SUPPLEMENTARY MATERIAL

Synthesis of derivatives of potent antitumor bistramide D and A leading to the first crystal structure of natural bistramide D

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Electronic Supplementary Information (ESI) available:

2D NMR spectroscopic data of BST Ada **3**, BST Dda **4** and BST Dda, 4-TBS **5**

Typical experimental procedures for **2**, **3**, **4** and **5**.

Table 1.
Comparison of ^{13}C NMR chemical shifts of natural bistramide D, with the hemisynthetic bistramide D (**2**), derivatives BST Ada (**3**), BST Dda (**4**) and BST Dda, 4-TBS (**5**).

Table 2.
Comparison of ^{1}H NMR chemical shifts of derivatives BST Ada (**3**), BST Dda (**4**) and BST Dda, 4-TBS (**5**).

spectroscopic data of 1H and 13C NMR for natural bistramide D

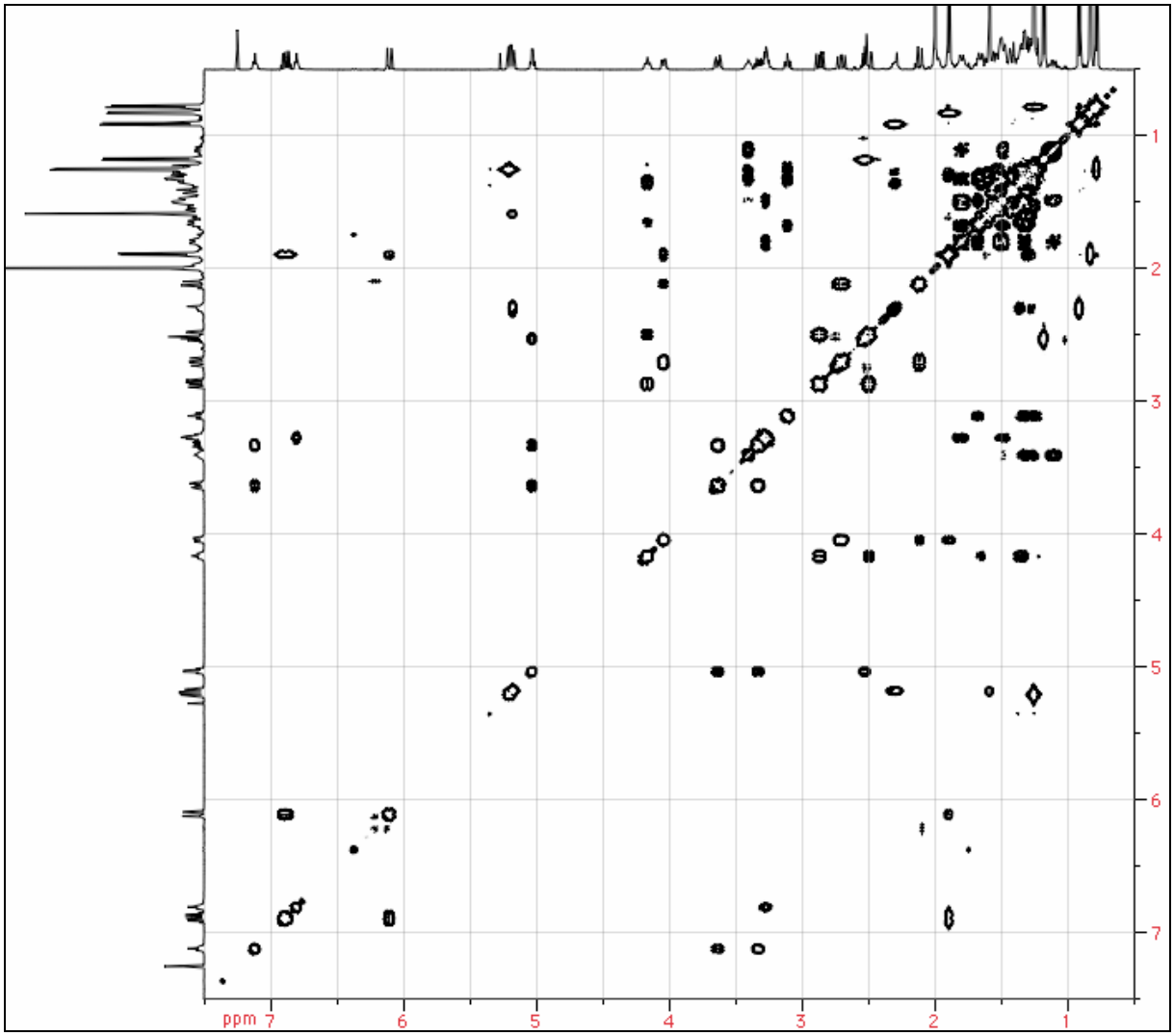
spectroscopic data of 1H and 13C NMR for hemisynthetic bistramide D; **2**

spectroscopic data of 1H and 13C NMR for bistramide Ada; **3**

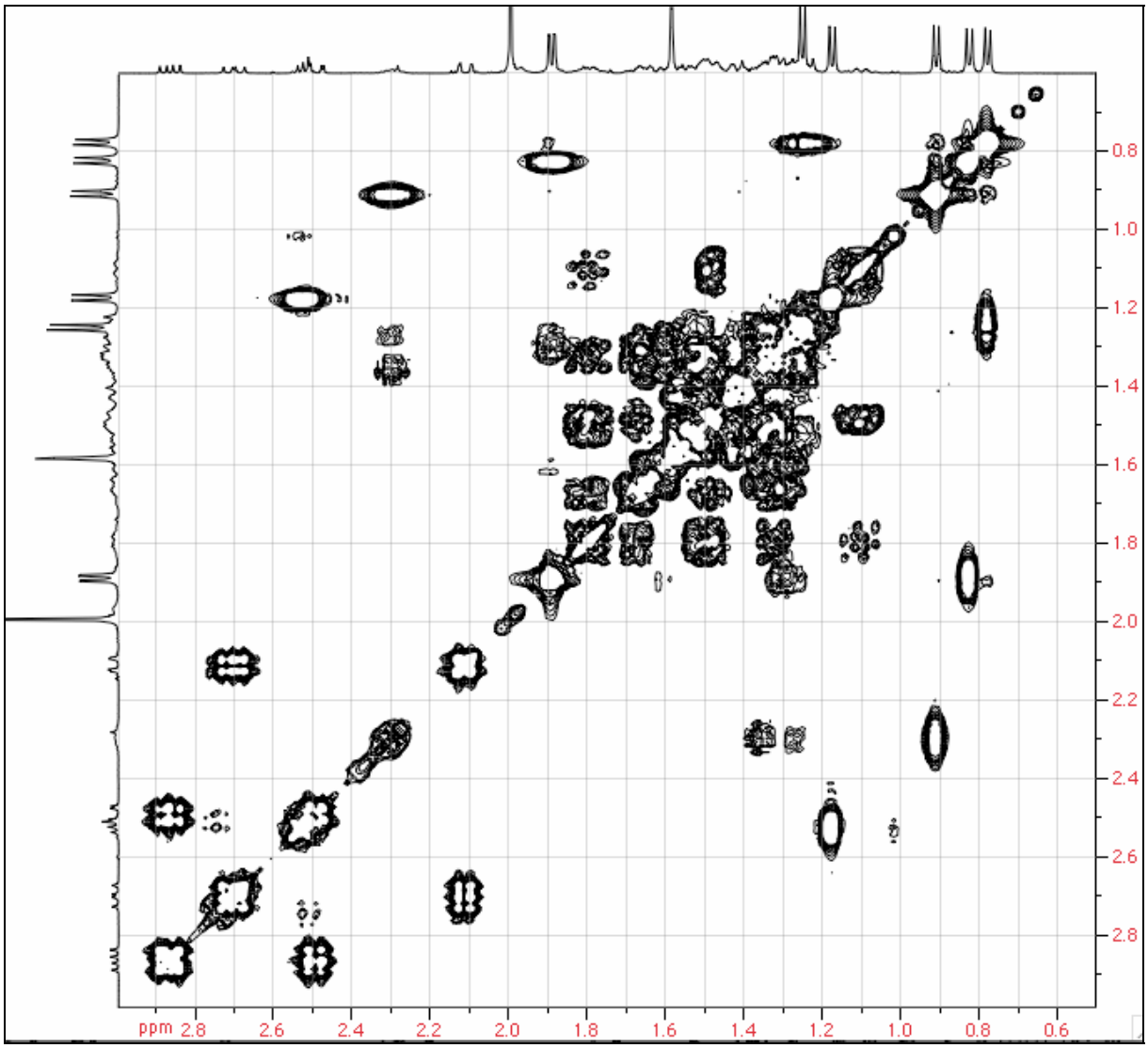
spectroscopic data of 1H and 13C NMR for bistramide Dda; **4**

spectroscopic data of 1H and 13C NMR for bistramide Dda, 4-TBS; **5**

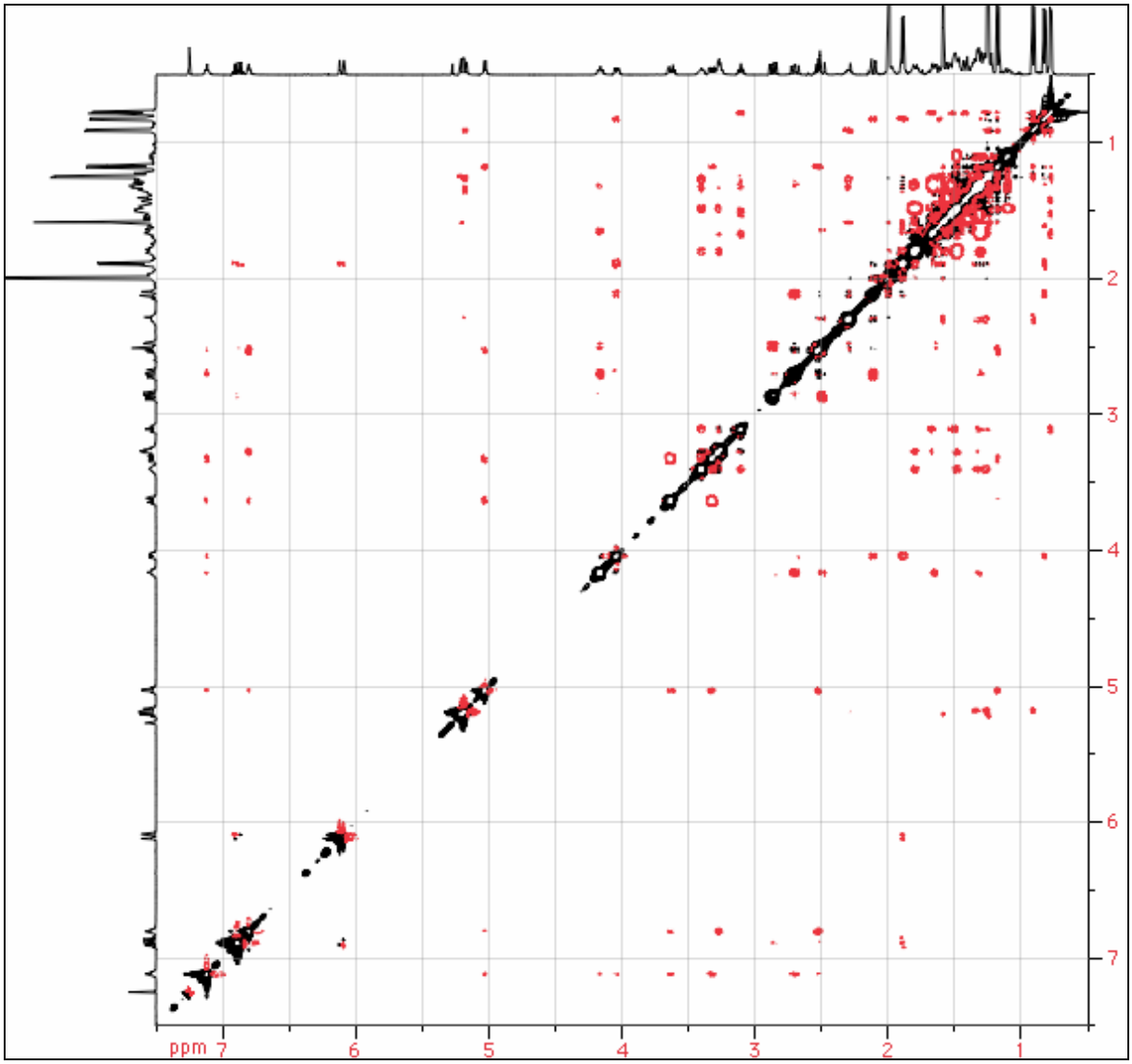
COSY spectrum of BST Ada : **3** (500 MHz; $CDCl_3$)



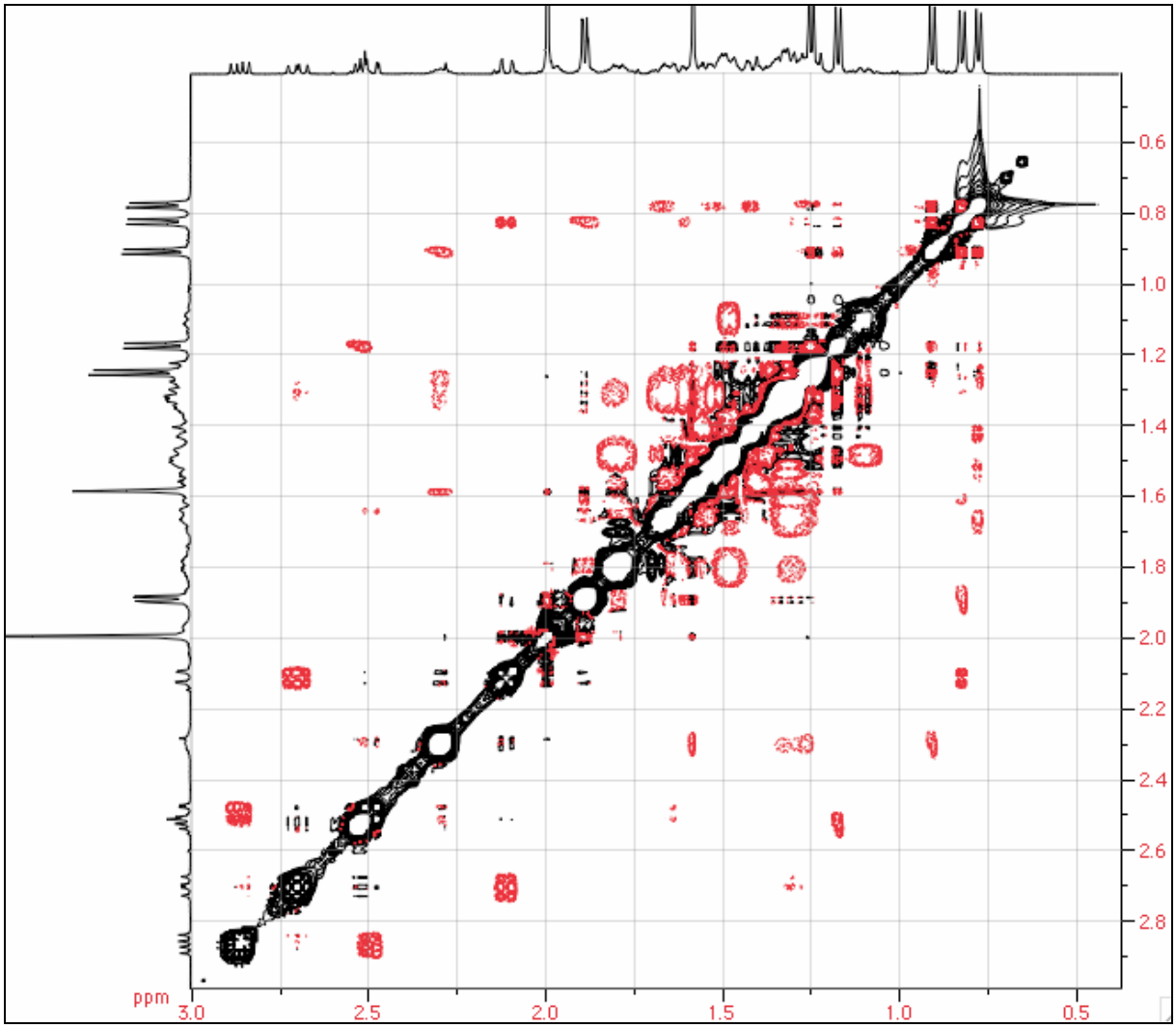
COSY spectrum of BST Ada : **3** (500 MHz; $CDCl_3$)



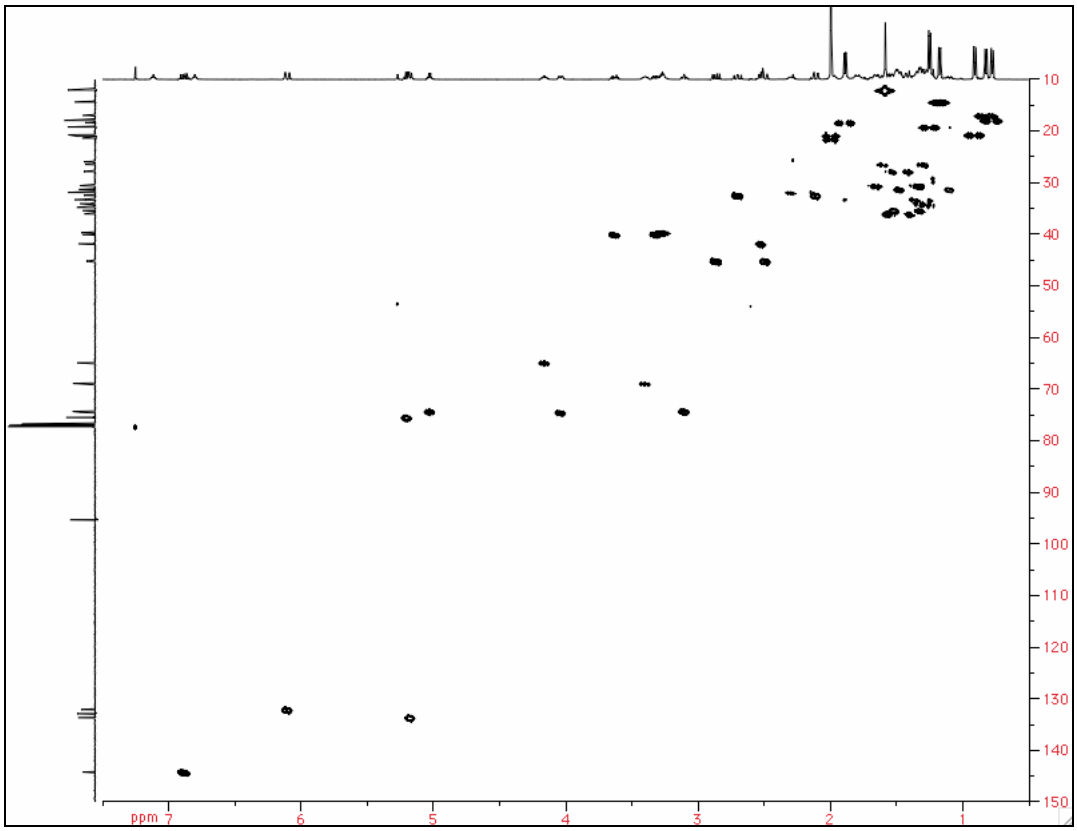
ROESY spectrum of BST Ada : **3** (500 MHz; $CDCl_3$)



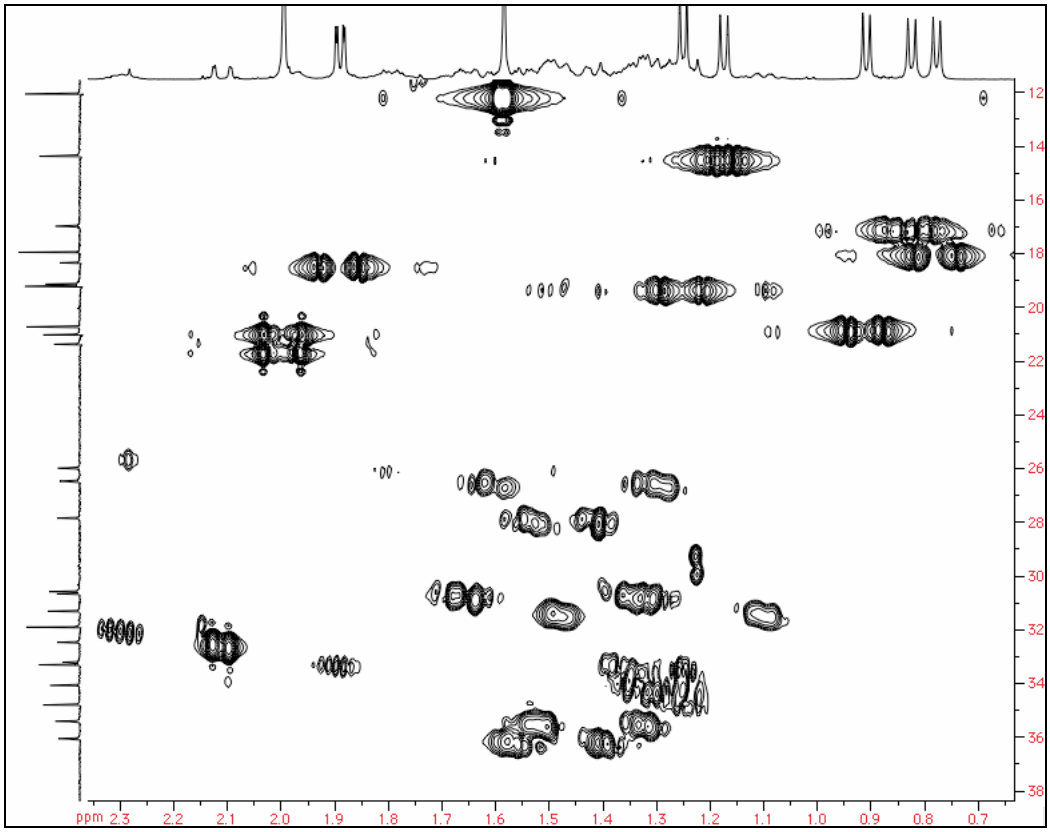
ROESY spectrum of BST Ada : **3** (500 MHz; $CDCl_3$)



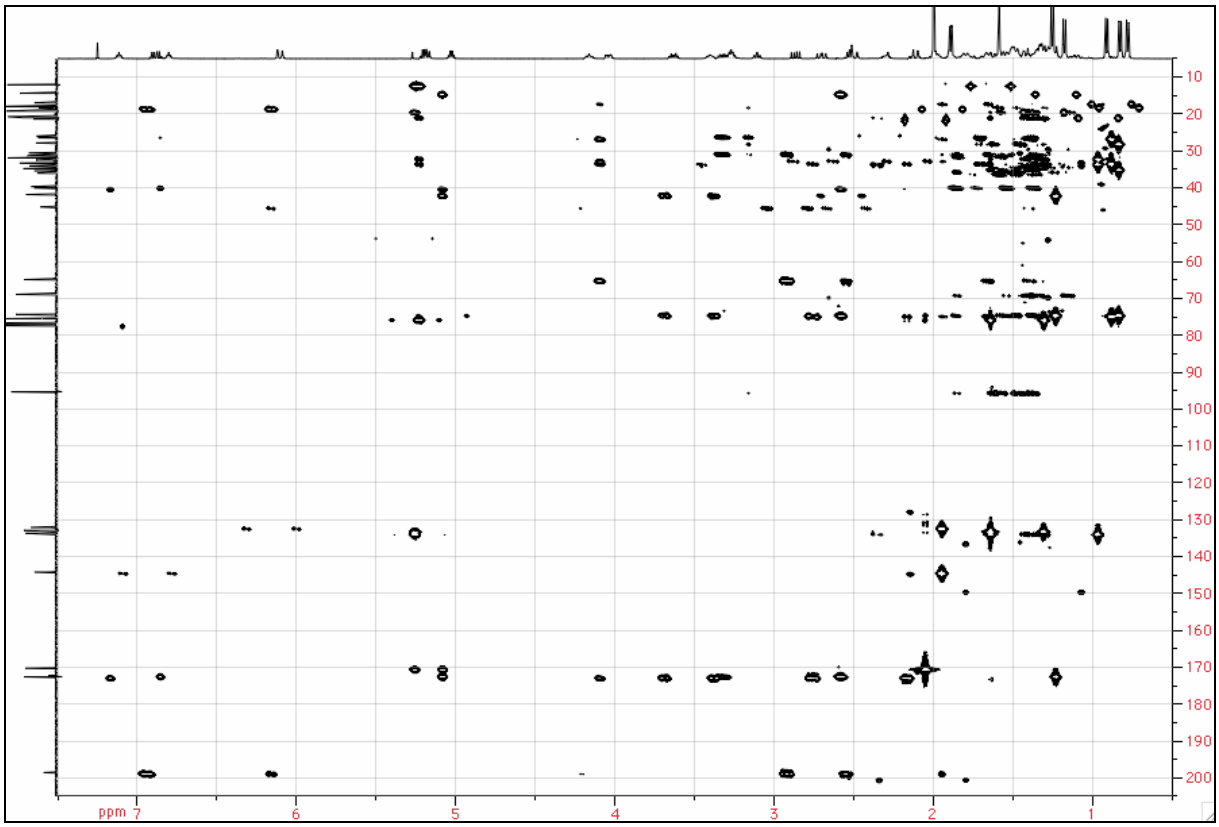
HMQC spectrum of BST Ada : **3** (500 MHz; $CDCl_3$)



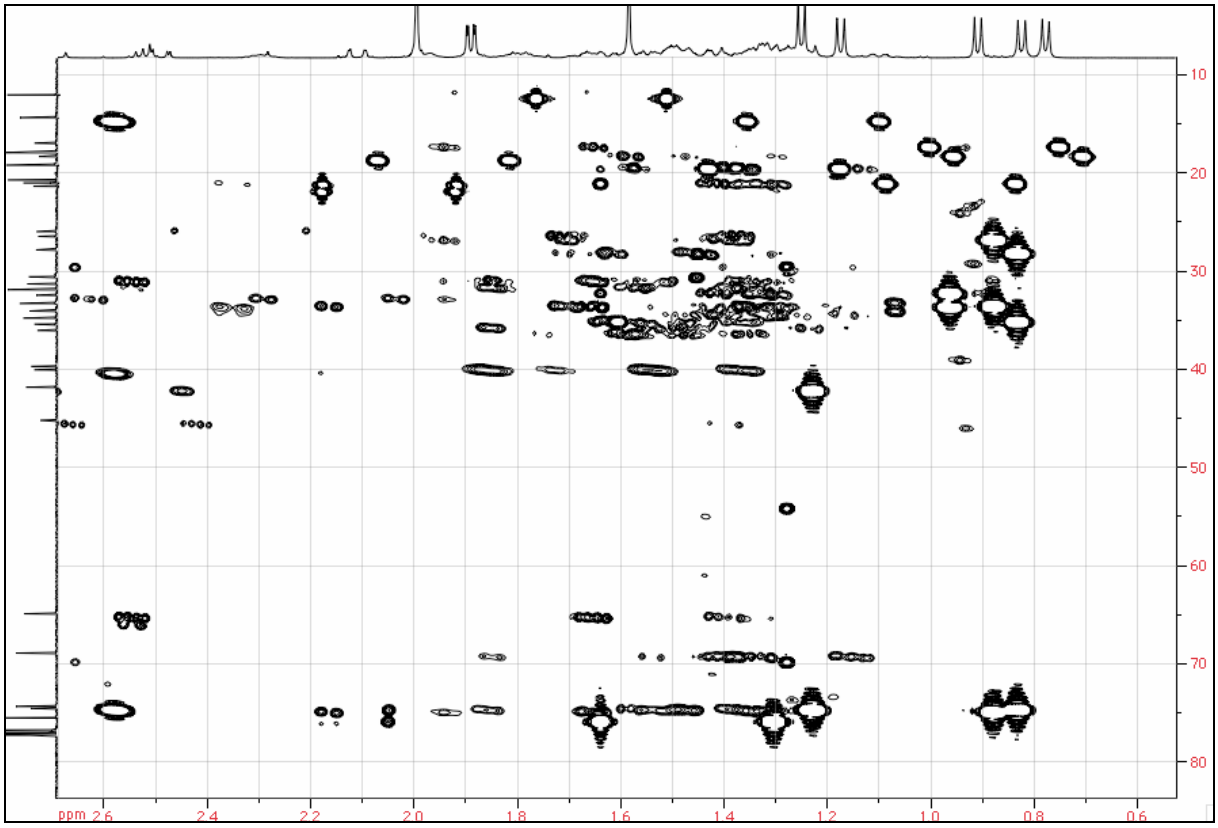
HMQC spectrum of BST Ada : **3** (500 MHz; $CDCl_3$)



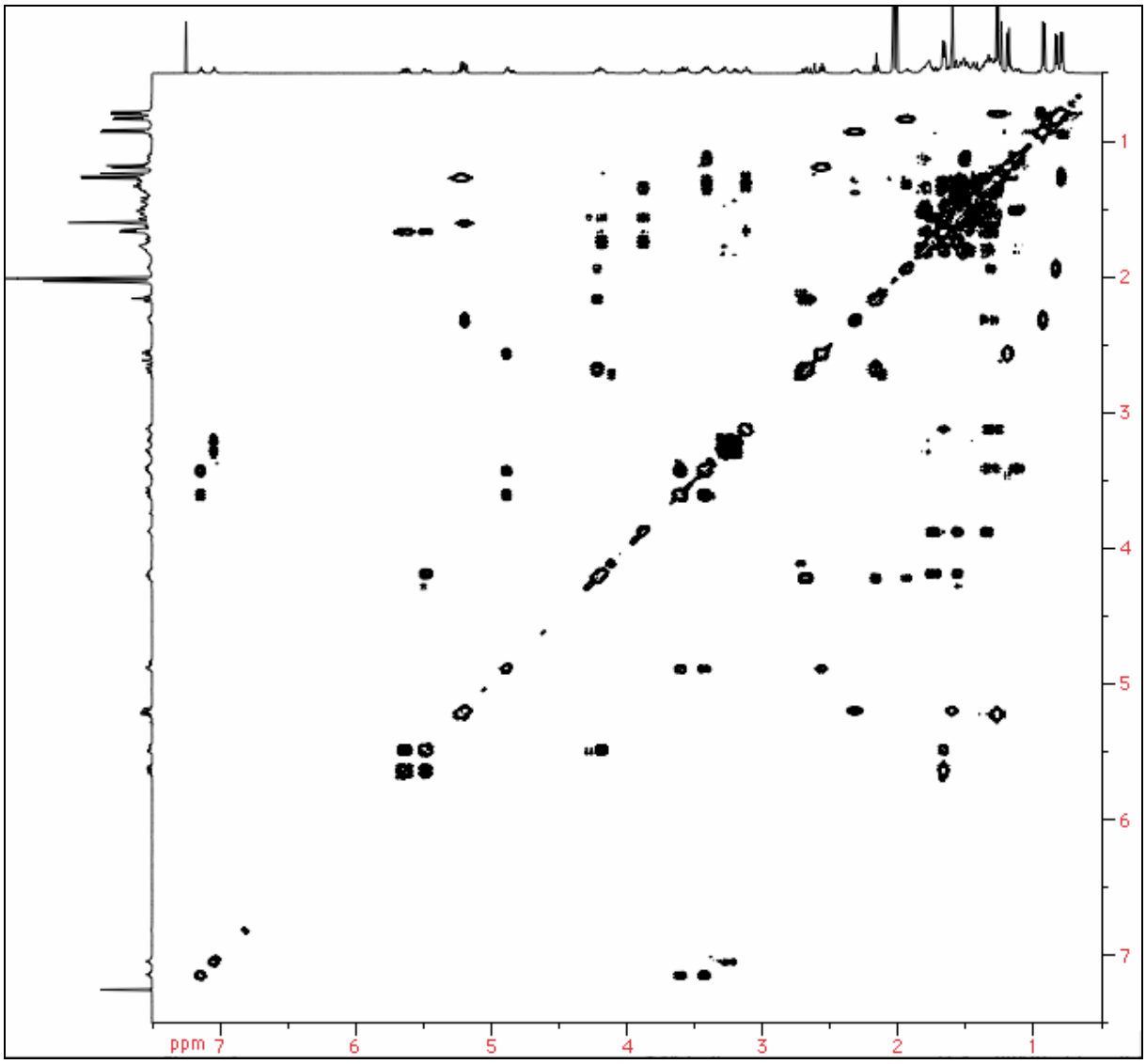
HMBC spectrum of BST Ada : **3** (500 MHz; $CDCl_3$)



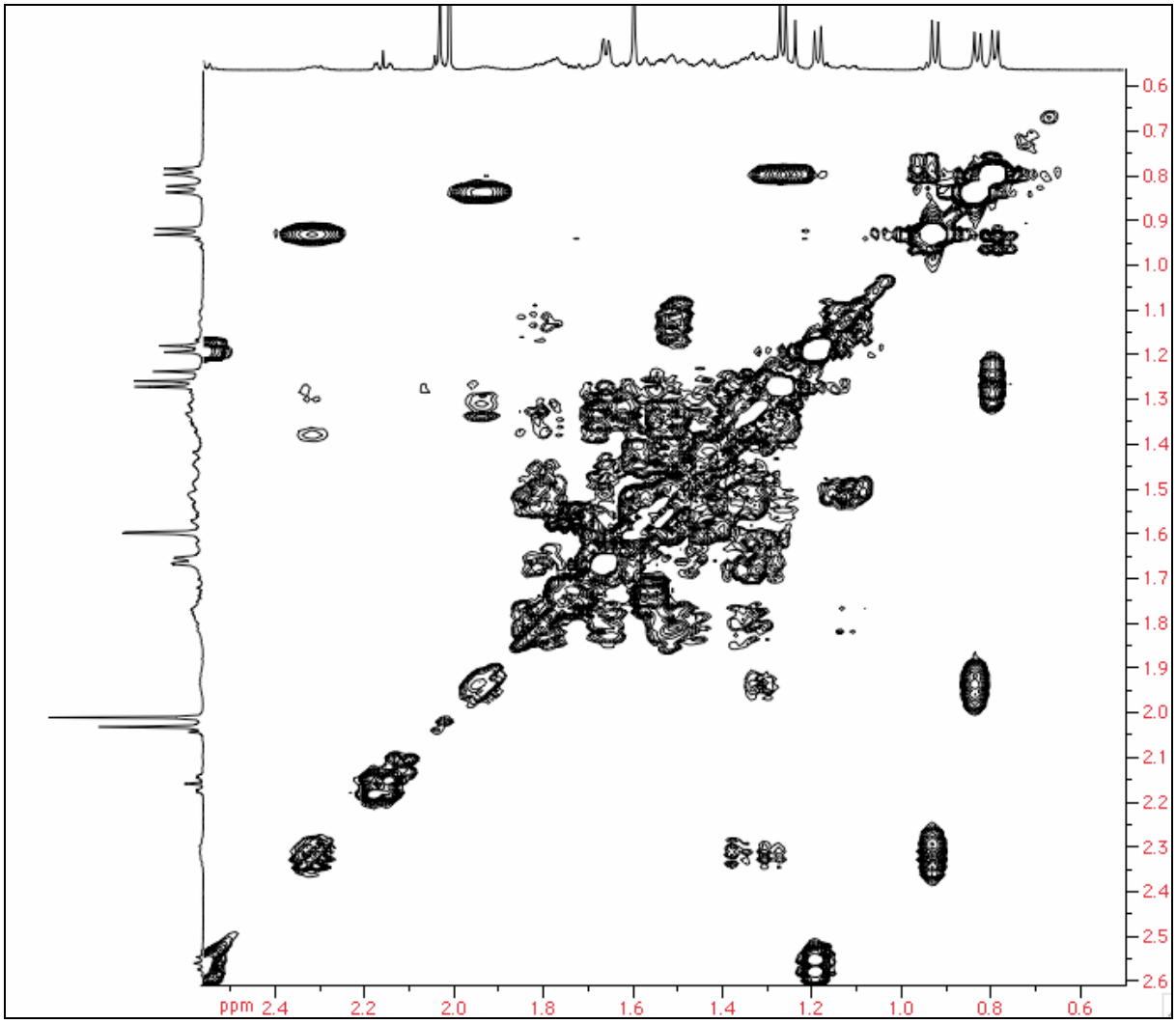
HMBC spectrum of BST Ada : **3** (500 MHz; $CDCl_3$)



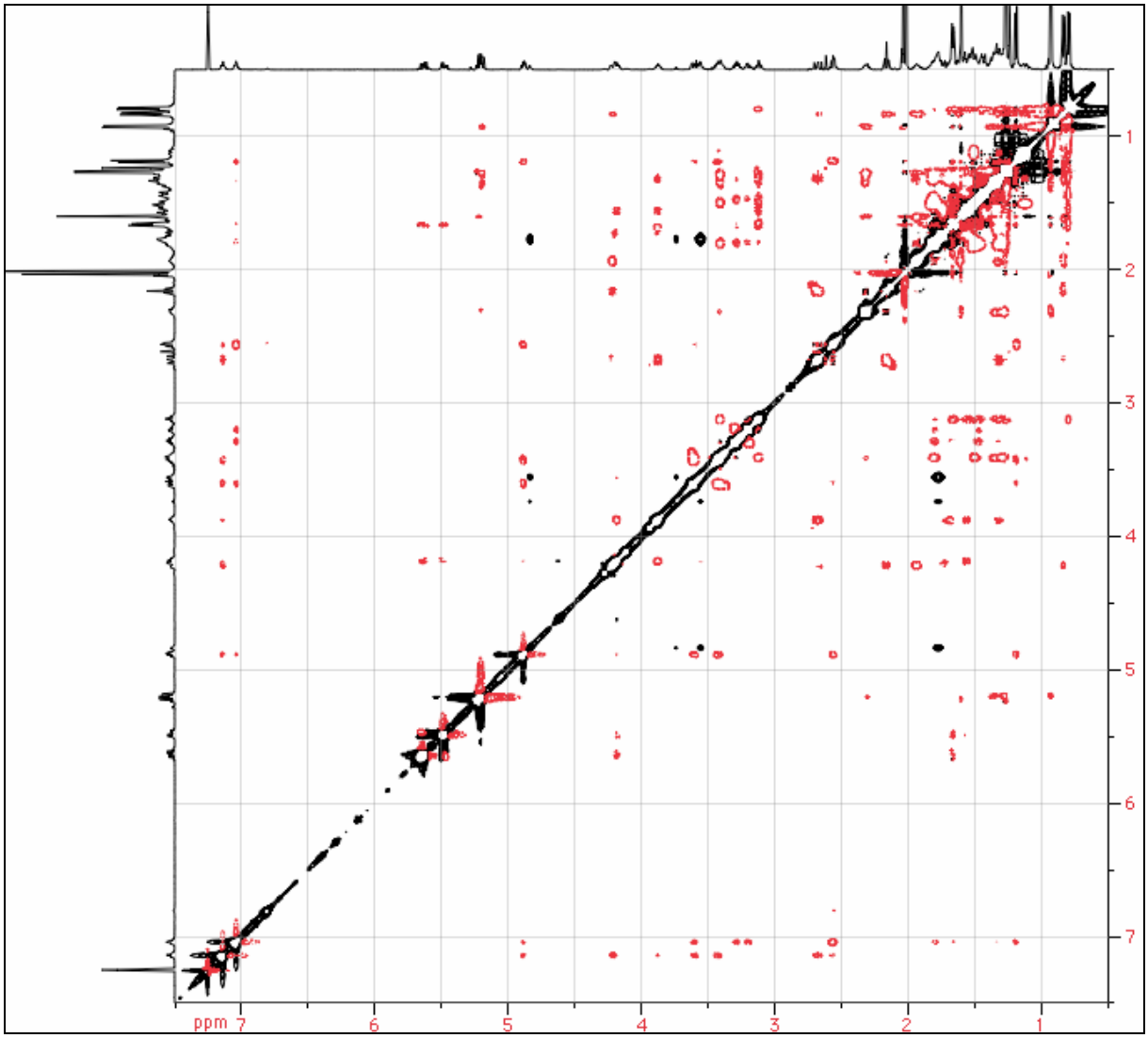
COSY spectrum of BST Dda : **4** (500 MHz; $CDCl_3$)



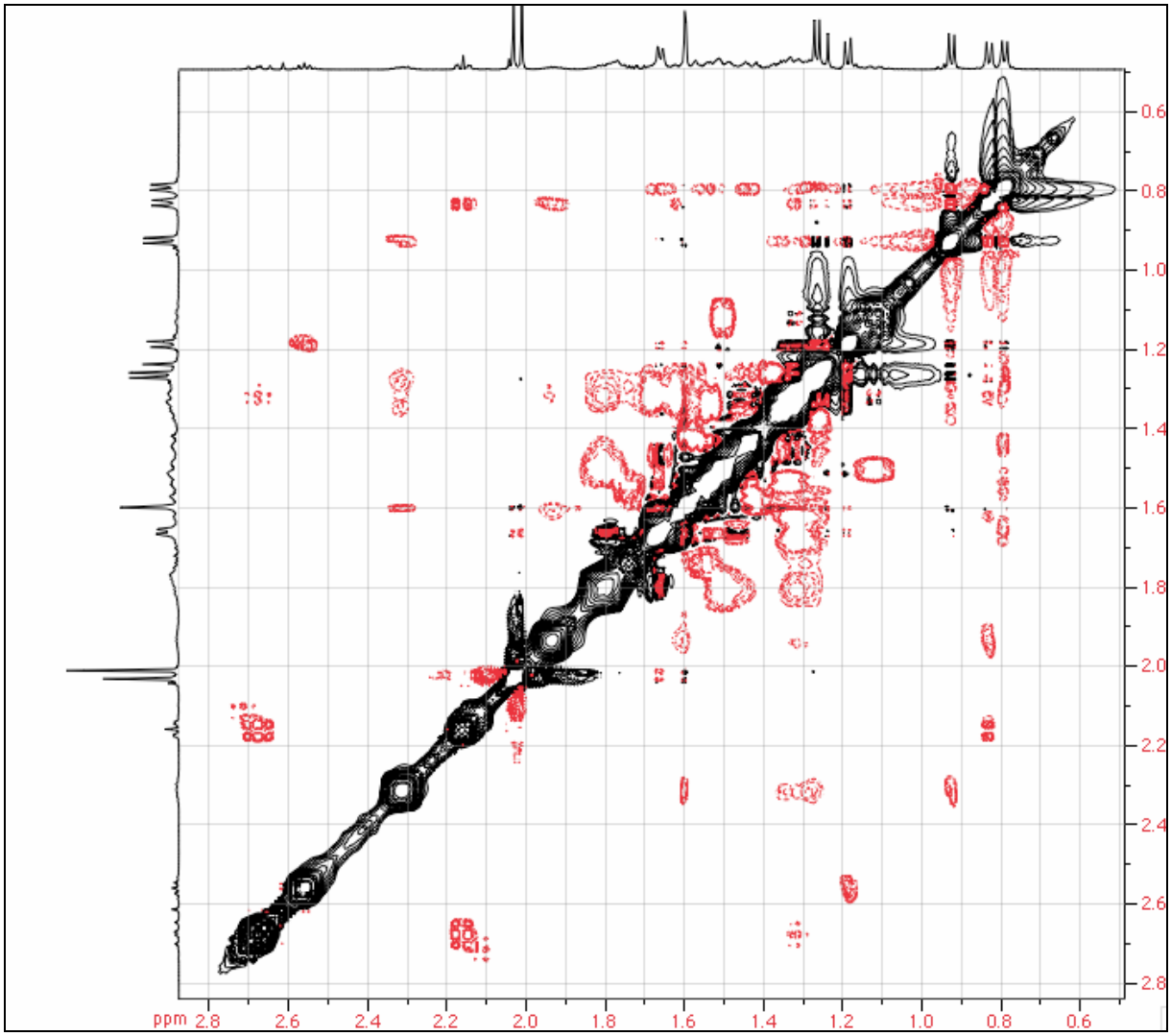
COSY spectrum of BST Dda : **4** (500 MHz; $CDCl_3$)



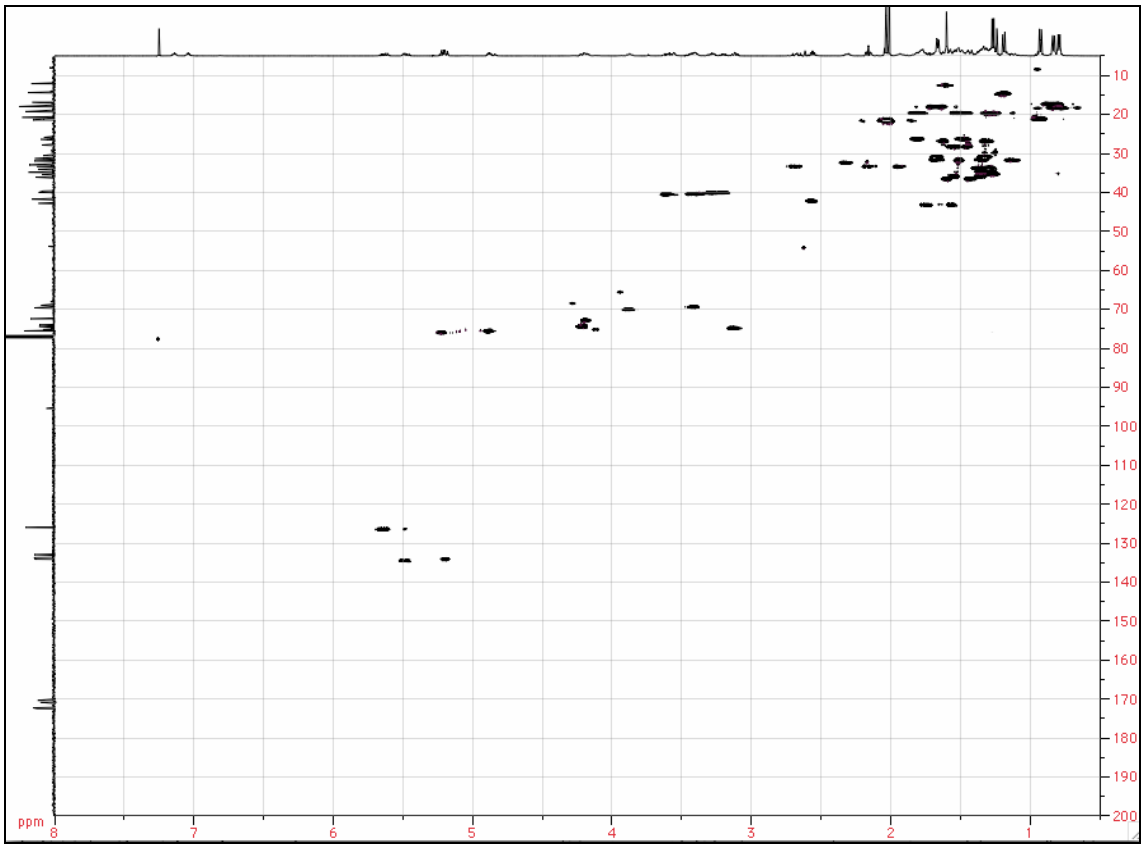
ROESY spectrum of BST Dda : **4** (500 MHz; $CDCl_3$)



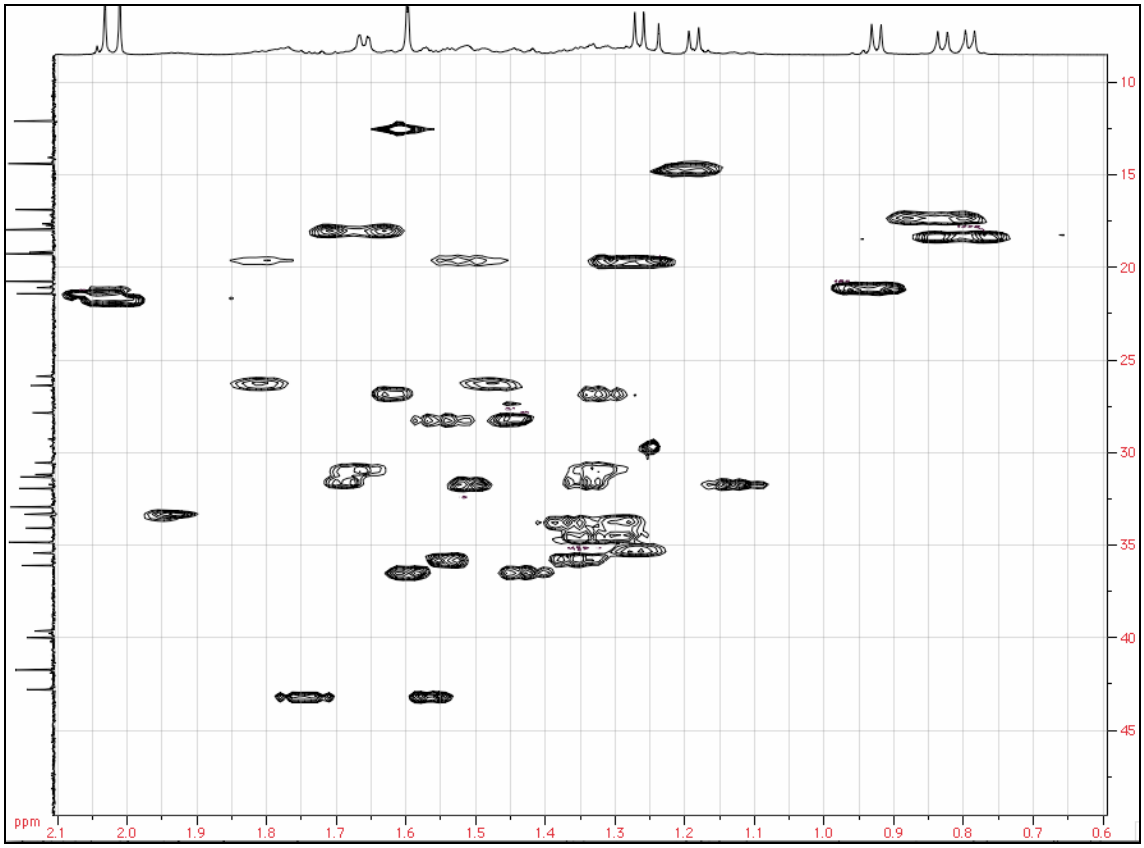
ROESY spectrum of BST Dda : **4** (500 MHz; $CDCl_3$)



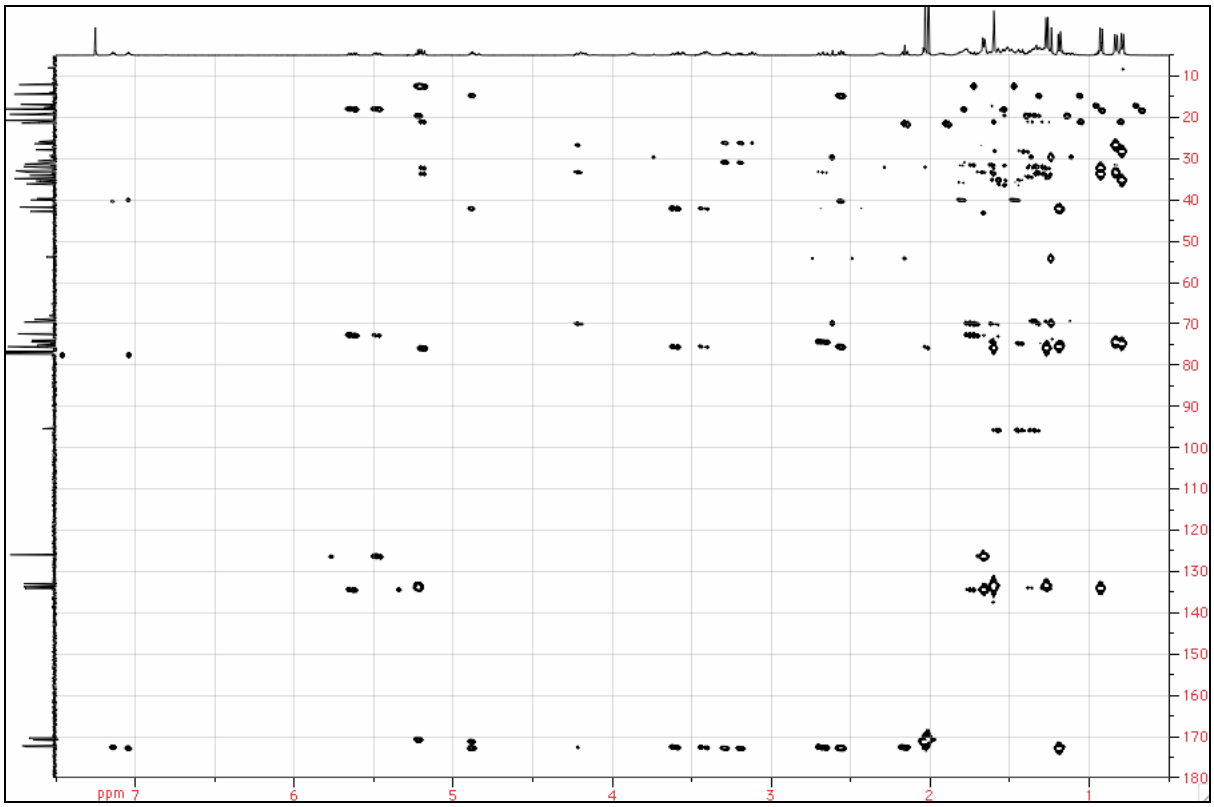
HSQC spectrum of BST Dda : **4** (500 MHz; $CDCl_3$)



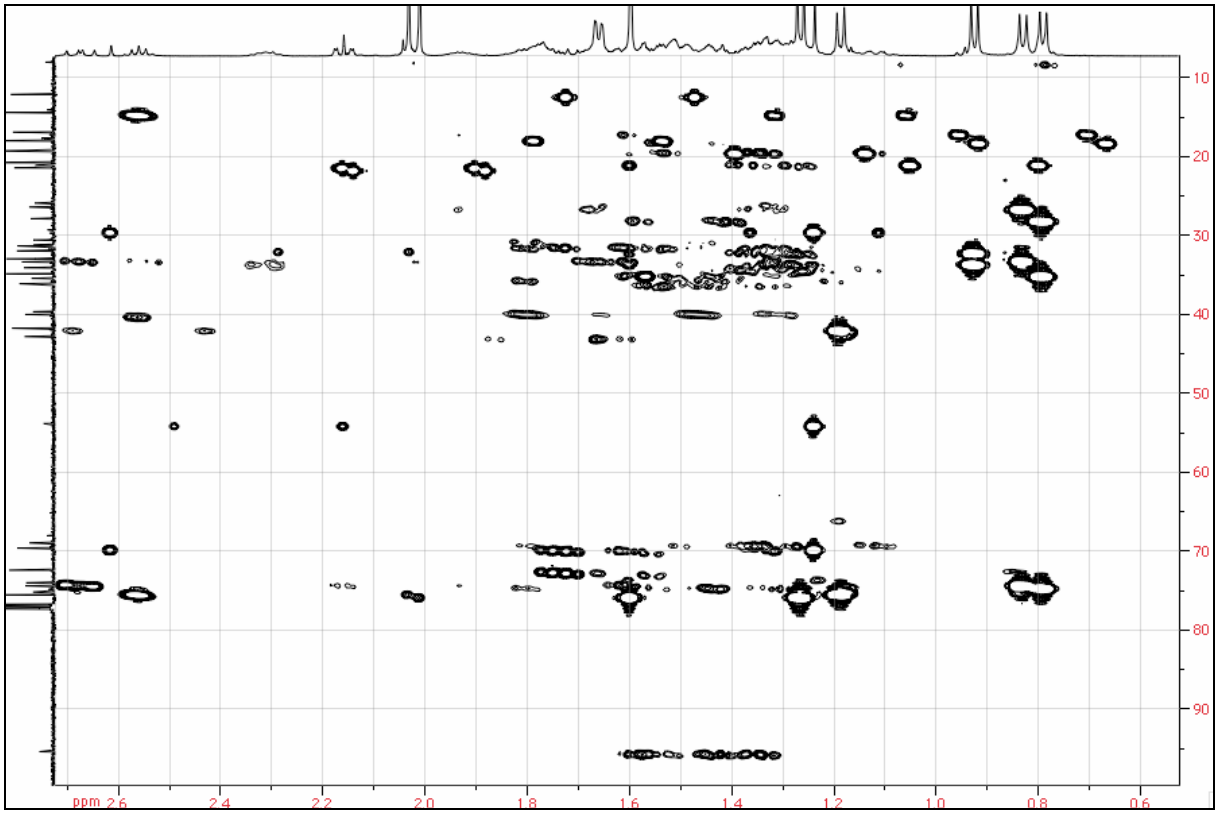
HSQC spectrum of BST Dda : **4** (500 MHz; $CDCl_3$)



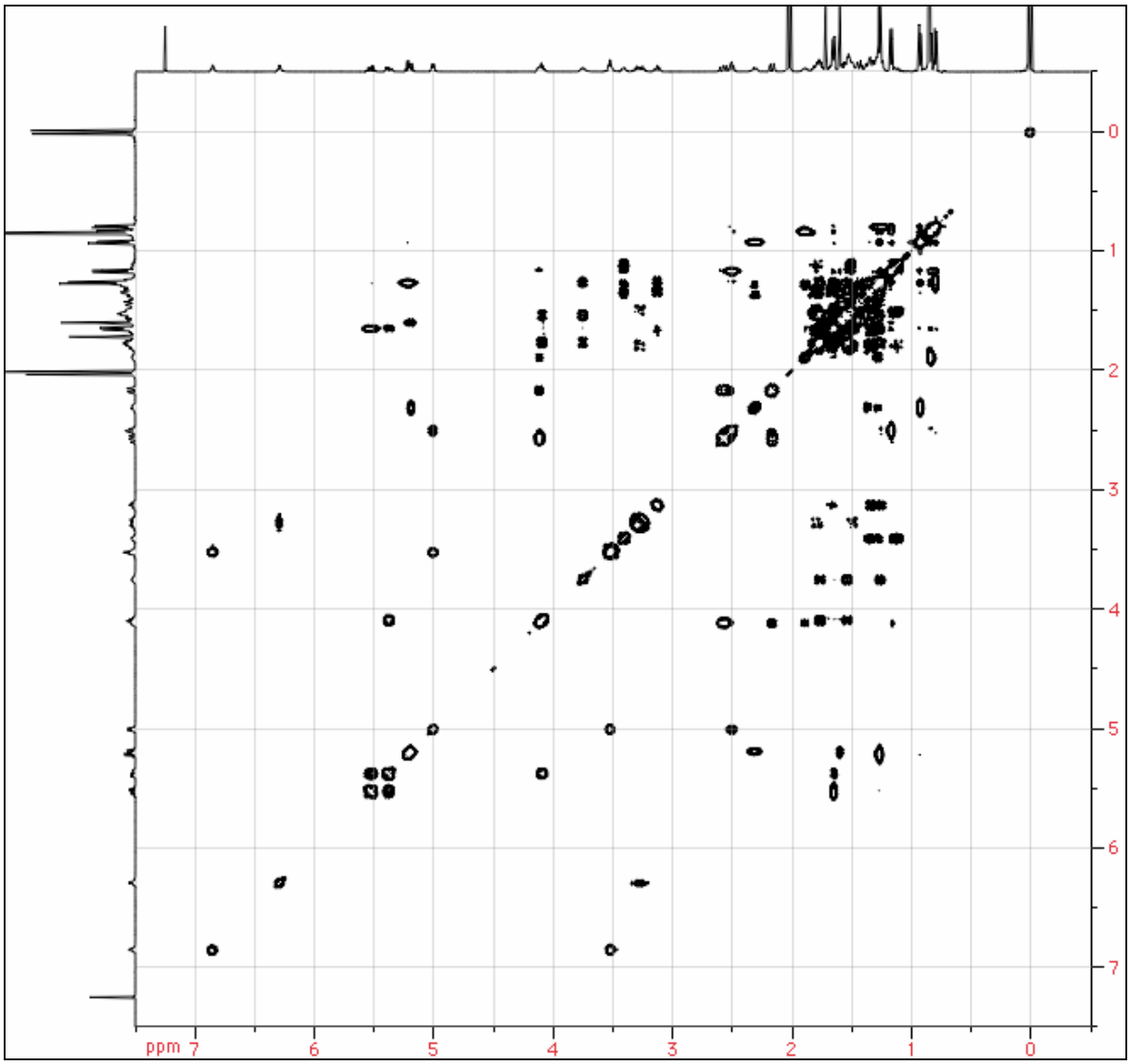
HMBC spectrum of BST Dda : **4** (500 MHz; $CDCl_3$)



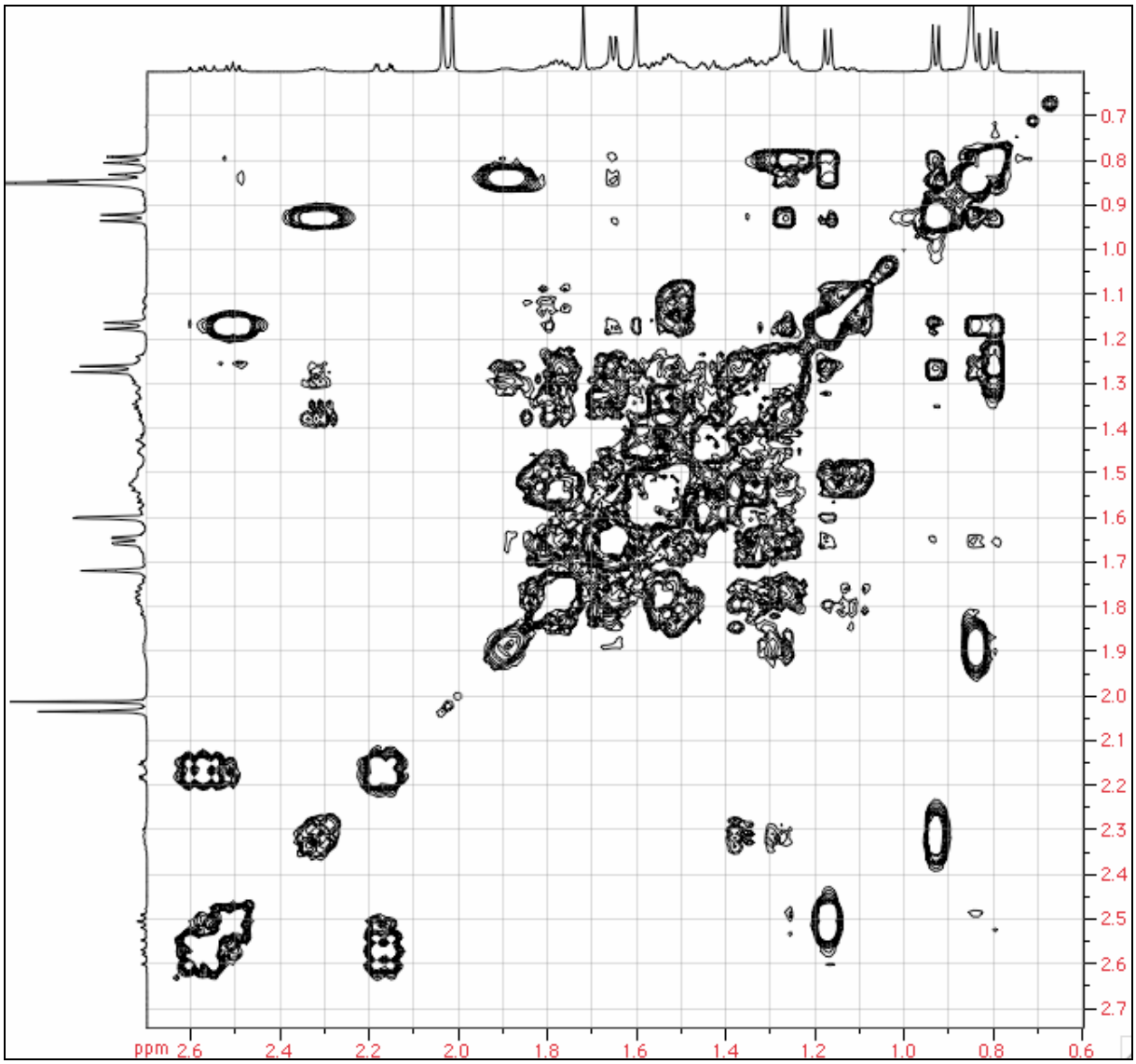
HMBC spectrum of BST Dda : **4** (500 MHz; $CDCl_3$)



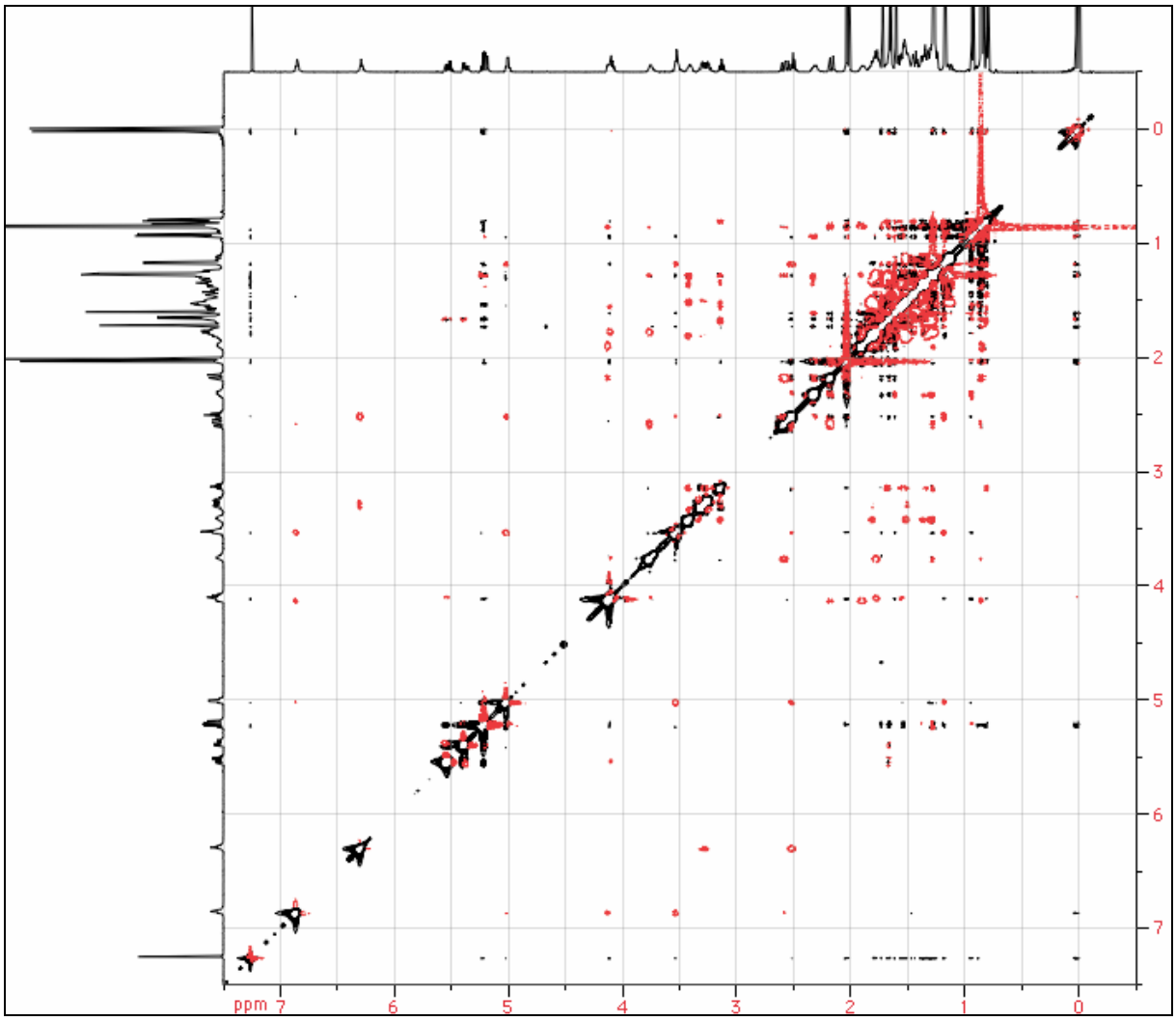
COSY spectrum of BST Dda, 4-TBS : **5** (500 MHz; $CDCl_3$)



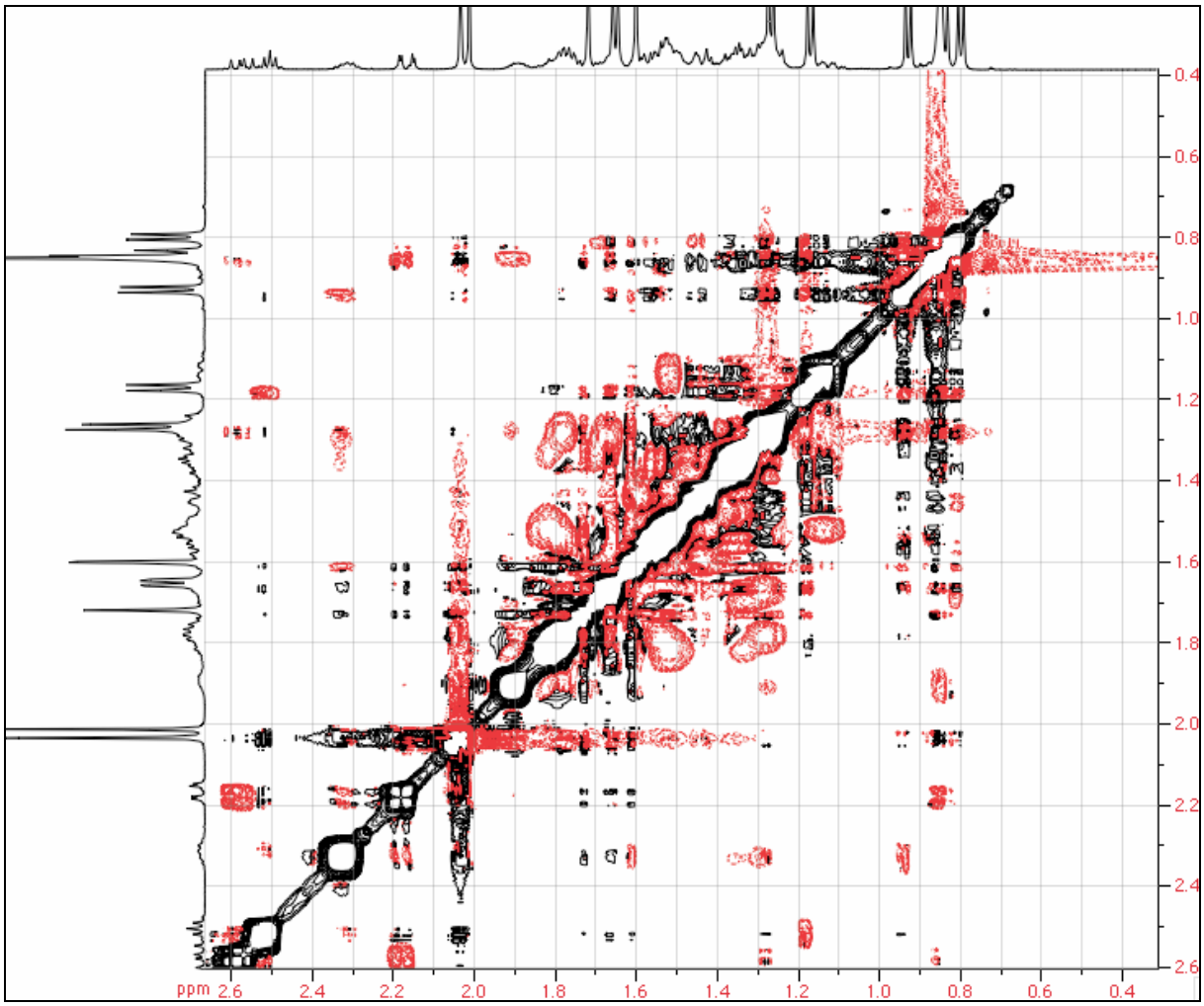
COSY spectrum of BST Dda, 4-TBS : **5** (500 MHz; $CDCl_3$)



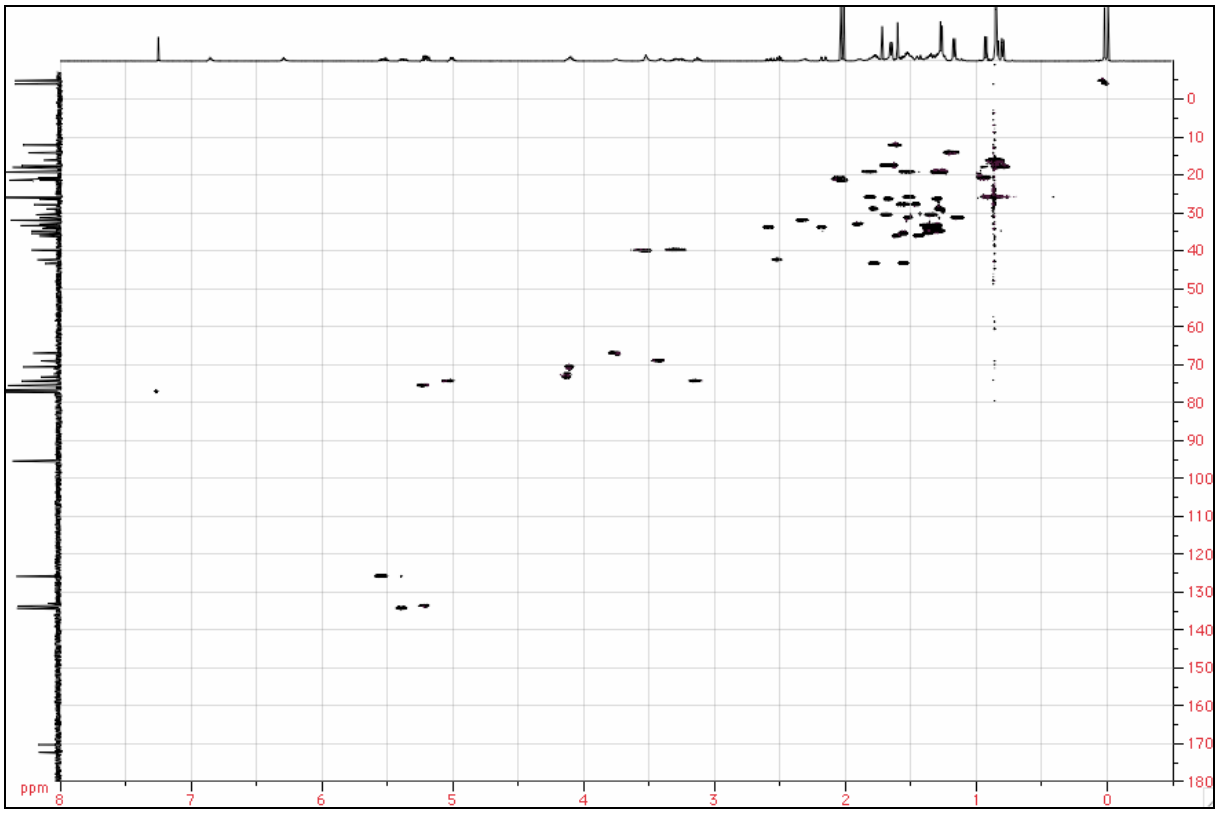
ROESY spectrum of BST Dda, 4-TBS : **5** (500 MHz; $CDCl_3$)



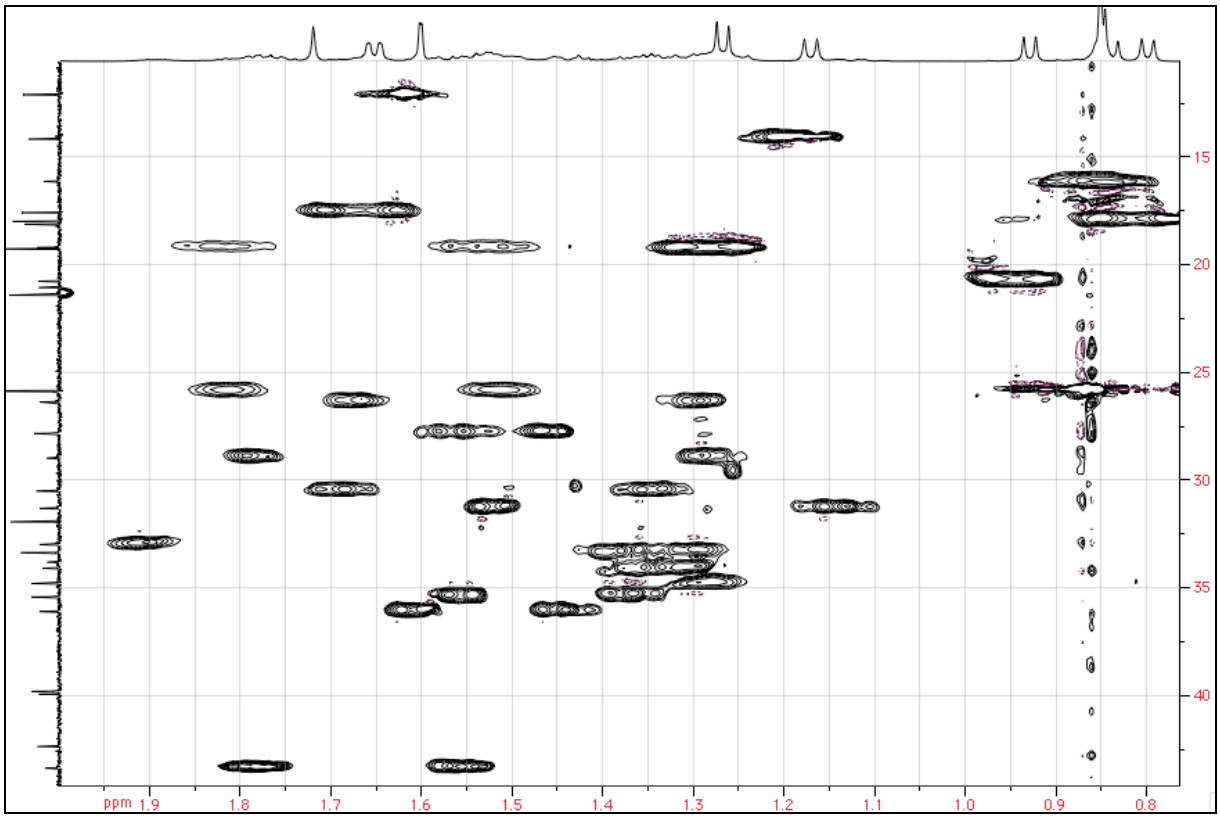
ROESY spectrum of BST Dda, 4-TBS : **5** (500 MHz; $CDCl_3$)



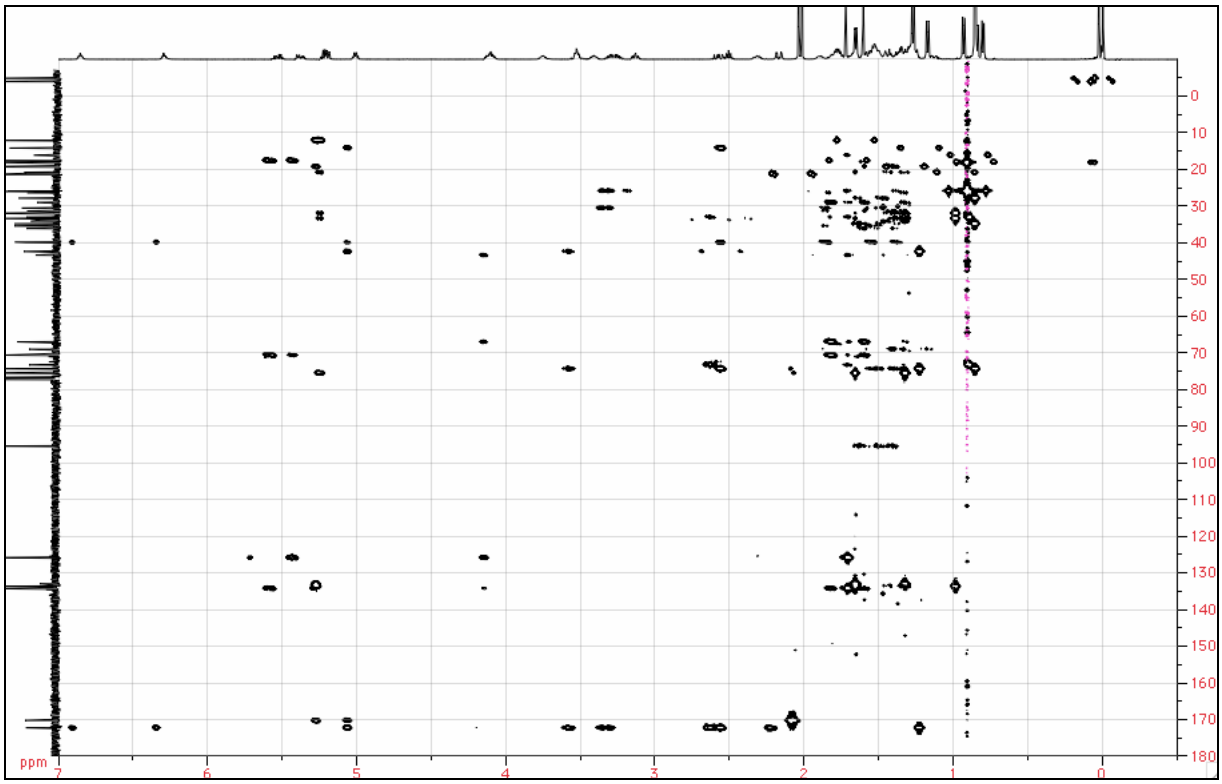
HSQC spectrum of BST Dda, 4-TBS : **5** (500 MHz; $CDCl_3$)



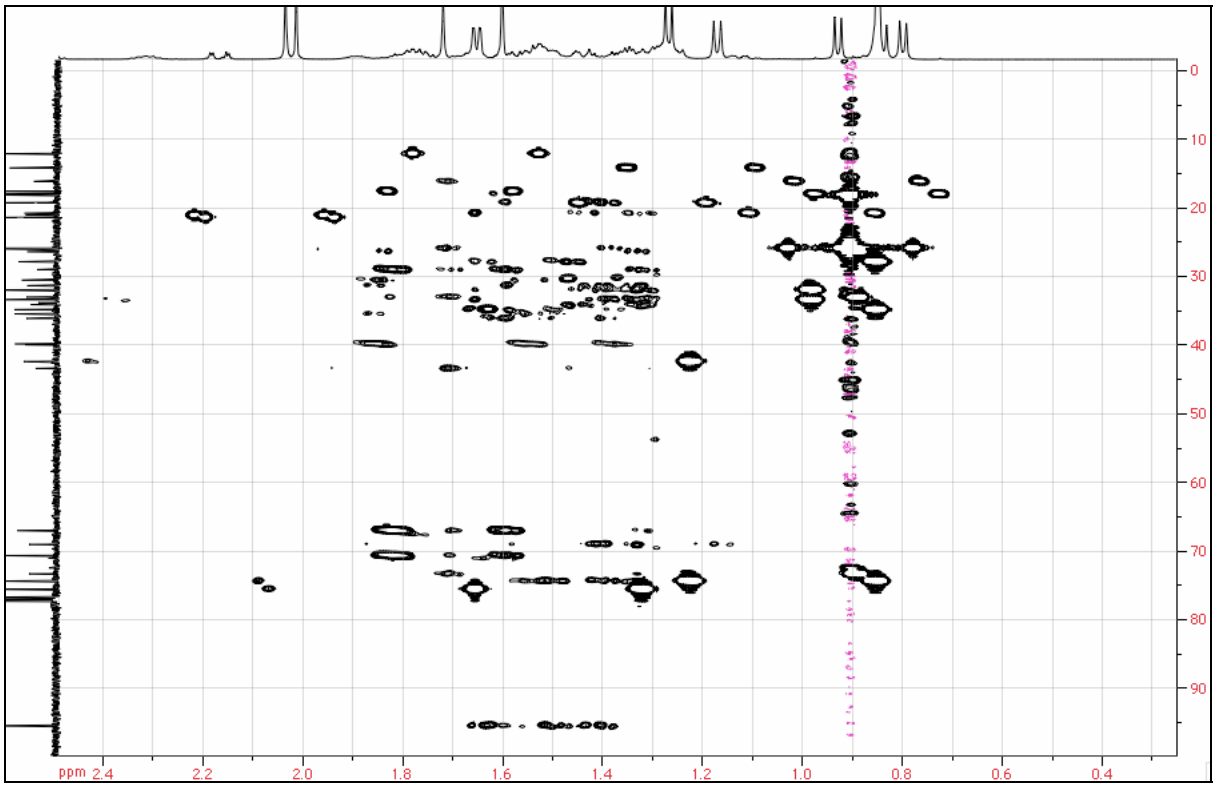
HSQC spectrum of BST Dda, 4-TBS : **5** (500 MHz; $CDCl_3$)



HMBC spectrum of BST Dda, 4-TBS : **5** (500 MHz; $CDCl_3$)



HMBC spectrum of BST Dda, 4-TBS : **5** (500 MHz; $CDCl_3$)

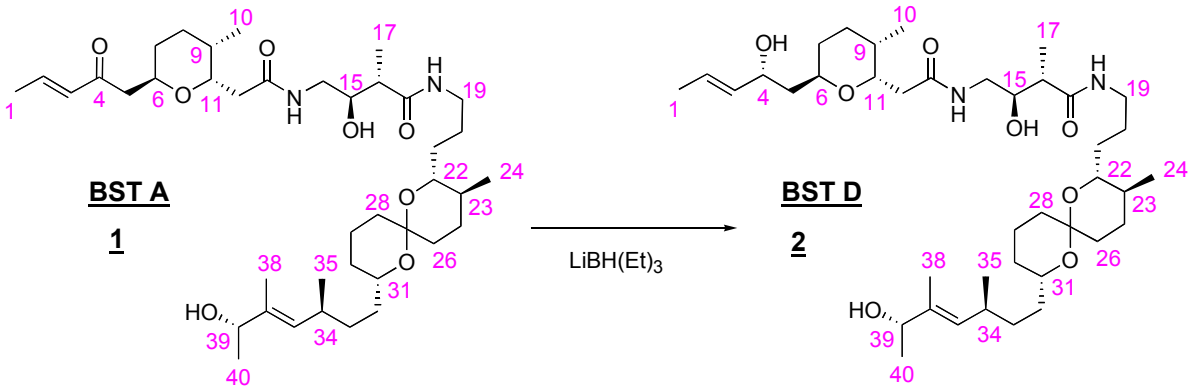


General Information.

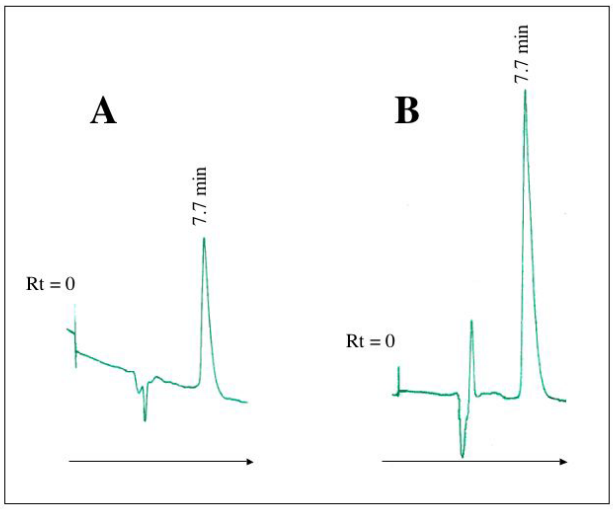
Reactions were carried out in oven or flame-dried glassware under an argon atmosphere, unless otherwise noted. All solvents used were of reagent grade. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone under argon immediately prior to use. Unless otherwise noted, reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm Merck pre-coated silica gel plates. Flash chromatography were performed with silica gel 60 (particle size 0.040-0.063 mm) supplied by Merck, Geduran. Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. 1H NMR, 13C NMR, COSY, ROESY, HSQC as well as HMBC spectra were measured on Bruker ARX-500 spectrometer using an internal deuterium lock at ambient temperature. If not otherwise noted, $CDCl_3$ (7.26 ppm relative to residual $CHCl_3$) is the solvent for all NMR experiments. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, ABX = an ABX system. Chemical shift are given in ppm and coupling constant are presented in Hz. Optical rotations [α] were recorded on a Polarimeter 241MC (Perkin Elmer) at a wavelength of 589 nm and are reported as follows: [α] D , concentration (*c* in g/100 mL) and solvent. Elemental analysis were collected at the Service de microanalyse of the University Louis Pasteur of Strasbourg.

Experimental section.

Stereoselective reduction of the bistramide A: BST D **2**.



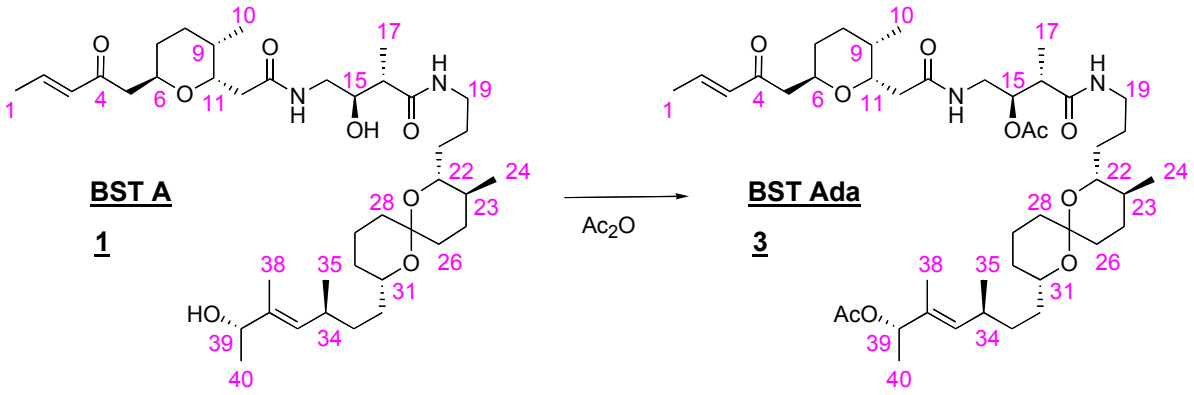
A solution of Super-Hydride® (1M in THF; 2.7 mL; 5.3 eq) was added dropwise at -78 °C to a precooled solution of bistramide A **1** (358 mg, 0.507 mmol) in dry THF (40 mL). The reaction was stirred for 1 h at this temperature and then quenched with a saturared aqueous solution of NH_4Cl (15 mL) and water (20 mL). The mixture was stirred at ambient temperature during 0.5 h. The organic layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic extracts were washed with water (15 mL), brine (15 mL) and then dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (acetone / CH_2Cl_2; 7:3) to afford the pure bistramide D **2** (254.4 mg; 70.9%).
The spectroscopic data (^{1}H and ^{13}C NMR) are in total agreement with the literature (J.F. Biard, C. Roussakis, J.M. Kornprobst, D. Gouiffes-Barbin, J.F. Verbist, P. Cotelle, M.P. Foster, C.M. Ireland and C. Debitus, J. Nat. Prod., 1994, 57 (10), 1336-45). See table 1 (below) for comparison of the ^{13}C NMR values.



HPLC chromatogram of natural bistramide D (**A**) and a mixture (50 / 50) of natural and synthetic bistramide D (**B**).

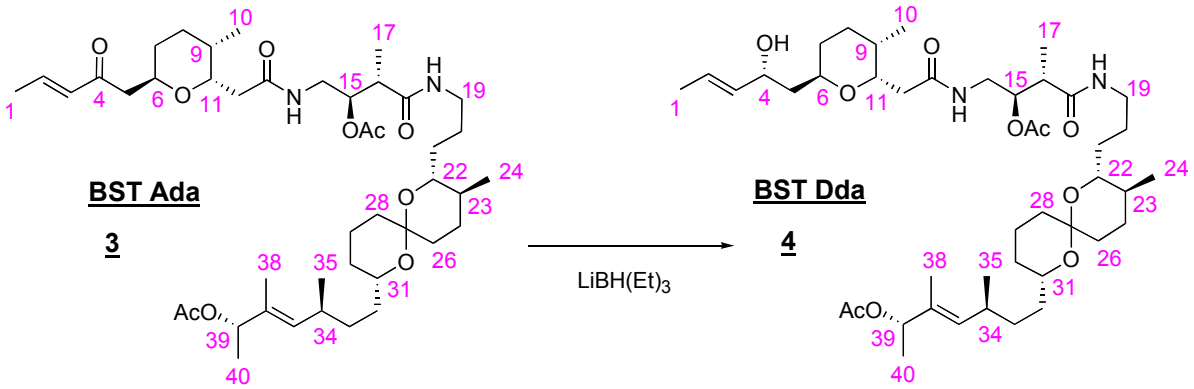
Each injection has a concentration of 250 µg / 25 µL in $CHCl_3$: MeOH (96 : 4) as solvant and eluant (1 mL / min). Silicagel column (Interchim Nucleosil 5 µm, 250 x 4.6 mm) was used and absorbance was monitored with a refractometer detector.

Acetylation of bistramide A into diacetylated product: BST Ada **3**.



Anhydride acetic (0.7 mL; 24 eq.) was added dropwise under argon to a solution of bistramide A **1** (217.5 mg; 0.308 mmol) in pyridine (3 mL). The resulting yellow mixture was stirred during 14 h at room temperature. Tlc analysis (acetone/ CH_2Cl_2 6:4) showed no more starting material so the reaction was diluted with diethylether (20 mL) and then quenched with a saturated aqueous solution of $CuSO_4$ (25 mL) and stirring was continue during 5 h at room temperature. The organic layer was washed with water (4 x 20 mL), brine (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to furnish **3** (223 mg; 91.9 %) as a white foam. Elemental analysis for $C_{44}H_{72}N_2O_{10}$ (789.050): calcd. C, 66.98; H, 9.20; N, 3.55. Found: C, 66.54; H, 9.26; N, 3.29.
For comparison with the other derivatives of the bistramides, the NMR spectroscopy data are given below in table 1 (for ^{13}C NMR) and in table 2 (for ^{1}H NMR).

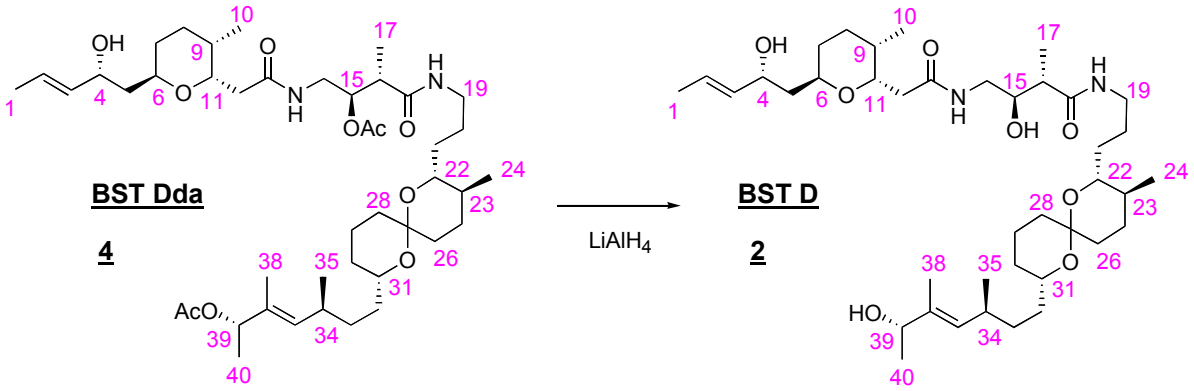
Stereoselective reduction of the diacetylated bistramide A: BST Dda **4**.



A solution of bistramide Ada **3** (127.9 mg; 0.162 mmole) in anhydrous THF (3.5 mL) was left under argon and stirred at -75°C during 30 min before adding dropwise the Super-Hydride® (1M in THF; 0.165; 1.02 eq). After 25 min at this temperature no starting material was observed (monitored by tlc, acetone/CH_2Cl_2 6:4). Thus the reaction was quenched by a saturated aqueous solution of NH_4Cl (10 mL) and the reaction flask was brought to room temperature for more 4 h. If no good separation is

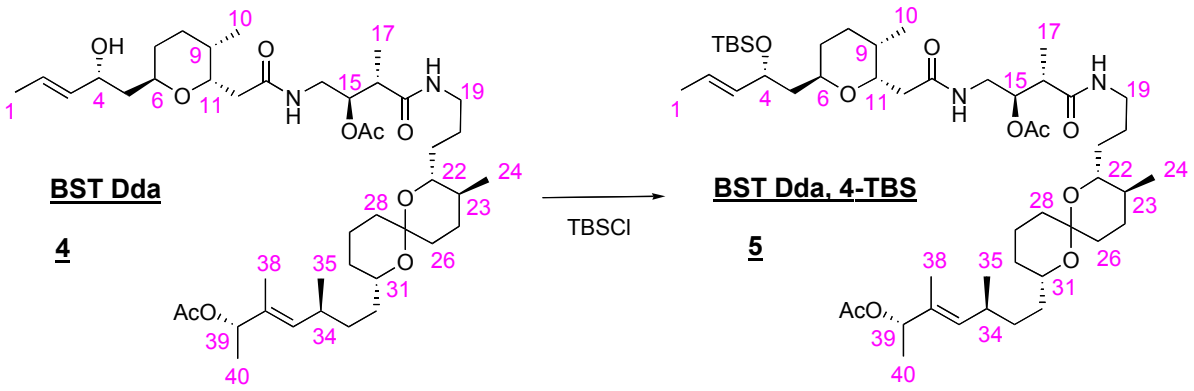
observed, it is recommanded to add a small quantity of HCl 5 % until pH 3-4, specially for gramme amount of starting material. The organic layer was concentrated under reduced pressure and the aqueous layer was extracted with AcOEt (4 x 15 mL). The combinated organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a white foam. The residue was purified by silica gel chromatography (eluant gradian, Acetone/CH_2Cl_2:1/3 to 1/1) to get **4** (92.9 mg; 72.5 %) as a white foam after evaporation of all the solvants. $[\alpha]_D^{20}$ + 10 (c 1.01 in CH_2Cl_2). Elemental analysis for $C_{44}H_{74}N_2O_{10}$ (791.066): calcd. C, 66.80; H, 9.43; N, 3.54. Found: C, 65.69; H, 9.50; N, 3.22.
For comparison with the other derivatives of the bistramides, the NMR spectroscopy data are given below in table 1 (for ^{13}C NMR) and in table 2 (for ^{1}H NMR).

Regioselective hydrolysis of bistramide Dda: BST D **2**.



A solution of $LiAlH_4$ (1M in Et_2O; 0.33 mL; 3.48 eq) was dropwise added at 0 °C into a solution of bistramide Dda **4** (75 mg; 0.095 mmol). A white precipitate was observed and the mixture was left for 16 h at room temperature. The reaction was then quenched with a saturated aqueous solution of NH_4Cl (3 mL) and water (3 mL) and stirring was continue during 6 h at ambient temperature. The aqueous layer was brought to pH 5 with HCl 10% and then extracted with Et_2O (3 x 10 mL). The combined organic extracts were washed with water (2 x 10 mL), brine (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the bistramide D **2** (54.1 mg; 80.9%) which exhibited the same NMR characteristics as the bistramide D obtained above in the stereoselective reduction of bistramide A **1**.
The spectroscopic data (^{1}H and ^{13}C NMR) are in total agreement with the literature (J.F. Biard, C. Roussakis, J.M. Kornprobst, D. Gouiffes-Barbin, J.F. Verbist, P. Cotelle, M.P. Foster, C.M. Ireland and C. Debitus, J. Nat. Prod., 1994, 57 (10), 1336-45).

Silylation of diacetylated bistramide Dda: BST Dda 4-TBS **5**.



Bistramide Dda **4** (270.8 mg; 0.342 mmole) and imidazole (76 mg; 3.26 eq) were dissolved under argon in DMF (5 mL) and then TBDMSCl (177 mg; 3.43 eq) was added int one portion. The mixture was stirred 16 h at room temperature before hydrolysis by water (30 mL) and diluted with diethylether (50 mL). Vigorous stirring was continued for 1.5 h and then the aqueous layer was extracted with diethylether (2 x15 mL). The combined organic layers were washed with water (6 x 50 mL), brine (15 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to obtain a crude product. The residue was purified by column chromatography on silica gel (AcOEt/CH_2Cl_2 1:1) to get **5** (231 mg; 74.6 %) as a white solid. In order to have some crystals of this compound for X-ray analysis we recrystallized it in hot pentane. Mp = 127-128 °C (pentane). $[\alpha]_D^{20}$ + 84 (c 1.19 in CH_2Cl_2). Elemental analysis for $C_{50}H_{88}N_2O_{10}$ Si (905.327): calcd. C, 66.33; H, 9.80; N, 3.09. Found: C, 66.20; H, 9.92; N, 2.85.

For comparison with the other derivatives, the NMR spectroscopy data are given below in table 1 (for ^{13}C NMR) and in table 2 (for ^{1}H NMR).

Table 1 : ^{13}C RMN (50 MHz, $CDCl_3$) for natural BST D and BST D (**2**)
^{13}C RMN (125 MHz, $CDCl_3$) for BST Ada (**3**), BST Dda (**4**) and BST Dda, 4-TBS (**5**)

natural BST D	BST D (**2**)	BST Ada (**3**)	BST Dda (**4**)	BST Dda, 4-TBS (**5**)
175.6; CO-18	175.9	198.5; CO	172.5; CONH-13	172.3; CONH-13
172.3; CO-13	172.4	172.5; CONH-13	172.3; CONH-18	172.2; CONH-18
137.1; C37	137.2	172.1; CONH-18	170.8; COOMe	170.5; OCOMe-39
134.0; C2	134.1	170.1; COOMe	170.4; COOMe	170.2; OCOMe-15
131.1; C36	131.2	144.9; C2	134.2; C3	134.2; C36
125.7; C3	125.8	133.5; C36	133.8; C36	133.7; C3
95.5; C27	95.5	132.7; C37	133.0; C37	132.9; C37
74.2; C11	74.3	132.0; C3	126.0; C2	125.9; C2
74.2; C22	74.3	95.2; C27	95.5; C27	95.4; C27
73.2; C39	73.3	75.4; C39	75.6; C39	75.6; C39
73.1; C15	73.2	74.4; C11	75.2; C15	74.3; C15 and C22
71.9; C4	72.0	74.2; C15 and C22	74.4; C22	73.3; C11
69.4; C6	69.4	68.7; C31	74.0; C11	70.6; C4
69.1; C31	69.2	64.7; C6	72.4; C4	69.0; C31
43.8; C14	43.9	45.0; C5	69.6; C6	66.9; C6
43.2; C16	43.2	41.6; C16	69.0; C31	43.4; C5
42.9; C5	42.9	39.8; C14	42.9; C5	42.3; C16
39.4; C19	39.4	39.6; C19	41.8; C16	39.9; C14
36.1; C26	36.1	35.9; C26	40.0; C14	39.8; C19
35.4; C28	35.5	35.3; C28	39.7; C19	36.1; C26
34.8; C23	34.9	34.7; C23	36.2; C26	35.4; C28
34.0; C32	34.1	33.9; C32	35.5; C28	34.8; C23
33.5; C33	33.6	33.2; C33	34.9; C23	34.1; C32
33.1; C12	33.1	33.1; C9	34.1; C33	33.6; C12
33.0; C9	32.9	32.3; C12	33.4; C32	33.4; C33
31.8; C34	31.9	31.8; C34	33.0; C9	33.0; C9
31.2; C30	31.3	31.2; C30	32.9; C12	31.9; C34
31.1; C7	31.2	30.5; C21	32.0; C34	31.3; C30
30.2; C21	30.3	30.4; C7	31.4; C30	30.5; C21
27.8; C25	27.9	27.7; C25	31.3; C7	29.0; C7
26.4; C8	26.5	26.3; C8	30.6; C21	27.8; C25
25.6; C20	25.7	25.9; C20	27.9; C25	26.3; C8
21.8; Me-40	21.8	21.3; OCOMe	26.4; C8	25.9; C20
20.9; Me-35	20.9	20.9; OCOMe	25.9; C20	25.8; tBu-Si
19.2; C29	19.3	20.6; Me-35	21.5; OCOMe	21.5; OCOMe
17.9; Me-24	18.0	19.1; Me-40	21.2; OCOMe	21.1; OCOMe
17.6; Me-1	17.7	19.0; C29	20.8; Me-35	20.8; Me-35
16.8; Me-10	17.0	18.2; Me-1	19.3; Me-40	19.2; Me-40
15.8; Me-17	15.9	17.8; Me-24	19.2; C29	19.1; C29
11.9; Me-38	12.0	16.9; Me-10	18.0; Me-24	18.1; C-Si
		14.3; Me-17	17.7; Me-1	18.0 Me-24
		11.9; Me-38	17.0; Me-10	17.6; Me-1
			14.5; Me-17	16.2; Me-10
			12.2 ; Me-38	14.2 ;Me-17
natural BST D	BST D (**2**)	BST Ada (**3**)	BST Dda (**4**)	BST Dda, 4-TBS (**5**)
				12.1; Me-38

				-3.9; Me-Si
				-4.9; Me-Si

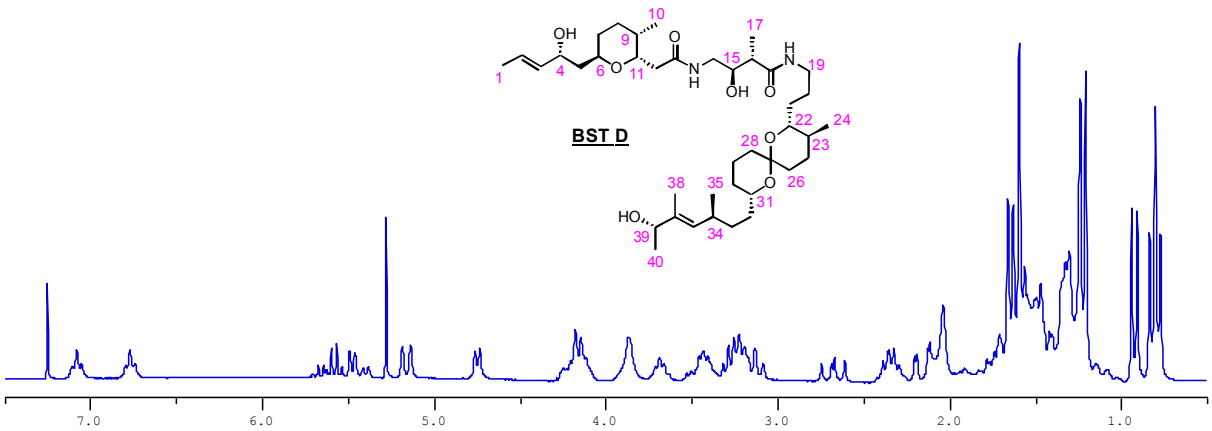
Table 2 : ^{1}H RMN (500 MHz, $CDCl_3$).

	BST Ada (**3**)		BST Dda (**4**)		BST Dda, 4-TBS (**5**)	
H	δ (ppm)	mult. $^{3}J_{H,H}$ (Hz)	δ (ppm)	mult. $^{3}J_{H,H}$ (Hz)	δ (ppm)	mult. $^{3}J_{H,H}$ (Hz)
1	1.90	dd 6.8, 1.6	1.66	dd 6.4, 1.4	1.66	dd 6.0, 1.6
2	6.92	dq 15.8, 6.9	5.64	dq 15.2, 6.4	5.53	ddq 15.4, 6.6, 1.1
3	6.12	dq 15.8, 1.6	5.49	ddq 15.2, 6.4, 1.5	5.38	ddq 15.4, 7.1, 1.6
4	-	-	4.21	m	4.09	dt 7.5, 5.4
5	2.88, 2.50	ABX 16.7, 9.0, 2.7	1.71, 1.56	m, m	1.77, 1.54	m, m
6	4.18	m	3.86	m	3.76	m
7	1.65, 1.34	m, m	1.49, 1.11	m, m	1.77, 1.27	m, m
8	1.58, 1.33	m, m	1.62, 1.31	m, m	1.66, 1.28	m, m
9	1.91	m	1.93	m	1.90	m
10	0.84	d 7.3	0.83	d 7.4	0.84	d 7.15
11	4.05	ddd 11.4, 4.9, 1.6	4.21	m	4.13	m
12	2.71, 2.12	ABX 15.0, 11.5, 1.6	2.67, 2.14	ABX 15.7, 11.8, 2.4	2.59, 2.17	ABX 6.4, 4.4, 1.1
13	-	-	-	-	-	-
14	3.65, 3.33	dt and dt 14.3, 5.8 and 15.1, 6.3	3.62, 3.43	m, m	3.53	dd 6.05 (c)
15	5.04	ddd 6.1 (d)	4.88	m	5.02	dt 7.1, 4.9
16	2.54	dq 6.8 (a)	2.57	dq 6.9 (a)	2.51	dq 7.1 (a)
17	1.18	d 7.0	1.20	d 6.9	1.17	d 7.1
18	-	-	-	-	-	-
19	3.29	m	3.29, 3.20	m, m	3.29	m
20	1.81, 1.50	m, m	1.80, 1.46	m, m	1.82, 1.52	m, m
21	1.66, 1.32	m	1.33, 1.65	m, m	1.67, 1.33	m, m
22	3.11	ddd 9.6 (b), 2.1	3.12	ddd 9.6 (b), 2.4	3.14	ddd 9.5 (b), 2.2
	BST Ada (**3**)		BST Dda (**4**)		BST Dda, 4-TBS (**5**)	

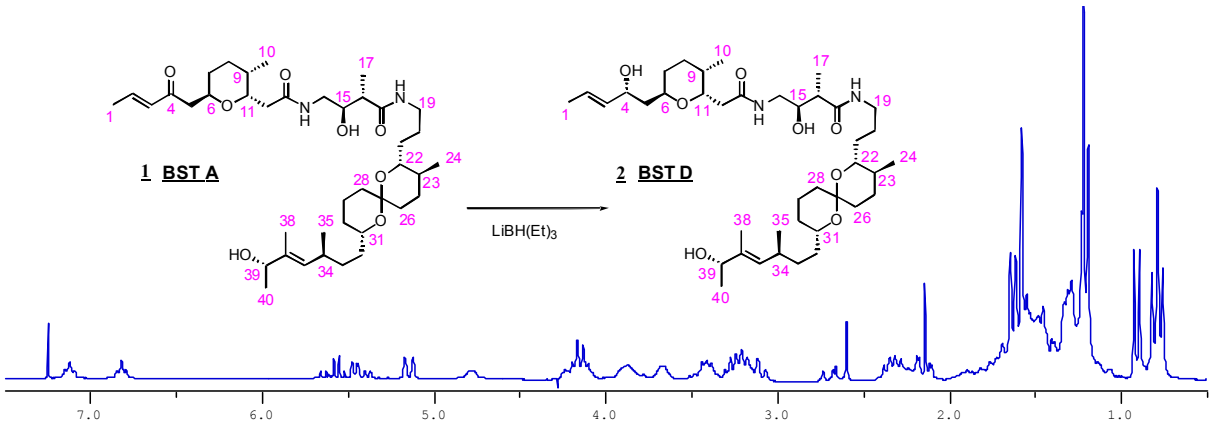
H	δ (ppm)	mult. $^3J_{H,H}$ (Hz)	δ (ppm)	mult. $^3J_{H,H}$ (Hz)	δ (ppm)	mult. $^3J_{H,H}$ (Hz)
23	1.26	m	1.24	m	1.27	m
24	0.78	d 6.4	0.80	d 6.8	0.80	d 6.6
25	1.52, 1.42	m, m	1.52, 1.41	m, m	1.55, 1.45	m, m
26	1.57, 1.40	m, m	1.58, 1.42	m, m	1.59, 1.43	m, m
27	-	-	-	-	-	-
28	1.52, 1.32	m, m	1.52, 1.32	m, m	1.54, 1.35	m, m
29	1.79, 1.48	m, m	1.77, 1.48	m, m	1.85, 1.54	m, m
30	1.48, 1.10	m, m	1.49, 1.11	m, m	1.50, 1.14	m, m
31	3.42	m	3.43	m	3.41	m
32	1.31, 1.24	m, m	1.33, 1.27	m, m	1.36, 1.29	m, m
33	1.35, 1.27	m, m	1.35, 1.28	m, m	1.37, 1.30	m, m
34	2.31	m	2.32	m	2.32	m
35	0.93	d 6.6	0.93	d 6.4	0.93	d 6.6
36	5.18	bd 8.9	5.20	bd 8.3	5.19	m
37	-	-	-	-	-	-
38	1.59	d 1.3	1.61	d 0.9	1.60	d 1.4
39	5.22	q 6.6	5.23	q 6.4	5.22	dq 6.6, 0.5
40	1.26	d 6.6	1.26	d 6.4	1.27	d 6.6
NH19	7.15	br t 6.2	7.04	br t 5.9	6.90	br t 5.8
NH14	6.85	br t 5.5	7.13	br t 5.7	6.35	br t 5.5
OAc	1.99	S (6H)	2.04, 2.02	2 x s	2.05, 2.03	2 x s
OH or TBDMS			3.56	br s	0.86 (tBu) 0.03 (MeSi) 0.001(MeSi)	3 x s

(a) : ($J_{16\text{-}15} = J_{16\text{-}17}$)
(b) : ($J_{22\text{-}21} = J_{22\text{-}23}$)
(c) : ($J_{14\text{-}15} = J_{14\text{-}NH}$)
(d) : ($J_{15\text{-}16} = J_{15\text{-}14a} = J_{15\text{-}14b}$)

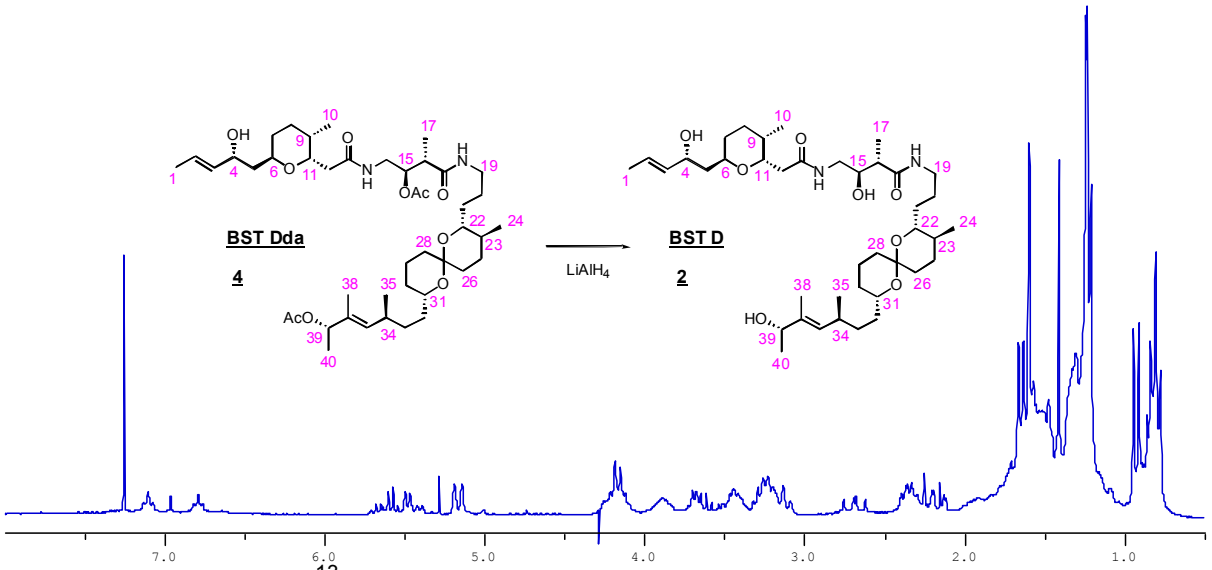
Natural bistramide D (^{1}H NMR, 200 MHz, $CDCl_3$)



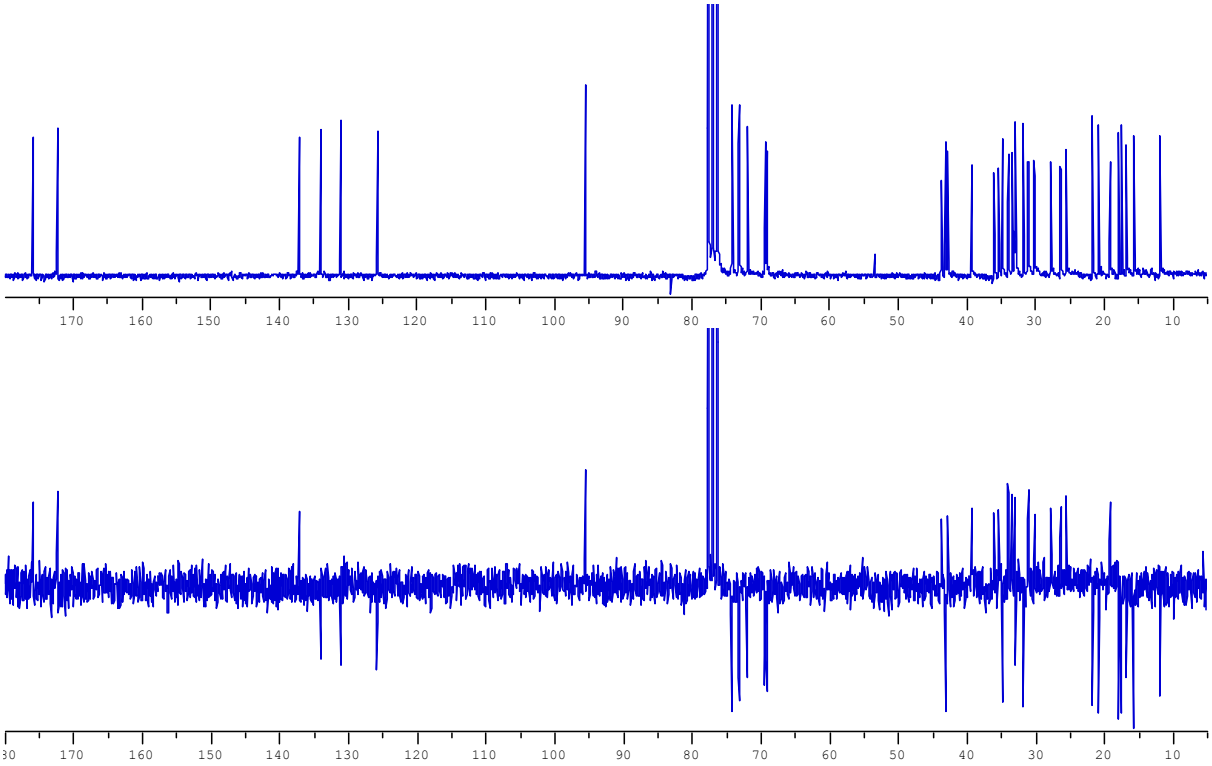
Hemisynthetic bistramide D, BST D : **2** (^{1}H NMR, 200 MHz, $CDCl_3$)



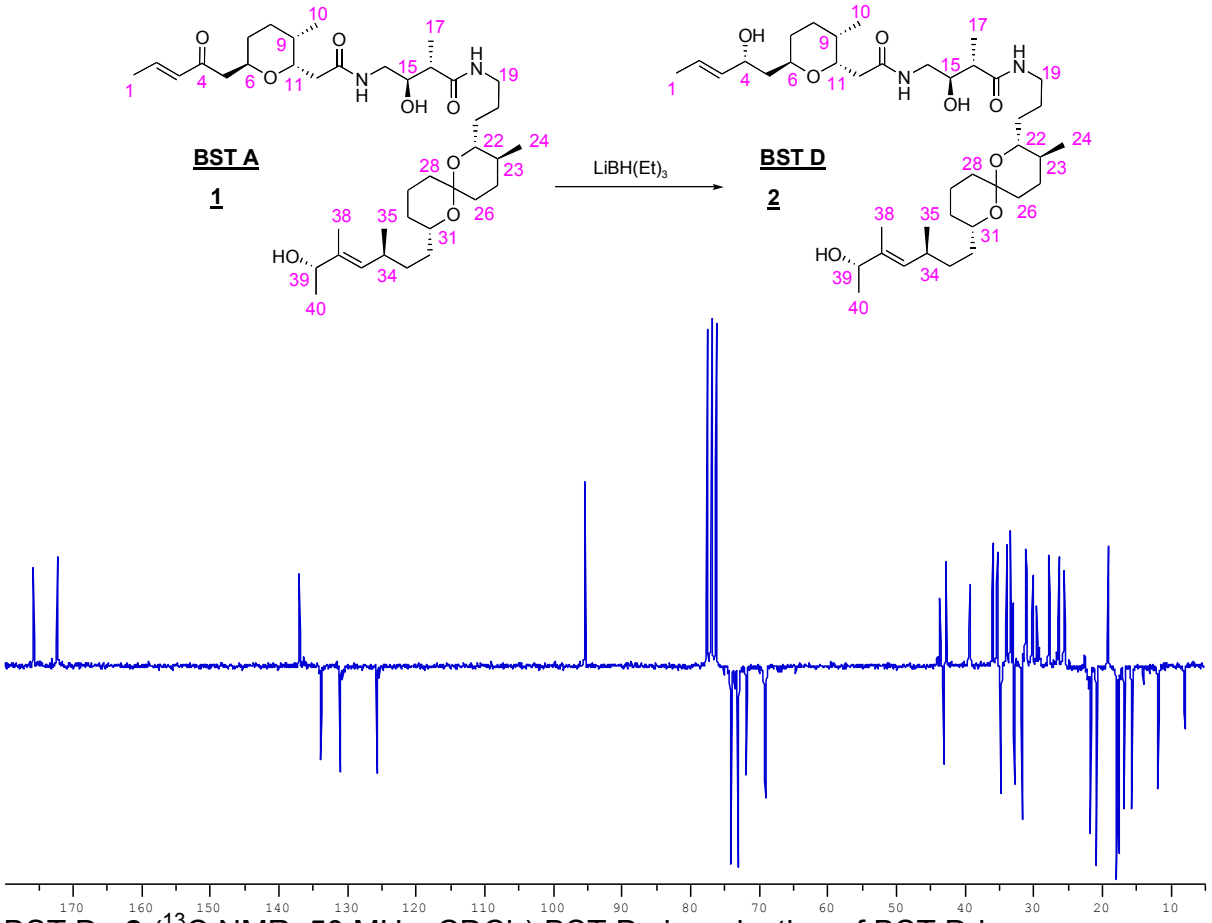
BST D : **2** (^{1}H NMR, 200 MHz, $CDCl_3$)



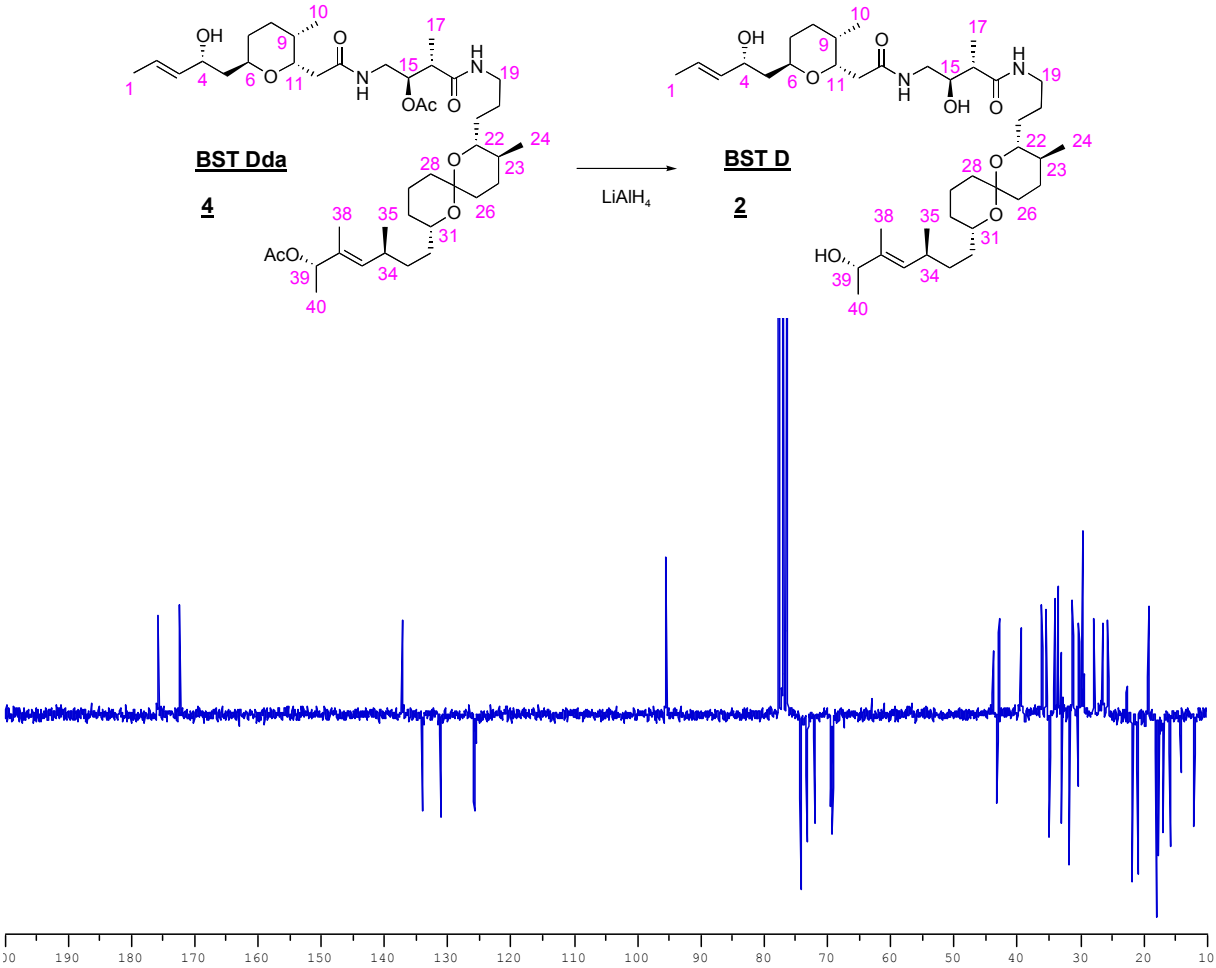
Natural bistramide D (^{13}C NMR, 50 MHz, $CDCl_3$)

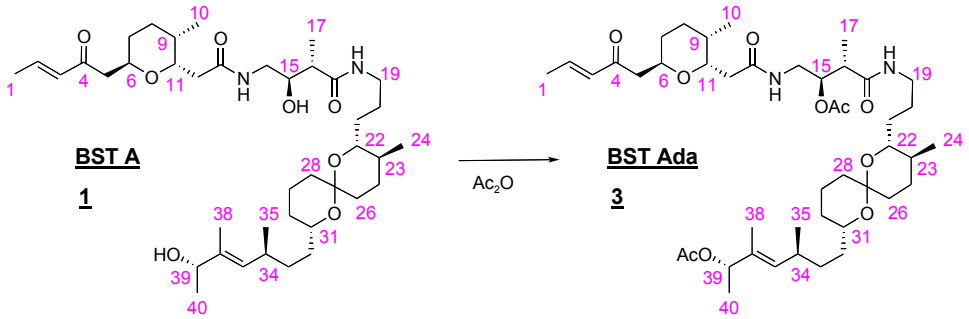


Hemisynthetic bistramide D, BST D : **2** (^{13}C NMR, 50 MHz, $CDCl_3$)

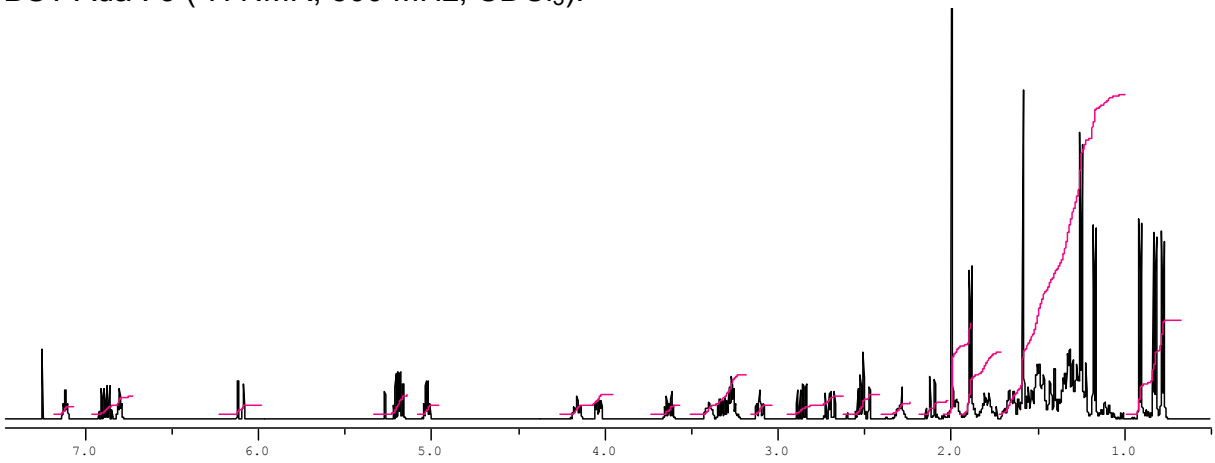


BST D : **2** (^{13}C NMR, 50 MHz, $CDCl_3$) BST D via reduction of BST Dda

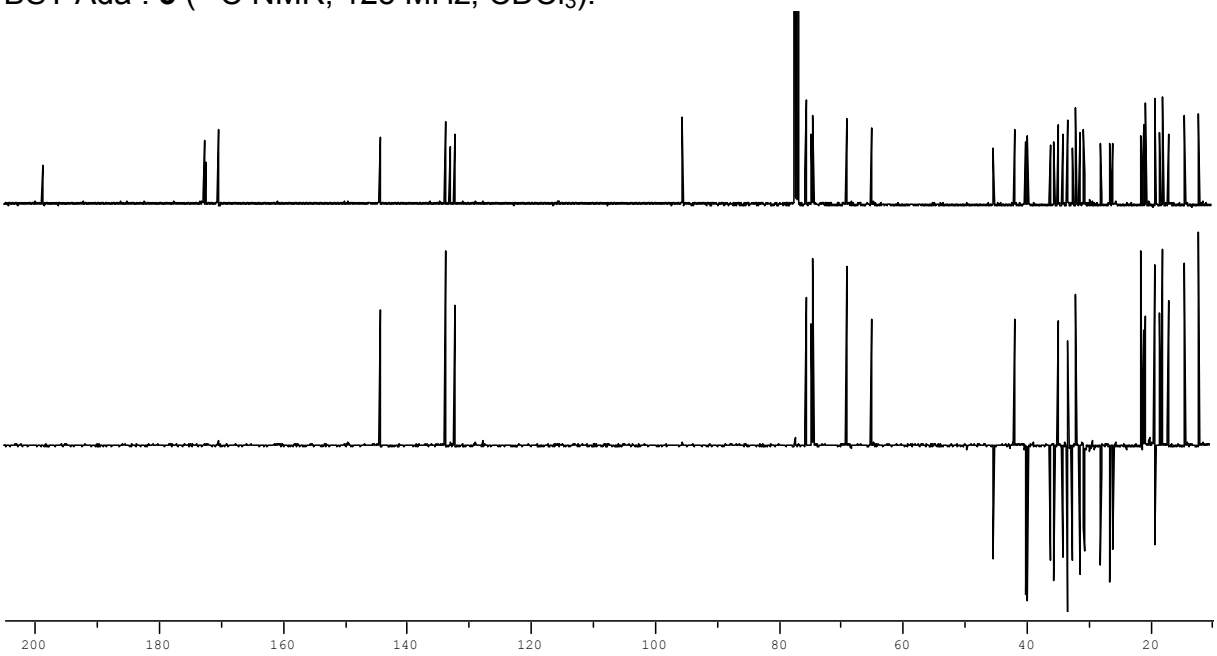

BST Dda
4
LiAlH4
BST D
2
OH
OAc
AcO
HO
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

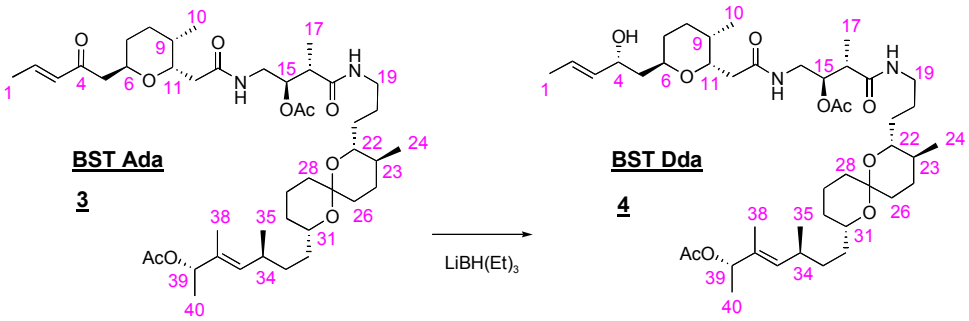


BST Ada : **3** (^{1}H NMR, 500 MHz, $CDCl_3$).

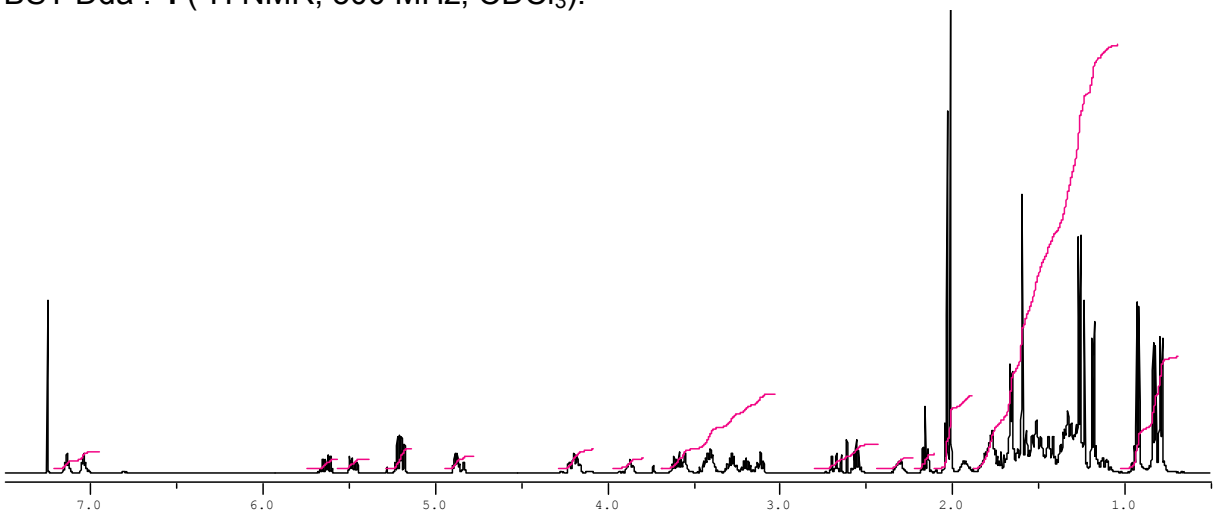


BST Ada : **3** (^{13}C NMR, 125 MHz, $CDCl_3$).

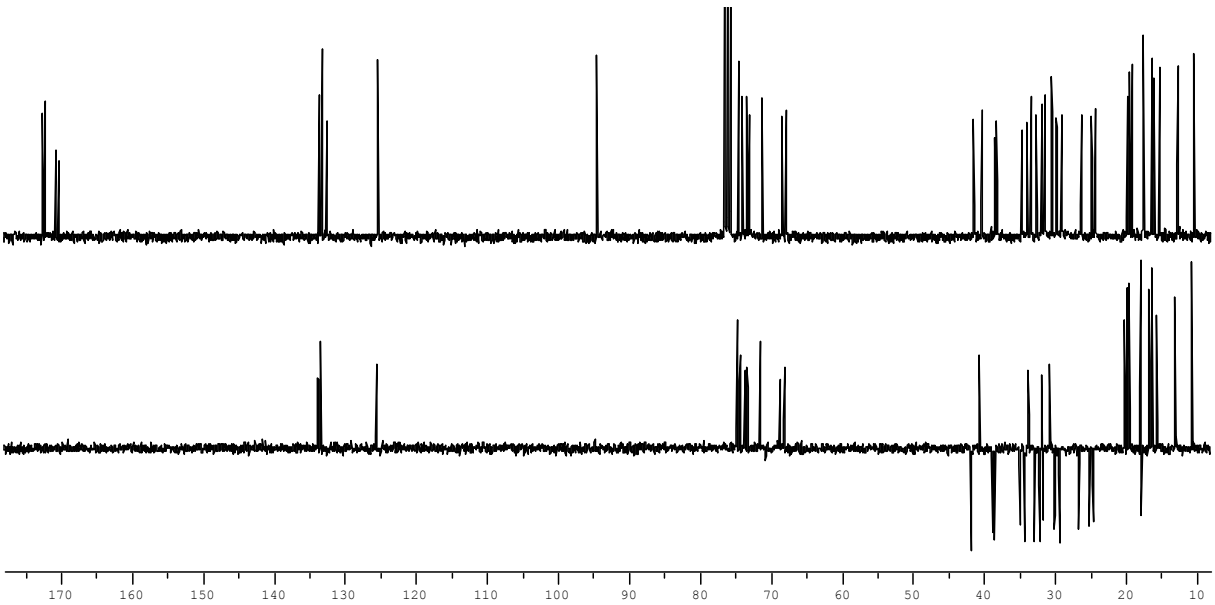


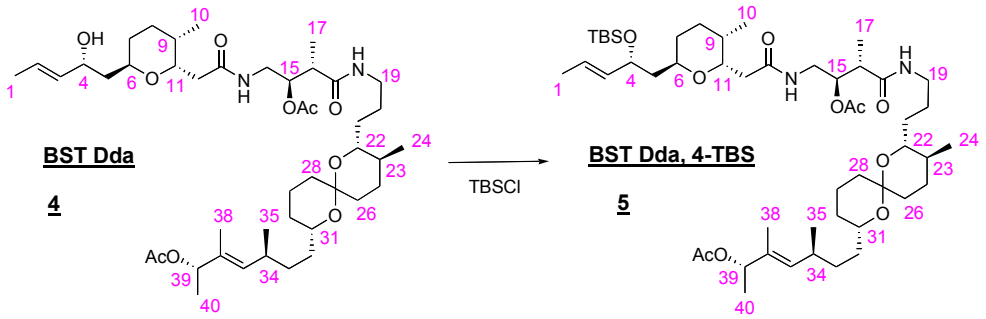


BST Dda : **4** (^{1}H NMR, 500 MHz, $CDCl_3$).

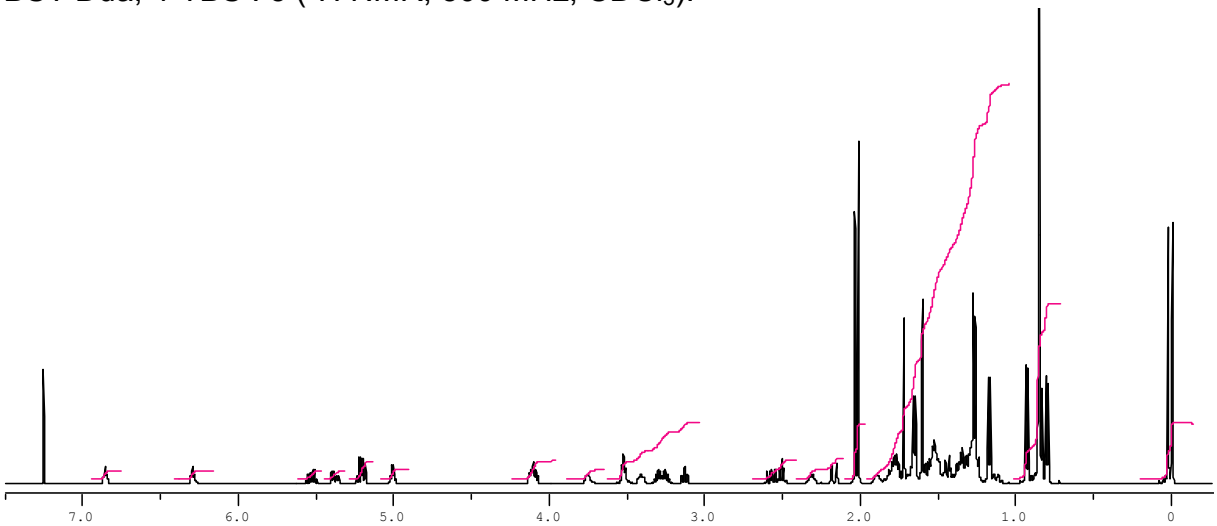


BST Dda : **4** (^{13}C NMR, 125 MHz, $CDCl_3$).

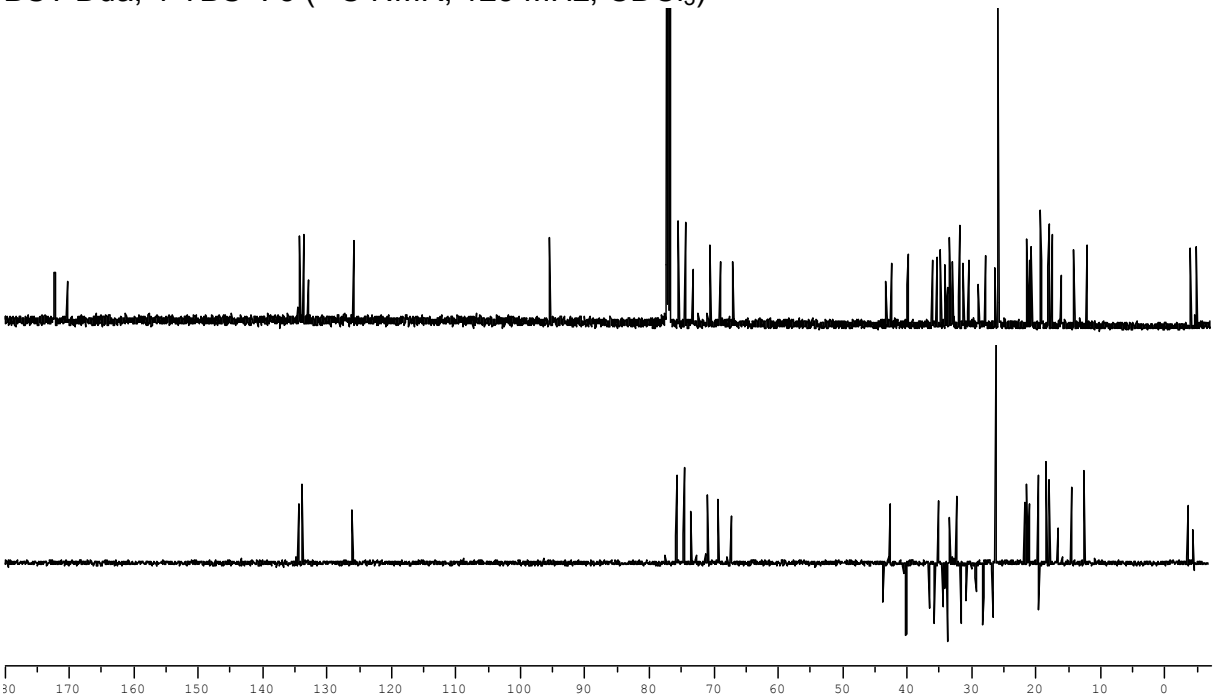




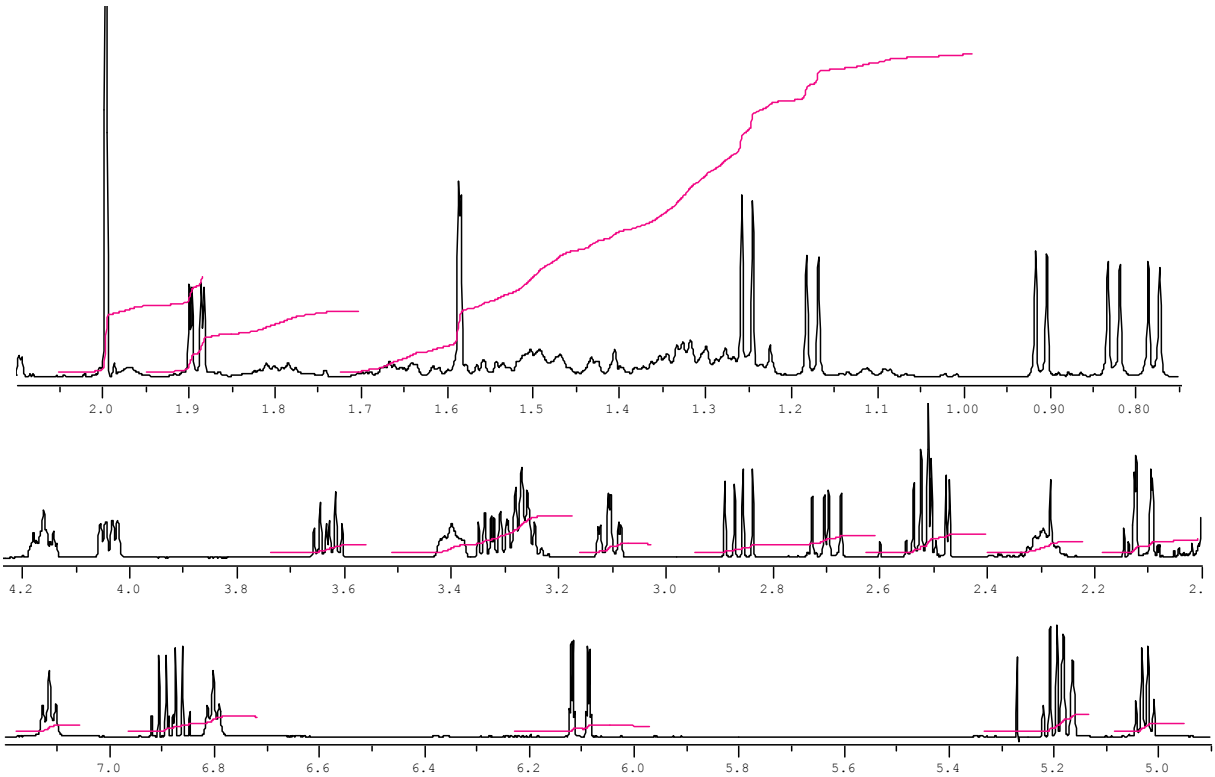
BST Dda, 4-TBS : **5** (^{1}H NMR, 500 MHz, $CDCl_3$).



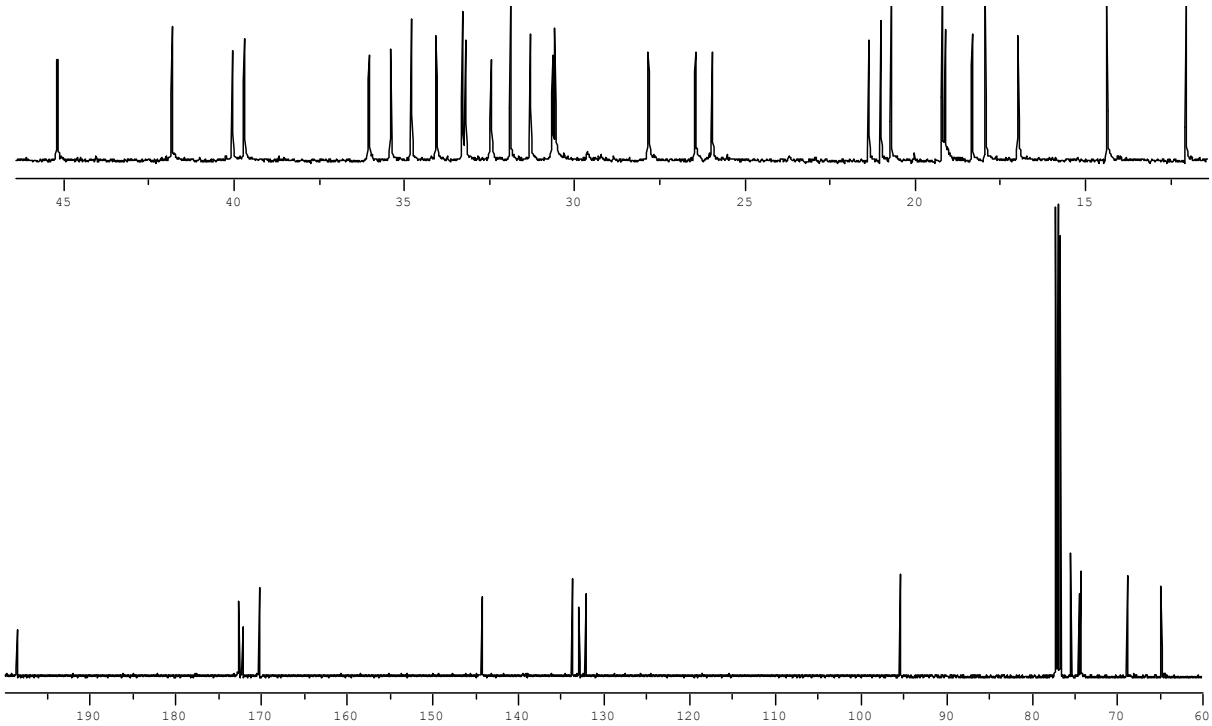
BST Dda, 4-TBS : **5** (^{13}C NMR, 125 MHz, $CDCl_3$)



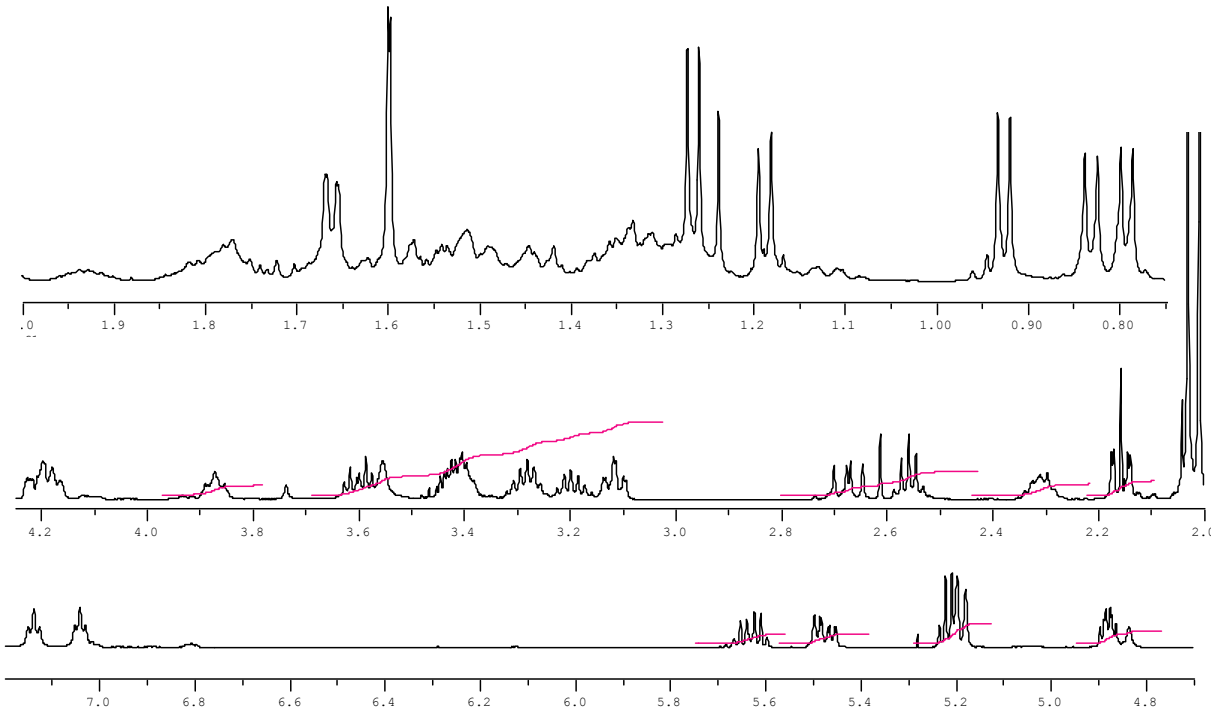
^{1}H NMR details of bistramide BST Ada : **3**



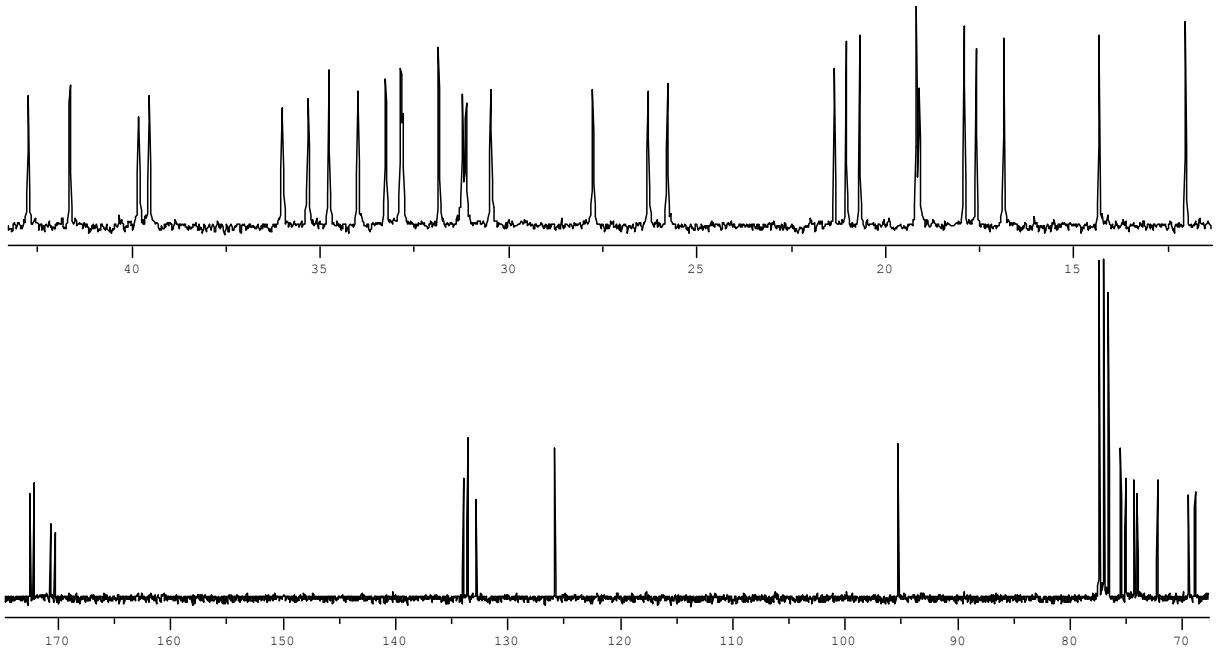
^{13}C NMR details of bistramide BST Ada : **3**



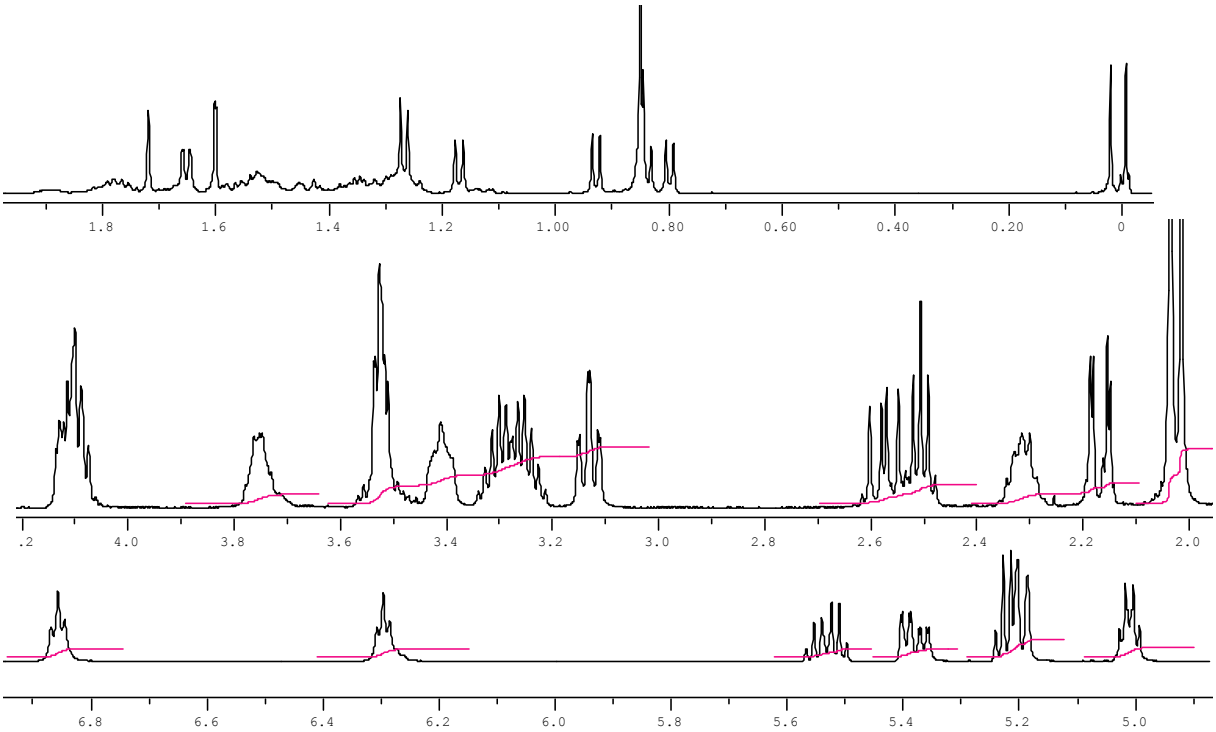
^{1}H NMR details of bistramide BST Dda : **4**



^{13}C NMR details of bistramide BST Dda : **4**



^{1}H NMR details of bistramide BST Dda, 4-TBS : **5**



^{13}C NMR details of bistramide BST Dda, 4-TBS : **5**