Discovery of G Protein-Biased Dopaminergics with a Pyrazolo[1,5-*a*]pyridine Substructure

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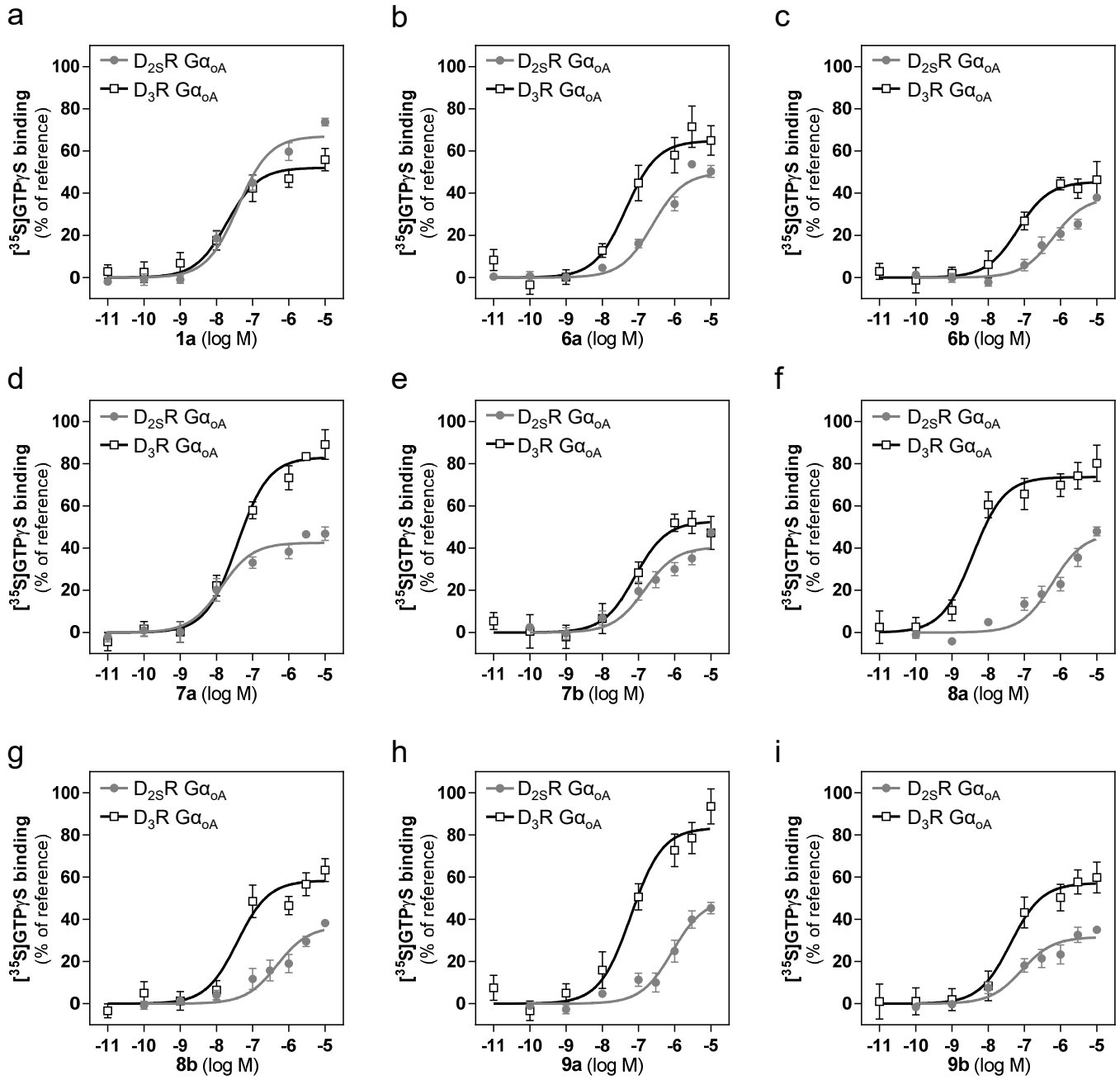
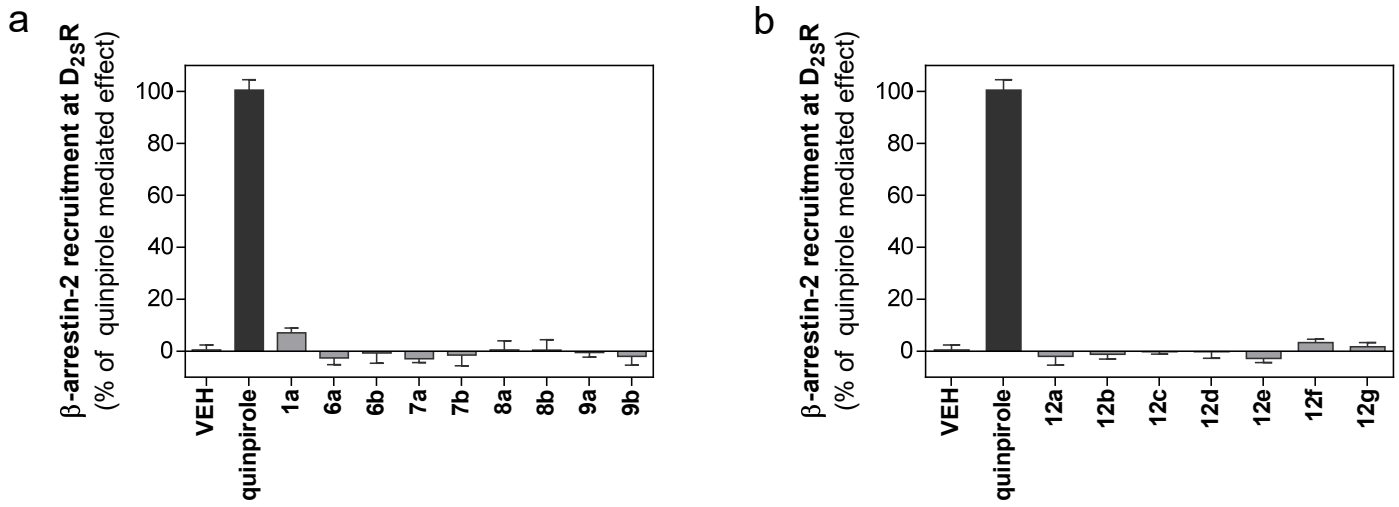
SUPPORTING INFORMATION

1) Supplementary Figure 1. [^{35}S]GTPγS binding experiments with compounds **1a** and **6a**,**b**-**9a**,**b**.



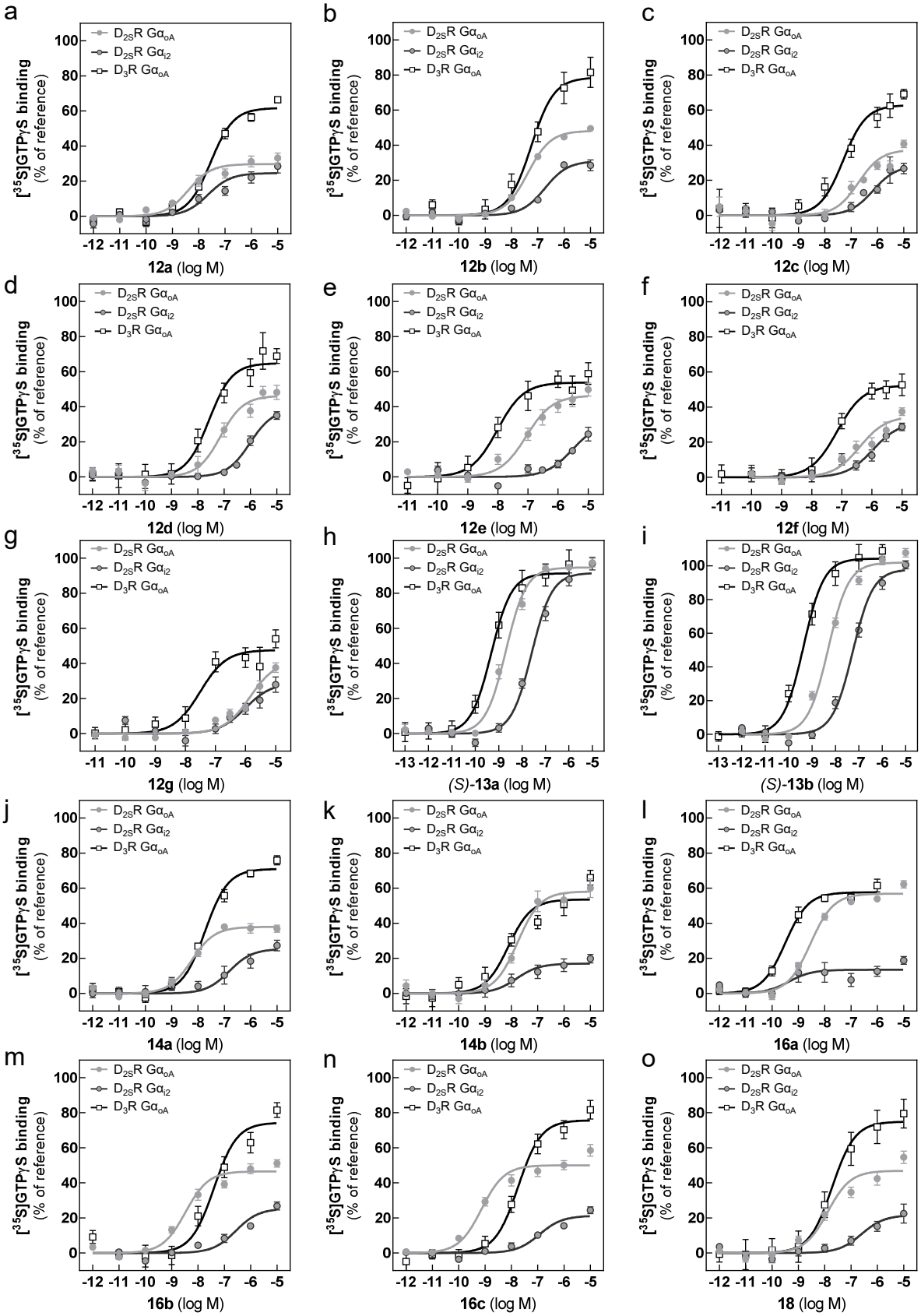
[^{35}S]GTPγS binding with membranes expressing $D_{2S}R$ or D_3R together with $G\alpha_{oA}$. Ligand stimulated nucleotide exchange was determined for the reference partial agonist **1a** (**a**) and the pyrazolo[1,5-*a*]pyridines **6a**,**b**-**9a**,**b** (**b**-**i**). While the reference compound **1a** shows almost equal potency for $D_{2S}R$ and D_3R activation, pyrazolo[1,5-*a*]pyridines comprising an amide moiety preferentially activate D_3R. Data represent mean ± S.E.M. from the pooled curve of three to seven independent experiments, each performed in triplicates.

2) Supplementary Figure 2. $D_{2S}R$-mediated β-arrestin-2 recruitment for **6a**,**b**-**9a**,**b** and **12a-g** examined using the PathHunter assay.



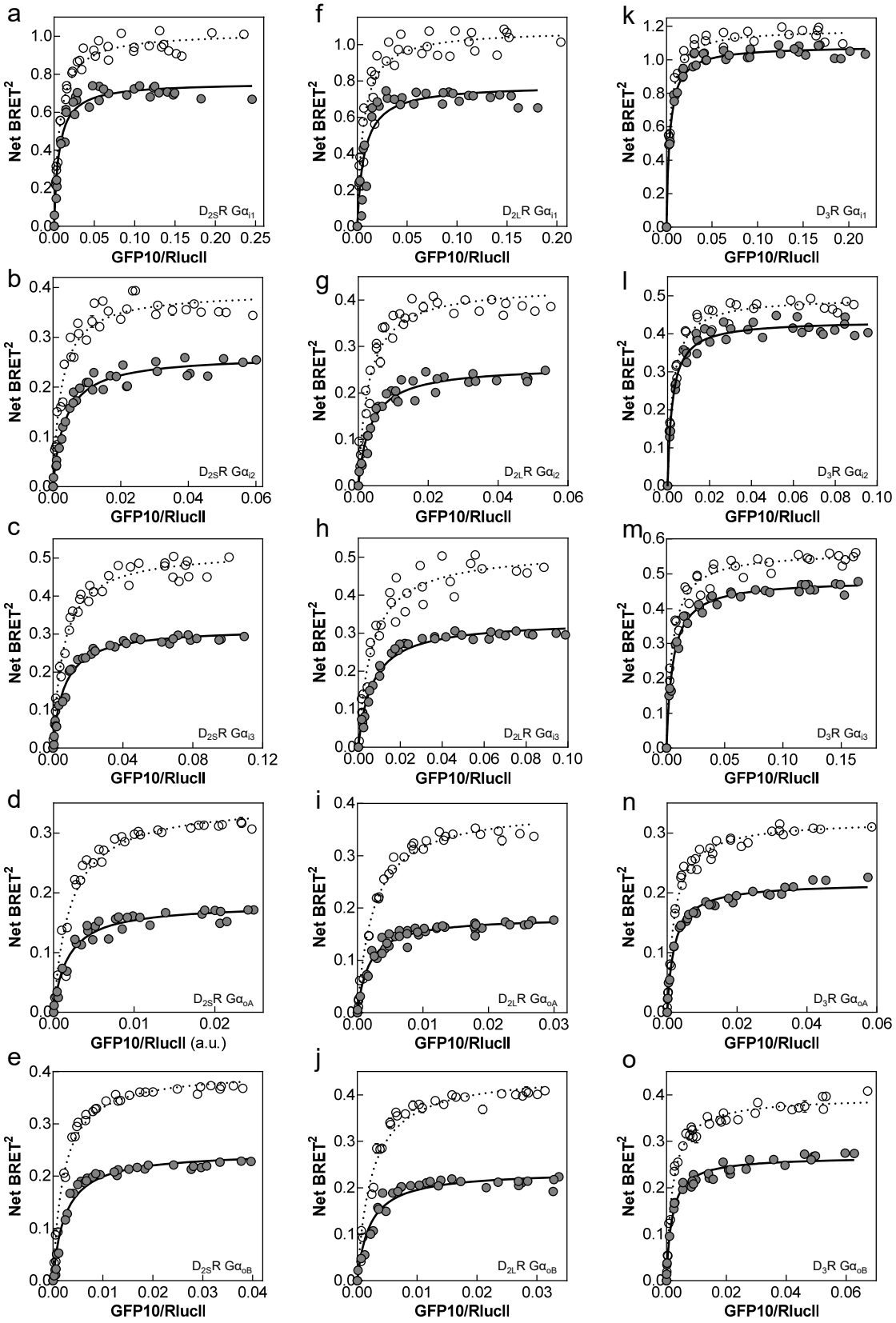
β-arrestin-2 recruitment at $D_{2S}R$ induced by treatment with the amide-spacer test compounds **6a**,**b**-**9a**,**b** (**a**) and 5-butoxypyrazolo[1,5-a]pyridines **12a-g** comprising a substituent in position 2 of the heterocycle (**b**) in comparison to the reference agonist quinpirole and the antipsychotic **1a**. All compounds were investigated at a concentration of 10μM using the DiscoveRx PathHunter assay. Data represent mean ± S.E.M. from the pooled results of at least two independent experiments, each performed in triplicates and normalized to vehicle conditions (VEH, PBS) and the maximum effect of quinpirole.

3) Supplementary Figure 3. [^{35}S]GTPγS binding experiments with butoxy-spacer compounds



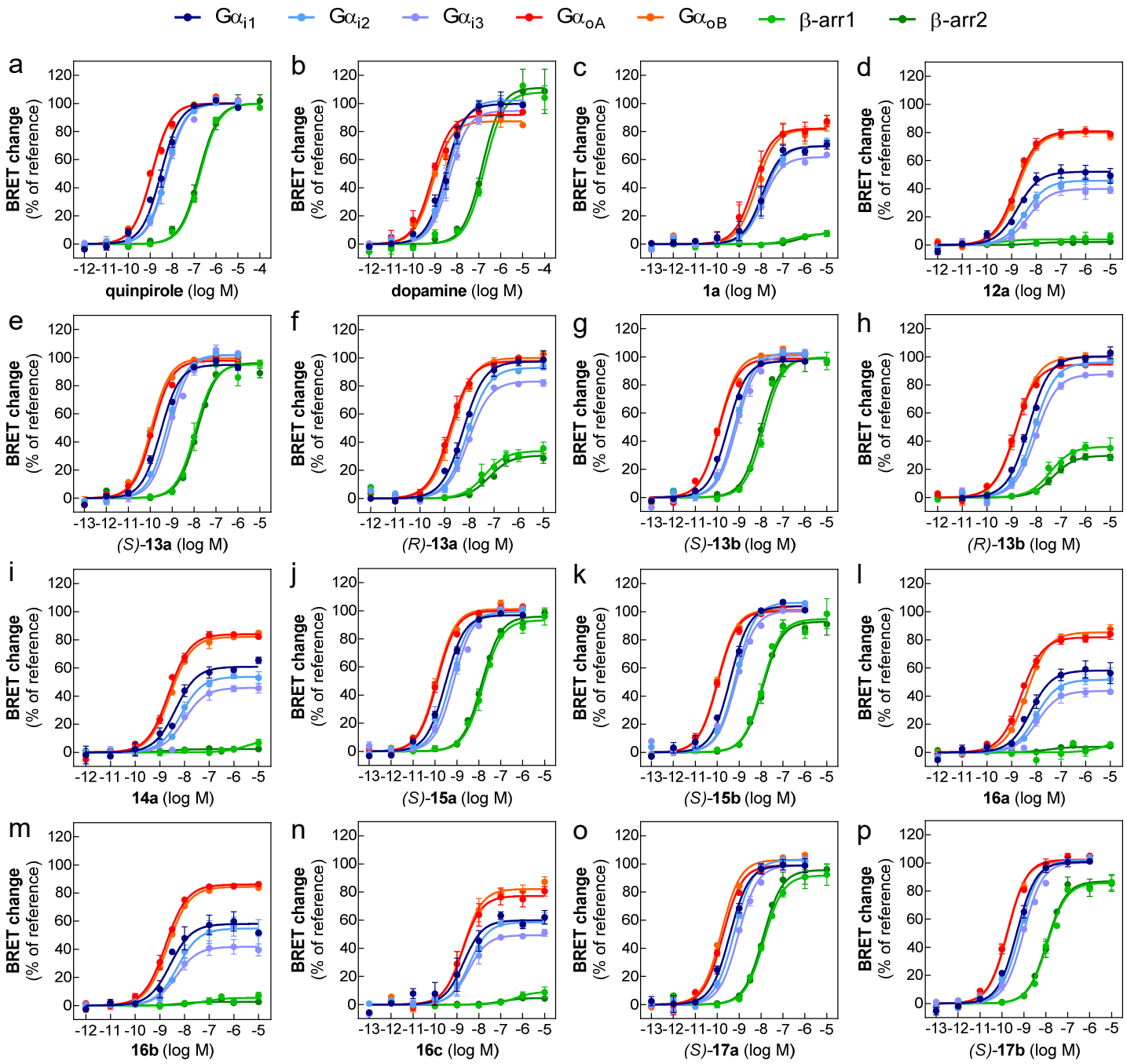
[^{35}S]GTPγS binding with membranes expressing $D_{2S}R$ or D_3R together with $G\alpha_{oA}$ or $G\alpha_{i2}$ and compounds **12a-g**, (*S*)-**13a**,**b**, **14a**,**b** and **16a-c**. Data represent mean ± S.E.M. from the pooled curve of four to ten independent experiments, each performed in triplicates.

4) Supplementary Figure 4. BRET² titrations for $D_{2S}R$, $D_{2L}R$ and D_3R



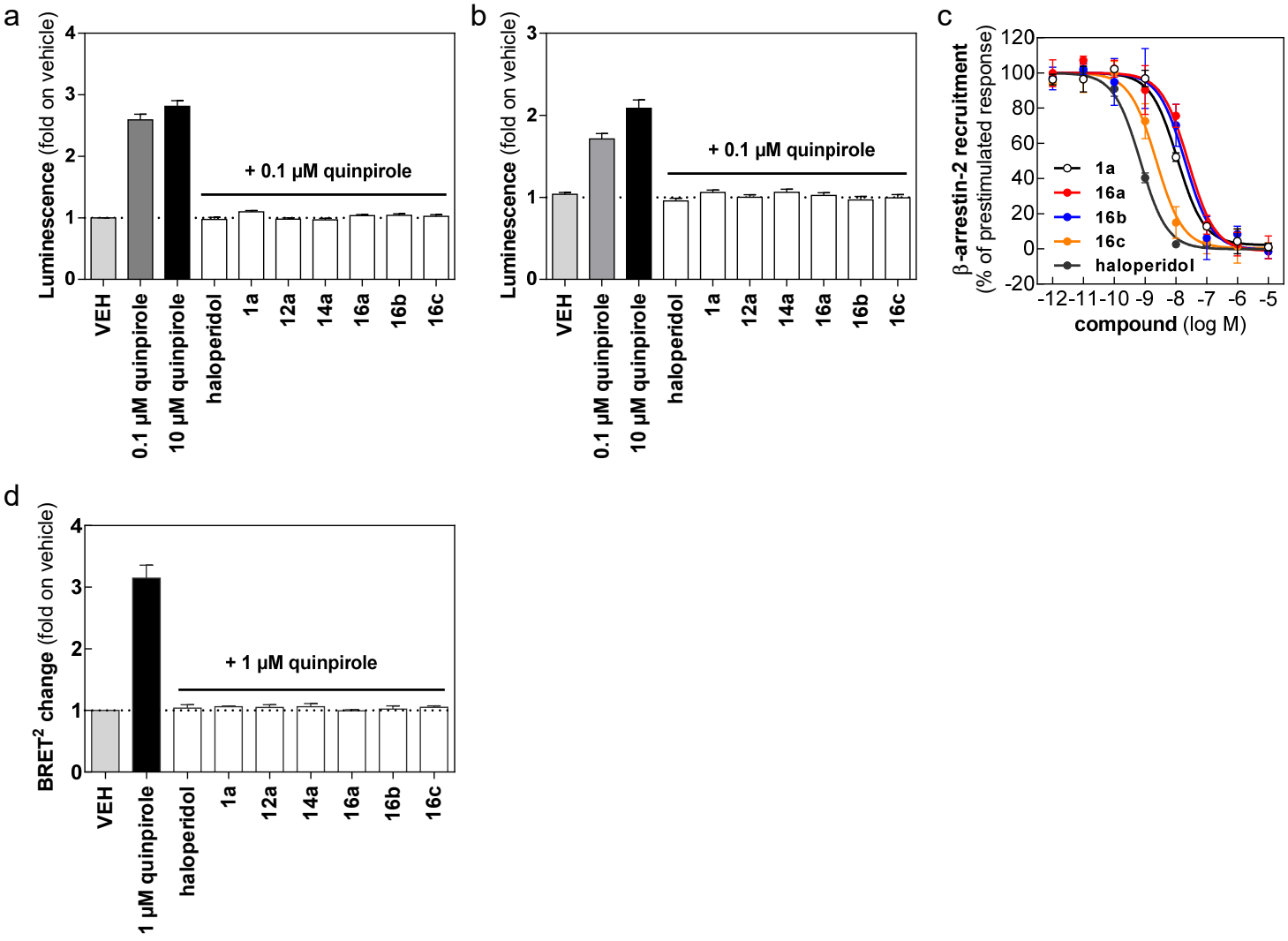
BRET titrations for $D_{2S}R$, $D_{2L}R$ and D_3R in combination with the different RLucII-$G\alpha_{i/o}$ variants, $G\beta_1$, and GFP10-$G\gamma_2$. BRET² was determined in the absence (open circles) and presence of 10 μM of the endogenous agonist dopamine (grey circles) at different transfection ratios of donor and acceptor cDNAs. Results represent mean ± S.E.M. of three independent experiments, each performed in quadruplicates.

5) Supplementary Figure 5. Dose-response curves for $D_{2S}R$ activation determined by BRET



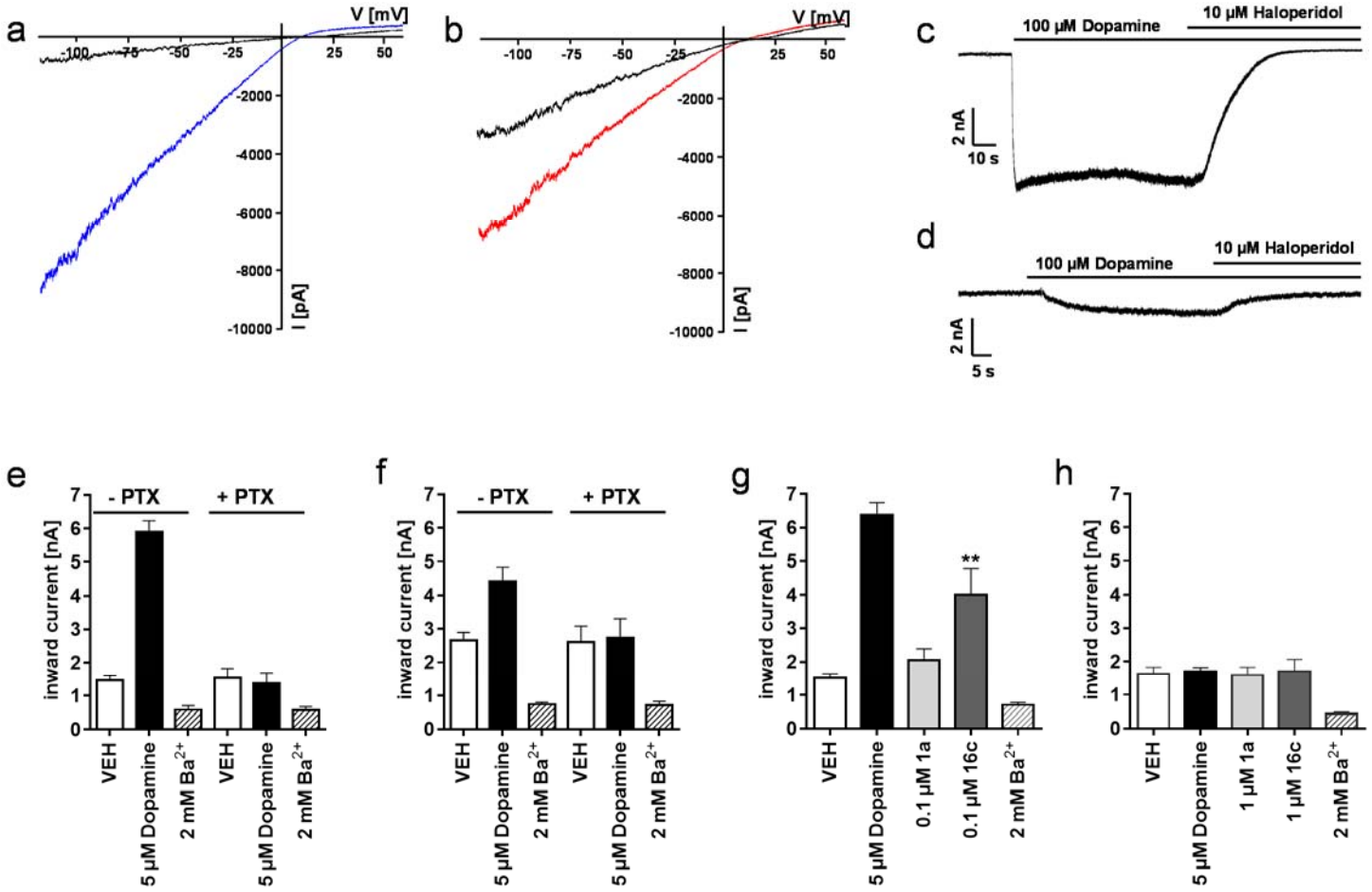
Dose-response curves for different signalling pathways upon ligand-stimulation at $D_{2S}R$ determined by BRET. Cells expressing FLAG-tagged $D_{2S}R$ together with the respective biosensors were stimulated for 10 minutes with the ligands at each concentration for the activation of different G proteins (RLucII-$G\alpha_{i/o}$ and GFP10-$G\gamma_2$) or 15 minutes for the recruitment of β-arrestins (RLucII-β-arr1/2 and CAAX-GFP10). Data are presented as mean ± S.E.M. derived from three to six independent experiments, with each concentration in duplicate.

6) Supplementary Figure 6. Inhibition of quinpirole-induced β-arrestin-2 recruitment



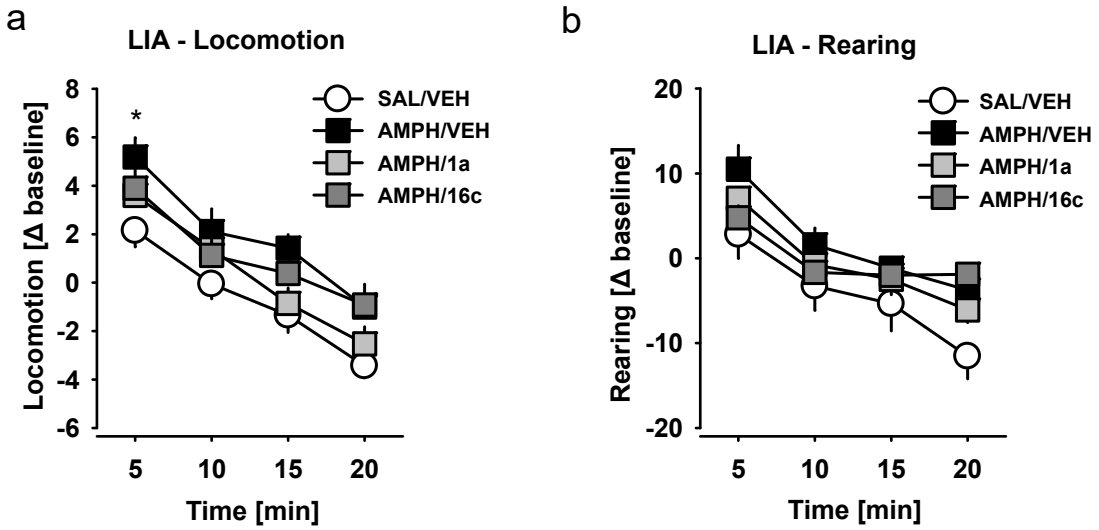
Inhibition of quinpirole-mediated β-arrestin recruitment examined by the PathHunter™ assay (**a-c**) or bioluminescence resonance energy transfer (**d**). In cells expressing either $D_{2S}R$ (**a**) or $D_{2L}R$ (**b**), quinpirole-induced β-arrestin-2 recruitment inhibited by coincubation with the antagonist haloperidol or test compounds **1a**, **12a**, **14a** or **16a-c** at a concentration of 10 µM. Inhibition was confirmed to be dose-dependent with highest potencies observed for haloperidol and the 2-methoxyphenylpiperazine **16c** (**c**). Antagonist properties for representative 2-methoxyphenylpiperazines were confirmed employing an assay based on enhanced bystander BRET, when cells were pre-treated with antagonist for 1 h and then challenged with the agonist for 15 min. (**d**). Data represent mean ± S.E.M. of three to six independent experiments, each performed in duplicate.

7) Supplementary Figure 7. $D_{2L}R$ or D_3R mediated activation of GIRK1/2 channels



$D_{2L}R$ or D_3R mediated activation of GIRK1/2 channels. GIRK1/2 activation is characterized by typical inward rectifying behaviour in HEK293T cells expressing either $D_{2L}R$ (**a**) or D_3R (**b**). Individual traces at the holding potential of -70 mV in high K^+ external solution show K^+-currents are evoked by stimulation with the endogenous agonist dopamine in $D_{2L}R$ (**c**) and D_3R (**d**) expressing HEK293T cells. In both cases, currents are inhibited by the D_2R/D_3R-antagonist haloperidol. Quantification from ramp currents (**e**-**h**) reveal that dopamine stimulates GIRK1/2 currents by means of $G\alpha_{i/o}$ activation, as overnight incubation with pertussis toxin (25 ng/mL) completely inhibits ligand-mediated activation of GIRK1/2 at $D_{2L}R$ (**e**) and D_3R (**f**). Pertussis toxin does not influence basal GIRK1/2 currents in both cases. (**g**) When tested at a concentration of 0.1µM, only **16c** but not **1a** evokes significant GIRK1/2 currents in $D_{2L}R$ expressing HEK293T cells. (**h**) In the presence of GIRK1/2 but absence of $D_{2L}R$ and D_3R, neither dopamine, nor **1a** or **16c** induce significant K^+-currents. Data represent mean ± S.E.M. from 4-30 individual cells, ** $p < 0.01$, one-way ANOVA with Tukey's test for multiple comparisons, VEH = vehicle.

8) Supplementary Figure 8. The effects of **16c** compared to **1a** on schizophrenia-like behavior in rats: LIA



The effects of **16c** compared to **1a** on schizophrenia-like behavior in rats. Animals received i.p. injections of amphetamine (AMPH) with a sensitization regimen or saline (SAL). Subsequently, the animals were continuously treated with **1a** (1.5 mg/kg/day), **16c** (1.5 mg/kg/day), or vehicle (VEH) for 7 days. Light-induced activity (LIA) was measured on day 5 of treatment. The effects of randomized light stimulation (10 x 30 sec) on the horizontal (**a**) and vertical (**b**) locomotion are shown in 5 min intervals. Light-induced behaviors are calculated as Δ baseline (vs. last 5 min of baseline). Values are shown as mean ± SEM. * $p < 0.05$ compared to SAL/VEH.

9) Supplementary Table 1. $D_{2L}R$ and D_3R activation characteristics determined by BRET.

	$D_{2L}R$ activation[†]														D_3R activation[†]			
	$G\alpha_{i1}$		$G\alpha_{i2}$		$G\alpha_{i3}$		$G\alpha_{oA}$		$G\alpha_{oB}$		β-arr1		β-arr2		$G\alpha_{oA}$		$G\alpha_{oB}$	
	EC_{50}[‡]	E_{max}[§]	EC_{50}[‡]	E_{max}[§]	EC_{50}[‡]	E_{max}[§]	EC_{50}[‡]	E_{max}[§]	EC_{50}[‡]	E_{max}[§]	EC_{50}[‡]	E_{max}[§]	EC_{50}[‡]	E_{max}[§]	EC_{50}[‡]	E_{max}[§]	EC_{50}[‡]	E_{max}[§]
quinpirole	5.9 ± 1.6	100 ± 0	7.4 ± 1.2	100 ± 0	8.8 ± 1.1	100 ± 1	2.0 ± 0.3	100 ± 0	1.7 ± 0.3	100 ± 1	137 ± 82	100 ± 1	113 ± 27	100 ± 1	1.4 ± 0.2	100 ± 1	1.2 ± 0.1	100 ± 1
dopamine	6.9 ± 1.7	103 ± 4	10.0 ± 2.5	101 ± 2	10.0 ± 1.2	93 ± 1	1.7 ± 0.3	94 ± 2	1.7 ± 0.3	93 ± 3	112 ± 35	102 ± 2	115 ± 39	115 ± 6	0.96 ± 0.29	83 ± 3	0.58 ± 0.20	80 ± 6
1a	29.8 ± 14.6	57 ± 1	30.0 ± 15.0	53 ± 2	28.4 ± 14.0	51 ± 2	15.2 ± 7.6	76 ± 5	18.2 ± 9.2	75 ± 4	n.d.[††]	< 10	n.d.[††]	< 15	46 ± 15	57 ± 3	220 ± 135	59 ± 3
12a	2.5 ± 0.7	33 ± 6	3.2 ± 0.2	27 ± 5	2.4 ± 0.7	30 ± 3	1.3 ± 0.1	71 ± 2	1.3 ± 0.1	68 ± 2	n.d.[††]	< 10	n.d.[††]	< 10	6.9 ± 2.5	44 ± 4	7.4 ± 0.9	47 ± 2
(*S*)-**13a**	0.67 ± 0.14	96 ± 1	1.1 ± 0.2	100 ± 1	1.2 ± 0.1	103 ± 3	0.18 ± 0.02	99 ± 3	0.15 ± 0.01	97 ± 1	18.5 ± 4.1	87 ± 5	7.4 ± 0.8	90 ± 6	4.8 ± 0.1	105 ± 2	3.4 ± 0.1	99 ± 1
(*R*)-**13a**	12.1 ± 0.9	94 ± 3	19.8 ± 2.1	84 ± 3	16.0 ± 1.2	78 ± 1	3.4 ± 0.5	93 ± 1	3.5 ± 0.7	92 ± 1	83 ± 10	34 ± 7	39 ± 12	30 ± 2	6.0 ± 0.2	82 ± 2	5.1 ± 0.2	82 ± 1
(*S*)-**13b**	0.74 ± 0.07	101 ± 1	1.1 ± 0.1	100 ± 1	1.1 ± 0.1	102 ± 1	0.21 ± 0.03	99 ± 1	0.16 ± 0.01	99 ± 1	6.4 ± 0.7	86 ± 3	6.7 ± 0.8	89 ± 5	3.9 ± 0.2	106 ± 1	2.5 ± 0.1	98 ± 1
(*R*)-**13b**	9.4 ± 1.4	94 ± 3	16.0 ± 0.4	87 ± 1	14.3 ± 2.2	81 ± 2	3.2 ± 0.4	93 ± 1	2.9 ± 0.1	93 ± 1	59 ± 22	32 ± 4	55 ± 6	36 ± 10	4.7 ±0.4	86 ± 4	3.7 ± 0.6	84 ± 1
14a	6.4 ± 0.9	40 ± 4	4.0 ± 1.5	33 ± 3	6.7 ± 2.2	34 ± 3	4.2 ± 1.8	71 ± 1	2.5 ± 0.3	67 ± 1	n.d.[††]	< 10	n.d.[††]	< 10	8.6 ± 1.9	38 ± 3	4.9 ± 0.3	39 ± 2
(*S*)-**15a**	0.82 ± 0.12	102 ± 2	1.2 ± 0.2	100 ± 1	0.99 ± 0.08	102 ± 2	0.21 ± 0.06	99 ± 2	0.23 ± 0.06	97 ± 1	9.5 ± 1.8	92 ± 6	8.0 ± 1.4	85 ± 4	4.1 ± 0.6	100 ± 1	2.6 ± 0.4	97 ± 4
(*S*)-**15b**	0.63 ± 0.08	106 ± 1	1.0 ± 0.2	104 ± 2	0.92 ± 0.08	105 ± 2	0.46 ± 0.32	100 ± 2	0.14 0.02	98 ± 1	7.3 ± 1.0	79 ± 6	6.8 ± 1.3	82 ± 10	3.5 ± 0.3	103 ± 2	2.3 ± 0.1	102 ± 1
16a	7.2 ± 0.9	39 ± 5	7.0 ± 1.5	35 ± 3	8.4 ± 0.5	34 ± 4	2.6 ± 0.6	74 ± 1	1.3 ± 0.1	67 ± 2	n.d.[††]	< 10	n.d.[††]	< 10	9.7 ± 0.1	35 ± 3	14.4 ± 4.8	42 ± 3
16b	4.2 ± 1.2	39 ± 7	3.6 ± 1.0	33 ± 4	3.7 ± 0.9	33 ± 1	1.2 ± 0.1	72 ± 1	1.8 ± 0.6	67 ± 2	n.d.[††]	< 10	n.d.[††]	< 10	4.1 ± 0.7	44 ± 3	6.4 ± 0.9	42 ± 3
16c	4.2 ± 1.9	42 ± 3	4.8 ± 1.5	42 ± 1	6.6 ± 1.0	38 ± 1	2.6 ± 0.8	72 ± 1	0.21 ± 0.2	67 ± 2	n.d.[††]	< 10	n.d.[††]	< 10	12.5 ± 4.1	44 ± 2	7.5 ± 2.3	45 ± 3
(*S*)-**17a**	0.72 ± 0.05	104 ± 4	0.95 ± 0.03	100 ± 2	1.33 ± 0.1	100 ± 1	0.24 ± 0.02	99 ± 1	0.23 ± 0.03	98 ± 1	7.7 ± 1.8	88 ± 10	6.0 ± 0.3	88 ± 3	4.4 ± 0.7	102 ± 1	2.9 ± 0.4	96 ± 1
(*S*)-**17b**	0.95 ± 0.11	105 ± 2	1.2 ± 0.1	101 ± 1	1.3 ± 0.2	100 ± 2	0.24 ± 0.02	100 ± 1	0.23 ± 0.03	98 ± 2	6.5 ± 1.9	82 ± 9	6.6 ± 1.1	84 ± 4	4.2 ± 0.6	99 ± 1	3.1 0.35	100 ± 1

[†]Data represent mean ± S.E.M. from three to seven independent experiments, each performed in duplicates. [‡]EC_{50} given in nM. [§]E_{max} relative to the effect of vehicle (0 %) and the saturating effect of quinpirole (100 %). [††]Not determined.

10) Supplementary Table 2. Quantification of ligand bias at D_2sR using the operational model of agonism [†]

comp.	$\log\left(\frac{\tau}{K_A}\right) G\alpha_{oA}$ (BRET)	$\Delta\log\left(\frac{\tau}{K_A}\right) G\alpha_{oA}$ (BRET)	$\log\left(\frac{\tau}{K_A}\right) \beta_{arr2}$ (PathHunter)	$\Delta\log\left(\frac{\tau}{K_A}\right) \beta_{arr2}$ (PathHunter)	$\Delta\Delta\log\left(\frac{\tau}{K_A}\right)^{\ddagger}$ $G\alpha_{oA}/\beta_{arr2}$	$10^{\Delta\Delta log\left(\frac{\tau}{K_A}\right)}$ bias factor
quinpirole	8.95 ± 0.06	0.00 ± 0.09	7.47 ± 0.04	0.00 ± 0.05	0.00 ± 0.10	1.00
1a	8.19 ± 0.07	-0.75 ± 0.10	n.d.	n.d.	n.d.	n.d.
12a	8.69 ± 0.09	-0.26 ± 0.11	n.d.	n.d.	n.d.	n.d.
(*S*)-**13a**	9.84 ± 0.07	0.89 ± 0.10	8.62 ± 0.05	1.15 ± 0.06	-0.25 ± 0.12	0.56
(*R*)-**13a**	8.76 ± 0.06	-0.19 ± 0.09	6.32 ± 0.13	-1.15 ± 0.13	0.96 ± 0.16[§]	9.09
(*S*)-**13b**	9.88 ± 0.07	0.94 ± 0.10	8.87 ± 0.05	1.40 ± 0.06	-0.46 ± 0.12	0.35
(*R*)-**13b**	8.78 ± 0.06	-0.17 ± 0.09	6.42 ± 0.14	-1.06 ± 0.14	0.88 ± 0.17[§]	7.65
14a	8.50 ± 0.08	-0.45 ± 0.10	n.d.	n.d.	n.d.	n.d.
(*S*)-**15a**	9.88 ± 0.07	0.94 ± 0.10	8.66 ± 0.06	1.19 ± 0.07	-0.25 ± 0.12.	0.56
(*S*)-**15b**	9.97 ± 0.07	1.02 ± 0.10	8.64 ± 0.06	1.17 ± 0.07	-0.15 ± 0.12	0.71
16a	8.44 ± 0.08	-0.50 ± 0.11	n.d.	n.d.	n.d.	n.d.
16b	8.65 ±0.08	-0.30 ± 0.10	n.d.	n.d.	n.d.	n.d.
16c	8.54 ± 0.08	-0.41 ± 0.10	n.d.	n.d.	n.d.	n.d.
(*S*)-**17a**	9.71 ± 0.08	0.76 ± 0.10	8.89 ± 0.06	1.41 ± 0.07	-0.65 ± 0.12[§]	0.22
(*S*)-**17b**	9.76 ± 0.08	0.82 ± 0.10	8.87 ± 0.08	1.39 ± 0.09	-0.58 ± 0.13[§]	0.26

[†]Data represent mean ± SEM calculated as detailed in the methods section. [‡]Negative values indicate preferential signaling via β-arrestin-2 recruitment, positive values indicate bias towards $G\alpha_{oA}$ signaling. [§]Significant bias ($p<0.05$) determined by one-way ANOVA followed by Dunnett's posthoc test. n.d. not determined.

11) Supplementary Results. The effects of **16c** and **1a** on schizophrenia-like behavior in rats.

In this study, we tested the antipsychotic efficiency of **16c**, and compared it with **1a**, an antipsychotic drug that is commonly used for the treatment of schizophrenia.[1] In order to induce schizophrenia-like alterations in animals, we used an amphetamine (AMPH)-sensitization regimen that has been developed by Peleg-Raibstein et al.,[2] and shown to effectively induce these alterations. AMPH administration reverses dopamine transporter activity and boosts the dopamine release from the nerve terminals, leading to elevated extracellular dopamine, especially in the striatum.[3, 4] This elevation is markedly increased when previously AMPH-sensitized animals are treated with AMPH, indicated by an increased locomotor response of these animals to a low dose AMPH challenge.[2, 5, 6] AMPH-sensitization may also cause disruptions in the pre-pulse inhibition (PPI), which is a commonly used paradigm to measure the sensorimotor gating system.[7] Attenuated PPI, which has been observed in schizophrenic patients,[8] can also be reversed by antipsychotic drug administration.[9] However, the evidence of PPI deficits following AMPH-sensitization in animal models is mixed.[10-12] Because of these inconsistent findings, instead of PPI, light-induced activity (LIA), a non-aversively motivated sensorimotor processing measure[13, 14] was investigated to assess the intactness of the sensorimotor gating system.[13, 15] After a six day pretreatment (sensitization) with escalating doses of AMPH or vehicle (0.9% saline), reference compound **1a** and test compound **16c** were continuously administered via an osmotic Alzet mini pump over the course of seven days. Five days after the mini pump implantation, the light-induced activity test was conducted to determine horizontal and vertical activities of animals induced by a white light stimulus. Seven days after the mini pump implantation, AMPH-induced hyperlocomotion was tested in an open field (OF). First, the baseline activity of the animals was assessed for 20 min. After this period, each animal was i.p. injected with 1.5 mg/kg AMPH, and their locomotor activities and anxiety-related behaviors were measured for 20 min.

Baseline Activity. Animals showed a clear habituation of horizontal locomotor activity to the OF test environment, which was reflected by a main effect of time ($F(3,120) = 60.539$, $p < 0.001$). The baseline locomotor activity was altered by the treatments (Figure 4a), which was supported by significant main effect of drug treatment ($F(3,40) = 11.458$, $p < 0.001$), and a significant treatment x time interaction ($F(9,120)= 2.202$, $p = 0.026$). Pre-planned comparisons revealed a decrement in the baseline locomotor activity in all treatment groups compared to SAL/VEH control group (AMPH/VEH: $p = 0.015$, AMPH/**1a**: $p = 0.015$, AMPH/**16c**: $p < 0.001$). Furthermore, when the AMPH-sensitized groups are compared, **16c** treatment further inhibited the baseline locomotor activity compared to VEH treatment ($p = 0.04$), whereas **1a** treatment did not alter it ($p > 0.05$).

Vertical activity (rearing) can provide measures of general physical motor abilities as well as degree of attention in the novelty of the environment. A clear effect of habituation was observed in the baseline rearing activity ($F(3,120) = 61.673$, $p < 0.001$). AMPH-sensitization changed the baseline rearing activity of animals, which was indicated by a significant effect for treatment ($F(3,40) = 10.588$, $p < 0.001$), and a significant time x

treatment interaction ($F(9,120) = 2.592$, $p = 0.009$). When SAL/VEH group is compared to the other treatment groups, pre-planned analyses revealed a significantly decreased rearing in all AMPH-pretreated groups (AMPH/VEH: $p = 0.03$, AMPH/**1a**: $p = 0.047$, AMPH/**16c**: $p < 0.001$, Figure 4b). Furthermore, **16c** treatment exacerbated the baseline rearing disruption induced by AMPH pretreatment ($p = 0.04$).

Central activity in the OF test is a useful parameter to estimate the anxiety level of animals,[16] and several comorbid anxiety disorders have been associated with AMPH abusers[17] and schizophrenia patients.[18, 19] Habituation to the test environment significantly reduced the central activity in all groups ($F(3,117) = 7.471$, $p < 0.001$). The significant effect of treatment ($F(3,39) = 6.014$, $p = 0.002$) indicated that AMPH-pretreatment induced alterations in the central duration (Figure 4c), yet a time x treatment interaction was not found ($F(9,117) = 0.529$, $p > 0.05$). Pre-planned comparisons evidently demonstrated that central duration in the AMPH-pretreated animals was reduced ($p = 0.011$), which could not be reversed by treatment with either **1a** ($p = 0.004$), or **16c** ($p = 0.019$).

Amphetamine-induced hyperactivity. The locomotor response to an acute AMPH-challenge (1.5 mg/kg, i.p.) was altered by the pre-treatments. There was a significant effect of time ($F(3,120) = 18.261$, $p < 0.001$), treatment ($F(3,40) = 6.239$, $p = 0.001$), and a time x treatment interaction ($F(9,120) = 4.495$, $p < 0.001$). Pre-planned analyses compared to SAL/VEH control group indicated a strong increment in AMPH-induced locomotion in the AMPH/VEH animals both at single time points (Figure 4d) and as area under the curve (AUC) total activity ($p = 0.001$, Figure 4g). This effect was attenuated by **1a** treatment ($p > 0.05$) and by **16c** treatment ($p > 0.05$). Furthermore, **16c** treatment successively reversed the hyper-locomotor activity induced by AMPH-sensitization; **16c**-treated animals had a significantly attenuated AUC total locomotor activity compared to AMPH/VEH group animals ($p = 0.03$, Figure 4g). This attenuation was also evident at single time points, as shown in Figure 4d. The rearing response to an acute AMPH injection was altered by the pre-treatments. We found significant effects of time ($F(3,120) = 20.197$, $p < 0.001$), treatment ($F(3,40) = 11.573$, $p < 0.001$), and time x treatment interaction ($F(9,120) = 2.304$, $p = 0.02$). Pre-planned comparisons with SAL/VEH group revealed an augmented rearing activity in AMPH/VEH group both at single time points (Figure 4e) and as AUC total activity ($p = 0.001$, Figure 4h). Treatment with **1a** as well as with **16c** reduced the overall AMPH-induced elevations in rearing behavior (AMPH/**1a**: $p = 0.008$, AMPH/**16c**: $p < 0.001$). Reduced rearing activity after **1a** and **16c** treatment was also demonstrated at single time points (Figure 4e). The central activity after an acute AMPH injection was different between treatment groups, which was revealed by significant effects of time ($F(3,117) = 11.912$, $p < 0.001$), treatment x time interaction ($F(9,117) = 5.157$, $p < 0.001$), and treatment ($F(3,39) = 2.967$, $p = 0.044$). Pre-planned analyses showed that compared to the SAL/VEH group, AMPH/VEH group animals spent less time at the center of the arena, shown as both activity at single time points ($p = 0.036$, Figure 4f) and AUC total activity (Figure 4i). This anxiogenic behavior was partially reversed by **16c** ($p > 0.05$ vs SAL/VEH), but a tendency for elevated anxiety was still present after **1a** treatment ($p = 0.063$ vs SAL/VEH). Furthermore, we compared the baseline and AMPH-induced central duration. Pairwise comparisons indicated that acute AMPH

injection led to an elevation in time spent at the center in all groups (VEH/VEH: $p = 0.001$, AMPH/**1a**: $p = 0.039$, AMPH/**16c**: $p = 0.003$), except for AMPH/VEH group ($p > 0.05$, data not shown).

Light induced activity (LIA). Light-stimulation, which has been shown to trigger locomotion,[13, 15, 20] can be used to investigate the sensorimotor gating properties of animals. The 20 min horizontal activity with light-stimulation yielded a significant effect of time ($F(3, 120) = 90.958$, $p < 0.001$), treatment ($F(3, 40) = 3.043$, $p = 0.04$), but no time x treatment interaction ($F(9,120) = 0.713$, $p > 0.05$). Pre-planned analyses denoted that the locomotion-inducing effects of light was exaggerated in the AMPH/VEH animals ($p = 0.03$). This effect was blocked after both **1a** and **16c** treatment (ps > 0.05, Supplementary Figure 2a). Light-induction elevates the locomotion most-strikingly at the first 5min interval, as shown in the literature.[13, 21] The treatment was effective at the first 5 min ($F(3,40) = 2.776$, $p = 0.054$). AMPH sensitization caused a significantly higher LIA compared to SAL/VEH group at the first time point ($p = 0.03$). This effect was partially reversed by both **1a** and **16c** (ps > 0.05). LIA was more pronounced in AMPH/VEH group and persistent for up to 3 test intervals (5min: $p < 0.001$, 10min: $p = 0.046$, 15min: $p = 0.028$). For all other treatment groups, LIA faded after 5min. Light-stimulation effects on rearing activity showed a significant effect of time ($F(3,120) = 106.274$, $p < 0.001$), but no effect of time x treatment interaction ($F(9,120) = 1.396$, $p > 0.05$). Although visual inspection of the data suggests that compared to the SAL/VEH group, AMPH/VEH group animals showed an elevated rearing activity at all single time points (Supplementary Figure 2b), this effect did not reach statistical significance($F(3,40) = 2.188$, $p > 0.05$), but showed a weak tendency for the first 5min interval ($p = 0.093$).

12) Supplementary Methods. Synthesis of compounds **3a**,**b**; **4a**,**b**; **5** and (*R*)/(*S*)-**11**.

Methyl 4-[4-(2,3-dichlorophenyl)-piperazin-1-yl]butyrate (3a). To a suspension of 1-(2,3-dichlorophenyl)piperazine hydrochloride (1.07 g, 3.99 mmol) in dry DMF (13.3 mL) was added methyl 4-bromobutyrate (0.51 mL, 3.99 mmol). Subsequently, triethylamine (1.67 mL, 12 mmol) was added dropwisely. After stirring at room temperature for 24 h, the mixture was diluted with water (100 mL) and extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (dichloromethane/dimethyl ethylamine 99.5:0.5) to yield **3a** as yellow oil (1.31 g, 98%). IR (NaCl): 2948, 2819, 1736, 1577, 1448, 1421, 1374, 1240, 1200, 1132, 1045, 1011, 968 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz, δ): 1.86 (quin, *J* = 7.3 Hz, 2 H), 2.38 (t, *J* = 7.3 Hz, 2 H), 2.44 (t, *J* = 7.3 Hz, 2 H), 2.59–2.67 (m, 4 H), 3.03–3.09 (m, 4 H), 3.68 (s, 3 H), 6.95 (dd, *J* = 6.9 Hz, 2.7 Hz, 1 H), 7.12–7.16 (m, 2 H). ^{13}C NMR ($CDCl_3$, 90 MHz, δ): 22.1, 32.0, 51.3, 51.5, 53.2, 57.6, 118.5, 124.5, 127.4, 127.5, 134.0, 151.3, 174.0. HPLC (system 1): t_R = 14.7 min, purity 99%. APCI-MS: *m/z* 331.7 [$M+H^+$].

Methyl 4-[4-(2-methoxyphenyl)piperazin-1-yl]butyrate (3b). Compound **3b** was prepared according to the protocol of **3a** using a suspension of 2-methoxyphenylpiperazine (932 mg, 4.85 mmol) and methyl 4-bromobutyrate (0.61 mL, 4.85 mmol) as well as triethylamine (2.01 mL, 14.6 mmol). Purification by flash chromatography (dichloromethane/0.5% dimethyl ethylamine and dichloromethane/0.25% methanol/0.5% dimethyl ethylamine) yielded **3b** as yellow oil (1.40 g, 88%). IR (NaCl): 2946, 2814, 1736, 1593, 1500, 1450, 1355, 1299, 1240, 1179, 1134, 1058, 1027, 962, 926 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz, δ): 1.86 (quin. *J* = 7.4 Hz, 2 H), 2.38 (t, *J* = 7.4 Hz, 2 H), 2.43 (t, *J* = 7.3 Hz, 2 H), 2.58–2.70 (m, 4 H), 3.02–3.16 (m, 4 H), 3.68 (s, 3 H), 3.86 (s, 3 H), 6.86 (dd, *J* = 8.1 Hz, 1.4 Hz, 1 H), 6.91 (ddd, *J* = 7.9 Hz, 7.0 Hz, 1.4 Hz, 1 H), 6.94 (dd, *J* = 7.9 Hz, 1.9 Hz, 1 H), 6.99 (ddd, *J* = 8.0 Hz, 7.1 Hz, 2.0 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 90 MHz, δ): 22.2, 32.1, 50.7, 51.5, 53.4, 55.3, 57.7, 111.2, 118.2, 121.0, 122.8, 141.4, 152.3, 174.0. HPLC (system 1): t_R = 11.4 min, purity 96%. APCI-MS: *m/z* 293.2 [$M+H^+$].

Sodium 4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyrate (4a).[22, 23] To a solution of **3a** (1.31 g, 3.93 mmol) in methanol (25 mL) was added 1 M NaOH (3.93 mL, 3.93 mmol). After stirring at 65 °C for 6 h, the solvent was evaporated and the residue was stirred in ethyl acetate (15 mL) at room temperature. The resulting precipitate was filtrated, washed various times with ethyl acetate and dried in vacuum to give **4a** as white solid (1.24 g, 93%). Mp: 133 °C. IR (ATR) 3275, 2931, 2818, 1567, 1449, 1405, 1374, 1245, 1181, 1007, 962 cm^{-1}. ^1H NMR (DMSO-d_6, 600 MHz, δ): 1.61 (quin, *J* = 7.5 Hz, 2 H), 1.88 (t, *J* = 7.4 Hz, 2 H), 2.29 (t, *J* = 7.5 Hz, 2 H), 2.44–2.50 (m, 4 H), 2.93–2.99 (m, 4 H), 7.13 (dd, *J* = 7.4 Hz, 2.1 Hz, 1 H), 7.26–7.31 (m, 2 H). ^{13}C NMR (DMSO-d_6, 90 MHz, δ): 23.7, 36.0, 51.0, 52.8, 58.3, 119.5, 124.2, 125.9, 128.4, 132.6, 176.8. HPLC (system 1): t_R = 15.4 min, purity 96%. APCI-MS: *m/z* 317.2 [$M+H^+$, free acid].

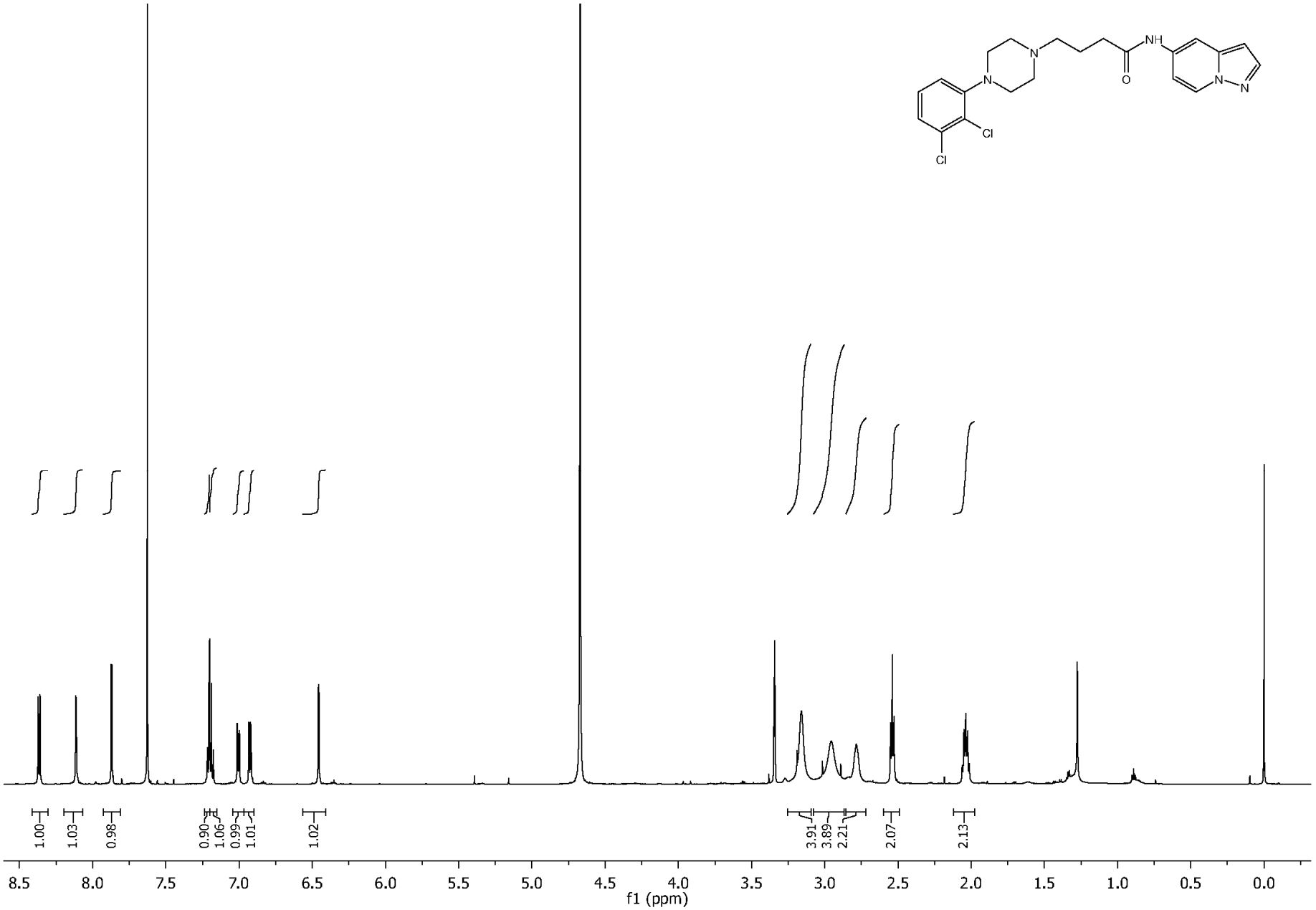
Sodium 4-[4-(2-methoxyphenyl)piperazin-1-yl]butyrate (4b).[22, 23] Compound **4b** was prepared according to the protocol of **4a** using a solution of **3b** (1.28 g, 4.35 mmol) in methanol (30 mL) and 1 M NaOH (4.36 mL, 4.36 mmol). After removal of the solvent and precipitation with ethyl acetate, **4b** was isolated as white solid (1.24 g, 94%). Mp: 181 °C. IR (ATR) 2946, 2816, 1566, 1499, 1412, 1237, 1135, 1026, 920, 750 cm^{-1}. ^{1}H NMR (DMSO-d_6, 600 MHz, δ): 1.57–1.63 (m, 2 H), 1.87 (t, J = 7.4 Hz, 2 H), 2.27 (t, J = 7.6 Hz, 2 H), 2.44–2.49 (m, 4 H), 2.91–2.97 (m, 4 H), 3.76 (s, 3 H), 6.84–6.95 (m, 4 H). ^{13}C NMR (DMSO-d_6, 90 MHz, δ): 23.8, 36.1, 50.1, 53.1, 55.3, 58.5, 111.9, 117.8, 120.8, 122.2, 141.4, 151.9, 176.6. HPLC (system 1): t_R = 11.0 min, purity 97%. HR-EIMS: [M^+] calcd for $C_{15}H_{21}N_2O_3$, 277.1552; found 277.1558.

5-Aminopyrazolo[1,5-a]pyridine (5). A solution of methyl 5-(tert-butoxycarbonylamino)pyrazolo[1,5-*a*]pyridine-3-carboxylate (390 mg, 1.34 mmol) in 48% hydrobromic acid (16 mL) was refluxed for 5 h. After cooling, the mixture was alkalized by addition of 5 M NaOH and extracted with dichloromethane. The combined organic layers were dried over $MgSO_4$ and evaporated. After drying under vacuum, **5** was isolated as light brown solid without further purification (169 mg, 95%). Mp: 122 °C. IR (NaCl) 3429, 3316, 3205, 1652, 1526, 1482, 1449, 1348, 1324, 1261, 1228, 1205, 1172, 1055, 918, 842 cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz, δ): 3.83 (bs, 2 H), 6.13 (d, J = 2.3 Hz, 1 H), 6.22 (dd, J = 7.6 Hz, 2.5 Hz, 1 H), 6.57 (d, J = 2.5 Hz, 1 H), 7.79 (d, J = 2.3 Hz, 1 H), 8.22 (d, J = 7.5 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 90 MHz, δ): 93.3, 96.5, 105.7, 129.1, 141.5, 142.6, 142.7. HPLC (system 1): t_R = 9.4 min, purity 95%. HR-EIMS: [M^+] calcd for $C_7H_7N_3$, 133.0640; found 133.0639. Further analytical data according to the literature[24]

(*S*)-6-(Propylamino)-5,6,7,8-tetrahydronaphthalen-1-ol ((*S*)-11). Commercially available (*S*)-5-methoxy-N-propyl-1,2,3,4-tetrahydronapththalen-2-amine (100 mg, 0.35 mmol) was refluxed in aqueous hydrobromic acid 48% for 16 h. After cooling it was basfied to pH 14 with 2 N KOH and extracted with ethylacetate. The organic layer was dired with Na_2SO_4 and cocentrated in vacuo to give crude *(S)*-**11**. The crude product was used for the following reactions without further purification. *(R)*-**11** was prepared under the same conditions starting from *(R)*-5-methoxy-N-propyl-1,2,3,4-tetrahydronapththalen-2-amine.

13) Supplementary Data. ^{1}H and ^{13}C NMR spectra of target compounds

^{1}H NMR **6a**



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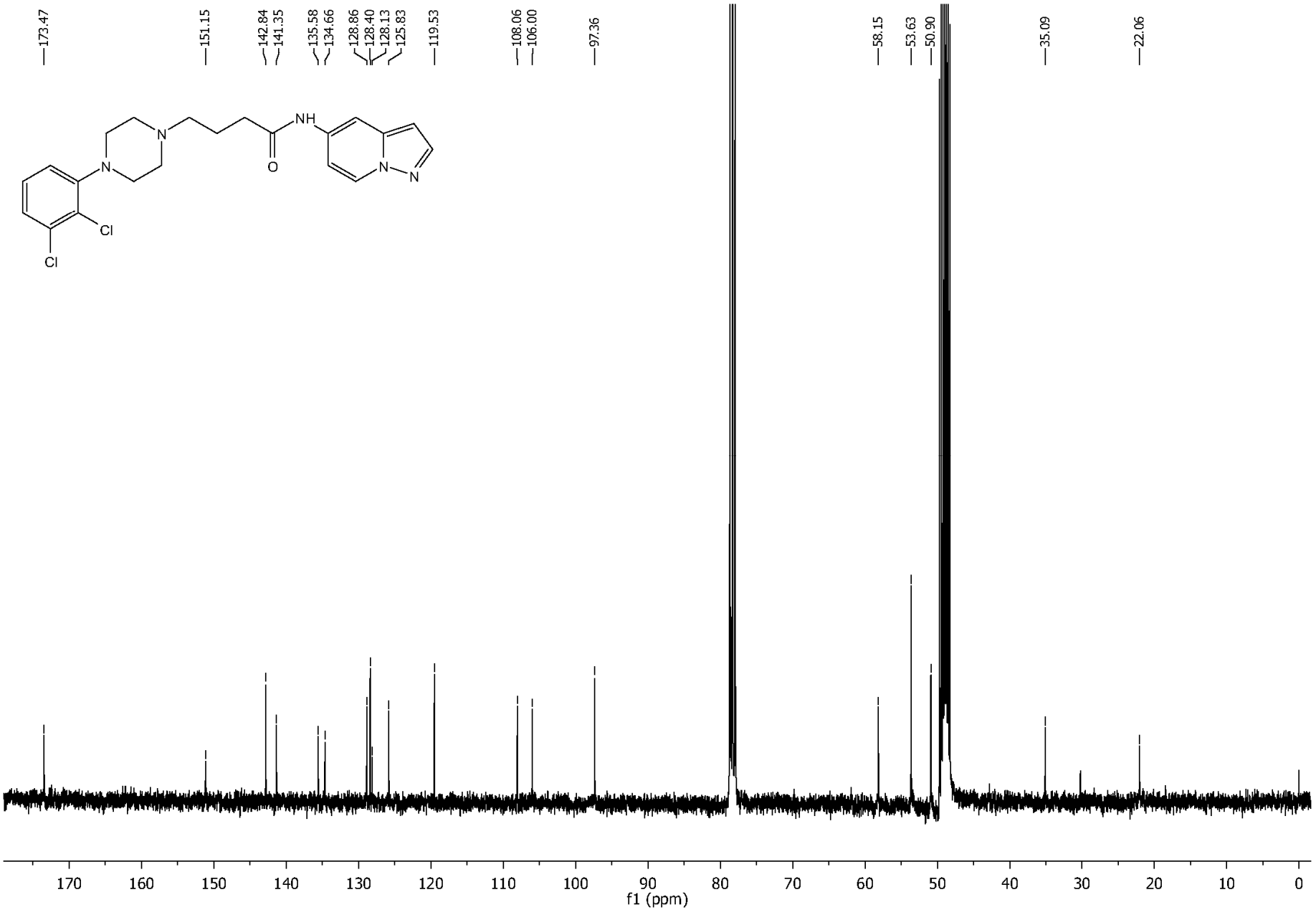
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50.90
35.09
22.06
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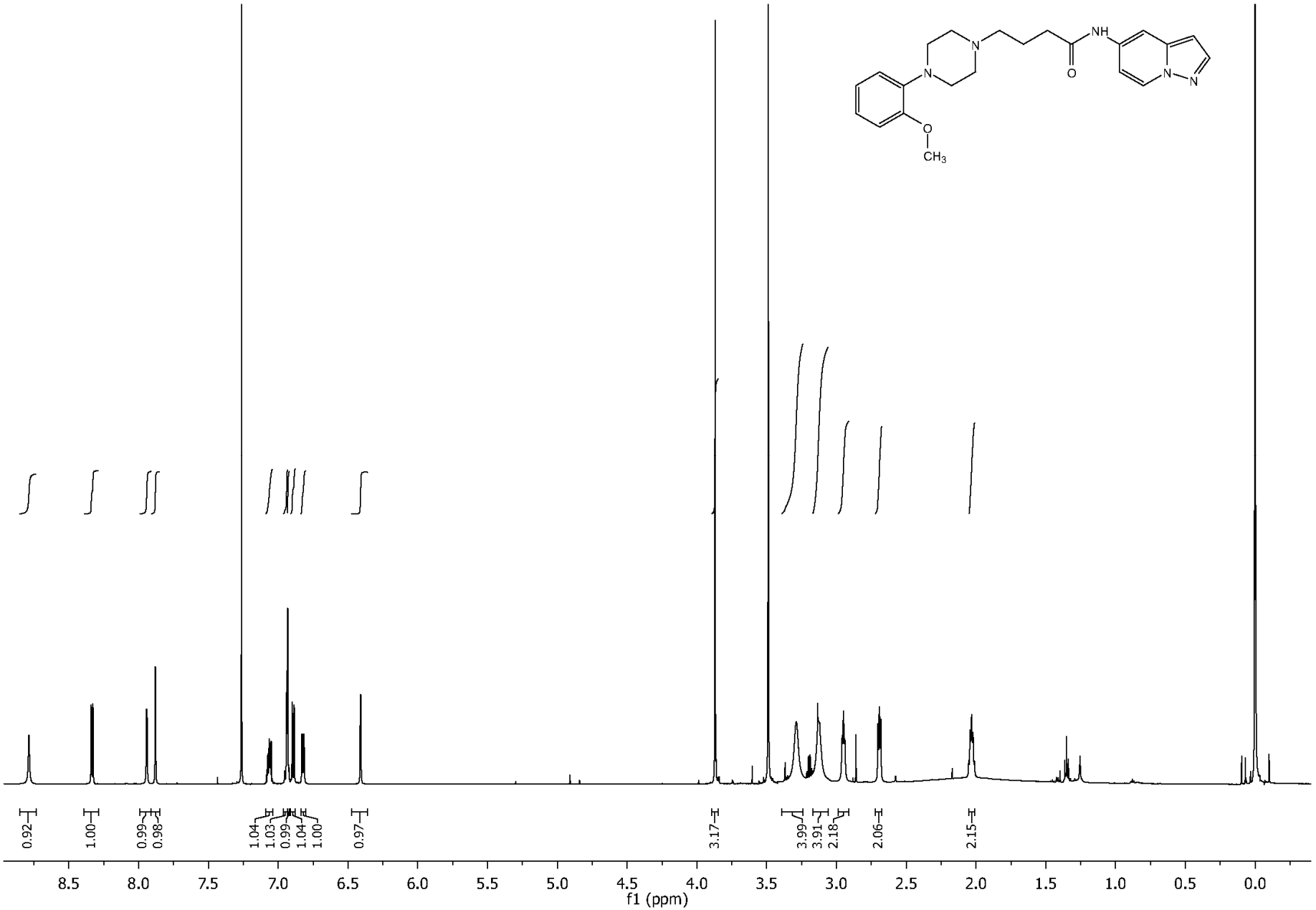
N
N
N
Cl
Cl
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR **6b**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0.99
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1.04
1.00
0.97
3.17
3.99
3.91
2.18
2.06
2.15
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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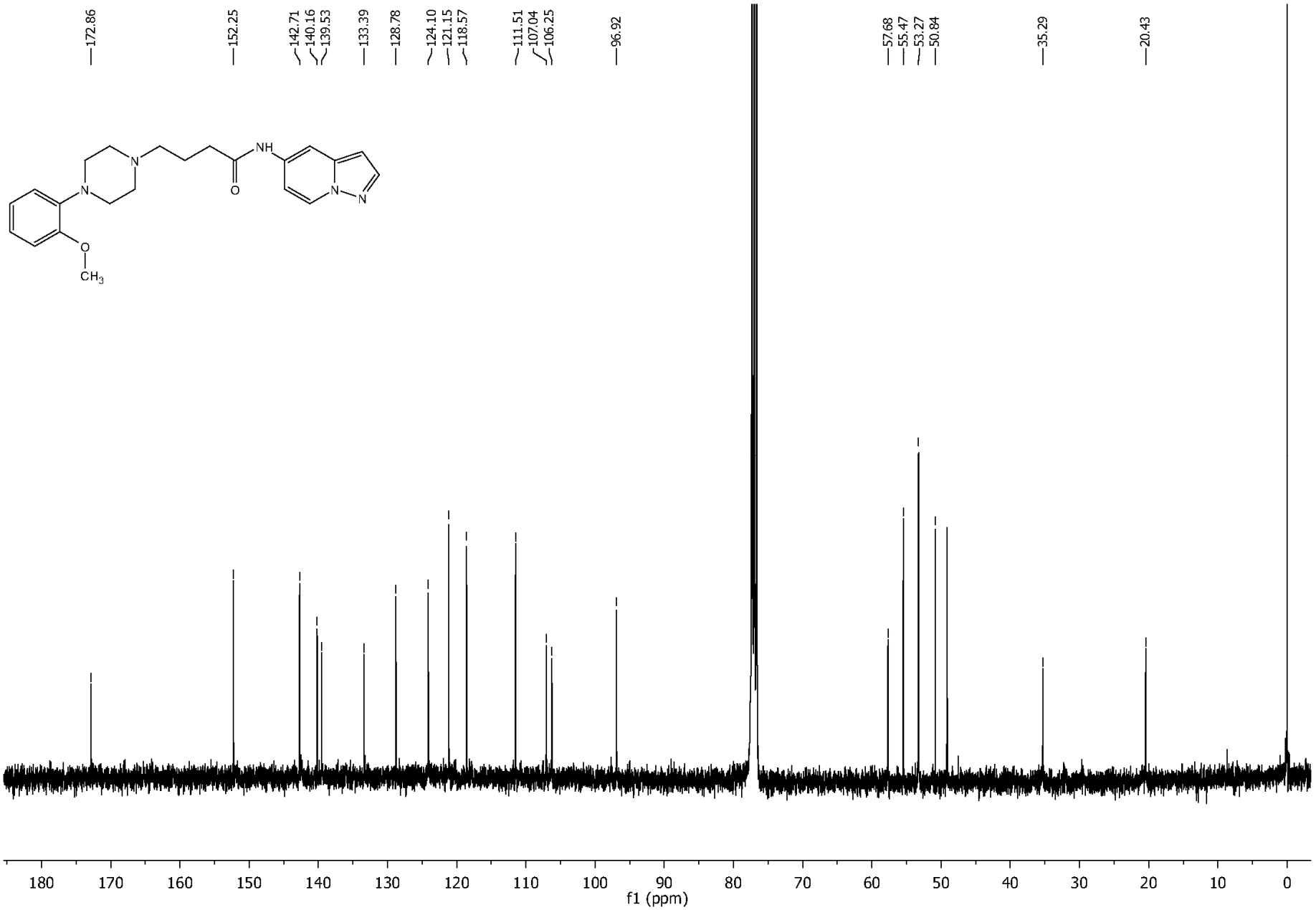
0.5
0.0
f1 (ppm)

^{13}C NMR **6b**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50.84
35.29
20.43
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N
N
N
O
CH3
180
170
160
150
140
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90
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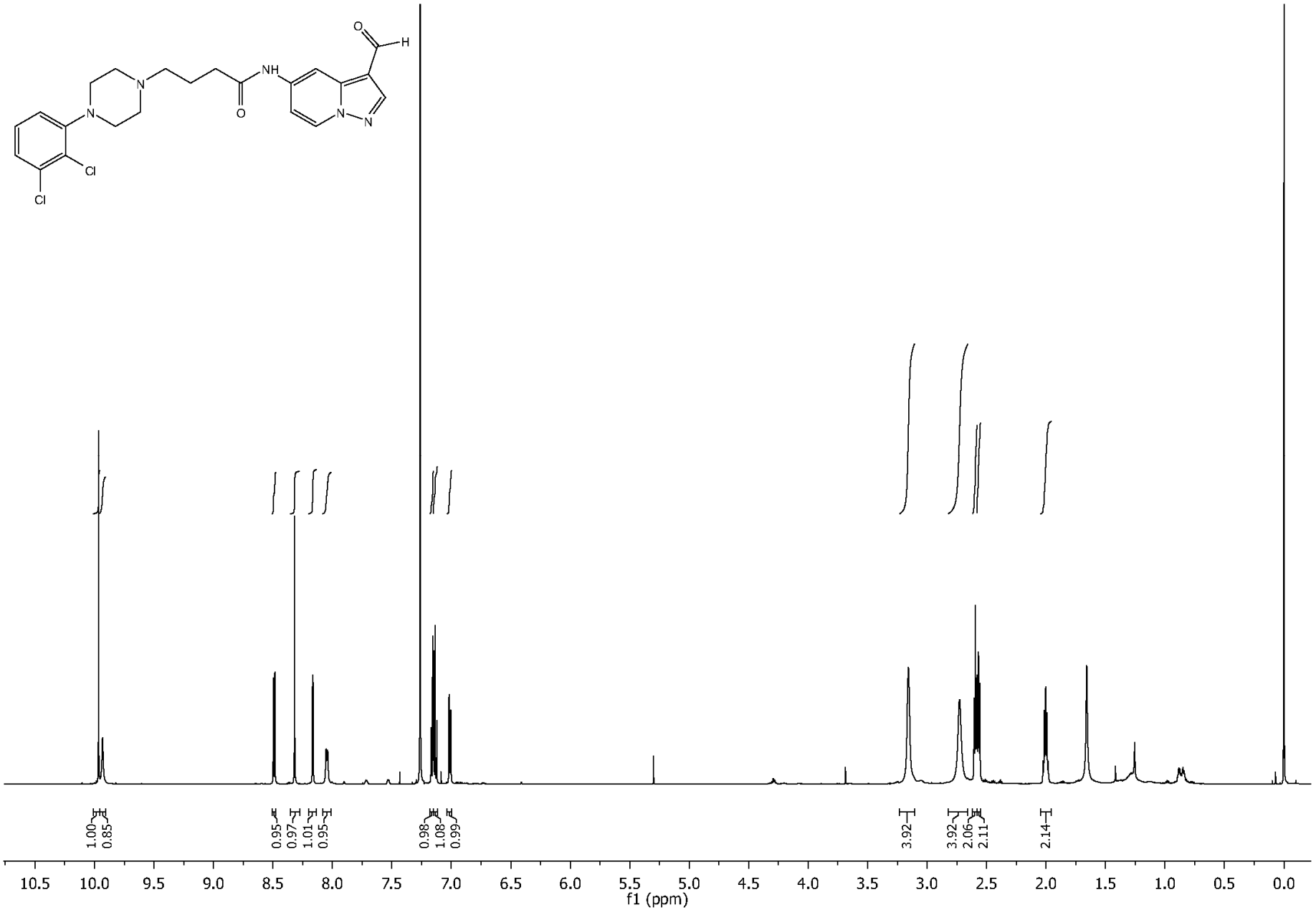
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR **7a**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1.08
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3.92
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2.11
2.14
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8.5
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7.5
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6.5
6.0
5.5
5.0
4.5
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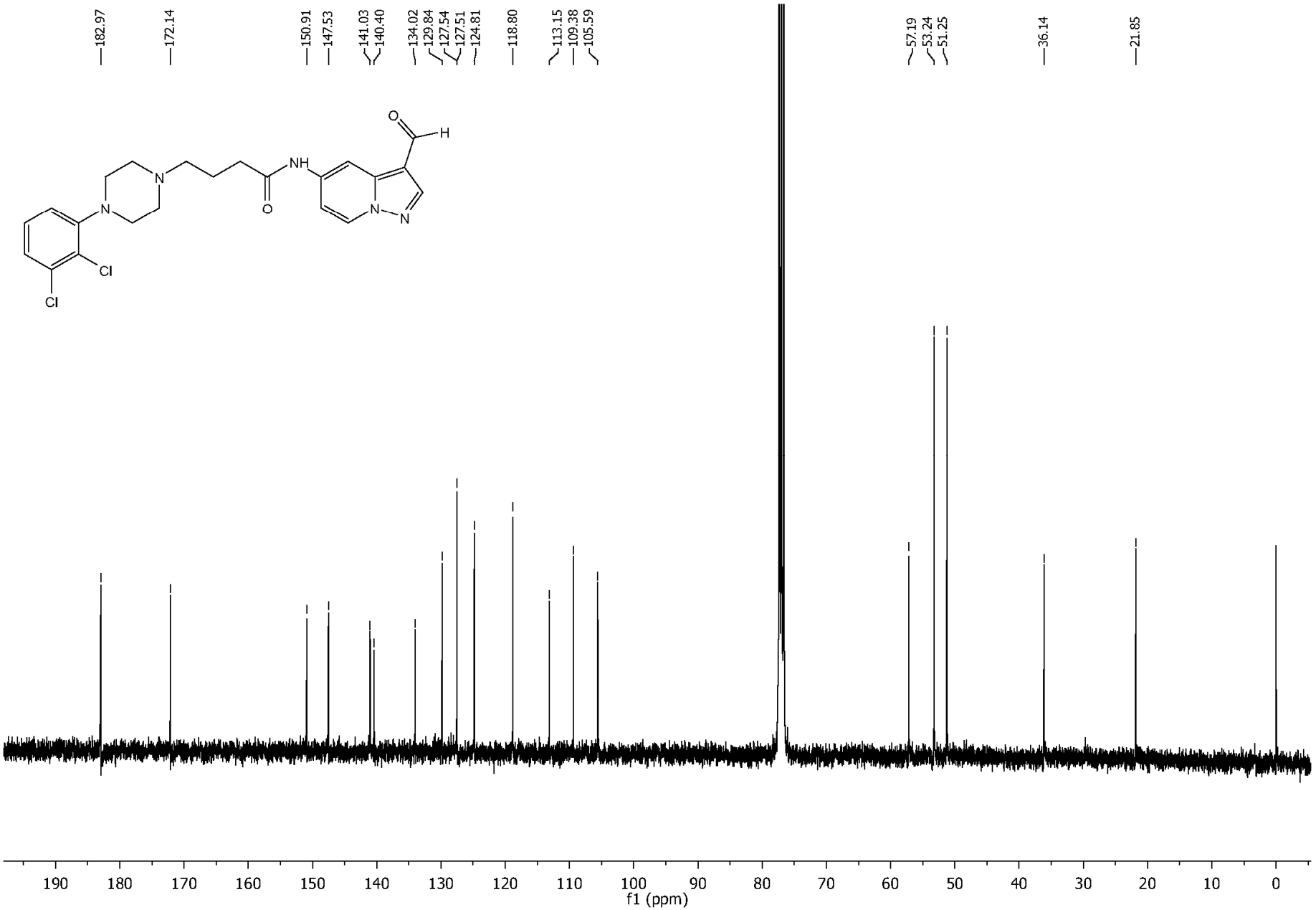
f1 (ppm)

^{13}C NMR **7a**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Cl
190
180
170
160
150
140
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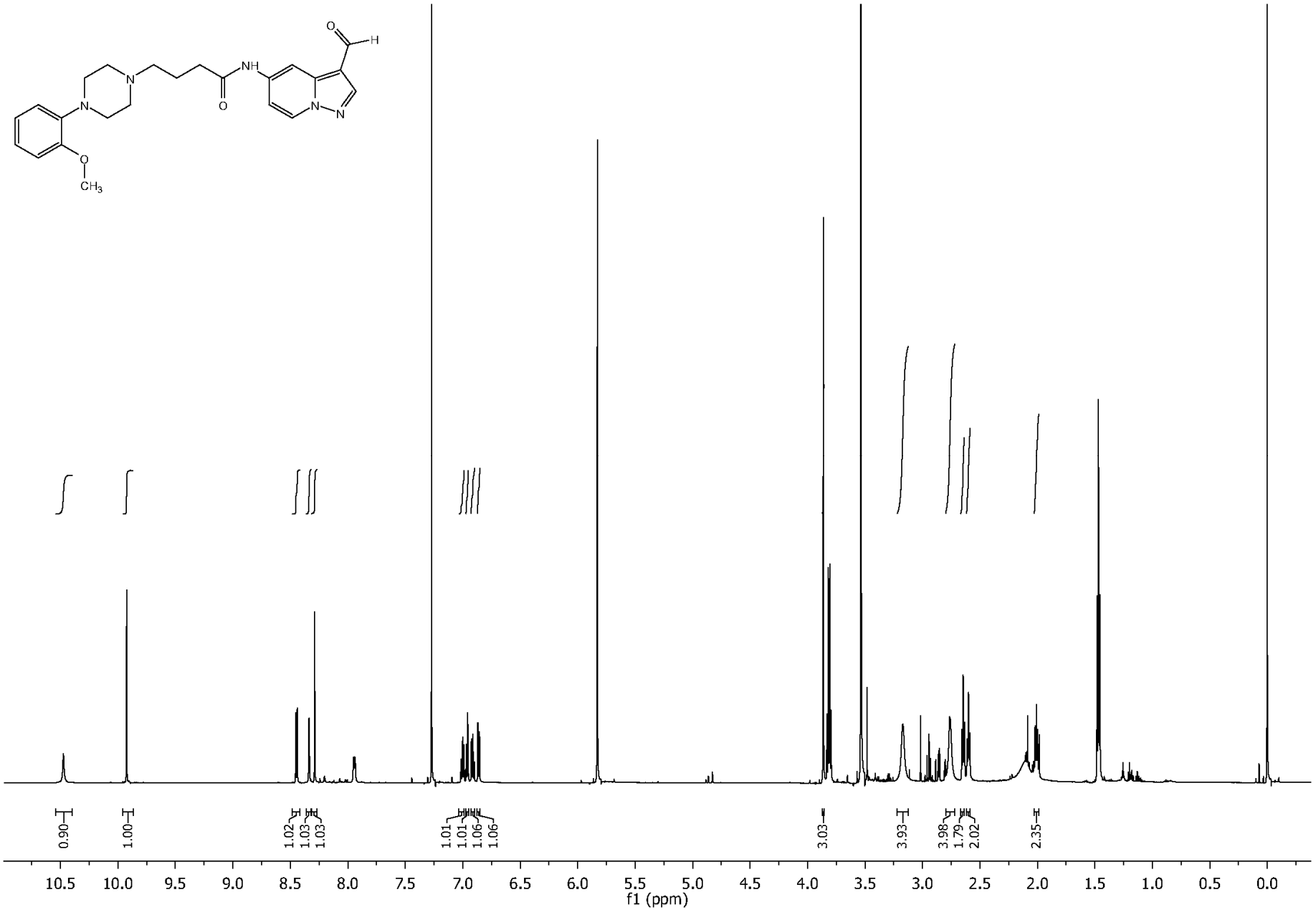
20
10
0
f1 (ppm)

^{1}H NMR **7b**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1.06
1.06
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3.98
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2.02
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7.5
7.0
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5.5
5.0
4.5
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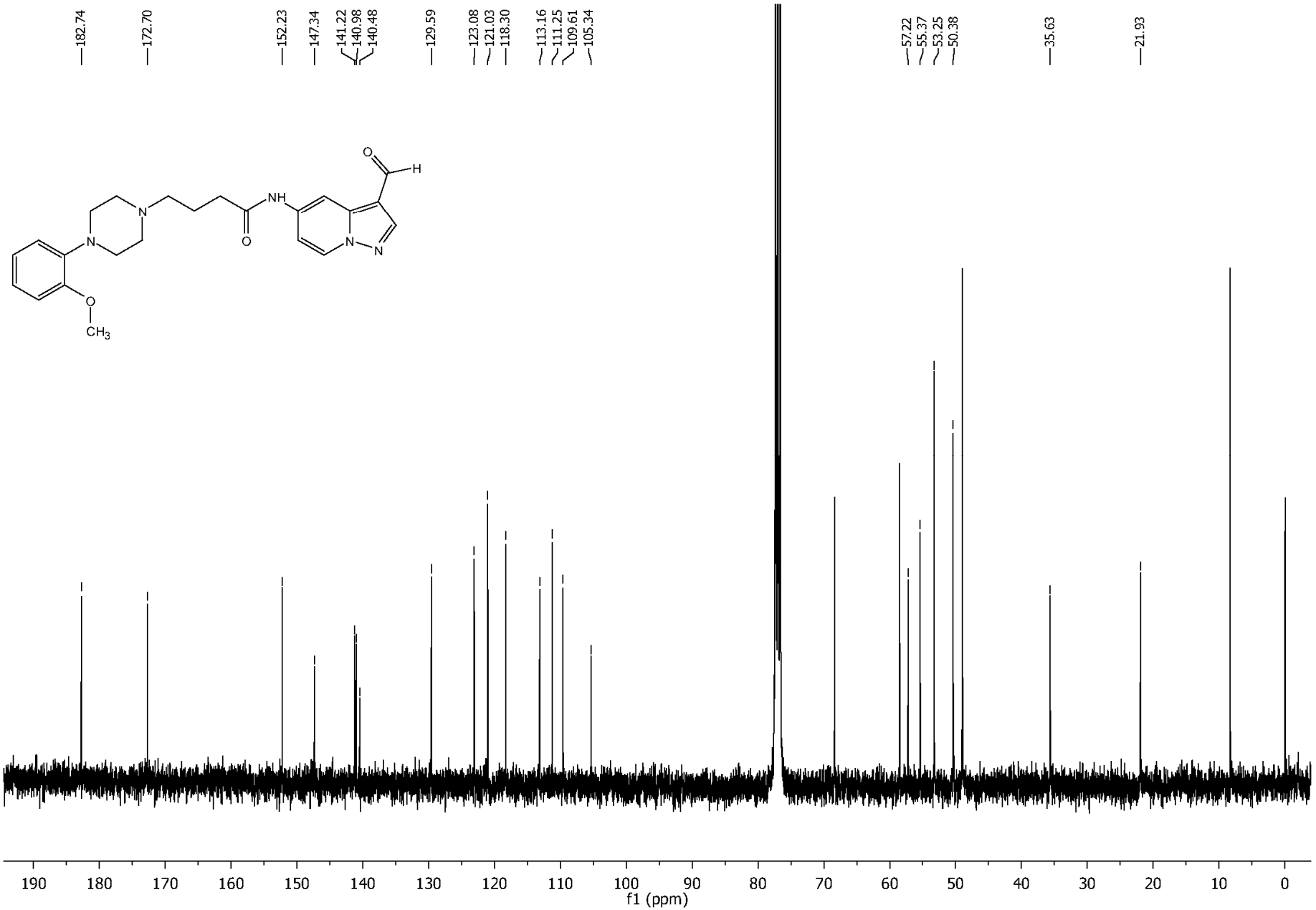
0.0
f1 (ppm)

^{13}C NMR **7b**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53.25
50.38
35.63
21.93
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O
O
CH3
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180
170
160
150
140
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90
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30
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10
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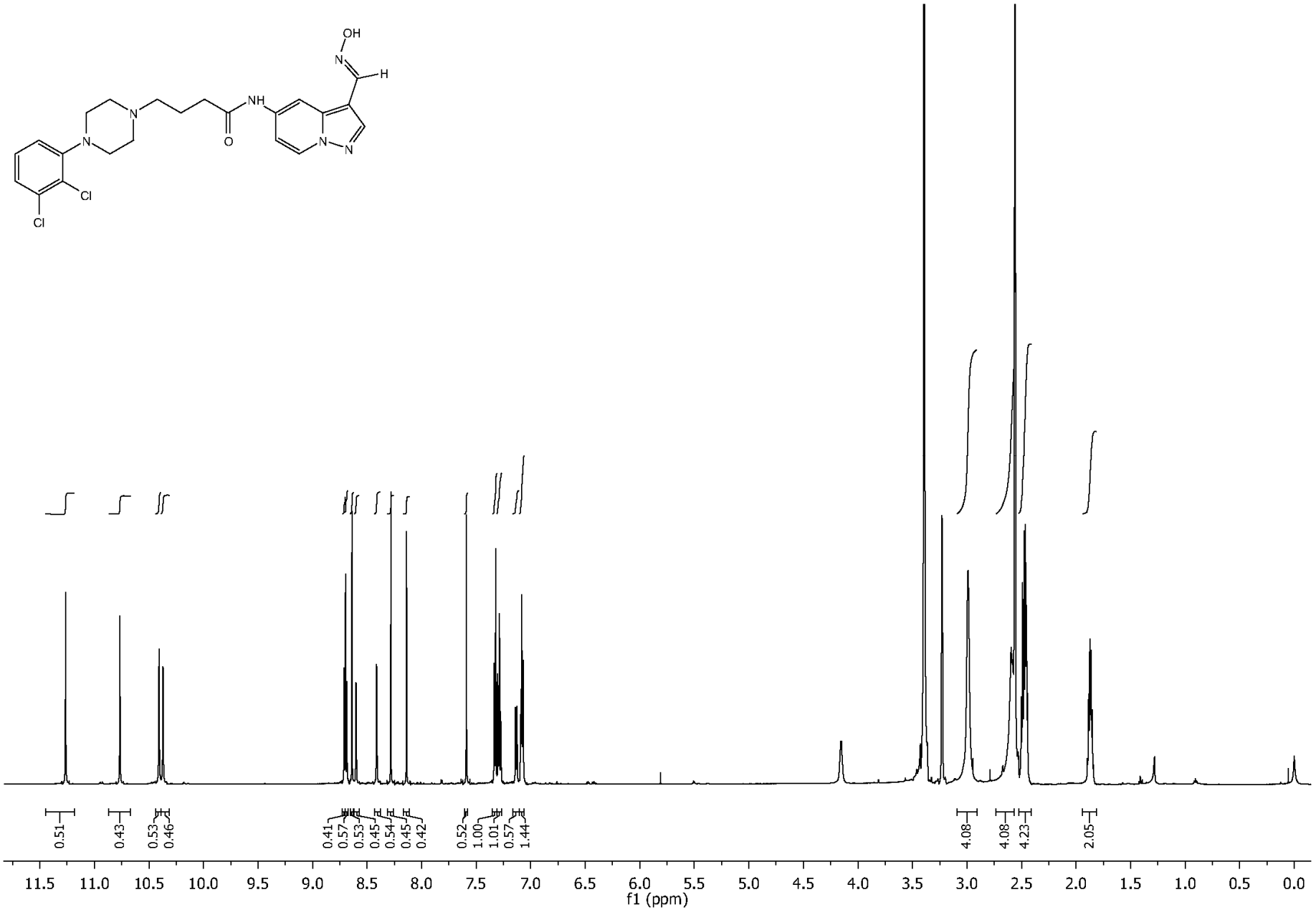
f1 (ppm)

^{1}H NMR **8a**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Cl
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0.54
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4.08
4.23
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11.0
10.5
10.0
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9.0
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6.0
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4.5
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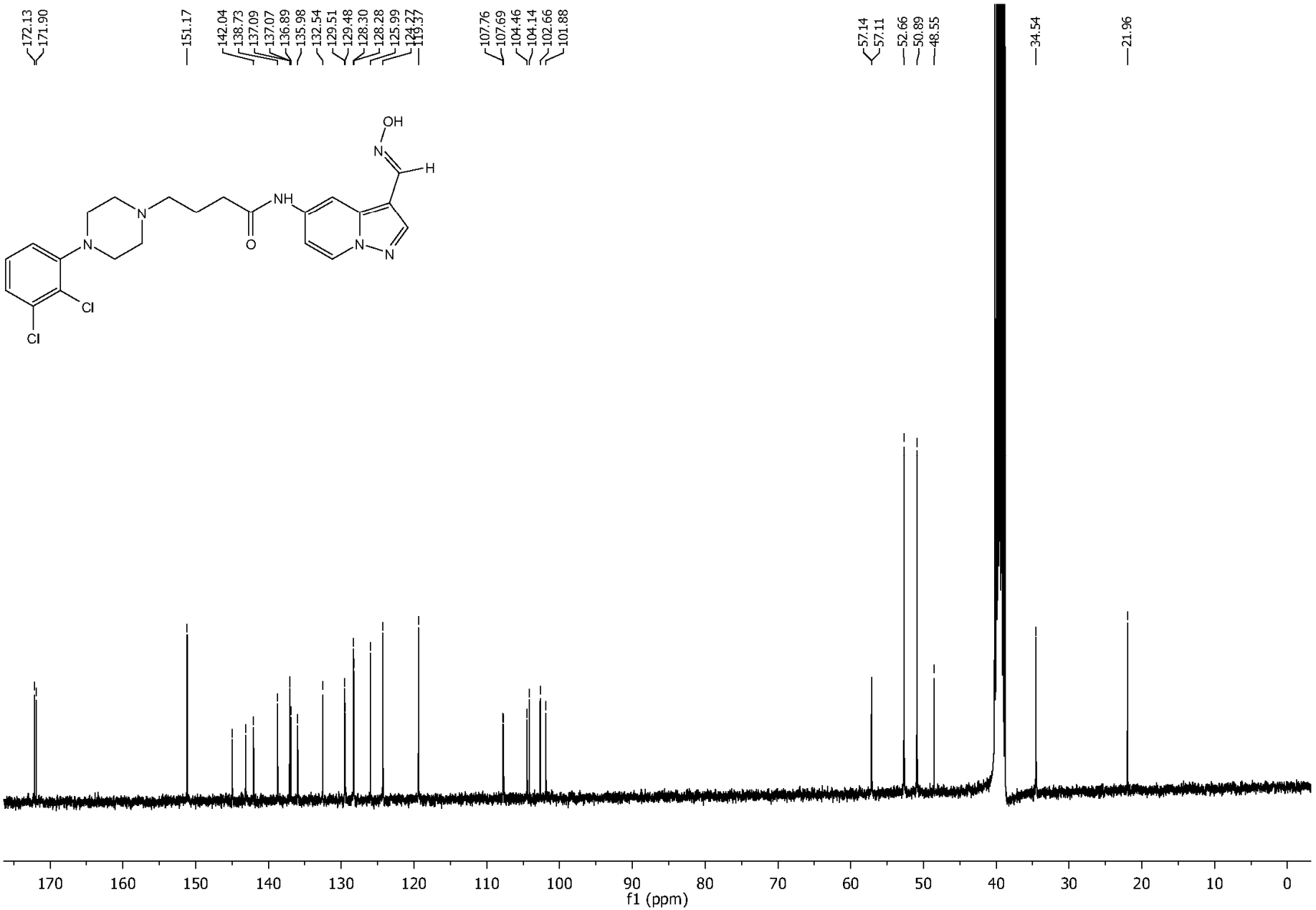
f1 (ppm)

^{13}C NMR **8a**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128.28
125.99
124.27
119.37
107.76
107.69
104.46
104.14
102.66
101.88
57.14
57.11
52.66
50.89
48.55
34.54
21.96
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N
N
Cl
Cl
170
160
150
140
130
120
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80
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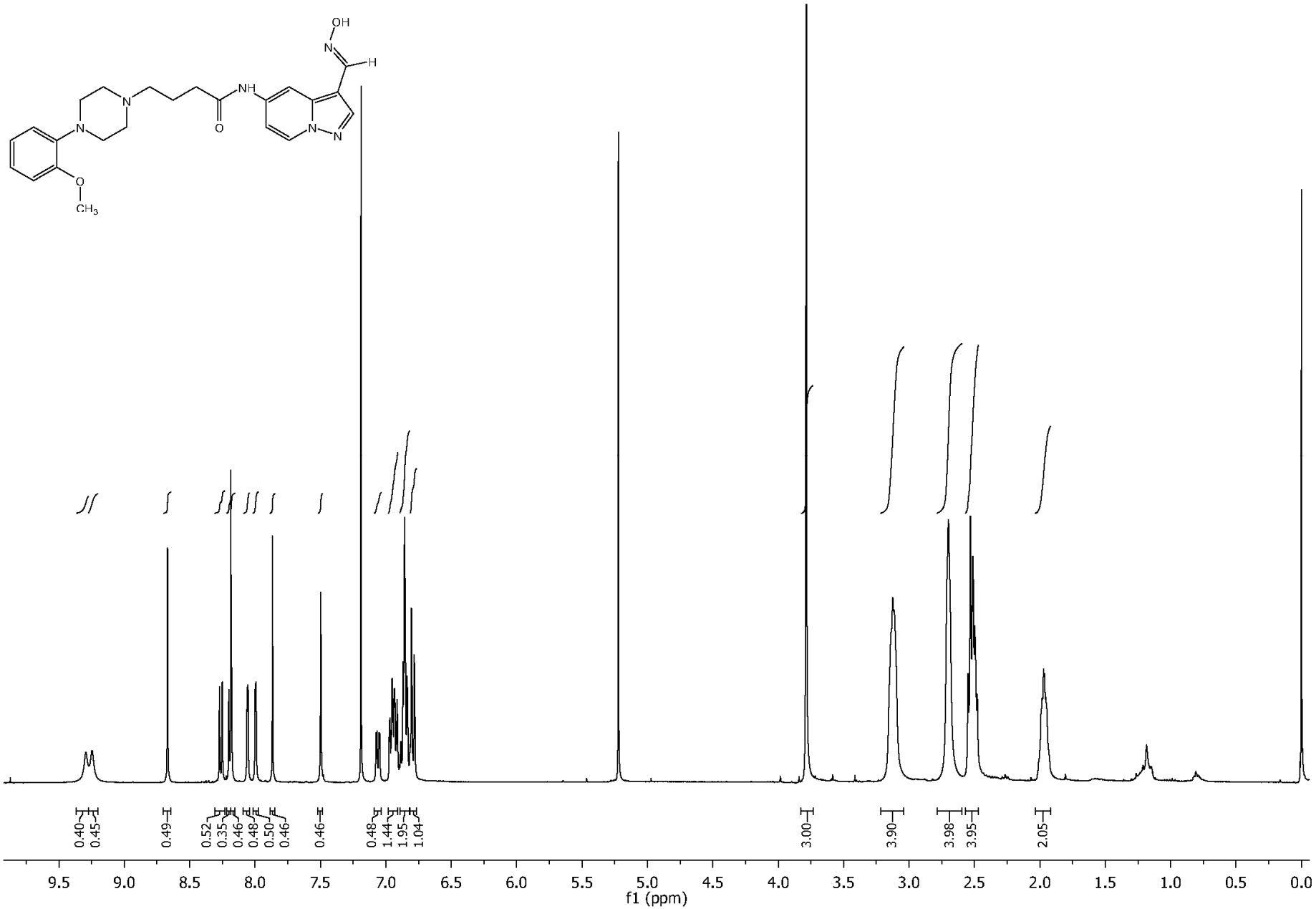
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR **8b**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0.50
0.46
0.46
0.48
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1.04
3.00
3.90
3.98
3.95
2.05
9.5
9.0
8.5
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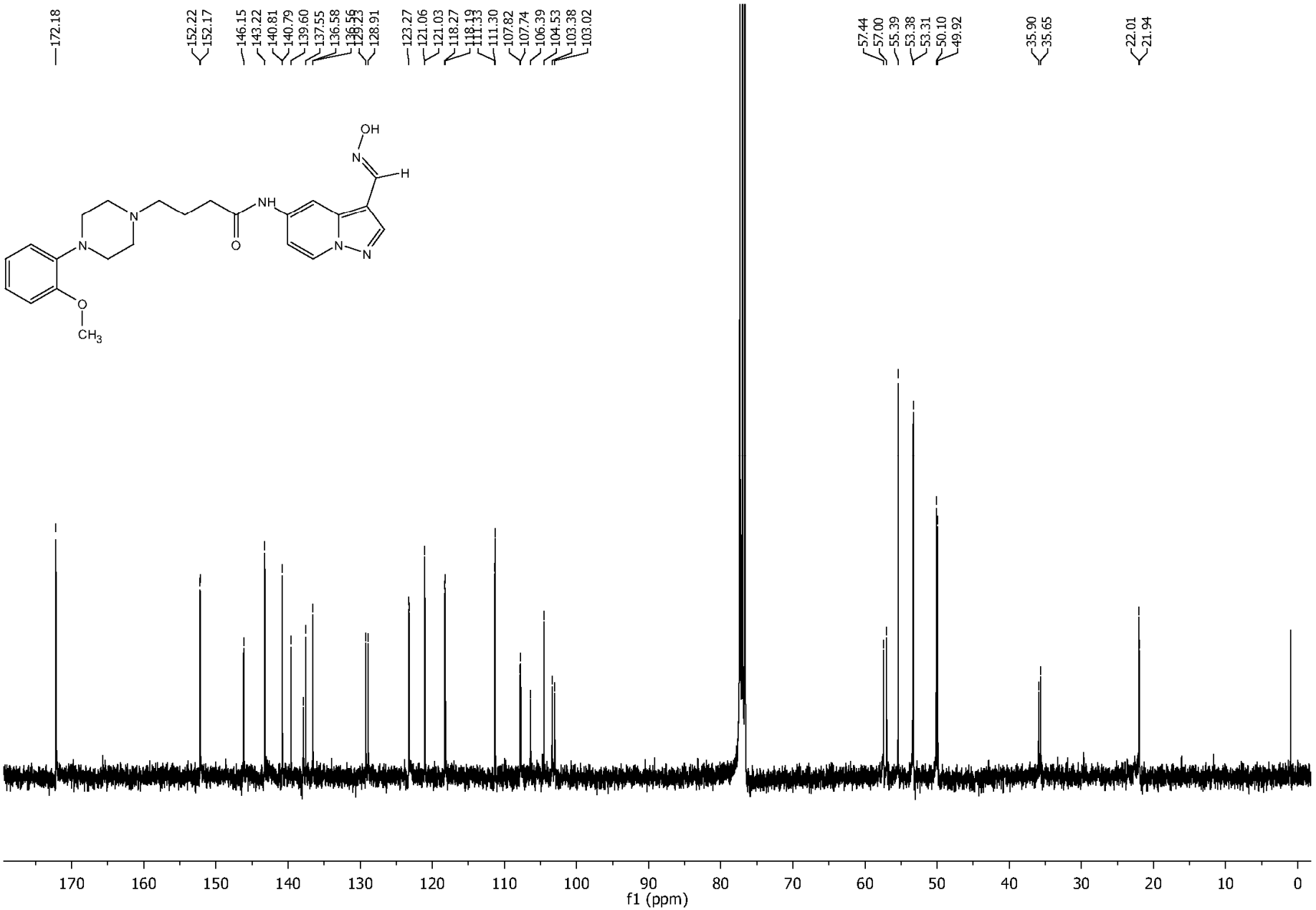
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{13}C NMR **8b**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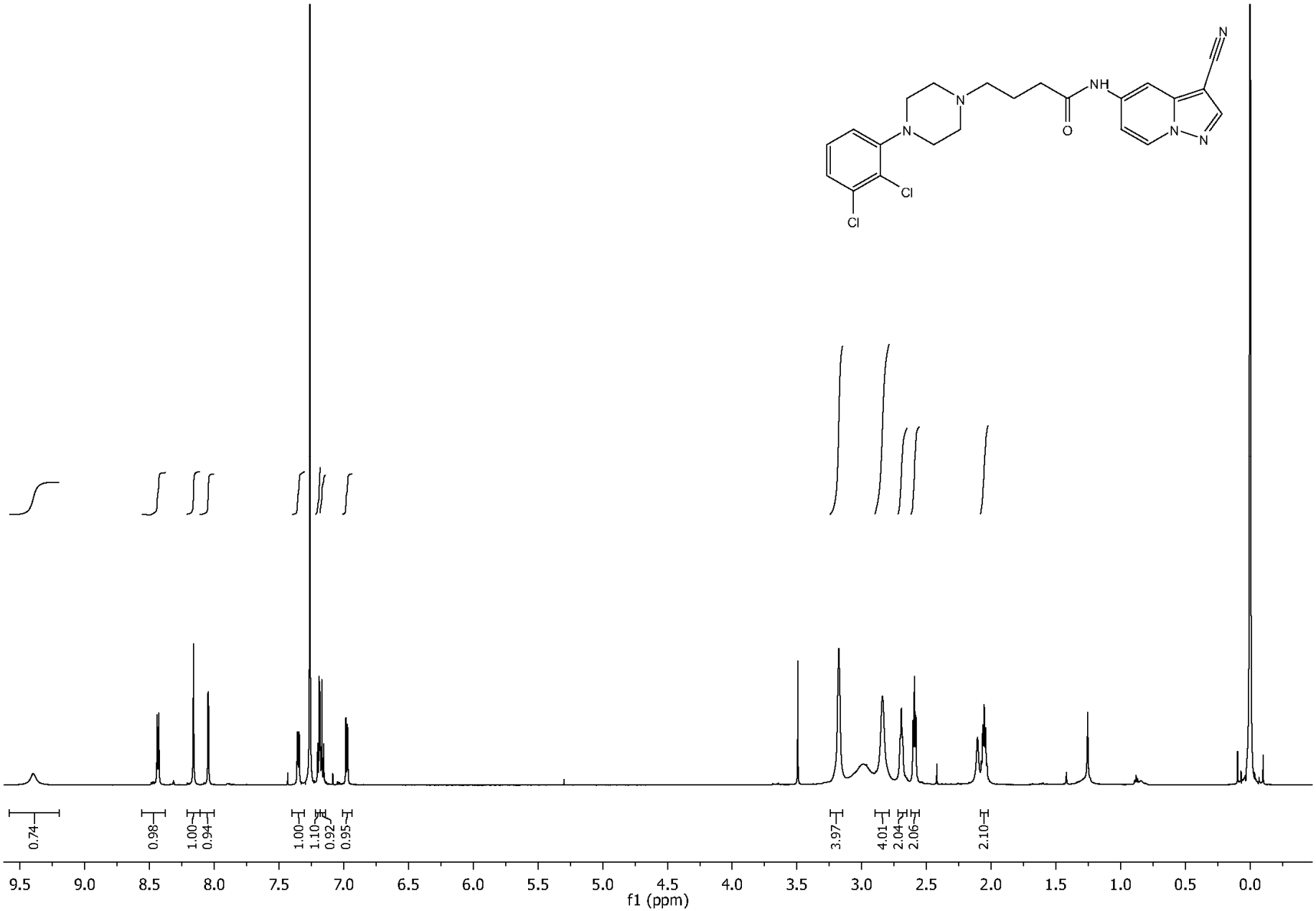
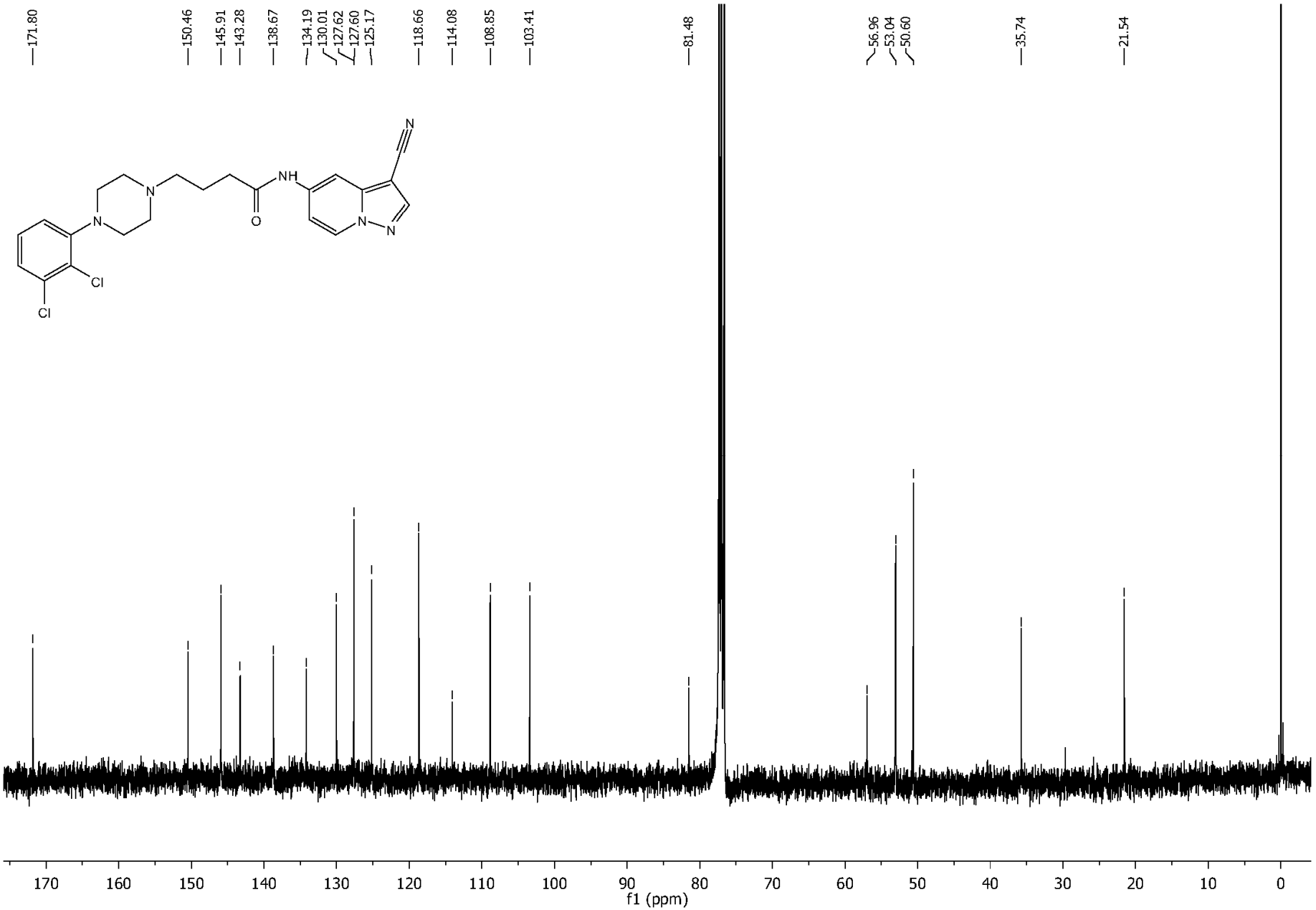
121.03
118.27
111.30
107.82
107.74
106.39
104.53
103.38
103.02
57.44
57.00
55.39
53.38
53.31
50.10
49.92
35.90
35.65
22.01
21.94
OH
N
H
NH
O
N
N
N
O
CH3
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

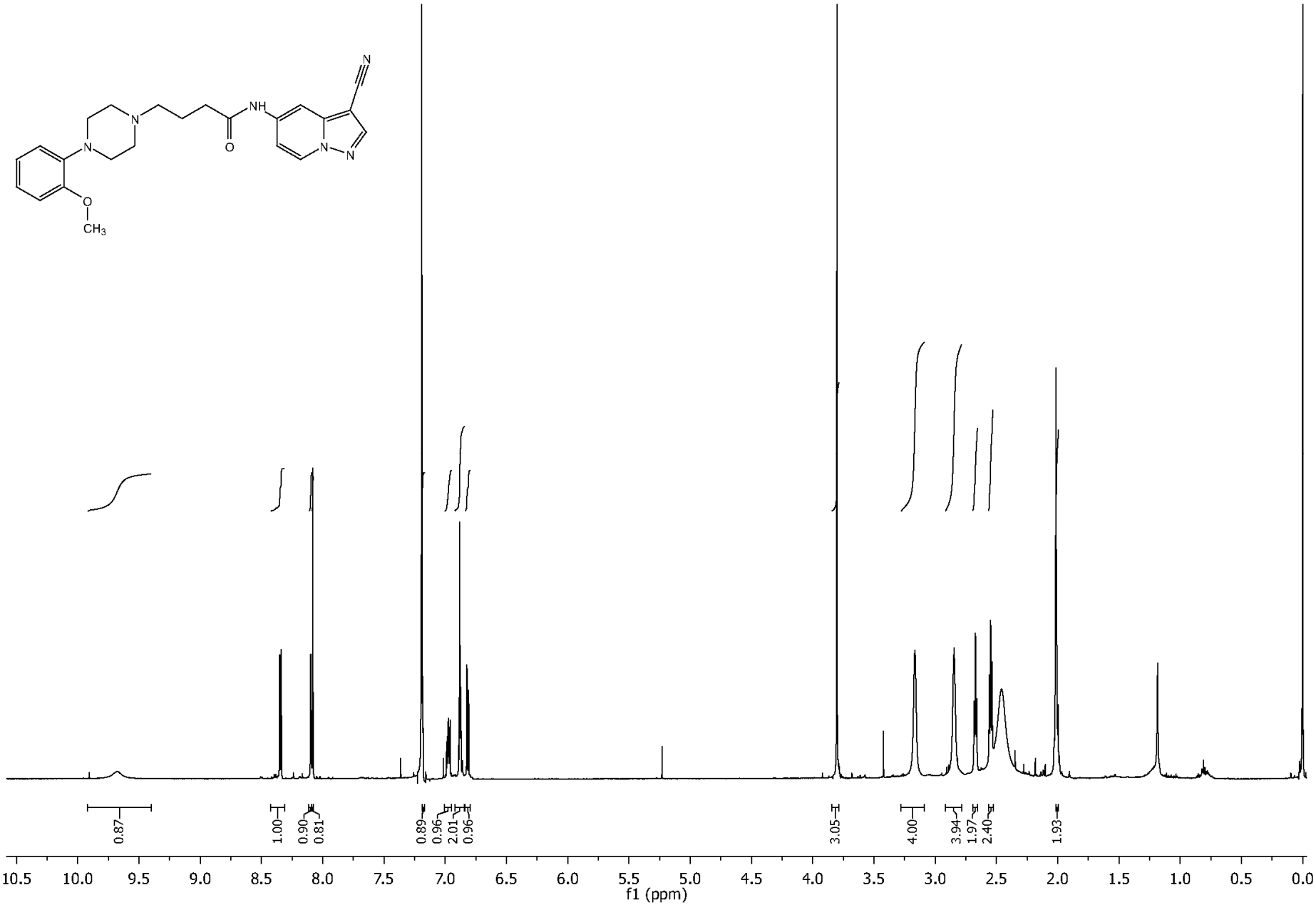
^{1}H NMR **9a**


N
NH
O
N
N
N
Cl
Cl
0.74
0.98
1.00
0.94
1.00
1.10
0.92
0.95
3.97
4.01
2.04
2.06
2.10
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

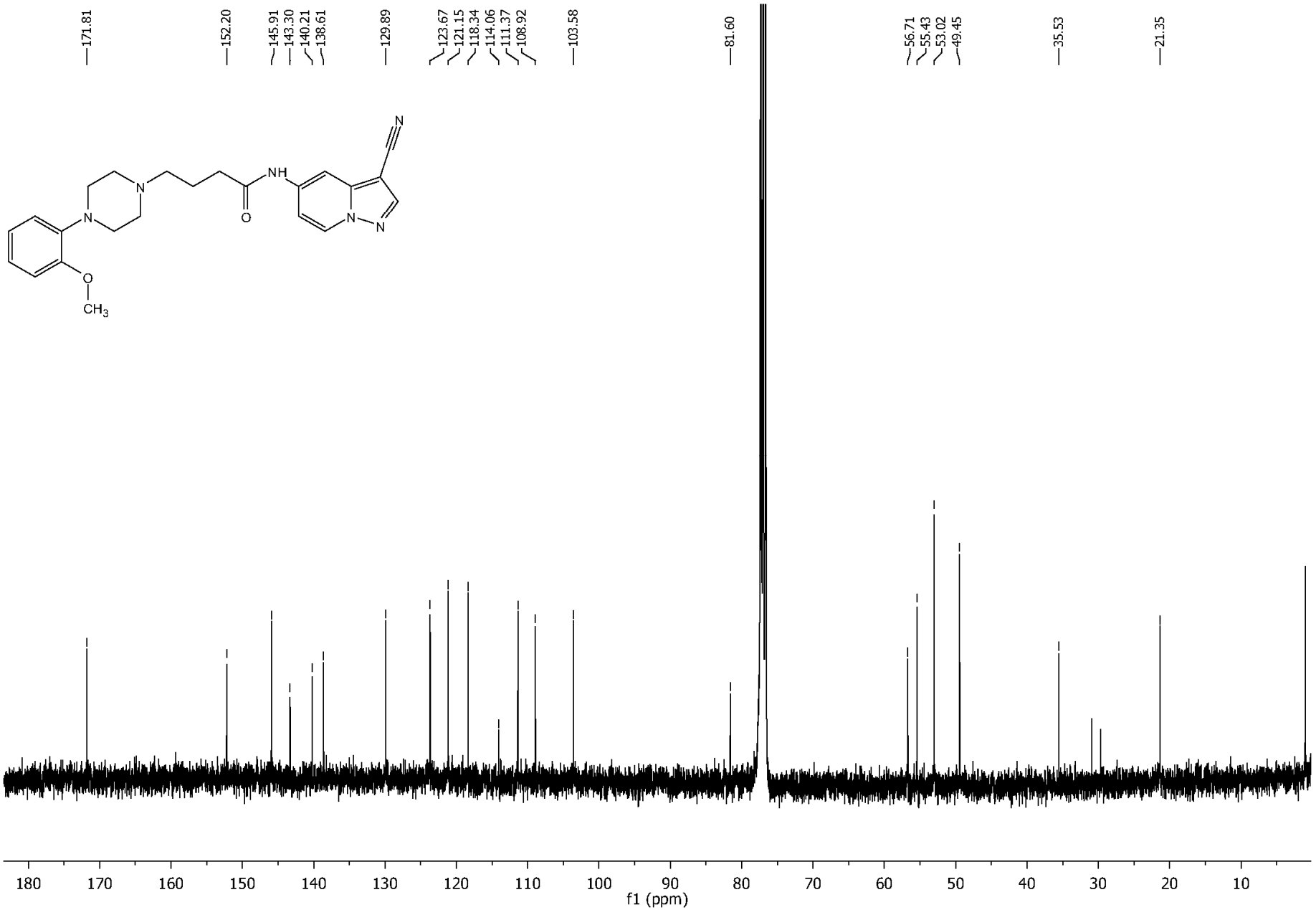
^{13}C NMR **9a**


171.80
150.46
145.91
143.28
138.67
134.19
130.01
127.62
127.60
125.17
118.66
114.08
108.85
103.41
81.48
56.96
53.04
50.60
35.74
21.54
N
NH
O
N
N
N
N
Cl
Cl
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

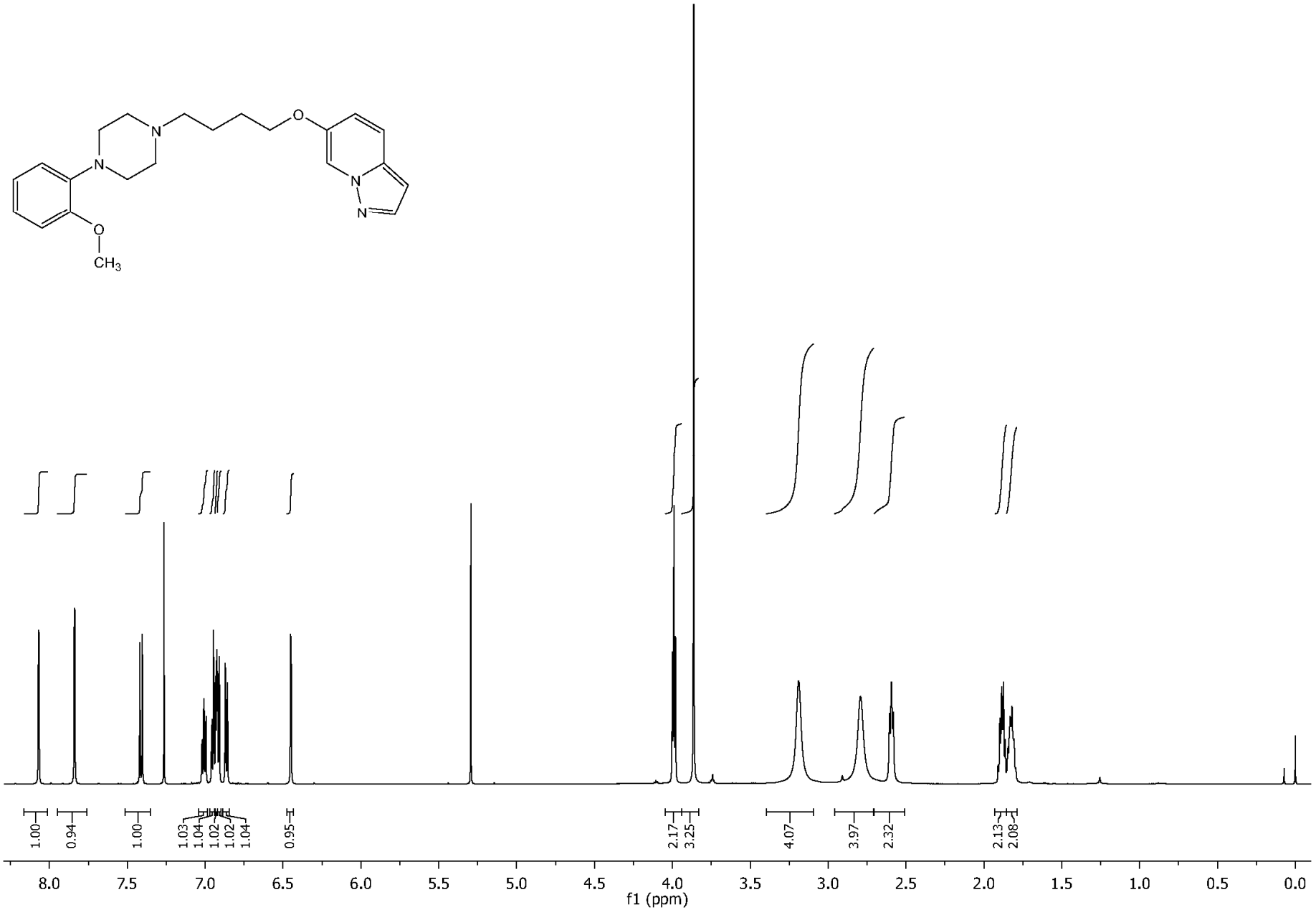
^{1}H NMR **9b**


N
NH
O
N
N
N
O
CH3
0.87
1.00
0.90
0.81
0.89
0.96
2.01
0.96
3.05
4.00
3.94
1.97
2.40
1.93
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

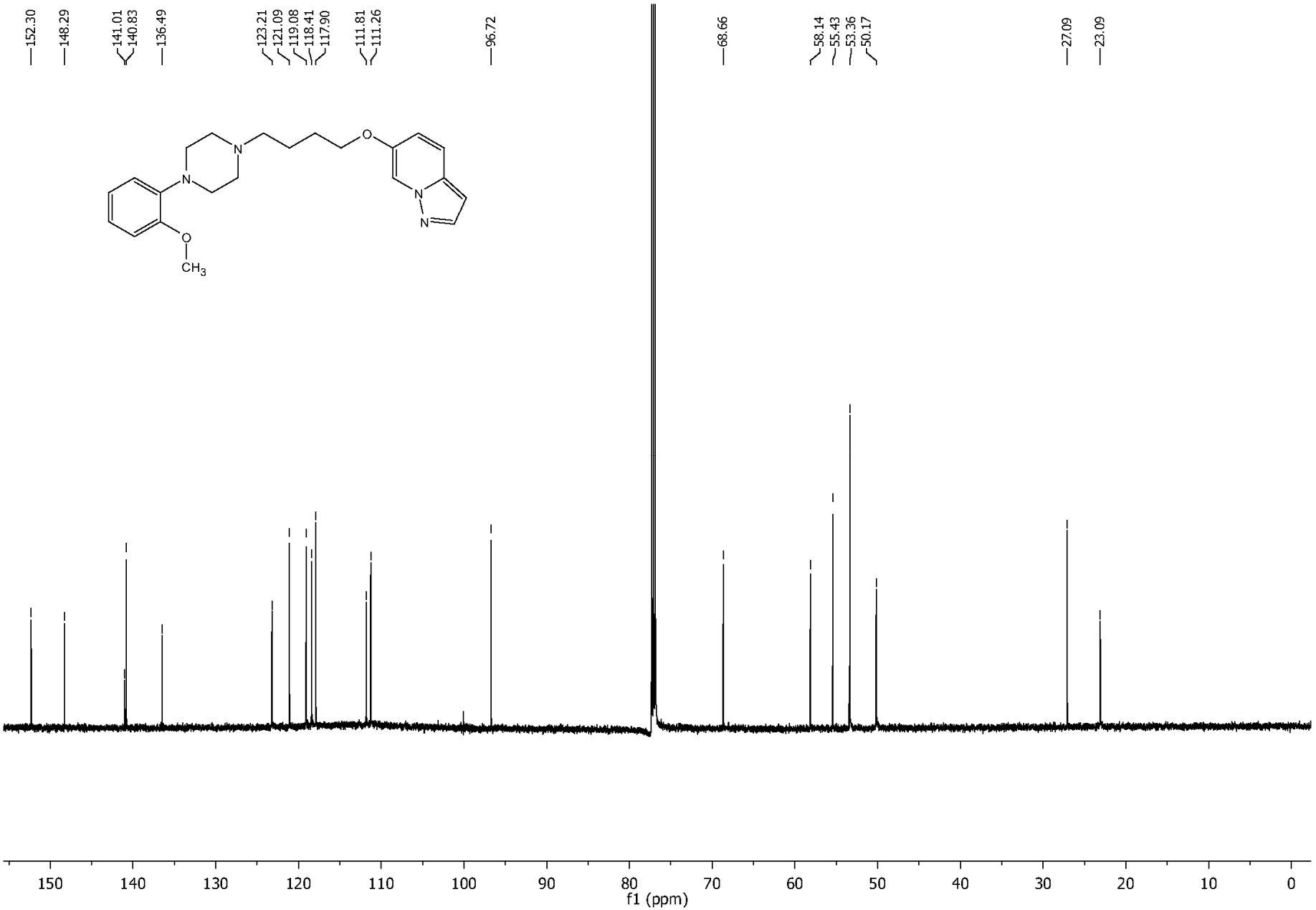
^{13}C NMR **9b**


171.81
152.20
145.91
143.30
140.21
138.61
129.89
123.67
121.15
118.34
114.06
111.37
108.92
103.58
81.60
56.71
55.43
53.02
49.45
35.53
21.35
N
NH
O
N
N
N
O
CH3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

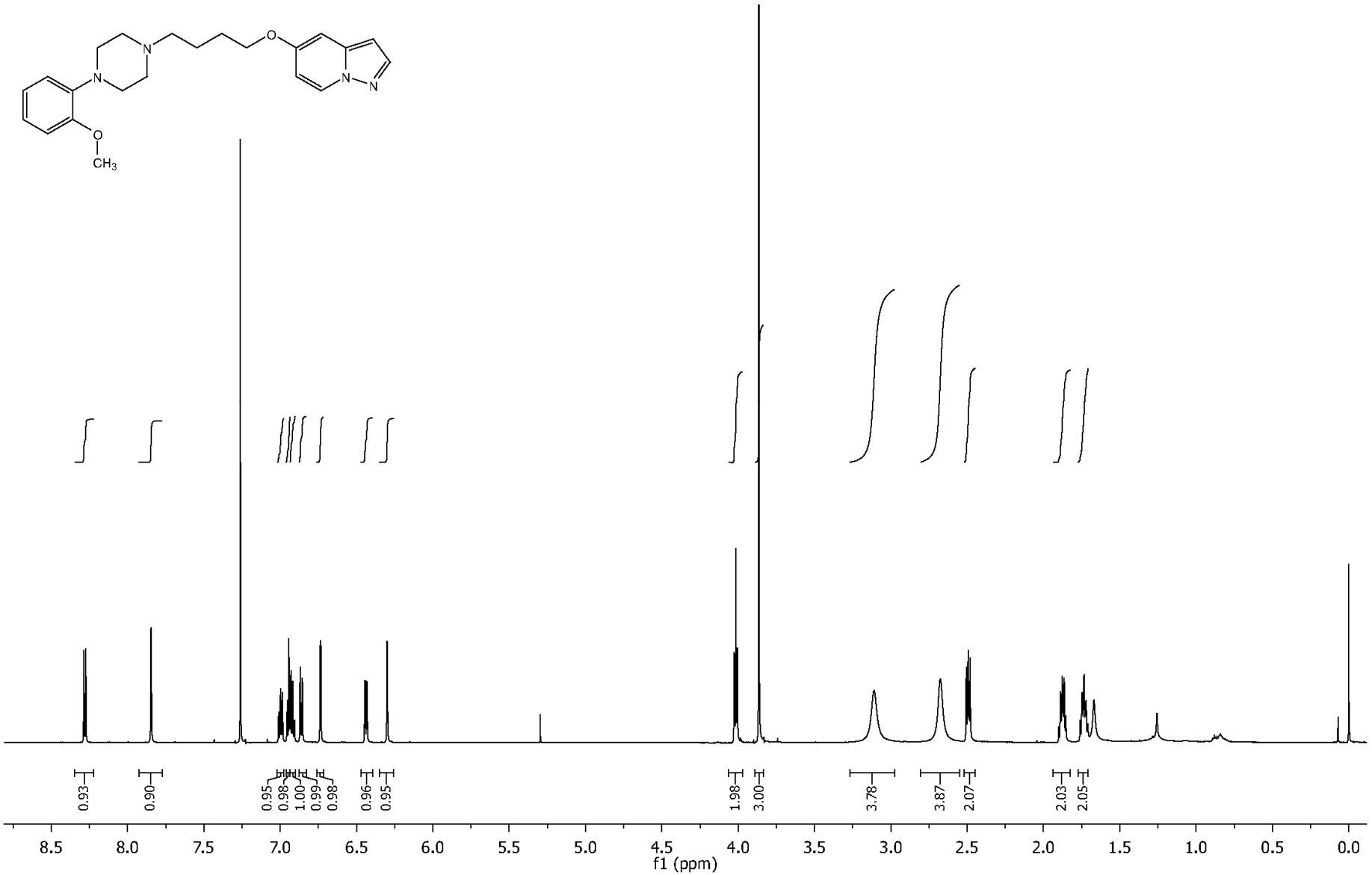
^{1}H NMR **12a**


O
N
N
N
O
CH3
1.00
0.94
1.00
1.03
1.04
1.02
1.02
1.04
0.95
2.17
3.25
4.07
3.97
2.32
2.13
2.08
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

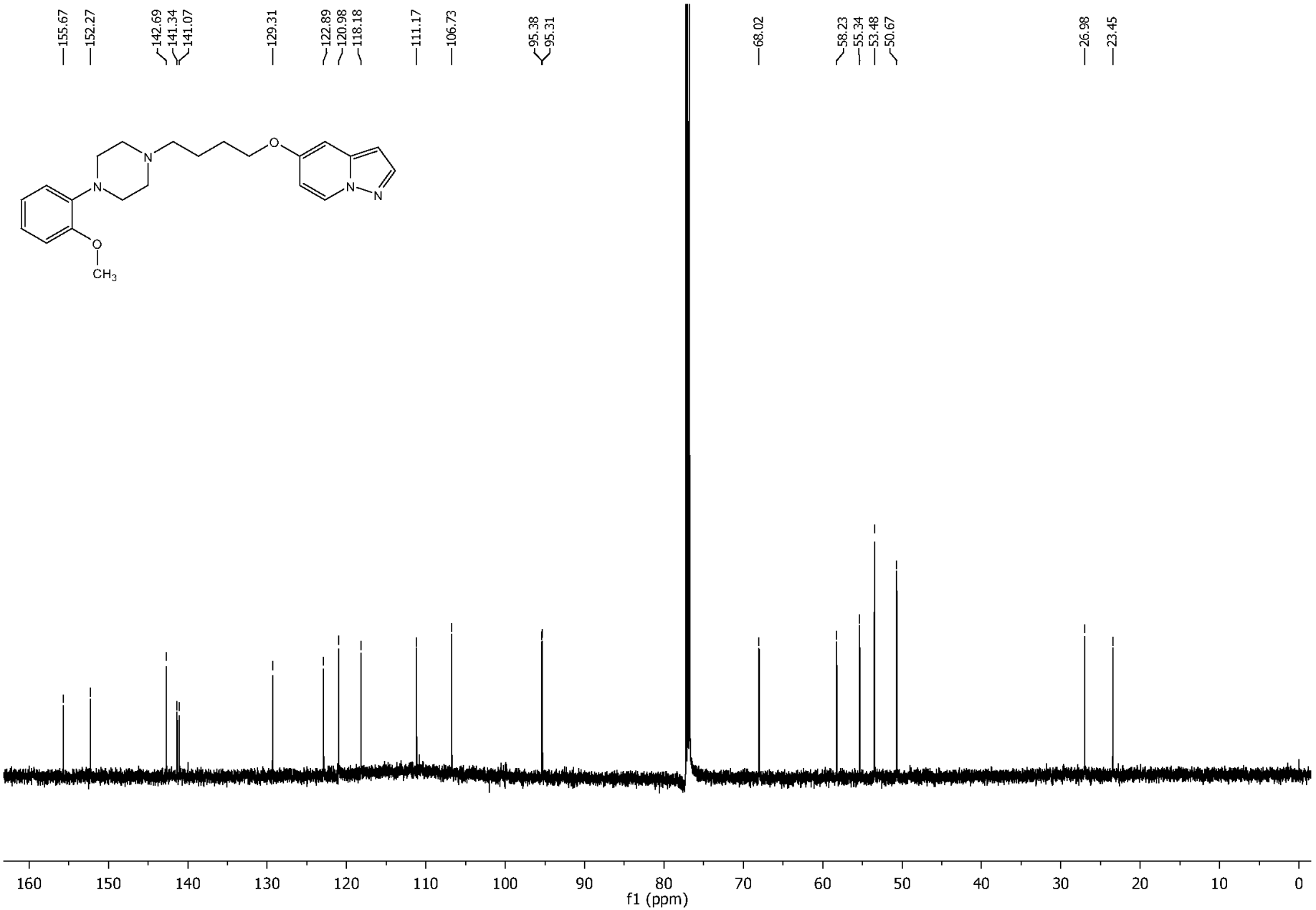
^{13}C NMR **12a**


152.30
148.29
141.01
140.83
136.49
123.21
121.09
119.08
118.41
117.90
111.81
111.26
96.72
68.66
58.14
55.43
53.36
50.17
27.09
23.09
N
N
O
O
CH3
N
N
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

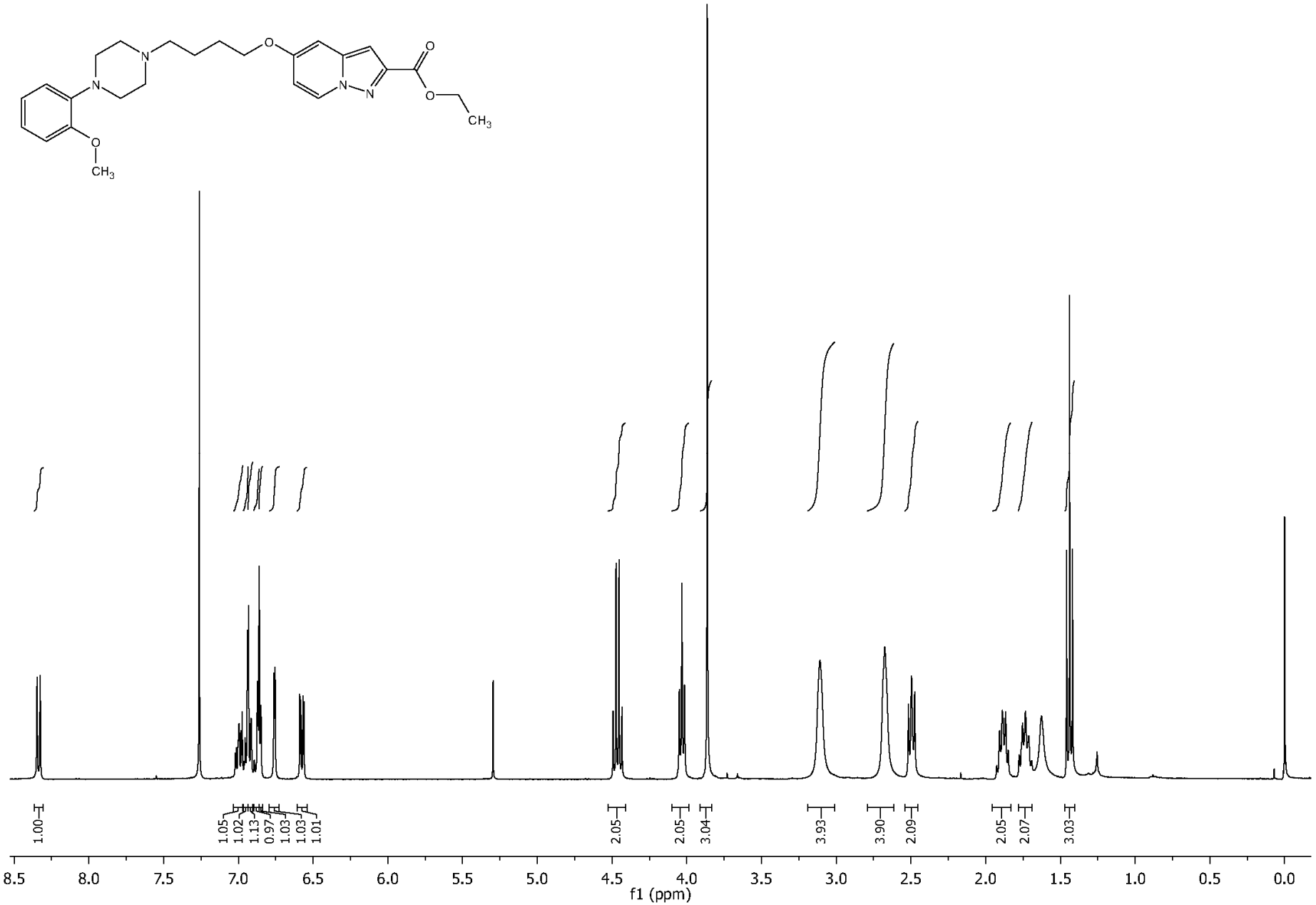
^{1}H NMR **12b**


O
N
N
N
N
O
CH3
0.93
0.90
0.95
0.98
1.00
0.99
0.98
0.96
0.95
1.98
3.00
3.78
3.87
2.07
2.03
2.05
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

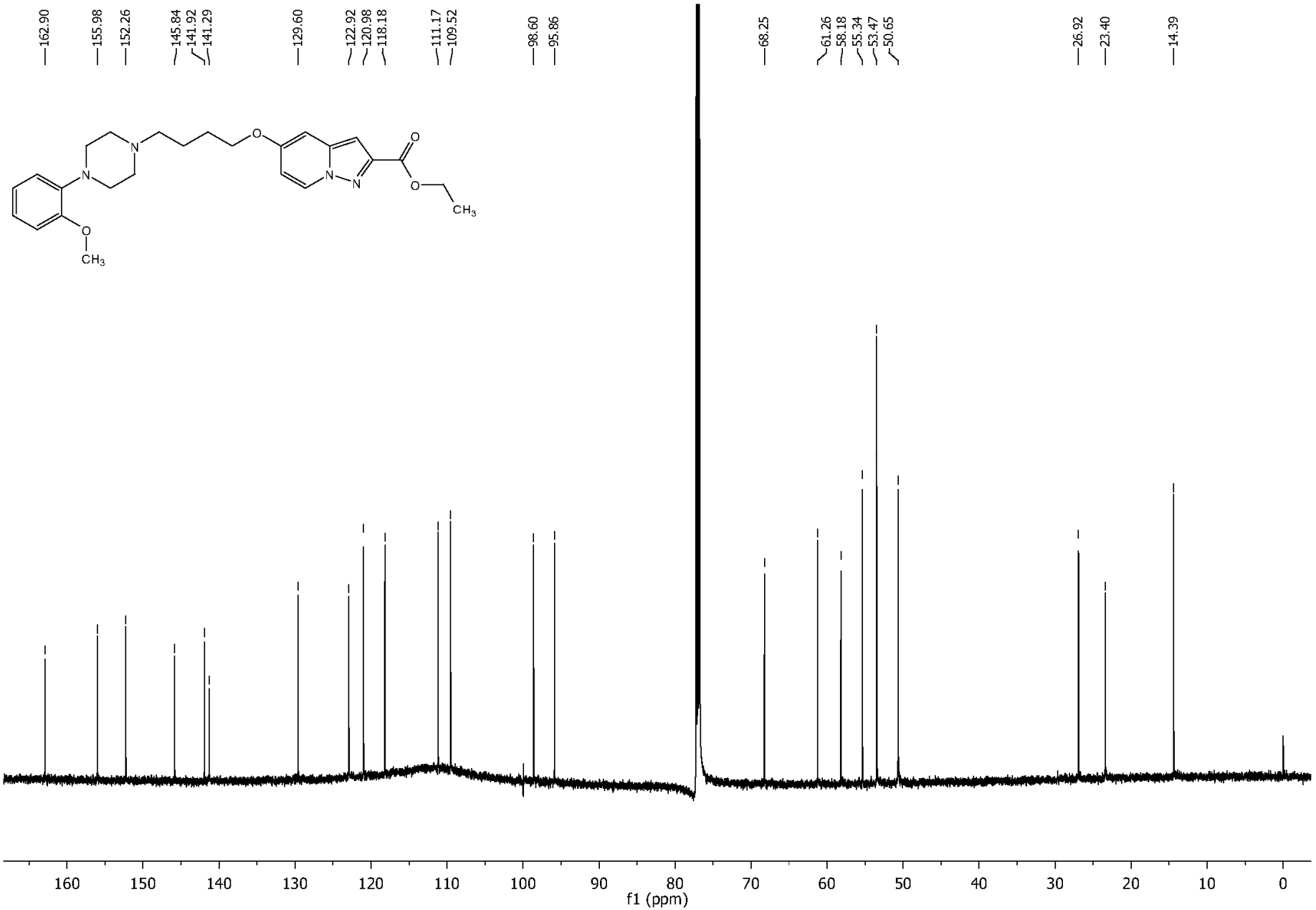
^{13}C NMR **12b**


155.67
152.27
142.69
141.34
141.07
129.31
122.89
120.98
118.18
111.17
106.73
95.38
95.31
68.02
58.23
55.34
53.48
50.67
26.98
23.45
O
N
N
N
N
O
CH3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

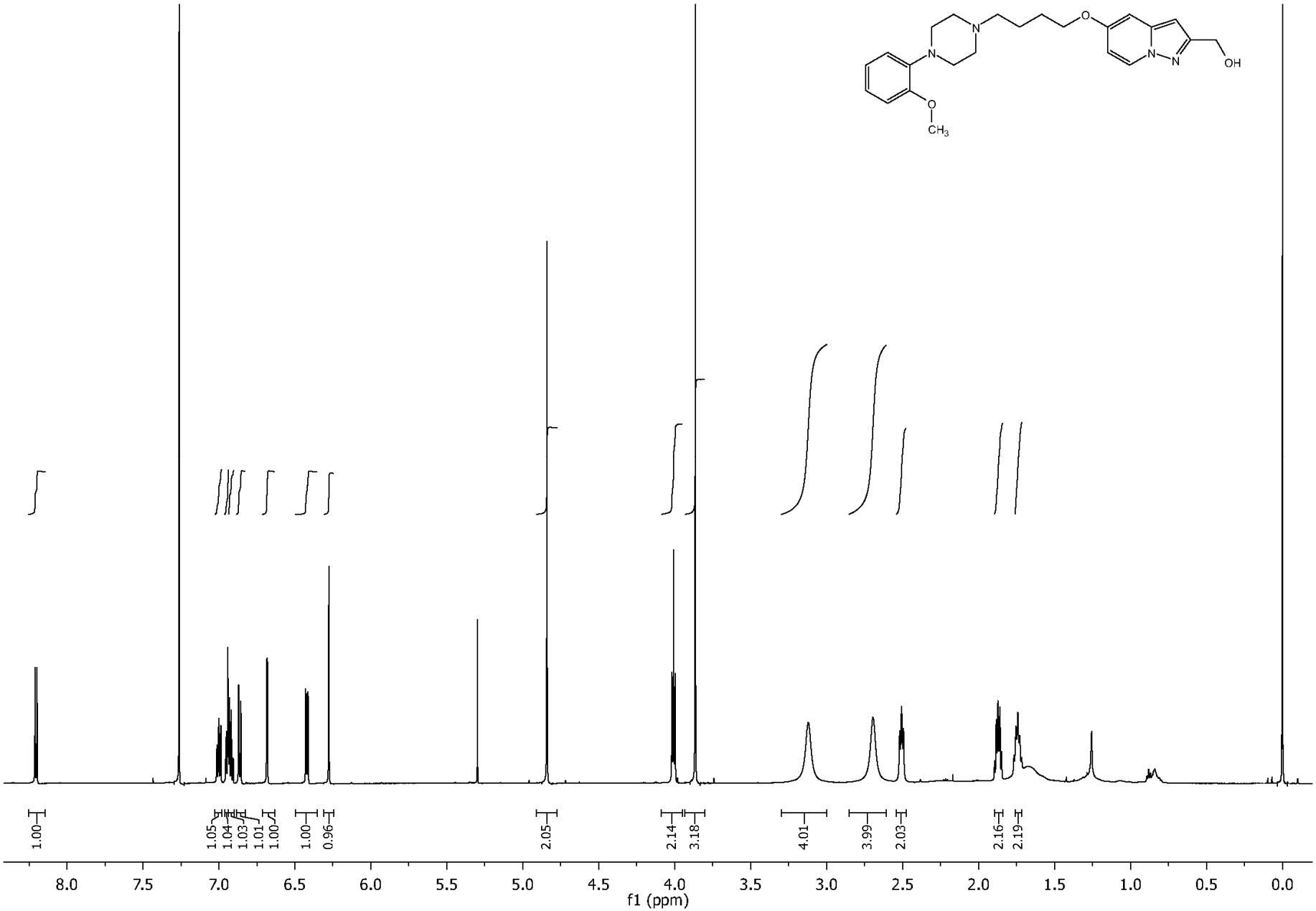
^{1}H NMR **12c**


1.00
1.05
1.02
1.13
0.97
1.03
1.03
1.01
2.05
2.05
3.04
3.93
3.90
2.09
2.05
2.07
3.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

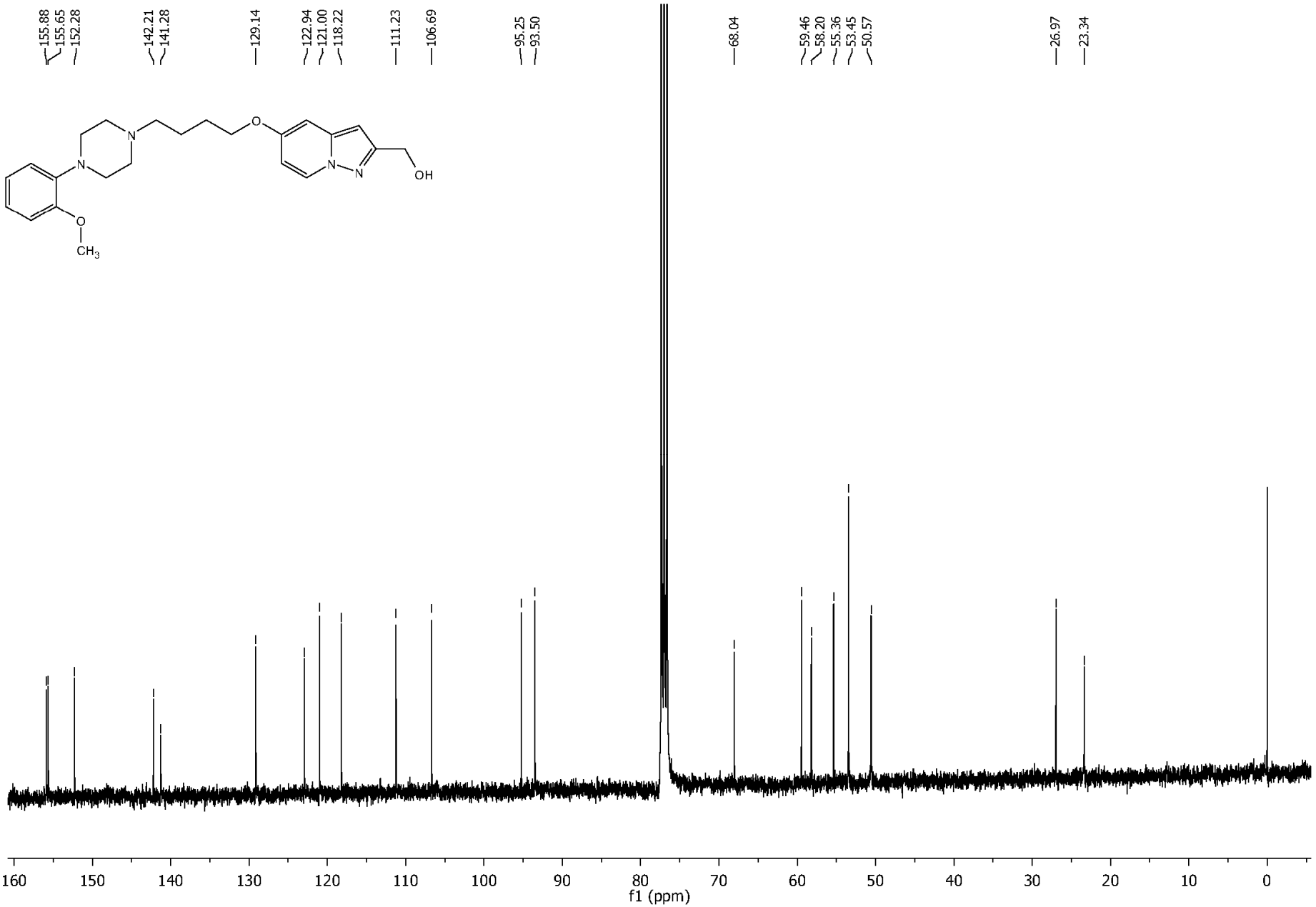
^{13}C NMR **12c**


162.90
155.98
152.26
145.84
141.92
141.29
129.60
122.92
120.98
118.18
111.17
109.52
98.60
95.86
68.25
61.26
58.18
55.34
53.47
50.65
26.92
23.40
14.39
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

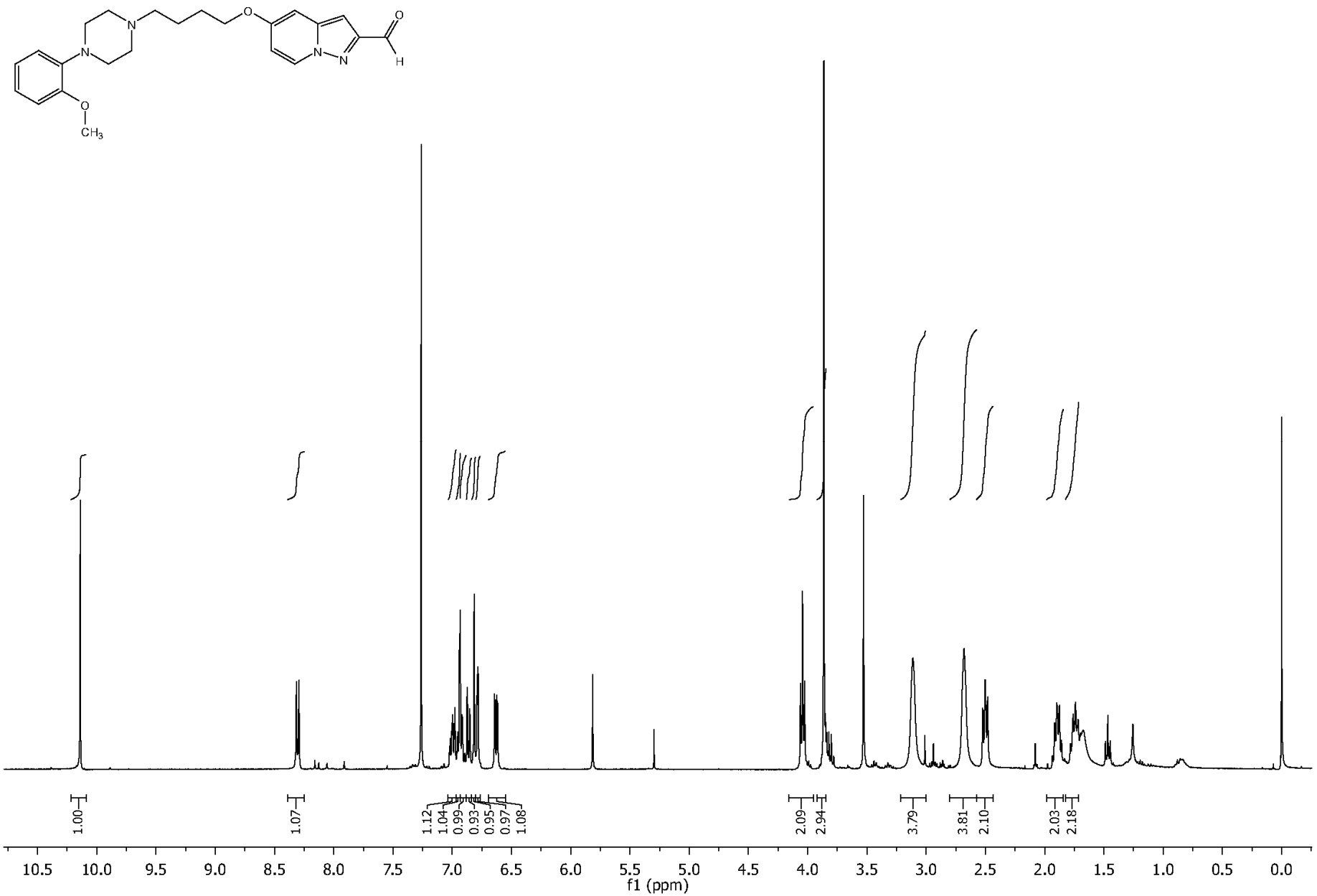
^{1}H NMR **12d**


1.00
1.05
1.04
1.03
1.01
1.00
1.00
0.96
2.05
2.14
3.18
4.01
3.99
2.03
2.16
2.19
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
N
N
O
O
CH3
N
N
OH

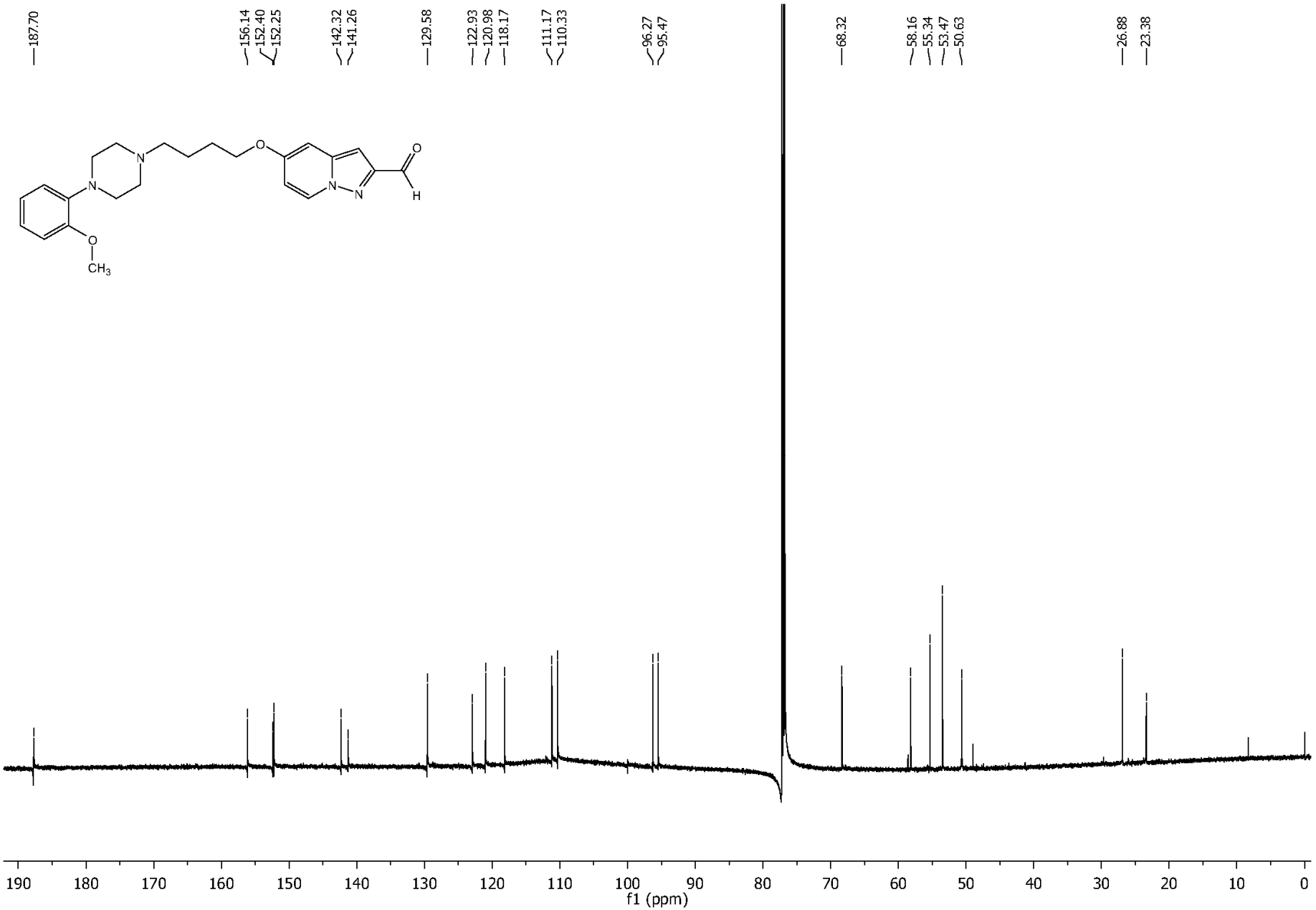
^{13}C NMR **12d**


155.88
155.65
152.28
142.21
141.28
129.14
122.94
121.00
118.22
111.23
106.69
95.25
93.50
68.04
59.46
58.20
55.36
53.45
50.57
26.97
23.34
N
N
O
N
N
OH
O
CH3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

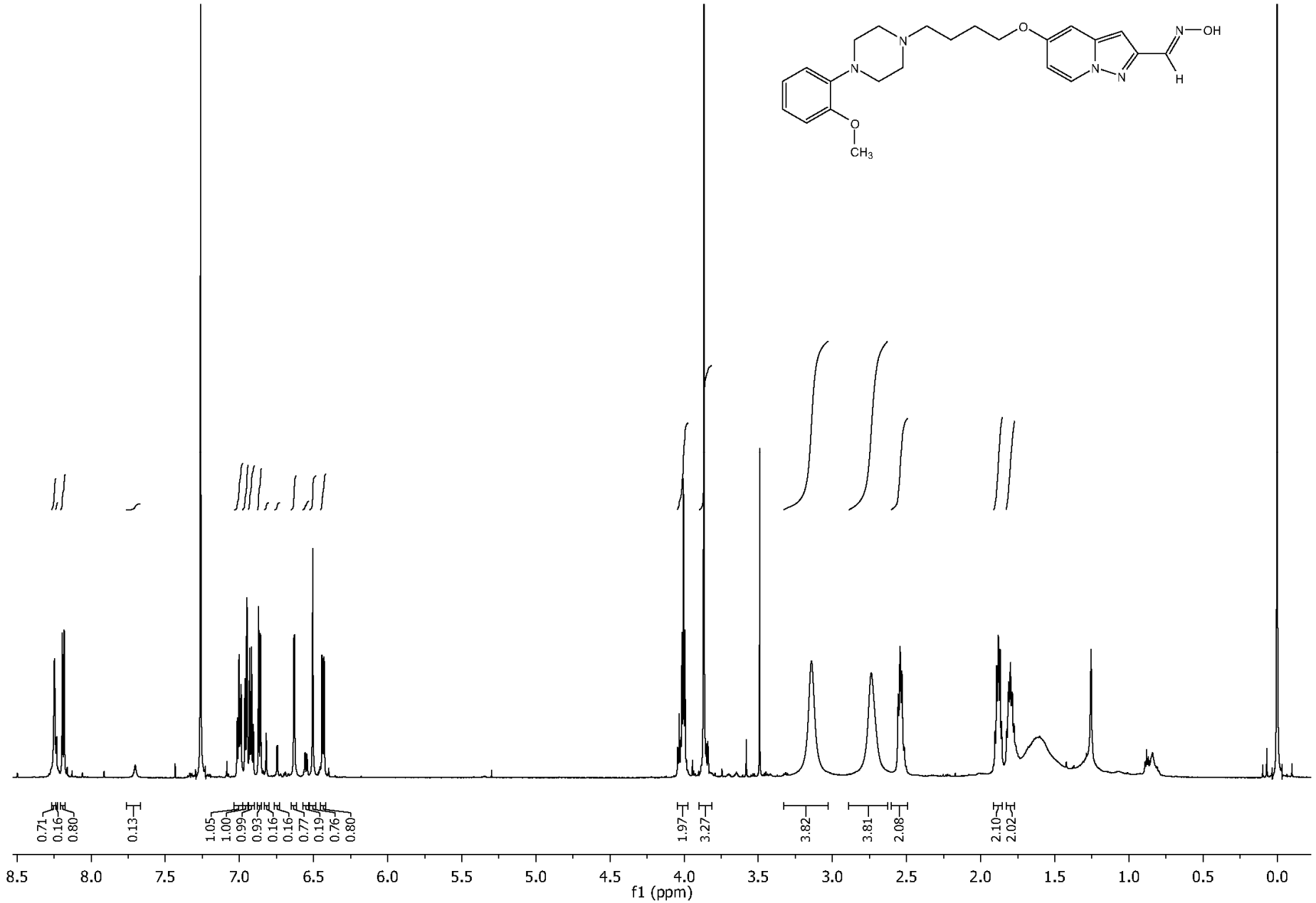
^{1}H NMR **12e**


1.00
1.07
1.12
1.04
0.99
0.93
0.95
0.97
1.08
2.09
2.94
3.79
3.81
2.10
2.03
2.18
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

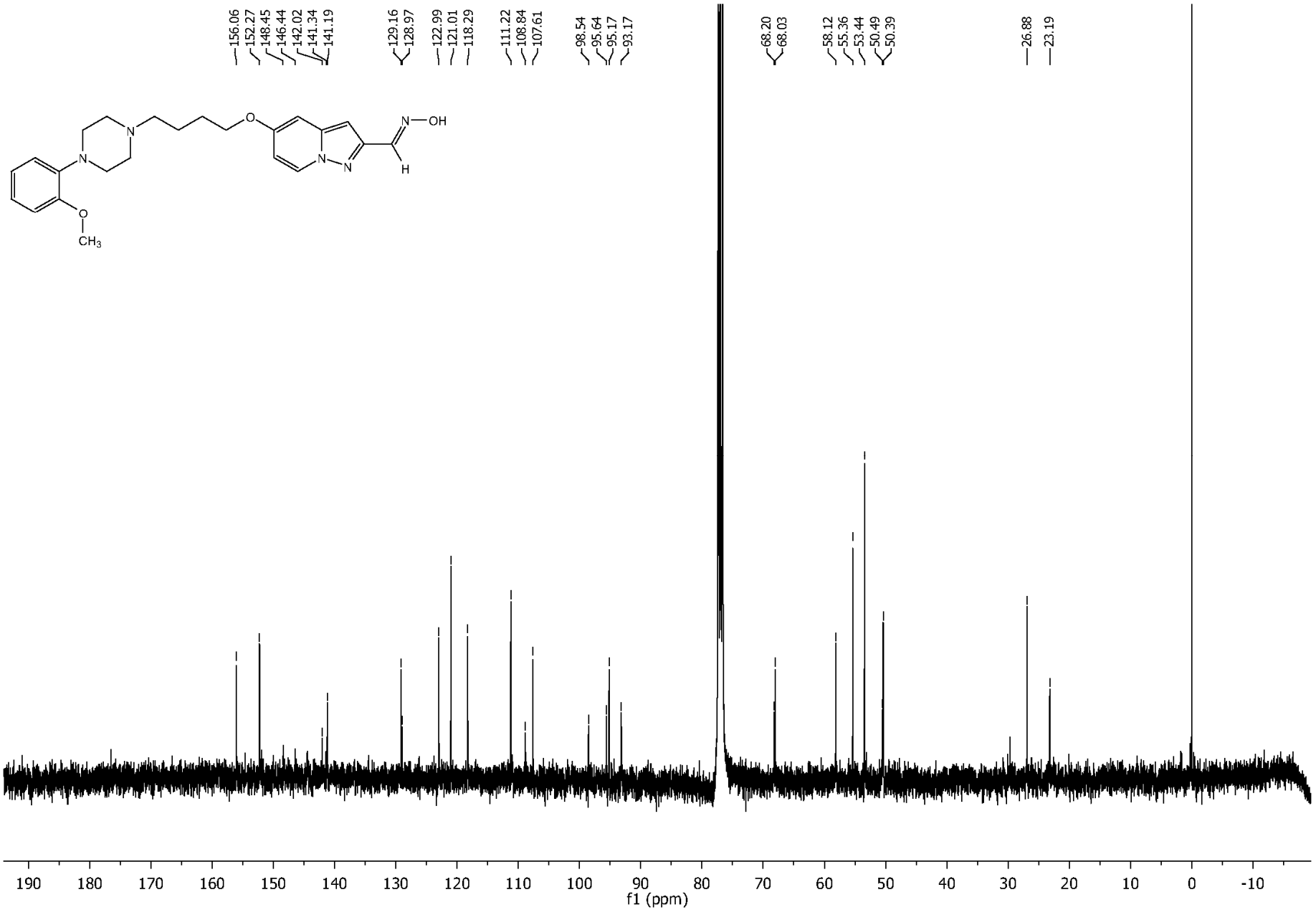
^{13}C NMR **12e**


187.70
156.14
152.40
152.25
142.32
141.26
129.58
122.93
120.98
118.17
111.17
110.33
96.27
95.47
68.32
58.16
55.34
53.47
50.63
26.88
23.38
O
N
N
N
N
O
O
H
CH3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

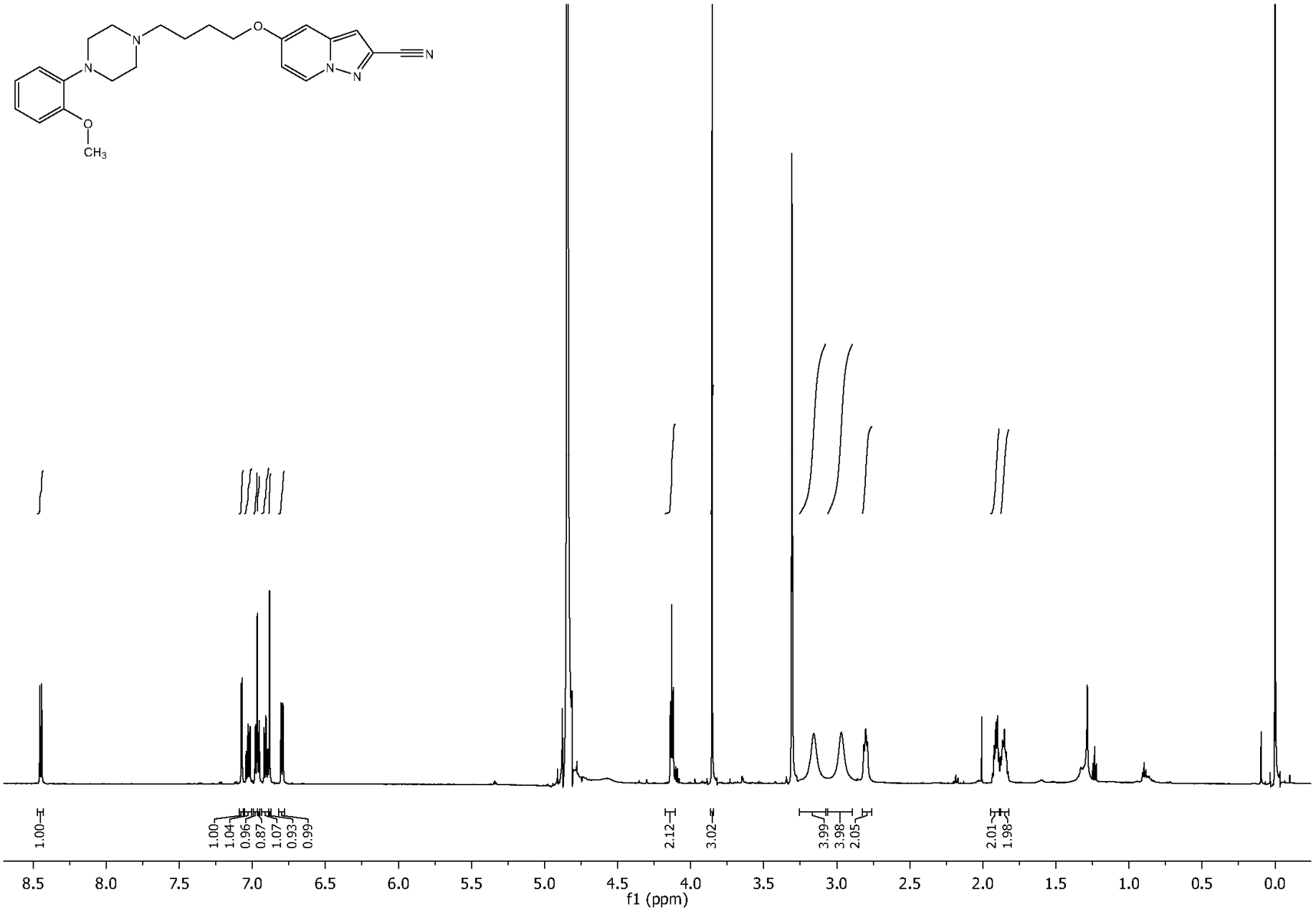
^{1}H NMR **12f**


N—OH
H
O
CH3
0.71
0.16
0.80
0.13
1.05
1.00
0.99
0.93
0.16
0.16
0.77
0.19
0.76
0.80
1.97
3.27
3.82
3.81
2.08
2.10
2.02
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

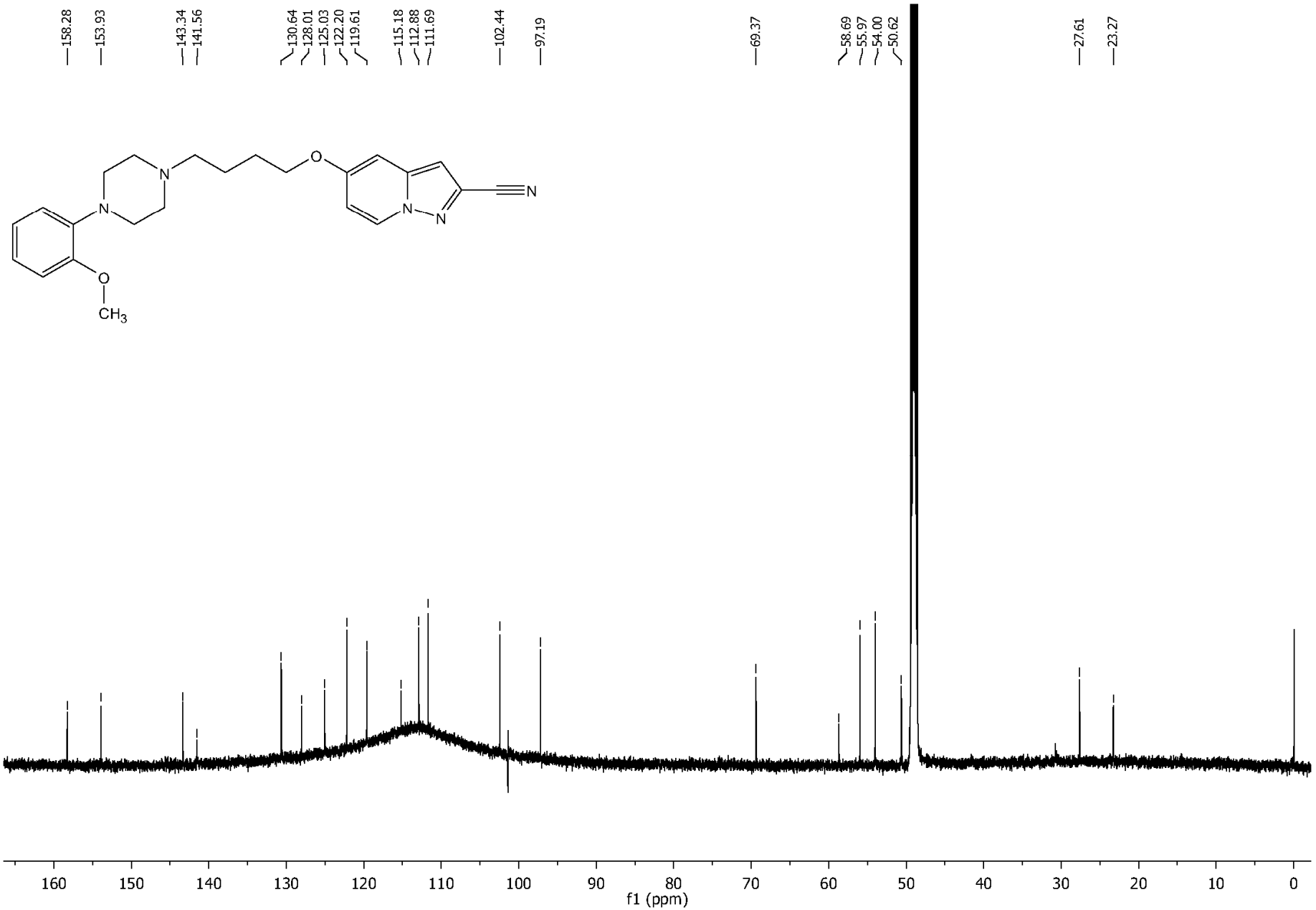
^{13}C NMR **12f**


156.06
152.27
148.45
146.44
142.02
141.34
141.19
129.16
128.97
122.99
121.01
118.29
111.22
108.84
107.61
98.54
95.64
95.17
93.17
68.20
68.03
58.12
55.36
53.44
50.49
50.39
26.88
23.19
N—OH
H
O
CH3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

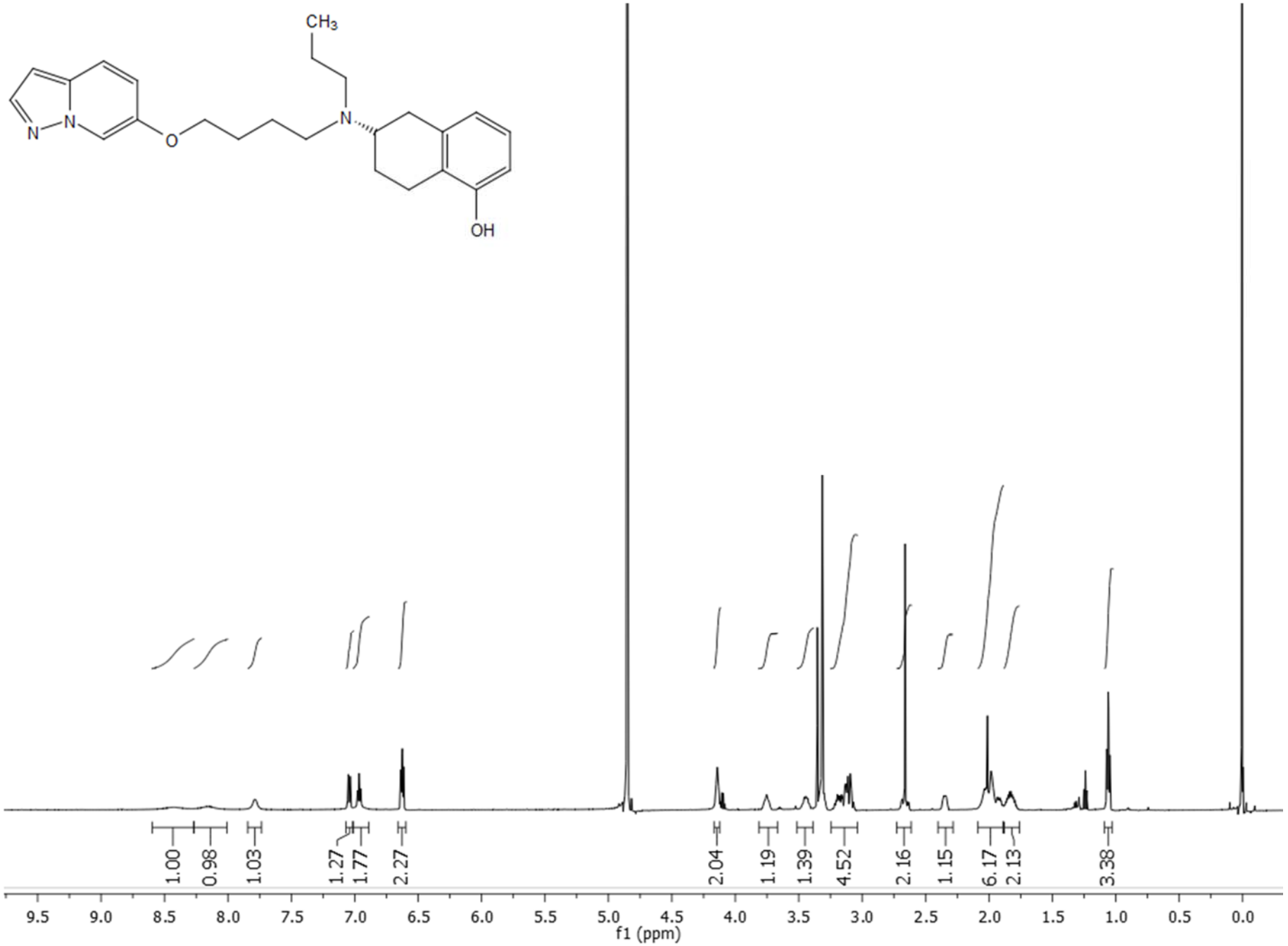
^{1}H NMR **12g**


1.00
1.00
1.04
0.96
0.87
1.07
0.93
0.99
2.12
3.02
3.99
3.98
2.05
2.01
1.98
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

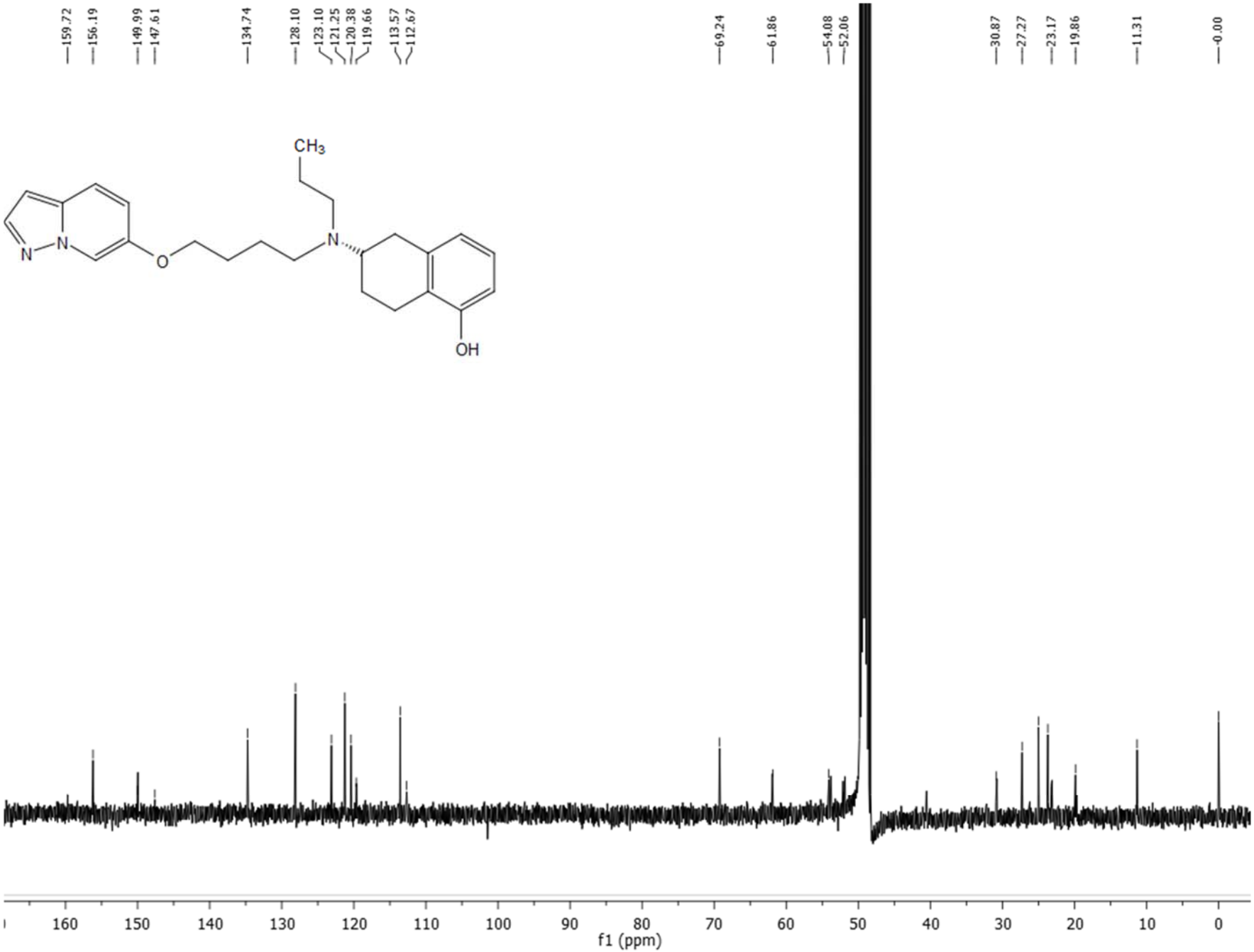
^{13}C NMR **12g**


158.28
153.93
143.34
141.56
130.64
128.01
125.03
122.20
119.61
115.18
112.88
111.69
102.44
97.19
69.37
58.69
55.97
54.00
50.62
27.61
23.27
N
N
O
N
N
N
O
CH3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

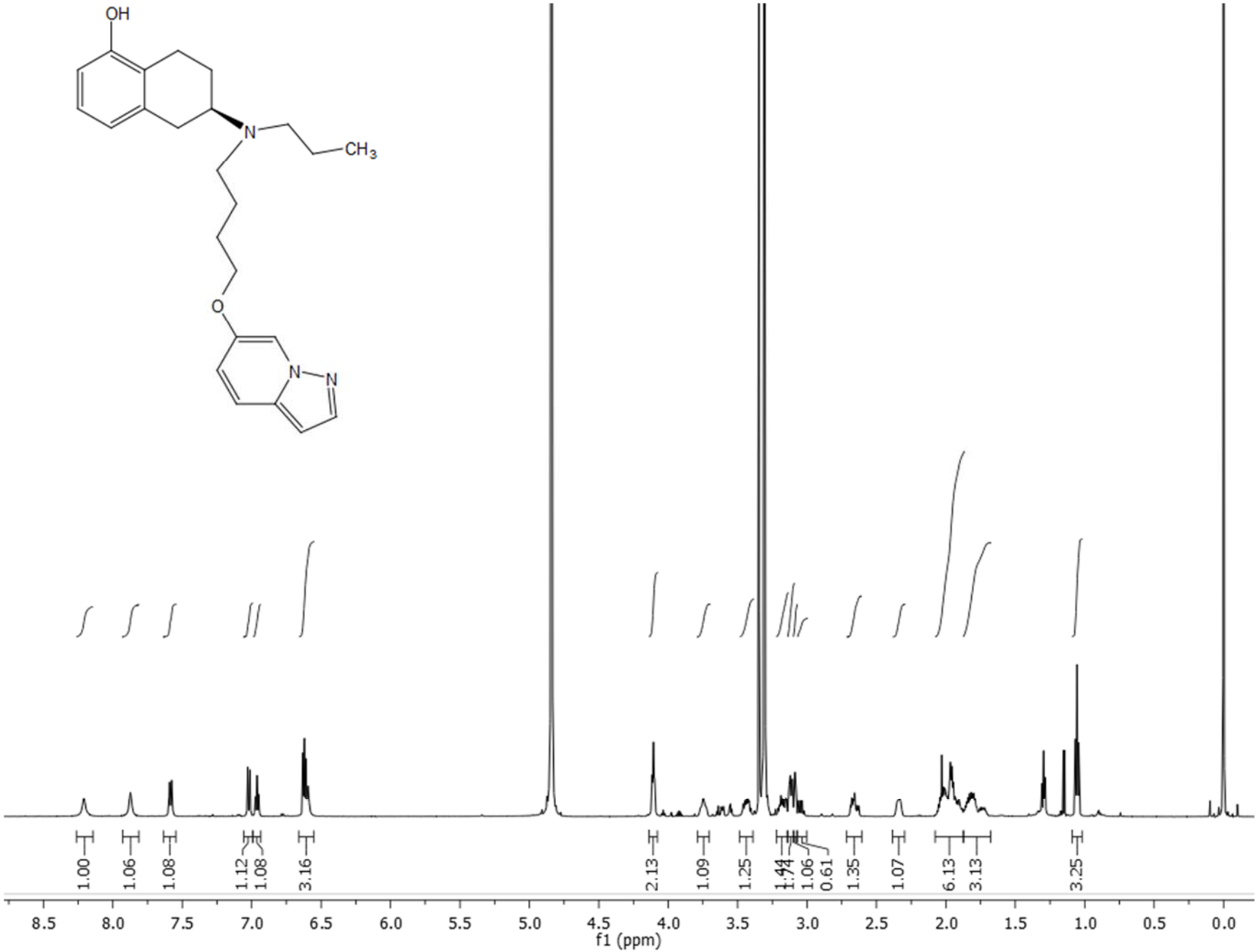
^{1}H NMR (*S*)-**13a**


CH3
N
N
O
N
OH
1.00
0.98
1.03
1.27
1.77
2.27
2.04
1.19
1.39
4.52
2.16
1.15
6.17
2.13
3.38
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

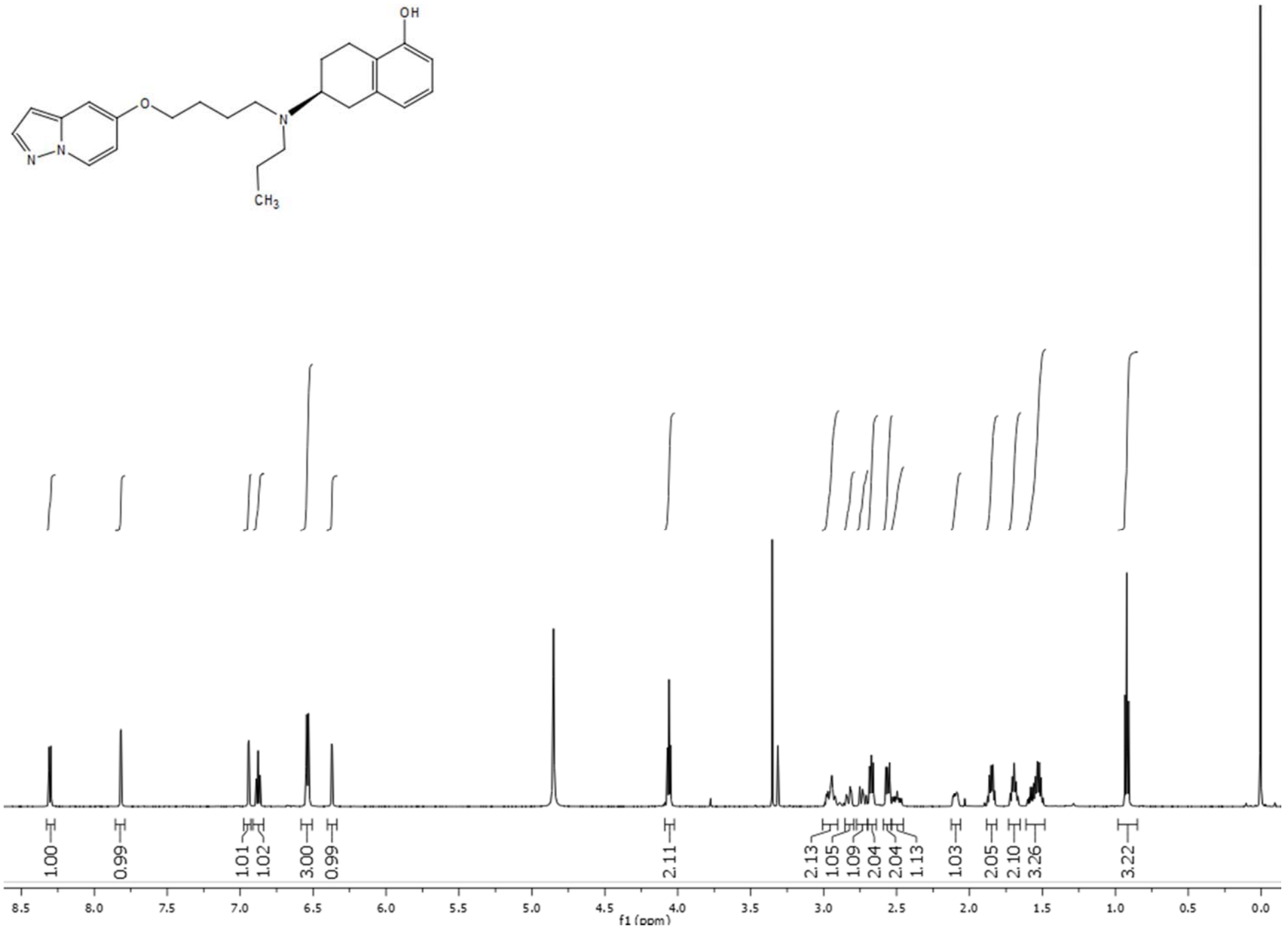
^{13}C NMR (*S*)-**13a**


159.72
156.19
149.99
147.61
134.74
128.10
123.10
121.25
120.38
119.66
113.57
112.67
69.24
61.86
54.08
52.06
30.87
27.27
23.17
19.86
11.31
0.00
CH3
N
N
O
N
OH
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR (*R*)-**13a**


OH
N
CH3
O
N
N
1.00
1.06
1.08
1.12
1.08
3.16
2.13
1.09
1.25
1.44
1.06
0.61
1.35
1.07
6.13
3.13
3.25
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

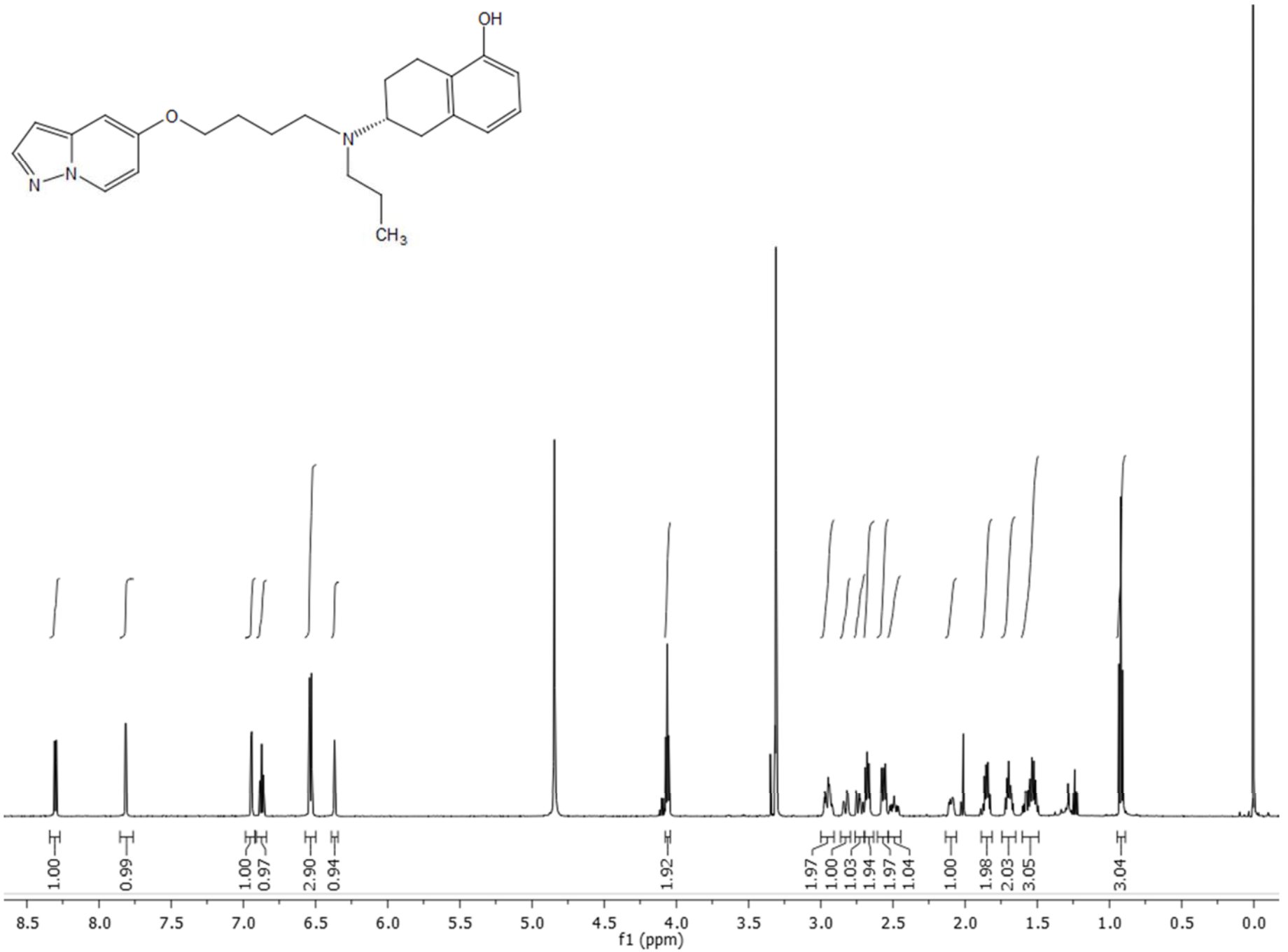
^{1}H NMR (*S*)-**13b**


OH
O
N
N
N
CH3
1.00
0.99
1.01
1.02
3.00
0.99
2.11
2.13
1.05
1.09
2.04
2.04
1.13
1.03
2.05
2.10
3.26
3.22
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

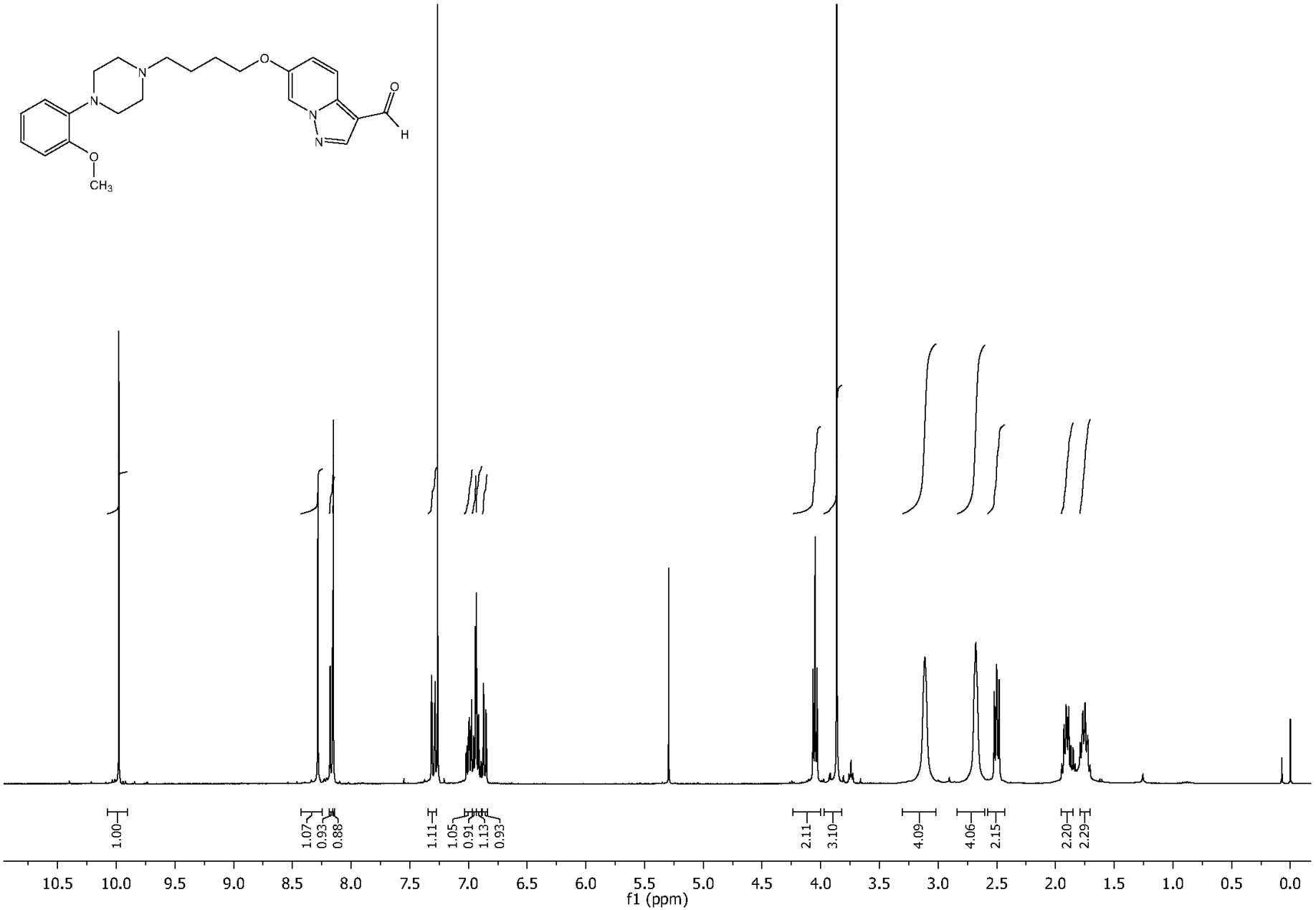
^{13}C NMR (*S*)-**13b**


157.53
155.93
143.34
142.93
138.65
129.98
127.24
124.36
121.57
112.70
108.47
96.80
96.76
69.38
58.24
53.75
51.30
33.14
28.05
25.82
24.82
22.54
12.28
0.00
OH
O
N
N
N
CH3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

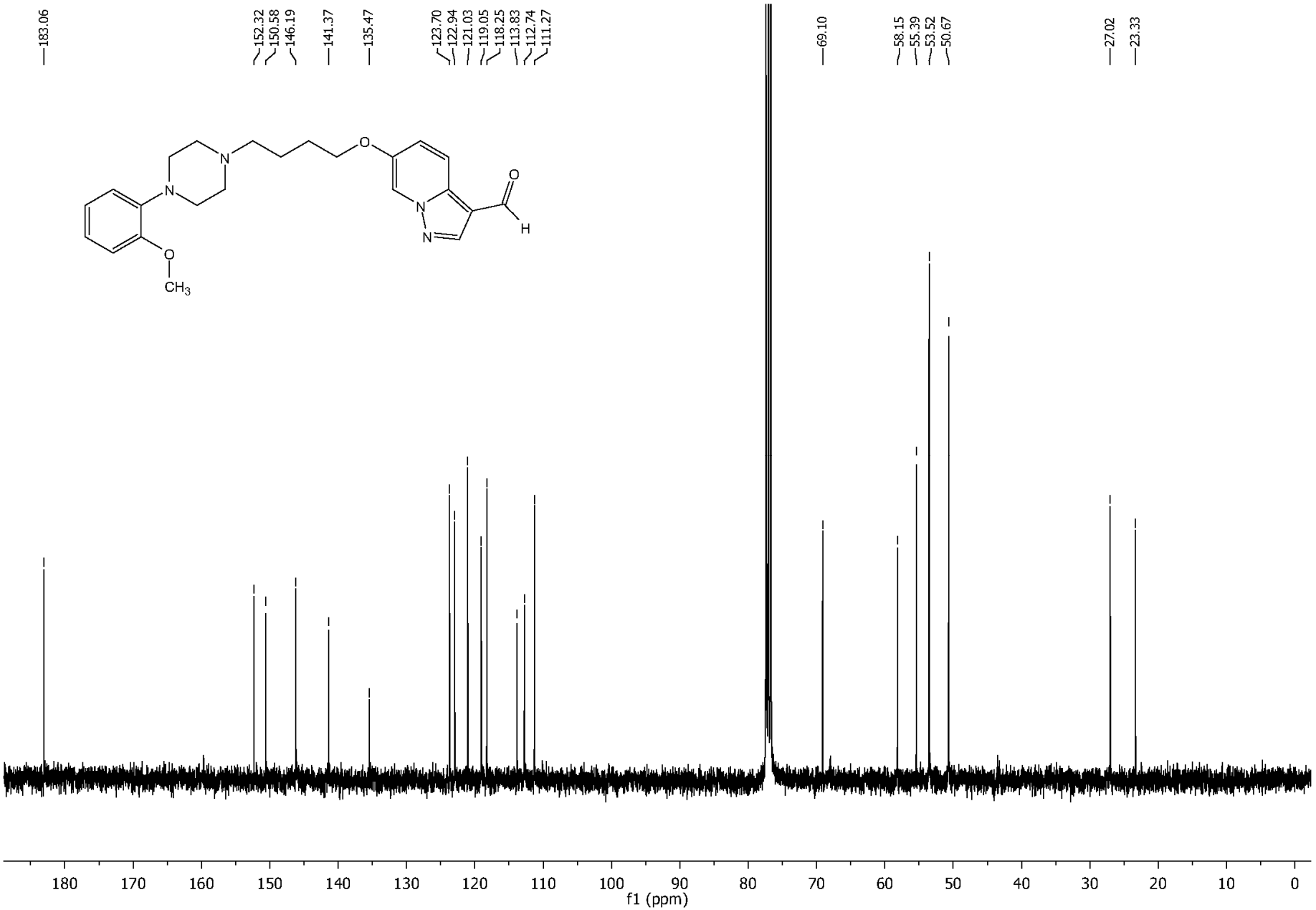
^{1}H NMR (*R*)-**13b**


OH
O
N
N
N
CH3
1.00
0.99
1.00
0.97
2.90
0.94
1.92
1.97
1.00
1.03
1.94
1.97
1.04
1.00
1.98
2.03
3.05
3.04
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

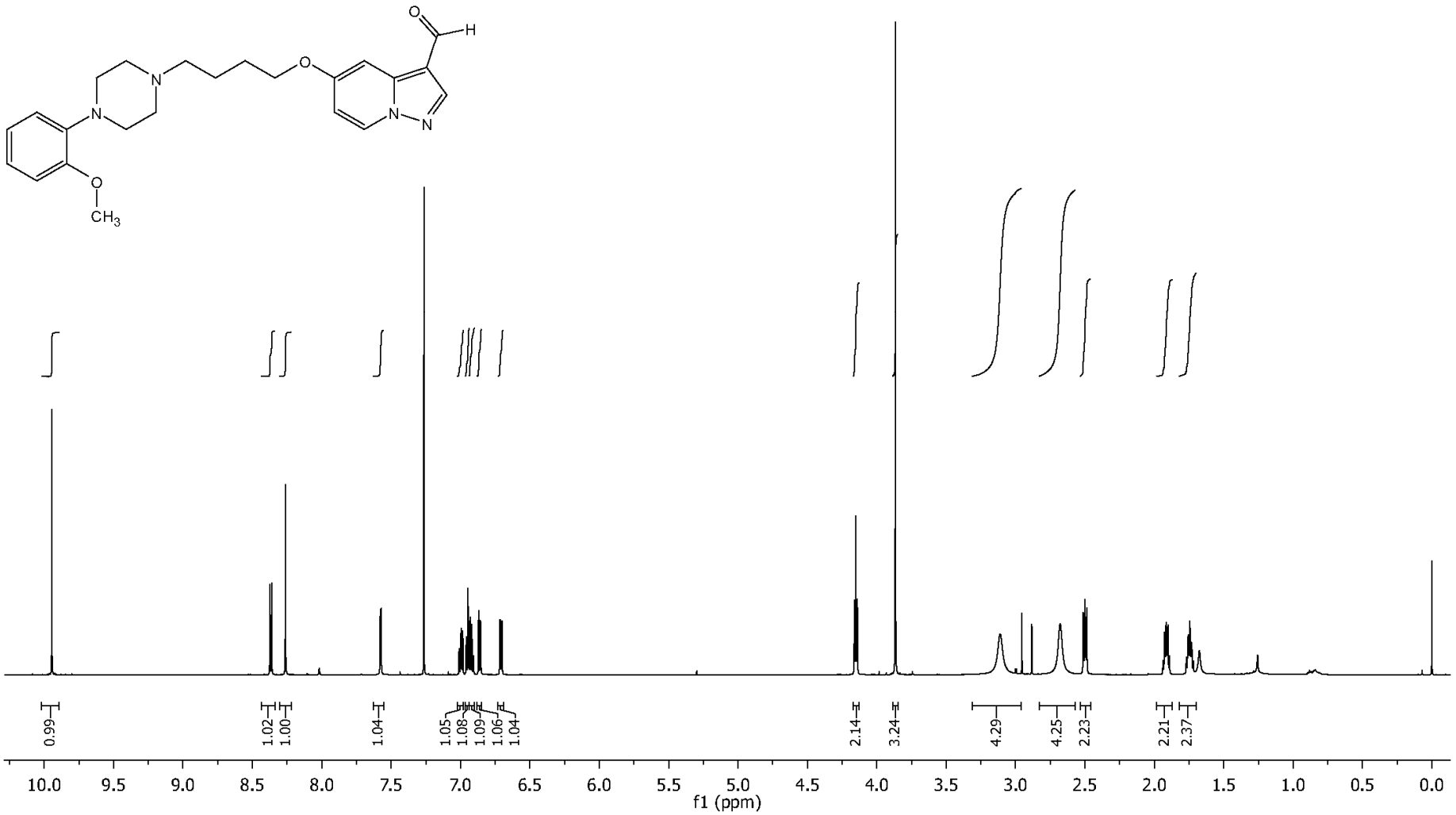
^{1}H NMR **14a**


1.00
1.07
0.93
0.88
1.11
1.05
0.91
1.13
0.93
2.11
3.10
4.09
4.06
2.15
2.20
2.29
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

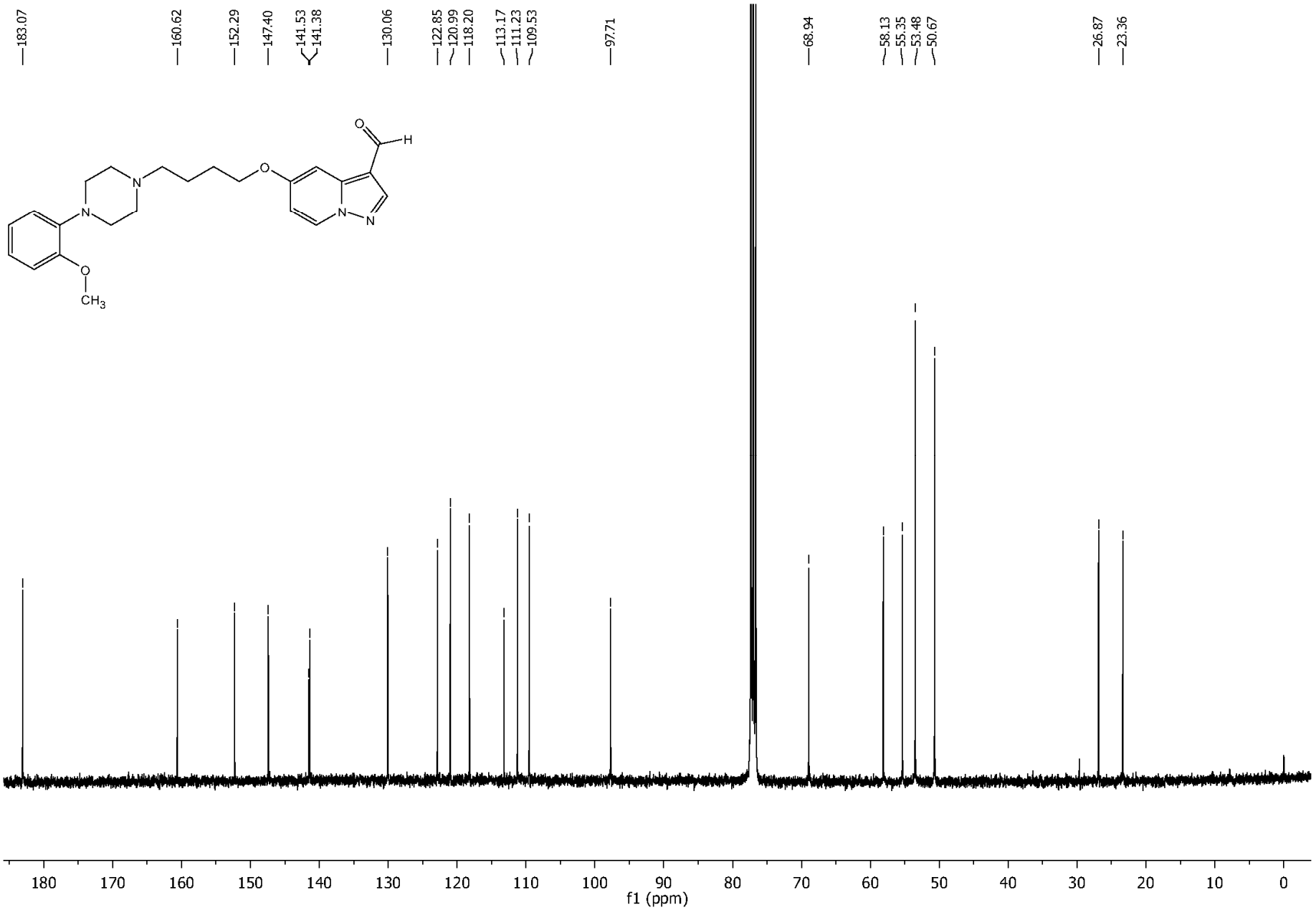
^{13}C NMR **14a**


183.06
152.32
150.58
146.19
141.37
135.47
123.70
122.94
121.03
119.05
118.25
113.83
112.74
111.27
69.10
58.15
55.39
53.52
50.67
27.02
23.33
O
N
N
O
O
N
N
H
CH3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

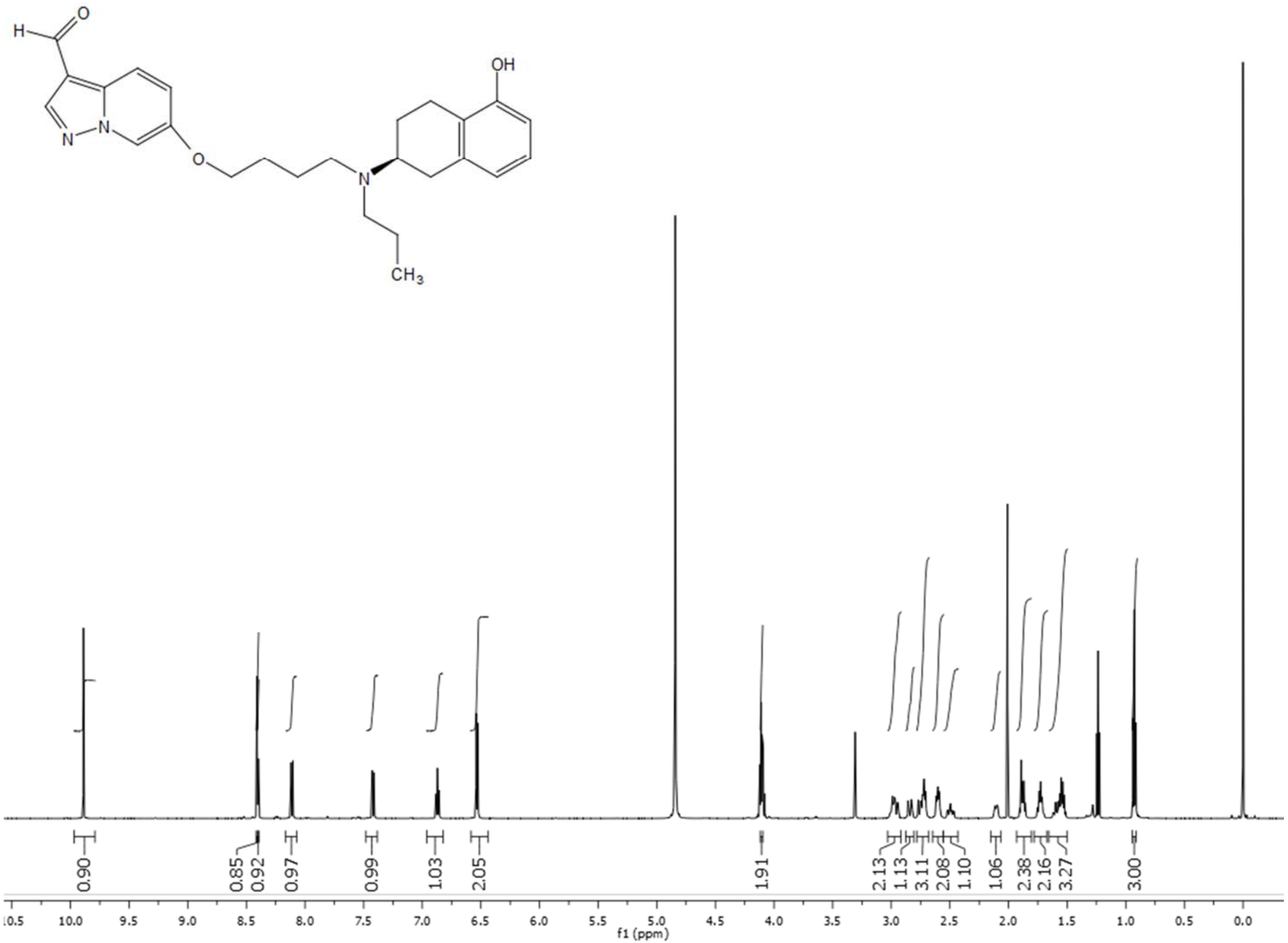
^{1}H NMR **14b**


O
H
N
O
N
N
N
O
CH3
0.99
1.02
1.00
1.04
1.05
1.08
1.09
1.06
1.04
2.14
3.24
4.29
4.25
2.23
2.21
2.37
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

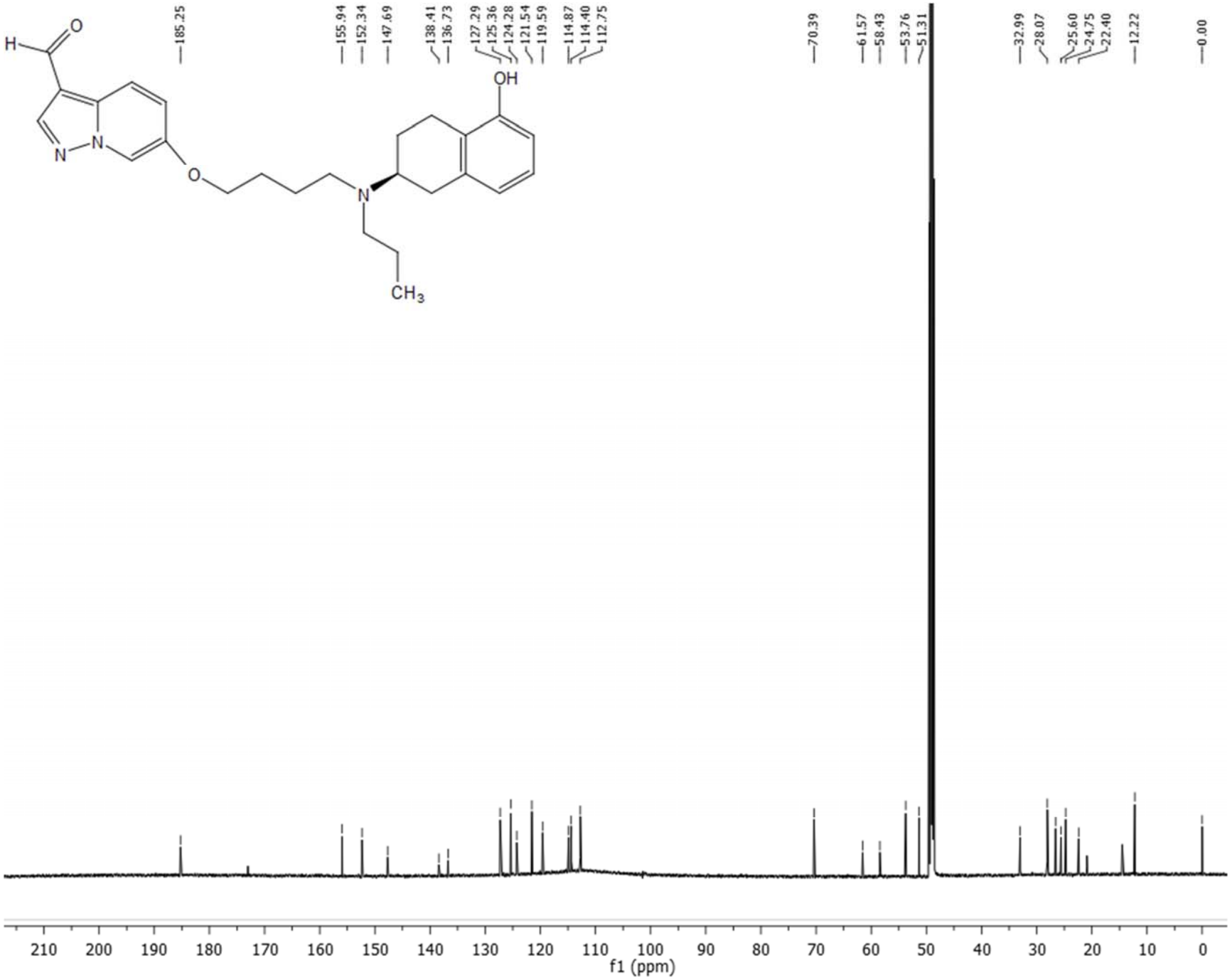
^{13}C NMR **14b**


183.07
160.62
152.29
147.40
141.53
141.38
130.06
122.85
120.99
118.20
113.17
111.23
109.53
97.71
68.94
58.13
55.35
53.48
50.67
26.87
23.36
O
H
N
N
O
N
N
O
CH3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

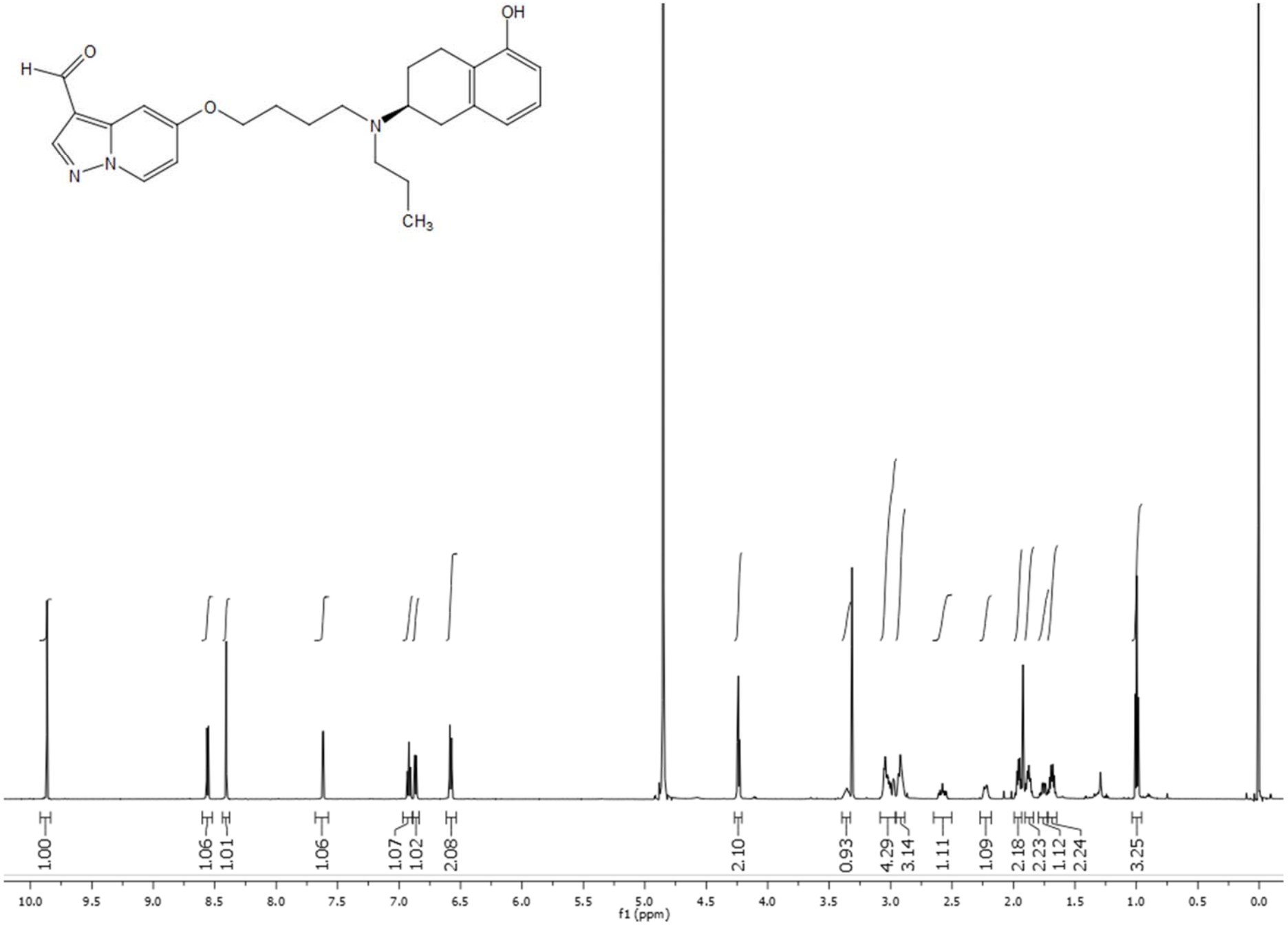
^{1}H NMR (*S*)-**15a**


O
H
OH
N
N
O
N
CH3
0.90
0.85
0.92
0.97
0.99
1.03
2.05
1.91
2.13
1.13
3.11
2.08
1.10
1.06
2.38
2.16
3.27
3.00
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

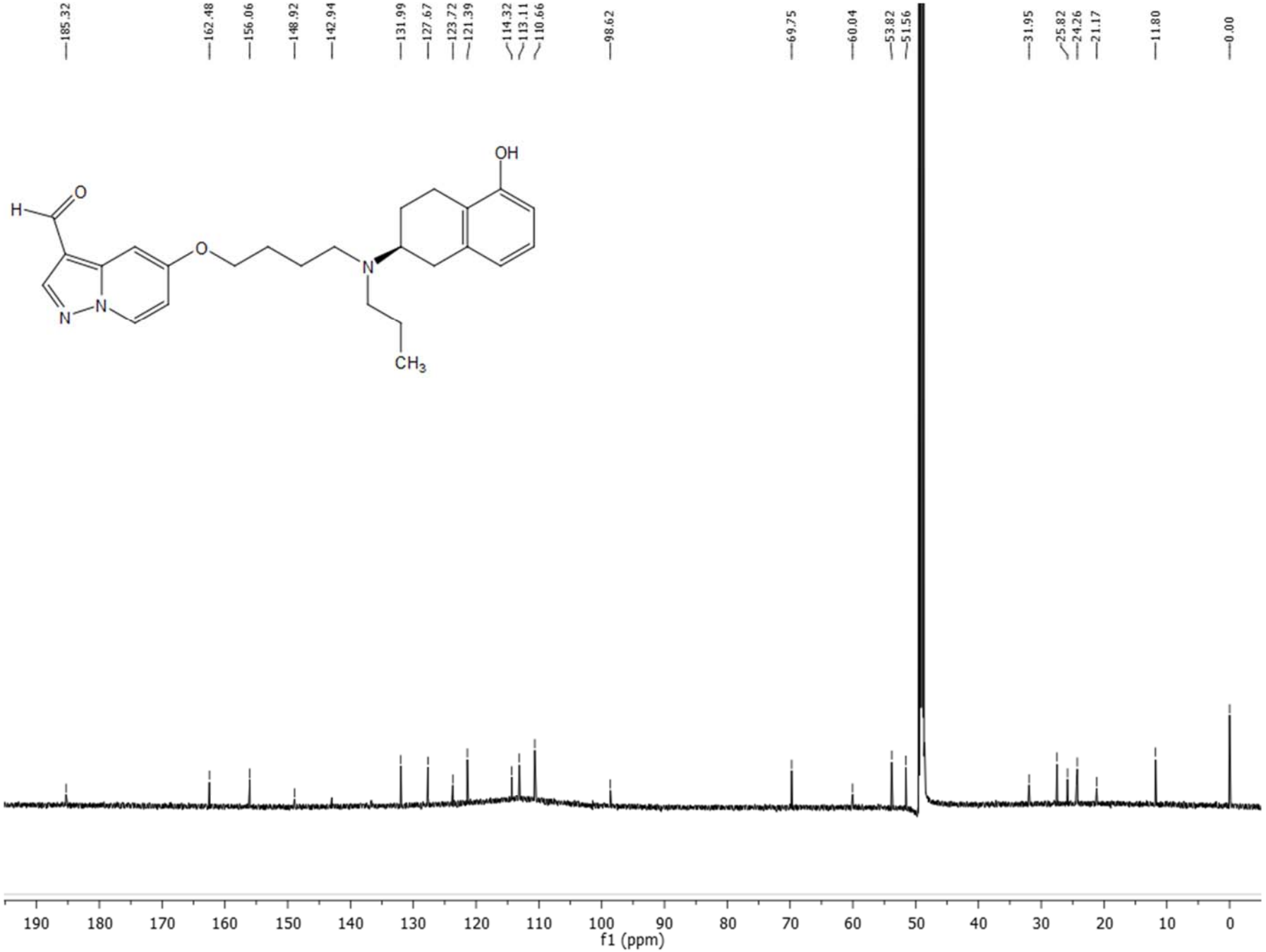
^{13}C NMR (*S*)-**15a**


185.25
155.94
152.34
147.69
138.41
136.73
127.29
125.36
124.28
121.54
119.59
114.87
114.40
112.75
70.39
61.57
58.43
53.76
51.31
32.99
28.07
25.60
24.75
22.40
12.22
0.00
H
O
N
N
O
N
OH
CH3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

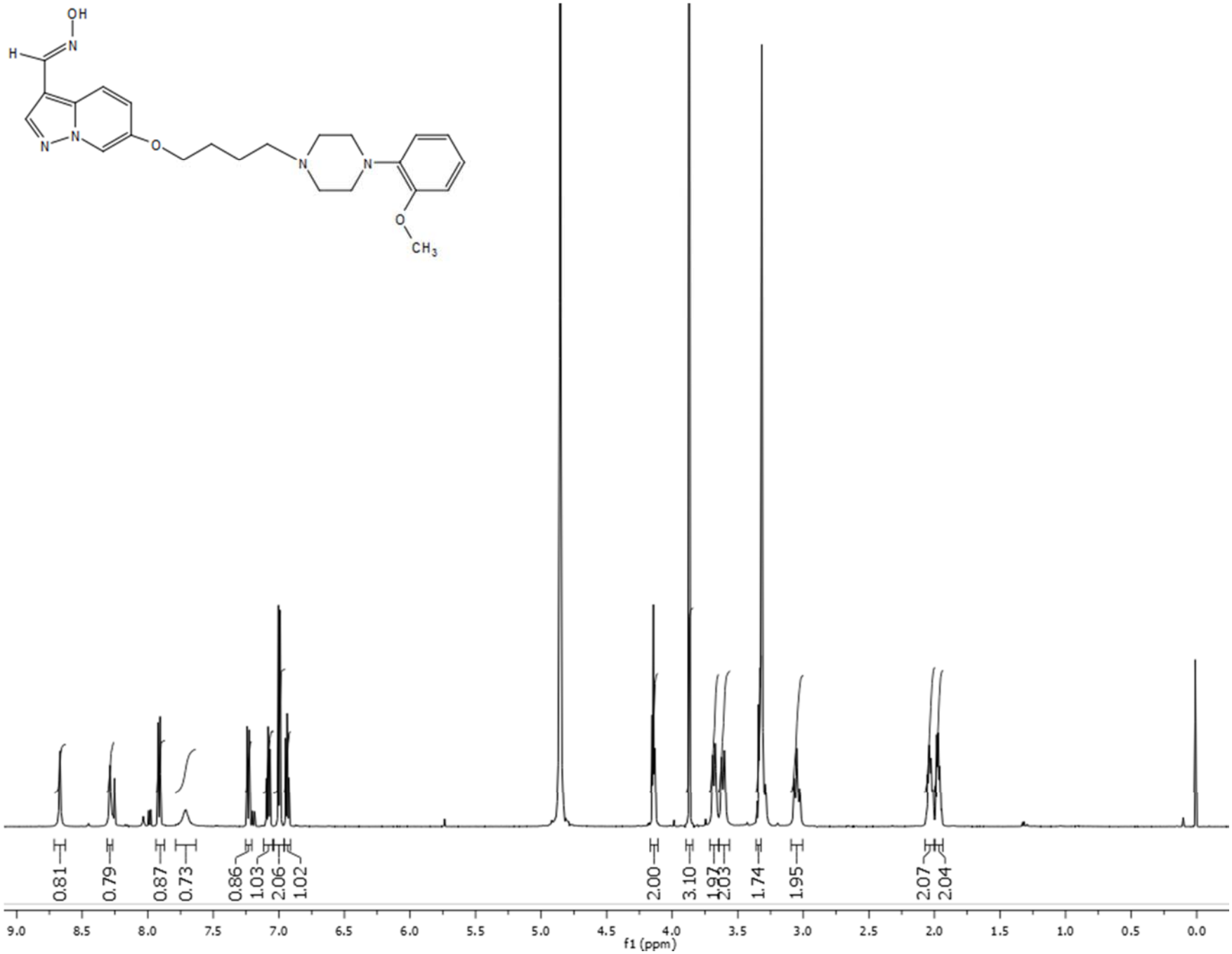
^{1}H NMR (*S*)-**15b**


OH
O
H
O
N
N
N
CH3
1.00
1.06
1.01
1.06
1.07
1.02
2.08
2.10
0.93
4.29
3.14
1.11
1.09
2.18
2.23
1.12
2.24
3.25
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

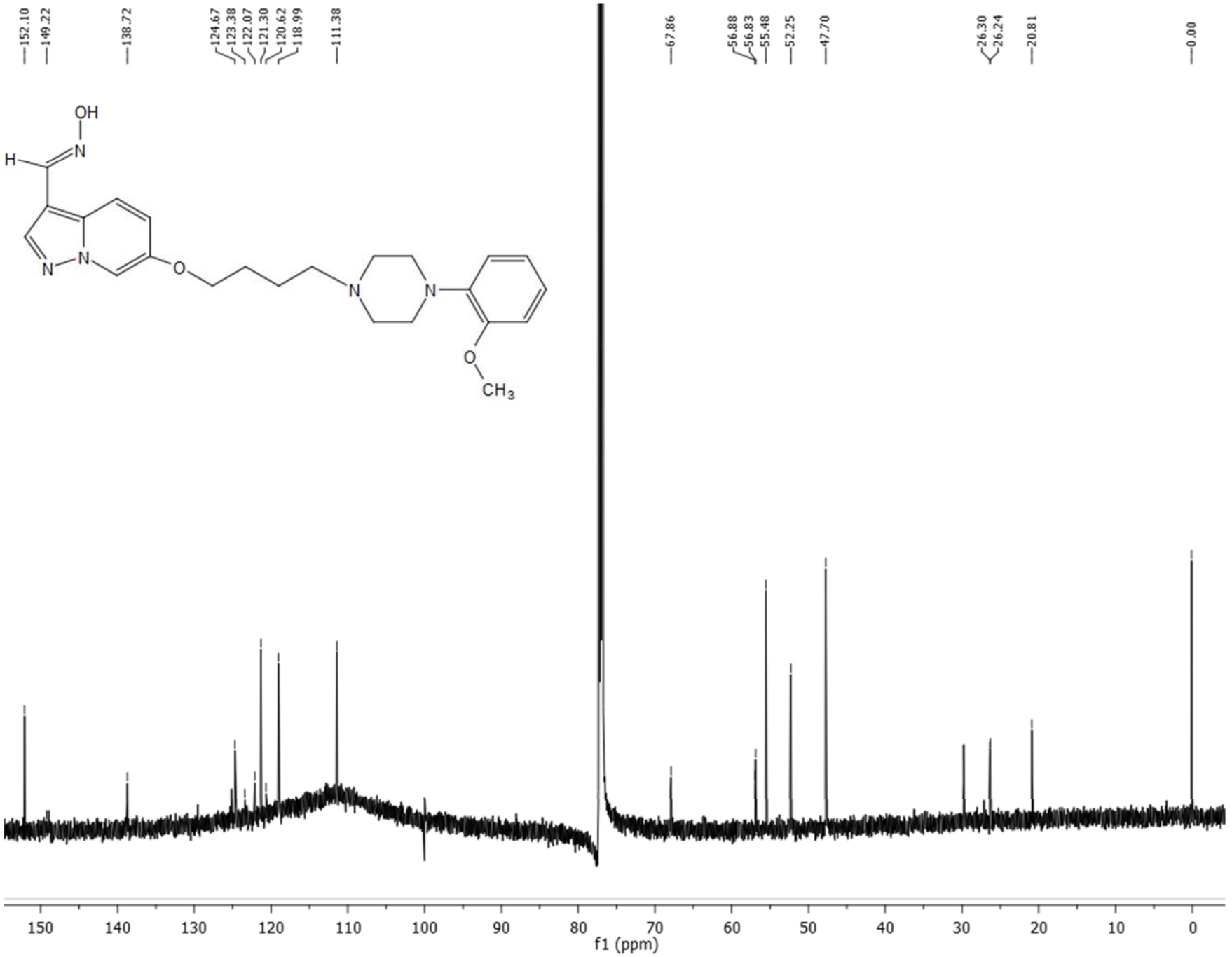
^{13}C NMR (*S*)-**15b**


185.32
162.48
156.06
148.92
142.94
131.99
127.67
123.72
121.39
114.32
113.11
110.66
98.62
69.75
60.04
53.82
51.56
31.95
25.82
24.26
21.17
11.80
0.00
OH
O
H
O
N
N
N
CH3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

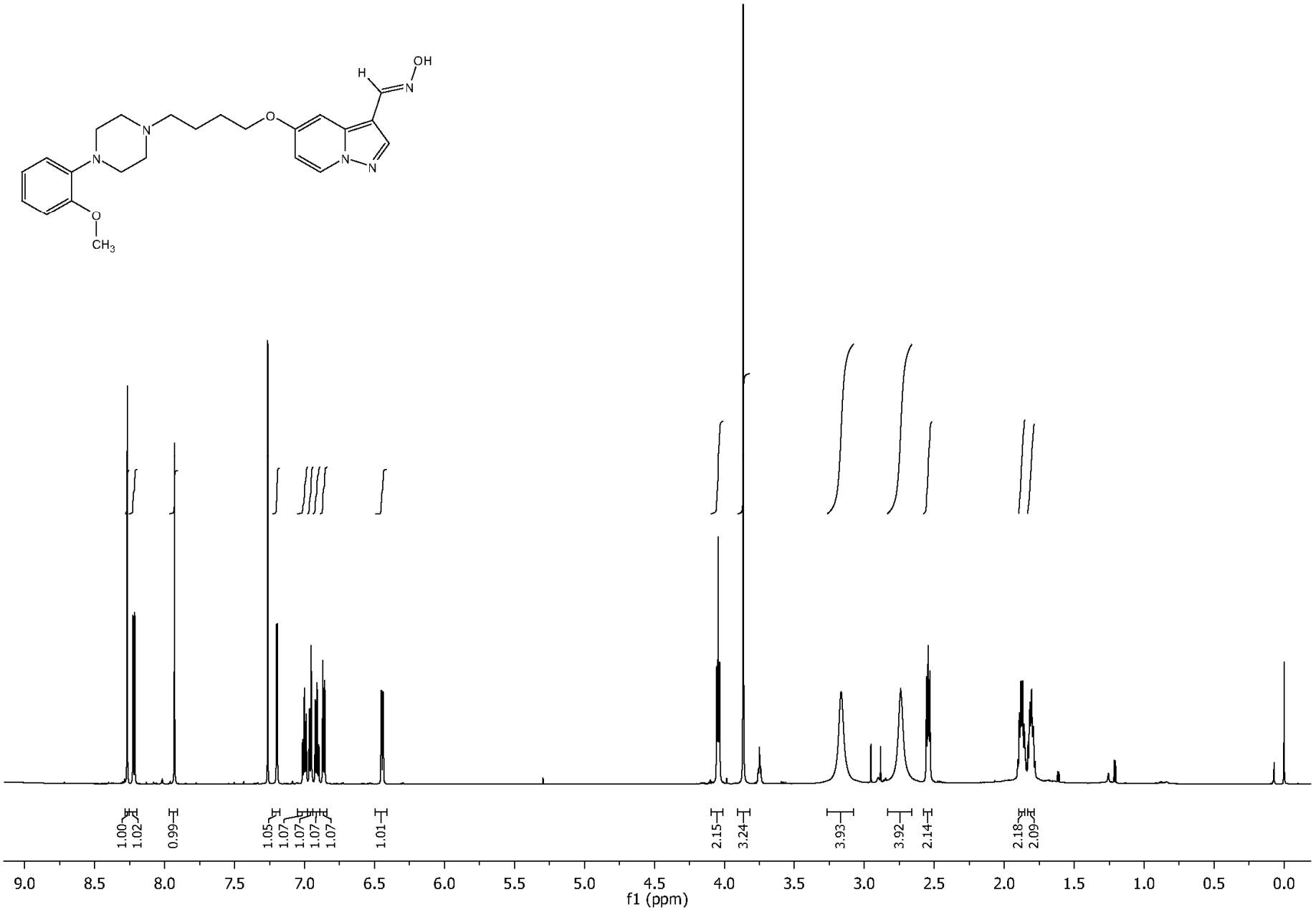
^{1}H NMR **16a**


OH
N
H
N
N
O
N
N
O
CH3
0.81
0.79
0.87
0.73
0.86
1.03
2.06
1.02
2.00
3.10
1.97
2.03
1.74
1.95
2.07
2.04
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

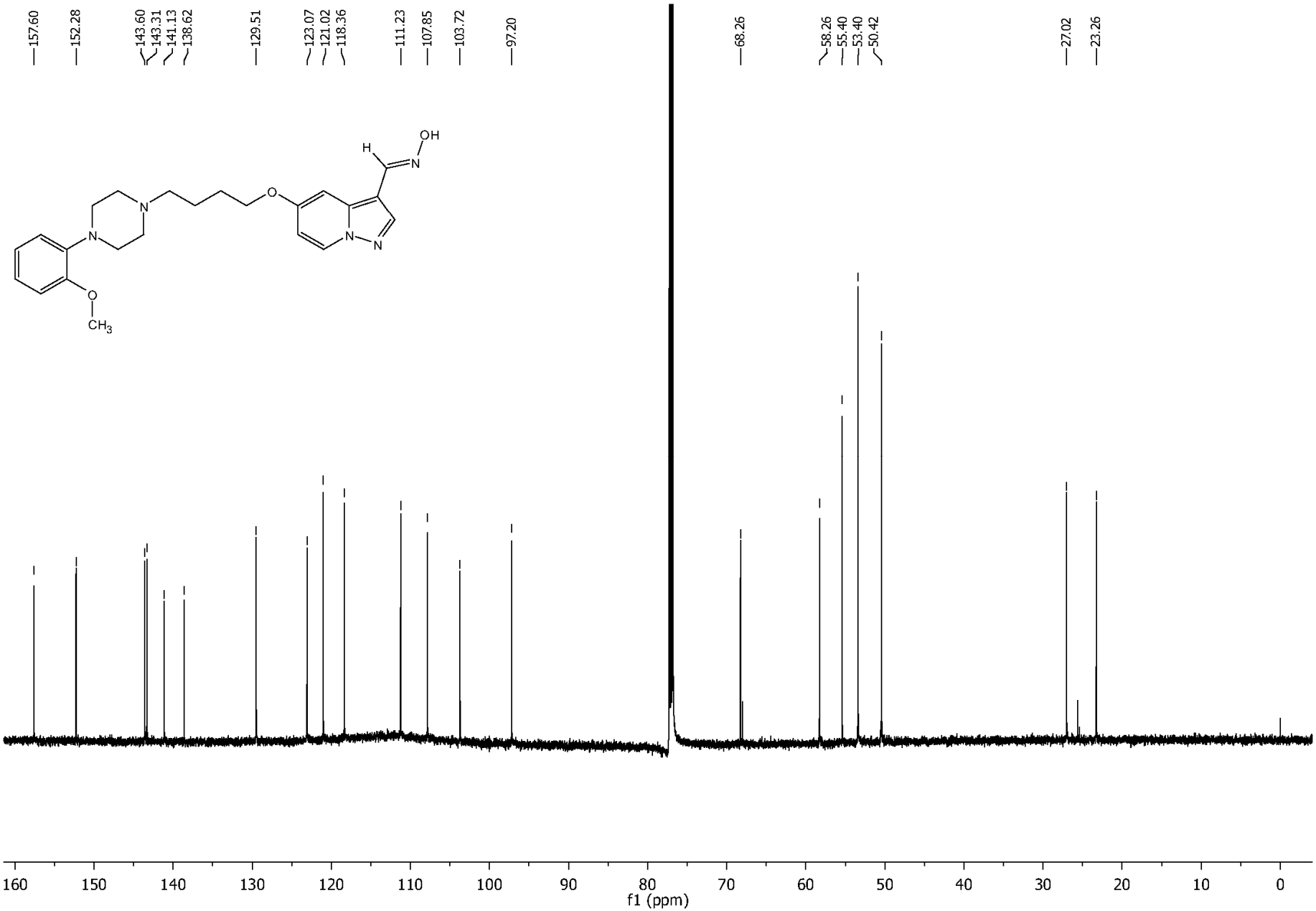
^{13}C NMR **16a**


152.10
149.22
138.72
124.67
123.38
122.07
121.30
120.62
118.99
111.38
67.86
56.88
56.83
55.48
52.25
47.70
26.30
26.24
20.81
0.00
OH
N
H
N
N
O
N
N
O
CH3
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

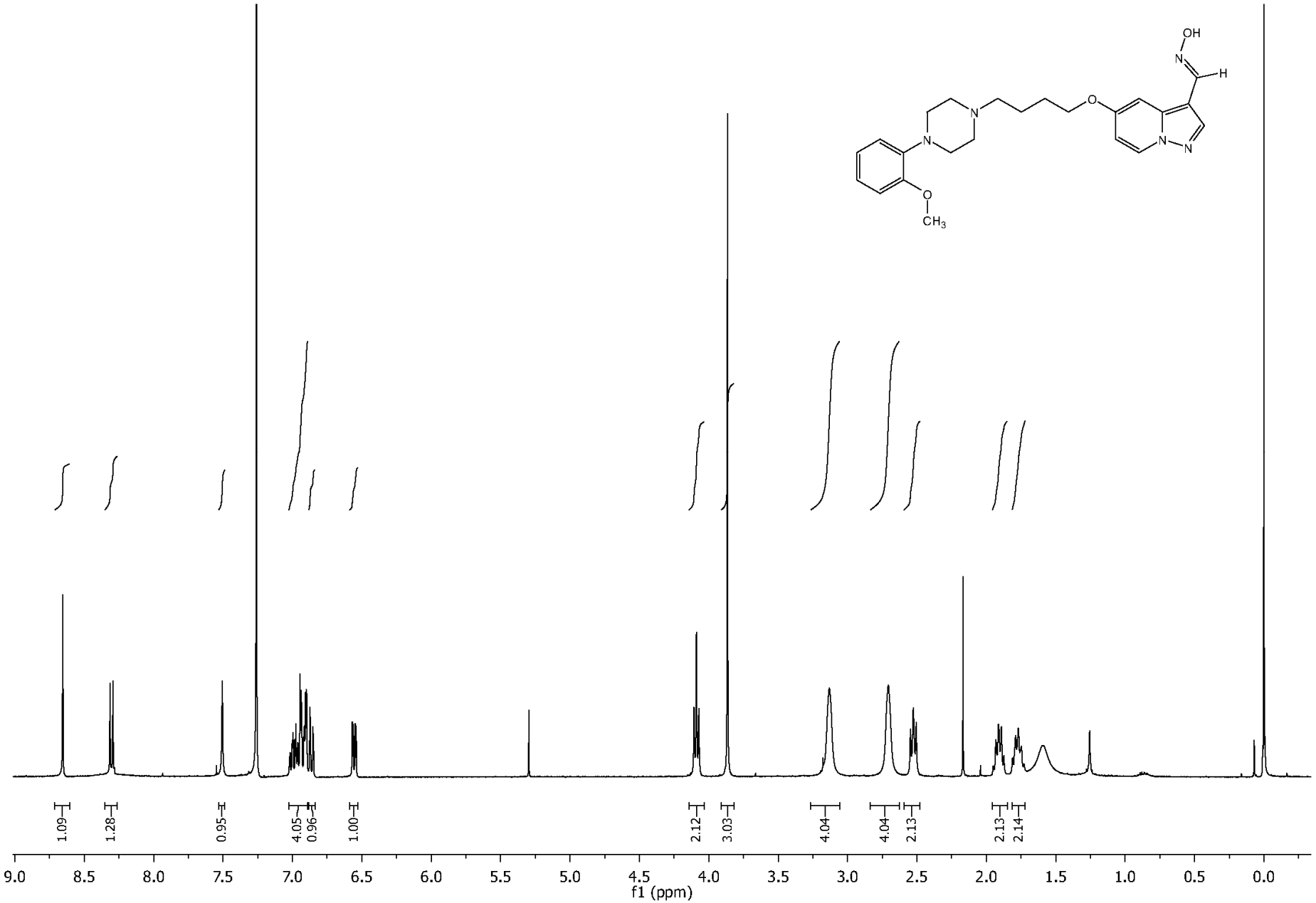
^{1}H NMR **16b**


OH
H
N
O
N
N
N
O
CH3
1.00
1.02
0.99
1.05
1.07
1.07
1.07
1.07
1.01
2.15
3.24
3.93
3.92
2.14
2.18
2.09
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

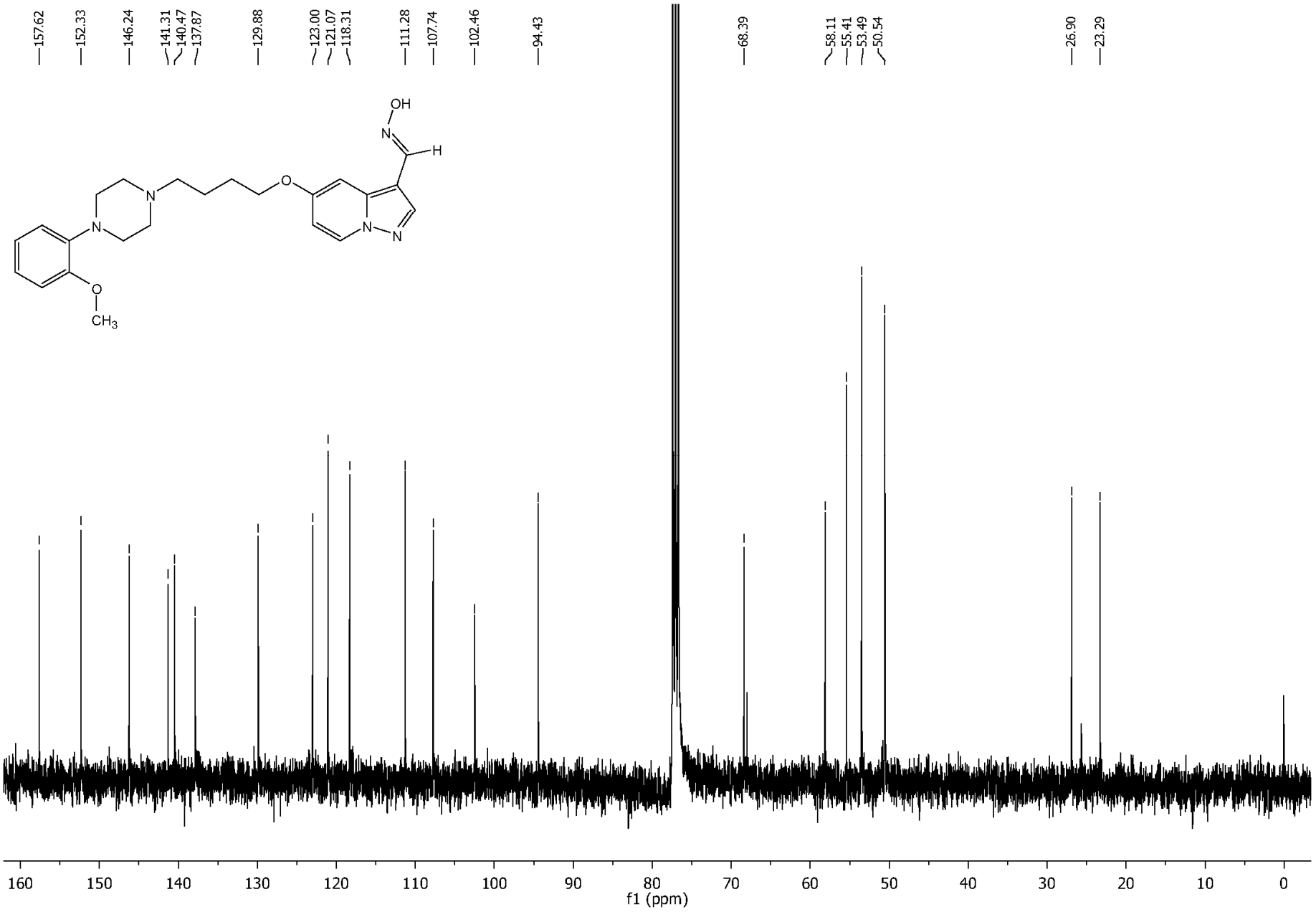
^{13}C NMR **16b**


157.60
152.28
143.60
143.31
141.13
138.62
129.51
123.07
121.02
118.36
111.23
107.85
103.72
97.20
68.26
58.26
55.40
53.40
50.42
27.02
23.26
OH
H
N
O
N
N
N
N
O
CH3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

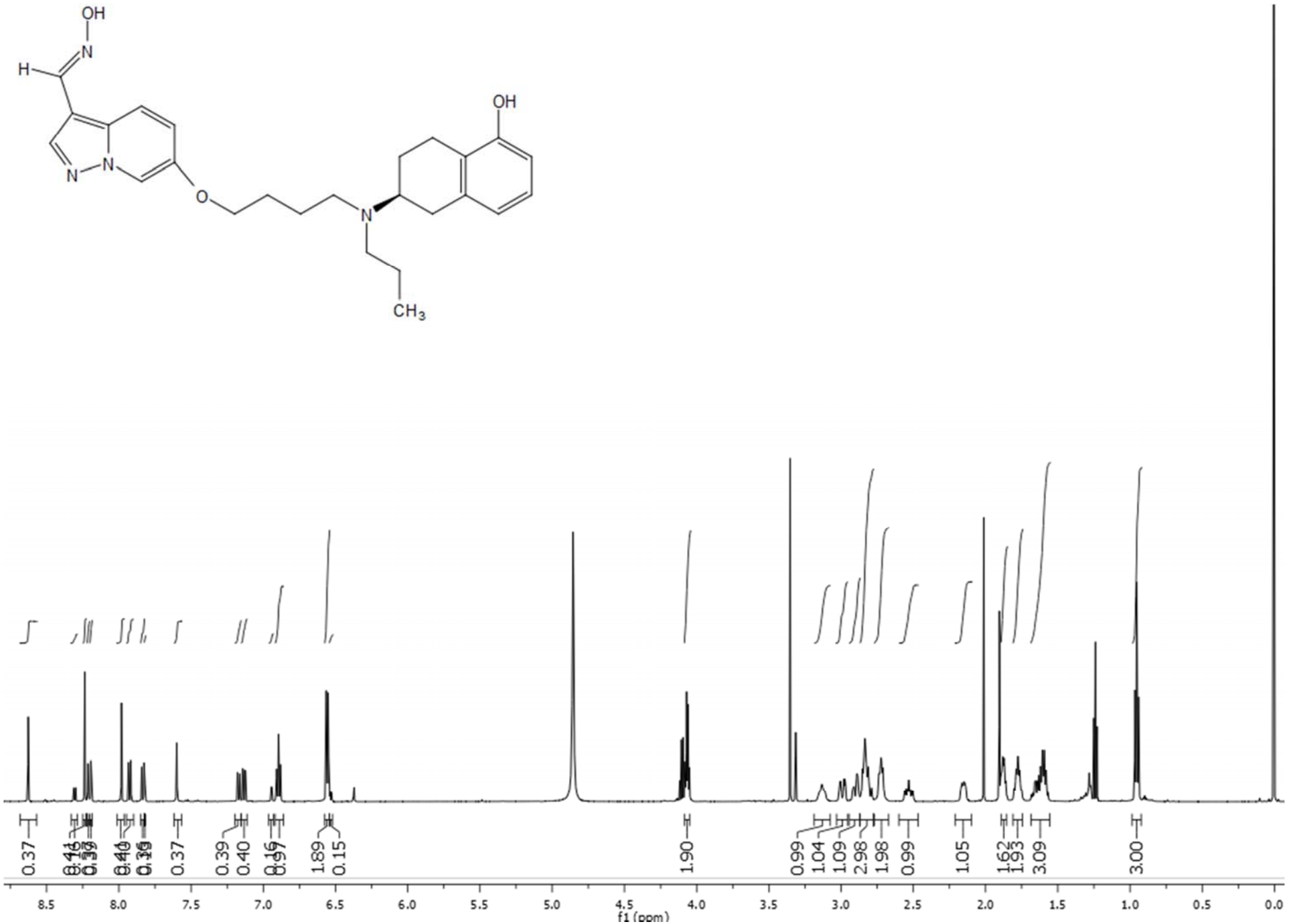
^{1}H NMR **16c**


OH
N
H
N
O
N
N
N
O
CH3
1.09
1.28
0.95
4.05
0.96
1.00
2.12
3.03
4.04
4.04
2.13
2.13
2.14
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

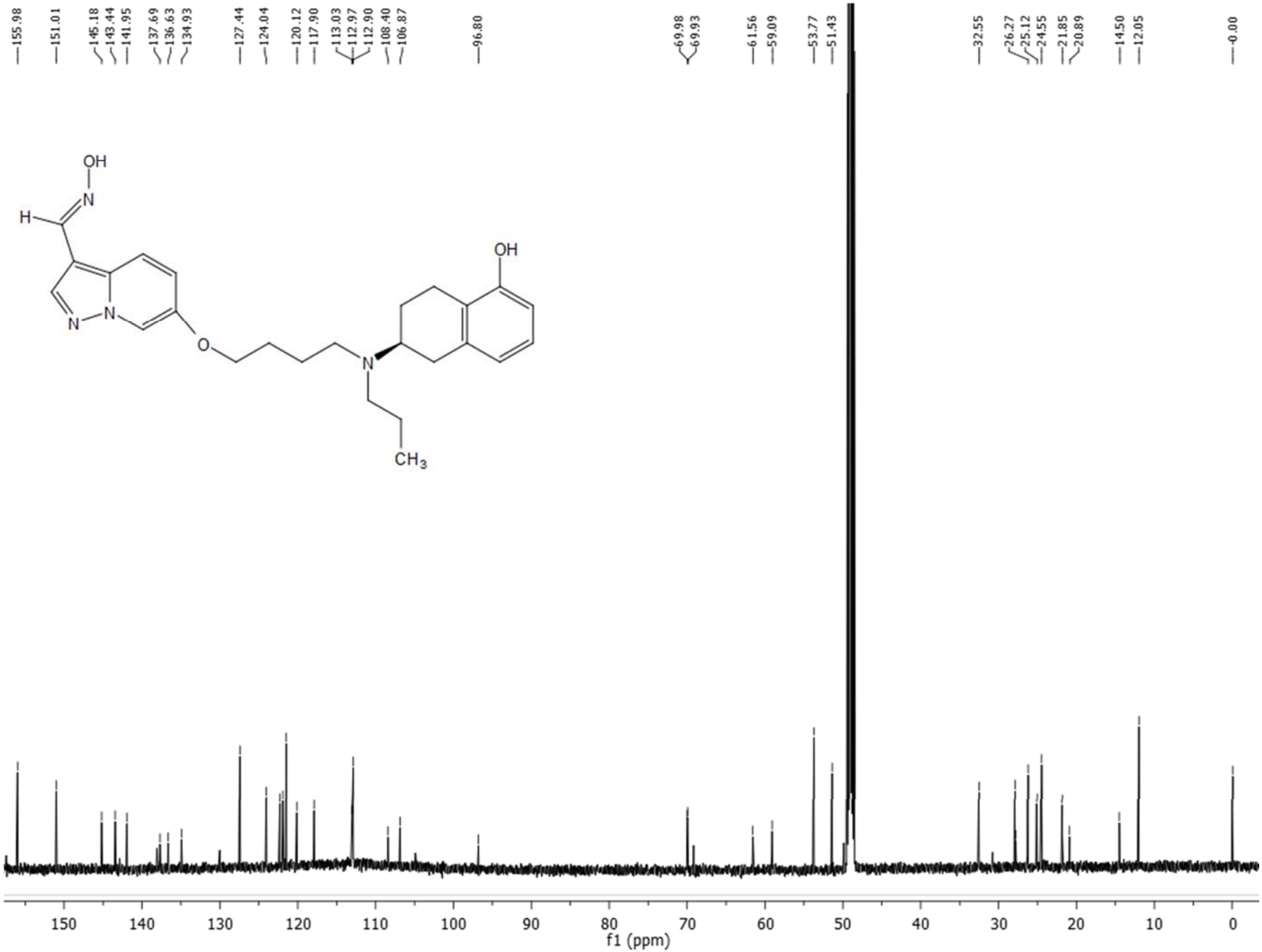
^{13}C NMR **16c**


157.62
152.33
146.24
141.31
140.47
137.87
129.88
123.00
121.07
118.31
111.28
107.74
102.46
94.43
68.39
58.11
55.41
53.49
50.54
26.90
23.29
OH
N
H
O
N
N
N
N
O
CH3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR (*S*)-**17a**


OH
N
H
N
N
O
N
OH
CH3
0.37
0.39
0.40
1.89
0.15
1.90
0.99
1.04
1.09
2.98
1.98
0.99
1.05
1.62
1.93
3.09
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

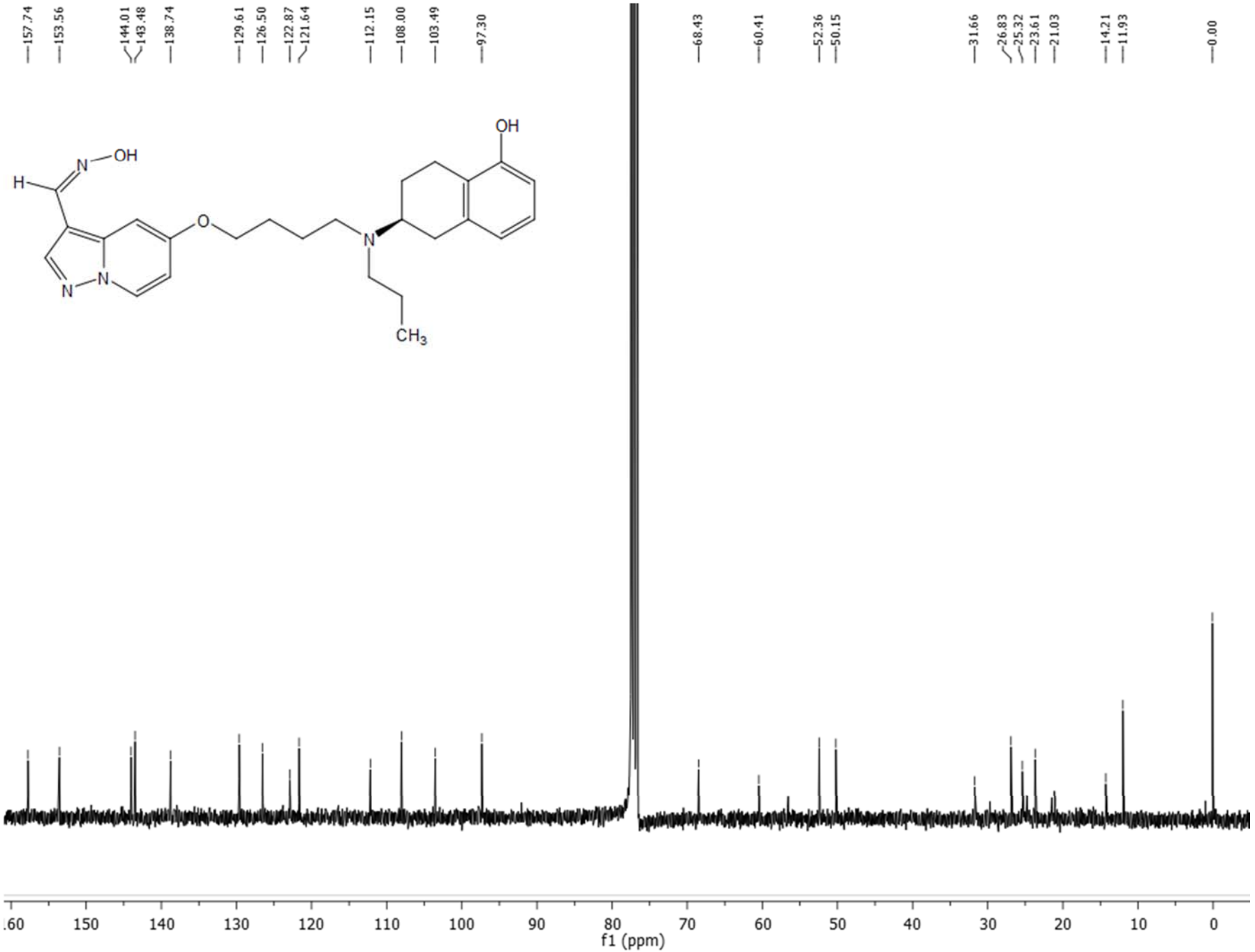
^{13}C NMR (*S*)-**17a**


155.98
151.01
145.18
143.44
141.95
137.69
136.63
134.93
127.44
124.04
120.12
117.90
113.03
112.97
112.90
108.40
106.87
96.80
69.98
69.93
61.56
59.09
53.77
51.43
32.55
26.27
25.12
24.55
21.85
20.89
14.50
12.05
0.00
OH
N
H
OH
N
N
O
N
CH3
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

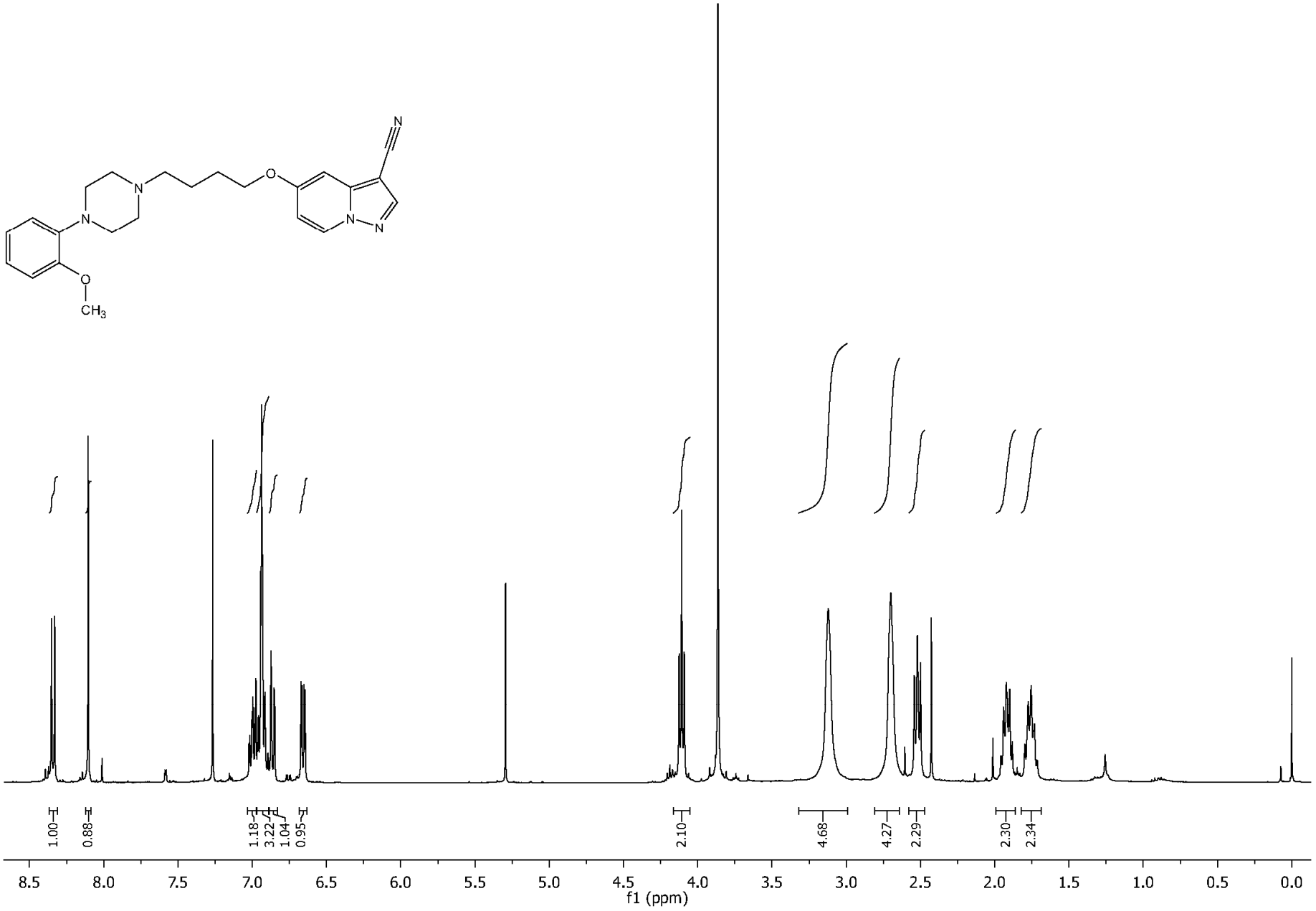
^{1}H NMR (*S*)-**17b**


OH
N
OH
H
O
N
N
N
CH3
0.89
0.96
0.95
1.01
1.08
1.07
1.07
0.95
2.00
1.06
1.26
1.29
1.28
2.08
3.54
1.17
2.32
3.54
2.41
2.95
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

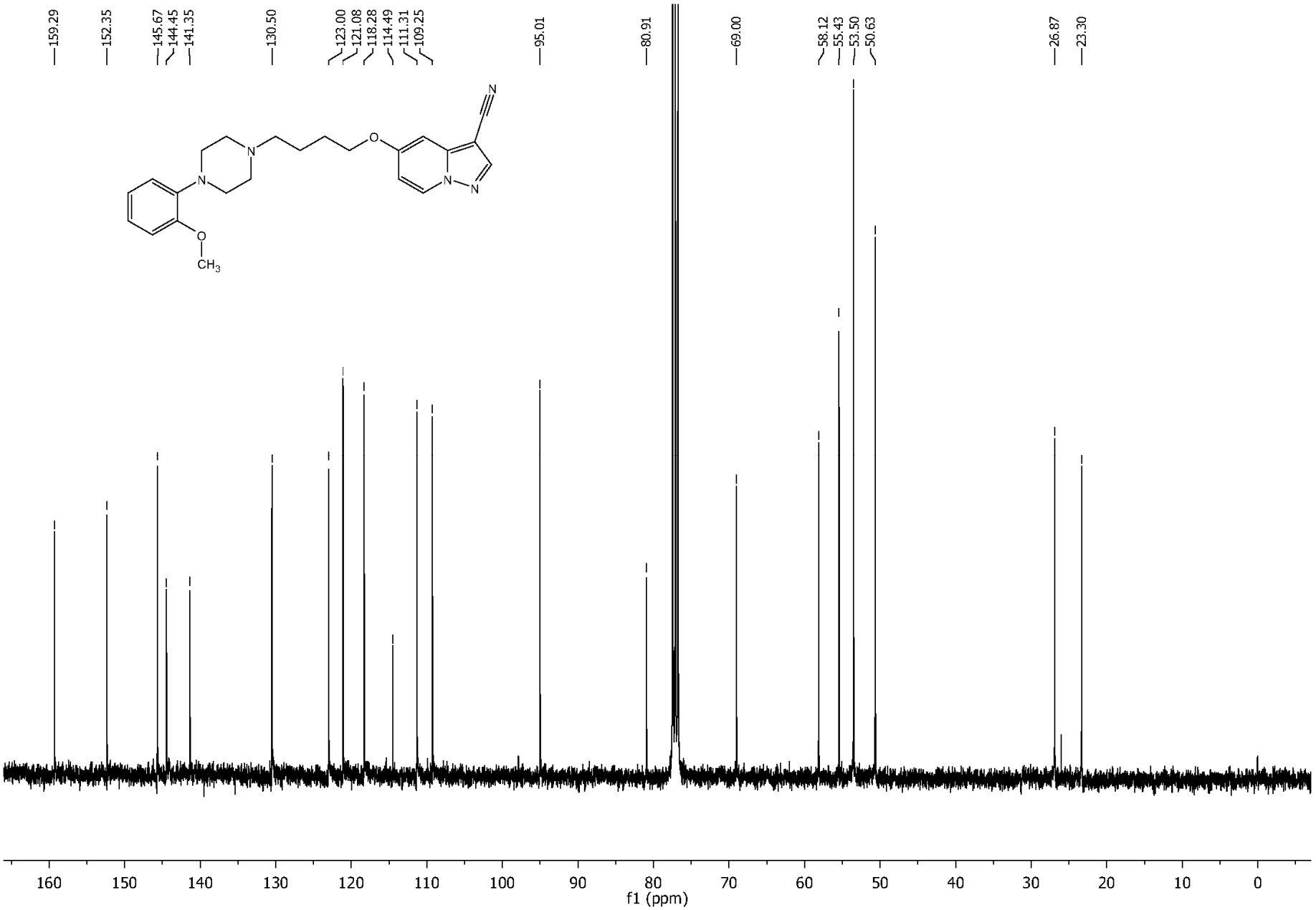
^{13}C NMR (*S*)-**17b**


157.74
153.56
144.01
143.48
138.74
129.61
126.50
122.87
121.64
112.15
108.00
103.49
97.30
68.43
60.41
52.36
50.15
31.66
26.83
25.32
23.61
21.03
14.21
11.93
0.00
OH
N
OH
H
O
N
N
N
CH3
f1 (ppm)

^{1}H NMR **18**


N
O
N
N
N
O
CH3
1.00
0.88
1.18
3.22
1.04
0.95
2.10
4.68
4.27
2.29
2.30
2.34
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{13}C NMR **18**


159.29
152.35
145.67
144.45
141.35
130.50
123.00
121.08
118.28
114.49
111.31
109.25
95.01
80.91
69.00
58.12
55.43
53.50
50.63
26.87
23.30
N
O
N
N
N
O
CH3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

14) Supplementary References.

1. Kane, J. M.; Carson, W. H.; Saha, A. R.; McQuade, R. D.; Ingenito, G. G.; Zimbroff, D. L.; Ali, M. W. Efficacy and Safety of Aripiprazole and Haloperidol Versus Placebo in Patients with Schizophrenia and Schizoaffective Disorder. *J. Clin. Psychiatr.* **2002,** *63*, 763-771.

2. Peleg-Raibstein, D.; Sydekum, E.; Feldon, J. Differential Effects on Prepulse Inhibition of Withdrawal from Two Different Repeated Administration Schedules of Amphetamine. *Int. J. Neuropsychopharmacol.* **2006,** *9*, 737-749.

3. Di Chiara, G.; Imperato, A. Drugs Abused by Humans Preferentially Increase Synaptic Dopamine Concentrations in the Mesolimbic System of Freely Moving Rats. *Proc. Natl. Acad. Sci. U.S.A.* **1988,** *85*, 5274-5278.

4. Heal, D. J.; Smith, S. L.; Gosden, J.; Nutt, D. J. Amphetamine, Past and Present – a Pharmacological and Clinical Perspective. *J. Psychopharm.* **2013,** *27*, 479-496.

5. Tenn, C. C.; Fletcher, P. J.; Kapur, S. A Putative Animal Model of the "Prodromal" State of Schizophrenia. *Biol. Psychiatry* **2005,** *57*, 586-593.

6. Robinson, T. E.; Becker, J. B. Enduring Changes in Brain and Behavior Produced by Chronic Amphetamine Administration: A Review and Evaluation of Animal Models of Amphetamine Psychosis. *Brain Res. Rev.* **1986,** *11*, 157-198.

7. Koch, M. The Neurobiology of Startle. *Prog. Neurobiol.* **1999,** *59*, 107-128.

8. Braff, D. L.; Grillon, C.; Geyer, M. A. Gating and Habituation of the Startle Reflex in Schizophrenic Patients. *Arch. Gen. Psychiatry* **1992,** *49*, 206-215.

9. Leumann, L.; Feldon, J.; Vollenweider, F. X.; Ludewig, K. Effects of Typical and Atypical Antipsychotics on Prepulse Inhibition and Latent Inhibition in Chronic Schizophrenia. *Biol. Psychiatry* **2002,** *52*, 729-739.

10. Featherstone, R. E.; Kapur, S.; Fletcher, P. J. The Amphetamine-Induced Sensitized State as a Model of Schizophrenia. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2007,** *31*, 1556-1571.

11. Tenn, C. C.; Kapur, S.; Fletcher, P. J. Sensitization to Amphetamine, but Not Phencyclidine, Disrupts Prepulse Inhibition and Latent Inhibition. *Psychopharmacology* **2005,** *180*, 366-376.

12. Murphy, C. A.; Fend, M.; Russig, H.; Feldon, J. Latent Inhibition, but Not Prepulse Inhibition, Is Reduced During Withdrawal from an Escalating Dosage Schedule of Amphetamine. *Behav. Neurosci.* **2001,** *115*, 1247-1256.

13. Amato, D.; Pum, M. E.; Groos, D.; Lauber, A. C.; Huston, J. P.; Carey, R. J.; de Souza Silva, M. A.; Müller, C. P. Neuropharmacology of Light-Induced Locomotor Activation. *Neuropharmacology* **2015,** *95*, 243-251.

14. Pum, M. E.; Huston, J. P.; Müller, C. P.; De Souza Silva, M. A. Light-Induced Activity in the Activity Box Is Not Aversively Motivated and Does Not Show Between-Trial Habituation. *Physiol. Behav.* **2009,** *96*, 434-439.

15. Pum, M. E.; Huston, J. P.; De Souza Silva, M. A.; Müller, C. P. Visual Sensory-Motor Gating by Serotonin Activation in the Medial Prefrontal and Occipital, but Not in the Rhinal, Cortices in Rats. *Neuroscience* **2008,** *153*, 361-372.

16. Prut, L.; Belzung, C. The Open Field as a Paradigm to Measure the Effects of Drugs on Anxiety-Like Behaviors: A Review. *Eur. J. Pharmacol.* **2003,** *463*, 3-33.

17. Antelman, S. M.; Chiodo, L. A. Amphetamine as a Stressor. In *Stimulants: Neurochemical, Behavioral and Clinical Perspectives*, Cresse, I., Ed. Raven Press: New York, 1983; pp 269-299.

18. Braga, R. J.; Petrides, G.; Figueira, I. Anxiety Disorders in Schizophrenia. *Compr. Psychiatry* **2004,** *45*, 460-468.

19. Buckley, P. F.; Miller, B. J.; Lehrer, D. S.; Castle, D. J. Psychiatric Comorbidities and Schizophrenia. *Schizophr. Bull.* **2009,** *35*, 383-402.

20. Godsil, B. P.; Fanselow, M. S. Light Stimulus Change Evokes an Activity Response in the Rat. *Anim. Learn. Behav.* **2004,** *32*, 299-310.

21. Pum, M. E.; Rubio, A. R.; Carey, R. J.; Silva, M. A. D. S.; Müller, C. P. The Effects of Cocaine on Light-Induced Activity. *Brain Res. Bull.* **2011,** *84*, 229-234.

22. Laxminarayan, B.; Prabhu Prasad, M.; Kouacou, A. Compositions, Synthesis and Methods of Using Piperazine Based Antipsychotic Agents. US 2009/0298819 A1, 2009.

23. Na, Y. H.; Hong, S. H.; Lee, J. H.; Park, W.-K.; Baek, D.-J.; Koh, H. Y.; Cho, Y. S.; Choo, H.; Pae, A. N. Novel Quinazolinone Derivatives as 5-Ht7 Receptor Ligands. *Biorg. Med. Chem.* **2008,** *16*, 2570-2578.

24. Kendall, J. D.; O'Connor, P. D.; Marshall, A. J.; Frederick, R.; Marshall, E. S.; Lill, C. L.; Lee, W. J.; Kolekar, S.; Chao, M.; Malik, A.; Yu, S.; Chaussade, C.; Buchanan, C.; Rewcastle, G. W.; Baguley, B. C.; Flanagan, J. U.; Jamieson, S. M.; Denny, W. A.; Shepherd, P. R. Discovery of Pyrazolo[1,5-a]Pyridines as P110alpha-Selective Pi3 Kinase Inhibitors. *Bioorg. Med. Chem.* **2012,** *20*, 69-85.