Electronic Supplementary Information

Materials and Methods

Origin, isolation and purification

A naphthenate deposit sample from an oilfield offshore West Africa was provided by Chevron Texaco. The naphthenic acids were isolated from the sample by the Acid-IER method[1]. The first step in that process is to wash the sample thoroughly in toluene to remove crude oil and oil-soluble fractions. This continued until an almost clear toluene phase was obtained. The remaining solid was then exposed to a 2:1 volume mixture of toluene and 1M HCl solution to dissolve the naphthenate. Hence, naphthenic acids chemically bound as naphthenate were converted to free acid monomers which dissolve into the toluene phase, leaving the counter-ions in the aqueous phase. The naphthenic acids were then selectively isolated from other potentially occurring polar compounds by use of the ion-exchange resin QAE Sephadex A-25. The solvent was removed from the naphthenic acids by evaporation on a rotary evaporator at 60 °C and further by drying in an oven at the same temperature.

NMR analyses

Structure determination of the 20-bis-16,16'-biphytane tetraacids (**1**) were performed at 298 K on a Bruker Avance 600 FT-NMR Spectrometer equipped with a TXI CryoProbe operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C. The sample was dissolved in dioxane-d_8 (0.6 ml, 99.8 atom %D), and the chemical shifts were calibrated against the solvent, 3.53 ppm for ^{1}H (dioxane-d_7) and 66.66 ppm for ^{13}C (dioxane-d_8). The following experiments were recorded (key experimental conditions in parenthesis): ^{1}H (30° flip-angle), ^{13}C (inverse-gated, 30° flip angle, 5.4 s repetition time), DEPT90, DEPT135, 1D ROESY (200 ms spin-lock, irradiation at 2.37, 2.19, 1.97, 0.87, 0.85 and 0.77 ppm), 1D TOCSY (40 and 80 ms mix time, irradiation at 2.37, 2.19, 1.97, 0.87, 0.85 and 0.77 ppm), ^{1}H-^{1}H COSY (magnitude mode, 45° flip angle), ^{1}H-^{1}H TOCSY (80 ms mix time), ^{1}H-^{1}H ROESY (200 and 450 ms spin-lock), ^{1}H-^{13}C HSQC ($^{1}J_{H,C}$ 135 Hz), ^{1}H-^{13}C HMBC ($^{n}J_{H,C}$ 8 Hz), ^{1}H-^{13}C HMSC ($^{1}J_{H,C}$ 145 Hz, $^{n}J_{H,C}$ 7 Hz), 2D HSQC-TOCSY ($^{1}J_{H,C}$ 125 Hz, 30 ms spin-lock), 2D HSQC-ROESY ($^{1}J_{H,C}$ 145 Hz, 300 ms spin-lock), 1,1-ADEQUATE ($^{1}J_{H,C}$ 145 Hz and $^{1}J_{C,C}$ 55 Hz), 1,n-ADEQUATE ($^{1}J_{H,C}$ 145 Hz and $^{n}J_{C,C}$ 7 Hz), 3D TOCSY-HSQC ($^{1}J_{H,C}$ 135 Hz, 35 ms spin-lock) and 3D NOESY-HSQC ($^{1}J_{H,C}$ 145 Hz, 300 ms mix time).

Key NMR spectra are given in Figs. S5 – S13.

For comparison with the spectra published by Baugh *et al*. (*10*), ^{1}H and ^{13}C NMR spectra of the 20-bis-16,16'-biphytane tetraacids (**1**) and the corresponding tetramethyl esters were recorded in $CDCl_3$ and dioxane-d_8 on a Bruker Avance DPX 400 FT-NMR Spectrometer, equipped with a DUL probe, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

The coupling constants of the protons at C-17 were determined by simulation of the multiplets at 1.95 and 0.77 ppm using the WinDaisy software from Bruker. These multiplets were well separated from other signals in the 1D TOCSY spectrum (Fig. S8) obtained by selective magnetisation of the proton at 2.16 ppm, which provided a 1D spectrum with no overlapping signals for these protons.

MS analysis
The positive mode electrospray ionisation mass spectrum (Fig. 13 below) of the permethylated 20-bis-16,16'-biphytanyl tetraacids was recorded on a Micromass QTOF 2W mass spectrometer. Positive mode APCI MS/MS spectra were also recorded, but structural fragmentation was not obtained with collision energies of 15, 20, 25 or 30 V. Accurate mass measurement (positive mode APCI) of the main component with 6 rings, gave *m*/*z* 1288.1370 (calc. 1288.1403 for $C_{84}H_{150}O_8$).

*Derivatisation of the tetraacid **1** to the permethyl ester*
An aliquote of the tetraacid **1** (ca. 20 mg) which had been purified by extraction into toluene as described above was dissolved in THF (5 ml). MeOH (5 ml) and BF_3-OMe_2 (1 ml) were added, and the solution refluxed for 5 min. Diethyl ether (15 ml) was added, and the solution washed with water (3x 10 ml) and saturated NaCl solution (2x 5 ml). The organic phase was dried over $MgSO_4$ and taken to dryness under reduced pressure. NMR (400 MHz) showed that 95% of the acid moieties were methylated. This sample was submitted to electrospray MS analysis.

MM2 Calculations
The MM2 program used here is incorporated in the ChemOffice2005 (Chem3D Ultra ver. 9.0.1) program package from CambridgeSoft, Cambridge, MA, USA.
Structures for the tetraacids (**1**) calculated by MM2 methods are given in Fig. 5 and Fig 3 here in Chem3D format.

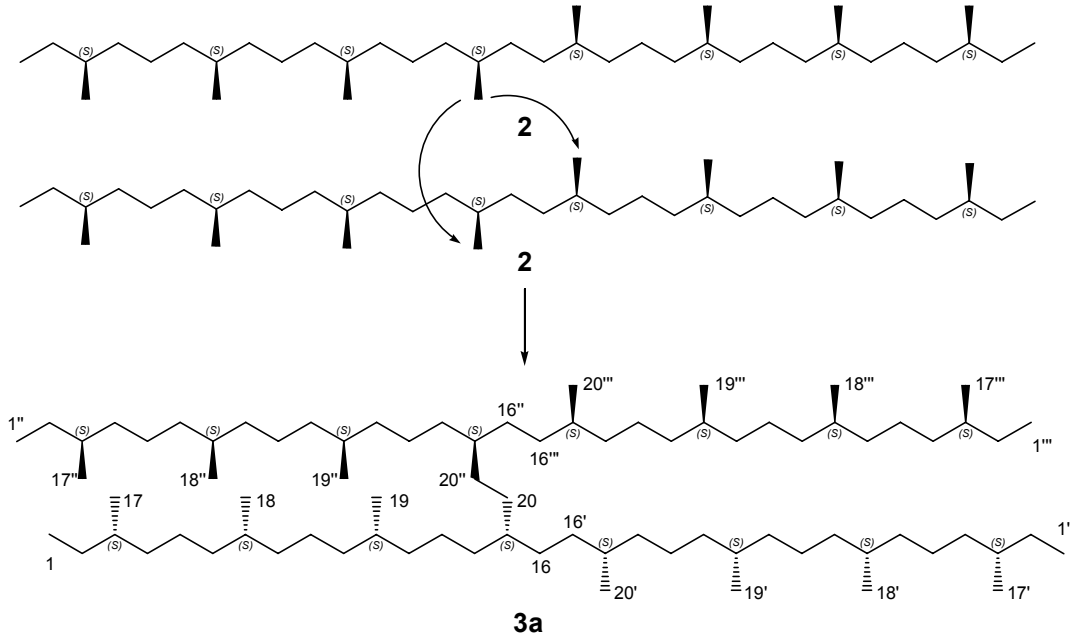

Fig. 1 Head-to-head coupling of two 16,16'-biphytane (**2**) moieties provides one stereoisomer of 20-bis-16,16'-biphytane (**3a**) regardless of whether the coupling, indicated by arrows, occurs between Me-20 and Me-20'' or Me-20' and Me-20''.

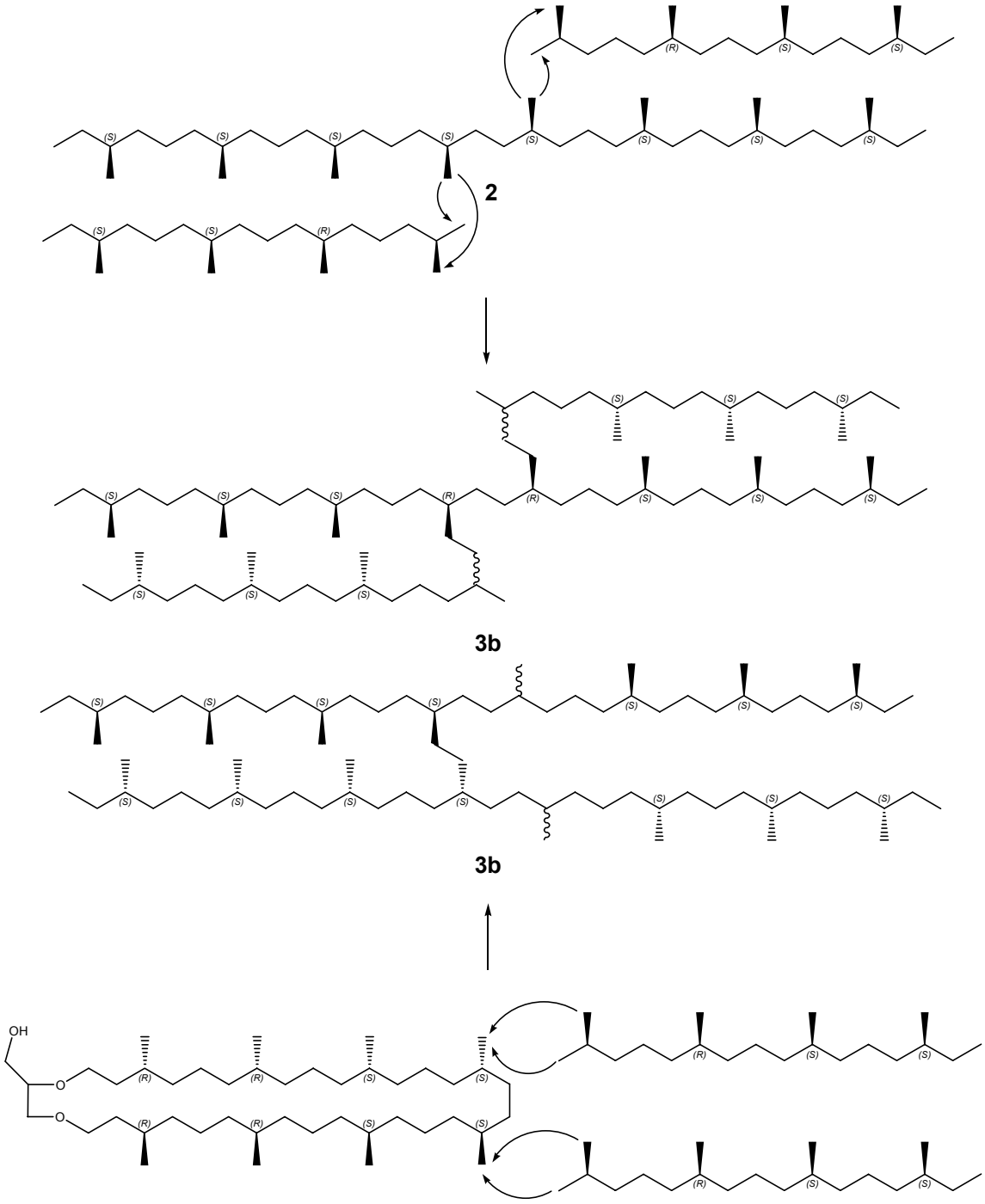

Fig. 2

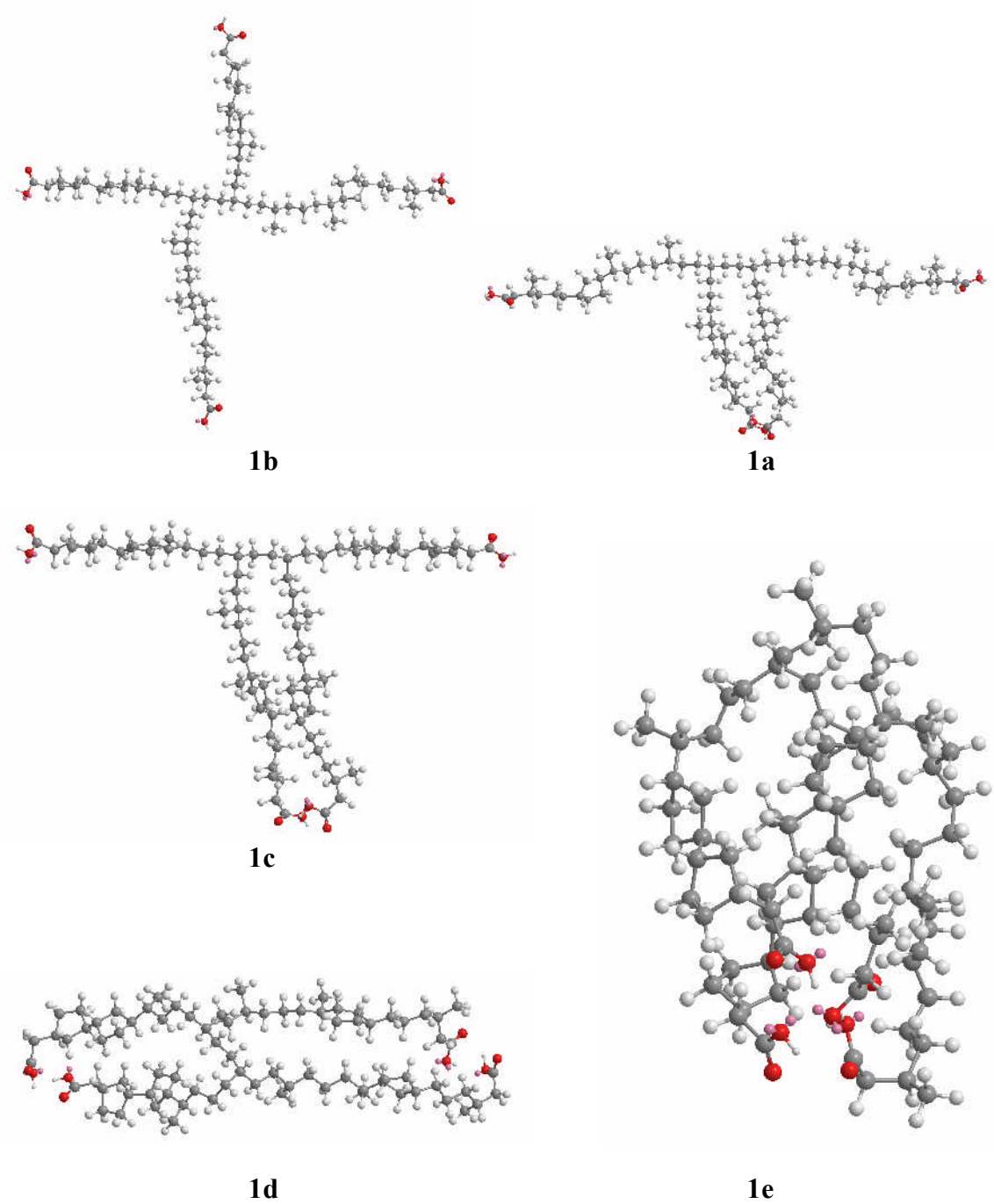

Fig. 3 Conformations calculated for (6:17,10:18,10’:18’,6’’:17’’,10’’:18’’,10’’’:18’’’)-hexacyclo-20-bis-16,16’-biphytane-1,1’,1’’,1’’’-tetracarboxylic acid (**1**), with stereochemistry preserved from 20-bis-16,16’-biphytane (**3**). Free energies of the conformations are given in Table 2 below. Structures can be viewed in 3D by Cambridgesoft Chem3D software.

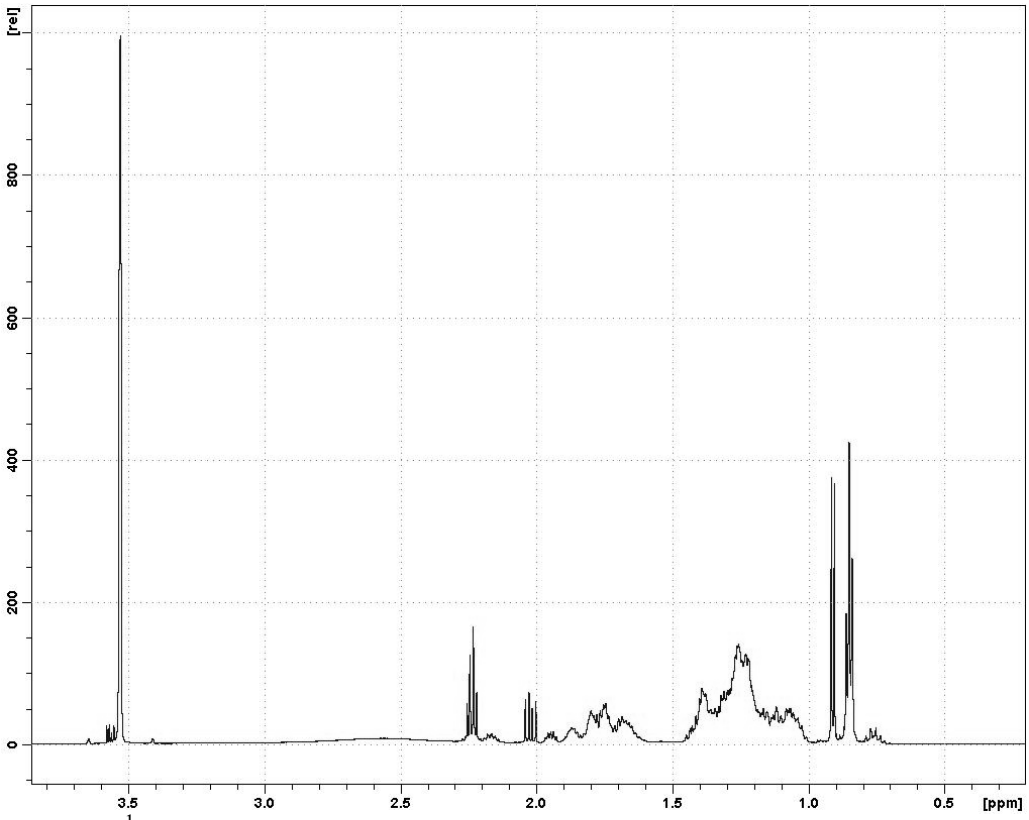

Fig. 4 600 MHz ^{1}H spectrum in dioxane-d_8 of the 20-bis-16,16'-biphytanyl tetraacids.

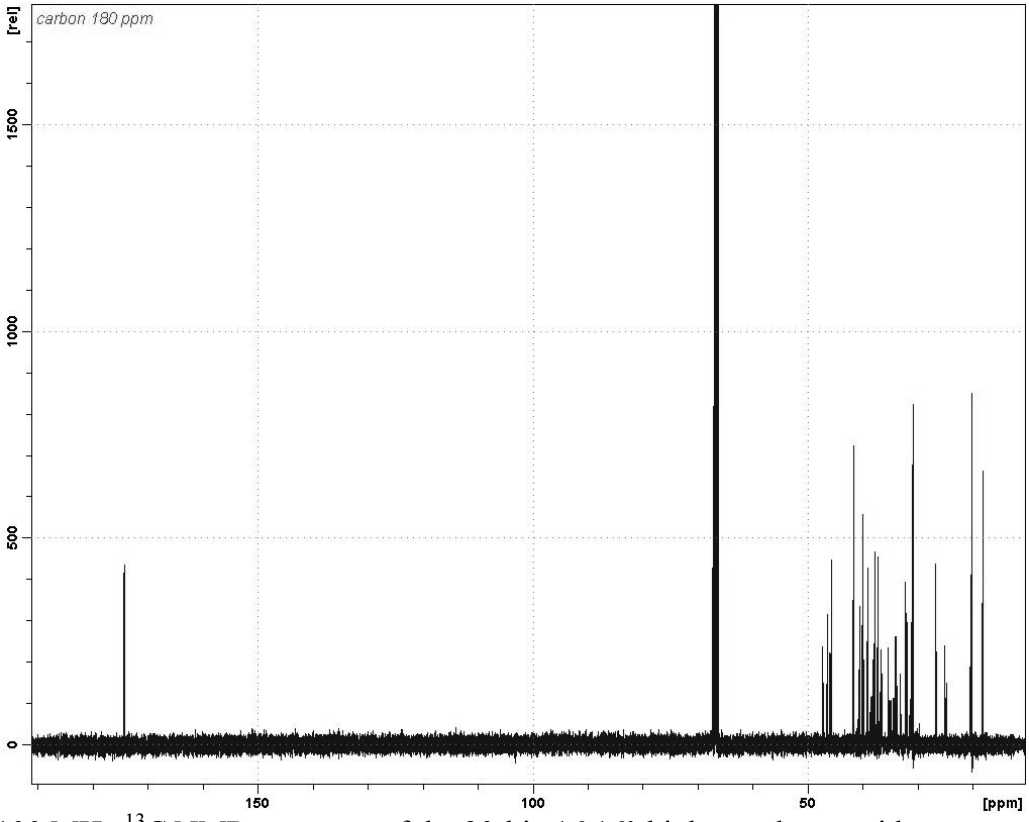

Fig. 5 100 MHz ^{13}C NMR spectrum of the 20-bis-16,16'-biphytanyl tetraacids.

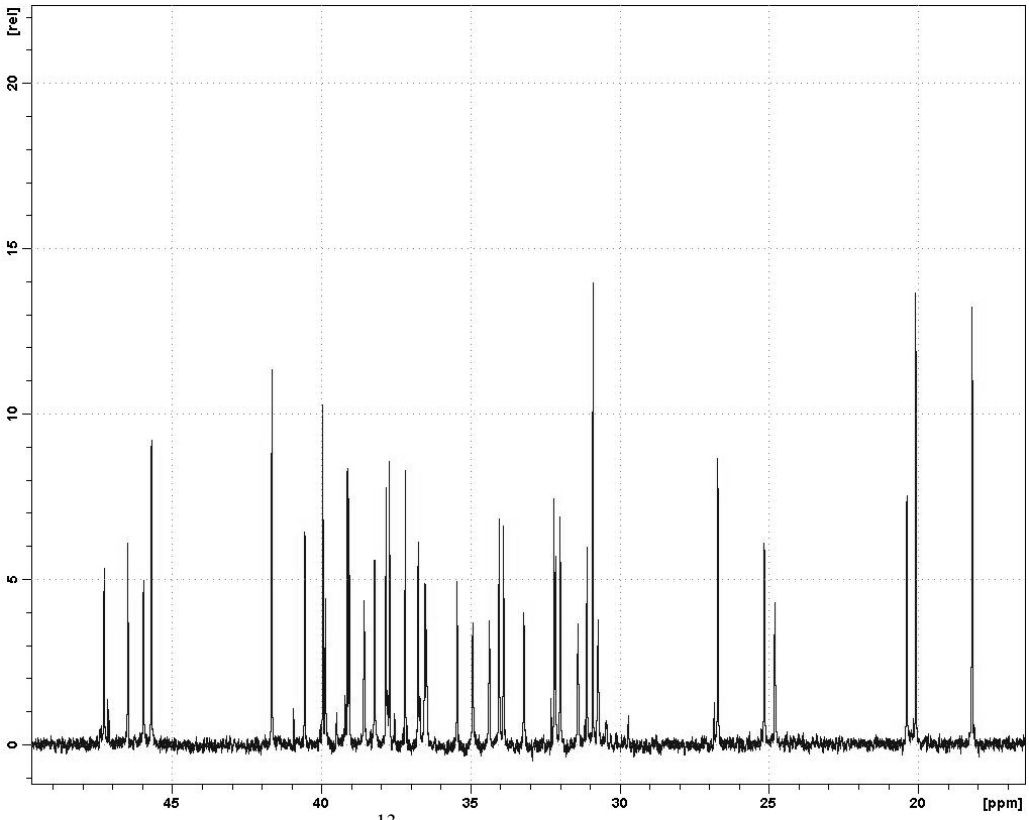

Fig. 6 Aliphatic region of 150 MHz ^{13}C spectrum (dioxane-d_8) of the 20-bis-16,16'-biphytanyl tetraacids.

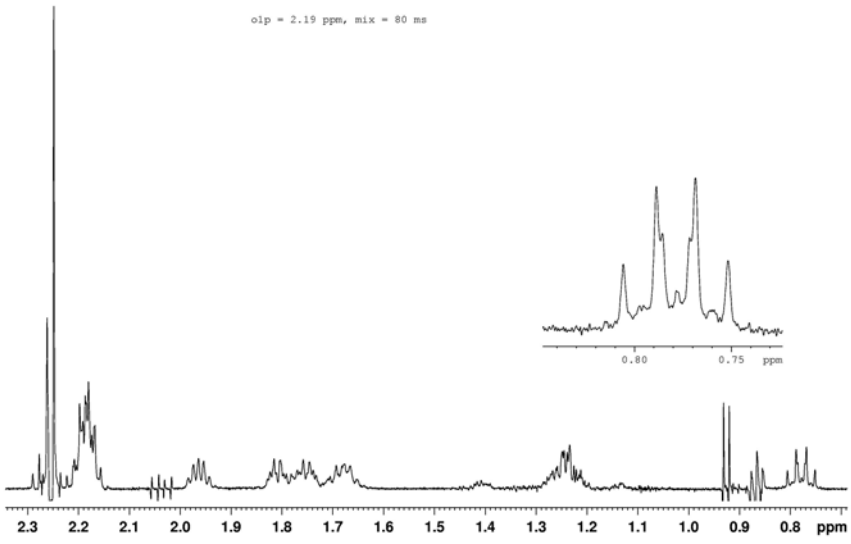

Fig. 7 1D TOCSY spectrum of the 20-bis-16,16'-biphytanyl tetraacids, with irradiation of the α-carbonyl protons at 2.16 ppm. Enlargement of the multiplet at 0.77 ppm inserted.

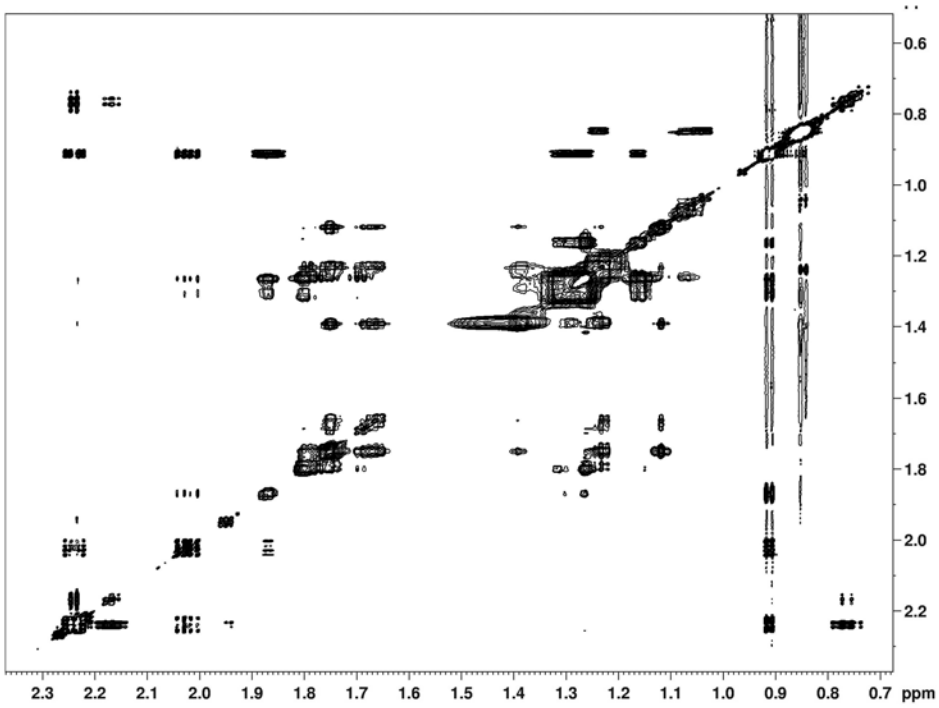

Fig. 8 2D ROESY spectrum (300 ms mix) of the 20-bis-16,16'-biphytanyl tetraacids.

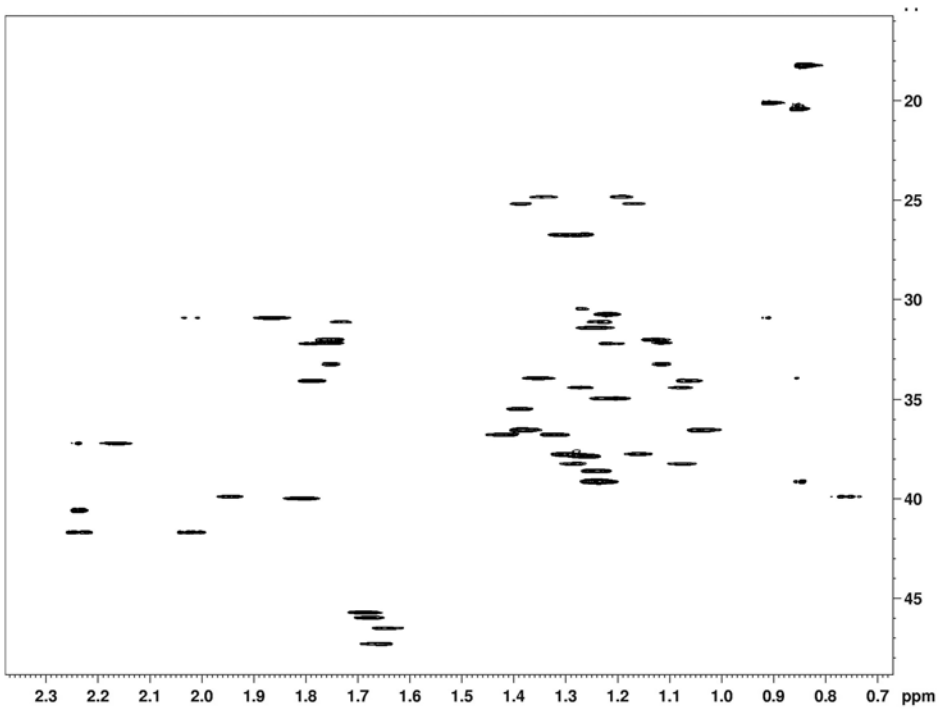

Fig. 9 ^{1}H-^{13}C HSQC spectrum of the 20-bis-16,16'-biphytanyl tetraacids.

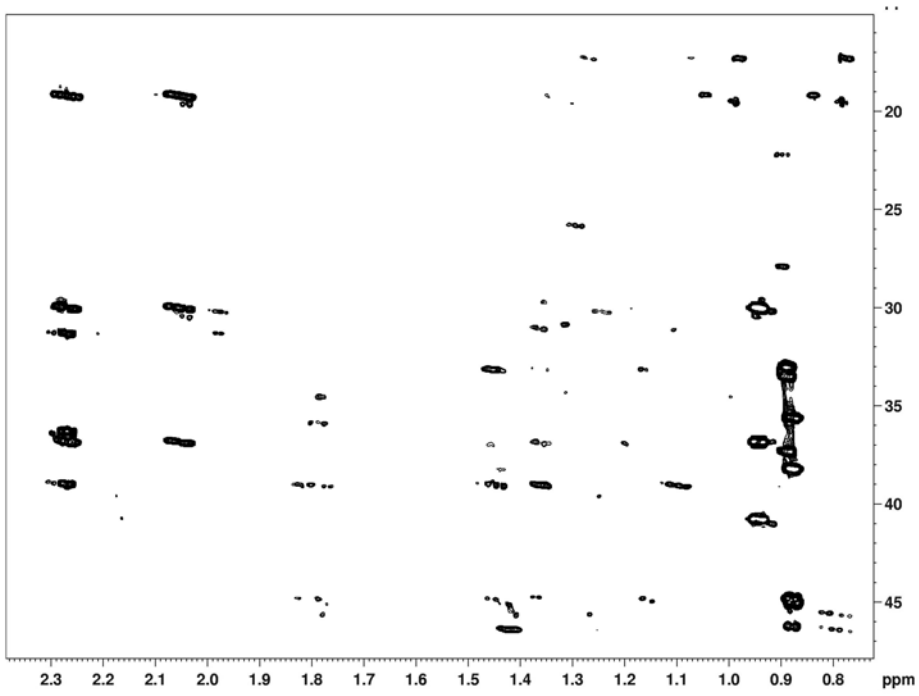

Fig. 10 Long-range part of the ^{1}H-^{13}C HMSC spectrum of the 20-bis-16,16'-biphytanyl tetraacids.

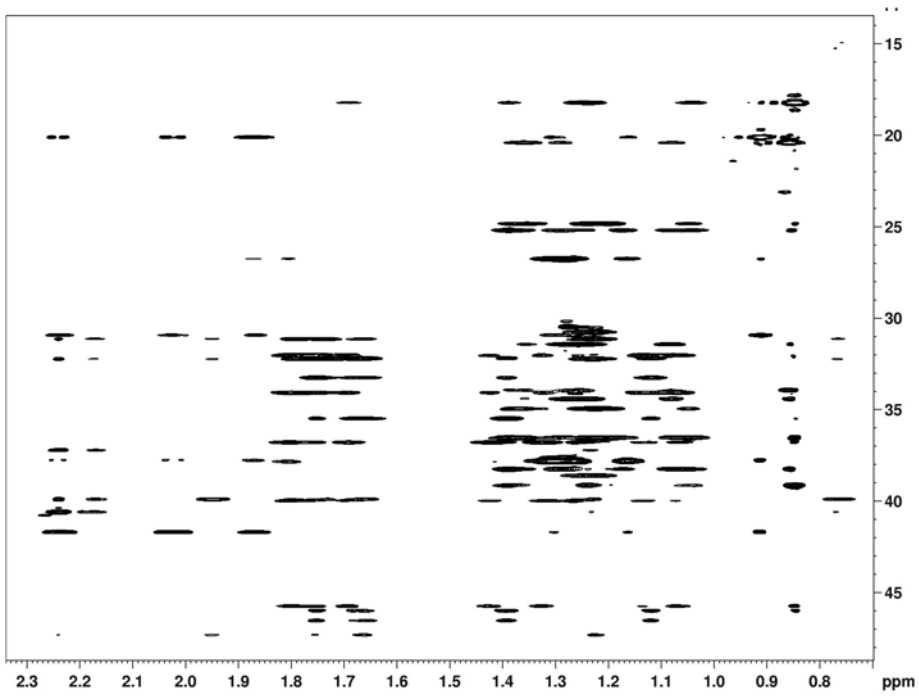

Fig. 11 2D HSQC-TOCSY spectrum of the 20-bis-16,16'-biphytanyl tetraacids.

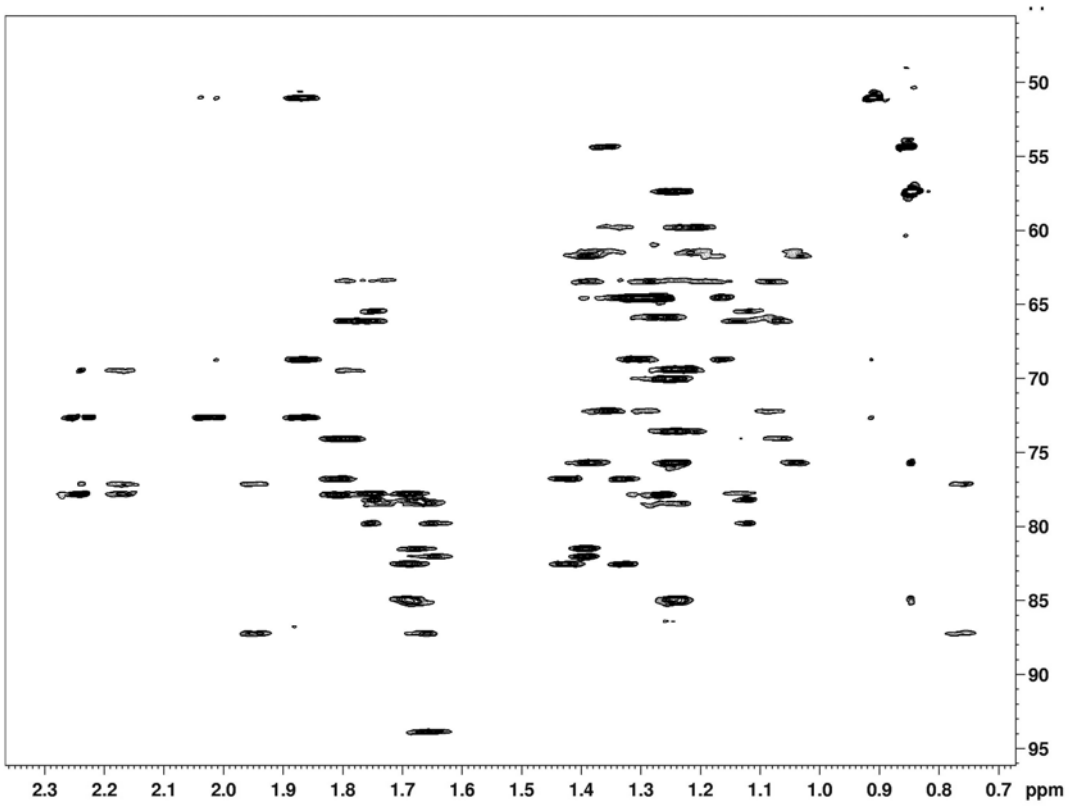

Fig. 12 1,1-ADEQUATE spectrum of the 20-bis-16,16'-biphytanyl tetraacids.

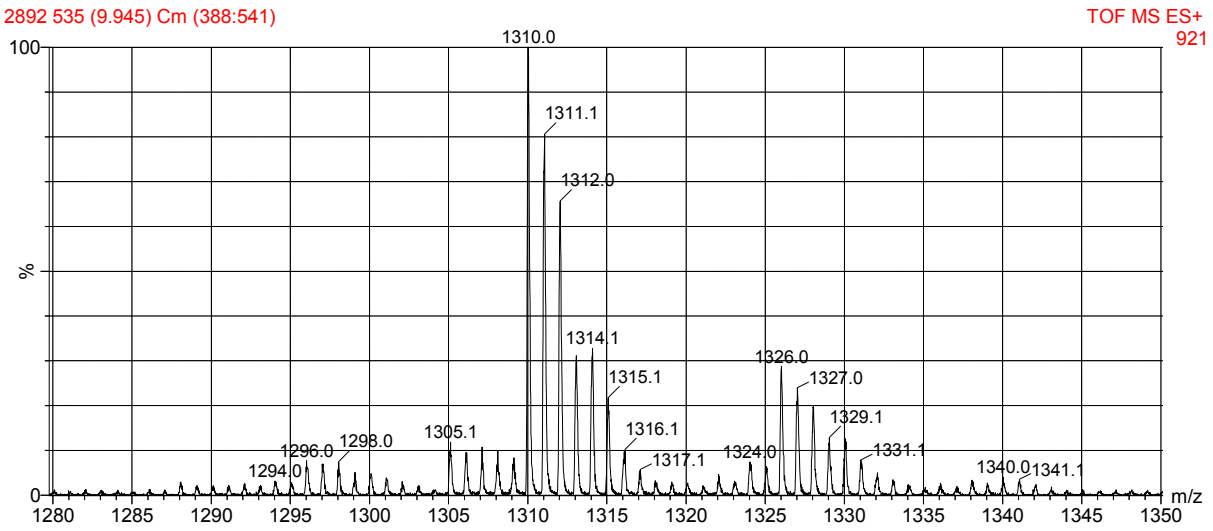

Fig. 13 ESI (positive) mass spectrum of the permethylated 20-bis-16,16'-biphytanyl tetraacids.

Table 1 ^{13}C- and ^{1}H-NMR data of **1**.

Carbon #	δ_C (ppm)				δ_H (ppm)
	CH_3	CH_2	CH	C	
1, 1"				174.37	
1', 1"'				174.36	
2, 2"		40.60			2.240
2', 2"'		41.70			2.236, 2.020
3, 3"			37.23		2.160
3', 3"'			30.94		1.870
4, 4"		32.25			1.794, 1.221
4', 4"'		37.76			1.300, 1.162
5, 5"		31.14			1.724, 1.233
5', 5"'		26.74			1.310, 1.270
6, 6"			47.31		1.657
6', 6"'		37.86			1.419, 1.324
7, 7"			46.53		1.648
7', 7"'			39.99		1.807
8, 8"		33.23			1.752, 1.126
8', 8"'		34.07			1.794, 1.064
9, 9"		32.18			1.751, 1.123
9', 9"'		32.02			1.751, 1.130
10, 10"			46.00		1.678
10', 10"'			45.74		1.691
11, 11"			39.10		1.236
11', 11"'			39.15		1.236
12, 12"		36.51			1.381, 1.038
12', 12"'		36.57			1.381, 1.038
13, 13"		24.86			1.342, 1.192
13', 13"'		25.20			1.380, 1.173
14, 14"		34.98			1.221
14', 14"'		38.26			1.290, 1.082
15, 15"			38.62		1.243
15', 15"'			33.94		1.236
16, 16"		31.46			1.249
16', 16"'		34.44			1.274, 1.083
17, 17"		39.89			1.942, 0.765
17', 17"'	20.12				0.910
18, 18"		35.48			1.396
18', 18"'		36.78			1.419, 1.324
19, 19"	18.22				0.844
19', 19"'	18.22				0.844
20, 20"		30.81			1.225
20', 20"'	20.41				0.854

Table 2 MM2 calculated free energies (kcal/mol) for conformations **1a-e** of tetraacid **1**. For structures, see Fig. 3 above.

Conformation	**1b**	**1a**	**1c**	**1d**	**1e**
Stretch	8.24	8.34	8.26	8.33	8.38
Bend	31.97	32.91	32.71	34.76	37.50
Stretch-Bend	1.44	1.54	1.51	1.61	1.70
Torsion	37.31	38.18	40.07	43.11	50.62
Non-1,4 VDW*	-21.70	-33.55	-40.74	-50.99	-89.11
1,4 VDW*	51.05	51.29	51.38	52.38	54.62
Dipole/Dipole	6.29	2.99	4.66	3.32	7.30
Total	114.60	101.70	97.85	92.52	70.71

*) van der Waals interaction

References

1. H. Mediaas, K. V. Grande, B. M. Hustad, A. Rasch, H. G. Rueslåtten, J. E. Vindstad, *Proceedings - SPE 5th International Oilfield Scale Symposium*, Aberdeen, SPE 80404, **2003**.