Supporting information

Diastereoselective synthesis of conformationally restricted KOR agonists

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1. Purity data of test compounds

1.1. Determination of purity by HPLC

Equipment: Pump: LPG-3600, degasser: DG-1210, autosampler: WPS 3000 PL, UV-detector: VWD-3400RS, interface: DIONEX UltiMate 3000, data acquisition: Chromeleon 7 (Thermo Fisher Scientific); column: LiChrospher® 60 RP-select B (5 µm), LiChroCART® 250-4 mm cartridge; flow rate: 1.0 mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: demineralized water with 0.05 % (V/V) trifluoroacetic acid, 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 %. Unless otherwise noted, the purity of all test compounds is higher than 95 %.

1.2. Determination of purity by quantitative NMR spectroscopy

The experimental conditions for the quantitative ^{1}H NMR analyses adhered to the guidelines of the Journal of Medicinal Chemistry.[1-3] An amount between 4 and 12 mg of analyte was weighed in an Eppendorf vial using the analytical scale. In the same vial an equimolar amount of 1,3,5-trimethoxybenzene (internal calibrant) was weighed. DMSO-d_6 (900 µL) was added using an Eppendorf pipette. The solution was transferred into a 5 mm standard NMR tube and submitted for the analysis. The NMR frequency for all the measurements was 600 MHz. The spectra were recorded at 25 °C.

Purity data of test compounds

compound	purity
33a	96 %
33b	95 %
35a	94 %
35b	98 %
36	99 %

2. NOE experiments

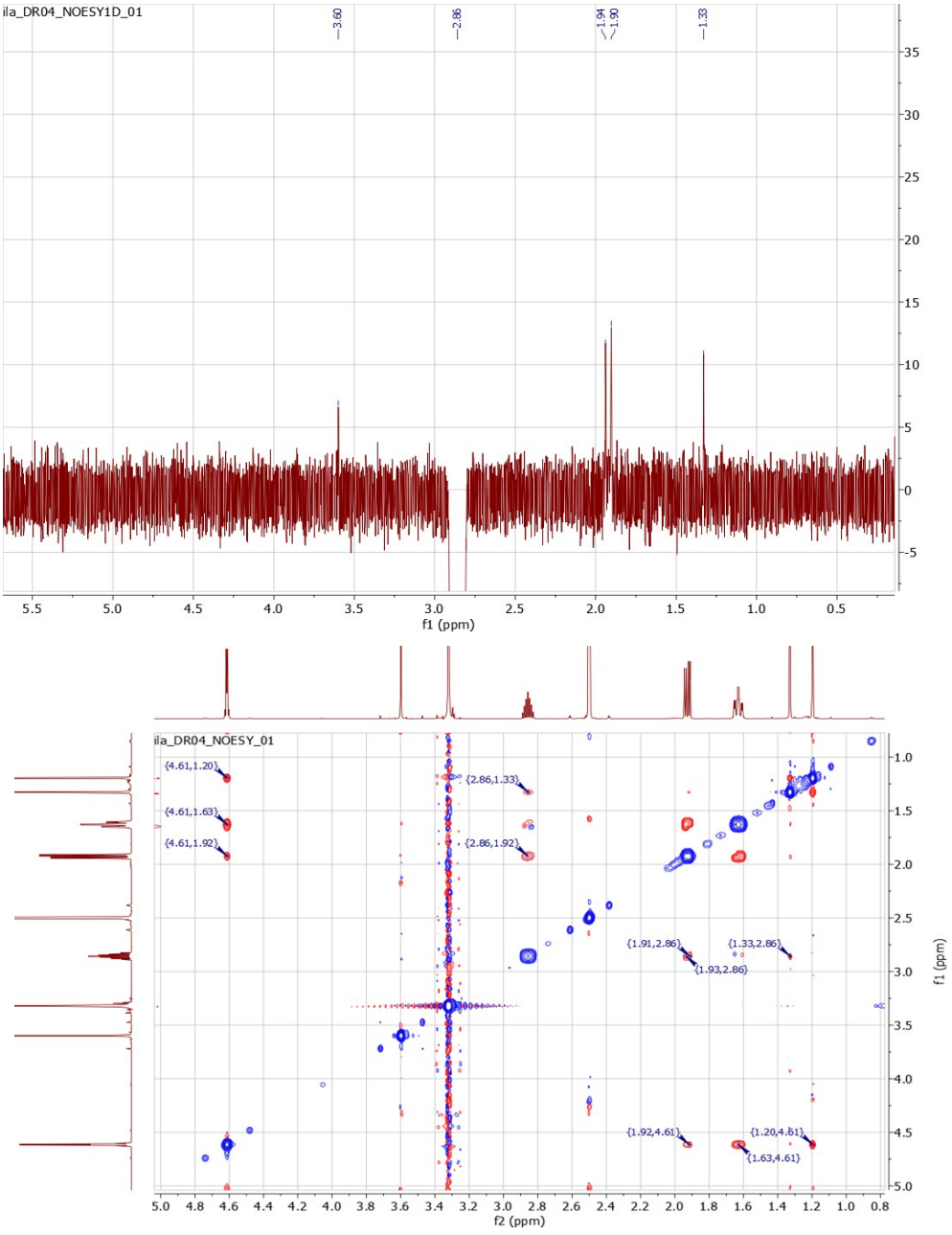


Figure S1: Top: NOE difference spectrum of ester **10**. Irradiation at 2.82 – 2.89 ppm (C*H*CO_2CH_3) led to an increased signal at 1.33 ppm (CH_3). Bottom: NOESY spectrum of ester **10**. Cross peaks between 2.86 ppm (C*H*CO_2CH_3) and 1.33 ppm (CH_3) on one side, and cross peaks between 4.61 ppm (OC*H*C*H*O) and 1.20 ppm (CH_3) on the other side of the molecule can be detected.

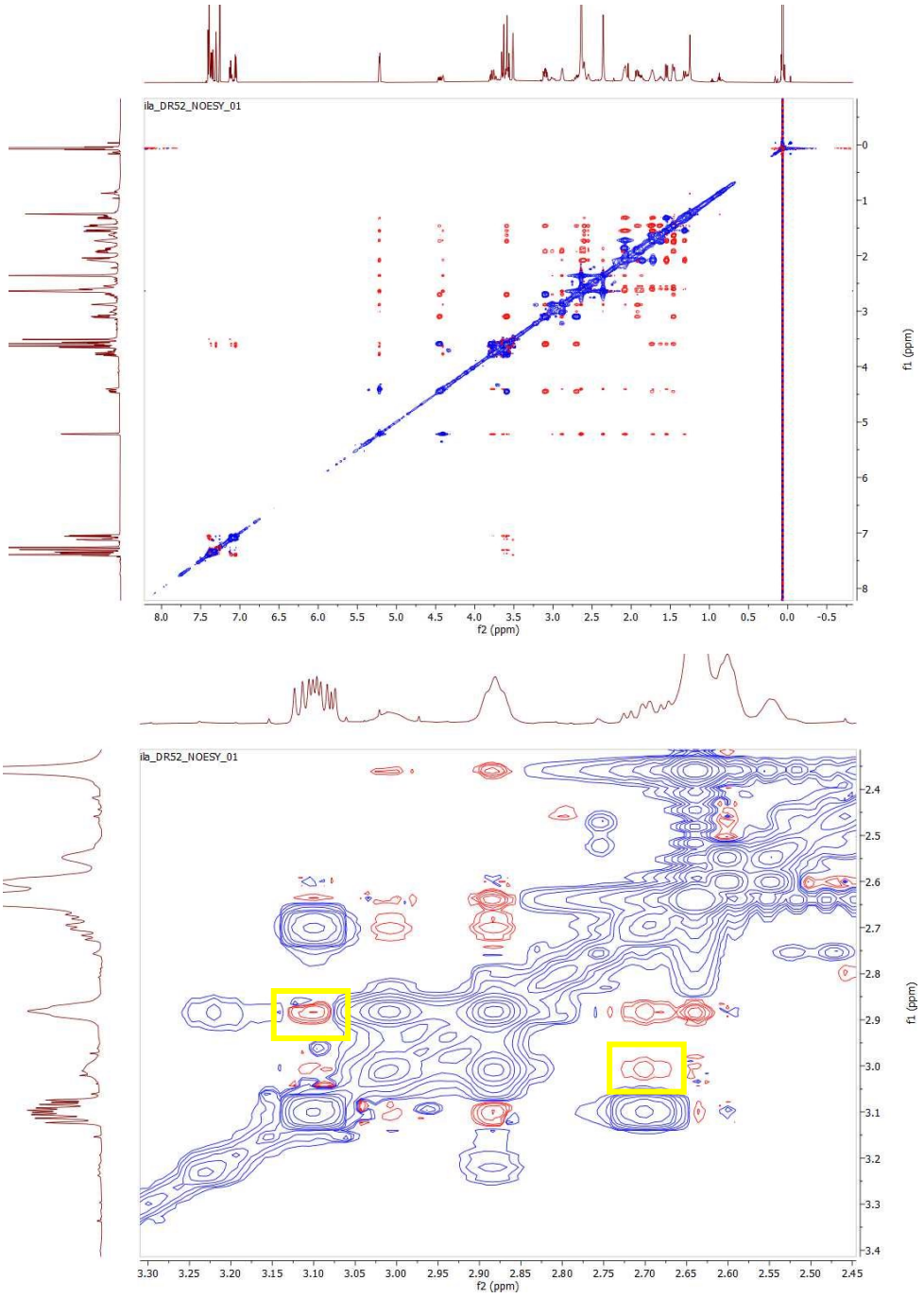


Figure S2: NOESY spectrum of bicyclic dimethylamine **35b**. Top: Complete NOESY spectrum. Bottom: Magnification of the region of interest. Cross peaks between 2.88 ppm (7-H) and 3.09 ppm (3-H_{2ax}) for the major rotational isomer, and cross peaks between 2.70 ppm (3-H_{2ax}) and 3.01 ppm (7-H) for the minor rotational isomer can be detected. The corresponding cross peaks are marked in yellow.

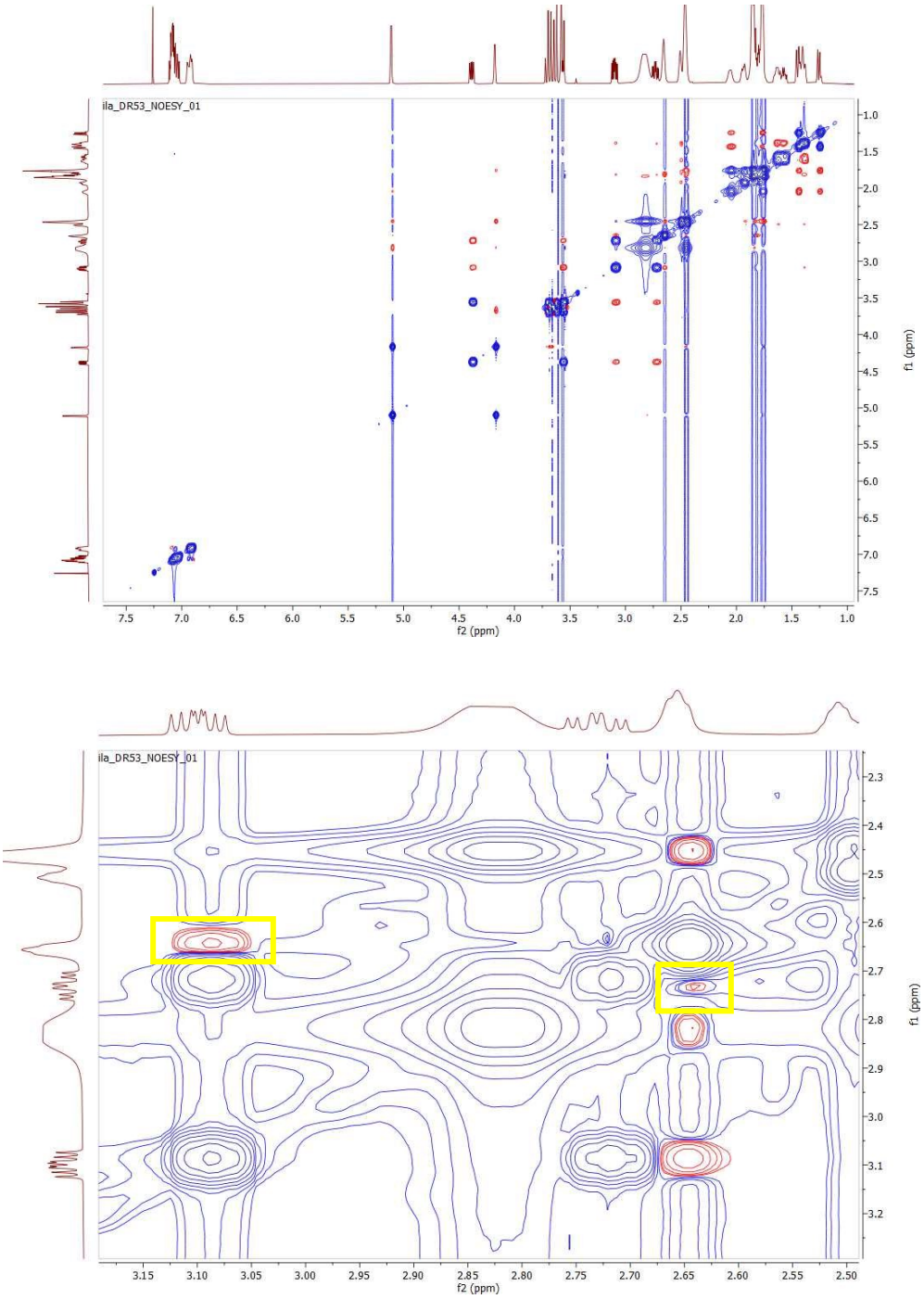
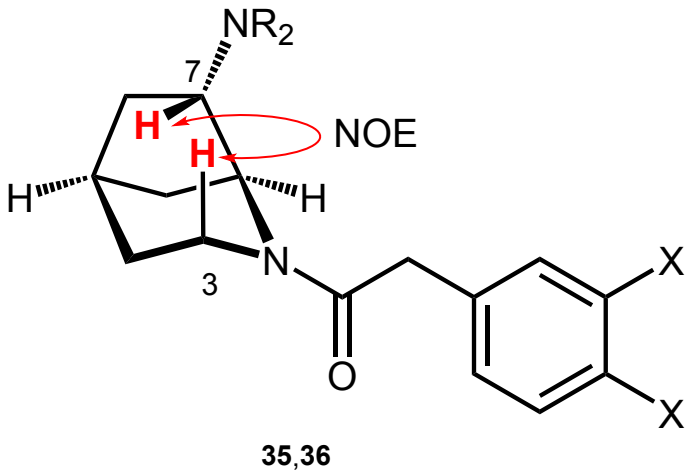


Figure S3: NOESY spectrum of bicyclic pyrrolidine **36**. Top: Complete NOESY spectrum. Bottom: Magnification of the region of interest. Cross peaks between 2.64 ppm (7-H) and 3.09 ppm (3-H_{2ax}) for the major rotational isomer, and cross peaks between 2.64 ppm (7-H) and 2.73 ppm (3-H_{2ax}) for the minor rotational isomer can be detected. The corresponding cross peaks are marked in yellow.



35,**36**

Figure S4: The proximity of 7-H and axially oriented 3-H led to a positive NOE.

3. Crystallography section

*X-Ray diffraction***:** Data sets for compound **28** were collected with a Nonius Kappa CCD diffractometer. Programs used: data collection, COLLECT;[4] data reduction Denzo-SMN;[5] absorption correction;[6] structure solution *SHELXT-2015*;[7] structure refinement *SHELXL-2015*[4] and graphics, *XP*.[8] *R*-values are given for observed reflections, and *w*R^2 values are given for all reflections.

*X-ray crystal structure analysis of **28** (dan9504):* A colorless prism-like specimen of $C_{19}H_{26}N_2O_4S$, approximate dimensions 0.180 mm x 0.200 mm x 0.240 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured. A total of 1689 frames were collected. The total exposure time was 22.38 h. The frames were integrated with the Bruker SAINT software package using a wide-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 10832 reflections to a maximum θ angle of 66.87° (0.84 Å resolution), of which 3169 were independent (average redundancy 3.418, completeness = 98.9%, R_{int} = 2.33%, R_{sig} = 2.31%) and 3057 (96.47%) were greater than 2σ(F^2). The final cell constants of a = 8.5936(2) Å, b = 9.1593(2) Å, c = 11.8002(3) Å, α = 97.4750(10)°, β = 95.7110(10)°, γ = 98.3790(10)°, volume = 904.42(4) $Å^3$, are based upon the refinement of the XYZ-centroids of 7670 reflections above 20 σ(I) with 7.611° < 2θ < 133.7°. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.914. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.6680 and 0.7350. The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group *P*-1, with Z = 2 for the formula unit, $C_{19}H_{26}N_2O_4S$. The final anisotropic full-matrix least-squares refinement on F^2 with 236 variables converged at R1 = 3.25%, for the observed data and wR2 = 8.42% for all data. The goodness-of-fit was 1.048. The largest peak in the final difference electron density synthesis was 0.370 $e^-/Å^3$ and the largest hole was -0.399 $e^-/Å^3$ with an RMS deviation of 0.049 $e^-/Å^3$. On the basis of the final model, the calculated density was 1.390 g/cm^3 and F(000), 404 e^-. CCDC number: 2063296.

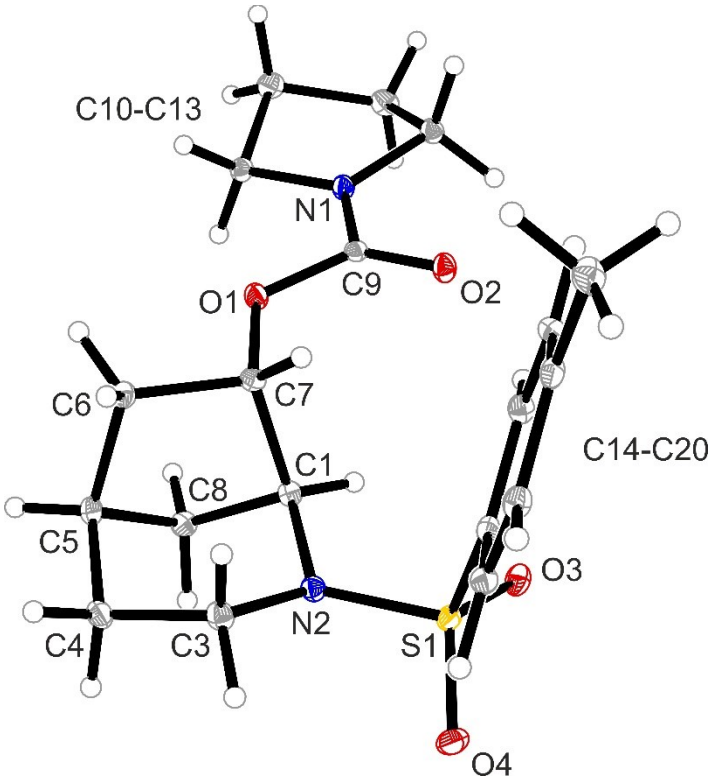


Figure S5: Crystal structure of bicyclic compound **28**.

Thermal ellipsoids are set at 30% probability.

4. Receptor binding studies

4.1 Materials

Guinea pig brains, rat brains and rat livers were commercially available (Harlan-Winkelmann, Borchen, 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).

4.2 Preparation of membrane homogenates from guinea pig brain[9-11]

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.

4.3 Preparation of membrane homogenates from rat brain[12-14]

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.

4.4 Preparation of membrane homogenates from rat liver[9-11]

2 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.

4.5 Protein determination

The protein concentration was determined by the method of Bradford,[15] modified by Stoscheck.[16] 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).

4.6 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.[17] The K_i values are given as mean value ± SEM from three independent experiments.

4.7. Performance of the KOR assay[12-14]

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.

4.8. Performance of the MOR assay[12-14]

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.

4.9. Performance of the DOR assay[12-14]

The assay was performed with the radioligand [^{3}H]DPDPE (69 Ci/mmol, BIOTREND). The thawed rat brain 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.

4.10. Performance of the σ_1 receptor assay[9-11]

The assay was performed with the radioligand [^{3}H]-(+)-pentazocine (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of guinea pig brain (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.[18]

4.11. Performance of the σ_2 receptor assay[9-11]

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.[19]

5. References

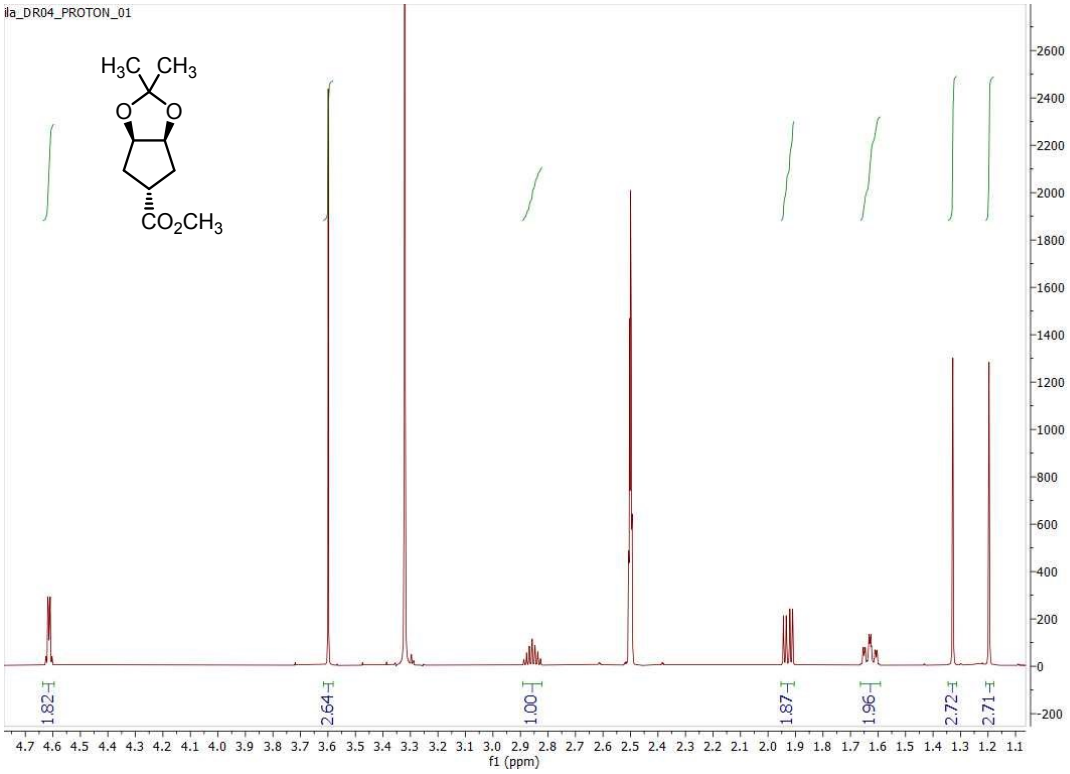
1. Pauli, G. F.; Chen, S.-N.; Simmler, C.; Lankin, D. C.; Gödecke, T.; Jaki, B. U.; Friesen, J. B.; McAlpine, J. B.; Napolitano, J. G. Importance of purity evaluation and the potential of quantitative ^{1}H NMR as a purity assay. *J. Med. Chem.* **2014,** *57*, 9220–9231.
2. Holzgrabe, U.; Deubner, R.; Schollmayer, C.; Waibel, B. Quantitative NMR spectroscopy--applications in drug analysis. *J. Pharm. Biomed. Anal.* **2005,** *38*, 806–812.
3. Bharti, S. K.; Roy, R. Quantitative 1H NMR spectroscopy. *TrAC Trends in Analytical Chemistry* **2012**, *35,* 5–26.
4. Hooft, R. W. W., Nonius B. V., *COLLECT, Program for Collecting Data on CCD Area Detectors*, **1998**, Delft, The Netherlands.
5. Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, 276, 307 – 326.
6. Otwinowski, Z.; Borek, D.; Majewski, W.; Minor, W. *Acta Crystallogr. Sect. A* **2003**, *59*, 228 – 234.
7. Sheldrick, G. M., *SHELXT – Integrated space-group and crystal-structure determination*, *Acta Cryst.,* **2015**, *A71*, 3-8.
8. *XP – Interactive molecular graphics, Version 5.1,* Bruker AXS Inc., Madison, Wisconsin, USA, **1998**.
9. Hasebein, P.; Frehland, B.; Lehmkuhl, K.; Fröhlich, R.; Schepmann, D.; Wünsch, B. Synthesis and pharmacological evaluation of like- and unlike-configured tetrahydro-2-benzazepines with the α-substituted benzyl moiety in the 5-position. *Org. Biomol. Chem.* **2014**, *12* (29), 5407–5426.
10. Meyer, C.; Neue, B.; Schepmann, D.; Yanagisawa, S.; Yamaguchi, J.; Würthwein, E.-U.; Itami, K.; Wünsch, B. Improvement of σ1 receptor affinity by late-stage C-H-bond arylation of spirocyclic lactones. *Bioorg. Med. Chem.* **2013**, *21* (7), 1844–1856.
11. Miyata, K.; Schepmann, D.; Wünsch, B. Synthesis and σ receptor affinity of regioisomeric spirocyclic furopyridines. *Eur. J. Med. Chem.* **2014***, 83*, 709–716.
12. Wittig, C.; Schepmann, D.; Soeberdt, M.; Daniliuc, C. G.; Wünsch, B. Stereoselective Synthesis of Conformationally Restricted KOR Agonists Based on the 2,5-diazabicyclo[2.2.2]octane Scaffold. *Org. Biomol. Chem.* **2017**, *15* (31), 6520–6540.
13. Geiger, C.; Zelenka, C.; Lehmkuhl, K.; Schepmann, D.; Englberger, W.; Wünsch, B.

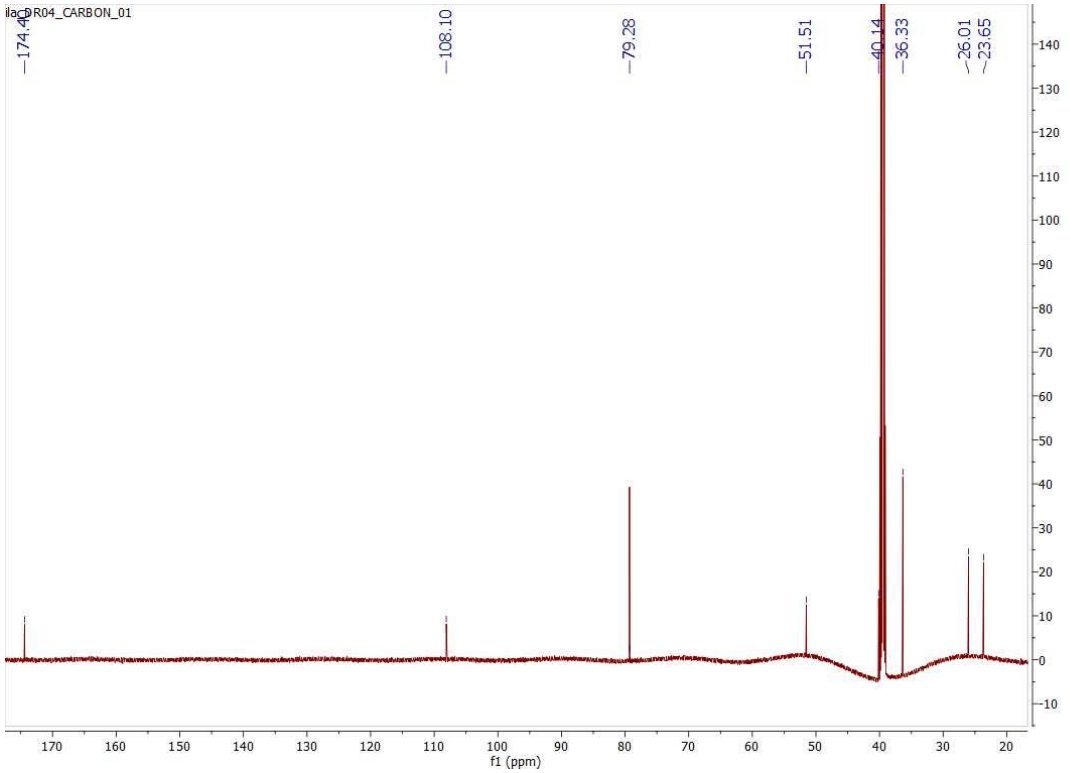
Conformationally Constrained K Receptor Agonists: Stereoselective Synthesis and Pharmacological Evaluation of 6,8-Diazabicyclo[3.2.2]nonane Derivatives. *J. Med. Chem* **2010**, *53*, 4212–4222.

14. Kracht, D.; Rack, E.; Schepmann, D.; Fröhlich, R.; Wünsch, B. Stereoselective Synthesis and Structure–affinity Relationships of Bicyclic κ Receptor Agonists. *Org. Biomol. Chem.* **2010**, *8(1),* 212–225.
15. Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.
16. Stoscheck, C. Quantitation of Protein. *Methods Enzymol.* **1990**, *182*, 50–68.
17. Yung-Chi, C.; Prusoff, W. H. Relationship between the Inhibition Constant (KI) and the Concentration of Inhibitor Which Causes 50 per Cent Inhibition (I50) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22* (23), 3099–3108.
18. DeHaven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. Characterization of the Binding of ([^{3}H]+)-Pentazocine to Sigma Recognition Sites in Guinea Pig Brain. *Eur. J. Pharmacol.* **1992**, *227* (4), 371–378.
19. Mach, R. H.; Smith, C. R.; Childers, S. R. Ibogaine Possesses a Selective Affinity for Sigma 2 Receptors. *Life Sci.* **1995**, *57* (4), PL57-62.

6. NMR spectra

10





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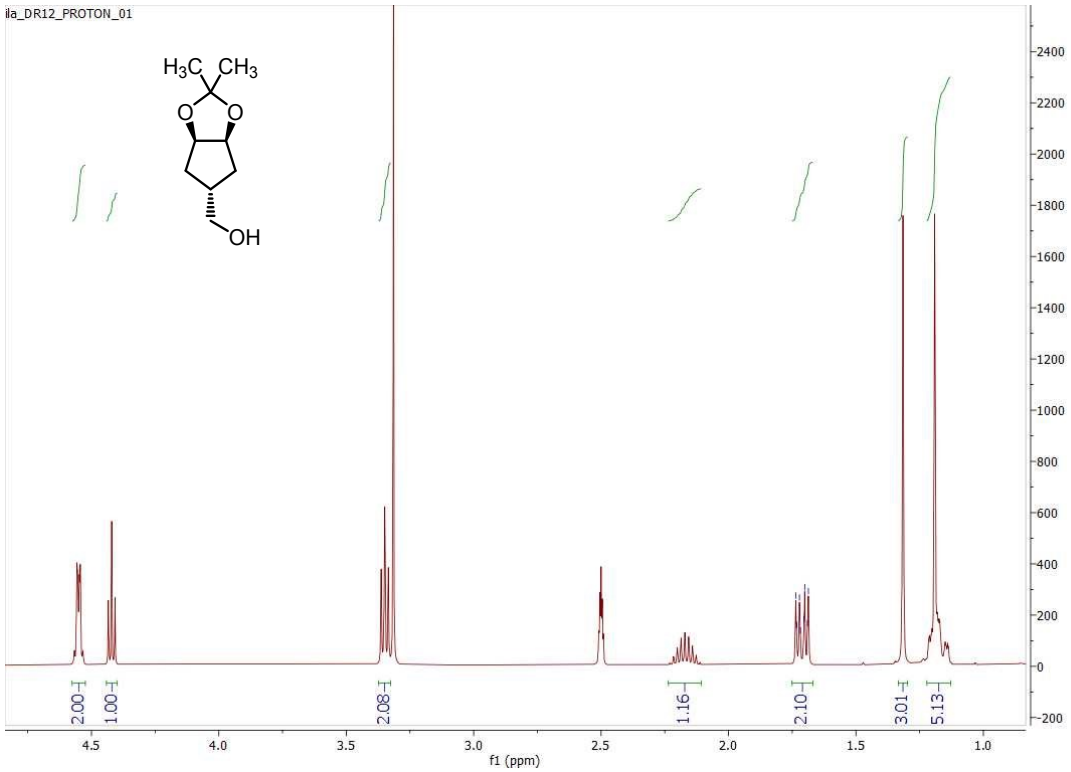
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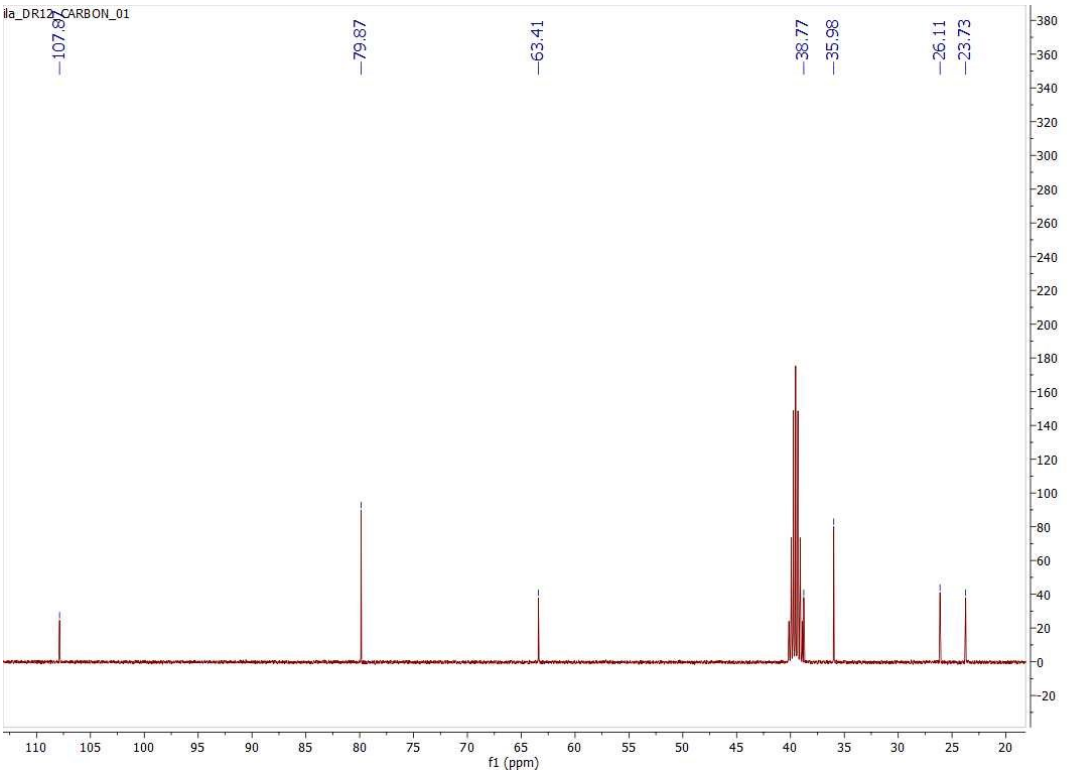
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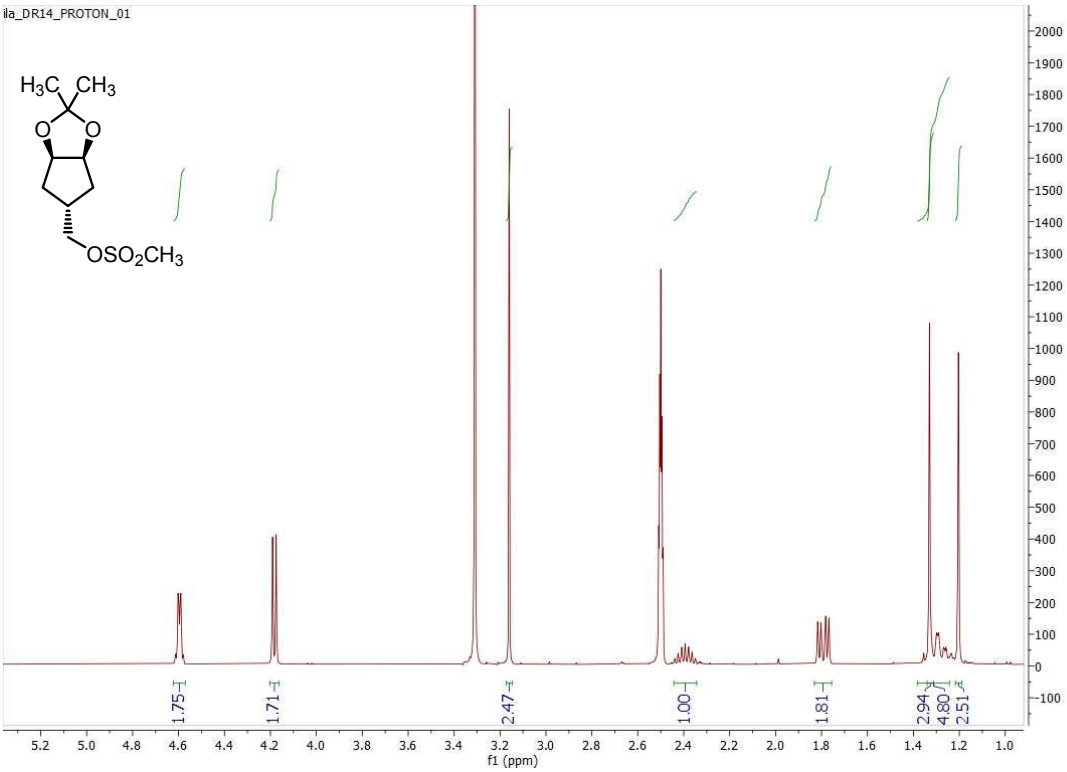
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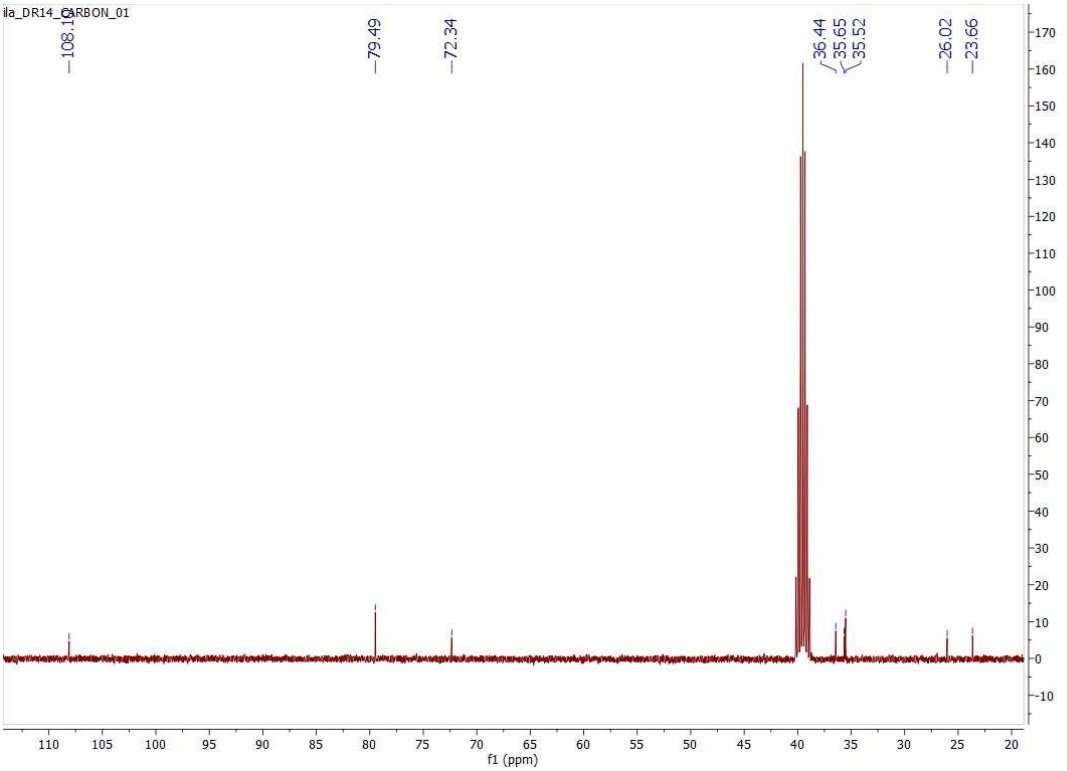
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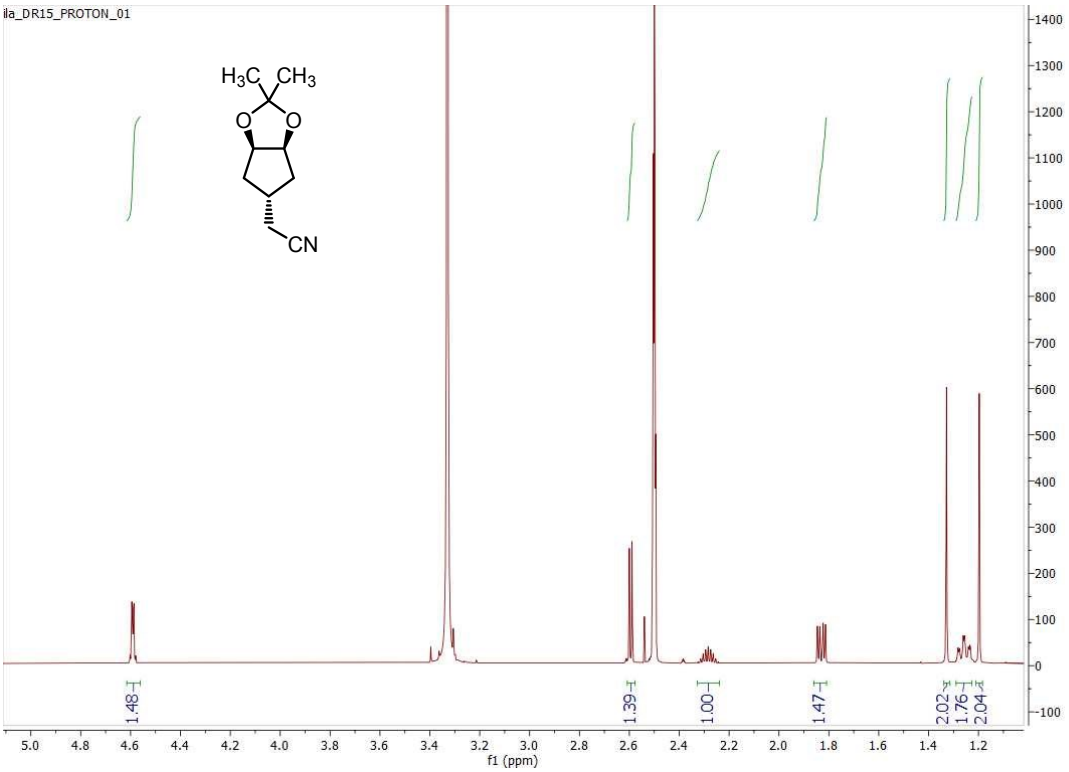
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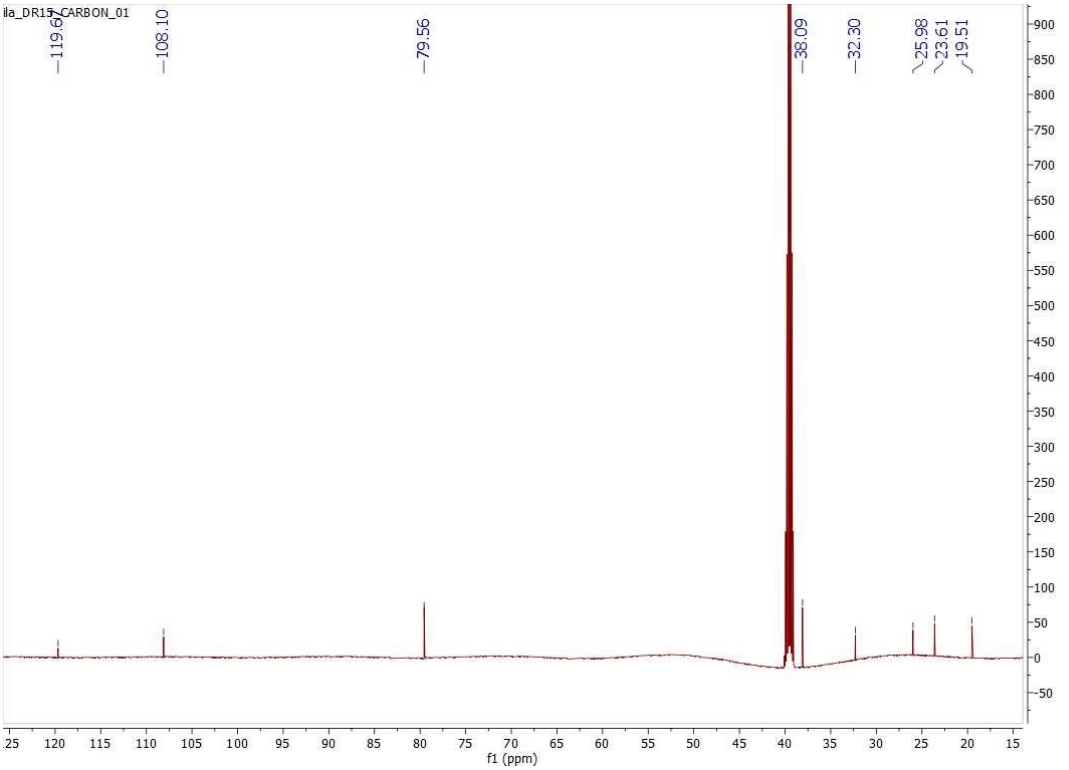
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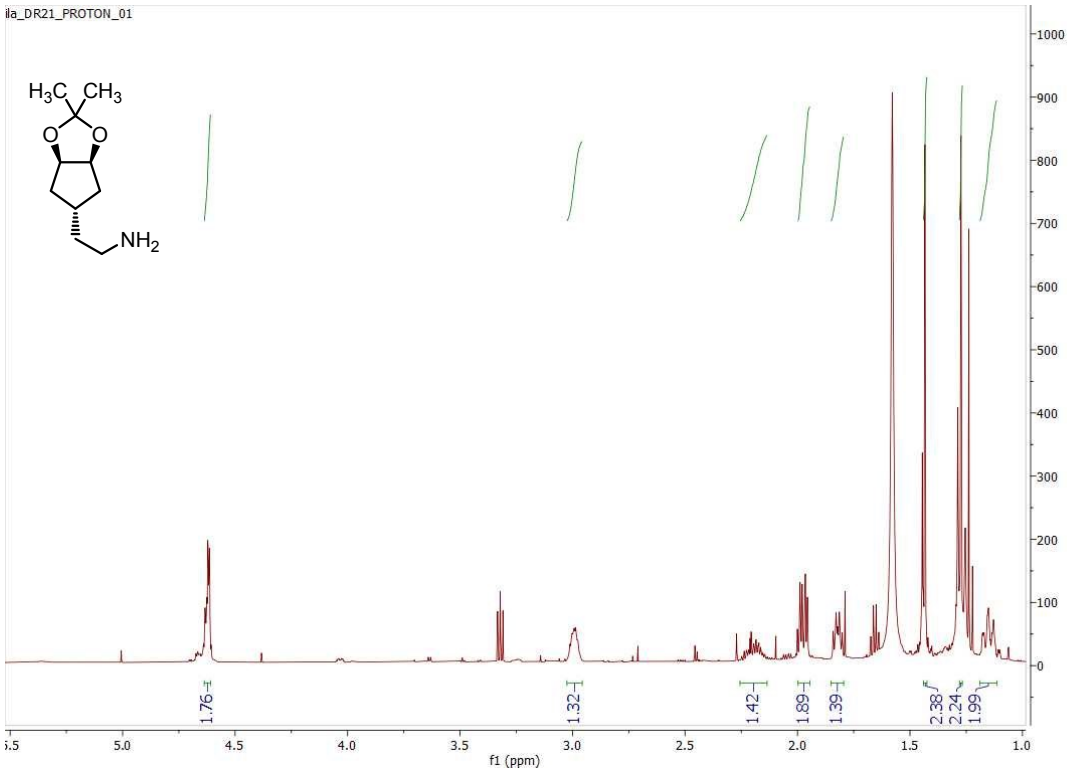
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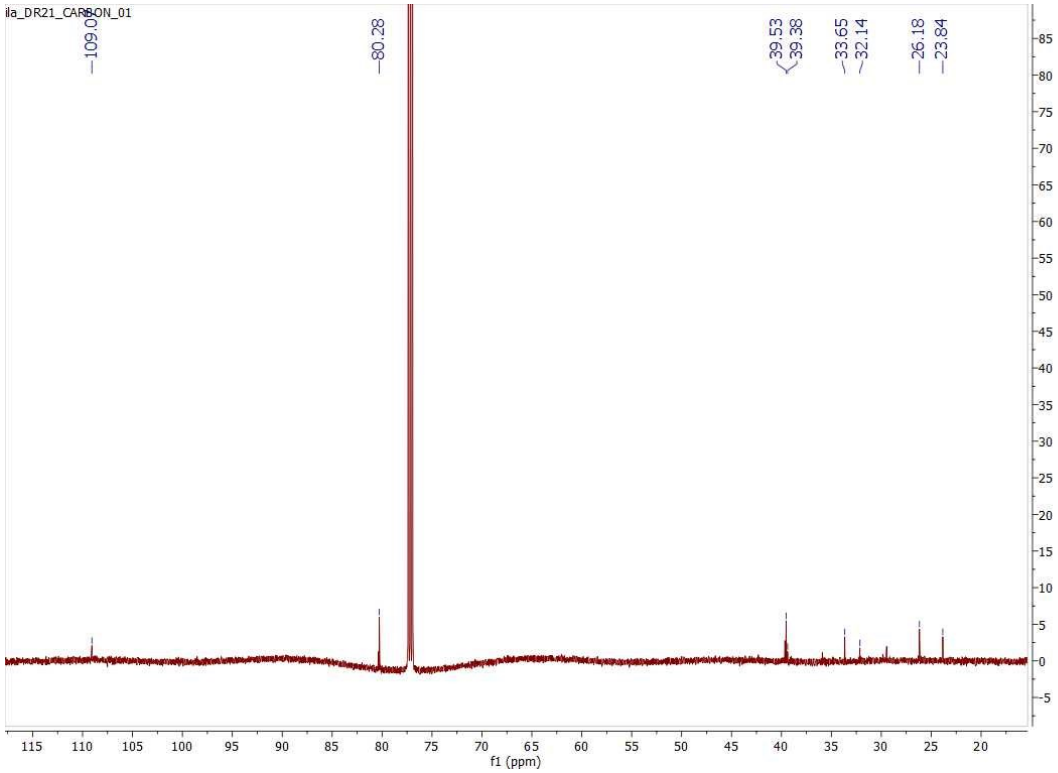
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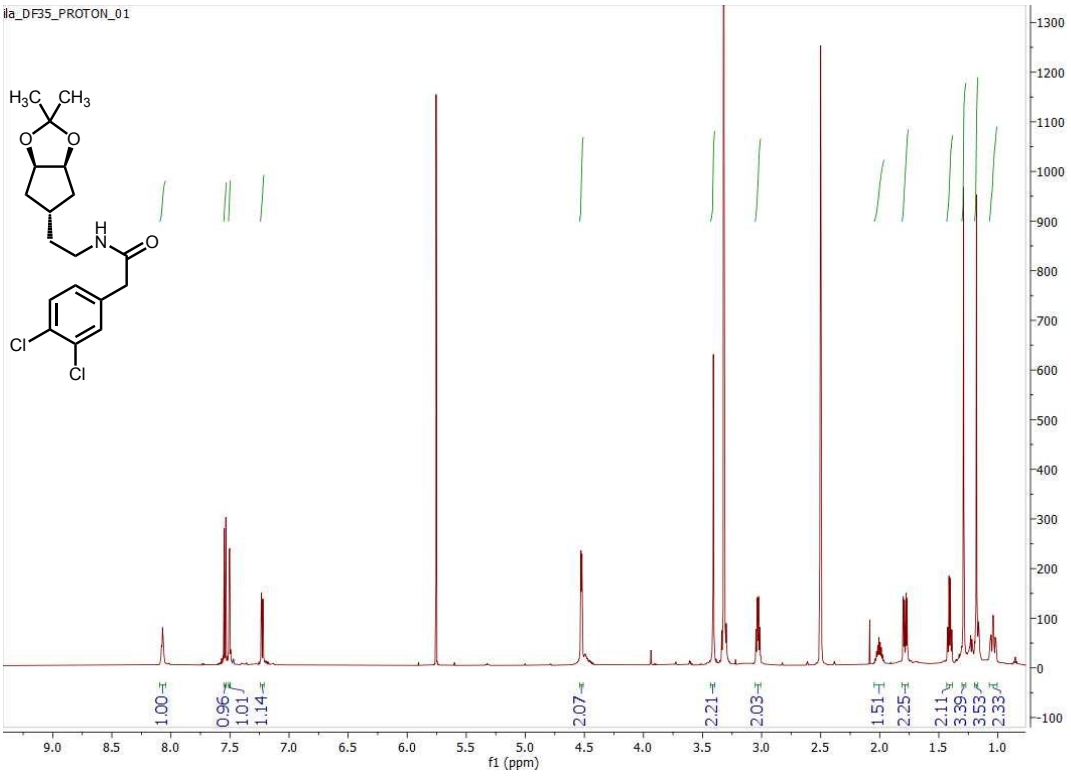
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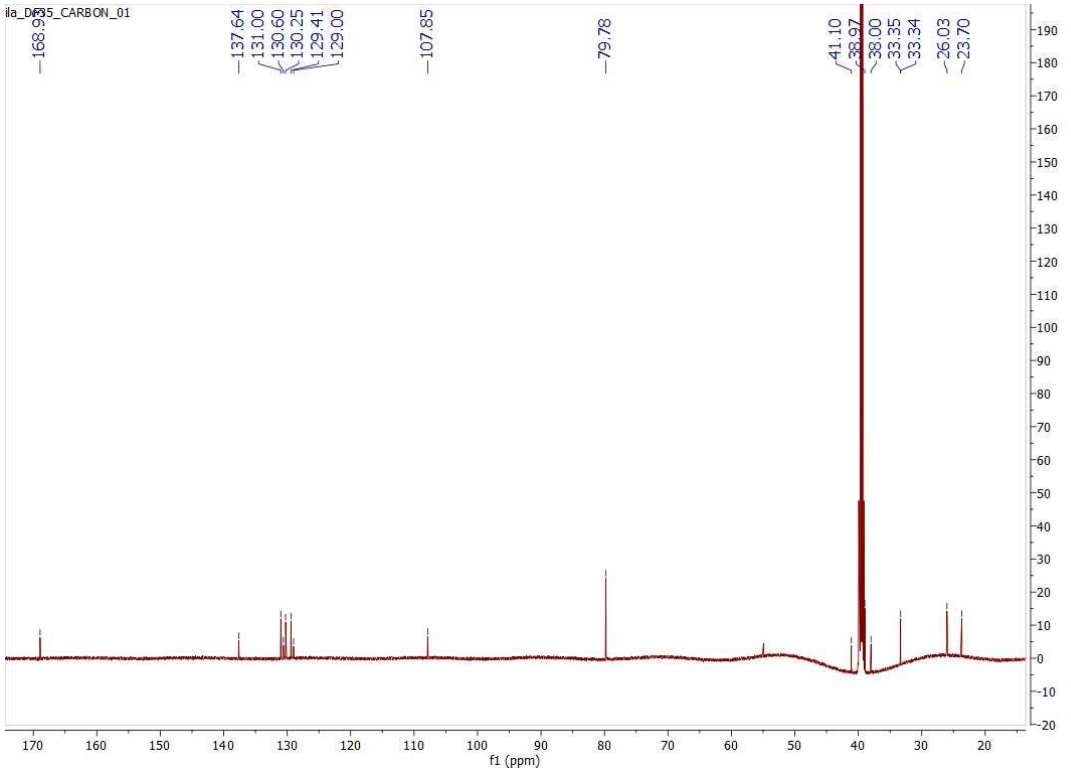
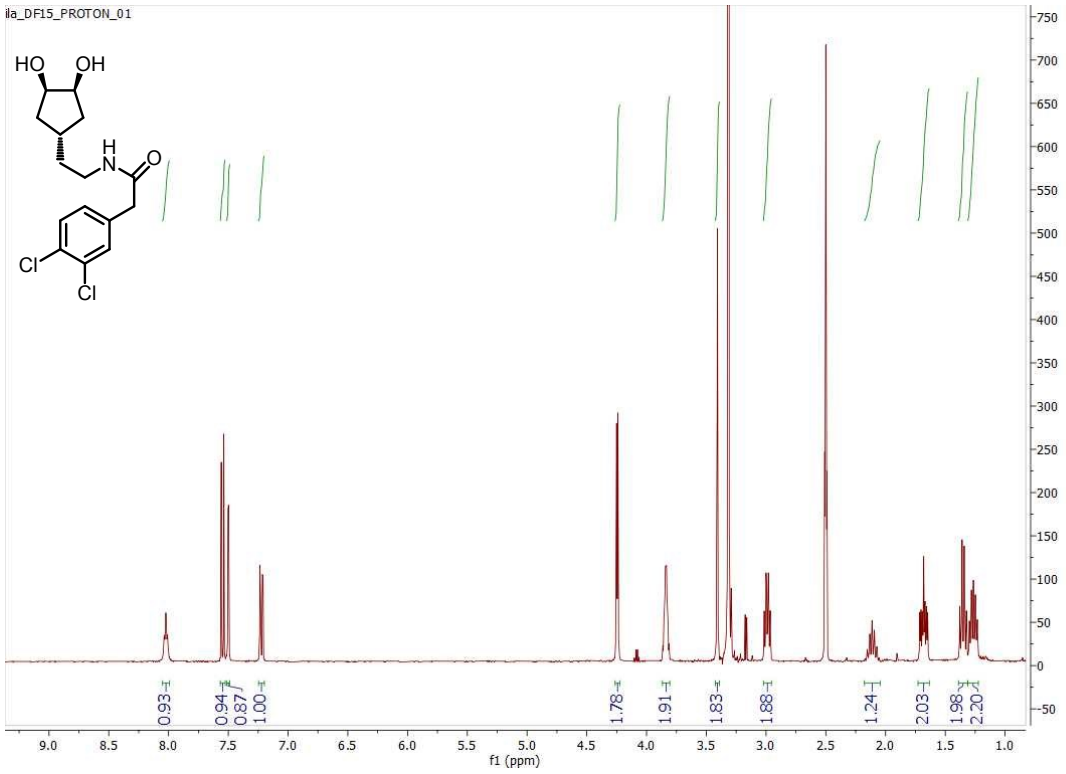
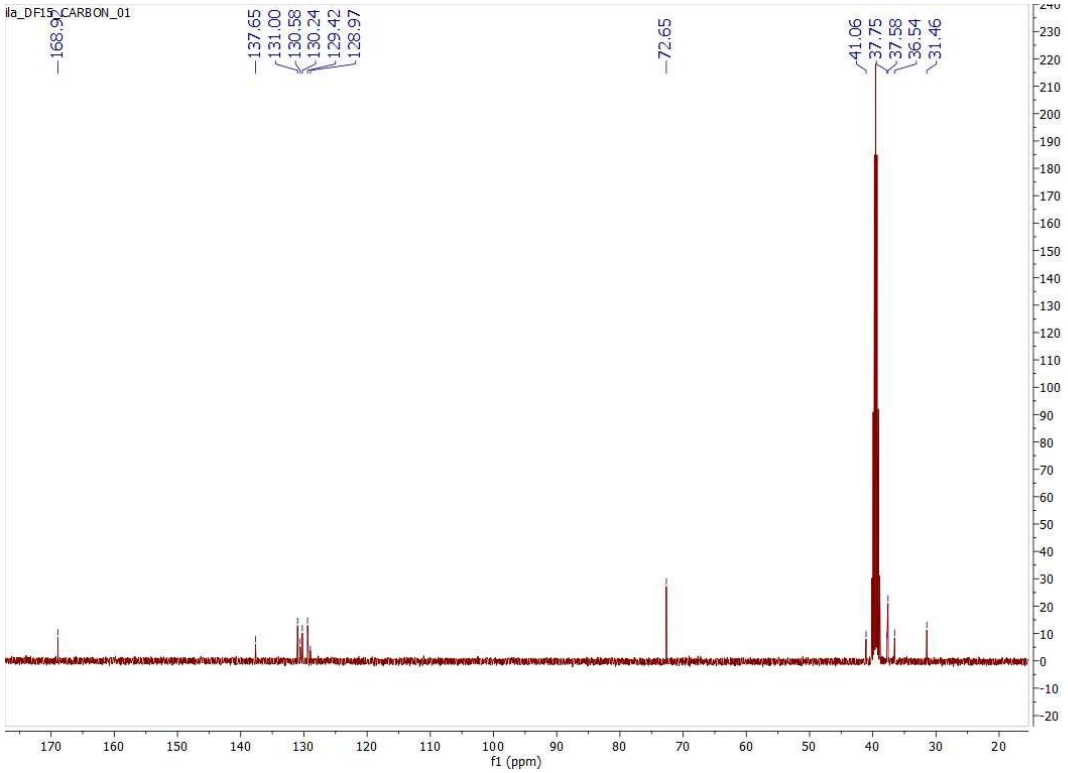
H3C CH3
O
O
H
N
O
Cl
Cl
1.00
0.96
1.01
1.14
2.07
2.21
2.03
1.51
2.25
2.11
3.39
3.53
2.33
f1 (ppm)

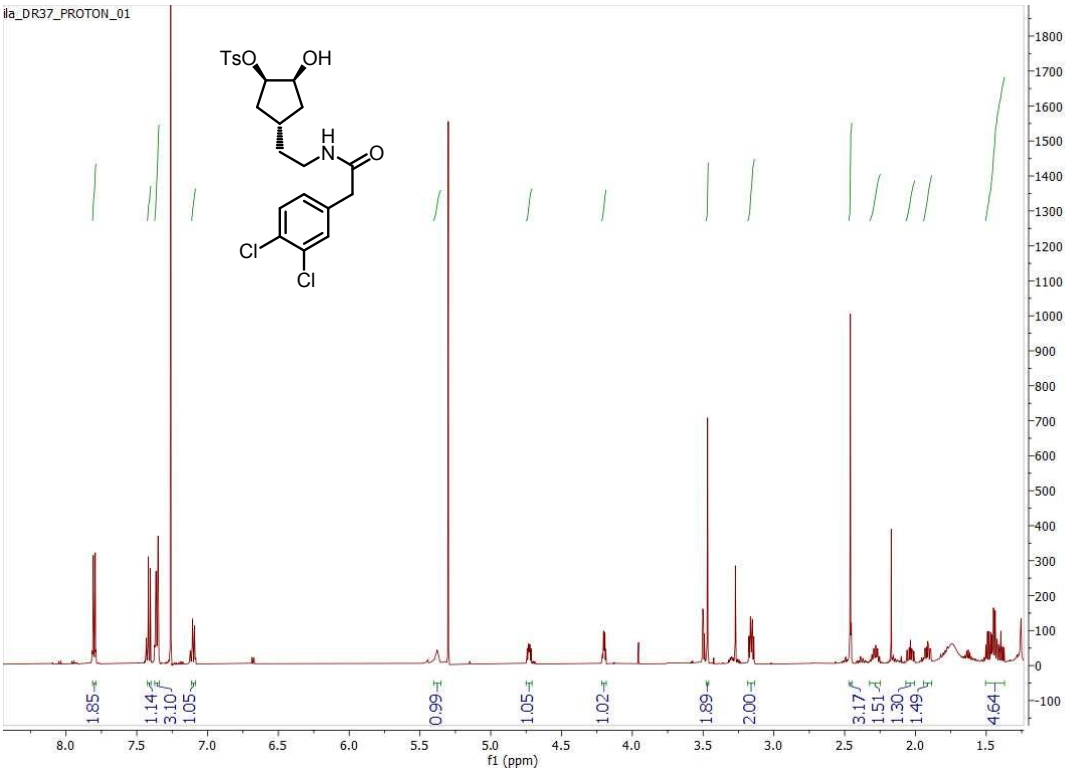

ila_DF35_CARBON_01
168.99
137.64
131.00
130.60
130.25
129.41
129.00
107.85
79.78
41.10
38.97
38.00
33.35
33.34
26.03
23.70
f1 (ppm)

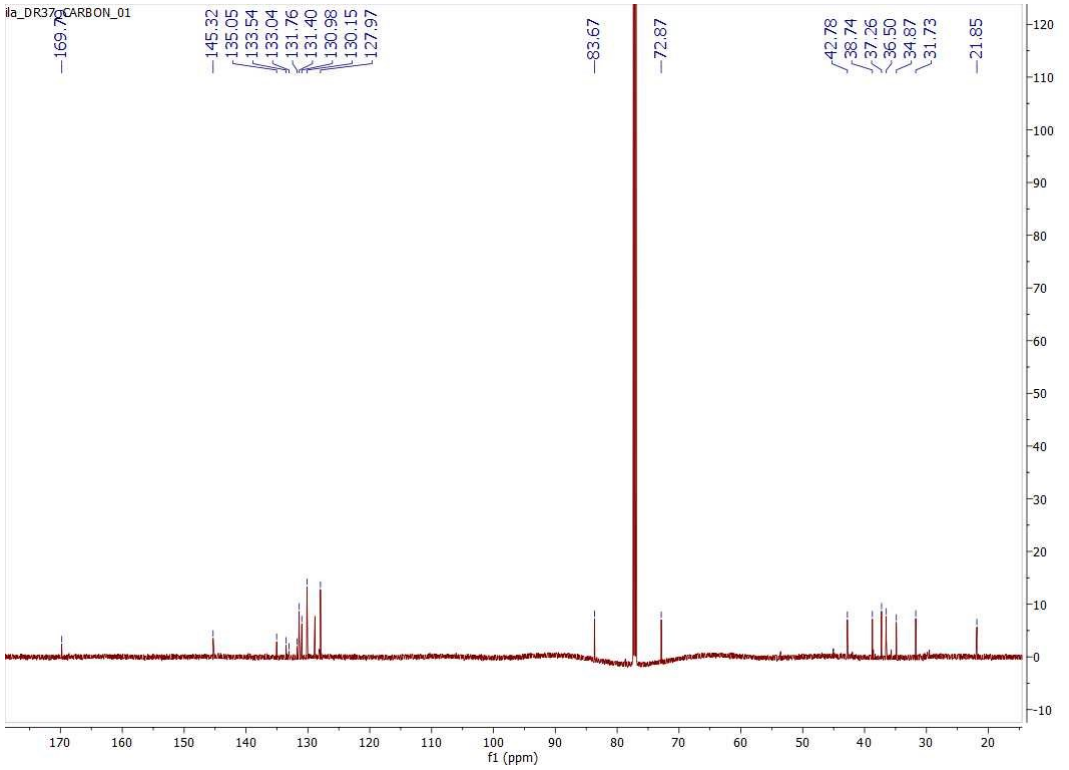
16


ila_DF15_PROTON_01
HO
OH
H
N
O
Cl
Cl
0.93
0.94
0.87
1.00
1.78
1.91
1.83
1.88
1.24
2.03
1.98
2.20
f1 (ppm)

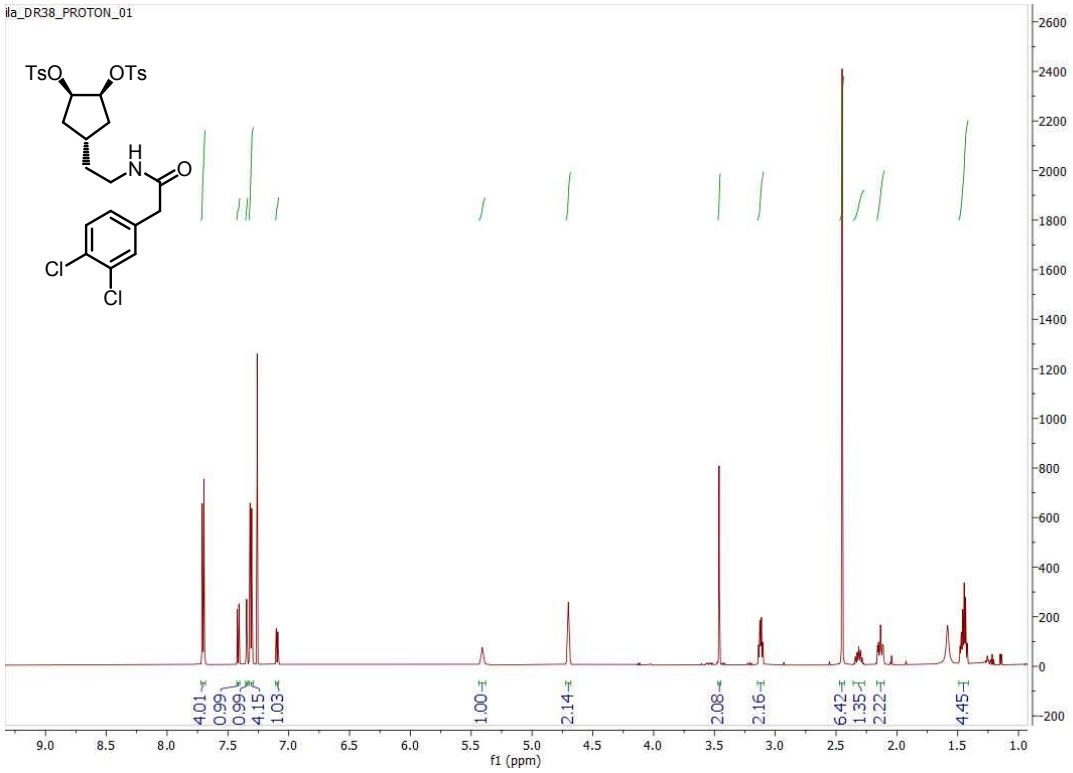

ila_DF15_CARBON_01
168.9
137.65
131.00
130.58
130.24
129.42
128.97
72.65
41.06
37.75
37.58
36.54
31.46
f1 (ppm)

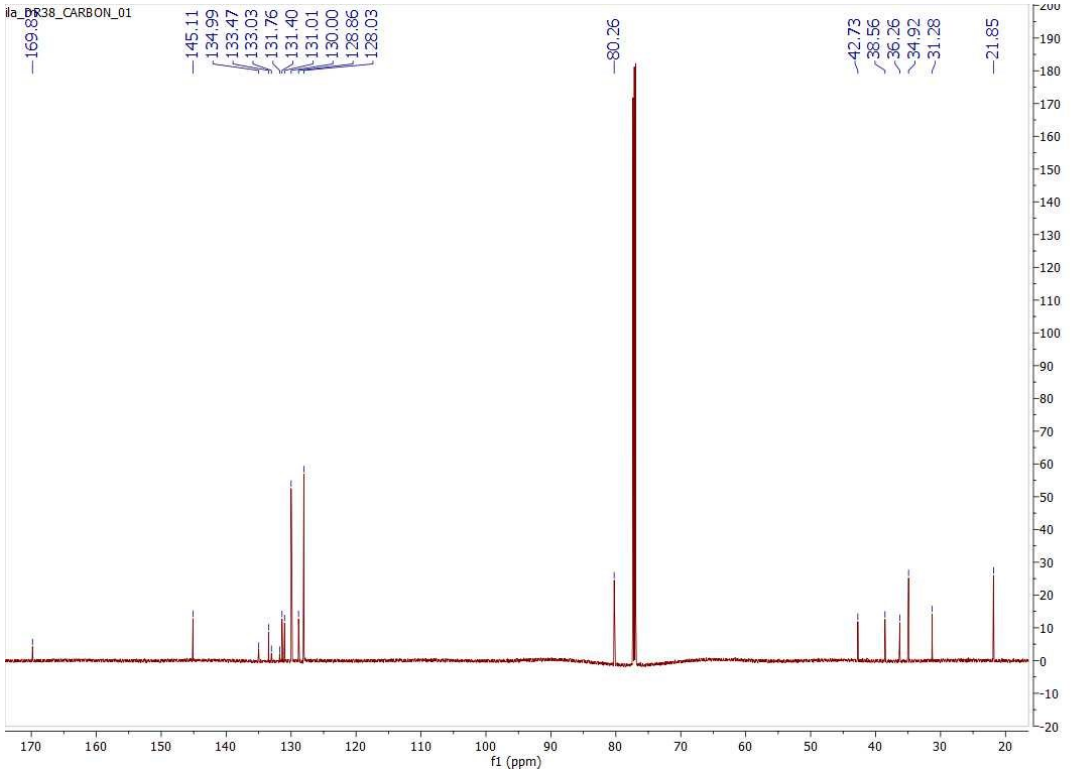
17


ila_DR37_PROTON_01
TsO
OH
H
N
O
Cl
Cl
1.85
1.14
3.10
1.05
0.99
1.05
1.02
1.89
2.00
3.17
1.51
1.30
1.49
4.64
f1 (ppm)

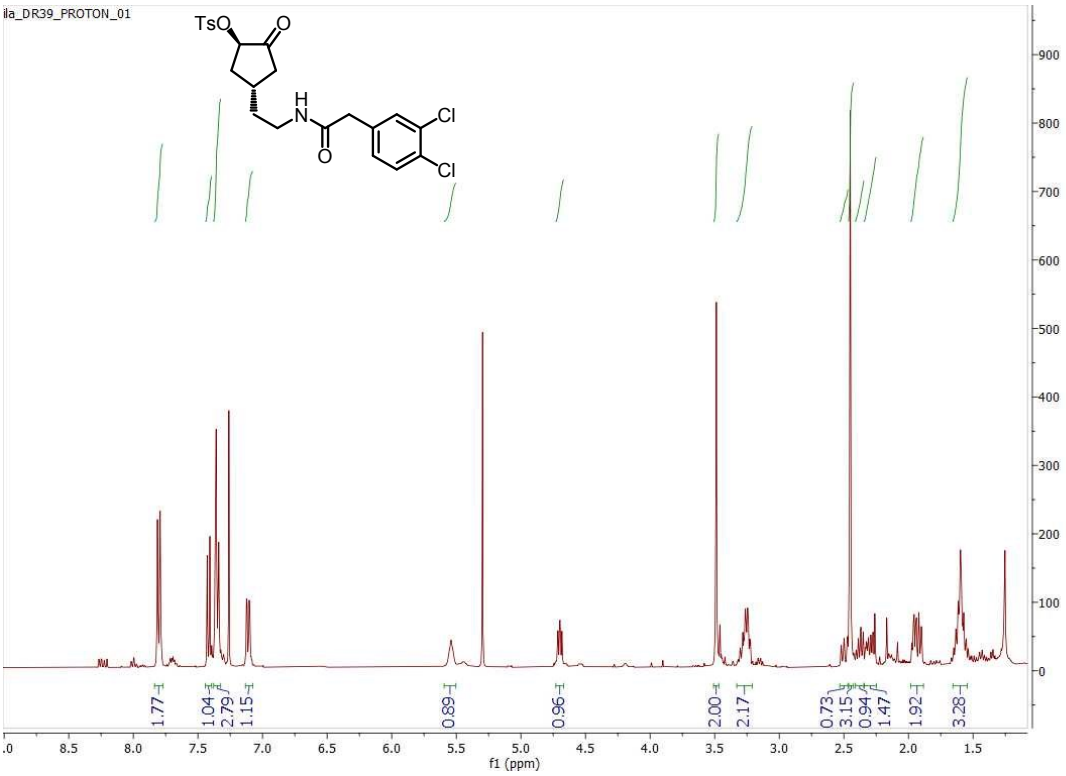

ila_DR37_CARBON_01
169.70
145.32
135.05
133.54
133.04
131.76
131.40
130.98
130.15
127.97
83.67
72.87
42.78
38.74
37.26
36.50
34.87
31.73
21.85
f1 (ppm)

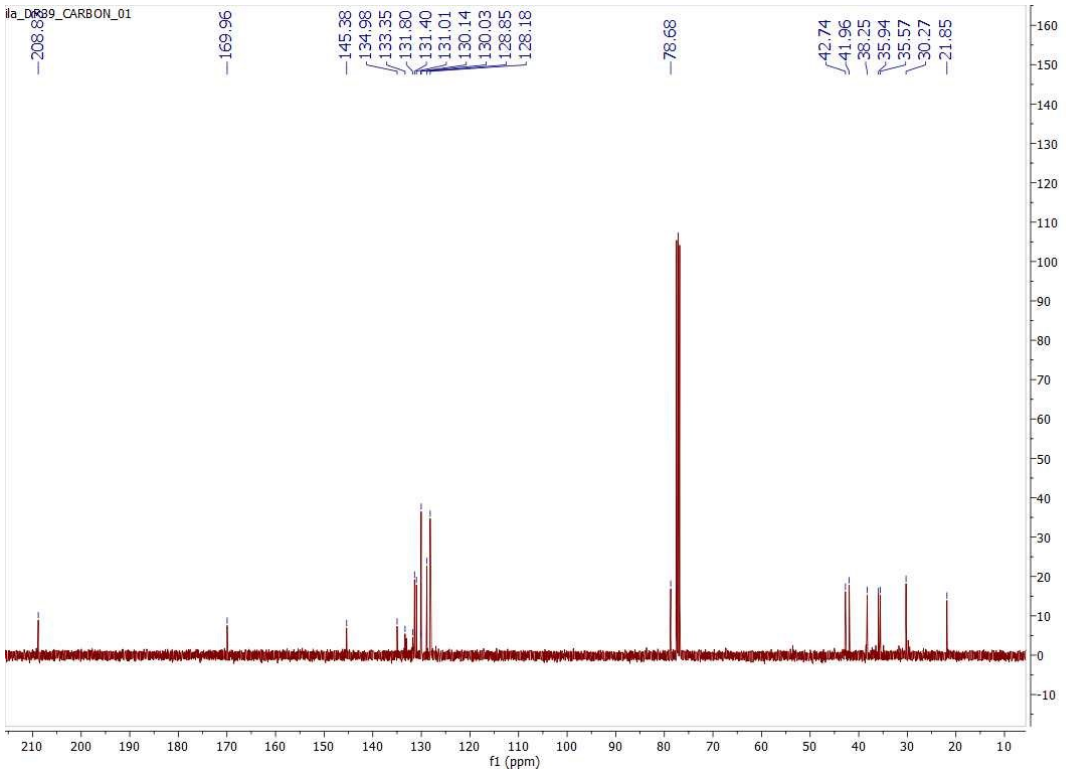
18


ila_DR38_PROTON_01
TsO
OTs
H
N
O
Cl
Cl
4.01
0.99
0.99
4.15
1.03
1.00
2.14
2.08
2.16
6.42
1.35
2.22
4.45
f1 (ppm)

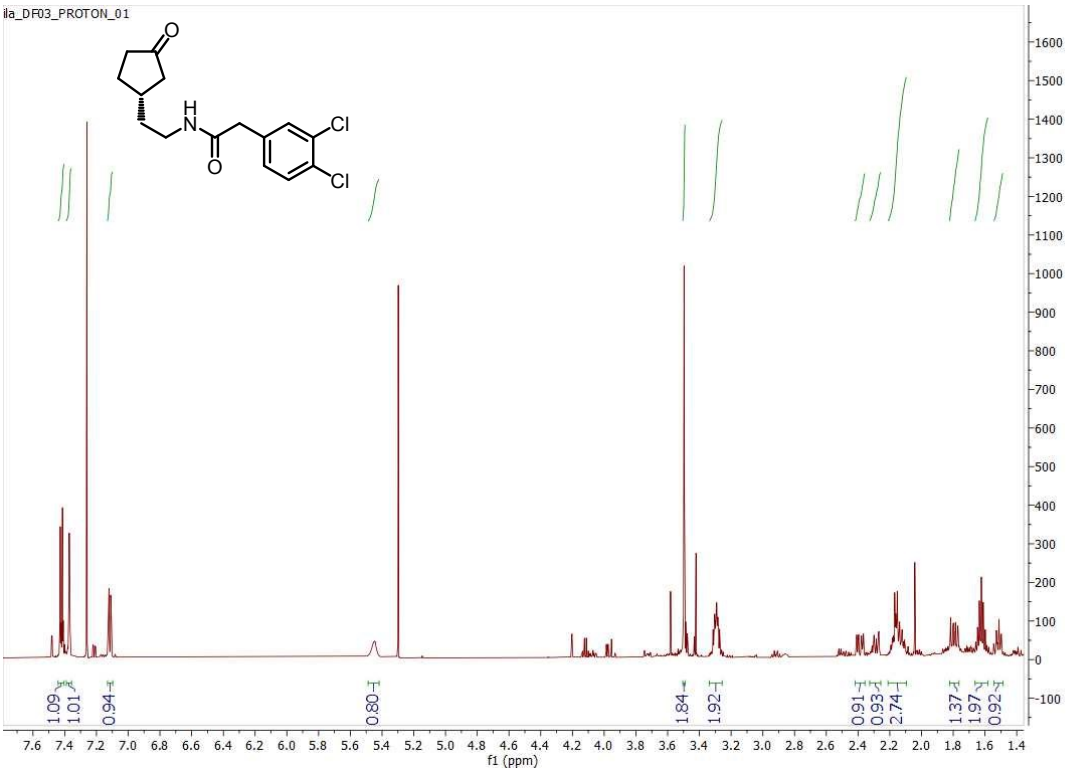

ila_DR38_CARBON_01
169.89
145.11
134.99
133.47
133.03
131.76
131.40
131.01
130.00
128.86
128.03
80.26
42.73
38.56
36.26
34.92
31.28
21.85
f1 (ppm)

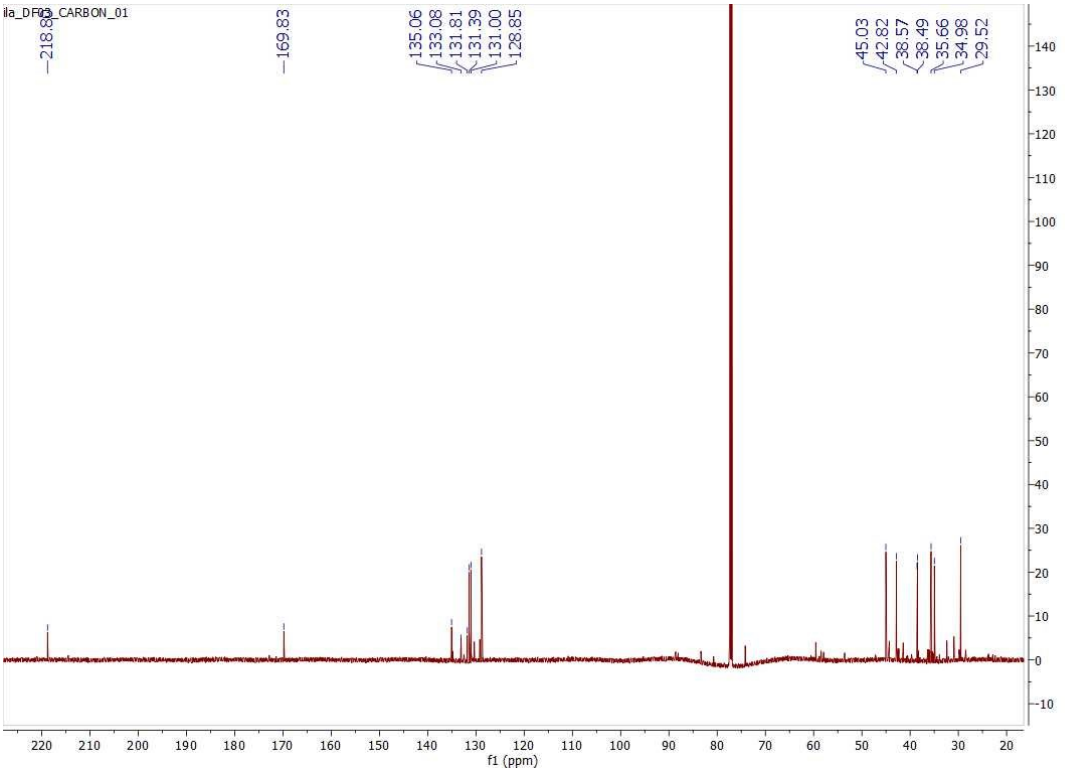
20


ila_DR39_PROTON_01
TsO
O
H
N
O
Cl
Cl
1.77
1.04
2.79
1.15
0.89
0.96
2.00
2.17
0.73
3.15
0.94
1.47
1.92
3.28
f1 (ppm)

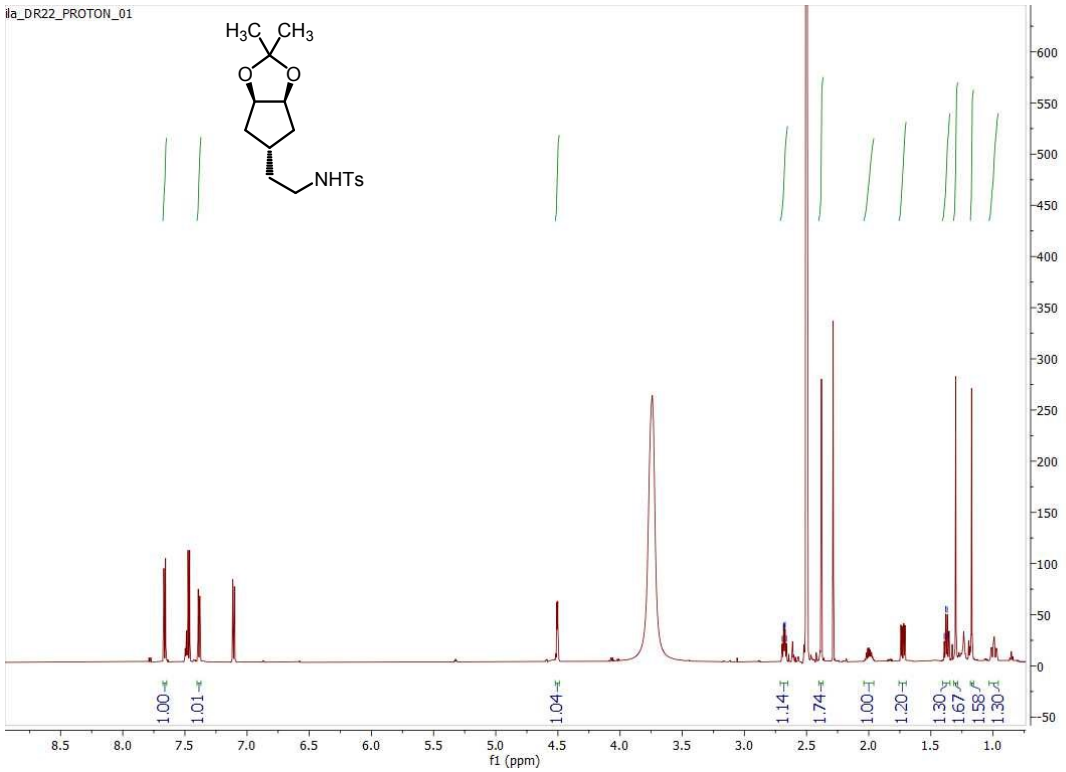

ila_DR39_CARBON_01
208.88
169.96
145.38
134.98
133.35
131.80
131.40
131.01
130.14
130.03
128.85
128.18
78.68
42.74
41.96
38.25
35.94
35.57
30.27
21.85
f1 (ppm)

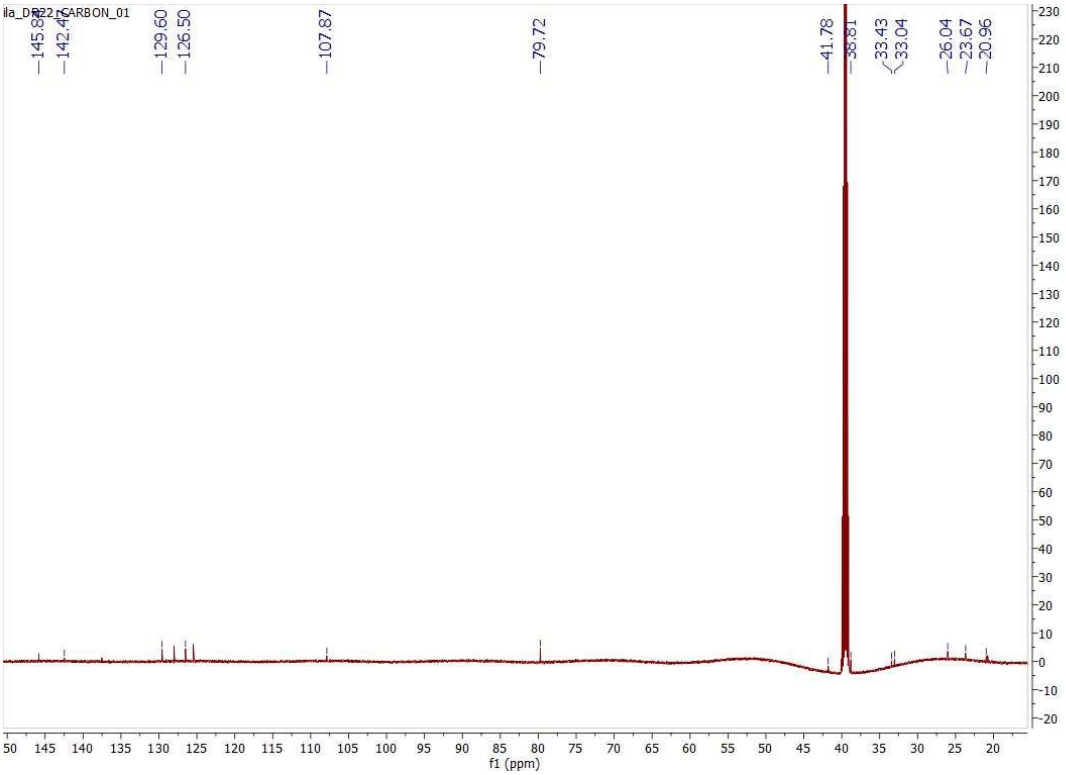
22


ila_DF03_PROTON_01
O
H
N
O
Cl
Cl
1.09
1.01
0.94
0.80
1.84
1.92
0.91
0.93
2.74
1.37
1.97
0.92
f1 (ppm)

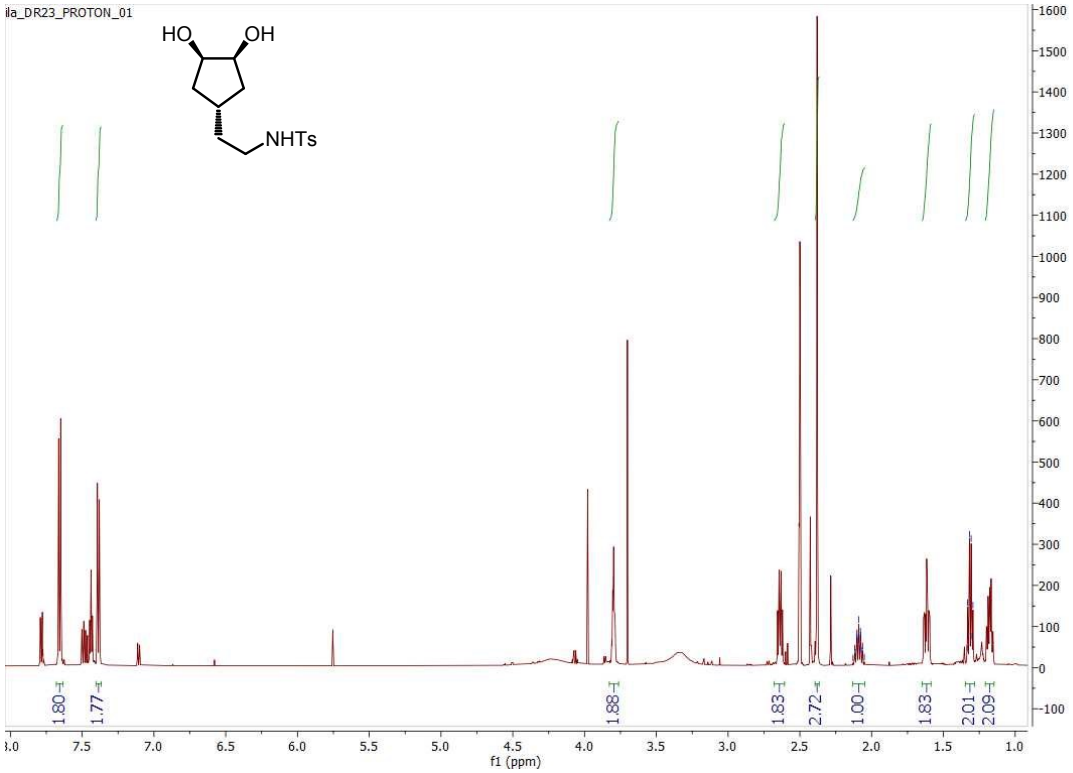

ila_DF03_CARBON_01
218.80
169.83
135.06
133.08
131.81
131.39
131.00
128.85
45.03
42.82
38.57
38.49
35.66
34.98
29.52
f1 (ppm)

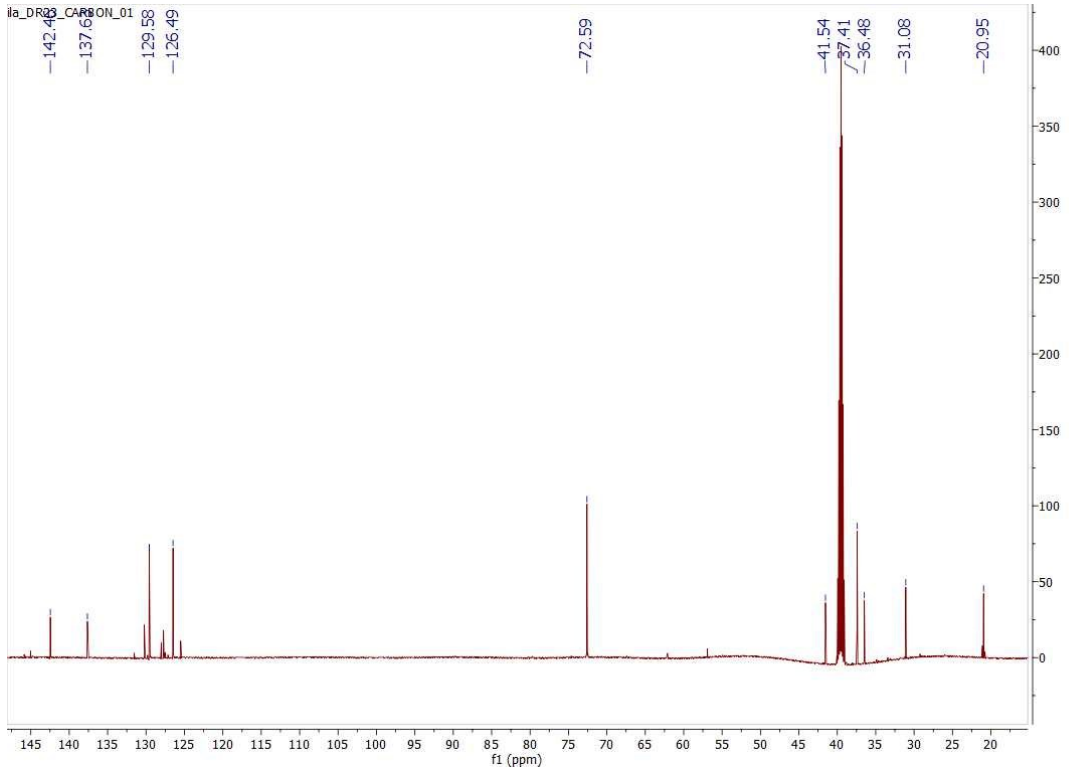
23


ila_DR22_PROTON_01
H3C CH3
O
O
NHTs
1.00
1.01
1.04
1.14
1.74
1.00
1.20
1.30
1.67
1.58
1.30
f1 (ppm)

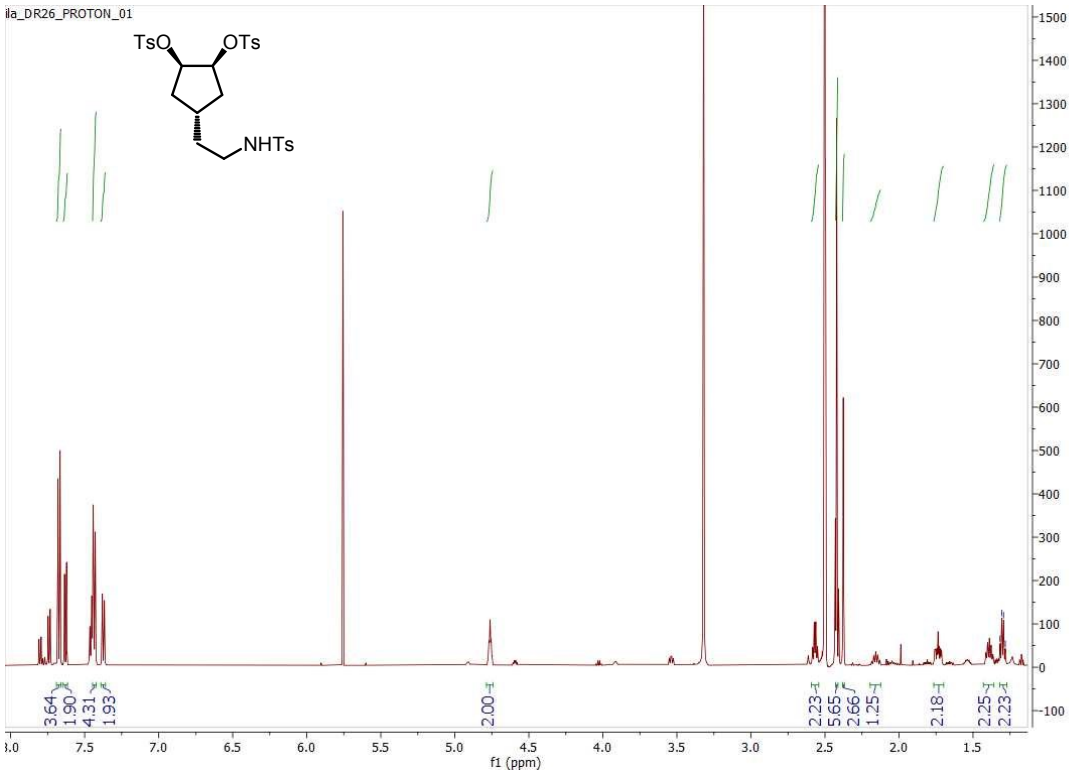

ila_DR22_CARBON_01
145.88
142.47
129.60
126.50
107.87
79.72
41.78
38.81
33.43
33.04
26.04
23.67
20.96
f1 (ppm)

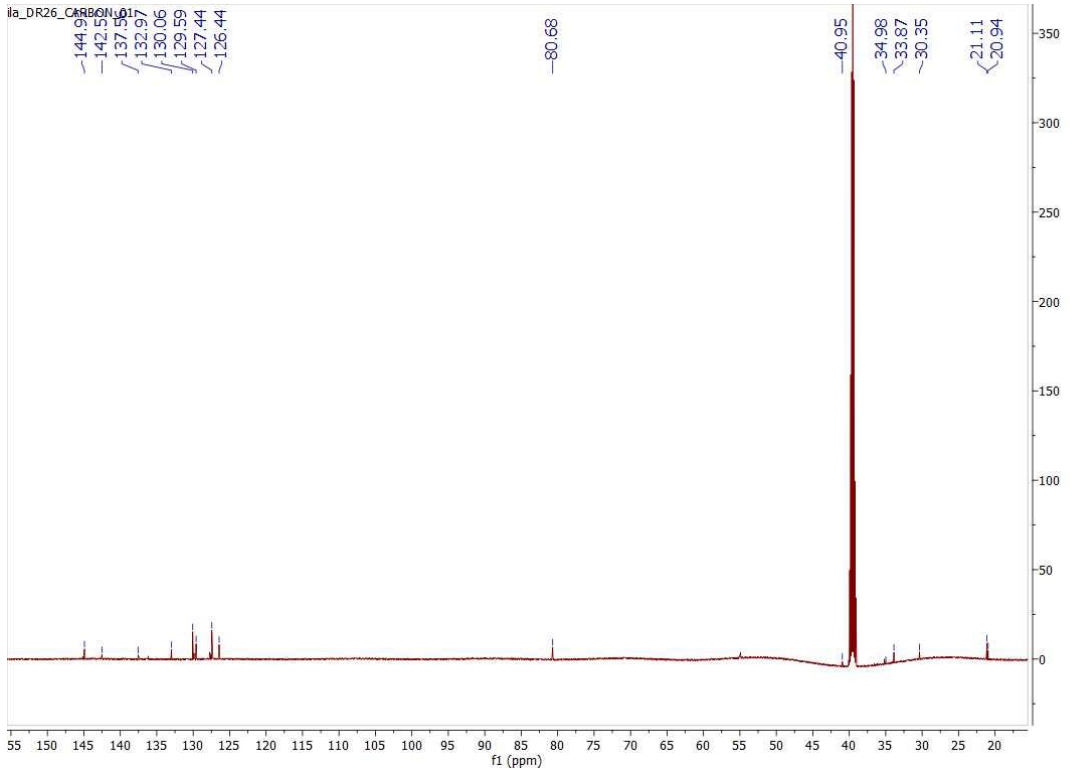
24


ila_DR23_PROTON_01
HO
OH
NHTs
1.80
1.77
1.88
1.83
2.72
1.00
1.83
2.01
2.09
f1 (ppm)

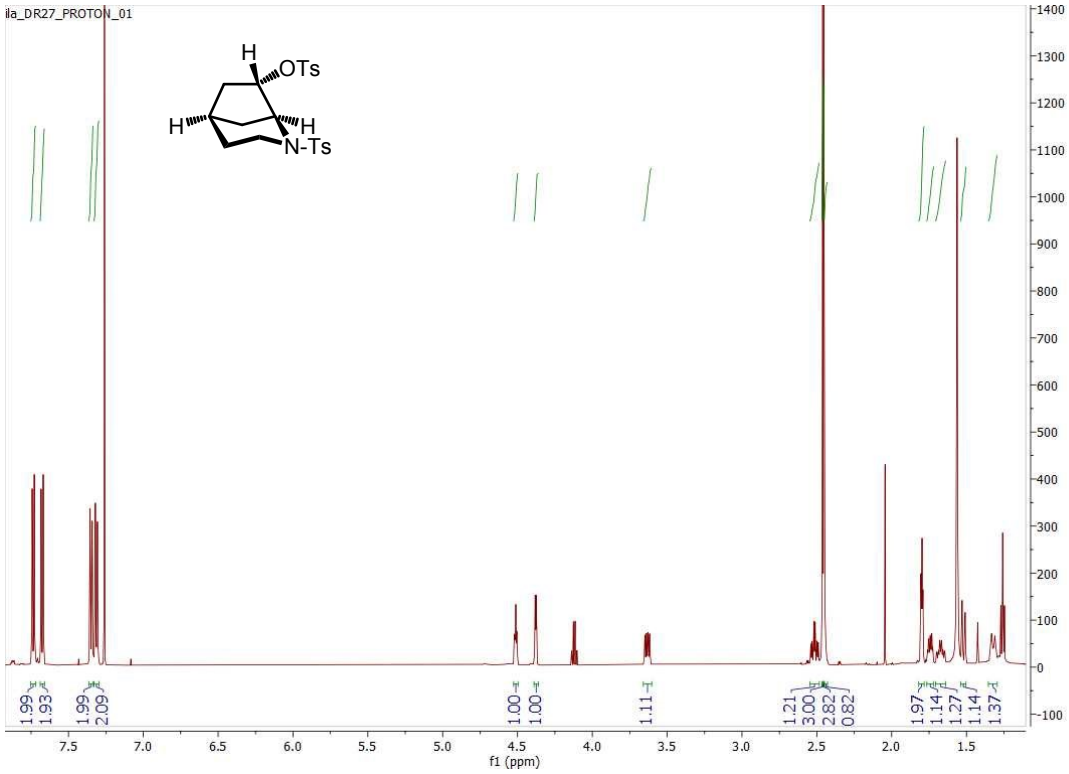

ila_DR23_CARBON_01
142.40
137.68
129.58
126.49
72.59
41.54
37.41
36.48
31.08
20.95
f1 (ppm)

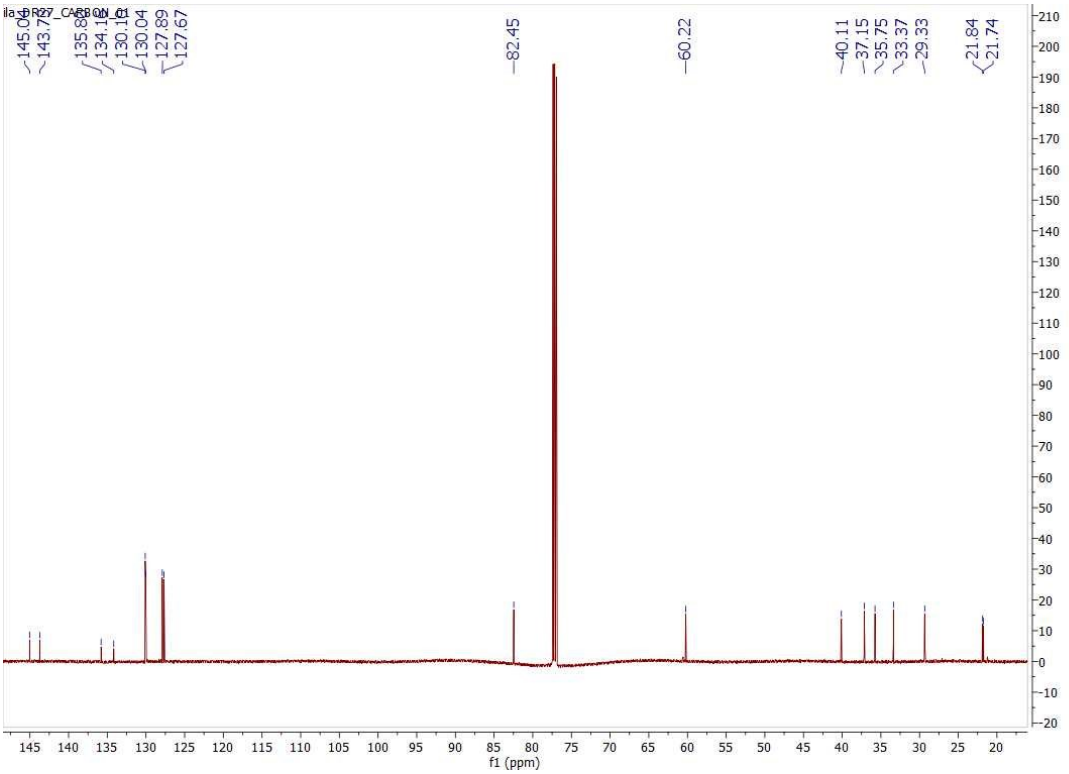
25


ila_DR26_PROTON_01
TsO
OTs
NHTs
3.64
1.90
4.31
1.93
2.00
2.23
5.65
2.66
1.25
2.18
2.25
2.23
f1 (ppm)

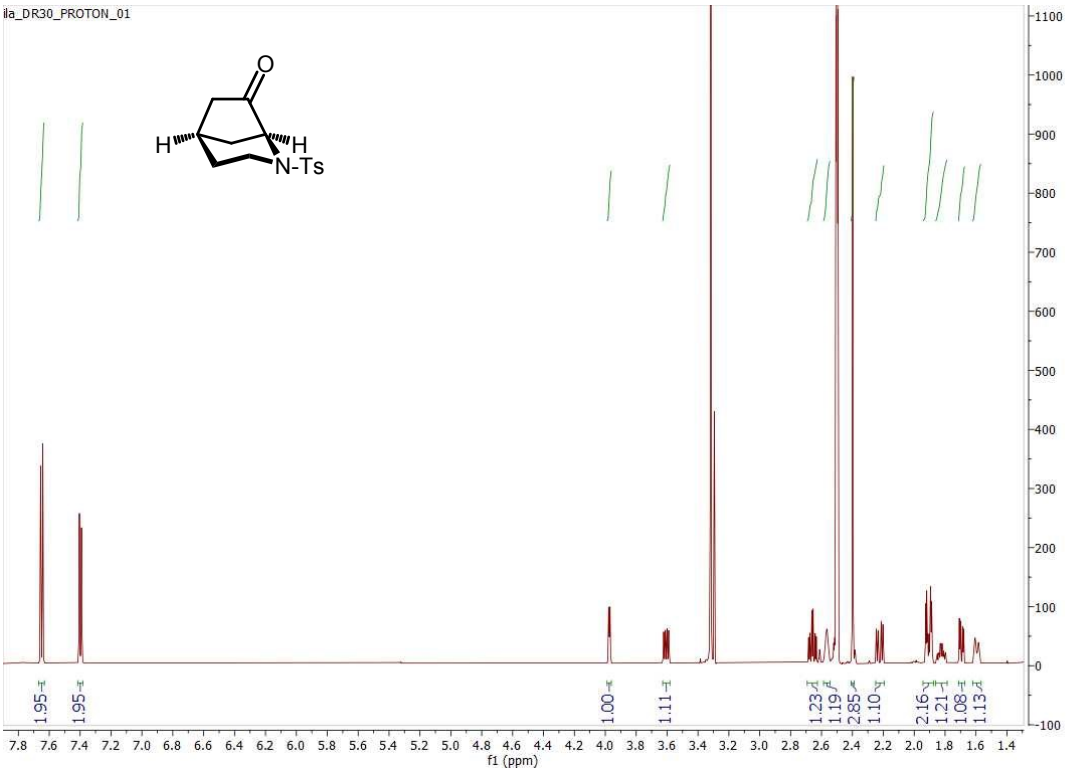

144.99
142.55
137.56
132.97
130.06
129.59
127.44
126.44
80.68
40.95
34.98
33.87
30.35
21.11
20.94
f1 (ppm)

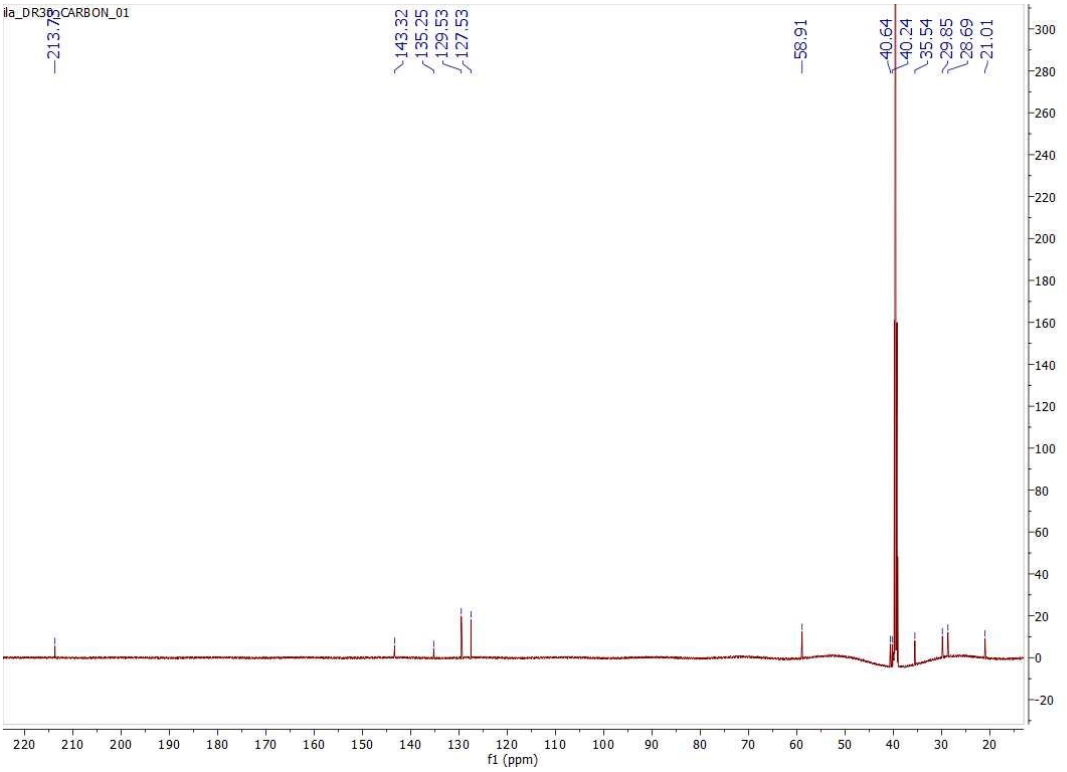
26


ila_DR27_PROTON_01
H
OTs
H
H
N-Ts
1.99
1.93
1.99
2.09
1.00
1.00
1.11
1.21
3.00
2.82
0.82
1.97
1.14
1.27
1.14
1.37
f1 (ppm)

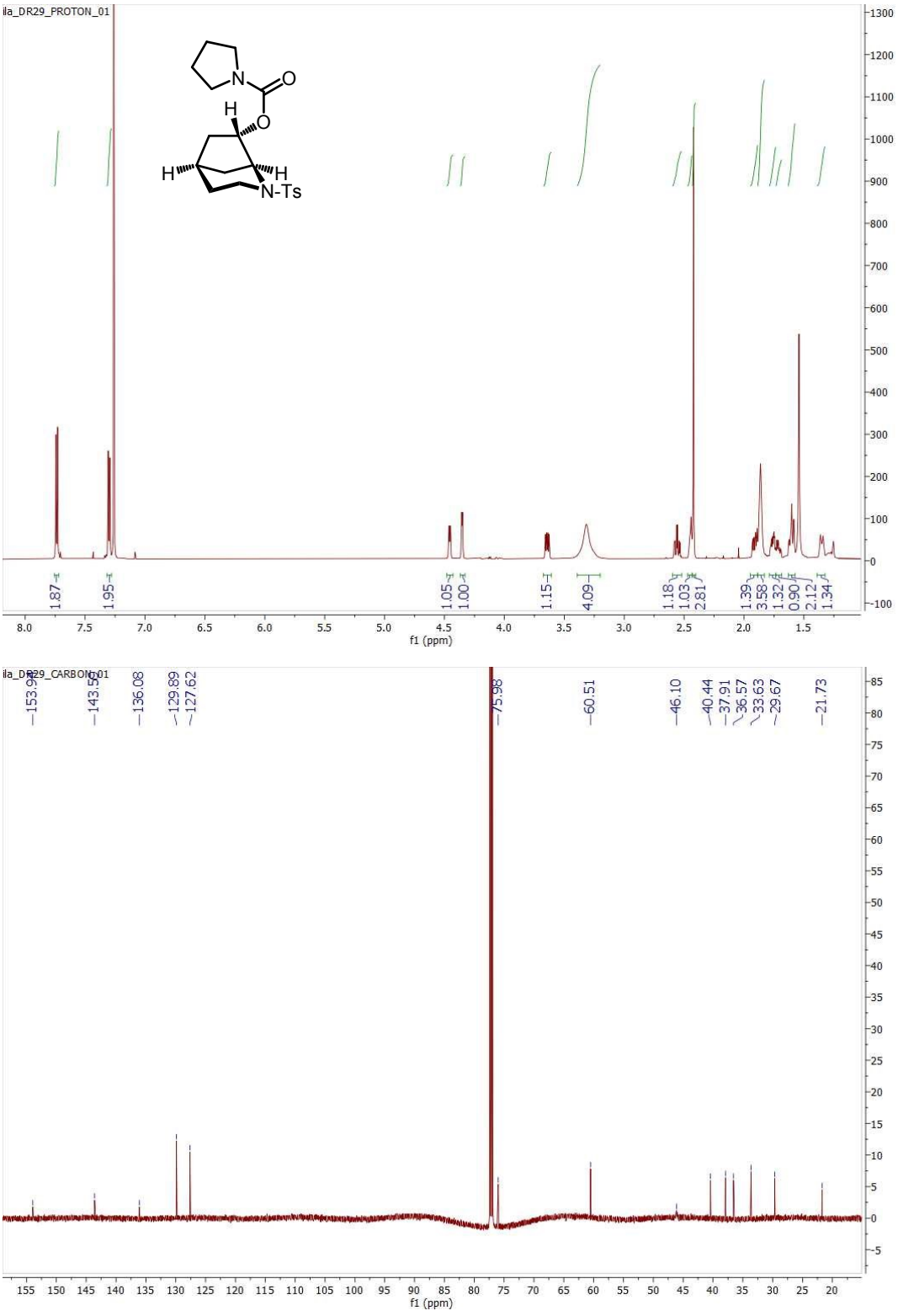

145.04
143.77
135.88
134.18
130.11
130.04
127.89
127.67
82.45
60.22
40.11
37.15
35.75
33.37
29.33
21.84
21.74
f1 (ppm)

27

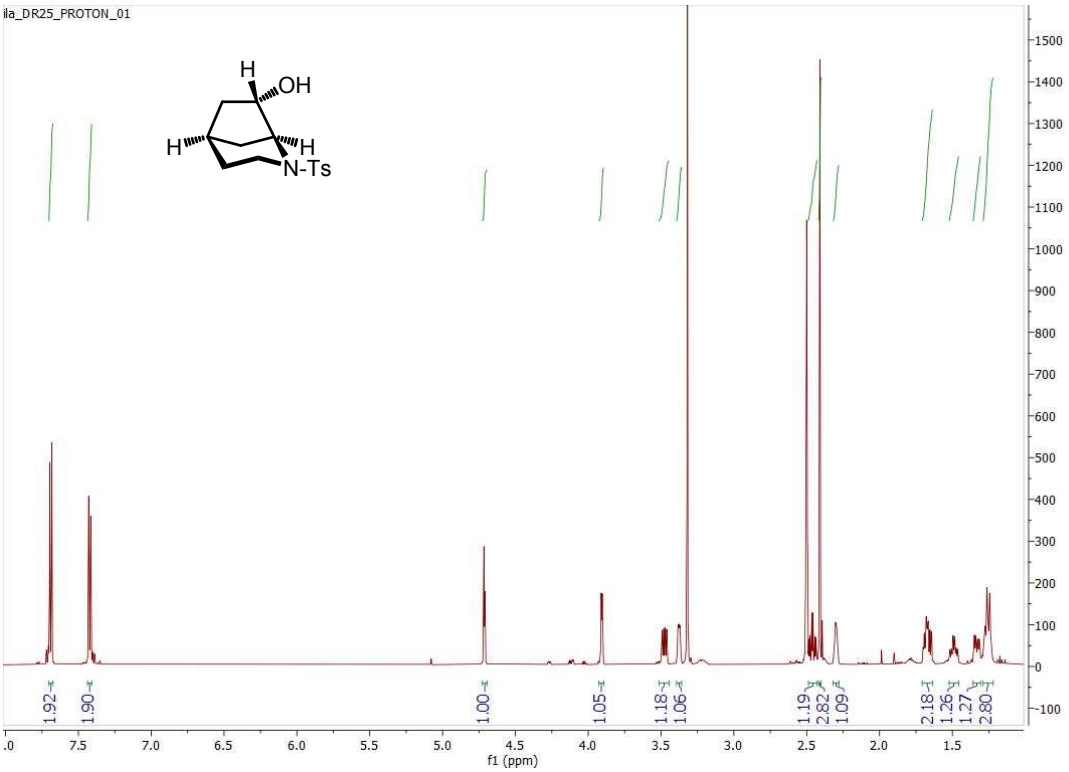

ila_DR30_PROTON_01
O
H
H
N-Ts
1.95
1.95
1.00
1.11
1.23
1.19
2.85
1.10
2.16
1.21
1.08
1.13
f1 (ppm)

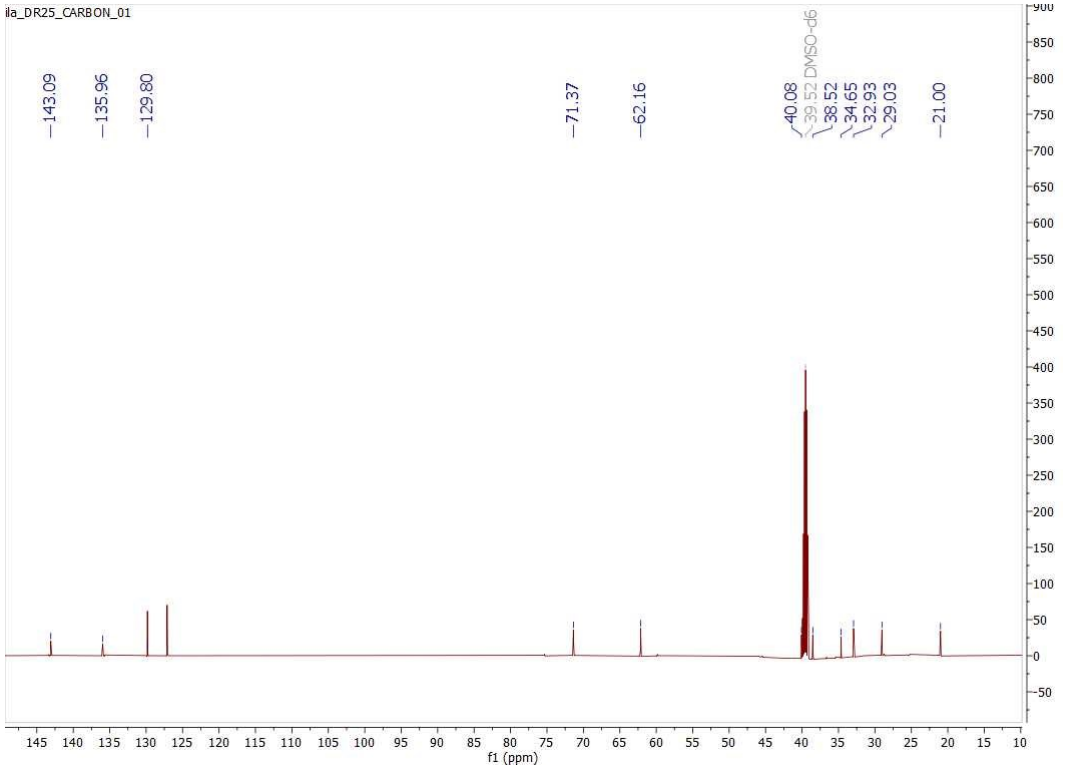

ila_DR30_CARBON_01
213.70
143.32
135.25
129.53
127.53
58.91
40.64
40.24
35.54
29.85
28.69
21.01
f1 (ppm)

28

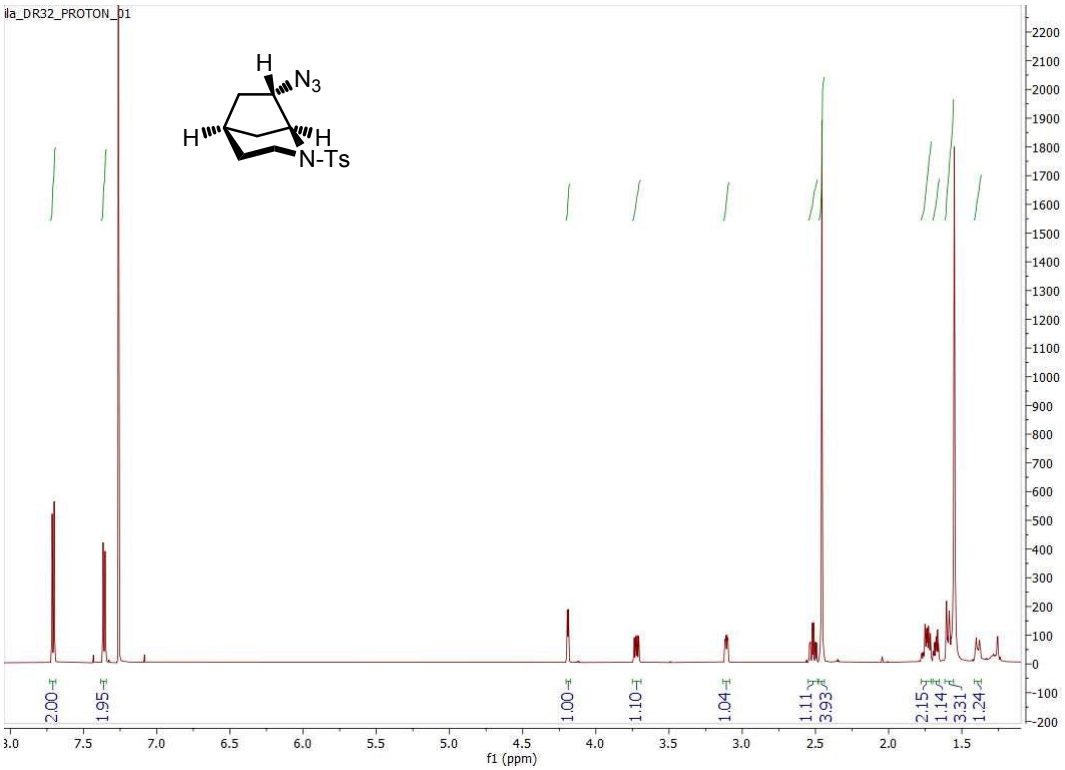

ila_DR29_PROTON_01
N
O
H
O
H
H
N-Ts
1.87
1.95
1.05
1.00
1.15
4.09
1.18
1.03
2.81
1.39
3.58
1.32
0.90
2.12
1.34
f1 (ppm)
ila_DR29_CARBON_01
153.98
143.50
136.08
129.89
127.62
75.98
60.51
46.10
40.44
37.91
36.57
33.63
29.67
21.73
f1 (ppm)

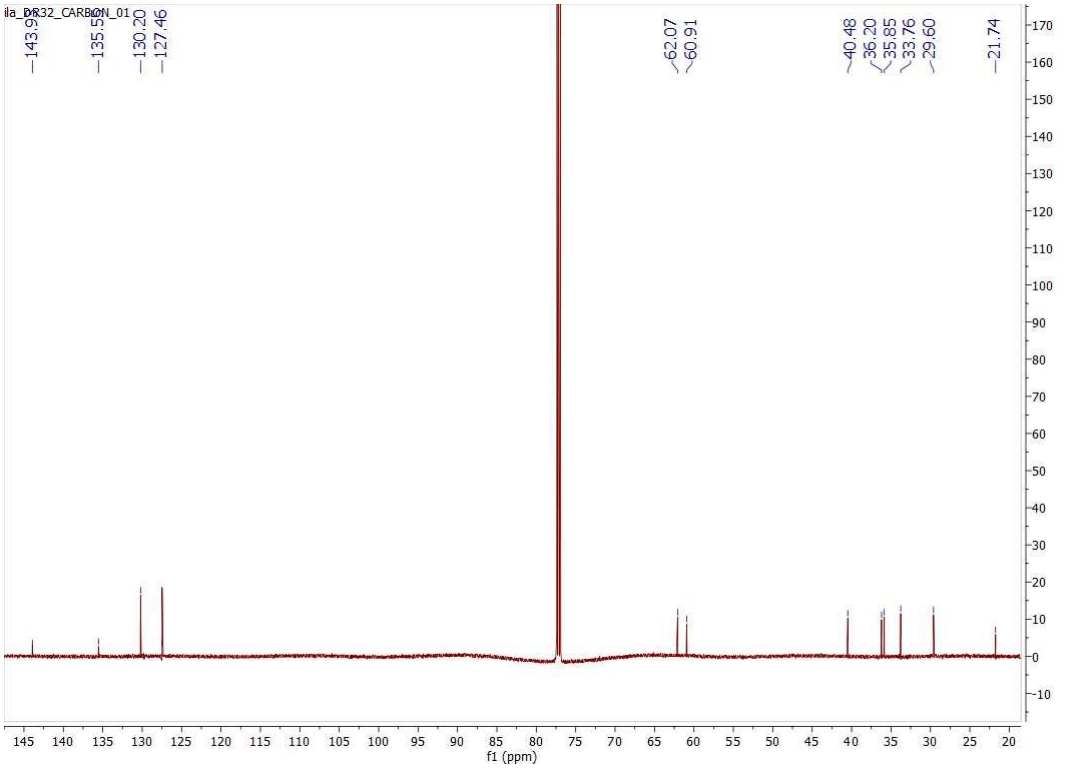
29


ila_DR25_PROTON_01
H
OH
H
H
N-Ts
1.92
1.90
1.00
1.05
1.18
1.06
1.19
2.82
1.09
2.18
1.26
1.27
2.80
f1 (ppm)

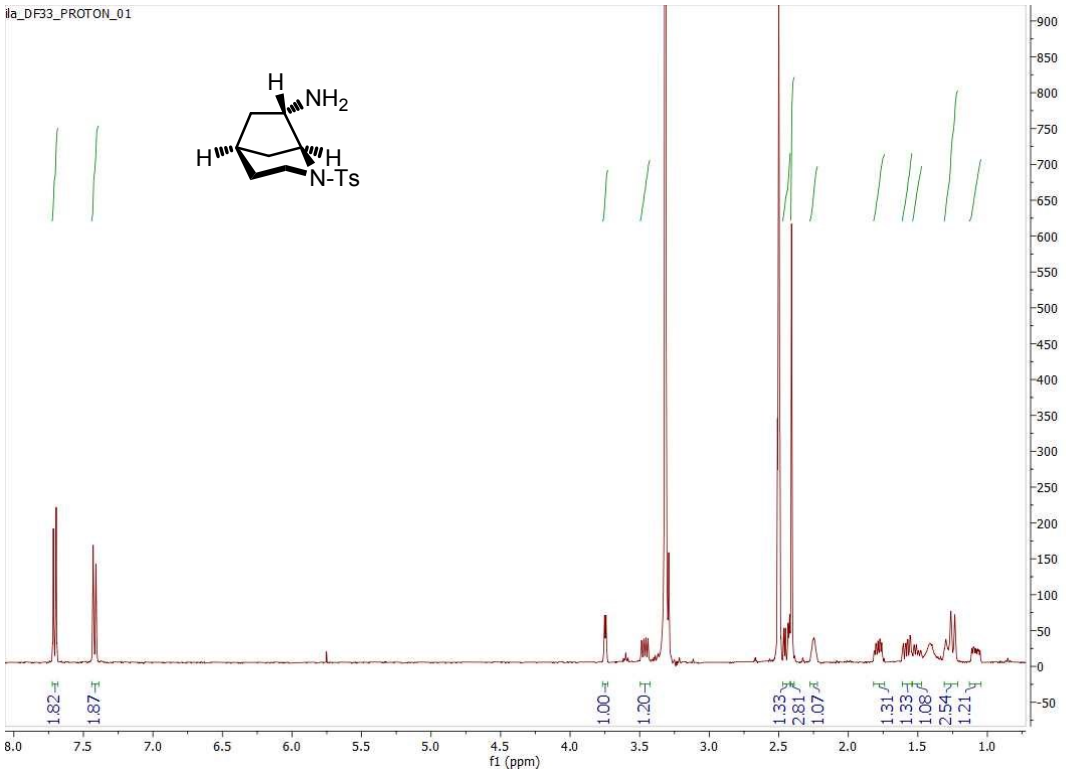

ila_DR25_CARBON_01
143.09
135.96
129.80
71.37
62.16
40.08
39.52 DMSO-d6
38.52
34.65
32.93
29.03
21.00
f1 (ppm)

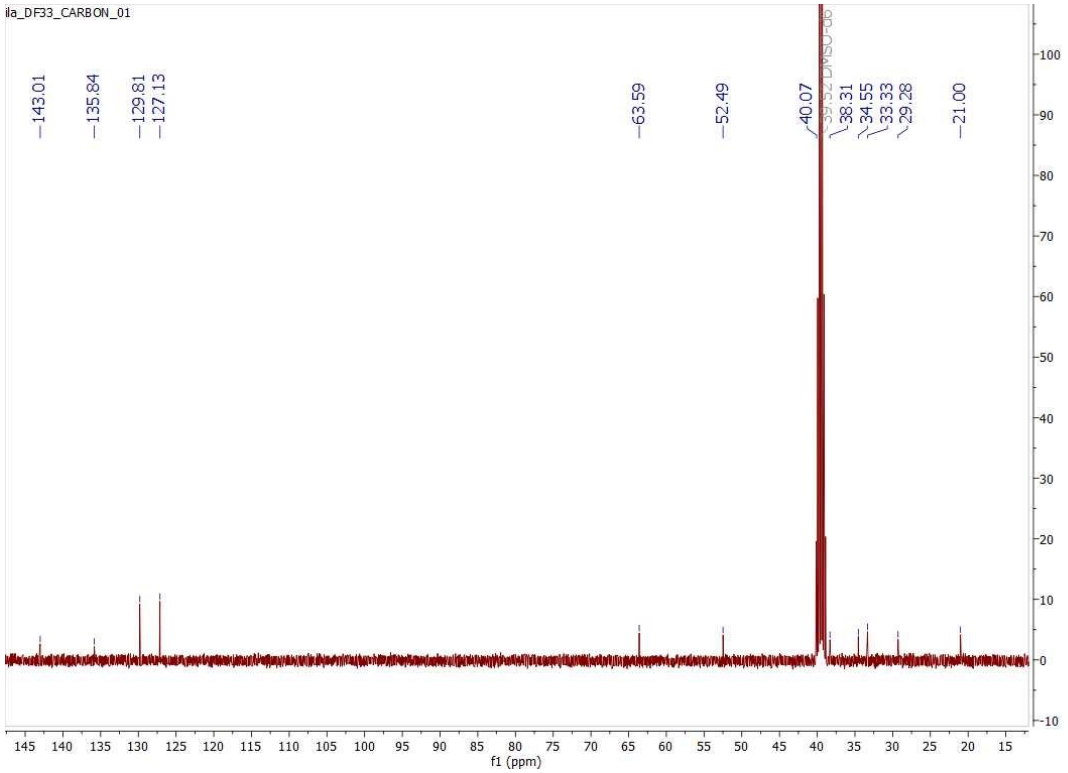
31


ila_DR32_PROTON_01
H
N3
H
H
N-Ts
2.00
1.95
1.00
1.10
1.04
1.11
3.93
2.15
1.14
3.31
1.24
f1 (ppm)

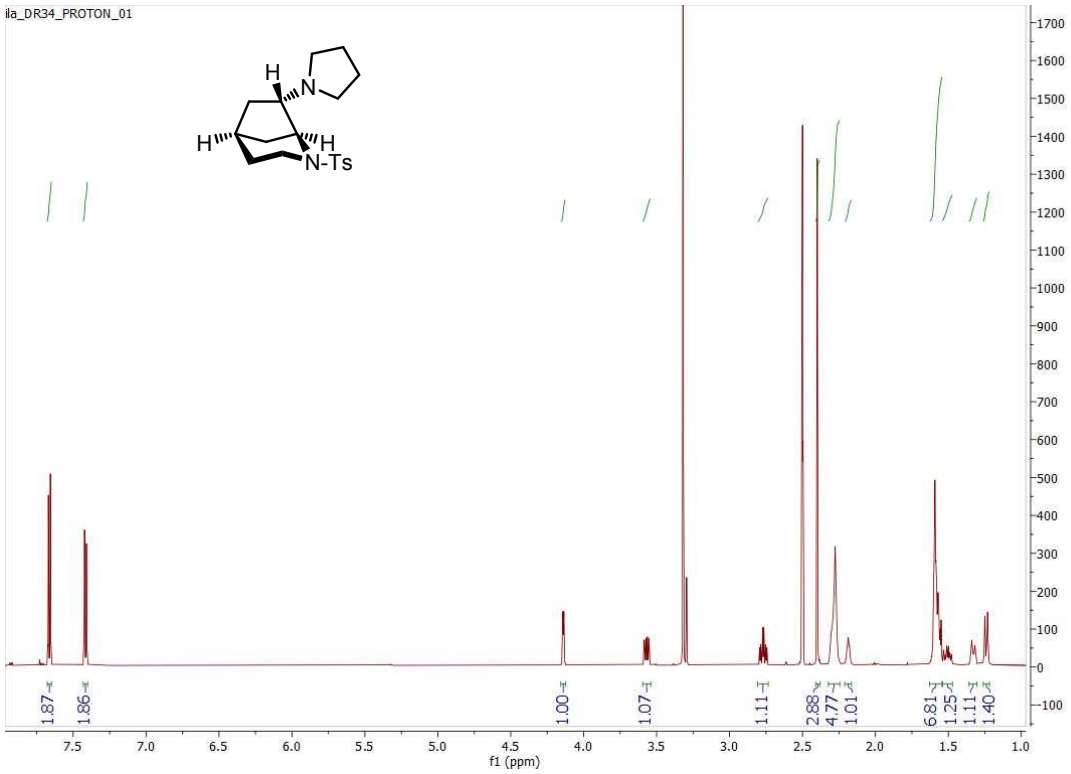

ila_DR32_CARBON_01
143.93
135.55
130.20
127.46
62.07
60.91
40.48
36.20
35.85
33.76
29.60
21.74
f1 (ppm)

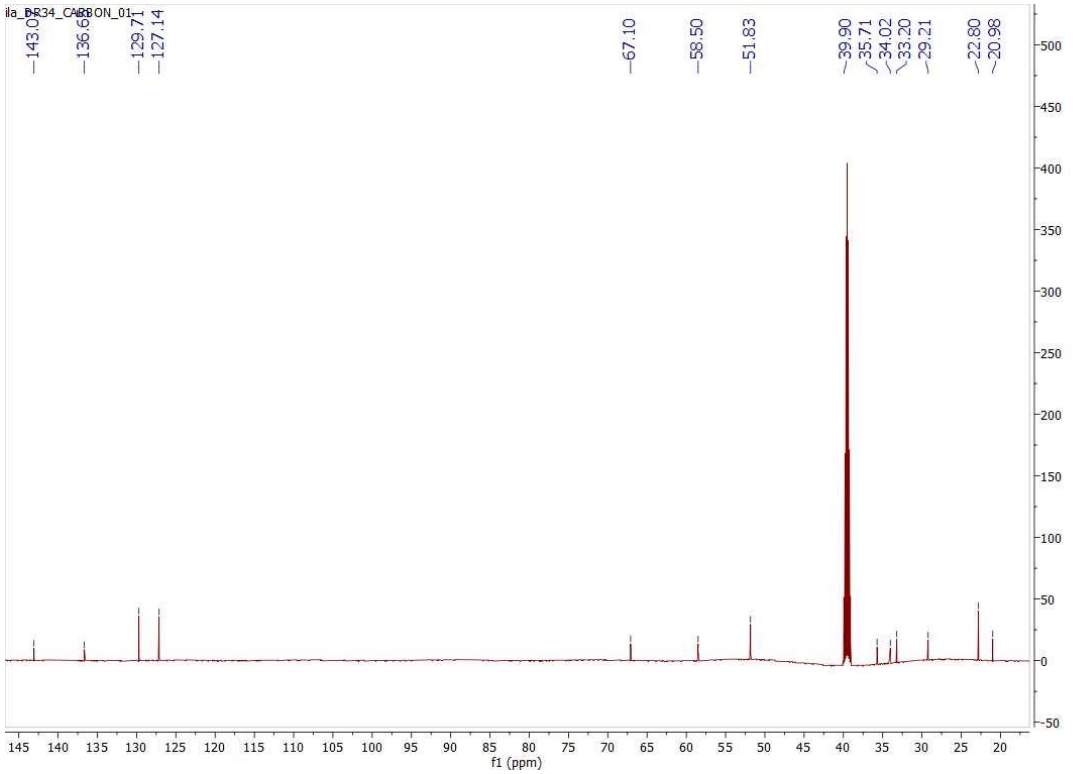
32


ila_DF33_PROTON_01
H
NH2
H
H
N-Ts
1.82
1.87
1.00
1.20
1.33
2.81
1.07
1.31
1.33
1.08
2.54
1.21
f1 (ppm)

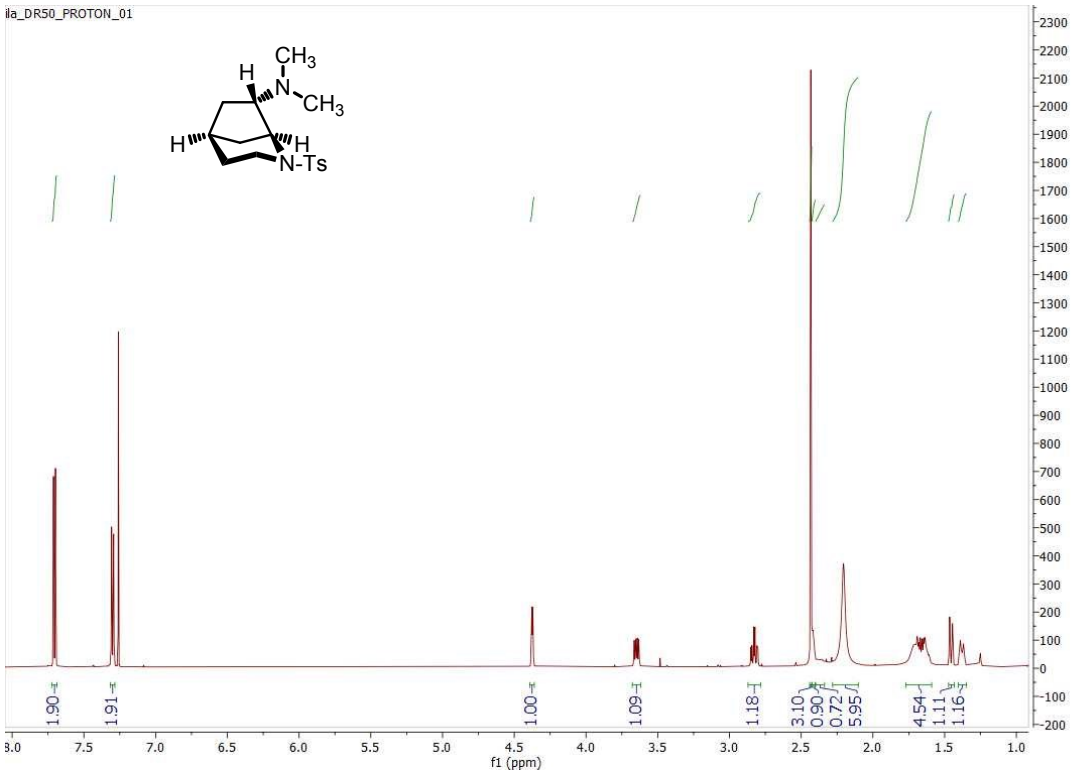

ila_DF33_CARBON_01
143.01
135.84
129.81
127.13
63.59
52.49
40.07
38.31
34.55
33.33
29.28
21.00
f1 (ppm)

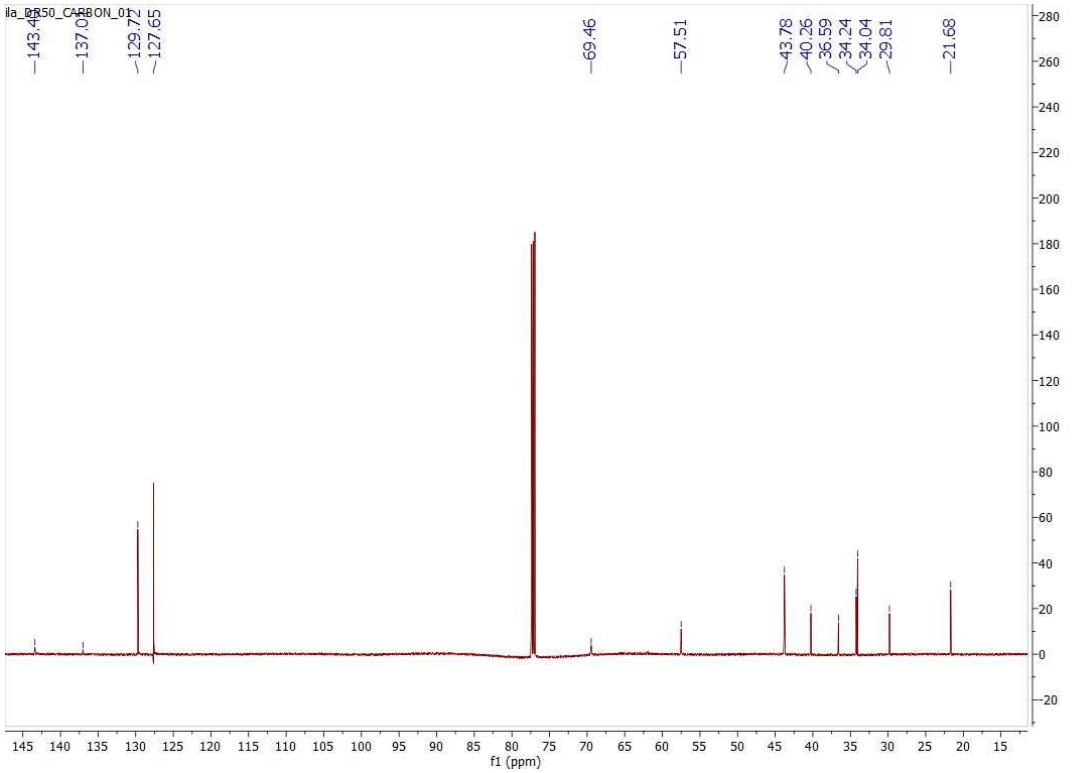
33a


ila_DR34_PROTON_01
H
N
H
H
N-Ts
1.87
1.86
1.00
1.07
1.11
2.88
4.77
1.01
6.81
1.25
1.11
1.40
f1 (ppm)

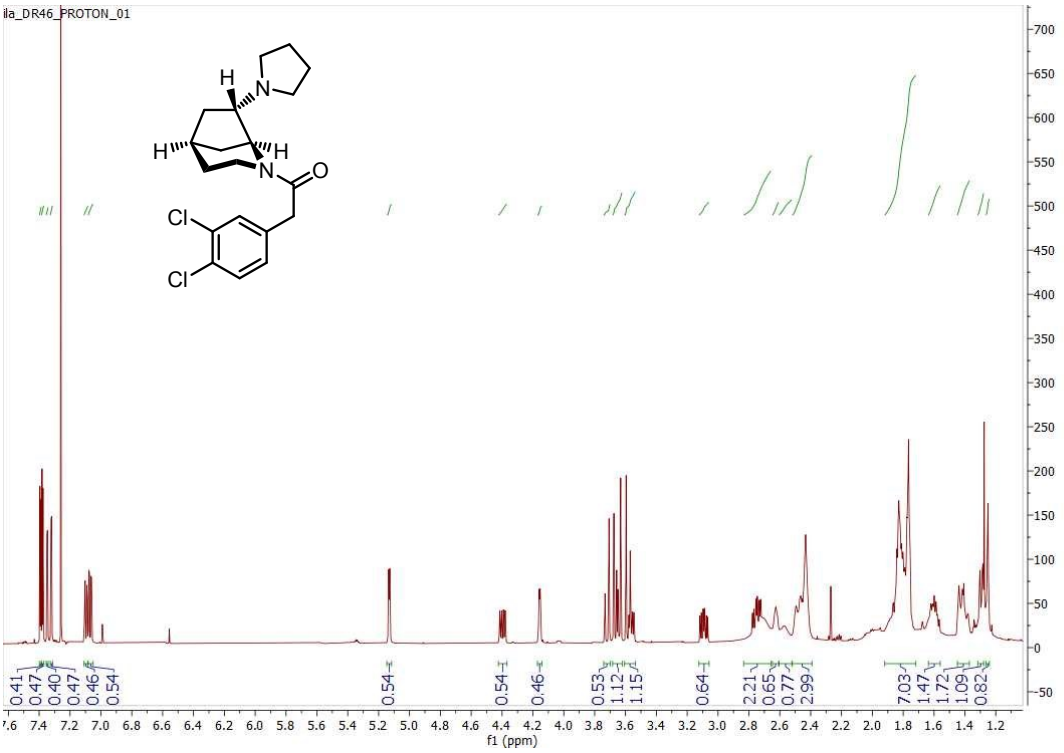

ila_DR34_CARBON_01
143.01
136.66
129.71
127.14
67.10
58.50
51.83
39.90
35.71
34.02
33.20
29.21
22.80
20.98
f1 (ppm)

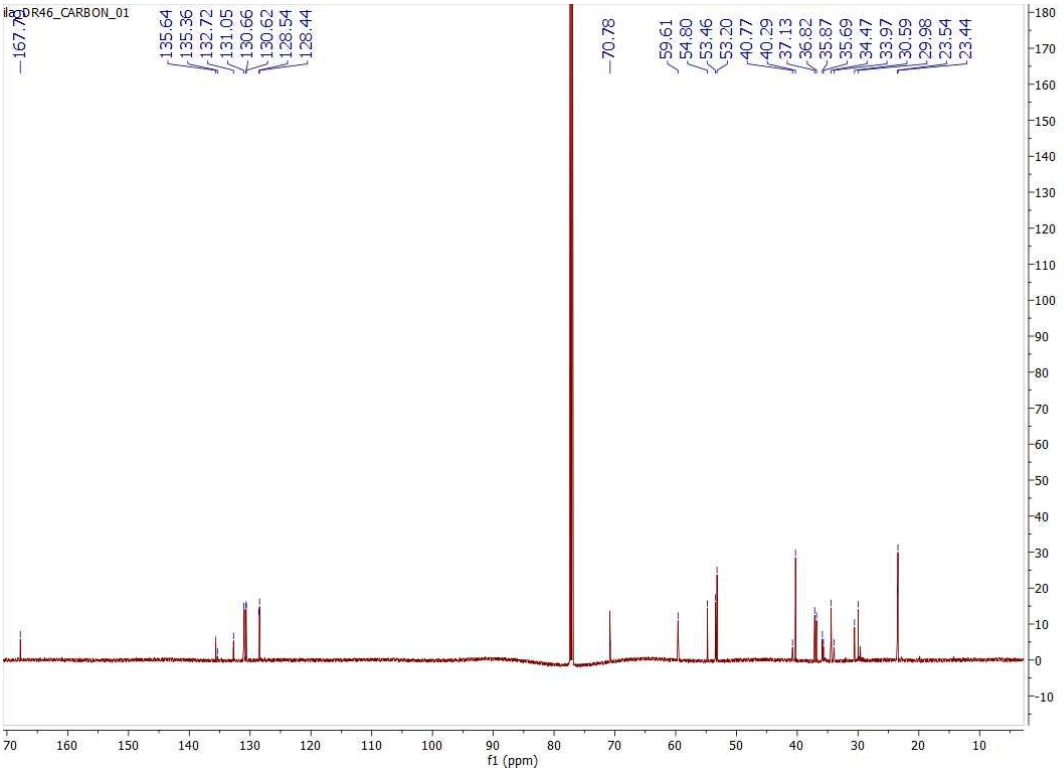
33b


ila_DR50_PROTON_01
CH3
H
N
CH3
H
H
N-Ts
1.90
1.91
1.00
1.09
1.18
3.10
0.90
0.72
5.95
4.54
1.11
1.16
f1 (ppm)

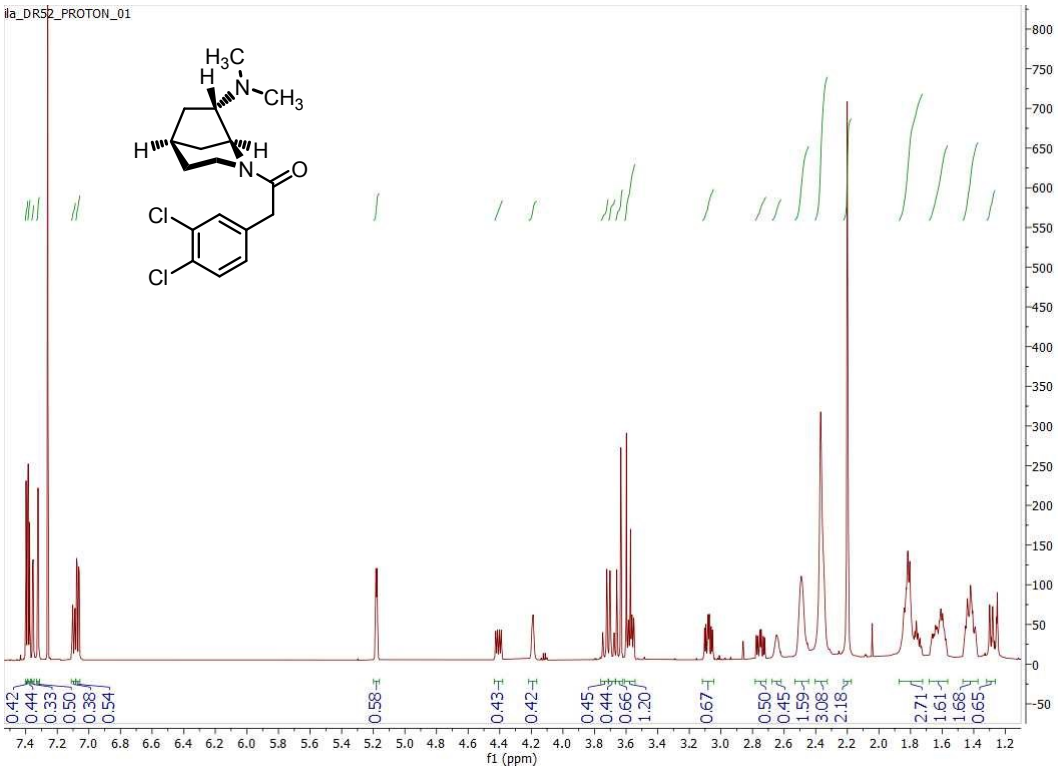

ila_DR50_CARBON_01
143.46
137.03
129.72
127.65
69.46
57.51
43.78
40.26
36.59
34.24
34.04
29.81
21.68
f1 (ppm)

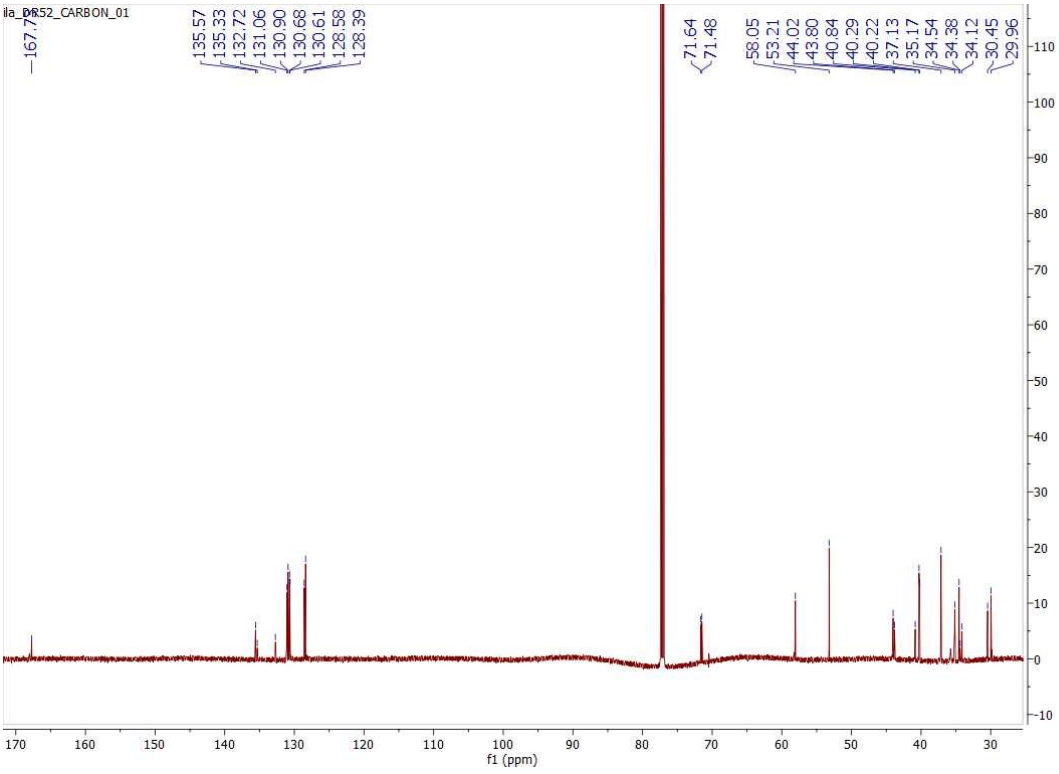
35a


ila_DR46_PROTON_01
H
N
H
H
N
O
Cl
Cl
0.41
0.47
0.40
0.47
0.46
0.54
0.54
0.54
0.46
0.53
1.12
1.15
0.64
2.21
0.65
0.77
2.99
7.03
1.47
1.72
1.09
0.82
f1 (ppm)

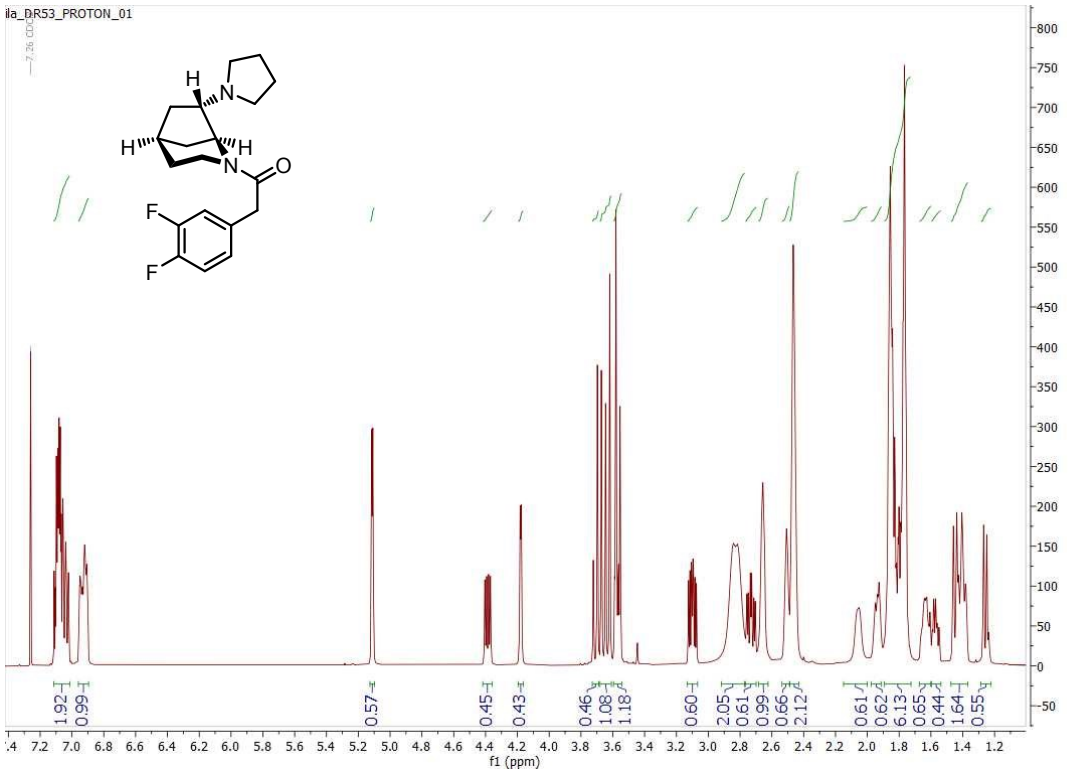

ila_DR46_CARBON_01
167.70
135.64
135.36
132.72
131.05
130.66
130.62
128.54
128.44
70.78
59.61
54.80
53.46
53.20
40.77
40.29
37.13
36.82
35.87
35.69
34.47
33.97
30.59
29.98
23.54
23.44
f1 (ppm)

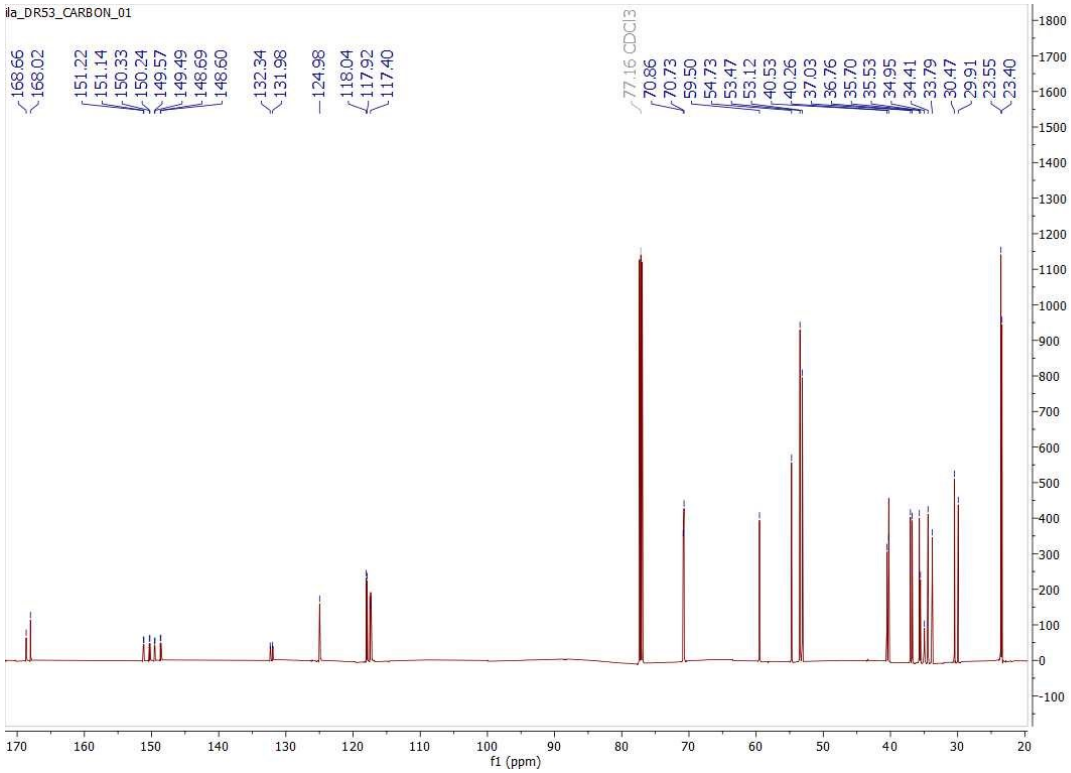
35b


ila_DR52_PROTON_01
H3C
H
N
CH3
H
H
N
O
Cl
Cl
0.42
0.44
0.33
0.50
0.38
0.54
0.58
0.43
0.42
0.45
0.44
0.66
1.20
0.67
0.50
0.45
1.59
3.08
2.18
2.71
1.61
1.68
0.65
f1 (ppm)


ila_DR52_CARBON_01
167.79
135.57
135.33
132.72
131.06
130.90
130.68
130.61
128.58
128.39
71.64
71.48
58.05
53.21
44.02
43.80
40.84
40.29
40.22
37.13
35.17
34.54
34.38
34.12
30.45
29.96
f1 (ppm)

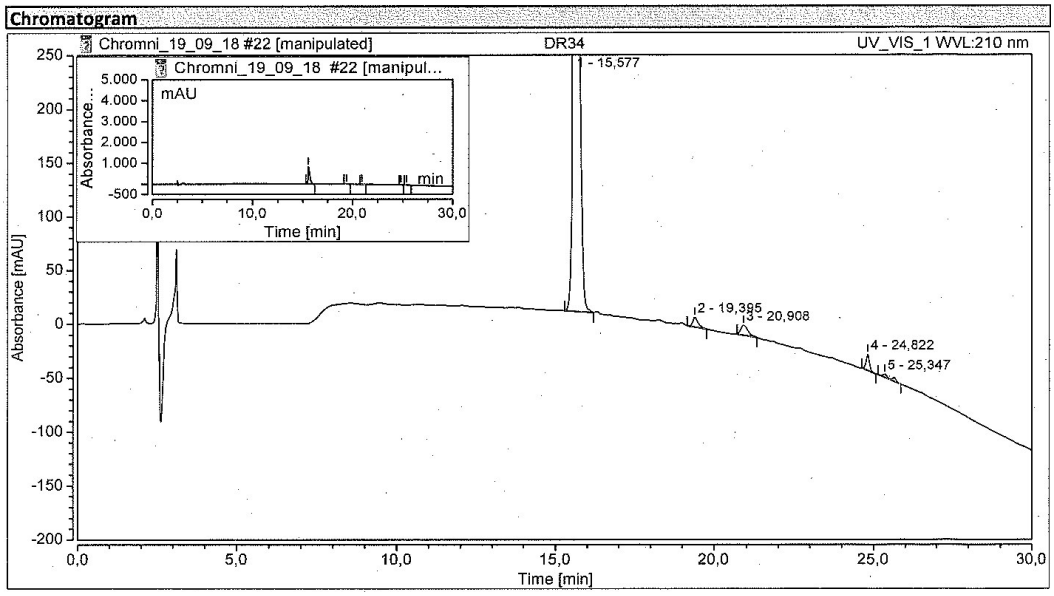
36


ila_DR53_PROTON_01
H
N
H
H
N
O
F
F
1.92
0.99
0.57
0.45
0.43
0.46
1.08
1.18
0.60
2.05
0.61
0.99
0.66
2.12
0.61
0.62
6.13
0.65
0.44
1.64
0.55
f1 (ppm)

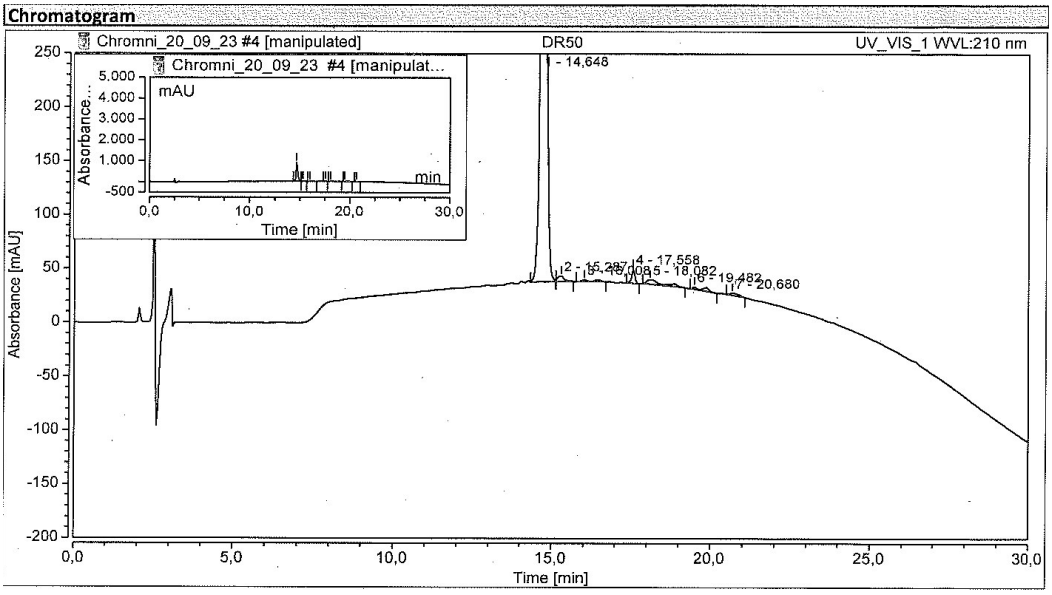

ila_DR53_CARBON_01
168.66
168.02
151.22
151.14
150.33
150.24
149.57
149.49
148.69
148.60
132.34
131.98
124.98
118.04
117.92
117.40
77.16 CDCl3
70.86
70.73
59.50
54.73
53.47
53.12
40.53
40.26
37.03
36.76
35.70
35.53
34.95
34.41
33.79
30.47
29.91
23.55
23.40
f1 (ppm)

7. HPLC chromatograms of test compounds

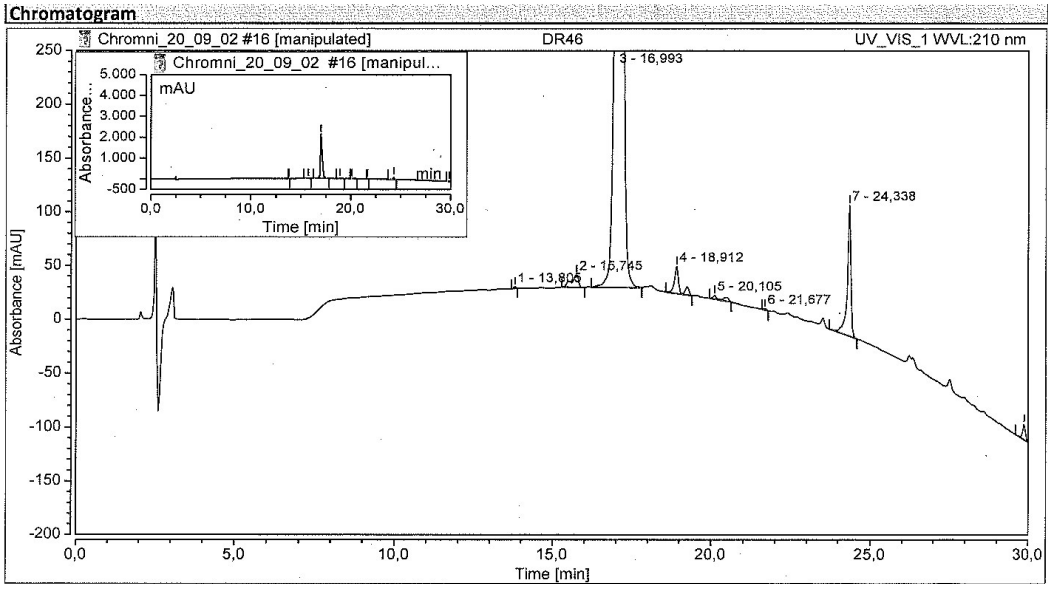
33a



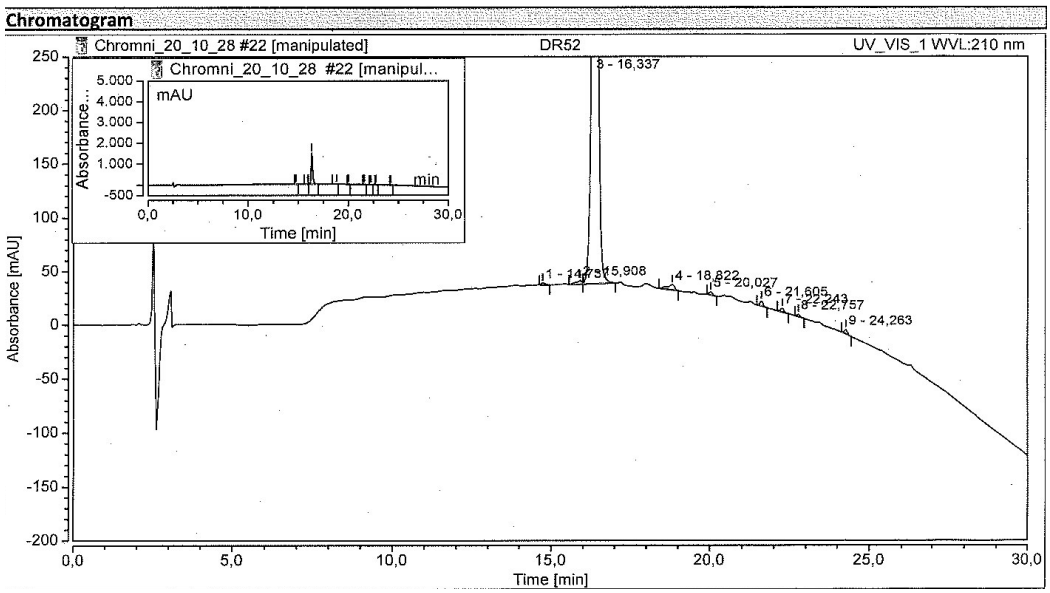
33b



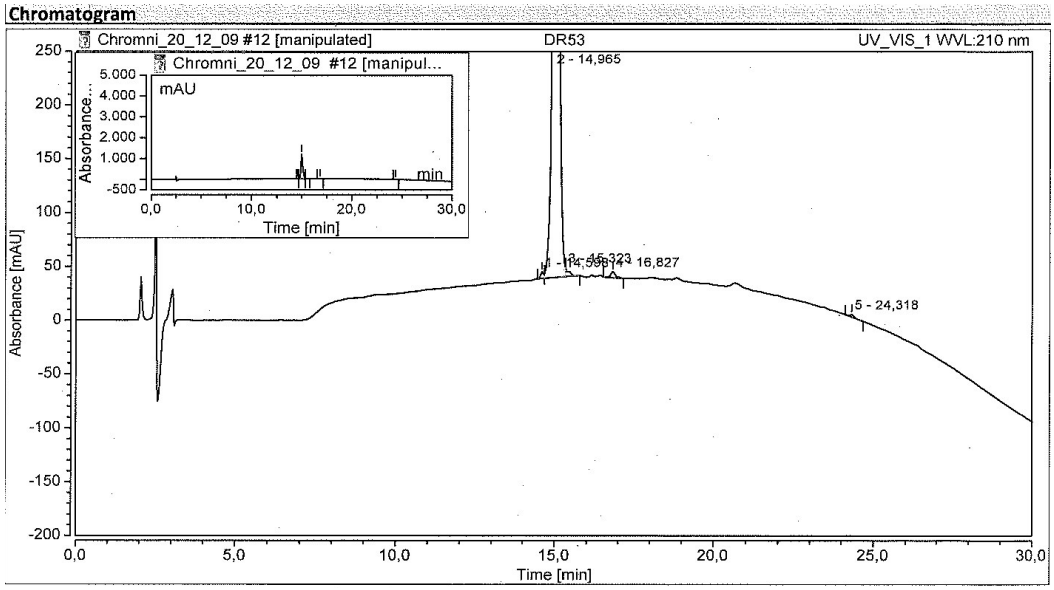
35a


Chromatogram
Chromni_20_09_02 #16 [manipulated]
DR46
UV_VIS_1 WVL:210 nm
Chromni_20_09_02 #16 [manipul...
mAU
min
Absorbance...
Time [min]
Absorbance [mAU]
3 - 16,993
7 - 24,338
4 - 18,912
2 - 15,745
1 - 13,805
5 - 20,105
6 - 21,677

35b


Chromatogram
Chromni_20_10_28 #22 [manipulated]
DR52
UV_VIS_1 WVL:210 nm
Chromni_20_10_28 #22 [manipul...
mAU
min
Absorbance...
Time [min]
Absorbance [mAU]
3 - 16,337
4 - 18,822
5 - 20,027
6 - 21,605
9 - 24,263

36


Chromatogram
Chromni_20_12_09 #12 [manipulated]
DR53
UV_VIS_1 WVL:210 nm
Chromni_20_12_09 #12 [manipul...
mAU
min
Absorbance...
Time [min]
2 - 14,965
16,827
5 - 24,318
Absorbance [mAU]
Time [min]