Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Assembly of ruthenium complexes on a double strand DNA using mismatch binding ligand

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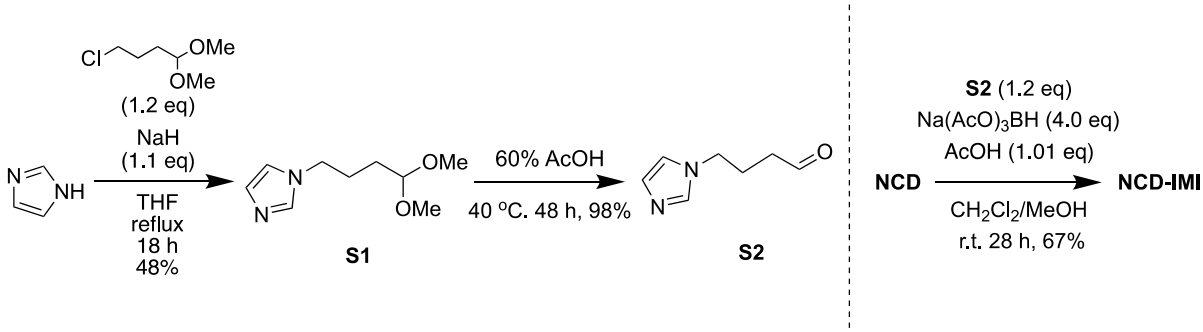
Supporting Information



Scheme S1
Preparation of **NCD**-IMI.

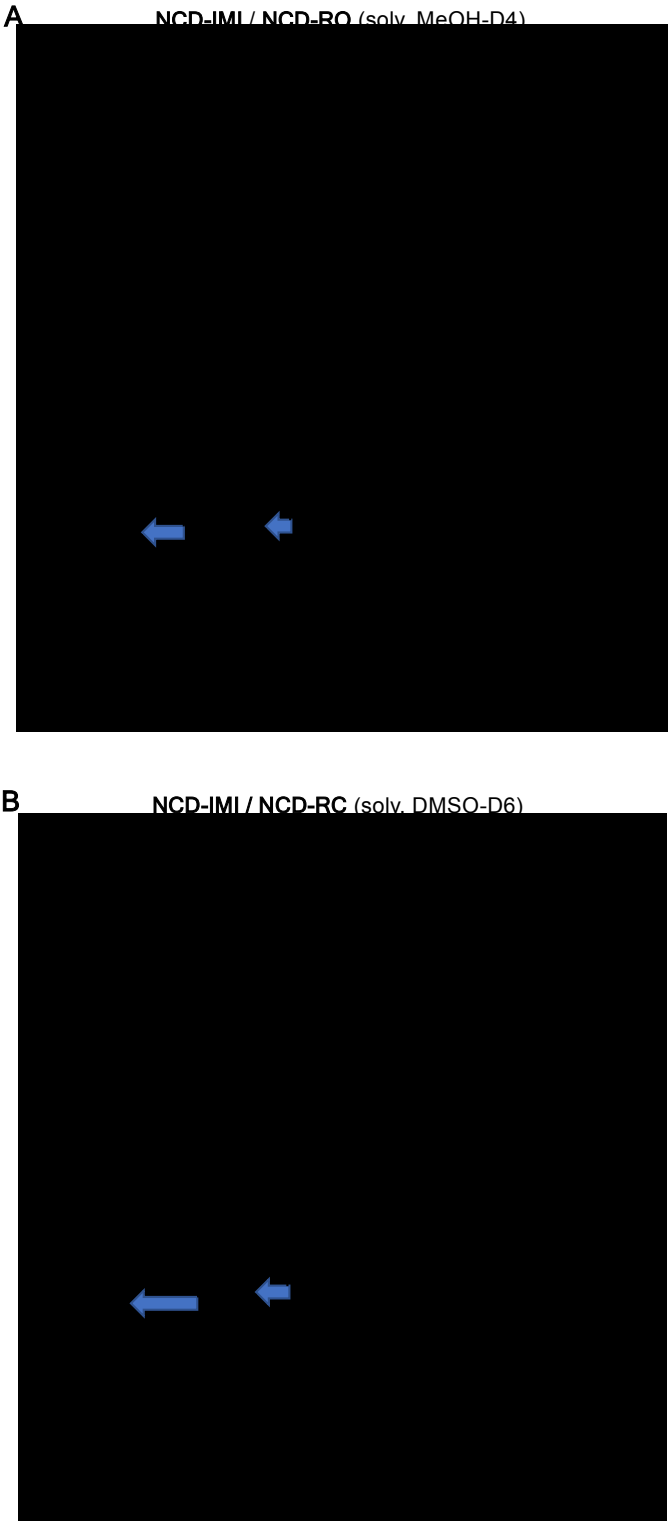
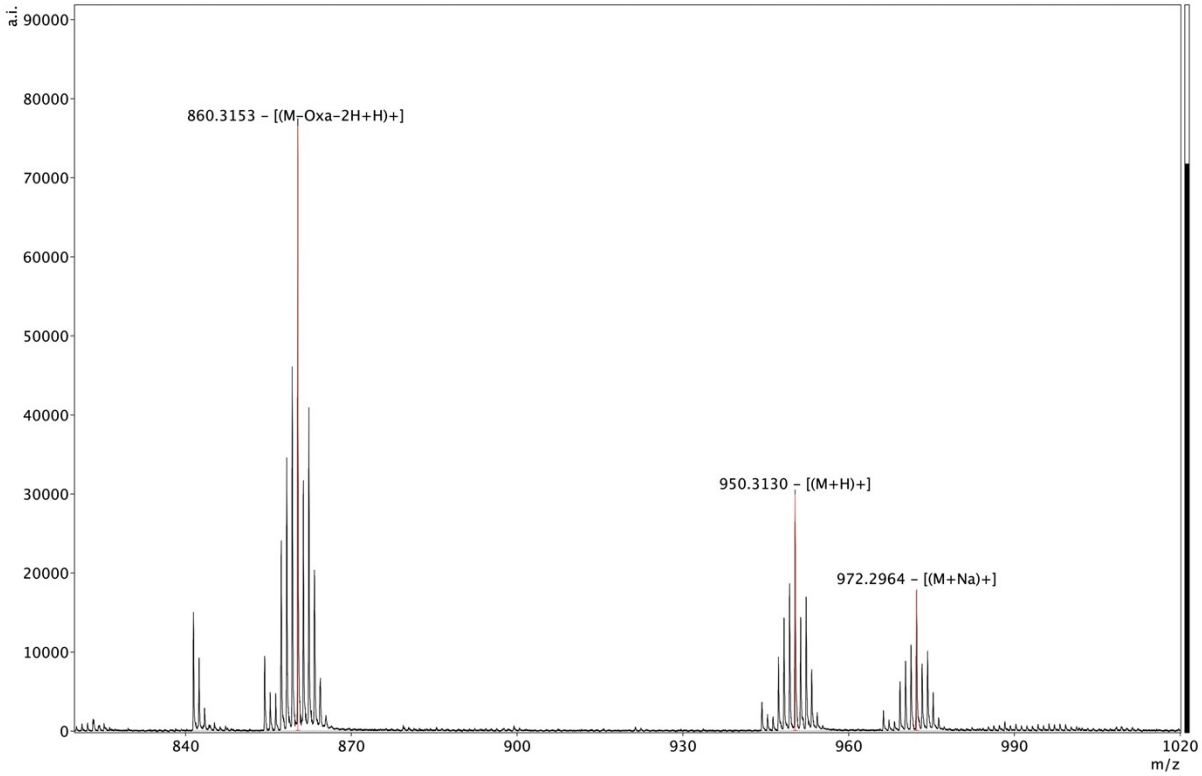


Fig. S1

Comparison of relevant peaks of ^{1}H-NMR (A) between **NCD-IMI** (top) and **NCD-RO** (bottom) in MeOH-D4 (B) between **NCD-IMI** (top) and **NCD-RC** (bottom) in DMSO-D6. The shifts of δ = 0.27 ppm (2H of imidazole) and δ = 0.13 ppm (5H of imidazole) for NCD-RO and δ = 0.33 ppm (2H of imidzaole) and δ = 0.16 ppm (5H of imidazole) for NCD-RC are shown arrows.



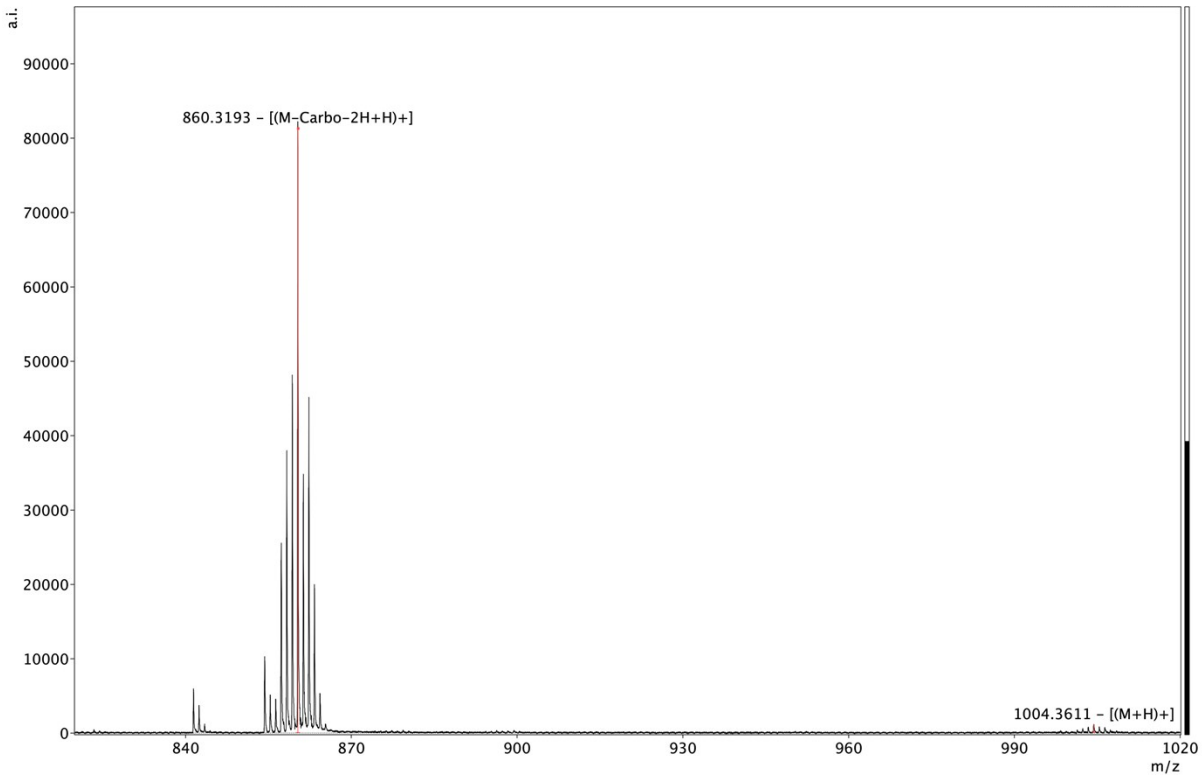


Fig. S2
HR-ESI-MS analysis of **NCD-RO** (top) and **NCD-RC** (bottom).

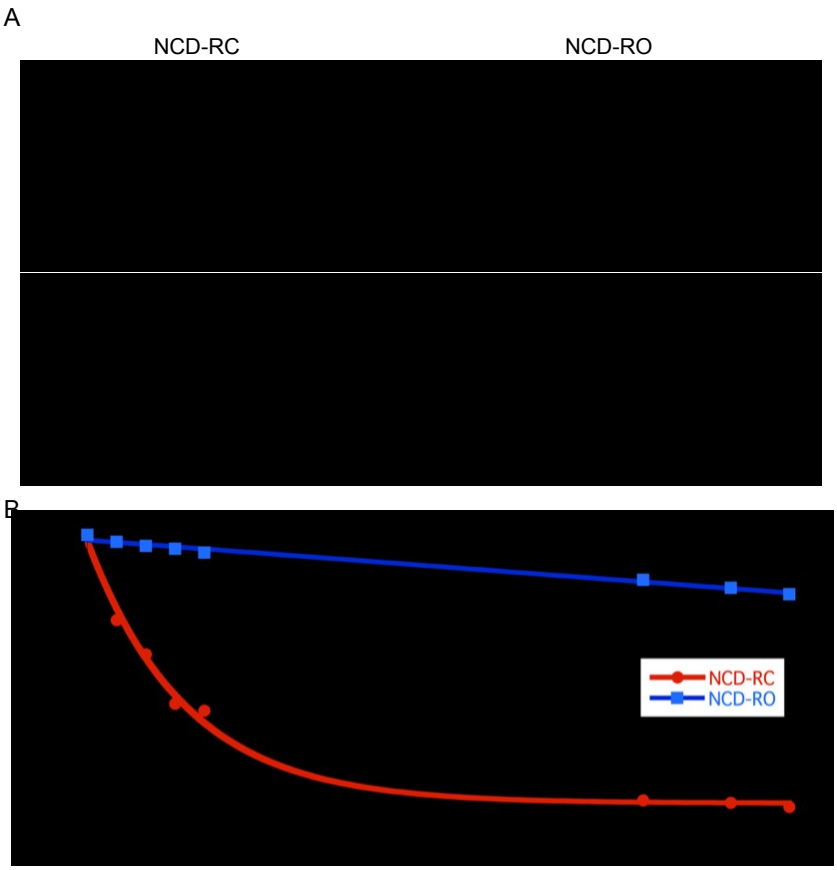


Fig. S3

The susceptibility of **NCD-RO** and **NCD-RC** in the presence of water analyzed by 1H-NMR. (A, left) The parts of 1H-NMR peak shifts of **NCD-RC** (10 mM) in mixed solution of D_2O and DMSO-d6 (1:4, v/v), and (A, right) that of **NCD-RO** (10 mM) in mixed solution of D_2O and methanol-d4 (1:4, v/v). Those solutions were kept still at room temperature, and were analyzed at the indicated time. (B) The relative peak integrals of p-cymene's peak (peak 1) compared with the internal standard (methanol) were plotted over the period. 1H-NMR was taken every hour up till 4 h, and additional data was taken at 19 h, 22 h, and 24 h.

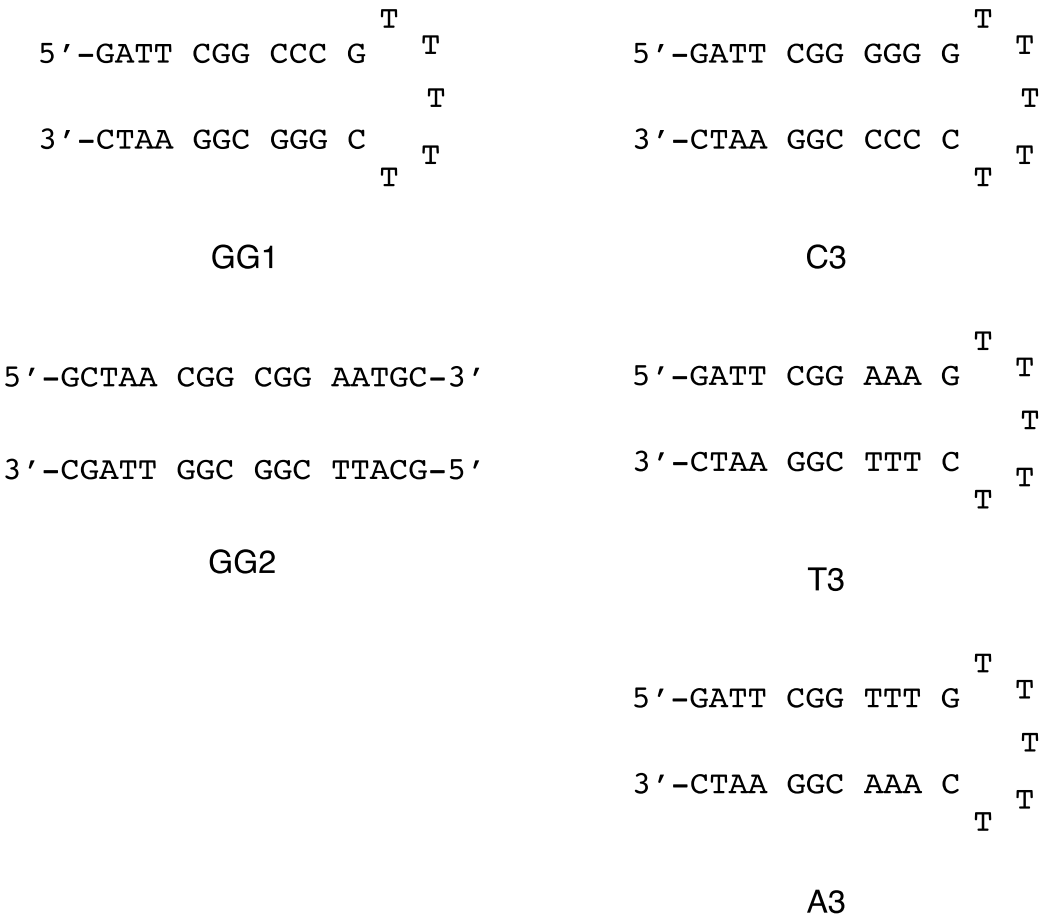
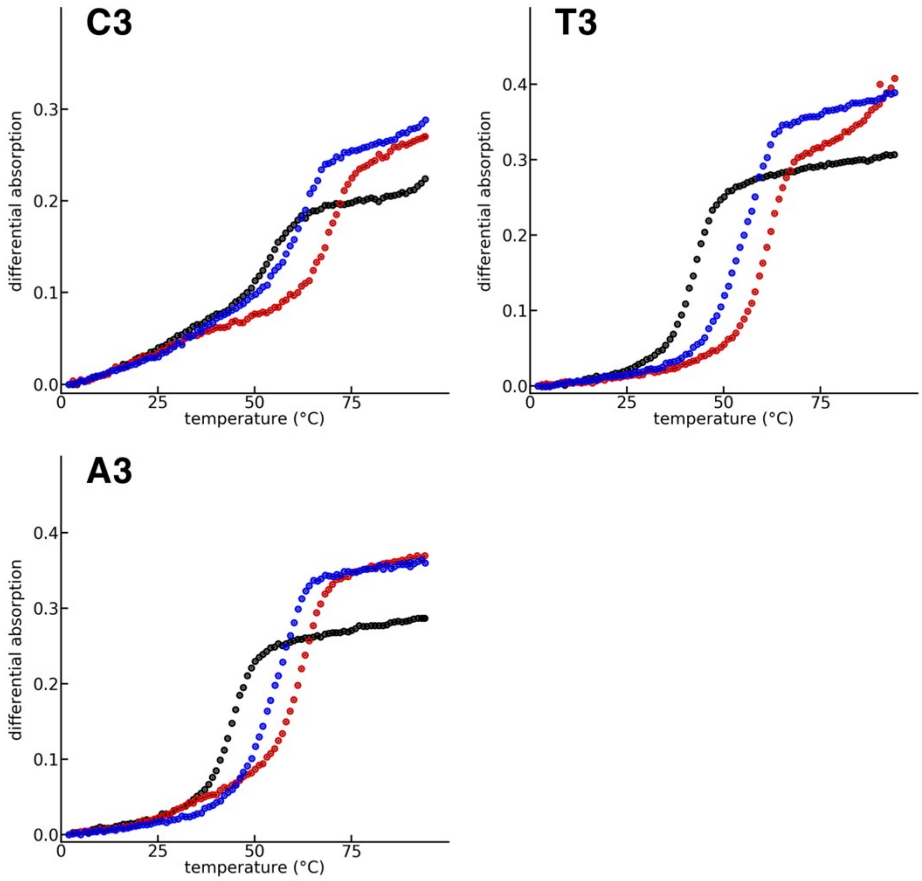


Fig. S4
The secondary structures of DNAs used in this study.



entry	compound	conc. (μM)	DNAs	T_m (°C)	SD	ΔT_m (°C)	TA (°C^{-1})
1	-	-	C3	54.2	0.2	-	0.009
2	**NCD**	20	C3	69.7	0.5	15.5	0.017
3	**NCD-RO**	20	C3	61.2	0.3	7.0	0.018
4	-	-	T3	42.3	0.4	-	0.022
5	**NCD**	20	T3	60.4	0.2	18.1	0.024
6	**NCD-RO**	20	T3	54.4	0.1	12.1	0.026
7	-	-	A3	43.9	0.2	-	0.020
8	**NCD**	20	A3	61.5	0.3	17.6	0.022
9	**NCD-RO**	20	A3	54.8	0.1	10.9	0.020

Fig. S5

T_m profiles for modified DNA (**C3**, **T3**, **A3**) in the absence or presence of compounds. UV-melting profiles for the 5 μM DNA in the absence or the presence of each compound were measured in 10 mM sodium phosphate buffer (pH 7.0) containing 4 mM NaCl; DNA only (black); DNA with **NCD** (red), DNA with **NCD-RO** (blue). The table below summarizes obtained T_m values and calculated ΔT_m and TA values for each DNA used. The DNA sequence used for this experiment is **C3** (5'-GATT CGG GGG G TTTTT C CCC CGG AATC-3'), **T3** (5'-GATT CGG AAA G TTTTT C TTT CGG AATC-3'), **A3** (5'-GATT CGG TTT G TTTTT C AAA CGG AATC-3') (The residues modified compared to **GG1** DNA are underlined)

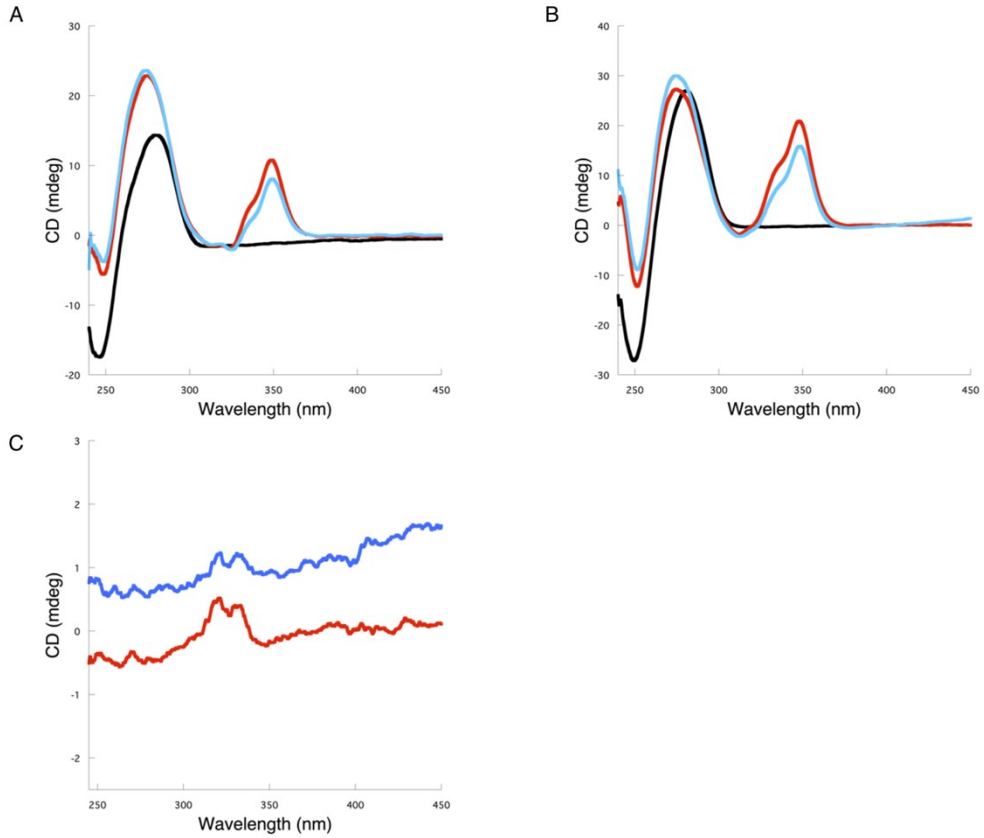


Fig. S6

CD spectrum change observed for DNA (Black) in presence or absence of **NCD** (red) or **NCD-RO** (blue). (A, top left) CD spectra of GG1 DNA (5 µM) in presence or absence of Ligand **NCD** or **NCD-RO** (20 µM), with DNA:Ligand molar ratio at 1:4. (B, top right) CD spectra of GG2 (5 µM) DNA in presence or absence of Ligand **NCD** or **NCD-RO** (40 µM), with DNA:Ligand molar ratio at 1:8. (C, bottom left) CD spectrum of Ligand **NCD** and **NCD-RO** (40 µM) in absence of DNA.

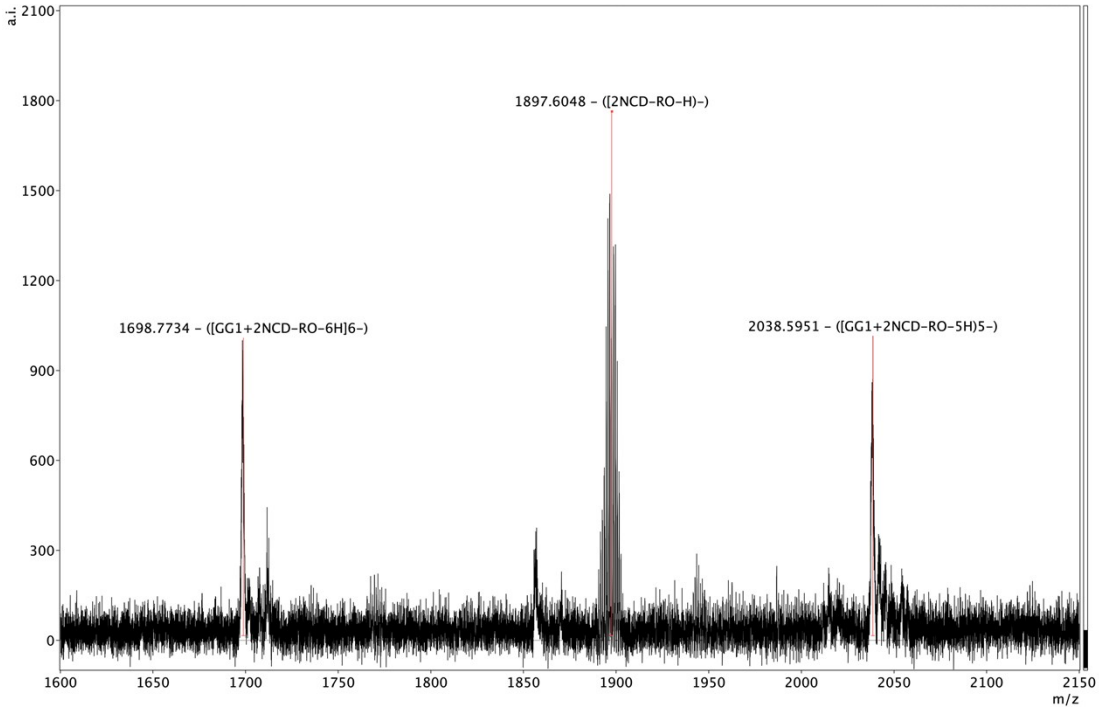


Fig. S7

Coldspray mass spectrometric analysis of GG1 DNA (20 µM) in presence of **NCD-RO** (80 µM) in NH_4OAc (100 mM) in MeOH/H2O (1:1, v/v). [(GG1+2NCD-RO-5H)$^{5-}$] calc. (m/z) 2038.5939, found 2038.5951; [(GG1+2NCD-RO-6H)$^{6-}$] calc. (m/z) 1698.6604, found 1698.7734.

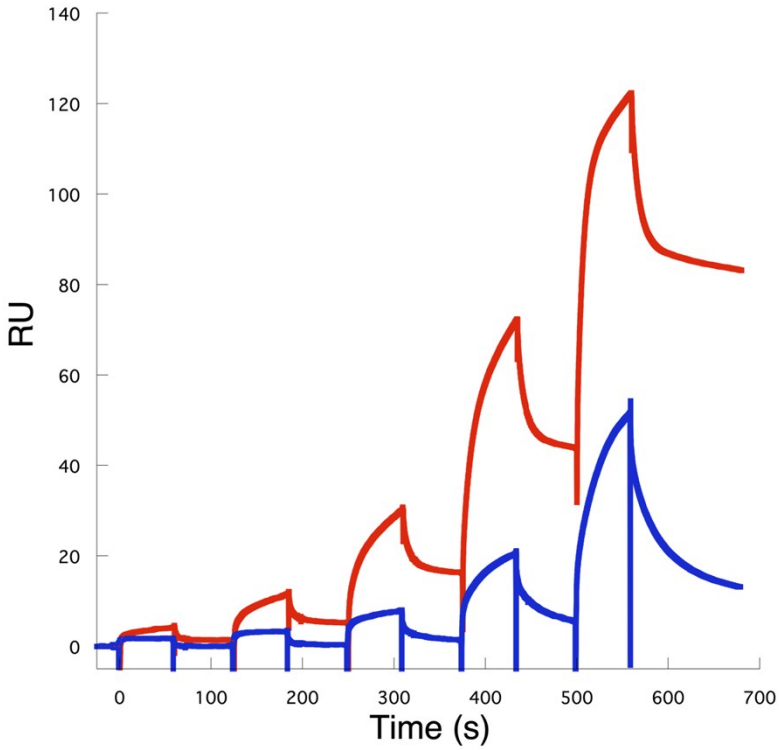


Fig. S8

Surface plasmon resonance (SPR) experiments determining the equilibrium binding constants of **NCD**(red) and **NCD-RO** (blue) towards immobilized CGG/CGG containing DNA time in HBS-EP$^+$ buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05 % v/v Surfactant P20, pH 7.4) with 5% DMSO content. The immobilized sequence used is: biotin-TEG-5'-CTAA CGG AATG TTTT CATT CGG TTAG-3' (The CGG/CGG region is underlined)

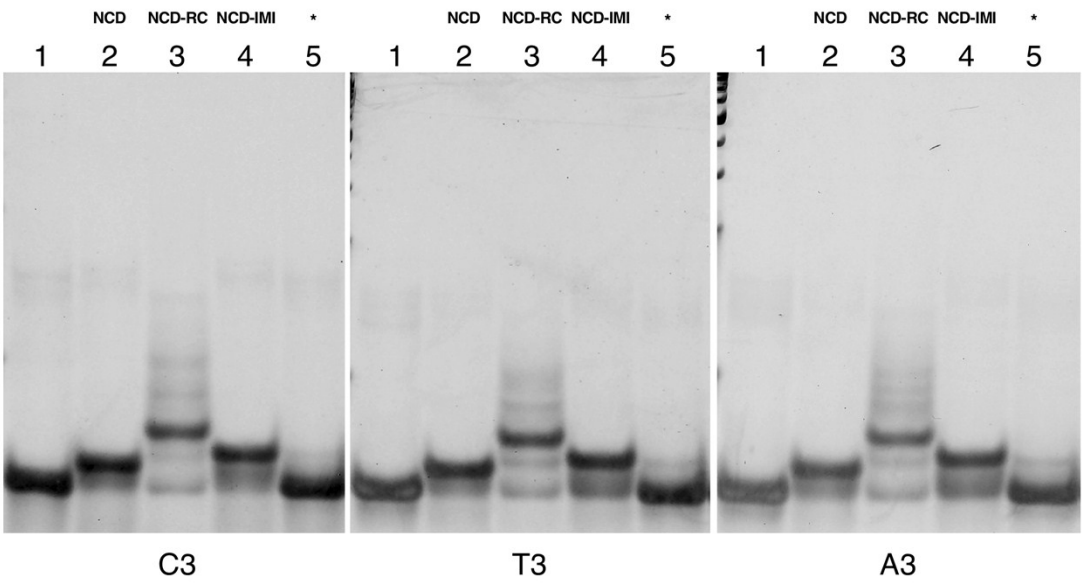


Fig. S9

Native PAGE analysis of respective DNAs (5 µM) in 10 mM NaCl, 10 mM sodium phosphate buffer (pH = 7.0) and 10% DMSO after incubation for 24 h under 37 °C in presence of respective ligands. Band shifting pattern of **C3, T3** and **A3** induced in the presence of different ligands at 1:8 (DNA:Ligand) ratio, **NCD** (lane 2), **NCD-RC** (lane 3), **NCD-IMI** (lane 4), **Carbo-RAI** (lane 5).

Experimental procedure

General. Reagents and solvents were purchased from standard suppliers and used without further purification. Reactions were monitored with TLC plate silica gel 60 F_{254}. Spots on TLC were monitored with UV, phosphomolybdic acid, ninhydrin, or anisaldehyde. C-300 Silica gel was used for silica gel chromatography. Circular dichroism (CD) measurements was conducted on J-725 CD spectropolarimeter (JASCO). T_m melting temperature measurements was conducted on UV-2700 UV-Vis spectrophotometer (SHIMADZU). CHEMCOBOND 5C_{18}-MS-II column (20 x 150 mm) was used for RP-HPLC. Surface plasmon resonance (SPR) measurements were conducted using Biacore T200 instrument (GE Healthcare). The CSI-TOF-MS was conducted using AccuTOF JMS-T100N mass spectrometer (JEOL) The multiplicity was expressed as follows; s = singlet, d = doublet, t = triplet, q = quintuplet, tt = triplet of triplets, m = multiplet. The chemical shifts are expressed in ppm relative to residual solvent as an internal standard; 7.26 ppm (^{1}H NMR) in chloroform-d, 77.16 ppm (^{13}C NMR) in chloroform-d, 2.50 ppm (^{1}H NMR) in DMSO-D6, 39.52 (^{13}C NMR) in DMSO-D6, 3.31 ppm (^{1}H NMR) in MeOH-D4, 49.00 ppm (^{13}C NMR) in MeOH-D4; coupling constants (*J* values) were represented in hertz.

SPR assay.

1) Sensor preparation: For the SPR assay, DNA containing 5'-CGG-3'/5'-CGG-3' sequence was immobilized to the SA sensor chip (BIAcore) being coated the surface with streptavidin. The surface of sensor chip SA was first washed three times with 50 mM NaOH and 1 M NaCl for 60 s with the flow rate of 30 µL min^{-1}. To the surface, a biotin-labeled DNA (biotin-TEG-5'-CTAA CGG AATG TTTT CATT CGG TTAG-3') (The CGG/CGG region is underlined) was reacted to the surface under the following conditions: 1 µM DNA in 10 mM HEPES, 500 mM NaCl, pH 7.4, the flow rate of 5 µL min^{-1} for the flow of 60 sec. The amount of DNA immobilized on sensor chip SA was about 672 RU.

2) SPR analysis protocol: SPR analysis for the binding of NCD and NCD-RO to the DNA-immobilized surface was carried out by subsequently flowing 0.0625, 0.125, 0.25, 0.5, and 1 µM of each compound for 60 sec of contacting time in HBS-EP$^+$ buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05 % v/v Surfactant P20, pH 7.4) (GE lifescience) containing 5 % DMSO with the flow rate of 30 µL min^{-1} at 25 °C followed by dissociation of bound compounds by flowing running buffer for 120 s. The flow path except sensor surface was then washed with 50 % aqueous solution of DMSO. Surface regeneration was carried out after assay of each compound using 1.2 mM NaOH, 0.2 M NaCl, and 0.1 mM EDTA solution, with a contact time of 180 sec and flow rate of 30 µL min^{-1}. Kinetic parameters of the binding of **NCD** and **NCD-RO** to the DNA-immobilized surface were obtained by using the single-cycle kinetics method.

Coldspray time-of-flight mass spectrometry: (CSI-TOF-MS)

The solution of **NCD-RO** (80 µM) containing the **GG1** DNA (20 µM), NH_4OAc (100 mM) in MeOH/H2O (1:1, v/v) was analyzed with an electrospray ionization mass spectrometer in the negative mode (orifice 1 voltage = -60 V). Spray temperature was fixed at -10 °C with a sample flow rate of 25 µl min^{-1}. Nitrogen gas was used as a de-solvation gas as well as a nebulizer.

Native polyacrylamide gel electrophoresis: (Native PAGE)

The samples were prepared under the stated conditions and were adjusted later to 10% glycerol content before loading. The electrophoresis was carried out using native polyacrylamide gel (16%, 19:1) in ice bath at 200 V and the gels were stained with SYBR Gold.

1-(4,4-dimethoxybutyl)-1*H*-imidazole (S1): 60% NaH oil suspension (184 mg, 4.62 mmol) was first dissolved in THF (3 mL) at 0 ^{o}C in ice bath under argon atmosphere prior to addition of imidazole (283 mg, 4.20 mmol) in THF (3 mL). The mixture was stirred for 1 h to allow the generated hydrogen gas to escape and was removed from the ice bath. 4-chloro-1,1-dimethoxybutane (734 µL, 5.00 mmol) was then added dropwise into the mixture. The resulting reaction mixture was then stirred and refluxed for 18 h. The reaction was then quenched via addition of water to the reaction mixture, water was added until all solids were dissolved. THF was then removed under reduced pressure and the remaining solution was extracted with chloroform (10 mL) three times before the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Resulting crude mixture was purified by column chromatography on silica using the elution solvent of chloroform/methanol = 20:1 (v/v). The product (372 mg, 2.02 mmol, 48%) was isolated as clear light-yellow liquid; 1H-NMR (600 MHz, CHLOROFORM-D) δ 7.46 (s, 1H), 7.06 (s, 1H), 6.90 (s, 1H), 4.32 (t, J = 5.5 Hz, 1H), 3.95 (t, J = 7.1 Hz, 2H), 3.29 (s, 6H), 1.84 (m, 2H), 1.58 (m, 2H); 13C-NMR (150 MHz, CHLOROFORM-D) δ 137.2, 129.6, 118.8, 104.2, 53.3, 46.9, 29.6, 26.3; HR-ESIMS calcd for $C_9H_{17}N_2O_2^+$ [$(M+H)^+$] 185.1285, found 185.1280.

4-(1*H*-imidazol-1-yl)butanal (S2): the previously obtained 1-(4,4-dimethoxybutyl)-1*H*-imidazole (128 mg, 0.697 mmol) was dissolved in acetic acid 60% aqueous solution (6 mL). The reaction mixture was then stirred for 48 h at 40 ^{o}C. Then the reaction was quenched and neutralized with Na_2CO_3 solution, and the product was extracted with dichloromethane. The organic layer was then washed with brine and dried over Na_2SO_4. Then the organic layer was condensed under reduced pressure providing light yellow liquid (94 mg, 0.68 mmol, 98%). The resulting substance was directly used for the reductive amination coupling of next step without further purification. 1H-NMR (600 MHz, CHLOROFORM-D) δ 9.76 (s, 1H), 7.46 (s, 1H), 7.07 (s, 1H), 6.90 (s, 1H), 4.00 (t, J = 6.9 Hz, 2H), 2.46 (t, J = 6.9 Hz, 2H), 2.10 (tt, J = 7.1, 6.9 Hz, 2H); 13C-NMR (150 MHz, CHLOROFORM-D) δ 200.6, 137.2, 130.0, 118.8, 45.9, 40.3, 23.5; HR-ESIMS calcd for $C_7H_{11}N_2O^+$ [$(M+H)^+$] 139.0866, found 139.0864.

((4-(1*H*-imidazol-1-yl)butyl)azanediyl)bis(propane-3,1-diyl)bis((7-methyl-1,8-naphthyridin-2-yl)carbamate) (NCD-IMI): To the solution of NCD (251 mg, 0.50 mmol) in dry dichloromethane (8 mL) was added 4-(1*H*-imidazol-1-yl)butanal (94 mg, 0.68 mmol) dissolved in dry methanol (5 mL). The mixture was allowed to stir for 20 min before sodium triacetoxyborohydride (423 mg, 2.00 mmol) and acetic acid (30 mg, 0.505 mmol) was added. The reaction solution was further stirred under room temperature for 28 h. The suspension was condensed under reduced pressure, and resuspended in chloroform (10 mL). The suspension was then washed with saturated sodium hydrogen carbonate solution and brine. Then the collected organic layer was dried over Na_2SO_4 and condensed under reduced pressure. The obtained crude product was purified with flash column chromatography on silica. Eluting solvent of chloroform/ methanol = 20 : 1 (v/v) gave **NCD-IMI** (209 mg, 0.335 mmol, 67%). 1H-NMR (600 MHz, DMSO-D6) δ 10.65 (s, 2H), 8.30 (d, J = 8.8 Hz, 2H), 8.19 (d, J = 8.1 Hz, 2H), 8.10 (d, J = 8.8 Hz, 2H), 7.57 (s, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.11 (s, 1H), 6.83 (s,

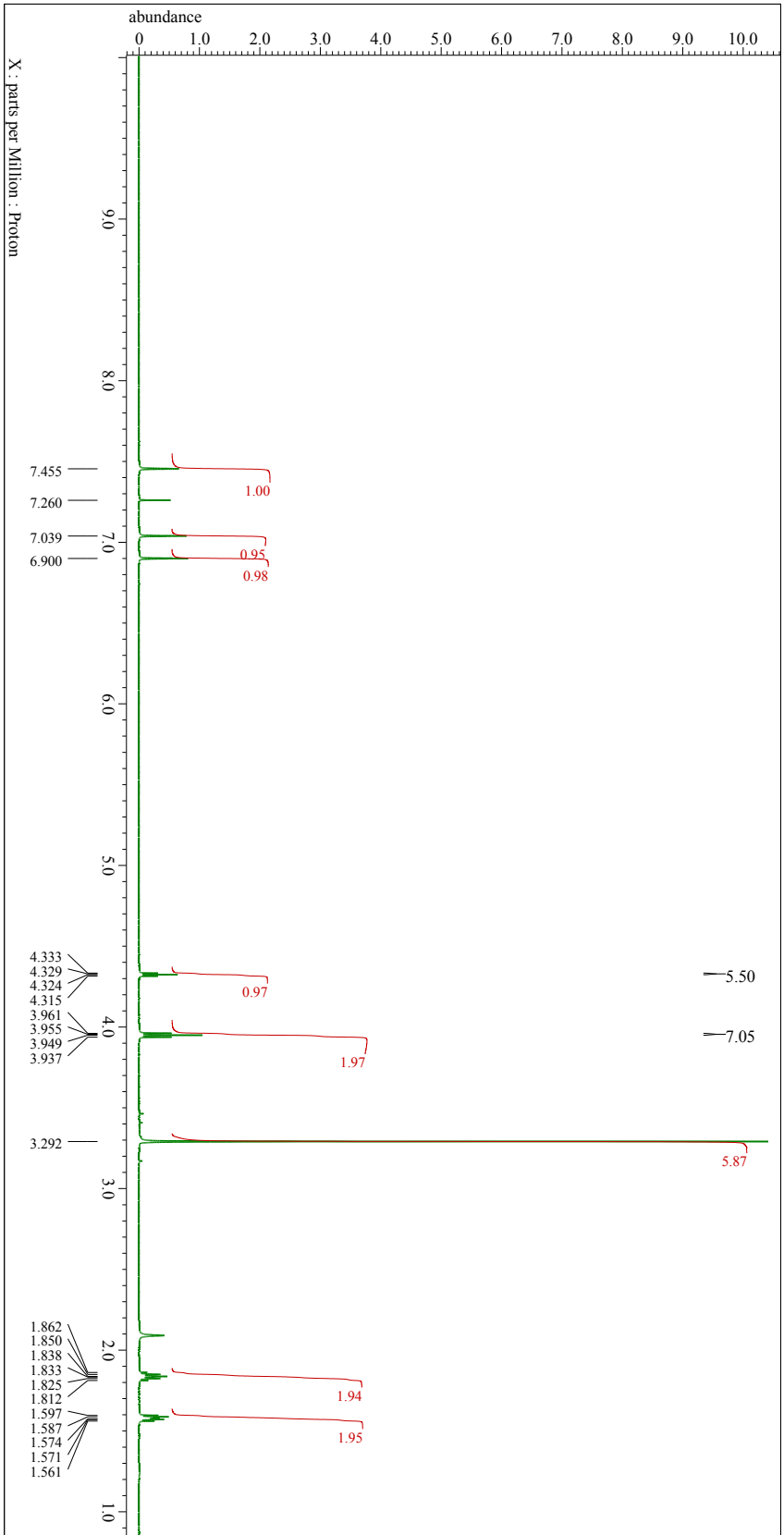
1H), 4.16 (t, J = 6.4 Hz, 4H), 3.92 (t, J = 7.1 Hz, 2H), 2.63 (s, 6H), 2.47 (t, J = 7.1 Hz, 4H), 2.37 (t, J = 6.9 Hz, 2H), 1.76 (m, 4H), 1.70 (m, 2H), 1.35 (m, 2H); 13C-NMR (175 MHz, DMSO-D6) δ 162.3, 154.5, 154.2, 153.9, 139.1, 137.1, 136.8, 128.3, 121.0, 119.2, 117.4, 112.8, 63.1, 52.8, 49.5, 45.9, 28.6, 25.2, 23.7; HR-ESIMS calcd for $C_{33}H_{40}N_9O_4^+$ [(M+H)$^+$] 626.3198 found 626.3192, calcd for $C_{33}H_{39}N_9O_4Na^+$ [(M+Na)$^+$] 648.3017, found 648.3010.

NCD-RO: [(*η*6-Cymene)RuCl(*μ*-Cl)]$_2$ (61 mg, 0.10 mmol) was mixed with silver oxalate (76 mg, 0.25 mmol) in water (15 mL) for 12 h in room temperature. The precipitate was filtered and removed. The filtrate was lyophilized to yield bright green-yellow solid (69 mg, 0.10 mmol, 99%) (HR-ESI-MS: calcd. for $C_{12}H_{14}O_4RuNa+$ 346.9833, found 346.9834). The solid was dissolved in 2 mL methanol to make a methanol stock solution of 50 mM. **NCD-RO** was then prepared *in situ* in methanol, where **NCD-IMI** (22 mg, 0.035 mmol) was dissolved in 350 µL methanol to make a 100 mM stock solution, **NCD-RO** was prepared by mixing 200 µL of **NCD-IMI** (13 mg, 0.02 mmol) and 200 µL of ruthenium stock solution under room temperature. Further purification of the compound was done via RP-HPLC, using 50 min linear gradient from 0 to 50% acetonitrile in 0.1% AcOH, with the yield of 34% (6.6 mg, 0.0069 mmol) and column chromatography. For the NMR, CD, HR-ESI-MS, T_m, and native-PAGE analysis, however, the prepared solution was used directly in the experiments without further purification. For NMR studies, 50 µL of both stock solutions was condensed under reduced pressure and re-dissolved in methanol-d4 instead before mixing and the volume was later adjusted to 400 µL using methanol-D4. 1H-NMR (600 MHz, METHANOL-D4) δ 8.17 (d, J = 8.9 Hz, 2H), 8.16 (d, J = 8.9 Hz , 2H), 8.10 (d, J = 8.3 Hz, 2H), 7.85 (s, 1H), 7.32 (d, J = 8.3 Hz, 2H), 7.24 (s, 1H), 6.91 (s, 1H), 5.72 (d, J = 6.2 Hz, 2H), 5.51 (d, J = 6.2 Hz, 2H), 4.29 (t, J = 6.2 Hz, 4H), 4.05 (t, J = 6.9 Hz, 2H), 2.76 (m, 1H), 2.69 (s, 6H), 2.60 (m, 4H), 2.48 (t, J = 6.9 Hz, 2H), 2.09 (s, 3H), 1.91 (m, 4H), 1.85 (m, 2H), 1.44 (m, 2H), 1.27 (d, J = 6.9 Hz, 6H); 13C-NMR (175 MHz, METHANOL-D4) δ 167.8, 163.9, 155.9, 155.5, 155.4, 140.5, 140.2, 138.7, 130.6, 122.4, 122.3, 119.2, 114.3, 102.3, 98.9, 83.9, 80.8, 64.8, 54.4, 51.1, 32.3, 29.9, 27.5, 25.2, 25.0, 22.7, 18.1; HR-ESI-MS: calcd for $C_{45}H_{53}N_9O_8RuNa^+$ [(M+Na)$^+$] 972.2953, found 972.2964; calcd for $C_{45}H_{54}N_9O_8Ru^+$ [(M+H)$^+$] 950.3133 found 950.3130; calcd for $C_{43}H_{52}N_9O_4Ru^+$ [(M-Oxa-2H+H)$^+$] 860.3180, found 860.3153.

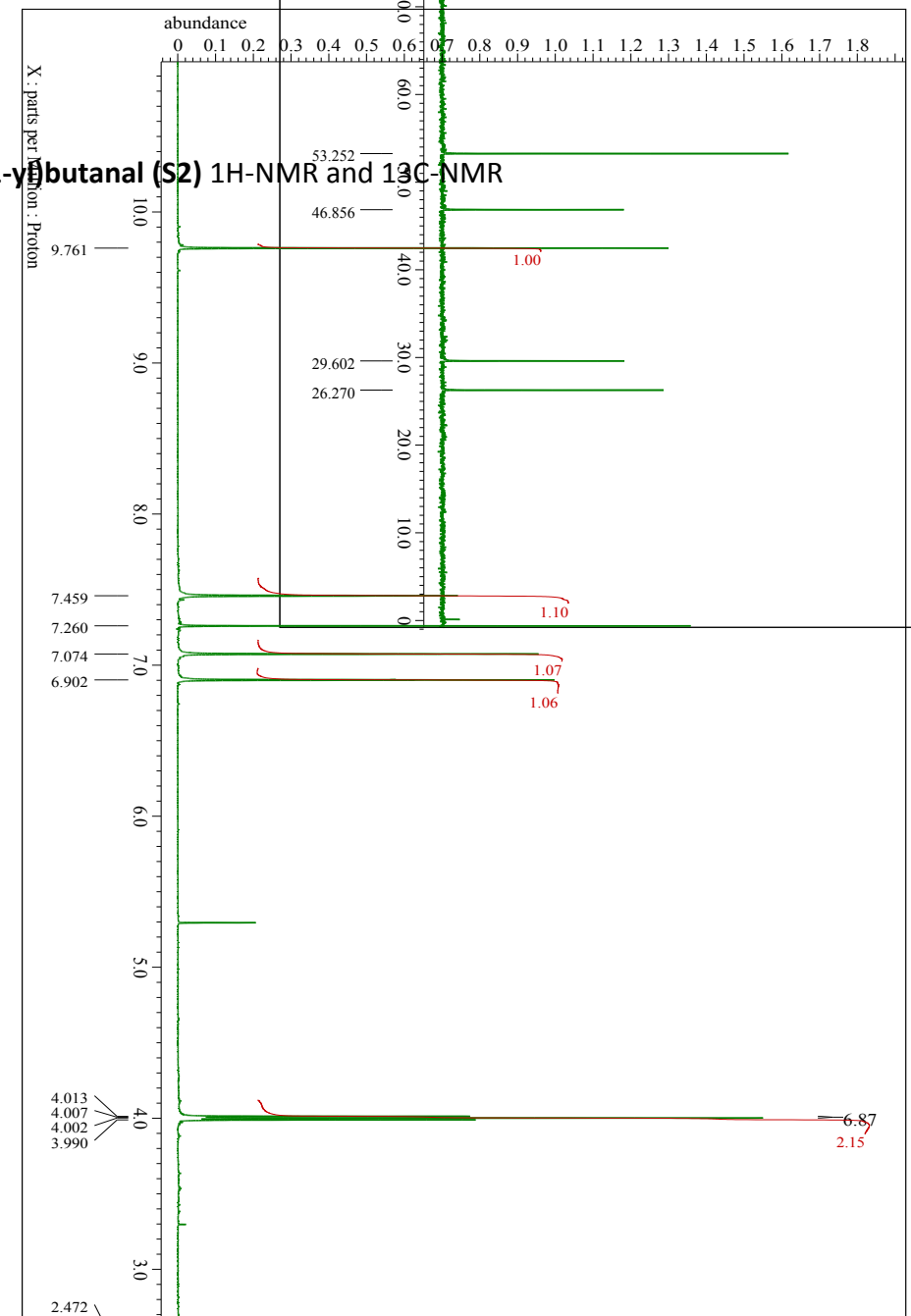
NCD-RC: [(*η*6-Cymene)RuCl(*μ*-Cl)]$_2$ (30 mg, 0.050 mmol) was mixed with silver 1,1-cyclobutanedicarbocylate (39 mg, 0.11 mmol) in acetonitrile (7 mL) for 18 h in room temperature, resulting in yellow pale solid. The solvent was removed under reduced pressure and the remaining solid was re-dissolved in methanol (15 mL) and filtered again to remove AgCl. The obtained filtrate was then condensed again to yield clear dark orange solid (30 mg, 0.040 mmol, 80%) (HR-ESI-MS: calcd. for $C_{16}H_{20}O_4RuNa^+$ 401.0303, found 401.0300). The solid was dissolved in 400 µL Chloroform to make a stock solution of 100 mM. Target compound was then prepared *in situ* in DMSO, where **NCD-IMI** (22 mg, 0.035 mmol) was dissolved in 350 µL methanol to make a 100 mM stock solution, **NCD-RC** was prepared by first condensing 5 µL of **NCD-IMI** and 2.5 µL previous ruthenium complex stock solution before re-dissolving the contents into the 5 µL of DMSO. The resulting solution was then mixed to prepare the stock **NCD-RC** solution, which was immediately used directly in the following experiments without further purification. For NMR experiments, 50 µL of **NCD-IMI** stock solution and 25 µL previous ruthenium complex, was condensed and re-dissolved into 50 µL DMSO-D6 instead before mixing and the volume was then adjusted to 400 µL using DMSO-D6. Due to the instability of the resulting complex, a fresh batch was made each time an experiment was

conducted and ^{13}C was also not obtained, as the complex would degrade in the process. 1H-NMR (600 MHz, DMSO-D6) δ 10.62 (s, 2H), 8.29 (d, J = 8.9 Hz, 2H), 8.17 (d, J = 8.1 Hz, 2H), 8.08 (d, J = 8.9 Hz, 2H), 7.90 (s, 1H), 7.34 (d, J = 8.1 Hz, 2H), 7.27 (s, 1H), 6.92 (s, 1H), 5.62 (d, J = 6.2 Hz, 2H), 5.44 (d, J = 6.0 Hz, 2H), 4.18 (t, J = 6.5 Hz, 4H), 3.99 (t, J = 7.1 Hz, 2H), 2.63 (m, 7H), 2.49(overlap with DMSO, confirmed by COSY), 2.39 (t, J = 7.1 Hz, 2H), 2.26 (m, 4H), 2.05 (s, 3H), 1.78 (m, 4H), 1.71 (m, 2H), 1.64 (m, 2H), 1.35 (m, 2H), 1.14(d, J = 6.9 Hz, 6H); HR-ESI-MS: calcd for $C_{49}H_{60}N_9O_8Ru^+$ [(M+H)$^+$] 1004.3603, found 1004.3611, calcd for $C_{43}H_{52}N_9O_4Ru^+$ [(M-Carbo-2H+H)$^+$] 860.3180, found 860.3193.

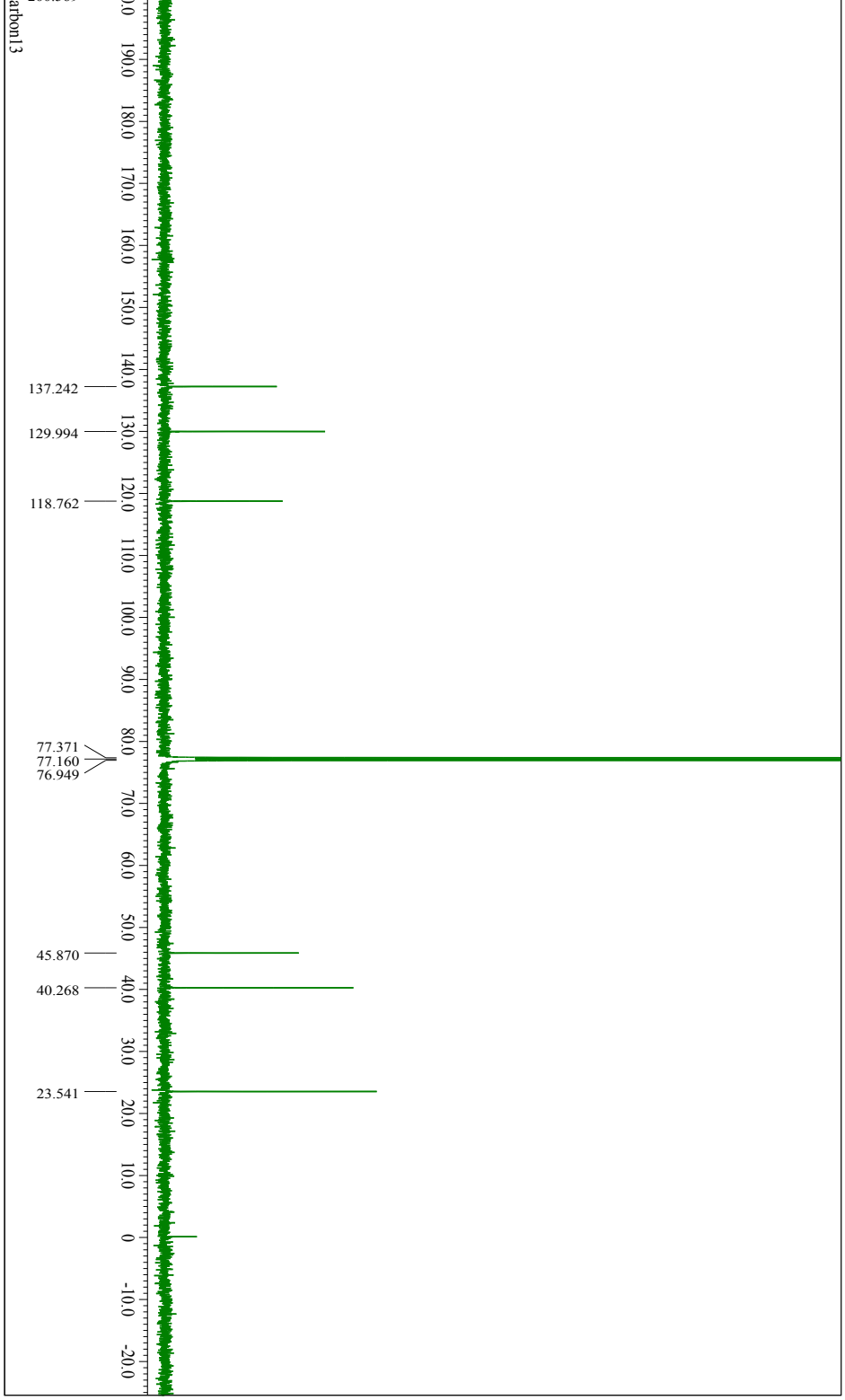
1-(4,4-dimethoxybutyl)-1*H*-imidazole (S1) 1H-NMR and 13C-NMR



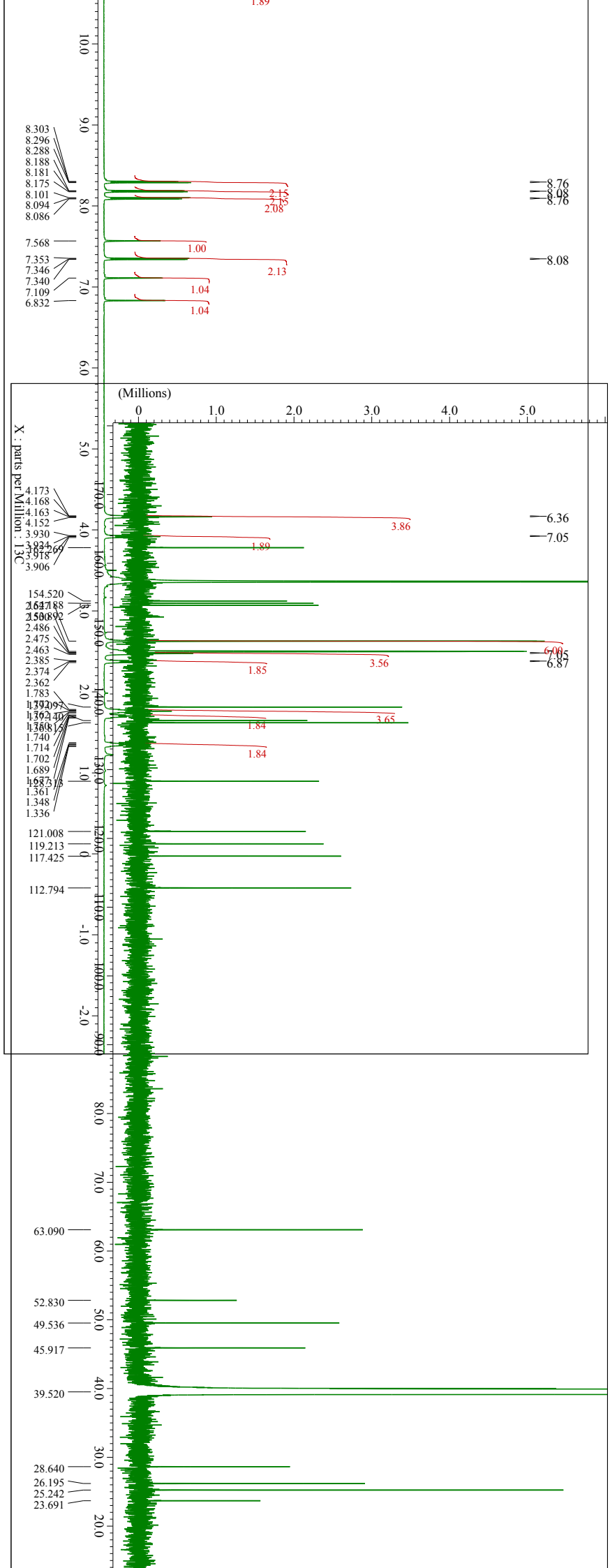
4-(1*H*-imidazol-1-yl)butanal (S2) 1H-NMR and 13C-NMR

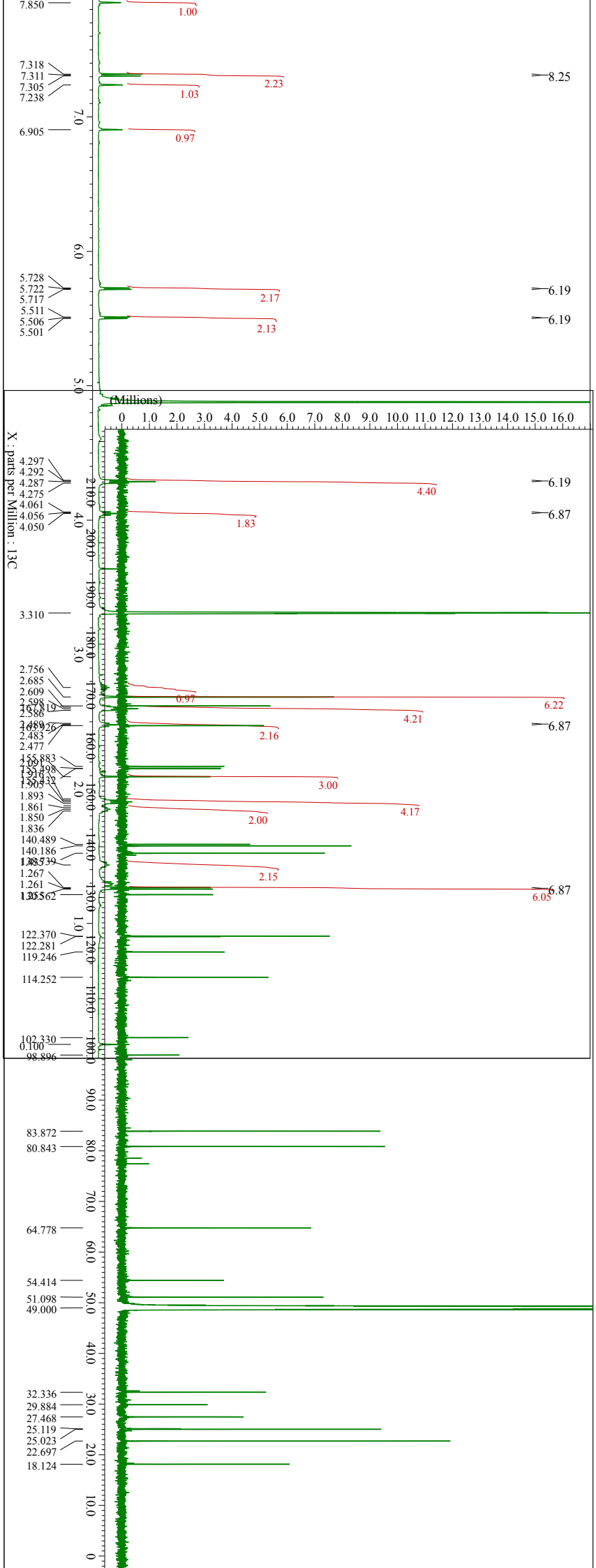


NCD-IMI 1H-NMR and 13C-NMR

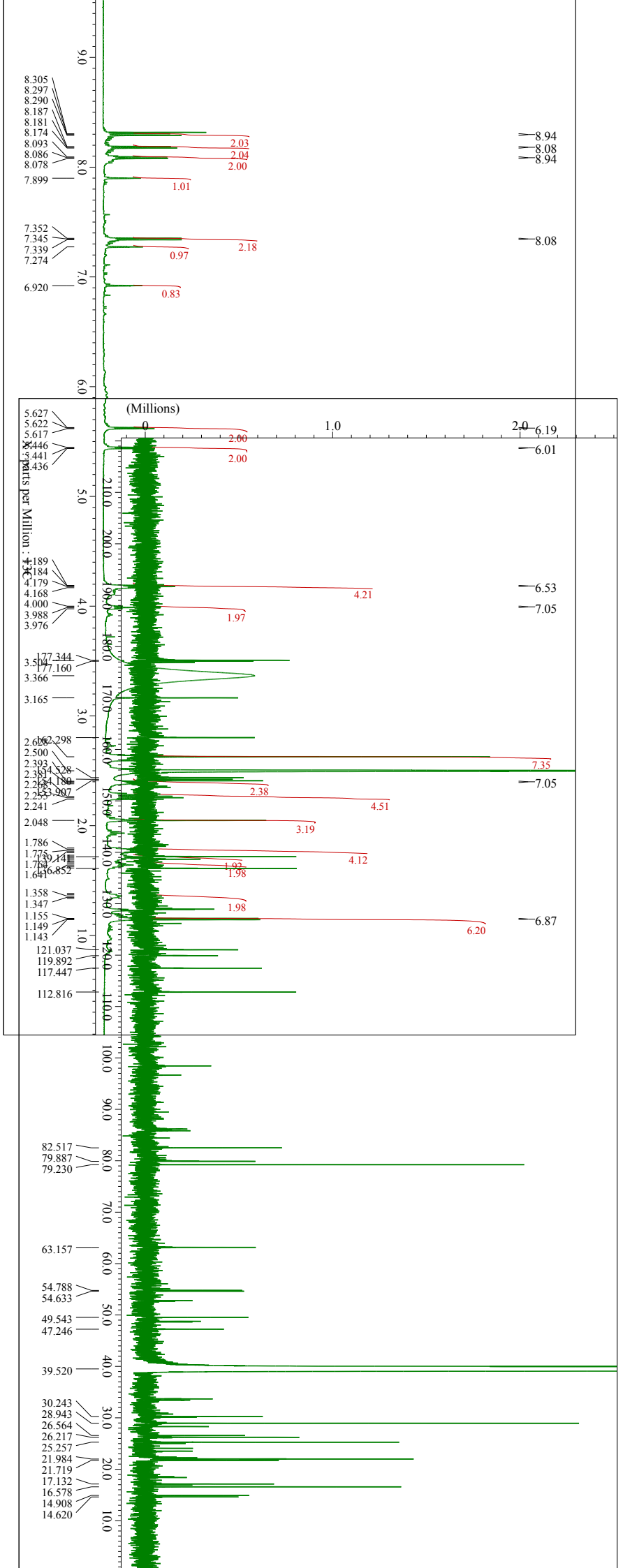


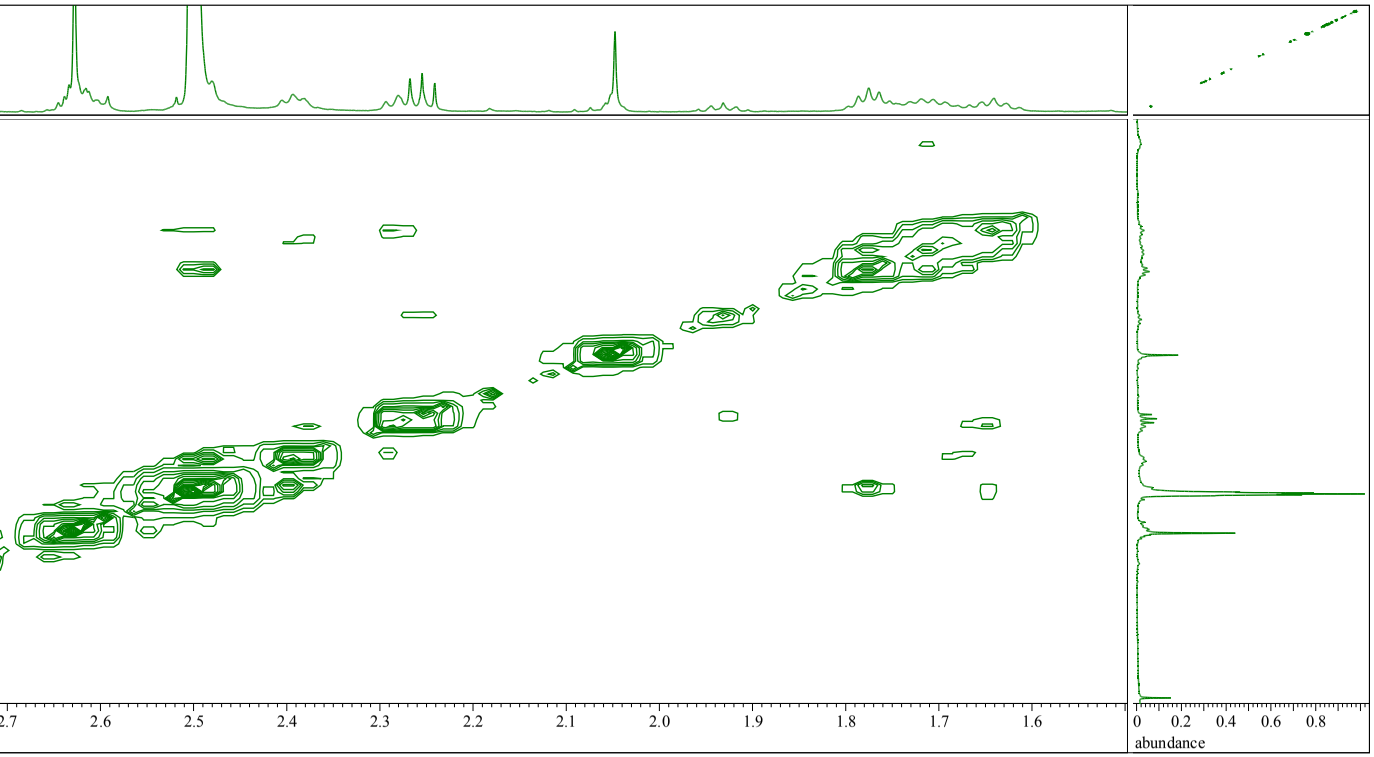
NCD-RO 1H-NMR and 13C-NMR





NCD-RC 1H-NMR and 13C-NMR


(Millions)
parts per Million : 1H
8.305
8.297
8.290
8.187
8.181
8.174
8.093
8.086
8.078
7.899
7.352
7.345
7.339
7.274
6.920
2.03
2.04
2.00
1.01
2.18
0.97
0.83
8.94
8.08
8.94
8.08
5.627
5.622
5.617
5.446
5.441
5.436
4.189
4.184
4.179
4.168
4.000
3.988
3.976
3.504
3.366
3.165
2.500
2.393
2.241
2.048
1.786
1.775
1.764
1.641
1.358
1.347
1.155
1.149
1.143
2.00
2.00
4.21
1.97
177.344
177.160
162.298
154.528
154.180
153.907
2.38
4.51
3.19
140.141
136.852
4.12
1.92
1.98
1.98
6.20
121.037
119.892
117.447
112.816
6.19
6.01
6.53
7.05
7.35
7.05
6.87
82.517
79.887
79.230
63.157
54.788
54.633
49.543
47.246
39.520
30.243
28.943
26.564
26.217
25.257
21.984
21.719
17.132
16.578
14.908
14.620


2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
0
0.2
0.4
0.6
0.8
abundance