Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Dimerization of conserved ascaroside building blocks generates species-specific male attractants in *Caenorhabditis* nematodes

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SUPPORTING INFORMATION **Pages**

Supporting Figures

Figure S1: Phylogeny of analyzed *Caenorhabditis* species [1] and occurrence of dominating ascaroside dimers as identified using ESI-(+)-MS/MS analysis.

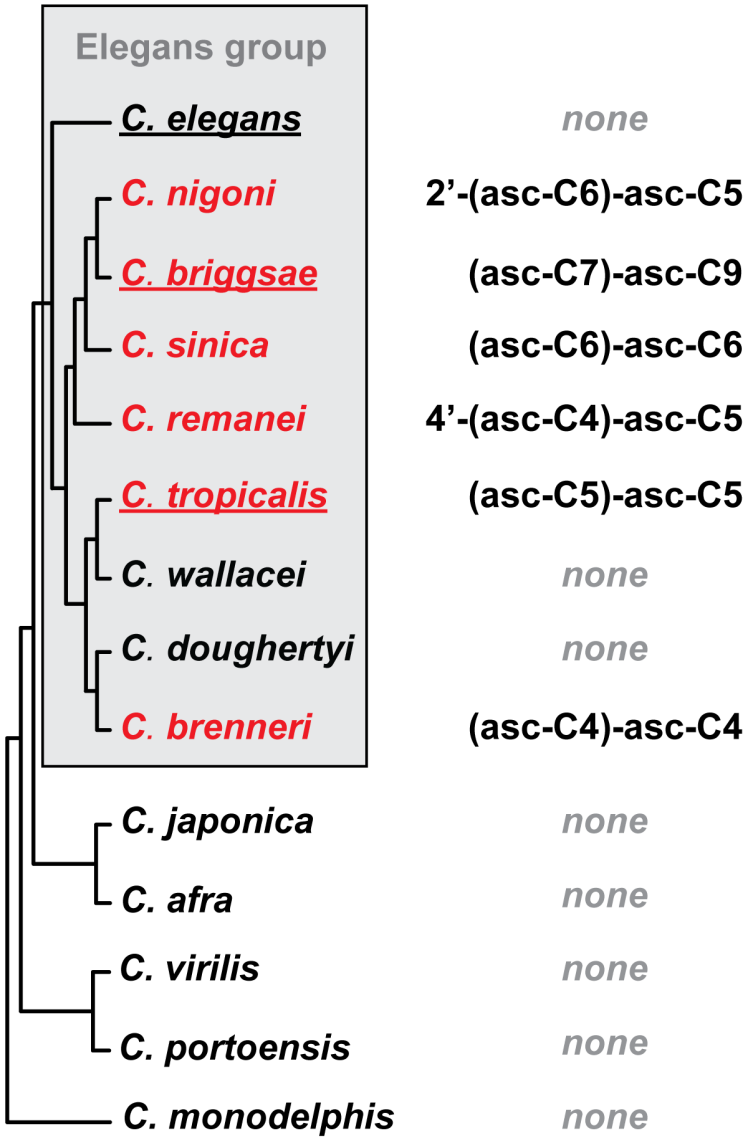


Figure S2a: HPLC-ESI-(+)-HR-MS/MS spectra of dimeric ascarosides from *Caenorhabditis spp.*

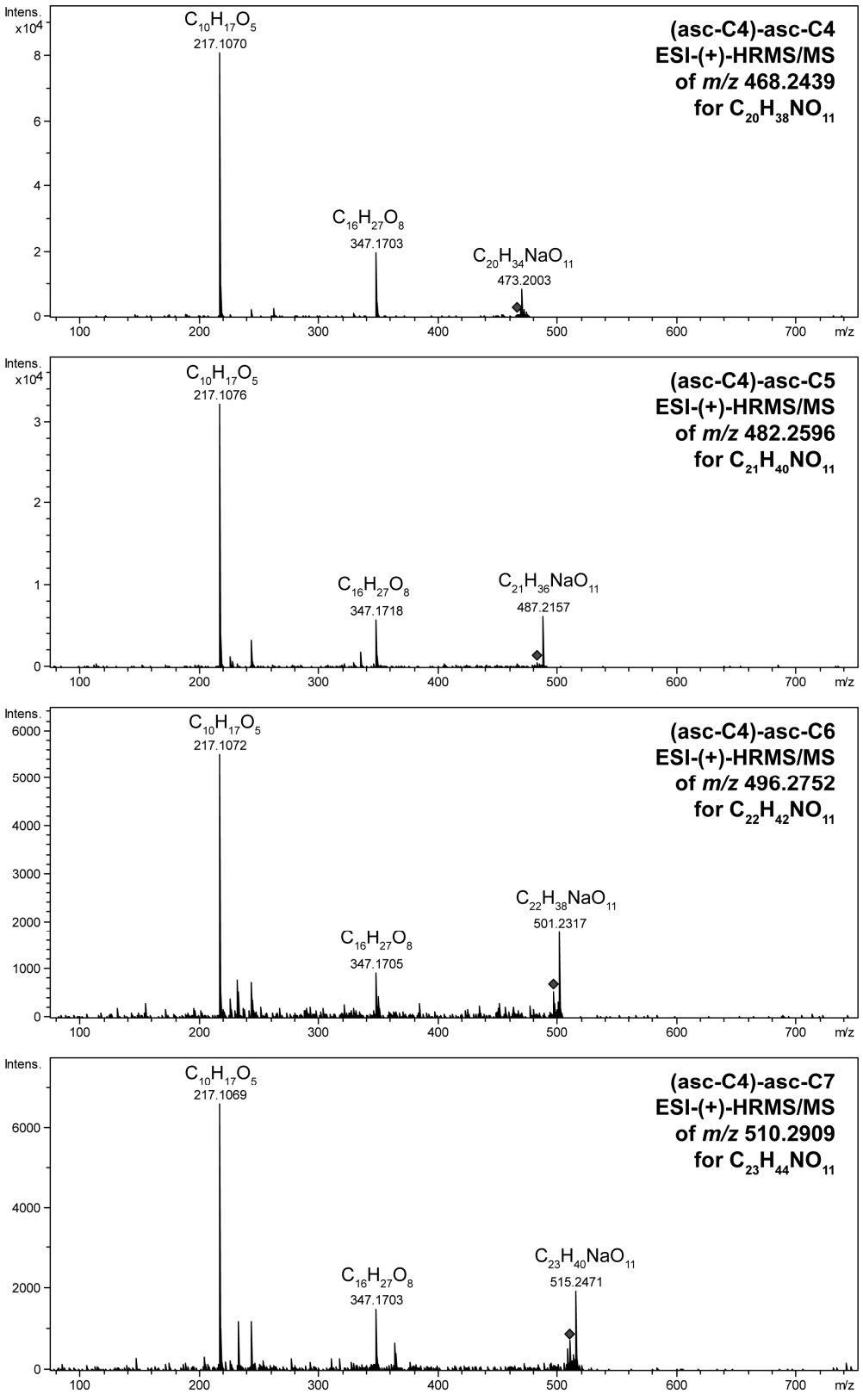


Figure S2b: HPLC-ESI-(+)-HR-MS/MS spectra of dimeric ascarosides from *Caenorhabditis spp.*

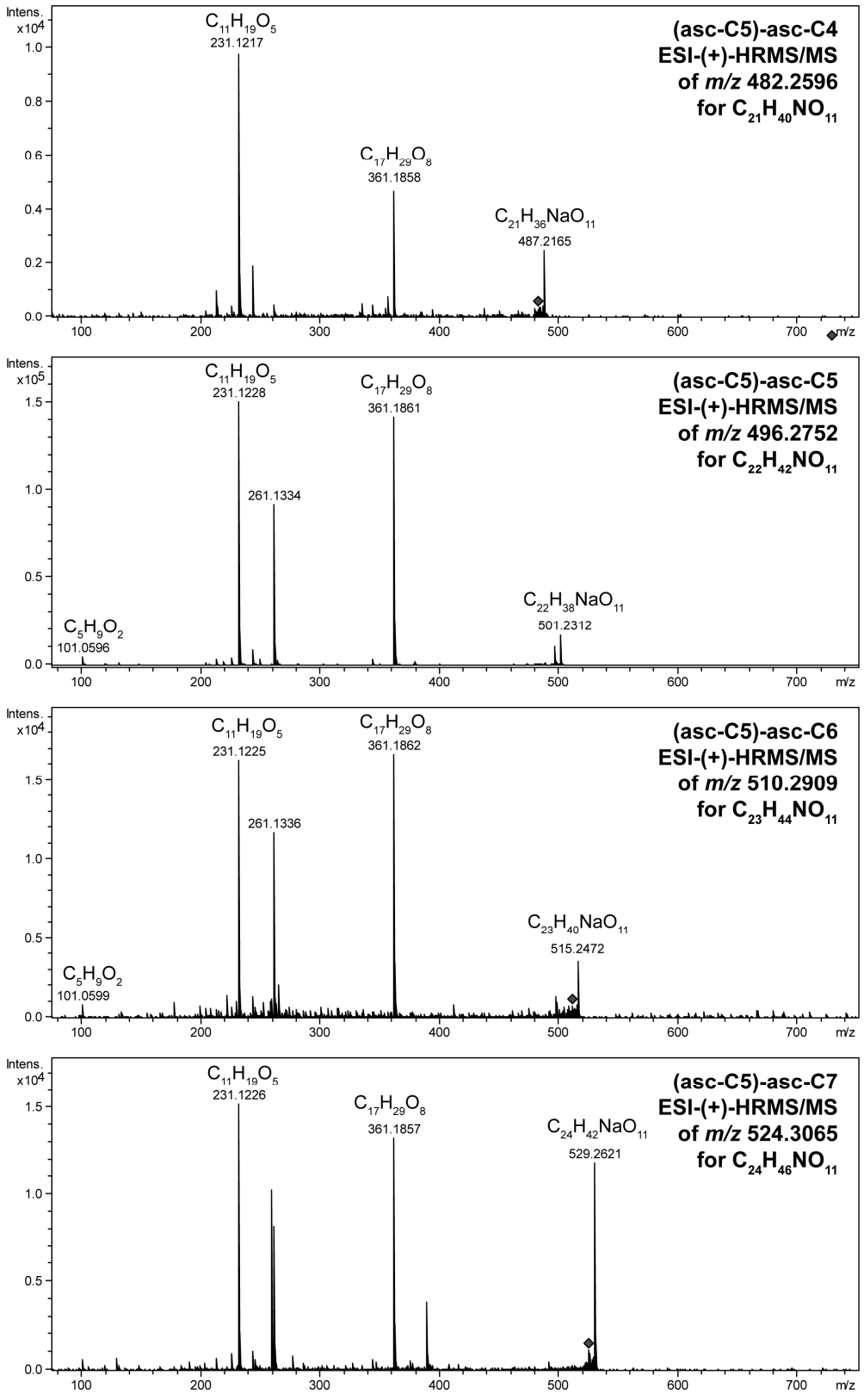


Figure S2c: HPLC-ESI-(+)-HR-MS/MS spectra of dimeric ascarosides from *Caenorhabditis spp.*

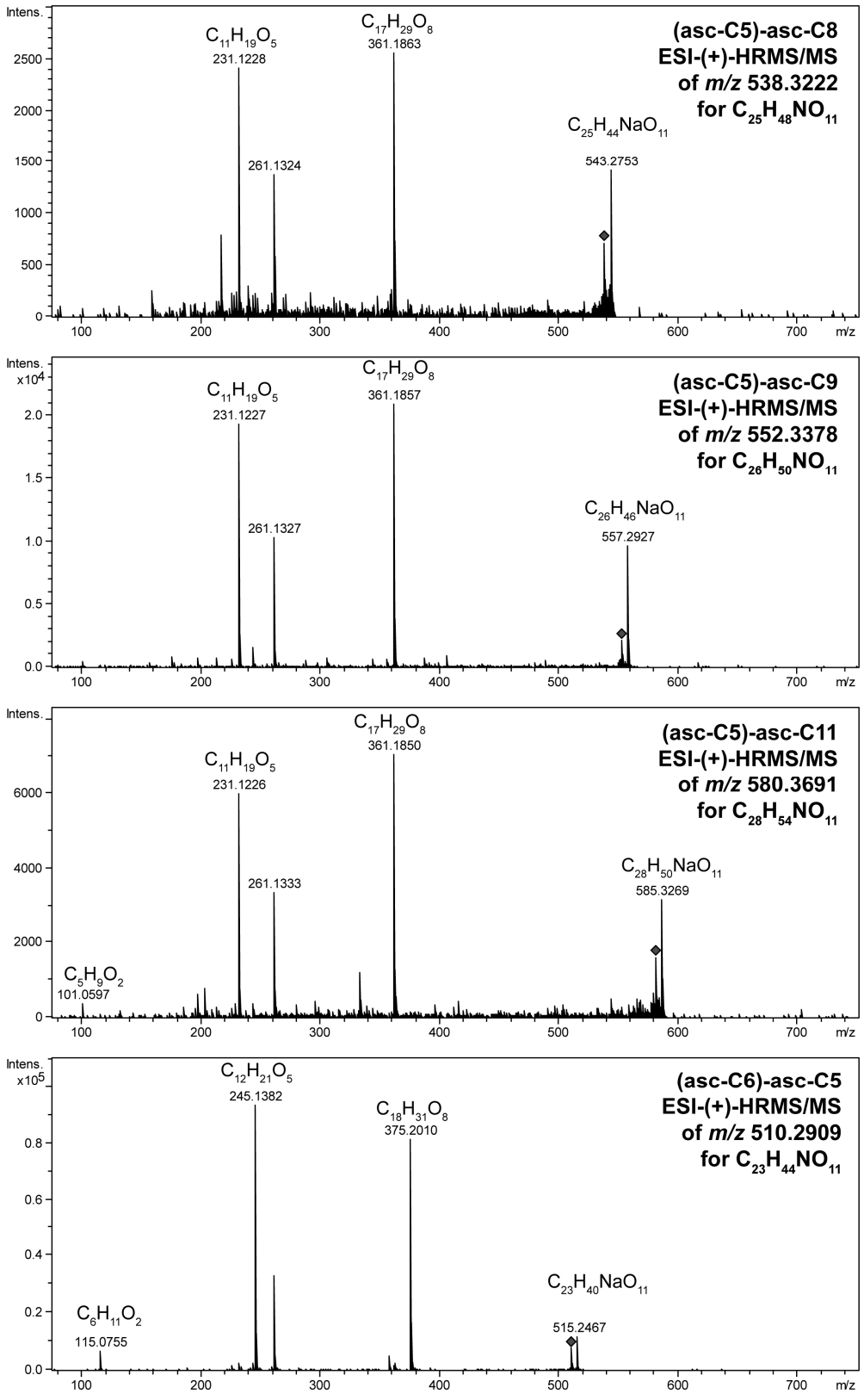


Figure S2d: HPLC-ESI-(+)-HR-MS/MS spectra of dimeric ascarosides from *Caenorhabditis spp.*

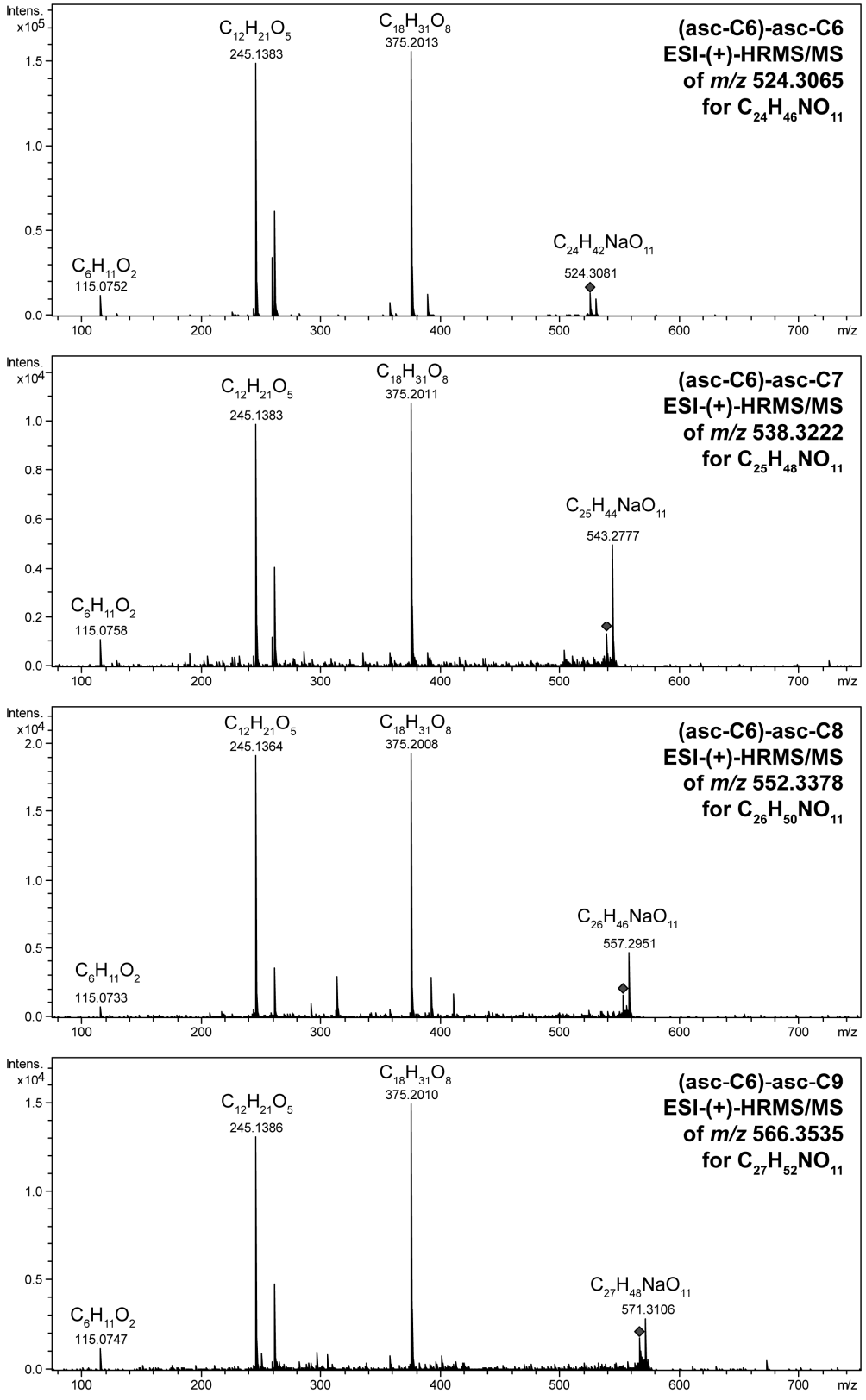


Figure S2e: HPLC-ESI-(+)-HR-MS/MS spectra of dimeric ascarosides from *Caenorhabditis spp.*

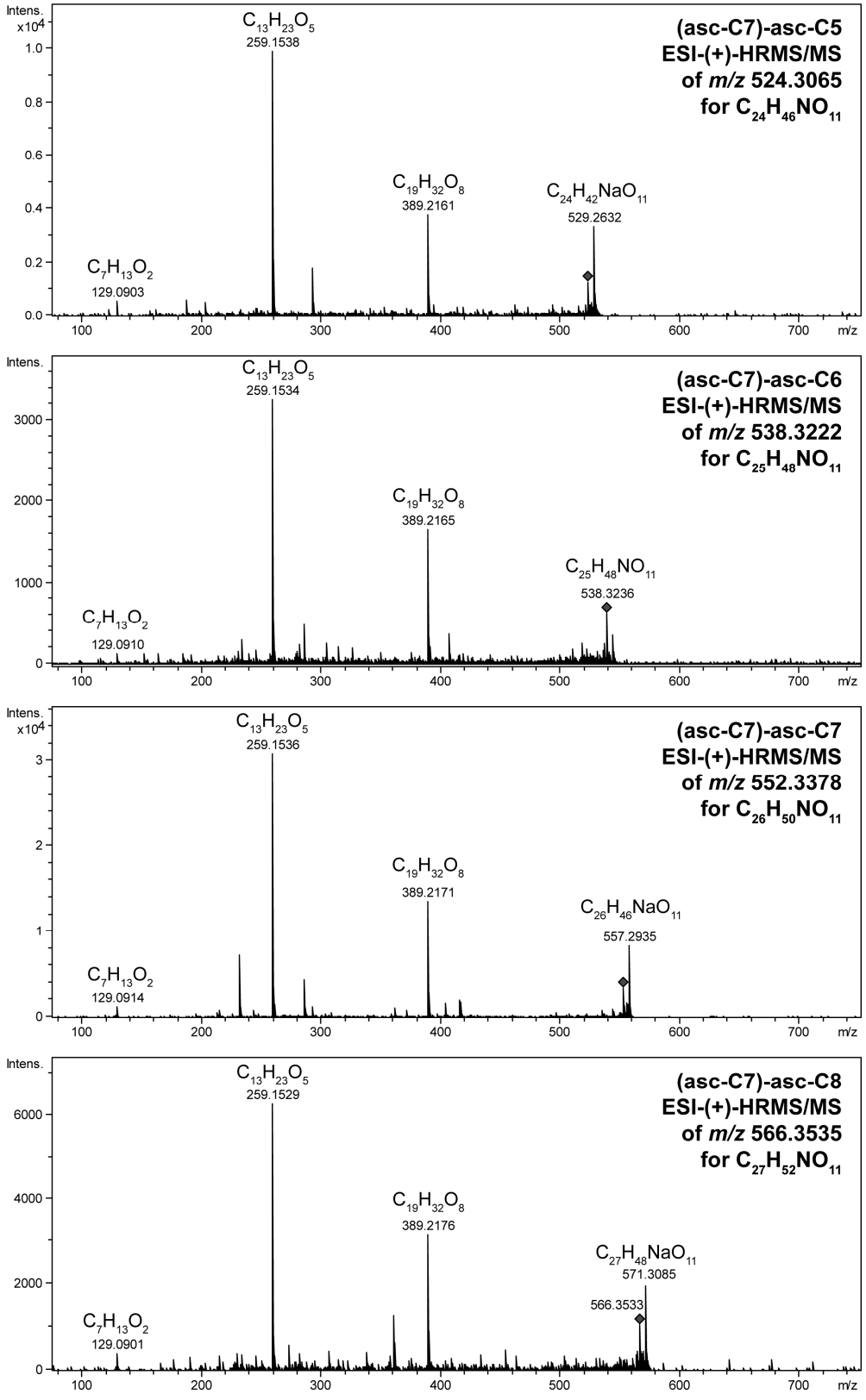


Figure S2f: HPLC-ESI-(+)-HR-MS/MS spectra of dimeric ascarosides from *Caenorhabditis spp.*

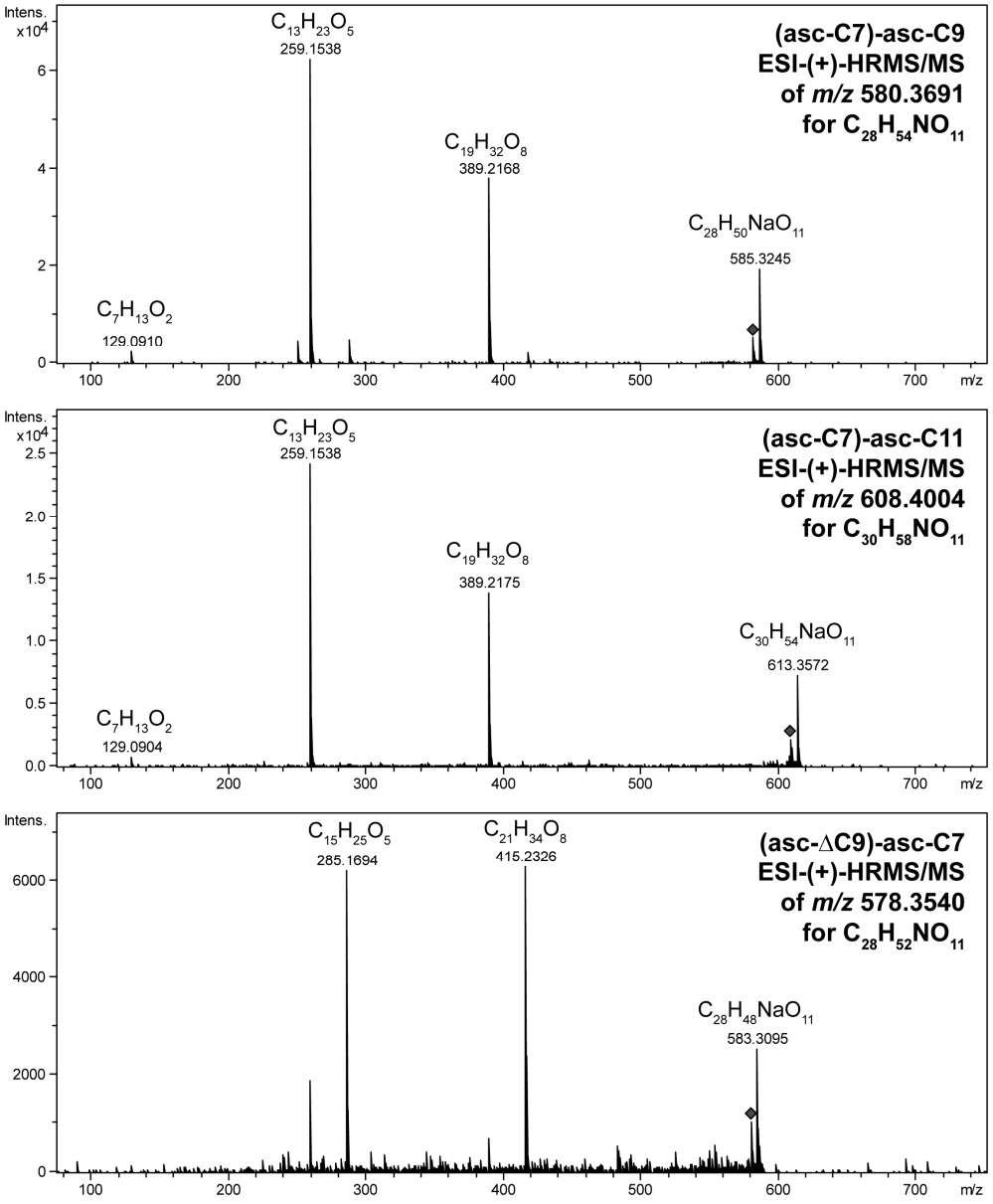


Figure S3a: Composition of ascaroside dimers in *C. remanei* PB4641 as deduced from HPLC-ESI-(+)-HR-MS/MS extracted ion chromatograms.

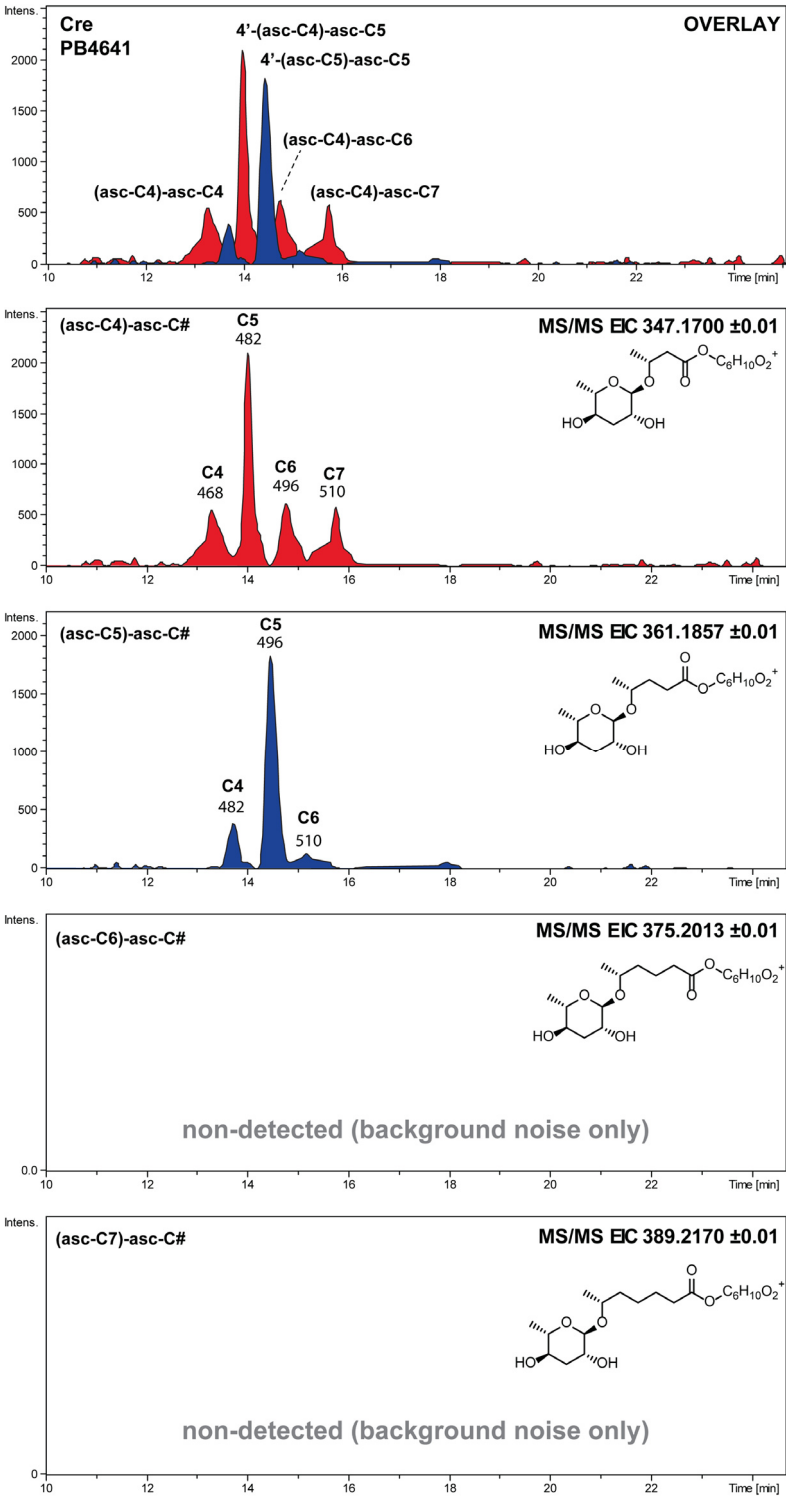


Figure S3b: Composition of ascaroside dimers in *C. nigoni* JU1422 as deduced from HPLC-ESI-(+)-HR-MS/MS extracted ion chromatograms.

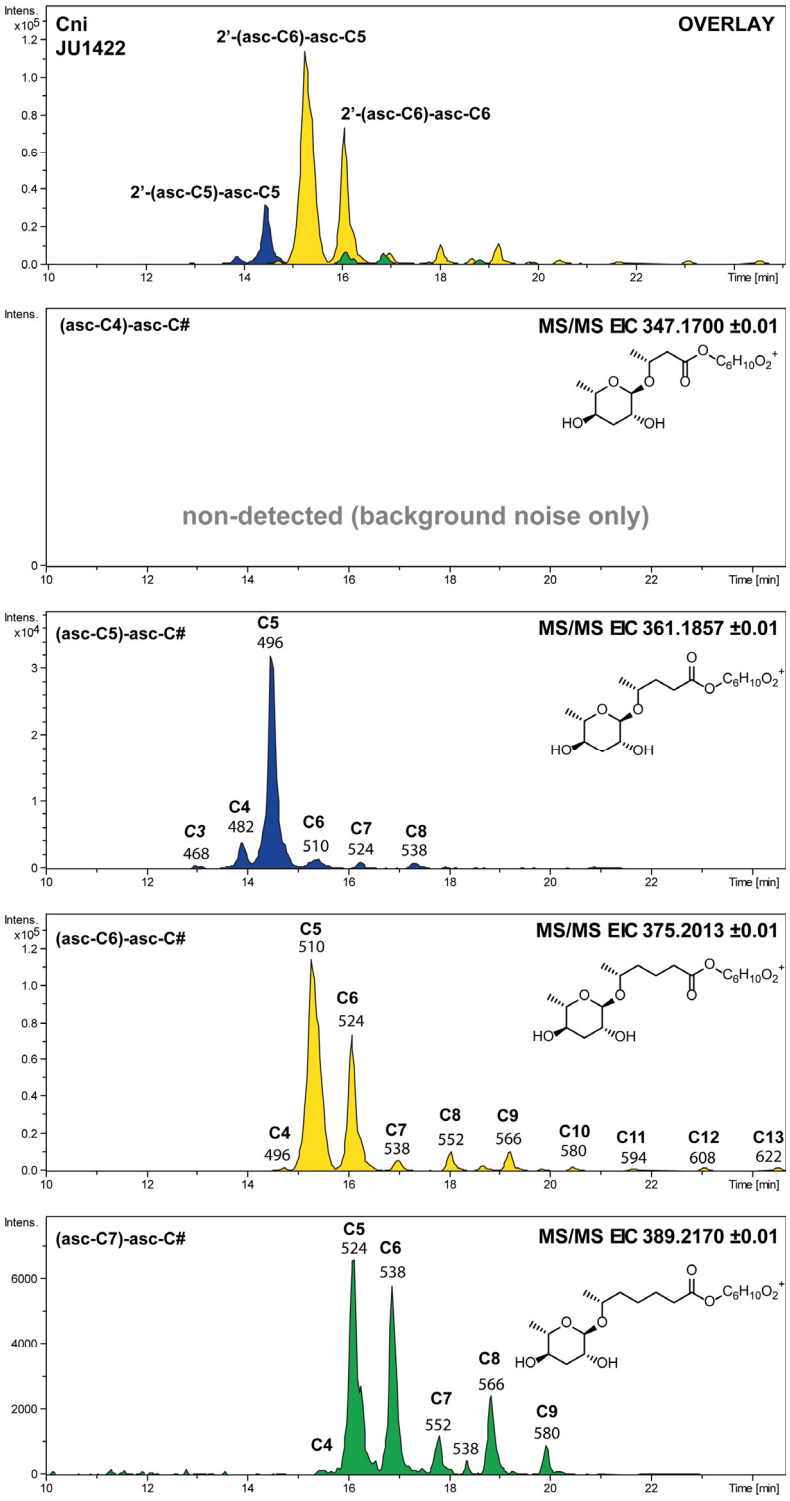


Figure S3c: Composition of ascaroside dimers in *C. brenneri* PB2801 as deduced from HPLC-ESI-(+)-HR-MS/MS extracted ion chromatograms.

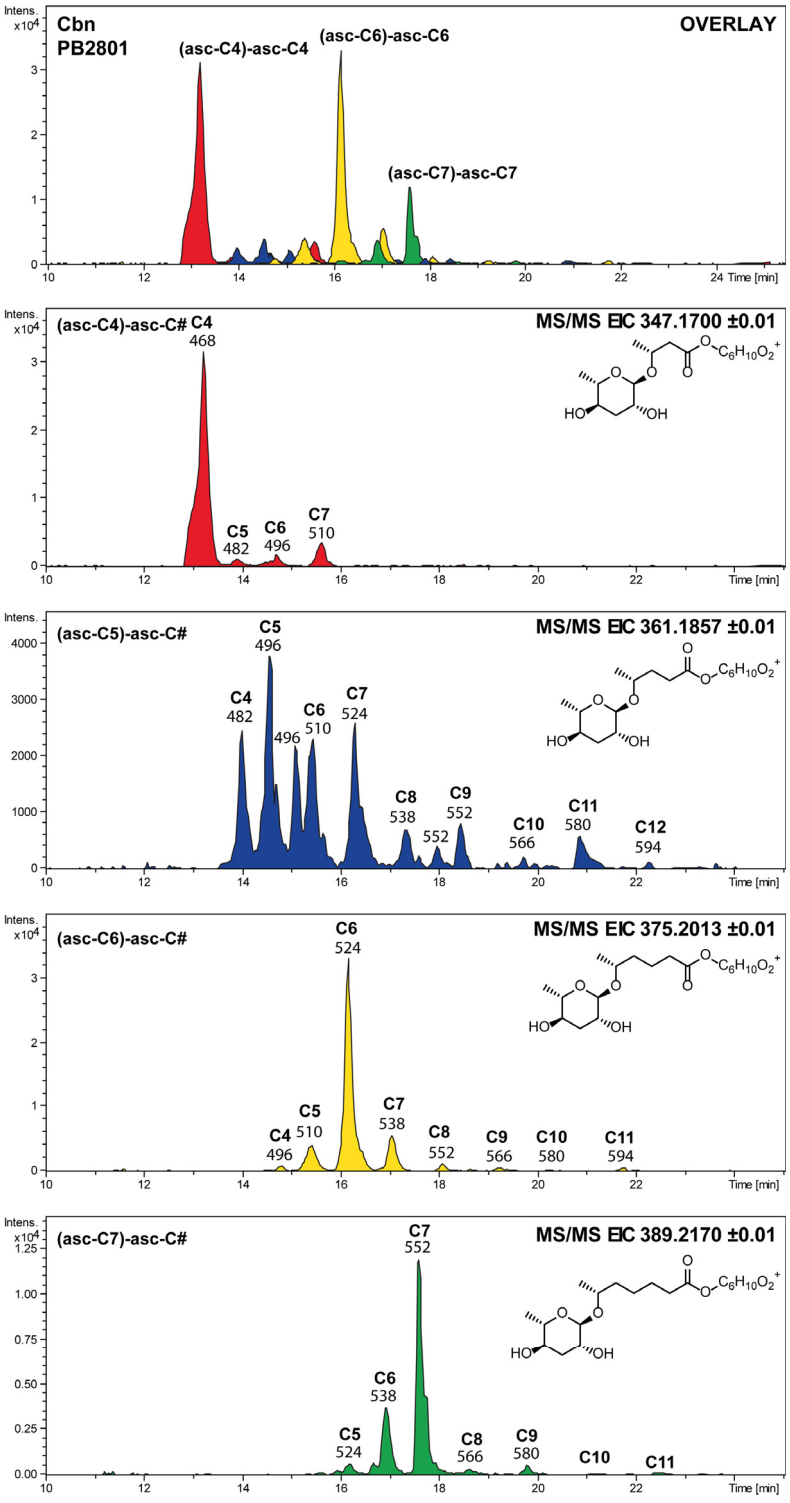


Figure S3d: Composition of ascaroside dimers in *C. tropicalis* JU1373 as deduced from HPLC-ESI-(+)-HR-MS/MS extracted ion chromatograms.

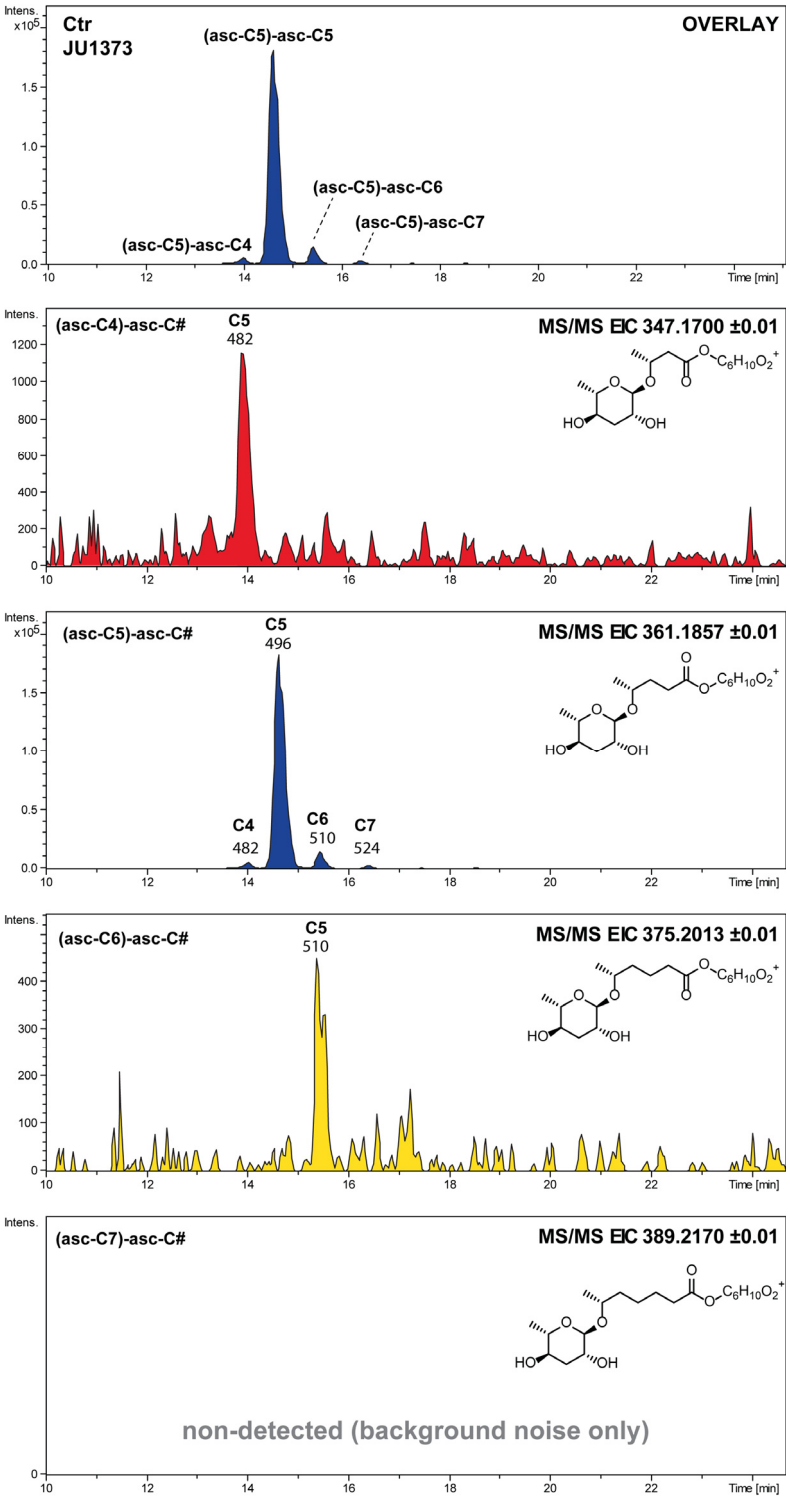


Figure S3e: Composition of ascaroside dimers in *C. sinica* JU727 as deduced from HPLC-ESI-(+)-HR-MS/MS extracted ion chromatograms.

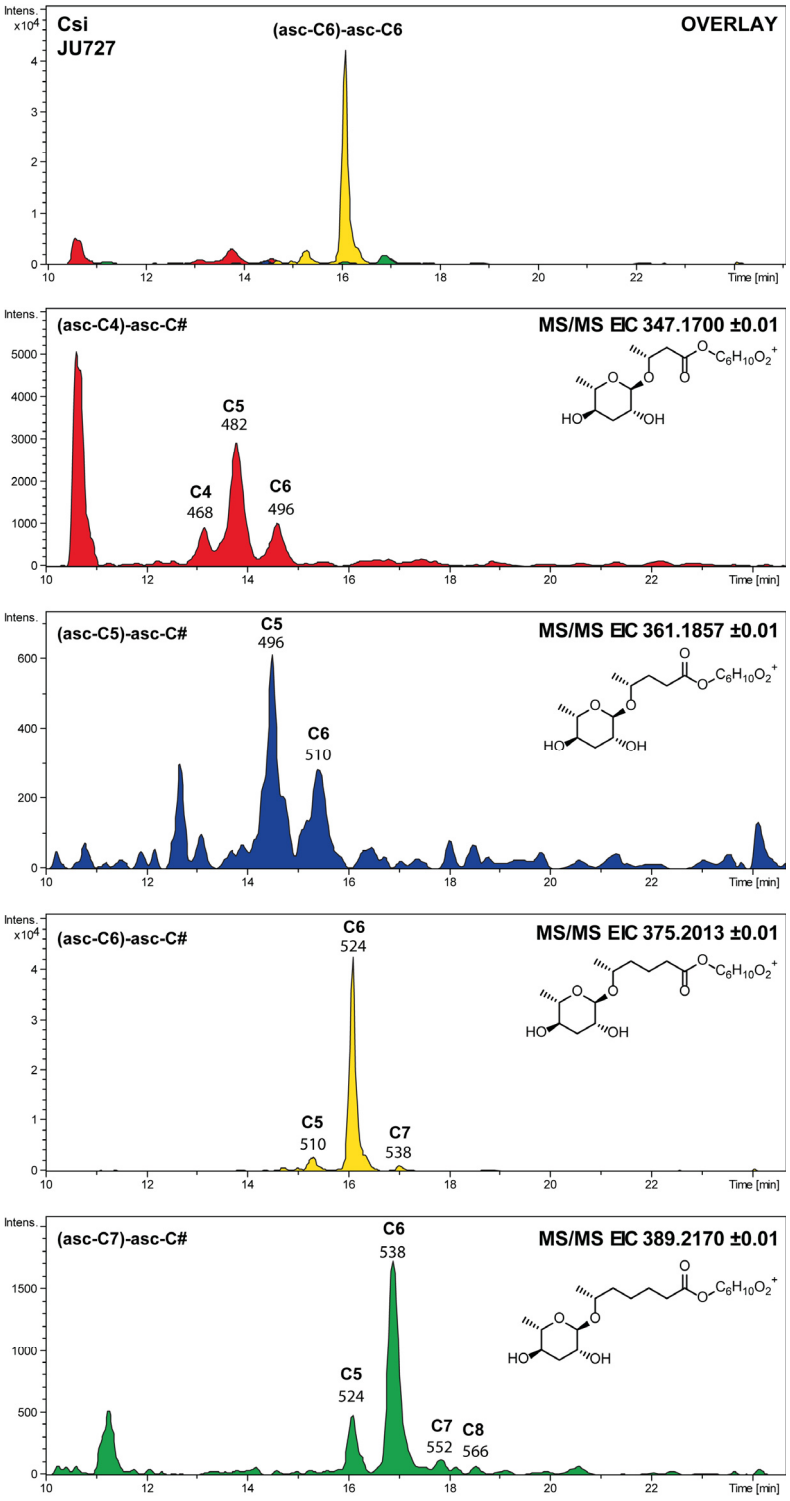


Figure S3f: Composition of ascaroside dimers in *C. briggsae* AF16 as deduced from HPLC-ESI-(+)-HR-MS/MS extracted ion chromatograms.

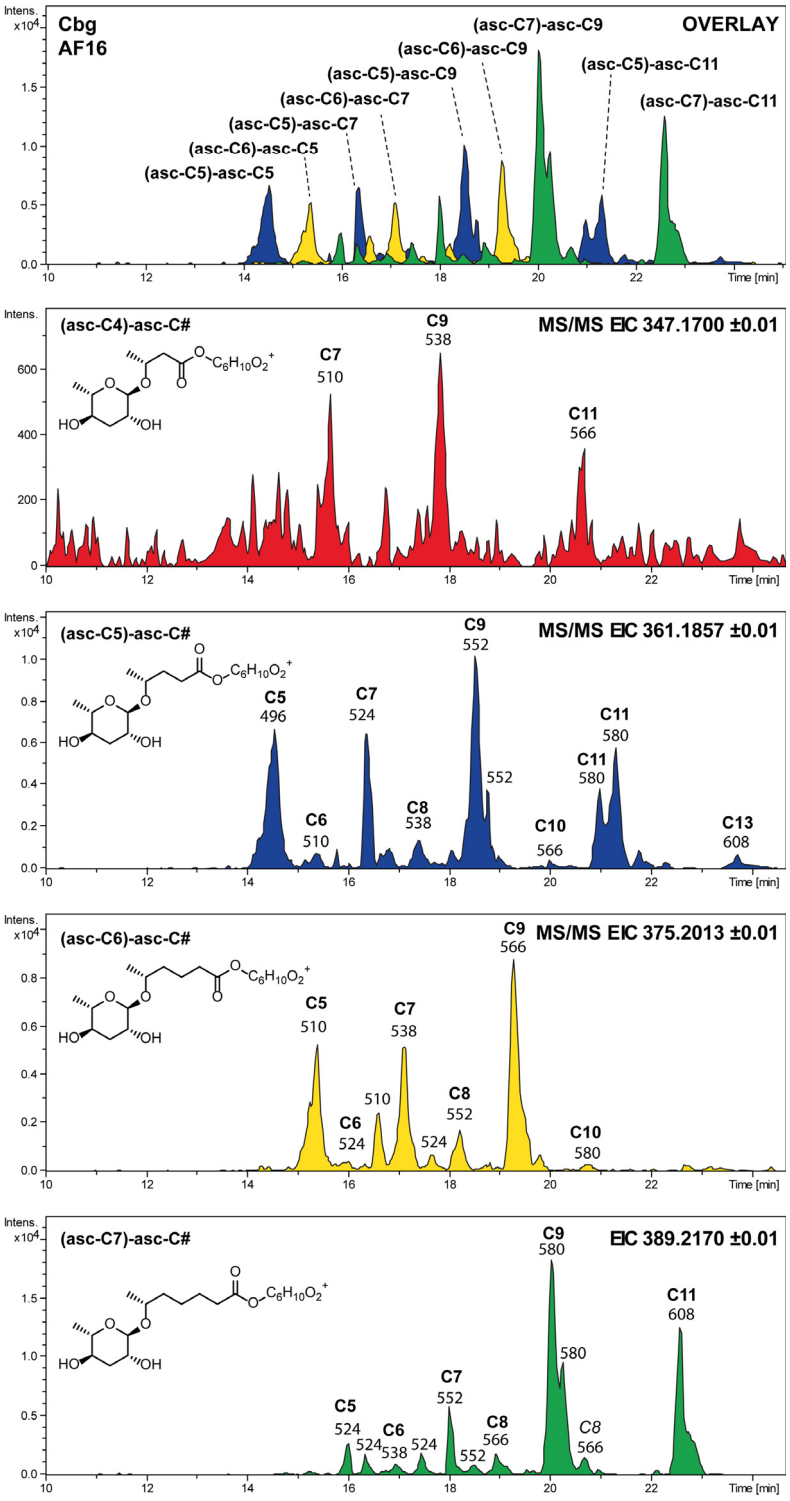
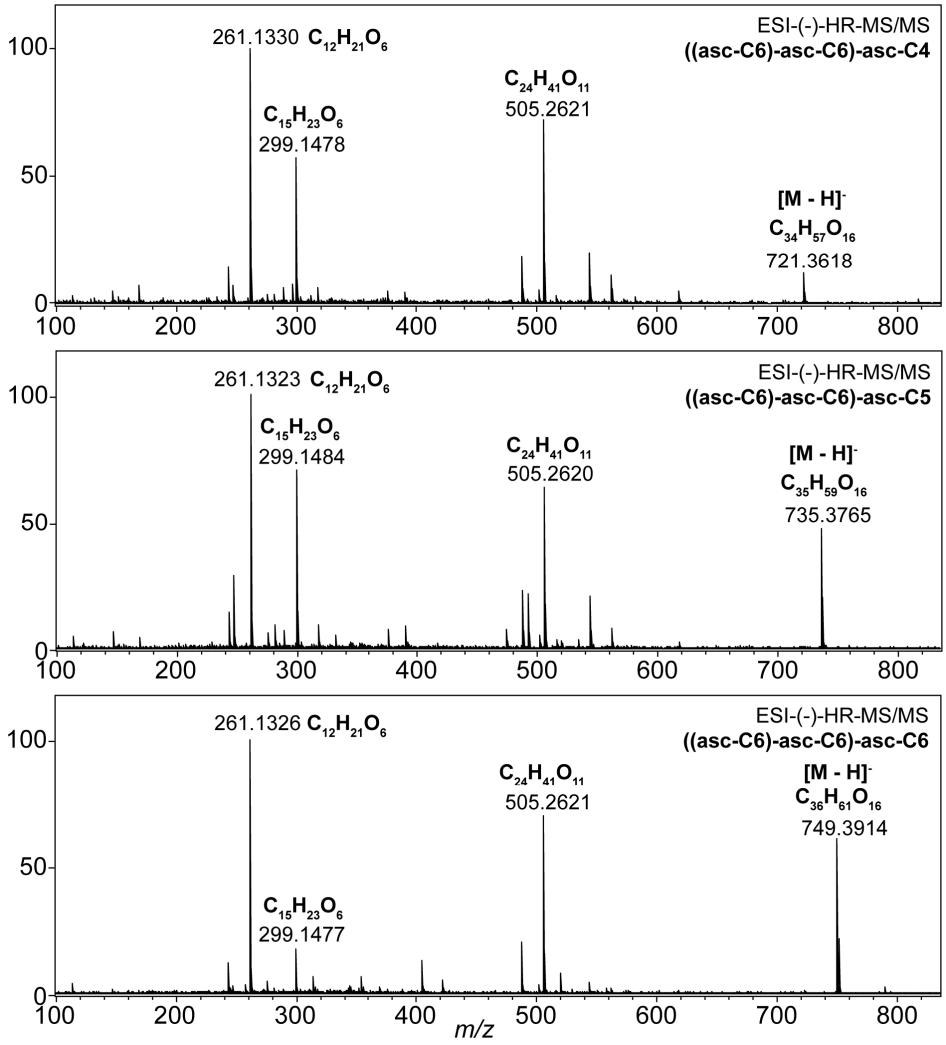
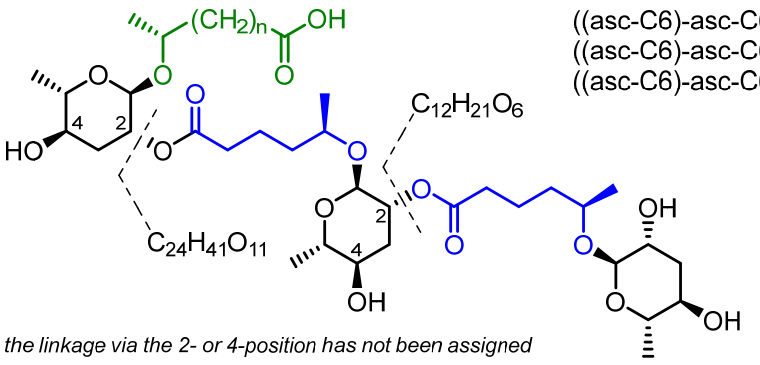


Figure S4a: HPLC-ESI-(-)-HR-MS/MS spectra and MS/MS fragmentation of trimeric ascarosides from *C. nigoni* JU1422.

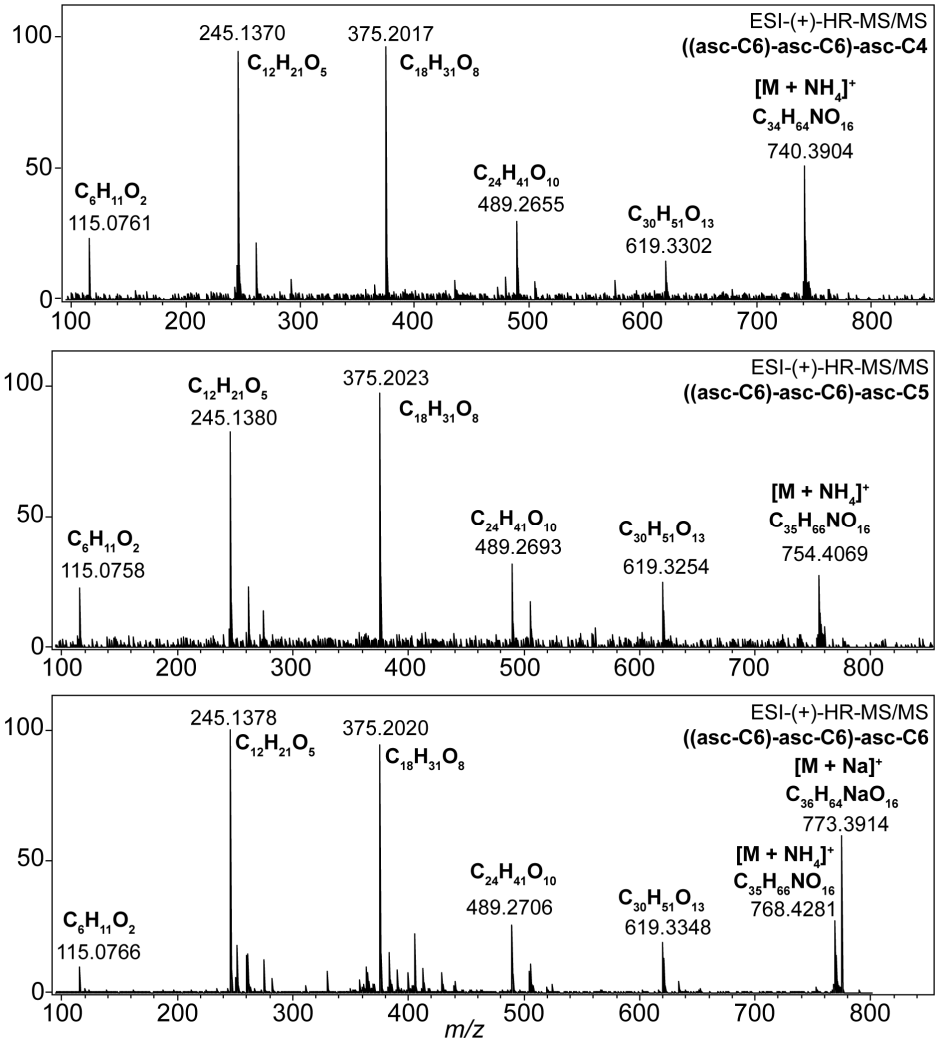




((asc-C6)-asc-C6)-asc-C4 (n = 1)
((asc-C6)-asc-C6)-asc-C5 (n = 2)
((asc-C6)-asc-C6)-asc-C6 (n = 3)

the linkage via the 2- or 4-position has not been assigned

Figure S4b: HPLC-ESI-(+)-HR-MS/MS spectra and MS/MS fragmentation of trimeric ascarosides from *C. nigoni* JU1422.



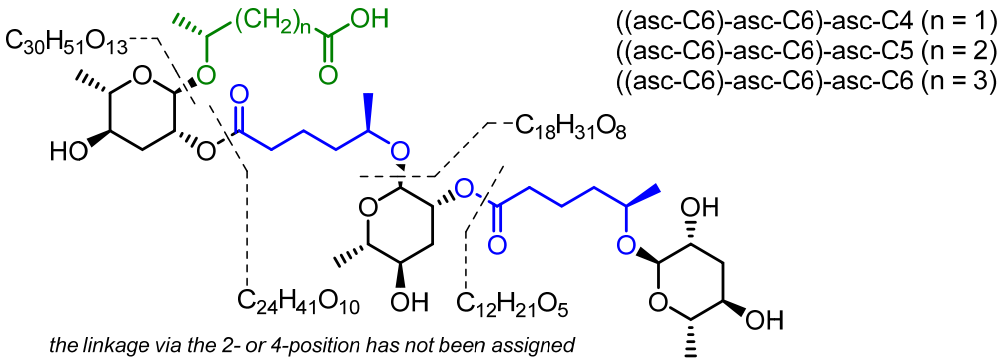


Figure S5: 400 MHz ^{1}H NMR spectra (in CD_3OD) showing the isolation of 4'-(asc-C4)-asc-C5 (**5**) from the *C. remanei* PB4641 exometabolome: (**A**) Partially enriched fraction from 1st solid phase extraction (SPE) on RP-C18; (**B**) enriched fraction from 2nd SPE on RP-C18ec; (**C**) pure 4'-(asc-C4)-asc-C5 (**5**) isolated by HPLC.

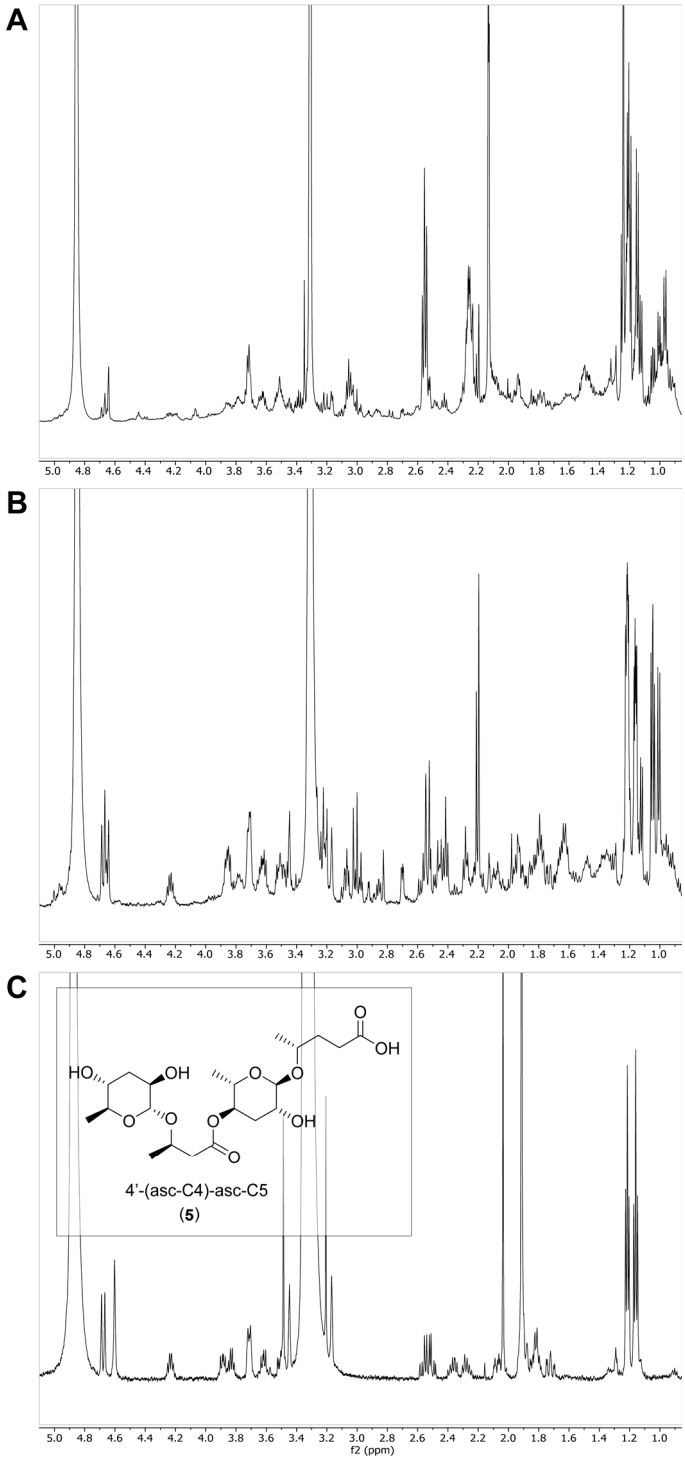


Figure S6a: 400 MHz *dqf*-COSY spectra (in CD_3OD) showing the isolation of 4'-(asc-C4)-asc-C5 (**5**) from the *C. remanei* PB4641 exometabolome. (**A**) Partially enriched fraction from 1st solid phase extraction (SPE) on RP-C18; (**B**) enriched fraction from 2nd SPE on RP-C18ec; (**C**) pure 4'-(asc-C4)-asc-C5 (**5**) isolated by HPLC; note that structure assignment is already possible after the 1st SPE fractionation step.

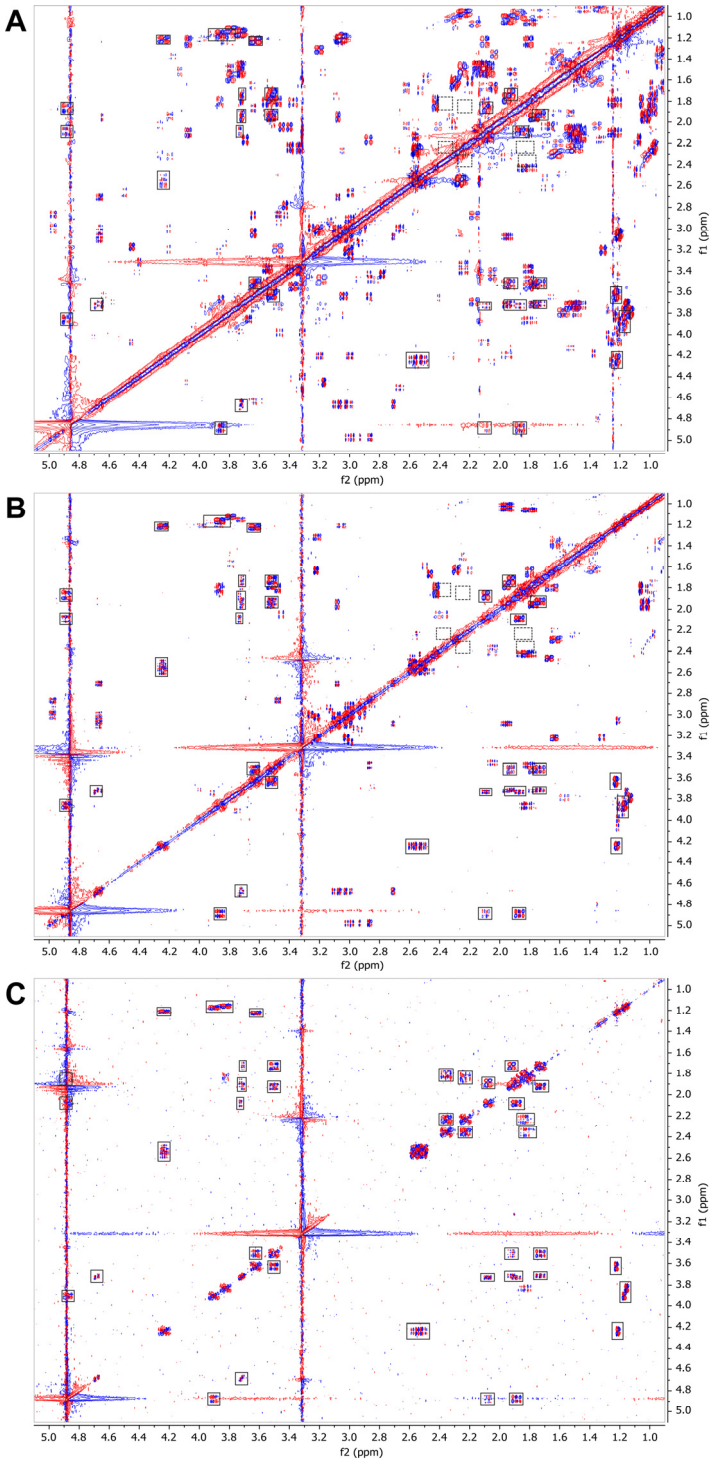


Figure S6b: Enlarged sections of 400 MHz *dqf*-COSY spectra (in CD_3OD) showing the isolation of 4'-(asc-C4)-asc-C5 (**5**) from the *C. remanei* PB4641 exometabolome. (**A**) Partially enriched fraction from 1st solid phase extraction (SPE) on RP-C18; (**B**) enriched fraction from 2nd SPE on RP-C18ec; (**C**) pure 4'-(asc-C4)-asc-C5 (**5**) isolated by HPLC; note that assignment of the 4-linkage is already possible after the 1st SPE fractionation step.

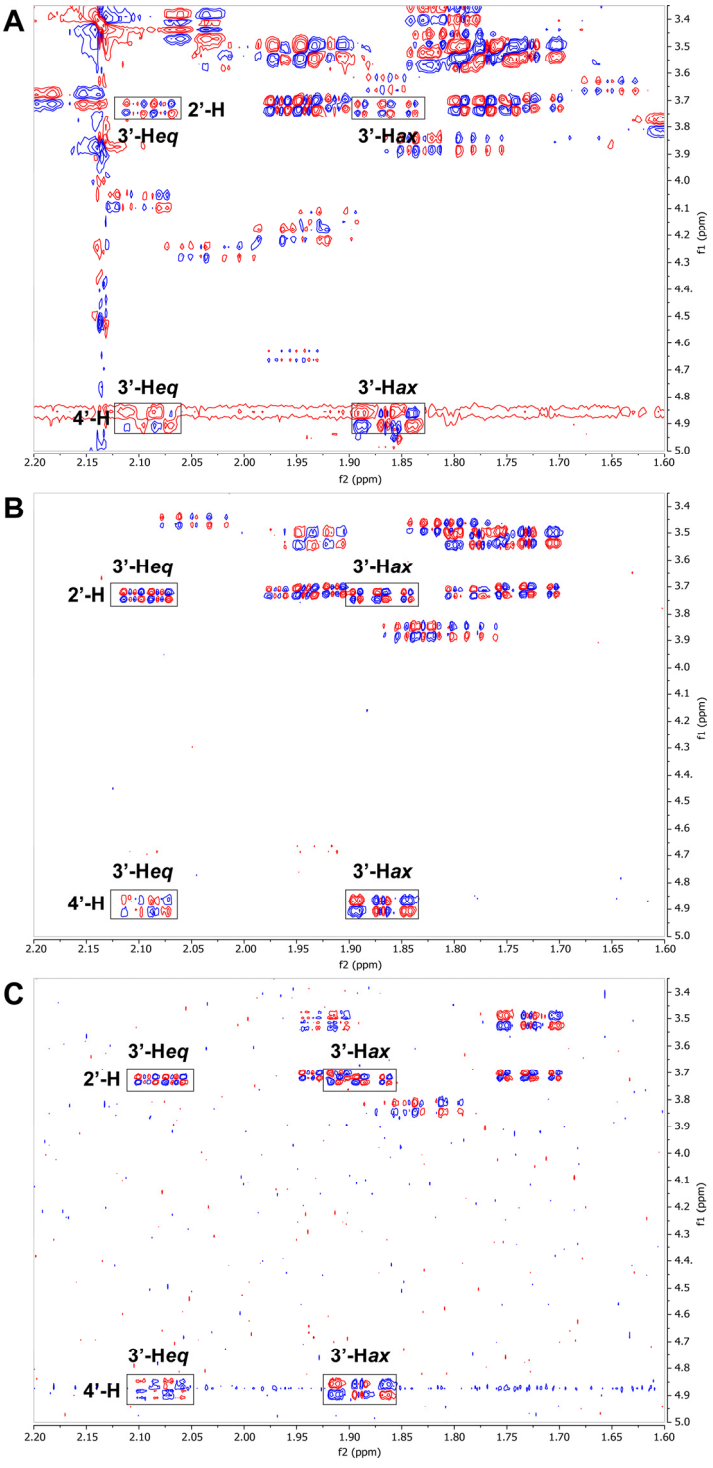


Figure S7: HPLC-ESI-(-)-HR-MS chromatograms showing the isolation of ascaroside dimers from *C. nigoni* strain JU1422: (**A**) & (**B**) partially enriched fractions from Solid Phase Extraction (SPE) on RP-C18; (**C**), (**D**) & (**E**) highly enriched HPLC fractions that were analyzed by *dqf*-COSY.

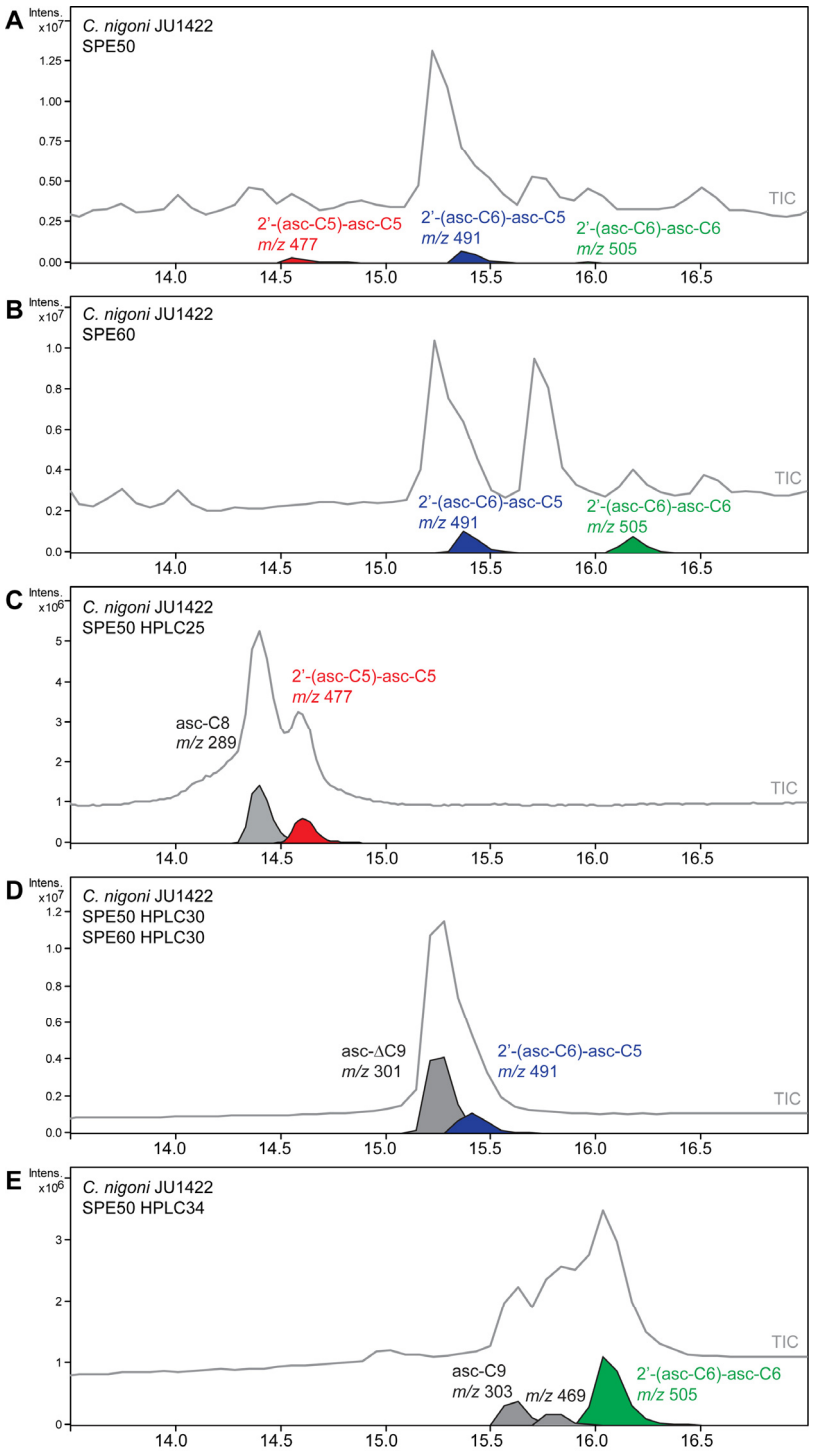


Figure S8: HPLC-ESI-(+)-HR-MS/MS spectra of (**A**) 2'-(asc-C5)-asc-C5 (**6**), (**B**) 2'-(asc-C6)-asc-C5 (**7**), and (**C**) 2'-(asc-C6)-asc-C6 (**8**) from *C. nigoni* JU1422.

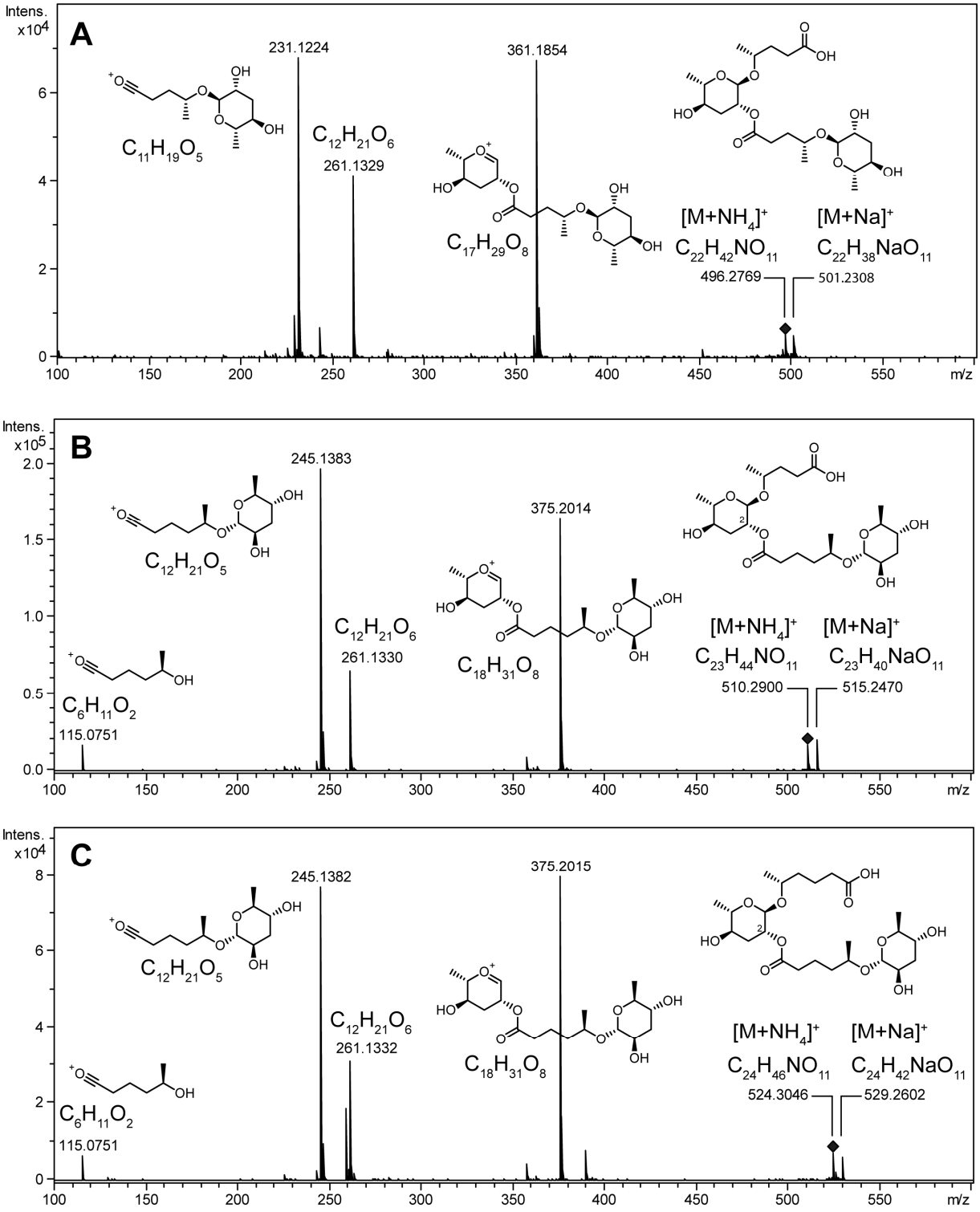


Figure S9a: Sections of the 400 MHz *dqf*-COSY spectra (in CD_3OD) of 2-linked ascaroside dimers (**A**) 2'-(asc-C5)-asc-C5 (**6**), (**B**) 2'-(asc-C6)-asc-C5 (**7**), and (**C**) 2'-(asc-C6)-asc-C6 (**8**) enriched from *C. nigoni* JU1422.

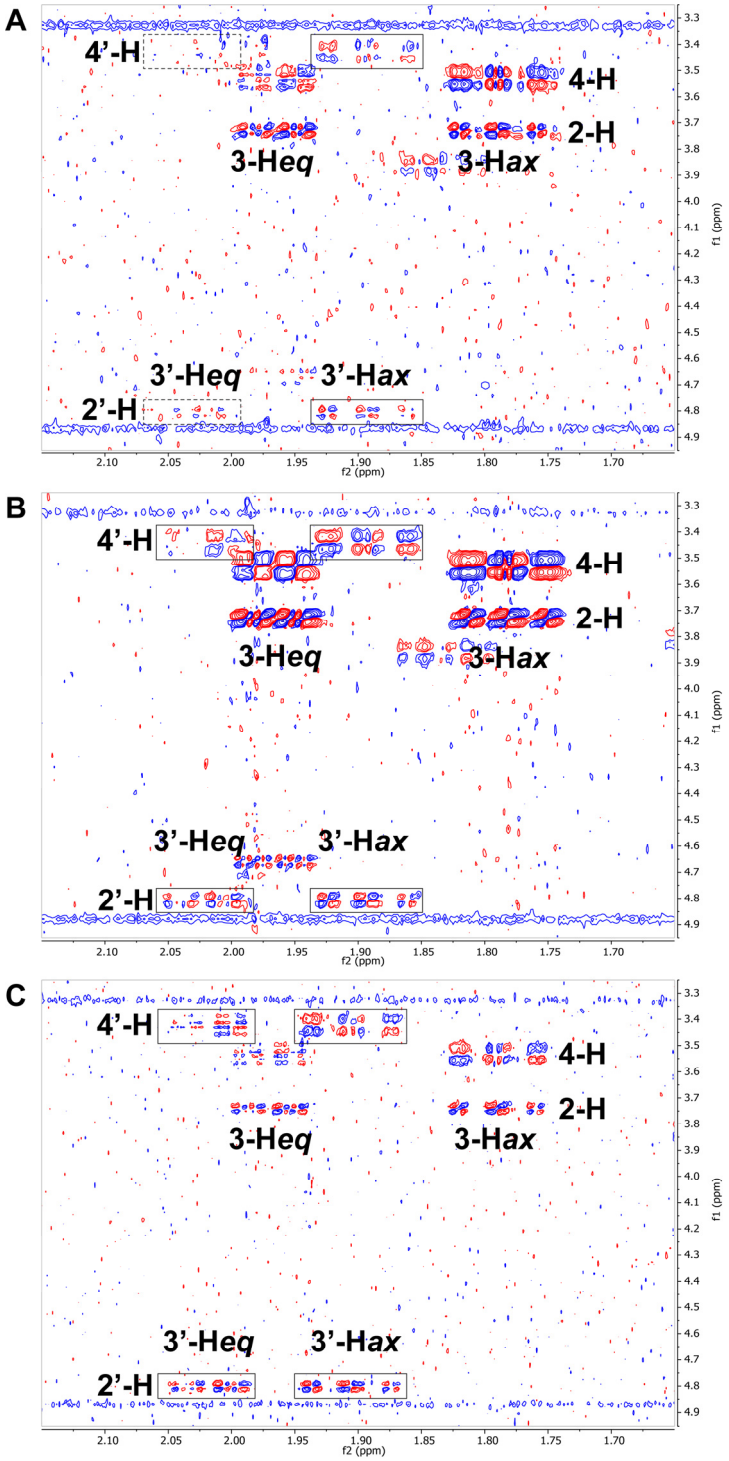


Figure S9b: Sections of the 400 MHz *dqf*-COSY spectra (in CD_3OD) of 2-linked ascaroside dimers (**A**) 2'-(asc-C5)-asc-C5 (**6**), (**B**) 2'-(asc-C6)-asc-C5 (**7**), and (**C**) 2'-(asc-C6)-asc-C6 (**8**) enriched from *C. nigoni* JU1422.

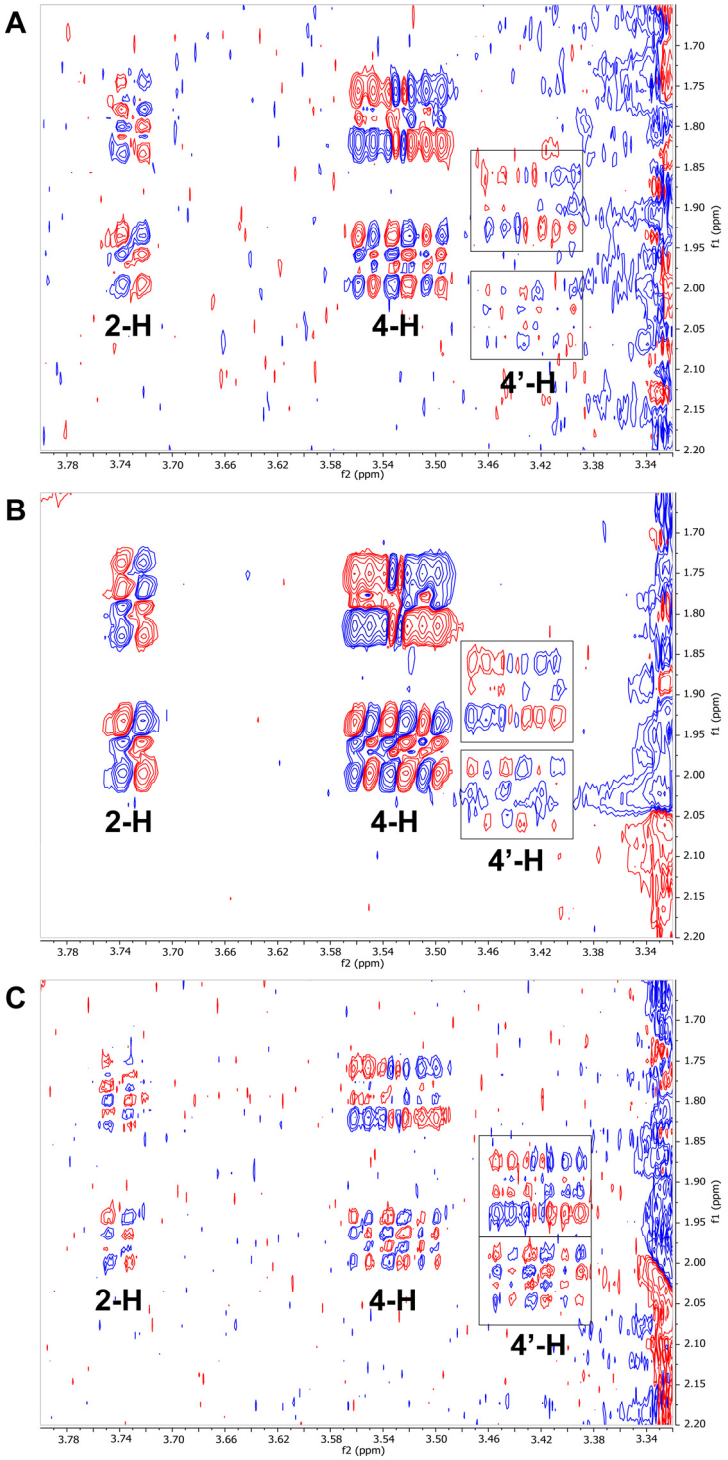


Figure S10a: Comparative analysis of the 400 MHz ^{1}H NMR spectra (in CD_3OD) of (**A**) synthetic 2'-(asc-C6)-asc-C6 (**8**) and (**B**) the natural product isolated from *C. nigoni* JU1422.

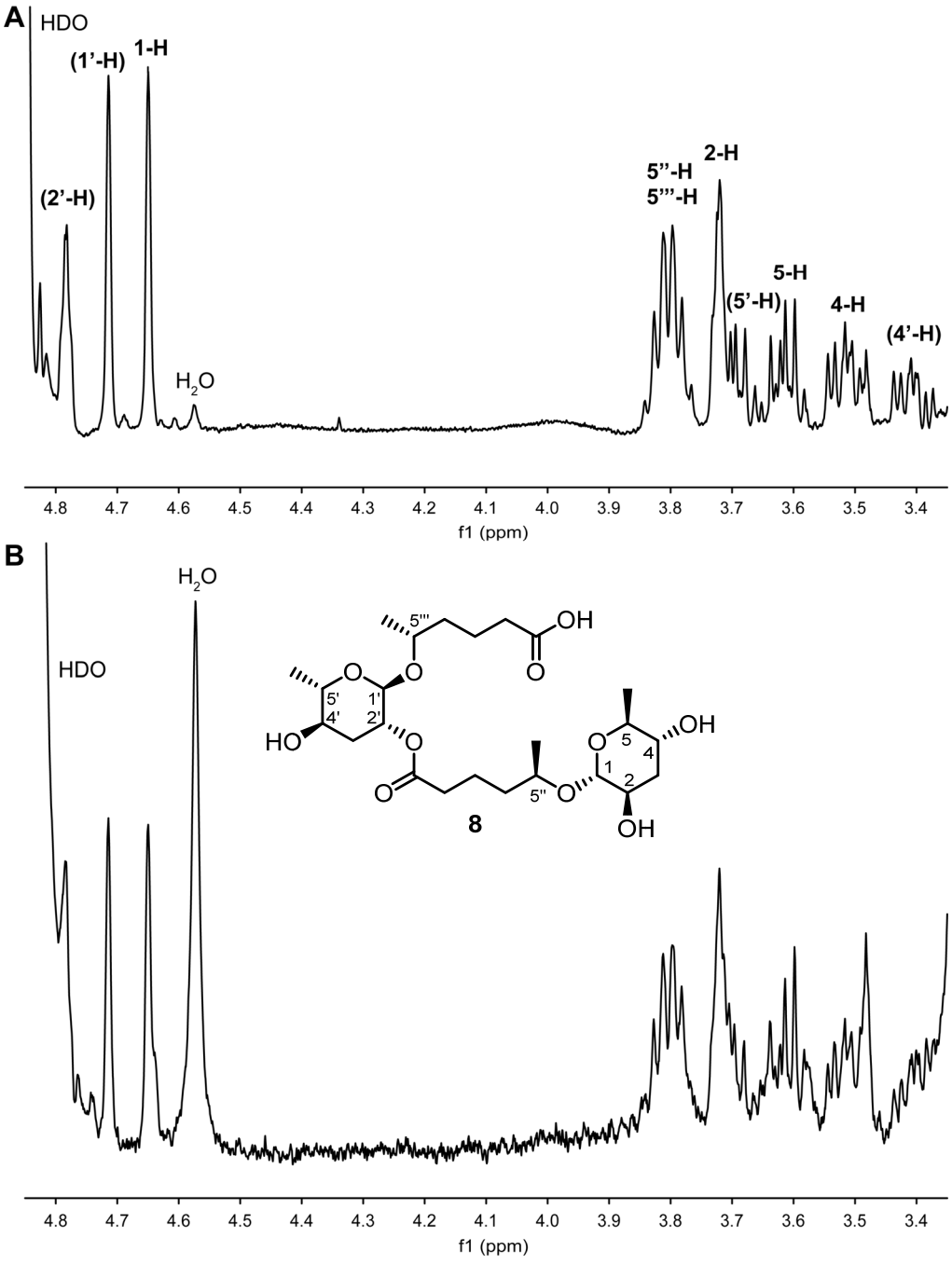


Figure S10b: Comparative analysis of the 400 MHz ^{1}H NMR spectra (in CD_3OD) of (**A**) synthetic 2'-(asc-C6)-asc-C6 (**8**) and (**B**) the natural product isolated from *C. nigoni* JU1422.

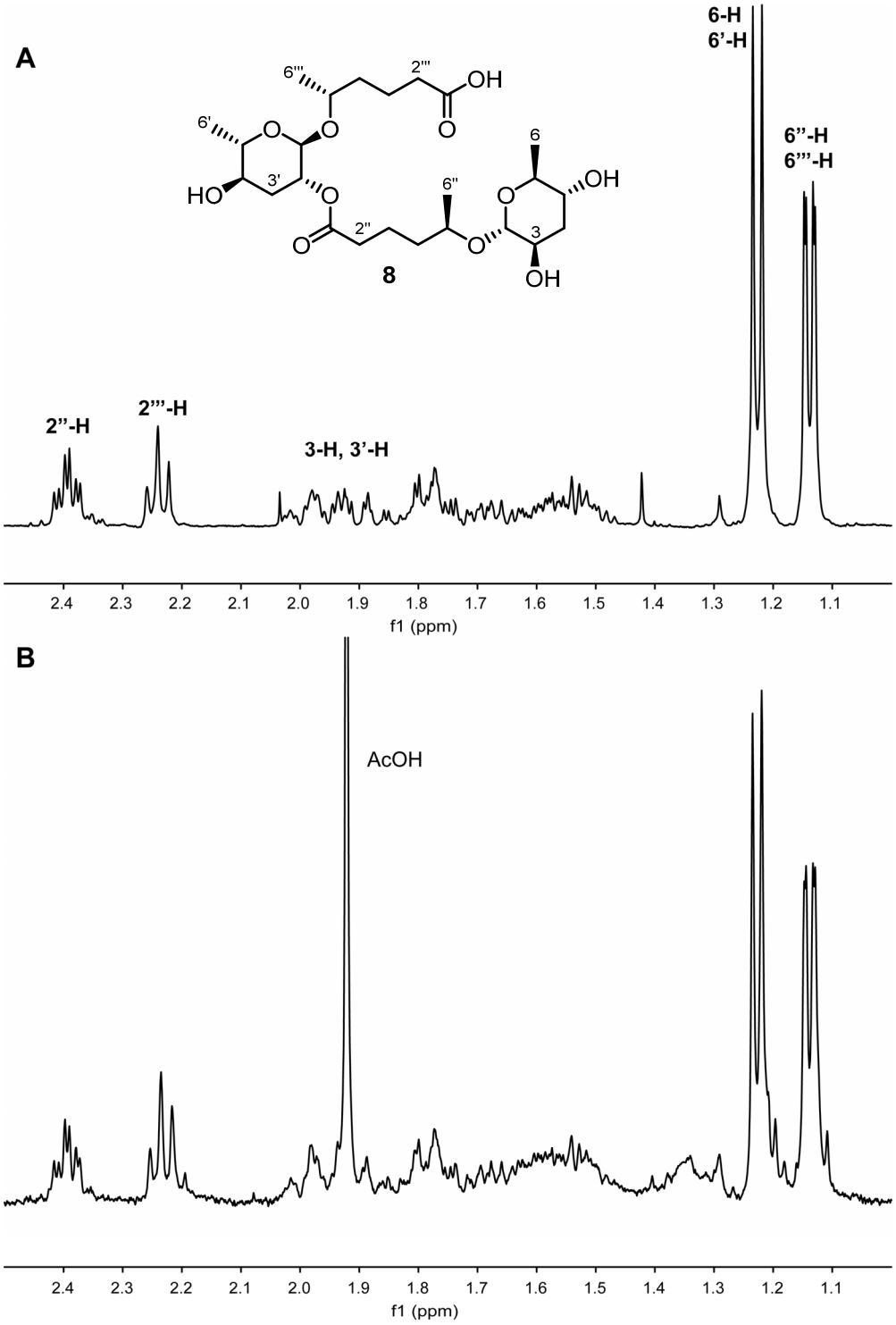


Figure S10c: Comparative analysis of the 400 MHz *dqf*-COSY spectra (in CD_3OD) of (**A**) synthetic 2'-(asc-C6)-asc-C6 (**8**) and (**B**) the natural product isolated from *C. nigoni* JU1422.

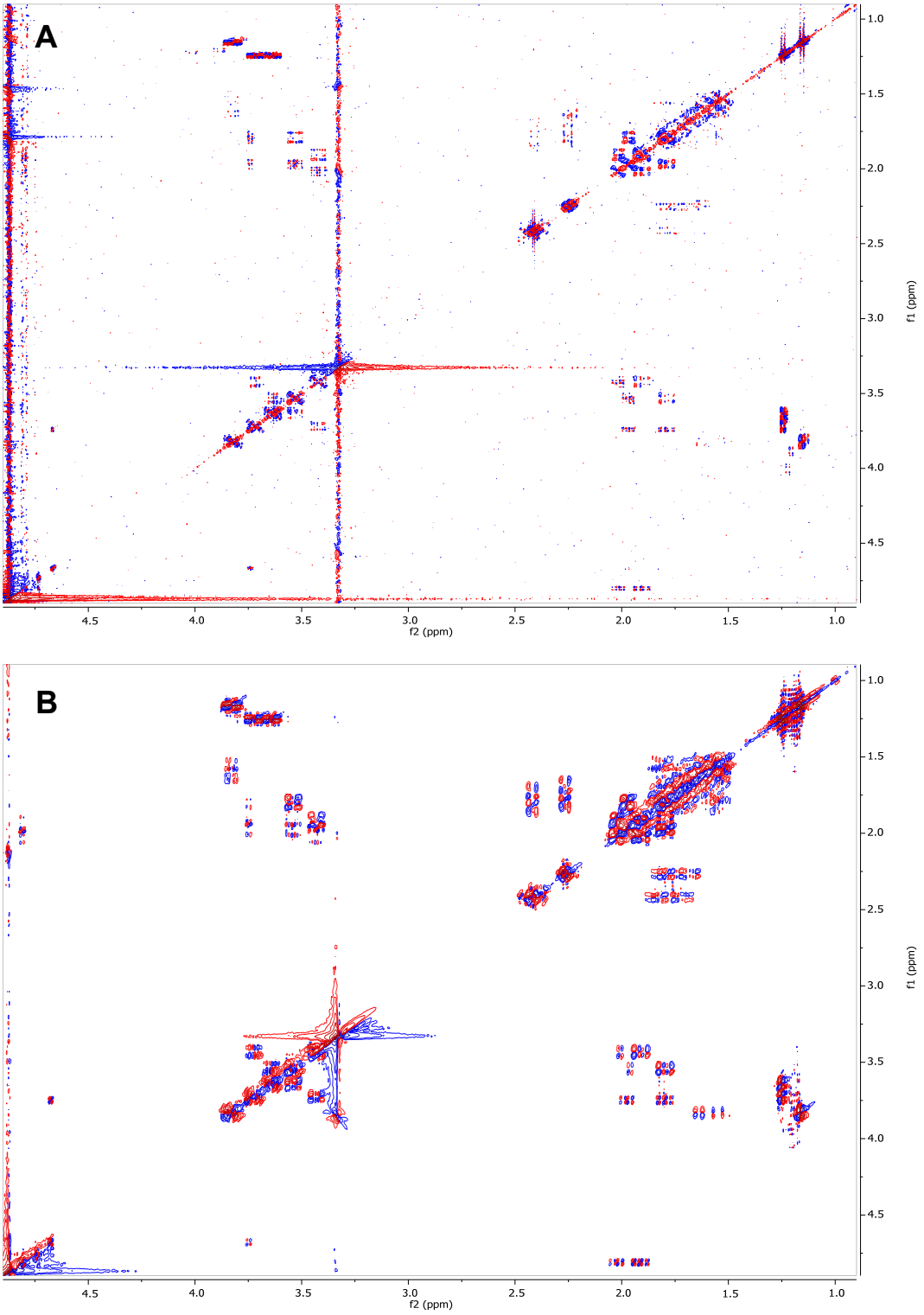


Figure S10d: Comparative analysis of the 400 MHz ^{1}H NMR spectra (in CD_3OD) of (**A**) enriched 2'-(asc-C6)-asc-C5 (**7**) from *C. nigoni* JU1422, (**B**) synthetic asc-ΔC9, and (**C**) synthetic 2'-(asc-C6)-asc-C5 (**7**).

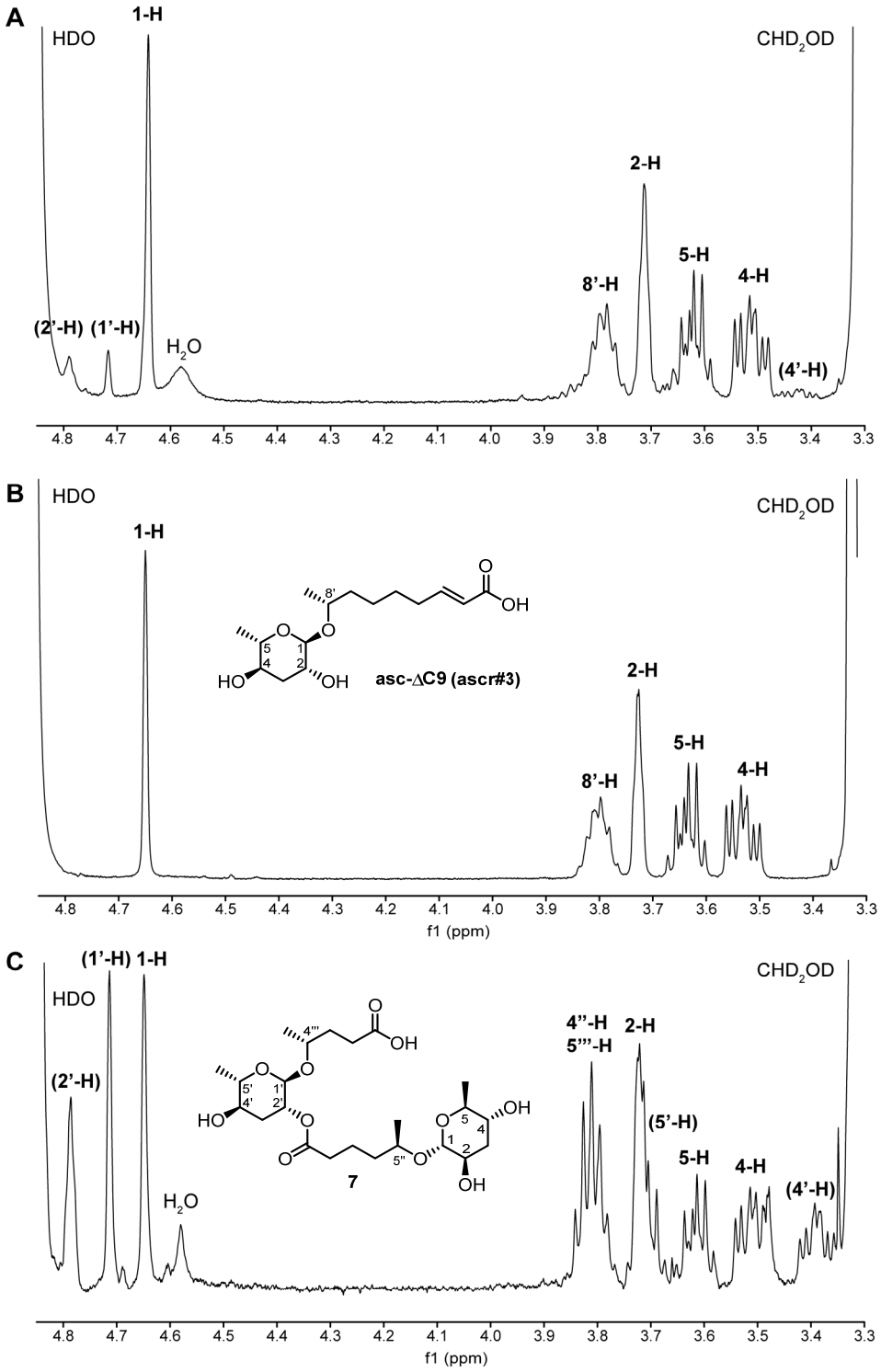
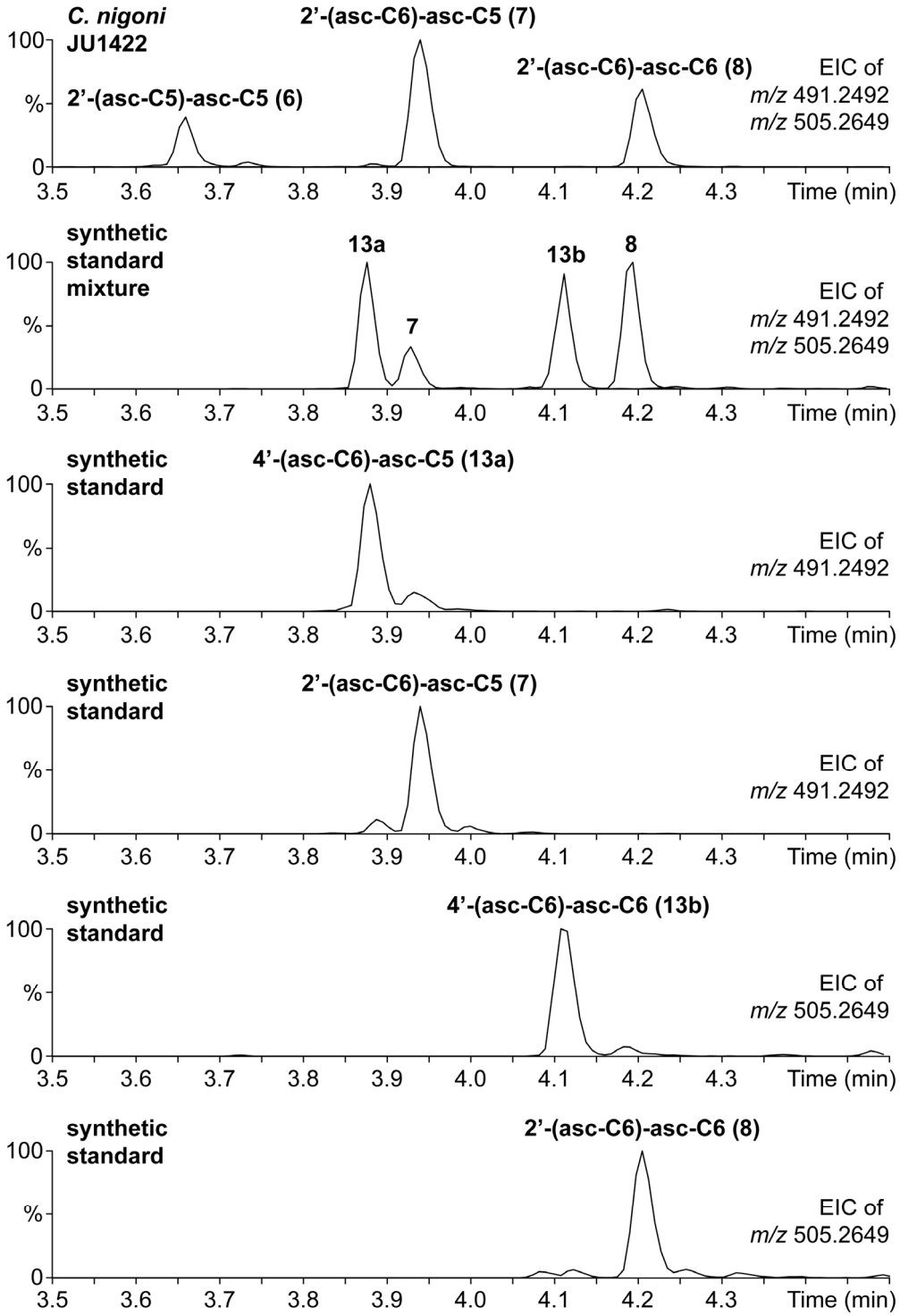


Figure S11: Comparative UPLC-HR-MS analysis of ascaroside dimers from *C. nigoni* JU1422 and synthetic standards of 2'-(asc-C6)-asc-C5 (**7**), 2'-(asc-C6)-asc-C6 (**8**), 4'-(asc-C6)-asc-C5 (**13a**), and 4'-(asc-C6)-asc-C6 (**13b**) confirms the structure assignment of the natural compounds as 2-linked **7** and **8**.



Supporting Tables

Table S1a. HPLC-ESI-HR-MS/MS data of ascaroside dimers from *Caenorhabditis* species.

			(asc-C#)-asc-C#		(asc-C#)-asc-C#		(asc-C#)-asc-C#		(asc-C#)-asc		asc-C#		C#	
			[M - H]$^-$		[M + Na]$^+$		[M + NH_4]$^+$		[M – agylcone]$^+$		[monomer-C≡O]$^+$		[agylcone]$^+$	
Fig.			*m/z*	formula	*m/z*	formula	*m/z*	formula	*m/z*	formula	*m/z*	formula	*m/z*	formula
S2a	**C4C4**	obs.	449.2033	$C_{20}H_{33}O_{11}$	473.2003	$C_{20}H_{34}NaO_{11}$	468.2447	$C_{20}H_{38}NO_{11}$	347.1703	$C_{16}H_{27}O_8$	217.1070	$C_{10}H_{17}O_5$	*nd*	$C_4H_7O_2$
		calc.	449.2028		473.1993		468.2439		347.1700		217.1071		87.0441	
S2a	**C4C5**	obs.	463.2176	$C_{21}H_{35}O_{11}$	487.2157	$C_{21}H_{36}NaO_{11}$	482.2608	$C_{21}H_{40}NO_{11}$	347.1718	$C_{16}H_{27}O_8$	217.1076	$C_{10}H_{17}O_5$	*nd*	$C_4H_7O_2$
		calc.	463.2185		487.2150		482.2596		347.1700		217.1071		87.0441	
S2a	**C4C6**	obs.	477.2348	$C_{22}H_{37}O_{11}$	501.2317	$C_{22}H_{38}NaO_{11}$	496.2765	$C_{22}H_{42}NO_{11}$	347.1705	$C_{16}H_{27}O_8$	217.1072	$C_{10}H_{17}O_5$	*nd*	$C_4H_7O_2$
		calc.	477.2341		501.2306		496.2752		347.1700		217.1071		87.0441	
S2a	**C4C7**	obs.	491.2501	$C_{23}H_{39}O_{11}$	515.2471	$C_{23}H_{40}NaO_{11}$	510.2914	$C_{23}H_{44}NO_{11}$	347.1703	$C_{16}H_{27}O_8$	217.1069	$C_{10}H_{17}O_5$	*nd*	$C_4H_7O_2$
		calc.	491.2498		515.2463		510.2909		347.1700		217.1071		87.0441	
S2b	**C5C4**	obs.	463.2197	$C_{21}H_{35}O_{11}$	487.2165	$C_{21}H_{36}NaO_{11}$	482.2609	$C_{21}H_{40}NO_{11}$	361.1858	$C_{17}H_{29}O_8$	231.1217	$C_{11}H_{19}O_5$	101.0609	$C_5H_9O_2$
		calc.	463.2185		487.2150		482.2596		361.1857		231.1227		101.1597	
S2b	**C5C5**	obs.	477.2335	$C_{22}H_{37}O_{11}$	501.2312	$C_{22}H_{38}NaO_{11}$	496.2760	$C_{22}H_{42}NO_{11}$	361.1861	$C_{17}H_{29}O_8$	231.1228	$C_{11}H_{19}O_5$	101.0596	$C_5H_9O_2$
		calc.	477.2341		501.2306		496.2752		361.1857		231.1227		101.1597	
S2b	**C5C6**	obs.	491.2495	$C_{23}H_{39}O_{11}$	515.2472	$C_{23}H_{40}NaO_{11}$	510.2917	$C_{23}H_{44}NO_{11}$	361.1862	$C_{17}H_{29}O_8$	231.1225	$C_{11}H_{19}O_5$	101.0599	$C_5H_9O_2$
		calc.	491.2498		515.2463		510.2909		361.1857		231.1227		101.1597	
S2b	**C5C7**	obs.	505.2664	$C_{24}H_{41}O_{11}$	529.2621	$C_{24}H_{42}NaO_{11}$	524.3066	$C_{24}H_{46}NO_{11}$	361.1857	$C_{17}H_{29}O_8$	231.1226	$C_{11}H_{19}O_5$	101.1245	$C_5H_9O_2$
		calc.	505.2654		529.2619		524.3065		361.1857		231.1227		101.1597	
S2c	**C5C8**	obs.	519.2807	$C_{25}H_{43}O_{11}$	543.2753	$C_{25}H_{44}NaO_{11}$	538.3204	$C_{25}H_{48}NO_{11}$	361.1863	$C_{17}H_{29}O_8$	231.1228	$C_{11}H_{19}O_5$	*nd*	$C_5H_9O_2$
		calc.	519.2811		543.2776		538.3222		361.1857		231.1227		101.1597	
S2c	**C5C9**	obs.	533.2975	$C_{26}H_{45}O_{11}$	557.2927	$C_{26}H_{46}NaO_{11}$	552.3370	$C_{26}H_{50}NO_{11}$	361.1857	$C_{17}H_{29}O_8$	231.1227	$C_{11}H_{19}O_5$	*nd*	$C_5H_9O_2$
		calc.	533.2967		557.2932		552.3378		361.1857		231.1227		101.1597	
-	**C5C10**	obs.	547.3103	$C_{27}H_{47}O_{11}$	571.3094	$C_{27}H_{48}NaO_{11}$	566.3544	$C_{27}H_{52}NO_{11}$	361.1865	$C_{17}H_{29}O_8$	231.1232	$C_{11}H_{19}O_5$	*nd*	$C_5H_9O_2$
		calc.	547.3124		571.3089		566.3535		361.1857		231.1227		101.1597	
S2c	**C5C11**	obs.	561.3288	$C_{28}H_{49}O_{11}$	585.3269	$C_{28}H_{48}NaO_{11}$	580.3712	$C_{28}H_{54}NO_{11}$	361.1850	$C_{17}H_{29}O_8$	231.1226	$C_{11}H_{19}O_5$	101.0697	$C_5H_9O_2$
		calc.	561.3280		585.3245		580.3691		361.1857		231.1227		101.1597	
-	**C5C12**	obs.	575.3431	$C_{29}H_{51}O_{11}$	599.3414	$C_{29}H_{52}NaO_{11}$	594.3855	$C_{28}H_{54}NO_{11}$	361.1864	$C_{17}H_{29}O_8$	231.1235	$C_{11}H_{19}O_5$	*nd*	$C_5H_9O_2$
		calc.	575.3437		599.3402		594.3848		361.1857		231.1227		101.1597	
-	**C5C13**	obs.	589.3604	$C_{30}H_{53}O_{11}$	613.3563	$C_{30}H_{54}NaO_{11}$	608.4012	$C_{28}H_{54}NO_{11}$	361.1871	$C_{17}H_{29}O_8$	231.1231	$C_{11}H_{19}O_5$	*nd*	$C_5H_9O_2$
		calc.	589.3593		613.3558		608.4004		361.1857		231.1227		101.1597	

Table S1b. HPLC-ESI-HR-MS/MS data of ascaroside dimers from *Caenorhabditis* species.

			(asc-C#)-asc-C#		(asc-C#)-asc-C#		(asc-C#)-asc-C#		(asc-C#)-asc		asc-C#		C#	
			$[M - H]^-$		$[M + Na]^+$		$[M + NH_4]^+$		$[M – agylcone]^+$		$[monomer\text{-}C{\equiv}O]^+$		$[agylcone]^+$	
Fig.			*m/z*	formula	*m/z*	formula	*m/z*	formula	*m/z*	formula	*m/z*	formula	*m/z*	formula
-	**C6C4**	obs.	477.2331	$C_{22}H_{37}O_{11}$	501.2305	$C_{22}H_{38}NaO_{11}$	496.2753	$C_{22}H_{42}NO_{11}$	375.2008	$C_{18}H_{31}O_8$	245.1385	$C_{12}H_{21}O_5$	115.0757	$C_6H_{11}O_2$
		calc.	477.2341		501.2306		496.2752		375.2013		245.1384		115.0754	
S2c	**C6C5**	obs.	491.2496	$C_{23}H_{39}O_{11}$	515.2567	$C_{23}H_{40}NaO_{11}$	510.2910	$C_{23}H_{44}NO_{11}$	375.2014	$C_{18}H_{31}O_8$	245.1382	$C_{12}H_{21}O_5$	115.0753	$C_6H_{11}O_2$
		calc.	491.2498		515.2463		510.2909		375.2013		245.1384		115.0754	
S2d	**C6C6**	obs.	505.2651	$C_{24}H_{41}O_{11}$	529.2622	$C_{24}H_{42}NaO_{11}$	524.3066	$C_{24}H_{46}NO_{11}$	375.2013	$C_{18}H_{31}O_8$	245.1383	$C_{12}H_{21}O_5$	115.0752	$C_6H_{11}O_2$
		calc.	505.2654		529.2619		524.3065		375.2013		245.1384		115.0754	
S2d	**C6C7**	obs.	519.2813	$C_{25}H_{43}O_{11}$	543.2777	$C_{25}H_{44}NaO_{11}$	538.3222	$C_{25}H_{48}NO_{11}$	375.2011	$C_{18}H_{31}O_8$	245.1383	$C_{12}H_{21}O_5$	115.0758	$C_6H_{11}O_2$
		calc.	519.2811		543.2776		538.3222		375.2013		245.1384		115.0754	
S2d	**C6C8**	obs.	533.2972	$C_{26}H_{45}O_{11}$	577.2951	$C_{26}H_{46}NaO_{11}$	552.3378	$C_{26}H_{50}NO_{11}$	375.2008	$C_{18}H_{31}O_8$	245.1364	$C_{12}H_{21}O_5$	115.0733	$C_6H_{11}O_2$
		calc.	533.2967		557.2932		552.3378		375.2013		245.1384		115.0754	
S2d	**C6C9**	obs.	547.3129	$C_{27}H_{47}O_{11}$	571.3106	$C_{27}H_{48}NaO_{11}$	566.3526	$C_{27}H_{52}NO_{11}$	375.2010	$C_{18}H_{31}O_8$	245.1386	$C_{12}H_{21}O_5$	115.0747	$C_6H_{11}O_2$
		calc.	547.3124		571.3089		566.3535		375.2013		245.1384		115.0754	
-	**C6C10**	obs.	561.3276	$C_{28}H_{49}O_{11}$	585.3252	$C_{28}H_{50}NaO_{11}$	580.3620	$C_{28}H_{54}NO_{11}$	375.2015	$C_{18}H_{31}O_8$	245.1382	$C_{12}H_{21}O_5$	*nd*	$C_6H_{11}O_2$
		calc.	561.3280		585.3245		580.3691		375.2013		245.1384		115.0754	
-	**C6C11**	obs.	575.3431	$C_{29}H_{51}O_{11}$	599.3389	$C_{29}H_{52}NaO_{11}$	580.3832	$C_{29}H_{56}NO_{11}$	375.2003	$C_{18}H_{31}O_8$	245.1385	$C_{12}H_{21}O_5$	*nd*	$C_6H_{11}O_2$
		calc.	575.3437		599.3402		594.3848		375.2013		245.1384		115.0754	
-	**C6C12**	obs.	589.3589	$C_{30}H_{53}O_{11}$	613.3546	$C_{30}H_{54}NaO_{11}$	608.3997	$C_{30}H_{58}NO_{11}$	375.2008	$C_{18}H_{31}O_8$	245.1387	$C_{12}H_{21}O_5$	*nd*	$C_6H_{11}O_2$
		calc.	589.3593		613.3558		608.4004		375.2013		245.1384		115.0754	
-	**C6C13**	obs.	603.3755	$C_{31}H_{55}O_{11}$	627.3698	$C_{31}H_{56}NaO_{11}$	622.4143	$C_{31}H_{60}NO_{11}$	375.2005	$C_{18}H_{31}O_8$	245.1375	$C_{12}H_{21}O_5$	*nd*	$C_6H_{11}O_2$
		calc.	603.3750		627.3715		622.4161		375.2013		245.1384		115.0754	
S2e	**C7C5**	obs.	505.2661	$C_{24}H_{41}O_{11}$	529.2632	$C_{24}H_{42}NaO_{11}$	524.3077	$C_{24}H_{46}NO_{11}$	389.2161	$C_{19}H_{33}O_8$	259.1538	$C_{13}H_{23}O_5$	129.0903	$C_7H_{13}O_2$
		calc.	505.2654		529.2619		524.3065		389.2170		259.1540		129.0910	
S2e	**C7C6**	obs.	519.2817	$C_{25}H_{43}O_{11}$	543.2791	$C_{25}H_{44}NaO_{11}$	538.3236	$C_{25}H_{48}NO_{11}$	389.2165	$C_{19}H_{33}O_8$	259.1534	$C_{13}H_{23}O_5$	129.0910	$C_7H_{13}O_2$
		calc.	519.2811		543.2776		538.3222		389.2170		259.1540		129.0910	
S2e	**C7C7**	obs.	533.2960	$C_{26}H_{45}O_{11}$	557.2935	$C_{26}H_{46}NaO_{11}$	552.3380	$C_{26}H_{50}NO_{11}$	389.2171	$C_{19}H_{33}O_8$	259.1536	$C_{13}H_{23}O_5$	129.0914	$C_7H_{13}O_2$
		calc.	533.2967		557.2932		552.3378		389.2170		259.1540		129.0910	
S2e	**C7C8**	obs.	547.3118	$C_{27}H_{47}O_{11}$	571.3085	$C_{27}H_{48}NaO_{11}$	533.3533	$C_{27}H_{52}NO_{11}$	389.2176	$C_{19}H_{33}O_8$	259.1529	$C_{13}H_{23}O_5$	129.0901	$C_7H_{13}O_2$
		calc.	547.3124		571.3089		566.3535		389.2170		259.1540		129.0910	
S2f	**C7C9**	obs.	561.3283	$C_{28}H_{49}O_{11}$	585.3245	$C_{28}H_{50}NaO_{11}$	580.3689	$C_{28}H_{54}NO_{11}$	389.2168	$C_{19}H_{33}O_8$	259.1538	$C_{13}H_{23}O_5$	129.0910	$C_7H_{13}O_2$
		calc.	561.3280		585.3245		580.3691		389.2170		259.1540		129.0910	
-	**C7C10**	obs.	575.3449	$C_{29}H_{51}O_{11}$	599.3407	$C_{29}H_{52}NaO_{11}$	594.3856	$C_{29}H_{56}NO_{11}$	389.2175	$C_{19}H_{33}O_8$	259.1548	$C_{13}H_{23}O_5$	*nd*	$C_7H_{13}O_2$
		calc.	575.3437		599.3402		594.3848		389.2170		259.1540		129.0910	
S2f	**C7C11**	obs.	589.3606	$C_{30}H_{53}O_{11}$	613.3572	$C_{30}H_{54}NaO_{11}$	608.4015	$C_{30}H_{58}NO_{11}$	389.2175	$C_{19}H_{33}O_8$	259.1538	$C_{13}H_{23}O_5$	129.0904	$C_7H_{13}O_2$
		calc.	589.3593		613.3558		608.4004		389.2170		259.1540		129.0910	
S2f	**ΔC9C7**	obs.	559.3128	$C_{28}H_{47}O_{11}$	583.3095	$C_{28}H_{48}NaO_{11}$	578.3544	$C_{28}H_{54}NO_{11}$	415.2326	$C_{21}H_{35}O_8$	285.1694	$C_{15}H_{25}O_5$	*nd*	$C_9H_{15}O_2$
		calc.	559.3124		583.3089		578.3540		415.2337		285.1707		155.1078	

Table S2. NMR data of natural ascaroside dimers (400 MHz, in CD_3OD) isolated from *C. remanei* (**5**) and *C. nigoni* (**6** - **8**).

	from *C. remanei* PB4641		**from *C. nigoni* JU1422**		
#	4'-(asc-C4)-asc-C5 (**5**)		2'-(asc-C5)-asc-C5 (**6**)	2'-(asc-C6)-asc-C5 (**7**)	2'-(asc-C6)-asc-C6 (**8**)
	^{1}H	^{13}C	^{1}H	^{1}H	^{1}H
1	-	182.1	-	-	-
2	2.22 *ddd* 14.9, 9.8, 6.1 2.35 *ddd* 15.1, 10.0, 6.4	35.2	2.32 *dt* 5.0, 7.2	2.32 *m*	2.24 *t* 7.2
3	1.83 *m*	35.0	1.80 *m*	1.80 *m*	1.65 *m* 1.76 *m*
4	3.82 *m*	72.2	3.83 *m*	3.83 *m*	1.57 *m*
5	1.15 *d* 6.1	18.7	1.14 *d* 6.1	1.14 *d* 6.2	3.80 *m*
6	- - -	- - -	- - -	- - -	1.14 *d* 6.3
1'	4.69 s	96.8	4.71 s	4.71 s	4.71 s
2'	3.72 *s.br*	69.2	4.79 *s.br*	4.79 *s.br*	4.78 *s.br*
3'	1.92 *ddd* 13.2, 11.4, 3.8 2.07 *dt* 12.6, 3.8	32.7	1.87 *ddd* 13.2, 11.4, 3.2 2.01 *dt* 13.1, 4.1	1.90 *ddd* 13.3, 11.2, 3.4 2.01 *dt* 13.2, 3.9	1.89 *ddd* 13.4, 11.5, 3.3 2.00 *dt* 13.3, 3.9
4'	4.87 *ddd* 11.3, 9.6, 4.5	71.4	3.41 *ddd* 11.4, 9.6, 4.6	3.40 *ddd* 11.5, 9.7, 4.9	3.40 *ddd* 11.5, 9.7, 4.9
5'	3.90 *dq* 9.6, 6.1	67.8	3.68 *dq* 9.7, 6.3	3.71 *dq* 9.7, 6.3	3.70 *dq* 9.7, 6.3
6'	1.16 *d* 6.1	18.0	1.23 *d* 6.2	1.22 *d* 6.3	1.22 *d* 6.3
1''	-	171.8	-	-	-
2''	2.50 *dd* 15.1, 5.4 2.56 *dd* 15.0, 7.5	43.1	2.39 *m*	2.39 *dt* 3.1, 7.4	2.39 *dt* 3.0, 7.5
3''	4.23 *m*	69.0	1.80 *m*	1.70 *m* 1.80 *m*	1.69 *m* 1.79 *m*
4''	1.21 *d* 6.2	18.7	3.80 *m*	1.56 *m*	1.57 *m*
5''	- - -	- - -	1.14 *d* 6.3	3.81 *m*	3.79 *m*
6''	- - -	- - -	- - -	1.14 *d* 6.3	1.14 *d* 6.3
1'''	4.67 s	97.1	4.65 s	4.65 s	4.65 s
2'''	3.70 *s.br*	69.3	3.72 *s.br*	3.73 *s.br*	3.72 *s.br*
3'''	1.72 *ddd* 13.0, 11.7, 3.7 1.92 *dt* 13.3, 3.9	35.6	1.77 *m* 1.96 *m*	1.77 *ddd* 13.3, 11.4. 3.0 1.96 *dt* 13.1, 4.1	1.77 *ddd* 13.0, 11.5. 3.1 1.95 *dt* 13.2, 3.9
4'''	3.51 *ddd* 11.6, 9.5, 4.8	67.9	3.52 *m*	3.52 *ddd* 11.3, 9.6, 4.7	3.51 *ddd* 11.5, 9.7, 4.9
5'''	3.62 *dq* 9.6, 6.2	71.0	3.62 *dq*	3.62 *dq* 9.5, 6.2	3.61 *dq* 9.7, 6.3
6'''	1.22 *d* 6.2	18.0	1.22 *d* 6.2	1.22 *d* 6.2	1.22 *d* 6.3

Table S3. NMR data of synthetic ascaroside dimers (400 MHz, in CD_3OD).

#	2'-(asc-C6)-asc-C5 (**7**)		2'-(asc-C6)-asc-C6 (**8**)		4'-(asc-C6)-asc-C5 (**13a**)		4'-(asc-C6)-asc-C6 (**13b**)	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	-	*nd*	-	*nd*	-	*nd*	-	*nd*
2	2.34 *m* 2.27 *m*	34.0	2.24 *t* 7.3	36.8	2.35 *m*	34.8	2.25 *t* 7.2	36.8
3	1.80 *m*	34.9	1.65 *m*, 1.76 *m*	22.6	1.80 *m*	33.2	1.67 *m*, 1.77 *m*	22.6
4	3.83 *m*	72.2	1.57 *m* [a]	37.4	3.85 *m*	71.7	1.57 *m* [a]	37.6
5	1.14 *d* 6.1	18.7	3.80 *m* [b]	72.2	1.16 *d* 6.2	18.5	3.80 *m* [b]	72.1
6	- - -	- - -	1.14 *d* 6.2	18.7	- - -	- - -	1.14 *d* 6.2	18.7
1'	4.71 *s*	94.1	4.71 *s*	94.3	4.69 *s*	97.2	4.69 *s*	97.3
2'	4.79 *s.br*	72.3	4.78 *s.br*	72.3	3.73 *s.br*	69.6	3.72 *s.br*	69.5
3'	1.90 *ddd* 13.4, 11.8, 3.4 2.01 *dt* 13.0, 3.8	33.4	1.88 *ddd* 13.4, 11.5, 3.0 2.00 *dt* 13.2, 3.6	33.1	1.85 *ddd* 13.3, 11.3, 3.0 2.04 *dt* 12.9 4.2	33.2	1.85 *ddd* 12.9, 11.4, 2.9 2.03 *dt* 12.8, 4.0	32.9
4'	3.40 *ddd* 11.4, 9.6, 4.4	68.2	3.41 *ddd* 11.3, 9.7, 4.7	68.5	4.87 *ddd* 11.3, 9.7, 4.5	71.1	4.86 *ddd* 11.4, 9.7, 4.6	71.0
5'	3.71 *dq* 9.6, 6.2	70.1	3.70 *dq* 9.5, 6.3	70.1	3.85 *dq* 9.7, 6.2	68.2	3.86 *dq* 9.7, 6.2	68.0
6'	1.23 *d* 6.1	17.9	1.22 *d* 6.3	17.8	1.14 *d* 6.3	18.5	1.14 *d* 6.2	18.7
1''	-	*nd*	-	*Nd*	-	*Nd*	-	*Nd*
2''	2.39 *dt* 3.2, 7.3	34.6	2.39 *dt* 3.1, 7.3	34.7	2.40 *t* 7.4	31.4	2.35 *dt* 5.2, 7.2	34.8
3''	1.70 *m*, 1.80 *m*	21.8	1.70 *m*, 1.79 *m*	22.6	1.67 *m*, 1.79 *m*	22.3	1.67 *m*, 1.78 *m*	22.6
4''	1.56 *m*	37.2	1.57 *m* [a]	37.4	1.56 *m*	37.3	1.57 *m* [a]	37.6
5''	3.81 *m*	72.2	3.79 *m* [b]	72.2	3.80 *m*	71.9	3.80 *m* [b]	72.1
6''	1.14 *d* 6.1	18.7	1.14 *d* 6.1	18.7	1.13 *d* 6.2	18.5	1.13 *d* 6.2	18.7
1'''	4.65 *s*	97.6	4.65 *s*	97.4	4.64 *s*	97.4	4.64 *s*	97.3
2'''	3.73 *s.br*	70.1	3.72 *s.br*	70.1	3.72 *s.br*	69.6	3.72 *s.br*	69.5
3'''	1.77 *ddd* 13.2, 11.1, 3.0 1.96 *dt* 13.2, 3.7	35.1	1.77 *ddd* 13.3, 11.2, 3.1 1.95 *dt* 13.4, 3.8	35.7	1.76 *ddd* 13.1, 11.1, 3.0 1.95 *dt* 13.0, 3.8	35.6	1.76 *ddd* 13.2, 11.2, 3.0 1.95 *dt* 13.2, 3.9	35.8
4'''	3.52 *ddd* 11.2, 9.5, 4.4	68.2	3.51 *ddd* 11.1, 9.6, 4.6	68.3	3.51 *ddd* 11.0, 9.5, 4.6	68.2	3.51 *ddd* 11.2, 9.4, 4.5	67.9
5'''	3.62 *dq* 9.3, 6.2	70.9	3.62 *dq* 9.5, 6.3	70.9	3.61 *dq* 9.4, 6.2	71.1	3.61 *dq* 9.5, 6.1	71.3
6'''	1.23 *d* 6.1	17.9	1.22 *d* 6.3	17.8	1.22 *d* 6.2	17.8	1.22 *d* 6.2	17.9

Table S4a. HPLC-ESI-(-)-HR-MS/MS data of trimeric ascarosides ((asc-C6)-asc-C6)-asc-C# from *C. nigoni* JU1422 (see Fig. S4a).

	ascaroside trimer	$[M - H]^-$	$[M - H]^-$	$[M - H]^-$	$[dimerC6C6]^-$		$[asc-C6]^-$
				obs. *m/z*	obs. *m/z*	obs. *m/z*	obs. *m/z*
		formula			**$C_{24}H_{41}O_{11}$**	**$C_{15}H_{23}O_6$**	**$C_{12}H_{21}O_6$**
			calc. *m/z*		**505.2654**	**299.1500**	**261.1344**
1	C6C6C4	**$C_{34}H_{57}O_{16}$**	721.3652	721.3618	505.2621	299.1478	261.1330
2	C6C6C5	**$C_{35}H_{59}O_{16}$**	735.3809	735.3765	505.2620	299.1484	261.1323
3	C6C6C6	**$C_{36}H_{61}O_{16}$**	749.3965	749.3914	505.2621	299.1477	261.1326

Table S4b. HPLC-ESI-(+)-HR-MS/MS data of trimeric ascarosides ((asc-C6)-asc-C6)-asc-C# from *C. nigoni* JU1422 (see Fig. S4b).

	ascaroside trimer	$[M + NH_4]^+$	$[M + NH_4]^+$	$[M + NH_4]^+$	$[M\text{-aglycone}]^+$	$[dimer\text{-}C{\equiv}O]^+$		$[monomer\text{-}C{\equiv}O]^+$	$[aglycone]^+$
				obs. *m/z*	obs. *m/z*	obs. *m/z*	obs. *m/z*	obs. *m/z*	obs. *m/z*
		formula			**$C_{30}H_{51}O_{13}$**	**$C_{24}H_{41}O_{10}$**	**$C_{18}H_{31}O_8$**	**$C_{12}H_{21}O_5$**	**$C_6H_{11}O_2$**
			calc. *m/z*		**619.3324**	**489.2694**	**375.2013**	**245.1384**	**115.0754**
1	C6C6C4	**$C_{34}H_{62}NO_{16}$**	**740.3912**	740.4063	619.3302	489.2664	375.2019	245.1370	115.0761
2	C6C6C5	**$C_{35}H_{64}NO_{16}$**	**754.4069**	754.4220	619.3254	489.2693	375.2023	245.1380	115.0758
3	C6C6C6	**$C_{36}H_{66}NO_{16}$**	**768.4281**	768.4376	619.3292	489.2732	375.2037	245.1367	115.0769

● **Supplementary NMR Spectra**

Figure S12: *dqf*-COSY (400 MHz, CD_3OD) of a RP-C18 SPE fraction enriched in 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* PB4641.

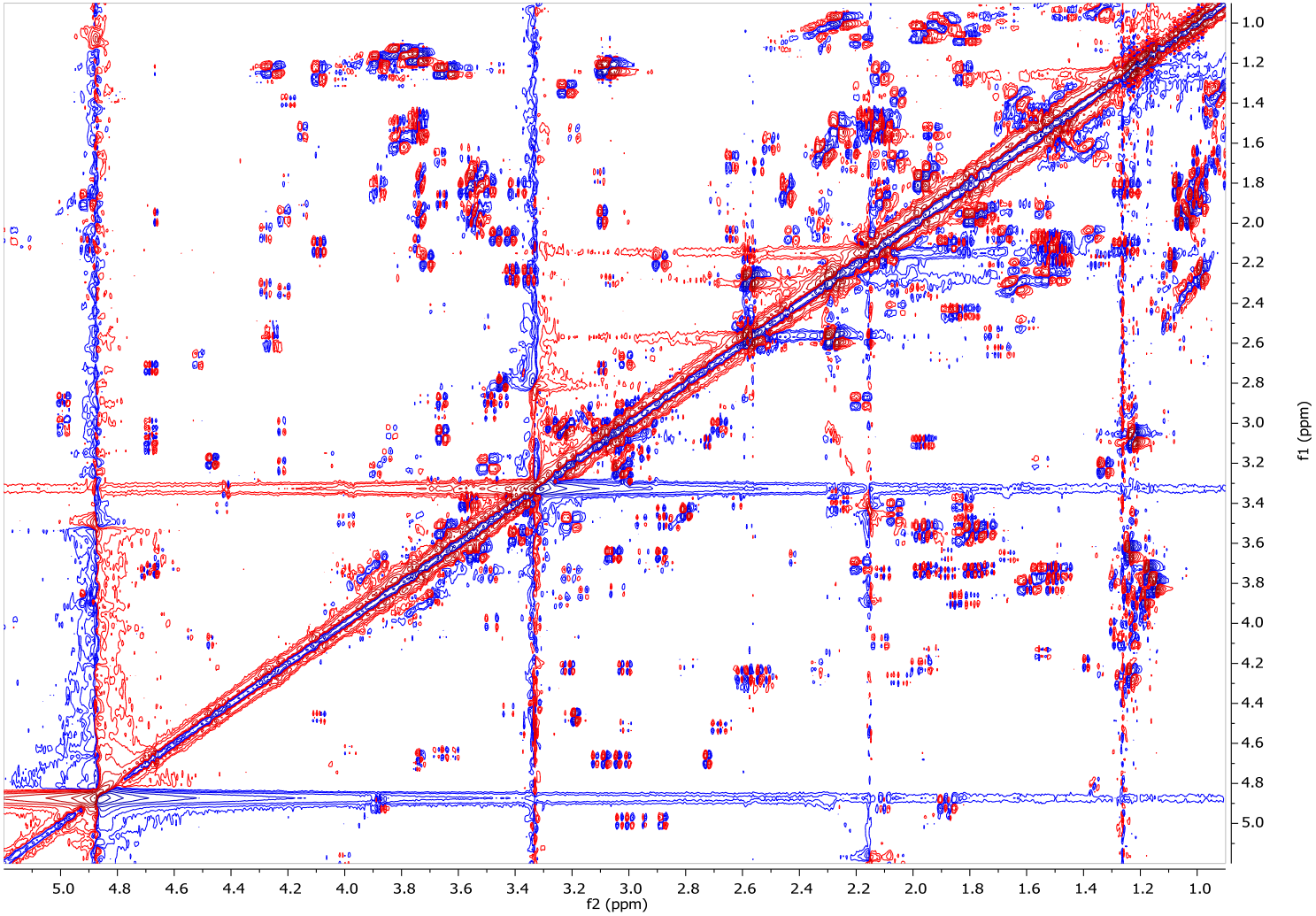


Figure S13: *dqf*-COSY (400 MHz, CD_3OD) of a RP-C18ec SPE fraction highly enriched in 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* PB4641.

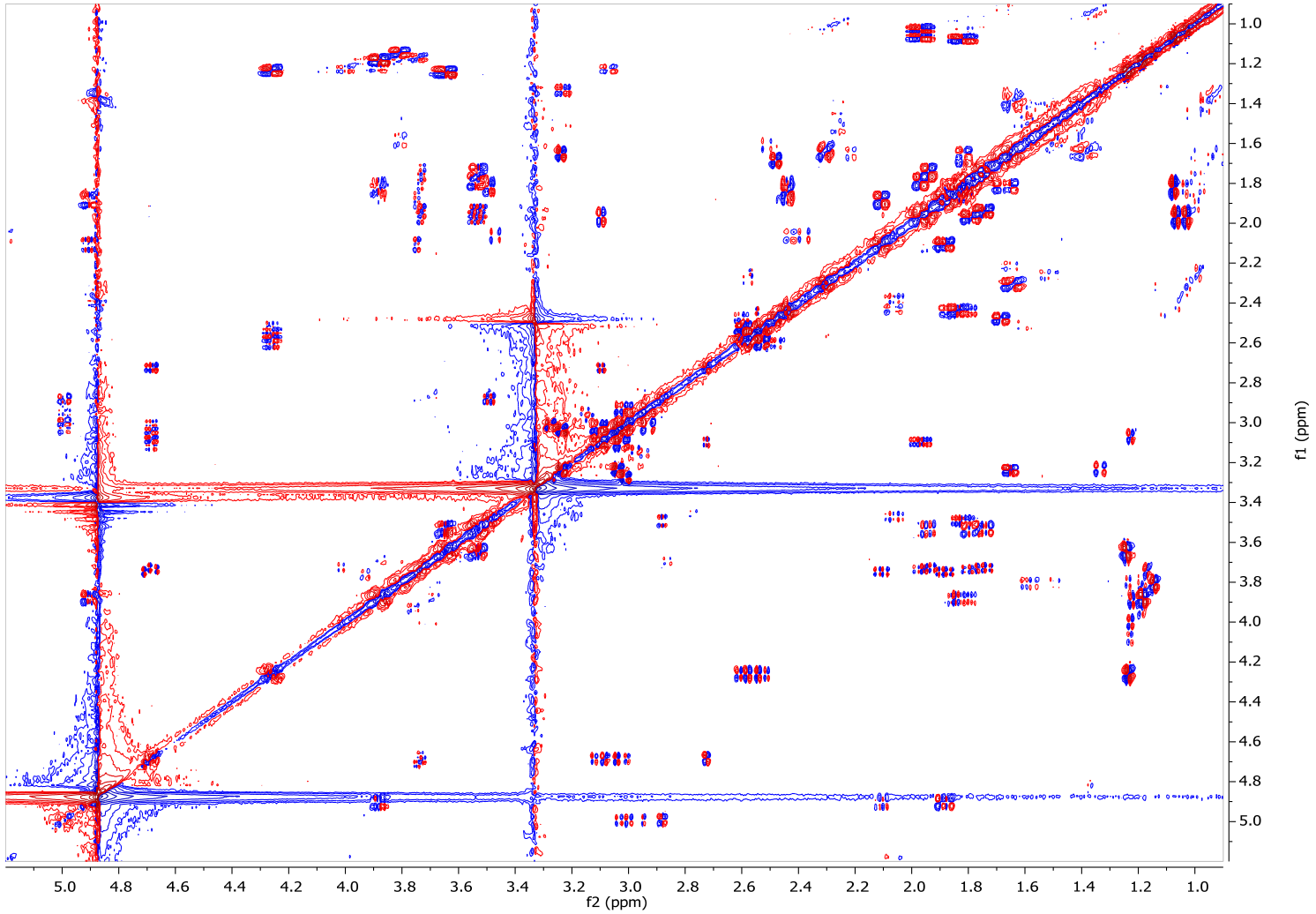


Figure S14: *dqf*-COSY (400 MHz, CD_3OD) of isolated 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* PB4641.

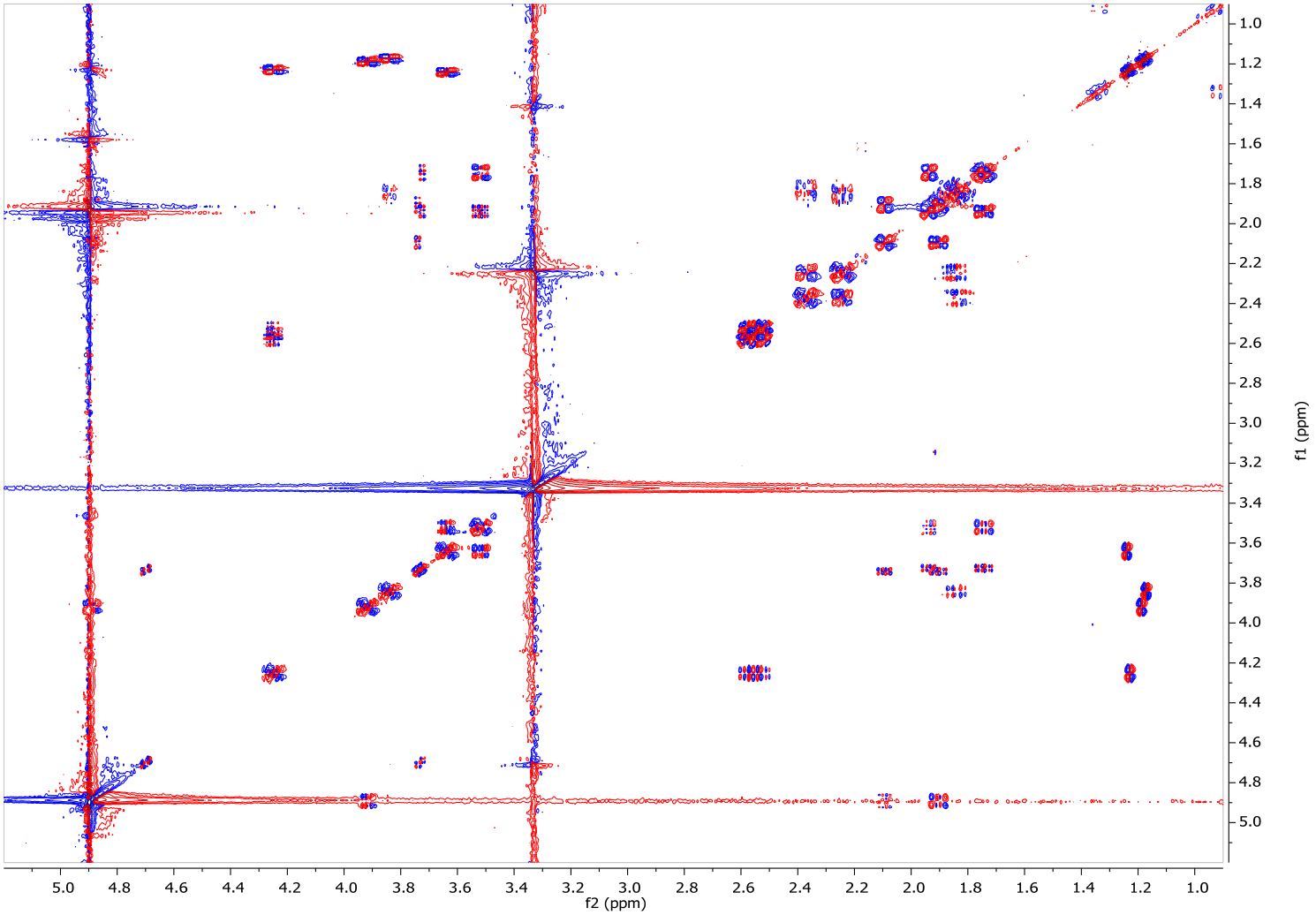


Figure S15: ^{1}H NMR (500 MHz, CD_3OD) of natural 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* PB4641.

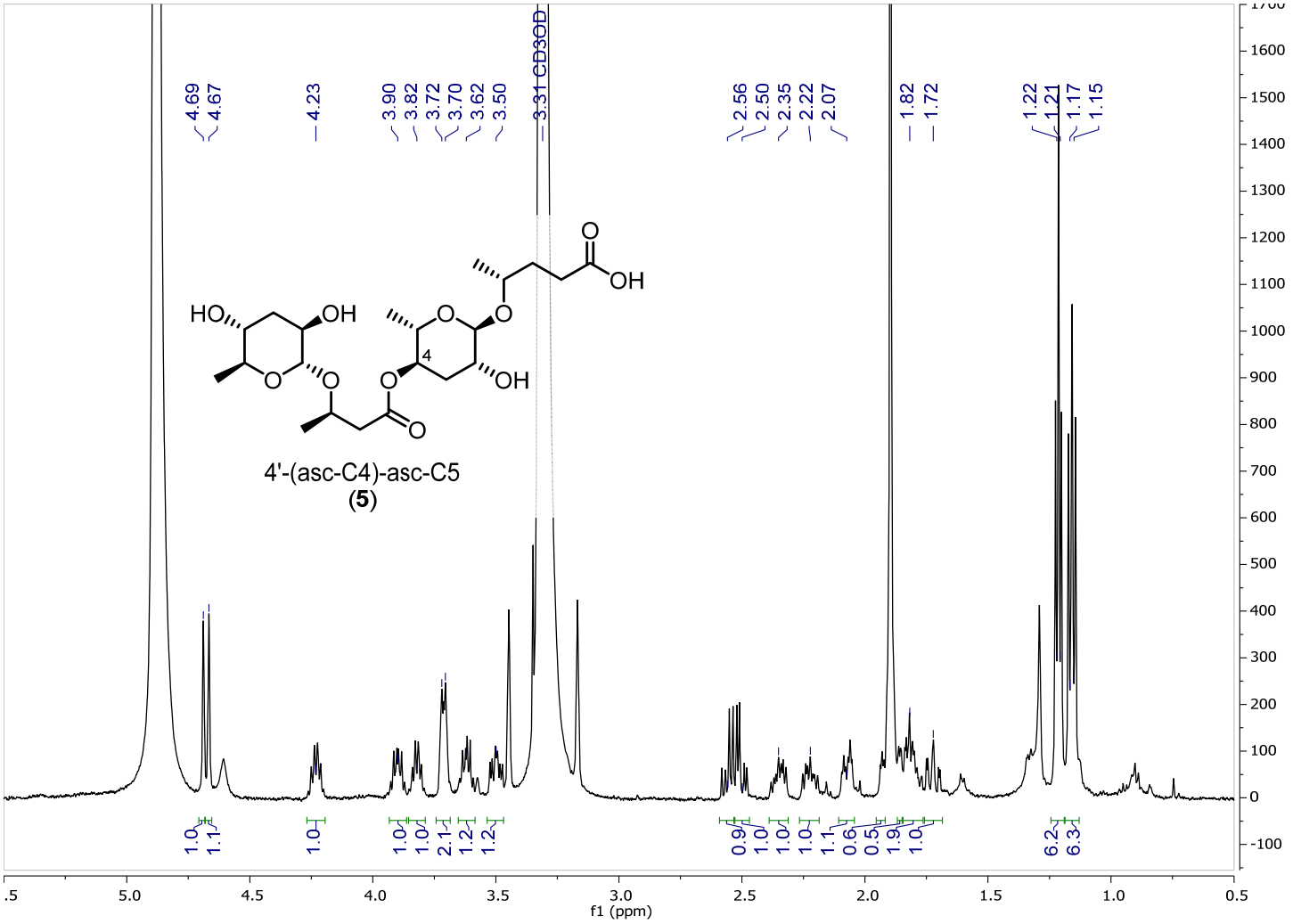


Figure S16: ^{1}H NMR (700 MHz, CD_3OD) of natural 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* PB4641.

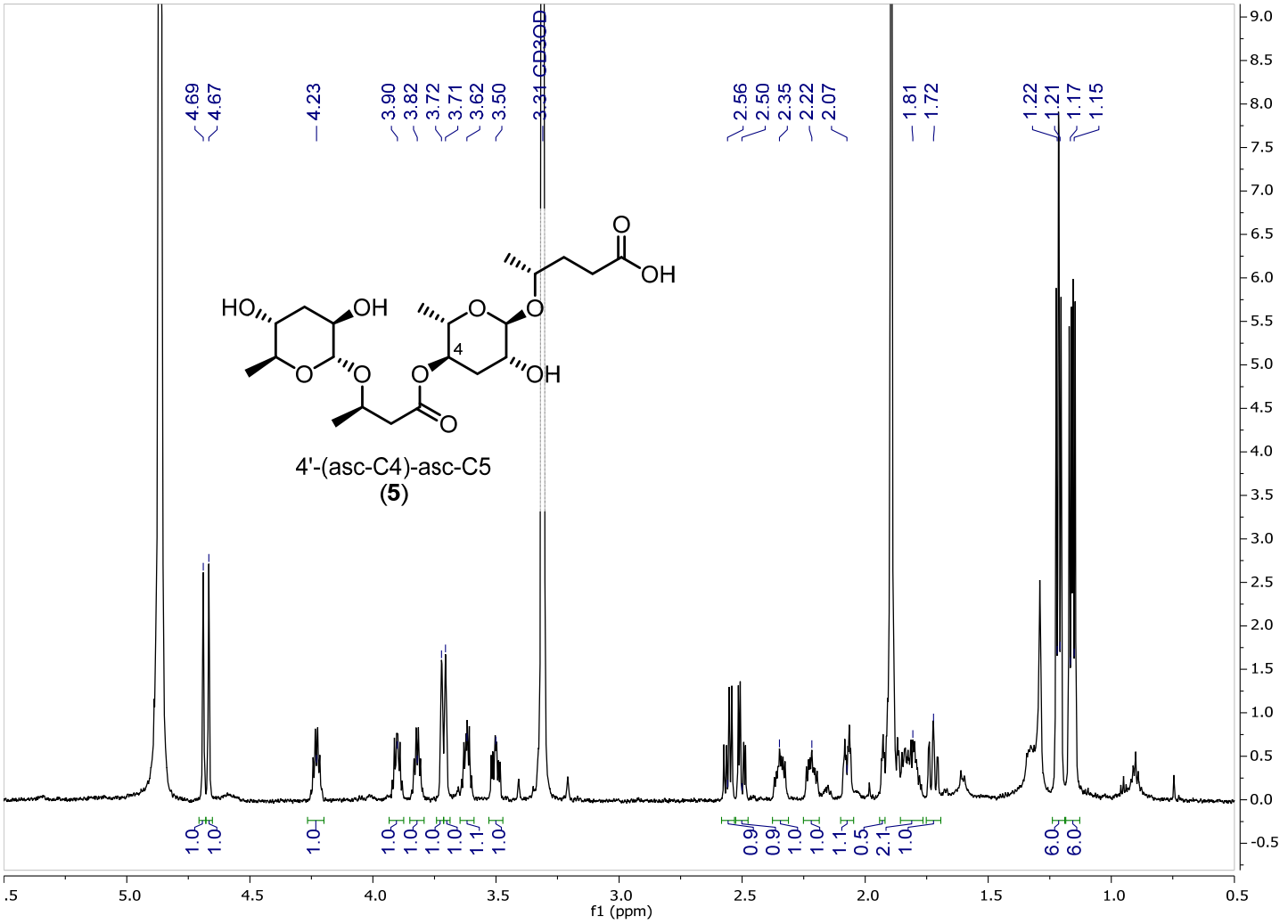


Figure S17: *dqf*-COSY (700 MHz, CD_3OD) of natural 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* PB4641.

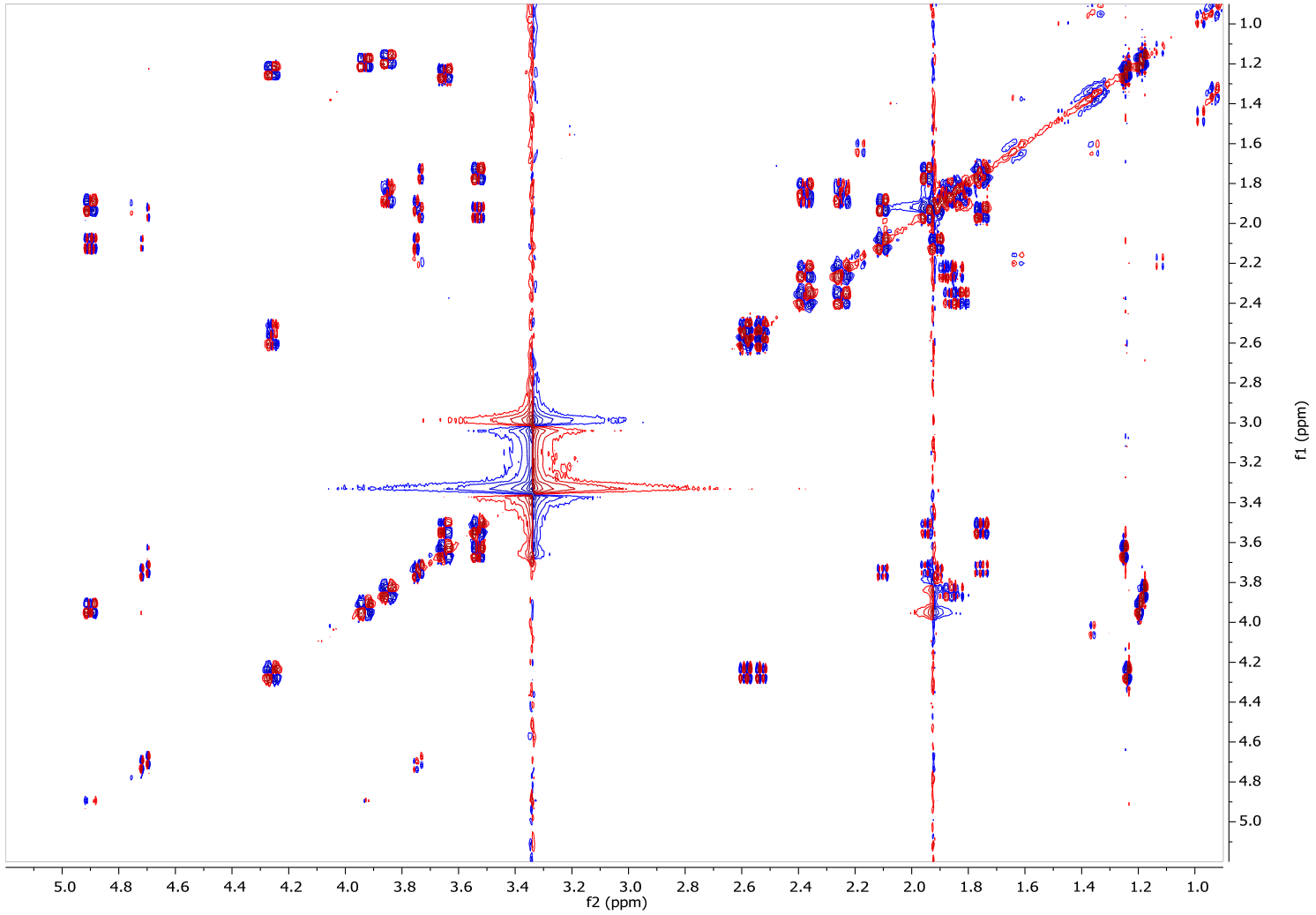


Figure S18: HSQC (700 MHz, CD_3OD) of natural 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* PB4641.

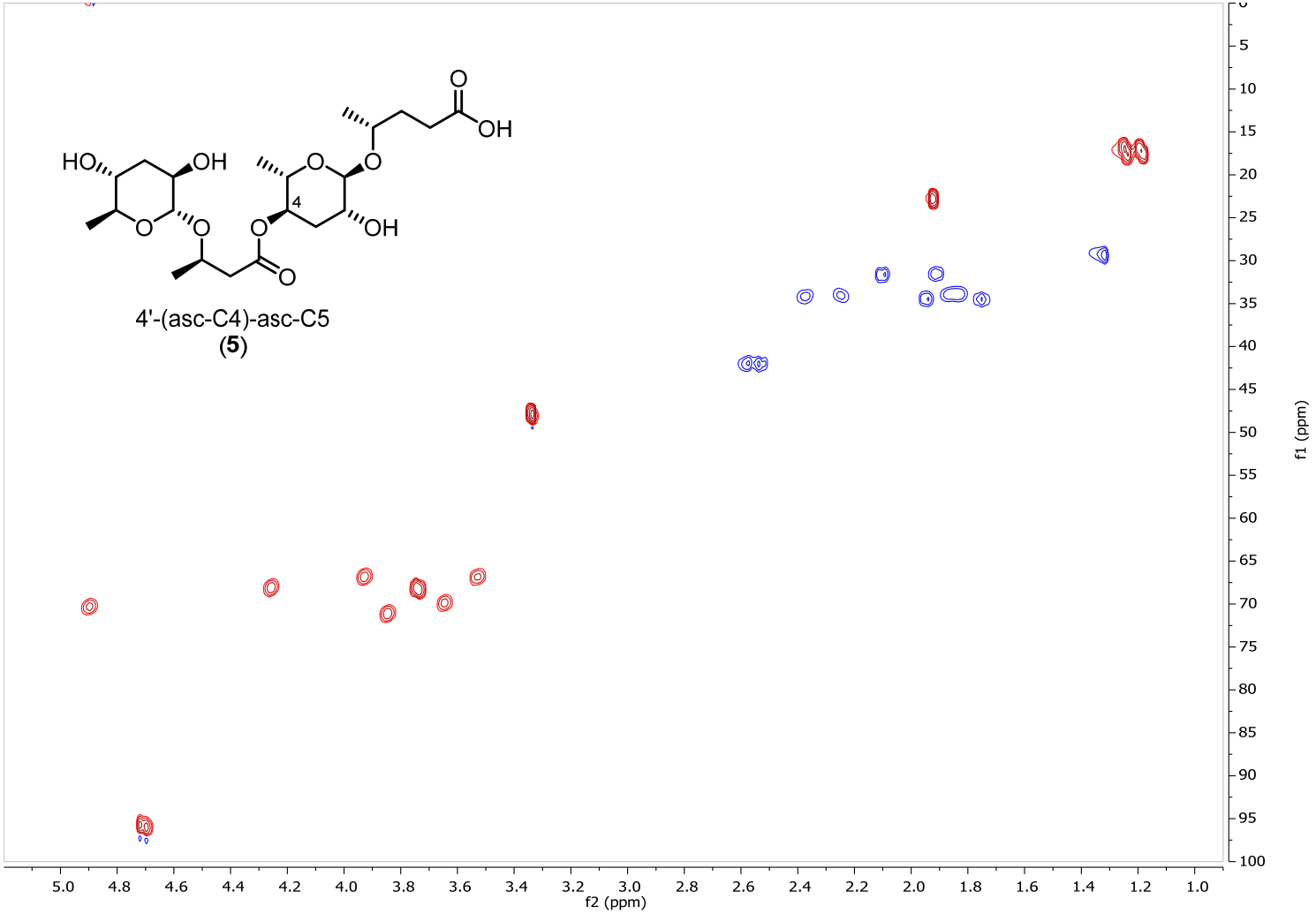


Figure S19: HMBC (700 MHz, CD_3OD) of natural 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* PB4641.

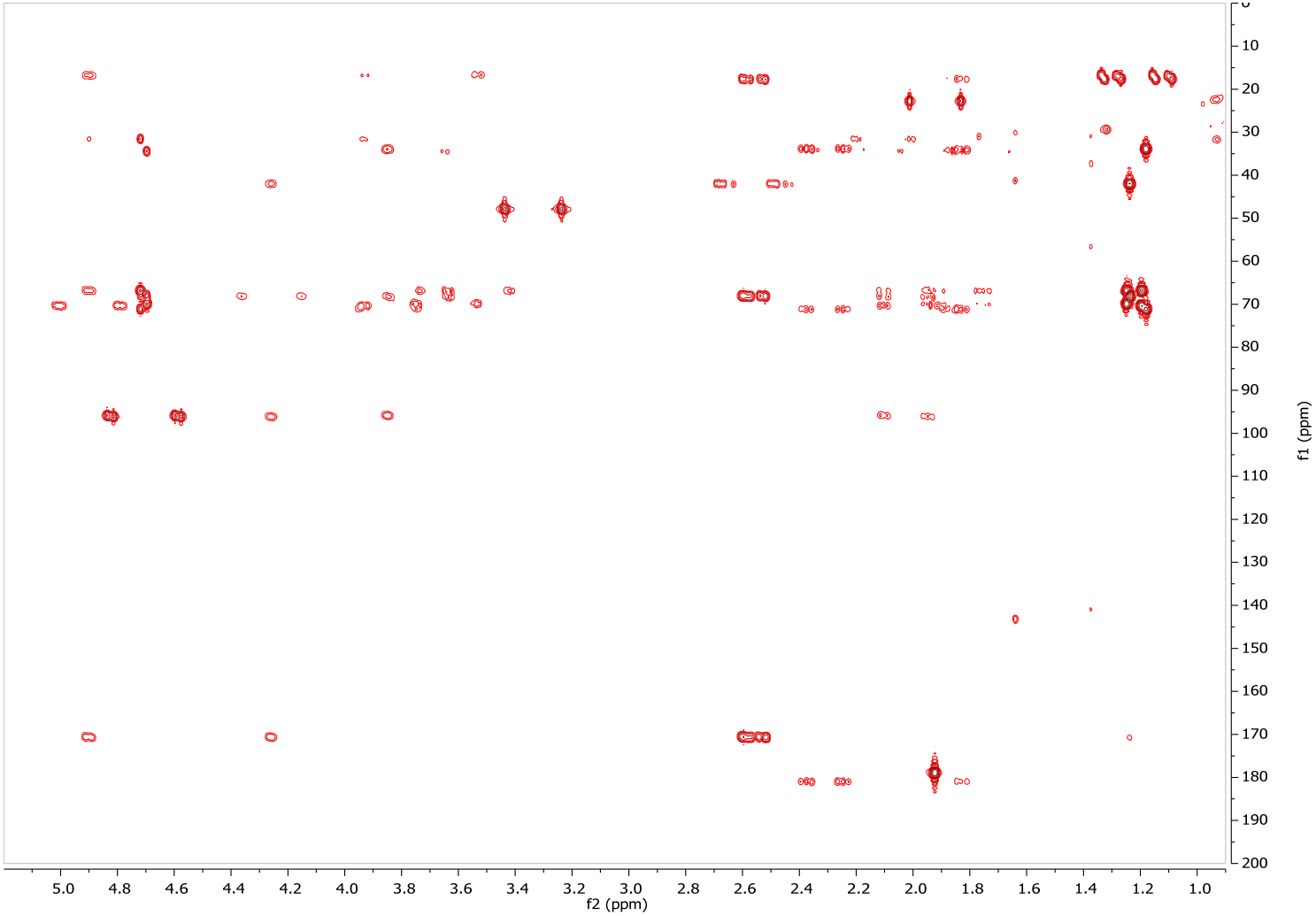


Figure S20: ^{1}H NMR (400 MHz, CD_3OD) of natural asc-C8 with minor amounts of 2'-(asc-C5)-asc-C5 (**6**) from *C. nigoni* JU1422.

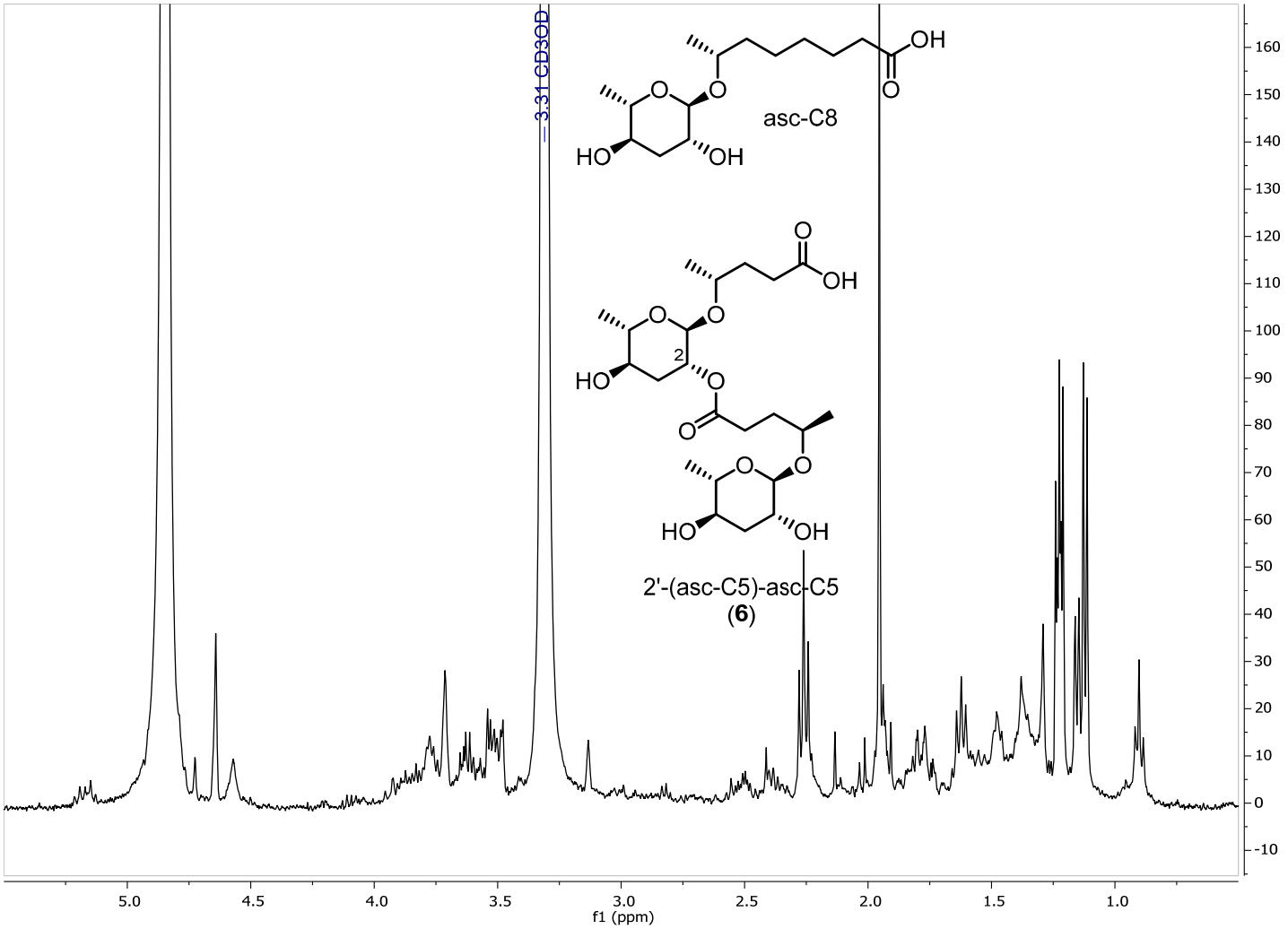


Figure S21: *dqf*-COSY (400 MHz, CD_3OD) of natural asc-C8 with minor amounts of 2'-(asc-C5)-asc-C5 (**6**) from *C. nigoni* JU1422.

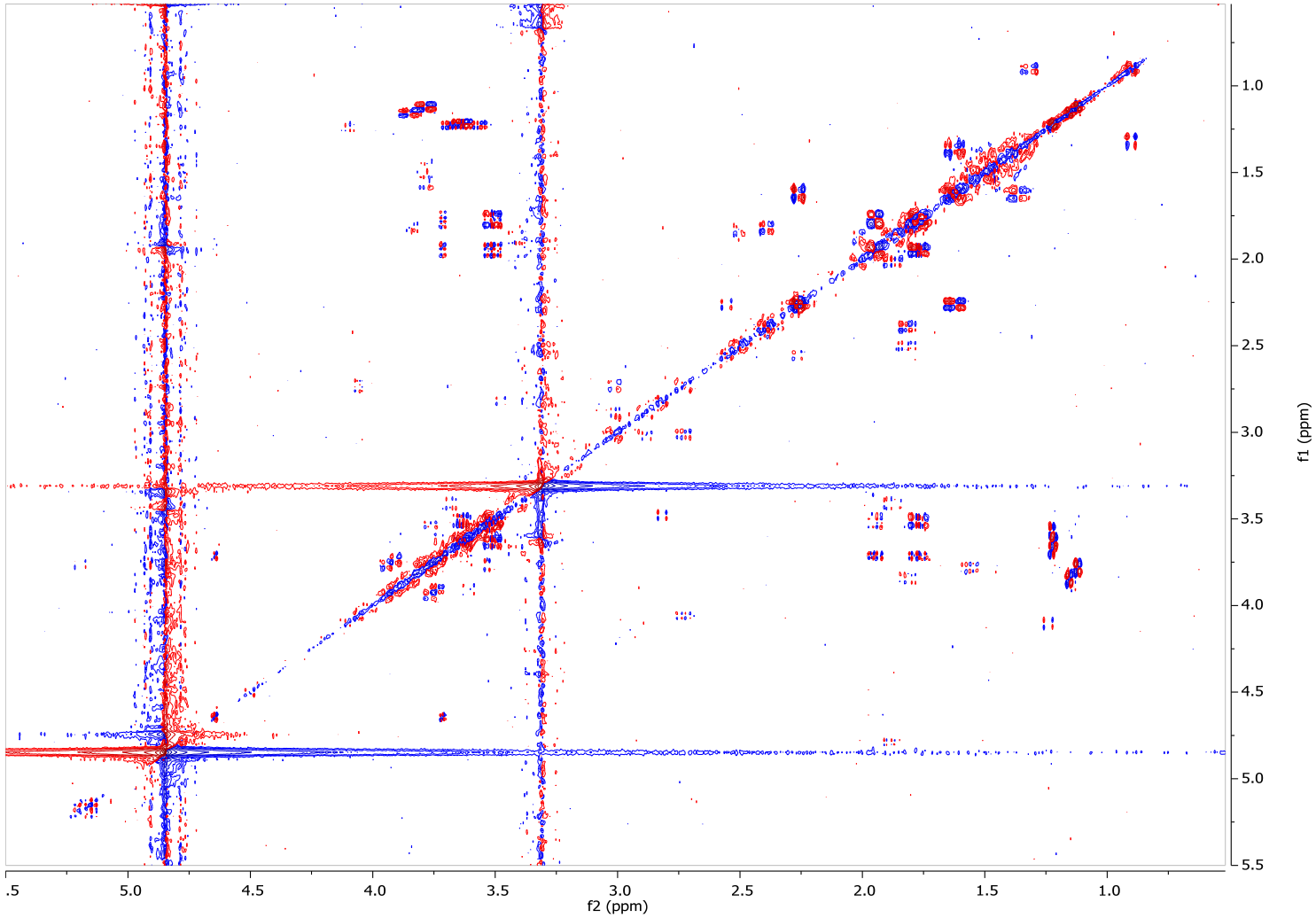


Figure S22: ^{1}H NMR (400 MHz, CD_3OD) of natural asc-ΔC9 with minor amounts of 2'-(asc-C6)-asc-C5 (**7**) from *C. nigoni* JU1422.

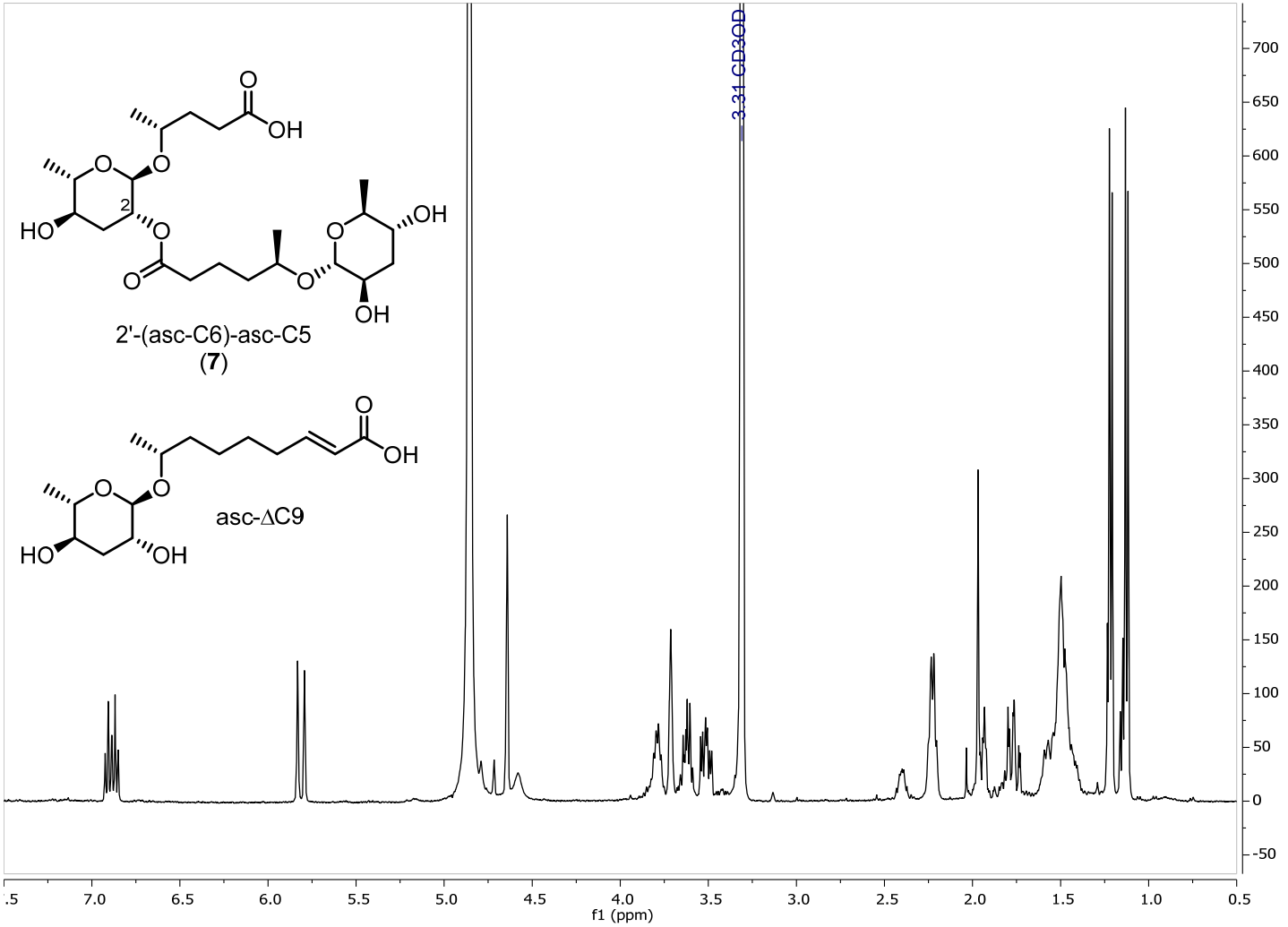


Figure S23: *dqf*-COSY (400 MHz, CD_3OD) of natural asc-ΔC9 with minor amounts of 2'-(asc-C6)-asc-C5 (**7**) from *C. nigoni* JU1422.

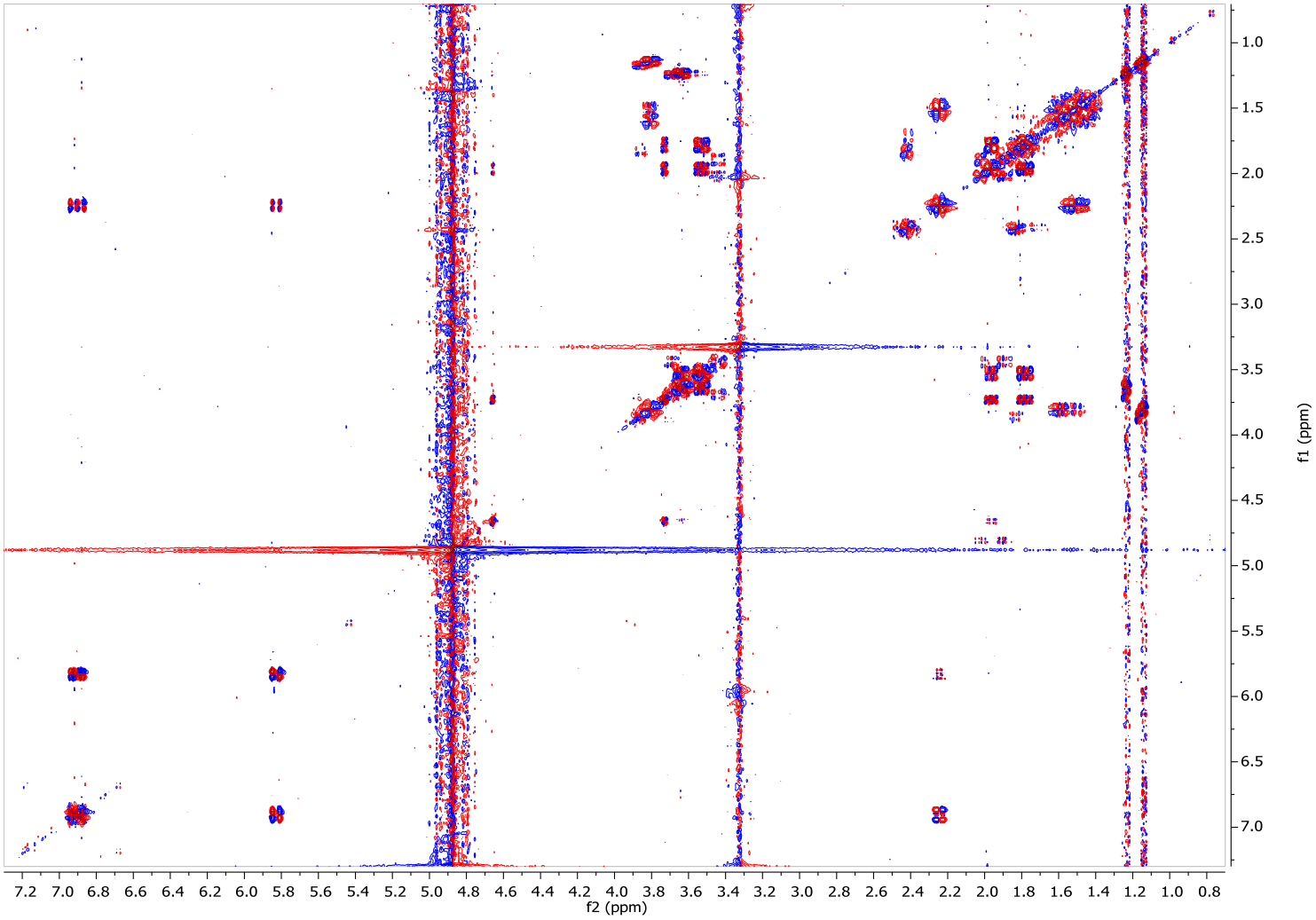


Figure S24: ^{1}H NMR (400 MHz, CD_3OD) of natural 2'-(asc-C6)-asc-C6 (**8**) from *C. nigoni* JU1422.

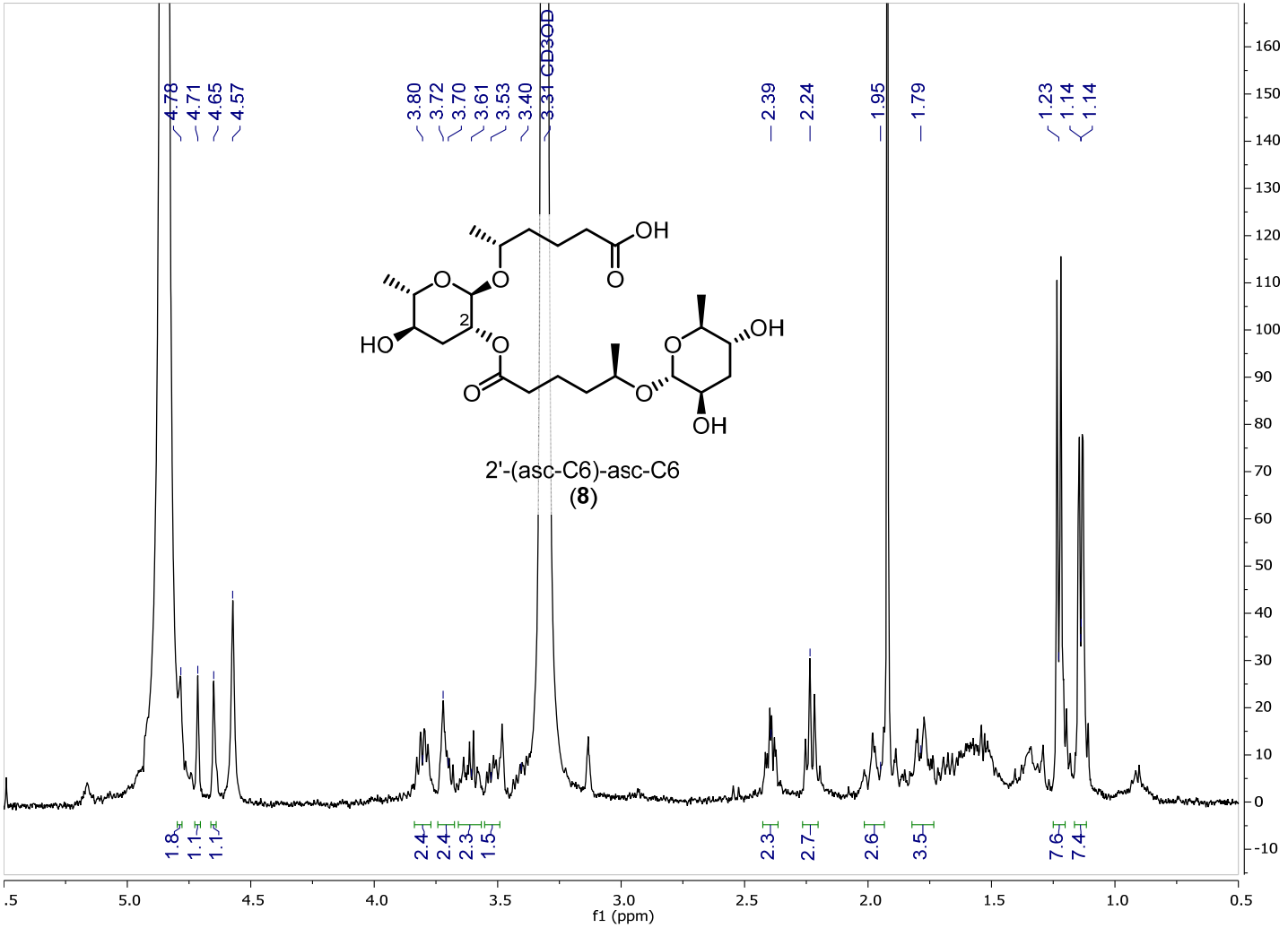


Figure S25: *dqf*-COSY (400 MHz, CD_3OD) of natural 2'-(asc-C6)-asc-C6 (**8**) from *C. nigoni* JU1422.

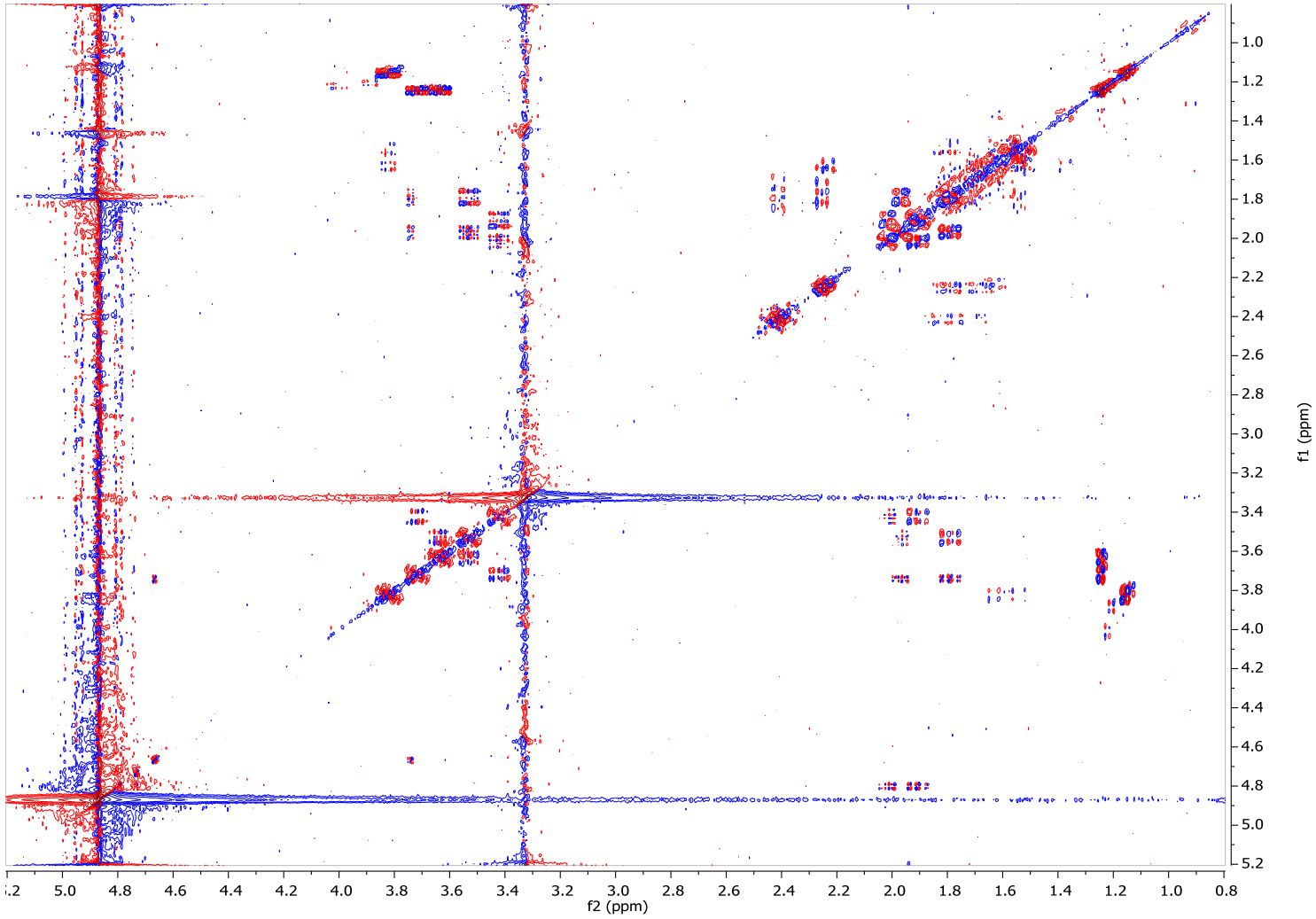


Figure S26: ^{1}H NMR (400 MHz, $CDCl_3$) of (3*R*)-3-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (**10a**).

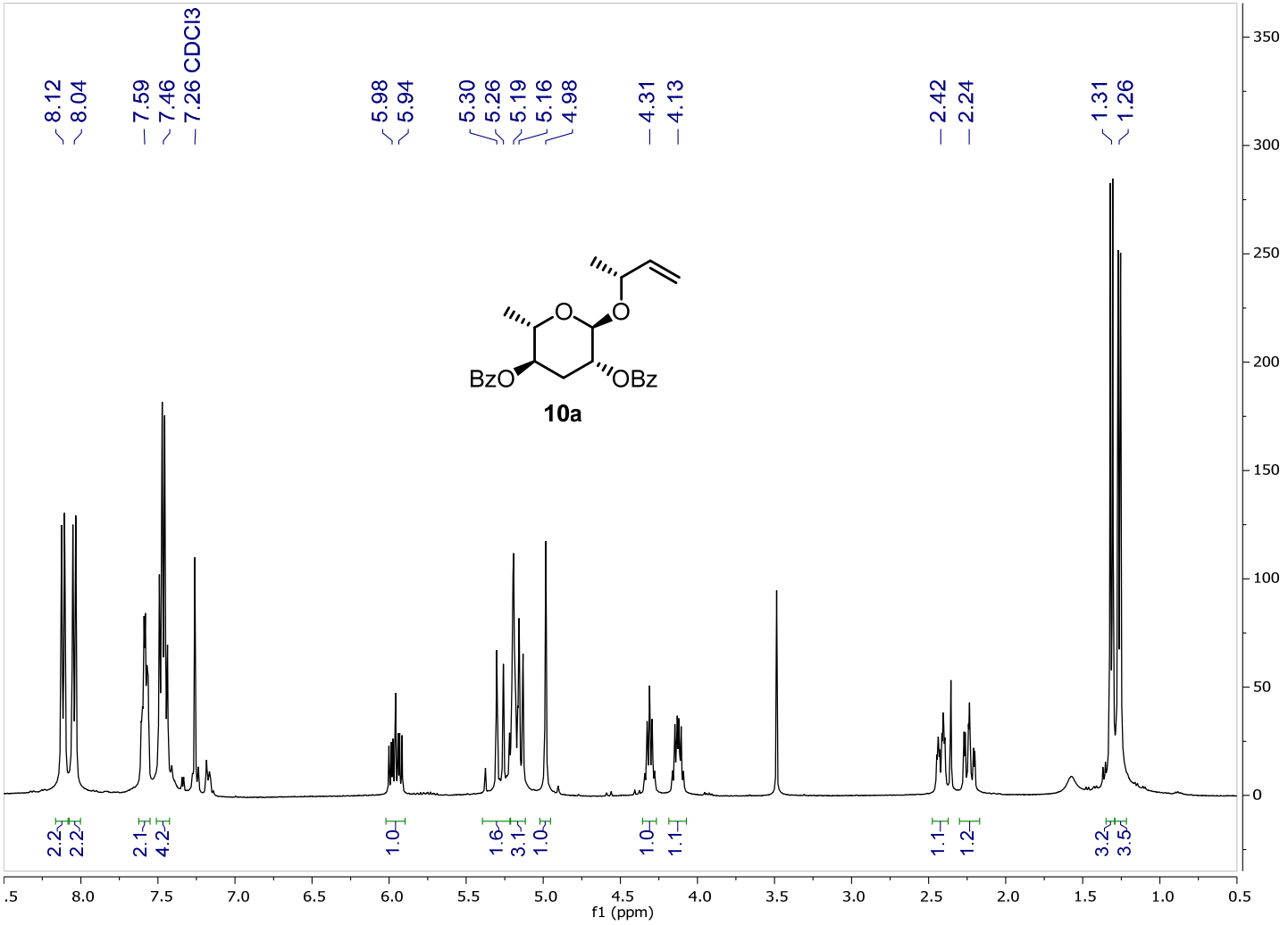


Figure S27: ^{13}C NMR (100 MHz, $CDCl_3$) of (3*R*)-3-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (**10a**).

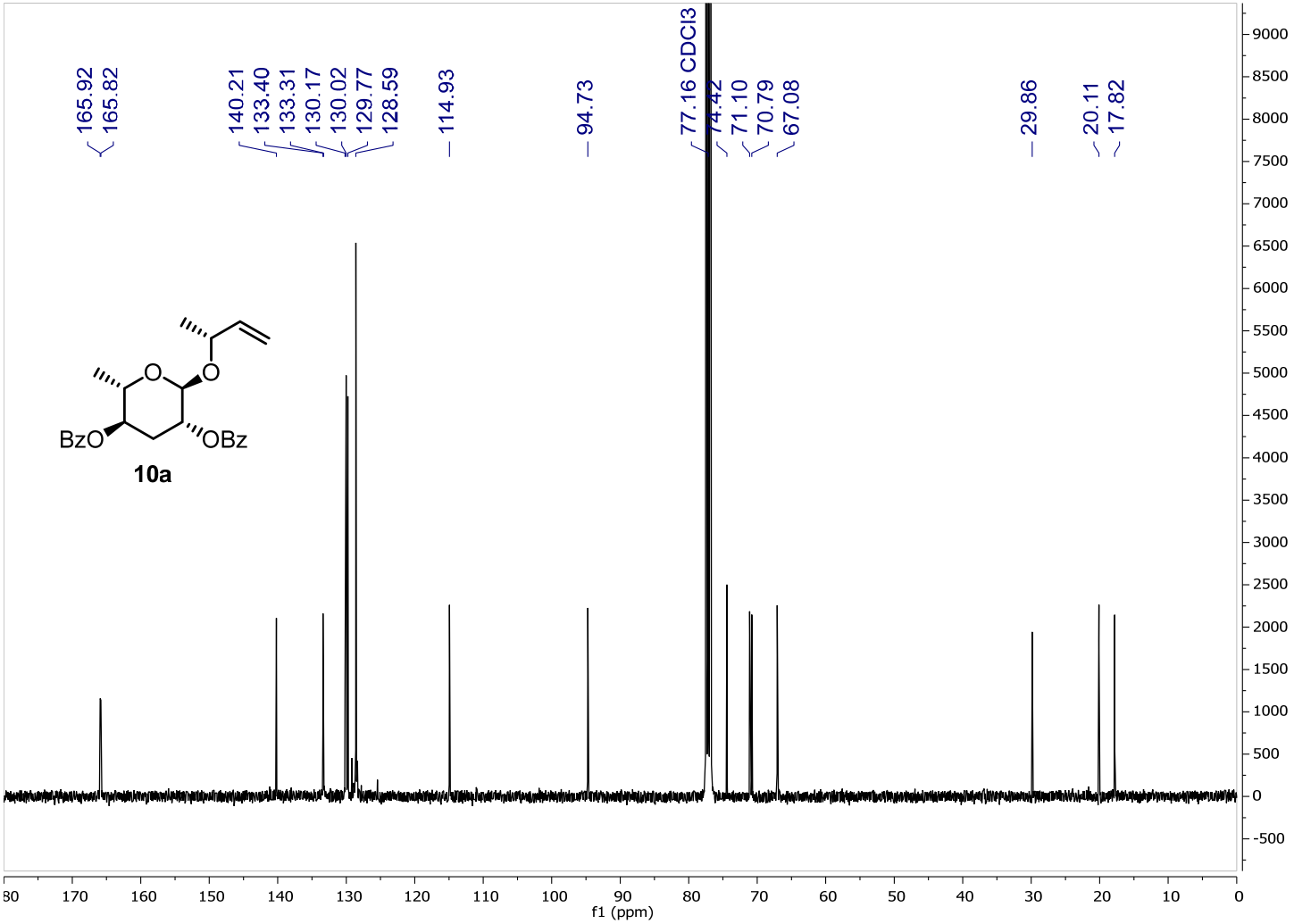


Figure S28: ^{1}H NMR (400 MHz, CD_3OD) of (4*R*)-4-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-pentene (**10b**).

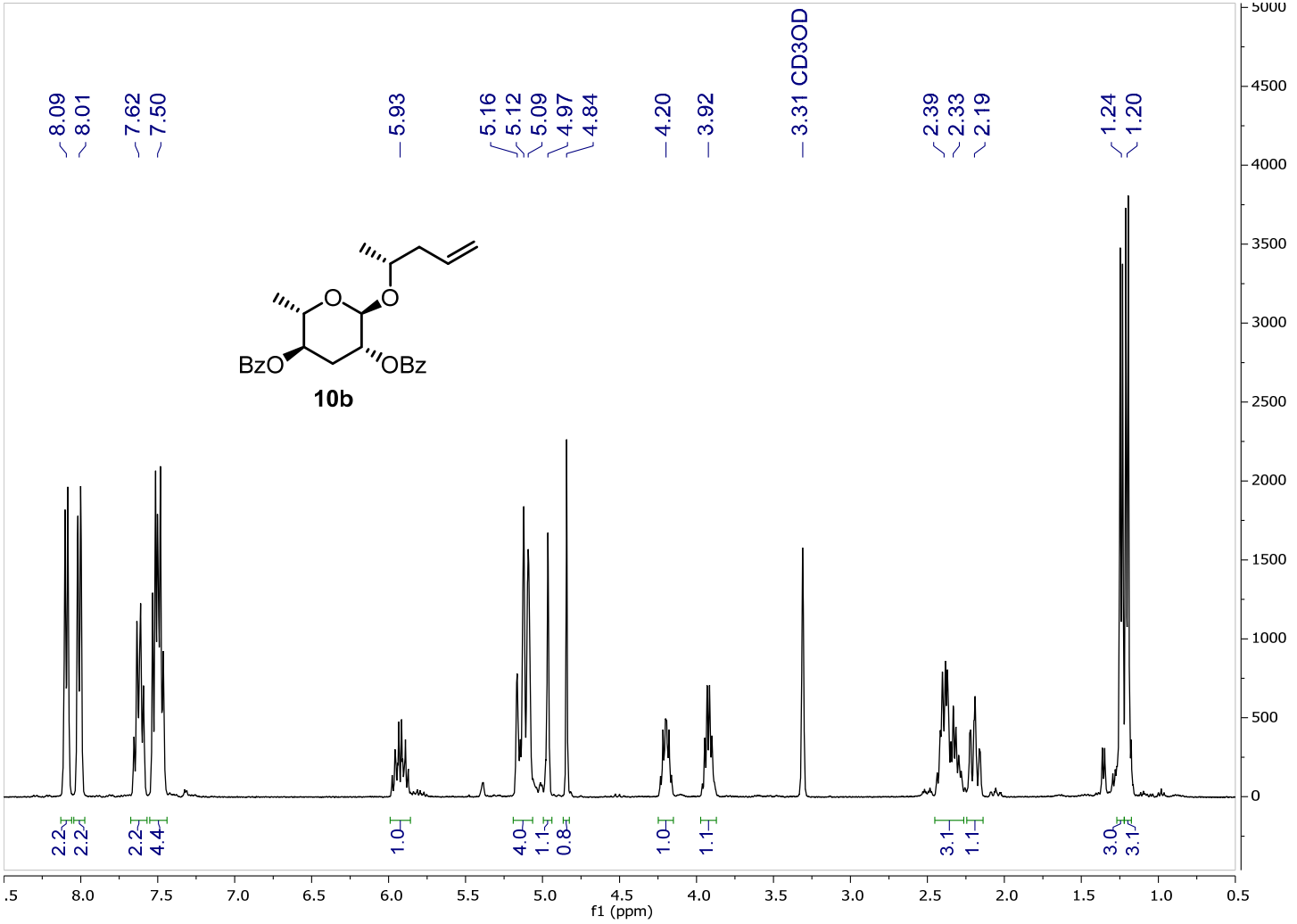


Figure S29: ^{13}C NMR (100 MHz, CD_3OD) of (4*R*)-4-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-pentene (**10b**).

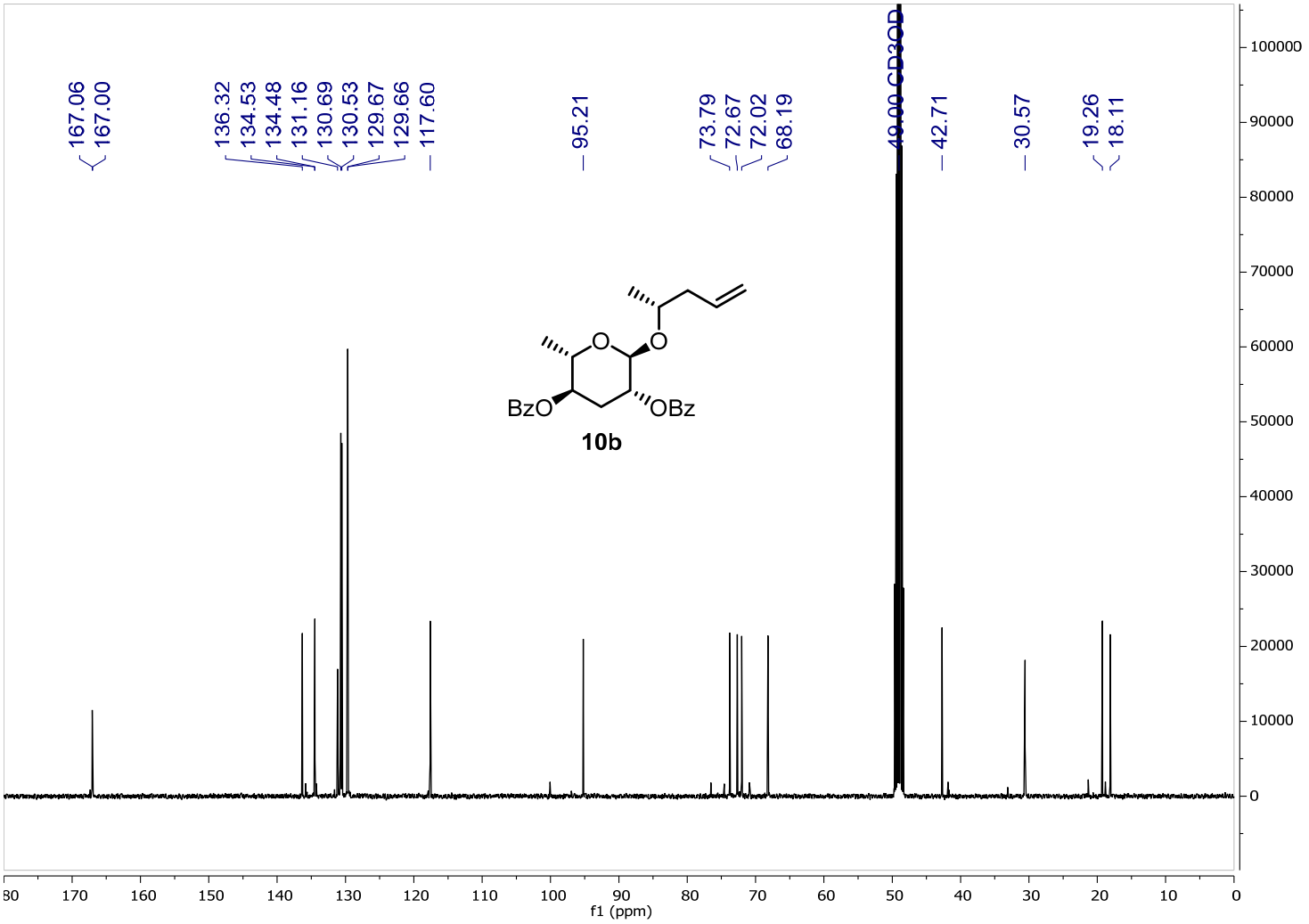


Figure S30: ^{1}H NMR (400 MHz, CD_3OD) of (3*R*)-3-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene.

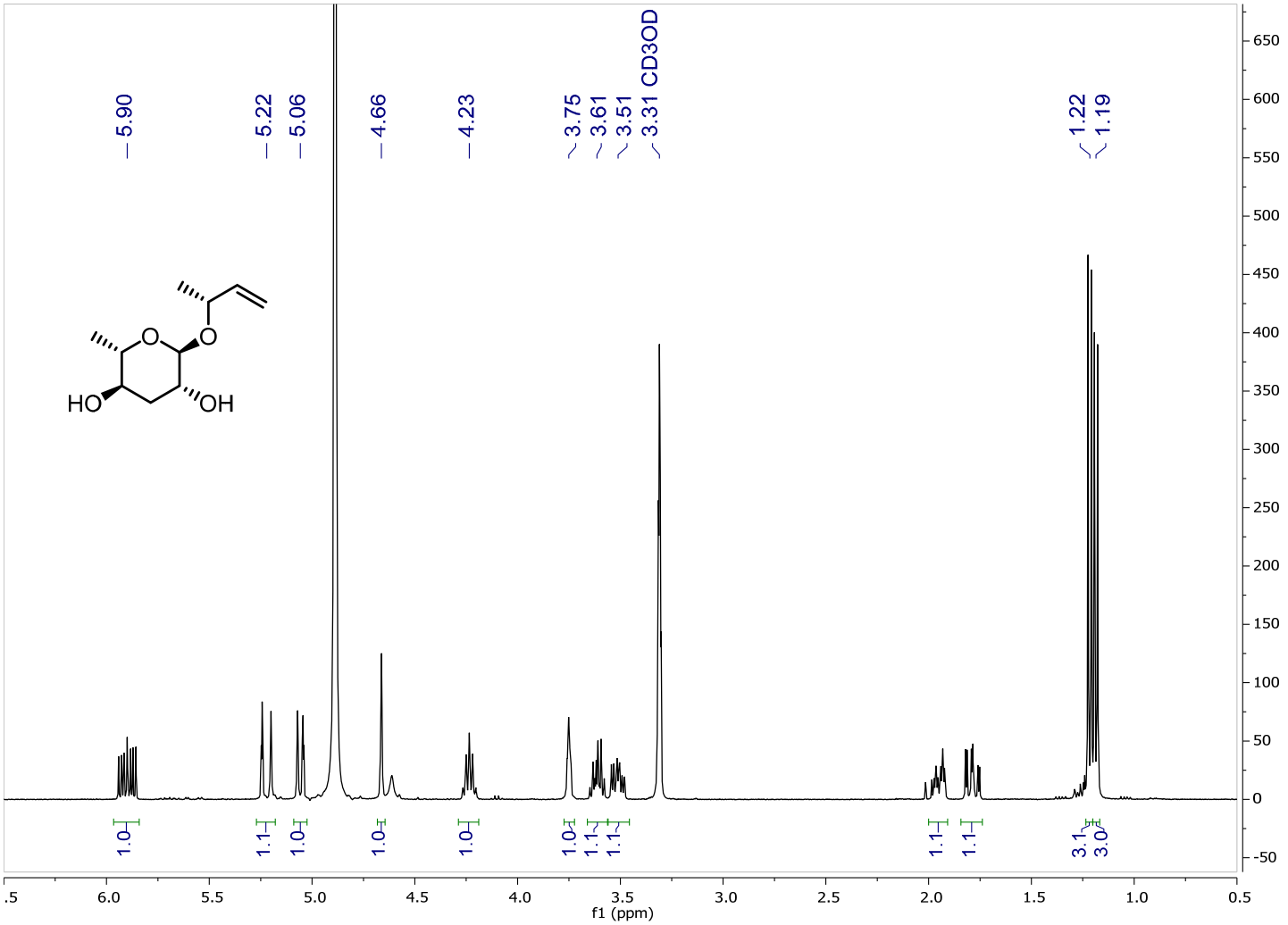


Figure S31: ^{1}H NMR (400 MHz, CD_3OD) of (4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-pentene.

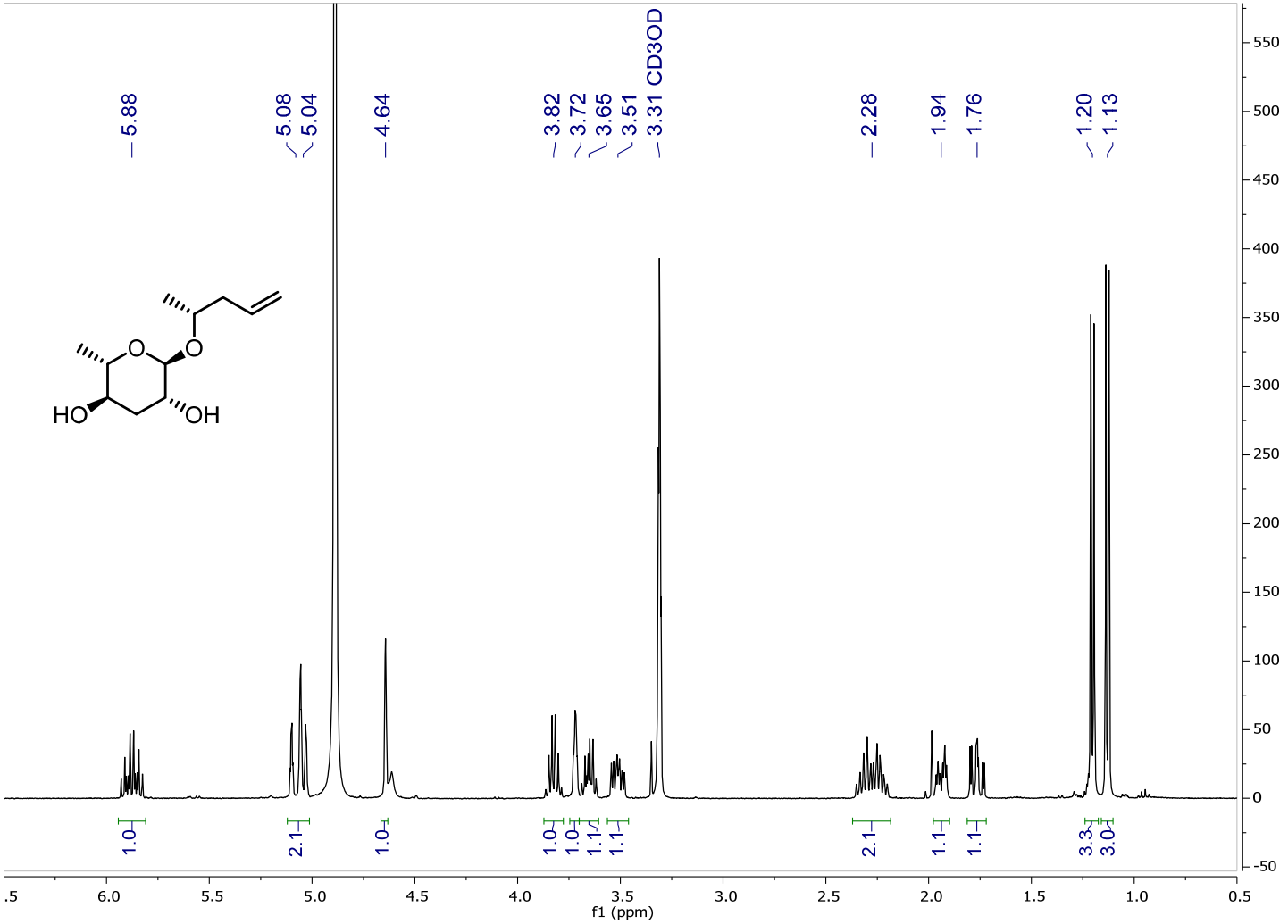


Figure S32: ^{1}H NMR (400 MHz, CD_3OD) of Benzyl (2*E*,4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-pentenoate (**11a**).

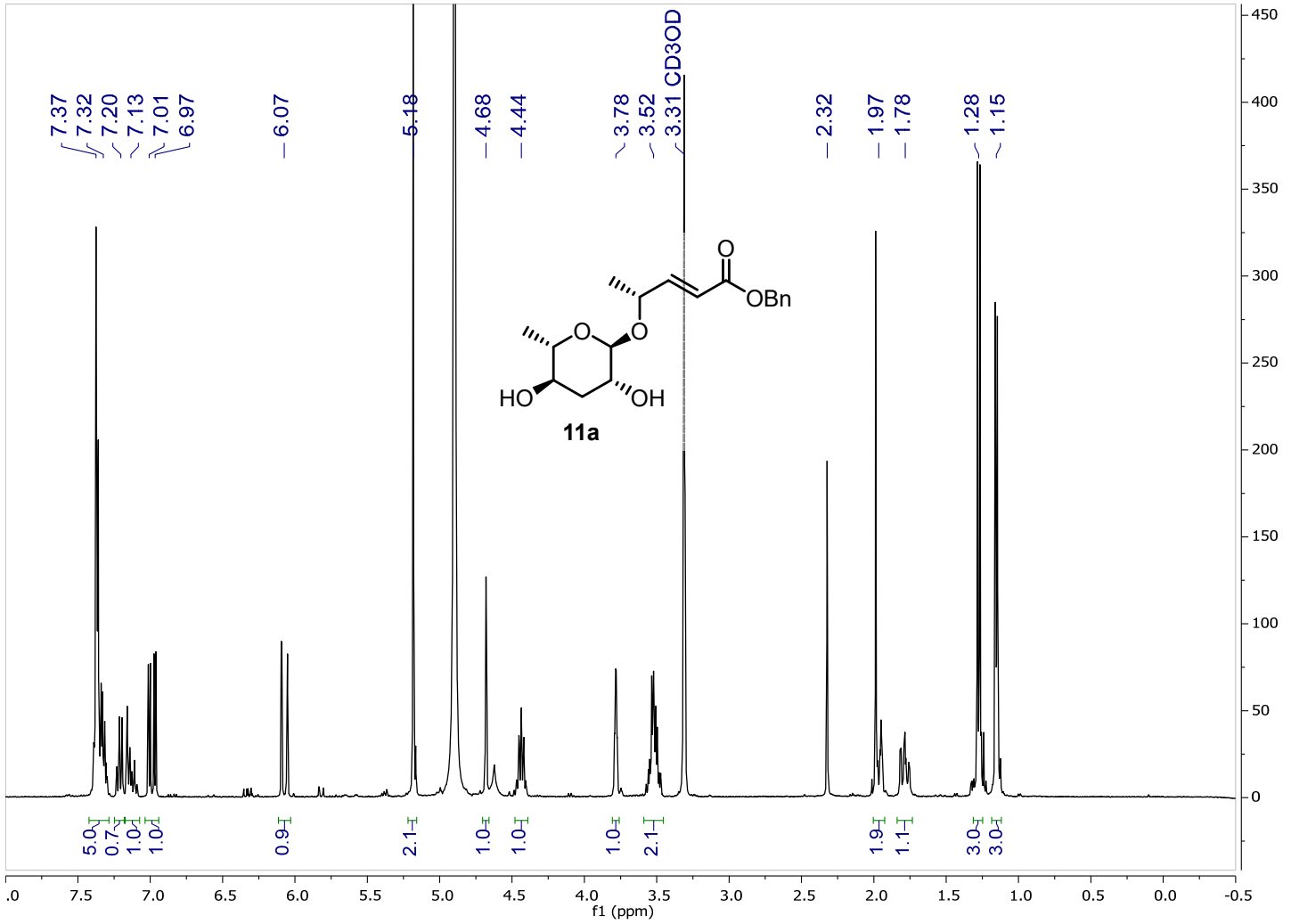


Figure S33: ^{13}C NMR (100 MHz, CD_3OD) of Benzyl (2*E*,4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-pentenoate (**11a**).

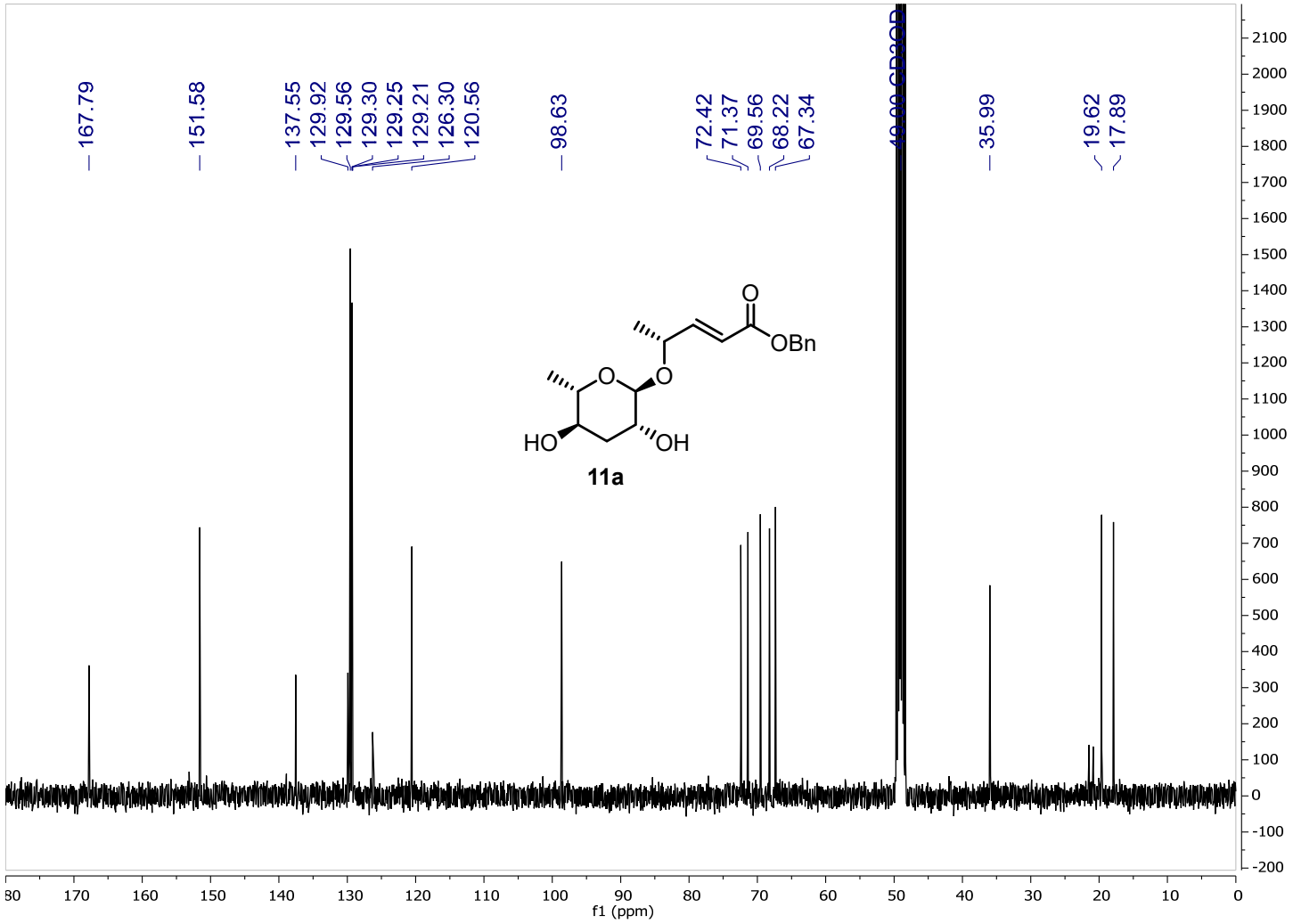


Figure S34: ^{1}H NMR (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate (**11b**).

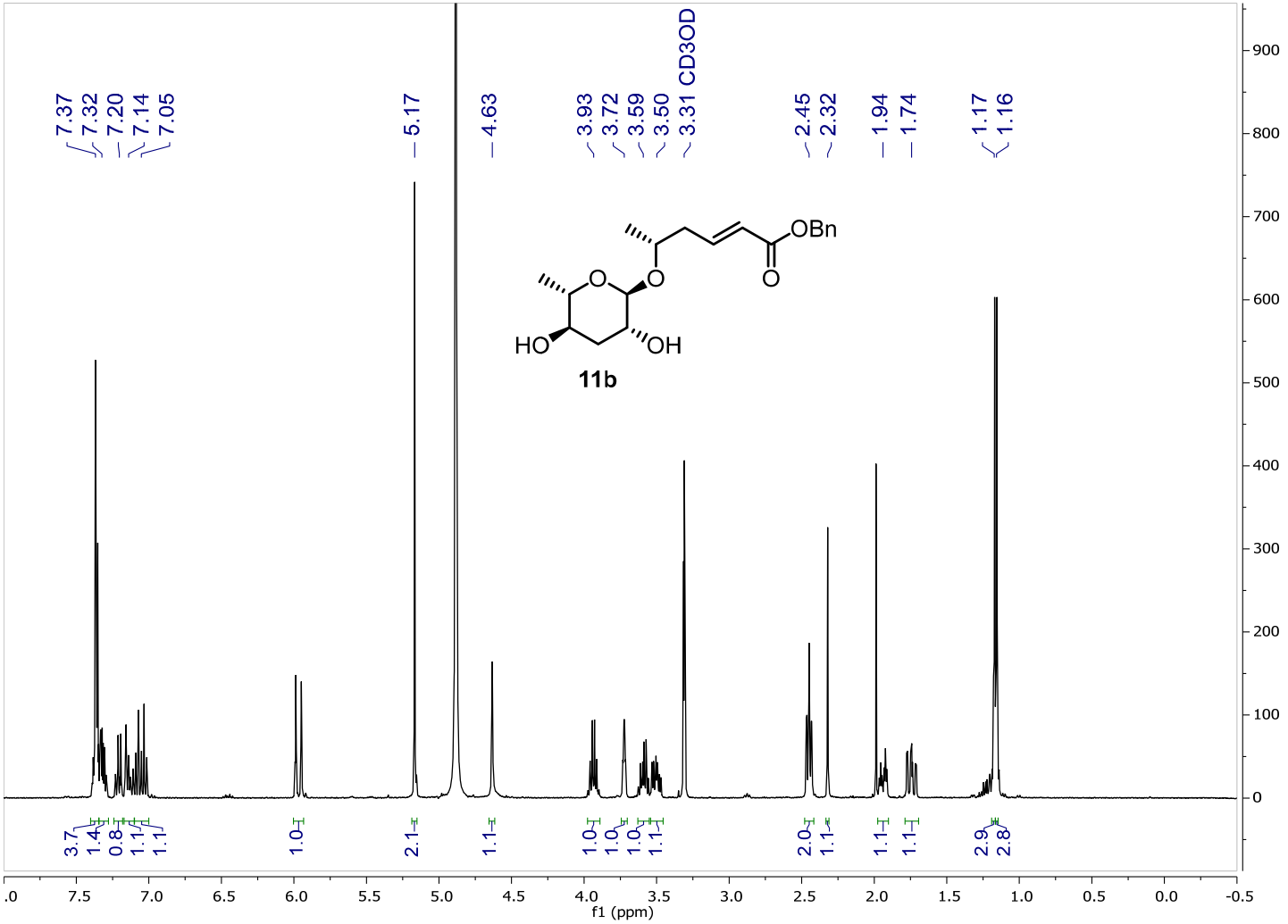


Figure S35: ^{13}C NMR (100 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate (**11b**).

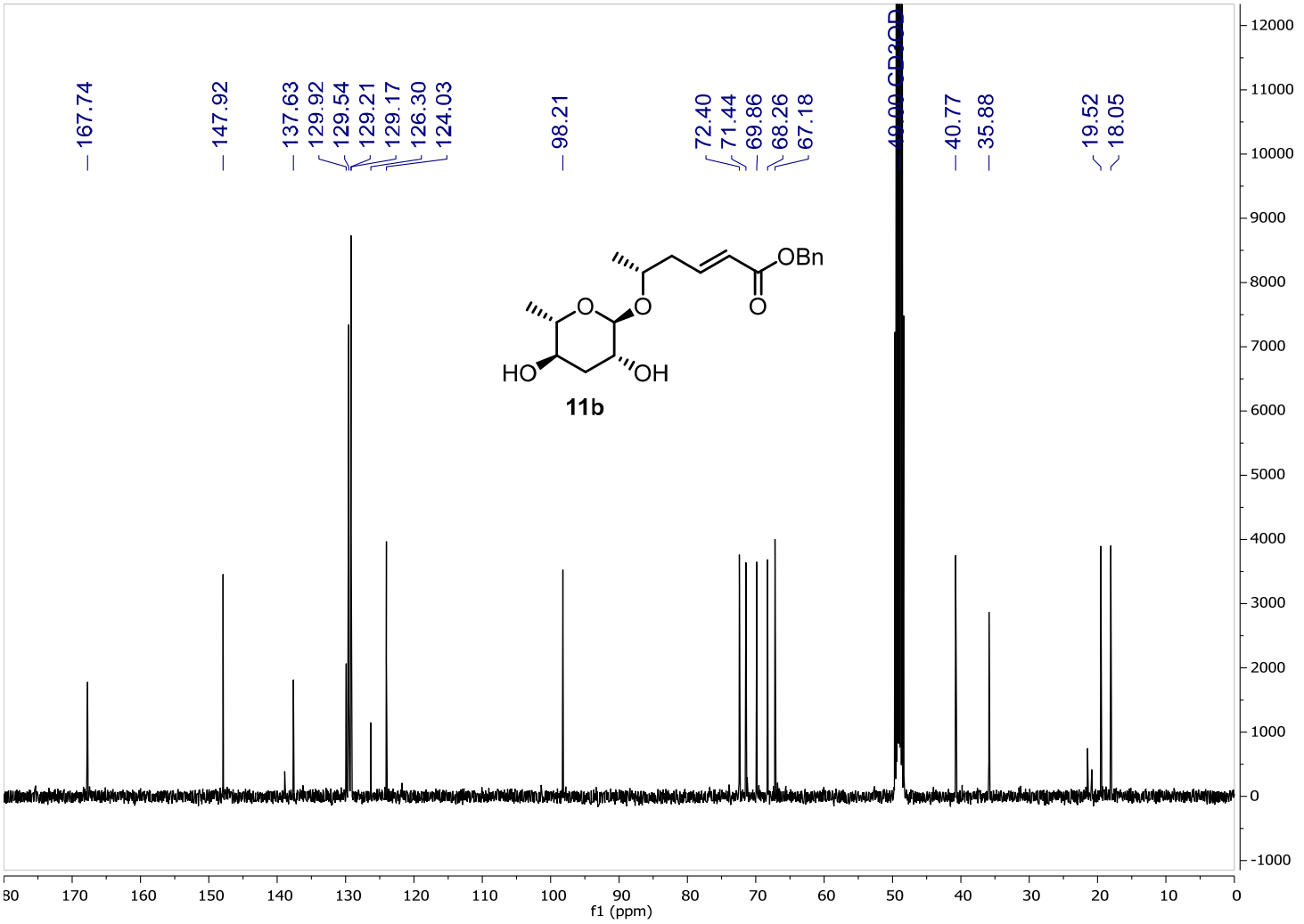


Figure S36: ^{1}H NMR (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate.

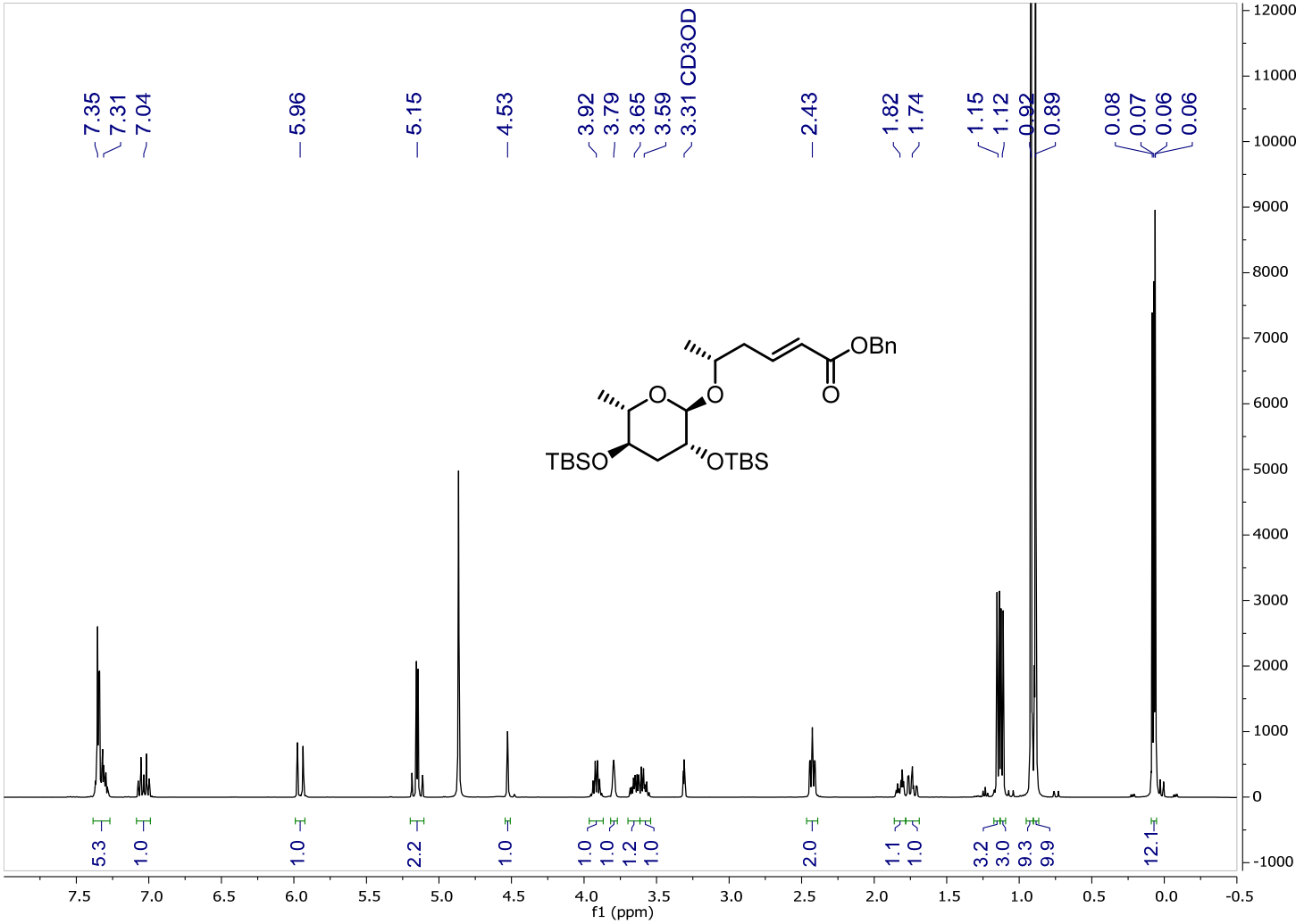


Figure S37: *dqf*-COSY (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate.

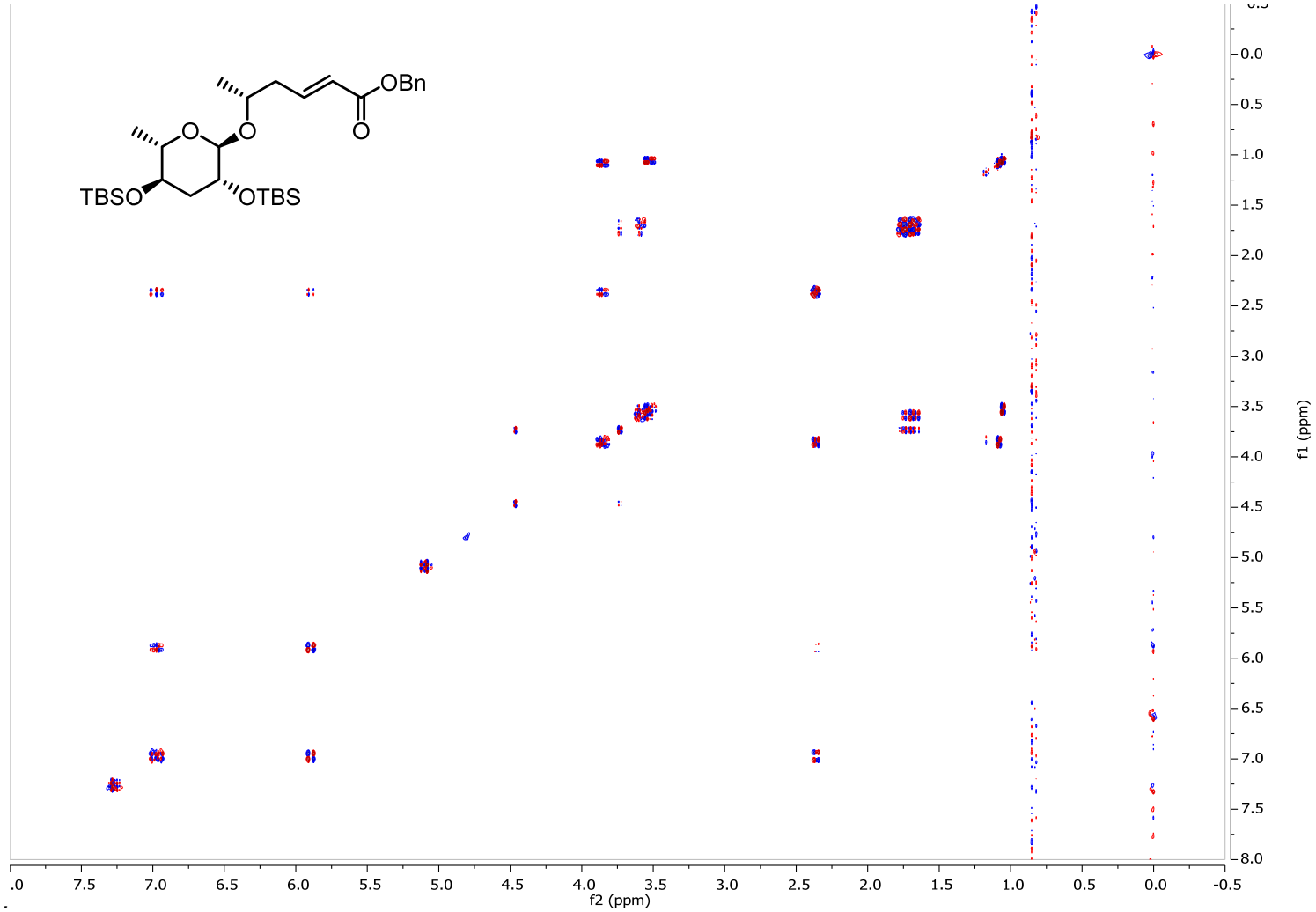


Figure S38: ^{13}C NMR (100 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate.

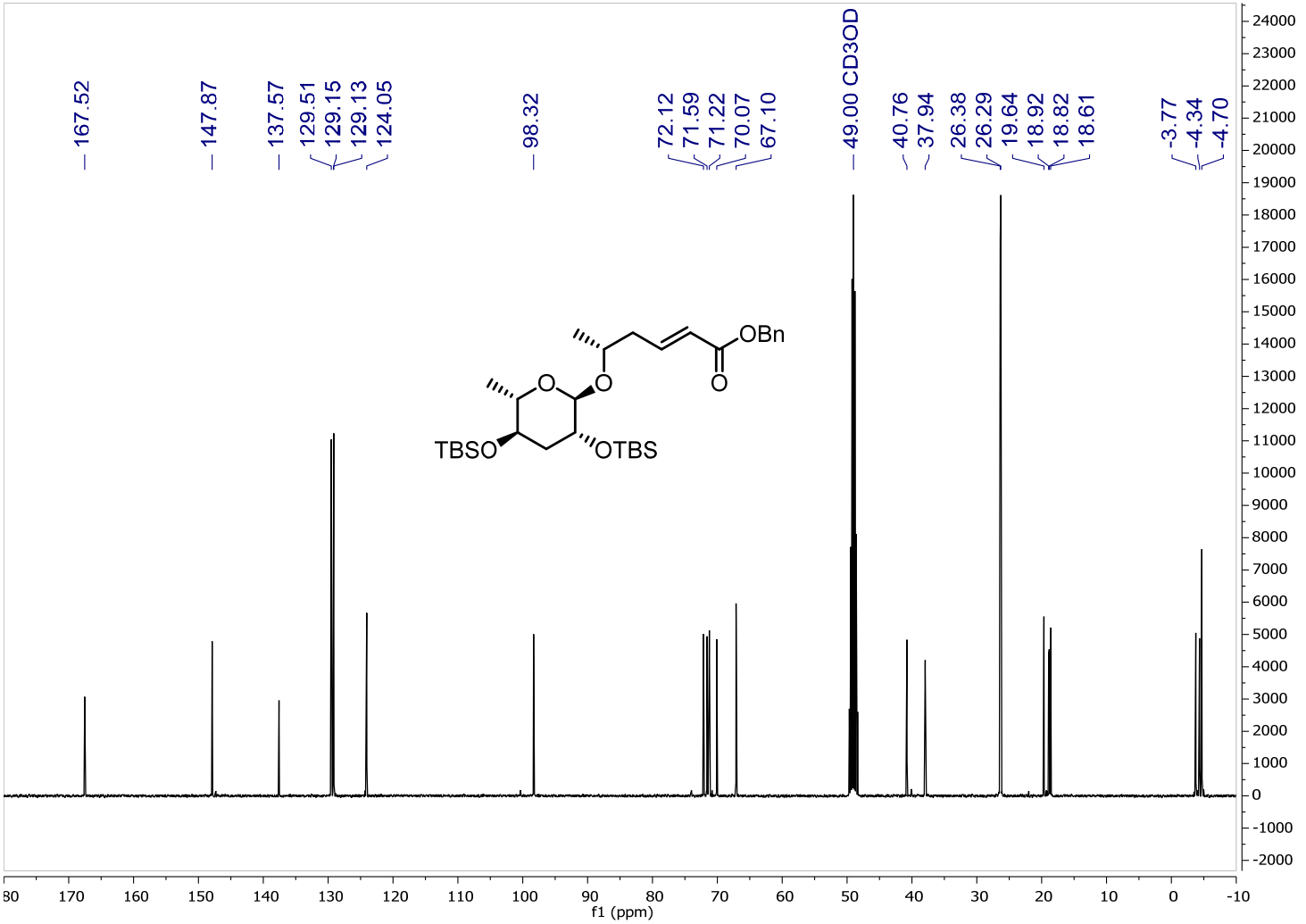


Figure S39: HSQC (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate.

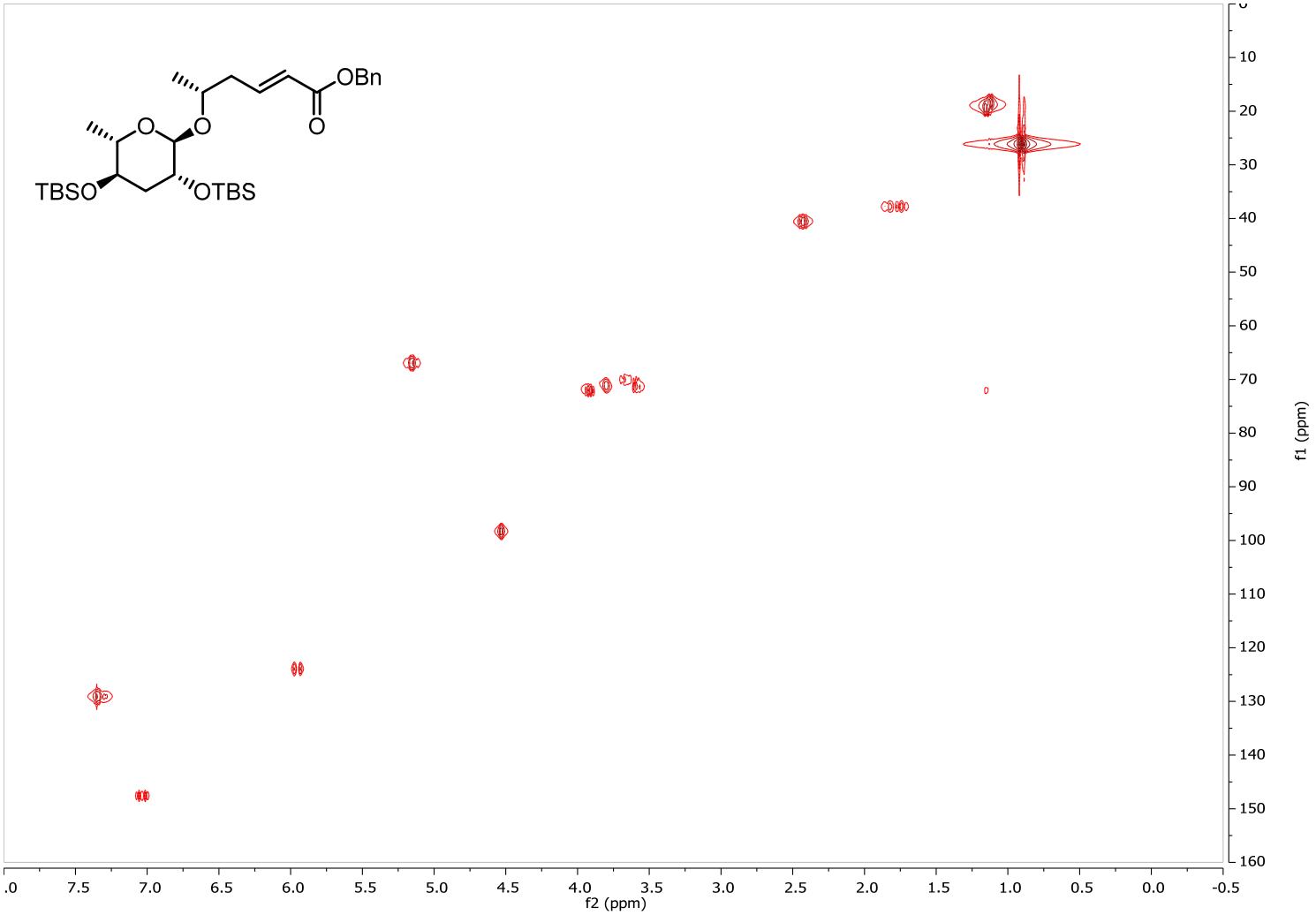


Figure S40: ^{1}H NMR (400 MHz, CD_3OD) of (5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-hexanoic acid (**12**).

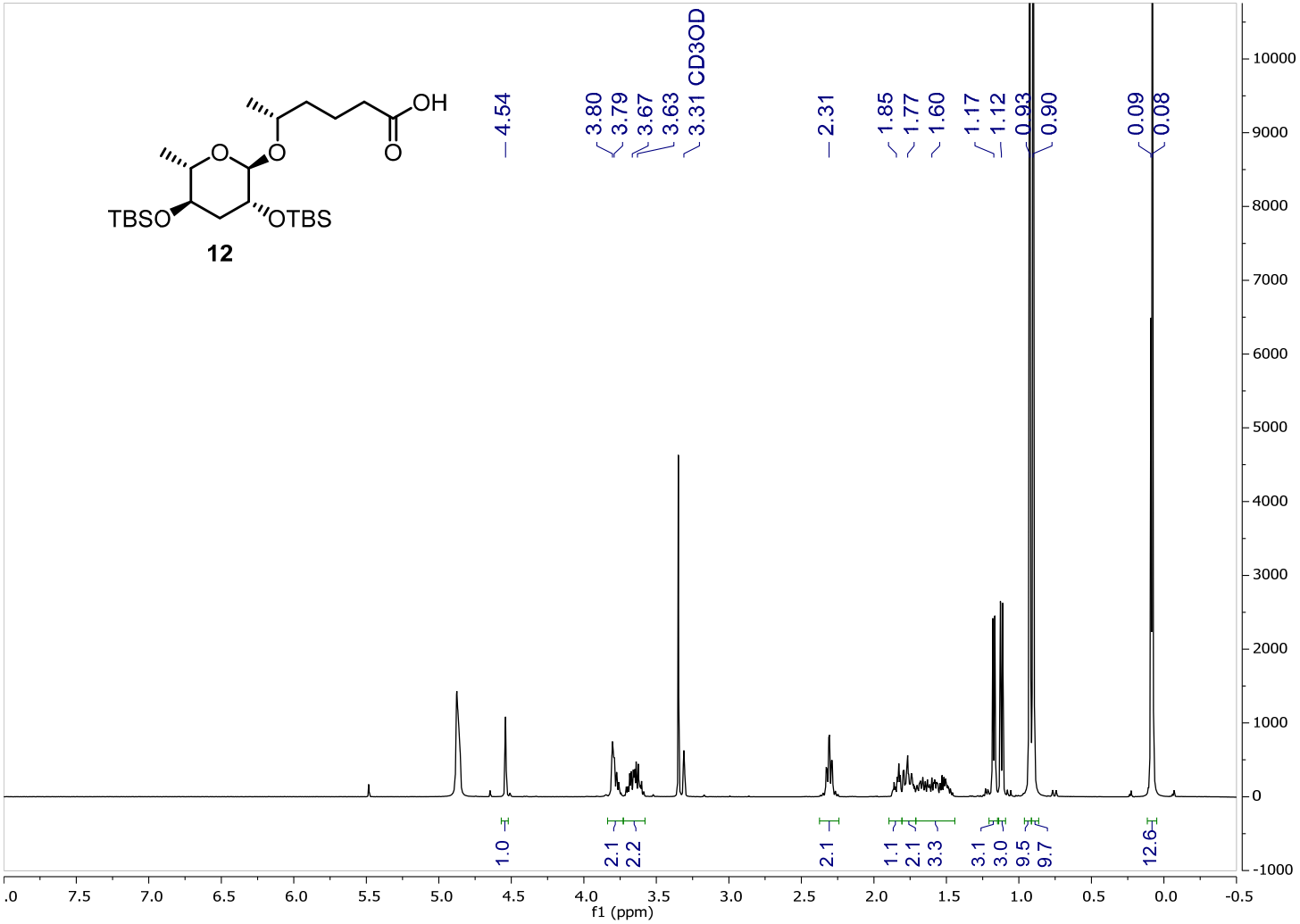


Figure S41: *dqf*-COSY (400 MHz, CD_3OD) of (5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-hexanoic acid (**12**).

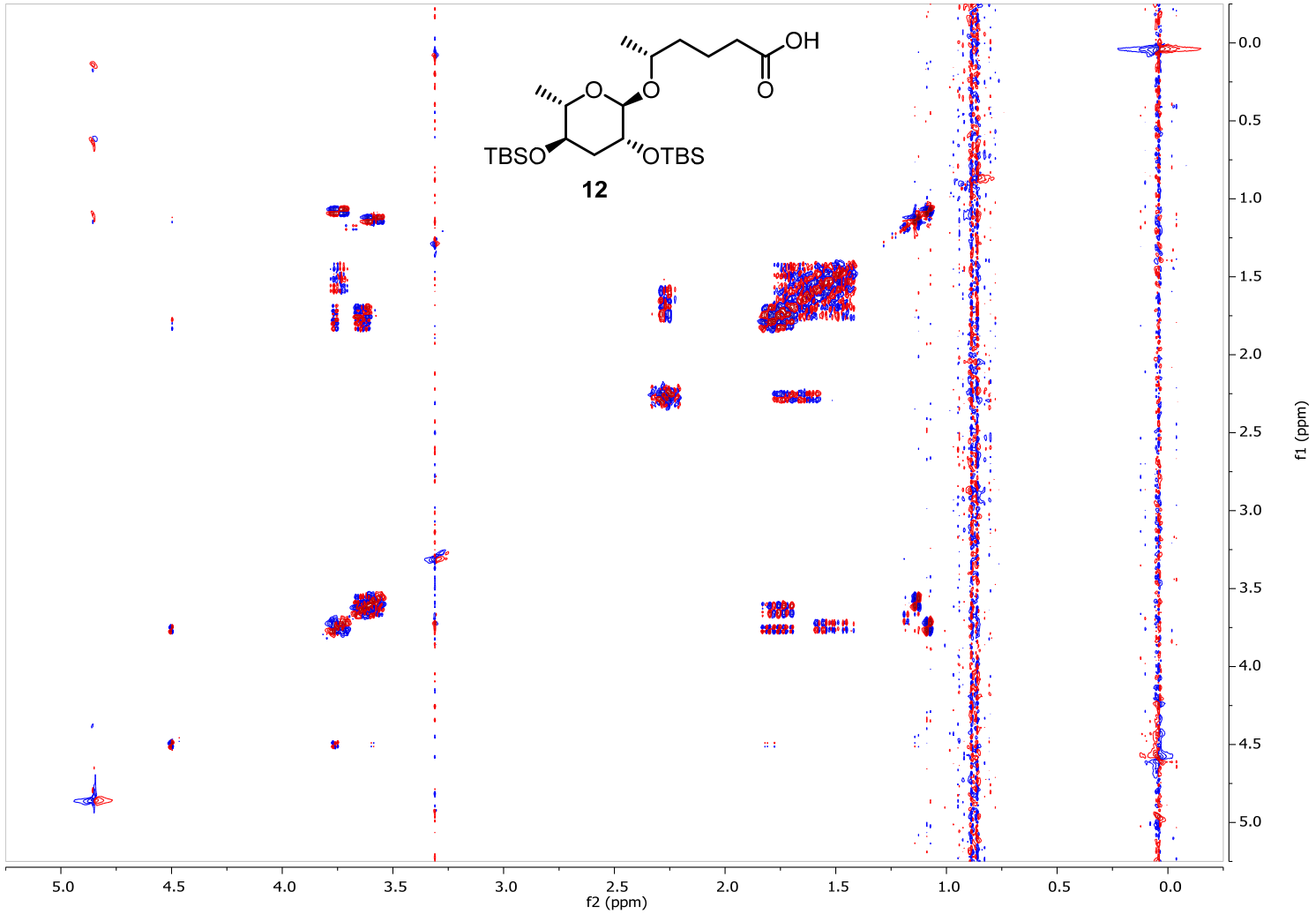


Figure S42: HSQC (400 MHz, CD_3OD) of (5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-hexanoic acid (**12**).

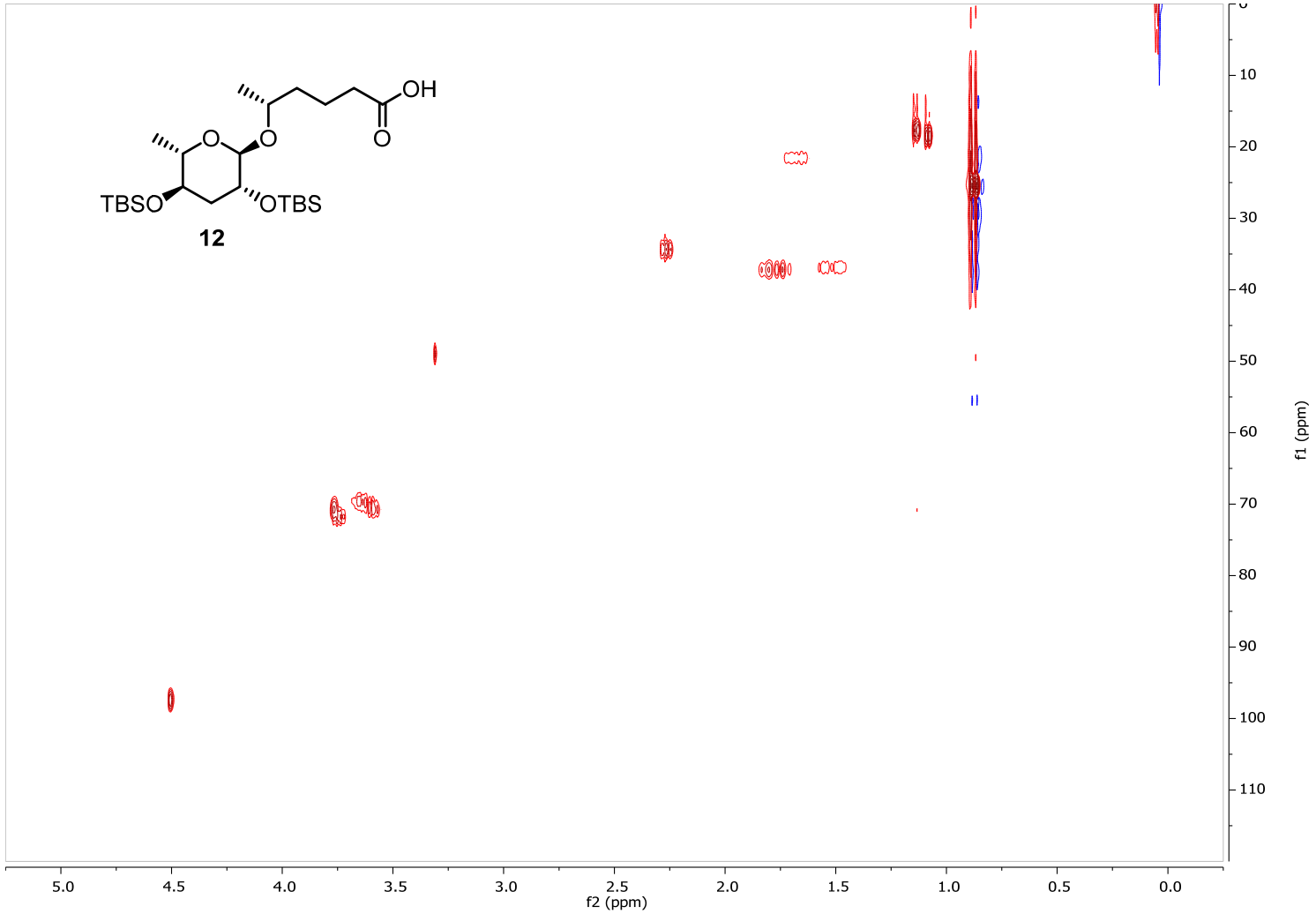


Figure S43: ^{1}H NMR (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2,4-di-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate.

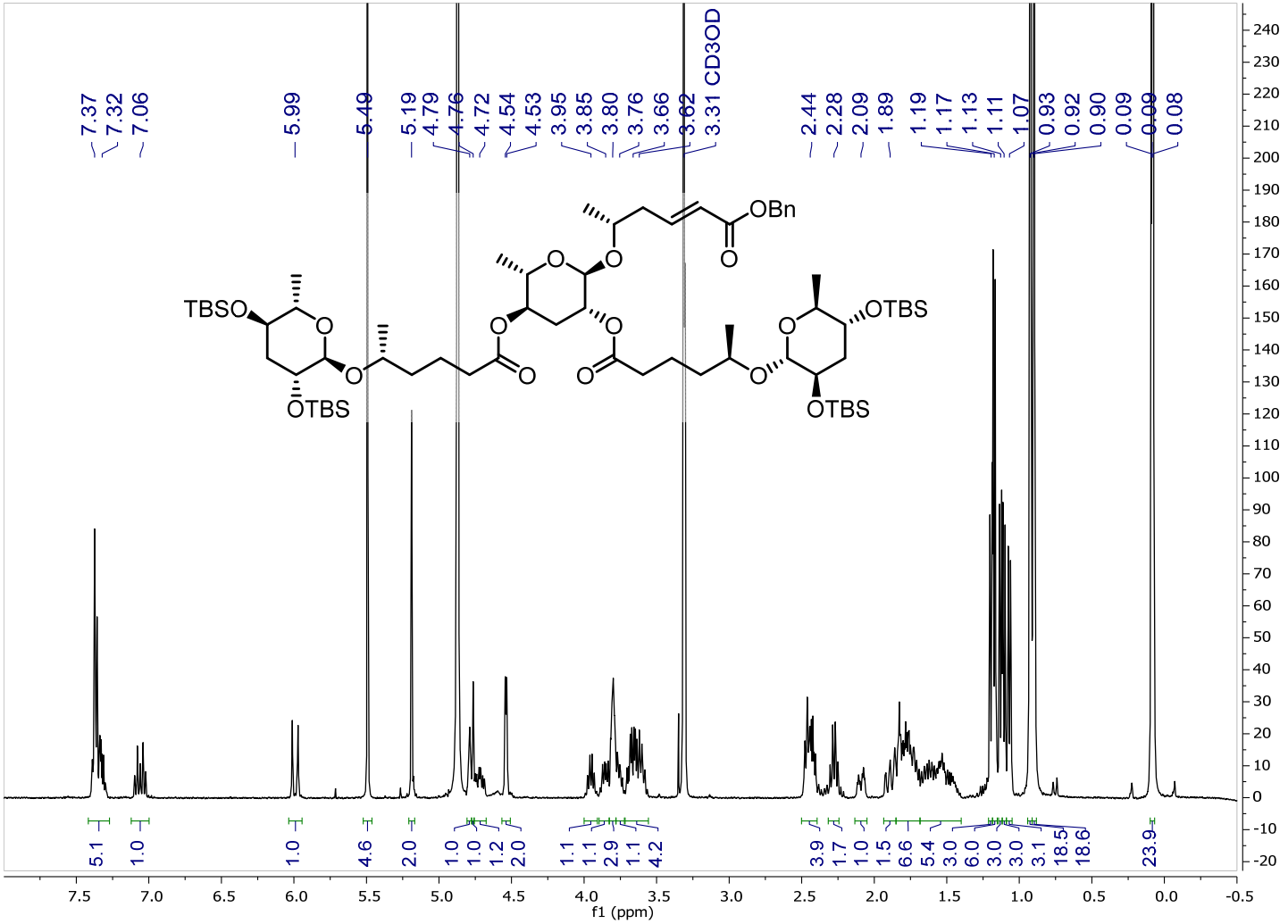


Figure S44: *dqf*-COSY (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2,4-di-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate.

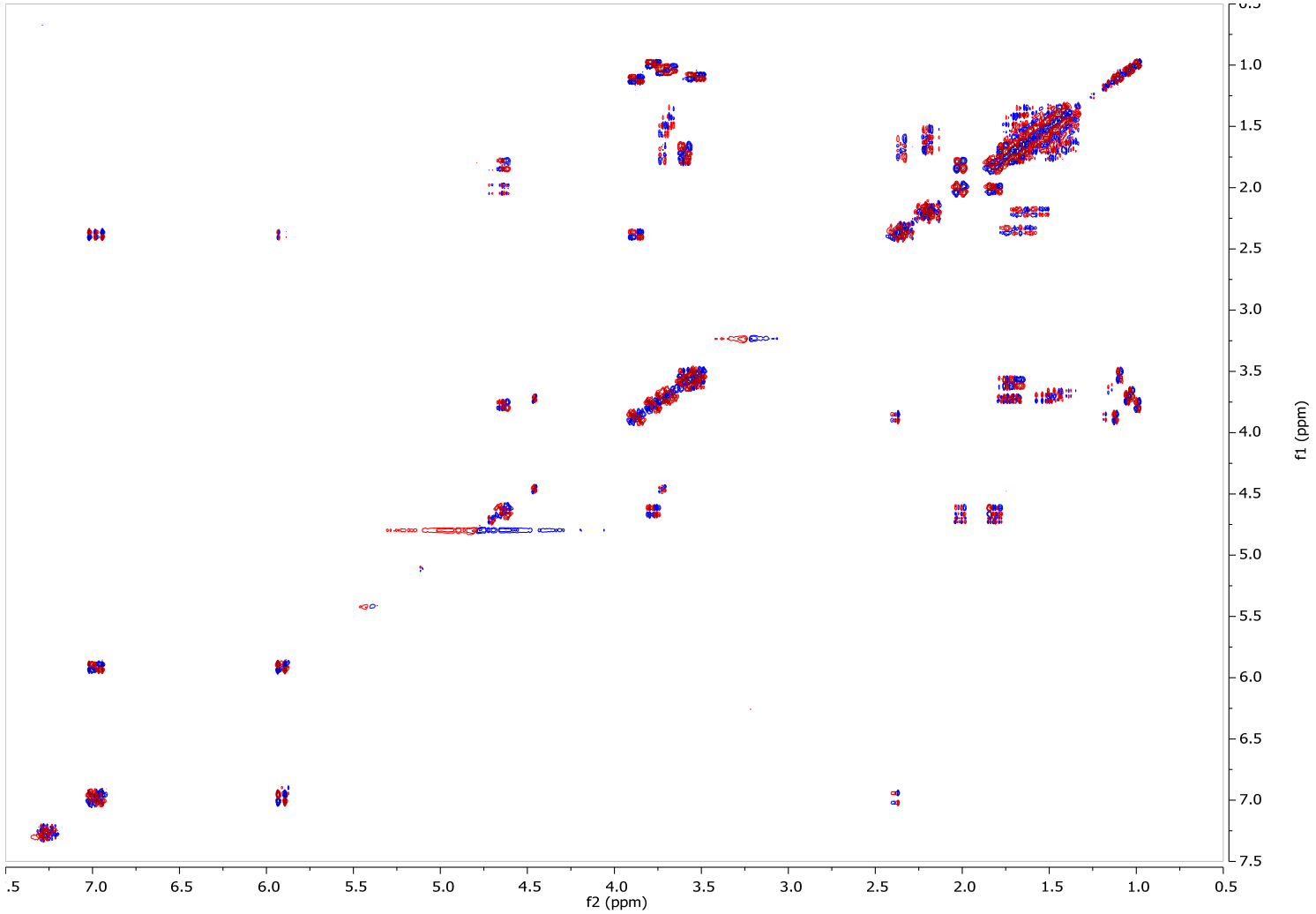


Figure S45: ^{1}H NMR (400 MHz, CD_3OD) of Benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate.

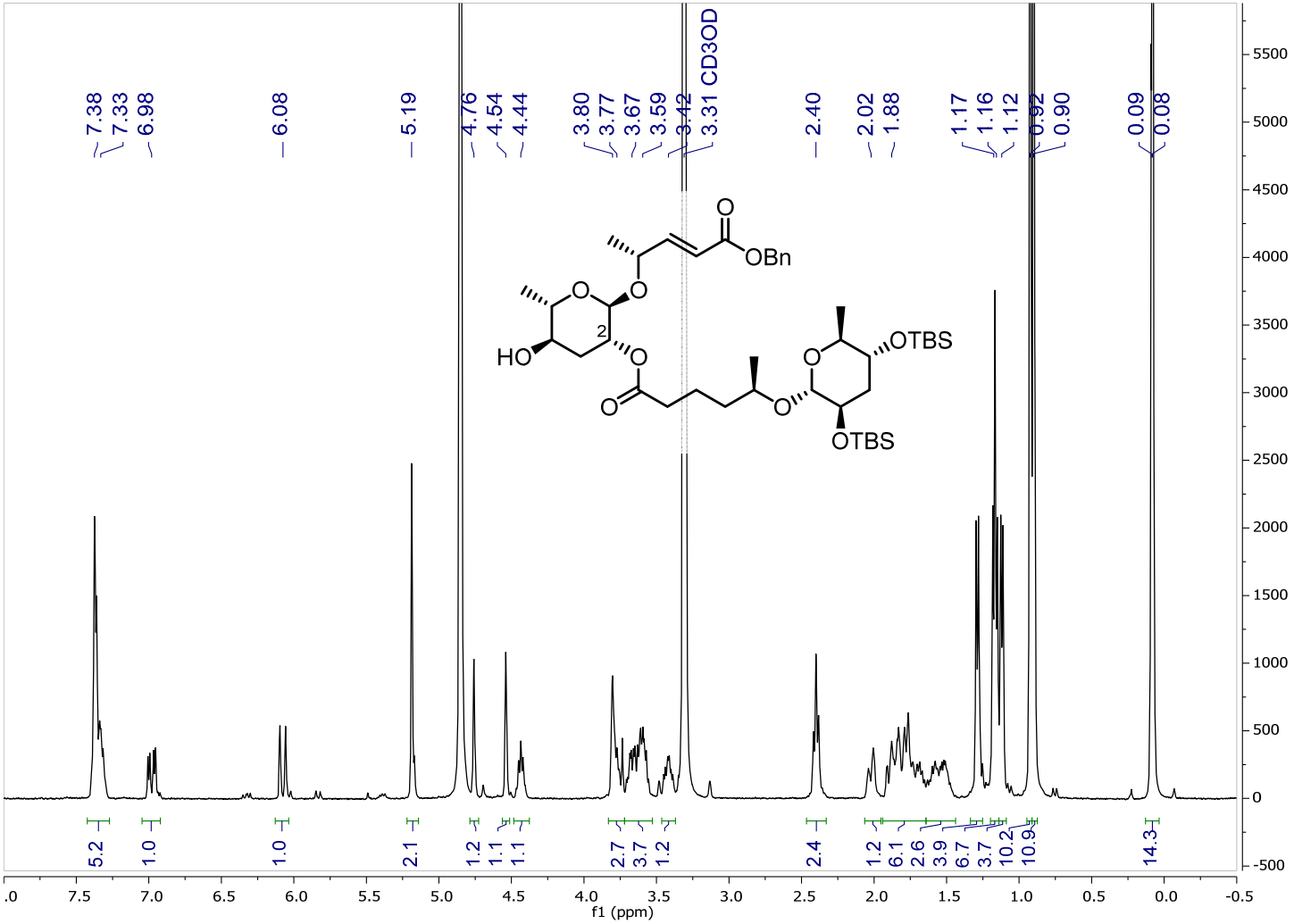


Figure S46: *dqf*-COSY (400 MHz, CD_3OD) of Benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate.

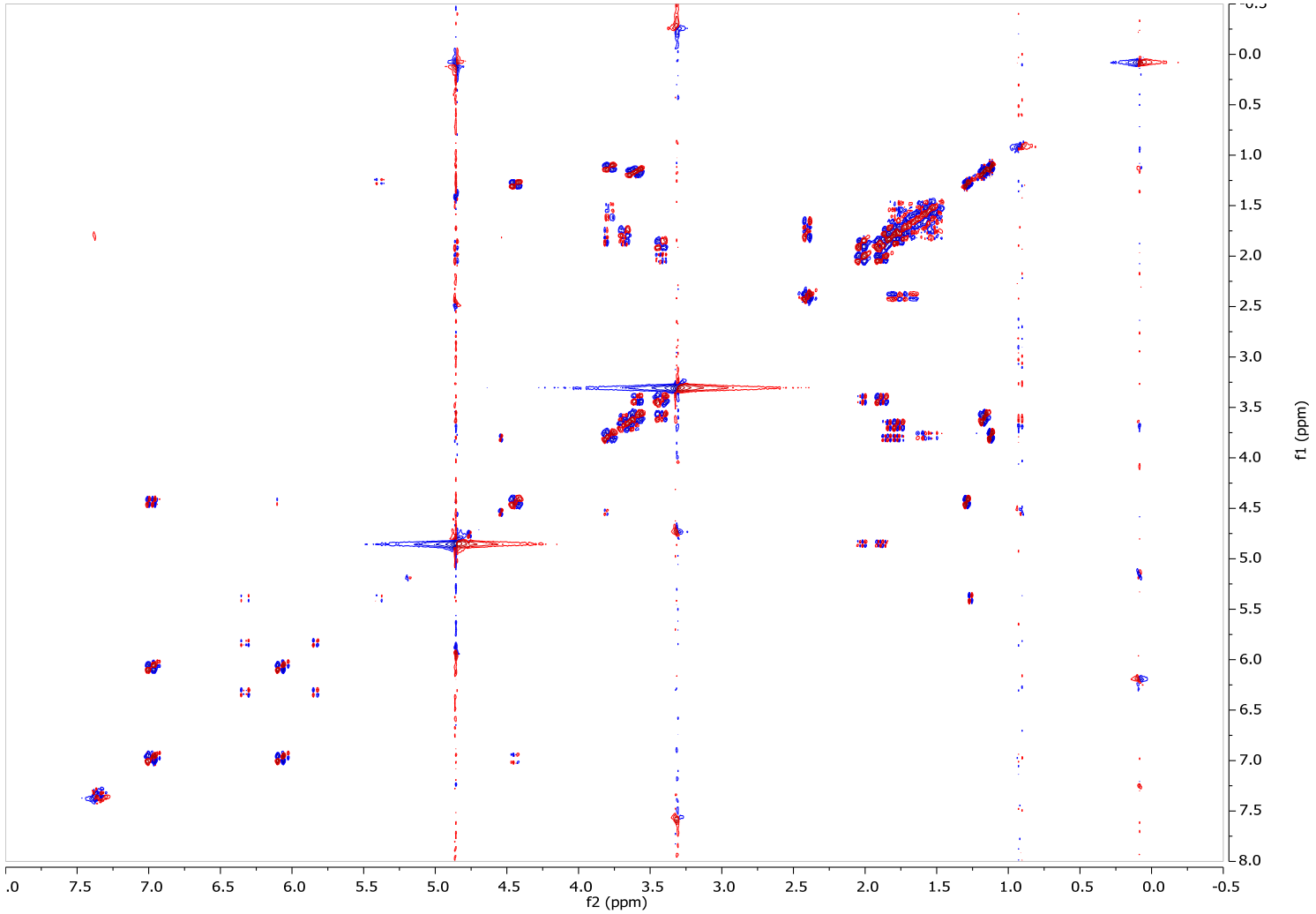


Figure S47: ^{1}H NMR (400 MHz, CD_3OD) of Benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate.

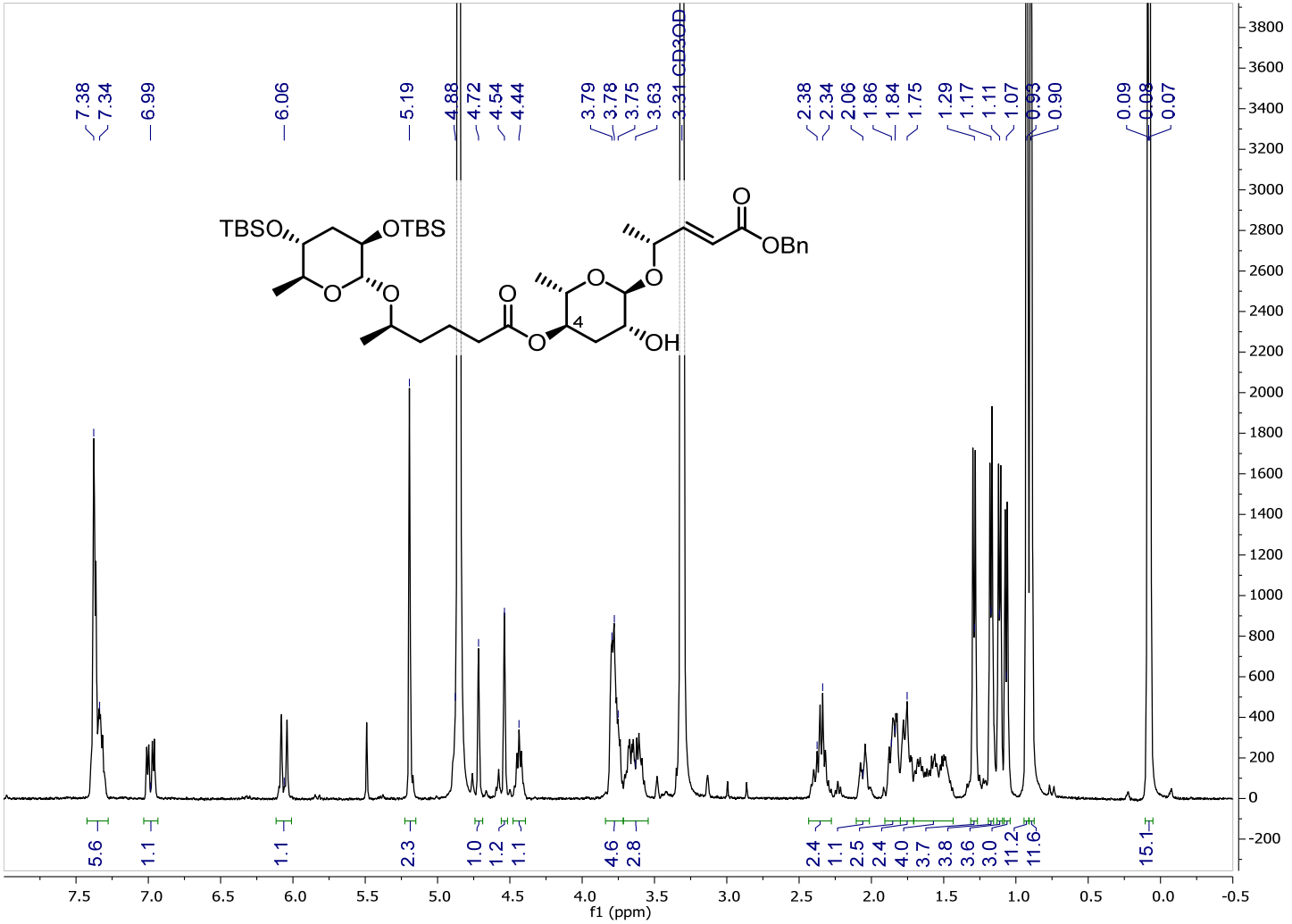


Figure S48: *dqf*-COSY (400 MHz, CD_3OD) of Benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate.

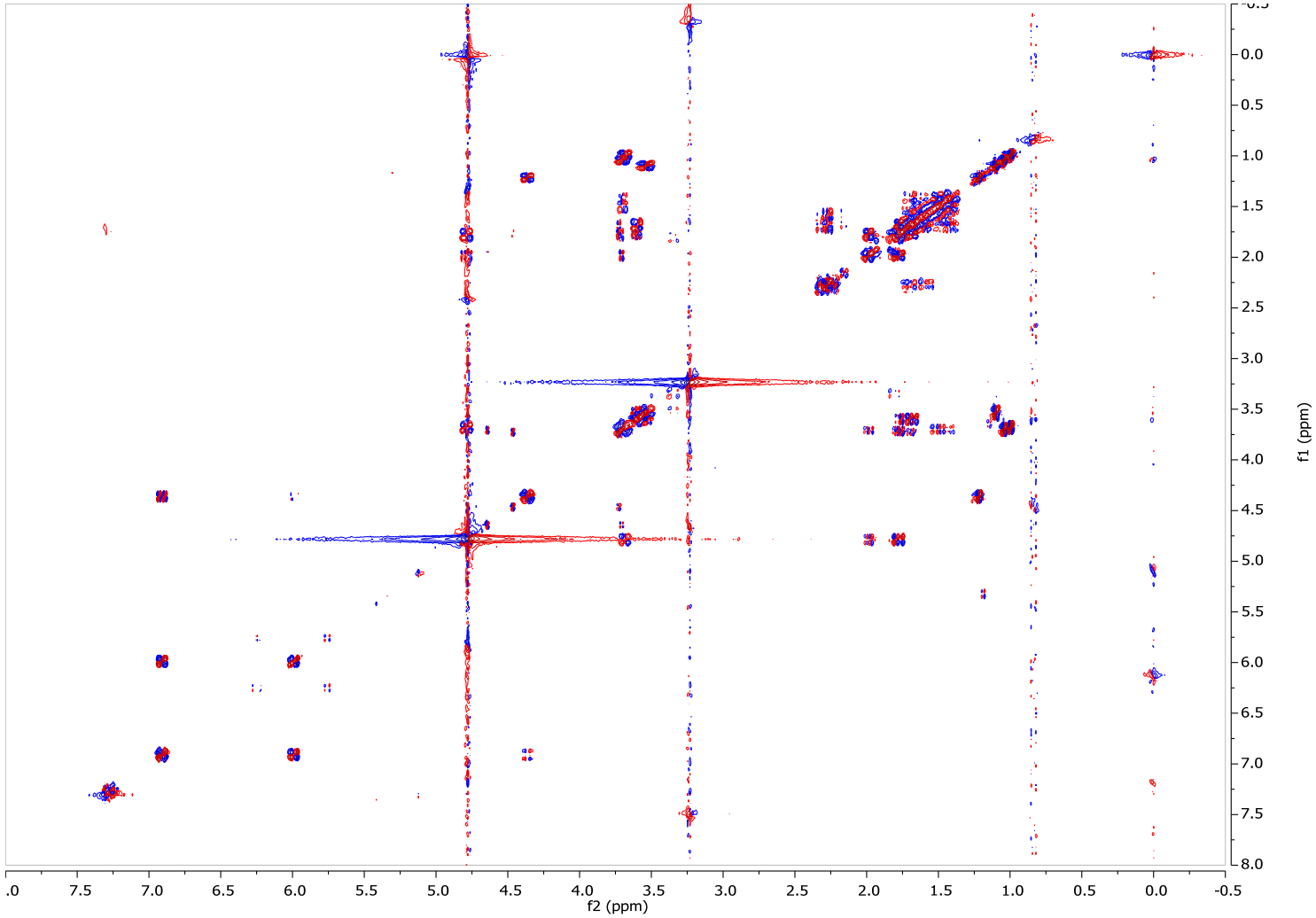


Figure S49: ^{1}H NMR (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate.

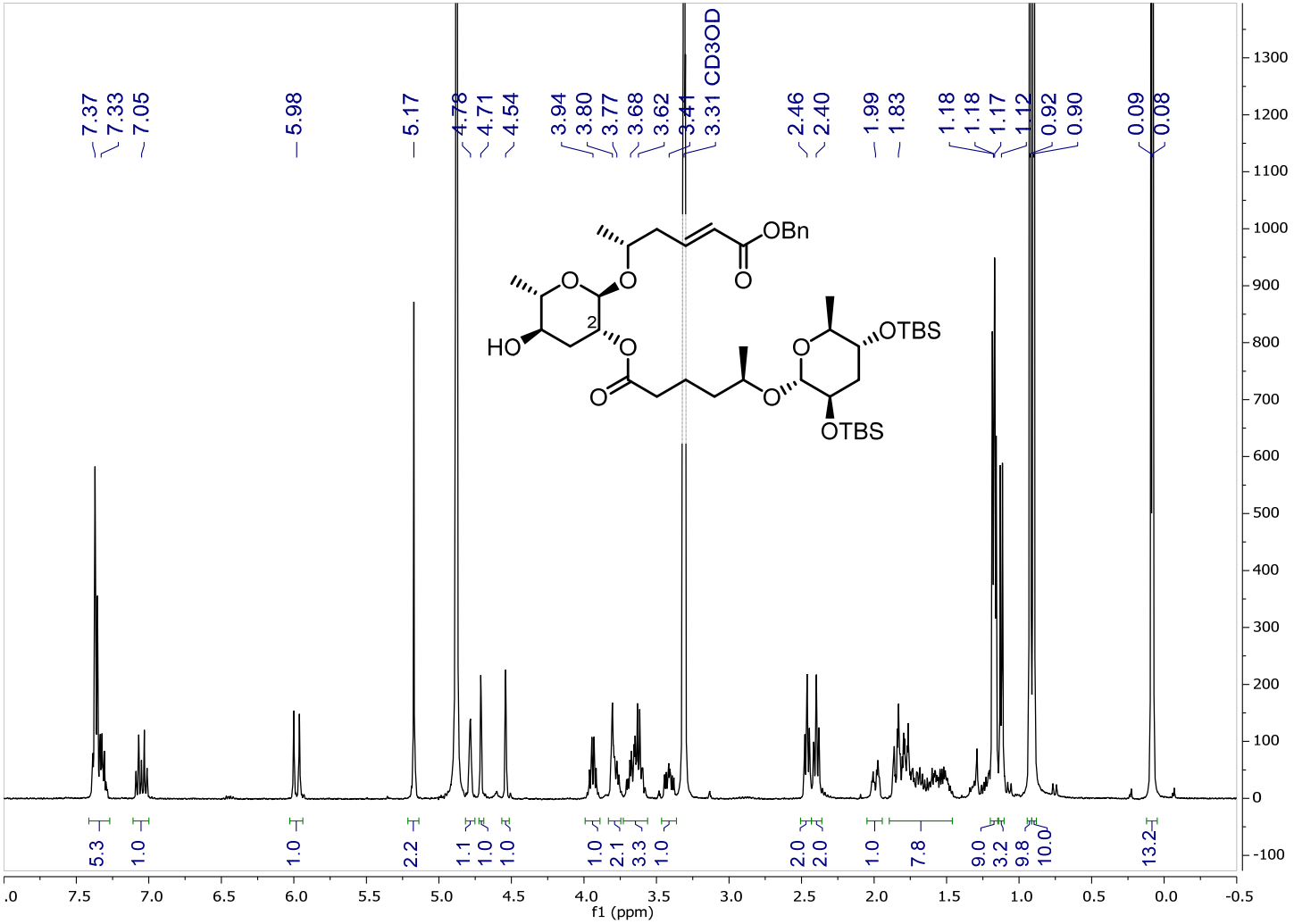


Figure S50: *dqf*-COSY (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate.

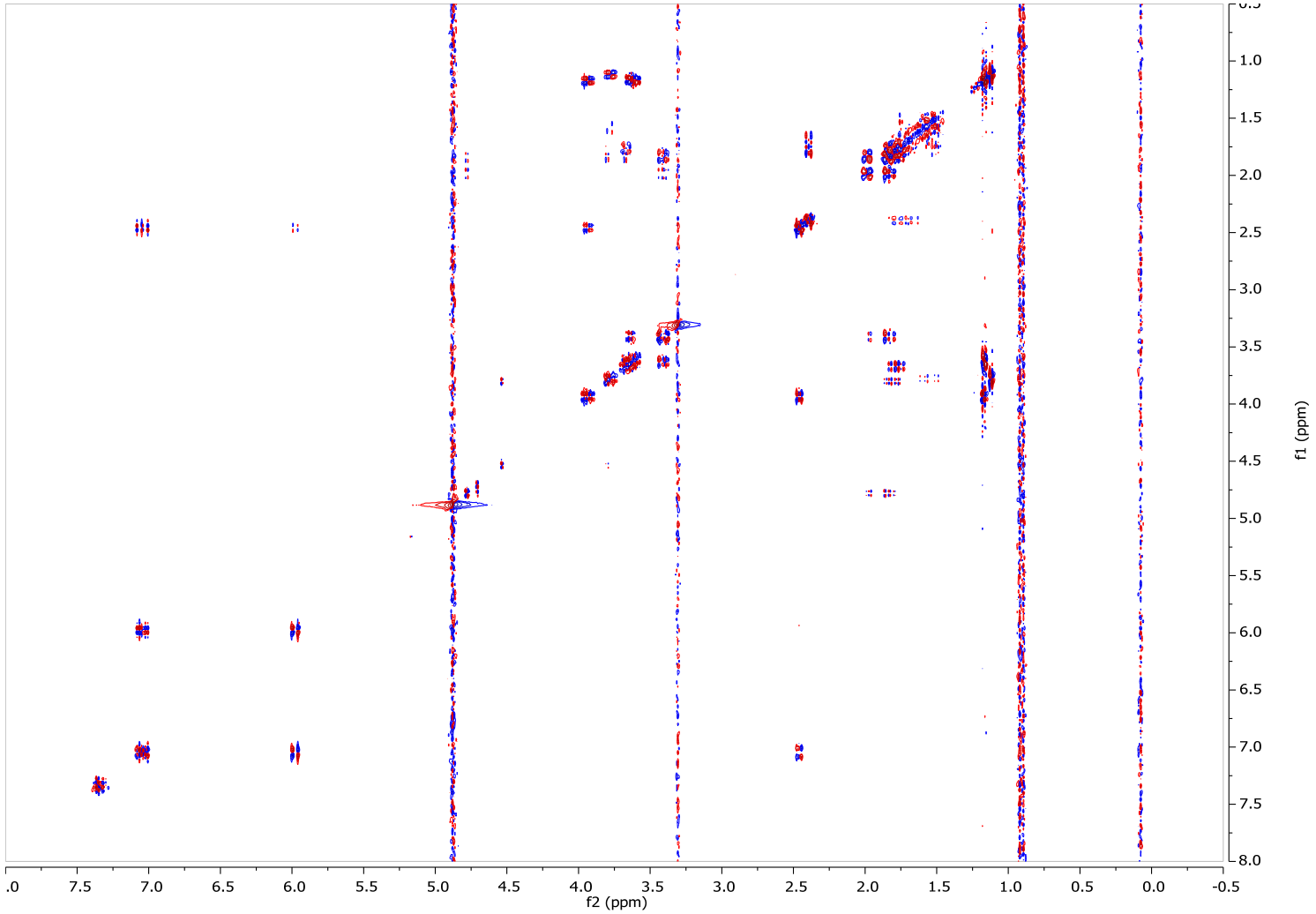


Figure S51: ^{1}H NMR (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate.

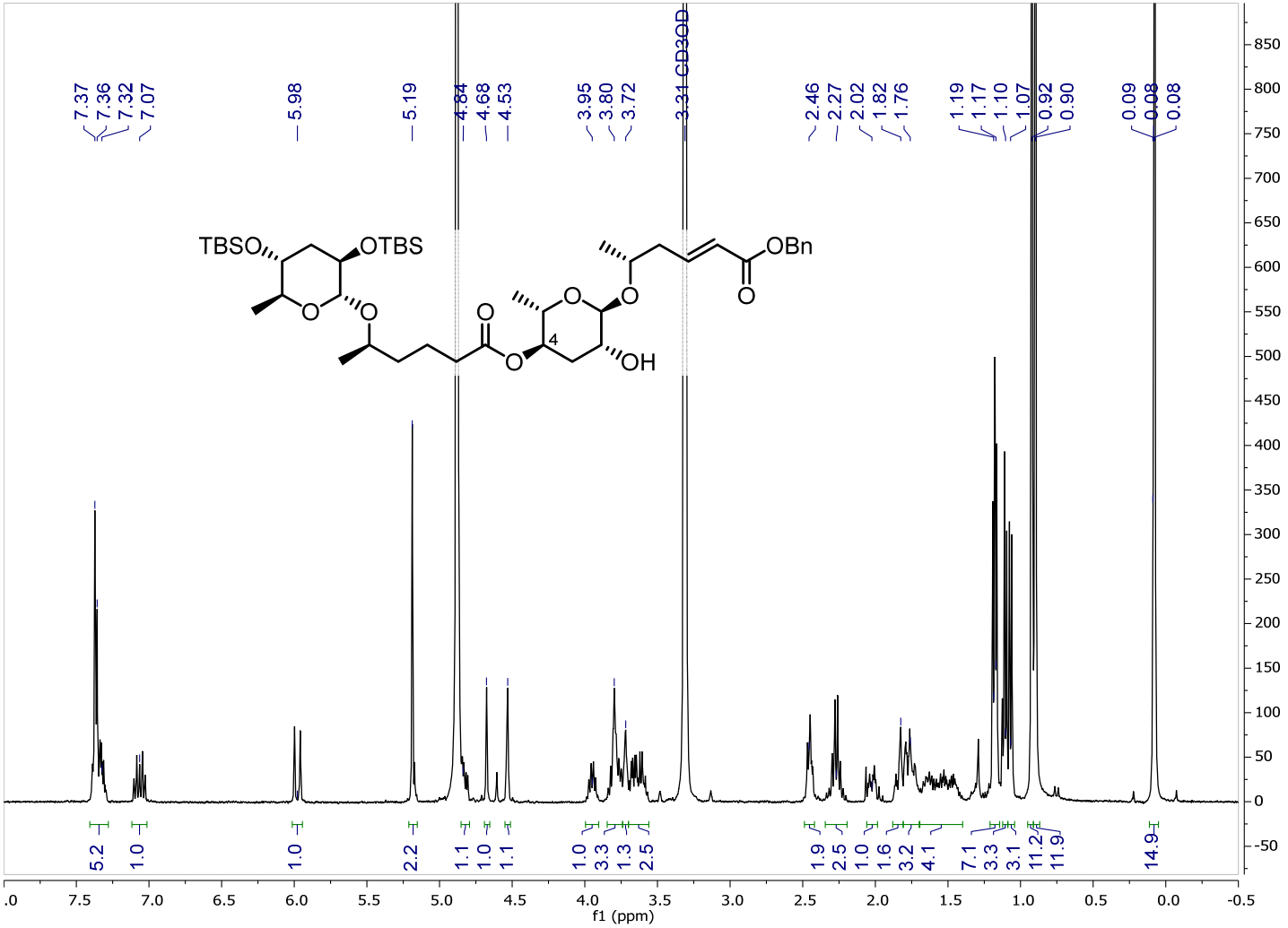


Figure S52: *dqf*-COSY (400 MHz, CD_3OD) of Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate.

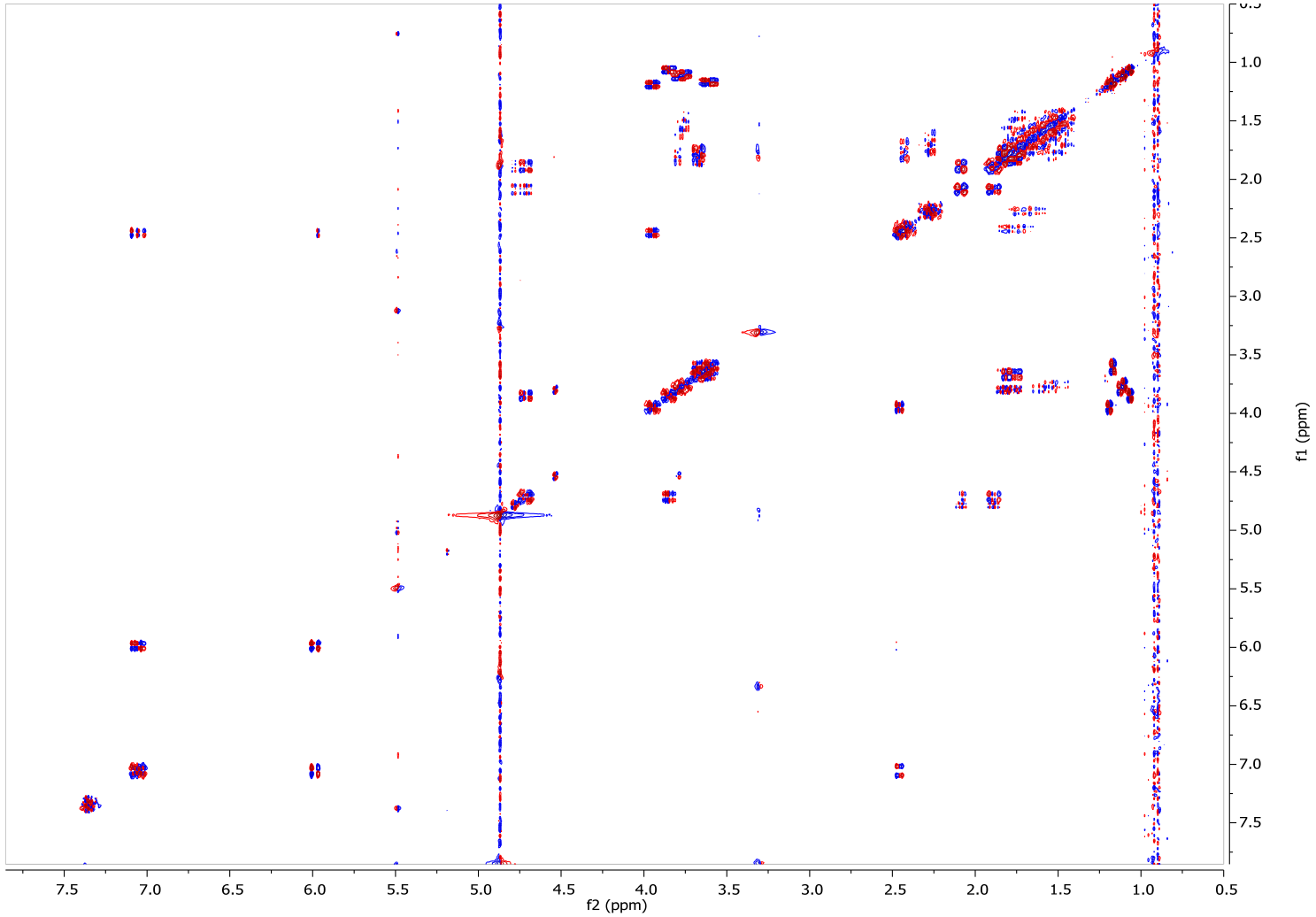


Figure S53: ^{1}H NMR (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid.

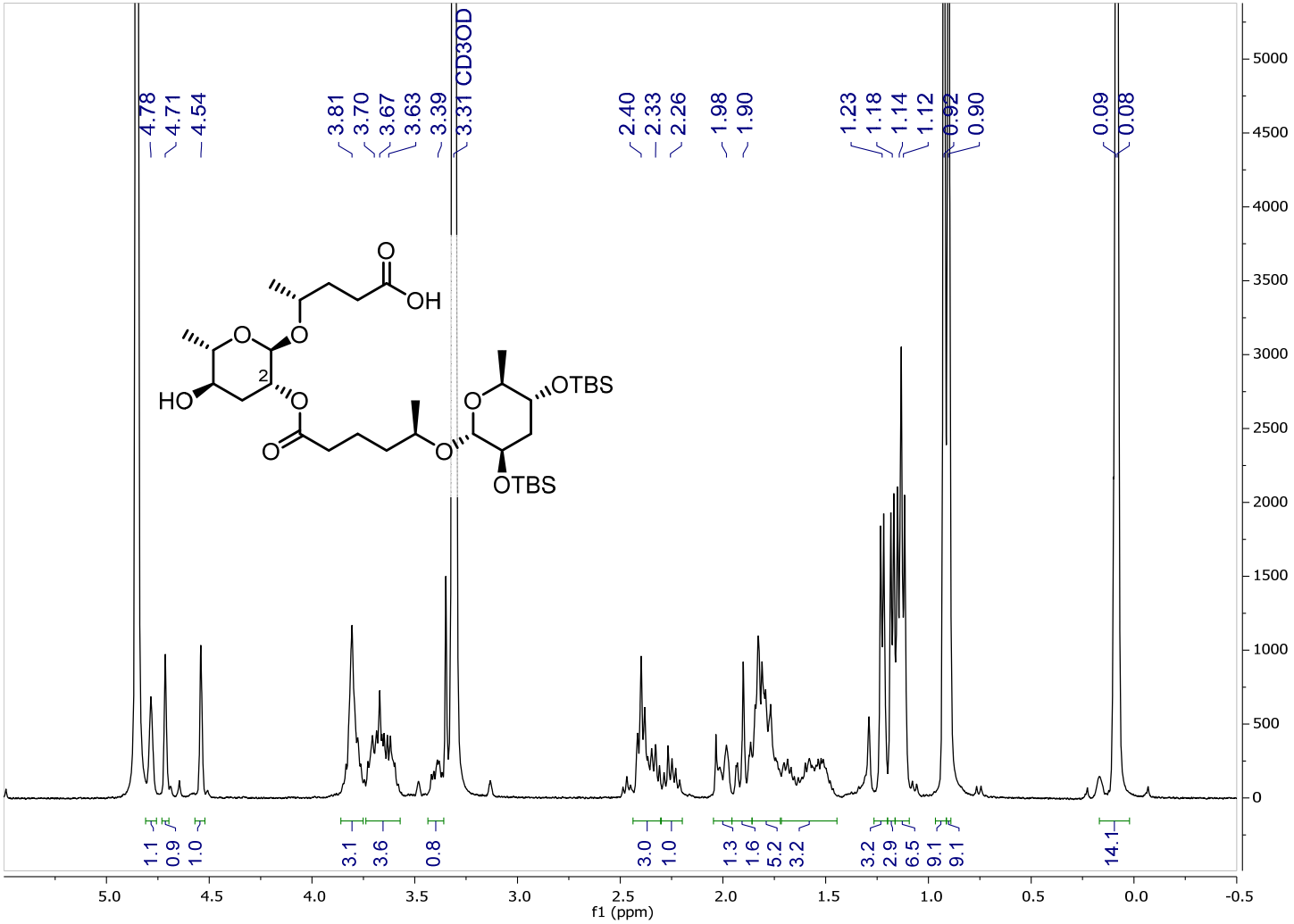


Figure S54: *dqf*-COSY (400 MHz, CD_3OD) of **(**4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid.

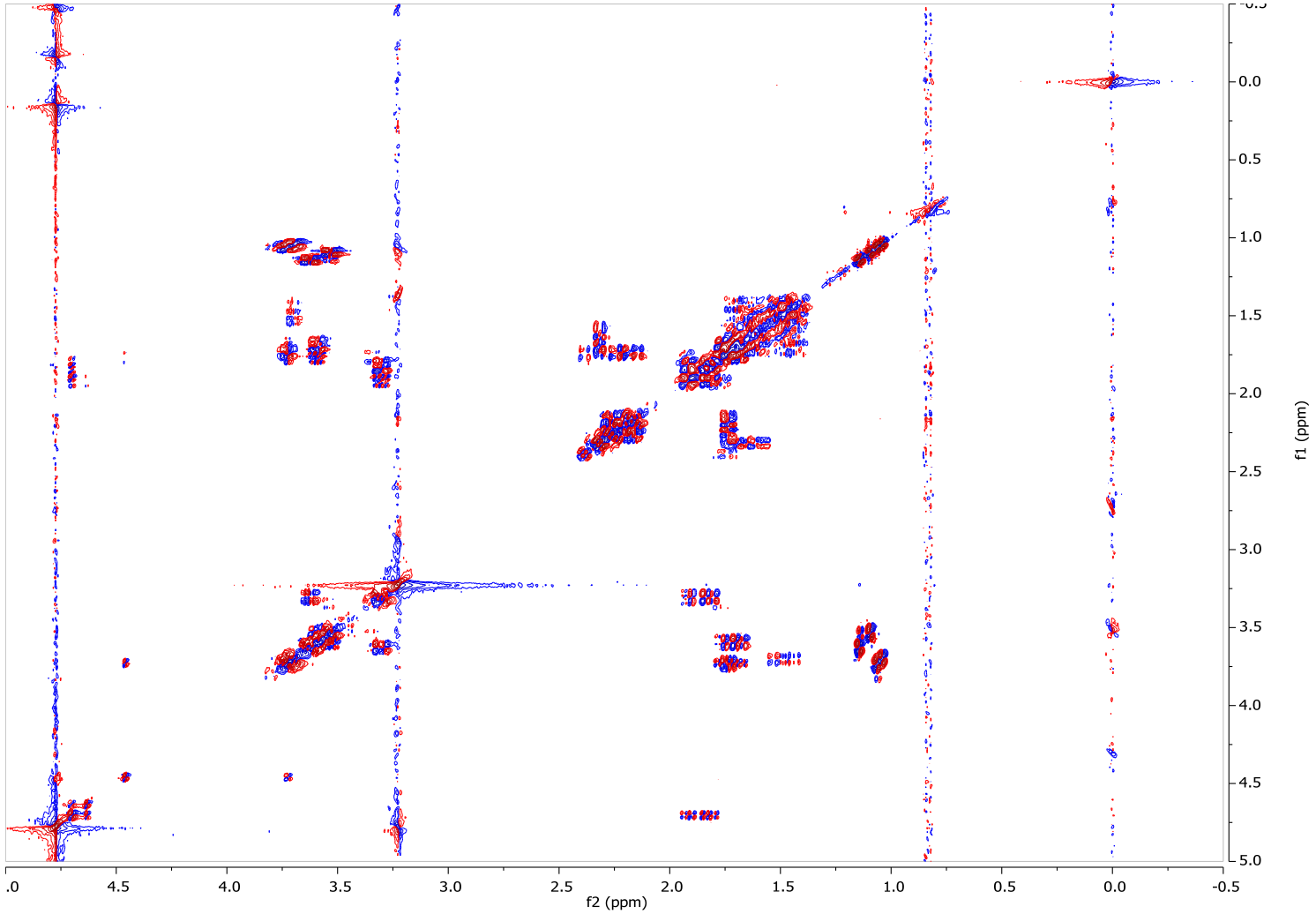


Figure S55: HSQC (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid.

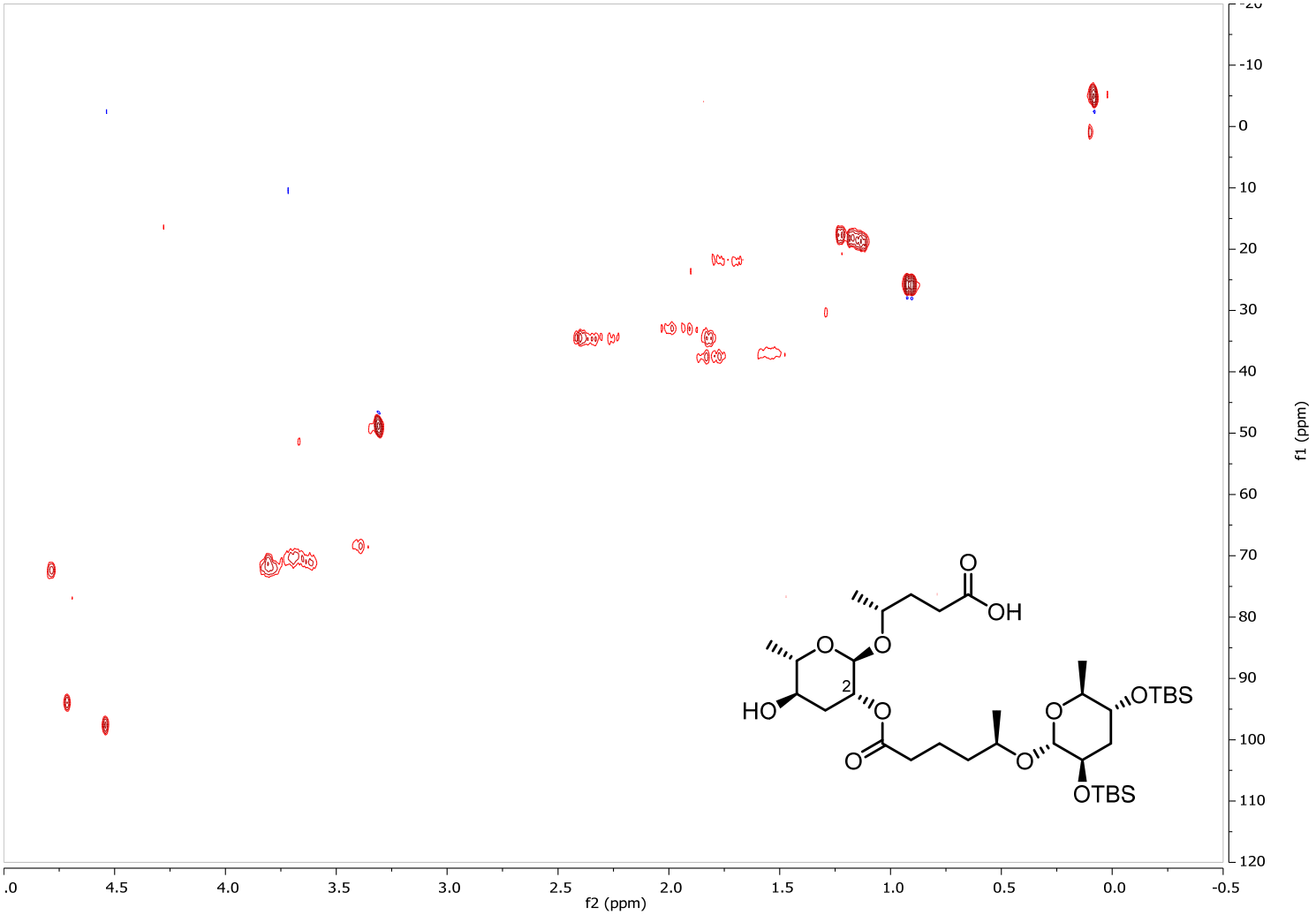


Figure S56: ^{1}H NMR (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid.

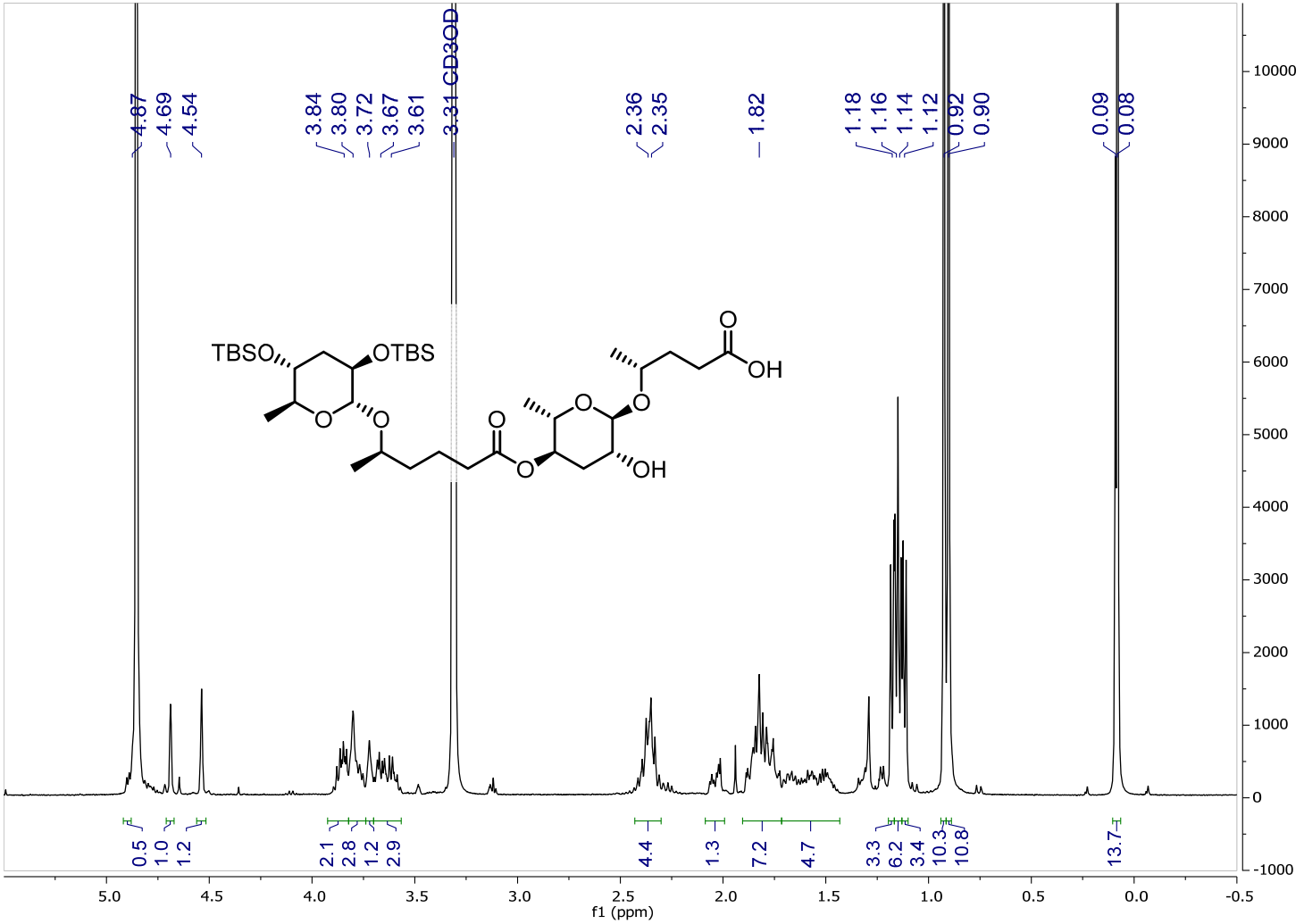


Figure S57: *dqf*-COSY (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid.

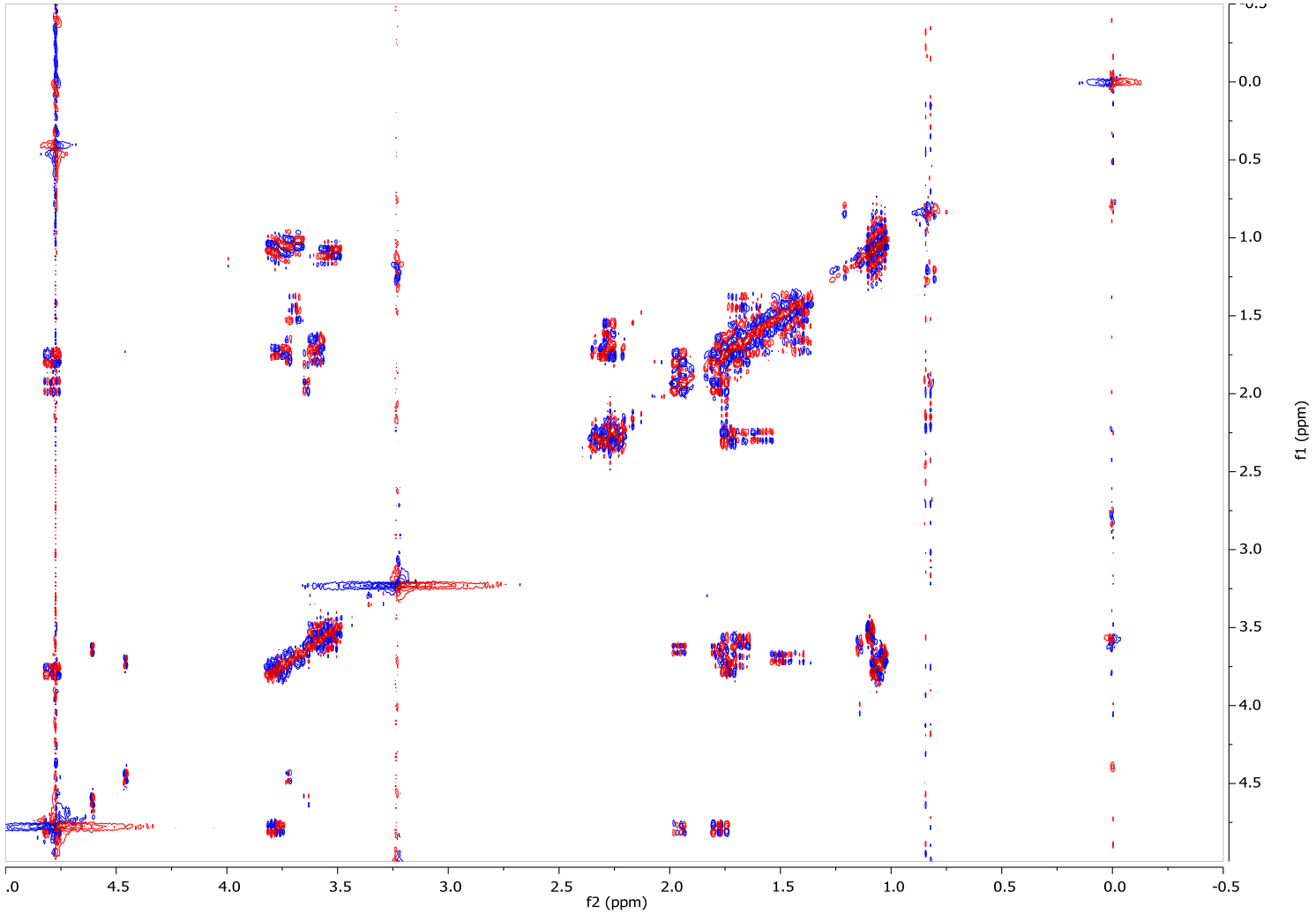


Figure S58: HSQC (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid.

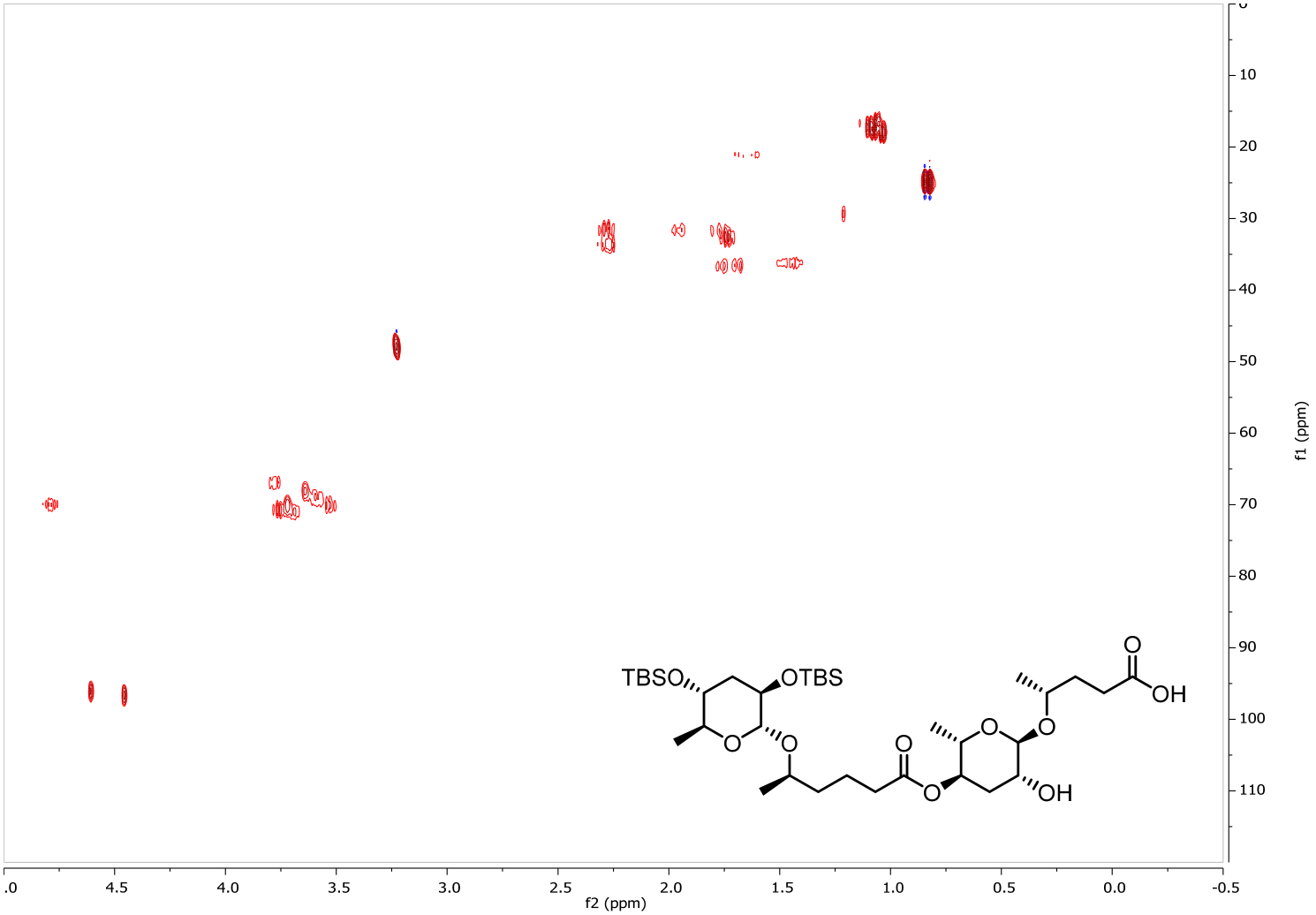


Figure S59: ^{1}H NMR (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid.

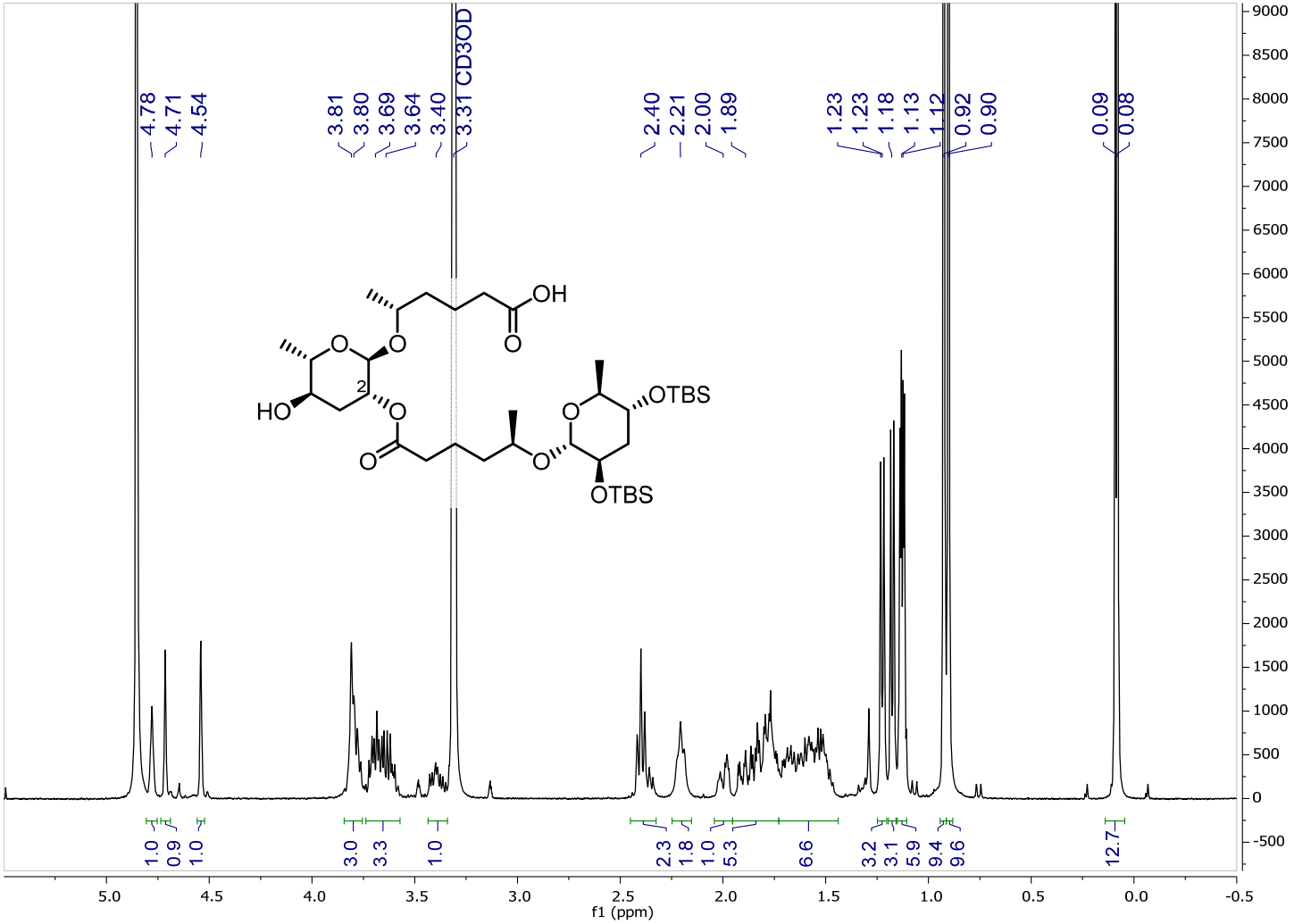


Figure S60: *dqf*-COSY (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid.

Figure S61: HSQC (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid.

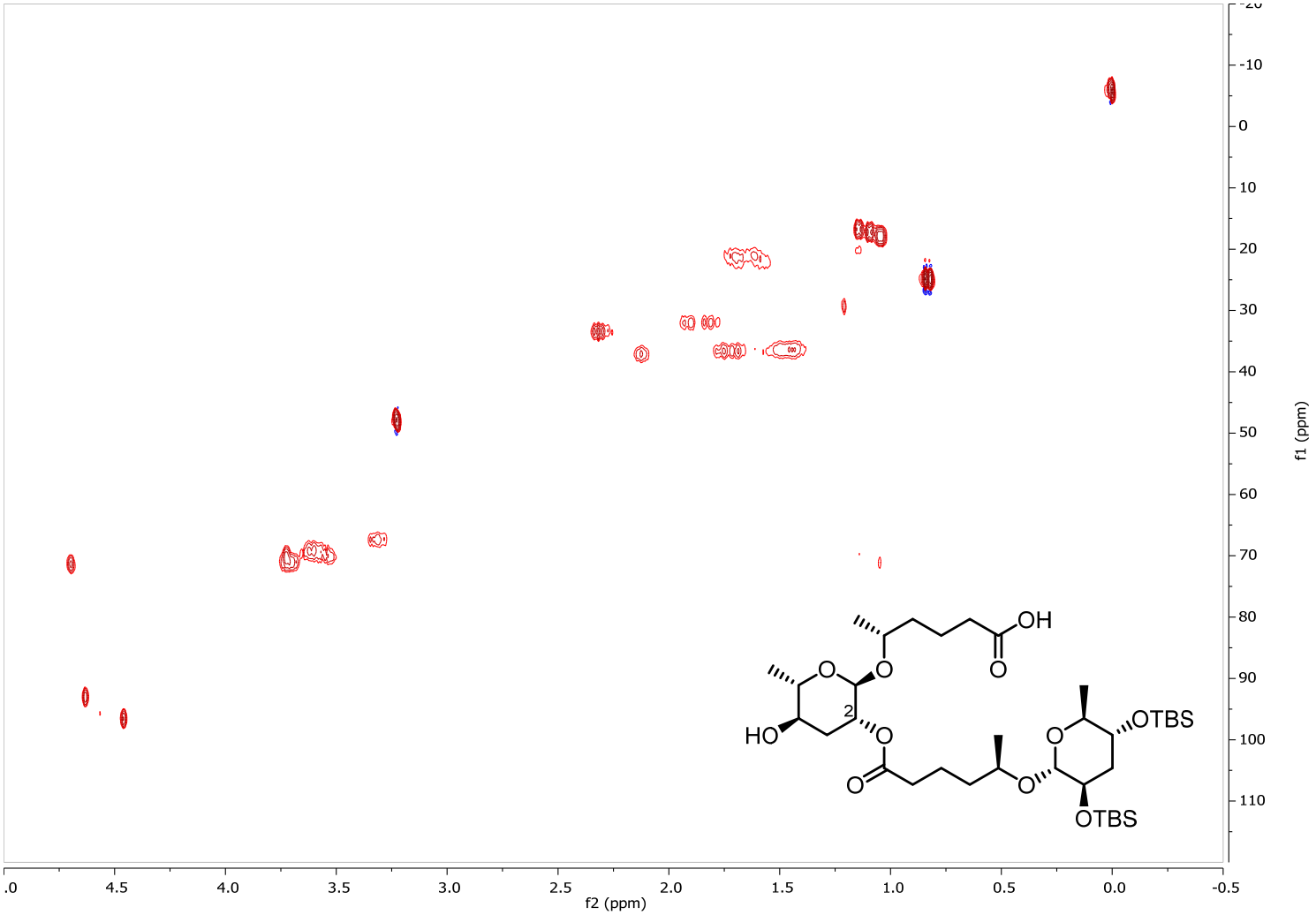


Figure S62: ^{1}H NMR (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic.

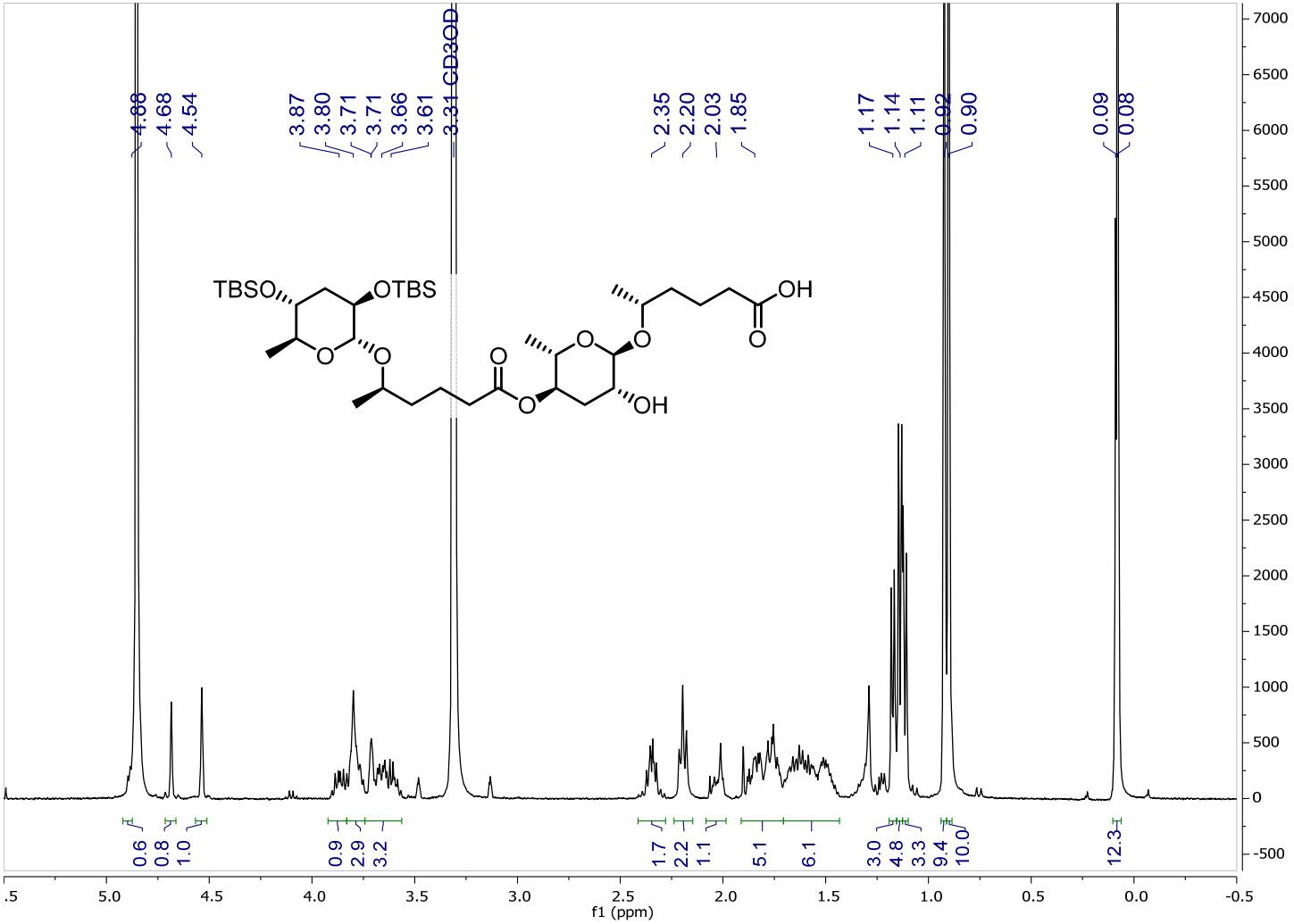


Figure S63: *dqf*-COSY (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic.

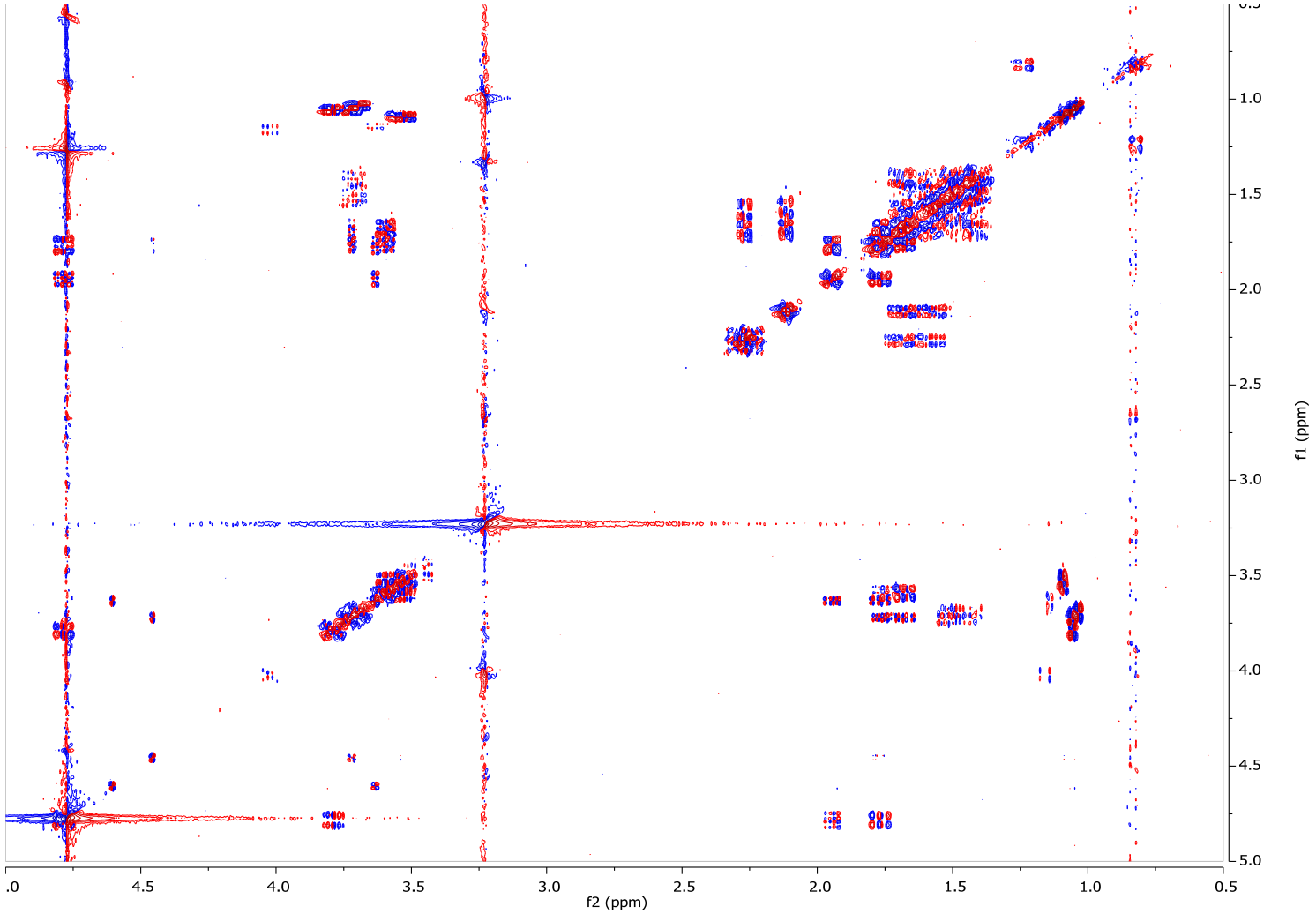


Figure S64: HSQC (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic.

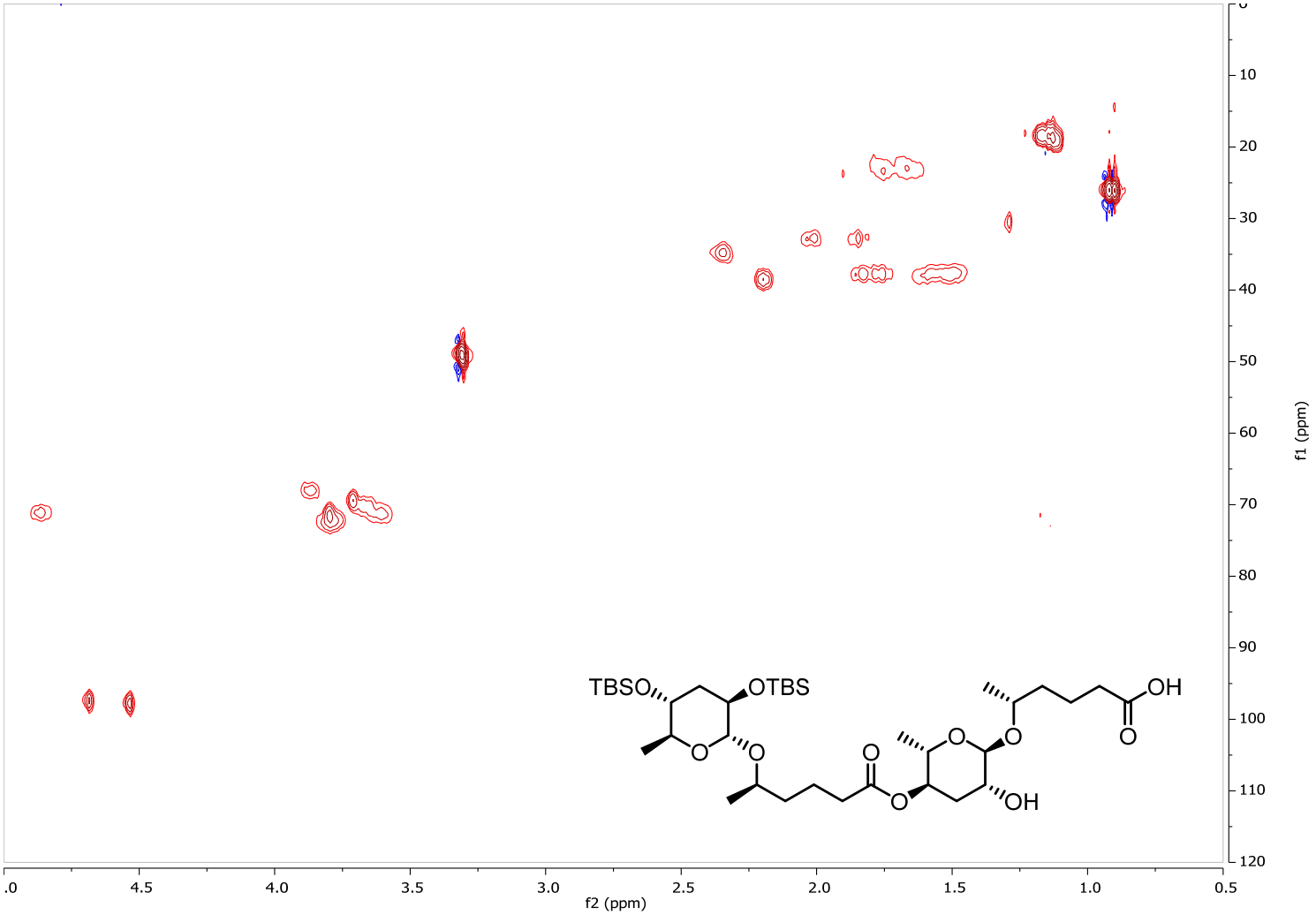


Figure S65: ^{1}H NMR (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (2'-(asc-C6)-asc-C5) (**7**).

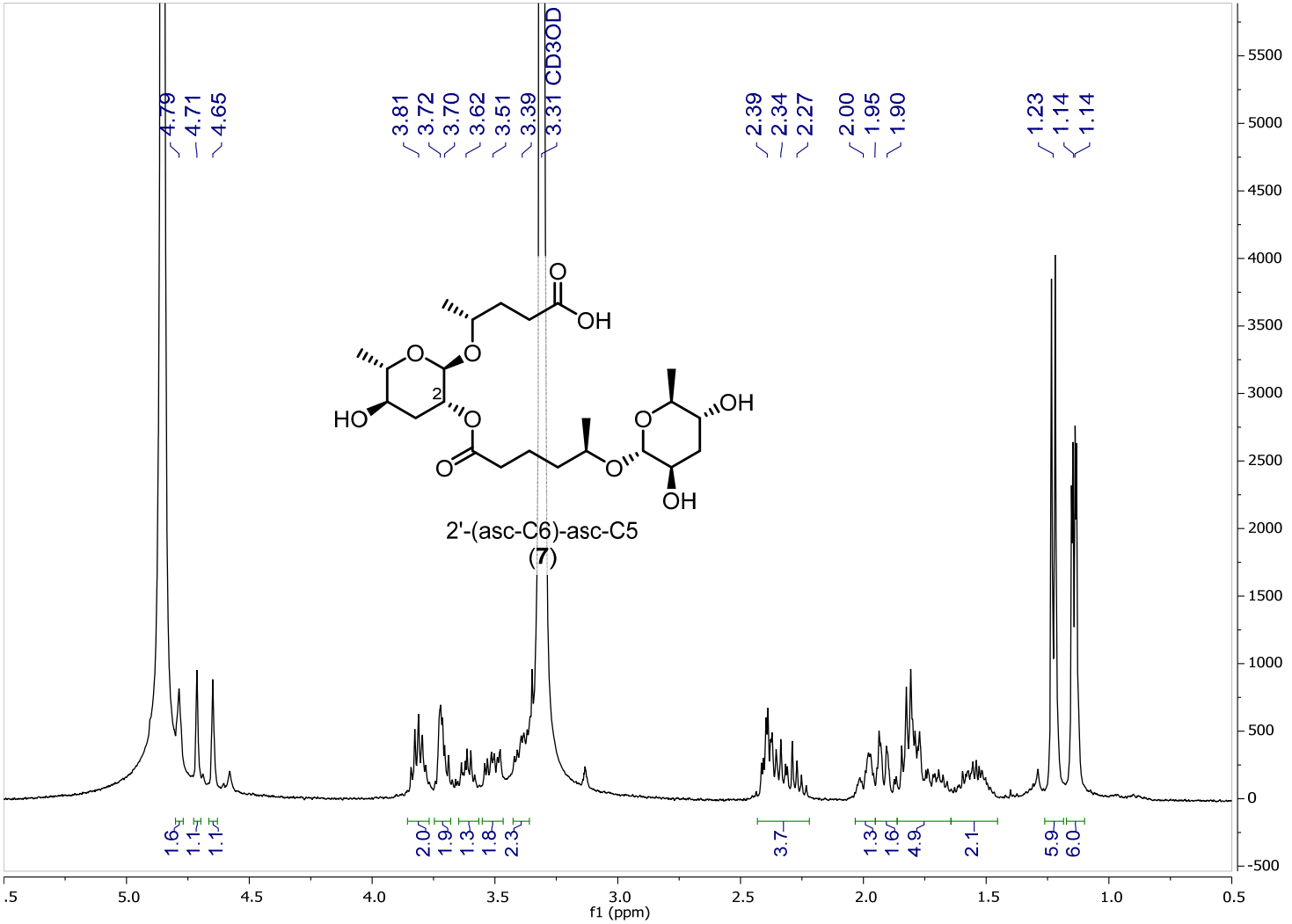


Figure S66: *dqf*-COSY (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid 2'-(asc-C6)-asc-C5) (**7**).

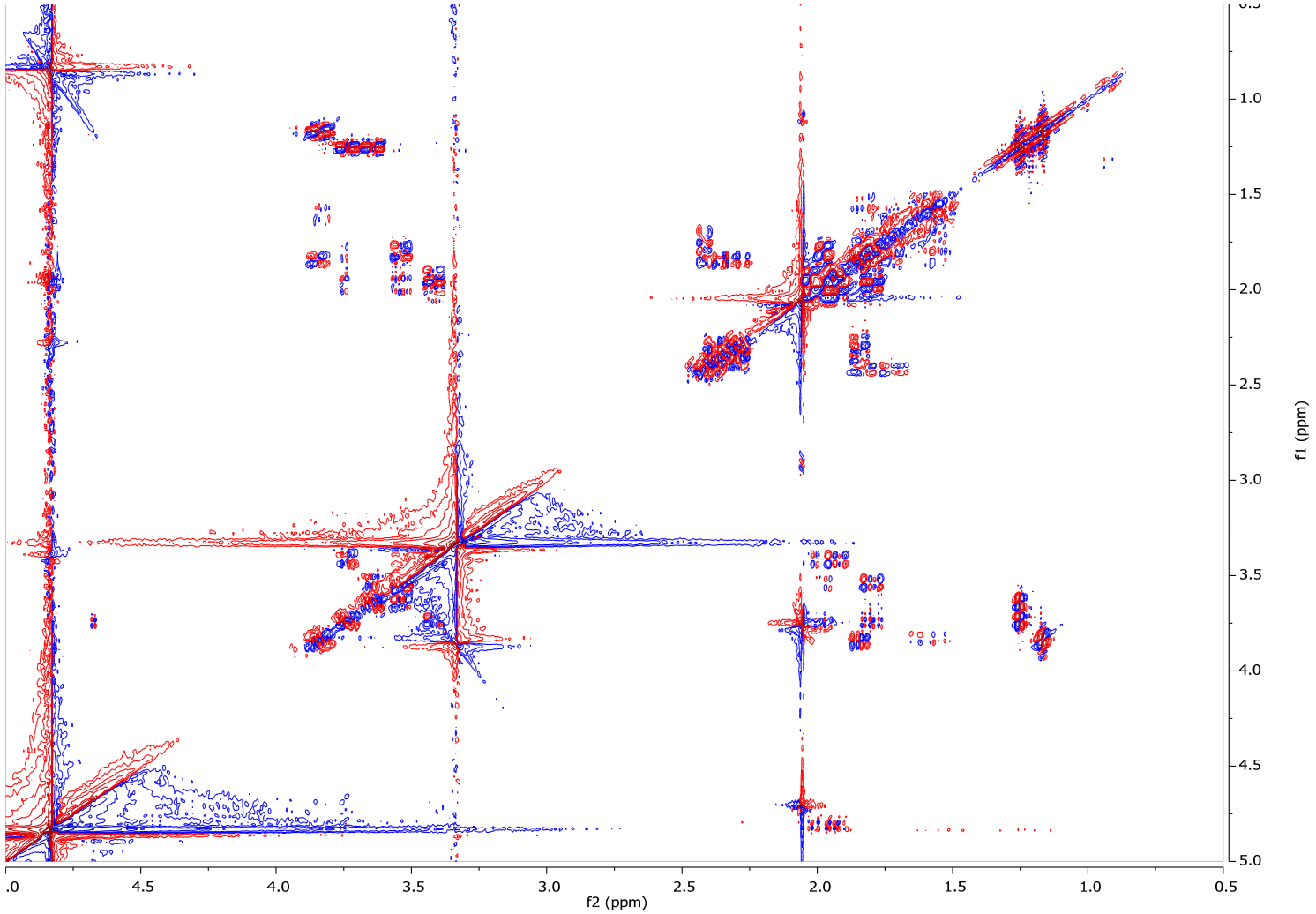


Figure S67: HSQC (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl] -α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (2'-(asc-C6)-asc-C5) (**7**).

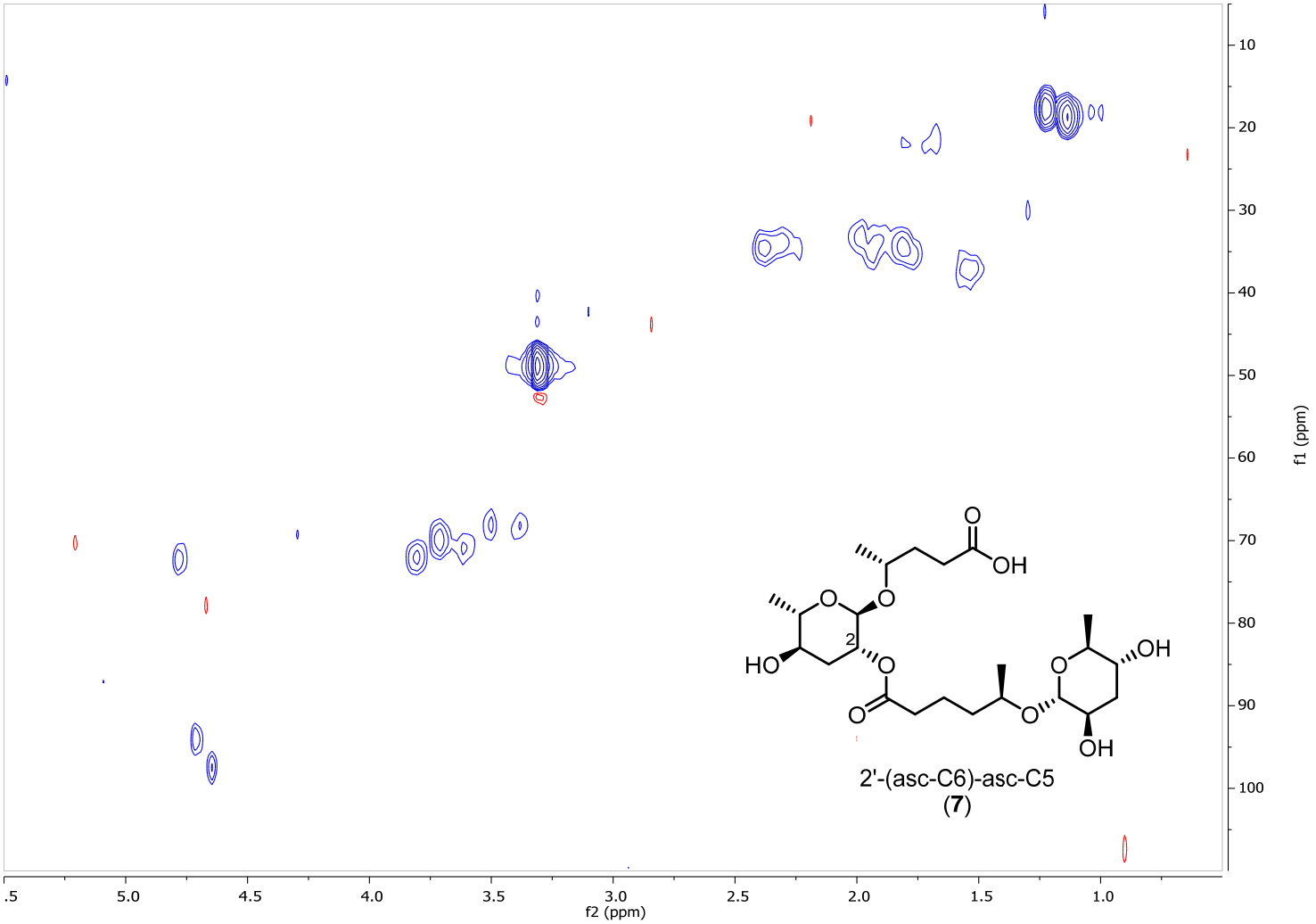


Figure S68: ^{1}H NMR (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (4'-(asc-C6)-asc-C5) (**13a**).

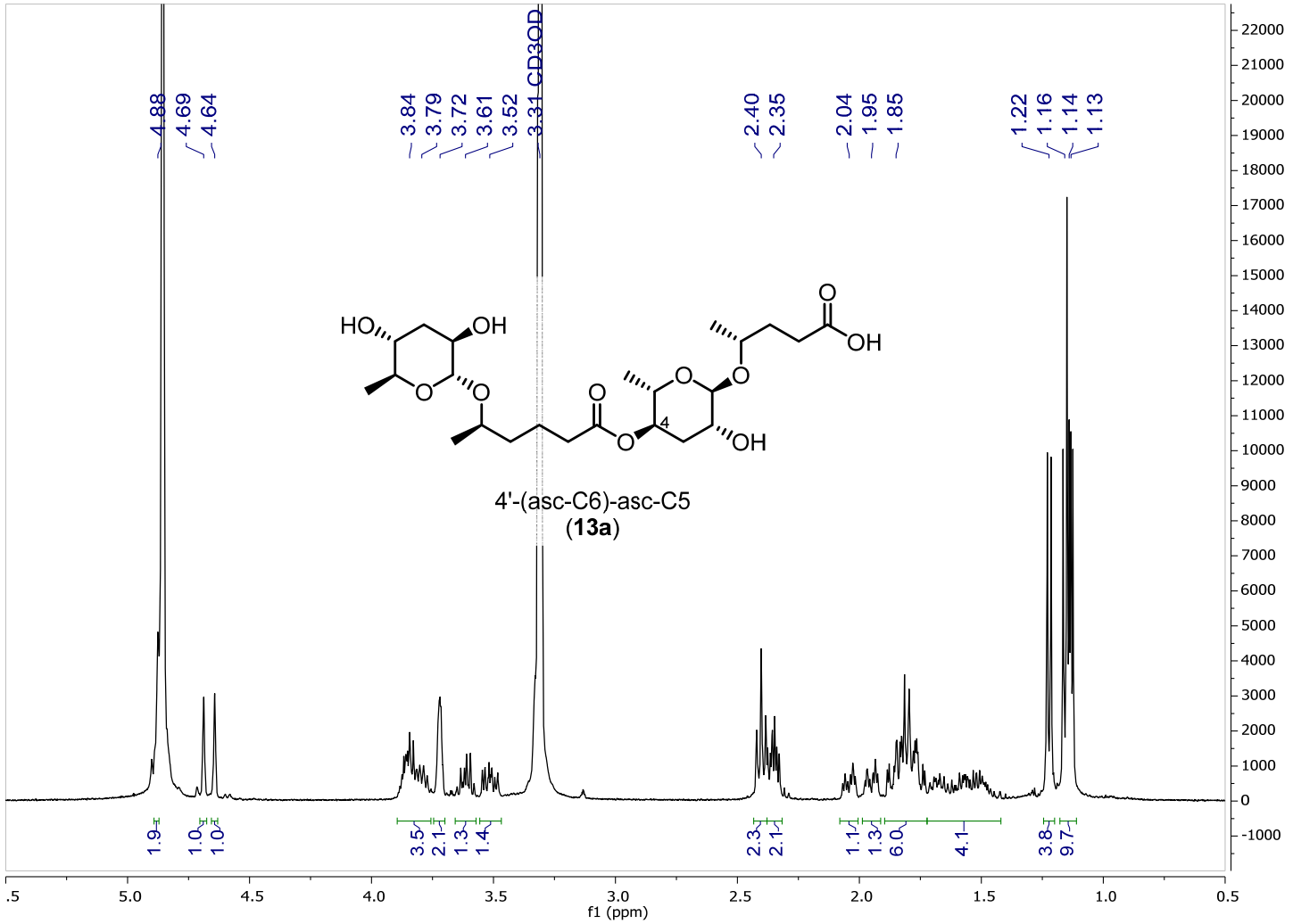


Figure S69: *dqf*-COSY (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (4'-(asc-C6)-asc-C5) (**13a**).

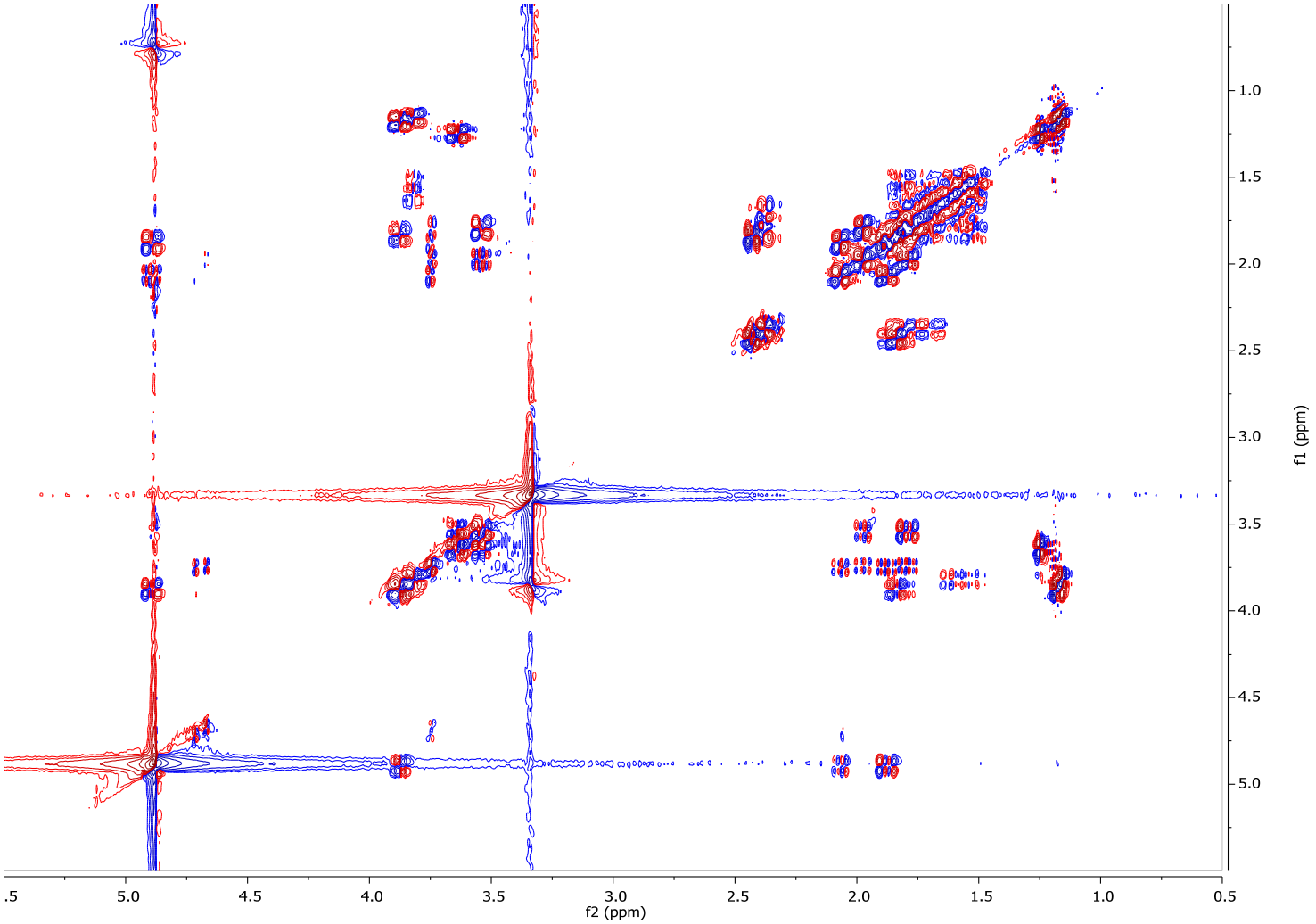


Figure S70: HSQC (400 MHz, CD_3OD) of (4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (4'-(asc-C6)-asc-C5) (**13a**).

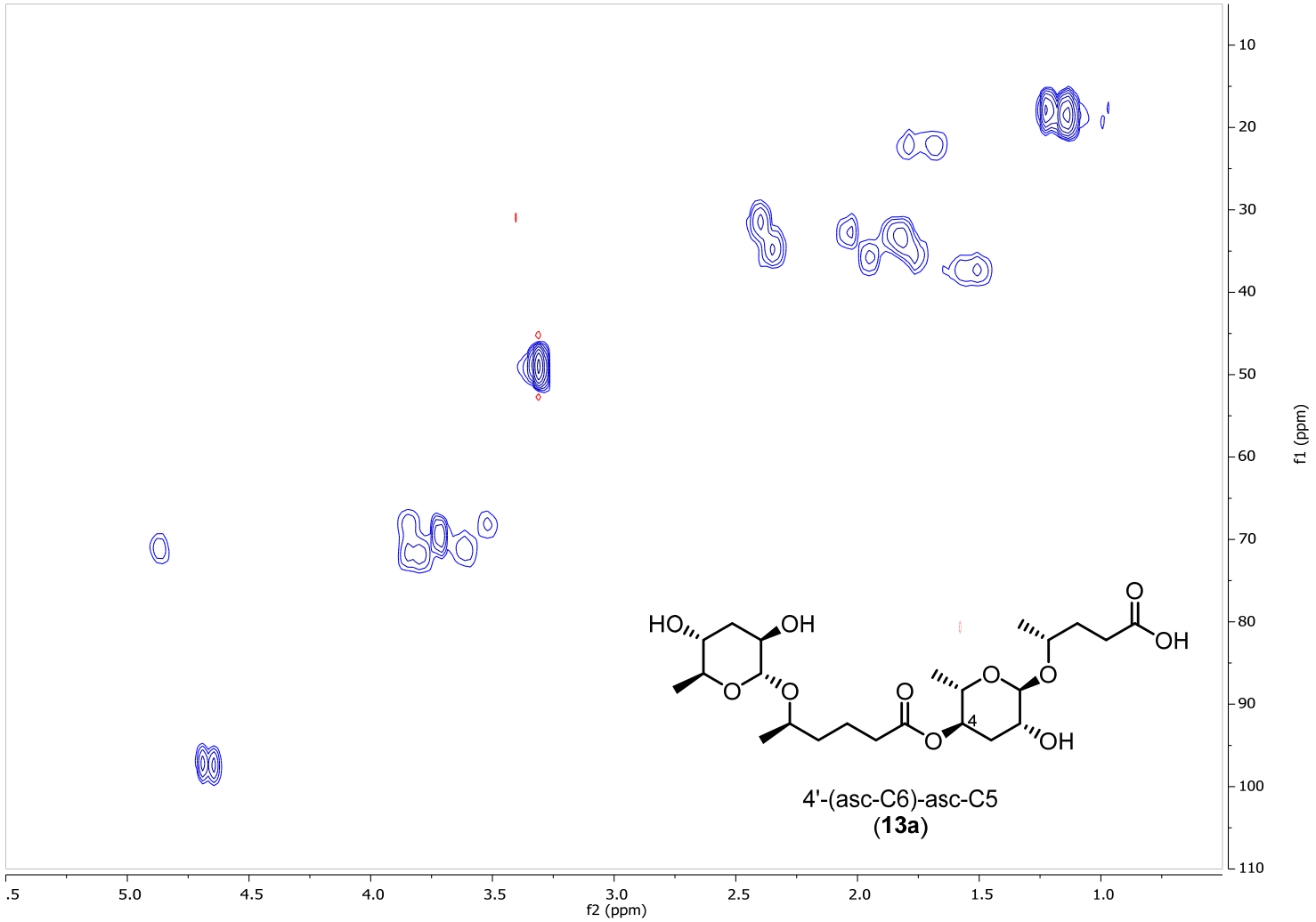


Figure S71: ^{1}H NMR (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (2'-(asc-C6)-asc-C6) (**8**).

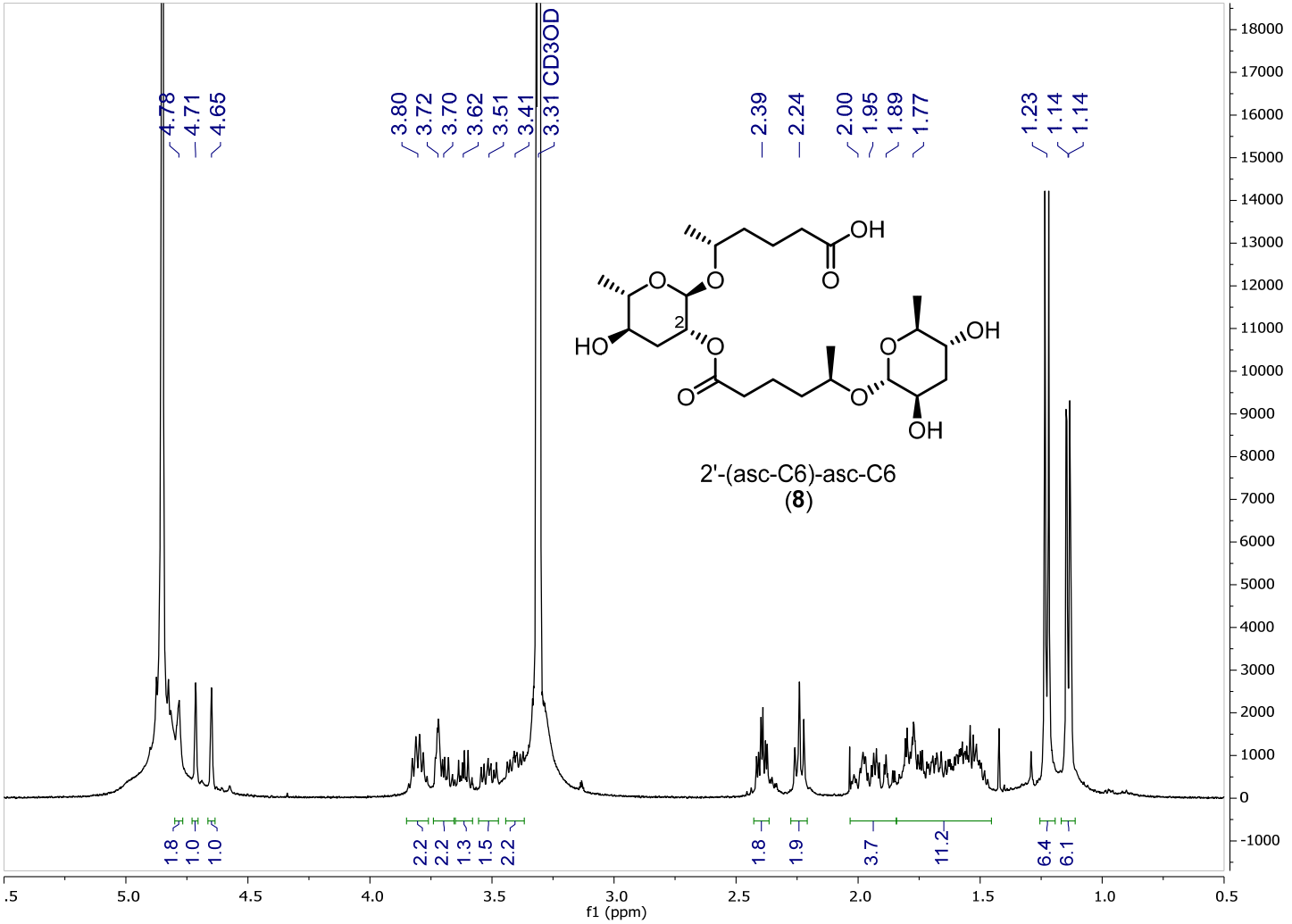


Figure S72: *dqf*-COSY (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (2'-(asc-C6)-asc-C6) (**8**).

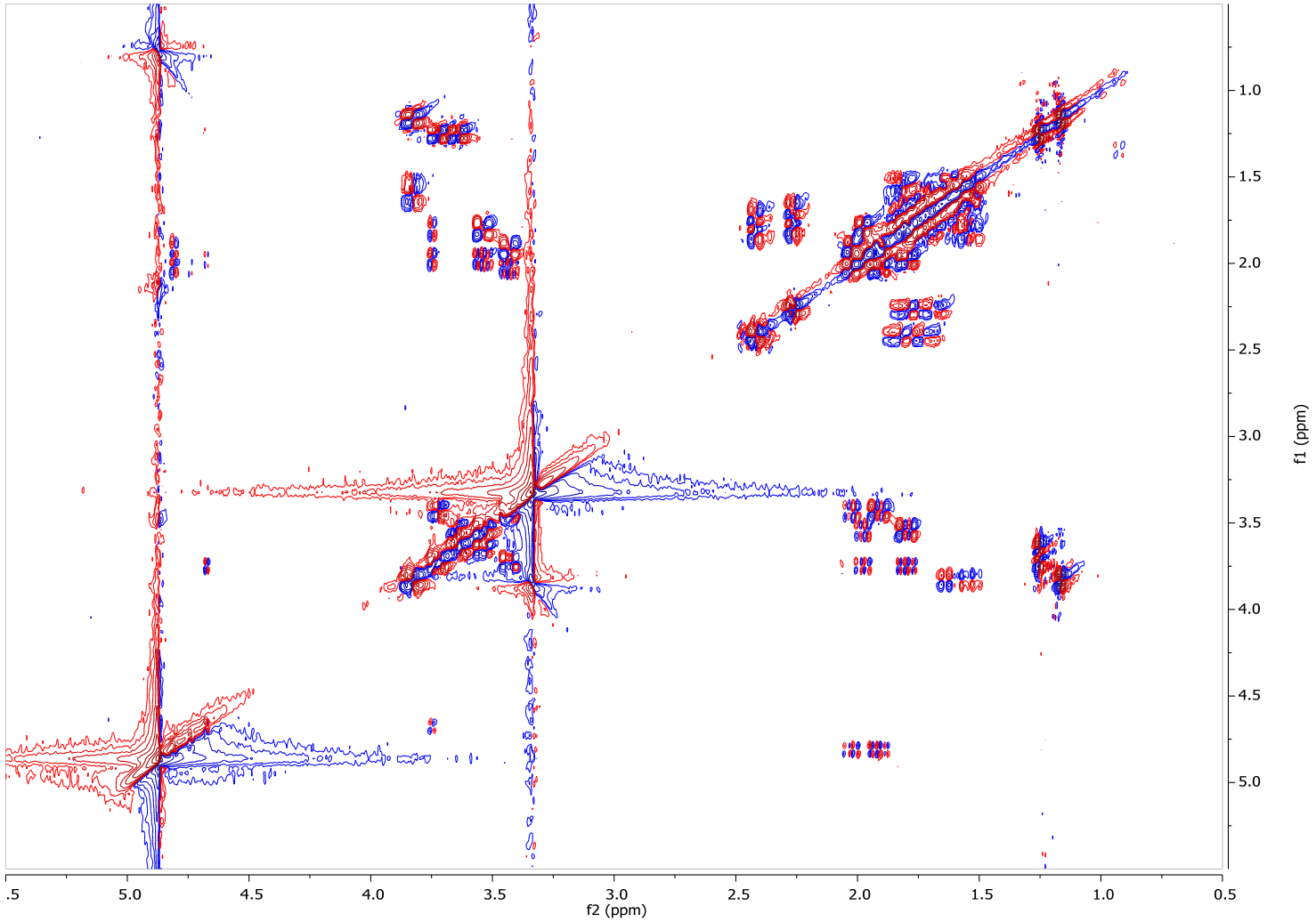


Figure S73: HSQC (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (2'-(asc-C6)-asc-C6) (**8**).

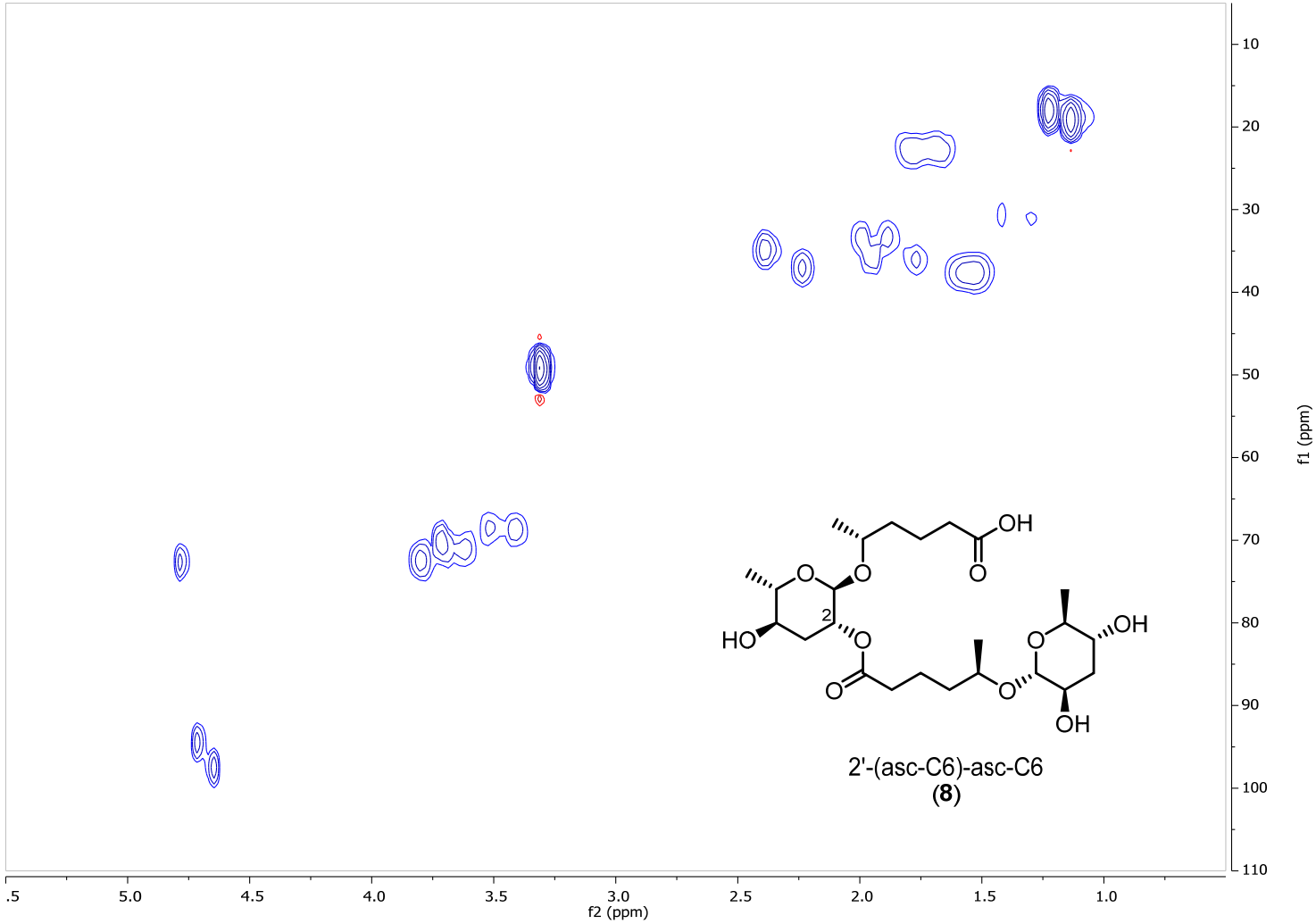


Figure S74: ^{1}H NMR (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (4'-(asc-C6)-asc-C6) (**13b**).

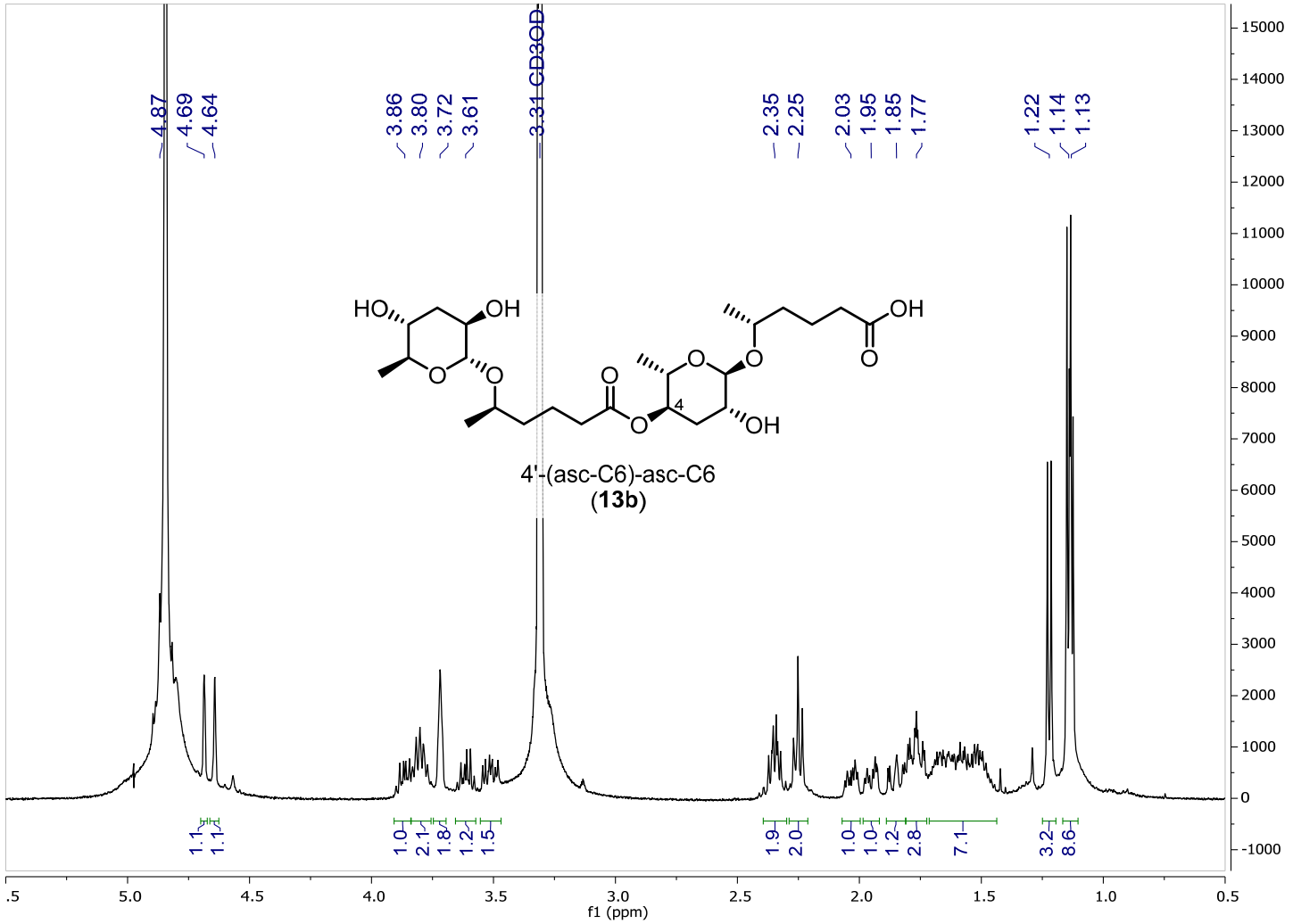


Figure S75: *dqf*-COSY (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (4'-(asc-C6)-asc-C6) (**13b**).

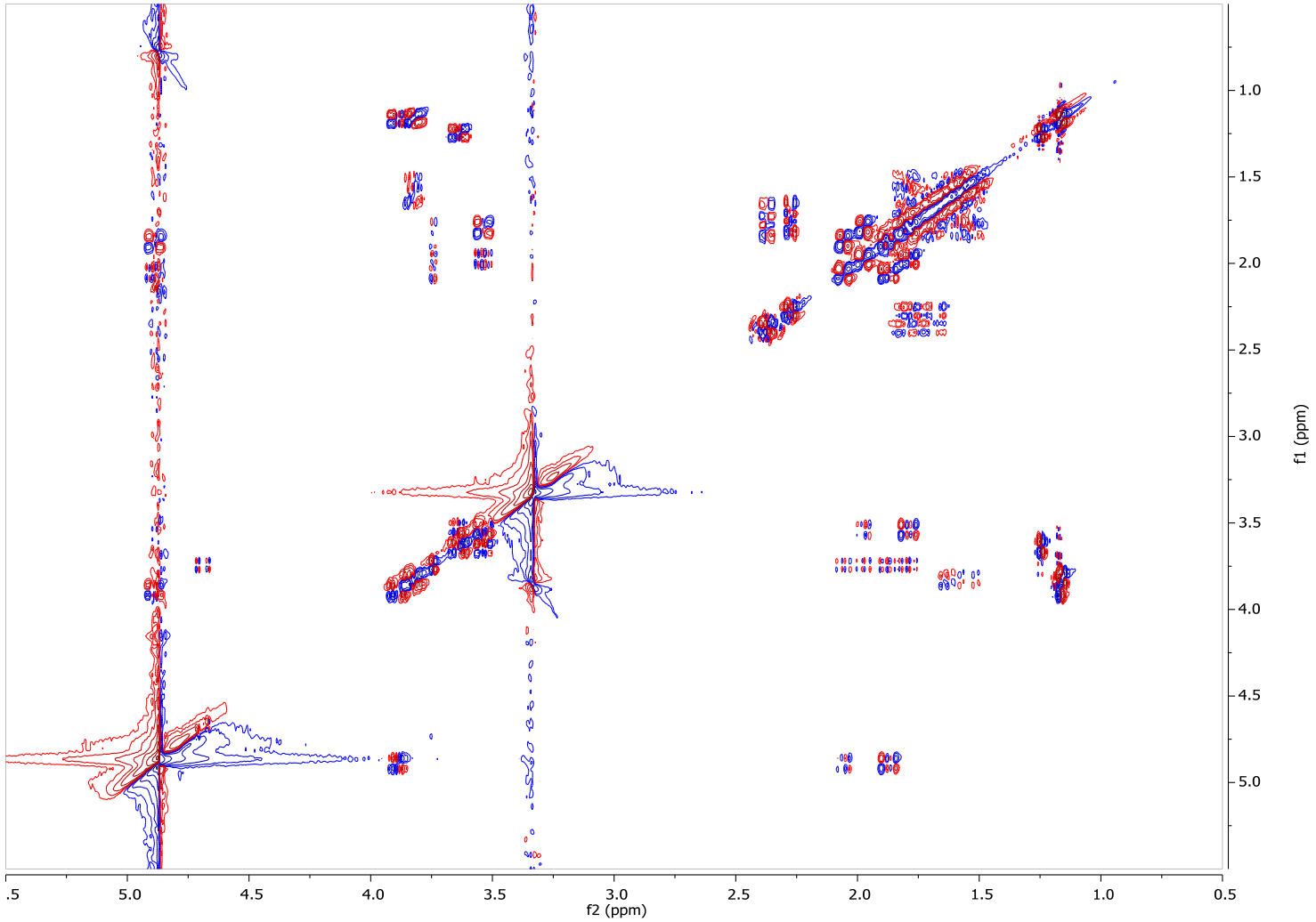
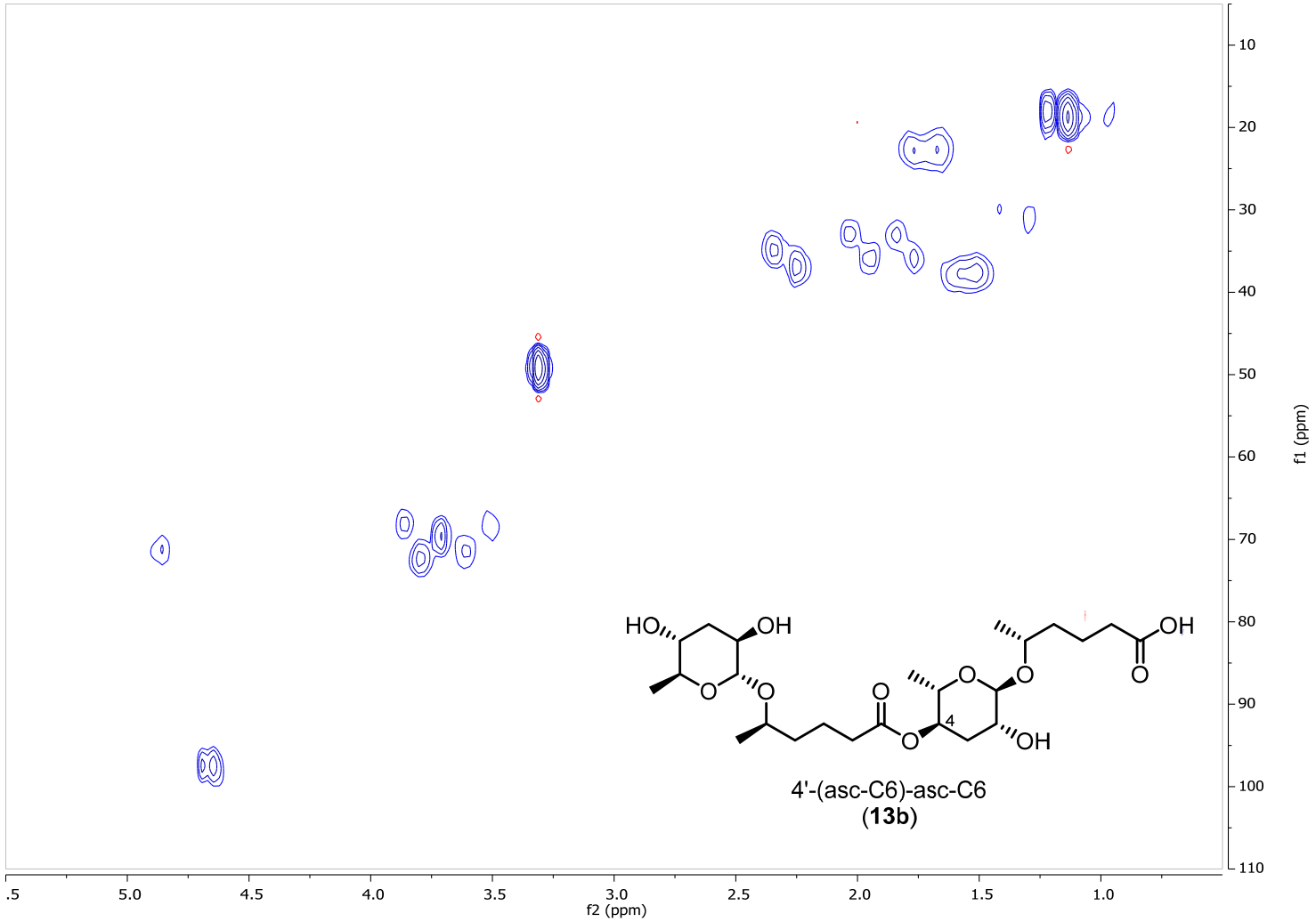


Figure S76: HSQC (400 MHz, CD_3OD) of (5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl] -α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (4'-(asc-C6)-asc-C6) (**13b**).



● **References**

[1] a) Kiontke, K. C.; Félix, M. A.; Ailion, M.; Rockman, M. V.; Braendle, C.; Pénigault J. B.; Fitch, D. H. A. *BMC Evol. Biol.* **2011**, *11*, 339; b) Félix, M. A.; Braendle, C.; Cutter, A. D. *PLoS One* **2014**, *9*, e94723; c) Slos, D.; Sudhaus, W.; Stevens, L.; Bert, W.; Blaxter, M. *BMC Zool.* **2018**, *2*, 4.