

SUPPORTING INFORMATION

Synthesis and evaluation of hetero- and homo-dimers of ribosome-targeting antibiotics: Antimicrobial activity, *in vitro* inhibition of translation, and drug resistance

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Table S1. Relative activity (%) for TOB and CAM derivatives compared to the parent drugs^a TOB (**1**) and CAM (**3**) against various drug-modifying enzymes.

Compound #	AAC(6')/APH(2'')	AAC(3)-IV	AAC(6')-Ib'	AAC(2')-Ic	Eis	ANT(4')
16	34 ± 1	200 ± 26	39 ± 11	244 ± 10	404 ± 9	90 ± 10
18	30 ± 1	143 ± 21	20 ± 5	165 ± 1	253 ± 1	38 ± 1
22	34 ± 2	142 ± 18	31 ± 4	156 ± 3	269 ± 2	28 ± 2
20	80 ± 3	184 ± 28	37 ± 4	203 ± 16	287 ± 14	63 ± 9
6	-- ^b	-- ^b	-- ^b	-- ^b	-- ^b	49 ± 4
Compound #	CPT	CNR	CAT _I			
13	51 ± 8	106 ± 41	-- ^b			
22	91 ± 21	117 ± 43	70 ± 2			
26	139 ± 21	220 ± 76	73 ± 3			
24	96 ± 12	174 ± 74	69 ± 4			
20	110 ± 12	76 ± 28	82 ± 2			
23	97 ± 14	133 ± 43	10 ± 1			
25	33 ± 7	10 ± 1	3 ± 1			

^aTOB (**1**) and CAM (**3**) activity were set at 100% for comparison purposes.

^b-- indicates not tested as the assay could not be utilized as it detects free thiols.

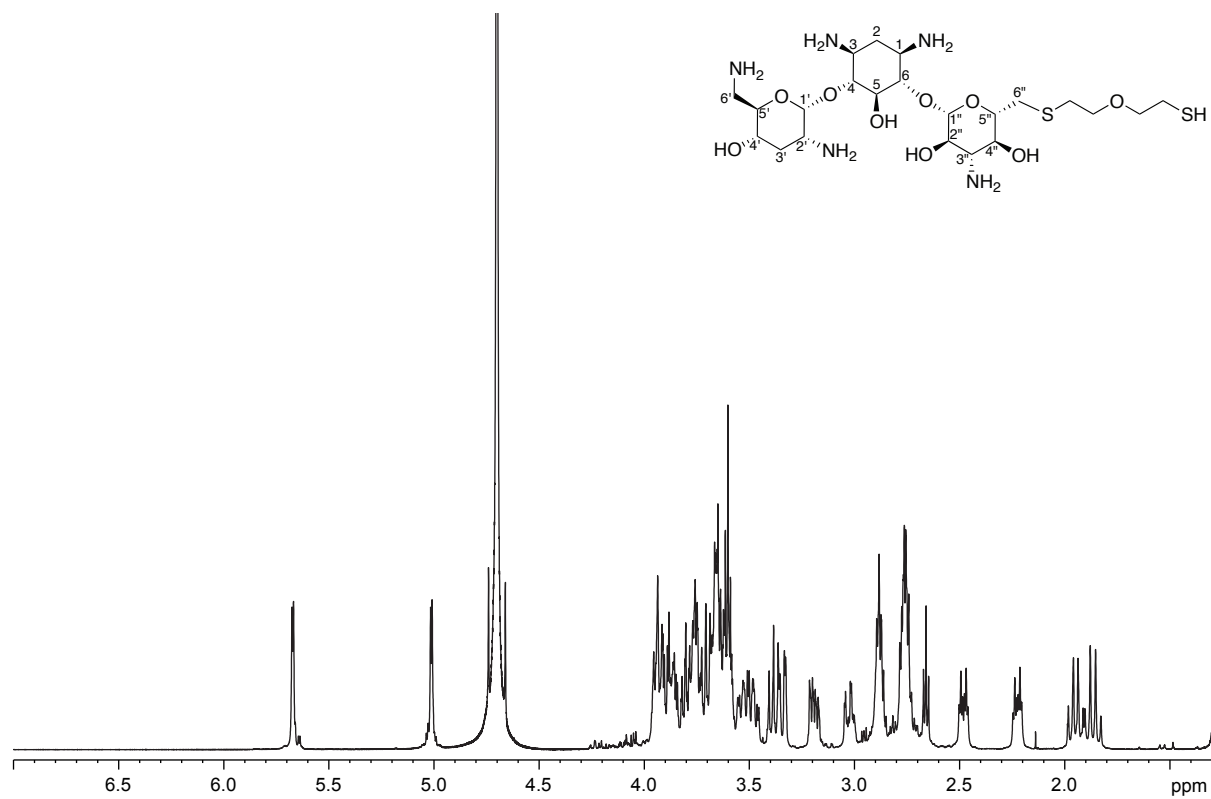


Fig. S1. ^1H NMR in D_2O for compound 6.

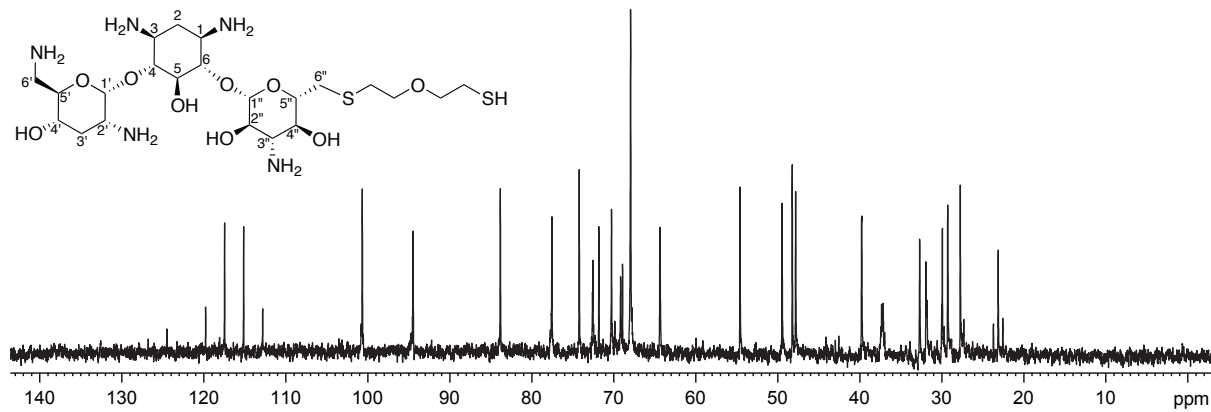


Fig. S2. ^{13}C NMR in D_2O for compound 6.

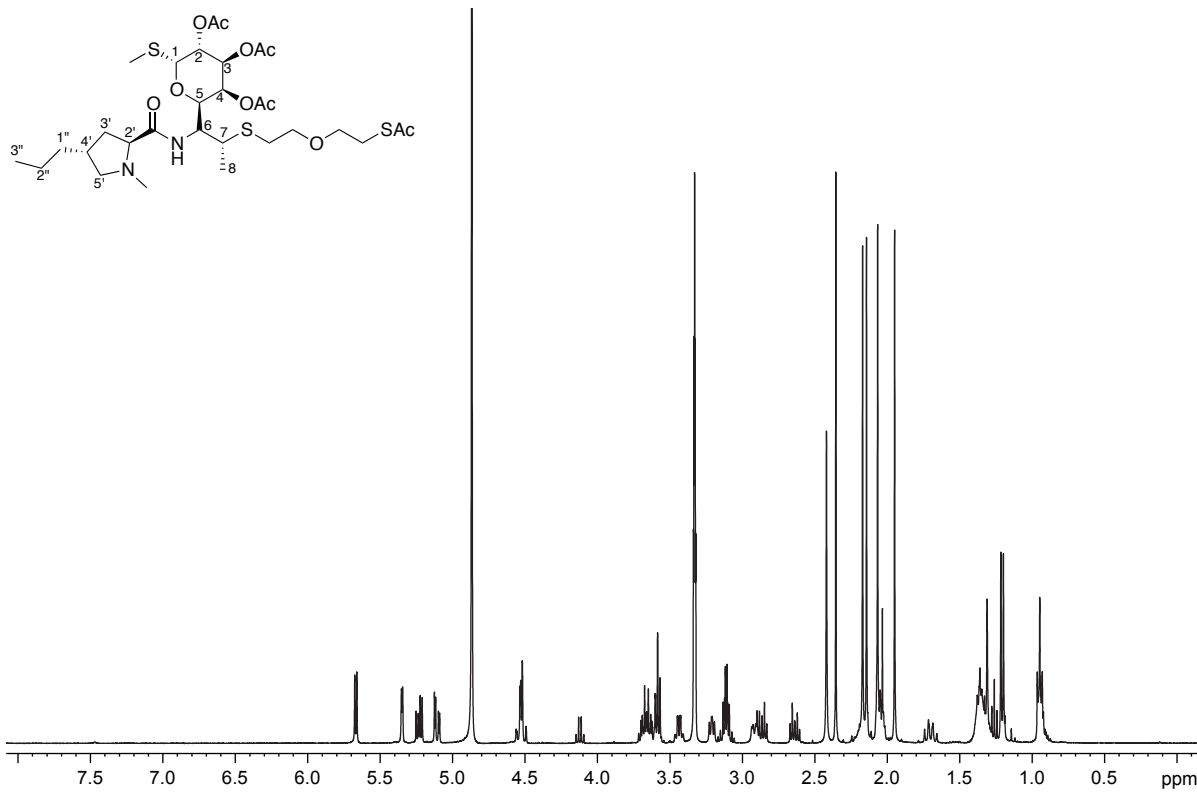


Fig. S3. ^1H NMR in CD_3OD for compound **8**.

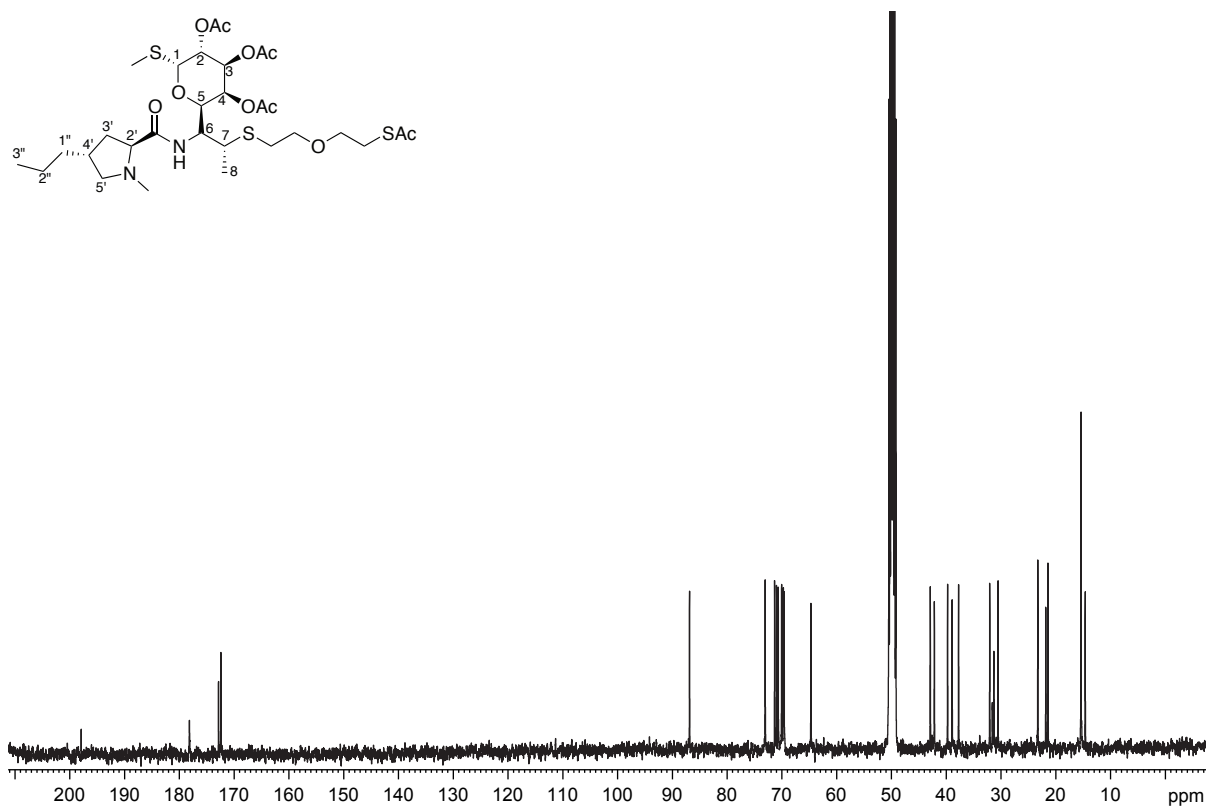


Fig. S4. ^{13}C NMR in CD_3OD for compound **8**.

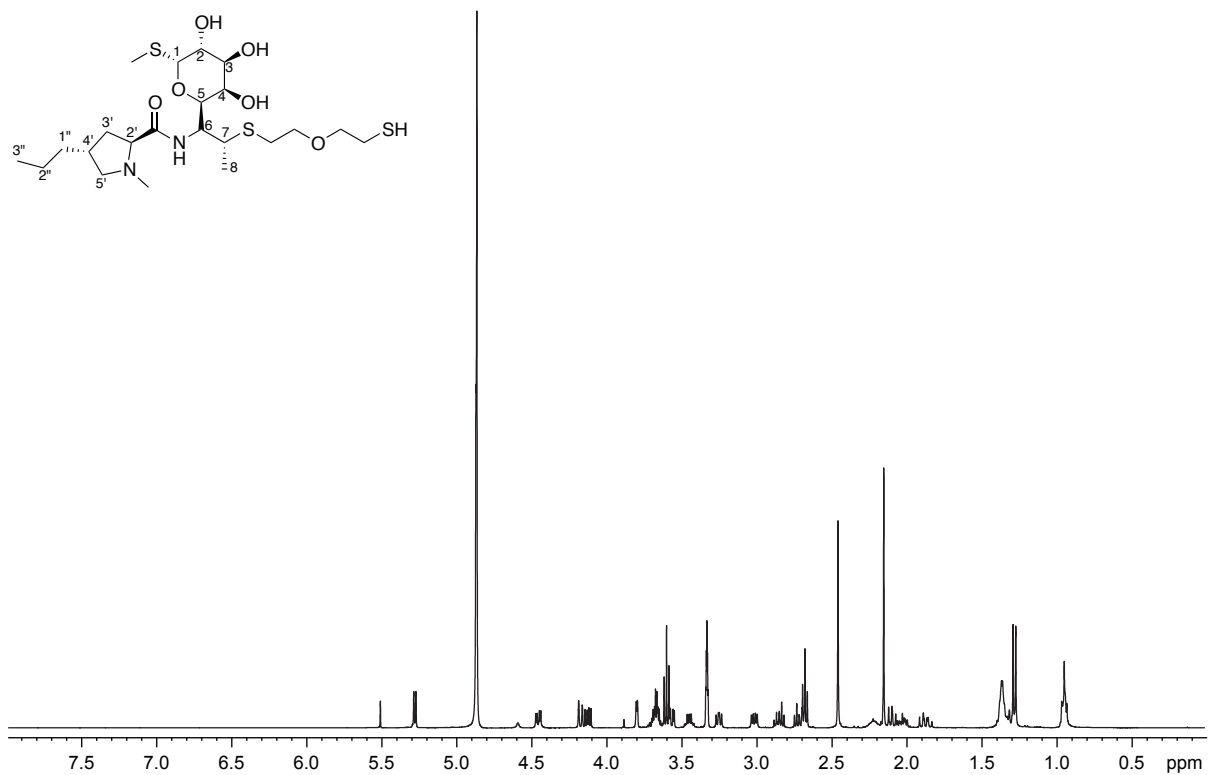


Fig. S5. ^1H NMR in CD_3OD for compound **9**.

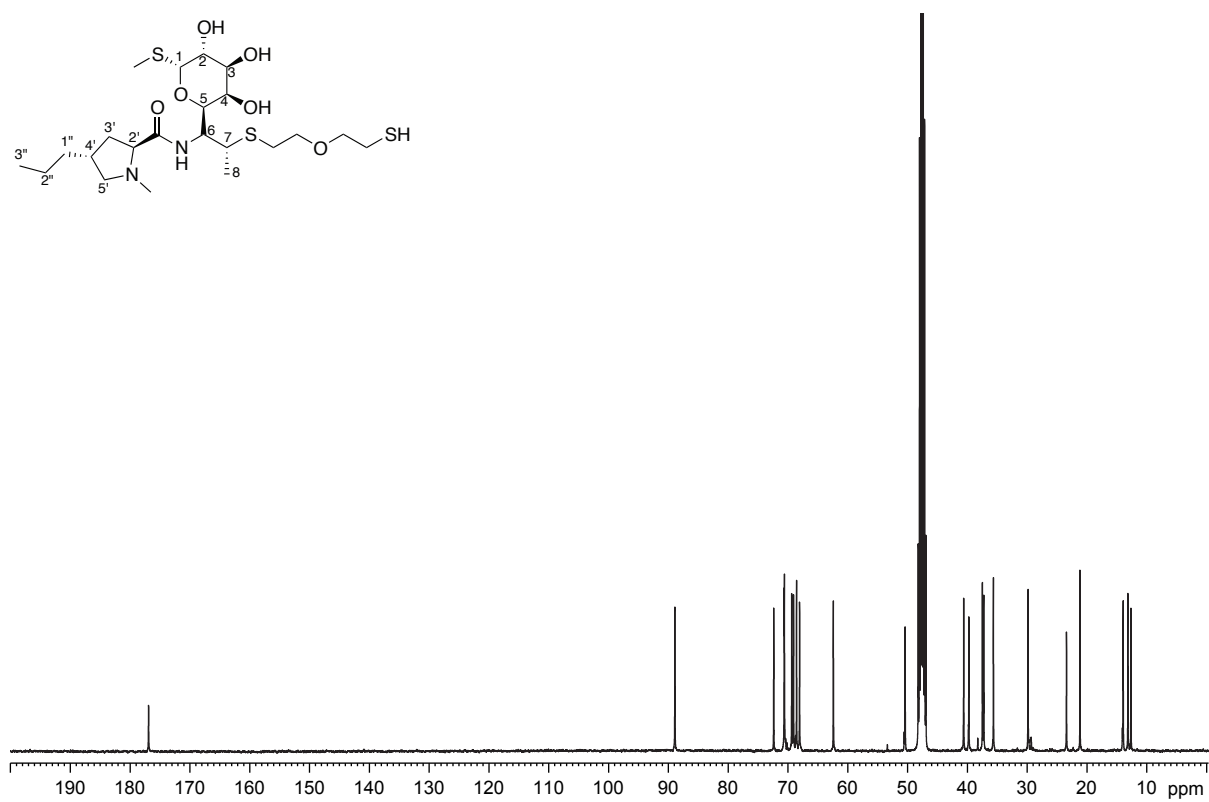


Fig. S6. ^{13}C NMR in CD_3OD for compound **9**.

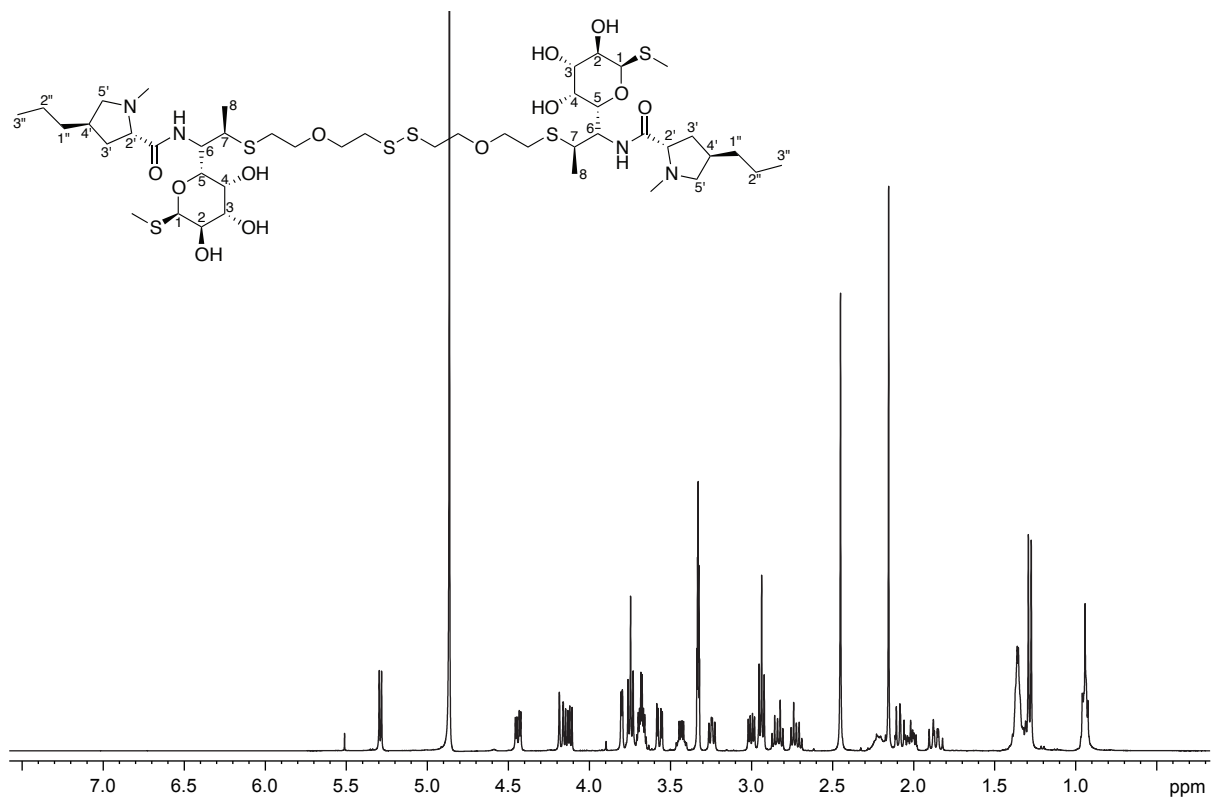


Fig. S7. ^1H NMR in CD_3OD for compound **10**.

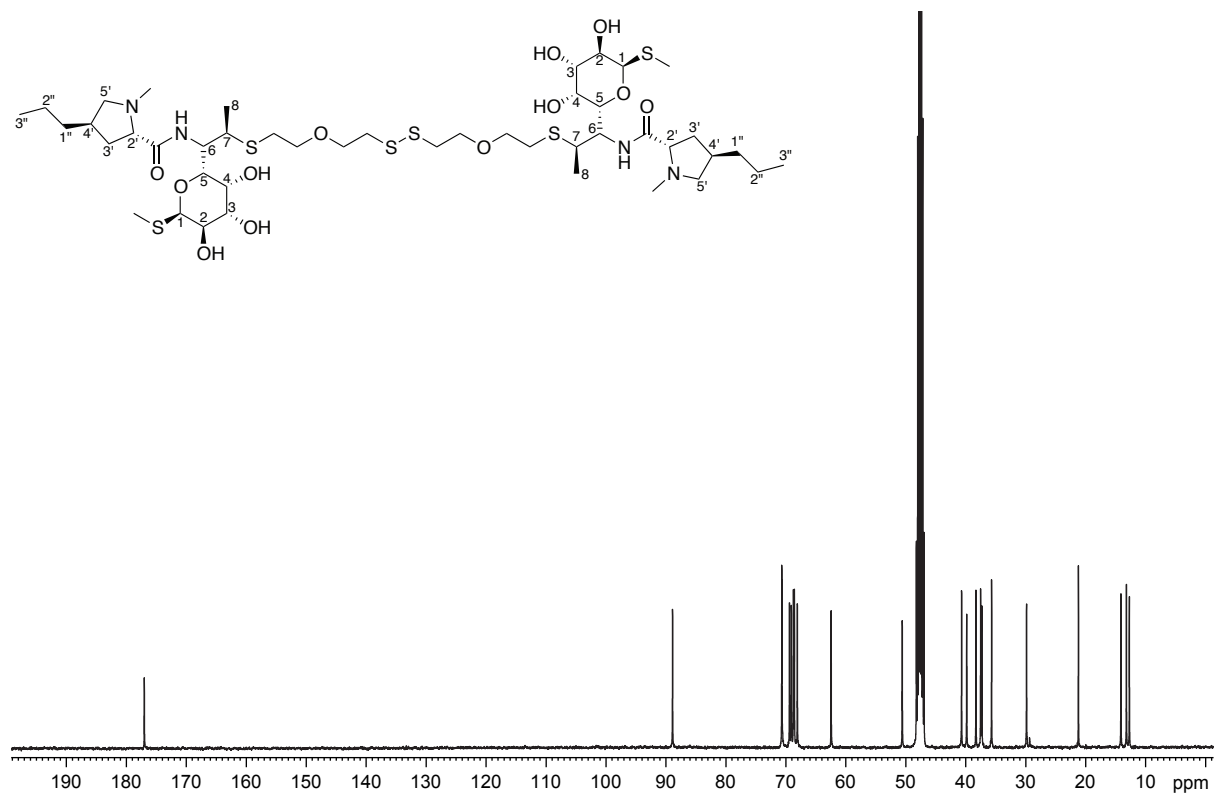
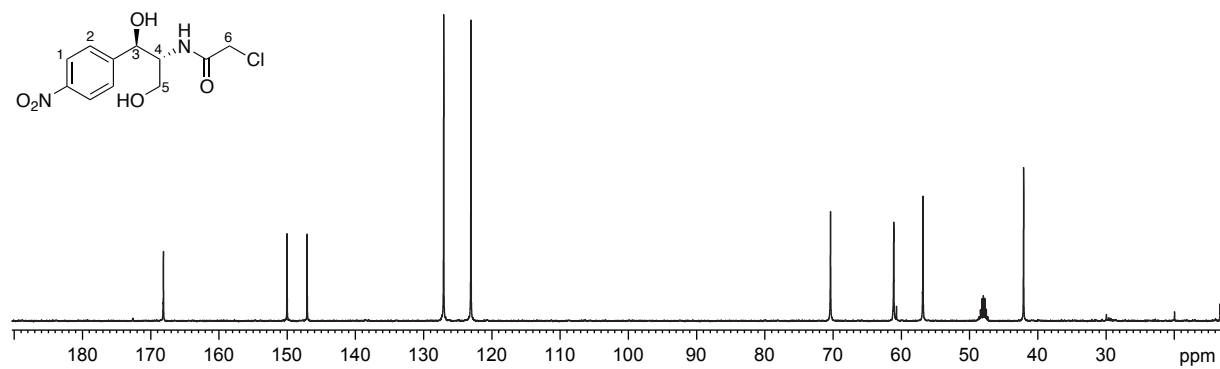
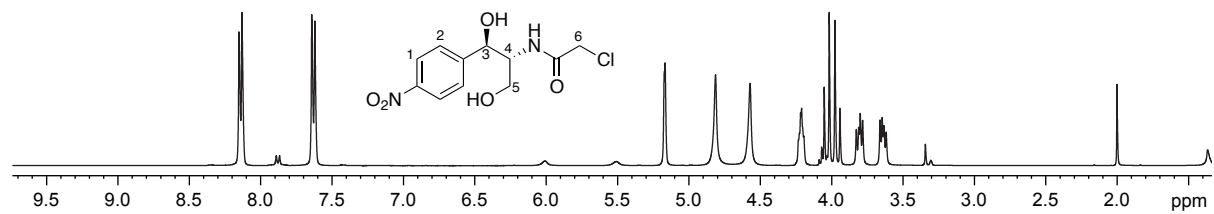


Fig. S8. ^{13}C NMR in CD_3OD for compound **10**.



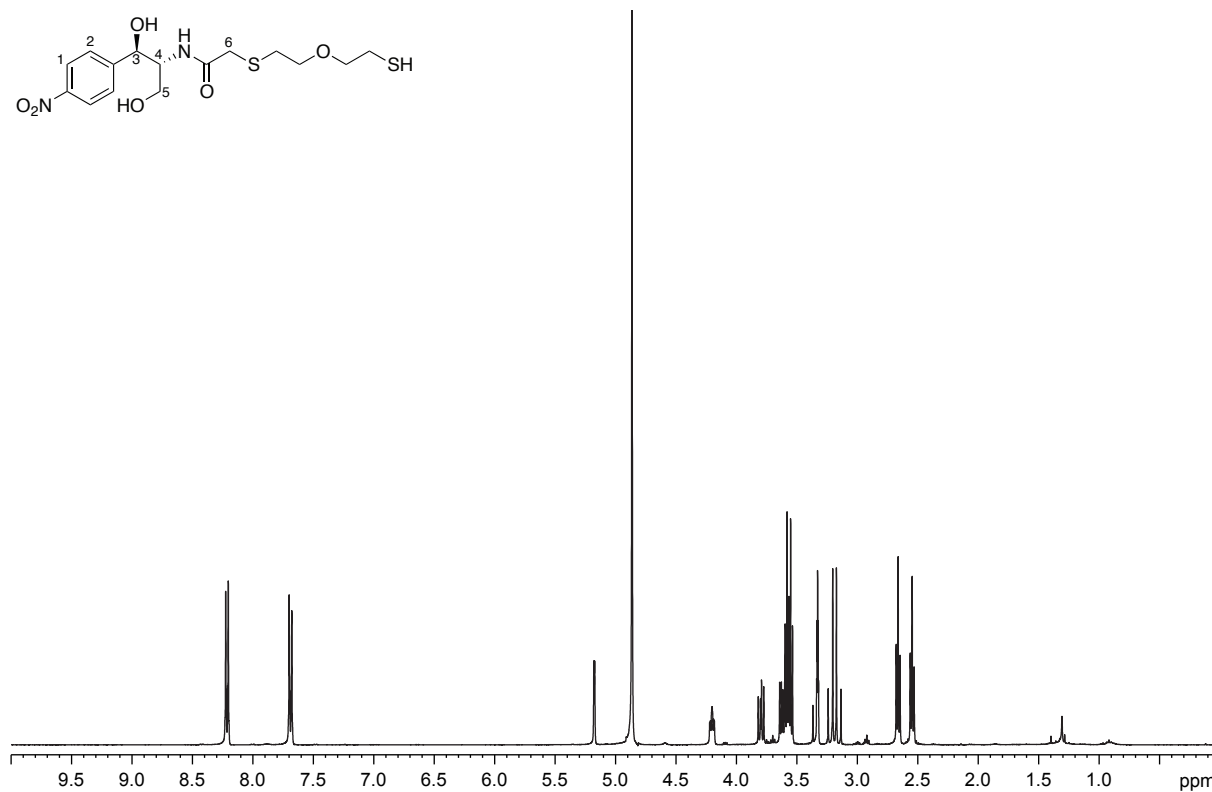
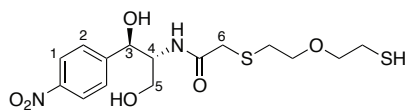


Fig. S11. ^1H NMR in CD_3OD for compound 13.

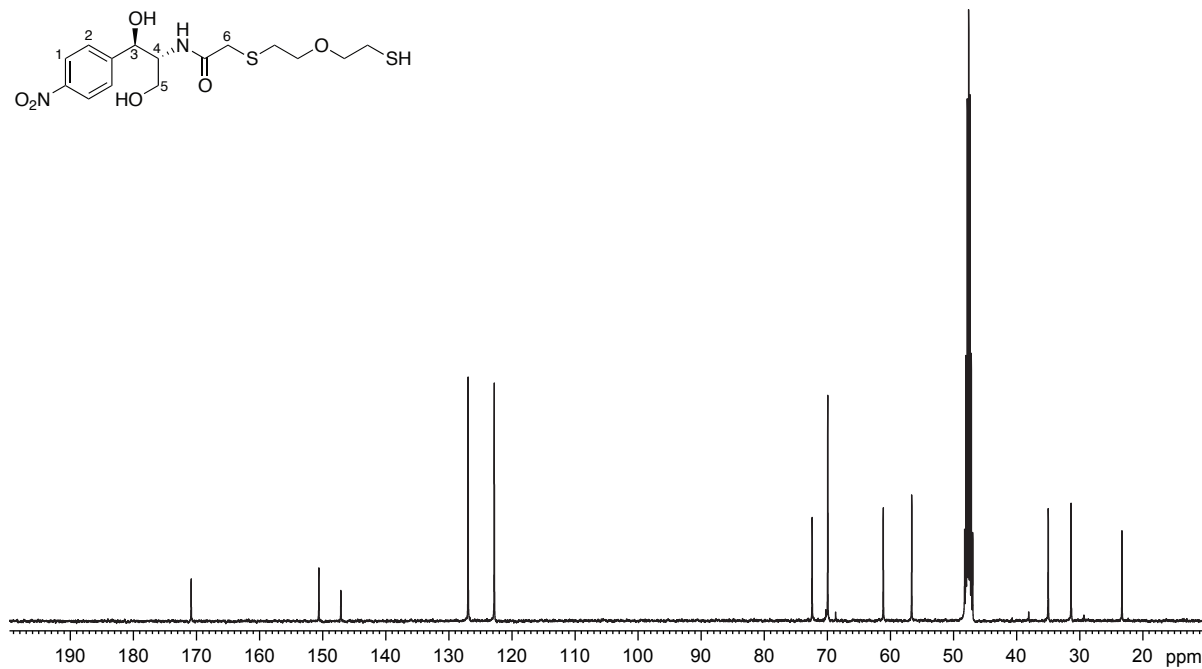
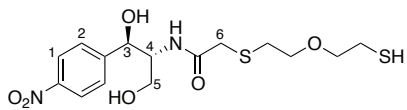
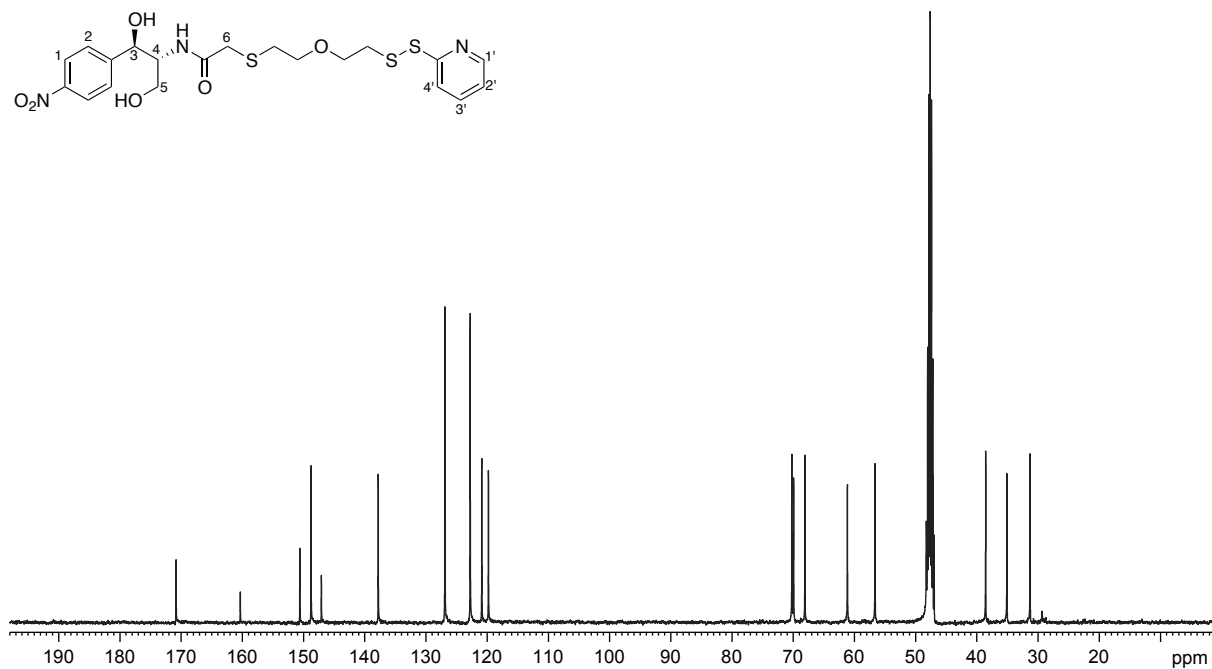
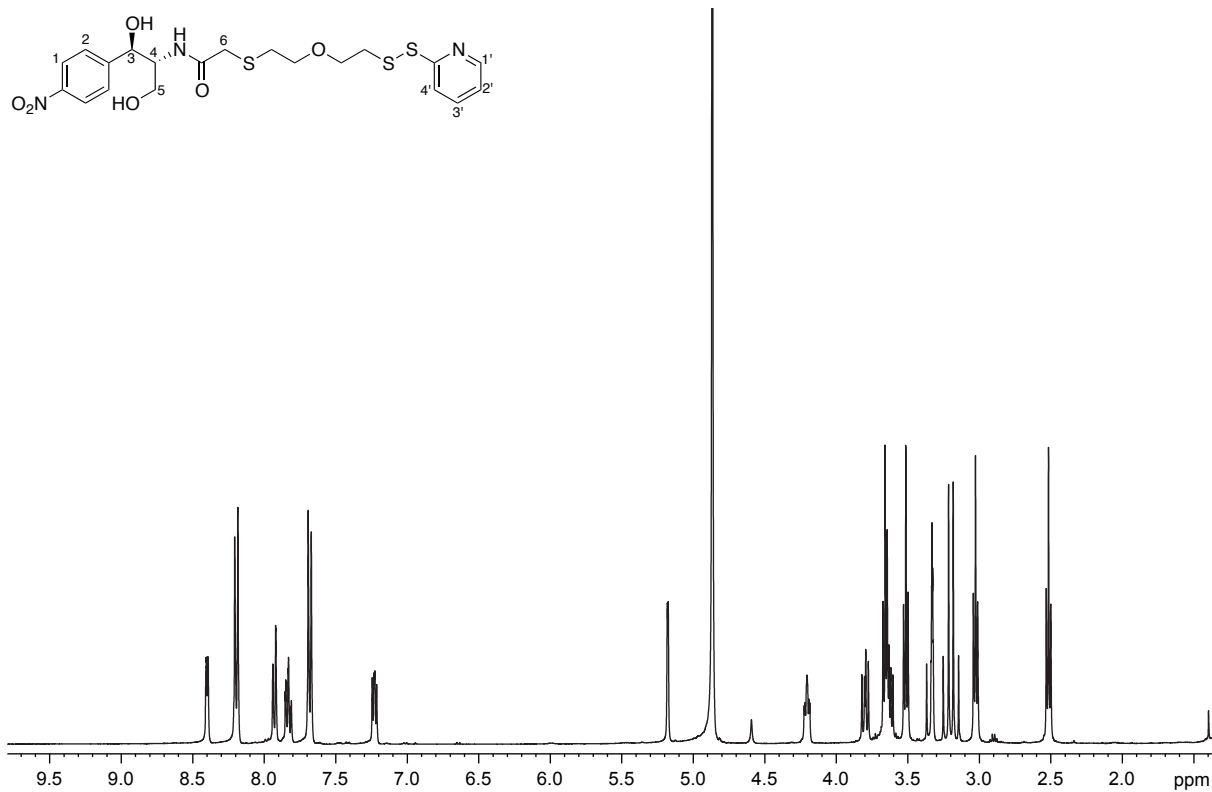


Fig. S12. ^{13}C NMR in CD_3OD for compound 13.



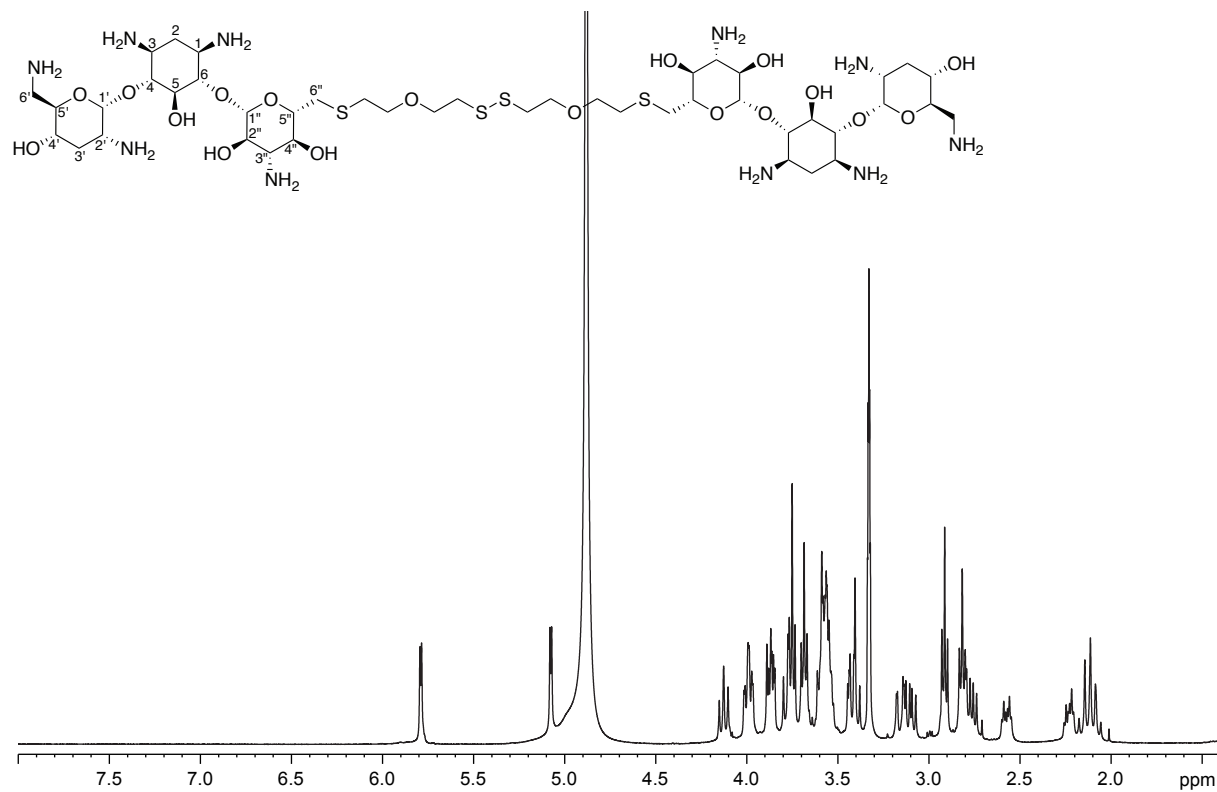


Fig. S15. ^1H NMR in CD_3OD for compound **16**.

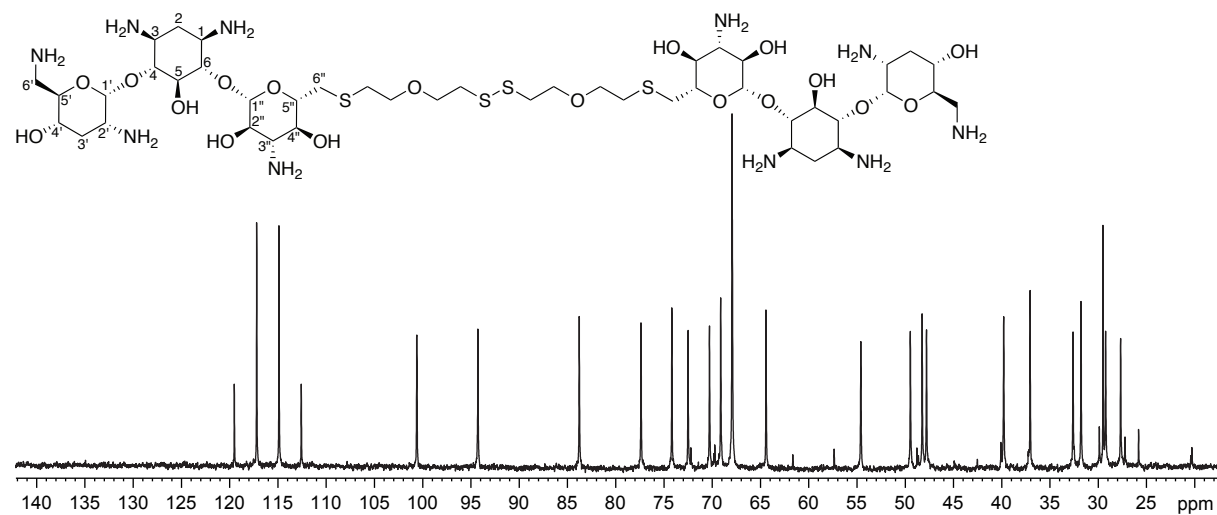


Fig. S16. ^{13}C NMR in CD_3OD for compound **16**.

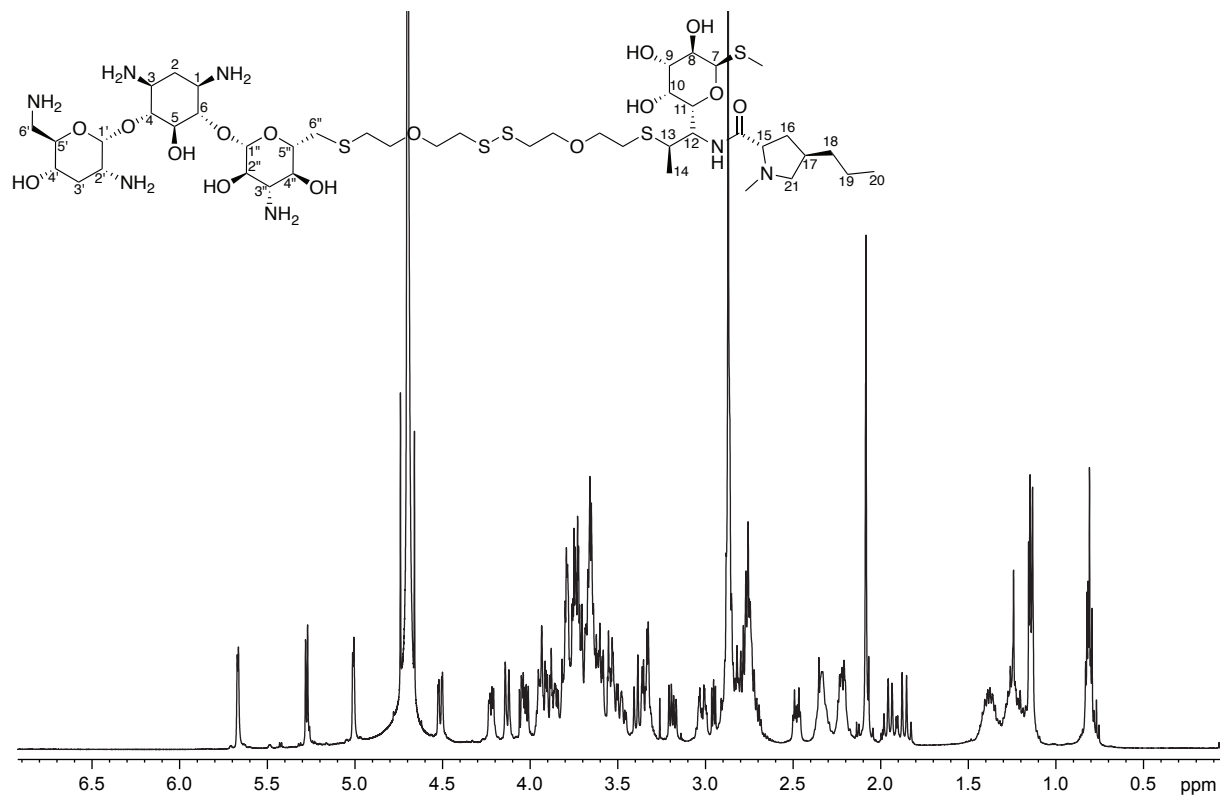


Fig. S17. ¹H NMR in D₂O for compound 18.

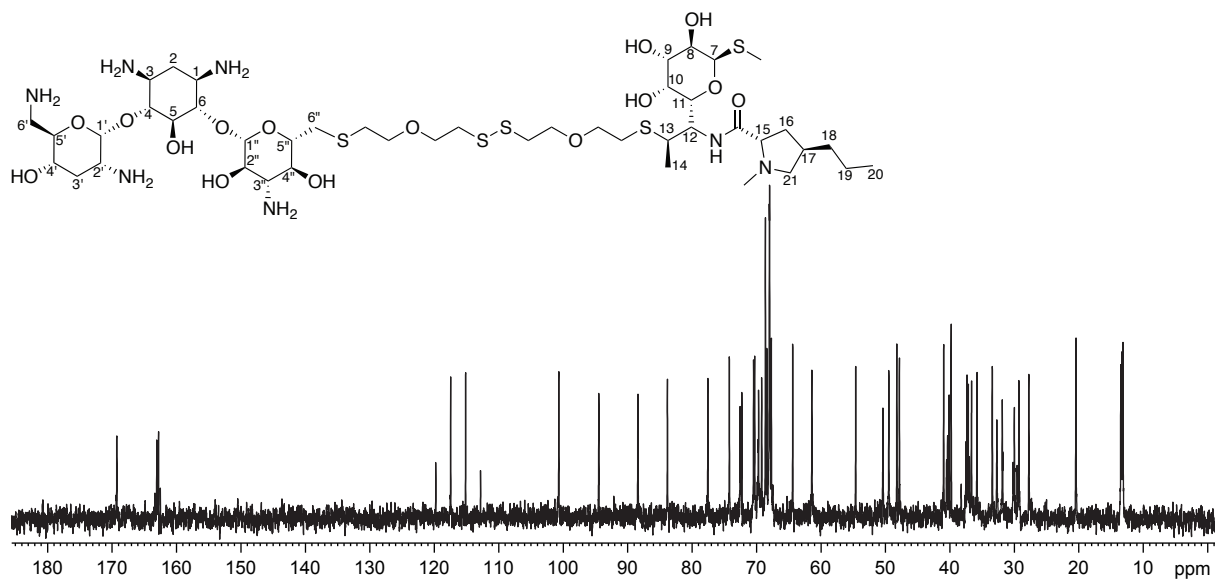


Fig. S18. ¹³C NMR in D₂O for compound 18.

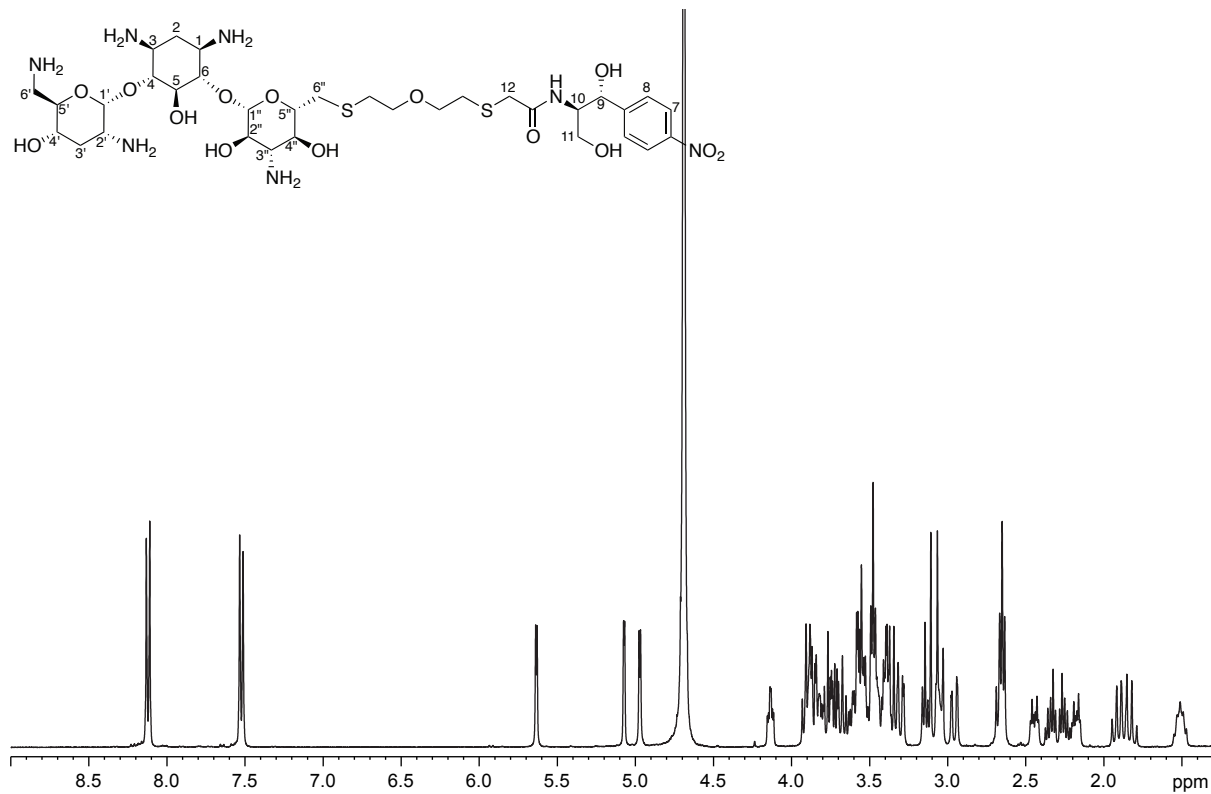


Fig. S19. ^1H NMR in D_2O for compound 20.

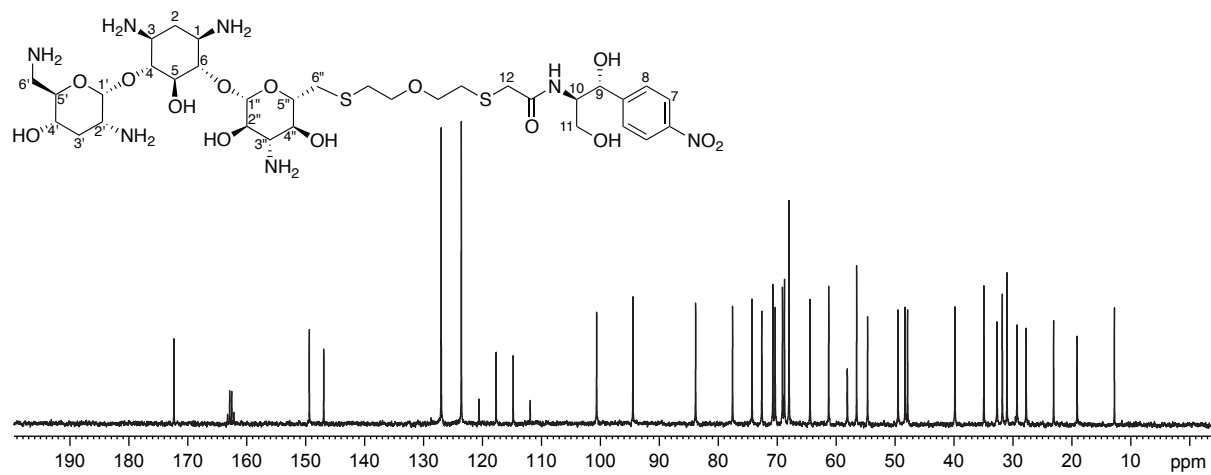


Fig. S20. ^{13}C NMR in D_2O for compound 20.

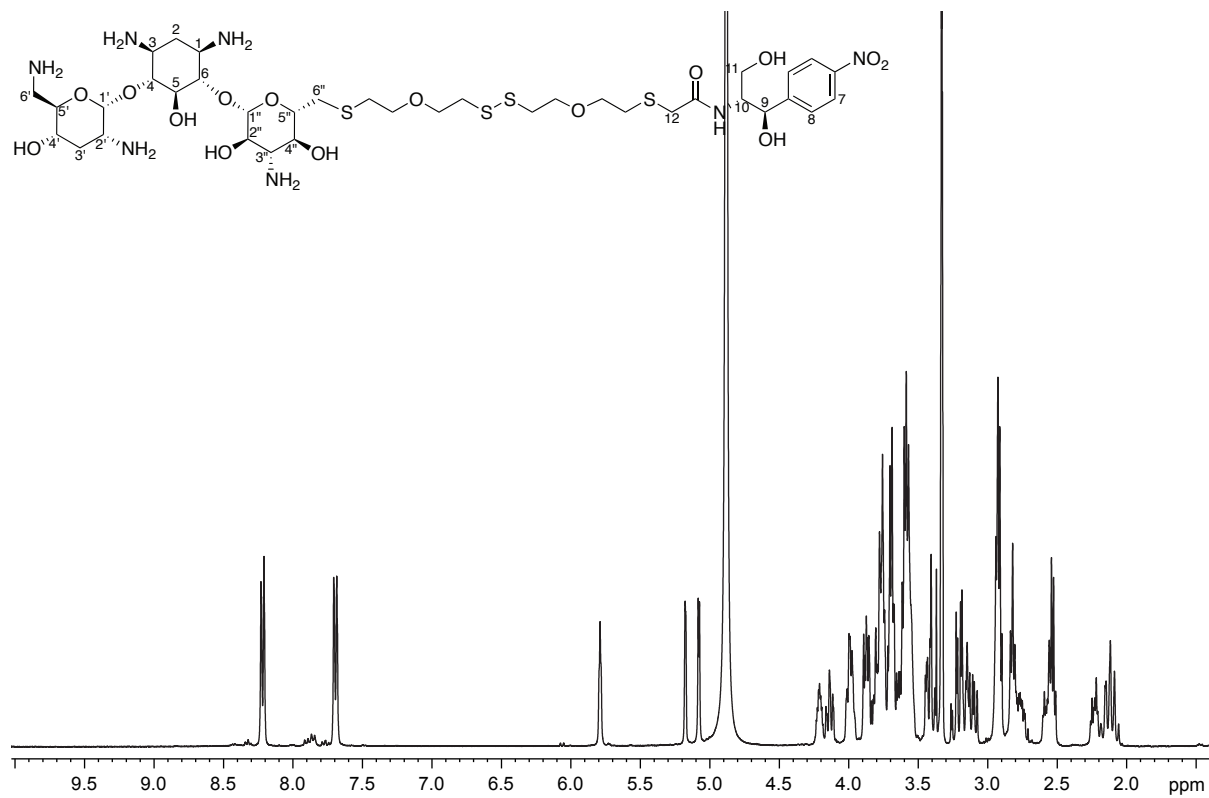


Fig. S21. ¹H NMR in CD₃OD for compound 22.

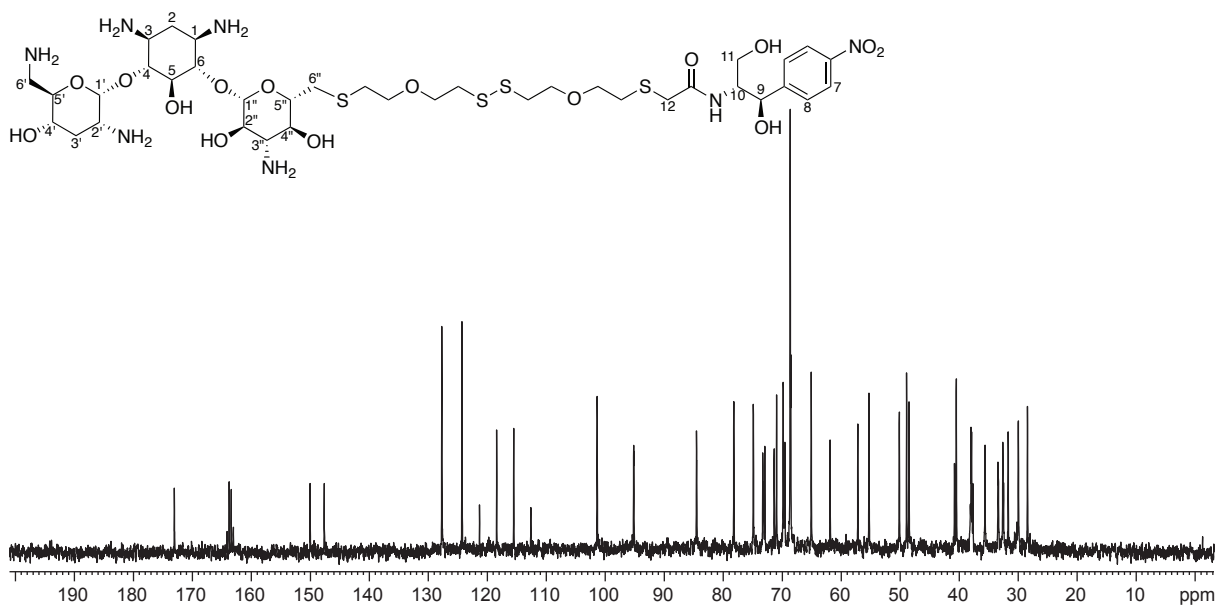


Fig. S22. ¹³C NMR in D₂O for compound 22.

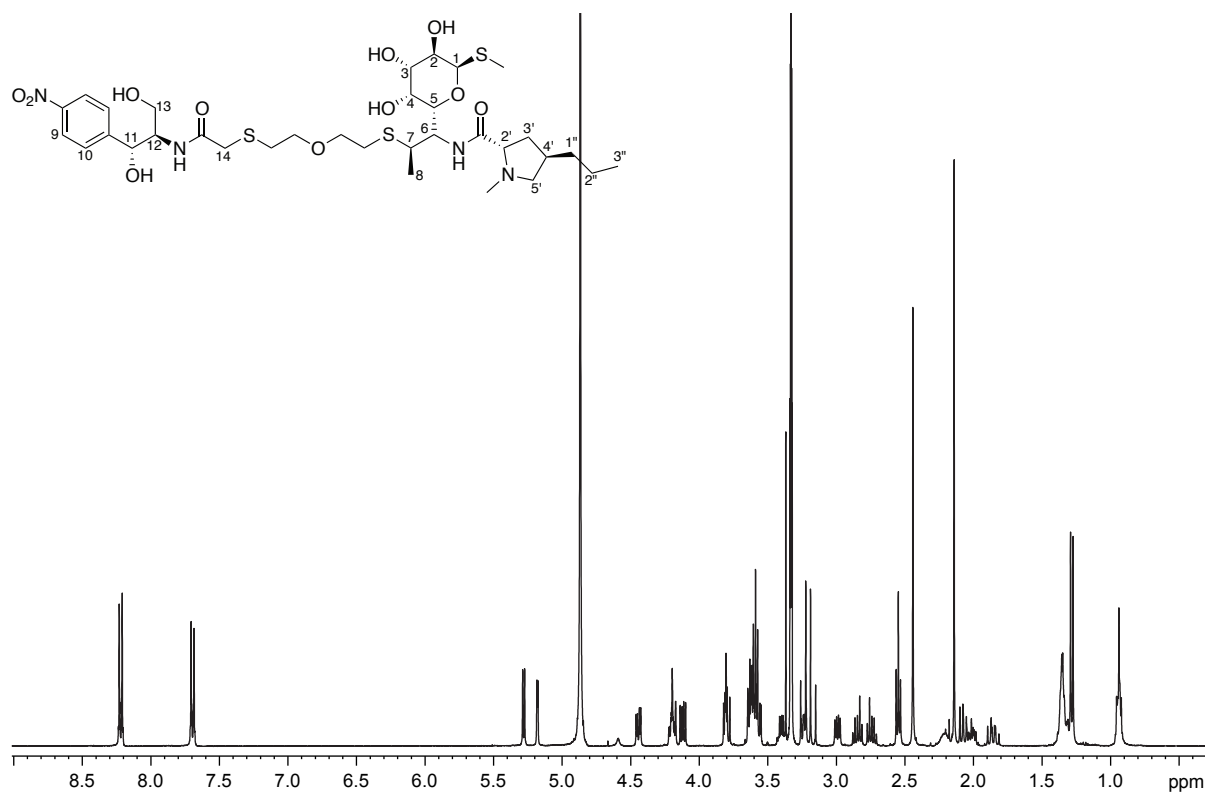


Fig. S23. ^1H NMR in CD_3OD for compound **23**.

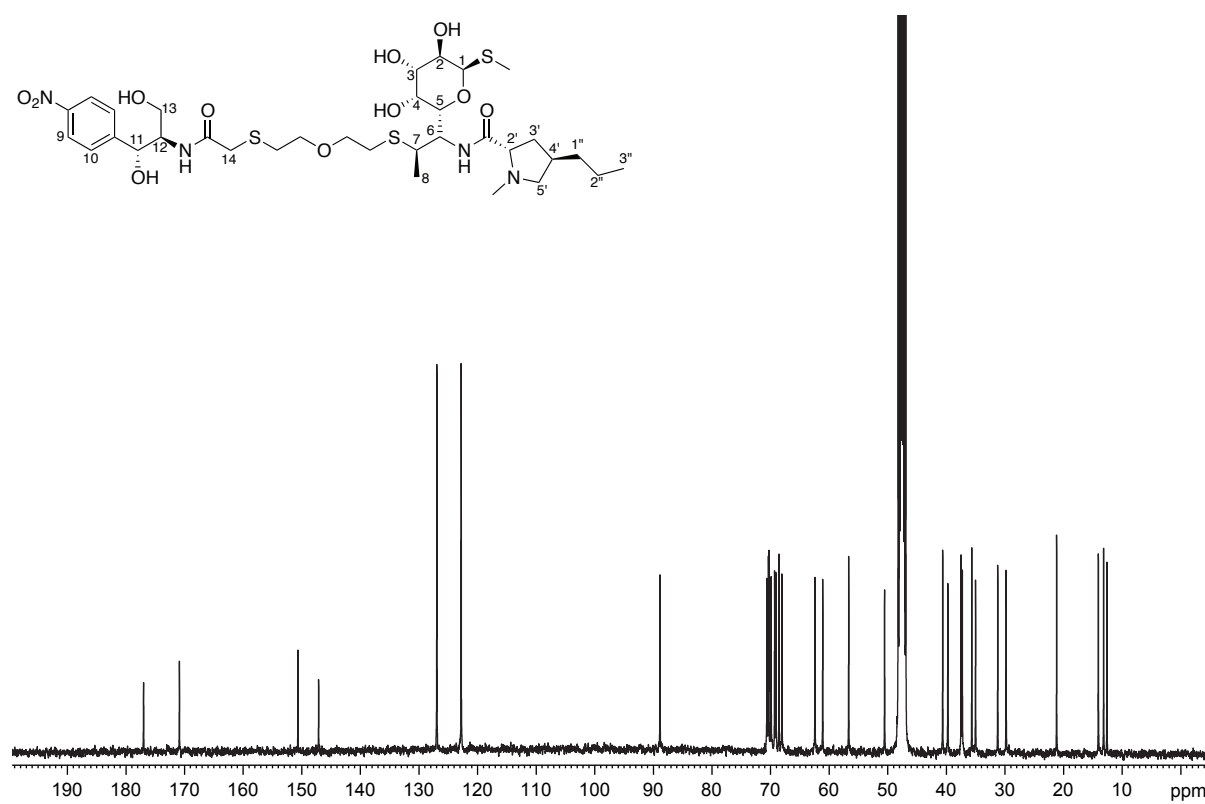


Fig. S24. ^{13}C NMR in CD_3OD for compound **23**.

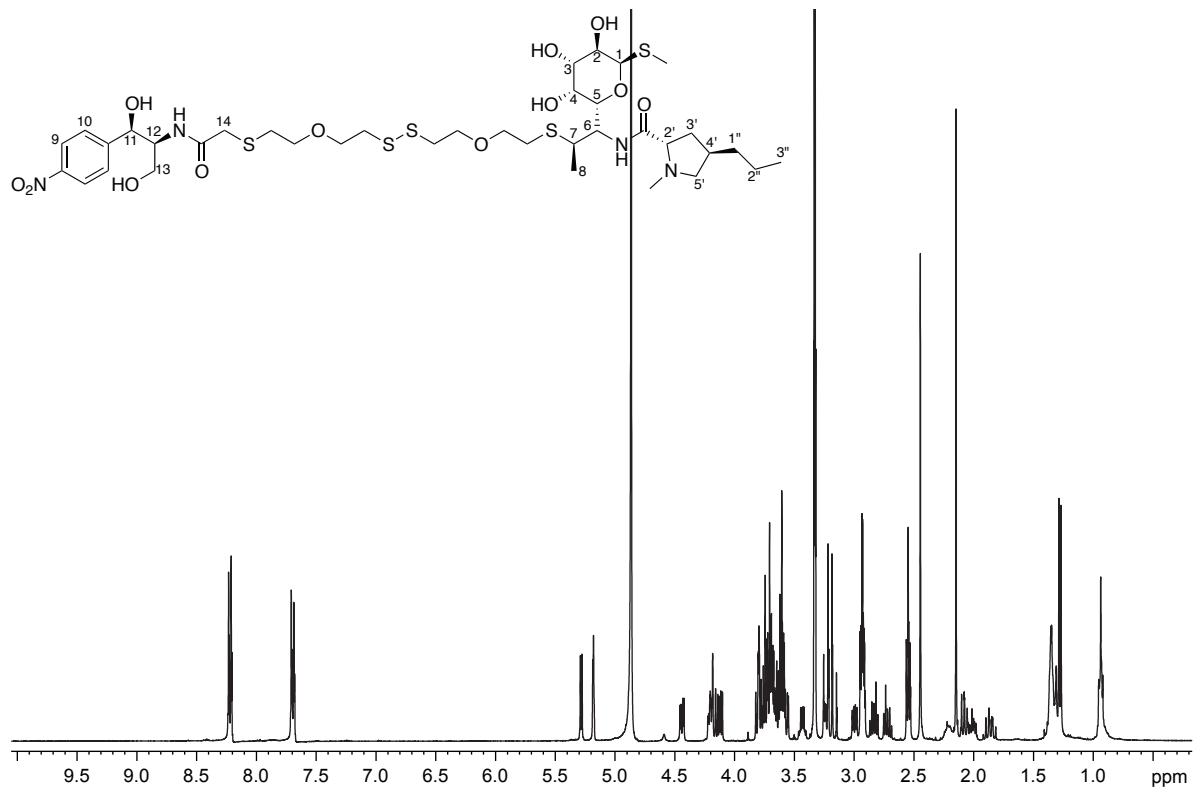


Fig. S25. ¹H NMR in CD₃OD for compound 24.

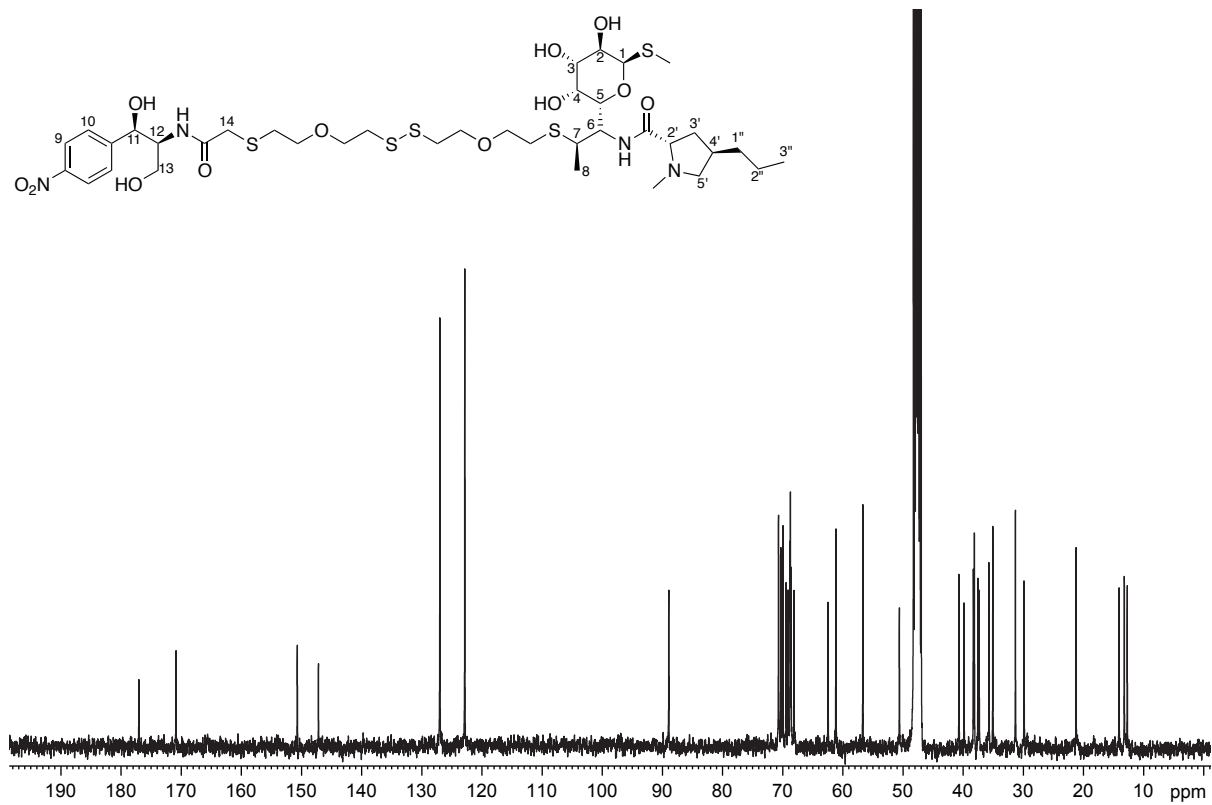


Fig. S26. ¹³C NMR in CD₃OD for compound compound 24.

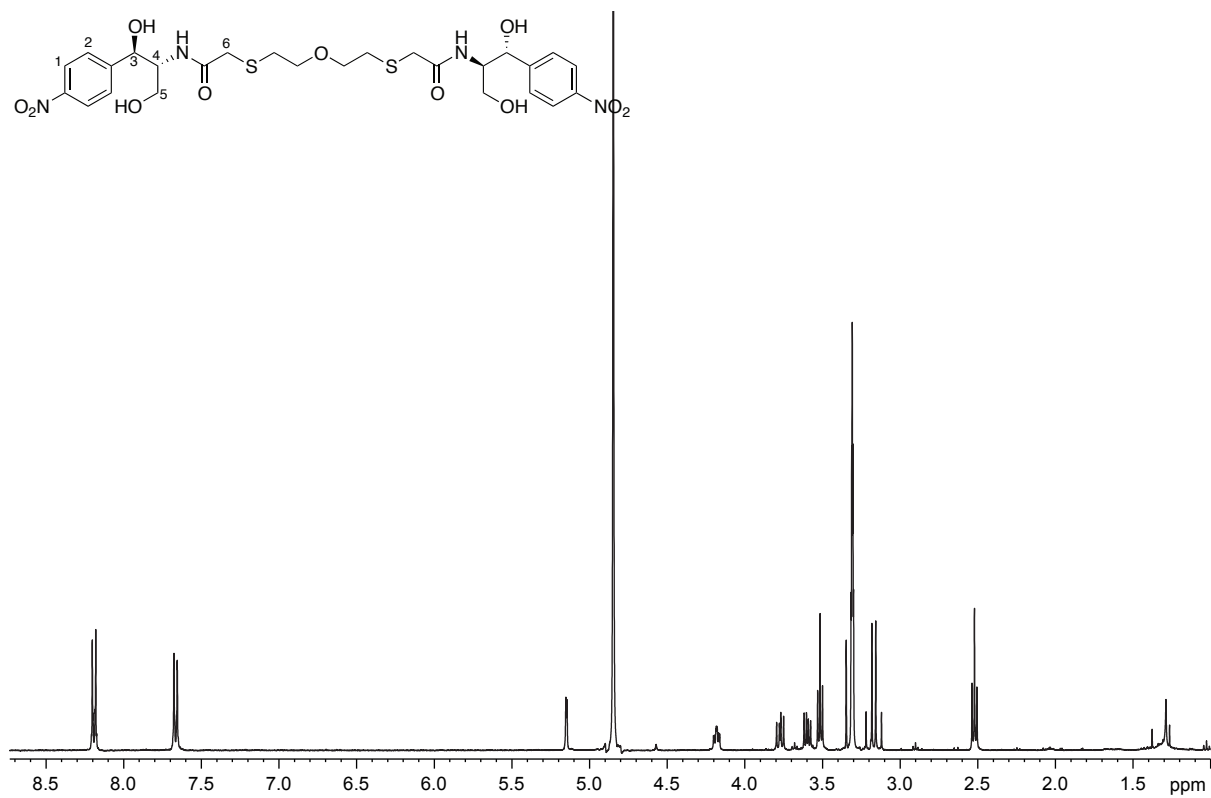


Fig. S27. ¹H NMR in CD₃OD for compound 25.

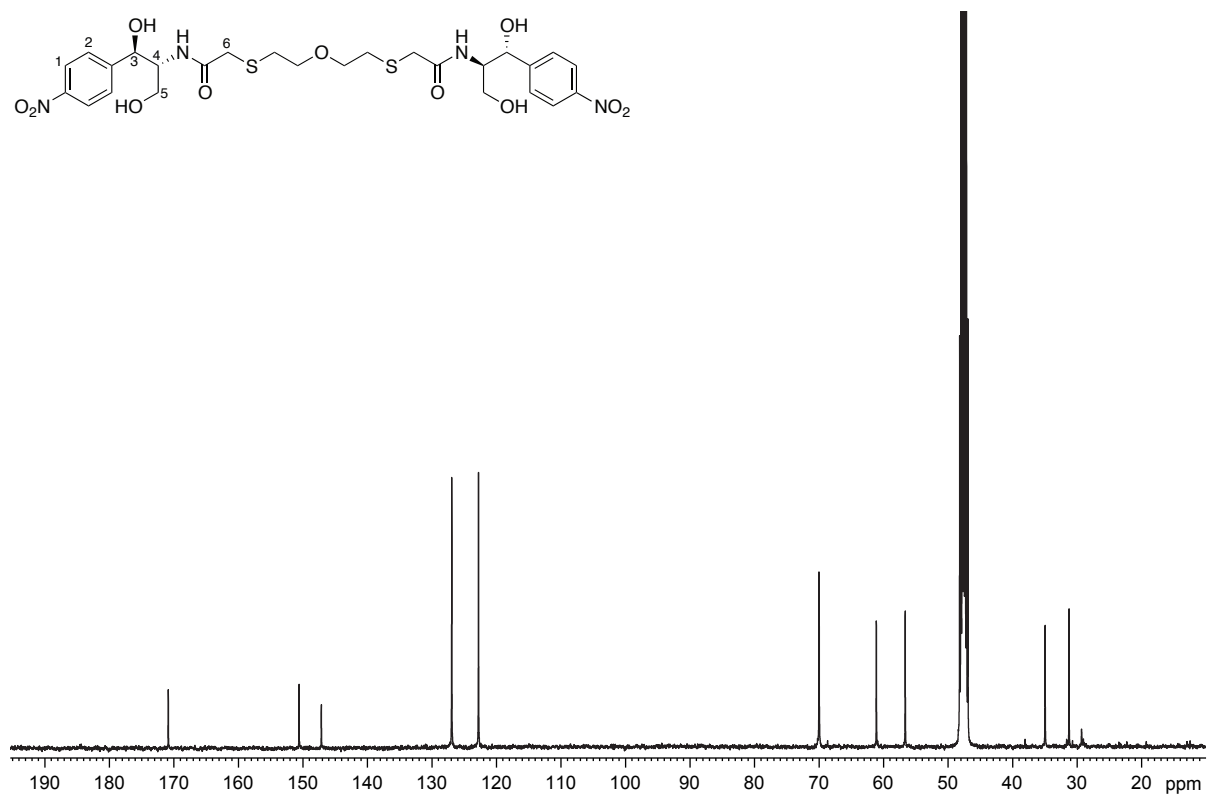


Fig. S28. ¹³C NMR in CD₃OD for compound 25.

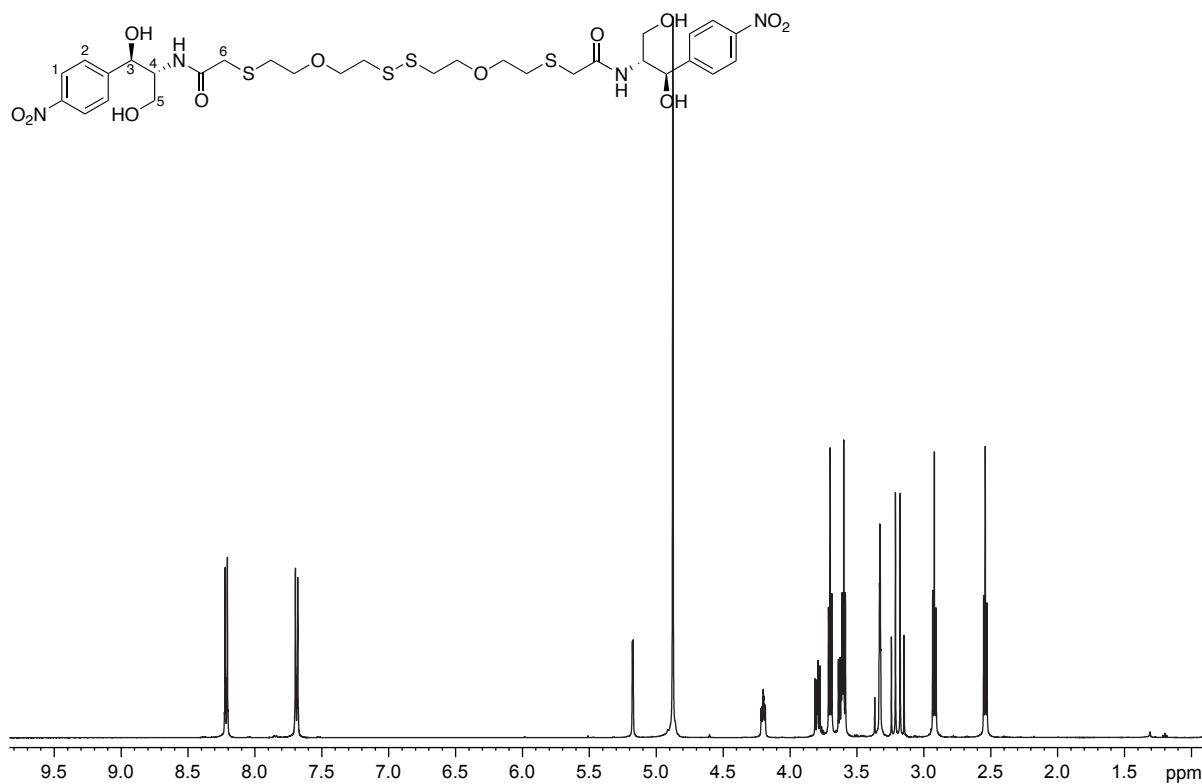


Fig. S29. ¹H NMR in CD₃OD for compound 26.

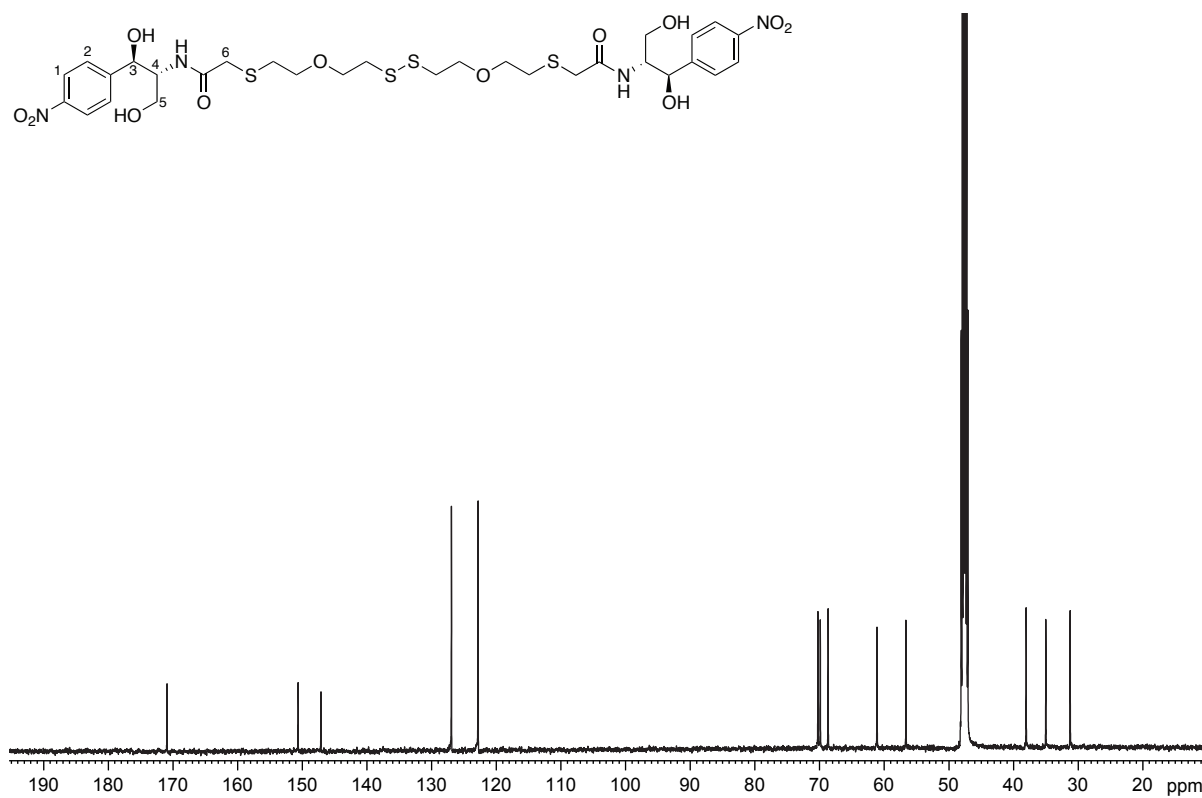


Fig. S30. ¹³C NMR in CD₃OD for compound 26.

Table S2. Purity of new dimers as determined by RP-HPLC (see Figs. S31-S39 below).

Compound #	Retention Time (min)	Purity (%)	λ max (nm)
10	13.19	97	210
16	14.36	97	294
18	9.82	96	245
20	8.08	99	345
22	9.70	>99	263
23	12.25	98	243
24	13.98	>99	295
25	13.06	98	374
26	15.24	97	384

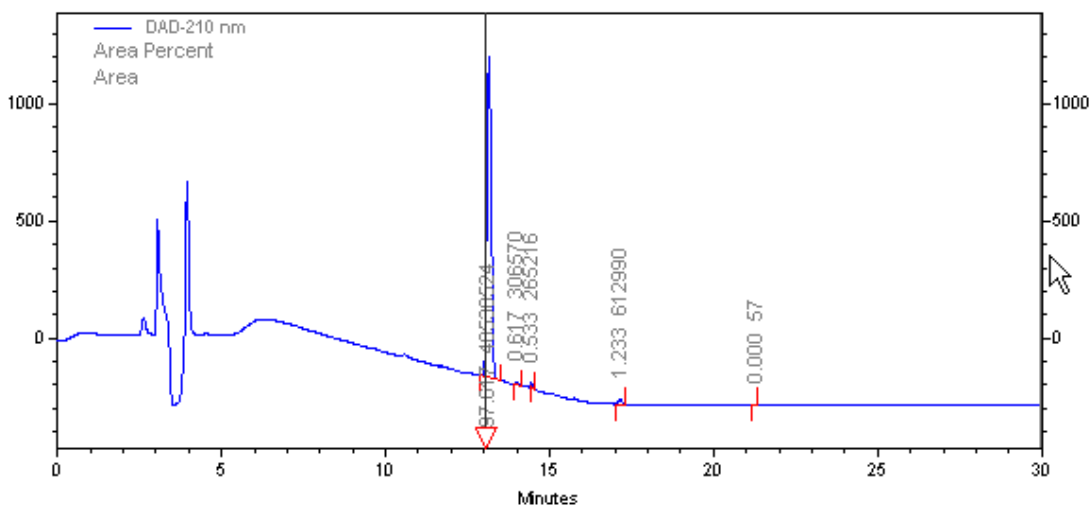


Fig. S31. RP-HPLC trace for compound 10.

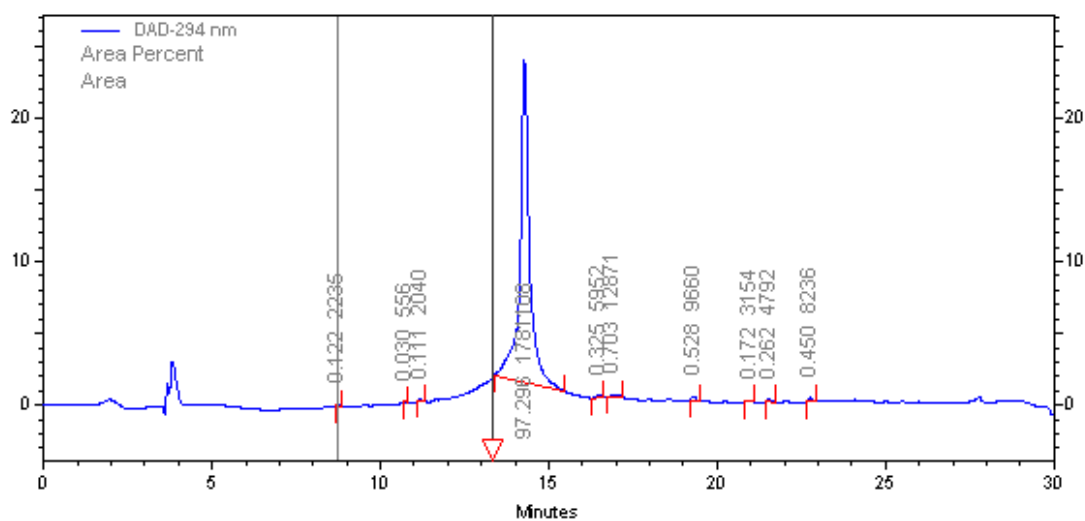


Fig. S32. RP-HPLC trace for compound 16.

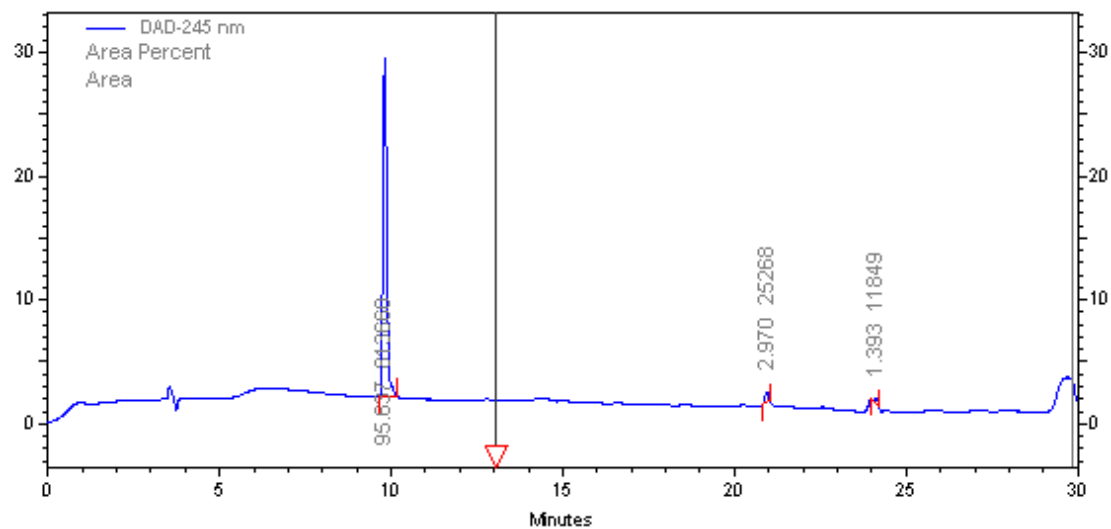


Fig. S33. RP-HPLC trace for compound 18.

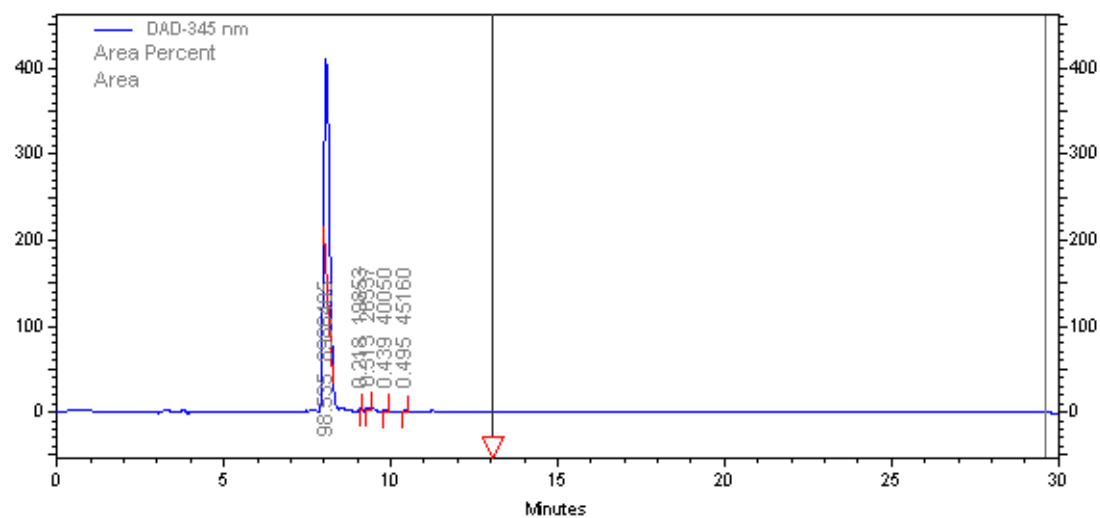


Fig. S34. RP-HPLC trace for compound 20.

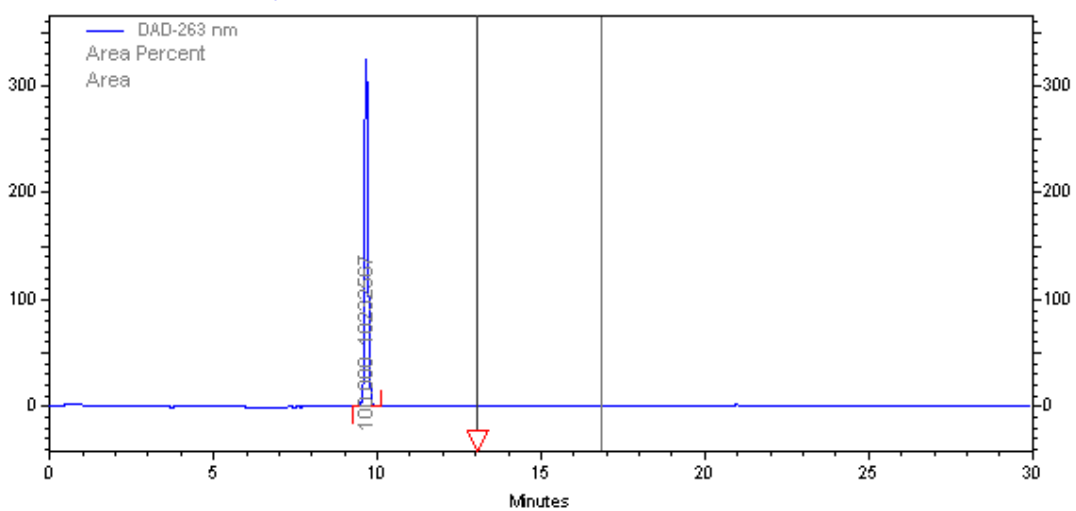


Fig. S35. RP-HPLC trace for compound 22.

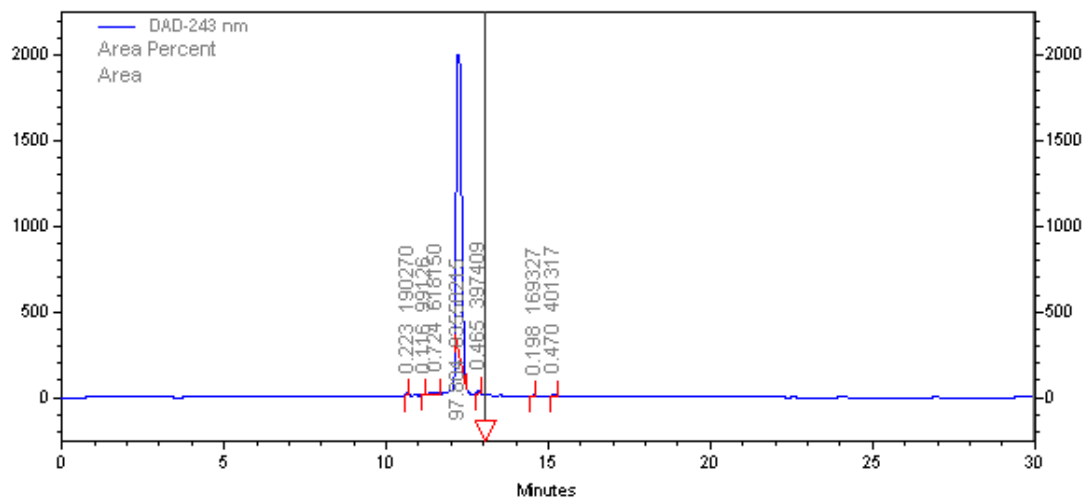


Fig. S36. RP-HPLC trace for compound 23.

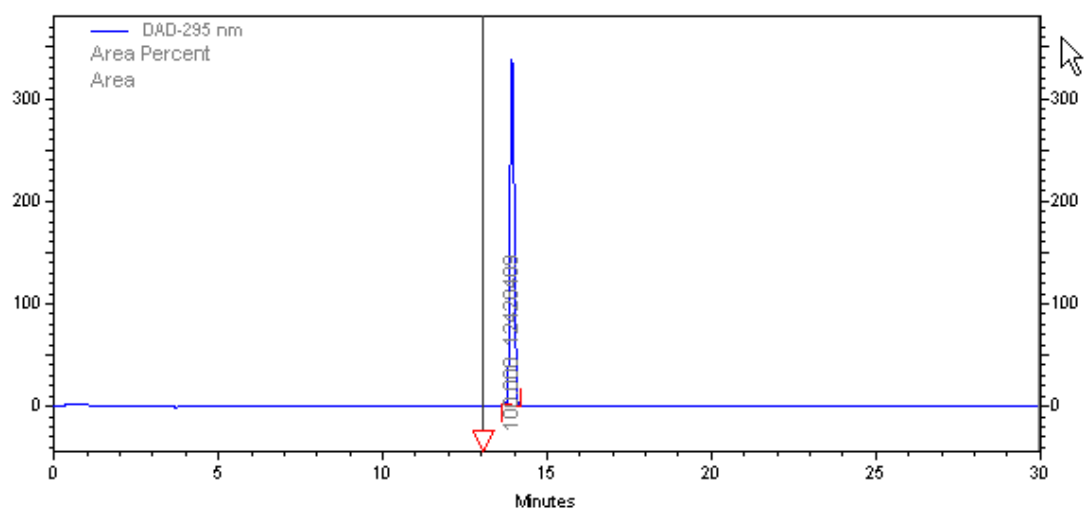


Fig. S37. RP-HPLC trace for compound 24.

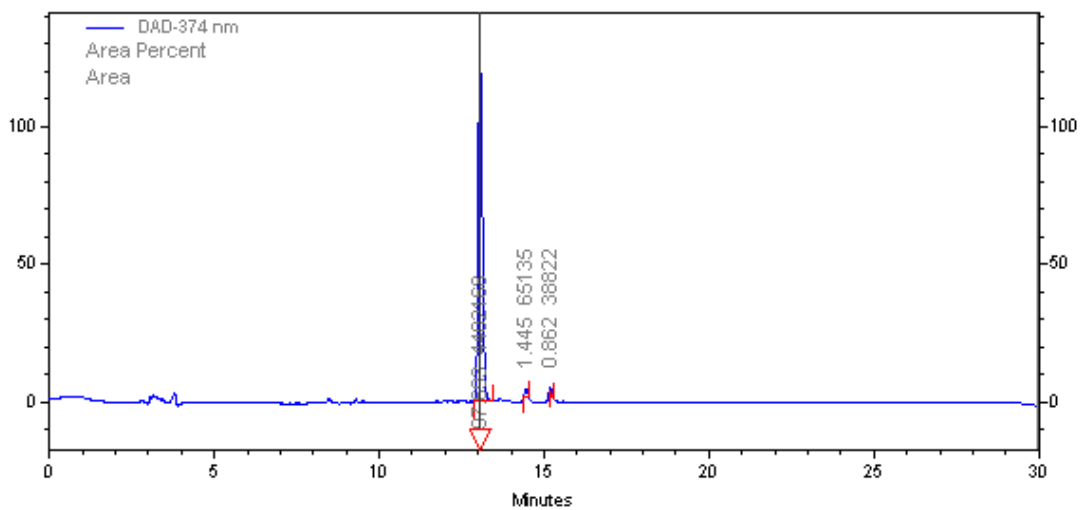


Fig. S38. RP-HPLC trace for compound 25.

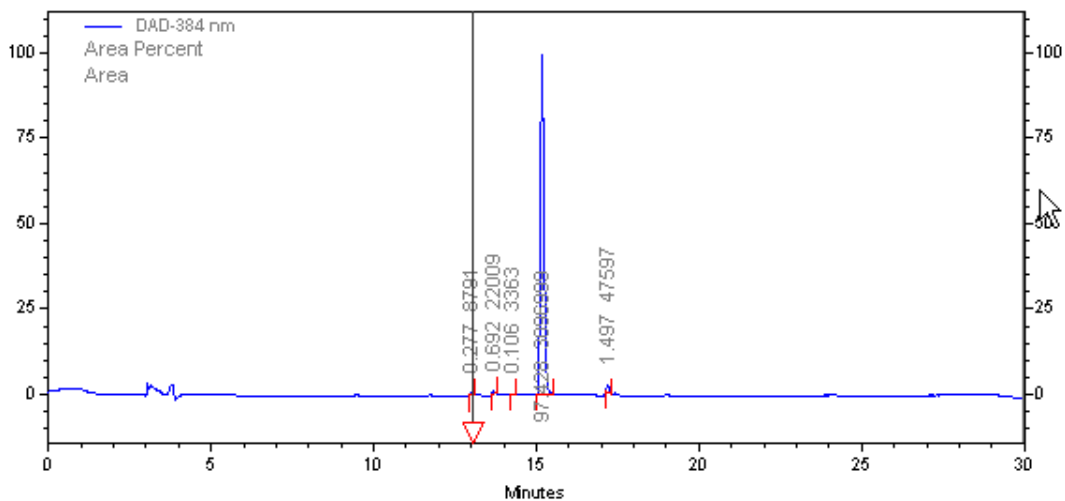


Fig. S39. RP-HPLC trace for compound **26**.

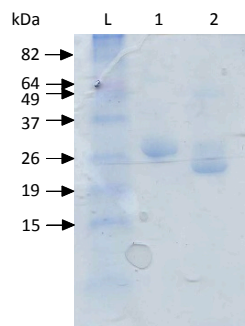


Fig. S40. Coomassie blue-stained 15% Tris-HCl SDS-PAGE gel showing the purified 29.2-kDa CPT (Lane 1) and 22.3-kDa CNR (Lane 2) CAM resistance enzymes. L = BenchMark™ Pre-Stained Ladder from Invitrogen. 6 μ g of each protein was loaded on the gel.