Immobilized and Reusable Cu(I) Catalyst for Metal Ion-Free Conjugation of Ligands to Fully Deprotected Oligonucleotides through Click Reaction

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Experimental Procedures:

Scheme 1

Synthesis of benzyl 15-azido 4,7,10,13-tetraoxapentadecanoate, Compound 2: To a solution of azido-PEG acid (**1**, 5.0 g, 17.18 mmol, 1.0 eq) and benzyl bromide (4.4 g, 25.77 mmol, 1.5 eq) in acetone was added K_2CO_3 (7.11 g, 51.54 mmol, 3.0 eq) as a solid, and the reaction was refluxed overnight. After completion of the reaction as judged by TLC, the reaction mixture was cooled to room temperature and filtered. The solvent was evaporated *in vacuo*, and the residue was dissolved in the ethyl acetate (EtOAc) and washed with water. The organic layer was collected, dried over anhydrous Na_2SO_4, concentrated, and purified by flash silica gel chromatography (eluent: hexane/EtOAc, 1:1) to obtain pure benzyl ester **2** as an oil (6.44 g, yield: 98 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.22 (m, 5H), 5.13 (s, 2H), 3.77 (t, J = 6.5, 2H), 3.71 – 3.53 (m, 14H), 3.37 (t, J = 5.1, 2H), 2.65 (t, J = 6.5, 2H); HRMS calc. for $C_{18}H_{27}N_3O_6$: 404.1798 (M+Na); found: 404.1798.

Synthesis of compound 4:

Method A: To a mixture of PEG-azide **2** (0.7 g, 1.83 mmol, 3.0 eq) and tris-propargylamine (0.08 g, 0.61 mmol, 1.0 eq) in *tert*-butanol/water (1:1) were added sodium ascorbate (0.036 g, 0.18 mmol, 0.3 eq) and $CuSO_4 \cdot 5H_2O$ (0.004 g, 0.018 mmol, 0.03 eq). The reaction was stirred at room temperature overnight. TLC indicated complete consumption of the starting materials. The reaction mixture was diluted with excess dichloromethane (DCM), and the organic layer was removed and washed with water. Combined organic fractions were dried over anhydrous $MgSO_4$, concentrated and purified by flash silica gel column chromatography (eluent: DCM/MeOH, 9:1) to obtain pure compound **4** as a pale green oil (0.69 g, 88%).

Method B: To a solution of PEG-azide (**2**, 3.0 g, 7.87 mmol, 3.0 eq) and tris-propargylamine **3** (0.343 g, 2.62 mmol, 1.0 eq) in *tert*-butanol/water (1:1) were added sodium ascorbate (0.155 g, 0.786 mmol, 0.3 eq) and $CuSO_4 \cdot 5H_2O$ (0.0195 g, 0.0786 mmol, 0.03 eq). The reaction mixture was microwave irradiated in a CEM Explorer-48 microwave synthesizer at 80 ^oC for 1 h with a ramp time of 20 min. TLC and LC-MS showed complete consumption of the starting materials after 1 h of irradiation. Workup and purification were performed as described in Method A to obtain a pure compound **4** as pale green oil (3.01 g, 90%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 3H), 7.41 – 7.19 (m, 15H), 5.12 (s, 6H), 4.52 (t, J = 5.3, 6H), 3.87 (t, J = 5.3, 6H), 3.76 (dd, J = 7.7, 5.2, 12H), 3.60 (d, J = 3.4, 36H), 2.64 (t, J = 6.5, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.59, 136.11, 128.75, 128.41, 128.36, 70.86, 70.79, 70.74, 70.66, 70.62, 69.59, 66.77, 66.49, 35.32. HRMS calc. for $C_{63}H_{90}N_{10}O_{18}$: 1275.6639; found: 1275.6620.

Synthesis of compound 5: To a solution of benzyl protected ligand (**4**, 0.6 g, 0.47 mmol) in methanol was added a catalytic amount of 10% Pd-C. The reaction was stirred under H_2 atmosphere at ambient temperature overnight. Completion of the reaction was confirmed by TLC. The reaction mixture was filtered through a small bed of celite. After washing with methanol several times, the combined washings were concentrated and dried under vacuum to obtain pure compound **5** as an oil (0.57 g, 97%), which was used for the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 3H), 4.57 (t, J = 5.1, 6H), 3.99 (s, 5H), 3.90 (t, J = 5.1, 6H), 3.75 (t, J = 6.1, 6H), 3.65 – 3.54 (m, 35H), 3.47 (s, 5H), 2.56 (t, J = 6.1, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 143.30, 124.47, 69.69, 69.60, 69.41, 68.83, 49.36, 46.93. HRMS calc. for $C_{42}H_{72}N_{10}O_{18}$: 1027.5050 (M+Na); found: 1027.5005.

Preparation of solid supported ligand 6: To a solution of compound **5** (0.88 g, 0.87 mmol), HBTU (0.16 g, 0.43 mmol), and DIPEA (0.24 mL, 1.13 mmol) in DMF was added aminomethyl polystyrene resin (230 μmmol/g, 1.52 g, 0.35 mmol), and the mixture was gently shaken on a wrist-action shaker at room temperature for 4 h. The resin was filtered and subsequently washed with DCM (1 vol.), DCM/methanol (9:1, 2 vol.), DCM (1 vol.), and ether (2 vol.) and dried under vacuum to obtain the solid support **6** (1.523 g).

Preparation of solid-supported ligand-Cu (I) complex 7: To a solution of tetrakis(acetonitrile)copper(I) hexafluorophosphate $[Cu(CH_3CN)_4PF_6]$ (0.08 g, 0.23 mmol) in DMF was added ligand **5** (0.5 g, 0.11 mmol). The suspension was gently shaken on a wrist-

action shaker at room temperature for 2 h. After filtration, the resin was washed with DCM (1 vol.), DCM/methanol (9:1, 2 vol.), DCM (1 vol.), and ether (2 vol.) and dried under vacuum to obtain the solid support **7** (0.507 g). Loading of the Cu(I) on the resin was analyzed by ICP/OES. Loading was 222 μmol/g, equivalent to ~97% of the total amine content on the resin.

Scheme 2

Preparation of Compound 11: Compound **11** (122 mg, 88%) was prepared under conditions similar to those used for synthesis of **12** using the alkyne **8** (0.07 g, 0.12 mmol, 1.0 eq), azide **9**[1] (0.066 g, 0.12 mmol, 1.0 eq), and Cu-complex **7** (0.05 g, 0.01 mmol, 0.1 eq) in DMF/DCM (2:1). ^{1}H NMR (400 MHz, CDCl3) δ 7.63 – 7.43 (m, 2H), 7.35 (d, J = 7.5 Hz, 2H), 7.31 – 7.14 (m, 8H), 6.88 – 6.73 (m, 4H), 6.04 – 5.86 (m, 1H), 5.50 (d, J = 20.8 Hz, 1H), 5.31 (t, J = 13.3 Hz, 1H), 4.86 – 4.62 (m, 3H), 4.61 – 4.34 (m, 2H), 4.34 – 4.16 (m, 4H), 3.76 (d, J = 0.8 Hz, 6H), 3.63 – 3.48 (m, 1H), 3.46 (dd, J = 10.7, 2.4 Hz, 1H), 3.40 – 3.23 (m, 1H), 3.09 (d, J = 6.1 Hz, 2H), 2.66 (q, J = 7.2 Hz, 6H), 2.44 – 2.06 (m, 2H), 2.04 – 1.61 (m, 8H), 1.62 – 1.18 (m, 21H), 1.17 – 0.99 (m, 15H), 1.01 – 0.78 (m, 15H), 0.64 (s, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 164.13, 159.21, 156.69, 151.28, 144.77, 144.51, 140.33, 136.06, 135.86, 135.76, 130.53, 128.55, 127.64, 123.12, 122.94, 113.83, 111.72, 89.53, 87.48, 82.15, 78.49, 77.85, 77.53, 77.21, 74.75, 74.50, 64.47, 63.53, 57.17, 56.62, 55.76, 50.78, 50.49, 46.33, 42.80, 41.08, 40.22, 40.00, 39.07, 37.48, 37.05, 36.67, 36.29, 32.39, 32.36, 30.57, 30.23, 28.72, 28.67, 28.50, 26.56, 26.50, 24.78, 24.32, 23.32, 23.06, 21.53, 19.82, 19.21, 12.35, 11.11. HRMS calc. for $C_{68}H_{92}N_6O_{10}$: 1161.6616 (M+Na); found: 1161.6630.

Preparation of Compound 12: To a mixture of alkyne **8** (0.024 g, 0.04 mmol, 1.0 eq) and 1-azido-docosane[1] (**10**, 0.014 g, 0.04 mmol, 1.0 eq) in DMF/DCM (2:1) was added Cu-complex **7** (0.02 g, 0.004 mmol, 0.1 eq), and the mixture was gently shaken on a wrist-action shaker overnight. Completion of the reaction was confirmed by TLC. The reaction mixture was filtered and washed with DCM (2 vol.). The filtrate was poured onto ice, and the product was extracted into DCM. The organic layer was washed with excess water, and then dried over anhydrous $MgSO_4$. The product was purified by flash silica gel column chromatography (eluent: DCM/MeOH, 95:5) to obtain pure compound **12** (36 mg, 99%). ^{1}H NMR (400 MHz, CDCl3) δ 8.15 – 7.88 (m, 2H), 7.49 (d, J = 14.9 Hz, 2H), 7.39 – 7.08 (m, 10H), 6.91 – 6.73 (m, 2H), 5.96 (d, J = 5.7 Hz, 1H), 5.34 (dd, J = 5.7, 3.3 Hz, 3H), 4.73 (dd, J = 29.2, 11.8 Hz, 2H), 4.29 (ddd, J

= 31.2, 29.1, 17.3 Hz, 3H), 3.79 (d, J = 1.4 Hz, 3H), 3.36 (ddd, J = 37.4, 30.9, 14.6 Hz, 2H), 2.26 – 2.10 (m, 3H), 1.98 (dd, J = 38.0, 32.3 Hz, 4H), 1.69 – 1.46 (m, 11H), 1.44 – 0.99 (m, 33H), 0.94 – 0.72 (m, 6H). ^{13}C NMR (101 MHz, CDCl3) δ 163.68, 158.95, 150.84, 144.47, 144.06, 135.84, 135.57, 135.46, 130.26, 128.27, 127.37, 122.62, 113.55, 111.48, 89.21, 87.25, 81.90, 78.29, 74.25, 64.16, 63.34, 55.48, 50.71, 32.14, 30.49, 29.92, 29.88, 29.83, 29.77, 29.61, 29.57, 29.22, 26.74, 22.90, 14.34, 12.03. HRMS calc. for $C_{56}H_{79}N_5O_8$: 972.5826 (M+Na); found: 972.5816 .

Scheme 3

Oligonucleotide Conjugates: A suspension of alkyne-oligonucleotide **13** (35.6 µmol in solution, 1.0 eq), azides **14**,[2] **15**,[1] **16**,[1] or **17** (71.0 µmol, 2.0 eq), and Cu-complex **7** (9.8 µmol, 0.2 eq) in DMF was gently shaken on a wrist-action shaker overnight at room temperature to yield the conjugate.

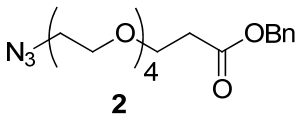
Table S1. Calculated and observed masses of click conjugates **18-21**

Alkyne	Mass	Azide	Mass	Product	Mass	
					Calc.	Found
13	7008.6	**14**	395.15	**18**	7403.75	7402.22
13	7008.6	**15**	334.15	**19**	7342.75	7342.10
13	7008.6	**16**	231.23	**20**	7239.83	7237.03
13	7008.6	**17**	2159.6	**21**	9168.20	9168.01

Preparation of Compound 17: The azido peptide **17** was synthesized on solid-phase using Fmoc peptide synthesis conditions. After the coupling of the last amino acid, 16-azidohexadecanoic acid[3] was coupled to the solid-support-bound peptide. Standard peptide deprotection and HPLC purification afforded the azido peptide **17**. MS calc. for $C_{95}H_{169}N_{39}O_{19}$: 2160.35; found: 2159.60.

References

1. T. Yamada, C. G. Peng, S. Matsuda, H. Addepalli, K. N. Jayaprakash, M. R. Alam, K. Mills, M. A. Maier, K. Charisse, M. Sekine, M. Manoharan, K. G. Rajeev, *J. Org. Chem.* 2011, **76**, 1198.
2. (a) R. Guezguez, K. Bougrin, K. El Akri, R. Benhida, *Tetrahedron Lett.* 2006, **47**, 4807; (b) A. Stimac, J. Kobe, *Carbohydrate Res.* 2000, **329**, 317.
3. H. C. Hang, E.-J. Geutjes, G. Grotenbreg, A. M. Pollington, M. J. Bijlmakers, H. L. Ploegh, *J. Am. Chem. Soc.* 2007, **129**, 2744.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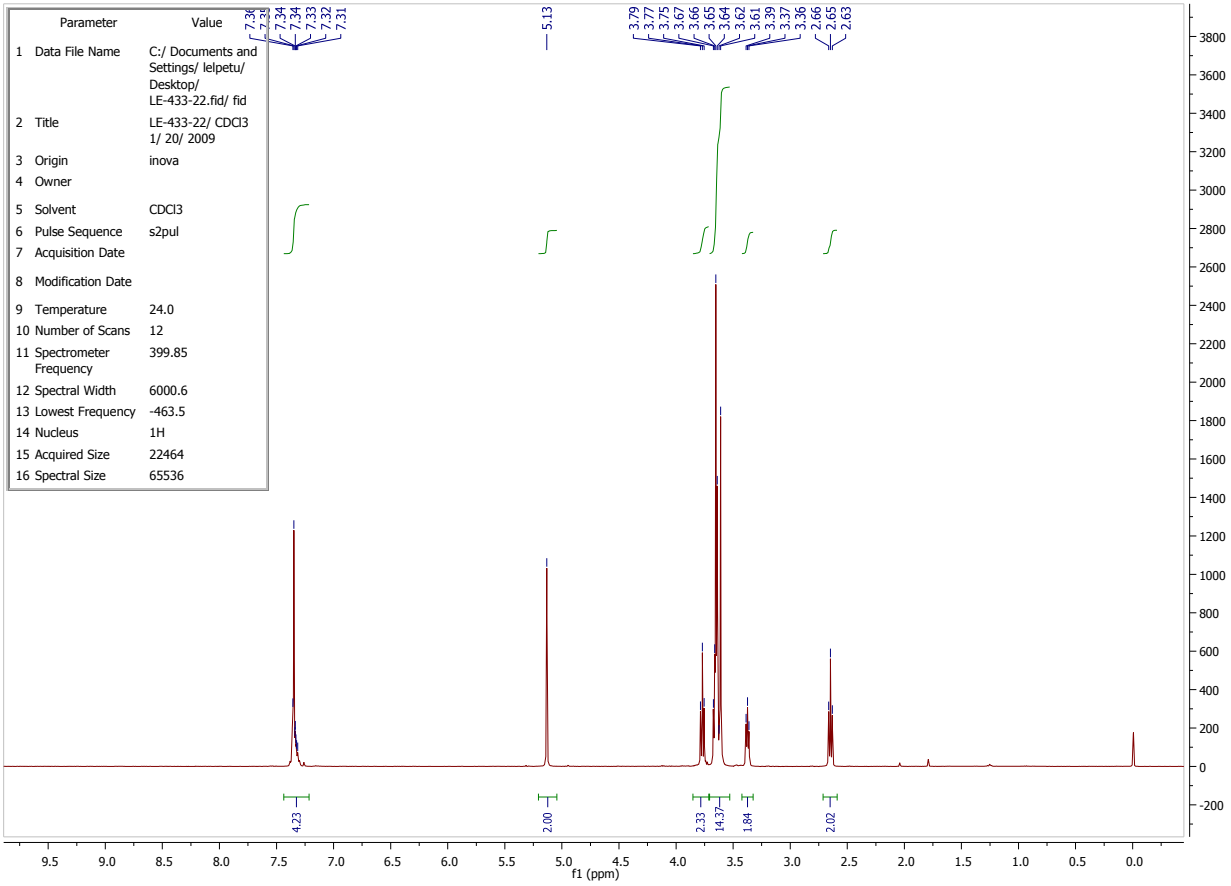
2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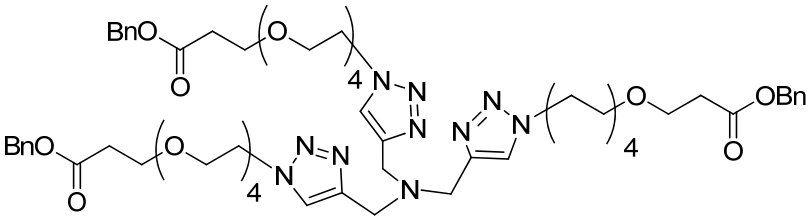

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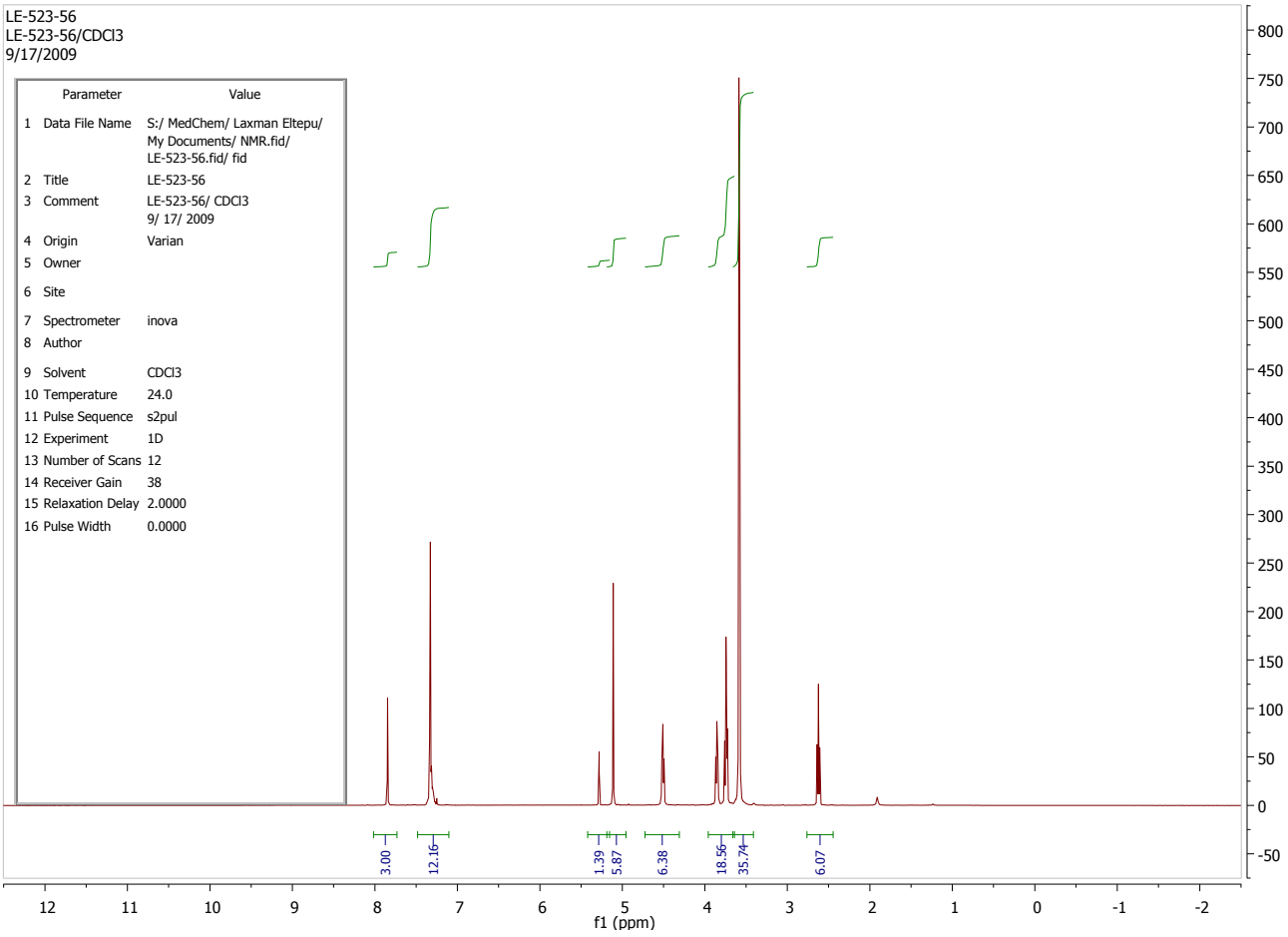
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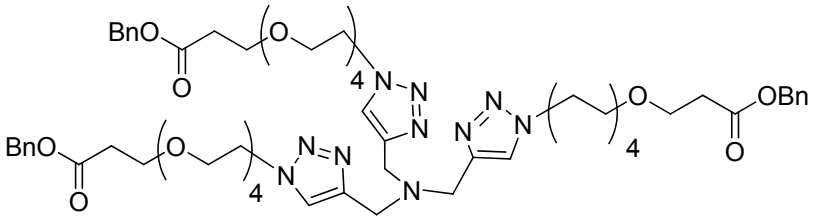

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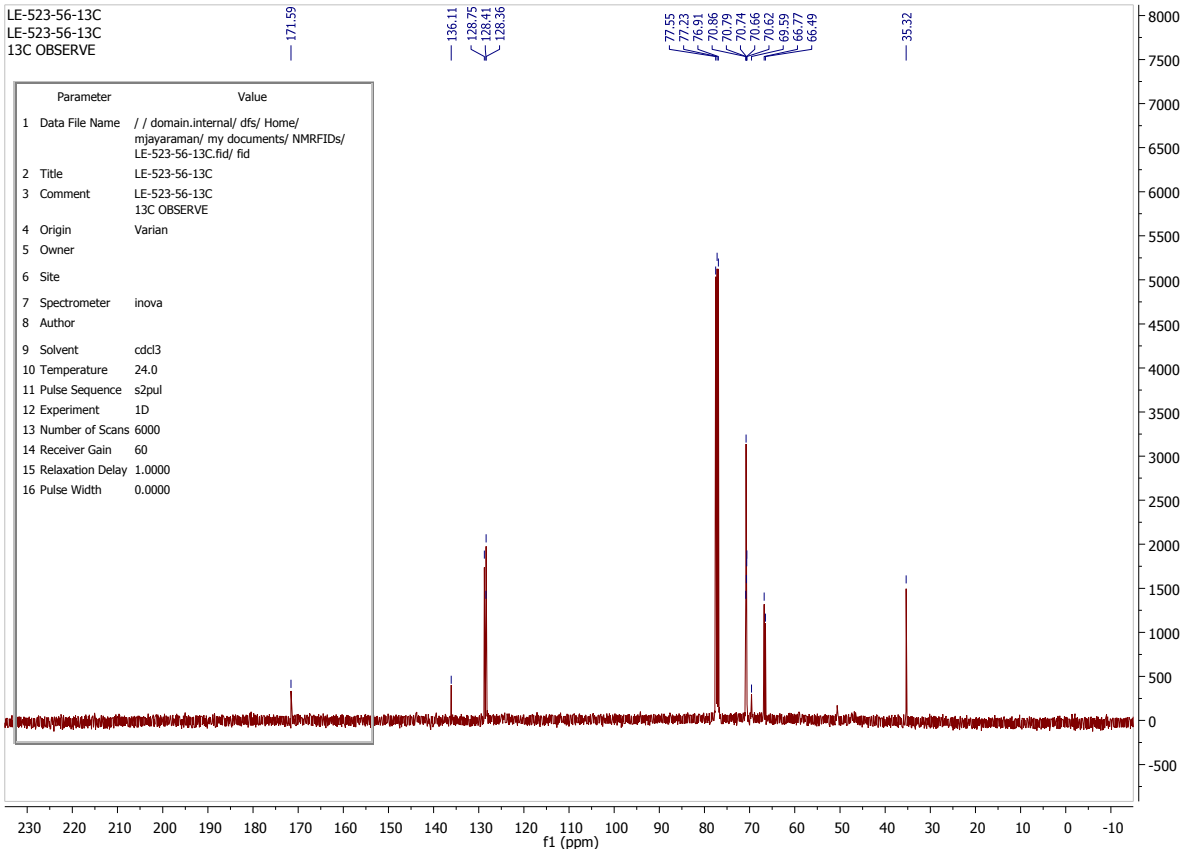
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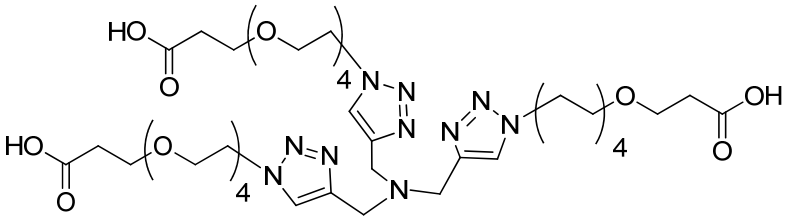

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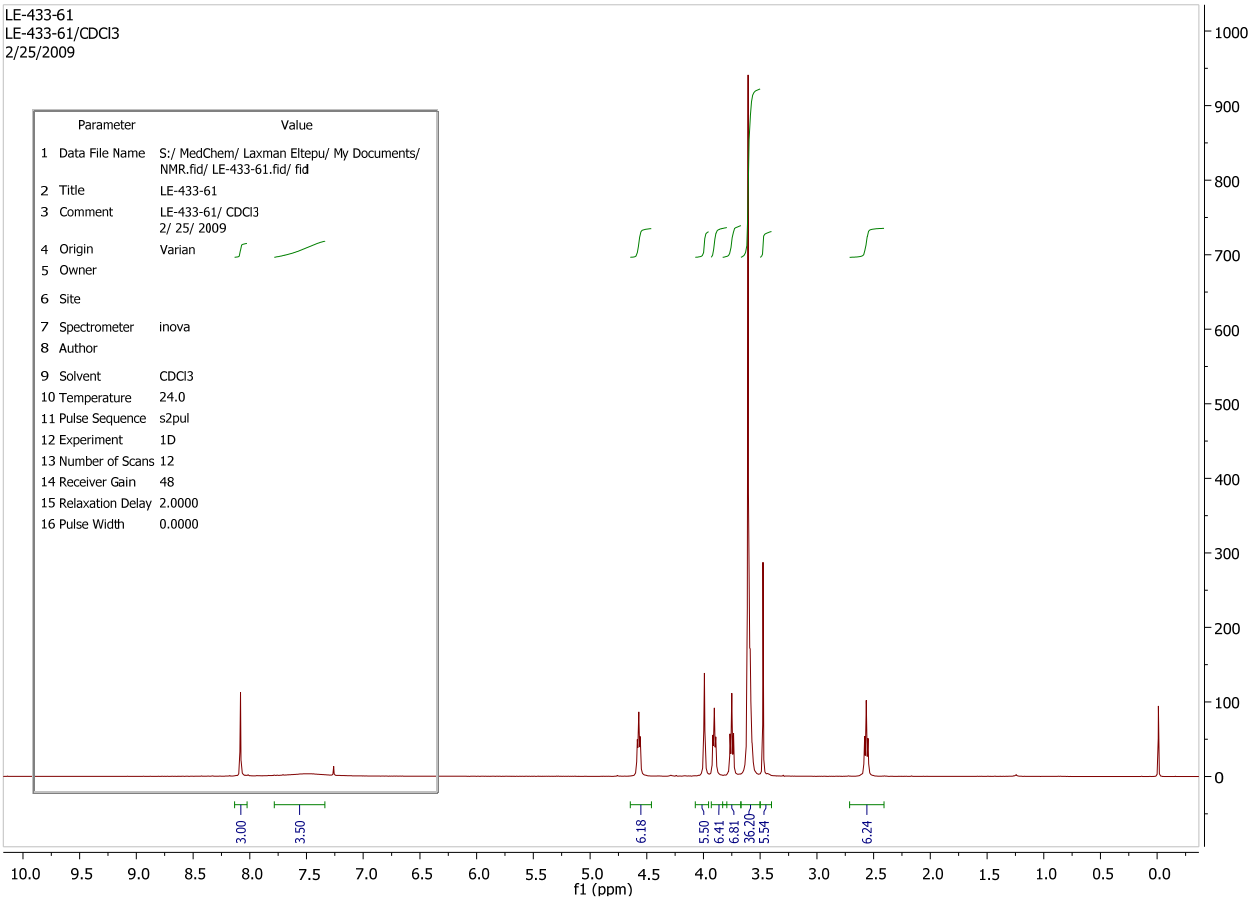
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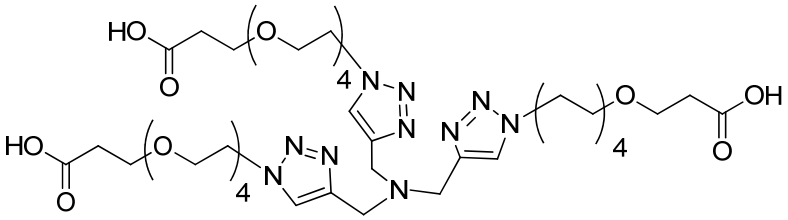

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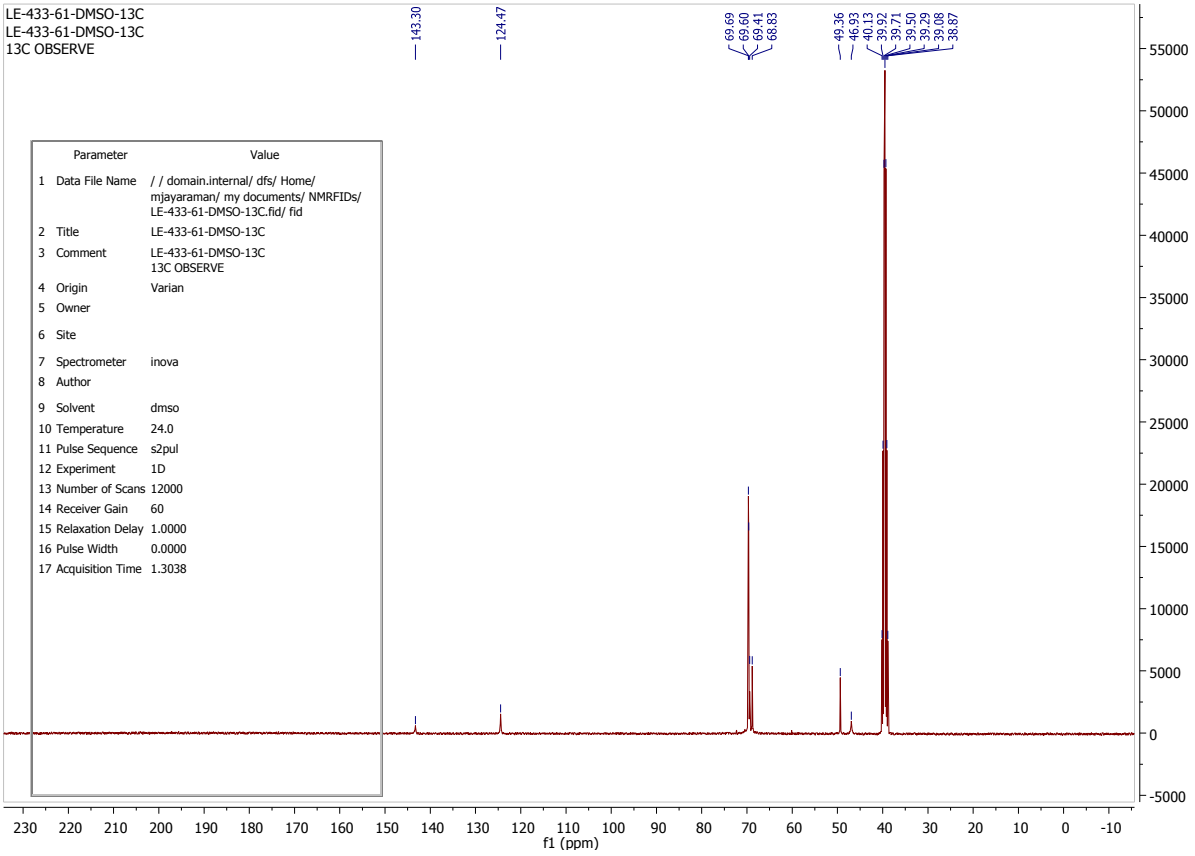
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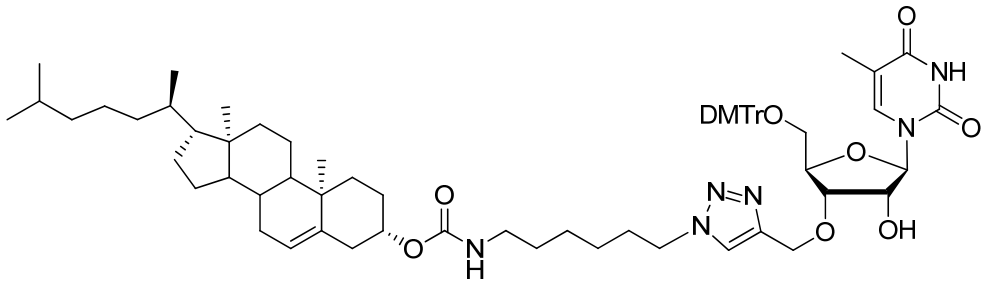
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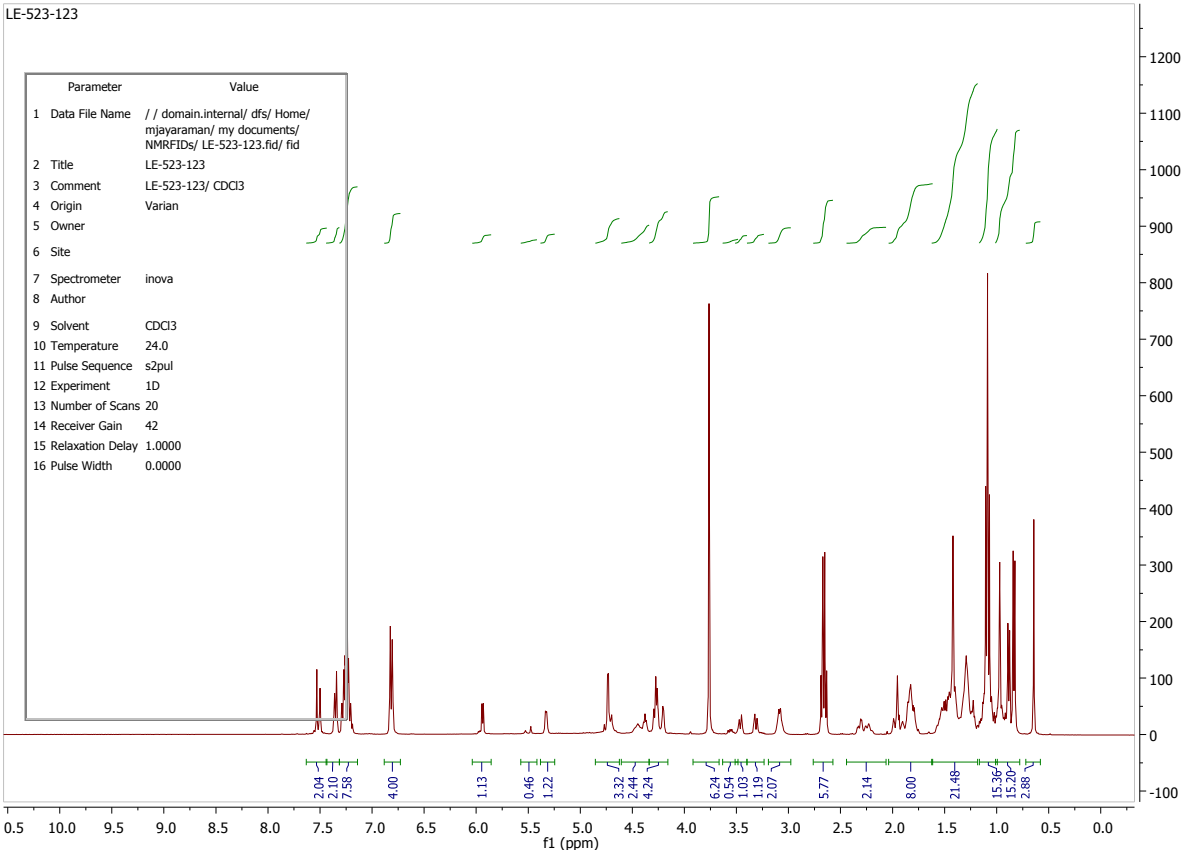
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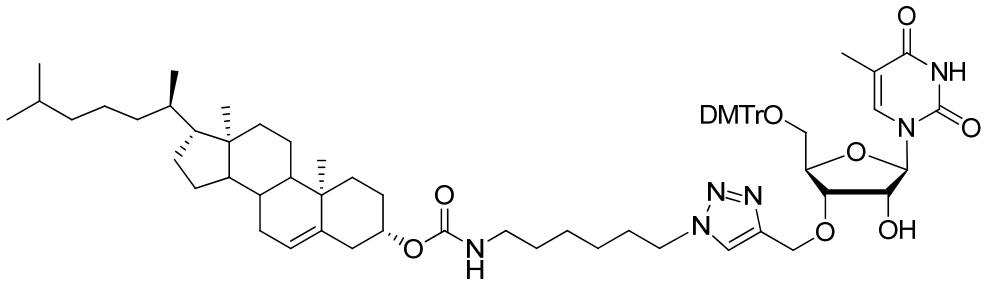
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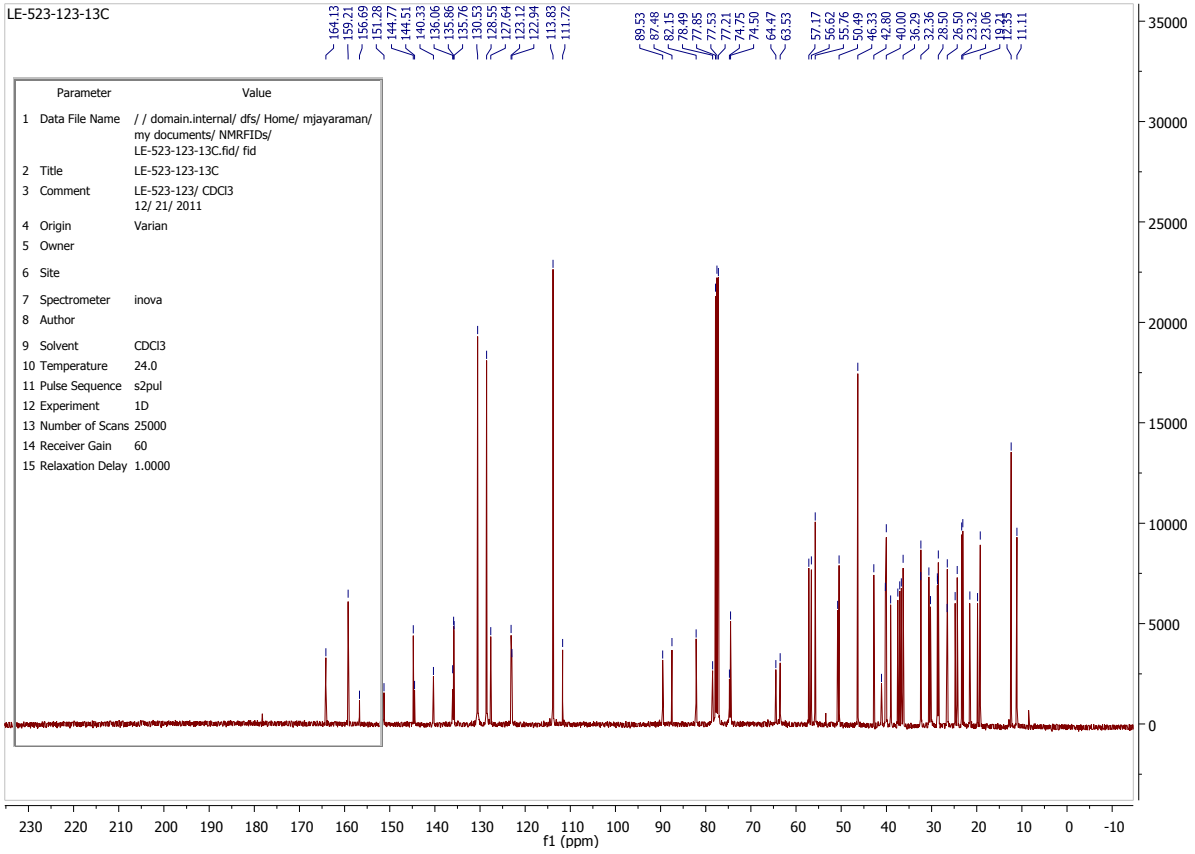
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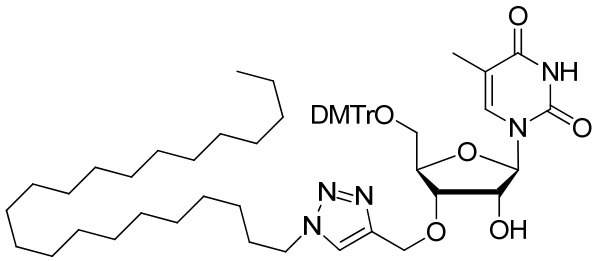
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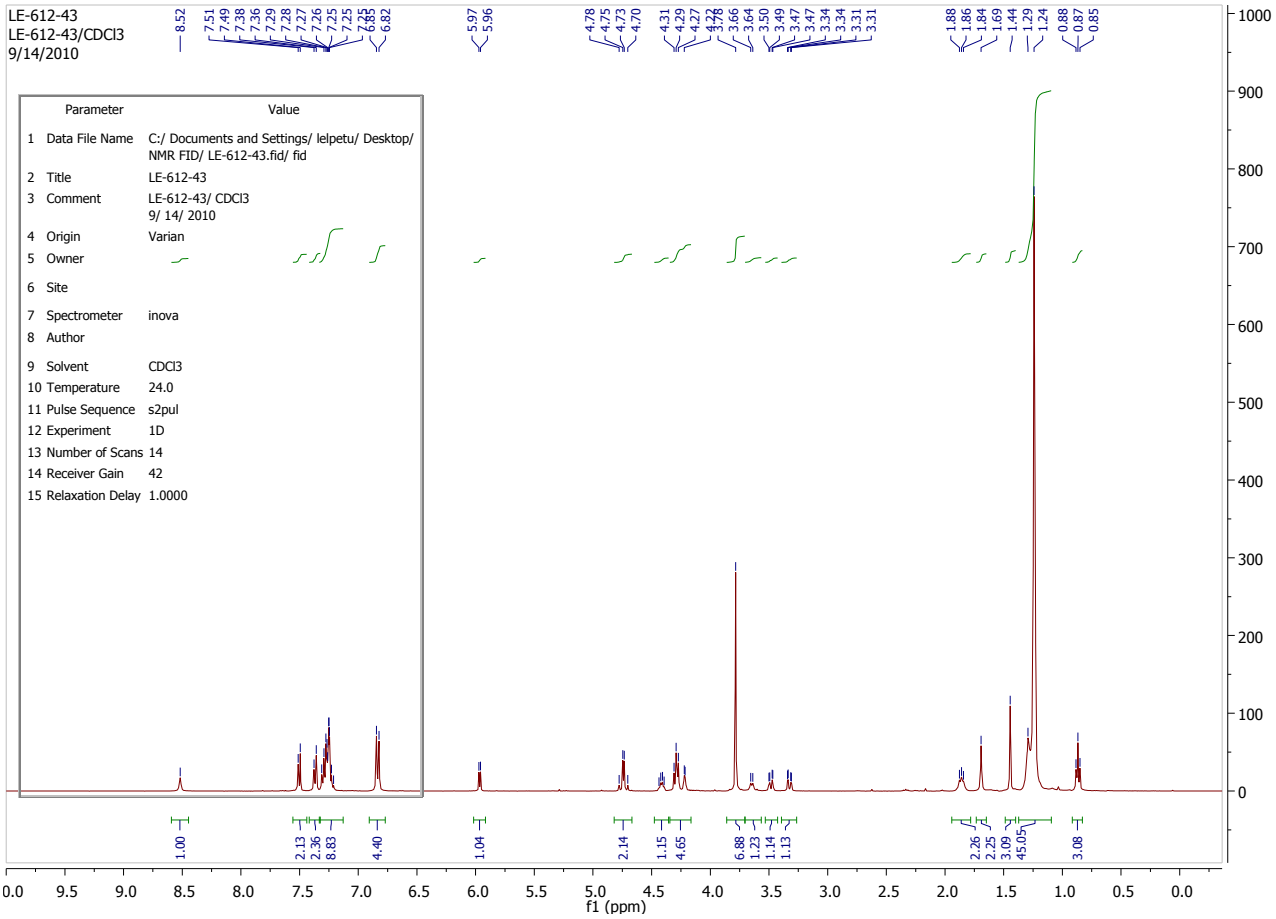
OH

12


LE-612-43
LE-612-43/CDCl3
9/14/2010
Parameter Value
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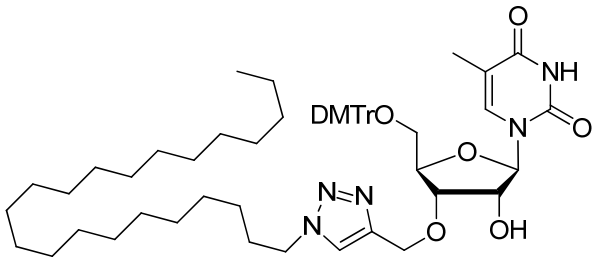
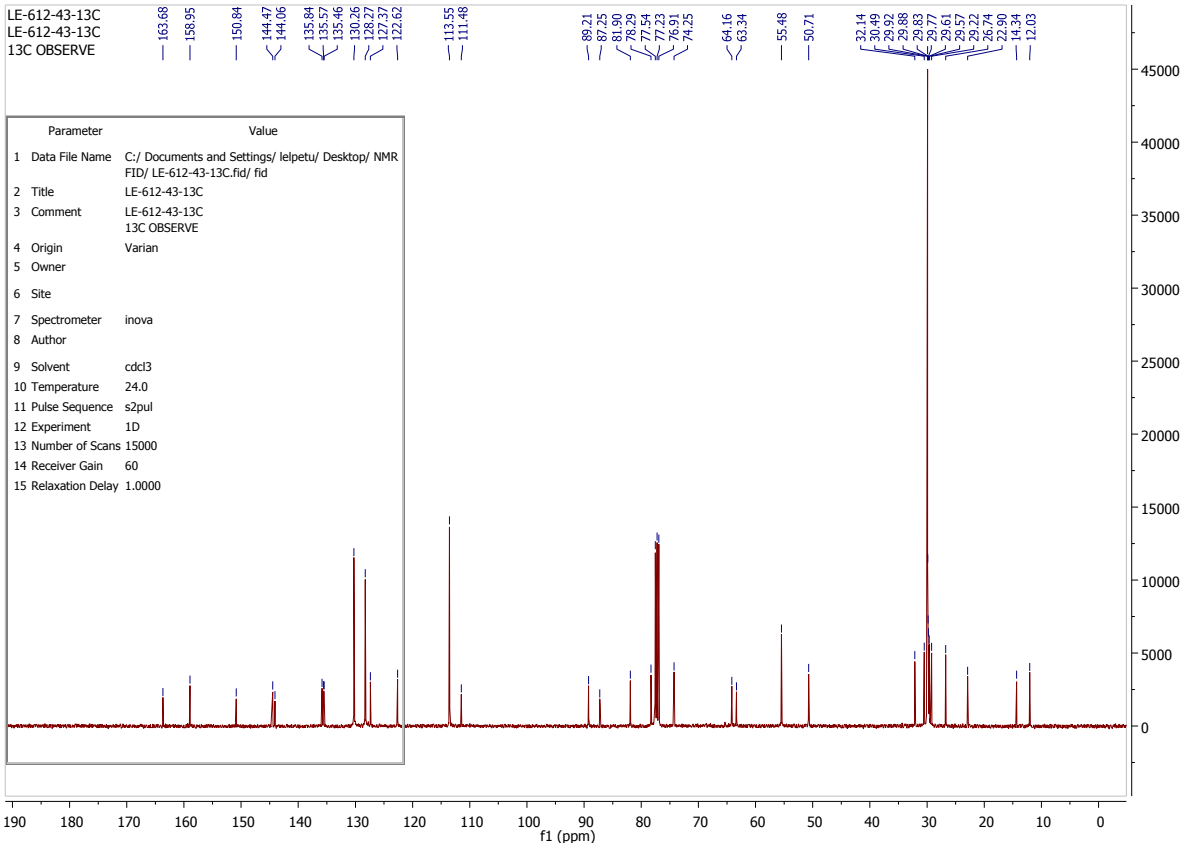
2 Title LE-612-43
3 Comment LE-612-43/ CDCl3 9/ 14/ 2010
4 Origin Varian
5 Owner
6 Site
7 Spectrometer inova
8 Author
9 Solvent CDCl3
10 Temperature 24.0
11 Pulse Sequence s2pul
12 Experiment 1D
13 Number of Scans 14
14 Receiver Gain 42
15 Relaxation Delay 1.0000
f1 (ppm)


O
NH
DMTrO
N
O
O
N=N
N
O
OH

12


LE-612-43-13C
LE-612-43-13C
13C OBSERVE
163.68
158.95
150.84
144.47
144.06
135.84
135.57
135.46
130.26
128.27
127.37
122.62
113.55
111.48
89.21
87.25
81.90
78.29
77.54
77.23
76.91
74.25
64.16
63.34
55.48
50.71
32.14
30.49
29.92
29.88
29.83
29.77
29.61
29.57
29.22
26.74
22.90
14.34
12.03
Parameter Value
1 Data File Name C:/ Documents and Settings/ lelpetu/ Desktop/ NMR FID/ LE-612-43-13C.fid/ fid
2 Title LE-612-43-13C
3 Comment LE-612-43-13C 13C OBSERVE
4 Origin Varian
5 Owner
6 Site
7 Spectrometer inova
8 Author
9 Solvent cdcl3
10 Temperature 24.0
11 Pulse Sequence s2pul
12 Experiment 1D
13 Number of Scans 15000
14 Receiver Gain 60
15 Relaxation Delay 1.0000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)