Supporting Information

Synthesis and structure affinity relationships of spirocyclic benzopyrans with exocyclic amino moiety

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1. Purity data of prepared compounds

compound	purity by HPLC	elemental analysis
3	96.6 %	
4a	94.7 %	
4b	97.1 %	
5a	96.0 %	
5b	98.7 %	
6	98.7 %	
7a	99.0 %	
7b	99.9 %	
8a	98.2 %	
8b	98.5 %	
10a	96.7 %	
10b	98.6 %	
11a	96.3 %	
11b	98.9 %	
14a[#]		$C_{21}H_{31}NO_2$ (329.5). calcd. C, 76.55, H, 9.48, N, 4.25 found C, 76.30, H, 9.44, N, 4.25.
14b[#]		$C_{21}H_{31}NO_2$ (329.5) calcd. C, 76.55, H, 9.48, N, 4.25 found C, 76.30, H, 9.44, N, 4.25.
15a[#]		$C_{22}H_{33}NO_2$ (343.6) calcd. C, 76.92, H, 9.68, N, 4.08 found C, 76.96, H, 9.74, N, 3.82.
15b[#]		$C_{22}H_{33}NO_2$ (343.6) calcd. C, 76.92, H, 9.68, N, 4.08 found C, 76.68, H, 9.80, N, 3.92.
17	98.3 %	
18	99.5 %	
19a	99.1 %	
19b	99.7 %	
20a	92.5 %	

20b	97.1 %	
21a	99.4 %	
21b	99.9 %	
22a	98.6 %	
22b	99.8 %	
23a[#]		$C_{25}H_{25}NO_2$ (323.5) calcd. C, 78.0, H, 7.80, N, 4.33 found C, 77.7, H, 8.02, N, 3.99.
23b[#]		$C_{25}H_{25}NO_2$ (323.5) calcd. C, 78.0, H, 7.80, N, 4.33 found C, 77.8, H, 7.95, N, 4.06.
24a	99.2 %	
24b	99.0 %	

[#] Not stable during the standard HPLC analysis.

2. Experimental Part chemistry

General

Moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography: Silica gel 60 F254 plates (Merck). Flash chromatography (fc): Silica gel 60, 40–43 µm (Merck); parentheses include: diameter of the column, eluent, R_f value. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Mercury Plus AS 400 NMR spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution; the assignments of ^{13}C and ^{1}H NMR signals were supported by 2D NMR techniques. MS: MAT GCQ (Thermo-Finnigan): EI, MAT LCQ (Thermo Finnigan): ESI, IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco) or FT/IR Prestige 21 (Shimadzu). Elemental analysis: CHNOS-Elementar Analysator Vario EL III (Elementar).

HPLC methods to determine the purity

The purity of the test compounds was determined by HPLC analysis. Merck Hitachi Equipment; UV detector: L-7400; autosampler:L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 µm); LiCroCART® 250-4 mm cartridge; pre-colimn LiChrospher® 60 RP-select B (5 µm) with LiCroCART® 4-4 mm cartridge; flow rate: 1.0 mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; temperature 20 °C.

HPLC, method A: Solvent A: demineralized H_2O with 0.05 % (v/v) trifluoroacetic acid; solvent B: acetonitrile with 0.05 % (v/v) trifluoroacetic acid: gradient elution (% A): 0-4 min: 90 %; 4-29 min: gradient from 90 % to 0 %; 29-31 min: 0 %; 31-31.5 min: gradient from 0 % to 90 %; 31.5-40 min: 90 %.

HPLC method B: Solvent A: demineralized H_2O with 0.05 % (v/v) trifluoroacetic acid; solvent B: methanol with 0.05 % (v/v) trifluoroacetic acid: gradient elution (% A): 0-1 min: 80 %; 1-22 min: gradient from 80 % to 0 %; 22-30 min: 0 %; 30-31.5 min: gradient from 0 % to 80 %; 31.5-40 min: 80 %.

The purity was calculated as ratio between the peak area of compound and the sum of areas of all detected peaks. According to these HPLC analyses the purity of all test compounds was >95 %. The purity of few compounds was determined by elemental analysis.

Synthesis of starting compounds

The synthesis of the following compounds is reported in reference[1]:
2-[2-(1-Hydroxy-4-oxocyclohexyl)phenyl]acetaldehyde dimethyl acetal (**2**);
3-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,1`-cyclohexan]-4`-one (**9**);
trans-*N*-Benzyl-3-methoxy-3,4-dihydrospiro-[[2]benzopyran-1,1`-cyclohexan]-4`-amine (**21a**);
cis-*N*-Benzyl-3-methoxy-3,4-dihydrospiro-[[2]benzopyran-1,1`-cyclohexan]-4`-amine (**21b**);
trans-*N*-Benzyl-3-methoxy-*N*-methyl-3,4-dihydrospiro-[[2]benzopyran-1,1`-cyclohexan]-4`-amine (**22a**);
cis-*N*-Benzyl-3-methoxy-*N*-methyl-3,4-dihydrospiro-[[2]benzopyran-1,1`-cyclohexan]-4`-amine (**22b**).
The synthesis of 2-bromobenzaldehyde dimethyl acetal (**12**) is reported in reference[2].

Synthetic procedures

Spiro[[2]benzopyran]-1,1'-cyclohexan-4'-one (3)

A solution of hydroxy acetal **2** (102 mg, 0.37 mmol) and *p*-toluenesulfonic acid monohydrate (12 mg, 0.06 mmol) in CH_2Cl_2 (8 mL) was stirred at rt for 6 d. Subsequently, CH_2Cl_2 (10 mL) was added and the mixture was washed with 0.2 M NaOH (10 mL) and H_2O (10 mL). The aqueous layer was re-extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 3 cm, cyclohexane/ethyl acetate = 4/1, 22 cm, 10 mL). R_f: (cyclohexane/ethyl acetate = 4/1, 0.38). Colorless solid, mp 104 °C, yield 78.5 mg (25 %). $C_{14}H_{14}O_2$ (214.3). MS (EI): m/z (%) = 214 [M, 13], 157 [M – CH_3 CH_2C^*=O, 100]. IR**:** ν (cm^{-1}) = 30583 (w, v, C-H, arom), 2960, 2926, 2901, 2851 (s, v, C-H, alkyl), 1702 (s, v, C=O), 1636 (s, v, C=C, conj. to arom), 1598, 1490 (w, v, C=C arom), 1447 (m, δ ,C-H, Alkyl), 754 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.09 („td", J = 13.8/4.9 Hz, 2H, (C*H*$_2$C*H*$_2$)$_2$C=O), 2.31 – 2.39 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$C=O), 2.61 – 2.69 (m, 2H, (C*H*$_2$CH$_2$)$_2$C=O), 2.84 („td", J= 14.4/6.3 Hz, 2H, (C*H*$_2$C*H*$_2$)$_2$C=O), 5.85

(d, J = 5.6 Hz, 1H, ArC*H*CHO), 6.58 (d, J = 5.7 Hz, 1H, ArCHC*H*O), 7.00 (dd, J = 7.2/1.6 Hz, 1H, Ar-H), 7.06 (dd, J = 7.5/1.2 Hz, 1H, Ar-H), 7.18 („td“, J = 7.4/1.6 Hz, 1H, Ar-H), 7.22 („td“, J = 7.3/1.4 Hz, 1H, Ar-H). Purity (HPLC method A): 96.6 %, t_R = 19.2 min.

***trans*-N-(Cyclohexylmethyl)spiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (4a) and**

***cis*-N-(Cyclohexylmethyl)spiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (4b)**

Under N_2, a mixture of ketone **3** (75 mg, 0.35 mmol), cyclohexylmethylamine (98 %, 63 mg, 0.54 mmol), acetic acid (20 µL, 0.45 mmol), $NaBH(OAc)_3$ (95 %, 141 mg, 0.63 mmol) and THF (7 mL) was stirred at rt for 3 h. Subsequently, 1 M NaOH (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and with Et_2O (20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2.5 cm, cyclohexane/ethyl acetate = 19/1 + 0.5 % *N,N*-dimethylethanamine, 15 cm, 10 mL). R_f (cyclohexane/ethyl acetate = 19/1 + 0.5 % *N,N*-dimethylethanamine, **4a**: R_f = 0.18, **4b**: R_f = 0.05).

4a: Colorless solid, mp 35 °C, yield 31 mg (28 %). $C_{21}H_{29}NO$ (311.5). MS (EI): m/z (%) = 311 [M, 44], 228 [M-cyclohexyl*, 79], 181 [$C_{13}H_9O)^+$, 40], 152 [CH_2=CHCH=HN^+CH_2 cyclohexyl, 60], 124 [($C_8H_{14}N)^+$, 100]. IR: ν (cm^{-1}) = 3061, 3027 (w, v, C-H, arom), 2920, 2849 (s, v, C-H, alkyl), 2802 (m, v, N-CH_2), 1629 (m, v, C=C, conj. to arom), 1601, 1486 (w, v, C=C, arom), 1449 (m, δ, C-H, Alkyl), 766, 746 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.92(„qd“, J = 11.9/2.9 Hz, 2H, NCH_2(cyclohexyl-*H*)), 1.11 – 1.32 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.38 – 1.50 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.55 – 1.64 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$CHN), 1.64 – 1.83 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.87 – 2.04 (m, 6H, (C*H*$_2$C*H*$_2$)$_2$CHN), 2.43 (d, J = 6.6 Hz, 2H, NC*H*$_2$(cyclohexyl-H), 2.87 („quint“, J = 3.0 Hz, 1H, 4'-H_e), 5.72 (d, J = 5.7, 1H, OCH=C*H*Ar), 6.49 (d, J = 5.7 Hz, 1H, OC*H*=CHAr), 6.89 – 6.94 (m, 1H, Ar-H), 7.12 – 7. 17 (m, 3H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC method A): 94.7 %, t_R = 19.5 min.

4b: Colorless solid, mp 85 °C, yield 57 mg (52 %). $C_{21}H_{29}NO$ (311.5). MS (EI): m/z (%) = 311 [M, 38], 228 [M-Cyclohexyl*, 100], 181[$C_{13}H_9O)^+$, 19], 152 [CH_2=CHCH=HN^+CH_2 Cyclohexyl, 41], 124[($C_8H_{14}N)^+$, 50]. IR: ν (cm^{-1}) = 3069, 3017 (w, v, C-H, arom), 2922, 2850 (s, v, C-H, alkyl), 2791 (m, v, N-CH_2), 1626 (m, v, C=C, conj. to arom), 1595, 1484 (w, v, C=C, arom), 1451 (m, δ, C-H, Alkyl), 769, 744 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.86 – 0.96 (m, 2H, NCH_2(Cyclohexyl-*H*)), 1.11 – 1.33 (m,

4H, NCH_2(cyclohexyl-*H*)), 1.40 – 1.50 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.54 – 1.87 (m, 10H, NCH_2(cyclohexyl-*H*) (4H), (C*H*$_2$C*H*$_2$)$_2$CHN) (6H)), 2.28 – 2.39 (m, 2H, 2'-H_e, 6'-H_e), 2.47 – 2.57 (m, 1H, 4'-H_a), 2.50 (d, J = 6.7 Hz, 2H, NC*H*$_2$(cyclohexyl-H)), 5.75 (d, J = 5.7 Hz, 1H, OCH=C*H*Ar), 6.47 (d, J = 5.6 Hz, 1H, OC*H*=CHAr), 6.92 – 6.94 (m, 1H, Ar-H), 7.05 – 7.07 (m, 1H, Ar-H), 7.12 – 7.19 (m, 2H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC method A): 97.1 %, t_R = 19.2 min.

trans-N-(Cyclohexylmethyl)-N-methylspiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (5a)

Under N_2, cyclohexylmethylamine **4a** (23.6 mg, 0.08 mmol) was dissolved in CH_2Cl_2 (5 mL). Formalin (37 %, stabilized with 10-15 % MeOH, 114 µL, 1.50 mmol) and $NaBH(OAc)_3$ (95 %, 27 mg, 0.12 mmol) were added and the reaction mixture was stirred at rt for 2 h. Subsequently, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and once Et_2O (20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane + 0.5% *N,N*-dimethylethanamine, 15 cm, 10 mL). R_f (cyclohexane + 0.5 % *N,N*-dimethylethanamine, 0.09, cyclohexane + 1 % N,N-dimethylethanamine, 0.35). Colorless oil, yield 20 mg (81 %). $C_{22}H_{31}NO$ (325.5). MS (EI): m/z (%) = 326 [MH, 10], 242 [M-cyclohexyl*, 100], 199 [M-H_3CN*CH_2cyclohexyl, 20], 181 [$C_{13}H_9O)^+$, 43], 171 [48], 138 [69]. IR: ν (cm^{-1}) = 3062, 3030 (w, v, C-H, arom), 2920, 2847 (s, v, C-H, alkyl), 2784 (m, v, N-CH_2), 1630 (m, v, C=C, conj. to arom), 1600, 1486 (w, v, C=C, arom), 1449 (m, δ, C-H, Alkyl), 766, 747 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.79 – 0.89 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.10 – 1.31 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.44 – 1.55 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.63 – 1.75 (m, 3H, NCH_2(cyclohexyl-*H*)(1H), (C*H*$_2$C*H*$_2$)$_2$CHN (2H)), 1.78 – 1.88 (m, 5H, NCH_2(cyclohexyl-*H*) (3H), (C*H*$_2$C*H*$_2$)$_2$CHN (2H)), 1.91 – 1.99 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$CHN), 2.03 – 2.12 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$CHN), 2.13 (d, J = 7.1 Hz, 2H, NC*H*$_2$(cyclohexyl-H), 2.17 (s, 3H, NCH_3), 2.24 („quint", J = 3.3 Hz, 1H, 4'-H_e), 5.72 (d, J = 5.6 Hz, 1H, OCH=C*H*Ar), 6.49 (d, J = 5.6 Hz, 1H, OC*H*=CHAr), 6.90 – 6.94 (m, 1H, Ar-H), 7.11 – 7.18 (m, 3H, Ar-H). Purity (HPLC method A): 96.0 %, t_R = 20.0 min.

cis-N-(Cyclohexylmethyl)-N-methylspiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (5b)

Under N_2, cyclohexylmethylamine **4b** (42.6 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (5 mL). Formalin (37 %, stabilized with 10-15 % MeOH, 205 µL, 2.70 mmol) and $NaBH(OAc)_3$ (95 %, 48 mg, 0.22 mmol) were added and the reaction mixture was stirred at rt for 2 h. Subsequently, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and once with Et_2O (20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane + 0.5% *N,N*-dimethylethanamine, 15 cm, 10 mL). R_f (cyclohexane + 0.5 % *N*,*N*-dimethylethanamine, 0.05, cyclohexane + 1 % *N,N*-dimethylethanamine, 0.12). Colorless solid, mp 73 °C, yield 40 mg (90 %). $C_{22}H_{31}NO$ (325.5). MS (EI): m/z (%) = 325 [M, 6], 242 [M-cyclohexyl*, 100], 181 [$C_{13}H_9O)^+$, 12]. IR: ν (cm^{-1}) = 3031 (w, v, C-H, arom), 2918, 2855 (s, v, C-H, alkyl), 2772 (m, v, N-CH_2), 1631 (m, v, C=C, conj. to arom), 1485 (w, v, C=C, arom), 1445 (m, δ, C-H, alkyl), 768, 748 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.79 – 0.92 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.11 – 1.32 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.35 – 1.47 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.52 – 1.84 (m, 10H, NCH_2(cyclohexyl-*H*) (4H), ($CH_2CH_2)_2$CHN) (6H)), 2.23 – 2.27 (m, 2H, ($CH_2CH_2)_2$CHN), 2.30 (s, 3H, NCH_3), 2.36 – 2.40 (m, 2H, NCH_2(cyclohexyl-H), 2.43 – 2.54 (m, 1H, 4'-H_a), 5.74 (d, J = 5.6 Hz, 1H, OCH=C*H*Ar), 6.51 (d, J = 5.6 Hz, 1H, OC*H*=CHAr), 6.92 – 6.94 (m, 1H, Ar-H), 7.05 – 7.09 (m, 1H, Ar-H), 7.12 – 7.18 (m, 2H, Ar-H). Purity (HPLC method A): 98.7 %, t_R = 20.2 min.

3-Ethoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-one (6)

A solution of hydroxy acetal **2** (474 mg, 1.70 mmol) and *p*-toluenesulfonic acid mono hydrate (29.3 mg, 0.15 mmol) in $CHCl_3$ (40 mL, stabilized with 1 % ethanol) was stirred at rt for 8 d. Subsequently, CH_2Cl_2 (40 mL) was added and the mixture was washed with 0.2 M NaOH (20 mL) and H_2O (20 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (∅ 4.5 cm, cyclohexane/ethyl acetate = 9/1, 20 cm, 30 mL). R_f (cyclohexane/ethyl acetate = 9/1, 0.09, cyclohexane/ethyl acetate = 4/1, 0.22). Colorless solid, mp 106 °C, yield 233 mg (53 %). $C_{16}H_{20}O_3$ (260.4). MS (EI): m/z (%) = 260 [M, 6], 214 [M – EtOH, 29], 203 [M – CH_3CH_2C*=O, 65], 157 [M – EtOH, - CH_3CH_2C*=O, 100]. IR: ν (cm^{-1}) = 2981, 2943, 2919, 2893 (s, v, C-H, alkyl), 2868 (m, v, OCH_2CH_3), 1703 (s, v, C=O), 1493 (w, v, C=C aromt), 1437 (m, δ, C-H alkyl), 768 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$):

δ (ppm) = 1.29 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 2.12 („td“, J = 14.2/4.9 Hz, 1H, $(CH_2CH_2)_2C=O$), 2.22 - 2.43 (m, 5H, $(CH_2CH_2)_2C=O$), 2.84 („td“, J = 14.5/6.4 Hz, 1H, $(CH_2CH_2)_2C=O$), 2.95 – 3.04 (m, 3H, $(CH_2CH_2)_2C=O$ (1H), $ArCH_2OCH_3$ (2H)), 3.62 (dq, J = 9.3/7.1 Hz, 1H, OCH_2CH_3), 3.64 (dq, J = 9.3/7.1 Hz, 1H, OCH_2CH_3), 5.07 (dd, J = 6.3/4.6 Hz, 1H, $ArCH_2CHOCH_3$), 7.06 – 7.10 (m, 1H, Ar-H), 7.12 – 7.16 (m, 1H, Ar-H), 7.18 – 7.23 (m, 2H, Ar-H). ^{13}C NMR ($CDCL_3$): δ (ppm) = 15.6 (1C, OCH_2CH_3), 35.7 (1C, $CH_2CHOCH_2CH_3$), 37.0 (1C, $(CH_2CH_2)_2C=O$), 37.7 (2C, $(CH_2CH_2)_2C=O$), 39.4 (1C, $(CH_2CH_2)_2C=O$), 64.7, (1C, OCH_2CH_3), 75.8 (1C, Spiro-C), 95.9 (1C, $CH_2CHOCH_2CH_3$), 124.5 (1C, arom), 127.0 (1C, arom), 127.5 (1C, arom), 129.8 (1C, arom), 132.0 (1C, arom), 140.0 (1C, arom), 211.8 (1C, *C*=O). Purity (HPLC method A): 98.7 %, t_R = 18.9 min.

trans-N-(Cyclohexylmethyl)-3-ethoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (7a) and _cis_-(Cyclohexylmethyl)-3-ethoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (7b)

Under N_2, a solution of ketone **6** (98 mg, 0.38 mmol), cyclohexylmethylamine (98 %, 65 mg, 0.56 mmol), acetic acid (22 µL, 0.39 mmol) and $NaBH(OAc)_3$ (95 %, 151 mg, 0.68 mmol) in THF (7 mL) was stirred at rt for 3 h. Subsequently, 1 M NaOH (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (3 cm, cyclohexane + 1 % *N*,*N*-dimethylethanamine, 20 cm, 10 mL). R_f (cyclohexane + 1 % *N*,*N*-dimethylethanamine, **7a**: R_f = 0.21, **7b**: R_f = 0.06). **7a:** Colorless oil, yield 50 mg (37 %). $C_{23}H_{35}NO_2$ (357.6). MS (EI): m/z (%) = 357 [M, 2], 312 [M-O*CH_2CH_3, 5], 274 [M-cyclohexyl*, 21], 228 [M-CH_3CH_2OH,-cyclohexyl*, 43], 199 [M-HN*CH_2cyclohexyl, 40], 181 [$(C_{13}H_9O)^+$, 100], 152 [CH_2=CHCH=HN^+CH_2 cyclohexyl, 87], 124 [$(C_8H_{14}N)^+$, 31]. IR: ν (cm^{-1}) = 3025 (w, ν, C-H, arom), 2920, 2849 (s, ν, C-H, alkyl), 2793 (m, ν, N-CH_2), 1489 (w, ν, C=C, arom), 1448 (m, δ, C-H, alkyl), 752 (s, δ, C-H, o-disubst. arom). ^{1}HNMR ($CDCl_3$): δ (ppm) = 0.91 – 1.01 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.13 – 1.34 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.29 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.41 – 1.52 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.57 – 1.87 (m, 8H, NCH_2(cyclohexyl-*H*) (4H), $(CH_2CH_2)_2CHN$) (4H)), 1.89 – 2.09 (m, 3H, $(CH_2CH_2)_2CHN$), 2.25 („td“, J = 13.4/3.6 Hz, 1H, 2'-H_a), 2.45 (d, J = 6.5 Hz, 2H, NCH_2(cyclohexyl-H), 2.87 – 2.96 (m, 3H, 4'-H_e (1H), $ArCH_2CHOCH_2CH_3$ (2H)), 3.60 (dq, J = 9.4/7.0 Hz, 1H,

OC*H*$_2$CH$_3$), 4.09 (dq, J = 9.4/7.0 Hz, 1H, OC*H*$_2$CH$_3$), 4.94 (dd, J = 6.8/4.4 Hz,1H, ArCH$_2$C*H*OCH$_2$CH$_3$), 7.05 – 7.10 (m, 1H, Ar-H), 7.11 – 7.22 (m, 3H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC method A): 99.0 %, t_R = 19.7 min.

7b: Colorless oil, yield 73 mg (54 %). $C_{23}H_{35}NO_2$ (357.6). MS (ESI): m/z (%) = 358 [MH, 2], 312 [M-O*CH$_2$CH$_3$, 5], 274 [M-cyclohexyl*, 11], 228 [M-CH$_3$CH$_2$OH,-cyclohexyl*, 100], 181 [$C_{13}H_9O)^+$, 49], 152 [CH$_2$=CH=HN$^+$CH$_2$cyclohexyl, 45], 124 [($C_8H_{14}N)^+$, 18]. IR: ν (cm^{-1}) = 3021 (w, v, C-H, arom), 2920, 2850 (s, v, C-H, alkyl), 1490 (w, v, C=C, arom), 1447 (m, δ, C-H, alkyl), 754 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.86 – 0.98 (m, 2H, NCH$_2$(cyclohexyl-*H*)), 1.11 – 1.33 (m, 4H, NCH$_2$(cyclohexyl-*H*)), 1.27 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 1.42 – 1.53 (m, 1H, NCH$_2$(cyclohexyl-*H*)), 1.55 – 1.86 (m, 9H, NCH$_2$(cyclohexyl-*H*) (4H), (C*H*$_2$C*H*$_2$)$_2$CHN) (5H)), 1.87 – 2.00 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$CHN), 2.12 (m, 1H, 2'-H$_e$), 2.52 – 2.60 (d, J = 6.9 Hz, 2H, NC*H*$_2$(cyclohexyl-H)), 2.51 – 2.60 (m, 1H, 4'-H$_a$), 2.88 – 2.96 (m, 2H, ArC*H*$_2$CHOCH$_2$CH$_3$), 3.57 (dq, J = 9.5/7.0 Hz, 1H, OC*H*$_2$CH$_3$), 4.10 (dq, J = 9.5/7.1 Hz, 1H, OC*H*$_2$CH$_3$), 4.93 (dd, J = 6.2/4.8 Hz, 1H, ArCH$_2$C*H*OCH$_2$CH$_3$), 7.06 – 7.12 (m, 2H, Ar-H), 7.13 – 7.21 (m, 2H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC method A): 99.9 %, t_R = 19.4 min.

trans-N-(Cyclohexylmethyl)-3-ethoxy-N-methyl-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (8a)

Under N$_2$, cyclohexylmethylamine **7a** (37.5 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (2.5 mL). Formalin (37 %, stabilized with 10-15 % MeOH, 157 µL, 2.10 mmol) and NaBH(OAc)$_3$ (95 %, 38 mg, 0.17 mmol) were added and the reaction mixture was stirred at rt for 2 h. Then, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (1 x 20 mL).The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2,5 cm, cyclohexane + 2 % *N*,*N*-dimethylethanamine, 24 cm, 10 mL). R_f (cyclohexane + 2 % *N*,*N*-dimethylethanamine: 0.50, cyclohexane + 1 % *N*,*N*-dimethylethylamine: 0.18). Colorless solid, mp 79 °C, yield 35 mg (90 %). $C_{24}H_{37}NO_2$ (371.6). MS (EI): m/z (%) = 371 [M, 2], 326 [M-*OCH$_2$CH$_3$, 3], 288 [M-cyclohexyl*, 100], 199 [M-CH$_3$N*CH$_2$cyclohexyl], 181 [$C_{13}H_9O)^+$, 80], 166 [CH$_2$=CHCH=N$^+$(-CH$_3$)-CH$_2$cyclohexyl, 52]. IR: ν (cm^{-1}) = 2920, 2848 (s, v, C-H, alkyl), 2782 (m, v, N-CH$_2$), 1487 (w, v, C=C, arom), 1447 (m, δ, C-H, alkyl), 752 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ

(ppm) = 0.81 – 0.95 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.11 – 1.34 (m, 3H, NCH_2(cyclohexyl-*H*)), 1.29 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.5 – 1.62 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.64 – 1.79 (m, 4H, NCH_2(cyclohexyl-*H*) (2H), (CH_2CH_2)$_2$CHN) (2H)), 1.82 – 1.93 (m, 5H, NCH_2(cyclohexyl-*H*) (2H), (CH_2CH_2)$_2$CHN (3H)), 1.93 – 2.07 (m, 2H, (CH_2CH_2)$_2$CHN), 2.16 (dd, J = 7.1/2.8 Hz, 2H, NCH_2(cyclohexyl-H)), 2.19 (s, 3H, NCH_3), 2.19 – 2.30 (m, 2H, (CH_2CH_2)$_2$CHN (1H), 4'-H_e (1H)), 2.89 (dd, J = 15.8/4.0 Hz, 1H, Ar$CH_2CH$$OCH_2CH_3$), 2.93 (dd, J = 16.0/7.5 Hz, 1H, Ar$CH_2CHOCH_2CH_3$), 3.60 (dq, J = 9.5/7.1 Hz, 1H, $ArCH_2CHOCH_2CH_3$), 4.09 (dq, J = 9.4/7.1 Hz, 1H, $ArCH_2CHOCH_2CH_3$), 4.94 (dd, J = 7.0/4.3 Hz, 1H, $ArCH_2CHOCH_2CH_3$), 7.06 – 7.08 (m, 1H, Ar-H), 7.13 – 7.22 (m, 3H, Ar-H). Purity (HPLC method A): 98.2 %, t_R = 19.57 min.

cis-N-(Cyclohexylmethyl)-3-ethoxy-N-methyl-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (8b)

Under N_2, cyclohexylmethylamine **7b** (57 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (3 mL). Formalin (37 %, stabilized with 10-15 % MeOH, 240 µL, 3.20 mmol) and $NaBH(OAc)_3$ (95 %, 57 mg, 0.26 mmol) were added and the reaction mixture was stirred at rt for 2 h. Then, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (1 x 20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane + 1 % *N*,*N*-dimethylethanamine, 15 cm, 10 mL). R_f (cyclohexane + 1 % *N*,*N*-dimethylethanamine: 0.16, cyclohexane + 2% *N*,*N*-dimethylethanamine: 0.27). Colorless oil, yield 54 mg (92 %). $C_{24}H_{37}NO_2$ (371.61). MS (EI): m/z (%) = 371 [M, 1], 288 [M-cyclohexyl*, 100], 242 [M-CH_3CH_2OH, -cyclohexyl*, 8], 181 [$(C_{13}H_9O)^+$, 27], 166 [CH_2=CHCH=N^+(-CH_3)-CH_2cyclohexyl, 20]. IR: ν (cm^{-1}) = 3024 (w, ν, C-H, arom), 2921, 2848 (s, ν, C-H, Alkyl), 2788 (m, ν, N-CH_2), 1490 (w, ν, C=C, arom), 1447 (m, δ, C-H, Alkyl), 754 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.79 – 0.92 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.10 – 1.33 (m, 3H, NCH_2(cyclohexyl-*H*)), 1.31 (t, J = 9.4 Hz, 3H, OCH_2CH_3), 1.37 – 1.48 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.55 – 1.84 (m, 9H, NCH_2(cyclohexyl-*H*) (5H), (CH_2CH_2)$_2$CHN) (4H)), 1.84 – 2.01 (m, 3H, (CH_2CH_2)$_2$CHN), 2.11 – 2.19 (m, 1H, (CH_2CH_2)$_2$CHN), 2.25 (d, J = 6.5 Hz, 2H, NCH_2(cyclohexyl-H)), 2.31 (s, 3H, NCH_3), 2.48 – 2.60 (m, 1H, 4'-H_a), 2.92 (d, J = 5.6 Hz, 2H, $ArCH_2CHOCH_2CH_3$), 3.63 (dq, J = 9.4/7.1 Hz, 1H, OCH_2CH_3), 4.09 (dq, J = 9.4/7.1 Hz,

1H, OC*H*$_2$CH$_3$), 4.95 (t, J = 5.6 Hz, 1H, ArCH$_2$C*H*OCH$_2$CH$_3$), 7.07 – 7.13 (m, 2H, Ar-H), 7.13 – 7.22 (m, 2H, Ar-H). Purity (HPLC method A): 98.5 %, t_R = 19.93 min.

trans-N-(Cyclohexylmethyl)-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (10a) and
cis-N-(Cyclohexylmethyl)-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (10b)

Under N_2, ketone **9** (146 mg, 0.59 mmol) was dissolved in THF (5 mL). Cyclohexylmethylamine (98 %, 89 mg, 0.77 mmol), acetic acid (34 µL, 0.60 mmol) and NaBH(OAc)$_3$ (95 %, 212 mg, 0.95 mmol) were added and the mixture was stirred at rt for 4 h. Subsequently, 1 M NaOH (15 mL) was added and the mixture was extracted with Et$_2$O (3 x 15 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 4 cm, cyclohexane + 2 % *N,N*-dimethylethanamine, 20 cm, 20 mL). R_f (cyclohexane + 2 % *N,N*-dimethylethanamine, **10a**: R_f = 0.24, **10b**: R_f = 0.05).

10a: Colorless solid, mp 56 °C, yield 75 mg (37 %). $C_{22}H_{33}NO_2$ (343.6). MS (ESI): m/z (%) = 344 [MH, 100]. FT-IR: ν (cm^{-1}) = 3063, 3032 (w, C-H, arom), 2963, 2915 (s, v, C-H, alkyl), 2845 (s, v, OCH$_3$), 2783 (m, v, N-CH$_2$), 1478 (w, C=C, arom), 1445 (m, δ, C-H, alkyl), 756 (s, δ, C-H, o-disubst. arom). ^{1}H NMR (CDCl$_3$): δ (ppm) = 0.90 – 1.03 (m, 2H, NCH$_2$(cyclohexyl-*H*)), 1.13 – 1.35 (m, 4H, NCH$_2$(cyclohexyl-*H*)), 1.40 – 1.53 (m, 1H, NCH$_2$(cyclohexyl-*H*)), 1.57 – 1.87 (m, 8H, NCH$_2$(cyclohexyl-*H*) (4H), (C*H*$_2$C*H*$_2$)$_2$CHN) (4H)), 1.90 – 2.12 (m, 3H, (C*H*$_2$C*H*$_2$)$_2$CHN), 2.25 („td“, J = 13.6/3.9 Hz, 1H, 2'H$_a$), 2.45 (d, J = 6.6 Hz, 2H, NC*H*$_2$(cyclohexyl-H), 2.86 – 2.95 (m, 3H, 4'-H$_e$ (1H), ArC*H*$_2$CHOCH$_3$ (2H)), 3.57 (s, 3H, OC*H*$_3$), 4.85 (dd, J = 6.7/4.2 Hz,1H, ArCH$_2$C*H*OCH$_3$), 7.05 – 7.10 (m, 1H, Ar-H), 7.12 – 7.24 (m, 3H, Ar-H). A signal for the NH-proton is not seen in the spectrum. (HPLC method A): 96.7 %, t_R = 19.0 min.

10b: Colorless solid, mp 95 °C, yield 107 mg (53 %). $C_{22}H_{33}NO_2$ (343.6). MS (ESI): m/z (%) = 344 [MH, 100]. FT-IR: ν (cm^{-1}) = 2918, 2851 (s, v, C-H, Alkyl), 1489 (w, v, C=C, arom), 1442 (m, δ, C-H, Alkyl), 766 (s, δ, C-H, o-disubst. arom).^{1}H NMR (CDCl$_3$): δ (ppm) = 0.86 – 0.98 (m, 2H, NCH$_2$(cyclohexyl-*H*)), 1.10 – 1.33 (m, 4H, NCH$_2$(cyclohexyl-*H*)), 1.42 – 1.53 (m, 1H, NCH$_2$(cyclohexyl-*H*)), 1.55 – 1.99 (m, 11 H, NCH$_2$(cyclohexyl-*H*) (4H), (C*H*$_2$C*H*$_2$)$_2$CHN) (7H)), 2.06 – 2.12 (m, 1H, 2'-H$_e$), 2.51 – 2.60 (m, 3H, NC*H*$_2$(cyclohexyl-H) (2H), 4'-H$_a$ (1H)), 2.89 (dd, J = 15.5/6.5 Hz, 1H, ArC*H*$_2$CHOCH$_3$), 2.94 (dd, J = 15.6/3.7 Hz, 1H, ArC*H*$_2$CHOCH$_3$), 3.56 (s, 3H, OC*H*$_3$),

4.85 (dd, J = 6.7/3.9 Hz, 1H, $ArCH_2CHOCH_3$), 7.07 – 7.13 (m, 2H, Ar-H), 7.13 – 7.22 (m, 2H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC method A): 98.6 %, t_R = 18.8 min.

trans-N-(Cyclohexylmethyl)-3-methoxy-N-methyl-3,4-dihydrospiro[2-benzopyran-1,1'-cyclohexan]-4'-amine (11a)

Under N_2, cyclohexylmethylamine **10a** (44 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (2.5 mL). Formalin (37 %, stabilized with 10-15 % MeOH, 190 µL, 2.55 mmol) and $NaBH(OAc)_3$ (95 %, 46 mg, 0.20 mmol) were added and the reaction mixture was stirred at rt for 2 h. Then, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (1 x 10 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane + 0.5 % *N*,*N*-dimethylethanamine, 20 cm, 10 mL). R_f (cyclohexane + 0.5 % *N*,*N*-dimethylethanamine: 0.09). Pale yellow solid, mp 60 °C, yield 41 mg (90 %). $C_{23}H_{35}NO_2$ (357.6). MS (ESI): m/z (%) = 358 [MH, 100]. IR: ν (cm^{-1}) = 3063, 3020 (w, C-H, arom), 2919 (s, v, C-H, alkyl), 2847 (s, v, OCH_3), 2781 (m, v, $N\text{-}CH_3$), 1604, 1489 (w, C=C, arom), 1445 (m, δ, C-H, alkyl), 752 (s, δ, C-H, o-disubst. arom). ^1H NMR ($CDCl_3$): δ (ppm) = 0.80 – 0.95 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.11 – 1.34 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.50 – 1.62 (m, 3H, NCH_2(cyclohexyl-*H*)), 1.65 – 1.80 (m, 4H, NCH_2(cyclohexyl-*H*) (2H), (CH_2CH_2)$_2$CHN) (2H)), 1.84 – 1.90 (m, 3H, (CH_2CH_2)$_2$CHN), 1.90 – 2.08 (m, 2H, (CH_2CH_2)$_2$CHN), 2.16 (dd, J = 7.2/1.5 Hz, 2H, NCH_2(cyclohexyl-H)), 2.20 – 2.33 (m, 5H, NCH_3 (3H), 4'-H_e (1H), 2'-H_a (1H)), 2.89 (dd, J = 15.7/7.0 Hz, 1H, $ArCH_2CHOCH_3$), 2.94 (dd, J = 15.8/4.0 Hz, 1H, $ArCH_2CHOCH_3$), 3.58 (s, 3H, OCH_3), 4.86 (dd, J = 6.6/4.3 Hz,1H, $ArCH_2CHOCH_3$), 7.05 – 7.09 (m, 1H, Ar-H), 7.12 – 7.24 (m, 3H, Ar-H). Purity (HPLC method A): 96.3 %, t_R = 18.94 min.

cis-N-(Cyclohexylmethyl)-3-methoxy-N-methyl-3,4-dihydrospiro[2-benzopyran-1,1'-cyclohexan]-4'-amine (11b)

Under N_2, cyclohexylmethylamine **10b** (46 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (2.5 mL). Formalin (37 %, stabilized with 10-15 % MeOH, 201 µL, 2.70 mmol) and $NaBH(OAc)_3$ (95 %, 48 mg, 0.22 mmol) were added and the reaction mixture was stirred at rt for 2 h. Then, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (1 x 10 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm,

cyclohexane + 1 % *N*,*N*-dimethylethanamine, 20 cm, 10 mL). R_f (cyclohexane + 1 % *N*,*N*-dimethylethanamine: 0.15). Pale yellow solid, mp 77 °C, yield 43 mg (89 %). $C_{23}H_{35}NO_2$ (357.6). MS (ESI): m/z (%) = 358 [MH, 100]. IR: ν (cm^{-1}) = 3061, 3015 (w, C-H, arom), 2921 (s, v, C-H, alkyl), 2840 (s, v, OCH_3), 2793 (m, v, N-CH_3), 1488 (w, C=C, arom), 1447 (m, δ, C-H, alkyl), 756 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.79 – 0.91 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.10 – 1.32 (m, 3H, NCH_2(cyclohexyl-*H*)), 1.36 – 1.49 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.57 – 1.75 (m, 6H, NCH_2(cyclohexyl-*H*) (5H), (CH_2CH_2)$_2$CHN (1H)), 1.76 – 1.84 (m, 3H, (CH_2CH_2)$_2$CHN), 1.85 – 2.01 (m, 3H, (CH_2CH_2)$_2$CHN), 2.09 – 2.17 (m, 1H, 2'-H_e), 2.25 (d, J = 6.8 Hz, 2H, NCH_2(cyclohexyl-H)), 2.31 (s, 3H, NCH_3), 2.49 – 2.59 (m, 1H, 4'-H_a), 2.89 (dd, J = 15.5/6.5 Hz, 1H, ArCH_2CHOCH_3), 2.94 (dd, J = 15.5/3.5 Hz, 1H, ArCH_2CHOCH_3), 3.59 (s, 3H, OCH_3), 4.86 (dd, J = 6.9/3.9 Hz, 1H, ArCH_2CHOCH_3), 7.06 – 7.22 (m, 4H, Ar-H). Purity (HPLC method A): 98.9 %, t_R = 18.99 min.

3-Methoxy-3*H*-spiro[[2]-benzofuran-1,1'-cyclohexan]-4'-one (13)

Under N_2, a solution of 2-bromobenzaldehyde dimethyl acetal (**12**, 257 mg, 1.11 mmol) in THF abs. (12 mL) was cooled to -78 °C. Subsequently, *n*-BuLi (1.6 M in *n*-hexane, 0.84 mL, 1.34 mmol) was added dropwise. After 20 min, cyclohexane-1,4-dione (250 mg, 2.23 mmol in THF abs., 2 mL) was added rapidly and the mixture was stirred at -78 °C for 20 min and 1 h at rt. Then, H_2O was added and the mixture was extracted with Et_2O (2x) and CH_2Cl_2 (2x). The organic layer was concentrated in vacuo and the residue was purified by fc (4 cm, cyclohexane/ethyl acetate = 2/1, 24 cm, 20 mL, R_f (cyclohexane/ethyl acetate = 2/1, 0.21). The isolated product (contaminated, 177 mg) was dissolved in THF, *p*-toluenesulfonic acid monohydrate (23 mg, 0.12 mmol) was added and the mixture was stirred at rt for 24 h. Subsequently, 0.2 M NaOH (20 mL) was added and the aqueous layer was extracted with Et_2O (3 x 20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 4.5 cm, cyclohexane/ethyl acetate = 6/1, 21 cm, 20 mL). R_f (cyclohexane/ethyl acetate = 6/1, 0.20). Colorless solid, mp 129 °C, yield 113 mg (44 %). $C_{14}H_{16}O_3$ (232.3). MS (EI): m/z (%) = 232 [M, 2], 201 [M – OCH_3, 17], 175 [M – CH_3CH_2C*=O,100]. HRMS-ESI (CH_3OH): m/z: $[M + Na]^+$ calculated for $C_{14}H_{16}O_3Na$: 255.0997 found: 255.9920; m/z $[2M + Na]^+$ calculated for ($C_{28}H_{32}O_6$)Na: 487.2097 found: 487.2091. IR: ν (cm^{-1}) = 2946, 2931(s, v, C-H, alkyl), 2853 (m, v, OCH_3), 1707 (s, v, C=O), 1483 (m, δ, C-H, C-H, alkyl), 769(s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.01

– 2.08 (m, 1H, (C*H*$_2$CH$_2$)$_2$C=O), 2.14 - 2.29 (m, 3H, (C*H$_2$*C*H$_2$*)$_2$C=O), 2.39– 2.46 (m, 2H, (CH$_2$C*H$_2$*)$_2$C=O), 2.90– 3.00 (m, 2H, (CH$_2$C*H$_2$*)$_2$C=O), 3.54 (s, 3H, OCH$_3$), 6.16 (s, 1H, ArC*H*OCH$_3$), 7.12 – 7.17 (m, 1H, Ar-H), 7.35 – 7.42 (m, 3H, Ar-H). ^{13}C NMR (CDCL$_3$): δ (ppm) = 37.9 (1C, (*C*H$_2$*C*H$_2$)$_2$C=O), 38.2 (1C, (*C*H$_2$*C*H$_2$)$_2$C=O), 38.6 (1C, (*C*H$_2$*C*H$_2$)$_2$C=O), 39.2 (1C, (*C*H$_2$*C*H$_2$)$_2$C=O), 55.3 (1C, O*C*H$_3$), 85.3 (1C, spiro-C), 106.5 (1C, Ar*C*HOCH$_3$), 120.8 (1C, arom), 123.6 (1C, arom), 128.8 (1C, arom), 129.9 (1C, arom), 137.8 (1C, arom), 145.8 (1C, arom), 211.7 (1C, C=O).

trans-N-(Cyclohexylmethyl)-3-methoxy-3*H*-spiro[[2]benzofuran-1,1'-cyclohexan]-4'-amine (14a) and _cis_-N-(Cyclohexylmethyl)-3-methoxy-3*H*-spiro[[2]benzofuran-1,1'-cyclohexan]-4'-amine (14b)

Under N_2, ketone **13** (90 mg, 0.39 mmol) was dissolved in THF (5 mL). Cyclohexylmethylamine (98 %, 69 mg, 0.58 mmol) in THF abs. (1 mL), acetic acid (25 µL, 0.44 mmol) and NaBH(OAc)$_3$ (95 %, 156 mg, 0.67 mmol) were added and the mixture was stirred at rt for 3 h. Subsequently, 1 M NaOH (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 3 cm, cyclohexane + 2 % *N,N*-dimethylethanamine, 20 cm, 10 mL). R_f (cyclohexane + 2 % *N,N*-dimethylethanamine, **14a**: R_f = 0.20, **14b**: R_f = 0.10).

14a: Colorless oil, yield 36 mg (28 %). $C_{21}H_{31}NO_2$ (329.5). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.94 („qd", J = 12.0/2.8 Hz, 2H, NCH$_2$(cyclohexyl-*H*)), 1.12 – 1.33 (m, 4H, NCH$_2$(cyclohexyl-*H*)), 1.41 – 1.53 (m, 1H, NCH$_2$(cyclohexyl-*H*)), 1.55 – 1.85 (m, 8H, NCH$_2$(cyclohexyl-*H*) (4H), (C*H$_2$*C*H$_2$*)$_2$CHN) (4H)), 1.93 – 2.10 (m, 4H, (C*H$_2$*C*H$_2$*)$_2$CHN), 2.47 (d, J = 6.4 Hz, 2H, NC*H$_2$*(cyclohexyl-H), 2.80 – 2.87 (m, 1H, 4'-H$_e$), 3.46 (s, 3H, OCH$_3$), 6.06 (s, 1H, ArC*H*OCH$_3$), 7.29 – 7.38 (m, 4H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Anal. calcd. for $C_{21}H_{31}NO_2$ (329.5). C, 76.55, H, 9.48, N, 4.25, found C, 76.30, H, 9.44, N, 4.25.

14b: Colorless oil, yield 86 mg (67 %). $C_{21}H_{31}NO_2$ (329.5). MS (ESI): m/z (%) = 330 [MH, 20], 298 [M-OCH$_3$, 17], 246 [M-cyclohexyl*, 20], 214 [100], 167 [93], 152 [CH$_2$=CHCH=HN$^+$CH$_2$cyclohexyl, 86]. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.85 – 0.99 (m, 2H, NCH$_2$(cyclohexyl-*H*)), 1.11 – 1.33 (m, 4H, NCH$_2$(cyclohexyl-*H*)), 1.41 – 1.52 (m, 1H, NCH$_2$(cyclohexyl-*H*)), 1.60 – 1.85 (m, 9H, NCH$_2$(cyclohexyl-*H*) (4H), (C*H$_2$*C*H$_2$*)$_2$CHN) (5H)), 1.85 – 1.97 (m, 3H, (C*H$_2$*C*H$_2$*)$_2$CHN), 2.49 – 2.61 (m, 1H, 4'-H$_a$), 2.52 (d, J = 7.4

Hz, 2H, NC*H*$_2$(cyclohexyl-H), 3.48 (s, 3H, OCH_3), 6.04 (s, 1H, ArC*H*OCH_3), 7.09 – 7.13 (m, 1H, Ar-H), 7.28 – 7.38 (m, 3H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Anal. calcd. for $C_{21}H_{31}NO_2$ (329.5). C, 76.55, H, 9.48, N, 4.25, found C, 76.54, H, 9.42, N, 4.38.

trans-N-(Cyclohexylmethyl)-3-methoxy-N-methyl-3*H*-spiro[[2]benzofuran-1,1'-cyclohexan]-4'-amine (15a)

Under N_2, cyclohexylmethylamine **14a** (21.8 mg, 0.07 mmol) was dissolved in CH_2Cl_2 (3 mL). Formalin (37 %, stabilized with 10-15 % MeOH, 99 µL, 1.30 mmol) and $NaBH(OAc)_3$ (95 %, 24 mg, 0.11 mmol) were added and the reaction mixture was stirred at rt for 2 h. Then, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (1 x 20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane + 1% *N*,*N*-dimethylethanamine, 15 cm, 10 mL). R_f (cyclohexane + 1 % *N*,*N*-dimethylethanamine: 0.16). Pale yellow oil, yield 22.5 mg (99 %). $C_{22}H_{33}NO_2$ (343.6). MS (EI): m/z (%) = 343 [M, 1], 312 [M-OCH_3, 7], 260 [M-cyclohexyl*, 100], 185 [36], 167 [*$CH_2CH_2CH{=}N^+(-CH_3)CH_2$cyclohexyl), 69]. IR: ν (cm^{-1}) = 3033 (w, v, C-H, arom), 2920, 2848 (s, v, C-H, alkyl), 2783 (m, v, N-CH_2), 1613, 1462 (w, v, C=C, arom), 1447 (m, δ, C-H, Alkyl), 751 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.82 – 0.92 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.12 – 1.33 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.45 – 1.53 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.60 – 1.78 (m, 4H, NCH_2(cyclohexyl-*H*) (2H), (C*H*$_2$C*H*$_2$)$_2$CHN) (2H)), 1.79 – 1.93 (m, 6H, NCH_2(cyclohexyl-*H*) (2H), (C*H*$_2$C*H*$_2$)$_2$CHN (4H)), 2.00 – 2.09 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$CHN), 2.21 (d, J = 7.0 Hz, 2H, NC*H*$_2$(cyclohexyl-H), 2.26 (s, 3H, NCH_3), 2.4 („quint", J = 5.4 Hz, 1H, 4'-H_e), 3.46 (s, 3H, OCH_3), 6.06 (s, 1H, ArC*H*OCH_3), 7.30 – 7.38 (m, 4H, Ar-H). Anal. calcd. for $C_{22}H_{33}NO_2$ (343.6). C, 76.92, H, 9.68, N, 4.08, found C, 76.96, H, 9.74, N, 3.82.

cis-N-(Cyclohexylmethyl)-3-methoxy-N-methyl-3*H*-spiro[[2]benzofuran-1,1'-cyclohexan]-4'-amine (15b)

Under N_2, cyclohexylmethylamine **14b** (64.6 mg, 0.20 mmol) was dissolved in CH_2Cl_2 (3 mL). Formalin (37 %, stabilized with 10-15 % MeOH, 294 µL, 3.92 mmol) and $NaBH(OAc)_3$ (95 %, 70 mg, 0.31 mmol) were added and the reaction mixture was stirred at rt for 2 h. Then, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (1 x 20 mL). The combined organic layers were dried

(K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane + 1% *N*,*N*-dimethylethanamine, 15 cm, 10 mL). R_f (cyclohexane + 1% *N*,*N*-dimethylethanamine: 0.12).Pale yellow oil, yield 65 mg (97 %). $C_{22}H_{33}NO_2$ (343.6). MS (EI): m/z (%) = 343 [M, 0.4], 312 [M-OCH_3, 3], 260 [M-cyclohexyl*, 100], 167 [*$CH_2CH_2CH=N^+(-CH_3)CH_2$cyclohexyl), 51]. IR: ν (cm^{-1}) = 2922, 2847 (s, v, C-H, alkyl), 2787 (m, v, N-CH_2), 1466 (w, v, C=C, arom), 1446 (m, δ, C-H, alkyl), 754 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.80 – 0.93 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.11 – 1.32 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.37 – 1.48 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.58 – 1.87 (m, 11 H, NCH_2(cyclohexyl-*H*) (4H), (CH_2CH_2)$_2$CHN) (7H)), 1.90 – 1.99 (m, 1H, 2'-H_e), 2.26 (d, J = 6.6 Hz, 2H, NCH_2(cyclohexyl-H)), 2.32 (s, 3H, NCH_3), 2.46 – 2.57 (m, 1H, 4'-H_a), 3.48 (s, 3H, OCH_3), 6.09 (s, 1H, Ar$CH(OCH_3)$), 7.11 – 7.13 (m, 1H, Ar-H), 7.29 – 7.37 (m, 3H, Ar-H). Anal. calcd. for $C_{22}H_{33}NO_2$ (343.6). C, 76.92, H, 9.68, N, 4.08, found C, 76.68, H, 9.80, N, 3.92.

3,4-Dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-one ethylene ketal (17)

Under N_2 and ice cooling, PBr_3 (3.17 mL, 0.11 g, 33.7.mmol) was added slowly to alcohol **16** (4.84 g, 24 mmol). Then, the mixture was heated to 80 °C for 4 h. The mixture was poured on ice, a saturated solution of $NaHCO_3$ (20 mL) was added and the mixture was stirred for 30 min at rt. The mixture was extracted with CH_2Cl_2 (4 x 60 mL) and the organic layer was washed with saturated solutions of $NaHCO_3$ (60 mL) and NaCl (60 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuo and the residue was purified by fc (∅ 8 cm, cyclohexane/ethyl acetate = 9/1, 20 cm, 65 mL, R_f (cyclohexane/ethyl acetate 9/1). Colorless oil (1-bromo-2-(2-bromoethyl)benzene), yield 4.58 g (72 %). $C_8H_8Br_2$ (264.0). MS (EI): m/z (%) = 266, 264, 262 [M, 12 (2*Br^{81}), 40(Br^{79}+Br^{81}) , 16(2*Br^{79})], 185, 183 [M (Br^{81} + Br^{79}) - Br, 86 ,100], 171, 169 [M(Br^{81} + Br^{79}) - CH_2Br, 24, 34], 104 [M(Br^{81} + Br^{79}) - Br_2, 65].]. IR: ν (cm^{-1}) = 3056 (w, v, C-H, arom), 2964 (s, v, C-H, alkyl), 1568, 1469 (w, v, C=C, arom), 1439 (m, δ, C-H alkyl), 1023 (s, ar-Br), 747 (s, o-disubst. arom), 661 (s, alkyl-Br). ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.30 (t, J = 7.6 Hz, 2H, ArCH_2CHBr), 3.60 (t, J = 7.6 Hz, 2H, ArCH_2CH_2Br), 7.10- 7.16 (m, 1H, Ar-H), 7.26 – 7.28 (m, 2H, Ar-H), 7.55 (d, J = 8.0 Hz, 1H, Ar-H). Purity (HPLC, method B): 99.7 %, t_R = 18.83 min.

Under N_2, a solution of 1-bromo-2-(2-bromoethyl)benzene (202 mg, 0.77 mmol) in THF (10 mL) was cooled to -88 °C. Subsequently, *n*-BuLi (1.6 M in *n*-hexane, 0.58 mL, 0.93 mmol) was added slowly. After stirring for 5 min at -88 °C, cyclohexane-1,4-dione

monoethylene ketal (0.168 g, 1.08 mmol in THF (2 mL) was added rapidly and the mixture was stirred at -88 °C for 5 min. and at rt for additional 1 h. Then, H_2O (10 mL) was added and the mixture was extracted with Et_2O (3 x 40mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 4 cm, cyclohexane/ethyl acetate = 9/1, 20 cm, 20 mL). R_f (cyclohexane/ethyl acetate = 9/1, 0.18). Colorless solid, mp 122 °C, yield 121 mg (61 %). $C_{16}H_{20}O_3$ (260.4). MS (ESI): m/z (%) = 261 [M+H,100]. IR: ν (cm^{-1}) = 2966, 2933, 2881 (s, v, C-H, alkyl), 2864 (m, v, OCH_2), 1488 (w, v, C=C arom), 762 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.60 – 1.65 (m, 2H, (C*H*$_2$CH$_2$)$_2$C=O), 1.94 – 2.07(m, 6H, (C*H*$_2$CH$_2$)$_2$C=O), 2.83 (t, J = 5.4 Hz, 2H,ArC*H*$_2$CH$_2$O), 3.91 (t, J = 5.6 Hz, 2H, ArCH$_2$C*H*$_2$O), 4.00 (s, 4H, OC*H*$_2$C*H*$_2$O), 7.06 – 7.10 (m, 1H, Ar-H), 7.12 – 7.16 (m, 1H, Ar-H), 7.16 – 7.20 (m, 2H, Ar-H). Purity (HPLC method A): 98.3 %, t_R = 19.4 min.

3,4-Dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-one (18)

A solution of ketal **17** (121 mg, 0.47 mmol) in Et_2O (4 mL) and 2 M HCl (4 mL) was heated to reflux for 48 h. Evaporated Et_2O was supplemented. Subsequently, H_2O (50 mL) and Et_2O (50 mL) were added and the mixture was extracted with Et_2O (3 x 50 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane/ethyl acetate = 9/1, 20 cm, 10 mL). R_f (cyclohexane/ethyl acetate = 9/1, 0.10, cyclohexane/ethyl acetate = 4/1, 0.23). Colorless solid, mp 134 °C, yield 93.5 mg (94 %). $C_{14}H_{16}O_2$ (216.3). MS (ESI): m/z (%) = 217 [M+H, 2], 159 [M – $CH_3CH_2C^*$=O, 100]. IR: ν (cm^{-1}) = 3048, 3020 (w, v, C-H, arom), 2953, 2923, 2861 (s, v, C-H, alkyl), 1703 (s, v, C=O), 1493 (w, v, C=C arom), 755 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.18 („td", J = 13.8/4.6 Hz, 2H, (C*H*$_2$CH$_2$)$_2$C=O), 2.26 - 2.34 (m, 4H, (C*H*$_2$C*H*$_2$)$_2$C=O), 2.86 („td", J = 14.3/6.3 Hz, 2H, (CH$_2$C*H*$_2$)$_2$C=O), 2.90 (t, J = 5.5 Hz, 2H, ArC*H*$_2$CH$_2$O), 4.01 (t, J = 5.6 Hz, 2H, ArCH$_2$C*H*$_2$O), 7.05 – 7.10 (m, 1H, Ar-H), 7.11 – 7.22 (m, 3H, Ar-H). Purity (HPLC method A): 99.5 %, t_R = 18.3 min.

***trans*-N-(Cyclohexylmethyl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (19a) and**

***cis*-N-(Cyclohexylmethyl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (19b)**

Under N_2, a solution of ketone **18** (85 mg, 0.39 mmol) in THF (5 mL) was treated with cyclohexylmethylamine (98 %, 68 mg, 0.59 mmol) dissolved in THF (2 mL), acetic acid (23 µL, 0.40 mmol) and $NaBH(OAc)_3$ (95 %, 158 mg, 0.71 mmol). The mixture was stirred at rt for 3 h. Subsequently, 1 M NaOH (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 3 cm, cyclohexane + 2 % *N,N*-dimethylethanamine, 20 cm, 10 mL). R_f (cyclohexane + 2 % *N,N*-dimethylethanamine, **19a**: R_f = 0.33, **19b**: R_f = 0.09).

19a: Colorless oil, yield 30 mg (24 %). $C_{21}H_{31}NO$ (313.5). MS (EI): m/z (%) = 314 [MH, 20], 230 [M-cyclohexyl*, 37], 183 [$C_{13}H_{11}O)^+$, 24], 201 [M-H_3CN*CH_2cyclohexyl, 31], 124 [($C_8H_{14}N)^+$, 15], 152 [CH_2=CHCH=HN^+CH_2cyclohexyl,100]. IR: ν (cm^{-1}) = 3019 (w, v, C-H, arom), 2919, 2849 (s, v, C-H, alkyl), 2793 (m, v, N-CH_2), 1489 (w, v, C=C, arom), 1448 (m, δ, C-H, alkyl), 751 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.90 – 1.00 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.13 – 1.34 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.43 – 1.52 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.53 – 1.62 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$CHN), 1.62 – 1.70 (m, 4H, NCH_2(cyclohexyl-*H*) (2H), (C*H*$_2$C*H*$_2$)$_2$CHN (2H)), 1.79 – 1.86 (m, 2H, NCH_2(cyclohexyl-*H*), 1.90 („tt", J = 13.8/3.4 Hz, 2H, 3'-H_a, 5'-H_a), 2.08 („td", J = 13.7/3.7 Hz, 2H, 2'-H_a, 6'-H_a), 2.44 (d, J = 6.6 Hz, 2H, NC*H*$_2$(cyclohexyl-H), 2.82 (t, J = 5.6 Hz, 2H, OCH_2C*H*$_2$Ar), 2.85 – 2.90 (m, 1H, 4'-H_e), 3.90 (t, J = 5.6 Hz, 2H, OC*H*$_2$$CH_2$Ar), 7.06 – 7.08 (m, 1H, Ar-H), 7.10 – 7.14 (m, 1H, Ar-H), 7.18 – 7.19 (m, 2H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC method A): 99.1 %, t_R = 19.0 min.

19b: Colorless oil, yield 89 mg (72 %). $C_{21}H_{31}NO$ (313.5). MS (ESI): m/z (%) = 314 [MH, 3], 230 [M-cyclohexyl*, 100], 183 [$C_{13}H_{11}O)^+$, 12], 152 [CH_2=CH=HN^+CH_2cyclohexyl, 66], 124 [($C_8H_{14}N)^+$, 8]. IR: ν (cm^{-1}) = 3018 (w, v, C-H, arom), 2919, 2849 (s, v, C-H, alkyl), 1489 (w, v, C=C, arom), 1448 (m, δ, C-H, alkyl), 753 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.84 – 0.98 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.10 – 1.31 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.41 – 1.51 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.52 – 1.62 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.63 – 1.84 (m, 8H, NCH_2(cyclohexyl-*H*)(2H), (C*H*$_2$C*H*$_2$)$_2$CHN(6H)), 1.94 – 2.03 (m, 2H, 2'-H_e, 6'-H_e), 2.50

(d, J = 6.7 Hz, 2H NC*H*$_2$(cyclohexyl-H), 2.50 – 2.59 (m, 1H, 4'-H$_a$), 2.82 (t, J = 5.5 Hz, 2H, OCH$_2$C*H*$_2$Ar), 3.88 (t, J = 5.6 Hz, 2H, OC*H*$_2$CH$_2$Ar), 7.07 – 7.19 (m, 4H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC method A): 99.7 %, t_R = 18.6 min.

trans-N-(Cyclohexylmethyl)-N-methyl-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (20a)

Under N_2, cyclohexylmethylamine **19a** (18.6 mg, 0.06 mmol) was dissolved in CH_2Cl_2 (2.5 mL). Formalin (37 %, stab. with 10-15 % MeOH, 89 µL, 1.19 mmol) and $NaBH(OAc)_3$ (95 %, 21 mg, 0.09 mmol) were added and the reaction mixture was stirred at rt for 2 h. Subsequently, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and with Et_2O (1 x 20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane + 1 % *N*,*N*-dimethylethanamine, 15 cm, 10 mL). R_f (cyclohexane + 1 % *N*,*N*-dimethylethanamine: 0.13). Colorless solid, yield 19 mg (97 %). $C_{22}H_{33}NO$ (327.6). MS (EI): m/z (%) = 327 [M, 4], 244 [M-cyclohexyl*, 100], 201 [M-H_3CN*CH_2cyclohexyl, 51], 183 [$C_{13}H_{11}O)^+$, 35], 166 [CH_2=CHCH=N^+(-CH_3)-(CH_2cyclohexyl), 39], 159 [1-Allyl[2]benzopyran, 36]. IR: ν (cm^{-1}) = 3062, 3021 (w, v, C-H, arom), 2919, 2848 (s, v, C-H, alkyl), 2782 (m, v, N-CH_2), 1489 (w, v, C=C, arom), 1447 (m, δ, C-H, alkyl), 751 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.82 – 0.95 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.12 – 1.34 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.50 – 1.78 (m, 7H, NCH_2(cyclohexyl-*H*) (3H), (C*H*$_2$C*H*$_2$)$_2$CHN) (4H), 1.78 – 1.93 (m, 5H, NCH_2(cyclohexyl-*H*) (2H), (C*H*$_2$C*H*$_2$)$_2$CHN (3H)), 2.05 – 2.16 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$C*H*N)), 2.15 (d, J = 7.2 Hz, 2H, NC*H*$_2$(cyclohexyl-H), 2.19 (s, 3H, NC*H*$_3$), 2.82 (t, J = 5.5 Hz, 2H, OCH$_2$C*H*$_2$Ar), 3.91 (t, J = 5.5 Hz, 2H, OC*H*$_2$CH$_2$Ar), 7.04 – 7.08 (m, 1H, Ar-H), 7.10 – 7.14 (m, 1H, Ar-H), 7.16 – 7.20 (m, 2H, Ar-H). Purity (HPLC method A): 92.5 %, t_R = 19.52 min.

cis-N-(Cyclohexylmethyl)-N-methyl-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (20b)

Under N_2, cyclohexylmethylamine **19b** (61 mg, 0.20 mmol) was dissolved in CH_2Cl_2 (2.5 mL). Formalin (37 %, stab. with 10-15% MeOH, 293 µL, 3.90 mmol) and $NaBH(OAc)_3$ (95 %, 70 mg, 0.31 mmol) were added and the reaction mixture was stirred at rt for 2 h. Then, H_2O (10 mL) was added and the mixture was extracted with

CH_2Cl_2 (3 x 20 mL) and Et_2O (1 x 20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 2 cm, cyclohexane + 2 % *N*,*N*-dimethylethanamine, 16 cm, 10 mL). R_f (cyclohexane + 2 % *N*,*N*-dimethylethanamine: 0.31). Colorless oil, yield 62 mg (97 %). $C_{22}H_{33}NO$ (327.6). MS (ESI): m/z (%) = 327 [M, 6], 244 [M-cyclohexyl*, 100], 183 [$C_{13}H_{11}O)^+$, 10], 166 [CH_2=CHCH=N^+(-CH_3)-CH_2cyclohexyl,16], 159 [1-Allyl[2]benzopyran, 15]. IR: ν (cm^{-1}) = 3062, 3019 (w, v, C-H, arom), 2920, 2848 (s, v, C-H, alkyl), 1489 (w, v, C=C, arom), 1448 (m, δ, C-H, alkyl), 753 (s, δ, C-H, o-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.79 – 0.93 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.10 – 1.31 (m, 4H, NCH_2(cyclohexyl-*H*)), 1.36 – 1.47 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.55 – 1.84 (m, 10 H, NCH_2(cyclohexyl-*H*) (4H), (C*H*$_2$C*H*$_2$)$_2$CHN) (6H)), 1.98 – 2.09 (m, 2H, 2'-H_e, 6'-H_e), 2.24 (d, J = 6.6 Hz, 2H, NC*H*$_2$(cyclohexyl-H)), 2.30 (s, 3H, NC*H*$_3$), 2.45 – 2.58 (m, 1H, 4'-H_a), 2.83 (t, J = 5.5 Hz, 2H, OCH_2C*H*$_2$Ar), 3.91 (t, J = 5.6 Hz, 2H, OC*H*$_2$$CH_2$Ar), 7.05 – 7.20 (m, 4H, Ar-H). Purity (HPLC method A): 99.1%, t_R = 19.42 min.

trans-N-Benzyl-3-methoxy-3*H*-spiro[[2]benzofuran-1,1'-cyclohexan]-4'-amine (23a) and

cis-N-Benzyl-3-methoxy-3*H*-spiro[[2]benzofuran-1,1'-cyclohexan]-4'-amine (23b)

Under N_2, a solution of ketone **13** (70.5 mg, 0.30 mmol), benzylamine (37 µL, 0.32 mmol), acetic acid (17 µL, 0.30 mmol) and $NaBH(OAc)_3$ (95 %, 98 mg, 0.44 mmol) in THF (5 mL) was stirred at rt for 4 h. Subsequently, 1 M NaOH (10 mL) was added and the mixture was extracted with Et_2O (10 mL) and CH_2Cl_2 (2 x 10 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 3.5 cm, cyclohexane + 2 % *N,N*-dimethylethanamine, 20 cm, 10 mL). R_f (cyclohexane + 2 % *N,N*-dimethylethanamine, **23a**: R_f = 0.16, **23b**: R_f = 0.09).

23a: Pale yellow oil, yield 35.3 mg (36 %). $C_{25}H_{25}NO_2$ (323.5). MS (ESI): m/z (%) = 324 [MH,100]. IR: ν (cm^{-1}) = 3028 (w, v, C-H, arom), 2925 (s, v, C-H, alkyl), 2849 (s, v, OCH_3), 2824 (m, v, N-CH_2), 1611, 1495 (w, C=C, arom), 1452 (m, δ, C-H, alkyl), 752 (s, δ,C-H, o-disubst. arom), 698 (s, δ, C-H, mono-subst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.55 – 1.62 (m, 1 H, (C*H*$_2$C*H*$_2$)$_2$CHN), 1.67 – 1.81 (m, 3H, (C*H*$_2$C*H*$_2$)$_2$CHN), 1.98 – 2.17 (m, 4H, (C*H*$_2$C*H*$_2$)$_2$CHN), 2.97 („quint", J = 3.9 Hz, 1H, 4'H_e), 3.46 (s, 3H, OC*H*$_3$), 3.83 (s, 2H, NC*H*$_2$Ar), 6.07 (s,1H, ArC*H*OCH_3), 7.25 – 7.29 (m, 1H, Ar-H), 7.29 – 7.40 (m, 8H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Anal. calcd. for $C_{25}H_{25}NO_2$ (323.5) C, 78.0, H, 7.80, N, 4.33, found C, 77.7, H, 8.02, N, 3.99.

23b: Pale yellow oil, yield 51.9 mg (53 %). $C_{25}H_{25}NO_2$ (323.5). MS (ESI): m/z (%) = 324 [MH,100]. IR: ν (cm^{-1}) = 3029 (w, v, C-H, arom), 2928 (s, v, C-H, alkyl), 2849 (s, v, OCH_3), 2820 (m, v, N-CH_2), 1604,1494 (w, C=C, arom), 1460 (m, δ, C-H, alkyl), 755 (s, δ, C-H, o-disubst. arom), 746, 697 (s, δ, C-H, mono-subst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.65 – 1.85 (m, 5H, (C*H*$_2$C*H*$_2$)$_2$CHN), 1.87 – 1.93 (m, 1H, (C*H*$_2$C*H*$_2$)$_2$CHN), 1.93 – 2.01 (m, 2H, (C*H*$_2$C*H*$_2$)$_2$CHN), 2.65 („tt“, J = 10.4/3.9 Hz, 1H, 4'-H_a), 3.49 (s, 3H, OCH_3), 3.90 (s, 2H, Ar-C*H*$_2$-NH), 6.05 (s, 1H, ArC*H*OCH_3), 7.08 – 7.12 (m, 1H, Ar-H), 7.24 – 7.29 (m, 1H, Ar-H), 7.30 – 7.38 (m, 7H, Ar-H). A signal for the NH-proton is not seen in the spectrum. Anal. calcd. for $C_{25}H_{25}NO_2$ (323.5) C, 78.0, H, 7.80, N, 4.33, found C, 77.8, H, 7.95, N, 4.06.

***trans*-N-Benzyl-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (24a) and**

***cis*-N-Benzyl-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (24b)**

Under N_2, ketone **18** (72.6 mg, 0.34 mmol) was dissolved in THF (5 mL). Benzylamine (56 µL, 0.50 mmol), acetic acid (19 µL, 0.34 mmol) and Na[$BH(OAc)_3$] (95 %, 135 mg, 0.60 mmol) were added and the mixture was stirred for 3.5 h at rt. Then, 1 M NaOH (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL) and Et_2O (2 x 20 mL). The combined organic layers were dried (K_2CO_3), concentrated in vacuo and the residue was purified by fc (∅ 3 cm, cyclohexane/ethyl acetate = 9/1 + 1 % *N*,*N*-dimethylethanamine, 20 cm, 10 mL). R_f (cyclohexane/ethyl acetate = 9/1 + 1% *N*,*N*-dimethylethanamine, **24a**: 0.29, **24b**: 0.08).

24a: Pale yellow oil, yield 27 mg (27 %). $C_{21}H_{25}NO$ (307.5). MS (ESI): m/z (%) = 308 [MH, 100]. IR: ν (cm^{-1}) = 3060, 3023 (m, v, C-H, arom), 2924, 2855 (s, v, C-H, alkyl), 1604, 1489 (w, v, C=C, arom), 1450 (s, δ , C-H, alkyl), 751 (s, δ, C-H, o-disubst. arom), 698 (s, δ, C-H, mono-subst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.59 – 1.74 (m, 4H, (C*H*$_2$C*H*$_2$)$_2$CHN), 1.94 („tt“, J = 13.6/3.4 Hz, 2H, (CH_2C*H*$_2$)$_2$CHN), 2.16 („tt“, J = 13.8/3.8 Hz, 2H, (C*H*$_2$$CH_2$)$_2$CHN), 2.83 (t, J = 5.4 Hz, 2H, OCH_2C*H*$_2$Ar), 3.0 („quint“, J = 2.8 Hz, 1H, 4'-H_e), 3.82 (s, 2H, NHC*H*$_2$Ar), 3.91 (t, J = 5.4Hz, 2H, OC*H*$_2$$CH_2$Ar), 7.07 – 7. 08 (m, 1H, Ar-H), 7.11 - 7.15 (m, 1H, Ar-H), 7.16 – 7.24 (m, 2H, Ar-H), 7.24 – 7.30 (m, 1H, Ar-H), 7.33– 7.43 (m, 4H, Ar-H). A signal for the NH-proton is not seen in the spectrum. ^{13}C NMR ($CDCL_3$): δ (ppm) = 26.2 (2C, 3'-C, 5'-C), 30.1 (1C, Ar*C*$H_2$$CH_2$O), 31.3 (2C, 2'-C, 6'-C), 50.8 (1C, 4'-C), 52.0 (1C, NH*C*H_2Ar), 59.0 (1C, ArCH_2*C*H_2O), 75.5 (1C, spiro-C), 126.0 (1C, arom), 126.2 (1C, arom), 126.4 (2C, arom), 127.1 (1C, arom),

128.5 (2C, arom), 128.7 (1C, arom), 129.0 (1C, arom) , 133.8 (1C, arom), 142.7 (1C, arom), 143.7 (1C, arom). Purity (HPLC method A): 99.2 %, t_R = 17.90 min.

24b: Pale yellow solid, mp 65 °C, yield 68 mg (66 %). $C_{21}H_{25}NO$ (307.5). MS (ESI): m/z (%) = 308 [MH, 100]. IR: ν (cm^{-1}) = 3060, 3025 (m, v, C-H, arom), 2925, 2855 (s, v, C-H, arom), 1603,1490 (w, v, C=C, arom), 1450 (m, δ, C-H, alkyl), 753 (s, δ, C-H, o-disubst. arom), 697 (s, δ, C-H, mono-subst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.59 – 1.76 (m, 4H, (C*H*$_2$C*H*$_2$)$_2$CHN), 1.80 – 1.88 (m, 2H, (CH$_2$C*H*$_2$)$_2$CHN), 1.96 – 2.04 (m, 2H, (C*H*$_2$CH$_2$)$_2$CHN), 2.64 („tt“, J = 10.9/3.9 Hz, 1H, 4'-H$_a$), 2.83 (t, J = 5.7 Hz, 2H, OCH$_2$C*H*$_2$Ar), 3.88 (s, 2H, NHC*H*$_2$Ar), 3.90 (t, J = 5.7 Hz, 2H, OC*H*$_2$CH$_2$Ar), 7.06 – 7.09(m, 2H, Ar-H), 7.11 - 7.19 (m, 2H, Ar-H), 7.23 – 7.30 (m, 1H, Ar-H), 7.31 – 7.38 (m, 4H, Ar-H). A signal for the NH-proton is not seen in the spectrum. ^{13}C NMR ($CDCL_3$): δ (ppm) = 29.0 (2C, 3'-C, 5'-C), 29.9 (1C, Ar*C*H$_2$CH$_2$O), 36.2 (2C, 2'-C, 6'-C), 51.2 (1C, NH*C*H$_2$Ar), 55.8 (1C, 4'-C), 58.9 (1C, ArCH$_2$*C*H$_2$O), 74.6 (1C, spiro-C), 125.3 (1C, arom), 126.2 (1C, arom), 126.2 (1C, arom), 127.1 (1C, arom), 128.3 (2C, arom), 128.7 (2C, arom), 129.1 (1C, arom), 133.9 (1C, arom), 141.1 (1C, arom), 142.8 (1C, arom). Purity (HPLC method A): 99.0 %, t_R = 17.6 min.

3. Receptor Binding Studies

Material

Guinea pig brains, rat brains and rat livers were commercially available (Harlan-Winkelmann, Borchen, Germany). Pig brains were a donation of the local slaughterhouse (Coesfeld, Germany). Homogenizers: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany) and Soniprep® 150, MSE, London, UK). Centrifuges: Cooling centrifuge model Eppendorf 5427R (Eppendorf, Hamburg, Germany) and High-speed cooling centrifuge model Sorvall® RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Multiplates: standard 96 well multiplates (Diagonal, Muenster, Germany). Shaker: self-made device with adjustable temperature and tumbling speed (scientific workshop of the institute). Harvester: MicroBeta® FilterMate 96 Harvester. Filter: Printed Filtermat Typ A and B. Scintillator: Meltilex® (Typ A or B) solid state scintillator. Scintillation analyzer: MicroBeta® Trilux (all Perkin Elmer LAS, Rodgau-Jügesheim, Germany).

Preparation of membrane homogenates from guinea pig brain

5 guinea pig brains were homogenized with the potter (500-800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 23,500 x g for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 7.4) and centrifuged again at 23,500 x g (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5-6 volumes of buffer and frozen (-80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

Preparation of membrane homogenates from rat liver

Two rat livers were cut into small pieces and homogenized with the potter (500-800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000 x g for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 8.0) and incubated at rt for 30 min. After the incubation, the suspension was centrifuged again at 31,000 x g for 20 min at 4 °C. The final pellet was resuspended in 5-6 volumes of buffer and stored at -80 °C in 1.5 mL portions containing about 2 mg protein/mL.

Preparation of membrane homogenates from rat brain

5 rat brains (species: Sprague Dawley rats) were homogenized with the potter (500-800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 23,500 x g for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 7.4) and centrifuged again at 23,500 x g (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5-6 volumes of buffer and frozen (-80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

Preparation of membrane homogenates from pig brain cortex

Fresh pig brain cortex was homogenized with the potter (500-800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000 x g for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of TRIS/EDTA buffer (5 mM TRIS/1 mM EDTA, pH 7.5) and centrifuged again at 31,000 x g (20 min, 4 °C). The final pellet was resuspended in 5-6 volumes of buffer and frozen (-80 °C) in 1.5 mL portions containing about 0.8 mg protein/mL.

Protein determination

The protein concentration was determined by the method of Bradford,[3] modified by Stoscheck.[4] The Bradford solution was prepared by dissolving 5 mg of Coomassie Brilliant Blue G 250 in 2.5 mL of EtOH (95 %, v/v). 10 mL deionized H_2O and 5 mL phosphoric acid (85 %, m/v) were added to this solution, the mixture was stirred and filled to a total volume of 50 mL with deionized water. The calibration was carried out using bovine serum albumin as a standard in 9 concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 and 4.0 mg /mL). In a 96 well standard multiplate, 10 µL of the calibration solution or 10 µL of the membrane receptor preparation were mixed with 190 µL of the Bradford solution, respectively. After 5 min, the UV absorption of the protein-dye complex at λ = 595 nm was measured with a plate reader (Tecan Genios®, Tecan, Crailsheim, Germany).

General procedures for the binding assays

The test compound solutions were prepared by dissolving approximately 10 µmol (usually 2-4 mg) of test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with the respective assay buffer. The filtermats were presoaked in 0.5 % aqueous polyethylenimine solution for 2 h at rt before use. All binding experiments were carried out in duplicates in the 96 well multiplates. The concentrations given are the final concentration in the assay. Generally, the assays were performed by addition of 50 µL of the respective assay buffer, 50 µL of test compound solution in various concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} mol/L), 50 µL of the corresponding radioligand solution and 50 µL of the respective receptor preparation into each well of the multiplate (total volume 200 µL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500-600 rpm at the specified temperature. Unless otherwise noted, the assays were terminated after 120 min by rapid filtration using the harvester. During the filtration, each well was washed five times with 300 µL of water. Subsequently, the filtermats were dried at 95 °C. The solid scintillator was melted on the dried filtermats at a temperature of 95 °C for 5 min. After solidifying of the scintillator at rt, the trapped radioactivity in the filtermats was measured with the scintillation analyzer. Each position on the filtermat corresponding to one well of the multiplate was measured for 5 min with the [^{3}H]-counting protocol. The overall counting efficiency was 20 %. The IC_{50} values were calculated with the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. Subsequently, the IC_{50} values were transformed into K_i values using the equation of Cheng and Prusoff.[5] The K_i values are given as mean value ± SEM from three independent experiments.

Performance of the binding assays

σ_1 receptor assay

The assay was performed with the radioligand [^{3}H]-(+)-pentazocine (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of guinea pig brain cortex (about 100 µg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and TRIS buffer (50 mM, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled (+)-pentazocine. The K_d value of (+)-pentazocine is 2.9 nM.[6]

σ_2 receptor assay

The assays were performed with the radioligand [^{3}H]di-*o*-tolylguanidine (specific activity 50 Ci/mmol; ARC, St. Louis, MO, USA). The thawed rat liver membrane preparation (about 100 µg protein) was incubated with various concentrations of the test compound, 3 nM [^{3}H]di-*o*-tolylguanidine and buffer containing (+)-pentazocine (500 nM (+)-pentazocine in TRIS buffer (50 mM TRIS, pH 8.0)) at rt. The non-specific binding was determined with 10 µM non-labeled di-*o*-tolylguanidine. The K_d value of di-*o*-tolylguanidine is 17.9 nM.[7]

MOR receptor assay

The assay was performed with the radioligand [^{3}H]DAMGO (51 Ci/mmol, Perkin Elmer). The thawed guinea pig brain membrane preparation (about 100 µg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]DAMGO, and TRIS-$MgCl_2$-buffer (50 mM TRIS, 8 mM $MgCl_2$, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled naloxone. The K_d value of DAMGO is 0.57 nM.

KOR receptor assay

The assay was performed with the radioligand [^{3}H]U-69,593 (55 Ci/mmol, BIOTREND). The thawed guinea pig brain membrane preparation (about 100 µg of the protein) was incubated with various concentrations of test compounds, 1 nM [^{3}H]U-69,593, and TRIS-$MgCl_2$-buffer (50 mM TRIS, 8 mM $MgCl_2$, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled U-69,593. The K_d value of U-69,593 is 0.69 nM.

DOR receptor assay

The assay was performed with the radioligand [^{3}H]DPDPE (69 Ci/mmol, BIOTREND). The thawed rat membrane preparation (about 75 µg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]DPDPE, and TRIS-$MgCl_2$-buffer (50 mM TRIS, 8 mM $MgCl_2$, , pH 7.4) supplemented with SIGMAFAST® protease inhibitor mix (Sigma Aldrich Biochemicals, Hamburg, Germany; 1 tablet dissolved in 100 mL of buffer) at 37 °C. The non-specific binding was determined with 10 µM unlabeled morphine. The $K_d$value of DPDPE is 0.65 nM.

PCP binding site of the NMDA receptor

The assay was performed with the radioligand [^{3}H]-(+)-MK-801 (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of pig brain cortex (about 100 µg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-MK-801, and TRIS/EDTA buffer (5 mM TRIS/1 mM EDTA, pH 7.5) at rt. The non-specific binding was determined with 10 µM unlabeled (+)-MK-801. The K_d value of (+)-MK-801 is 2.26 nM.[8]

4. Inhibition of CYP enzymes[9]

CYP inhibition assay was carried out in a robotic liquid handling system (Packard Multiprobe II, Perkin-Elmer). All incubations were performed in duplicate and individually for each test compound. Compounds were incubated in 96-well plates at 37 ºC at final concentrations of 1 µM. The inhibition potential of test compounds was evaluated using fluorescent probe substrates and recombinant human cytochrome P450 isoenzymes (rhCYP1A2, 2C9, 2C19, 2D6 and 3A4). Selective known inhibitors were screened alongside the test compounds as positive controls for the assay. Method including composition of incubation media, time of incubation and substrates used per each isoform are described in detail elsewhere.[9] Fluorescence per well was measured using a fluorescence plate reader. Results were expressed as percentage of inhibition.

5. Cytotoxicity[10]

Assay: Compound-induced cytotoxicity was analyzed in human HepG2 cells using two different colorimetric methodologies: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and RN (Neutral Red Uptake). Briefly, 3 x 10^4 cells/well were seeded in 96 well plates containing culture media: MEM (GIBCO) supplemented with 10 % FBS (Sigma), 2 mM L-glutamine (Sigma), 1 % non-essential amino acids (Sigma), 1 % pyruvate (Sigma) and 100 U/µg/mL of penicillin/streptomycin (Sigma). Plates were incubated at 37 ºC and 5 % CO_2 for approximately 24 h. At this time, cells were treated with 1, 10 and 100 µM DMSO solutions of the test compounds during 20 h in serum-free culture media, using the same incubation conditions as mentioned above. Afterwards, compounds were washed out with PBS (Sigma). For MTT assay,

MTT solution (Sigma, 100 µL) at a final concentration of 0.5 mg/ml was added to each well and cells were incubated at 37 ºC and 5 % CO_2. After 4 h, MTT solution was removed and DMSO (100 µL) was added to dissolve formazan crystals. Light absorption was then measured at 550 nm (SpectraMax® 340PC384, Molecular Devices). For Red Neutral Uptake, RN solution (Sigma, 100 µL) at a final concentration of 50 µg/mL was added to the well and cells were incubated at 37 ºC and 5 % CO_2 for 3 h. Then, RN solution was removed by filtration, washing of each well with PBS and 100 µL of a solution of 50 % ethanol and 1 % of acetic acid in water was performed to extract the Neutral Red. Likewise, light absorption was then measured at 550 nm. The lowest compound concentration (1, 10 or 100 µM) producing more than 50 % of cytotoxicity, compared to non-treated cells, was taken as IC_{50} value. Each compound was analyzed in duplicates in two different assays (n = 4).

6. In vitro metabolic stability in human liver microsomes[11]

Assay: The assay was carried out in a robotic liquid handling system (Freedom Evo, Tecan). All incubations were performed individually and in duplicates for each test compound. Compounds (1 µM) were incubated in 96-well plates at 37 ºC during 1 h under standard incubation conditions: sodium-potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), the NADPH-regenerating system and CYP content (0.3 nmol/mL). At defined time points (0, 10, 20, 40 and 60 min) aliquots of the reaction mixture were stopped with an equal volume of cold acetonitrile. Upon centrifugation of the resulting mixture, supernatants were analyzed by a generic UPLC-MS/MS method. Metabolic stability was determined by the disappearance of compound over time. Ln-linear plots of the % of compound remaining based on chromatographic peak area versus time were plotted, and the slope was calculated by linear fitting of the curve. Results were expressed as percentage of test compound remaining at the end of the incubation period. Terfenadine was used as positive control in all assays.

7. Table S1

Experimental binding free energies (ΔG_{exp}), computational binding free energies (ΔG_{comp}), computational enthalpic contribution (ΔH_{comp}) and computational entropic contributions ($-T\Delta S_{comp}$) for the entire set of the 28 bioactive ligands in complex with σ_1 receptor.

Compound	ΔG_{exp} (kcal/mol)	ΔG_{comp} (kcal/mol)	ΔH_{comp} (kcal/mol)	$-T\Delta S_{comp}$ (kcal/mol)
4a	-9.45	-9.38	-21.35	11.97
4b	-9.89	-9.56	-21.54	11.98
5a	-9.98	-9.72	-21.76	12.04
5b	-10.42	-9.83	-21.79	11.96
7a	-7.83	-9.01	-20.78	11.77
7b	-8.79	-9.04	-20.89	11.85
8a	-9.31	-9.21	-21.28	12.07
8b	-9.68	-9.36	-21.31	11.95
10a	-9.17	-9.88	-21.81	11.93
10b	-9.88	-9.99	-21.96	11.97
11a	-10.25	-10.21	-22.27	12.06
11b	-10.54	-10.63	-22.74	12.11
14a	-9.17	-9.40	-21.03	11.63
14b	-9.14	-9.57	-21.15	11.58
15a	-9.69	-9.52	-21.08	11.56
15b	-9.67	-9.61	-21.13	11.52
19a	-9.31	-9.39	-20.90	11.51
19b	-9.21	-9.53	-21.01	11.48
20a	-9.69	-9.61	-21.05	11.44
20b	-10.25	-9.67	-21.07	11.40
21a	-7.19	-8.24	-19.38	11.14
21b	-7.92	-8.31	-19.47	11.16
22a	-8.69	-8.69	-19.68	10.99
22b	-9.03	-8.81	-19.93	11.12
23a	-7.62	-8.31	-19.52	11.21
23b	-8.60	-8.35	-19.58	11.23
24a	-7.63	-8.28	-19.12	10.84
24b	-8.21	-8.33	-19.21	10.88

*The ΔG_{exp} values were obtained from the corresponding $K_i\sigma_1$ values using the relationship $\Delta G_{exp} = -RT \ln(1/K_i\sigma_1)$. Errors on the calculated total binding free energy and its components within 5%.

8. References

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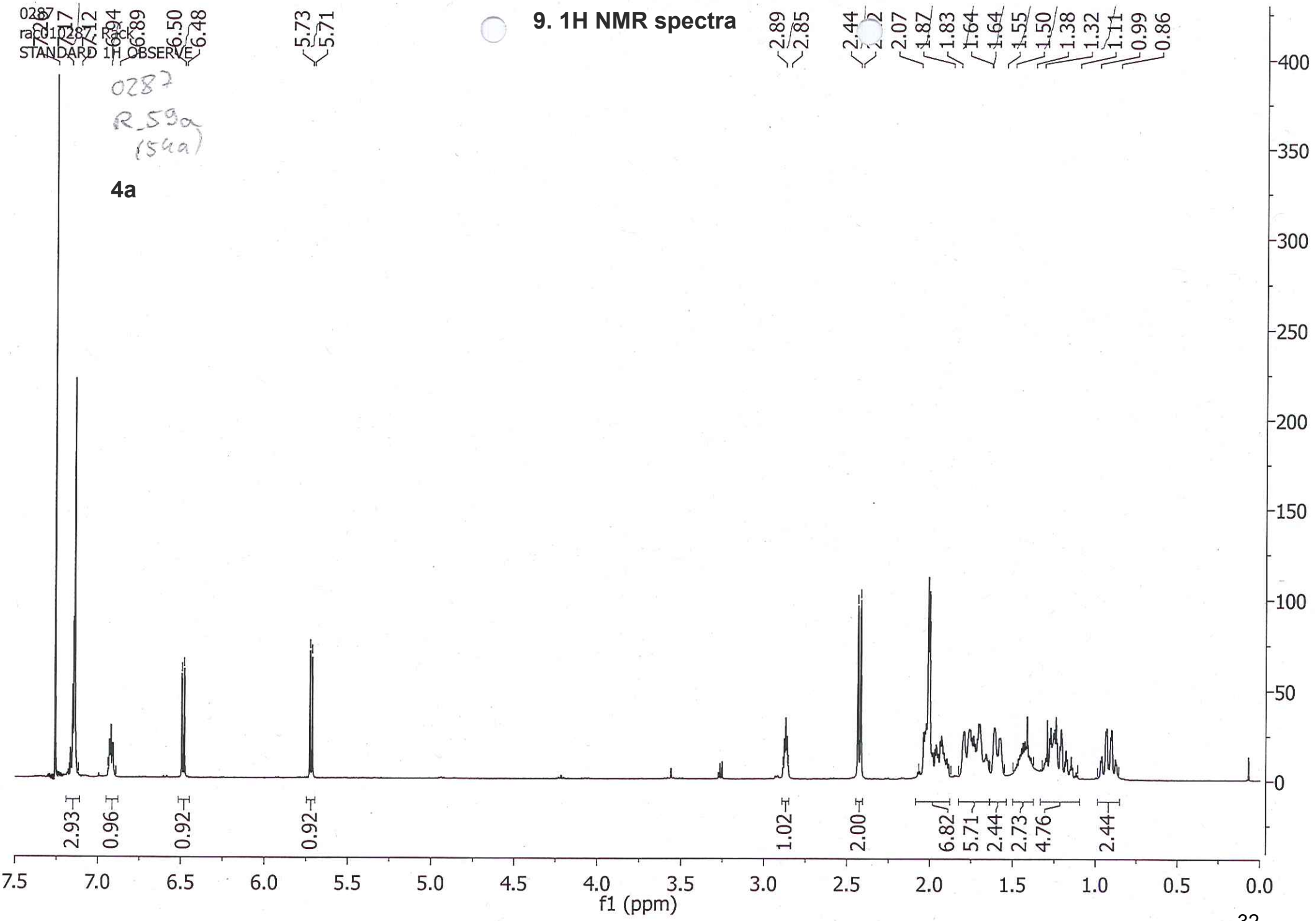
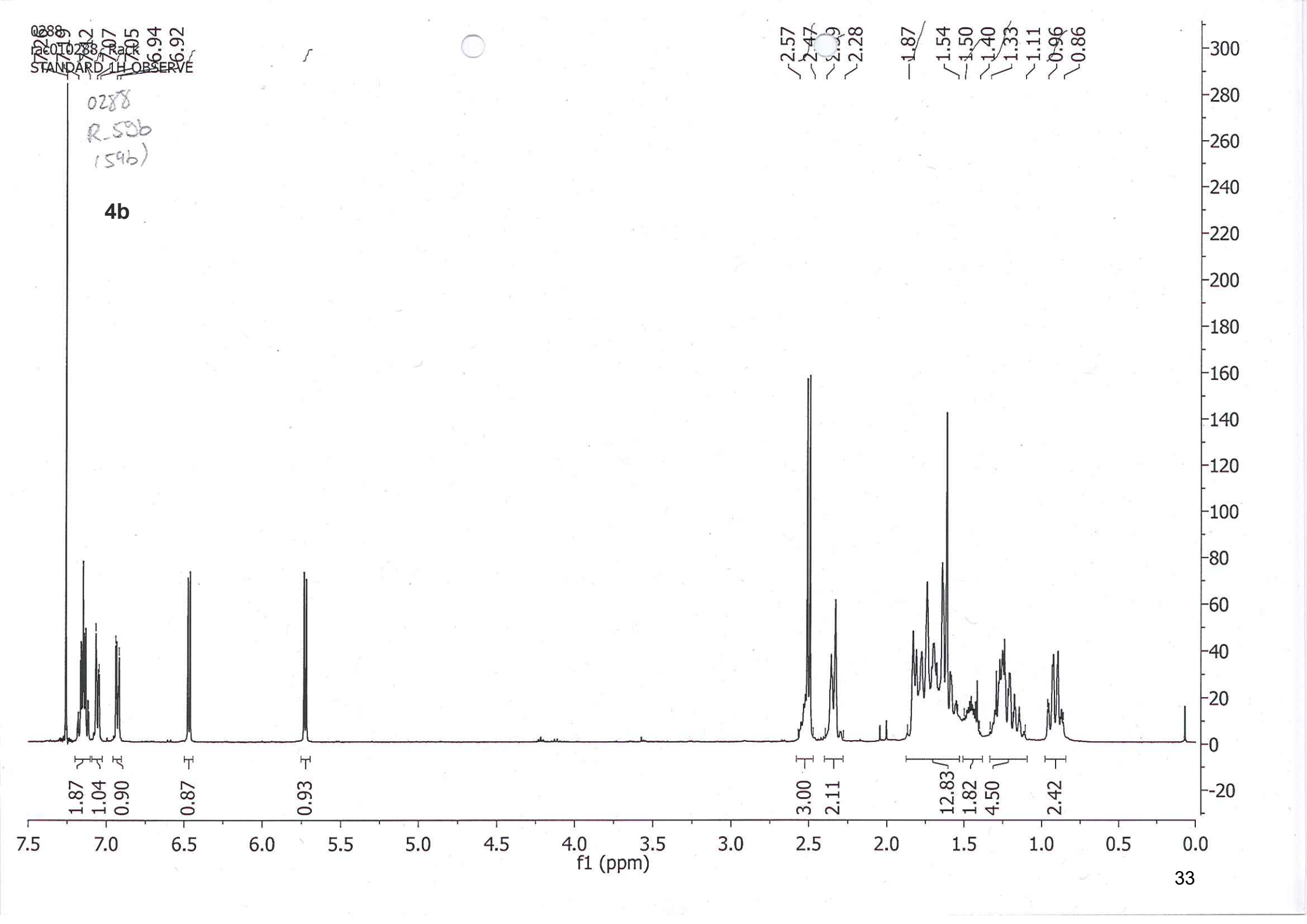
9. 1H NMR spectra

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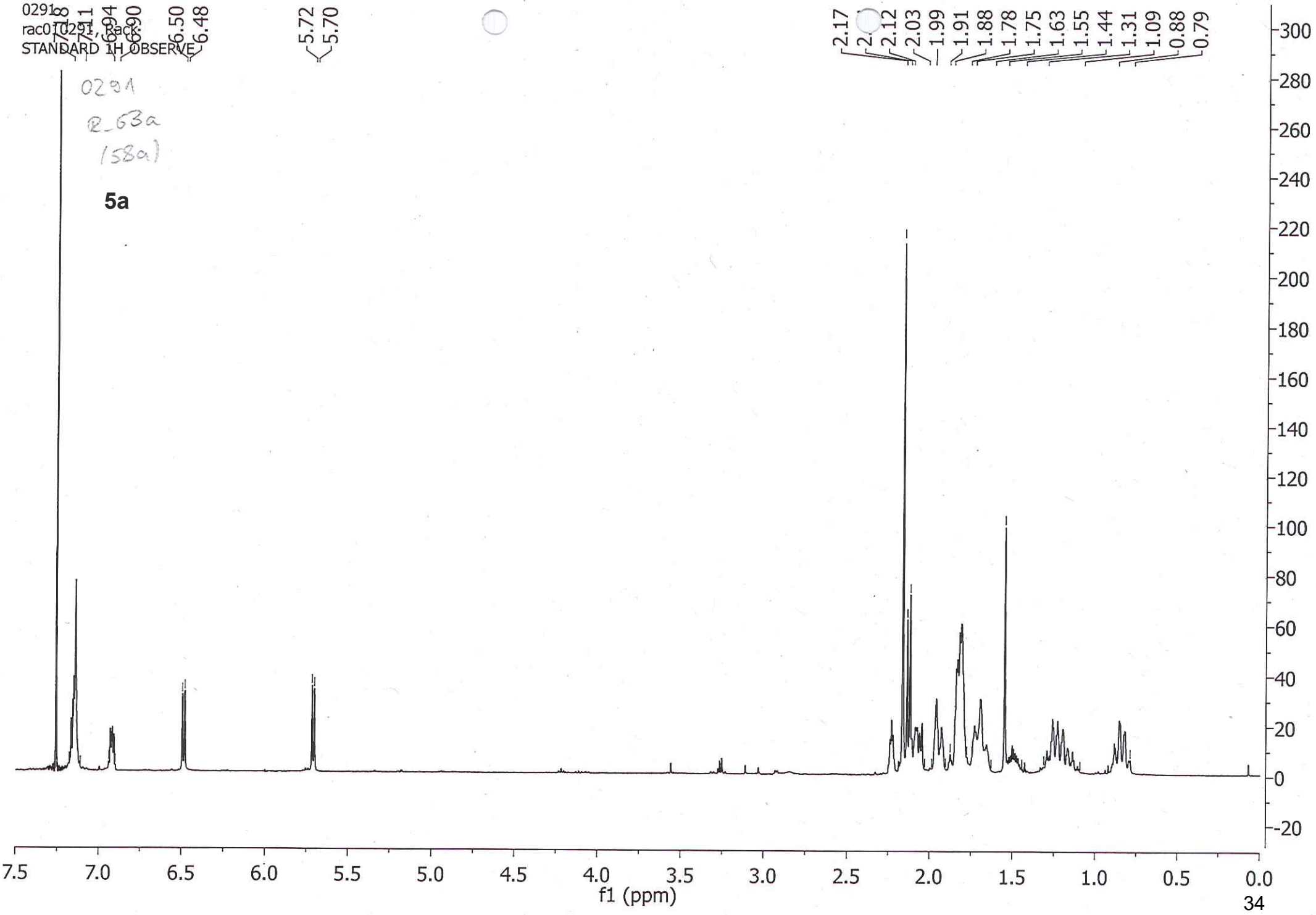
R_596
(596)
4b
7.29
7.22
7.07
7.05
6.94
6.92
2.57
2.47
2.39
2.28
1.87
1.54
1.50
1.40
1.33
1.11
0.96
0.86
1.87
1.04
0.90
0.87
0.93
3.00
2.11
12.83
1.82
4.50
2.42
f1 (ppm)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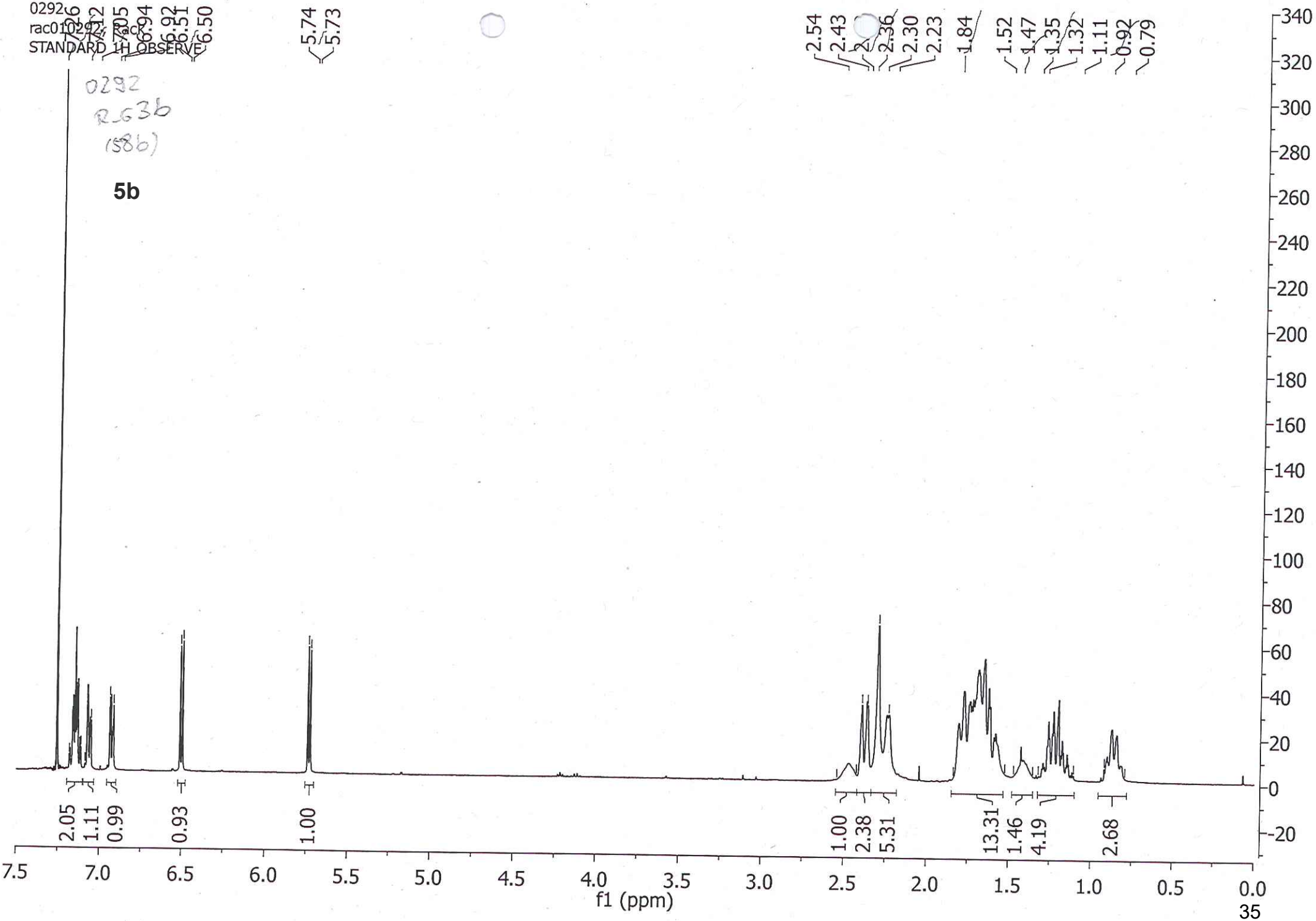
7.11
6.94
6.90
6.50
6.48
5.72
5.70
2.17
2.12
2.03
1.99
1.91
1.88
1.78
1.75
1.63
1.55
1.44
1.31
1.09
0.88
0.79
0291
R_63a
(58a)
5a
f1 (ppm)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rac010292
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RG36
(586)
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7.26
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7.05
6.94
6.92
6.51
6.50
5.74
5.73
2.54
2.43
2.36
2.30
2.23
1.84
1.52
1.47
1.35
1.32
1.11
0.92
0.79
2.05
1.11
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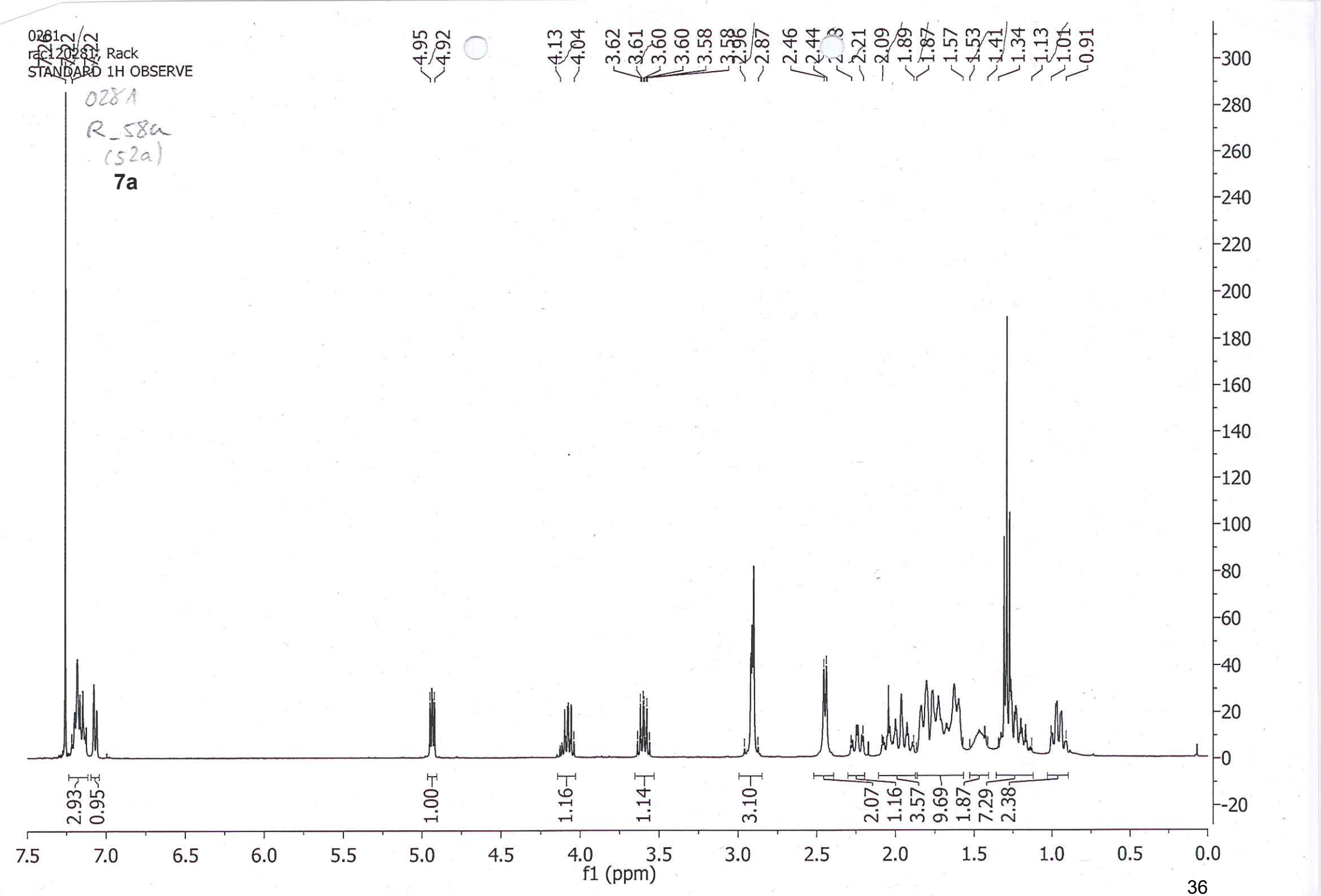
1.00
1.00
2.38
5.31
13.31
1.46
4.19
2.68
f1 (ppm)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Tac120281, Rack
STANDARD 1H OBSERVE
0281
R_58a
(52a)
7a
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4.92
4.13
4.04
3.62
3.61
3.60
3.60
3.58
3.58
2.98
2.87
2.46
2.44
2.21
2.09
1.89
1.87
1.57
1.53
1.41
1.34
1.13
1.01
0.91
2.93
0.95
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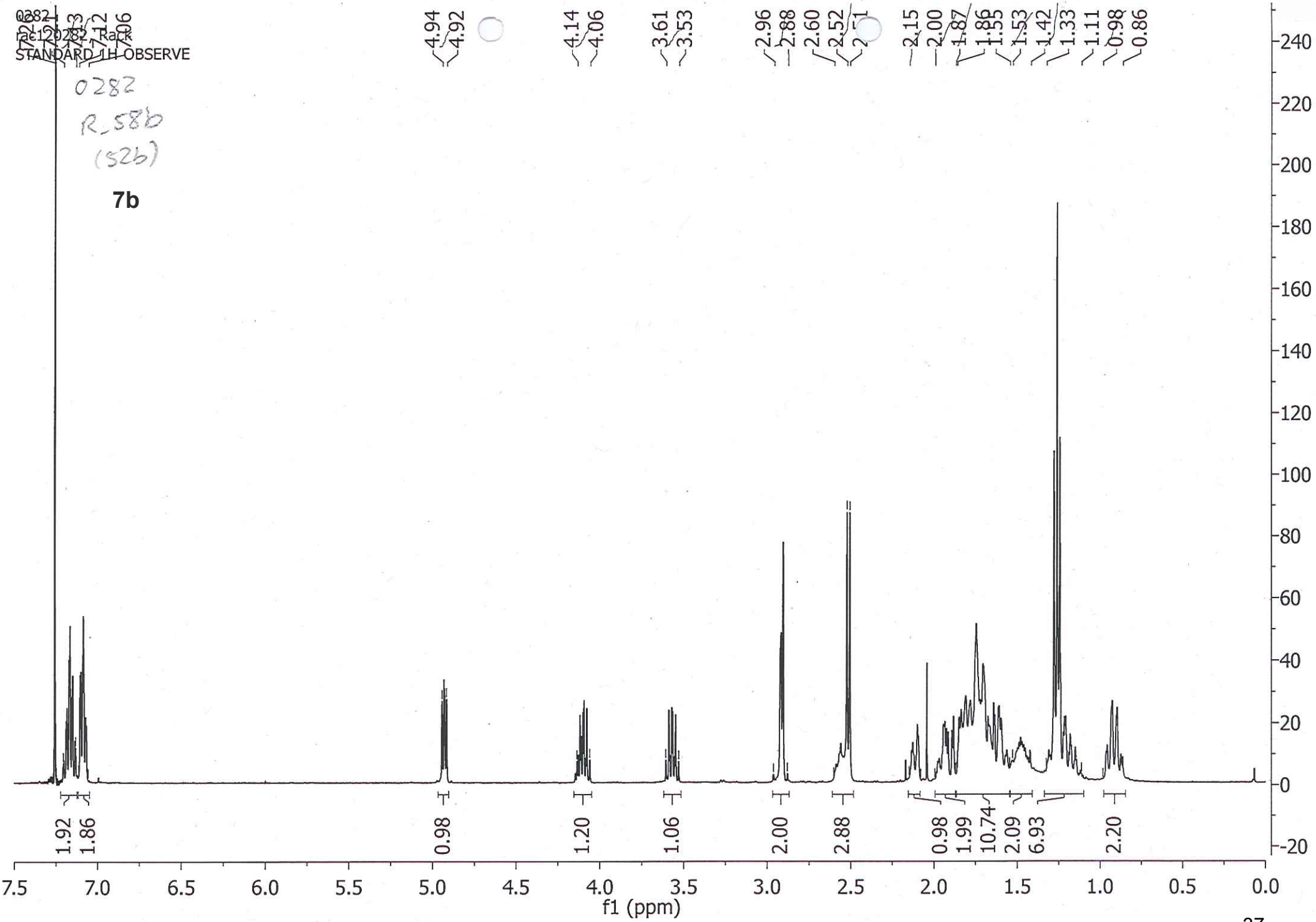
2.38
f1 (ppm)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0282
R_58b
(52b)
7b
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7.23
7.12
7.06
4.94
4.92
4.14
4.06
3.61
3.53
2.96
2.88
2.60
2.52
2.51
2.15
2.00
1.87
1.86
1.55
1.53
1.42
1.33
1.11
0.98
0.86
1.92
1.86
0.98
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1.99
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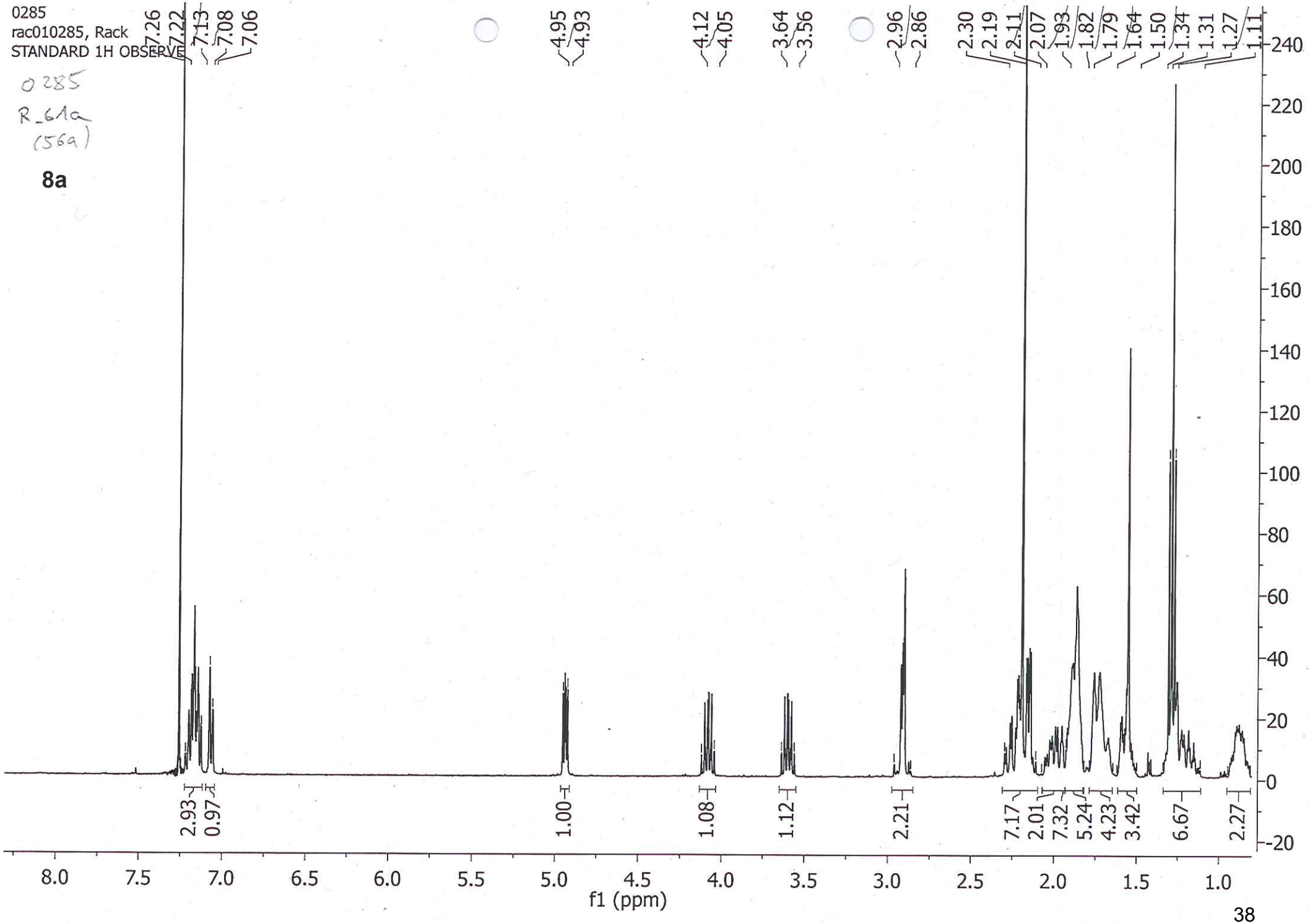
2.20
f1 (ppm)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R_61a
(56a)
8a
7.26
7.22
7.13
7.08
7.06
4.95
4.93
4.12
4.05
3.64
3.56
2.96
2.86
2.30
2.19
2.11
2.07
1.93
1.82
1.79
1.64
1.50
1.34
1.31
1.27
1.11
2.93
0.97
1.00
1.08
1.12
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7.17
2.01
7.32
5.24
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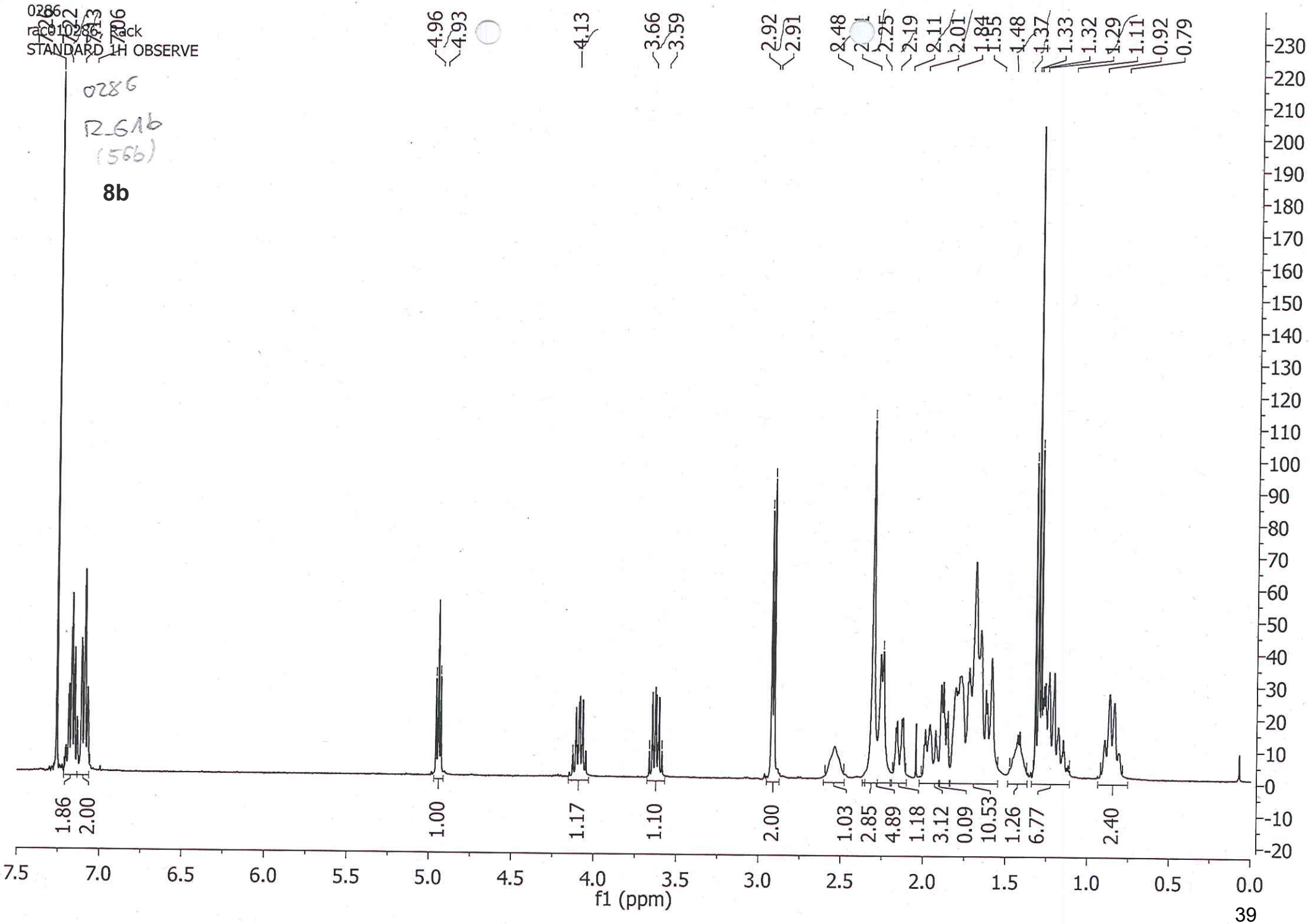
3.42
6.67
2.27
f1 (ppm)


0286
rac010286, rack
STANDARD 1H OBSERVE
0286
R_616
(566)
8b
7.22
7.13
7.06
4.96
4.93
4.13
3.66
3.59
2.92
2.91
2.48
2.25
2.19
2.11
2.01
1.84
1.55
1.48
1.37
1.33
1.32
1.29
1.11
0.92
0.79
1.86
2.00
1.00
1.17
1.10
2.00
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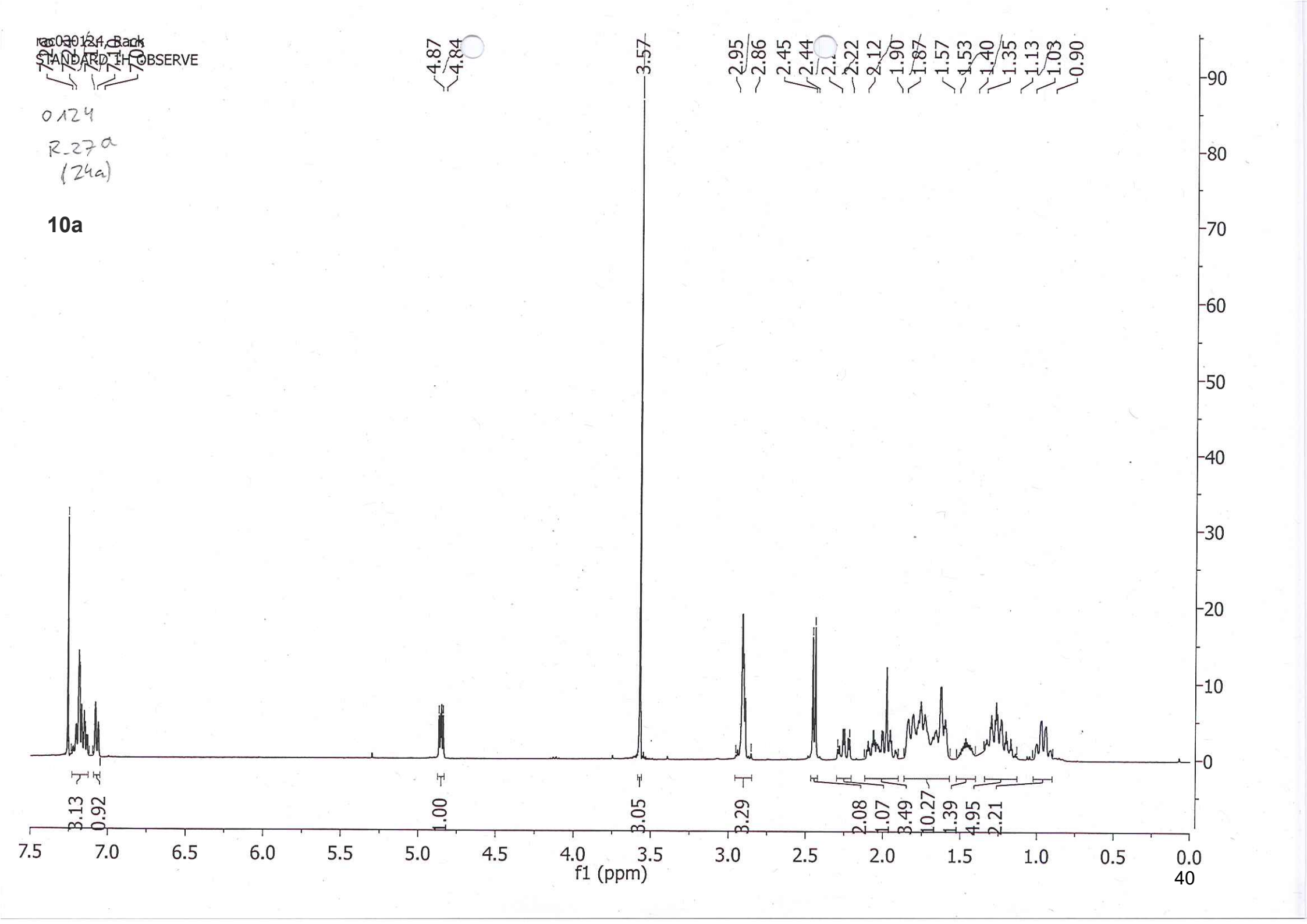
f1 (ppm)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STANDARD 1H OBSERVE
0124
R_27a
(24a)
10a
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4.84
3.57
2.95
2.86
2.45
2.44
2.22
2.12
1.90
1.87
1.57
1.53
1.40
1.35
1.13
1.03
0.90
3.13
0.92
1.00
3.05
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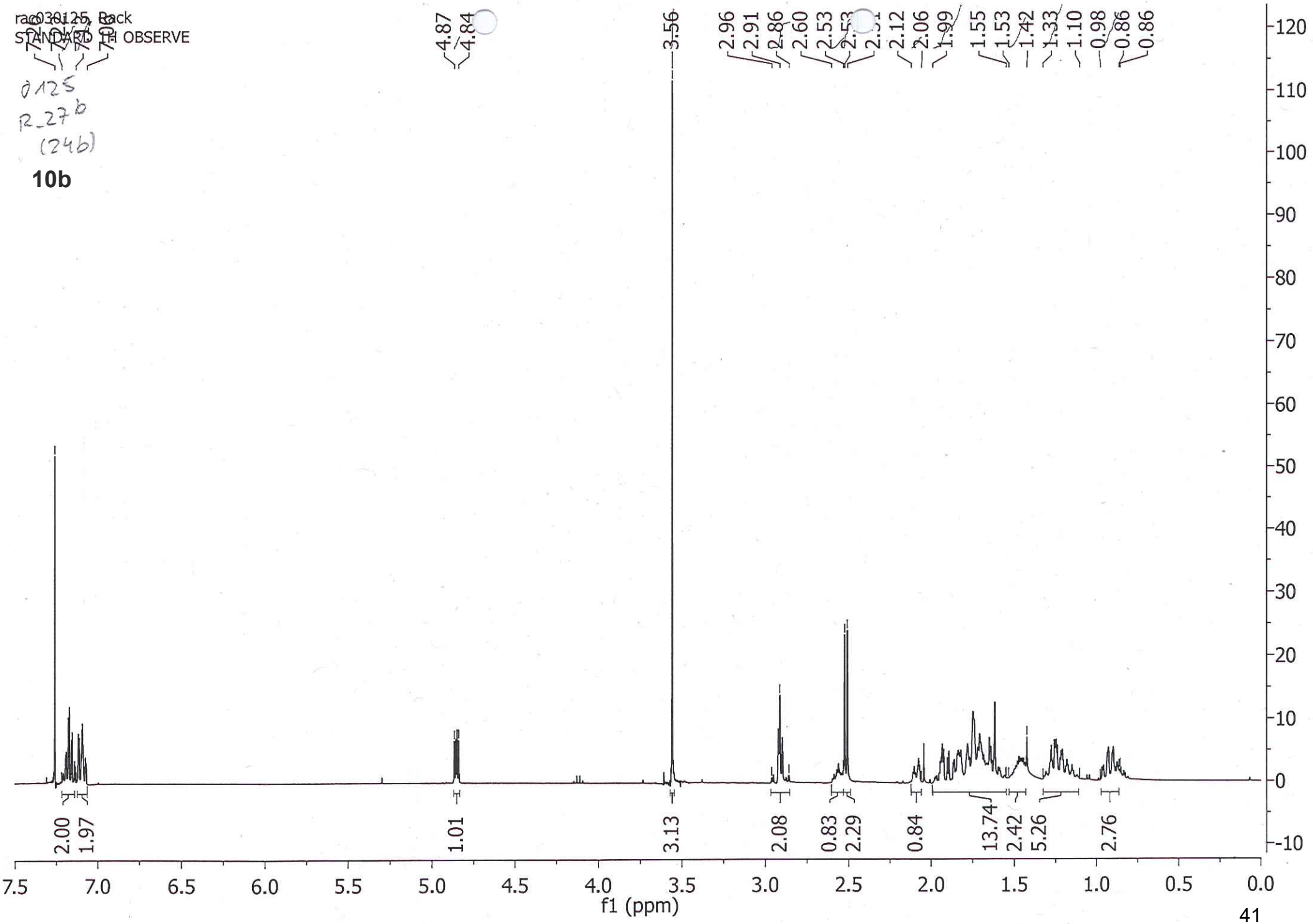
2.08
1.07
3.49
10.27
1.39
4.95
2.21
f1 (ppm)
40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0125
R_27b
(24b)
10b
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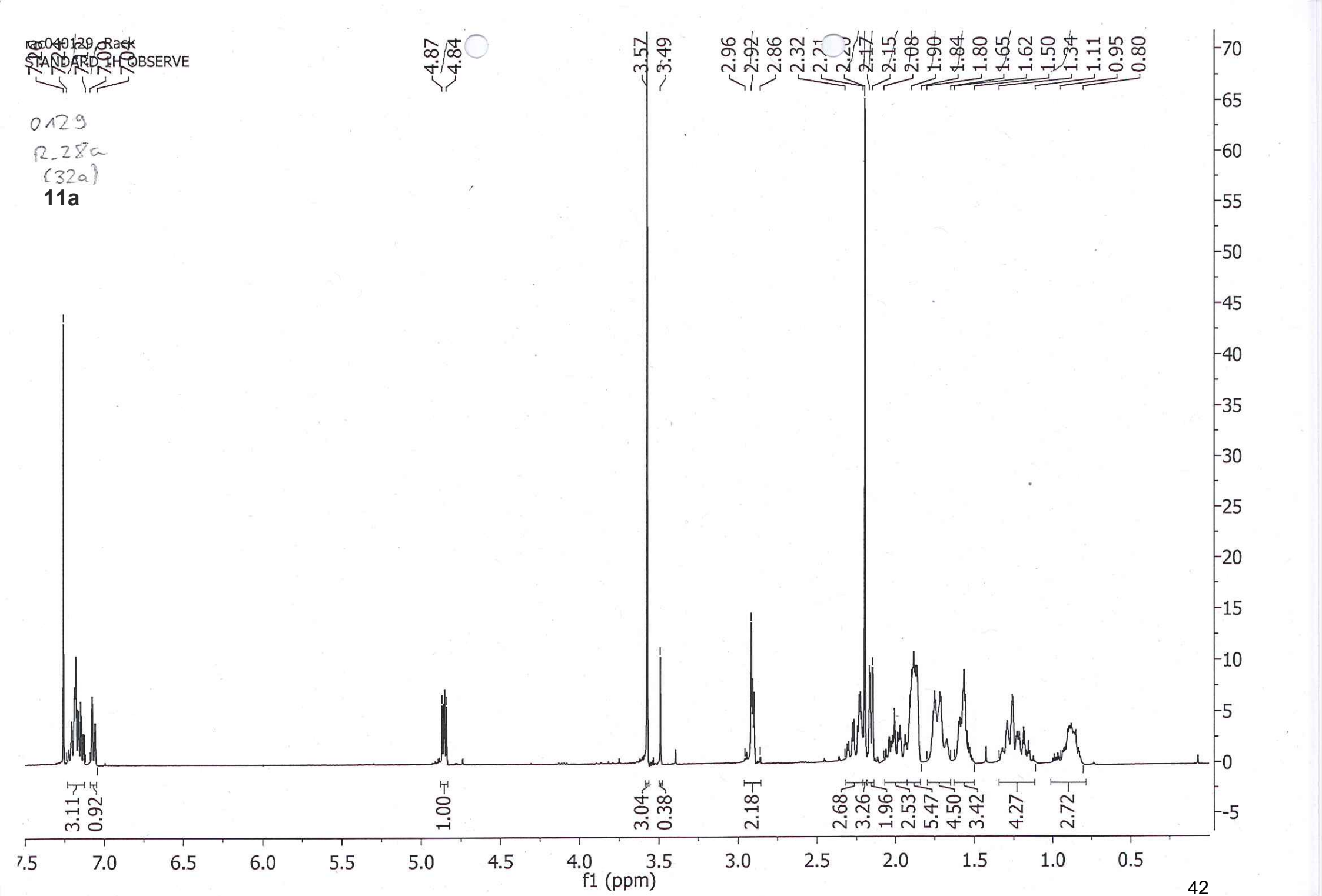
4.84
3.56
2.96
2.91
2.86
2.60
2.53
2.12
2.06
1.99
1.55
1.53
1.42
1.33
1.10
0.98
0.86
0.86
2.00
1.97
1.01
3.13
2.08
0.83
2.29
0.84
13.74
2.42
5.26
2.76
f1 (ppm)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R_28a
(32a)
11a
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4.84
3.57
3.49
2.96
2.92
2.86
2.32
2.17
2.15
2.08
1.90
1.84
1.80
1.65
1.62
1.50
1.34
1.11
0.95
0.80
3.11
0.92
1.00
3.04
0.38
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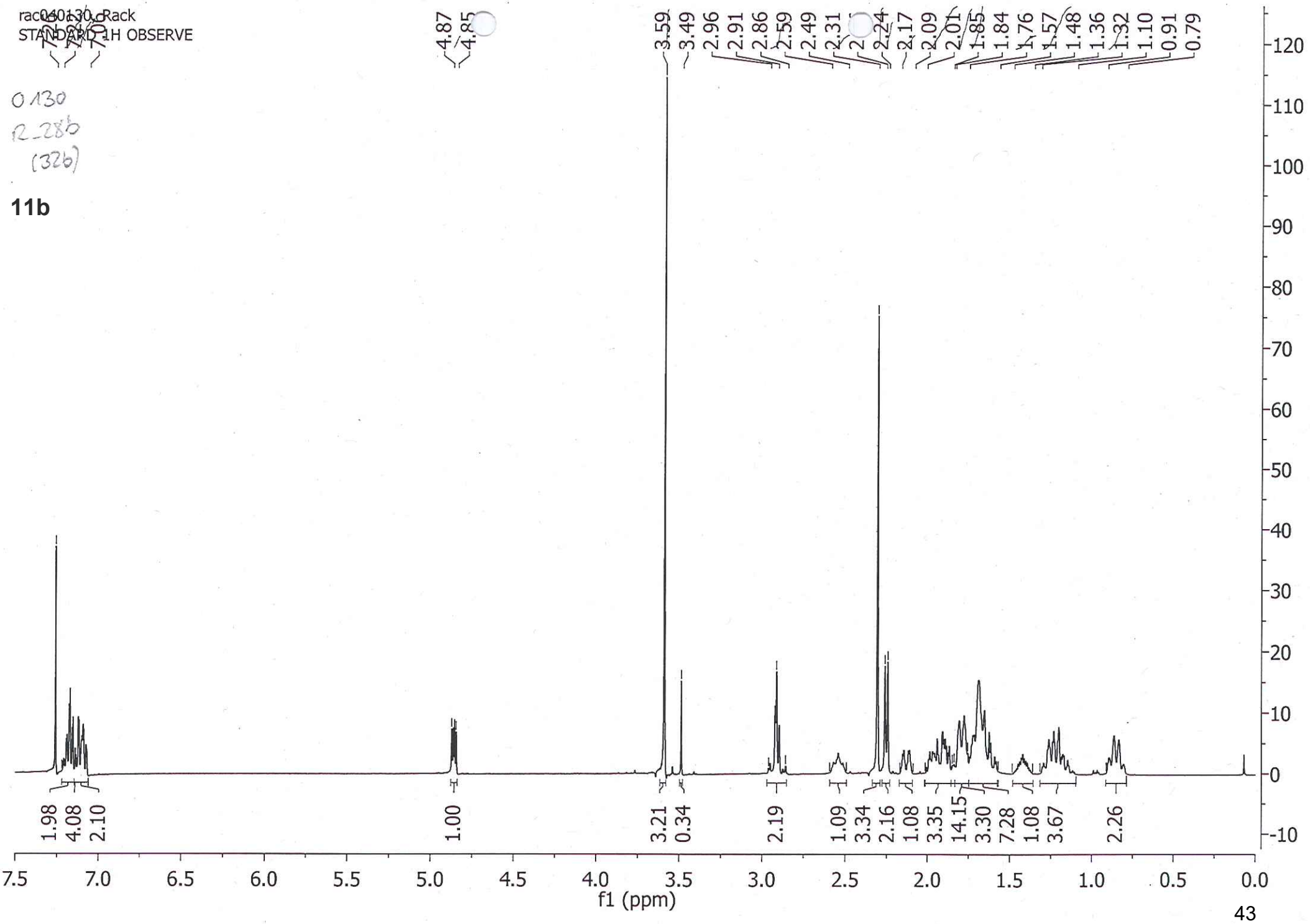
5.47
4.50
3.42
4.27
2.72
f1 (ppm)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(32b)
11b
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7.24
7.06
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4.85
3.59
3.49
2.96
2.91
2.86
2.59
2.49
2.31
2.24
2.17
2.09
2.01
1.85
1.84
1.76
1.57
1.48
1.36
1.32
1.10
0.91
0.79
1.98
4.08
2.10
1.00
3.21
0.34
2.19
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3.34
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1.08
3.35
14.15
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1.08
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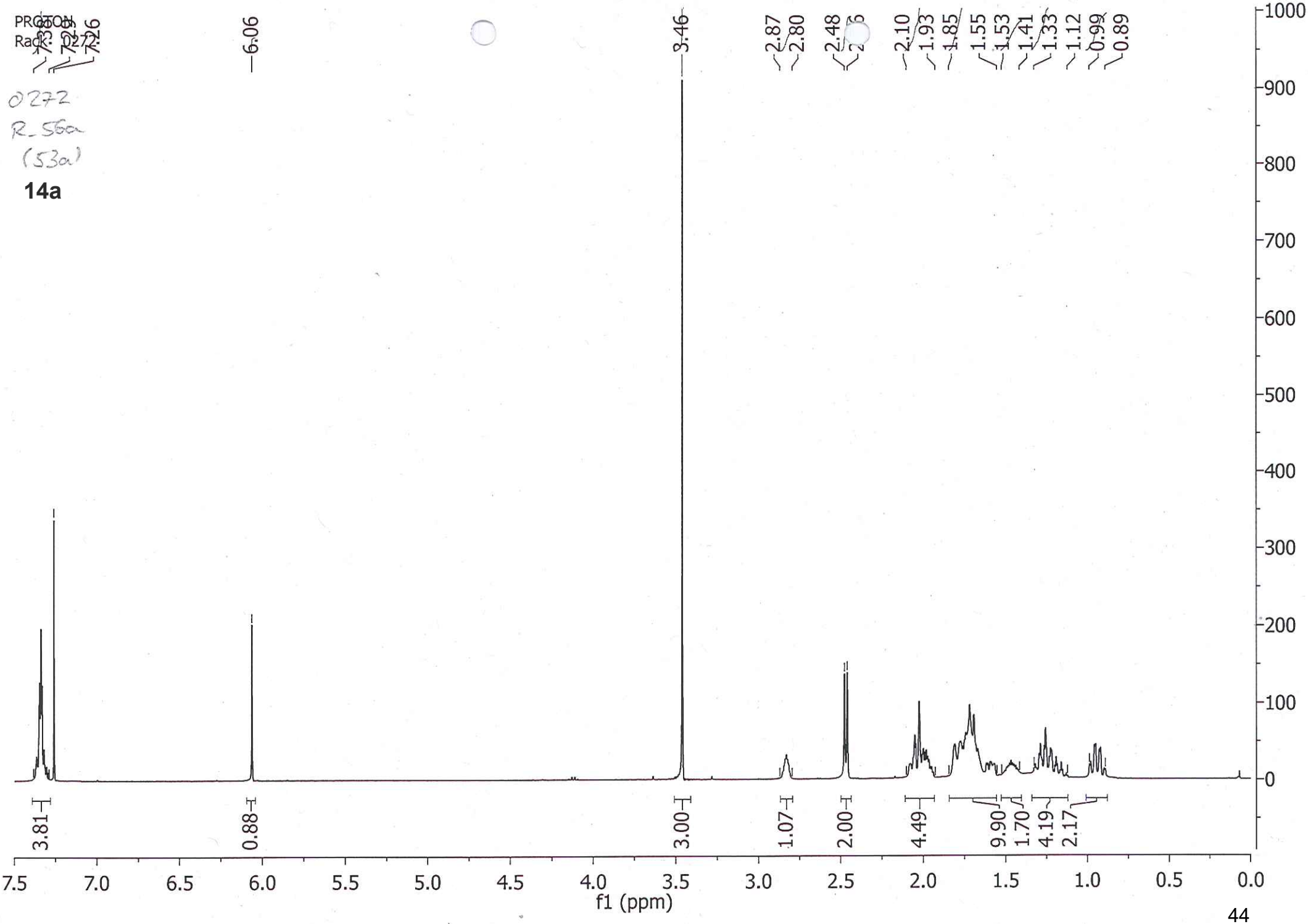
2.26
f1 (ppm)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0272
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7.29
7.27
7.26
6.06
3.46
2.87
2.80
2.48
2.10
1.93
1.85
1.55
1.53
1.41
1.33
1.12
0.99
0.89
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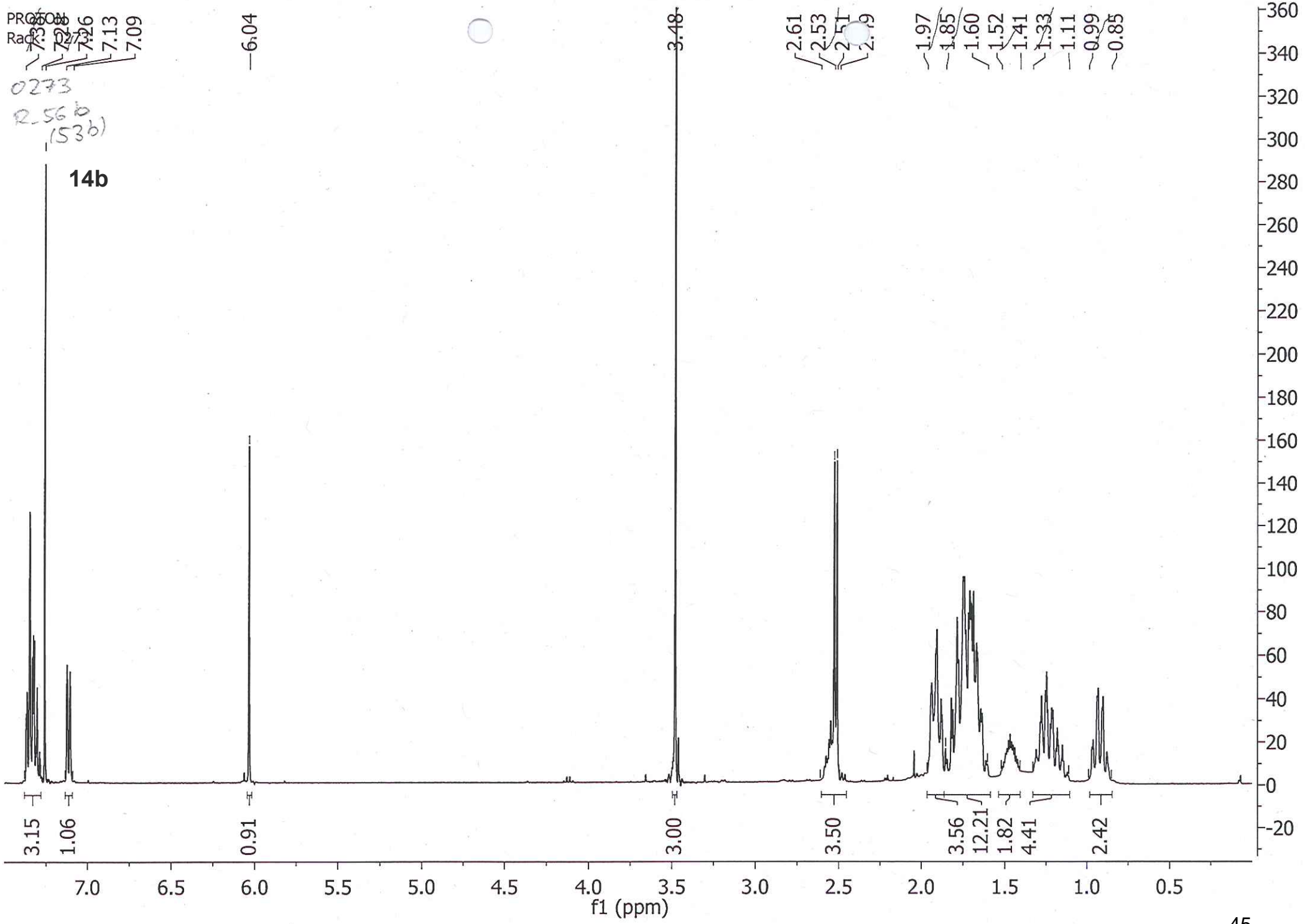
1.07
2.00
4.49
9.90
1.70
4.19
2.17
f1 (ppm)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R_56 b
(536)
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7.28
7.26
7.13
7.09
6.04
3.48
2.61
2.53
2.51
1.97
1.85
1.60
1.52
1.41
1.33
1.11
0.99
0.85
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0.91
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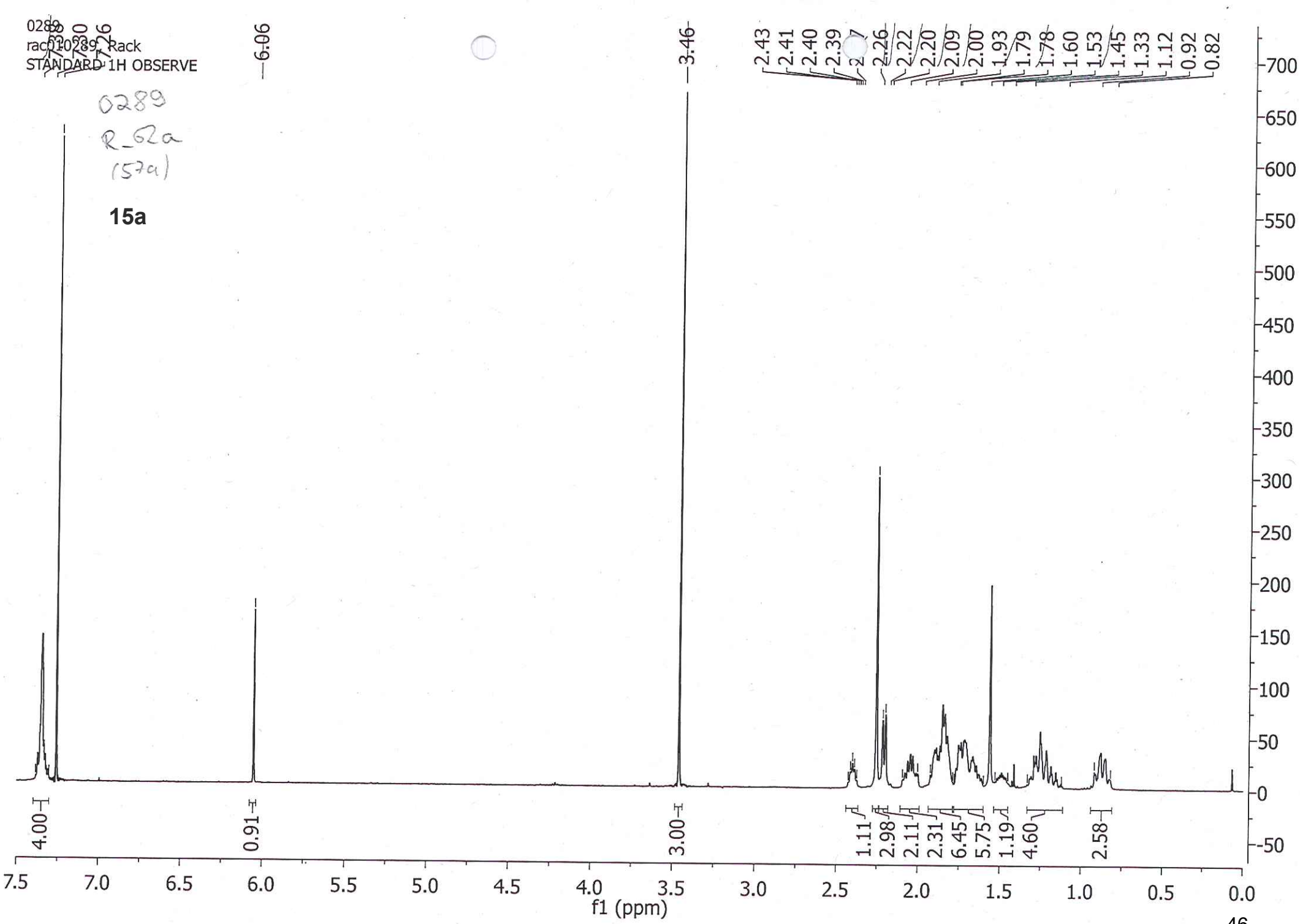
4.41
2.42
f1 (ppm)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7.20
6.06
3.46
2.43
2.41
2.40
2.39
2.26
2.22
2.20
2.09
2.00
1.93
1.79
1.78
1.60
1.53
1.45
1.33
1.12
0.92
0.82
4.00
0.91
3.00
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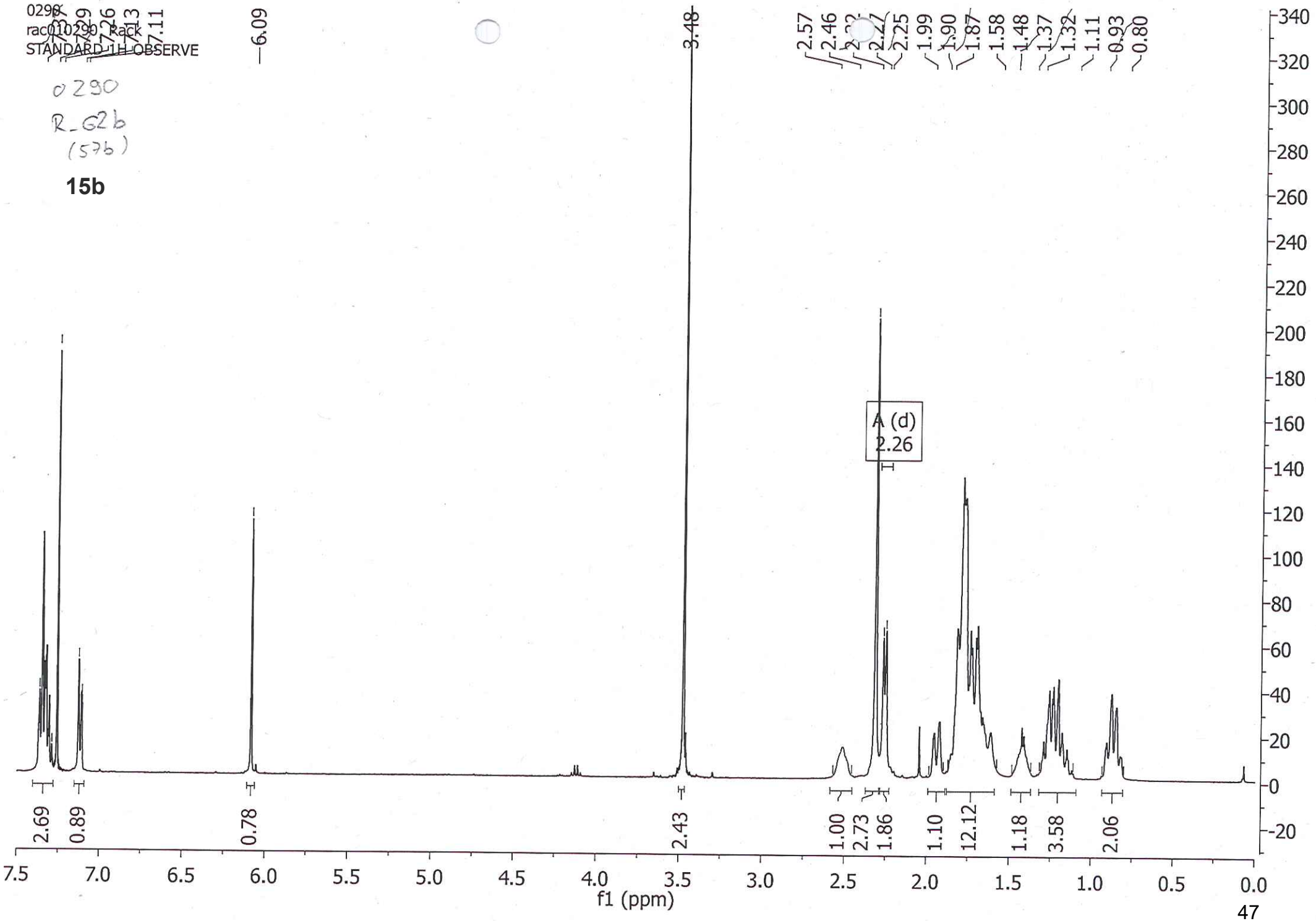
f1 (ppm)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2.27
2.25
1.99
1.90
1.87
1.58
1.48
1.37
1.32
1.11
0.93
0.80
A (d)
2.26
2.69
0.89
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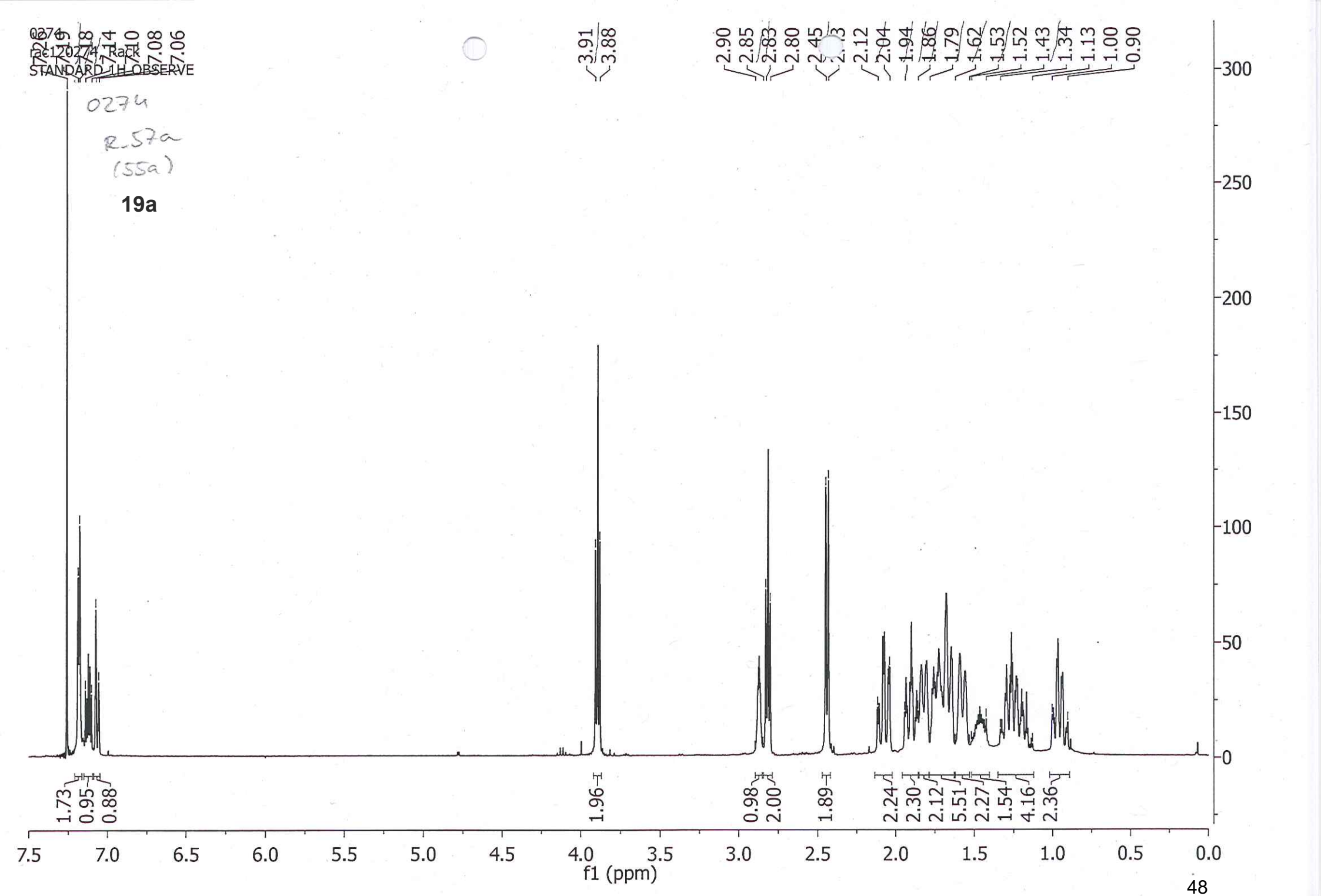
1.18
3.58
2.06
f1 (ppm)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Tac120274 Rack
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R.57a
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7.14
7.10
7.08
7.06
3.91
3.88
2.90
2.85
2.83
2.80
2.45
2.13
2.12
2.04
1.94
1.86
1.79
1.62
1.53
1.52
1.43
1.34
1.13
1.00
0.90
1.73
0.95
0.88
1.96
0.98
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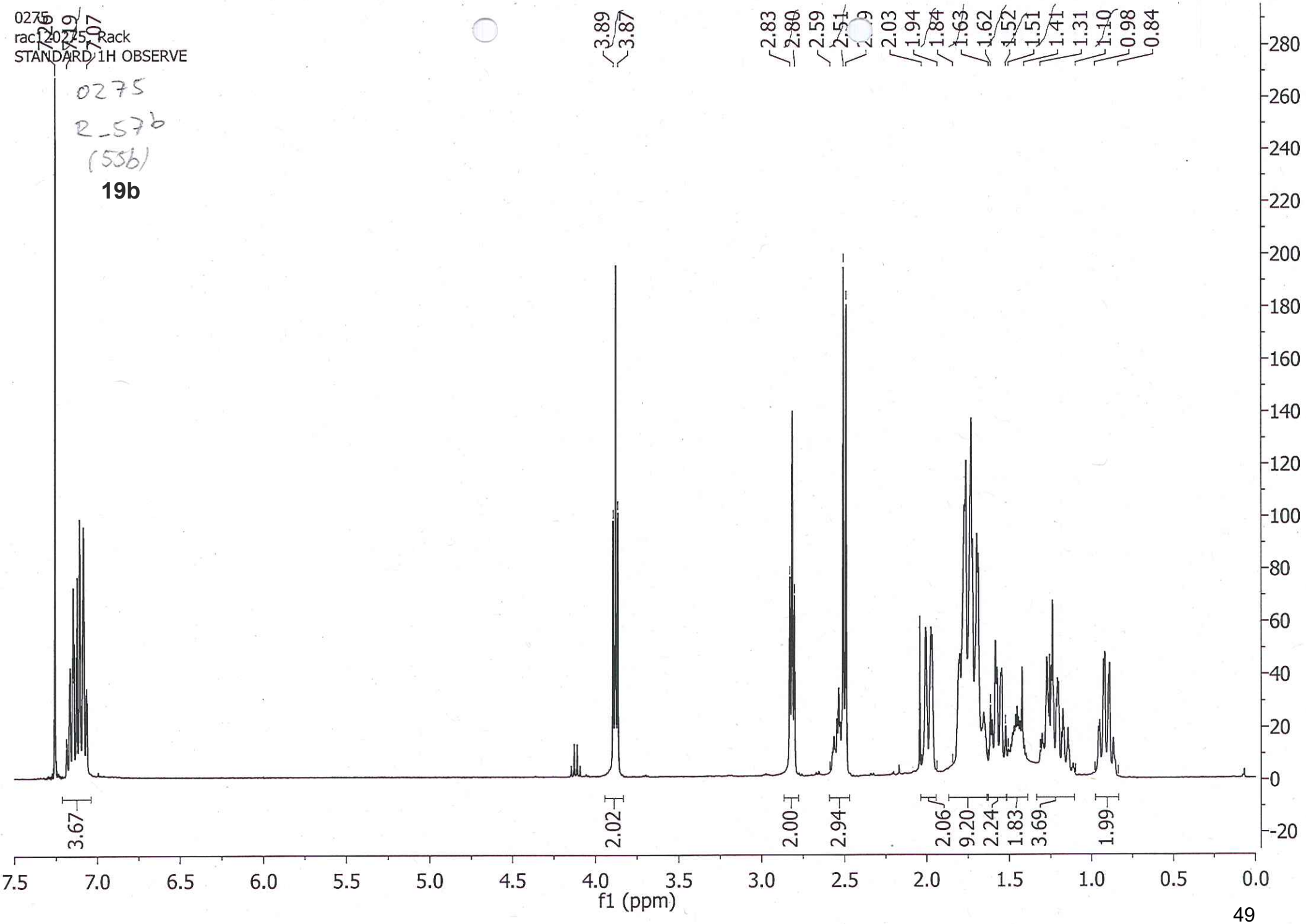
f1 (ppm)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3.87
2.83
2.80
2.59
2.51
2.03
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1.63
1.62
1.52
1.51
1.41
1.31
1.10
0.98
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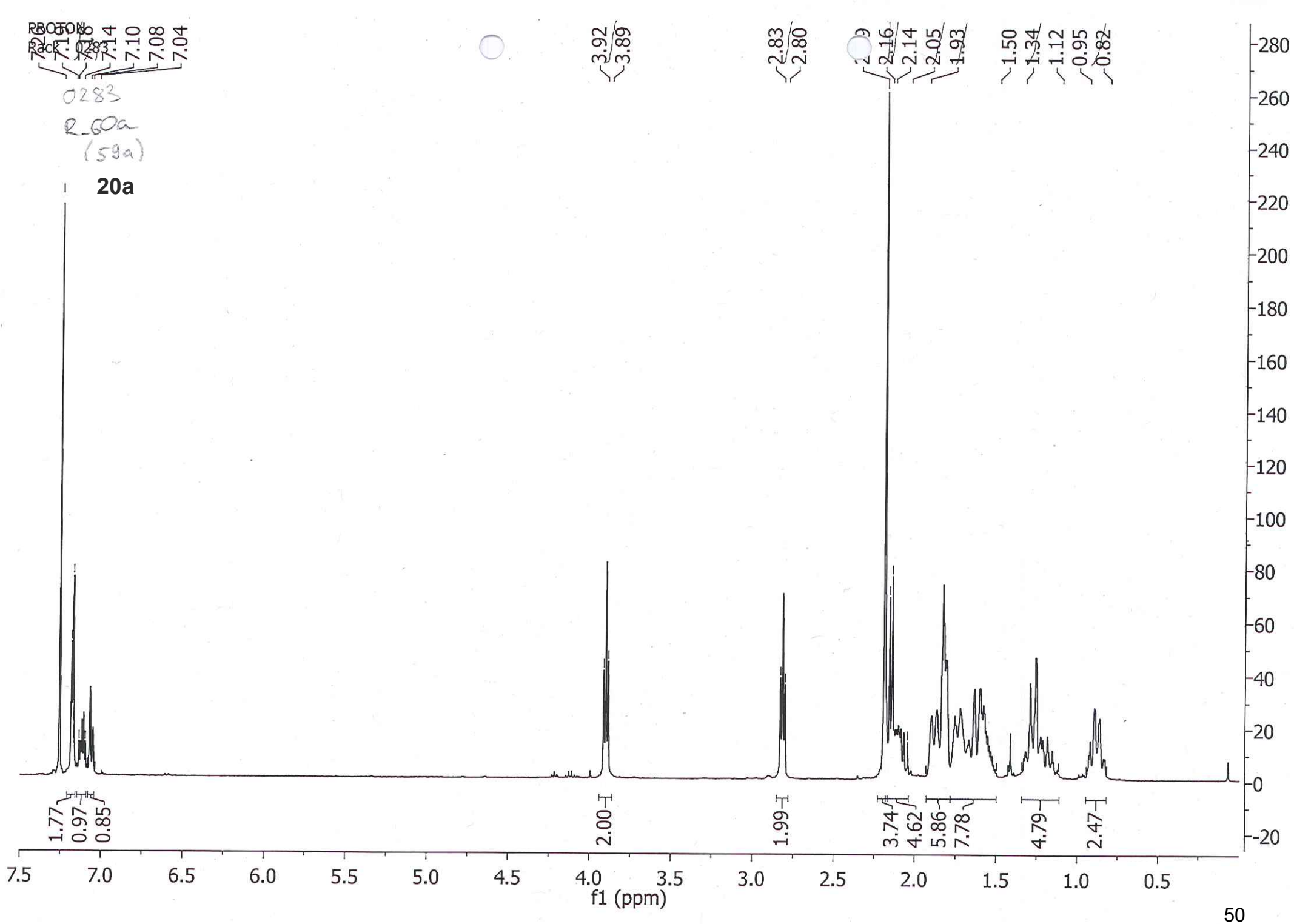
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7.04
3.92
3.89
2.83
2.80
2.16
2.14
2.05
1.93
1.50
1.34
1.12
0.95
0.82
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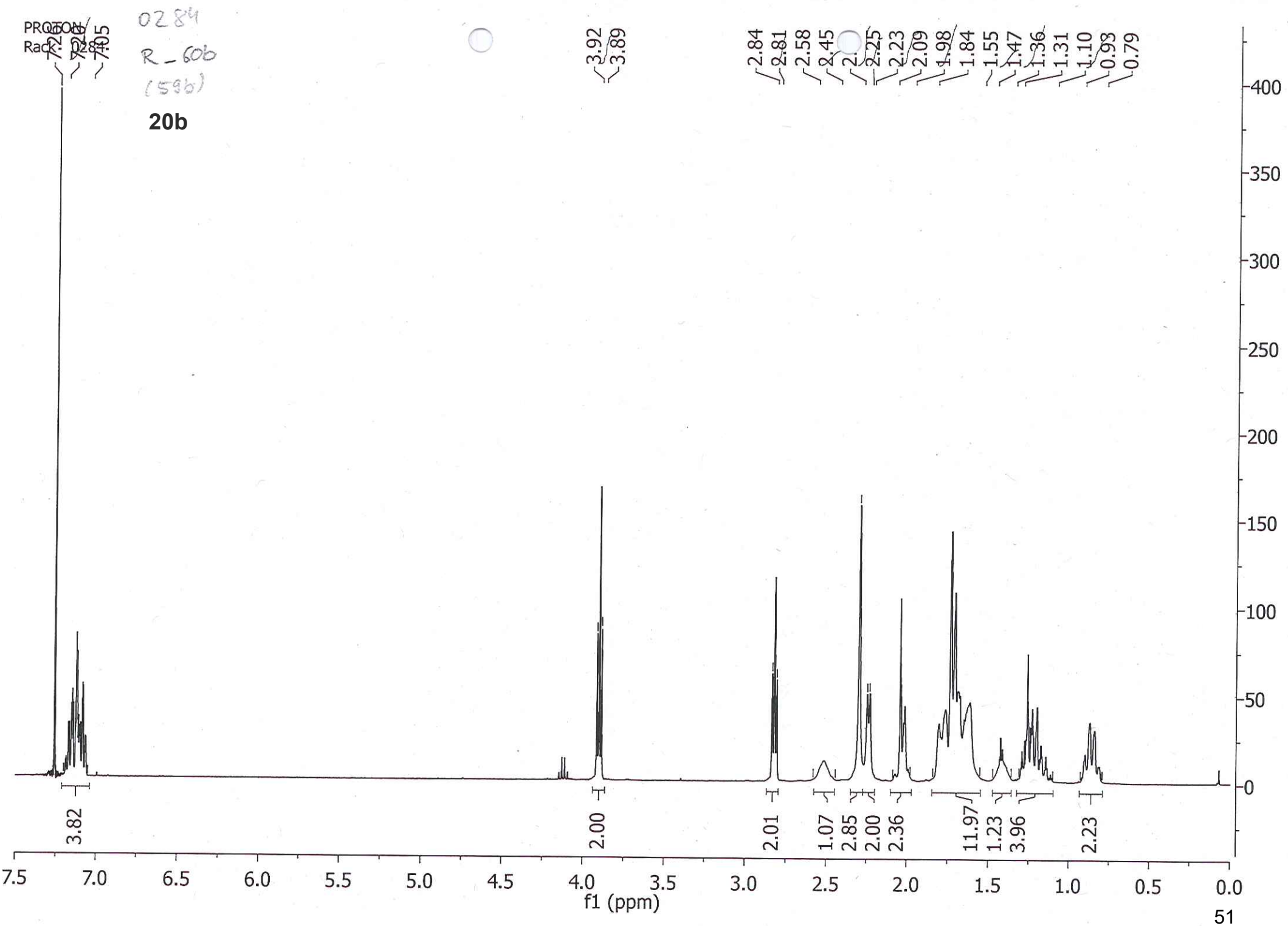
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3.89
2.84
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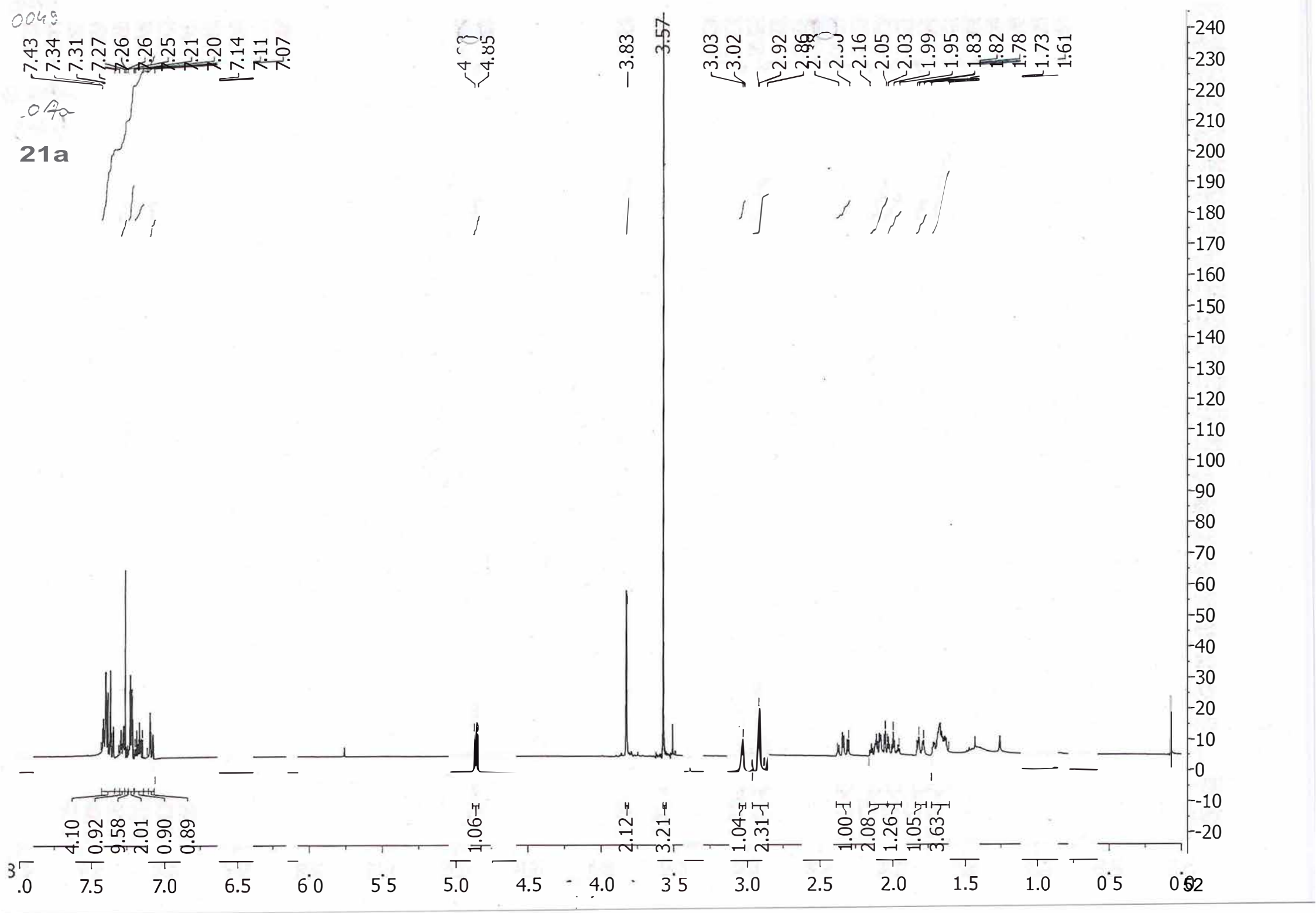
2.58
2.45
2.25
2.23
2.09
1.98
1.84
1.55
1.47
1.36
1.31
1.10
0.93
0.79
3.82
2.00
2.01
1.07
2.85
2.00
2.36
11.97
1.23
3.96
2.23
f1 (ppm)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7.14
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3.83
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2.92
2.16
2.05
2.03
1.99
1.95
1.83
1.82
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9.58
2.01
0.90
0.89
1.06
2.12
3.21
1.04
2.31
1.00
2.08
1.26
1.05
3.63
7.5
7.0
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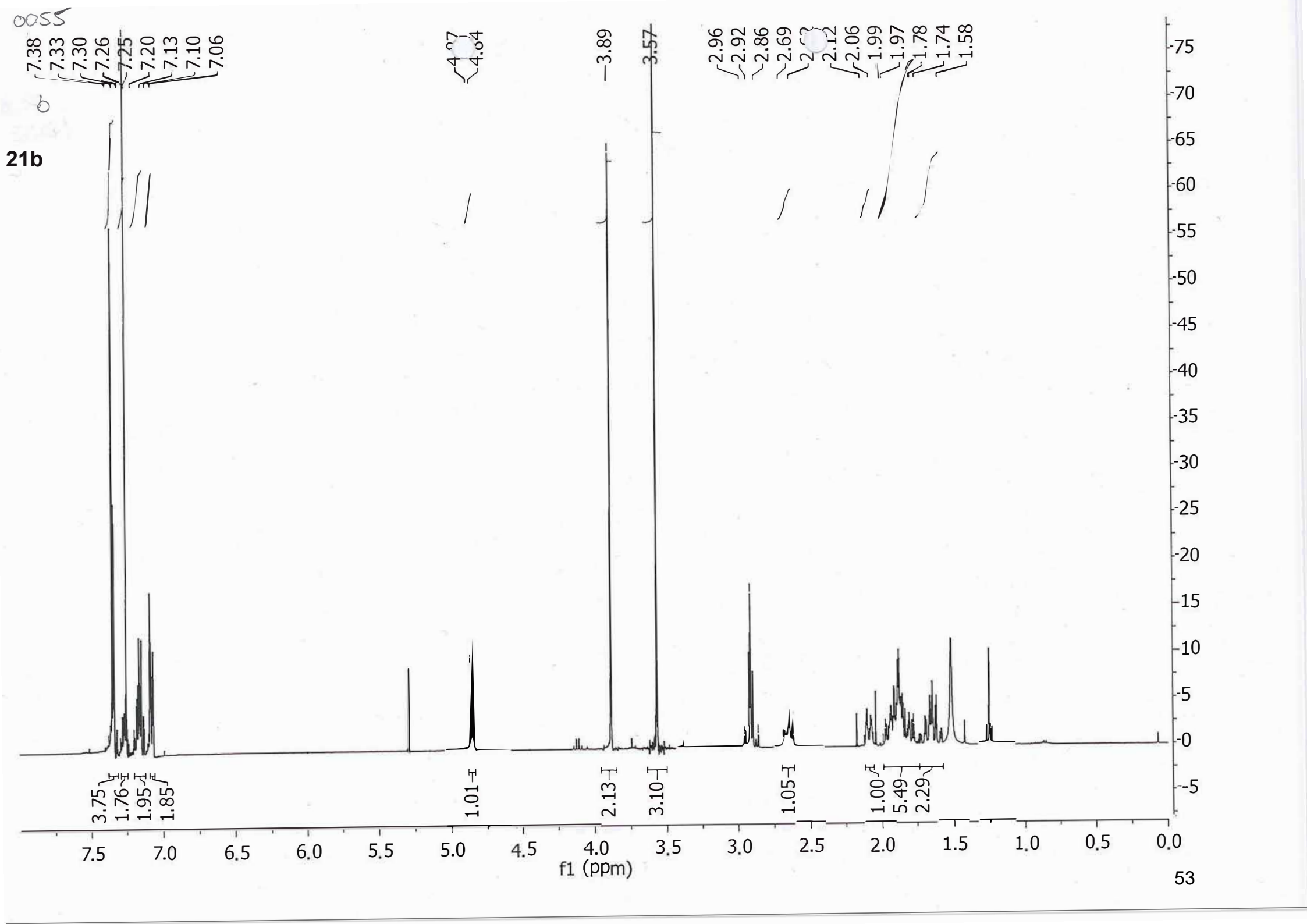
0055
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7.38
7.33
7.30
7.26
7.25
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7.13
7.10
7.06
4.87
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3.89
3.57
2.96
2.92
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2.69
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2.06
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1.74
1.58
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1.76
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1.01
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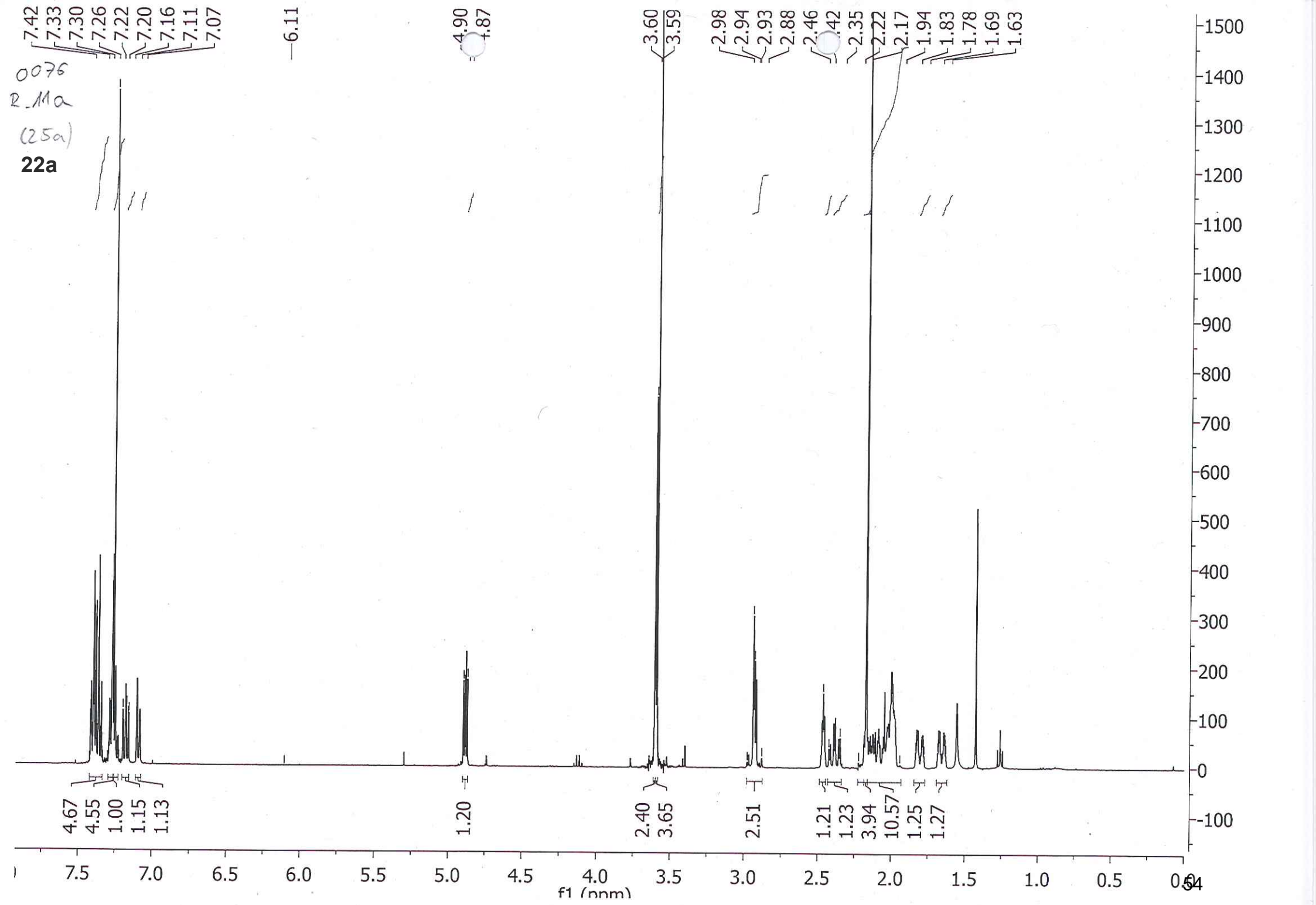
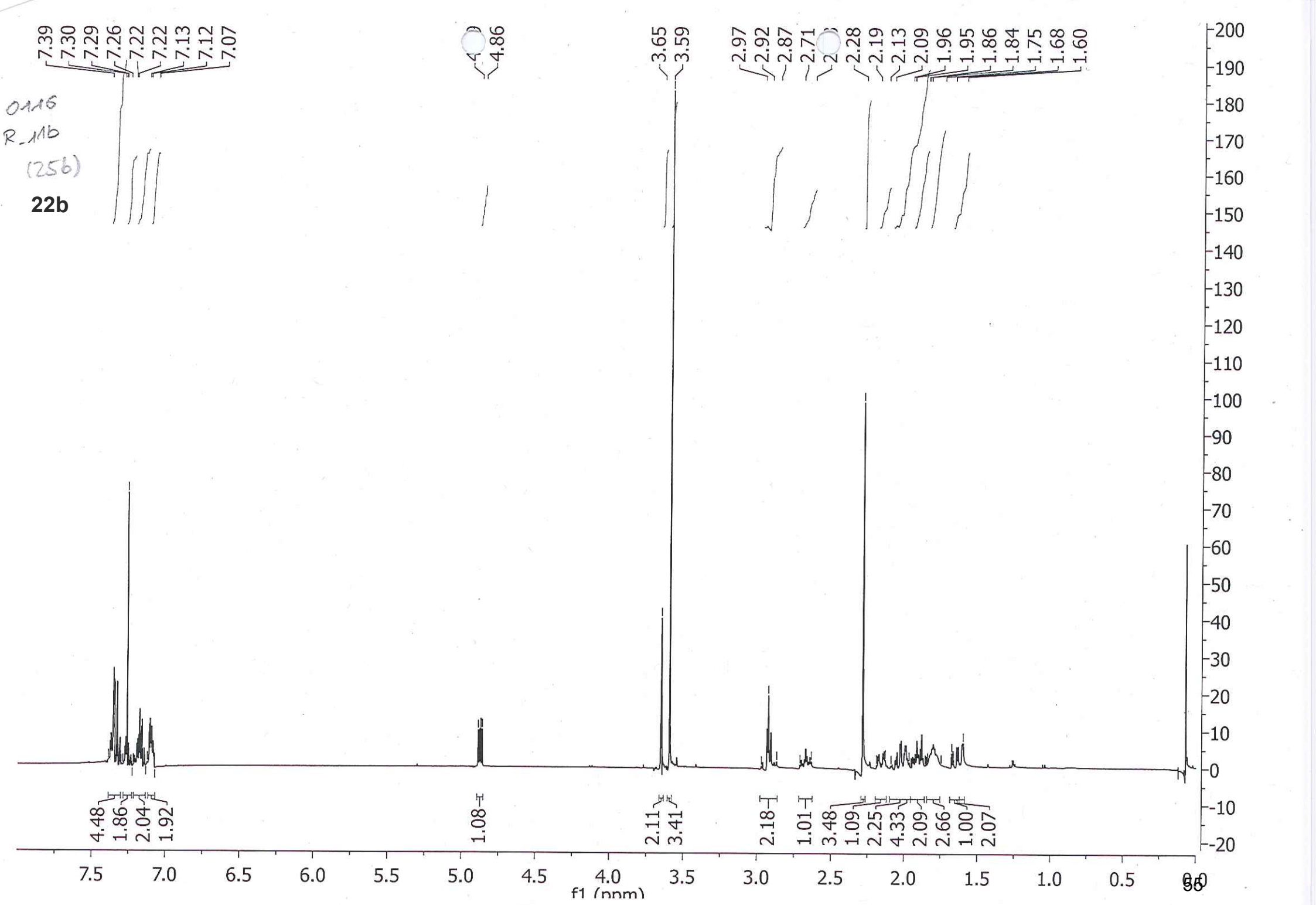
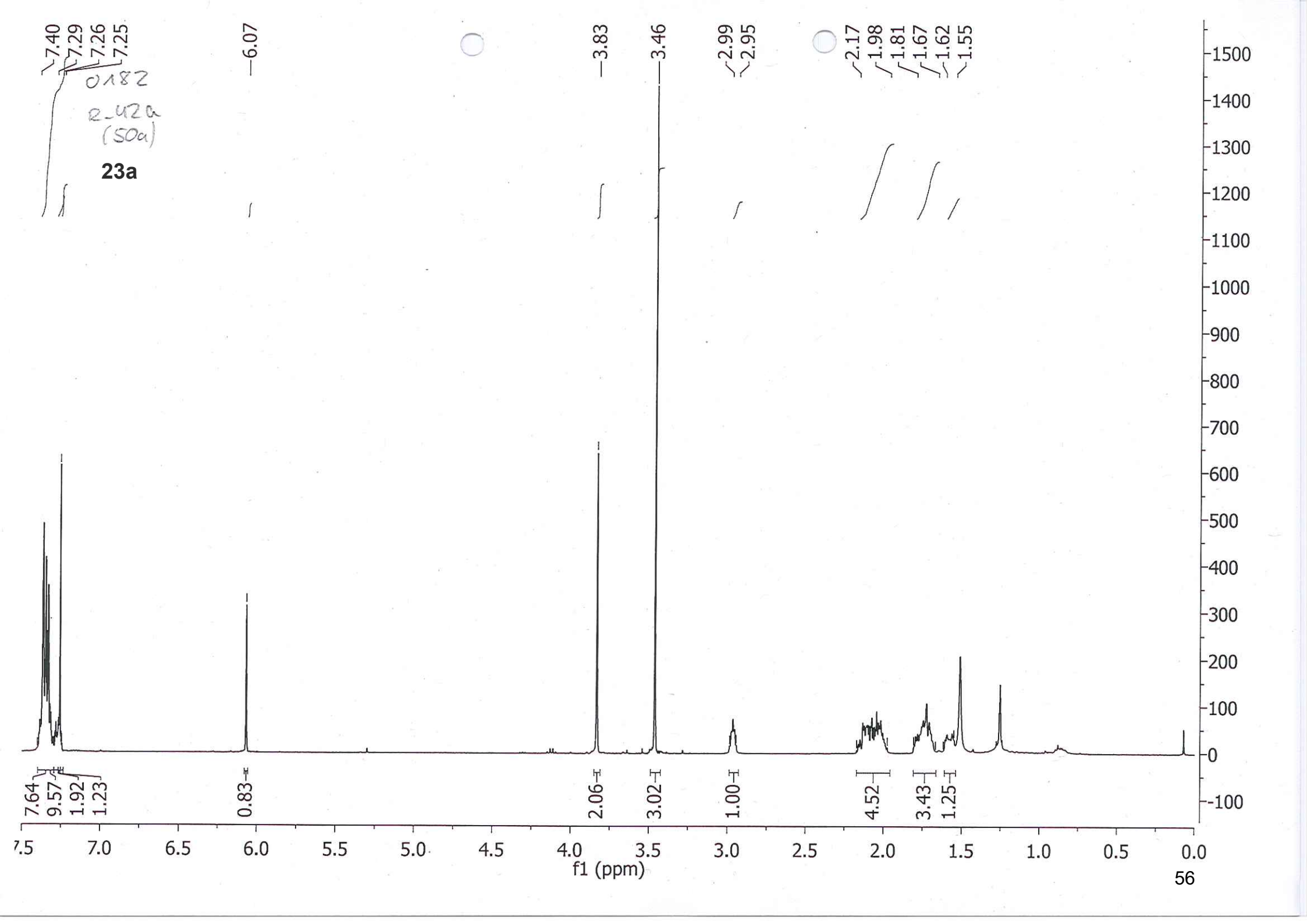
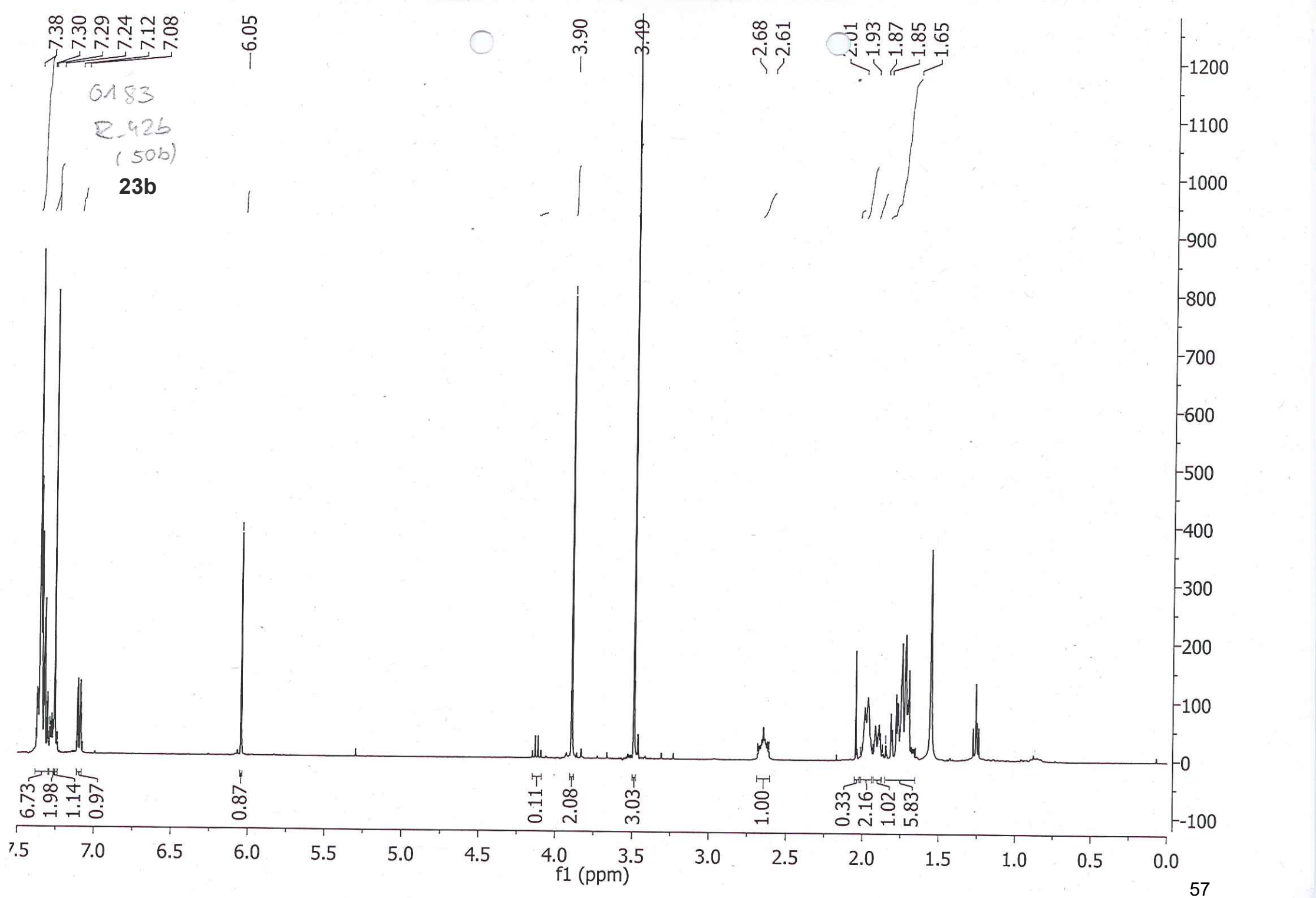
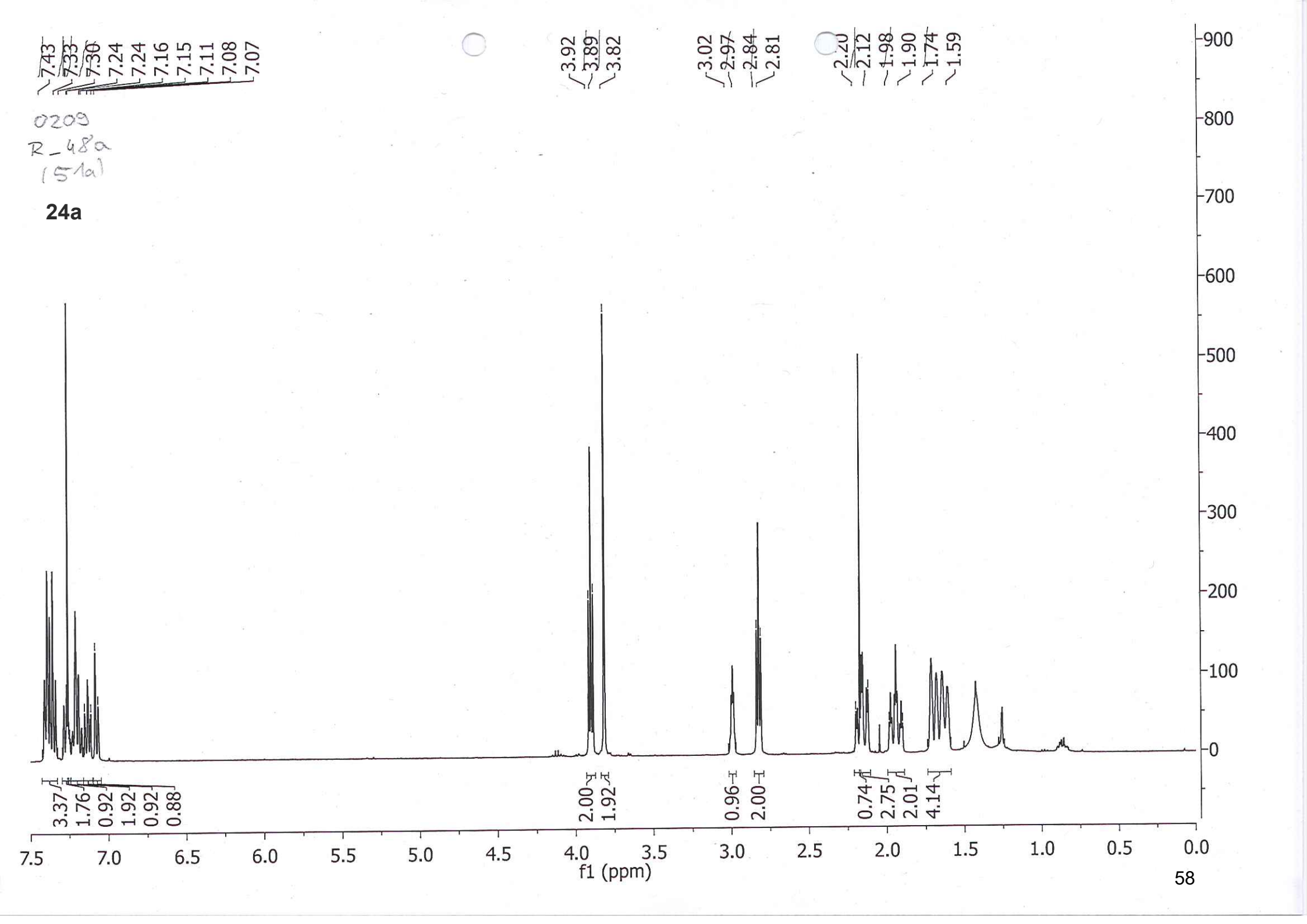
3.10
1.05
1.00
5.49
2.29
f1 (ppm)
53

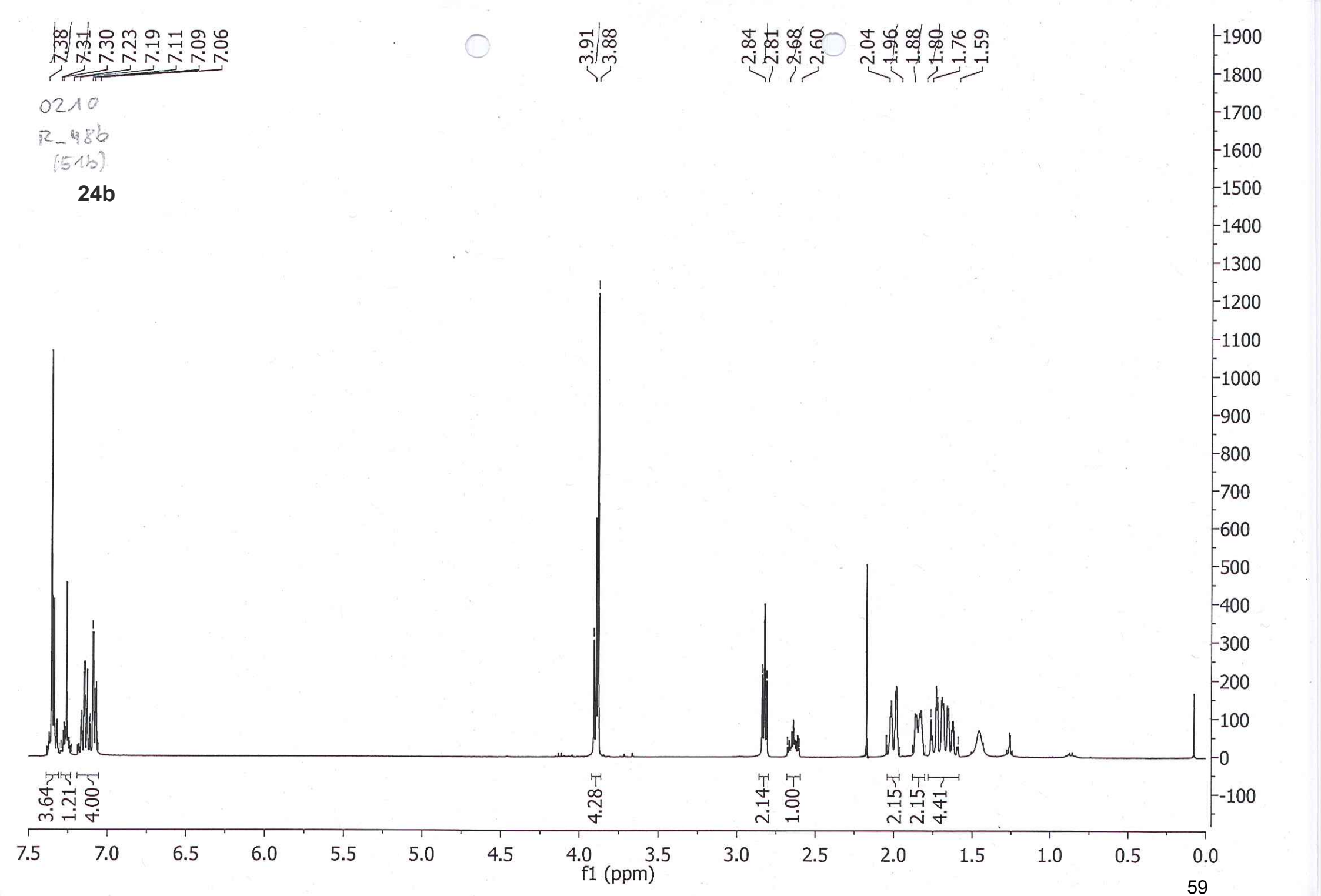

0076
R_11a
(25a)
22a
7.42
7.33
7.30
7.26
7.22
7.20
7.16
7.11
7.07
6.11
4.90
4.87
3.60
3.59
2.98
2.94
2.93
2.88
2.46
2.42
2.35
2.22
2.17
1.94
1.83
1.78
1.69
1.63
4.67
4.55
1.00
1.15
1.13
1.20
2.40
3.65
2.51
1.21
1.23
3.94
10.57
1.25
1.27
f1 (ppm)


7.39
7.30
7.29
7.26
7.22
7.22
7.13
7.12
7.07
4.86
3.65
3.59
2.97
2.92
2.87
2.71
2.28
2.19
2.13
2.09
1.96
1.95
1.86
1.84
1.75
1.68
1.60
0116
R_116
(256)
22b
4.48
1.86
2.04
1.92
1.08
2.11
3.41
2.18
1.01
3.48
1.09
2.25
4.33
2.09
2.66
1.00
2.07
f1 (ppm)


7.40
7.29
7.26
7.25
6.07
3.83
3.46
2.99
2.95
2.17
1.98
1.81
1.67
1.62
1.55
0182
R_42a
(50a)
23a
7.64
9.57
1.92
1.23
0.83
2.06
3.02
1.00
4.52
3.43
1.25
f1 (ppm)


7.38
7.30
7.29
7.24
7.12
7.08
6.05
3.90
3.49
2.68
2.61
2.01
1.93
1.87
1.85
1.65
0153
R_926
(50b)
23b
6.73
1.98
1.14
0.97
0.87
0.11
2.08
3.03
1.00
0.33
2.16
1.02
5.83
f1 (ppm)


7.43
7.33
7.30
7.24
7.24
7.16
7.15
7.11
7.08
7.07
3.92
3.89
3.82
3.02
2.97
2.84
2.81
2.20
2.12
1.98
1.90
1.74
1.59
0209
R-48a
(51a)
24a
3.37
1.76
0.92
1.92
0.92
0.88
2.00
1.92
0.96
2.00
0.74
2.75
2.01
4.14
f1 (ppm)


7.38
7.31
7.30
7.23
7.19
7.11
7.09
7.06
3.91
3.88
2.84
2.81
2.68
2.60
2.04
1.96
1.88
1.80
1.76
1.59
0210
R_486
(51b)
24b
3.64
1.21
4.00
4.28
2.14
1.00
2.15
2.15
4.41
f1 (ppm)

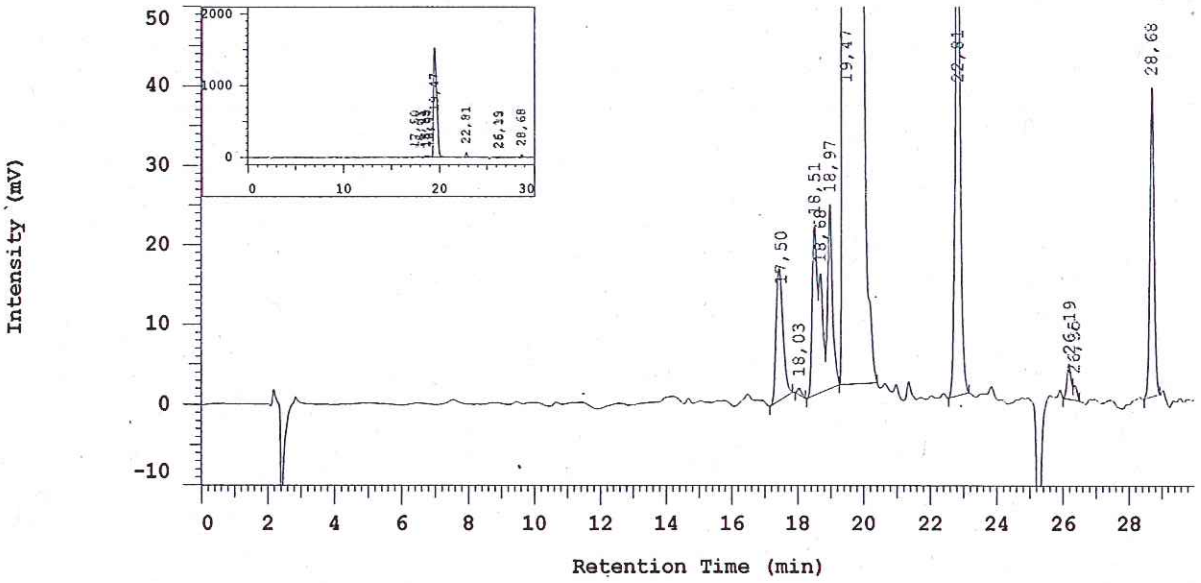
(54a)

4a

HPLC

Analyzed: 14.01.10 14:27
Reported: 14.01.10 17:34
Processed: 14.01.10 17:34

Data Path: D:\WIN32APP\HSM\Chromni\DATA\1042\
Application: Chromni
Series:1042
Sample Name: R_59a
Vial Number: 24
Injection from this vial: 1 of 1
Vial Type: UNK
Volume: 5,0 ul
Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	17,50	233238	0,662	MC
2	18,03	7159	0,020	MC
3	18,51	210003	0,596	MC
4	18,68	138741	0,394	MC
5	18,97	228569	0,649	MC
6	19,47	33335763	94,675	MC
7	22,81	674122	1,915	MC
8	26,19	38208	0,109	MC
9	26,35	13412	0,038	MC
10	28,68	331331	0,941	MC
		35210546	100,000	

Peak rejection level: 0

(546)

4b

HPLC

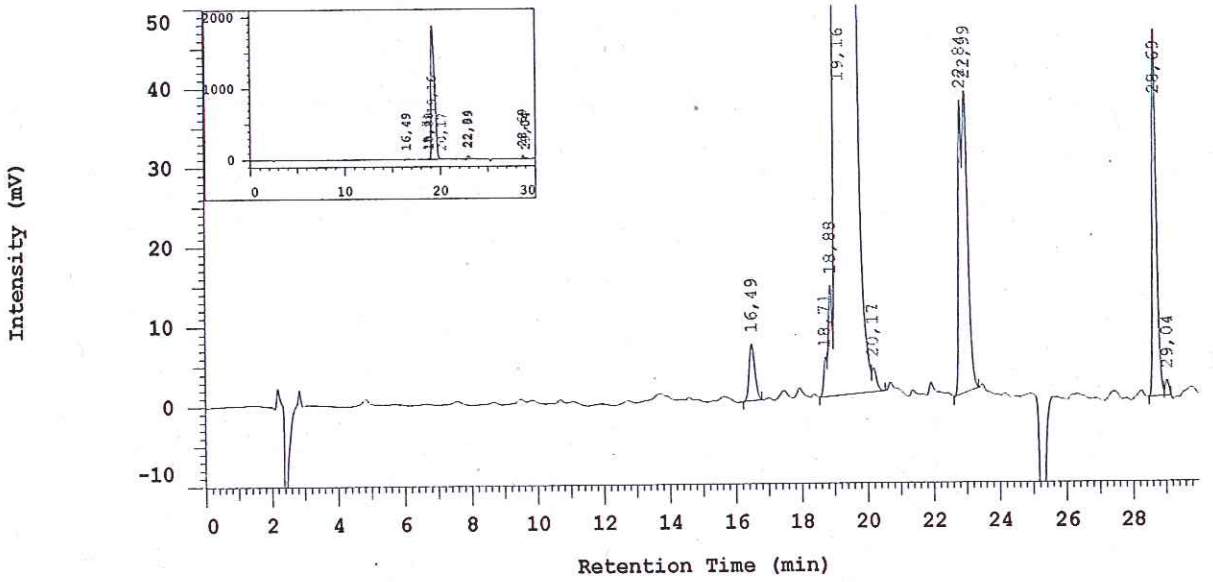
Analyzed: 14.01.10 15:08

Data Path: D:\WIN32APP\HSM\Chromni\DATA\1043\
Application: Chromni
Sample Name: R_59b
Injection from this vial: 1 of 1

Reported: 14.01.10 17:22
Processed: 14.01.10 17:22

Series:1043
Vial Number: 25
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	16,49	84462	0,184	MC
2	18,71	29379	0,064	MC
3	18,88	106082	0,231	VV
4	19,16	44541600	97,104	VV
5	20,17	29949	0,065	MC
6	22,84	254986	0,556	MC
7	22,99	412261	0,899	MC
8	28,69	396118	0,864	MC
9	29,04	15010	0,033	MC
		45869847	100,000	

Peak rejection level: 0

(58a)

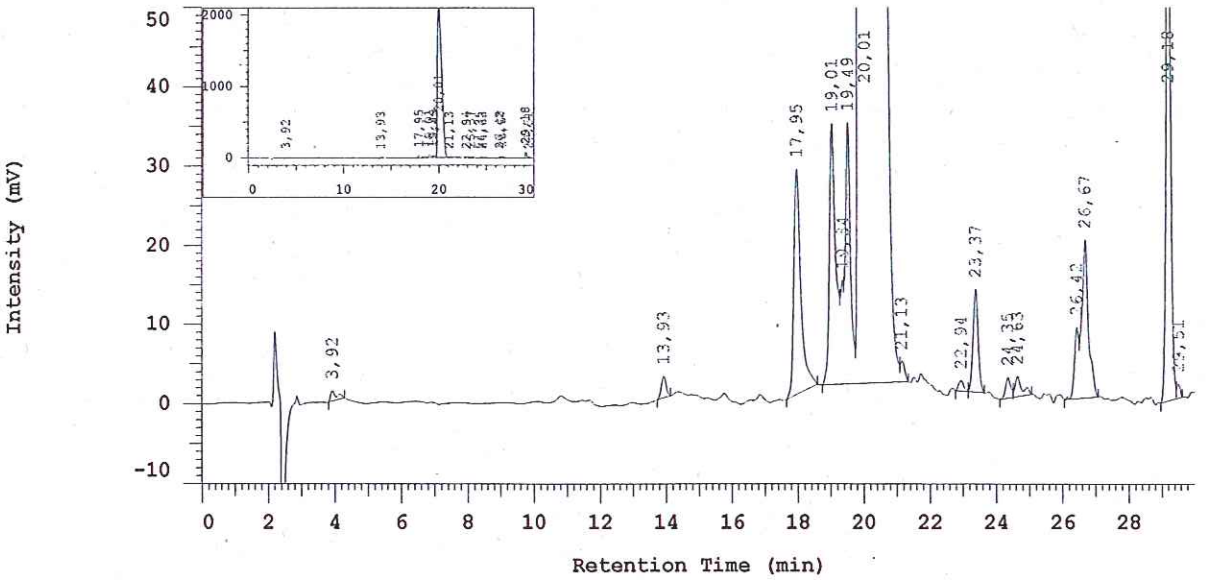
5a

HPLC

Analyzed: 21.01.10 01:47
Reported: 21.01.10 10:58
Processed: 21.01.10 10:58

Data Path: D:\WIN32APP\HSM\Chromni\DATA\1068\
Application: Chromni
Series:1068
Sample Name: R_63a
Vial Number: 8
Injection from this vial: 1 of 1
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	3,92	16077	0,025	MC
2	13,93	26683	0,041	BB
3	17,95	414680	0,635	MC
4	19,01	422427	0,646	MC
5	19,34	87903	0,135	MC
6	19,49	354747	0,543	MC
7	20,01	62743718	96,007	MC
8	21,13	25071	0,038	MC
9	22,94	14416	0,022	BB
10	23,37	135767	0,208	BB
11	24,35	27120	0,041	BB
12	24,63	35647	0,055	BB
13	26,42	77406	0,118	BV
14	26,67	273139	0,418	VB
15	29,18	686159	1,050	MC
16	29,51	12126	0,019	MC
		65353086	100,000	

Peak rejection level: 0

(58b)

5b

HPLC

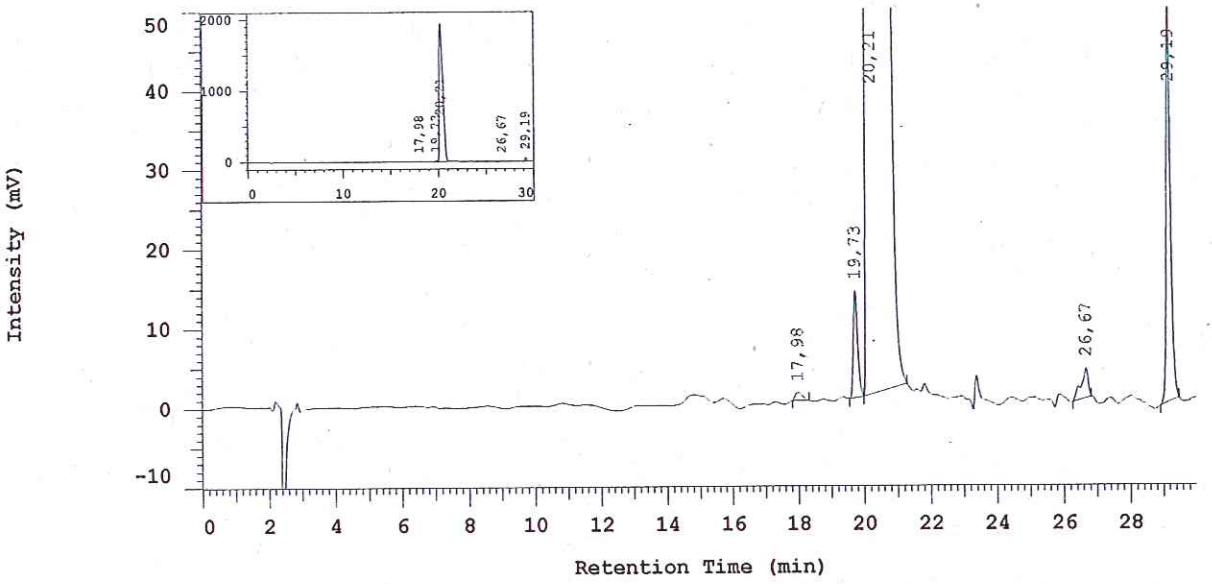
Analyzed: 21.01.10 02:28

Reported: 21.01.10 11:00
Processed: 21.01.10 11:00

Data Path: D:\WIN32APP\HSM\Chromni\DATA\1069\
Application: Chromni
Sample Name: R_63b
Injection from this vial: 1 of 1

Series:1069
Vial Number: 9
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	17,98	15828	0,031	MC
2	19,73	118899	0,236	BB
3	20,21	49836108	98,733	MC
4	26,67	52980	0,105	MC
5	29,19	451769	0,895	MC
		50475584	100,000	

Peak rejection level: 0

(52a)

7a

HPLC

Analyzed: 16.12.09 21:16

Reported: 02.08.10 13:38
Processed: 17.12.09 13:11

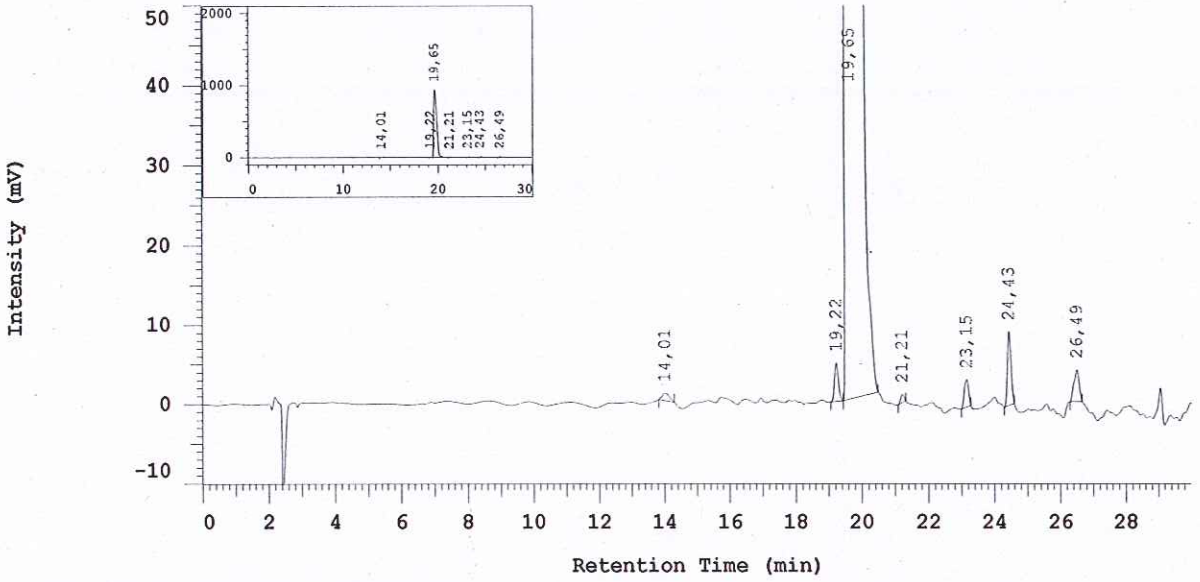
Data Path: D:\WIN32APP\HSM\Chromni\DATA\0953\
Application: Chromni

Sample Name: R_58a

Injection from this vial: 1 of 1

Series:0953
Vial Number: 4
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Solvent A: Wasser + 0,05%TFA

Developed by: Jens
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	14,01	14875	0,077	MC
2	19,22	38980	0,201	MC
3	19,65	19222091	98,958	MC
4	21,21	6869	0,035	MC
5	23,15	30527	0,157	MC
6	24,43	67671	0,348	MC
7	26,49	43571	0,224	MC
		19424584	100,000	

Peak rejection level: 0

(52b)

7b

HPLC

Analyzed: 16.12.09 21:58

Reported: 17.12.09 13:14
Processed: 17.12.09 13:14

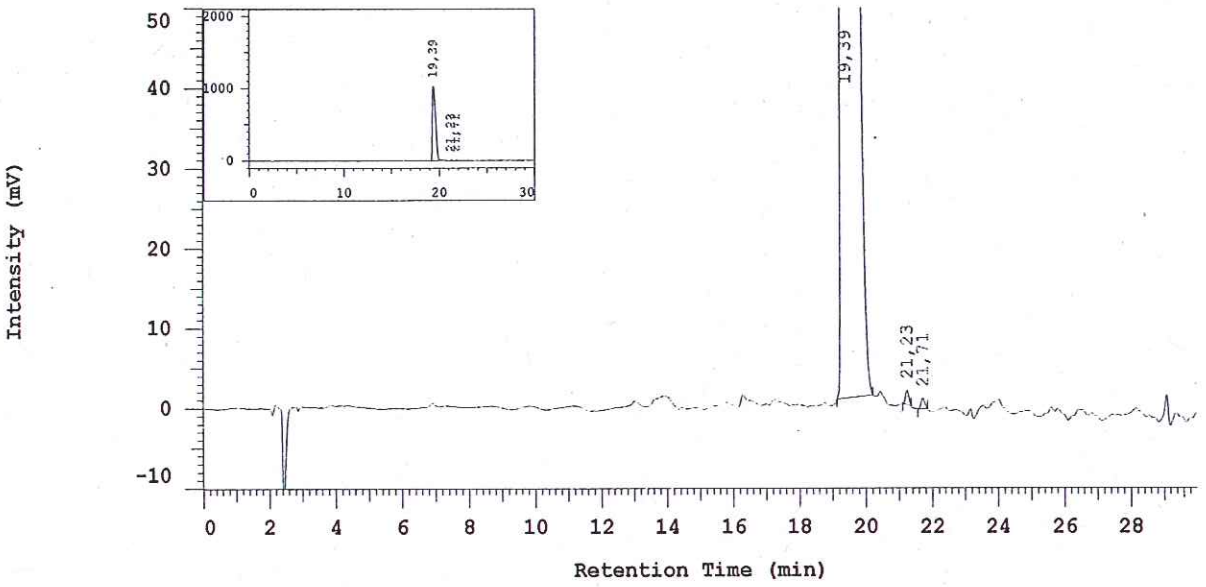
Data Path: D:\WIN32APP\HSM\Chromni\DATA\0954\
Application: Chromni

Sample Name: R_58b

Injection from this vial: 1 of 1

Series:0954
Vial Number: 5
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	19,39	22167977	99,902	MC
2	21,23	12108	0,055	MC
3	21,71	9567	0,043	BB
		22189652	100,000	

Peak rejection level: 0

(56a)

8a

HPLC

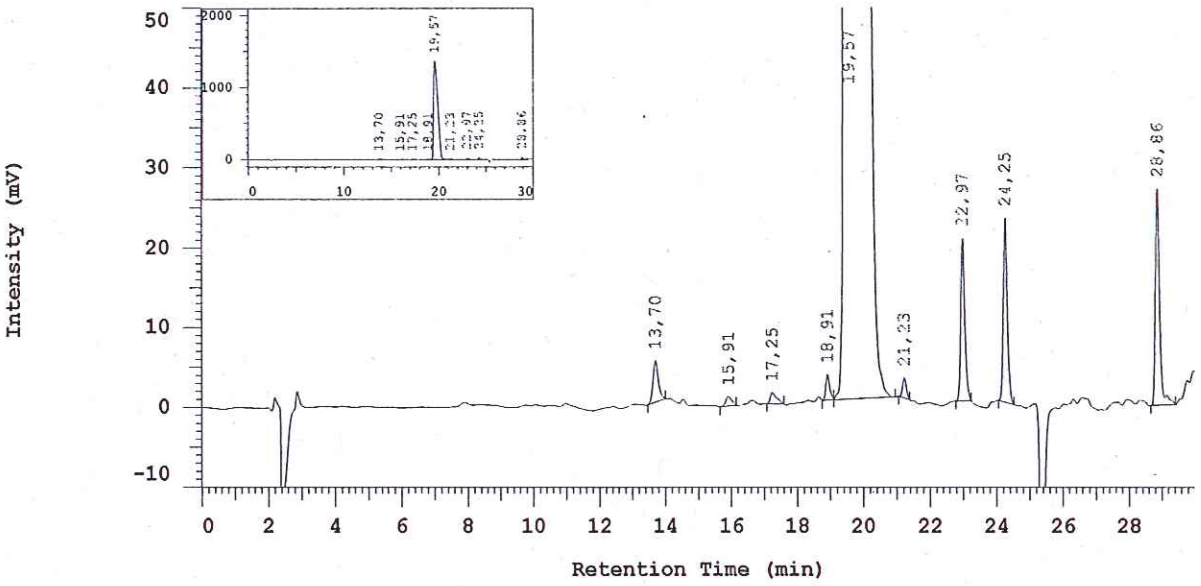
Analyzed: 13.01.10 23:55

Reported: 14.01.10 10:45
Processed: 14.01.10 10:45

Data Path: D:\WIN32APP\HSM\Chromni\DATA\1021\
Application: Chromni
Sample Name: R_61a
Injection from this vial: 1 of 1

Series:1021
Vial Number: 7
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	13,70	58163	0,143	MC
2	15,91	15257	0,038	MC
3	17,25	18602	0,046	MC
4	18,91	25123	0,062	BB
5	19,57	39829011	98,178	BB
6	21,23	19840	0,049	BB
7	22,97	168762	0,416	BB
8	24,25	189035	0,466	BB
9	28,86	244486	0,603	BB
		40568279	100,000	

Peak rejection level: 0

(56b)

8b

HPLC

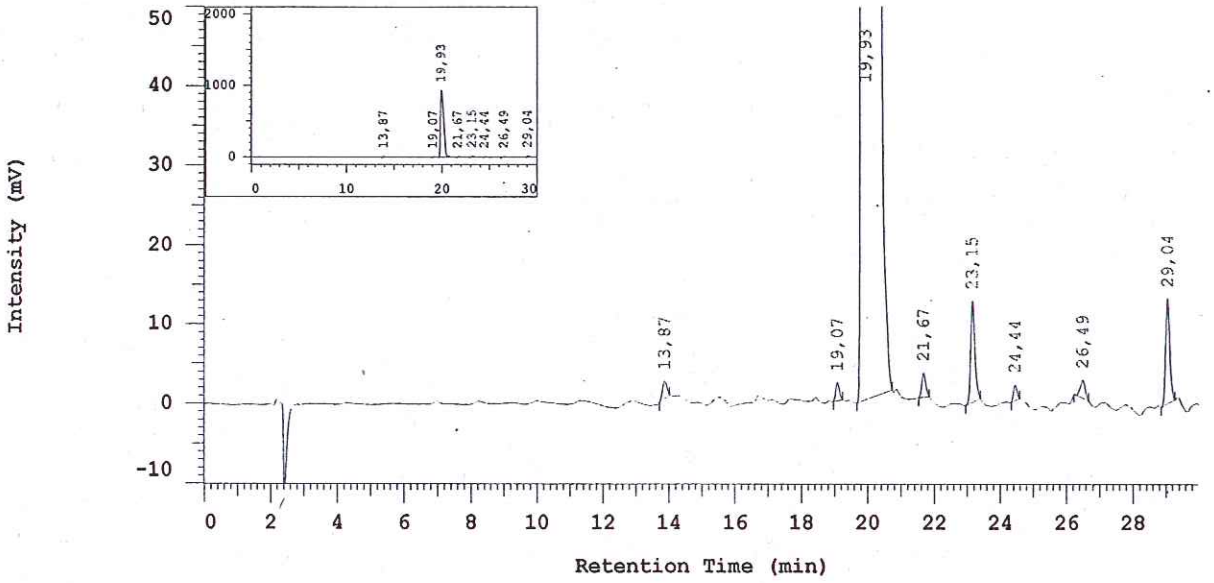
Analyzed: 07.01.10 02:21

Reported: 07.01.10 16:24
Processed: 07.01.10 16:24

Data Path: D:\WIN32APP\HSM\Chromni\DATA\0987\
Application: Chromni
Sample Name: R_61b
Injection from this vial: 1 of 1

Series:0987
Vial Number: 11
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	13,87	19714	0,095	MC
2	19,07	17731	0,085	MC
3	19,93	20467212	98,469	MC
4	21,67	24684	0,119	MC
5	23,15	109487	0,527	BB
6	24,44	13766	0,066	MC
7	26,49	22556	0,109	MC
8	29,04	110234	0,530	MC
		20785384	100,000	

Peak rejection level: 0

(24a)

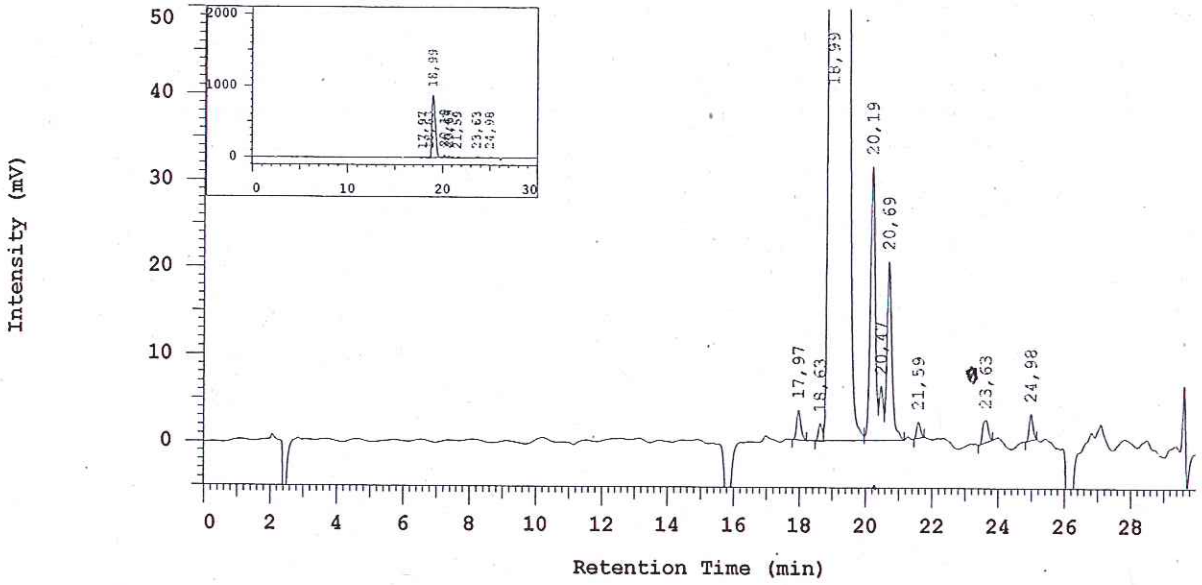
HPLC

10a

Analyzed: 27.03.08 05:08
Reported: 27.03.08 09:54
Processed: 27.03.08 09:54

Data Path: D:\WIN32APP\HSM\Christoph\DATA\1151\
Application: Christoph
Series:1151
Sample Name: R_27.1a
Vial Number: 14
Injection from this vial: 1 of 1
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	17,97	29484	0,142	MC
2	18,63	15000	0,072	BB
3	18,99	20055654	96,665	BB
4	20,19	321527	1,550	MC
5	20,47	51834	0,250	MC
6	20,69	208166	1,003	MC
7	21,59	14119	0,068	BB
8	23,63	28980	0,140	BB
9	24,98	22907	0,110	BB
		20747671	100,000	

Peak rejection level: 0

(24b)

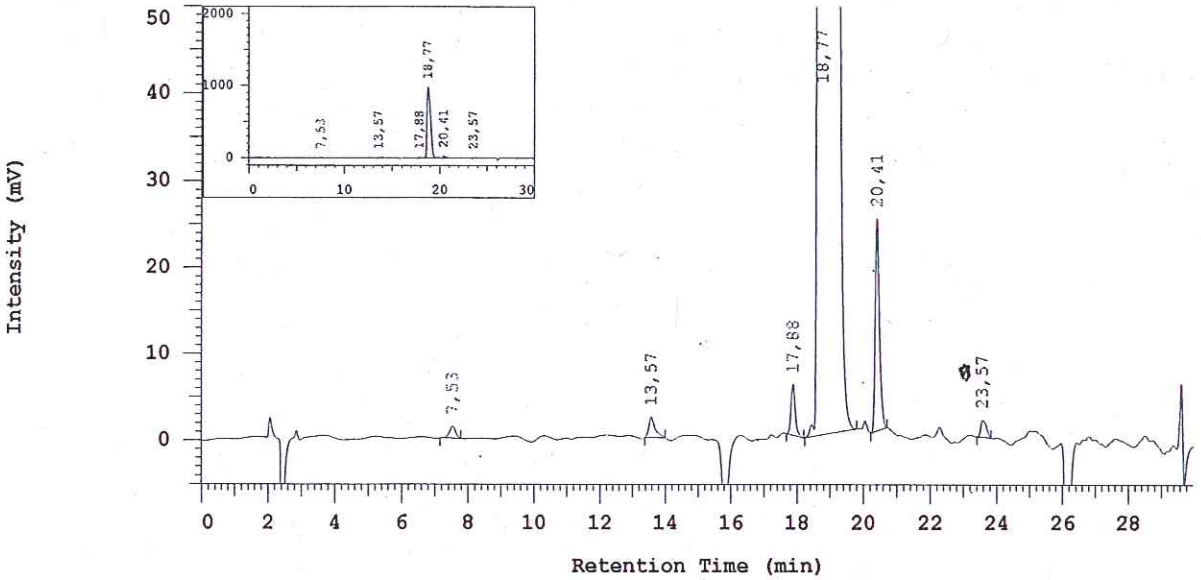
10b

HPLC

Analyzed: 27.03.08 05:49
Reported: 27.03.08 09:55
Processed: 27.03.08 09:55

Data Path: D:\WIN32APP\HSM\Christoph\DATA\1152\
Application: Christoph
Series:1152
Sample Name: R_27.1b
Vial Number: 15
Injection from this vial: 1 of 1
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	7,53	17399	0,072	MC
2	13,57	31034	0,129	MC
3	17,88	49586	0,206	BB
4	18,77	23754438	98,608	MC
5	20,41	214237	0,889	BB
6	23,57	23031	0,096	MC
		24089725	100,000	

Peak rejection level: 0

(396)

11a

HPLC

Analyzed: 03.04.08 06:06

Reported: 03.04.08 11:05
Processed: 03.04.08 11:05

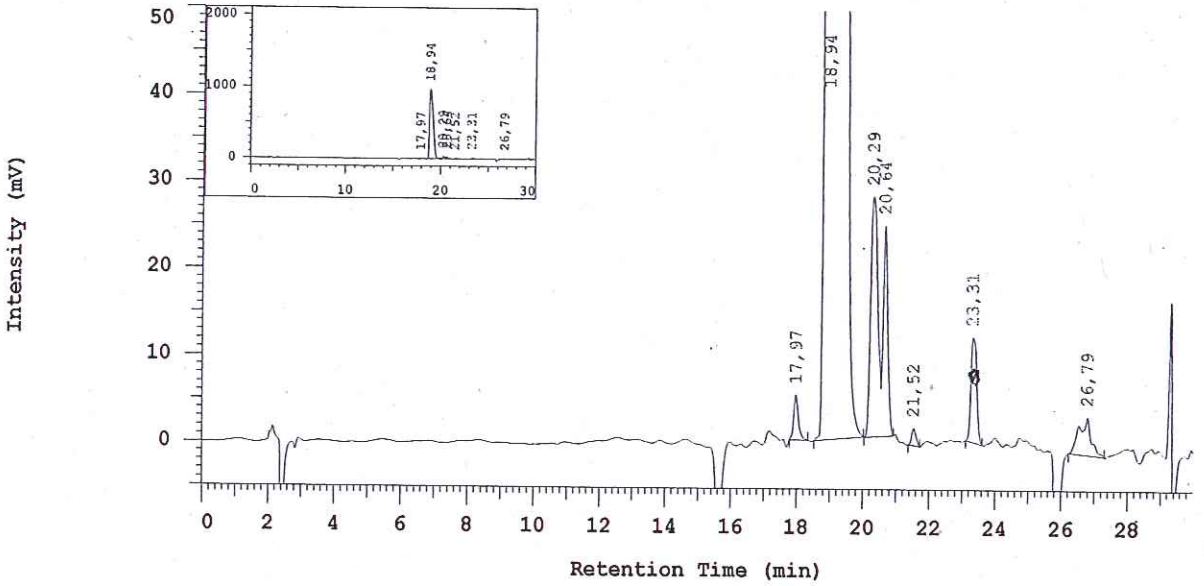
Data Path: D:\WIN32APP\HSM\Christoph\DATA\1172\
Application: Christoph

Sample Name: R_28.1a

Injection from this vial: 1 of 1

Series:1172
Vial Number: 14
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Solvent A: Wasser + 0,05%TFA

Developed by: Jens
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	17,97	48375	0,189	MC
2	18,94	24695846	96,265	MC
3	20,29	406221	1,583	MC
4	20,64	230095	0,897	MC
5	21,52	17788	0,069	MC
6	23,31	144273	0,562	BB
7	26,79	111300	0,434	MC
		25653898	100,000	

Peak rejection level: 0

(32b)

11b

HPLC

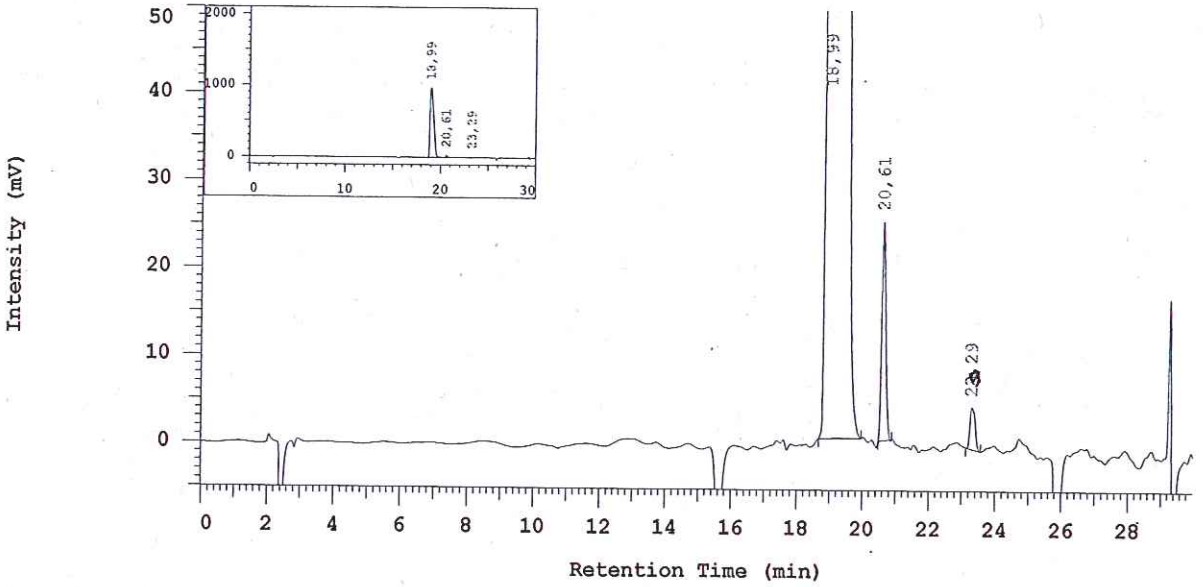
Analyzed: 03.04.08 06:48

Reported: 03.04.08 11:06
Processed: 03.04.08 11:06

Data Path: D:\WIN32APP\HSM\Christoph\DATA\1173\
Application: Christoph
Sample Name: R_28.1b
Injection from this vial: 1 of 1

Series:1173
Vial Number: 15
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	18,99	24533420	98,967	BB
2	20,61	205765	0,830	MC
3	23,29	50368	0,203	BB
		24789553	100,000	

Peak rejection level: 0

(55a)

19a

HPLC

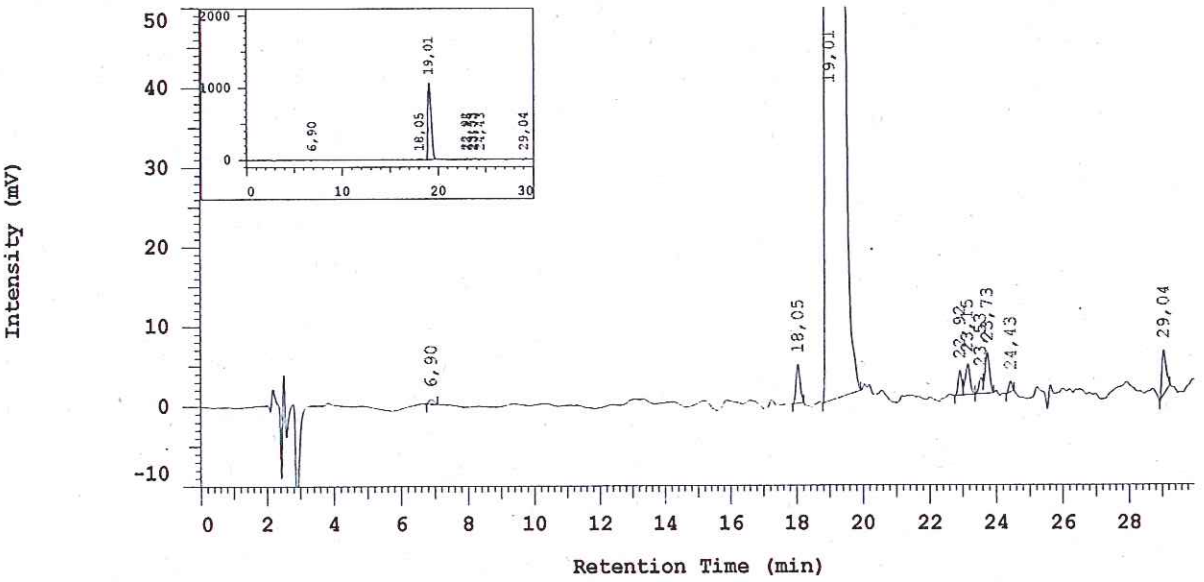
Analyzed: 10.12.09 03:57

Data Path: D:\WIN32APP\HSM\Chromni\DATA\0944\
Application: Chromni
Sample Name: R.57a
Injection from this vial: 1 of 1

Reported: 10.12.09 10:53
Processed: 10.12.09 10:53

Series:0944
Vial Number: 12
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	6,90	5954	0,025	MC
2	18,05	38906	0,165	MC
3	19,01	23295238	99,068	MC
4	22,92	21678	0,092	MC
5	23,15	36456	0,155	MC
6	23,53	14478	0,062	MC
7	23,73	45688	0,194	MC
8	24,43	7713	0,033	BB
9	29,04	48254	0,205	MC
		23514365	100,000	

Peak rejection level: 0

(55b)

19b

HPLC

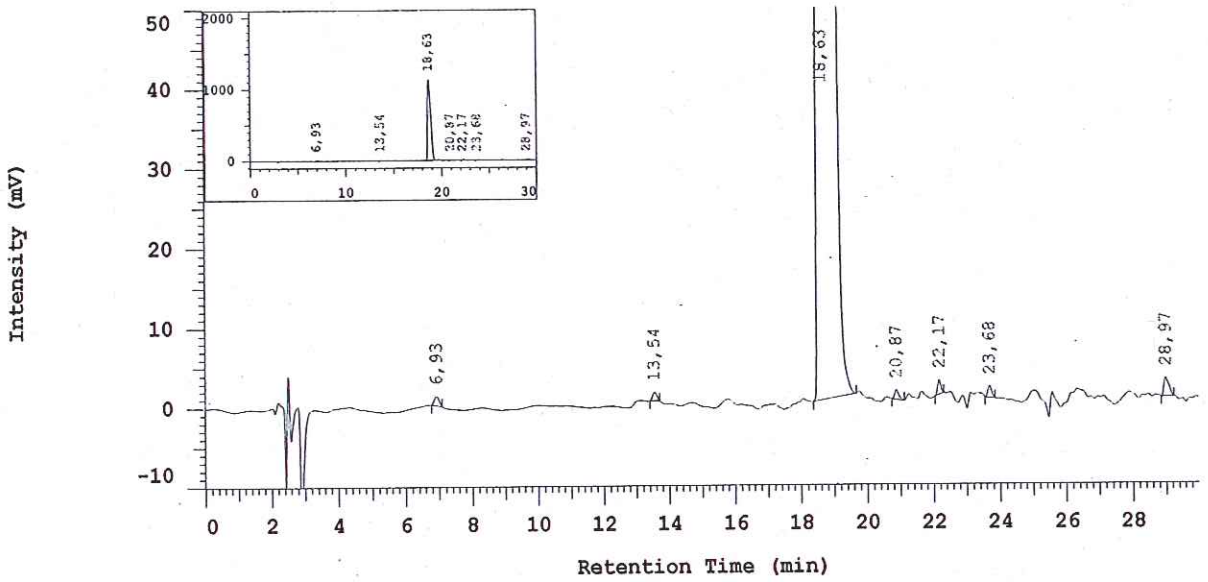
Analyzed: 10.12.09 04:39

Reported: 10.12.09 10:57
Processed: 10.12.09 10:57

Data Path: D:\WIN32APP\HSM\Chromni\DATA\0945\
Application: Chromni
Sample Name: R.57b
Injection from this vial: 1 of 1

Series:0945
Vial Number: 13
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	6,93	11410	0,046	MC
2	13,54	9867	0,039	BB
3	18,63	24936696	99,682	MC
4	20,87	10259	0,041	BB
5	22,17	11646	0,047	BB
6	23,68	11521	0,046	BB
7	28,97	24880	0,099	BB
		25016279	100,000	

Peak rejection level: 0

(59a)

20a

HPLC

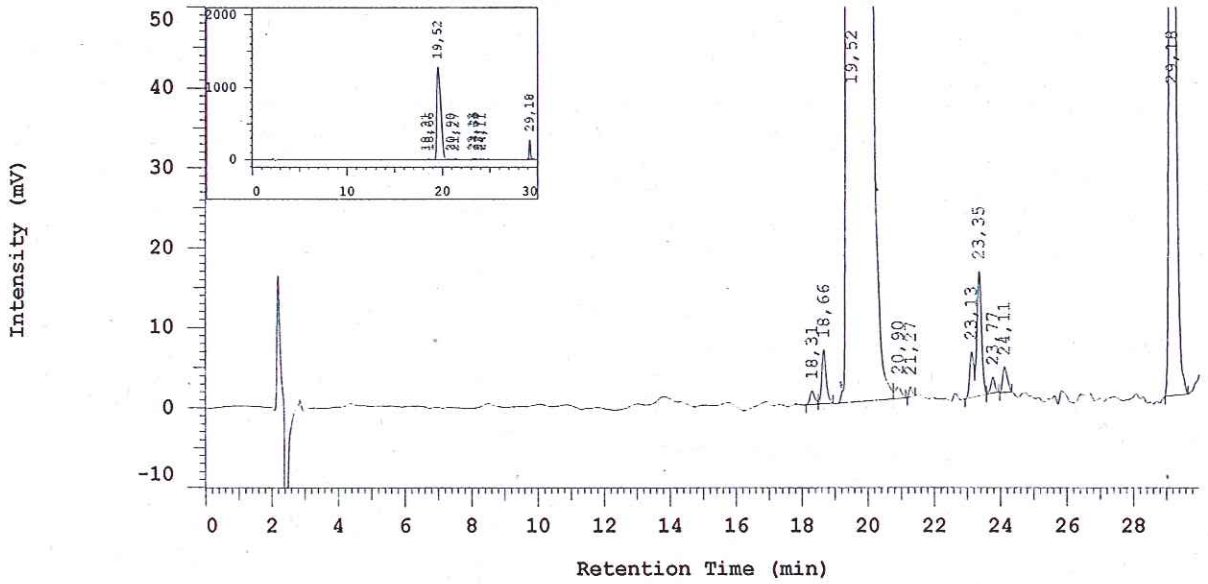
Analyzed: 21.01.10 00:24

Reported: 21.01.10 10:57
Processed: 21.01.10 10:56

Data Path: D:\WIN32APP\HSM\Chromni\DATA\1066\
Application: Chromni
Sample Name: R_60a
Injection from this vial: 1 of 1

Series:1066
Vial Number: 7
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	18,31	16520	0,043	MC
2	18,66	60906	0,158	MC
3	19,52	35619420	92,536	MC
4	20,90	19569	0,051	MC
5	21,27	7747	0,020	MC
6	23,13	46700	0,121	MC
7	23,35	145812	0,379	MC
8	23,77	16058	0,042	MC
9	24,11	30382	0,079	MC
10	29,18	2529376	6,571	MC
		38492490	100,000	

Peak rejection level: 0

(59b)

20b

HPLC

Analyzed: 07.01.10 00:58

Reported: 07.01.10 16:21
Processed: 07.01.10 16:21

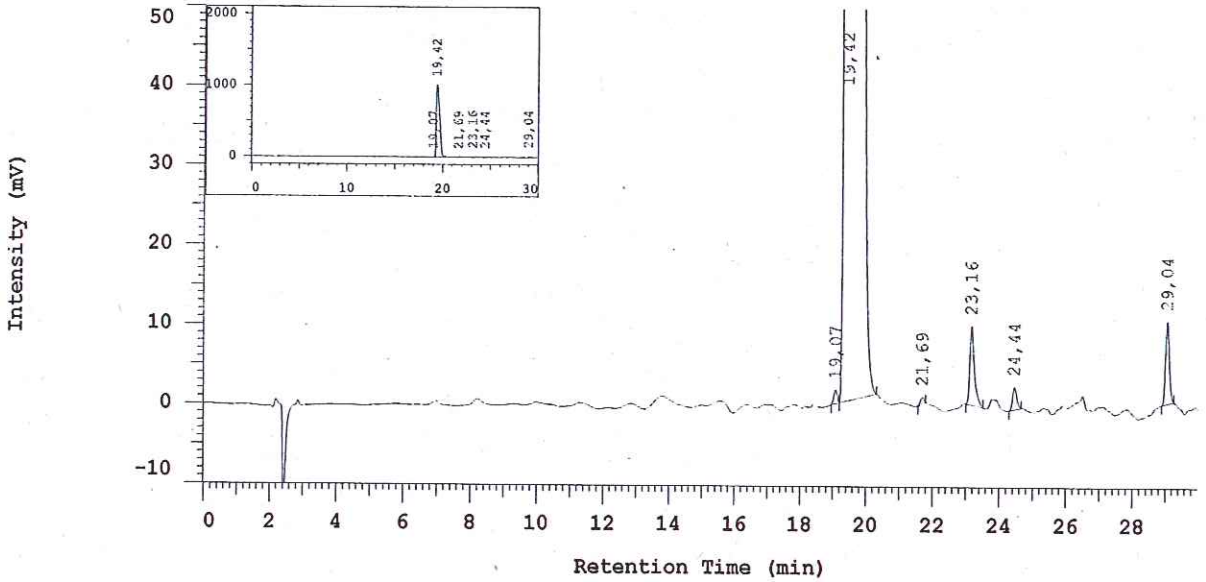
Data Path: D:\WIN32APP\HSM\Chromni\DATA\0985\
Application: Chromni

Sample Name: R_60b

Injection from this vial: 1 of 1

Series:0985
Vial Number: 9
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	19,07	11865	0,051	BB
2	19,42	22856195	99,061	BB
3	21,69	5499	0,024	MC
4	23,16	92703	0,402	MC
5	24,44	23177	0,100	MC
6	29,04	83368	0,361	MC
		23072807	100,000	

Peak rejection level: 0

(11a)

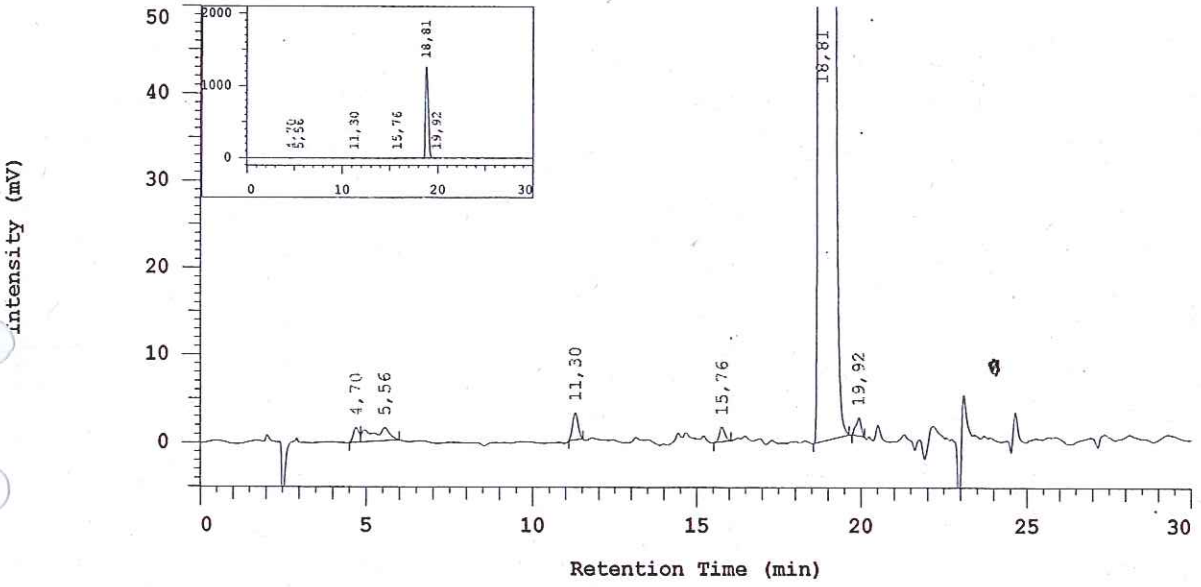
21a

HPLC

Analyzed: 01.06.07 02:00
Reported: 01.06.07 13:34
Processed: 01.06.07 13:34

Data Path: D:\WIN32APP\HSM\Christoph\DATA\0013\
Application: Christoph
Series:0013
Sample Name: R7.3a
Vial Number: 13
Injection from this vial: 1 of 1
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	4,70	18471	0,077	MC
2	5,56	58131	0,241	MC
3	11,30	35225	0,146	MC
4	15,76	18362	0,076	MC
5	18,81	23987840	99,368	MC
6	19,92	22322	0,092	MC
		24140351	100,000	

Peak rejection level: 0

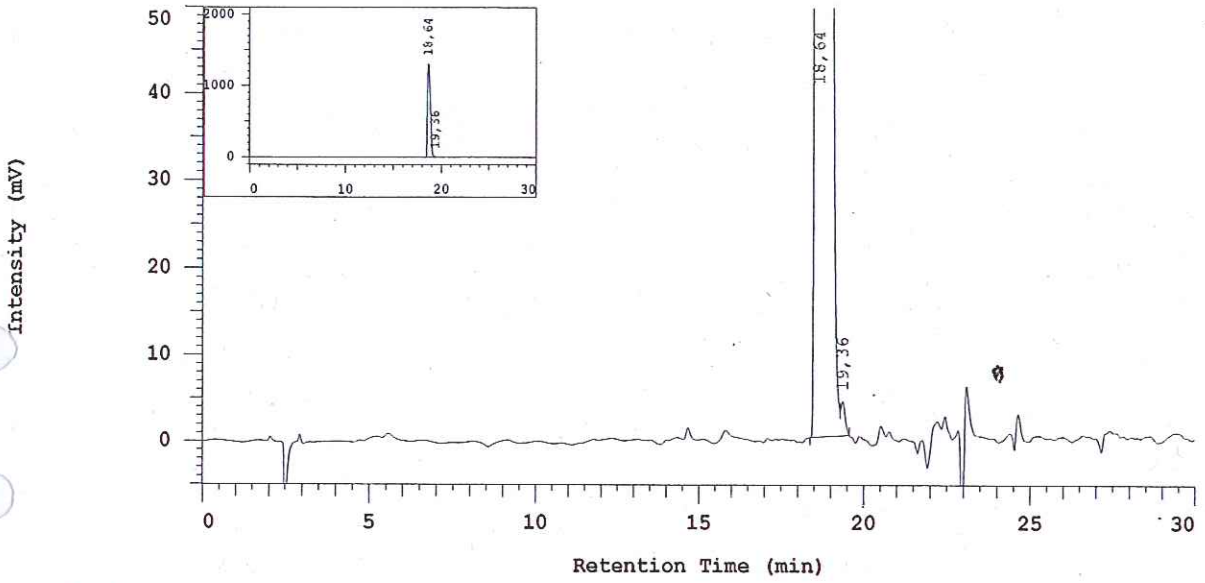
(11b)

21b

HPLC

Analyzed: 01.06.07 02:42
Reported: 01.06.07 13:37
Processed: 01.06.07 13:37

Data Path: D:\WIN32APP\HSM\Christoph\DATA\0014\
Application: Christoph
Series:0014
Sample Name: R7.3b
Vial Number: 14
Injection from this vial: 1 of 1
Vial Type: UNK
Volume: 5,0 ul
Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	18,64	25859494	99,854	MC
2	19,36	37937	0,146	MC
		25897431	100,000	

Peak rejection level: 0

(25a)

22a

HPLC

Analyzed: 31.08.07 10:10

Reported: 31.08.07 11:34
Processed: 31.08.07 11:34

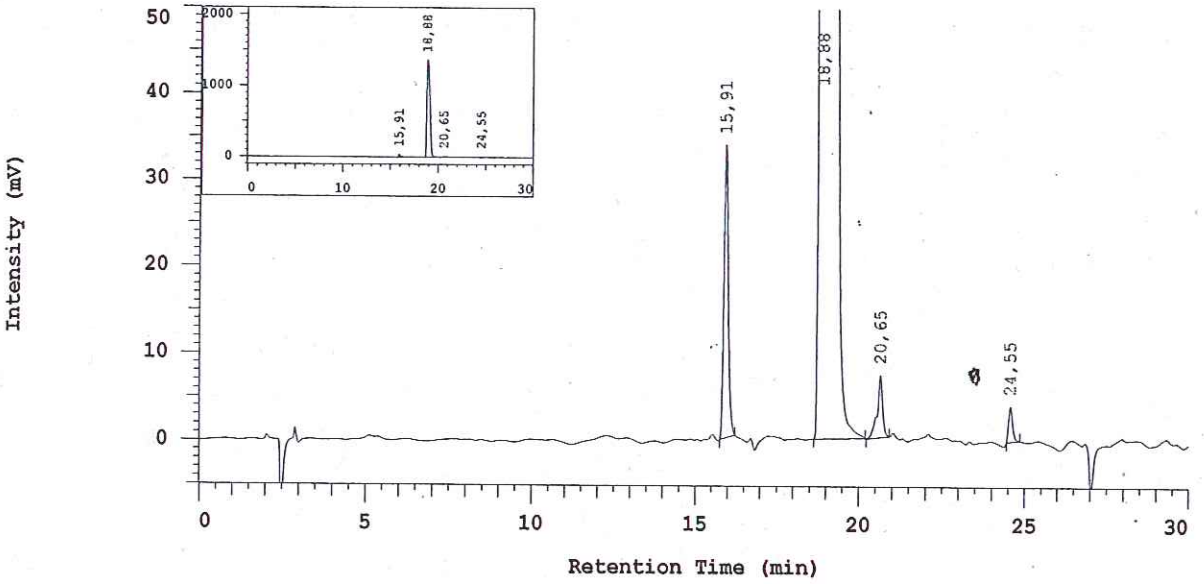
Data Path: D:\WIN32APP\HSM\Christoph\DATA\0455\
Application: Christoph

Sample Name: R11.1a

Injection from this vial: 1 of 1

Series:0455
Vial Number: 21
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010

Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	15,91	295972	1,030	MC
2	18,88	28322745	98,579	MC
3	20,65	79561	0,277	MC
4	24,55	32636	0,114	MC
		28730914	100,000	

Peak rejection level: 0

(25b)

22b

HPLC

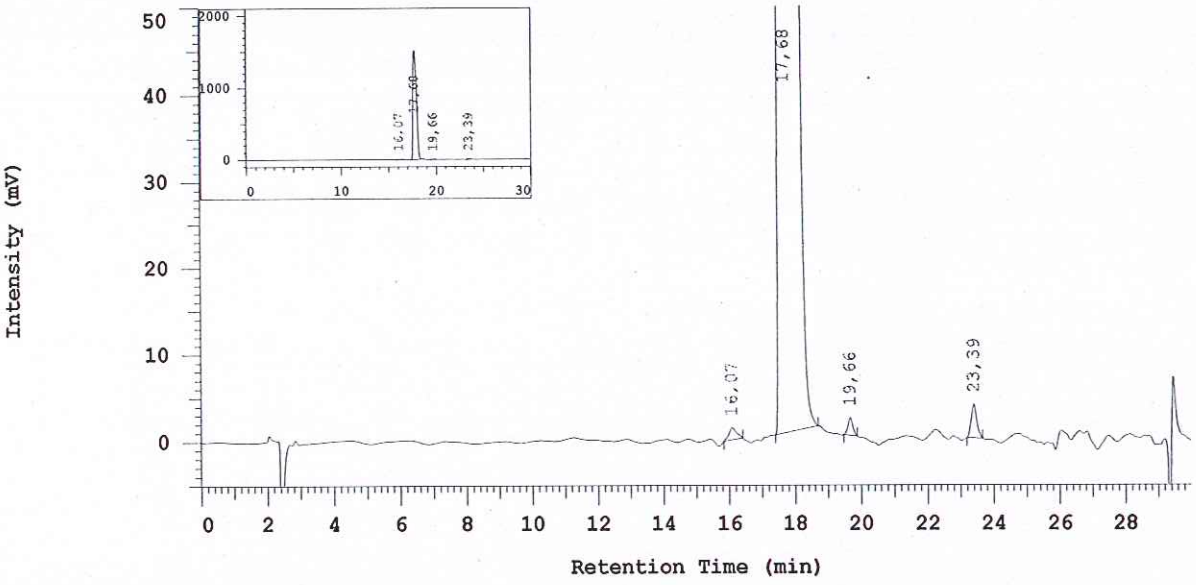
Analyzed: 06.03.08 03:09

Reported: 30.07.10 13:22
Processed: 06.03.08 15:29

Data Path: D:\WIN32APP\HSM\Christoph\DATA\1041\
Application: Christoph
Sample Name: R_11.2b
Injection from this vial: 1 of 1

Series:1041
Vial Number: 11
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Solvent A: Wasser + 0,05%TFA

Developed by: Jens
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	16,07	21925	0,059	MC
2	17,68	36991052	99,768	MC
3	19,66	20089	0,054	MC
4	23,39	43862	0,118	MC
		37076928	100,000	

Peak rejection level: 0

15 1a)

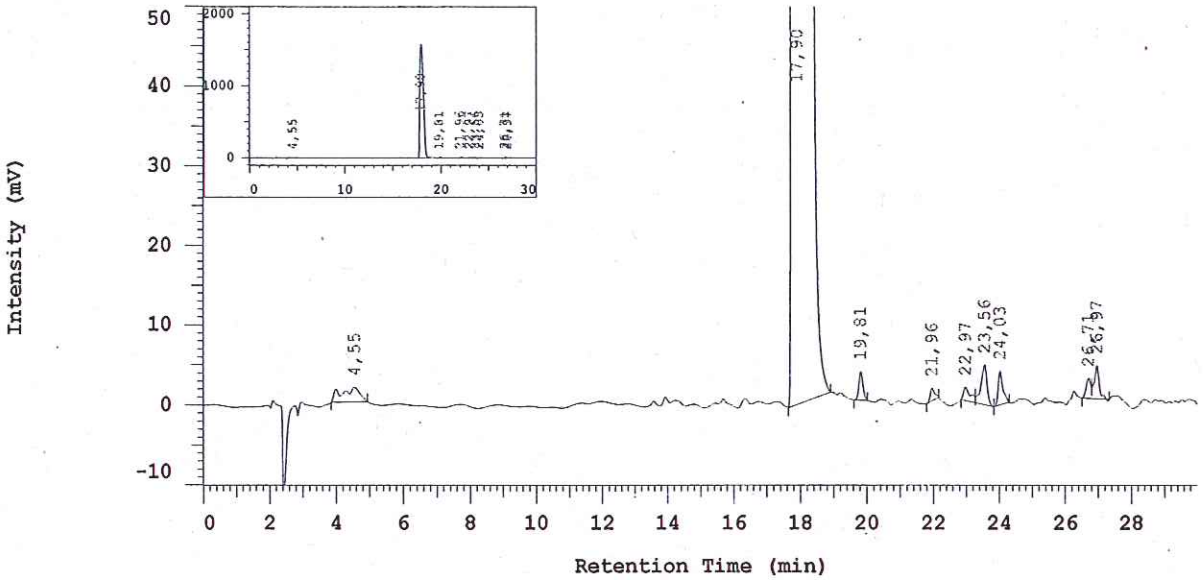
24a

HPLC

Analyzed: 19.02.09 03:34
Reported: 19.02.09 10:06
Processed: 19.02.09 10:06

Data Path: D:\WIN32APP\HSM\Christoph\DATA\2046\
Application: Christoph
Series:2046
Sample Name: R_48.1a
Vial Number: 11
Injection from this vial: 1 of 1
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	4,55	67743	0,180	BB
2	17,90	37245113	99,189	BB
3	19,81	29714	0,079	BB
4	21,96	13811	0,037	BB
5	22,97	24523	0,065	BB
6	23,56	62800	0,167	BB
7	24,03	40042	0,107	BB
8	26,71	23495	0,063	BV
9	26,97	42551	0,113	VB
		37549792	100,000	

Peak rejection level: 0

(51b)

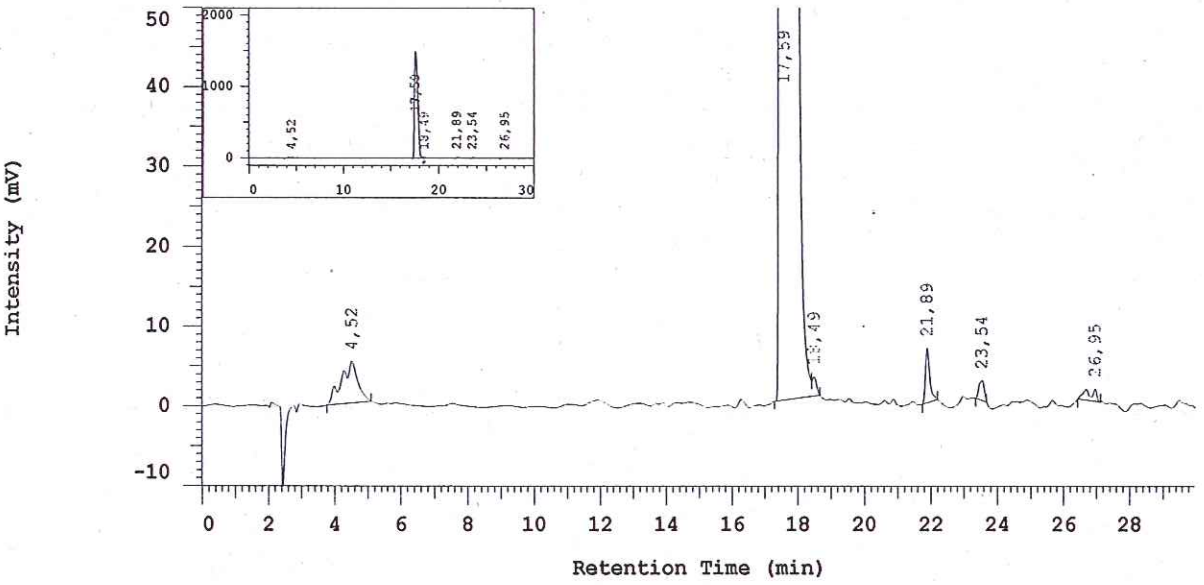
24b

HPLC

Analyzed: 19.02.09 04:57
Reported: 19.02.09 10:07
Processed: 19.02.09 10:07

Data Path: D:\WIN32APP\HSM\Christoph\DATA\2048\
Application: Christoph
Series:2048
Sample Name: R_48.1b
Vial Number: 12
Injection from this vial: 1 of 1
Vial Type: UNK
Volume: 5,0 ul

Chrom Type: HPLC Channel : 1



Acquisition Method: Chromni
Blank Subtr Sample Name: ACN
Column Type: 010
Developed by: Jens
Solvent A: Wasser + 0,05%TFA
Solvent B: ACN + 0,05%TFA

No.	RT	Area	Conc 1	BC
1	4,52	175238	0,536	MC
2	17,59	32393225	99,039	BV
3	18,49	21450	0,066	MC
4	21,89	60516	0,185	MC
5	23,54	27298	0,083	MC
6	26,95	29949	0,092	BB
		32707676	100,000	

Peak rejection level: 0