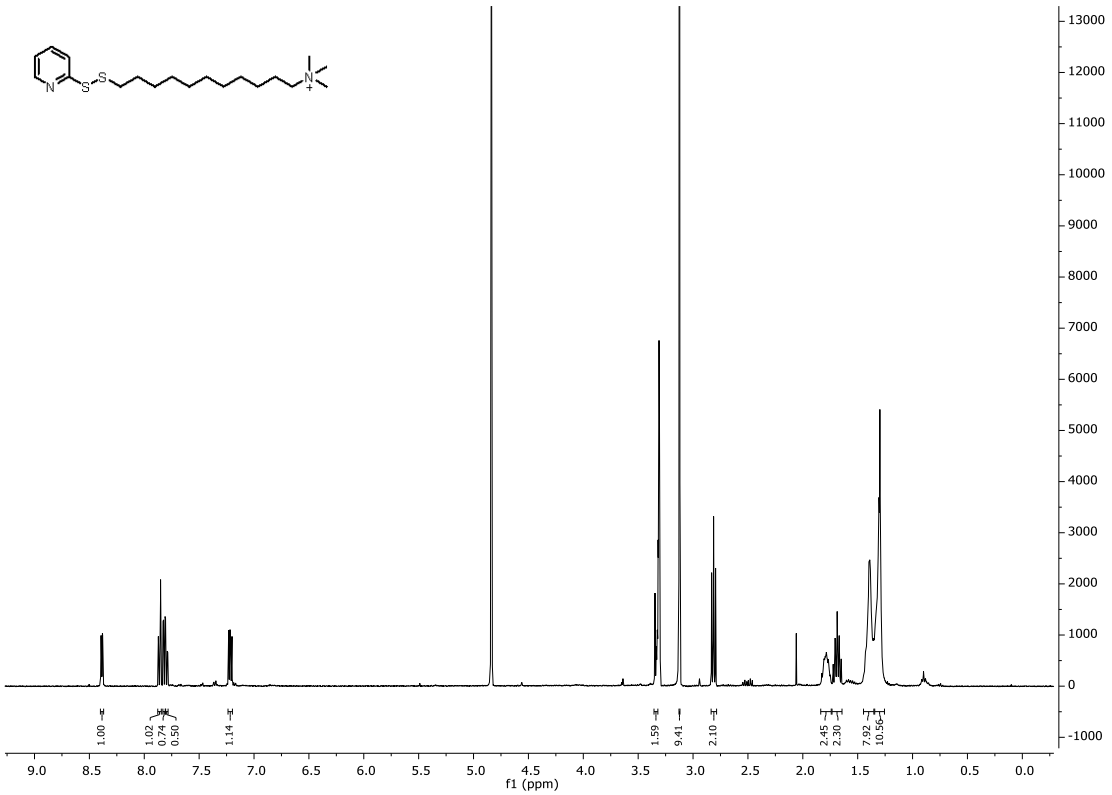


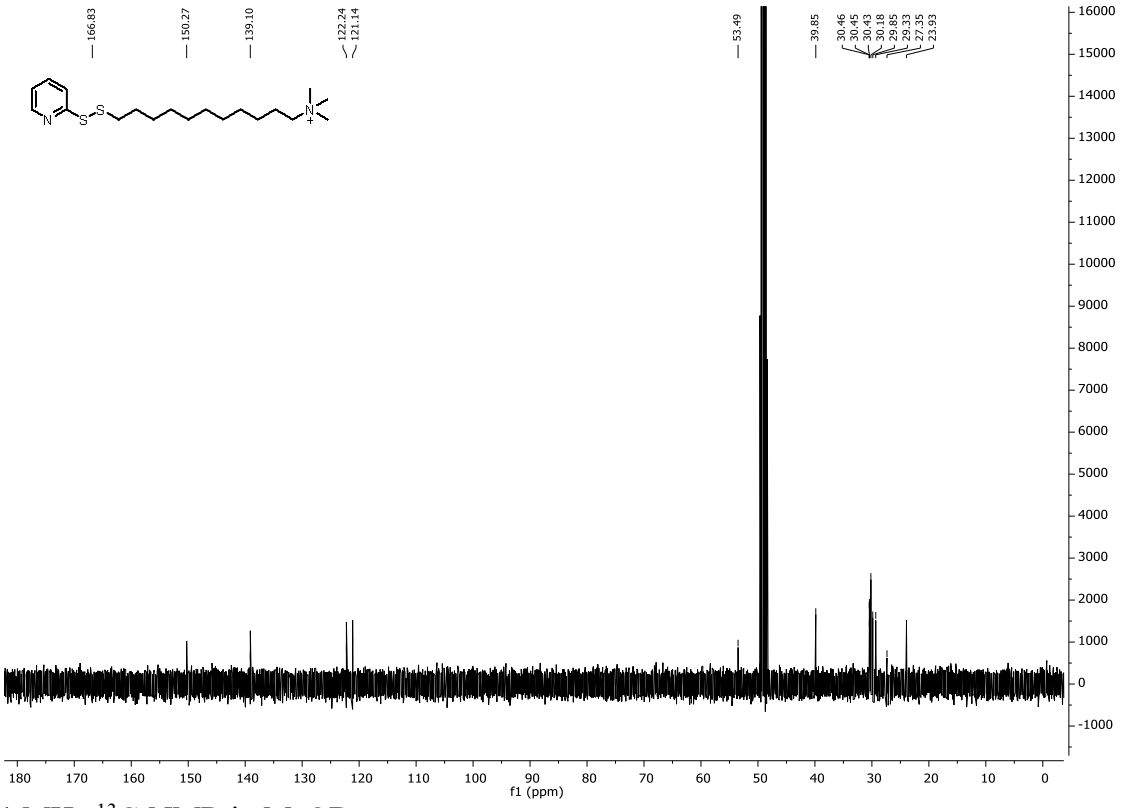
500 MHz ^{1}H-NMR in MeOD



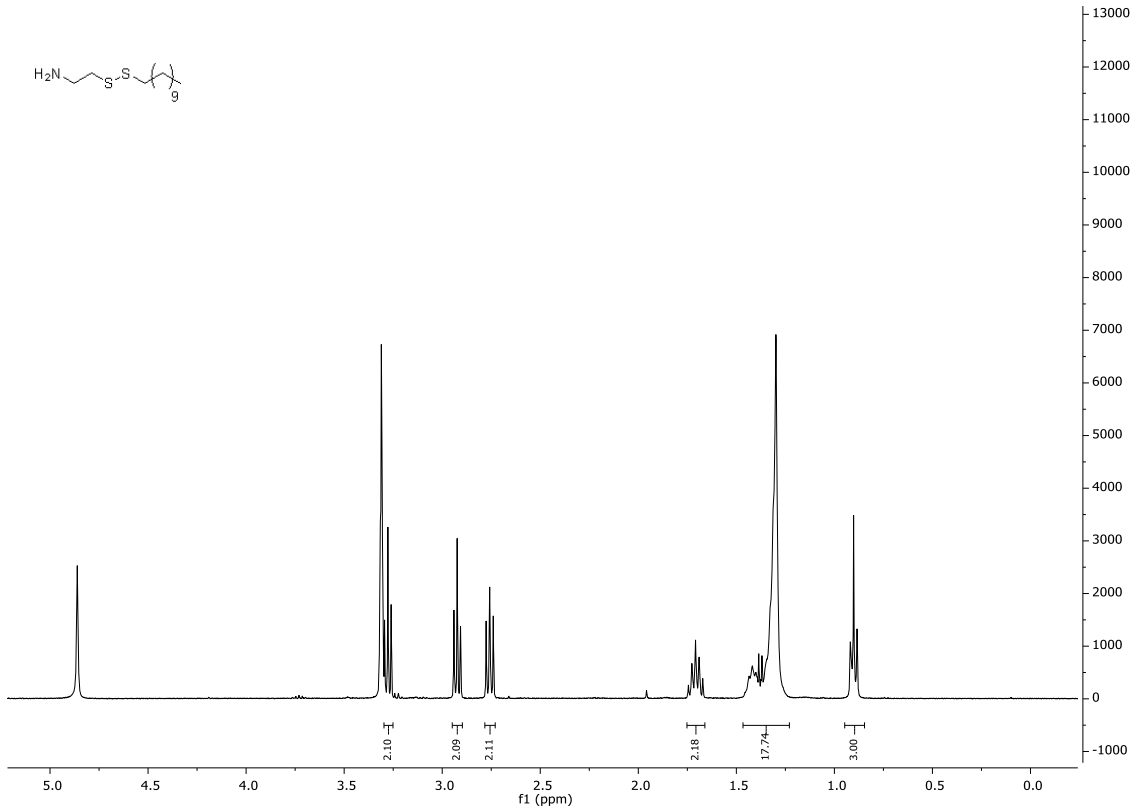
126 MHz ^{13}C-NMR in MeOD



400 MHz ^{1}H-NMR in MeOD



101 MHz ^{13}C-NMR in MeOD



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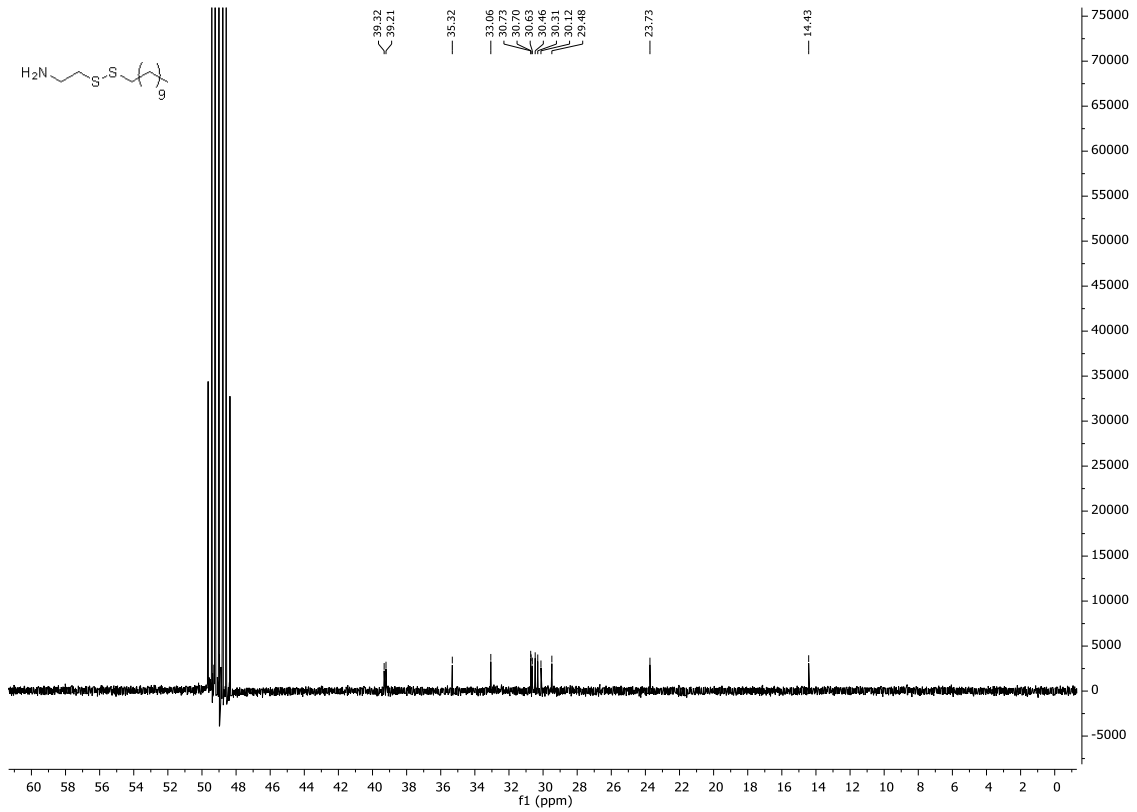

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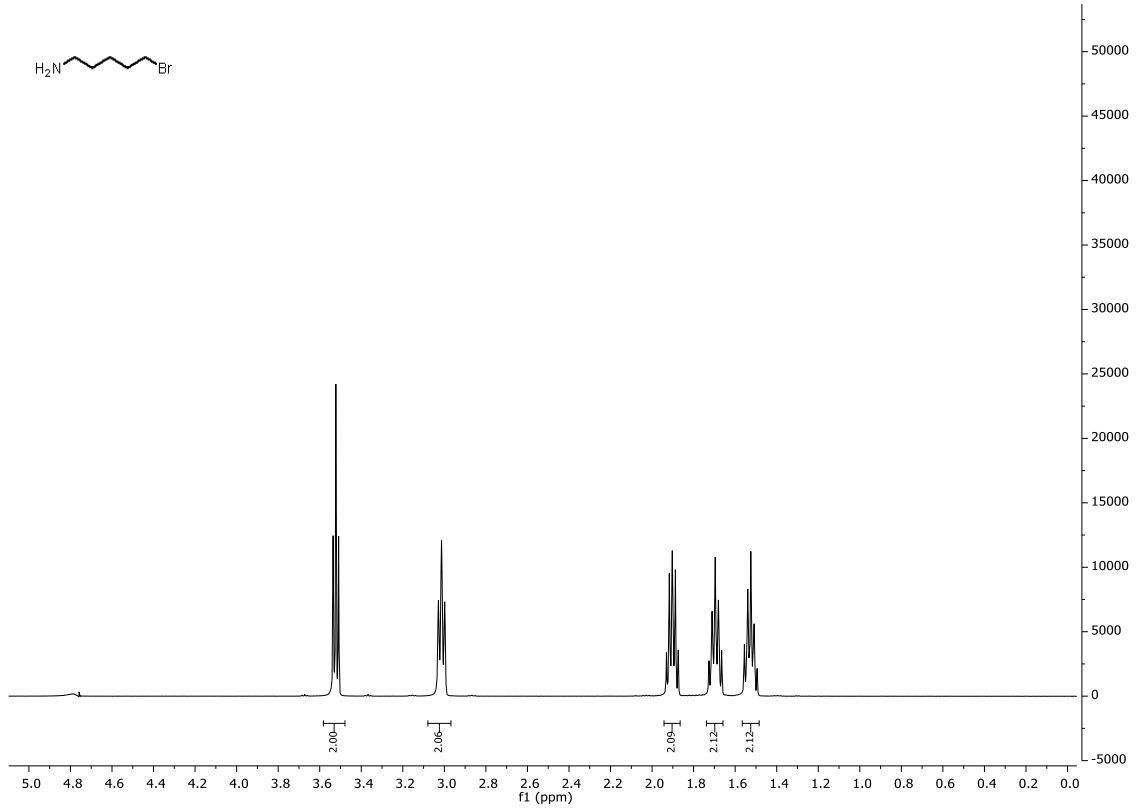
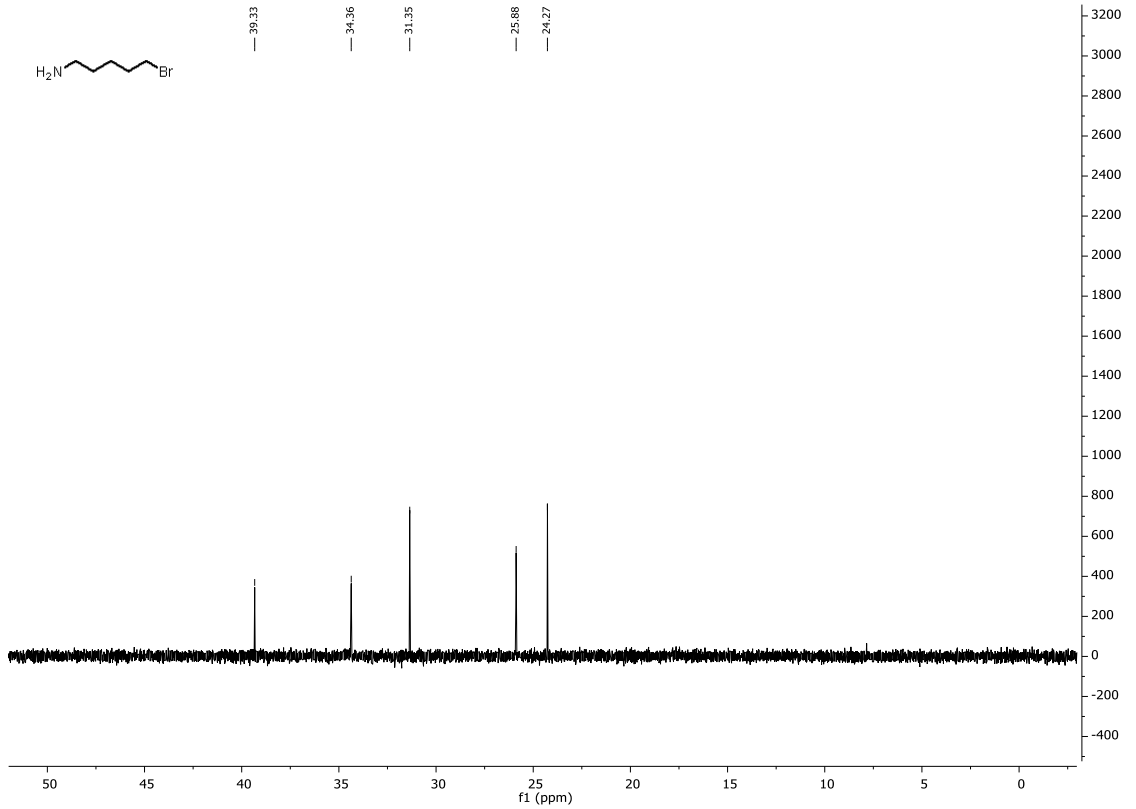
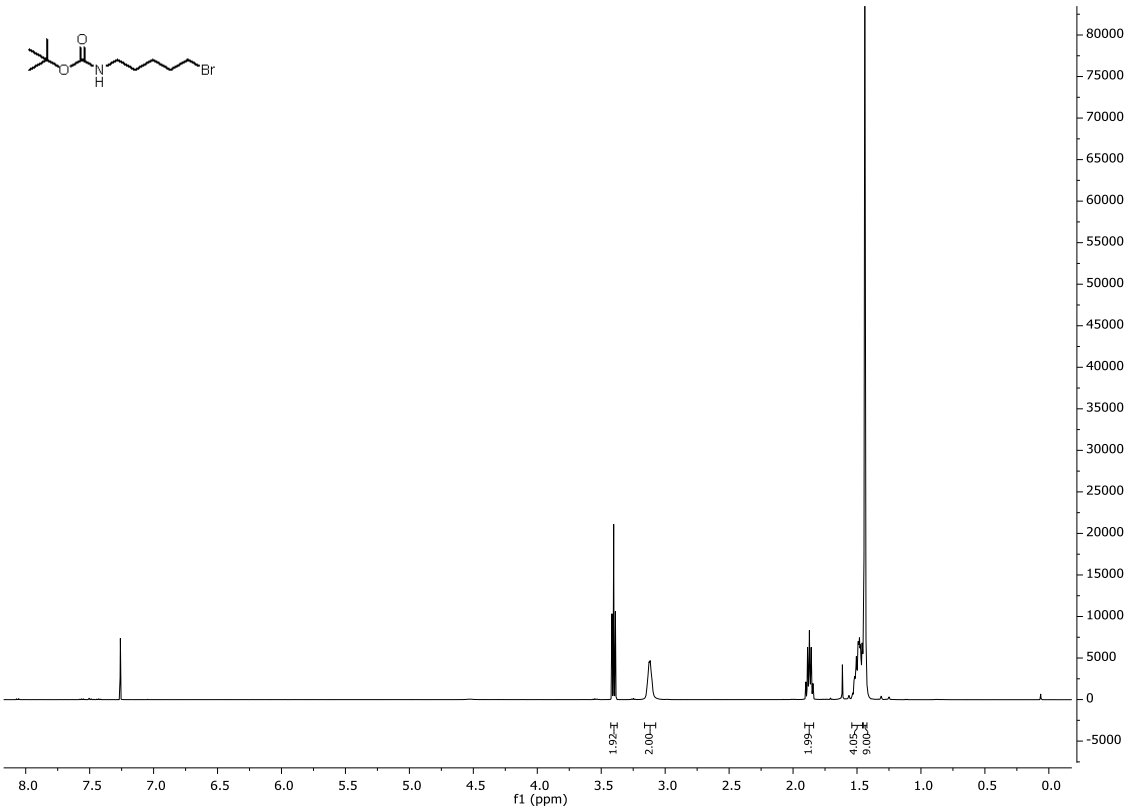


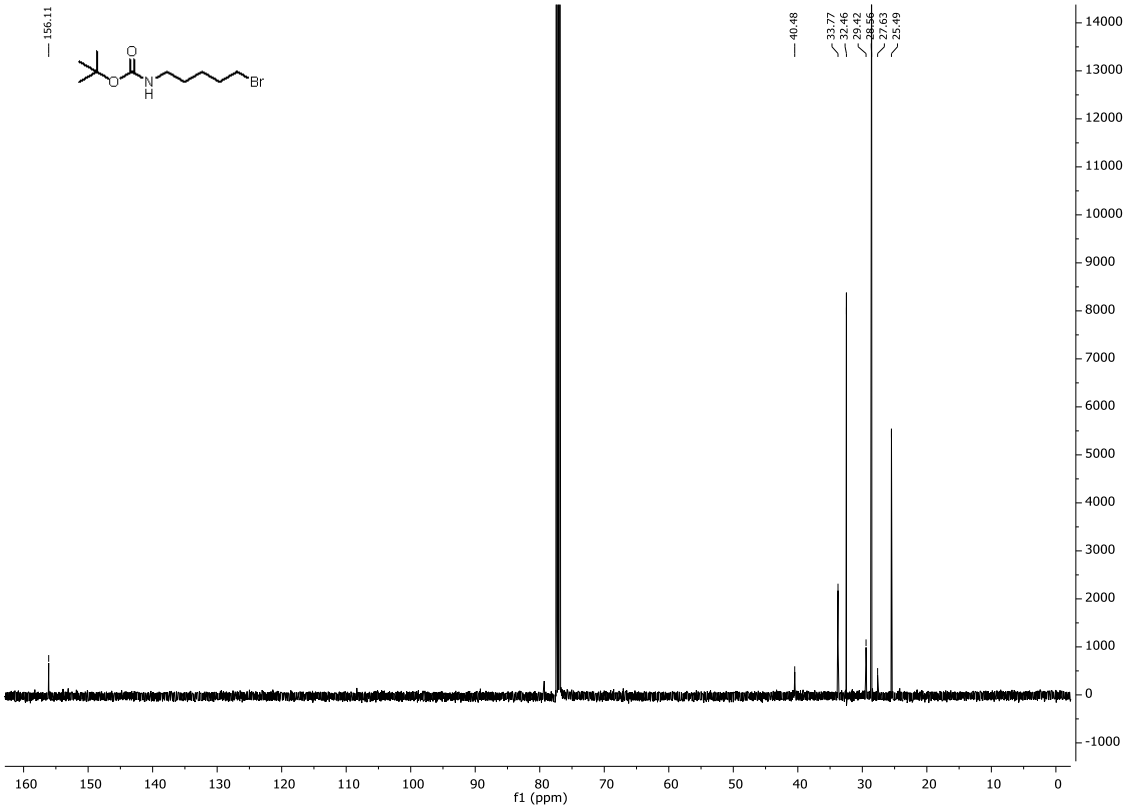
500 MHz ^{1}H-NMR in D_2O



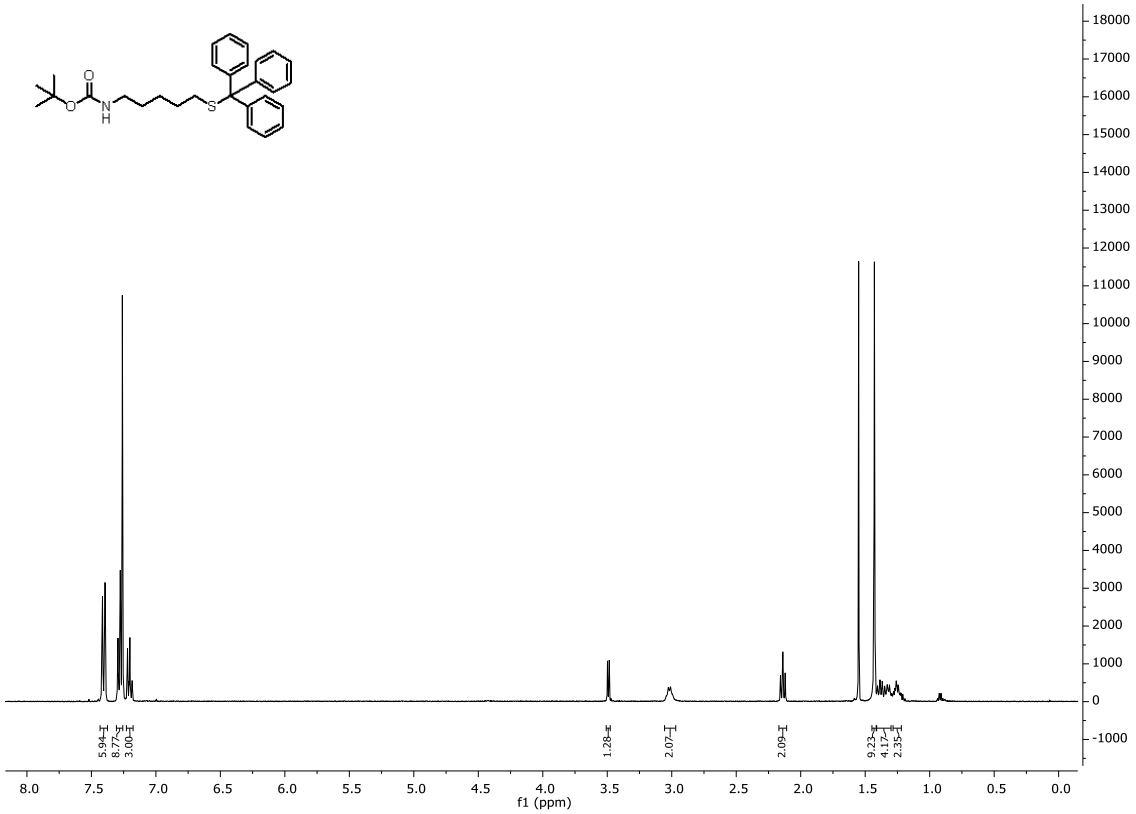
126 MHz ^{13}C-NMR in D_2O



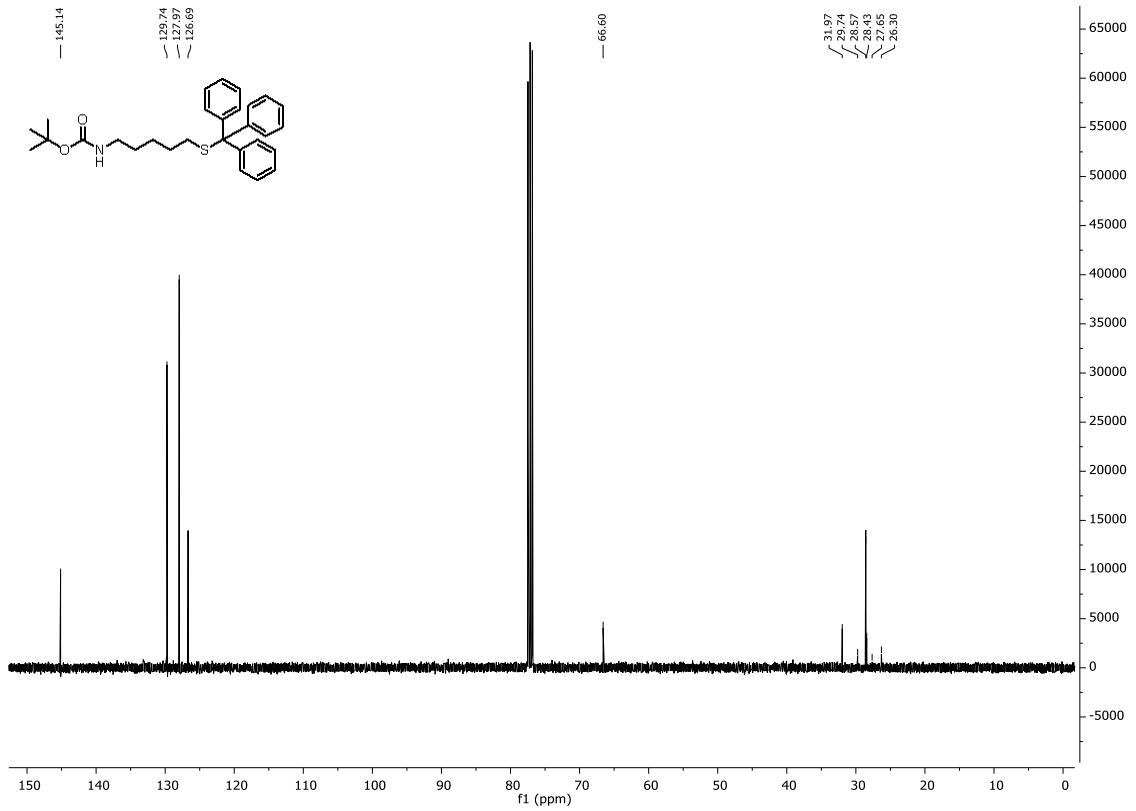
500 MHz ^{1}H-NMR in $CDCl_3$



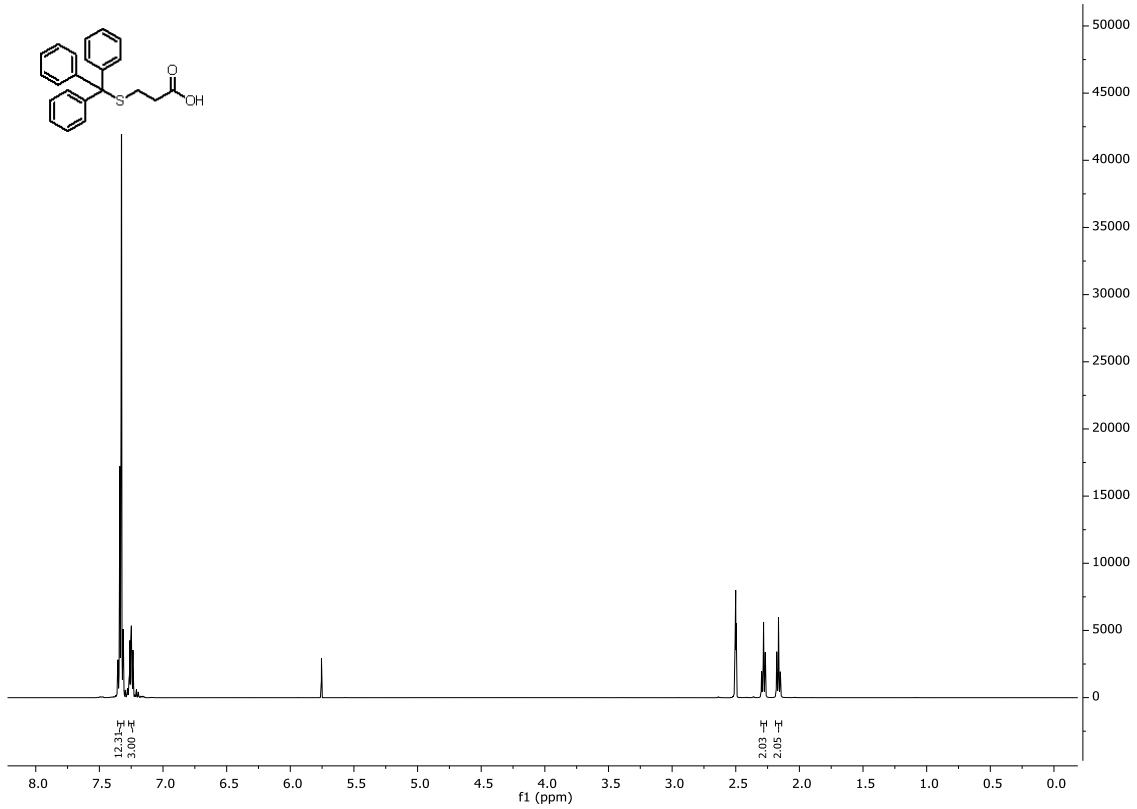
126 MHz ^{13}C-NMR in $CDCl_3$



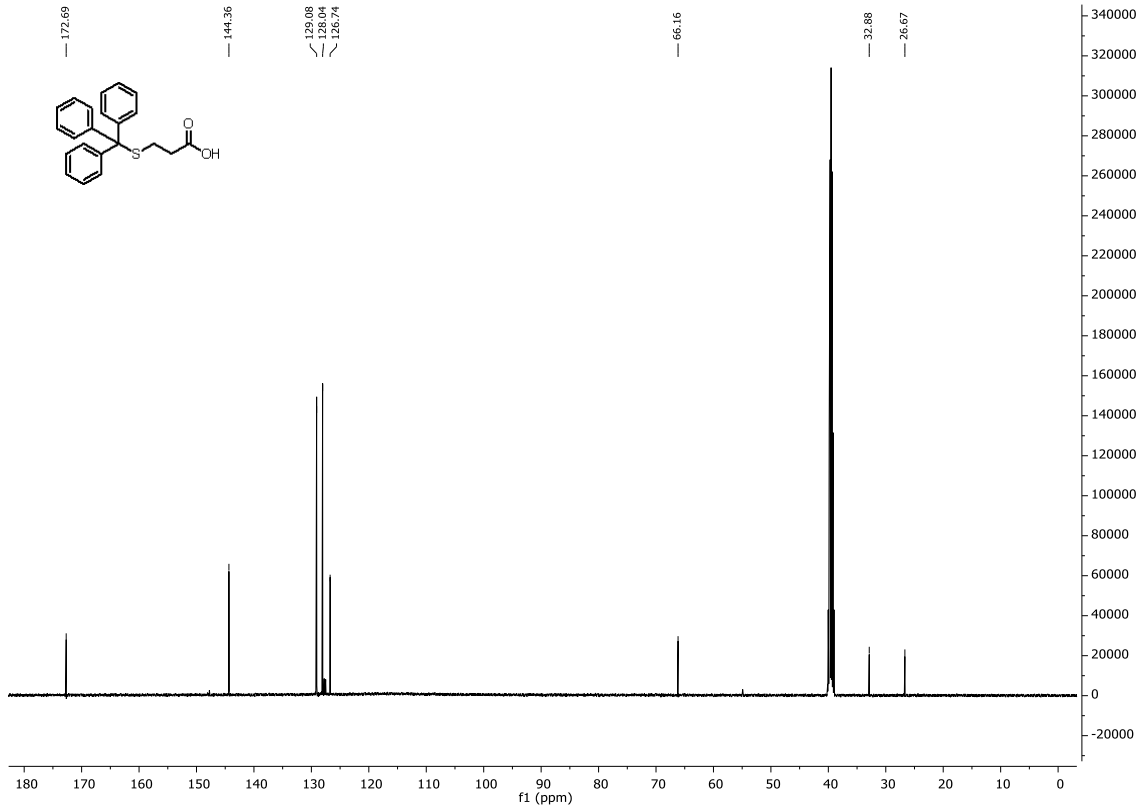
400 MHz ^{1}H-NMR in $CDCl_3$



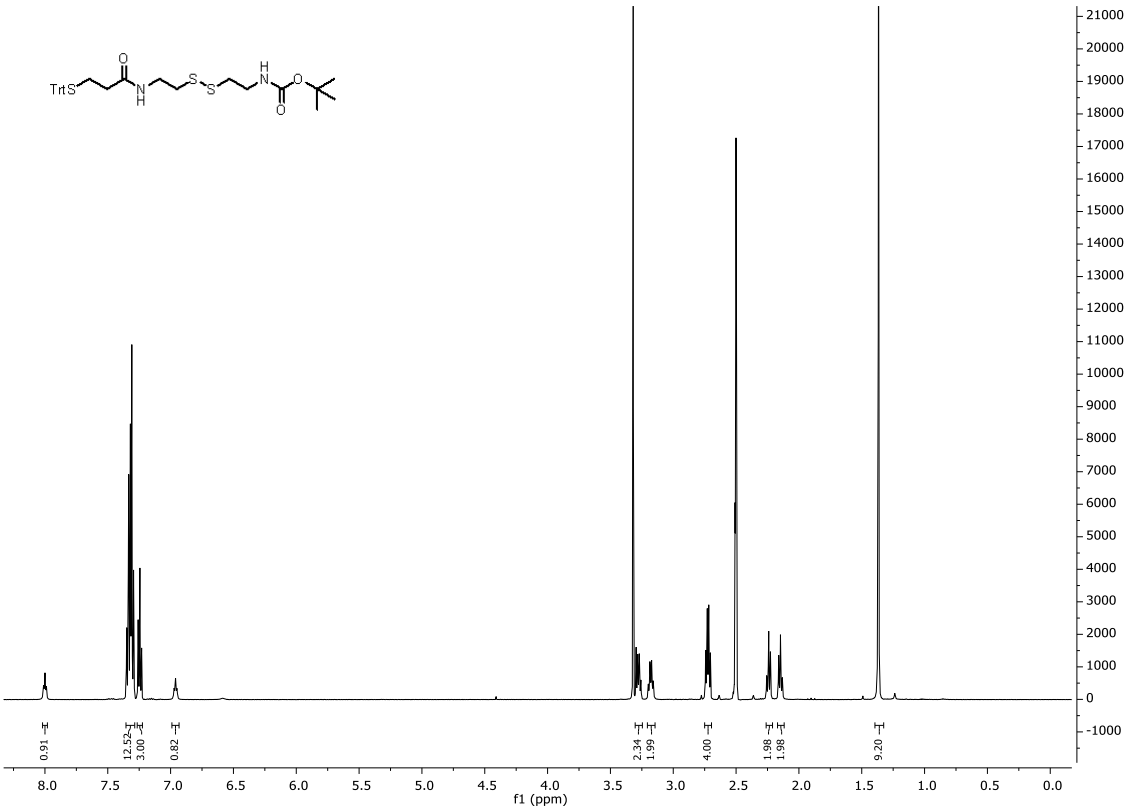
101 MHz ^{13}C-NMR in $CDCl_3$



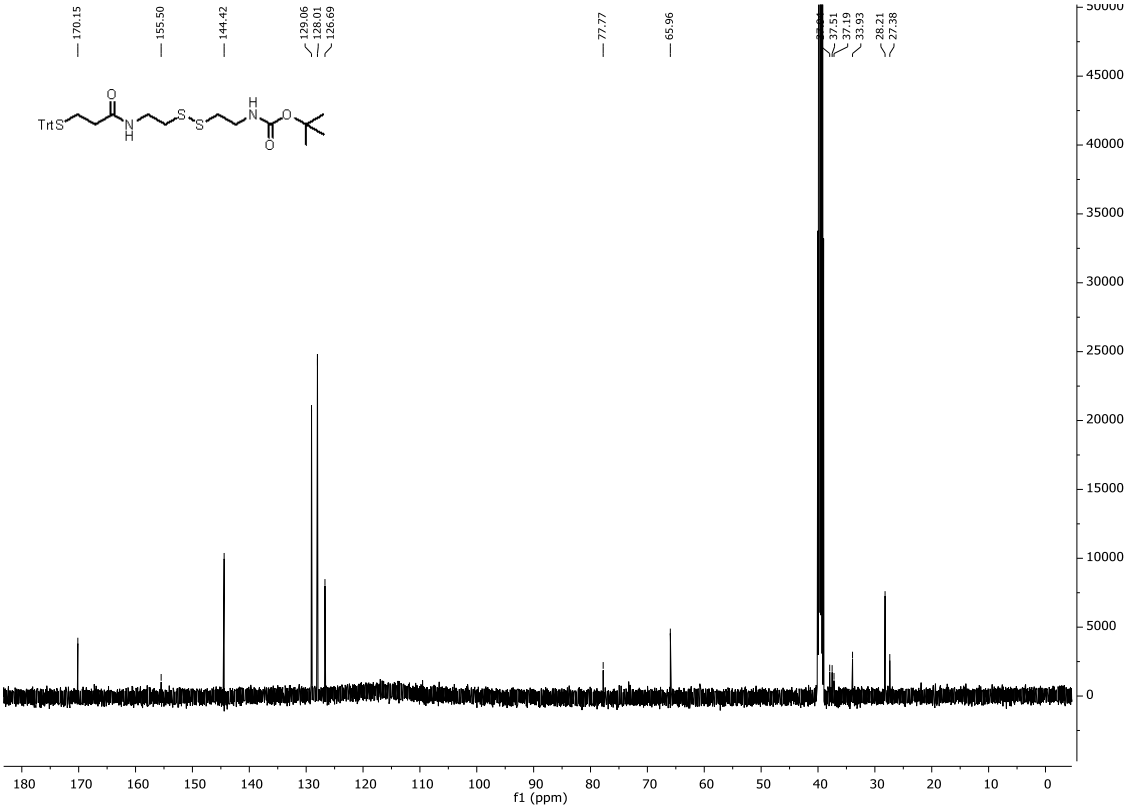
500 MHz ^{1}H-NMR in DMSO-d_6



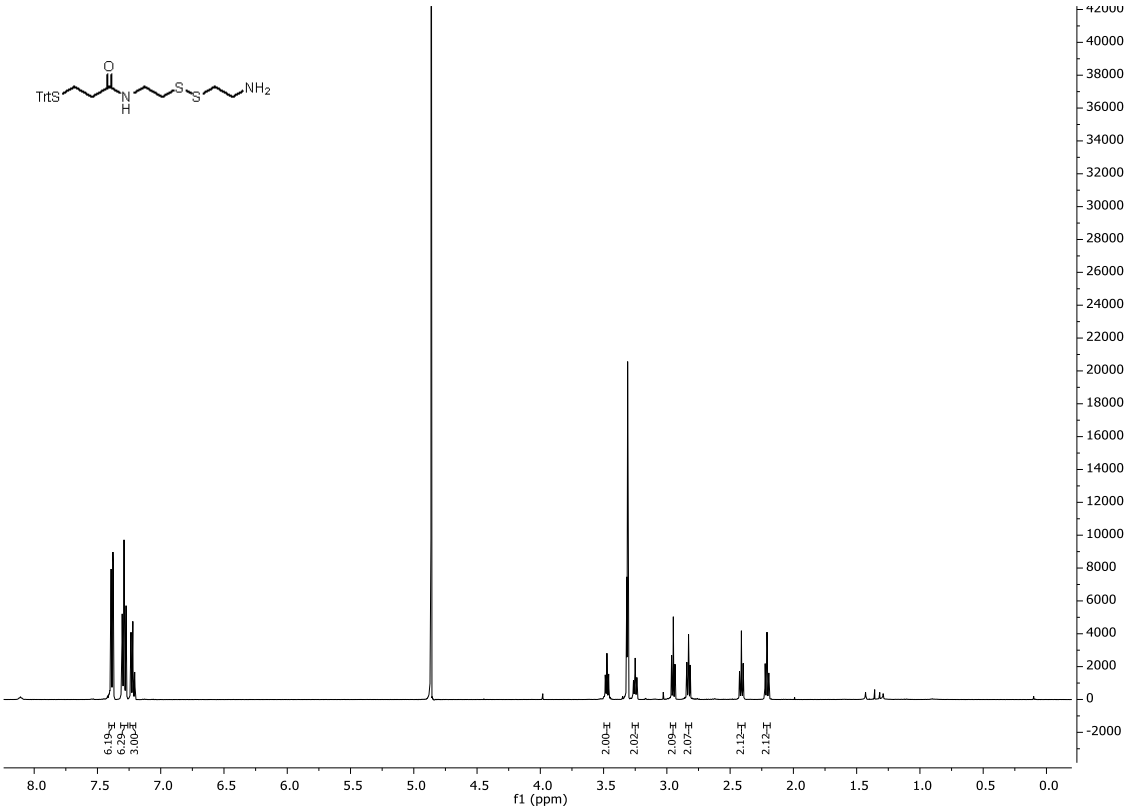
126 MHz ^{13}C-NMR in DMSO-d_6



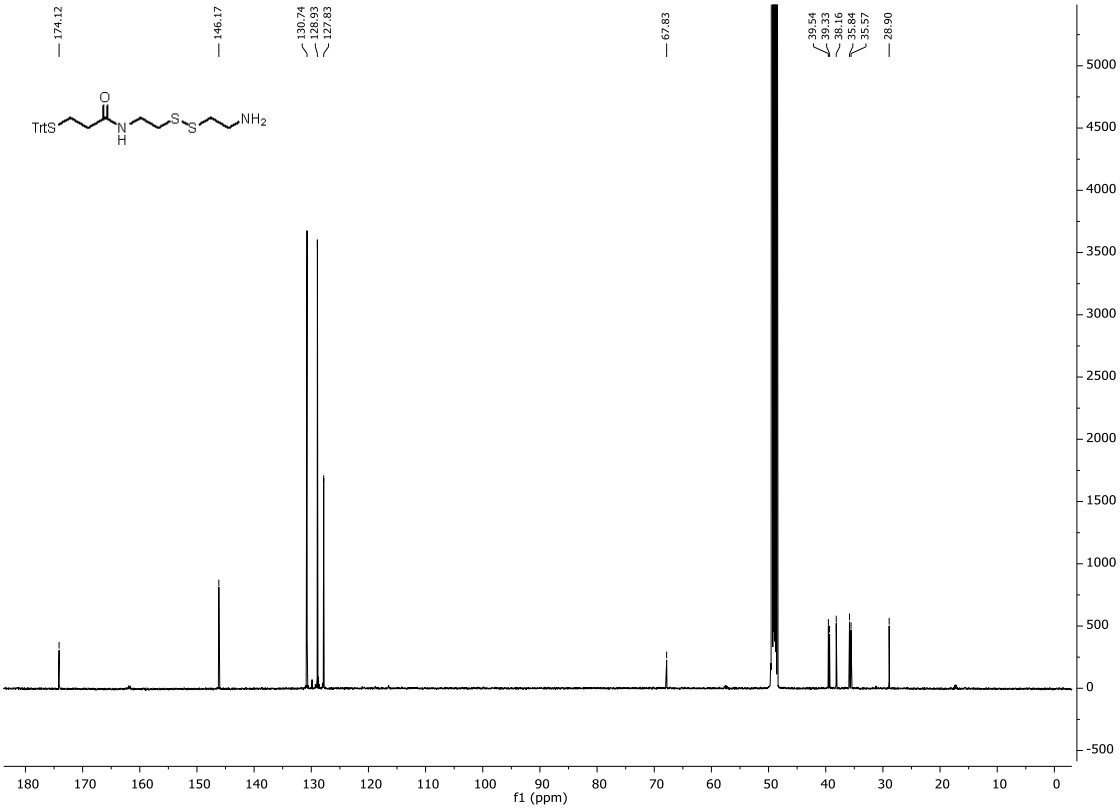
500 MHz ^{1}H-NMR in DMSO-d_6



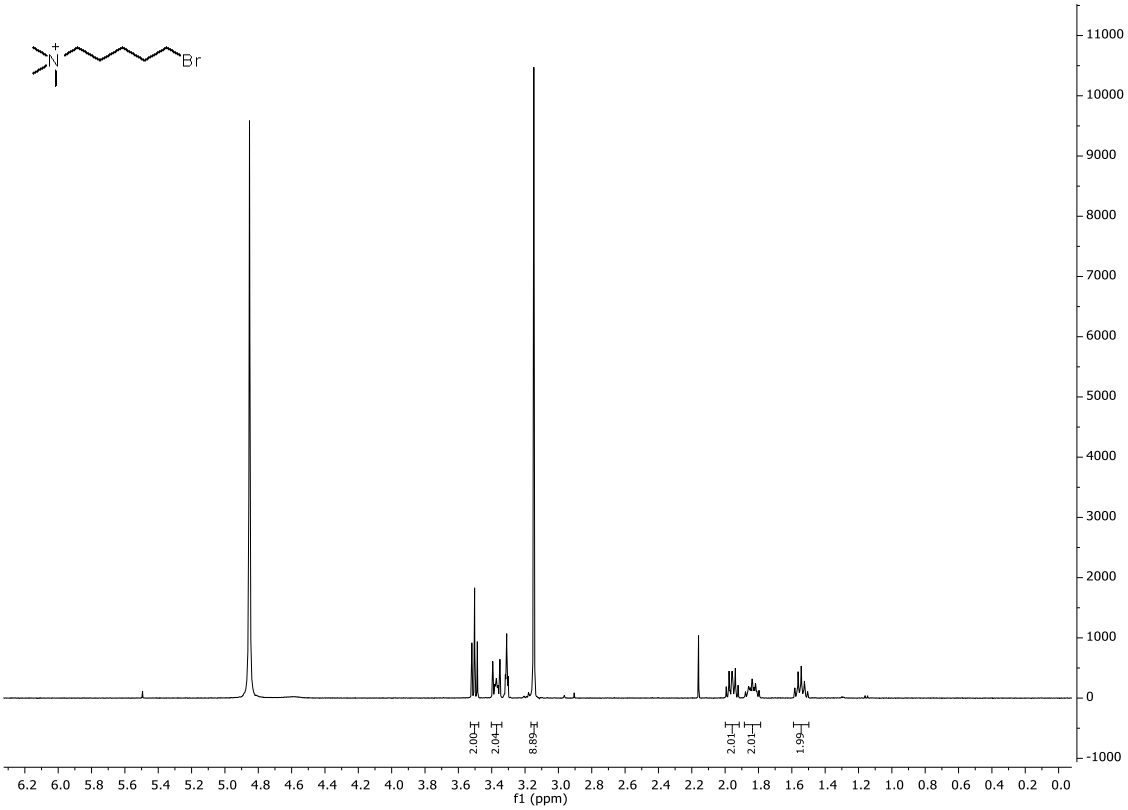
126 MHz ^{13}C-NMR in DMSO-d_6



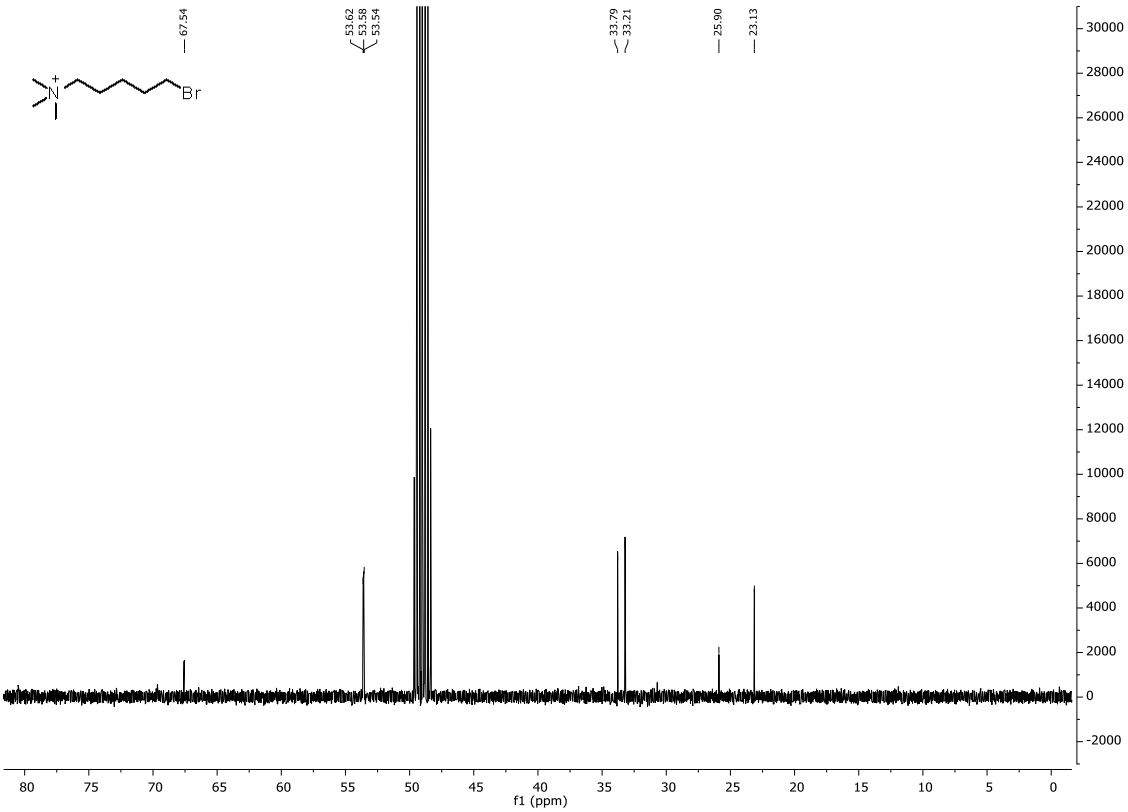
500 MHz ^{1}H-NMR in MeOD



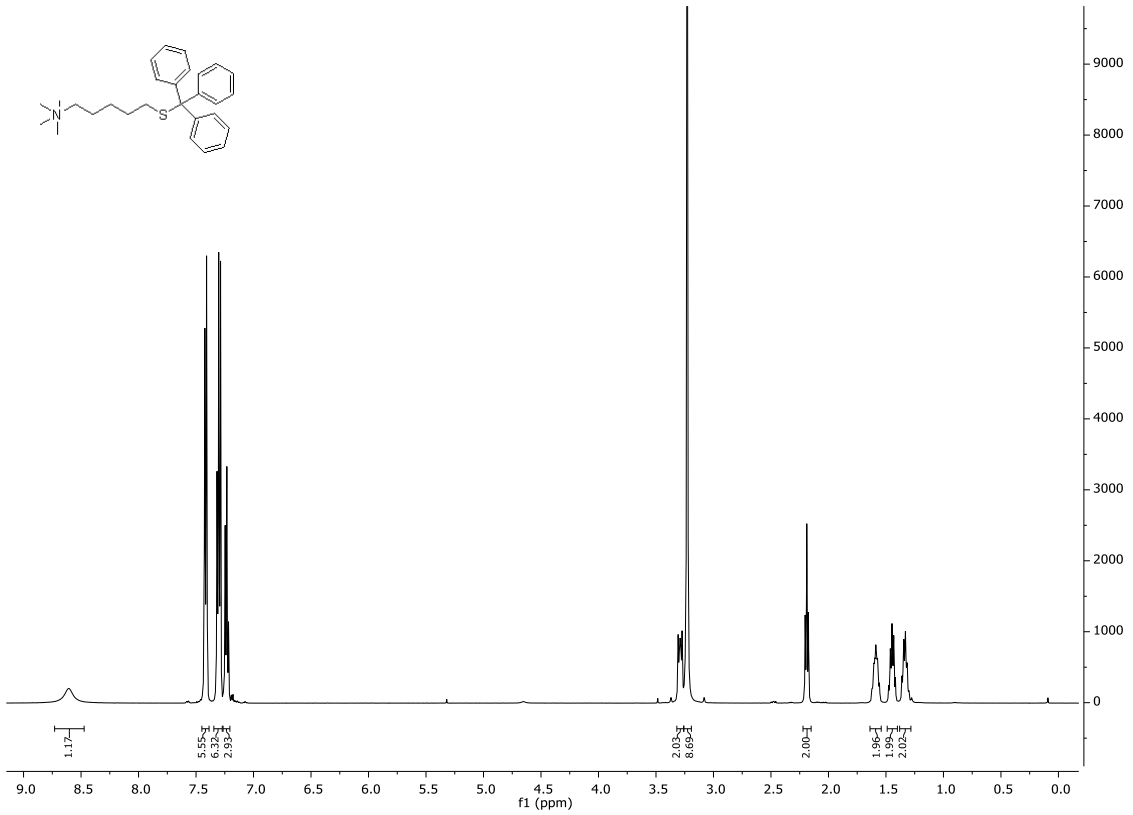
126 MHz ^{13}C-NMR in MeOD



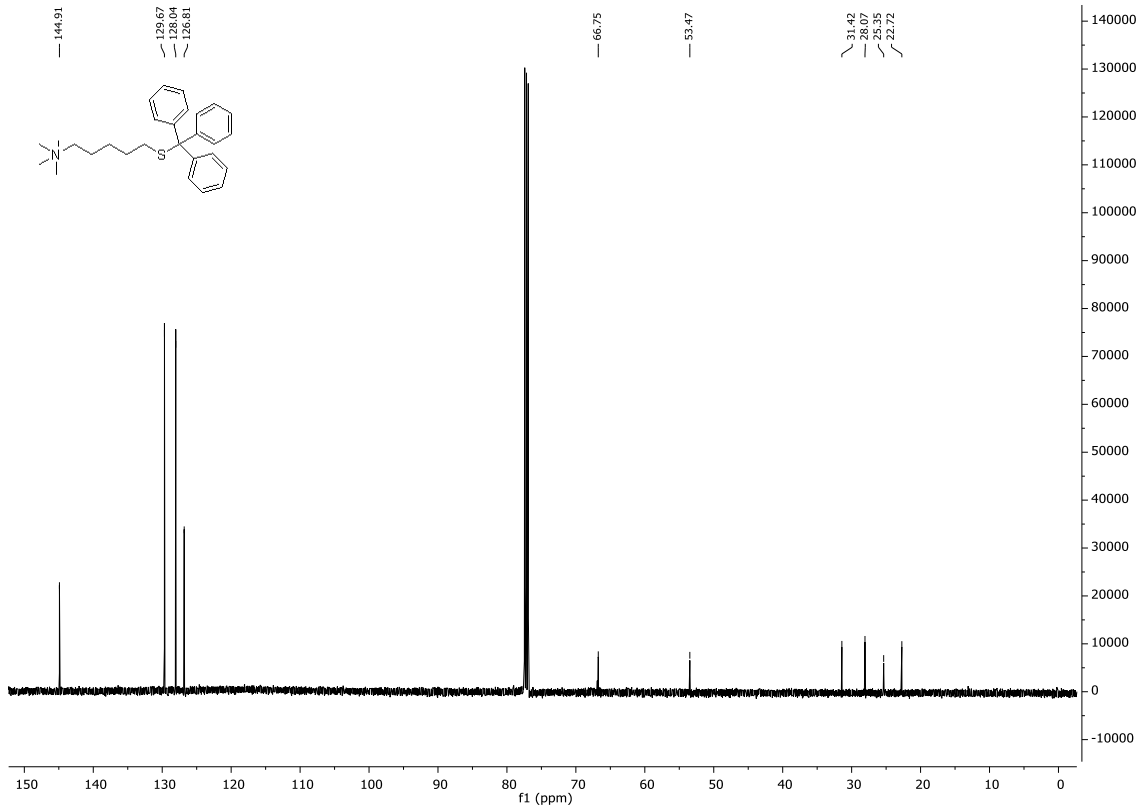
500 MHz ^{1}H-NMR in MeOD



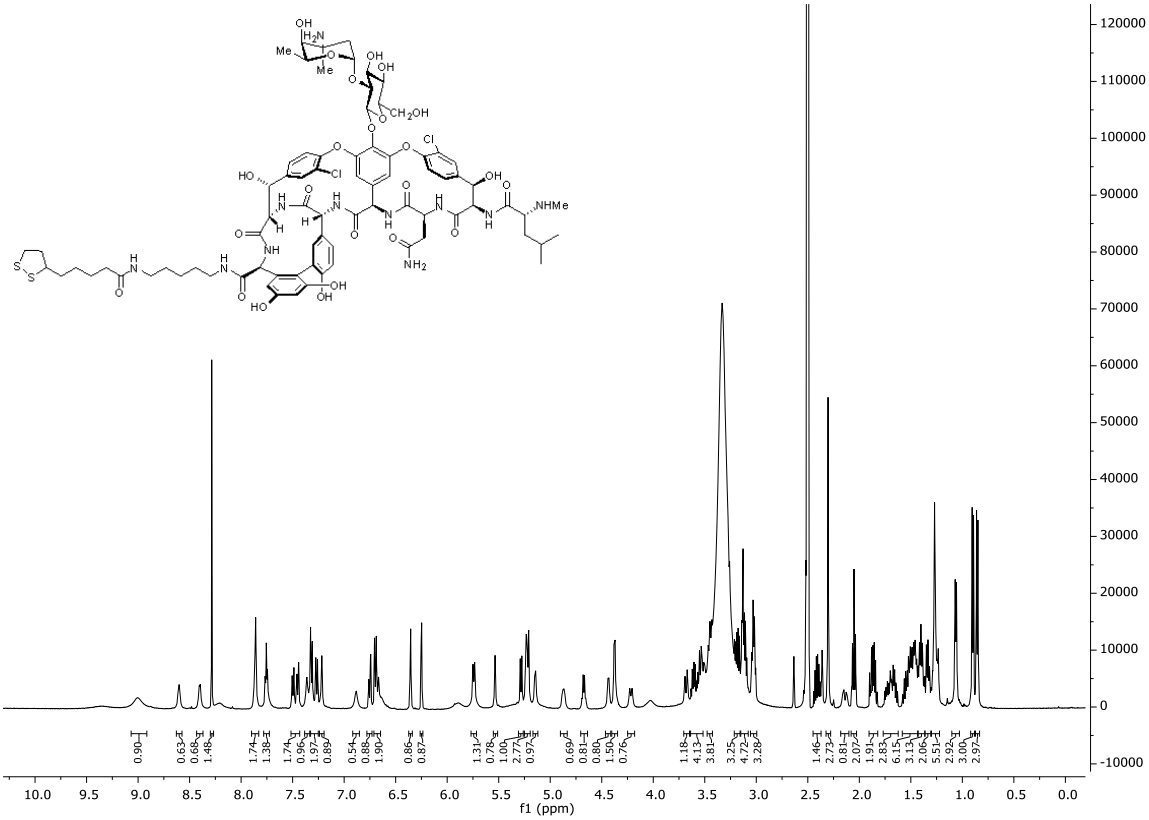
126 MHz ^{13}C-NMR in MeOD



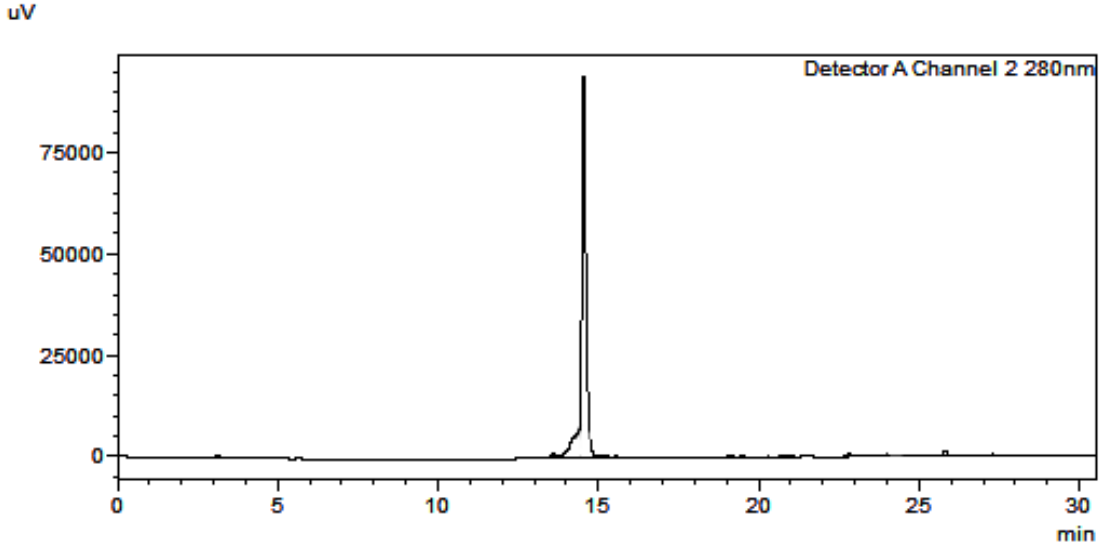
500 MHz ^{1}H-NMR in $CDCl_3$



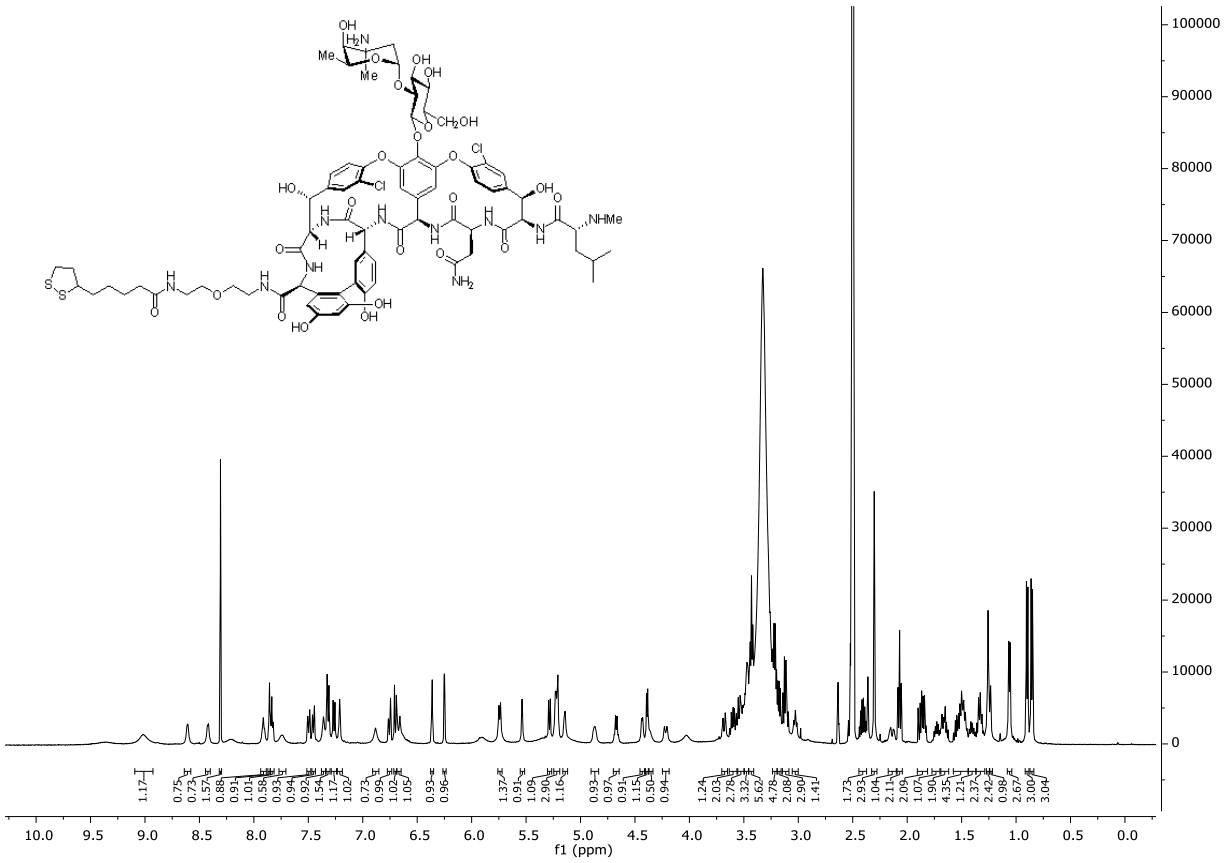
126 MHz ^{13}C-NMR in $CDCl_3$



500 MHz ^{1}H-NMR in DMSO-d_6 of **1a**



HPLC chromatogram of compound **1a**



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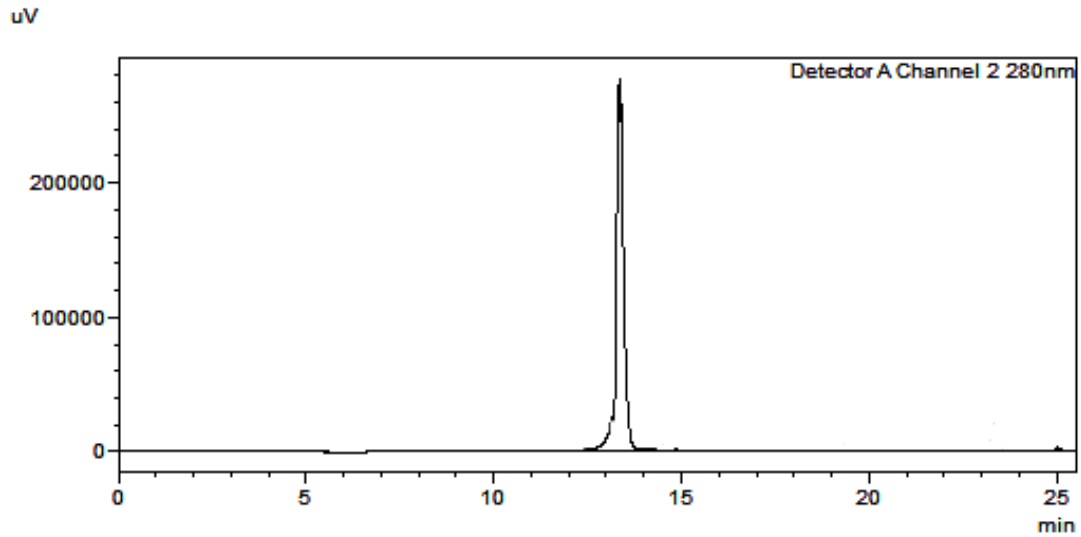

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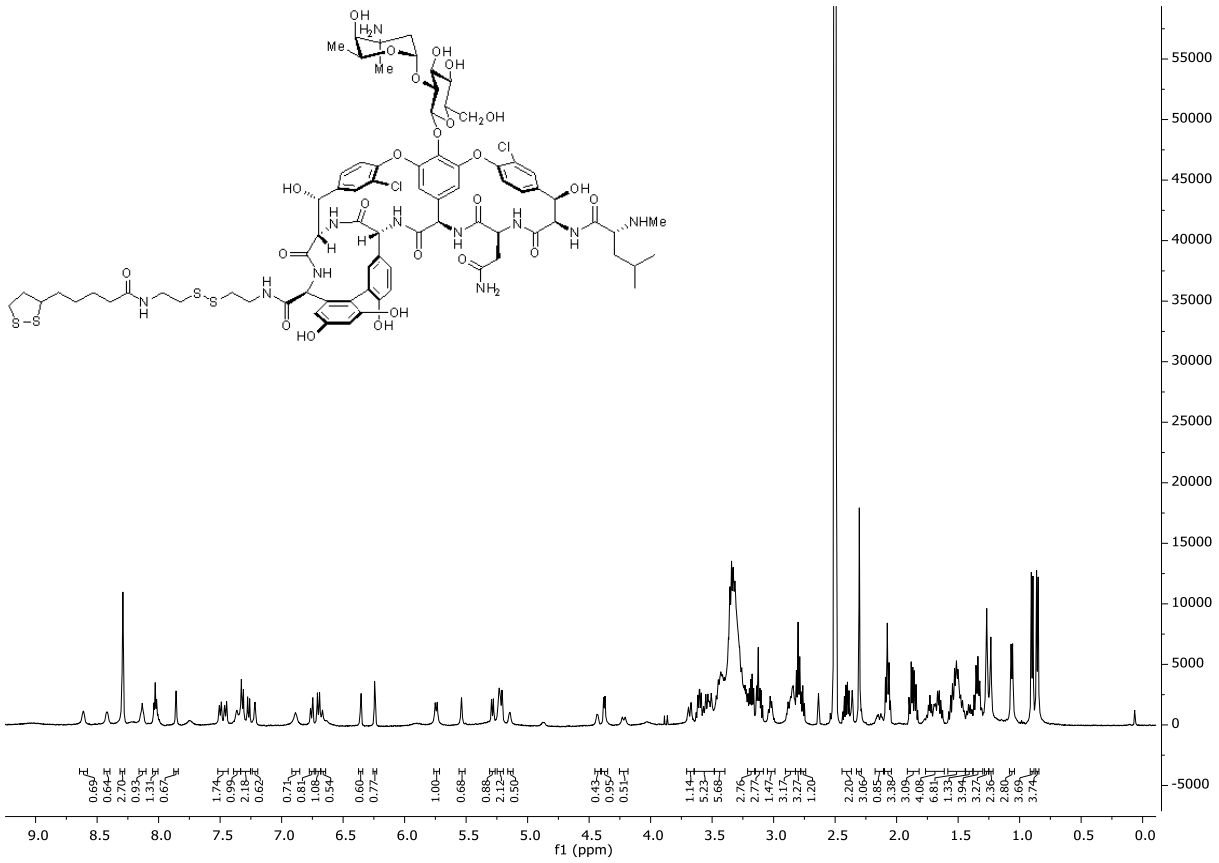
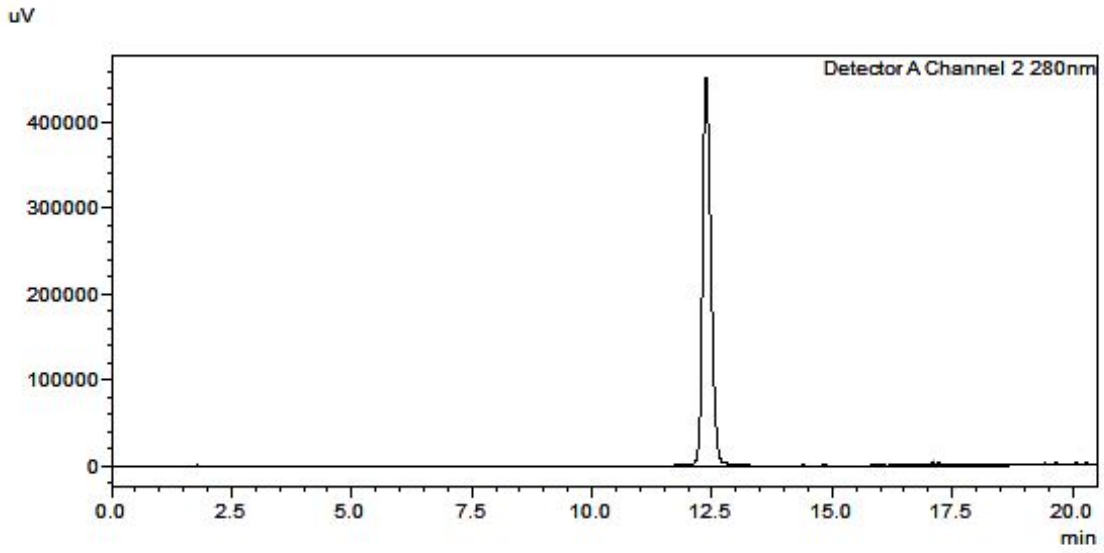
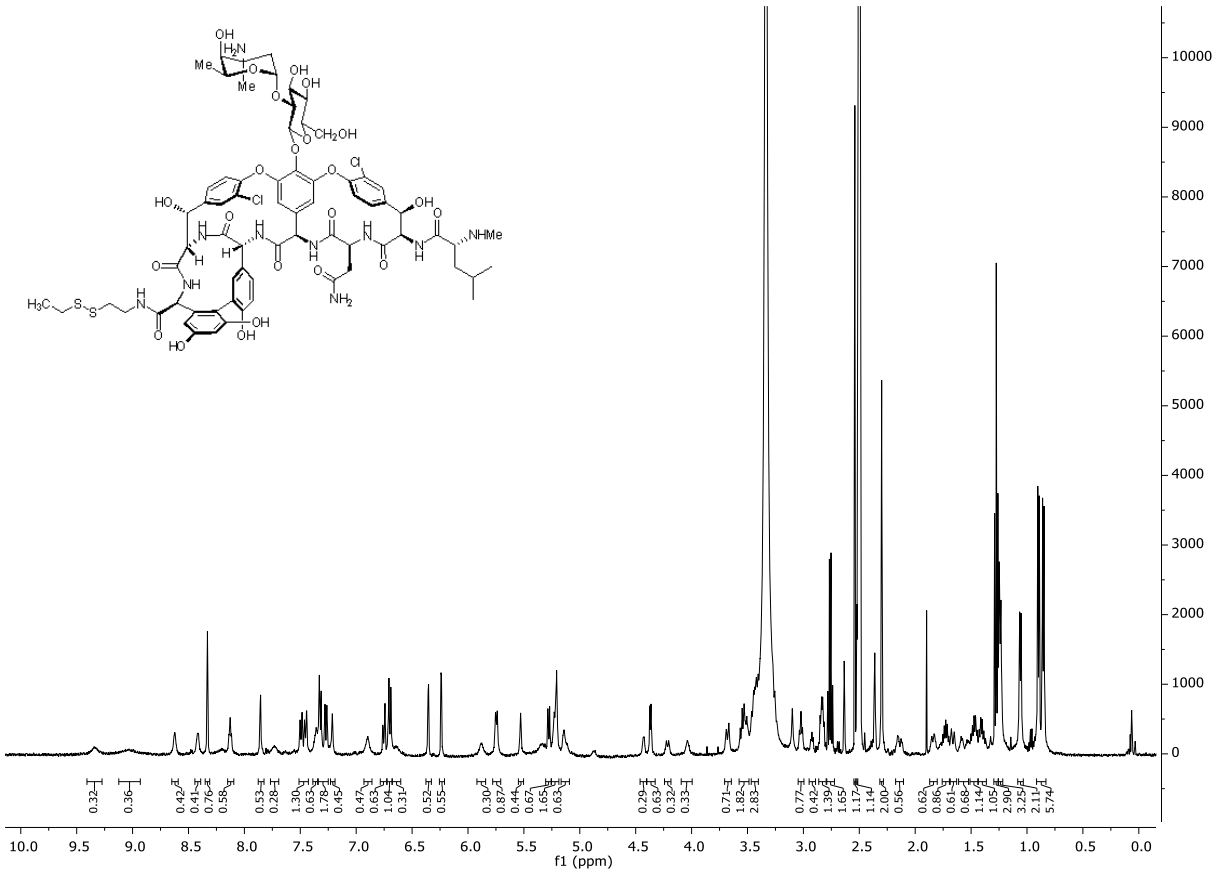


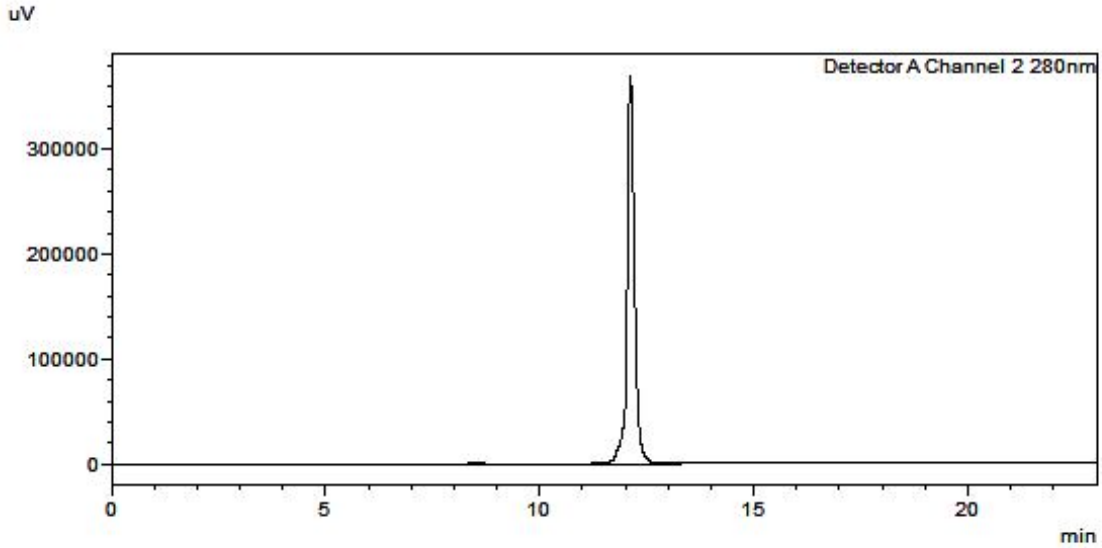
500 MHz ^{1}H-NMR in DMSO-d_6 of **1c**



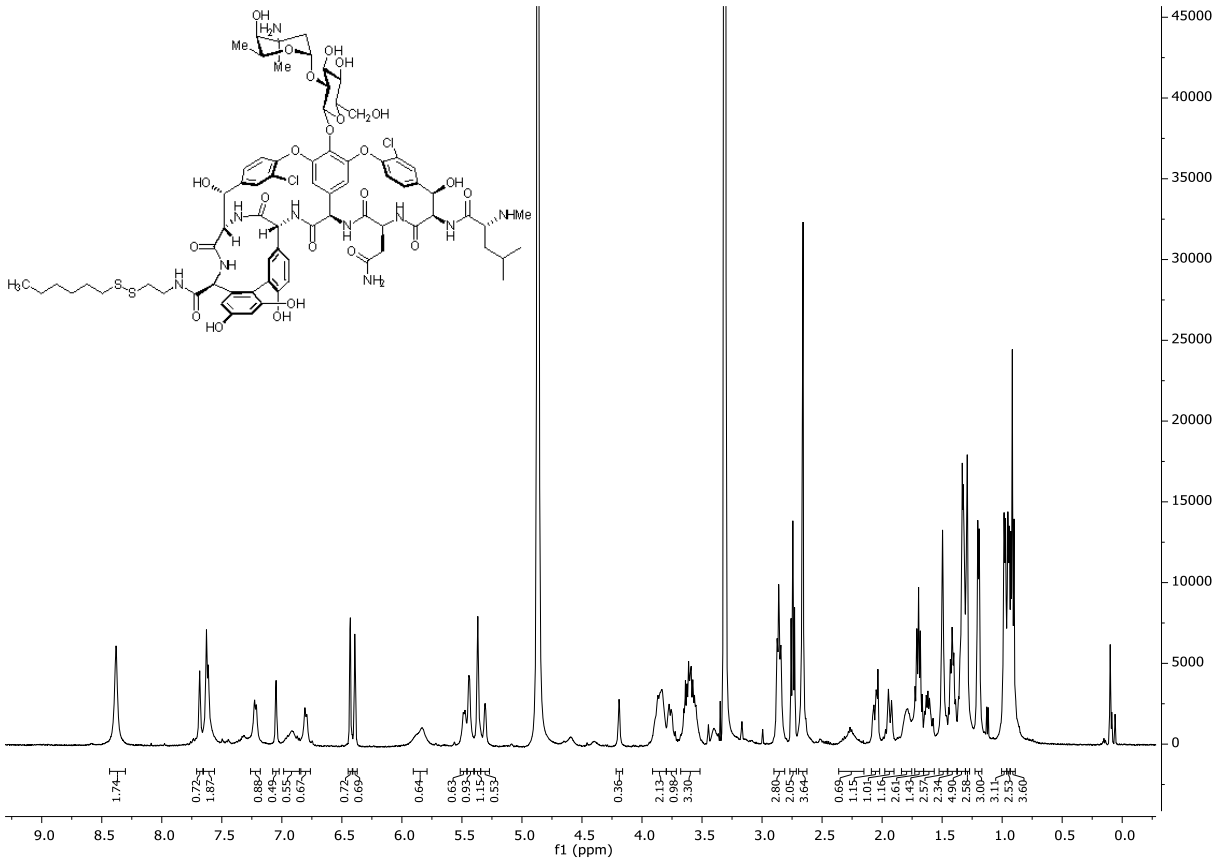
HPLC chromatogram of compound **1c**



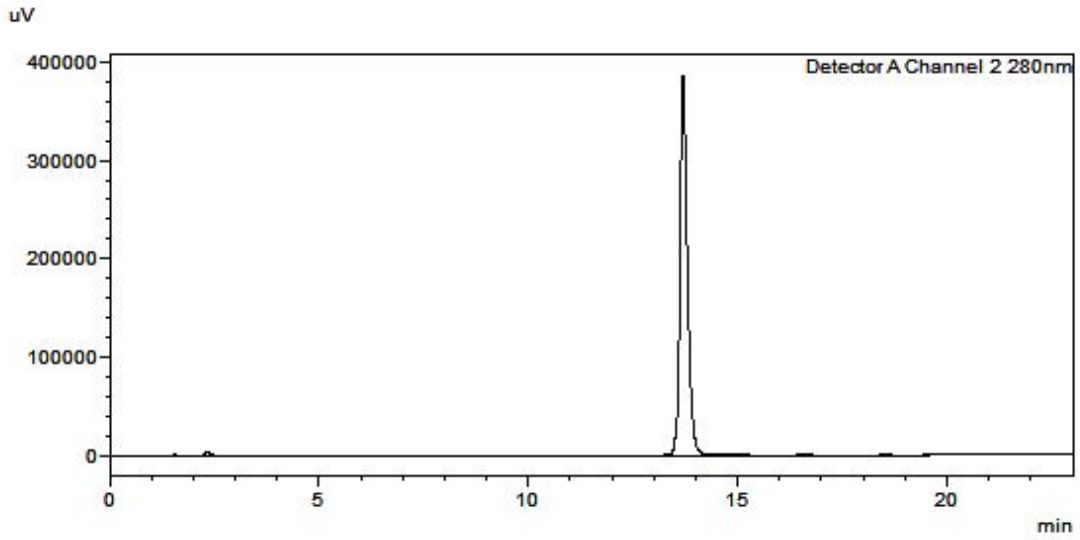
500 MHz ^{1}H-NMR in DMSO-d_6 of **2a**



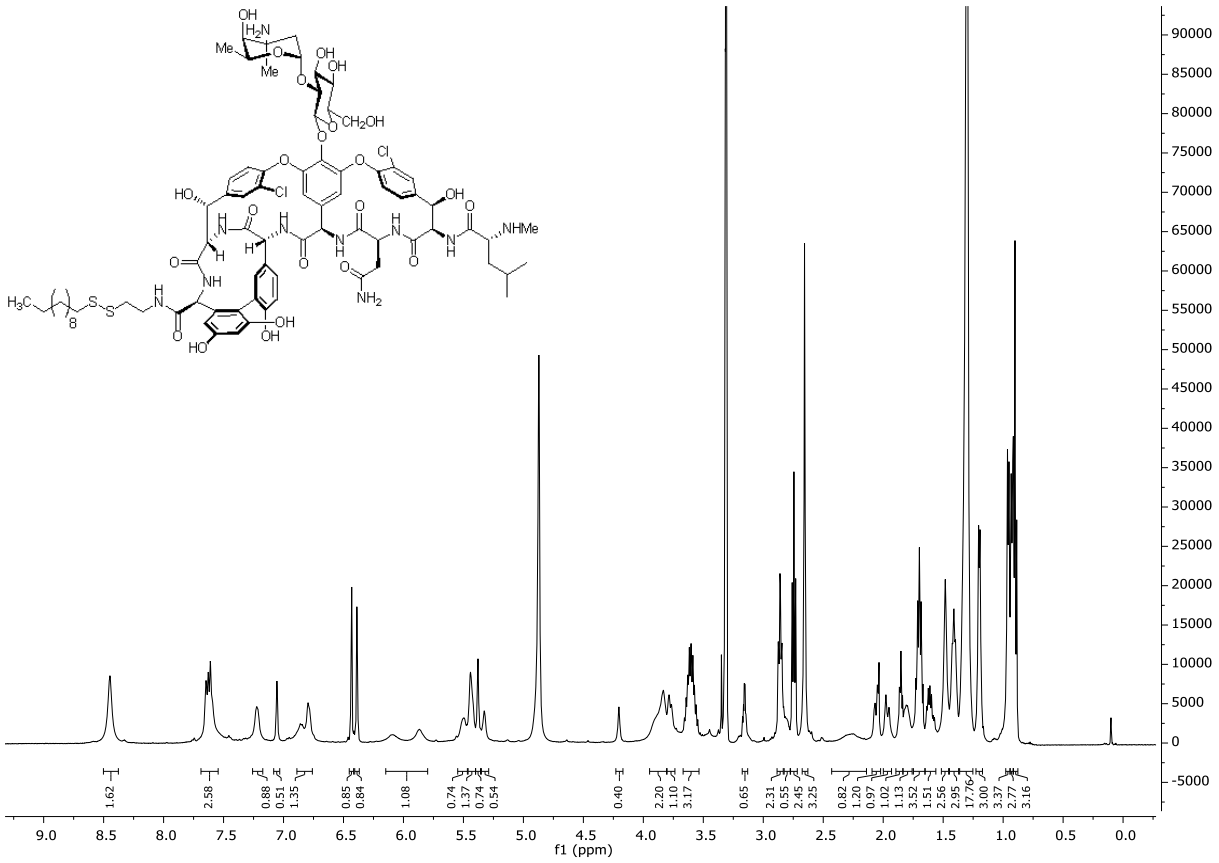
HPLC chromatogram of compound **2a**



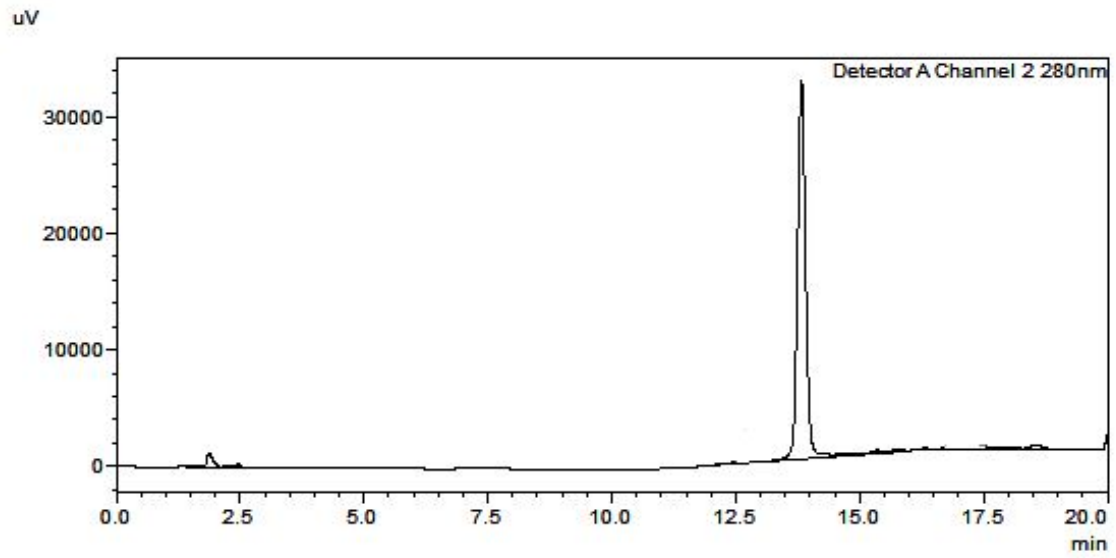
500 MHz ^{1}H-NMR in MeOD of **2b**



HPLC chromatogram of compound **2b**



500 MHz ^{1}H-NMR in MeOD of **2c**



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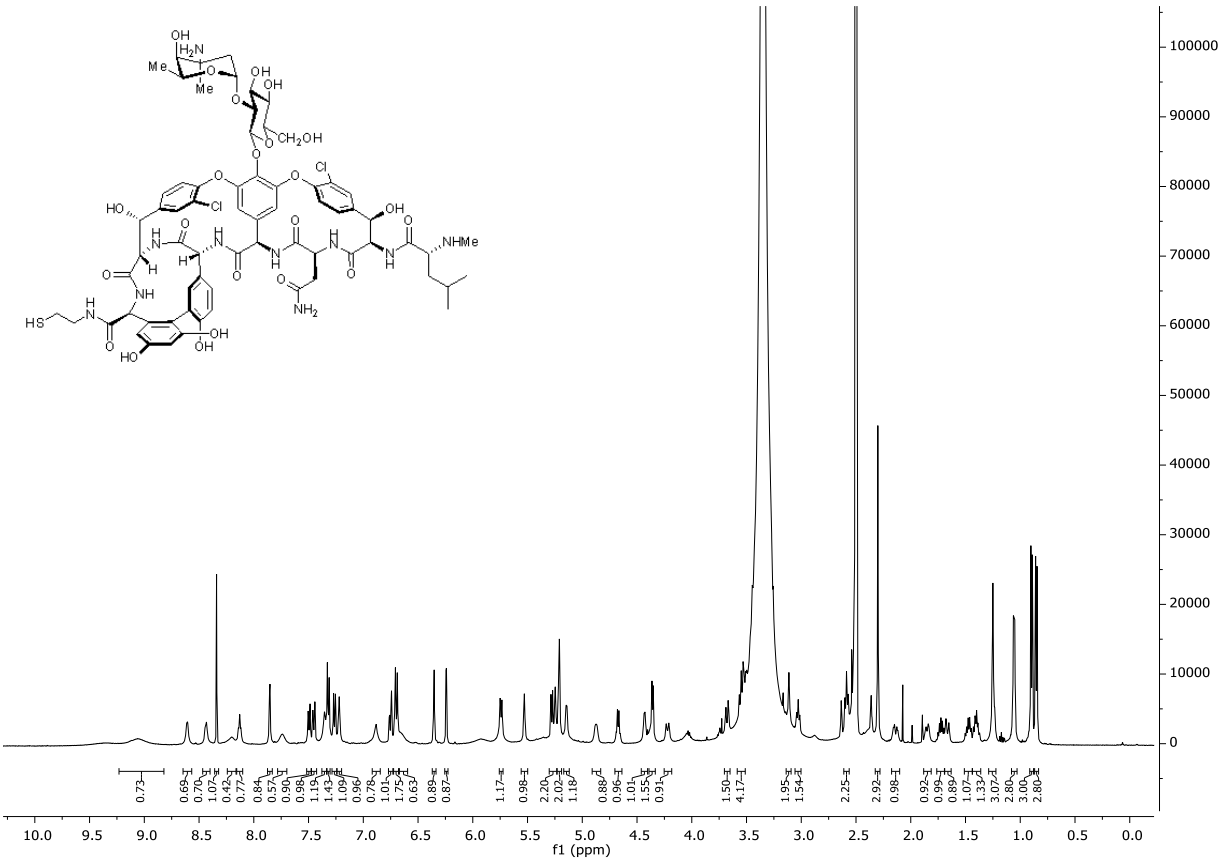
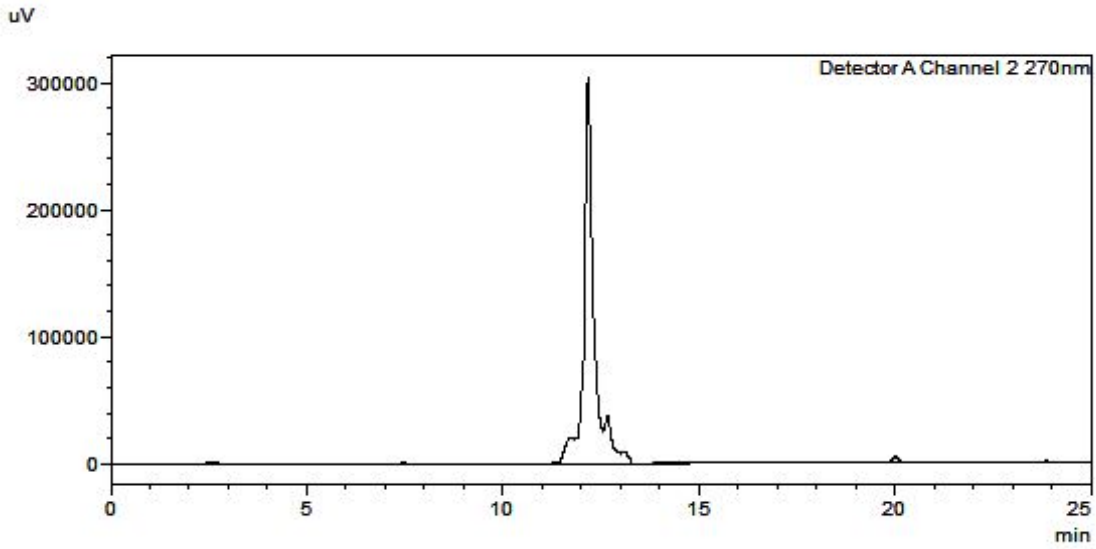


500 MHz ^{1}H-NMR in DMSO-d_6 of **4a**



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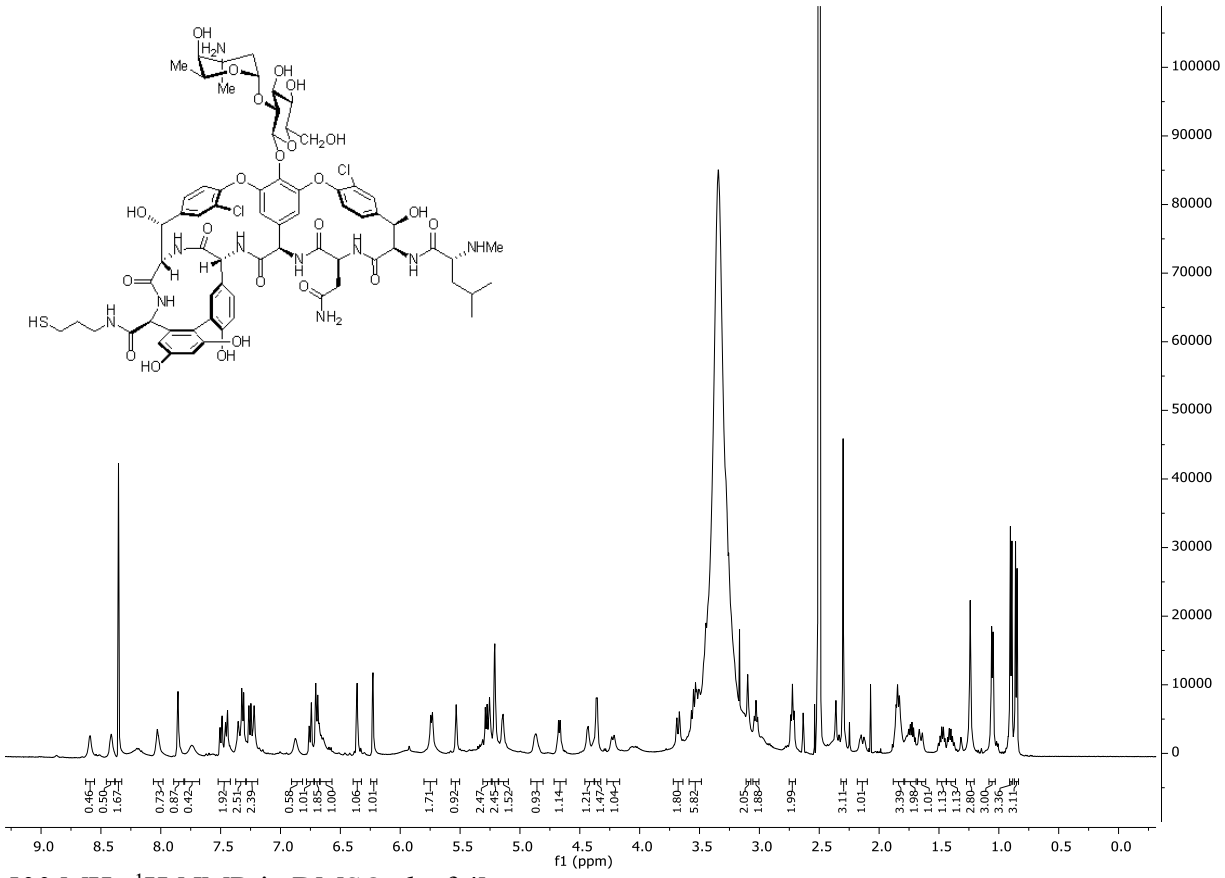

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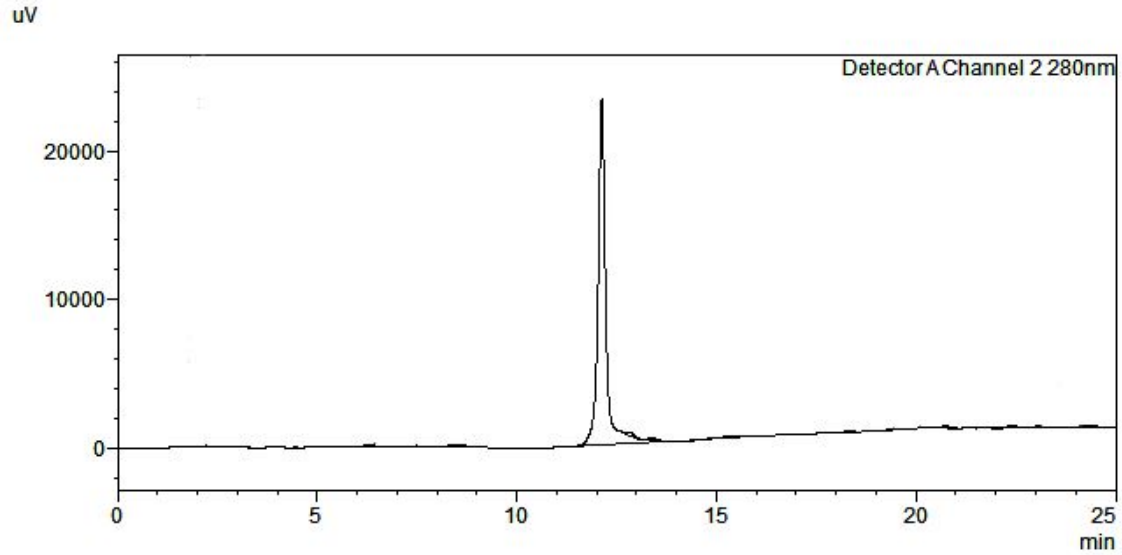

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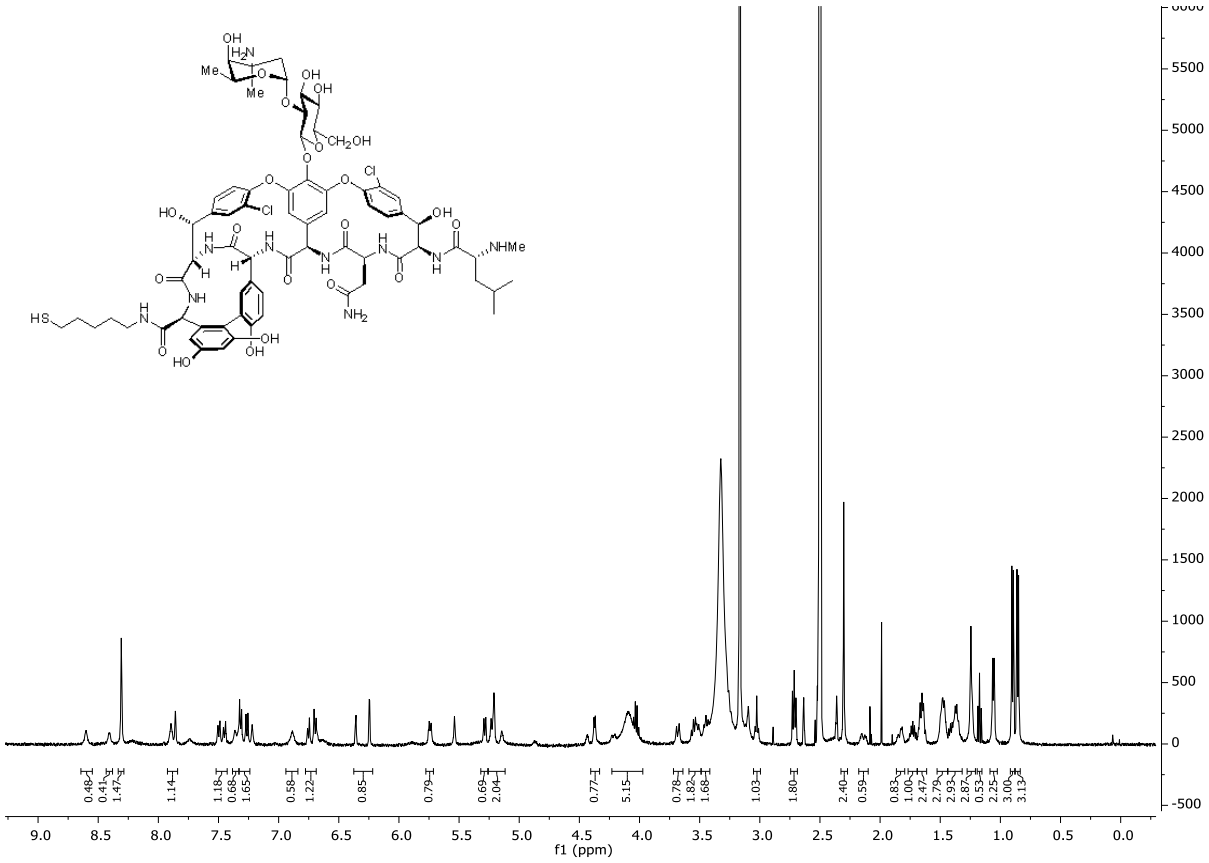
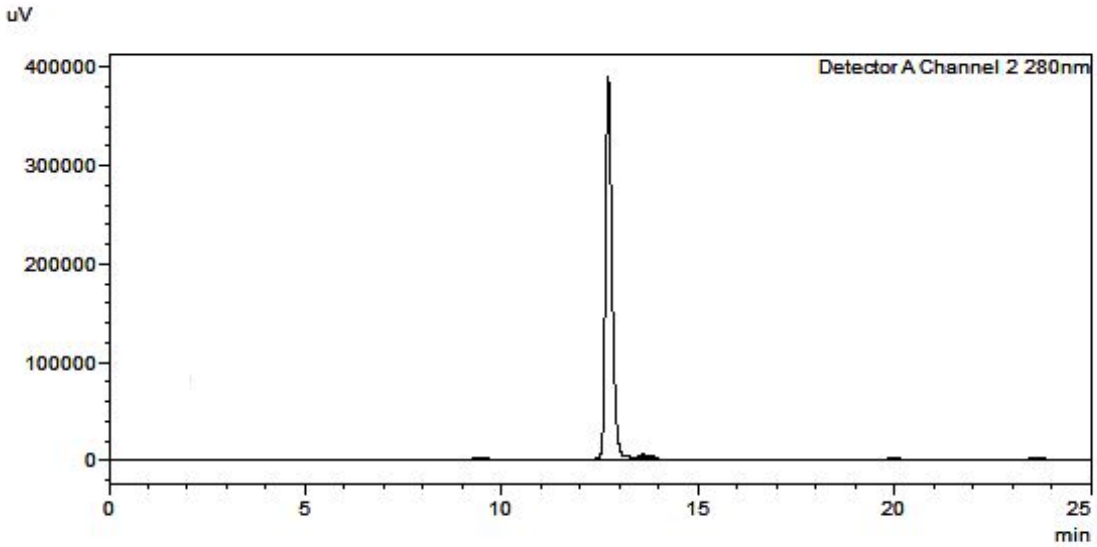


500 MHz ^{1}H-NMR in DMSO-d_6 of **4c**



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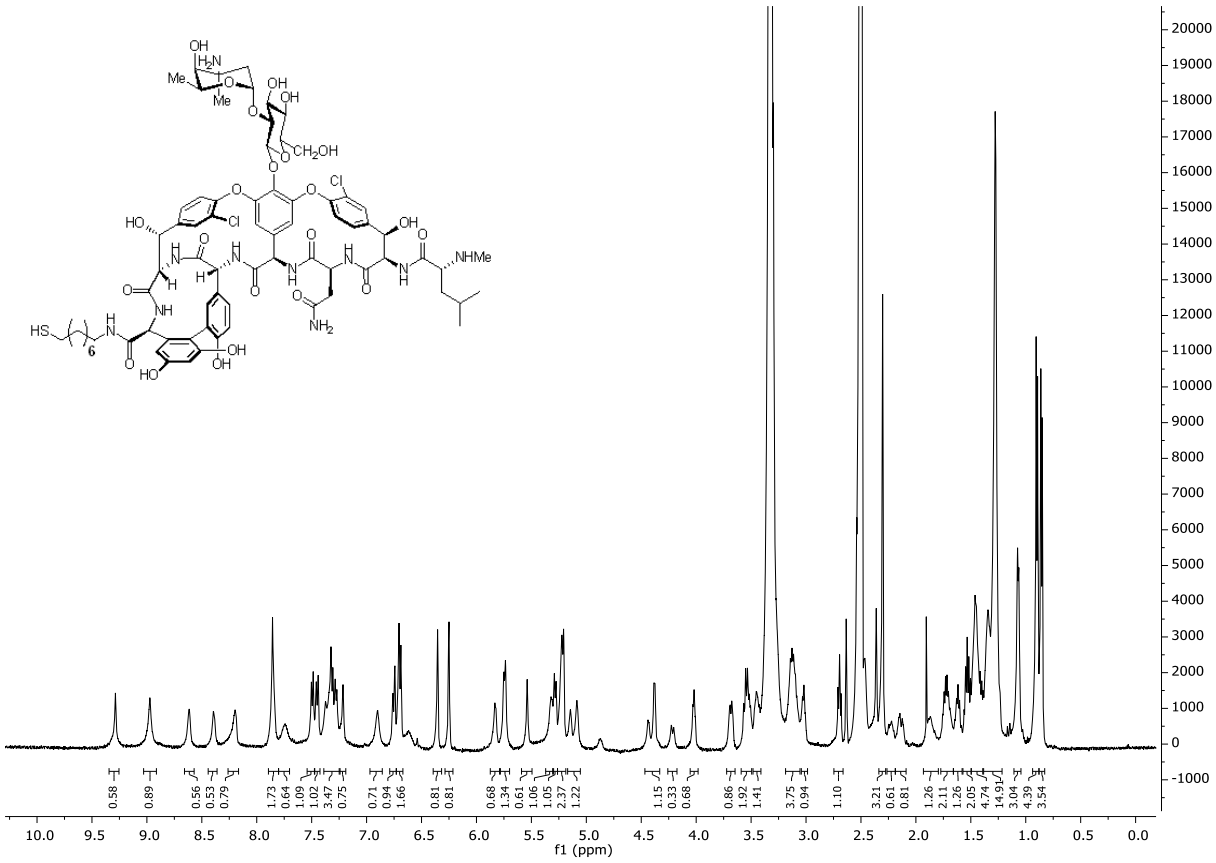
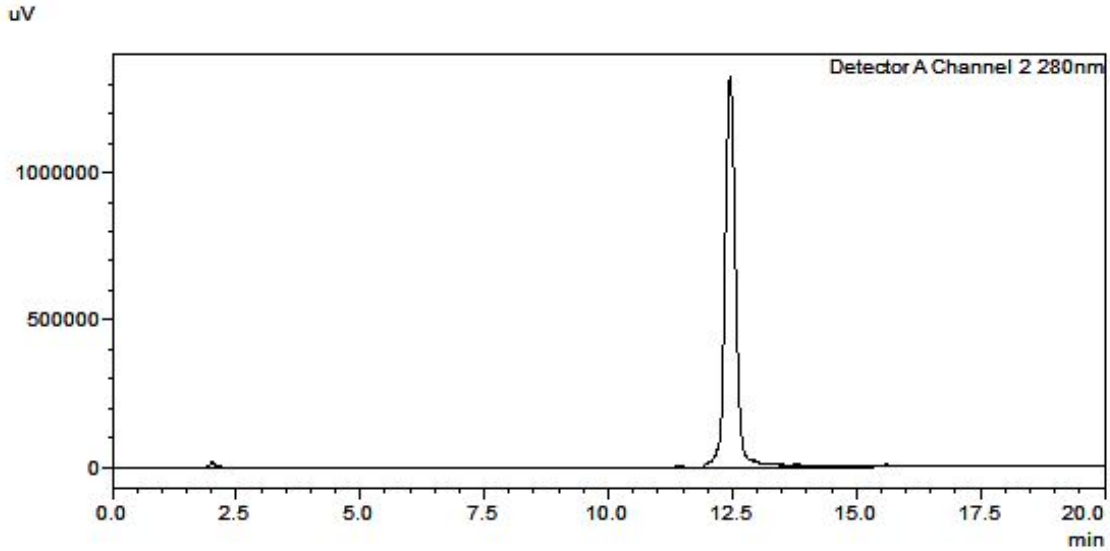
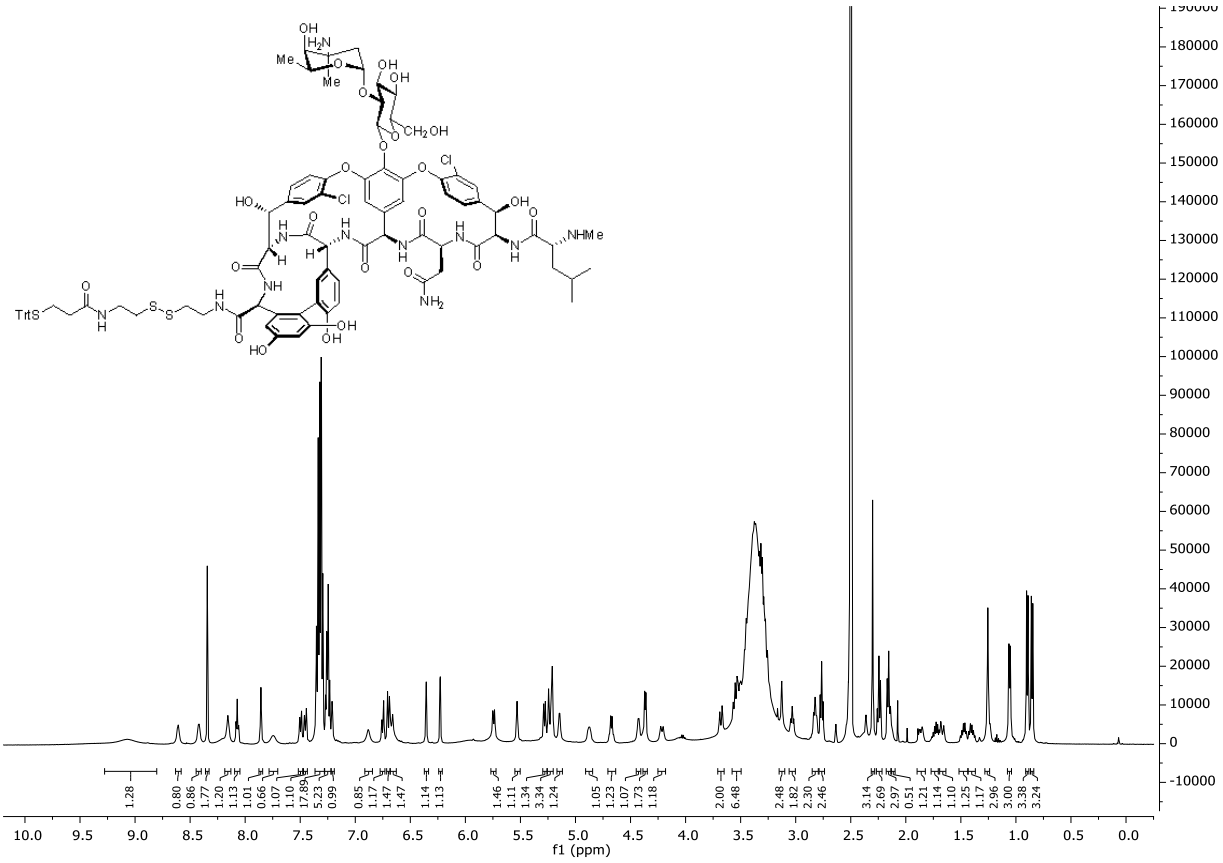


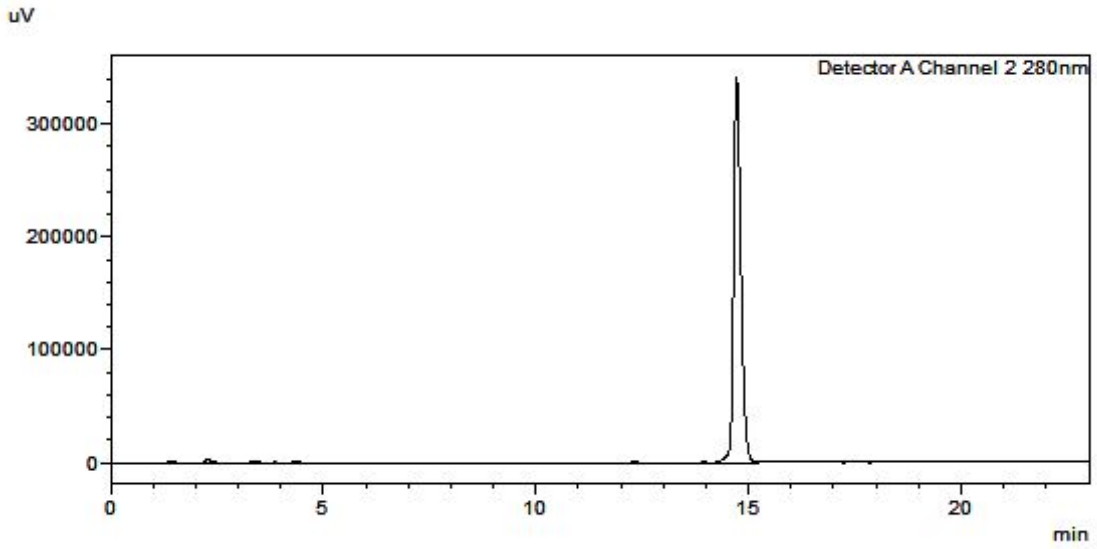
500 MHz ^{1}H-NMR in DMSO-d_6 of **4d**



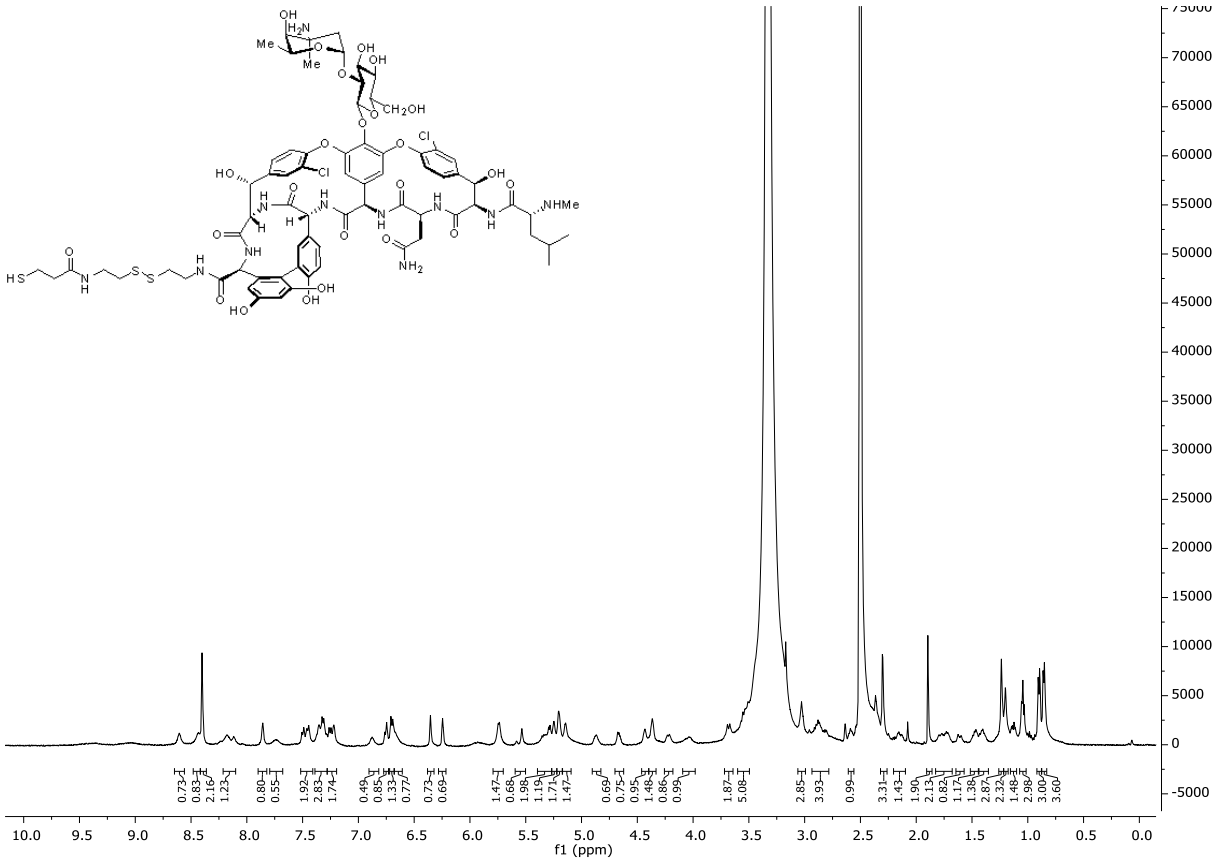
HPLC chromatogram of compound **4d**



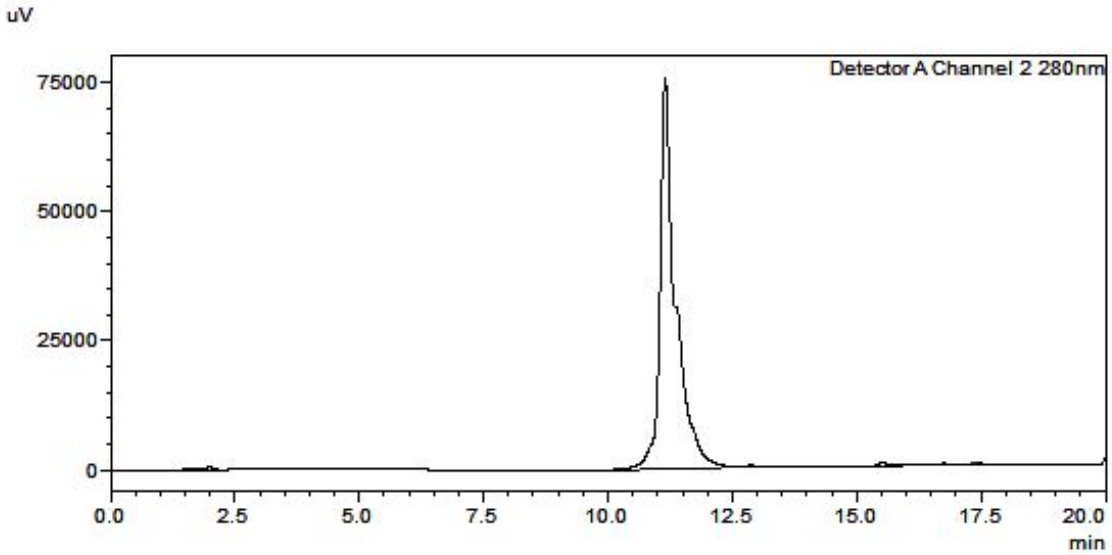
500 MHz ^{1}H-NMR in DMSO-d_6 of **3a**



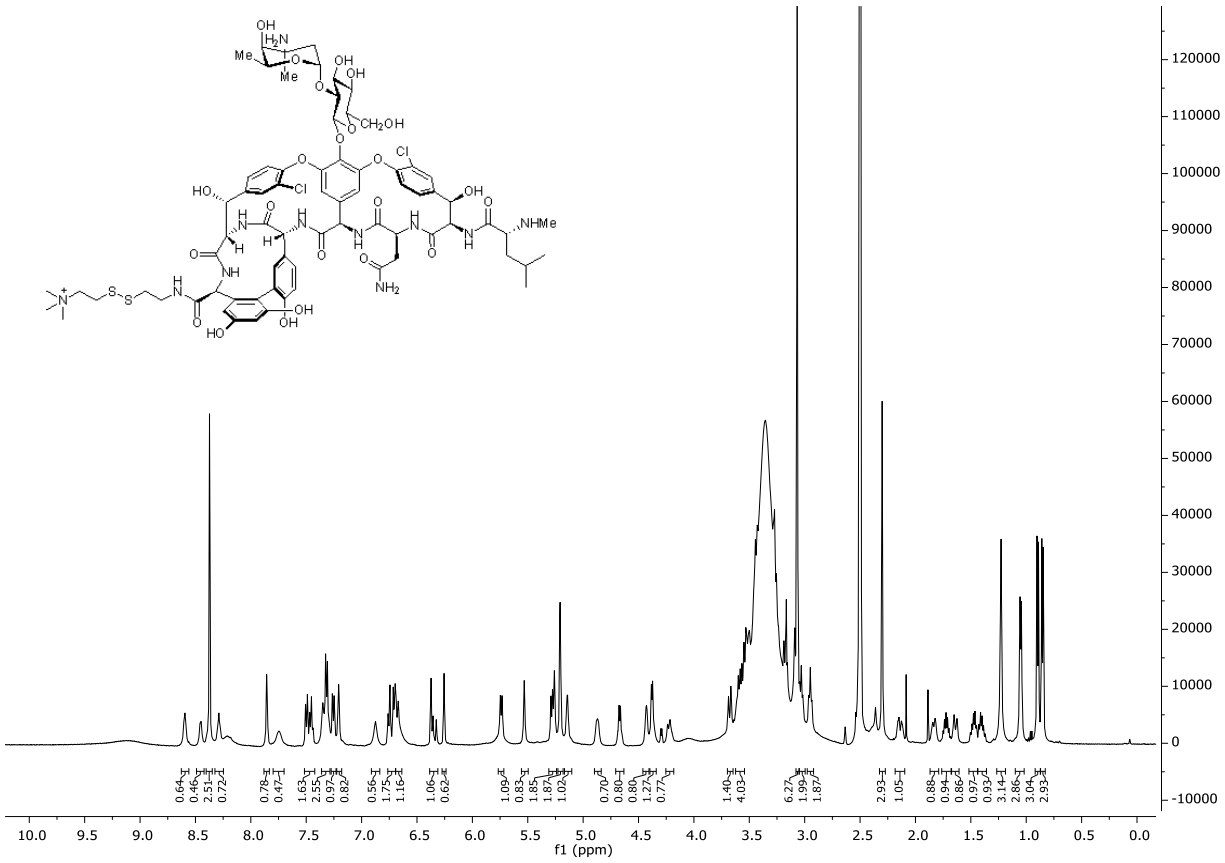
HPLC chromatogram of compound **3a**



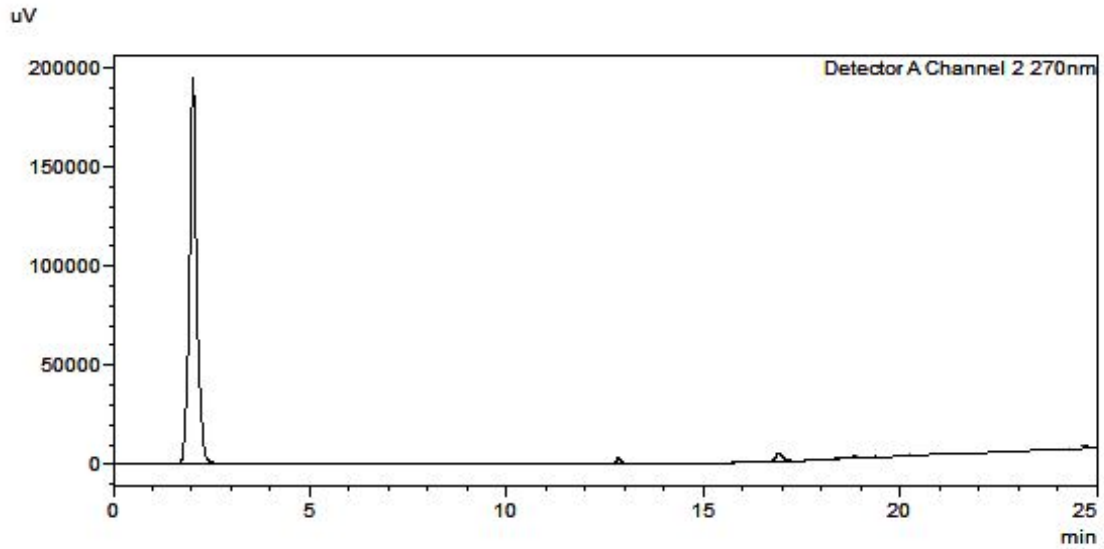
500 MHz ^{1}H-NMR in DMSO-d_6 of **3b**



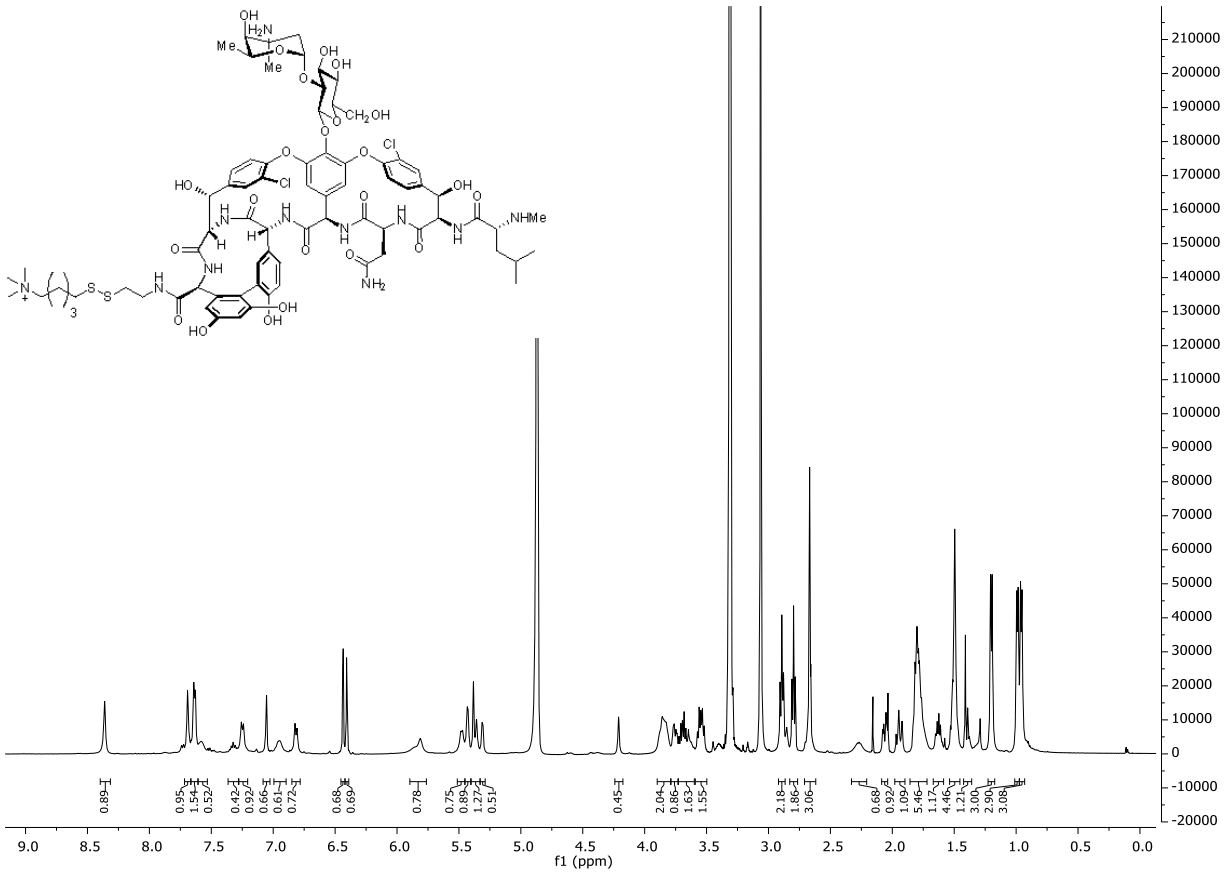
HPLC chromatogram of compound **3b**



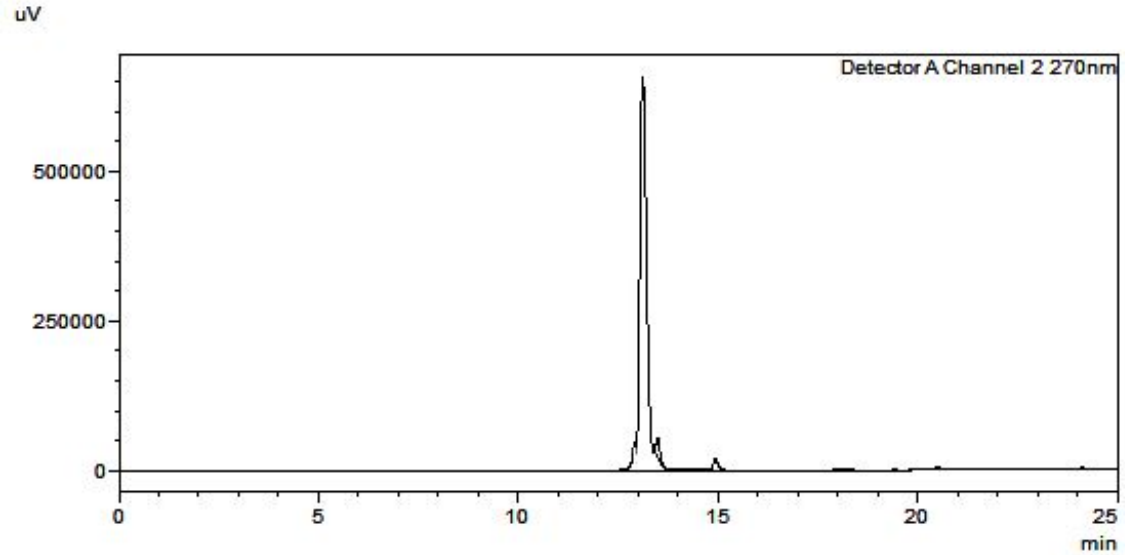
500 MHz ^{1}H-NMR in DMSO-d_6 of **5a**



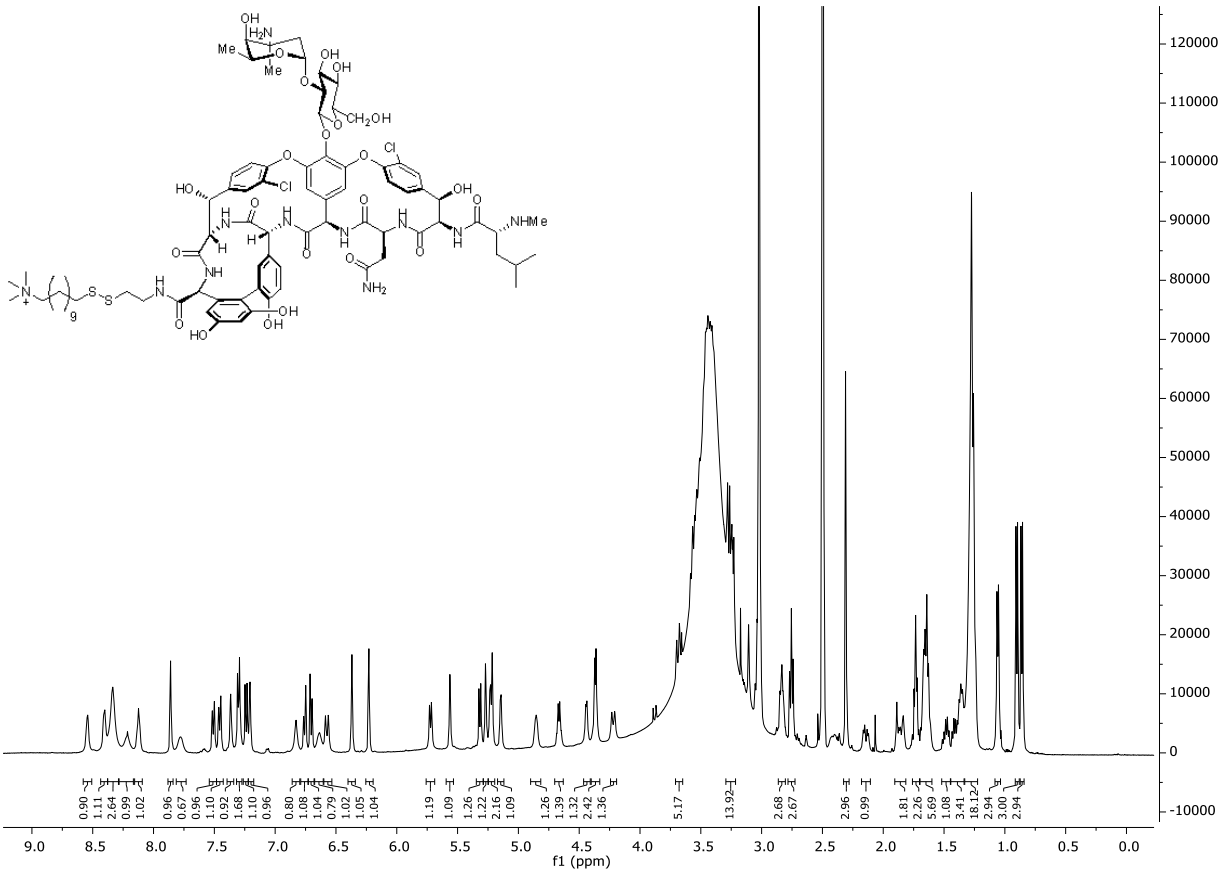
HPLC chromatogram of compound **5a**



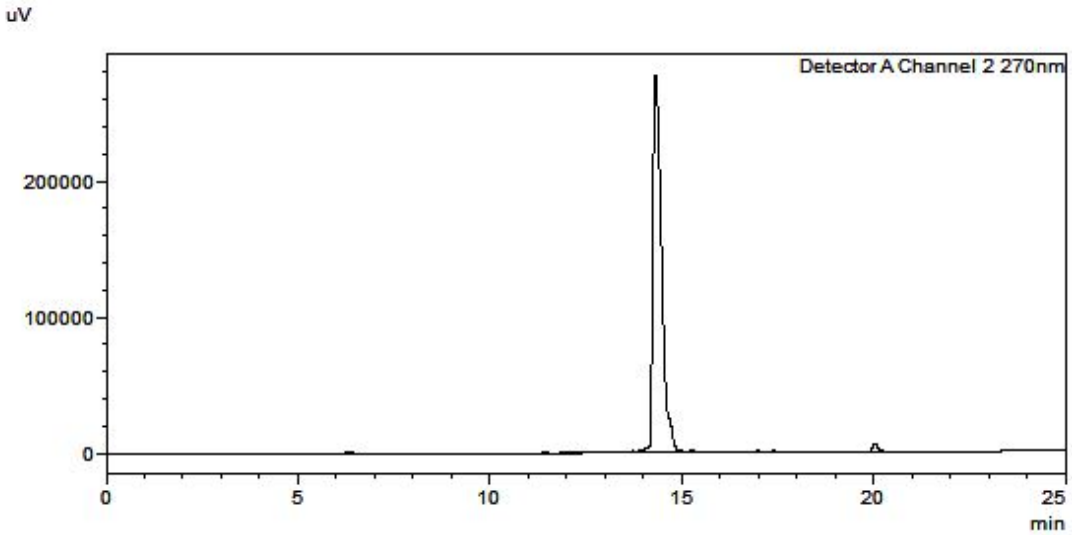
500 MHz ^{1}H-NMR in MeOD of **5b**



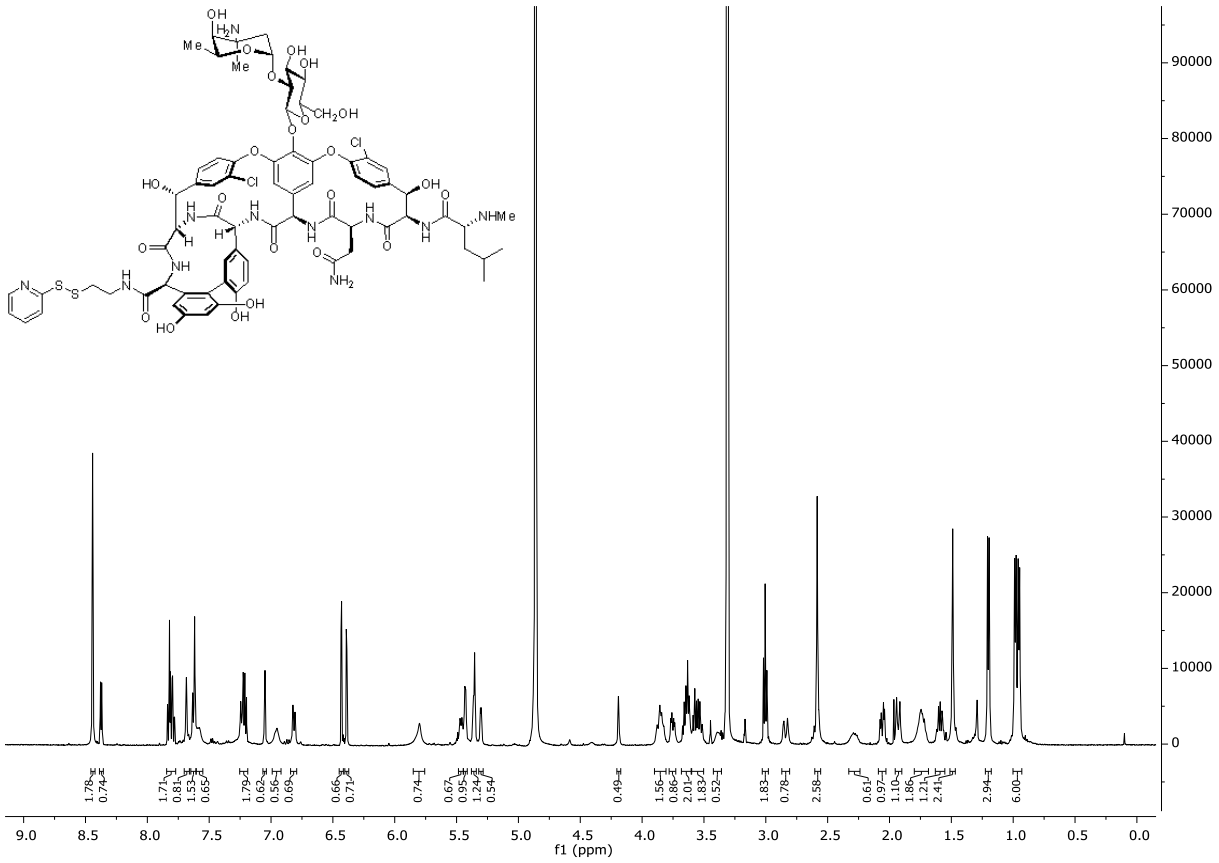
HPLC chromatogram of compound **5b**



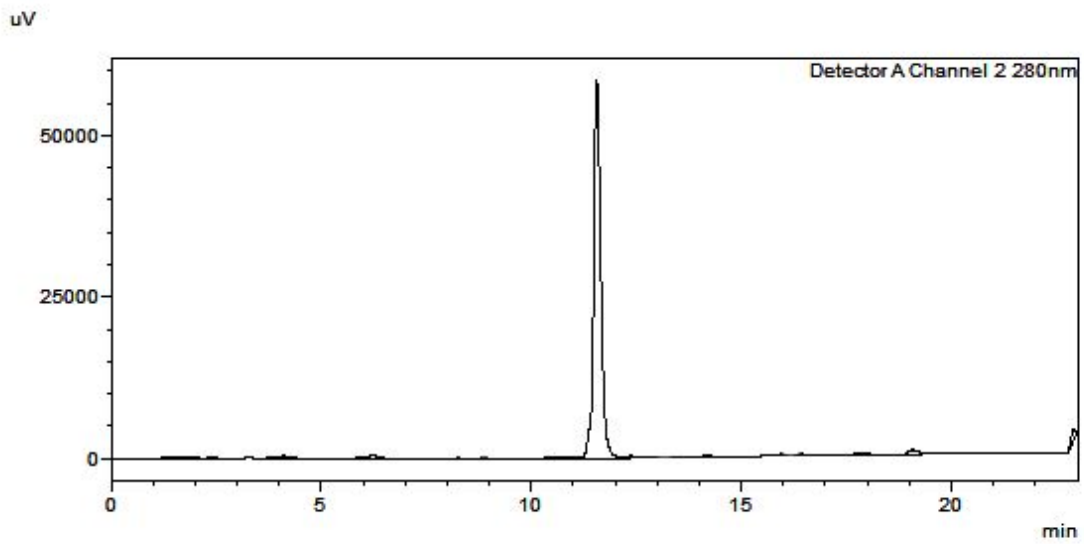
500 MHz ^{1}H-NMR in DMSO-d_6 of **5c**



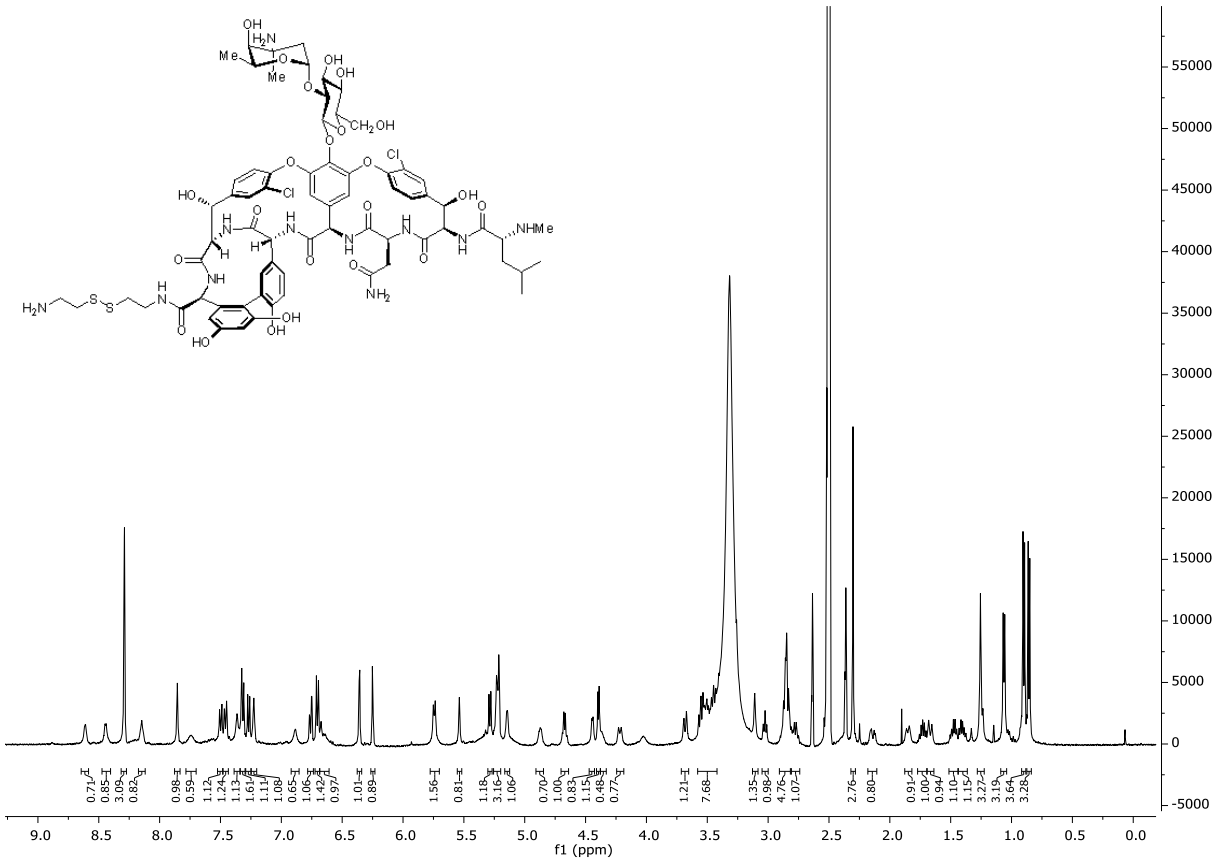
HPLC chromatogram of compound **5c**



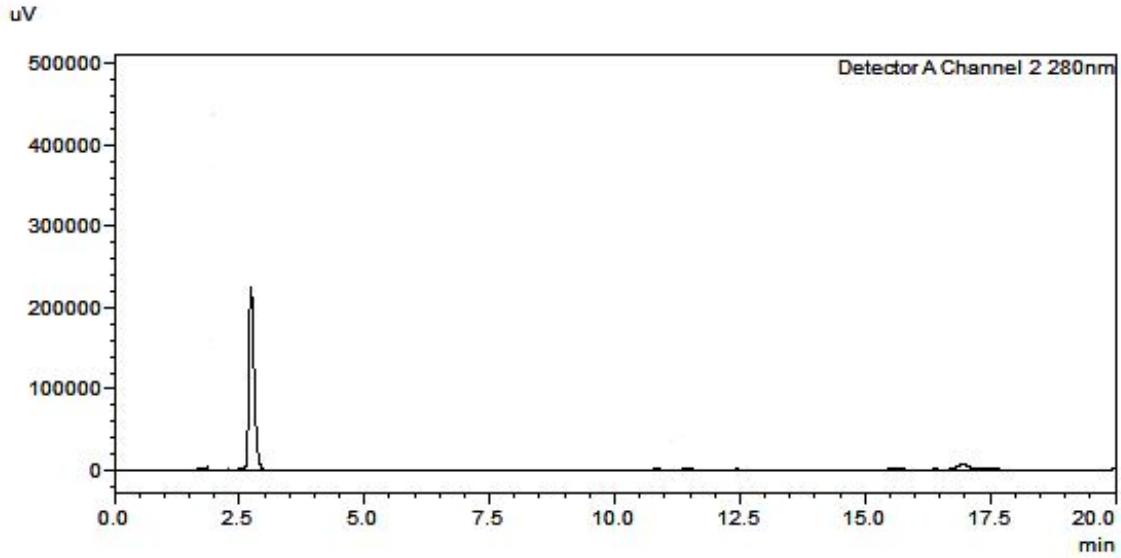
500 MHz ^{1}H-NMR in MeOD of **6**



HPLC chromatogram of compound **6**



500 MHz ^{1}H-NMR in DMSO-d_6 of **7**



HPLC chromatogram of compound **7**

IV. Microbiological assays

Bacterial Strains, Media, Reagents from University of Zurich.

Bacillus subtilis (*B. subtilis* ATCC 6633), *Staphylococcus aureus* (*S. aureus* ATCC 25922, ATCC 29213, ATCC 43300 (MRSA)), *Enterococcus faecalis* (*E. faecalis* ATCC 51299 (VanB)), *Enterococcus faecium* (*E. faecium* ATCC 700221 (VanA)), *Enterococcus gallinarum* (*E. gallinarum* ATCC 700425 (VanC)), *Escherichia coli* (*E. coli* ATCC 25922, K12 MG1622), *Acinetobacter baumannii* (*A. baumannii* ATCC 19606), Pseudomonas aeruginosa (*P. aeruginosa* ATCC 27853, PAO1) was purchased from either the German Collection of Microorganisms and Cell Cultures (DSMZ) or the American Type Culture Collection (ATCC). The bacteria culture was stored at –80 ºC, and new cultures were prepared by streaking on Mueller Hinton (MH) Agar, Luria-Bertani (LB) Agar, Trypsic Soy (TS) Agar or Brain Heart Infusion (BHI) Agar plates. The overnight culture was prepared by inoculating a single colony into a sterile tube (15 mL) containing the broth (5 mL) and the cultures were shaken (200 rcf/min) overnight at 37 ºC. The conditions for growth of each bacteria strain are shown in Table S1.

Table S1. Conditions for the bacterial growth

Bacteria strain	Agar	Broth
B. subtilis	MH	MHB
S. aureus	LB	LB
E. faecalis	TS	TS
E. faecium	BHI	BHI
E. gallinarum	TS	TS
E. coli	LB	LB
A. baumannii	LB	LB
P. aeruginosa	LB	LB

Test Organisms from Micromyx (USA)

Test organisms were either reference strains from the American Type Culture Collection (ATCC; Manassas, VA) or clinical isolates from the Micromyx collection (MMX; Kalamazoo, MI). Upon receipt at Micromyx, the isolates were streaked under suitable conditions onto agar medium appropriate to each organism and were incubated for 18-24 h at 35 °C. Colonies harvested from these growth plates were resuspended in the appropriate medium containing a cryoprotectant. Aliquots of each suspension were then frozen at -80 °C. Prior to testing, the aerobic isolates were streaked from frozen vials onto trypticase soy agar with 5% sheep blood (BAP; Becton Dickinson [BD]; Lot No. 0267570; Sparks, MD) and were incubated at 35 °C for 20 – 24 h. The anaerobic isolates were streaked onto Brucella Agar supplemented with

hemin, Vitamin K1 and 5% sheep blood (Hardy Diagnostics; Lot No. 132444; Santa Maria, CA;) and incubated anaerobically at 35 °C for 44 – 48 h.

Broth Microdilution MIC Assay from Zurich University

The MIC assay for *B. subtilis* ATCC 6633, *S. aureus* (ATCC 25923, 29213, 43300), *E. faecalis* ATCC 51299, *E. faecium* ATCC 700221, *E. gallinarum* ATCC 700425, *E. coli* (ATCC 25922, K12 MG1622), *P. aeruginosa* ATCC 27853 and PAO1 was carried out according to the EUCAST standard protocol.[11]

The turbidity of the overnight culture of *B. subtilis* ATCC 6633, *S. aureus* (ATCC 25923, 29213, 43300), *E. coli* (ATCC 25922, K12 MG1622), *P. aeruginosa* ATCC 27853 and PAO1 was adjusted to McFarland standard 0.5 (OD = 0.08 – 1.1), and then diluted 1 to 200 for *B. subtilis*, 1:150 for *E. coli*, and 1:100 for the *S. aureus* and *P. aeruginosa* in cation-adjusted Mueller-Hinton-II broth (MHB). The turbidity of the overnight culture *E. faecalis* ATCC 51299, *E. faecium* ATCC 700221, and *E. gallinarum* ATCC 700425 was adjusted to McFarland standard 0.5 and then diluted 1 to 100 in Brain Heart Infusion Broth. The compounds were dissolved either in water or water/5% DMSO at a concentration of 1 mg/mL. In 96-wells microtiter plates, two-fold serial dilutions of the compounds (ranging from 64 µg/mL to 0.06 µg/mL) were prepared in MHB or BHI depending on the strain to a final volume of 50 µl. 0.002% Tween 80 was added for testing vancomycin derivatives. The bacterial suspension (50 µl) was added into each well on the microtiter plate for inoculation, corresponding to approximately $5x10^5$ CFU/mL. The microplates were shaken (200 rcf/min) overnight at 37 °C. The MIC was read and recorded as the lowest concentration of drug that inhibited visible growth.

Broth Microdilution MIC Assay from Micromyx (USA)

Test Media

The medium employed for aerobic testing in the broth microdilution MIC assay was cationadjusted Mueller Hinton broth (CAMHB; BD; Lot No. 9024807). For the testing of Streptococcus pneumoniae, CAMHB was supplemented with 5% laked horse blood (LHB; Hemostat; Lot No. 595853; Dixon, CA). The medium employed for anaerobic testing in the broth microdilution MIC assay was Brucella Broth (BD; Lot No. 7306905), supplemented with hemin (Sigma; Lot No. SLBP5720V), Vitamin K (Sigma; Lot No. MKCB9432) and 5% LHB.

MIC assay

The MIC assay method followed the procedure described by the CLSI[12] and employed automated liquid handlers (Multidrop 384, Labsystems, Helsinki, Finland; Biomek 3000 and

Biomek FX, Beckman Coulter, Fullerton CA) to conduct serial dilutions and liquid transfers. The wells in columns 2 through 12 in a standard 96-well microdilution plate (Costar) were filled with 150 µL of the appropriate diluent (DMSO for University of Zurich test agents; H_2O for vancomycin, levofloxacin, and metronidazole). The drugs (300 µL at 101X the desired top concentration in the test plates) were dispensed into the appropriate well in column 1 of the mother plates. The Biomek 2000 was used to make serial 2-fold dilutions through column 11 in the "mother plate". The wells of column 12 contained no drug and were the organism growth control wells. The daughter plates for testing of all isolates were loaded with 190 µL per well of CAMHB for aerobes and supplemented Brucella broth with 5% LHB for anaerobes using the Multidrop 384. The daughter plates were prepared on the Biomek FX instrument which transferred 2 µL of 101X drug solution from each well of a mother plate to the corresponding well of each daughter plate in a single step. A standardized inoculum of each organism was prepared per CLSI methods (1-3). For all aerobic isolates, suspensions were prepared in PBS to equal the turbidity of a 0.5 McFarland standard. These suspensions were further diluted 1:20 in CAMHB (CAMHB with LHB for Streptococcus pneumoniae). The inoculum for each organism was dispensed into sterile reservoirs (Beckman Coulter 372788), and the Biomek 3000 was used to deliver 10 µL of standardized inoculum into each well resulting in a final cell density of approximately 5 x 10^5 CFU/mL. Daughter plates were placed on the Biomek 3000 work surface in reverse orientation so that inoculation took place from low to high drug concentration. Plates were stacked four high, covered with a sterile lid on the top plate, placed in plastic bags, and incubated at 35 °C in ambient atmosphere for approximately 16-20 hr. For all anaerobe isolates, suspensions were prepared in supplemented Brucella broth supplemented with hemin and Vitamin K to equal the turbidity of a 0.5 McFarland standard. These suspensions were further diluted 1:10 in supplemented Brucella broth with 5% LHB. The inoculum was dispensed into sterile reservoirs (Beckman Coulter) and transferred by hand in the Bactron Anaerobe chamber so that inoculation took place from low to high drug concentration.

A 10 µL aliquot of inoculum was delivered into each well. Inoculated daughter plates were stacked and placed in an anaerobic box with GasPak sachets (BD; Lot No. 9030752), covered with a sterile lid on the top plate, and incubated at 35-37 °C for 46 – 48 hrs. Following incubation, the microplates were removed from the incubator and viewed from the bottom using a plate viewer. For each of the test compounds, an un-inoculated solubility control plate for each test medium was observed for evidence of drug precipitation. The MIC was read and recorded as the lowest concentration of drug that inhibited visible growth.

Table S2. MIC results for vancomycin derivatives

	MIC (µg/mL)								
	VAN	**1a**	**1b**	**1c**	**2a**	**2b**	**2c**	**3a**	**3b**
Gram-positive strains									
S. aureus ATCC 25923 (VSSA)	1-2	2	4	2	1	0.12-0.25	1	0.5	8
S. aureus ATCC 29213 (VSSA)	0.5-1	0.5-2	2	0.5-2	1	0.12-0.25	1	0.5-1	8
S. aureus ATCC 33591 (MRSA)	1-2	2	n.d.	0.5	n.d.	0.12	0.5	1	n.d.
S. aureus ATCC 43300 (MRSA)	1-2	2	2	2	1	0.12-0.25	1-2	0.5-1	4
S. aureus ATCC NRS1 (VISA, Mu50)	8	1	n.d.	2	n.d.	0.5	0.5	1	n.d.
S. aureus NRS2 (hVISA, Mu3)	1	1	n.d.	1	n.d.	0.25	0.5	1	n.d.
S. aureus NRS24 (hVISA)	16-32	32	n.d.	2	n.d.	0.5	1	1	n.d.
E. faecalis ATCC 29212 (VSE)	2	0.5	n.d.	0.5	n.d.	0.25	1	2	n.d.
E. faecalis MMX 0486 (VanA)	>32	>32	n.d.	>32	n.d.	16	2	8	n.d.
E faecalis ATCC 51299 (VanB)	16	4	4	4	4-8	0.5	2	2-4	2
E. faecium ATCC 700221 (VanA)	128-256	32	32	32	64	16-32	4	64	16-64
E. faecium MMX 0485 (VanA)	>128	16	n.d.	8	n.d.	8	1	2	n.d.
E. gallinarum ATCC 700425 (VanC)	8-16	8	8	8	8	4-8	4	4	1-2
S. pneumoniae ATCC 29212	0.12-0.5	0.06	n.d.	0.25	n.d.	0.06	0.25	0.25	n.d.
S. epidermidis ATCC 12228	1	1	n.d.	0.5	n.d.	0.12	0.25	0.5	n.d.
B. fragilis ATCC 25285	16	>16	n.d.	>16	n.d.	>16	16	>16	n.d.
C. perfringens ATCC *13124*	0.5	0.5	n.d.	0.5	n.d.	0.5	1	1	n.d.
C. difficile ATCC 700057	0.25-0.5	0.12	n.d.	0.5	n.d.	0.25	2	0.5	n.d.

C. difficile ATCC BAA-1805 (rt 027)	0.25	0.06	n.d.	0.25	n.d.	0.25	2	0.5	n.d.
C. difficile MMX 5680 (rt 027)	0.25	0.12	n.d.	0.5	n.d.	0.5	4	1	n.d.
C. difficile ATCC 43255 (rt 087)	0.12-0.5	0.06	n.d.	0.5	n.d.	0.5	0.12	1	n.d.
C. difficile MMX 8260 (rt 017)	0.25	0.12	n.d.	0.5	n.d.	0.25	2	0.5	n.d.
C. difficile MMX 8264 (rt 027)	0.25	0.12	n.d.	0.5	n.d.	0.5	2	1	n.d.
C. difficile ATCC 9689 (rt 001)	0.25-0.5	0.12	n.d.	1	n.d.	0.5	2	1	n.d.
C. difficile MMX 8282 (rt 017)	0.25	0.12	n.d.	0.5	n.d.	0.5	2	1	n.d.
C. difficile MMX 8290 (rt 078)	0.25	0.12	n.d.	0.5	n.d.	0.25	2	0.5	n.d.
C. difficile ATCC BAA-1875 (rt 078)	0.25	0.12	n.d.	0.5	n.d.	0.25	2	1	n.d.
B. subtilis ATCC 6633	0.12-0.25	0.25	0.25-0.5	0.06	0.125	0.03	0.5-2	0.12-0.5	0.06-0.12
Gram-negative strains									
E. coli ATCC 25922	256	128	128	128	128	128	>128	>128	>128
E. coli MMX 0121 (Δ*tolC*)	>32	>32	n.d.	>32	n.d.	>32	>32	>32	n.d.
A. baumanni ATCC 19606	128	64	64	64	64	64	>128	>128	>128
P. aureginosa ATCC 27853	512	256	256	256	>128	>128	>128	>128	>128
M. catarrhalis ATCC 25238	>32	32	n.d.	16	n.d.	2	2	1	n.d.
M. catarrhalis ATCC 8193	>32	4	n.d.	8	n.d.	2	0.5	2	n.d.

VSSA - vancomycin-sensitive *Staphylococcus aureus*; MRSA - methicilin-resistant *Staphylococcus aureus*; VISA - vancomycin-intermediate *Staphylococcus aureus*; hVISA – heterogenous vancomycin-intermediate *Staphylococcus aureus*; VSE – vancomycin – susceptible *Enterococcus faecalis*; Vancomycin-resistant (Van A or VanB) *Enterococcus faecalis*; Vancomycin-resistant (Van A) *Enterococcus faecium*; Vancomycin-resistant (Van C) *Enterococcus gallinarum*; rt - Ribotype

Table S2. *Continue*

	MIC (µg/mL)									
	VAN	**4a**	**4b**	**4c**	**4d**	**5a**	**5b**	**5c**	**6**	**7**
Gram-positive strains										
S. aureus ATCC 25923 (VSSA)	1-2	4	4	8	4	1	1	1	4	1-2
S. aureus ATCC 29213 (VSSA)	0.5-1	4	1-4	2-8	2-4	0.5-1	0.5-2	0.25-0.5	2-4	0.25-1
S. aureus ATCC 33591 (MRSA)	1-2	n.d.	>32	4	2	2	2	1	2	0.5
S. aureus ATCC 43300 (MRSA)	1-2	4	4	8	4	0.5	0.5-1	0.5	2-4	1-2
S. aureus ATCC NRS1 (VISA, Mu50)	8	n.d.	8	2	2	0.5	1	0.25	4	1
S. aureus NRS2 (hVISA, Mu3)	1	n.d.	16	2	2	0.5	1	0.5	2	0.25
S. aureus NRS24 (hVISA)	16-32	n.d.	>32	>32	2	>32	>32	8	4	1
E. faecalis ATCC 29212 (VSE)	2	n.d.	0.25	1	1	1	1	0.5	1	0.5
E. faecalis ATCC 29212 (VanA)	>32	n.d.	>32	>32	>32	>32	>32	>32	>32	>32
E faecalis ATCC 51299 (VanB)	16	2-4	4	1-2	8	16	8-16	2	16	8
E. faecium ATCC 700211 (VanA)	128-256	32	32	32	64	64	64	32	32-64	64
E. faecium MMX 0485 (VanA)	>128	n.d.	1	1	2	32	16	16	8	8
E. gallinarum ATCC 700425 (VanC)	8-16	2	1-2	0.5-1	8	4	4	2	8-16	8
S. pneumoniae ATCC 29212	0.12-0.5	n.d.	≤0.03	≤0.03	0.5	0.25	0.06	0.12	0.5	≤0.03
S. epidermidis ATCC 12228	1	n.d.	4	1	1	0.5	0.5	0.25	2	0.25
B. fragilis ATCC 25285	16	n.d.	>16	>16	>16	>16	16	>16	16	16
C. perfringens ATCC 13124	0.5	n.d.	0.06	0.5	2	1	0.5	1	0.5	0.5
C. difficile ATCC 700057	0.25-0.5	n.d.	0.25	0.25	4	0.5	0.5	0.5	0.5	0.25

C. difficile ATCC BAA1805 (rt 027)	0.25	n.d.	0.06	0.06	4	0.25	0.25	0.25	0.5	0.25
C. difficile MMX 5680 (rt 027)	0.25	n.d.	0.25	0.25	4	0.5	0.5	1	0.5	0.5
C. difficile ATCC 43255 (rt 087)	0.12-0.5	n.d.	0.12	0.06	4	≤0.015	≤0.015	1	0.5	0.5
C. difficile MMX 8260 (rt 017)	0.25	n.d.	0.12	0.25	1	0.5	0.5	0.5	0.5	0.5
C. difficile MMX 8264 (rt 027)	0.25	n.d.	0.06	0.12	1	0.5	0.25	0.5	0.5	0.25
C. difficile ATCC 9689 (rt 001)	0.25-0.5	n.d.	0.12	0.25	4	0.5	0.5	0.5	0.5	0.5
C. difficile MMX 8282 (rt 017)	0.25	n.d.	0.12	0.25	1	0.5	0.5	0.5	0.5	0.5
C. difficile MMX 8290 (rt 078)	0.25	n.d.	0.12	0.25	1	0.5	0.5	0.5	0.5	0.5
C. difficile ATCC BAA-1875 (rt 078)	0.25	n.d.	0.12	0.25	1	0.5	0.5	0.5	0.5	0.5
B. subtilis ATCC 6633	0.12-0.25	0.03-0.12	0.03	0.25	0.5	0.06-0.12	0.06-0.12	0.06-0.12	0.25-0.5	0.06
Gram-negative strains										
E. coli ATCC 25922	256	128	128	128	128	64	64	64	64	64
E. coli MMX 0121 (Δ*tolC*)	>32	n.d.	>32	>32	>32	>32	>32	>32	>32	>32
A. baumanni ATCC 19606	128	>128	128	64	64	128	128	64	64	32
P. aureginosa ATCC 27853	512	>128	>128	>128	>128	>128	>128	128	>128	128
M. catarrhalis ATCC 25238	>32	n.d.	>32	>32	4	>32	>32	8	>32	>32
M. catarrhalis ATCC 8193	>32	n.d.	16	4	8	2	2	1	>32	>32

VSSA - vancomycin-sensitive *Staphylococcus aureus*; MRSA - methicilin-resistant *Staphylococcus aureus*; VISA - vancomycin-intermediate *Staphylococcus aureus*; hVISA – heterogenous vancomycin-intermediate *Staphylococcus aureus*; VSE – vancomycin – susceptible *Enterococcus faecalis*; Vancomycin-resistant (Van A or VanB) *Enterococcus faecalis*; Vancomycin-resistant (Van A) *Enterococcus faecium*; Vancomycin-resistant (Van C) *Enterococcus gallinarum*; rt - Ribotype

Defining conditions for biofilm inhibition assay.[13,14]

6 bacterial strains (Table S2) were prepared for testing. Fresh cultures were prepared from stock by subculturing bacterial strains on fresh LB or BHI plates and incubated at 37 °C for 16-20 h. The single colonies were chosen, and the culture was growing in Trypsic Soy broth or BHI broth (for *Enterococcus* strains) for 20-24 h at 37 °C with shaking 200 chf/min.

Table S3. Bacterial strains tested for the biofilm formation

Bacterial Strain	ATCC number	Profile
S. aureus	29213	Weak β-lactamase producing strain
S. aureus	43300	MRSA
B. subtilis	6633	
E. faecium	700221	VRE, VanA
E. faecalis	51299	VRE, VanB
E. gallinarum	700425	VRE, VanC

Biofilm production.

The overnight culture was diluted in Trypsic soy broth to OD_{600} = 0.1 – 0.12 corresponding to $10x10^8$ cell/mL. The broths used for the experiment were prepared: Muller-Hilton broth (MHB), Trypsic Soy (TS), Trypsic Soy supplemented with 1% glucose (TSG), Trypsic Soy supplemented with 2% glucose (TS2G), Brain Heart Infusion (BHI), BHI supplemented with 1% glucose (BHIG). The adjusted to OD_{600} cells were diluted 1:100 with corresponding medium. An aliquot of 100 µL of bacterial suspension per well was dispensed into a 96-well round bottom microplate. Microplates were then incubated at 37 °C for 24 h (*S. aureus, B. subtilis*) or 48 h (*Enterococcus* strains).

Assessment of biofilm biomass by crystal violet.

An aliquot of 0.130 µL of 0.1% solution of crystal violet (CV) in water was added in each well contained biofilm. The microplates were incubated for 10 min at rt, and the plates were rinsed 2-3 times with sterile water. The plates were left to dry for 2 h at rt. Next, 0.130 µL of 30% AcOH in water were added to each well of the microplate to solubilize the CV. The plates were incubated for 10 min at rt. The obtained solutions were transferred into a new flat bottom 96-well plate. The absorbance was quantified at 570 nm using 30% AcOH in water as the blank by a plate reader.

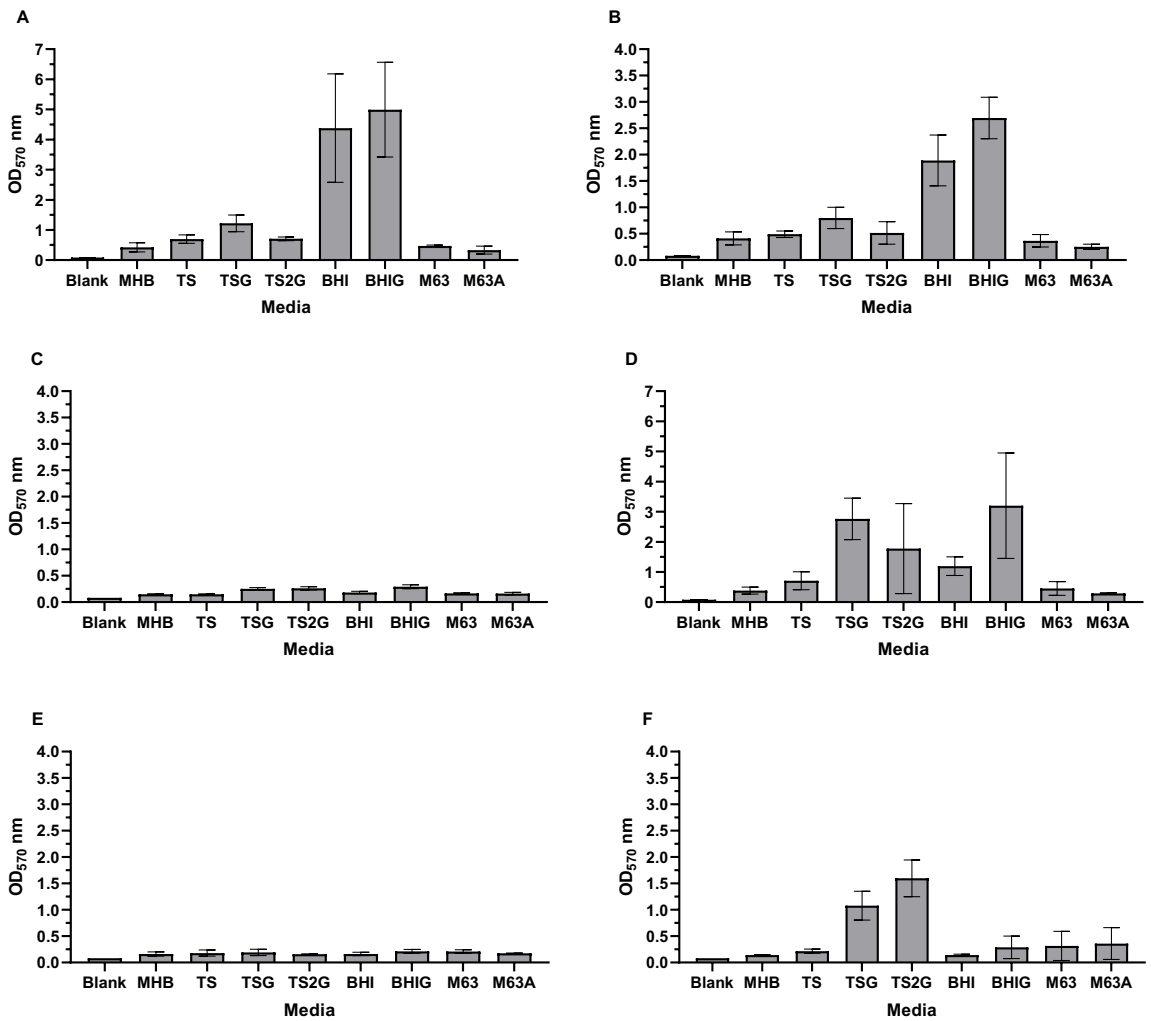


Figure S1. Assessment of biofilm biomass by crystal violet staining of ten bacterial strains: (**A**) *S. aureus* ATCC 29213, (**B**) *S. aureus* MRSA ATCC 43300, (**C**) *E. gallinarum* ATCC 700221, (**D**) *E. faecalis* ATCC 51299 (**E**) *E. faecium* ATCC 700221, (**F**) *B. subtilis* ATCC 6633. Strains were incubated in eight different media. Error bars represent standard deviation of two independent experiments in triplicate.

Assessment of metabolic activity of biofilm cells by resazurin.

Biofilm production was performed using BHIG for *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, and *E. faecalis* ATCC 51299. A stock of resazurin (Sigma-Aldrich) was prepared at 1 mg/mL in sterile PBS and DMSO, respectively. The solutions were filter-sterilized and stored at 4 °C in the darkness. Three concentrations of resazurin solution for Gram-positive bacteria were investigated: 2 µg/mL, 4 µg/mL and 8 µg/mL at 37 °C. The diluted resazurin solution in PBS were prepared right before the assay. For the assay, firstly, biofilm was carefully washed with 200 µL of sterile water. Next, 100 µL of diluted resazurin solution was added into each well containing biofilm, along with its respective negative controls (un-inoculated broth, three wells). Microplates were placed in a plate reader to measure kinetic of the reaction. The relative fluorescence units (RFU) (λ_{Ex} = 530 nm and λ_{Em} = 590 nm for resazurin) was measured every

2 min for 2 h. The experiment was performed twice in triplicates. The optimal conditions were chosen based on the analysis of quality parameters (Z' > 0.50) calculated by the equations.

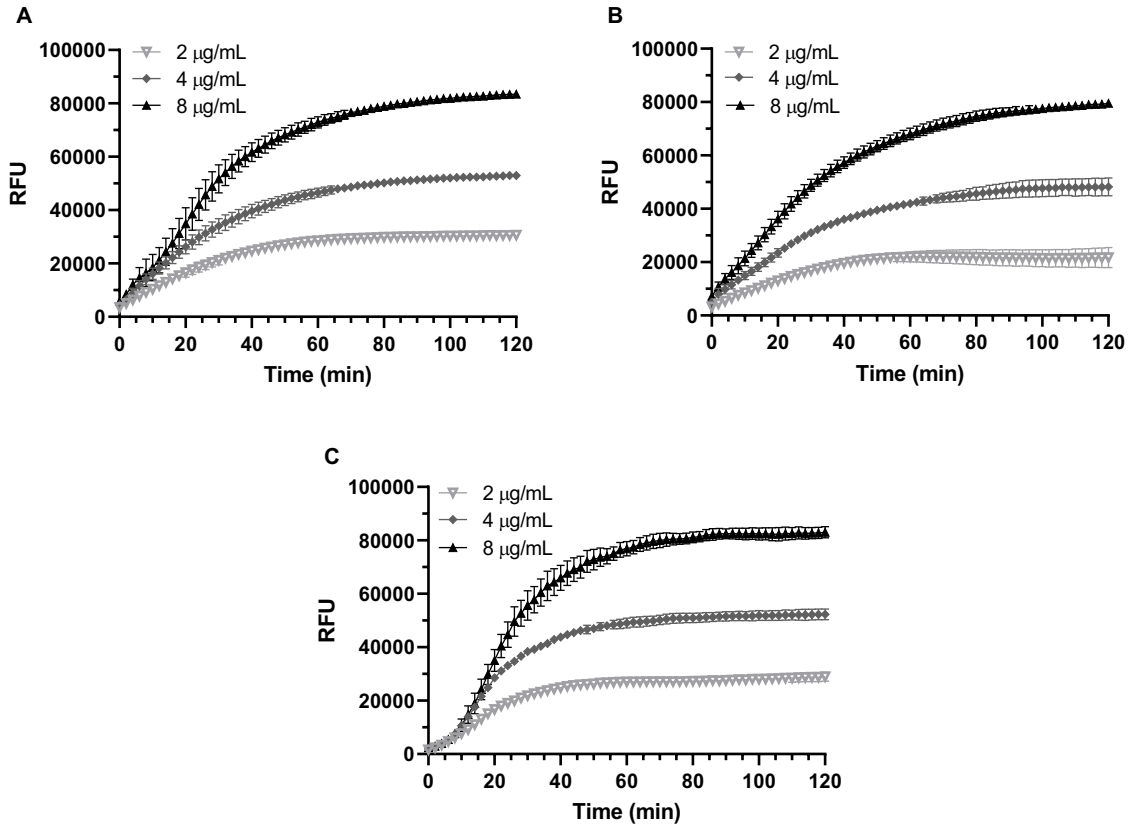


Figure S2. Assessment of metabolic activity of biofilm cells by resazurin, using three concentrations of solutions (2, 4, and 8 µg/mL): **(A)** *S. aureus* ATCC 29213, **(B)** *S. aureus* ATCC 43300 (MRSA), **(C)** *E. faecalis* ATCC 51299 (VanB), incubated at 37 ºC. Relative fluorescence measurements were taken every 2 min for 2 h. Error bars represent standard deviation of two independent experiments in triplicate.

Calculation of data viability for the experiments of metabolic activity of biofilm cells.

Statistical quality parameters were calculated according to the method described before.[15,16] The data was combined, and the Mean value and SD was calculated by Prism from 6 data points. Then obtained data was used for determining assay performance or sensitivity.

X_{max} – means of maximal signal

X_{min} – means of minimal signal

SD_{max} – the standard deviation of maximal signal

SD_{min} – the standard deviation of minimal signal

Signal to noise $S{:}N = \frac{X_{max} - X_{min}}{\sqrt{SD_{max}{}^2 - SD_{min}{}^2}}$

Signal to background $S{:}B = \frac{X_{max}}{X_{min}}$

Signal window $SW = \frac{X_{max} - X_{min} - 6(SD_{max} - SD_{min})}{SD_{max}}$

Z' factor $Z' = 1 - \frac{6SD_{max} - 6SD_{min}}{|X_{max} - X_{min}|}$

Table S4. Optimal conditions for the assessment of metabolic activity of biofilm cells

Bacterial strains	Medium	Dye	Concentration (µg/mL)	Incubation time	Temperature (°C)
S. aureus ATCC 29213	BHIG	Resazurin	8	60	37
S. aureus ATCC 43300	BHIG	Resazurin	4	40	37
E. faecalis ATCC 51299	BHIG	Resazurin	4	40	37

Table S5. Calculation of quality parameters for metabolic activity of biofilm cells assay

Bacterial strains	Resazurin concentration (µg/mL)	Time (min)	10	20	30	40	50	60	70	80	90	100	110	120
		Quality parameters												
S. aureus ATCC 29213	2	S/N	10.39	9.56	11.84	20.87	42.62	138.38	162.26	62.22	55.78	51.79	42.62	43.14
		S/B	12.71	19.72	24.37	28.22	30.57	31.86	32.36	32.58	32.26	32.37	32.29	31.98
		SW	7.65	6.76	9.06	18.08	39.07	107.30	119.04	56.69	51.08	47.55	39.14	39.63
		Z'	0.36	0.32	0.44	0.67	0.82	0.91	0.91	0.86	0.85	0.85	0.82	0.82
	4	S/N	6.94	9.56	11.51	14.99	20.54	27.43	33.83	54.18	82.03	88.56	121.94	109.54
		S/B	10.45	16.73	21.43	24.45	26.54	27.86	28.70	29.17	29.34	29.27	29.21	29.11
		SW	4.09	6.66	8.60	12.10	17.67	24.58	30.97	50.91	76.58	81.86	107.16	96.24
		Z'	0.09	0.35	0.46	0.58	0.69	0.76	0.80	0.87	0.90	0.91	0.92	0.91
	8	S/N	3.27	5.41	9.39	16.79	24.89	33.33	56.03	89.10	162.46	327.59	369.76	118.80
		S/B	8.21	15.33	2.32	26.28	28.59	30.07	31.06	31.52	31.73	31.77	31.63	31.45
		SW	0.34	2.48	6.48	13.93	22.06	30.45	52.39	81.45	129.90	171.67	169.20	97.31
		Z'	-0.88	-0.13	0.34	0.62	0.74	0.79	0.87	0.90	0.93	0.94	0.93	0.91
S. aureus ATCC 43300	2	S/N	8.09	14.43	19.05	21.29	16.65	11.38	8.46	2.28	6.67	6.32	5.85	5.44
		S/B	9.87	14.36	17.60	19.56	20.23	20.00	19.17	18.35	17.51	16.79	16.24	15.93
		SW	5.21	11.57	16.20	18.45	13.80	8.50	5.55	4.37	3.77	3.42	2.95	2.53
		Z'	0.23	0.56	0.66	0.70	0.62	0.45	0.27	0.15	0.07	0.02	-0.06	-0.14
	4	S/N	9.07	14.67	34.81	44.99	39.32	36.56	28.52	19.14	15.87	14.97	14.81	14.55
		S/B	9.37	13.64	16.68	18.02	18.48	18.54	18.43	17.99	17.63	17.10	16.48	15.93
		SW	6.33	11.47	30.16	36.79	32.14	29.63	23.56	15.91	12.96	12.10	11.89	11.61
		Z'	0.27	0.50	0.74	0.76	0.73	0.70	0.65	0.54	0.47	0.43	0.41	0.39
	8	S/N	6.77	11.85	20.44	27.07	31.12	33.97	40.28	57.82	-	-	-	-
		S/B	8.82	13.72	17.10	18.70	19.36	19.51	19.42	19.11	18.57	18.00	17.43	18.89
		SW	3.92	9.13	17.57	23.43	26.37	27.74	30.62	35.95	46.48	57.08	61.25	59.03
		Z'	0.06	0.43	0.63	0.68	0.69	0.70	0.70	0.71	0.73	0.74	0.73	0.72

E. faecalis ATCC 51299	2	S/N	15.13	37.16	34.68	55.19	30.18	63.81	55.20	41.88	28.33	20.36	16.90	16.29
		S/B	8.82	18.21	22.80	24.79	25.19	25.06	24.44	23.87	23.65	23.37	23.29	23.17
		SW	12.22	34.27	31.80	52.05	27.33	59.79	51.47	38.68	25.47	17.57	14.11	13.51
		Z'	0.59	0.83	0.81	0.87	0.78	0.88	0.86	0.83	0.76	0.67	0.61	0.59
	4	S/N	11.96	31.94	33.09	40.01	36.76	25.21	27.06	27.93	28.07	27.16	27.08	24.38
		S/B	7.10	18.25	23.39	25.65	26.35	26.51	26.24	25.86	25.50	24.97	24.44	24.04
		SW	9.10	29.02	30.16	37.09	33.86	22.30	24.17	25.05	25.19	24.30	24.23	21.52
		Z'	0.47	0.81	0.81	0.84	0.83	0.75	0.77	0.77	0.77	0.76	0.76	0.74
	8	S/N	3.67	8.00	9.73	13.50	18.21	28.18	28.68	38.34	43.91	35.10	35.64	39.17
		S/B	4.86	15.47	24.03	27.94	30.25	31.42	32.33	32.28	32.31	31.91	31.57	31.35
		SW	0.73	5.03	6.77	10.56	15.30	25.31	25.82	35.44	40.88	32.19	32.71	36.13
		Z'	-0.67	0.24	0.38	0.55	0.66	0.77	0.77	0.82	0.84	0.81	0.81	0.82

Determination of Minimum Biofilm Inhibitory Concentration (MBIC)

Vancomycin derivatives stock solutions were prepared in sterile water or water+5% DMSO to a concentration of 1 mg/mL. MBIC assay was performed by the broth microdilution method in 96-well round bottom microplate adapted from Clinical and Laboratory Standards Institute (CLSI) guideline.[17]

Bacterial suspension was diluted with BHIG broth to obtain an inoculum of 1 x 10^6 CFU/mL. Two-fold serial dilutions of the compounds (ranging from 128 µg/mL to 0.5 µg/mL) were prepared in BHIG to a final volume of 50 µL. The bacterial suspension (50 µL) was added into each well on the microtiter plate for inoculation, corresponding to approximately 5x10^5 CFU/mL. The plates were incubated without shaking for 24 h at 37 °C, and MBIC was defined by performing the optimized resazurin assay (Table S3). CFUs were also determined after resazurin assay completion, in order to compare the results. The experiments were performed in triplicates.

Table S6. MBIC results for vancomycin derivatives

Bacterial strain	Antibiotic	MIC (µg/mL)	MBIC (µg/mL)	
			Resazurin (decrease in RFU)	CFU/mL (growth inhibition)
S. aureus ATCC 29213	**VAN**	1	2 (100%)	2 (100%)
	1a	2	4 (100%)	4 (100%)
	1b	4	4 (97%)	4 (100%)
	1c	2	4 (99%)	4 (100%)
	2a	1	4 (99%)	4 (100%)
	2b	0.25	1 (100%)	1 (100%)
	2c	0.25	2 (99%)	2 (100%)
	3a	0.5	1 (99%)	1 (100%)
	3b	8	16 (97%)	16 (100%)
	4a	4	8 (100%)	8 (100%)
	4b	4	16 (99%)	16 (100%)
	4c	8	32 (98%)	32 (100%)
	4d	4	8 (97%)	8 (100%)
	5a	1	4 (100%)	4 (100%)
	5b	2	1 (99%)	1 (100%)
	5c	0.5	2 (99%)	2 (100%)
	6	4	8 (100%)	8 (100%)
	7	1-2	2 (96%)	2 (100%)
S. aureus ATCC 43300 (MRSA)	**VAN**	1-2	2 (100%)	2 (100%)
	1a	2	4 (100%)	4 (100%)
	1b	2	4 (99%)	4 (100%)
	1c	2	4 (99%)	4 (100%)
	2a	1	4 (100%)	4 (100%)
	2b	0.125 – 0.5	1 (100%)	1 (100%)
	2c	0.125	2 (99%)	2 (100%)

	3a	0.5-1	1 (98%)	1 (100%)
	3b	4	8 (100%)	8 (100%)
	4a	4	8 (99%)	8 (100%)
	4b	4	8 (100%)	8 (100%)
	4c	8	16 (99%)	16 (100%)
	4d	4	8 (97%)	8 (100%)
	5a	0.5	4 (99%)	4 (100%)
	5b	0.5-1	1 (97%)	1 (100%)
	5c	0.5	2 (94%)	2 (100%)
	6	2-4	16 (95%)	16 (100%)
	7	1-2	4 (99%)	4 (100%)

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