

**Bromophycoic acids: Bioactive natural products from
a Fijian red alga *Callophycus* sp.**

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Table 1. Additional bioactivity of bromophycoic acids A-E (1-5).

	Antifungal MIC ($\mu\text{g/mL}$)	Breast Cancer Cell Lines IC ₅₀ (μM)									Colon Cancer Cell Lines IC ₅₀ (μM)			Prostate Cancer Cell Line IC ₅₀ (μM)	Ovarian Cancer Cell Line IC ₅₀ (μM)
		AU565	H3396	HCC1143	HCC70	MDA-MB-468	ZR-75-1	KPL4	MCF-7	T47D	HCT116	SW403	LS174T	LNCaP-FGC	PA-1
bromophycoic acid A (1)	>50	23.1	>50.0	>50.0	>50.0	>50.0	31.9	35.7	>50.0	29.4	49.1	22.0	44.5	10.6	49.1
bromophycoic acid B (2)	25.0	25	>25.0	>25.0	>25.0	17.1	>25.0	23.1	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	21.7
bromophycoic acid C (3)	25.0	17.0	>25.0	>25.0	11.4	12.1	>25.0	>25.0	>25.0	>25.0	6.7	9.7	12.8	6.2	12.4
bromophycoic acid D (4)	>50	5.6	4.7	7.0	5.1	7.1	14.5	9.2	5.4	21.6	5.3	17.5	4.1	5.4	2.0
bromophycoic acid E (5)	>50	7.0	5.0	24.6	5.3	>25.0	23.7	7.3	>25	21.9	19.0	>25.0	22.1	4.9	14.5

Table 2. Red alga of the class Florideophyceae for phylogenetic analysis and corresponding GenBank accession numbers for the small subunit ribosomal RNA (18S rRNA) and cytochrome *c*.

Subclass	Order	Species	GenBank Accessions	
			18S rRNA	COI
Rhodomeniophycidae	Ceramiales	<i>Laurencia majuscula</i>	GU223784	HQ422617
		<i>Leveillea jungermannioides</i>	GU223793	HQ423107
		<i>Polysiphonia fucoides</i>	AF427530	HM573496
	Gelidiales	<i>Gelidium pusillum</i>	U32564	HQ412446
		<i>Pterocladia caerulescens</i>	EF191193	HQ412475
		Gigartinales	<i>Callophycus densus</i>	FJ660609
	<i>Callophycus serratus</i>		FJ660613	JX135006
	<i>Callophycus oppositifolius</i>		AY437654	
	<i>Dumontia contorta</i>			AY971161
	<i>Gibsmithia dotyi</i>		AF317108	HQ422673
	<i>Hennedya crispa</i>		AY437652	
	<i>Hypnea flexicaulis</i>		EU240850	FN823052
	Gracilariales	<i>Portieria hornemanii</i>	AF317118	HQ422692
		<i>Gracilaria vermiculophylla</i>	GQ292853	EF434926
	Rhodymeniales	<i>Champia parvula</i>	EF190546	HQ422763
		<i>Coelothrix irregularis</i>	EU086456	HQ422592
<i>Rhodomenia leptophylla</i>		U09621	HM033088	
Corallinophycidae	Corallinales	<i>Corallina elongata</i>	EU095607	FM180065
		<i>Haliptilon squamatum</i>	EU095617	FM180090
		<i>Jania rubens</i>	EU095621	FM180064
Hildenbrandiophycidae	Hildenbrandiales	<i>Hildenbrandia rubra</i>	AF076995	HQ422942

Table 3. Results of similarity searches of G-0807 small subunit ribosomal RNA (18S rRNA) and cytochrome *c* oxidase subunit 1 (COI) from BLAST search in the GenBank nucleotide database.

	Similar species	GenBank accession	E-value	Max identity
18S rRNA	<i>Callophycus oppositifolius</i>	AY437654	0	99%
	<i>Hennedyia crispa</i>	AY437652	0	99%
	<i>Mychodeophyllum papillitectum</i>	AY437687	0	98%
	<i>Areschougia congesta</i>	U09613	0	98%
	<i>Calviclonium ovatum</i>	AY437650	0	98%
	<i>Acrotylus australis</i>	AY437647	0	98%
	<i>Tichocarpus crinitus</i>	AY437698	0	98%
	<i>Schmitziella endophloea</i>	AY437697	0	98%
	<i>Erythroclonium angustatum</i>	AY437655	0	98%
	<i>Mychodea marginifera</i>	AY437685	0	98%
	<i>Rhabdonia verticillata</i>	AY437656	0	98%
	<i>Mychodea pusilla</i>	AY437686	0	98%
	<i>Mychodea acanthymenia</i>	EF033589	0	98%
	<i>Austroclonium charoides</i>	AY437666	0	98%
	<i>Furcellaria lumbricalis</i>	Z14141	0	98%
	<i>Mychodea carnosa</i>	U33135	0	98%
	<i>Nizymenia australis</i>	U09616	0	98%
	<i>Dicranema revolutum</i>	AY437678	0	98%
	<i>Phacelocarpus peperocarpus</i>	U09617	0	97%
	<i>Caulacanthus okamurae</i>	AY437663	0	97%
COI	<i>Dumontia contorta</i>	AY971161	5e-164	88%
	<i>Predaea sp.</i>	HQ422703	5e-164	88%
	<i>Kallymeniaceae sp.</i>	JQ255028	2e-162	88%
	<i>Euthora timburtonii</i>	GU140181	2e-162	88%
	<i>Acrosymphyton taylorii</i>	HQ422907	1e-160	88%
	<i>Pugetia cryptica</i>	JF903305	2e-152	87%

Table 4. COSY correlations for bromophycoic acids A-E (1-5). For diastereotopic protons with dissimilar chemical shifts, the proton whose chemical shift is listed first in Table 1 of the main article is termed “a” and the other is “b”. “NA” (not applicable) indicates that no proton signal exists for that position.

¹ H at Position # COSY correlations observed between protons listed on far left and those below:					
	1	2	3	4	5
3	-	5a, 5b	5a, 5b		5a, 5b
5a	5b, 6	3, 5b, 6	3, 5b, 6	5b, 6	3, 5b, 6
5b	5a, 6	3, 5a, 6	3, 5a, 6	5a, 6	3, 5a, 6
6	5a, 5b	5a, 5b	5a, 5b	5a, 5b	5a, 5b, 21a, 21b
8a	8b	8b	8b, 22b	8b	NA
8b	8a	8a, 22b	8a, 22b	8a	NA
9a	NA	NA	NA	NA	9b, 10a, 10b
9b	NA	NA	NA	NA	9a, 10a, 10b
10a	11a, 11b	10b, 11a, 11b	10b, 11a, 11b,	-	9a, 9b, 10a, 11
10b	NA	10a, 11a, 11b	10a, 11a, 11b	-	9a, 9b, 10a, 11
11a	10, 11b, 12	10a, 10b, 11b, 12	10a, 10b, 11b, 12	11b, 12	10a, 10b
11b	10, 11a, 12	10a, 10b, 11a, 12	10a, 10b, 11a, 12	11a, 12	NA
12	11a, 11b	11a, 11b	11a, 11b	11a, 11b	NA
13a	NA	NA	NA	NA	13b, 14a, 14b
13b	NA	NA	NA	NA	13a, 14a, 14b
14a	15a, 15b	15, 16	15, 16	15a, 15b	13a, 13b, 14b, 15
14b	-	-	-	-	13a, 13b, 14a, 15
15a	14, 15b, 16	14, 16	14, 16	14, 15b	14a, 14b, 17, 27
15b	14, 15a, 16	NA	NA	14, 15a	NA
16	15a, 15b	14, 15a	14, 15	-	NA
17	NA	NA	NA	NA	15
18a	-	-	-	27	19
18b	NA	NA	NA	27	NA
19	20	20	20	20	18
20	19	19	19	19	NA
21a	NA	NA	NA	NA	6, 21b, 22
21b	NA	NA	NA	NA	6, 21a
22a	22b	22b	22b	22b, 23a, 23b	21a
22b	22a, 23	8b, 22a, 23a, 23b	8a, 8b, 22a, 23b	22a, 23a, 23b	-
23a	22b	22b, 23b, 24	23b	22a, 22b	-
23b	NA	22b, 23a	22b, 23a	22b	NA
24	-	-	-	-	-
25	-	-	-	-	-
26	-	-	-	-	-
27	-	-	-	18a, 18b	15

Table 5. HMBC correlations for bromophycoic acids A-E (1-5). For diastereotopic protons with dissimilar chemical shifts, the proton whose chemical shift is listed first in Table 1 of the main article is termed “a” and the other is “b”. “NA” (not applicable) indicates that no proton signal exists for that position.

¹ H at Position # HMBC Correlations observed between protons listed on far left and carbons at positions listed below:					
	1	2	3	4	5
3	1, 5, 19, 21	1, 5, 19, 21	1, 5, 19, 21	1, 5, 19, 21	1, 5, 18, 20
5a	3, 4, 7, 21	4, 7, 21	4, 7, 21	4, 7	4, 6, 20, 21
5b	3, 4, 6, 7, 21	4, 6, 7, 21	4, 6, 7, 21	3, 4, 6, 7	3, 4, 6, 7, 20
6	5, 7, 21	5, 7	5, 7	7	5, 7, 24
8a	-	6, 7, 9, 10	6, 7, 25	6, 7, 9, 25	N/A
8b	6, 7, 9, 22, 24, 25	6, 7, 9, 22, 24	7, 9, 22, 24, 25	7	N/A
9a	NA	NA	NA	NA	8, 11
9b	NA	NA	NA	NA	8, 11, 25
10a	-	-	-	-	12
10b	-	12	12, 25	-	11, 12
11a	9, 10, 12	9	9	-	9, 10, 12, 13, 26
11b	10, 12	-	-	-	N/A
12	11, 13, 26	11, 13, 26	11, 26	26	N/A
13a	NA	NA	NA	NA	11, 12
13b	NA	NA	NA	NA	11, 12, 14
14	9, 12, 13, 16, 23, 24	12, 13, 15, 16, 24, 26	12, 13, 15, 16, 24, 26	-	-
14b	-	-	-	-	15
15a	16, 17	14, 16, 17	14, 16, 17	16	17, 27
15b	16, 17	NA	NA	16	N/A
16	14, 15, 18, 27	14, 15, 17, 18, 27	14, 15, 17, 18, 27	NA	N/A
17	NA	NA	NA	NA	15, 16, 27
18a	14, 16, 17, 27	16, 17, 27	16, 17, 27	16, 27	1, 3, 20
18b	NA	-	-	16, 17, 27	NA
19	1, 3, 21	1, 3, 21	1, 3, 21	1, 3, 21	1, 2, 4, 20
20	2, 3, 4, 21	2, 4, 21	2, 4, 21	2, 4	NA
21a	NA	NA	NA	NA	-
21b	NA	NA	NA	NA	6
22a	-	-	24	-	-
22b	7, 8, 23, 24	-	-	-	-
23a	-	-	-	-	12, 13
23b	-	-	-	22, 24	NA
24	-	-	9, 10, 13, 26	13, 9, 22, 26	6, 7, 8
25	8, 9, 10, 24	8, 9, 10, 24	6, 8, 9, 10, 24	8, 9, 10, 24	7, 8, 9, 23
26	12, 13, 14, 24	12, 13, 14, 24	12, 13, 14, 15, 24	12, 13, 14, 24	1, 12, 13, 23
27	14, 16, 17, 18	16, 17, 18	16, 17, 18	16, 17, 18	15, 16, 17

Table 6. Observed NOEs from NOESY and ROESY NMR experiments, for bromophycoic acids A-E (**1-5**). For diastereotopic protons with dissimilar chemical shifts, the proton whose chemical shift is listed first in Tables 1 of the main article is termed “a” and the other is “b”. Only NOEs important to determinations of stereochemistry are listed.

¹ H at Position #	NOE observed between protons listed on far left and protons at positions listed below:				
	1	2	3	4	5
3	5b	5a, 5b, 6		5a, 5b	5a, 5b
5a	6, 8b, 25	3, 6, 8b, 25	6	3, 6, 25	3, 24
5b	3, 6, 8b, 22b, 25	3, 8b	8b	3, 6, 8b	3, 6, 21b
6	5a, 5b, 22b, 23, 25	3, 5a, 22b, 23a, 25	5a, 22b, 23a, 25	5a, 5b, 22b, 23a, 25	5b, 21b, 25
8a					
8b	5a, 5b, 25	5a, 5b	5b	5b	
9a					24
9b					
10a	12, 24		12		9b, 11
10b		11a, 11b			25, 26
11a	12, 14	10b, 12, 24	12	12	10a, 23, 24
11b	25, 26	10b, 25, 26	25	25, 26	
12	10a, 11a, 14, 24	11a, 24	10a, 11a, 24	11a	
13a					
13b					
14a	11a, 12				
14b					
15a					
15b					
16					
17					
18a					
18b					
19					
20					
21a					
21b					5b, 6
22a					
22b	5b, 6, 23	6	6	6, 23a	
23a	6, 22b, 25, 26	6, 25, 26	6	6, 22b	11, 24
23b					
24	10a, 12	11a, 12	12		5a, 9a, 11, 23
25	5a, 5b, 6, 8b, 11b, 23, 26	5a, 6, 11b, 23a, 26	6, 11b, 26	5a, 6, 11b	6, 10b
26	11b, 23, 25	11b, 23a, 25	25	11b	10b
27					

Table 7. HSQC-TOCSY correlations for bromophycoic acid E (**5**). For diastereotopic protons with dissimilar chemical shifts, the proton whose chemical shift is listed first in Table 1 of the main article is termed “a” and the other is “b”.

¹ H at Position #	HSQC-TOCSY observed between protons listed on far left and carbons at positions listed below:
3	3, 5, 7, 19
5a	5, 6
5b	5, 6
6	5, 6
8a	
8b	
9a	9, 10, 11
9b	9, 10, 11
10a	9, 10, 11
10b	9, 10, 11
11a	9, 10, 11
11b	
12	
13a	13, 14
13b	13, 14
14	13, 14, 15
14b	13, 14, 15
15a	13, 14, 15, 17
15b	
16	
17	
18a	18, 19
18b	
19	18, 19
20	
21a	6, 22, 23
21b	6, 22, 23
22a	21, 22, 23
22b	
23a	21, 22, 23
23b	
24	
25	
26	
27	

Figure 1. ^1H NMR spectrum of bromophycoic acid A (**1**) (500 MHz; $\text{DMSO-}d_6$).

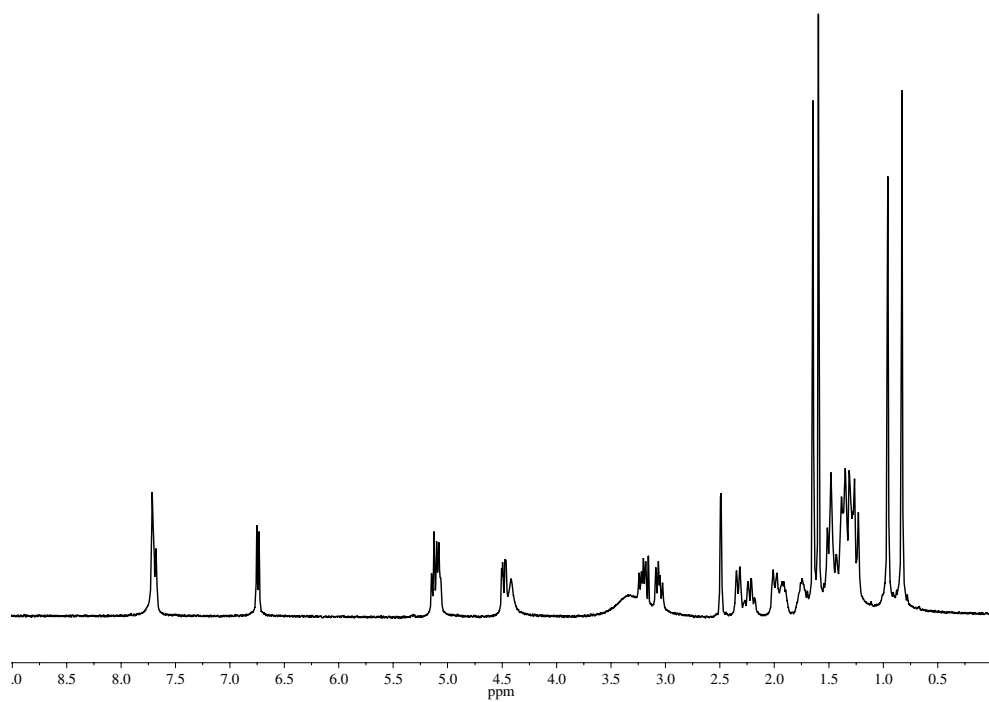


Figure 2. ^{13}C NMR spectrum of bromophycoic acid A (**1**) (125 MHz; $\text{DMSO-}d_6$).

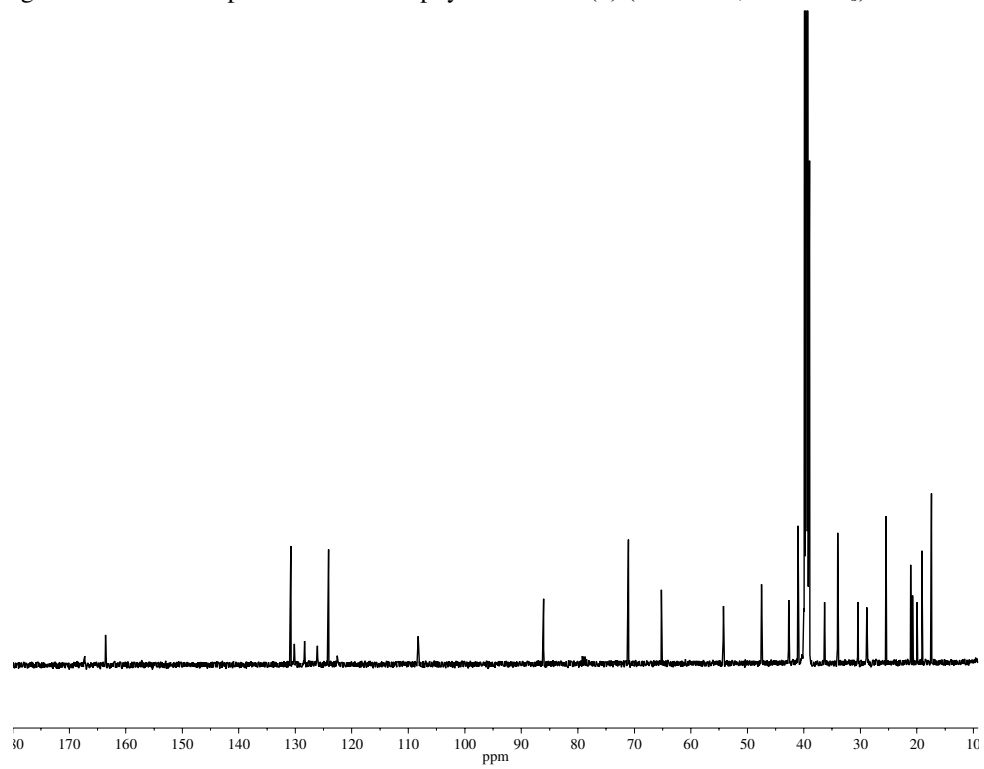


Figure 3. HSQC NMR spectrum of bromophycoic acid A (**1**) (DMSO- d_6).

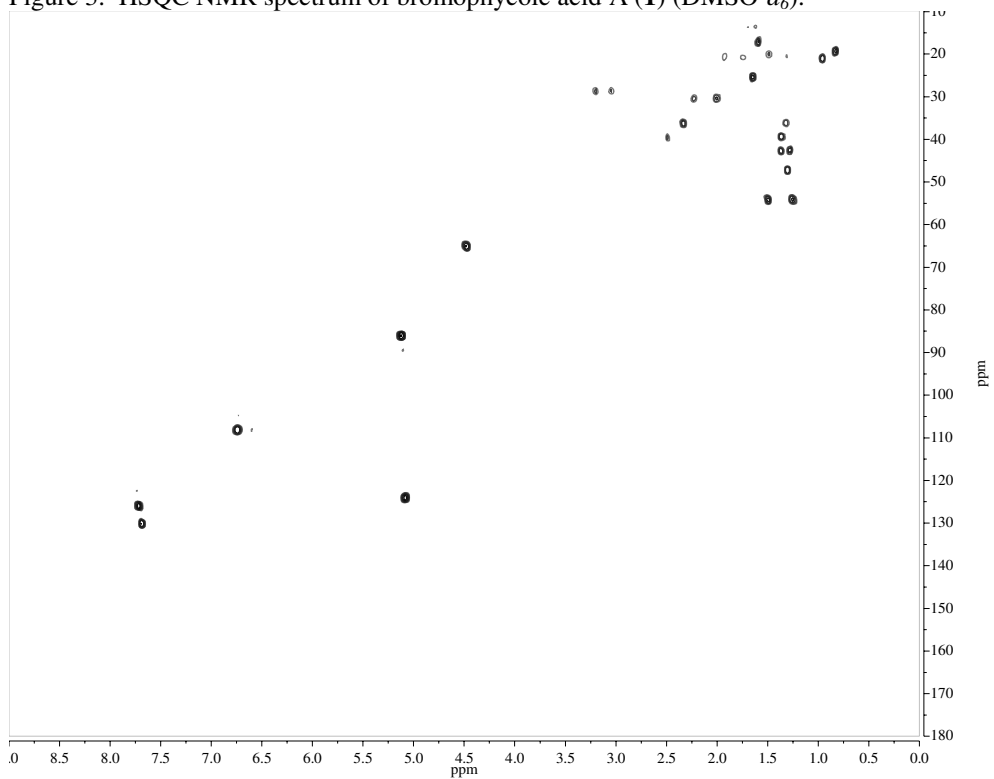


Figure 4. ^1H COSY NMR spectrum of bromophycoic acid A (**1**) (500 MHz; DMSO- d_6).

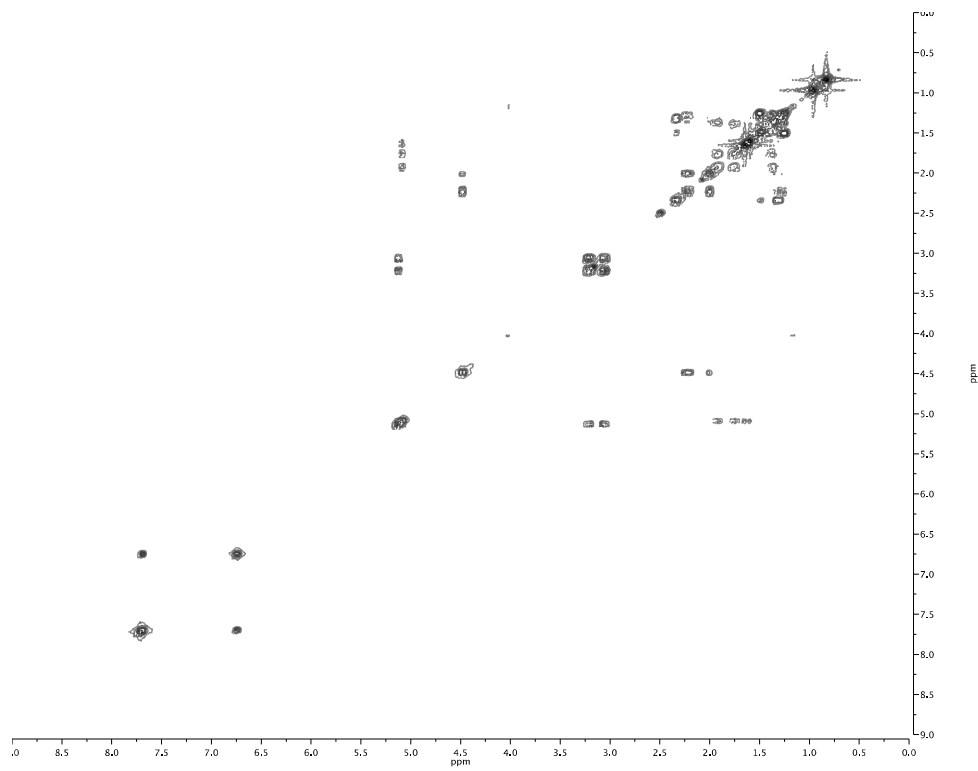


Figure 5. HMBC NMR spectrum of bromophycoic acid **(1)** (DMSO- d_6).

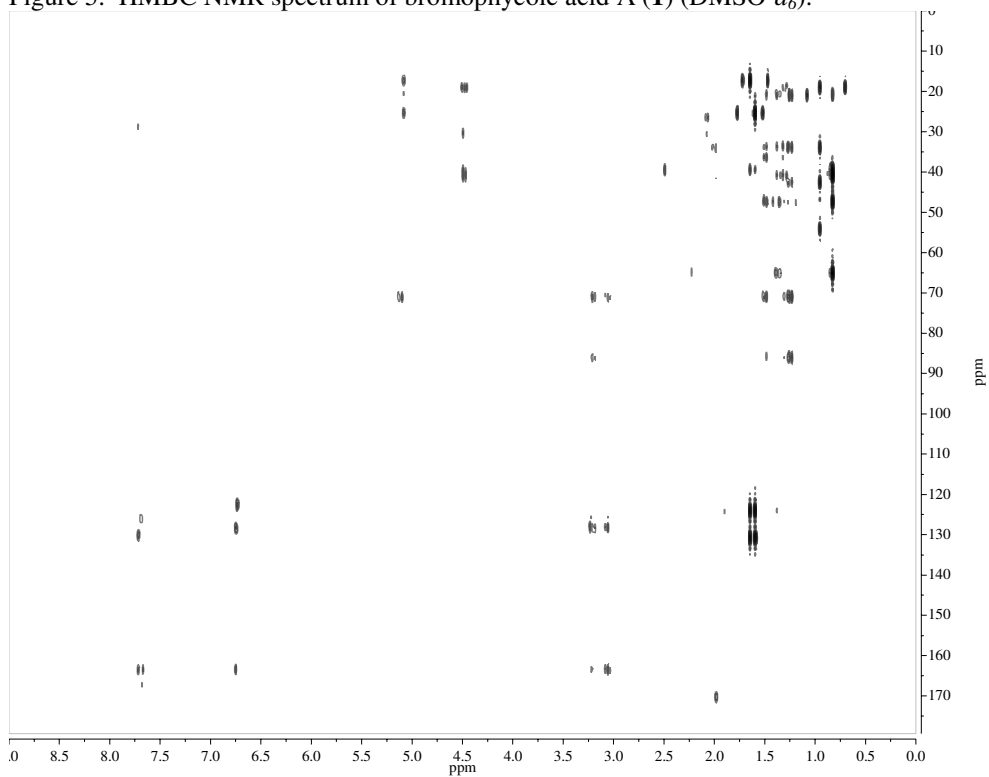


Figure 6. ROESY NMR spectrum of bromophycoic acid **(1)** (DMSO- d_6).

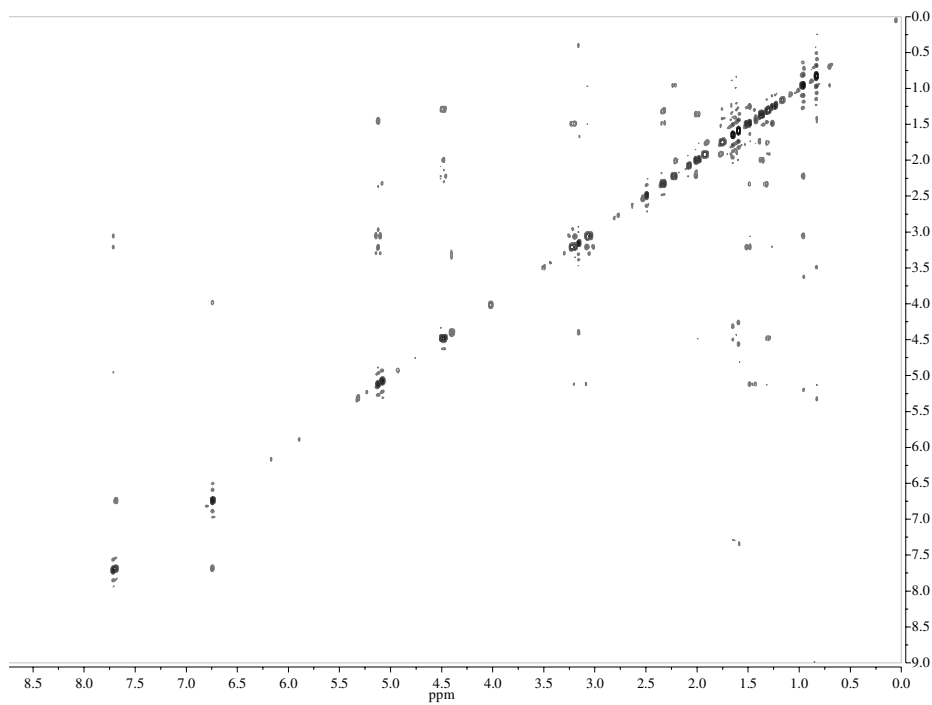


Figure 7. ^1H NMR spectrum of bromophycoic acid **(2)** (500 MHz; $\text{DMSO-}d_6$).

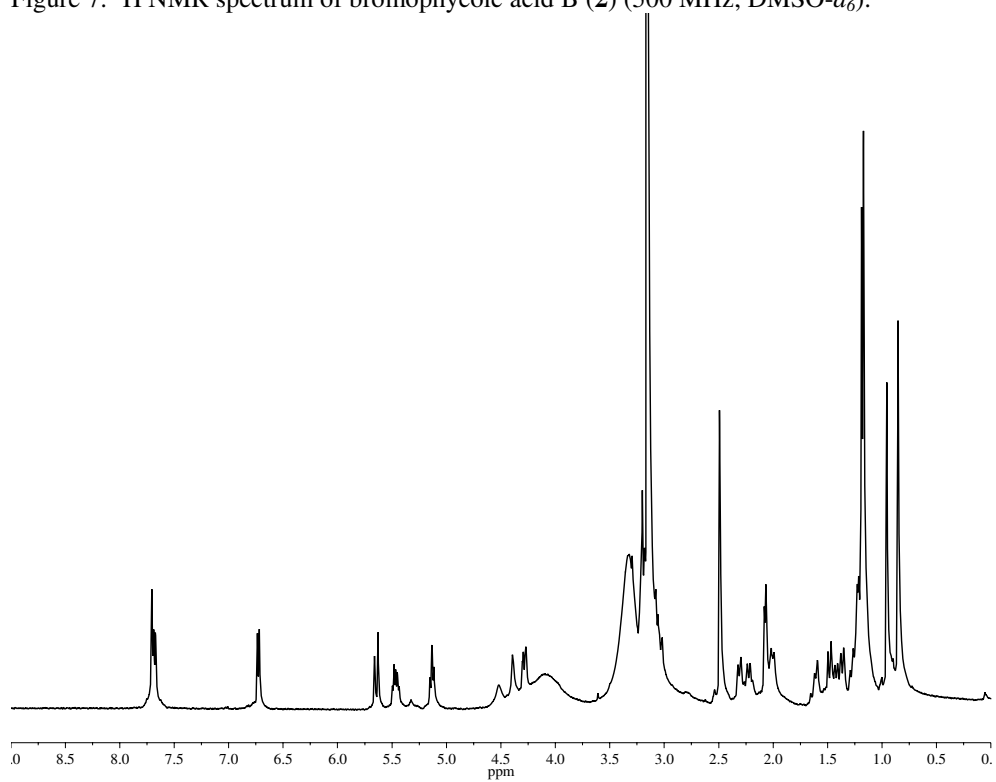


Figure 8. ^{13}C DEPT-135 NMR spectrum of bromophycoic acid **(2)** (125 MHz; $\text{DMSO-}d_6$).

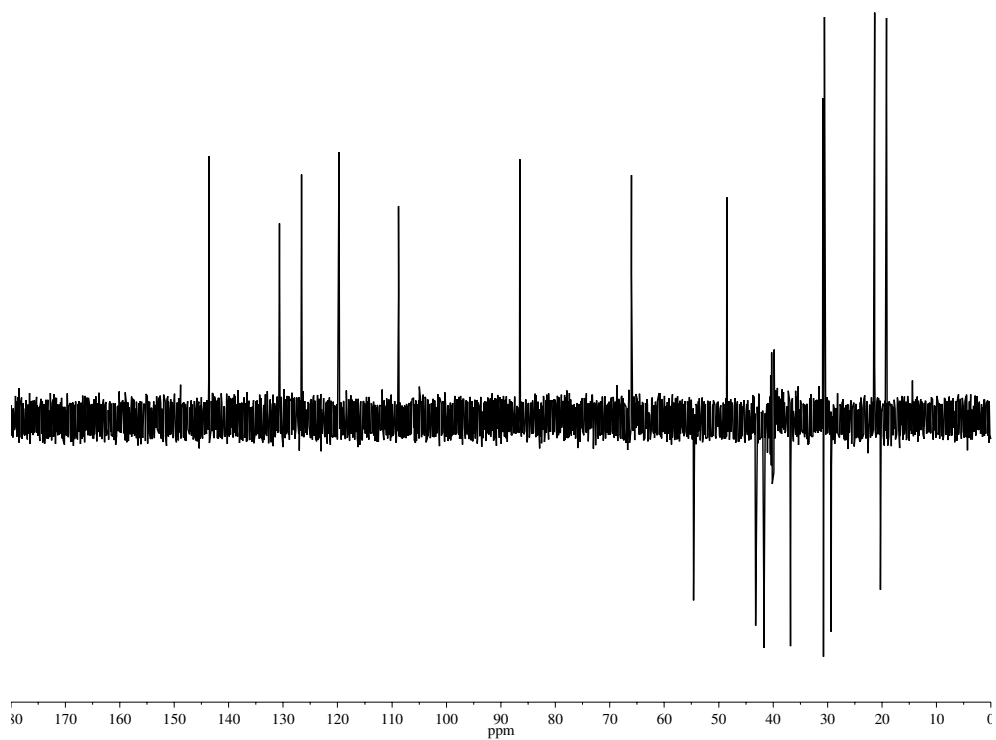


Figure 9. HSQC NMR spectrum of bromophycoic acid B (**2**) (DMSO- d_6).

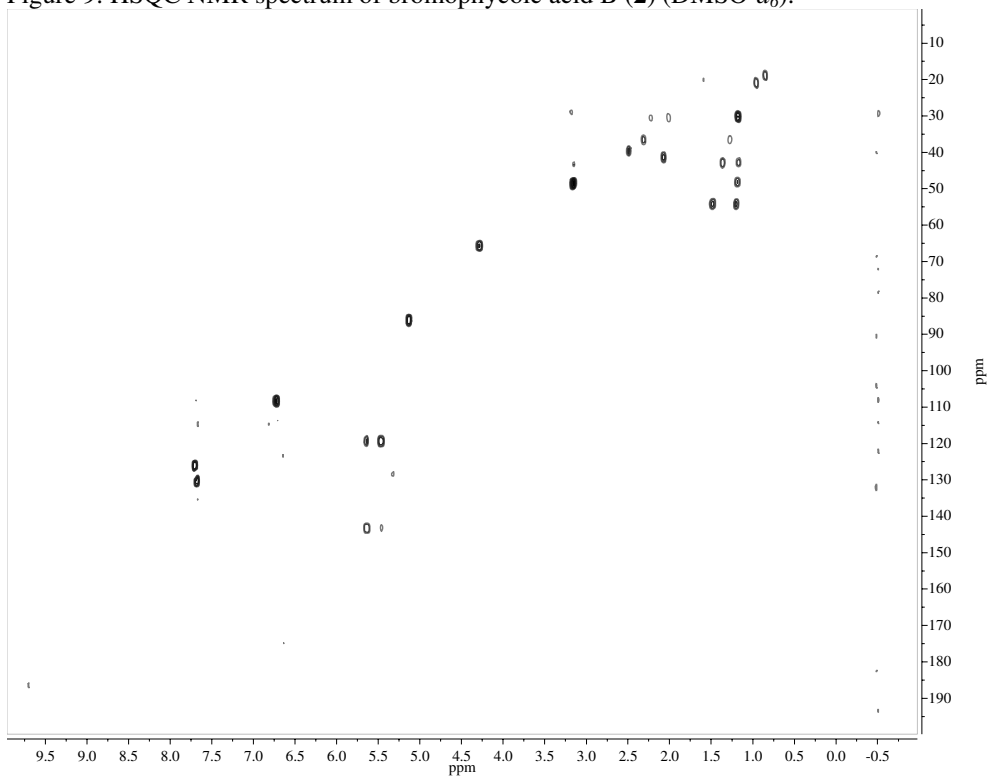


Figure 10. ^1H COSY NMR spectrum of bromophycoic acid B (**2**) (500 MHz; DMSO- d_6).

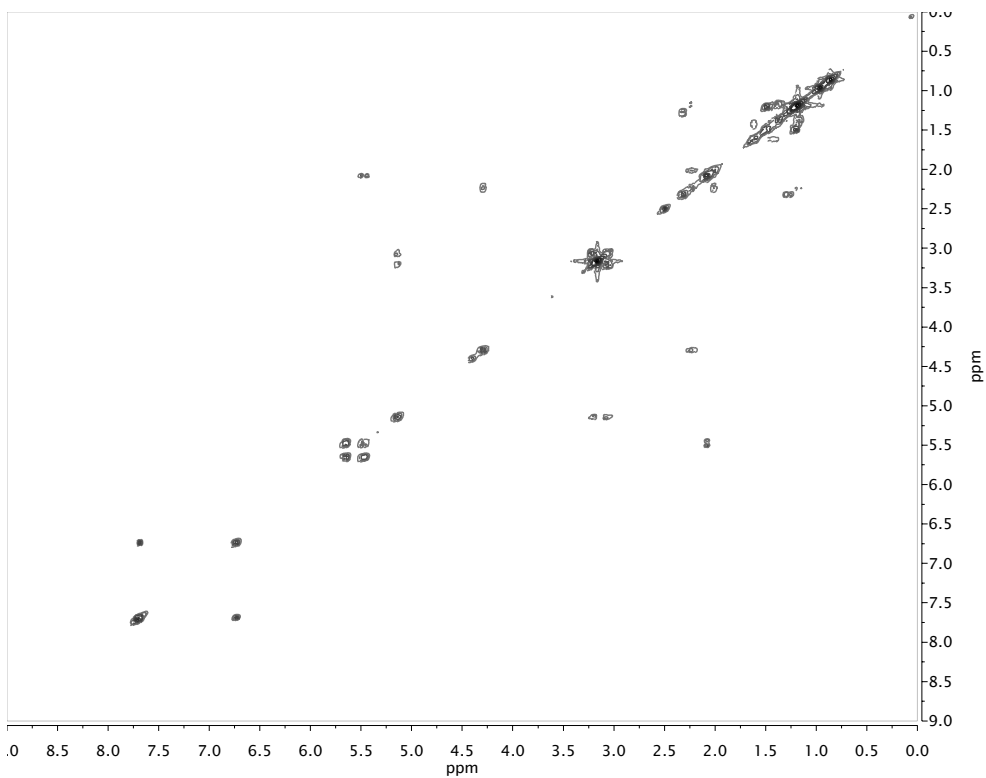


Figure 11. HMBC NMR spectrum of bromophycoic acid B (**2**) (DMSO- d_6).

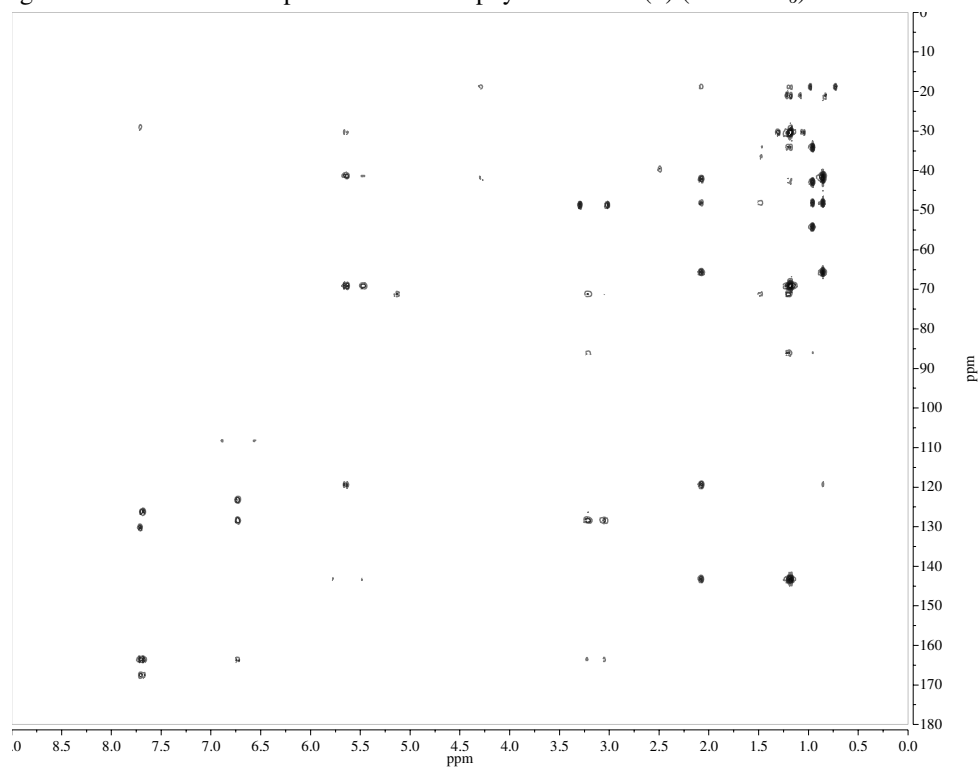


Figure 12. ROESY NMR spectrum of bromophycoic acid B (**2**) (DMSO- d_6).

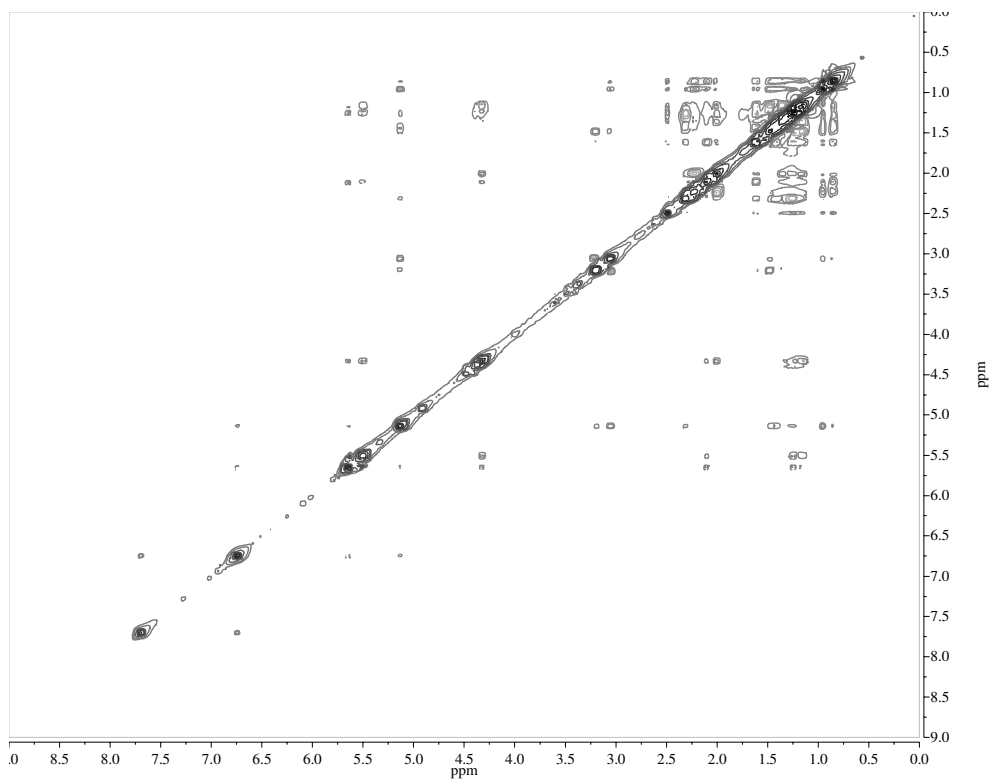


Figure 13. ^1H NMR spectrum of bromophycoic acid **3** (125 MHz; $\text{DMSO-}d_6$).

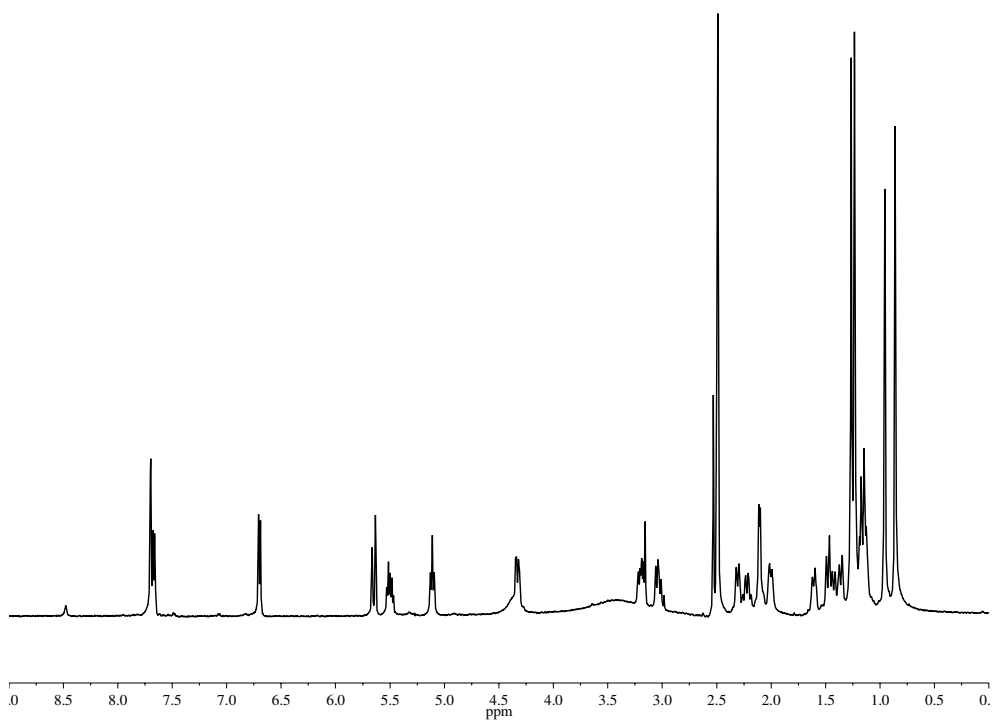


Figure 14. ^{13}C NMR spectrum of bromophycoic acid **3** (125 MHz; $\text{DMSO-}d_6$).

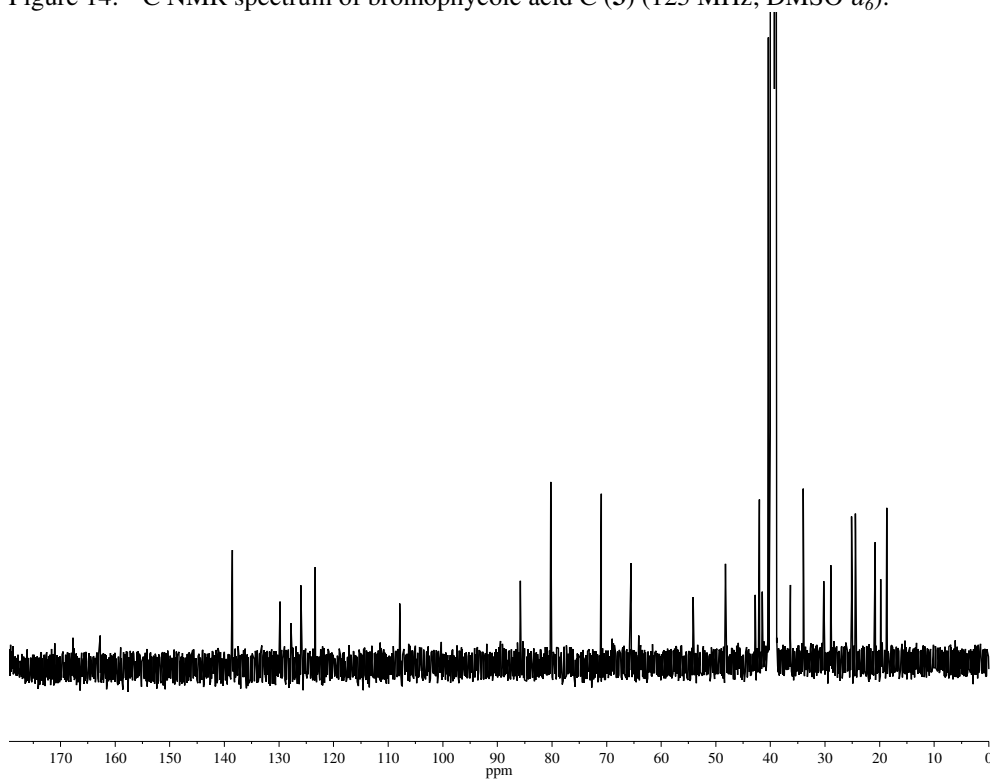


Figure 15. HSQC NMR spectrum of bromophycoic acid **3** (DMSO- d_6).

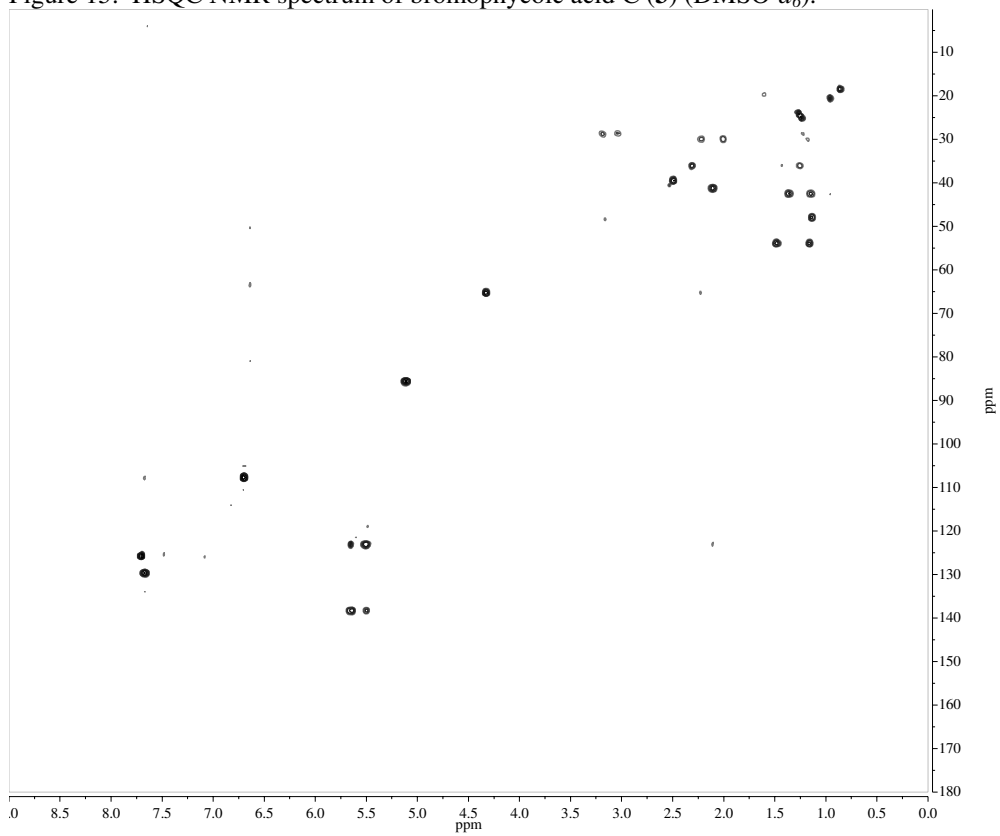


Figure 16. ^1H COSY NMR spectrum of bromophycoic acid **3** (500 MHz; DMSO- d_6).

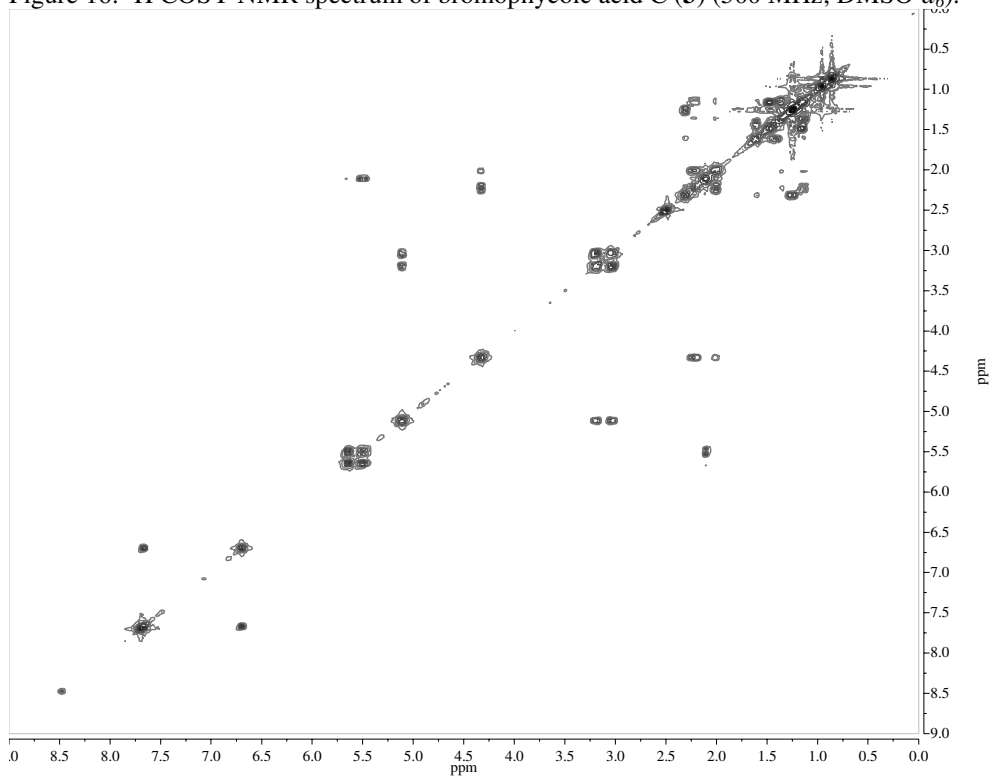


Figure 17. HMBC NMR spectrum of bromophycoic acid **3** (DMSO- d_6).

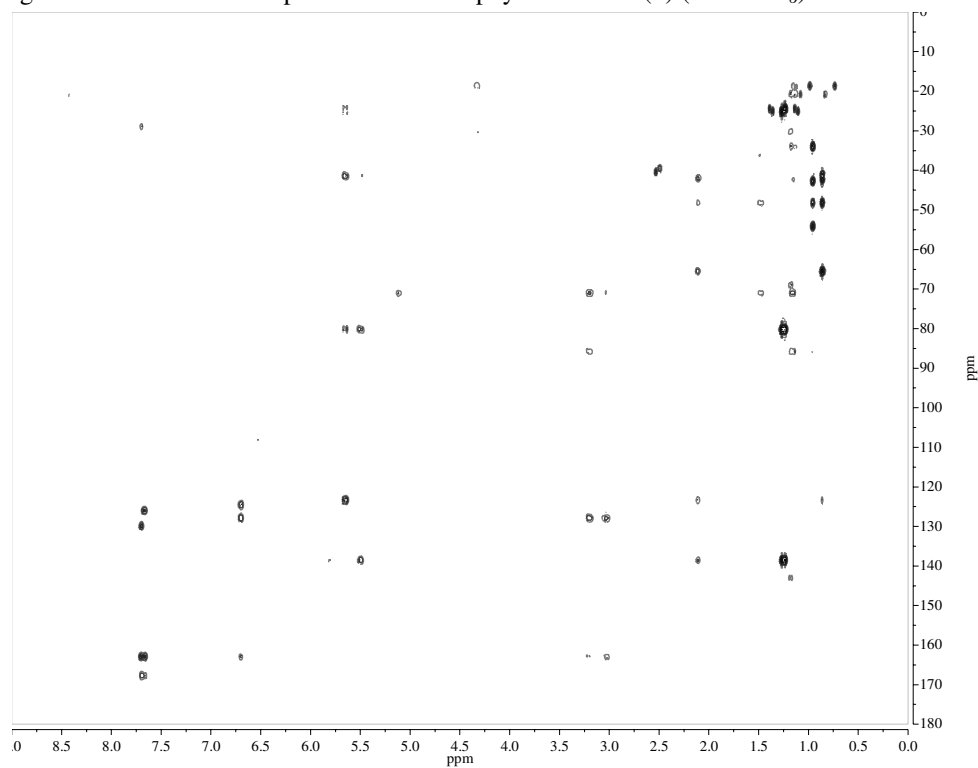


Figure 18. ROESY NMR spectrum of bromophycoic acid **3** (DMSO- d_6).

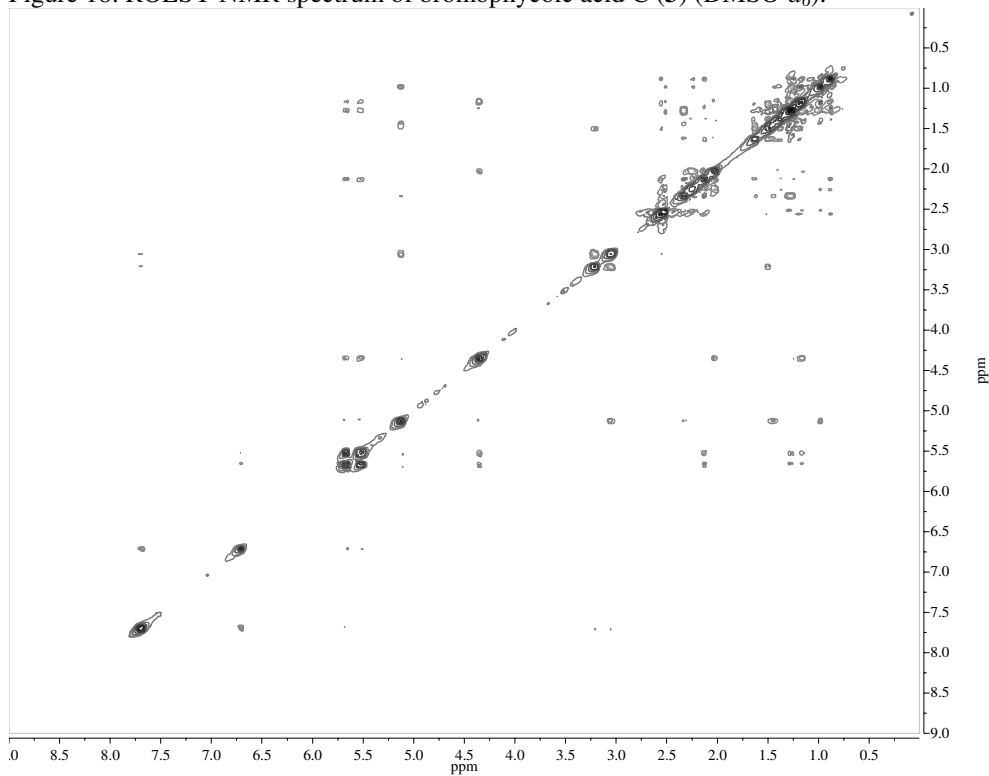


Figure 19. ^1H NMR spectrum of bromophycoic acid D (**4**) (500 MHz; $\text{DMSO-}d_6$).

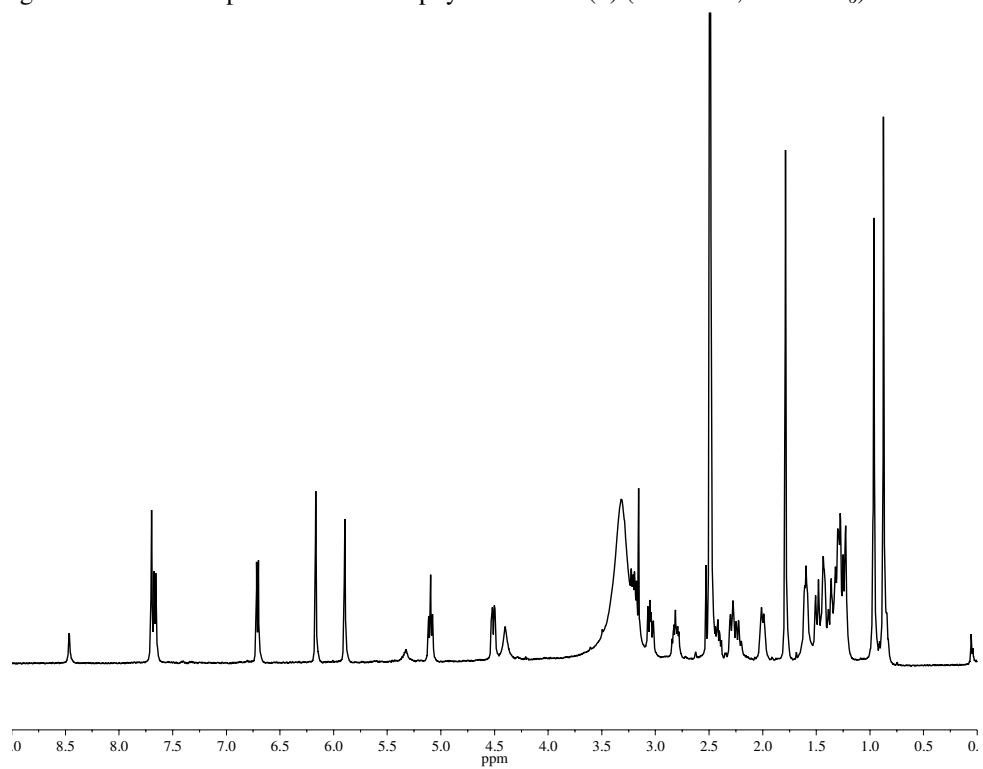


Figure 20. ^{13}C DEPT-135 NMR spectrum of bromophycoic acid D (**4**) (125 MHz; $\text{DMSO-}d_6$).

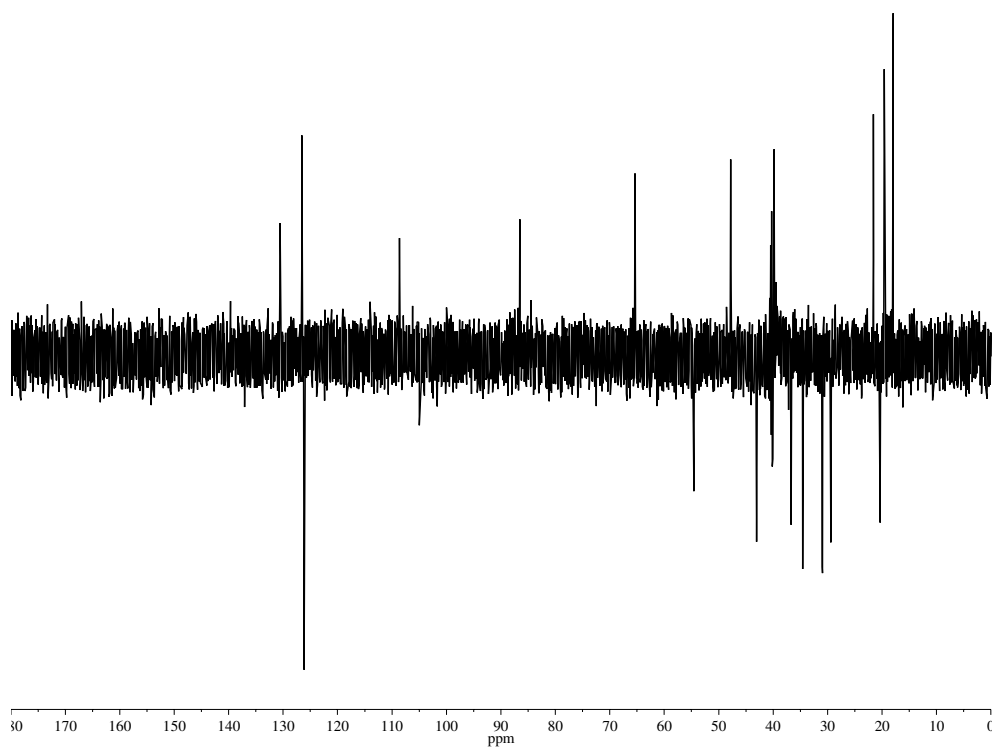


Figure 21. HSQC NMR spectrum of bromophycoic acid D (**4**) (DMSO- d_6).

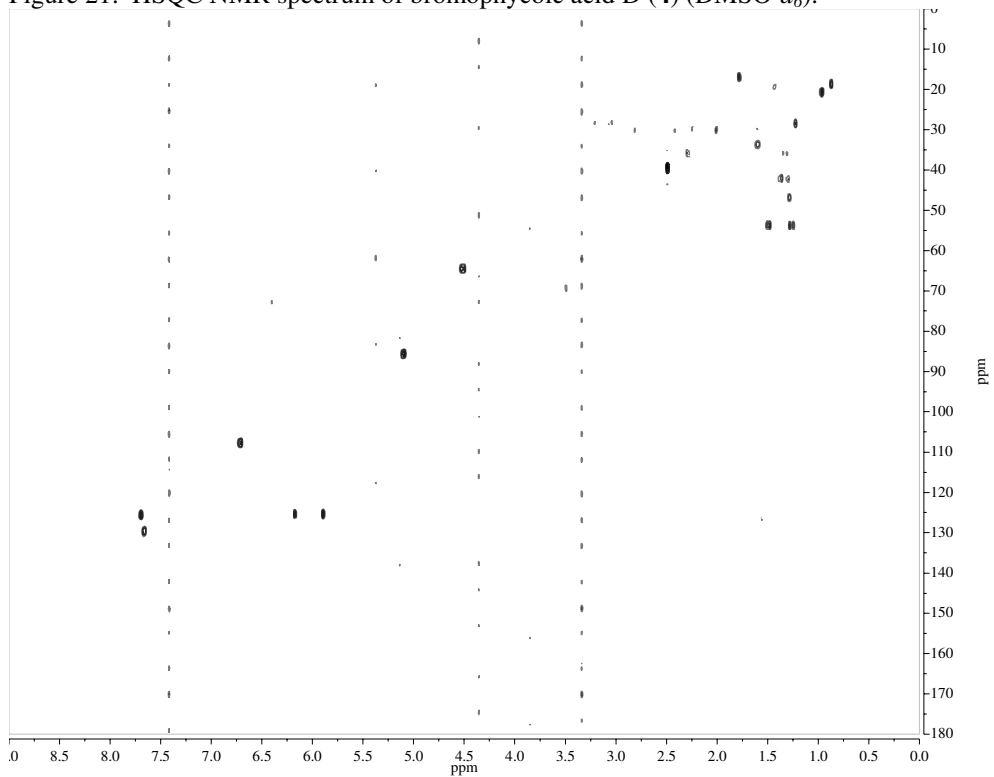


Figure 22. ^1H COSY NMR spectrum of bromophycoic acid D (**4**) (500 MHz; DMSO- d_6).

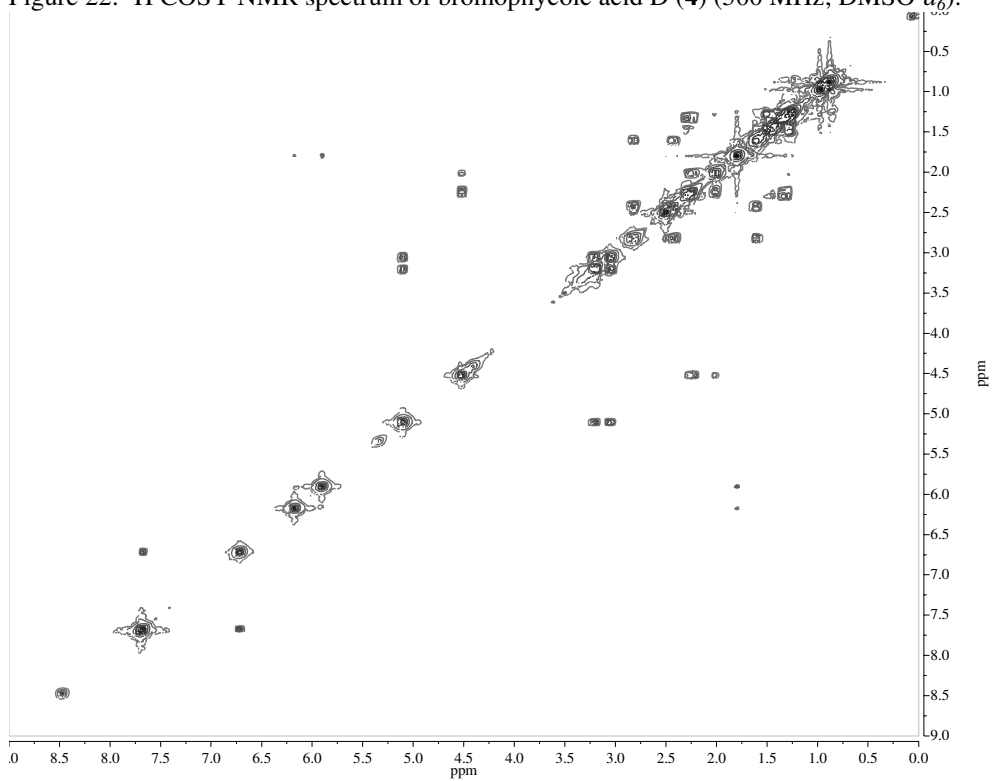


Figure 23. HMBC NMR spectrum of bromophycoic acid D (4) (DMSO- d_6).

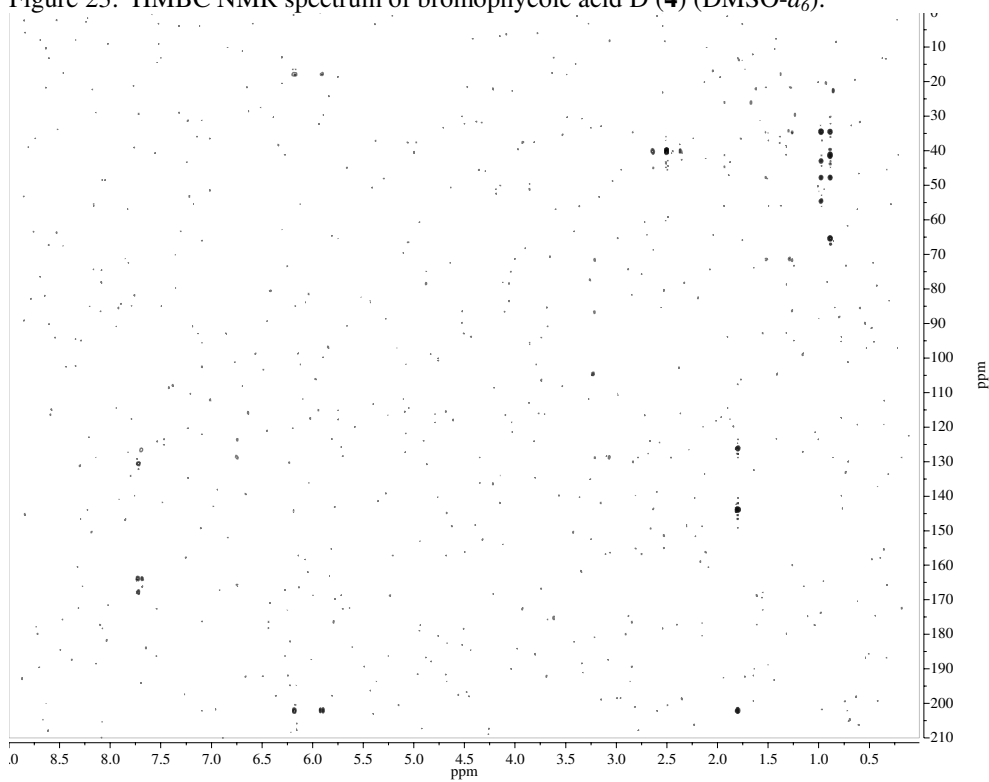


Figure 24. ROESY NMR spectrum of bromophycoic acid D (4) (DMSO- d_6).

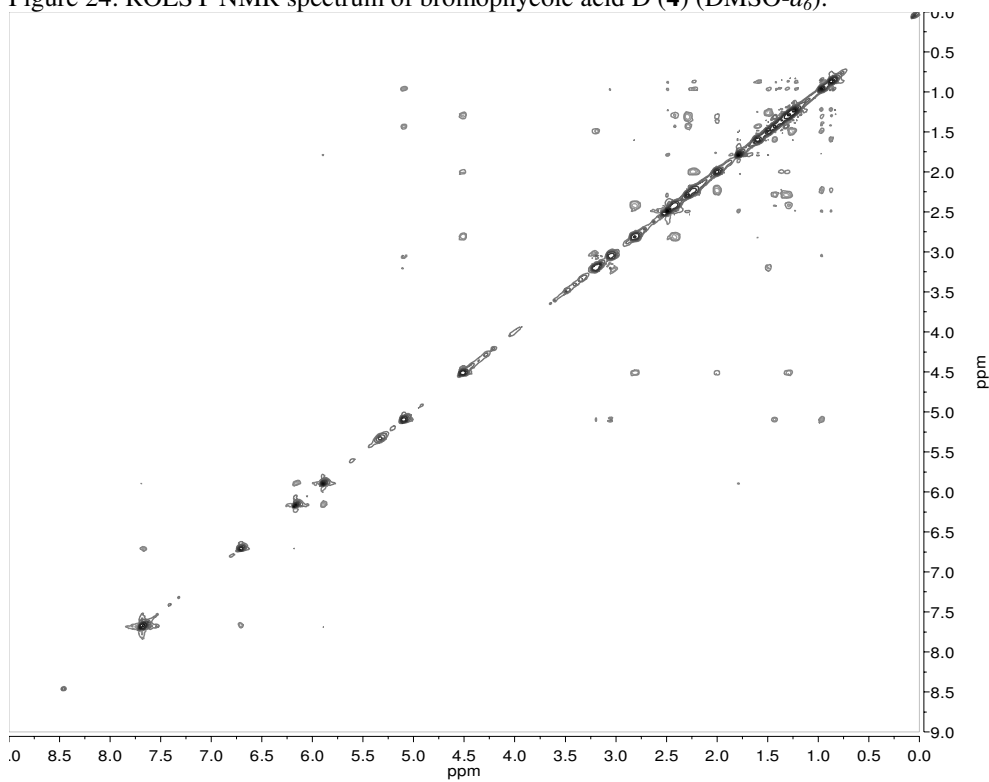


Figure 25. ^1H NMR spectrum of bromophycoic acid **(5)** (500 MHz; CDCl_3).

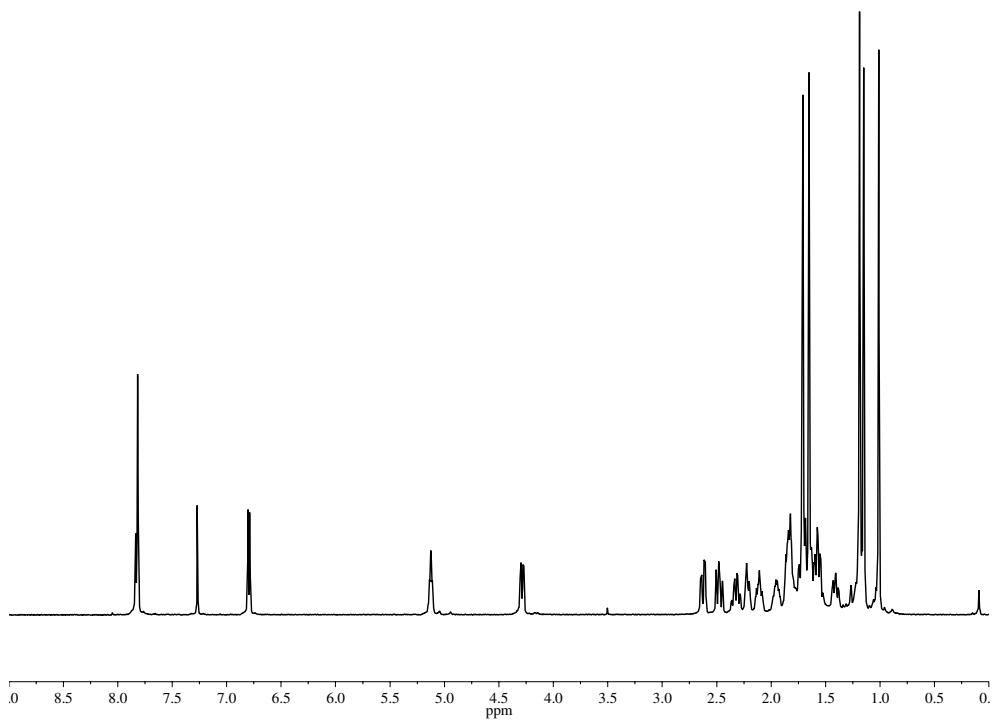


Figure 26. ^{13}C NMR spectrum of bromophycoic acid **(5)** (125 MHz; CDCl_3).

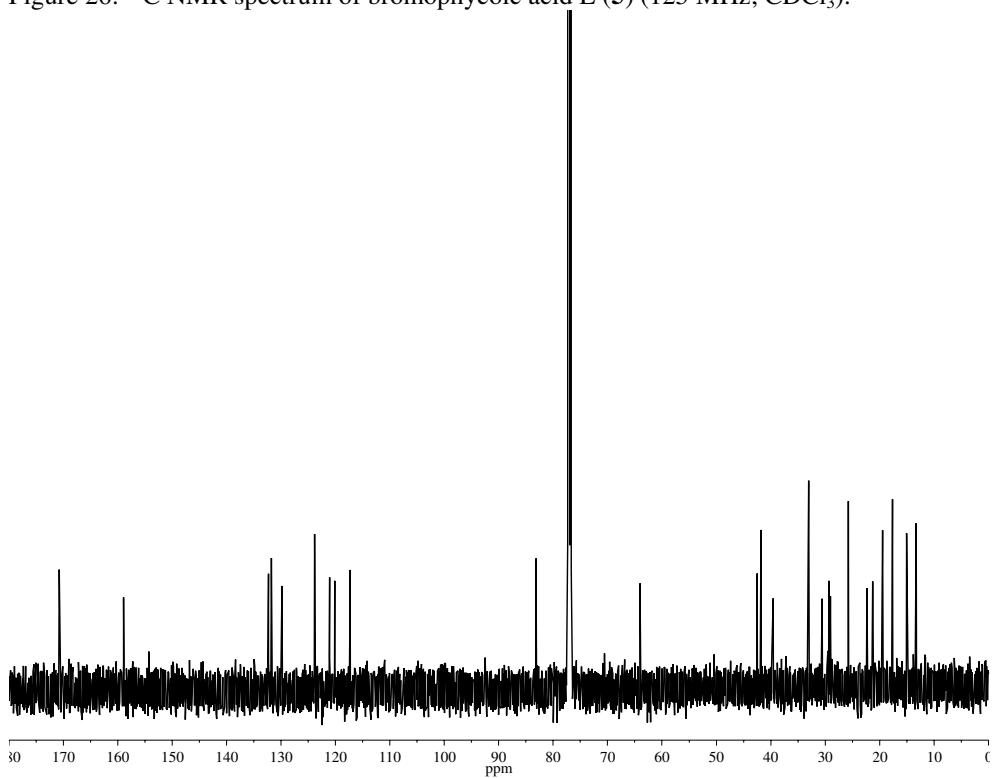


Figure 27. DEPT-135 spectrum of bromophycoic acid E (**5**) (125 MHz; CDCl₃).

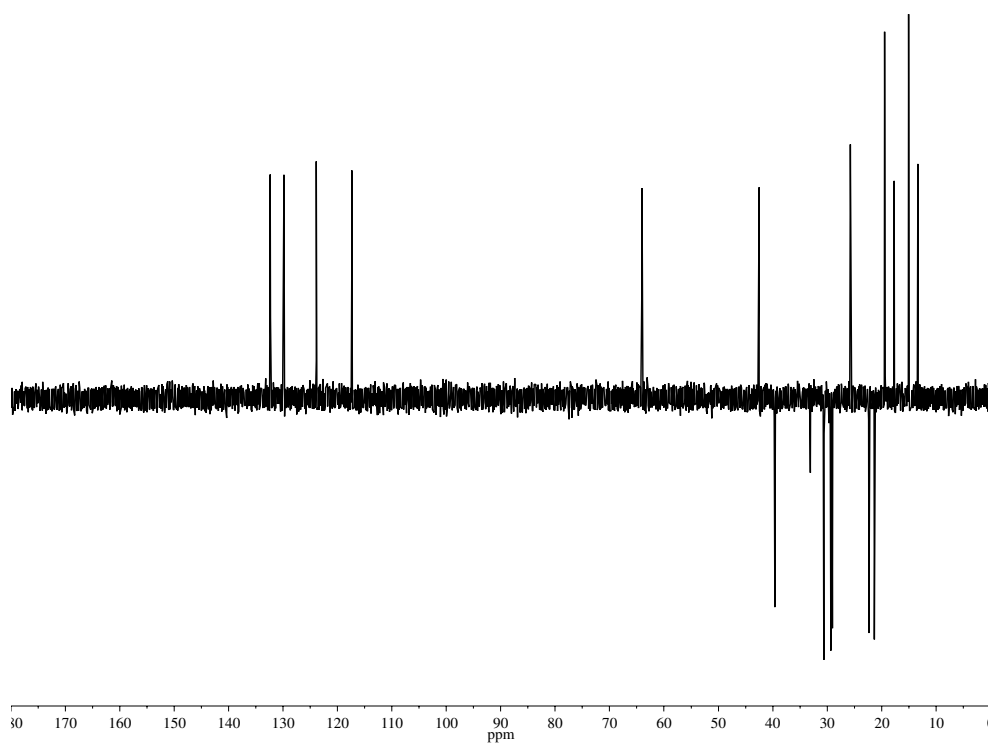


Figure 28. HSQC NMR spectrum of bromophycoic acid E (**5**) (CDCl₃).

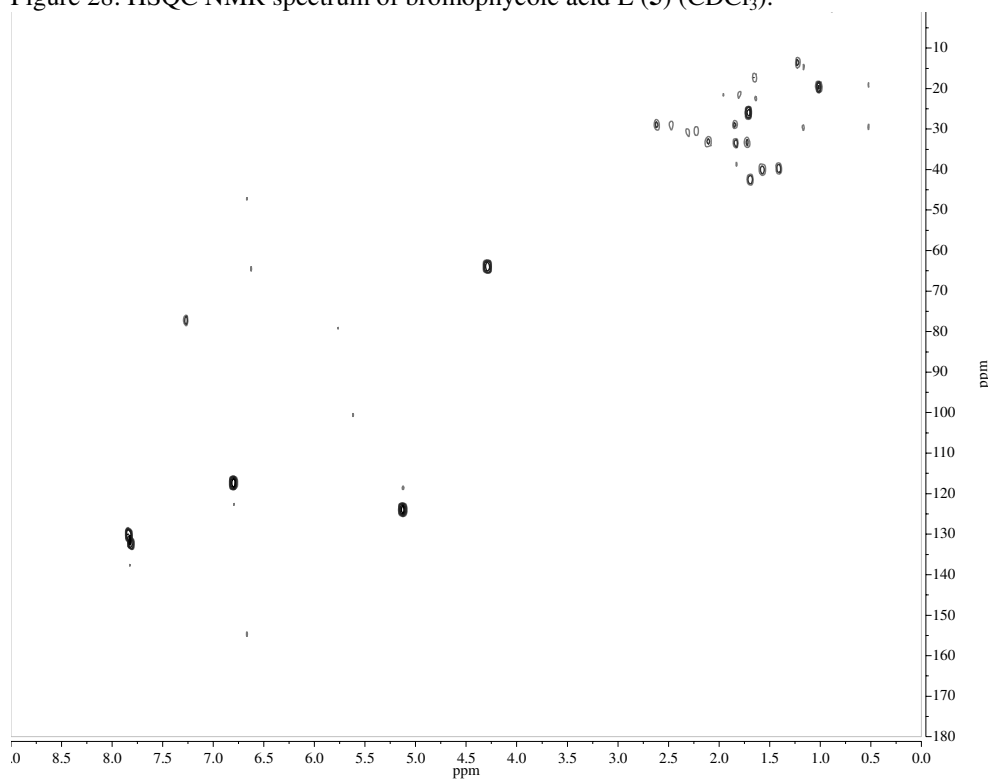


Figure 29. ^1H COSY NMR spectrum of bromophycoic acid E (**5**) (500 MHz; CDCl_3).

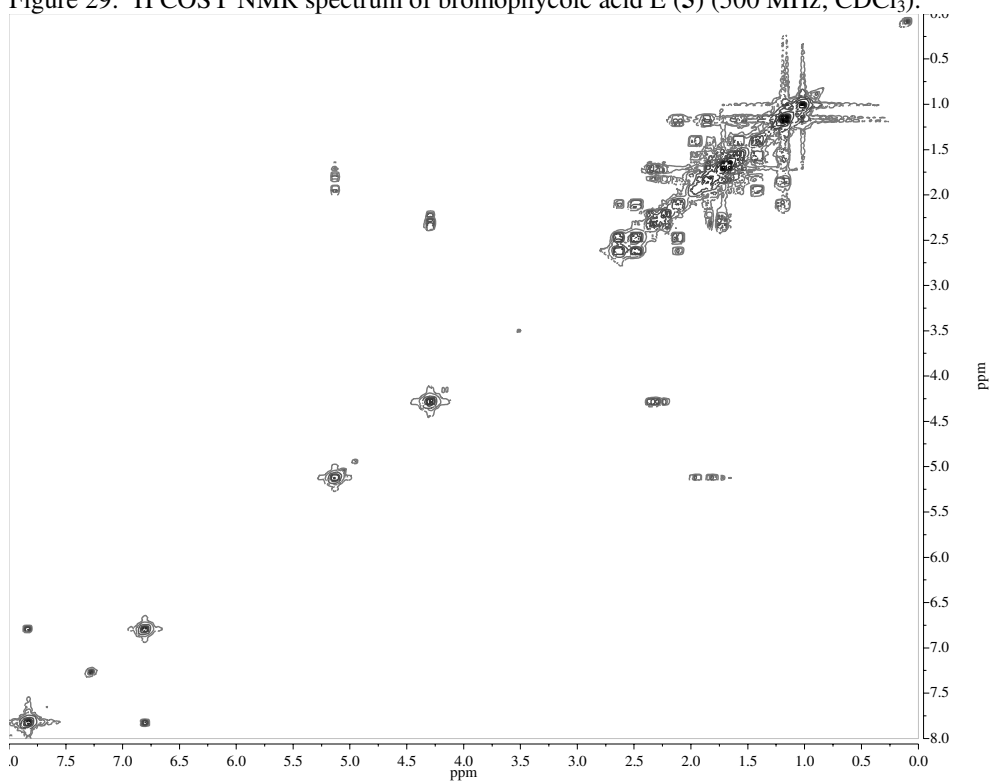


Figure 30. HMBC NMR spectrum of bromophycoic acid E (**5**) (CDCl_3).

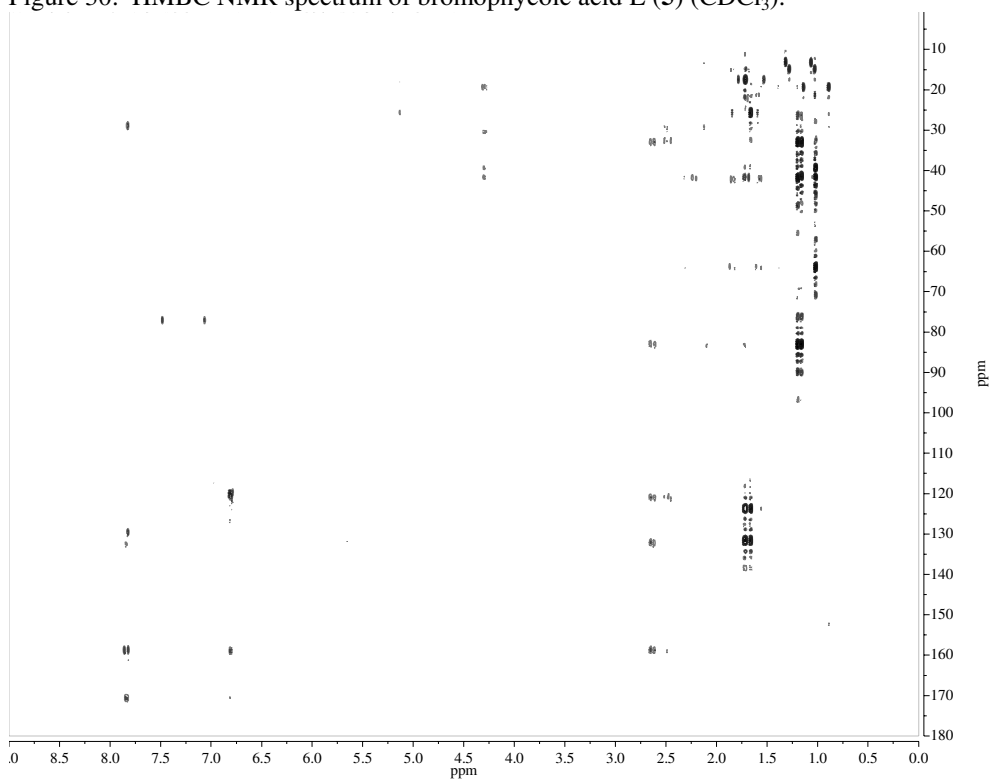


Figure 31. HSQC-TOCSY NMR spectrum of bromophycoic acid E (**5**) (CDCl_3).

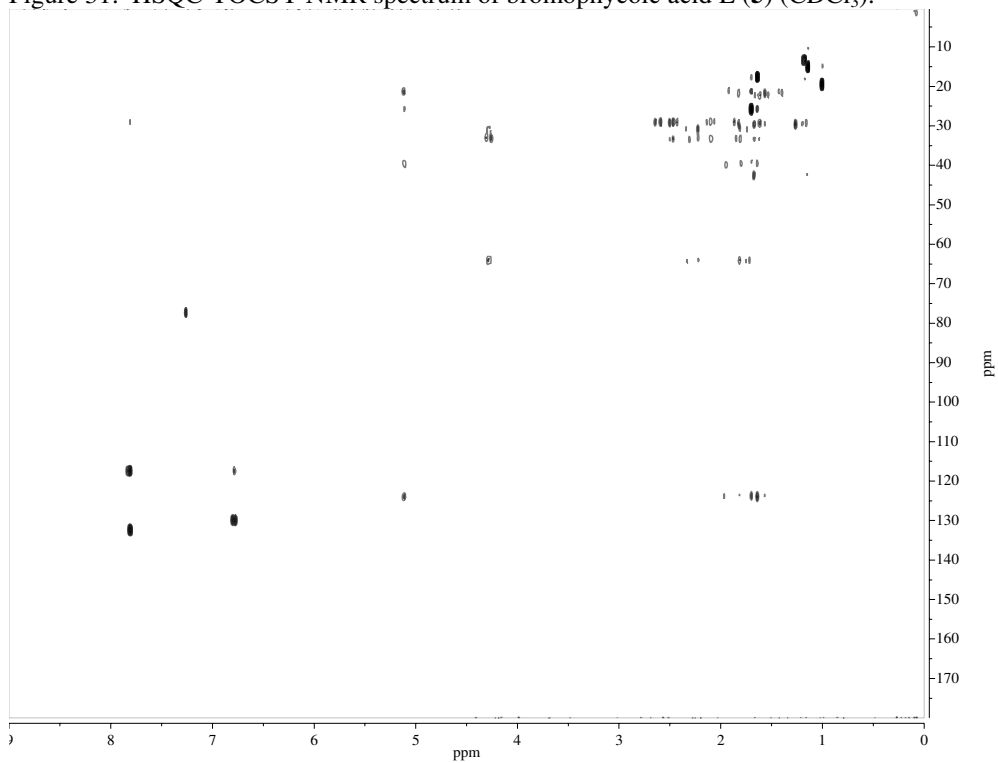


Figure 32. ROESY NMR spectrum of bromophycoic acid E (**5**) (CDCl_3).

