Stereochemical Determination of Dictyostatin, a Novel Microtubule-Stabilising Macrolide from the Marine Sponge *Corallistidae sp.*

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General experimental:

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 700 spectrometer operating at a proton frequency of 700.03 MHz. Proton spectra were referenced to 3.34 ppm for residual CHD_2OD. The NOESY experiment was acquired in approximately 4 hours with a mixing time of 600 msec. Acquisition parameters included 4K data points in F_2 and 768 increments with 8 scans per increment. The data was zero filled to 1K in F_1 for processing. The HSCQ-HECADE experiment was acquired in approximately 75 hours with a TOCSY mixing time of 60 msec and a *J*(scale) factor of 1. The acquisition parameters included 8K data points in F_2 and 768 increments with 108 scans per increment. The data was zero-filled to 16K in F_2 and 2K in F_1 for processing.
Molecular modelling studies were performed on potential diastereomers of dictyostatin using Macromodel (version 7.2) and the MM2* force field, together with the generalised Born/Surface area (GB/SA) water solvent model. Structures were subjected to a minimisation procedure to the nearest local minimum prior to the generation of new low energy conformers by Monte Carlo searching (10000 steps). All conformations within 50 kJ mol^{-1} of the lowest energy structure were recorded. The normal set-up protocol was employed, with experiments sampling batches of 1000 to 2000 structures.

Table 1. ^{1}H- NMR data and NOESY Correlations (700 MHz, CD_3OD)

Proton No.	Chemical Shift (ppm) and Multiplicity (H-z)	NOESY Correlations[s]
2	5.55 (d, 11.5)	H-3 (s)
3	6.64 (dd, 11.5, 11.5)	H-2 (s), H-5 (s)
4	7.20 (dd, 15.7, 11.5)	Me-6 (vs), H-6 (m), Me-20 (m), H-23 (w), H-8b (w), H-20 (w)
5	6.17 (dd, 15.7, 6.7)	H-3 (s), H-6 (s), H-7 (m), Me-6 (w)
6	2.59 (ddq (6.7, 4.0, 6.9)	H-5 (s), H-7 (s), H-4 (m)
7	4.04 (ddd, 10.6, 4.0, 2.7)	H-6 (s), H-8b (s), H-5 (m), Me-6 (m), H-9 (w)
8a	1.49 (ddd, 14.0, 10.6, 3.3)	H-9 (s), H-6 (w), H-10 (w), H-12 (w)
8b	1.41 (ddd, 14.0, 10.1, 2.7)	H-7 (s), H-6 (m), H-9 (m), H-10 (m)
9	4.64 (dddd, 10.1, 9.5, 3.3, 0.8)	H-8a (s), H-12 (vs), H-8b (m), Me-12 (m)
10	5.40 (dd, 11.1, 9.5)	H-11 (s), H-8b (m), H-8a (w)
11	5.55 (ddd, 11.1, 11.1, 0.8)	H-14 (s), H-13 (w), Me-12 (w)
12	2.75 (ddq, 11.1, 3.1, 7.0)	H-9 (vs), H-13 (s), H-15a (vs), H-11 (w)
13	3.09 (dd, 8.0, 3.1)	H-12 (s), H-14 (s), H-15b (s), Me-12 (s), H-11 (w), H-15a (m), Me-14 (m)
14	1.59 (dddq, 11.2, 8.0, 3.8, 6.5)	H-11 (s), H-13 (w)
15a	1.24 (ddd, 13.8, 10.3, 3.8)	H-12 (vs), H-17b (s), H-13 (w)
15b	0.89 (ddd, 13.8, 11.2, 3.8)	H-13 (s)
16	1.53 (m)	
17a	1.57 (m)	
17b	0.69 (dddd, 12.8, 12.8, 9.0, 4.7)	H-20 (vs), H-15a (s), H-18a (s) H-19 (s)
18a	1.83 (dddd, 12.8, 12.8, 5.8, 4.7)	H-19 (s), H-17b (s), H-21 (s), H-15b (s)
18b	1.10 (m)	
19	3.34 (ddd, 2.0, 5.8, 5.8)	H-20 (s), H-22 (s), H-17b (m), H-18a (m), H-21 (w), H-25 (w)
20	1.88 (ddq, 5.8, 5.1, 6.9)	H-19 (s), H-21 (s), H-17b (s), Me-22 (s), H-18a (m), H-23 (w), H-4 (w)
21	5.13 (dd, 6.9, 5.1)	H-18a (s), H-20 (s), Me-22 (s), H-21 (s), Me-20 (m), H-19 (w)
22	3.16 (ddq, 10.6, 6.9, 6.8)	H-25 (vs), Me-20 (s), H-19 (s), H-21 (m), H-23 (w)
23	5.32 (dd, 11.1, 10.6)	H-24 (vs), Me-22 (s), H-21 (s), H-20 (m), H-22 (w)
24	6.05 (dd, 11.1, 11.1)	H-23 (vs), H-26a (s), H-25 (w)
25	6.70 (ddd, 16.8, 11.1, 10.3)	H-22 (vs), H-19 (w)
26a	5.24 (dd, 16.8, 2.1)	H-24 (s), H-26b (s)
26b	5.14 (dd, 10.3, 2.1)	H-26a (s) H-25 (m)
Me-6	1.14 (d, 6.9)	
Me-12	1.12 (d, 7.0)	
Me-14	0.94 (d, 6.5)	H-13 (m)
Me-16	0.92 (d, 6.6)	H-15a (m)
Me-20	1.06 (d, 6.9)	H-22 (s), H-4 (m), H-19 (m), H-21 (m)
Me-22	1.00 (d, 6.8)	

[a] definitions: (s) = strong, (m) = medium, (w) = weak.

Table 2. Relevant Homonuclear and Heteronuclear Coupling Constants (700 MHz, CD_3OD)

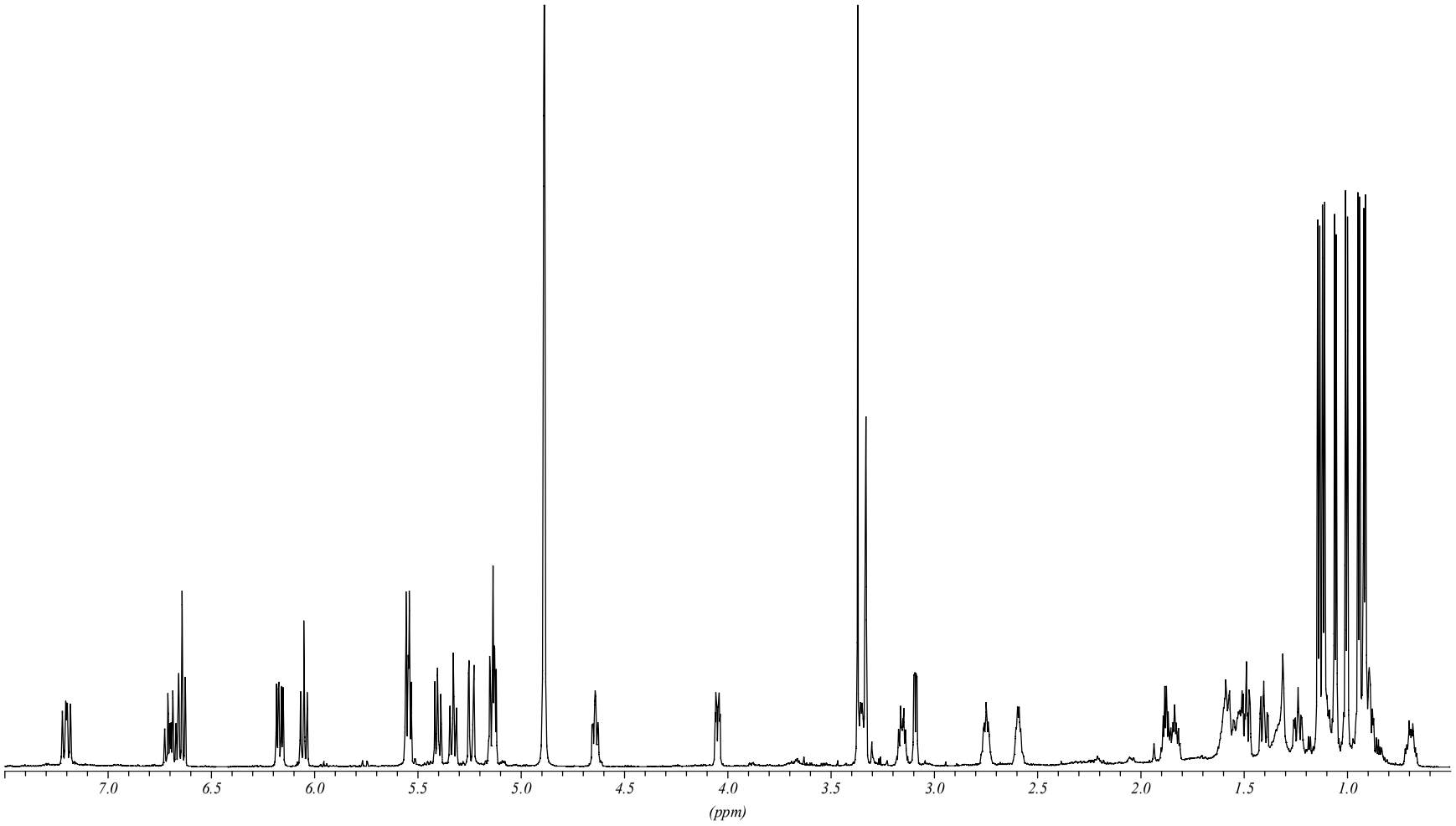
Proton No.	Chemical Shift (ppm) and Multiplicity (Hz)	$^2J_{H,C}$ (Hz)[a]	$^3J_{H,C}$ (Hz)[a]
2	5.55 (d, 11.5)		C-4 (9.8)
3	6.64 (dd, 11.5, 11.5)		C-5 (5.7)
4	7.20 (dd, 15.7, 11.5)		C-2 (3.1), C-6 (5.0)
5	6.17 (dd, 15.7, 6.7)		C-3 (8.6), Me-6 (5.5)
6	2.59 (ddq (6.7, 4.0, 6.9)	C-7 (5.9)	
7	4.04 (ddd, 10.6, 4.0, 2.7)		C-9 (2.8), Me-6 (4.9)
8a	1.49 (ddd, 14.0, 10.6, 3.3)	C-7 (6.1), C-9 (1.0)	C-10 (0.8)
8b	1.41 (ddd, 14.0, 10.1, 2.7)	C-7 (0.5), C-9 (6.0)	C-10 (3.8)
9	4.64 (dddd, 10.1, 9.5, 3.3, 0.8)		C-7 (2.3), C-11 (3.7)
10	5.40 (dd, 11.1, 9.5)	C-9 (0.6)	C-8 (1.5), C-12 (8.7)
11	5.55 (ddd, 11.1, 11.1, 0.8)		C-9 (11.1), Me-12 (2.2)
12	2.75 (ddq, 11.1, 3.1, 7.0)	C-13 (1.1)	C-10 (3.5)
13	3.09 (dd, 8.0, 3.1)		C-15 (1.4), Me-12 (2.2), Me-14 (3.4)
14	1.59 (dddq, 11.2, 8.0, 3.8, 6.5)	C-13 (5.7)	
15a	1.24 (ddd, 13.8, 10.3, 3.8)		C-13 (2.7), Me-14 (8.2), Me-16 (2.7)
15b	0.89 (ddd, 13.8, 11.2, 3.8)		C-13 (0.8), Me-16 (3.3)
16	1.53 (m)		
17a	1.57 (m)		
17b	0.69 (dddd, 12.8, 12.8, 9.0, 4.7)		
18a	1.83 (dddd, 12.8, 12.8, 5.8, 4.7)		
18b	1.10 (m)	C-19 (6.1)	
19	3.34 (ddd, 2.0, 5.8, 5.8)		Me-20 (4.3)
20	1.88 (ddq, 5.8, 5.1, 6.9)	C-21 (2.9)	
21	5.13 (dd, 6.9, 5.1)		C-23 (0.2), Me-20 (4.7), Me-22 (1.9)
22	3.16 (ddq, 10.6, 6.9, 6.8)	C-21 (6.9)	
23	5.32 (dd, 11.1, 10.6)		C-21 (2.1), Me-22 (3.8)
24	6.05 (dd, 11.1, 11.1)		C-22 (9.0)
25	6.70 (ddd, 16.8, 11.1, 10.3)		C-23 (4.2)
26a	5.24 (dd, 16.8, 2.1)		
26b	5.14 (dd, 10.3, 2.1)		

[a] Couplings determined by HSQC-HECADE at both 800 MHz and 700 MHz.

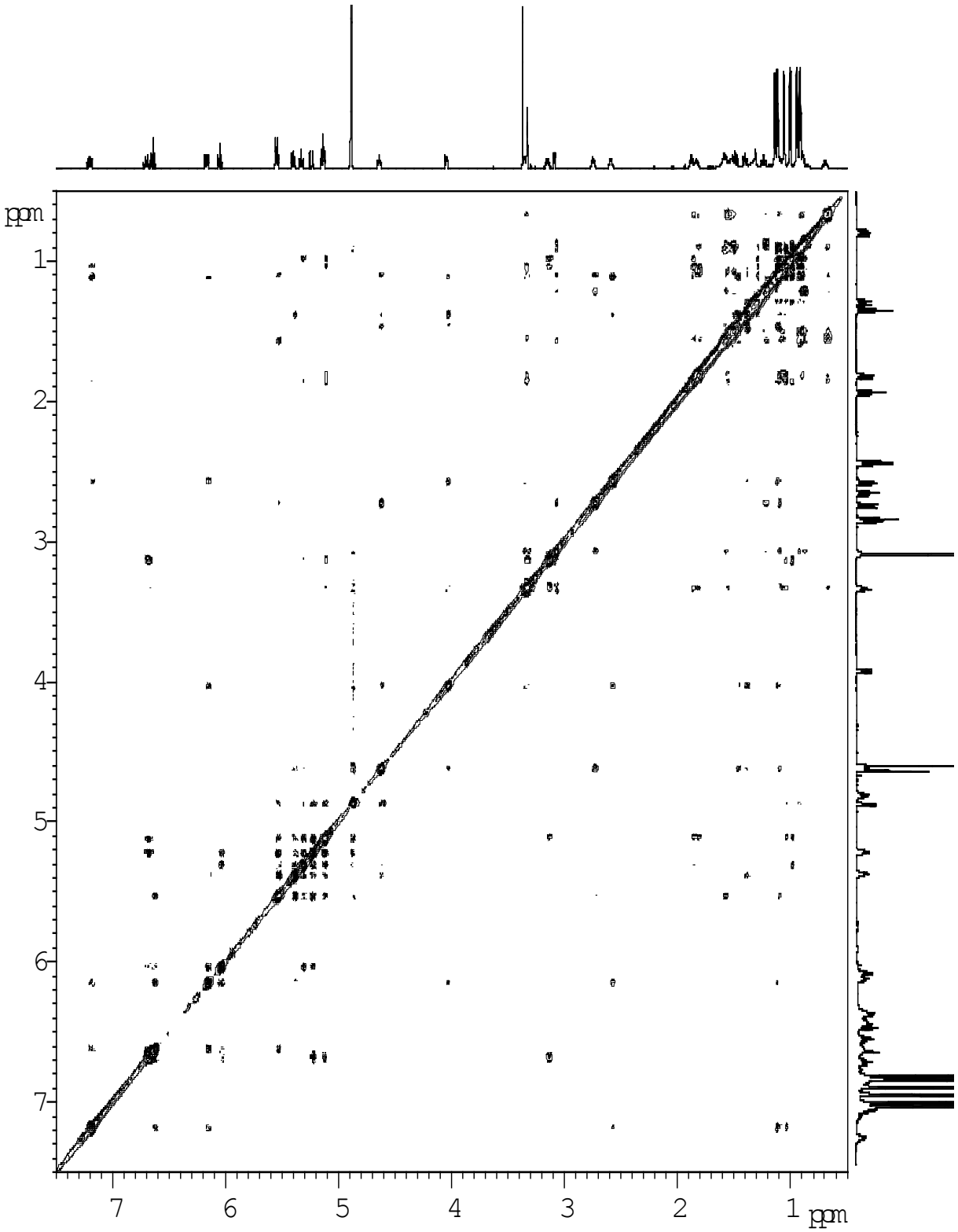
Table 3. Dihedral angles for lowest energy conformation of dictyostatin (5), calculated and measured $^{3}J_{H,H}$ coupling constants in CD_3OD.

H-H fragment	Dihedral angle	Calc. $^{3}J_{H,H}$ (Hz)	Exp. $^{3}J_{H,H}$ (Hz)
H5 to H6	174.9	11.1	6.7
H6 to H7	59.3	1.8	4.0
H7 to H8a	164.6	11.3	10.6
H7 to H8b	79.7	1.0	2.7
H8a to H9	46.2	4.0	3.3
H8b to H9	68.9	1.4	10.1
H9 to H10	129.0	5.6	9.5
H10 to H11	0.3	9.4	11.1
H11 to H12	178.6	11.2	11.1
H12 to H13	65.6	1.2	3.1
H13 to H14	167.6	9.8	8.0
H14 to H15a	61.1	3.0	3.8
H14 to H15b	176.4	12.3	11.2
H15a to H16	174.3	12.2	10.3
H15b to H16	60.2	3.2	3.8
H16 to H17a	61.7	3.0	Sm
H16 to H17b	178.5	12.3	9.0
H17a to H18a	172.8	12.8	12.8
H17a to H18b	71.3	1.6	Sm
H17b to H18a	56.4	3.7	4.7
H17b to H18b	172.2	12.8	12.8
H18a to H19	170.4	11.7	5.8
H18b to H19	55.0	2.5	2.0
H19 to H20	61.6	1.5	5.8
H20 to H21	171.1	9.9	4.9

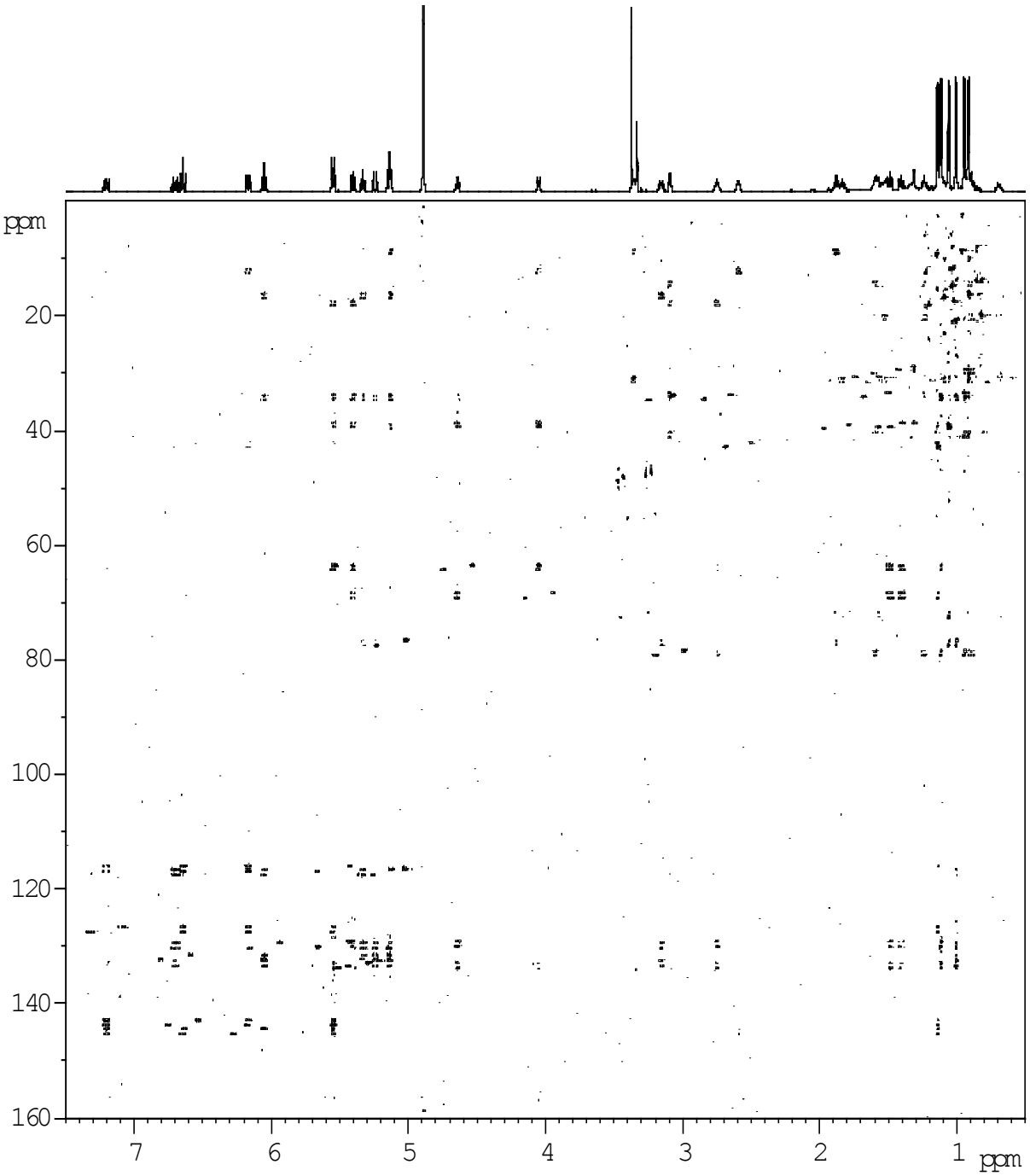
^{1}H NMR Spectum of dictyostatin (5) in CD_3OD (700 MHz)



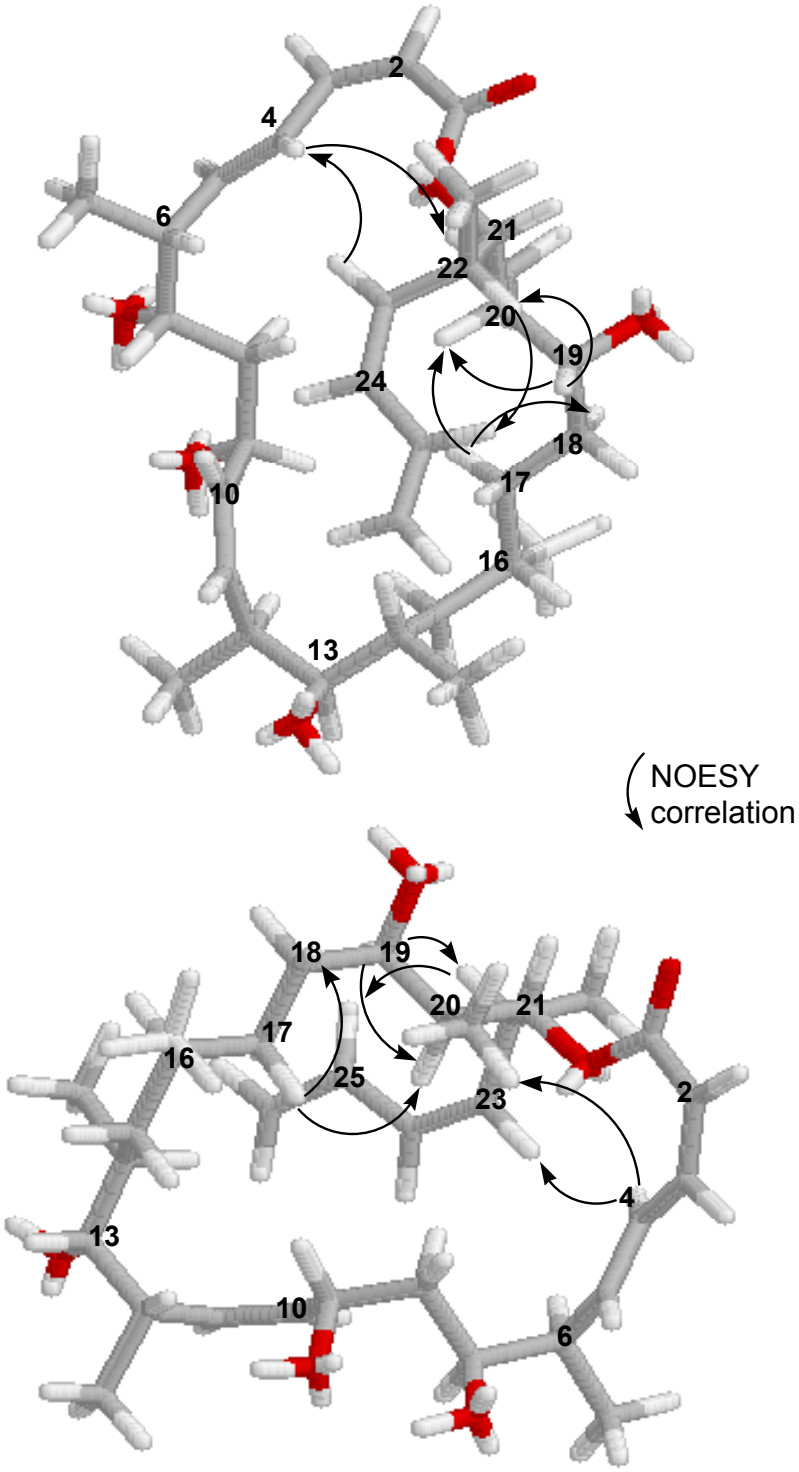
NOESY Spectum of dictyostatin (5) in CD_3OD (700 MHz)



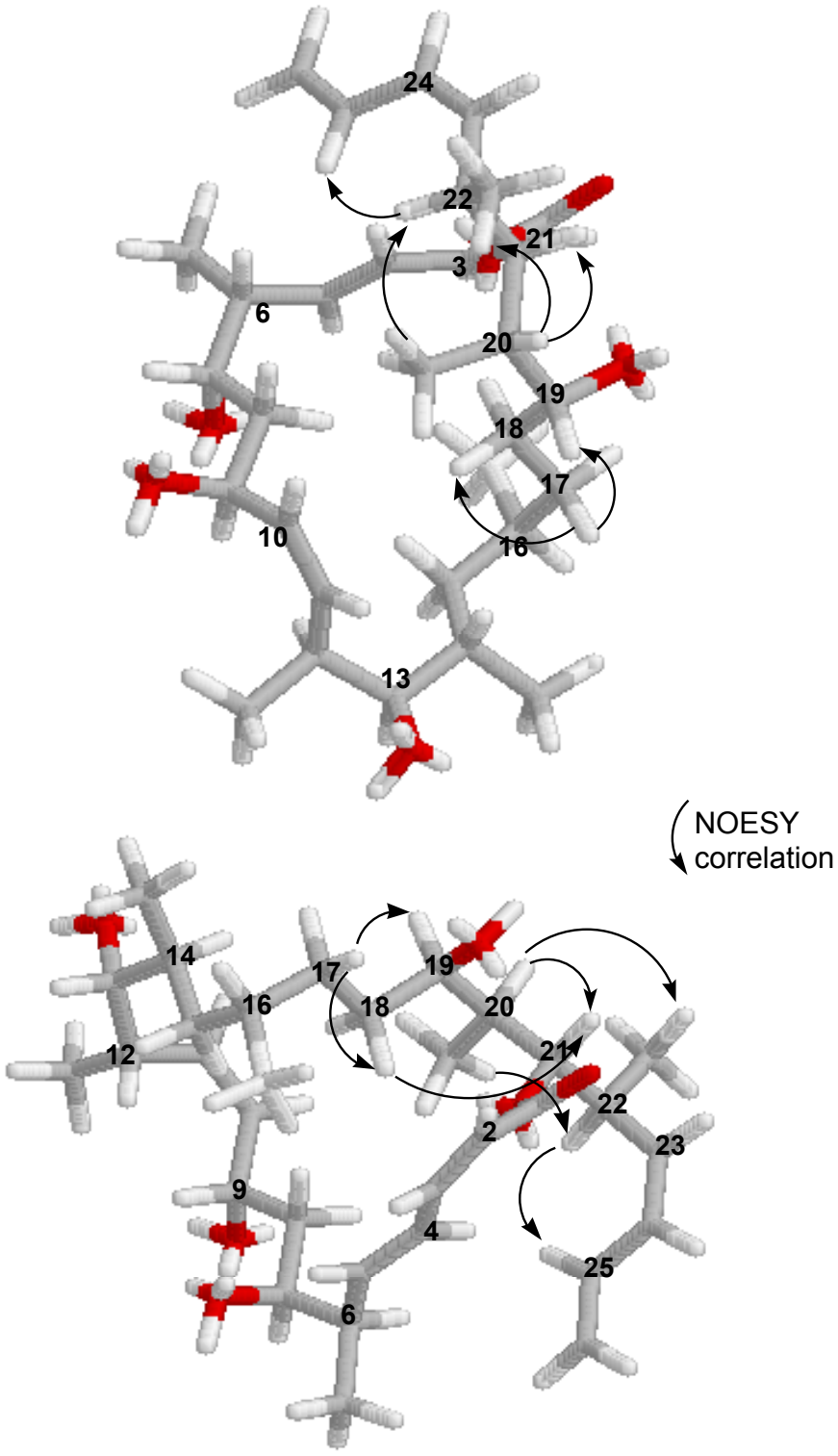
HSQC-HECADE Spectum of dictyostatin (5) in CD_3OD (700 MHz)



Perspective drawings of the lowest energy conformation of dictyostatin (5) generated by Macromodel V 8.0



Perspective drawings of the s-cis conformation of dictyostatin (4B) generated by Macromodel V 8.0



PDB output-file for the lowest energy conformation of dictyostatin (5)

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HETATM    3  C5  UNK     1     -24.897  54.220  30.500  1.00  0.00           C
HETATM    4  C6  UNK     1     -25.350  52.020  29.243  1.00  0.00           C
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HETATM    6  O2  UNK     1     -24.583  49.874  29.995  1.00  0.00           O
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HETATM   82  H45 UNK     1     -27.730  56.941  24.612  1.00  0.00           H
HETATM   83  H46 UNK     1     -26.831  57.090  26.161  1.00  0.00           H
HETATM   84  H50 UNK     1     -24.365  54.511  32.622  1.00  0.00           H
HETATM   85  H51 UNK     1     -23.000  54.763  31.476  1.00  0.00           H
HETATM   86  H52 UNK     1     -24.288  56.006  31.629  1.00  0.00           H
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HETATM   90 1H16 UNK     1     -28.528  50.485  23.532  1.00  0.00           H
HETATM   91 2H16 UNK     1     -29.370  50.434  25.100  1.00  0.00           H
HETATM   92  H1  UNK     1     -26.841  53.978  31.584  1.00  0.00           H
HETATM   93  H2  UNK     1     -26.622  55.750  29.071  1.00  0.00           H
HETATM   94  H3  UNK     1     -28.820  55.613  31.239  1.00  0.00           H
HETATM   95  H4  UNK     1     -30.483  56.064  29.608  1.00  0.00           H
HETATM   96  H14 UNK     1     -27.096  48.441  23.869  1.00  0.00           H
HETATM   97  H15 UNK     1     -27.167  50.340  26.305  1.00  0.00           H
HETATM   98 1H14 UNK     1     -29.257  48.160  25.033  1.00  0.00           H
HETATM   99 2H14 UNK     1     -28.203  46.784  25.441  1.00  0.00           H
HETATM  100 3H14 UNK     1     -28.437  48.109  26.637  1.00  0.00           H
CONECT    1    2    3    4   49
CONECT    2    1   87   39   40
CONECT    3    1   31   50   32
CONECT    4    1    5   51   52
CONECT    5    4    6    7   53
CONECT    6    5   54   41   42
CONECT    7    5    8   55
CONECT    7    8
CONECT    8    7    9   56
CONECT    8    7
CONECT    9    8   10   24   57
CONECT   10    9   11   25   58
CONECT   11   10   12   26   59
```

```
CONECT   12   11   13   88   89
CONECT   13   12   14   38   96
CONECT   14   13   15   60   97
CONECT   15   14   16   90   91
CONECT   16   15   17   27   61
CONECT   17   16   18   28   62
CONECT   18   17   19   29   63
CONECT   19   18   20   30   64
CONECT   20   19   21   65
CONECT   20   21
CONECT   21   20   22   66
CONECT   21   20
CONECT   22   21   23   67
CONECT   22   23
CONECT   23   22   68   69
CONECT   23   22
CONECT   24    9   70   71   72
CONECT   25   10   73   43   44
CONECT   26   11   74   75   76
CONECT   27   16   77   45   46
CONECT   28   17   78   79   80
CONECT   29   18   36   47   48
CONECT   30   19   81   82   83
CONECT   31    3   84   85   86
CONECT   49    1
CONECT   50    3
CONECT   51    4
CONECT   52    4
CONECT   53    5
CONECT   54    6
CONECT   55    7
CONECT   56    8
CONECT   57    9
CONECT   58   10
CONECT   59   11
CONECT   60   14
CONECT   61   16
CONECT   62   17
CONECT   63   18
CONECT   64   19
CONECT   65   20
CONECT   66   21
CONECT   67   22
CONECT   68   23
CONECT   69   23
CONECT   70   24
CONECT   71   24
CONECT   72   24
CONECT   73   25
CONECT   74   26
CONECT   75   26
CONECT   76   26
CONECT   77   27
CONECT   78   28
CONECT   79   28
CONECT   80   28
CONECT   81   30
CONECT   82   30
CONECT   83   30
CONECT   84   31
```

```
CONECT   85   31
CONECT   86   31
CONECT   32    3   33   92
CONECT   32   33
CONECT   33   32   34   93
CONECT   33   32
CONECT   34   33   35   94
CONECT   34   35
CONECT   35   34   36   95
CONECT   35   34
CONECT   36   29   35   37
CONECT   36   37
CONECT   37   36
CONECT   37   36
CONECT   87    2
CONECT   88   12
CONECT   89   12
CONECT   38   13   98   99  100
CONECT   90   15
CONECT   91   15
CONECT   92   32
CONECT   93   33
CONECT   94   34
CONECT   95   35
CONECT   96   13
CONECT   97   14
CONECT   98   38
CONECT   99   38
CONECT  100   38
CONECT   39    2
CONECT   40    2
CONECT   41    6
CONECT   42    6
CONECT   43   25
CONECT   44   25
CONECT   45   27
CONECT   46   27
CONECT   47   29
CONECT   48   29
END
```