Supporting Information

A Comprehensive Study of Okaspirodiol: Characterization, Total Synthesis and Biosynthesis of a New Metabolite from *Streptomyces* sp.

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Contents

1. General Experimental Methods

^1H NMR spectra were recorded at 250, 300 or 600 MHz. Chemical shifts in CD_2Cl_2, CD_3OD or [D_6]-acetone are reported as δ values relative to $CHDCl_2$ (δ 5.32), CHD_2OD (δ 3.35) or [D_5]-acetone (δ 2.05) as internal reference unless stated otherwise. – ^{13}C NMR spectra were recorded at 62.9, 75.5 or 150.8 MHz. Chemical shifts in CD_2Cl_2 or [D_6]-acetone are reported as δ values relative to CD_2Cl_2 (δ 53.37) or [D_6]-acetone (δ 29.84); the multiplicity of the signals was determined by the APT or DEPT technique. – Chemical shift assignments were made using COSY, HSQC and if necessary HMBC spectra. Spectra were recorded at ambient temperature and with normal detection (300MHz) or inverse detection (600 MHz). 600 MHz COSY spectra were collected using a gCOSY pulse sequence. HSQC spectra were collected using a gHSQC pulse sequence. ^{13}C HMBC spectra were collected using a gHMBC pulse sequence. 600 MHz NOESY spectra were recorded using a NOESY pulse sequence. – HRMS used a preselected ion peak matching at R >> 10000 to be within ± 2 ppm of the exact masses. – Optical rotations are referenced to the sodium D line (589 nm). – Melting points are uncorrected. – Solvents for extraction and chromatography were of technical grade and distilled before use. – All moisture sensitive reactions were carried out under dry nitrogen or argon in oven- and/or flame-dried glassware. – Column chromatography: silica gel 60 (0.040–0.063 mm, Machery&Nagel), Sephadex LH-20 (Pharmacia). – Flash column chromatography: silica gel 60 (0.025–0.040 mm, Machery&Nagel). – TLC: silica gel 60 F_{254} precoated plates (Merck, 0.25 mm). – Fermentations were carried out in a 2L fermentation vessel; medium A: 10 g/L malt extract, 4 g/L glucose, 4 g/L yeast extract, culture plates: additional 10 g/L agar. – Methyl lithium was titrated according to the method of Suffert.[1] – Ethyl ether was distilled from sodium benzophenone ketyl, dichloromethane and DMF were distilled from CaH_2. (2*S*,3*S*)-3-Benzyloxymethyl-2-[(tetrahydro-2*H*-pyran-2-yl)oxy]-4-butanolide (**10**)[2] and (*S*)-2-[(tetrahydro-2*H*-pyran-2-yl-oxy]-4-pentyne (**12**)[3] were prepared as described in the literature.

(1) Suffert, J. *J. Org. Chem.* **1989**, *54*, 509–510.

(2) Ueki, T.; Kinoshita, T. *Org. Biomol. Chem.* **2004**, *2*, 2777–2785.

(3) White, J. D.; Somers, T. C.; Reddy, G. N. *J. Org. Chem.* **1992**, *57*, 4991–4998.

2. Procedures for the isomerization of 4 and analytical data of isomers 4a–4c

Condition A: Storage of the spiroacetal **4** (86.9 mg, 0.430 mmol) in $CDCl_3$ (0.4 mL) for 48 h at rt led to an isomerization. After concentration in vacuo and column chromatography on silica gel (25 g, CH_2Cl_2/MeOH 9:1) 24.1 mg (28%) of **4** (R_f 0.42), 38.8 mg (45%) of **4a** (R_f 0.40), 15.9 mg (18%) of **4c** (R_f 0.28) and 4.1 mg (5%) of **4b** (R_f 0.25) were obtained as colorless solids.

Condition B: Okaspirodiol (**4**) (1.5 mg, 7.5 µmol) was dissolved in CD_3OD (100 µL) and concentrated hydrochloric acid (1 µL) was added. The resulting mixture was kept at rt and the equilibration process was monitored by TLC (CH_2Cl_2:MeOH 9:1) and ^{13}C NMR spectroscopy. After standing at rt for 48 h, no further conversion was observed.

Condition C: Okaspirodiol (**4**) (1.5 mg, 7.5 µmol) was dissolved in CD_2Cl_2 (100 µL) and (+)-10 camphorsulfonic acid (1 mg) was added. The resulting mixture was kept at rt and the equilibration process was monitored by TLC (CH_2Cl_2:MeOH 9:1) and ^{13}C NMR spectroscopy. After standing at rt for 48 h, no further conversion was observed.

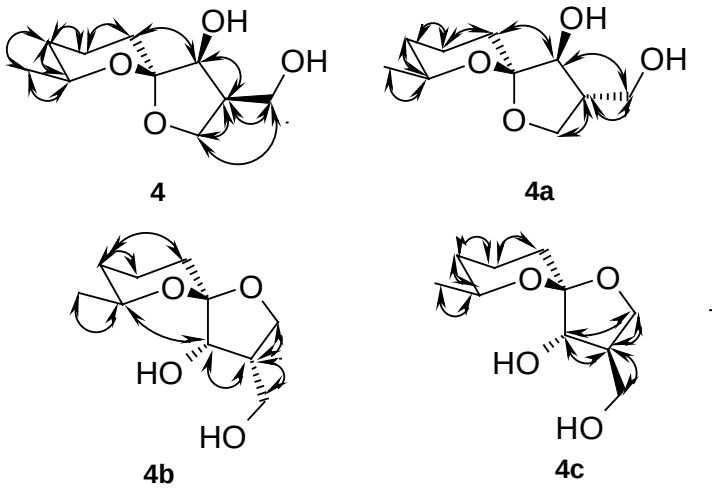
(3*R*,4*S*,5*S*,7*S*)-3-Hydroxymethyl-7-methyl-1,6-dioxaspiro[4.5]decan-4-ol (4a). Mp 85 °C; $[\alpha]_D^{20}$ – 112 (*c* 1.0, MeOH); ^1H NMR (600 MHz**,** CD_3OD) δ 1.14 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.21 (m_c, 1 H, 8-H), 1.45 (m_c, 1 H, 10-H), 1.58 (m_c, 1 H, 8-H), 1.64–1.75 [m, 2 H, 9(10)-H], 1.79 (m_c, 1 H, 9-H), 2.40 (m_c, 1 H, 3-H), 3.42 (d, ^3J = 8.5 Hz, 1 H, 4-H), 3.56 (dd, ^3J = 7.5, ^2J = 11.0 Hz, 1 H, 2-H), 3.65 (dd, ^3J = 7.0, ^2J = 8.8 Hz, 1 H, C*H*$_2$OH), 3.75 (dd, ^3J = 4.0, ^2J = 11.0 Hz, 1 H, 2-H), 3.91 (ddq, ^3J = 2.0, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H), 3.98 (t, J = 8.5 Hz, 1 H, C*H*$_2$OH); ^1H NMR (300 MHz, CD_2Cl_2) δ 1.11 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.15–1.30 (m, 1 H, 8-H), 1.48–1.75 [m, 5 H, 8(9,10)-H], 2.35 (m_c, 1 H, 3-H), 2.70 (s, br, 2 H, OH), 3.40–3.80 [m, 4 H, 2(4)-H, C*H*$_2$OH], 3.85–4.10 (m, 2 H, 7-H, C*H*$_2$OH); ^{13}C NMR (75.5 MHz, CD_2Cl_2) δ 20.1 (C-9), 21.9 (CH_3), 30.3 (C-10), 32.8 (C-8), 47.1 (C-3), 63.5 (C-2), 66.8 (CH_2OH), 67.2 (C-7), 79.1 (C-4), 102.9 (C-5); IR (cm^{-1}, KBr) 3425, 2939, 2538, 1444, 1383, 1199, 1137, 1106; MS (ESI) *m/z* (%) 225.1 (90) $[M+Na]^+$, 256.5 (100), 426.8 (50) $[2M+Na]^+$.

(3*S*,4*S*,5*R*,7*S*)-3-Hydroxymethyl-7-methyl-1,6-dioxaspiro[4.5]decan-4-ol (4b). Mp 98 °C; $[\alpha]_D^{20}$ +60 (*c* 0.5, MeOH); ^1H NMR (600 MHz**,** CD_3OD) δ 1.15 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.17 (m_c, 1 H, 8-H), 1.38 (m_c, 1 H, 8-H), 1.57–1.66 [m, 2 H, 9(10)-H], 1.77 (m_c, 1 H, 9-H), 2.12 (m_c, 1 H, 10-H), 2.74 (m_c, 1 H, 3-H), 3.62 (dd, ^3J = 7.0, ^2J = 11.0 Hz, 1 H, 2-H), 3.66 (dd, ^3J = 9.5, ^2J = 8.0 Hz, 1 H, C*H*$_2$OH), 3.71 (ddq, ^3J = 2.0, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H), 3.82 (dd, ^3J = 7.5, ^2J = 11.0 Hz, 1 H, 2-H), 4.04 (dd, ^3J = 9.0, ^2J = 8.0 Hz, 1 H, C*H*$_2$OH), 4.32 (d, J = 4.5 Hz, 1 H, 4-H); ^1H NMR (300 MHz, CD_2Cl_2) δ 1.12 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.15–1.30 (m, 1 H, 8-H), 1.42–1.61 [m, 3 H, 8(9)-H], 1.71–1.80 (m, 1 H, 10-H), 2.00–2.10 (m, 1 H, 10-H), 2.55 (s, br, 1 H, OH), 2.72 (m_c, 1 H, 3-H), 2.80 (s, br, 1 H, OH), 3.51–3.70 (m, 1 H, 7-H), 3.71 (t, J = 9.0 Hz, 1 H, C*H*$_2$OH), 3.78–3.90 (m, 2 H, 2-H), 4.00 (t, J = 9.0 Hz, 1 H, C*H*$_2$OH), 4.49 (d, ^3J = 6.1 Hz, 1 H, 4-H); ^{13}C NMR (75.5 MHz, CD_2Cl_2) δ 21.1 (C-9),

22.1 (CH_3), 27.9 (C-10), 32.5 (C-8), 43.5 (C-3), 60.3 (C-2), 67.4 (CH_2OH), 71.2 (C-7), 72.9 (C-4), 109.8 (C-5); IR (cm^{-1}, KBr) 3426, 2934, 1635, 1458, 1381, 1205, 1062; MS (ESI) *m/z* (%) 225.1 (100) $[M+Na]^+$.

(3*R*,4*S*,5*R*,7*S*)-3-Hydroxymethyl-7-methyl-1,6-dioxaspiro[4.5]decan-4-ol (4c). Mp 65 °C; $[\alpha]_D^{20}$ +24 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz**,** CD_3OD) δ 1.14 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.17 (m_c, 1 H, 8-H), 1.39 (m_c, 1 H, 8-H), 1.57–1.68 [m, 2 H, 9(10)-H], 1.76 (m_c, 1 H, 9-H), 2.05 (m_c, 1 H, 10-H), 2.34 (m_c, 1 H, 3-H), 3.59 (dd, ^3J = 8.0, ^2J = 10.5 Hz, 1 H, 2-H), 3.67–3.73 (m, 3 H, 2(7)-H, CH_2OH), 4.11 (t, J = 8.5 Hz, 1 H, CH_2OH), 4.14 (d, ^3J = 2.0 Hz, 1 H, 4-H); ^{1}H NMR (300 MHz, CD_2Cl_2) δ 1.12 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.15–1.30 (m, 1 H, 8-H), 1.40–1.61 [m, 3 H, 8(9)-H], 1.72–1.82 (m, 1 H, 10-H), 1.95–2.10 (m, 1 H, 10-H), 2.36 (m_c, 1 H, 3-H), 2.61 (s, br, 1 H, OH), 2.90 (s, br, 1 H, OH), 3.60–3.75 [m, 4 H, 2(7)-H, CH_2OH], 4.10 (t, J = 9.0 Hz, 1 H, CH_2OH), 4.19 (m_c, 1 H, 4-H); ^{13}C NMR (75.5 MHz, CD_2Cl_2) δ 20.6 (C-9), 21.8 (CH_3), 28.3 (C-10), 32.2 (C-8), 51.1 (C-3), 63.5 (C-2), 67.8 (CH_2OH), 71.2 (C-7), 75.9 (C-4), 109.1 (C-5); IR (cm^{-1}, KBr) 3422, 2939, 1653, 1445, 1381, 1194, 1162; MS (ESI) *m/z* (%) 203.2 (30) $[M+H]^+$, 225.1 (80) $[M+Na]^+$, 427.2 (100) $[2M+Na]^+$.

3. Observed cross peaks from NOESY experiments



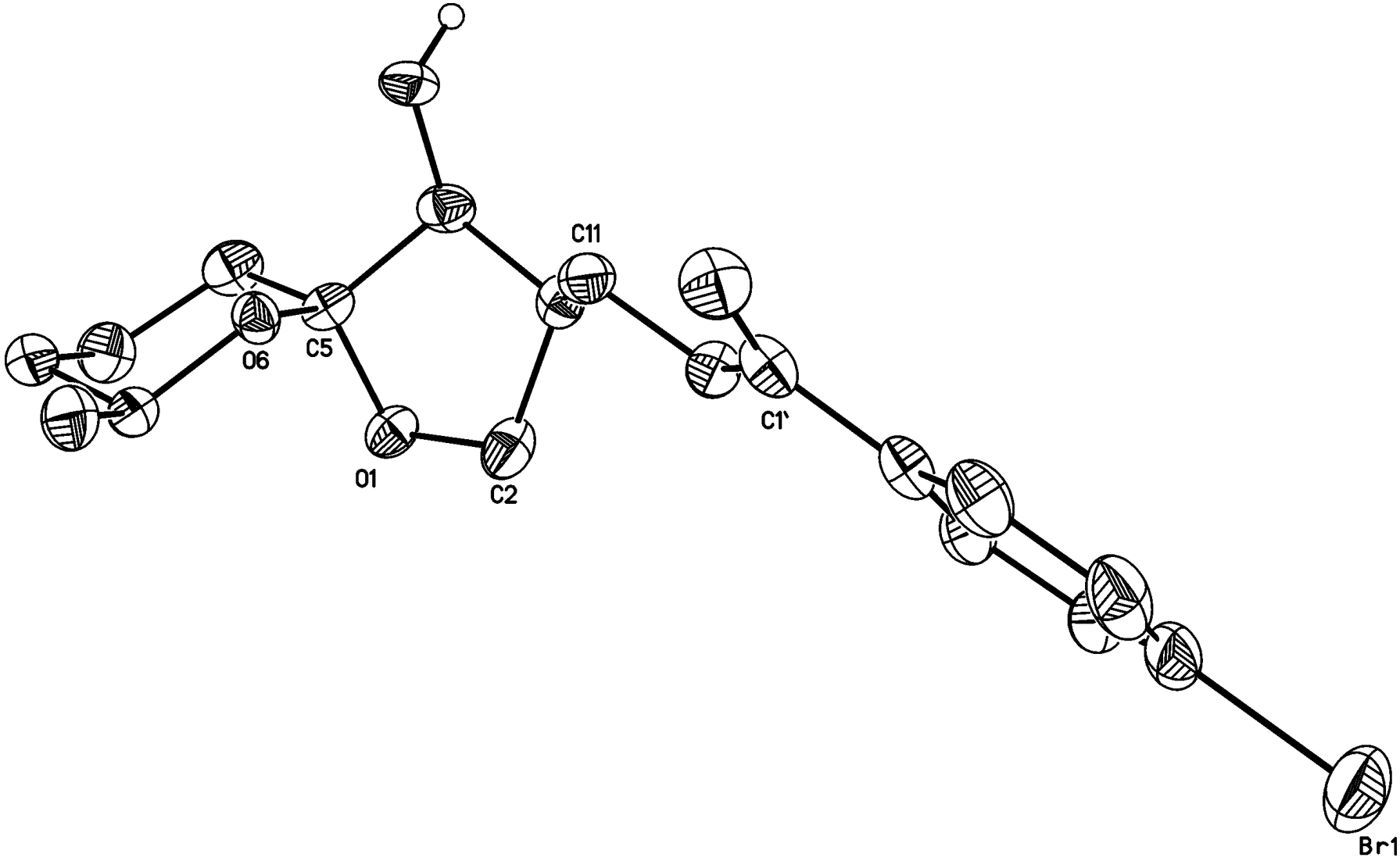
The signal assignment in **4c** is complicated by extensive signal overlaps, especially for the protons of the five membered ring.

4. HMBC and COSY correlations for okaspirodiol (4)

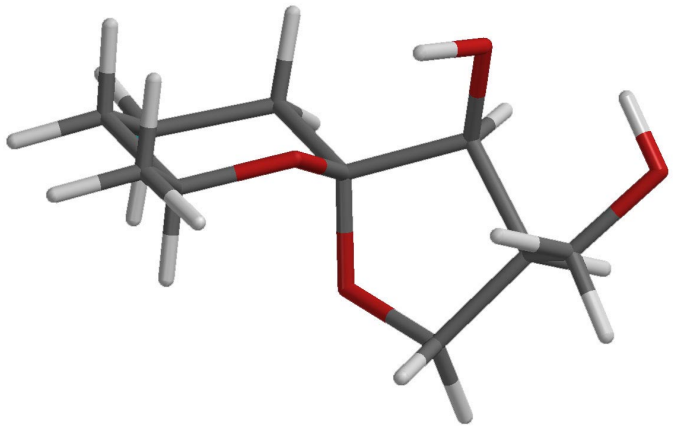
Carbon atom	δ_C [ppm]	HMBC	COSY
2	68.9	3.76 (2-H_a), 3.74 (11-H_b), 3.69 (11-H_a)	a) 3.98 (2-H_b), 2.49 (3-H) b) 3.76 (2-H_a) 2.49 (3-H)
3	43.2	3.98 (2-H_b), 3.84 (4-H), 3.76 (2-H_a), 3.74 (11-H_b), 3.69 (11-H_a)	3.98 (2-H_b), 3.84 (4-H), 3.76 (2-H_a), 3.74 (11-H_b), 3.69 (11-H_a)
4	77.8	3.98 (2-H_b), 3.76 (2-H_a), 3.74 (11-H_b), 3.69 (11-H_a), 2.49 (3-H)	2.49 (3-H)
5	103.9	3.98 (2-H_b), 2.49 (3-H), 1.73 (10-H_b), 1.67 (9-H_a), 1.48 (10-H_a)	-
7	67.9	1.73 (10-H_b), 1.67 (9-H_a), 1.21 (8-H_a), 1.14 (12-H)	1.58 (8-H_b), 1.21 (8-H_a), 1.14 (12-H)
8	33.4	1.80 (9-H_b), 1.73 (10-H_b), 1.67 (9-H_a), 1.48 (10-H_a), 1.14 (12-H)	a) 3.93 (7-H), 1.80 (9-H_b), 1.67 (9-H_a), 1.21 (8-H_a) b) 1.80 (9-H_b), 1.67 (9-H_a), 1.58 (8-H_b)
9	21.0	1.73 (10-H_b), 1.48 (8-H_b), 1.21 (8-H_a)	a) 1.80 (9-H_b), 1.73 (10-H_b), 1.58 (8-H_b), 1.48 (10-H_a), 1.21 (8-H_a) b) 1.73 (10-H_b), 1.67 (9-H_a), 1.58 (8-H_b), 1.48 (10-H_a), 1.21 (8-H_a)
10	31.4	3.84 (4-H), 1.80 (9-H_b), 1.67 (9-H_a), 1.58 (8-H_b)	a) 1.80 (9-H_b), 1.67 (9-H_a), 1.73 (10-H_b) b)1.80 (9-H_b), 1.67 (9-H_a), 1.48 (10-H_a)
11	62.1	3.98 (2-H_b), 3.84 (4-H), 3.76 (2-H_a)	a) 3.74 (11-H_b), 2.49 (3-H), b) 3.69 (11-H_a), 2.49 (3-H)
12	22.0	1.21 (8-H_a)	3.93 (7-H)

Spectra were recorded on a 500 MHz spectrometer, COSY and ^{1}H NMR in acetone-d_6, HMBC and ^{13}C NMR in methanol-d_4.

5. ORTEP drawing of the *p*-bromobenzoate 5 of okaspirodiol



6. Computational Data



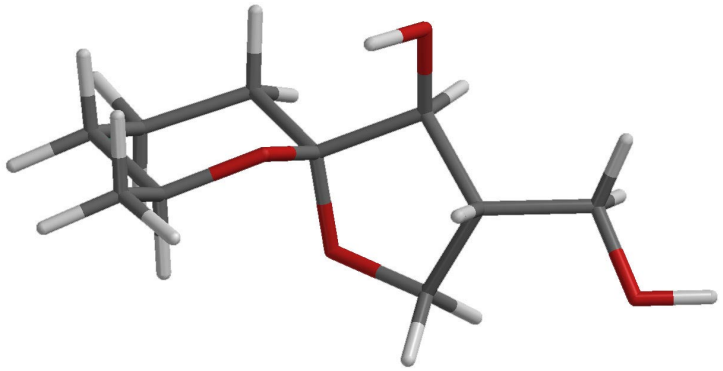
Term	ZPE [kJ/mol]	Enthalpy [kJ/mol]	Entropy [kJ/mol]	Cv [kJ/mol]
Total Vibrations	742.5559	27.5236	166.4359	196.8729
Ideal Gas		2.4789		
Translation		3.7184	174.9558	12.4716
Rotation		3.7184	131.79787	12.4716
Totals		**779.9953**	473.1904	221.8162
Gibb's Free Energy (H - TS)		638.9135		

Standard thermodynamic quantities at 298.15 K and 1.00 atm.

a) Structure, Total Energies and Cartesian coordinates for okaspirodiol (**4**)

Atom		X	Y	Z
C	1	0.0199007	0.0177633	0.0302675
C	2	0.0284798	-0.0012032	0.0351600
C	3	-0.0152552	-0.0229763	0.0280214
C	4	-0.0254535	0.0240326	-0.0235461
C	5	0.0358443	-0.0144030	-0.0245619
O	6	-0.0872416	-0.1017662	0.0729712
H	7	-0.0132998	0.0117922	-0.1716477
H	8	-0.0005005	0.0260184	-0.1717565
H	9	0.0213094	-0.0217157	0.1681547
H	10	0.0043932	-0.0001225	0.1720908
H	11	0.1225845	0.1068901	0.0655647
H	12	0.1496453	-0.0447814	-0.0756785
H	13	-0.0491981	0.1624910	-0.0492364
O	14	0.1093425	0.0922617	-0.1476742
C	15	-0.0238046	-0.0275498	-0.0334841
H	16	-0.0307641	0.1731999	0.0412020
C	17	0.0310686	-0.0355359	0.0036558
H	18	-0.1288694	0.1188236	-0.0185676
C	19	-0.0310101	0.0017918	0.0392707
H	20	-0.0731408	0.0896770	-0.1434218
H	21	0.0273294	-0.1655383	-0.0490839
O	22	-0.1258165	0.0723438	0.1950837
H	23	0.1251387	-0.1589551	-0.0151463
C	24	0.0243655	0.0426021	-0.0115611
H	25	-0.1063723	-0.0533829	-0.1345289
H	26	0.1140425	-0.1206570	0.0185691
O	27	-0.2444668	-0.0629105	0.0115977
H	28	0.1170244	0.0589456	0.1516499
C	29	0.0008558	0.0136662	-0.0082822
H	30	-0.1366651	-0.0994563	-0.0579722
H	31	0.1475620	-0.0623174	-0.0727338
H	32	0.0129719	-0.0190277	0.1756239

b) Structure, Total Energies and Cartesian coordinates for isomer **4a**

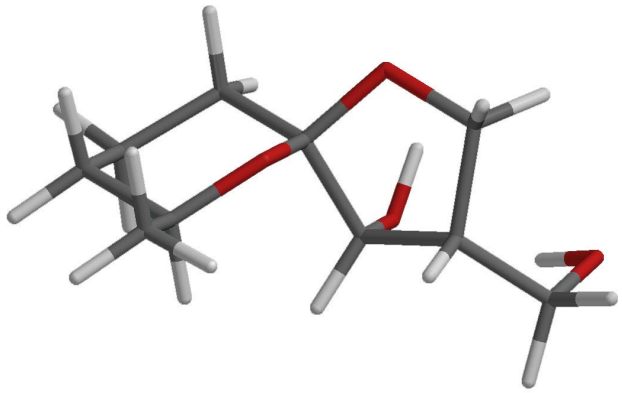


Term	ZPE [kJ/mol]	Enthalpy [kJ/mol]	Entropy [kJ/mol]	Cv [kJ/mol]
Total Vibrations	738.7749	29.2508	178.5163	202.0069
Ideal Gas		2.4789		
Translation		3.7184	174.9558	12.4716
Rotation		3.7184	132.5164	12.4716
Totals		**777.9415**	485.9886	226.9501
Gibb's Free Energy (H - TS)		633.0440		

Standard thermodynamic quantities at 298.15 K and 1.00 atm.

Atom		X	Y	Z
C	1	0.0083987	0.0263136	0.0216150
C	2	0.0353555	0.0069169	0.0295670
C	3	-0.0073072	-0.0220951	0.0314082
C	4	-0.0309600	0.0171797	-0.0222556
C	5	0.0326788	-0.0113567	-0.0262931
O	6	-0.0619413	-0.1067316	0.0970292
H	7	-0.0342447	-0.0089981	-0.1682859
H	8	-0.0233098	0.0083179	-0.1717256
H	9	0.0439500	-0.0008882	0.1646969
H	10	0.0225008	0.0169401	0.1691838
H	11	0.1089712	0.1301832	0.0409347
H	12	0.1443159	-0.0288742	-0.0901650
H	13	-0.0791641	0.1471048	-0.0559972
O	14	0.0881089	0.0655010	-0.1658968
C	15	-0.0092243	-0.0347324	-0.0232749
H	16	-0.0654992	0.1634235	0.0082008
C	17	-0.0152769	0.0427092	-0.0116504
C	18	0.0067246	-0.0059665	0.0404185
H	19	-0.1093946	0.1120453	-0.0860576
H	20	0.0148521	-0.1509192	-0.1006525
O	21	-0.1168799	0.0979105	0.2040355
H	22	0.1362879	-0.1561493	0.0005368
C	23	-0.0018575	0.0116826	-0.0092565
H	24	-0.1246333	-0.1237820	-0.0303145
H	25	0.1455154	-0.0464273	-0.0861033
H	26	0.0362880	0.0023278	0.1729619
C	27	-0.0089387	-0.0036911	-0.0004486
H	28	-0.0411966	0.1590657	-0.0353510
H	29	-0.0348670	-0.0251212	0.1673785
O	30	0.1180831	-0.1395413	-0.1350893
H	31	-0.2134853	0.0237341	0.0287287
H	32	0.0361494	-0.1660820	0.0421223

c) Structure, Total Energies and Cartesian coordinates for isomer **4b**

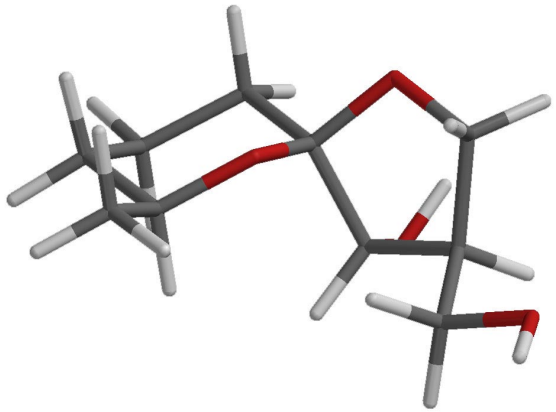


Term	ZPE [kJ/mol]	Enthalpy [kJ/mol]	Entropy [kJ/mol]	Cv [kJ/mol]
Total Vibrations	742.6325	27.4222	161.1249	197.3138
Ideal Gas		2.4789		
Translation		3.7184	174.9558	12.4716
Rotation		3.7184	132.5164	12.4716
Totals		**779.9704**	468.0191	222.2571
Gibb's Free Energy (H - TS)		640.4305		

Standard thermodynamic quantities at 298.15 K and 1.00 atm.

Atom		X	Y	Z
C	1	-0.0196095	0.0186690	0.0469256
C	2	-0.0335710	0.0118578	-0.0211685
C	3	0.0059435	-0.0277374	-0.0329346
C	4	0.0378291	0.0148945	0.0181317
C	5	-0.0280716	-0.0074104	0.0469501
O	6	0.0347639	-0.1197784	-0.1119611
H	7	0.0601144	-0.0088695	0.1533472
H	8	0.0580711	0.0114678	0.1604717
H	9	-0.0767259	-0.0012206	-0.1541319
H	10	-0.0598563	0.0139771	-0.1640222
H	11	-0.1053760	0.1397079	-0.0226876
H	12	-0.1250289	-0.0139439	0.1185988
H	13	0.0930325	0.1453378	0.0285576
C	14	-0.0014827	-0.0204555	-0.0319540
O	15	-0.0105199	0.0973432	-0.1806880
C	16	-0.0118299	-0.0129254	0.0360635
H	17	0.1544707	0.0724643	-0.0611567
C	18	0.0537526	0.0359896	0.0121770
H	19	-0.0755230	-0.1510772	0.0313676
C	20	0.0034083	0.0112606	0.0103867
H	21	-0.0723076	-0.0403081	0.1634139
H	22	-0.0358286	-0.1486376	-0.0983795
O	23	0.0341838	0.0802477	0.2205164
H	24	0.0448862	0.0798759	-0.1857425
C	25	-0.0565978	0.0021721	-0.0159790
H	26	-0.0130615	-0.0330118	0.1670741
H	27	0.0901289	-0.1384737	-0.0723548
O	28	0.2275026	-0.0025598	-0.1025324
H	29	-0.0937844	0.1552975	0.0835716
H	30	-0.0672771	0.0016446	-0.1653008
H	31	0.1181176	-0.1334779	0.0110372
H	32	-0.1297533	-0.0323203	0.1124031

d) Structure, Total Energies and Cartesian coordinates for isomer **4c**

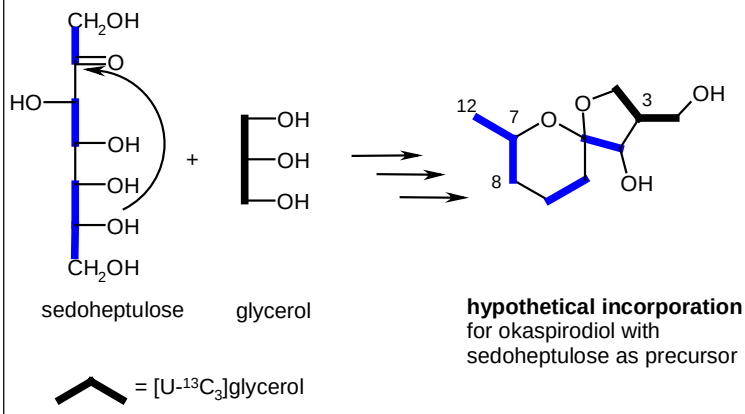


Term	ZPE [kJ/mol]	Enthalpy [kJ/mol]	Entropy [kJ/mol]	Cv [kJ/mol]
Total Vibrations	738.9499	29.0760	175.7837	202.2328
Ideal Gas		2.4789		
Translation		3.7184	174.9558	12.4716
Rotation		3.7184	132.5164	12.4716
Totals		**777.9416**	482.8877	227.1760
Gibb's Free Energy (H - TS)		633.9687		

Atom		X	Y	Z
C	1	0.0342035	0.0123018	-0.0452986
C	2	0.0208514	-0.0012149	0.0320996
C	3	-0.0182238	-0.0220313	0.0329459
C	4	-0.0235341	0.0257051	-0.0232505
C	5	0.0291403	-0.0197214	-0.0424565
O	6	-0.0743927	-0.0869156	0.1086315
H	7	-0.0412276	-0.0003750	-0.1594297
H	8	-0.0335316	0.0174047	-0.1670632
H	9	0.0549174	-0.0140833	0.1626350
H	10	0.0430536	0.0066089	0.1691961
H	11	0.1438783	0.0976062	0.0302381
H	12	0.1245987	-0.0646363	-0.1009933
H	13	-0.0337492	0.1656315	-0.0460013
C	14	-0.0151872	-0.0196627	0.0382669
O	15	0.0227612	0.1016213	0.1761546
C	16	0.0111672	-0.0098903	-0.0354495
H	17	-0.1261062	0.1259108	0.0355353
C	18	-0.0001245	0.0143614	0.0052069
C	19	0.0022962	0.0127233	-0.0132476
H	20	0.0703625	-0.0726755	-0.1515421
H	21	-0.0277397	-0.1414816	0.1088704
O	22	0.0011611	0.0741252	-0.2301041
H	23	-0.0339057	0.1050125	0.1749877
H	24	0.0455803	-0.0075879	0.1725467
H	25	-0.1523255	-0.0871448	-0.0195271
H	26	0.1224769	-0.0832767	-0.0938028
H	27	-0.1470469	0.0401698	-0.0932468
C	28	0.0091043	0.0205117	-0.0030216
H	29	0.1344937	-0.0416750	0.0621747
H	30	0.0049130	-0.0439347	-0.1659049
O	31	-0.1907402	0.1218121	0.1047365
H	32	0.0428751	-0.2251994	-0.0238863

Standard thermodynamic quantities at 298.15 K and 1.00 atm.

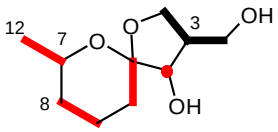
7. Exclusion of a sedoheptulose-based biosynthesis



sedoheptulose glycerol

= [U-$^{13}C_3$]glycerol

hypothetical incorporation pattern
for okaspirodiol with
sedoheptulose as precursor

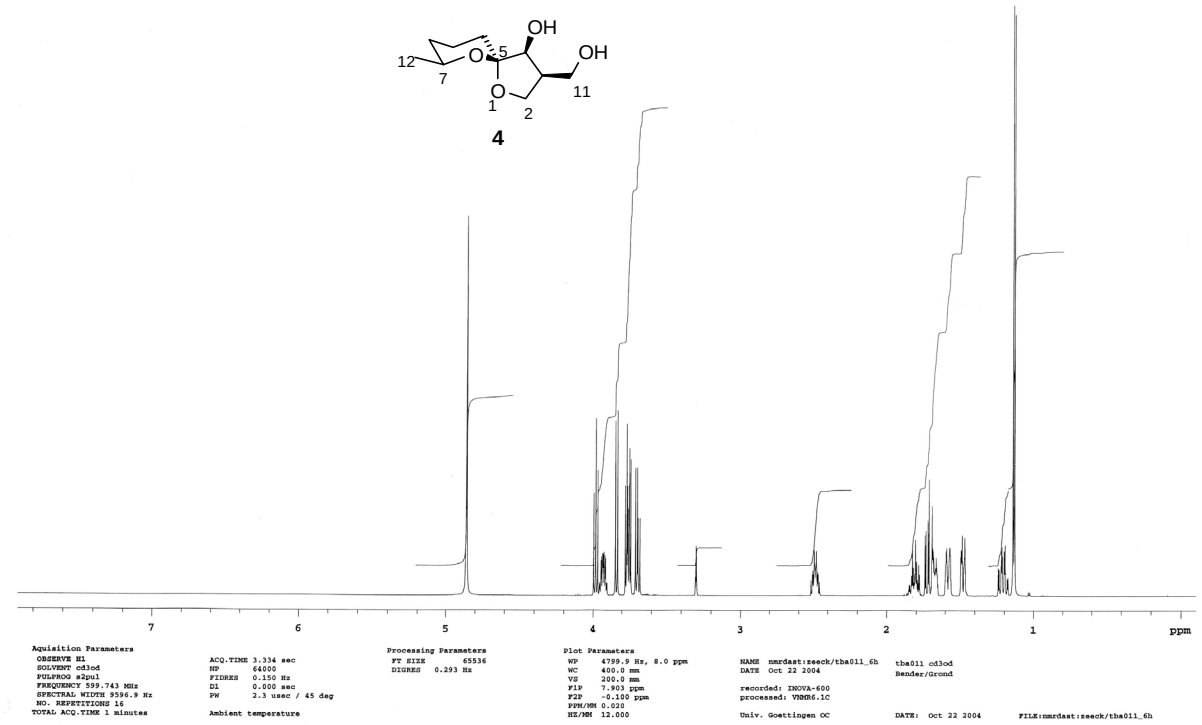


observed incorporation pattern
of okaspirodiol
from [U-$^{13}C_3$]glycerol feeding

The alternative hypothesis for the biosynthesis of okaspirodiol with sedoheptulose and glycerol as precursors is not supported by the observed incorporation pattern!

8. Spectra

a) ^{1}H, ^{13}C NMR and NOESY spectra of okaspirodiol (**4**) with extentions (CD_3OD)



Aquisition Parameters
OBSERVE H1
SOLVENT cd3od
PULPROG s2pul
FREQUENCY 599.743 MHz
SPECTRAL WIDTH 9596.9 Hz
NO. REPETITIONS 16
TOTAL ACQ.TIME 1 minutes

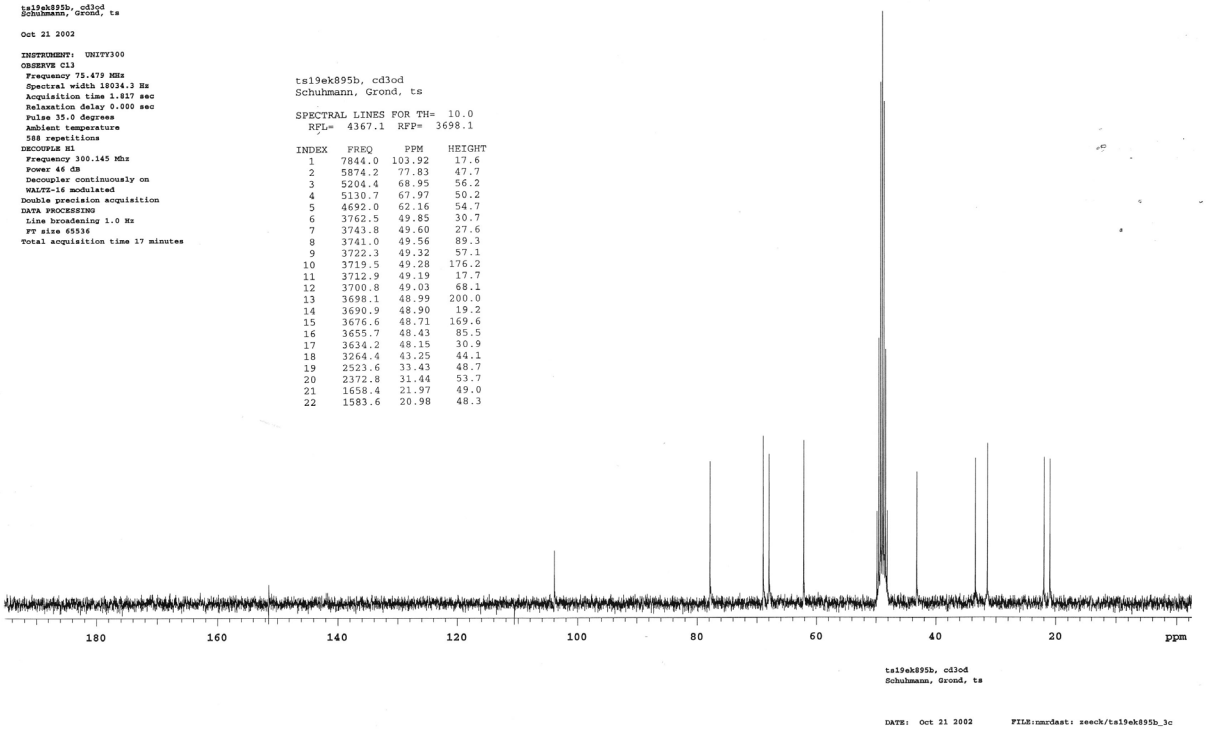
ACQ.TIME 3.334 sec
NP 64000
FIDRES 0.150 Hz
D1 0.000 sec
PW 2.3 usec / 45 deg
Ambient temperature

Processing Parameters
FT SIZE 65536
DIGRES 0.293 Hz

Plot Parameters
WP 4799.9 Hz, 8.0 ppm
WC 400.0 mm
VS 200.0 mm
F1P 7.903 ppm
F2P -0.100 ppm
PPM/MM 0.020
HZ/MM 12.000

NAME nmrdast:zeeck/tba011_6h
DATE Oct 22 2004
recorded: INOVA-600
processed: VNMR6.1C
Univ. Goettingen OC

tba011 cd3od
Bender/Grond
DATE: Oct 22 2004
FILE:nmrdast:zeeck/tba011_6h



ts19ek895b, cd3od
Schuhmann, Grond, ts

Oct 21 2002

INSTRUMENT: UNITY300
OBSERVE C13
Frequency 75.479 MHz
Spectral width 18034.3 Hz
Acquisition time 1.817 sec
Relaxation delay 0.000 sec
Pulse 35.0 degrees
Ambient temperature
588 repetitions
DECOUPLE H1
Frequency 300.145 Mhz
Power 46 dB
Decoupler continuously on
WALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total acquisition time 17 minutes

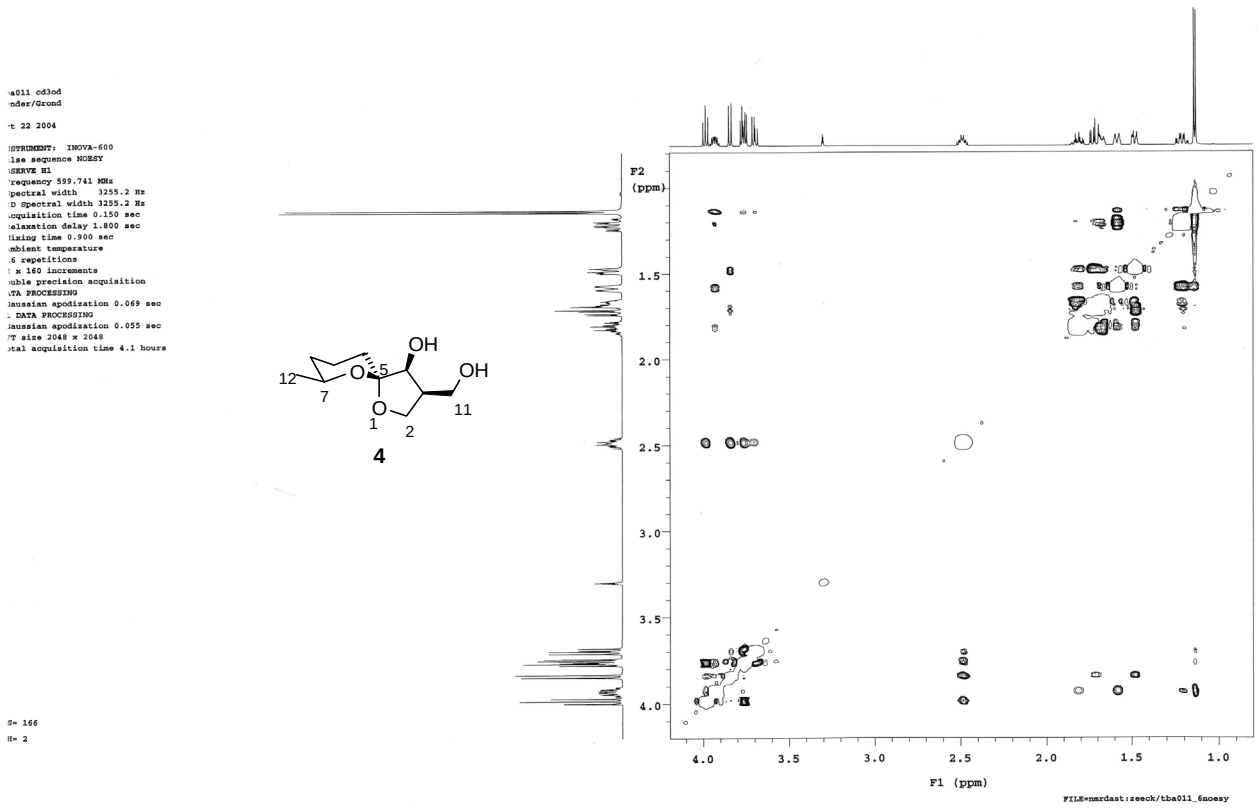
ts19ek895b, cd3od
Schuhmann, Grond, ts

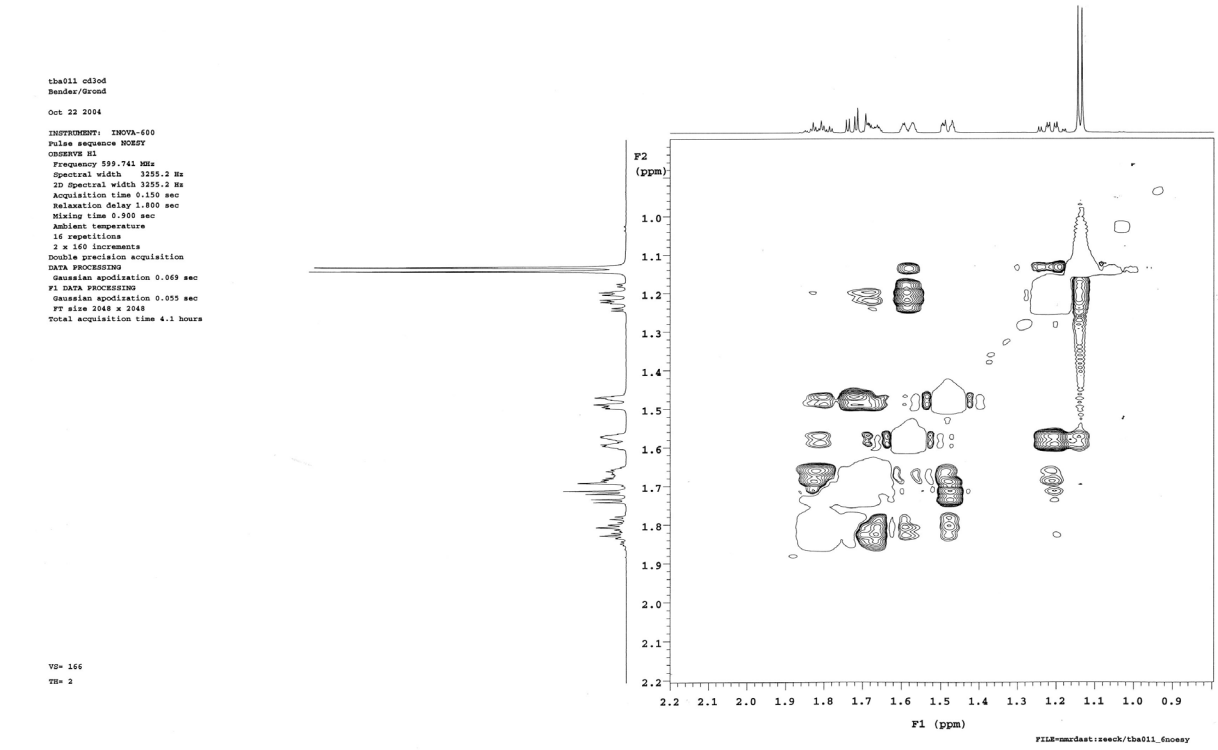
SPECTRAL LINES FOR TH= 10.0
RFL= 4367.1 RFP= 3698.1

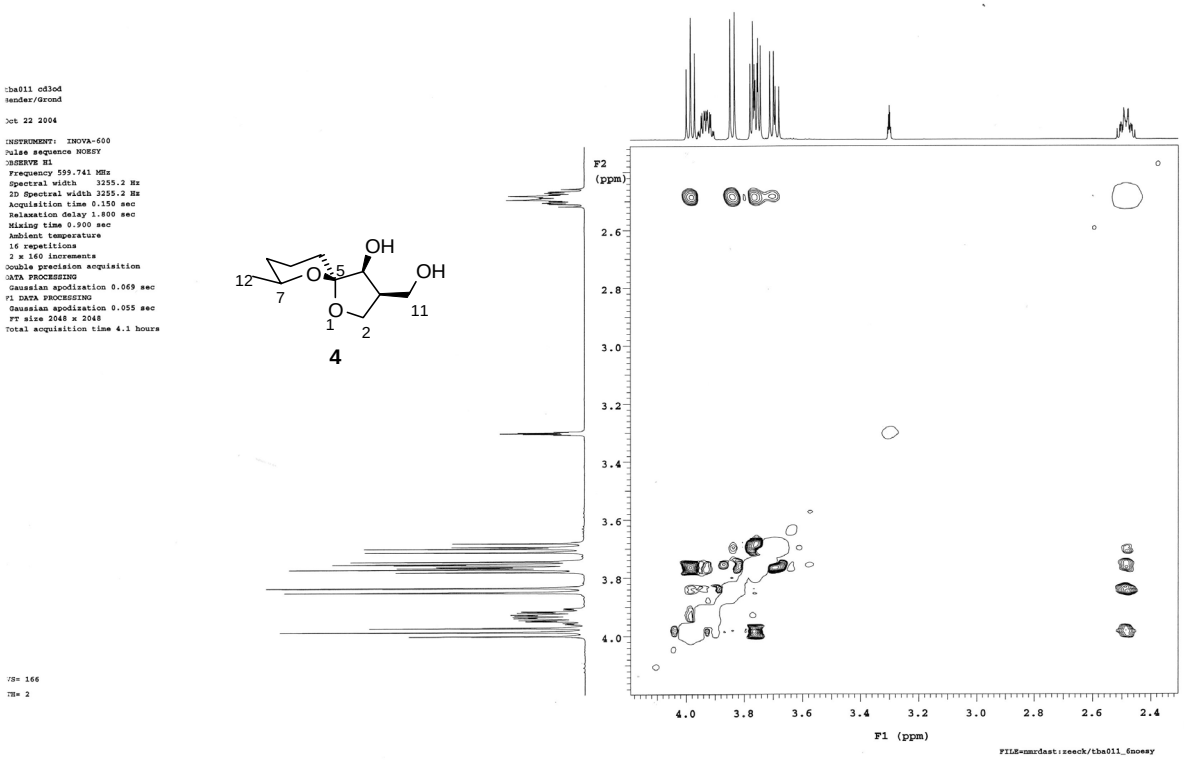
INDEX	FREQ	PPM	HEIGHT
1	7844.0	103.92	17.6
2	5874.2	77.83	47.7
3	5204.4	68.95	56.2
4	5130.7	67.97	50.2
5	4692.0	62.16	54.7
6	3762.5	49.85	30.7
7	3743.8	49.60	27.6
8	3741.0	49.56	89.3
9	3722.3	49.32	57.1
10	3719.5	49.28	176.2
11	3712.9	49.19	17.7
12	3700.8	49.03	68.1
13	3698.1	48.99	200.0
14	3690.9	48.90	19.2
15	3676.6	48.71	169.6
16	3655.7	48.43	85.5
17	3634.2	48.15	30.9
18	3264.4	43.25	44.1
19	2523.6	33.43	48.7
20	2372.8	31.44	53.7
21	1658.4	21.97	49.0
22	1583.6	20.98	48.3

ts19ek895b, cd3od
Schuhmann, Grond, ts

DATE: Oct 21 2002
FILE:nmrdast: zeeck/ts19ek895b_3c

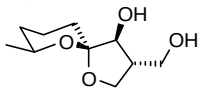

a011 cd3od
nder/Grond
t 22 2004
STRUMENT: INOVA-600
lse sequence NOESY
SERVE H1
requency 599.741 MHz
pectral width 3255.2 Hz
D Spectral width 3255.2 Hz
cquisition time 0.150 sec
elaxation delay 1.800 sec
ixing time 0.900 sec
mbient temperature
6 repetitions
x 160 increments
uble precision acquisition
TA PROCESSING
aussian apodization 0.069 sec
DATA PROCESSING
aussian apodization 0.055 sec
T size 2048 x 2048
tal acquisition time 4.1 hours
S= 166
H= 2
OH
OH
O
O
12
7
5
1
2
11
4
F2 (ppm)
F1 (ppm)
FILE=nmrdast:zeeck/tba011_6noesy


tba011 cd3od
Bender/Grond
Oct 22 2004
INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.741 MHz
Spectral width 3255.2 Hz
2D Spectral width 3255.2 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.055 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours
VS= 166
TH= 2
F2 (ppm)
F1 (ppm)
FILE=nmrdast:zeeck/tba011_6noesy


tba011 cd3od
sender/grond
Oct 22 2004
INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.741 MHz
Spectral width 3255.2 Hz
2D Spectral width 3255.2 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.055 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours
NS= 166
TH= 2
OH
OH
O
O
12
7
5
1
2
11
4
F2
(ppm)
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
F1 (ppm)
FILE=nmrdast:zeeck/tba011_6noesy

b) ^{1}H NMR, ^{13}C NMR and NOESY spectra of isomer **4a** with extentions (CD_3OD)

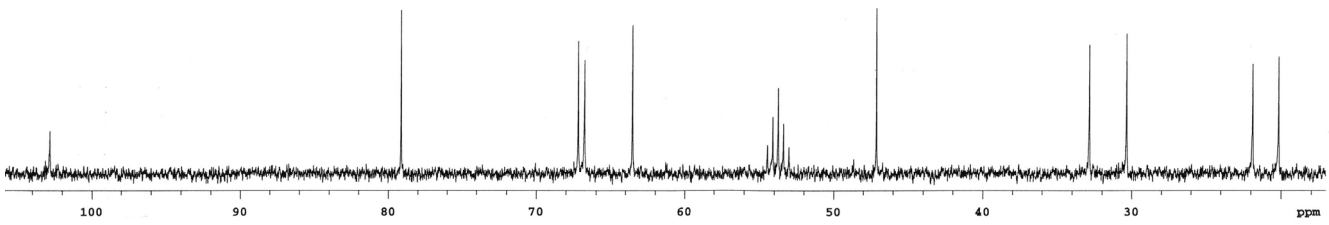
.



4a


```
Aquisition Parameters                                  Processing Parameters                Plot Parameters
 OBSERVE H1                                             FT SIZE      65536                   WP     4799.9 Hz, 8.0 ppm
 SOLVENT cd3od                                          DIGRES   0.293 Hz                    WC     400.0 mm
 PULPROG s2pul                                                                               VS     200.0 mm
 FREQUENCY 599.743 MHz                                                                      F1P    7.903 ppm
 SPECTRAL WIDTH 9596.9 Hz                                                                   F2P    -0.100 ppm
 NO. REPETITIONS 16                                                                         PPM/MM 0.020
TOTAL ACQ.TIME 1 minutes                                                                    HZ/MM  12.000

ACQ.TIME 3.334 sec                                                                          NAME  nmrdast:zeeck/ts19ek8953_6h ts19ek8953  cd3od
NP       64000                                                                              DATE  Oct 22 2004                 Schuhmann/Grond
FIDRES   0.150 Hz                                                                                                            recorded: INOVA-600
D1       0.000 sec                                                                                                           processed: VNMR6.1C
PW       2.3 usec / 45 deg                                                                  Univ. Goettingen OC            DATE:  Oct 22 2004      FILE:nmrdast:zeeck/ts19ek8953_6h
Ambient temperature
```

```
ts19ek8953, cd2cl2
Schuhmann, Grond, ts

May 24 2004

INSTRUMENT  MERCURY-300
OBSERVE C13
 Frequency 75.478 MHz
 Spectral width 18761.7 Hz
 Acquisition time 1.706 sec
 Relaxation delay 0.000 sec
 Pulse width 35.0 degrees
 Ambient temperature
 No. repetitions 335
DECOUPLE H1
 Frequency 300.140 Mhz
 Power 40 dB
 Decoupler continuously on
 WALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
 Line broadening 1.0 Hz
 FT size 131072
Total acquisition time 9 minutes
```



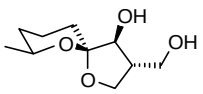

```
ts19ek8953, cd2cl2
Schuhmann, Grond, ts

DATE:  May 24 2004      FILE:nmrdast:zeeck/ts19ek8953_3c
```

ts19ek8953 cd3od
Schuhmann/Grond

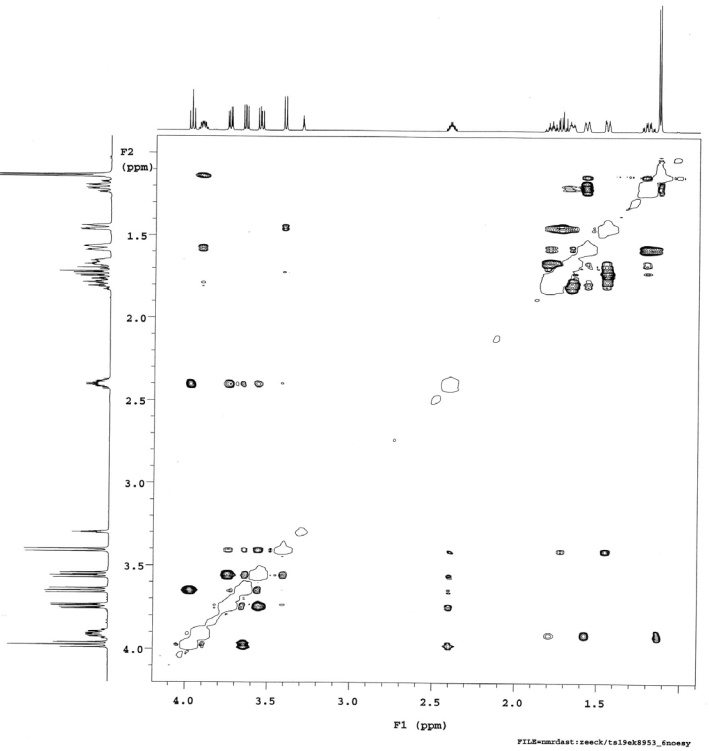
Oct 22 2004

INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.741 MHz
Spectral width 2759.4 Hz
2D Spectral width 2759.4 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.037 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours

VS= 345
TH= 2



4a



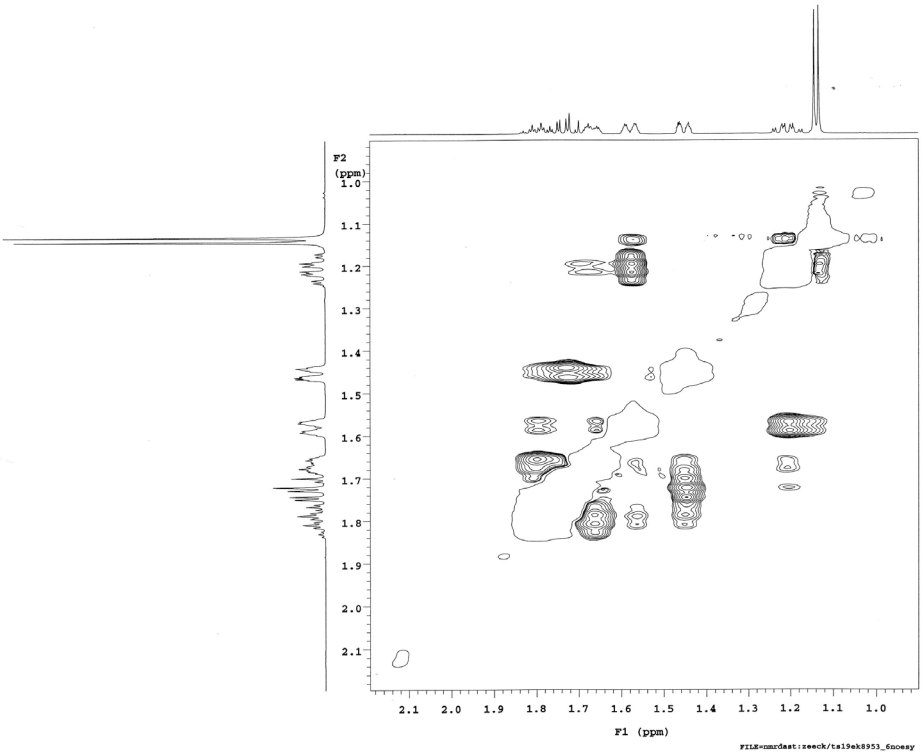
FILE=nmrdast:zeeck/ts19ek8953_6noesy

s19ek8953 cd3od
chuhmann/Grond

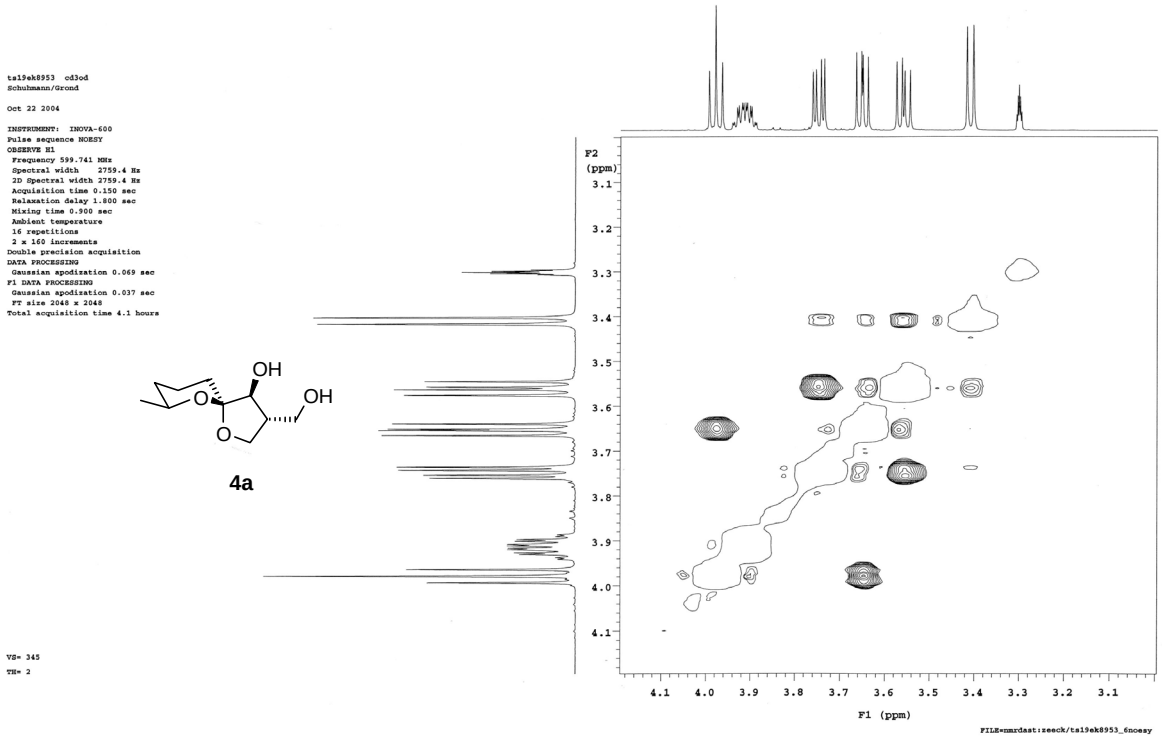
ct 22 2004

NSTRUMENT: INOVA-600
ulse sequence NOESY
BSERVE H1
Frequency 599.741 MHz
Spectral width 2759.4 Hz
2D Spectral width 2759.4 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
ouble precision acquisition
ATA PROCESSING
Gaussian apodization 0.069 sec
'1 DATA PROCESSING
Gaussian apodization 0.037 sec
FT size 2048 x 2048
otal acquisition time 4.1 hours

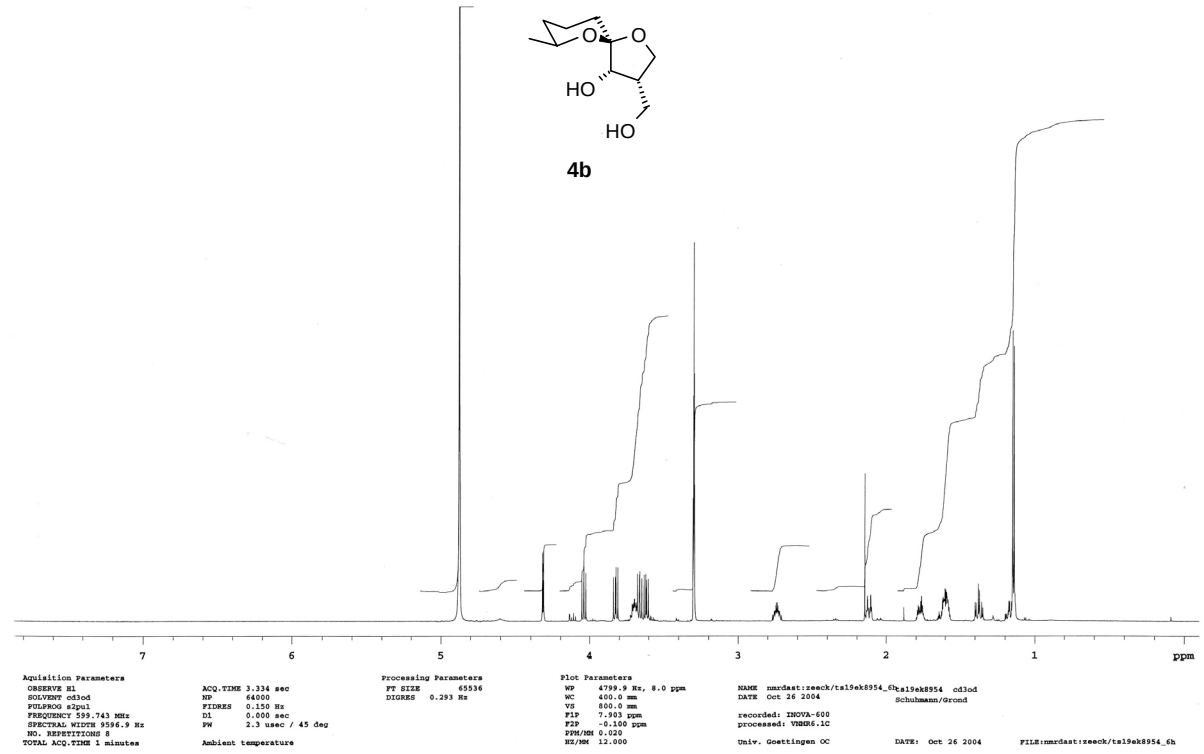
VS= 345
TH= 2



FILE=nmrdast:zeeck/ts19ek8953_6noesy


ts19ek8953 cd3od
Schuhmann/Grond
Oct 22 2004
INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.741 MHz
Spectral width 2759.4 Hz
2D Spectral width 2759.4 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.037 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours
OH
OH
O
O
4a
VS= 345
TH= 2
F2 (ppm)
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1
F1 (ppm)
FILE=nmrdast:zeeck/ts19ek8953_6noesy

c) ^{1}H NMR, ^{13}C NMR and NOESY spectra of isomer **4b** with extentions (CD_3OD)



Aquisition Parameters
OBSERVE H1
SOLVENT cd3od
PULPROG s2pul
FREQUENCY 599.743 MHz
SPECTRAL WIDTH 9596.9 Hz
NO. REPETITIONS 8
TOTAL ACQ.TIME 1 minutes

ACQ.TIME 3.334 sec
NP 64000
FIDRES 0.150 Hz
D1 0.000 sec
PW 2.3 usec / 45 deg
Ambient temperature

Processing Parameters
FT SIZE 65536
DIGRES 0.293 Hz

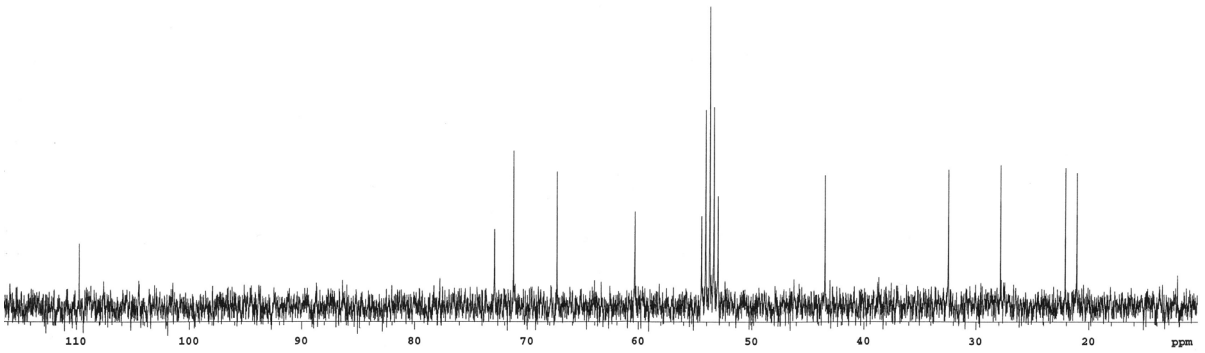
Plot Parameters
WP 4799.9 Hz, 8.0 ppm
WC 400.0 mm
VS 800.0 mm
F1P 7.903 ppm
F2P -0.100 ppm
PPM/MM 0.020
HZ/MM 12.000

NAME nmrdast:zeeck/ts19ek8954_6h ts19ek8954 cd3od
DATE Oct 26 2004 Schuhmann/Grond
recorded: INOVA-600
processed: VNMR6.1C
Univ. Goettingen OC
DATE: Oct 26 2004 FILE:nmrdast:zeeck/ts19ek8954_6h

ts19ek8954, cd2c12
Schuhmann, Grond, ts

May 24 2004

INSTRUMENT MERCURY-300
OBSERVE C13
Frequency 75.478 MHz
Spectral width 18761.7 Hz
Acquisition time 1.706 sec
Relaxation delay 0.000 sec
Pulse width 35.0 degrees
Ambient temperature
No. repetitions 452
DECOUPLE H1
Frequency 300.140 MHz
Power 40 dB
Decoupler continuously on
WALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total acquisition time 12 minutes



ts19ek8954, cd2c12
Schuhmann, Grond, ts

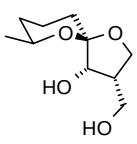
DATE: May 24 2004 FILE:nmrdast:zeeck/ts19ek8954_3c

ts19ek8954 cd3od
Schuhmann/Grond

Oct 26 2004

INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.741 MHz
Spectral width 3109.5 Hz
2D Spectral width 3109.5 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.088 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours

VS= 77
TH= 2



4b

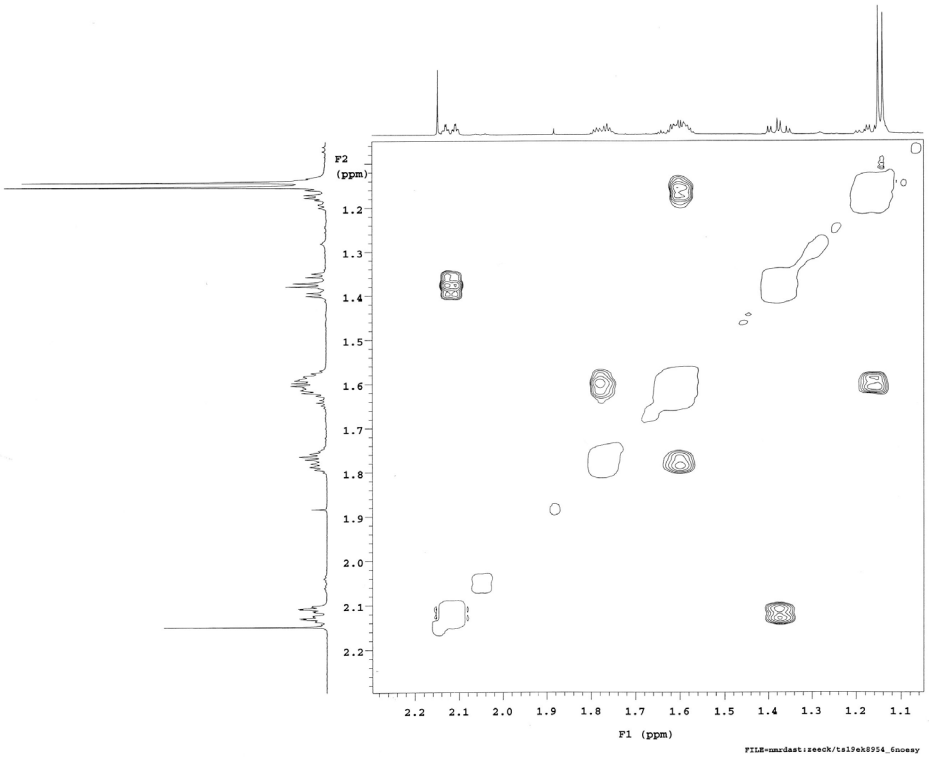
FILE=nmrdast:zeeck/ts19ek8954_6noesy

ts19ek8954 cd3od
Schuhmann/Grond

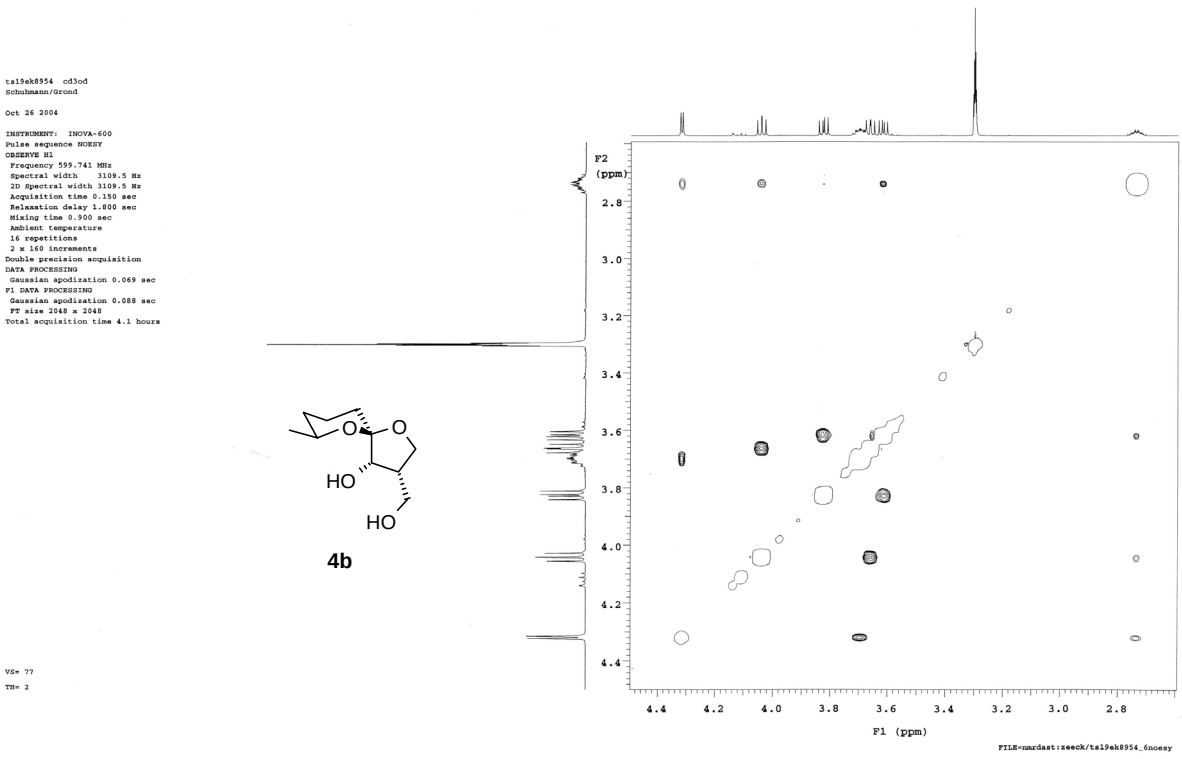
Oct 26 2004

INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.741 MHz
Spectral width 3109.5 Hz
2D Spectral width 3109.5 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.088 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours

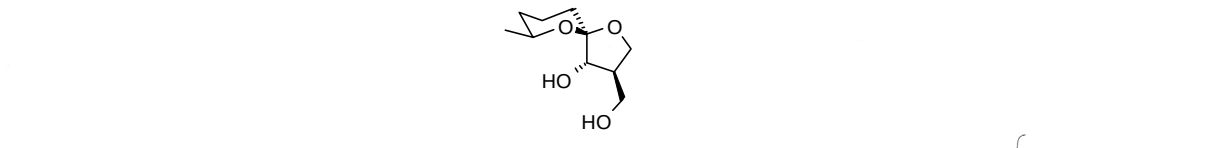
VS= 77
TH= 2



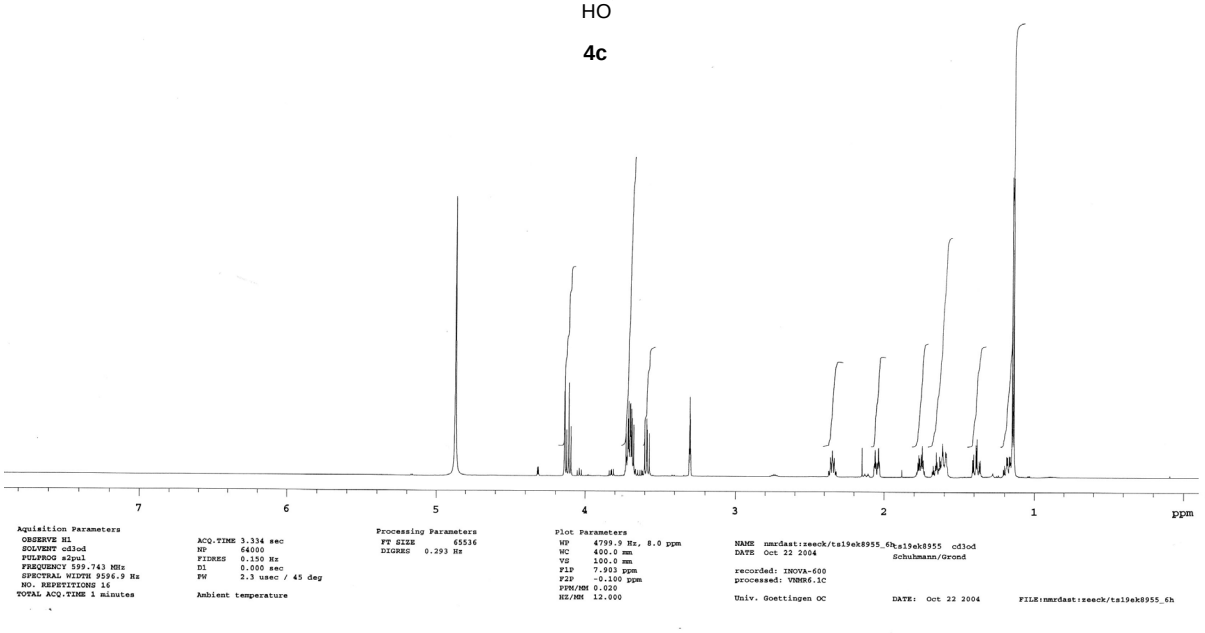
FILE=nmrdast:zeeck/ts19ek8954_6noesy


ts19ek8954 cd3od
Schuhmann/Grond
Oct 26 2004
INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.741 MHz
Spectral width 3109.5 Hz
2D Spectral width 3109.5 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.088 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours
VS= 77
TH= 2
HO
HO
4b
F2 (ppm)
F1 (ppm)
FILE=nmrdast:zeeck/ts19ek8954_6noesy

d) ^{1}H NMR, ^{13}C NMR and NOESY spectra of isomer **4c** with extentions (CD_3OD)



4c



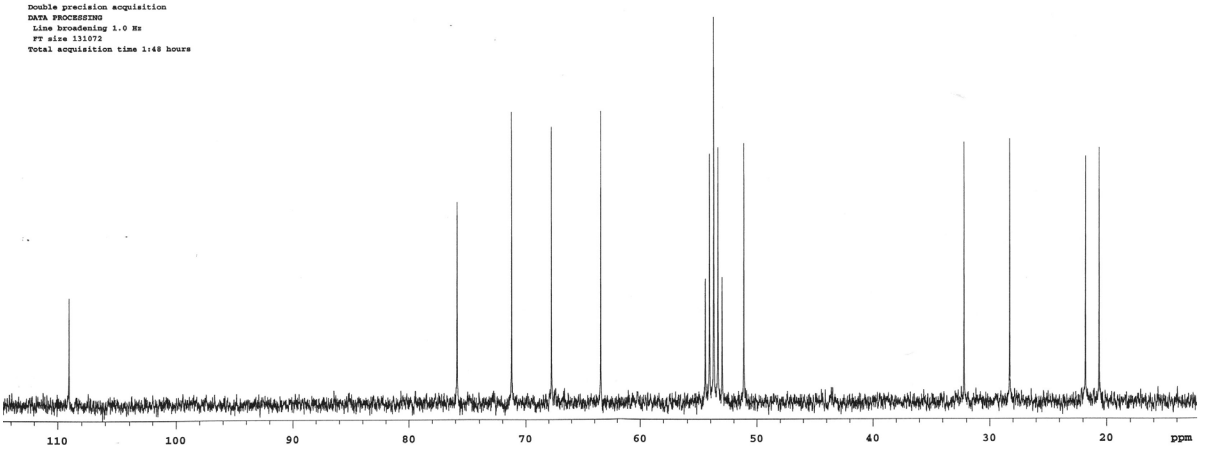
Aquisition Parameters
OBSERVE H1
SOLVENT cd3od
PULPROG s2pul
FREQUENCY 599.743 MHz
SPECTRAL WIDTH 9596.9 Hz
NO. REPETITIONS 16
TOTAL ACQ.TIME 1 minutes

ACQ.TIME 3.334 sec
NP 64000
FIDRES 0.150 Hz
D1 0.000 sec
PW 2.3 usec / 45 deg
Ambient temperature

Processing Parameters
FT SIZE 65536
DIGRES 0.293 Hz

Plot Parameters
WP 4799.9 Hz, 8.0 ppm
WC 400.0 mm
VS 100.0 mm
F1P 7.903 ppm
F2P -0.100 ppm
PPM/MM 0.020
HZ/MM 12.000

NAME nmrdast:zeeck/ts19ek8955_6h ts19ek8955 cd3od
DATE Oct 22 2004 Schuhmann/Grond
recorded: INOVA-600
processed: VNMR6.1C
Univ. Goettingen OC
DATE: Oct 22 2004
FILE:nmrdast:zeeck/ts19ek8955_6h



ts19ek8955, cd2c12
Schuhmann, Grond, fs
4,6 mg

Jul 27 2004

INSTRUMENT MERCURY-300
OBSERVE C13
Frequency 75.478 MHz
Spectral width 18761.7 Hz
Acquisition time 1.706 sec
Relaxation delay 0.000 sec
Pulse width 35.0 degrees
Ambient temperature
No. repetitions 3827
DECOUPLE H1
Frequency 300.140 Mhz
Power 40 dB
Decoupler continuously on
WALTZ-16 modulated
Double precision acquisition
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total acquisition time 1:48 hours

ts19ek8955, cd2c12
Schuhmann, Grond, fs
4,6 mg

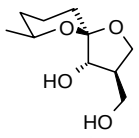
DATE: Jul 27 2004
FILE:nmrdast:zeeck/ts19ek8955_3c

ts19ek8955 cd3od
Schuhmann/Grond

Oct 22 2004

INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.742 MHz
Spectral width 2637.1 Hz
2D Spectral width 2637.1 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.112 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours

VS= 288
TH= 2



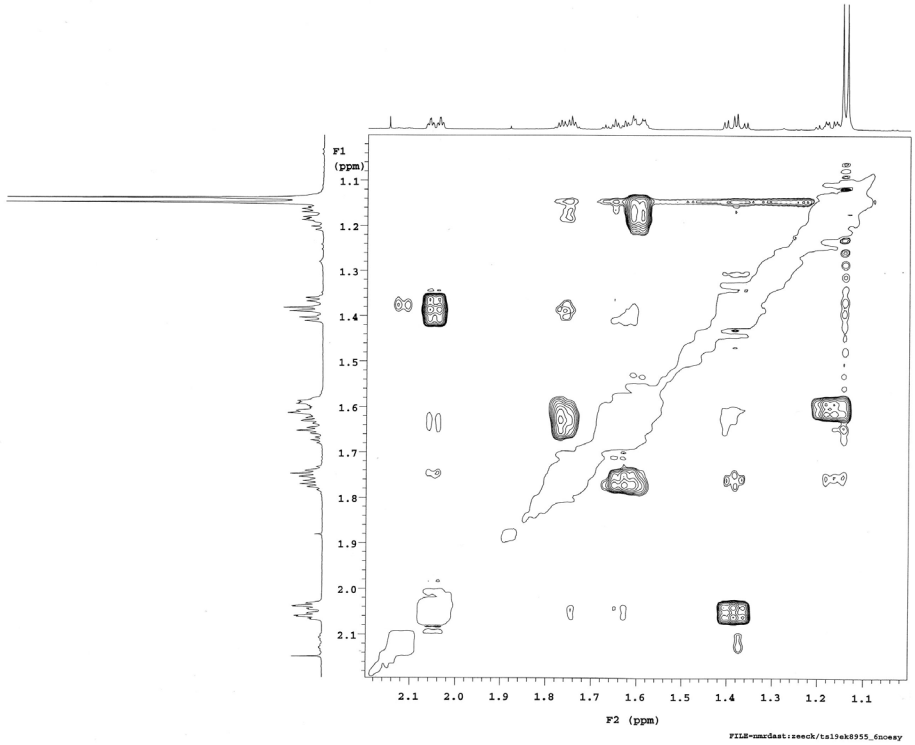
4c

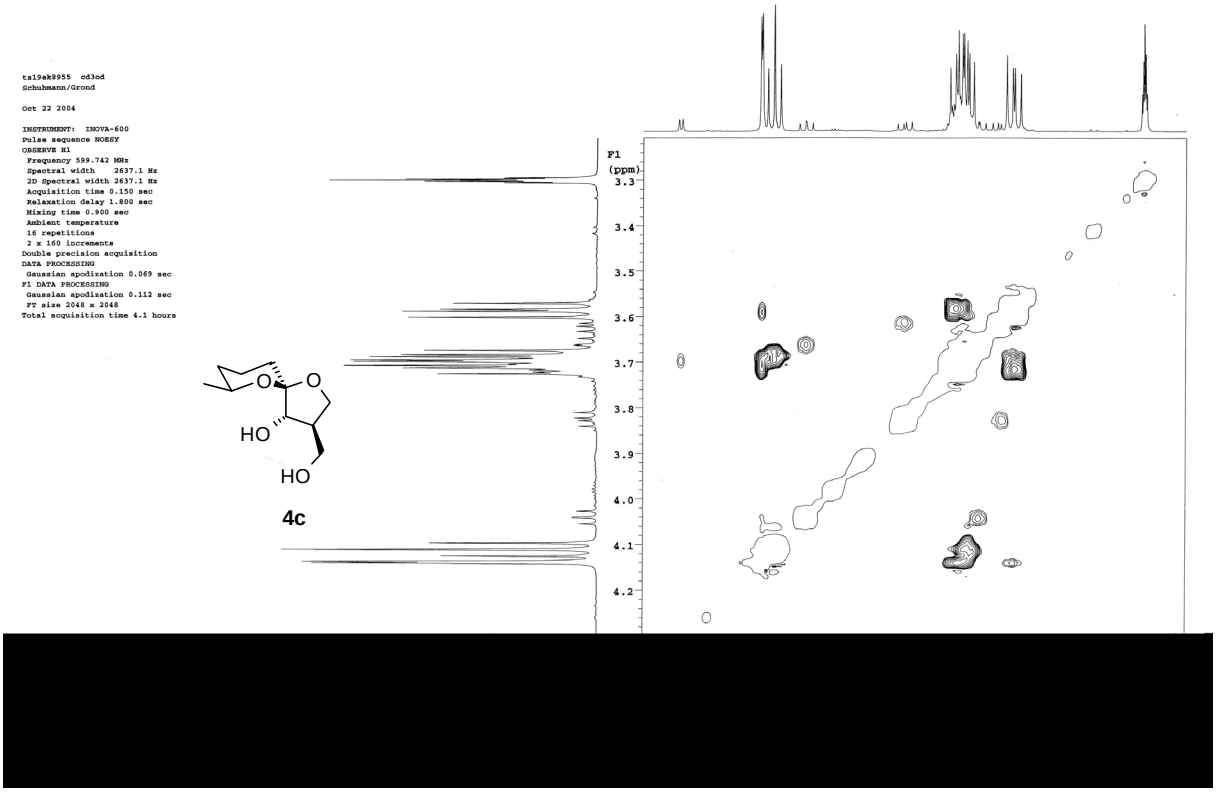
ts19ek8955 cd3od
Schuhmann/Grond

Oct 22 2004

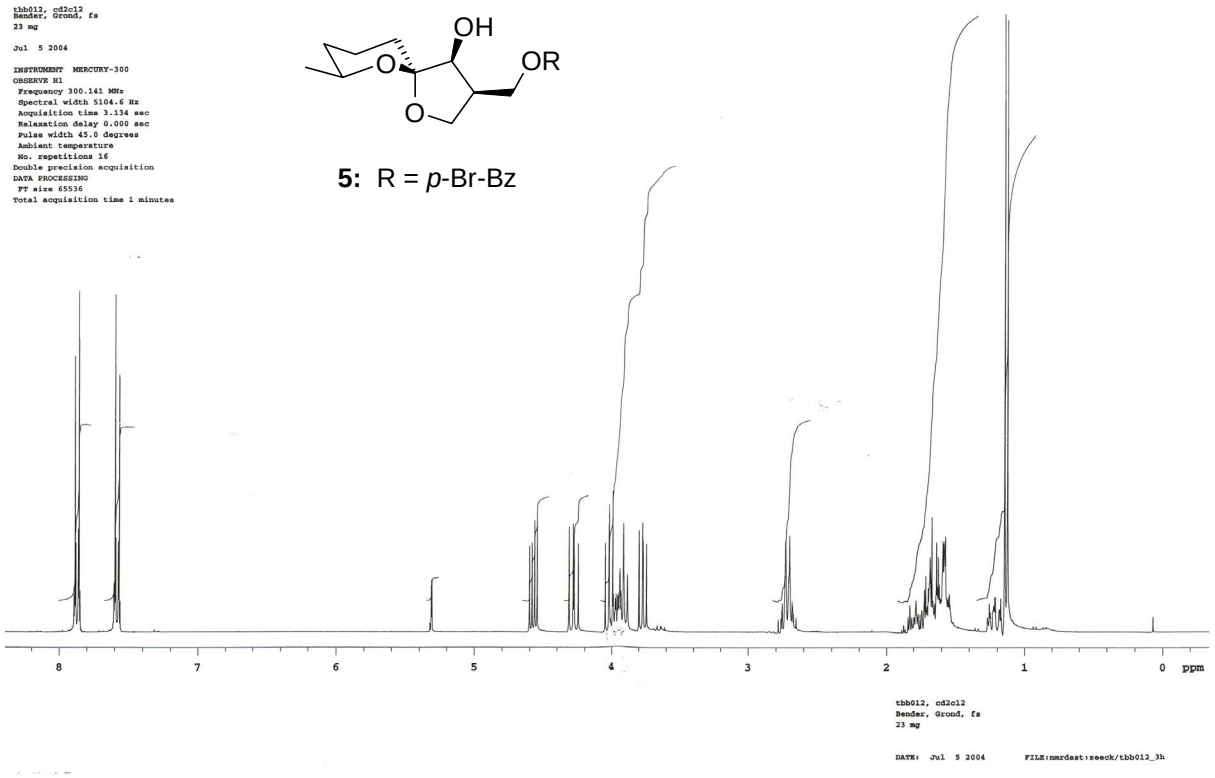
INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.742 MHz
Spectral width 2637.1 Hz
2D Spectral width 2637.1 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.112 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours

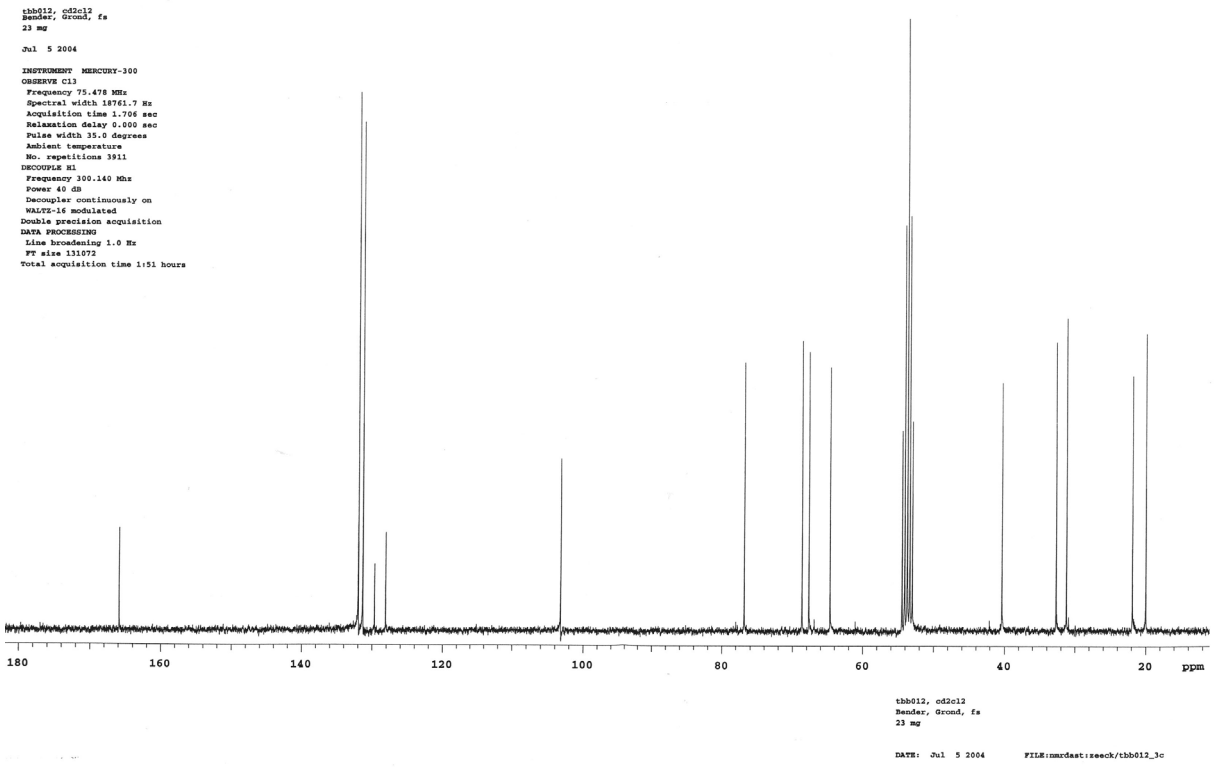
VS= 288
TH= 2



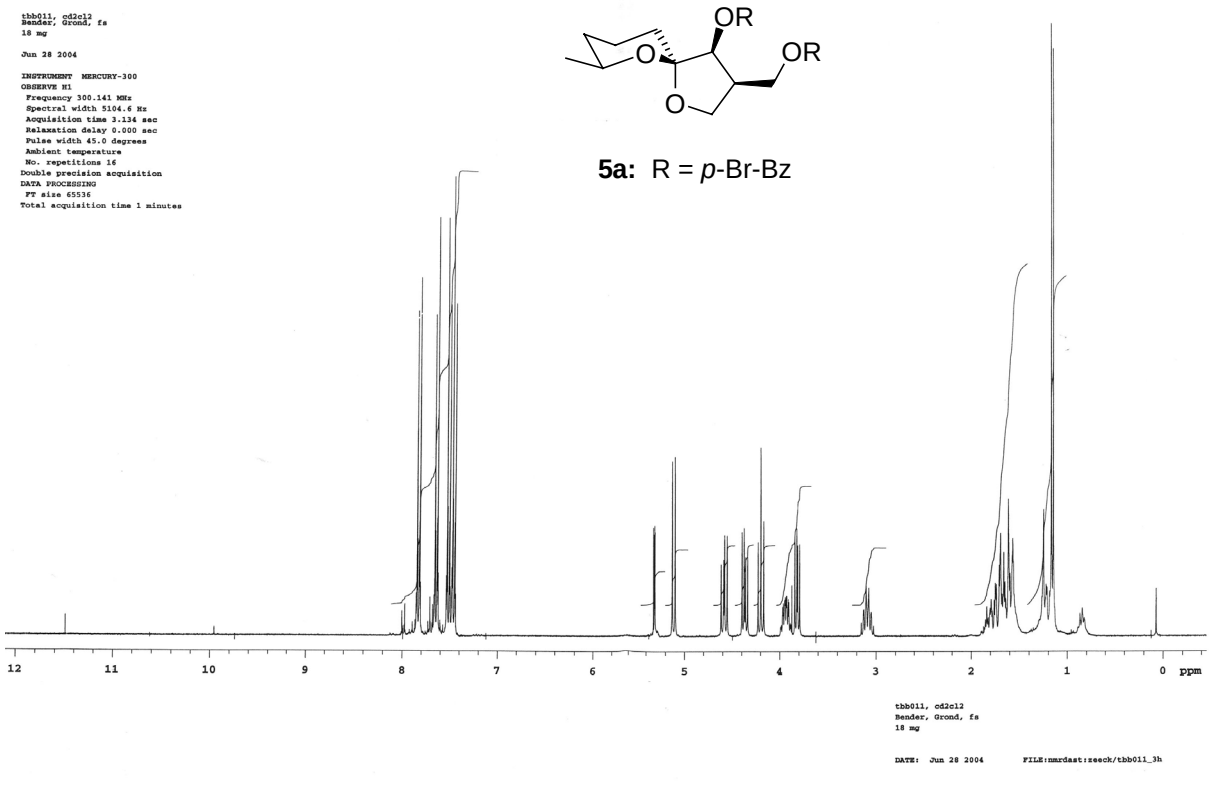

ts19ek8955 cd3od
Schuhmann/Grond
Oct 22 2004
INSTRUMENT: INOVA-600
Pulse sequence NOESY
OBSERVE H1
Frequency 599.742 MHz
Spectral width 2637.1 Hz
2D Spectral width 2637.1 Hz
Acquisition time 0.150 sec
Relaxation delay 1.800 sec
Mixing time 0.900 sec
Ambient temperature
16 repetitions
2 x 160 increments
Double precision acquisition
DATA PROCESSING
Gaussian apodization 0.069 sec
F1 DATA PROCESSING
Gaussian apodization 0.112 sec
FT size 2048 x 2048
Total acquisition time 4.1 hours
O
O
HO
HO
4c
F1
(ppm)
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2

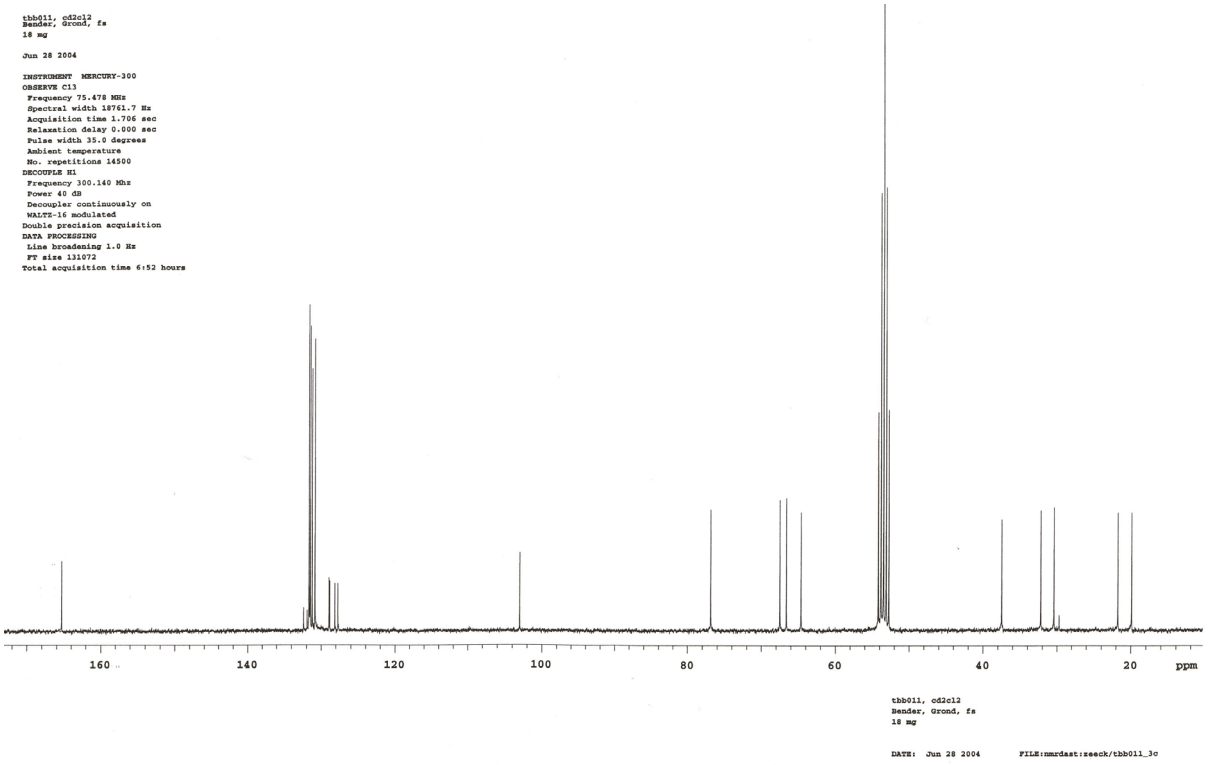
e) ^{1}H NMR and ^{13}C NMR spectra of **5** (CD_2Cl_2)



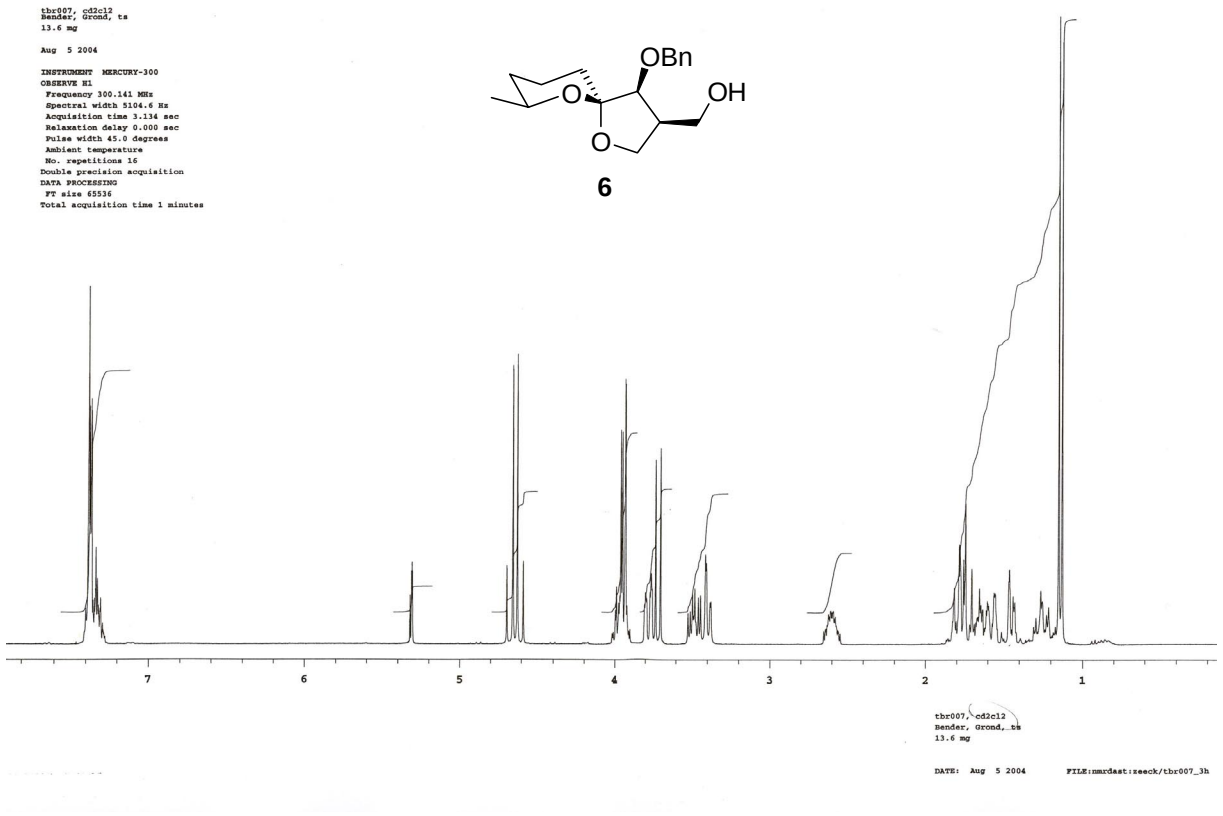


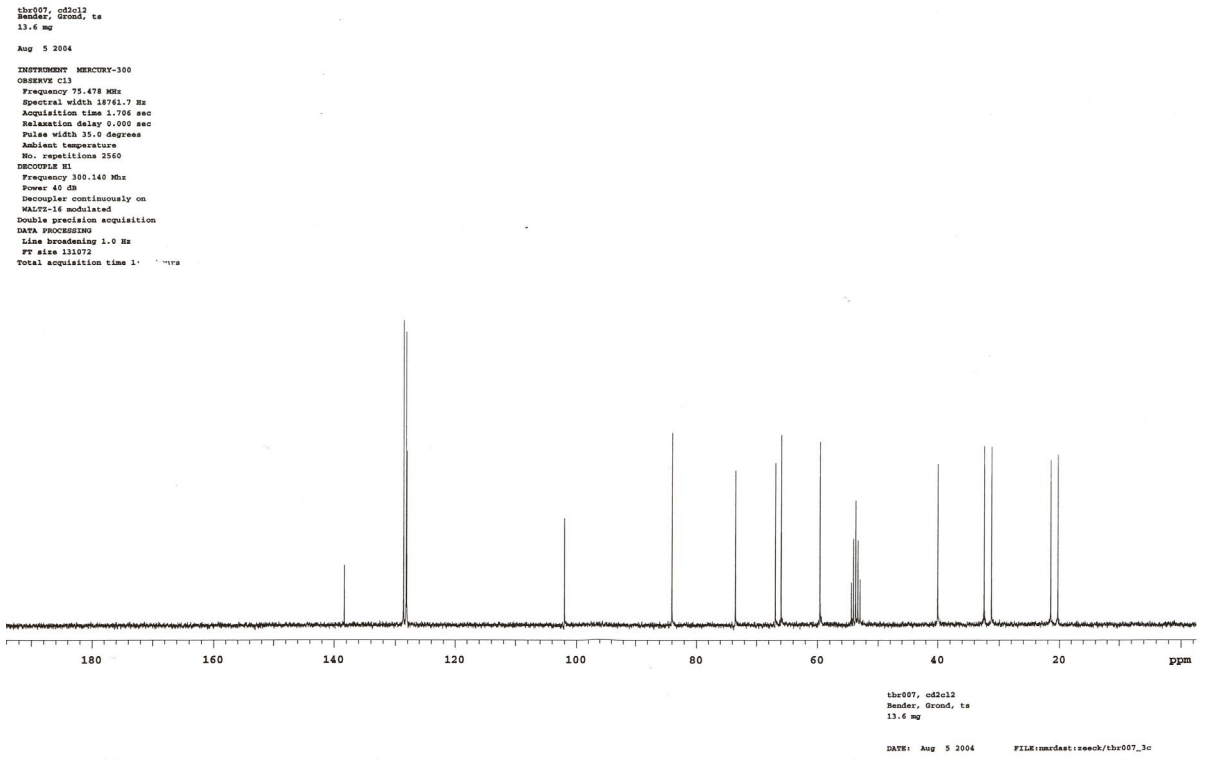
f) ^{1}H NMR and ^{13}C NMR spectra of **5a** (CD_2Cl_2)



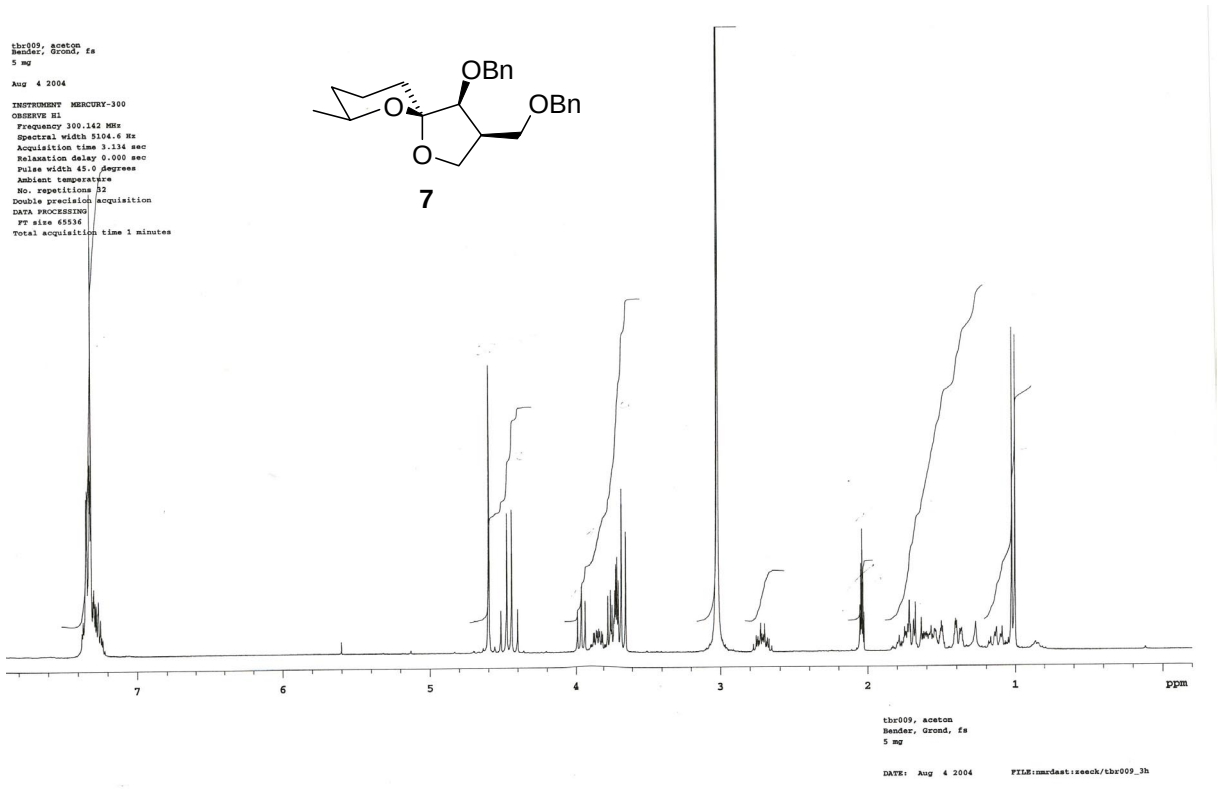


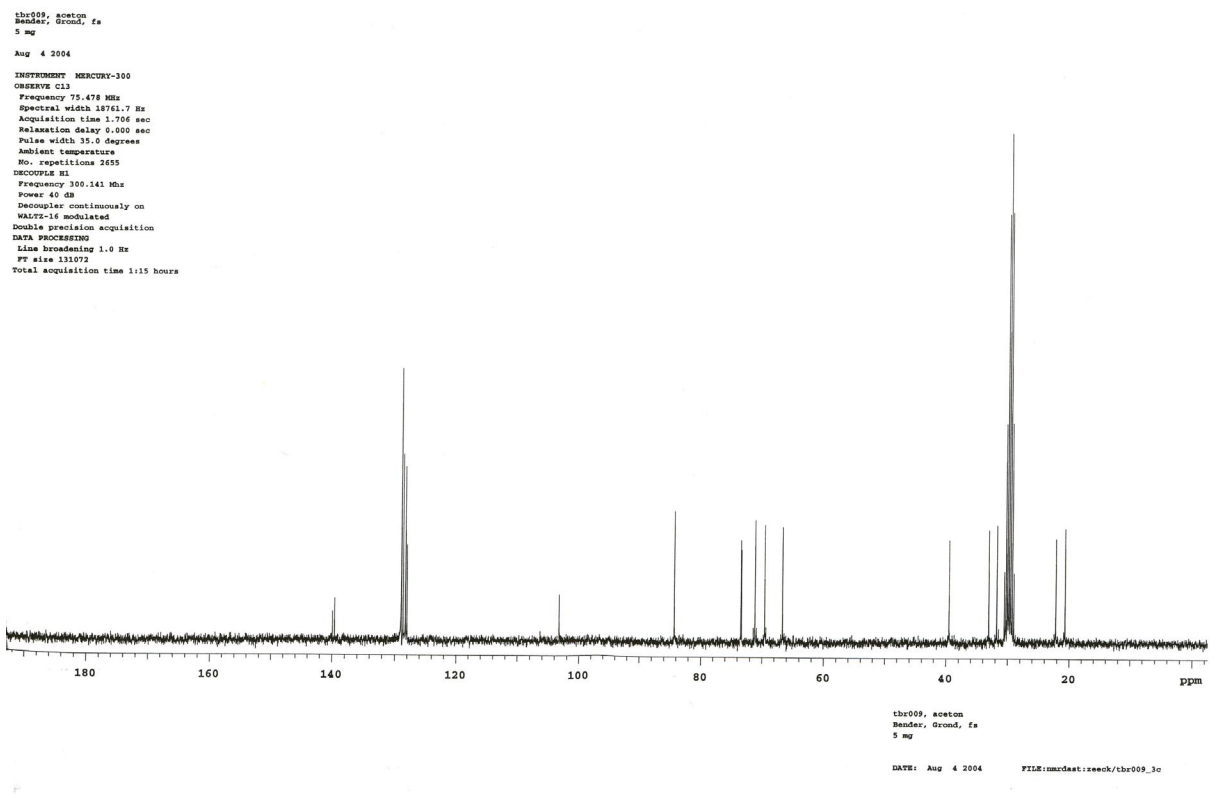
g) ^{1}H NMR and ^{13}C NMR spectra of **6** (CD_2Cl_2)



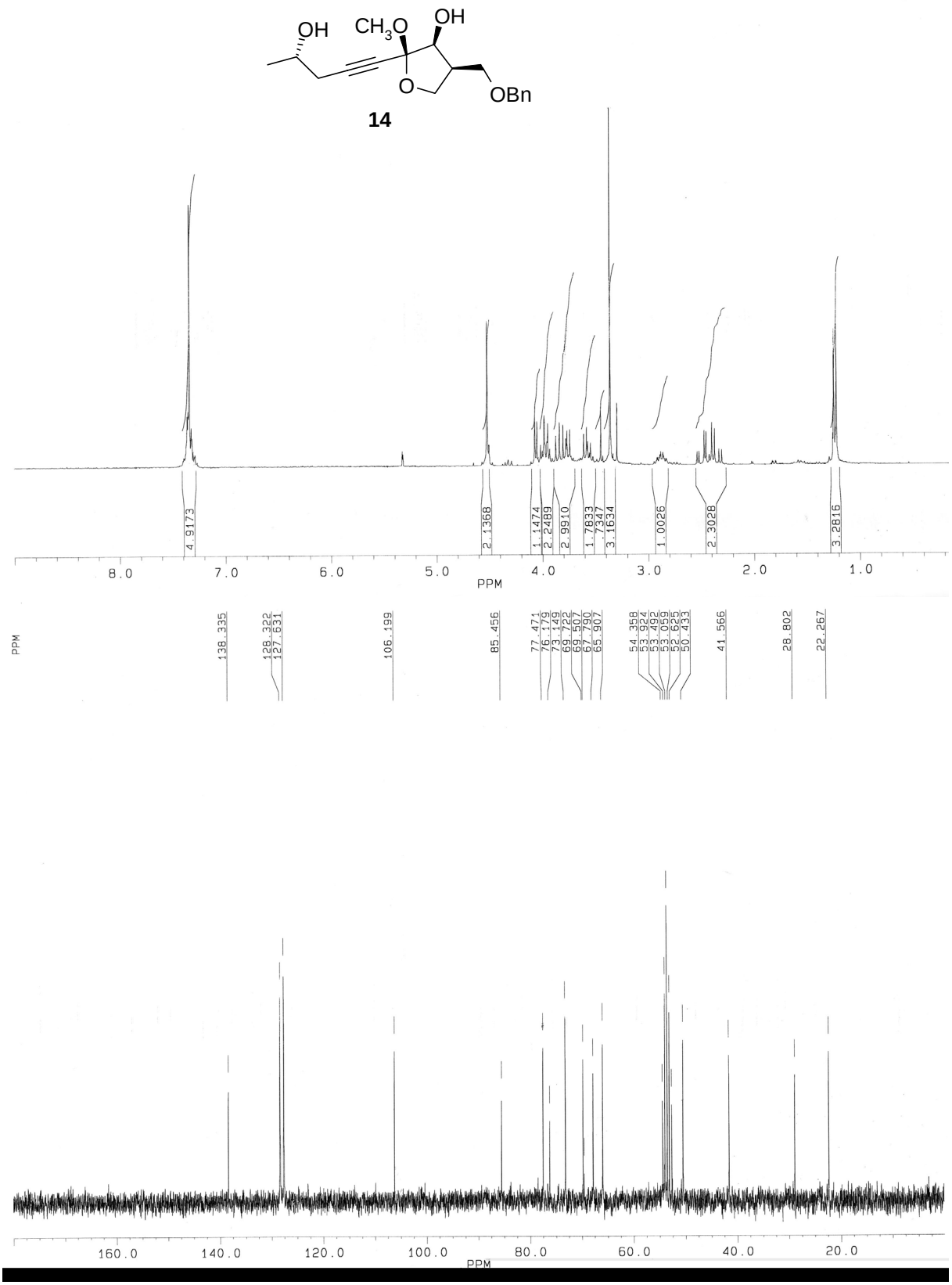


h) ^{1}H NMR and ^{13}C NMR spectra of **7** (CD_3COCD_3)

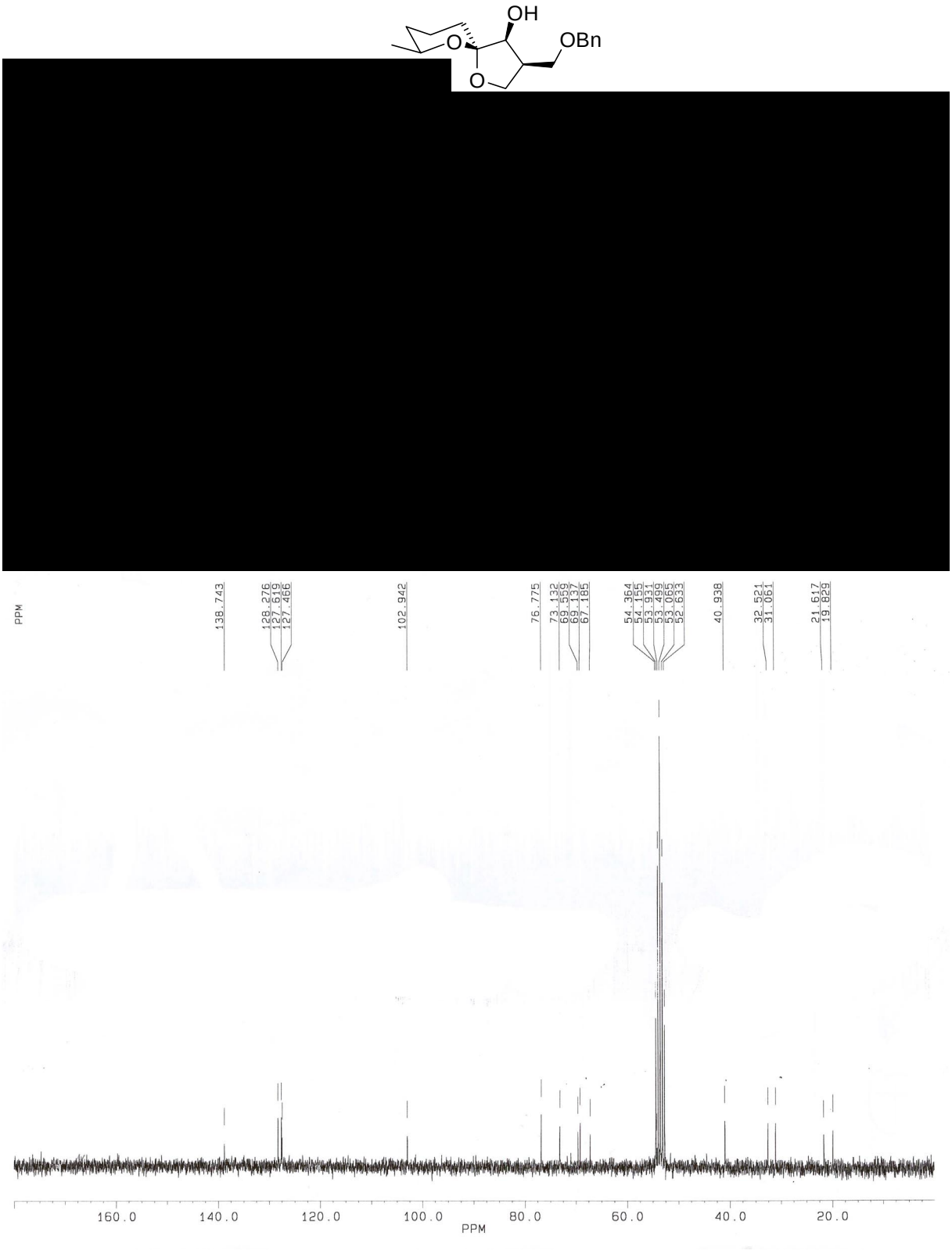




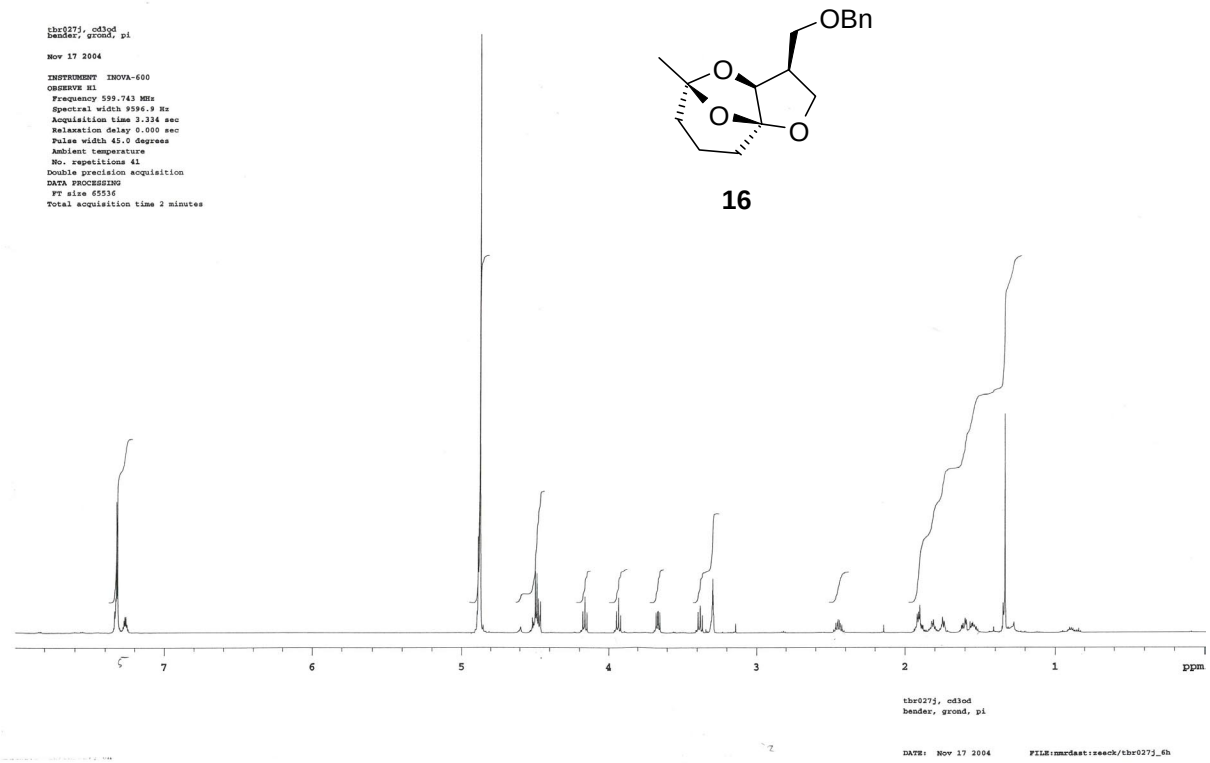
i) ^{1}H NMR and ^{13}C NMR spectra of **14** (CD_2Cl_2)

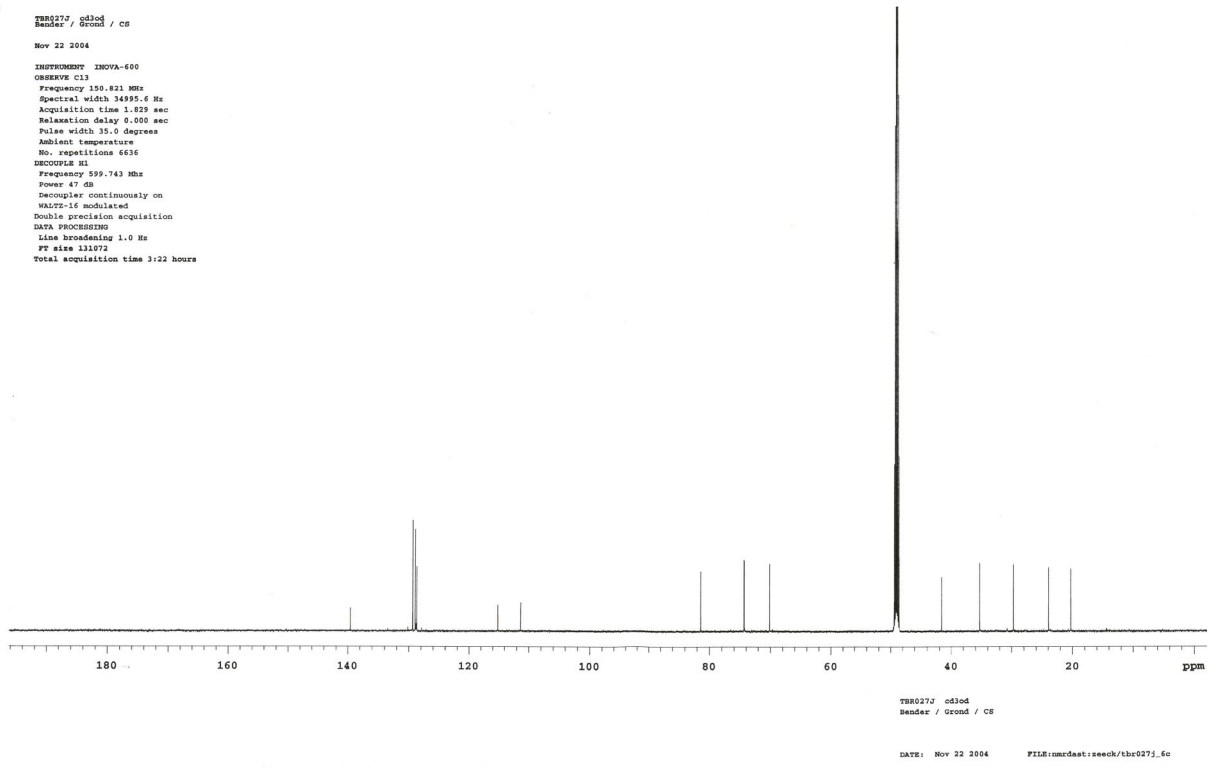


j) ^{1}H NMR and ^{13}C NMR spectra of **15** (CD_2Cl_2)



k) ^{1}H NMR and ^{13}C NMR spectra of **16** (CD_2Cl_2)





l) ^{13}C NMR of **4** obtained from the [U-$^{13}C_3$]glycerol feeding experiment