Reversible covalent c-Jun N-terminal kinase inhibitors targeting a specific cysteine by precision-guided Michael-acceptor warheads

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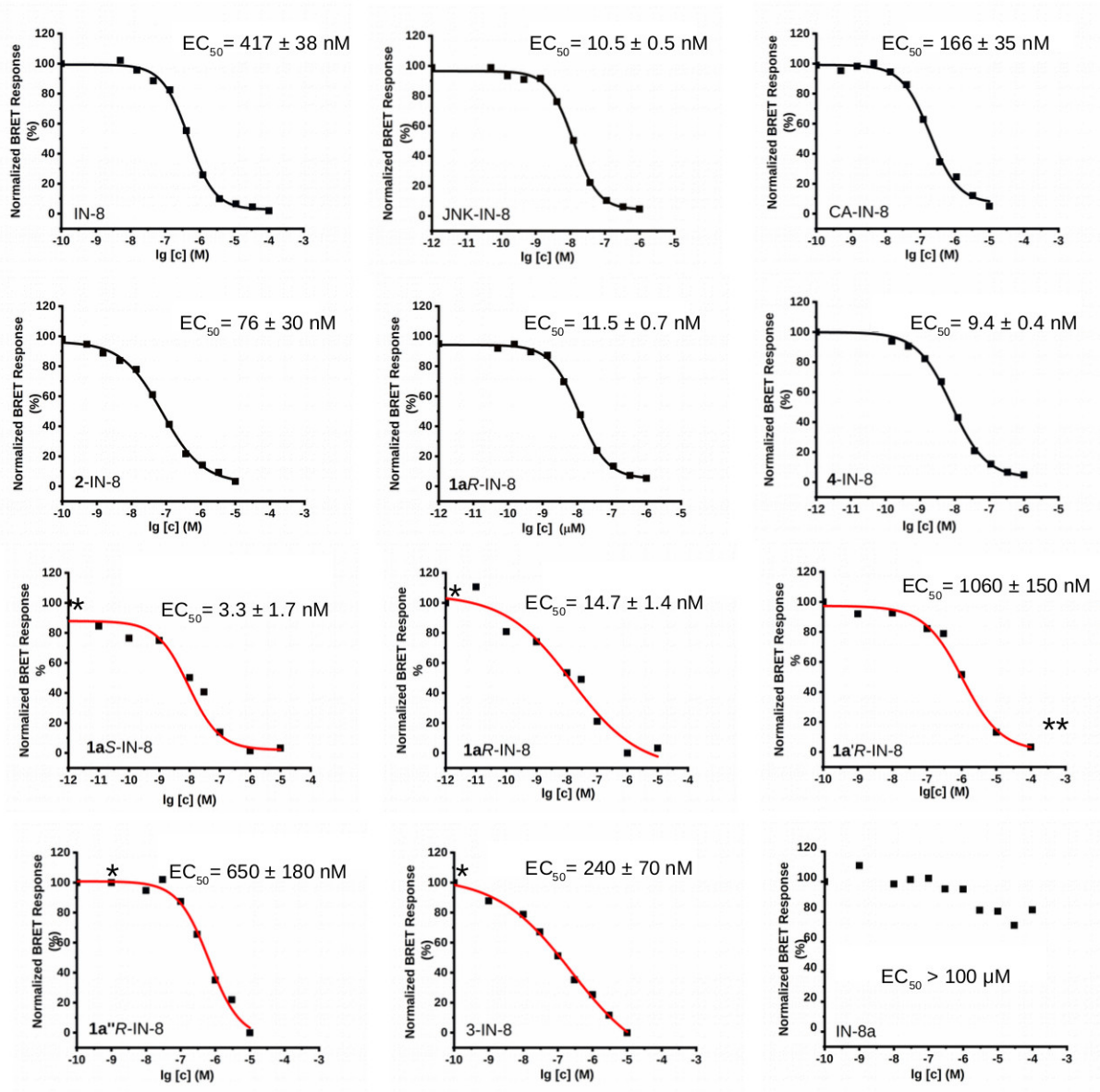
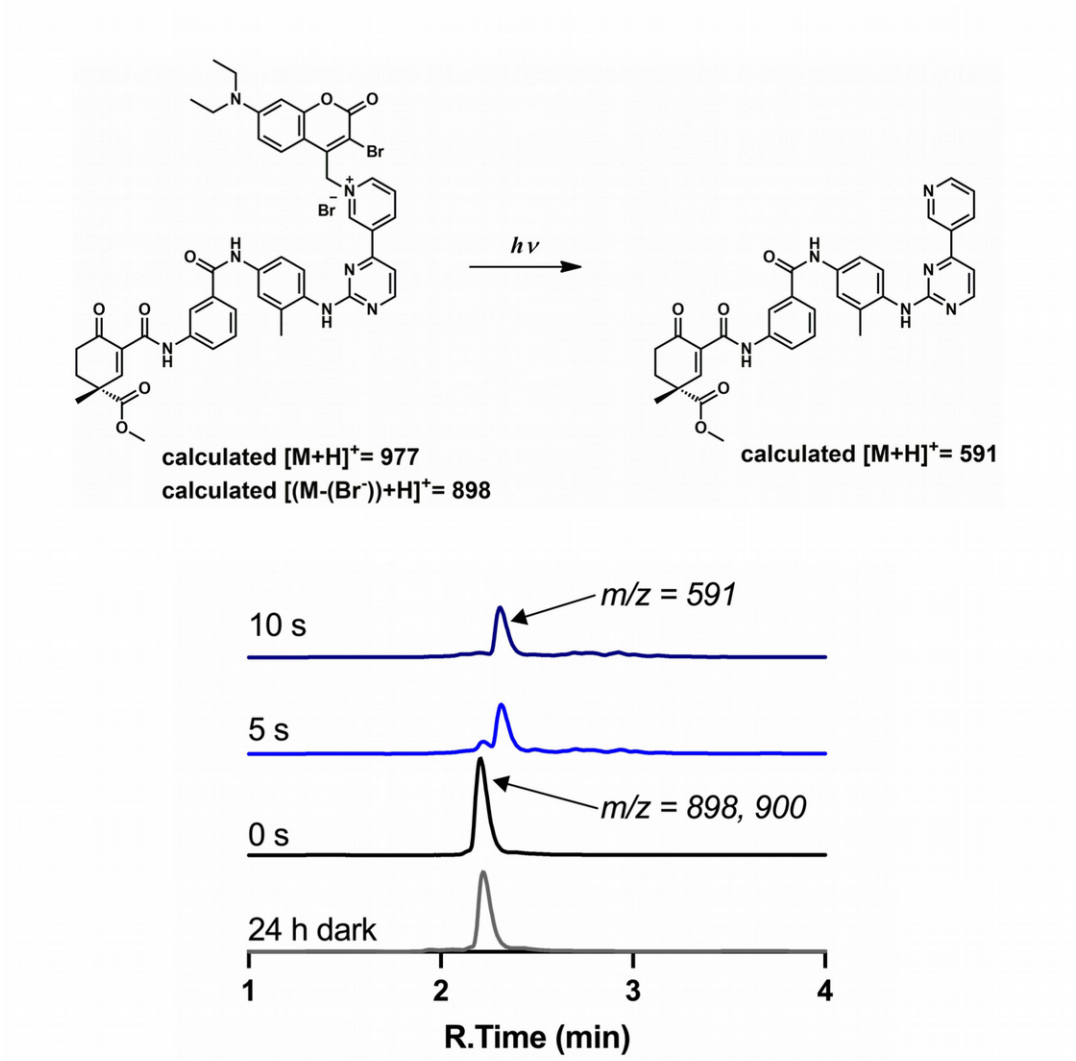
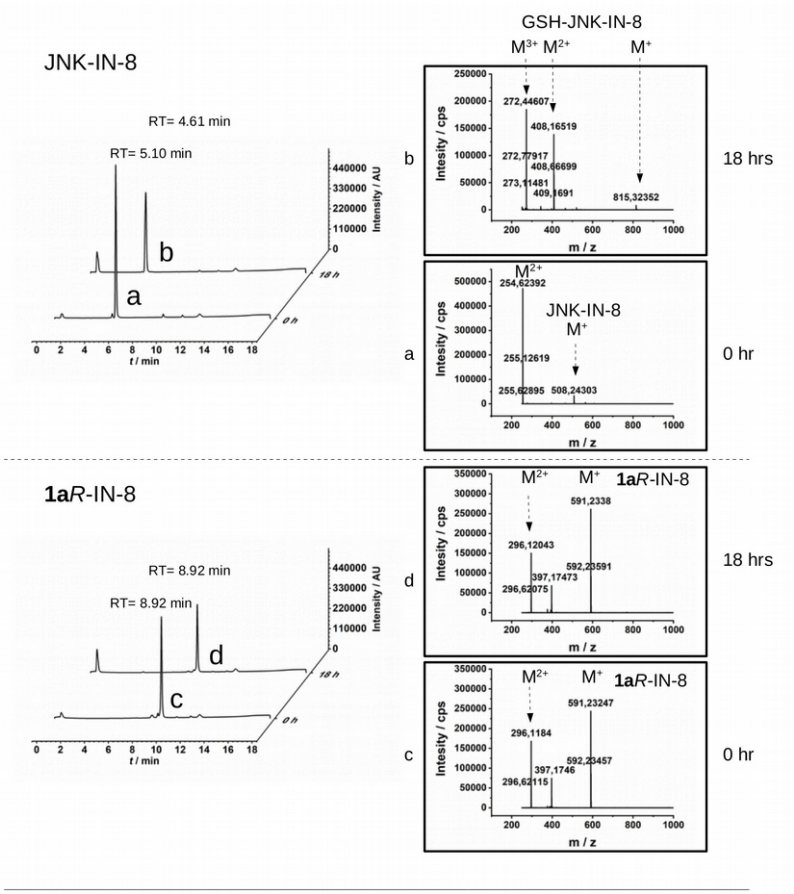
Supplementary Material



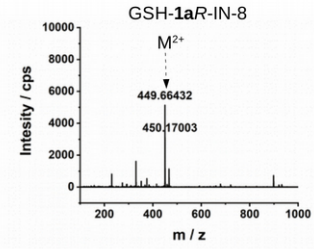
Supplementary Figure 1. NanoBRET target engagement assays
Panels show results of the NanoBRET target engagement assay measured by Reaction Biology (USA) (first 6 panels) or by own experiments (last 6 panels) using the same methodology. Duplicate measurements, data show the mean value. EC_{50} values show the mean and the parameter error estimate from weighted least squares method (* denotes a data point with only DMSO added and ** denotes a data point with no tracer added, background). Note that the two panels in the middle contain measurements for the same compound (**1a***R*-IN-8) determined by Reaction Biology Corp., as a service provider, or by an own experiment, and both measurement gave a similar EC_{50} value. IN-8a repeatedly gave very weak target engagement with the Nanoluc-JNK1 construct, in contrast to IN-8. This may be due the extended *N*-acetyl group in the former compound compared to the latter's amine next to the ATP-binding moiety (see Table 1). Source data are provided as a source Date file.



Supplementary Figure 2. Uncaging of Photocaged 1a*R*-IN-8 in vitro
Partial LC-MS chromatograms at 254 nm of the photo-uncaging of Photocaged **1a*R*-IN-8** (0.1 mM in water-MeCN 95:5) upon blue light irradiation (450 nm). Note that the compound contains one covalently linked Br but it is also complexed to Br^- and the molecular mass of the Photocaged **1a*R*-IN-8** compound without the bromide ion is 898 (and a mass of 900 corresponds to the compound with the heavier natural Br isotope).

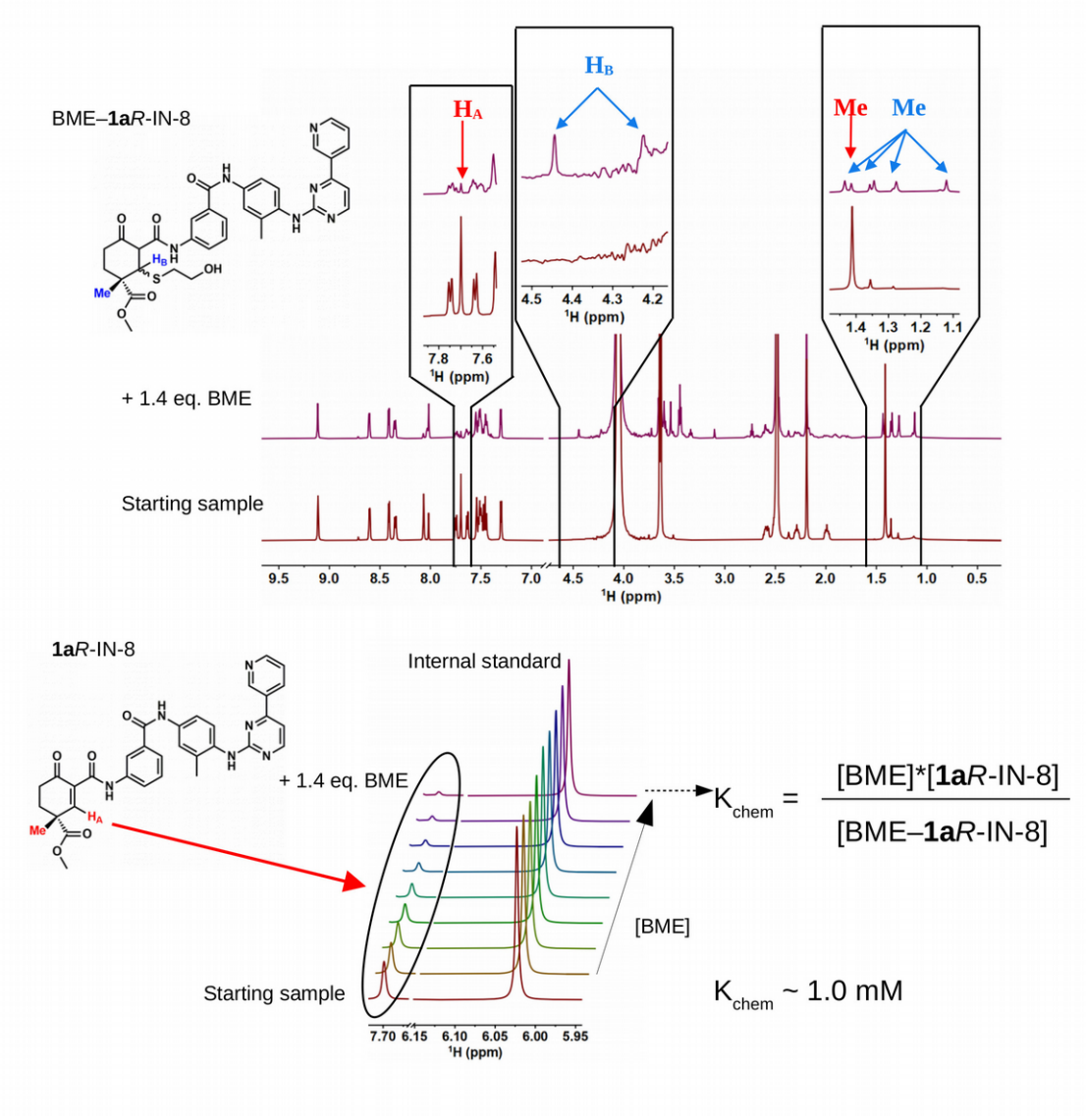


Detection of the GSH–**1a***R*-IN-8 adduct with a shorter LC step



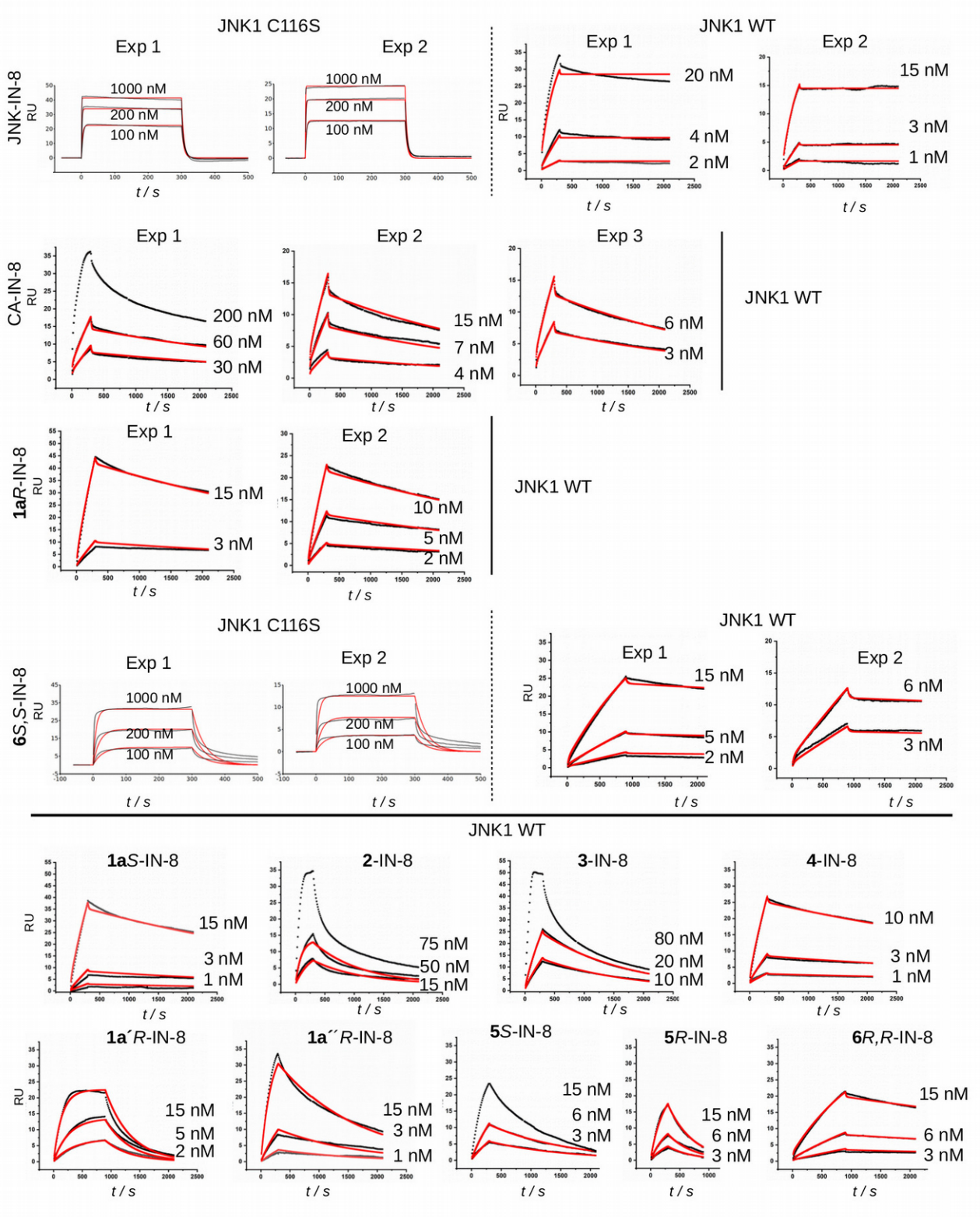
Supplementary Figure 3. Electrospray mass spectrometric characterization of JNK-IN-8 and 1a*R*-IN-8 incubated with 10 mM GSH

The GSH-adduct formation is apparent in the different elution profile in the total ion-chromatograms for JNK-IN-8 incubated with GSH for 18 hours versus no incubation (0 hr) as well as in the corresponding mass spectra (RT: retention time; M+, M2+, M3+ indicate the mass of differently charged molecular ions). The GSH–**1a***R*-IN-8 adduct could not be detected with this method that worked well for the GSH–JNK-IN-8 adduct, however after shortening the HPLC separation time, using a shorter (guard) column, the GSH–**1a***R*-IN-8 adduct also became detectable. These results suggest that **1a***R*-IN-8 does not form an irreversible GSH adduct; and the reversible adduct is short-lived. 50 µM compound was incubated with 10 mM GSH in PBS (pH ~ 7.4) for 30 minutes before subjected to this shorter LC-MS analysis.



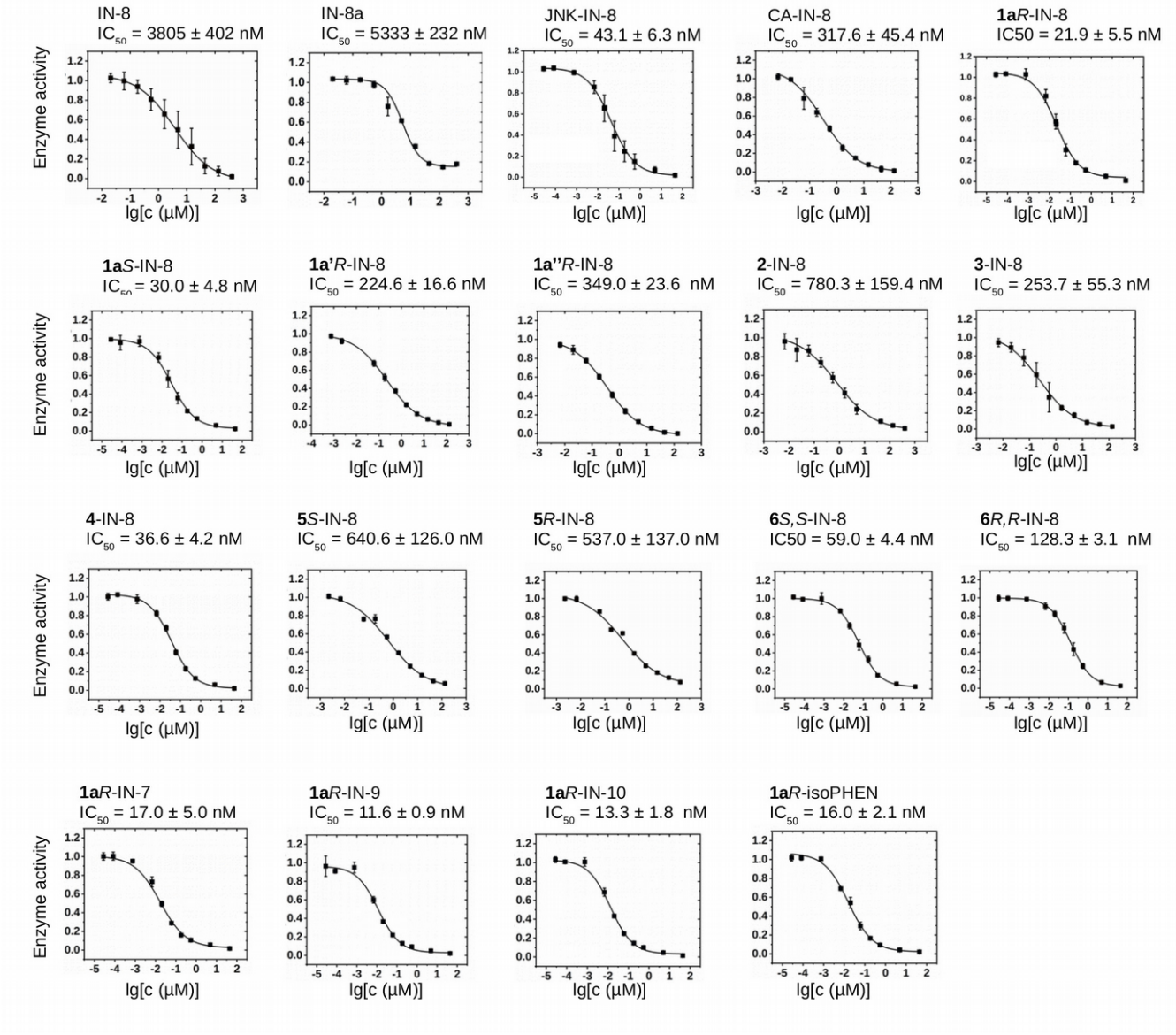
Supplementary Figure 4. NMR analysis of covalent adduct formation between 1a*R*-IN-8 and BME and K_{chem} calculation

The structure of the BME–**1a***R*-IN-8 adduct or **1a***R*-IN-8 highlights H_B or H_A at C3 and the methyl group at C4 showing changes in the ^{1}H NMR spectra. Upper panels show the spectra of the initial **1a***R*-IN-8 sample (13.1 mM) and after mixing it with 1.4 molar equivalent BME. Lower panels show the results of the ^{1}H NMR titration experiment where increasing amounts of BME was added to the starting **1a***R*-IN-8 sample. ^{1}H NMR spectra were recorded in PBS (D_2O; pH ~ 7.2) with 75% DMSO. The position of the 6.02 ppm protons from the reference compound was used as the internal standard important to be able to calculate K_{chem} (see Supplementary Note 2). The K_{chem} value was calculated from the concentration of the relevant components (taken from the +1.4 eq. BME spectrum), assuming that the system reached equilibrium and the decrease of the H_A signal directly correlates with adduct formation (albeit the corresponding peaks for the products could not be monitored due to being at overlapping positions).

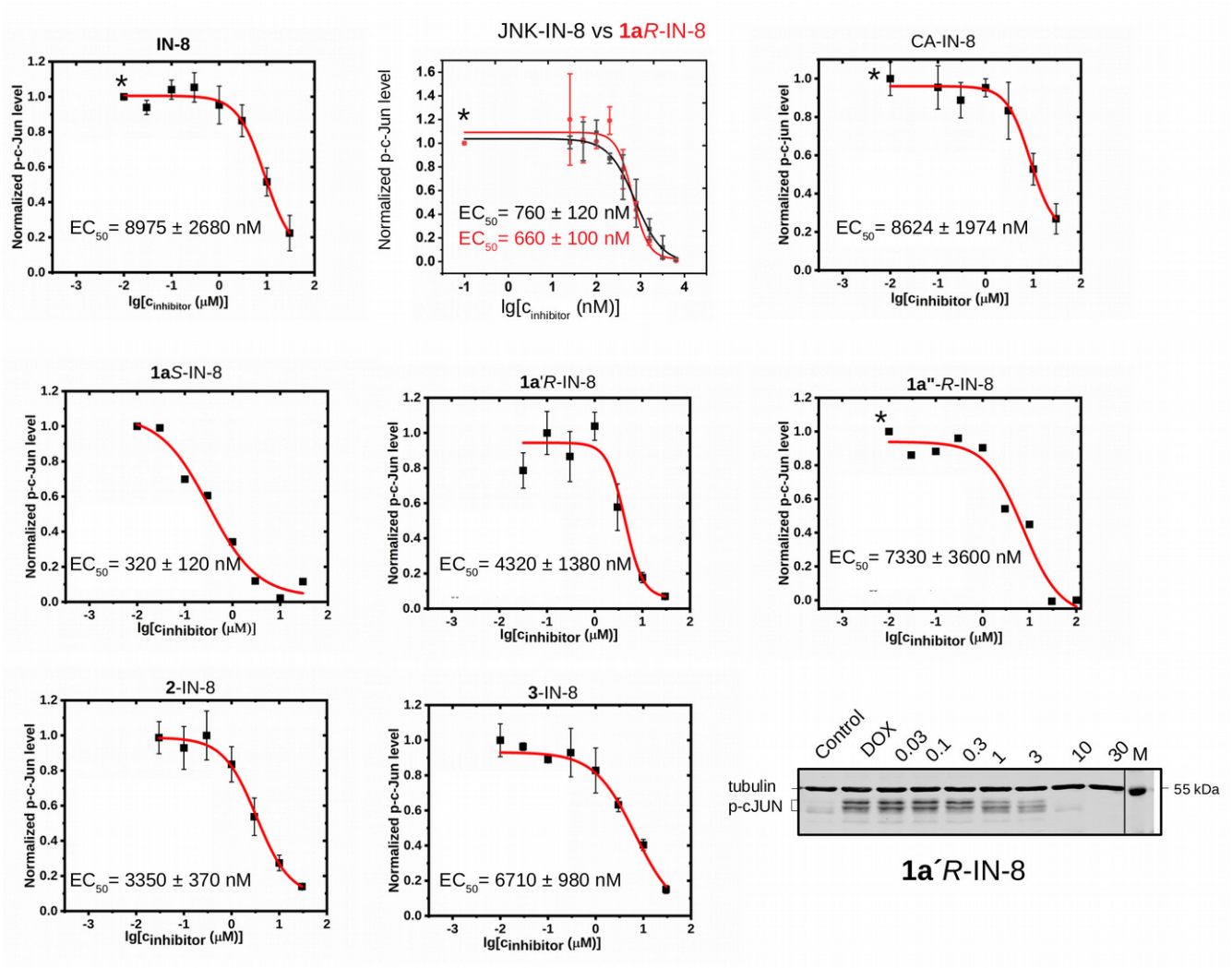


Supplementary Figure 5. SPR kinetic binding curves of different inhibitors targeting Cys116 in JNK1 including parallel experiments

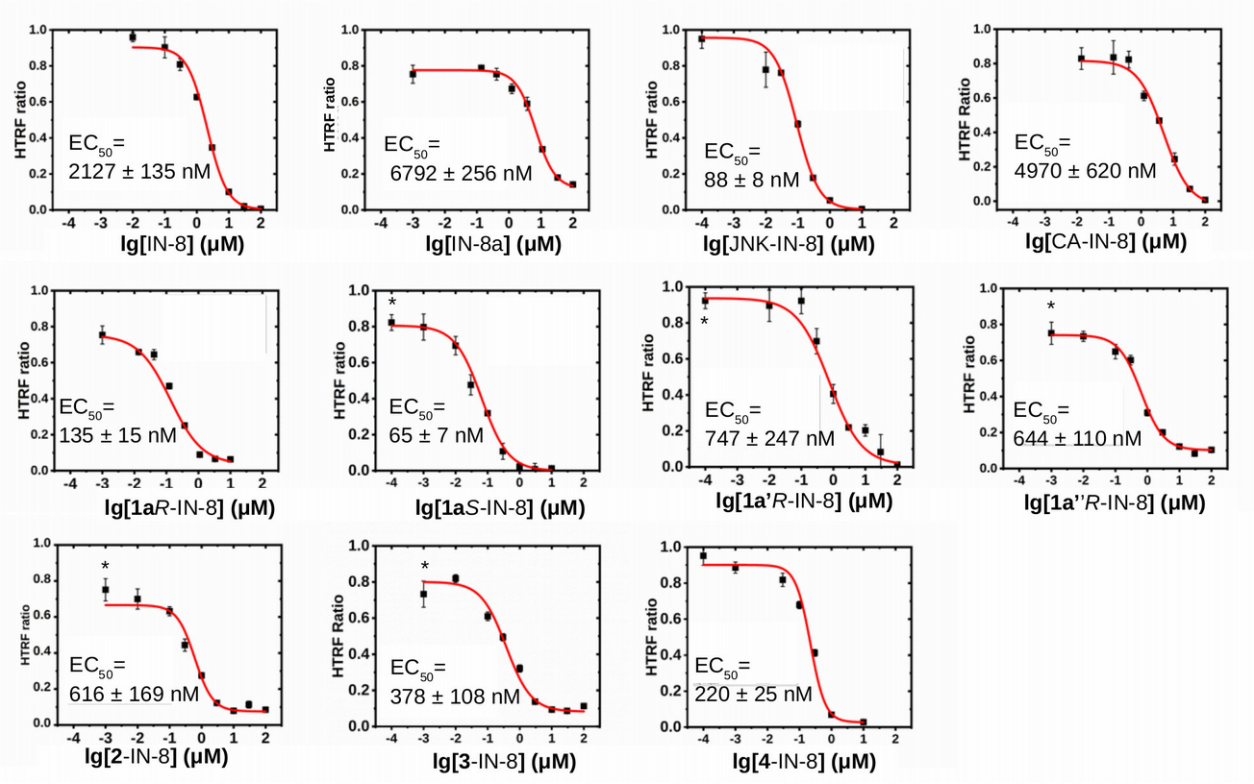
The experimental kinetic binding curves are shown in black, and the calculated plots (in red) are based on a 2-step reversible kinetic binding scheme (see Fig. 3) with the final $k_{1\text{-}4}$ values listed in Supplementary Table 1 (experiment 1, Exp 1). (For some of the compounds with fast on-rates the kinetic binding curve corresponding to the highest inhibitor concentration injection was not used in the numerical fit.) One or two additional measurements was also carried out with JNK-IN-8, CA-IN-8, **1a***R*-IN-8, and **6***S,S*-IN-8 (in addition to Exp1 to assess precision: Exp2 and Exp3). One extra experiment was also carried out for JNK-IN-8 and **6***S,S*-IN-8 on the JNK1 C116S surface (shown left of the dashed lines), in addition to the JNK1 WT surface. The comparison of the $k_{1\text{-}4}$ values determined in Exp2 and Exp3 are listed in the Methods with some discussion about precision and on caveats of this complex analysis. Note that the Response Units (RU) on the SPR sensorgrams may differ because the JNK1 immobilization level on the chip varied, and this was intentionally made different (within a reasonable range) in parallel experiments.



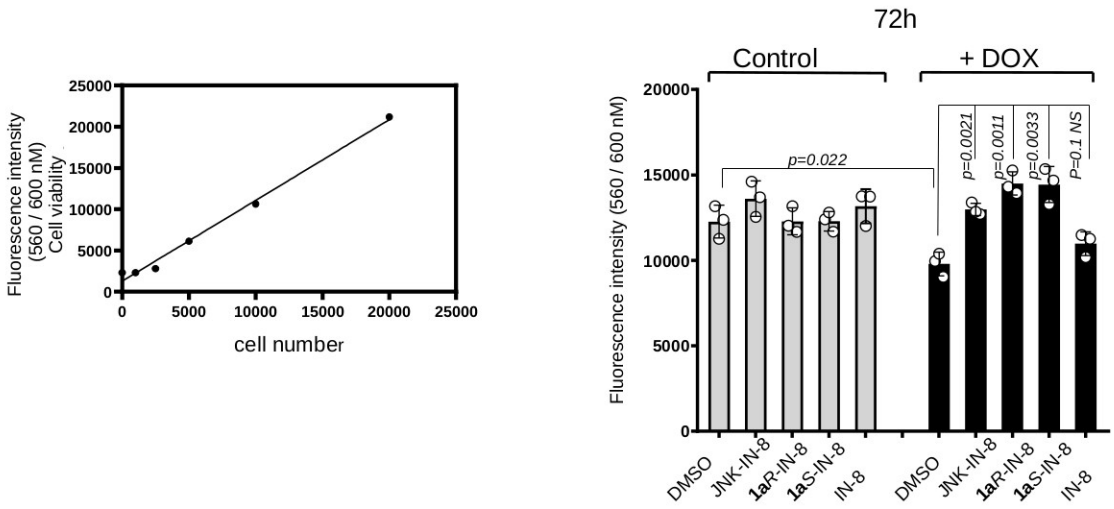
Supplementary Figure 6. In vitro kinase activity measurements with the PhALC assay
Panels show PhALC assay results with JNK1 in the presence of 10 mM GSH**.** Data show the mean and error bars show SD (n=3; independent experiments). IC_{50} vales show the mean with parameter error estimates from weighted least squares method. Source data are provided as a source Date file.



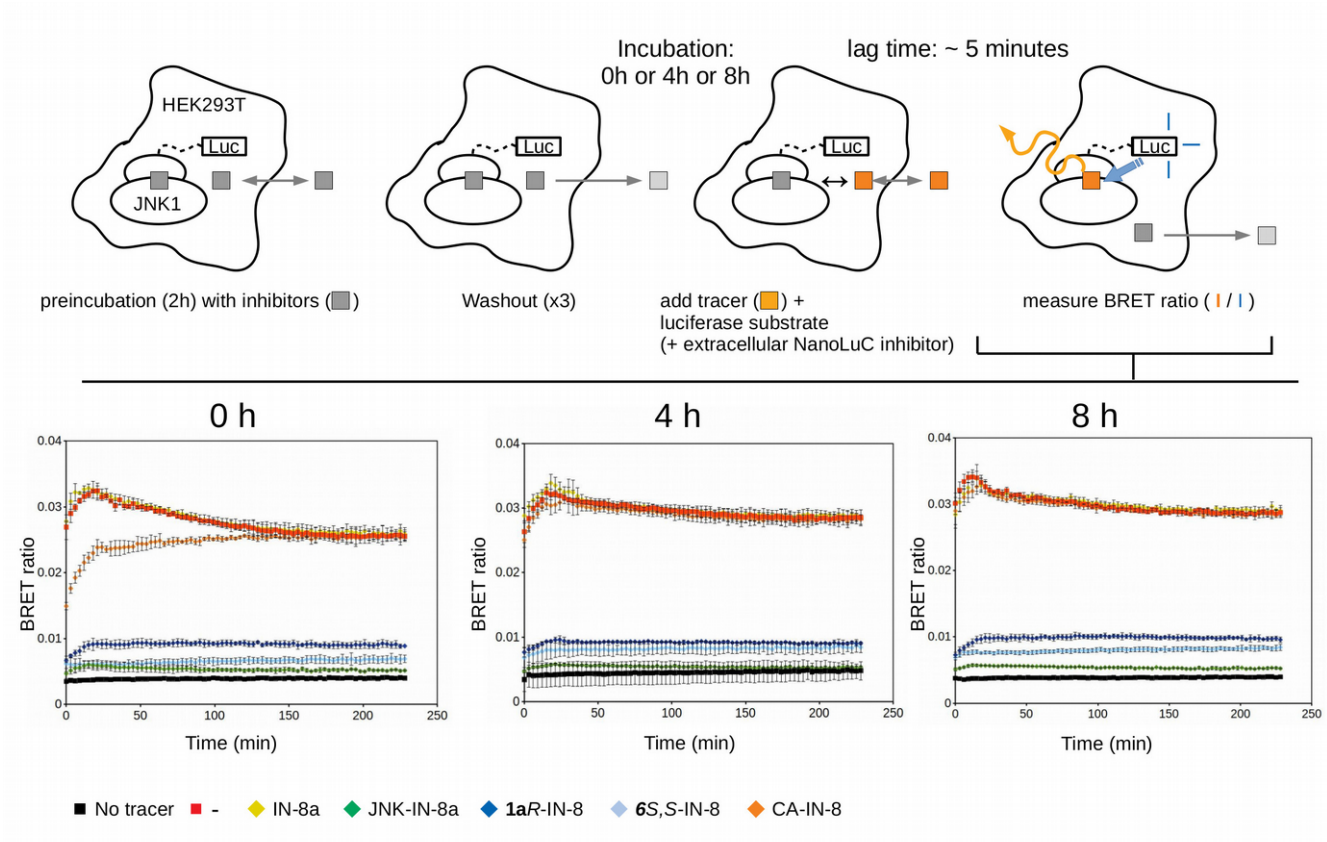
Supplementary Figure 7. p-c-Jun IC_{50} measurements by quantitative western blots
EC_{50} of compounds on JNK mediated c-Jun(Ser73) phosphorylation in doxycycline stimulated SH-SY5Y MKK7 ACT cells after 6 hours (n=3; data show the mean and error bars show SD calculated based on three independent measurements; but n=1 for **1a***S*-IN-8 and **1a"***R*-IN-8). The phosphorylation of c-Jun was determined by using quantitative western blots using a phospo-c-Jun specific(Ser73) antibody. This WB signal was divided by the WB signal of the load control (anti-tubulin), and this corrected WB signal was normalized to the phospho-c-Jun signal measured in cells that were not treated by any inhibitor but induced by doxycycline (DOX) (and an example of the raw WB for **1a'***R*-IN-8 is shown in the last panel; "Control": no DOX stimulation; 0.03-30 µM compound were added with DOX; see Source data for the other western blots). EC_{50} vales show the mean with parameter error estimates from weighted least squares method or the EC_{50} based on one measurement with error parameter estimate from least squares method. "*" denotes values obtained with DOX but untreated with any inhibitor. Source data are provided as a source Date file.



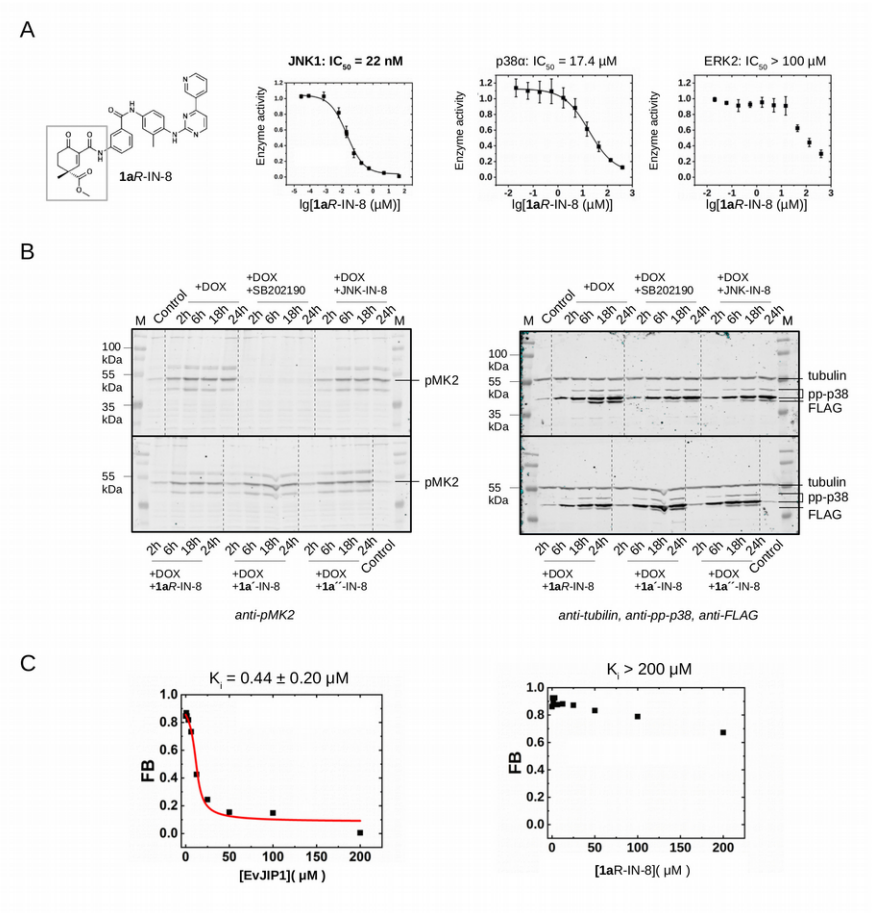
Supplementary Figure 8. Results of the HTRF p-c-Jun(Ser63) assay
SH-SY5Y MKK7 ACT cells allowing specific activation of JNK. For the determination of JNK inhibitor mediated c-Jun phosphorylation, cells were stimulated with 3 µg/mL doxycycline (DOX), co-administered with different amounts of inhibitors, and samples were subjected to HTRF ratio measurements using the HTRF Phospho-c-Jun(Ser63) Detection Kit (PerkinElmer) after 6 hours. EC_{50} values show the mean and the error indicates the parameter estimate from weighted least squares method (n=3, independent experiments; data show the mean and error bars show SD). * indicates control with only DMSO added (and this point is artificially set to correspond to an inhibitor concentration of 1 nM). Source data are provided as a source Date file.



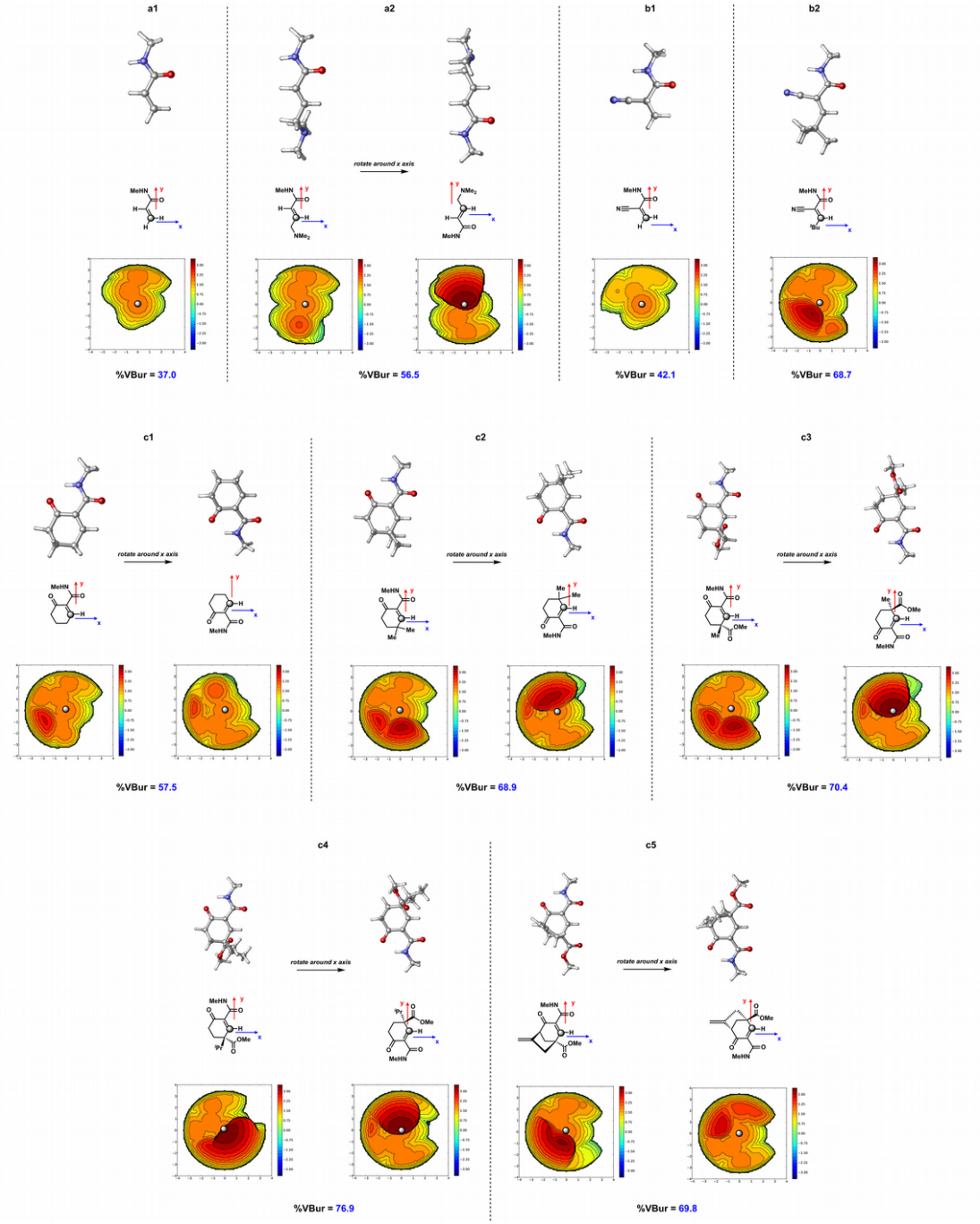
Supplementary Figure 9. Cell survival of SH-SY5Y MKK7 ACT cells
Cell viability was measured by monitoring the reducing power of living cells. The latter is a cell health indicator and can be monitored by fluorescence intensity measurements after addition of a resazurin-based solution (PrestoBlue). The left panel shows the linearity of the fluorescence signal with the cell number of untreated SH-SY5Y MKK7 ACT cells (data show the mean value). The right panel shows the results of an experiment where cells were uninduced (Control) or induced with doxycycline (+DOX) and treated with no inhibitor (DMSO) or with different inhibitors in 1 μM concentration. Cell viability was measured 72 hours after doxycycline and inhibitor co-administration. Notice that doxycycline treatment reduces cell viability but cells are well-protected by composite inhibitors containing covalent warheads (JNK-IN-8, **1a***R*-IN-8, or **1a***S*-IN-8). (Data show the mean and error bars show SD, n=3; p-values were calculated based on two-sided, unpaired t-test; NS: not significant, $p > 0.05$.) Source data are provided as a source Date file.



Supplementary Figure 10. Details of cellular wash-out experiments monitored by nanoBRET
HEK293T cells were transfected with the NanoLuc-JNK1 expression plasmid (not shown), preincubated with the inhibitors for 2 hours, washed out 3 times and incubated without inhibitor for 0, 4 or 8 hours before adding the fluorescent tracer (0.4 µM K10) and luciferase substrate (along with the extracellular NanoLuc inhibitor to block potential extracellular luciferase activity), and the BRET ratio was measured for 4 hours. Note that the lag time of the measurement after the addition of the tracer compound binding into the JNK1 ATP-pocket is about 5 minutes. The panels below show the raw data of the BRET ratio measurements (emission at 650 nM / emission at 450 nM; n=3; data show the mean and error bars show SD). The BRET ratio values on Fig 4.d were taken from the 0 time point shown at each panel. (The upward trend of the BRET ratio plot up to ~ 15 minutes may indicate that the tracer needs some time to reach its equilibrium level in the JNK1 ATP pocket, while the slight decrease for samples with high BRET ratio may reflect some degree of luciferase substrate depletion by time.)

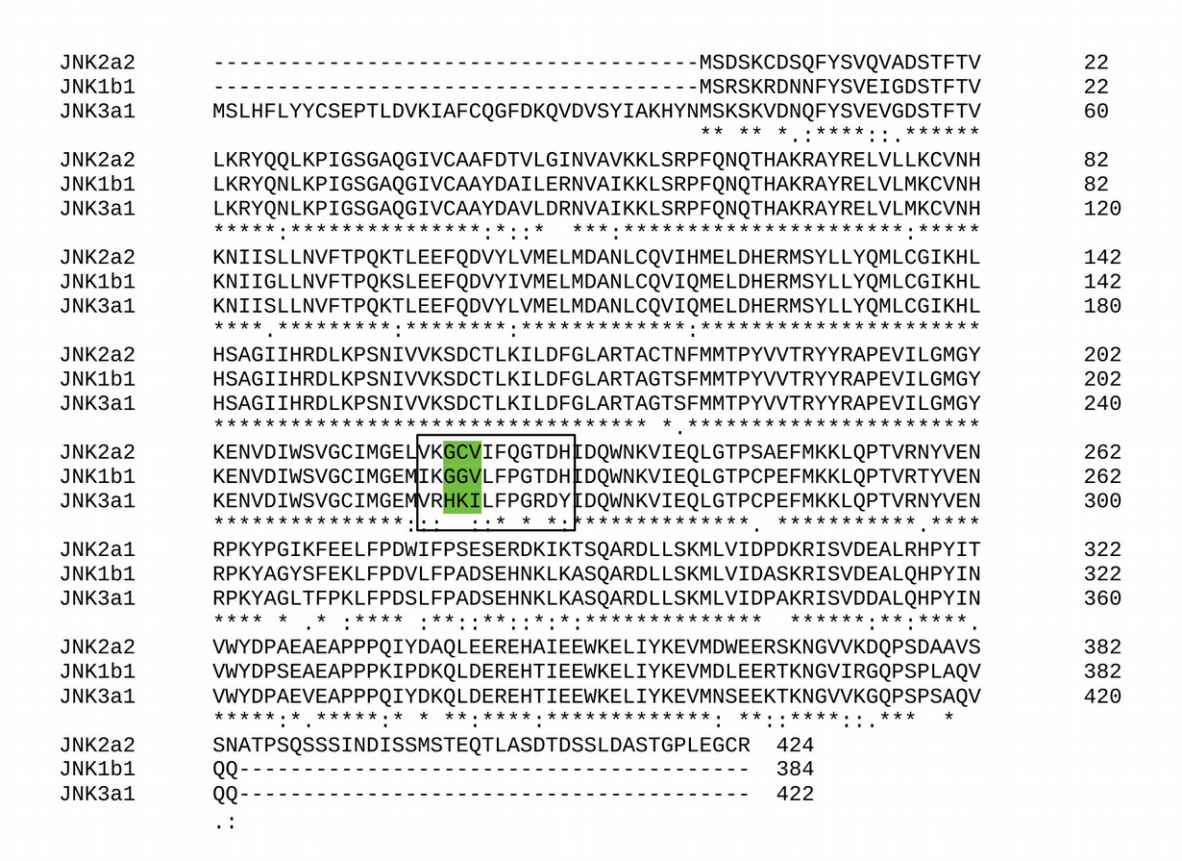


Supplementary Figure 11. MAPK specificity of composite JNK inhibitors
(**A**) JNK specificity of **1a***R*-IN-8 in comparison to ERK2 and p38α in the PhALC assay (n=3, independent experiments; Data show the mean and error bars show SD.)
(**B**) JNK inhibitors do not affect the p38 MAPK pathway. HEK293T MKK6EE cells (allowing p38-specific activation by doxycycline inducible expression of the constitutively active upstream activator kinase, MKK6EE, with a FLAG-tag) were stimulated for the indicated time by doxycycline (DOX) and cells were simultaneously treated with SB202190 (1 μM), a known p38-specific ATP competitive inhibitor, or with different JNK inhibitors (3 μM). The panel on the left shows the western blot results with a phospho-MK2-specific antibody, while the panel on the right shows the western blot results using three antibodies (anti-tubulin – as the load control, anti-pp-p38 – recognizing the activated form of p38 kinases, and anti-FLAG – monitoring the expression level of MKK6EE-FLAG). MK2, also known as MAPKAPK2, is a specific substrate of p38.
(**C**) **1a***R*-IN-8 does not interfere with docking peptide (EvJIP1) binding. A fluorescence polarization-based protein-peptide binding assay was used to detect the binding of the fluorescently labeled EvJIP1 peptide in the MAPK docking groove harboring Cys163 (FB: fraction bound). The panel on the left shows how the unlabeled EvJIP1 peptide competes the labeled peptide off, and the panel on the right shows that the inhibitor does not interfere with docking motif binding, and hence does not target Cys163 located in the MAPK docking groove. The measurements were taken ~ 30 minutes after all components were added. Data show the mean value based on three independent experiments (n=3). Source data are provided as a source Date file.



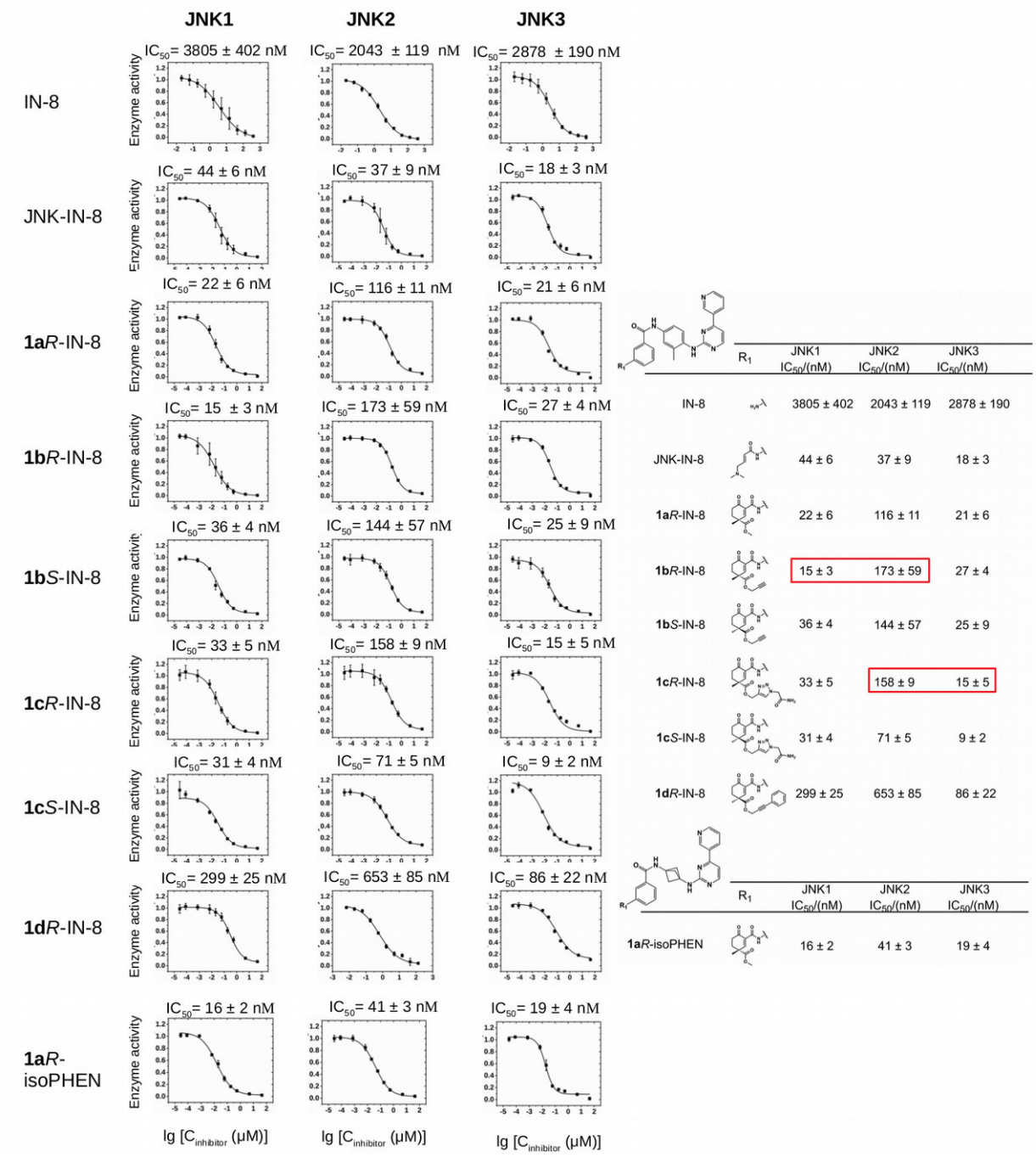
Supplementary Figure 12. More complete topographic steric maps of simplified warheads and buried volume calculation (%Vbur)

Steric maps are derived from the DFT-optimized structures (see Supplementary Note 1). The isocontour scheme is in Å, and a coloring scheme from dark red to deep blue is used to display sterically encumbered regions around the reactive center. The grey dot shown at the center of the xy plane represents the reactive carbon atom in the Michael acceptor, and the steric map is viewed down the z-axis. Comparison of the steric maps of the simplified warheads indicates that the establishment of the cyclic form and incorporation of pendant groups notably increase the steric congestion near the reactive center.



Supplementary Figure 13. Multiple sequence alignment of human JNK isoforms (JNK1b1, JNK2a2 and JNK3a1)

Residues corresponding to exon 6 are boxed in the sequence alignment below. This short region varies among JNK isoforms and the three residues (GGV in JNK1b1) displaying the greatest variation among the examined JNK isoforms are colored green.

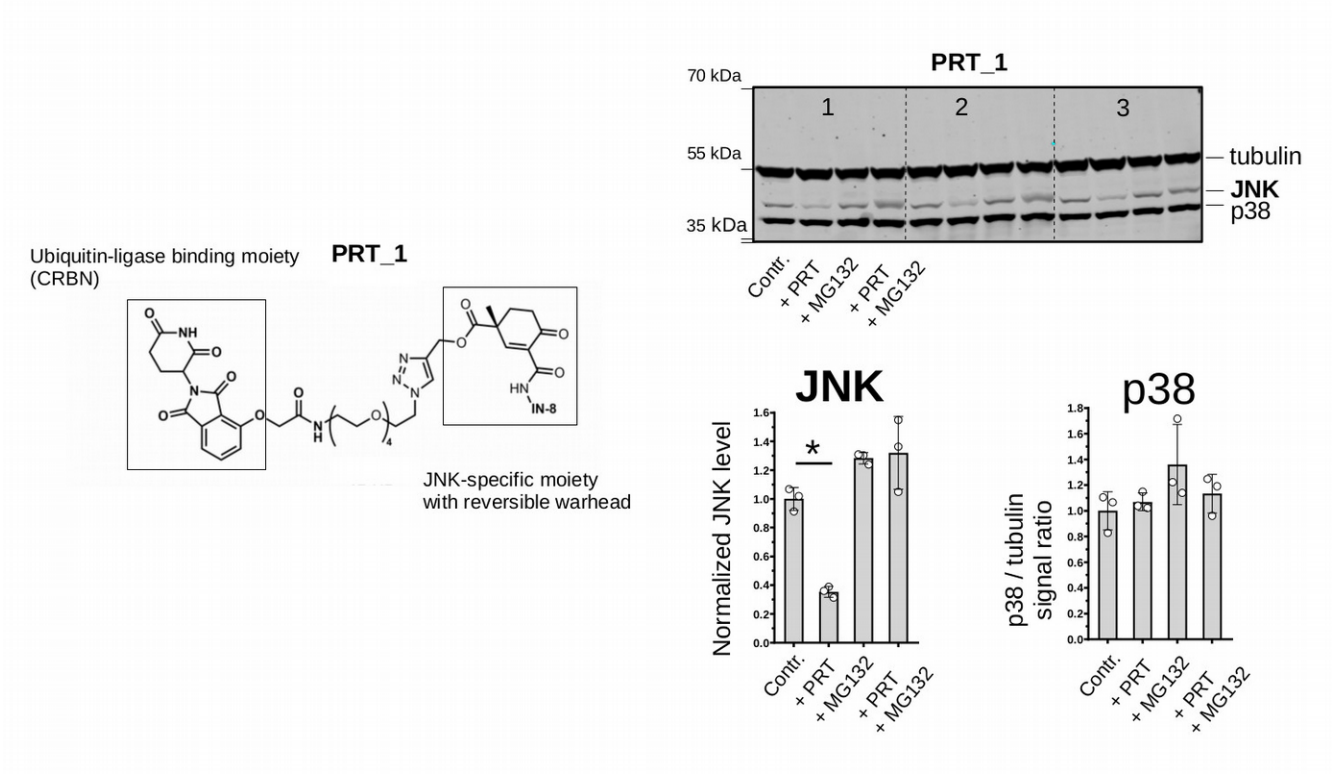


	R1	JNK1 IC50/(nM)	JNK2 IC50/(nM)	JNK3 IC50/(nM)
IN-8		3805 ± 402	2043 ± 119	2878 ± 190
JNK-IN-8		44 ± 6	37 ± 9	18 ± 3
1aR-IN-8		22 ± 6	116 ± 11	21 ± 6
1bR-IN-8		15 ± 3	173 ± 59	27 ± 4
1bS-IN-8		36 ± 4	144 ± 57	25 ± 9
1cR-IN-8		33 ± 5	158 ± 9	15 ± 5
1cS-IN-8		31 ± 4	71 ± 5	9 ± 2
1dR-IN-8		299 ± 25	653 ± 85	86 ± 22

	R1	JNK1 IC50/(nM)	JNK2 IC50/(nM)	JNK3 IC50/(nM)
1aR-isoPHEN		16 ± 2	41 ± 3	19 ± 4

Supplementary Figure 14. JNK isoform selectivity of cyclohexenone warhead containing JNK inhibitors

Panels show PhALC assay results with JNK1, JNK2 and JNK3 (JNK1b1, JNK2a2 and JNK3a1). Data show the mean and error bars show SD (n=3). IC_{50} values show the mean and the parameter estimate from weighted least squares method. The table on the right shows the summary of these measurements, where compounds with >10-fold selectivity for JNK1 vs JNK2 or JNK3 vs JNK2 are boxed in red. Source data are provided as a source Date file.



Supplementary Figure 15. A JNK-specific PROTAC design
A JNK PROTAC comprised of a JNK-specific binding moiety (as in **1a***R*-IN-8) and an E3 ubiquitin ligase binding moiety (CRBN) connected by a PEG-based linker. The western blot (WB) panel shows the results of three parallel experiments (n=3; 1,2,3). The levels of JNK1 and p38 were monitored using JNK- or p38-specific antibodies and a tubulin-specific antibody was used as the load control. HeLa cells were untreated ("Contr."), treated with **PRT_1** (+PRT), with a proteosome inhibitor (+MG132), or with both (PRT+MG132). **PRT_1** and MG132 were used in 10 μM concentration and treatment was for 24 hours. The plots below show the quantitative summary of normalized JNK1 level changes or the p38/tubulin WB signal ratio. JNK1 levels were normalized to the JNK1/tubulin WB signal ratio of the "Contr.". Data show the mean value and error bars show SD based on three independent experiments (*: $p < 0.05$; two-sided, unpaired t-test; n=3). Source data are provided as a source Date file.

Supplementary Table 1. Values of $k_{1\text{-}4}$, K_i [(k_2/k_1) / $(1+ (k_3/k_4)$] and the energy contribution of reversible bond formation (ΔΔG) (see Fig. 3 and Supplementary Fig. 5**).**

	JNK-IN-8	CA-IN-8	**1a***R*-IN-8	**1a***S*-IN-8	**2**-IN-8	**3**-IN-8	**4**-IN-8
k_1	1.5E+6	1.1E+5	1.9E+5	2.9E+5	1.9E+5	1.3E+5	2.5E+5
k_2	0.15360	0.09983	0.03172	0.05346	0.10230	0.04122	0.03529
k_3	0.02952	0.01140	0.02617	0.02464	0.00615	0.03598	0.02385
k_4	-	0.00026	0.00034	0.00029	0.00111	0.00129	0.00028
K_i	-	20.6	2.1	2.1	81.0	10.7	1.6
ΔΔG	-	-2.25	-2.57	-2.64	-1.11	-1.99	-2.64
	1a'*R*-IN-8	**1a''***R*-IN-8	**5***S*-IN-8	**5***R*-IN-8	**6***S*,*S*-IN-8	**6***R*,*R*-IN-8	
k_1	3.4E+5	3.9E+5	3.0E+5	3.11E+05	1.64E+5	3.76E+5	k_1 ($M^{-1} \cdot s^{-1}$)
k_2	0.05520	0.06206	0.10800	0.12720	0.03460	0.07970	k_2, k_3, k_4 (s^{-1})
k_3	0.60000	0.36693	0.05831	0.09699	0.01064	0.01045	K_i (nM)
k_4	0.02831	0.00508	0.00117	0.00420	0.00006	0.00016	ΔΔG
K_i	7.4	2.2	7.1	17.0	1.1	3.1	(kcal / mol)
ΔΔG	-1.83	-2.54	-2.33	-1.88	-3.10	-2.50	

Supplementary Table 2. Results of the Wild Type Kinase Panel (Reaction Biology Corp, USA; 340 human kinases) with **1a***R*-IN-8 (BD837) used at 1 μM concentration. (Values show the remaining kinase activity (%) compared to control, no inhibitor added; duplicate measurements; kinases for which inhibition was over 50 % are highlighted in orange.)

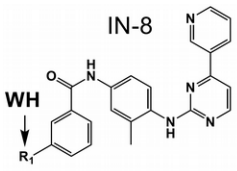
#	Kinase Name	Kinase Family*	BD837 1.00E-06	BD837 1.00E-06	Mean 1.00E-06
1	ABL1	TK	107	99	103
2	ABL2	TK	88	88	88
3	ACK1	TK	85	75	80
4	ACVR1	TKL	112	104	108
5	ACVR1B	TKL	111	112	111
6	ACVR2A	TKL	96	98	97
7	ACVR2B	TKL	92	73	82
8	ACVRL1	TKL	87	83	85
9	AKT1	AGC	91	85	88
10	AKT2	AGC	85	82	84
11	AKT3	AGC	101	91	96
12	ALK	TK	90	80	85
13	AMPKalpha1	CAMK	94	131	113
14	ARAF-Y279Y	TKL	100	93	97
15	ARK5	CAMK	105	96	100
16	ASK1	STE	95	102	99
17	AuroraA	OTHER	90	82	86
18	AuroraB	OTHER	85	80	82
19	AuroraC	OTHER	90	87	88
20	AXL	TK	109	97	103
21	BLK	TK	95	87	91
22	BMPR1A	TKL	106	96	101
23	BMPR1B	TKL	94	92	93
24	BMX	TK	94	91	92
25	BRAF	TKL	87	78	83
26	BRK	TK	92	91	91
27	BRSK1	CAMK	120	117	118
28	BRSK2	CAMK	107	99	103
29	BTK	TK	99	93	96
30	BUB1B	OTHER	91	80	86
31	CAMK1D	CAMK	94	93	93
32	CAMK2A	CAMK	100	105	102
33	CAMK2B	CAMK	107	84	95
34	CAMK2D	CAMK	97	90	93
35	CAMK2G	CAMK	117	104	110
36	CAMK4	CAMK	93	95	94
37	CAMKK1	OTHER	102	104	103
38	CAMKK2	OTHER	92	82	87
39	CDC42BPA	AGC	110	89	99
40	CDC42BPB	AGC	98	93	96
41	CDC7/DBF4	OTHER	96	87	91
42	CDK1/CycA2	CMGC	105	94	99
43	CDK1/CycB1	CMGC	102	89	95
44	CDK1/CycE1	CMGC	94	82	88
45	CDK10/CycQ	CMGC	88	87	88
46	CDK11B 357-795/CycK	CMGC	81	84	83
47	CDK12/CycK	CMGC	103	97	100
48	CDK13/CycK	CMGC	102	90	96
49	CDK16/CycY	CMGC	90	96	93
50	CDK17p35NCK	CMGC	91	84	87
51	CDK18/CycY	CMGC	104	101	103
52	CDK19/CycC	CMGC	62	61	62
53	CDK2/CycA2	CMGC	95	93	94
54	CDK2/CycD1	CMGC	96	85	90
55	CDK2/CycE1	CMGC	87	90	89
56	CDK20/CycH	CMGC	96	91	94
57	CDK20/CycT1	CMGC	103	92	98
58	CDK3/CycC	CMGC	98	95	96
59	CDK3/CycE1	CMGC	106	91	98
60	CDK4/CycD1	CMGC	104	101	102
61	CDK4/CycD2	CMGC	86	87	86
62	CDK4/CycD3	CMGC	92	87	90
63	CDK5/p25NCK	CMGC	104	91	98
64	CDK5/p35NCK	CMGC	99	94	96
65	CDK6/CycD1	CMGC	95	89	92
66	CDK6/CycD2	CMGC	108	100	104
67	CDK6/CycD3	CMGC	97	95	96
68	CDK7/CycH/MAT1	CMGC	97	93	95
69	CDK8/CycC	CMGC	58	54	56
70	CDK9/CycK	CMGC	85	90	88
71	CDK9/CycT1	CMGC	88	83	85
72	CHK1	CAMK	93	87	90
73	CHK2	CAMK	89	90	89
74	CK1alpha1	CK1	91	95	93
75	CK1delta	CK1	82	79	81
76	CK1epsilon	CK1	113	105	109
77	CK1gamma1	CK1	97	95	96
78	CK1gamma2	CK1	87	72	80
79	CK1gamma3	CK1	96	88	92
80	CK2alpha1	OTHER	97	92	95
81	CK2alpha2	OTHER	103	95	99
82	CLK1	CMGC	82	80	81
83	CLK2	CMGC	124	109	117
84	CLK3	CMGC	102	97	99
85	CLK4	CMGC	96	85	91
86	COT	STE	91	88	90
87	CSF1R	TK	94	84	89
88	CSK	TK	96	87	91
89	DAPK1	CAMK	113	105	109
90	DAPK2	CAMK	99	93	96
91	DAPK3	CAMK	110	96	103
92	DCAMKL2	CAMK	102	89	95
93	DDR2	TK	105	104	104
94	DMPK	AGC	97	95	96
95	DNAPK	ATYPICAL	105	96	101
96	DYRK1A	CMGC	90	91	90
97	DYRK1B	CMGC	91	87	89
98	DYRK2	CMGC	95	87	91
99	DYRK3	CMGC	95	90	93
100	DYRK4	CMGC	104	90	97
101	EEF2K	ATYPICAL	88	85	86
102	EGFR	TK	109	88	99
103	EIF2AK2	OTHER	76	64	70
104	EIF2AK3	OTHER	94	91	93
105	EPHA1	TK	90	84	87
106	EPHA2	TK	101	86	94
107	EPHA3	TK	78	74	76
108	EPHA4	TK	92	73	83
109	EPHA5	TK	118	110	114
110	EPHA6	TK	94	87	91
111	EPHA7	TK	95	81	88
112	EPHA8	TK	82	81	81
113	EPHB1	TK	111	91	101
114	EPHB2	TK	98	98	98
115	EPHB3	TK	102	96	99
116	EPHB4	TK	91	75	83
117	ERBB2	TK	83	90	87
118	ERBB4	TK	100	89	95
119	ERK1	CMGC	96	88	92
120	ERK2	CMGC	98	86	92
121	ERK5	CMGC	82	93	87
122	ERK7	CMGC	114	104	109
123	FAK	TK	69	62	65
124	FER	TK	78	70	74
125	FES	TK	78	66	72
126	FGFR1	TK	85	81	83
127	FGFR2	TK	78	70	74
128	FGFR3	TK	86	101	94
129	FGFR4	TK	102	86	94
130	FGR	TK	83	77	80
131	FLT3	TK	92	79	86
132	FRK	TK	101	94	98
133	FYN	TK	102	69	86
134	GRK2	AGC	102	95	99
135	GRK3	AGC	116	102	109
136	GRK4	AGC	104	93	98
137	GRK5	AGC	103	87	95
138	GRK6	AGC	112	107	109
139	GRK7	AGC	102	95	99
140	GSG2	OTHER	89	80	84
141	GSK3alpha	CMGC	96	104	100
142	GSK3beta	CMGC	103	89	96
143	HCK	TK	101	93	97
144	HIPK1	CMGC	103	99	101
145	HIPK2	CMGC	96	89	93
146	HIPK3	CMGC	97	86	92
147	HIPK4	CMGC	95	93	94
148	HRI	OTHER	97	98	98
149	IGF1R	TK	103	87	95
150	IKKalpha	OTHER	97	94	96
151	IKKbeta	OTHER	86	87	86
152	IKKepsilon	OTHER	99	89	94
153	INSR	TK	102	101	101
154	INSRR	TK	90	88	89
155	IRAK1	TKL	86	84	85
156	IRAK4	TKL	104	103	103
157	ITK	TK	104	97	100
158	JAK1	TK	91	93	92
159	JAK2	TK	92	96	94
160	JAK3	TK	99	93	96
161	JNK1	CMGC	2	2	2
162	JNK2	CMGC	7	7	7
163	JNK3	CMGC	1	1	1
164	KIT	TK	71	66	69
165	LCK	TK	102	104	103
166	LIMK1	TKL	45	43	44
167	LIMK2	TKL	93	77	85
168	LKB1/MO25a/STRADa	CAMK	103	101	102
169	LRRK2	TKL	89	80	85
170	LTK	TK	86	85	85
171	LYN	TK	108	84	96
172	MAP3K1	STE	81	85	83
173	MAP3K10	STE	74	70	72
174	MAP3K11	STE	97	98	98
175	MAP3K7/MAP3K7IP1	STE	93	93	93
176	MAP3K9	STE	103	99	101
177	MAP4K2	STE	88	85	87
178	MAP4K4	STE	97	90	93
179	MAP4K5	STE	110	92	101
180	MAPKAPK2	CAMK	96	78	87
181	MAPKAPK3	CAMK	97	82	89
182	MAPKAPK5	CAMK	95	88	92
183	MARK1	CAMK	92	90	91
184	MARK2	CAMK	92	84	88
185	MARK3	CAMK	99	93	96
186	MARK4	CAMK	94	87	91
187	MASTL	AGC	94	95	94
188	MATK	TK	110	65	87
189	MEK1	STE	102	99	101
190	MEK2	STE	94	90	92
191	MEK5	STE	98	83	91
192	MEKK2	STE	98	91	94
193	MEKK3	STE	93	95	94
194	MELK	CAMK	82	67	74
195	MERTK	TK	95	89	92
196	MET	TK	99	102	101
197	MINK1	STE	92	87	89
198	MKK4	STE	132	131	132
199	MKK6 SDTD	STE	87	94	90
200	MKK7	STE	110	114	112
201	MKNK1	CAMK	97	97	97
202	MKNK2	CAMK	108	104	106
203	MLK4	TKL	116	109	113
204	MST1	STE	95	91	93
205	MST2	STE	98	83	90
206	MST3	STE	104	101	103
207	MST4	STE	106	97	101
208	MTOR	ATYPICAL	111	116	113
209	MUSK	TK	91	97	94
210	MYLK	CAMK	73	88	80
211	MYLK2	CAMK	84	90	87
212	MYLK3	CAMK	83	75	79
213	NDR1	AGC	95	88	91
214	NDR2	AGC	111	89	100
215	NEK1	OTHER	92	92	92
216	NEK11	OTHER	98	101	99
217	NEK2	OTHER	103	98	100
218	NEK3	OTHER	103	89	96
219	NEK4	OTHER	96	100	98
220	NEK6	OTHER	103	95	99
221	NEK7	OTHER	116	113	115
222	NEK9	OTHER	85	83	84
223	NIK	STE	106	89	98
224	NLK	CMGC	94	91	93
225	p38alpha	CMGC	101	88	95
226	p38beta	CMGC	86	83	85
227	p38delta	CMGC	113	105	109
228	p38gamma	CMGC	101	83	92
229	PAK1	STE	89	85	87
230	PAK2	STE	66	71	68
231	PAK3	STE	92	96	94
232	PAK4	STE	95	89	92
233	PAK6	STE	93	94	94
234	PAK7	STE	89	85	87
235	PASK	CAMK	94	88	91
236	PBK	OTHER	133	83	108
237	PDGFRalpha	TK	102	92	97
238	PDGFRbeta	TK	91	84	88
239	PDK1	AGC	94	90	92
240	PHKG1	CAMK	96	93	94
241	PHKG2	CAMK	98	89	94
242	PIM1	CAMK	97	89	93
243	PIM2	CAMK	104	95	99
244	PIM3	CAMK	103	90	96
245	PKA	AGC	72	71	71
246	PKCalpha	AGC	108	89	98
247	PKCbeta1	AGC	95	82	88
248	PKCbeta2	AGC	92	82	87
249	PKCdelta	AGC	107	93	100
250	PKCepsilon	AGC	97	88	93
251	PKCeta	AGC	116	94	105
252	PKCgamma	AGC	105	81	93
253	PKCiota	AGC	97	93	95
254	PKCmu	AGC	93	87	90
255	PKCnu	AGC	79	76	77
256	PKCtheta	AGC	103	91	97
257	PKCzeta	AGC	100	92	96
258	PKMzeta	AGC	91	93	92
259	PKN3	AGC	93	82	88
260	PLK1	OTHER	104	96	100
261	PLK3	OTHER	96	83	90
262	PRK1	AGC	102	110	106
263	PRK2	AGC	95	87	91
264	PRKD2	CAMK	91	87	89
265	PRKG1	AGC	90	84	87
266	PRKG2	AGC	97	100	99
267	PRKX	AGC	110	94	102
268	PYK2	TK	85	74	80
269	RAF1 YDYD	TKL	75	85	80
270	RET	TK	101	83	92
271	RIPK2	TKL	88	94	91
272	RIPK4	TKL	95	93	94
273	RIPK5	TKL	89	84	86
274	ROCK1	AGC	99	97	98
275	ROCK2	AGC	95	88	92
276	RON	TK	91	86	89
277	ROS	TK	96	93	95
278	RPS6KA1	AGC	109	100	105
279	RPS6KA2	AGC	104	87	95
280	RPS6KA3	AGC	96	82	89
281	RPS6KA4	AGC	100	93	96
282	RPS6KA5	AGC	93	90	91
283	RPS6KA6	AGC	87	74	81
284	S6K	AGC	108	96	102
285	S6Kbeta	AGC	102	98	100
286	SAK	OTHER	56	67	61
287	SGK1	AGC	81	78	80
288	SGK2	AGC	127	116	121
289	SGK3	AGC	113	100	106
290	SIK1	CAMK	100	97	98
291	SIK2	CAMK	97	82	89
292	SIK3	CAMK	93	91	92
293	SLK	STE	97	90	94
294	SNARK	CAMK	94	88	91
295	SNK	OTHER	103	91	97
296	SRC	TK	84	71	77
297	SRMS	TK	101	96	98
298	SRPK1	CMGC	81	83	82
299	SRPK2	CMGC	89	89	89
300	STK17A	CAMK	76	72	74
301	STK23	CAMK	99	89	94
302	STK25	STE	88	90	89
303	STK33	CAMK	96	91	94
304	STK39	STE	100	88	94
305	SYK	TK	96	84	90
306	TAOK2	STE	96	90	93
307	TAOK3	STE	96	97	96
308	TBK1	OTHER	106	99	102
309	TEC	TK	81	83	82
310	TGFBR1	TKL	103	99	101
311	TGFBR2	TKL	69	65	67
312	TIE2	TK	86	81	83
313	TLK1	AGC	110	86	98
314	TLK2	AGC	97	88	92
315	TNK1	TK	40	35	38
316	TRKA	TK	95	54	75
317	TRKB	TK	83	71	77
318	TRKC	TK	100	94	97
319	TSF1	OTHER	71	78	75
320	TSK2	CAMK	104	94	99
321	TSSK1	CAMK	102	93	97
322	TTBK1	CK1	106	100	103
323	TTBK2	CK1	105	102	104
324	TTK	OTHER	79	75	77
325	TXK	TK	94	96	95
326	TYK2	TK	95	97	96
327	TYRO3	TK	90	88	89
328	ULK2	OTHER	101	105	103
329	VEGFR1	TK	96	94	95
330	VEGFR2	TK	87	79	83
331	VEGFR3	TK	99	85	92
332	VRK1	CK1	88	80	84
333	VRK2	CK1	104	100	102
334	WEE1	OTHER	103	92	97
335	WNK1	OTHER	95	82	88
336	WNK2	OTHER	96	89	93
337	WNK3	OTHER	96	101	98
338	YES	TK	77	68	72
339	ZAK	TKL	100	97	99
340	ZAP70	TK	87	90	68	79
Selectivity Score (< 50 % residual activity):					0.015

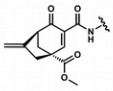
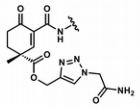
Supplementary Table 3. The table shows data on the effect of JNK-IN-8[1] and **1a***R*-IN-8 used in 1 µM concentration. The table lists only those kinases that had < 50% remaining "activity" in the presence of the inhibitor (meaning that they were bound well by JNK-IN-8; apart from TNK1 and LIMK1 which were inhibited more than 50 % in the in vitro substrate phosphorylation test used in the **1a***R*-IN-8 experiment).

Kinase	% control JNK-IN-8[#]	% activity **1a***R*-IN-8[!]	Kinase	% control JNK-IN-8[#]	% activity **1a***R*-IN-8[!]	Kinase	% control JNK-IN-8[#]	% activity **1a***R*-IN-8[!]
JNK3	0	1	FAK	11	65	CK2α2	39	99
JNK1	0	2	IRAK1	13	85	TTK	46	77
JNK2	0	7	MYLK2	13	87	MST2	48	90
PRKX	0	102	INSR	14	101	SGK3	48	106
KIT	4	69	CK1δ	18	81	ROCK2	49	92
HIPK4	4	94	p38β	20	85	…		
PDGFRβ	5	88	DYRK2	20	91	…		
MKNK2	7	106	STK17A	24	74	TNK1	60	38
CS1FR	9	89	CDK7	39	95	LMK1	74	44

([#] Data from Zhang et al., 2012; off-target kinases with < 50% remaining "activity" (namely % of control signal compared to when no inhibitor was added) is colored in gray; [!] Data from this study; average of two experiments; N=2). Note that the values in the different columns cannot be directly compared but we used this comparison to choose a limited set of off-target kinases to be tested with the same assay; see text and Fig. 5b)

Supplementary Table 4. Comparative pharmacokinetic analysis of the warhead designs
The metabolic stability of composite JNK inhibitors containing different warheads (WH) were analyzed using rat primary hepatocytes and blood plasma. The cyclic warheads had differently functionalized C4 (see black arrowhead shown at the structure of **2**-IN-8). The table below shows the results of hepatic in vivo clearance prediction calculated from the in vitro PK values (Cl_{int}) measured in primary rat hepatocytes as well as the elimination half-life values ($t_{1/2}$) of the intact compounds in rat blood plasma. The data show the mean with SD values determined from samples obtained from three different animals (n=3). ('-' not available, not measured; 'stable' indicates that there was no change detected in compound concentration after 120 minutes; * indicates that $t_{1/2}$ is only estimated because decrease of compound concentration after 120 minutes did not reach 50% of the initial concentration;** indicates that no parent compound was detected at the first sampling time point (10-min); Cl_{int}, Cl_H, E_H, and F denote measured intrinsic clearance (ml/min/kg), predicted hepatic clearance, hepatic extraction ratio, and bioavailability, respectively; the cut-off value was 0.3 between the low (slow) and intermediate extraction compounds. Source data are provided as a Source Data file.



			JNK-IN-8	**2**-IN-8	**1a***R*-IN-8	**6***S,S*-IN-8	**1c***R*-IN-8	**5***S*-IN-8	PK_test
									
liver (rat)	in vitro PK	Cl_{int} (ml/min/kg)	15.1 ± 4.9	-	294.2 ± 130.0	-	-	-	501.0 ± 254.6
	predicted in vivo PK	Cl_H (ml/min/kg)	10.1 ± 2.3	-	28.4 ± 1.8	-	-	-	29.6 ± 1.6
		E_H	0.18 ± 0.04	-	0.51 ± 0.03	-	-	-	0.54 ± 0.03
		F (%)	81.6 ± 4.1	-	48.5 ± 3.2	-	-	-	46.4 ± 2.9
			Slow	-	**Intermediate**	-	-	-	**Intermediate**
blood (rat)	stability in plasma	$t_{1/2}$ (min)	347.0 ± 40.0*	**Stable**	17.9 ± 8.9	3.1 ± 1.1	44.7 ± 3.9	275.8 ± 20.9*	<10**

SUPPLEMENTARY NOTE 1 (on the topographic steric map calculations)

To gain insight into steric environment of the reactive centre, we performed DFT calculations with the *Gaussian 16* suite of programs (Revision A.03).[2] The original molecules were truncated by replacing the directing noncovalent moiety with a Me-group. The geometry optimizations were performed using dispersion-corrected, range-separated hybrid ωB97X-D exchange-correlation functional with implicit SMD continuum solvation model (water; ε = 78.3553).[3–5] Several conformers were examined for each ligand, and we report only the most stable forms herein. The relative stabilities of the conformers were estimated based on the computed Gibbs free energy. The Gibbs free energies were obtained by combining ωB97X-D/Def2TZVPP/SMD(H_2O) electronic energies ($\boldsymbol{E_0'}$) with the thermal and entropic contributions computed at the ωB97X-D/Def2SVP/SMD(H_2O) level (T = 298.15 K).[6] The thermal and entropic contributions were estimated within the ideal gas−rigid rotor−harmonic oscillator (RRHO) approximation. The formula that was used to calculate the free energy is: $G = E_0' + (G_0 - E_0) + \Delta G_{conc}$, where E_0 and G_0 denote electronic and Gibbs free energies obtained with the Def2SVP basis set. The value of ΔG_{conc} (0.0030119 Hartree at 298.15 K ≈ 1.89 kcal/mol) corresponds to concentration correction to the Gibbs free energy when shifting from ideal gas standard state (p = 1 atm) to the standard concentration in solution phase (c = 1 mol/dm^3).

To quantify the steric encumbrance around the reactive carbon atom, we applied buried volume descriptor (%V_{Bur}) using *SambVca 2.1* web application.[7] The buried volume measures the space occupied by the adjacent atoms in the sphere of the reactive center. The space taken by a given atom is calculated based on the Bondi radius of the atom. For calculation of the buried volume, the most important parameters from a practical standpoint are the following: (1) The atoms coordinated to the center of the sphere was the reactive carbon atom. (2) Applied sphere radius 3.5 Å (3) The atomic radii were scaled by 1.17 (4) The H atoms in the calculations were included in the calculations of the buried volume.

Computed energy components of the reported structures

Structure	$\boldsymbol{E_0'}$(H_2O)	$\boldsymbol{G_0}$(H_2O)	$\boldsymbol{E_0}$(H_2O)	$\boldsymbol{G}$(H_2O)
a1	-286.6396	-286.2385	-286.3159	-286.5592
a2	-459.9381	-459.2548	-459.4267	-459.7632
b1	-378.8785	-378.3780	-378.4512	-378.8023
b2	-536.1530	-535.3802	-535.5597	-535.9705
c1	-516.7407	-516.0230	-516.1702	-516.5905
c2	-595.3765	-594.5222	-594.7225	-595.1732
c3	-783.9560	-782.8749	-783.0855	-783.7423
c4	-862.5899	-861.3720	-861.6367	-862.3223
c5	-860.1415	-858.9711	-859.1932	-859.9164

Supplementary Table 5. Summary of energy data (given in Hartree) computed for optimized structures at the ωB97X-D/Def2SVP/SMD(H_2O) level of theory. Note that G contains concentration correction. For the definition of various energy components, see Computational details section (vide supra).

Cartesian coordinates of the reported structures

Cartesian coordinates of the optimized geometries are given below in standard XYZ format (units are in Å). The first line is the molecule name (as defined above in **Supplementary Table 5.**), the second line indicates total number of atoms.

13
a1

C	-4.267660	0.775928	0.625459
O	-3.444446	0.218518	-0.107075
C	-5.739941	0.575290	0.485359
H	-6.393974	1.114691	1.177515
N	-3.895570	1.612020	1.610398
H	-4.610601	2.038761	2.187787
C	-2.506200	1.905253	1.876918
H	-2.445844	2.614528	2.709936
H	-1.949603	0.995309	2.149099
H	-2.017181	2.352631	0.998236
C	-6.243868	-0.231866	-0.448065
H	-5.585761	-0.769238	-1.137348
H	-7.321345	-0.380039	-0.551908

24
a2

C	-4.328470	0.572255	0.776920
O	-3.603217	-0.423072	0.672429
C	-5.749190	0.590062	0.334652
H	-6.312477	1.520925	0.459927
N	-3.877515	1.726812	1.300505
H	-4.510121	2.515406	1.365774
C	-2.520088	1.871497	1.772707
H	-2.380816	2.891639	2.147624
H	-2.301739	1.165026	2.588253
H	-1.793948	1.692289	0.965057
C	-6.326301	-0.488751	-0.201857
H	-5.728901	-1.400137	-0.321715
C	-7.757732	-0.565520	-0.628822
H	-8.224932	0.441875	-0.563405
H	-8.288091	-1.204586	0.098125
N	-7.925472	-1.155358	-1.949534
C	-7.336911	-0.308788	-2.972251
H	-7.454970	-0.773388	-3.962003
H	-7.804104	0.699465	-3.012124
H	-6.260571	-0.171192	-2.793006
C	-9.333805	-1.373008	-2.223857
H	-9.773158	-2.042410	-1.469645
H	-9.921135	-0.429242	-2.222427
H	-9.460530	-1.843019	-3.210095

14
b1

C -4.215164 1.055198 0.188714
O -3.636839 -0.026995 0.133952
C -5.724644 1.116663 0.139668
N -3.569432 2.223864 0.294415
H -4.090302 3.093351 0.332196
C -2.124564 2.280227 0.349612
H -1.815618 3.327972 0.429423
H -1.739765 1.729901 1.220974
H -1.676388 1.846642 -0.556710
C -6.442784 -0.010007 0.045123
H -5.924843 -0.970773 0.003326
C -6.384855 2.391200 0.197448
N -6.904801 3.426793 0.244513
H -7.533430 0.005316 0.009061

26

b2

C -4.232085 1.053584 0.193036
O -3.644253 -0.025576 0.145247
C -5.740745 1.114583 0.142554
N -3.585083 2.223380 0.291756
H -4.106191 3.092661 0.323040
C -2.140608 2.280375 0.347766
H -1.832165 3.328729 0.422674
H -1.755265 1.735066 1.222136
H -1.690326 1.842901 -0.555732
C -6.434589 -0.036164 0.039743
H -5.801557 -0.929648 0.001558
C -6.368875 2.401679 0.205056
N -6.833179 3.464115 0.256581
C -7.930516 -0.250782 -0.034224
C -8.493685 0.432739 -1.293592
H -9.572292 0.226345 -1.369883
H -8.360979 1.524087 -1.265679
H -8.006621 0.048097 -2.202524
C -8.194798 -1.759944 -0.118532
H -7.800685 -2.279840 0.768399
H -9.275805 -1.956432 -0.177696
H -7.718298 -2.195361 -1.010486
C -8.612946 0.311441 1.225621
H -8.205168 -0.153708 2.135961
H -8.494046 1.401087 1.310938
H -9.691252 0.094104 1.184056

22

c1

C -1.800983 -1.434201 0.253909
C -0.320515 -1.321475 0.341384
C 0.354739 -0.225238 0.753395
C -0.398783 1.007468 1.106263

C	-1.884807	0.993105	0.845214
C	-2.514505	-0.373434	1.080912
H	0.274642	-2.189562	0.041965
H	-2.068684	-1.342552	-0.816163
H	-2.103661	-2.451447	0.544808
H	-2.343796	1.781268	1.457142
H	-2.009296	1.287125	-0.213505
H	-2.434360	-0.631743	2.149599
H	-3.584878	-0.346049	0.834581
O	0.141951	2.017156	1.543357
C	1.859658	-0.317888	0.795188
O	2.444211	-1.337499	0.412995
N	2.520222	0.745921	1.268333
H	1.960821	1.549477	1.549648
C	3.961101	0.766358	1.353036
H	4.277996	1.729100	1.769903
H	4.336594	-0.038202	2.003844
H	4.425161	0.643381	0.362209

28

c2

C	-1.896443	-1.195577	-0.035993
C	-0.404318	-1.164440	0.122406
C	0.320575	-0.216404	0.754287
C	-0.366922	0.961984	1.344962
C	-1.845164	1.086509	1.081375
C	-2.551002	-0.258522	0.989274
H	0.150472	-1.989166	-0.338171
H	-2.274698	1.726497	1.864010
H	-1.936437	1.642411	0.131200
H	-2.520004	-0.744664	1.978449
H	-3.612103	-0.114090	0.736457
O	0.223072	1.829590	1.979183
C	1.817662	-0.406797	0.791504
O	2.350989	-1.365902	0.222959
N	2.527081	0.503587	1.469261
H	2.006983	1.267368	1.898589
C	3.965084	0.423997	1.569647
H	4.325567	1.268562	2.167802
H	4.282776	-0.511076	2.056001
H	4.441260	0.467761	0.577954
C	-2.389207	-2.632335	0.179383
H	-3.485852	-2.669248	0.089687
H	-1.960081	-3.315031	-0.569988
H	-2.115268	-3.001487	1.179670
C	-2.214225	-0.763437	-1.481336
H	-1.700469	-1.412762	-2.206378
H	-3.297784	-0.841876	-1.659470
H	-1.904143	0.273688	-1.677028

31

c3

C	-1.945765	-1.157473	0.086630
C	-0.441987	-1.131223	0.142864
C	0.312170	-0.212001	0.775308
C	-0.338594	0.963148	1.419763
C	-1.827216	1.098112	1.231588
C	-2.538325	-0.245055	1.175551
H	0.080701	-1.947194	-0.365634
H	-2.214931	1.734247	2.038224
H	-1.969382	1.654935	0.287359
H	-2.426069	-0.762355	2.141438
H	-3.614493	-0.106749	1.004675
O	0.288458	1.813483	2.037358
C	1.808878	-0.423023	0.749615
O	2.301653	-1.379135	0.143000
N	2.556374	0.464524	1.414747
H	2.067223	1.230057	1.875554
C	3.996106	0.363003	1.455564
H	4.391964	1.186590	2.060371
H	4.317914	-0.588995	1.904581
H	4.432617	0.426263	0.446888
C	-2.434084	-2.603238	0.266826
H	-3.531339	-2.635410	0.301035
H	-2.086442	-3.245122	-0.555927
H	-2.045445	-3.008521	1.212339
C	-2.359532	-0.643453	-1.294159
O	-1.601788	-0.140805	-2.094746
O	-3.655650	-0.796422	-1.516786
C	-4.162097	-0.330040	-2.767095
H	-5.235778	-0.543916	-2.757269
H	-3.992803	0.750536	-2.870561
H	-3.678637	-0.860473	-3.598775

37

c4

C	-1.905402	-1.184990	0.119339
C	-0.402802	-1.133127	0.186361
C	0.330047	-0.177188	0.789081
C	-0.345062	1.010323	1.381677
C	-1.835528	1.105682	1.185426
C	-2.517788	-0.253931	1.179374
H	0.147967	-1.951229	-0.286344
H	-2.238825	1.763784	1.966380
H	-1.987323	1.624034	0.220507
H	-2.398880	-0.720808	2.167643
H	-3.595057	-0.143518	1.000758
O	0.262745	1.898913	1.964100
C	1.829642	-0.365728	0.785795
O	2.342188	-1.346469	0.236936

N	2.558201	0.568828	1.405937
H	2.052543	1.347193	1.825911
C	3.998545	0.488827	1.469522
H	4.376481	1.351132	2.030226
H	4.326643	-0.431436	1.976531
H	4.446599	0.499555	0.464042
C	-2.408082	-2.658179	0.299549
H	-3.498330	-2.577801	0.438895
C	-2.310816	-0.705623	-1.275616
O	-1.531403	-0.408382	-2.153951
O	-3.626105	-0.663243	-1.426284
C	-4.121918	-0.277246	-2.707204
H	-5.213574	-0.315419	-2.633327
H	-3.795402	0.742081	-2.954645
H	-3.770389	-0.975279	-3.479525
C	-1.812524	-3.316100	1.544558
H	-2.281490	-4.298353	1.704273
H	-0.729250	-3.482817	1.432054
H	-1.972327	-2.721844	2.455400
C	-2.152307	-3.526869	-0.933031
H	-2.504507	-4.551899	-0.745469
H	-2.684014	-3.158412	-1.823438
H	-1.079260	-3.586313	-1.174688

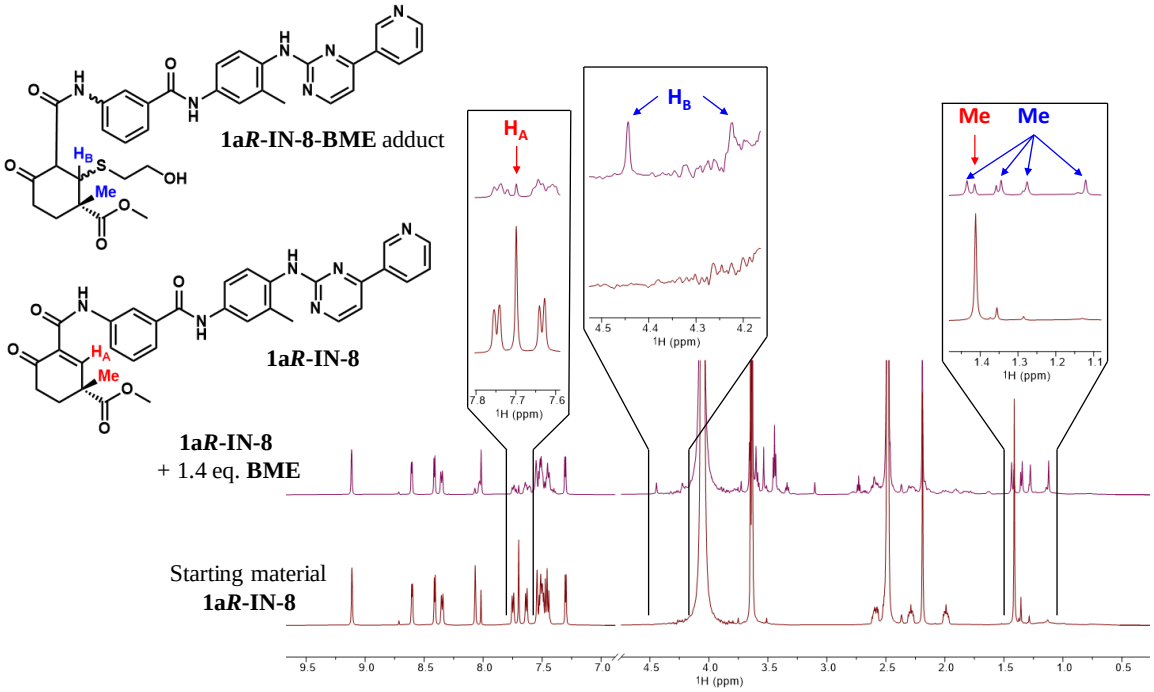
33

c5

C	-1.925685	-0.672865	-0.026240
C	-0.460348	-0.417562	-0.215418
C	0.303174	0.213917	0.699827
C	-0.337409	0.678322	1.962473
C	-1.800722	0.302006	2.113615
C	-2.529540	0.492282	0.781766
H	-2.218342	0.864934	2.956228
H	-3.611102	0.354828	0.911928
H	-2.344799	1.469077	0.313458
O	0.256171	1.276731	2.847046
C	1.766508	0.394079	0.387957
O	2.248403	-0.040840	-0.662307
C	3.911289	1.276183	1.114260
C	-2.092765	-1.859626	0.973113
H	-3.127257	-2.230810	0.913910
H	-1.414961	-2.696574	0.756275
C	-2.611468	-0.935759	-1.354646
O	-2.042709	-1.060243	-2.415892
O	-3.926033	-1.023404	-1.218003
C	-4.689886	-1.288295	-2.394023
H	-5.735706	-1.327774	-2.072825
H	-4.551034	-0.485218	-3.130658
H	-4.393442	-2.249539	-2.835657
C	-1.838681	-1.211732	2.317077

C	-1.644021	-1.832180	3.479606
H	-1.468892	-1.266752	4.399100
H	-1.661108	-2.923498	3.549112
H	0.012991	-0.784538	-1.129155
N	2.498175	1.044087	1.300373
H	2.019073	1.363208	2.140653
H	4.104116	1.866547	0.205517
H	4.296120	1.828679	1.978761
H	4.465822	0.329247	1.026964

SUPPLEMENTARY NOTE 2 (on the NMR experiment of the BME–1a*R*-IN-8 adduct formation)



Supplementary Fig. 16. The ^{1}H NMR spectra of **1a*R*-IN-8** before (lower spectrum) and after (upper spectrum) the addition of 1.4 eq. **BME** in DMSO-d_6 –PBS (D_2O) buffer. The disappearance of the H_A alkene hydrogen and the emergence of H_B signals indicating the Michael adduct formation. The presence of four newly appeared methyl groups (instead of the anticipated two signals due to diastereomer formation) can be explained by the hindered rotation of the amide group.

SUPPLEMENTARY NOTE 3 (on the LC-MS analysis for the PK measurements)

Mass spectrometric analyis

The quantitation of the parent compounds in primary hepatocytes were identified using a Triple TOF 5600+ hybrid Quadrupole-TOF LC/MS/MS system (Sciex, Framingham, MA, USA) equipped with a DuoSpray Ion source coupled with a Shimadzu Prominence LC20 UFLC (Shimadzu, Japan) system consisting of quaternary pump, an autosampler and a thermostated column compartment, a UV-VIS detector and a communication module. Data acquisition was performed using Analyst TF software version 1.7.1 (Sciex , Framingham, MA, USA).

Chromatographic separation was performed on a XSelect CSH C18 (150 x 4.6 mm, 3.5µ) HPLC column (Waters, MA, USA). Gradient elution was applied by using water containing 0.1% formic acid (eluent A) and acetonitrile containing 0.1% formic acid (eluent B). The initial composition of solvents was 5% of eluent B and this was kept for 0.5 min. A linear gradient was applied by 5 min to reach 95% of eluent B. This was held for 1 min and the initial solvent composition was set back by 0.5 min followed by a 3 min equilibration part. The flow rate was 1 mL/min. 5 µL of samples were injected. The column temperature was set to 40°C.

Data were acquired in positive electrospray mode. The mass range was set to m/z 150-1000 with an accumulation time of 0.25 s. Source conditions were: spray voltage: 5000V, nebulizer gas (GS1), drying gas (GS2) and curtain gas (CUR) values were set to 40, 45 and 45 arbitrary unit, respectively. The declustering potential (DP) was set to 80V and source temperature was set to 450°C. The resolution of the instrument was above 25000 over the entire mass range.

Peak View SoftwareTM V.2.2 (version 2.2, Sciex, Framingham, MA, USA) was used for the data processing.

Quantitation of parent compounds in serum samples

The quantitation of the parent compounds was performed in two separate batches on two different MS system. The reason of this change in mass spectrometer was the failure of the high voltage power supply the repair of which required longer time.

The first batch was run on the Triple TOF 5600+ hybrid Quadrupole-TOF LC/MS/MS system (Sciex, Framingham, MA, USA) equipped with a DuoSpray Ion source coupled with a Shimadzu Prominence LC20 UFLC (Shimadzu, Japan) system consisting of quaternary pump,

an autosampler and a thermostated column compartment, a UV-VIS detector and a communication module. Data acquisition was performed using Analyst TF software version 1.7.1 (Sciex , Framingham, MA, USA).

The same MS conditions were used as described above in case of primary hepatocyte experiments.

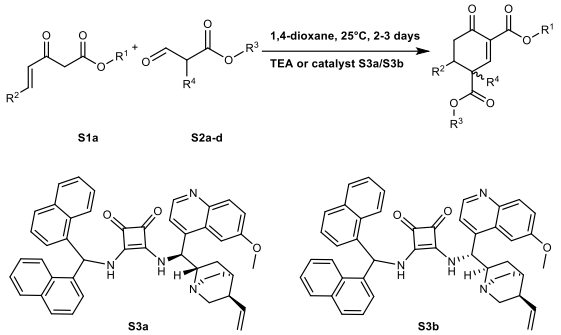
The second batch was, however, measured on a Sciex 6500QTrap (Sciex, Framingham, MA, USA) hybrid quadrupole-ion trap mass spectrometer equipped with Turbo-V ion source. HPLC separations were carried out on an Agilent 1100 system consisting of a binary pump, an autosampler and a column compartment. Analyst 1.6.3. (Sciex, Framingham, MA, USA) software was used for controlling the instrument and for data processing. Quantitation was performed by using the Sciex Multiquant software.

In case of triple quadrupole mass spectrometer, a sensitive MRM experiment was used with two optimized MRM transitions for each parent molecule.

The same chromatographic method was used for both batches by using Kinetex EVO C18 (50 x 2.1 mm, 5µm) HPLC column (Phenomenex, Torrance, CA, USA). Gradient elution was applied by using water containing 0.1% formic acid (eluent A) and acetonitrile containing 0.1% formic acid (eluent B). The initial composition of solvents was 5% of eluent B and kept for 0.5 min. A linear gradient was applied by 4 min to reach 95% of eluent B. This was held for 0.5 min and the initial solvent composition was set back by 0.2 min followed by a 2.8 min equilibration part. The flow rate was 0.6 mL/min. 2 µL of samples were injected. The column temperature was ambient.

SUPPLEMENTARY NOTE 4 (on the synthesis of the compounds)

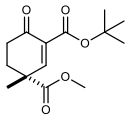
General procedure of synthesizing cyclohexenone warhead compounds:



Compound **S1a** (1.0 eq.), compound **S2a-d** (1.1 eq.) and the catalyst (triethylamine (0.10 eq.) for racemic mixtures or catalyst **S3a** or **S3b** (0.02 eq.) for the enantiomerically enriched compounds respectively) was dissolved in 1,4-dioxane (1.0 M) and the reaction mixture was stirred at 25°C for 2-3 days. When the reaction was completed removal of the volatile compounds under reduced pressure was followed by purification by flash chromatography on silica gel (hexanes 0-25% ethyl acetate) giving the products as a yellow oil.

Compounds **S1a**, **S2a-c**, **S3a**, **S3b** were synthesized according to literature procedures.[8–10]

Preparation of 1-(*tert*-butyl) 3-methyl 3-methyl-6-oxocyclohex-1-ene-1,3-dicarboxylate (S4, S4*R*/S4*S*):



Following the **General Procedure A** using: **S1a** (2.50 g, 14.7 mmol), **S2a** (1.88 g, 16.2 mmol) and **S3a**/**S3b** (201.0 mg, 0.29 mmol) in 1,4-dioxane (15 mL, 1.0 M) at 25°C for 2 days. After removal of the volatile compounds under reduced pressure, purification by flash chromatography on silica gel (hexanes 0-25% ethyl acetate) gave the product as a yellow oil (2.25 g, 57% for **S4**, 2.81 g, 71% for **S4*R***, 2.78 g, 71% for **S4*S***).

ee: 99% with catalyst **S3a**

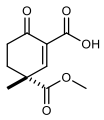
ee: -89% with catalyst **S3b**

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{14}H_{21}O_5$ 269.1379 found 269.1383

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31 (s, 1H); 3.73 (s, 3H); 2.53 – 2.45 (m, 2H); 2.44 – 2.37 (m, 1H); 2.00 – 1.90 (m, 1H); 1.48 (s, 9H); 1.45 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 193.6; 173.9; 163.7; 153.9; 133.3; 82.2; 52.9; 44.2; 35.4; 32.1; 28.1; 24.7;

Preparation of 3-(methoxycarbonyl)-3-methyl-6-oxocyclohex-1-ene-1-carboxylic acid (S5, S5*R*/S5*S*):



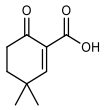
Trifluoroacetic acid (811 µL, 10.53 mmol, 3 eq.) was added to a solution of compound **S4**/**S4*R***/**S4*S*** (942 mg, 3.51 mmol, 1 eq.) in methylene chloride (20 mL) and then the mixture was stirred at 25°C for 3 hours. The reaction was monitored by TLC and when it was completed the reaction mixture was evaporated. The remaining trifluoroacetic acid was removed by redissolving the mixture in toluene and evaporating to dryness at 50°C for three times. Compound **S5**/**S5*R***/**S5*S*** was obtained as a yellow oil in quantitative yield (745 mg) and was used in the next step without further purification.

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{10}H_{13}O_5$ 213.0757 found 213.0759

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (s, 1H), 3.79 (s, 3H), 2.78–2.65 (m, 2H), 2.58–2.53 (m, 1H), 2.08–2.02 (m, 1H), 1.56 (s, 1H).

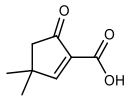
^{13}C NMR (125 MHz, $CDCl_3$): δ 200.1, 172.6, 165.2, 163.4, 126.0, 53.2, 45.4, 34.6, 31.8, 24.5;

Preparation of 3,3-dimethyl-6-oxocyclohex-1-ene-1-carboxylic acid (S6):



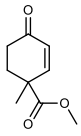
Compound **S6** was prepared according to the literature procedures.[11,12]

Preparation of 3,3-dimethyl-5-oxocyclopent-1-ene-1-carboxylic acid (S7):



Compound **S7** was prepared according to the literature procedures.[13–15]

Preparation of methyl 1-methyl-4-oxocyclohex-2-enecarboxylate (S8):



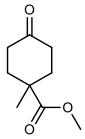
To methyl vinyl ketone (500 µL, 6.00 mmol, 1 eq.), dissolved in 6 mL of 1,4-dioxane, was added **S2a** (770.0 mg, 6.60 mmol, 1.1 eq.) and triethylamine (170 µL, 1.5 mmol, 0.25 eq.). The reaction mixture was stirred at 25°C and monitored by TLC. Upon completion, p-toluenesulfonic acid (630 mg, 3.3 mmol, 0.55 eq.) along with 3 mL of 1,4-dioxane was added, and the reaction was stirred at 100°C until complete conversion of the intermediate product. After removal of the solvent under reduced pressure, the residue was purified by flash column chromatography on silica gel (hexanes 0-25% ethyl acetate) to afford compound **S8** as a yellow oil (131 mg, 51%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_9H_{13}O_3$ 169.0859 found 169.0865

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.81 (d, *J* = 10.2 Hz, 1H), 5.89 (d, *J* = 10.2 Hz, 1H), 3.67 (s, 3H), 2.50 – 2.33 (m, 3H), 1.95 – 1.85 (m, 1H), 1.37 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 198.1, 174.4, 151.5, 128.6, 52.5, 43.7, 34.4, 32.4, 24.7;

Preparation of dimethyl 3-methyl-5-oxocyclopent-1-ene-1,3-dicarboxylate (S14):

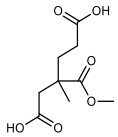


Compound **S8** (517 mg, 3.07 mmol, 1 eq.) was dissolved in ethyl acetate (3.11 mL) in a vial under a nitrogen atmosphere. 20.0 mg of 10% palladium on carbon was added and the reaction mixture was purged with hydrogen with a use of a balloon. After purging the atmosphere, three hydrogen balloons were attached to the vial and the mixture was vigorously stirred for 16 hours at 25°C. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate and filtered through a pad of Celite which was washed two times with ethyl acetate. The solvent was evaporated under reduced pressure affording compound **S9** as a colorless oil without further purification (463 mg, 88%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_9H_{15}O_3$ 171.1021 found 171.1023

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.76 (s, 3H), 2.47 – 2.37 (m, 4H), 2.36 – 2.27 (m, 2H), 1.75 – 1.63 (m, 2H), 1.31 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 210.9, 176.8, 52.2, 42.5, 38.5, 35.3, 25.7;

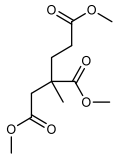


To a suspension of compound **S9** (400.0 mg, 2.35 mmol, 1 eq.) and $KMnO_4$ (742.8 mg, 4.70 mmol, 2 eq.) in water (14 mL) was added a solution of NaOH (35.0 mg, 0.869 mmol, 0.36 eq.) in water (2 mL). The reaction was stirred at 25°C for 16 h, over which time the solid dissolved. A saturated aqueous $NaHSO_3$ solution was added until the purple color disappeared. The reaction mixture was filtered through a pad of Celite which was washed two times with diethyl ether to remove the brown solid. The filtrate was acidified with conc. HCl to pH=1. The reaction was washed with diethyl ether three times, and the combined extracts were dried over anhydrous Na_2SO_4, the solvent was evaporated under reduced pressure, affording compound **S10** as a colorless oil without further purification (240.0 mg, 47%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_9H_{15}O_6$ 219.0868 found 219.0871

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.69 (s, 3H), 2.77 (d, *J* = 16.5 Hz, 1H), 2.58 (d, *J* = 16.4, 1.4 Hz, 1H) 2.38 (t, *J* = 8.1 Hz, 2H), 2.03 (ddd, *J* = 14.1, 9.2, 6.8 Hz, 1H), 1.94 (ddd, *J* = 14.1, 9.4, 6.5 Hz, 1H), 1.30 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 179.5, 177.4, 175.9, 52.4, 43.3, 42.5, 33.3, 29.4, 21.7;

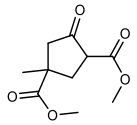


To compound **S10** (240.0 mg, 1.10 mmol, 1 eq.) in toluene (2.5 mL) was added MeOH (245µL, 6.05 mmol, 5.5 eq.) and cc. H_2SO_4 (91 µL, 1.70 mmol, 1.6 eq.). The reaction was stirred for 16 hours at 90°C and after cooling to 25°C the organic solvent was removed under reduced pressure. The residue was diluted with ethyl acetate and washed with a saturated $NaHCO_3$ solution and brine two times. The organic layer was dried over anhydrous Na_2SO_4, the solvent was evaporated under reduced pressure affording compound **S11** as a yellow oil without further purification (163.0 mg, 60%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{11}H_{19}O_6$ 247.1181 found 247.1189

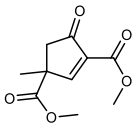
^{1}H NMR (500 MHz, $CDCl_3$): δ 3.70 (s, 3H), 3.67 (s, 3H), 3.66 (s, 3H), 2.76 (d, *J* = 15.9 Hz, 1H), 2.46 (d, *J* = 15.9 Hz, 1H), 2.38 – 2.23 (m, 2H), 2.01 (ddd, *J* = 14.0, 10.3, 6.0 Hz, 1H), 1.89 (ddd, *J* = 14.0, 10.2, 6.4 Hz, 1H), 1.27 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 175.9, 173.5, 171.4, 52.2, 51.8, 51.7, 43.7, 42.7, 33.9, 29.5, 21.7;



A solution of KO^{t}Bu (111.4 mg, 0.993 mmol, 1.5 eq.) in anhydrous THF (1 mL) was cooled to 0˚C and compound **S11** in anhydrous THF (1.5 mL) was added dropwise. The reaction was allowed to stir at 25°C for 1.5 hours, during which time it turned brown. Glacial acetic acid (0.20 mL) was added, resulting in a brownish solution containing a white precipitate. A solution of 0.40 g Na_2HPO_4 in 1.45 mL of water was added, causing the suspension to become homogeneous. The organic layer was separated, and the aqueous layer was washed with chloroform three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, the solvent was evaporated under reduced pressure. The resulting crude product was purified by flash chromatography (hexanes: ethyl acetate) to give compound **S12** as inseparable diastereomers as a yellow oil (50.0 mg, 35%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{10}H_{15}O_5$ 215.0919 found 215.0922



To a solution of compound **S12** (50.0 mg, 0.233 mmol, 1 eq.) in methylene chloride (0.1 mL) at 0°C was added pyridine (23µL, 0.280 mmol, 1.2 eq.). After 5 minutes, a solution of phenylselenyl chloride (53.6 mg, 0.280 mmol, 1.2 eq.) in methylene chloride (0.1 mL) was added dropwise. After 16 hours the reaction mixture was quenched with a saturated aqueous solution of ammonium chloride and washed twice with ethyl acetate. The combined organic layers were washed with water two times and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure affording **S13** intermediate.

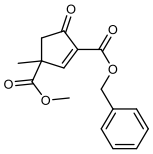
To a solution of the above prepared **S13** in methylene chloride (1 mL) was added m-CPBA (138.1 mg, 0.560 mmol, 2.4 eq.) at 0°C in small amounts. After 16 hours, water was added and the mixture was washed three times with methylene chloride. The organic layers were washed with a 5% $NaHCO_3$ solution and brine two times, dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The resulting crude product was purified by flash chromatography (hexanes: ethyl acetate) to give compound **S14** as a yellow oil (26.3 mg, 53%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{10}H_{13}O_5$ 213.0762 found 213.0763

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (s, 1H), 3.82 (s, 3H), 3.73 (s, 3H), 3.11 (d, *J* = 18.9 Hz, 1H), 2.41 (d, *J* = 18.9 Hz, 1H), 1.53 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 200.2, 172.9, 171.9, 161.9, 135.6, 53.2, 52.3, 49.0, 47.2, 24.4;

Preparation of 1-benzyl 3-methyl 3-methyl-5-oxocyclopent-1-ene-1,3-dicarboxylate (S15):



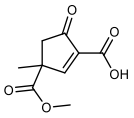
To a solution of compound **S14** (120.0 mg, 0.566 mmol, 1 eq.) in toluene (2.4 mL) was added benzyl-alcohol (588 µL, 5.66 mmol, 10 eq.) and the resulting mixture was stirred at 110 °C for 48 hours. After the starting material was fully consumed the volatiles were evaporated under reduced pressure. The crude product was purified by flash chromatography (hexanes: ethyl acetate) to give compound **S15** as a yellow oil (72.0 mg, 44%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{16}H_{17}O_5$ 289.1075 found 289.1079

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.43 (m, 2H), 7.40 – 7.31 (m, 3H), 5.28 (d, *J* = 2.3 Hz, 2H), 3.74 (s, 3H), 3.14 (d, *J* = 18.9 Hz, 1H), 2.42 (d, *J* = 18.9 Hz, 1H), 1.54 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 200.1, 172.8, 171.6, 161.1, 135.4, 135.3, 128.6, 128.43, 128.41, 66.8, 53.1, 48.9, 47.0, 24.3;

Preparation of 3-(methoxycarbonyl)-3-methyl-5-oxocyclopent-1-ene-1-carboxylic acid (S16):



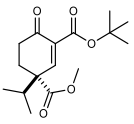
To a solution of compound **S15** (450.0 mg, 1.56 mmol, 1 eq.) in methylene chloride (9 mL) was added TFA (1.19 mL, 15.6 mmol, 10 eq.) and 98% sulfuric acid (10 drops, 0.5 mL) and the resulting mixture was stirred at 25°C for 10 minutes. After the starting material was consumed, the reaction mixture was diluted with 150 mL of water and was washed with methylene chloride three times. The combined organic layers were washed two times with brine, dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure affording compound **S16** as an off-white solid (309.0 mg, 99 %) and was used in the next step without further purification.

HRMS (ESI-TTOF) m/z $[M+Na]^+$ calculated for $C_9H_{10}O_5Na$ 221.0425 found 221.0428

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.48 (s, 1H), 3.78 (s, 3H), 3.32 (d, *J* = 19.6 Hz, 1H), 2.57 (d, *J* = 19.6 Hz, 1H), 1.61 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 206.2, 175.0, 172.1, 161.1, 133.1, 53.3, 49.7, 46.4, 24.0;

Preparation of 1-(*tert*-butyl) 3-methyl 3-isopropyl-6-oxocyclohex-1-ene-1,3-dicarboxylate (S17*R*/S17*S*):



Following the **General Procedure A** using: **S1a** (1.00 g, 5.88 mmol), **S2b** (931.7 mg, 6.46 mmol) and catalyst **S3a**/**S3b** (80.5 mg, 0.118 mmol) in 1,4-dioxane (5.9 mL, 1.0 M) at 25°C for 3 days. After removal of the volatile compounds under reduced pressure, purification by flash chromatography on silica gel (hexanes 0-25% ethyl acetate) gave the product (**S17*R***/**S17*S***) as a colorless oil (0.883 g, 51% for **S17*R***, 1.442 g, 83% for **S17*S***).

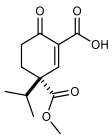
ee: 99+% with catalyst **S3a** (**S17*R***)
ee: -99+% with catalyst **S3b** (**S17*S***)

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{16}H_{25}O_5$ 297.1696 found 297.1687

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (d, *J* = 1.9 Hz, 1H), 3.72 (s, 3H), 2.57 – 2.41 (m, 2H), 2.35 (dddd, *J* = 13.6, 5.0, 4.0, 1.9 Hz, 1H), 2.23 (sept., *J* = 6.9 Hz, 1H), 1.95 (ddd, *J* = 13.7, 12.7, 5.1 Hz, 1H), 1.49 (s, 9H), 0.92 (dd, *J* = 13.1, 6.9 Hz, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 194.2, 172.7, 163.8, 154.0, 134.1, 52.6, 52.1, 36.0, 35.8, 28.2, 25.5, 17.8, 17.6;

Preparation of 3-isopropyl-3-(methoxycarbonyl)-6-oxocyclohex-1-ene-1-carboxylic acid (S18*R*/S18*S*):



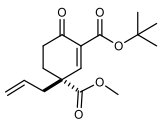
Trifluoroacetic acid (312 µL, 4.05 mmol, 3 eq.) was added to a solution of compound **S17*R*/S17*S*** (400.0 mg, 1.35 mmol, 1 eq.) in methylene chloride (10.4 mL) and then the mixture was stirred at 25°C for 3 hours. The reaction was monitored by TLC and when it was completed the reaction mixture was evaporated. The remaining trifluoroacetic acid was removed by redissolving the mixture in toluene and evaporating to dryness at 50°C three times. Compound **S18*R*/S18*S*** was obtained as a yellow oil in quantitative yield (324.0 mg) and was used in the next step without further purification.

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{12}H_{17}O_5$ 241.1070 found 241.1062

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.27 (d, *J* = 1.9 Hz, 1H), 3.76 (s, 3H), 2.73 – 2.64 (m, 2H), 2.51 – 2.41 (m, 1H), 2.35 (sept., *J* = 6.9 Hz, 1H), 2.04 (ddd, *J* = 13.8, 10.5, 7.7 Hz, 1H), 0.98 (dd, *J* = 12.8, 6.9 Hz, 6H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 202.6, 171.5, 165.4, 163.6, 53.4, 53.0, 36.1, 35.0, 25.4, 18.0, 17.6;

Preparation of 1-(*tert*-butyl) 3-methyl-3-allyl-6-oxocyclohex-1-ene-1,3-dicarboxylate (S19*R*/S19*S*):



Following the **General Procedure A** using: **S1a** (14.3 g, 83.8 mmol), **S2c** (13.1 g, 92.2 mmol) and catalyst **S3a**/**S3b** (1.15 g, 1.68 mmol) in 1,4-dioxane (84 mL, 1.0 M) at 25°C for 2 days. After removal of the volatile compounds under reduced pressure, purification by flash chromatography on silica gel (hexanes 0-25% ethyl acetate) gave the product as a yellow oil (12.1 g, 49% for **S19*R***, 18.4 g, 75% for **S19*S***).

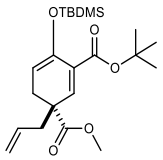
ee: 99+% with catalyst **S3a** (**S19*R*)**
ee: -99+% with catalyst **S3b** (**S19S**)

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{16}H_{23}O_5$ 295.1540 found 295.1543

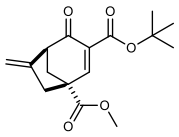
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (s, *J* = 4.7 Hz, 1H), 5.72 – 5.62 (m, 1H), 5.19 – 5.11 (m, 2H), 3.73 (s, 3H), 2.52 (ddt, *J* = 16.2, 11.3, 5.4 Hz, 4H), 2.47 – 2.34 (m, 1H), 2.03 – 1.95 (m, 1H), 1.51 – 1.49 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 193.7, 172.6, 163.7, 152.8, 133.9, 131.5, 120.2, 82.3, 52.7, 48.2, 42.8, 35.4, 29.8, 28.1;

Preparation of 3-(*tert*-butyl) 1-methyl 6-methylene-4-oxobicyclo[3.2.1]oct-2-ene-1,3-dicarboxylate (S20*R*,*R*/S20*S*,*S*):



A flame-dried vial was charged with an anhydrous toluene solution (3.5 mL) of compound **S19*R*/S19*S*** (1.00 g, 3.40 mmol, 1 eq.) and triethylamine (940 µL, 6.79 mmol, 2 eq.) under a nitrogen atmosphere. The reaction mixture was cooled to 0°C then TBDMSOTf (936 µL, 4.08 mmol, 1.2 eq.) was added dropwise, and it was stirred at 0°C for 2 hours. When the reaction was completed, the mixture was diluted with water and extracted with n-Hexane three times. The combined organic phases were washed two times with a saturated $NaHCO_3$ solution and once with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The silyl-enol ether intermediate was obtained as a colorless oil (1.307 g, 94%) and was used without further purification in the next step.



To an anhydrous dimethyl sulfoxide (18 mL) solution of the intermediate were added 4Å molecular sieves, and $Pd(OAc)_2$ (71.8mg, 320.0 mg, 0.1 eq.), and the atmosphere was charged with 3 bars of O_2. The reaction mixture was stirred at 60°C for 18 hours. After completion, the reaction mixture was cooled to 25°C and 50 mL of diethyl-ether was added and it was stirred for a further 1 hour. The reaction mixture was filtered through a pad of Celite then it was washed with water twice. The aqueous phase was extracted with 30 mL of diethyl-ether three times, then the combined organic phases were washed three times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. Purification by flash chromatography on silica gel (hexanes, 0-25% ethyl acetate) to give the product as a white solid (214,8 mg, 46% for **S20*S*,*S***, 179.0 mg, 38% for **S20*R*,*R***).

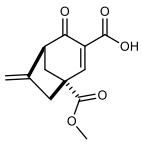
ee: 99+% with catalyst **S3a** (**S20*S*,*S***)
ee: -99+% with catalyst **S3b** (**S20*R*,*R***)

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{16}H_{21}O_5$ 293.1383 found 293.1377

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03 (d, *J* = 1.9 Hz, 1H), 5.39 – 5.33 (m, 1H), 5.14 (d, *J* = 1.2 Hz, 1H), 3.82 (s, 3H), 3.60 (dt, *J* = 4.8, 1.0 Hz, 1H), 3.01 – 2.92 (m, 1H), 2.73 – 2.60 (m, 1H), 2.41 – 2.25 (m, 2H), 1.51 (s, 9H).

^{13}C NMR (125 MHz, CDCl3): δ 192.0, 163.0, 155.7, 142.0, 131.0, 114.0, 82.2, 58.5, 52.9, 51.8, 42.2, 41.1, 28.2;

Preparation of 1-(methoxycarbonyl)-6-methylene-4-oxobicyclo[3.2.1]oct-2-ene-3-carboxylic acid (S21*S*,*S*/S21*R*,*R*):

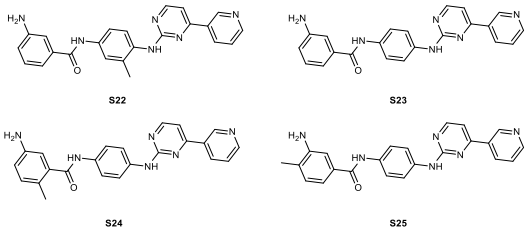


Trifluoroacetic acid (158 µL, 2.05 mmol, 3 eq.) was added to a solution of compound **S20*S,S*/S20*R,R*** (200.0 mg, 0.684 mmol, 1 eq.) in methylene chloride (4.0 mL) and then the mixture was stirred at 25°C for 3 hours. The reaction was monitored by TLC and when it was completed the reaction mixture was evaporated. The remaining trifluoroacetic acid was removed by redissolving the mixture in toluene and evaporating to dryness at 50°C three times. Compound **S21*S,S*/S21*R,R*** was obtained as a yellow oil in quantitative yield (160.0 mg) and was used in the next step without further purification.

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{12}H_{13}O_5$ 237.0757 found 237.0762

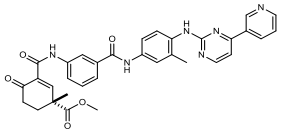
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.80 (d, *J* = 2.0 Hz, 1H), 5.45 (t, *J* = 1.7 Hz, 1H), 5.29 – 5.24 (m, 1H), 3.85 (s, 3H), 3.76 (d, *J* = 4.9 Hz, 1H), 3.07 (dt, *J* = 16.1, 2.6 Hz, 1H), 2.68 – 2.62 (m, 1H), 2.45 (ddd, *J* = 11.8, 4.9, 2.1 Hz, 1H), 2.36 (dd, *J* = 11.9, 2.5 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 199.6, 171.3, 164.5, 163.1, 140.4, 124.8, 116.0, 57.0, 53.1, 52.6, 42.5, 40.1;



Compounds **S22** (IN-8), **S23** (IN-7), **S24** (IN-9), and **S25** (IN-10) were synthesized according to the literature procedures.[1]

Preparation of methyl 1-methyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (1a*R*-IN-8 and 1a*S*-IN-8):



Compound **S5*R*/S5*S*** (87.5 mg, 0.412 mmol, 1.5 eq.) was dissolved in anhydrous dimethylformamide (1.125 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (156.0 mg, 0.412 mmol, 1.5 eq.) and *N*-methylmorpholine (91 µL, 0.825 mmol, 3.0 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (1.125 mL) of compound **S22** (109 mg, 0.275 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated

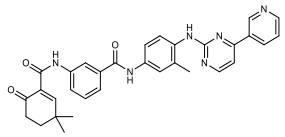
under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). The target compound was obtained as a yellow solid (41.8 mg, 26% for **1a*R*-IN-8** 40.2 mg, 25% for **1a*S*-IN-8**).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{33}H_{31}N_6O_5$ 591.2355 found 591.2377

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.86 (s, 1H), 9.22 (d, *J* = 2.3 Hz, 1H), 8.75 – 8.64 (m, 1H), 8.47 (d, *J* = 5.1 Hz, 1H), 8.37 – 8.32 (m, 1H), 8.26 (s, 1H), 8.21 (t, *J* = 1.9 Hz, 1H), 8.09 (s, 1H), 7.99 (d, *J* = 8.7 Hz, 1H), 7.83 – 7.73 (m, 1H), 7.67 (d, *J* = 7.7 Hz, 1H), 7.62 (d, *J* = 2.5 Hz, 1H), 7.53 – 7.50 (m, 1H), 7.46 – 7.39 (m, 2H), 7.14 (d, *J* = 5.1 Hz, 1H), 6.98 (s, 1H), 3.77 (s, 3H), 2.77 – 2.57 (m, 2H), 2.57 – 2.50 (m, 1H), 2.36 (s, 3H), 2.03 (ddd, *J* = 14.4, 9.9, 5.0 Hz, 1H), 1.55 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 199.6, 173.4, 165.3, 162.7, 162.0, 161.1, 160.8, 159.3, 151.6, 148.6, 138.4, 136.1, 134.6, 134.2, 134.0, 132.9, 130.4, 129.6, 129.1, 123.8, 123.7, 123.1, 122.7, 118.8, 118.7, 108.1, 53.1, 45.2, 36.0, 32.1, 24.8, 18.4;

Preparation of methyl 1-methyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (2-IN-8):



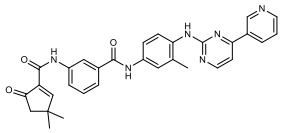
Compound **S11** (85.0 mg, 0.504 mmol, 2 eq.) was dissolved in anhydrous dimethylformamide (0.935 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (192.0 mg, 0.504 mmol, 2 eq.) and *N*-methylmorpholine (111 µL, 1.009 mmol, 4.0 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (0.935 mL) of compound **S22** (100 mg, 0.252 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **2-IN-8** was obtained as a yellow solid (31.0 mg, 22%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{32}H_{31}N_6O_3$ 547.2457 found 547.2457

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.94 (s, 1H), 9.22 (s, 1H), 8.74 – 8.64 (m, 1H), 8.46 (d, *J* = 5.1 Hz, 1H), 8.34 (dt, *J* = 8.0, 1.9 Hz, 1H), 8.18 (d, *J* = 1.8 Hz, 2H), 8.07 (s, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.78 (ddd, *J* = 8.1, 2.1, 1.1 Hz, 1H), 7.65 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.62 (d, *J* = 2.5 Hz, 1H), 7.52 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.44 – 7.38 (m, 2H), 7.13 (d, *J* = 5.2 Hz, 1H), 7.05 (s, 1H), 2.63 (dd, *J* = 7.5, 6.2 Hz, 2H), 2.34 (s, 3H), 1.92 (t, *J* = 6.8 Hz, 2H), 1.26 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 200.5, 170.4, 165.4, 162.6, 161.4, 161.0, 159.2, 151.5, 148.5, 138.5, 136.0, 134.7, 134.2, 133.9, 132.8, 130.4, 129.6, 127.5, 123.8, 123.6, 123.5, 123.1, 122.6, 118.74, 118.71, 108.0, 35.7, 35.4, 34.3, 27.3, 18.4;

Preparation of 3-(3,3-dimethyl-5-oxocyclopent-1-ene-1-carboxamido)-*N*-(3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)benzamide (3-IN-8):



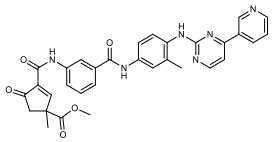
Compound **S25** (77.8 mg, 0.504 mmol, 2 eq.) was dissolved in anhydrous dimethylformamide (1.10 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (191.3 mg, 0.504mmol, 2 eq.) and *N*-methylmorpholine (111 µL, 1.01 mmol, 4 eq.) were added and the mixture was stirred at 25°C. After 30 minutes a DMF solution (1.10 mL) of compound **S22** (100.0 mg, 0.252 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 16 hours. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **3-IN-8** was obtained as a yellow solid (39.5 mg, 29%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{31}H_{29}N_6O_3$ 533.2301 found 533.2303

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.06 (s, 1H), 9.22 (s, 1H), 8.73 – 8.65 (m, 1H), 8.46 (d, *J* = 5.1 Hz, 1H), 8.38 (d, *J* = 6.3 Hz, 1H), 8.35 (d, *J* = 8.1 Hz, 1H), 8.18 (d, *J* = 4.5 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.80 (dd, J = 8.0, 2.0 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 2.5 Hz, 1H), 7.52 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.43 – 7.39 (m, 2H), 7.13 (d, *J* = 4.5 Hz, 2H), 3.90 (s, 1H), 2.55 (s, 3H), 2.34 (s, 3H), 1.31 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 207.6, 181.5, 165.3, 162.6, 161.0, 159.2, 159.1, 151.2, 148.3, 138.1, 136.1, 134.9, 134.5, 134.3, 133.9, 130.6, 129.6, 125.8, 124.8, 123.6, 123.4, 123.3, 122.7, 118.8, 118.7, 108.0, 51.5, 39.1, 27.5, 18.4;

Preparation of methyl 1-methyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclopent-2-ene-1-carboxylate (4-IN-8):



Compound **S34** (30.0 mg, 0.151 mmol, 1 eq.) was dissolved in anhydrous dimethylformamide (0.375 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (57.4 mg, 0.151 mmol, 1 eq.) and *N*-methylmorpholine (75 µL, 0.681 mmol, 4.5 eq.) were added and the mixture was stirred at 25°C. After 30 minutes a DMF solution (0.375 mL) of compound **S22** (90.0 mg, 0.227 mmol, 1.5 eq.) was added and the reaction mixture was stirred at 25°C for 16 hours. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution and two times with brine. The organic phase was dried

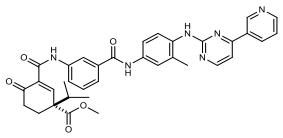
over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **4-IN-8** was obtained as a yellow solid (22.2 mg, 25%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{32}H_{29}N_6O_5$ 577.2193 found 577.2193

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.31 (s, 1H), 8.80 (s, 1H), 8.55 – 8.5 (m, 1H), 8.50 (s, 1H), 8.45 – 8.42 (m, 1H), 8.24-8.22 (m, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.73 (d, *J* = 1.8 Hz, 1H), 7.72 – 7.71 (m, 1H), 7.67 – 7.65 (m, 1H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.59 (d, *J* = 2.3 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.24 (d, *J* = 5.6 Hz, 1H), 3.78 (s, 3H), 3.34 (d, *J* = 19.3 Hz, 1H), 2.59 (d, *J* = 19.3 Hz, 1H), 2.37 (s, 3H), 1.62 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 205.5, 173.3, 172.5, 165.7, 158.6, 158.5, 137.0, 136.9, 136.1, 136.0, 135.8, 129.6, 125.0, 123.7, 123.6, 122.8, 122.6, 119.1, 119.0, 118.7, 118.6, 107.1, 53.2, 49.8, 49.6, 49.4, 49.3, 49.1, 49.0, 47.3, 24.1, 18.2;

Preparation of methyl 1-isopropyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (5*R*-IN-8 and 5*S*-IN-8):



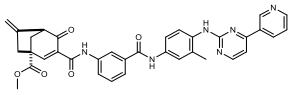
Compound **S21*R*/S21*S*** (13.9 mg, 0.058 mmol, 1.25 eq.) was dissolved in anhydrous dimethylformamide (0.154 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (21.9 mg, 0.058 mmol, 1.25 eq.) and *N*-methylmorpholine (13 µL, 0.116 mmol, 2.5 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (0.154 mL) of compound **S22** (20.0 mg, 0.046 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). The target compound was obtained as a yellow solid (15.6 mg, 55% for **5*R*-IN-8,** 7.7 mg, 27 % for **5*S*-IN-8**).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{35}H_{35}N_6O_5$ 619.2663 found 619.2668

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.91 (s, 1H), 9.26 (s, 1H), 8.73 (s, 1H), 8.48 (d, *J* = 5.1 Hz, 1H), 8.35 (d, J = 7.9 Hz, 1H), 8.27 (d, *J* = 1.8 Hz, 1H), 8.25 (s, 1H), 8.02 – 7.96 (m, 2H), 7.79 (dt, *J* = 8.0, 1.5 Hz, 1H), 7.69 – 7.66 (m, 1H), 7.62 (d, *J* = 2.5 Hz, 1H), 7.52 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.45 (t, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 5.1 Hz, 1H), 6.98 (s, 1H), 5.29 (s, 1H), 3.76 (s, 3H), 2.76 – 2.57 (m, 2H), 2.51 – 2.41 (m, 1H), 2.37 (s, 3H), 2.04 (td, *J* = 13.3, 5.4 Hz, 1H), 0.98 (dd, *J* = 14.0, 6.9 Hz, 6H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 200.2, 172.3, 165.3, 162.8, 162.1, 161.0, 160.8, 159.0, 151.4, 148.4, 138.5, 136.1, 134.9, 134.2, 133.9, 130.5, 129.9, 129.7, 123.7, 123.2, 122.7, 118.8, 118.7, 108.1, 53.2, 52.9, 36.5, 36.1, 25.6, 18.5, 18.0, 17.7;

Preparation of methyl 3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-6-methylene-4-oxobicyclo[3.2.1]oct-2-ene-1-carboxylate (6*S*,*S*-IN-8 and 6*R*,*R*-IN-8):



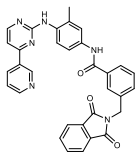
Compound **S24*S*,*S*/S24*R*,*R*** (15.0 mg, 0.064 mmol, 1.25 eq.) was dissolved in anhydrous dimethylformamide (0.170 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (24.1 mg, 0.064 mmol, 1.25 eq.) and *N*-methylmorpholine (14 µL, 0.127 mmol, 2.5 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (0.170 mL) of compound **S22** (22 mg, 0.051 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). The target compound was obtained as a yellow solid (5.7 mg, 18% for **6*S*,*S*-IN-8**, 9.7 mg, 31% for **6*R*,*R*-IN-8**).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{35}H_{31}N_6O_5$ 615.2350 found 615.2350

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.68 (s, 1H), 9.25 (s, 1H), 8.80 (d, *J* = 1.8 Hz, 1H), 8.74 (s, 1H), 8.49 (d, *J* = 5.2 Hz, 1H), 8.41 (dt, *J* = 8.0, 1.8 Hz, 1H), 8.23 (t, *J* = 2.0 Hz, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 7.93 (s, 1H), 7.81 – 7.77 (m, 1H), 7.68 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.63 (d, *J* = 2.5 Hz, 1H), 7.18 (d, *J* = 5.2 Hz, 1H), 5.43 (s, 1H), 5.22 (s, 1H), 3.86 (s, 3H), 3.74 (d, *J* = 4.7 Hz, 1H), 3.05 (dt, *J* = 16.1, 2.7 Hz, 1H), 2.67 (d, *J* = 16.1 Hz, 1H), 2.45 – 2.40 (m, 1H), 2.39 (s, 3H), 2.04 (s, 2H), 1.68 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 197.8, 172.2, 165.3, 161.7, 160.7, 151.1, 148.1, 141.5, 138.4, 136.1, 135.3, 134.4, 133.7, 130.7, 129.7, 128.1, 125.2, 123.73, 123.65, 123.4, 122.7, 118.74, 118.71, 115.1, 108.0, 58.5, 53.1, 52.7, 42.6, 40.6, 32.4, 29.8, 26.6, 23.6, 18.5;

Preparation of 3-((1,3-dioxoisoindolin-2-yl)methyl)-*N*-(3-methyl-4-((4-pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)benzamide (S26):



3-((1,3-dioxoisoindolin-2-yl)methyl)benzoic acid (0.140 g, 0.50 mmol, 1.25 eq.) was dissolved in anhydrous DMF (3 mL) under a nitrogen atmosphere and HBTU (0.192 g, 0.50 mmol,1.25 eq.) and *N*-methyl-morpholine (504 µl, 0.455 g, 1.5 mmol) were added to the solution. Then the reaction mixture was stirred for 10 minutes and 2-methyl-*N*-(4-(pyridin-3-yl)pyrimidin-2-

yl)benzene-1,4-diamine (0.110 g, 0.40 mmol, 1 eq.) was added. After that, the reaction mixture was stirred for 16 hours at 25°C. After that, the reaction mixture was diluted with ethyl acetate and was washed with a 10% citric acid solution two times and brine four times. The organic phase was dried over Na_2SO_4. Then it was filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using methylene chloride: methanol = 9:1 as eluent. Compound **S26** was obtained as yellow crystals (120.0 mg, 44%).

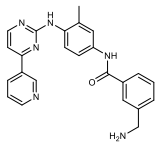
m.p. 118-123 ºC.

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{32}H_{25}N_6O_3$ 541.1988 found 541.1984

^{1}H NMR (500 MHz, CD_3OD): δ 9.25 (s, 1H), 8.71 (d, *J* = 5.0 Hz, 1H), 8.46 (d, *J* = 5.3 Hz, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.31 (s, 1H), 8.13 (s, 1H),7.99 (d, *J* = 7.8 Hz, 1H), 7.95 (s, 1H), 7.84-7.80 (m, 6H), 7.70-7.68 (m, 5H), 7.65-7.60 (m, 3H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.51-7.49 (m, 2H), 7.42-7.37 (m, 3H), 7.33-7.30 (m, 1H), 7.16 (d, *J* = 5.3 Hz, 1H), 4.88 (s, 2H), 2.34 (s, 3H).

^{13}C NMR (125 MHz, CD_3OD): δ 169.5, 168.1, 162.5, 160.5, 158.3, 150.1, 147.3, 137.1, 136.8, 136.2, 135.7, 134.2, 133.2, 132.2, 131.5, 131.0, 130.1, 129.6, 129.3, 128.9, 126.7, 125.2, 124.0, 123.6, 122.8, 118.8, 118.5, 110.5, 107.7, 41.5, 38.7.

Preparation of 3-(aminomethyl)-*N*-(3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)benzamide (S27):



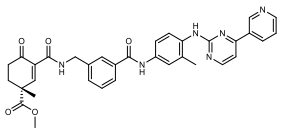
S26 (0.120 g, 0.222 mmol, 1 eq.) was dissolved in methanol (2 mL) and hydrazine hydrate (35 µl, 0.036 g, 0.710 mmol, 3.5 eq.) was added to the solution. After that the reaction mixture was stirred at 25°C for 48 hours. The white suspension formed was filtered and the filtrate was evaporated under reduced pressure. Compound **S27** was obtained as yellow crystals (20.0 mg, 22%) and was used for next step without further purification.

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{24}H_{23}N_6O$ 411.1933 found 411.1936

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.22 (s, 1H), 8.68 (d, J = 4.9 Hz, 1H), 8.45 (d, J = 5.2 Hz, 1H), 8.31 (d, J = 7.3 Hz, 1H), 8.24 (s, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.88 (s, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.62 (s, 1H), 7.52 – 7.47 (m, 1H), 7.45 (d, J = 7.4 Hz, 1H), 7.41 – 7.37 (m, 2H), 5.29 (s, 1H), 3.93 (s, 2H), 2.32 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 165.8, 162.7, 161.1, 159.3, 151.6, 148.6, 135.5, 134.6, 134.3, 134.0, 132.8, 130.8, 130.5, 129.0, 126.1, 125.9, 123.8, 123.2, 122.7, 118.8, 108.1, 18.4;

Preparation of methyl 1-methyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)benzyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (1a'*R*-IN-8):



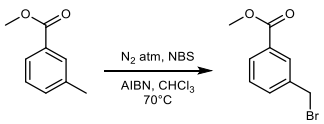
Compound **S5*R*** (23.3mg, 0.110 mmol, 1.5 eq.) was dissolved in anhydrous dimethylformamide (0.25 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (41.6 mg, 0.110mmol, 1.5 eq.) and *N*-methylmorpholine (24 µL, 0.220 mmol, 3 eq.) were added and the mixture was stirred at 25°C. After 30 minutes a DMF solution (0.25 mL) of compound **S27** (30.0 mg, 0.073mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **1a'*R*-IN-8** was obtained as a yellow solid (13.3 mg, 30%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{34}H_{33}N_6O_5$ 605.2506 found 605.2509

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.09 (t, *J* = 6.3 Hz, 1H), 8.47 (d, *J* = 8.0 Hz, 2H), 8.17 (s, 1H), 7.98 – 7.88 (m, 2H), 7.84 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 2.3 Hz, 1H), 7.56 – 7.48 (m, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 5.0 Hz, 1H), 4.64 (t, *J* = 6.1 Hz, 2H), 3.76 (s, 3H), 2.69 – 2.57 (m, 1H), 2.54 – 2.47 (m, 1H), 2.38 (s, 3H), 2.04 – 1.95 (m, 1H), 1.52 (s, 3H).

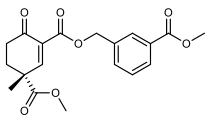
^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 199.1, 173.4, 165.4, 164.3, 162.8, 160.8, 160.0, 157.6, 139.1, 135.7, 135.4, 134.5, 133.1, 131.1, 131.0, 129.1, 128.9, 126.2, 126.0, 123.5, 122.4, 118.4, 107.6, 52.8, 44.9, 43.2, 35.8, 32.0, 24.6, 18.3;

Preparation of 3-methoxycarbonylbenzyl bromide (S28):



3-methoxycarbonylbenzyl bromide was prepared according to the literature procedures.[16]

Preparation of 1-(3-methoxycarbonyl)benzyl) 3-methyl (*R*)-3-methyl-6-oxocyclohex-1-ene-1,3-dicarboxylate (S29):



Compound **S5*R*** (0.100 g, 0.50 mmol, 1.3 eq.) was dissolved in anhydrous DMF (2 mL) under a nitrogen atmosphere and K_2CO_3 (0.064 g, 0.47 mmol, 1.2 eq.) was added to the solution and the mixture was stirred for 45 minutes. Then **S28** (0.09 g, 0.39 mmol, 1 eq.) was added to the

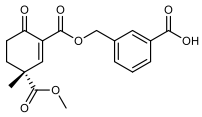
reaction mixture and was stirred for 24 hours at 25°C. After that, the reaction mixture was diluted with ethyl acetate and was washed with brine four times. The combined organic phases were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using hexane: ethyl acetate = 3:1 as eluent. Compound **S29** was obtained as a yellow oil (40.0 mg, 23%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{19}H_{21}O_7$ 361.1287 found 361.1293

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08 (br, 1H), 8.01 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.7 Hz, 1H), 7.54 (s, 1H), 7.46 (t, *J* = 7.7 Hz, 1H), 5.30 (s, 2H), 3.93 (s, 3H), 3.76 (s, 3H), 2.59 – 2.55 (m, 2H), 2.52 – 2.45 (m, 1H), 2.04 – 1.98 (m, 1H), 1.49 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 193.2, 173.6, 166.8, 164.1, 156.3, 136.1, 132.8, 131.6, 130.6, 129.6, 129.4, 128.8, 66.5, 53.0, 52.3, 44.5, 35.4, 32.4, 24.6;

Preparation of (*R*)-3-(((3-methoxycarbonal)-3-methyl-6-oxocyclohex-1-ene-1-carbonyl)oxy)methyl)benzoic acid (S30):



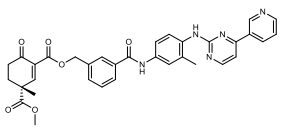
Compound **S29** (0.04 g, 0.10 mmol, 1 eq.) was dissolved in THF:MeOH:H_2O (3:1:1) (0.6 : 0.2 : 0.2 mL) and $LiOH{\cdot}H_2O$ (0.014 g, 0.33 mmol, 3.3 eq.) was added. Then the reaction mixture was stirred for 16 hours at 25°C. After that, the reaction mixture was diluted with distilled water and was washed with methylene chloride two times. The aqueous phase was acidified with a 10% citric acid solution and was washed with methylene chloride three times. The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Compound **S30** was obtained as a yellow oil (15.0 mg, 43%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{18}H_{19}O_7$ 347.1130 found 347.1145

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 7.7 Hz, 1H), 7.56 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 1H), 5.32 (s, 2H), 3.77 (s, 3H), 2.60-2.56 (m, 2H), 2.53 – 2.46 (m, 1H), 2.04 – 1.98 (m, 1H), 1.50 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 193.3, 173.7, 170.9, 164.2, 156.5, 136.3, 133.6, 131.6, 130.2, 130.0, 129.8, 129.1, 66.5, 53.1, 44.6, 35.5, 32.1, 24.7;

Preparation of 3-methyl 1-(3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)benzyl) (*R*)-3-methyl-6-oxocyclohex-1-ene-1,3-dicarboxylate (1a"*R*-IN-8):



Compound **S30** (0.02 g, 0.06 mmol, 1 eq.) was dissolved in anhydrous DMF (1 mL) under a nitrogen atmosphere and HBTU (0.022 g, 0.06 mmol, 1 eq.) and *N*-methyl-morpholine (0.052 g, 1.74 mmol) were added to the solution. Then the reaction mixture was stirred for 10 minutes

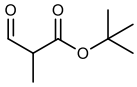
and 2-methyl-*N*-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,4-diamine (0.016 g, 0.06 mmol, 1 eq.) was added. After that, the reaction mixture was stirred for 16 hours at 25°C. Then the reaction mixture was diluted with ethyl acetate and washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over Na_2SO_4. Then it was filtered and evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **1a''*R*-IN-8** was obtained as a yellow oil (3.0 mg, 8%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{34}H_{32}N_5O_6$ 606.2352 found 606.2369

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.47 (s, 1H), 8.42 (d, *J* = 7.8 Hz, 1H), 8.38 (s, 1H), 8.14 (s, 1H), 7.94 (d, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.74 (s, 1H), 7.64 (d, *J* = 8.9Hz, 1H), 7.56 (s, 1H), 7.54 – 7.48 (m, 2H), 7.18 (d, *J* = 5.3 Hz, 1H), 5.39 (s, 2H), 3.77 (s, 3H), 2.69 – 2.56 (m, 2H), 2.54 – 2.49 (m, 1H), 2.38 (s, 3H), 2.10 – 1.94 (m, 1H), 1.52 (s, 3H).

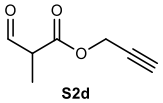
^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 194.1, 173.5, 172.8, 165.2, 164.6, 163.1, 160.4, 157.9, 156.5, 150.8, 147.9, 136.5, 135.4, 135.0, 133.3, 132.2, 131.2, 130.5, 129.2, 127.5, 125.7, 123.8, 122.4, 118.5, 107.7, 66.3, 53.1, 44.5, 35.5, 32.1, 24.7, 18.5;

Preparation of tert-butyl 2-methyl-3-oxopropanoate (S31):



Compound **S31** was synthesized according to literature procedures.[17]

Preparation of prop-2-yn-1-yl 2-methyl-3-oxopropanoate (S2d):



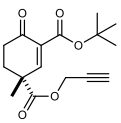
S2d

Compound **S31** (1.00 g, 6.321 mmol, 1 eq.) was dissolved in toluene (30 mL) then propargyl alcohol (0.547 mL, 9.482 mmol, 1.5 eq.) was added, and the mixture was heated to 110°C where it was kept for until full conversion was achieved (1 hour). The mixture was cooled to 25°C and then concentrated under reduced pressure at 30°C. Compound **S2d** was obtained by vacuum distillation as colorless oil (624.0 mg, 68%).

^{1}H NMR (500 MHz, $CDCl_3$) enol-form: δ 11.04 (d, *J* = 12.8 Hz, 1H), 7.03 (dq, *J* = 12.8, 1.2 Hz, 1H), 4.79 (d, *J* = 2.5 Hz, 2H), 2.50 (t, *J* = 2.5 Hz, 1H), 1.72 (d, *J* = 1.2 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) enol form: δ 196.6, 171.5, 99.6, 75.6, 75.1, 51.8, 12.1;

Preparation of 1-(*tert*-butyl) 3-(prop-2-yn-1-yl) 3-methyl-6-oxocyclohex-1-ene-1,3-dicarboxylate (S32*R*/S32*S*):



Following the **General Procedure A** using: **S1a** (300.0 mg, 1.76 mmol), **S2d** (271.7 mg, 1.94 mmol) and catalyst **S3a**/**S3b** (24.14 mg, 0.0353 mmol) in 1,4-dioxane (1.8 mL, 1.0 M) at 25°C for 3 days. After removal of the volatile compounds under reduced pressure, purification

by flash chromatography on silica gel (hexanes 0-25% ethyl acetate) gave the product as a colorless oil (442.7 mg, 86% for **S32*R*** 433.0 mg, 84% for **S32*S***).

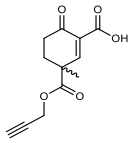
ee: 92% with catalyst **S3a** (**S32*R*)**
ee: -87% with catalyst **S3b** (**S32*S*)**

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{16}H_{21}O_5$ 293.1388 found 293.1393

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (s, 1H), 4.74 (dd, *J* = 5.6, 2.5 Hz, 2H), 2.58 – 2.53 (m, 2H), 2.50 (s, 1H), 2.48 – 2.43 (m, 1H) 2.04 – 1.96 (m, 1H), 1.52 (s, 9H), 1.50 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 199.5, 172.7, 163.7, 153.3, 133.7, 82.4, 75.6, 53.2, 44.2, 35.3, 32.0, 28.5, 28.2, 24.5;

Preparation of 3-methyl-6-oxo-3-((prop-2-yn-1-yloxy)carbonyl)cyclohex-1-ene-1-carboxylic acid (S33*R*/S33*S*):



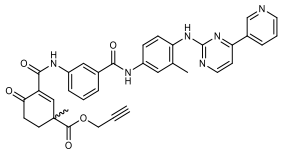
Trifluoroacetic acid (159 µL, 2.06 mmol, 3 eq.) was added to a solution of compound **S32*R*/S32*S*** (201 mg, 0.69 mmol, 1 eq.) in methylene chloride (5 mL) and then the mixture was stirred at 25°C. The reaction was monitored by TLC and when it was completed (3 h) the reaction mixture was evaporated. The remaining trifluoroacetic acid was removed by redissolving the mixture in toluene and evaporating to dryness at 50°C under reduced pressure which was repeated three times. Compound **S33R/S33S** was obtained as a yellow oil in quantitative yield (162 mg) and was used in the next step without further purification.

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{12}H_{13}O_5$ 237.0757 found 237.0750

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (s, 1H), 4.80 – 4.77 (m, 2H), 2.83 – 2.66 (m, 2H), 2.59 (dddd, *J* = 13.1, 6.6, 5.4, 1.2 Hz, 1H), 2.54 (t, *J* = 2.5 Hz, 1H), 2.10 (ddd, *J* = 14.5, 9.9, 5.2 Hz, 1H), 1.60 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 201.9, 171.5, 164.5, 163.3, 151.2, 76.7, 76.1, 53.5, 45.3, 34.5, 31.7, 24.3;

Preparation of prop-2-yn-1-yl 1-methyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (1b*R*-IN-8 and 1b*S*-IN-8):



Compound **S33*R*/S33*S*** (39.3 mg, 0.166 mmol, 2 eq.) was dissolved in anhydrous dimethylformamide (0.400 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (63.1 mg, 0.166 mmol, 2 eq.) and *N*-methylmorpholine (37 µL, 0.333 mmol, 4 eq.) were added and the mixture was stirred at 25°C. After 30 minutes a DMF solution (0.400 mL) of compound **S22** (33.0 mg, 0.083 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed

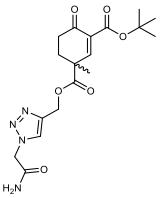
the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). The target compound was obtained as a yellow solid (23.0 mg, 45% for **1b*R*-IN-8**, 18.9 mg, 37% **1b*S*-IN-8**).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{35}H_{31}N_6O_5$ 615.2355 found 615.2382

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.84 (s, 1H), 9.24 (s, 1H), 8.72 (s, 1H), 8.47 (d, *J* = 5.1 Hz, 1H), 8.35 (d, *J* = 7.9 Hz, 1H), 8.25 (s, 1H), 8.20 (s, 1H), 8.10 (s, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.62 (d, *J* = 2.3 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 1H), 7.14 (d, *J* = 5.0 Hz, 1H), 7.04 (s, 1H), 4.75 (t, *J* = 2.8 Hz, 2H), 2.75 – 2.62 (m, 2H), 2.61 – 2.52 (m, 1H), 2.35 (s, 3H), 2.06 (td, *J* = 8.9, 4.3 Hz, 1H), 1.57 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 199.5, 172.2, 165.3, 162.7, 161.3, 161.2, 160.7, 159.2, 151.5, 148.5, 138.3, 136.1, 134.7, 134.1, 134.0, 130.4, 129.6, 129.3, 123.7, 123.6, 123.1, 122.7, 118.8, 118.7, 108.1, 75.9, 53.6, 53.4, 45.2, 35.9, 32.0, 24.5, 18.4;

Preparation of 3-((1-(2-amino-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl) 1-(*tert*-butyl) 3-methyl-6-oxocyclohex-1-ene-1,3-dicarboxylate (S34*R*/S34*S*):



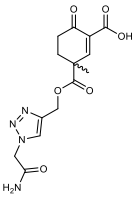
To a solution of compound **S32*R*** /**S32*S*** (80.0 mg, 0.274mmol, 1 eq.) and 2-azidoacetmide (41.1 mg, 0.410 mmol, 1.5 eq.) in methylene chloride (0.5 mL) was added a solution of sodium-ascorbate (27.1 mg, 0.137 mmol, 0.5 eq.) and copper(II) sulfate pentahydrate (6.80 mg, 0.027 mmol, 0.1 eq.) in water (0.5 mL) and methanol (0.25 mL). The reaction mixture was stirred vigorously at 25°C for 1 hour. When the starting material was consumed, the reaction mixture was diluted with 5 mL methylene chloride and washed with water two times. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using methylene chloride: methanol = 9:1 as eluent. The target compound was isolated as a yellow solid (50.0 mg, 47% for **S34*R***, 53.1 mg, 50% for **S34*S***).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{18}H_{25}N_4O_6$ 393.1774 found 393.1779

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 (s, 1H), 7.30 (s, 1H), 5.32 (s, 2H), 5.08 (s, 2H), 2.52 – 2.47 (m, 2H), 2.46 – 2.40 (m, 1H), 2.02 – 1.93 (m, 1H), 1.51 (s, 9H), 1.47 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 193.9, 173.2, 167.6, 163.7, 153.9, 133.3, 125.8, 82.5, 58.6, 53.5, 52.2, 44.2, 35.2, 32.0, 28.1, 24.4;

Preparation of 3-(((1-(2-amino-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)-3-methyl-6-oxocyclohex-1-ene-1-carboxylic acid (S35*R*/S35*S*):



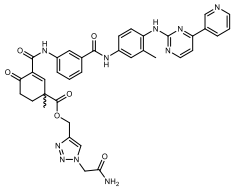
To a solution of compound **S34*R*/S34*S*** (53.1 mg, 0.135 mmol, 1 eq.) in methylene chloride (0.65 mL) was added TFA (31 µL, 0.406 mmol, 3 eq.) and the reaction mixture was stirred at 25°C for 3 hours. The reaction was monitored by TLC and when it was completed the reaction mixture was evaporated. The remaining trifluoroacetic acid was removed by redissolving the mixture in toluene and evaporating at 50°C for three times. Compound **S35*R*/S35*S*** was obtained as a yellow oil in quantitative yield (45.5 mg) and was used in the next step without further purification.

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{14}H_{17}N_4O_6$ 337.1148 found 337.1154

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.82 (s, 1H), 5.22 (s, 2H), 5.03 (s, 2H), 2.58 (dt, *J* = 12.0, 5.8 Hz, 2H), 2.47 – 2.36 (m, 1H), 1.97 (ddd, *J* = 14.3, 9.1, 5.5 Hz, 1H), 1.44 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 200.7, 172.2, 167.9, 164.2, 163.4, 142.1, 126.8, 125.9, 58.7, 51.9, 45.1, 34.4, 31.5, 23.9;

Preparation of (1-(2-amino-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl 1-methyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (1c*R*-IN-8 and 1c*S*-IN-8):



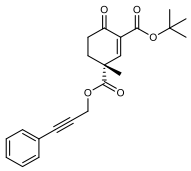
Compound **S35*R*/S35*S*** (45.2 mg, 0.134 mmol, 1.5 eq.) was dissolved in anhydrous dimethylformamide (0.550 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (50.9 mg, 0.134 mmol, 1.5 eq.) and *N*-methylmorpholine (39 µL, 0.358 mmol, 4 eq.) were added and the mixture was stirred at 25°C. After 30 minutes a DMF solution (0.550 mL) of compound **S22** (35.5 mg, 0.089 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). The target compound was obtained as a yellow solid (19.3 mg, 30% for **1c*R*-IN-8**, 16.6 mg, 26% for **1c*S*-IN-8**).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{37}H_{35}N_{10}O_6$ 715.2741 found 715.2763

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.78 (s, 1H), 9.13 (s, 1H), 8.61 (s, 1H), 8.39 (d, *J* = 5.2 Hz, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.13 (s, 1H), 8.11 (d, *J* = 2.8 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.65 (d, *J* = 7.7 Hz, 1H), 7.59 (s, 1H), 7.51 (t, *J* = 8.6 Hz, 2H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.09 (d, *J* = 5.2 Hz, 1H), 5.25 (s, 2H), 5.00 (s, 2H), 2.59 (ddd, *J* = 14.8, 11.4, 6.7 Hz, 2H), 2.45 (dt, *J* = 12.9, 6.1 Hz, 1H), 2.29 (s, 3H), 1.98 (ddd, *J* = 14.3, 9.4, 6.1 Hz, 1H), 1.48 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 199.3, 172.7, 167.6, 166.2, 162.5, 161.5, 161.0, 160.9, 159.0, 150.8, 148.0, 142.3, 137.3, 136.1, 135.2, 134.7, 133.6, 132.0, 129.4, 129.1, 125.8, 125.0, 124.4, 123.9, 123.5, 122.9, 121.5, 119.4, 118.9, 107.8, 58.7, 45.1, 35.6, 31.8, 24.2, 18.2, 18.0;

Preparation of 1-(*tert*-butyl) 3-(3-phenylprop-2-yn-1-yl) (*R*)-3-methyl-6-oxocyclohex-1-ene-1,3-dicarboxylate (S36*R*):



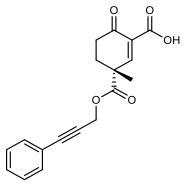
Into a flame-dried vial under a nitrogen atmosphere was added $Pd(PPh_3)_2Cl_2$ (7.2 mg, 0.010 mmol, 0.06 eq.) and CuI (3.6 mg, 0.019 mmol, 0.11 eq.). To this vial was added a solution of compound **S32*R*** (50.0 mg, 0.171 mmol, 1 eq.) and iodobenzene (34.9 mg, 0.171 mmol, 1 eq.) in anhydrous tetrahydrofuran (0.375 mL) and diisopropylamine (0.375 mL). The reaction mixture was stirred at 25°C for 4 hours and when the starting material was consumed it was filtered through a pad of Celite then evaporated. The residue was purified by flash column chromatography on silica gel using hexane: ethyl acetate = 1:1 as eluent to afford compound **S36*R*** as a yellow oil (31.7 mg, 50%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{22}H_{24}O_5Na$ 391.1515 found 391.1514

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45 (d, *J* = 1.6 Hz, 1H), 7.43 (d, *J* = 1.9 Hz, 1H), 7.39 (s, 1H), 7.35 – 7.33 (m, 2H), 7.32 – 7.29 (m, 1H), 5.02 (d, *J* = 15.5 Hz, 1H), 4.97 (d, *J* = 15.5 Hz, 1H)2.65 – 2.55 (m, 1H), 2.55 – 2.47 (m, 2H), 2.09 – 1.98 (m, 1H), 1.53 (s, 3H), 1.52 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 193.5, 172.8, 163.7, 153.5, 133.7, 132.0, 129.1, 128.5, 122.0, 87.2, 82.3, 82.3, 54.1, 44.3, 35.4, 32.1, 28.2, 24.5;

Preparation of (*R*)-3-methyl-6-oxo-3-(((3-phenylprop-2-yn-1-yl)oxy)carbonyl)cyclohex-1-ene-1-carboxylic acid (S37*R*):



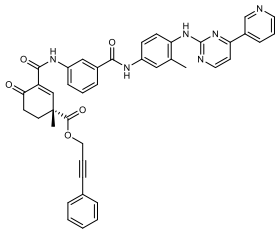
To a solution of compound **S36*R*** (31.7 mg, 0.086 mmol, 1 eq.) in methylene chloride (0.80 mL) was added TFA (40 µL, 0.516 mmol, 6 eq.) and the reaction mixture was stirred at 25°C for 3 hours. The reaction was monitored by TLC and when it was completed the reaction mixture was evaporated. The remaining trifluoroacetic acid was removed by redissolving the mixture in toluene and evaporating to dryness under reduced pressure at 50°C which was repeated three times. Compound **S37*R*** was obtained as a brown oil (25.3 mg, 94%).

HRMS (ESI-TTOF) m/z $[M\text{-}H^+]^-$ calculated for $C_{18}H_{15}O_5$ 311.0919 found 311.0899

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (s, 1H), 7.48 – 7.39 (m, 2H), 7.37 – 7.29 (m, 2H), 7.35 – 7.28 (m, 1H), 4.98 (d, *J* = 2.7 Hz, 2H), 2.82 – 2.66 (m, 2H), 2.68 – 2.51 (m, 1H), 2.08 (m, 1H), 1.59 (d, *J* = 0.8 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 202.0, 171.6, 164.8, 163.4, 132.0, 129.2, 128.5, 126.2, 121.8, 87.6, 81.9, 54.5, 45.4, 34.6, 31.8, 24.4;

Preparation of 3-phenylprop-2-yn-1-yl (*R*)-1-methyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (1d*R*-IN-8):



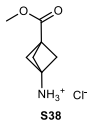
Compound **S37*R*** (25.3 mg, 0.081 mmol, 1.25 eq.) was dissolved in anhydrous dimethylformamide (0.350 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (30.7 mg, 0.081 mmol, 1.25 eq.) and *N*-methylmorpholine (18 µL, 0.162 mmol, 2.5 eq.) were added and the mixture was stirred at 25°C. After 30 minutes a DMF solution (0.350 mL) of compound **S22**(25.7 mg, 0.065 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **1d*R*-IN-8** was obtained as a yellow solid (10.9 mg, 24%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{41}H_{35}N_6O_5$ 691.2668 found 691.2684

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.22 (s, 1H), 8.63 (s, 1H), 8.46 (d, *J* = 8.0 Hz, 1H), 8.42 (d, *J* = 5.2 Hz, 1H), 8.16 (s, 1H), 8.15-8.13 (m, 1H), 7.85 – 7.7 (m, 1H), 7.70 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.64 – 7.62 (m, 2H), 7.57 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.57 – 7.49 (m, 2H), 7.55 – 7.50 (m, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.42 – 7.41 (m, 1H), 7.40-7.39 (m, 1H), 7.34 – 7.30 (m, 4H), 7.28 (d, *J* = 5.3 Hz, 1H), 5.03 (s, 2H), 2.57 – 2.44 (m, 2H), 2.17 – 2.09 (m, 1H), 2.06 – 2.01 (m, 1H), 1.59 (s, 3H).

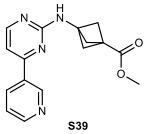
^{13}C NMR (125 MHz, $CDCl_3$): δ 200.1, 173.7, 168.0, 163.5, 162.7, 162.6, 161.4, 160.1, 153.5, 151.6, 148.9, 139.1, 137.2, 136.5, 136.4, 135.2, 133.9, 132.69, 132.66, 130.9, 130.2, 129.9, 129.8, 129.4, 126.1, 124.8, 124.1, 123.1, 120.7, 120.1, 108.4, 87.7, 83.4, 54.8, 46.1, 36.3, 32.8, 24.4, 18.5;

Preparation of 3-((3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)bicyclo[1.1.1]pentan-1-yl)carbamoyl)benzenaminium chloride (S45):



S38

Compound **S38** was synthesized according to the literature procedures from bicyclo[1.1.1]pentane-1,3-dicarboxylic acid.[18]



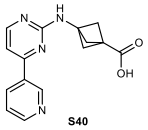
S39

2-chloro-4-(pyridin-3-yl)pyrimidine (541.0 mg, 2.823 mmol, 1 eq.) was dissolved in anhydrous 1,4-dioxane (18 mL) and $PdOAc_2$ (58.87 mg, 0.282 mmol, 0.1 eq.), BINAP (351.6 mg, 0.565 mmol, 0.2 eq.), Cs_2CO_3 (2.073 g, 8.469 mmol, 3 eq.) and compound **S38** (0.752 g, 4.235 mmol, 1.5 eq.) were added to the solution. After that the reaction mixture was refluxed for 1.5 hours. The reaction mixture was cooled down to 25°C and filtered through Celite and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using hexanes:ethyl acetate as eluent. Compound **S39** was obtained as an off-white solid (0.460 g, 55%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{16}H_{17}N_4O_2$ 297.1351found 297.1356

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.24 (d, *J* = 2.5 Hz, 1H), 8.71 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.38 (d, *J* = 5.2 Hz, 1H), 8.31 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.42 (ddd, *J* = 7.9, 4.8, 0.9 Hz, 1H), 7.07 (d, J = 5.2 Hz, 1H), 5.68 (s, 1H), 3.72 (s, 3H), 2.50 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 162.5, 162.5, 159.0, 151.5, 148.7, 134.5, 133.1, 123.7, 107.4, 54.4, 51.9, 47.0, 36.3;



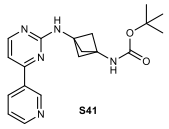
S40

Compound **S39** (273.0 mg, 0.921 mmol, 1 eq.) was dissolved in a mixture of THF and H_2O (7:3, 1.70:0.730 mL), and $LiOH \cdot H_2O$ (57.98 mg, 1.382 mmol, 1.5 eq.) was added to the solution. The mixture was stirred for 1.5 h at 25°C and then diluted with water (10 mL) and washed with methylene chloride (5mL) two times. The aqueous phase was acidified to pH=7 with a 10% HCl solution and the resulting white precipitate was filtered and dried under reduced pressure affording compound **S40** (0.208 g, 80%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{15}H_{15}N_4O_2$ 283.1195 found 283.1199

^{1}H NMR (500 MHz, DMSO-d_6): δ 12.37 (s, 1H), 9.28 (d, *J* = 2.2 Hz, 1H), 8.70 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.46 – 8.37 (m, 2H), 8.05 (s, 1H), 7.57 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.32 (d, *J* = 5.2 Hz, 1H), 2.33 (s, 6H).

^{13}C NMR (125 MHz, DMSO-d_6): δ 171.0, 162.3, 161.2, 159.3, 151.2, 147.9, 134.2, 132.5, 123.9, 106.6, 53.4, 46.3, 36.2;



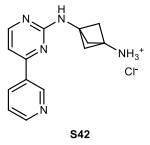
S41

To compound **S40** (150.0 mg, 0.531 mmol, 1 eq), in anhydrous toluene (2.5 mL), was added TEA (222 µL, 1.594 mmol, 3.0eq) and *tert*-butanol (176 µL, 1.860 mmol, 3.5 eq.) to this mixture was added DPPA (219.3 mg, 0.797 mmol, 1.5 eq.). The reaction mixture was stirred at 100°C for 16 hours. After the reaction was completed, the mixture was cooled to 25°C, and it was concentrated under reduced pressure to remove the solvent. Water was added to the system, the brownish-white precipitate was filtered, washed with water, and dried under reduced pressure to afford the Boc-protected amine compound **S41** as a yellow powder (39.3 mg, 21%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{19}H_{24}N_5O_2$ 354.1930 found 354.1935

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.11 (s, 1H), 8.56 (d, *J* = 4.6 Hz, 1H), 8.29 – 8.19 (m, 2H), 7.38 (dd, *J* = 8.0, 4.9 Hz, 1H), 6.98 (d, *J* = 5.2 Hz, 1H), 3.64 (s, 6H), 2.40 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 162.2, 162.1, 158.6, 156.3, 153.2, 150.6, 147.9, 135.0, 133.3, 124.0, 106.9, 54.5, 45.5, 45.2.



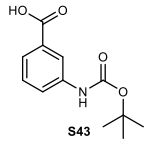
S42

Compound **S41** (50.0 mg, 141.5 mmol, 1 eq.) was added 8M solution of HCl in 1,4-dioxane (885 µL, 7.073 mmol, 50 eq.) and the mixture was stirred at 25°C for 1 h. After the starting material was consumed the reaction mixture was evaporated under reduced pressure, suspended in diethyl ether, filtered, and washed two times with diethyl ether affording compound **S42** as a yellow solid (38.4 mg, 94%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{14}H_{17}N_5$ 254.1405 found 254.1413

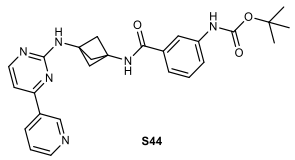
^{1}H NMR (500 MHz, DMSO-d_6+$CDCl_3$): δ 9.47 (d, *J* = 2.0 Hz, 1H), 9.10 – 9.02 (m, 2H), 9.00 (d, *J* = 5.5 Hz, 1H), 8.52 (dd, *J* = 5.3, 1.7 Hz, 1H), 8.11 (t, *J* = 3.6, 3.0 Hz, 1H), 7.48 (d, *J* = 5.3 Hz, 1H), 2.39 (s, 3H), 2.36 (s, 3H).

^{13}C NMR (125 MHz, DMSO-d_6+$CDCl_3$): δ 154.8, 141.7, 135.3, 129.2, 126.8, 120.09, 120.05, 107.1, 106.7, 66.3, 54.1, 53.3, 44.8, 42.9;



S43

Compound **S43** was synthesized according to the literature procedure.[19]



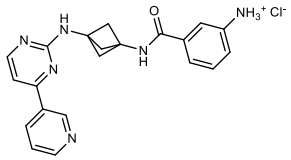
S44

Compound **S43** (47.3 mg, 0.199 mmol, 1.5 eq.) was dissolved in anhydrous dimethylformamide (0.500 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (75.6 mg, 0.199 mmol, 1.5 eq.) and *N*-methylmorpholine (51 µL, 0.465 mmol, 3.5 eq.) were added and the mixture was stirred at 25°C. After 30 minutes compound **S42** (38.4 mg, 0.133 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 16 hours. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by flash column chromatography on silica gel using hexanes:ethyl acetate as eluent, affording compound **S44** as an off-white solid (24.9 mg, 40%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{26}H_{29}N_6O_3$ 473.2301 found 473.2318

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.08 (s, 1H), 8.53 (d, *J* = 3.8 Hz, 1H), 8.31 (d, *J* = 8.3 Hz, 1H), 8.24 (d, *J* = 5.2 Hz, 1H), 7.63 (s, 1H), 7.40 – 7.35 (m, 3H), 7.22 (t, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 5.1 Hz, 1H), 2.49 (s, 6H), 1.41 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 168.6, 162.2, 162.1, 158.6, 153.7, 150.6, 147.8, 138.8, 135.1, 134.9, 133.4, 129.1, 124.0, 121.8, 117.0, 106.8, 80.6, 54.7, 46.0, 45.5, 28.2;



S45

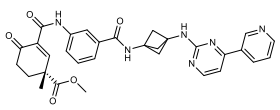
To compound **S44** (24.9 mg, 0.053 mmol, 1 eq.) was added an 8M solution of HCl in 1,4-dioxane (329 µL, 2.635 mmol, 50 eq.) and the mixture was stirred at 25°C for 1 h. After the starting material was consumed the reaction mixture was evaporated under reduced pressure, suspended in diethyl ether, filtered, and washed two times with diethyl ether affording compound **S45** as a yellow solid (16.9 mg, 78%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{21}H_{22}N_6O$ 373.1776 found 373.1787

^{1}H NMR (500 MHz, $CDCl_3$+DMSO-d_6): δ 9.07 (d, *J* = 2.0 Hz, 1H), 8.50-8.47 (m, 3H), 8.11 (d, *J* = 5.6 Hz, 1H), 7.53 (bs, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 7.0 Hz, 1H), 7.05 (t, *J* = 7.9 Hz, 1H), 7.0 (br, 1H), 2.18 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$+DMSO-d_6) partial: δ 164.4, 141.0, 134.7, 130.4, 128.0, 125.8, 125.7, 124.8, 121.5, 105.6, 65.3, 53.5, 44.4, 44.2;

Preparation of methyl (*R*)-1-methyl-4-oxo-3-((3-((3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)bicyclo[1.1.1]pentan-1-yl)carbamoyl)phenyl)carbamoyl)cyclohex-2-ene-1-carboxylate (1a*R*-isoPHEN):



Compound **S5*R*** (13.2 mg, 0.062 mmol, 1.5 eq.) was dissolved in anhydrous dimethylformamide (0.100 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (23.5

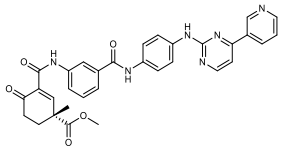
mg, 0.062 mmol, 1.5 eq.) and *N*-methylmorpholine (18 µL, 0.165 mmol, 4 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (0.150 mL) of compound **S45** (16.9 mg, 0.041 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **1a*R*-isoPHEN** was obtained as a yellow oil (12.9 mg, 55%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{31}H_{31}N_6O_5$ 567.2355 found 567.2351

^{1}H NMR (500 MHz, $CDCl_3$+CD_3OD): δ 9.14 (br, 1H), 8.60 (br, 1H), 8.36 (d, *J* = 8.2 Hz, 1H), 8.30 (d, *J* = 5.2 Hz, 1H), 8.19 (s, 1H), 8.04 (t, *J* = 1.9 Hz, 1H), 7.59 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.53 (ddd, J = 8.1, 2.1, 1.1 Hz, 1H), 7.44 – 7.41 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 5.1 Hz, 1H), 3.73 (s, 3H), 2.65 – 2.61 (m, 2H), 2.56 (s, 6H), 2.50 – 2.45 (m, 1H), 2.27 – 2.21 (m, 1H), 1.51 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$+CD_3OD) partial: δ 199.4, 174.2, 173.4, 168.2, 162.3, 162.2, 161.9, 160.9, 158.7, 150.6, 147.9, 137.3, 135.3, 135.1, 129.3, 128.8, 124.1, 123.6, 119.0, 106.8, 54.7, 52.9, 46.0, 45.1, 42.6, 35.7, 31.8, 24.4;

Preparation of methyl (*R*)-1-methyl-4-oxo-3-((3-((4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)cyclohex-2-ene-1-carboxylate (1a*R*-IN-7):



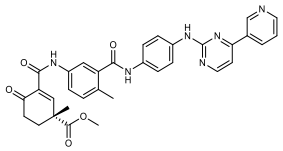
Compound **S5*R*** (50.0 mg, 0.236 mmol, 1.25 eq.) was dissolved in anhydrous dimethylformamide (0.625 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (89.4 mg, 0.236 mmol, 1.25 eq.) and *N*-methylmorpholine (83 µL, 0.754 mmol, 4.0 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (0.625 mL) of compound **S23** (72.1 mg, 0.189 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **1a*R*-IN-7** was obtained as a yellow solid (27.1 mg, 25%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{32}H_{29}N_6O_5$ 577.2193 found 577.2183

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.13 (s, 1H), 8.64 – 8.54 (m, 1H), 8.39 (d, *J* = 5.1 Hz, 1H), 8.34 (d, *J* = 8.2 Hz, 1H), 8.16 (s, 1H), 8.08 (s, 1H), 7.64 (d, *J* = 7.7 Hz, 2H), 7.62 – 7.60 (m, 2H), 7.54 (d, *J* = 7.6 Hz, 2H), 7.45 – 7.39 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.09 (d, *J* = 5.1 Hz, 1H), 3.70 (s, 3H), 2.60 (dt, *J* = 11.8, 5.8 Hz, 2H), 2.44 (dt, *J* = 12.4, 5.7 Hz, 1H), 1.97 (ddd, *J* = 14.4, 9.5, 5.5 Hz, 1H), 1.47 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 199.4, 173.4, 166.2, 162.2, 162.0, 161.0, 160.2, 158.9, 150.6, 147.8, 137.3, 136.1, 135.9, 135.3, 133.4, 133.2, 129.4, 128.8, 124.4, 124.1, 123.8, 121.4, 120.1, 119.3, 107.9, 52.9, 45.1, 35.7, 31.8, 24.4;

Preparation of methyl (R)-1-methyl-3-((4-methyl-3-((4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (1a*R*-IN-9):



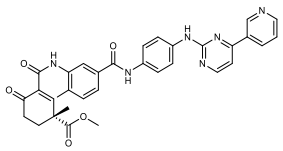
Compound **S5*R*** (50.0 mg, 0.236 mmol, 1.25 eq.) was dissolved in anhydrous dimethylformamide (0.625 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (89.4 mg, 0.236 mmol, 1.25 eq.) and *N*-methylmorpholine (83 µL, 0.754 mmol, 4.0 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (0.625 mL) of compound **S24** (74.7 mg, 0.189 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **1a*R*-IN-9** was obtained as a yellow solid (26.4 mg, 24%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{33}H_{31}N_6O_5$ 591.2355 found 591.2362

^{1}H NMR (500 MHz, $CDCl_3$): δ 11.83 (s, 2H), 10.73 (s, 1H), 9.40 (s, 1H), 8.85 (s, 1H), 8.71 (d, *J* = 8.1 Hz, 1H), 8.46 – 8.38 (m, 1H), 8.21 (s, 1H), 8.14 (s, 1H), 7.91 (s, 1H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.68 – 7.59 (m, 4H), 7.44 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.29 (d, *J* = 5.5 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 3.76 (s, 3H), 2.75 – 2.59 (m, 2H), 2.51 (dt, *J* = 12.6, 5.8 Hz, 1H), 2.44 (s, 3H), 2.02 (ddd, *J* = 14.3, 9.7, 5.0 Hz, 1H), 1.53 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 199.7, 173.4, 167.8, 162.0, 160.8, 139.3, 136.6, 135.7, 134.9, 134.0, 133.1, 132.0, 129.0, 122.5, 121.6, 121.1, 119.2, 107.2, 53.1, 45.2, 35.9, 32.1, 24.7, 19.5;

Preparation of methyl (*R*)-1-methyl-3-((2-methyl-5-((4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (1a*R*-IN-10):



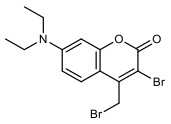
Compound **S5*R*** (16.1 mg, 0.076 mmol, 1.25 eq.) was dissolved in anhydrous dimethylformamide (0.200 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (28.7 mg, 0.076 mmol, 1.25 eq.) and *N*-methylmorpholine (27 µL, 0.242 mmol, 4.0 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (0.200 mL) of compound **S25** (24.0 mg, 0.061 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **1a*R*-IN-10** was obtained as a yellow solid (4.4 mg, 12%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{33}H_{31}N_6O_5$ 591.2355 found 591.2359

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.82 (s, 1H), 9.31 (s, 1H), 8.72 (d, J = 1.9 Hz, 1H), 8.50 (d, J = 5.2 Hz, 2H), 8.43 (d, *J* = 8.0 Hz, 2H), 8.30 (s, 1H), 8.07 (s, 1H), 7.58 (s, 1H), 7.51 (s, 1H), 7.72 – 7.61 (m, 2H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 4.8 Hz, 1H), 3.79 (s, 3H), 2.78 – 2.64 (m, 2H), 2.56 (dt, *J* = 12.5, 5.8 Hz, 1H), 2.44 (s, 3H), 2.06 (ddd, *J* = 14.3, 10.0, 5.0 Hz, 1H), 1.57 (s, 3H).

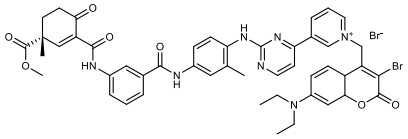
^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 199.9, 173.4, 165.5, 162.8, 162.2, 160.8, 160.2, 158.7, 151.3, 148.3, 136.6, 135.8, 135.1, 133.7, 133.5, 132.2, 131.2, 129.3, 124.5, 124.1, 121.3, 120.3, 119.4, 108.2, 53.2, 45.3, 36.0, 32.2, 24.8, 18.4.

Preparation of 3-bromo-4-(bromomethyl)-7-(diethylamino)-2*H*-chromen-2-one (S46):



Compound **S46** was synthesized according to the literature procedure.[20]

Preparation of 1-((3-bromo-7-(diethylamino)-2-oxo-4a,8a-dihydro-2*H*-chromen-4-yl)methyl)-3-(2-((4-(3-((*R*)-3-(methoxycarbonyl)-3-methyl-6-oxocyclohex-1-ene-1-carboxamido)benzamido)-2-methylphenyl)amino)pyrimidin-4-yl)pyridin-1-ium bromide (Photocaged 1a*R*-IN-8):

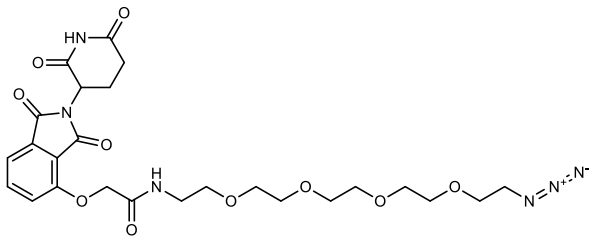


1a*R*-IN-8 (5.4 mg, 0.0091 mmol, 1 eq.) was dissolved in HPLC grade acetonitrile (0.5 mL) in a flame-dried, brown vial. To this solution was added **S46** (4.1 mg, 0.011 mmol, 1.15 eq.) and the reaction mixture was stirred at 60°C in the dark (avoiding decomposition) for 16 hours. The reaction mixture was evaporated, then the resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **Photocaged 1a*R*-IN-8** was obtained as a yellow solid (3.0 mg, 40%).

HRMS (ESI-TTOF) m/z $[M]^+$ calculated for $C_{47}H_{47}N_7O_7Br$ 898.2558 found 898.2567

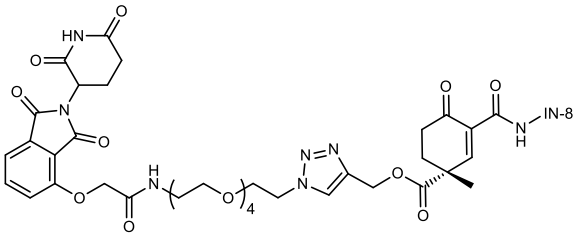
^{1}H NMR (500 MHz, $CDCl_3$): δ 10.80 (s, 1H), 9.97 (s, 1H), 9.06 (s, 1H), 8.96 (d, *J* = 8.5 Hz, 2H), 8.48 (d, *J* = 4.9 Hz, 1H), 8.19 (d, *J* = 4.9 Hz, 2H), 8.04 (t, *J* = 7.1 Hz, 1H), 7.92 (d, *J* = 9.2 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.66 (s, 1H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.41 (q, *J* = 6.5, 5.0 Hz, 2H), 6.64 (d, *J* = 7.3 Hz, 1H), 6.47 (s, 2H), 6.42 (d, *J* = 2.5 Hz, 1H), 3.76 (s, 3H), 3.34 (q, *J* = 7.2 Hz, 4H), 2.76 (s, 3H), 2.71 – 2.59 (m, 2H), 2.51 (dt, *J* = 12.9, 6.1 Hz, 1H), 2.24 (s, 3H), 2.02 (td, *J* = 14.3, 9.7, 5.4 Hz, 2H), 1.91 (s, 3H), 1.54 (s, 3H), 1.14 (t, *J* = 7.0 Hz, 6H).

***N*-(14-azido-3,6,9,12-tetraoxatetradecyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (S47):**



Compound **S47** was purchased from BroadPharm, USA.

Preparation of (1-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-17-yl)-1*H*-1,2,3-triazol-4-yl)methyl (1*R*)-1-methyl-3-((3-((3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)carbamoyl)phenyl)carbamoyl)-4-oxocyclohex-2-ene-1-carboxylate (PRT_1):



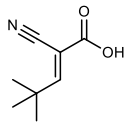
To a solution of compound **1b*R*-IN-8** (32.0 mg, 0.520 mmol, 1.5 eq.) and compound **S47** (20.0 mg, 0.347 mmol, 1 eq.) in methylene chloride (0.139 mL) was added a solution of sodium-ascorbate (3.4 mg, 0.017 mmol, 0.5 eq.) and copper(II) sulfate pentahydrate (0.87 mg, 0.0035 mmol, 0.1 eq.) in water (0.139 mL) and methanol (0.069 mL). The reaction mixture was stirred vigorously at 25°C for 1 hour. When the starting material was consumed, the reaction mixture was diluted with 2.5 mL methylene chloride and washed with water two times. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure then the resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). **PRT_1** was isolated as a yellow oil (14.6 mg, 35%).

HRMS (ESI-TTOF) m/z $[M]^+$ calculated for $C_{60}H_{63}N_{12}O_{15}$ 1191.4530 found 1191.4475

^{1}H NMR (600 MHz, $CDCl_3$): δ 10.84 – 10.81 (m, 1H), 9.74 – 9.63 (m, 1H), 9.23 (s, 1H), 8.70 (s, 1H), 8.48 (d, *J* = 5.2 Hz, 1H), 8.46 (d, *J* = 3.7 Hz, 1H), 8.34 (dt, *J* = 8.0, 1.9 Hz, 1H), 8.23 (s, 1H), 8.25 – 8.20 (m, 1H), 7.94 (d, *J* = 8.7 Hz, 1H), 7.82 (s, 1H), 7.73 – 7.72 (m, 1H), 7.70 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.67 (ddd, *J* = 8.3, 7.3, 1.0 Hz, 1H), 7.65 – 7.63 (m, 1H), 7.62 – 7.56 (m, 1H), 7.51 (dd, *J* = 8.2, 3.1 Hz, 1H), 7.46 (d, *J* = 7.3 Hz, 1H), 7.46 – 7.40 (m, 2H), 7.14 (d, *J* = 5.1 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H), 7.11 (s, 1H), 5.34 – 5.25 (m, 2H), 5.02 – 4.94 (m, 1H), 4.63 – 4.54 (m, 2H), 4.51 (t, *J* = 5.1 Hz, 2H), 3.83 (t, *J* = 5.0 Hz, 2H), 3.66 – 3.53 (m, 14H), 3.53 – 3.49 (m, 2H), 2.87 – 2.81 (m, 1H), 2.80 – 2.73 (m, 2H), 2.71 – 2.57 (m, 2H), 2.53 – 2.46 (m, 1H), 2.33 (s, 3H), 2.15 – 2.09 (m, 1H), 2.05 – 1.97 (m, 1H), 1.52 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 199.6, 199.5, 172.7, 171.62, 171.59, 168.71, 168.70, 166.9, 166.8, 166.7, 166.7, 165.8, 165.2, 162.6, 161.79, 161.77, 160.9, 160.64, 160.62, 159.0, 154.3, 151.4, 148.4, 141.9, 138.00, 137.99, 136.8, 136.00, 135.99, 134.6, 134.3, 133.7, 133.6, 132.7, 130.3, 130.2, 129.44, 129.43, 129.02, 129.01, 125.1, 124.0, 123.9, 123.7, 123.5, 123.97, 122.95, 122.57, 122.55, 119.3, 118.7, 118.61, 118.59, 117.9, 117.2, 107.9, 70.55 – 70.22 (m), 69.4, 69.2, 67.80, 67.79, 59.0, 50.3, 49.3, 45.1, 39.0, 35.7, 31.9, 31.4, 24.3, 22.7, 18.3;

Preparation of (*E*)-2-cyano-4,4-dimethylpent-2-enoic acid (S48):



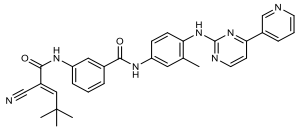
Pivaldehyde (2.03 g, 23.5 mmol, 2 eq.) was added to a solution of 2-cyanoacetic acid (1.0 g, 11.8 mmol) in pyridine (5 mL). Pyrrolidine (201 mg, 2.82 mmol, 0.24 eq.) was added, and the reaction mixture was stirred for 18 hours at 25°C. The mixture was then poured onto cc. HCl (6 mL) and water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated under reduced pressure to give compound **S48** as a white solid (1.53 g, 85%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_8H_{12}NO_2$ 154.0868 found 154.0864

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.72 (s, 1H), 1.34 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 174.3, 166.7, 113.6, 105.3, 35.6, 28.9;

Preparation of (*E*)-3-(2-cyano-4,4-dimethylpent-2-enamido)-*N*-(3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)benzamide (CA-IN-8):

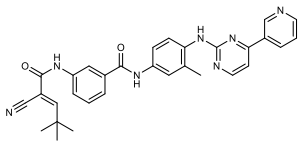


Compound **S48** (77.3 mg, 0.504 mmol, 2 eq.) was dissolved in anhydrous dimethylformamide (1.0 mL) in a flame-dried vial under a nitrogen atmosphere, HBTU (191.3 mg, 0.504 mmol, 2 eq.) and *N*-methylmorpholine (111 µL, 1.01 mmol, 4.0 eq.) were added and the mixture was stirred at 25°C. After 30 minutes an anhydrous DMF solution (1.0 mL) of compound **S22** (100 mg, 0.252 mmol, 1 eq.) was added and the reaction mixture was stirred at 25°C for 3 days. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was diluted with ethyl acetate, washed two times with a 5% citric acid solution, two times with a saturated aqueous Na_2CO_3 solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, then evaporated under reduced pressure. The resulting crude product was purified by preparative RP-HPLC using a gradient method on a Gemini® 5µm C18 110 Å column (H_2O:MeCN = 95:5 (0.1 % HCOOH) to 100% MeCN (0.1 % HCOOH)). Compound **CA-IN-8** was obtained as a yellow solid (40.2 mg, 25%).

HRMS (ESI-TTOF) m/z $[M+H]^+$ calculated for $C_{31}H_{30}N_7O_2$ 532.2460 found 532.2448

The structure was found to be in an equilibrium between the acyclic and cyclic forms (see below) in $CDCl_3$ in a 1:0.76 ratio.

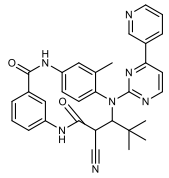
Acyclic form:



^{1}H NMR (500 MHz, $CDCl_3$): δ 9.24 (s, 1H), 8.68 (s, 1H), 8.45 (d, *J* = 5.2 Hz, 1H), 8.34 – 8.30 (m, 1H), 8.23 (s, 1H) 8.19 (s, 1H), 8.07 (t, *J* = 1.9 Hz, 1H), 7.99 (t, *J* = 8.0 Hz, 1H), 7.78 (s, 1H), 7.77 – 7.75 (m, 1H), 7.72 – 7.67 (m, 1H), 7.57 (d, *J* = 2.5 Hz, 1H), 7.50 – 7.45 (m, 2H), 7.42 (t, *J* = 6.5 Hz, 1H), 7.11 (dd, *J* = 5.2, 1.45 Hz, 1H), 7.02 (s, 1H), 2.32 (s, 3H), 1.33 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 170.9, 165.2, 162.7, 161.0, 159.2, 158.6, 151.5, 148.6, 137.5, 136.4, 134.4, 134.1, 133.8, 132.6, 130.4, 124.2, 124.0, 123.8, 123.7, 123.1, 122.8, 119.4, 118.9, 115.7, 108.1, 106.6, 35.2, 29.1, 18.4.

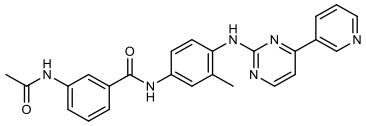
Cyclic form:



^{1}H NMR (500 MHz, $CDCl_3$): δ 9.24 (s, 1H), 8.67 (s, 1H), 8.45 (d, *J* = 5.2 Hz, 1H), 8.34 – 8.30 (m, 1H), 8.23 (s, 1H), 8.07 (t, *J* = 1.9 Hz, 1H), 7.99 (t, *J* = 8.0 Hz, 1H), 7.84 (t, *J* = 1.8 Hz, 1H), 7.77 – 7.75 (m, 1H), 7.72 – 7.67 (m, 1H), 7.60 (d, J = 2.5 Hz, 1H), 7.50 – 7.45 (m, 2H), 7.42 (t, *J* = 6.5 Hz, 1H), 7.11 (dd, *J* = 5.2, 1.45 Hz, 1H), 4.33 (d, *J* = 2.9 Hz, 1H) , 3.83 (d, *J* = 2.9 Hz, 1H), 2.32 (s, 3H), 1.03 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 164.7, 162.7, 161.0, 159.2, 156.4, 151.5, 148.6, 137.5, 136.4, 134.4, 134.0, 133.9, 132.8, 130.4, 124.1, 124.0, 123.8, 123.7, 123.1, 122.9, 119.9, 118.9, 114.2, 108.1, 66.4, 38.8, 34.6, 26.0, 18.4.

Preparation of 3-acetamido-*N*-(3-methyl-4-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)benzamide (IN-8a):



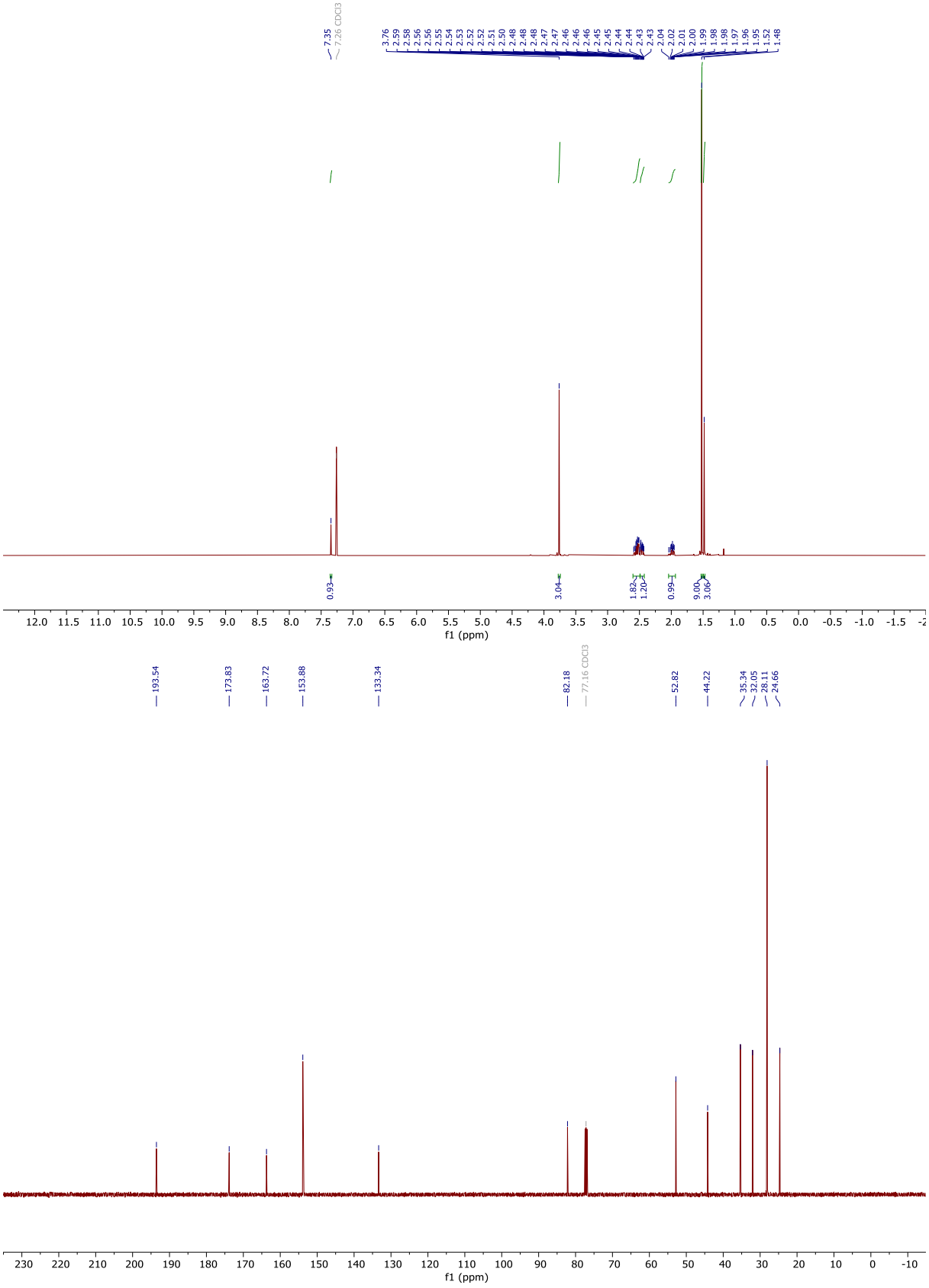
To a solution of **S22** (10.0 mg, 0.025 mmol, 1 eq.) in glacial acetic acid (0.125 mL), was added acetic anhydride dropwise (2.4 µL, 0.025 mmol, 1 eq.) dissolved in glacial acetic acid (0.125 mL) and the reaction mixture was stirred at 25°C for 2 hours. The reaction was monitored by HPLC-MS and when the starting material was consumed the reaction mixture was evaporated, then dissolved in ethyl acetate, the organic phase was washed four times with a saturated aqueous $NaHCO_3$ solution, and two times with brine. The organic phase was dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated under reduced pressure to give compound **IN-8a** as a yellow solid(6.8 mg, 62 %).

HRMS (ESI-TTOF) m/z $[M]^+$ calculated for $C_{25}H_{23}N_6O_2$ 439.1877 found 439.1869

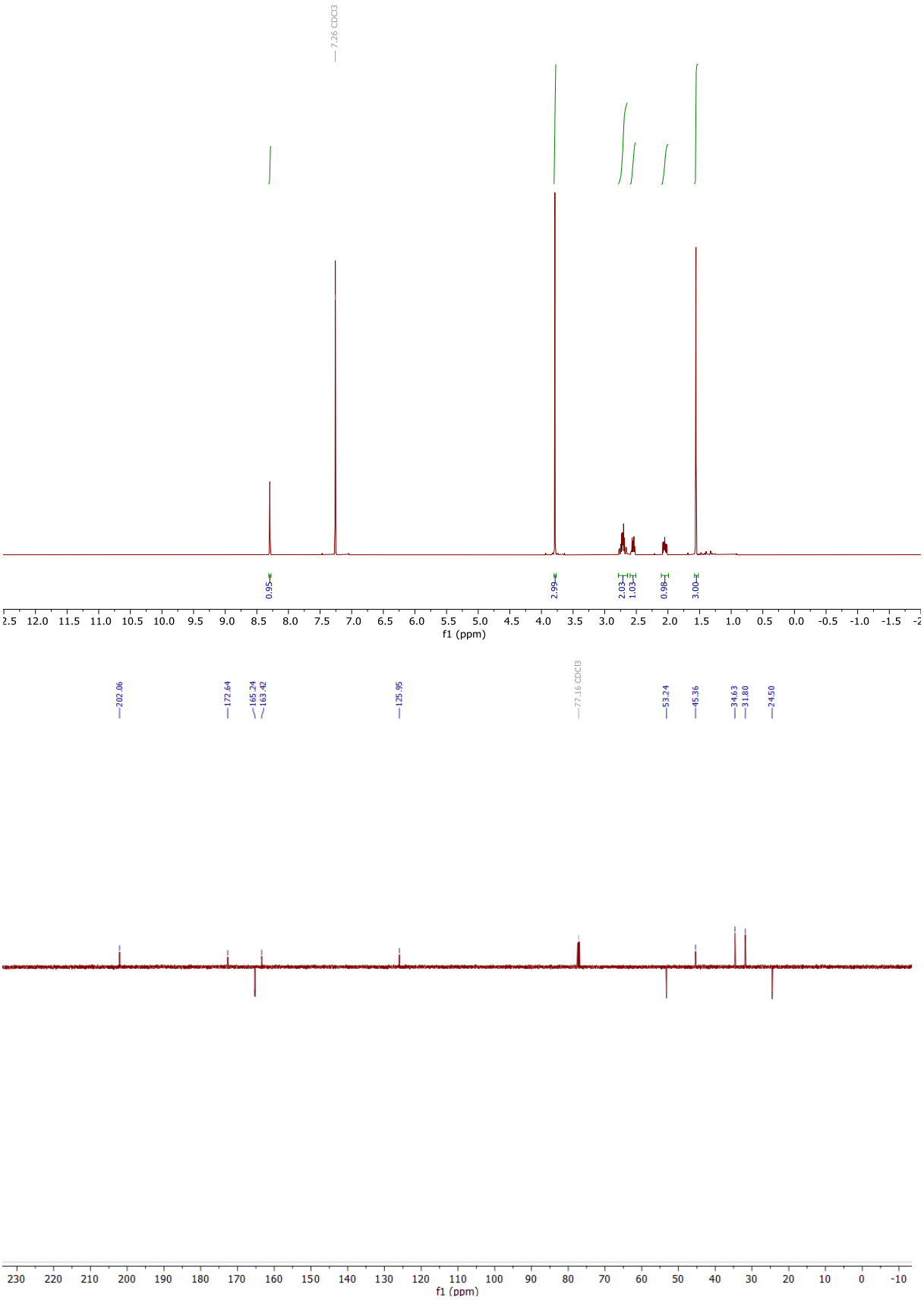
^{1}H NMR (500 MHz, $CDCl_3$): δ 10.17 (s, 1H), 10.13 (s, 1H), 9.24 (d, *J* = 2.1 Hz, 1H), 8.92 (s, 1H), 8.69 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.49 (d, J = 5.1 Hz, 1H), 8.39 (dd, *J* = 6.0, 3.9 Hz, 1H), 8.08 (s, 1H), 7.82 (d, *J* = 8.2 Hz, 1H), 7.66 – 7.61 (m, 2H), 7.57 – 7.52 (m, 1H), 7.48 – 7.45 (m, 1H), 7.42 – 7.39 (m, 1H), 2.25 (s, 3H), 2.08 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) partial: δ 168.5, 165.3, 161.5, 161.4, 159.4, 151.4, 148.1, 139.5, 135.8, 135.7, 134.2, 133.6, 132.8, 132.3, 128.7, 125.6, 123.8, 122.2, 121.8, 118.5, 118.2, 107.3, 24.0, 18.4;

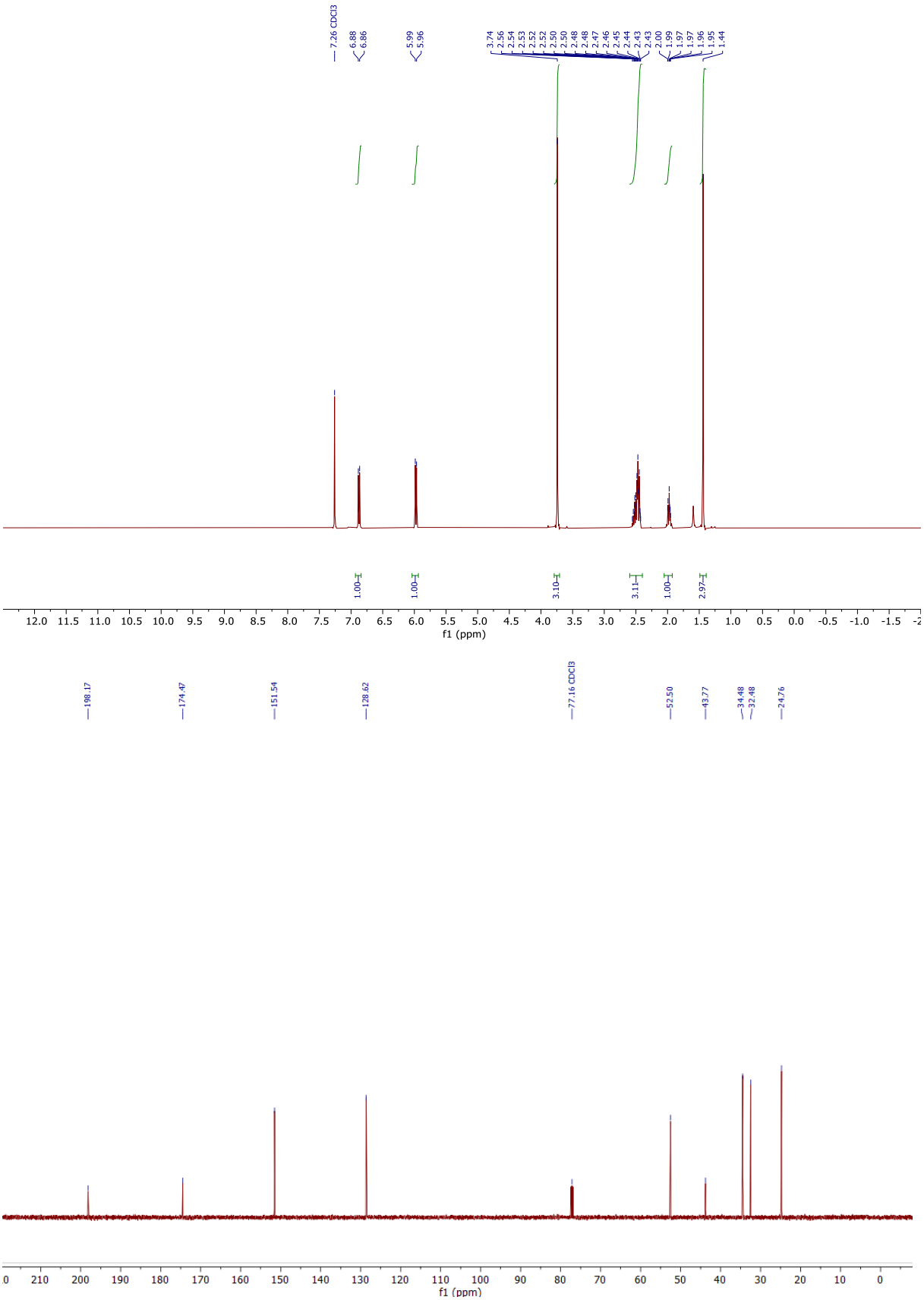
NMR Spectra



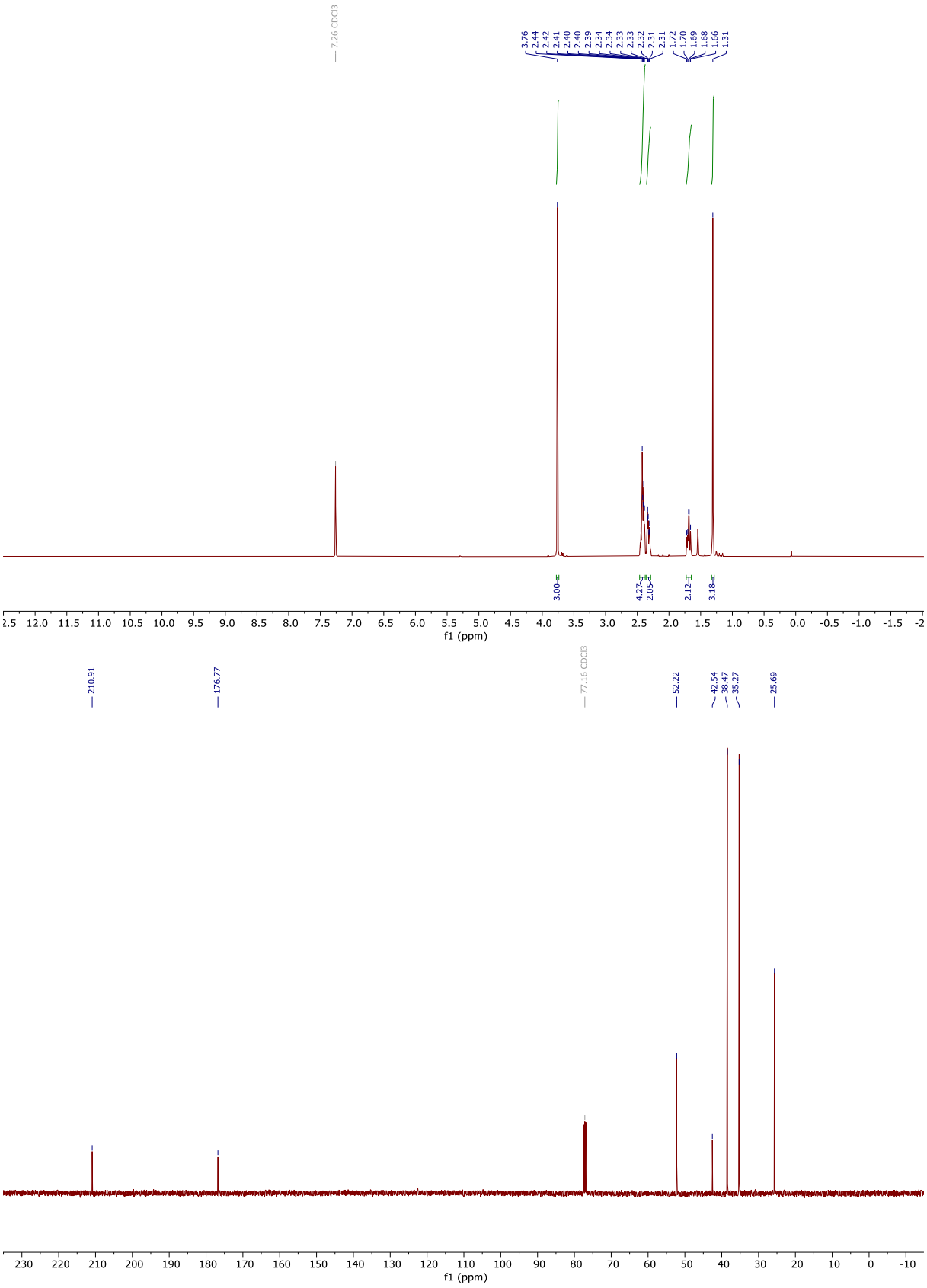
Supplementary Fig. 17. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **4,/4*R*, 4S** in $CDCl_3$



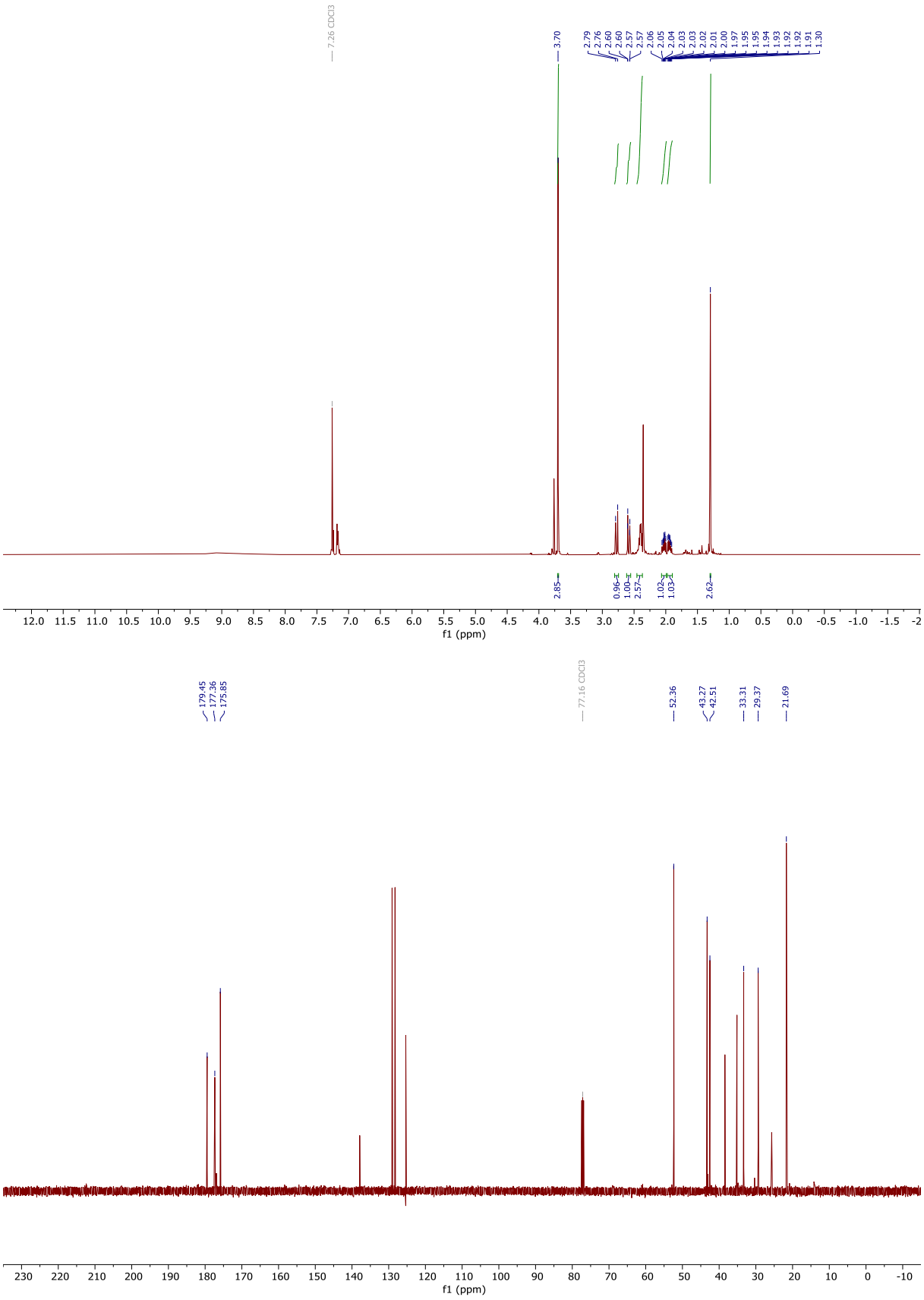
Supplementary Fig. 18. The ^{1}H-NMR (500 MHz) and APT-NMR (125 MHz) spectra of compound **5**, **5*R*/5*S*** in $CDCl_3$



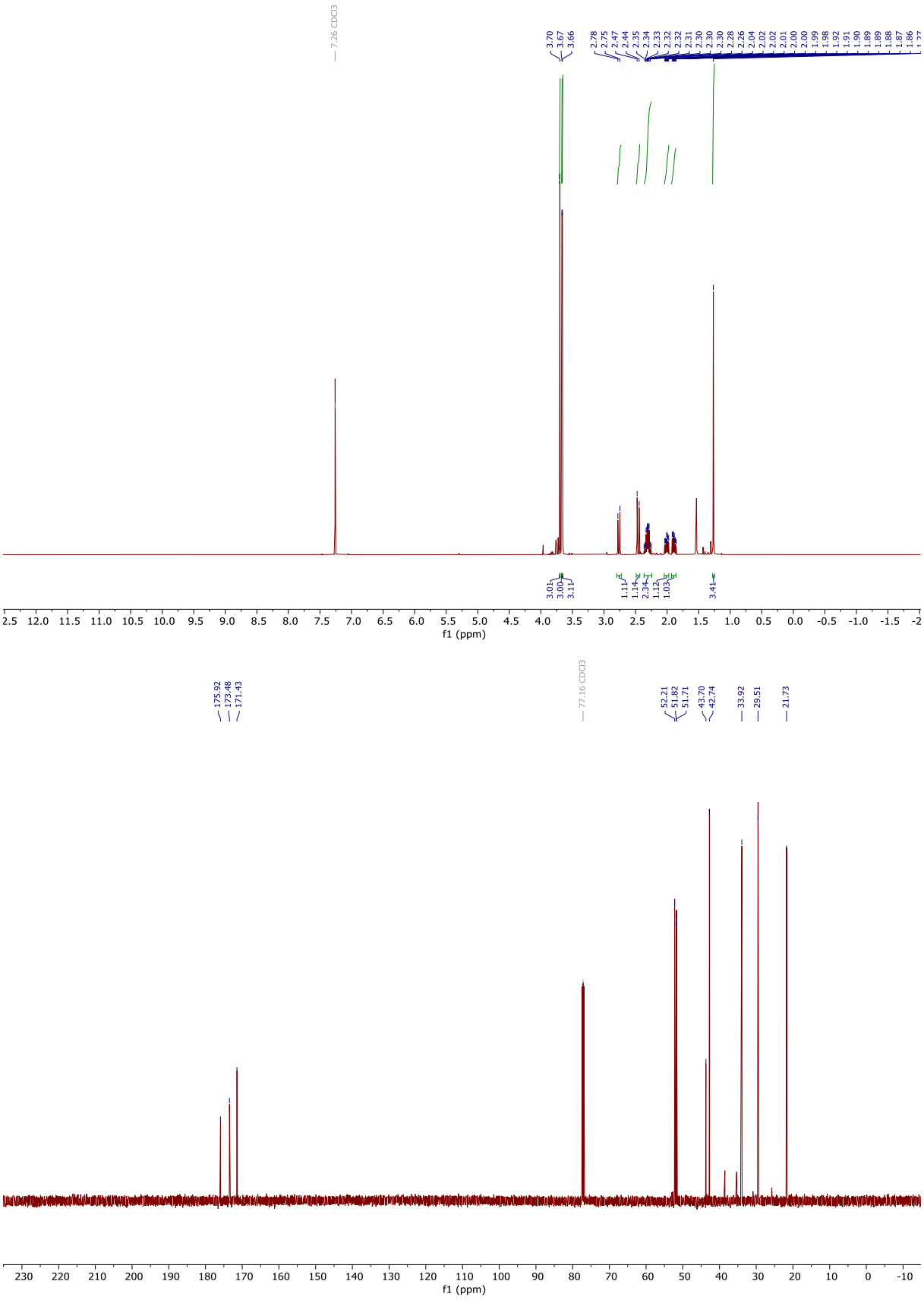
Supplementary Fig. 19. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S8** in $CDCl_3$



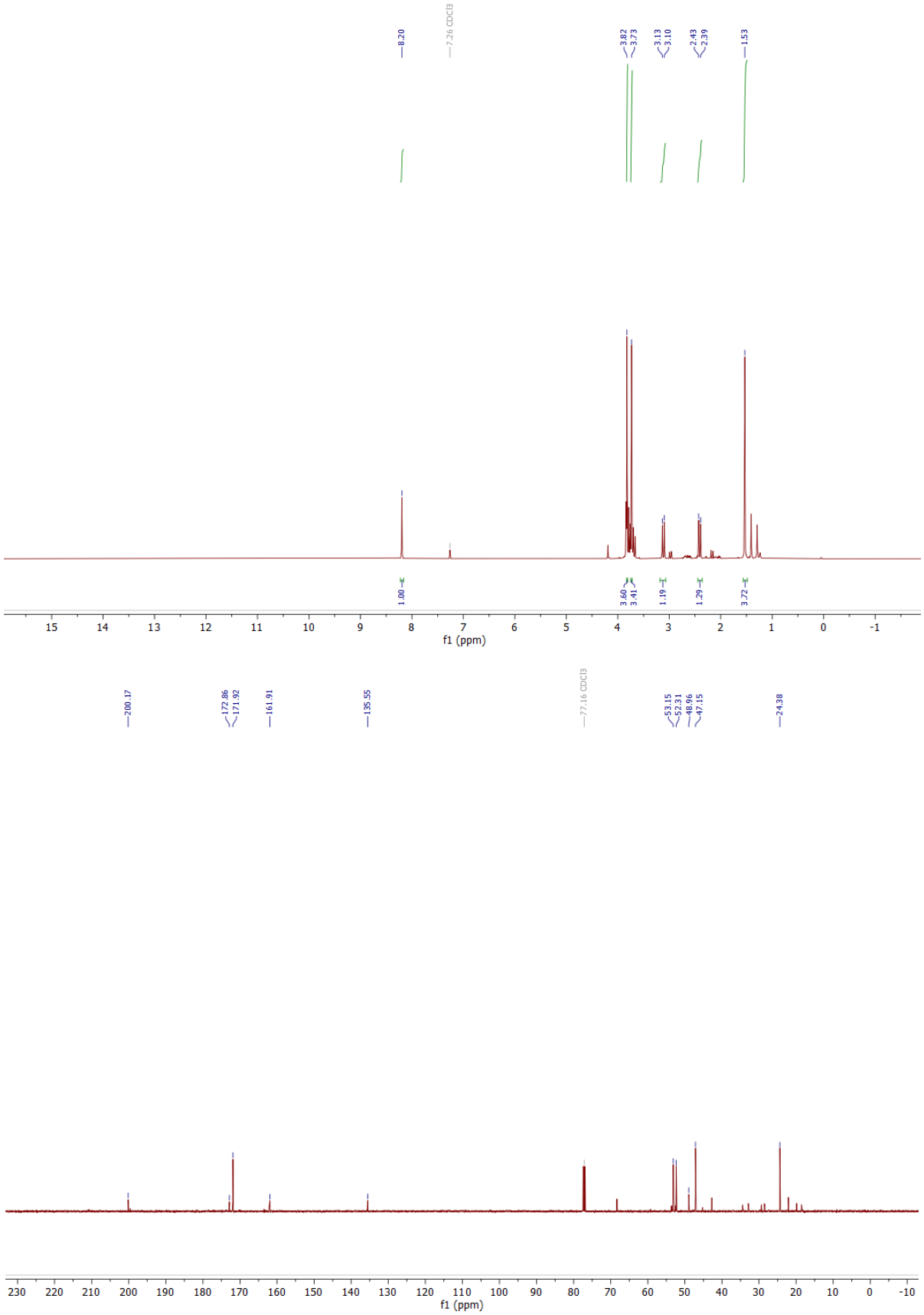
Supplementary Fig. 20. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S9** in $CDCl_3$



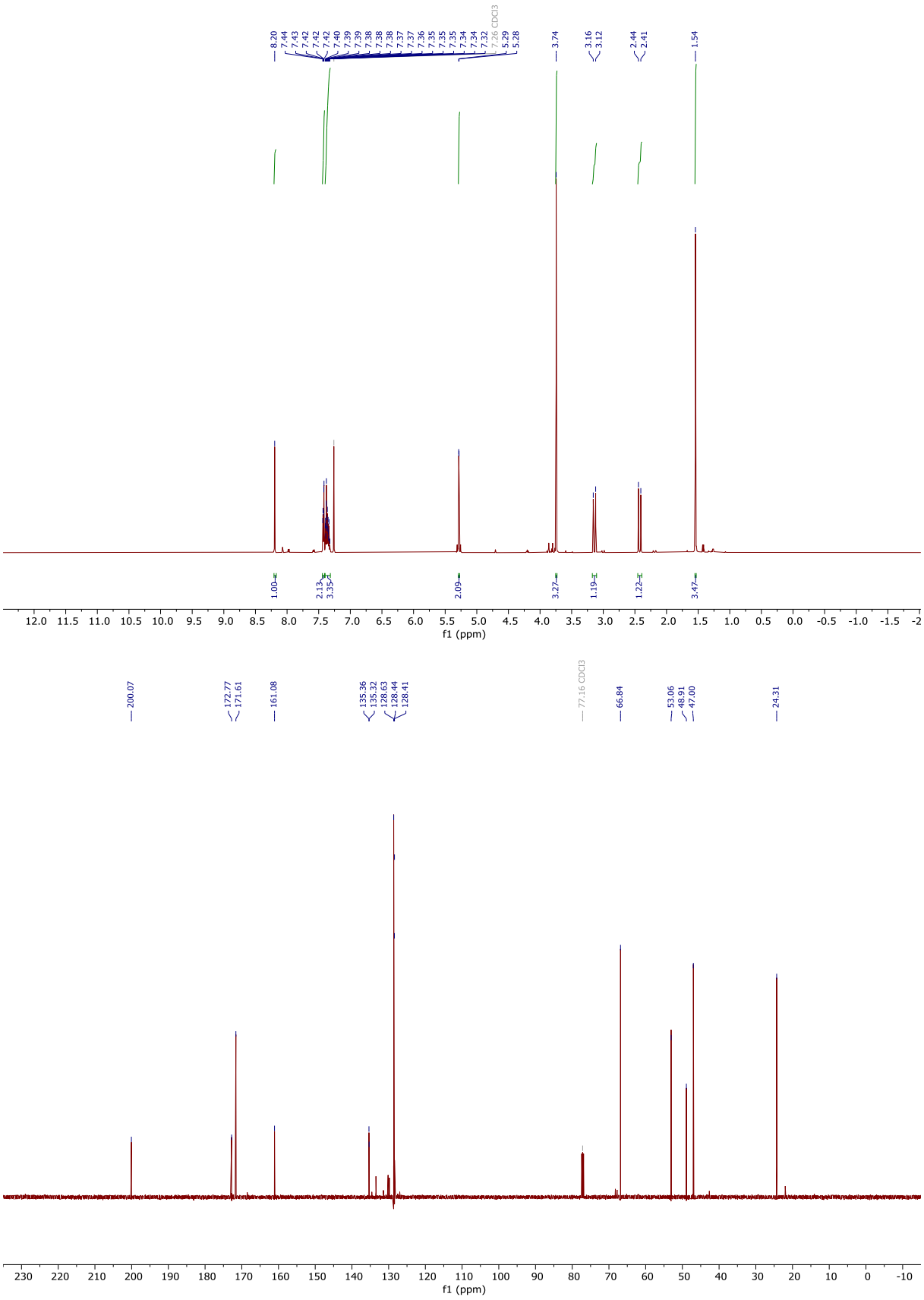
Supplementary Fig. 21. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S10** in $CDCl_3$



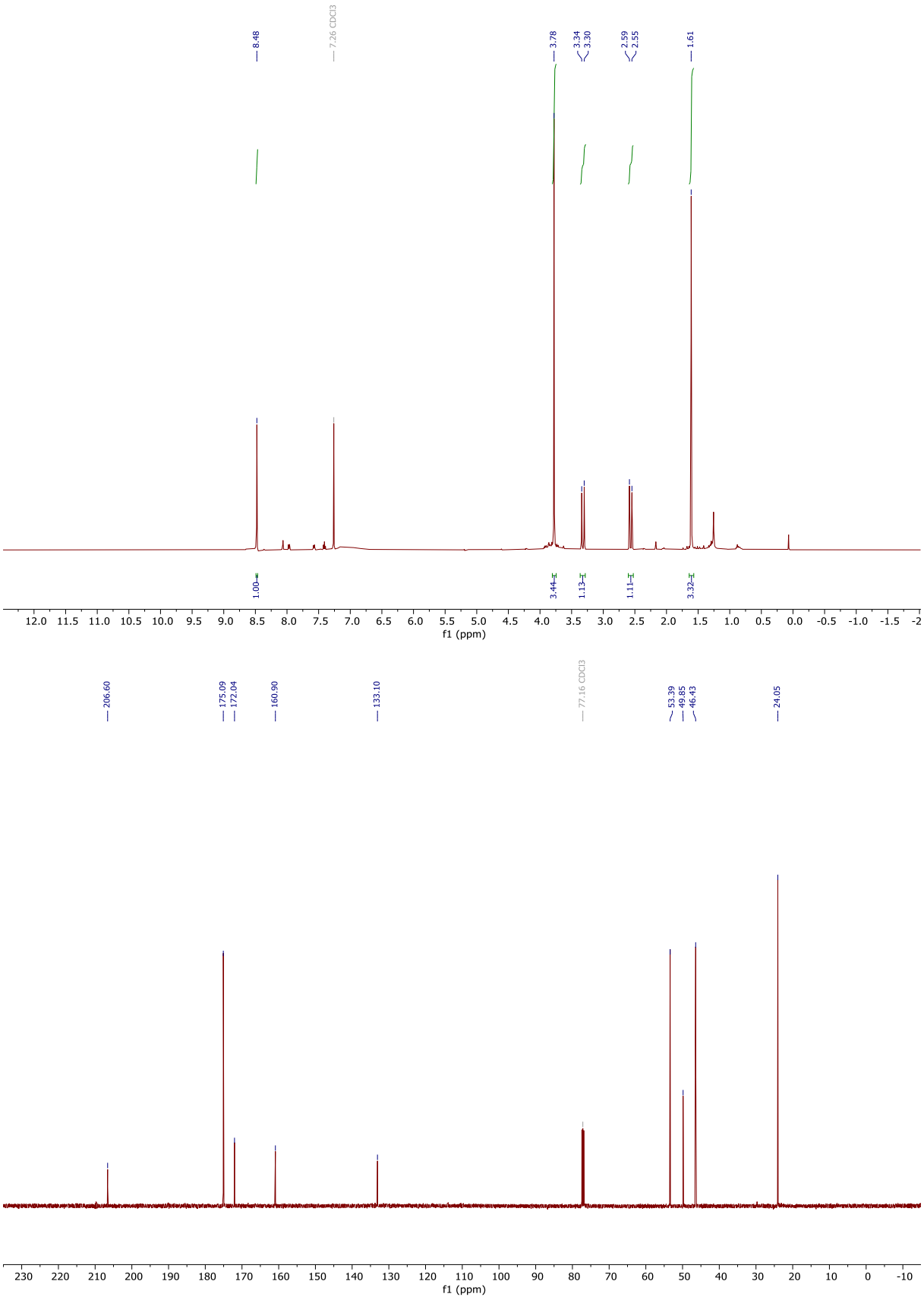
Supplementary Fig. 22. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S11** in $CDCl_3$



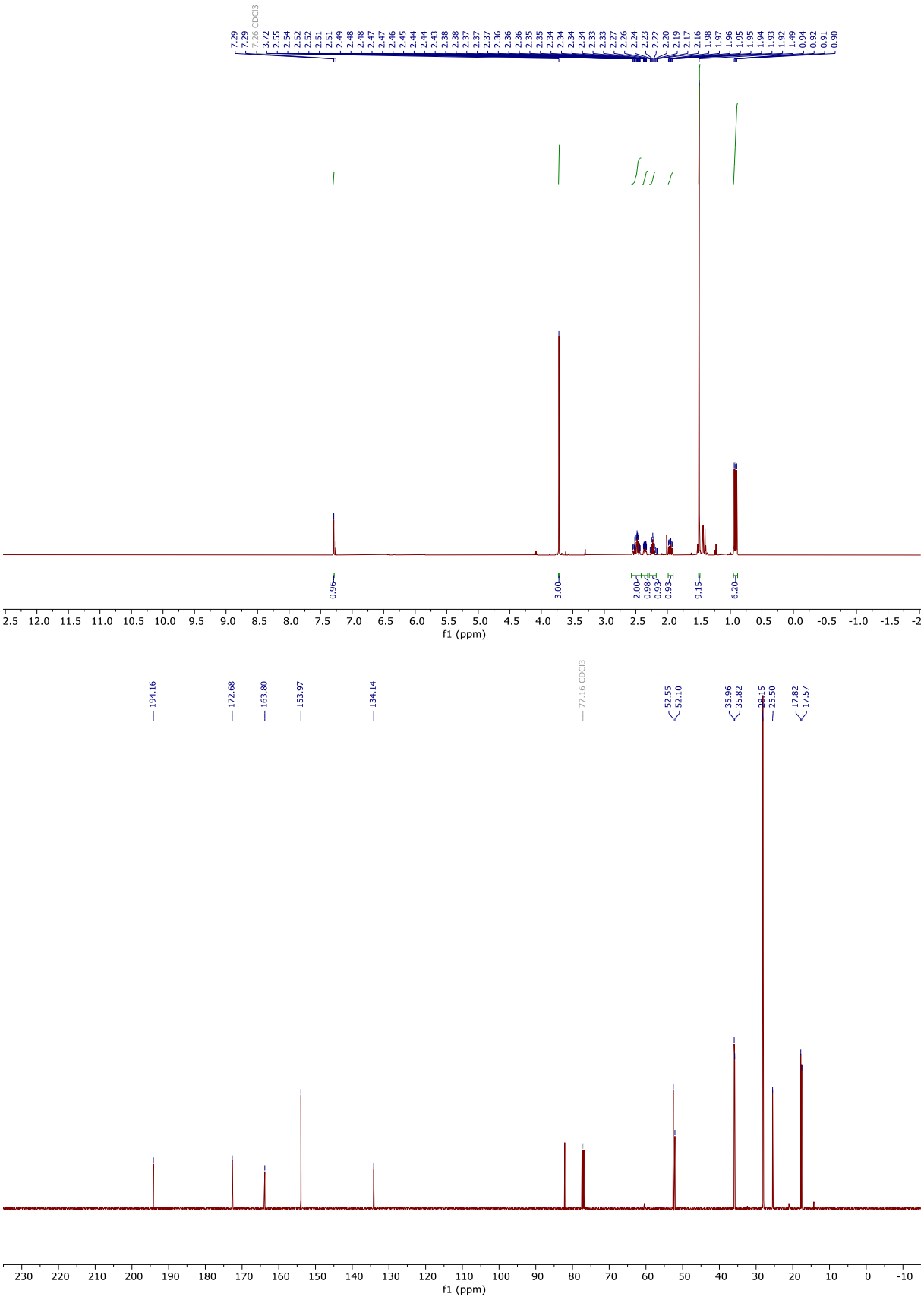
Supplementary Fig. 23. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S14** in $CDCl_3$



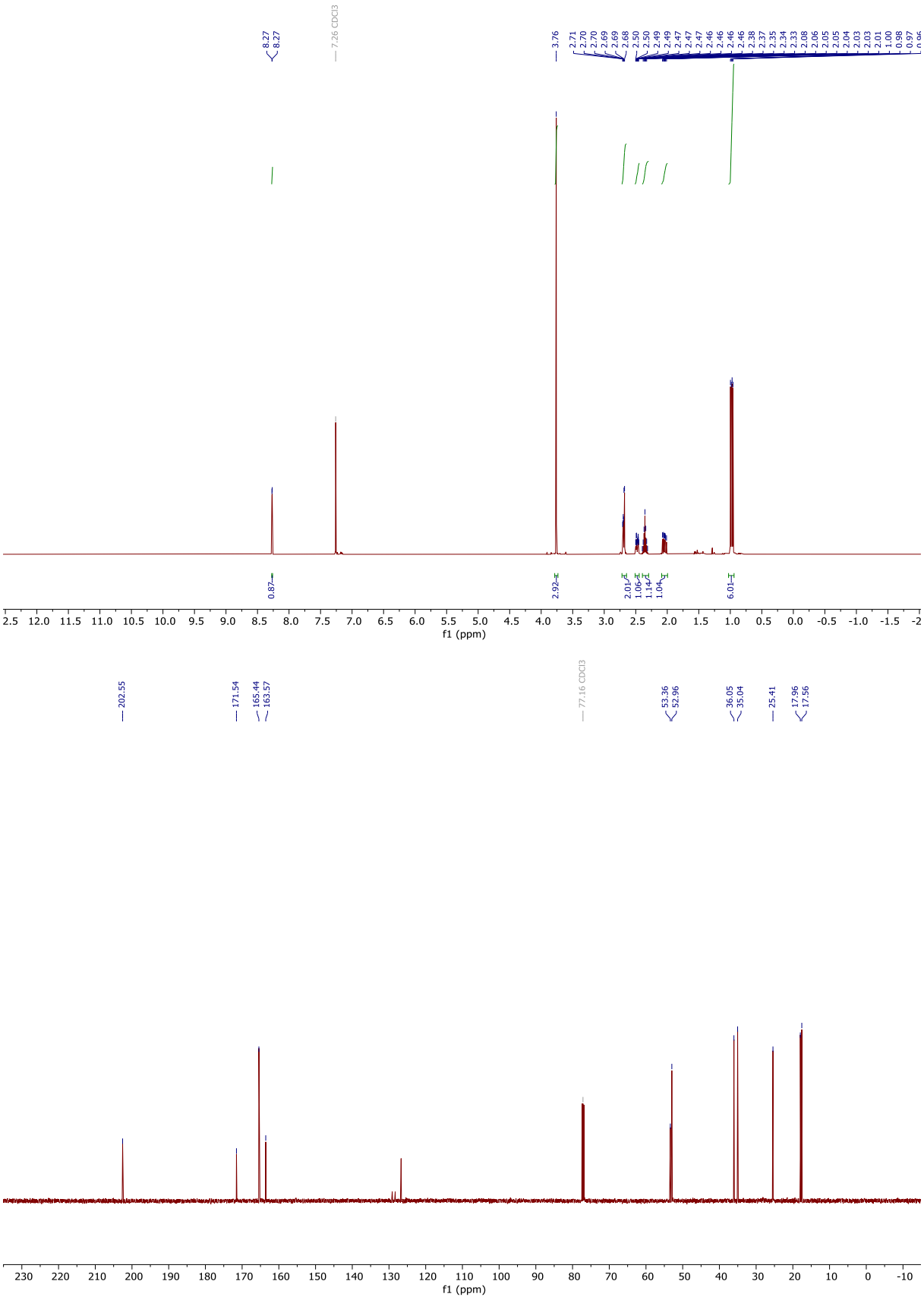
Supplementary Fig. 24. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S15** in $CDCl_3$



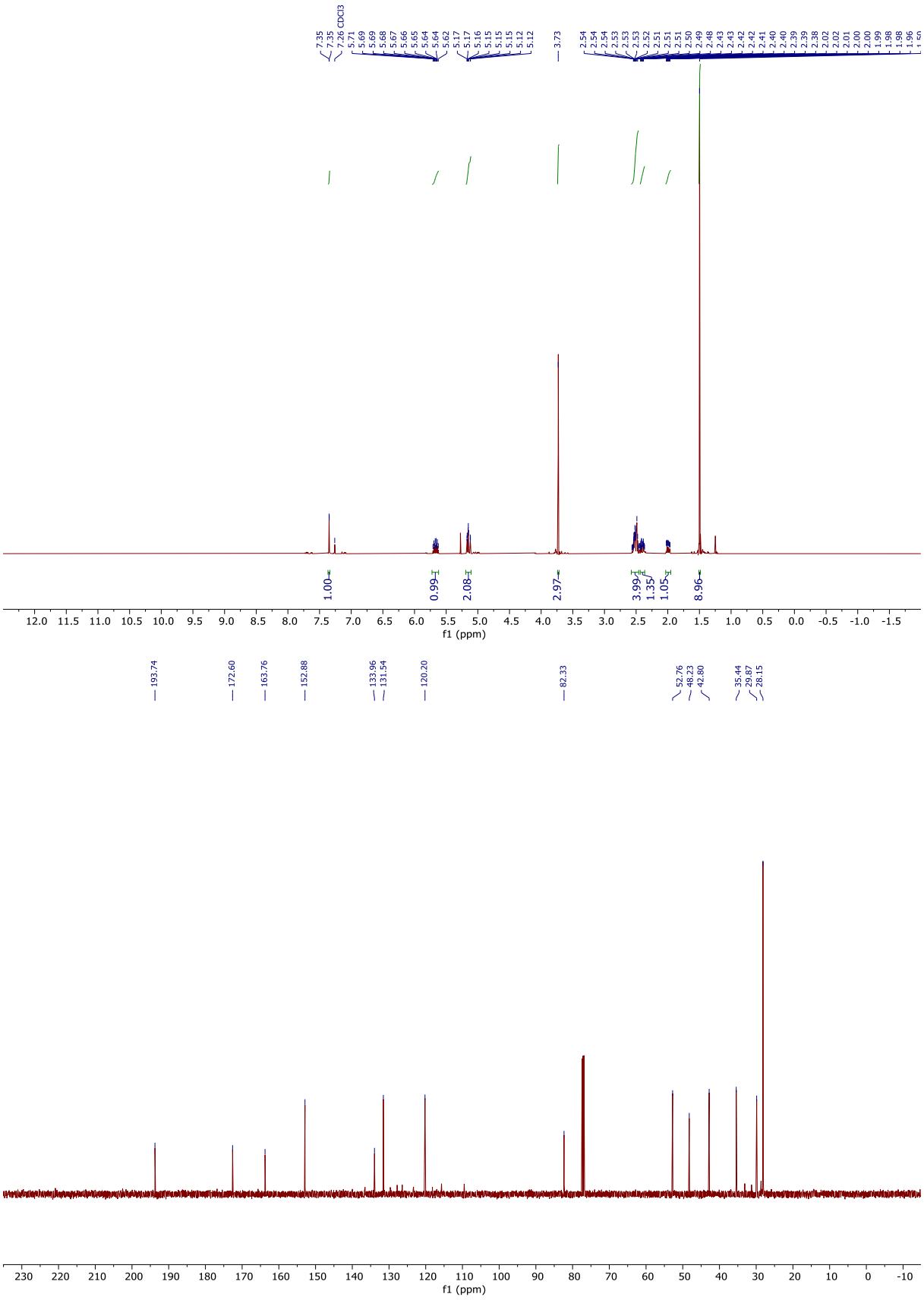
Supplementary Fig. 25. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S16** in $CDCl_3$



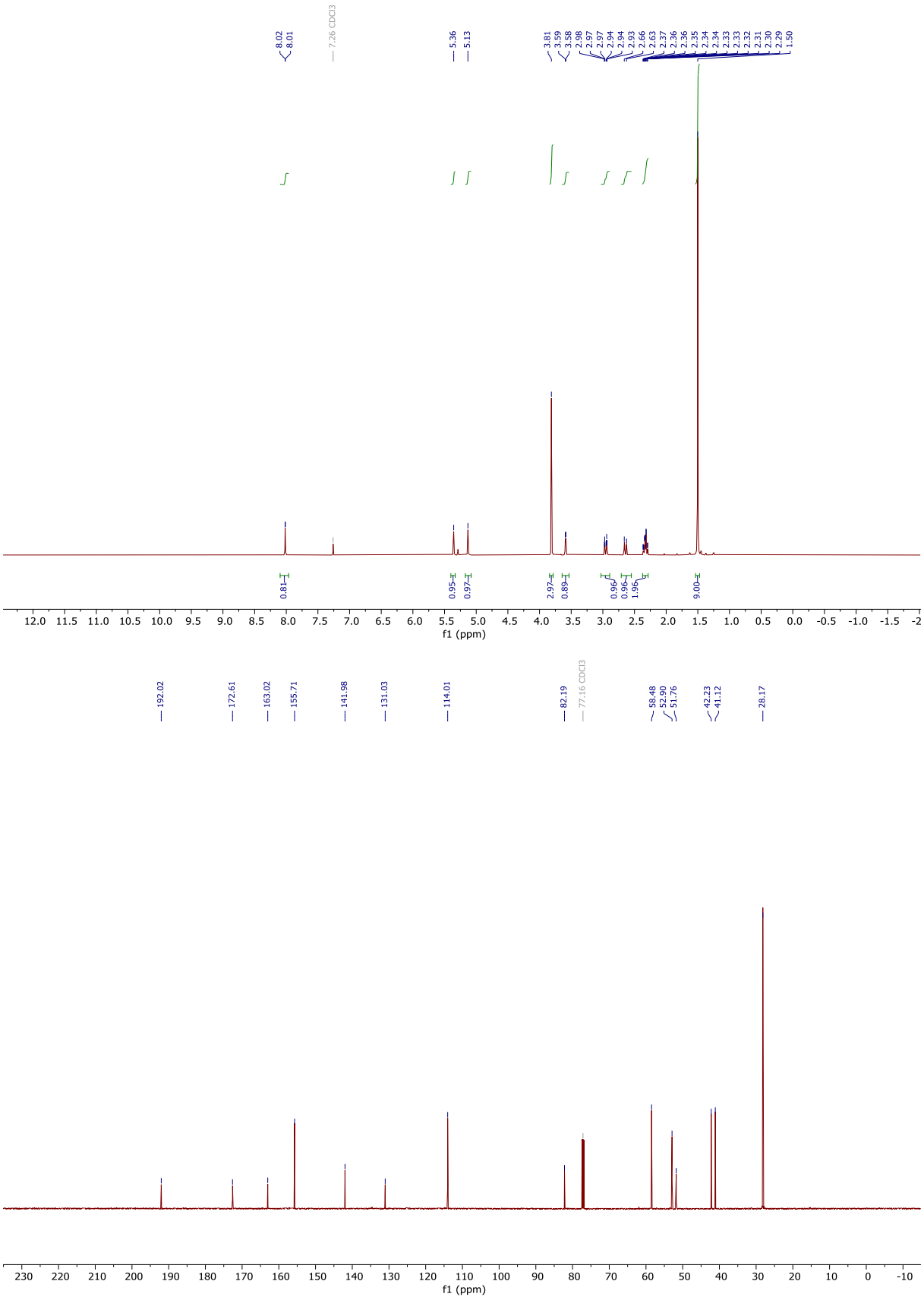
Supplementary Fig. 26. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S17*R***/**S17*S*** in $CDCl_3$



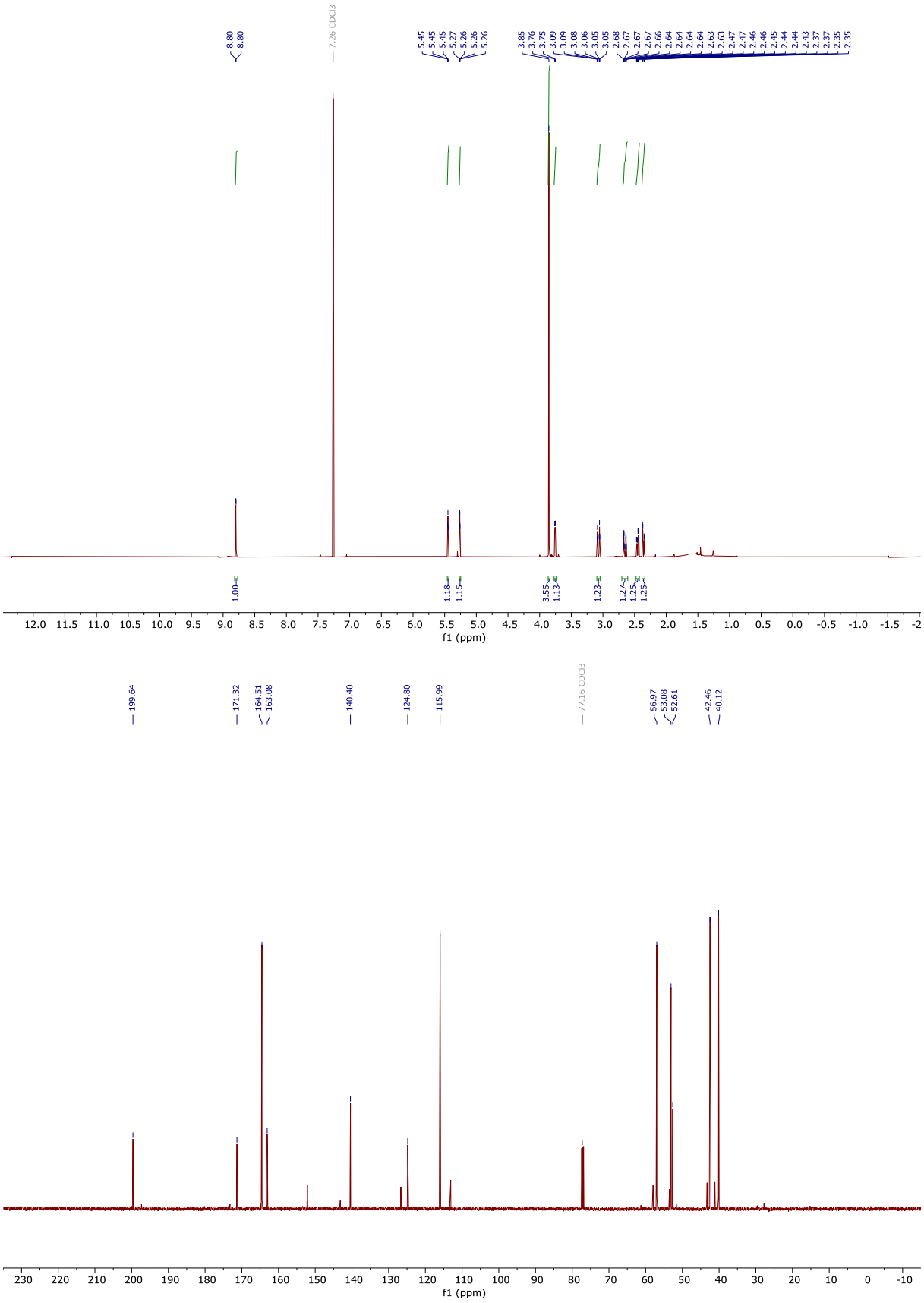
Supplementary Fig. 27. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S18*R***/**S18*S*** in $CDCl_3$



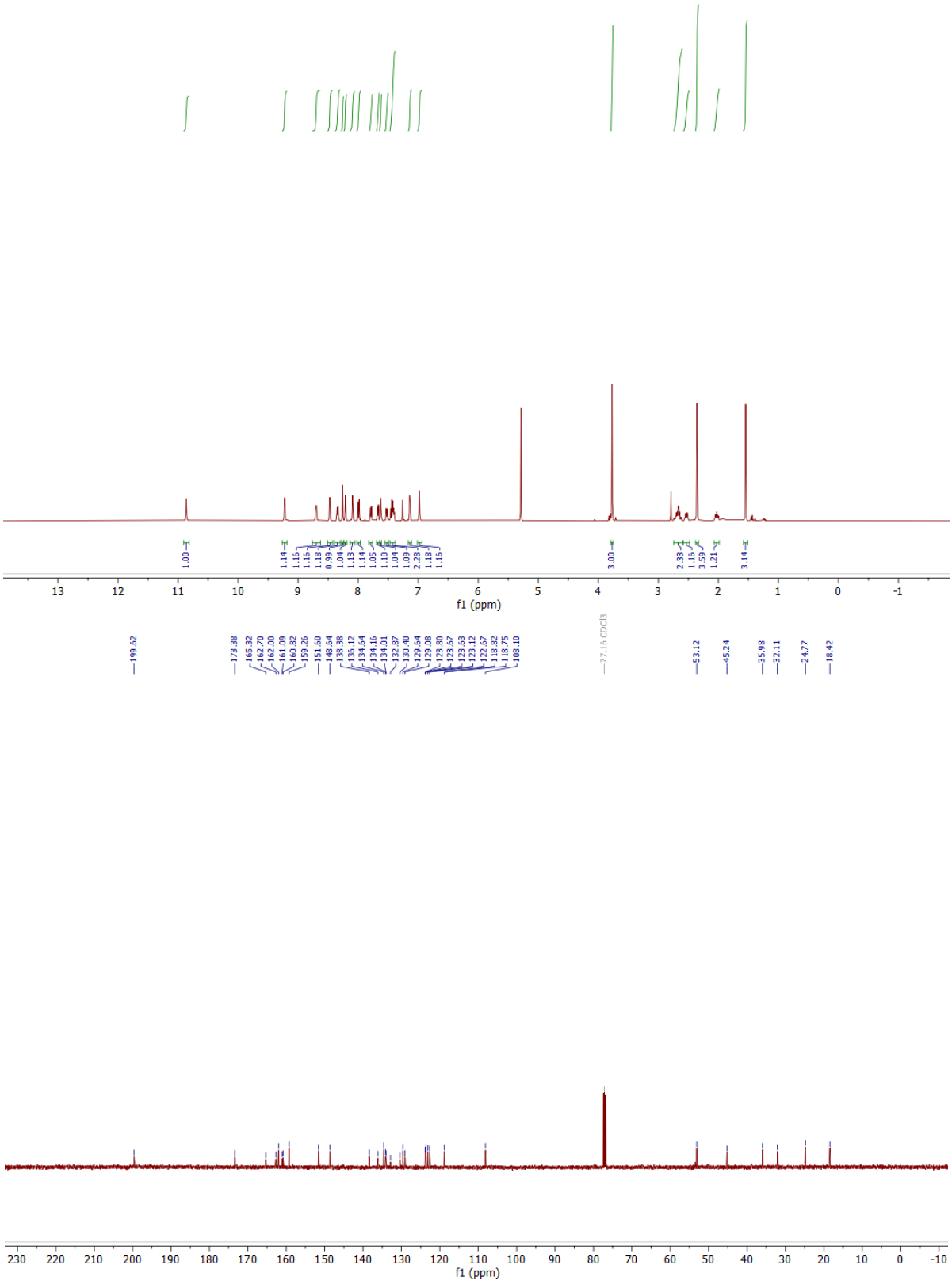
Supplementary Fig. 28. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S19*R*/S19*S*** in $CDCl_3$



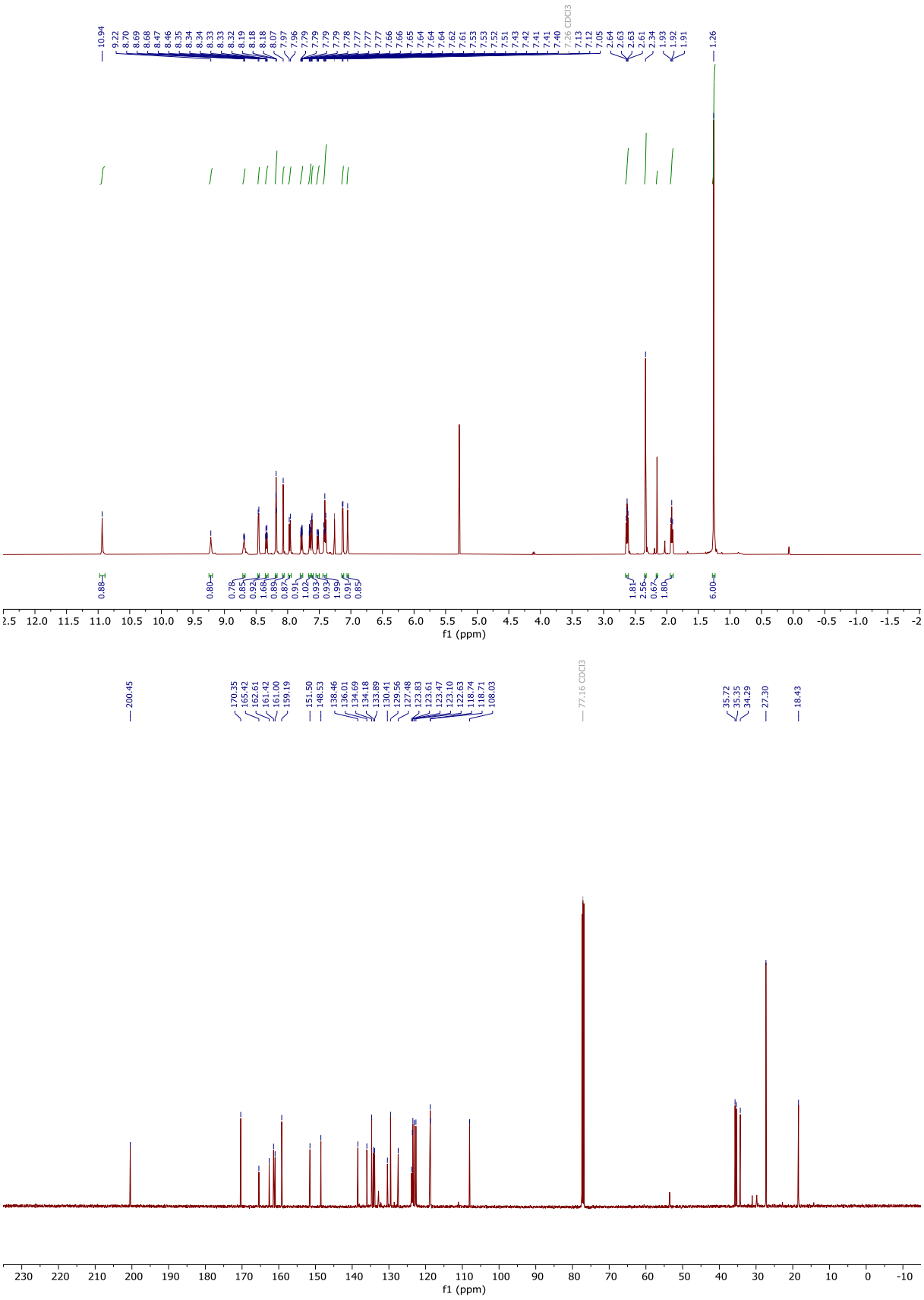
Supplementary Fig. 29. The ^{1}H-NMR (500 MHz) and APT-NMR (125 MHz) spectra of compound **S20*R*,*R*/S20*S*,*S*** in $CDCl_3$



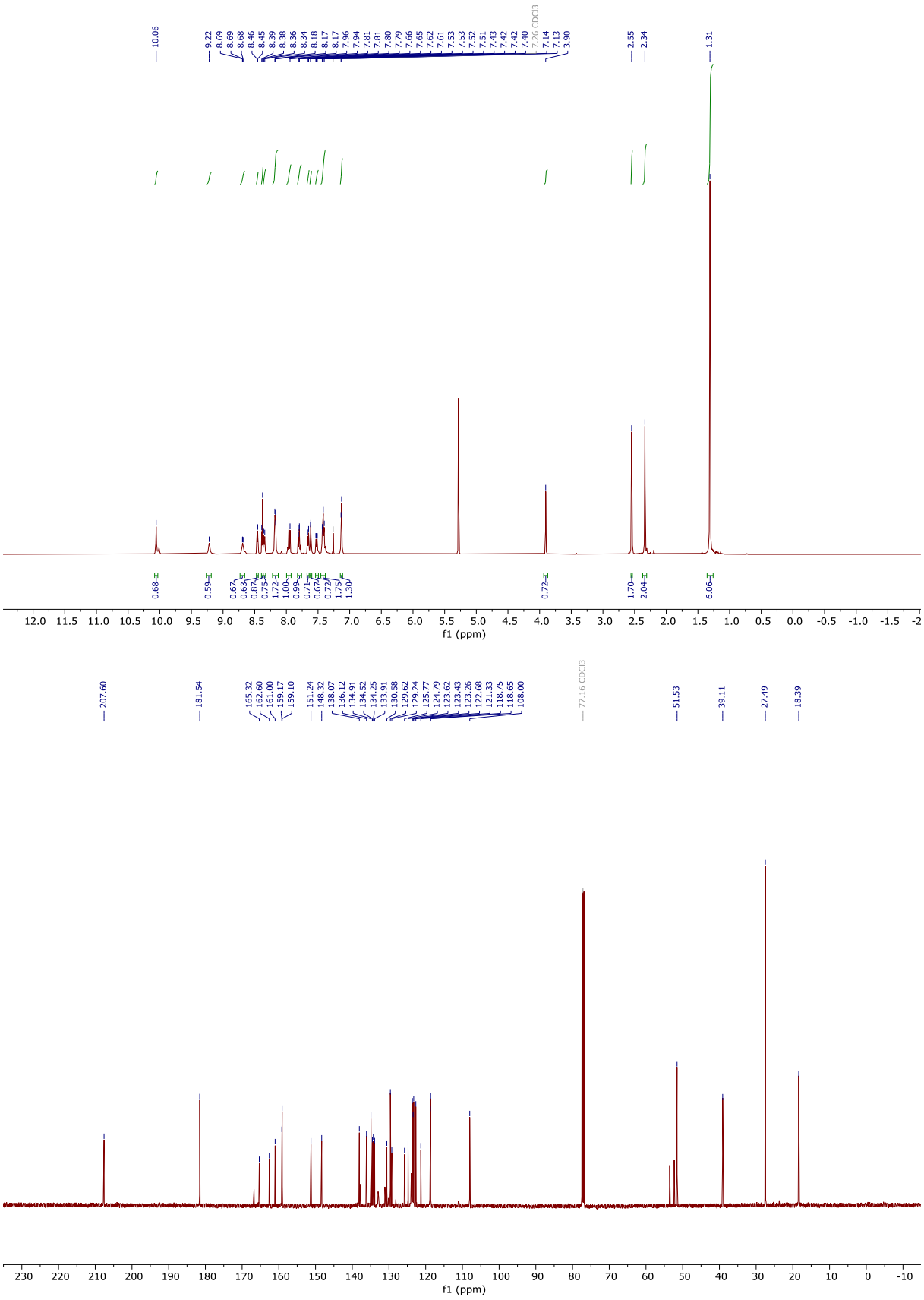
Supplementary Fig. 30. The ^{1}H-NMR (500 MHz) and APT-NMR (125 MHz) spectra of compound **S21*R*,*R*/S21*S*,*S*** in $CDCl_3$



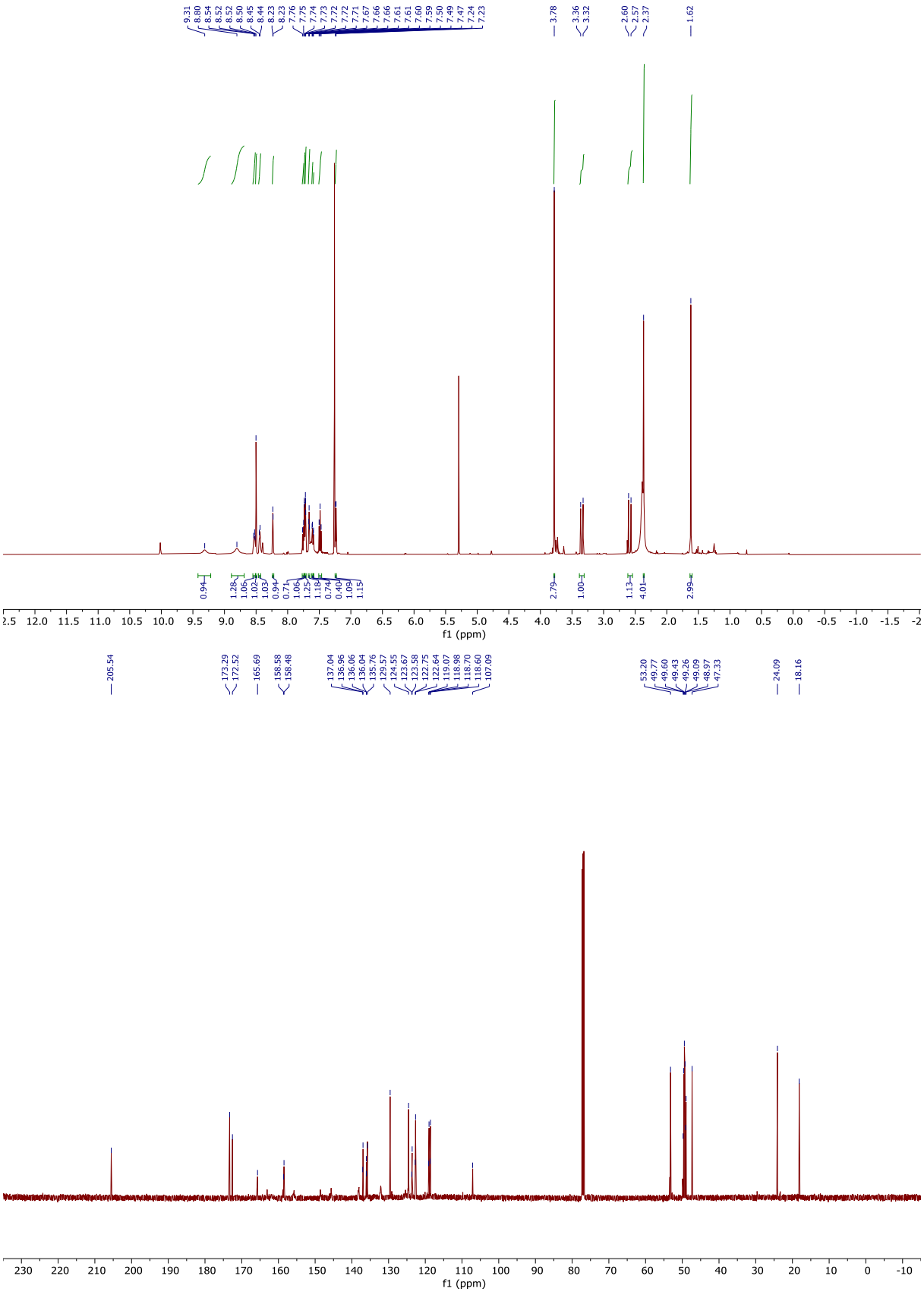
Supplementary Fig. 31. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1a*R*-IN-8**/**1a*S*-IN-8** in $CDCl_3$



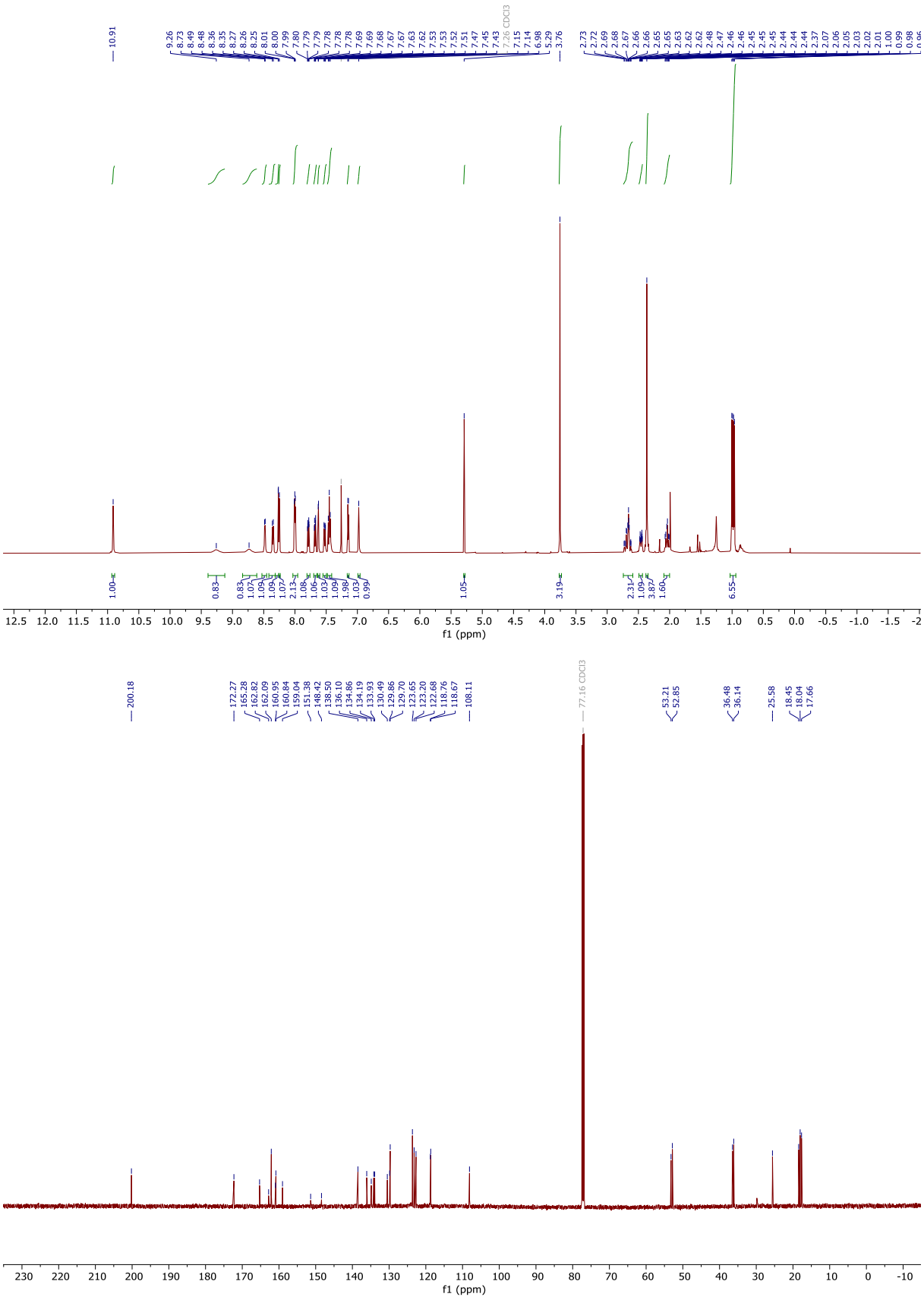
Supplementary Fig. 32. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **2-IN-8** in $CDCl_3$



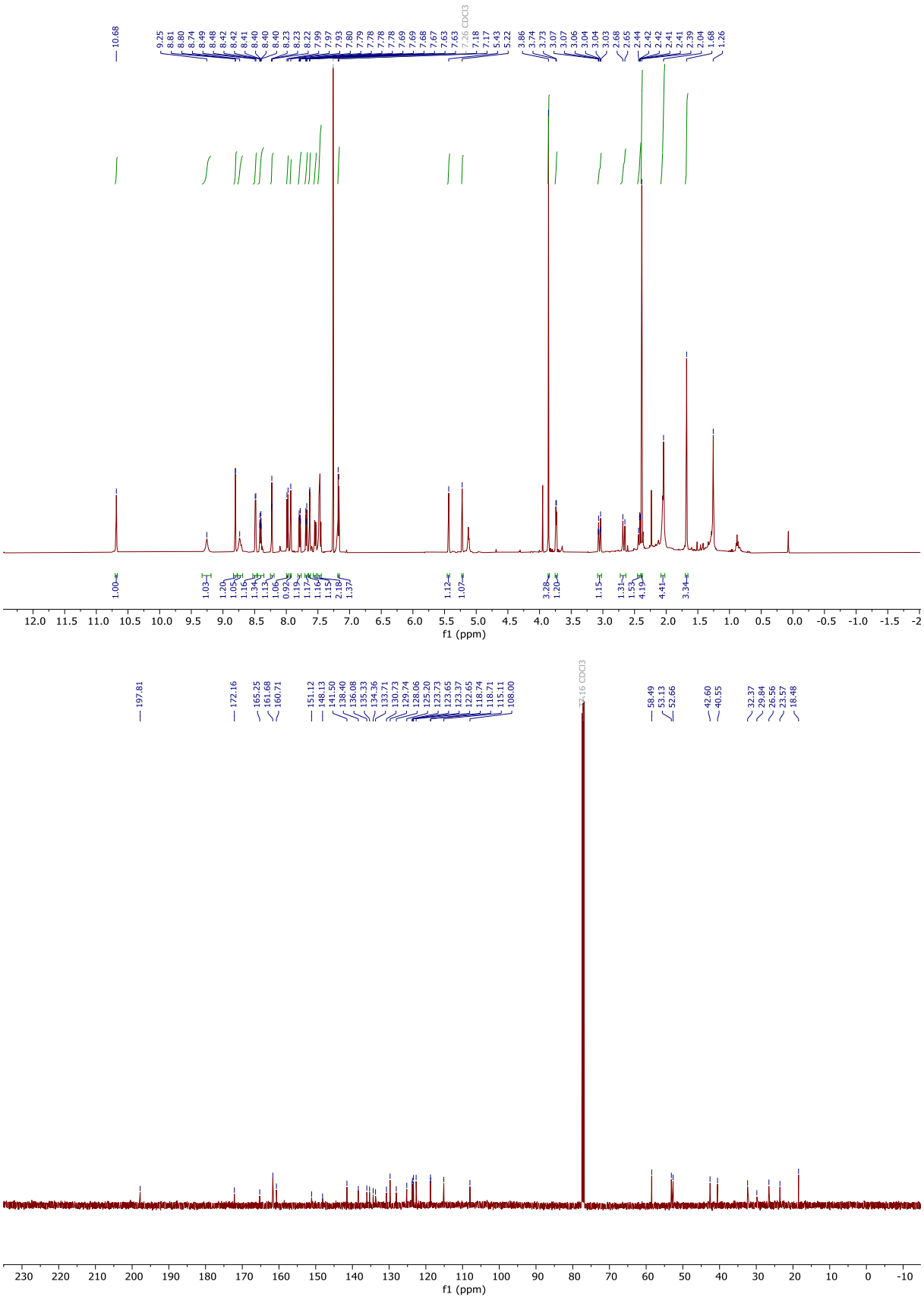
Supplementary Fig. 33. The ^{1}H-NMR (500 MHz) and APT-NMR (125 MHz) spectra of compound **3-IN-8** in $CDCl_3$



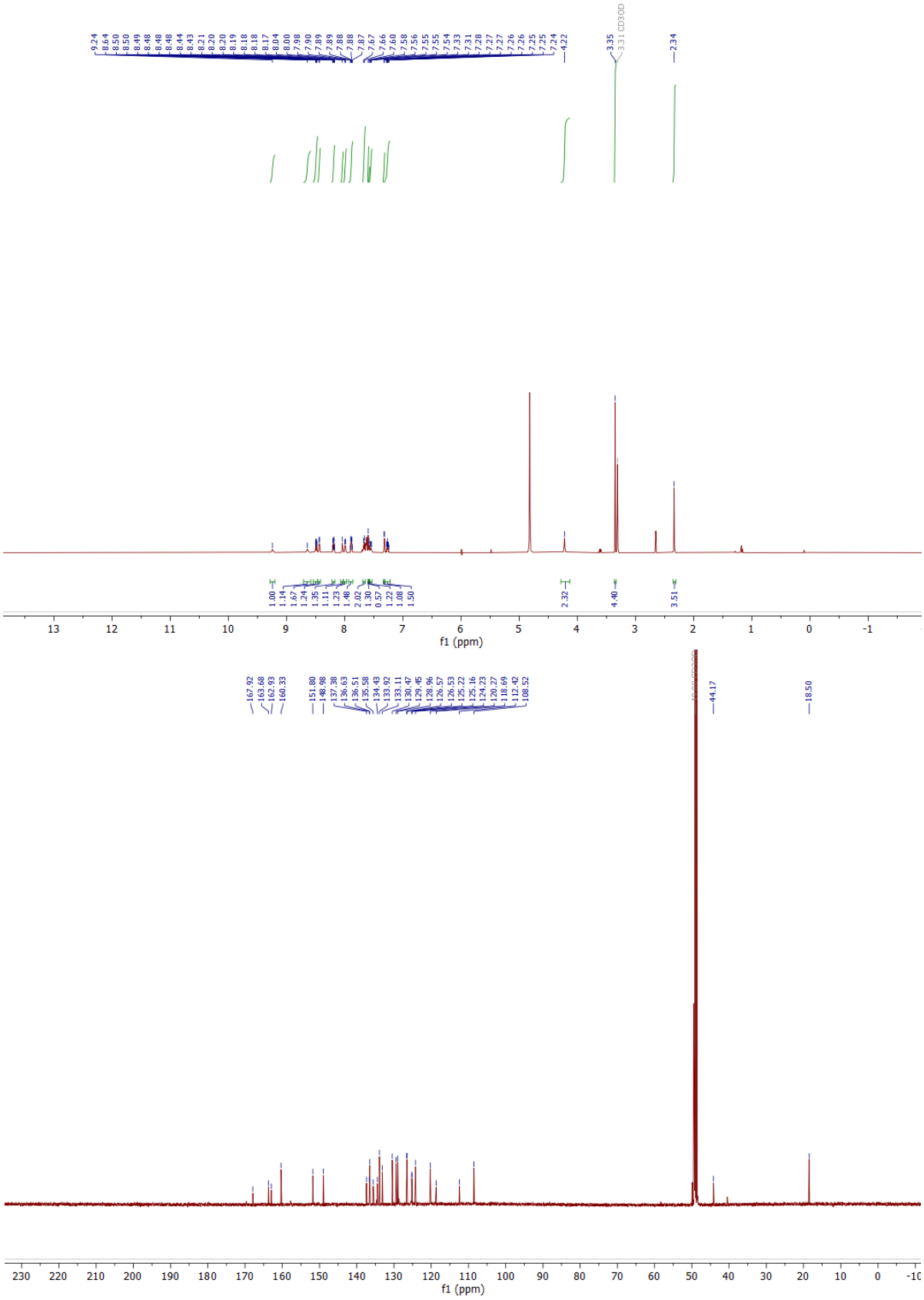
Supplementary Fig. 34. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **4-IN-8** in $CDCl_3$



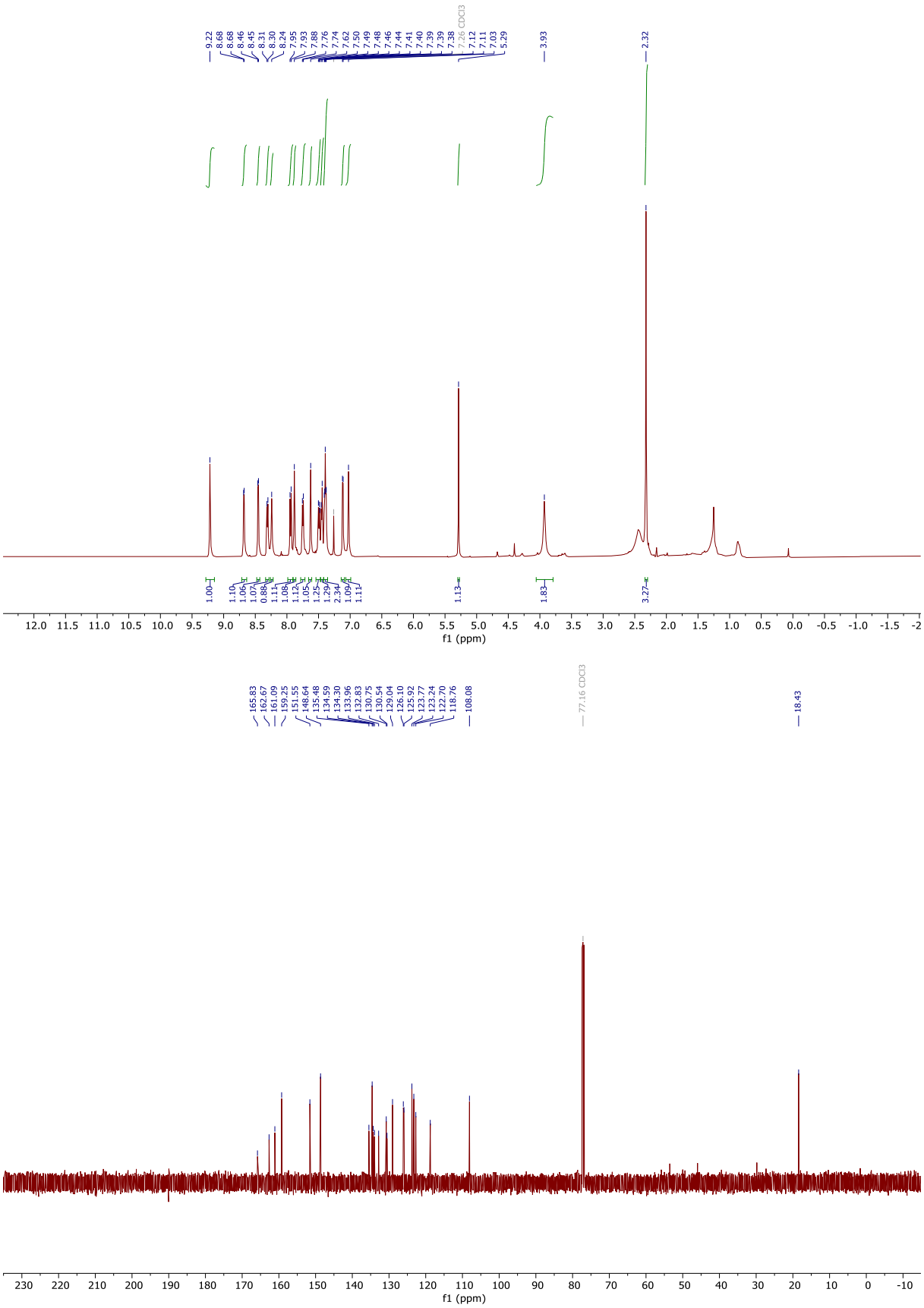
Supplementary Fig. 35. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **5*R*-IN-8**/**5*S*-IN-8** in $CDCl_3$



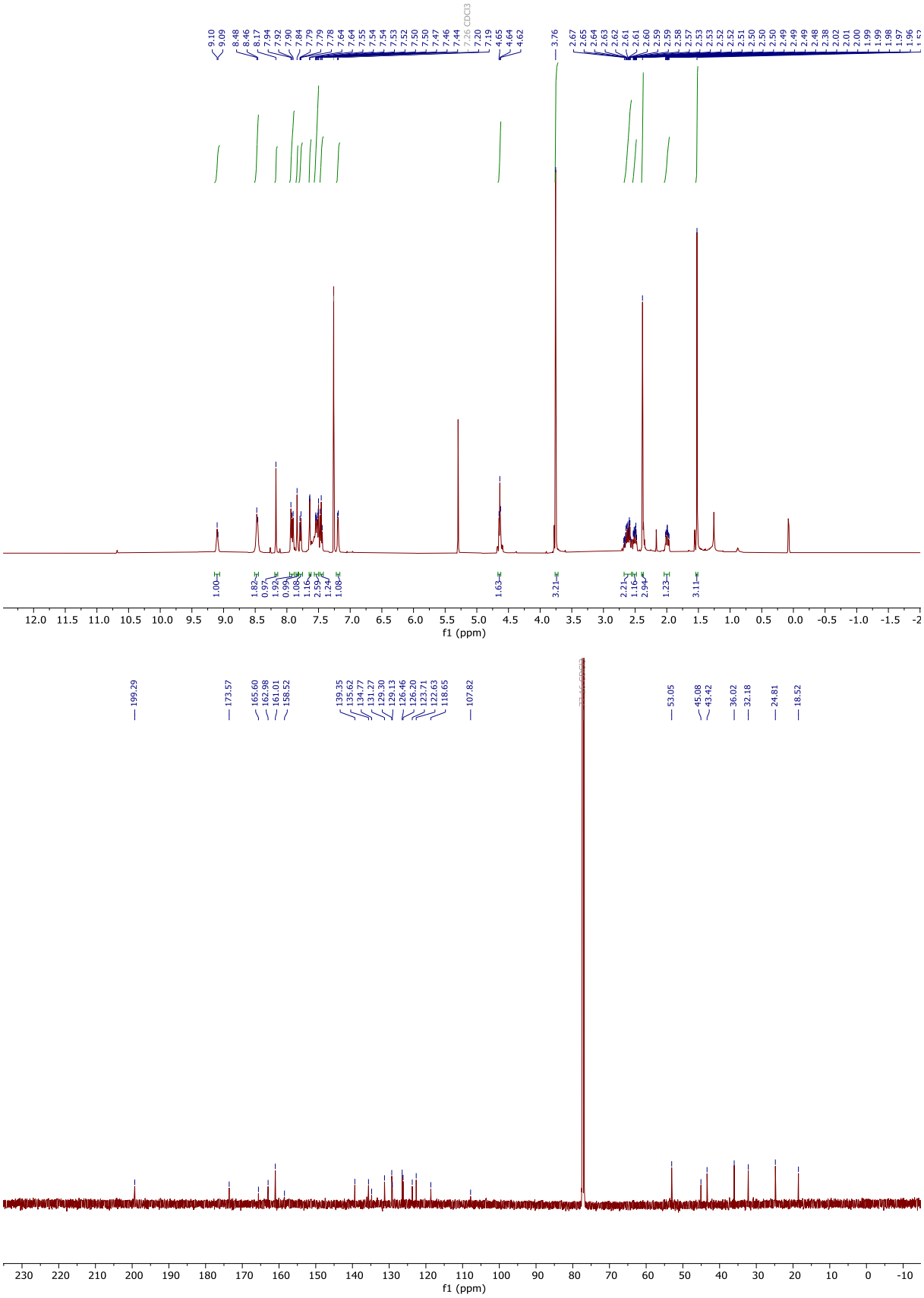
Supplementary Fig. 36. The ^{1}H-NMR (500 MHz) and APT-NMR (125 MHz) spectra of compound **6*S*,*S*-IN-8**/**6*R*,*R*-IN-8** in $CDCl_3$



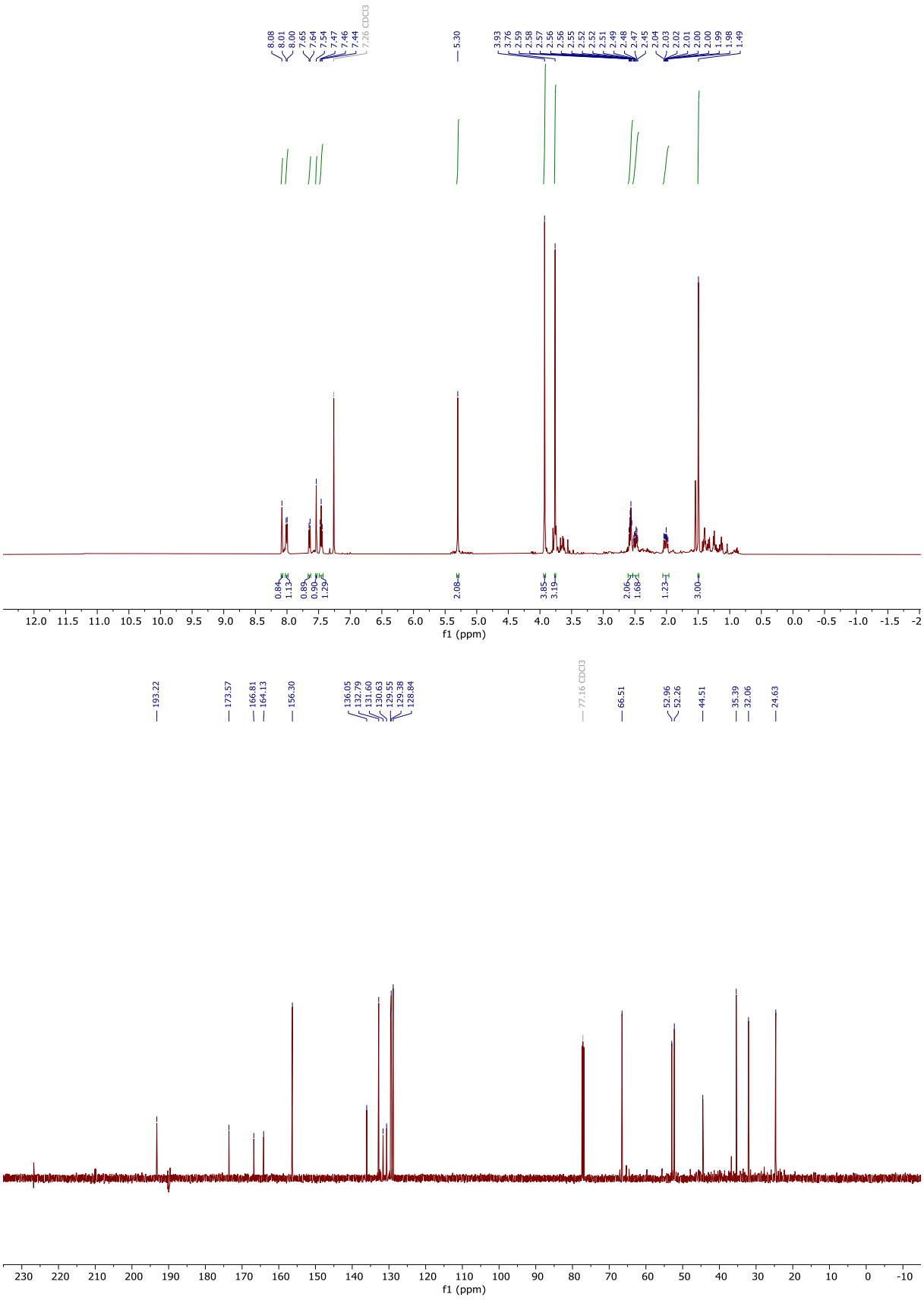
Supplementary Fig. 37. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S26** in CD_3OD



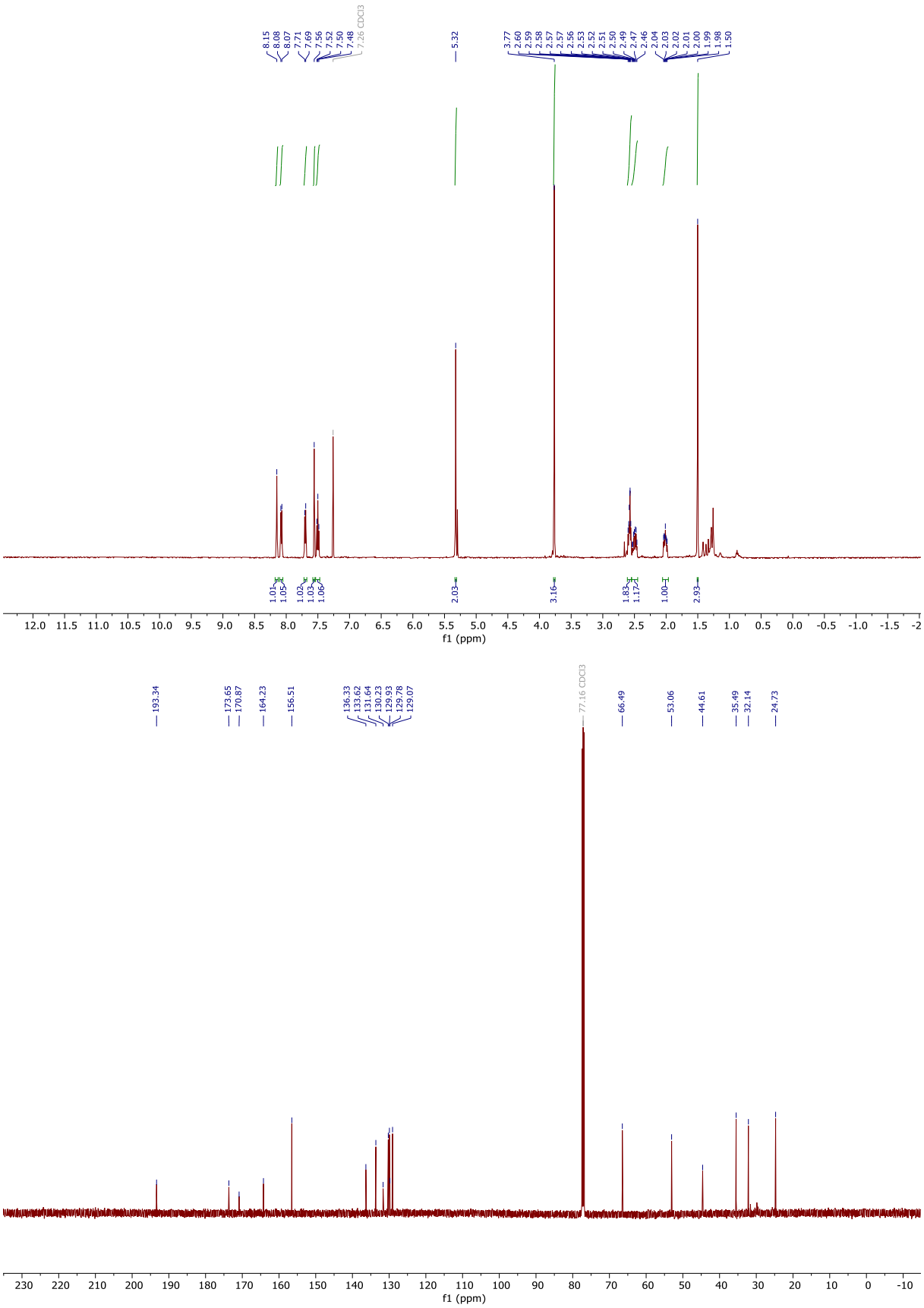
Supplementary Fig. 38. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S27** in $CDCl_3$



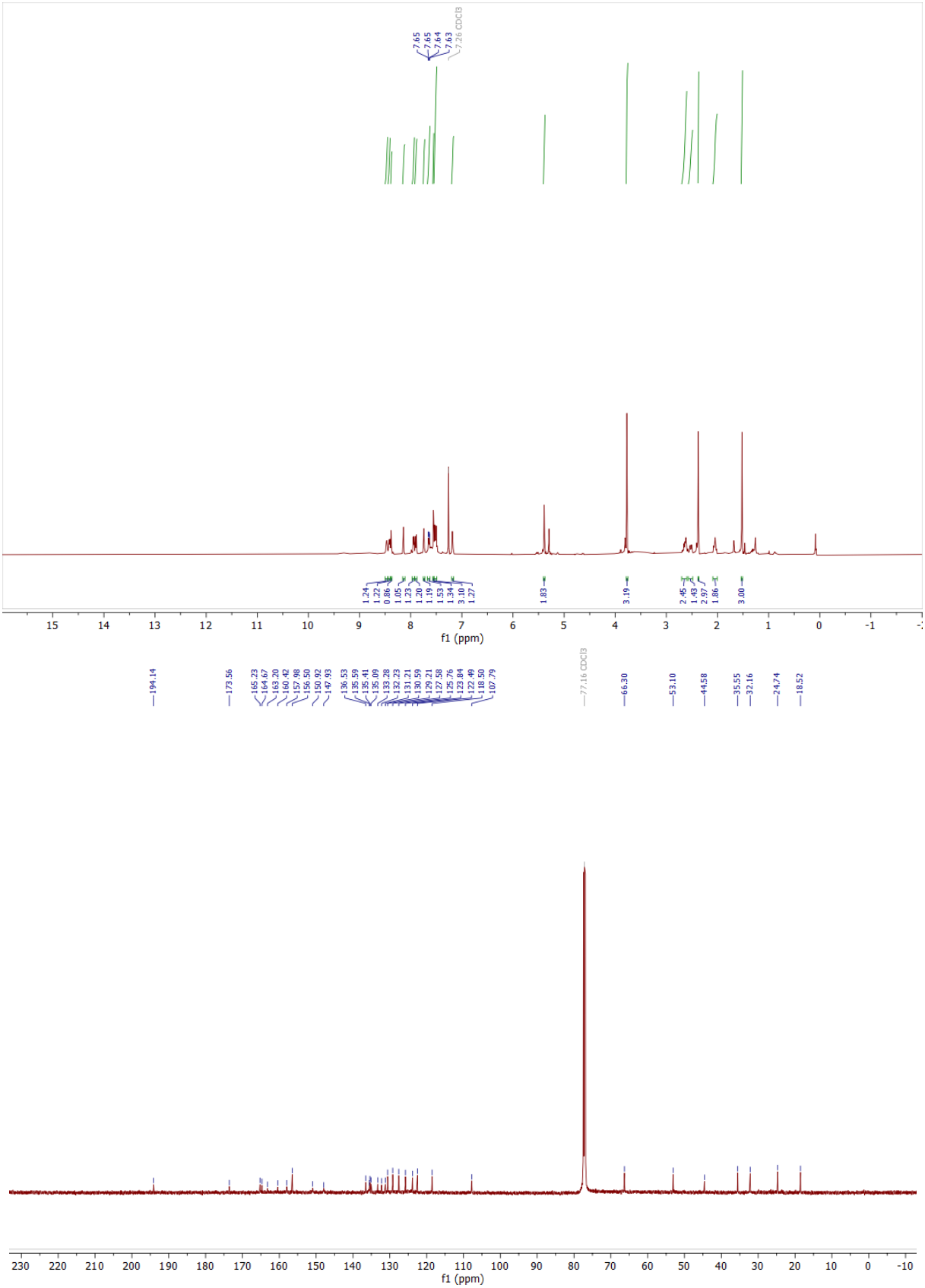
Supplementary Fig. 39. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1a'*R*-IN-8** in $CDCl_3$



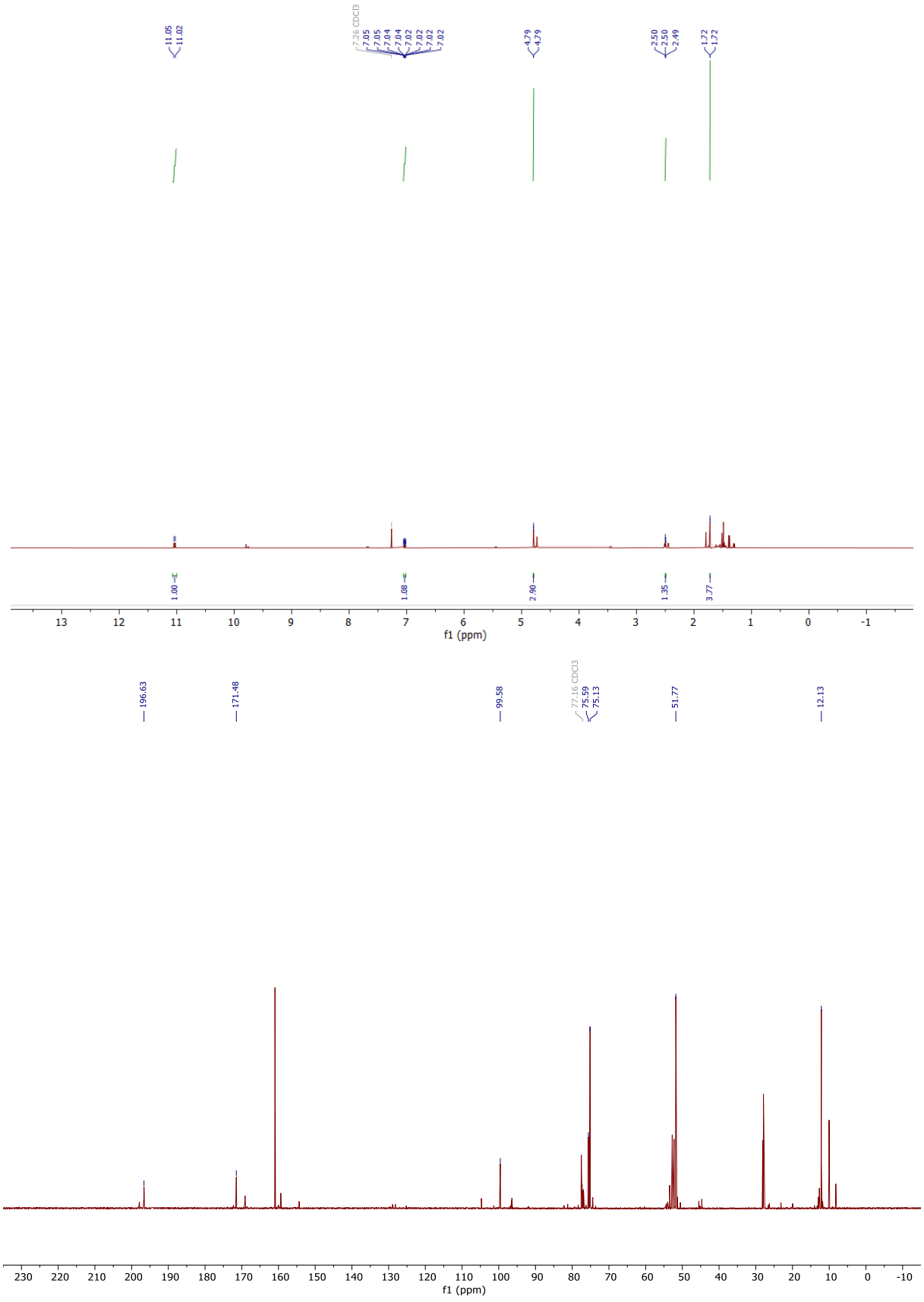
Supplementary Fig. 40. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S29** in $CDCl_3$



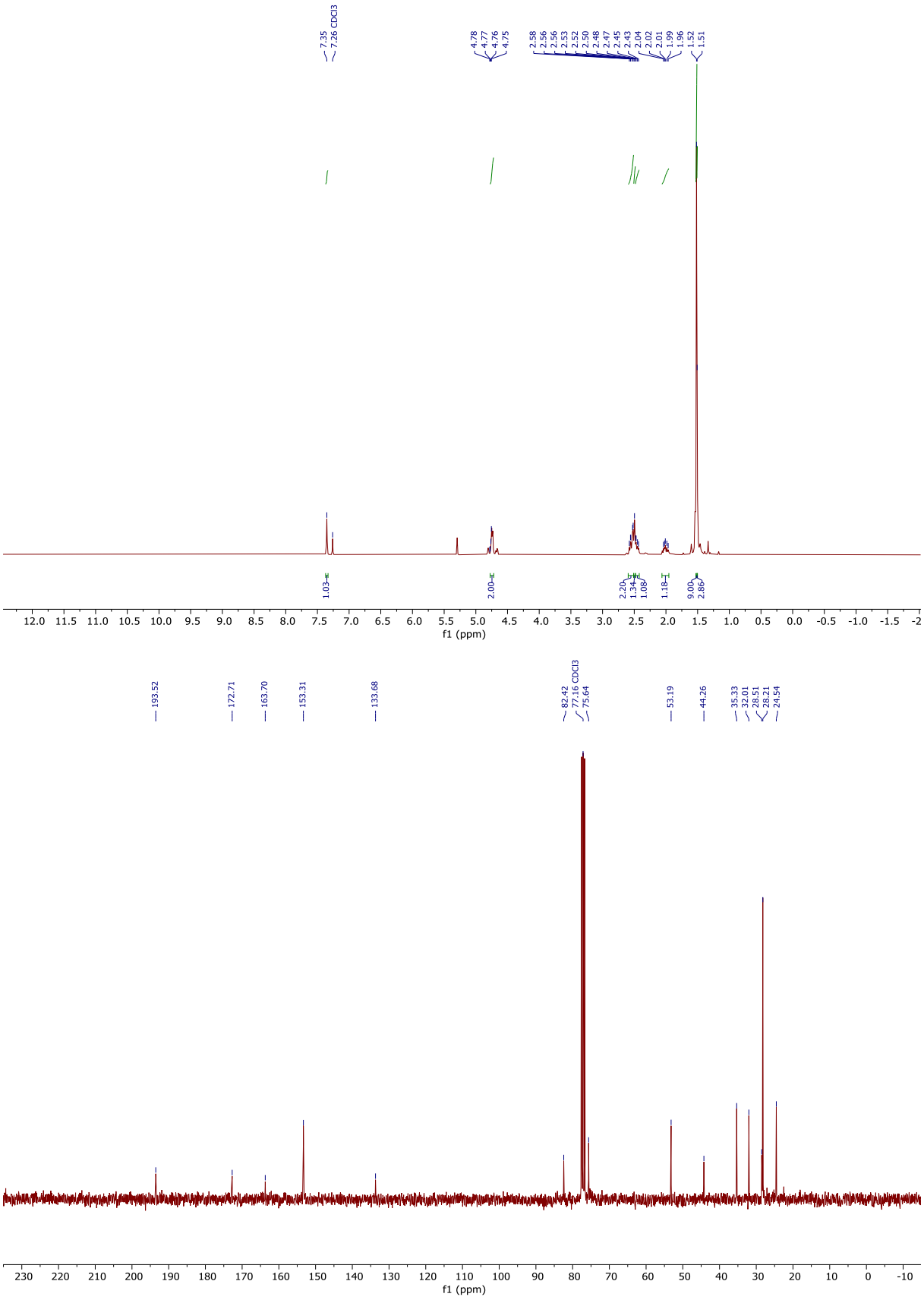
Supplementary Fig. 41. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S30** in $CDCl_3$



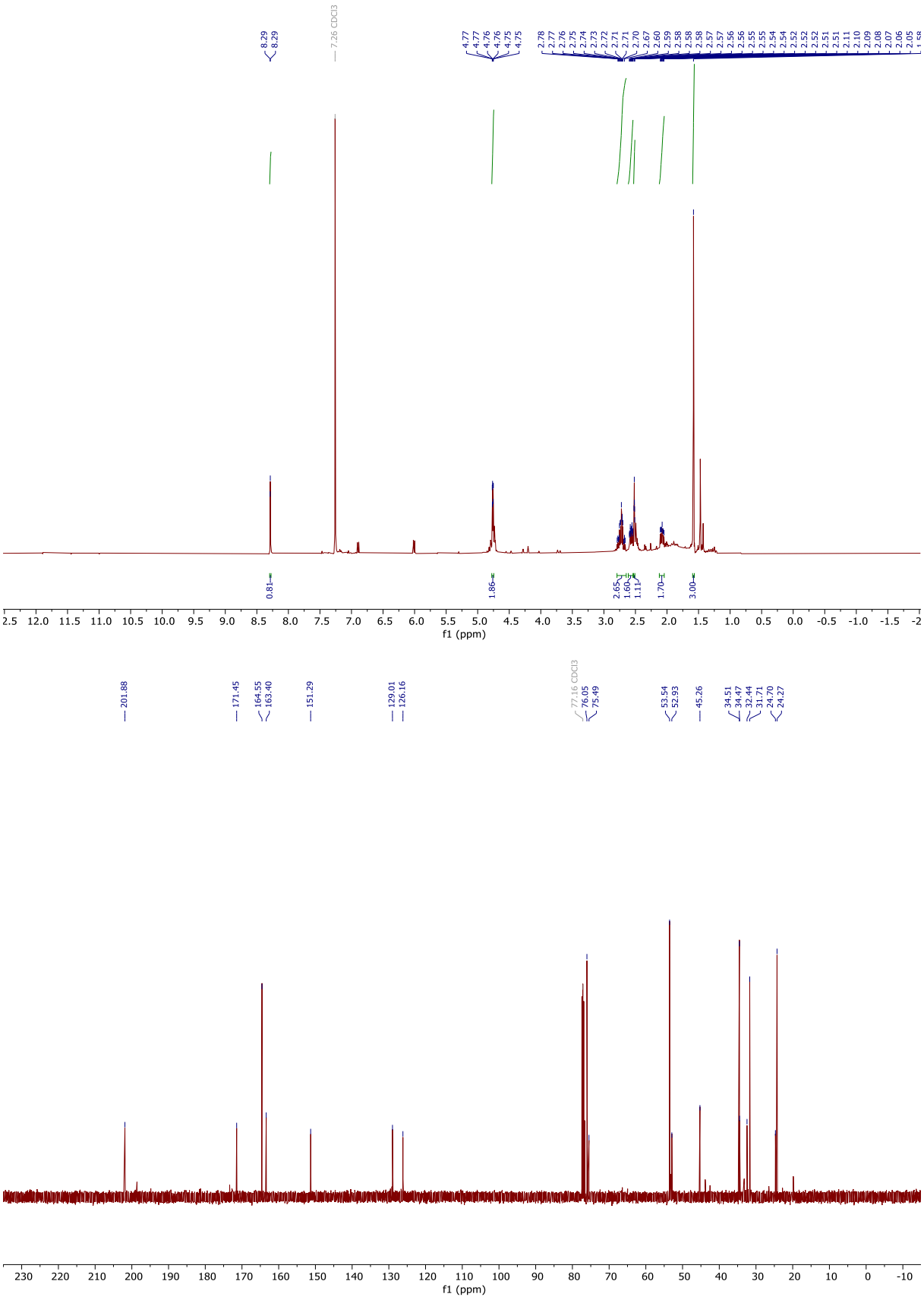
Supplementary Fig. 42. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1a"*R*-IN-8** in $CDCl_3$



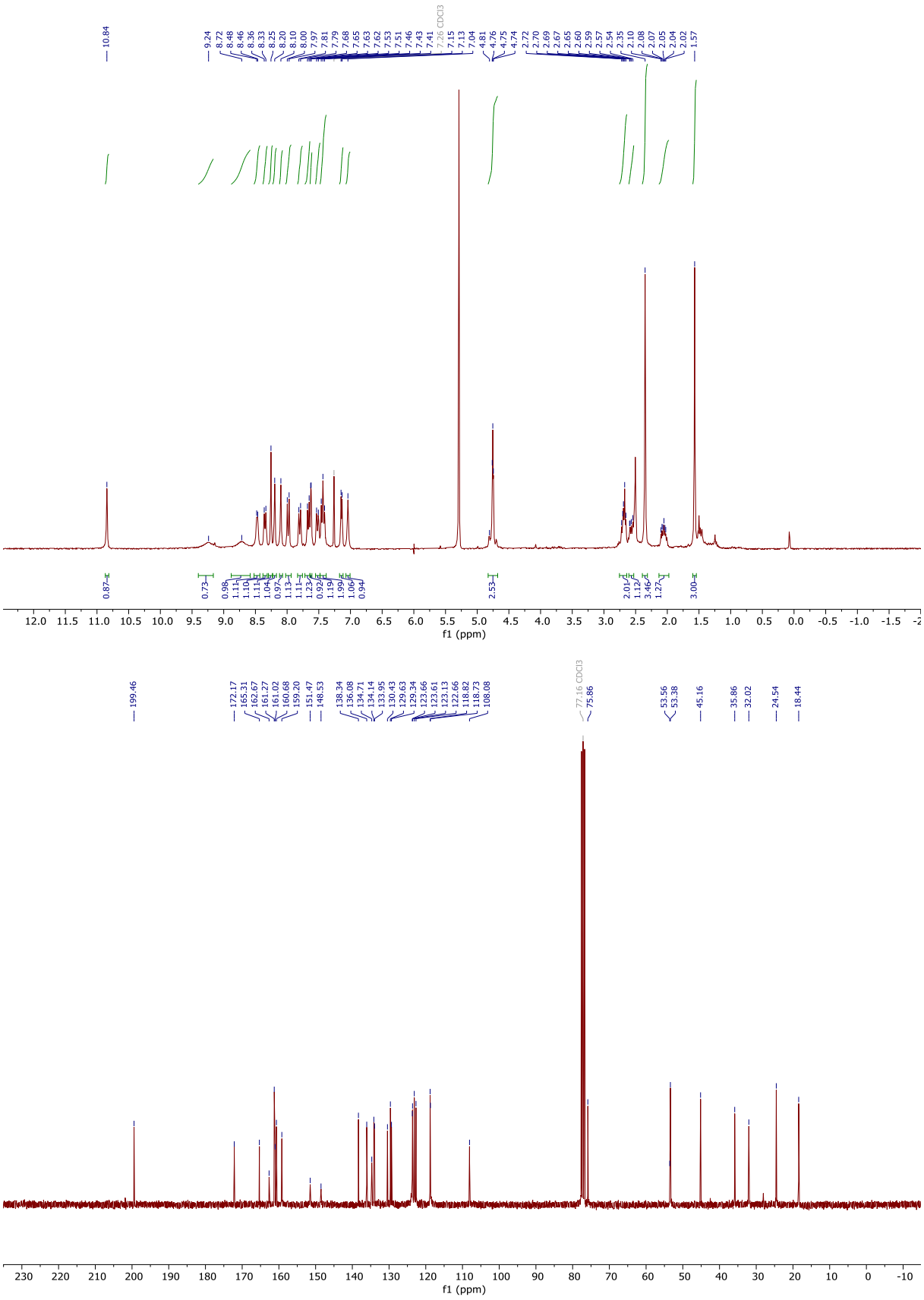
Supplementary Fig. 43. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S2d** in $CDCl_3$



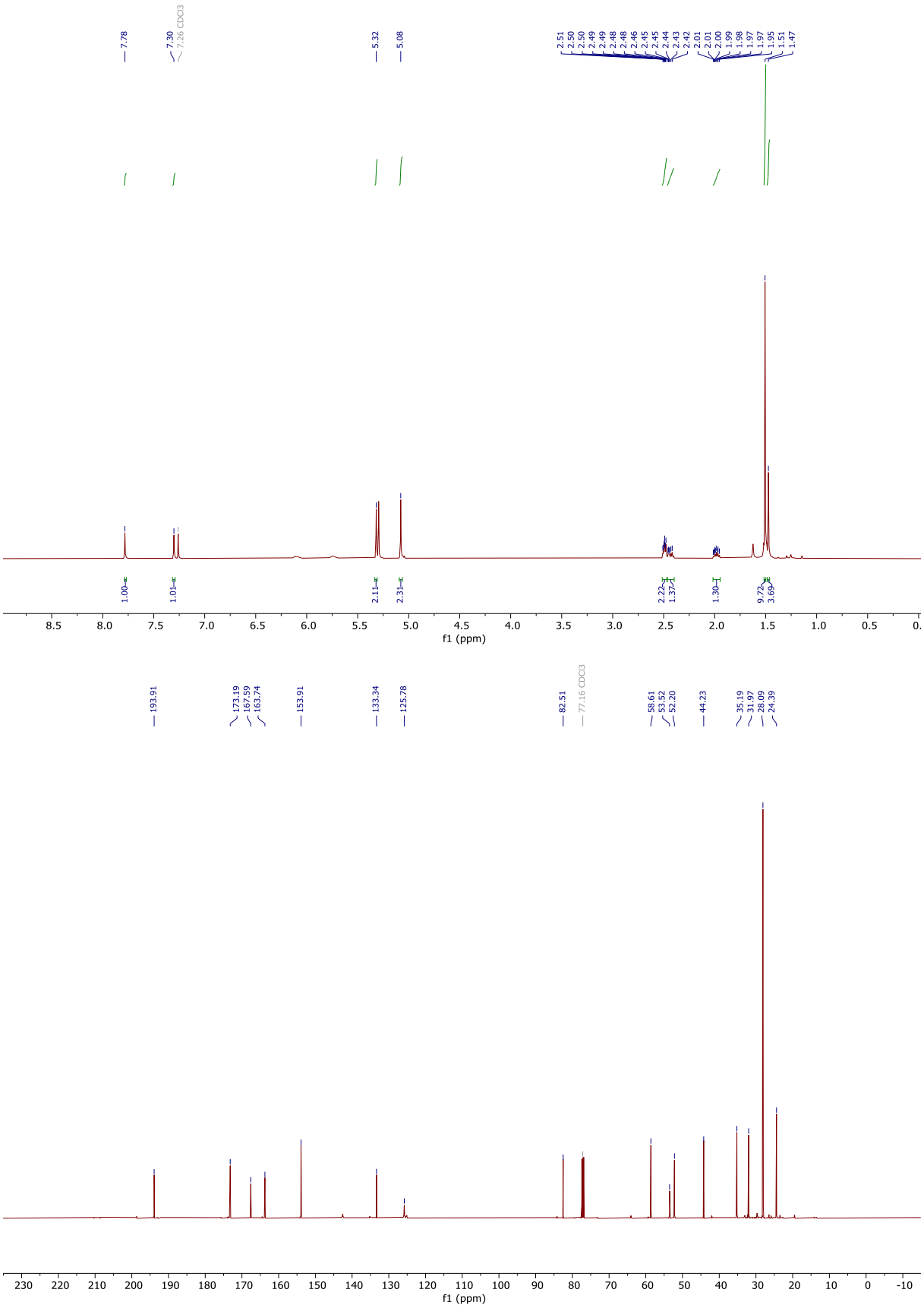
Supplementary Fig. 44. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S32*R*/S32*S*** in $CDCl_3$



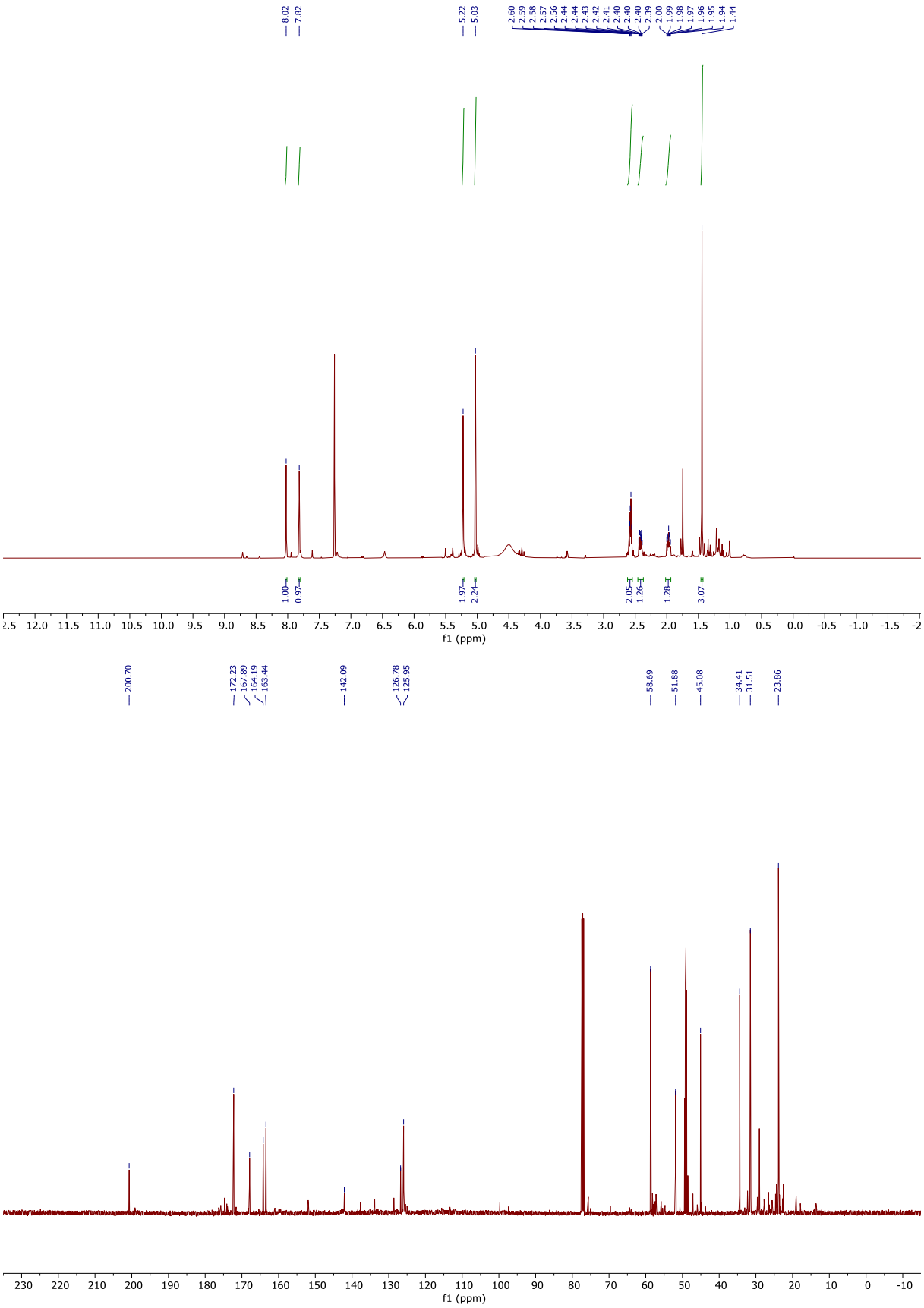
Supplementary Fig. 45. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S33*R*/S33*S*** in $CDCl_3$



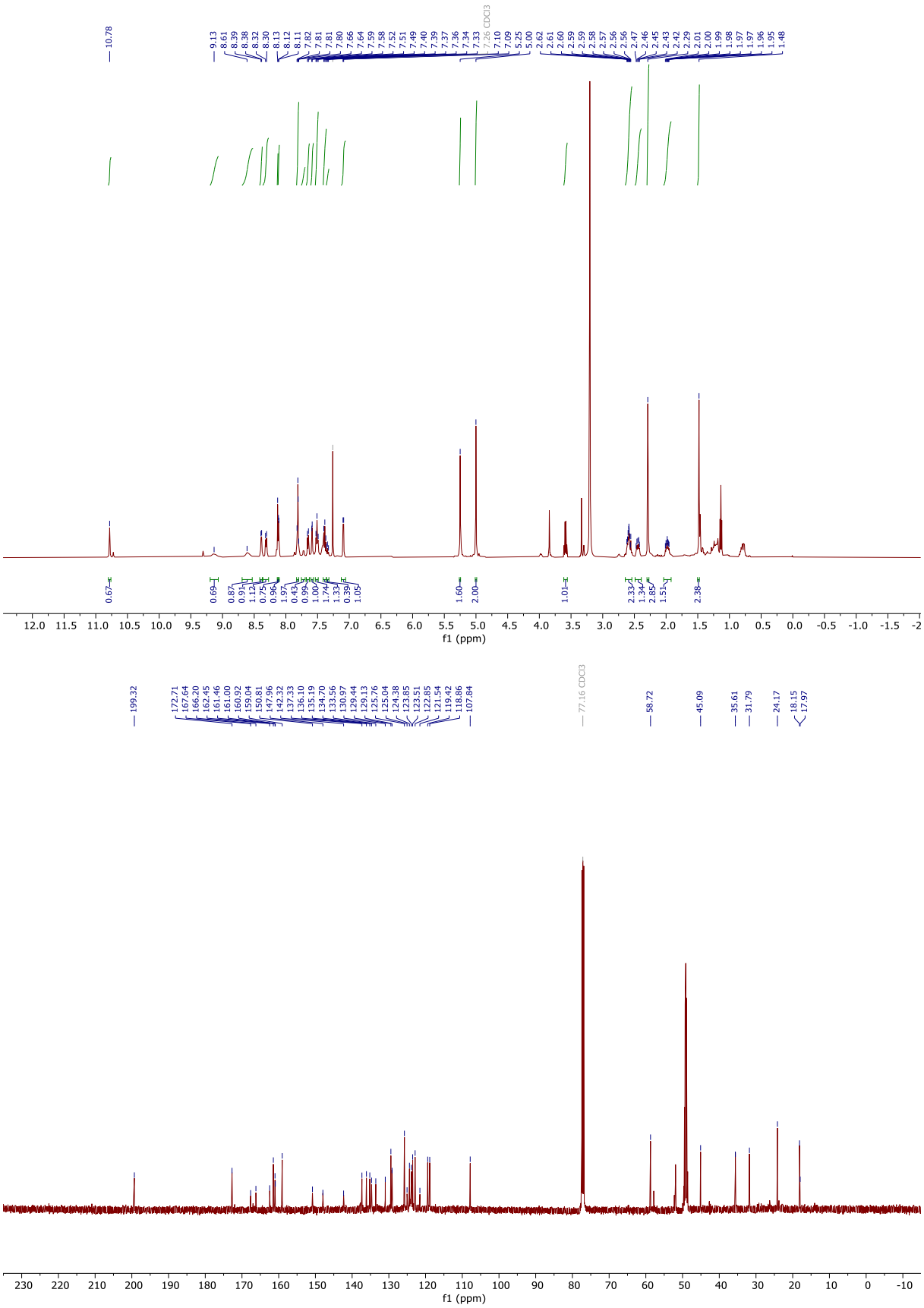
Supplementary Fig. 46. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1b*R*-IN-8**/**1b*S*-IN-8** in $CDCl_3$



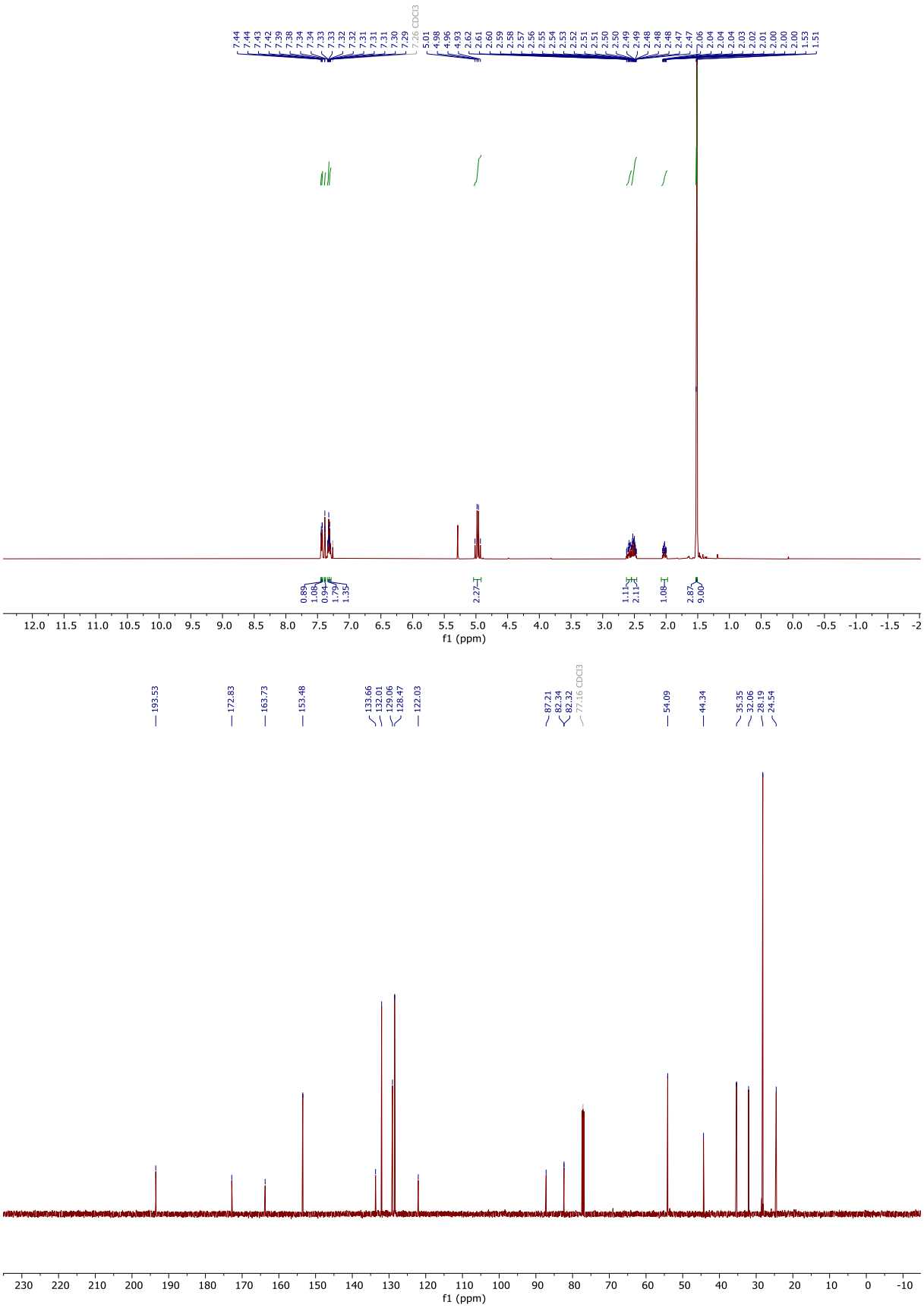
Supplementary Fig. 47. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S34*R*/S34*S*** *in* $CDCl_3$



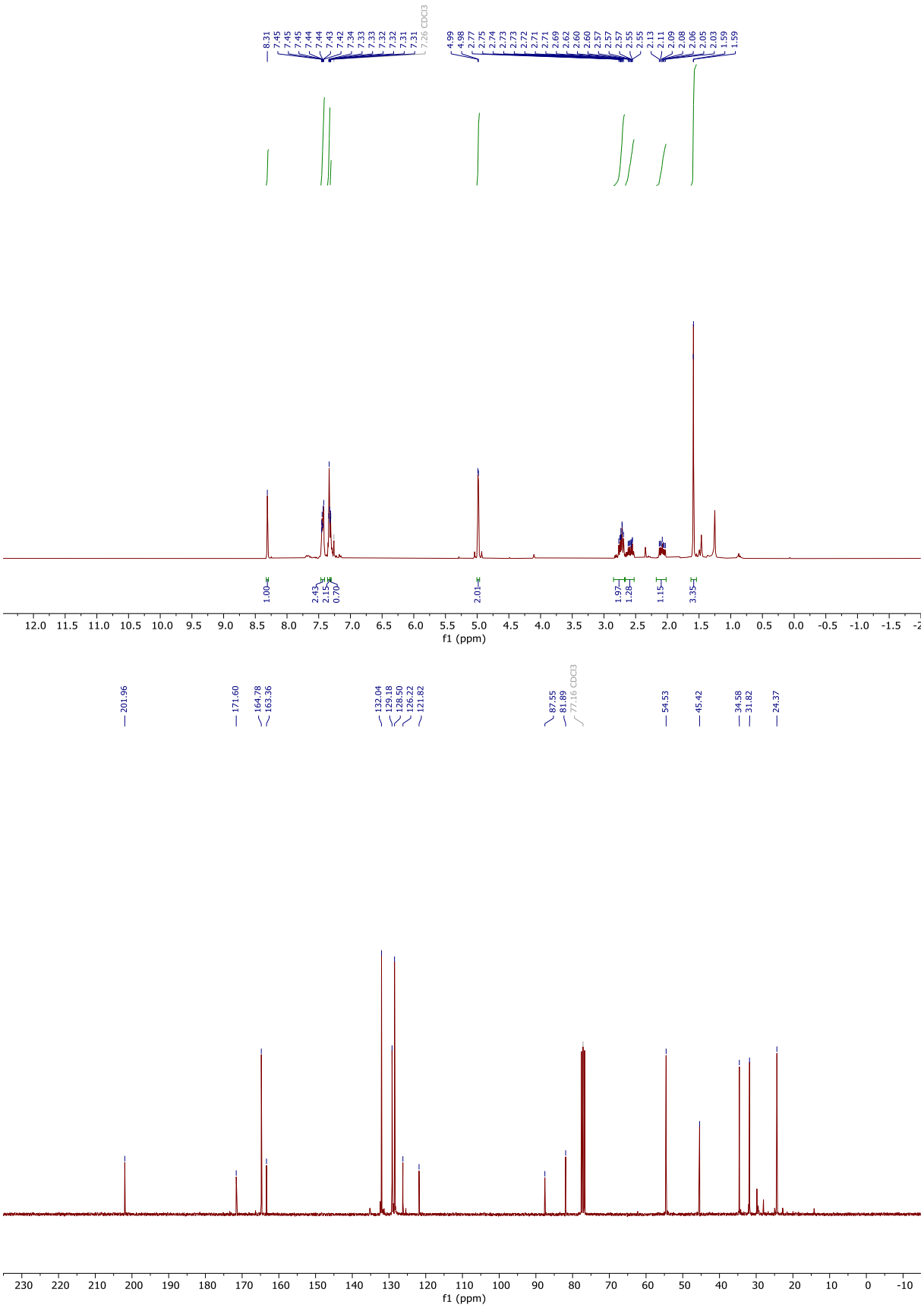
Supplementary Fig. 48. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S35*R*/S35*S*** in $CDCl_3$



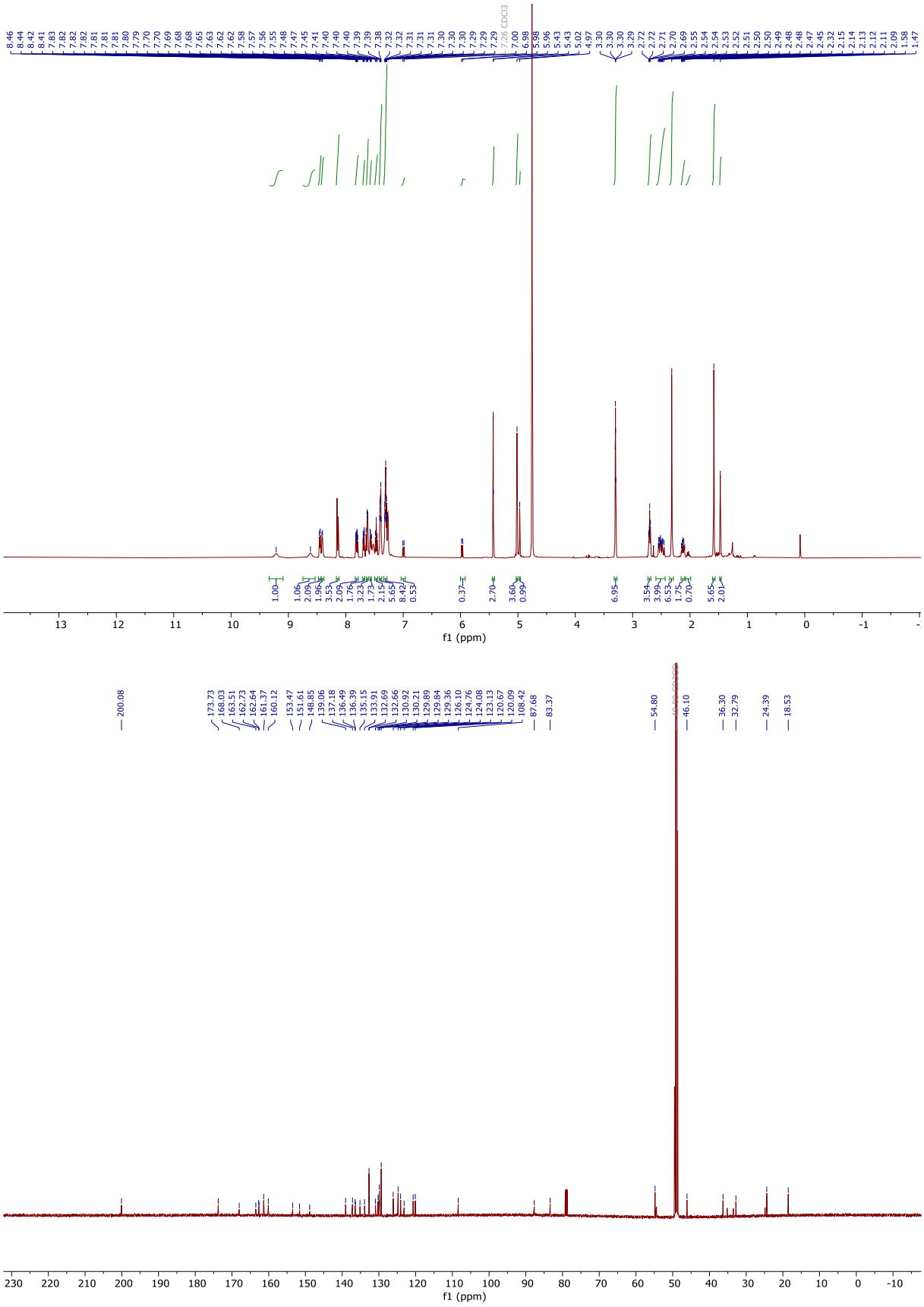
Supplementary Fig. 49. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1c*R*-IN-8**/**1c*S*-IN-8** in $CDCl_3$



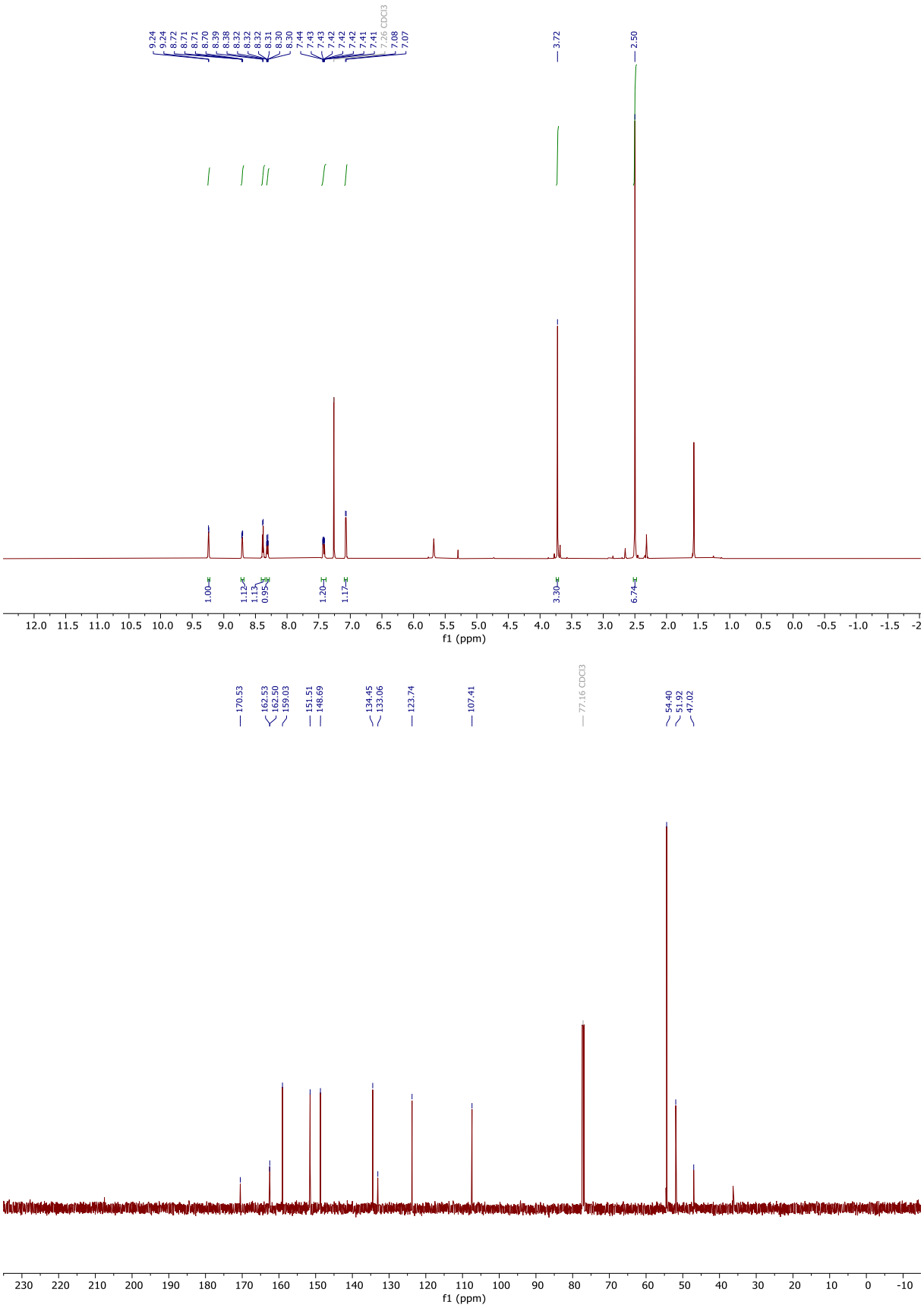
Supplementary Fig. 50. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S36*R*** in $CDCl_3$



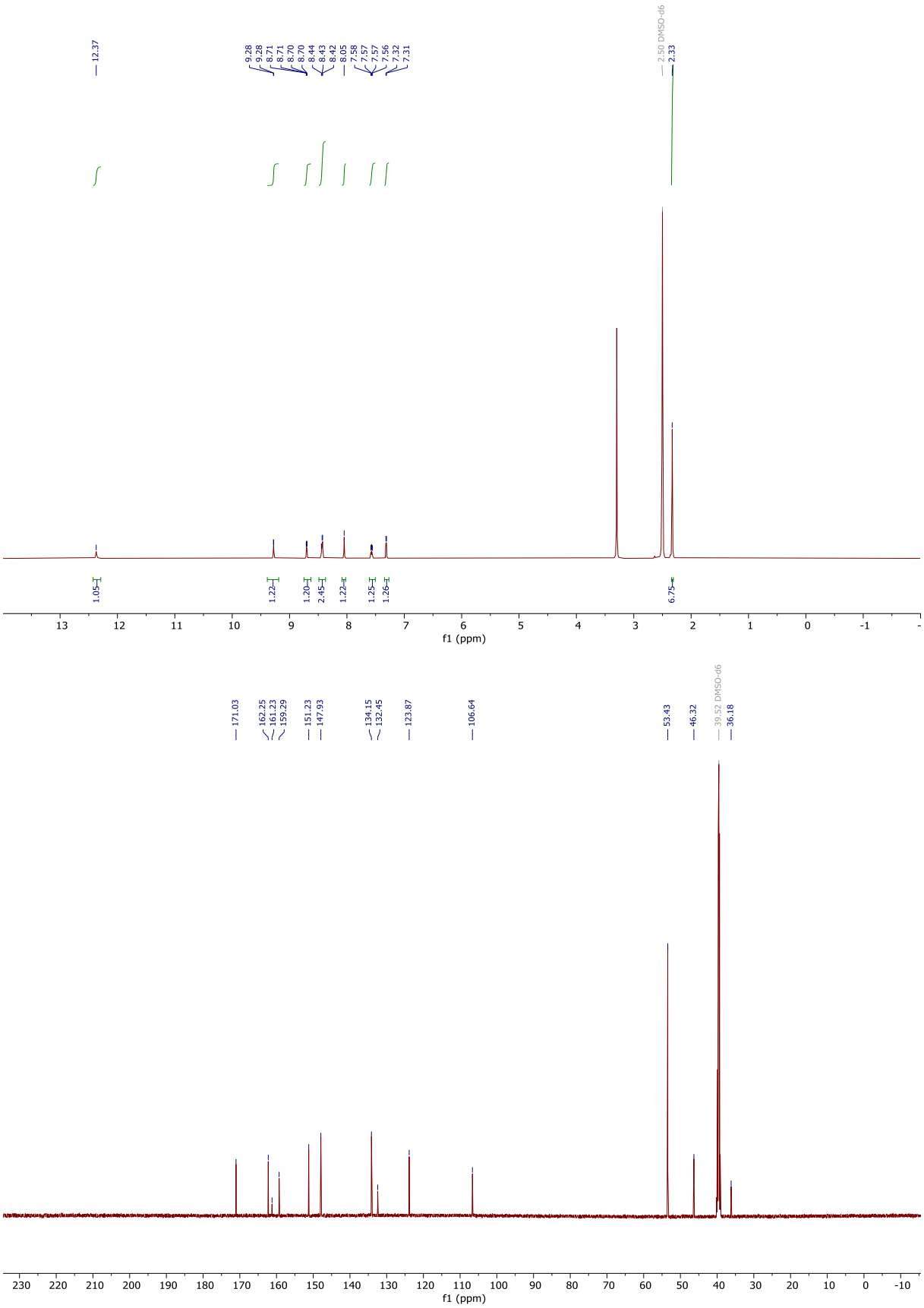
Supplementary Fig. 51. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S37*R*** in $CDCl_3$



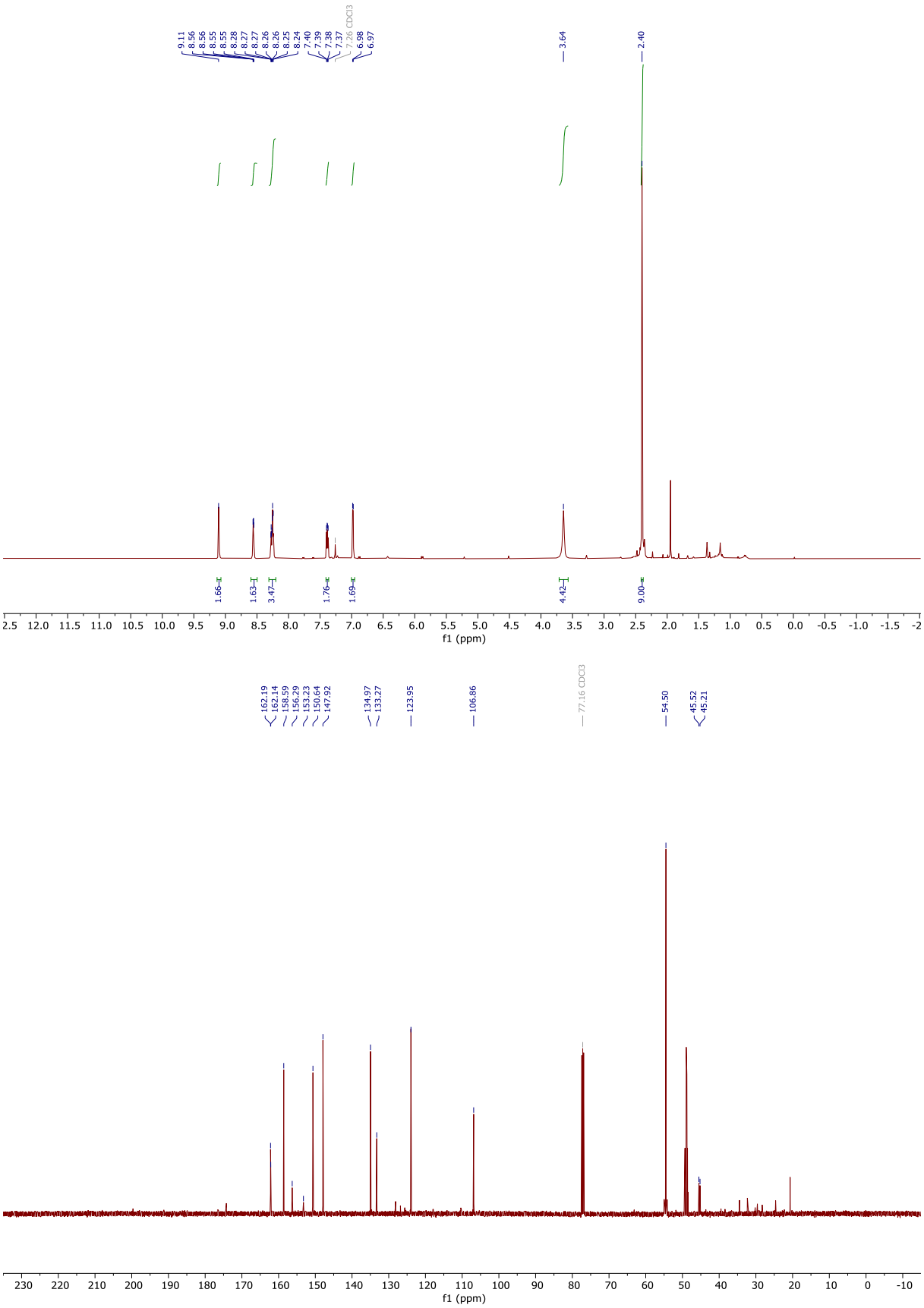
Supplementary Fig. 52. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1d*R*-IN-8** in CD_3OD+$CDCl_3$



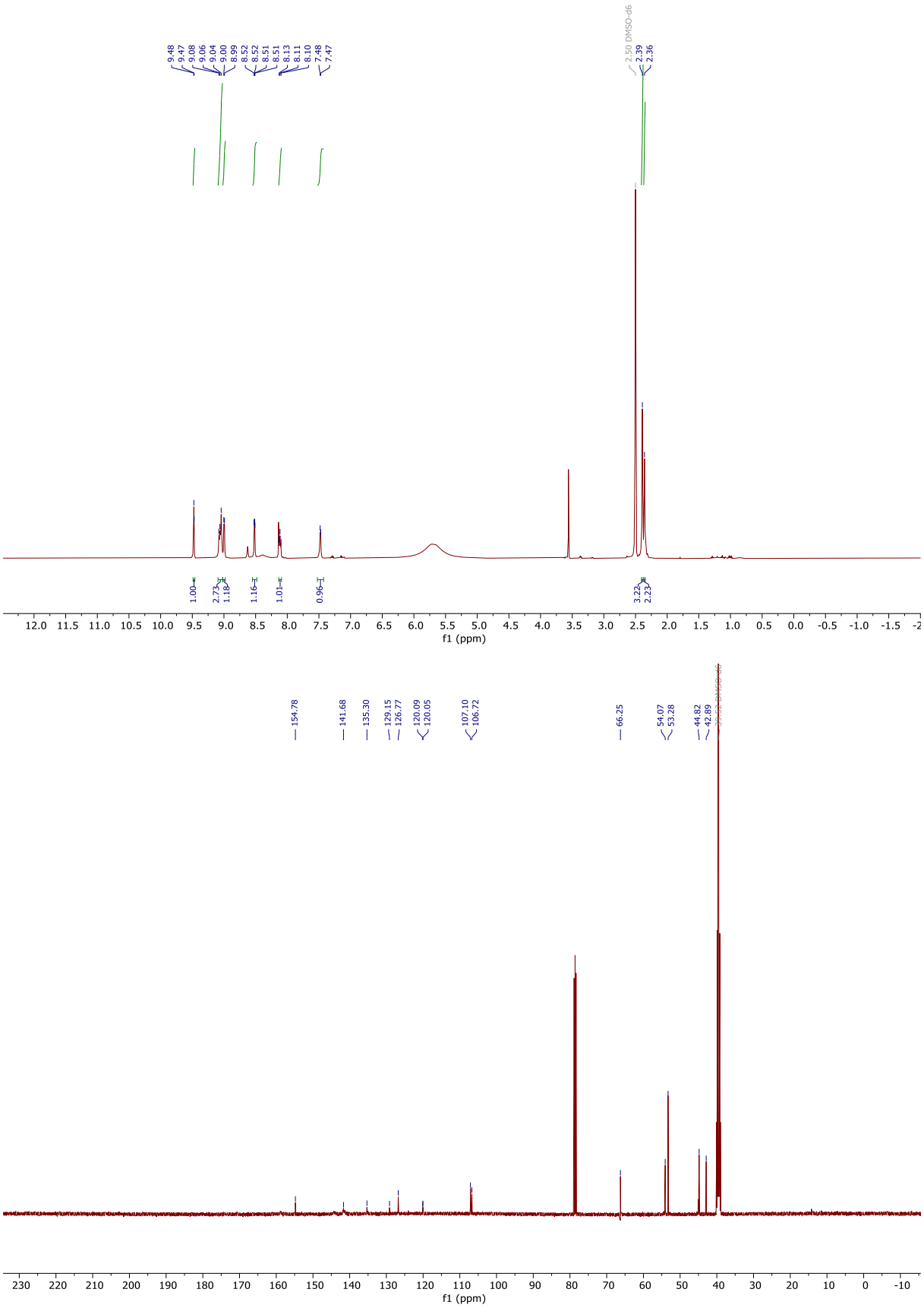
Supplementary Fig. 53. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S39** in $CDCl_3$



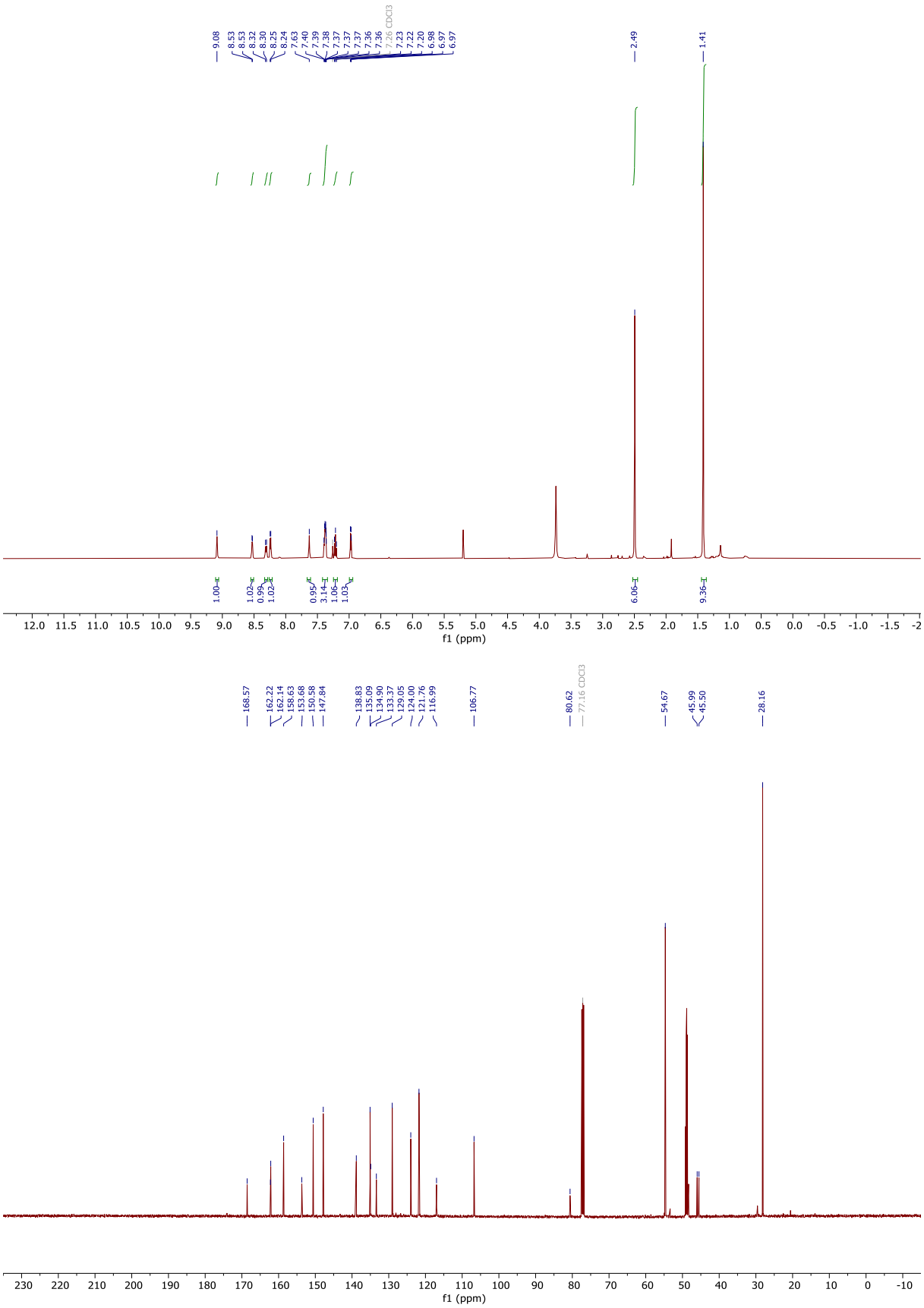
Supplementary Fig. 54. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S40** in DMSO-d_6



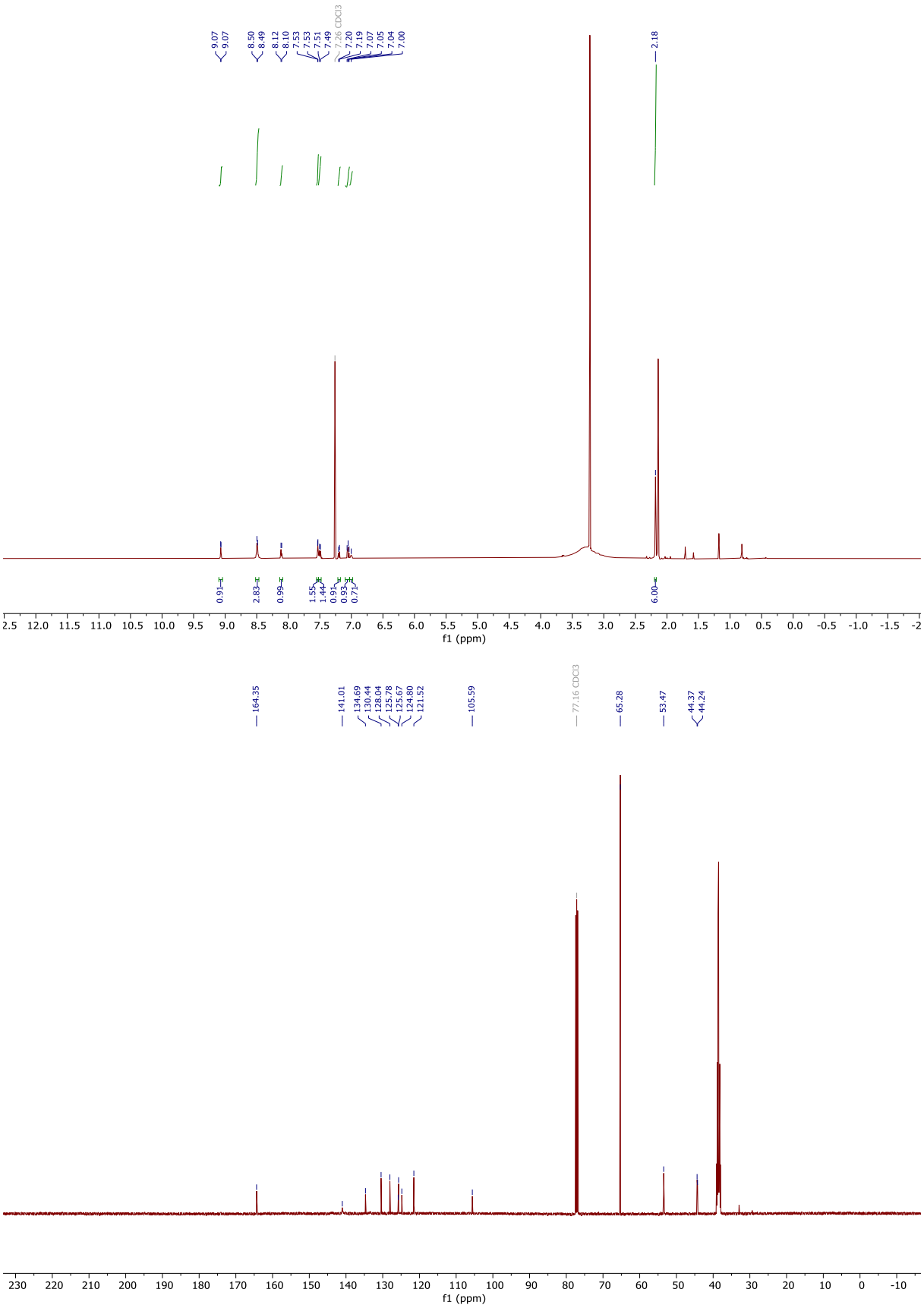
Supplementary Fig. 55. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S41** in $CDCl_3$



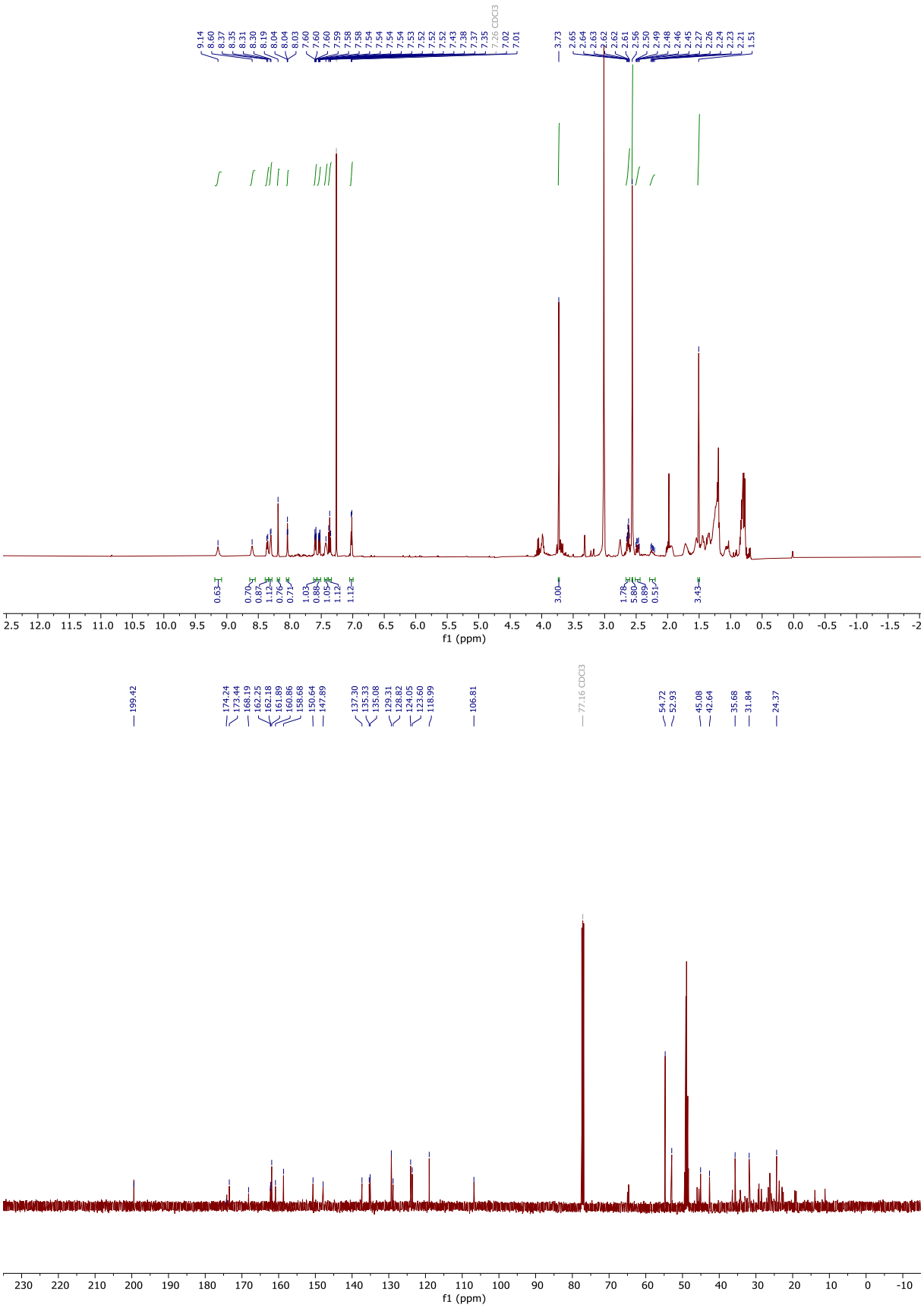
Supplementary Fig. 56. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S42** in DMSO-d_6+$CDCl_3$



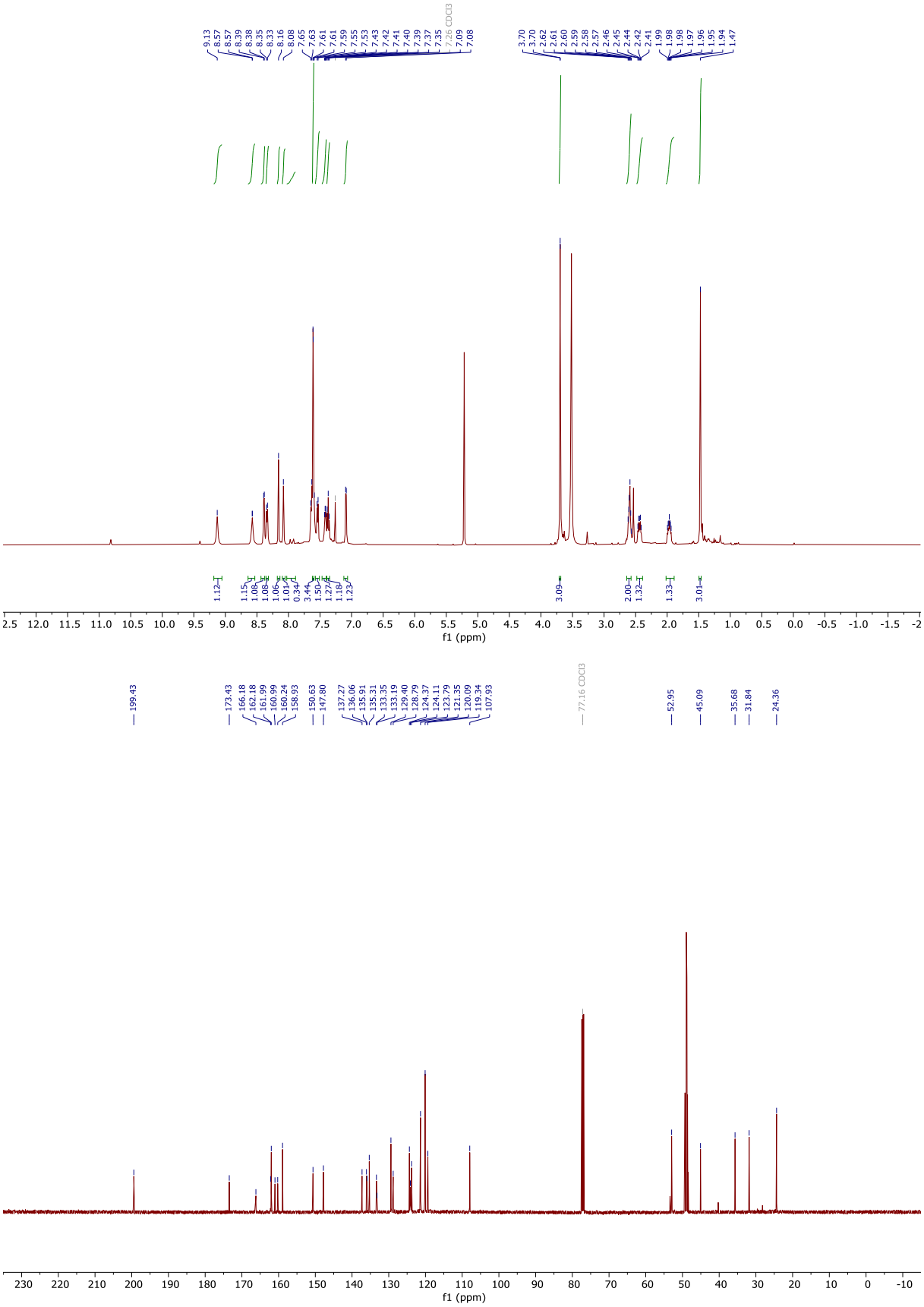
Supplementary Fig. 57. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S44** in $CDCl_3$



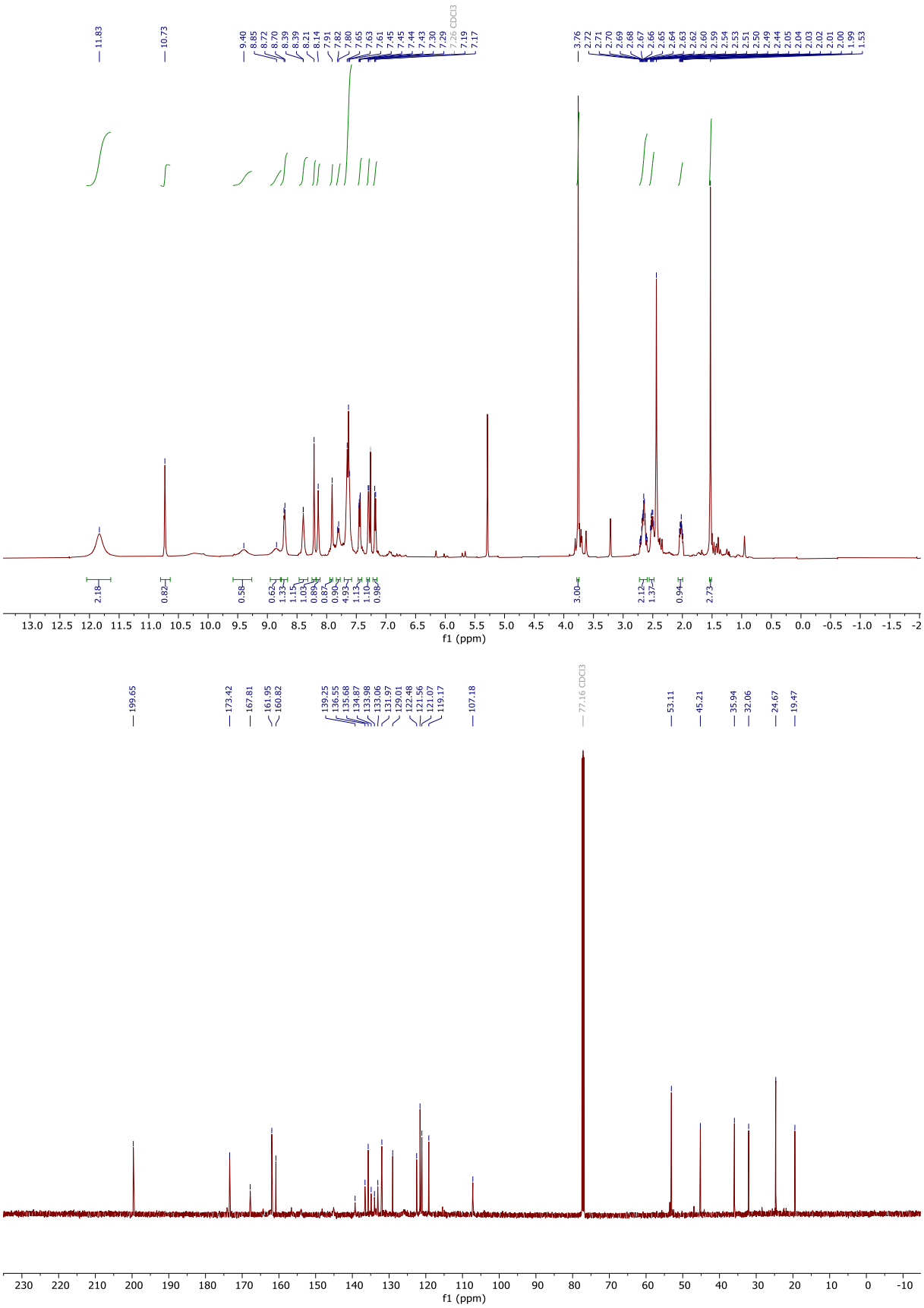
Supplementary Fig. 58. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S45** in $CDCl_3$+DMSO-d_6



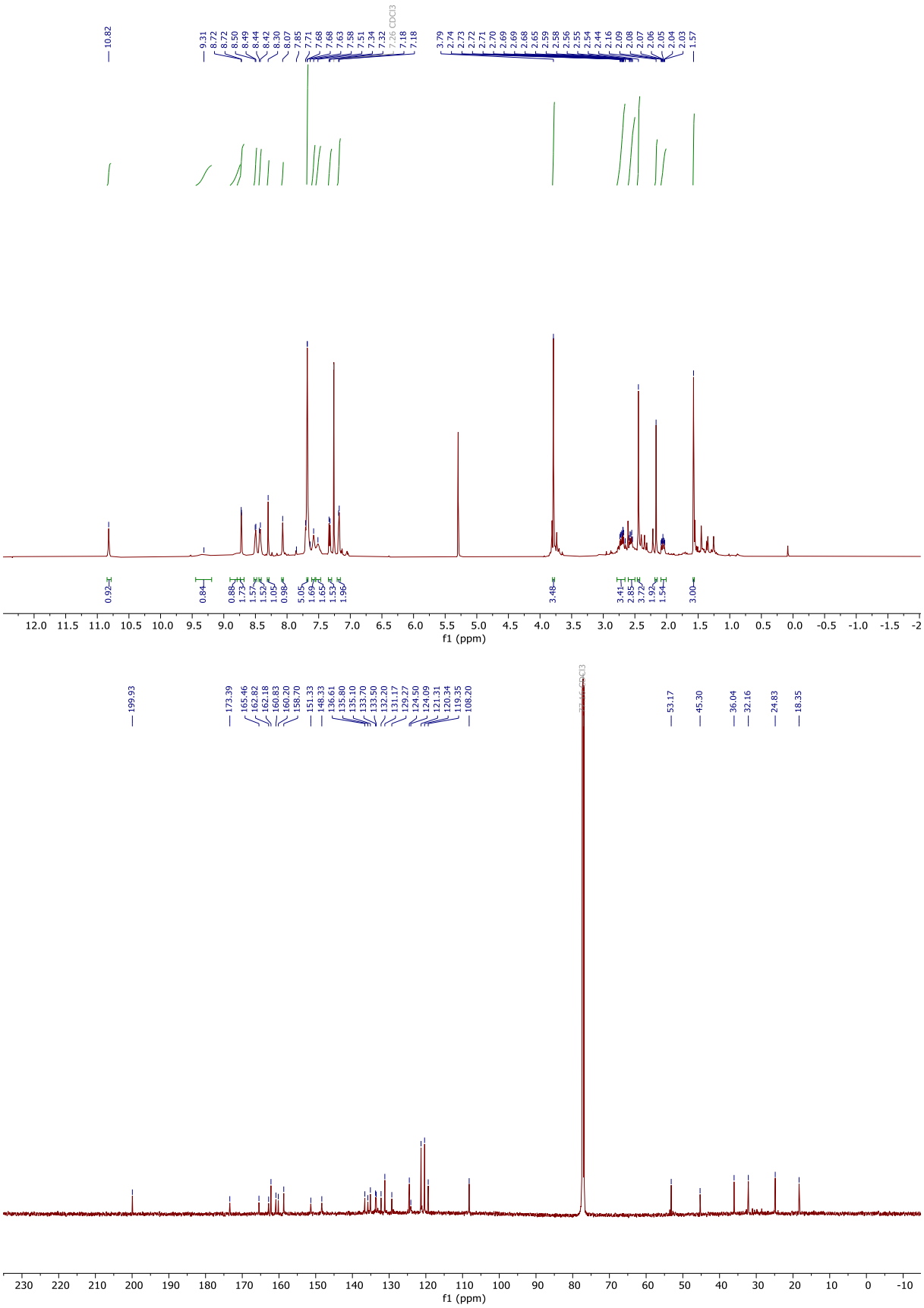
Supplementary Fig. 59. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1a*R*-isoPHEN** in $CDCl_3$+CD_3OD



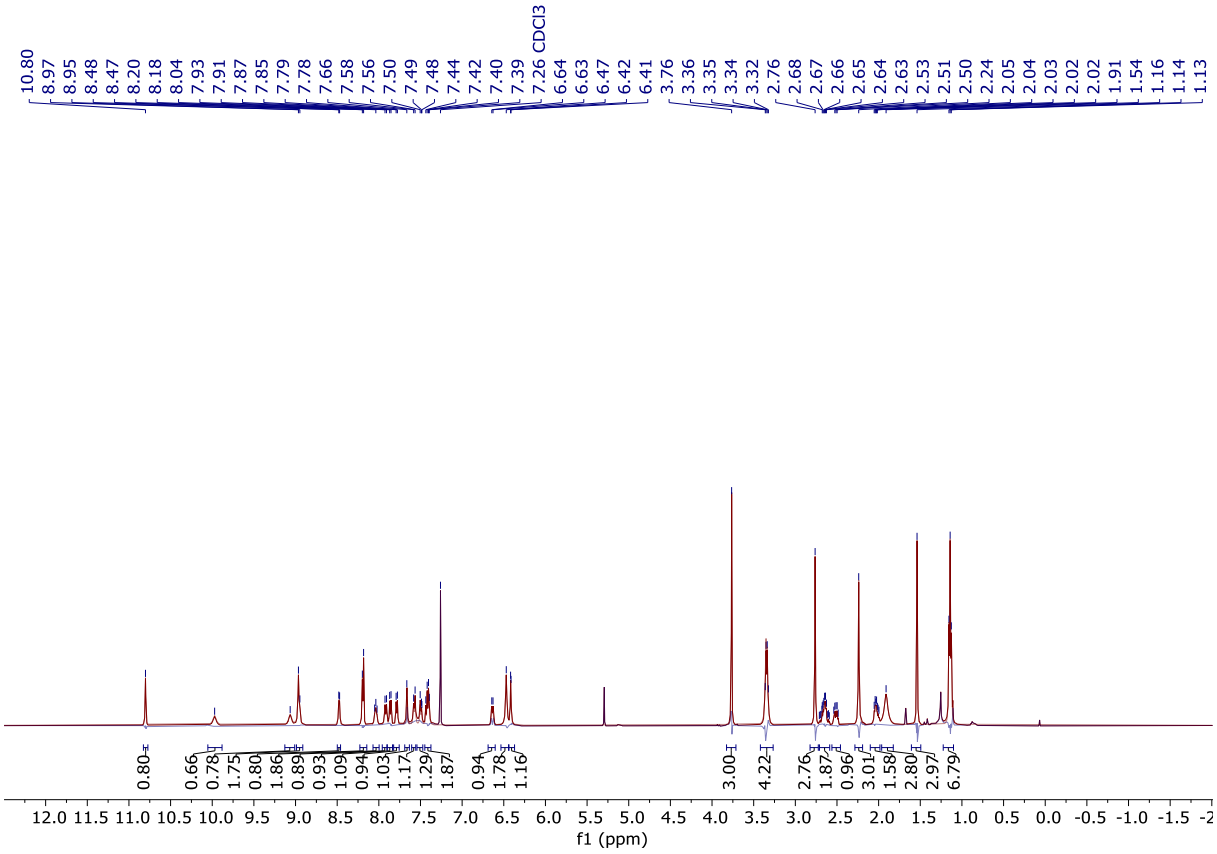
Supplementary Fig. 60. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1a*R*-IN-7** in $CDCl_3$



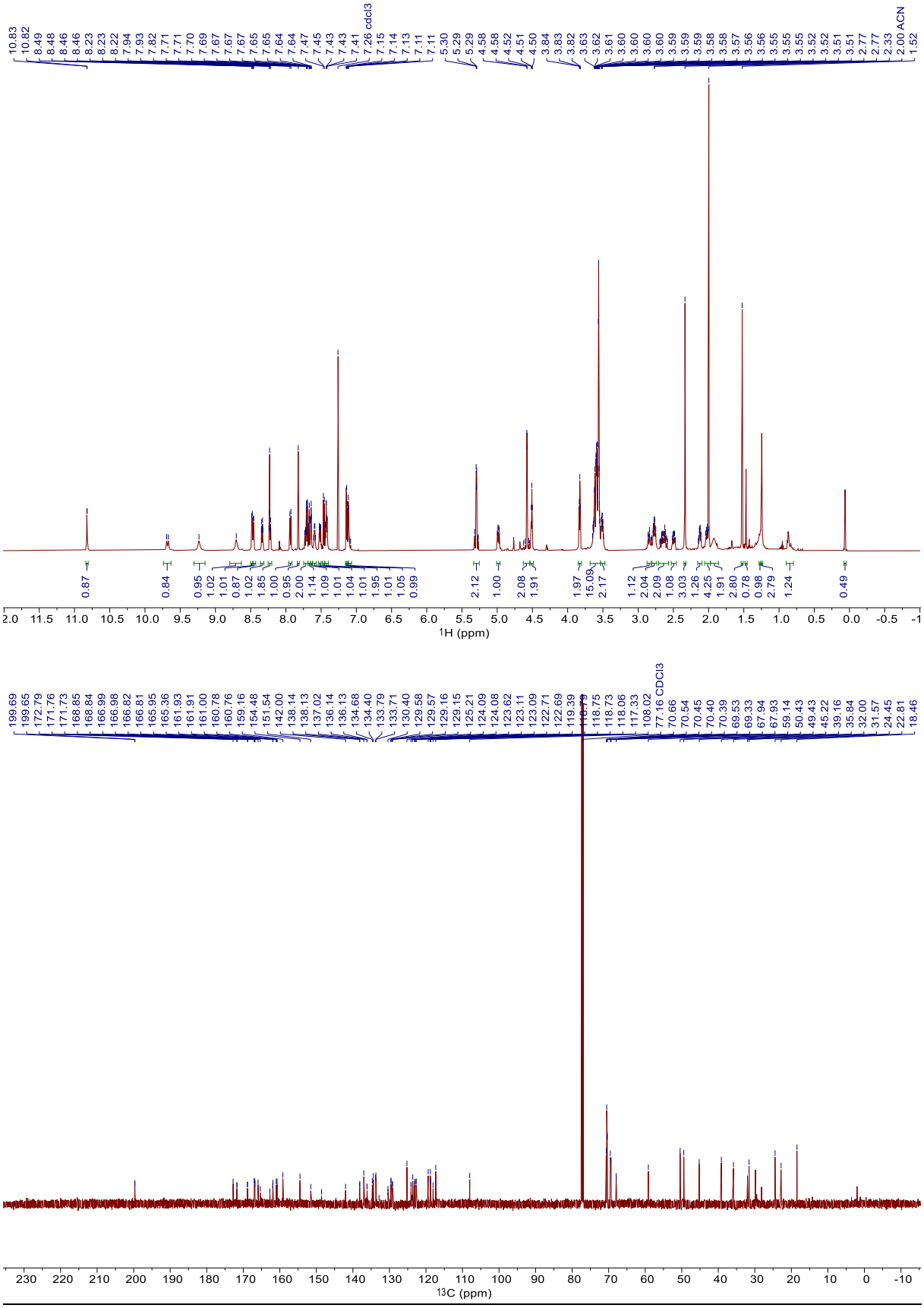
Supplementary Fig. 61. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1a*R*-IN-9** in $CDCl_3$



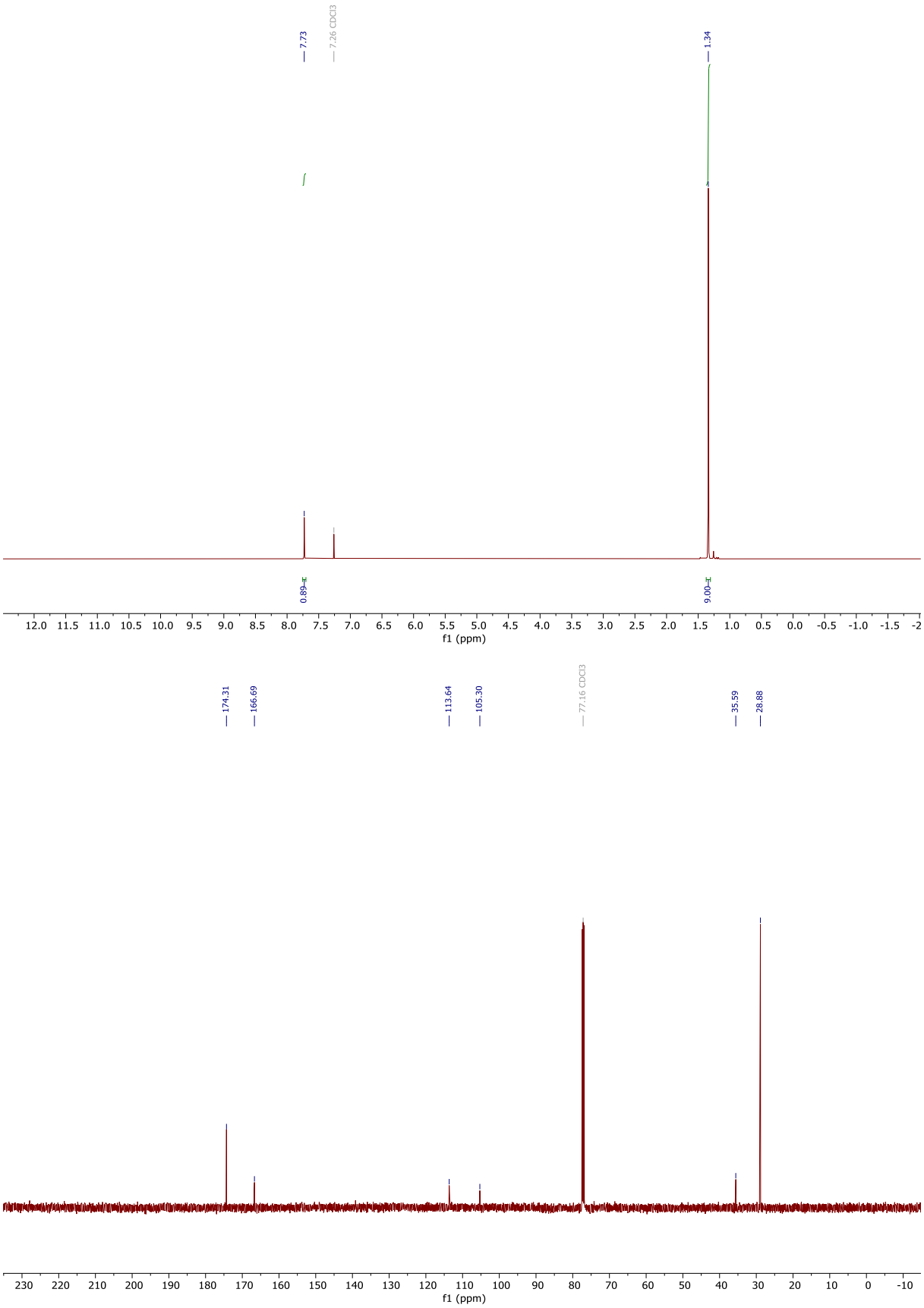
Supplementary Fig. 62. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **1a*R*-IN-10** in $CDCl_3$



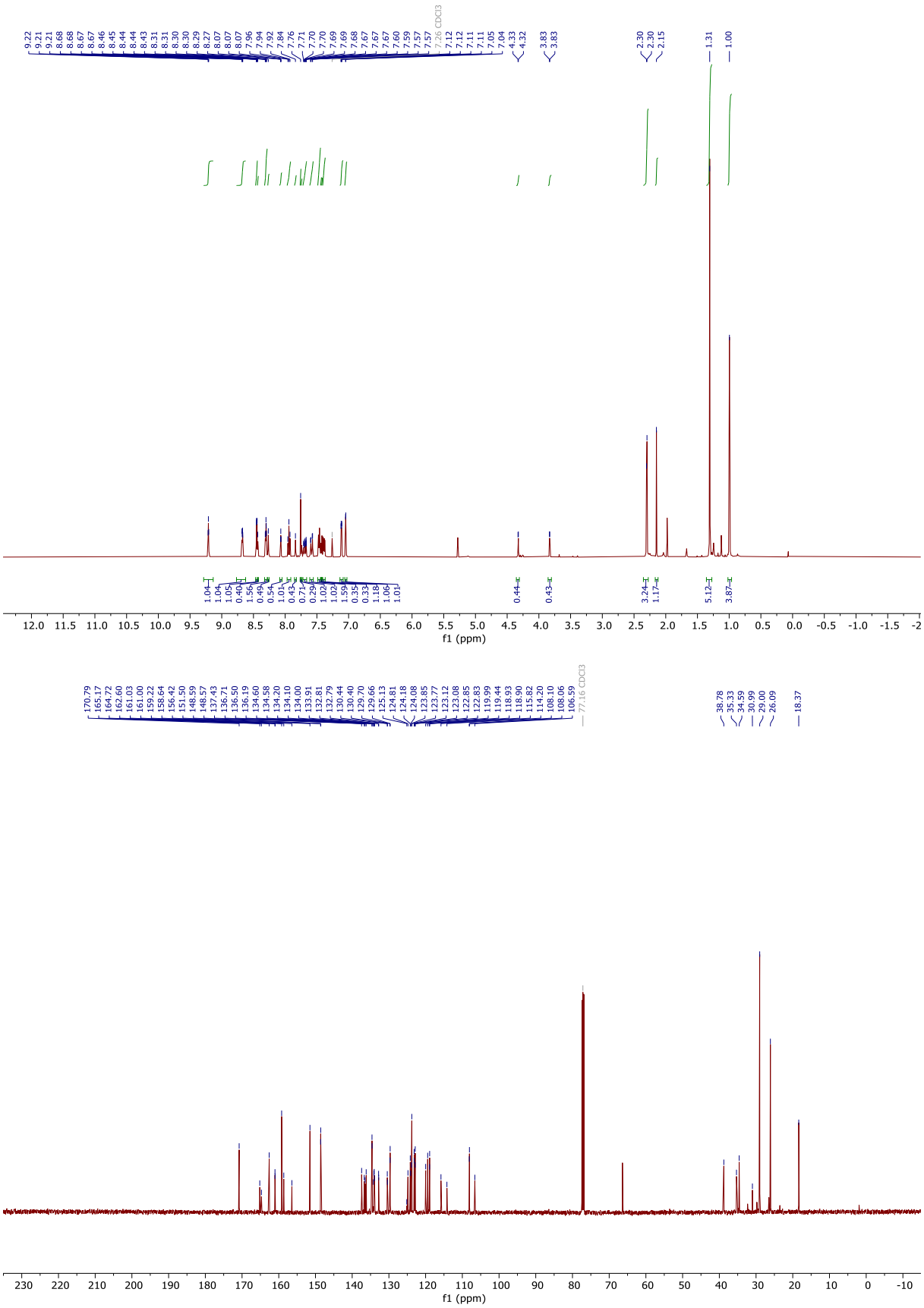
Supplementary Fig. 63. The ^{1}H-NMR (500 MHz) spectrum of compound **Photocaged 1a*R*-IN-8** in $CDCl_3$



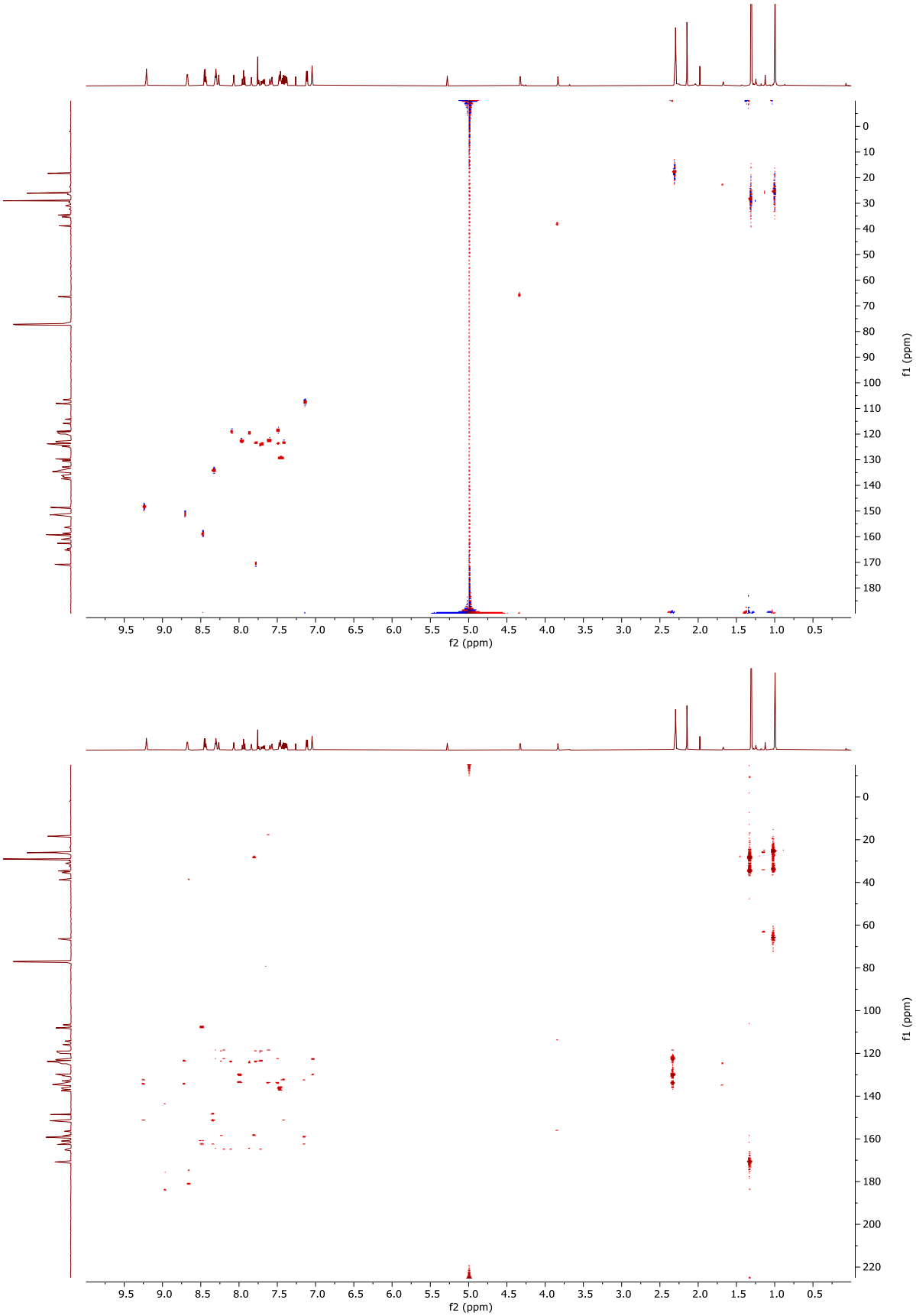
Supplementary Fig. 64. The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (151 MHz) spectra of compound **PRT_1 i**n $CDCl_3$



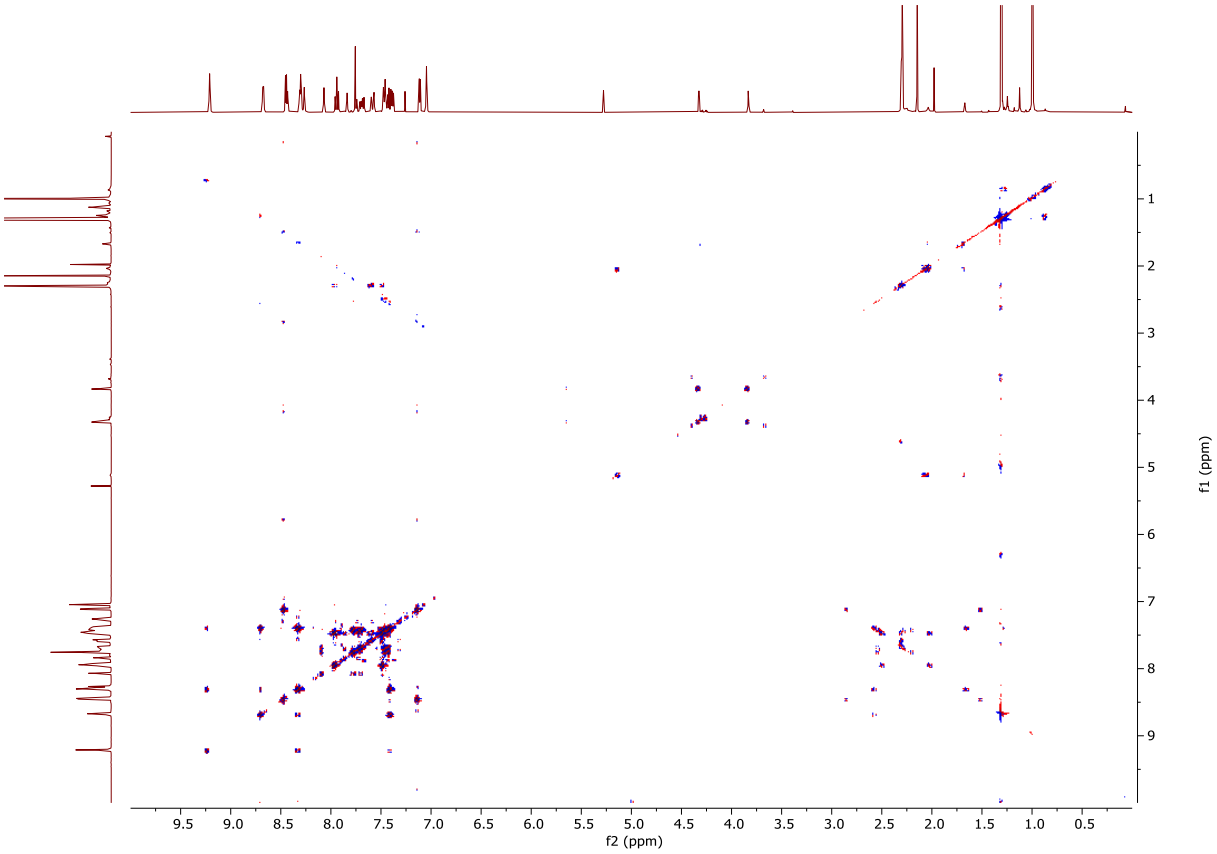
Supplementary Fig. 65. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **S48** in $CDCl_3$



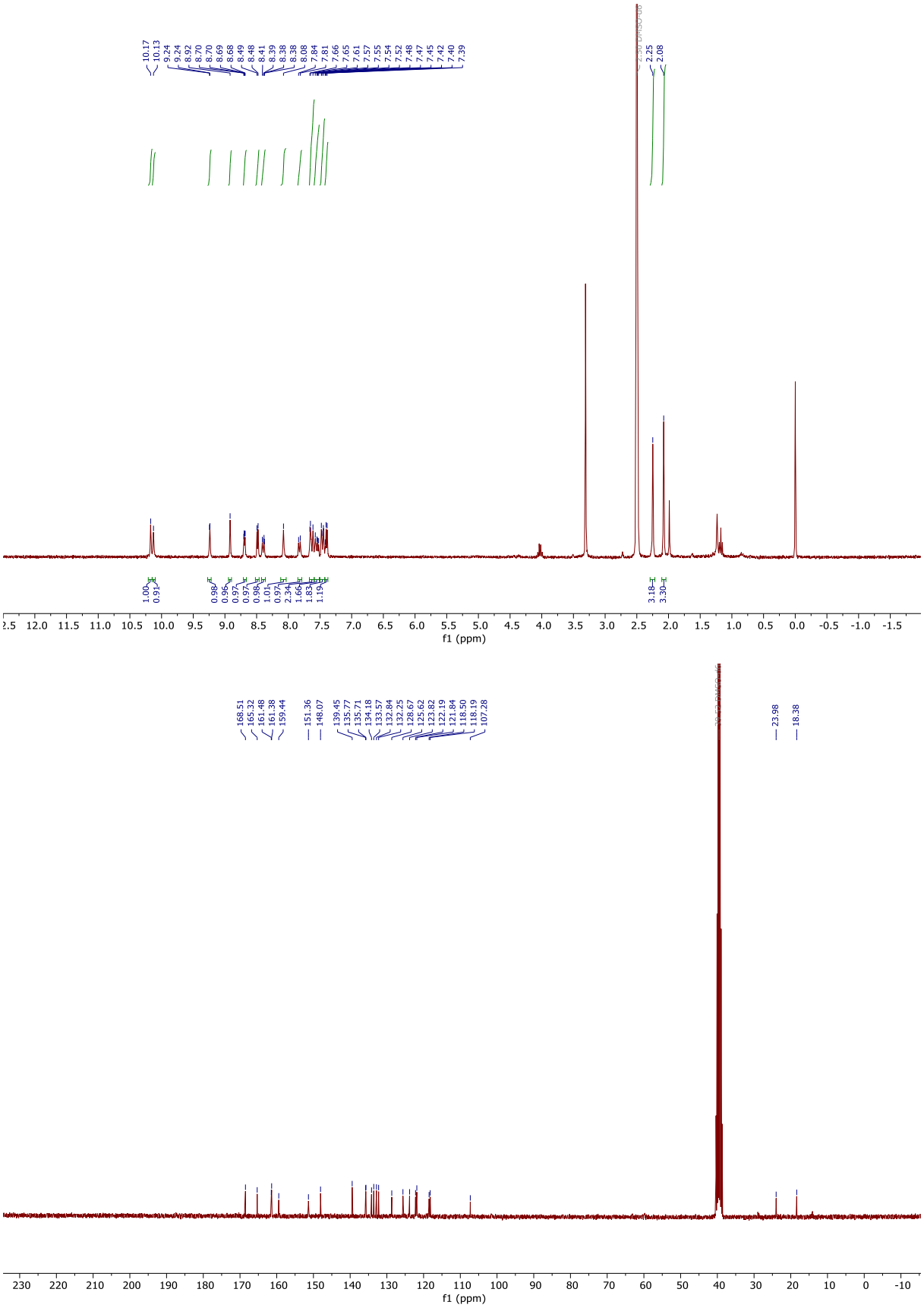
Supplementary Fig. 66. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **CA-IN-8** in $CDCl_3$



Supplementary Fig. 67. The HSQC and HMBC spectra of compound **CA-IN-8** in $CDCl_3$

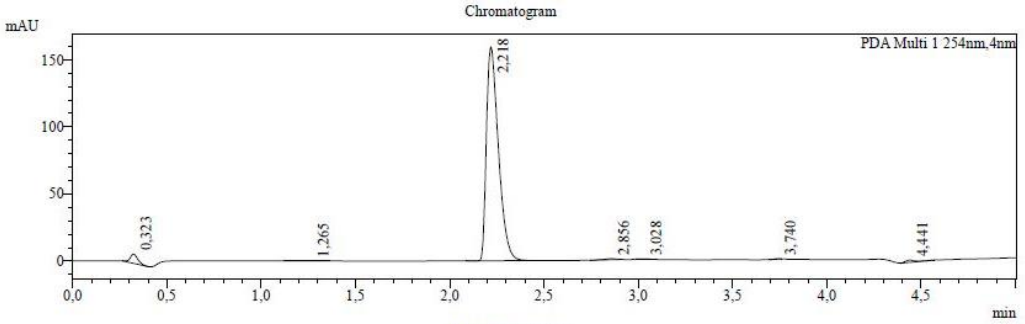


Supplementary Fig. 68. The COSY spectrum of compound **CA-IN-8** in $CDCl_3$



Supplementary Fig. 69. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of compound **IN-8a** in $CDCl_3$

High-performance liquid chromatography chromatograms

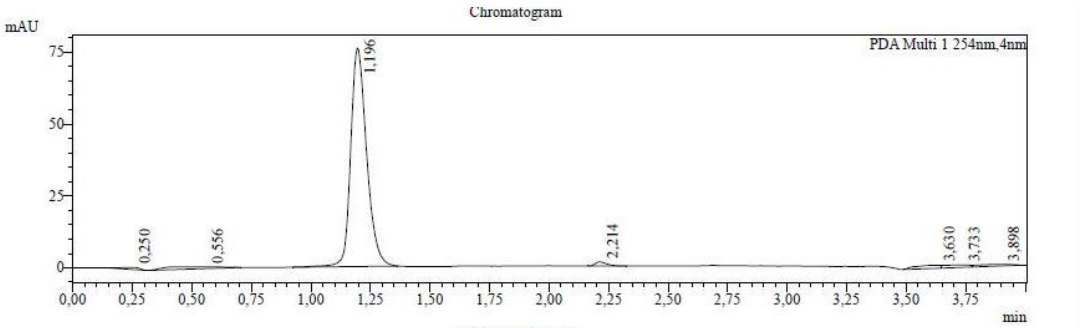


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,323	19161	2,603	6851	4,038	
2	1,265	1199	0,163	171	0,101	
3	2,218	704557	95,706	159625	94,096	
4	2,856	4114	0,559	878	0,517	
5	3,028	471	0,064	199	0,118	
6	3,740	742	0,101	306	0,181	
7	4,441	5921	0,804	1609	0,949	
Total		736165	100,000	169640	100,000	

Supplementary Fig. 70. The high-performance liquid chromatography chromatogram of compound **S4**/**S4*R***/**S4*S***

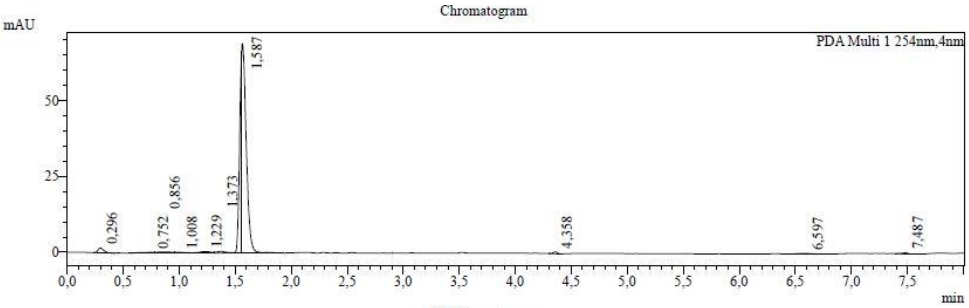


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,250	3592	0,909	709	0,873	
2	0,556	12689	3,213	642	0,790	
3	1,196	353210	89,424	76067	93,600	
4	2,214	4696	1,189	1402	1,725	
5	3,630	8382	2,122	1064	1,309	
6	3,733	6547	1,658	860	1,058	
7	3,898	5868	1,486	524	0,645	
Total		394984	100,000	81268	100,000	

Supplementary Fig. 71. The high-performance liquid chromatography chromatogram of compound **S5**/**S5*R***/**S5*S***

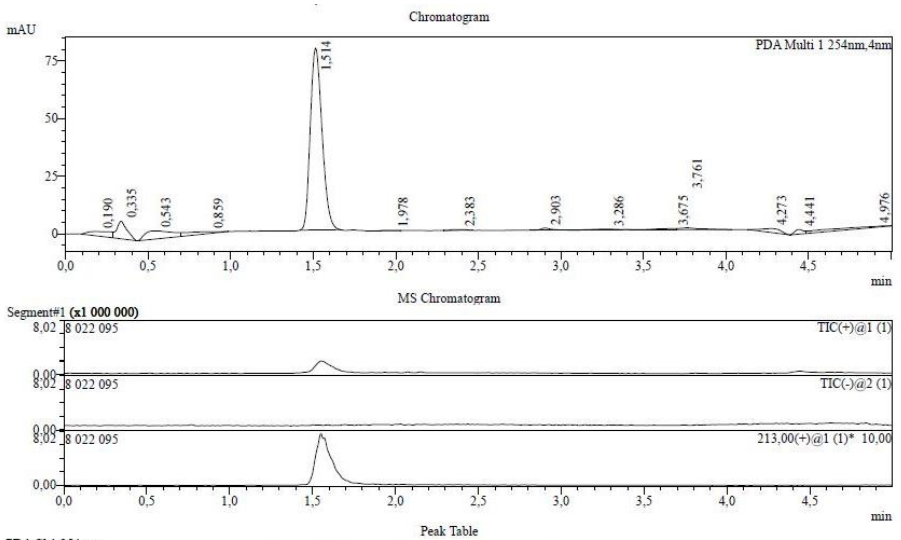


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,296	5432	1,808	1535	2,595	
2	0,752	2504	0,833	281	0,475	
3	0,856	3629	1,208	399	0,674	
4	1,008	1874	0,623	292	0,494	
5	1,229	3533	1,176	456	0,771	
6	1,373	98299	32,708	530	0,896	
7	1,587	181214	60,298	54491	92,113	RT:1,587
8	4,358	1550	0,516	612	1,035	
9	6,597	1004	0,334	124	0,209	
10	7,487	1492	0,497	436	0,738	
Total		300531	100,000	59156	100,000	

Supplementary Fig. 72. The high-performance liquid chromatography chromatogram of compound **S8**

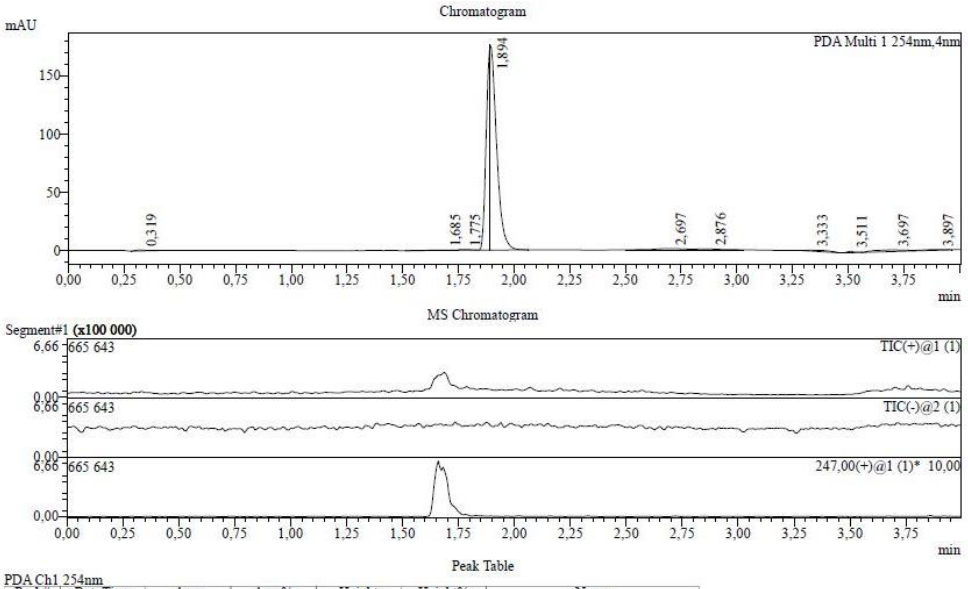


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,190	19900	3,537	1891	1,893	
2	0,335	32437	5,766	7678	7,686	
3	0,543	40076	7,124	3538	3,542	
4	0,859	15309	2,721	840	0,840	
5	1,514	390264	69,375	78893	78,976	
6	1,978	1489	0,265	251	0,252	
7	2,383	2416	0,429	385	0,386	
8	2,903	3681	0,654	892	0,892	
9	3,286	3871	0,688	340	0,340	
10	3,675	4462	0,793	500	0,501	
11	3,761	6348	1,128	812	0,813	
12	4,273	13902	2,471	1694	1,695	
13	4,441	7489	1,331	2015	2,017	
14	4,976	20895	3,714	166	0,166	
Total		562539	100,000	99895	100,000	

Supplementary Fig. 73. The high-performance liquid chromatography chromatogram of compound **S9**

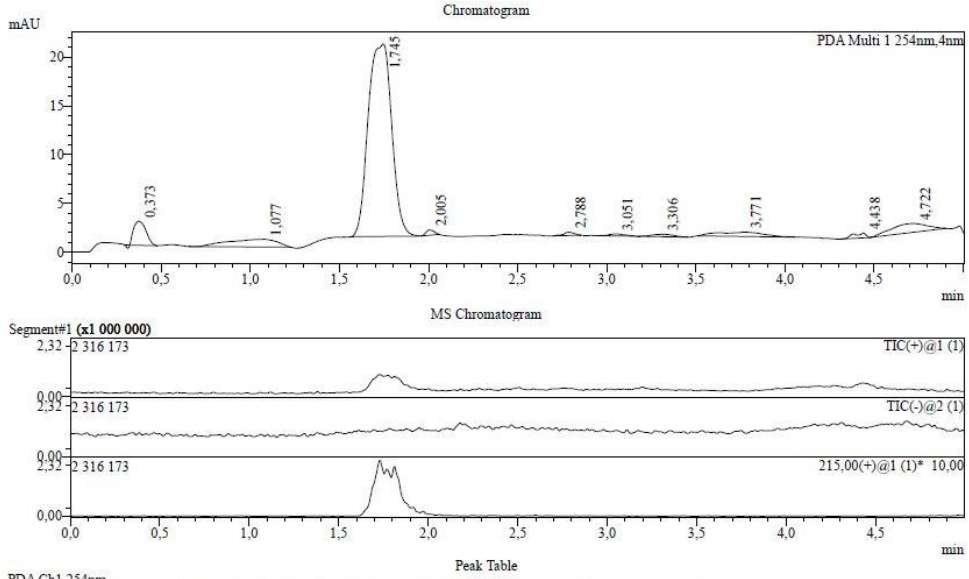


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,319	2708	0,433	809	0,439	
2	1,685	3184	0,509	355	0,192	
3	1,775	246099	39,362	675	0,366	
4	1,894	321070	51,353	176343	95,608	
5	2,697	12899	2,063	1265	0,686	
6	2,876	9286	1,485	965	0,523	
7	3,333	7327	1,172	764	0,414	
8	3,511	2645	0,423	1204	0,653	
9	3,697	14643	2,342	1361	0,738	
10	3,897	5364	0,858	703	0,381	
Total		625224	100,000	184444	100,000	

Supplementary Fig. 74. The high-performance liquid chromatography chromatogram of compound **S11**

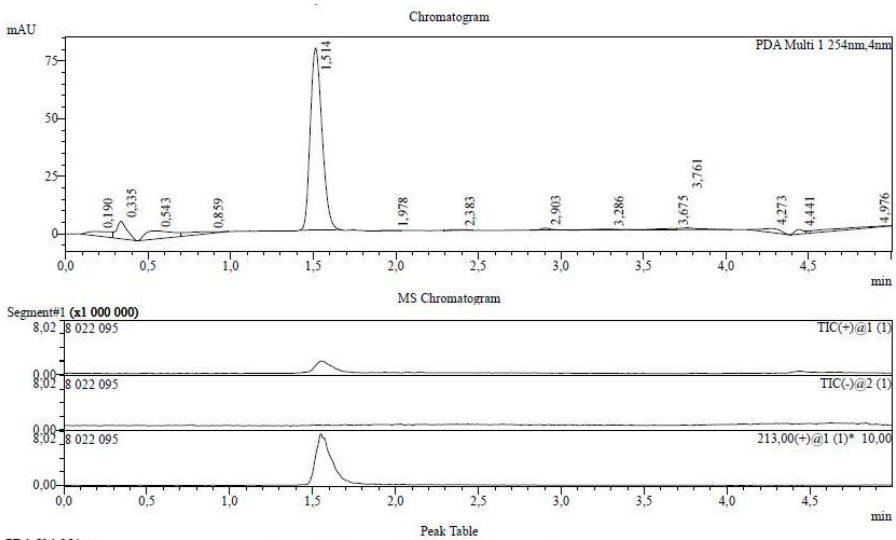


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,373	11635	4,885	2468	9,413	
2	1,077	15367	6,451	793	3,023	
3	1,745	181003	75,989	19764	75,386	
4	2,005	1764	0,741	559	2,131	
5	2,788	1372	0,576	339	1,294	
6	3,051	1235	0,518	208	0,793	
7	3,306	2057	0,864	255	0,973	
8	3,771	7995	3,356	449	1,713	
9	4,438	2486	1,043	488	1,860	
10	4,722	13283	5,577	895	3,413	
Total		238196	100,000	26217	100,000	

Supplementary Fig. 75. The high-performance liquid chromatography chromatogram of compound **S12**

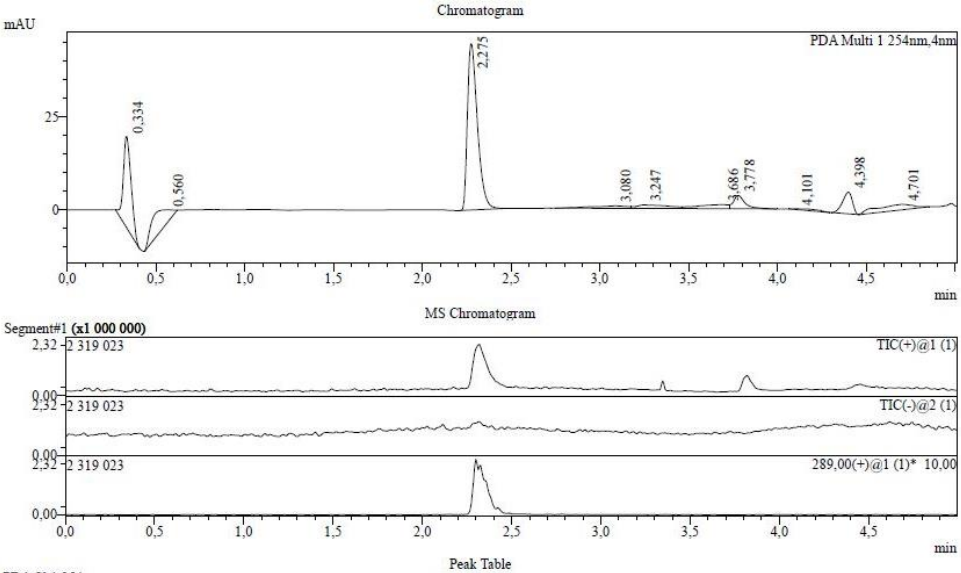


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,190	19900	3,537	1891	1,893	
2	0,335	32437	5,766	7678	7,686	
3	0,543	40076	7,124	3538	3,542	
4	0,859	15309	2,721	840	0,840	
5	1,514	390264	69,375	78893	78,976	
6	1,978	1489	0,265	251	0,252	
7	2,383	2416	0,429	385	0,386	
8	2,903	3681	0,654	892	0,892	
9	3,286	3871	0,688	340	0,340	
10	3,675	4462	0,793	500	0,501	
11	3,761	6348	1,128	812	0,813	
12	4,273	13902	2,471	1694	1,695	
13	4,441	7489	1,331	2015	2,017	
14	4,976	20895	3,714	166	0,166	
Total		562539	100,000	99895	100,000	

Supplementary Fig. 76. The high-performance liquid chromatography chromatogram of compound **S14**

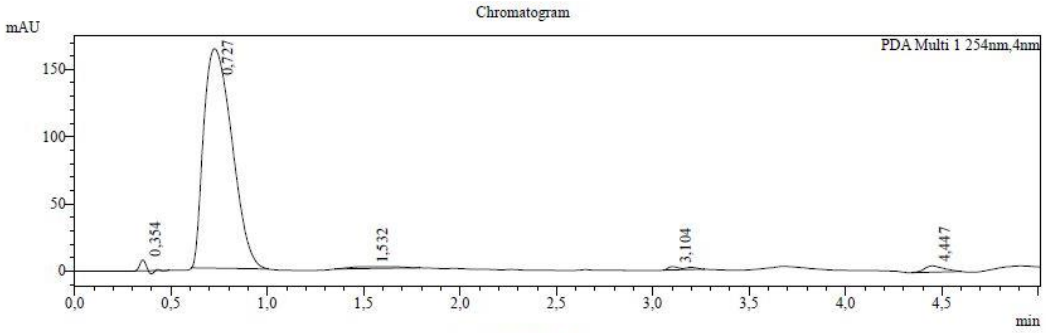


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,334	73392	18,811	24464	28,332	
2	0,560	41958	10,754	3586	4,153	
3	2,275	175805	45,060	44609	51,663	
4	3,080	9234	2,367	633	0,733	
5	3,247	11826	3,031	947	1,096	
6	3,686	12985	3,328	1085	1,257	
7	3,778	15995	4,100	3559	4,121	
8	4,101	3489	0,894	208	0,241	
9	4,398	22284	5,712	5812	6,731	
10	4,701	23184	5,942	1444	1,672	
Total		390154	100,000	86347	100,000	

Supplementary Fig. 77. The high-performance liquid chromatography chromatogram of compound **S15**

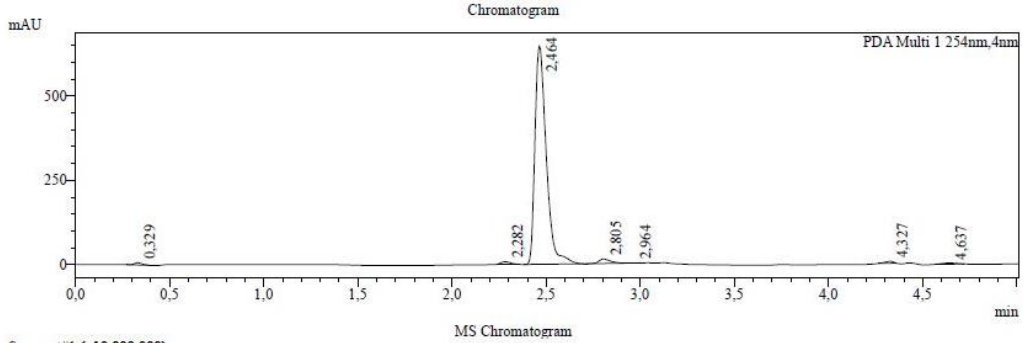


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,354	13672	0,778	8008	4,454	
2	0,727	1672894	95,149	163358	90,855	
3	1,532	23645	1,345	1438	0,800	
4	3,104	15956	0,908	2445	1,360	
5	4,447	32023	1,821	4552	2,532	
Total		1758191	100,000	179801	100,000	

Supplementary Fig. 78. The high-performance liquid chromatography chromatogram of compound **S16**

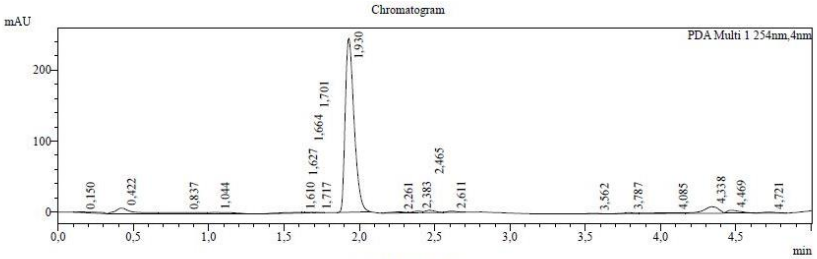


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,329	18704	0,658	6635	0,969	
2	2,282	24594	0,866	7291	1,065	
3	2,464	2703766	95,180	648755	94,761	
4	2,805	60389	2,126	13504	1,973	
5	2,964	1228	0,043	443	0,065	
6	4,327	16678	0,587	5292	0,773	
7	4,637	15314	0,539	2701	0,395	
Total		2840673	100,000	684620	100,000	

Supplementary Fig. 79. The high-performance liquid chromatography chromatogram of compound **S17*R*/S17*S***

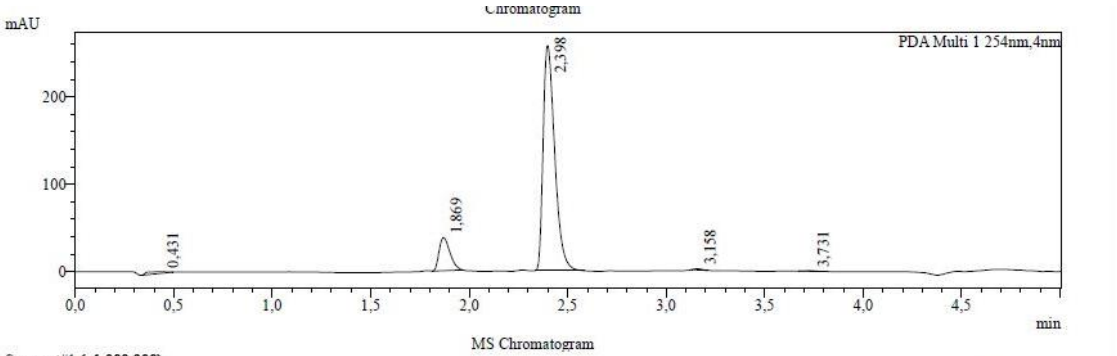


PDA Ch1 254nm

Peak Table

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,150	12461	0,945	691	0,241	
2	0,422	64146	4,865	7994	2,785	
3	0,837	36261	2,750	1928	0,672	
4	1,044	25696	1,949	2130	0,742	
5	1,610	11971	0,908	931	0,324	
6	1,627	1048	0,079	843	0,294	
7	1,664	1973	0,150	660	0,230	
8	1,701	402	0,031	424	0,148	
9	1,717	663	0,050	337	0,117	
10	1,930	997763	75,670	244836	85,311	
11	2,261	11326	0,859	1554	0,541	
12	2,383	9595	0,728	2234	0,778	
13	2,465	14722	1,117	3386	1,180	
14	2,611	7364	0,558	1837	0,640	
15	3,562	596	0,045	189	0,066	
16	3,787	7232	0,548	1363	0,475	
17	4,085	15786	1,197	886	0,309	
18	4,338	65204	4,945	9287	3,236	
19	4,469	25557	1,938	4089	1,425	
20	4,721	8813	0,668	1397	0,487	
Total		1318579	100,000	286993	100,000	

Supplementary Fig. 80. The high-performance liquid chromatography chromatogram of compound **S18*R*/S18*S***

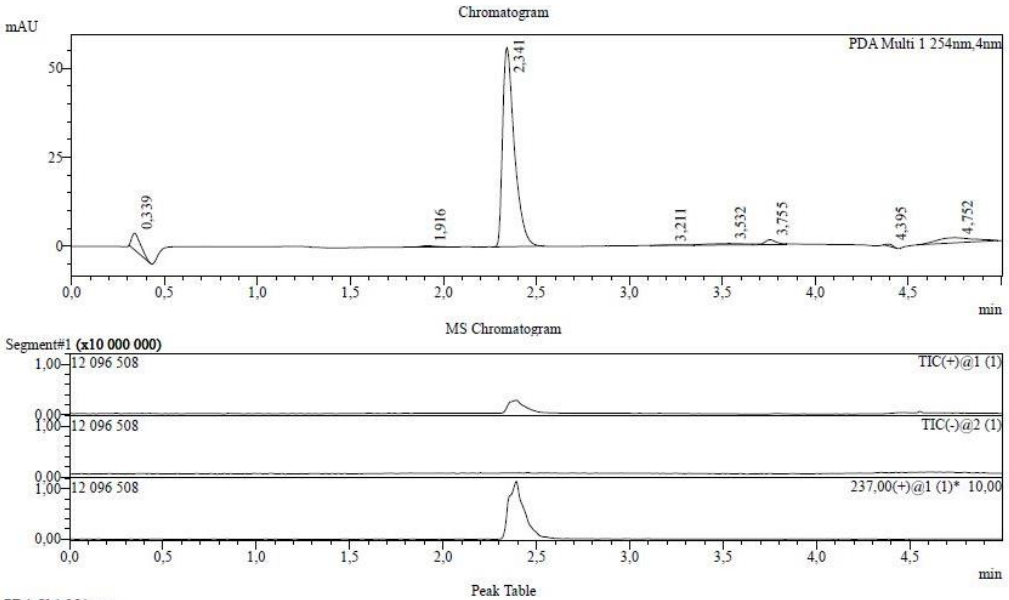


PDA Ch1 254nm

Peak Table

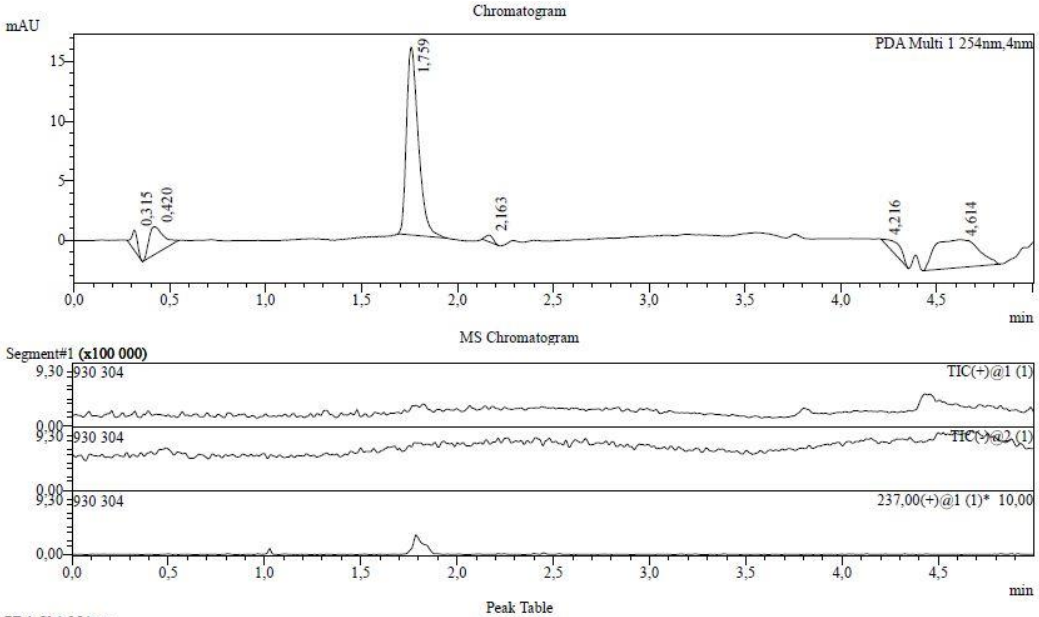
Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,431	16675	1,368	1972	0,660	
2	1,869	144523	11,858	37725	12,624	
3	2,398	1052522	86,362	257319	86,107	
4	3,158	3486	0,286	1378	0,461	
5	3,731	1531	0,126	443	0,148	
Total		1218737	100,000	298837	100,000	

Supplementary Fig. 81. The high-performance liquid chromatography chromatogram of compound **S19*R*/S19*S***



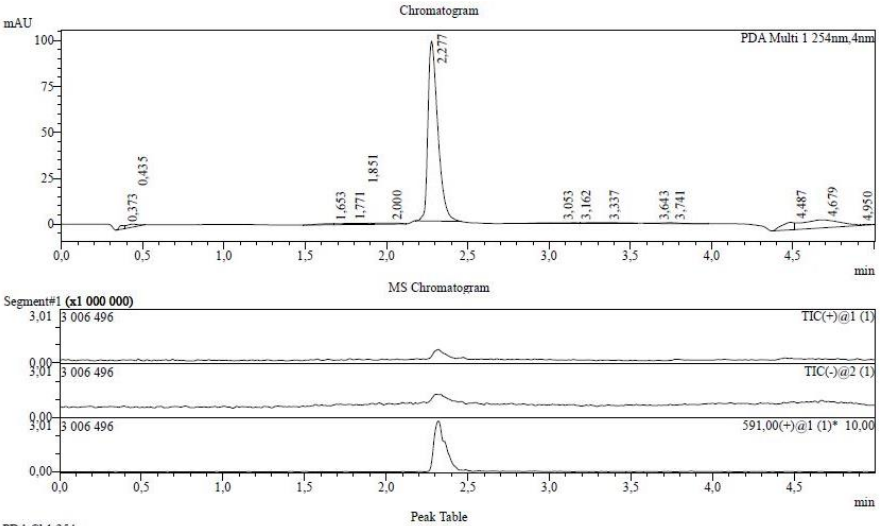
Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,339	15244	5,109	4645	7,154	
2	1,916	1725	0,578	326	0,501	
3	2,341	246595	82,638	56042	86,328	
4	3,211	1444	0,484	146	0,225	
5	3,532	5590	1,873	384	0,592	
6	3,755	6216	2,083	1377	2,121	
7	4,395	1252	0,420	564	0,869	
8	4,752	20338	6,816	1434	2,209	
Total		298404	100,000	64918	100,000	

Supplementary Fig. 82. The high-performance liquid chromatography chromatogram of compound **S20*R*,*R*/S20*S*,*S***



Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,315	3288	2,589	1717	7,531	
2	0,420	12246	9,643	2329	10,212	
3	1,759	67742	53,342	15745	69,050	
4	2,163	1464	1,153	545	2,389	
5	4,216	5114	4,027	127	0,557	
6	4,614	37142	29,247	2340	10,261	
Total		126995	100,000	22803	100,000	

Supplementary Fig. 83. The high-performance liquid chromatography chromatogram of compound **S21*S*,*S*/S21*R*,*R***

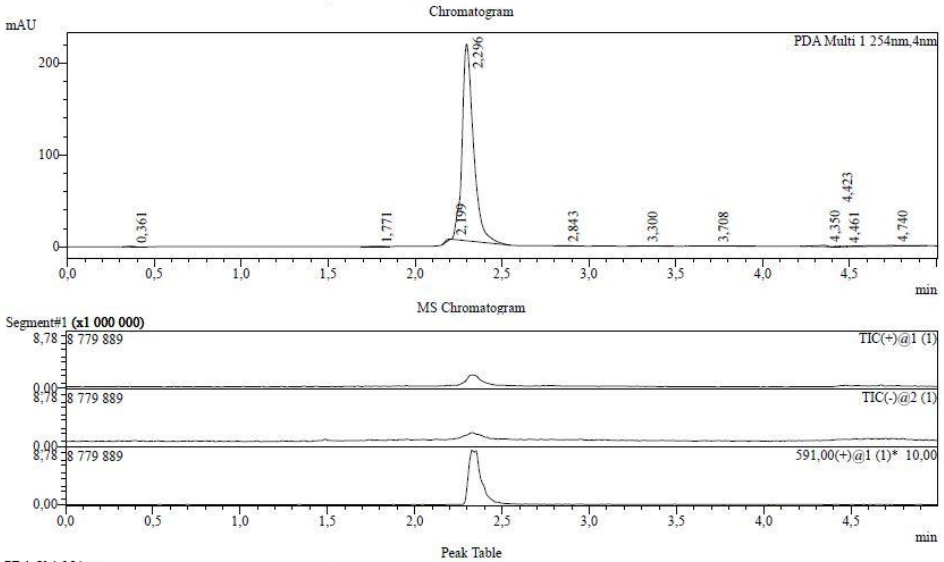


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,373	4745	0,842	1998	1,736	
2	0,435	7917	1,404	1599	1,389	
3	1,653	4283	0,760	580	0,504	
4	1,771	6213	1,102	704	0,611	
5	1,851	2940	0,522	606	0,527	
6	2,000	5296	0,940	564	0,490	
7	2,277	415435	73,693	97913	85,073	
8	3,053	3981	0,706	413	0,359	
9	3,162	2729	0,484	484	0,421	
10	3,337	10038	1,781	575	0,499	
11	3,643	1531	0,272	290	0,252	
12	3,741	4211	0,747	601	0,522	
13	4,487	22609	4,011	4055	3,524	
14	4,679	70695	12,540	4326	3,759	
15	4,950	1117	0,198	387	0,336	
Total		563740	100,000	115093	100,000	

Supplementary Fig. 84. The high-performance liquid chromatography chromatogram of compound **1a*R*-IN-8**

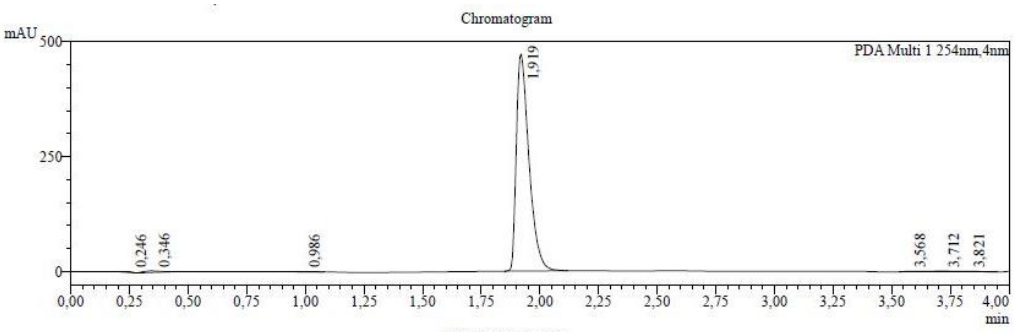


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,361	2445	0,239	753	0,343	
2	1,771	1285	0,126	265	0,121	
3	2,199	3961	0,387	538	0,245	
4	2,296	982654	96,102	214006	97,343	
5	2,843	1280	0,125	241	0,109	
6	3,300	1026	0,100	128	0,058	
7	3,708	3259	0,319	279	0,127	
8	4,350	6982	0,683	1545	0,703	
9	4,423	1197	0,117	598	0,272	
10	4,461	1531	0,150	678	0,308	
11	4,740	16895	1,652	818	0,372	
Total		1022514	100,000	219848	100,000	

Supplementary Fig. 85. The high-performance liquid chromatography chromatogram of compound **1a*S*-IN-8**

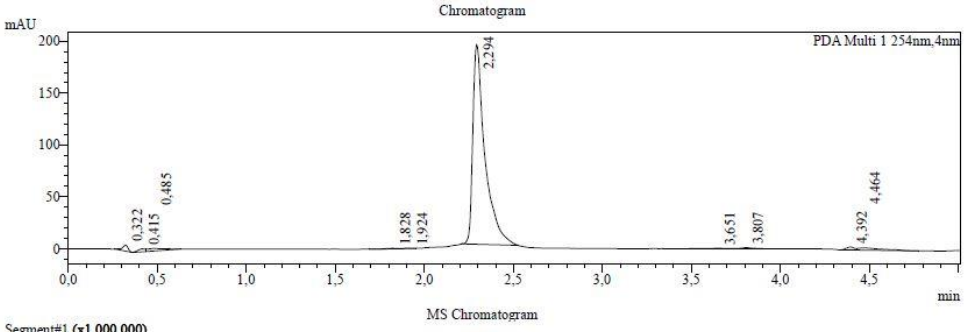


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,246	4177	0,228	1937	0,401	
2	0,346	18477	1,006	3597	0,744	
3	0,986	6288	0,342	545	0,113	
4	1,919	1771089	96,466	471743	97,567	
5	3,568	4445	0,242	1526	0,316	
6	3,712	19832	1,080	2291	0,474	
7	3,821	11673	0,636	1870	0,387	
Total		1835981	100,000	483509	100,000	

Supplementary Fig. 86. The high-performance liquid chromatography chromatogram of compound **2-IN-8**

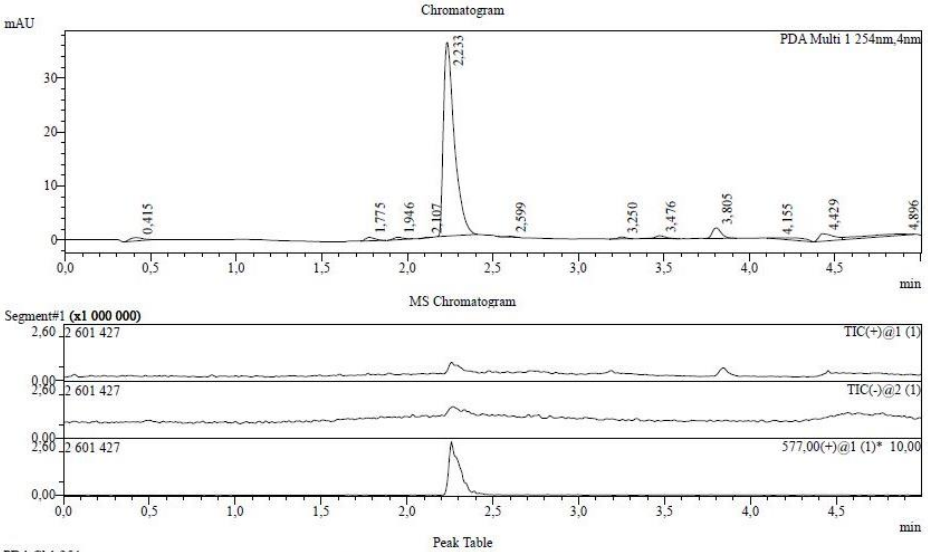


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0.322	12472	1.256	5941	2.817	
2	0.415	8556	0.862	2784	1.320	
3	0.485	15305	1.541	2175	1.031	
4	1.828	1779	0.179	386	0.183	
5	1.924	947	0.095	229	0.109	
6	2.294	914000	92.036	192883	91.455	
7	3.651	3547	0.357	356	0.169	
8	3.807	5817	0.586	1076	0.510	
9	4.392	8825	0.889	2804	1.329	
10	4.464	21843	2.199	2269	1.076	
Total		993089	100.000	210904	100.000	

Supplementary Fig. 87. The high-performance liquid chromatography chromatogram of compound **3-IN-8**

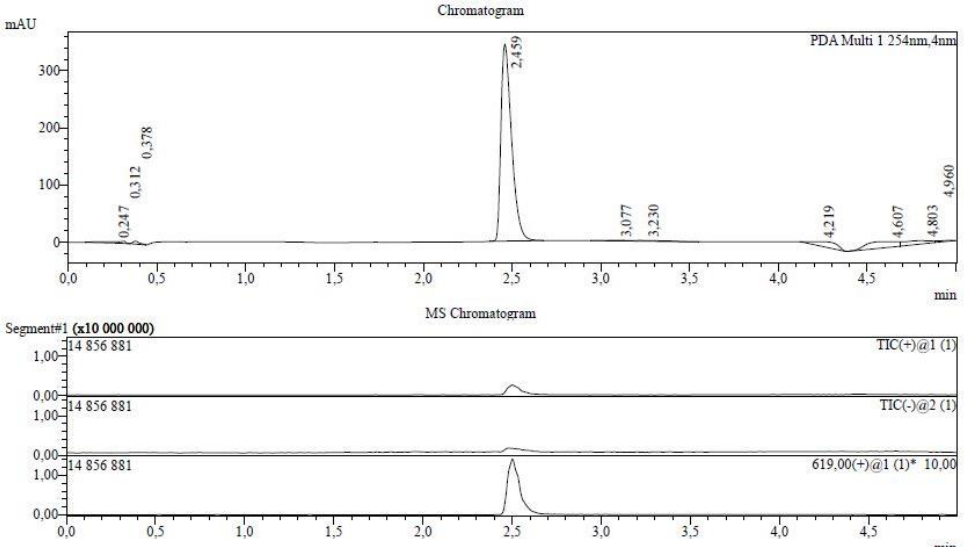


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,415	3429	1,714	619	1,455	
2	1,775	2542	1,270	669	1,572	
3	1,946	1666	0,833	441	1,036	
4	2,107	214	0,107	99	0,232	
5	2,233	160218	80,091	35968	84,515	
6	2,599	374	0,187	145	0,340	
7	3,250	1095	0,547	333	0,783	
8	3,476	2089	1,045	510	1,199	
9	3,805	7003	3,501	1949	4,579	
10	4,155	4670	2,334	182	0,428	
11	4,429	8517	4,258	1403	3,298	
12	4,896	8227	4,113	240	0,563	
Total		200045	100,000	42558	100,000	

Supplementary Fig. 88. The high-performance liquid chromatography chromatogram of compound **4-IN-8**

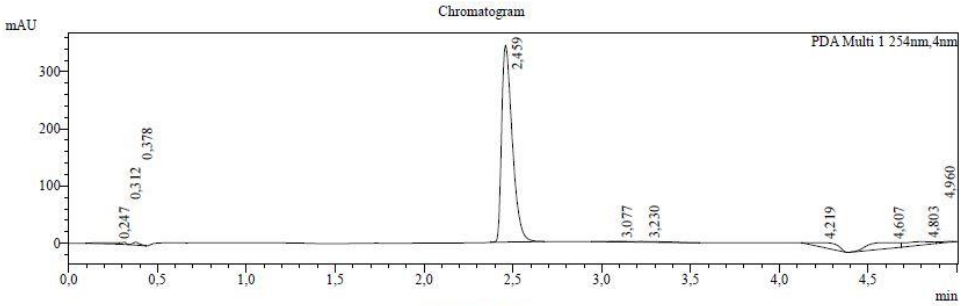


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,247	15086	0,840	1976	0,517	
2	0,312	7452	0,415	3959	1,036	
3	0,378	12975	0,723	5078	1,329	
4	2,459	1432415	79,789	344946	90,269	
5	3,077	9365	0,522	956	0,250	
6	3,230	16811	0,936	1293	0,338	
7	4,219	88028	4,903	6593	1,725	
8	4,607	137992	7,686	10324	2,702	
9	4,803	64745	3,606	5751	1,505	
10	4,960	10382	0,578	1254	0,328	
Total		1795251	100,000	382130	100,000	

Supplementary Fig. 89. The high-performance liquid chromatography chromatogram of compound **5*R*-IN-8**

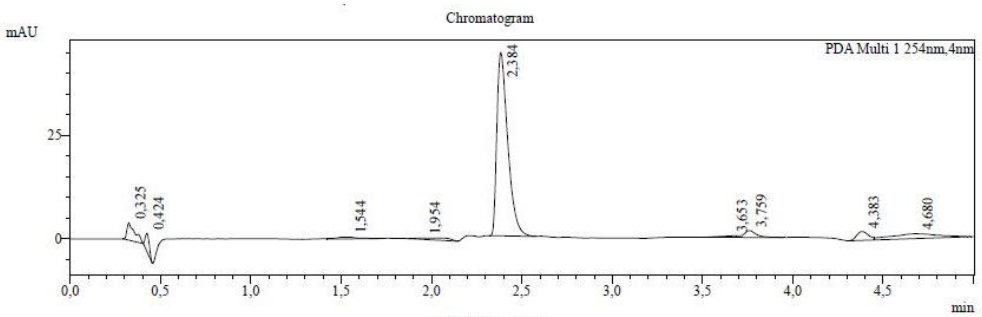


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,247	15086	0,840	1976	0,517	
2	0,312	7452	0,415	3959	1,036	
3	0,378	12975	0,723	5078	1,329	
4	2,459	1432415	79,789	344946	90,269	
5	3,077	9365	0,522	956	0,250	
6	3,230	16811	0,936	1293	0,338	
7	4,219	88028	4,903	6593	1,725	
8	4,607	137992	7,686	10324	2,702	
9	4,803	64745	3,606	5751	1,505	
10	4,960	10382	0,578	1254	0,328	
Total		1795251	100,000	382130	100,000	

Supplementary Fig. 90. The high-performance liquid chromatography chromatogram of compound **5*S*-IN-8**

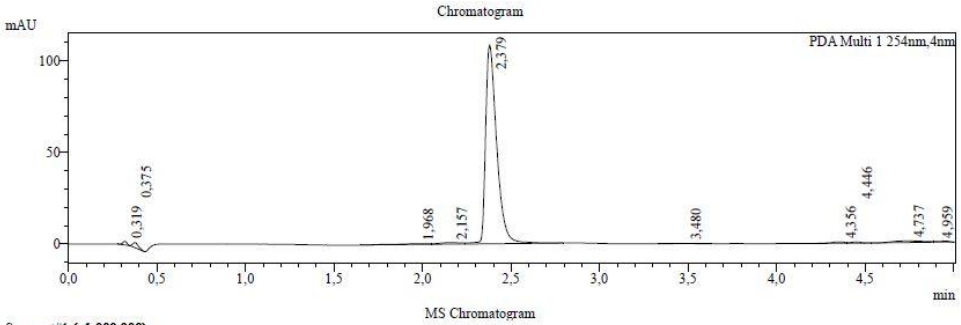


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,325	12721	4,969	4258	7,207	
2	0,424	6186	2,416	4335	7,337	
3	1,544	3936	1,537	445	0,752	
4	1,954	5277	2,061	337	0,569	
5	2,384	186439	72,822	44402	75,144	
6	3,653	2161	0,844	335	0,566	
7	3,759	7804	3,048	1654	2,799	
8	4,383	9788	3,823	2138	3,618	
9	4,680	21709	8,479	1186	2,008	
Total		256021	100,000	59089	100,000	

Supplementary Fig. 91. The high-performance liquid chromatography chromatogram of compound **6*R*,*R*-IN-8**

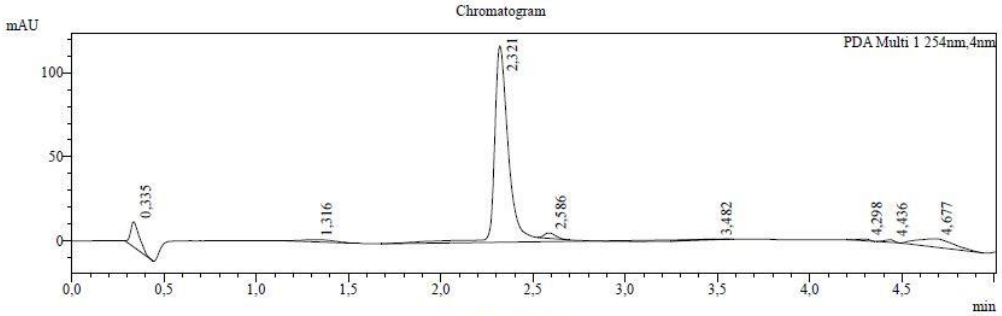


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,319	3120	0,607	2035	1,733	
2	0,375	5884	1,145	2782	2,369	
3	1,968	5554	1,080	421	0,359	
4	2,157	7282	1,417	813	0,692	
5	2,379	470979	91,616	108465	92,378	
6	3,480	1851	0,360	196	0,167	
7	4,356	3593	0,699	745	0,634	
8	4,446	2755	0,536	621	0,529	
9	4,737	11033	2,146	906	0,772	
10	4,959	2026	0,394	430	0,367	
Total		514077	100,000	117414	100,000	

Supplementary Fig. 92. The high-performance liquid chromatography chromatogram of compound **6*S*,*S*-IN-8**

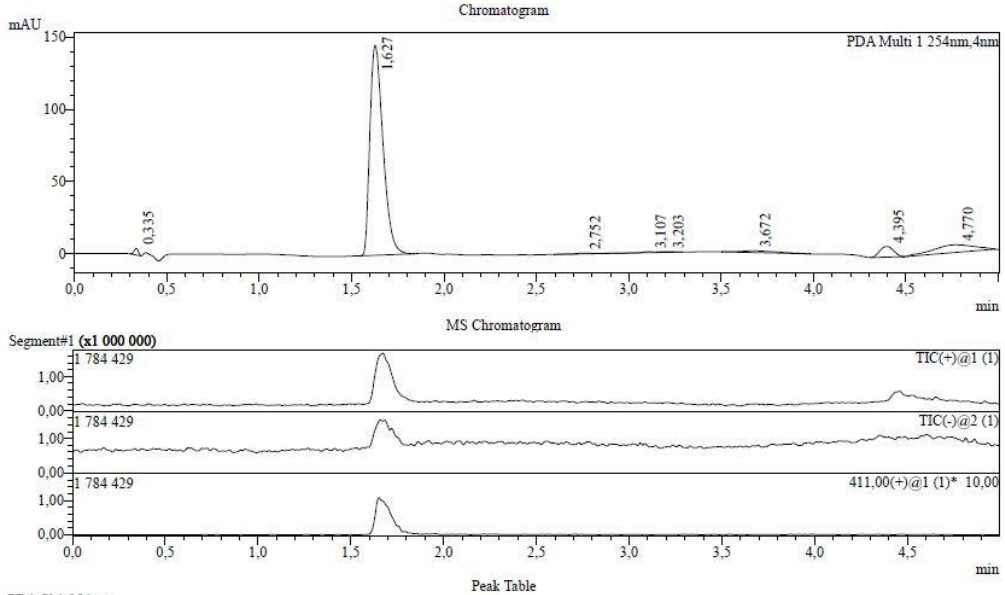


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,335	49063	6,262	15054	10,413	
2	1,316	16076	2,052	1331	0,921	
3	2,321	620919	79,250	116985	80,919	
4	2,586	13612	1,737	3244	2,244	
5	3,482	5338	0,681	389	0,269	
6	4,298	4473	0,571	1021	0,706	
7	4,436	5356	0,684	1536	1,062	
8	4,677	68656	8,763	5011	3,466	
Total		783493	100,000	144570	100,000	

Supplementary Fig. 93. The high-performance liquid chromatography chromatogram of compound **S26**

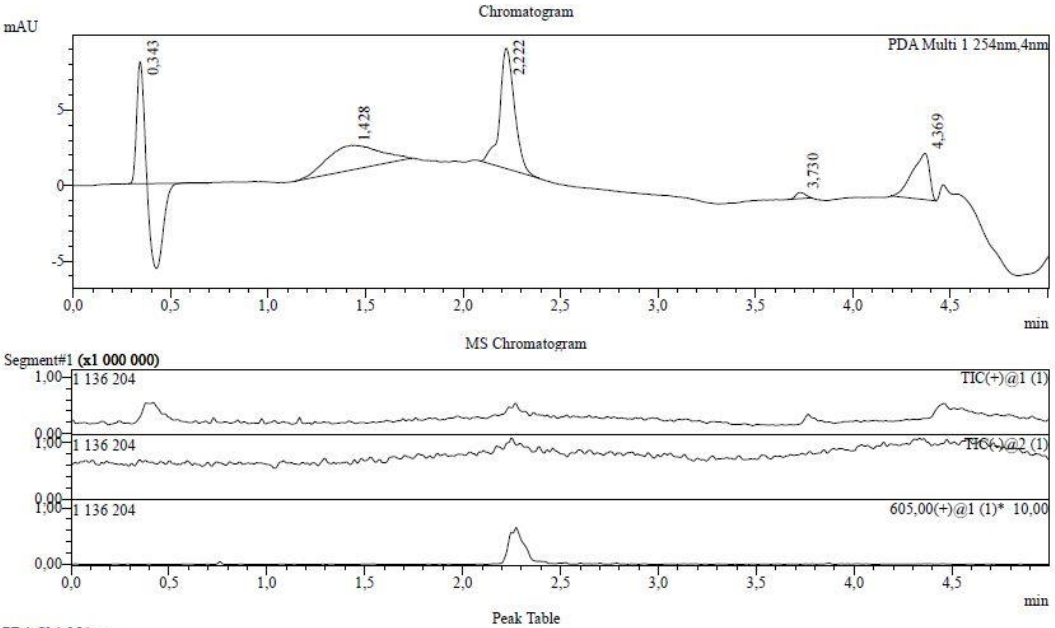


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,335	6752	0,773	4691	2,825	
2	1,627	719446	82,389	145870	87,827	
3	2,752	3052	0,349	526	0,317	
4	3,107	2999	0,343	649	0,391	
5	3,203	2436	0,279	495	0,298	
6	3,672	16361	1,874	1160	0,699	
7	4,395	38344	4,391	7429	4,473	
8	4,770	83840	9,601	5268	3,172	
Total		873231	100,000	166089	100,000	

Supplementary Fig. 94. The high-performance liquid chromatography chromatogram of compound **S27**

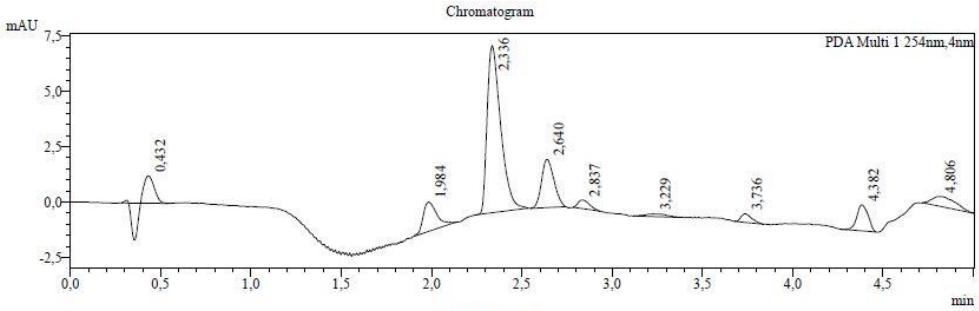


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,343	-4721	-5,360	8116	38,242	
2	1,428	30394	34,506	1650	7,775	
3	2,222	42111	47,808	7974	37,574	
4	3,730	1426	1,618	399	1,881	
5	4,369	18874	21,427	3083	14,528	
Total		88084	100,000	21222	100,000	

Supplementary Fig. 95. The high-performance liquid chromatography chromatogram of compound **1a'*R*-IN-8**

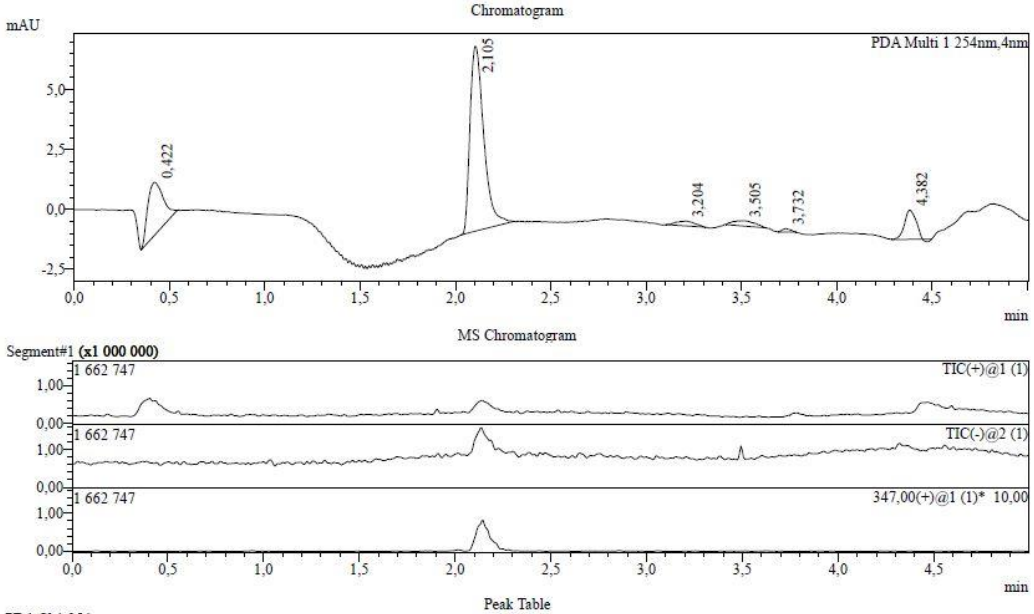


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,432	1284	1,793	1244	8,403	
2	1,984	6944	9,696	1332	8,997	
3	2,336	39540	55,209	7553	51,004	
4	2,640	10421	14,550	2178	14,704	
5	2,837	1682	2,348	391	2,643	
6	3,229	1010	1,411	124	0,834	
7	3,736	1594	2,226	387	2,615	
8	4,382	5035	7,031	1172	7,916	
9	4,806	4108	5,736	427	2,885	
Total		71618	100,000	14809	100,000	

Supplementary Fig. 96. The high-performance liquid chromatography chromatogram of compound **S29**

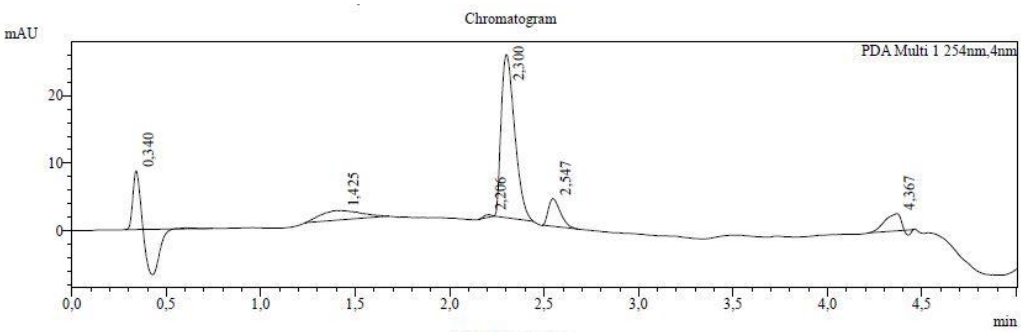


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,422	12587	21,009	2210	18,901	
2	2,105	38874	64,883	7715	65,978	
3	3,204	1378	2,300	204	1,744	
4	3,505	1698	2,835	213	1,821	
5	3,732	422	0,704	141	1,209	
6	4,382	4954	8,269	1210	10,348	
Total		59913	100,000	11694	100,000	

Supplementary Fig. 97. The high-performance liquid chromatography chromatogram of compound **S30**

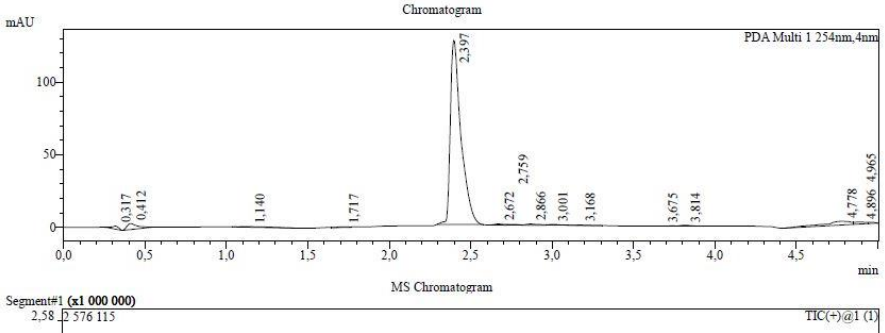


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,340	-8193	-4,925	8736	21,084	
2	1,425	20709	12,448	1408	3,398	
3	2,206	1166	0,701	388	0,936	
4	2,300	120668	72,529	24227	58,473	
5	2,547	18184	10,930	4111	9,922	
6	4,367	13836	8,316	2564	6,188	
Total		166371	100,000	41433	100,000	

Supplementary Fig. 98. The high-performance liquid chromatography chromatogram of compound **1a”*R*-IN-8**

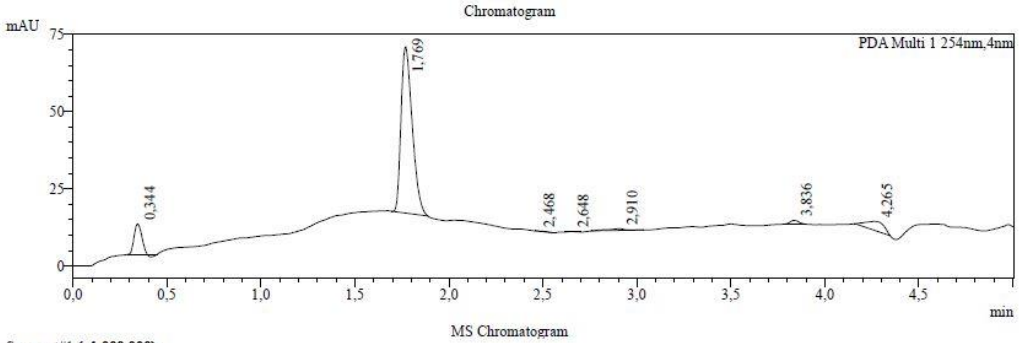


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,317	5374	0,804	2005	1,411	
2	0,412	19066	2,853	4117	2,896	
3	1,140	5846	0,875	342	0,241	
4	1,717	2287	0,342	445	0,313	
5	2,397	575670	86,145	126796	89,185	
6	2,672	2755	0,412	693	0,488	
7	2,759	2007	0,300	513	0,361	
8	2,866	4661	0,698	827	0,582	
9	3,001	3458	0,517	647	0,455	
10	3,168	2121	0,317	344	0,242	
11	3,675	1095	0,164	128	0,090	
12	3,814	2329	0,349	618	0,435	
13	4,778	32675	4,890	2631	1,851	
14	4,896	7174	1,074	1333	0,938	
15	4,965	1735	0,260	732	0,515	
Total		668253	100,000	142172	100,000	

Supplementary Fig. 99. The high-performance liquid chromatography chromatogram of compound **S32*R*/S32*S***

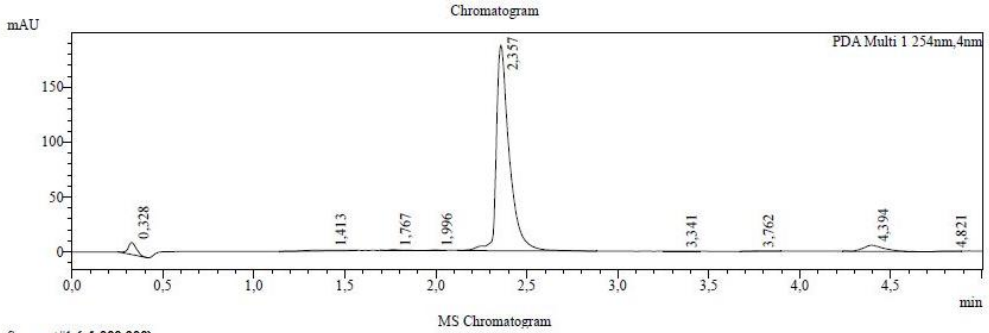


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,344	26227	9,438	9829	14,323	
2	1,769	223070	80,276	53933	78,589	
3	2,468	332	0,119	74	0,108	
4	2,648	413	0,149	177	0,258	
5	2,910	4004	1,441	526	0,767	
6	3,836	3842	1,383	1181	1,721	
7	4,265	19992	7,194	2906	4,235	
Total		277879	100,000	68626	100,000	

Supplementary Fig. 100. The high-performance liquid chromatography chromatogram of compound **S33*R*/S33*S***

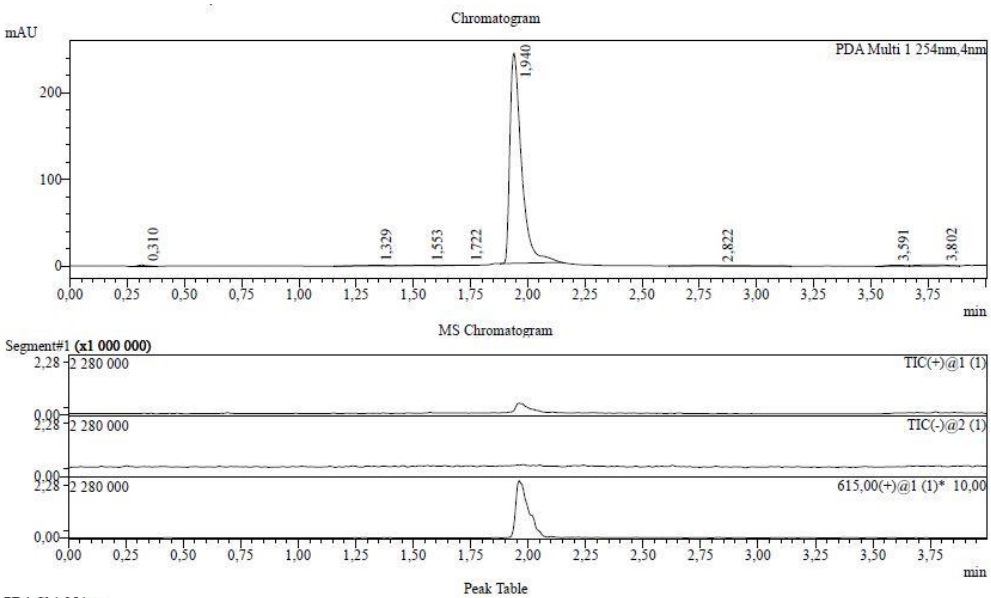


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,328	34105	3,302	10945	5,294	
2	1,413	9567	0,926	623	0,301	
3	1,767	3440	0,333	687	0,332	
4	1,996	2331	0,226	608	0,294	
5	2,357	932331	90,268	187480	90,692	
6	3,341	1309	0,127	172	0,083	
7	3,762	2908	0,282	354	0,171	
8	4,394	44931	4,350	5596	2,707	
9	4,821	1929	0,187	257	0,124	
Total		1032851	100,000	206722	100,000	

Supplementary Fig. 101. The high-performance liquid chromatography chromatogram of compound **1b*R*-IN-8**

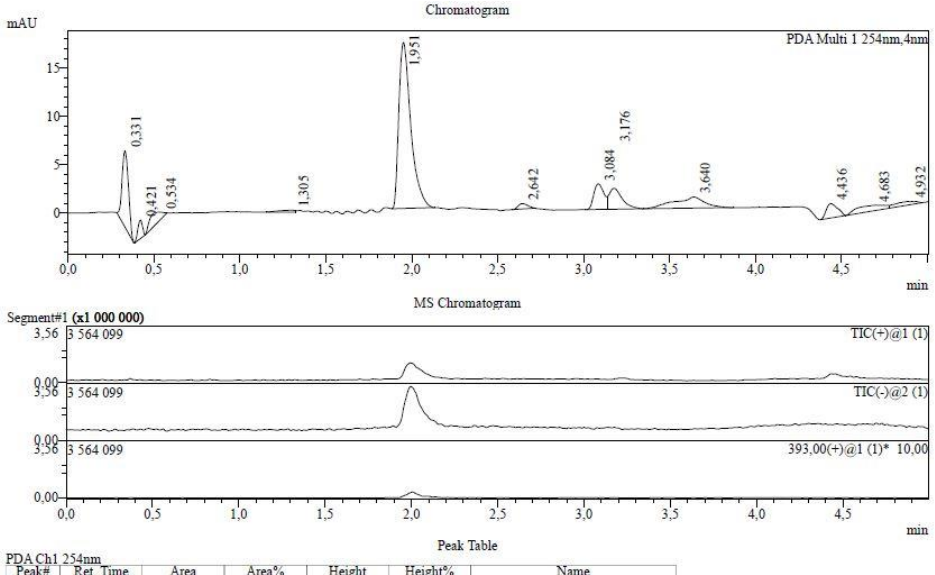


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,310	4959	0,551	1628	0,655	
2	1,329	6190	0,688	722	0,290	
3	1,553	2745	0,305	263	0,106	
4	1,722	496	0,055	141	0,057	
5	1,940	859363	95,461	243019	97,711	
6	2,822	7220	0,802	489	0,196	
7	3,591	6854	0,761	1099	0,442	
8	3,802	12392	1,377	1351	0,543	
Total		900220	100,000	248711	100,000	

Supplementary Fig. 102. The high-performance liquid chromatography chromatogram of compound **1b*S*-IN-8**

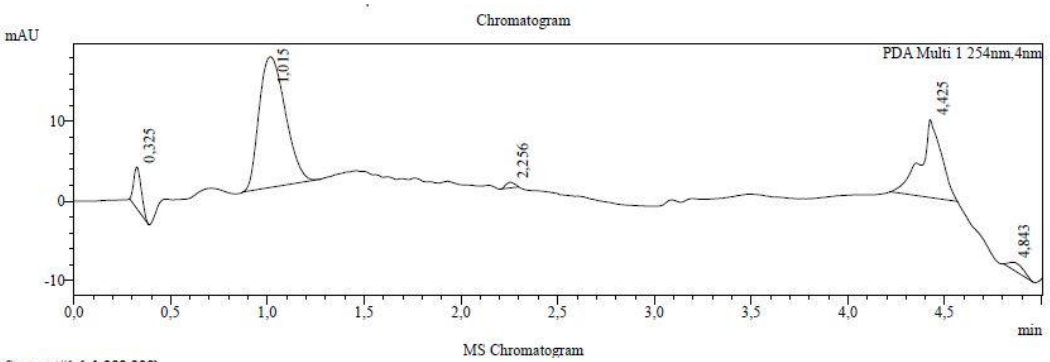


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,331	19945	11,634	7906	21,435	
2	0,421	3725	2,172	1910	5,178	
3	0,534	6484	3,782	834	2,260	
4	1,305	1369	0,799	218	0,591	
5	1,951	83467	48,686	17188	46,599	
6	2,642	2160	1,260	560	1,518	
7	3,084	11021	6,428	2645	7,171	
8	3,176	11359	6,626	2162	5,863	
9	3,640	14596	8,514	1156	3,135	
10	4,436	6878	4,012	1490	4,039	
11	4,683	7092	4,137	571	1,548	
12	4,932	3343	1,950	244	0,662	
Total		171440	100,000	36885	100,000	

Supplementary Fig. 103. The high-performance liquid chromatography chromatogram of compound **S34*R*/S34*S***

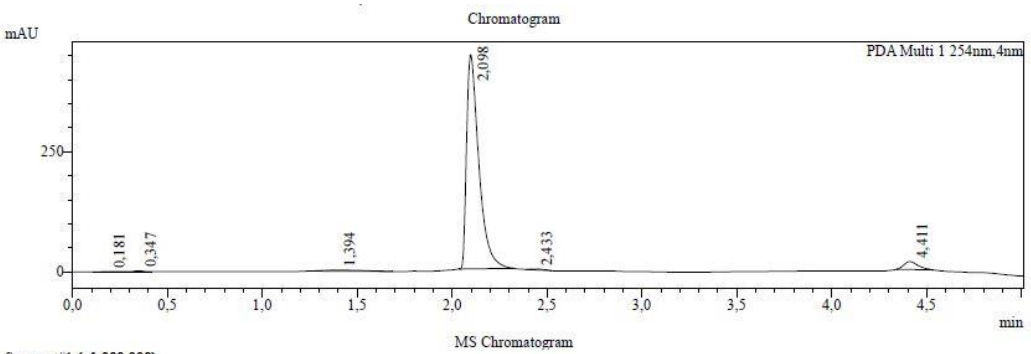


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0.325	13772	5,474	5247	15,916	
2	1.015	155575	61,838	16436	49,859	
3	2.256	2286	0,909	701	2,125	
4	4.425	74725	29,702	9796	29,715	
5	4.843	5227	2,078	786	2,385	
Total		251586	100,000	32965	100,000	

Supplementary Fig. 104. The high-performance liquid chromatography chromatogram of compound **S35*R*/S35*S***

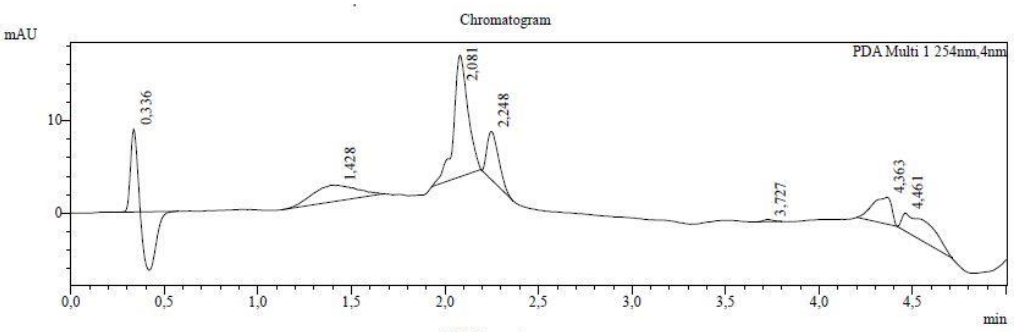


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0.181	1356	0,063	237	0,051	
2	0.347	9360	0,436	2313	0,493	
3	1.394	34329	1,601	2169	0,462	
4	2.098	2004708	93,475	446111	95,069	
5	2.433	10091	0,471	1984	0,423	
6	4.411	84813	3,955	16435	3,502	
Total		2144656	100,000	469249	100,000	

Supplementary Fig. 105. The high-performance liquid chromatography chromatogram of compound **1c*R*-IN-8**

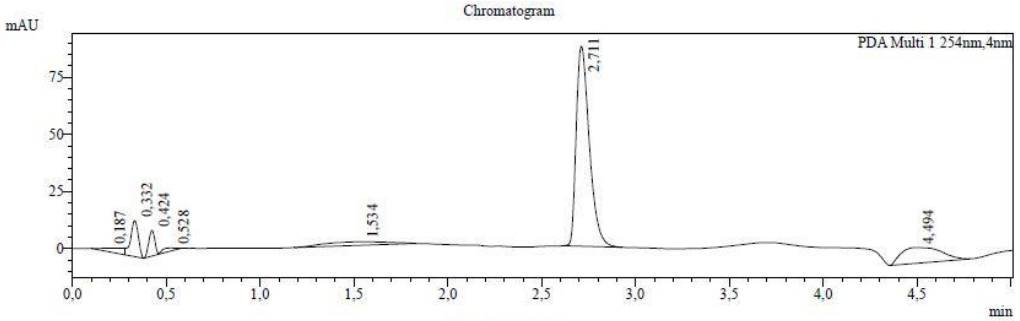


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,336	-7242	-4,462	8993	26,371	
2	1,428	29108	17,935	1714	5,027	
3	2,081	70475	43,425	13132	38,507	
4	2,248	22275	13,725	5195	15,232	
5	3,727	851	0,524	225	0,659	
6	4,363	20208	12,452	2899	8,501	
7	4,461	26616	16,400	1945	5,703	
Total		162291	100,000	34103	100,000	

Supplementary Fig. 106. The high-performance liquid chromatography chromatogram of compound **1c*S*-IN-8**

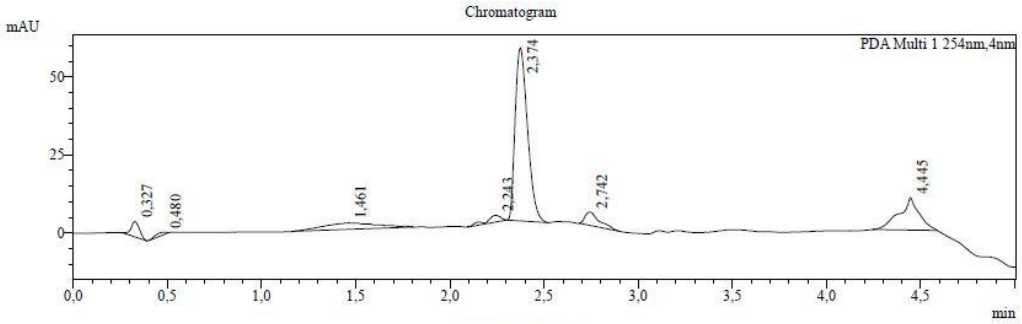


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,187	15310	2,321	1428	1,134	
2	0,332	44801	6,792	15854	12,588	
3	0,424	25525	3,870	11243	8,926	
4	0,528	8039	1,219	1255	0,997	
5	1,534	37212	5,642	1509	1,198	
6	2,711	426522	64,666	87743	69,667	
7	4,494	102165	15,490	6915	5,490	
Total		659574	100,000	125946	100,000	

Supplementary Fig. 107. The high-performance liquid chromatography chromatogram of compound **S36*R***

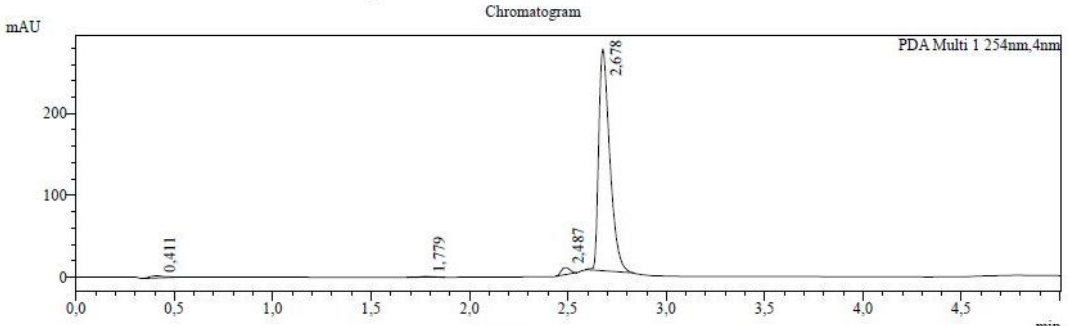


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,327	14866	3,502	4843	6,055	
2	0,480	4156	0,979	698	0,873	
3	1,461	41468	9,768	2090	2,613	
4	2,243	11650	2,744	2084	2,606	
5	2,374	253154	59,631	55551	69,459	
6	2,742	22727	5,353	4301	5,378	
7	4,445	76514	18,023	10409	13,016	
Total		424534	100,000	79976	100,000	

Supplementary Fig. 108. The high-performance liquid chromatography chromatogram of compound **S37*R***

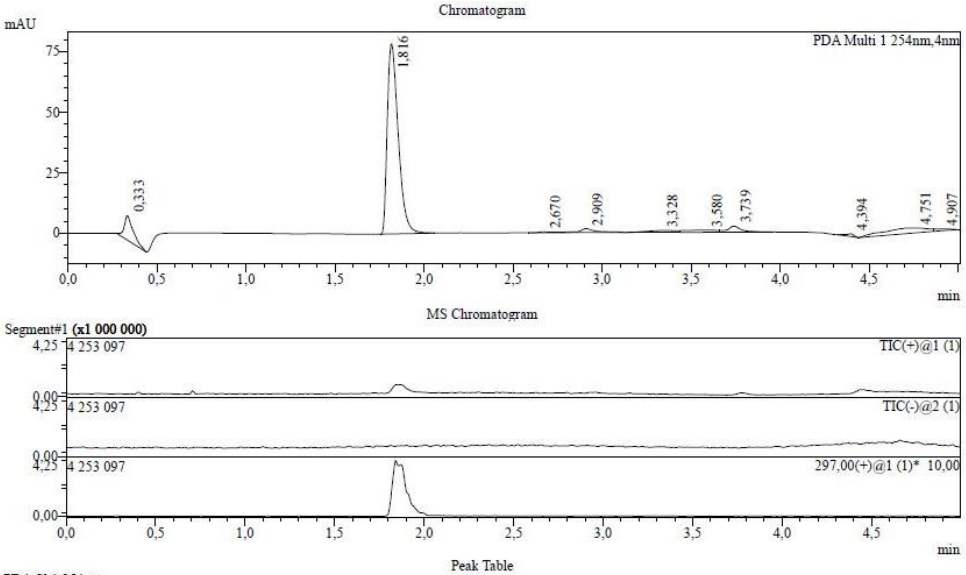


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,411	13602	1,226	2525	0,889	
2	1,779	4601	0,415	1125	0,396	
3	2,487	26689	2,406	8596	3,028	
4	2,678	1064230	95,953	271659	95,687	
Total		1109122	100,000	283906	100,000	

Supplementary Fig. 109. The high-performance liquid chromatography chromatogram of compound **1d*R*-IN-8**

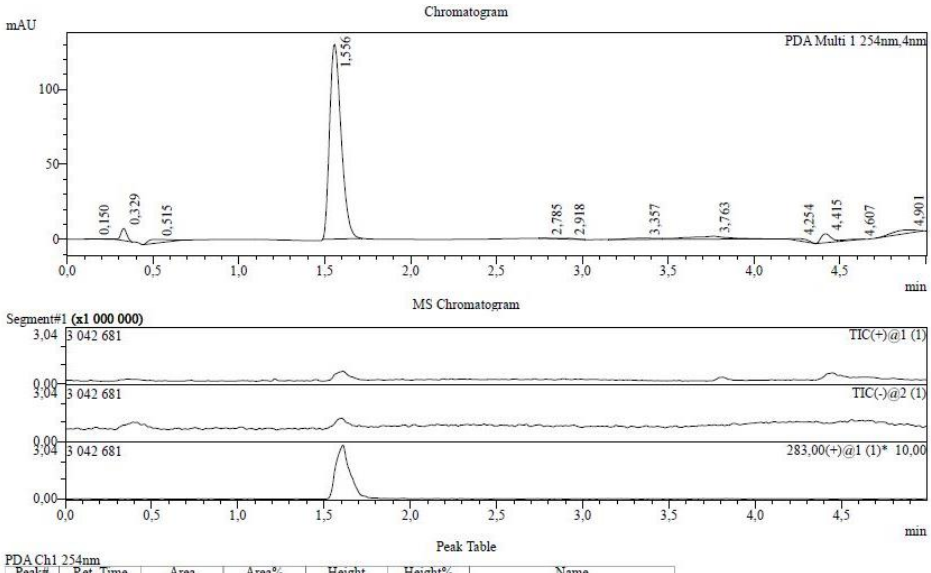


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,333	34908	7,495	10036	10,188	
2	1,816	335513	72,038	78332	79,516	
3	2,670	1493	0,321	337	0,342	
4	2,909	11961	2,568	1614	1,638	
5	3,328	9301	1,997	768	0,780	
6	3,580	11684	2,509	957	0,972	
7	3,739	13951	2,995	2502	2,540	
8	4,394	3208	0,689	1086	1,102	
9	4,751	39219	8,421	2115	2,147	
10	4,907	4503	0,967	764	0,775	
Total		465742	100,000	98511	100,000	

Supplementary Fig. 110. The high-performance liquid chromatography chromatogram of compound **S39**

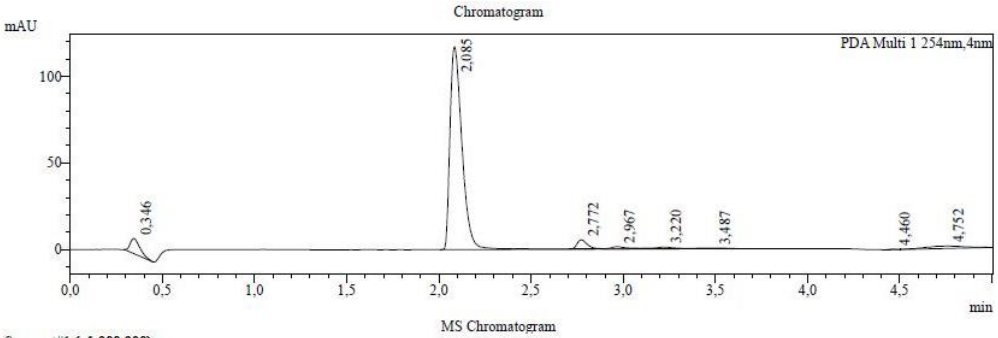


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,150	1640	0,216	222	0,143	
2	0,329	18646	2,460	8119	5,256	
3	0,515	21037	2,776	2544	1,647	
4	1,556	609051	80,355	130191	84,274	
5	2,785	1363	0,180	242	0,156	
6	2,918	2530	0,334	626	0,405	
7	3,357	10491	1,384	878	0,568	
8	3,763	31211	4,118	1899	1,229	
9	4,254	8805	1,162	1300	0,841	
10	4,415	23828	3,144	5920	3,832	
11	4,607	5244	0,692	387	0,251	
12	4,901	24103	3,180	2159	1,398	
Total		757949	100,000	154486	100,000	

Supplementary Fig. 111. The high-performance liquid chromatography chromatogram of compound **S40**

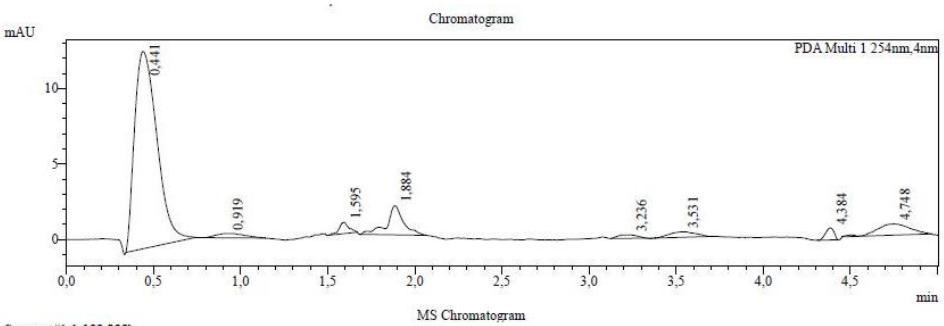


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,346	31696	4,983	8863	6,511	
2	2,085	540365	84,955	117497	86,310	
3	2,772	20361	3,201	5315	3,905	
4	2,967	7240	1,138	1290	0,948	
5	3,220	8640	1,358	1016	0,747	
6	3,487	5273	0,829	398	0,293	
7	4,460	1168	0,184	344	0,253	
8	4,752	21318	3,352	1409	1,035	
Total		636062	100,000	136134	100,000	

Supplementary Fig. 112. The high-performance liquid chromatography chromatogram of compound **S41**



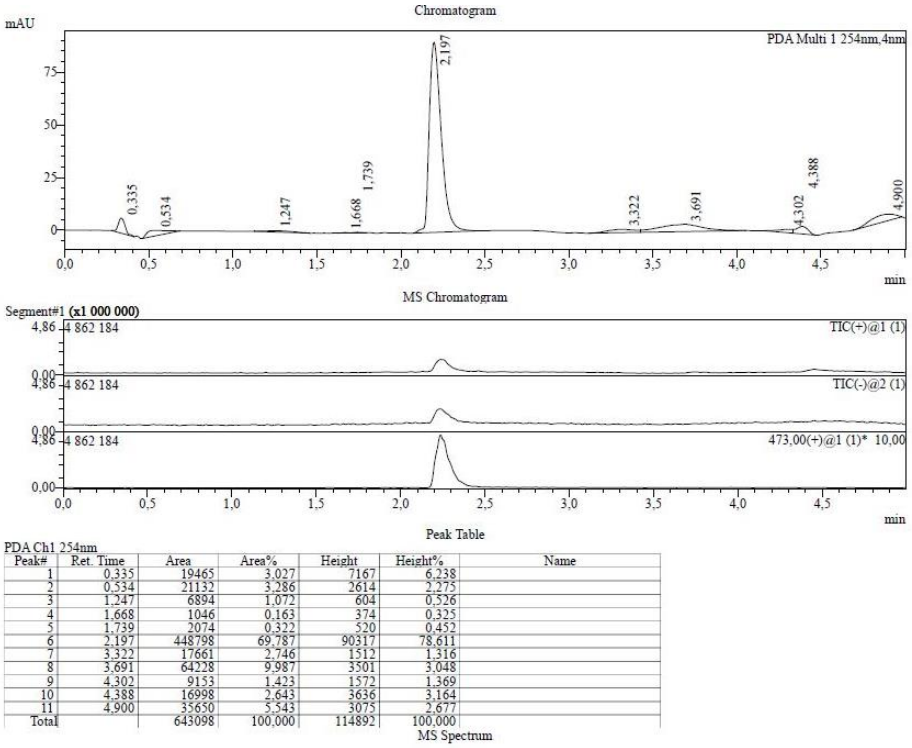
Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,441	117073	75,955	13063	71,857	
2	0,919	3289	2,134	291	1,599	
3	1,595	2926	1,898	748	4,117	
4	1,884	12827	8,322	1920	10,564	
5	3,236	1688	1,095	232	1,274	
6	3,531	3794	2,461	365	2,005	
7	4,384	2705	1,755	806	4,433	
8	4,748	9832	6,379	754	4,150	
Total		154134	100,000	18179	100,000	

MS Spectrum

Supplementary Fig. 113. The high-performance liquid chromatography chromatogram of compound **S42**



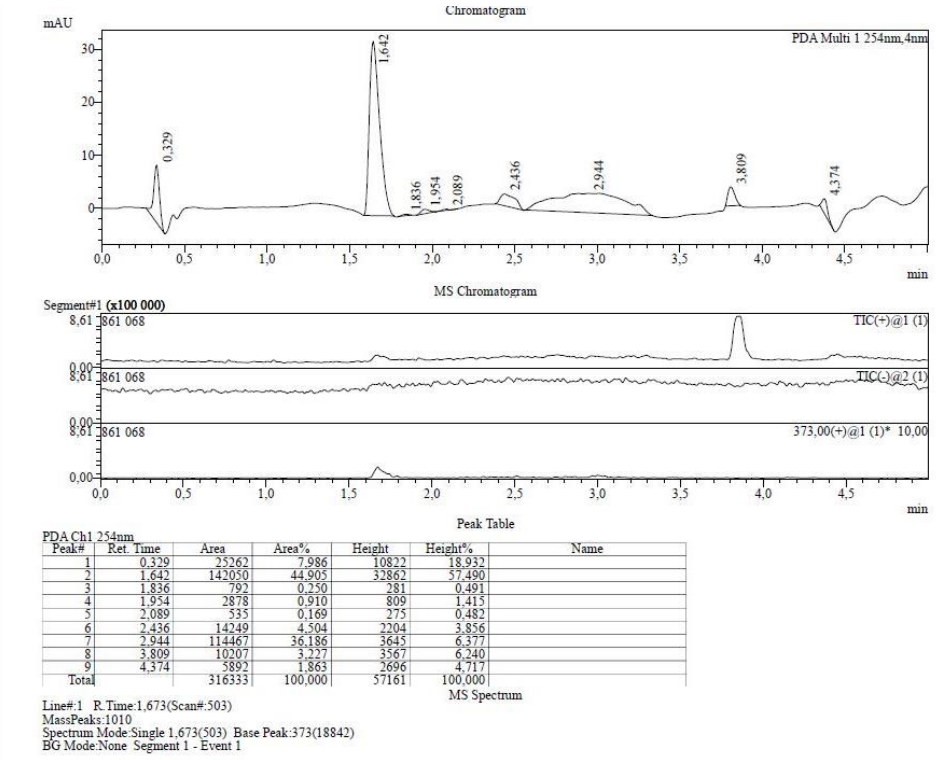
Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,335	19465	3,027	7167	6,238	
2	0,534	21132	3,286	2614	2,275	
3	1,247	6894	1,072	604	0,526	
4	1,668	1046	0,163	374	0,325	
5	1,739	3074	0,322	520	0,452	
6	2,197	448798	69,787	90317	78,611	
7	3,322	17661	2,746	1512	1,316	
8	3,691	64228	9,987	3501	3,048	
9	4,302	9153	1,423	1572	1,369	
10	4,388	16998	2,643	3636	3,164	
11	4,900	35650	5,543	3075	2,677	
Total		643098	100,000	114892	100,000	

MS Spectrum

Supplementary Fig. 114. The high-performance liquid chromatography chromatogram of compound **S44**



Peak Table

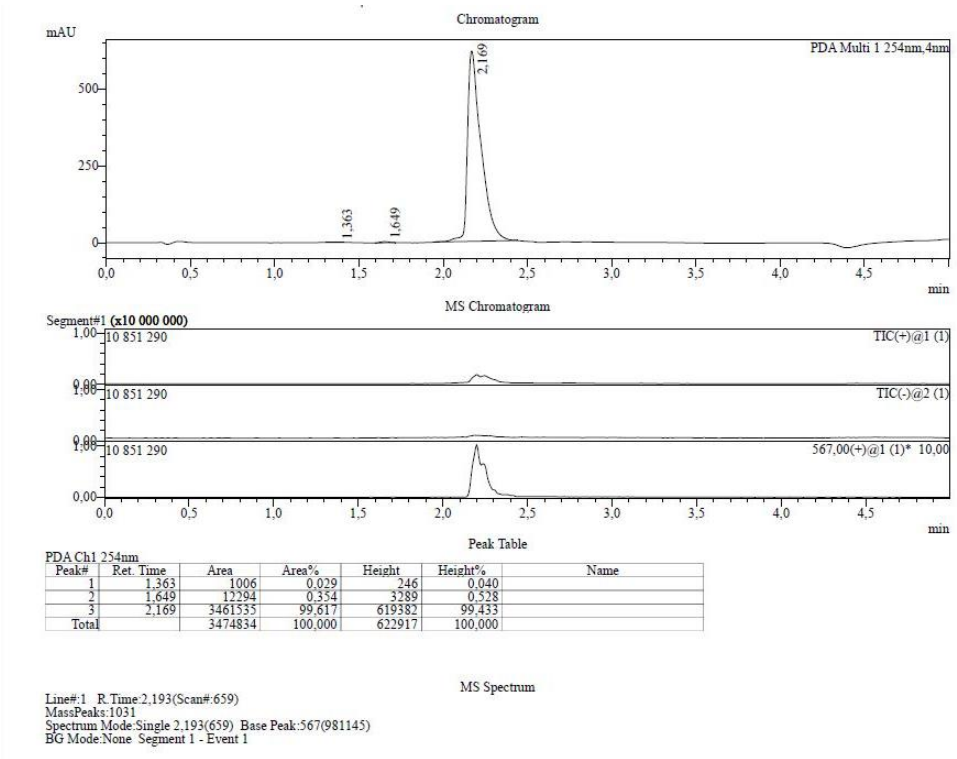
PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,329	25262	7,986	10822	18,932	
2	1,642	142050	44,905	32862	57,490	
3	1,836	792	0,250	281	0,491	
4	1,954	2878	0,910	809	1,415	
5	2,089	535	0,169	275	0,482	
6	2,436	14249	4,504	2204	3,856	
7	2,944	114467	36,186	3645	6,377	
8	3,809	10207	3,227	3567	6,240	
9	4,374	5892	1,863	2696	4,717	
Total		316333	100,000	57161	100,000	

MS Spectrum

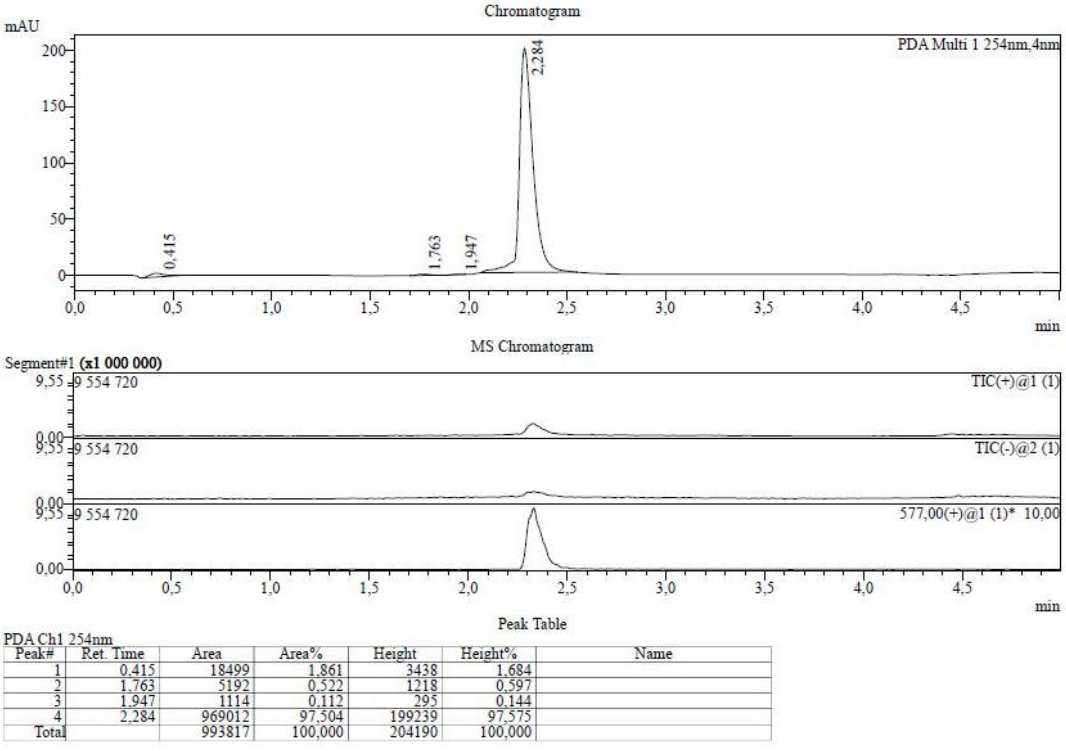
Line#:1 R.Time:1,673(Scan#:503)
MassPeaks:1010
Spectrum Mode:Single 1,673(503) Base Peak:373(18842)
BG Mode:None Segment 1 - Event 1

Supplementary Fig. 115. The high-performance liquid chromatography chromatogram of compound **S45**



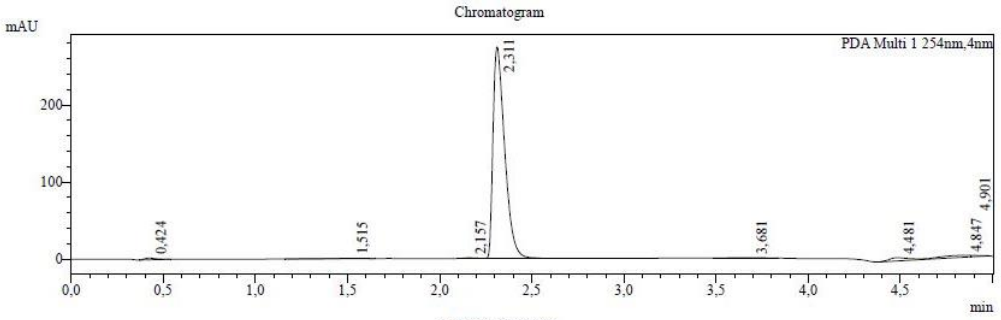
Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	1,363	1006	0,029	246	0,040	
2	1,649	12294	0,354	3289	0,528	
3	2,169	3461535	99,617	619382	99,433	
Total		3474834	100,000	622917	100,000	

Supplementary Fig. 116. The high-performance liquid chromatography chromatogram of compound **1a*R*-isoPHEN**



Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,415	18499	1,861	3438	1,684	
2	1,763	5192	0,522	1218	0,597	
3	1,947	1114	0,112	295	0,144	
4	2,284	969012	97,504	199239	97,575	
Total		993817	100,000	204190	100,000	

Supplementary Fig. 117. The high-performance liquid chromatography chromatogram of compound **1a*R*-IN-7**

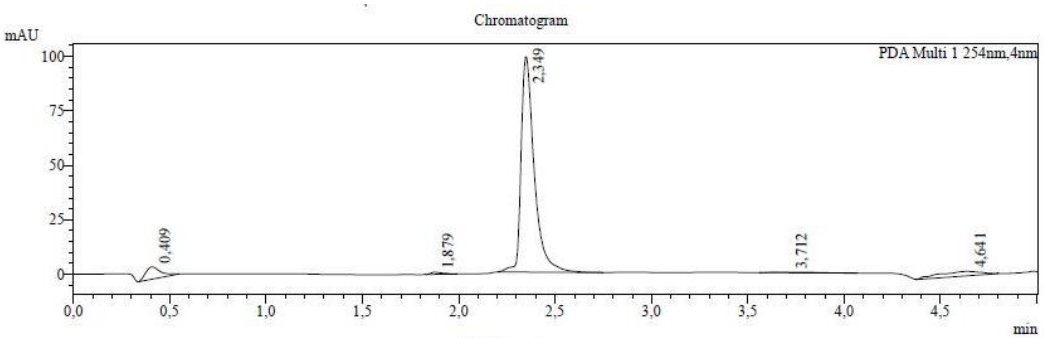


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,424	9446	0,728	2119	0,742	
2	1,515	5439	0,419	302	0,106	
3	2,157	1745	0,135	472	0,165	
4	2,311	1203956	92,817	274248	96,058	
5	3,681	5709	0,440	489	0,171	
6	4,481	32322	2,492	4166	1,459	
7	4,847	33601	2,590	2251	0,788	
8	4,901	4905	0,378	1454	0,509	
Total		1297123	100,000	285502	100,000	

Supplementary Fig. 118. The high-performance liquid chromatography chromatogram of compound **1a*R*-IN-9**

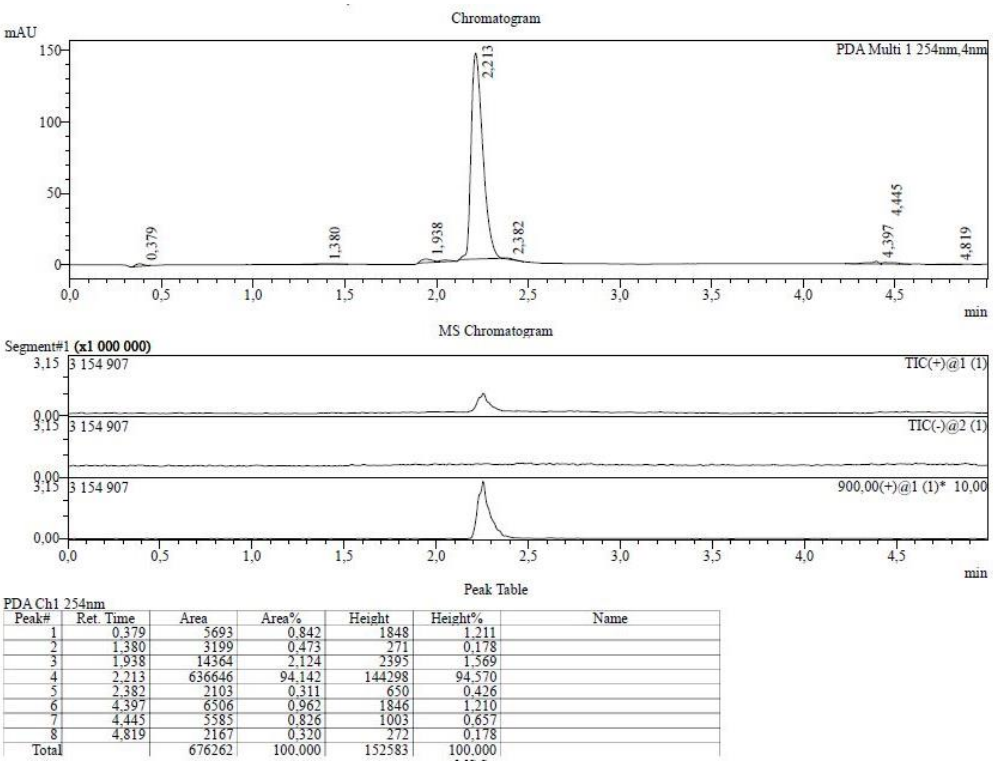


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,409	31221	5,742	5685	5,272	
2	1,879	4075	0,750	941	0,873	
3	2,349	470655	86,558	98914	91,739	
4	3,712	4560	0,839	323	0,299	
5	4,641	33231	6,111	1959	1,817	
Total		543742	100,000	107821	100,000	

Supplementary Fig. 119. The high-performance liquid chromatography chromatogram of compound **1a*R*-IN-10**

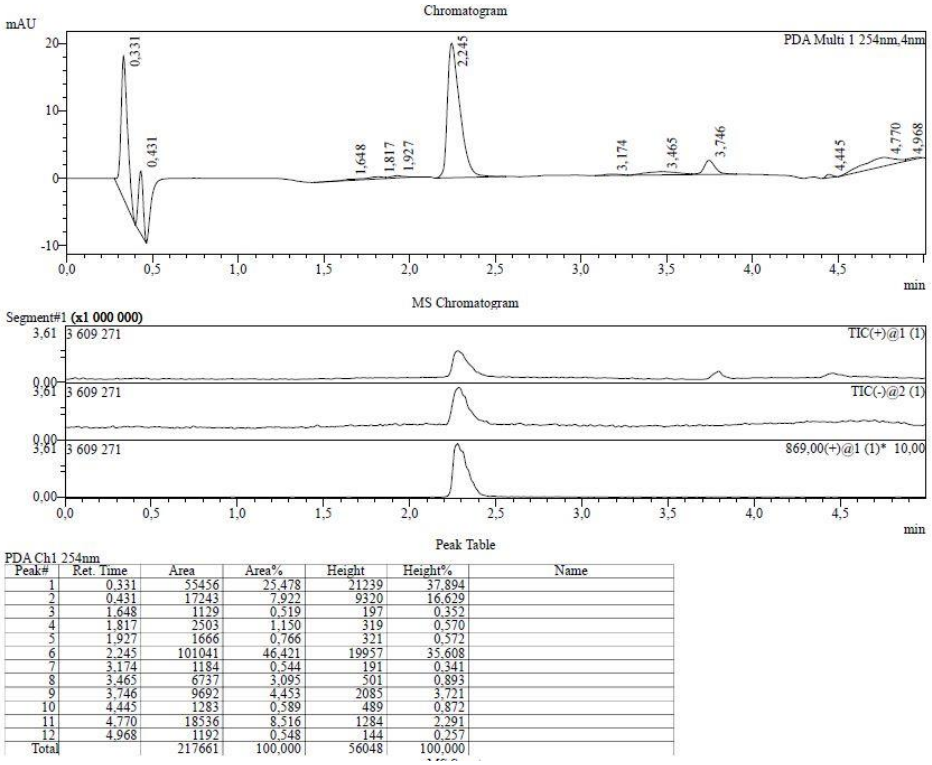


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,379	5693	0,842	1848	1,211	
2	1,380	3199	0,473	271	0,178	
3	1,938	14364	2,124	2395	1,569	
4	2,213	636646	94,142	144298	94,570	
5	2,382	2103	0,311	650	0,426	
6	4,397	6506	0,962	1846	1,210	
7	4,445	5585	0,826	1003	0,657	
8	4,819	2167	0,320	272	0,178	
Total		676262	100,000	152583	100,000	

Supplementary Fig. 120. The high-performance liquid chromatography chromatogram of compound **Photocaged 1a*R*-IN-8**

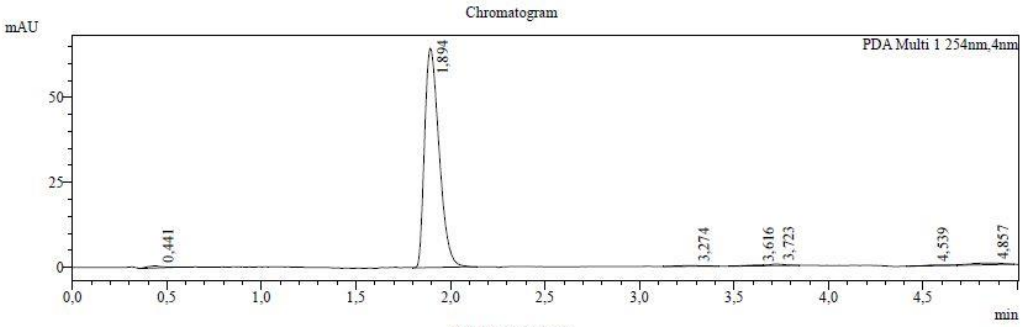


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,331	55456	25,478	21239	37,894	
2	0,431	17243	7,922	9320	16,629	
3	1,648	1129	0,519	197	0,352	
4	1,817	2503	1,150	319	0,570	
5	1,927	1666	0,766	321	0,572	
6	2,245	101041	46,421	19957	35,608	
7	3,174	1184	0,544	191	0,341	
8	3,465	6737	3,095	501	0,893	
9	3,746	9692	4,453	2085	3,721	
10	4,445	1283	0,589	489	0,872	
11	4,770	18536	8,516	1284	2,291	
12	4,968	1192	0,548	144	0,257	
Total		217661	100,000	56048	100,000	

Supplementary Fig. 121. The high-performance liquid chromatography chromatogram of compound **PRT_1**

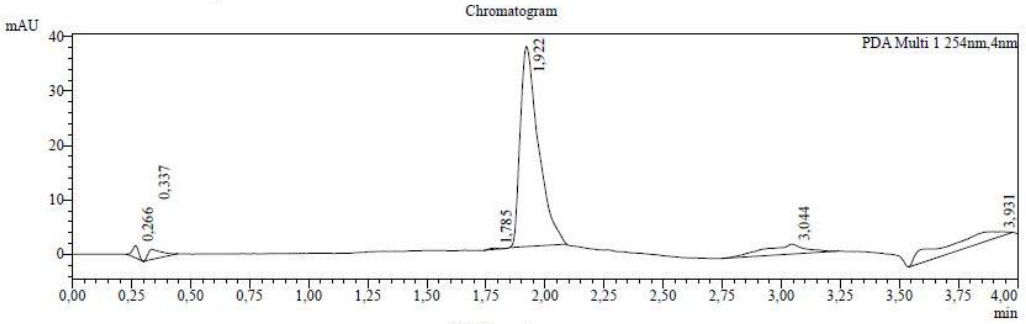


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0.441	2925	0.807	556	0.833	
2	1.894	345361	95.234	64656	96.754	
3	3.274	2098	0.579	235	0.352	
4	3.616	1468	0.405	209	0.313	
5	3.723	2469	0.681	451	0.675	
6	4.539	2220	0.612	230	0.345	
7	4.857	6105	1.683	487	0.729	
Total		362646	100.000	66826	100.000	

Supplementary Fig. 122. The high-performance liquid chromatography chromatogram of compound **S48**

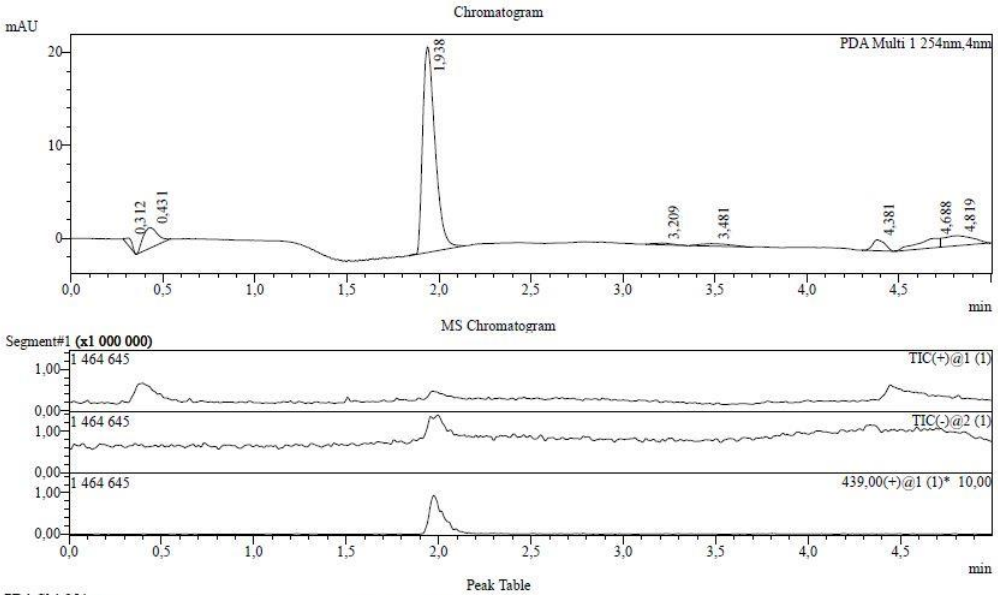


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,266	3850	1,417	2293	5,241	
2	0,337	7416	2,730	1849	4,228	
3	1,785	654	0,241	235	0,536	
4	1,922	197247	72,604	36739	83,982	
5	3,044	22744	8,372	1806	4,128	
6	3,931	39763	14,636	825	1,885	
Total		271675	100,000	43746	100,000	

Supplementary Fig. 123. The high-performance liquid chromatography chromatogram of compound **CA-IN-8**



Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%	Height	Height%	Name
1	0,312	1737	1,169	790	2,744	
2	0,431	12331	8,294	2184	7,591	
3	1,938	104018	69,965	22105	76,811	
4	3,209	1255	0,844	188	0,654	
5	3,481	2983	2,006	260	0,905	
6	4,381	5497	3,698	1174	4,079	
7	4,688	9158	6,160	1004	3,488	
8	4,819	11693	7,865	1073	3,728	
Total		148671	100,000	28778	100,000	

Supplementary Fig. 124. The high-performance liquid chromatography chromatogram of compound **IN-8a**

SUPPLEMENTARY REFERENCES

1. Zhang, T. *et al.* Discovery of Potent and Selective Covalent Inhibitors of JNK. *Chem Biol* **19**, 140–154 (2012).

2. Gaussian 16, Revision A.03, Frisch, M. J. *et al.* Gaussian, Inc., Wallingford CT, 2016.

3. Chai, J. Da & Head-Gordon, M. Long-range corrected hybrid density functionals with damped atom–atom dispersion corrections. *Physical Chemistry Chemical Physics* **10**, 6615–6620 (2008).

4. Chai, J. Da & Head-Gordon, M. Systematic optimization of long-range corrected hybrid density functionals. *Journal of Chemical Physics* **128**, 84106 (2008).

5. Marenich, A. V., Cramer, C. J. & Truhlar, D. G. Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *Journal of Physical Chemistry B* **113**, 6378–6396 (2009).

6. Weigend, F. & Ahlrichs, R. Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. *Physical Chemistry Chemical Physics* **7**, 3297–3305 (2005).

7. Falivene, L. *et al.* Towards the online computer-aided design of catalytic pockets. *Nature Chemistry 2019 11:10* **11**, 872–879 (2019).

8. Berkes, B. *et al.* Expedient and Diastereodivergent Assembly of Terpenoid Decalin Subunits having Quaternary Stereocenters through Organocatalytic Robinson Annulation of Nazarov Reagent. *Chemistry – A European Journal* **22**, 18101–18106 (2016).

9. Varga, S. *et al.* Total Syntheses of (−)-Minovincine and (−)-Aspidofractinine through a Sequence of Cascade Reactions. *Angewandte Chemie International Edition* **59**, 13547–13551 (2020).

10. McIver, E. G. *et al.* Synthesis and structure–activity relationships of a novel series of pyrimidines as potent inhibitors of TBK1/IKKε kinases. *Bioorg Med Chem Lett* **22**, 7169–7173 (2012).

11. Flaugh, M. E., Crowell, T. A. & Farlow, D. S. Acid-catalyzed annelation of .alpha.-alkylaldehydes and .alpha.,.beta.-unsaturated ketones. A one-pot synthesis of 4,4-dimethyl-2-cyclohexen-1-one. *J Org Chem* **45**, 5399–5400 (1980).

12. Deny, L. J. *et al.* Bis-Michael Acceptors as Novel Probes to Study the Keap1/Nrf2/ARE Pathway. *J Med Chem* **59**, 9431–9442 (2016).

13. Schultz, A. G. & Sundararaman, P. Stereochemical control in cyclofunctionalization of olefinic alcohols and olefinic phenols with benzeneselenenyl chloride. *J Org Chem* **49**, 2455–2462 (1984).

14. Peelen, T. J., Chi, Y., English, E. P. & Gellman, S. H. Synthesis of 4,4-Disubstituted 2-Aminocyclopentanecarboxylic Acid Derivatives and Their Incorporation into 12-Helical β-Peptides. *Org Lett* **6**, 4411–4414 (2004).

15. Meyer, C., Piva, O. & Pete, J. P. [2+2] Photocycloadditions and Photorearrangements of 2-Alkenylcarboxamido-2-cycloalken-1-ones. *Tetrahedron* **56**, 4479–4489 (2000).

16. Schierle, S. *et al.* Dual Farnesoid X Receptor/Soluble Epoxide Hydrolase Modulators Derived from Zafirlukast. *ChemMedChem* **15**, 50–67 (2020).

17. El-Mansy, M. F., Kang, J. Y., Lingampally, R. & Carter, R. G. Proline Sulfonamide-Catalyzed, Domino Process for Asymmetric Synthesis of Amino- and Hydroxy-Substituted Bicyclo[2.2.2]octanes. *European J Org Chem* **2016**, 150–157 (2016).

18. Kokhan, S. O., Valter, Y. B., Tymtsunik, A. V., Komarov, I. V. & Grygorenko, O. O. Bicyclo[1.1.1]pentane-Derived Building Blocks for Click Chemistry. *European J Org Chem* **2017**, 6450–6456 (2017).

19. Mu, F. *et al.* Design, Synthesis, and Biological Evaluation of a Series of Lavendustin A Analogues That Inhibit EGFR and Syk Tyrosine Kinases, as Well as Tubulin Polymerization. *J Med Chem* **44**, 441–452 (2001).

20. Tang, X. J. *et al.* Photorelease of Pyridines Using a Metal-Free Photoremovable Protecting Group. *Angewandte Chemie International Edition* **59**, 18386–18389 (2020).