Supplementary material to the article

Semi-synthetic puwainaphycin/minutissamide cyclic lipopeptides with improved antifungal activity and limited cytotoxicity

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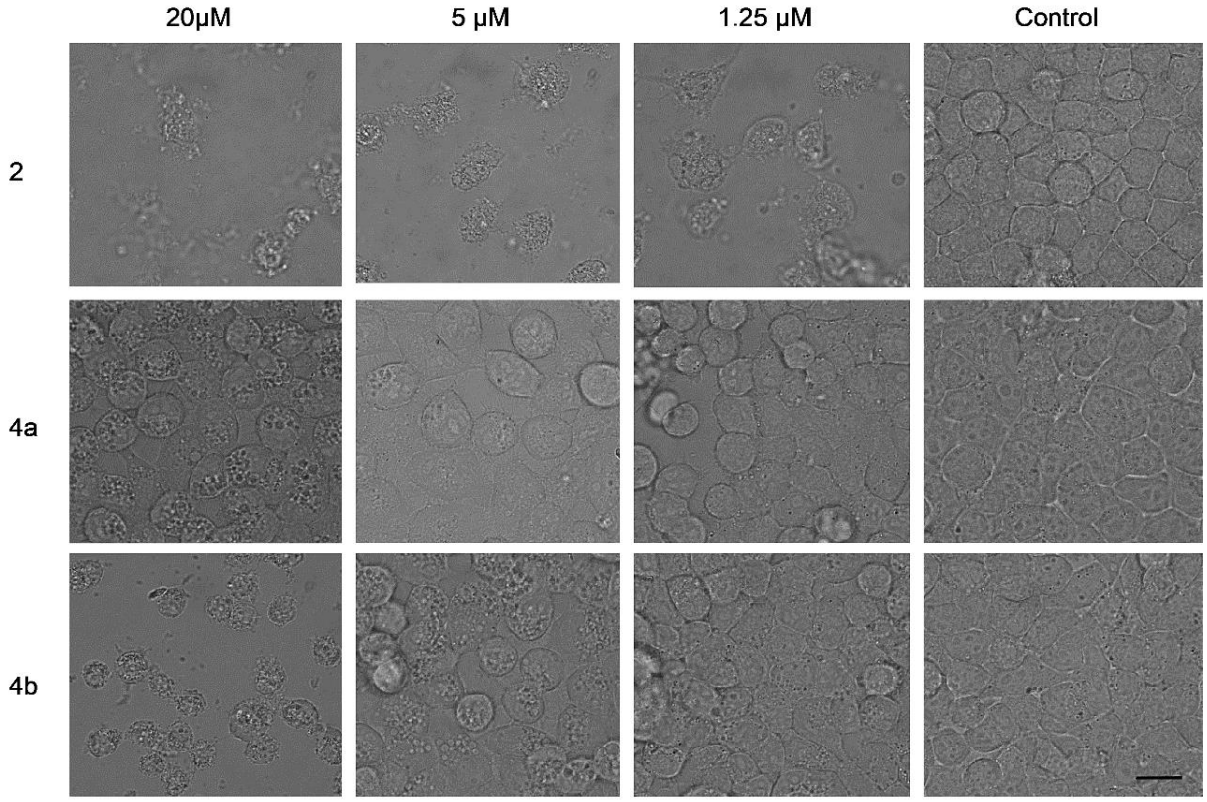


Figure S1. The morphology of HeLa cells treated by semi-synthetic PUW/MINs (4a and 4b) and naturally occurring PUW F (2) used as reference. Semi-synthetic compound **4b** induced clear membrane burst comparable to natural compounds **2** only at highest concentration tested (20 μM). At concentration 5 μM and 1.25 μM of **4a** cytoplasmic vacuolation is observed, however, no membrane permeabilization effect was recorded. Under treatment of **4a** the cells appeared stressed (cytoplasmic vacuolation) but with not membrane burst at the end of the experiments. Scale bar represents 20 μm. Vehicle treated cells are depicted as control.

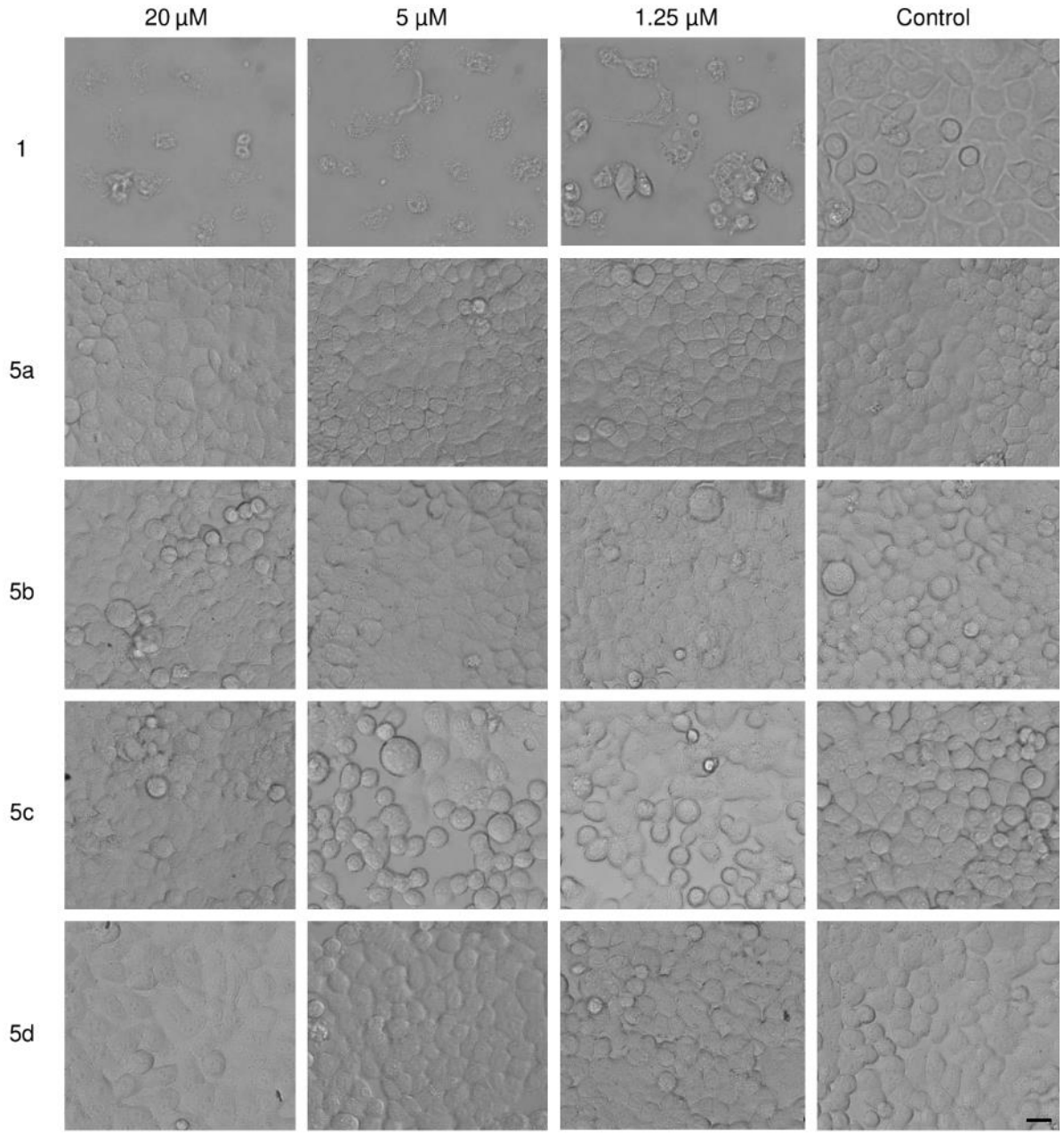


Figure S2. The morphology of HeLa cells treated by semi-synthetic PUW/MINs (5a-5c) and naturally occurring MIN A (1) used as reference. In case of all esterified variants (**5b-5d**) no morphology alteration has been observed. Scale bar represents 20 µm.

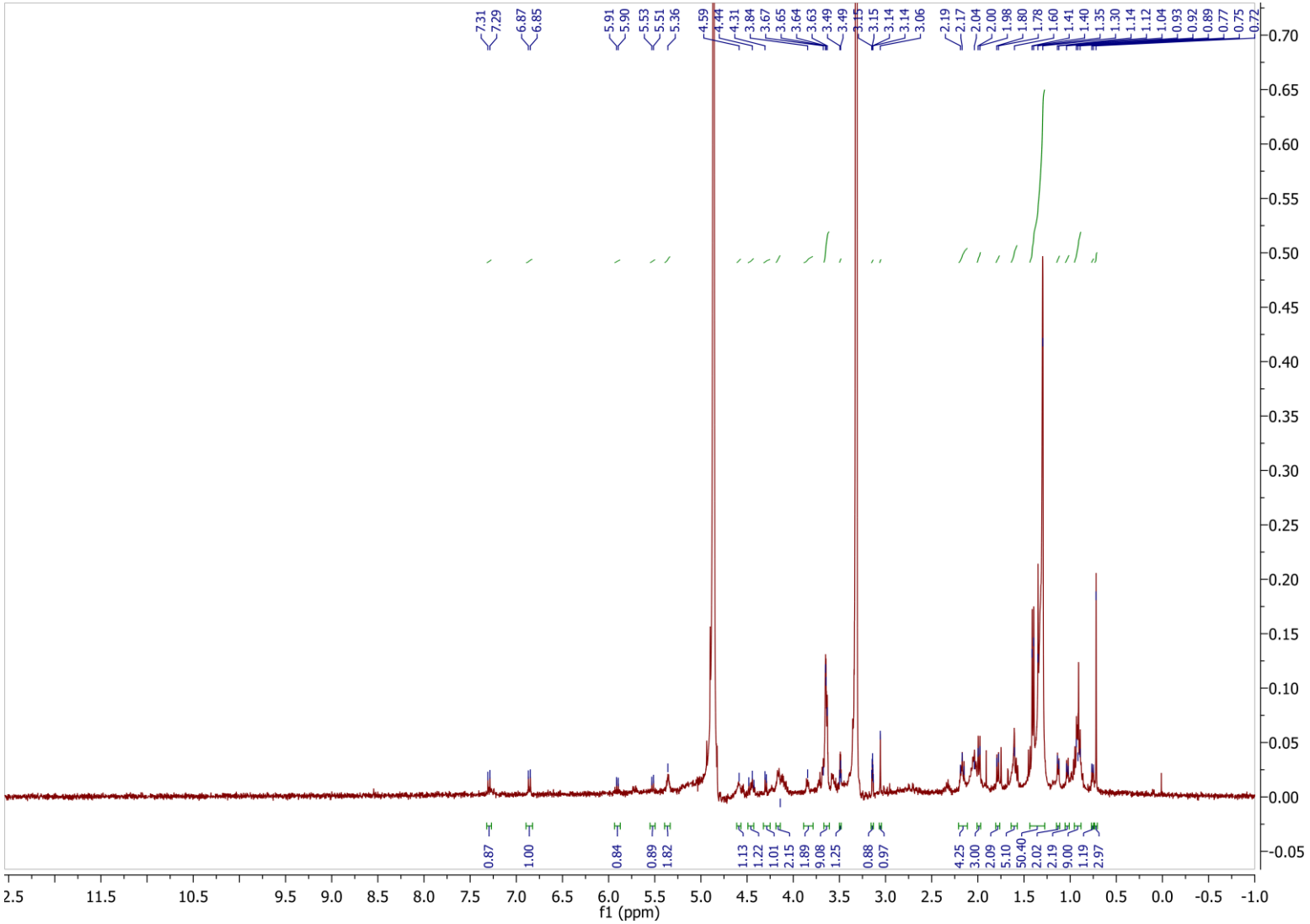


Figure S3. ^{1}H NMR spectrum of compound **4a**

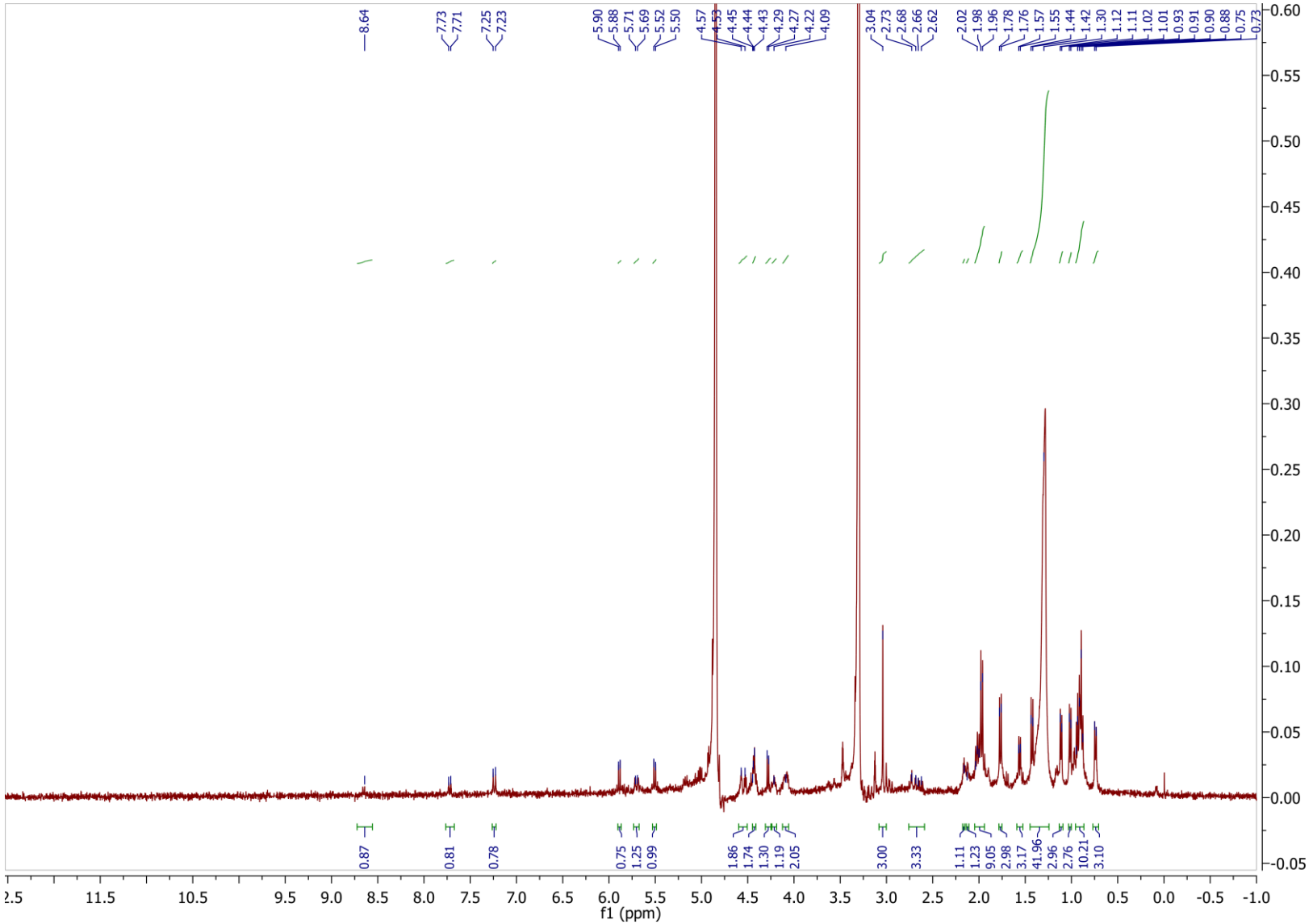


Figure S4. [1]H NMR spectrum of compound **4b**

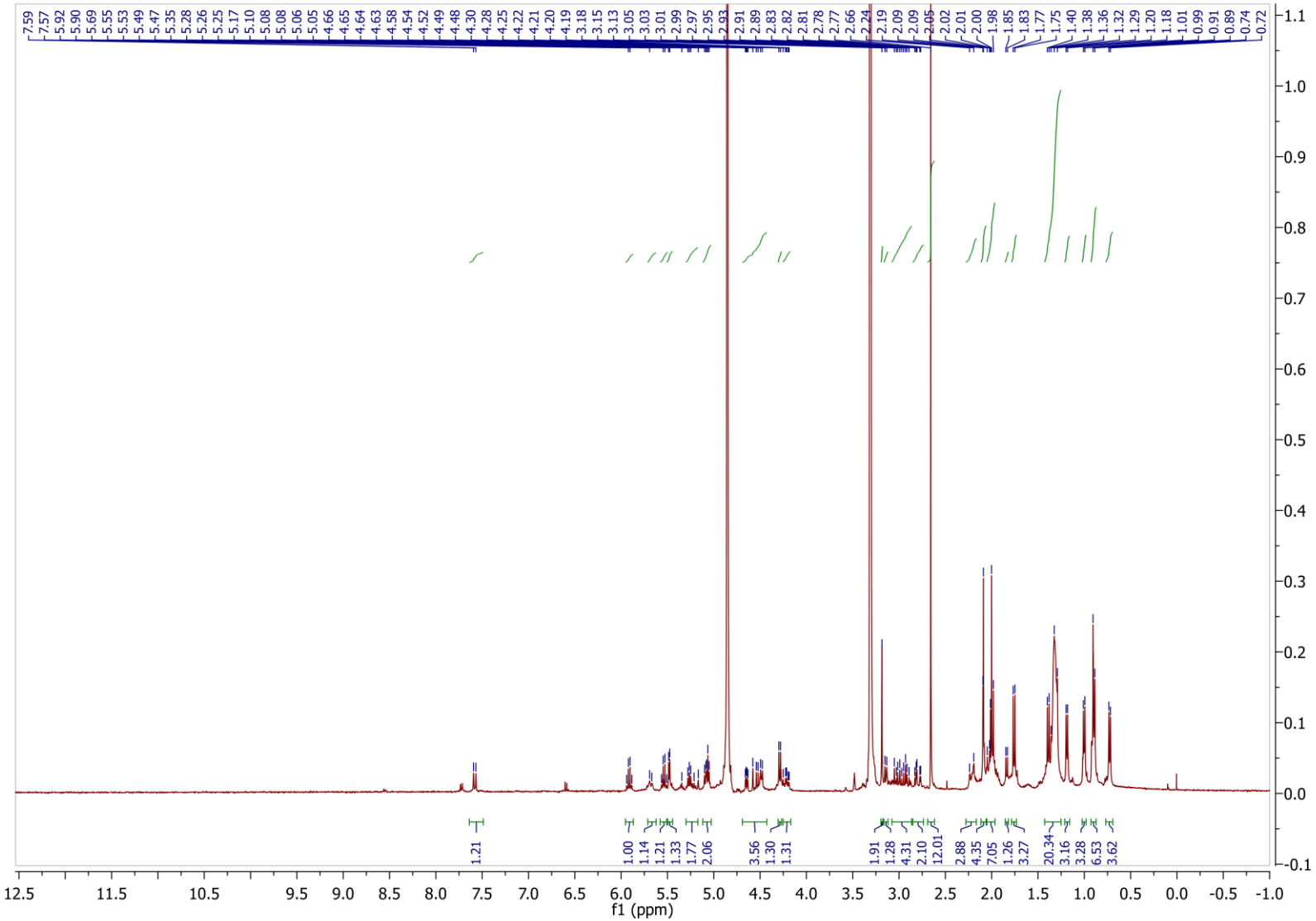


Figure S5. ^{1}H NMR spectrum of compound **5a**

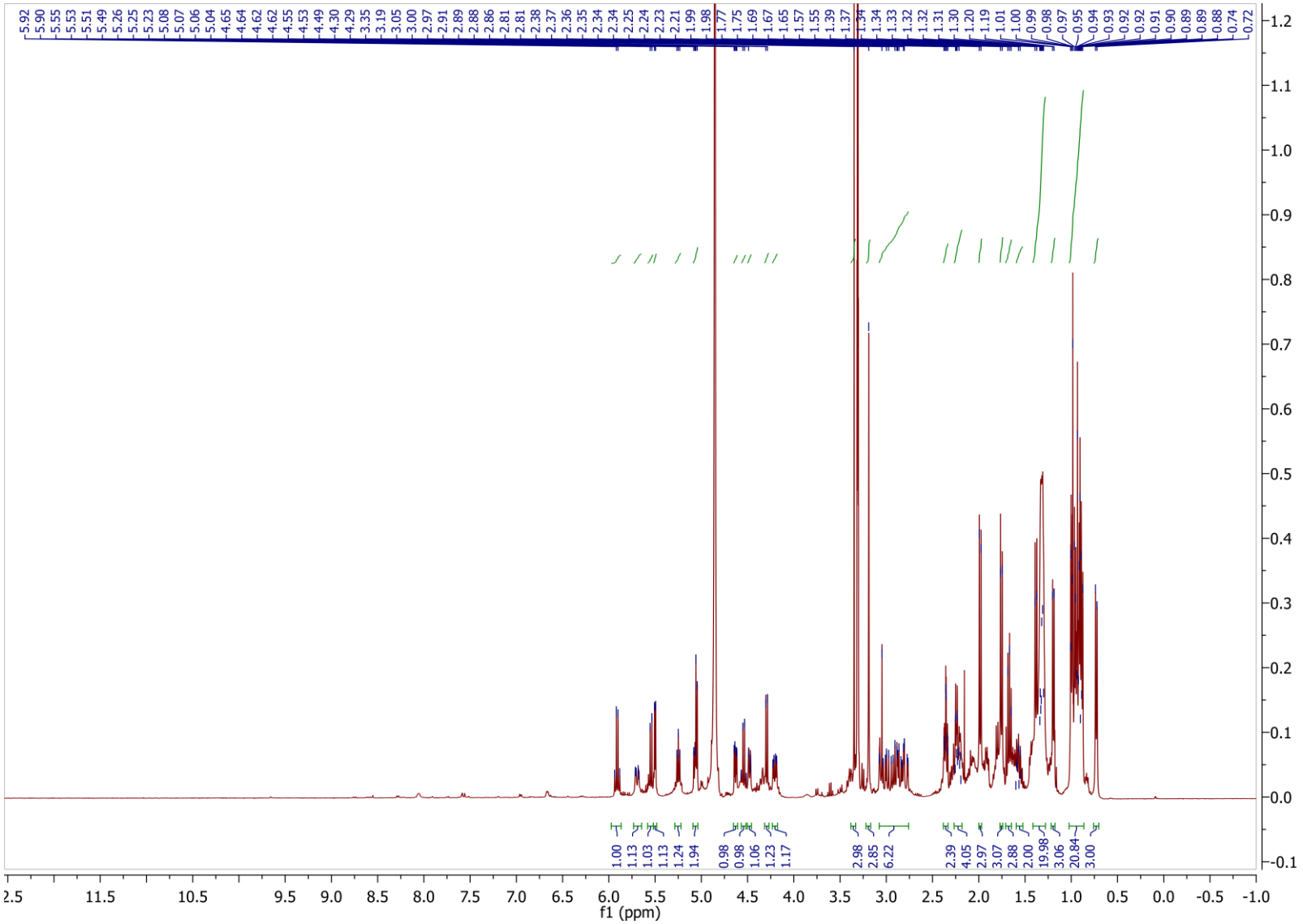


Figure S6. ^{1}H NMR spectrum of compound **5b**

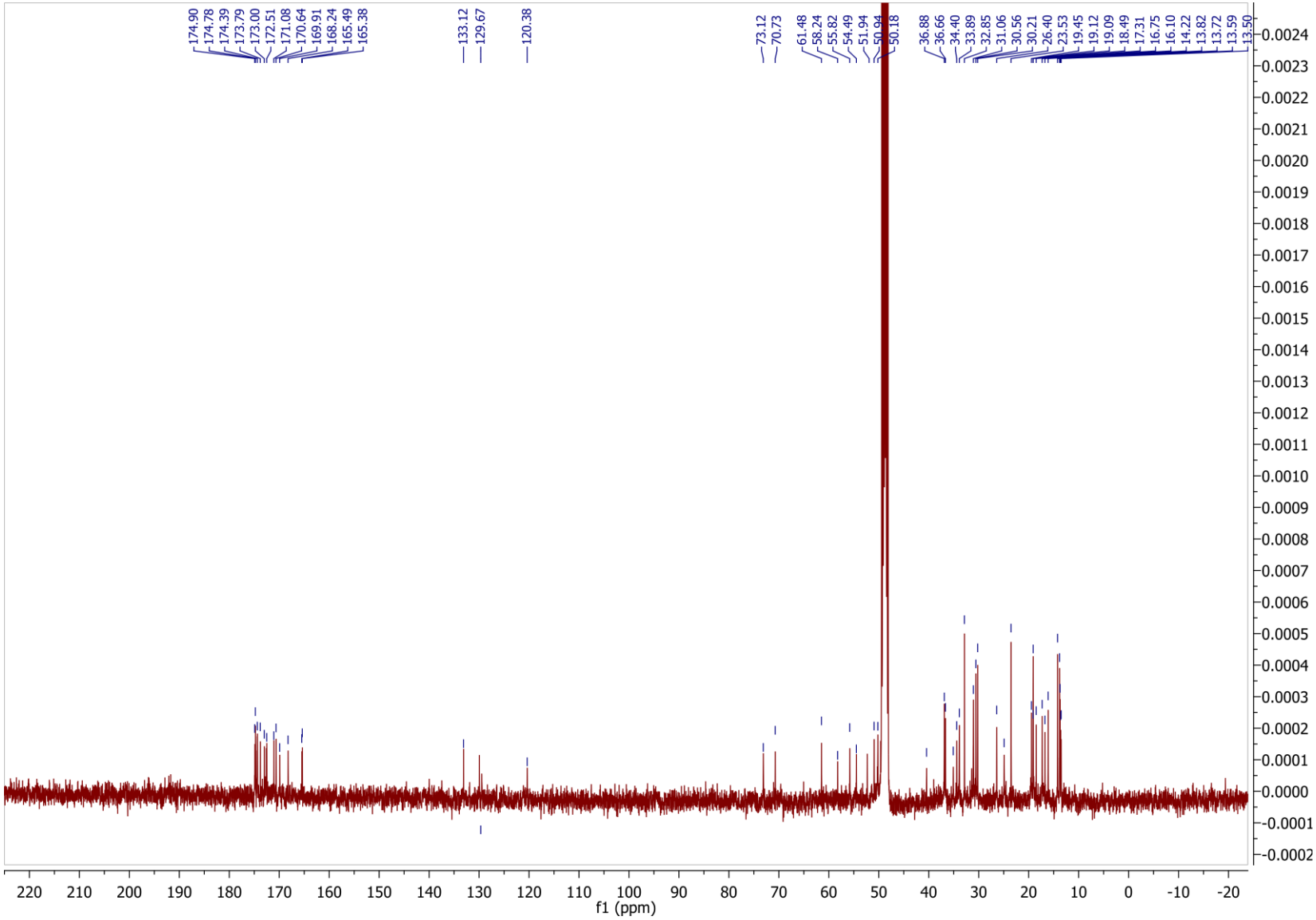


Figure S7. ^{13}C NMR spectrum of compound **5b**

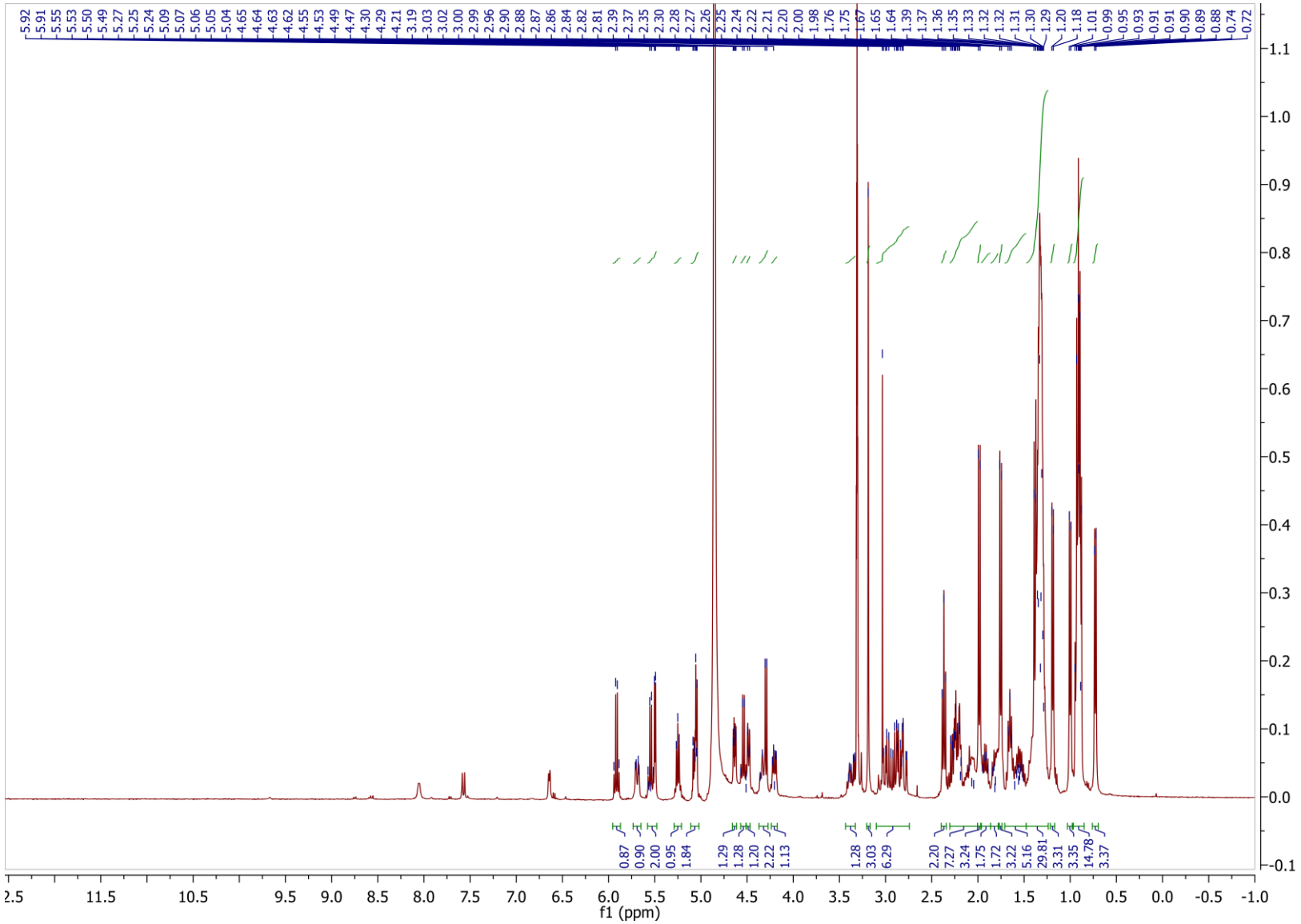


Figure S8. ^{1}H NMR spectrum of compound **5c**

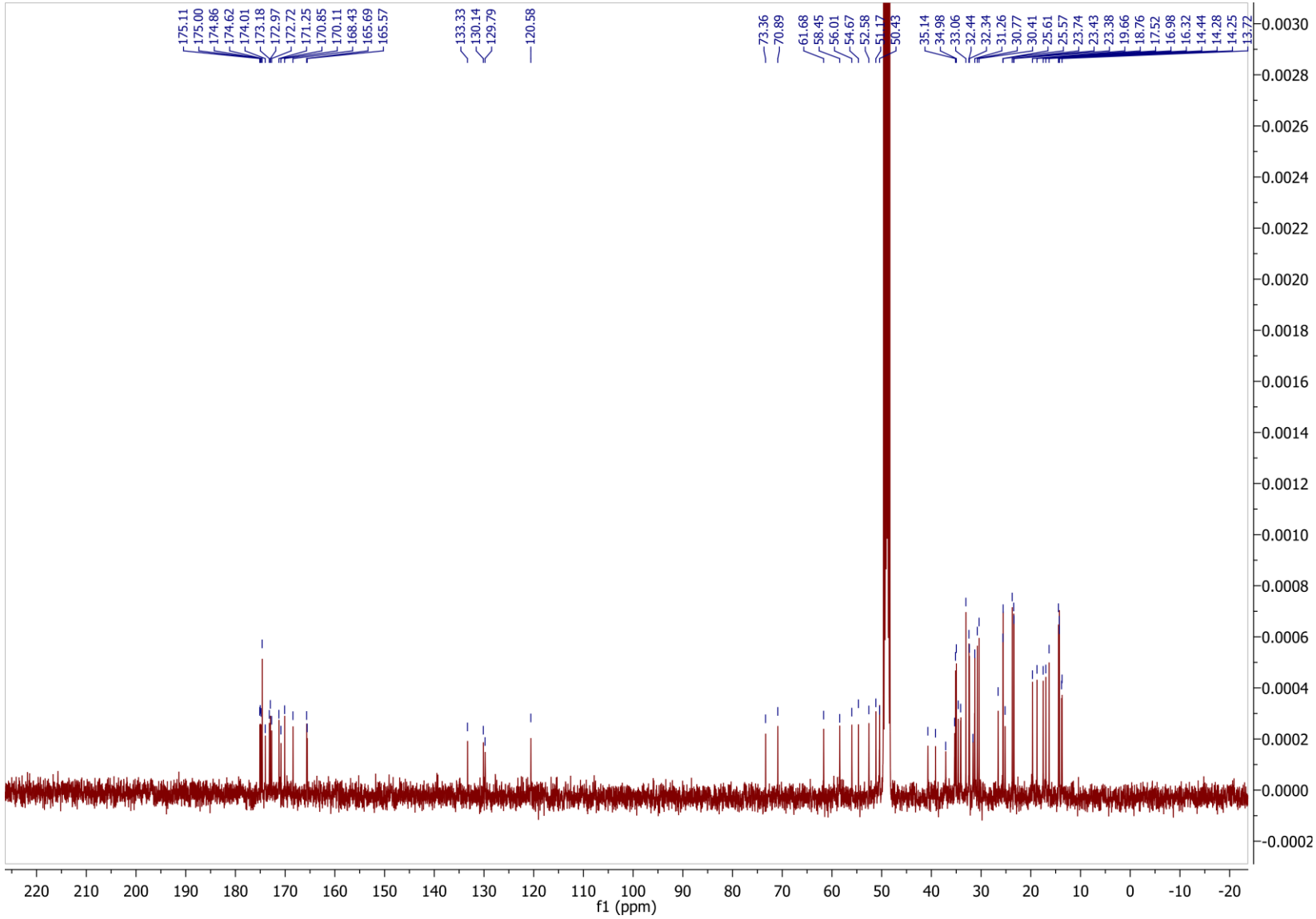


Figure S9. ^{13}C NMR spectrum of compound **5c**

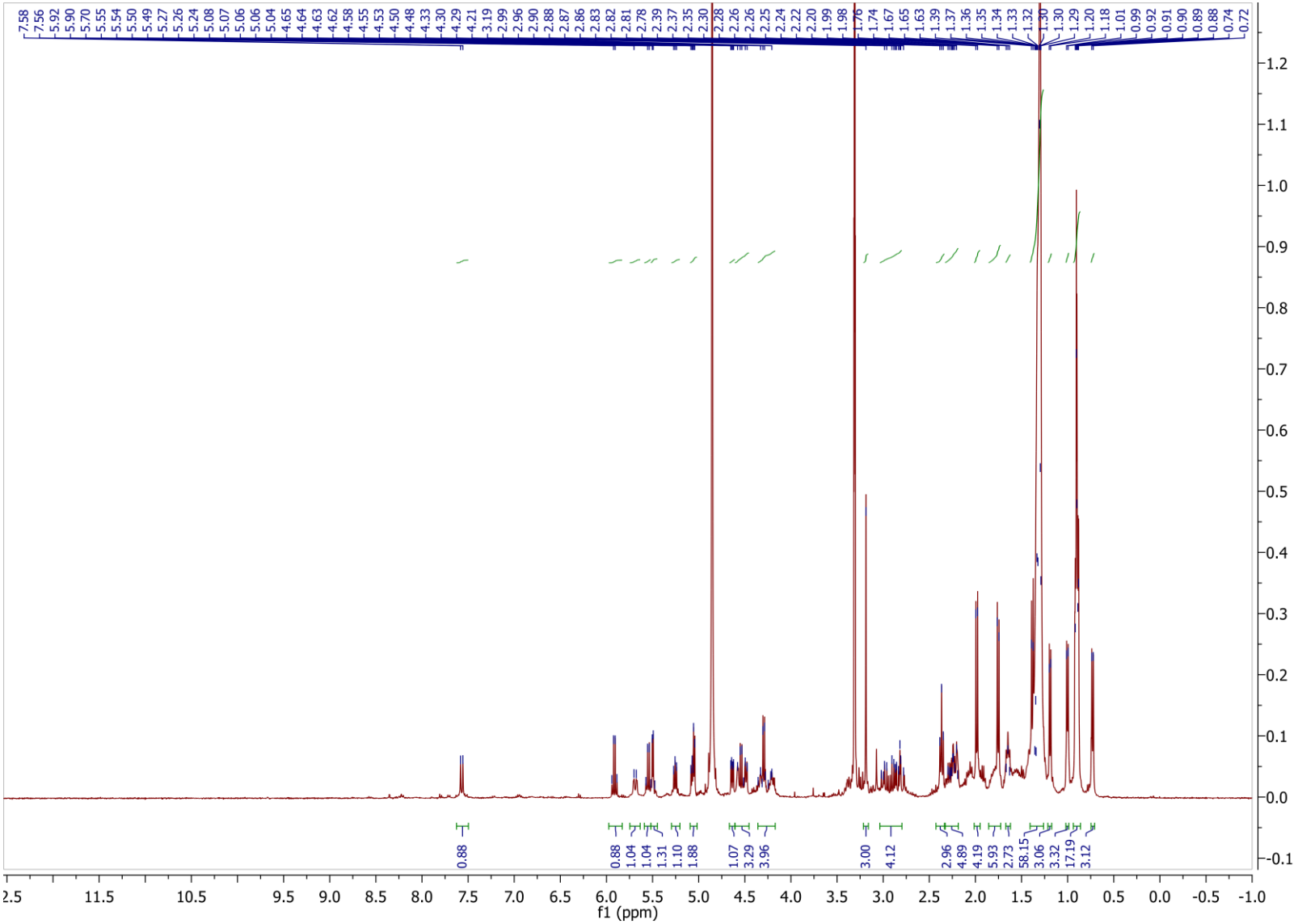


Figure S10. ^{1}H NMR spectrum of compound **5d**

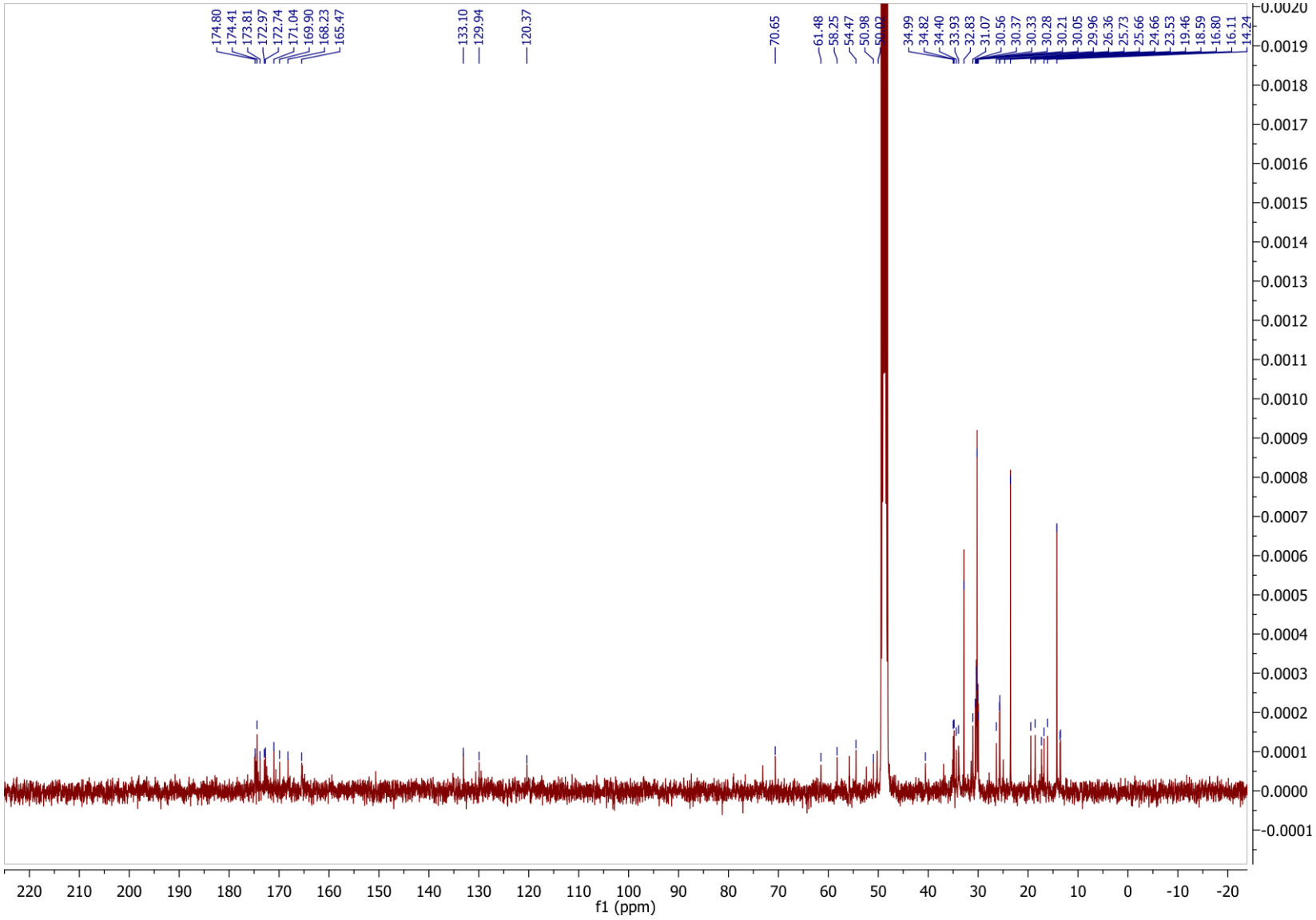
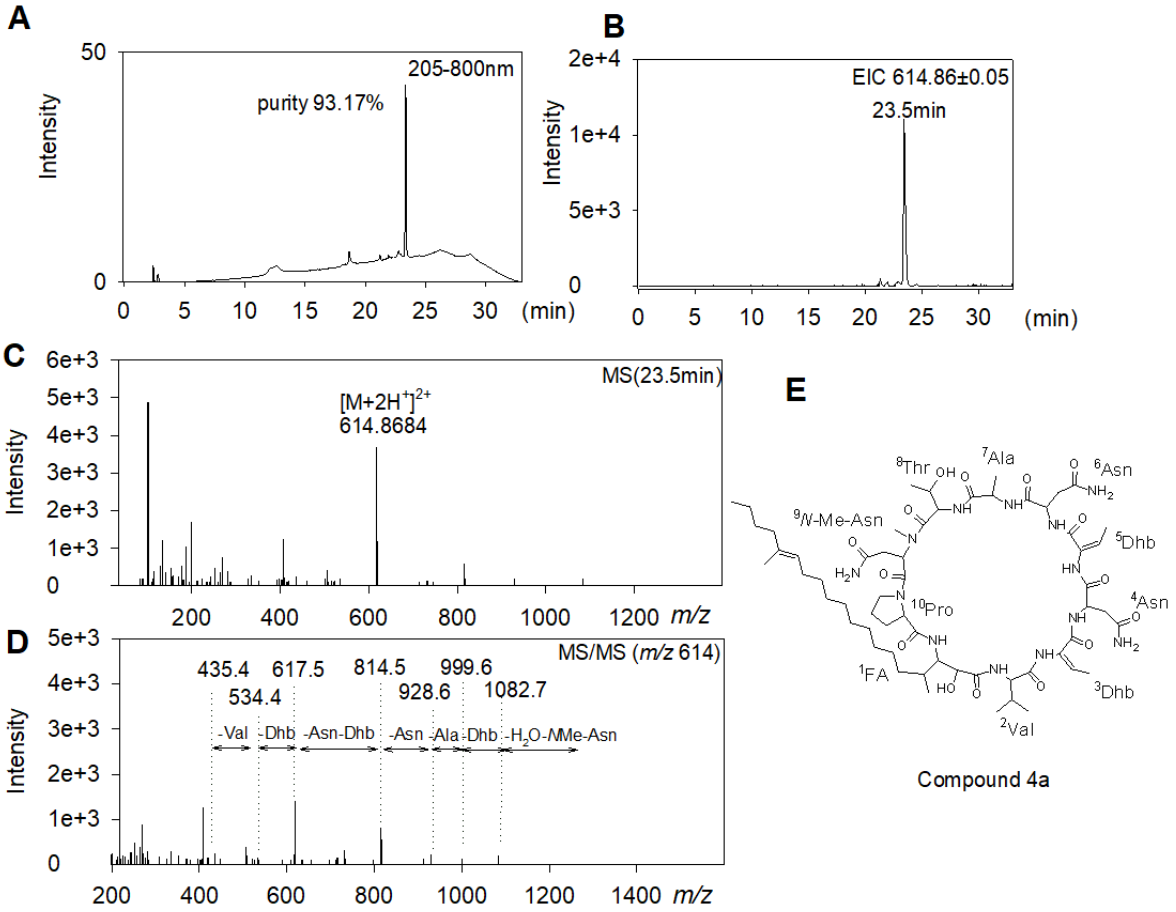


Figure S11. ^{13}C NMR spectrum of compound **5d**



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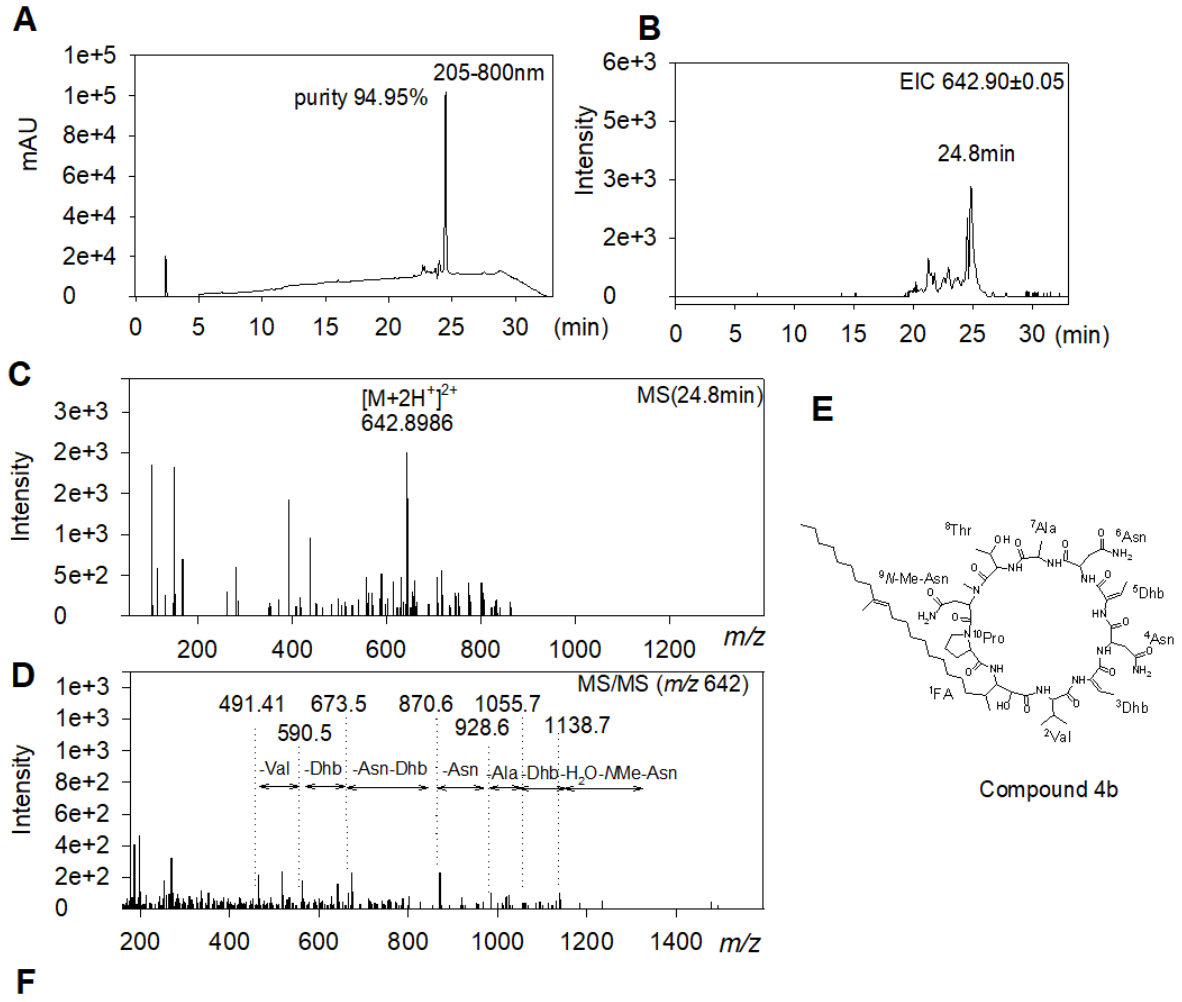
Sequence	Summ Formula	m/z	z	error
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Thr-*N* MeAsn	$C_{59}H_{97}N_{13}O_{15}+2H^+$	614.8684	2	+0.4ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb-*N* MeAsn	$C_{59}H_{95}N_{13}O_{14}+H^+$	n.a.		n.a.
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb	$C_{54}H_{87}N_{11}O_{12}+H^+$	1082.6714	1	-9.8ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala	$C_{50}H_{82}N_{10}O_{11}+H^+$	999.6253	1	-1.6ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn	$C_{47}H_{77}N_9O_{10}+H^+$	928.5897	1	-3.3ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb	$C_{43}H_{71}N_7O_8+H^+$	814.5462	1	-3.1ppm
Pro-FA[1)]-Val-Dhb-Asn	$C_{39}H_{66}N_6O_7+H^+$	n.a.		n.a.
Pro-FA[1)]-Val-Dhb	$C_{35}H_{60}N_4O_5+H^+$	617.4687	1	-8.3ppm
Pro-FA[1)]-Val	$C_{31}H_{55}N_3O_4+H^+$	534.4325	1	-11.1ppm
Pro-FA[1)]	$C_{26}H_{46}N_2O_3+H^+$	435.3557	1	+5.6ppm

[1)] FA = 3-amino-2-hydroxy-15-methyl-nonadec-14-enoic acid

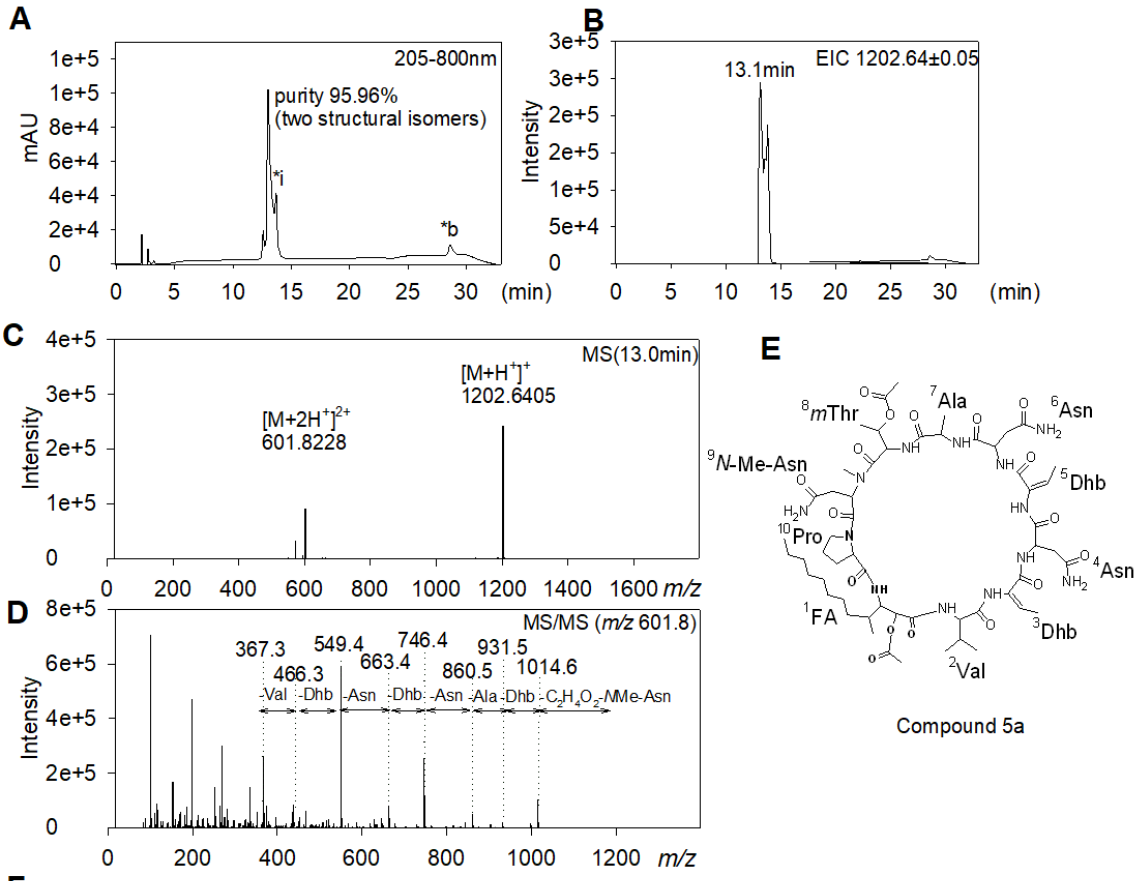
Figure S12. HPLC-HRMS analysis of compound **4a**. A) UV/VIS chromatogram 205-800nm. Purity was estimated based on the UV/VIS peak integral. Impurities between 17 a 23 min represent minor polyethylene glycol contaminants originating from the purification process. B) EIC chromatogram *m/z* 614.86 ±0.05 corresponding to $[M+2H^+]^{2+}$ of compound **4a**. C) Full MS spectrum at 23.5 min. D) MS/MS spectrum of *m/z* 614 corresponding to $[M+2H^+]^{2+}$ of compound **4a**. After initial water loss, all amino acids are consecutively cleaved until formation of diagnostic fragments Pro-Fa-Val-Dhb (617 Da) and Pro-FA (435 Da). E) The structure of compound **4a**. F) MS/MS data interpretation - sum formula, measured m/z, charge and error are depicted. n.a. - not available or signal below interpretable intensity.



Sequence	Summ Formula	m/z	z	error
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Thr-*N* MeAsn	$C_{63}H_{105}N_{13}O_{15}+2H^+$	642.8986	2	+2.0ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb-*N* MeAsn	$C_{63}H_{103}N_{13}O_{14}+H^+$	n.a.		n.a.
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb	$C_{58}H_{95}N_{11}O_{12}+H^+$	1138.7293	1	-5.1ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala	$C_{54}H_{90}N_{10}O_{11}+H^+$	1055.6942	1	-7.5ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn	$C_{51}H_{85}N_9O_{10}+H^+$	984.6459	1	+3.4ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb	$C_{47}H_{79}N_7O_8+H^+$	870.6044	1	+2.2ppm
Pro-FA[1)]-Val-Dhb-Asn	$C_{43}H_{74}N_6O_7+H^+$	n.a.		n.a.
Pro-FA[1)]-Val-Dhb	$C_{39}H_{77}N_4O_5+H^+$	673.5267	1	-0.7ppm
Pro-FA[1)]-Val	$C_{35}H_{63}N_3O_4+H^+$	590.49	1	-1.5ppm
Pro-FA[1)]	$C_{30}H_{54}N_2O_3+H^+$	491.4168	1	+8.0ppm

[1)] FA = 3-amino-2-hydroxy-15-methyl-tricosan-14-enoic acid

Figure S13: HPLC-HRMS analysis of compound **4b**. A) UV/VIS chromatogram 205-800 nm. Purity was estimated based on the UV/VIS peak integral. Impurities between 20 a 24 min represent minor polyethylene glycol contaminants originating from the purification process. B) EIC chromatogram m/z 642.90 ±0.05 corresponding to $[M+2H^+]^{2+}$ of compound **4b**. C) Full MS spectrum at 24.8 min. D) MS/MS spectrum of m/z 642 corresponding to $[M+2H^+]^{2+}$ of compound **4b**. After initial water loss, all amino acids are consecutively cleaved until formation of diagnostic fragments Pro-Fa-Val-Dhb (674 Da) and Pro-FA (491 Da). E) The structure of compound **4b**. F) MS/MS data interpretation - sum formula, measured m/z, charge and error are depicted. n.a. - not available or signal below interpretable intensity.



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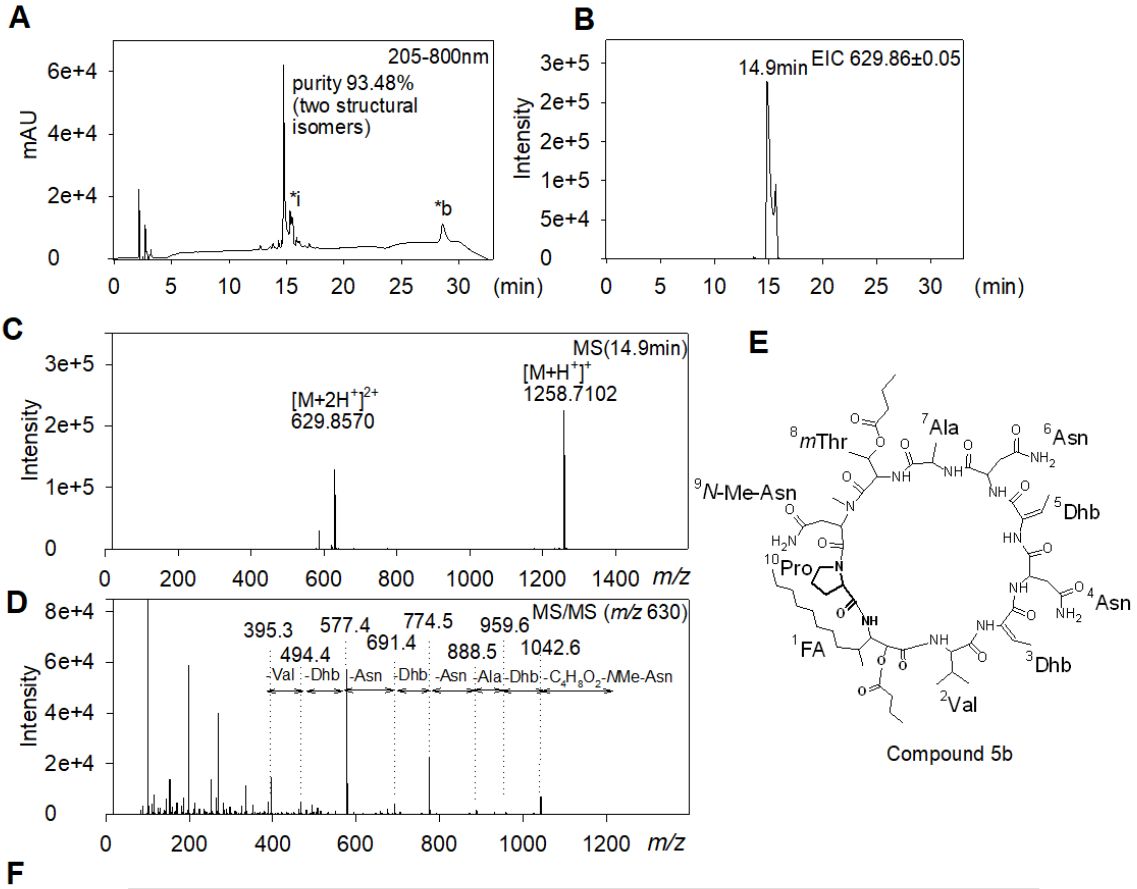
Sequence	Summ Formula	*m/z*	z	error
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn-Ala-*m*Thr[2]-*N*MeAsn	$C_{55}H_{86}N_{13}O_{17}+H^+$	1202.6399	1	+1.3ppm
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb-*N*MeAsn	$C_{53}H_{82}N_{13}O_{15}+H^+$	n.a.		n.a.
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb	$C_{48}H_{75}N_{11}O_{13}+H^+$	1014.5607	1	+1.2ppm
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn-Ala	$C_{44}H_{70}N_{10}O_{12}+H^+$	931.5248	1	+0.2ppm
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn	$C_{41}H_{65}N_9O_{11}+H^+$	860.4865	1	+1.3pm
Pro-FA[1]-Val-Dhb-Asn-Dhb	$C_{37}H_{59}N_7O_9+H^+$	746.4440	1	+0.9ppm
Pro-FA[1]-Val-Dhb-Asn	$C_{33}H_{54}N_6O_8+H^+$	663.4072	1	+0.5ppm
Pro-FA[1]-Val-Dhb	$C_{29}H_{48}N_4O_6+H^+$	549.3643	1	+0.6ppm
Pro-FA[1]-Val	$C_{25}H_{43}N_3O_5+H^+$	466.3274	1	+0.3ppm
Pro-FA[1]	$C_{20}H_{34}N_2O_4+H^+$	367.2592	1	+0.1ppm

1) FA = *O*-acetyl-3-amino-2-hydroxy-4-methyl-dodecanoic acid
2) *m*Thr = *O*-acetyl-Thr

Figure S14: HPLC-HRMS analysis of compound **5a**. A) UV/VIS chromatogram 205-800 nm. Purity was estimated based on the UV/VIS peak integral. Impurities at 12 min represent side products (monoacylated MIN A) C) Full MS spectrum at 13.0 min. D) MS/MS spectrum of m/z 602 corresponding to $[M+2H^+]^{2+}$ of compound **5a**. After initial carboxylic acid loss ($-CH_3COOH$), all amino acids are consecutively cleaved until formation of diagnostic fragments Pro-Fa-Val-Dhb (549 Da) and Pro-FA (367 Da). E) The structure of compound **5a**. F) MS/MS data interpretation - sum formula, measured m/z, charge and error are depicted. n.a. - not available or signal below interpretable intensity. *b indicates system contaminants (mostly from C_{18} column), *i shows peak of structural isomer of the main compound



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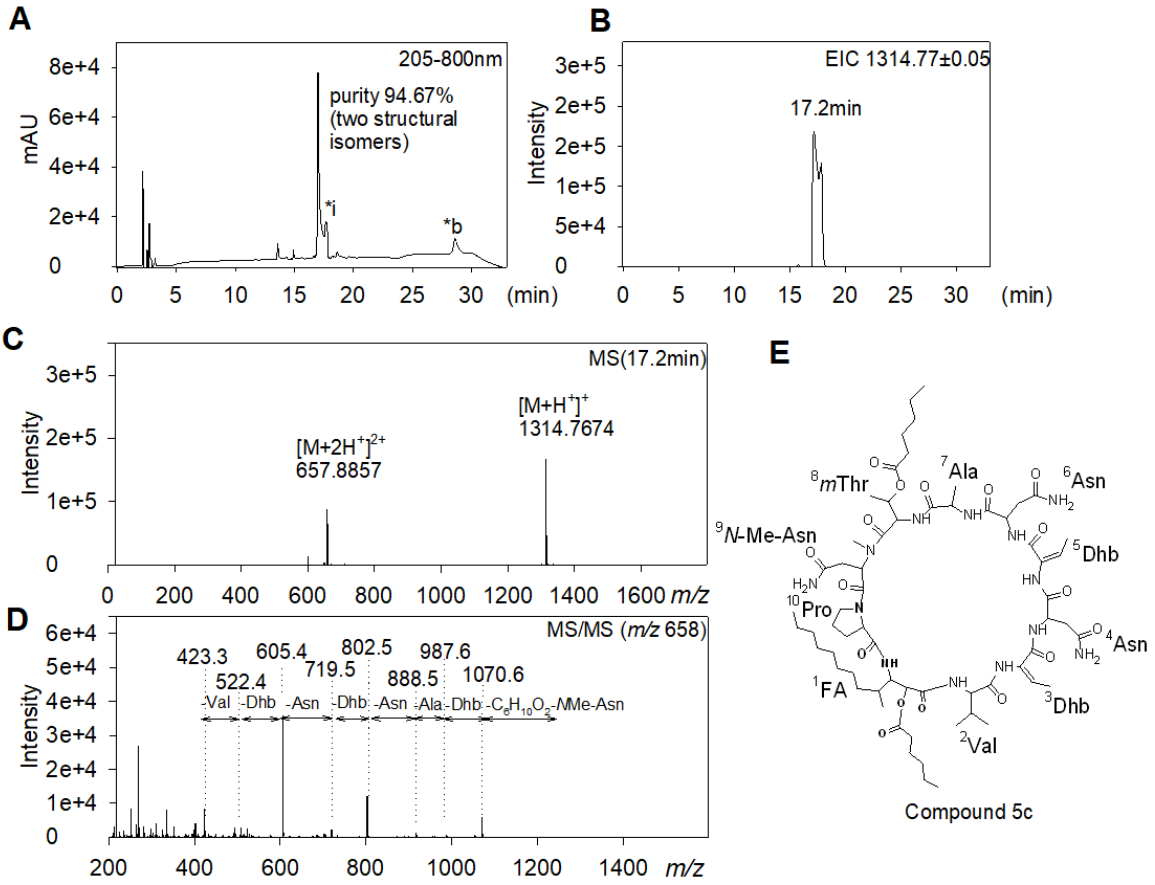
Sequence	Summ Formula	*m/z*	z	error
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn-Ala-*m*Thr[2]-*N*MeAsn	$C_{59}H_{95}N_{13}O_{17}+2H^+$	629.857	2	-2.0ppm
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb-*N*MeAsn	$C_{55}H_{87}N_{13}O_{15}+H^+$	n.a.		n.a.
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb	$C_{50}H_{79}N_{11}O_{13}+H^+$	1042.5953	1	-2.0ppm
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn-Ala	$C_{46}H_{74}N_{10}O_{12}+H^+$	959.5613	1	-5.4ppm
Pro-FA[1]-Val-Dhb-Asn-Dhb-Asn	$C_{43}H_{69}N_9O_{11}+H^+$	888.5194	1	-0.6ppm
Pro-FA[1]-Val-Dhb-Asn-Dhb	$C_{39}H_{63}N_7O_9+H^+$	774.4785	1	-3.2ppm
Pro-FA[1]-Val-Dhb-Asn	$C_{35}H_{58}N_6O_8+H^+$	691.4388	1	-0.2ppm
Pro-FA[1]-Val-Dhb	$C_{31}H_{52}N_4O_6+H^+$	577.3974	1	-2.5ppm
Pro-FA[1]-Val	$C_{27}H_{47}N_3O_5+H^+$	494.3591	1	-0.5ppm
Pro-FA[1]	$C_{22}H_{38}N_2O_4+H^+$	395.2906	1	-0.5ppm

1) FA = *O*-butyl-3-amino-2-hydroxy-4-methyl-dodecanoic acid
2) *m*Thr = *O*-butyl-Thr

Figure S15: HPLC-HRMS analysis of compound **5b**. A) UV/VIS chromatogram 205-800 nm. Purity was estimated based on the UV/VIS peak integral. Impurities between 12 min and 14 min represent side products (monoacylated variants of MIN A) B) EIC chromatogram m/z 629.86 ±0.05 corresponding to $[M+2H^+]^{2+}$ of compound **5b**. C) Full MS spectrum at 14.9 min. D) MS/MS spectrum of m/z 630 corresponding to $[M+2H^+]^{2+}$ of compound **5b**. After initial carboxylic acid loss ($-CH_3(CH_2)_2COOH$), all amino acids are consecutively cleaved until formation of diagnostic fragments Pro-Fa-Val-Dhb (577 Da) and Pro-FA (395 Da). E) The structure of compound **5b**. F) MS/MS data interpretation - sum formula, measured m/z, charge and error are depicted. n.a. - not available or signal below interpretable intensity. *b indicates system contaminants (mostly from C_{18} column), *i shows peak of structural isomer of the main compound



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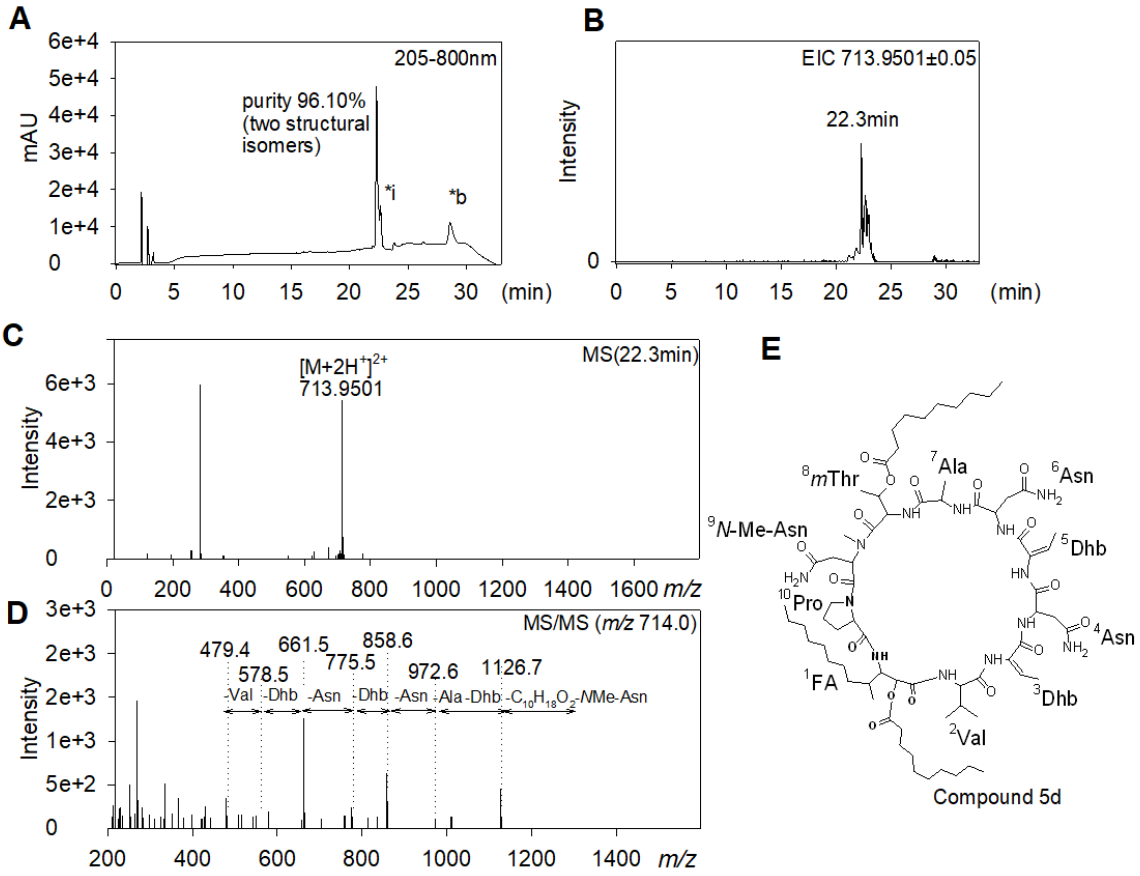
Sequence	Summ Formula	*m/z*	z	error
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-*m*Thr[2)]-*N*MeAsn	$C_{63}H_{103}N_{13}O_{17}+H^+$	1314.7674	1	-0.5ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb-*N*MeAsn	$C_{59}H_{95}N_{13}O_{15}+H^+$	n.a.		n.a.
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb	$C_{52}H_{83}N_{11}O_{13}+H^+$	1070.6257	1	-1.1ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala	$C_{48}H_{78}N_{10}O_{12}+H^+$	987.5813	1	+6.2ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn	$C_{45}H_{73}N_9O_{11}+H^+$	916.5451	1	+5.6ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb	$C_{41}H_{67}N_7O_9+H^+$	802.5067	1	+0.7ppm
Pro-FA[1)]-Val-Dhb-Asn	$C_{37}H_{62}N_6O_8+H^+$	719.4688	1	+1.9ppm
Pro-FA[1)]-Val-Dhb	$C_{33}H_{56}N_4O_6+H^+$	605.4261	1	+2.0ppm
Pro-FA[1)]-Val	$C_{29}H_{51}N_3O_5+H^+$	522.388	1	+4.1ppm
Pro-FA[1)]	$C_{24}H_{42}N_2O_4+H^+$	423.3217	1	+0.0ppm

1) FA = *O*-hexyl-3-amino-2-hydroxy-4-methyl-dodecanoic acid
2) *m*Thr = *O*-hexyl-Thr

Figure S16: HPLC-HRMS analysis of compound **5c**. A) UV/VIS chromatogram 205-800 nm. Purity was estimated based on the UV/VIS peak integral. Impurities between 13-17 min represent side products (monoacylated variants) B) EIC chromatogram m/z 1314.77 ±0.05 corresponding to $[M+H^+]^+$ of compound **5c**. C) Full MS spectrum at 17.2 min. D) MS/MS spectrum of m/z 658 corresponding to $[M+2H^+]^{2+}$ of compound **5c**. After initial carboxylic acid loss ($-CH_3(CH_2)_4COOH$), all amino acids are consecutively cleaved until formation of diagnostic fragments Pro-Fa-Val-Dhb (605 Da) and Pro-FA (423 Da). E) The structure of compound **5c**. F) MS/MS data interpretation - sum formula, measured m/z, charge and error are depicted. n.a. - not available or signal below interpretable intensity. *b indicates system contaminants (mostly from C_{18} column), *i shows peak of structural isomer of the main compound



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Sequence	Summ Formula	*m/z*	z	error
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-*m*Thr[2)]-*N*MeAsn	$C_{69}H_{111}N_{13}O_{17}+2H^+$	713.9501	2	-0.7ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb-*N*MeAsn	$C_{63}H_{103}N_{13}O_{15}+H^+$	n.a.		n.a.
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala-Dhb	$C_{56}H_{91}N_{11}O_{13}+H^+$	1126.6951	1	-7.2ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn-Ala	$C_{52}H_{86}N_{10}O_{12}+H^+$	n.a.		n.a.
Pro-FA[1)]-Val-Dhb-Asn-Dhb-Asn	$C_{49}H_{81}N_9O_{11}+H^+$	972.617	1	-4.3ppm
Pro-FA[1)]-Val-Dhb-Asn-Dhb	$C_{45}H_{75}N_7O_9+H^+$	858.5653	1	+5.4ppm
Pro-FA[1)]-Val-Dhb-Asn	$C_{41}H_{70}N_6O_8+H^+$	775.5381	1	-6.8ppm
Pro-FA[1)]-Val-Dhb	$C_{37}H_{64}N_4O_6+H^+$	661.4949	1	-7.7ppm
Pro-FA[1)]-Val	$C_{33}H_{59}N_3O_5+H^+$	578.4594	1	-11.6ppm
Pro-FA[1)]	$C_{28}H_{50}N_2O_4+H^+$	479.3854	1	-2.2ppm

[1)] FA = *O*-decyl-3-amino-2-hydroxy-4-methyl-dodecanoic acid
[2)] *m*Thr = *O*-decyl-Thr

Figure S17: HPLC-HRMS analysis of compound **5d**. A) UV/VIS chromatogram 205-800 nm. Purity was estimated based on the UV/VIS peak integral. Impurities at 14 min represent undescribed modification of MIN A. B) EIC chromatogram m/z 713.95 ±0.05 corresponding to $[M+H^+]^+$ of compound **5d**. C) Full MS spectrum at 22.3 min. D) MS/MS spectrum of m/z 714 corresponding to $[M+2H^+]^{2+}$ of compound **5d**. After initial carboxylic acid loss ($-CH_3(CH_2)_8COOH$), all amino acids are consecutively cleaved until formation of diagnostic fragments Pro-Fa-Val-Dhb (661 Da) and Pro-FA (479 Da). E) The structure of compound **5d**. F) MS/MS data interpretation - sum formula, measured m/z, charge and error are depicted. n.a. - not available or signal below interpretable intensity. *b indicates system contaminants (mostly from C_{18} column), *i shows peak of structural isomer of the main compound