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GRAPHICAL ABSTRACT

Synthesis and cytotoxic activity evaluation of some novel 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones in human cancer cells

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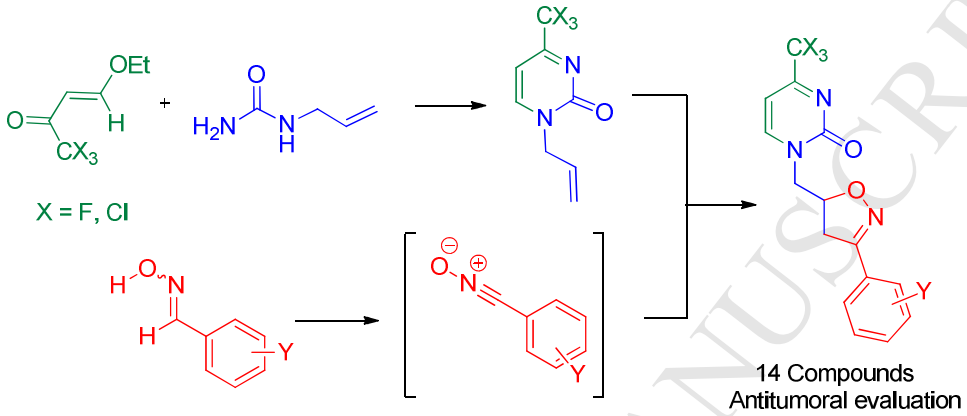

Synthesis and cytotoxic activity evaluation of some novel 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones in human cancer cells

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Abstract

The synthesis of a series of 14 new 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones from the 1,3-dipolar cycloaddition reaction of 1-allyl-4-(trihalomethyl)pyrimidin-2(1*H*)-ones with aryl nitrile oxides is described. Also, the antiproliferative activity of the title compounds was tested against five human tumoral cell lines: MCF-7 breast cancer cell line, ER+ (estrogen receptor positive); HepG-2 (hepatoma); T-24 (bladder cancer); HCT-116 cell (colorectal carcinoma); and CACO-2. The preliminary results are promising, since three compounds presented IC_{50} values below 2 μM, as well as moderate to high selectivity.

Keywords: 4,5-dihydroisoxazoles; pyrimidin-2-ones; nitrile oxides; antiproliferative activity; human tumoral cell

1. Introduction

Cancer is a complex disease that involves molecular, cellular, and tissual [1] factors and, in most cases, is caused by continuous exposure to mutagens and carcinogens such as sunlight, toxic chemicals, ionizing energies, etc. [2]. Worldwide, there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (with 5 years or less of diagnosis) in 2012. Fifty-seven percent (8 million) of new cancer cases, 65 % (5.3 million) of the cancer deaths, and 48 % (15.6 million) of the cancer cases with 5 years or less of diagnosis occurred in less developed regions [3].

In general, antineoplastic agents act primarily in the cell division process and affect both normal and abnormal cells [4]. Consequently, there are often severe side effects associated with their administration. The most common side effects are alopecia, dry mouth, dyspepsia, hematological disorders, and fatigue [5,6].

In order to overcome such drawbacks and, ultimately, to improve patients' health and wellness, several studies are being conducted to develop new anticancer agents that have superior activity and/or have fewer side effects than the currently used drugs [7]. In this context, pyrimidine nucleosides and their derivatives are considered to be an important molecular template in the development of new and improved antiproliferative agents. Several structural changes in both the nitrogenated base and the furanose ring have been proposed, with the aim of developing leading compounds or enhancing the antitumor activity of existing ones.

As a result of these studies, a series of DNA bases and nucleoside analogues have been developed and have been used in various types of cancer treatment. Among these, one can highlight 5-fluorouracil, 6-mercaptopurine and cytarabine (Figure 1) [8-10].

Figure 1

Unfortunately, these drugs present a wide range of unwanted side effects and limited efficacy. Thus, the development of new, more effective and less toxic agents remains an ongoing challenge.

Since structural modifications of a parent compound or a class of compounds are often used to improve drug candidates, we envisioned synthesizing a series of trihalomethyl-pyrimidine sugar-modified nucleoside derivatives and evaluating their cytotoxic effect on several cancer cell lines. The central idea was: i) to replace the pentose ring with a 4-5-dihydroisoxazole in order to improve the stability in physiological pH; ii) to use a methylenic linkage between the pyrimidinone and the sugar-based heterocycle, thus preventing the acidic hydrolysis which is often observed in the glycosidic bond; and iii) to insert a trihalomethyl group into the pyrimidinone moiety, due to the well-known improvements in lipophilicity and stability, which are often associated with such groups [11,12] (Figure 2).

Figure 2

2. Results and discussion

2.1. Chemistry

Scheme 1 outlines the preparation of 1-(3-aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones **8-9a–g** in a convergent and efficient way from the 1,3-dipolar [3+2] cycloaddition reaction between *N*1-allylpyrimidinones **4-5** and nitrile

oxides (**7a-g**). The *N*1-allylpyrimidinones **4-5** were obtained from the cyclocondensation reaction of enones **1-4** with allylurea **(3)** in methanol and in the presence of hydrochloric acid [13]. The hydroxymoyl chlorides were prepared from the reaction of aldoximes with *N*-chlorosuccinimide (NCS) and hydrochloric acid in THF and were converted in situ to the corresponding nitrile oxides **7a-g** by using trimethylamine [14]. Addition of the *N*1-allylpyrimidin-2-ones **4-5** to the nitrile oxides **7a-g** furnished the desired trihalomethyl-pyrimidine sugar-modified nucleoside analogues **8-9a-g** at good yields, after a 4 h period under reflux.

Scheme 1

Although similar synthetic procedures have been applied to access other modified nucelosides and nucleobases, our proposal brings some interesting improvements [13a,15]. The highly regioselective synthesis of 1-allylpyrimidinones **4-5** in a single step is one of these improvements. This straightforward method, which has already been reported by our research group, avoids a five-step procedure that would involve the following: pyrimidinone cyclization with 1,3-dielectrophiles and urea; protection of pyrimidinone nitrogen atoms; pyrimidinone *N*-1 deprotection; *N*1-allylation; and pyrimidinone *N*-3 deprotection. Additionally, the *N*-allylation procedure of our choice is simpler and more efficient than the Mitsunobu-type conditions previously described [16].

Pyrimidinone **4** and oxime **6a** were selected for the optimization of reaction conditions (Table 1). The method described by Bujak [17] was adapted for our substrates and we established that the best reaction condition was THF reflux for 4 h (Entry 6, Table 1).

Table 1

Once the best reaction condition has been established, the synthesis of all compounds of the series is performed. It was observed that neither the oxime aryl substituents nor the trihalomethyl group of the pyrimidinone significantly influenced the reaction yields. These results show the method's versatility and prove that it can be used to access a collection of sugar-modified nucleoside derivatives similar to compounds **8–9a–g**.

Table 2

It has long been established that 1,3-dipolar cycloadditions between nitrile oxides and alkenes may form two different regioisomers, with each one being a pair of enatiomers (Figure 3) [18]. The regiochemistry of these reactions has been extensively studied by Houk and Sustmann, and it essentially depends on steric and electronic effects [19].

Figure 3

The regiochemistry of the products **8-9** was assigned based on the ^{1}H and ^{13}C NMR spectra and 2D HMBC experiments. The ^{1}H and ^{13}C NMR spectra of the synthesized products **8-9** show a characteristic chemical shift pattern of 3,5-substituted isoxazolines. For compound **8d,** for example, the isoxazolinic hydrogen H-5' appears as a multiplet at δ = 5.12 ppm and the two methylene carbons neighbor to the stereogenic center, comprising four diastereotopic hydrogens, show four doublets of doublets. Two of these double doublets, belonging to the hydrogens attached to the exocyclic methylene, appear at δ = 4.41 and 3.97 ppm and the other two doublets of doublets, at δ = 3.61 and 3.28 ppm, for the endocyclic methylene hydrogens. In the ^{13}C NMR spectrum, the regiochemistry of the 1,3-dipolar cycloaddition reaction could be confirmed by the presence of the C-4' and C-5' carbons, at δ = 76.7 and 37.7 ppm, respectively [20]. In the HMBC spectrum, a cross-peak was not observed between the exocyclic H-7 with the endocyclic carbon C-3' (4 bonds), but a cross-peak of the endocyclic methylene hydrogens with the sp^2 carbon of the isoxazolinic ring was observed. This also shows

that the regioisomer obtained is the 3,5-isoxazoline because, if its 3,4-substituted isomer were obtained, one would observe a cross-peak between H-7 and C-3'. Compound **8d** was also confirmed by x-ray diffraction (Figure 4). All products of the series **8-9a-g** showed the same ^{1}H- and ^{13}C-NMR spectral pattern, which is consistent with 3,5-disubstituted isoxazolines, as described for compound **8d**.

Figure 4

2.2. Biological studies

The in vitro cytotoxic activity of some trihalomethyl-pyrimidine sugar-modified nucleoside derivatives against a wide range of cancer cell lines is presented in Table 3. The antiproliferative effects were tested using the XTT assay and were quantified in terms of IC_{50} values. The lower the IC_{50} value, the higher the antiproliferative activity [7]. The ratio between the cytotoxic parameters found in HEK-293 non tumor derived cell line and those observed in tumoral cell lines can be considered as a measurement of compound selectivity index (SI). SI ratios between 3 and 6 refer to moderate selectivity, and ratios higher than six indicate high selectivity, whereas compounds that do not fulfill either of these criteria are rated as non-selective [6,11]. The SI value is also shown in Table 3. Of the twelve compounds tested, compounds **8a**, **8e**, and **9c** stand out due to their low IC_{50} concentrations (IC_{50} < 2 μM) and moderate to high selectivity (entries 1, 4, and 9, Table 3).

Table 3

Our results suggest that **8a** exerted the highest cytotoxicity against HepG-2 cells, with an IC_{50} value of 1.04 μM and inhibition 14 times higher than normal cells (HEK-293). More importantly, **8a** was non-cytotoxic against HEK-293 non-tumor derived cell lines.

Additionally, **8e** and **9c** also showed significant inhibitory activity, in human cancer cell line T-24 and CACO-2, respectively (Table 3).

In comparison with mitoxantrone, **8a**, **8e**, and **9c** demonstrated lower IC_{50} values in tumor cell lines. Considering both cytotoxicity and the selectivity index, **8a** displayed the best profile in HepG-2 cells. Such characteristics are very important because hepatocellular carcinoma (HCC) is a common malignant disease that is often associated with chemoresistance [12]. Further studies with **8a**, using other biological models, are currently being performed in our laboratories and should provide a better understanding of the mechanisms underlying these effects in human HCC cell lines.

4. Conclusion

This study showed an elegant and efficient synthesis of a new series of trihalomethyl-pyrimidine sugar-modified nucleoside analogues from the 1,3-dipolar [3+2] cycloaddition reaction of 6-substituted 1-allyl-4-(trihalomethyl)pyrimidin-2(1*H*)-ones with different nitrile oxides, derived from the corresponding aryl oximes. The characterization and regiochemistry of the products were assigned based on the ^{1}H and 13C NMR spectra and 2D HMBC experiments, and subsequently confirmed by x-ray diffraction. In vitro antiproliferative activity of these compounds evaluated against five human cancer cell lines (MCF-7, HepG-2, T-24, HCT-116, and CACO-2) showed that three of the compounds (**8a**, **8e**, and **9c**) exhibited high antiproliferative activity (IC_{50} less than 1.40 μM) and appeared to be less toxic against normal cells. In particular, **8a** was three times more selective than MXT standard anticancer drugs. Overall, our results for the first time highlight the potential of novel 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones obtained from a simple and easy-to-obtain synthetic procedure. These novel elements could be employed as therapeutic

drugs for HCC treatment, thus providing the rationale for future experimental use in humans.

5. Experimental Protocols

All the products described in this article were fully characterized by melting point, ^{1}H and ^{13}C NMR elemental analysis, and 2D NMR experiments. All melting points were determined on a Kofler Reichert Thermovar or on a MQAPF-301 apparatus, and are uncorrected. The CHN microanalyses were performed on a Perkin Elmer 2400 elemental analyzer. High resolution mass spectra were recorded on a Bruker QTOF spectrometer in ESI-mode. ^{1}H- and ^{13}C-NMR spectra and 2D NMR experiments were registered on a Bruker DPX 400 (^{1}H at 400.13 MHz and ^{13}C at 100.62 MHz) in $CDCl_3$ or DMSO-d_6,using TMS as the internal reference.

5.1. Chemistry

General: The 4-alkoxy-4-substituted-but-3-enones (**1–2**) were prepared according to the literature [21]. The *N*1-allylpyrimidinones **6–9** were obtained from the cyclocondensation reaction of enones **1**–**2** with allylurea (**3**) in methanol and in the presence of hydrochloric acid, in accordance with previous studies [13]. The oximes **6a**–**g** were obtained by the reaction of the corresponding aldehyde with hydroxylamine hydrochloride, in accordance with the method described in the literature [22].

*5.1.2. General procedure for the synthesis of 1-(3-aryl-4,5-dihydroisoxazol-5-yl)methyl-4-trihalomethyl-1H-pyrimidin-2-ones (**8-9**)*

To a solution of oximes **6a–g** (1.2 mmol) in anhydrous THF (8 mL), *N*-Chlorosuccinimide (1.3 mmol, 0.173 g) and hydrochloric acid 37 % (1 mmol, 0.1 mL) were added and the mixture was stirred at room temperature for 2 h. The pyrimidinones **4–5** (1.0 mmol) were added to the reaction flask, followed by the dropwise addition of

triethylamine (3.9 mmol, 0.55 mL), and the temperature was raised to reflux for 4 h. The reaction product was isolated by extraction with dichloromethane (3 x 20 mL) and the combined organic phases were further extracted with 3 % hydrochloric acid solution (1 × 20 mL), then dried under anhydrous sodium sulfate, filtered, and finally, the solvent was evaporated in a rotary evaporator. The products were isolated as stable solids and they were purified by recrystallization from chloroform or ethanol, in accordance with the description of the synthesis of each compound.

*5.1.2.1. 1-((3-Phenyl-4,5-dihydroisoxazol-5-yl)methyl)-4-(trichloromethyl) pyrimidin-2(1H)-one (**8a**).* Yellow crystal, 98% yield, m.p. 195–197 ºC (ethanol); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.44 (d, *J* = 6.9, 1H, H-6), 7.67 – 7.63 (m, 2H, Ar), 7.47 – 7.43 (m, 3H, Ar), 7.04 (d, *J* = 6.9, 1H, H-5), 5.14 – 5.07 (m, 1H, H-5'), 4.25 (dd, *J* = 13.6, 3.8, 1H, H-7), 4.10 (dd, *J* = 13.6, 7.9, 1H, H-7), 3.62 (dd, *J* = 17.3, 10.6, 1H, H-4'), 3.33 (dd, *J* = 17.3, 6.1, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 170.5 (C-4), 157.0 (C-3'), 154.2 (C-2), 153.8 (C-6), 130.2, 128.9, 128.7, 126.6 (Ar), 98.5 (C-5), 95.3 (CCl_3), 76.9 (C-5'), 53.3 (C-7), 37.7 (C-4'); Anal. Calcd. for $C_{15}H_{12}Cl_3N_3O_2$ (371.00): C, 48.35; H, 3.25, N, 11.28%. Found: C, 48.72; H, 3.23; N, 11.31%.

*5.1.2.2. 1-((3-(4-Fluorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trichloromethyl) pyrimidin-2(1H)-one (**8b**).* Beige powder, 83% yield, m.p. 205–207 ºC (chloroform); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.45 (d, *J* = 6.9, 1H, H-6), 7.75 – 7.69 (m, 2H, Ar), 7.30 (t, *J* = 8.9, 2H, Ar), 7.06 (d, *J* = 6.9, 1H, H-5), 5.14 – 5.06 (m, 1H, H-5'), 4.24 (dd, *J* = 13.6, 3.7, 1H, H-7), 4.08 (dd, *J* = 13.6, 8.1, 1H, H-7), 3.62 (dd, *J* = 17.3, 10.6, 1H, H-4'), 3.39 – 3.35 (m, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 170.6 (C-4), 163.17 (d, *J* = 248.1, Ar),156.2 (C-3'), 154.2 (C-2), 154.0 (C-5), 163.2 (d, *J* = 248.1),

129.1 (d, *J* = 8.6), 125.5 (d, *J* = 3.1), 115.9 (d, *J* = 22.0) (Ar), 98.6 (C-5), 95.4 (CCl_3), 77.1 (C-5'), 53.3 (C-7), 37.8; Anal. Calcd. for $C_{15}H_{11}Cl_3FN_3O_2$ (388.99): C, 46.12; H, 2.84, N, 10.76%. Found: C, 46.27; H, 2.80; N, 11.20%

*5.1.2.3. 1-((3-(2-Toluyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trichloromethyl) pyrimidin-2(1H)-one (**8c**).* White powder, 97% yield, m.p. 202–205 ºC (chloroform); ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 8.07 (d, *J* = 7.0, 1H, H-6), 7.33 – 7.20 (m, 4H, Ar), 6.91 (d, *J* = 6.9, 1H, H-5), 5.14 – 5.05 (m, 1H, H-5'), 4.41 (dd, *J* = 13.6, 2.5, 1H, H-7), 3.97 (dd, *J* = 13.6, 7.6, 1H, H-7), 3.61 (dd, *J* = 17.1, 10.6, 1H, H-4'), 3.28 (dd, *J* = 17.1, 6.2, 1H, H-4'), 2.50 (s, 3H, Me); ^{13}C NMR (101 MHz, $CDCl_3$/TMS) δ 172.1 (C-4), 158.2 (C-3'), 155.1 (C-2), 151.5 (C-6), 137.9, 131.7, 129.97, 129.0, 127.6, 126.1 (Ar), 99.8 (C-5), 95.1 (CCl_3), 76.6 (C-5'), 53.7 (C-7), 40.7 (C-4'), 22.9 (Me); HRMS-ESI: [M + H] calcd. for $C_{16}H_{15}Cl_3N_3O_2$: 386.0230; found: 386.0227.

*5.1.2.4. 1-((3-(4-Toluyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trichloromethyl) pyrimidin-2(1H)-one (**8d**).* Brown crystal, 80% yield, m.p. 209–211 ºC (ethanol); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.46 (d, *J* = 6.9, 1H, H-6), 7.56 (d, *J* = 8.0, 2H, Ar), 7.27 (d, *J* = 8.2, 2H, Ar), 7.06 (d, *J* = 6.9, 1H, H-5), 5.12 – 5.00 (m, 1H, H-5'), 4.24 (dd, *J* = 13.5, 3.6, 1H, H-7), 4.06 (dd, *J* = 13.6, 8.2, 1H, H-7), 3.60 (dd, *J* = 17.3, 10.7, 1H, H-4'), 3.35 – 3.25 (m, 1H, H-4'), 2.34 (s, 3H, Me); ^{13}C NMR (100 MHz, DMSO-d_6/TMS) δ 170.5 (C-4), 156.8(C-3'), 154.1 (C-2), 153.8 (C-6), 140.0, 129.2, 126.5, 126.1 (Ar), 98.4 (C-5), 95.3 (CCl_3), 76.7 (C-5'), 53.2 (C-7), 37.7 (C-4'), 20.8 (Me); Anal. Calcd. for $C_{16}H_{14}Cl_3N_3O_2$ (385.02): C, 49.70; H, 3.65, N, 10.87%. Found: C, 49.32; H, 3.71; N, 10.72%

*5.1.2.5. 1-((3-(2-Hydroxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trichloromethyl) pyrimidin-2(1H)-one (**8e**).* Beige powder, 61% yield, m.p. 216–218 ºC (ethanol); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 9.78 (s, 1H, OH), 8.46 (d, *J* = 6.9, 1H, H-6), 7.44 (dd, *J* = 7.8, 1.5, 1H, Ar), 7.36 – 7.29 (m, 1H, Ar), 7.05 (d, *J* = 6.9, 1H, H-5), 6.93 (ddd, *J* = 15.0, 8.0, 1.6, 2H, Ar), 5.14 – 5.05 (m, 1H, H-5'), 4.26 (dd, *J* = 13.6, 3.7, 1H, H-7), 4.12 (dd, *J* = 13.6, 7.9, 1H, H-7), 3.71 (dd, *J* = 17.6, 10.5, 1H, H-4'), 3.44 (dd, *J* = 17.6, 6.1, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 170.5 (C-4), 158.0 (Ar), 156.0 (C-3'), 154.1 (C-2), 153.8 (C-6), 131.5, 129.0, 119.3, 116.3, 114.6 (Ar), 98.4 (C-5), 95.3 (CCl_3), 76.2 (C-5'), 53.0 (C-7), 39.0 (C-4'); Anal. Calcd. for $C_{15}H_{12}Cl_3N_3O_3$ (386.99): C, 46.36; H, 3.11, N, 10.81%. Found: C, 46.50; H, 3.00; N, 10.37%

*5.1.2.6. 1-((3-(2-Methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trichloromethyl) pyrimidin-2(1H)-one (**8f**).* White powder, 99% yield, m.p. 124–126 ºC (chloroform); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.44 (d, *J* = 6.9, 1H, H-6), 7.51 – 7.40 (m, 2H, Ar), 7.11 (d, *J* = 8.3, 1H, Ar), 7.06 (d, *J* = 6.9, 1H, H-5), 6.98 (t, *J* = 7.5, 1H, Ar), 5.10 – 5.02 (m, 1H, H-5'), 4.21 (dd, *J* = 13.7, 3.6, 1H, H-7), 4.13 (dd, *J* = 13.6, 7.1, 1H, H-7), 3.83 (s, 3H, OMe), 3.60 (dd, *J* = 17.8, 10.7, 1H, H-4'), 3.38 – 3.32 (m, 1H. H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 170.5 (C-4), 157.3 (Ar), 156.1 (C-3'), 154.2 (C-2), 154.0 (C-6), 131.6, 128.9, 120.5, 117.9, 112.1 (Ar), 98.5 (H-5), 95.4 (CCl_3), 76.9 (C-5'), 55.7 (OMe), 53.1 (C-7), 40.0 (C-4'). Anal. Calcd. for $C_{16}H_{14}Cl_3N_3O_3$ (401.01): C, 47.73; H, 3.50, N, 10.44%. Found: C, 47.85; H, 3.53; N, 10.21%

*5.1.2.7. 1-((3-(4-Methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trichloromethyl) pyrimidin-2(1H)-one (**8g**).* White powder, 94% yield, m.p. 219–221 ºC (chloroform); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.45 (d, *J* = 6.9, 1H, H-6), 7.60 (d, *J* = 8.9, 2H,

Ar), 7.05 (d, *J* = 6.9, 1H, H-5), 7.01 (d, *J* = 8.9, 2H, Ar), 5.11 – 4.99 (m, 1H, H-5'), 4.23 (dd, *J* = 13.5, 3.7, 1H, H-7), 4.05 (dd, *J* = 13.6, 8.1, 1H, H-7), 3.80 (s, 3H, OMe), 3.58 (dd, *J* = 17.2, 10.5, 1H, H-4'), 3.30 (dd, *J* = 17.2, 5.9, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 170.4 (C-4), 160.7 (Ar), 156.4 (C-3'), 154.1 (C-2), 153.8 (C-6), 128.2, 121.3, 114.13 (Ar), 98.3 (C-5), 95.3 (CCl3), 76.5 (C-5'), 55.2 (OMe), 53.2 (C-7), 37.9 (C-4'); Anal. Cal. for $C_{16}H_{14}Cl_3N_3O_3$ (401.01): C, 47.73; H, 3.50, N, 10.44%. Found: C, 47.77; H, 3.66; N, 10.55%

*5.1.2.8. 1-((3-Phenyl-4,5-dihydroisoxazol-5-yl)methyl)-4-(trifluoromethyl)pyrimidin-2(1H)-one (**9a**).* White powder, 92% yield, m.p. 181–182 ºC (ethanol); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.57 (d, *J* = 6.6, 1H, H-6), 7.67 (dd, *J* = 6.6, 3.1, 2H, Ar), 7.49 – 7.43 (m, 3H, Ar), 6.90 (d, *J* = 6.6, 1H, H-5), 5.16 – 5.06 (m, 1H, H-5'), 4.28 (dd, *J* = 13.5, 3.8, 1H, H-7), 4.11 (dd, *J* = 13.5, 8.1, 1H, H-7), 3.62 (dd, *J* = 17.3, 10.6, 1H, H-4'), 3.39 – 3.31 (m, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 161.6 (q, *J* = 35.3, C-4), 157.2 (C-3'), 155.5 (C-6), 154.7 (C-2), 130.5, 129.1, 129.0, 126.9 (Ar), 119.7 (q, *J* = 277.5, CF_3), 99.0 (d, *J* = 1.8, C-5), 77.0 (C-5'), 53.9 (C-7), 37.9 (C-4'); Anal. calcd. for $C_{15}H_{12}F_3N_3O_2$ (323.09): C, 55.73; H, 3.74; N, 13.00%. Found: C, 55.79; H, 3.75, N, 12.96%.

*5.1.2.9. 1-((3-(4-Fluorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trifluoromethyl) pyrimidin-2(1H)-one (**9b**).* White powder, 84% yield, m.p. 186–187 ºC (ethanol); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.54 (d, *J* = 6.6, 1H, H-6), 7.72 (dd, *J* = 8.6, 5.5, 2H, Ar), 7.28 (t, *J* = 8.8, 2H, Ar), 6.87 (d, *J* = 6.6, 1H, H-5), 5.14 – 5.07 (m, 1H, H-5'), 4.27 (dd, *J* = 13.6, 3.9, 1H, H-7), 4.12 (dd, *J* = 13.5, 7.9, 1H, H-7), 3.61 (dd, *J* = 17.3, 10.6, 1H, H-4'), 3.34 (dd, *J* = 17.3, 6.2, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-

d_6/TMS) δ 163.1 (d, *J* = 248.2, Ar), 161.4 (q, *J* = 35.5, C-4), 156.1 (C-3'), 155.1 (C-6), 154.4 (C-2), 128.9 (d, *J* = 8.7, Ar), 125.44 (d, *J* = 3.2, Ar), 119.4 (q, *J* = 277.4, CF_3), 115.69 (d, *J* = 22.0, Ar), 98.7 (q, *J* = 2.0, C-5), 76.9 (C-5'), 53.5 (C-7), 37.7 (C-4'); Anal. calcd. for $C_{15}H_{11}F_4N_3O_2$ (341.08): C, 52.79; H, 3.25; N, 12.31. Found: C, 52.91; H, 3.17; N, 12.21%.

*5.1.2.10. 1-((3-(2-Toluyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trifluoromethyl) pyrimidin-2(1H)-one (**9c**).* White powder, 99% yield, m.p. 142–143 ºC (ethanol); ^{1}H NMR (400 MHz, DMSO) δ 8.58 (d, *J* = 6.6, 1H, H-6), 7.43 (d, *J* = 7.5, 1H, Ar), 7.36 – 7.25 (m, 3H, Ar), 6.90 (d, *J* = 6.6, 1H, H-5), 5.11 – 5.02 (m, 1H, H-5'), 4.28 (dd, *J* = 13.5, 3.8, 1H, H-7), 4.15 (dd, *J* = 13.5, 7.6, 1H, H-7), 3.68 (dd, *J* = 17.2, 10.6, 1H, H-4'), 3.37 (dd, *J* = 17.2, 5.8, 1H, H-4'), 2.44 (s, 3H, Me); ^{13}C NMR (101 MHz, DMSO) δ 161.4 (q, *J* = 35.4, C-4), 157.7 (C-3'), 155.3 (C-6), 154.5 (C-2), 136.9, 131.2, 129.4, 129.1, 128.0, 125.9 (Ar), 119.4 (q, *J* = 277.5, CF_3), 98.8 (d, *J* = 1.7, C-5), 75.8 (C-5'), 53.5 (C-7), 40.2 (C-4'), 22.0 (Me); Anal. calcd. for $C_{16}H_{14}F_3N_3O_2$ (337.10): C, 56.97; H, 4.18; N, 12.46%. Found: C, 56.52; H, 4.22; N, 12.35%.

*5.1.2.11. 1-((3-(4-Toluyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trifluoromethyl) pyrimidin-2(1H)-one (**9d**).* White powder, 86% yield, m.p. 205–207 ºC (ethanol); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.51 (d, *J* = 6.6, 1H, H-6), 7.53 (d, *J* = 8.1, 2H, Ar), 7.25 (d, *J* = 8.0, 2H, Ar), 6.87 (d, *J* = 6.6, 1H, H-5), 5.10 – 5.02 (m, 1H, H-5'), 4.26 (dd, *J* = 13.6, 3.7, 1H, H-7), 4.08 (dd, *J* = 13.6, 8.0, 1H, H-7), 3.58 (dd, *J* = 17.3, 10.6, 1H, H-4'), 3.30 (dd, *J* = 17.3, 6.1, 1H, H-4'), 2.33 (s, 3H, Me); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 161.6 (q, *J* = 35.3, C-4), 157.1 (C-3'), 155.3 (C-6), 154.7 (C-2), 140.3, 129.5, 126.8, 126.2 (Ar), 119.6 (q, *J* = 277.5, CF_3), 99.0 (q, *J* = 1.8, C-5), 76.8

(C-5'), 53.9 (C-7), 38.0 (C-4'), 21.0 (Me); Anal. calcd. for $C_{16}H_{14}F_3N_3O_2$ (337.10): C, 56.97; H, 4.18; N, 12.46%. Found: C, 57.18; H, 4.03; N, 12.48%

*5.1.2.12. 1-((3-(2-Hydroxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trifluoromethyl) pyrimidin-2(1H)-one (**9e**).* Beige powder, 55% yield, m.p. 212–213 ºC (ethanol); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 9.73 (s, 1H, OH), 8.54 (d, *J* = 6.6, 1H, H-6), 7.44 (dd, *J* = 7.8, 1.6, 1H, Ar), 7.32 (ddd, *J* = 8.3, 7.3, 1.7, 1H, Ar), 6.98 – 6.88 (m, 2H, Ar), 6.87 (d, *J* = 6.6, 1H, H-6), 5.13 – 5.03 (m, 1H, H-5'), 4.28 (dd, *J* = 13.6, 3.8, 1H, H-7), 4.14 (dd, *J* = 13.6, 7.9, 1H, H-7), 3.71 (dd, *J* = 17.6, 10.5, 1H, H-4'), 3.44 (dd, *J* = 17.6, 6.1, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 161.3 (q, *J* = 35.2, C-4), 157.9 (Ar), 156.0 (C-3'), 155.0 (C-6), 154.3 (C-2), 131.4, 128.9 (Ar), 119.3 (q, *J* = 277.5, CF_3) 119.2, 116.2, 114.5 (Ar), 98.6 (d, *J* = 1.7, C-5), 76.1 (C-5'), 53.3 (C-7), 38.9 (C-4'); Anal. calcd. for $C_{15}H_{12}F_3N_3O_3$ (339.08): C, 53.10; H, 3.57; N, 12.39%. Found: C, 52.77; H, 3.76; N, 12.08%

*5.1.2.13. 1-((3-(2-Methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trifluoromethyl) pyrimidin-2(1H)-one (**9f**).* White powder, 92% yield, m.p. 142–143 ºC (chloroform); ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.54 (d, *J* = 6.6, 1H, H-6), 7.50 (d, *J* = 7.6, 1H, Ar), 7.43 (t, *J* = 7.9, 1H, Ar), 7.10 (d, *J* = 8.4, 1H, Ar), 6.97 (t, *J* = 7.5, 1H, Ar), 6.90 (d, *J* = 6.6, 1H, H-5), 5.09 – 5.01 (m, 1H, H-5'), 4.23 (dd, *J* = 13.5, 3.5, 1H, H-7), 4.13 (dd, *J* = 13.6, 7.3, 1H, H-7), 3.82 (s, 3H, OMe), 3.60 (dd, *J* = 17.8, 10.6, 1H, H-4'), 3.38 – 3.30 (m, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 161.42 (q, *J* = 35.4, C-4) 157.3 (Ar), 156.2 (C-3'), 155.4 (C-6), 154.6 (C-2), 131.7, 129.0, 120.5 (Ar), 119.51 (q, *J* = 277.5, CF_3), 117.9, 112.2 (Ar), 98.8 (d, *J* = 1.8, C-5), 76.8 (C-5'), 55.7 (OMe), 53.5

(C-7), 40.1 (C-4'); Anal. calcd. for $C_{16}H_{14}F_3N_3O_3$ (353.10): C, 54.39; H, 3.99; N, 11.89%. Found: C, 54.42; H, 3.89; N, 11.92%.

*5.1.2.14. 1-((3-(4-Methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-(trifluoromethyl) pyrimidin-2(1H)-one (**9g**).* White powder, yield 99%, m.p. 188–190 ºC (chloroform). ^{1}H NMR (400 MHz, DMSO-d_6/TMS) δ 8.56 (d, *J* = 6.6, 1H, H-6), 7.60 (d, *J* = 8.6, 2H, Ar), 7.01 (d, *J* = 8.6, 2H, Ar), 6.90 (d, *J* = 6.6, 1H, H-5), 5.09 – 5.01 (m, 1H, H-5'), 4.26 (dd, *J* = 13.5, 3.7, 1H, H-7), 4.08 (dd, *J* = 13.5, 8.1, 1H, H-7), 3.80 (s, 3H, OMe), 3.58 (dd, *J* = 17.2, 10.5, 1H, H-4'), 3.35 – 3.27 (m, 1H, H-4'); ^{13}C NMR (101 MHz, DMSO-d_6/TMS) δ 161.4 (q, *J* = 35.4, C-4), 160.8 (Ar), 156.5 (C-3'), 155.3 (C-6), 154.5 (C-2), 128.3, 121.3 (Ar), 119.48 (q, *J* = 277.3, CF_3), 114.2 (Ar), 98.8 (d, *J* = 1.6, C-5), 76.4 (C-5'), 55.3 (OMe), 53.7 (C-7), 37.9 (C-4'); Anal. calcd. for $C_{16}H_{14}F_3N_3O_3$ (353.10): C, 54.39; H, 3.99; N, 11.89%. Found: C, 54.42; H, 3.86; N, 11.87%.

5.2. Biologic activities

5.2.1. General

Dulbecco's modified Eagle's medium (DMEM); Roswell Park Memorial Institute medium (RPMI-1640); phosphate-buffered saline (PBS) — Na_2HPO_4, KH_2PO_4, and KCl, pH 7.4; and mitoxantrone (MXT) were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS) and penicillin/streptomycin were obtained from Gibco-BRL (Grand Island, NY, USA). The Cell Proliferation Kit II (XTT) was acquired from Roche (Basel, Switzerland). All other reagents were of analytical grade.

The human cell lines MCF-7 (breast adenocarcinoma), HepG-2 (hepatoma), T-24 (bladder cancer), CACO-2 (colorectal adenocarcinoma), and HEK-293 (human embryonic kidney) were obtained from the Rio de Janeiro Cell Bank (Rio de Janeiro,

RJ, Brazil). The HCT-116 cell (colorectal carcinoma) was kindly provided by Dr. Annette K. Larsen — Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France. All cell lines except MCF-7 were grown in DMEM supplemented with 10 % or 20 % (for CACO-2) FBS, 100 units.mL^{-1} penicillin, and 100 μg.mL^{-1} streptomycin, at 37 °C in a humidified atmosphere of 5 % CO_2. MCF-7 cells were maintained in RPMI-1640 supplemented with 20 % FBS, in the same conditions as described above.

5.2.2. Cytotoxic activity of 1-(3-aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1H-pyrimidin-2-ones ***8-9a-g***

The cytotoxic potential of the 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones **8-9a-g** as evaluated by the XTT assay in human tumor cell lines as well as in HEK-293 normal epithelial cells. Cells (1×10^4 cells) were seeded on 96-well plates in growth medium and incubated overnight. Afterwards, these compounds (0.1, 0.5, 1.0, 2.5, 5.0, and 10 μM) were added to each well and incubated for 24 h. Mitoxantrone, which is a cytostatic anthracenedione that intercalates in DNA and increases the incidence of double-strand breaks by stabilizing the cleavable complex of topoisomerase II and DNA, was used as the positive control [23]. At the end of each treatment, cell viability was assessed according to the manufacturer's instructions. Briefly, after discarding the medium, 1 mL of XTT labeling mixture was added to the cells and incubated for 2 h at 37 °C. Absorbance was measured with a SpectraMax reader (Bio-Rad, USA) at a test wavelength of 492 nm (A_{492}) and a reference wavelength of 690 nm (A_{690}). The final result corresponds to A_{492}-A_{690}. The absorbance of negative control cells was set as 100 % viability, and the values for treated cells were calculated as a percentage of the control cells [7].

The IC_{50} (50 % inhibitory concentration) values and their 95 % confidence intervals (CI 95 %) were obtained by nonlinear regression, using the GraphPad Prism v5 program (Intuitive Software for Science, San Diego, CA, USA). The SI was calculated by IC_{50} in HEK-293 cells/IC_{50} in tumoral cells. All experiments were independently repeated at least three times, with triplicate samples for each treatment. Results are expressed as means ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA), and means were compared using the Tukey test, with $P \leq 0.05$ considered to be statistically significant.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

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Captions:

Table 1. Optimization of the reaction conditions for the synthesis of compound **8a**

Table 2. Optimized reaction conditions and yields for the synthesis of compounds **8-9a-g**

Table 3. Antiproliferative activity of 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones **compounds on human cell lines**

Figure 1. Structure of 5-fluorouracil (a), da 6-mercaptopurine (b) e da cytarabine (c)

Figure 2. Structural modifications in the deoxycytidine nucleoside feature

Figure 3. Different types of HOMO-LUMO interactions that control regioselectivity in 1,3-dipolar cycloaddition reactions [18]

Figure 4. ORTEP of compound **8d**

Scheme 1. Convergent synthesis of 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones (**8-9a-g)**

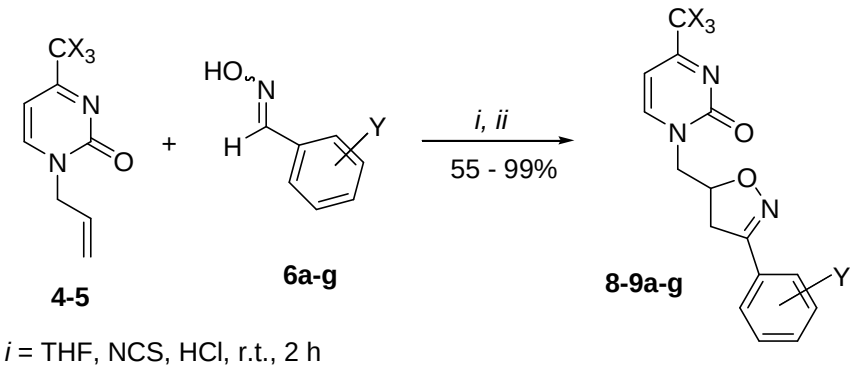
Table 1. Optimization of the reaction conditions for the synthesis of compound **8a**

Entry	Solvent	Temp.	Time (h)	Product (%)	Yield (%)
1	DCM	r.t.	20	**4** + **6a** + **8a**	-[a]
3	DCM	r.t.	36	**4** + **6a** + **8a**	-[a]
2	DCM	Reflux	20	**4** + **6a** + **8a**	-[a]
4	DCE	Reflux	20	**4** + **6a** + **8a**	-[b]
5	THF	r.t.	20	**8a**	86
6	THF	Reflux	4	**8a**	98

[a] Proportion of **8a**:**4** was 1:0.4 determined by ^{1}H NMR integrals.

[b] Proportion of **8a**:**4** was 1:0.2 determined by ^{1}H NMR integrals.

Table 2. Optimized reaction conditions and yields for the synthesis of compounds **8-9a-g**



4-5 **6a-g** **8-9a-g**

i = THF, NCS, HCl, r.t., 2 h
ii = Et_3N, reflux, 4 h.

Reactants	Product	Y	X	Yield (%)
4 + 6a	**8a**	H	Cl	98
4 + 6b	**8b**	4-F	Cl	83
4 + 6c	**8c**	2-Me	Cl	97
4 + 6d	**8d**	4-Me	Cl	80
4 + 6e	**8e**	2-OH	Cl	61
4 + 6f	**8f**	2-OMe	Cl	99
4 + 6g	**8g**	4-OMe	Cl	94
5 + 6a	**9a**	Ph	F	92
5 + 6b	**9b**	4-F	F	84
5 + 6c	**9c**	2-Me	F	99
5 + 6d	**9d**	4-Me	F	86
5 + 6e	**9e**	2-OH	F	55
5 + 6f	**9f**	2-OMe	F	92
5 + 6g	**9g**	4-OMe	F	99

Table 3. Antiproliferative activity of 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones compounds on human cell lines

Entry	Compd.	IC_{50} (μM)[a] Selectivity index (SI)[b]					
		HEK-293	***MCF-7***	***HepG-2***	***T-24***	***HCT-116***	***CACO-2***
1	**8a**	14.63	7.09	1.04 (SI: 14.06)	5.10	4.15	> 10
2	**8b**	5.01	> 10	> 10	> 10	> 10	> 10
3	**8d**	3.59	> 10	> 10	> 10	7.21	4.95
4	**8e**	6.12	6.02	6.77	8.50	5.27	1.37 (SI: 4.47)
5	**8f**	19.41	4.30	> 10	> 10	> 10	> 10
6	**8g**	5.19	5.89	8.21	9.02	6.91	5.84
7	**9a**	2.42	> 10	> 10	> 10	> 10	> 10
8	**9b**	5.99	> 10	> 10	> 10	> 10	> 10
9	**9c**	10.85	7.99	6.03	1.29 (SI: 8.4)	8.54	4.98
10	**9d**	27.79	> 10	5.99	> 10	8.96	> 10
11	**9f**	10.79	> 10	7.03	> 10	> 10	> 10
12	**9g**	8.43	> 10	7.87	> 10	> 10	> 10
14	**MXT**[c]	2.88	0.87	3.50	2.50	0.61	2.40

[a]Drug concentration required to inhibit the cell growth by 50% after 24 h of incubation. [b]Selectivity index (*in vitro*): IC_{50} in HEK-293 cells/IC_{50} in tumoral cells. Data represent mean ± three separate experiments. [c]Mitoxantrone (MXT) was used as positive control.

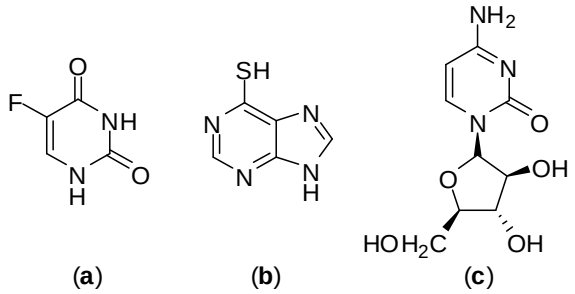


Figure 1. Structure of 5-fluorouracil (a), da 6-mercaptopurine (b) e da cytarabine (c)

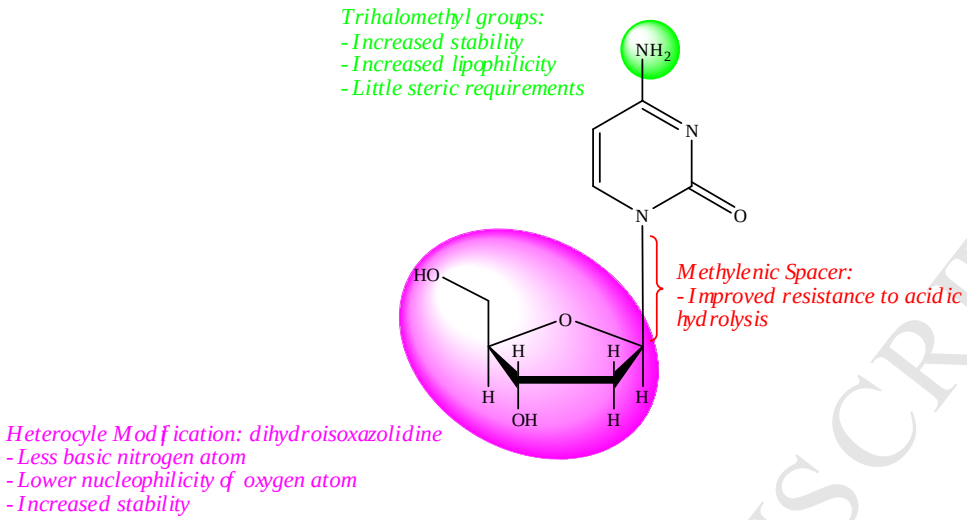


Figure 2. Structural modifications in the deoxycytidine nucleoside feature

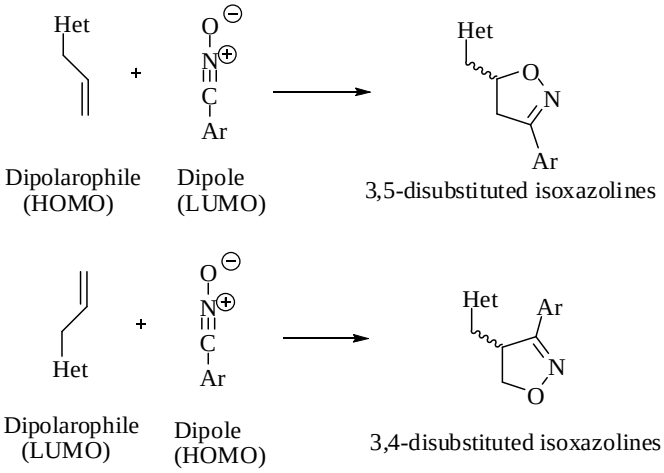


Figure 3. Different types of HOMO-LUMO interactions that control regioselectivity in 1,3-dipolar cycloaddition reactions [18]

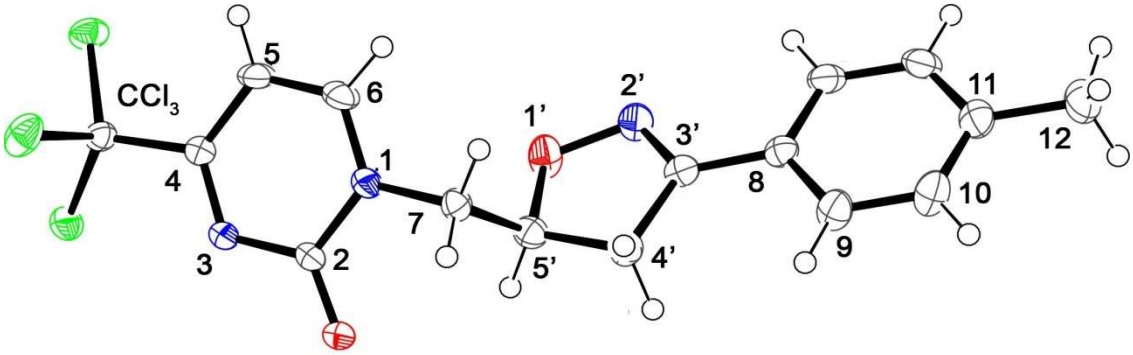
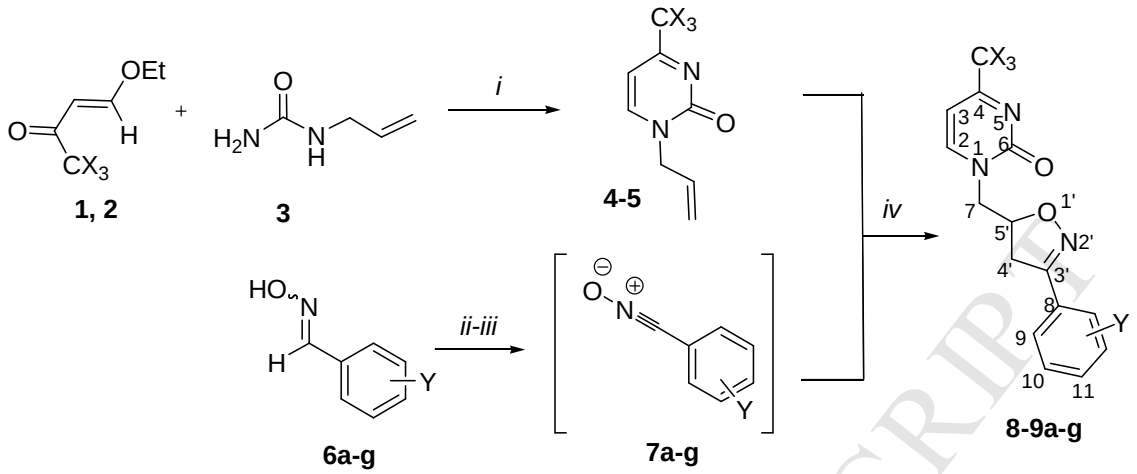


Figure 4. ORTEP of compound **8d**



X, Y: See Table 2 for the definition of X anf Y

Reaction conditions:
i) MeOH, HCl, reflux, 20 h
ii) THF, NCS (1.3 equiv.), HCl (1 equiv.), r.t., 2 h
iii) Et_3N (3.9 equiv.)
iv) Reflux 4 h

Scheme 1. Convergent synthesis of 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones (**8-9a-g)**

Highlights:

1. Synthesis of dihydroisoxazolmethyl-pyrimidines from dipolar cycloaddition reaction.

2. Antiproliferative activity of dihydroisoxazol-5-yl-(methyl)-1*H*-pyrimidin-2-ones.

3. Antiproliferative activity of pyrimidines against five human tumoral cell lines.

4. Three derivatives showed high activity agains *HepG-2, T-24*, and *CACO-2*.

Synthesis and cytotoxic activity evaluation of some novel 1-(3-(aryl-4,5-dihydroisoxazol-5-yl)methyl)-4-trihalomethyl-1*H*-pyrimidin-2-ones in human cancer cells

Marcio M. Lobo, Cassiana M. Viau, Josiane M. dos Santos, Helio G. Bonacorso, Marcos A. P. Martins, Simone S. Amaral, Jenifer Saffi, Nilo Zanatta

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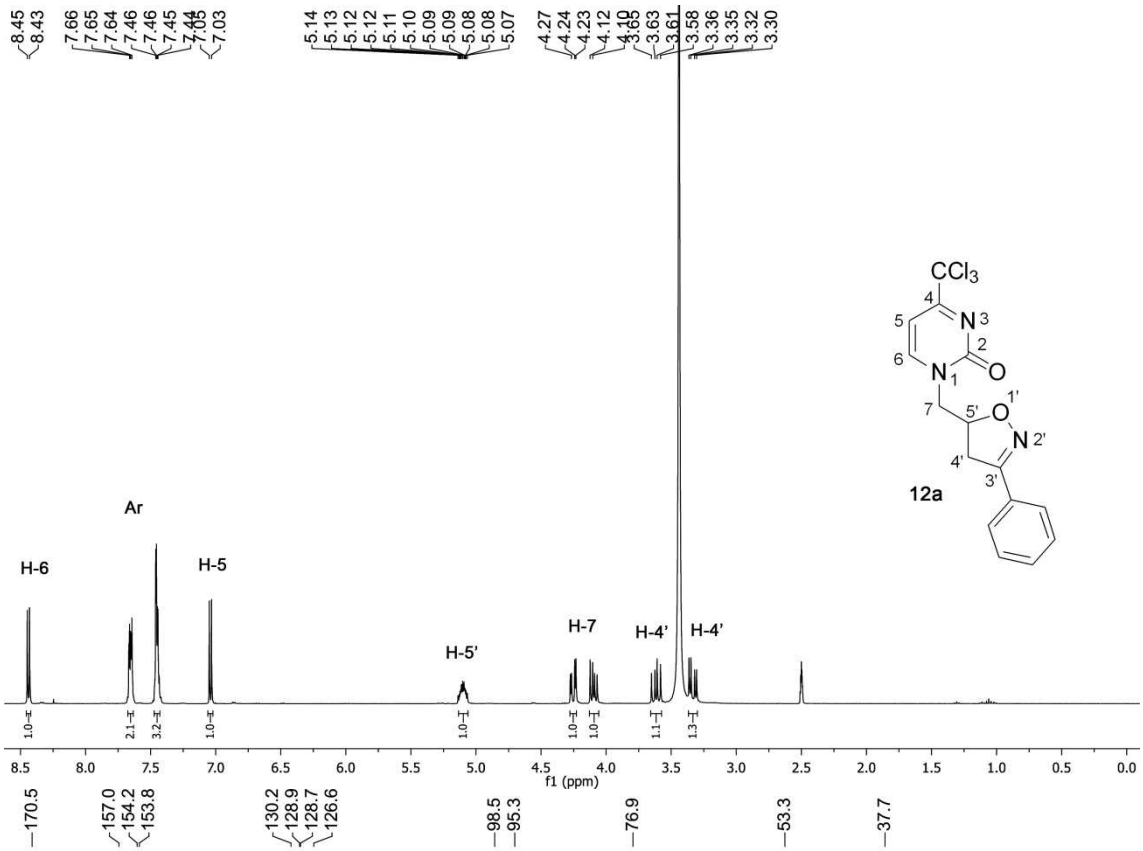
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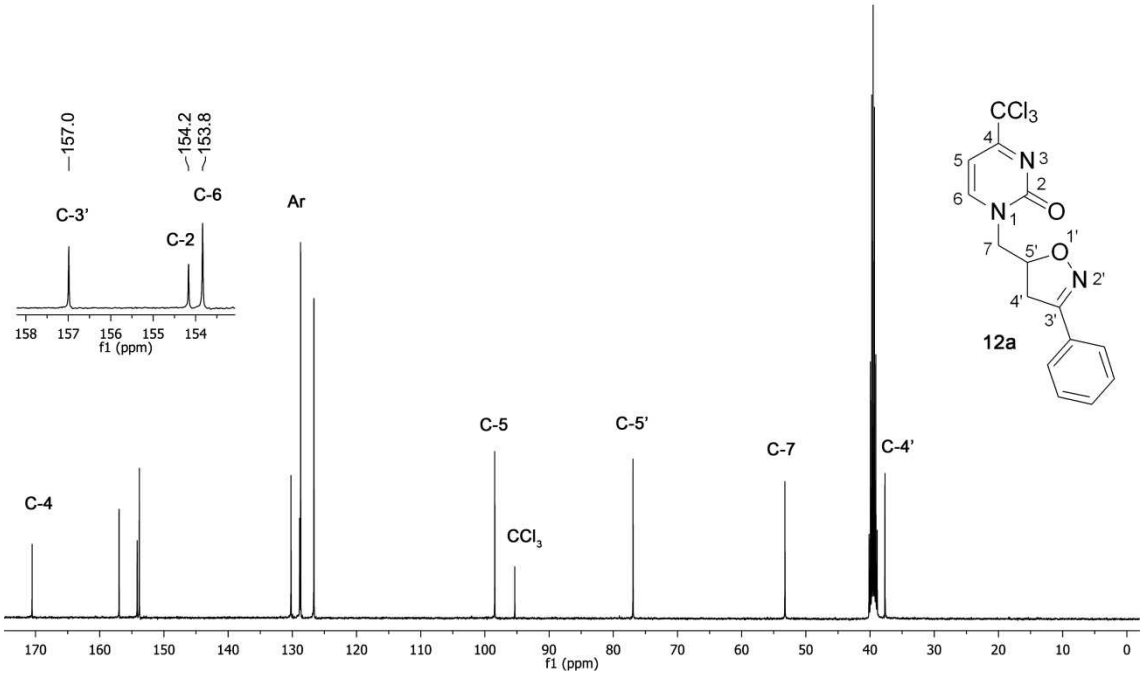
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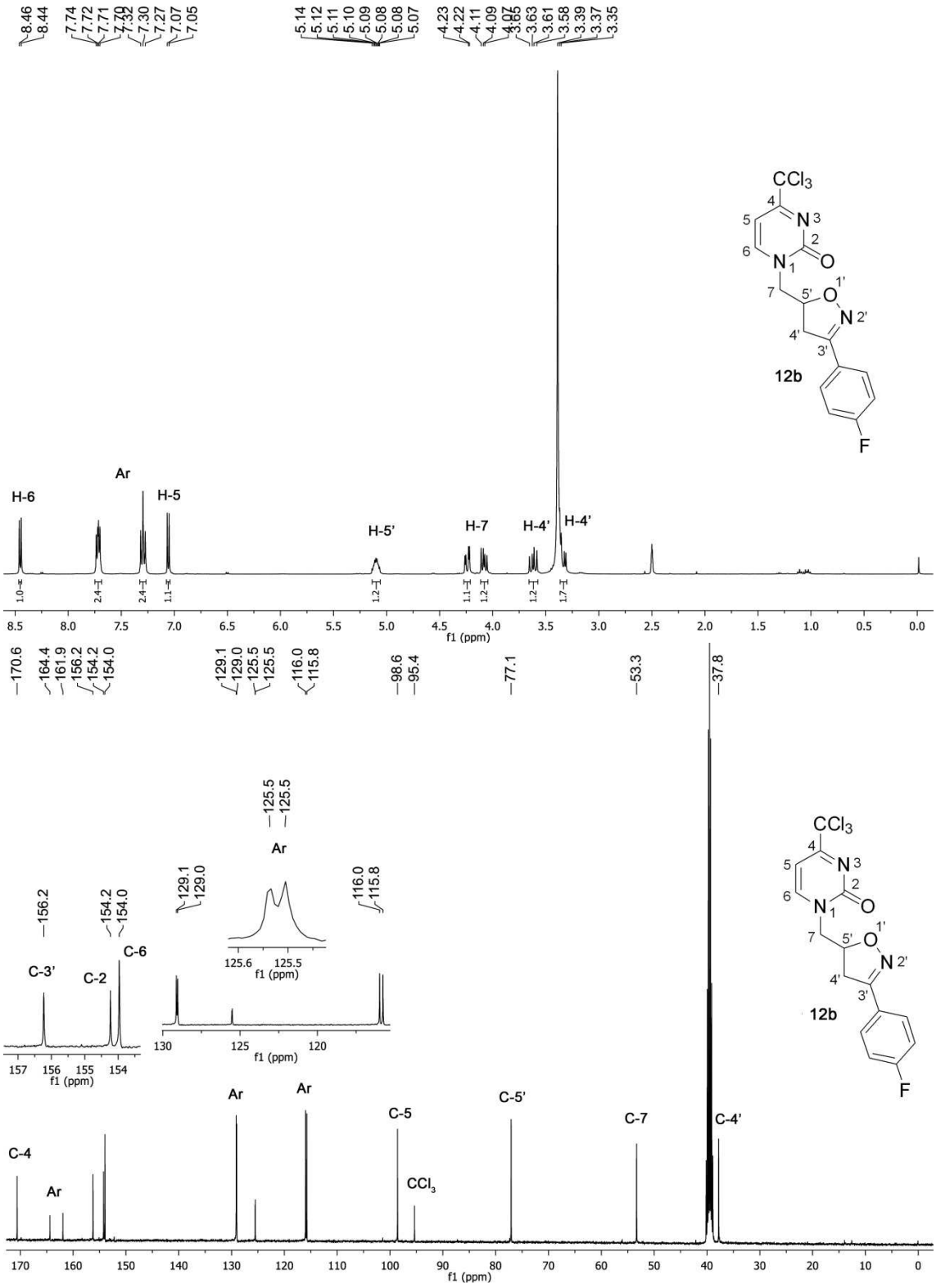
f1 (ppm)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H-5'
H-7
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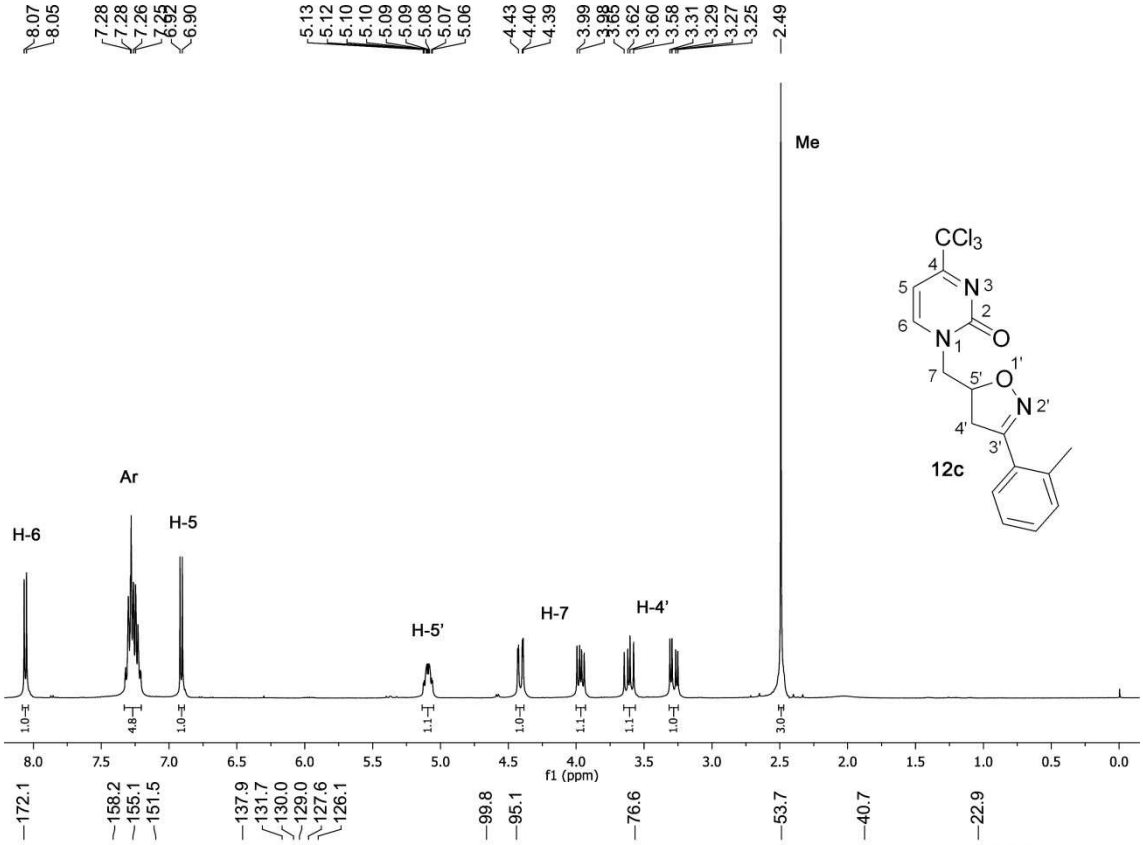
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H-5'
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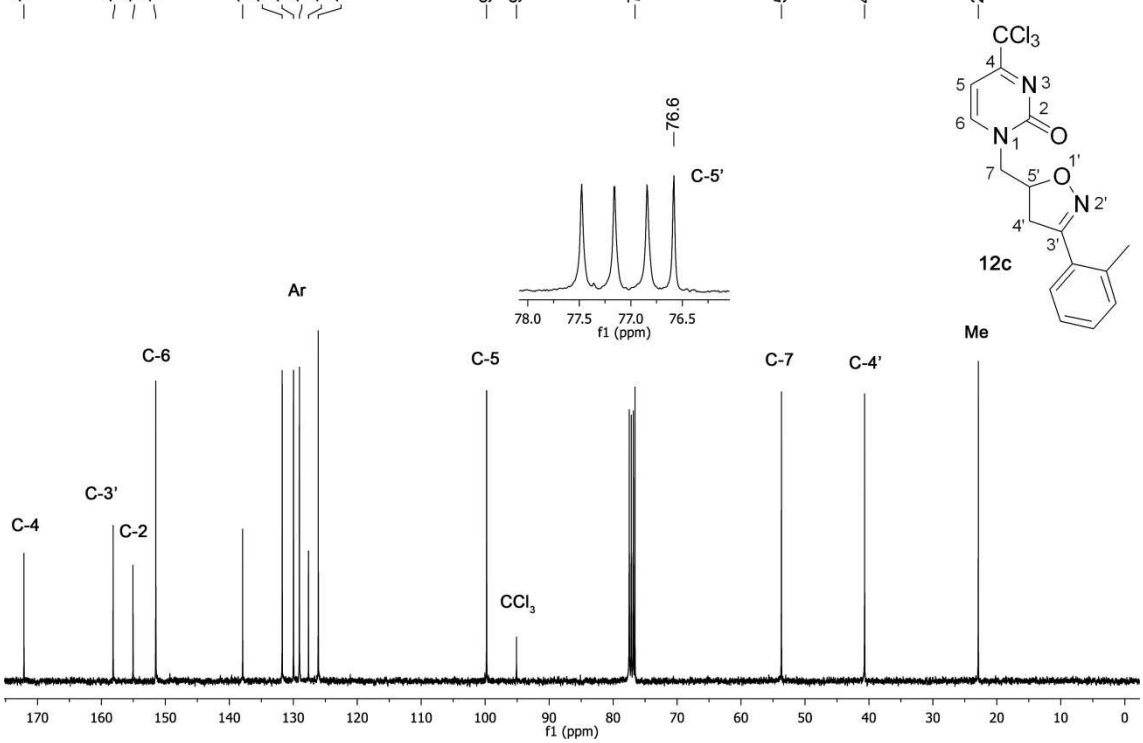
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12c
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C-5'
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76.5
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C-5
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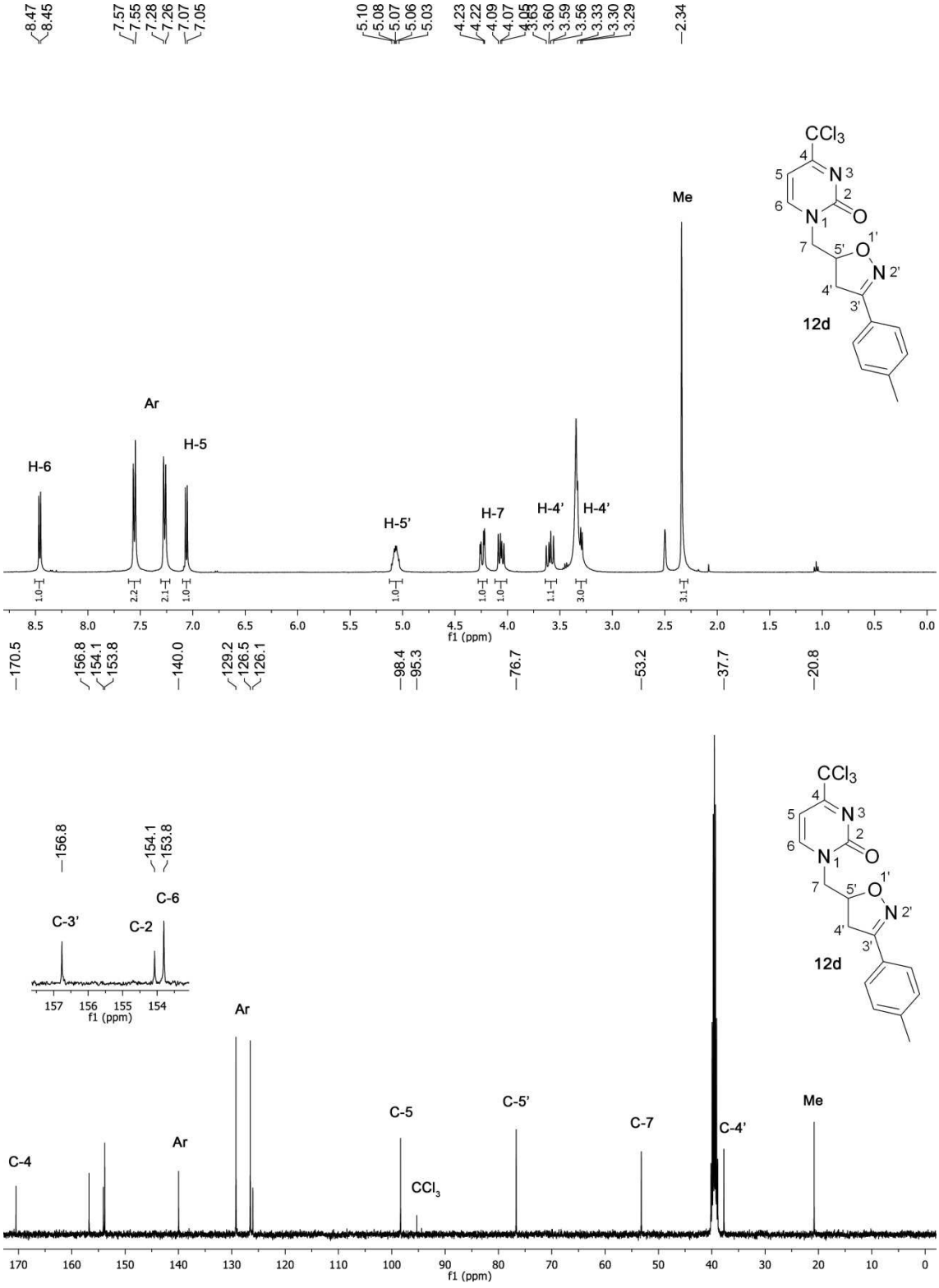
f1 (ppm)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H-5
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H-7
H-4'
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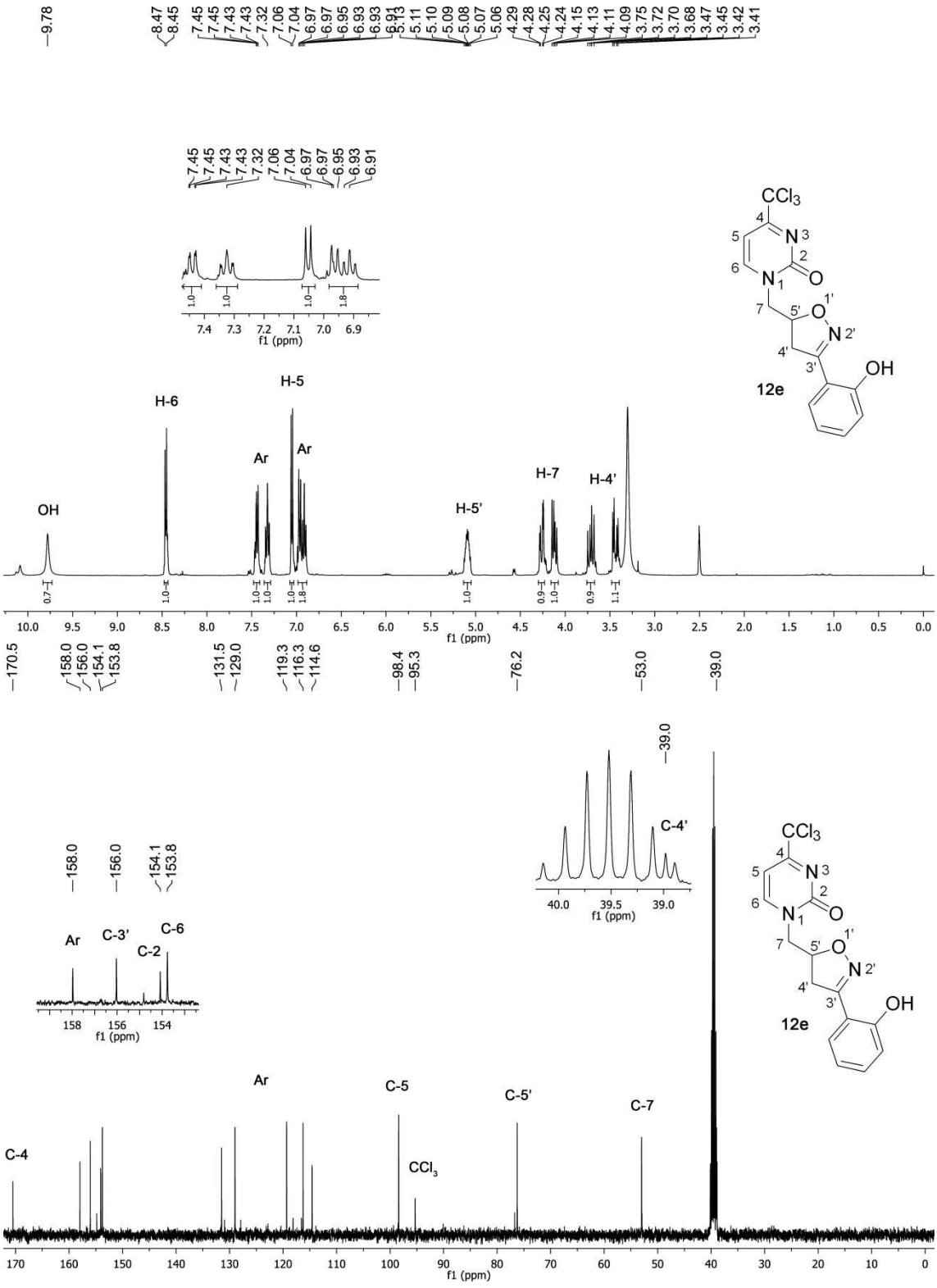
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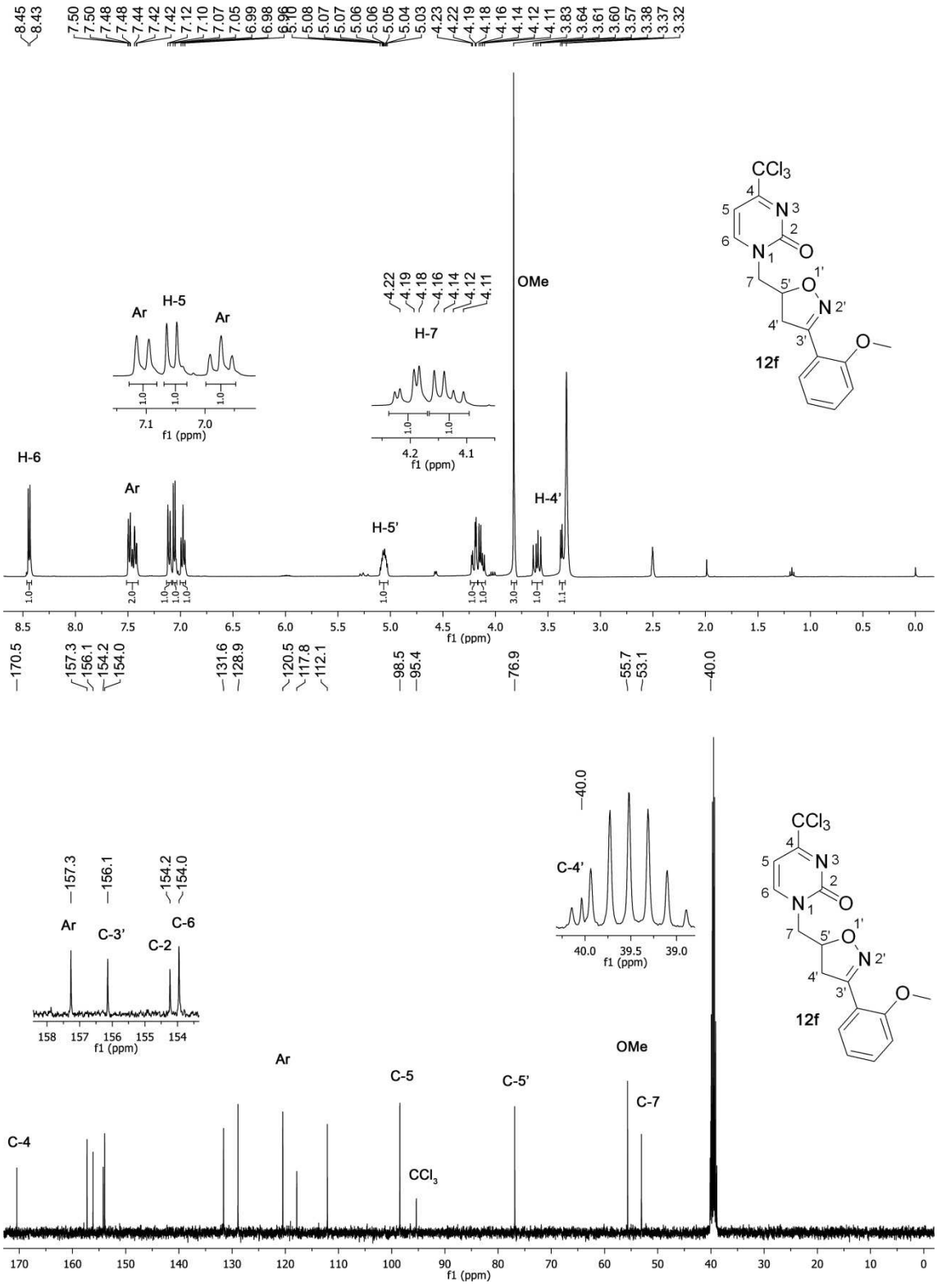
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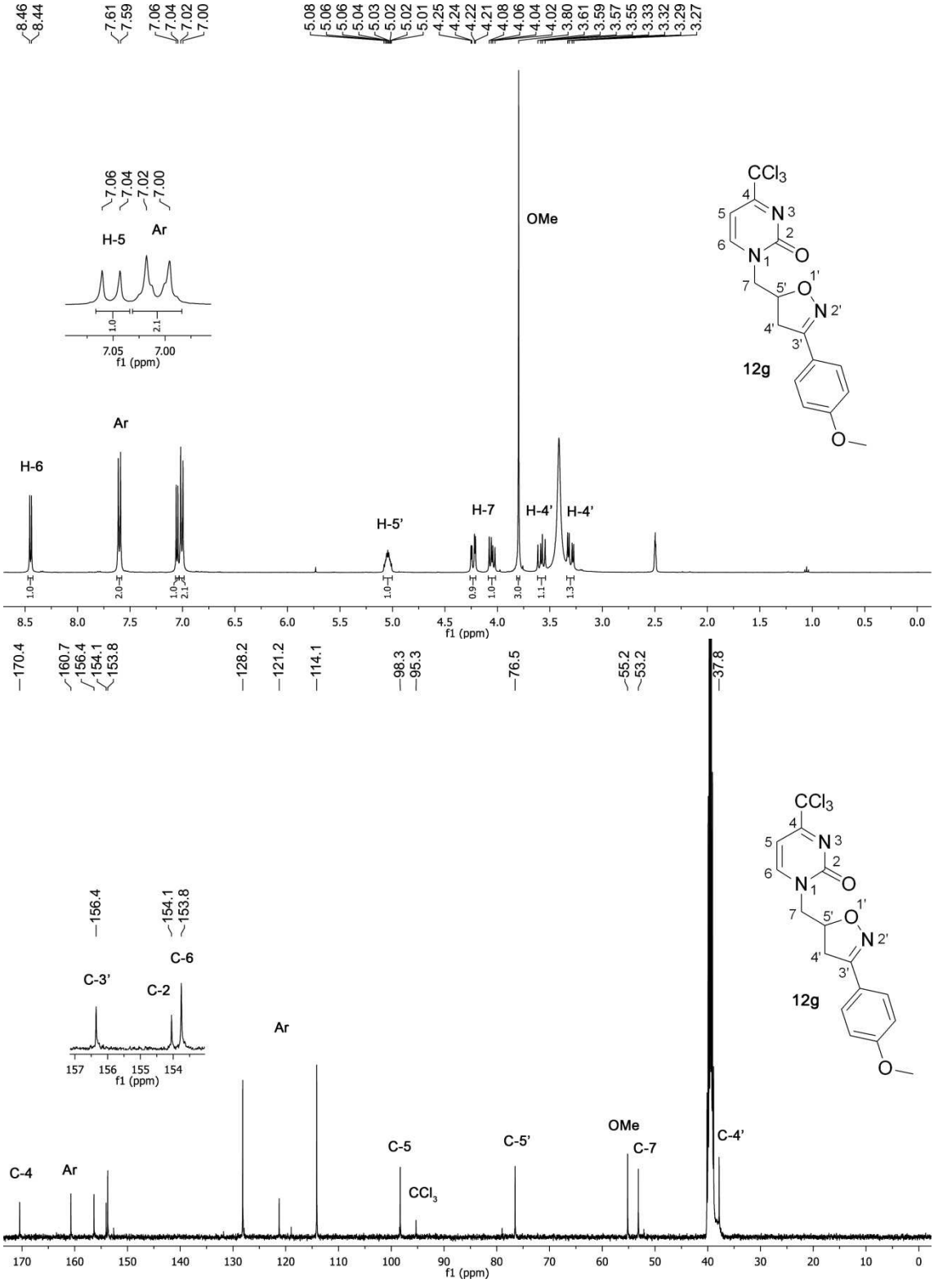
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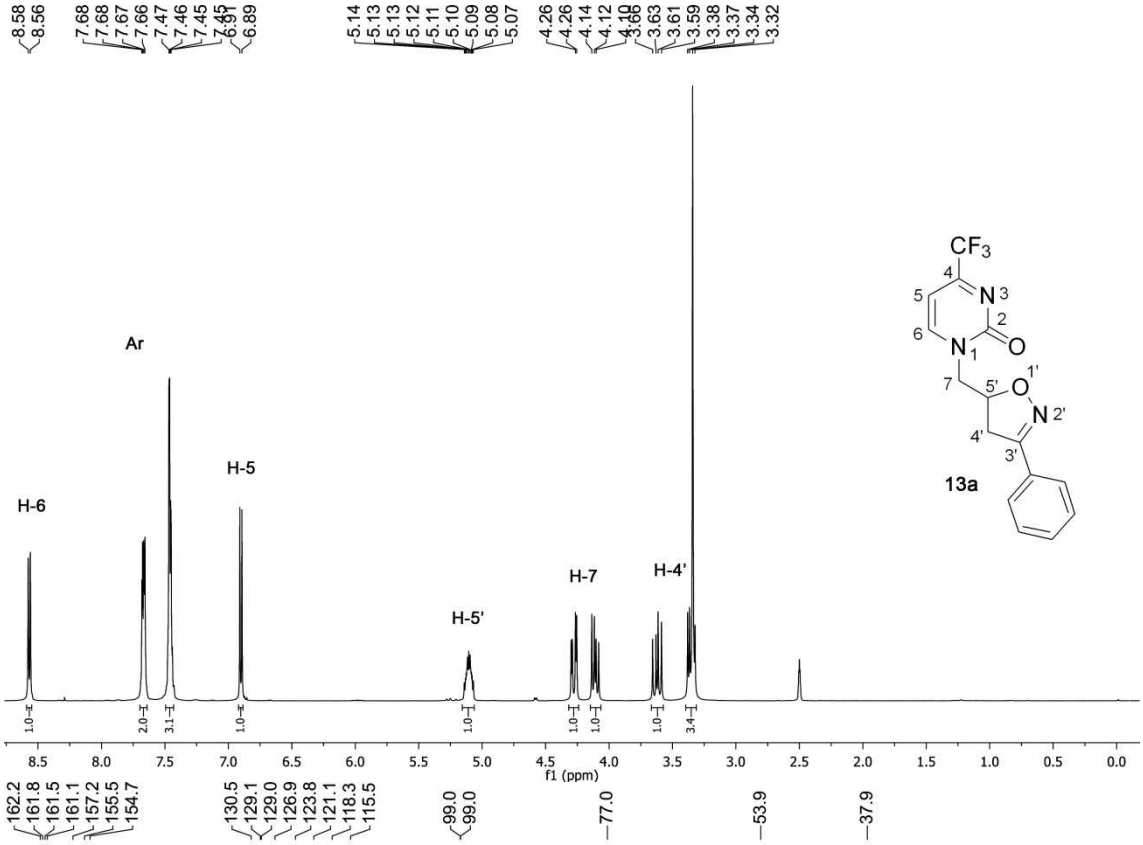
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H-5
H-5'
H-7
H-4'
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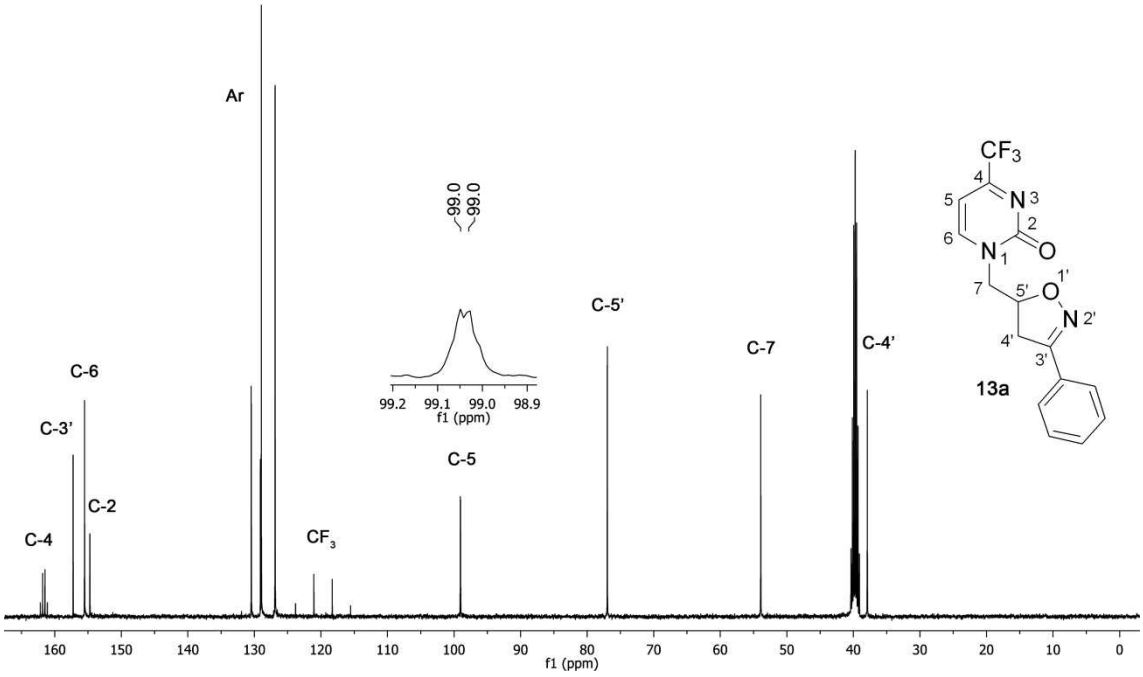
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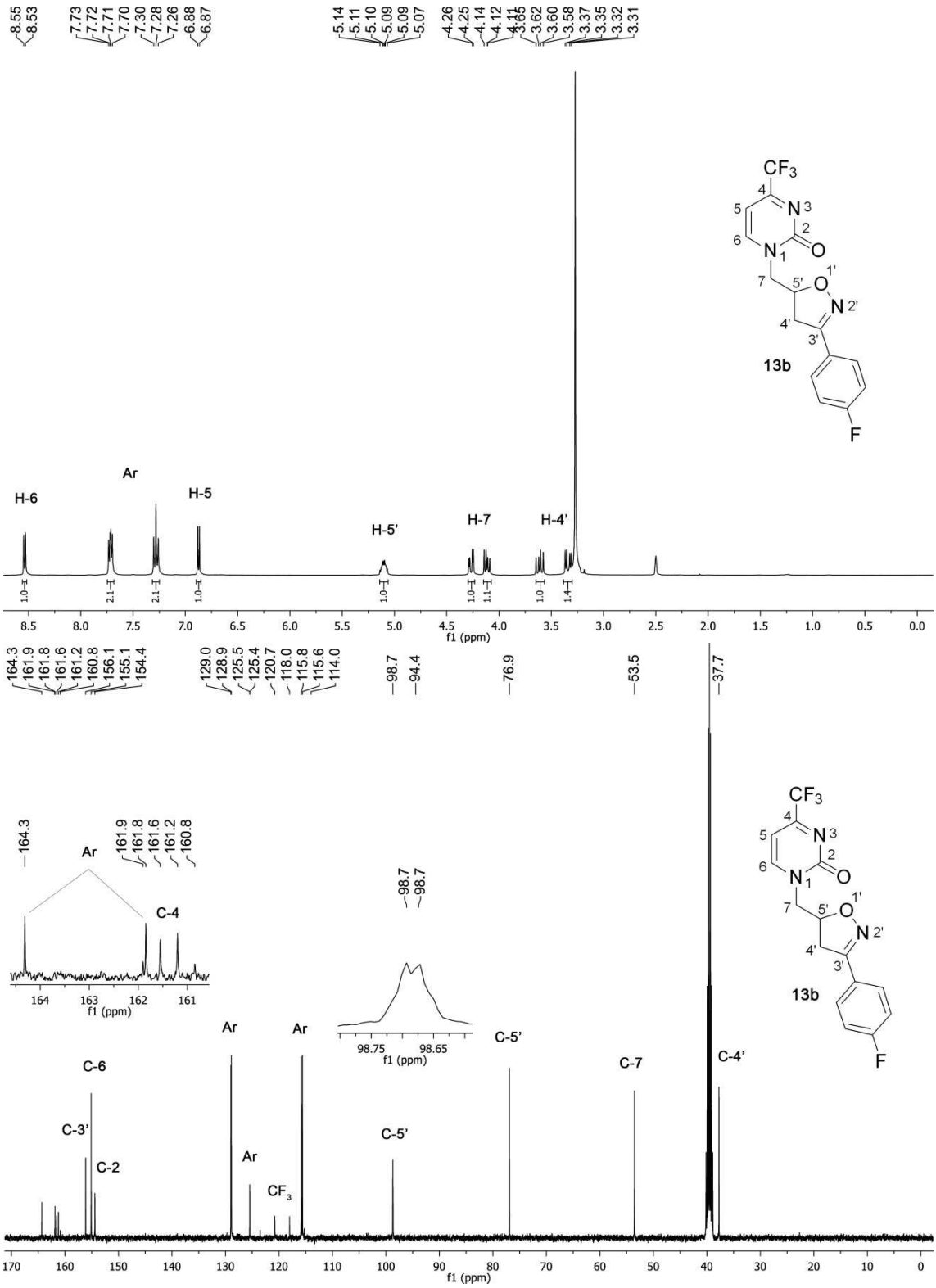
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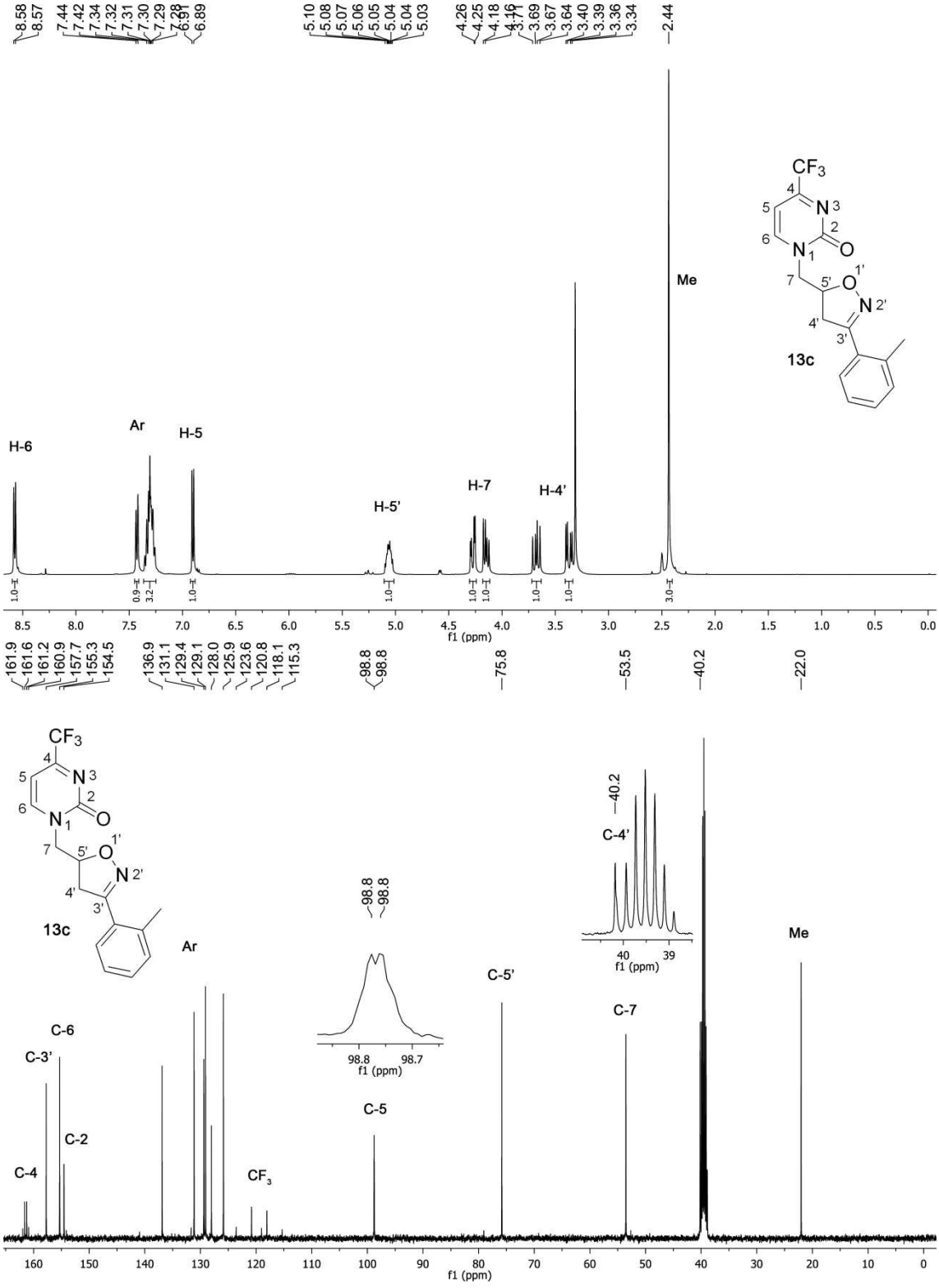
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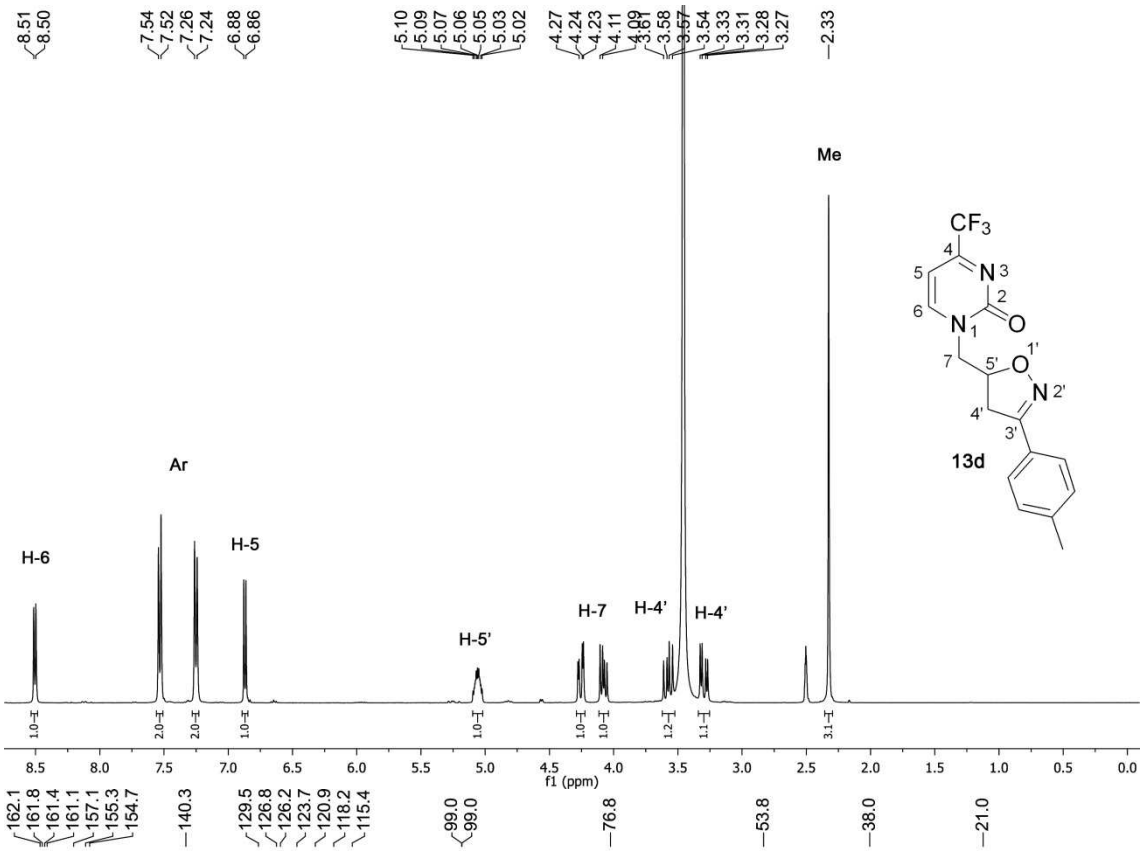
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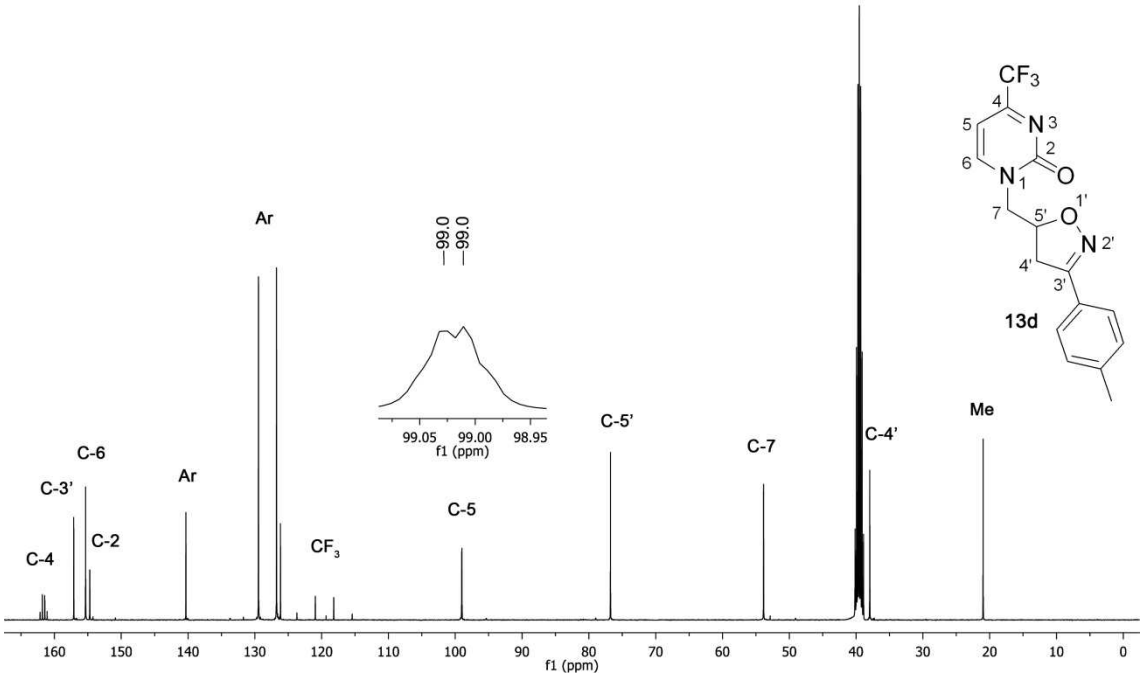
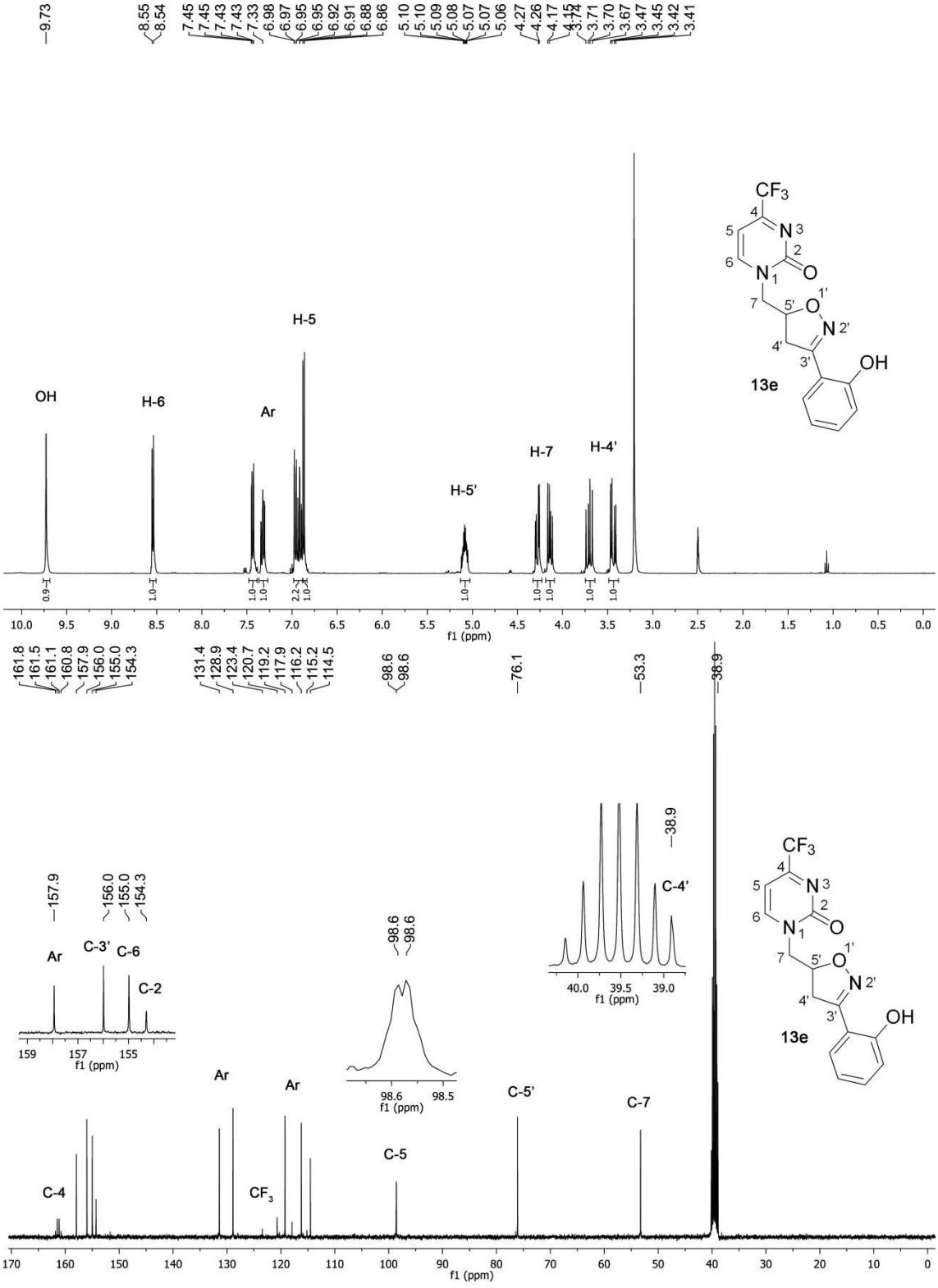
99.0
76.8
53.8
38.0
21.0
CF3
13d


Ar
99.0
99.0
99.05
99.00
98.95
f1 (ppm)
C-6
C-3'
C-2
C-4
Ar
CF3
C-5
C-5'
C-7
C-4'
Me
f1 (ppm)
CF3
13d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8.54
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7.43
7.43
7.33
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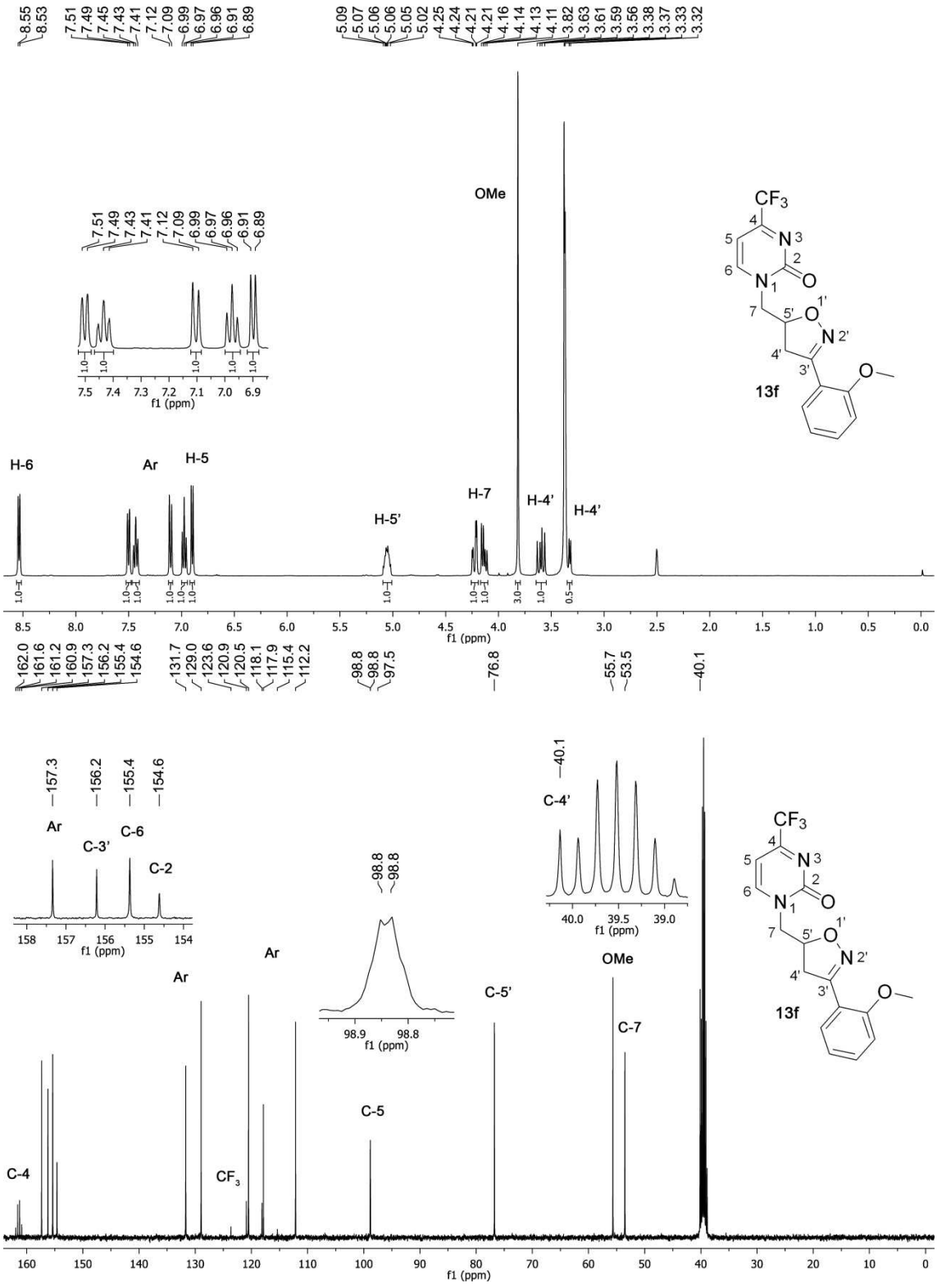
6.97
6.95
6.95
6.92
6.91
6.88
6.86
5.10
5.10
5.09
5.08
5.07
5.07
5.06
4.27
4.26
4.17
3.71
3.70
3.67
3.47
3.45
3.42
3.41
OH
H-6
Ar
H-5
H-5'
H-7
H-4'
f1 (ppm)
CF3
13e
161.8
161.5
161.1
160.8
157.9
156.0
155.0
154.3
131.4
128.9
123.4
120.7
119.2
117.9
116.2
115.2
114.5
98.6
98.6
76.1
53.3
38.9
C-4'
C-3'
C-6
C-2
Ar
C-4
CF3
C-5
C-5'
C-7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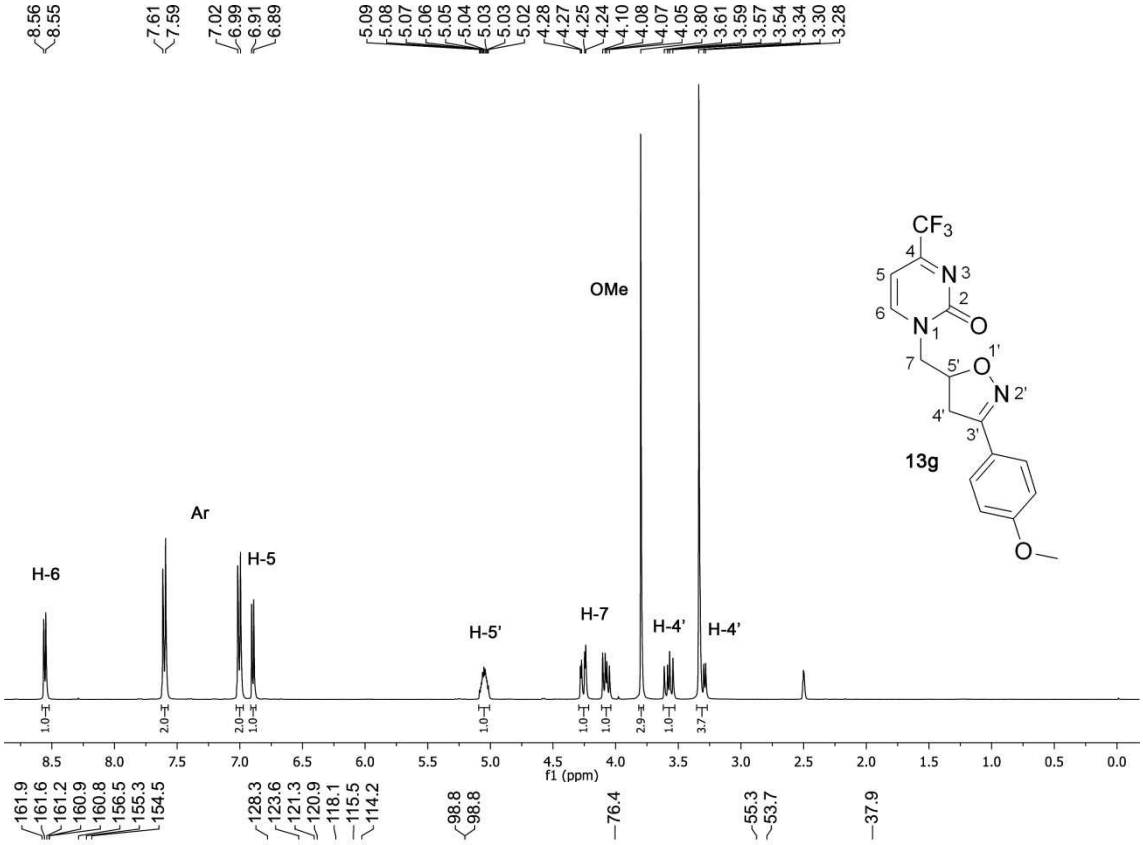
7.12
7.09
6.99
6.97
6.96
6.91
6.89
5.09
5.07
5.06
5.06
5.05
5.02
4.25
4.24
4.21
4.21
4.16
4.14
4.13
4.11
3.82
3.63
3.61
3.59
3.56
3.38
3.37
3.33
3.32
OMe
H-6
Ar
H-5
H-5'
H-7
H-4'
H-4'
f1 (ppm)
162.0
161.6
161.2
160.9
157.3
156.2
155.4
154.6
131.7
129.0
123.6
120.9
120.5
118.1
117.9
115.4
112.2
98.8
98.8
97.5
76.8
55.7
53.5
40.1
Ar
C-3'
C-6
C-2
C-4'
C-5'
C-7
C-5
C-4
CF3
13f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8.55
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6.91
6.89
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5.08
5.07
5.06
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5.04
5.03
5.03
5.02
4.28
4.27
4.25
4.24
4.10
4.08
4.07
4.05
3.80
3.61
3.59
3.57
3.54
3.34
3.30
3.28
OMe
Ar
H-6
H-5
H-5'
H-7
H-4'
H-4'
13g
f1 (ppm)
161.9
161.6
161.2
160.9
160.8
156.5
155.3
154.5
128.3
123.6
121.3
120.9
118.1
115.5
114.2
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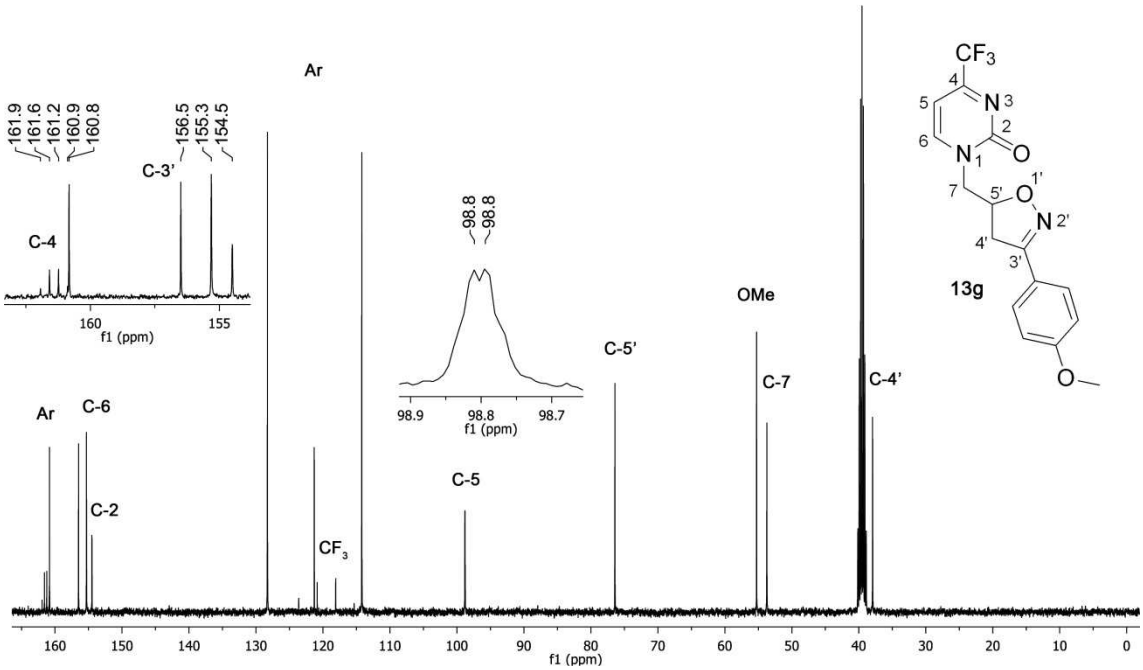
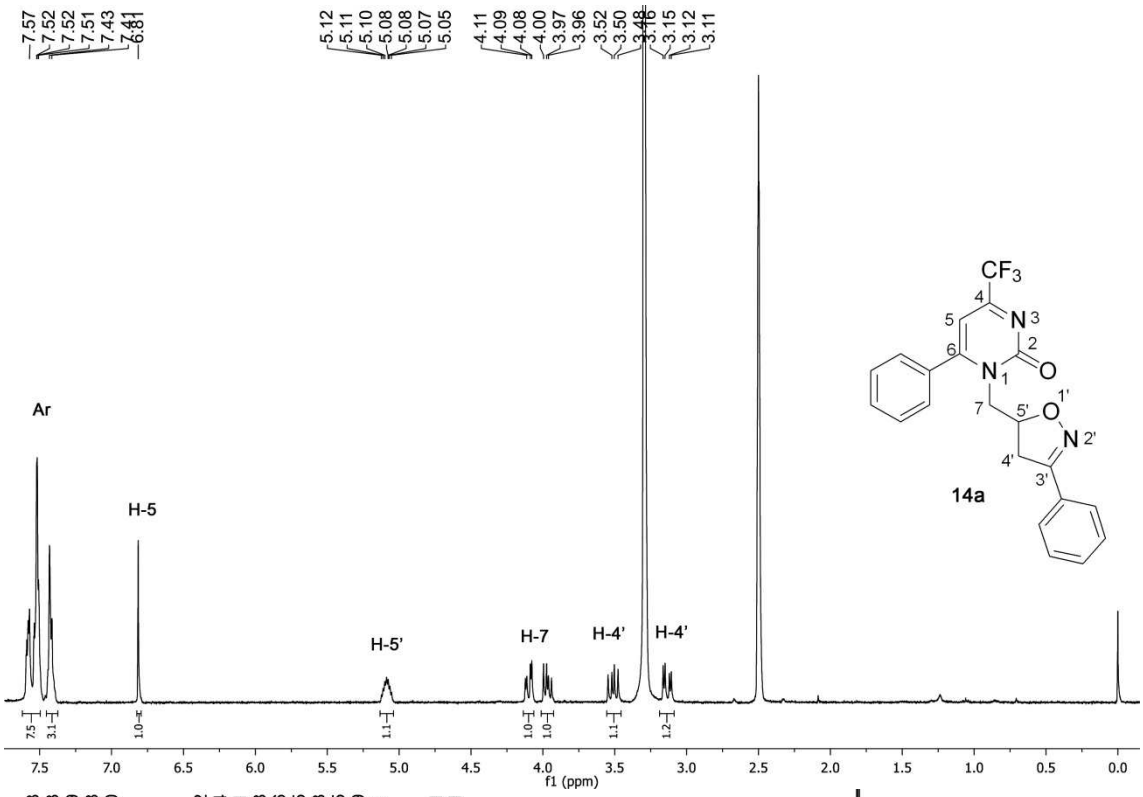
98.8
76.4
55.3
53.7
37.9


Ar
C-3'
C-4
C-6
C-2
CF3
C-5
C-5'
C-7
OMe
C-4'
13g
f1 (ppm)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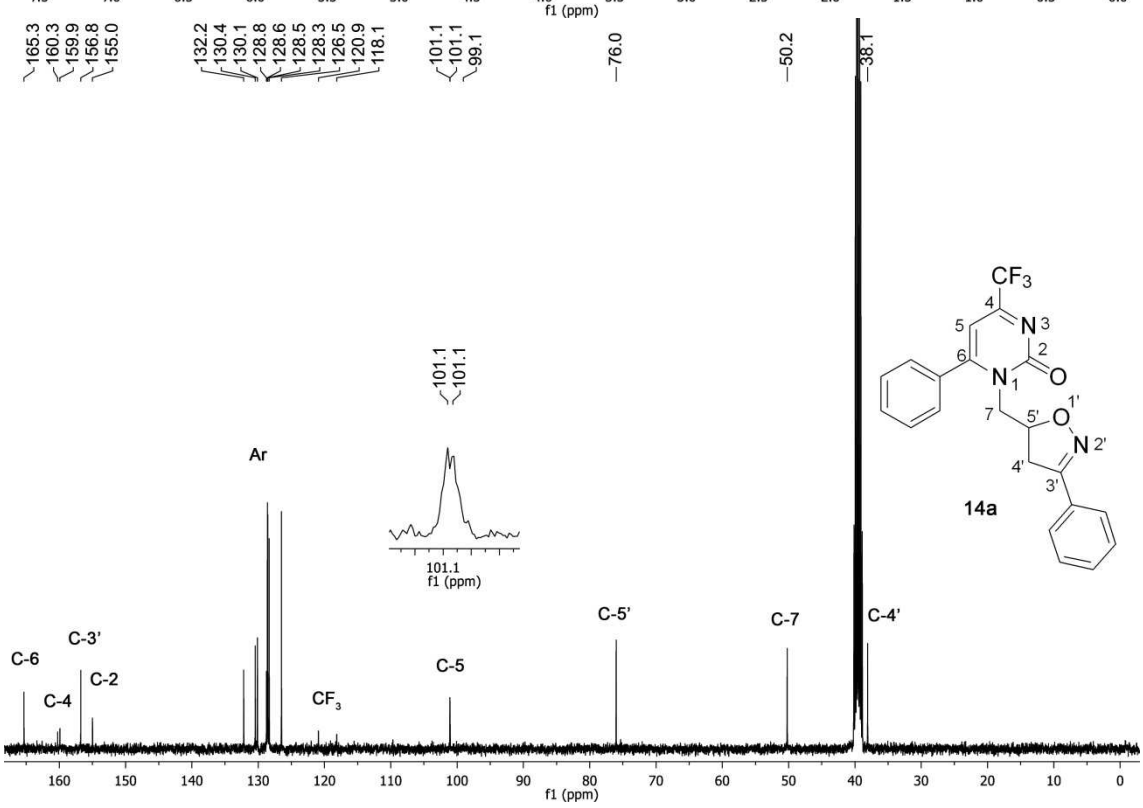
7.52
7.51
7.43
7.41
6.81
5.12
5.11
5.10
5.08
5.08
5.07
5.05
4.11
4.09
4.08
4.00
3.97
3.96
3.52
3.50
3.48
3.16
3.15
3.12
3.11
Ar
H-5
H-5'
H-7
H-4'
H-4'
7.5
3.1
1.0
1.1
1.0
1.0
1.1
1.2
f1 (ppm)
CF3
14a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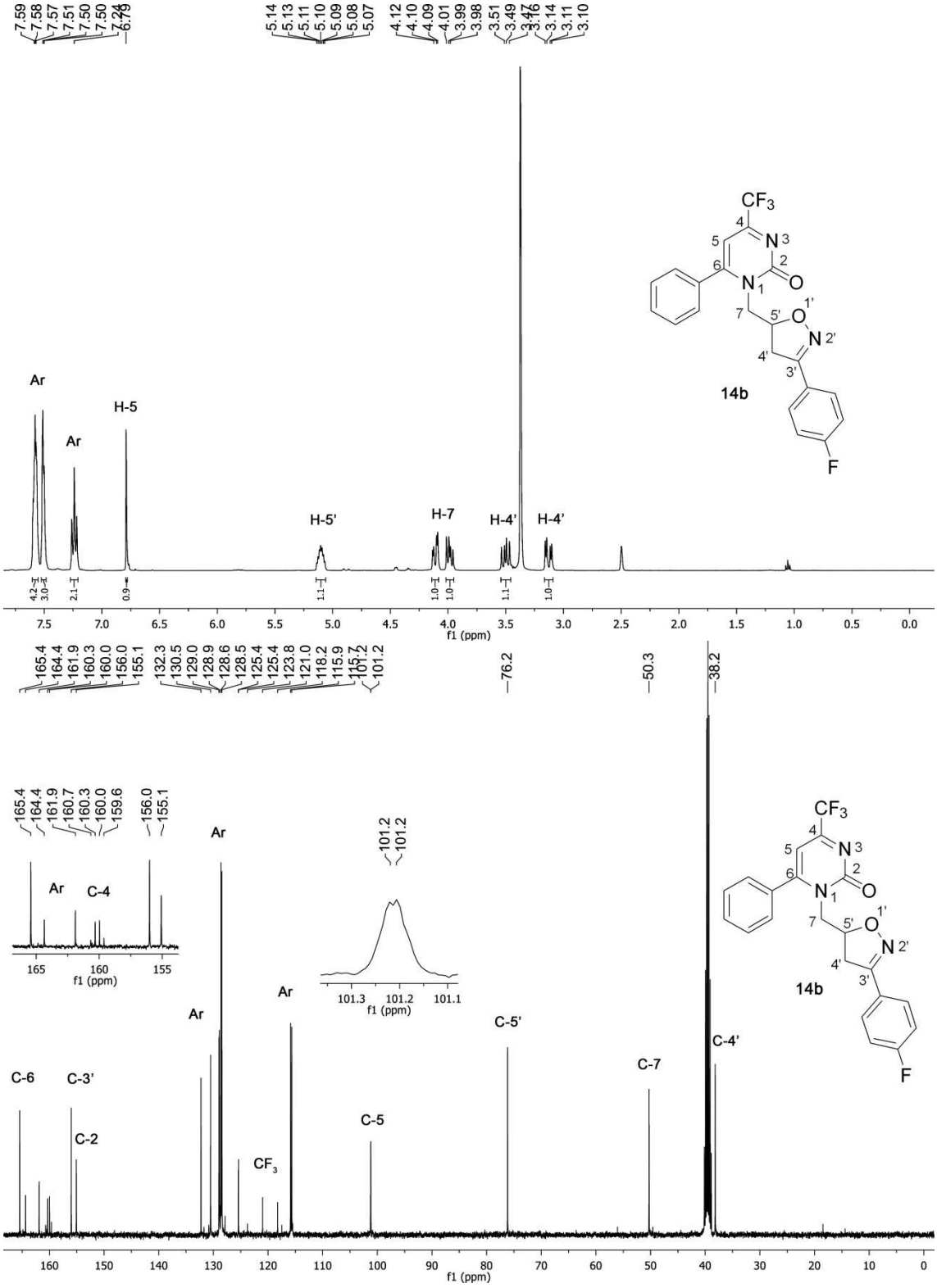
160.3
159.9
156.8
155.0
132.2
130.4
130.1
128.8
128.6
128.5
128.3
126.5
120.9
118.1
101.1
101.1
99.1
76.0
50.2
38.1
101.1
101.1
f1 (ppm)
Ar
C-6
C-4
C-3'
C-2
CF3
C-5
C-5'
C-7
C-4'
f1 (ppm)
CF3
14a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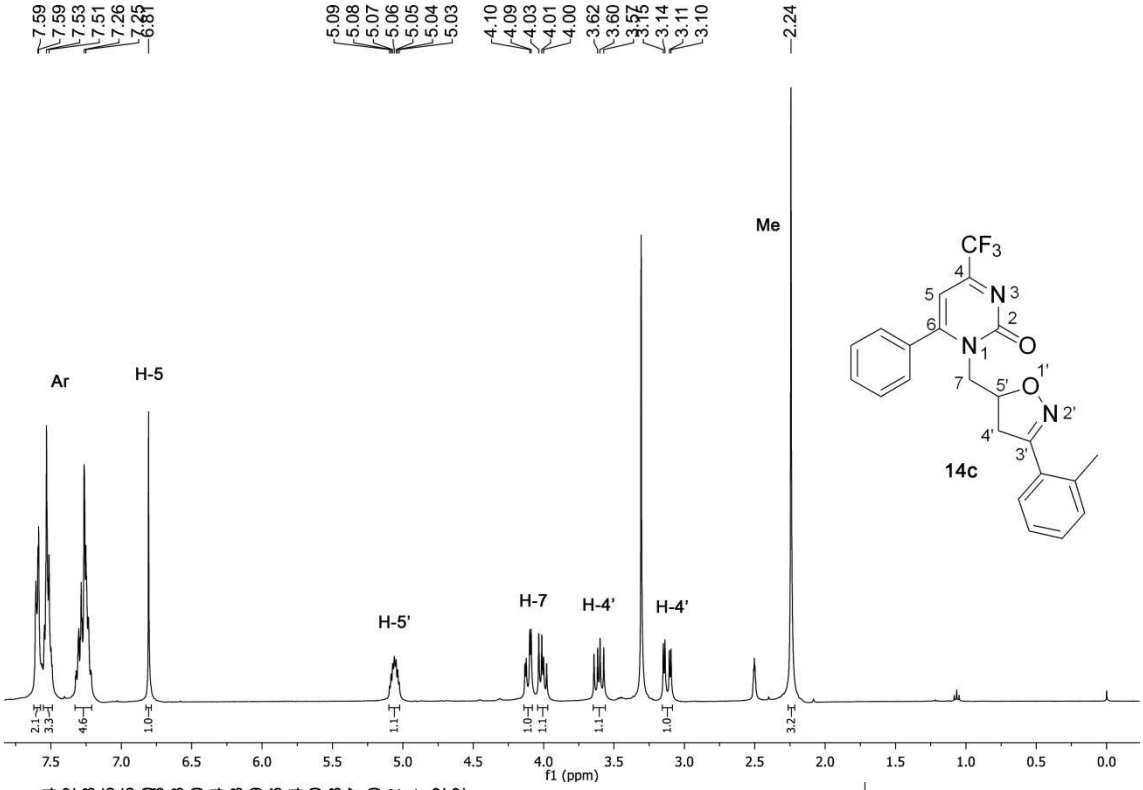
7.50
7.24
6.78
5.14
5.13
5.11
5.10
5.09
5.08
5.07
4.12
4.10
4.09
4.01
3.99
3.98
3.51
3.49
3.47
3.16
3.14
3.11
3.10
Ar
Ar
H-5
H-5'
H-7
H-4'
H-4'
4.2
3.0
2.1
0.9
1.1
1.0
1.0
1.1
1.0
f1 (ppm)
CF3
14b
F
165.4
164.4
161.9
160.3
160.0
156.0
155.1
132.3
130.5
129.0
128.9
128.6
128.5
125.4
125.4
123.8
121.0
118.2
115.9
115.7
101.2
101.2
76.2
50.3
38.2
Ar
C-4
165
160
155
f1 (ppm)
101.2
101.2
101.3
101.2
101.1
f1 (ppm)
Ar
Ar
Ar
CF3
C-6
C-3'
C-2
C-5
C-5'
C-7
C-4'
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.53
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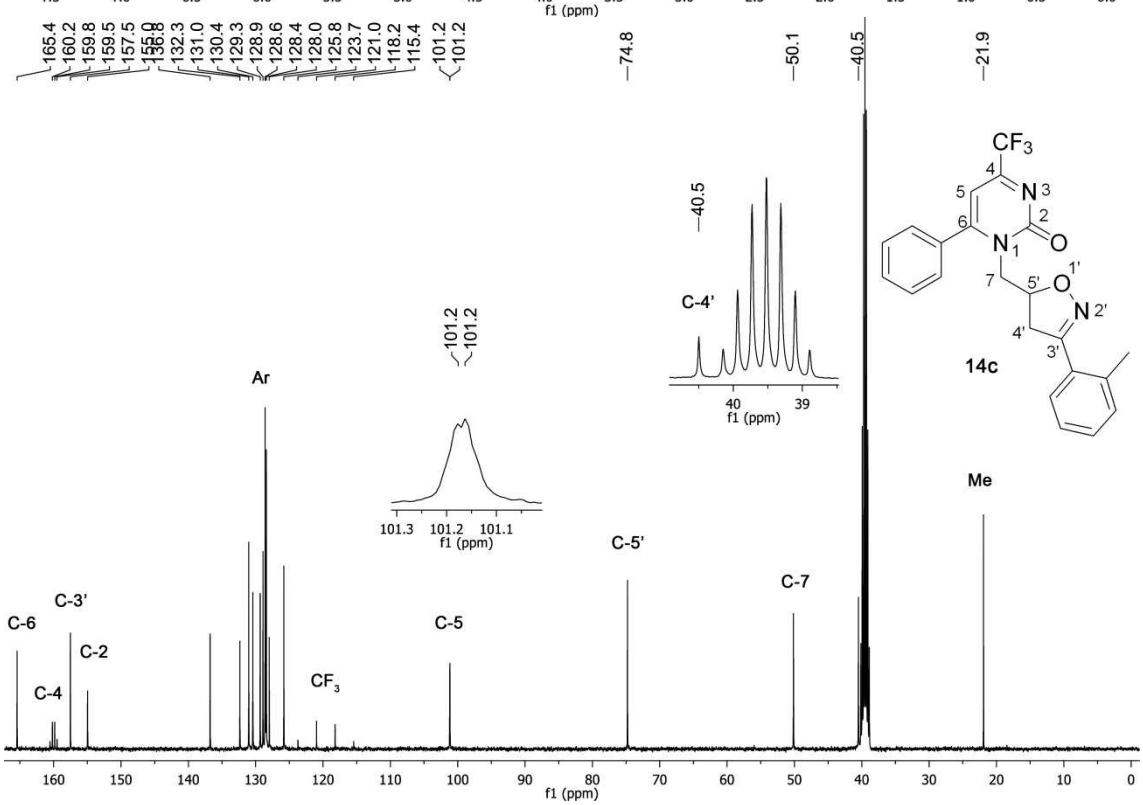
7.26
7.25
6.81
5.09
5.08
5.07
5.06
5.05
5.04
5.03
4.10
4.09
4.03
4.01
4.00
3.62
3.60
3.57
3.15
3.14
3.11
3.10
2.24
Ar
H-5
H-5'
H-7
H-4'
H-4'
Me
CF3
N
O
14c
f1 (ppm)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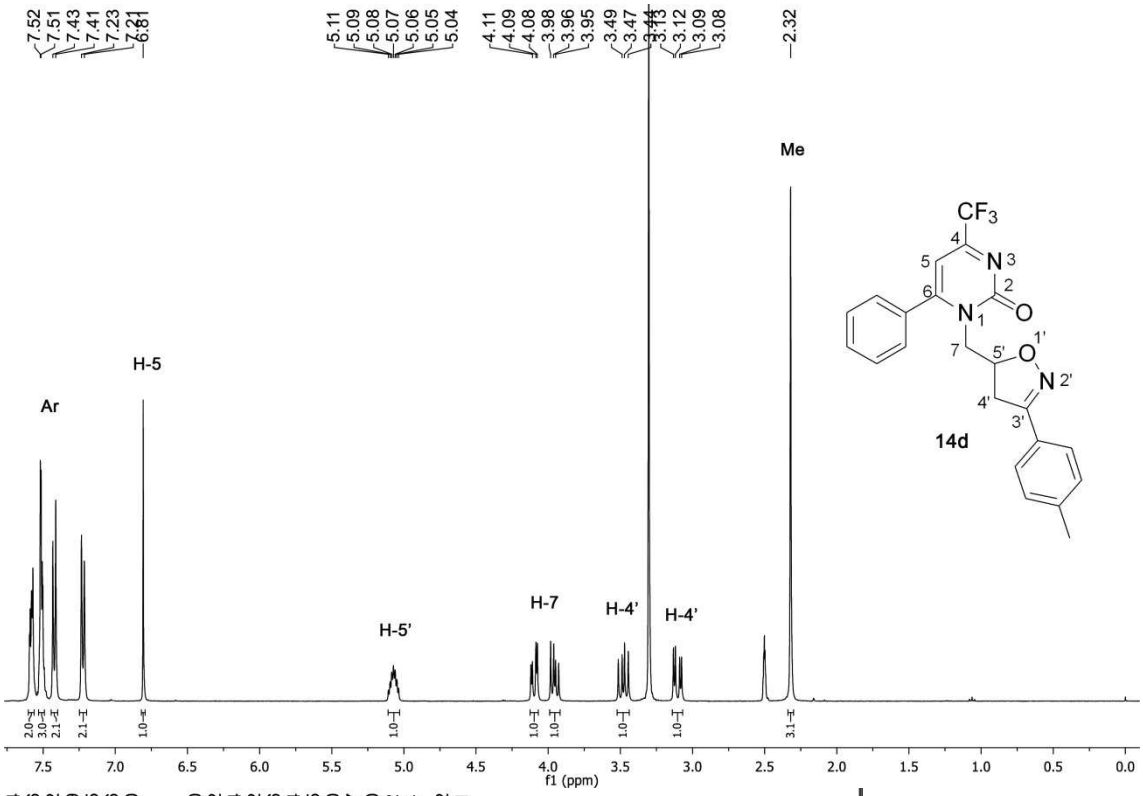
159.8
159.5
157.5
132.3
131.0
130.4
129.3
128.9
128.6
128.4
128.0
125.8
123.7
121.0
118.2
115.4
101.2
101.2
74.8
50.1
40.5
21.9
C-4'
C-6
C-3'
C-2
C-4
Ar
CF3
C-5
C-5'
C-7
Me
14c
f1 (ppm)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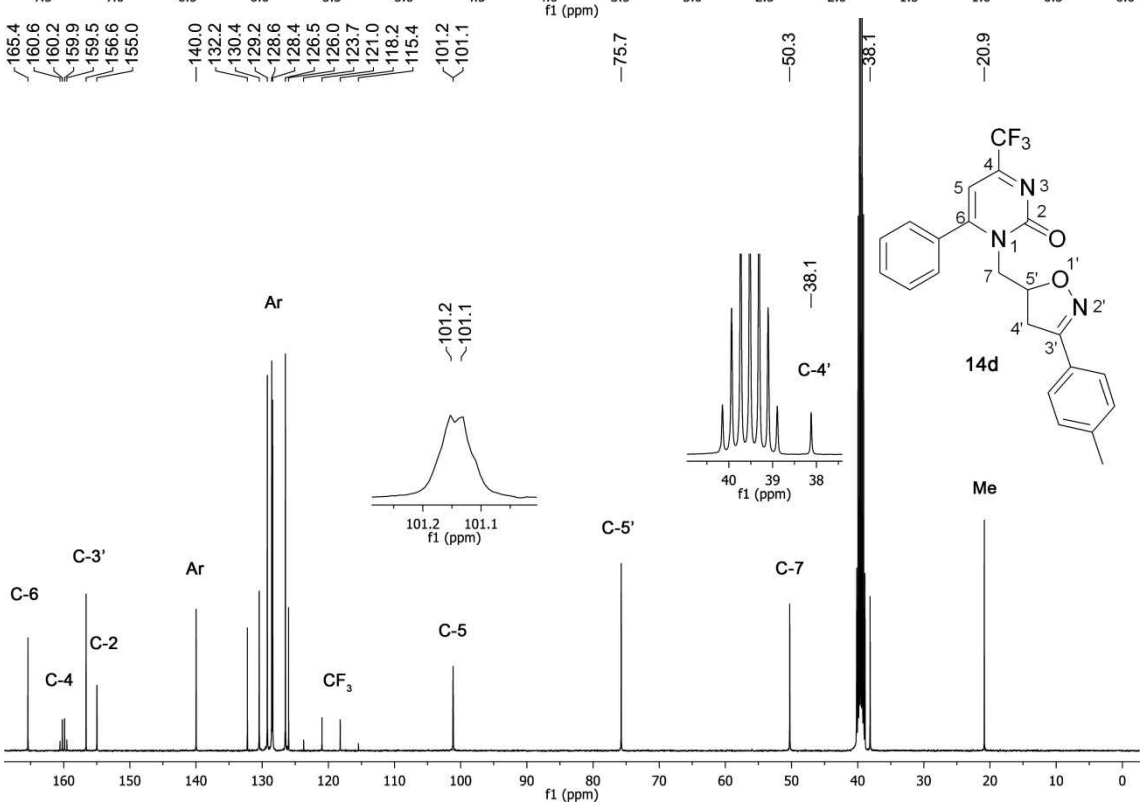
7.43
7.41
7.23
6.81
5.11
5.09
5.08
5.07
5.06
5.05
5.04
4.11
4.09
4.08
3.98
3.96
3.95
3.49
3.47
3.12
3.09
3.08
2.32
Me
H-5
Ar
H-5'
H-7
H-4'
H-4'
CF3
14d
f1 (ppm)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160.6
160.2
159.9
159.5
156.6
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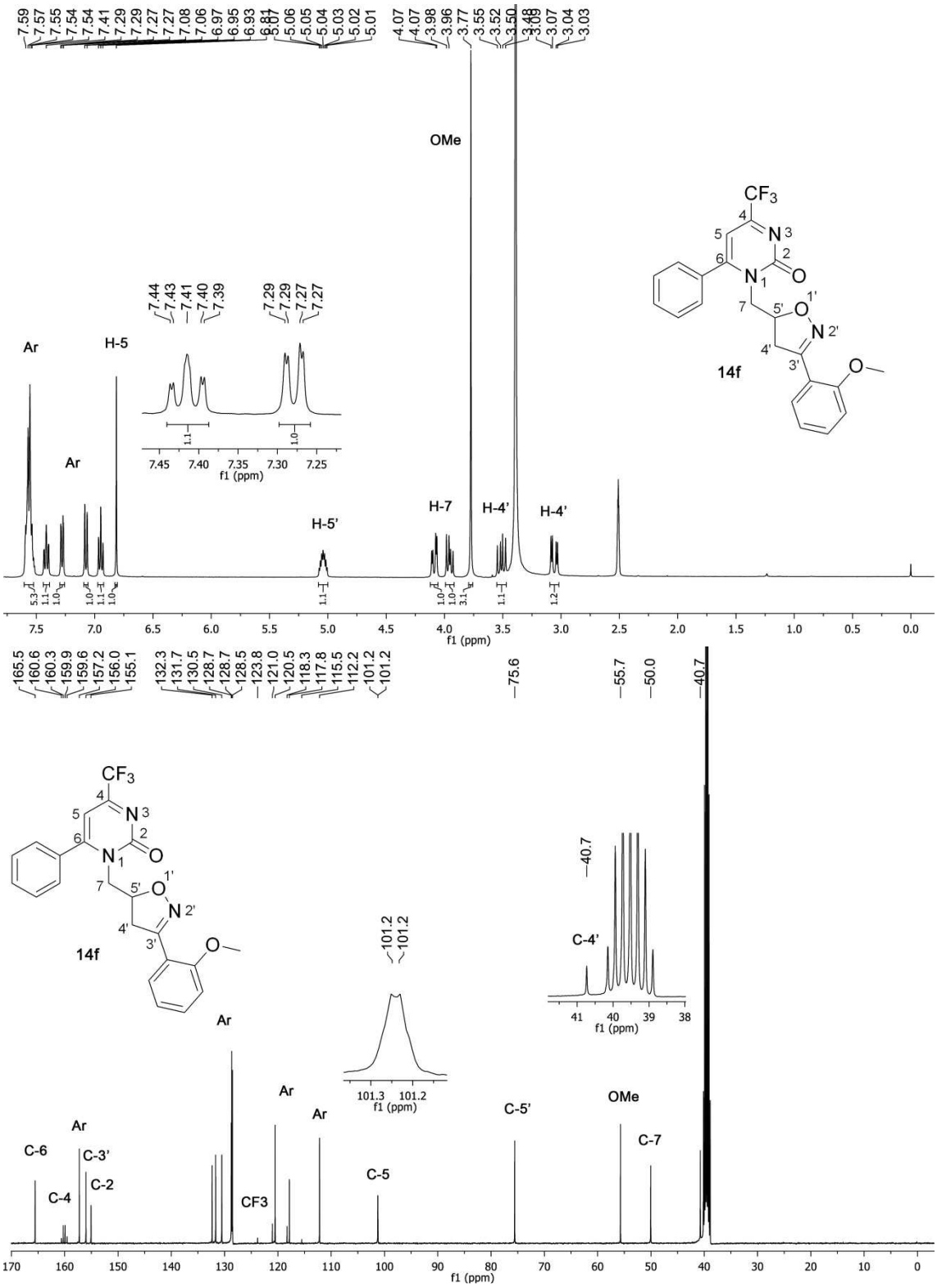
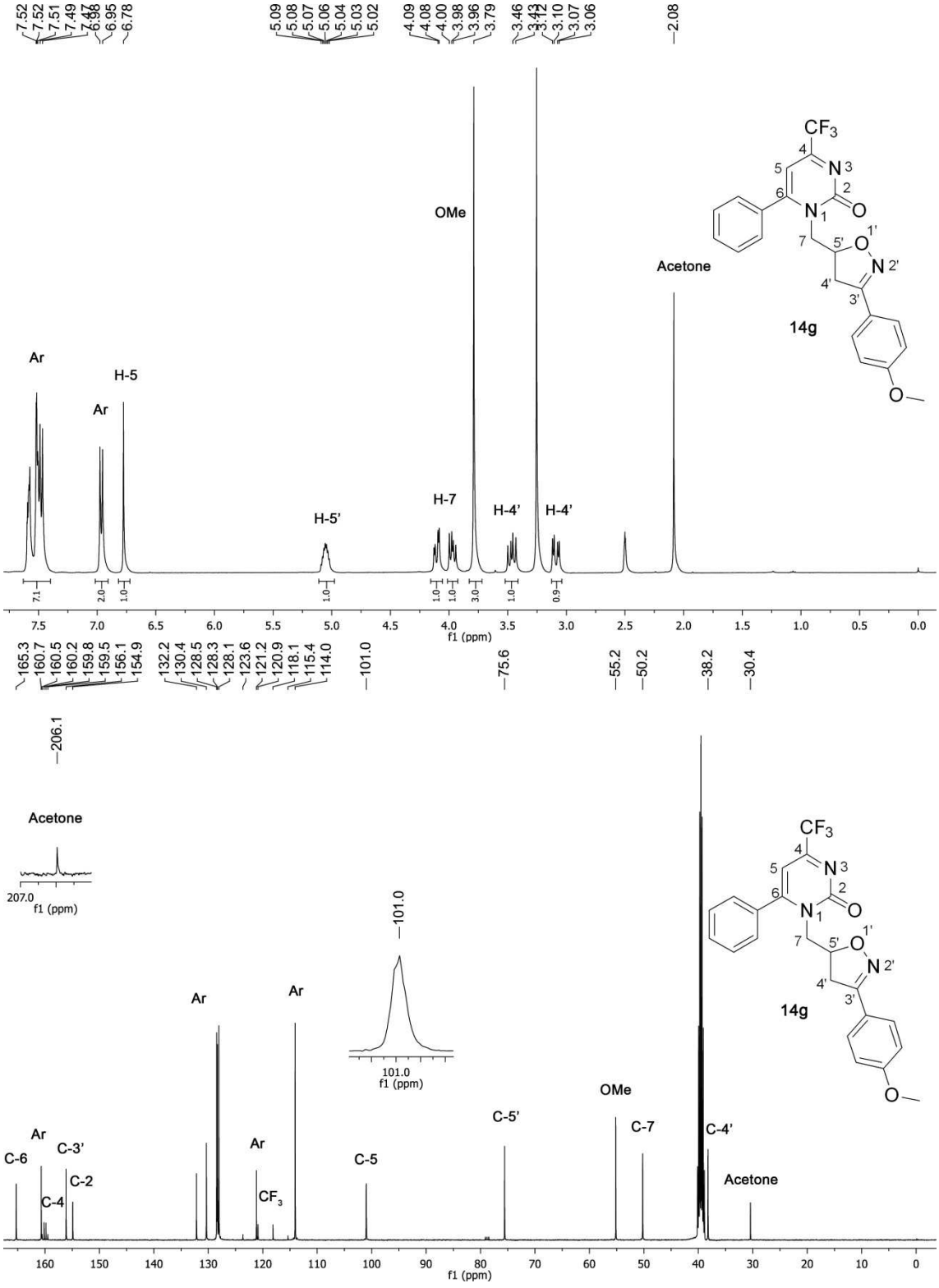
140.0
132.2
130.4
129.2
128.6
128.4
126.5
126.0
123.7
121.0
118.2
115.4
101.2
101.1
75.7
50.3
38.1
20.9
Ar
C-3'
C-6
C-4
C-2
Ar
CF3
C-5
C-5'
C-7
Me
C-4'
14d
f1 (ppm)


OMe
Ar
H-5
Ar
H-5'
H-7
H-4'
H-4'
14f
f1 (ppm)
C-6
C-4
Ar
C-3'
C-2
CF3
Ar
Ar
C-5
C-5'
OMe
C-7
C-4'
14f
f1 (ppm)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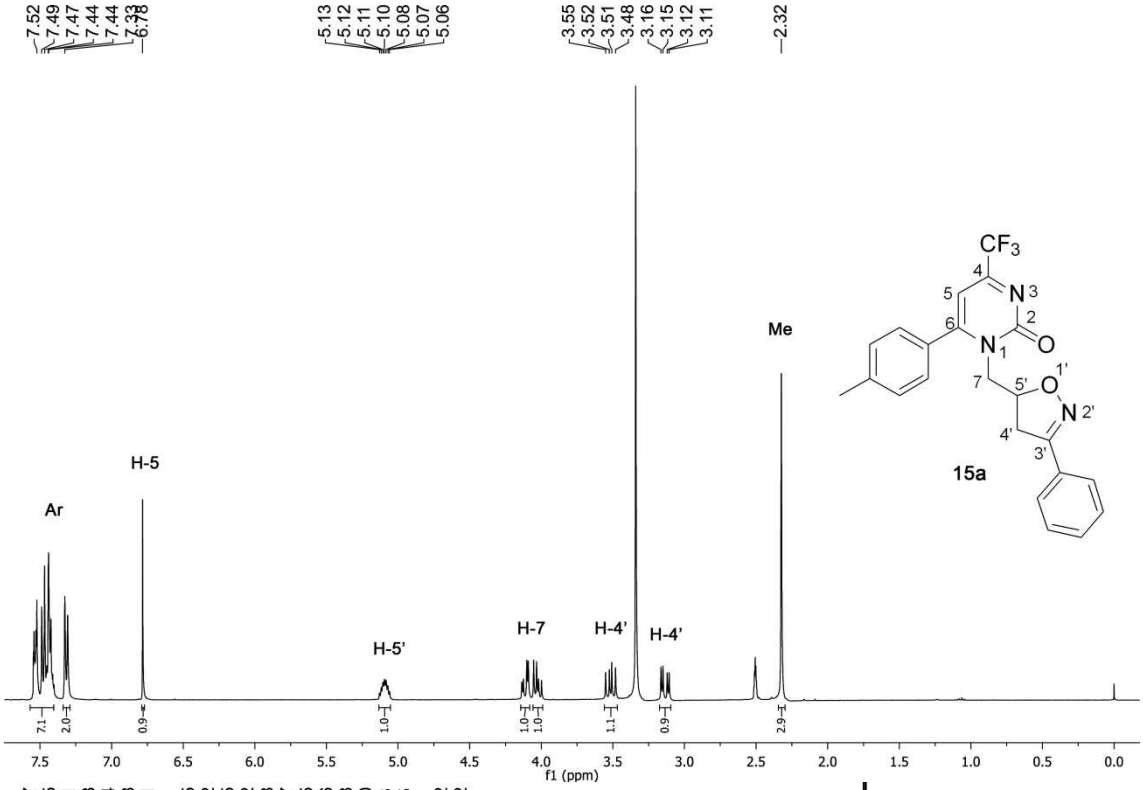
6.78
5.09
5.08
5.07
5.06
5.04
5.03
5.02
4.09
4.08
4.00
3.98
3.96
3.79
3.46
3.10
3.07
3.06
2.08
OMe
Acetone
Ar
H-5
Ar
H-5'
H-7
H-4'
H-4'
CF3
14g
f1 (ppm)
165.3
160.7
160.5
160.2
159.8
159.5
156.1
154.9
132.2
130.4
128.5
128.3
128.1
123.6
121.2
120.9
118.1
115.4
114.0
101.0
75.6
55.2
50.2
38.2
30.4
206.1
Acetone
207.0
f1 (ppm)
101.0
101.0
f1 (ppm)
Ar
Ar
Ar
C-6
Ar
C-3'
C-2
C-4
CF3
C-5
C-5'
OMe
C-7
C-4'
Acetone
14g
f1 (ppm)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7.49
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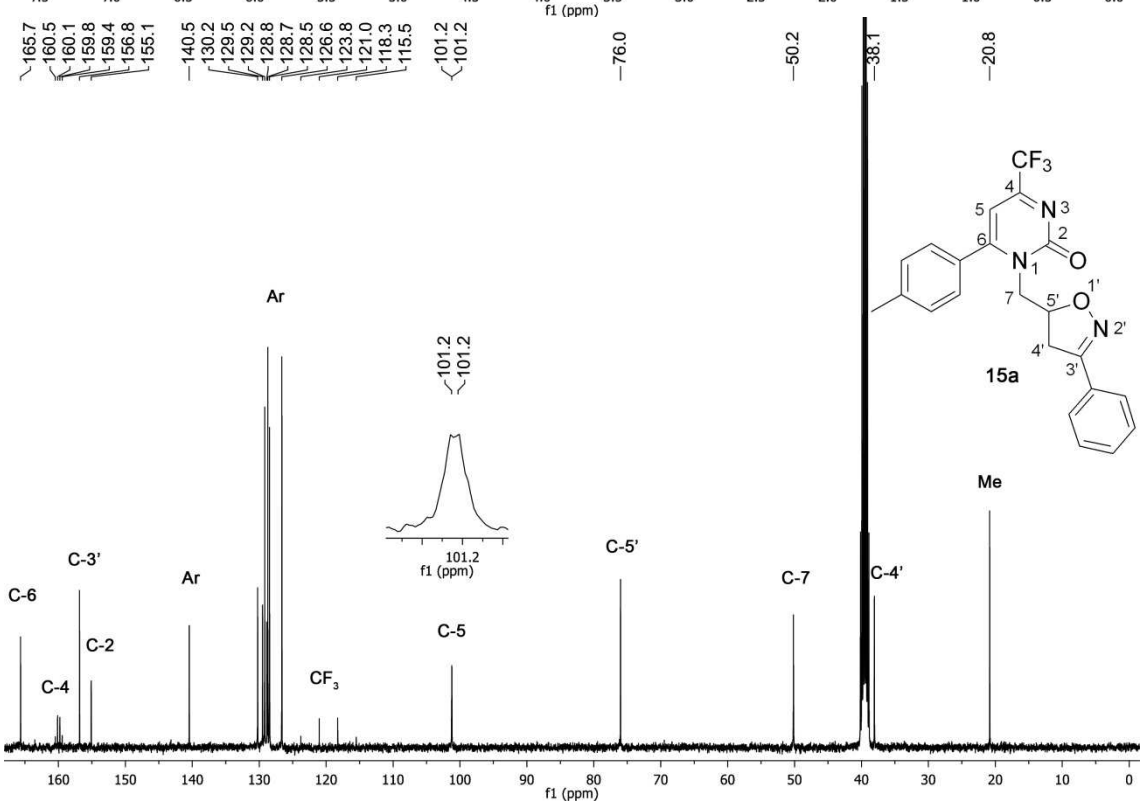
7.44
7.44
7.38
6.78
5.13
5.12
5.11
5.10
5.08
5.07
5.06
3.55
3.52
3.51
3.48
3.16
3.15
3.12
3.11
2.32
Ar
H-5
H-5'
H-7
H-4'
H-4'
Me
7.1
2.0
0.9
1.0
1.0
1.0
1.1
0.9
2.9
f1 (ppm)
CF3
15a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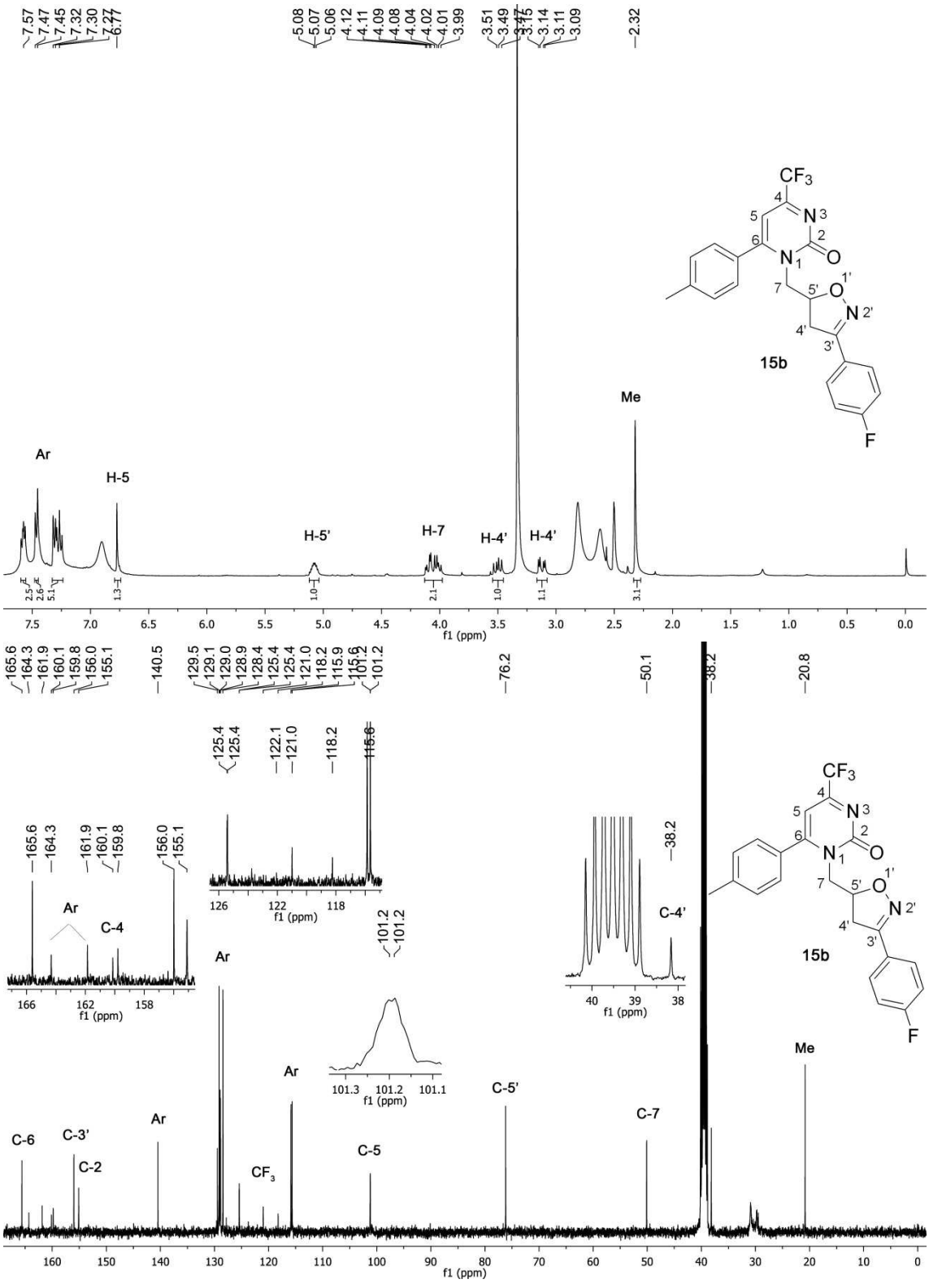
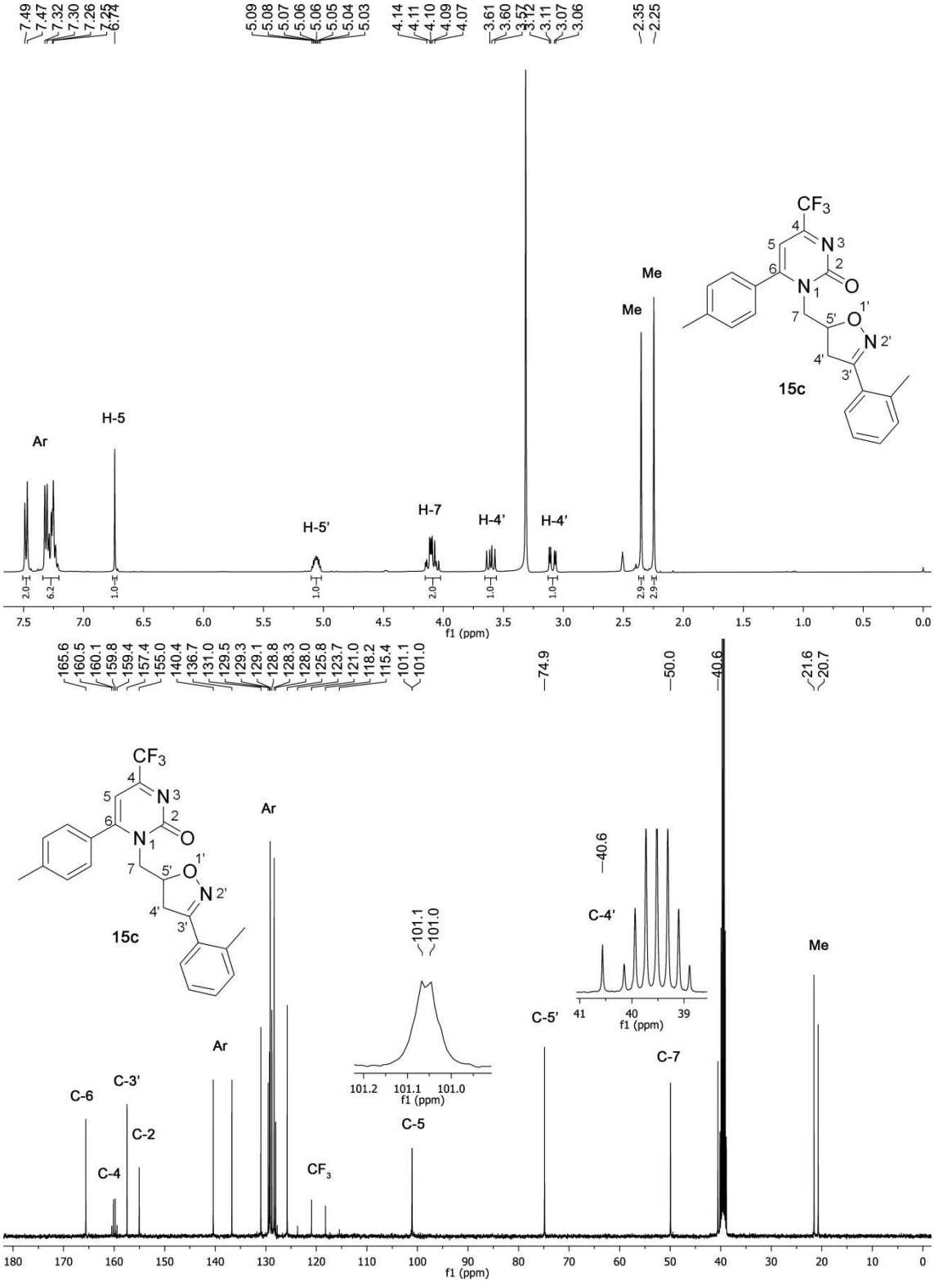
160.1
159.8
159.4
156.8
155.1
140.5
130.2
129.5
129.2
128.8
128.7
128.5
126.6
123.8
121.0
118.3
115.5
101.2
101.2
76.0
50.2
38.1
20.8
Ar
101.2
101.2
f1 (ppm)
C-3'
C-6
Ar
C-2
C-4
CF3
C-5
C-5'
C-7
C-4'
Me
f1 (ppm)
CF3
15a


15b
Ar
H-5
H-5'
H-7
H-4'
H-4'
Me
f1 (ppm)
C-6
C-3'
C-2
Ar
Ar
CF3
Ar
C-5
C-5'
C-7
Me
C-4
C-4'
15b
f1 (ppm)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6.74
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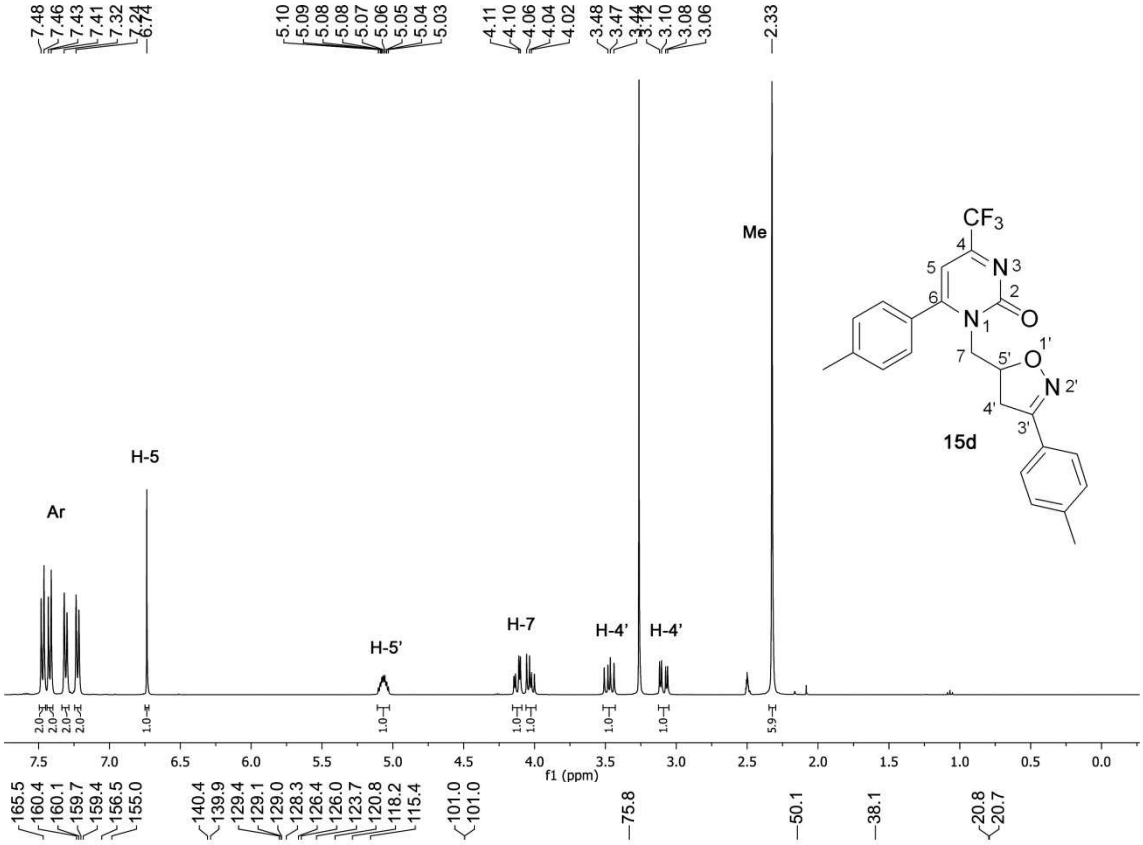
5.08
5.07
5.06
5.06
5.05
5.04
5.03
4.14
4.11
4.10
4.09
4.07
3.61
3.60
3.12
3.11
3.07
3.06
2.35
2.25
CF3
Me
Me
Ar
H-5
H-5'
H-7
H-4'
H-4'
15c
f1 (ppm)
165.6
160.5
160.1
159.8
159.4
157.4
155.0
140.4
136.7
131.0
129.5
129.3
129.1
128.8
128.3
128.0
125.8
123.7
121.0
118.2
115.4
101.1
101.0
74.9
50.0
40.6
21.6
20.7
Ar
C-6
C-3'
C-2
C-4
Ar
CF3
101.1
101.0
C-5
C-5'
C-7
40.6
C-4'
Me
15c
f1 (ppm)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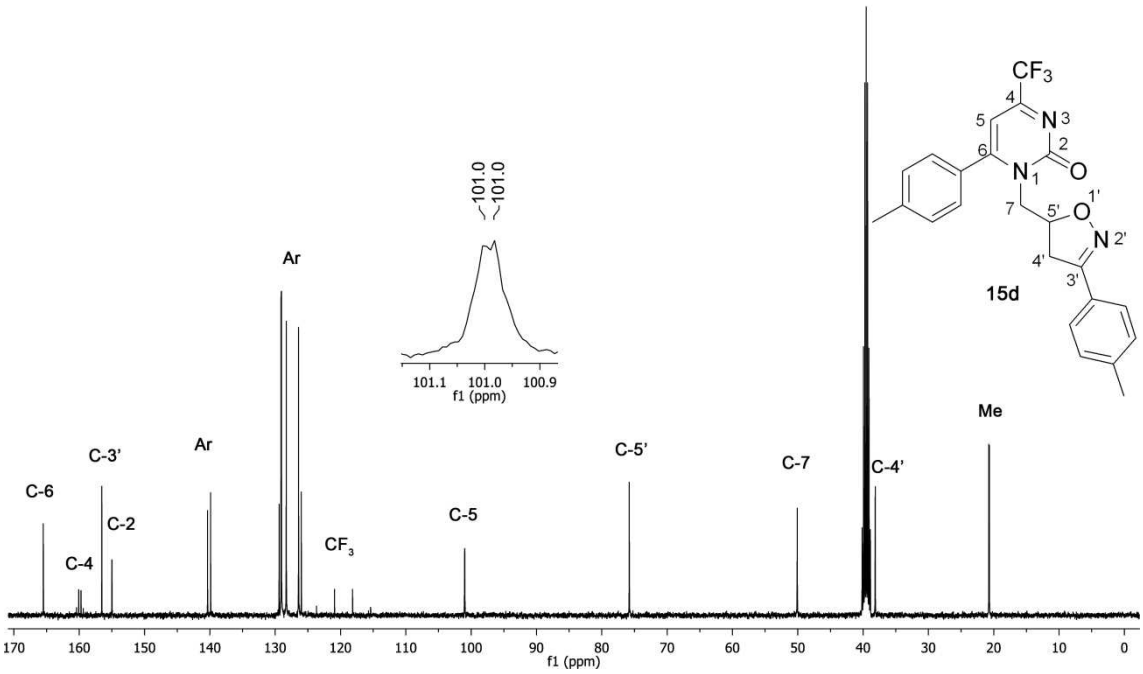
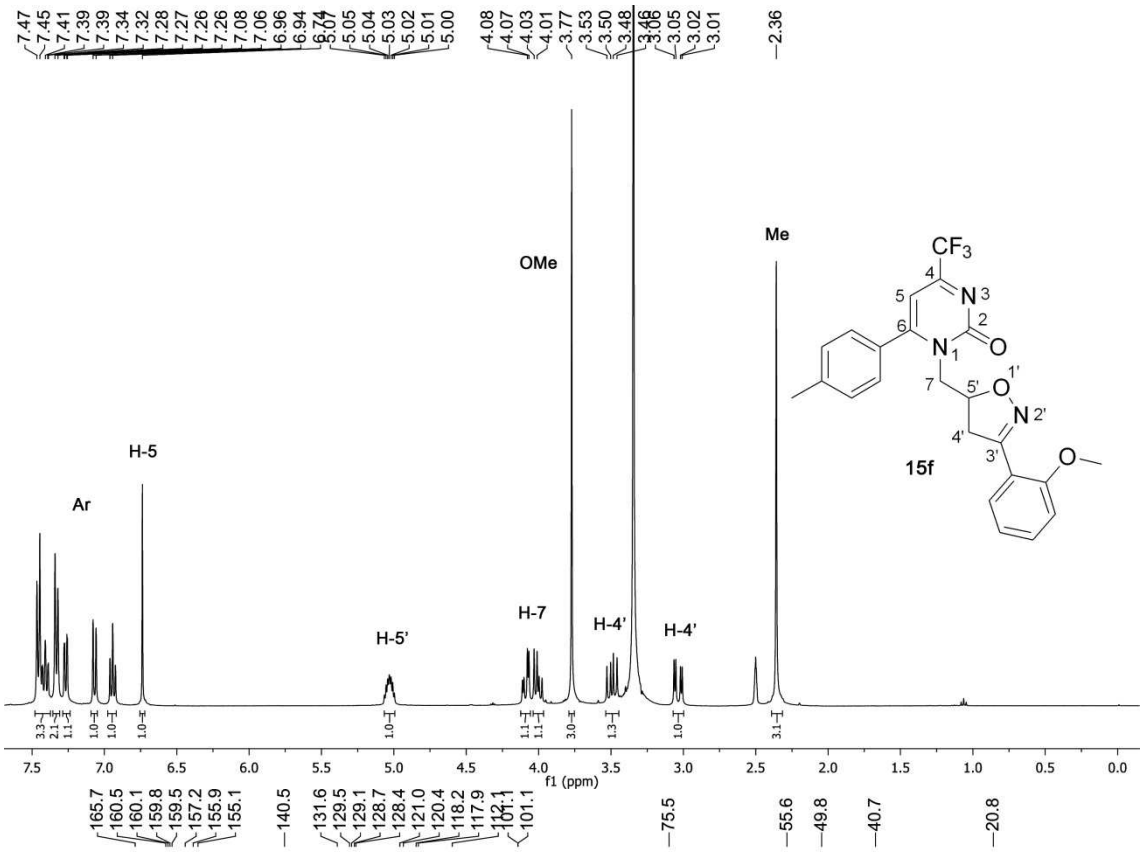
5.09
5.08
5.08
5.07
5.06
5.05
5.04
5.03
4.11
4.10
4.06
4.04
4.02
3.48
3.47
3.44
3.42
3.10
3.08
3.06
2.33
Me
CF3
H-5
Ar
H-5'
H-7
H-4'
H-4'
15d
f1 (ppm)
165.5
160.4
160.1
159.7
159.4
156.5
155.0
140.4
139.9
129.4
129.1
129.0
128.3
126.4
126.0
123.7
120.8
118.2
115.4
101.0
101.0
75.8
50.1
38.1
20.8
20.7


CF3
15d
101.0
101.0
101.1
101.0
100.9
f1 (ppm)
Ar
Ar
C-6
C-4
C-3'
C-2
CF3
C-5
C-5'
C-7
C-4'
Me
f1 (ppm)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7.45
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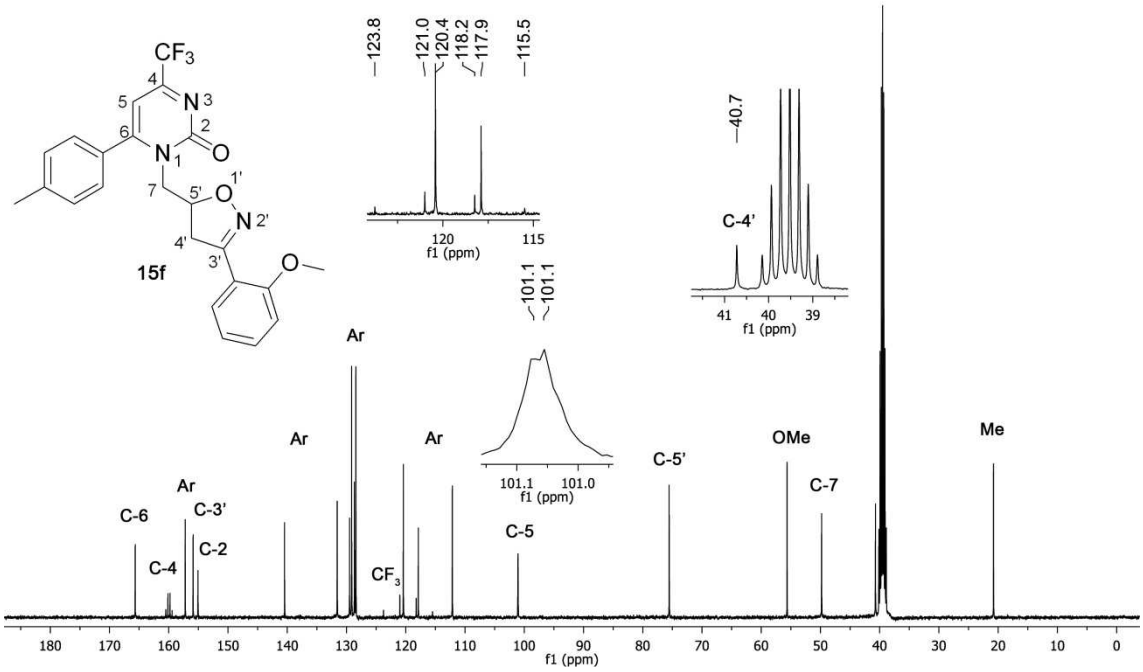
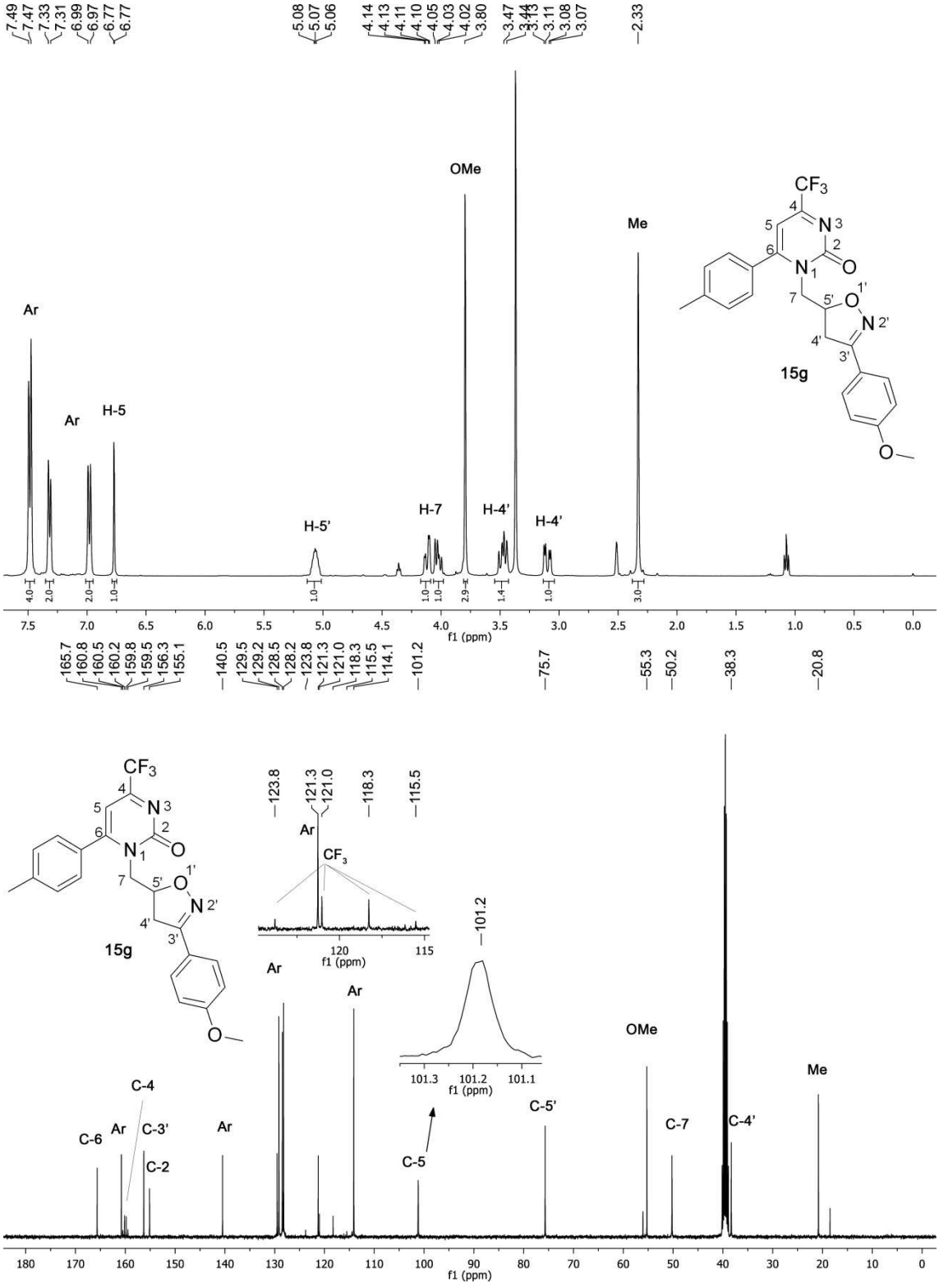
7.39
7.39
7.34
7.32
7.28
7.27
7.26
7.26
7.08
7.06
6.96
6.94
6.74
6.74
5.07
5.05
5.04
5.03
5.02
5.01
5.00
4.08
4.07
4.03
4.01
3.77
3.53
3.50
3.48
3.46
3.06
3.05
3.02
3.01
2.36
OMe
Me
H-5
Ar
H-5'
H-7
H-4'
H-4'
CF3
15f
f1 (ppm)
165.7
160.5
160.1
159.8
159.5
157.2
155.9
155.1
140.5
131.6
129.5
129.1
128.7
128.4
121.0
120.4
118.2
117.9
112.1
101.1
75.5
55.6
49.8
40.7
20.8


CF3
15f
123.8
121.0
120.4
118.2
117.9
115.5
101.1
101.1
40.7
C-4'
Ar
Ar
Ar
C-6
C-4
C-3'
C-2
CF3
C-5
C-5'
OMe
C-7
Me
f1 (ppm)


7.49
7.47
7.33
7.31
6.99
6.97
6.77
6.77
5.08
5.07
5.06
4.14
4.13
4.11
4.10
4.05
4.03
4.02
3.80
3.47
3.44
3.13
3.11
3.08
3.07
2.33
OMe
Me
Ar
Ar
H-5
H-5'
H-7
H-4'
H-4'
CF3
15g
f1 (ppm)
165.7
160.8
160.5
160.2
159.8
159.5
156.3
155.1
140.5
129.5
129.2
128.5
128.2
123.8
121.3
121.0
118.3
115.5
114.1
101.2
75.7
55.3
50.2
38.3
20.8
C-4
C-6
Ar
C-3'
C-2
Ar
Ar
Ar
C-5
C-5'
OMe
C-7
C-4'
Me