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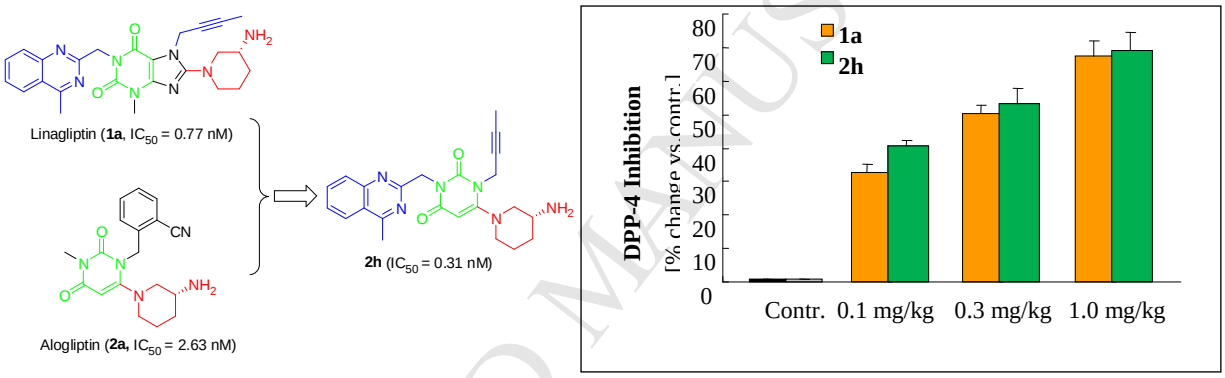
Graphical Abstract

A highly potent DPP-4 inhibitor **2h** (IC_{50} = 0.31 nM) has been identified by hybrid compound design on the basis of SAR analysis and binding modes of linagliptin and alogliptin.



Discovery of Highly Potent DPP-4 Inhibitors by Hybrid Compound Design Based on Linagliptin and Alogliptin

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Abastract

Highly potent DPP-4 inhibitors have been identified by hybrid compound design based on linagliptin and alogliptin. The most promising compound **2h** (IC_{50} = 0.31 nM) exhibited 8.5-fold and 2.5-fold more potent activity than that of alogliptin (IC_{50} = 2.63 nM) and linagliptin (IC_{50} = 0.77 nM), respectively. Compound **2h** had a good inhibition selectivity for DPP-4 over DPP-8/9 and thus was selected for further biological evaluation, including oral glucose tolerance, plasma DPP-4 inhibitory activity, pharmacokinetic profile, acute toxicity and hERG inhibition. The assay results showed that **2h** displayed significant *in vivo* glucose-lowering effect and low risk of toxicity. Further studies are expected to confirm **2h** as a potential drug candidate for the treatment of type 2 diabetes.

Keywords

DPP-4 inhibitors; hybrid compound design; linagliptin; alogliptin; diabetes.

1. Introduction

Human glucagon-like peptide-1 (GLP-1), an incretin resulted from selective cleavage of the proglucagon molecule and mainly secreted by ileal L-cells, is a potent antihyperglycemic hormone. It exerts glucose lowering effect via multi-functions: stimulating insulin secretion and increasing insulin sensitivity, decreasing glucagon release, inhibiting acid secretion and gastric emptying, etc [1-3]. Most importantly, the ability of GLP-1 to enhance insulin secretion is glucose-dependent [4]. Nevertheless, the active forms GLP-1-(7-37) NH_2 and GLP-1-(7-36) NH_2, once existing in the circulation, quickly lose activity due to rapid degradation by the enzyme dipeptidyl peptidase-4 (DPP-4) [5, 6]. Inhibition of DPP-4 has been proved to prolong and enhance the activity of GLP-1, and thus validated as an effective approach to treatment of type 2 diabetes [7, 8]. To date, seven DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin, linagliptin, anagliptin, teneligliptin and alogliptin) have been approved for the treatment of diabetes. Yet, the enthusiasm

in developing new DPP-4 inhibitors remains strong since current drugs still have some undesirable side effects [9, 10].

Among the marketed DPP-4 inhibitors, linagliptin and alogliptin (Figure 1) have the same structure fragment (*R*)-3-aminopiperidine, which has been identified to provide key hydrogen bonding interactions with Glu205 and Glu206 of DPP-4 [11, 12]. However, a systematic study on the structure-activity relationship of this fragment still remains to be explored, particularly, it would be interesting to replace this fragment with other amino groups. In this regard, we designed and synthesized a series of novel analogues **1b~1j** and **2b~2d** with diverse amino groups to replace the (3*R*)-aminopiperidine moiety (Figure 2). Moreover, we reasoned that hybrid compound design based on the privileged fragments of linagliptin and alogliptin might result in more potent DPP-4 inhibititors. Thus, after careful comparison of the binding modes of linagliptin and alogliptin, we used the quinazolinylmethyl and 2-butynyl groups of linagliptin to take the places of the N_3-methyl and N_1-orthocyanobenzyl groups of alogliptin, respectively, while (3*R*)-aminopiperidine group was remained or displaced by other amines (**2e~2k**, Figure 3). Upon screening of compounds **2e~2k**, a couple of compounds were found to be highly potent DPP-4 inhibititors, and encouraged by this finding, more derivatives along this line (**2l~2v**, Figure 3) were synthesized and evaluated. During this study, very recently Xie et al also reported their discovery of highly potent DPP-4 inhibitors by this hybrid strategy [13]. Herein, we present our study results.

2. Result and discussion

2.1. Chemistry

Compounds **1a**-**1j** were prepared according to the procedure depicted in Scheme 1 [11]. 8-Bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione was selectively alkylated at *N*-7 by 1-bromo-2-butyne, 3-bromo-1-propyne or 3-bromo-1-propene to give the corresponding *N*-alkylated purinediones **3a**-**3c**, respectively, which further reacted with 2-chloromethyl-4-methylquinazoline to afford the purinediones **4a**-**4c**. Amination of **4a**-**4c** gave the target compounds **1a**-**1j**.

The synthetic route to compounds **2a**-**2v** is outlined in Scheme 2 [12]. In brief, 6-chlorouracil was selectively alkylated at *N*-1 by alkyl halides under mild conditions (DIPEA and r.t.) to give the corresponding alkylated uracils **5a**-**5d**, which were further alkylated at *N*-3 by alkyl halides under strong conditions (NaH/LiBr/80 ^{o}C) to afford the dialkylated uracils **6a**-**6o**. Amination of **6a**-**6o** in the presence of 4 Å molecule sieve furnished the target compounds **2a**-**2v**.

*2.2. Biological evaluation and prelimilary SAR analysis of **1a-1j** and **2a-2d***

The DPP-4 inhibitory activity was screened for analogues **1b**-**1i** and **2b**-**2d** at 10 μM and 100 nM, with **1a** (linagliptin) and **2a** (alogliptin) as the positive controls. The compounds with good inhibition rates at 100 nM were further selected to determine their IC_{50} values. As shown in Tables 1~2, all the analogues without (3*R*)-aminopiperidine substituent exhibited poor DPP-4 inhibitory activity, and the compounds with (*S*)-configuration were generally less active than those with (*R*)-configuration (*e.g.* **1h** *vs* **1i**; **2c** *vs* **2d**), implying that (3*R*)-aminopiperidine was a predominant fragment for the potency.

2.3. Comparison of the binding modes of linagliptin and alogliptin

The crystallographic structures of DPP-4 complexed with linagliptin and alogliptin (PDB codes: 2RGU and 3G0B) were aligned using PyMol [14]. It was revealed that alogliptin structurally overlaped well with the right

part of linagliptin (Figure 4). The uracil ring of alogliptin and the purinedione skeleton of linagliptin lay nearly in a plane, both forming hydrogen bonding interactions with Ser630 and π-π stacks with Tyr547. The 2-cyanobenzyl group of alogliptin and the 2-butynyl group of linagliptin inserted into the S1 pocket with their distant carbons almost completely overlapping.

Interestingly, although both two primary amino groups formed hydrogen bonding interactions with Glu205/206, it was found that the primary amino group of alogliptin took an axial conformation, but not equatorial like in linagliptin. With computational study, Zhang *et al* explained that the alogliptin molecule energy with an axial NH_2 was lower than that with an equatorial NH_2, because the axial NH_2 could make intromolecular hydrogen bonding interactions with the cyanobenzyl CN [12]. This speculation seems discord with a very recent crystallography study [15]. In this crystallography study, both axial NH_2 and equatorial NH_2 were observed in the crystals of alogliptin, indicating that the energy gap between the axial and equatorial NH_2 is small. Why only axial NH_2 was observed in alogliptin binding to DPP-4? One reason might be that the alogliptin molecule with equatorial NH_2 was induced to take an axial conformation for fitting the Glu205/Glu206 residues of DPP-4. Another possibility is that it could not interact with Glu205/Glu206 at all. Taken together, it might be not easy for the alogliptin molecule with equatorial NH_2 to interact with DPP-4, therefore causing alogliptin less potent than linagliptin. Moreover, it was clearly disclosed that the quinazoline skeleton of linagliptin paralell faced Trp629, indicating that linagliptin had π-π stacks not only with Tyr547 but also with Trp629. This is probably one main factor leading to that linagliptin is more potent than alogliptin.

*2.4. Discovery of highly potent DPP-4 inhibitors **2h** and **2i***

Based on the preliminary SAR study and the binding mode analysis, hybrid compounds **2e**-**2k** were designed, synthesized and evaluated for their DPP-4 inhibitory activity. As shown in Table 3, compound **2h** and its (*S*)-entiomer **2i** exhibited highly potent DPP-4 inhibitory activity, with IC_{50} of 0.31 nM and 0.35 nM, respectively, while the others except **2j** (IC_{50} = 12.7 nM) showed poor or no activity. It seems that a proper combination of R_3 and R_4 substituents had profound effect on potency (*e.g* **2e** *vs* **2h**; **2f** *vs* **2j**; **2h** *vs* **2k**). For R_2 substitution, 3-aminopiperidine was found to be critical for the potency.

To predict the possible interactions of **2h** and **2i** binding to DPP-4, molecular docking of **2h** and **2i** into the inhibitor binding site of DPP-4 was performed based on the DPP-4 co-crystal structure (PDB code: 2RGU) by the genetic algorithm of GOLD 3.0.1. As expected, apart from keeping hydrogen bonding interactions with Glu205 and Glu206, and π-π stacks with Try547, **2h** and **2i** probably have additional π-π stacks with Trp629 (Figure 5), thus leading to be 7.5-fold and 8.5-fold more potent than alogliptin, respectively.

2.5. Further compound design and SAR analysis

Based on the discovery of **2h** and **2i**, compounds **2l**-**2v** were designed and synthesized, mainly to probe the effect of diverse substituents of R_3 aromatic groups on th epotency. As shown in Table 4, most of compounds **2l**-**2v** displayed DPP-4 inhibitory activity to some degree. Among them, **2l**, **2s** and **2u** showed quite potent activity with IC_{50} < 4 nM. Moreover, there was a very clear tendency that the R_3 substituent with electron withdrawn ability benefited the DPP-4 inhibitory activity (**2l** *vs* **2m**/**2n**, **2o** *vs* **2p**~**2s**). As for the R_4 substituents, the allyl group could be an alternative substituent for 2-butynyl to keep th epotency (**2l** *vs* **2u**), while the 2-propynyl group obviously reduced the activity (**2l** *vs* **2t**).

2.6. Selectivity for DPP-4 over DPP-8 and DPP-9

Inhibition selectivity for DPP-4 over DPP-8 and DPP-9 [16] was tested for 8 compounds with DPP-4 IC_{50} < 12 nM. The DPP-8/9 inhibition rates of 8 compounds at 100 μM and 25 μM are listed in Table 5, with **1a** (linagliptin) and **2a** (alogliptin) as the controls. As shown in Table 5, except that **1h** and **2t** at 100 μM exhibited DPP-8 inhibtion rates >33% and >27%, the other 6 compounds were proved to be highly selective for DPP-4 over DPP-8 and DPP-9.

*2.7. Further in vitro and in vivo evaluation of **2h***

The effect of **2h** on glucose tolerance was tested in *db*/*db* diabetic mice, with **1a** (linagliptin) as the positive control (Figures 6, 7). As shown in Figure 7, by single oral administration of **2h** 45 min prior to glucose load, the plasma glucose excursion was remarkably reduced in a dose-dependent manner from 0.1 mg/kg to 1.0 mg/kg. Compared with **1a** (Figure 6), the improvement effect of **2h** on glucose tolerance was observed. For example, the plasma glucose level 30 min after glucose load was suppressed to below 24 mM by only 0.1 mg/kg dose of **2h** (Figure 7), while it was almost not affected by the same dose of **1a** (Figure 6). In addition, the inhibition rates of plasma glucose $AUC_{0\rightarrow120\ min}$ by 0.1 mg/kg and 0.3 mg/kg doses of **2h** were much higher than that by the same doses of **1a** (Figure 8). However, at a 1.0 mg/kg dose, the inhibition rate of $AUC_{0\rightarrow120\ min}$ by **2h** was equal to that by **1a**. Moreover, the improvement of glucose tolerance by **2h** was proved to correlate with decreased DPP-4 activity (Figure 9).

Preliminary pharmacokinetic profile of **2h** was studied in SD rats. The selected parameters including half life ($t_{1/2}$), time to the maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), area under the plasma concentration time curve ($AUC_{0-\infty}$), mean residence time (MRT) and oral bioavailability (*F*), are listed in Table 6. The results showed that **2h** was orally available.

The safety of **2h** was evaluated by acute toxicity study and hERG channel inhibitory activity test. Compound **2h** was proved to have wide safety margin (LD_{50} = 1.63 g/kg) and low risk of hERG channel inhibition (IC_{50} > 100 μM).

3. Conclusion

Several highly potent selective DPP-4 inhibitors have been discovered based on structure activity relationship study and hybrid compound design, with the marketed drugs linagliptin and alogliptin as lead compounds. Among them, **2h** (IC_{50} = 0.31 nM) and **2i** (IC_{50} = 0.35 nM) showed the best DPP-4 inhibitory activity, with more potent activity than both linagliptin (IC_{50} = 0.77 nM) and alogliptin (IC_{50} = 2.63 nM). Preliminary efficacy, pharmacokinetics and safety studies identified **2h** as a potential drug candidate for the treatment of type 2 diabetes.

4. Experiments

4.1. Chemistry

4.1.1. General

All commercially available solvents and reagents were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300/500 and 75/125 MHz respectively on an ACF * 300Q Bruker or ACF* 500Q Bruker spectrometer with Me_4Si as the internal reference. Low-resolution and high-resolution mass spectra (LRMS and HRMS) were given with electron impact mode. The mass analyzer type used for the HRMS measurements was TOF. Reactions were monitored by TLC on silica gel 60 F254 plates (Qingdao

Ocean Chemical Company, China). Column chromatography was carried out on silica gel (200−300 mesh, Qingdao Ocean Chemical Company, China). Optical rotation datas were recorded on Jasco p-1020 Polarimeter. The optical purity of the key compounds were determined by analytical HPLC (Equipment: Agilent 1100 system with a VWD G1314A UV detector; Column: Chiralpak IC 4.6 mm × 250 mm).

*4.1.2. 8-Bromo-3-methyl-7-(prop-2-ynyl)-1H-purine-2,6(3H,7H)-dione (**3b**)*

To a mixture of 8-bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (244 mg, 1 mmol) and DIPEA (277 μl, 1.3 mmol) in DMF (4 mL) was added 3-bromo-1-butyne (93 μl, 1.2 mmol). The reaction mixture was stirred overnight at r. t. The precipitate was collected by filtration, washed with water and EtOH, and dried to give **3b** as a white solid (212 mg, 75%). mp: 275-276 ^{o}C. IR (KBr, cm^{-1}): 3447, 3263, 3154, 3017, 2833, 2129, 1685, 1598, 1531, 1436, 1366, 1288, 1204, 878, 748, 566. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.33 (s, 1H), 5.10 (d, J = 2.1 Hz, 2H), 3.52 (s, 1H), 3.32 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 153.8, 150.4, 149.2, 127.8, 108.1, 76.8, 76.6, 36.1, 28.5. HRMS (ESI) calcd for $C_9H_8N_4O_2Br$ $[M+H]^+$ 282.9831, found 282.9834.

*4.1.3. 8-Bromo-7-(but-2-ynyl)-3-methyl-1H-purine-2,6(3H,7H)-dione (**3a**)*

Following a similar procedure for the preparation of **3b**, **3a** was prepared starting from 8-bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione and 1-bromo-2-butyne. White solids (5 g, 85%). mp: 256-260 ^{o}C. IR (KBr, cm^{-1}): 3480, 3160, 3028, 2836, 1723, 1685, 1531, 1362, 1202, 863, 746, 553. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.32 (s, 1H), 5.06 (s, 2H), 3.32 (s, 3H), 1.80 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 153.8, 150.5, 149.2, 127.7, 108.1, 81.8, 72.3, 36.5, 28.5, 3.0. HRMS (ESI) calcd for $C_{10}H_{10}N_4O_2Br$ $[M+H]^+$ 296.9987, found 296.9989.

*4.1.4. 7-Allyl-8-bromo-3-methyl-1H-purine-2,6(3H,7H)-dione (**3c**)*

Following a similar procedure for the preparation of **3b**, **3c** was prepared starting from 8-bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione and 3-bromo-1-butene. White solids (420 mg, 86%). mp: 238~240 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.04 (br s, 1H), 5.95 (ddt, J = 22.3, 10.7, 5.6 Hz, 1H), 5.29 (d, J = 10.2 Hz, 1H), 5.22 (d, J = 17.1 Hz, 1H), 4.94 (d, J = 5.3 Hz, 2H), 3.52 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.6, 152.4, 150.7, 130.6, 127.7, 119.4, 114.4, 49.2, 29.1. MS (ESI) m/z 285.0 $[M+1]^+$.

*4.1.5. 8-Bromo-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-7-(prop-2-ynyl)-1H-purine-2,6(3H,7H)-dione (**4b**)*

To a suspension of **3b** (849 mg, 3 mmol) and Na_2CO_3 (382 mg, 3.6 mmol) in DMF (36 mL) was added 2-chloromethyl-4-methylquinazoline (636 mg, 3.3 mmol). The reaction mixture was stirred at 80 ^{o}C for 12 h. After cooling to r. t., the reaction mixture was diluted with DCM, washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by flash chromatography (petroleum ether/ethyl acetate, 1:1) to give **4b** as a white solid (1.05 g, 80%). mp: 228-232 ^{o}C. IR (KBr, cm^{-1}): 3455, 3264, 3196, 1709, 1670, 1614, 1567, 1534, 1437, 1399, 1362, 1205, 1189, 949, 769. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02 (d, J = 8.3 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.77 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 5.56 (s, 2H), 5.19 (d, J = 2.1 Hz, 2H), 3.60 (s, 3H), 2.89 (s, 3H), 2.42 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.7, 160.4, 154.2, 151.3, 149.9, 148.6, 133.3, 128.8, 127.6, 126.8, 124.8, 123.2, 108.7, 75.6, 74.3, 46.4, 36.5, 29.9, 21.7. HRMS (ESI) calcd for $C_{19}H_{16}N_6O_2Br$ $[M+H]^+$ 439.0518, found 439.0522.

*4.1.6. 8-Bromo-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**4a**)*

Following a similar procedure for the preparation of **4b**, **4a** was prepared starting from **3a** and 2-chloromethyl-4-methylquinazoline. White solids (1.1 g, 85%). mp: 229-231 ^{o}C. IR (KBr, cm^{-1}): 3447, 3162, 1704, 1672, 1566, 1540, 1400, 1367, 763. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.78 (t, J = 7.2 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 5.58 (s, 2H), 5.14 (d, J = 2.3 Hz, 2H), 3.60 (s, 3H), 2.90 (s, 3H), 1.80 (t, J = 2.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.6, 160.5, 154.2, 151.4, 149.9, 148.5, 133.3, 128.9, 127.5, 126.8, 124.8, 123.1, 108.6, 82.3, 71.3, 46.4, 37.1, 29.8, 21.7, 3.5. HRMS (ESI) calcd for $C_{20}H_{18}N_6O_2Br$ $[M+H]^+$ 453.0675, found 453.0678.

*4.1.7. 7-Allyl-8-bromo-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**4c**)*

Following a similar procedure for the preparation of **4b**, **4c** was prepared starting from **3c** and 2-chloromethyl-4-methylquinazoline. White solids (450 mg, 72%). mp: 210-214 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (d, J = 8.3 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.77 (t, J = 7.6 Hz, 1H), 7.54 (t, J = 7.2 Hz, 1H), 5.96 (ddt, J = 17.2, 10.7, 5.5 Hz, 1H), 5.56 (s, 2H), 5.27 (d, J = 10.3 Hz, 1H), 5.20 (d, J = 17.2 Hz, 1H), 4.98 (d, J = 5.5 Hz, 2H), 3.63 (d, J = 16.5 Hz, 3H), 2.88 (s, 3H). MS (ESI) *m/z* 441.1 $[M+1]^+$.

*4.1.8. (R)-8-(3-Aminopiperidin-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-7-(prop-2-ynyl)-1H-purine-2,6(3H,7H)-dione (**1i**)*

A mixture of **4b** (88 mg, 0.2 mmol), (*R*)-3-(*N*-Boc-amino)piperidine (44 mg, 0.22 mmol) and K_2CO_3 (55 mg, 0.4 mmol) in DMF (6 mL) was stirred at 75 °C for 6 h. After cooling to r. t., the mixture was poured into water (12 mL) and extracted with DCM (3×10 mL). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate, 1:1) to give the Boc precusor of **1i** as a colorless syrup (80 mg, 72%), which was dissolved in DCM (2 mL), and TFA (390 μL) was added. The solution was stirred at room temperature for 3 h and then poured into ice-cold water (4 mL). The organic phase was separated, and the aqueous phase was basified with K_2CO_3 and extracted with DCM (2×10 mL). The organic layers were combined and washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (DCM/MeOH/TEA, 100:0.5:0.5) to give pure **1i** as a white solid (51 mg, 85%). mp: 164-167 ^{o}C. $[\alpha]_D^{20}$ +17.641 (*c* 0.034, MeOH). IR (KBr, cm^{-1}): 3441, 3135, 1701, 1655, 1570, 1517, 1400, 1283, 762. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, J = 8.3 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.72 (t, J = 7.6 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 5.53 (s, 2H), 4.91 (d, J = 2.1 Hz, 2H), 3.76 – 3.42 (m, 5H), 3.08 (m, 2H), 2.94 – 2.87 (m, 1H), 2.84 (s, 3H), 2.37 (t, J = 2.2 Hz, 1H), 2.26 (s, 2H), 2.06 – 1.93 (m, 1H), 1.91-1.79 (m, 1H), 1.73 (ddd, J = 20.0, 11.9, 6.9 Hz, 1H), 1.43-1.26 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 161.0, 156.3, 154.4, 151.7, 149.8, 148.1, 133.1, 128.8, 126.6, 124.7, 123.0, 76.6, 73.4, 58.2, 50.4, 47.2, 46.2, 45.8, 35.2, 33.4, 29.6, 23.2, 21.6, 9.9. HRMS (ESI) calcd for $C_{24}H_{27}N_8O_2$ $[M+H]^+$ 459.2257, found 459.2261.

*4.1.9. (S)-8-(3-Aminopiperidin-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-7-(prop-2-ynyl)-1H-purine-2,6(3H,7H)-dione (**1h**)*

Following a similar procedure for the preparation of **1i**, **1h** was prepared starting from **4b** and (*S*)-3-(*N*-Boc-amino)piperidine. White solids (42 mg, 84%). $[\alpha]_D^{20}$ -18.182 (*c* 0.011, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, J = 8.3 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.78-7.69 (m, 1H), 7.50 (t, J = 7.6 Hz, 1H), 5.52 (d, J = 11.6 Hz, 2H), 4.94 (d, J = 1.9 Hz, 2H), 3.75-3.61 (m, 1H), 3.62-3.45 (m, 4H), 3.24-3.06 (m, 2H), 3.02-2.80 (m, 4H), 2.38 (t, J = 2.3 Hz, 1H), 2.24 (br s, 3H), 1.93 (ddd, J = 13.6, 10.9, 6.0 Hz, 2H), 1.74 (ddd, J = 19.7, 11.7, 8.0 Hz, 1H), 1.49 – 1.36 (m, 1H).

*4.1.10. (R)-8-(3-Aminopiperidin-1-yl)-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**1a**)*

Following a similar procedure for the preparation of **1i**, **1a** was prepared starting from **4a** and (*R*)-3-(*N*-Boc-amino)piperidine. Light yellow solids (2.8 g, 80%). mp: 132-135 ^{o}C. $[\alpha]_{D}^{20}$ -15.972 (*c* 0.144, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, *J* = 8.1 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.82-7.69 (m, 1H), 7.52 (m, 1H), 5.71 (d, *J* = 6.1 Hz, 1H), 5.57 (s, 2H), 4.91 (q, 2H), 3.88 (s, 1H), 3.56 (s, 4H), 3.34 (s, 3H), 2.88 (s, 3H), 1.80 (m, 7H), 1.45 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 161.1, 155.8, 155.1, 154.4, 151.8, 149.9, 147.6, 133.1, 128.9, 126.6, 124.7, 123.1, 104.5, 81.3, 72.8, 54.5, 50.8, 46.2, 46.0, 35.5, 29.6, 29.4, 28.3, 22.0, 21.7, 3.6. HRMS (ESI) calcd for $C_{26}H_{29}N_8O_2$ $[M+H]^+$ 485.2413, found 485.2415. [11]

*4.1.11. (R)-8-(7-Amino-5-azaspiro[2.4]heptan-5-yl)-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**1b**)*

Following a similar procedure for the preparation of **1i**, **1b** was prepared starting from **4a** and (*R*)-*N*-Boc-5-azaspiro[2.4]heptan-7-amine. White solids (25 mg, 78%). mp: 130-132 ^{o}C. $[\alpha]_{D}^{20}$ +10.000 (*c* 0.030, MeOH). IR (KBr, cm^{-1}): 3448, 3179, 1693, 1655, 1619, 1571, 1524, 1400, 1237, 1137, 948, 762. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, *J* = 8.1 Hz, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.80-7.71 (m, 1H), 7.52 (m, 1H), 5.56 (s, 2H), 5.07 (d, *J* = 2.2 Hz, 2H), 4.09 (dd, *J* = 10.1, 5.4 Hz, 1H), 4.00 (d, *J* = 9.7 Hz, 1H), 3.68 (dd, *J* = 10.1, 3.4 Hz, 1H), 3.58 (s, 1H), 3.54 (s, 3H), 3.17 (dd, *J* = 5.1, 3.6 Hz, 1H), 2.88 (s, 3H), 1.79 (t, *J* = 2.1 Hz, 3H), 0.88-0.77 (m, 1H), 0.76-0.60 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 161.3, 154.5, 153.9, 151.9, 149.9, 149.1, 133.0, 128.8, 126.4, 124.6, 123.0, 103.4, 81.4, 73.6, 58.0, 55.9, 55.3, 46.0, 35.2, 29.4, 27.4, 21.6, 10.9, 5.3, 3.6. HRMS (ESI) calcd for $C_{26}H_{29}N_8O_2$ $[M+H]^+$ 485.2413, found 485.2415.

*4.1.12. (S)-8-(7-Amino-5-azaspiro[2.4]heptan-5-yl)-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**1c**)*

Following a similar procedure for the preparation of **1i**, **1c** was prepared starting from **4a** and (*S*)-*N*-Boc-5-azaspiro[2.4]heptan-7-amine. White solids (30 mg, 79%). $[\alpha]_{D}^{20}$ -10.638 (*c* 0.094, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.01 (d, *J* = 8.2 Hz, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.76 (t, *J* = 7.6 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 5.56 (s, 2H), 5.07 (s, 2H), 4.11 (dd, *J* = 10.2, 5.3 Hz, 1H), 4.02 (d, *J* = 9.6 Hz, 1H), 3.72 (dd, *J* = 10.2, 2.9 Hz, 1H), 3.61-3.52 (m, 4H), 3.20 (d, *J* = 3.4 Hz, 1H), 2.88 (s, 3H), 1.89 (s, 3H), 1.79 (s, 3H), 0.92-0.84 (m, 1H), 0.76-0.62 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.3, 161.4, 154.4, 154.1, 151.8, 150.0, 149.0, 133.0, 128.9, 126.5, 124.7, 123.1, 103.3, 81.5, 73.7, 57.8, 56.1, 55.4, 46.1, 35.3, 29.5, 27.3, 21.7, 11.5, 5.5, 3.6.

*4.1.13. 8-(7-Amino-5-azaspiro[2.4]heptan-5-yl)-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**1d**)*

Following a similar procedure for the preparation of **1i**, **1d** was prepared starting from **4a** and *N*-Boc-5-azaspiro[2.4]heptan-7-amine. White solids (32 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, *J* = 8.1 Hz, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.80-7.71 (m, 1H), 7.52 (dd, *J* = 11.1, 4.0 Hz, 1H), 5.56 (s, 2H), 5.07 (d, *J* = 2.2 Hz, 2H), 4.09 (dd, *J* = 10.1, 5.4 Hz, 1H), 4.00 (d, *J* = 9.7 Hz, 1H), 3.68 (dd, *J* = 10.1, 3.4 Hz, 1H), 3.58 (s, 1H), 3.54 (s, 3H), 3.17 (dd, *J* = 5.1, 3.6 Hz, 1H), 2.88 (s, 3H), 1.79 (t, *J* = 2.1 Hz, 3H), 0.88-0.77 (m, 1H), 0.76-0.60 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3, 161.3, 154.5, 153.9, 151.9, 149.9, 133.0, 128.8, 126.4, 124.6, 123.0, 103.4, 81.4, 73.6, 57.9, 55.9, 55.3, 46.0, 35.2, 29.5, 27.4, 21.6, 10.9, 5.3, 3.6.

*4.1.14. (S)-8-[2-(Aminomethyl)pyrrolidin-1-yl]-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**1e**)*

Following a similar procedure for the preparation of **1i**, **1e** was prepared starting from **4a** and (*S*)-2-(*N*-Boc-aminomethyl)pyrrolidine. White solids (30 mg, 52%). $[\alpha]_D^{20}$ +6.250 (*c* 0.016, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, *J* = 7.7 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.81-7.71 (m, 1H), 7.58-7.47 (m, 1H), 5.56 (d, *J* = 1.1 Hz, 2H), 5.30 (s, 1H), 4.77 (dd, *J* = 18.0, 2.3 Hz, 1H), 4.42 (dd, *J* = 10.5, 5.9 Hz, 1H), 3.84 (dt, *J* = 14.2, 7.1 Hz, 1H), 3.73-3.48 (m, 4H), 3.06-2.81 (m, 5H), 2.22-2.04 (m, 2H), 2.04-1.85 (m, 2H), 1.78 (t, *J* = 2.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 161.3, 155.5, 154.1,151.9, 149.9, 149.1, 133.1, 128.9, 126.6, 124.7, 123.1, 103.4, 81.0, 73.3, 62.7, 51.1, 46.2, 44.3, 35.4, 29.6, 28.1, 25.3, 21.7, 3.5. HRMS (ESI) calcd for $C_{25}H_{29}N_8O_2$ $[M+H]^+$ 473.2413, found 473.2417.

*4.1.15. (R)-8-(3-Aminopyrrolidin-1-yl)-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**1f**)*

A mixture of **4a** (181 mg, 0.4 mmol), (*R*)-3-aminopyrrolidine dihydrochloride (70 mg, 0.44 mmol) and K_2CO_3 (110 mg, 0.8 mmol) in DMF (4 mL) was stirred at 75 ℃ for 6 h. After cooling to r. t., the mixture was poured into water (4 mL) and extracted with DCM (3×10 mL). The organic layers were combinded and washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (DCM/MeOH/TEA, 100:1:0.5) to give pure **1f** as a light yellow solid (128 mg, 70%). mp: 236-240 ^{o}C. $[\alpha]_D^{20}$ -2.410 (*c* 0.083, MeOH). IR (KBr, cm^{-1}): 3441, 3168, 2360, 2341, 1697, 1655, 1621, 1565, 1524, 1400, 1235, 945, 762. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, *J* = 8.2 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 1H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 5.55 (s, 2H), 5.05 (d, *J* = 2.2 Hz, 2H), 4.05-3.84 (m, 2H), 3.84-3.65 (m, 2H), 3.65-3.40 (m, 4H), 2.88 (d, *J* = 10.9 Hz, 3H), 2.19 (dt, *J* = 12.8, 7.1 Hz, 1H), 1.96 – 1.66 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3, 161.3, 154.6, 153.9, 151.9, 149.9, 149.2, 133.0, 128.8, 126.5, 124.7, 123.0, 103.2, 81.3, 73.7, 57.8, 51.1, 47.9, 46.1, 35.2, 34.6, 29.5, 21.7, 3.6. HRMS (ESI) calcd for $C_{24}H_{27}N_8O_2$ $[M+H]^+$ 459.2257, found 459.2260.

*4.1.16. (S)-8-(3-Aminopyrrolidin-1-yl)-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**1g**)*

Following a similar procedure for the preparation of **1f**, **1g** was prepared starting from **4c** and (*S*)-3-aminopiperidine. Light yellow solid (120 mg, 65%). $[\alpha]_D^{20}$ +4.000 (*c* 0.025, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (d, *J* = 8.2 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.73 (t, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.4 Hz, 1H), 5.54 (s, 2H), 5.04 (s, 2H), 3.89 (dd, *J* = 10.0, 5.7 Hz, 2H), 3.81 – 3.58 (m, 2H), 3.59-3.36 (m, 4H), 2.85 (s, 3H), 2.17 (dt, *J* = 12.6, 6.2 Hz, 1H), 1.87-1.62 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3, 161.3, 154.6, 153.9, 151.9, 149.9, 149.2, 133.0, 128.8, 126.5, 124.7, 123.0, 103.2, 81.3, 73.7, 57.8, 51.1, 47.9, 46.1, 35.2, 34.6, 29.5, 21.7, 3.6. MS (ESI) *m*/*z* 481.2 $[M+Na]^+$.

*4.1.17. (R)-7-Allyl-8-(3-aminopiperidin-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6(3H,7H)-dione (**1j**)*

Following a similar procedure for the preparation of **1f**, **1j** was prepared starting from **4c** and (*R*)-3-aminopiperidine. Yellow solids (50 mg, 78%). mp: 176-180 ^{o}C. $[\alpha]_D^{20}$ -17.391 (*c* 0.115, MeOH). ^{1}H NMR (300 MHz, MeOD): δ 7.99 (d, *J* = 8.3 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 6.06 (ddd, *J* = 22.0, 10.2, 5.0 Hz, 1H), 5.54 (s, 2H), 5.31-5.03 (m, 2H), 4.73 (d, *J* = 4.8 Hz, 2H), 3.56 (s, 3H), 3.53-3.48 (m, 1H), 3.42-3.37 (m, 1H), 3.09-2.94 (m, 2H), 2.86 (s, 3H), 2.83-2.76 (m, 1H), 1.99-1.95 (m, 2H), 1.91-1.78 (m, 3H), 1.38-1.24(m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 161.1, 156.5, 149.9, 133.3,

133.1, 128.8, 126.6, 124.8, 123.1, 117.0, 104.8, 58.5, 50.8, 47.7, 47.3, 46.3, 33.4, 29.7, 23.3, 21.7. HRMS (ESI) calcd for $C_{24}H_{29}N_8O_2$ $[M+H]^+$ 461.2408, found 461.2409.

*4.1.18. 1-(But-2-ynyl)-6-chloropyrimidine-2,4(1H,3H)-dione (**5b**)*

To a suspension of 6-chlorouracil (17.58 g, 120 mmol) in DMF (60 mL) were added DIPEA (27.2 mL, 156 mmol) and 1-bromo-2-butyne (12 mL, 132 mmol). The reaction mixture was stirred overnight at r. t. Water was added. The precipitate was collected by filtration, washed with water and EtOH, and dried to give **5b** as a light yellow solid (21 g, 88%). mp: 216-217 ^oC. IR (KBr, cm^{-1}): 3176, 3072, 3046, 1707, 1595, 1440, 1405, 1342, 1293, 1231, 1166, 1133, 1015, 951, 857, 830. ^1H NMR (300 MHz, $CDCl_3$): δ 9.36 (s, 1H), 5.90 (s, 1H), 4.75 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.8, 149.6, 147.2, 103.1, 81.6, 71.9, 36.0, 3.4. HRMS (ESI) calcd for $C_8H_8N_2O_2Cl$ $[M+H]^+$ 199.0274, found 199.0276.

*4.1.19. 6-Chloro-1-(2-cyanobenzyl)pyrimidine-2,4(1H,3H)-dione (**5a**)*

Following a similar procedure for the preparation of **5b**, **5a** was prepared starting from 6-chlorouracil and 2-cyanobenzyl bromide. White solids (1.5 g, 58%). ^1H NMR (500 MHz, DMSO): δ 11.78 (s, 1H), 7.88 (dd, J = 7.7, 0.8 Hz, 1H), 7.74-7.68 (m, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 6.06 (s, 1H), 5.33 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.0, 150.4, 146.3, 139.7, 133.8, 133.3, 128.3, 126.5, 116.8, 109.5, 102.8, 46.8.

*4.1.20. 6-Chloro-1-(prop-2-ynyl)pyrimidine-2,4(1H,3H)-dione (**5c**)*

Following a similar procedure for the preparation of **5b**, **5c** was prepared starting from 6-chlorouracil and 1-bromo-2-propyne. Yellow solids (512 mg, 70%). mp: 184 ^oC. IR (KBr, cm^{-1}): 3297, 3028, 1706, 1592, 1444, 1406, 1381, 1331, 1137, 1019, 953, 830, 681. ^1H NMR (500 MHz, $CDCl_3$): δ 9.15 (brs, 1H), 5.96 (s, 1H), 4.83 (d, J = 2.4 Hz, 2H), 2.38 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.5, 149.4, 146.9, 103.3, 77.2, 73.6, 35.4. HRMS (ESI) calcd for $C_7H_6N_2O_2Cl$ $[M+H]^+$ 185.0118, found 185.0120.

*4.1.21. 1-Allyl-6-chloropyrimidine-2,4(1H,3H)-dione (**5d**)*

Following a similar procedure for the preparation of **5b**, **5d** was prepared starting from 6-chlorouracil and 1-bromo-2-propene. White solids (1.4 g, 75%). mp: 122-125 ^oC. IR (KBr, cm^{-1}): 3011, 2848, 2803, 1669, 1588, 1456, 1413, 1373, 1332, 1158, 1093, 1019, 996, 933, 813, 754, 660. ^1H NMR (300 MHz, $CDCl_3$): δ 10.24 (brs, 1H), 5.99-5.78 (m, 2H), 5.35-5.28 (m, 1H), 5.25 (d, J = 10.6 Hz, 1H), 4.66 (d, J = 5.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.6, 150.1, 147.8, 130.7, 118.8, 102.8, 47.9. HRMS (ESI) calcd for $C_7H_8N_2O_2Cl$ $[M+H]^+$ 187.0274, found 187.0273.

*4.1.22. 1-(But-2-ynyl)-6-chloro-3-[(4-methylquinazolin-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (**6c**)*

To a suspension of **5b** (14.6 g, 73.5 mmol) and LiBr (5.6 g, 58.8 mmol) in DMF (150 mL) was added NaH (60%, 3.82 g, 95.5 mmol) in portions under nitrogen at 0 °C. The mixture was stirred for 0.5 h. 2-Chloromethyl-4-methylquinazoline (15.6 g, 81 mmol) was added. The mixture was stirred overnight at 80 °C. The mixture was evaporated and azeotroped with water in vacuo to remove most of the DMF. The crude product was suspended in the mixture of hot EtOAc (100 mL) and isopropyl ether (200 mL). The suspension was stirred for 30 min and allowed to stand at -20 °C for 1 h. The formed precipitate was collected by filtration, washed with water, EtOH and isopropyl ether, and dried to give **6c** as a yellow brown solid (21 g, 88%). mp: 153 °C. IR (KBr, cm^{-1}): 3434, 3106, 1716, 1661, 1608, 1572, 1435, 1397, 1202, 821, 768; ^1H NMR (300 MHz, $CDCl_3$): δ 8.08 (d, J = 8.3 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 7.85 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H),

7.60 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 6.12 (s, 1H), 5.54 (s, 2H), 4.89 (dd, *J* = 4.5, 2.2 Hz, 2H), 2.95 (s, 3H), 1.89 (t, *J* = 2.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.6, 160.6, 159.6, 150.8, 149.8, 145.4, 133.4, 128.8, 126.9, 124.8, 123.1, 102.6, 81.1, 72.2, 46.7, 36.8, 21.7, 3.5. HRMS (ESI) calcd for $C_{18}H_{16}N_4O_2Cl$ $[M+H]^+$ 355.0962, found 355.0965.

*4.1.23. 6-Chloro-1-(2-cyanobenzyl)-3-methyl-pyrimidine-2,4(1H,3H)-dione (**6a**)*

Following a similar procedure for the preparation of **6c**, **6a** was prepared starting from **5a** and methyl iodide. White solids (386 mg, 70%). IR (KBr, cm^{-1}): 3463, 3096, 2227, 1708, 1654, 1604, 1441, 1398, 1203, 1093, 1030, 981, 942, 838, 766, 521. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, *J* = 7.7 Hz, 1H), 7.47 (dd, *J* = 11.1, 4.4 Hz, 1H), 7.30 (t, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 7.9 Hz, 1H), 5.86 (s, 1H), 5.35 (s, 2H).

*4.1.24. 6-Chloro-1-(2-cyanobenzyl)-3-[(4-methylquinazolin-2-yl)methyl]-pyrimidine-2,4(1H,3H)-dione (**6b**)*

Following a similar procedure for the preparation of **6c**, **6b** was prepared starting from **5a** and 2-chloromethyl-4-methylquinazoline. White solids (500 mg, 50%). mp: 204-206 ^{o}C. IR (KBr, cm^{-1}): 2224, 1713, 1608, 1569, 1436, 1426, 1209, 814,764. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.3 Hz, 1H), 7.92-7.76 (m, 2H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.56 (q, *J* = 8.2 Hz, 2H), 7.39 (t, *J* = 7.9 Hz, 2H), 6.10 (s, 1H), 5.55 (s, 2H), 5.51 (s, 2H), 2.89 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.7, 160.3, 159.4, 151.4, 149.7, 145.4, 139.2, 133.5, 133.3, 133.0, 128.4, 128.2, 127.0, 126.3, 125.0, 123.1, 116.6, 110.9, 103.0, 47.5, 46.5, 21.7. HRMS (ESI) calcd for $C_{22}H_{17}N_5O_2Cl$ $[M+H]^+$ 418.1071, found 418.1074.

*4.1.25 1-(But-2-ynyl)-6-chloro-3-methylpyrimidine-2,4(1H,3H)-dione (**6d**)*

Following a similar procedure for the preparation of **6c**, **6d** was prepared starting from **5b** and methyl iodide. Yellow solids (250 mg, 98%). mp: 133 ^{o}C. IR (KBr,cm^{-1}): 3078, 2304, 2231, 1716, 1653, 1603, 1446, 1427, 1332, 1202, 1172, 980, 948, 860, 751, ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.04 (s, 1H), 4.87 (q, 2H), 3.42 (s, 3H), 1.90 (t, *J* = 2.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.7, 150.6, 145.0, 102.4, 81.3, 77.4, 77.0, 76.6, 72.0, 36.8, 28.4, 3.6. HRMS (ESI) calcd for $C_9H_{10}N_2O_2Cl$ $[M+H]^+$ 213.0431, found 213.0433.

*4.1.26. 1-(But-2-ynyl)-6-chloro-3-(quinoxalin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**6e**)*

Following a similar procedure for the preparation of **6c**, **6e** was prepared starting from **5b** and 2-chloromethylquinoxaline. Red oil (1.4 g, 82%). IR (KBr, cm^{-1}): 3450, 1715, 1668, 1609, 1494, 1431, 1342, 1203, 762. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (s, 1H), 8.06 (dd, *J* = 6.3, 3.5 Hz, 1H), 8.00 (dd, *J* = 6.3, 3.5 Hz, 1H), 7.73-7.66 (m, 2H), 6.04 (s, 1H), 5.47 (s, 2H), 4.79 (d, *J* = 2.4 Hz, 2H), 1.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.2, 150.4, 150.3, 145.6, 143.9, 141.7, 141.7, 129.8, 129.4, 129.1, 129.0, 102.3, 81.3, 71.8, 44.6, 36.8, 3.4. HRMS (ESI) calcd for $C_{17}H_{14}N_4O_2Cl$ $[M+H]^+$ 341.0805, found 341.0808.

*4.1.27. 1-(But-2-ynyl)-6-chloro-3-(naphthalen-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**6f**)*

Following a similar procedure for the preparation of **6c**, **6f** was prepared starting from **5b** and 2-bromomethylnaphthalene. Colorless syrup (1.3 g, 77%). IR (KBr, cm^{-1}): 3451, 2921, 1710, 1665, 1607, 1432, 1331, 1275, 749. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.83-7.78 (m, 1H), 7.75 (dd, *J* = 11.5, 5.3 Hz, 2H), 7.64 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.45-7.36 (m, 2H), 5.90 (s, 1H), 5.21 (d, *J* = 5.1 Hz, 2H), 4.60 (d, *J* = 2.1 Hz, 2H), 1.78 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.8, 149.8, 144.6, 133.4, 132.7, 132.4, 127.8, 127.6, 127.4, 127.0, 126.6, 125.5, 125.5, 101.9, 80.7, 71.9, 44.5, 36.2, 3.0. HRMS (ESI) calcd for $C_{19}H_{16}N_2O_2Cl$ $[M+H]^+$ 339.0900, found 339.0902.

*4.1.28. 1-(But-2-ynyl)-6-chloro-3-(naphthalen-1-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**6g**)*

Following a similar procedure for the preparation of **6c**, **6g** was prepared starting from **5b** and 1-bromomethylnaphthalene. White solids (1.4 g, 83%). mp: > 300 °C. IR (KBr, cm^{-1}): 3461, 1717, 1686, 1608, 1436, 1074, 783, 472. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.29 (d, J = 8.6 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.81 (dd, J = 6.7, 2.5 Hz, 1H), 7.63-7.57 (m, 1H), 7.56-7.51 (m, 1H), 7.46-7.40 (m, 2H), 6.06 (s, 1H), 5.65 (s, 2H), 4.80 (d, J = 2.3 Hz, 2H), 1.86 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.6, 150.6, 145.5, 133.8, 131.3, 131.2, 128.7, 128.2, 126.3, 125.6, 125.4, 125.2, 123.4, 102.6, 81.4, 72.0, 42.6, 36.9, 3.6. HRMS (ESI) calcd for $C_{19}H_{16}N_2O_2Cl$ $[M+H]^+$ 339.0900, found 339.0902.

*4.1.29. 3-Benzyl-1-(but-2-ynyl)-6-chloropyrimidine-2,4(1H,3H)-dione (**6h**)*

Following a similar procedure for the preparation of **6c**, **6h** was prepared starting from **5b** and benzyl chloride. White solids (1 g, 69%). mp: 82-85 ^{o}C. IR (KBr, cm^{-1}): 3103, 2235, 1706, 1661, 1602, 1423, 1326, 1192, 990, 828, 552,449; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47-7.41 (m, 2H), 7.29-7.24 (m, 2H), 7.24-7.19 (m, 1H), 5.92 (s, 1H), 5.05 (s, 2H), 4.74-4.67 (m, 2H), 1.78 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.1, 150.2, 144.9, 136.0, 128.9, 128.1, 128.1, 128.1, 127.5, 102.3, 81.0, 71.9, 44.6, 36.5, 3.3. HRMS (ESI) calcd for $C_{15}H_{14}N_2O_2Cl$ $[M+H]^+$ 289.0744, found 289.0746.

*4.1.30. 1-(But-2-ynyl)-6-chloro-3-(2-cyanobenzyl)pyrimidine-2,4(1H,3H)-dione (**6i**)*

Following a similar procedure for the preparation of **6c**, **6i** was prepared starting from **5b** and 2-cyanobenzyl bromide. White solids (1.1 g, 71%). mp: 162-164 °C. IR (KBr, cm^{-1}): 3085, 2225, 1712, 1666, 1600, 1429, 1413, 1355, 1320, 846,765. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.65 (d, J = 7.8 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 6.01 (s, 1H), 5.33 (s, 2H), 4.78 (d, J = 2.2 Hz, 2H), 1.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.2, 150.2, 145.9, 139.6, 132.9, 132.8, 127.8, 127.4, 117.1, 112.0, 102.3, 81.5, 71.8, 43.1, 36.9, 3.5. HRMS (ESI) calcd for $C_{16}H_{13}N_3O_2Cl$ $[M+H]^+$ 314.0696, found 314.0697.

*4.1.31. 1-(But-2-ynyl)-6-chloro-3-(3-chlorobenzyl)pyrimidine-2,4(1H,3H)-dione (**6j**)*

Following a similar procedure for the preparation of **6c**, **6j** was prepared starting from **5b** and 3-chlrobenzyl bromide. White solids (1.2 g, 75%). mp: 118 °C; IR (KBr, cm^{-1}): 3099, 1700, 1648, 1608, 1429, 1343, 1201, 1181, 1000, 965, 845, 759; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43 (s, 1H), 7.33 (t, J = 4.3 Hz, 1H), 7.21 (d, J = 4.3 Hz, 2H), 5.95 (s, 1H), 5.03 (s, 2H), 4.74 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.0, 150.2, 145.2, 138.0, 134.0, 129.4, 128.8, 127.8, 127.1, 102.3, 81.3, 71.8, 44.1, 36.7, 3.4. HRMS (ESI) calcd for $C_{15}H_{13}N_2O_2Cl_2$ $[M+H]^+$ 323.0354, found 323.0357.

*4.1.32. 1-(But-2-ynyl)-6-chloro-3-(3-fluorobenzyl)pyrimidine-2,4(1H,3H)-dione (**6k**)*

Following a similar procedure for the preparation of **6c**, **6k** was prepared starting from **5b** and 3-fluorobenzyl chloride. White solids (870 mg, 57%). mp: 98-100 °C. IR (KBr, cm^{-1}): 3117, 1707, 1663, 1605, 1518, 1436, 1346, 1197, 752. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 (d, J = 9.9 Hz, 2H), 7.16 (d, J = 9.8 Hz, 1H), 6.95 (t, J = 7.0 Hz, 1H), 5.96 (s, 1H), 5.07 (s, 2H), 4.75 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.3, 160.2, 150.4, 145.4, 138.4, 129.9, 129.8, 124.7, 124.6, 116.0, 115.7, 114.8, 114.5, 102.5, 81.4, 71.9, 44.9, 44.4, 36.8, 29.6, 3.5. HRMS (ESI) calcd for $C_{15}H_{13}N_2O_2ClF$ $[M+H]^+$ 307.0641, found 307.0644.

*4.1.33. 1-(But-2-ynyl)-6-chloro-3-(4-nitrobenzyl)pyrimidine-2,4(1H,3H)-dione (**6l**)*

Following a similar procedure for the preparation of **6c**, **6l** was prepared starting from **5b** and 4-nitrobenzyl bromide. White solids (1.0 g, 60%). mp: 156-160 °C. IR (KBr, cm^{-1}): 3117, 2299, 2228, 1707, 1663, 1605, 1522, 1435, 1345, 1316, 1197, 994, 827, 752, 595. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31-8.10 (m, 2H), 7.62 (d, J = 8.6 Hz, 2H), 6.00 (s, 1H), 5.17 (s, 2H), 4.94 – 4.72 (m, 2H), 1.82 (d, J = 2.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.2, 150.3, 145.8, 143.2, 129.8, 123.6, 102.4, 81.6, 71.7, 44.2, 37.0, 3.5. HRMS (ESI) calcd for $C_{15}H_{13}N_3O_4Cl$ $[M+H]^+$ 334.0597, found 334.0595.

*4.1.34. 6-Chloro-3-[(4-methylquinazolin-2-yl)methyl]-1-(prop-2-ynyl)pyrimidine-2,4(1H,3H)-dione (**6m**)*

Following a similar procedure for the preparation of **6c**, **6m** was prepared satrting from **5c** and 2-chloromethyl-4-methylquinazoline. White solids (300 mg, 22%). mp: 230 °C. IR (KBr, cm^{-1}): 3280, 1720, 1709, 1660, 1611, 1573, 1502, 1439, 1393, 815, 767; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, J = 8.3 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.80 (t, J = 7.6 Hz, 1H), 7.55 (t, J = 7.1 Hz, 1H), 6.08 (s, 1H), 5.47 (s, 2H), 4.89 (d, J = 2.4 Hz, 2H), 2.90 (s, 3H), 2.36 (t, J = 2.3 Hz, 1H). HRMS (ESI) calcd for $C_{17}H_{14}N_4O_2Cl$ $[M+H]^+$ 341.0805, found 341.0807.

4.1.35. 1-Allyl-6-chloro-3-[(4-methylquinazolin-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (***6n***)

Following a similar procedure for the preparation of **6c**, **6n** was prepared starting from **5d** and 2-chloromethyl-4-methylquinazoline. Light yellow solids (1.1 g, 65%). mp: 127-130 °C. IR (KBr, cm^{-1}): 3075, 2962, 1714, 1656, 1604, 1569, 1502, 1442, 1341, 1203, 1097, 988, 927, 763. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, J = 8.3 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.77 (m, 1H), 7.58-7.46 (m, 1H), 6.03 (s, 1H), 5.90 (m, 1H), 5.48 (s, 2H), 5.29 (dd, J = 14.9, 10.4 Hz, 2H), 4.84-4.64 (m, 2H), 2.86 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.7, 160.7, 159.7, 151.0, 149.8, 145.9, 133.4, 131.0, 128.7, 126.9, 124.9, 123.1, 117.7, 102.4, 48.4, 46.5, 21.7. HRMS (ESI) calcd for $C_{17}H_{16}N_4O_2Cl$ $[M+H]^+$ 343.0962, found 343.0963.

*4.1.36. 6-Chloro-1,3-di(prop-2-ynyl)pyrimidine-2,4(1H,3H)-dione (**6o**)*

Following a similar procedure for the preparation of **6c**, **6o** was prepared starting from **5c** and 1-bromo-2-propyne. Light yellow solids (680 mg, 80%). mp: 160 °C. IR (KBr, cm^{-1}): 3304, 3256, 3099, 1712, 1672, 1609, 1427, 1332, 1207, 1184, 1138, 1112, 1009, 969, 936, 850, 760. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.01 (s, 1H), 4.86 (d, J = 2.4 Hz, 2H), 4.69 (d, J = 2.4 Hz, 2H), 2.38 (s, 1H), 2.20 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.1, 149.6, 145.3, 102.7, 77.3, 76.3, 73.5, 71.1, 36.2, 30.9. HRMS (ESI) calcd for $C_{10}H_8N_2O_2Cl$ $[M+H]^+$ 223.0274, found 223.0275

*4.1.37. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-[(4-methylquinazolin-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (**2h**)*

A mixture of **6c** (8 g, 22.5 mmol), (*R*)-3-aminopiperidine dihydrochloride (4.67 g, 27 mmol), $NaHCO_3$ (9.45g, 112.5 mmol) and activated 4 Å MS (2.6 g) in isopropanol (150 mL) was stirred at 100 °C for 2 h. The reaction mixture was filtered through Celite and concentrated in vacuo. The residue was diluted with DCM, washed with water and saturated brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (DCM/MeOH/TEA, 100:0.5:0.5) to give pure **2h** as a light yellow solid (8 g, 85%). mp: 78-81 ^{o}C. $[\alpha]_D^{20}$ -1.923 (*c* 0.102, MeOH). IR (KBr, cm^{-1}): 3402, 2924, 1704, 1651, 1440, 1228, 763, 565. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 8.3 Hz, 1H), 7.76 (t, J = 7.5 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 5.47 (s, 2H), 5.32 (s, 1H), 4.58 (d, J = 1.9 Hz, 2H), 3.39 (d, J = 10.9 Hz, 1H), 3.27 (d, J = 11.7 Hz, 1H), 3.13-2.98 (m, 1H), 2.98-2.82 (m, 3H), 2.76 (dd, J = 15.4, 6.2 Hz, 1H), 2.65-2.47 (m, 1H), 2.05-1.86 (m, 5H), 1.80 (s, 3H), 1.76-1.62 (m, 1H), 1.41-1.20 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ

168.3, 162.9, 160.3, 159.4, 152.4, 149.7, 133.1, 128.6, 126.6, 124.7, 122.9, 89.0, 79.7, 73.7, 59.1, 51.3, 47.4, 46.2, 35.3, 32.9, 23.0, 21.5, 3.4. HRMS (ESI) calcd for $C_{23}H_{27}N_6O_2$ $[M+H]^+$ 419.2195, found 419.2199.

*4.1.38. (R)-6-(3-Aminopiperidin-1-yl)-1-(2-cyanobenzyl)-3-methylpyrimidine-2,4(1H,3H)-dione (**2a**)*

Following a similar procedure for the preparation of **2h**, **2a** was prepared starting from **6a** and (*R*)-3-aminopiperidine dihydrochloride. White solid (2.93 g, 80%). $[\alpha]_D^{20}$ +6.000 (*c* 0.100, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (d, *J* = 7.6 Hz, 1H), 7.56 (t, *J* = 7.7 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 7.9 Hz, 1H), 5.38 (s, 1H), 5.35 – 5.25 (m, 2H), 3.31 (s, 3H), 3.05 (d, *J* = 11.2 Hz, 1H), 3.00-2.86 (m, 2H), 2.61 (t, *J* = 10.5 Hz, 1H), 2.41 (t, *J* = 9.6 Hz, 1H), 1.94 (dd, *J* = 9.8, 5.3 Hz, 1H), 1.83-1.72 (m, 1H), 1.60 (m, 4H), 1.35-1.12 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.0, 159.7, 152.6, 140.7, 133.1, 133.0, 127.8, 126.6, 117.0, 110.7, 90.4, 63.3, 59.3, 52.9, 51.8, 47.2, 46.2, 33.2, 29.6, 27.8, 23.1. [12]

*4.1.39. (R)-6-(7-Amino-5-azaspiro[2.4]heptan-5-yl)-1-(2-cyanobenzyl)-3-methylpyrimidine-2,4(1H,3H)-dione (**2b**)*

Following a similar procedure for the preparation of **2h**, **2b** was prepared starting from **6a** and (*R*)-5-azaspiro[2.4]heptan-7-amine dihydrochloride. Colorless syrup (60 mg, 81%). $[\alpha]_D^{20}$ +40.000 (*c* 0.035, MeOH). IR (KBr, cm^{-1}): 3416, 2925, 2223, 1695, 1643, 1450, 1316, 1157, 1024, 803, 768. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (d, *J* = 7.6 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.24 (d, *J* = 7.9 Hz, 1H), 5.31 (q, *J* = 17.1 Hz, 2H), 5.25 (s, 1H), 3.51 (dd, *J* = 9.6, 5.5 Hz, 1H), 3.40 (d, *J* = 9.3 Hz, 1H), 3.31 (s, 3H), 3.09 (t, *J* = 4.6 Hz, 1H), 2.96 (dd, *J* = 9.6, 3.9 Hz, 1H), 2.91 (d, *J* = 9.3 Hz, 1H), 1.53 (brs, 2H), 0.79-0.71 (m, 1H), 0.67-0.59 (m, 1H), 0.56 (dt, *J* = 10.3, 5.3 Hz, 1H), 0.52-0.43 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 162.8, 156.8, 153.1, 140.6, 133.1, 132.8, 127.7, 126.4, 116.8, 110.2, 84.5, 59.7, 57.1, 55.4, 48.3, 27.7, 27.0, 10.8, 5.4. HRMS (ESI) calcd for $C_{19}H_{22}N_5O_2$ $[M+H]^+$ 352.1774, found 352.1777.

*4.1.40. (R)-6-(3-Aminopyrrolidin-1-yl)-1-(2-cyanobenzyl)-3-methylpyrimidine-2,4(1H,3H)-dione (**2c**)*

Following a similar procedure for the preparation of **2h**, **2c** was prepared satrting from **6a** and (*R*)-3-aminopyrrolidine dihydrochloride. White solids (114 mg, 84%). $[\alpha]_D^{20}$ +10.204 (*c* 0.049, MeOH). IR (KBr, cm^{-1}): 3367, 2223, 1696, 1642, 1446, 1275, 1260, 769, 759, 742. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, *J* = 7.7 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 5.23 (s, 2H), 5.18 (s, 1H), 3.66-3.47 (m, 1H), 3.40-3.17 (m, 5H), 3.07 (m, 1H), 2.78 (dd, *J* = 9.7, 4.6 Hz, 1H), 2.05 (td, *J* = 13.0, 6.6 Hz, 1H), 1.70-1.56 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.8, 156.8, 153.2, 140.7, 133.2, 132.9, 127.8, 126.5, 117.0, 110.2, 84.5, 59.5, 50.7, 49.7, 48.5, 34.2, 27.8. HRMS (ESI) calcd for $C_{17}H_{20}N_5O_2$ $[M+H]^+$ 326.1617, found 326.1620.

*4.1.41. (S)-6-(3-Aminopyrrolidin-1-yl)-1-(2-cyanobenzyl)-3-methylpyrimidine-2,4(1H,3H)-dione (**2d**)*

Following a similar procedure for the preparation of **2h**, **2d** was prepared starting from **6a** and (*S*)-3-aminopyrrolidine dihydrochloride. White solids (91 mg, 82%). $[\alpha]_D^{20}$ -10.000 (*c* 0.097, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.60 (d, *J* = 7.6 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 7.9 Hz, 1H), 5.21 (s, 2H), 5.17 (s, 1H), 3.58 (dd, *J* = 10.6, 5.3 Hz, 1H), 3.49 (q, *J* = 7.2 Hz, 1H), 3.32 – 3.16 (m, 5H), 3.05 (m, 1H), 2.85 (dd, *J* = 9.7, 4.6 Hz, 1H), 2.06-2.01 (dt, *J* = 13.1, 6.5 Hz, 1H), 1.72-1.69 (m, 1H).

*4.1.42. (R)-6-(3-Aminopiperidin-1-yl)-1-(2-cyanobenzyl)-3-[(4-methylquinazolin-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (**2e**)*

Following a similar procedure for the preparation of **2h**, **2e** was prepared starting from **6b** and (*R*)-3-aminopyrrolidine dihydrochloride. White solids (90 mg, 88%). mp: 128-132 ^{o}C. $[\alpha]_D^{20}$-3.571 (*c* 0.112, MeOH). IR (KBr, cm^{-1}): 2944, 1706, 1653, 1569, 1436, 1225, 761. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (d, *J* = 8.2 Hz, 1H), 7.85 (d, *J* = 8.3 Hz, 1H), 7.83-7.76 (m, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.52 (q, *J* = 7.2 Hz, 2H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.32 (s, 1H), 5.49 (s, 1H), 5.46 (s, 2H), 5.30 (d, *J* = 20.5 Hz, 2H), 3.88 (brs, 2H), 3.25 (d, *J* = 9.7 Hz, 1H), 3.05 (d, *J* = 9.6 Hz, 1H), 2.96 (d, *J* = 11.7 Hz, 1H), 2.85 (s, 3H), 2.63 (d, *J* = 10.5 Hz, 2H), 2.02 (d, *J* = 9.0 Hz, 1H), 1.82-1.69 (m, 1H), 1.66-1.53 (m, 1H), 1.35 (m, 1H).^{13}C NMR (126 MHz, $CDCl_3$): δ 168.4, 162.8, 160.0, 159.6, 152.5, 149.5, 140.6, 133.2, 133.0, 132.7, 128.2, 127.5, 126.6, 126.4, 124.8, 122.8, 116.9, 110.2, 90.7, 57.4, 51.6, 47.1, 45.8, 31.6, 29.4, 22.9, 21.5. HRMS (ESI) calcd for $C_{27}H_{28}N_7O_2$ $[M+H]^+$ 482.2304, found 482.2308.

*4.1.43. (R)-6-(7-Amino-5-azaspiro[2.4]heptan-5-yl)-1-(2-cyanobenzyl)-3-[(4-methylquinazolin-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (**2f**)*

Following a similar procedure for the preparation of **2h**, **2f** was prepared starting from **6b** and (*R*)-5-azaspiro[2.4]heptan-7-amine dihydrochloride. Light yellow solids (50 mg, 88%). mp: 65-70 ^{o}C. $[\alpha]_D^{20}$+45.556 (*c* 0.090, MeOH). IR (KBr, cm^{-1}): 3428, 2944, 2873, 2223, 1697, 1650, 1571, 1449, 1437, 1312, 1295, 763. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, *J* = 8.1 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.75 (t, *J* = 7.5 Hz, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 4.6 Hz, 2H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.34-7.24 (m, 1H), 5.44 (s, 2H), 5.40-5.20 (m, 3H), 3.60-3.45 (m, 1H), 3.39 (d, *J* = 9.0 Hz, 1H), 3.04 (s, 1H), 2.93 (dd, *J* = 16.8, 7.2 Hz, 2H), 2.83 (s, 3H), 0.69 (d, *J* = 3.3 Hz, 1H), 0.63-0.47 (m, 2H), 0.44 (d, *J* = 4.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3, 162.5, 160.3, 156.8, 153.2, 149.6, 140.6, 133.2, 133.0, 132.6, 128.4, 127.5, 126.6, 126.3, 124.8, 122.9, 116.9, 109.9, 84.8, 59.7, 57.0, 55.3, 47.7, 45.9, 45.9, 21.6, 10.5, 5.3. HRMS (ESI) calcd for $C_{28}H_{28}N_7O_2$ $[M+H]^+$ 494.2304, found 494.2308.

*4.1.44. (R)-6-(3-Aminopyrrolidin-1-yl)-1-(2-cyanobenzyl)-3-[(4-methylquinazolin-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (**2g**)*

Following a similar procedure for the preparation of **2h**, **2g** was prepared starting from **6b** and (*R*)-3-aminopyrrolidine dihydrochloride. White solids (82 mg, 88%). mp: 113-120 ^{o}C. $[\alpha]_D^{20}$+1.562 (*c* 0.131, MeOH). IR (KBr, cm^{-1}): 3446, 3255, 2925, 1710, 1653, 1436, 1227, 763. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.87-7.78 (m, 1H), 7.63 (d, *J* = 7.6 Hz, 1H), 7.54 (dd, *J* = 10.3, 3.8 Hz, 2H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 5.50 (s, 2H), 5.38 (s, 2H), 5.34 (s, 1H), 3.72-3.60 (m, 1H), 3.35 (ddd, *J* = 15.3, 9.5, 6.4 Hz, 2H), 3.29-3.14 (m, 1H), 2.90 (s, 5H), 2.13 (td, *J* = 12.9, 6.5 Hz, 1H), 1.71 (dt, *J* = 18.8, 6.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.4, 162.6, 160.5, 156.9, 153.3, 149.8, 140.8, 133.2, 133.1, 132.7, 128.5, 127.6, 126.7, 126.6, 124.9, 123.1, 117.0, 110.1, 85.1, 59.6, 50.8, 49.8, 47.8, 46.1, 34.4, 21.7. HRMS (ESI) calcd for $C_{26}H_{26}N_7O_2$ $[M+H]^+$ 468.2148, found 468.2150.

*4.1.45. (S)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-[(4-methylquinazolin-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (**2i**)*

Following a similar procedure for the preparation of **2h**, **2i** was prepared starting from **6c** and (*S*)-3-aminopyrrolidine dihydrochloride. Light yellow oil (144 mg, 83%). $[\alpha]_D^{20}$+1.923 (*c* 0.104, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.76 (t, *J* = 7.6 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 5.46 (s, 2H), 5.32 (s, 1H), 4.58 (s, 2H), 3.29 (d, *J* = 9.3 Hz, 1H), 3.11 (s, 1H), 2.86 (s, 3H), 2.76 (s,

1H), 2.64 (s, 1H), 2.02 (d, *J* = 9.2 Hz, 1H), 1.95-1.84 (m, 1H), 1.79 (s, 3H), 1.72-1.66 (m, 1H), 1.40-1.33 (m, 1H).

*4.1.46. (R)-6-(7-Amino-5-azaspiro[2.4]heptan-5-yl)-1-(but-2-yn-1-yl)-3-((4-methylquinazolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**2j**)*

Following a similar procedure for the preparation of **2h**, **2j** was prepared starting from **6c** and (*R*)-5-azaspiro[2.4]heptan-7-amine dihydrochloride. Colorless syrup (100 mg, 78%). IR (KBr, cm^{-1}): 3390, 2922, 1699, 1652, 1646, 1572, 1444, 1398, 1294, 1201, 784; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.3 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.77 (t, *J* = 7.6 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 1H), 5.59-5.41 (m, 2H), 5.21 (s, 1H), 4.63 (q, 2H), 3.92-3.72 (m, 2H), 3.36 (dd, *J* = 10.1, 3.2 Hz, 1H), 3.22 (m, 2H), 2.89 (s, 3H), 2.66 (brs, 2H), 1.80 (s, 3H), 0.90 (dd, *J* = 8.9, 4.9 Hz, 1H), 0.79-0.60 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 162.8, 160.7, 156.4, 152.9, 149.9, 133.1, 128.8, 126.6, 124.8, 123.1, 83.4, 80.0, 73.9, 59.1, 57.1, 55.8, 46.3, 37.4, 26.9, 21.7, 11.6, 5.6, 3.6. HRMS (ESI) calcd for $C_{24}H_{27}N_6O_2$ $[M+H]^+$ 431.2195, found 419.2199.

*4.1.47. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-methylpyrimidine-2,4(1H,3H)-dione (**2k**)*

Following a similar procedure for the preparation of **2h**, **2k** was prepared starting from **6d** and (*R*)-3-aminopyrrolidine dihydrochloride. Colorless syrup (70 mg, 98%). $[\alpha]_D^{20}$ -4.167 (*c* 0.144, MeOH). IR (KBr, cm^{-1}): 3369, 2937, 1700, 1620, 1608, 1436, 1376, 1220, 1098, 798. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.17 (s, 1H), 4.48 (d, *J* = 1.8 Hz, 2H), 3.24 (s, 4H), 3.15 (d, *J* = 10.2 Hz, 1H), 3.07-2.92 (m, 1H), 2.64 (s, 1H), 2.45 (s, 1H), 1.98-1.87 (m, 1H), 1.86-1.78 (m, 1H), 1.76 (s, 5H), 1.70-1.56 (m, 1H), 1.25 (d, *J* = 10.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.0, 159.1, 152.2, 88.9, 79.9, 73.7, 59.1, 51.4, 47.4, 35.2, 32.9, 29.5, 27.6, 23.0, 3.4. HRMS (ESI) calcd for $C_{14}H_{21}N_4O_2$ $[M+H]^+$ 277.1665, found 277.1667.

*4.1.48. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-(quinoxalin-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**2l**)*

Following a similar procedure for the preparation of **2h**, **2l** was prepared starting from **6e** and (*R*)-3-aminopyrrolidine dihydrochloride. Brown solids (1.3 g, 92%). mp: 68-72 ^{o}C. $[\alpha]_D^{20}$ -0.800 (*c* 0.125, MeOH). IR (KBr, cm^{-1}): 3359, 2942, 2855, 1705, 1654, 1606, 1437, 1371, 1226, 1111, 764. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.82 (s, 1H), 8.13-7.99 (m, 2H), 7.76-7.59 (m, 2H), 5.47 (s, 2H), 5.32 (s, 1H), 4.56 (d, *J* = 2.3 Hz, 2H), 3.39 (d, *J* = 13.7 Hz, 1H), 3.27 (d, *J* = 11.9 Hz, 1H), 3.13-3.02 (m, 1H), 2.77 (s, 1H), 2.59 (s, 1H), 2.26 (brs, 2H), 2.06-1.95 (m, 1H), 1.95-1.84 (m, 1H), 1.79 (dt, *J* = 12.4, 2.3 Hz, 3H), 1.76-1.63 (m, 1H), 1.33 (dt, *J* = 16.9, 7.2 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.7, 159.6, 152.3, 151.5, 144.1, 141.9, 141.7, 129.8, 129.3, 129.3, 129.0, 89.0, 80.3, 73.5, 58.9, 51.5, 47.5, 44.4, 35.7, 32.8, 23.1, 3.6. HRMS (ESI) calcd for $C_{22}H_{25}N_6O_2$ $[M+H]^+$ 405.2039, found 405.2043.

*4.1.49. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-(naphthalen-2-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**2m**)*

Following a similar procedure for the preparation of **2h**, **2m** was prepared starting from **6f** and (*R*)-3-aminopyrrolidine dihydrochloride. White solids (1 g, 87%). mp: 58-62 ^{o}C. $[\alpha]_D^{20}$ +0.840 (*c* 0.119, MeOH). IR (KBr, cm^{-1}): 3359, 2921, 2852, 1700, 1653, 1600, 1436, 1370, 1224, 1093, 922, 781, 564. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 3H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.42 -7.21 (m, 2H), 5.15 (d, *J* = 11.0 Hz, 3H), 4.38 (s, 2H), 3.09 (dd, *J* = 24.1, 9.7 Hz, 2H), 2.80 (s, 1H), 2.50 (t, *J* = 9.5 Hz, 1H), 2.40-2.20 (m, 1H), 2.03-1.58 (m, 3H), 1.48 (d, *J* = 10.2 Hz, 1H), 1.11 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.8, 159.3, 152.2,

134.7, 133.2, 132.7, 127.9, 127.5, 127.1, 125.9, 125.7, 88.9, 80.1, 73.9, 58.9, 51.3, 47.4, 44.3, 35.5, 32.9, 29.6, 23.1, 3.6. HRMS (ESI) calcd for $C_{24}H_{27}N_4O_2$ $[M+H]^+$ 403.2134, found 403.2137.

*4.1.50. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-(naphthalen-1-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**2n**)*

Following a similar procedure for the preparation of **2h**, **2n** was prepared starting from **6g** and (*R*)-3-aminopyrrolidine dihydrochloride. Colorless syrup (300 mg, 75%). $[\alpha]_D^{20}$ -0.962 (*c* 0.119, MeOH). IR (KBr, cm^{-1}): 3428, 2935, 1704, 1653, 1600, 1437, 1375, 1226, 766, 566. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.74 – 7.65 (m, 1H), 7.48 (ddd, *J* = 22.1, 11.3, 4.3 Hz, 2H), 7.39-7.26 (m, 2H), 5.58 (s, 2H), 5.23 (s, 1H), 4.47 (d, *J* = 2.2 Hz, 2H), 3.16 (dd, *J* = 24.2, 11.6 Hz, 2H), 2.99 – 2.78 (m, 1H), 2.59 (t, *J* = 10.2 Hz, 1H), 2.49-2.28 (m, 1H), 1.91-1.79 (m, 1H), 1.79-1.70 (m, 4H), 1.64 (d, *J* = 10.8 Hz, 3H), 1.62-1.46 (m, 1H), 1.21-1.08 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.0, 159.5, 152.4, 133.7, 132.2, 131.3, 128.6, 127.8, 126.1, 125.6, 125.2, 124.9, 123.6, 89.0, 80.1, 73.8, 59.1, 51.4, 47.4, 41.9, 35.6, 33.0, 23.2, 3.6. HRMS (ESI) calcd for $C_{24}H_{27}N_4O_2$ $[M+H]^+$ 403.2134, found 403.2137.

*4.1.51. (R)-6-(3-Aminopiperidin-1-yl)-3-benzyl-1-(but-2-ynyl)pyrimidine-2,4(1H,3H)-dione (**2o**)*

Following a similar procedure for the preparation of **2h**, **2o** was prepared starting from **6h** and (*R*)-3-aminopyrrolidine dihydrochloride. Colorless syrup (81 mg, 38%). $[\alpha]_D^{20}$ -0.806 (*c* 0.124, MeOH). IR (KBr, cm^{-1}): 3442, 2940, 1701, 1653, 1602, 1437, 1275, 1225, 763, 749. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54-7.45 (m, 2H), 7.31-7.25 (m, 2H), 7.25-7.20 (m, 1H), 5.23 (s, 1H), 5.08 (s, 2H), 4.51 (d, *J* = 2.0 Hz, 2H), 3.30 (d, *J* = 10.6 Hz, 1H), 3.19 (d, *J* = 11.2 Hz, 1H), 3.00 (td, *J* = 8.8, 4.3 Hz, 1H), 2.69 (t, *J* = 9.9 Hz, 1H), 2.48 (s, 1H), 2.02-1.91 (m, 1H), 1.89-1.82 (m, 1H), 1.81 (t, *J* = 2.3 Hz, 3H), 1.71-1.63 (m, 3H), 1.32-1.24 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.7, 159.2, 152.1, 137.0, 129.0, 128.1, 127.2, 89.0, 80.0, 73.6, 59.1, 51.3, 47.3, 44.1, 35.3, 33.0, 23.0, 3.4. HRMS (ESI) calcd for $C_{20}H_{25}N_4O_2$ $[M+H]^+$ 353.1978, found 353.1980.

*4.1.52. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-(2-cyanobenzyl)pyrimidine-2,4(1H,3H)-dione (**2p**)*

Following a similar procedure for the preparation of **2h**, **2p** was prepared starting from **6i** and (*R*)-3-aminopyrrolidine dihydrochloride. Light yellow solid (400 mg, 90%). $[\alpha]_D^{20}$ +1.316 (*c* 0.154, MeOH). IR (KBr, cm^{-1}): 3418, 2946, 2224, 1703, 1653, 1601, 1437, 1227, 763. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (d, *J* = 6.1 Hz, 1H), 7.28 (s, 1H), 7.10 (s, 1H), 7.03 (d, *J* = 6.5 Hz, 1H), 5.07 (s, 2H), 5.04 (s, 1H), 4.33 (s, 2H), 3.14 (d, *J* = 8.4 Hz, 1H), 3.04 (s, 1H), 2.80 (s, 1H), 2.53 (s, 1H), 2.34 (s, 1H), 1.81 (m, 2H), 1.69-1.38 (m, 5H), 1.14 (d, *J* = 39.1 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.7, 159.1, 151.4, 139.9, 132.2, 132.0, 126.8, 126.3, 116.6, 110.7, 87.7, 79.5, 73.0, 58.3, 52.1, 46.7, 41.7, 35.1, 32.3, 22.4, 2.8. HRMS (ESI) calcd for $C_{21}H_{24}N_5O_2$ $[M+H]^+$ 378.1930, found 378.1933.

*4.1.53. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-(3-chlorobenzyl)pyrimidine-2,4(1H,3H)-dione (**2q**)*

Following a similar procedure for the preparation of **2h**, **2q** was prepared starting from **6j** and (*R*)-3-aminopyrrolidine dihydrochloride. Colorless syrup (1 g, 84%). $[\alpha]_D^{20}$ -1.531 (*c* 0.196, MeOH). IR (KBr, cm^{-1}): 3442, 2941, 1701, 1652, 1600, 1433, 1225, 765. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45 (s, 1H), 7.38-7.33 (m, 1H), 7.25-7.17 (m, 2H), 5.23 (s, 1H), 5.04 (s, 2H), 4.52 (d, *J* = 2.1 Hz, 2H), 3.31 (d, *J* = 10.3 Hz, 1H), 3.21 (d, *J* = 11.1 Hz, 1H), 3.08-2.94 (m, 1H), 2.70 (t, *J* = 7.2 Hz, 1H), 2.51 (d, *J* = 8.9 Hz, 1H), 2.05-1.92 (m, 1H), 1.92-1.78 (m, 4H), 1.77-1.58 (m, 1H), 1.29 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.2, 159.1, 151.7,

138.7, 133.5, 129.1, 128.5, 127.1, 126.8, 88.5, 79.8, 73.3, 58.8, 51.0, 47.1, 43.3, 35.2, 32.7, 22.8, 3.2. HRMS (ESI) calcd for $C_{20}H_{24}N_4O_2Cl$ $[M+H]^+$ 387.1588, found 387.1590.

*4.1.54. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-(3-fluorobenzyl)pyrimidine-2,4(1H,3H)-dione (**2r**)*

Following a similar procedure for the preparation of **2h**, **2r** was prepared starting from **6k** and (*R*)-3-aminopyrrolidine dihydrochloride. Colorless syrup (810 mg, 77%). $[\alpha]_D^{20}$ +0.508 (*c* 0.197, MeOH). IR (KBr, cm^{-1}): 3449, 2923, 2854, 1700, 1653, 1600, 1437, 1226, 765. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.00 (s, 2H), 6.93 (d, *J* = 9.8 Hz, 1H), 6.67 (d, *J* = 6.0 Hz, 1H), 5.02 (s, 1H), 4.83 (s, 2H), 4.30 (s, 2H), 3.10 (d, *J* = 10.8 Hz, 1H), 3.00 (d, *J* = 11.5 Hz, 1H), 2.77 (s, 1H), 2.48 (t, *J* = 10.4 Hz, 1H), 2.39-2.20 (m, 1H), 1.75 (s, 3H), 1.62 (d, *J* = 21.3 Hz, 4H), 1.55-1.34 (m, 1H), 1.06 (d, *J* = 8.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.5, 162.0, 160.3, 158.9, 151.5, 139.0, 138.9, 129.1, 129.0, 123.8, 123.8, 115.0, 114.8, 113.7, 113.4, 88.1, 79.5, 73.2, 58.4, 50.7, 46.8, 43.0, 35.0, 32.4, 22.6, 2.9. HRMS (ESI) calcd for $C_{20}H_{24}N_4O_2F$ $[M+H]^+$ 371.1883, found 371.1886.

*4.1.55. (R)-6-(3-Aminopiperidin-1-yl)-1-(but-2-ynyl)-3-(4-nitrobenzyl)pyrimidine-2,4(1H,3H)-dione (**2s**)*

Following a similar procedure for the preparation of **2h**, **2s** was prepared starting from **6l** and (*R*)-3-aminopyrrolidine dihydrochloride. Yellow oil (800 mg, 85%). $[\alpha]_D^{20}$ -0.769 (*c* 0.130, MeOH). IR (KBr, cm^{-1}): 3455, 2940, 2855, 1696, 1649, 1604, 1521, 1434, 1343, 1226, 1108, 763. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (dd, *J* = 8.6, 3.2 Hz, 2H), 7.49-7.27 (m, 2H), 5.00 (d, *J* = 3.0 Hz, 1H), 4.90 (s, 2H), 4.29 (s, 2H), 3.11 (d, *J* = 11.2 Hz, 1H), 3.01 (d, *J* = 11.3 Hz, 1H), 2.77 (d, *J* = 3.0 Hz, 1H), 2.49 (t, *J* = 10.7 Hz, 1H), 2.30 (t, *J* = 9.8 Hz, 1H), 1.67 (dd, *J* = 39.8, 21.5 Hz, 7H), 1.49-1.32 (m, 1H), 1.06 (d, *J* = 10.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.8, 159.0, 151.4, 146.4, 143.9, 128.9, 122.7, 87.9, 79.6, 73.0, 58.4, 50.7, 46.8, 42.9, 35.1, 32.4, 22.5, 2.9. HRMS (ESI) calcd for $C_{20}H_{24}N_5O_4$ $[M+H]^+$ 398.1828, found 398.1832.

*4.1.56. (R)-6-(3-Aminopiperidin-1-yl)-3-[(4-methylquinazolin-2-yl)methyl]-1-(prop-2-ynyl)pyrimidine-2,4(1H,3H)-dione (**2t**)*

Following a similar procedure for the preparation of **2h**, **2t** was prepared starting from **6m** and (*R*)-3-aminopyrrolidine dihydrochloride. Colorless syrup (50 mg, 85%). $[\alpha]_D^{20}$ -0.833 (*c* 0.121, MeOH). IR (KBr, cm^{-1}): 3419, 3244, 2927, 2853, 1700, 1653, 1609, 1569, 1436, 1226, 806, 764. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.72 (t, *J* = 7.6 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 1H), 5.41 (s, 2H), 5.30 (s, 1H), 4.61 (s, 2H), 3.34 (d, *J* = 10.1 Hz, 1H), 3.20 (s, 1H), 3.05 (d, *J* = 8.7 Hz, 1H), 2.82 (s, 3H), 2.71 (dd, *J* = 14.1, 7.0 Hz, 1H), 2.55 (s, 1H), 2.33 (s, 2H), 2.31 (d, *J* = 2.0 Hz, 1H), 1.97 (d, *J* = 9.0 Hz, 1H), 1.91-1.79 (m, 1H), 1.67 (dt, *J* = 14.0, 10.5 Hz, 1H), 1.21 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.4, 162.8, 160.2, 159.2, 152.4, 149.7, 133.2, 128.6, 126.7, 124.7, 123.0, 89.5, 78.5, 72.1, 63.1, 58.9, 52.8, 51.4, 46.2, 34.8, 32.8, 21.6. HRMS (ESI) calcd for $C_{22}H_{25}N_6O_2$ $[M+H]^+$ 405.2039, found 405.2041.

*4.1.57. (R)-1-Allyl-6-(3-aminopiperidin-1-yl)-3-((4-methylquinazolin-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**2u**)*

Following a similar procedure for the preparation of **2h**, **2u** was prepared starting from **6n** and (*R*)-3-aminopyrrolidine dihydrochloride. Light yellow solid (714 mg, 61%). mp: 82-84 ^oC. $[\alpha]_D^{20}$ +1.875 (*c* 0.162, MeOH). IR (KBr, cm^{-1}): 3456, 2995, 1769, 1759, 1654, 1383, 1247, 1055, 749. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.77 (t, *J* = 7.6 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 5.96 (m, 1H), 5.47 (s, 2H), 5.37 (s, 1H), 5.32-5.11 (m, 2H), 4.51 (d, *J* = 2.5 Hz, 2H), 3.24 (d, *J* = 9.7 Hz, 1H), 3.12 (d, *J* = 12.0 Hz, 1H), 3.01 (td, *J* = 9.0, 4.4 Hz, 1H), 2.87 (s, 3H), 2.76-2.61 (m, 1H), 2.47 (t, *J* = 10.0 Hz, 1H), 1.94

(m, 5H), 1.75-1.57 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 163.1, 160.4, 160.1, 152.8, 149.8, 133.2, 132.9, 128.6, 126.6, 124.7, 123.0, 116.2, 89.6, 59.4, 51.6, 47.4, 46.9, 46.1, 33.3, 23.1, 21.6, 7.9. HRMS (ESI) calcd for $C_{22}H_{27}N_6O_2$ $[M+H]^+$ 407.2195, found 407.2197.

*4.1.58. (R)-6-(3-Aminopiperidin-1-yl)-1,3-di(prop-2-yn-1-yl)pyrimidine-2,4(1H,3H)-dione (**2v**)*

Following a similar procedure for the preparation of **2h**, **2v** was prepared starting from **6o** and (*R*)-3-aminopyrrolidine dihydrochloride. Colorless syrup (85 mg, 98%). $[\alpha]_D^{20}$-1.911 (*c* 0.157, MeOH). IR (KBr, cm^{-1}): 3284, 2940, 2855, 1704, 1653, 1605, 1436, 1374, 1342, 1227, 764, 749; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.16 (s, 1H), 4.56 (d, *J* = 2.2 Hz, 2H), 4.53 (dd, *J* = 6.7, 2.1 Hz, 2H), 3.22 (d, *J* = 10.7 Hz, 1H), 3.12 (d, *J* = 11.1 Hz, 1H), 3.01- 2.89 (m, 1H), 2.64 (t, *J* = 9.8 Hz, 1H), 2.45 (s, 1H), 2.32 (s, 1H), 2.10 (t, *J* = 2.2 Hz, 1H), 1.90 (d, *J* = 12.8 Hz, 3H), 1.83-1.75 (m, 1H), 1.66-1.55 (m, 1H), 1.23 (dd, *J* = 19.4, 9.2 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.5, 159.4, 151.4, 89.1, 78.3, 78.3, 72.6, 70.6, 59.1, 51.4, 47.4, 35.0, 33.0, 30.2, 23.1. HRMS (ESI) calcd for $C_{15}H_{19}N_4O_2$ $[M+H]^+$ 287.1508, found 287.1510.

4.2. In vitro assay for inhibition of DPP-4, DPP-8 and DPP-9

The DPP-4 Drug Discovery Kit (Enzo Life Sciences International, Inc.) was used for the assay of inhibition of DPP-4 activity, which was determined by measuring the rate of hydrolysis of a surrogate substrate, H-Gly-Pro-7-amino-4-methylcoumarin (H-Gly-Pro-AMC). The DPP-4 inhibitor P32/98A was selected as the reference inhibitor. The 500 μM substrate solution was diluted with assay buffer (50 mM Tris, pH=7.5) to give a 5 μM substrate solution. 10 μL of appropriately diluted solutions of the test compounds and 25 μl of assay buffer were added to 96-well microtiter plates, followed by addition of 15 μL of assay buffer containing 0.26 MU of recombinant human DPP-4. The reaction was initiated by addition of 50 μL of 5 μM substrate solution. After incubation at room temperature for 10 min, fluorescence was measured using an excitation wavelength of 380 nm and an emission wavelength of 460 nm by a SpectraMax M5 microplate reader. A fluorescence standard curve for 7-amino-4-methylcoumarin (AMC) was generated using 0.12-15 μM of AMC buffer solutions. The inhibitory rate relative to the control without inhibitor was calculated and IC_{50} values were determined by nonlinear regression. By the similar principle, the assays for inhibition of DPP-8 and DPP-9 activity were performed using DPP-8 and DPP-9 fluorescent activity assay kits (Cat: 80208 and 80209, BPS).

4.3. Molecular docking

The crystal structures of linagliptin (**1a**) and alogliptin (**2a**) were extracted from the X-ray crystallographical structures of DPP-4 (PDB codes: 2RGU and 3G0B) and used as the templates to construct the 3D structures of **2h** and **2i** in Sybyl 6.9. After hydrogens were added and Gasteiger-Hückel charges were assigned, the structures of **2h** and **2i** were further optimized with Tripos force field. The protein structure was prepared by removal of all ligands and waters from the X-ray crystallographical structure of DPP-IV (PDB code: 2RGU) and protonated. The optimized structures of **2h** and **2i** were docked into the inhibitor binding pocket of DPP-4 A chain with GOLD 3.0.1. The residues within a radius of 10 Å around the original ligand were selected to define the active site. Default parameters were set. Maximum 10 solutions were adapted for each compound and ranked according to Goldscore. Computational interaction modes of the best solutions and atom distances were pictured with PyMol 1.3.

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Figure captions

Figure 1. Structures of linagliptin (**1a**) and alogliptin (**2a**).

Figure 2. Compounds designed based on **1a** and **2a** with diverse amino groups.

Figure 3. Hybrid design based on **1a** and **2a**.

Figure 4. Binding mode comparison of linaliptin and alogliptin in DPP-4.

Figure 5. Predicted docking interactions of **2h**/**2i** binding to DPP-4.

Figure 6. Effect of **1a** (linagliptin) on glucose tolerance in *db*/*db* mice.

Figure 7. Effect of **2h** on glucose tolerance in *db*/*db* mice.

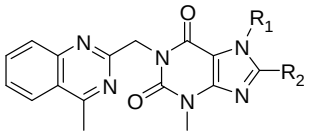
Figure 8. Effect of **1a** (linagliptin) and **2h** on plasma glucose $AUC_{0\rightarrow 120\ min.}$

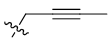
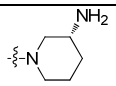
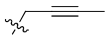
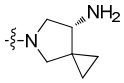
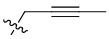
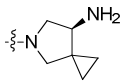
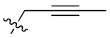
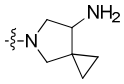
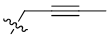
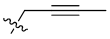
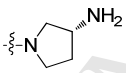
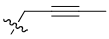
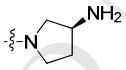
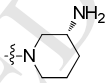
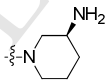
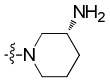
Figure 9. Effect of **1a** (linagliptin) and **2h** on plasma DPP-4 activity.

Scheme 1. Synthesis of compounds **1a**-**1j**.

Scheme 2. Synthesis of compounds **2a**-**2v**.

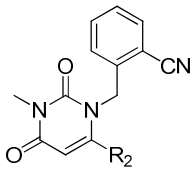
Table 1. DPP-4 inhibitory activity of **1a-1j**.

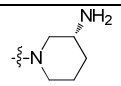
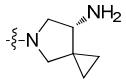
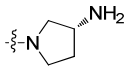
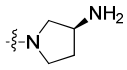


Cpd	R_1	R_2	Inhibition (%)		IC_{50} (nM)
			10 μM	100 nM	
1a			94.8	96.8	0.77
1b			94.7	5.7	NT [a]
1c			66.0	-17.4	NT [a]
1d			94.4	13.1	NT [a]
1e			91.3	39.2	NT [a]
1f			94.5	25.8	NT [a]
1g			91.0	13.6	NT [a]
1h			97.1	92.2	1.33
1i			93.0	27.6	NT [a]
1j			NT	NT	3.77

[a] NT means “not tested”.

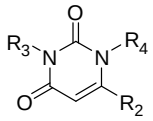
Table 2. DPP-4 inhibitory activity of **2a**-**2d**.

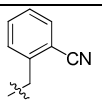
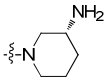
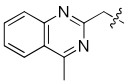
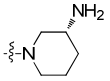
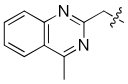
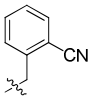
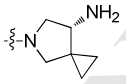
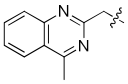
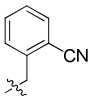
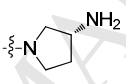
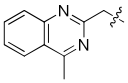
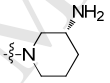
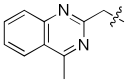
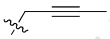
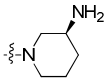
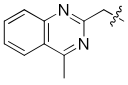
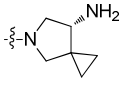
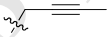
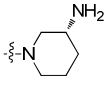


Cpd	R_2	Inhibition (%)		IC_{50} (nM)
		10 μM	100 nM	
2a		102.2	86.5	2.63
2b		93.0	-0.26	NT [a]
2c		97.3	23.9	NT [a]
2d		57.9	-0.96	NT [a]

[a] NT means “not tested”.

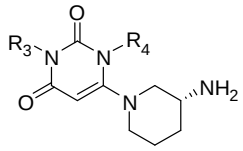
Table 3. DPP-4 inhibitory activity of **2e-2k.**

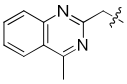
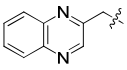
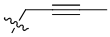
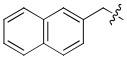
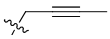
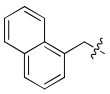
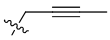
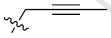
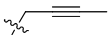
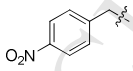
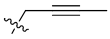
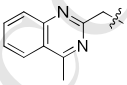
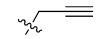
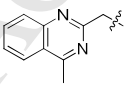
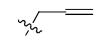
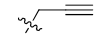


Cpd	R_3	R_4	R_2	Inhibition (%) 100 nM	IC_{50} (nM)
2a	$-CH_3$			86.5	2.63
2e				34.8	NT [a]
2f				-0.3	NT [a]
2g				3.1	NT [a]
2h				95.9	0.31
2i				102.5	0.35
2j				71.8	12.7
2k	$-CH_3$			-2.1	NT [a]

[a] NT means “not tested”.

Table 4. DPP-4 inhibitory activity of compounds **2l-2v**.



Cpd	R_3	R_4	Inhibition (%) 100 nM	IC_{50} (nM)
2h			95.9	0.31
2l			98.6	3.6
2m			21.8	NT [a]
2n			43.2	164
2o			51.3	65.9
2p			87.7	7.6
2q			66.5	66.4
2r			61.1	30.8
2s			89.7	2.5
2t			83.6	11.4.
2u			92.6	3.8
2v			29.7	NT [a]

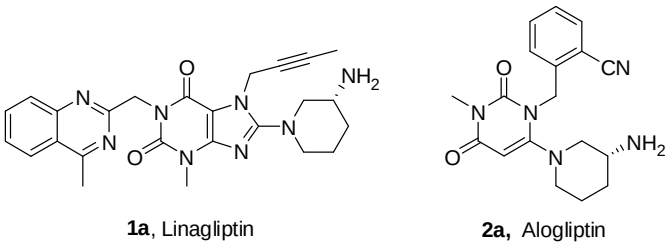
[a] NT means “not tested”.

Table 5. Selectivity for DPP-4 over DPP-8 and DPP-9.

Cpd	DPP-4 IC_{50} (nM)	DPP-8 inhibition (%)		DPP-9 inhibition (%)	
		100 μM	25 μM	100 μM	25 μM
1a (linagliptin)	0.77	19.6	6.1	9.5	4.2
1h	1.33	33.6	14.6	23.1	5.9
2a (alogliptin)	2.63	-1.4	3.1	7.0	8.6
2h	0.31	2.9	4.0	8.7	11.1
2i	0.35	16.2	5.7	0.1	5.8
2l	3.6	2.8	-3.6	9.9	3.1
2p	7.6	-1.0	4.1	5.2	9.5
2s	2.5	9.7	1.1	-2.4	4.9
2t	11.4	27.2	11.8	8.0	8.6
2u	3.8	10.1	1.5	9.0	3.6

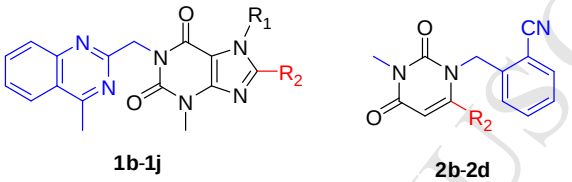
Table 6. Selected pharmacokinetics parameters for **2h** in rats.

Dose (5 mg/kg)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/mL)	$AUC_{0-\infty}$ (μg·h/mL)	$MRT_{0-\infty}$ (h)	*F* (%)
iv	3.8±2.5	0.1±0.0	2.75±0.49	2.37±0.48	1.4±0.4	/
oral	7.5±NA	5.5±4.3	0.04±0.02	0.36±NA	9.7±NA	15.1



1a, Linagliptin **2a,** Alogliptin

Figure 1.



1b-1j **2b-2d**

Figure 2.

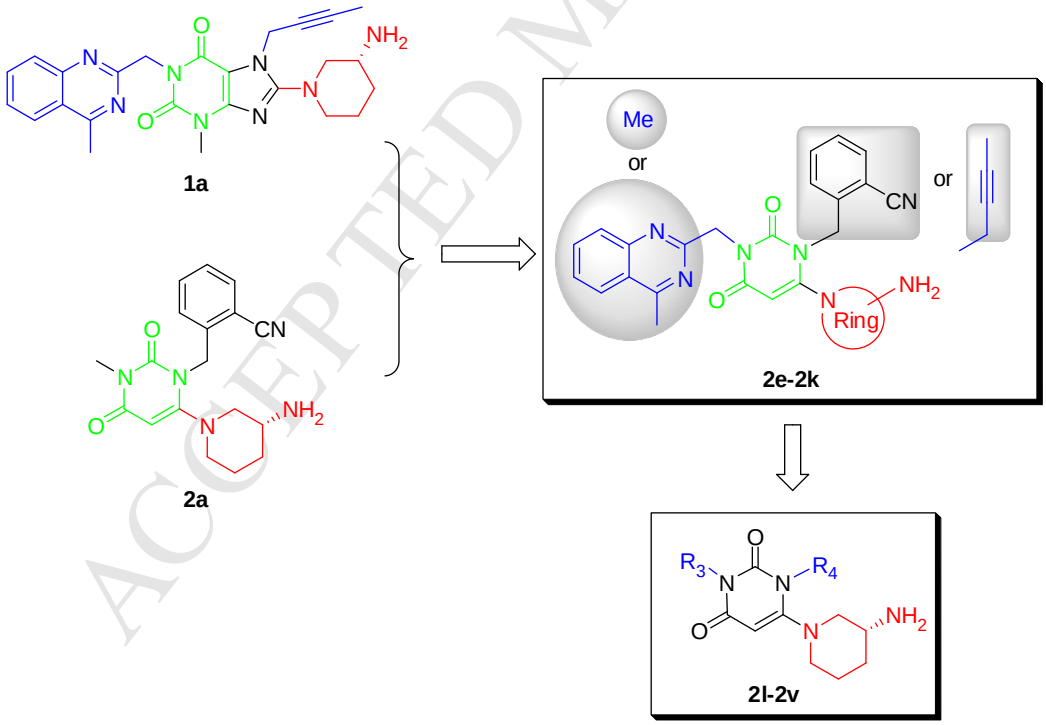
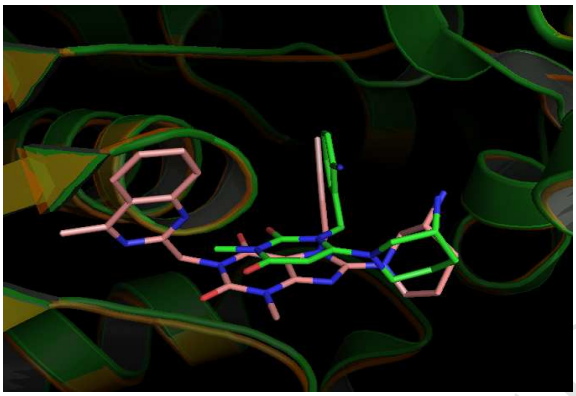
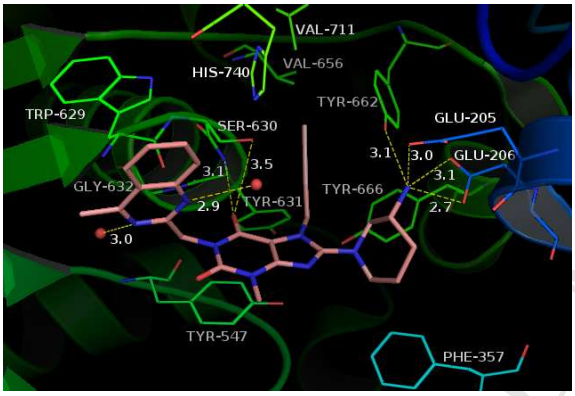
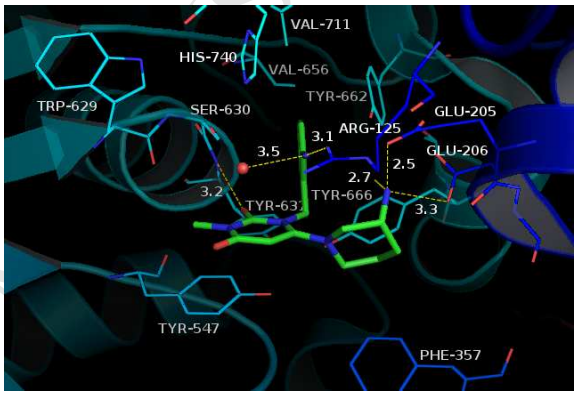


Figure 3.



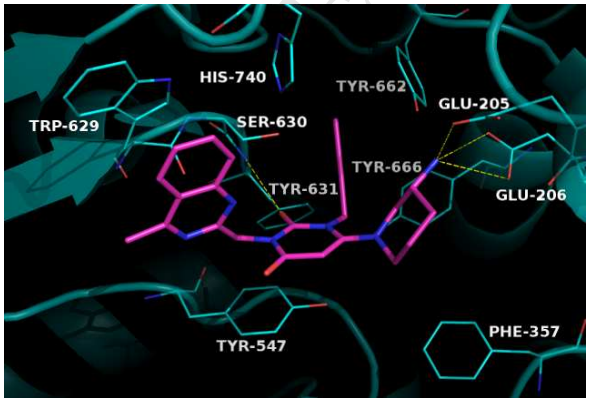
A) Structural alignment of linagliptin (pink) and alogliptin (green).

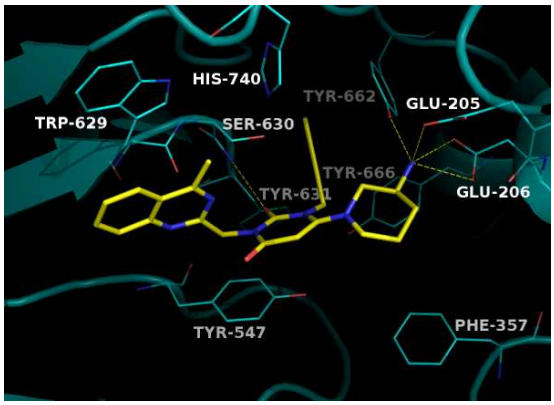




B) Interactions of linagliptin (pink) binding to DPP-4. C) Interactions of alogliptin (green) binding to DPP-4.

Figure 4.





A) **2h** (purple) binding to DPP-4. B) **2i** (green) binding to DPP-4.

Figure 5.

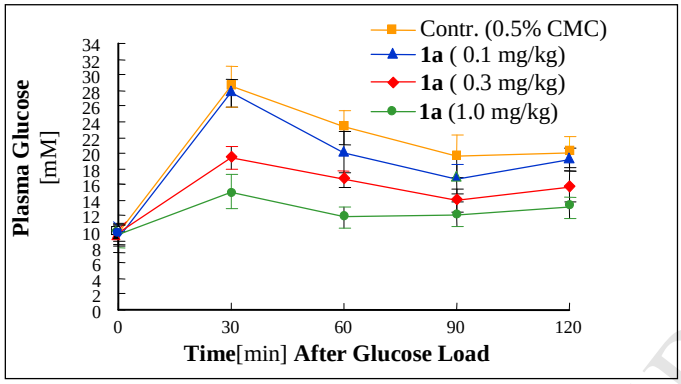


Figure 6.

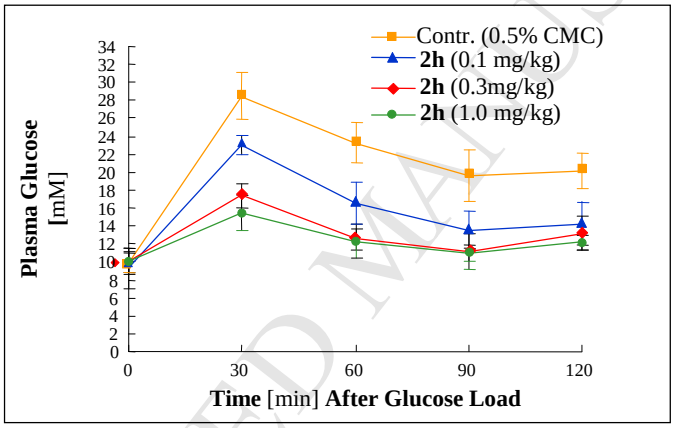


Figure 7.

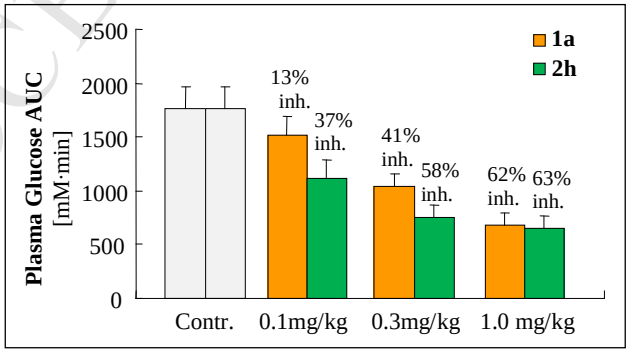


Figure 8.

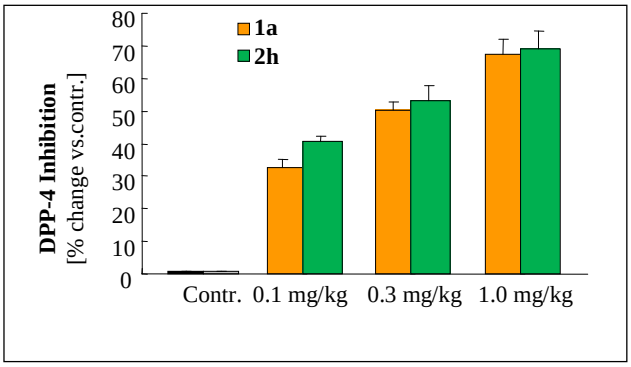
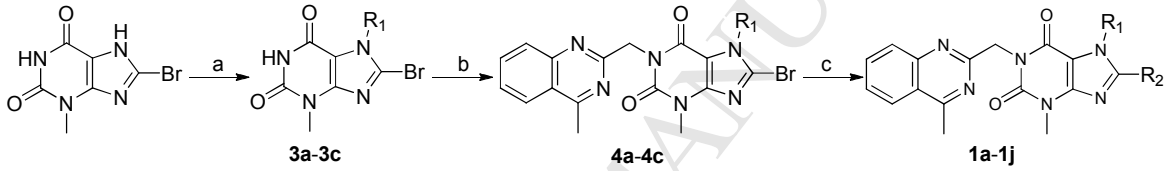


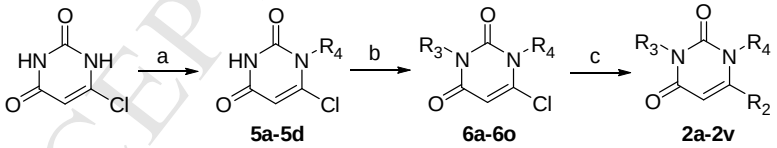
Figure 9.

Scheme 1.



Reagents and conditions: a) R_1X, DIPEA, DMF, r.t.; b) 2-chloromethyl-4-methylquinazoline, Na_2CO_3, DMF, 80 ^oC; c) Method A: *N*-Boc amine, K_2CO_3, DMF, 75 ^oC; then TFA, DCM, r.t.; Method B: amine hydrochloride, K_2CO_3, DMF, 75 ^oC.

Scheme 2.



Reagents and conditions: a) R_4X, DIPEA, DMF, r.t; b) R_3X, NaH, LiBr, DMF, 80 ^oC; c) Amine, $NaHCO_3$, 4 Å MS, *i*-PrOH, reflux.

Highlights

- We identified several highly potent DPP-4 inhibitors by hybrid compound design.
- The most promising compound **2h** was more potent than linagliptin and alogliptin.
- Compound **2h** had a good inhibition selectivity for DPP-4 over DPP-8/9.
- Compound **2h** displayed significant glucose-lowering effect and good safety profile.
- Compound **2h** exhibited a potential as drug candidate for treating type 2 diabetes.

Supporting Information

Discovery of Highly Potent DPP-4 Inhibitors by Hybrid Compound Design Based on Linagliptin and Alogliptin

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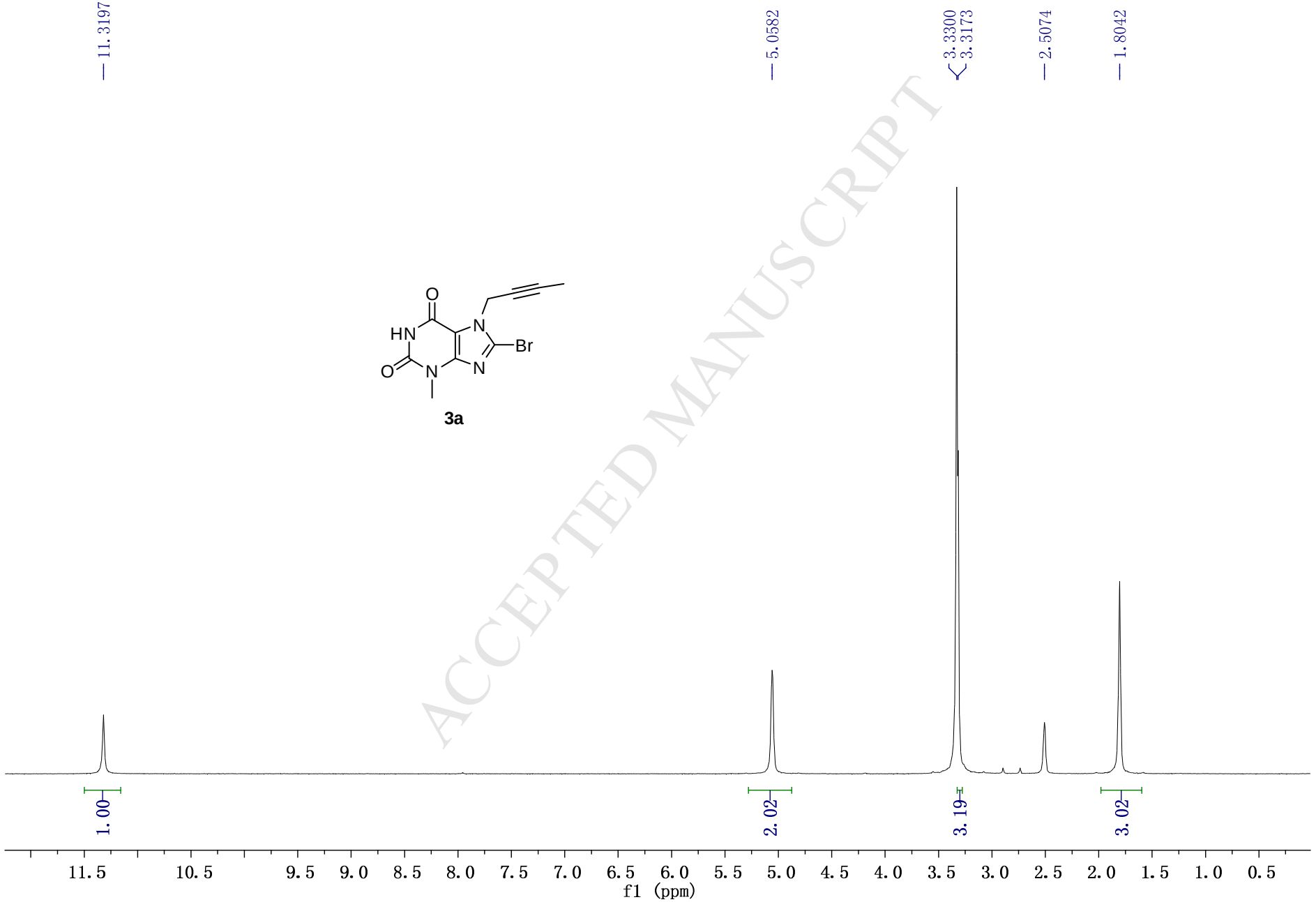
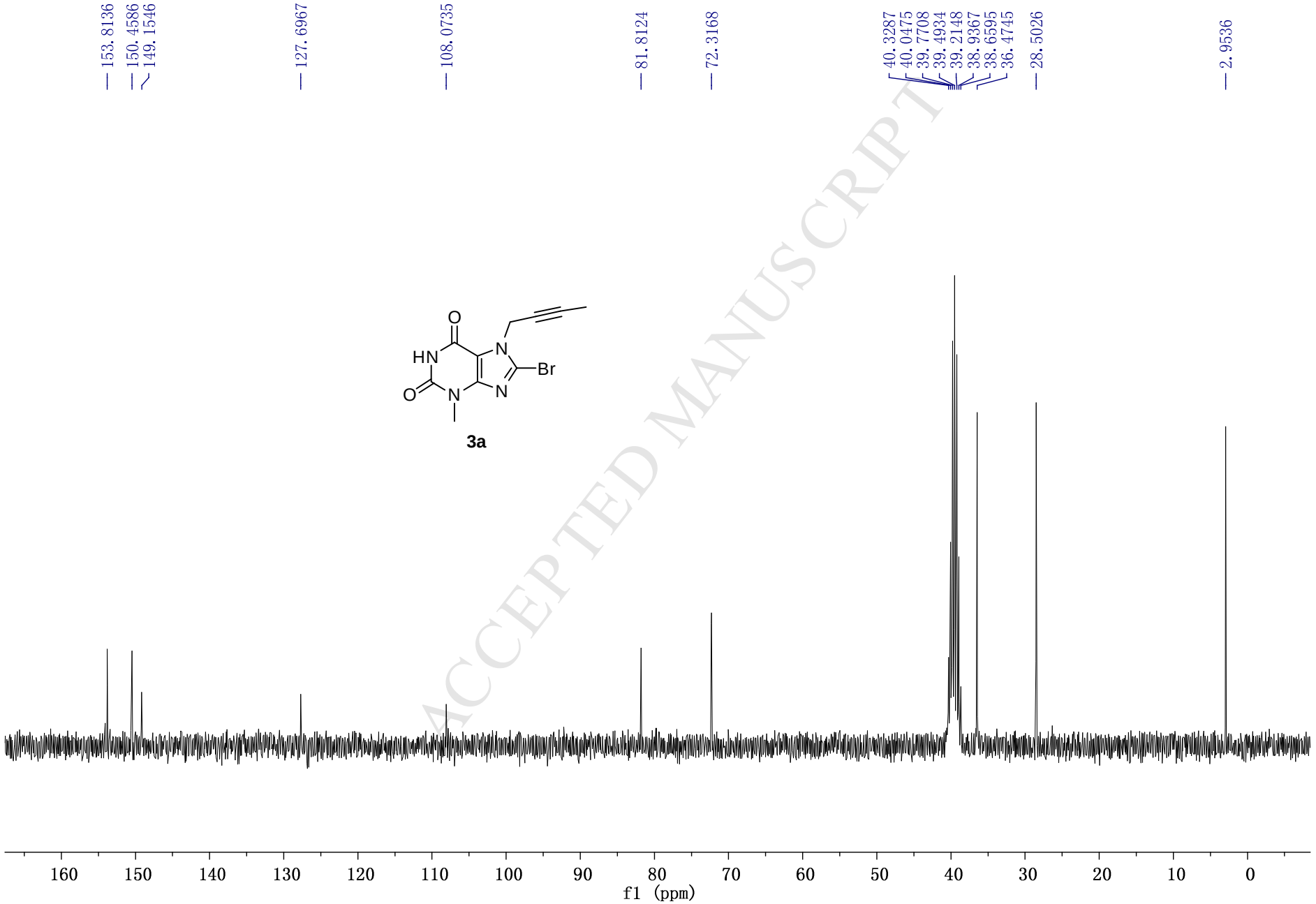


3a

^{13}C NMR spectrum of **3a**



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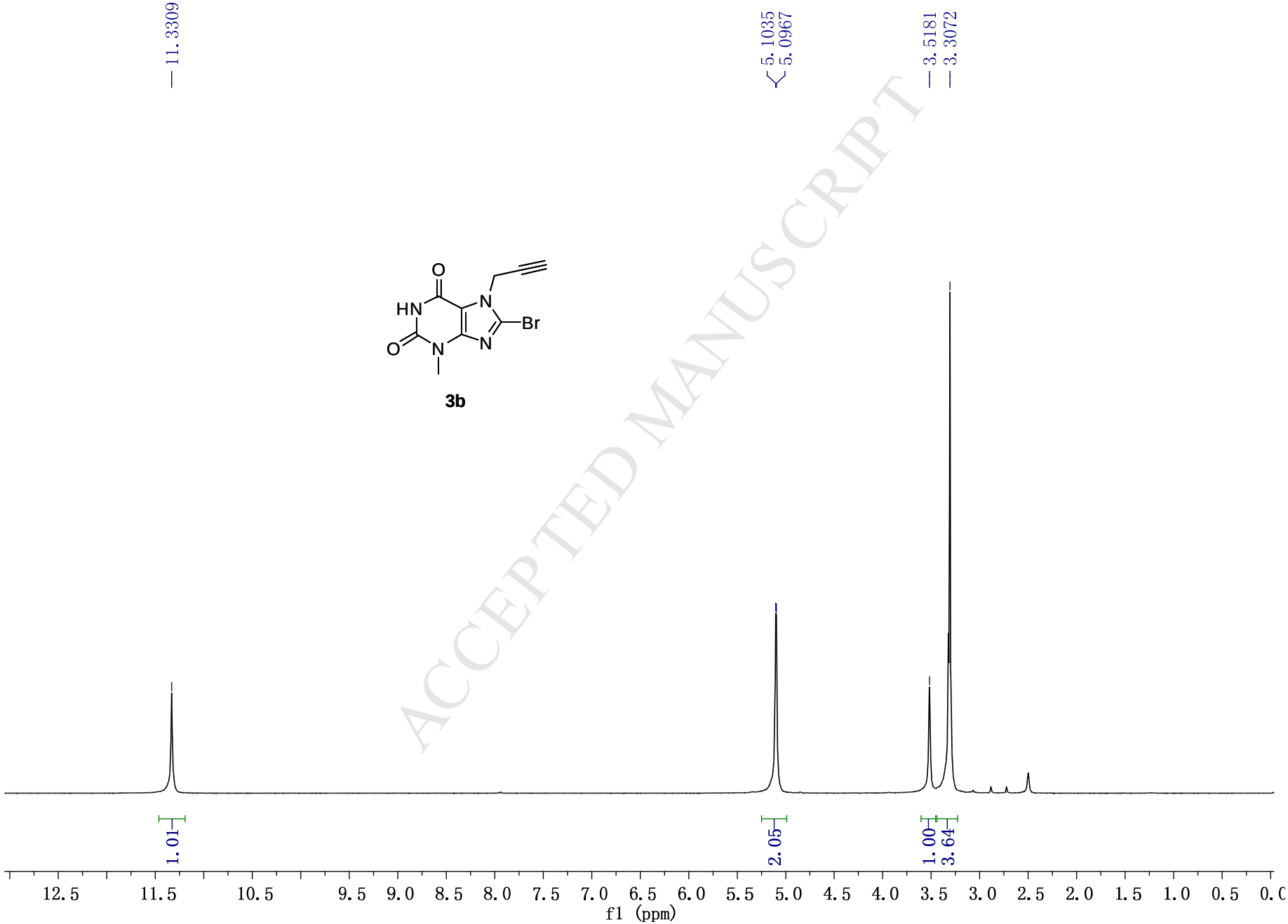

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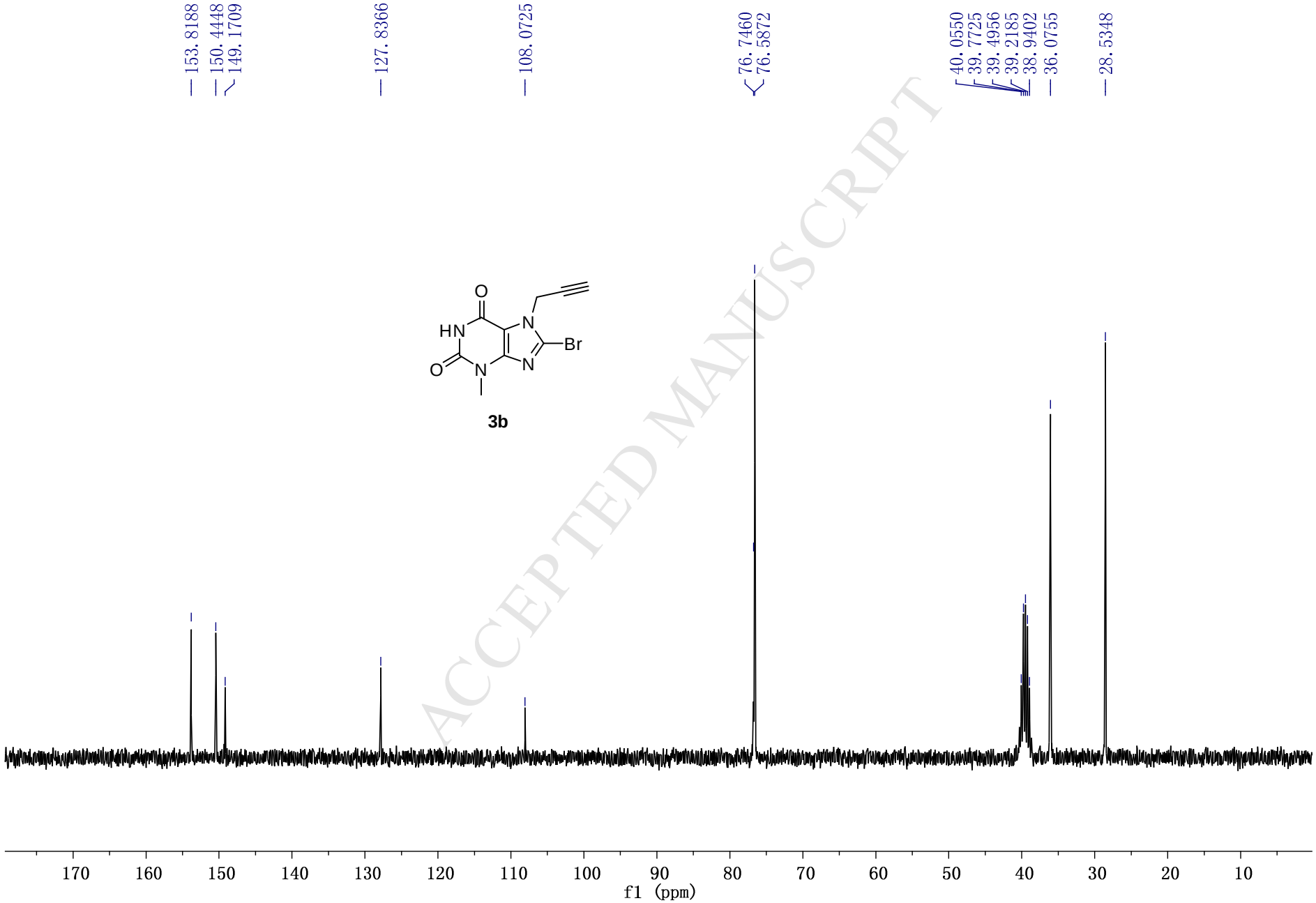

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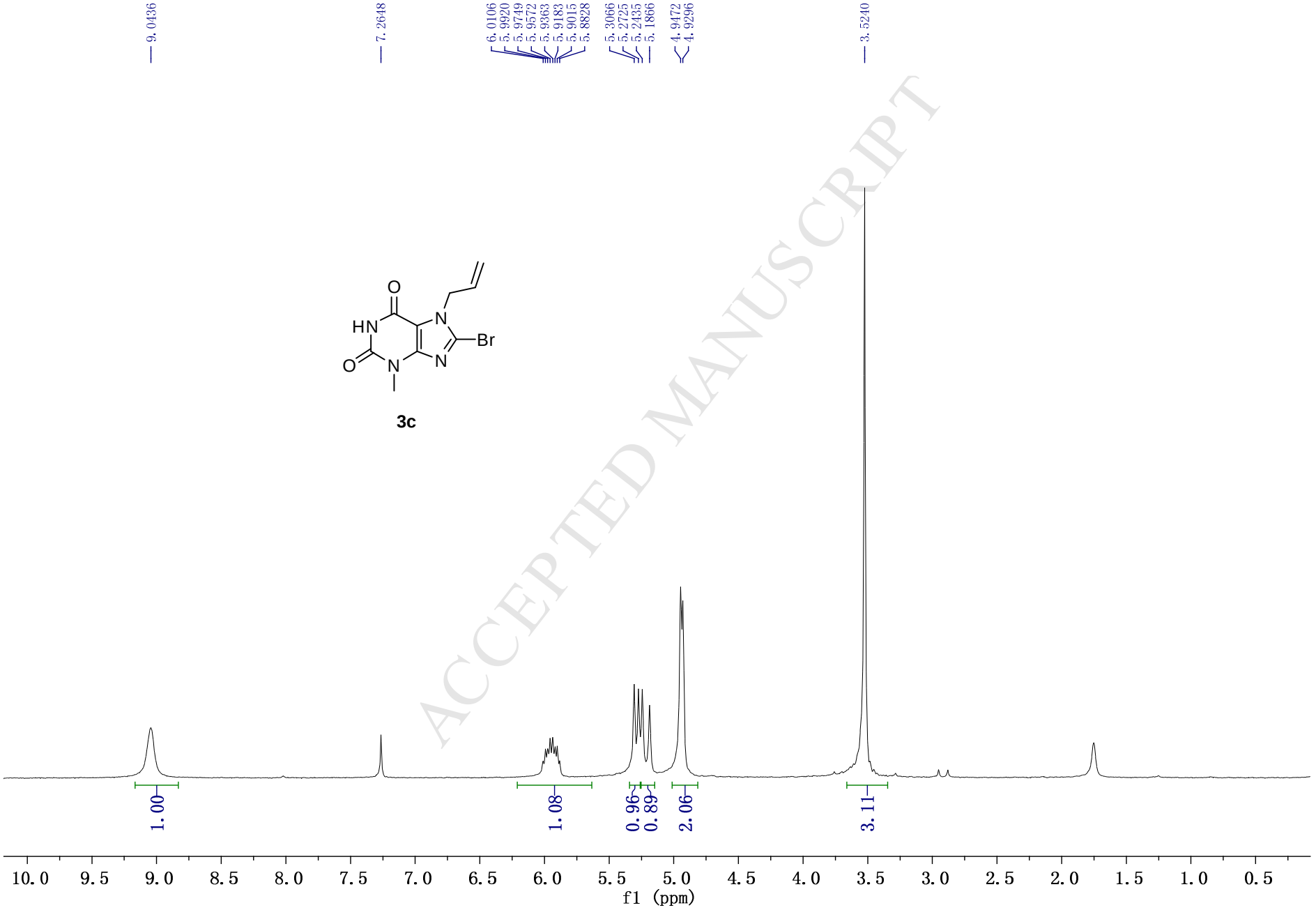

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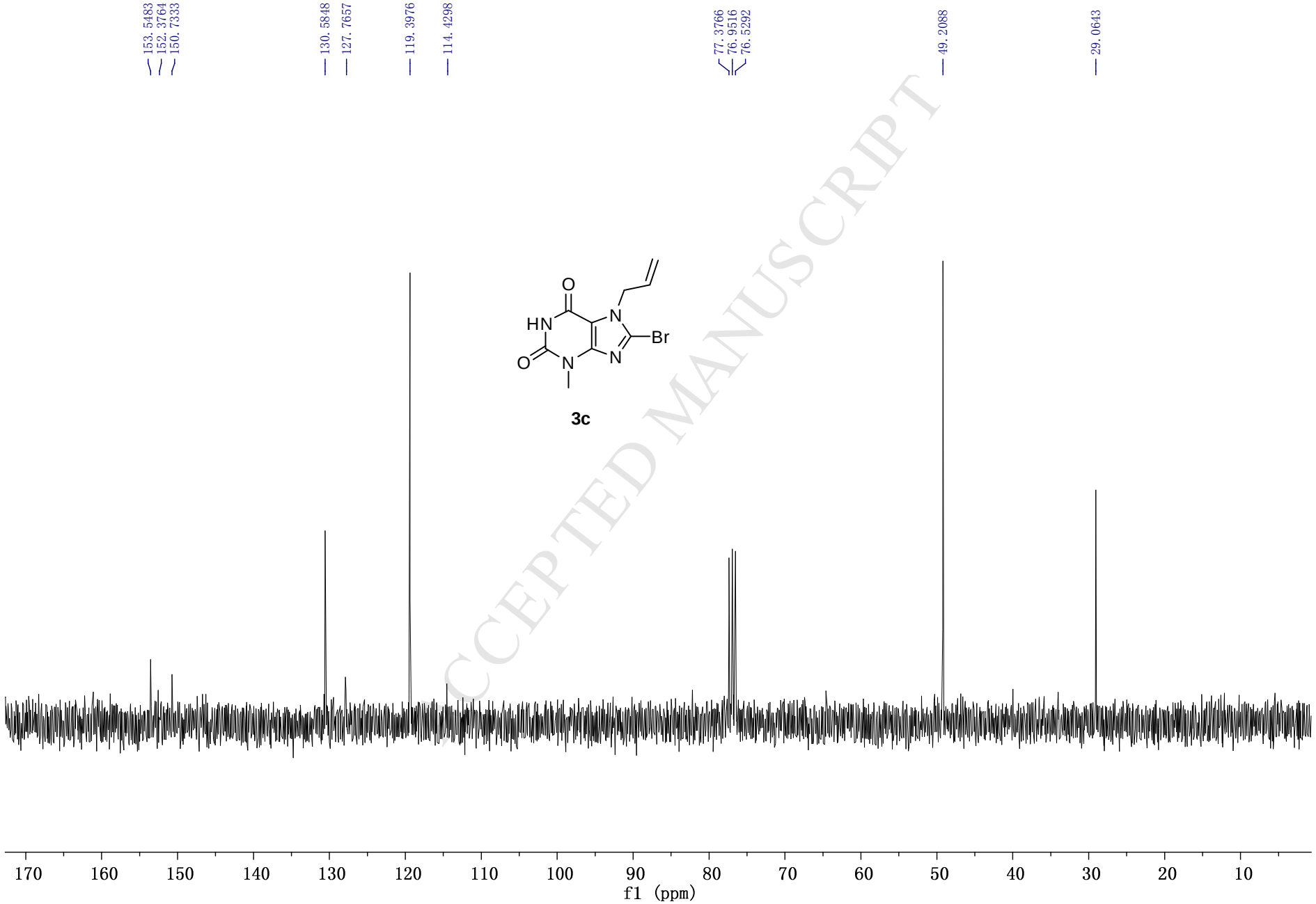

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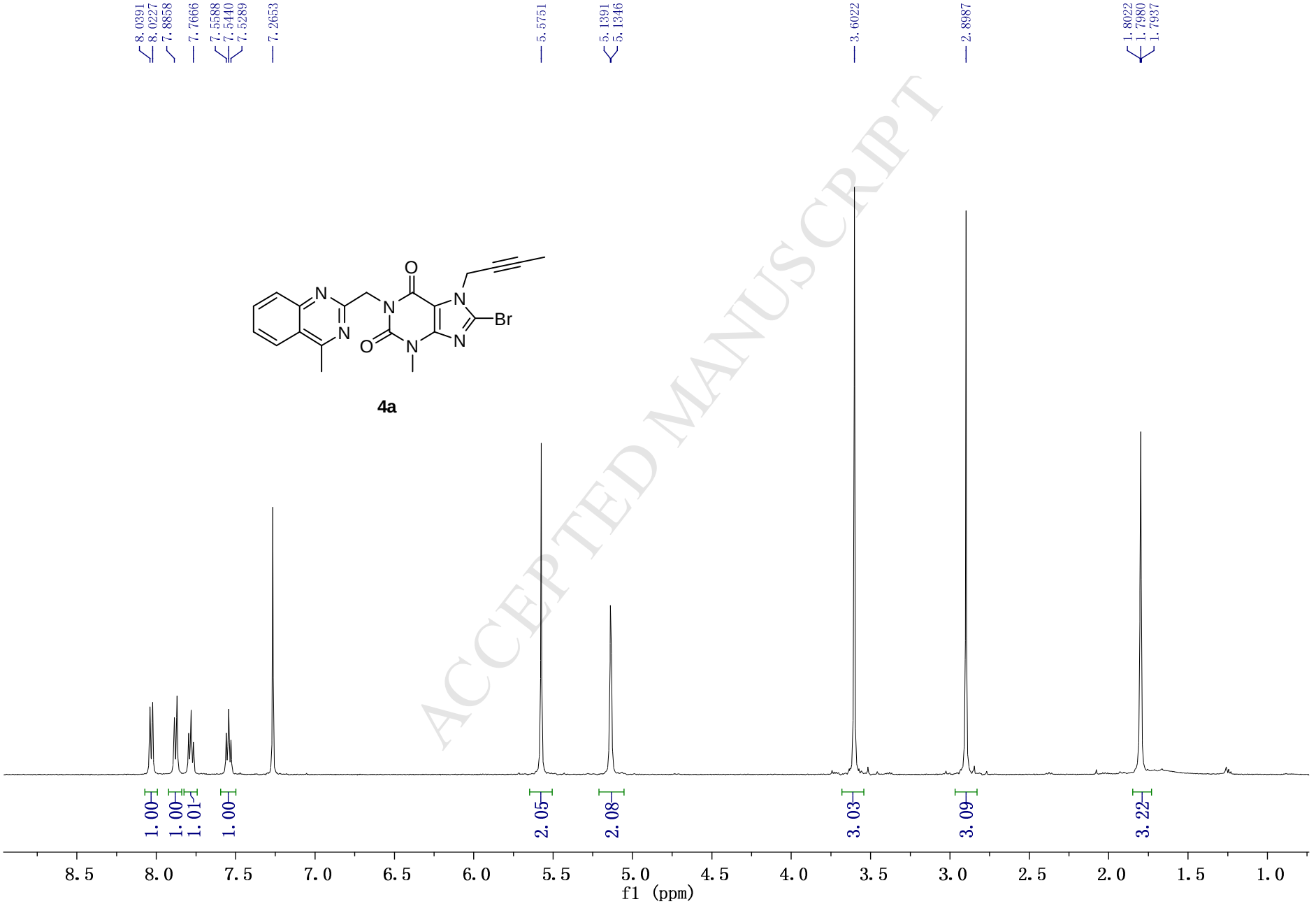

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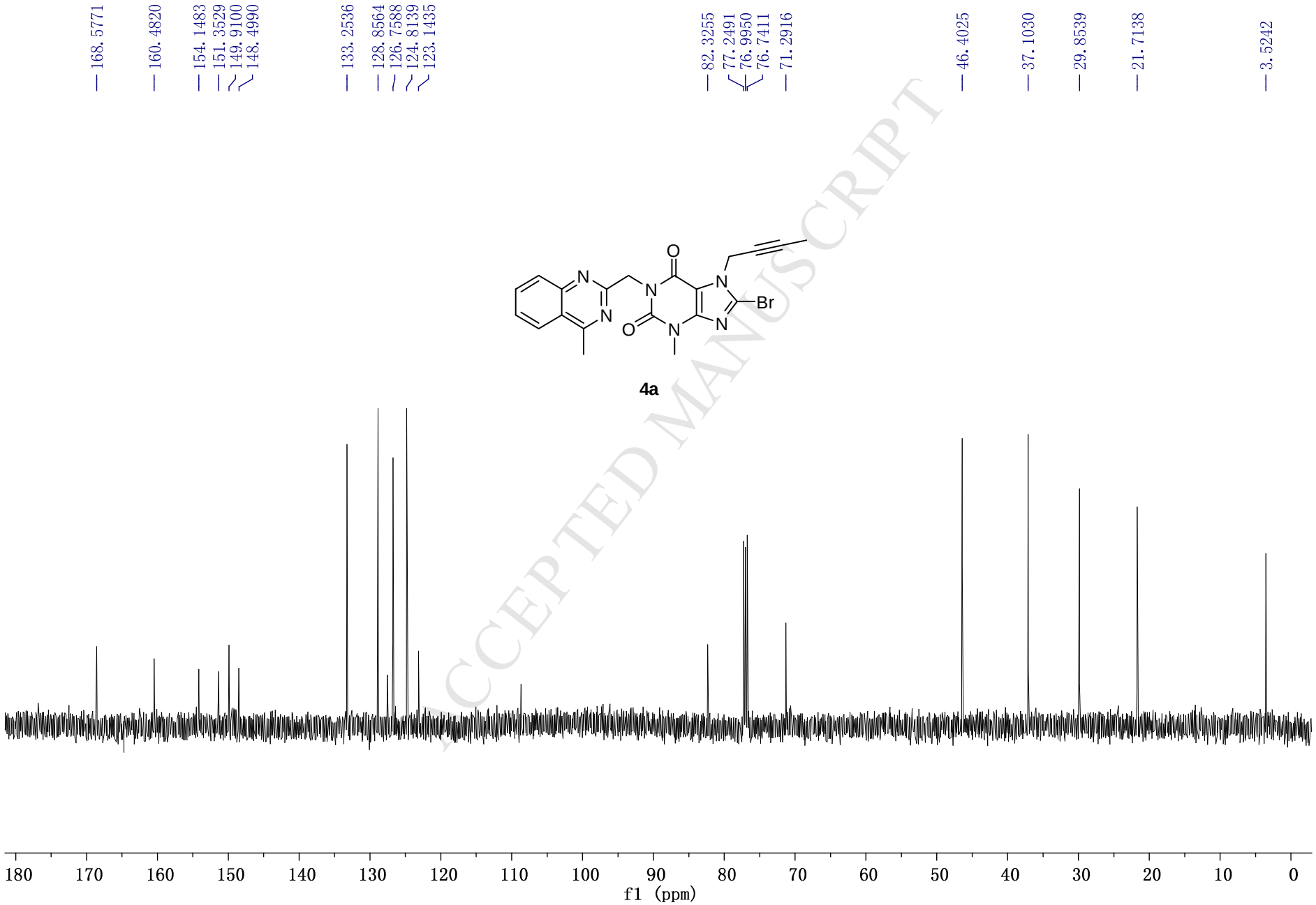

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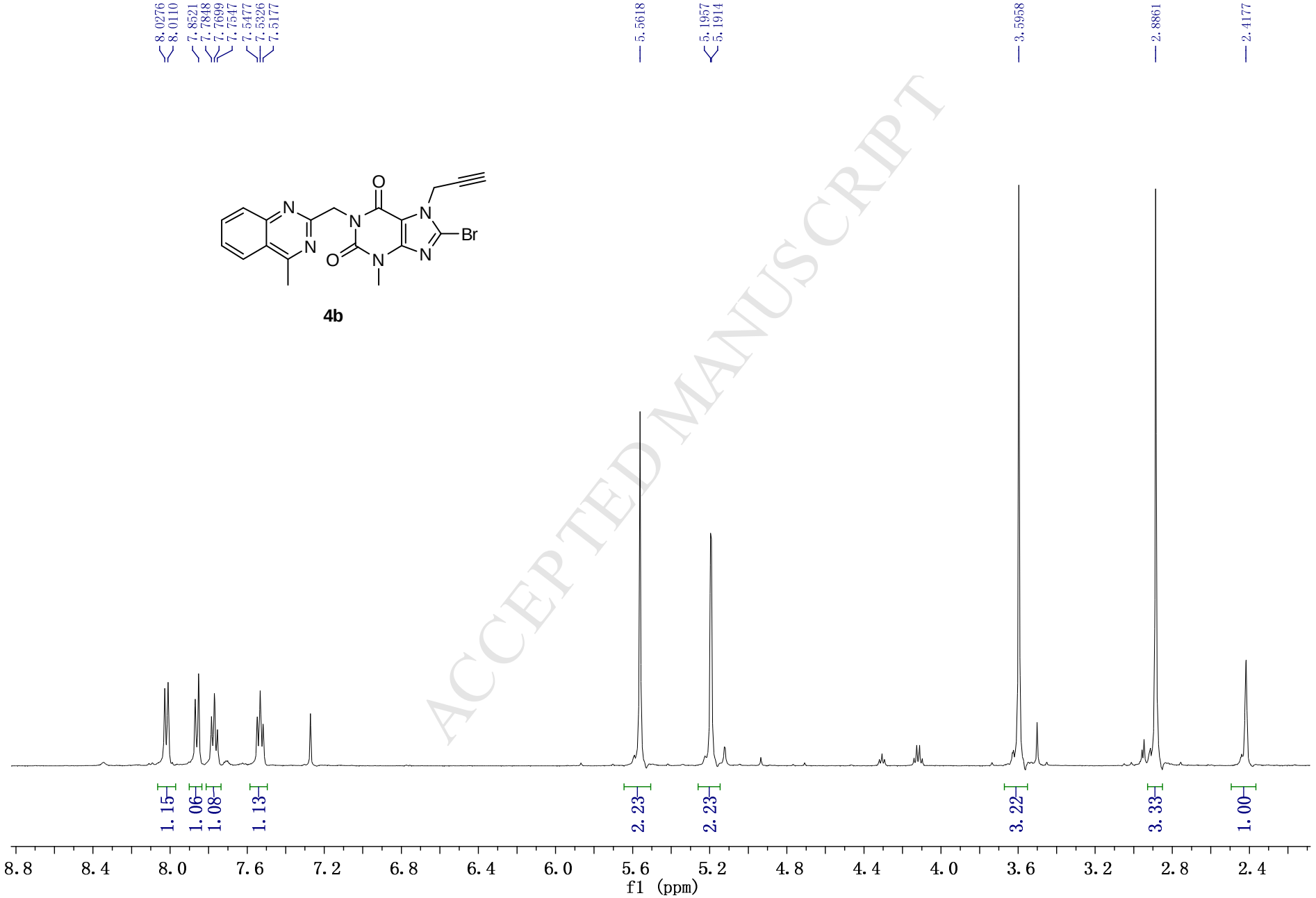

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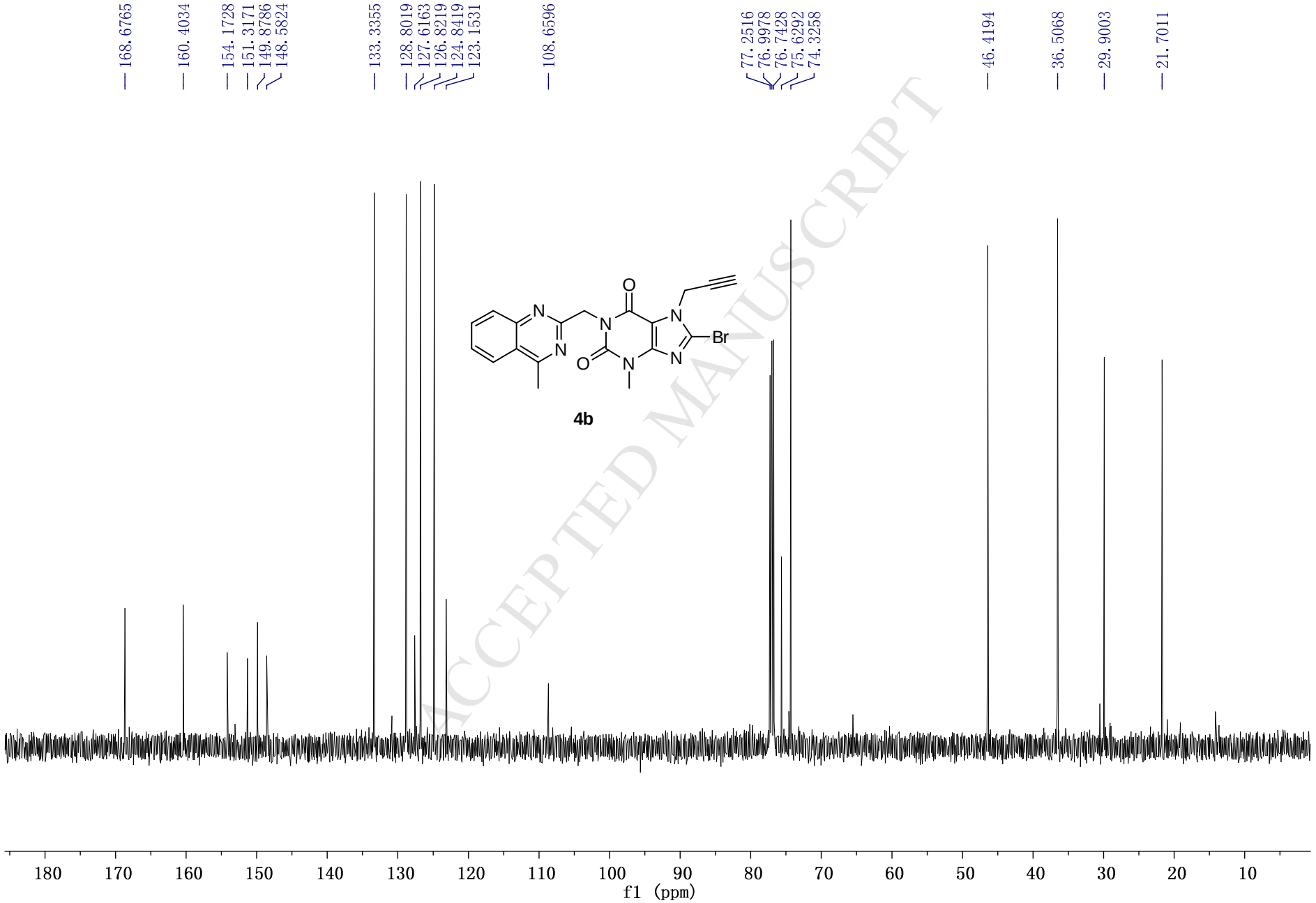

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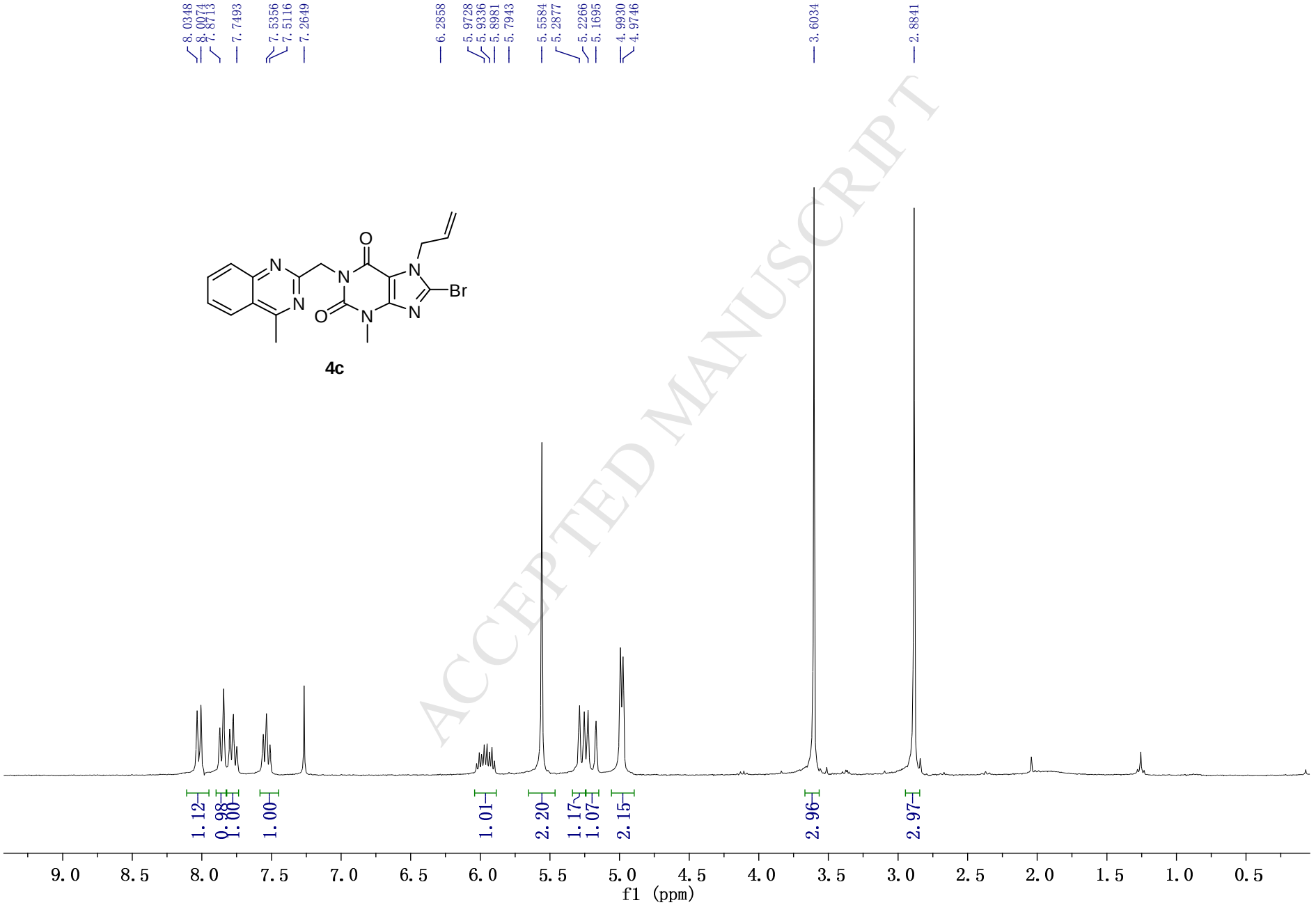

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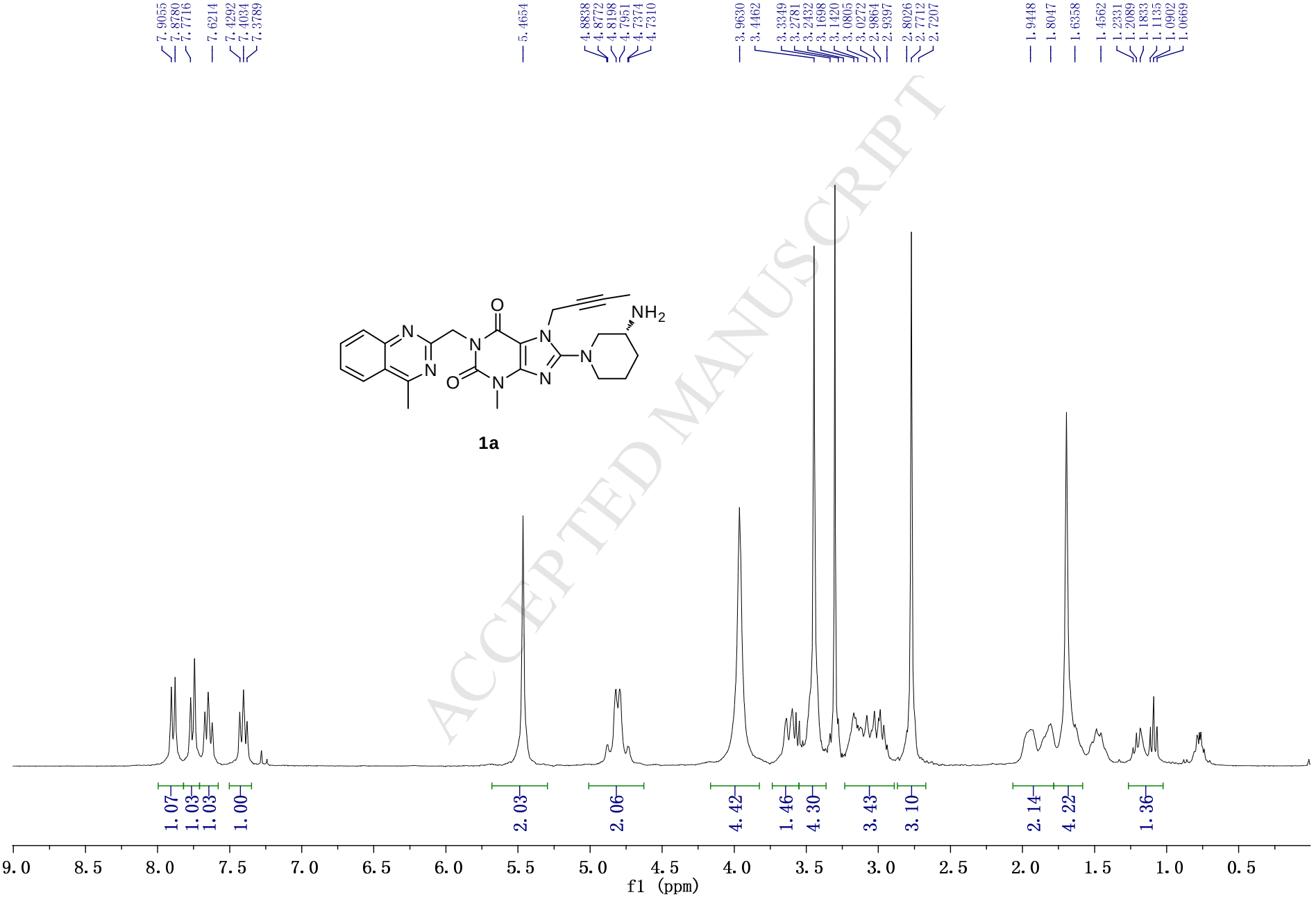

1a

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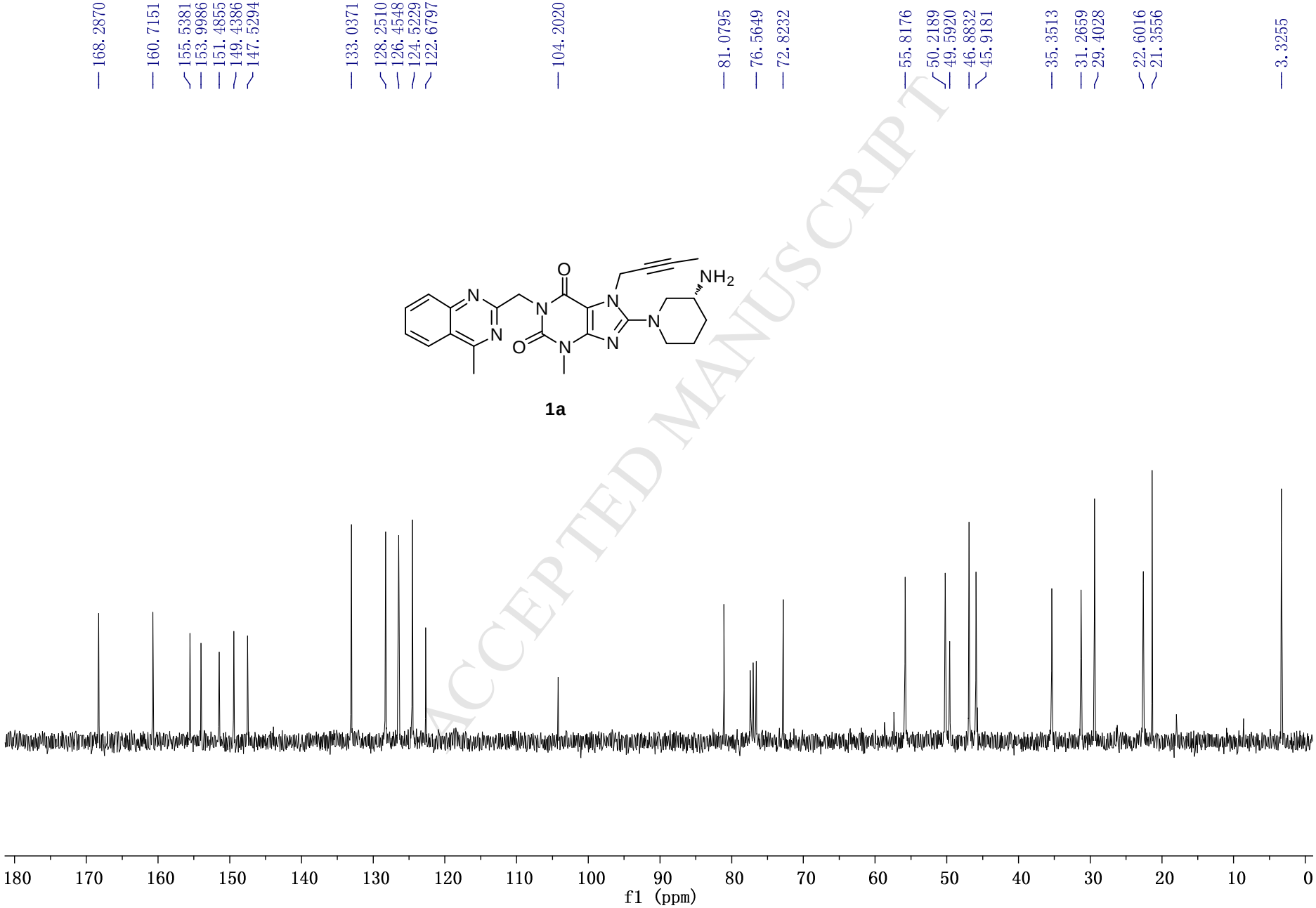

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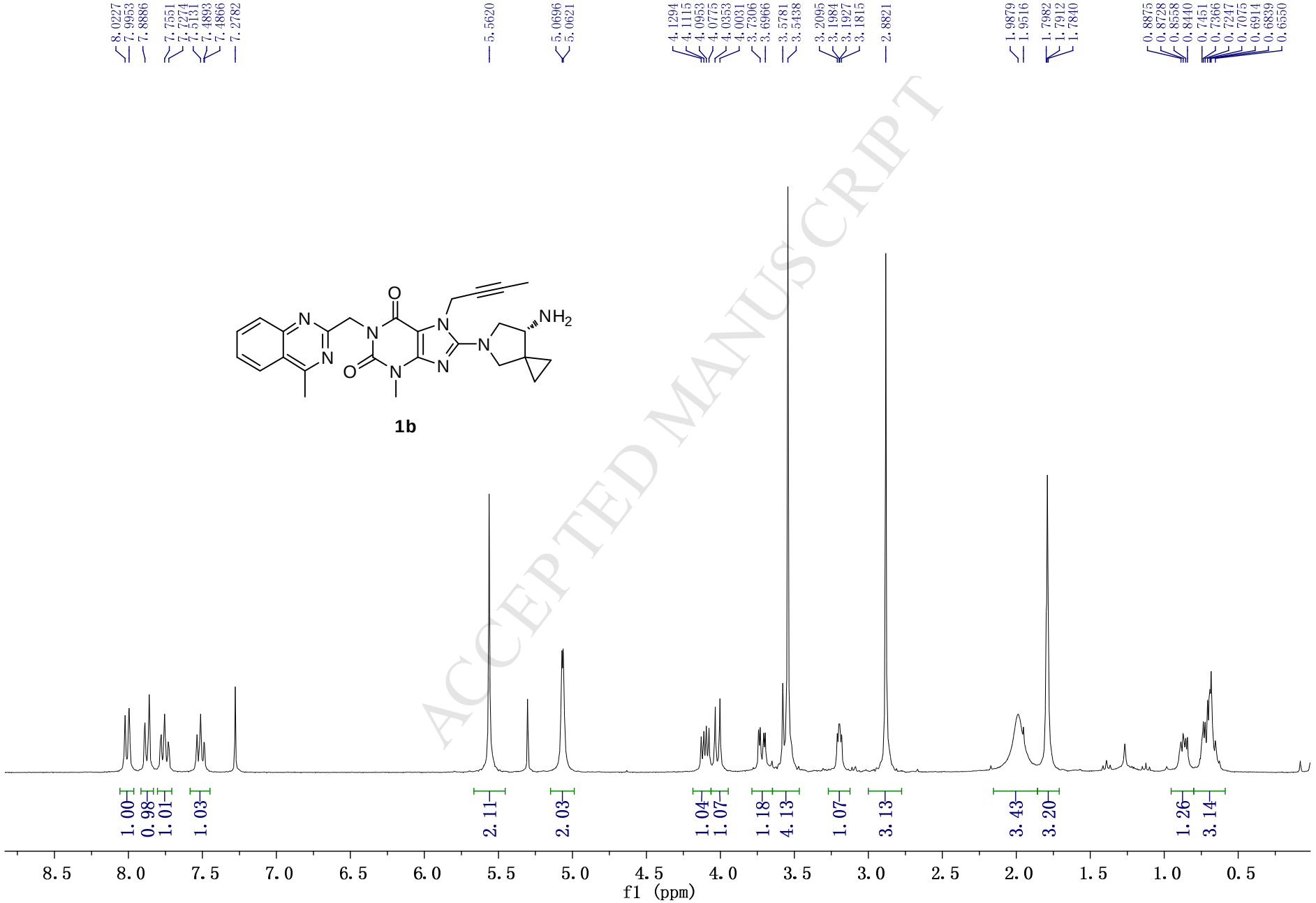

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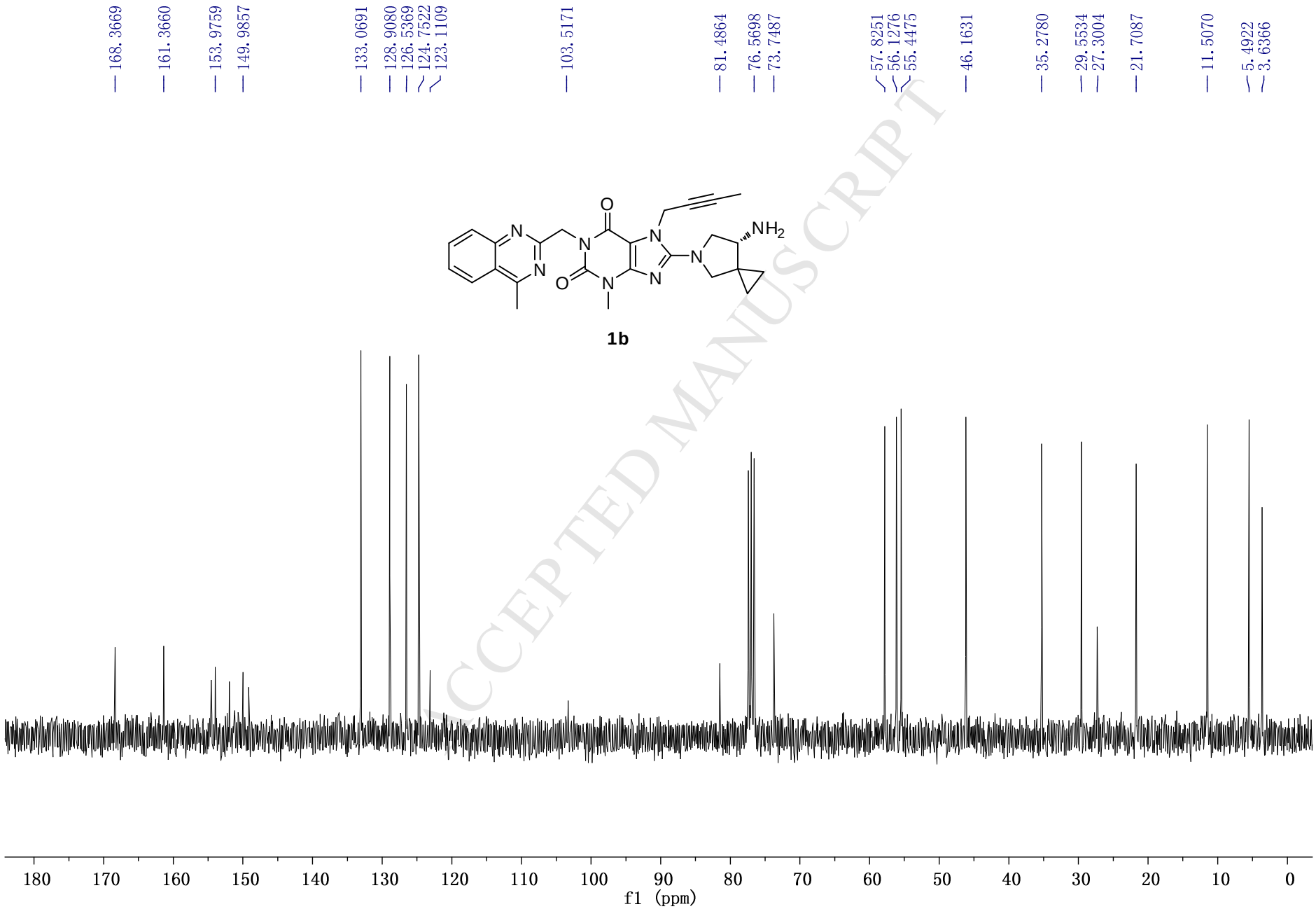

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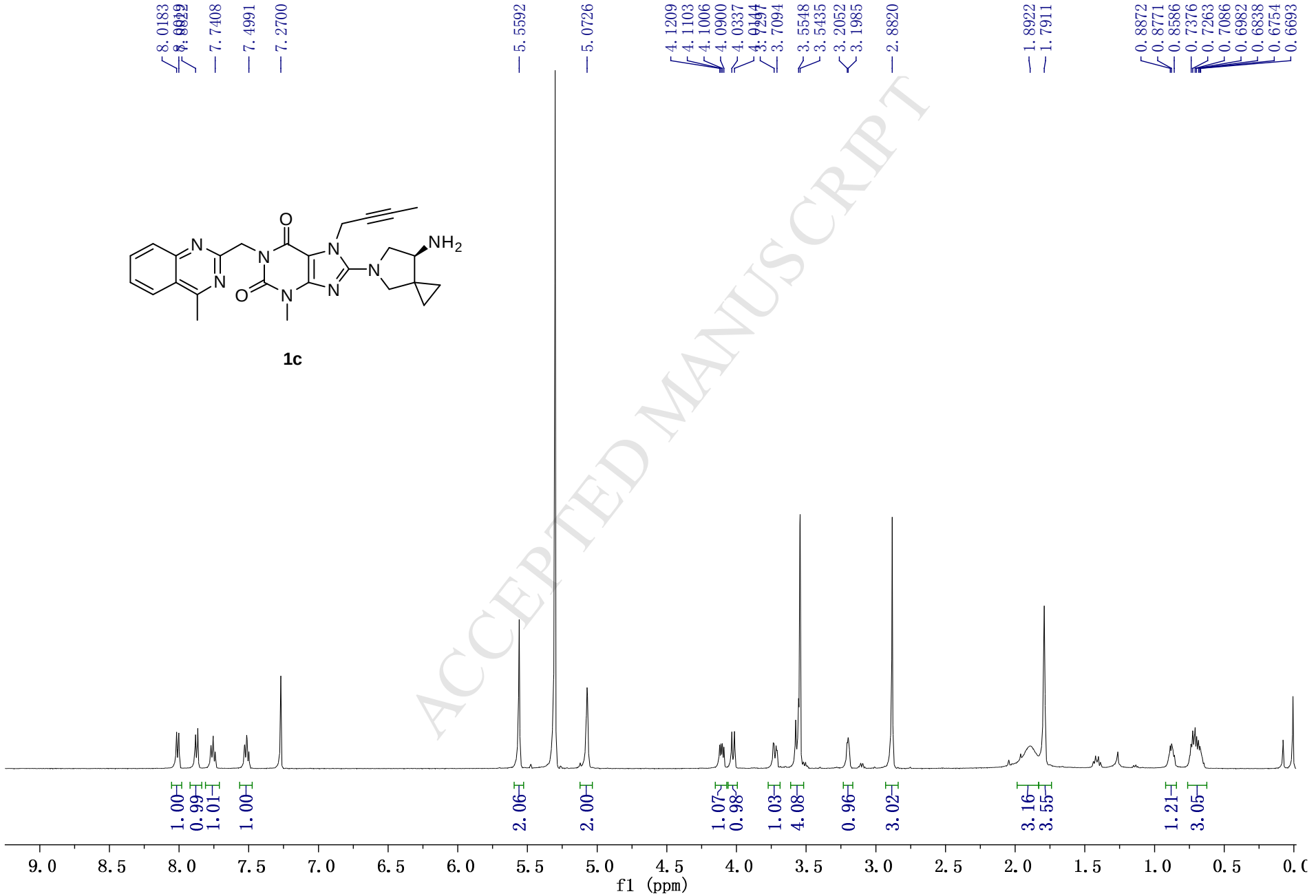

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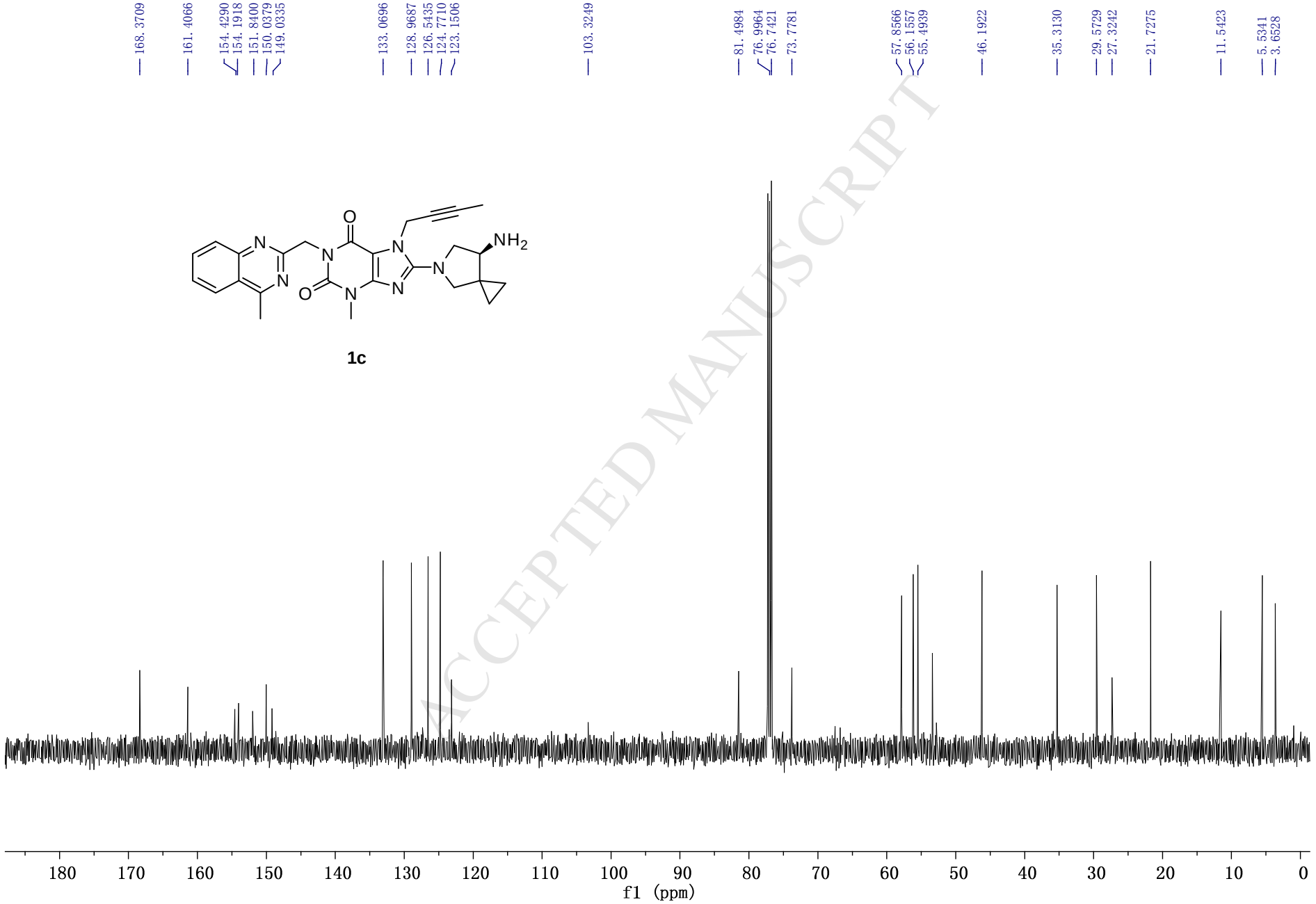

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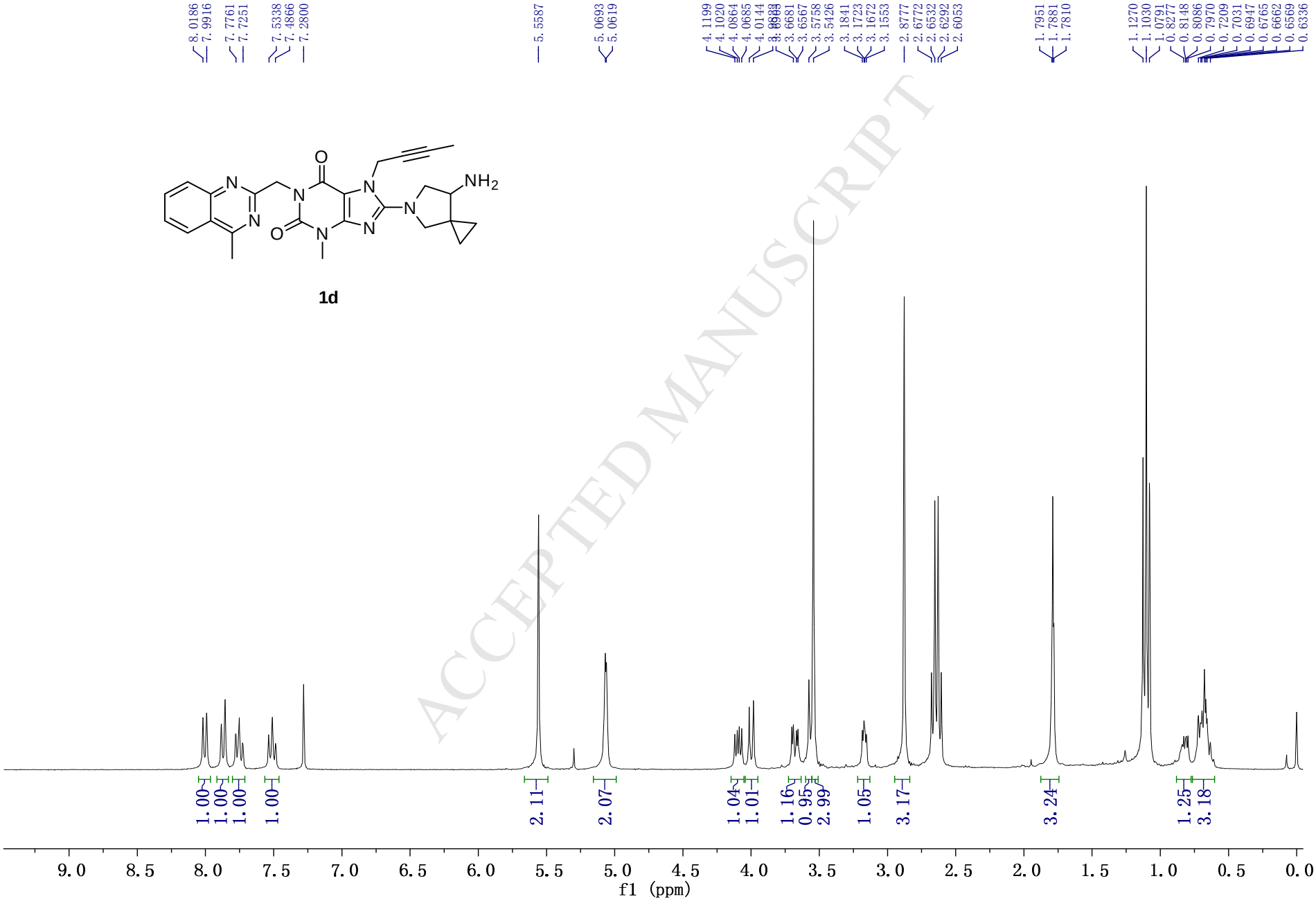

1d

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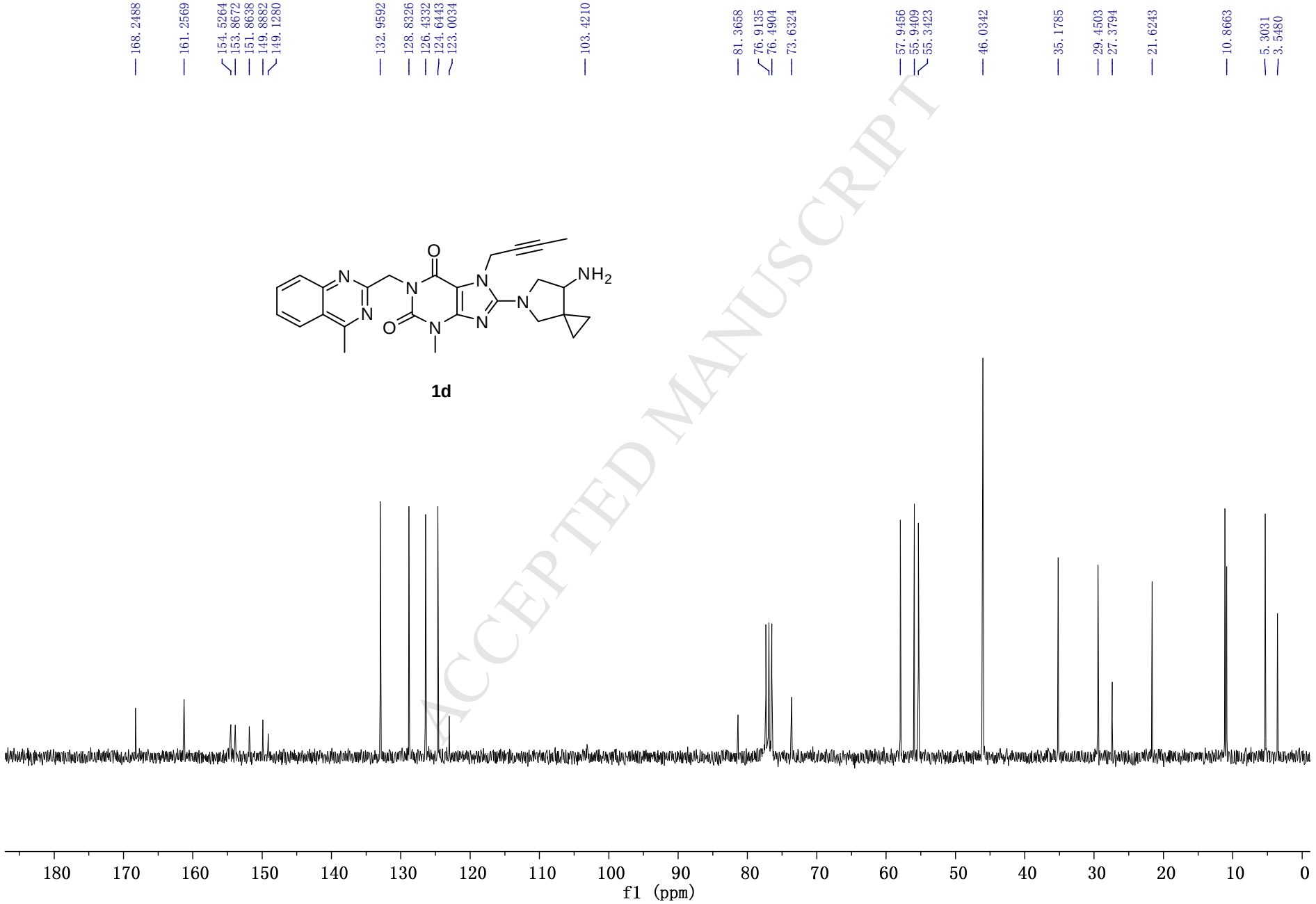

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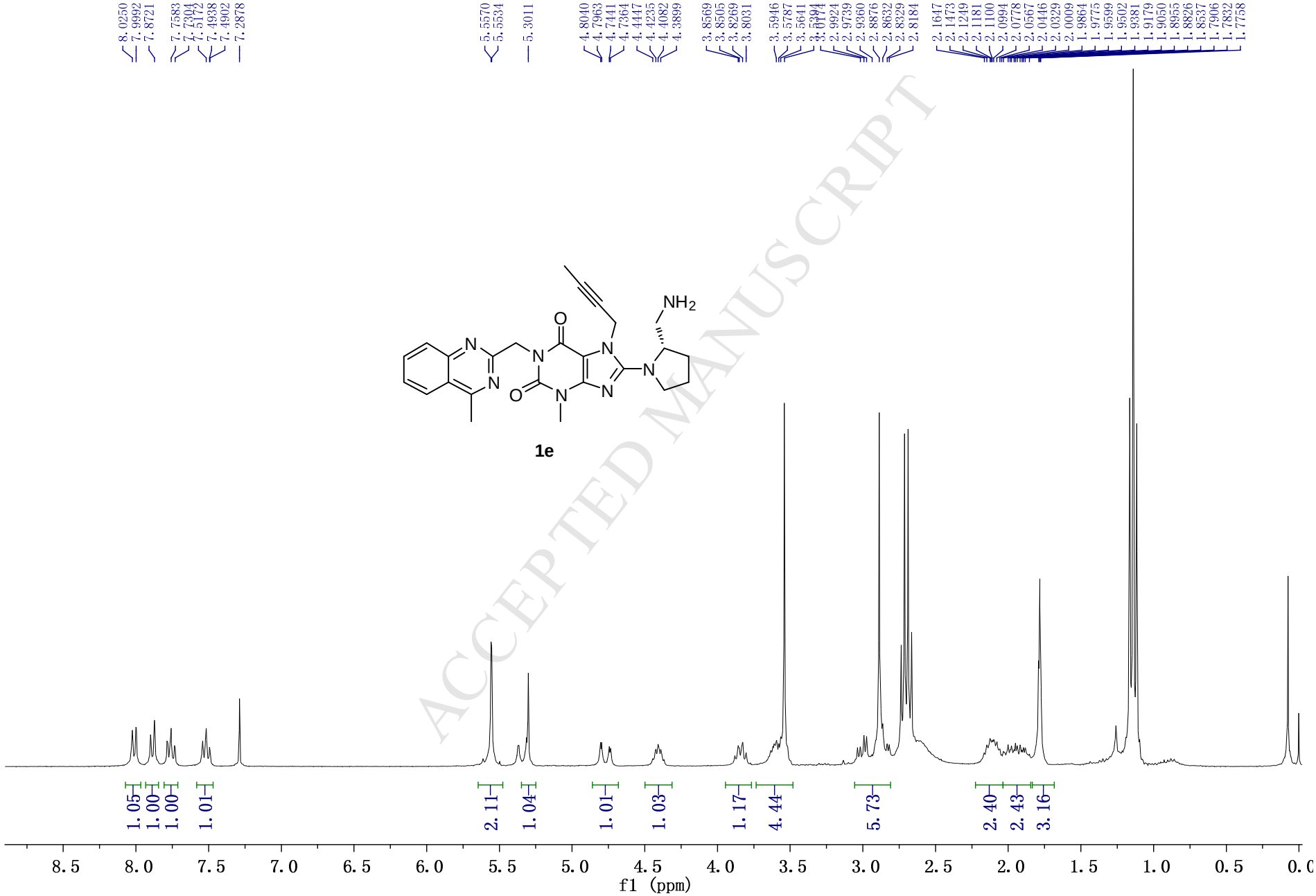

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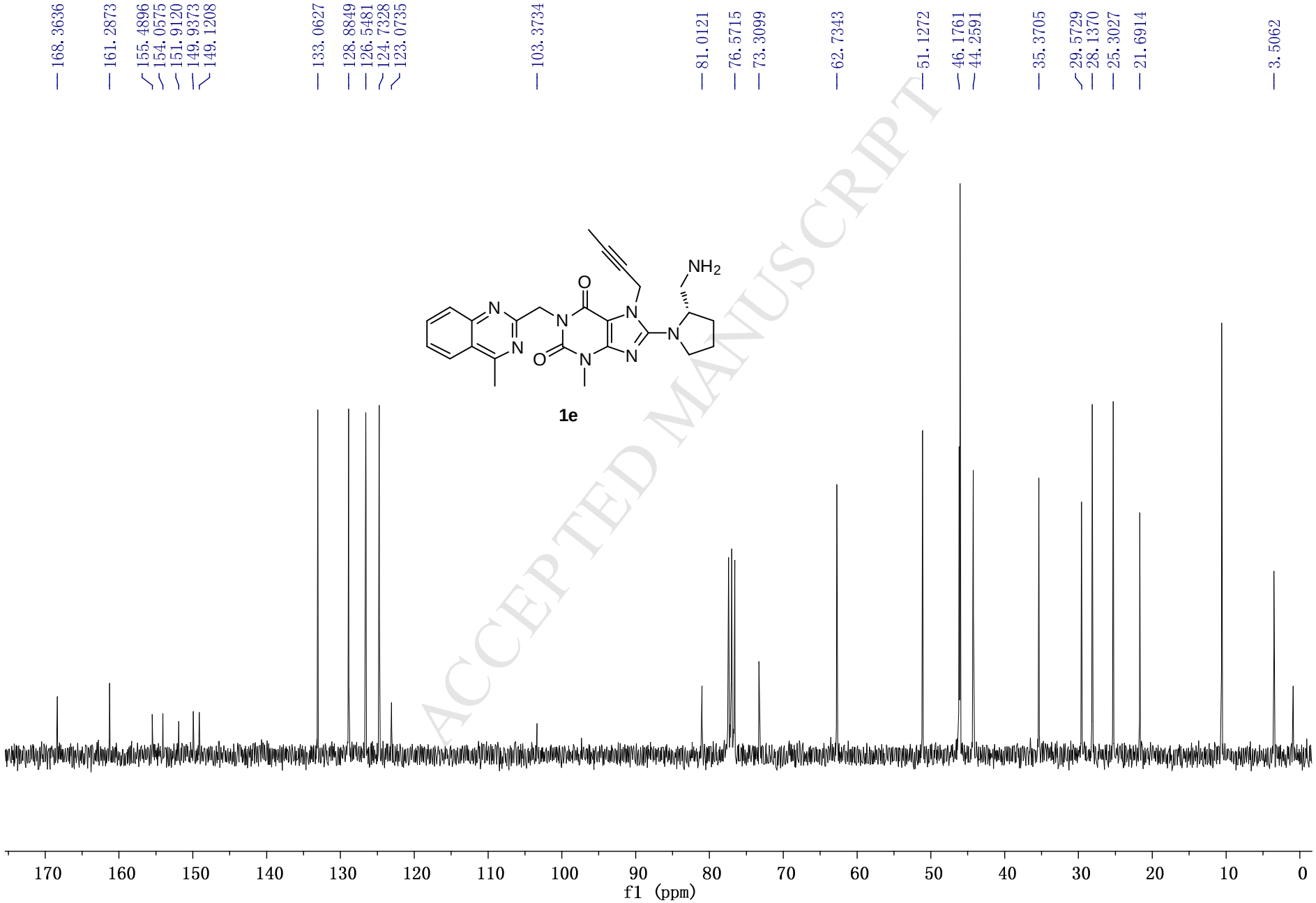

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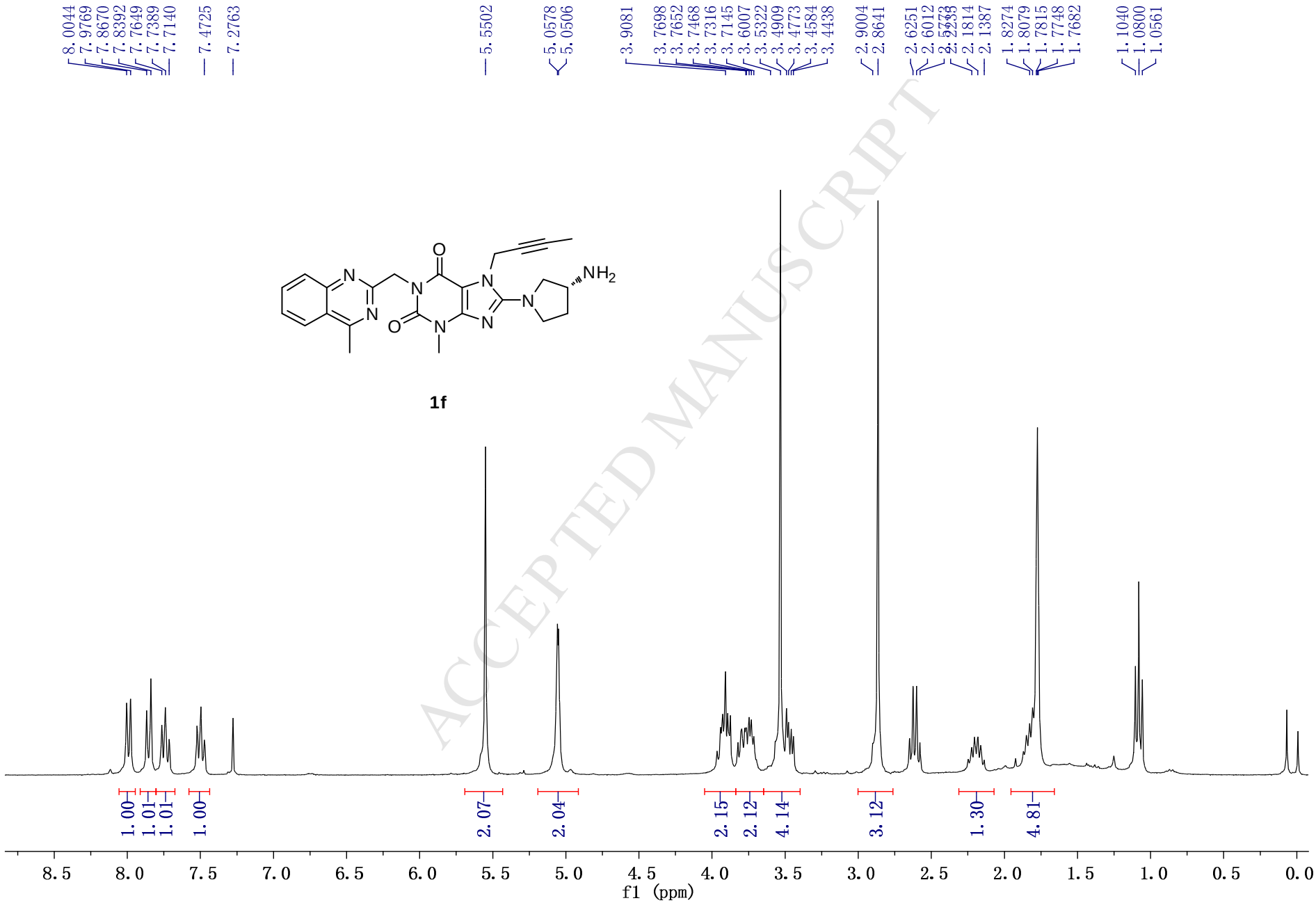

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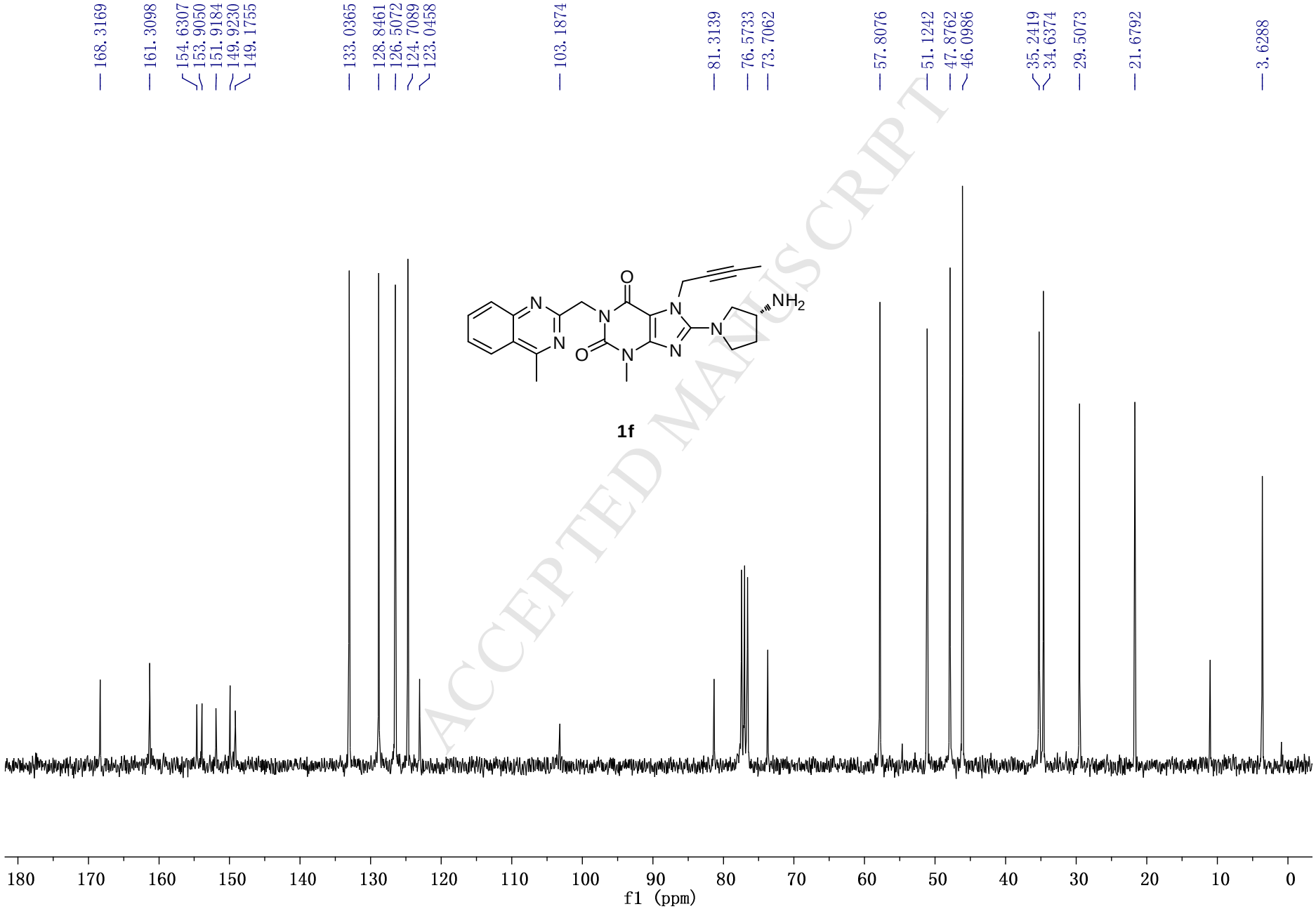

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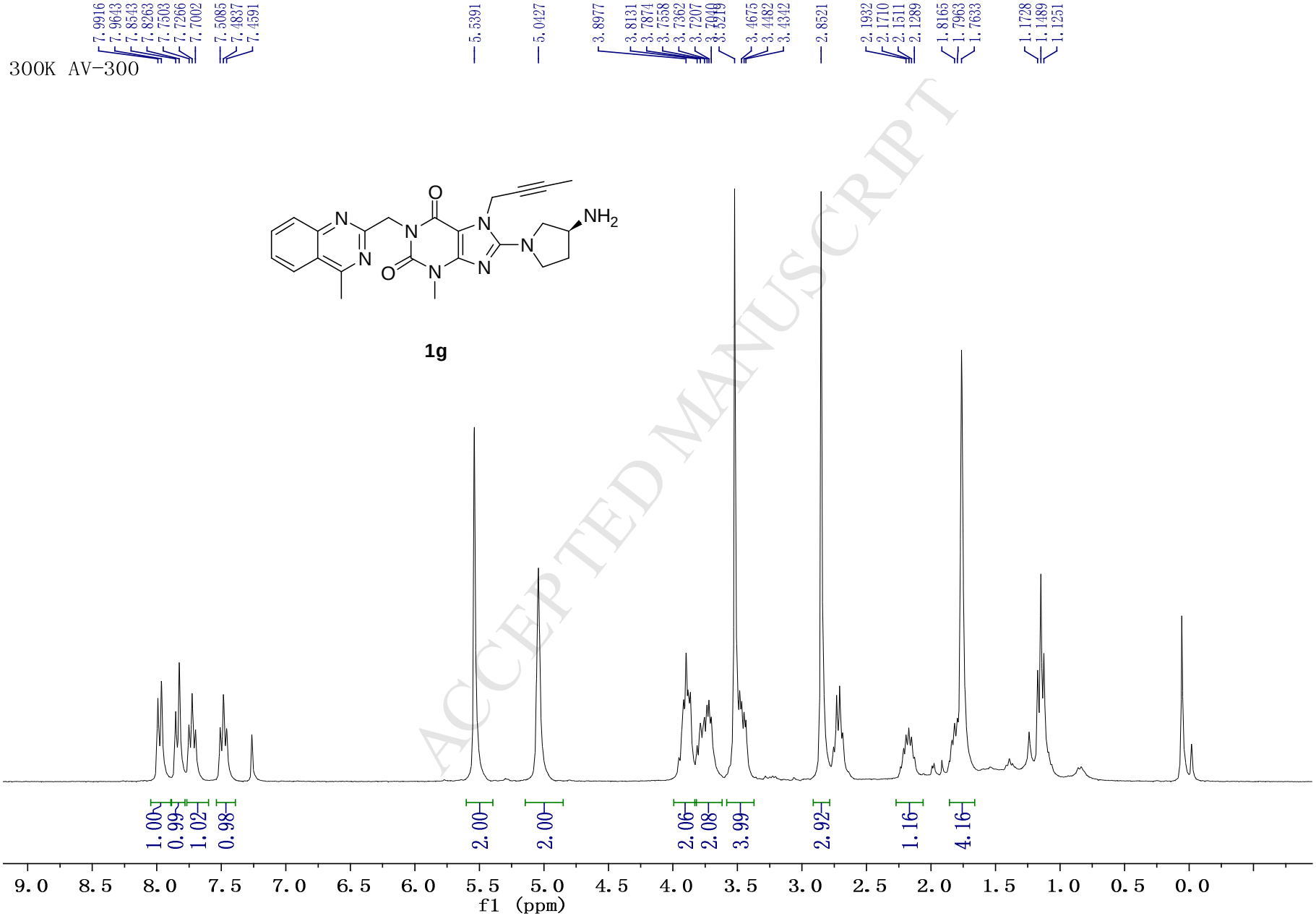

^{13}C NMR spectrum of **1g**

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132.9973
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126.4779
124.6945
123.0485
81.2925
76.5559
73.7060
57.8162
51.1248
47.8793
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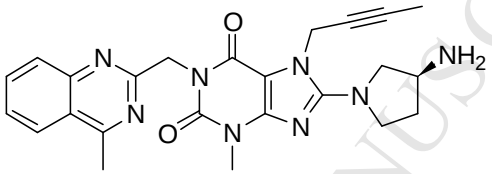
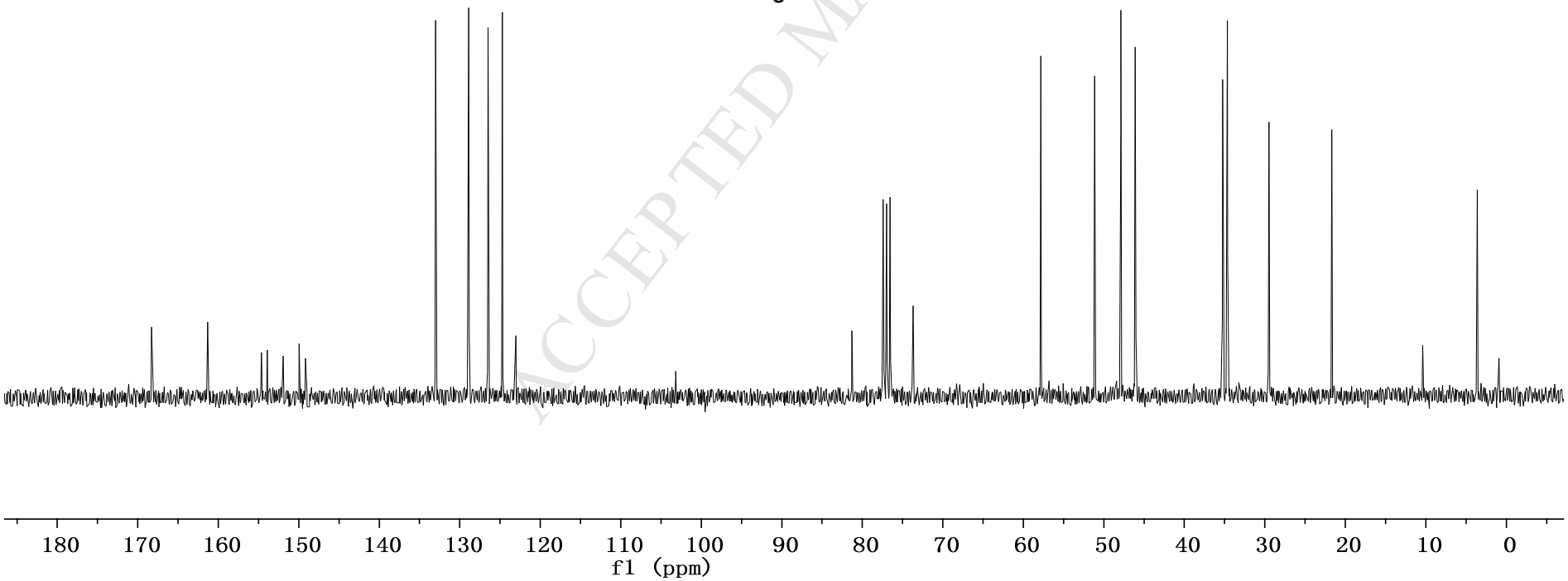
21.6746
10.3899
3.6212

CDC13 300K AV-300



1g



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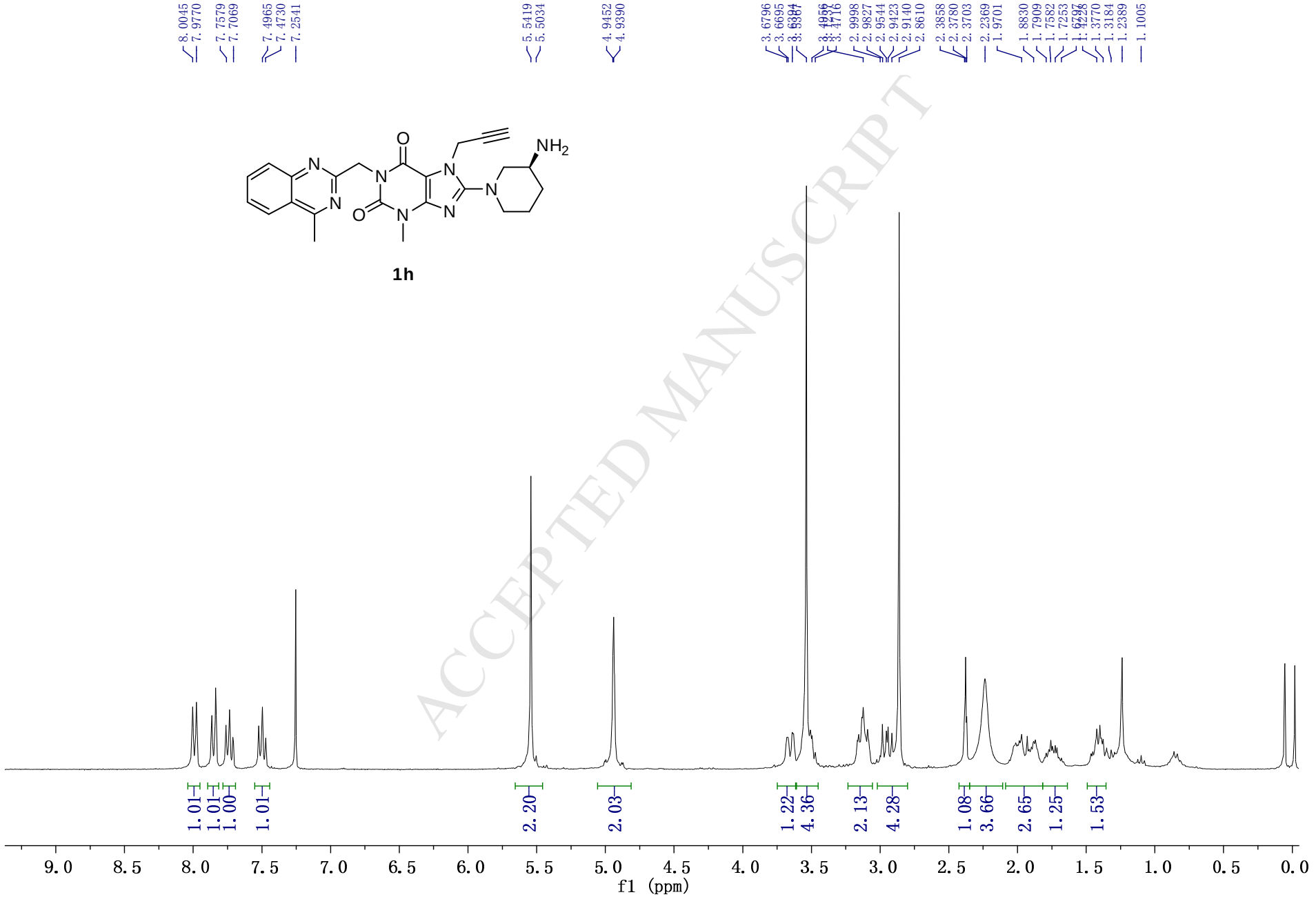

1h

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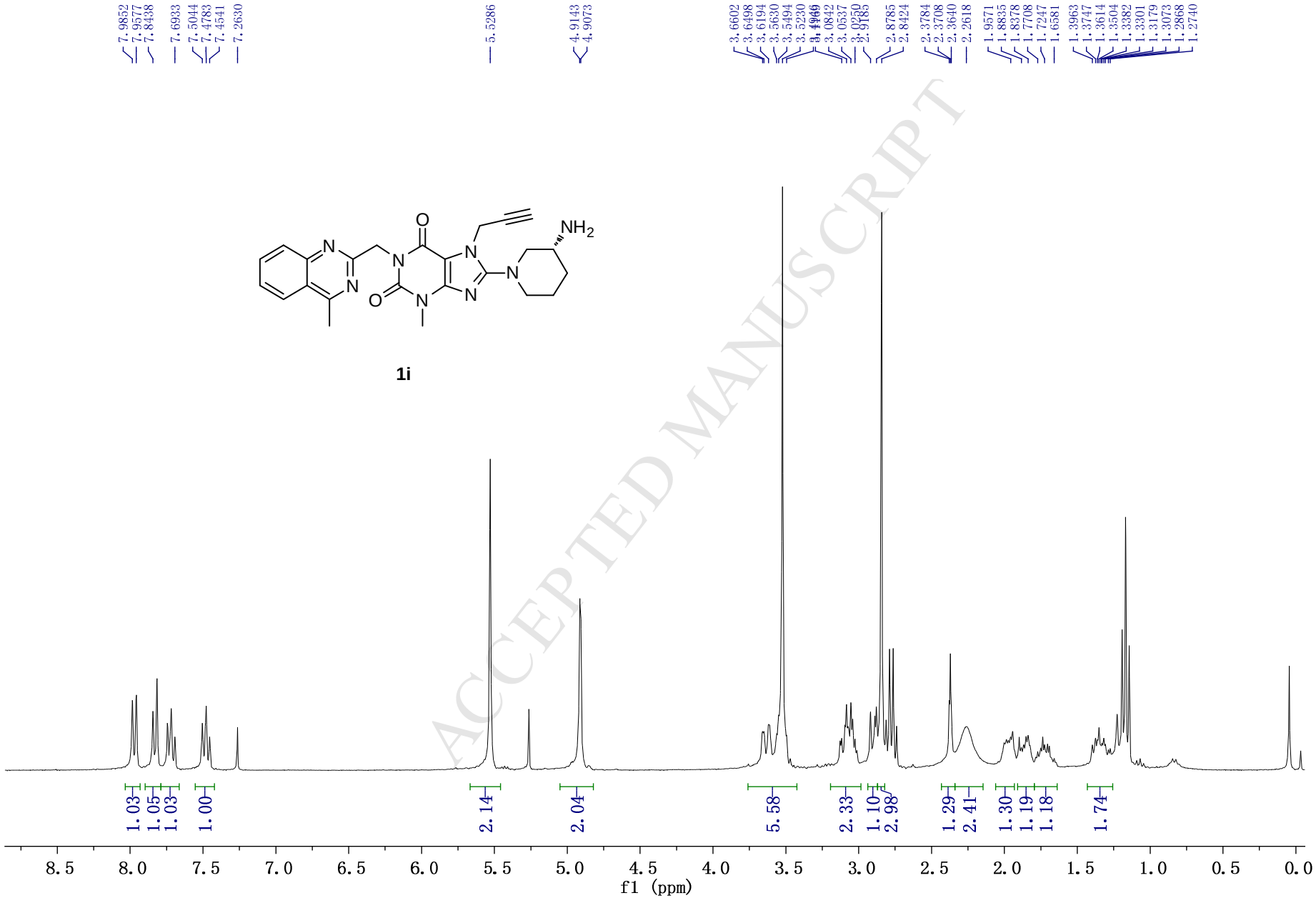

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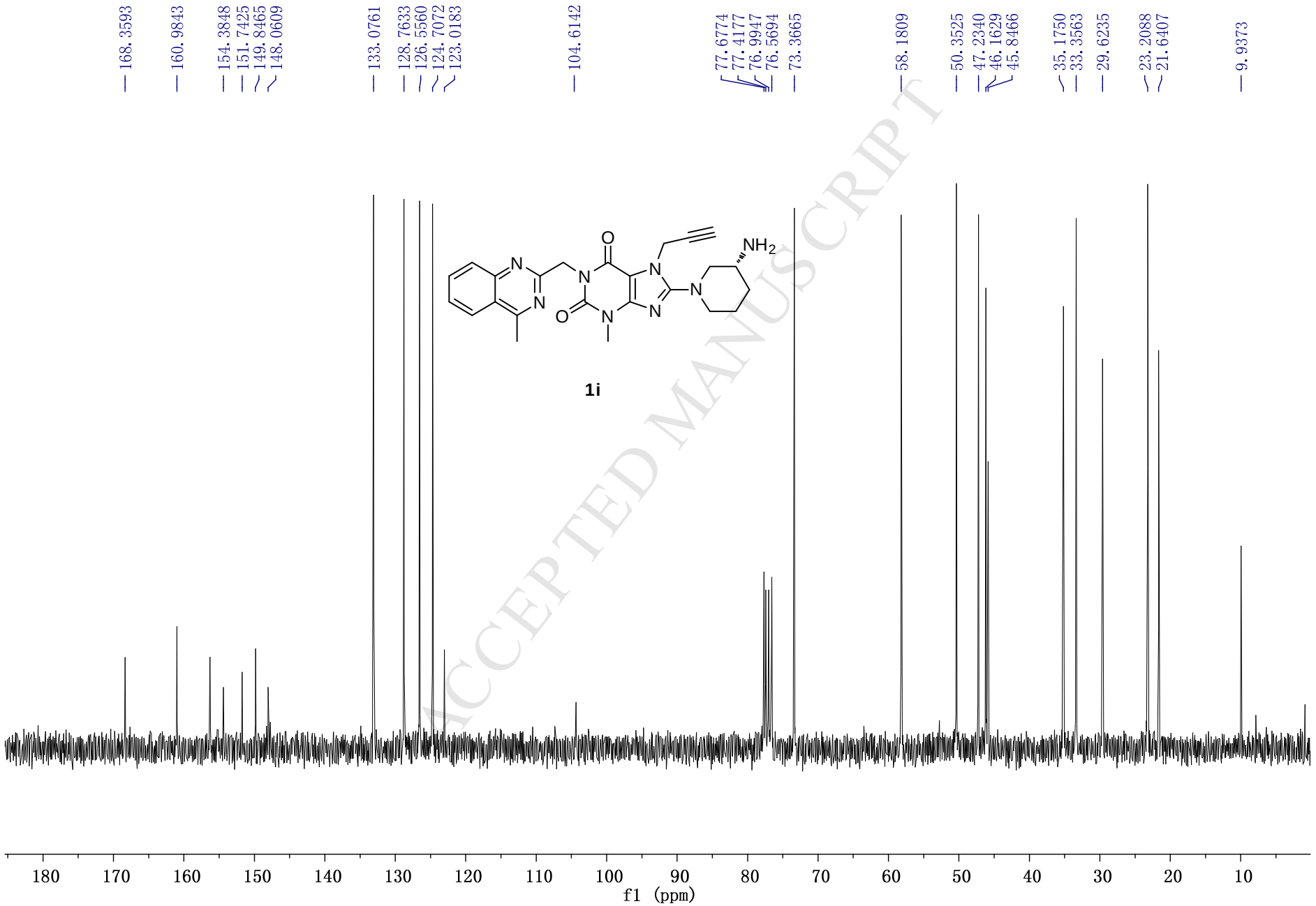

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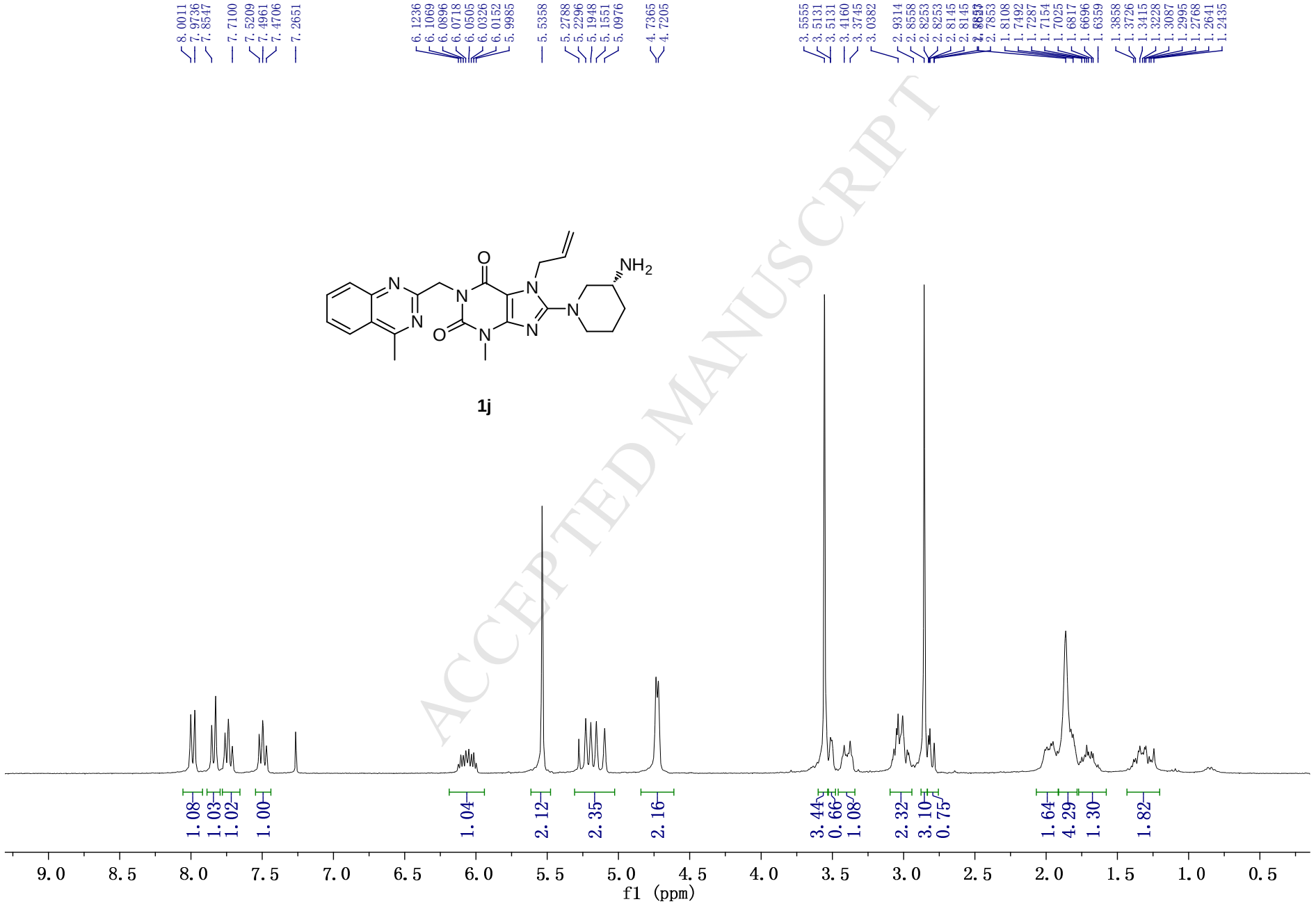

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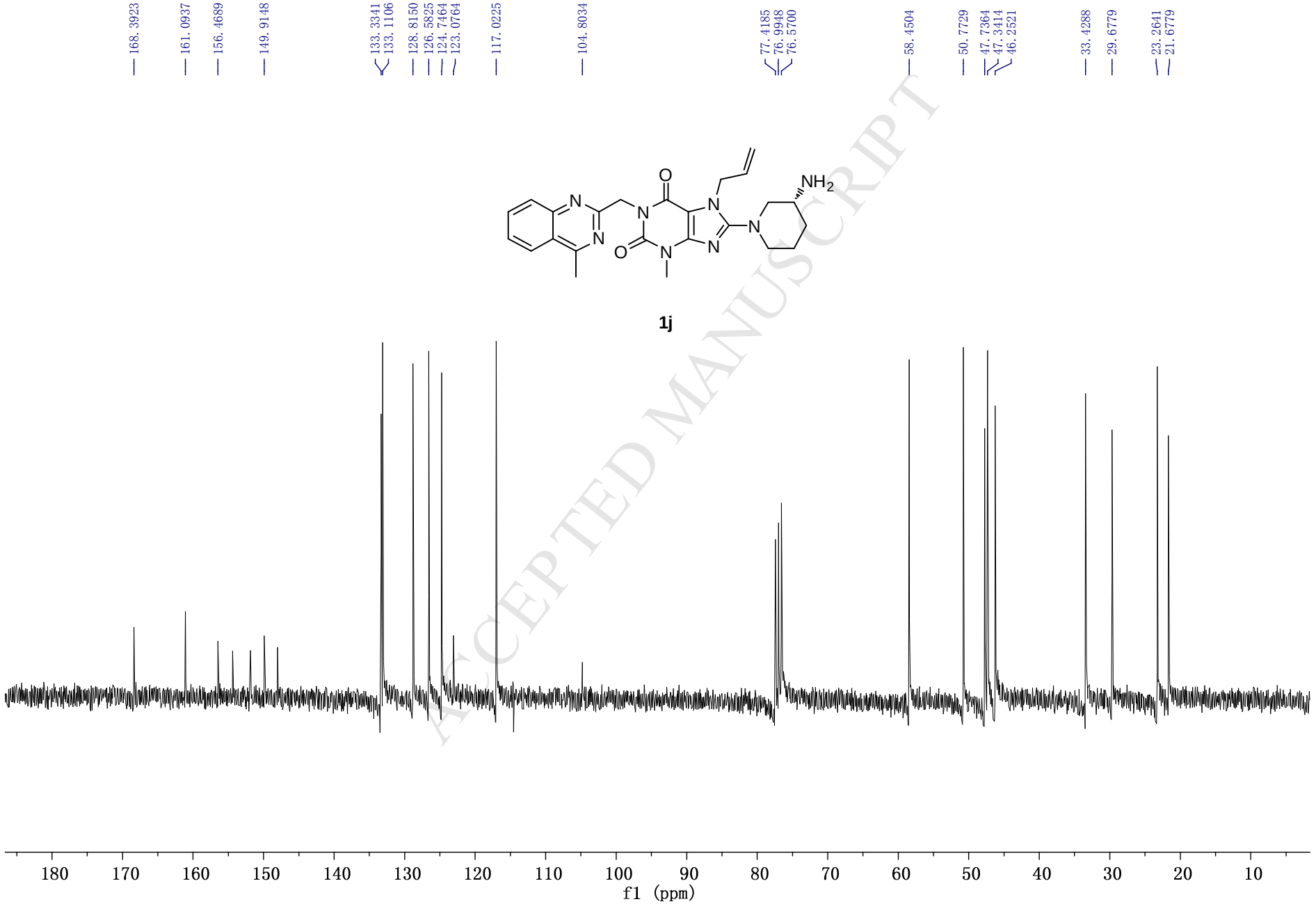

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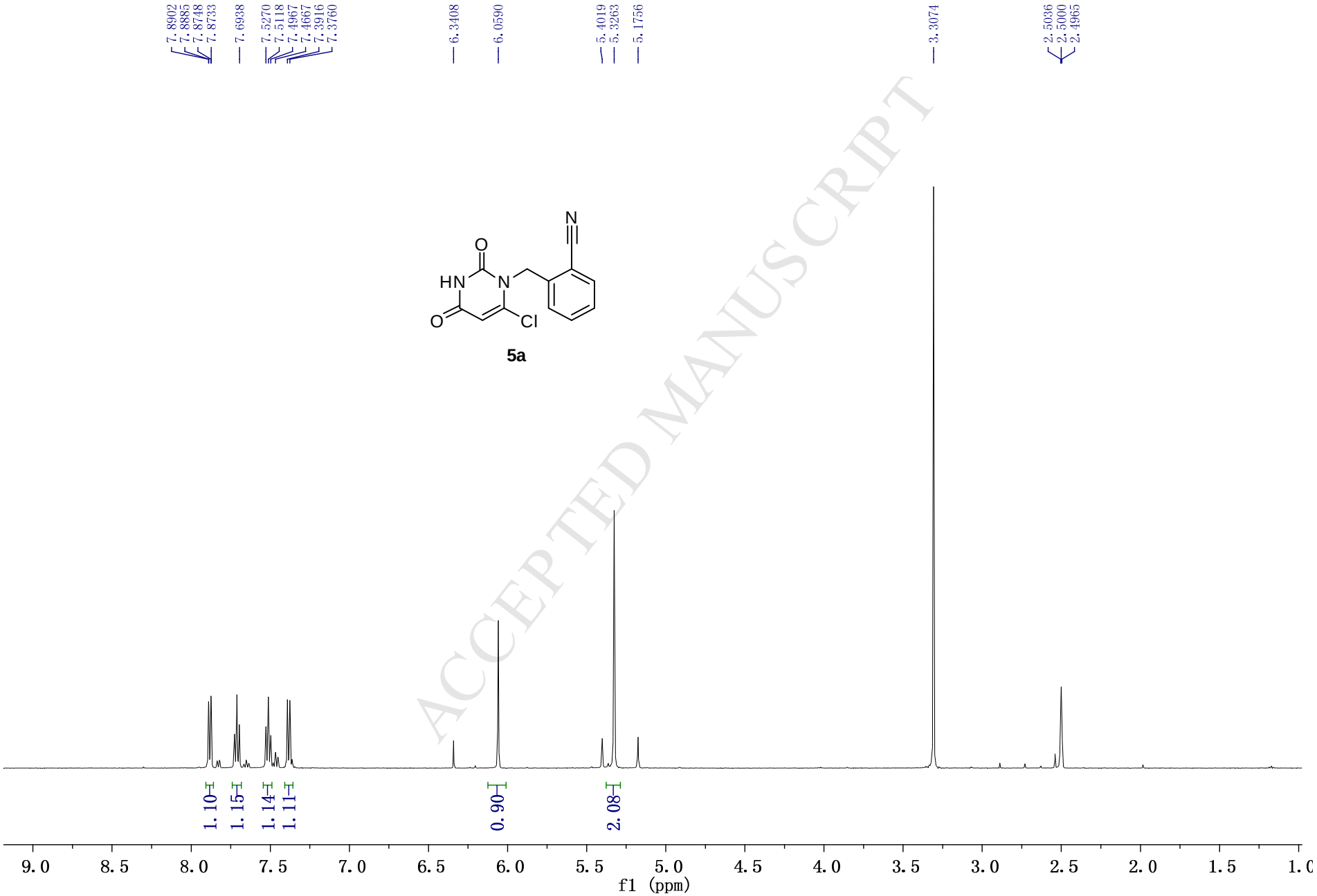

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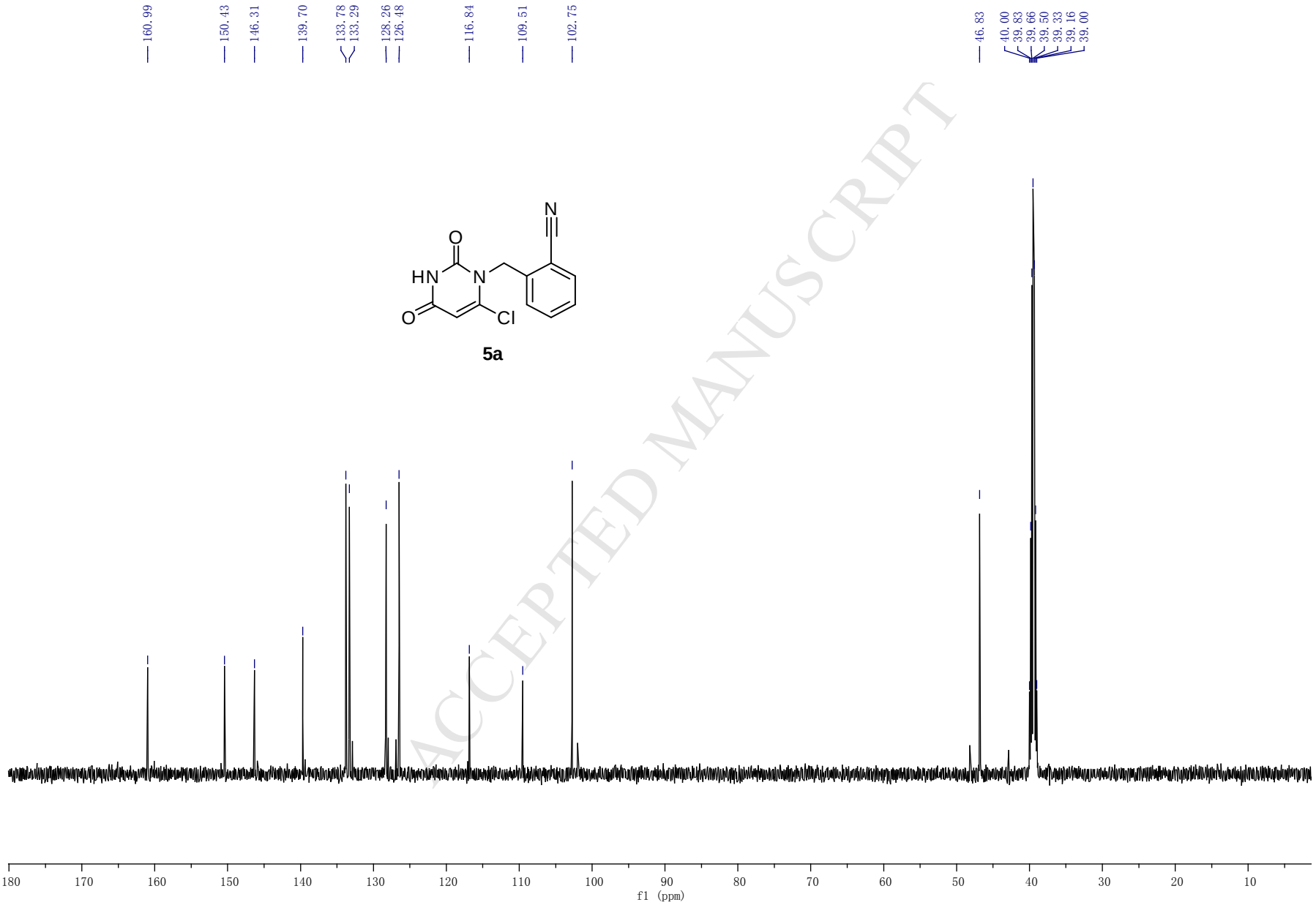

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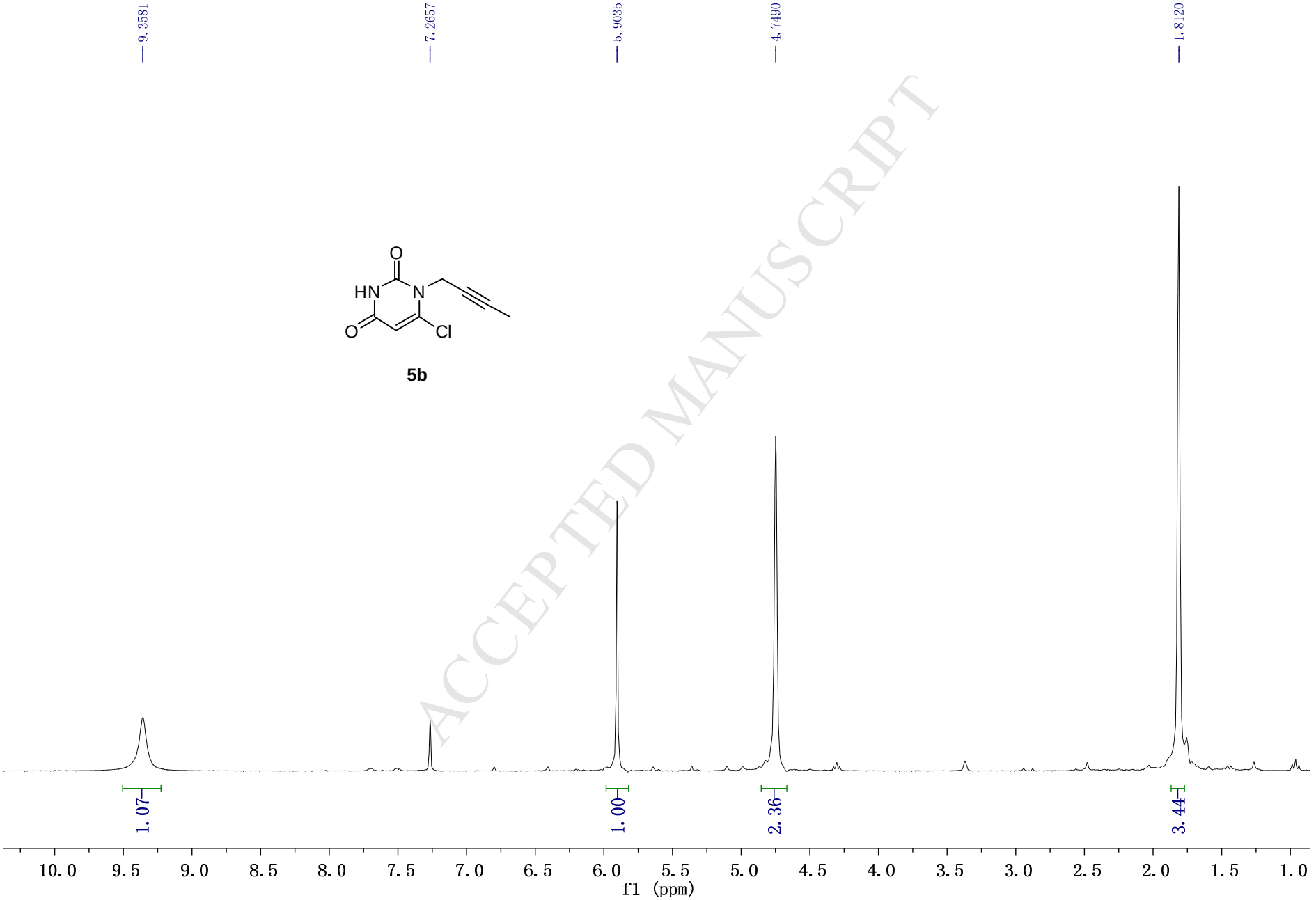

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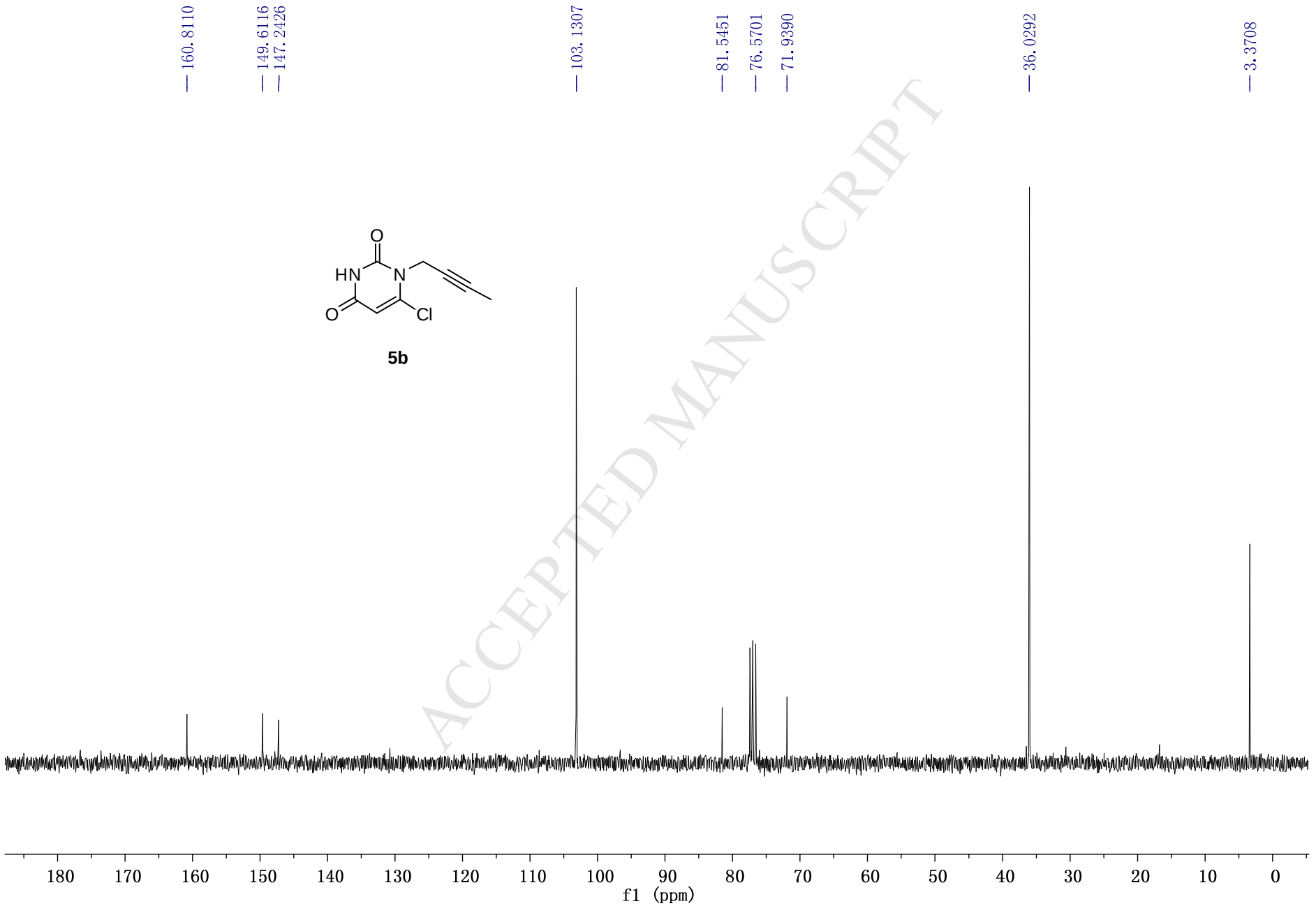

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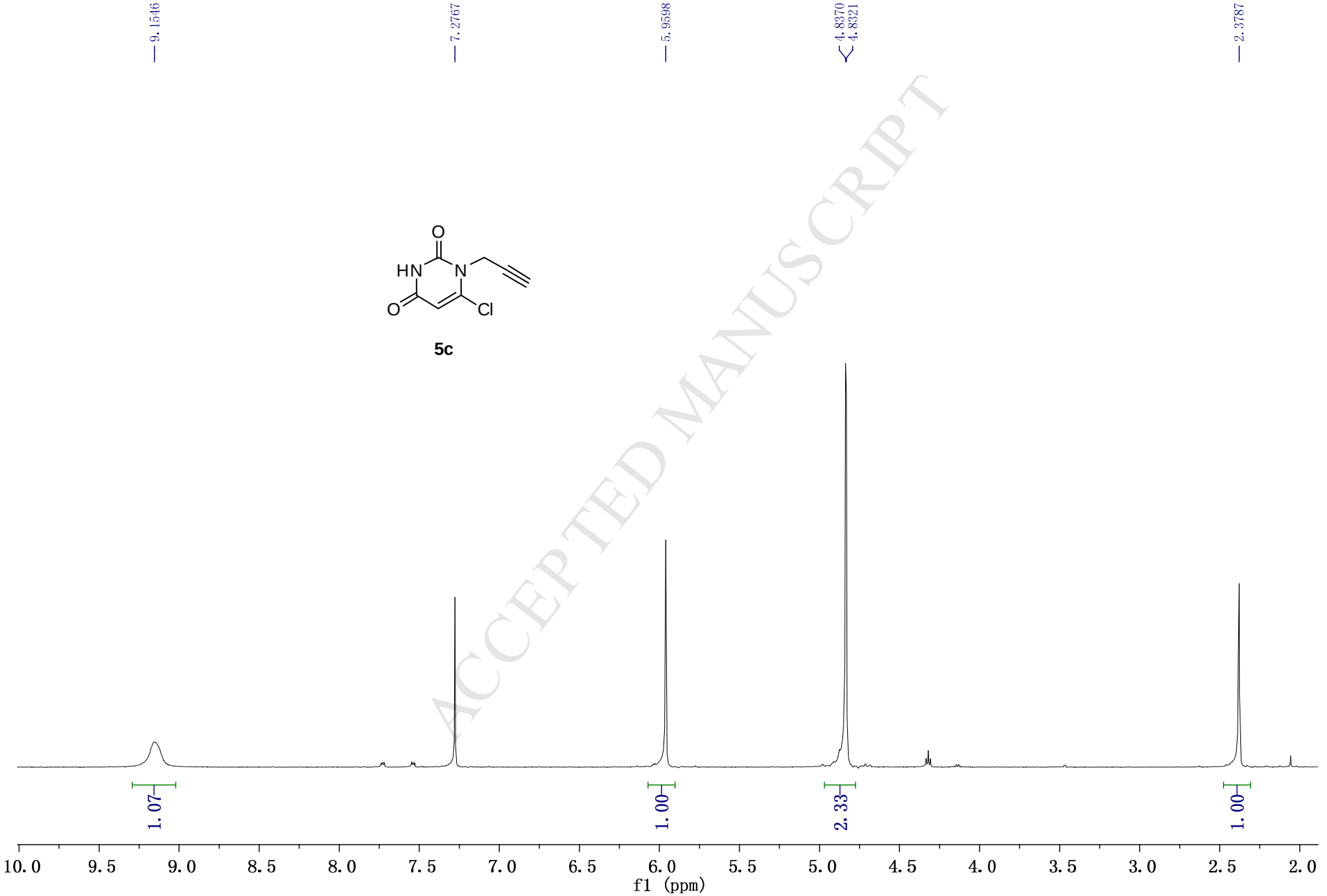

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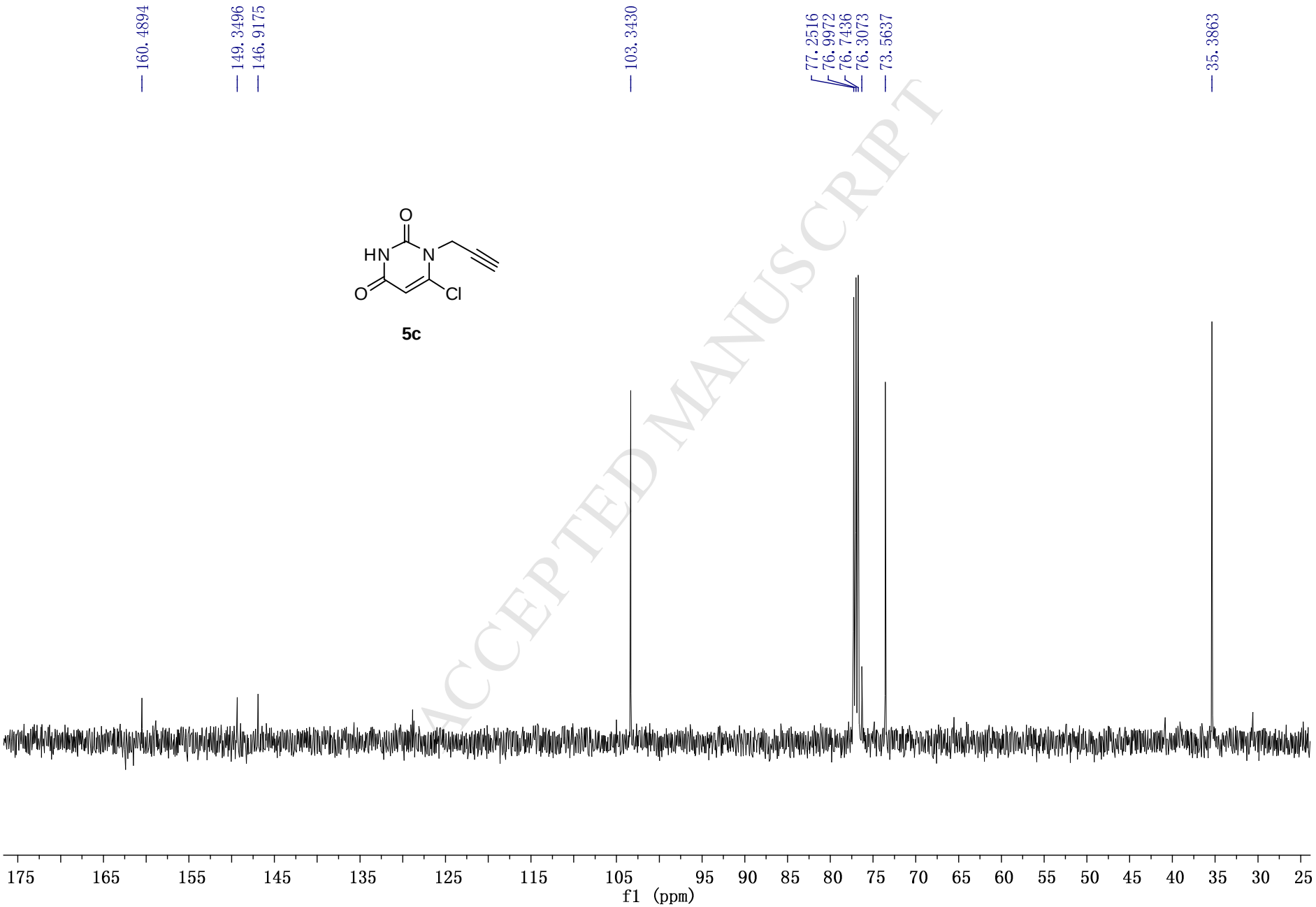

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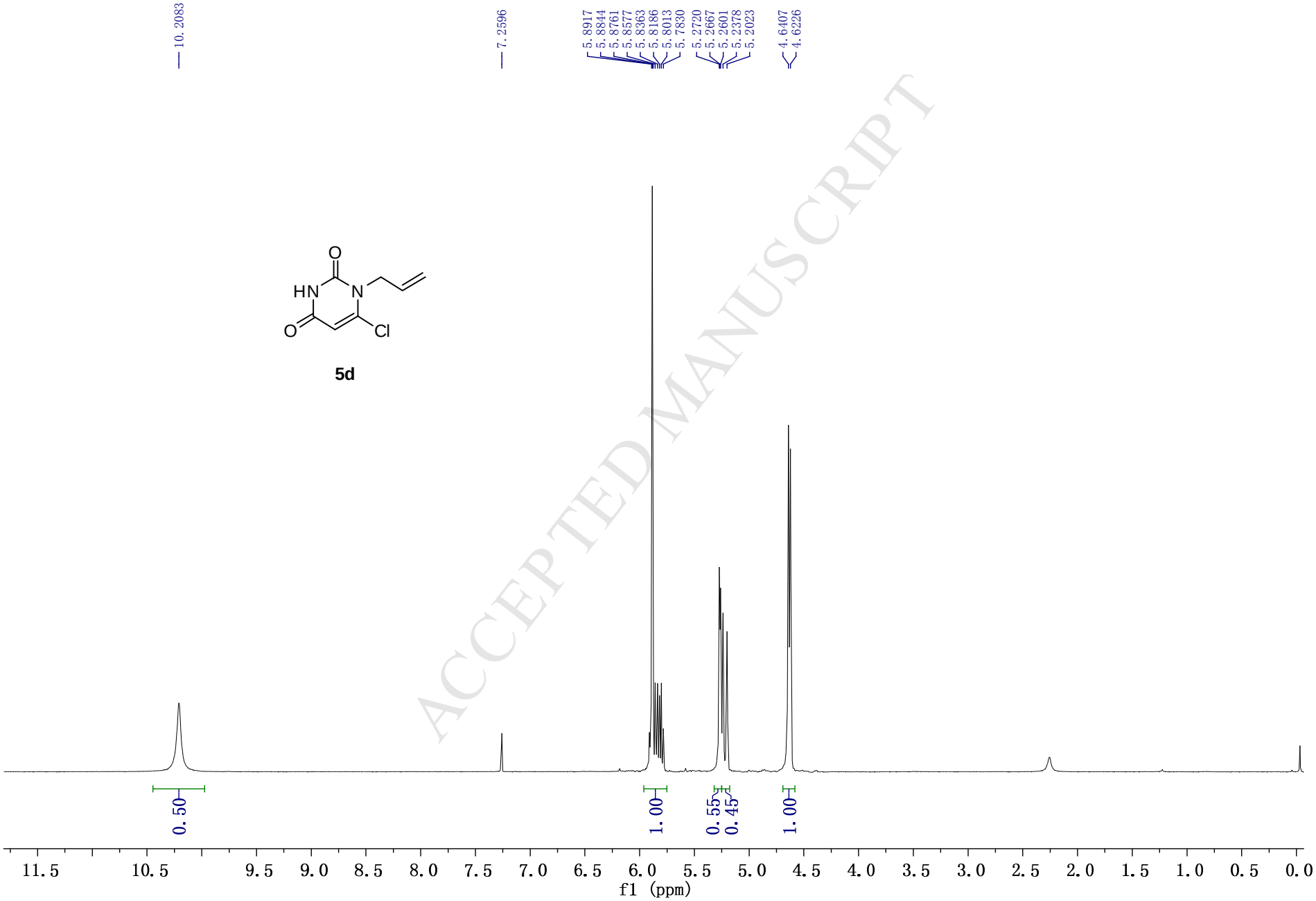

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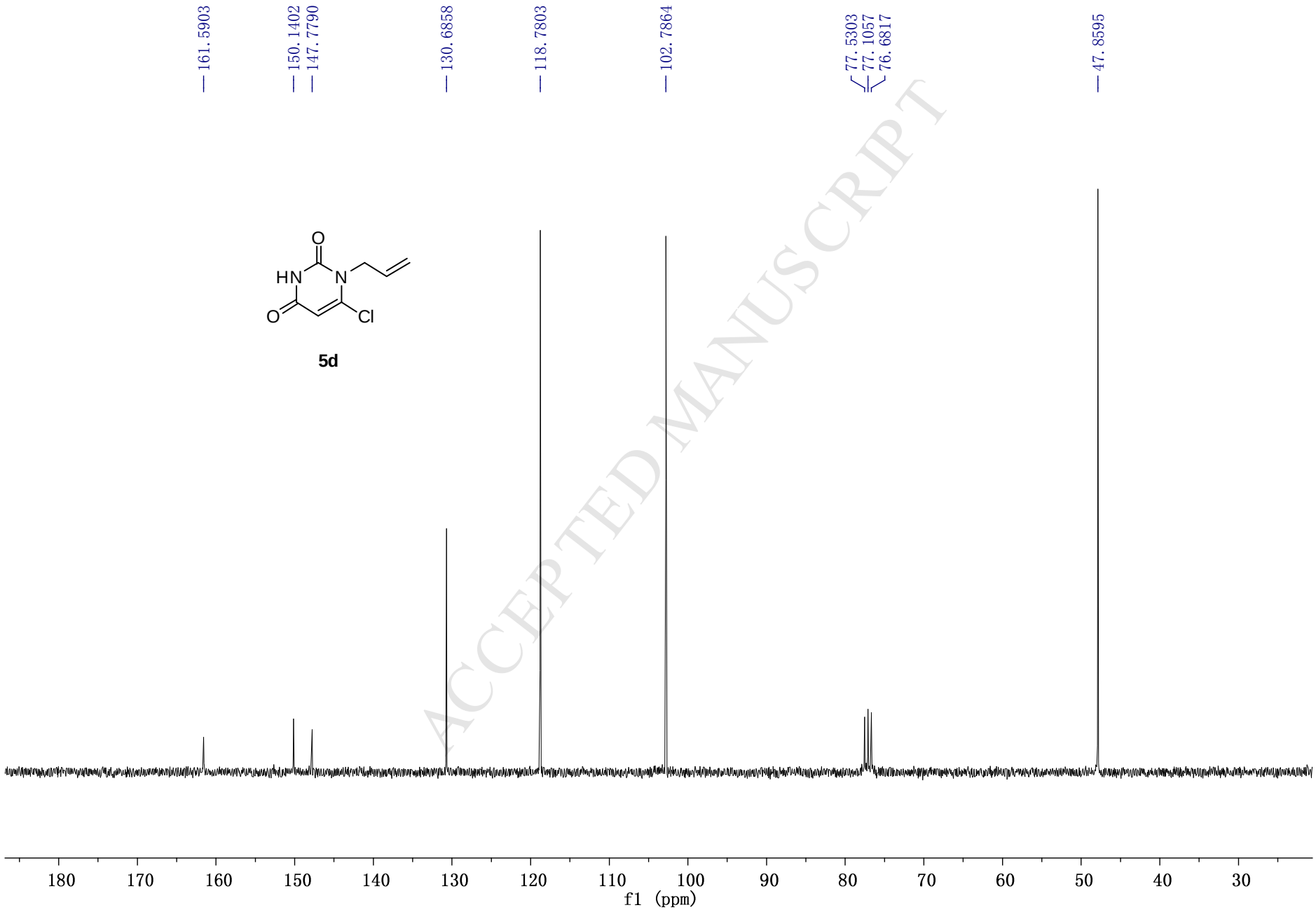

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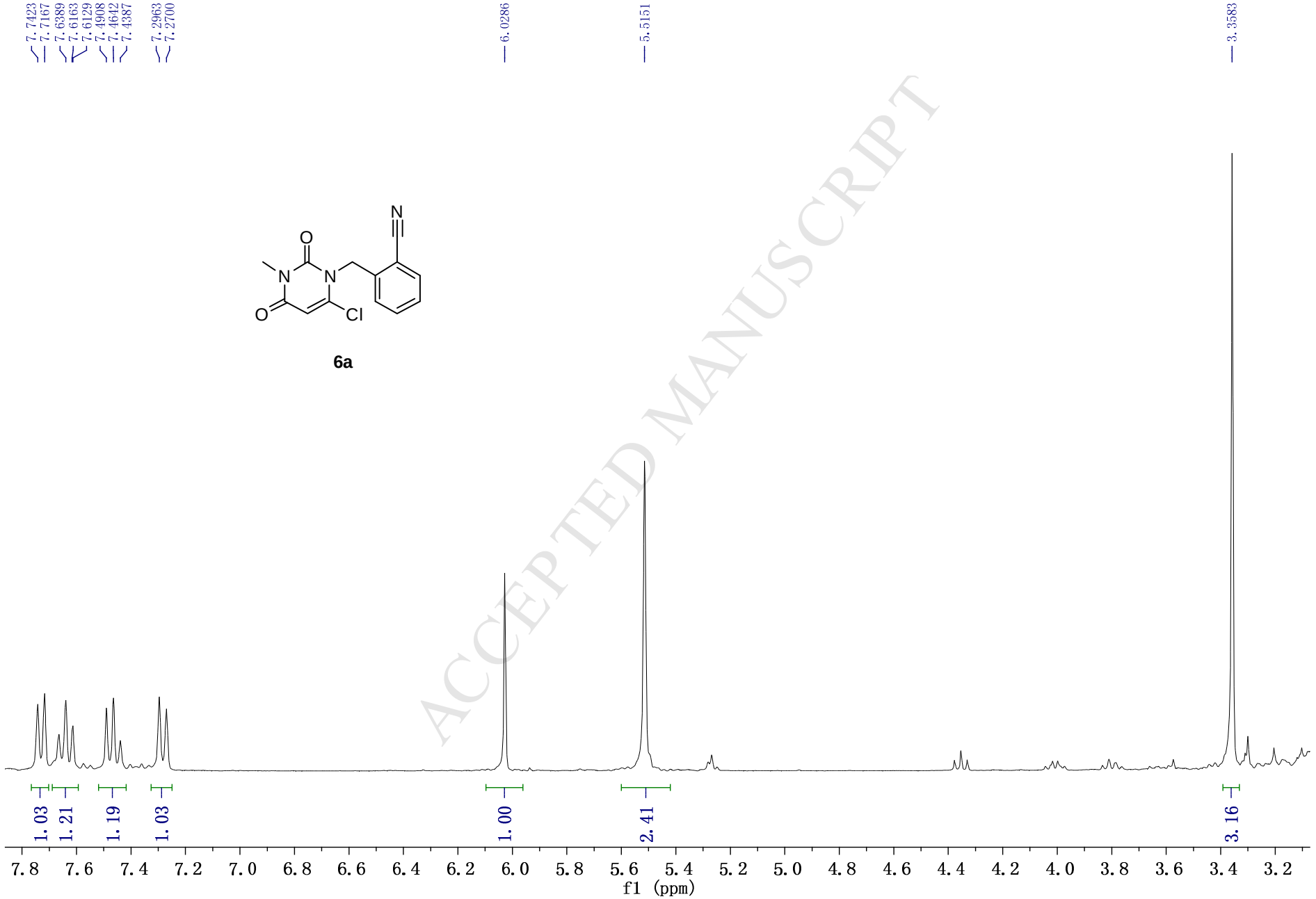

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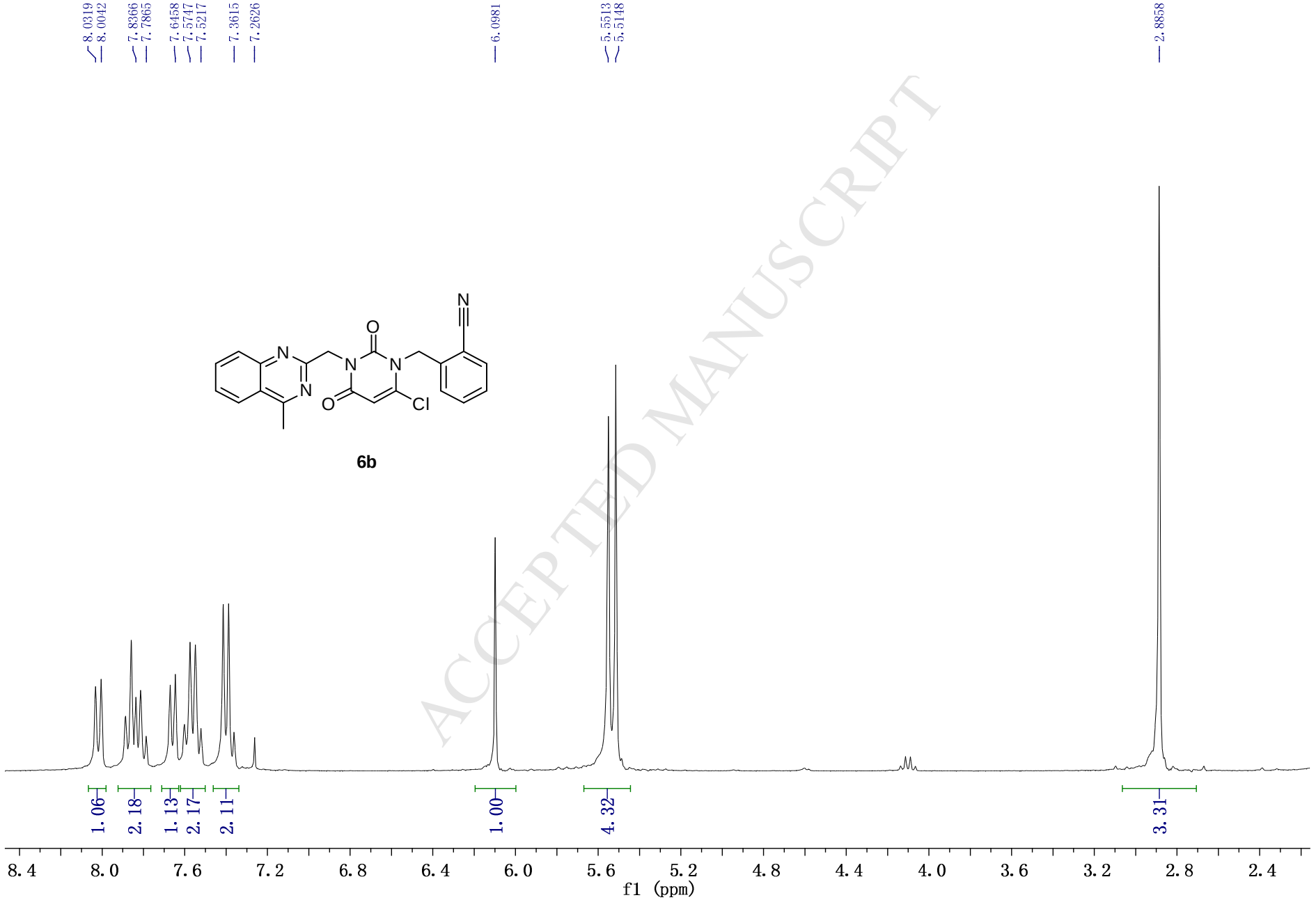

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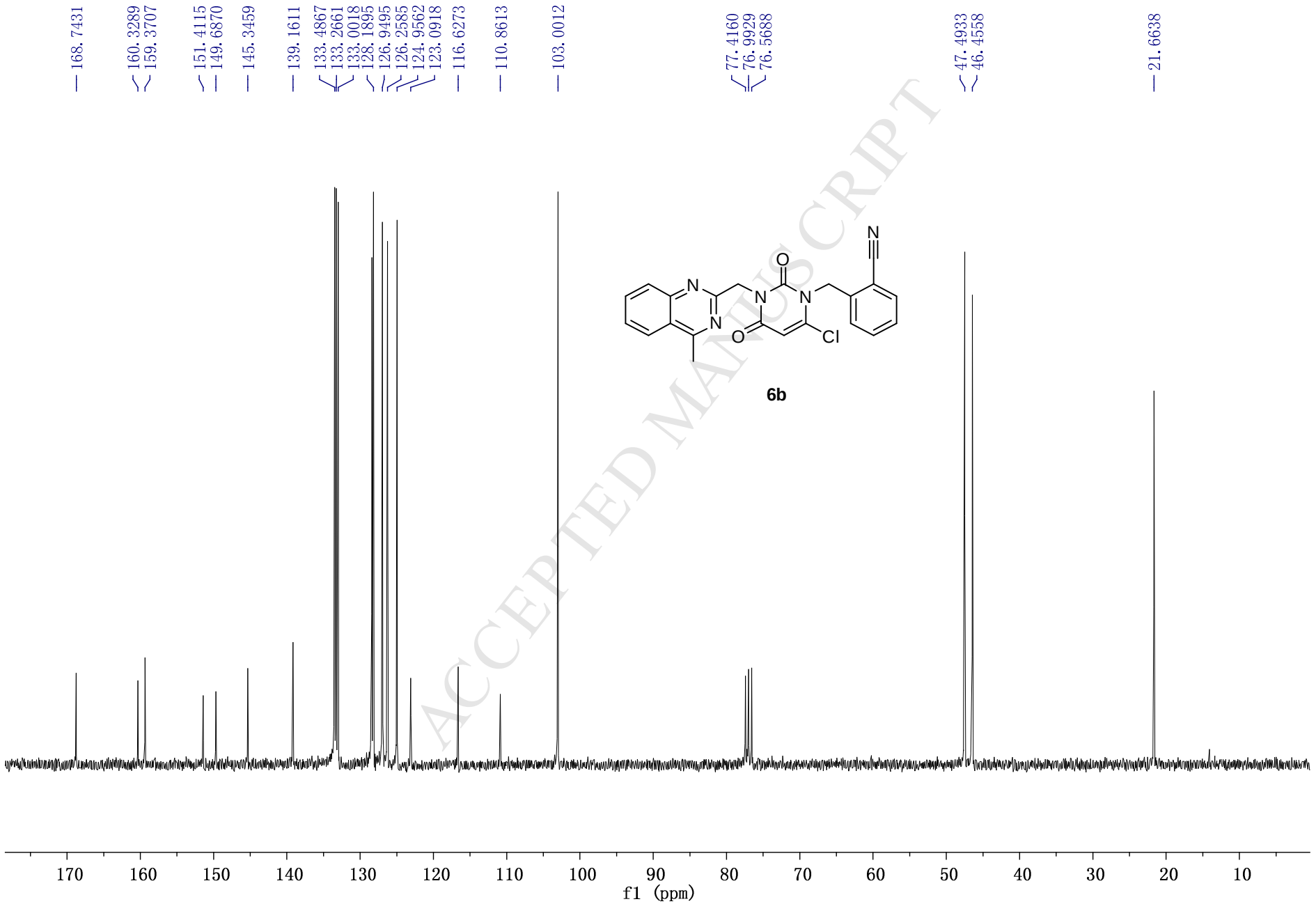

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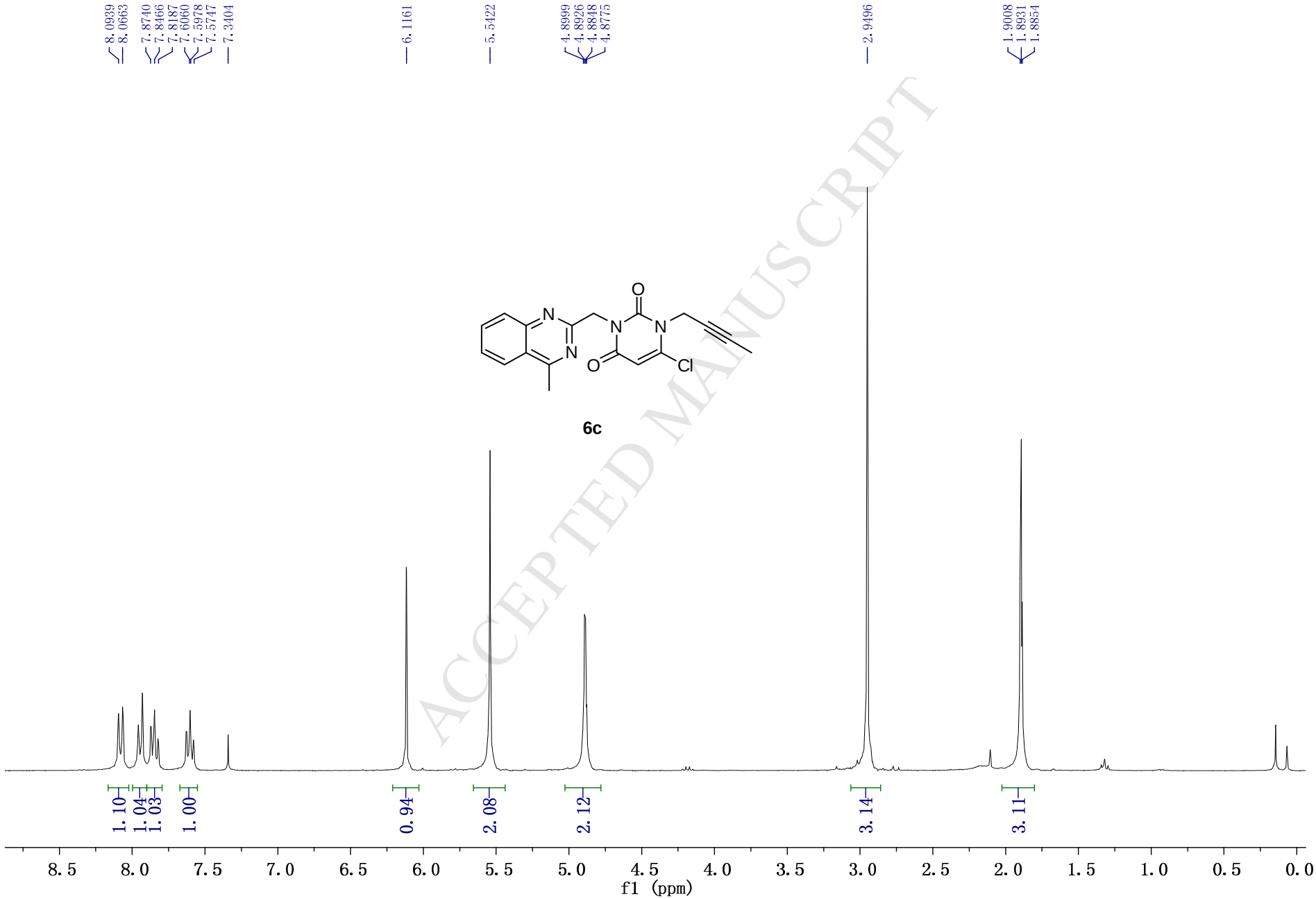

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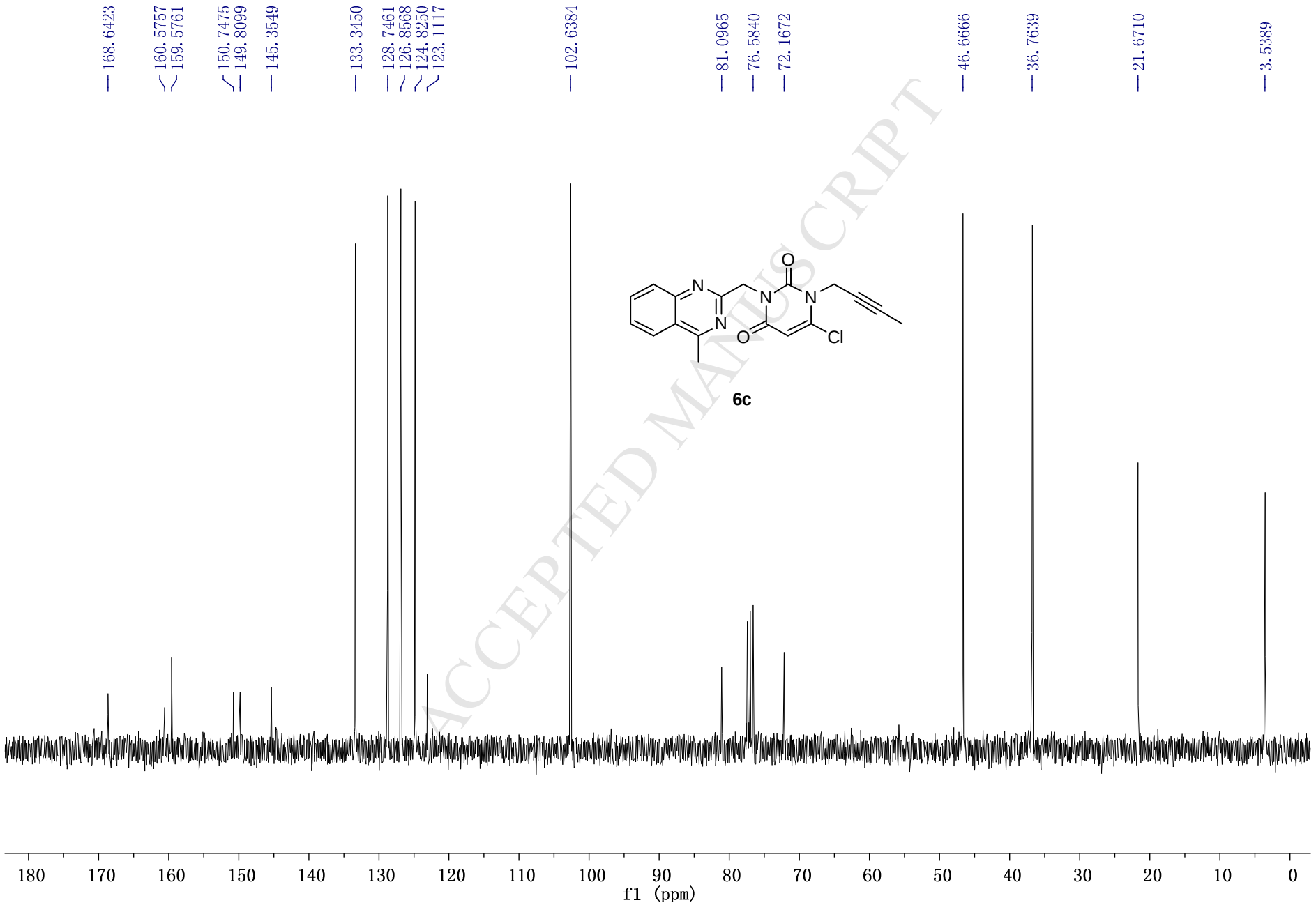

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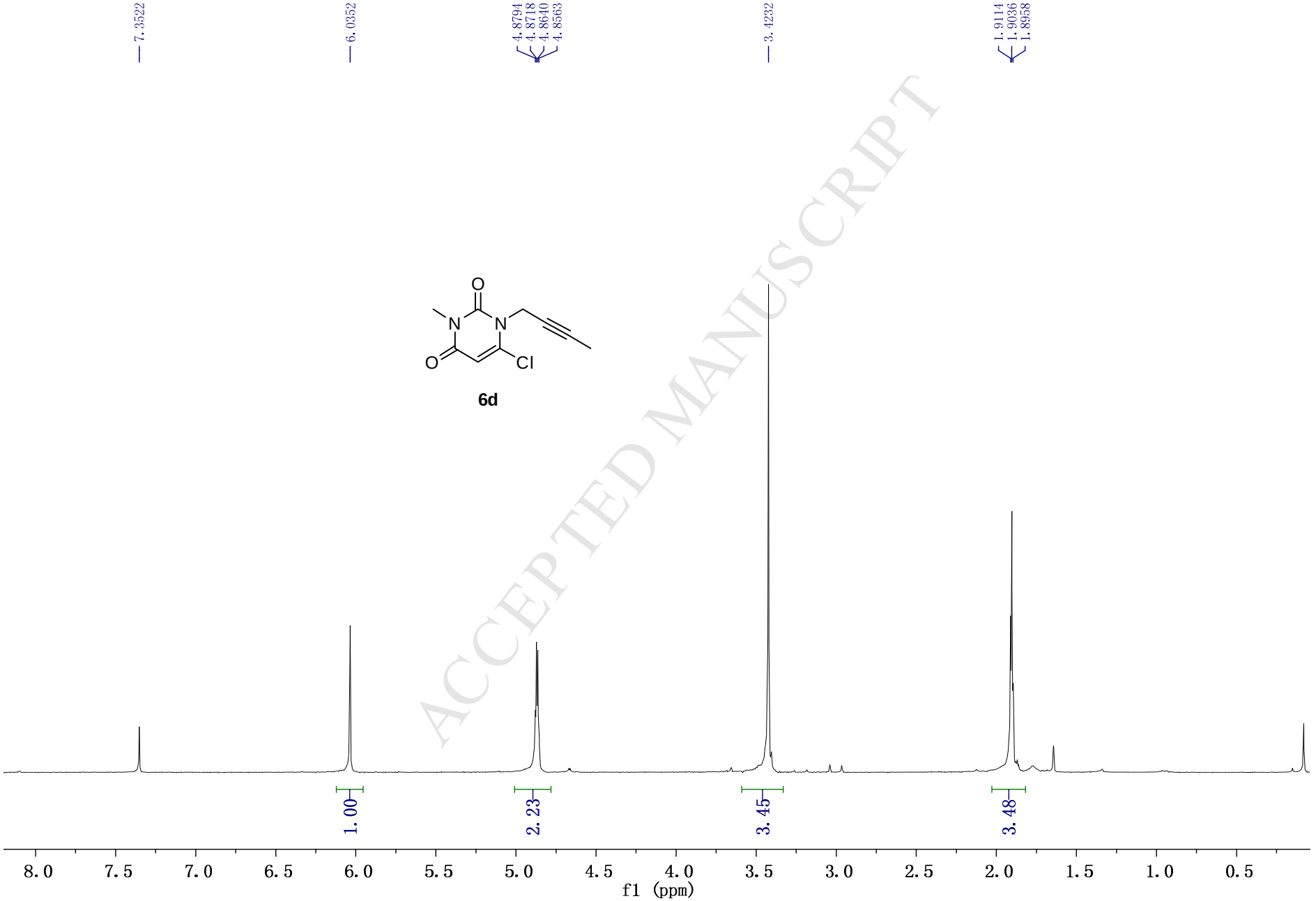

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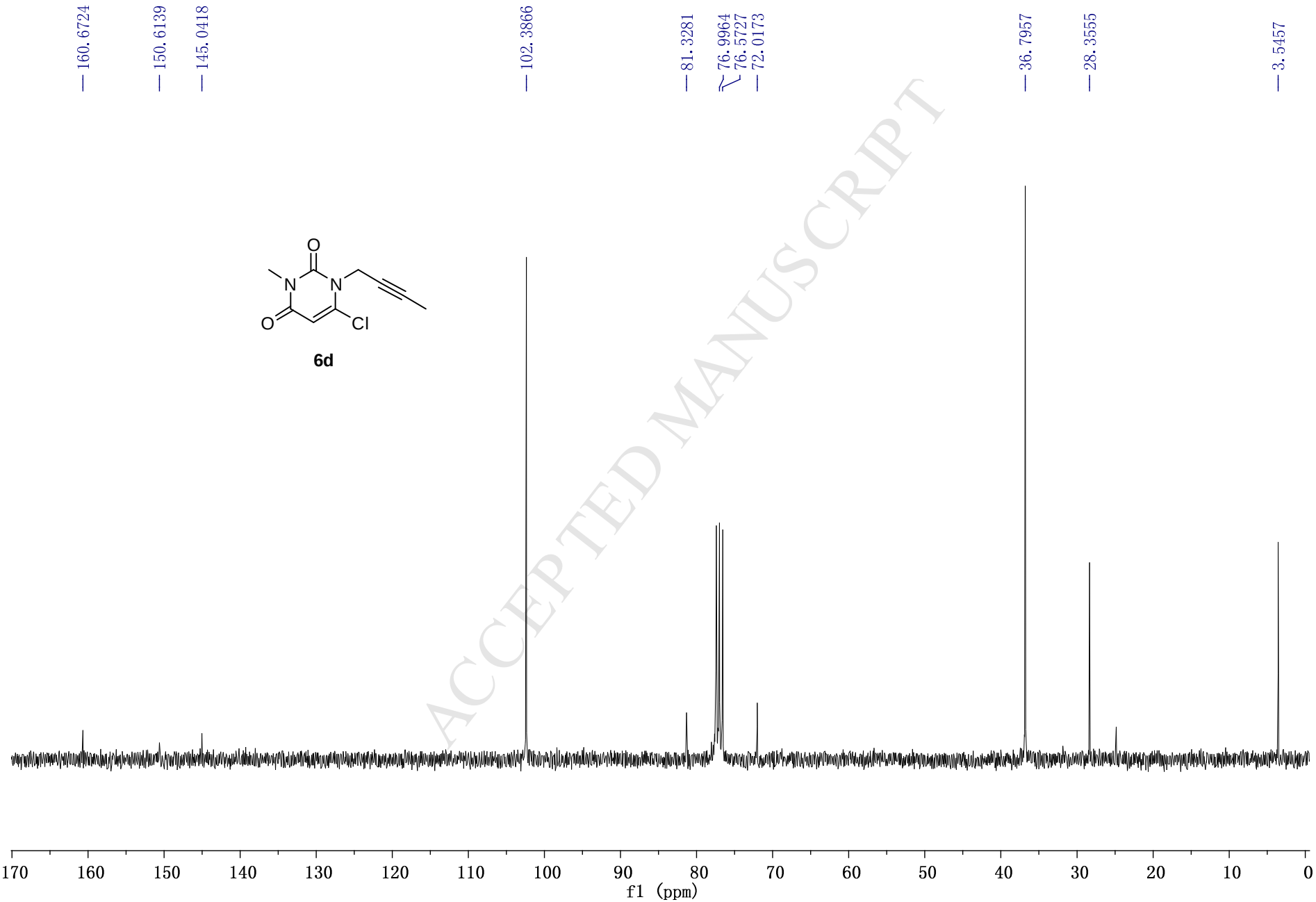

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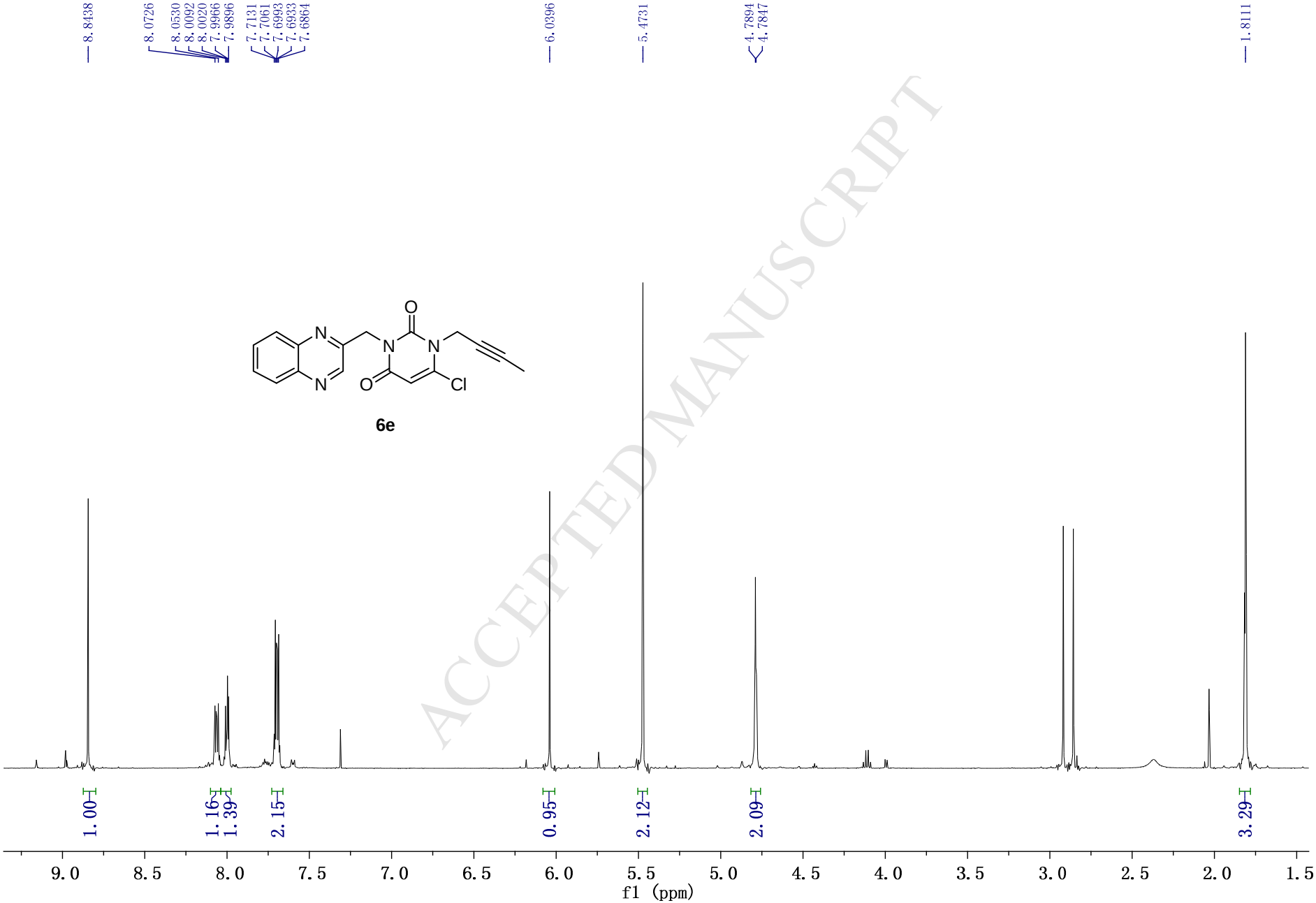

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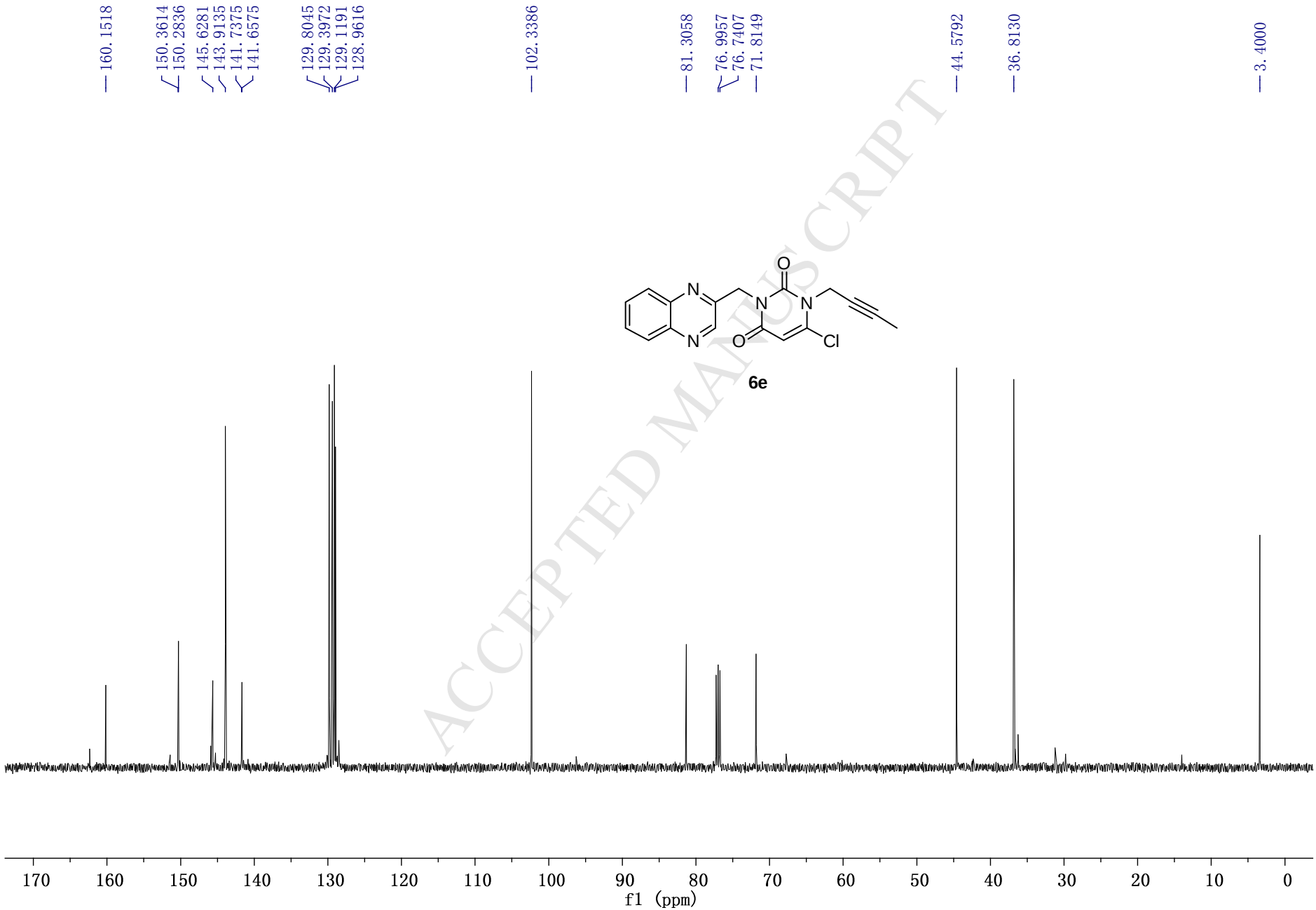

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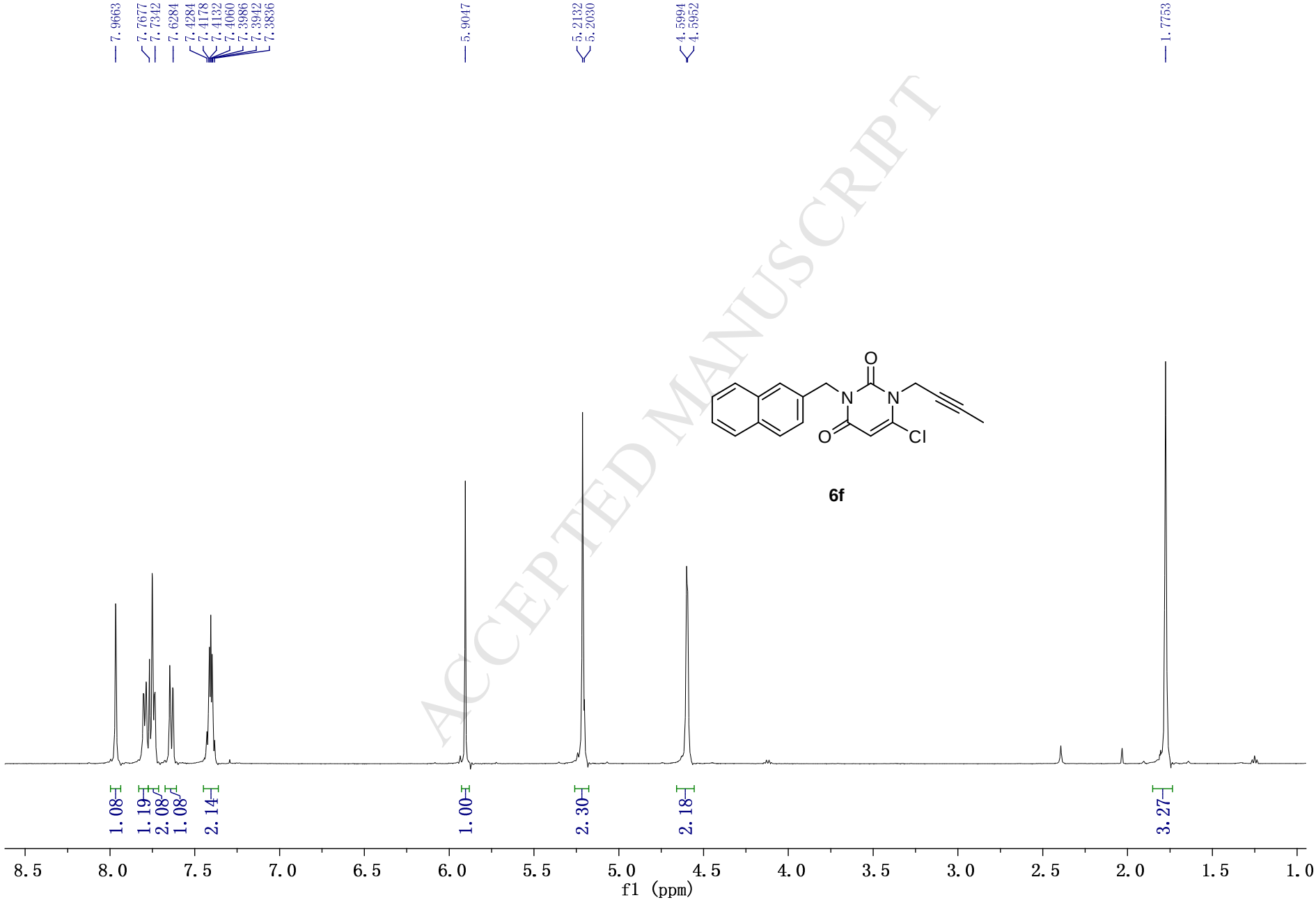
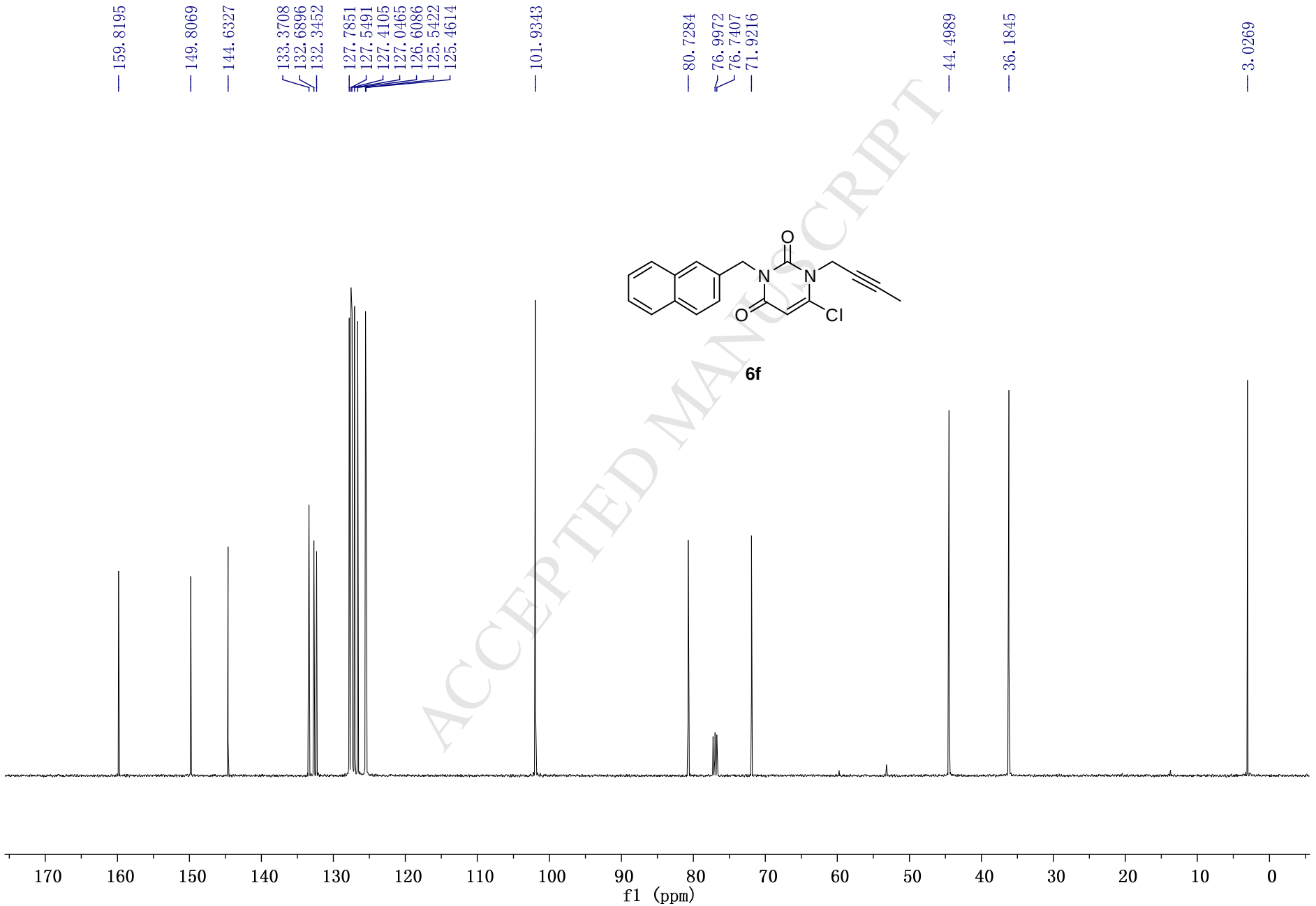


6f

^{13}C NMR spectrum of **6f**



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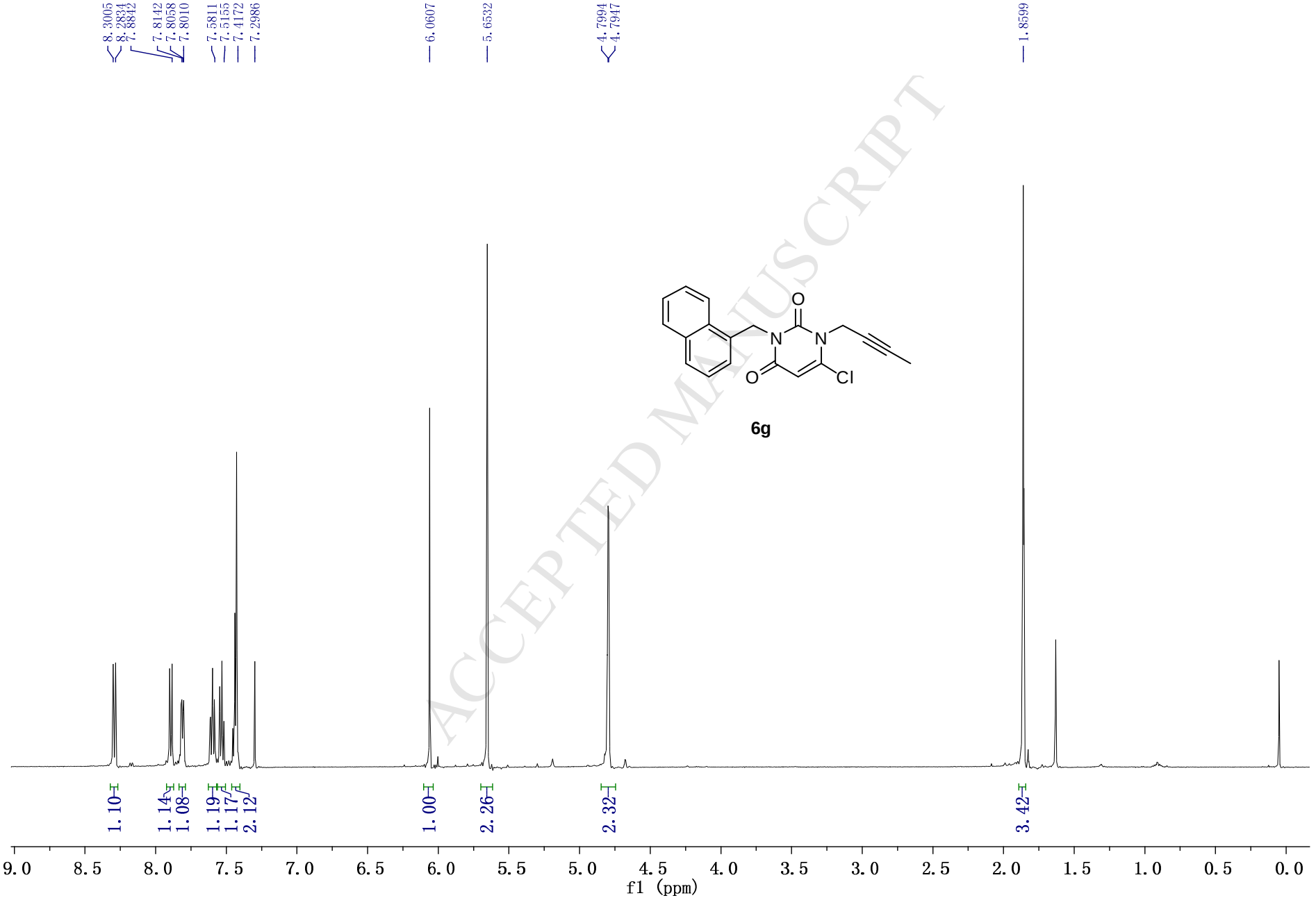

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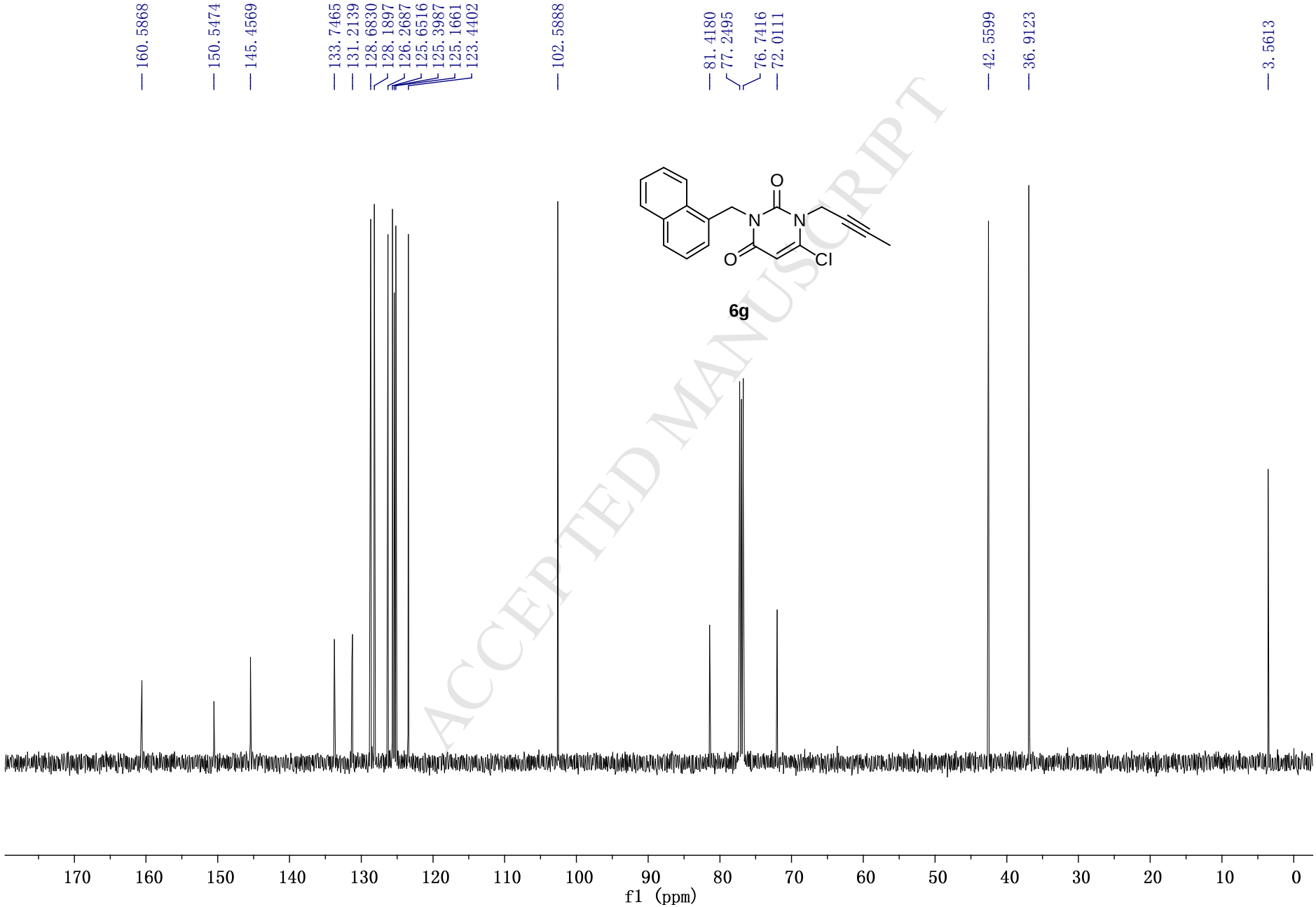

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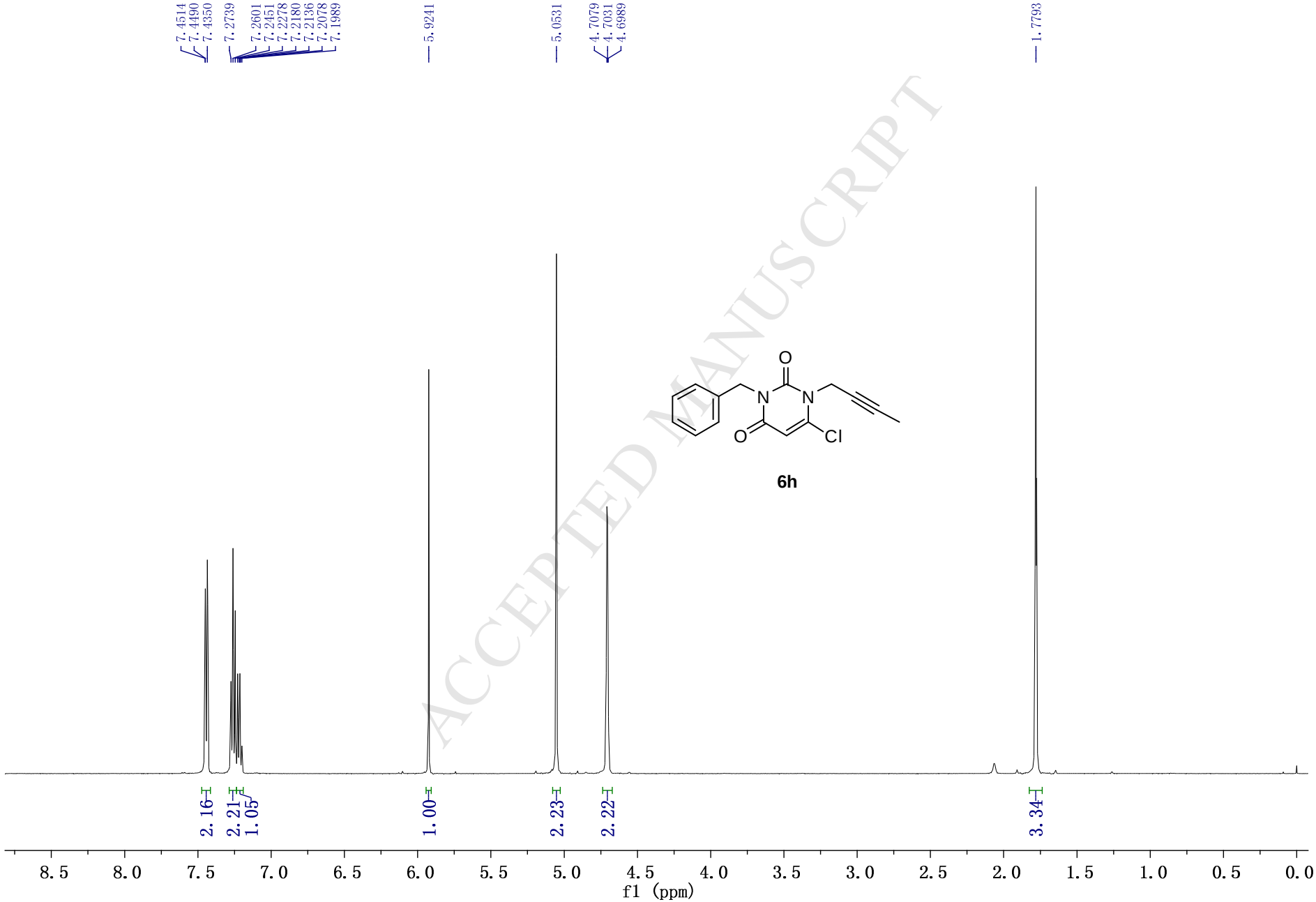

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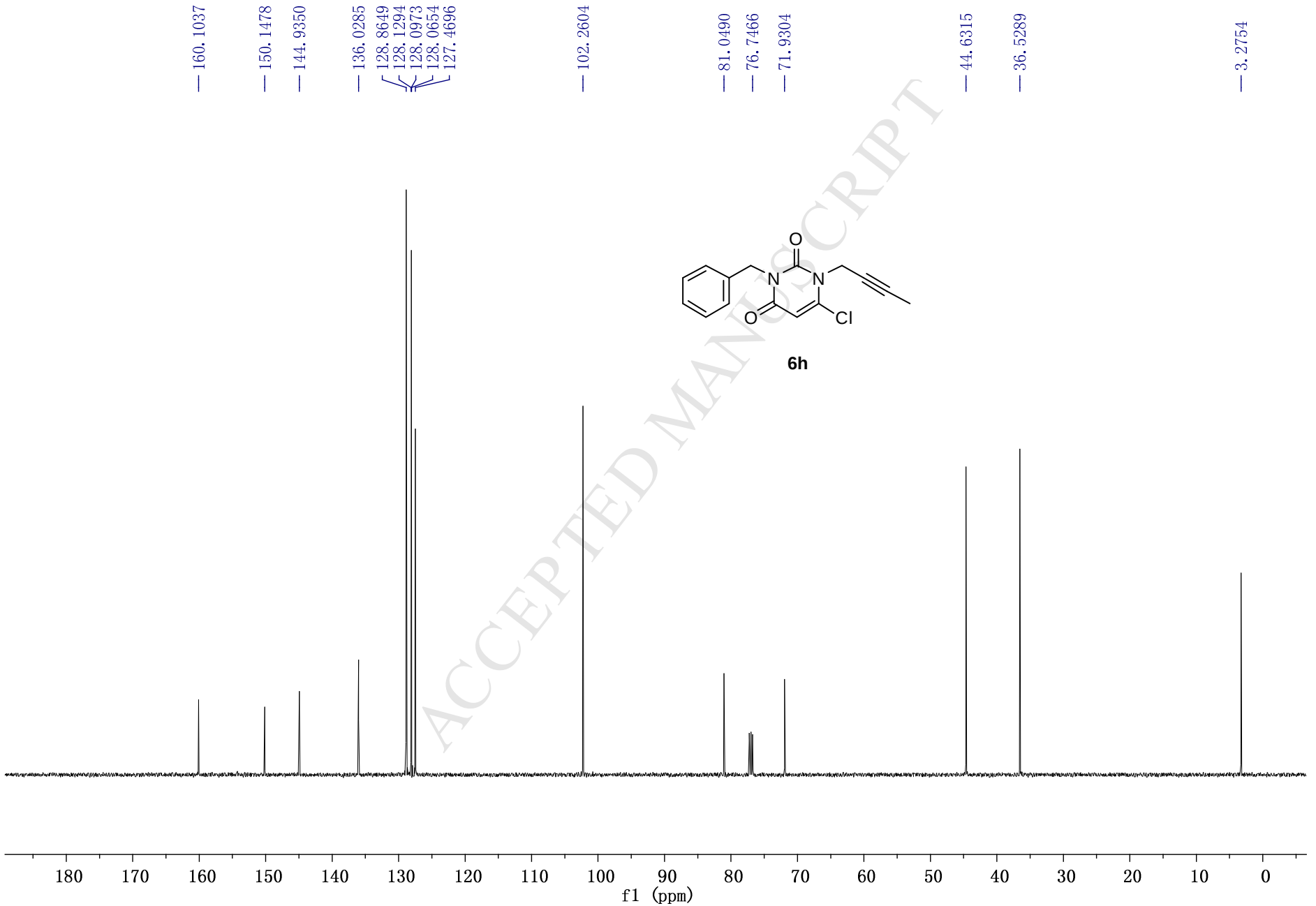
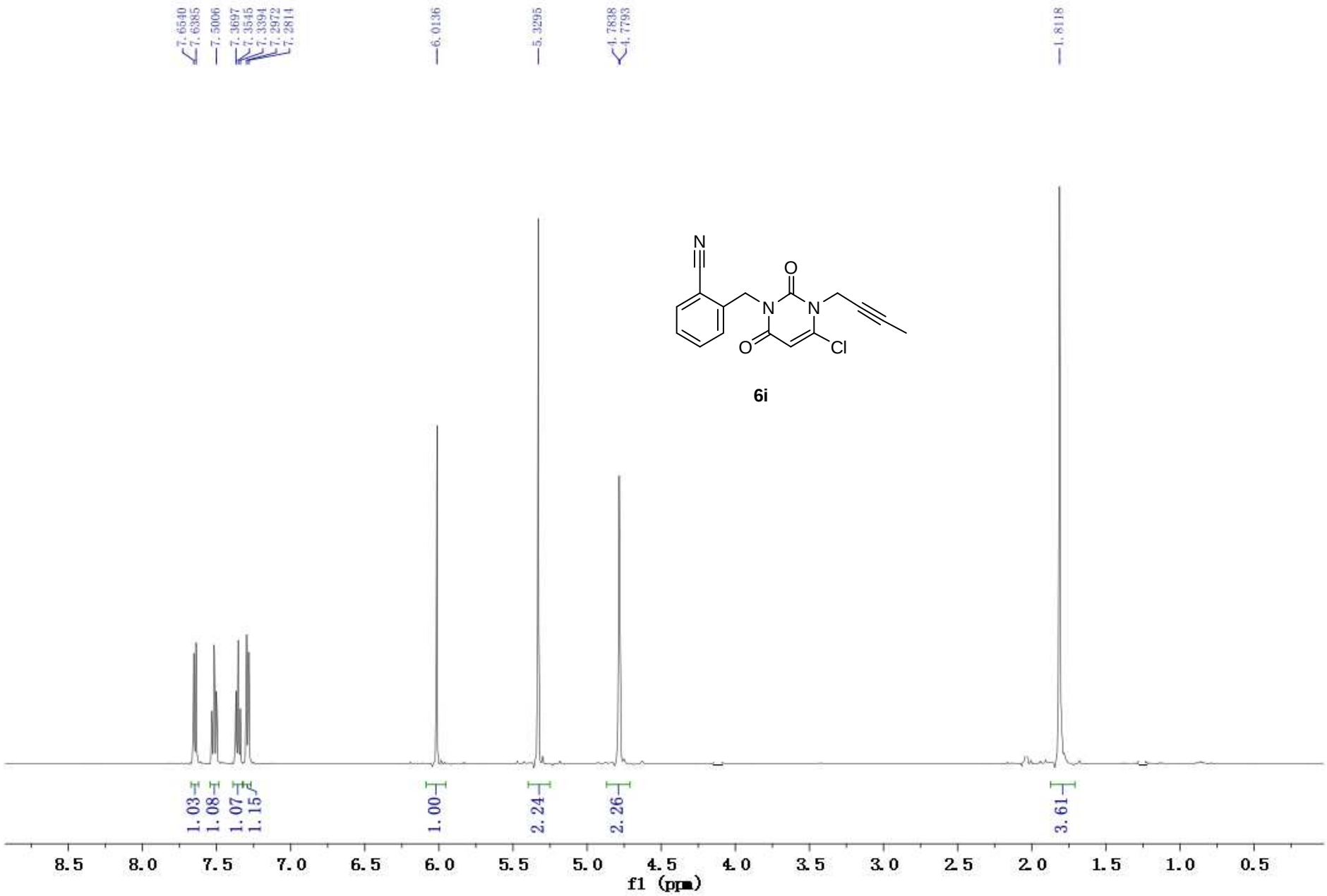
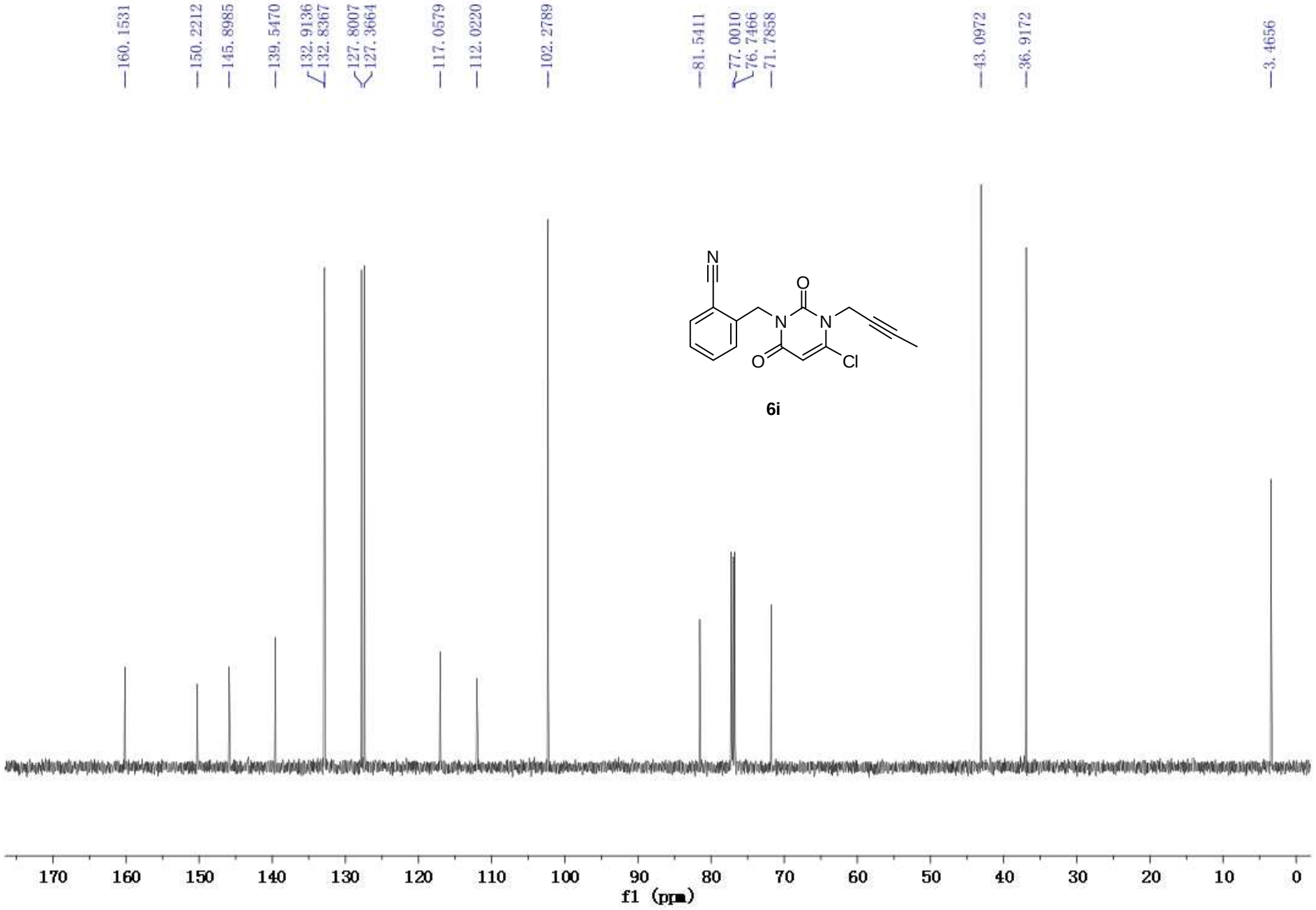


^{1}H NMR spectrum of **6i**

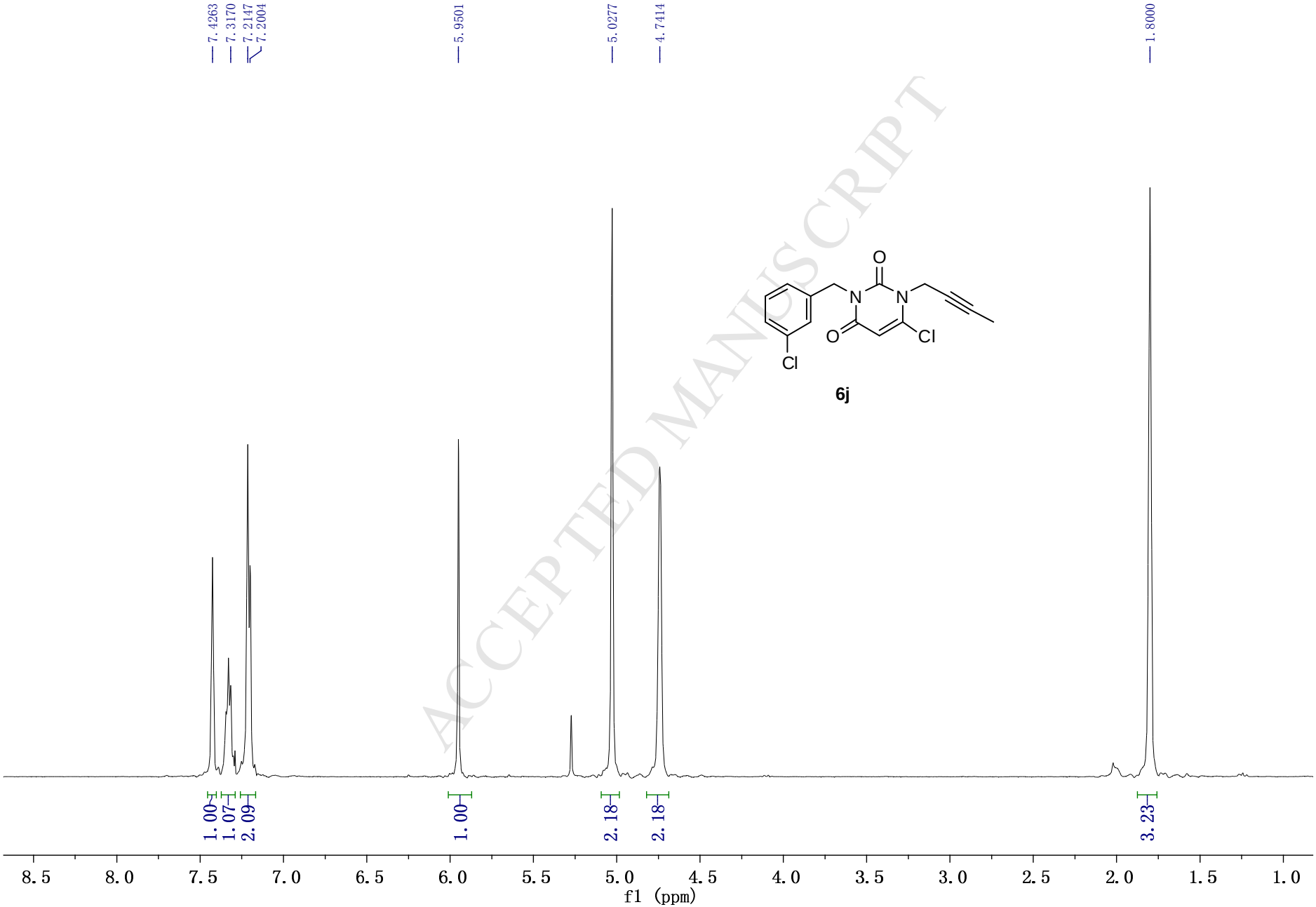


6i

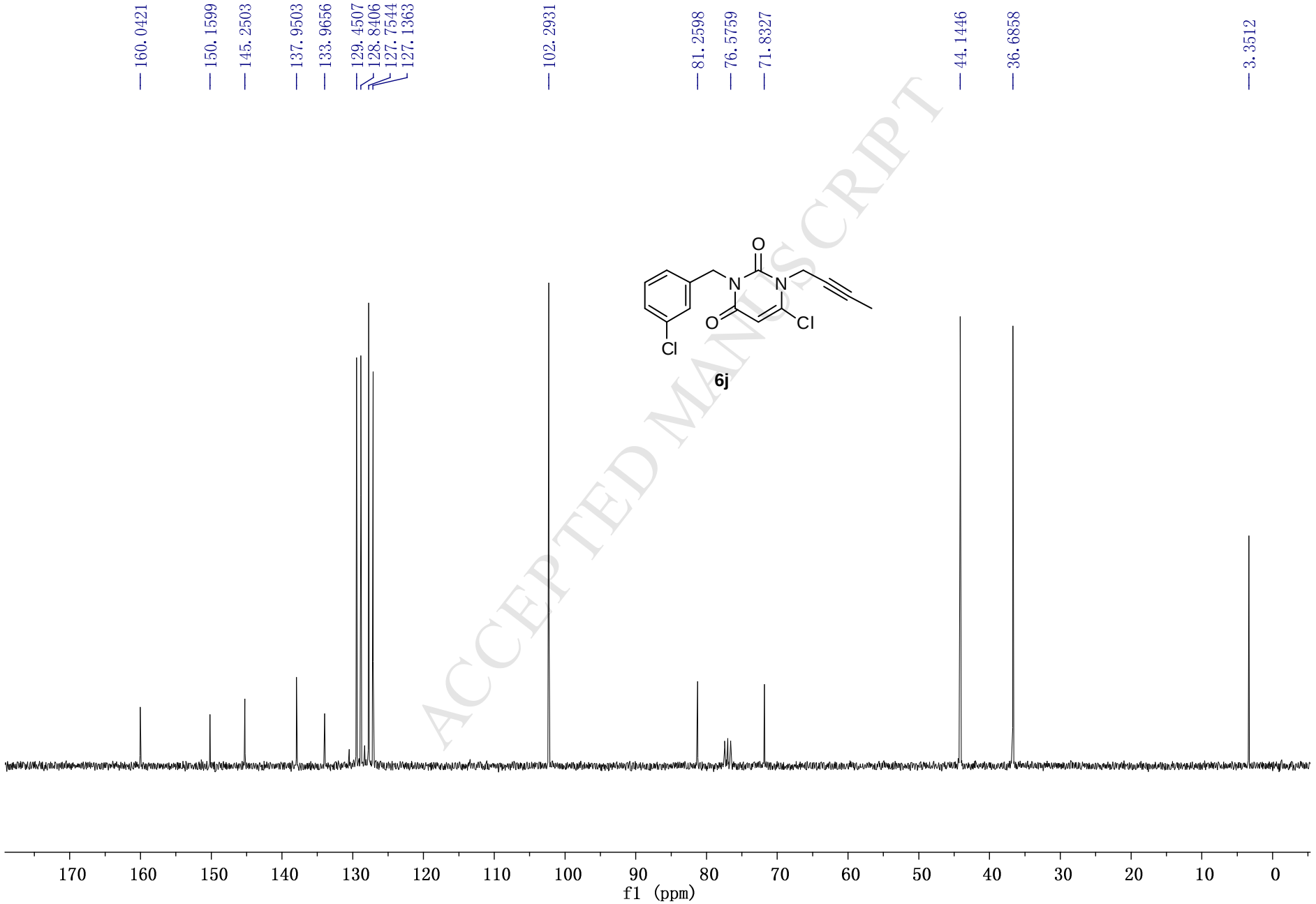
^{13}C NMR spectrum of **6i**



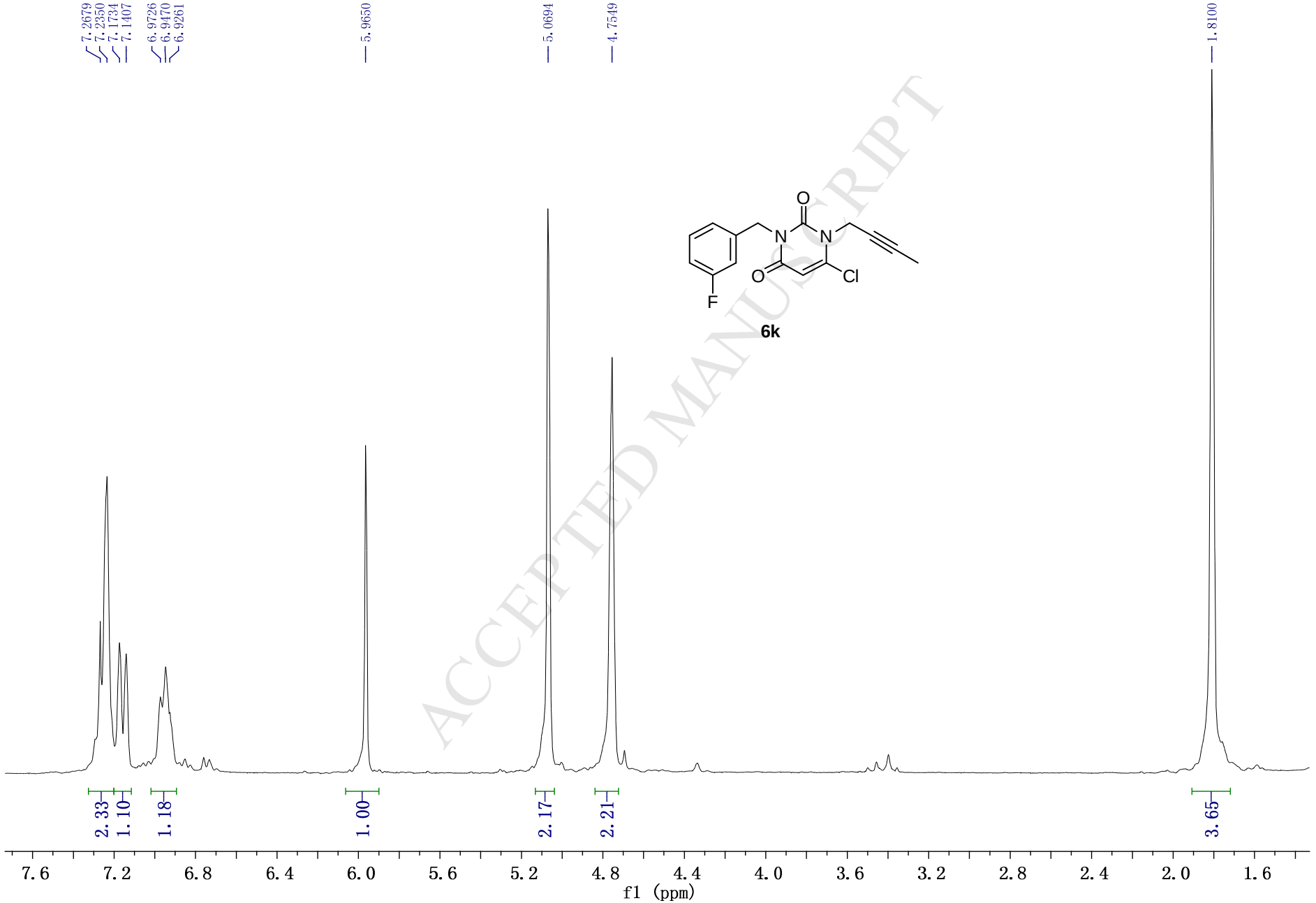
^{1}H NMR spectrum of **6j**



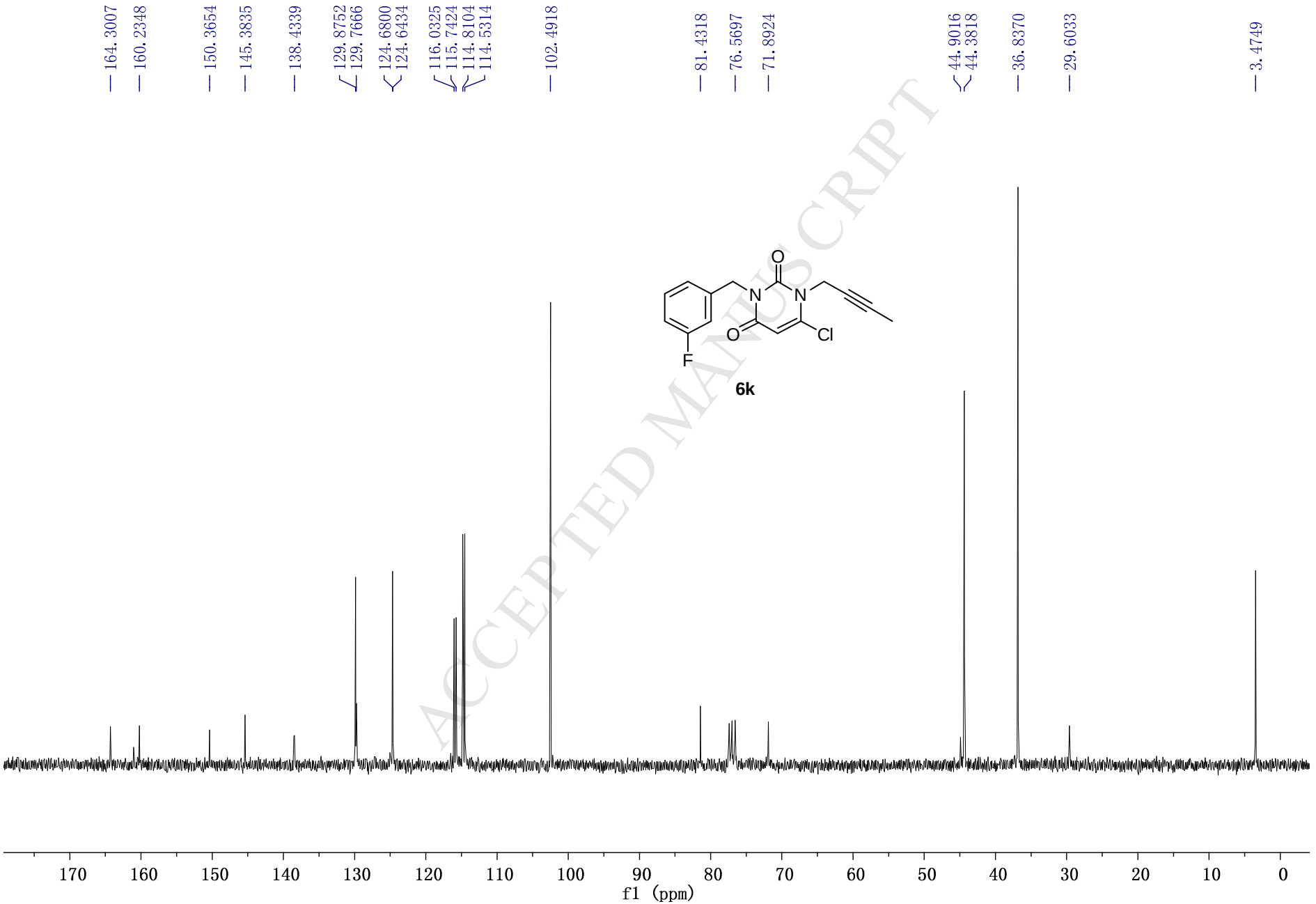
^{13}C NMR spectrum of **6j**



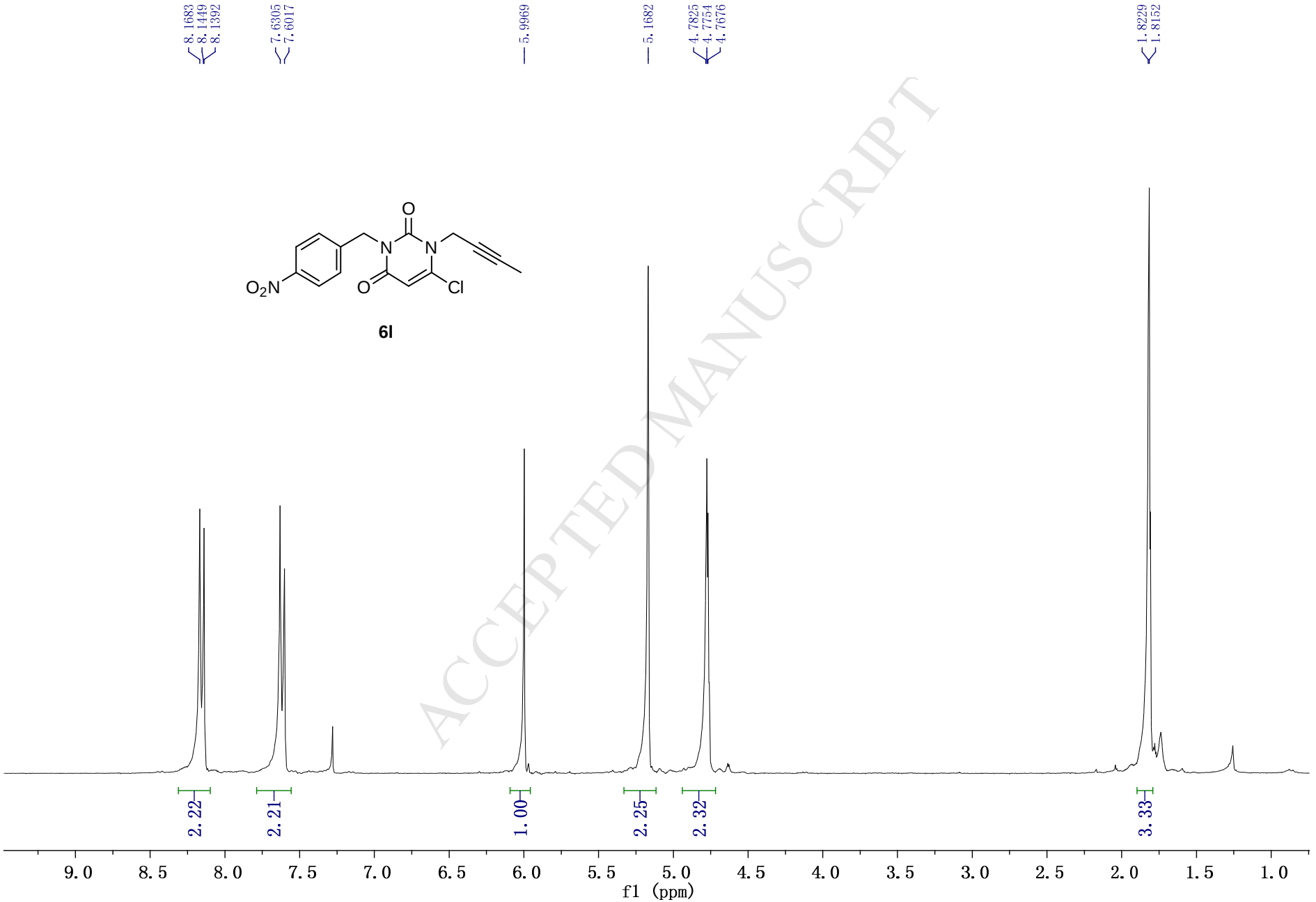
^{1}H NMR spectrum of **6k**



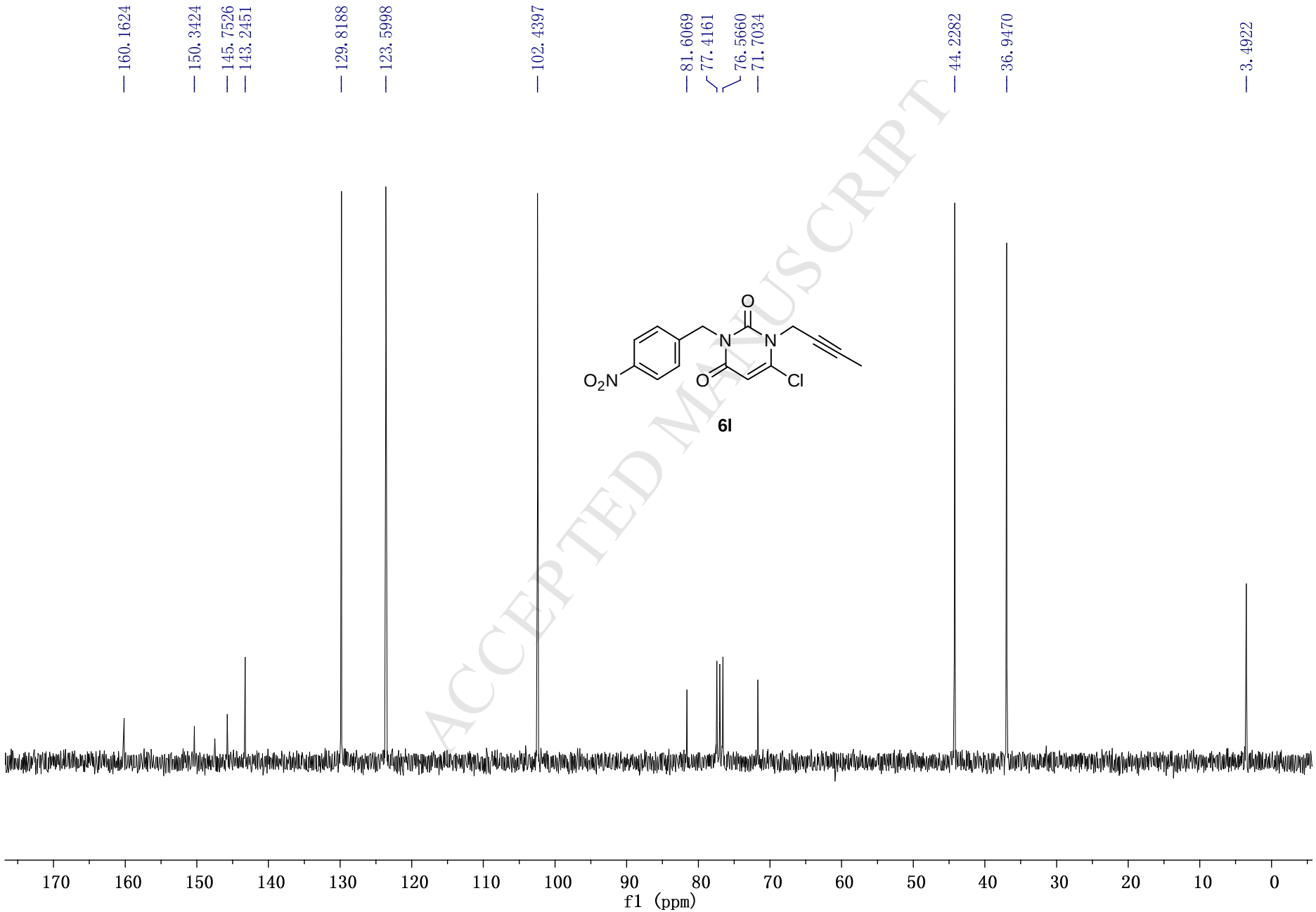
^{13}C NMR spectrum of **6k**



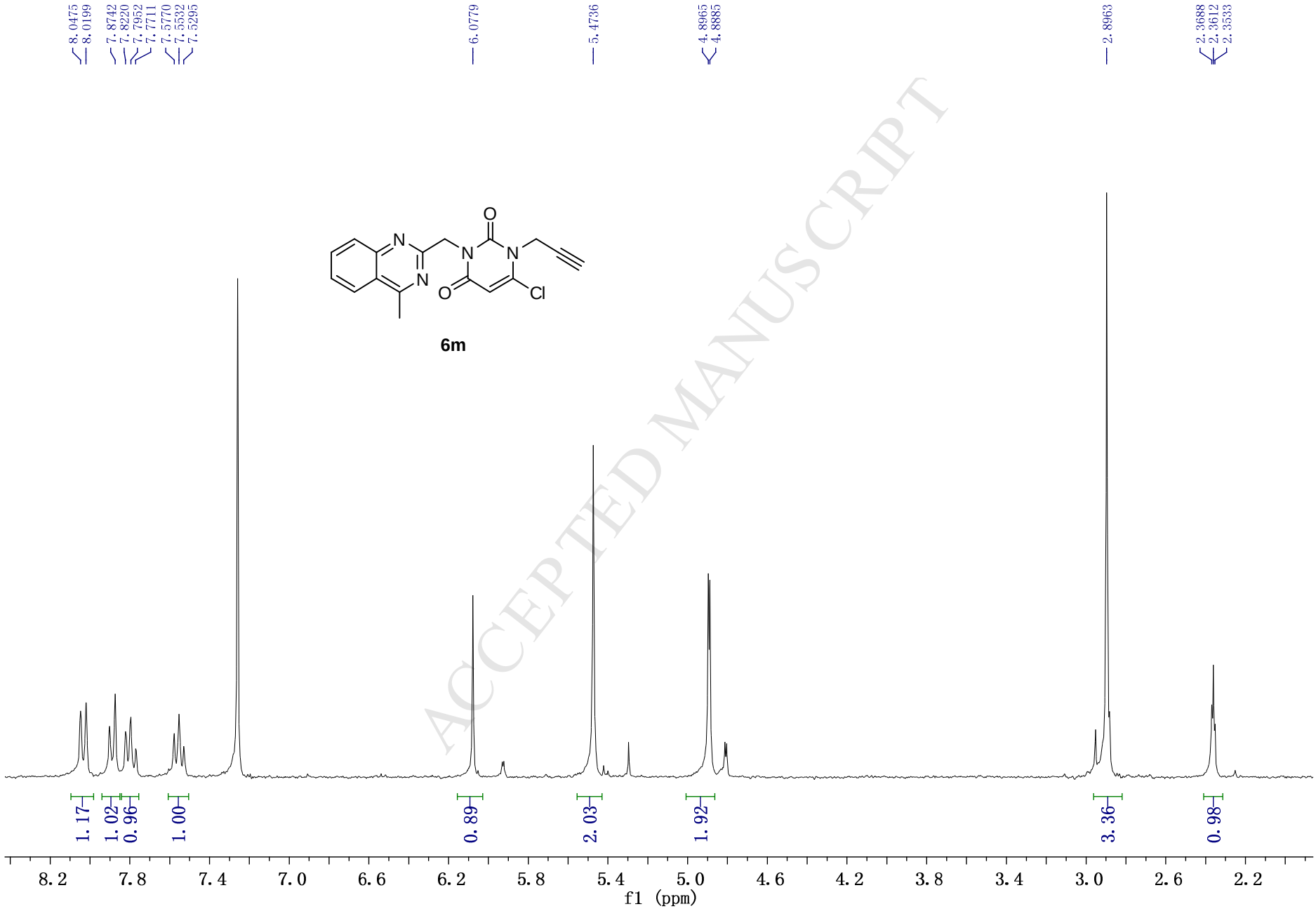
^{1}H NMR spectrum of **6l**



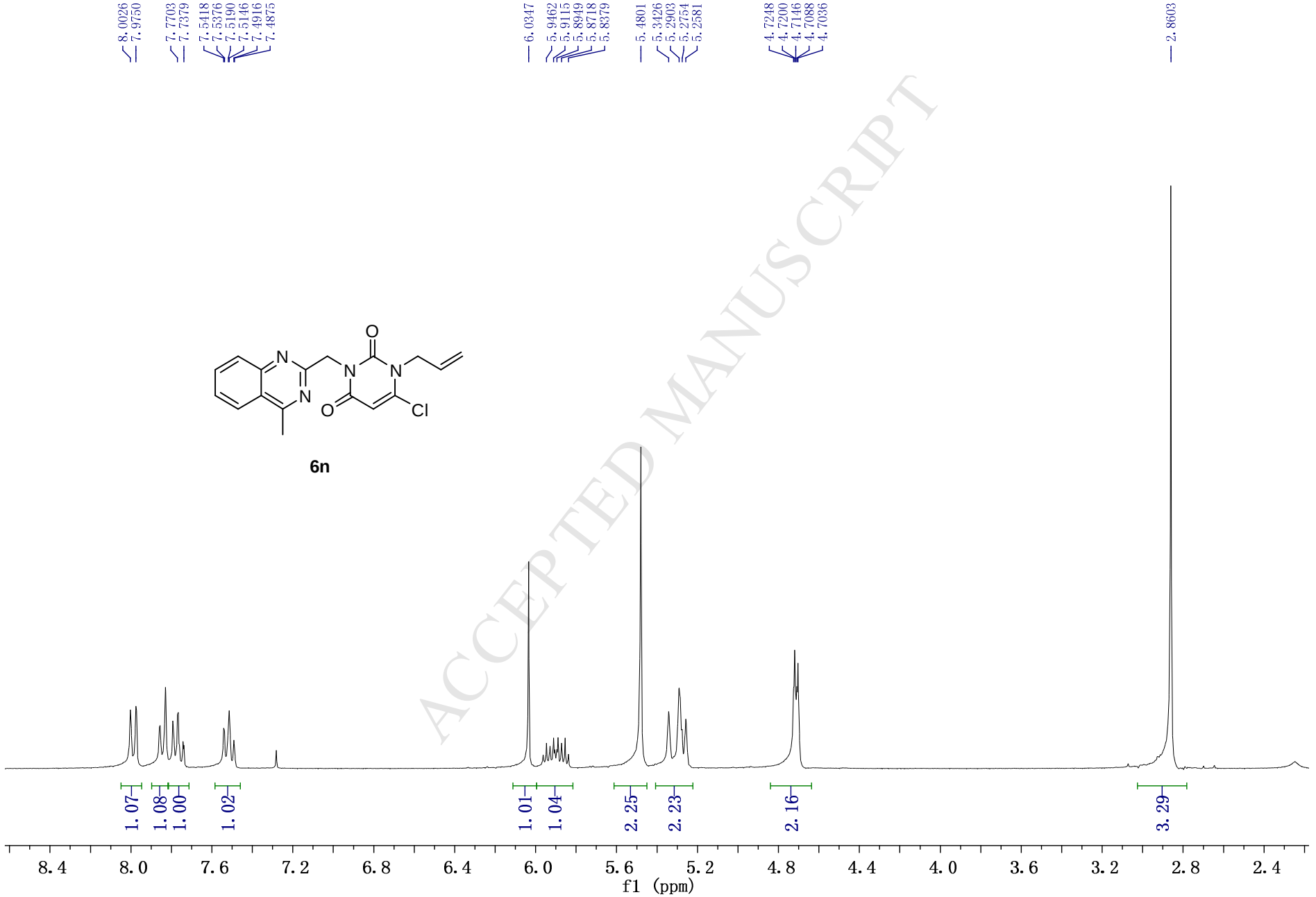
^{13}C NMR spectrum of **6l**



^{13}C NMR spectrum of **6m**

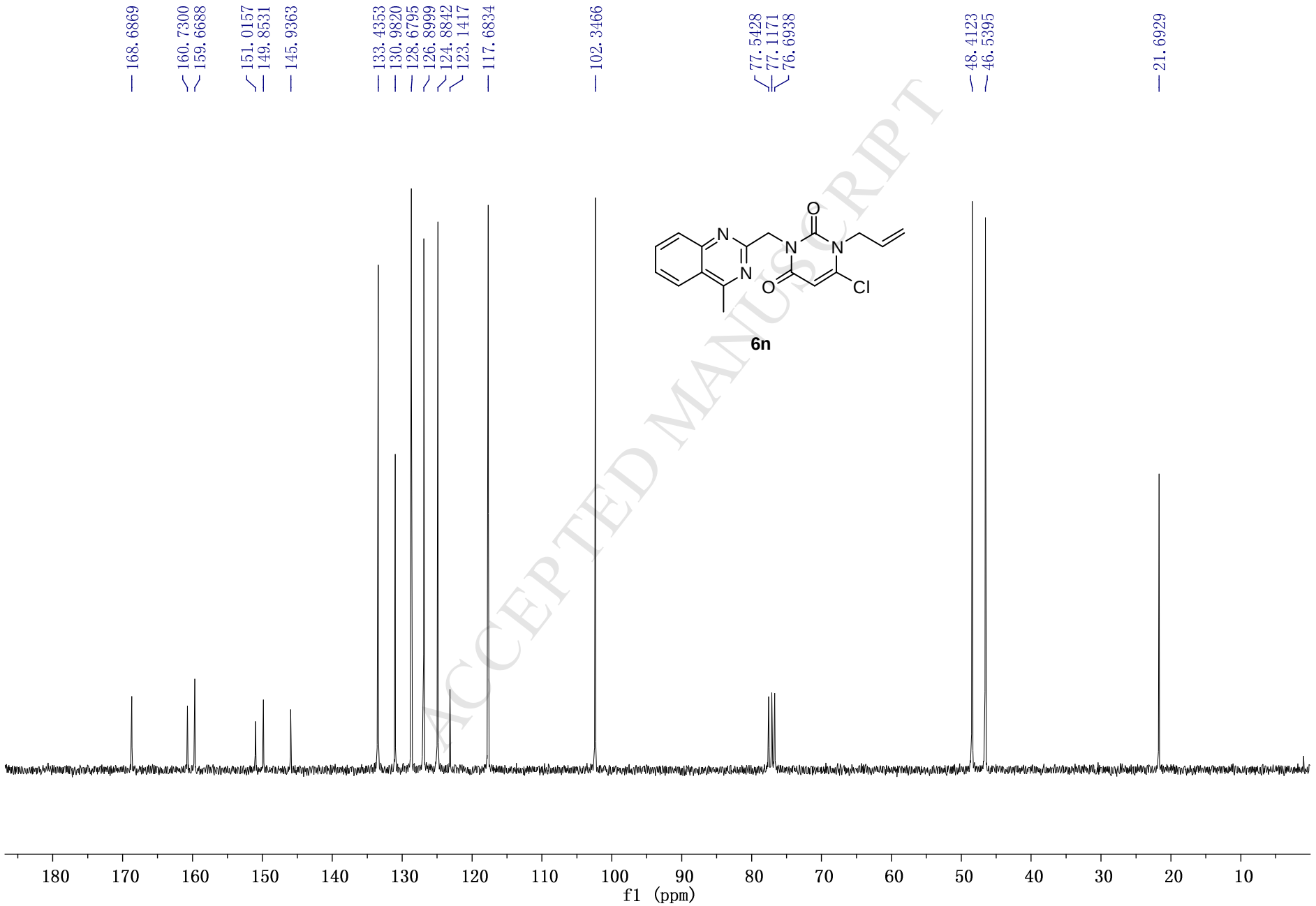


^{1}H NMR spectrum of **6n**

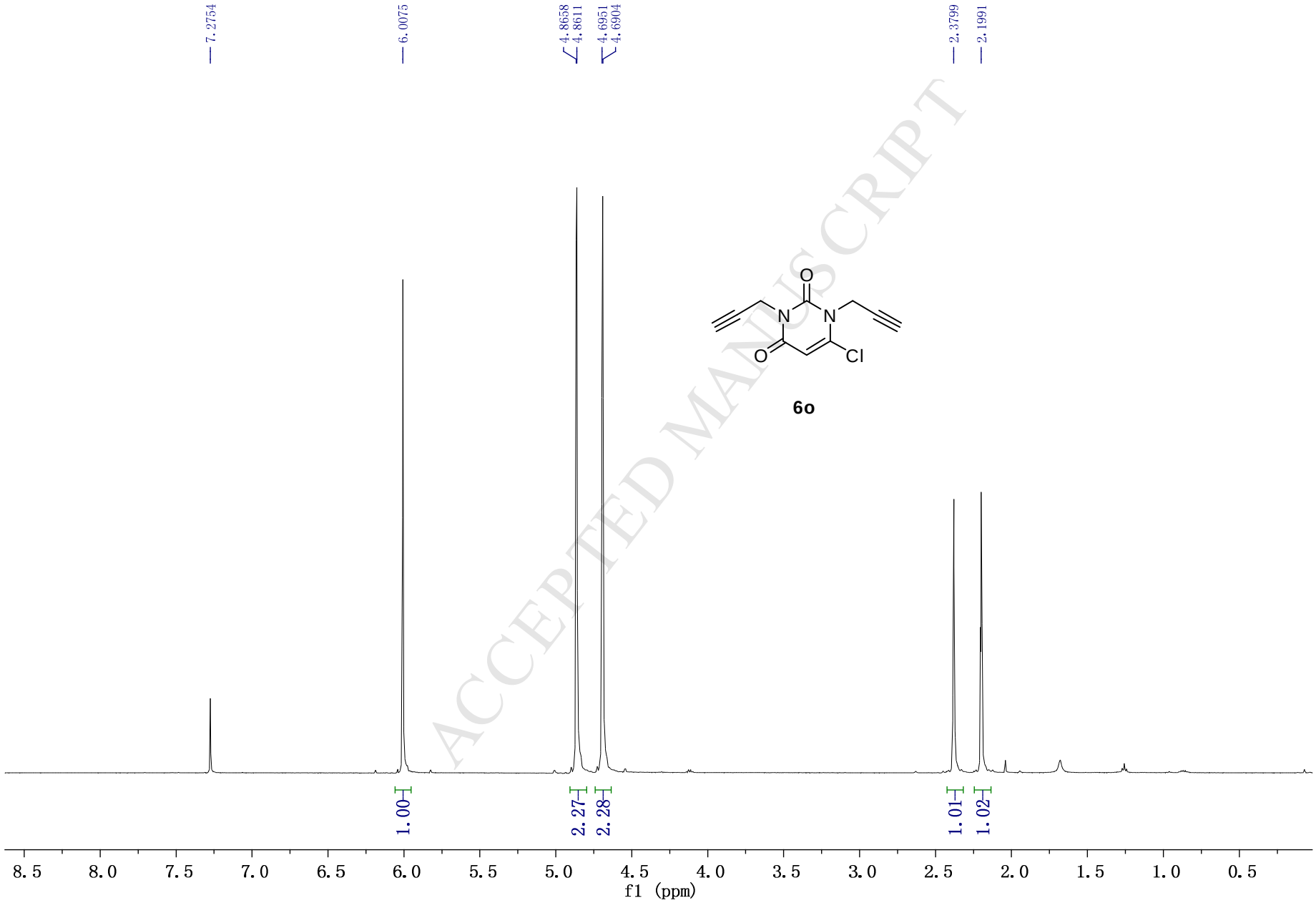


6n

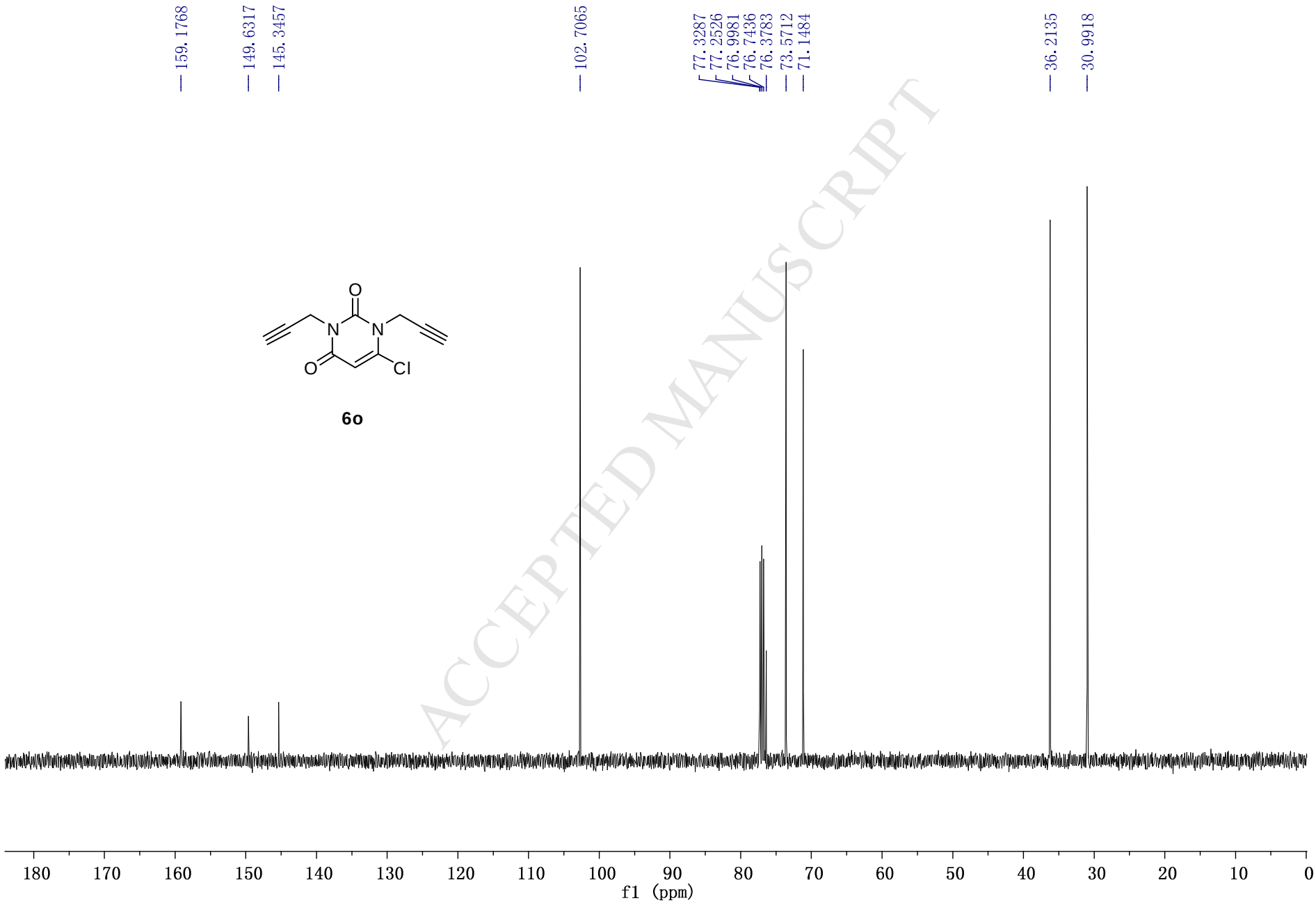
^{13}C NMR spectrum of **6n**



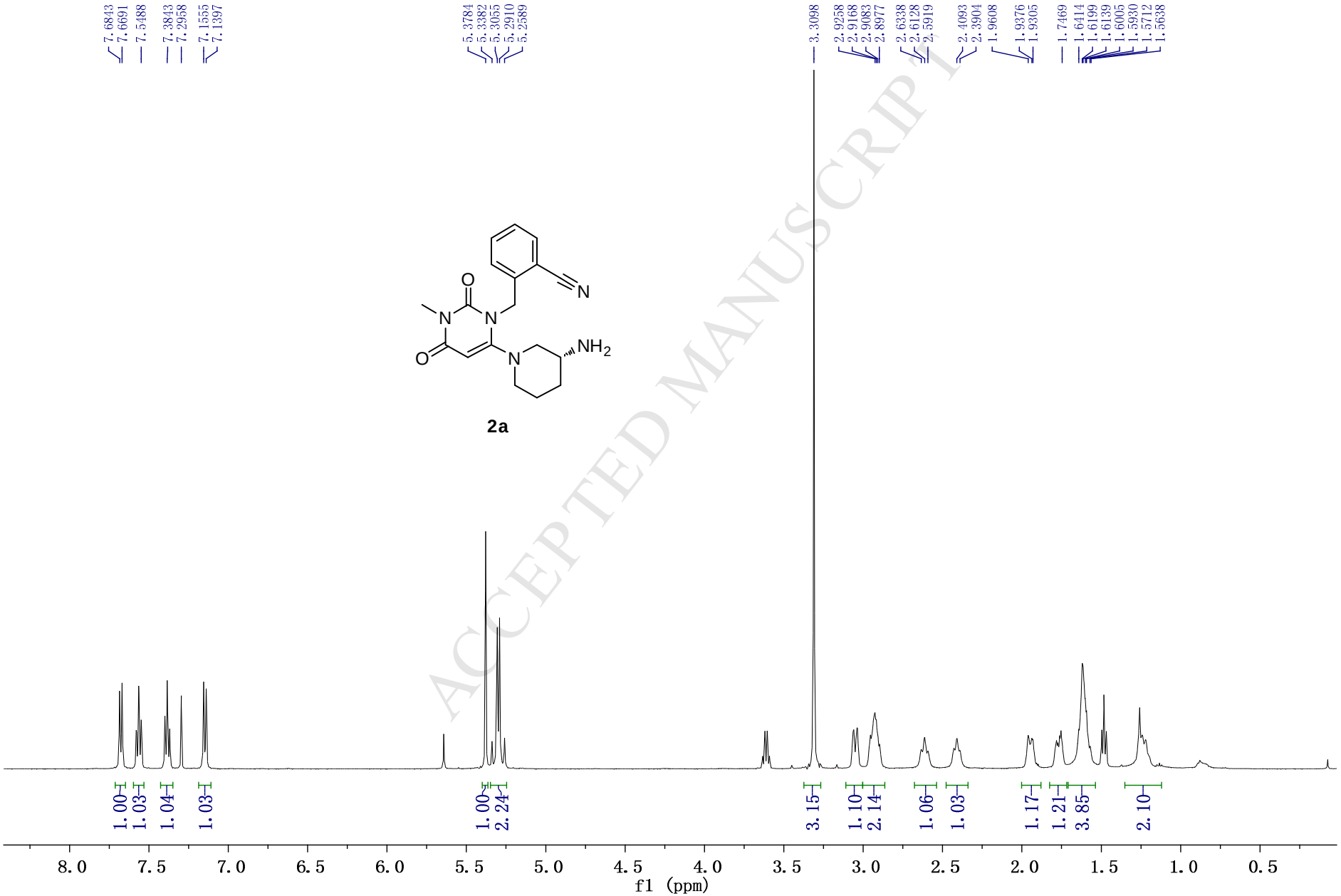
^{1}H NMR spectrum of **6o**



^{13}C NMR spectrum of **6o**

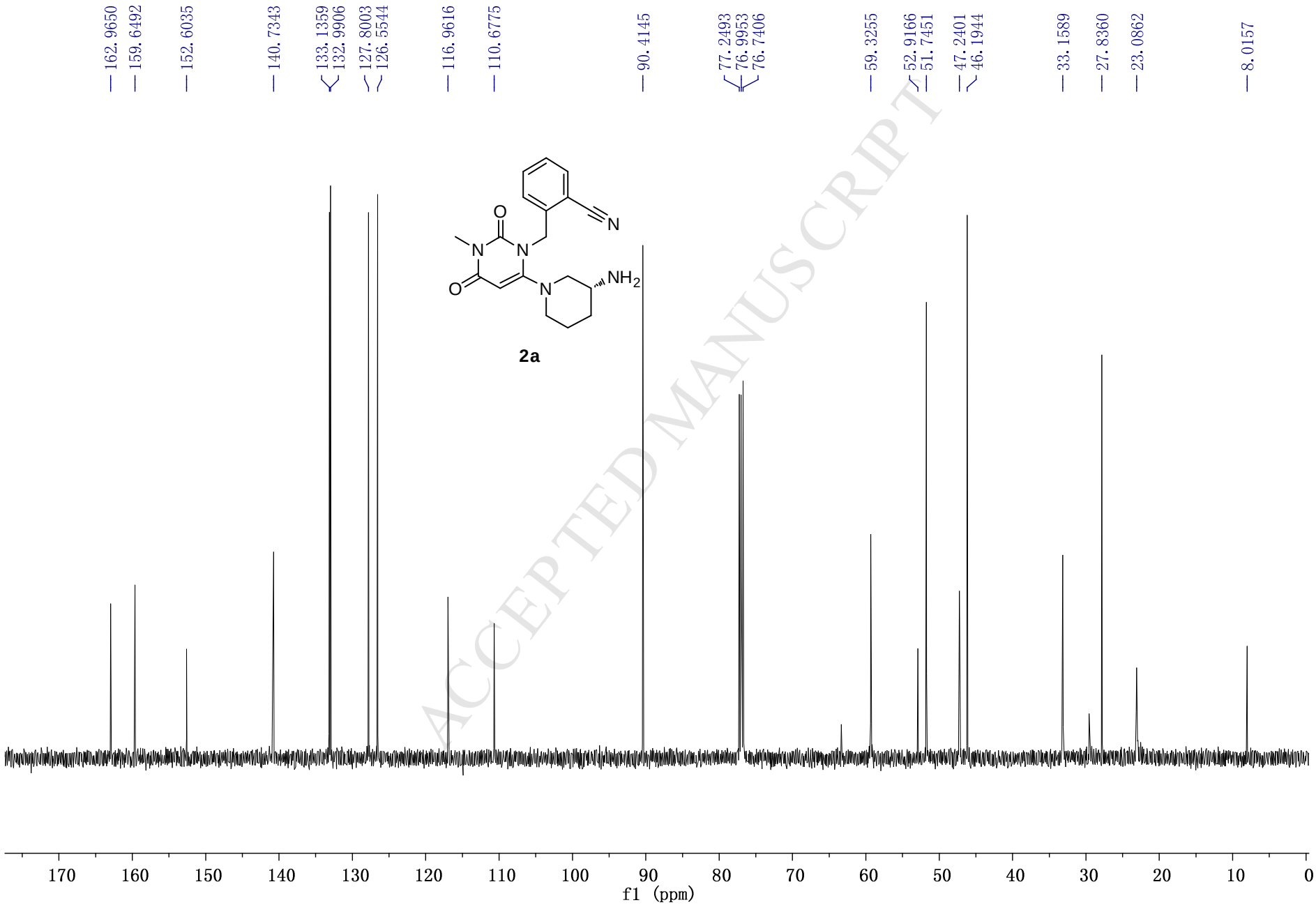


^{1}H NMR spectrum of **2a**

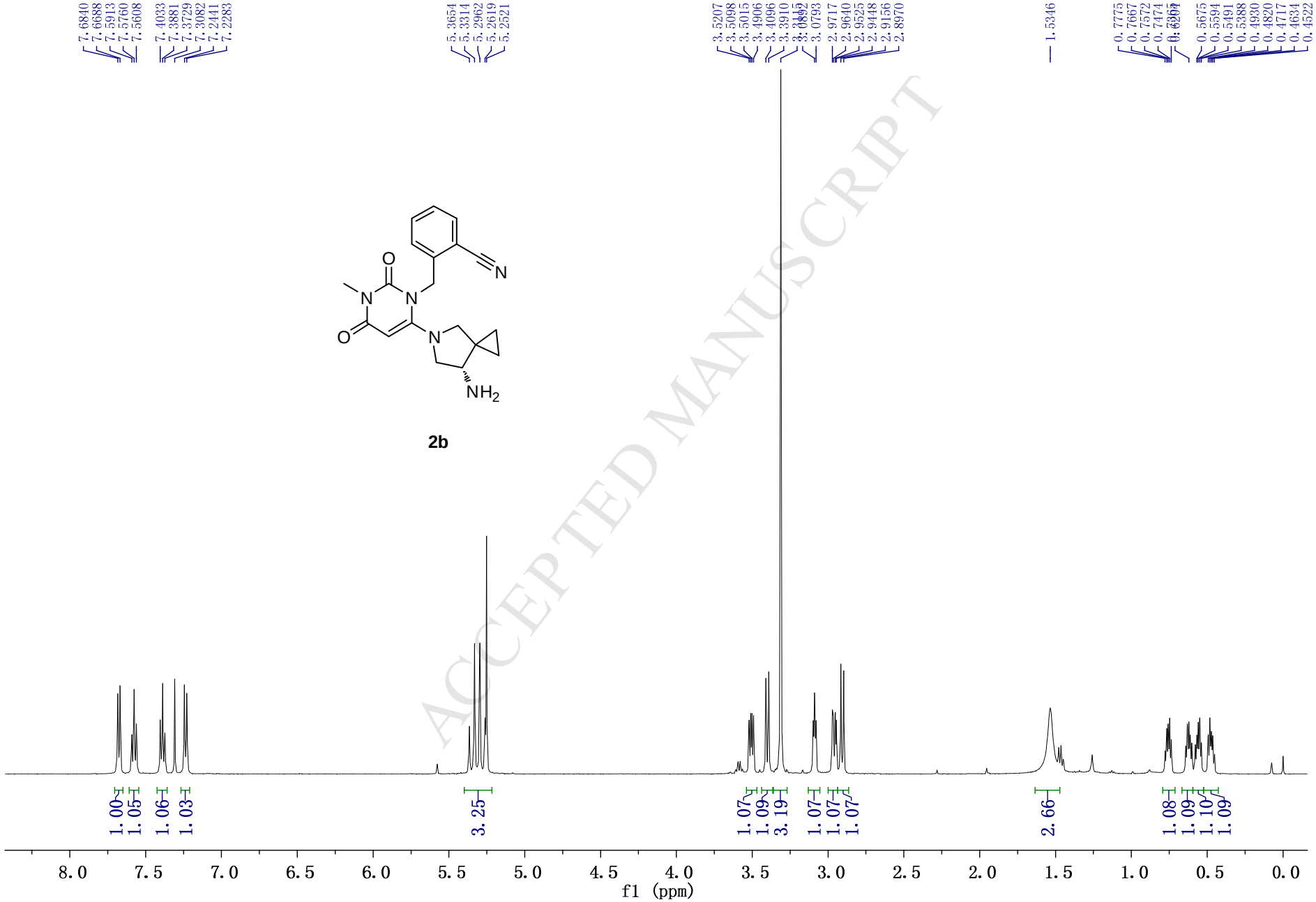


2a

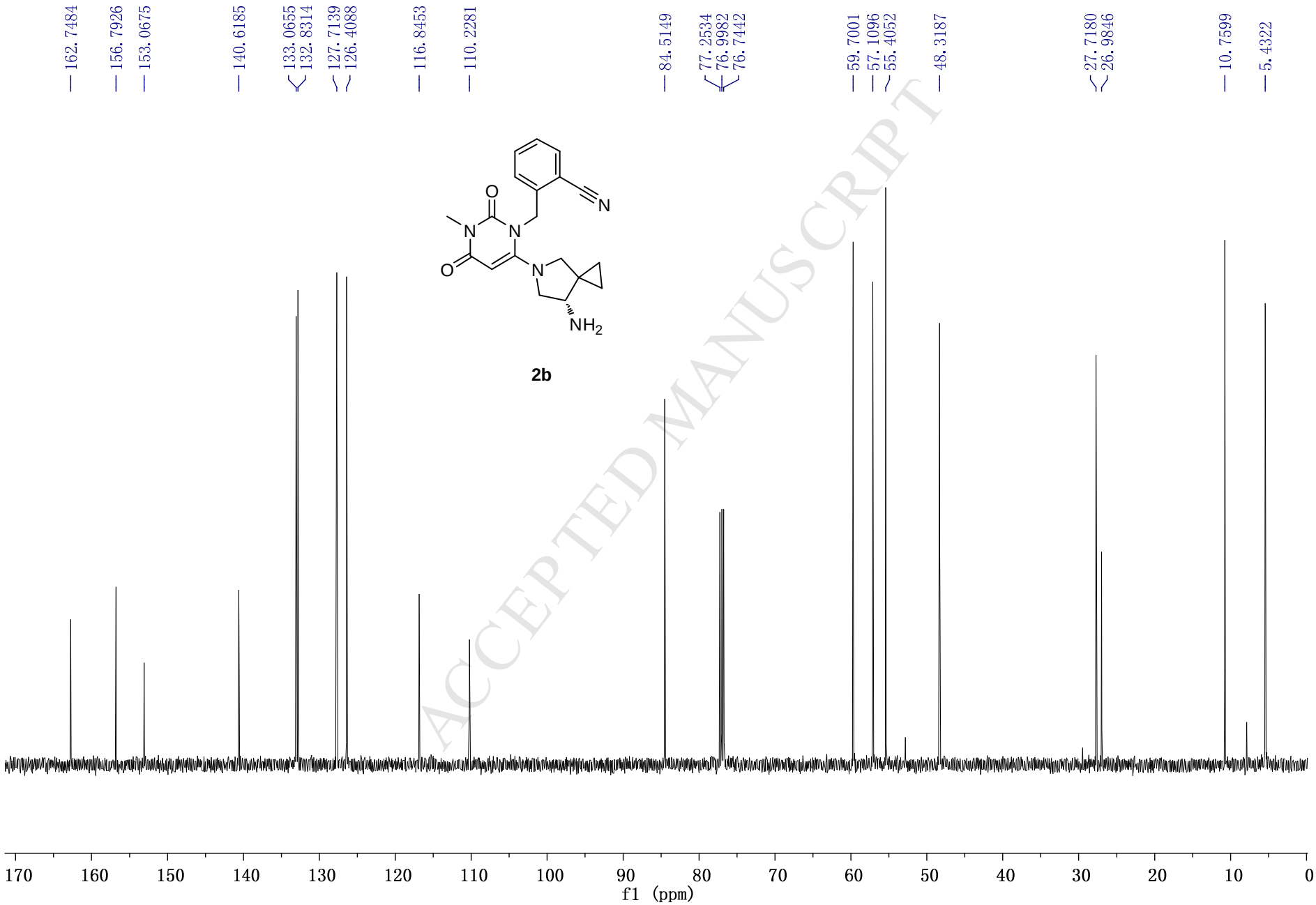
^{13}C NMR spectrum of **2a**



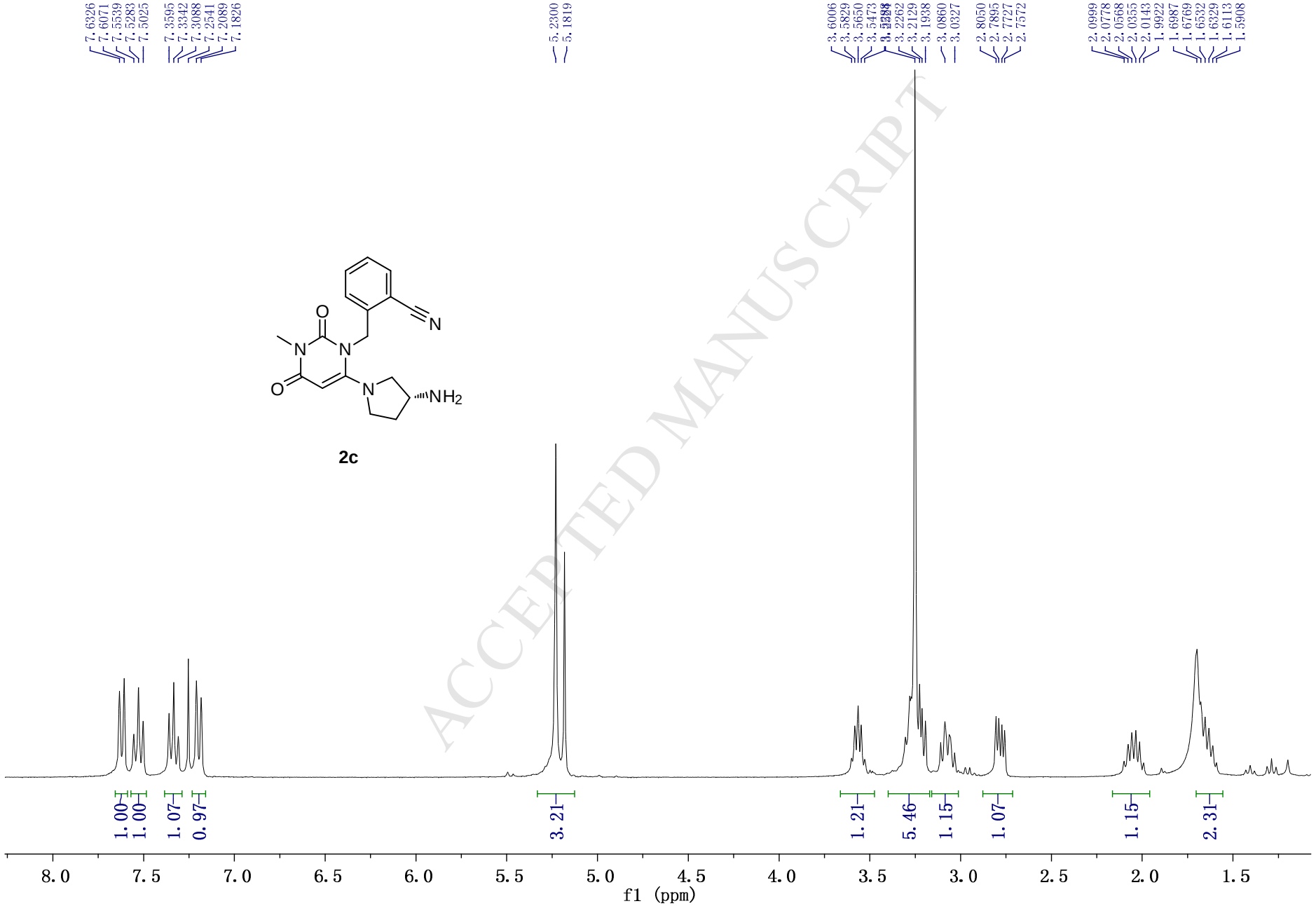
^{1}H NMR spectrum of **2b**



^{13}C NMR spectrum of **2b**

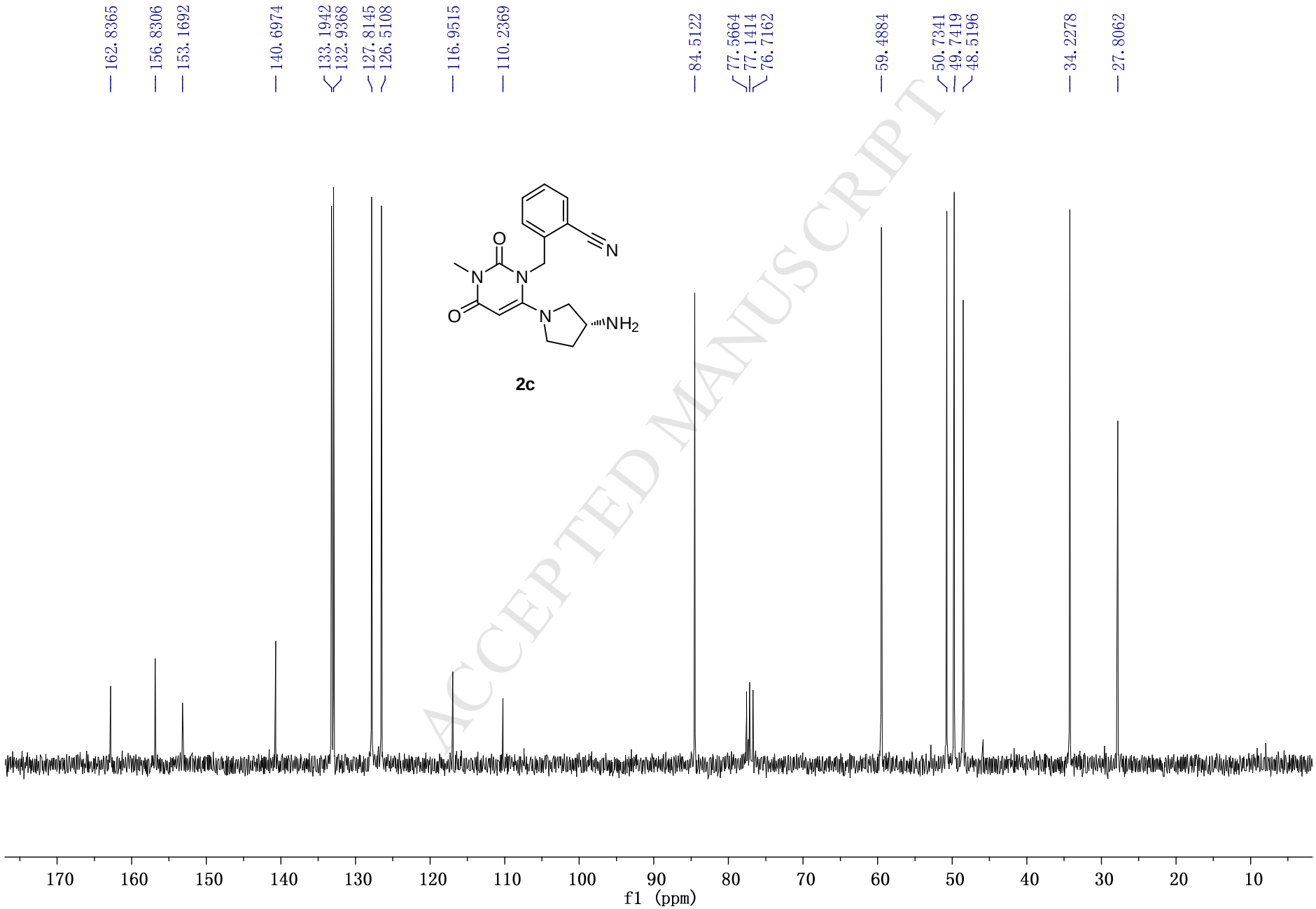


^{1}H NMR spectrum of **2c**

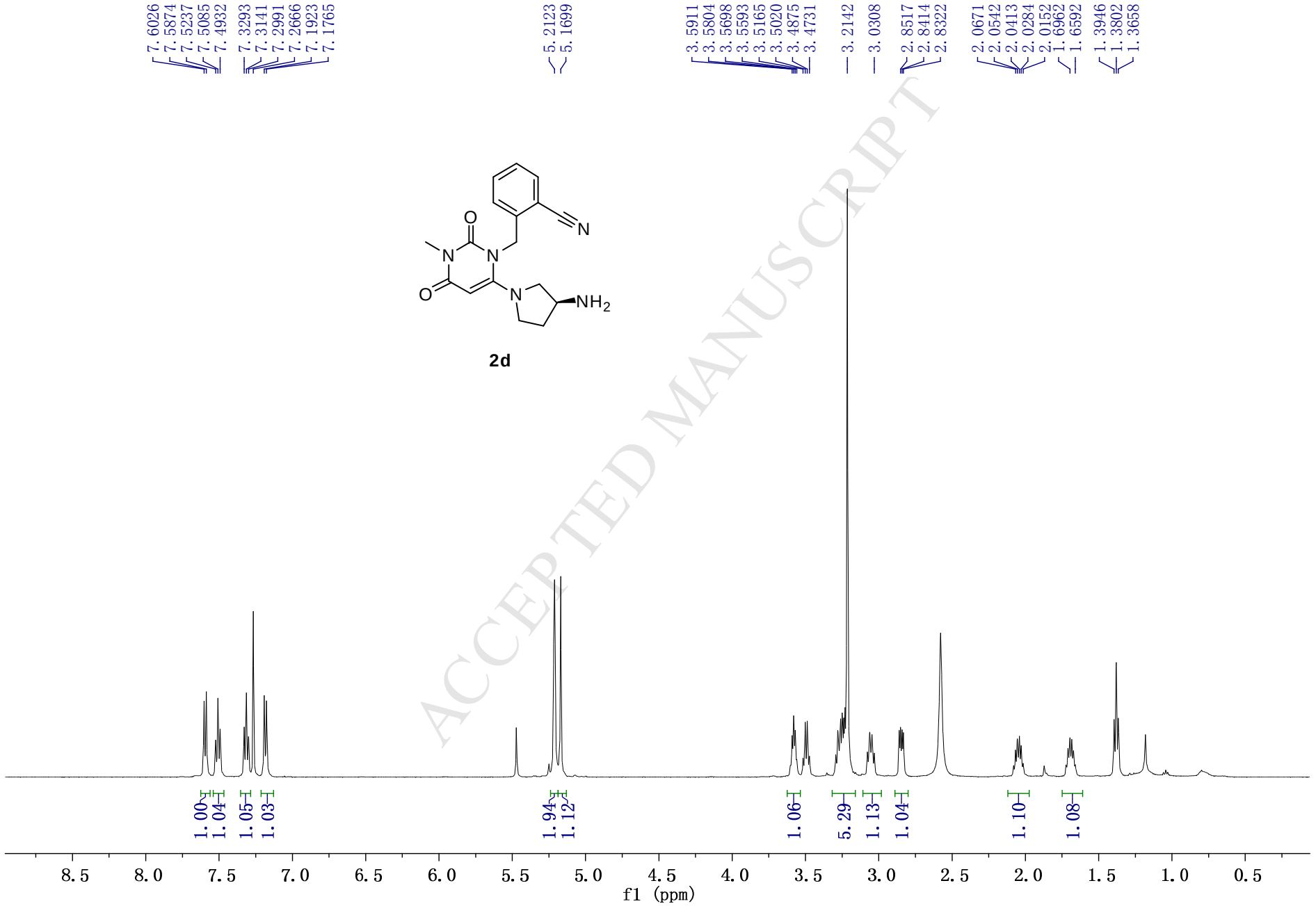


2c

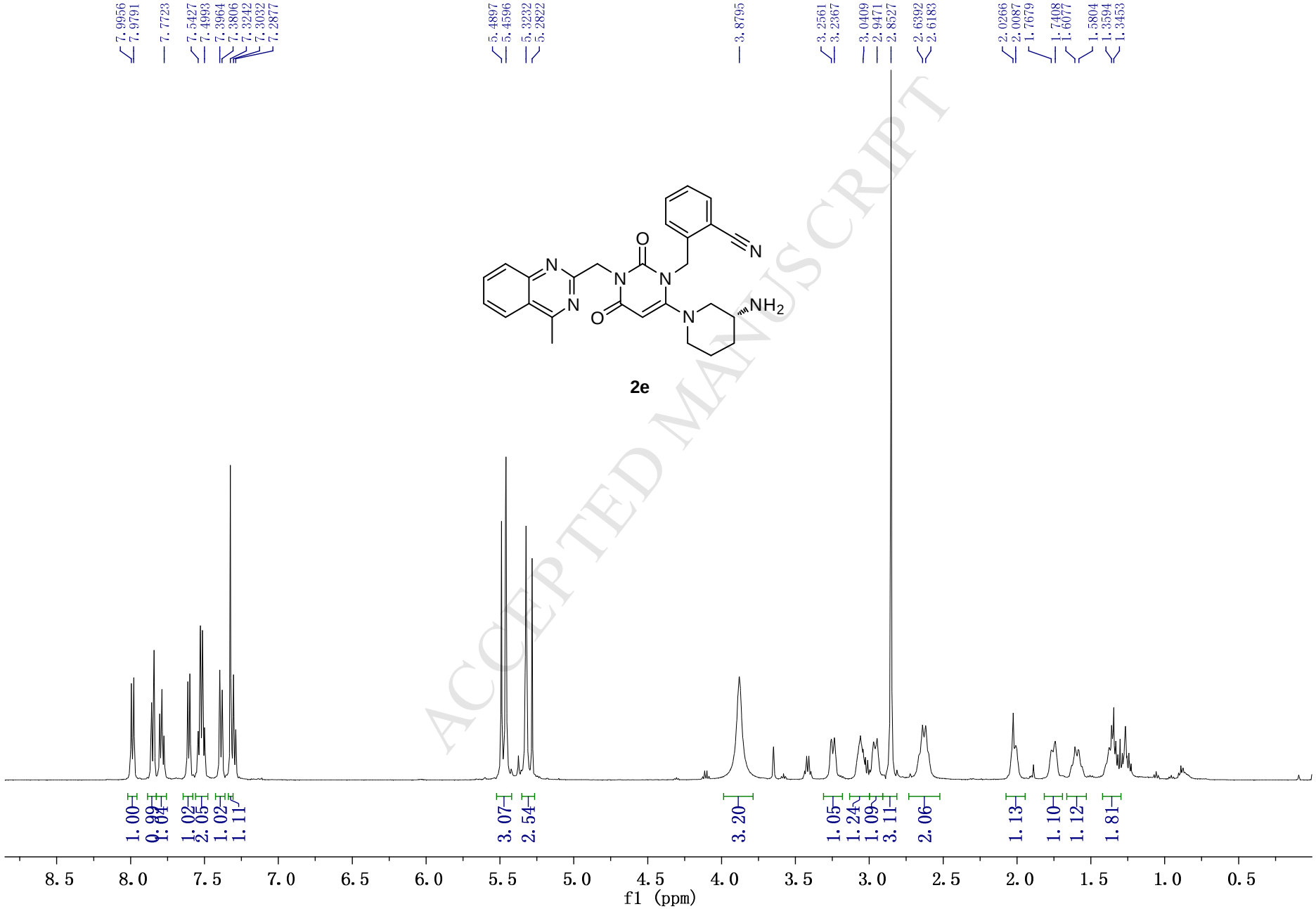
^{13}C NMR spectrum of **2c**



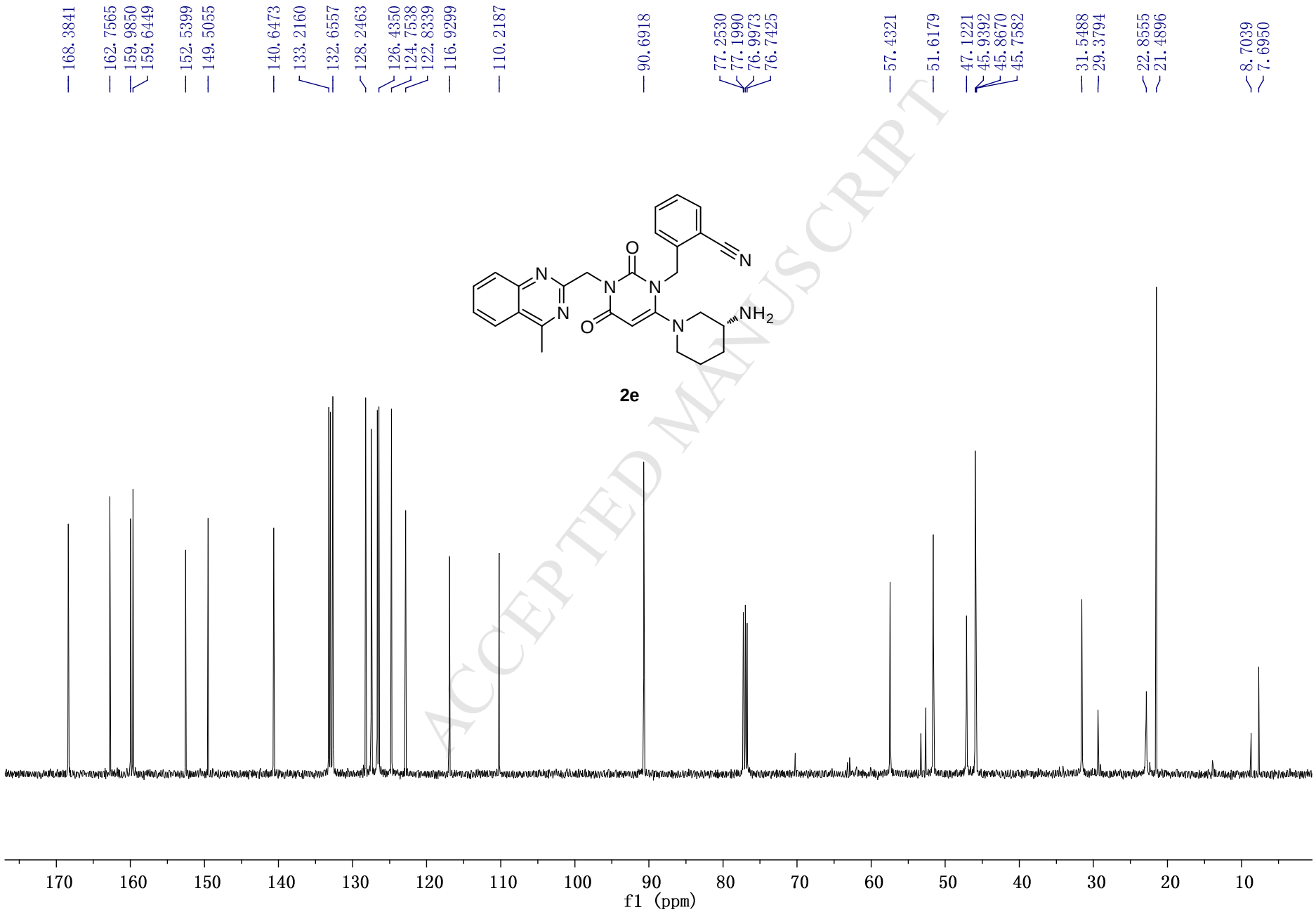
^{1}H NMR spectrum of **2d**



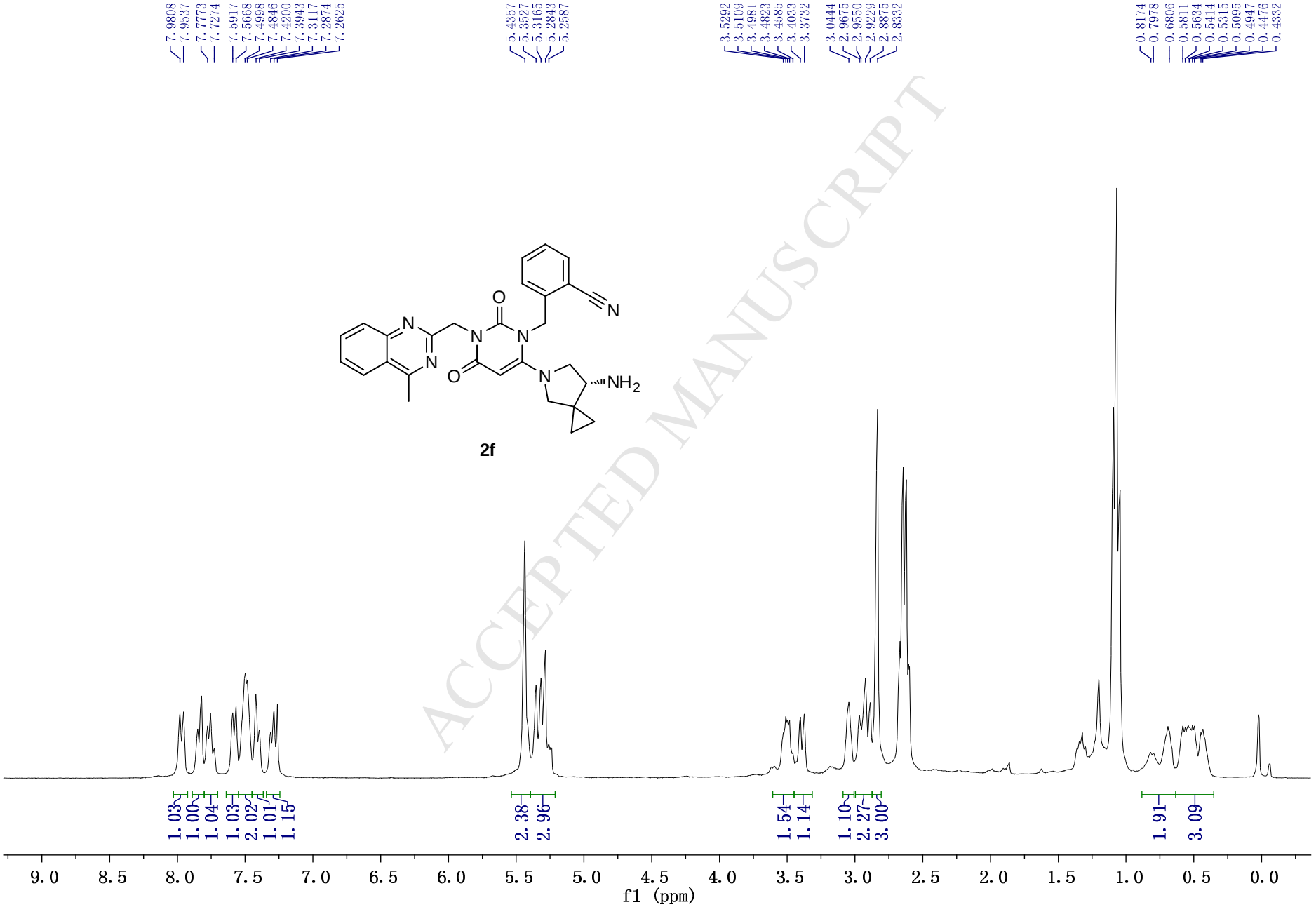
^{1}H NMR spectrum of **2e**



^{13}C NMR spectrum of **2e**

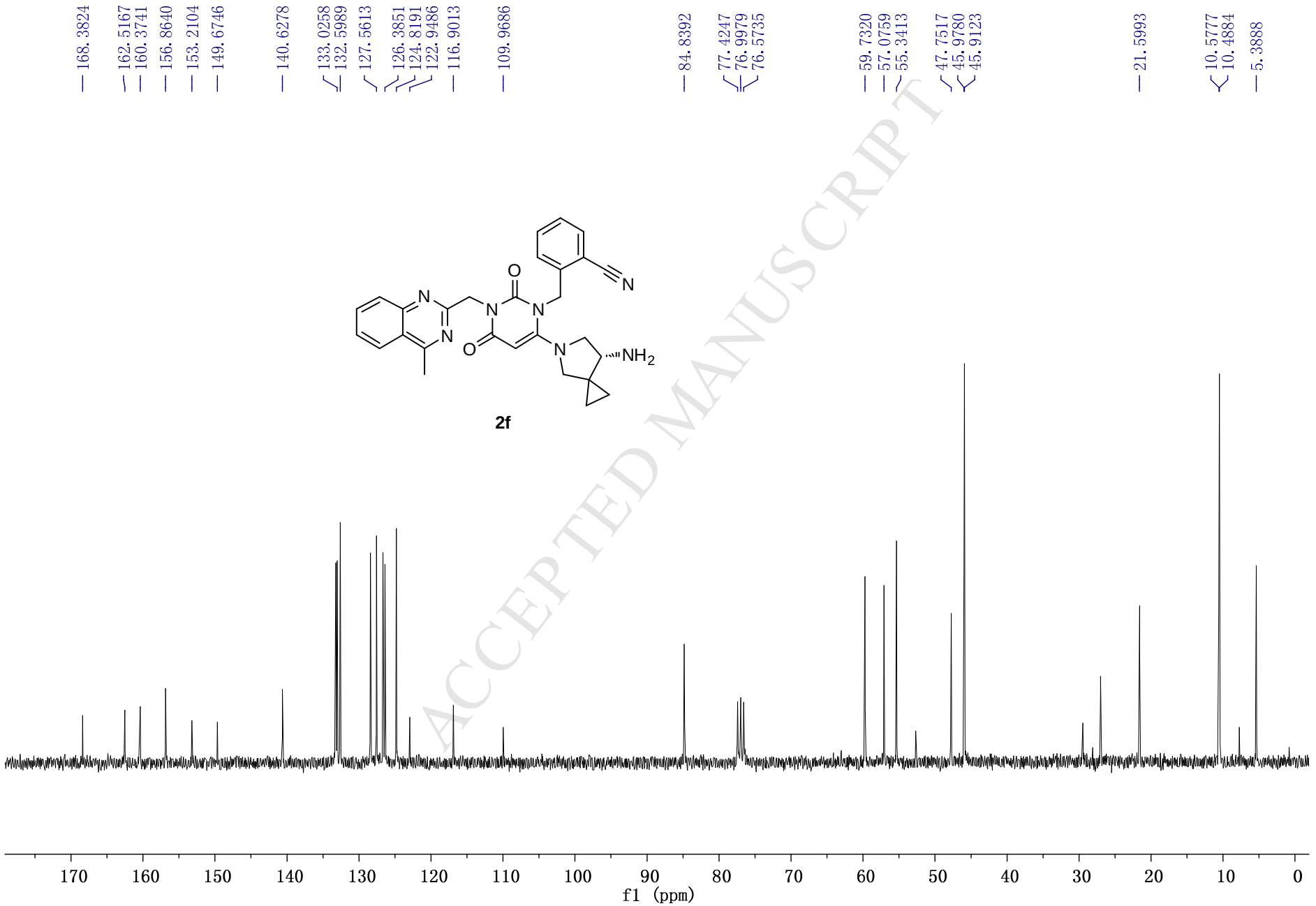


^{1}H NMR spectrum of **2f**

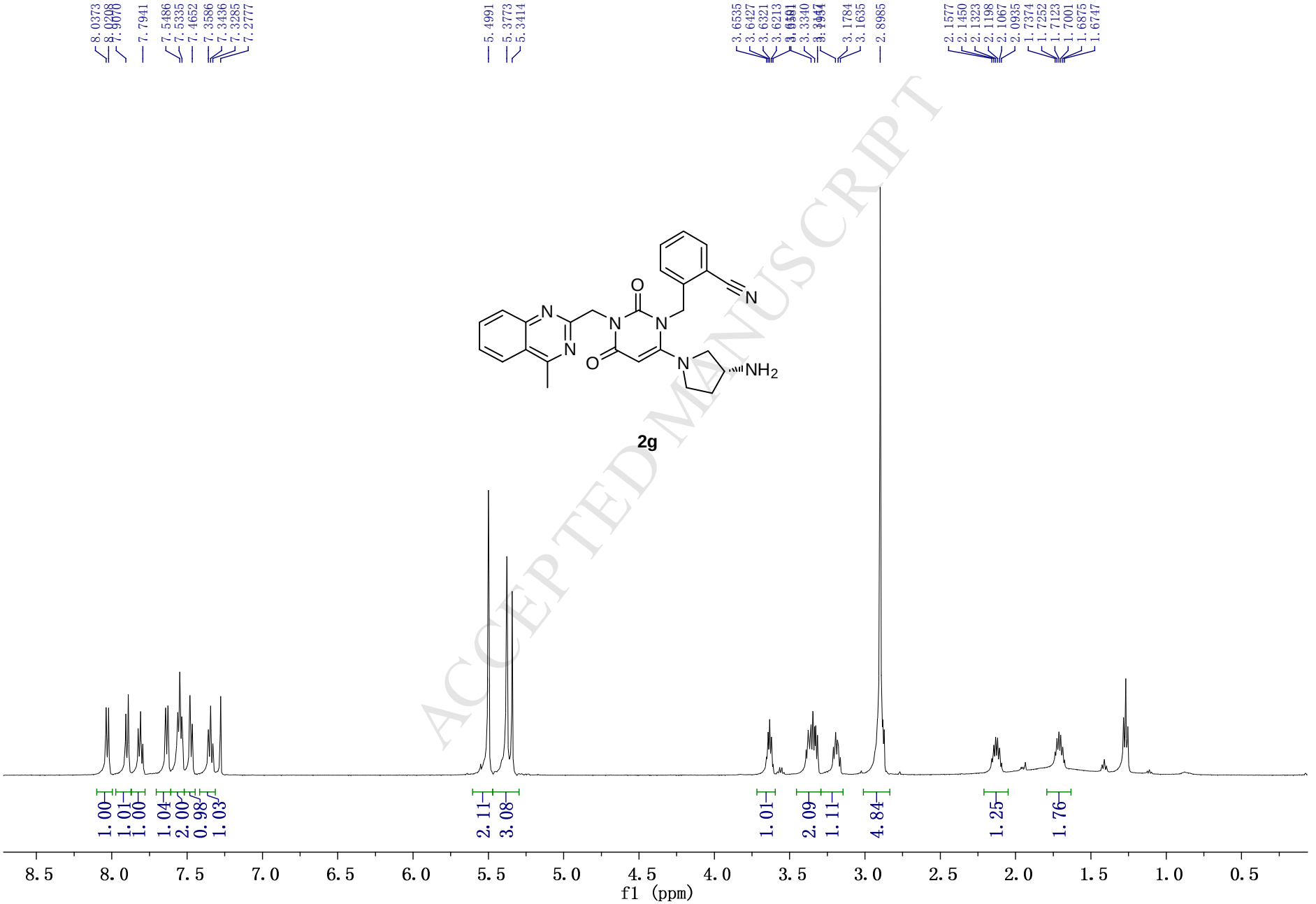


2f

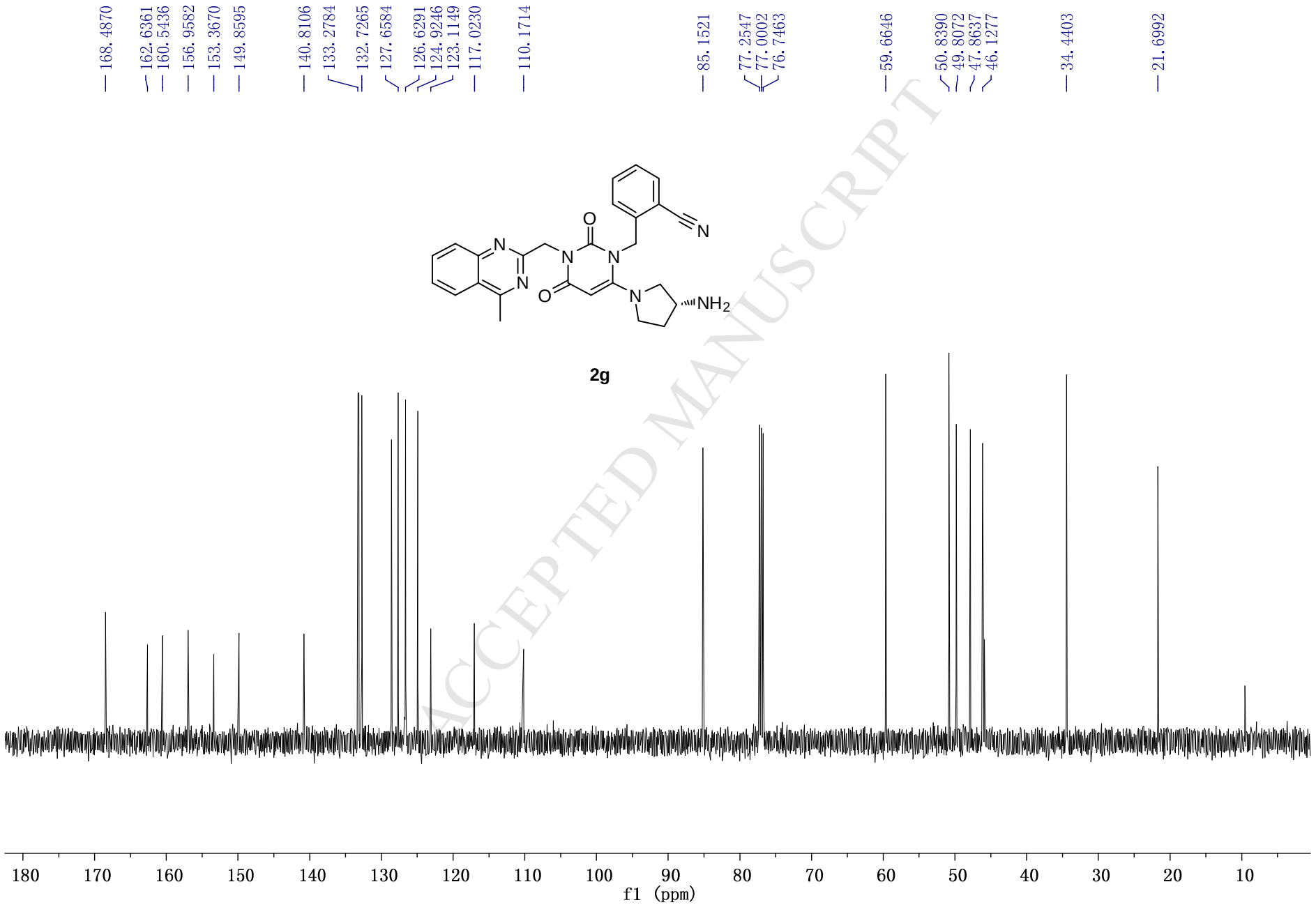
^{13}C NMR spectrum of **2f**



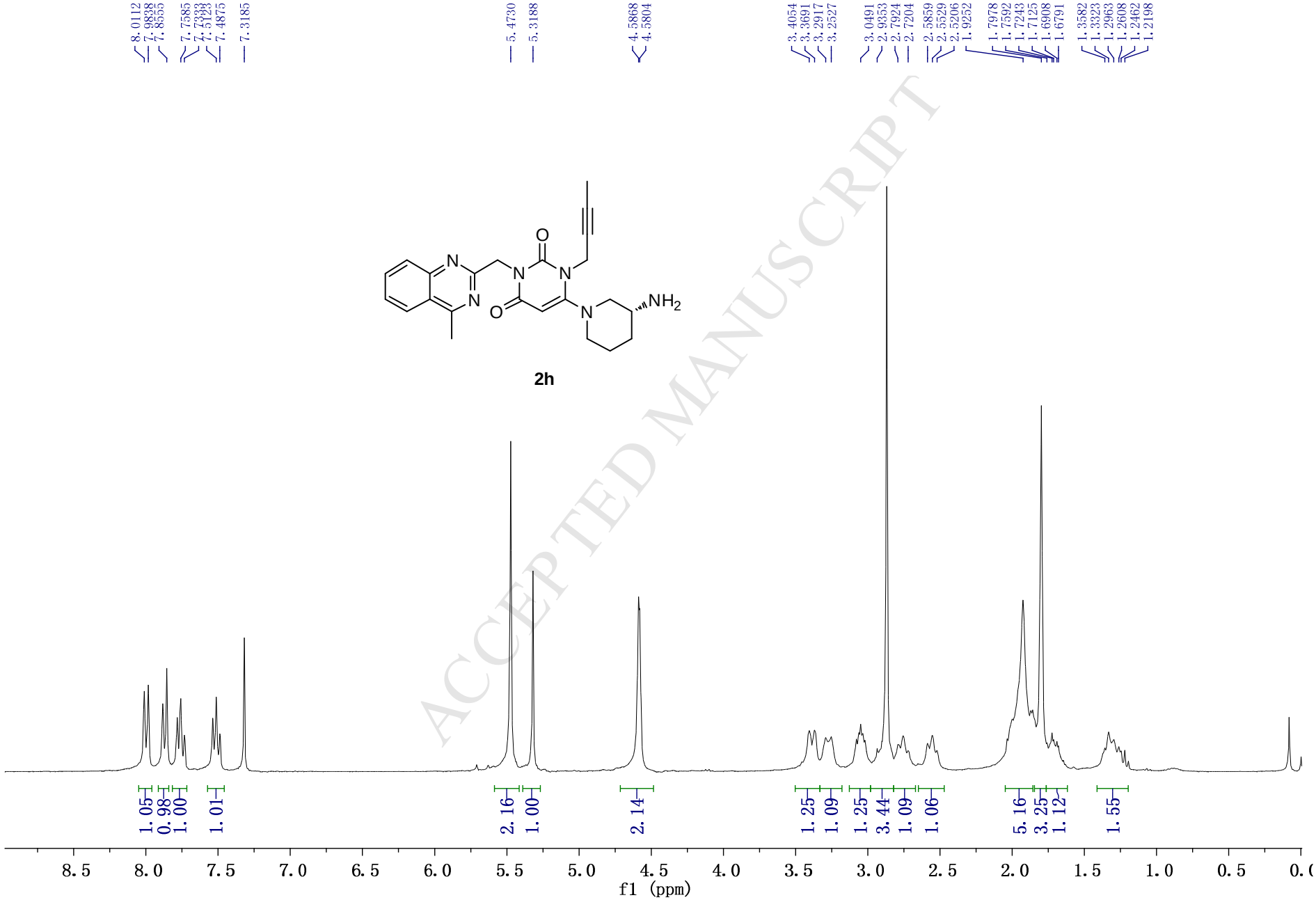
^{1}H NMR spectrum of **2g**



^{13}C NMR spectrum of **2g**

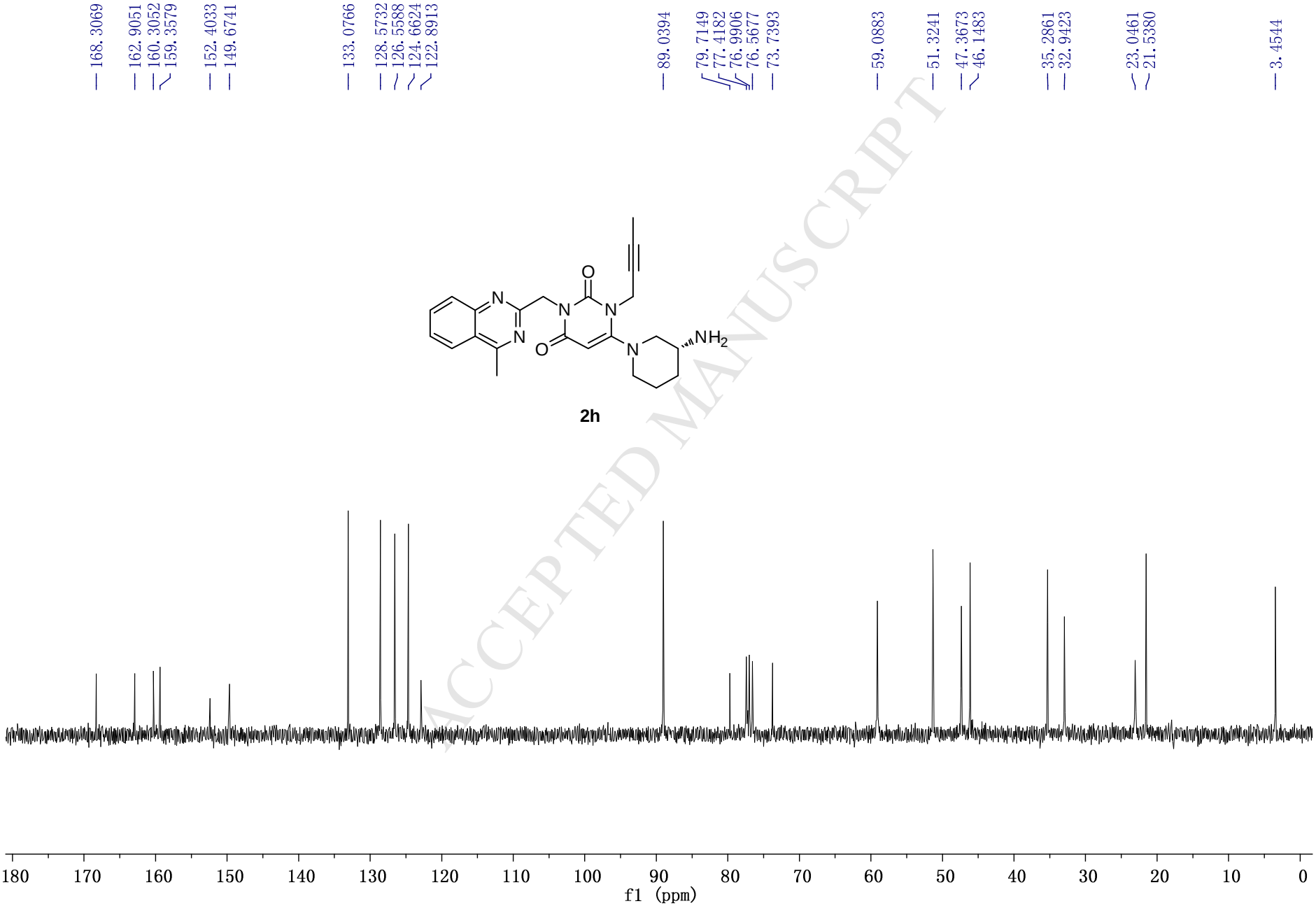


^{1}H NMR spectrum of **2h**

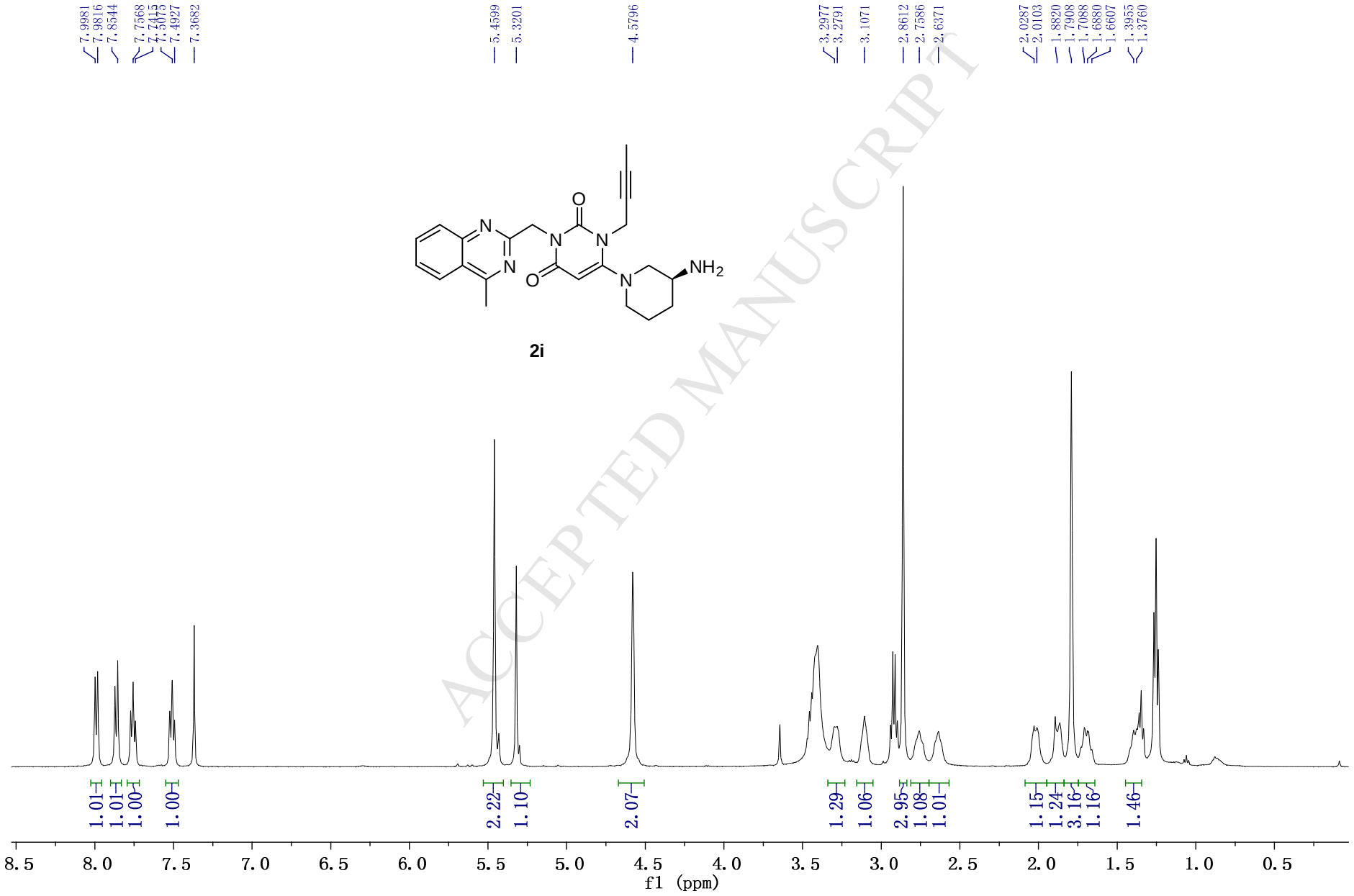


2h

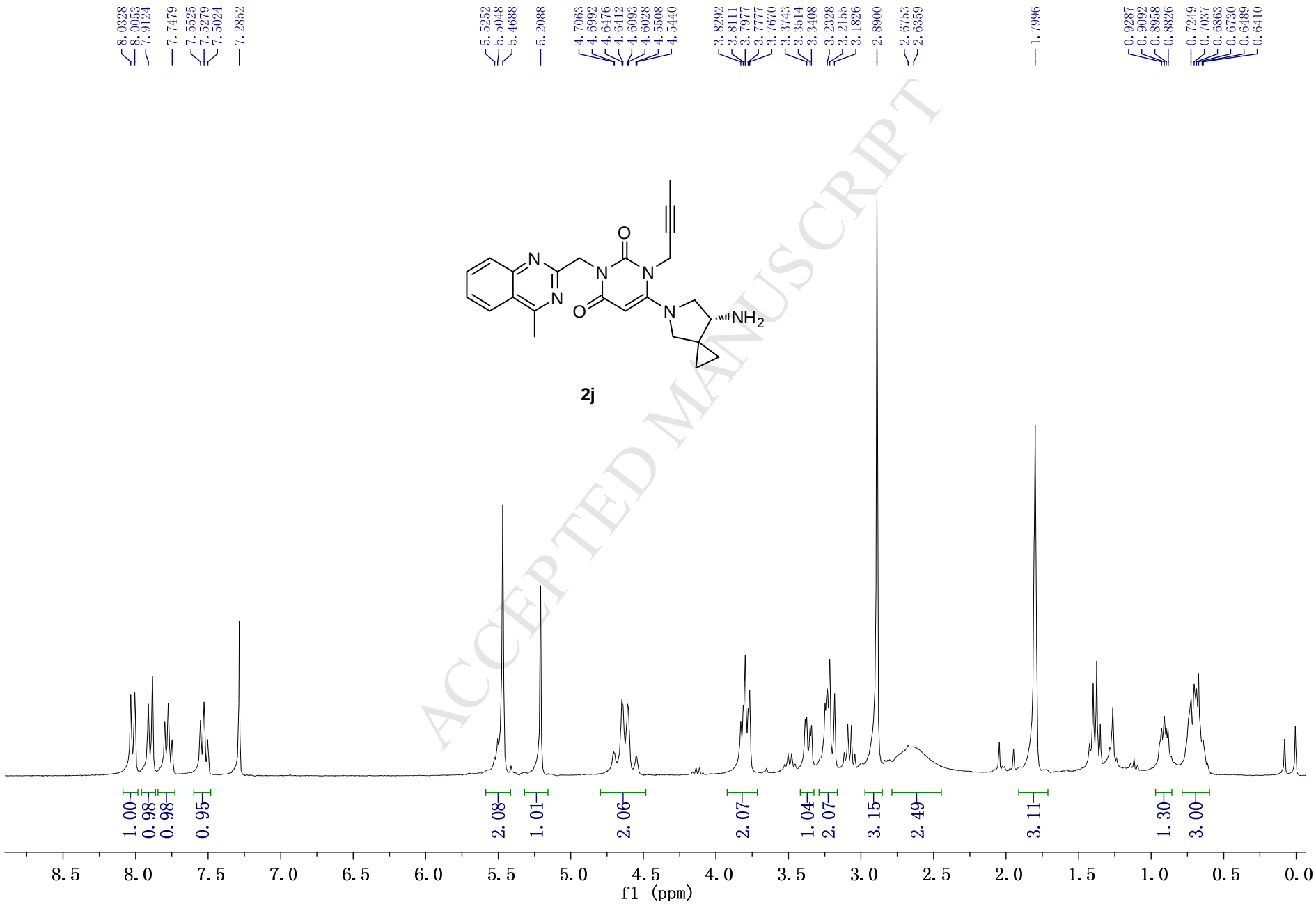
^{13}C NMR spectrum of **2h**



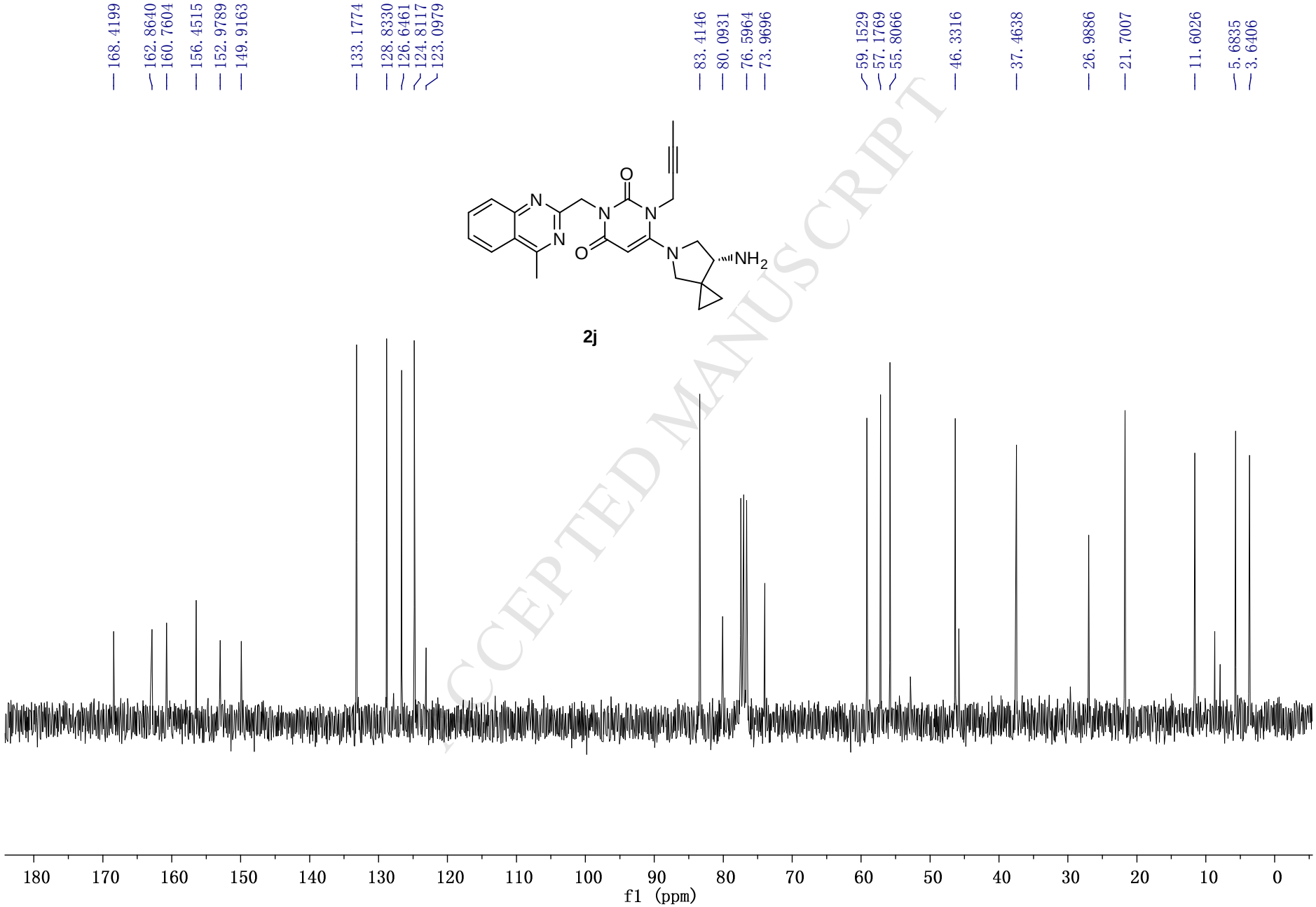
^{1}H NMR spectrum of **2i**



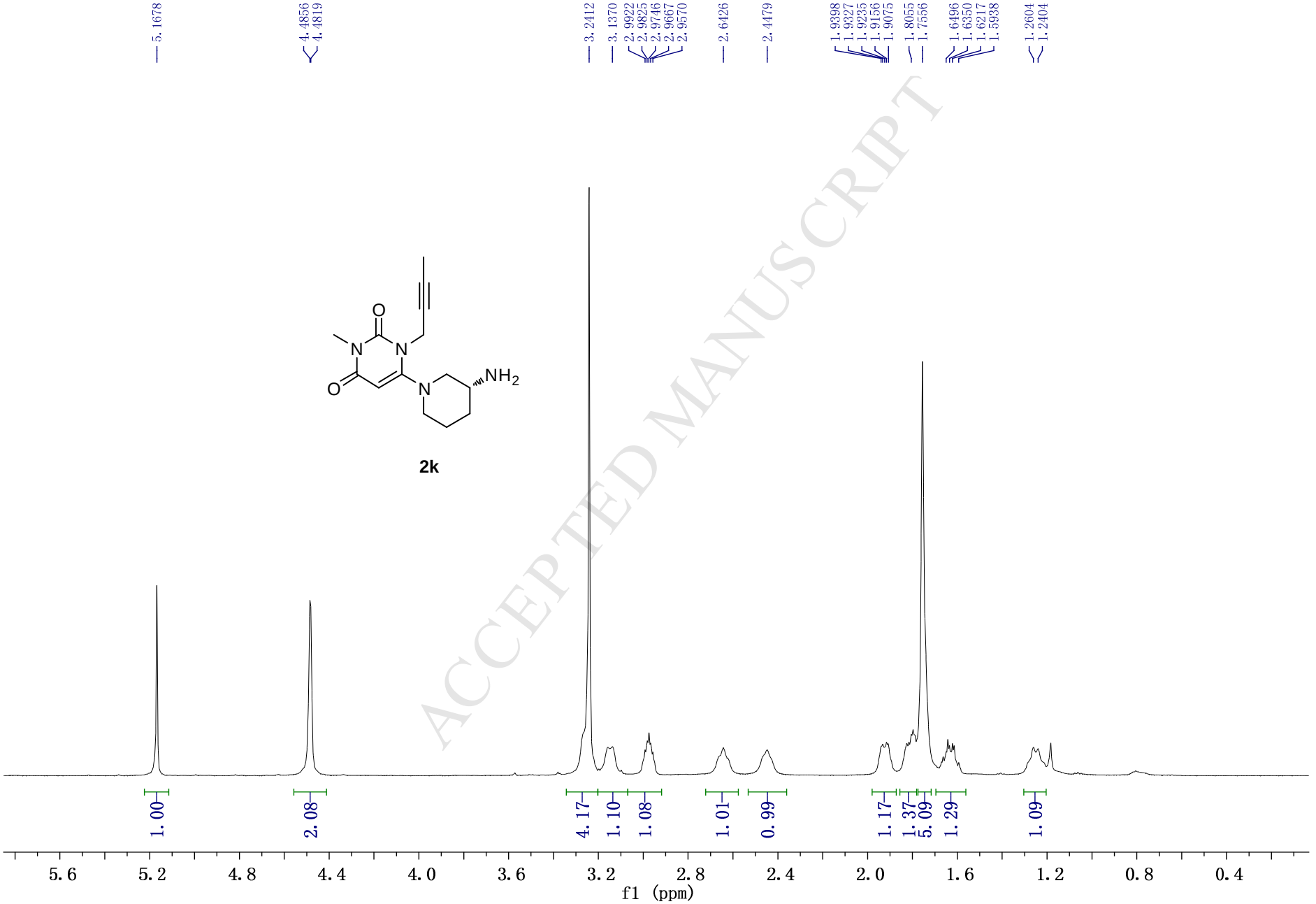
^{1}H NMR spectrum of **2j**



^{13}C NMR spectrum of **2j**

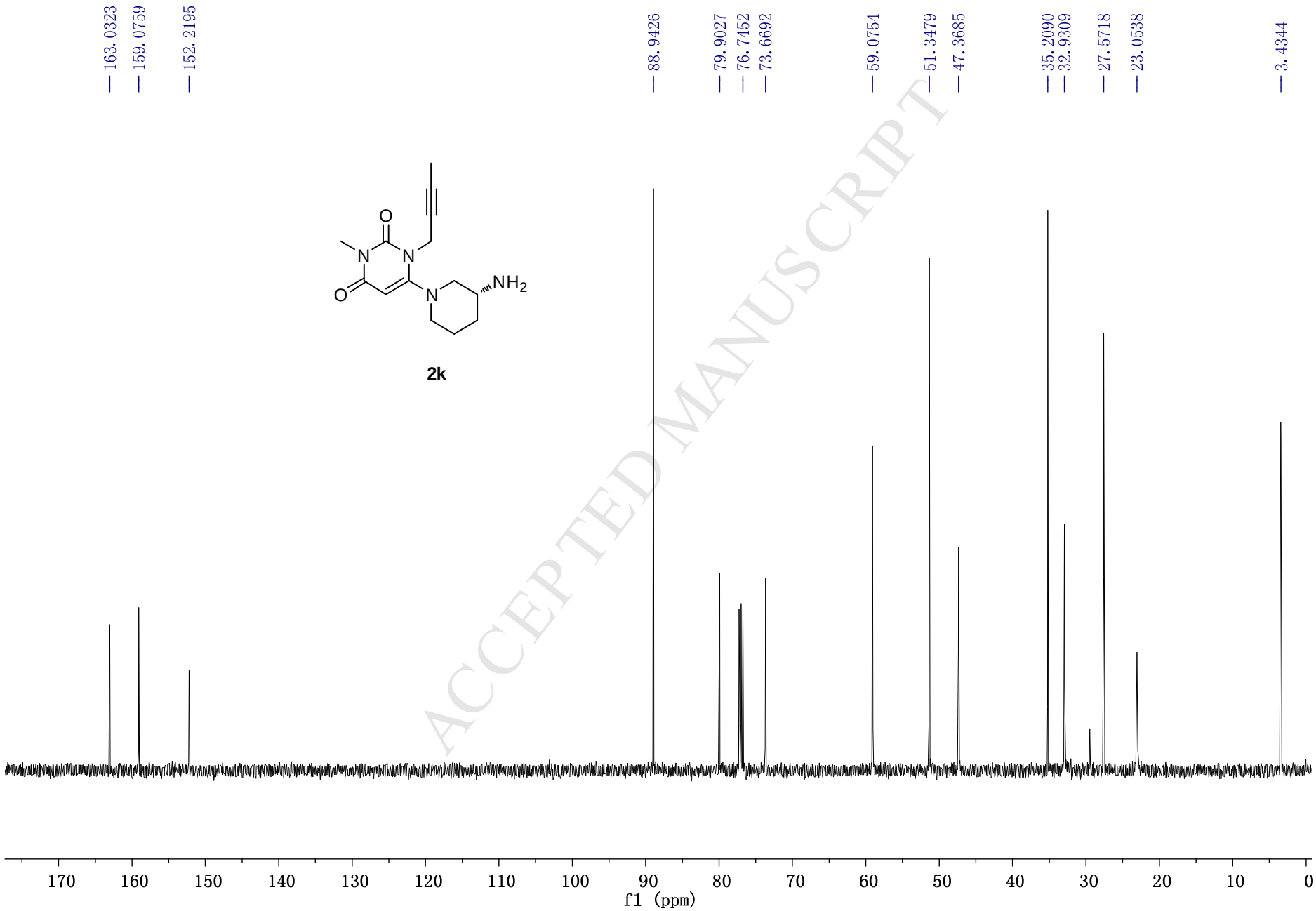


^{1}H NMR spectrum of **2k**

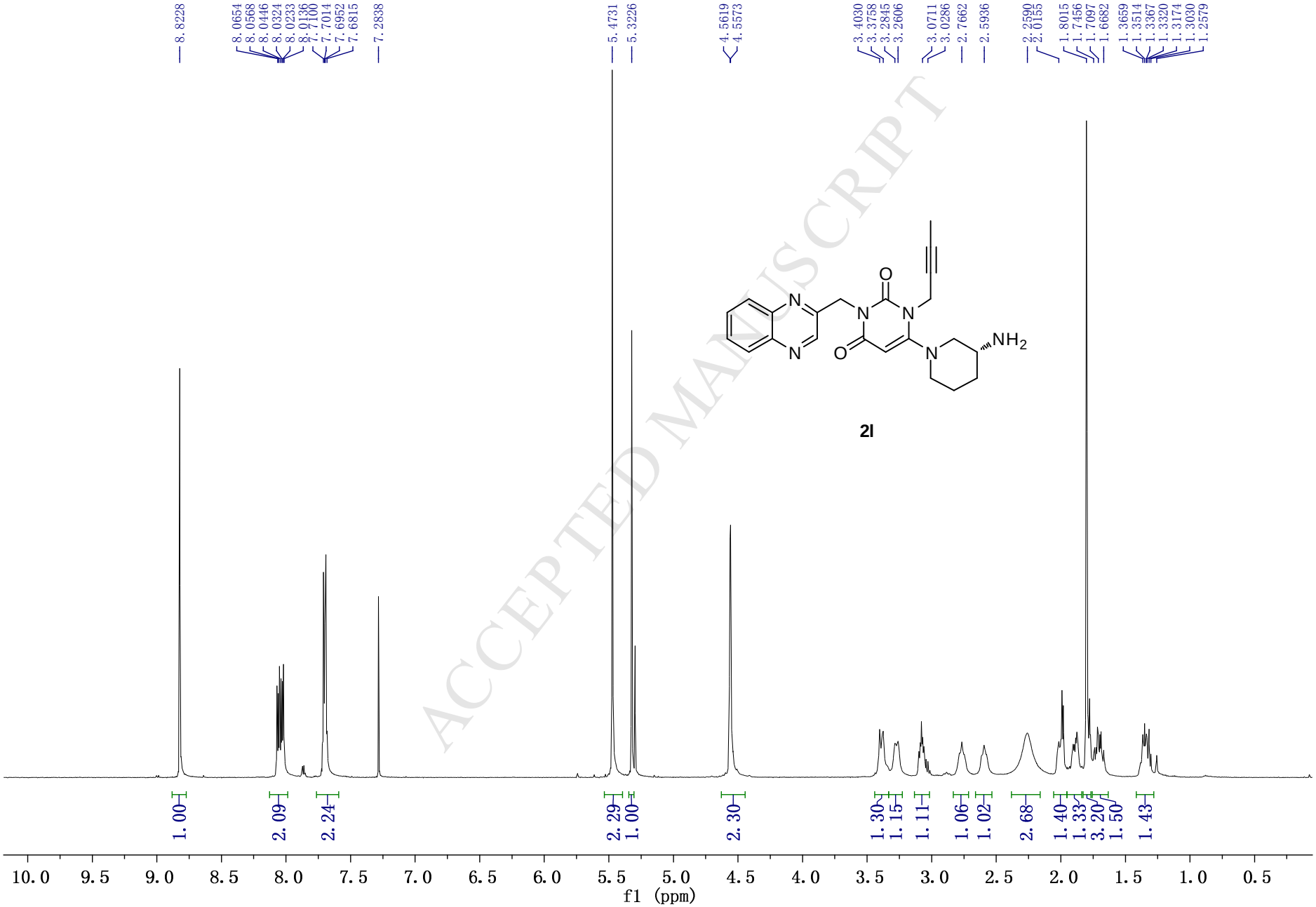


2k

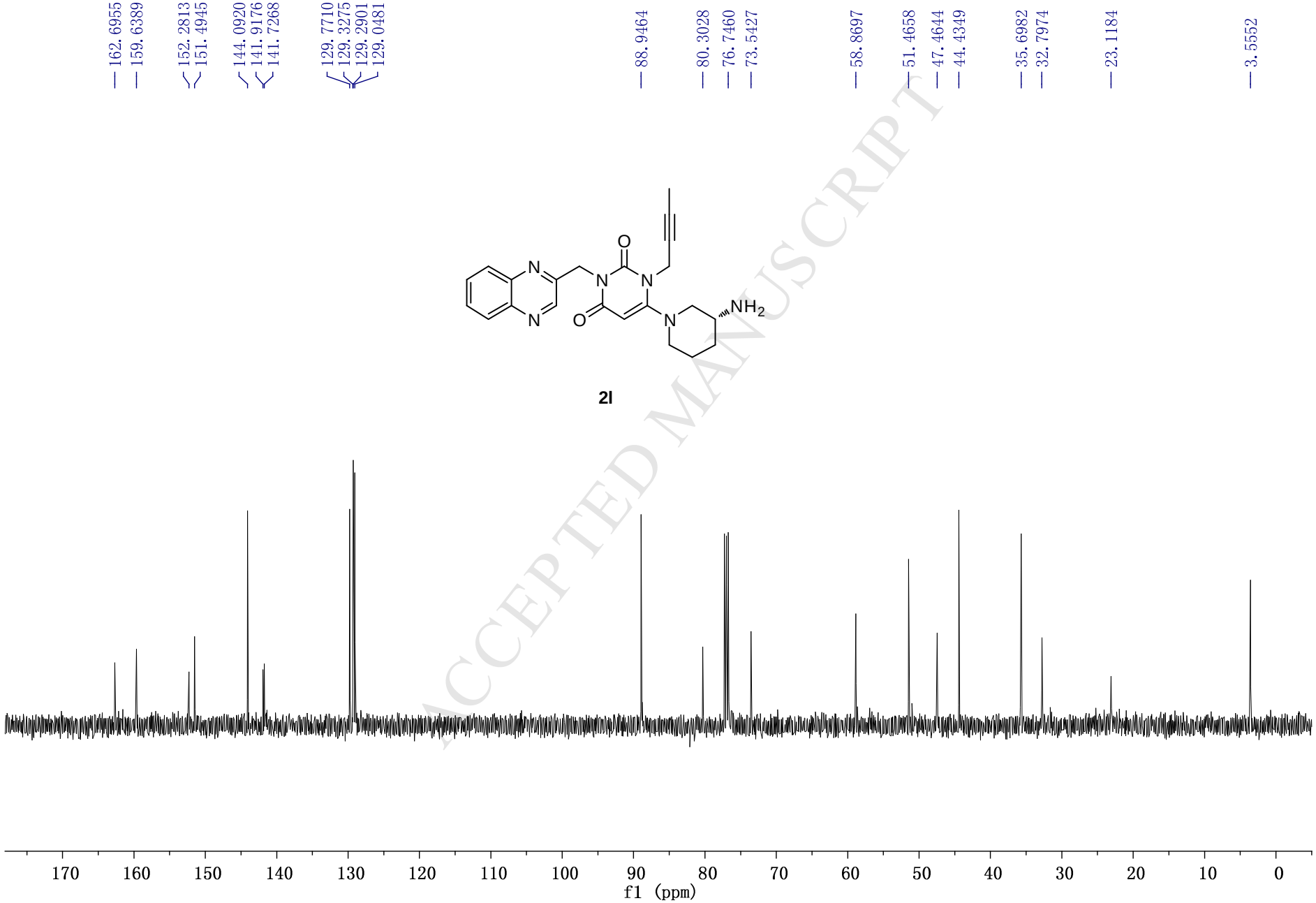
^{13}C NMR spectrum of **2k**



^{1}H NMR spectrum of **2l**

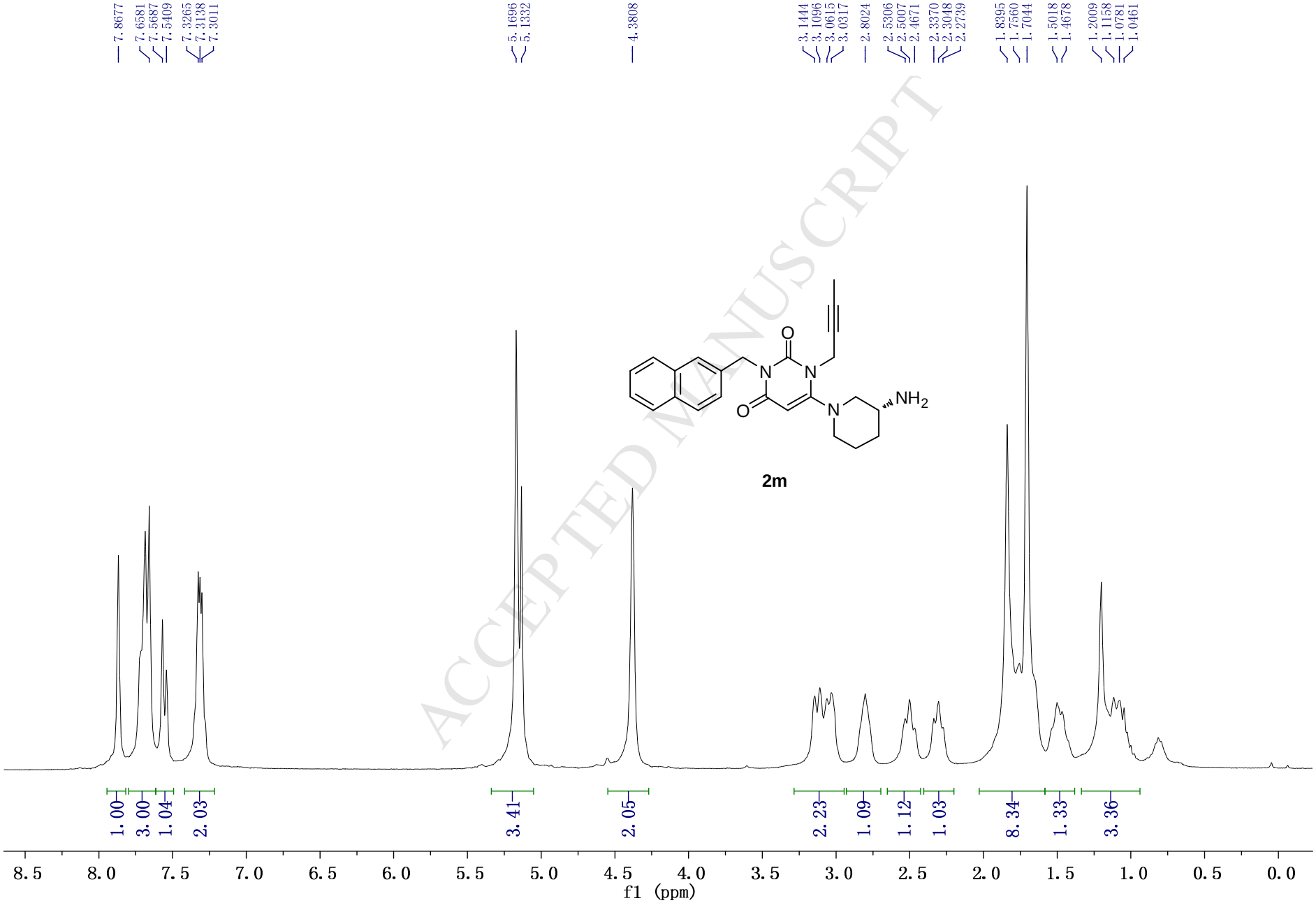


^{13}C NMR spectrum of **2l**



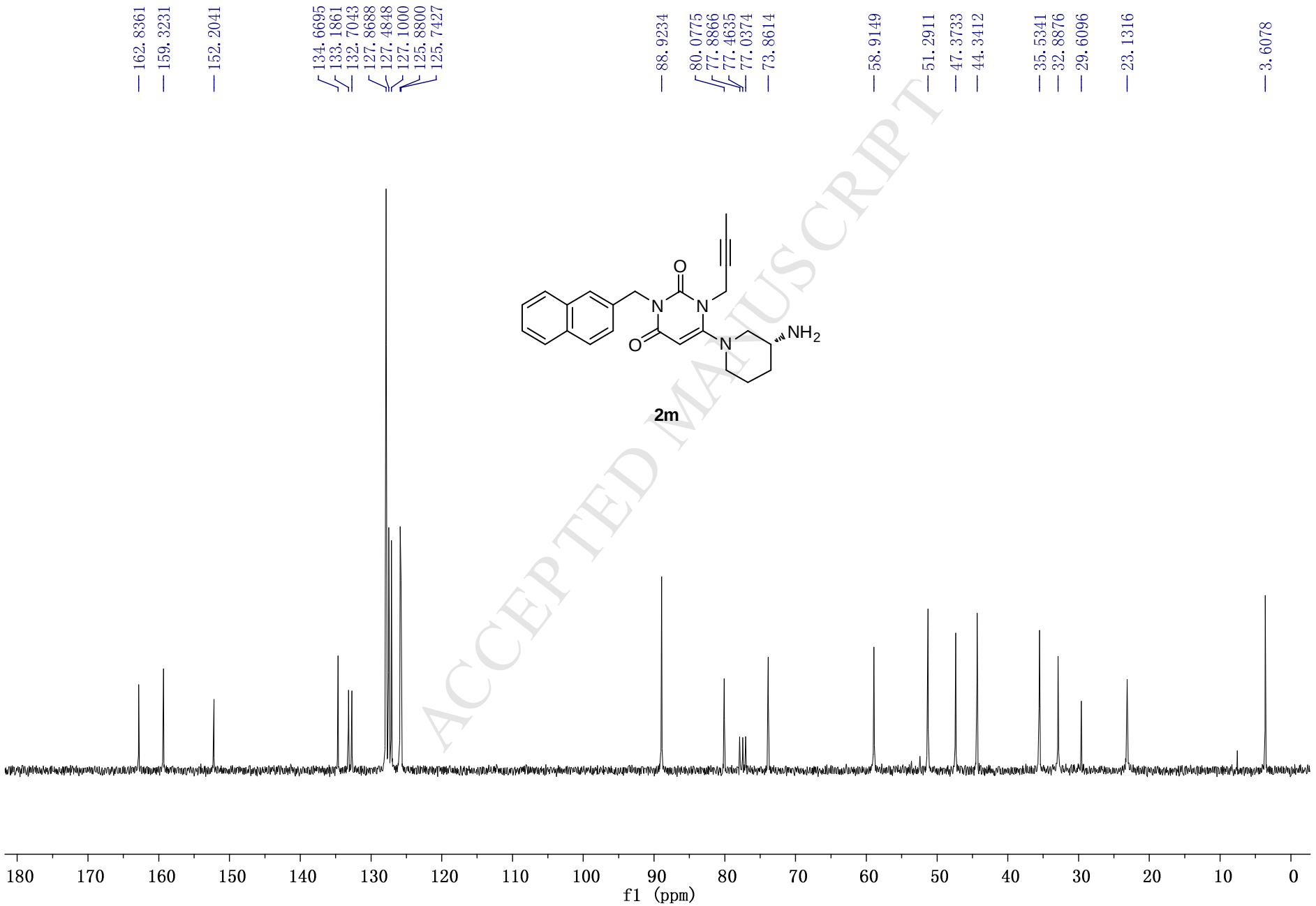
2l

^{1}H NMR spectrum of **2m**

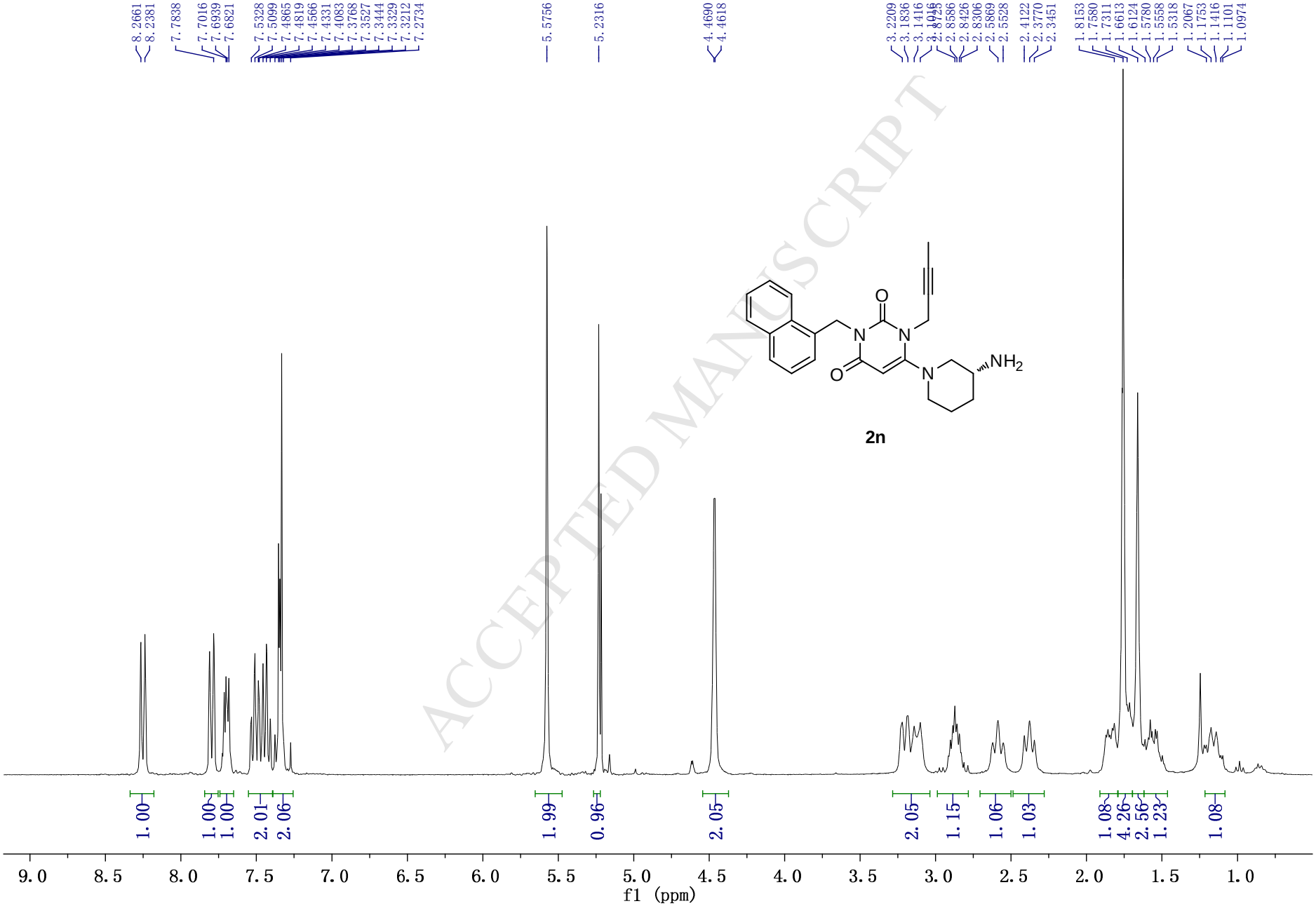


2m

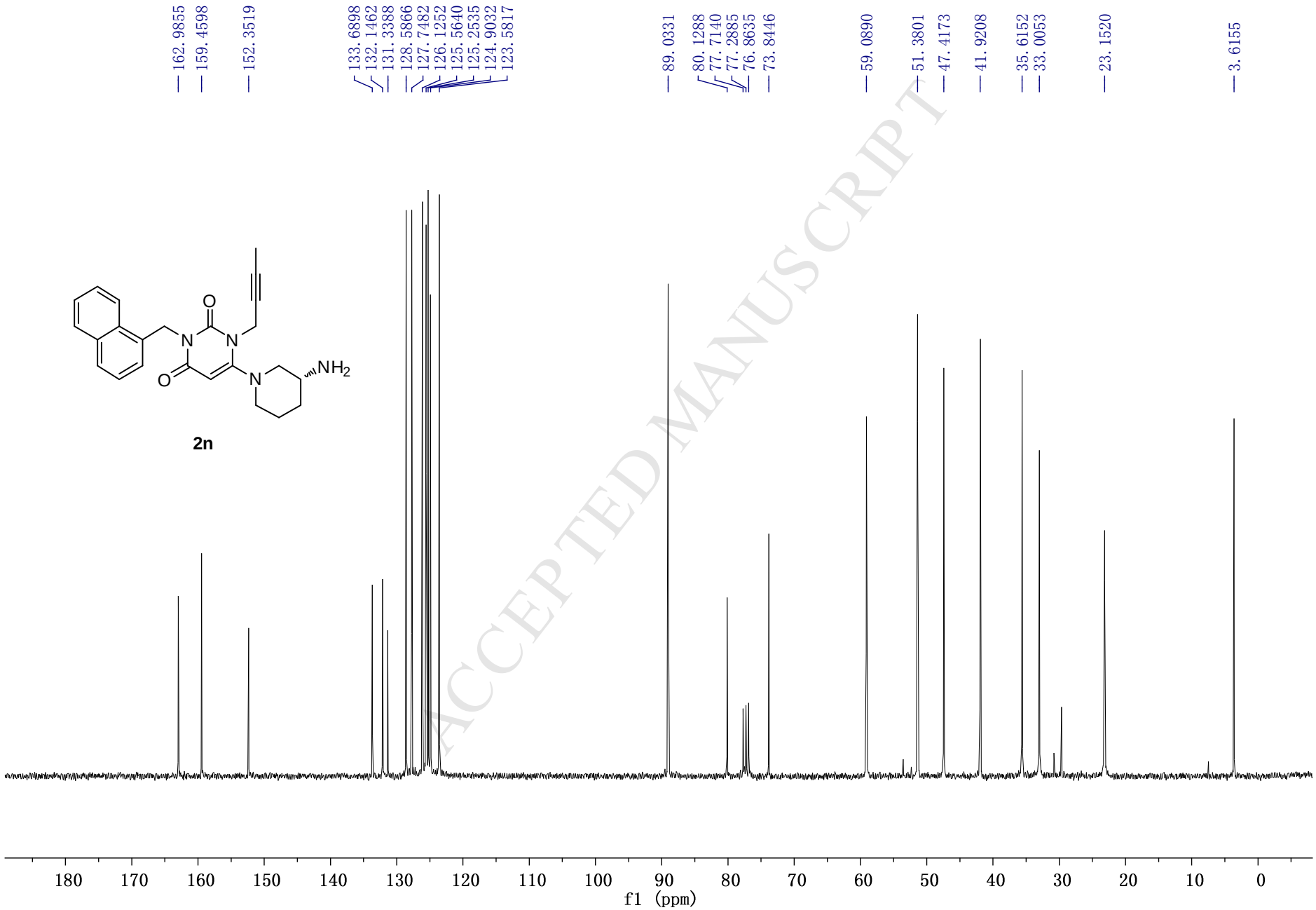
^{13}C NMR spectrum of **2m**



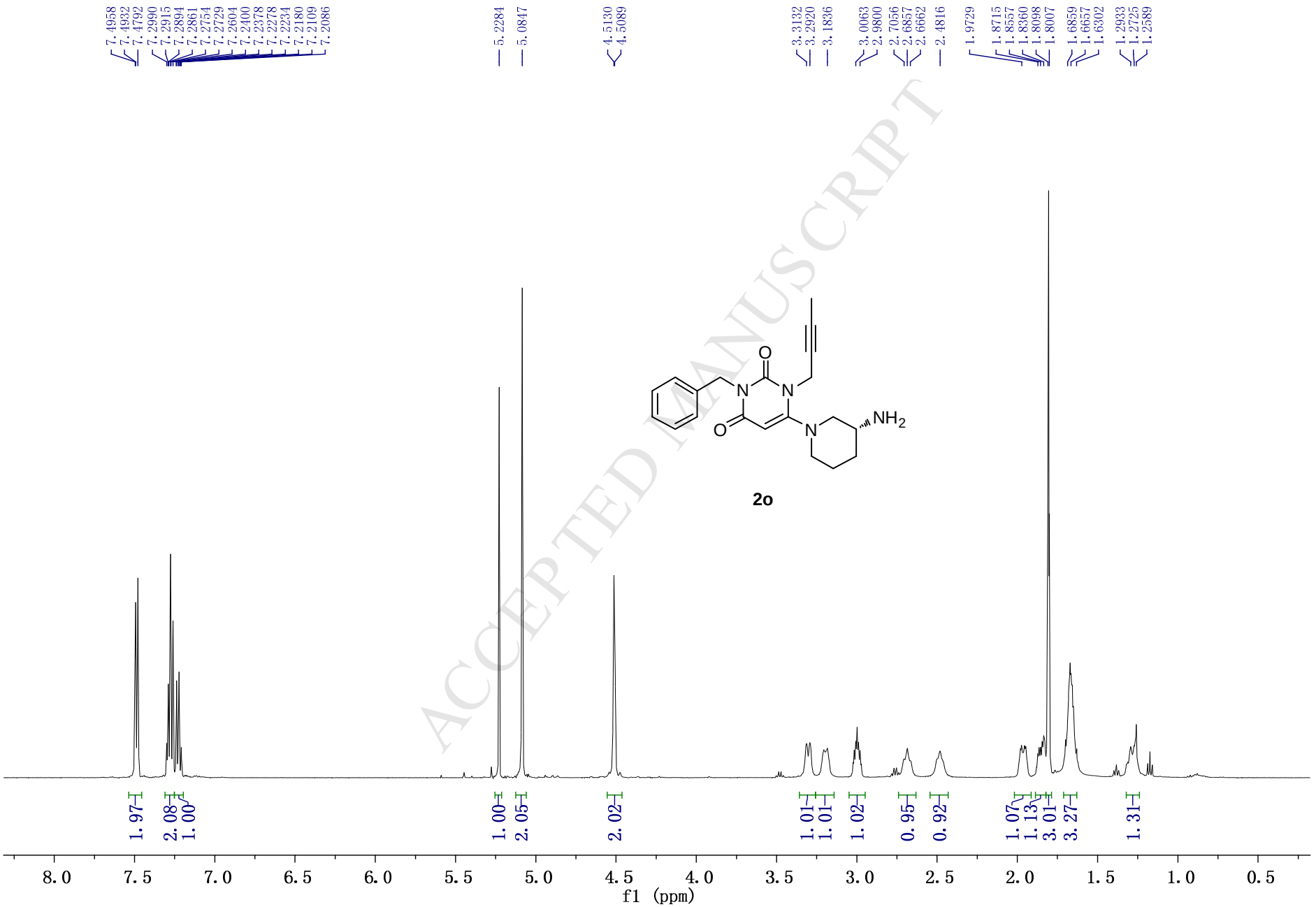
^{1}H NMR spectrum of **2n**



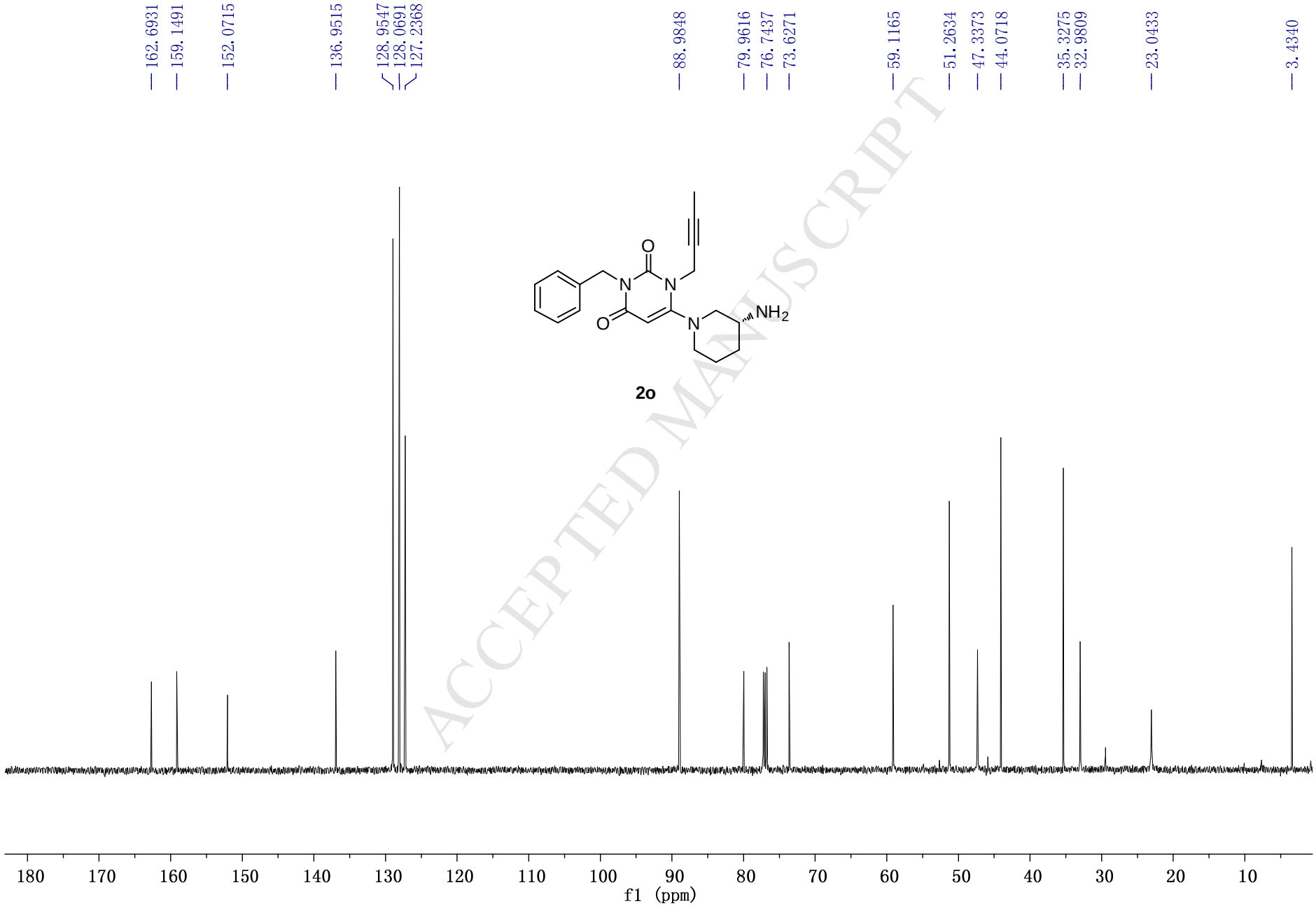
^{13}C NMR spectrum of **2n**



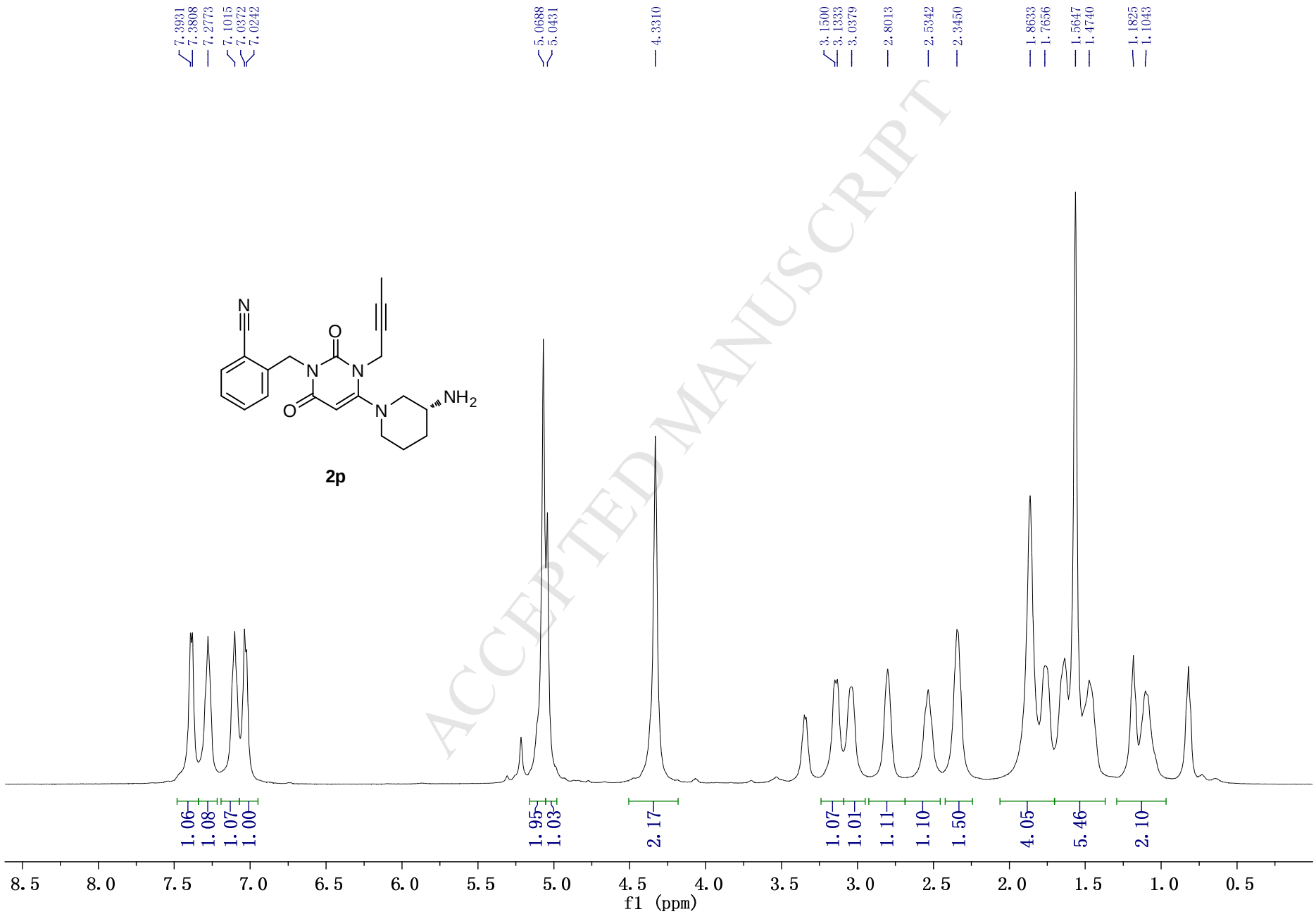
^{1}H NMR spectrum of **2o**



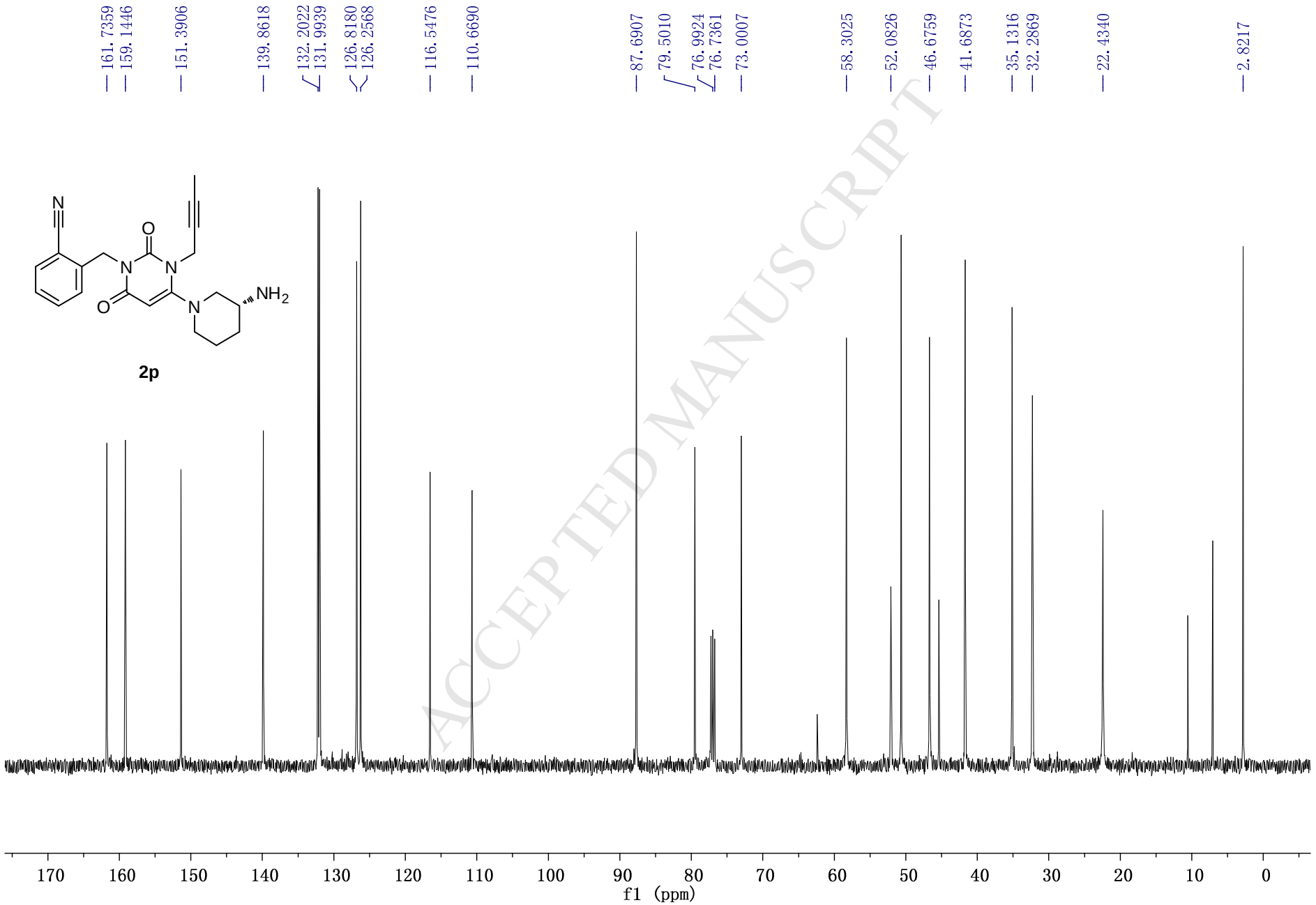
^{13}C NMR spectrum of **2o**



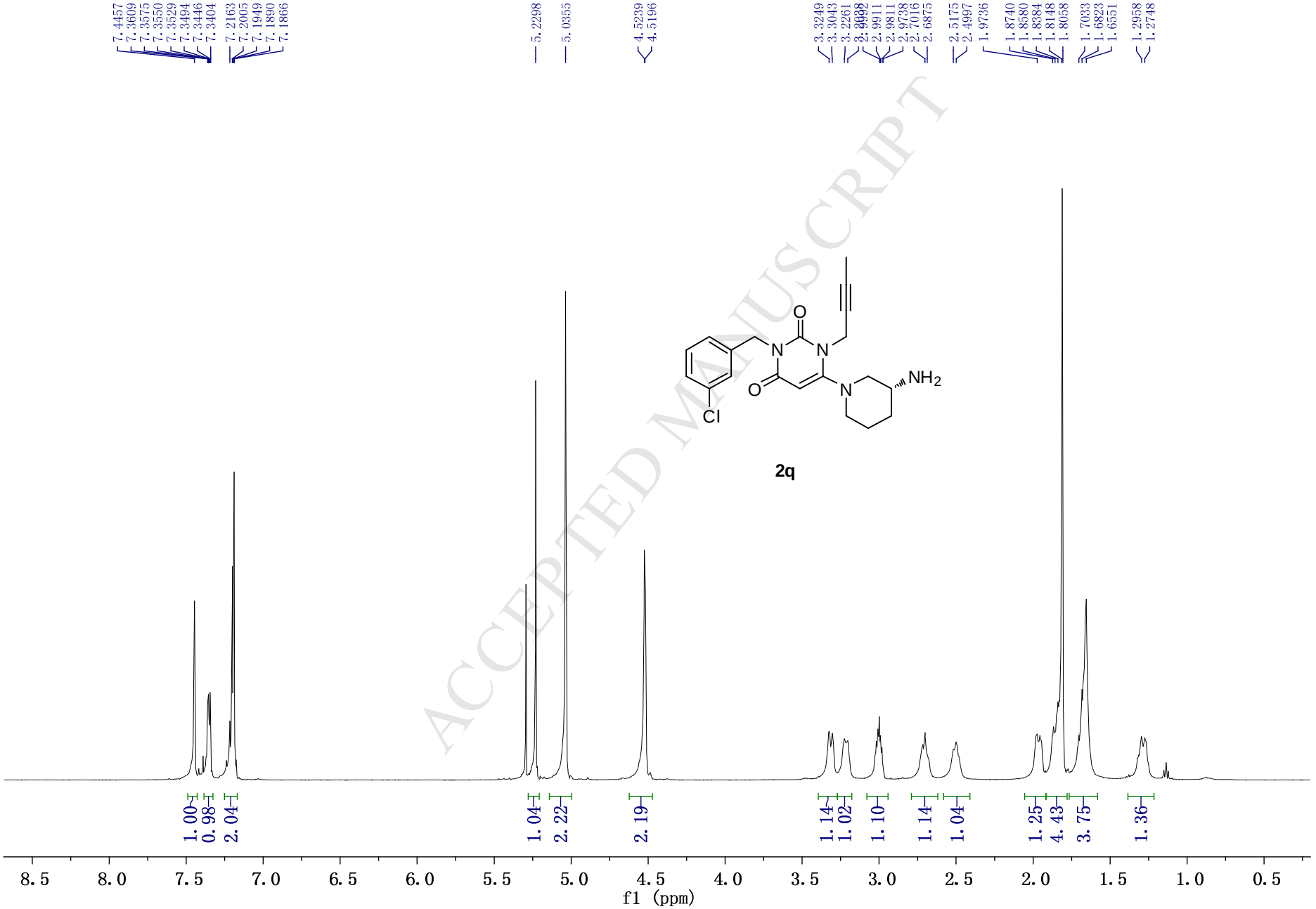
^{1}H NMR spectrum of **2p**



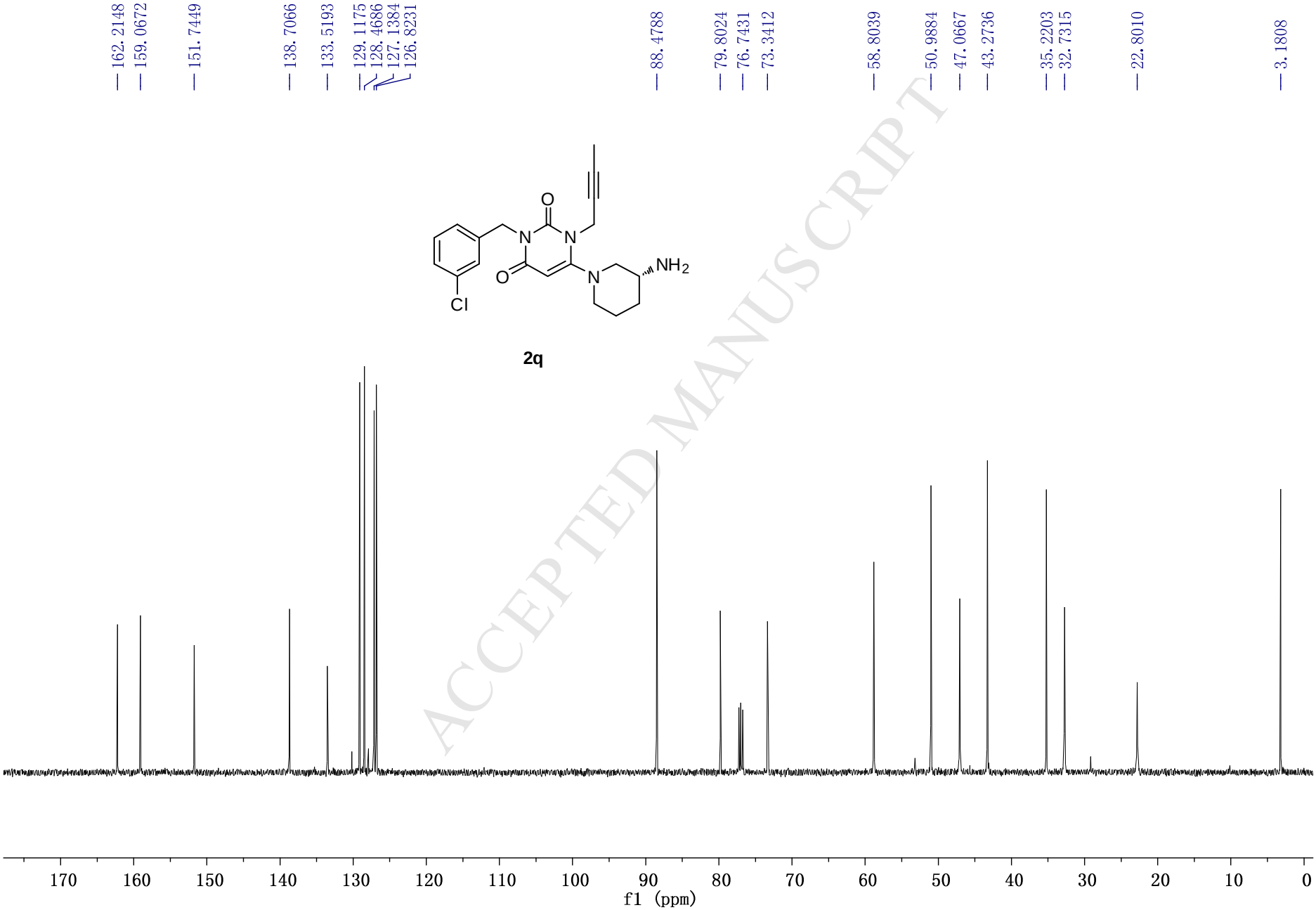
^{13}C NMR spectrum of **2p**



^{1}H NMR spectrum of **2q**

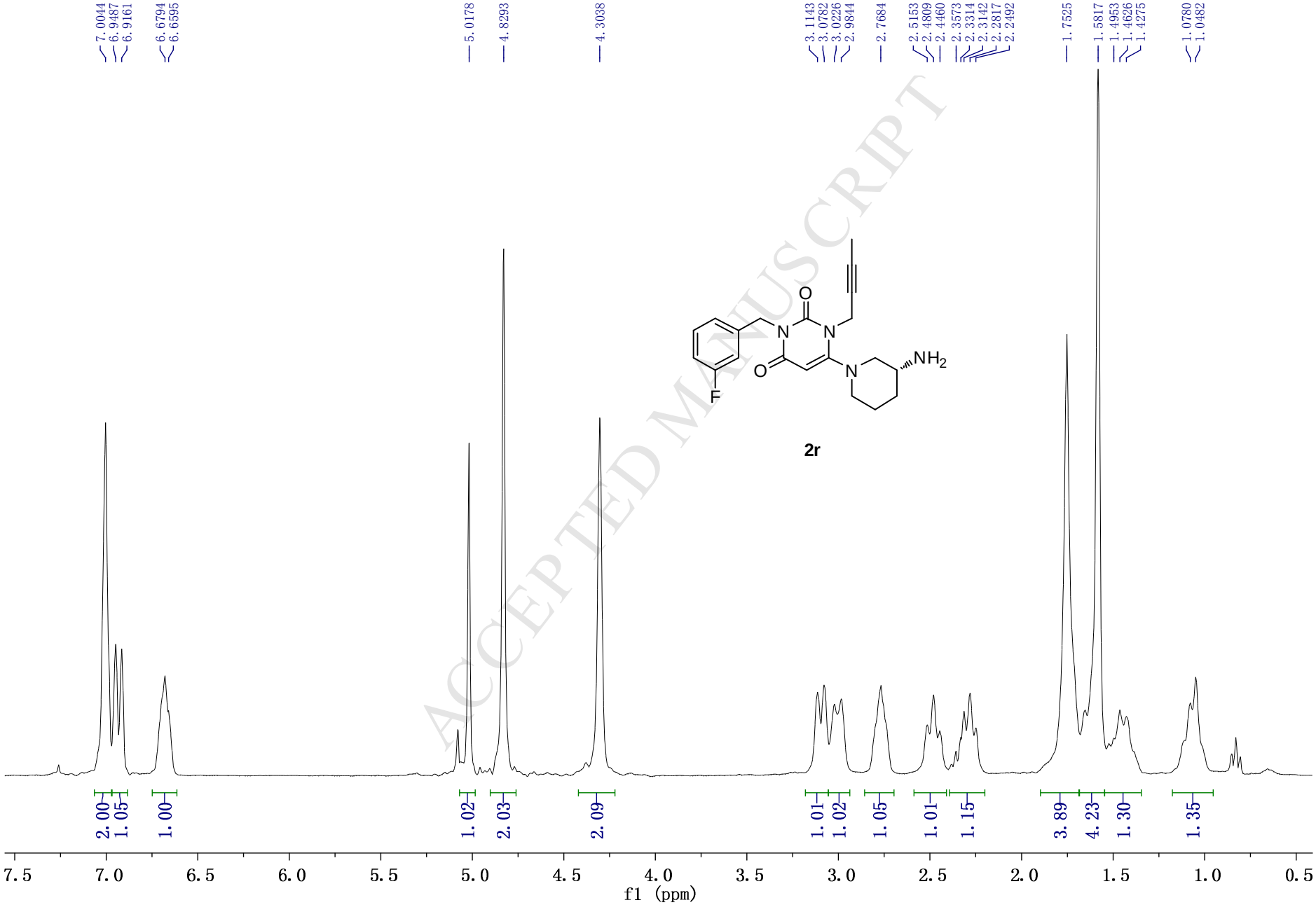


^{13}C NMR spectrum of **2q**

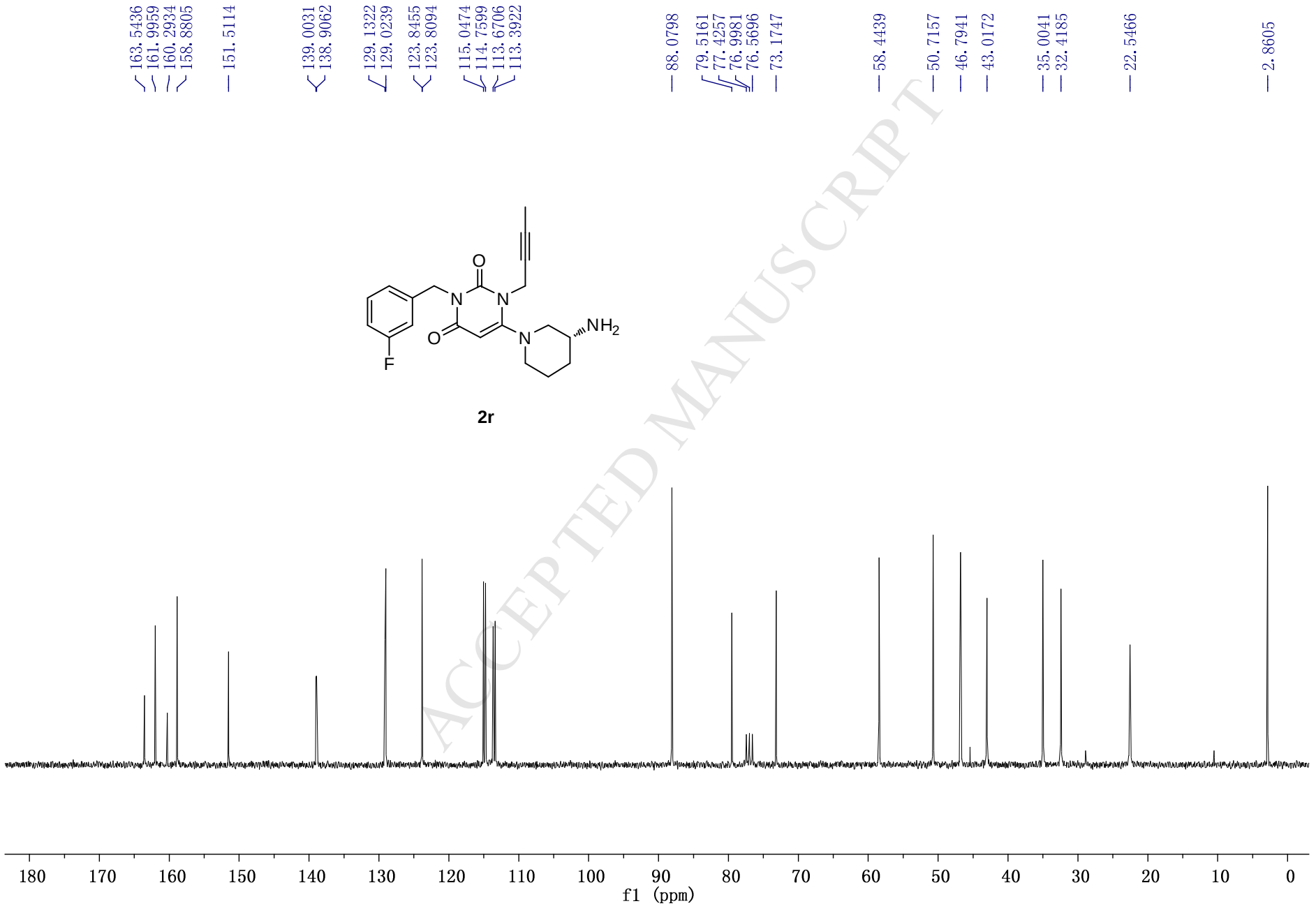


2q

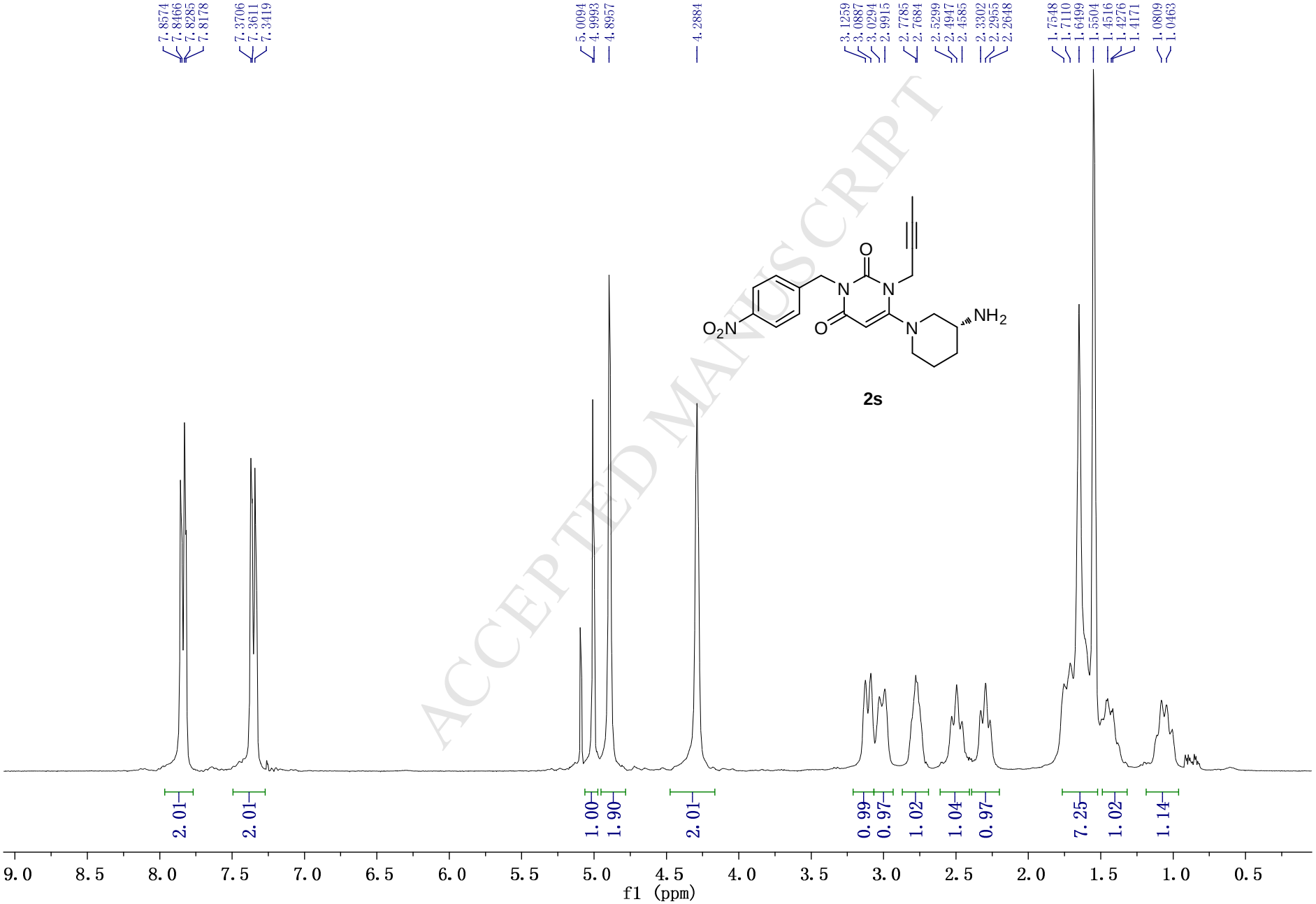
^{1}H NMR spectrum of **2r**



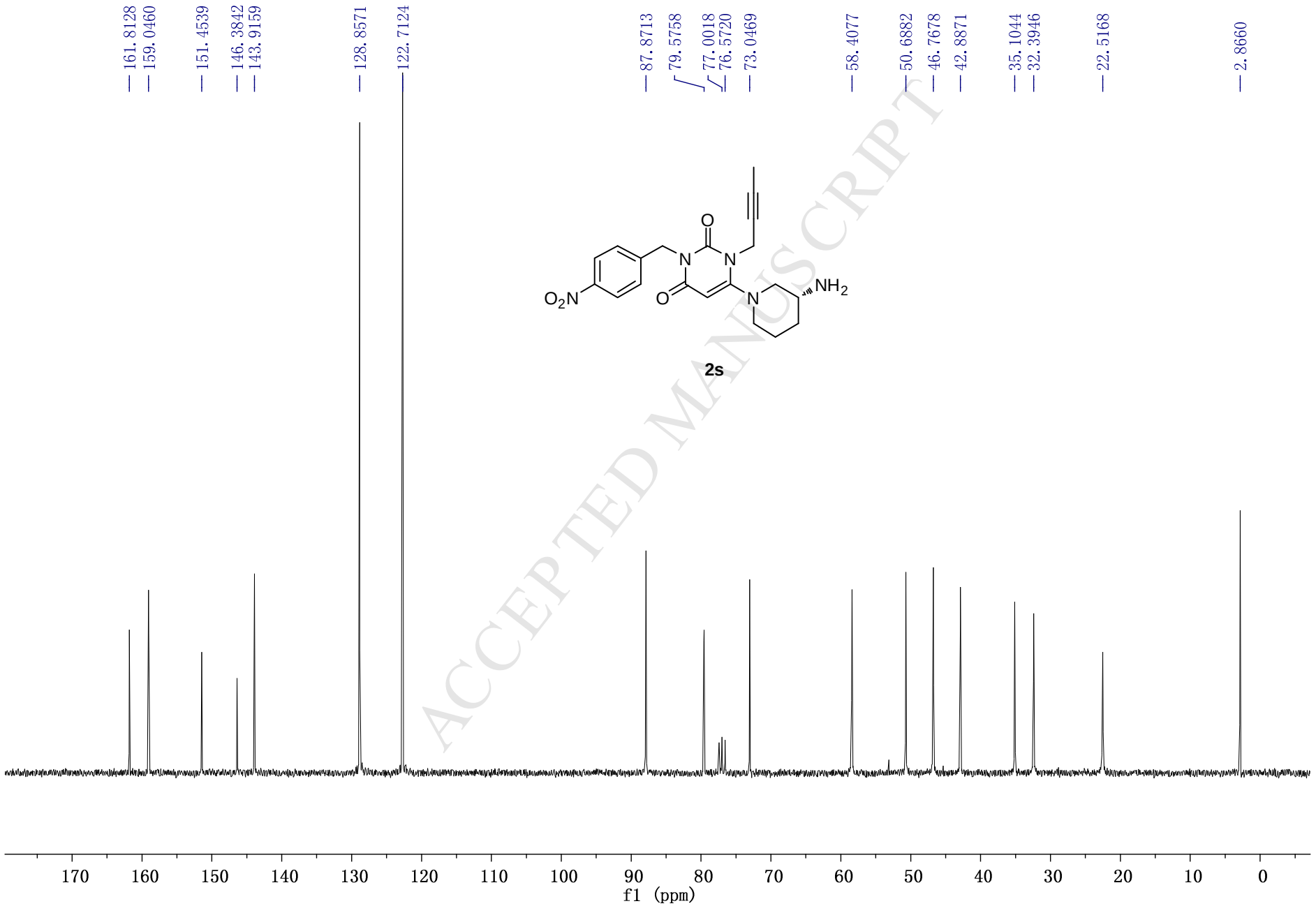
^{13}C NMR spectrum of **2r**



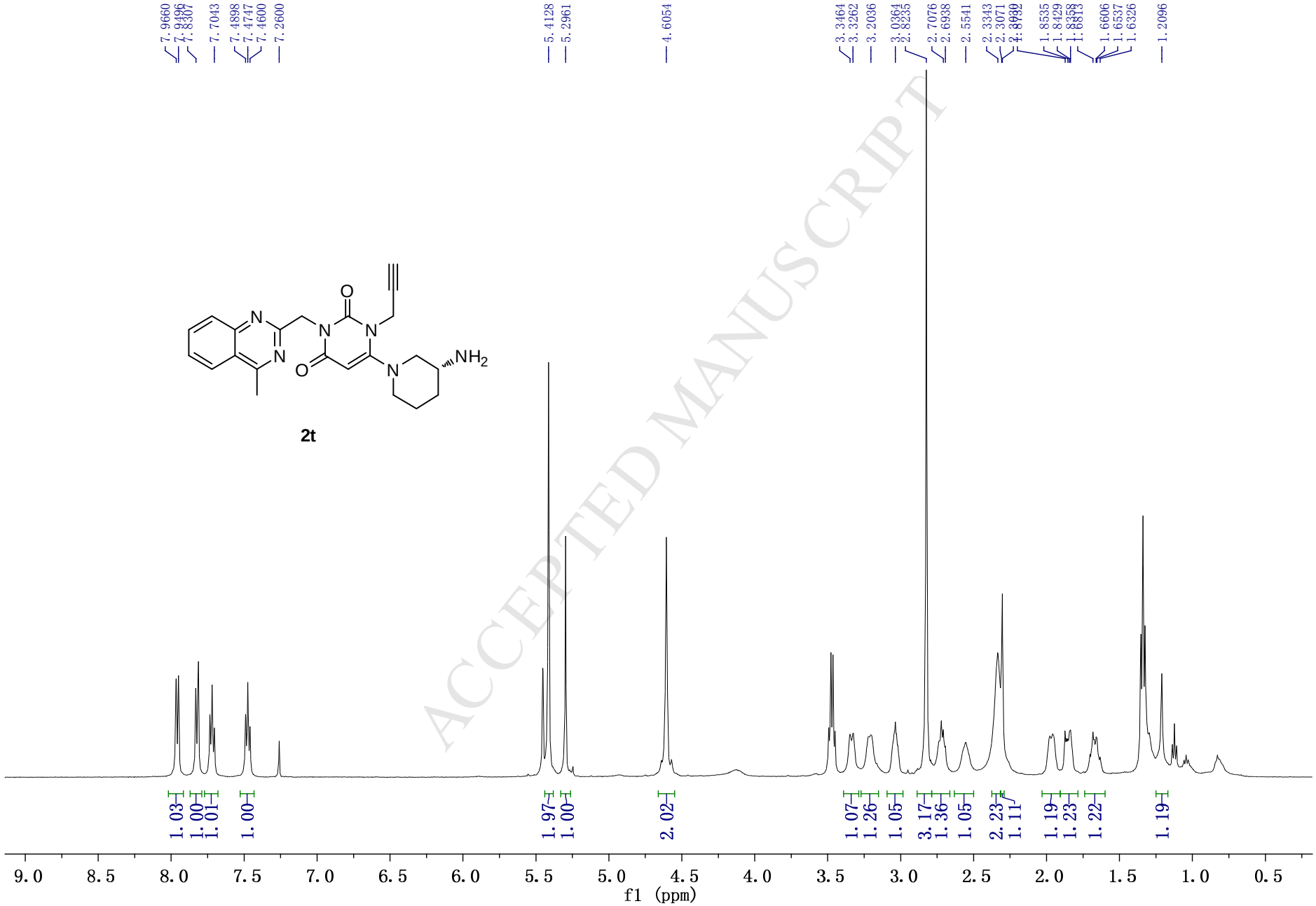
^{1}H NMR spectrum of **2s**



^{13}C NMR spectrum of **2s**

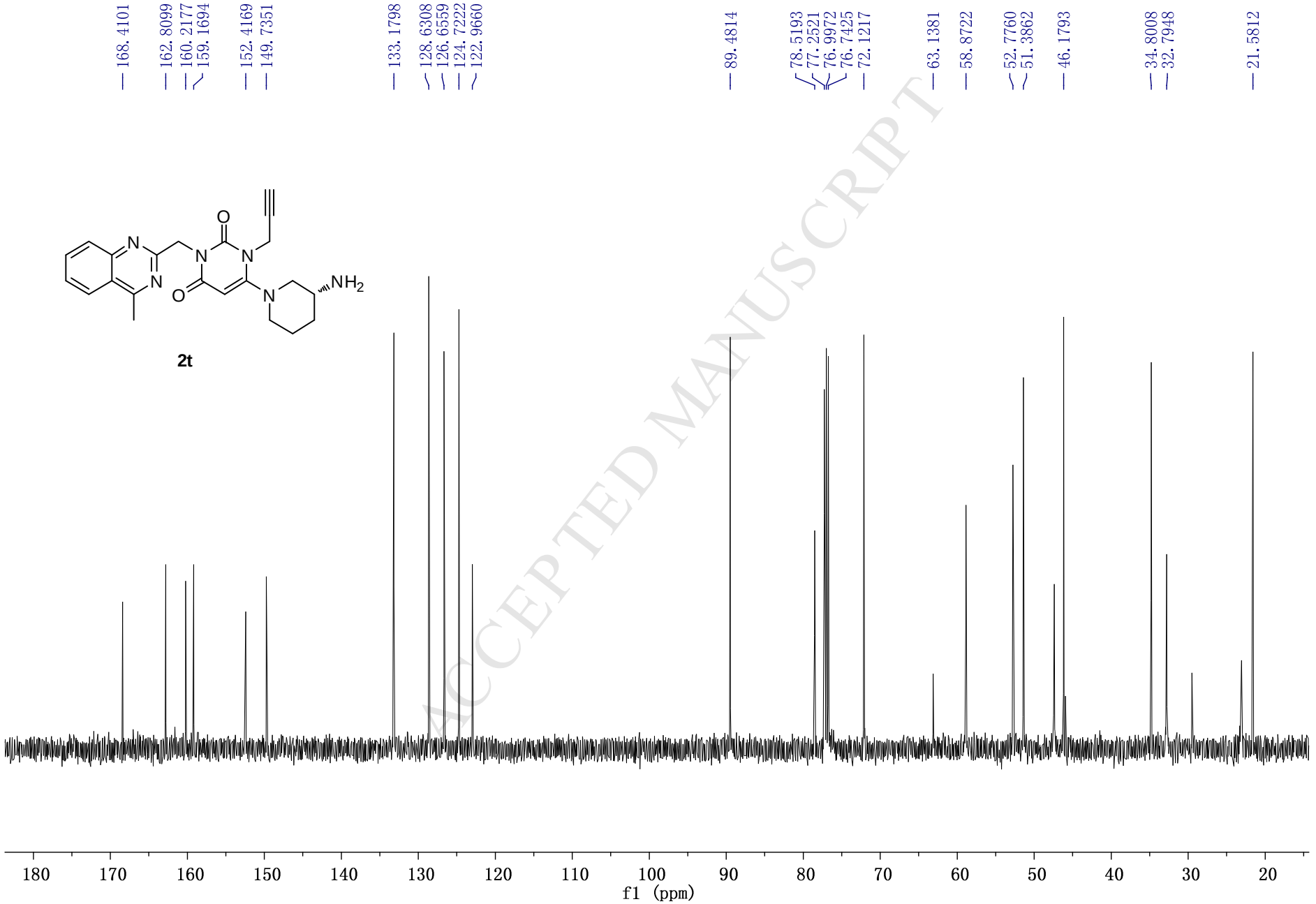


^{1}H NMR spectrum of **2t**

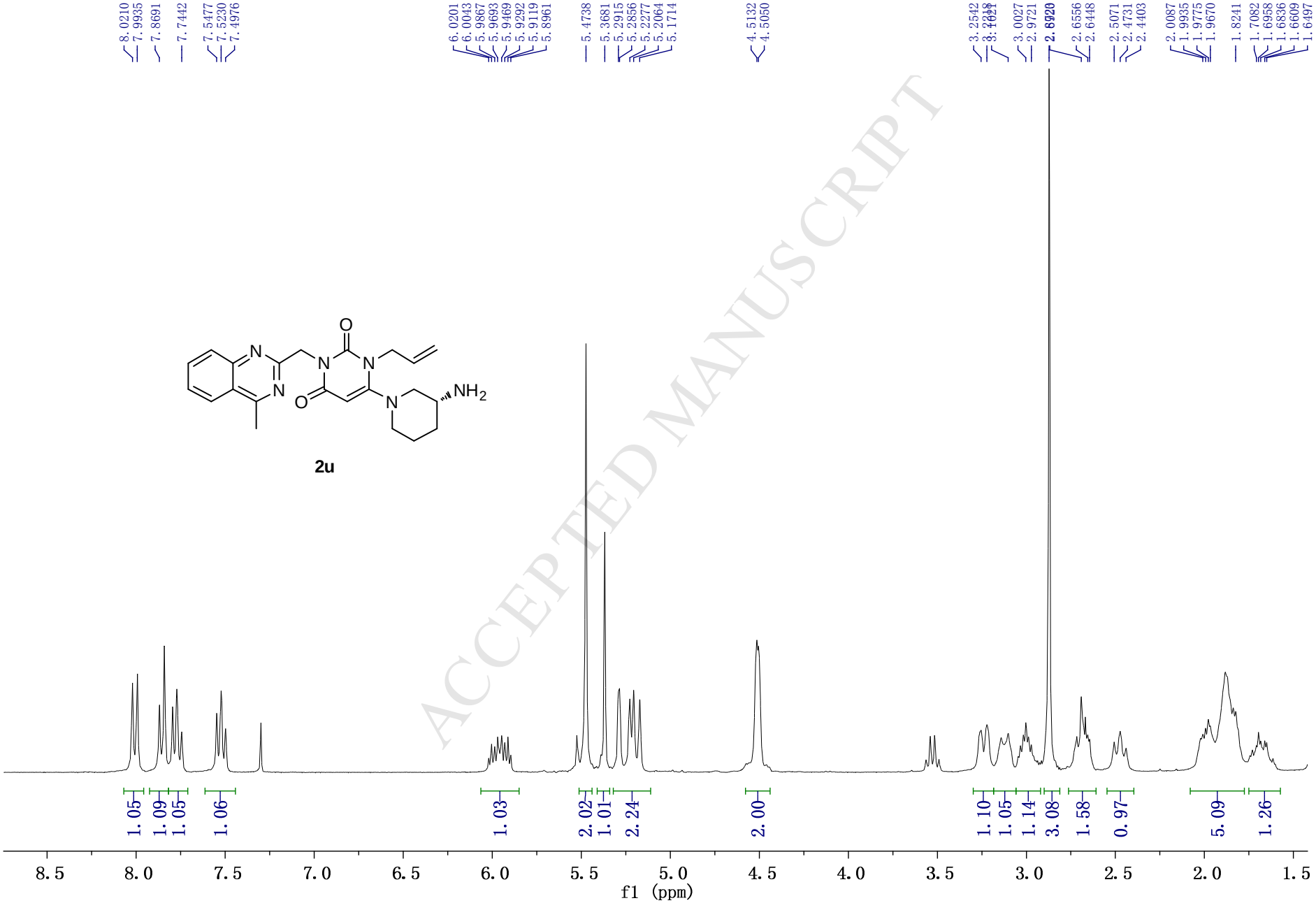


2t

^{13}C NMR spectrum of **2t**

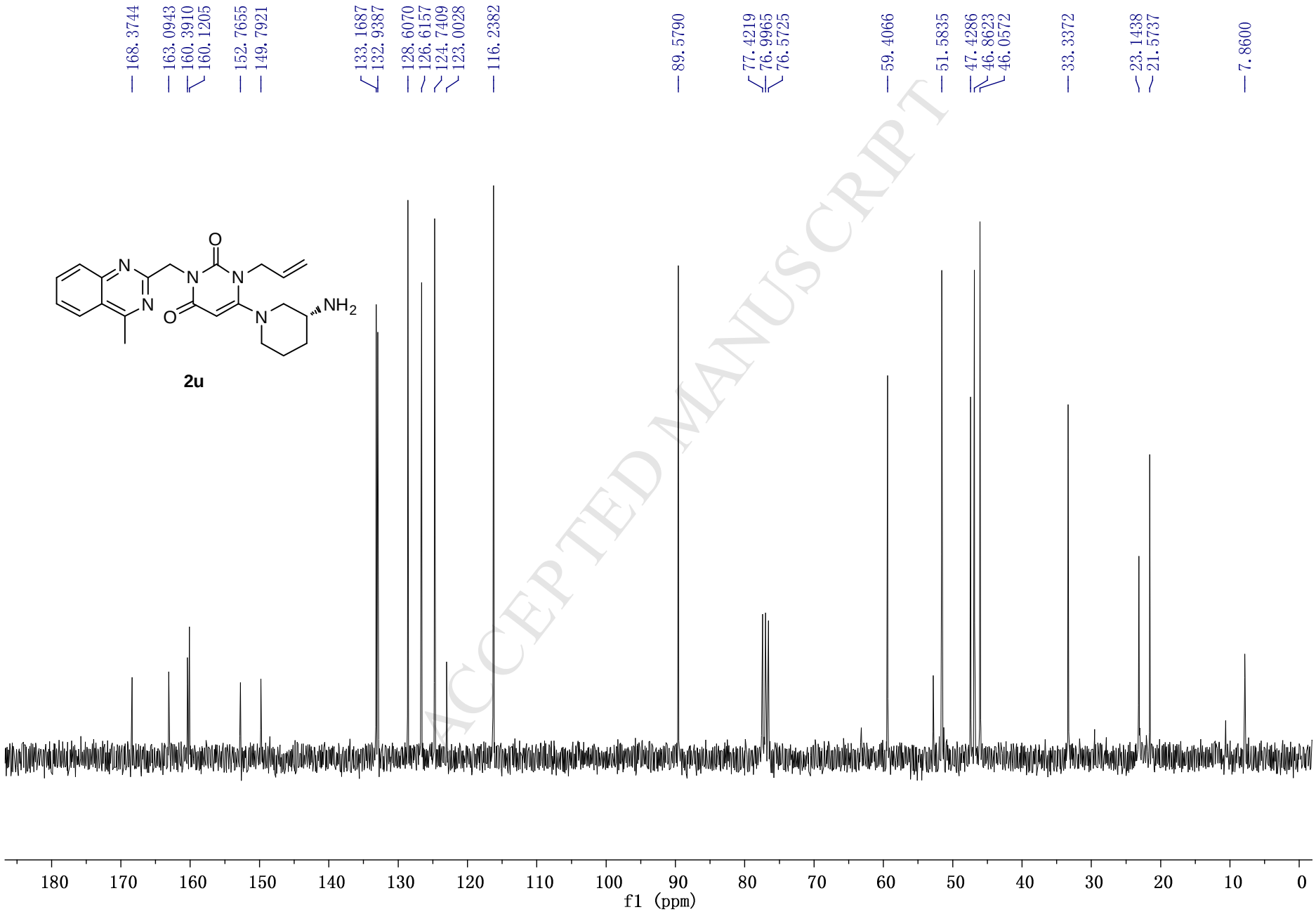


^{1}H NMR spectrum of **2u**

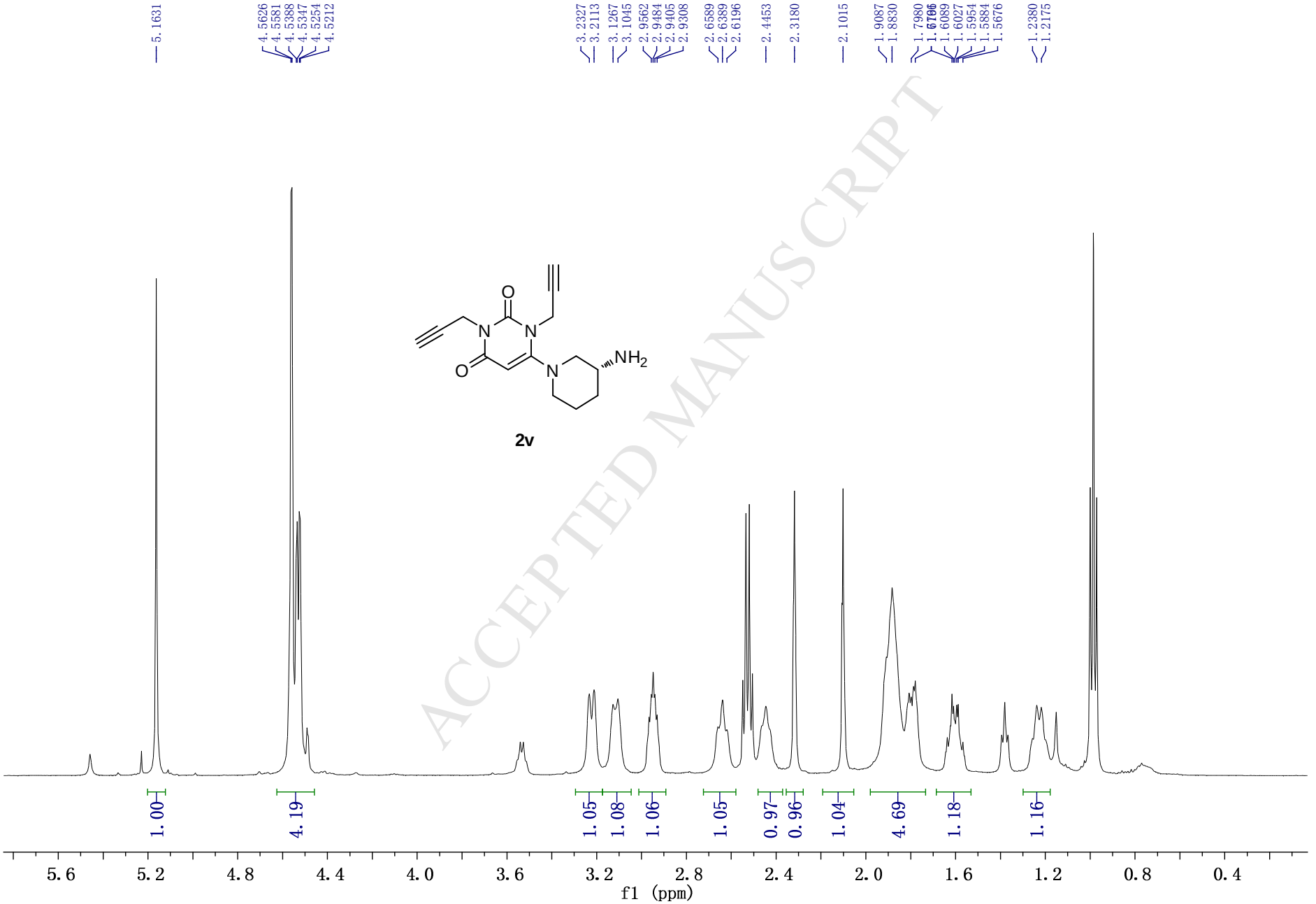


2u

^{13}C NMR spectrum of **2u**



^{1}H NMR spectrum of **2v**



^{13}C NMR spectrum of **2v**