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Graphical abstract

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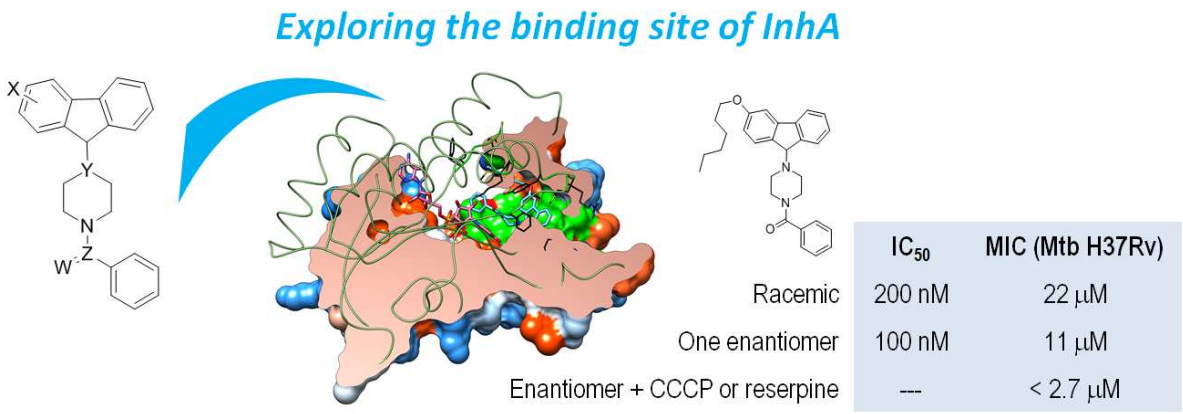

	IC_{50}	MIC (Mtb H37Rv)
Racemic	200 nM	22 μM
One enantiomer	100 nM	11 μM
Enantiomer + CCCP or reserpine	---	< 2.7 μM

Design, synthesis and evaluation of new GEQ derivatives as inhibitors of InhA enzyme and *Mycobacterium tuberculosis* growth

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Keywords: Medicinal chemistry; inhibitors; InhA; *Mycobacterium tuberculosis*; efflux pumps.

ABSTRACT

A series of fluorene-based derivatives was synthesized and evaluated for inhibiting both InhA and *Mycobacterium tuberculosis* growth. These compounds were inspired by the previously reported Genz-10850 molecule, a good InhA inhibitor, but with a poor activity against *M. tuberculosis* growth. Structure-activity relationships were performed by introducing the following chemical modifications: 1) the piperazine ring; 2) the amide group; 3) the aryl moiety; and 4) the fluorene moiety. Among these new derivatives, one of them was more effective against both the InhA activity and mycobacterial growth, compared to the hit compound. Docking studies were also performed to rationalize activities of these derivatives. Furthermore, we showed for the first time that efflux pump inhibitors potentiated the efficacy of Genz-10850 (GEQ) derivatives against *M. tuberculosis* growth, demonstrating that these compounds could be substrates of some efflux pumps.

1. Introduction

Tuberculosis remains one of the leading infectious diseases around the world with 9 million new cases and 1.5 million deaths in 2013.[1] One-third of the world population has been estimated as infected by *Mycobacterium tuberculosis*, the causative pathogen of tuberculosis. With the resurgence of the disease related to the HIV-coinfection and to resistance to current clinical treatment, there is a growing need to find new antitubercular drugs.[1,2] In the last few years, *M. tuberculosis* enzymes involved in the fatty acid synthase type II (FAS-II) system have been identified and validated as relevant drug targets.[3-5] Among the FAS-II enzymes, InhA, a *trans*-2-enoyl-ACP reductase, is one of the most druggable target in tuberculosis field. Different classes of direct InhA inhibitors, including 4-hydroxy-2-pyridone derivatives, have been recently reported in the literature with potent bactericidal activity against *M. tuberculosis* strains.[6,7] Among all these inhibitors, Genz-10850 (also called **GEQ)** has been identified as a very promising inhibitor of InhA (Figure 1), after *in vitro* screening of a library of 500,000 compounds.[8] Later, He *et al.* synthesized a series of **GEQ** derivatives with InhA inhibitory activities ranging from nanomolar to micromolar.[9] Compound **1** (Figure 1) was one of these derivatives and it exhibited an IC_{50} value in the low nanomolar range. These molecules have poor activity against *M. tuberculosis,* showing a MIC above 125 µM because of their low permeability or the activation of efflux pumps.[9] Therefore, chemical modification of these inhibitors was performed in order to improve their low biological activity.

In the present work, we reported the synthesis and the evaluation of new **GEQ** analogues with an improved inhibitory activity against both InhA and *M. tuberculosis* growth. Furthermore, we showed that efflux pumps inhibitors potentiate their effectiveness against *M. tuberculosis* growth.

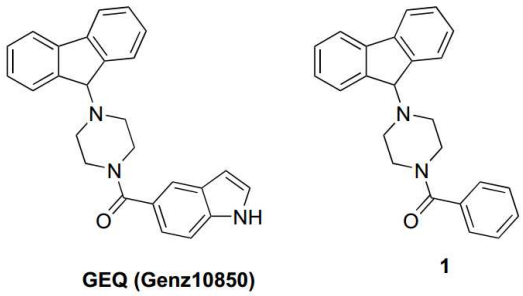


Figure 1. **GEQ** and its analogue **1** as inhibitors of InhA

2. Results and discussion

2.1. Design of the GEQ derivatives. During the development of **GEQ** analogues by Ortiz de Montellano *et al.*, a significant improvement was performed by replacing the indole ring with a phenyl group (Figure 1).[9] The reported IC_{50} toward InhA inhibition for benzoyl analogue **1** was lower than that of **GEQ**. Consequently compound **1** remained as our reference molecule all along this study.

To ensure similar hydrophobic interactions in the binding site of InhA, the global scaffold of the compound **1** was retained. The proposed modifications were performed in an attempt to improve both inhibition of InhA and the *M. tuberculosis* membrane permeability (Figure 2).

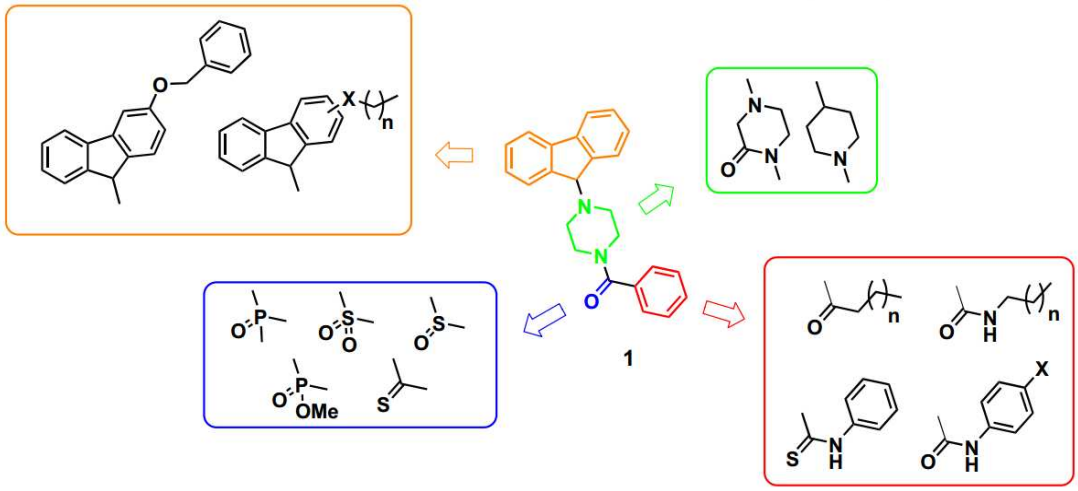


Figure 2. Structural modifications proposed from the compound **1**

Modification of the central core.

The piperazine central core was replaced with a piperidine in order to overcome the eventual protonation of the nitrogen atom (Figure 2). We are consequently expected to improve activity of the lead molecule concerning its inhibitory action toward mycobacterial growth. **GEQ** analogues bearing a succinimide central core have been recently reported by our group.[10] These analogues displayed good inhibitory activity against InhA protein and some of them displayed a significantly increased activity against *M. tuberculosis* growth, with a MIC of 5.4 µM for the best compound. Similarly, the replacement of the piperazine ring with piperazinone was proposed in order to enhance the interactions with the InhA enzyme.

Modification of the amide group.

Key interaction has been described between the oxygen atom of the piperazine amide of **GEQ** and the hydroxyl group of the Tyr158 residue of InhA.[11] Consequently, in an attempt to strengthen this interaction, investigations were performed around the carbonyl group of the

lead molecule **1** through introduction of isosteric groups. Then, the carbonyl group of the amide moiety in compound **1** was replaced by various functional groups to gain insights into the specificity of the binding site. Then, sulfonyl- , sulfinyl-, phosphonyl- and phosphinamide were also introduced. In order to gain insights into settled hydrogen bond, urea and thiourea analogues were also prepared. Furthermore, thioamide compounds could be synthesized and evaluated. The length of the C=S bond coupled with the larger size of the sulfur atom might better be accommodated with the hydroxyl group of the tyrosine.[12]

Modification of the aryl moiety.

Previously, succinimide **GEQ** analogues, bearing a long alkyl chain (C_8 and C_9) instead of the indole moiety, were found to be efficient against InhA.[10] In a similar manner, derivatives with a piperazine central core and a fatty alkyl chain were synthesized with either an amide or an (thio)urea linker.

Modification of the fluorene moiety.

GEQ molecule and the C_{16} fatty acyl substrate analogue displayed a similar binding mode within the active site of the InhA protein (PDB-1P44 [8] and PDB-1BVR [13]). Indeed, interactions with the key residue Tyr158 were maintained in both situations and hydrophobic contacts guide either the fluorene fragment or the long alkyl chain in the upper part of the cavity. Superposition of both molecules within the binding pocket highlights the possibility of substitution on the fluorene moiety (Figure 3). In a similar strategy, Tonge and coworkers have developed analogues of triclosan through introduction of an alkyl chain.[14] In fact, the 5-hexyl substituted diphenyl ether analogue of triclosan exhibits a 10^3-fold better activity.[15,16] Consequently, we focused our attention on the synthesis of 2- and 3-alkyl-substituted fluorene derivatives. The length of the chain and the nature of the linker were also investigated. Kuo and coworkers also evidenced, in a similar fashion, the high potency of GEQ derivative substituted with a benzyl amide group on the position 2 of the fluorene moiety.[8] Consequently, a benzyl ether group was also covalently attached to the fluorene in order to explore the whole cavity.

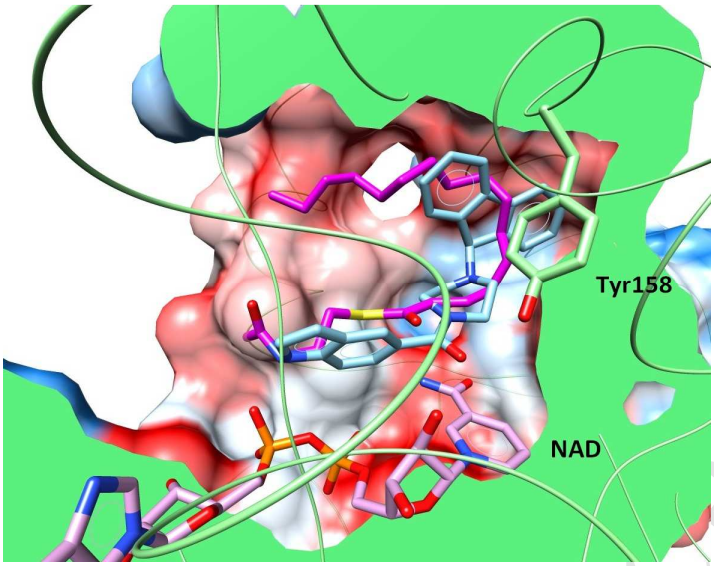
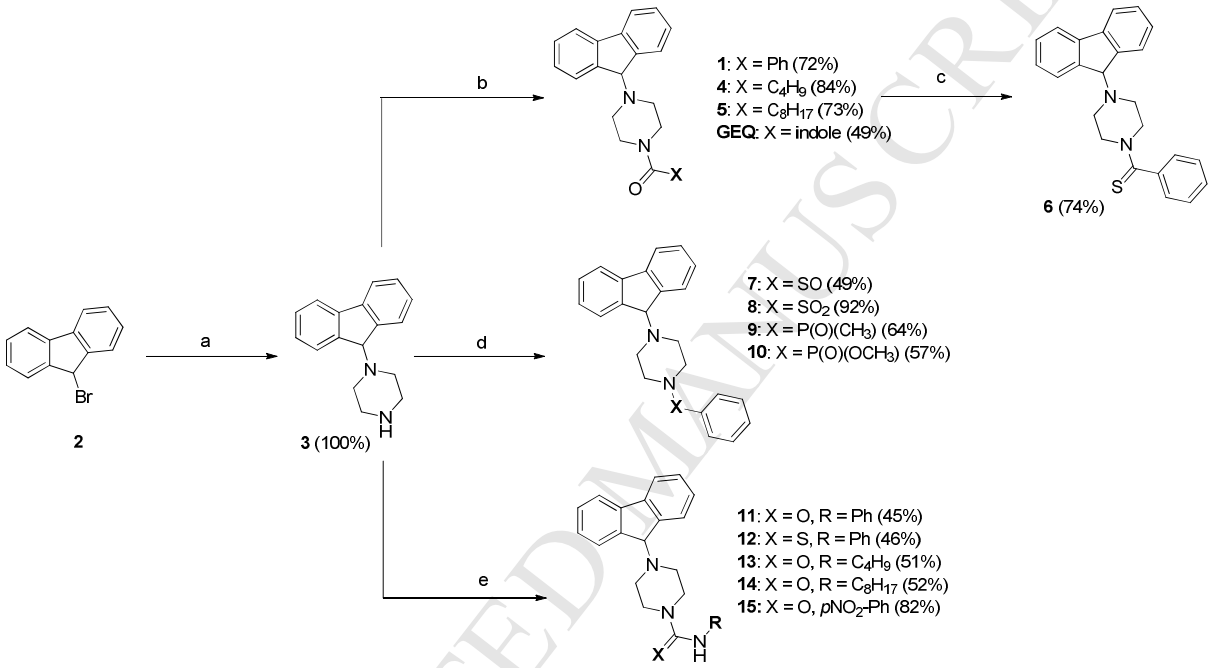


Figure 3. Superimposition of GEQ (blue) and substrate analogue (purple) molecules in the InhA binding site (PDB IDs respectively 1P44 [8] and 1BVR [13]). Colouring according the kd Hydrophobicity [17] scale for each amino acid from dodger blue to the most hydrophilic, to white, to orange red for the most hydrophobic.

2.2. Chemistry

The first strategy described in scheme 1 was based upon an *N*-acylation reaction onto the 1-(9*H*-fluoren-9-yl)piperazine **3**. The key intermediate **3** was synthesized in a quantitative yield via the reaction of 9-bromofluorene **2** and piperazine as previously reported.[18] Both reference compounds **GEQ** and **1** were *de novo* synthesized to serve as a positive control for the subsequent *in situ* enzyme inhibition. The coupling between the indole carboxylic acid and the piperazine moiety was performed using peptide coupling agents HOBt, EDC.HCl and DIPEA as base to afford the **GEQ** molecule in a similar manner as previously reported.[9] To yield compound **1**, the piperazine synthon **3** was coupled to benzoyl chloride reagent in the presence of TEA as a base, in a relatively good yield (72%). Similarly, valeryl chloride and nonanoyl chloride were engaged in the same coupling reaction to introduce an alkyl chain and provide compounds **4** and **5**. Compound **1** was subsequently involved in a thionation reaction with Lawesson's reagent as described by Coppola *et al.* to introduce the thioamide function in compound **6**.[19] To gain insights into the specificity of the binding site, compounds **7-10** were prepared in a similar fashion with respectively benzenesulfinic chloride, benzene sulfonyl chloride, [20] phenylphosphinic dichloride and methylphenylphosphinic chloride. To get the phosphonamide **10**, commercially available phenyl phosphonic dichloride, was coupled to compound **3** then, after overnight stirring at room temperature, methanol was

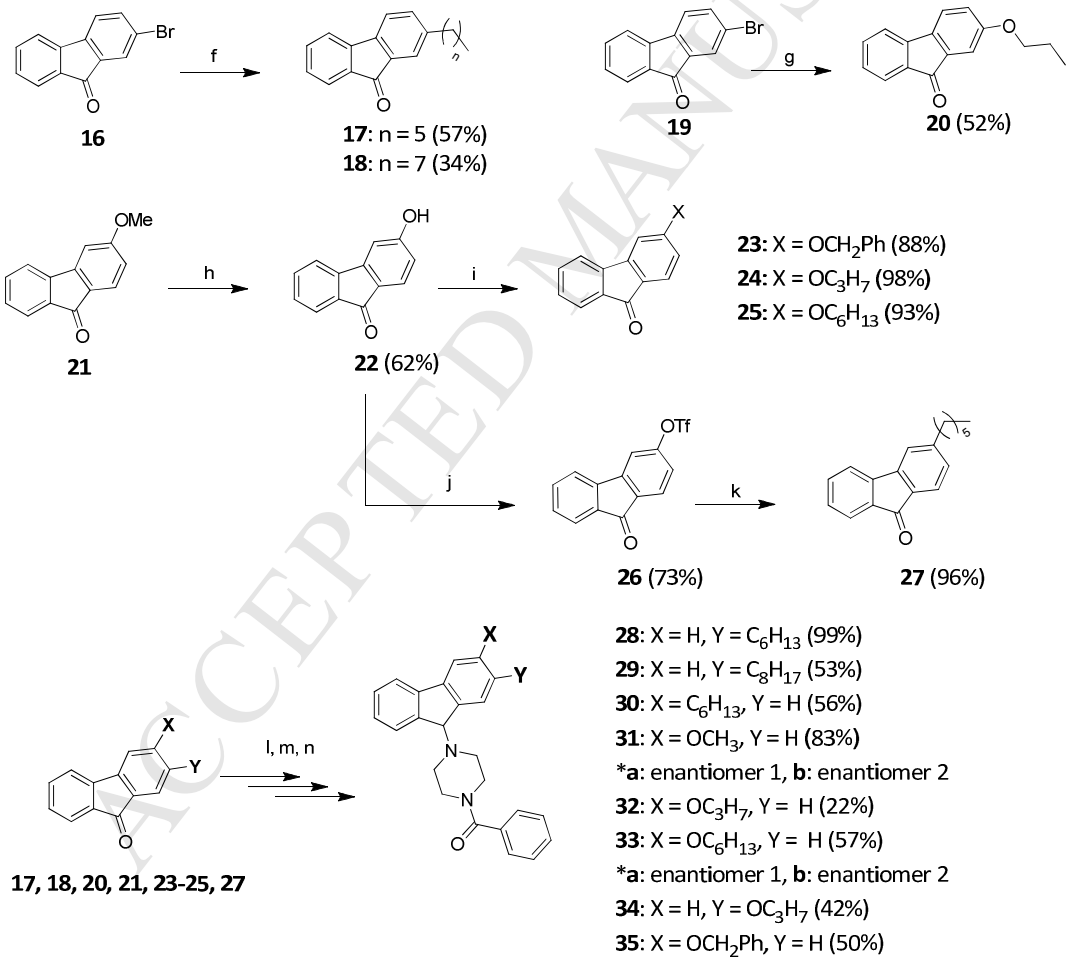
added to quench the reaction and to substitute the last chlorine atom with a methoxy group.[21] The benzenesulfinic chloride reagent was obtained after activation of the corresponding benzenesulfinic acid sodium salt with thionyl chloride.[22] Compounds **7**, **9** and **10** were obtained as racemic mixture of *R*- and *S*- derivatives and were directly engaged in enzymatic assay to evaluate their inhibitory potency. The ureas **11**, **13-15** and thiourea **12** were synthesized in a one-step reaction from compound **3** and the corresponding isocyanate [23] or isothiocyanate.[24]



Scheme 1. General synthetic procedure for replacement of the benzamide moiety. Reagents and reaction conditions: a. TEA, piperazine, THF, reflux 6 h; b. RCOCl, TEA, DCM (for **GEQ** compound: RCOOH, HOBt, DIPEA, DMF); c. From compound **1**, Lawesson's reagent, toluene; f. RNCX, CH_3CN, 18 h; d. PhXCl, TEA, DCM (for compound **8**, MeOH was added in a second time); e. RCNX, CH_3CN, 18 h.

For substituted fluorene derivatives, the key fluorenone intermediates (**17**, **18**, **20**, **21**, **23-25**, **27**) were synthesized according to various routes presented in Scheme 2. 2-Bromo-fluorenone was engaged in a Suzuki coupling reaction with 1-hexene and 1-octene after hydroboration of those last two reagents with 9-BBN to afford 2-alkyl-fluorenone intermediates **17** and **18** in yields similar to those previously described.[25] Intermediate **20** was obtained using a pallado-catalyzed reaction in the presence of cesium carbonate and *t*BuXPhos, with propan-1-ol as coupling partner.[26] The 3-methoxy-fluorenone **21** was prepared according to a previously reported method which involved a Suzuki coupling reaction between ethyl-2-bromobenzoate and 3-methoxyphenylboronic acid followed by methanesulfonic-acid-

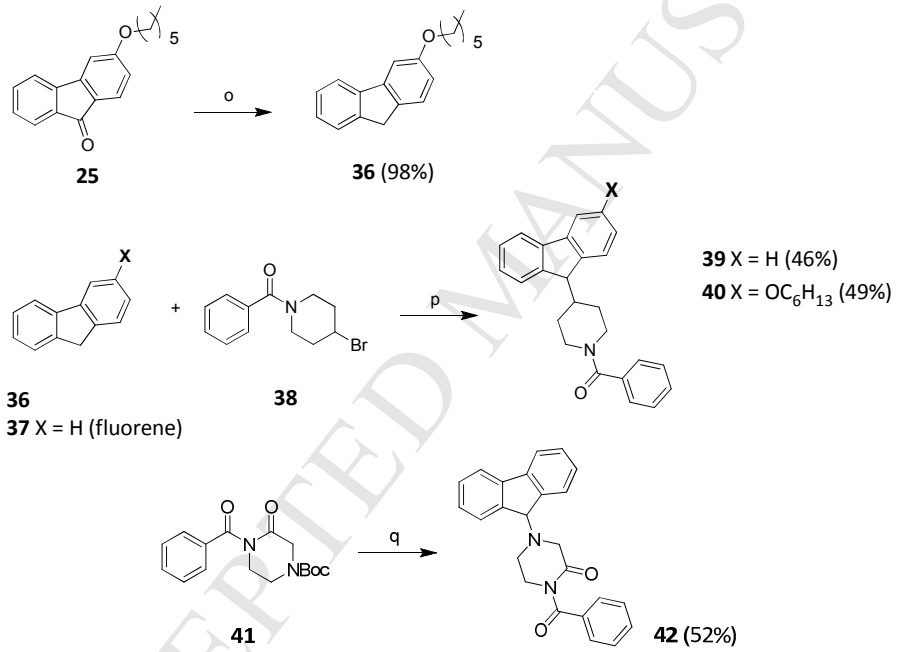
catalyzed ring closure.[27] The 3-methoxy intermediate was partially engaged in a methyl ether deprotective reaction using the couple HBr/HOAc to give the corresponding 3-hydroxyl-fluorenone **22**.[28] *O*-alkylation of the hydroxyl intermediate with benzyl, propyl and hexyl bromide permitted us to achieve the 3-substituted fluorenones with an ether linker (**23-25**). In a second time, conversion of intermediate **22** to its triflate derivative **26** allowed the introduction of a hexyl chain in the position C2 after a Suzuki reaction, as previously described by Spencer *et al.*[25] These fluorenone intermediates were respectively engaged in a two-steps reaction implying a ketone reduction using sodium borohydride followed by a specific conversion of the alcohols to the alkyl bromides using phosphorus tribromide. The intermediates were obtained in good to excellent yields without purification. Commercially available 1-benzoylpiperazine was finally coupled to the different 9-bromofluorenes to afford the final products **28-35**.



Scheme 2. Synthesis of fluorene substituted derivatives. Reagents and reaction conditions: f. (1) 1-Hexene or 1-octene, 9-BBN, THF, 0 °C, 2 h then RT, 3 h; (2) Cs_2CO_3, $AsPh_3$, $Pd(dppf)Cl_2$,THF, DMF, H_2O, 85 °C, 18 h; g. $Pd(OAc)_2$, *t*BuXPhos, Cs_2CO_3, propan-1-ol, toluene, 80 °C, 18 h; h. HBr HOAc, 6 h, reflux; i. RBr, K_2CO_3, DMF; j. Tf_2O, 2.6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, -78 °C, 1 h, 0 °C, 1.5 h; k. (1) 1-Hexene, 9-BBN,

THF, 0 °C, 2 h, RT, 3 h (2) K_3PO_4, Pd(dppf)Cl_2, THF, 16 h, reflux; l. MeOH, $NaBH_4$, 0.5 h, RT; m. DCM, PBr_3, 0 °C, 2 h; n. 1-benzoylpiperazine, K_2CO_3, DMF.

The synthesis of the derivatives **39** and **40** bearing a piperidine as a central core is described in Scheme 3. Firstly, the decarbonylation reaction of the *O*-alkylated fluorenone **25** in the presence of hydrazine led to the fluorene intermediate **36**.[29] Then, this compound and commercially available fluorene **37** were engaged in a deprotonation reaction with *n*BuLi and were coupled with *N*-benzoyl-4-bromopiperidine. The piperazinone core was also introduced to replace the piperazine central ring by using the previously synthesized *N*-Boc protected intermediate **41**. Boc deprotection with TFA, followed directly by a coupling reaction with 9-bromofluorene in the presence of triethylamine afforded the target compound **42**.[30]



Scheme 3. Replacement of the piperazine central core by piperidine and piperazinone. Reagents and reaction conditions: o.(1) H_2NNH_2, diethylene glycol, 10 min; (2) KOH, reflux, 4 h; p. *n*BuLi, THF, -78 °C → RT; q. (1) TFA, DCM, 0 °C to RT, 18 h; (2) 9-bromo-fluorene, TEA, DCM, 18 h, RT.

2.3. Inhibitory InhA activities

The new compounds were tested for their capacity to inhibit the reduction of the substrate double bond by NADH in the presence of InhA. The assays were performed in triplicate in the presence of the substrate analogue 2-*trans*-dodecenoyl-CoA and the percentage of InhA inhibition was determined by measuring the conversion of the NADH cofactor to its oxidized form NAD^+ by means of the decreasing of the absorbance at 340 nm. The molecules were

firstly tested at 50 µM and 10 µM for some of them due to solubility problem. For the more potent compounds, IC_{50} were determined using the 4-parameter curve-fitting software XLFit (IDBS) with at least six points. The results are reported in Tables **1** and **2**.

As references, **GEQ** compound and its phenyl analogue **1** were firstly tested on InhA protein to confirm their activity and for comparison (Table 1). Both compounds showed a complete InhA inhibition at 50 µM and IC_{50} in the nanomolar range, as expected [9,11]. Compound **1** demonstrated the most potent inhibitory activity by comparison with **GEQ**.

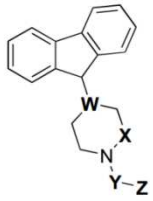
Investigations around the piperazine amide bond revealed an impressive decrease of activity for these derivatives. Indeed, replacing the oxygen atom with sulfur completely dropped the activity of the molecule (compound **6**, Table 1). Additionally, no InhA inhibitory activities were observed for sulfinamide **7**, sulfonamide **8**, phosphinamide **9** and phosphonamide **10** derivatives. These results demonstrate that the amide bond is critically required for good inhibitory activity by its interaction with the hydroxyl group of Tyr158. Nonetheless, the derivative **11** containing a urea functional group instead of the amide bond showed a relatively good InhA inhibitory activity with about 84% inhibition at 50 µM and IC_{50} = 1.18 µM. An additional nitro substituent in the phenyl group at the *para* position totally abolished the activity (compound **15**, Table **1**). This result highlighted either the possibility of steric hindrance or the necessity of an electron-rich aromatic ring to maintain interaction with the pyrophosphate moiety of the NADH molecule. Moreover, compound **12** bearing a thiourea linker, demonstrated a relatively good activity at 50 µM with 57% inhibition but still far from the reference compound **1**.

Alkyl chains were also introduced to replace the phenyl ring maintaining either the amide or the urea as a linker to the piperazine ring. Firstly, we could observe a decrease in activity correlated with a longer chain length (compounds **13**, **14** and **4**, **5;** Table 1). The poor activity noticed for long alkyl chain derivatives is in accordance with Tonge and coworkers binding site description.[6] They described this region of the binding site as size-limited and surrounded by both polar and non polar groups. Indeed, both compounds **13** and **4** exhibited about 70% of activity at 50 µM (Table 1) toward InhA protein.

Efforts were also furnished to replace the piperazine ring by either piperidine (compound **39**, Table 1) or piperazinone (compound **42**, Table 1). The additional keto bond in compound **42** abolished the inhibitory activity and comforted the suggested steric clash in the case of carbon substitution of the piperazine ring.[8] Removal of the nitrogen atom in the piperazine ring into compound **39** led to a potent InhA inhibitor with similar potency at 50 µM than the reference

compound **1**. IC_{50} was determined and revealed a sub-micromolar activity for the piperidine derivative.

Table 1. Inhibitory potencies of compounds **1**, **3**-**5**, **6**-**15**, **39**, **42** against InhA



Compound	W	X	Y	Z	InhA inhibition (%)		IC_{50} (µM)
					50 µM	10 µM	
GEQ	N	CH_2	CO	Indole	87	81	0.86 ± 0.16
1	N	CH_2	CO	Ph	94	94	0.49 ± 0.06
3	N	CH_2	H	-	7	-	-
4	N	CH_2	CO	C_4H_9	75	45	-
5	N	CH_2	CO	C_8H_{17}	47	22	-
6	N	CH_2	CS	Ph	31	-	-
7	N	CH_2	SO	Ph	14	-	-
8	N	CH_2	SO_2	Ph	12	-	-
9	N	CH_2	$PO(OCH_3)$	Ph	17	-	-
10	N	CH_2	$PO(CH_3)$	Ph	17	-	-
11	N	CH_2	CONH	Ph	84	79	1.18 ± 0.15
12	N	CH_2	CSNH	Ph	57	32	-
13	N	CH_2	CONH	C_4H_9	68	42	-
14	N	CH_2	CONH	C_8H_{17}	34	-	-
15	N	CH_2	CONH	$p$$NO_2$Ph	33	-	-
39	CH	CH_2	CO	Ph	93	88	0.94 ± 0.11
42	N	CO	CO	Ph	26	-	-

Substitutions on the fluorene have already been performed and revealed that, generally, halogen and nitro substituents and even some bulky groups were well tolerated concerning the InhA inhibitory activity.[8] We subsequently investigated compounds bearing fluorene substituted with alkyl chains on either C-2- or C-3 position to mimic the substrate and we introduced for some of them, an ether linker. The biological activities against the InhA protein are reported in Table 2.

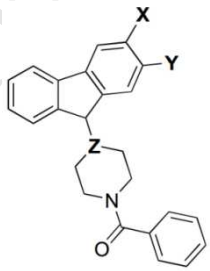
Hexyl and octyl chains were subsequently attached on the C-2 position of fluorene and the corresponding derivatives revealed around 50% inhibition at 50 µM. However, no enhancement was observed compared to the reference compound (compounds **28** and **29**, Table 2). A shorter chain seems to be privileged in this position. Lately, compound **27** holding a similar 6-carbons length alkyl chain on the C-3 position of the fluorene revealed a real gain of activity compared to compounds **28** and **30** at the C-2 position. Ether-linked derivatives have been particularly studied. First results on the C-3 position of the fluorene revealed a significant improvement of the activity with the increasing length of the alkyl chain (compounds **31**-**33**, Table 2). The rank order of InhA inhibitory potency was **31** (OC_1) < **32**

(OC_3) < **33** (OC_6). The hexyloxy derivative **33** exhibited an IC_{50} of 0.20 µM, about 2.5 times lower than reference compound **1**. The additional alkyl chain partly mimicking the substrate would enhance the activity of the molecule and would probably ensure constructive hydrophobic interactions within the active site. The C3-alkyl chain substitution attached *via* an ether linkage was also investigated at position 2 of the fluorene. No significant difference was observed concerning the substituted position for ether-linked derivatives with such a carbon length (compounds **32** and **34**, Table 2). With respectively IC_{50} of 1.00 and 1.20 µM, compounds substituted in positions C-2- and C-3- appear equivalent as inhibitors.

In order to explore the whole cavity and to strengthen hydrophobic interactions with the substrate binding loop, a benzyl substituent was covalently attached through an ether linker to the C-3 position of the fluorene. Enzymatic assays also revealed good activity with an IC_{50} estimated at 1.0 µM (compound **35** Table 2).

Then compound **40** bearing both a piperidine central core and a 3-hexyloxy substituent, was tested. Compared to the unsubstituted derivative **39**, no difference of activity was observed (compounds **39** Table 1 and **40** Table 2). Both compounds exhibited IC_{50} in the micromolar range. Nevertheless, it is important to note that compound **40** is in a racemic mixture due to the asymmetric C-9 on the fluorene moiety. We can subsequently envision that only one of the two enantiomers is biologically active.

Table 2. Inhibitory potencies of compounds **28**-**35**, **40** against InhA



Compound	X	Y	Z	InhA inhibition (%)		IC_{50} (µM)
				50 µM	10 µM	
GEQ	-	-	-	87	81	0.86 ± 0.16
1	H	H	N	94	94	0.49 ± 0.06
28	H	C_6H_{13}	N	48	45	-
29	H	C_8H_{17}	N	54	27	-
30	C_6H_{13}	H	N	60*****	52	≈ 1.0*
31	OCH_3	H	N	92	74	2.69 ± 0.20
32	OC_3H_7	H	N	91	84	1.01 ± 0.14
33	OC_6H_{13}	H	N	79*	70	**0.20 ± 0.04**
34	H	OC_3H_7	N	88	83	1.30 ± 0.10
35	OCH_2Ph	H	N	63*	57*	≈ 1.0*
40	OC_6H_{13}	H	CH	64*	71	1.05 ± 0.19

* Due to solubility problem. IC_{50} was estimated by direct inhibition measurement to obtain about 50% inhibition

2.4. Enantiomeric separation by chiral supercritical fluid chromatography

Each substituted fluorene derivatives present an asymmetric carbon at the 9-position of the fluorene. Consequently, two compounds bearing two different alkyl chains, namely **31** and the more potent **33** were engaged into enantiomeric separation to analyze the contribution of both enantiomers according to their activity against the InhA protein. Chiral chromatography was performed on a Supercritical Fluid Chromatography (SFC) equipment (Prep80Q from Waters) with the AD-H column (from Chiral Technologies) and methanol as co-solvent. Compounds **33a** (rt = 10.4 min)/**33b** (rt = 16.4 min) and **31a** (rt = 10.5 min)/**31b** (rt = 12.6 min) were obtained with an excellent enantiopurity (> 98.5%). However, none of the isolates were able to crystallize, so the absolute configuration of the chiral carbon was not determined.

2.5. Biological activities for enantiopure compounds against the InhA protein

First enzymatic assays were performed for compounds **31a/31b** and **33a/33b** respectively at 5 µM and 250 nM to evaluate their inhibitory activity against InhA protein. IC_{50} was measured for the most potent molecules (Table 3). For the methoxy derivative **31**, the racemic mixture exhibits 60% inhibitory activity at 5 µM. While the first isolated enantiomer (rt = 10.5 min) did not inhibit InhA activity (compound **31a** Table 3), the second one (rt = 12.6 min, compound **31b**, Table 3) was much more potent (IC_{50} estimated at 2.07 µM) and was responsible for the overall activity.

The most interesting result was observed for the hexyloxy derivative. Indeed, the first enantiomer **33a** was not successfully efficient on InhA inhibitory activity whereas the other one **33b** inhibited at 71%. Consequently, IC_{50} of 102 nM was measured for compound **33b**, which displays the best inhibitory activity for this series of molecules.

Table 3 inhibitory activities of compounds **31a**, **31b**, **33a** and **33b** after enantiomeric separation

Compound	X	Y	Z	Ligand concentration	InhA Inhibition (%)	IC_{50} (µM)
GEQ	-	-	-	-	-	0.86 ± 0.16
1	H	H	N	-	-	0.49 ± 0.06
31	OCH_3	H	N	5 µM	60	2.69 ± 0.20
31a	OCH_3	H	N	5 µM	11	-
31b	OCH_3	H	N	5 µM	68	2.07 ± 0.29
33	OC_6H_{13}	H	N	250 nM	53	0.20 ± 0.04
33a	OC_6H_{13}	H	N	250 nM	0	-
33b	OC_6H_{13}	H	N	250 nM	71	**0.102 ± 0.004**

2.6. Computational study

Molecular docking was performed in an attempt to determine the interaction network between the newly synthesized compounds and the receptor, the InhA protein. To model the binding mode of these compounds, we performed a docking study with the InhA protein that was crystallized with **GEQ** ligand (PDB ID: 1P44, chain a) using Molegro Virtual Docker (MVD). For compounds (**7**, **10**, **28**, **29**, **30**, **31**, **32**, **33**, **34**, **35**, **39**, **40**) bearing an asymmetric carbon atom, each enantiomer was docked individually. The final (filtered) results were expressed as ligand efficiency (LE) indices [31] rather than RAW docking scores. The docking and filtering procedures gives at least two LE descriptor values per compound: two values for the same molecule (if not asymmetric) or one value per enantiomer. These results are given in the Table S1 of supplementary materials. In order to compare experimental results and theoretical LE values, the InhA inhibition percent at 50 µM (PI50) was used, PI50 values being available for each compound.

In the case of InhA, the protein is characterized by *i)* a wide and flexible binding site; *ii)* some structural transitions giving opening/closing of a minor and major portal; *iii)* a cofactor (NAD^+/NADH) to be taken into account in calculations. These elements (not exhaustive) reduce the production of correlations between descriptors issued directly (not using a QSAR approach) from docking studies *vs.* experimental data, even for derivatives that keep an essential feature (i.e. fluorene group) of a single compound.

According to this context, only a coarse-grained approach seems to be relevant for the analysis of this **GEQ**-focused ligand collection. The PI50 results were classified in 3 groups: *group1* for compounds giving upper than 75% of inhibitory activity; *group3* for compounds that exhibits PI50 values lesser than 30% and *group2* for intermediate compounds and the docking results were analyzed using this classification. Then, these tree classes were confronted to LE descriptors, and we found that *group1* was clearly related to best scores and *group3* to worst scores (Figure S1 of supplementary materials). This result is limited but interesting because it gives insights to improve post-docking filter results. Especially since we found that the calculated values of LogP (octanol/water) for each compound do not seem to be correlated with LE descriptors.

Then, analysis of selected poses after docking study allowed us to understand the biological activities of our compounds regarding their structure.

Sulfinamide derivative **7** showed poor inhibitory activity against InhA (PI50 = 14%) and docking study revealed, for compound with *S* configuration, a disfavored orientation (figure

4A) as well as its enantiomer *R*. To maintain the hydrogen bond with the Tyr158 residue, the sulfinamide tetrahedral linker forced the piperazine ring to shift 1.1 Å which induced the fluorene group to rotate 110° and to clash with Met199 residue. For thioamide derivative **6**, the overall binding mode is quite similar to the reference molecule **1**, even if its enzymatic activity at 50 µM was evaluated at 31%. Indeed, for thioamide **6**, the C-S bond is longer and the sulfur atom bigger than the corresponding carbonyl in GEQ which induced steric hindrance with the Tyr158 side chain (Figure 4B).

Compounds bearing an alkyl chain on the fluorene moiety (**28**, **29** and **30**) demonstrated a modest activity on the InhA protein up to IC_{50} (InhA) value of 1.0 µM. Their corresponding *R* enantiomers displayed a consistent orientation within the InhA binding site wherein the additional alkyl chain is oriented onto the major portal mimicking, as predicted, the substrate analogue. Interestingly, in the case of compound **32**, bearing a 3-propoxy substituent on the fluorene moiety, both enantiomers disclose a consistent orientation. Indeed, similar to the latter compounds, the *R* enantiomer orientated the alkyl chain within the hydrophobic substrate cavity surrounding with Met103, Ile202 and Leu207 residue (Figure 4C). On the other hand, the *S* enantiomer oriented the alkyl chain upon the minor portal with close interaction with hydrophobic residues Met155, Leu218 and Trp222 (Figure 4D).

For benzyloxy derivative **35**, the best docking pose allows us to envisage the possibility of binding only for compound with configuration *R* (Figure 4E) with the benzyl moiety sandwiched between Met103 and Ile202 residues. Nonetheless, the binding site of limited size seems to disfavor such interaction which leads consequently to an increase of docking score values and correlates with the lower activity compared to the reference molecule **1**.

Concerning the best molecule **33**, only the *R*-enantiomer compound displayed a consistent orientation within the InhA binding site wherein the additional alkyl chain is oriented onto the major portal (Figure 4F). This result supports the biological activity found for only enantiomer **33b**. Even though the geometry of the enantiopure compound **33b** was not determined, we can suggest, with caution, that C-9 carbon atom is of *R* configuration.

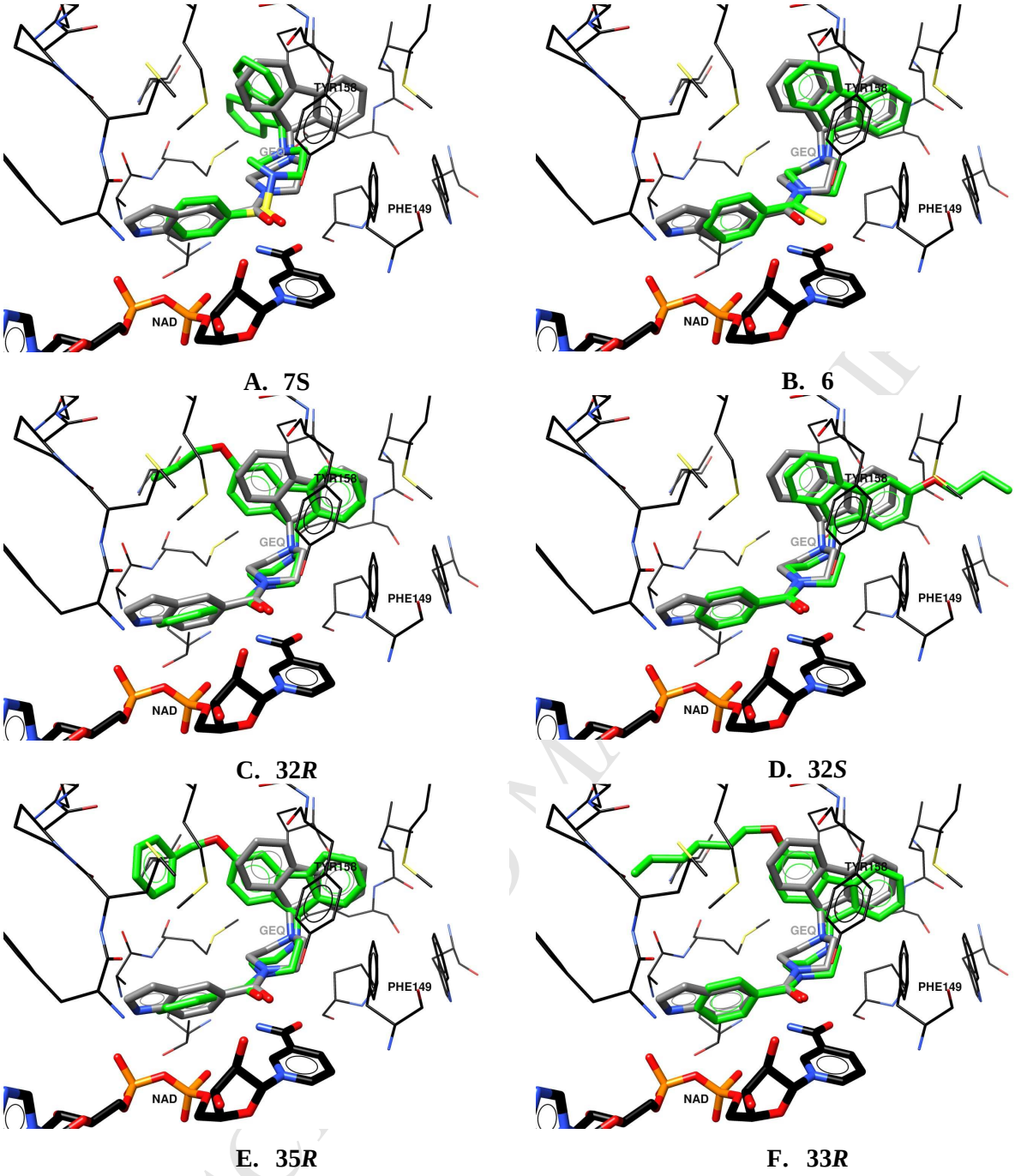


Figure 4. Selected docking conformations of compounds **7** (**A.**), **6** (**B.**), **32** (**C.** with *R* configuration and **D.** with *S* configuration), **35** (**E.** *R*-enantiomer) **and 33** (**F.** *R*-enantiomer). The best pose for each compound (**green**) was depicted in the InhA binding site, as well NADH cofactor molecule (**black**), GEQ (**grey**) for comparison and flexible residues as defined in the experimental part.

2.7. Inhibition of Mycobacterium tuberculosis (H37Rv) growth

The activities of the synthesized compounds were evaluated by measuring the inhibition of *M. tuberculosis* (H37Rv) growth. Isoniazid and **GEQ** molecules were used as control. Results are reported in Table 4.

Table 4 : MIC determination for *M. tuberculosis* H37Rv strain

Compound	MIC (µM)	Compound	MIC (µM)
GEQ	50.1	**INH**	0.4
1	14.1	**29**	> 21.4
3	> 39.9	**30**	22.8
4	29.9	**31**	26.0
5	12.8	**31a**	> 26.0
6	13.5	**31b**	> 26.0
7	> 26.7	**32**	24.2
8	> 25.6	**33**	22.0
9	> 24.7	**33a**	>22.0
10	> 25.7	**33b**	11.0
11	> 27.1	**34**	> 24.2
12	> 25.9	**35**	> 21.7
13	14.3	**39**	28.3
14	> 24.7	**40**	88.2
15	12.1	**42**	> 27.1
28	22.8		

GEQ molecule did not display any substantial inhibitory activity against *M. tuberculosis* growth (MIC > 40 µM). Consistent with this, GEQ molecule has been evaluated against *M. tuberculosis* growth through CFU counting method and using the Alamar Blue Assay, giving an MIC value above 125 µM.[8] Furthermore, we measured a MIC value of 14.1 µM for the benzoyl derivative **1**, while He *et al.* reported an MIC above 125 µM.[9]

The majority of the compounds displayed better activities than GEQ. The first family of compounds related to central core modifications revealed poor MIC values for piperidine derivative **39** and piperazinone derivative **42** as well (MIC values: **39**, 28.3 µM; **42**, > 27.1 µM).

As shown in Table 4, investigation around the amide bond showed modest antimycobacterial activity. The thioamide analogue **6** displayed MIC in a similar range (13.5 µM) as compound **1**. The sulfin-, sulfon-, phosphin- and phosphonamide derivatives (compounds **7**-**10**) presented MICs values above 25.0 µM. The urea and thiourea derivatives **11** and **12** did not demonstrate better activities against *M. tuberculosis* growth. Interestingly, compound **15** bearing an additional nitro group on the phenyl ring exhibited MIC value in a similar range as compound **1** (MIC = 12.1 µM).

All the derivatives possessing an alkyl chain instead of the benzoyl moiety showed MIC values ranged from 12.8 to above 24.9 µM; among them, compound **5** was the most active (MIC = 12.8 µM).

The class of racemic compounds bearing an alkyl chain on the fluorene moiety (**28**-**35**) displayed weak *in vitro* activities against *M. tuberculosis* with MIC values superior to 20 µM. In addition, the racemic mixture **33** exhibited MIC value of 22.0 µM. Interestingly, the corresponding enantiopure compound **33b**, showing the best inhibition of InhA enzymatic activity and the lowest MIC (11.0 µM). Interestingly, the other enantiomer showed a MIC superior to 22.0 µM. These results eventually suggest a binding specificity of this enantiomer as observed with InhA binding assays.

2.8. Biological activity in the presence of efflux pump inhibitors

In some bacteria, such as in *M. tuberculosis*, efflux pumps have been described as a possible mechanism for intrinsic and acquired drug resistance.[32] While He *et al.* suggested the possibility that GEQ compounds are actively extruded out of the bacterial cell by efflux, no studies have been performed to confirm this resistance mechanism.[9] In order to elucidate the role of the efflux pumps in the poor effectiveness of the **GEQ** compound and its derivatives **1**, **33b** and **40**, their activity was determined in the presence of already known efflux inhibitors (reserpine, verapamil or carbonyl cyanide *m*-chlorophenylhydrazone (CCCP)). Reserpine is a calcium channel blocker and plant alkaloid that inhibits P-glycoprotein in eukaryotic cells.[33] Moreover, reserpine reduced resistance to isoniazid in some *M. tuberculosis* strains.[34] Verapamil is a calcium channel blocker that inhibits P-glycoprotein and also several bacterial ABC efflux pumps. CCCP is uncoupler of the proton motive force that inhibits the efflux of several drugs[35], but it is described as a substrate of some efflux pumps.[36] The efflux pump inhibitors, at the used concentration, did not inhibit *M. tuberculosis* growth. Results are reported in Table 5.

Table 5 : MIC determination for some compounds against *M. tuberculosis* H37Rv in the presence of efflux pump inhibitors

	Efflux pump inhibitors[a]			
	Ø	Reserpine	Verapamil	CCCP
Compounds	MIC (µM)			
GEQ	50.1	25.4	< 12.7	< 12.7
1	14.1	56.5	56.5	< 3.5
33b	11.0	< 2.7	11.0	< 2.7

40	88.2	22.1	44.1	nd

[a] Reserpine, verapamil and CCCP were respectively added at 3.0, 40.0 and 7.5 µg/mL final concentration.
[b] nd for not determined

The activity of **GEQ** compound increased more than four-fold in combination with verapamil and CCCP, underlying the possibility that some transporter could extrude it out of the cells, in agreement with its poor activity.

CCCP increased the effectiveness of the *N*-benzoyl derivative **1** more than five-fold. Reserpine and verapamil decreased the sensitivity to this compound at the used concentration; it could be hypothesized that these combination influenced either the permeability of the compound or its binding with the target.

The efflux inhibitor verapamil did not have effect with our best derivative **33b**, whilst in combination with the other two efflux inhibitors its activity was increased more than four-fold. Interestingly, the same behaviour was observed with MmpL7 transporter that pumps out of the cell, isoniazid. [37] It could be hypothesized that the same transporter effluxes compound **33b** because both molecules have the same target. In the case of compound **40** with a piperidine moiety, MICs improved four-fold in the presence of reserpine and two-fold with verapamil.

These results confirmed the possibility that **GEQ** and its derivatives could be effluxed out of the mycobacterial cell by some efflux pump. The chemical diversity on the fluorene moiety should be enlarged in order to obtain compounds bearing different substitutions to either improve uptake by mycobacteria or to avoid efflux pumps.

3. Conclusion

This work describes the synthesis and the evaluation of twenty five **GEQ** analogues. All these compounds were evaluated for the inhibition of InhA enzymatic activity and against *Mycobacterium tuberculosis* growth. Thus, we observed that the *N*-benzoyl-piperazine central core is of key importance to ensure good inhibition of InhA enzymatic activity. The majority of the compounds displayed higher activities than **GEQ** against *M. tuberculosis* growth.

Among them, compound **33b** bearing an additional hexyloxy chain on the fluorene moiety displayed improved activity against both InhA enzyme (IC_{50} up to 102 nM) and *M. tuberculosis* growth (MIC = 11 µM). In addition, its activity improved in combination of

some efflux inhibitors. These results suggest that the poor biological activity against *M. tuberculosis* of **GEQ** and its derivatives could depend on the efflux of these molecules by some mycobacterial transporters.

Further work will focus on optimization of drug uptake with the aim of producing a candidate series for the treatment of tuberculosis. Moreover, the research for more effective efflux inhibitors that could be used in combination with conventional antibiotics could be another challenge to pursue.

4. Experimental section

4.1. General condition

All chemicals were obtained from Aldrich-Sigma or Acros Organics and used without further purification. Anhydrous solvents were freshly distilled before use or were obtained from the M.Braun Solvent Purification System (MB-SPS-800). Optical rotations were measured using a sodium D line on a P-2000 series Jasco, PTC-262 polarimeter. The melting points were determined on a Mettler Toledo MP50 melting point system and are uncorrected. Infra-red spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR Spectrometer. ^{1}H NMR spectra were recorded on a Bruker spectrometer at 300 MHz using $CDCl_3$, DMSO-d6 or CD_3OD as the solvent. For ^{1}H NMR the residual proton signal of the deuterated solvent was used as an internal reference: $CDCl_3$ δ = 7.29 ppm, DMSO δ = 2.50 ppm and CD_3OD δ = 3.31 ppm. ^{13}C NMR spectra were recorded on a Bruker spectrometer at 75 MHz. Mass spectra (DCI/NH_3) were obtained on a DSQ Thermo Fisher Scientific. For the MS–ESI a Dionex ultimate 3000 UPLC system with a ABSciex Q TRAP 4500 was used. High-resolution mass spectra (HRMS) were recorded on a UPLC Xevo G2 Q-TOF Waters using electrospray ionization methods. The desired product was purified by flash column chromatography with puriFlash 430 system using puriFlash® columns from Interchim. The purity of title compounds was evaluated by reverse phase LC-MS on a UPLC Acquity system (from Waters) equipped with a photodiode array detector and a simple quadripole detector. The Acquity CSH C18 1.7 µm (2.1 mm × 50 mm) column was used as a stationary phase. MilliQ water (with 0.02% HCOOH) and acetonitrile (with 0.02% HCOOH) were respectively employed as solvents A and B with a flow rate of 0.6 mL/min. Purity was estimated at λ = 212 nm and two elution methods were followed and will be mentioned in each case. For the method 1, gradient was 5 min run from 2% to 98% B and then returned to initial conditions. For method 2, the gradient was 10% B during 1 min and the percentage of B went from 10 to 30 for 1 to 10 min, and the

percentage of B reached 100% 10 to 12 minutes and then returned to initial conditions. The enzymatic evaluation was performed on a Cary Bio 100.

4.2. Chemistry

*4.2.1. 1-(9H-Fluoren-9-yl)piperazine (**3**).* A solution of 9-bromofluorene **2** (8.2 mmol, 2.0 g, 1.0 eq), triethylamine (1.2 mmol, 165 µL, 0.15 eq) and piperazine (61.1 mmol, 5.27 g, 7 eq) in dry THF (50.0 mL) was refluxed under argon for 6 hours. The reaction mixture was then filtered off and the filtrate was concentrated under vacuum pressure. The resulting crude product was purified by flash chromatography (isocratic 95/5 dichloromethane/methanol) to afford a white powder (2.04 g, 100%). TLC R_f: 0.12 (dichloromethane/methanol 97/3); mp: 133.6 °C; IR (cm^{-1}): 738, 805, 1007, 1138, 1325, 1448, 2831, 2940, 3294. ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.46 (m, 4H); 2.66 (m, 4H); 4.86 (s, 1H); 7.32 (td, *J* = 1.5 Hz, 3.5 Hz, 2H); 7.40 (t, *J* = 7.5 Hz, 2H); 7.63 (d, *J* = 7.2 Hz, 2H), 7.83 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d6) δ (ppm): 46.7 (2 x CH_2), 50.4 (2 x CH_2), 70.2 (CH), 120.4 (2 x CH), 126.3 (2 x CH), 127.5 (2 x CH), 128.5 (2 x CH), 140.9 (2 x C), 144.4 (2 x C). MS (ESI) m/z: 251.3 [M +H^+]. HRMS (ESI): for $C_{17}H_{19}N_2$ [M+H^+]: calcd: 251.1551; found: 251.1548.

4.2.2. **(***4-(9H-Fluoren-9-yl)piperazin-1-yl)(1H-indol-5-yl)methanone (**GEQ**).* In a round bottom flask submerged in a bath of ice were added indole-5-carboxylic acid (0.80 mmol, 129 mg, 1.0 eq), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride, EDC.HCl, (0.88 mmol, 168 mg, 1.1 eq) and 1-hydroxybenzotriazole, HOBt, (0.88 mmol, 119 mg, 1.1 eq) in DMF (5.0 mL). 1-(9*H*-fluoren-9-yl)piperazine (0.80 mmol, 200 mg, 1.0 eq) and DIPEA (2.00 mmol, 330 µL, 2.5 eq) were subjoined to the reaction mixture and were stirred overnight at room temperature. HCl 1N was added and the aqueous phase was neutralized with KOH 2 M and the product was extracted with ethyl acetate (3×). The organic phase was dried over magnesium sulphate, filtered and concentrated under vacuum pressure. The resulting crude product was purified by flash chromatography (gradient 80/20 to 20/80 petroleum ether/ethyl acetate) to afford a colorless solid (154 mg, 49%). HPLC: method 1, rt = 2.45 min, purity 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.30 – 3.05 (bs, 4 H); 3.35 – 3.45 (bs, 4H); 4.92 (s, 1 H); 6.51 (t, *J* = 2.4 Hz, 1 H); 7.16 (td, *J* = 1.5 Hz, 4.8 Hz, 1 H); 7.20 (t, *J* = 8.7 Hz, 2 H); 7.34 (td, *J* = 1.2 Hz, 7.2 Hz, 2 H); 7.42 (t, *J* = 7.2 Hz, 2 H); 7.64 – 7.70 (m, 3 H); 7.72 (d, *J* = 7.5 Hz, 2 H); 9.13 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 49.2 (broad, 4 X CH_2); 69.9 (CH); 102.8 (CH); 111.2 (CH); 119.9 (2 x CH); 120.1 (CH); 121.1 (CH); 125.7 (CH); 126.0 (2 x CH); 126.6 (C); 127.2 (2 x CH); 128.4 (2 x CH); 136.5 (C); 141.1 (C); 143.3 (C); 172.2 (C). MS (DCI/CH_4) m/z: 394.19 [M+H^+]. HRMS (DCI/CH_4): for $C_{26}H_{24}N_3O$ [M+H^+]: calcd: 394.1919; found: 394.1912.

*4.2.3. General procedure for **1**, **4**, **5**, **8**, **9** and **10**.*

1-(9*H*-Fluoren-9-yl)piperazine **3** (1.0 eq) was dissolved in dry dichloromethane. Triethylamine (1.2 eq) was added and the reaction mixture was stirred at room temperature for 10 minutes. Benzoyl chloride (1.1 eq) was slowly added to reaction mixture previously cooled with an ice bath. After complete addition the reaction mixture was stirred overnight at room temperature. A saturated aqueous solution of sodium hydrogenocarbonate was added and the product was extracted with dichloromethane (3×). The organic phase was washed with a large amount of water, dried over magnesium sulphate, filtered and concentrated under vacuum pressure. The resulting crude product was purified by flash chromatography as indicated in each case to afford the desired compound.

*4.2.3.1. (4-(9H-Fluoren-9-yl)piperazin-1-yl)(phenyl)methanone (**1**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.40 mmol, 100 mg), triethylamine (0.48 mmol, 65 µL) and benzoyl chloride (0.44 mmol, 51 µL). The crude product was purified

by flash chromatography (isocratic 50/50 petroleum ether/ethyl acetate) to afford a white solid (102 mg, 72%). TLC R_f: 0.82 (dichloromethane/methanol 97/3); mp: 186 °C; IR (cm^{-1}): 709, 741, 1005, 1275, 1426, 1636, 2826, 2880, 2906. HPLC: method 1, rt = 2.72 min, purity 99%. ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.55 (m, 4H); 3.59 (m, 4H); 5.00 (s, 1H); 7.31-7.48 (m, 9 H); 7.65 (d, *J* = 7.2 Hz, 2H), 7.84 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d6) δ (ppm): 48.6 (2 x CH_2), 49.4 (2 x CH_2), 69.6 (CH), 120.5 (2 x CH); 126.3 (2 x CH); 127.4 (2 x CH); 127.7 (2 x CH); 128.7 (2 x CH); 128.8 (2 x CH); 129.9 (CH); 136.3 (C); 140.9 (2 x C); 143.8 (2 x C); 169.4 (C). MS (ESI) m/z: 355.2 [M+H$^+$]. 377.2 [M+Na]. HRMS (ESI): for $C_{24}H_{23}N_2O$ [M+H$^+$]: calcd: 355.1812; found: 355.1810.

*4.2.3.2. 1-(4-(9H-Fluoren-9-yl)piperazin-1-yl)pentan-1-one (**4**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.40 mmol, 100 mg), triethylamine (0.48 mmol, 65 µL) and valeryl chloride (0.48 mmol, 65 µL). The crude product was purified by flash chromatography (isocratic 80/20 petroleum ether/ethyl acetate) to afford a white solid (112 mg, 84%). TLC R_f: 0.32 (dichloromethane/methanol 95/5); mp: 70 °C; IR (cm^{-1}): 672, 741, 999, 1137, 1196, 1203, 1219, 1447, 1637, 2817, 2868, 2927. HPLC: method 2, rt = 2.10 min, purity 96%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.93 (t, *J* = 7.2 Hz, 3 H); 1.33 (sex, *J* = 7.5 Hz, 2 H); 1.60 (quin, *J* = 7.5 Hz, 2 H); 2.28 (dd, *J* = 6.6 Hz, 8.4 Hz, 2 H); 2.49 (t, *J* = 4.8 Hz, 2 H); 2.78 (t, *J* = 4.8 Hz, 2 H); 3.41 (t, *J* = 4.8 Hz, 2 H); 3.66 (t, *J* = 4.8 Hz, 2 H); 4.90 (s, 1 H); 7.32 (td, *J* = 1.2 Hz Hz, 7.2 Hz, 2 H); 7.41 (t, *J* = 7.5 Hz, 2 H); 7.64 (d, *J* = 7.5 Hz, 2 H); 7.72 (d, *J* = 7.8 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 13.9 (CH_3); 22.6 (CH_2); 27.4 (CH_2); 33.0 (CH_2); 42.3 (CH_2); 46.3 (CH_2); 48.4 (CH_2); 49.7 (CH_2); 69.9 (CH); 119.8 (2 x CH); 125.9 (2 x CH); 127.2 (2 x CH); 128.3 (2 x CH); 141.1 (2 x C); 143.5 (2 x C); 171.7 (C). MS (DCI/CH_4) m/z: 363.24 [M+$C_2H_5^+$], 335.21[M+H$^+$], 165.07 [M-169]. HRMS (DCI/CH_4): for $C_{22}H_{27}N_2O$ [M+H$^+$]: calcd: 335.2123; found: 335.2115.

*4.2.3.3. 1-(4-(9H-Fluoren-9-yl)piperazin-1-yl)nonan-1-one (**5**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.40 mmol, 100 mg), triethylamine (0.48 mmol, 65 µL) and nonanoyl chloride (0.48 mmol, 86 µL). The crude product was purified by flash chromatography (isocratic 80/20 petroleum ether/ethyl acetate) to afford a white solid (114 mg, 73%). TLC R_f: 0.62 (dichloromethane/methanol 95/5); mp: 88 °C; IR (cm^{-1}): 673, 740, 1001, 1137, 1196, 1228, 1320, 1448, 1639, 2818, 2851, 2919. HPLC: method 2, rt = 5.04 min, purity 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.90 (t, *J* = 7.2 Hz, 3 H); 1.20 – 1.42 (m, 10 H); 1.35 – 1.67 (m, 2 H); 2.28 (t, *J* = 7.2 Hz, 2 H); 2.49 (t, *J* = 4.8 Hz, 2 H); 2.77 (t, *J* = 4.8 Hz, 2 H); 3.41 (t, *J* = 4.8 Hz, 2 H); 3.66 (t, *J* = 4.8 Hz, 2 H); 4.90 (s, 1 H); 7.32 (td, *J* = 1.5 Hz, 7.5 Hz, 2 H); 7.41 (t, *J* = 7.5 Hz, 2 H); 7.64 (d, *J* = 7.5 Hz, 2 H); 7.72 (d, *J* = 7.2 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 25.4 (CH_2); 29.2 (CH_2); 29.4 (CH_2); 29.5 (CH_2); 31.8 (CH_2); 33.3 (CH_2); 70.0 (CH); 119.8 (2 x CH); 125.9 (2 x CH); 127.1 (2 x CH); 128.3 (2 x CH); 141.1 (2 x C); 143.5 (2 x C); 171.7 (C). MS (DCI/CH_4) m/z: 419.31 [M+$C_2H_5^+$], 391.27 [M+H$^+$], 165.07 [M-225]. HRMS (DCI/CH_4): for $C_{26}H_{35}N_2O$ [M+H$^+$]: calcd: 391.2749; found: 391.2751.

*4.2.3.4. 1-(9H-Fluoren-9-yl)-4-(phenylsulfonyl)piperazine (**8**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.32 mmol, 80 mg), triethylamine (0.38 mmol, 52 µL) and benzene sulfonyl chloride (0.38 mmol, 49 µL). The crude product was purified by flash chromatography (isocratic 85/15 petroleum ether/ethyl acetate) to afford a white solid (115 mg, 92%), TLC R_f: 0.39 (petroleum ether/ethyl acetate 80/20); mp: 161 °C; IR (cm^{-1}): 575, 646, 669, 690, 736, 939, 1001, 1113, 1128, 1171, 1292, 1320, 1348, 1447, 2836, 2887, 2942. HPLC: method 1, rt = 3.66 min, purity 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.68-2.78 (m, 4 H); 2.99-3.10 (m, 4 H); 4.82 (s, 1 H); 7.28 (td, *J* = 1.2 Hz, 7.5 Hz, 2 H); 7.40 (t, *J* = 7.5 Hz, 2 H); 7.51-7.70 (m, 5 H); 7.71 (d, *J* = 7.5 Hz, 2 H); 7.75-7.81 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 46.8 (2 x CH_2); 48.1 (2 x CH_2); 69.8 (CH); 119.9 (2 x CH); 125.8 (2 x CH); 127.2 (2 x CH); 127.8 (2 x CH); 128.4 (2 x CH); 129.1 (2 x CH); 132.8 (CH); 136.0 (C); 141.0 (2 x C); 143.2 (2 x C). MS (DCI/CH_4) m/z: 391.15 [M+H$^+$], 249.14 [M-141 ($PhSO_2$)], 165.07 [M-225 (fluorene)]. HRMS (DCI/CH_4): $C_{23}H_{23}N_2O_2S$ [M+H$^+$]: calcd: 391.1480; found: 391.1491.

*4.2.3.5. (4-(9H-Fluoren-9-yl)piperazin-1-yl)(phenyl)phosphinate (**10**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.80 mmol, 100 mg), triethylamine (0.80 mmol, 188 µL) and phenylphosphinic dichloride (0.96 mmol, 132 µL). Tetrazole (0.04 mmol, 3 mg) was also put in the reaction mixture and methanol (1.0 mL) was added after overnight stirring to quench the

reaction and formed the desired product. The crude product was purified by flash chromatography (isocratic 95/5 petroleum ether/ethyl acetate) to afford a yellow oil (92 mg, 57%). TLC R_f: 0.75 (dichloromethane/MeOH 90/10). IR (cm^{-1}): 551, 561, 643, 669, 695, 730, 798, 910, 968, 999, 1034, 1131, 1231, 1300, 1438, 1450, 1675, 1715, 2845, 2945, 3060. HPLC: method 1, rt = 2.23 min, purity 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.61 (t, *J* = 5.1 Hz, 4 H); 3.09 - 3.18 (m, 4 H); 3.76 (d, *J* = 11.1 Hz, 3 H); 4.83 (s, 1 H); 7.30 (t, *J* = 7.2 Hz, 2 H); 7.40 (t, *J* = 7.5 Hz, 2 H); 7.42 – 7.56 (m, 3 H); 7.56 – 7.63 (m, 2 H); 7.67 – 7.78 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 44.8 (d, *J* = 2.2 Hz, 2 x CH_2); 49.3 (d, *J* = 5.8 Hz, 2 x CH_2); 51.0 (d, *J* = 6.1 Hz, CH_3); 70.3 (CH); 119.7 (CH); 119.8 (CH); 125.8 (CH); 125.9 (CH); 127.0 (CH); 127.1 (CH); 128.2 (2 x CH); 128.4 (d, *J* = 14.1 Hz, 2 CH); 130.2 (d, *J* = 172.4 Hz, C); 131.3 (d, *J* = 9.4 Hz, 2 x CH); 131.7 (d, *J* = 3.0 Hz, CH); 141.0 (C); 141.1 (C); 143.6 (C); 143.7 (C). ^{31}P{H} NMR (121.5 MHz, $CDCl_3$): 23.0 ppm. ^{31}P{H} NMR (121.5 MHz, $CDCl_3$): 23.0 ppm. MS (DCI/CH_4) m/z: 405.176 [M+H$^+$], 239.10 [M-165], 165.07 [M-239]. HRMS (DCI/CH_4): for $C_{24}H_{26}N_2O_2P$ [M+H$^+$]: calcd: 405.1732; found: 405.1730.

*4.2.4. (4-(9H-Fluoren-9-yl)piperazin-1-yl)(phenyl)methanethione (**6**).* A mixture of (4-(9*H*-fluoren-9-yl)piperazin-1-yl)(phenyl)methanone **1** (0.28 mmol, 100 mg, 1.0 eq) and Lawesson's reagent (0.14 mmol, 57 mg, 0.5 eq) in 10 mL of toluene was refluxed for 8 hours. Another half equivalent of Lawesson's reagent (0.5 eq) was added and reaction mixture was refluxed overnight. Then, the solvent was removed under vacuum pressure and the residue was dissolved in dichloromethane. The solution was washed with 8% aqueous sodium hydrogenocarbonate and was dried over magnesium sulphate. The solvent was removed under reduced pressure and the residue flash chromatographed (100% dichloromethane) to afford a yellow solid (77 mg, 74%). TLC R_f: 0.60 (petroleum ether/ethyl acetate 80/20); mp: 204 °C; IR (cm^{-1}): 618, 670, 700, 738, 762, 919, 928, 932, 1003, 1040, 1140, 1224, 1254, 1290, 1435, 1469, 1488, 2755, 2880. HPLC: method 1, rt = 3.87 min, purity 97%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.46 (t, *J* = 5.4 Hz, 2 H); 3.02 (t, *J* = 5.4 Hz, 2 H); 3.53 (t, *J* = 5.4 Hz, 2 H); 4.49 (t, *J* = 5.4 Hz, 2 H); 4.94 (s, 1 H); 7.22 – 7.28 (m, 2 H); 7.29 – 7.33 (m, 3 H); 7.34 (td, *J* = 1.5 Hz, 7.5 Hz, 2 H); 7.43 (t, *J* = 7.2 Hz, 2 H); 7.65 (d, *J* = 7.2 Hz, 2 H); 7.72 (d, *J* = 7.5 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 48.3 (CH_2); 49.3 (CH_2); 50.1 (CH_2); 52.7 (CH_2); 69.5 (CH); 119.9 (2 x CH); 125.8 (2 x CH); 125.9 (2 x CH); 127.3 (2 x CH); 128.4 (2 x CH); 128.5 (2 x CH); 128.6 (CH); 141.1 (2 x C); 142.9 (2 x C); 143.1 (C); 200.4 (C). MS (DCI/CH_4) m/z: 399.19 [M+Na], 371.16 [M+H$^+$], 165.01 [fluorene]. HRMS (DCI/CH_4): for $C_{24}H_{23}N_2S$ [M+H$^+$]: calcd: 371.1582; found: 371.1567.

*4.2.5. 1-(9H-Fluoren-9-yl)-4-(phenylsulfinyl)piperazine (**7**).* To a suspension of benzenesulfinic acid sodium salt (0.86 mmol, 158 mg, 1 eq) in anhydrous dichloromethane (5.0 mL), was added dropwise thionyl chloride (1.15 mmol, 83 µL, 1.2 eq) at 4 °C. After the addition was finished, stirring was continued for an additional 2 hours at room temperature. The mixture obtained was then directly added into a cooled solution of 1-(9*H*-fluoren-9-yl)piperazine **3** (7.99 mmol, 200 mg, 1.0 eq) and triethylamine (0.96 mmol, 129 µL, 1.2 eq) in anhydrous dichloromethane (5.0 mL). Once the addition was finished, the reaction mixture was stirred overnight at room temperature. Brine was added and the aqueous phase was extracted with dichloromethane. The organic phase was dried over magnesium sulphate, filtered and concentrated under vacuum pressure. The resulting crude product was purified by flash chromatography (isocratic 60/40 petroleum ether/ethyl acetate) to afford a white solid (147 mg, 49%). TLC R_f: 0.39 (petroleum ether/ethyl acetate 60/40); mp: 156 °C; IR (cm^{-1}): 560, 640, 669, 689, 701, 742, 756, 906, 1003, 1056, 1086, 1122, 1321, 1442, 2823, 2841, 2941. HPLC: method 1, rt = 2.72 min, purity 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.58-2.80 (m, 4 H); 2.92-3.05 (m, 2 H); 3.10-3.25 (m, 2 H); 4.86 (s, 1 H); 7.32 (dd, *J* = 1.2 Hz, 5.7 Hz, 1 H); 7.34 (dd, *J* = 1.2 Hz, 5.4 Hz, 1 H); 7.41 (td, *J* = 3.0 Hz, 6.6 Hz, 2 H); 7.46-7.56 (m, 3 H); 7.61 (d, *J* = 7.2 Hz, 1 H); 7.63-7.69 (m, 3 H); 7.71 (d, *J* = 7.5 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 46.5 (2 x CH_2); 49.1 (2 x CH_2); 69.9 (CH); 119.7 (2 x CH); 125.8 (2 x CH); 126.1 (2 x CH); 127.1 (2 x CH); 128.2 (2 x CH); 128.8 (2 x CH); 130.8 (CH); 141.0 (2 x C), 142.8 (2 x C); 143.6 (C). MS (DCI/CH_4) m/z: 375.15 [M+H$^+$], 249.14 [M-125 (PhSO)], 165.07 [M-209 (fluorene)]. HRMS (DCI/CH_4): for $C_{23}H_{23}N_2O_2$ [M+H$^+$]: calcd: 375.1531 found: 375.1536.

4.2.6. *(4-(9*H*-Fluoren-9-yl)piperazin-1-yl)(methyl)(phenyl)phosphine oxide (**9**).* Reagents: 1-(9H-fluoren-9-yl)piperazine **3** (0.80 mmol, 200 mg), triethylamine (0.96 mmol, 129 µL) and methylphenylphosphinic chloride (0.96 mmol, 132 µL). The crude product was purified by flash chromatography (isocratic 95/5 petroleum ether/ethyl acetate) to a white solid (204 mg, 64%). TLC R_f: 0.19 (dichloromethane/MeOH 95/5); mp: 178 °C; IR (cm^{-1}): 620, 639, 666, 692, 713, 737, 883, 901, 965, 998, 1117, 1138, 1186, 1300, 1319, 1377, 1437, 1449, 2839, 2908, 2931. HPLC: method 1, rt = 1.91 min, purity 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.68 (d, $J_{p,H}$ = 13.8 Hz, 3 H); 2.63 (t, *J* = 4.5 Hz, 4 H); 2.94-3.16 (m, 4 H); 4.84 (s, 1 H); 7.31 (tt, *J* = 1.2 Hz, 7.5 Hz, 2 H); 7.39 (t, *J*= 7.5 Hz, 1 H); 7.43-7.55 (m, 3 H); 7.63 (t, *J* = 6.3 Hz, 1 H); 7.69 (d, *J* = 7.5 Hz, 1 H); 7.74 (dd, *J* = 1.8 Hz, 12.0 Hz, 1 H); 7.76 (dd, *J* = 1.5 Hz, 12.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (d, *J* = 92.3 Hz, CH_3); 44.8 (2 x CH_2); 49.3 (d, *J* = 7.7 Hz, 2 x CH_2); 70.2 (d, *J* = 1.0 Hz, CH); 119.8 (2 x CH); 125.8 (d, *J* = 4.1 Hz, 2 x CH); 127.1 (2 x CH); 128.1 (2 x CH); 128.4 (d, *J* = 12.3 Hz, 2 x CH); 131.1 (d, *J* = 9.6 Hz, 2 x CH); 131.6 (d, J = 2.7 Hz, CH); 131.7 (C); 133.4 (C); 140.9 (2 x C); 143.5 (C) . ^{31}P NMR (121.5 MHz, $CDCl_3$) δ (ppm): 35.32. MS (DCI/CH_4) m/z: 389.18 [M+H^+]. HRMS (DCI/CH_4): for $C_{24}H_{26}N_2OP$ [M+H^+]: calcd: 389.1783; found: 389.1784.

4.2.7. General procedure for **11**-**15**. The corresponding isocyanate or isothiocyanate (1.0 eq) was dissolved in dry acetonitrile under argon. A solution of 1-(9*H*-fluoren-9-yl)piperazine **3** (1.0 eq) in dry acetonitrile was added to the reaction mixture. After overnight stirring, a precipitate was isolated and washed with acetonitrile. The resulting crude product was purified by flash chromatography to afford the title compound.

*4.2.7.1. 4-(9H-Fluoren-9-yl)-N-phenylpiperazine-1-carboxamide (**11**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.40 mmol, 100 mg) and phenyl isocyanate (0.40 mmol, 48 µL). The crude product was purified by flash chromatography (dry-load, gradient 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a white solid (67 mg, 45%). TLC R_f: 0.63 (dichloromethane/MeOH 90/10); mp: 223 °C; IR (cm^{-1}): 527, 691, 742, 872, 944, 972, 995, 1144, 1199, 1209, 1246, 1300, 1312, 1325, 1365, 1439, 1504, 1521, 1596, 1654, 2821, 2947, 3408. HPLC: method 1, rt = 2.25 min, purity 95%. ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.55 (t, *J* = 5.1 Hz, 4 H); 3.43 (t, *J* = 4.8 Hz, 4 H); 5.01 (s, 1 H); 6.93 (tt, *J* = 1.2 Hz, 7.2 Hz, 1 H); 7.18 – 7.26 (m, 2 H); 7.34 (td, *J* = 1.2 Hz, 7.5 Hz, 2 H); 7.29 – 7.46 (m, 4 H); 7.65 (d, *J* = 7.5 Hz, 2 H); 7.86 (d, *J* = 7.5 Hz, 2 H); 8.44 (s, 1 H). ^{13}C NMR (75 MHz, DMSO-d6) δ (ppm): 44.9 (2 x CH_2); 49.0 (2 x CH_2); 69.6 (CH); 120.0 (2 x CH); 120.5 (2 x CH); 122.1 (CH); 126.3 (2 x CH); 127.7 (2 x CH); 128.7 (2 x CH); 128.8 (2 x CH); 140.9 (2 x CH); 141.0 (C); 143.9 (2 x C); 155.3 (C). MS (DCI/CH_4) m/z: 370.19 [M+H^+], 251.15 [M-119], 165.07 [M-205]. HRMS (DCI/CH_4): for $C_{24}H_{24}N_3O$ [M+H^+]: calcd: 370.1919; found: 370.1913.

*4.2.7.2. 4-(9H-Fluoren-9-yl)-N-phenylpiperazine-1-carbothioamide (**12**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.40 mmol, 100 mg) and phenyl isothiocyanate (0.40 mmol, 48 µL). The crude product was purified by flash chromatography (dry-load, gradient 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a white solid (71 mg, 46%). TLC R_f:

0.71 (dichloromethane/methanol 90/10); mp: 206 °C; IR (cm^{-1}): 525, 701, 737, 746, 997, 1037, 1123, 1219, 1261, 1301, 1315, 1399, 1439, 1496, 1518, 1591, 2814, 3306. HPLC: method 2, rt = 2.86 min, purity 98%. ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.60 (t, *J* = 4.8 Hz, 4 H); 3.88 (t, *J* = 4.5 Hz, 4 H); 5.04 (s, 1 H); 7.05 – 7.13 (m, 1 H); 7.23 – 7.32 (m, 4 H); 7.36 (td, *J* = 1.2 Hz, 7.5 Hz, 2 H); 7.43 (t, *J* = 7.5 Hz, 2 H); 7.66 (d, *J* = 7.2 Hz, 2 H); 7.87 (d, *J* = 7.5 Hz, 2 H); 9.24 (s, 1 H). ^{13}C NMR (75 MHz, DMSO-d6) δ (ppm): 48.8 (2 x CH_2); 49.2 (2 x CH_2); 69.3 (CH); 120.6 (2 x CH); 124.7 (CH); 125.6 (2 x CH); 126.3 (2 x CH); 127.7 (2 x CH); 128.4 (2 x CH); 128.8 (2 x CH); 141.0 (2 x CH); 141.5 (C); 143.8 (2 x C); 181.8 (C). MS (DCI/CH_4) m/z: 386.17 [M+H^+], 251.15 [M-134], 165.07 [M-220], 136.02 [M-249]. HRMS (DCI/CH_4): for $C_{24}H_{24}N_3S$ [M+H^+]: calcd: 386.1691; found: 386.1690.

*4.2.7.3. N-Butyl-4-(9H-fluoren-9-yl)piperazine-1-carboxamide (**13**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.40 mmol, 100 mg) and butyl isocyanate (0.48 mmol, 54 µL). The crude product was purified by flash chromatography (isocratic, petroleum ether/ethyl acetate 70/30) to afford a white solid (72 mg, 51%). TLC R_f: 0.83 (dichloromethane/methanol 90/10); mp: 174 °C; IR (cm^{-1}): 737, 758, 848, 939, 1001, 1141, 1208, 1264, 1304, 1327, 1411, 1447, 1538, 1613, 2822, 2861, 2928, 2956, 3353. HPLC: method 1, rt = 2.05 min, purity 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.93 (t, *J* = 7.2 Hz, 3 H); 1.26 – 1.41 (m, 2 H); 1.41 – 1.54 (m, 2 H); 2.63 (t, *J* = 4.8 Hz, 4 H); 3.17 – 3.26 (m, 2 H); 3.34 (t, *J* = 4.8 Hz, 4 H); 4.45 (t, *J* = 5.7 Hz, 1 H); 4.88 (s, 1 H); 7.31 (td, *J* = 1.2 Hz, 7.5 Hz, 2 H); 7.41 (t, *J* = 7.5 Hz, 2 H); 7.63 (d, *J* = 7.2 Hz, 2 H); 7.71 (d, *J* = 7.5 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 13.9 (CH_3); 20.1 (CH_2); 32.4 (CH_2); 40.7 (CH_2); 44.5 (2 x CH_2); 48.8 (2 x CH_2); 70.0 (CH); 119.8 (2 x CH); 126.0 (2 x CH); 127.1 (2 x CH); 128.2 (2 x CH); 141.0 (2 x C); 143.6 (2 x C); 158.0 (C). MS (DCI/CH_4) m/z: 350.22 [M+H^+], 251.16 [M-98], 184.15 [M-165], 165.07 [M-184], 143.12 [M-206]. HRMS (DCI/CH_4): for $C_{22}H_{28}N_3O$ [M+H^+]: calcd: 350.2232; found: 350.2228.

*4.2.7.4. 4-(9H-Fluoren-9-yl)-N-octylpiperazine-1-carboxamide (**14**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.40 mmol, 100 mg) and octyl isocyanate (0.48 mmol, 85 µL). The crude product was purified by flash chromatography (isocratic, petroleum ether/ethyl acetate 70/30) to afford a white solid (85 mg, 52%). TLC R_f: 0.83 (dichloromethane/methanol 90/10); mp: 139 °C; IR (cm^{-1}): 618, 738, 844, 1006, 1127, 1143, 1177, 1206, 1260, 1301, 1326, 1406, 1444, 1466, 1540, 1616, 2851, 2925, 3298. HPLC: method 1, rt = 3.12 min, purity 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.86 – 0.95 (m, 3 H); 1.22 – 1.38 (m, 10 H); 1.49 (quint, *J* = 6.6 Hz, 2 H); 2.64 (t, *J* = 4.8 Hz, 4 H); 3.16 – 3.25 (m, 2 H); 3.35 (t, *J* = 4.8 Hz, 4 H); 4.36 (s, 1 H); 4.89 (s, 1 H); 7.31 (td, *J* = 1.2 Hz, 7.2 Hz, 2 H); 7.41 (t, *J* = 7.2 Hz, 2 H); 7.64 (d, *J* = 7.5 Hz, 2 H); 7.72 (d, *J* = 7.5 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 27.0 (CH_2); 29.2 (CH_2); 29.3 (CH_2); 30.3 (CH_2); 31.8 (CH_2); 41.0 (CH_2); 44.5 (2 x CH_2); 48.7 (2 x CH_2); 70.0 (CH); 119.8 (2 x CH); 126.0 (2 x CH); 127.1 (2 x CH); 128.2 (2 x CH); 141.0 (2 x C); 143.6 (2 x C); 157.7 (C). MS (DCI/CH_4) m/z: 406.28 [M+H^+], 251.16 [M-154], 240.21 [M-165], 199.18 [M-206], 165.07 [M-240]. HRMS (DCI/CH_4): for $C_{26}H_{36}N_3O$ [M+H^+]: calcd: 406.2858; found: 406.2840.

*4.2.7.5. 4-(9H-Fluoren-9-yl)-N-(4-nitrophenyl)piperazine-1-carboxamide (**15**).* Reagents: 1-(9*H*-fluoren-9-yl)piperazine **3** (0.40 mmol, 100 mg) and 4-nitrophenylisocyanate (0.40 mmol, 65 mg). The crude product was purified by flash chromatography chromatography (dry-load, gradient 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a white solid (82 mg, 82%). TLC R_f: 0.71 (dichloromethane/methanol 90/10); mp: 263 °C; IR (cm^{-1}): 620, 672, 690, 739, 807, 842, 1001, 1110, 1237, 1299, 1328, 1424, 1497, 1541, 1599, 1611, 1639, 2827, 2913, 3341. HPLC: method 1, rt = 2.68 min, purity 95%. ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.57 (t, *J* = 5.1 Hz, 4 H); 3.47 (t, *J* = 5.1 Hz, 4 H); 5.02 (s, 1 H); 7.34 (td, *J* = 1.5 Hz, 7.5 Hz, 2 H); 7.43 (t, *J* = 7.2 Hz, 2 H); 7.65 (d, *J* = 7.2 Hz, 2 H); 7.67 – 7.73 (m, 2 H); 7.86 (d, *J* = 7.5 Hz, 2 H); 8.10 – 8.19 (m, 2 H); 9.18 (s, 1 H). ^{13}C NMR (75 MHz, DMSO-d6) δ (ppm): 45.1 (2 x CH_2); 49.0 (2 x CH_2); 69.6 (CH); 118.7 (2 x CH); 120.6 (2 x CH); 125.2 (2 x CH); 126.3 (2 x CH); 127.7 (2 x CH); 128.7 (2 x CH); 141.0 (2 x C); 141.2 (C); 143.8 (2 x CH); 148.0 (C); 154.3 (C). MS (DCI/CH_4) m/z: 415.186 [M+H^+]. HRMS (DCI/CH_4): for $C_{24}H_{23}N_4O_2$ [M+H^+]: calcd: 415.1770; found: 415.1779.

*4.2.8. General procedure for compounds **17** and **18**.* To a solution of the corresponding alkene (1.0 eq), in anhydrous tetrahydrofuran was added 9-BBN (1.2 eq) at 0°C. The mixture was stirred at 0°C for 2 hours and at RT for 3 hours more, and then was introduced into a mixture of 2-bromofluorenone **16** (1.25 eq), cesium carbonate (3.75 eq), triphenylarsine (0.25 eq) and Pd(dppf)Cl_2 (0.25 eq) in a mixture of 8 mL of THF, 8 mL DMF and 2 mL H_2O. The resulting mixture was heated at 85 °C overnight, cooled and passed through a short pad of silica gel with 4:1 petroleum ether/ethyl acetate. The solvent was evaporated and the residue was dissolved in ethyl acetate, washed with brine, dried over magnesium sulfate, filtered and evaporated. The residue was purified by flash chromatography (gradient, 100% petroleum ether to 95:5 petroleum ether/ethyl acetate in 15 minutes) to afford the title compound.

*4.2.8.1. 2-Hexyl-9H-fluoren-9-one (**17**).* Reagents: 1-hexene (1.7 mmol, 211 µL), 9-BBN (2.05 mmol, 4.1 mL of 0.5 M solution in THF), 2-bromofluorenone **16** (2.1 mmol, 550 mg), cesium carbonate (6.4 mmol, 2.07 g), triphenylarsine (0.42 mmol, 130 mg) and Pd(dppf)Cl_2 (0.42 mmol, 346 mg). A yellow oil was obtained (257 mg, 57%). TLC R_f: 0.57 (petroleum ether/ethyl acetate 97/3). IR (cm^{-1}): 736, 766, 831, 958, 1106, 1176, 1291, 1457, 1602, 1713, 2854, 2925. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.85-0.98 (m, 3 H); 1.25-1.45 (m, 6 H); 1.53-1.73 (m, 2 H); 2.66 (t, *J* = 7.5 Hz, 2 H); 7.25-7.34 (m, 2 H); 7.44 (d, *J* = 7.8 Hz, 1 H); 7.47-7.53 (m, 3 H); 7.66 (dt, *J* = 0.9 Hz, 7.5 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.6 (CH_2); 28.8 (CH_2); 31.2 (CH_2); 31.7 (CH_2); 35.8 (CH_2); 120.0 (CH); 120.2 (CH); 124.3 (CH); 124.4 (CH); 128.6 (CH); 134.4 (C); 134.5 (C); 134.7 (CH); 134.7 (CH); 142.1 (C); 144.5 (C); 144.7 (C); 194.3 (C). MS (DCI/CH_4) m/z: 265.16 [M+H^+]. HRMS (DCI/CH_4): for $C_{19}H_{20}O$ [M+H^+]: calcd: 265.1592; found: 265.1599.

*4.2.8.2. 2-Octyl-9H-fluoren-9-one (**18**).* Reagents: 1-Octene (1.7 mmol, 267 µL), 9-BBN (2.05 mmol, 4.1 mL of 0.5 M solution in THF), 2-bromofluorenone **16** (2.1 mmol, 550 mg), cesium carbonate (6.4 mmol, 2.07 g), triphenylarsine (0.42 mmol, 130 mg,) and Pd(dppf)Cl_2 (0.42 mmol, 346 mg). A yellow oil was obtained (170 mg, 34%). TLC R_f: 0.72 (petroleum ether/ethyl acetate 95/5). IR (cm^{-1}): 650, 737, 765, 831, 963, 1106, 1176, 1291, 1457, 1603, 1714, 2853, 2923. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.91 (t, *J* = 6.6 Hz, 3 H); 1.22-1.43 (m, 10 H); 1.65 (q, *J* = 7.5 Hz, 2 H); 2.64 (t, *J* = 7.5 Hz, 2 H); 7.25-7.34 (m, 2 H); 7.45 (d, *J* = 7.8 Hz, 1 H); 7.49 (dd, *J* = 0.9 Hz, 3.3 Hz, 1 H); 7.51 (d, *J* = 0.9 Hz, 1 H); 7.52 (s, 1 H); 7.67 (dt, *J* = 0.9 Hz, 7.5 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 29.2 (CH_2); 29.3 (CH_2); 29.5 (CH_2); 31.2 (CH_2); 31.9 (CH_2); 35.8 (CH_2); 120.0 (CH); 120.2 (CH); 124.2 (CH); 124.4 (CH); 128.6 (CH); 134.7 (C); 134.5 (C); 134.6 (CH); 134.7 (CH); 142.0 (C); 144.5 (C); 144.7 (C); 194.1 (C). MS (DCI/CH_4) m/z: 293.19 [M+H^+]. HRMS (DCI/CH_4): for $C_{21}H_{25}O$ [M+H^+]: calcd: 293.1905; found: 293.1906.

*4.2.9. 2-Propoxy-9H-fluoren-9-one (**20**).* An oven-dried 5 mL round-bottom flask was charged with Pd(OAc)$_2$ (0.011 mmol, 2.6 mg, 0.01 eq), ligand *t*BuXPhos (0.023 mmol, 9.8 mg, 0.02 mmol) and Cs_2CO_3 (1.74 mmol, 566 mg, 1.5 eq). The round-bottom flask was sealed with septum, evacuated and back-filled with argon. Toluene (2.0 mL), 2-bromo-9-fluorenone **19** (1.16 mmol, 300 mg, 1.0 eq) and propan-1-ol (3.47 mmol, 260 µL, 3.0 eq) were added to the reaction mixture and was stirred overnight at 80 °C. Brine was added and the product was extracted with ethyl acetate (3×). The organic phase was dried over magnesium sulfate, filtered and concentrated under vacuum pressure. The resulting crude product was purified by flash chromatography (petroleum ether/diethyl ether 97/3 in 20 minutes) to afford a yellow solid (134 mg, 52%). TLC R_f: 0.35 (petroleum ether/Et_2O 97/3); mp: 61 °C; IR (cm^{-1}): 741, 766, 837, 996, 1012, 1130, 1247, 1296, 1455, 1489, 1599, 1716, 2875, 2940. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.08 (t, *J* = 7.2 Hz, 3 H); 1.85 (sex, *J* = 7.2 Hz, 2 H); 3.99 (t, *J* = 6.6 Hz, 2 H); 6.99 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.21 (d, *J* = 2.4 Hz, 1 H); 7.21 (td, *J* = 1.5 Hz, 7.2 Hz, 1 H); 7.37 – 7.49 (m, 3 H); 7.61 (dt, *J* = 0.9 Hz, 7.2 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 10.5 (CH_3); 22.5 (CH_2); 70.1 (CH_2); 109.9 (CH); 119.5

(CH); 120.8 (CH); 121.3 (CH); 124.3 (CH); 127.8 (CH); 134.3 (C); 134.8 (CH); 135.9 (C); 136.8 (C); 145.0 (C); 160.4 (C); 194.0 (C). MS (DCI/CH_4) m/z: 239.11 [M+H^+]. HRMS (DCI/CH_4): for $C_{16}H_{15}O_2$ [M+H^+]: calcd: 239.1072; found: 239.1084.

*4.2.10. 3-Hydroxy-9H-fluoren-9-one (**22**).* A mixture of 3-methoxyfluorenone **21** (1.1 mmol, 226 mg, 1.0 eq), acetic acid (26 mmol, 1.5 mL, 25 eq), and HBr 47% (2.5 mL) was heated under reflux for 6 hours. After cooling, the reaction mixture was poured into water (100 mL) and extracted with ethyl acetate. The organic phase was dried over magnesium sulfate, filtered and concentrated under vacuum pressure. The residue was purified by flash chromatography (petroleum ether:ethyl acetate 80:20 in 15 minutes) to afford a yellow solid (130 mg, 62%). TLC R_f: 0.42 (petroleum ether/ethyl acetate 70/30); mp: 238 °C; IR (cm^{-1}): 670, 736, 829, 870, 926, 1102, 1204, 1298, 1388, 1445, 1586, 1611, 1676, 3082. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 6.69 (dd, *J* = 2.1 Hz, 8.1 Hz, 1 H); 7.05 (d, *J* = 2.1 Hz, 1 H); 7.31-7.38 (m, 1 H); 7.48-7.61 (m, 4 H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 107.9 (CH); 114.7 (CH); 120.0 (CH); 123.0 (CH); 125.3 (C); 126.1 (CH); 129.0 (CH); 134.2 (CH); 135.1 (C); 143.4 (C); $C_{13}H_9O_2$ [M+H^+]: calcd: 197.0603; found: 197.0610.

*4.2.11. General procedure for compounds **23**-**25**.* 3-Hydroxy-9-fluorenone **22** (1.0 eq) was dissolved in DMF (5.0 mL), potassium carbonate (1.5 eq) and the corresponding alkyl or aryl bromine reagent (0.95 eq) were added. The mixture was heated to 80°C and stirred overnight. The reaction mixture was then cooled and 1 N hydrochloric acid (5.0 mL) was added dropwise thereto in a water bath to quench the reaction. The mixture was extracted with dichloromethane and washed once with 1 N hydrochloric acid and 4 times with water. The organic layer was dried over magnesium sulfate, filtered and concentrated under vacuum pressure to afford the title compound. No further purification was needed.

*4.2.11.1. 3-(Benzyloxy)-9H-fluoren-9-one (**23**).* Reagents: 3-Hydroxy-9-fluorenone **22** (0.54 mmol, 105 mg), potassium carbonate (0.81 mmol, 111 mg) and benzyl bromide (0.64 mmol, 77 µL). A yellow solid was obtained (105 mg, 88%). TLC R_f: 0.35 (petroleum ether/ethyl acetate); mp: 124 °C; IR (cm^{-1}): 693, 735, 767, 839, 1023, 1099, 1183, 1204, 1237, 1285, 1306, 1381, 1450, 1491, 1583, 1610, 1734, 2853, 2922, 3305. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.17 (s, 2 H); 6.82 (dd, *J* = 2.1 Hz, 8.1 Hz, 1 H); 7.10 (d, *J* = 2.1 Hz, 1 H); 7.27 – 7.33 (m, 1 H); 7.38 – 7.52 (m, 7 H); 7.62 (d, *J* = 8.1 Hz, 1 H); 7.64 (dt, *J* = 0.6 Hz, 7.5 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 70.5 (CH_2); 108.0 (CH); 113.8 (CH); 120.2 (CH); 123.9 (CH);126.3 (CH);127.4 (C); 127.6 (2 x CH); 128.4 (CH); 128.8 (2 x CH); 129.3 (CH); 134.2 (CH); 135.3 (C); 136.1 (C); 143.4 (C); 147.0 (C); 164.5 (C); 192.5 (C). MS (DCI/CH_4) m/z: 287.11 [M+H^+]. HRMS (DCI/CH_4): for $C_{20}H_{15}O_2$ [M+H^+]: calcd: 287.1072; found: 287.1074.

*4.2.11.2. 3-Propoxy-9H-fluoren-9-one (**24**).* Reagents: 3-Hydroxy-9-fluorenone **22** (0.41 mmol, 80 mg), potassium carbonate (0.61 mmol, 85 mg) and bromopropane (0.39 mmol, 35 µL). A yellow solid was obtained (95 mg, 98%). TLC R_f: 0.09 (petroleum ether/ethyl acetate 80/20); mp: 55 °C; IR (cm^{-1}): 538, 616, 642, 670, 731, 762, 826, 859, 924, 1007, 1013, 1109, 1203, 1218, 1293, 1370, 1447, 1590, 1603, 1616, 1705, 2879, 2938, 2967. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.07 (t, *J* = 7.5 Hz, 3 H); 1.85 (sep, *J* = 7.2 Hz, 2 H); 4.02 (t, *J* = 6.6 Hz, 2 H); 6.72 (dd, *J* = 2.1 Hz, 8.1 Hz, 1 H); 7.01 (d, *J* = 2.4 Hz, 1 H); 7.28 (dq, *J* = 3.3 Hz, 5.1 Hz, 1 H); 7.43-7.48 (m, 2 H); 7.60 (d, *J* = 8.1 Hz, 1 H); 7.62 (dt, *J* = 1.2 Hz, 7.2 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 10.5 (CH_3); 22.5 (CH_2); 70.1 (CH_2); 107.6 (CH); 113.5 (CH); 120.1 (CH); 123.9 (CH); 126.3 (CH); 127.0 (C); 129.3 (CH); 134.1 (CH); 135.4 (C); 143.5 (C); 147.0 (C); 165.1 (C); 192.6 (C). MS (DCI/CH_4) m/z: 239.10 [M+H^+]. HRMS (DCI/CH_4): for $C_{16}H_{15}O_2$ [M+H^+]: calcd: 239.1072; found: 239.1060.

*4.2.11.3. 3-Hexyloxy-9H-fluoren-9-one (**25**).* Reagents: 3-Hydroxy-9-fluorenone **22** (0.41 mmol, 80 mg), potassium carbonate (0.61 mmol, 85 mg) and bromohexane (0.39 mmol, 54 µL). A brown, solid was obtained (106 mg, 93%). TLC R_f: 0.81 (petroleum ether/ethyl acetate 80/20); mp: 84 °C; IR (cm^{-1}): 677, 728, 737, 765, 846, 990, 1017, 1097, 1188, 1233,

1298, 1452, 1587, 1605, 1696, 2861, 2935, 2951. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.96 (t, *J* = 6.9 Hz, 3 H); 1.32-1.45 (m, 4 H); 1.46-1.58 (m, 2 H); 1.86 (q, *J* = 6.6 Hz, 2 H); 4.09 (t, *J* = 6.3 Hz, 2 H); 6.76 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.05 (d, *J* = 2.1 Hz, 1 H); 7.32 (dq, *J* = 3.0 Hz, 5.4 Hz, 1 H); 7.45-7.52 (m, 2 H); 7.63 (d, *J* = 8.1 Hz, 1 H); 7.66 (dt, *J* = 1.2 Hz, 7.2 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.6 (CH_2); 25.7 (CH_2); 29.1 (CH_2); 31.6 (CH_2); 68.6 (CH_2); 107.6 (CH); 113.5 (CH); 120.1 (CH); 123.9 (CH); 126.3 (CH); 127.0 (C); 129.3 (CH); 134.1 (CH); 135.4 (C); 143.5 (C); 147.0 (C); 165.1 (C); 192.6 (C). MS (DCI/CH_4) m/z: 281.15 [M+H$^+$]. HRMS (DCI/CH_4): for $C_{19}H_{21}O_2$ [M+H$^+$]: calcd: 281.1542; found: 281.1543.

*4.2.12. 9-Oxo-9H-fluoren-6-yl trifluoromethanesulfonate (**26**).* To a solution of 3-hydroxy-9-fluorenone **22** (2.0 mmol, 300 mg, 1.0 eq) and of 2,6-di-*tert*-butyl-4-methylpyridine (5.1 mmol, 1.05 g, 2.5 eq) in anhydrous dichloromethane (15.0 mL) was added triflic anhydride (2.45 mmol, 411 µL, 1.2 eq) dropwise at -78 °C. The mixture was stirred at -78 °C for 1 hour and at 0 °C for 1.5 hours, and then was evaporated. The yellow residue was directly purified by flash chromatography (isocratic petroleum ether/ethyl acetate 95/5 in 15 minutes) to afford a yellow solid (491 mg, 73%). TLC R_f: 0.90 (petroleum ether/ethyl acetate 90/10). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.18 (dd, *J* = 2.1 Hz, 8.1 Hz, 1 H); 7.34-7.40 (m, 1 H); 7.41 (d, *J* = 2.1 Hz, 1 H); 7.54 (dd, *J* = 0.9 Hz, 2.4 Hz, 1 H); 7.55 (d, *J* = 0.9 Hz, 1 H); 7.69 (dt, *J* = 0.9 Hz, 7.2 Hz, 1 H); 7.73 (d, *J* = 8.4 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 114.1 (CH); 116.8 (C); 121.1 (CH); 121.8 (CH); 124.9 (CH); 126.1 (CH); 130.5 (CH); 133.8 (C); 134.4 (C); 135.3 (CH); 142.6 (C); 147.3 (C); 154.0 (C); 191.6 (C). MS (DCI/CH_4) m/z: 329.01 [M+H$^+$]. HRMS (DCI/CH_4): for $C_{14}H_8F_3O_4S$ [M+H$^+$]: calcd: 329.0100; found: 329.0095.

*4.2.13. 3-Hexyl-9H-fluoren-9-one (**27**).* To a solution 1-hexene (0.34 mmol, 43 µL, 1.0 eq) in anhydrous THF (3.0 mL) was added 9-BBN (0.41 mmol, 812 µL of a 0.5 M solution in THF, 1.2 eq) at 0 °C. The mixture was stirred at 0°C for 2 hours and at room temperature for 2 hours additional, and then was introduced to a mixture of 9-oxo-9*H*-fluoren-6-yl trifluoromethanesulfonate **26** (0.31 mmol, 100 mg, 0.9 eq), K_3PO_4 (0.47 mmol, 100 mg, 1.4 eq) and Pd(dppf)Cl_2,CH_2Cl_2 (0.02 mmol, 20 mg, 0.07 eq) in anhydrous THF (3.0 mL). The resulting mixture was heated at reflux overnight, cooled and passed through a short pad of silica gel with petroleum ether:ethyl acetate 4:1. The solvent was evaporated and the residue was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a yellow oil (77 mg, 96%). TLC R_f: 0.59 (petroleum ether/ethyl acetate 95/5). IR (cm^{-1}): 677, 737, 764, 838, 919, 1111, 1193, 1297, 1422, 1448, 1601, 1613, 1708, 2855, 2925. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.88 – 0.98 (m, 3 H); 1.27-1.45 (m, 6 H); 1.62 – 1.75 (m, 2 H); 2.69 (t, *J* = 7.2 Hz, 2 H); 7.12 (dd, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.30 (td, *J* = 1.5 Hz, 7.2 Hz, 1 H); 7.34 – 7.38 (m, 1 H); 7.49 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.52 – 7.54 (m, 1 H); 7.60 (d, *J* = 7.5 Hz, 1 H); 7.67 (td, *J*= 1.2 Hz Hz, 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.6 (CH_2); 29.0 (CH_2); 31.2 (CH_2); 31.7 (CH_2); 36.6 (CH_2); 120.1 (CH); 120.6 (CH); 124.2 (CH); 124.4 (CH); 129.0 (CH); 129.1 (CH); 132.1 (C); 134.4 (CH); 134.8 (C); 144.4 (C); 144.8 (C); 151.0 (C); 193.7 (C). MS (DCI/CH_4) m/z: 293.19 [M+$C_2H_5^+$], 265.16[M+H$^{+]}$. HRMS (DCI/CH_4): for $C_{19}H_{21}O$ [M+H$^+$]: calcd: 265.1592; found: 265.1599.

*4.2.14. General procedure for **28-35**.* To a mixture of 1-benzoylpiperazine (1.0 eq) and potassium carbonate (2.0 eq) in dimethylformamide was added dropwise a solution of the corresponding substituted 9-bromo-9*H*-fluorene (1.0 eq) in dimethylformamide (The synthesis of 9-bromo-9H-fluorene derivatives is reported in supporting information). After stirring for 24 hours at room temperature, solvent was removed and the crude residue was dissolved in diethyl ether, washed with brine, dried over magnesium sulfate, filtered and concentrated under vacuum. The residue was purified by flash chromatography as indicated in each case to afford the title compound.

*4.2.14.1. (4-(2-Hexyl-9H-fluoren-9-yl)piperazin-1-yl)(phenyl)methanone (**28**).* Reagents: 1-Benzoylpiperazine (0.15 mmol, 29 mg), potassium carbonate (0.30 mmol, 42 mg) and 9-bromo-2-hexyl-9*H*-fluorene (0.15 mmol, 50 mg). The crude product

was purified by flash chromatography (isocratic, petroleum ether/ethyl acetate 80/20) to afford a yellow oil (66 mg, 99%). TLC R_f: 0.34 (petroleum ether/ethyl acetate 80/20). IR (cm^{-1}): 697, 708, 739, 766, 1001, 1137, 1256, 1277, 1426, 1455, 1632, 1674, 1715, 2854, 2925. HPLC: method 2, rt = 2.67 min purity 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.93 (t, *J* = 7.2 Hz, 3 H); 1.24-1.48 (m, 6 H); 1.63-1.76 (m, 2 H); 2.44 (s, 2 H); 2.72 (t, *J* = 7.8 Hz, 2 H); 2.91 (s, 2 H); 3.38 (s, 2 H); 3.84 (s, 2 H); 4.88 (s, 1 H); 7.23 (dd, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.29 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.34 – 7.42 (m, 6 H); 7.46 (s, 1 H); 7.61 (d, *J* = 7.5 Hz, 1 H); 7.63 (d, *J* = 7.5 Hz, 1 H); 7.67 (d, *J* = 7.5 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 29.0 (CH_2); 31.8 (2 x CH_2); 36.2 (CH_2); 43.0 (CH_2); 48.5 (CH_2); 48.8 (CH_2); 49.7 (CH_2); 69.9 (CH); 119.5 (CH); 119.6 (CH); 125.9 (CH); 126.0 (CH); 126.7 (CH); 127.1 (2 x CH); 128.3 (CH); 128.4 (2 x CH); 128.5 (CH); 129.6 (CH); 135.9 (C); 138.7 (C); 141.2 (C); 142.4 (C); 143.3 (C); 143.6 (C); 170.3 (C). MS (DCI/CH_4) m/z: 438.27 [M]. HRMS (DCI/CH_4): for $C_{30}H_{34}N_2O$ [M]: calcd: 438.2671; found: 438.2660.

*4.2.14.2. (4-(2-Octyl-9H-fluoren-9-yl)piperazin-1-yl)(phenyl)methanone (**29**).* Reagents: 1-Benzoylpiperazine (0.16 mmol, 30 mg), potassium carbonate (0.31 mmol, 43 mg) and 9-bromo-2-octyl-9*H*-fluorene (0.16 mmol, 55 mg). The crude product was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a yellow oil (38 mg, 53%). TLC R_f: 0.10 (petroleum ether/ethyl acetate 90/10). IR (cm^{-1}): 697, 708, 740, 765, 828, 1001, 1015, 1141, 1154, 1255, 1277, 1302, 1425, 1455, 1634, 1715, 2853, 2923. HPLC: method 2, rt = 7.63 min, purity 97%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.82 – 1.01 (m, 3 H); 1.23-1.47 (m, 10 H); 1.59-1.78 (m, 2 H); 2.43 (s, 2 H); 2.72 (t, *J* = 7.8 Hz, 2 H); 2.92 (s, 2 H); 3.38 (s, 2 H); 3.85 (s, 2 H); 4.88 (s, 1 H); 7.23 (d, *J* = 7.8 Hz, 1 H); 7.30 (td, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.34 – 7.43 (m, 6 H); 7.47 (s, 1 H); 7.61 (d, *J* = 7.5 Hz, 1 H); 7.63 (d, *J* = 7.5 Hz, 1 H); 7.67 (d, *J* = 7.1 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3); 22.7 (CH_2); 29.3 (CH_2); 29.4 (CH_2); 29.5 (CH_2); 31.8 (CH_2); 31.9 (CH_2); 36.2 (CH_2); 43.0 (CH_2); 48.4 (CH_2); 48.5 (CH_2); 49.7 (CH_2); 69.9 (CH); 119.5 (CH); 119.6 (CH); 125.8 (CH); 125.9 (CH); 126.7 (CH); 127.1 (2 x CH); 128.3 (CH); 128.4 (2 x CH); 128.5 (CH); 129.6 (CH); 135.9 (C); 138.7 (C); 141.2 (C); 142.4 (C); 143.3 (C); 143.6 (C); 170.3 (C). MS (DCI/CH_4) m/z: 467.31 [M+H^+], 277.20 [M-189]. HRMS (DCI/CH_4): for $C_{32}H_{39}N_2O$ [M+H^+]: calcd: 467.3062; found: 467.3063.

*4.2.14.3. (4-(3-Hexyl-9H-fluoren-9-yl)piperazin-1-yl)(phenyl)methanone (**30**).* Reagents: 1-Benzoylpiperazine (0.08 mmol, 15 mg), potassium carbonate (0.15 mmol, 20 mg) and 9-bromo-3-hexyl-9*H*-fluorene (0.08 mmol, 25 mg). The crude product was purified by flash chromatography (isocratic, petroleum ether/ethyl acetate 70/30 in 15 minutes) to afford a yellow oil (18 mg, 56%). TLC R_f: 0.11 (petroleum ether/ethyl acetate 80/20). IR (cm^{-1}): 630, 674, 697, 708, 739, 768, 787, 1001, 1015, 1142, 1155, 1255, 1277, 1301, 1424, 1447, 1633, 2854, 2925. HPLC: method 2, rt = 2.56 min, purity 97%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.85 – 1.00 (m, 3 H); 1.29-1.48 (m, 6 H); 1.62-1.77 (m, 2 H); 2.46 (s, 2 H); 2.72 (t, *J* = 7.5 Hz, 2 H); 2.86 (s, 2 H); 3.38 (s, 2 H); 3.82 (s, 2 H); 4.87 (s, 1 H); 7.15 (dd, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.32 (td, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.35 – 7.48 (m, 6 H); 7.53 (s, 1 H); 7.55 (d, *J* = 5.4 Hz, 1 H), 7.64 (d, *J* = 7.5 Hz, 1 H); 7.70 (d, *J* = 7.5 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.6 (CH_2); 29.1 (CH_2); 31.7 (CH_2); 31.8 (CH_2); 36.1 (CH_2); 43.0 (CH_2); 48.4 (CH_2); 48.8 (CH_2); 49.5 (CH_2); 69.7 (CH); 119.7 (CH); 119.8 (CH); 125.6 (CH); 125.9 (CH); 127.0 (CH); 127.1 (2 x CH); 127.5 (CH); 128.2 (CH); 128.4 (2 x CH); 129.6 (CH); 135.9 (C); 140.7 (C); 141.1 (C); 141.2 (C); 143.3 (C); 143.8 (C); 170.3 (C). MS (DCI/CH_4) m/z: 438.26 [M+H^+], 249.16 [M-188]. HRMS (DCI/CH_4): for $C_{30}H_{34}N_2O$ [M+H^+]: calcd: 438.2671; found: 438.2674.

*4.2.14.4. (4-(3-Methoxy-9H-fluoren-9-yl)piperazin-1-yl)(phenyl)methanone (**31**).* Reagents: 1-Benzoylpiperazine (0.20 mmol, 38 mg), potassium carbonate (0.40 mmol, 55 mg) and 9-bromo-3-methoxy-9*H*-fluorene (0.20 mmol, 55 mg). The crude product was purified by flash chromatography (isocratic, petroleum ether/ethyl acetate 80/20 in 15 minutes) to afford a brown solid (64 mg, 83%). TLC R_f: 0.17 (petroleum ether/ethyl acetate 80/20); mp: 90 °C; IR (cm^{-1}): 615, 632, 669, 709, 739, 769, 846, 1000, 1016, 1031, 1169, 1212, 1277, 1427, 1453, 1489, 1577, 1628, 2830, 2934. HPLC: method 2, rt = 1.94

min, purity 97%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.44 (s, 2 H); 2.85 (s, 2 H); 3.37 (s, 2 H); 3.82 (s, 2 H); 3.91 (s, 3H); 4.85 (s, 1 H); 6.88 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.33 (td, *J* = 1.2 Hz, 7.2 Hz, 1 H); 7.36 – 7.45 (m, 7 H); 7.54 (d, *J* = 8.4 Hz, 1 H); 7.64 (d, *J* = 6.6 Hz, 1 H); 7.68 (d, *J* = 6.9 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 42.9 (CH_2); 48.5 (CH_2); 48.7 (CH_2); 49.5 (CH_2); 69.4 (CH); 105.2 (CH); 113.1 (CH); 119.8 (CH); 125.8 (CH); 126.6 (CH); 127.1 (2 x CH); 127.3 (CH); 128.3 (CH); 128.4 (2 x CH); 129.6 (CH); 135.4 (C); 135.9 (C); 140.9 (C); 142.6 (C); 144.4 (C); 160.3 (C); 170.3 (C). MS (DCI/CH_4) m/z: 384.18 [M]. HRMS (DCI/CH_4): for $C_{25}H_{24}N_2O_2$ [M]: calcd: 384.1838; found: 384.1835.

*4.2.14.5. Phenyl(4-(3-propoxy-9H-fluoren-9-yl)piperazin-1-yl)methanone (**32**).* Reagents: 1-Benzoylpiperazine (0.23 mmol, 43 mg), potassium carbonate (0.45 mmol, 62 mg) and 9-bromo-3-propoxy-9*H*-fluorene (0.23 mmol, 68 mg). The crude product was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a brown oil (20 mg, 22%). TLC R_f: 0.20 (petroleum ether/ethyl acetate 70/30). IR (cm^{-1}): 670, 709, 727, 768, 907, 980, 1001, 1016, 1142, 1192, 1256, 1277, 1427, 1448, 1490, 1577, 1628, 2855, 2928. HPLC: method 2, rt = 3.40 min, purity 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.11 (t, *J* = 7.5 Hz, 3 H); 1.89 (quint, *J* = 7.5 Hz, 2 H); 2.44 (s, 2 H); 2.86 (s, 2 H); 3.37 (s, 2 H); 3.80 (s, 2 H); 4.04 (t, *J* = 6.6 Hz, 1 H); 4.86 (s, 1 H); 6.87 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.24 (d, *J* = 2.4 Hz, 1 H); 7.32 (td, *J* = 1.2 Hz, 7.2 Hz, 1 H); 7.36 – 7.45 (m, 6 H); 7.53 (d, *J* = 8.4 Hz, 1 H); 7.63 (d, *J* = 7.2 Hz, 1 H); 7.67 (d, *J* = 7.2 Hz, 1 H).^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 10.6 (CH_3); 22.7 (CH_2); 48.6 (2 x CH_2); 49.5 (2 x CH_2); 69.4 (CH); 69.8 (CH_2); 105.8 (CH); 113.6 (CH); 119.7 (CH); 125.8 (CH); 126.6 (CH); 127.1 (2 x CH); 127.2 (CH); 128.3 (CH); 128.4 (2 x CH); 129.6 (CH); 135.2 (C); 135.9 (C); 141.0 (C); 142.5 (C); 144.4 (C); 159.9 (C); 170.3 (C). MS (DCI/CH_4) m/z: 441.25 [M+$C_2H_5^+$], 412.22 [M], 223.11 [M-189]. HRMS (DCI/CH_4): for $C_{27}H_{28}N_2O_2$ [M]: calcd: 412.2151; found: 412.2158.

*4.2.14.6. (4-(3-(Hexyloxy)-9H-fluoren-9-yl)piperazin-1-yl)(phenyl)methanone (**33**).* Reagents: 1-Benzoylpiperazine (0.21 mmol, 40 mg), potassium carbonate (0.42 mmol, 58 mg) and 9-bromo-3-hexyloxy-9*H*-fluorene (0.21 mmol, 63 mg). The crude product was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a brown oil (54 mg, 57%). TLC R_f: 0.20 (petroleum ether/ethyl acetate 70/30). IR (cm^{-1}): 615, 633, 670, 708, 734, 768, 788, 847, 1000, 1016, 1142, 1190, 1256, 1277, 1301, 1426, 1449, 1490, 1578, 1630, 2857, 2929. HPLC: method 2, rt = 5.36 min, purity 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.91 – 1.02 (m, 3 H); 1.33-1.47 (m, 4 H); 1.47-1.61 (m, 2 H); 1.87 (quin, *J* = 6.9 Hz, 2 H); 2.44 (s, 2 H); 2.86 (s, 2 H); 3.37 (s, 2 H); 3.82 (s, 2 H); 4.07 (t, *J* = 6.6 Hz, 2 H); 4.85 (s, 1 H); 6.87 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.24 (d, *J* = 2.4 Hz, 1 H); 7.32 (td, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.36 – 7.45 (m, 6 H); 7.52 (d, *J* = 8.4 Hz, 1 H); 7.63 (d, *J* = 5.2 Hz, 1 H); 7.67 (d, *J* = 6.9 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 25.8 (CH_2); 29.4 (CH_2); 31.7 (CH_2); 42.9 (CH_2); 48.5 (CH_2); 48.6 (CH_2); 49.5 (CH_2); 68.3 (CH_2); 69.4 (CH); 105.8 (CH); 113.6 (CH); 119.7 (CH); 125.8 (CH); 126.6 (CH); 127.1 (2 x CH); 127.2 (CH); 128.2 (CH); 128.4 (2 x CH); 129.6 (CH); 135.2 (C); 135.9 (C); 141.0 (C); 142.5 (C); 144.4 (C); 159.9 (C); 170.3 (C). MS (DCI/CH_4) m/z: 454.26 [M], 265.16 [M-189]. HRMS (DCI/CH_4): for $C_{30}H_{34}N_2O_2$ [M]: calcd: 454.2620; found: 454.2607.

*4.2.14.7. Phenyl(4-(2-propoxy-9H-fluoren-9-yl)piperazin-1-yl)methanone (**34**).* Reagents: 1-Benzoylpiperazine (0.06 mmol, 11 mg), potassium carbonate (0.12 mmol, 16 mg) and 9-bromo-2-propoxy-9*H*-fluorene (0.06 mmol, 18 mg). The crude product was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a yellow oil (10 mg, 42%). TLC R_f: 0.34 (petroleum ether/ethyl acetate 70/30). IR (cm^{-1}): 708, 738, 766, 824, 1002, 1141, 1277, 1454, 1632, 1716, 2868, 2927, 3047, 3449. HPLC: method 2, rt = 4.12 min, purity 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.12 (t, *J* = 7.5 Hz, 3 H); 1.89 (sex, *J* = 6.6 Hz, 2 H); 2.47 (s, 2 H); 2.89 (s, 2 H); 3.38 (s, 2 H); 3.83 (s, 2 H); 4.03 (t, *J* = 6.6 Hz, 2 H); 4.85 (s, 1 H); 6.95 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.20 (s, 1 H); 7.25 (t, *J* = 6.9 Hz, 1 H); 7.33 – 7.44 (m, 6 H); 7.56 – 7.64 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 10.3 (CH_3); 22.7 (CH_2); 29.7 (CH_2); 42.8 (CH_2); 48.6 (CH_2); 49.6 (CH_2); 69.9 (CH); 77.2 (CH_2); 112.4 (CH); 114.5 (CH); 119.0 (CH); 120.5 (CH); 125.8 (CH); 125.9 (CH); 127.1

(2 x CH); 128.3 (CH); 128.4 (2 x CH); 129.6 (CH); 133.8 (C); 135.9 (C); 141.2 (C); 142.9 (C); 145.3 (C); 159.1 (C); 170.3 (C). MS (DCI/CH_4) m/z: 413.22. HRMS (DCI/CH_4): $C_{27}H_{29}N_2O_2$ [M+H^+]: calcd: 413.2229; found: 413.2235.

*4.2.14.8. (4-(3-(Benzyloxy)-9H-fluoren-9-yl)piperazin-1-yl)(phenyl)methanone (**35**).* Reagents: 1-Benzoylpiperazine (0.23 mmol, 44 mg), potassium carbonate (0.46 mmol, 64 mg) and 3-(benzyloxy)-9-bromo-9*H*-fluorene (0.23 mmol, 81 mg). The crude product was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a yellow oil (54.3 mg, 50%). TLC R_f: 0.40 (petroleum ether/ethyl acetate 80/20). IR (cm^{-1}): 697, 710, 770, 1001, 1017, 1186, 1257, 1278, 1427, 1448, 1488, 1578, 1628, 2851, 2920. HPLC: method 2, rt = 3.65 min, purity 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.45 (bs, 2 H); 2.86 (bs, 2 H); 3.38 (bs, 2 H); 3.83 (bs, 2 H); 4.86 (s, 1 H); 5.18 (s, 2 H); 6.97 (dd, *J* = 2.4 Hz, 8.1 Hz, 1 H); 7.34 (td, *J* = 2.1 Hz, 7.8 Hz, 2 H); 7.37 – 7.48 (m, 9 H); 7.51 (s, 1 H); 7.53 (t, *J* = 8.4 Hz, 2 H); 7.65 (t, *J* = 8.1 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 42.9 (CH_2); 48.6 (2 x CH_2); 49.5 (CH_2); 69.4 (CH_2); 70.4 (CH_2); 106.3 (CH); 113.8 (CH); 119.8 (CH); 125.9 (CH); 126.6 (CH); 127.1 (2 x CH); 127.4 (CH); 127.6 (2 x CH); 128.1 (CH); 128.3 (CH); 128.4 (2 x CH); 128.7 (2 x CH); 129.6 (CH); 135.7 (C); 135.9 (C); 137.0 (C); 140.9 (C); 142.6 (C); 144.4 (C); 159.5 (C); 170.3 (C). MS (DCI/CH_4) m/z: 461.22 [M+H^+]. HRMS (DCI/CH_4): for $C_{31}H_{29}N_2O_2$ [M+H^+]: calcd: 461.2229; found: 461.2235.

*4.2.15. 3-(Hexyloxy)-9H-fluorene (**36**).* A mixture of 3-(hexyloxy)-9*H*-fluoren-9-one **25** (97.8 mg, 0.34 mmol, 1.0 eq) and hydrazine hydrate (247 µL) was stirred on 3.7 mL diethylene glycol for 10 minutes, then 40% KOH solution (365 µL) was added dropwise, and the mixture was refluxed for 4 hours. Brine was added and the product was extracted three times with ethyl acetate, dried over magnesium sulfate and concentrated under vacuum pressure. The crude product was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a yellow powder (89 mg, 98%). TLC R_f: 0.48 (petroleum ether/ethyl acetate 97/3); mp: 111 °C; IR (cm^{-1}): 730; 765, 808, 851, 900, 1033, 1186, 1211, 1243, 1280, 1306, 1325, 1399, 1450, 1474, 1492, 1578, 1608, 2855, 2871, 2887, 2921, 2947. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.0 (t, *J* = 9.0 Hz, 3H); 1.28-1.49 (m, 4H); 1.52-1.70 (m, 2H);1.69 (quint, *J* = 6.0 Hz, 2H); 3.88 (s, 2H); 4.11 (t, *J* = 6.6 Hz, 2H); 6.93 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H); 7.36 (td, *J* = 1.2 Hz, 7.2 Hz, 1H); 7.38 (d, *J* = 2.4 Hz, 1H); 7.43 (t, *J* = 7.5 Hz, 1H); 7.46 (t, *J* = 8.4 Hz, 1H); 7.58 (d, *J* = 7.2 Hz, 1H); 7.81 (d, *J* = 7.2 Hz, 1H). . ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 25.9 (CH_2); 29.5 (CH_2); 31.7 (CH_2); 36.2 (CH_2); 68.4 (CH_2); 105.7 (CH); 113.8 (CH); 119.8 (CH); 125.1 (CH); 125.5 (CH); 126.7 (CH); 126.8 (CH); 135.2 (C); 141.8 (C); 143.0 (C); 144.3 (C); 158.8 (C). MS (DCI/CH_4) m/z: 267.18 [M+H^+]. HRMS (DCI/CH_4): for $C_{19}H_{23}O$ [M+H^+]: calcd 267.1749; found 267.1761.

*4.2.16. General procedure for **39** and **40**.* Reagent **38** was synthesized according to a procedure reported in supporting information. The corresponding fluorene (1.0 eq) was dissolved in dry tetrahydrofuran (2.5 mL) under argon. *n*-Butyl lithium solution (1.6 M in hexanes, 1.0 eq) was added slowly to the reaction mixture at room temperature. The reaction mixture was then cooled to -78°C and a solution of *N*-benzoyl-4-bromopiperidine **38** (1.0 eq) in dry tetrahydrofuran (5.0 mL) was added dropwise. The reaction mixture was then warmed slowly to room temperature and let stirring overnight at room temperature. Saturated solution of ammonium chloride (20.0 mL) was then added and the product was extracted three times with ethyl acetate. The organic phase was then washed with brine, dried over magnesium sulfate and concentrated under vacuum pressure. Preparative scale liquid chromatography

with Xbridge C18 column 5 µm (19 x 150 mm) was achieved on AutoPurification HPLC/PDA System (from Waters). The mixture water/acetonitrile as gradient eluant was needed to separate unreacted starting compound and product. The AutoPurification HPLC System included 2767 Sample Manager, 2545 Binary Gradient Module, System Fluidics Organizer, 2489 UV/Visible Detector and MassLynx Software with the FractionLynx Application Manager.

*4.2.16.1. (4-(9H-Fluoren-9-yl)piperidin-1-yl)(phenyl)methanone (**39**).* Reagents: Fluorene **37** (0.37 mmol, 62 mg), *n*-butyl lithium solution (1.6 M in hexanes, 0.37 mmol, 233 µL) and *N*-benzoyl-4-bromopiperidine **38** (0.37 mmol, 100 mg). The crude product was purified by HPLC to afford a yellow oil (60 mg, 46%). TLC R_f: 0.16 (petroleum ether/ethyl acetate). IR (cm^{-1}): 696, 707, 727, 908, 968, 1167, 1276, 1290, 1325, 1376, 1446, 1575, 1611, 2858, 2940. HPLC: method 2, rt = 4.22 min, purity 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.06 – 1.46 (m, 2H); 1.48 – 1.90 (m, 2 H); 2.39 – 2.54 (m, 1 H); 2.65 – 2.87 (m, 1 H); 2.87 – 3.08 (m, 1 H); 3.60 – 3.89 (m, 1 H); 4.02 (d, *J* = 3.3 Hz, 1 H); 4.67 – 4.97 (m, 1 H); 7.31 – 7.48 (m, 9 H); 7.56 (s, 2 H); 7.79 (d, *J* = 7.2 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 28.7 (CH_2); 29.0 (CH_2); 41.5 (CH); 43.3 (CH_2); 48.6 (CH_2); 52.1 (CH); 119.9 (2 x CH); 124.6 (CH); 125.1 (CH); 126.9 (2 x CH_2); 127.0 (2 x CH); 127.4 (2 x CH); 128.5 (2 x CH); 129.8 (CH); 135.4 (C); 141.6 (2 x C); 145.0 (C); 145.4 (C); 170.8 (C). MS (DCI/CH_4) m/z: 354.18 [M+H^+]. HRMS (DCI/CH_4): for $C_{25}H_{24}NO$ [M+H^+]: calcd: 354.1858; found: 354.1855.

*4.2.16.2. (4-(3-(Hexyloxy)-9H-fluoren-9-yl)piperidin-1-yl)(phenyl)methanone (**40**).* Reagents: 3-(Hexyloxy)-9*H*-fluorene **36** (0.21 mmol, 59 mg), *n*-butyl lithium solution (1.6 M in hexanes, 0.25 mol, 156 µL) and *N*-benzoyl-4-bromopiperidine **38** (0.21 mmol, 56 mg). The crude product was purified by HPLC to afford a yellow oil (49 mg, 49%). TLC R_f: 0.32 (petroleum ether/EtOAC). IR (cm^{-1}): 707, 735, 771, 970, 1022, 1051, 1204, 1237, 1284, 1370, 1448, 1491, 1578, 1629, 1707, 2856, 2930, 3057. HPLC: method 1, rt = 1.21 min, purity 96%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.96 (t, *J* = 6.9 Hz, 3 H); 1.22 (bs, 2 H); 1.32-1.45 (m, 4 H); 1.47-1.58 (m, 2 H); 1.67 (bs, 2 H); 1.87 (quint, *J* = 6.9 Hz, 2 H); 2.30-2.47 (m, 1 H); 2.74 (bs, 1H); 2.91 (bs, 1 H); 3.70 (bs, 1H); 3.95 (d, *J* = 3.0 Hz, 1 H); 4.08 (t, *J* = 6.6 Hz, 2 H); 4.81 (bs, 1 H); 6.90 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H); 7.26-7.47 (m, 9 H); 7.52 (bs, 1 H); 7.72 (d, *J* = 7.2 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 25.8 (CH_2); 28.3 (CH_2); 29.0 (CH_2); 29.4 (CH_2); 31.7 (CH_2); 41.6 (CH); 42.8 (CH_2); 48.4 (CH_2); 51.5 (CH); 68.3 (CH_2); 105.7 (CH); 113.7 (CH); 119.8 (CH); 125.1 (CH); 125.6 (CH); 126.8 (2 x CH); 127.0 (CH); 127.2 (CH); 128.4 (2 x CH); 129.5 (CH); 136.2 (C); 141.6 (C); 142.9 (C); 144.7 (C); 146.5 (C); 159.1 (C); 170.3 (C). MS (DCI/CH_4) m/z: 454.27 [M+H^+]. HRMS (DCI/CH_4): for $C_{31}H_{36}NO_2$ [M+H^+]: calcd: 454.2746; found: 454.2741.

*4.2.17. 1-Benzoyl-4-(9H-fluoren-9-yl)piperazin-2-one (**42**).* Reagent **41** was synthesized according to a procedure reported in supporting information. A solution of *tert*-butyl 4-benzoyl-3-oxopiperazine-1-carboxylate **41** (0.24 mmol, 72 mg, 1.0 eq) in anhydrous dichloromethane (5.0 mL) was treated with trifluoroacetic acid (3.55 mmol, 264 µL, 15.0 eq) at 0°C. The solution was allowed to warm to room temperature and stirred overnight at room temperature. The solvent was removed under vacuum pressure and the crude mixture was used as such (without further purification) in the following step. The crude *N*-Boc deprotected compound was dissolved in anhydrous dichloromethane (5.0 mL) and triethylamine (0.47 mmol, 64 µL, 2.0 eq) was added. After 30 minutes stirring at room temperature, a solution of 9-bromo-fluorene (0.47 mmol, 116 mg, 2.0 eq) in anhydrous dichloromethane (2.0 mL) was added to the cooled reaction mixture. After overnight stirring, brine (20.0 mL) was added and the compound was extracted with dichloromethane. The organic phase was dried over magnesium sulfate, filtered and concentrated under vacuum pressure. The residue was purified by flash chromatography (gradient, 100%

petroleum ether to 100% ethyl acetate in 15 minutes) to afford the title compound as a yellow solid (46 mg, 52%). TLC R_f: 0.41 (petroleum ether/ethyl acetate 80/20); mp: 175.6; IR (cm^{-1}): 571, 622, 701, 742, 949, 1014, 1075, 1152, 1236, 1278, 1323, 1395, 1449, 1600, 1681, 1711, 3062. HPLC: method 2, rt = 1.32 min, purity 87%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.12 (dd, *J* = 3.9 Hz, 5.7 Hz, 2 H); 3.40 (s, 2 H); 3.86 (dd, *J* = 3.9 Hz, 6.9. Hz, 2 H); 5.01 (s, 1 H); 7.37 (td, *J* = 1.2 Hz, 7.5 Hz, 2 H); 7.41 (tt, *J* = 1.5 Hz, 7.8 Hz, 2 H); 7.44 – 7.61 (m, 5 H); 7.67 (d, *J* = 7.5 Hz, 2 H); 7.76 (d, *J* = 7.5 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 45.8 (CH_2); 46.8 (CH_2); 54.2 (CH_2); 68.4 (CH); 120.2 (CH); 125.9 (CH); 127.5 (CH); 128.0 (CH); 128.1 (CH); 128.8 (CH); 131.8 (CH); 135.8 (C); 141.3 (C); 142.0 (C); 169.6 (C); 173.8 (C). MS (DCI/CH_4) m/z: 369.16 [M+H$^+$]. HRMS (DCI/CH_4): for $C_{24}H_{21}N_2O_2$ [M+H$^+$]: calcd: 369.1603; found: 369.1620.

4.2.18. Chiral chromatography. Supercritical fluid chromatography (SFC) preparative scale was performed on a Purification Prep 80 system (from Waters) with a chiralpak AD-H 5 µm (10 x 250) mm column for enantiomeric separation of compounds **31** and **33**. Those racemic mixtures were respectively eluted with 20% and 25% methanol at 15 mL/min (P_{out} = 100 bar, oven temperature = 40 °C). For analytical chromatography, a SFC-Piclab Analytic Picsolution instrument was used with an AD-H 5 µm (4.6 x 250) mm column. Each analysis was performed at 4 mL/min (P_{out} = 100 bar, oven temperature = 35 °C) in an identical co-solvent percentage than in purification step.

The retention times for each enantiomers are the following: **31a**: rt = 10.5 min; **31b**, rt = 12.6 min; **33a**: rt = 10.4 min; **33b**: rt = 16.4 min. Additional informations are reported in supporting information document.

4.3. Biology

*4.3.1. InhA expression and purification***.** The production and purification of the InhA-6xHis protein from a protease-deficient strain of *E. coli* BL21(DE3) transformed with the pHAT5/*inhA* plasmid were performed as followed. 1 mL of the bacteria was grown in 100 mL of LB medium containing ampicillin (100mg/mL) at 37°C. After 4 h, the solution was rediluted in 1 L of the same medium and re-grown at 37°C. When the proper concentration (OD_{595} = 0.6-0.8) was reached, protein expression was induced for overnight incubation with 1 mM isopropyl-β-D-galactopyranoside (IPTG) at 20°C. Cells were harvested by centrifugation at 6,000 g for 30 min at 4°C. The dry pellet was kept at -80°C for several months. Thawed cells (1.5 g) were sonicated in 20 mL lysis buffer (300 mM NaCl, 10 mM imidazole, 50 mM sodium phosphate buffer, pH 8.0). After centrifugation at 10,000 g for 45 min at 4 °C, the supernatant was applied onto a nickel-chelated His-Trap HP 1 mL column (GE Healthcare) previously equilibrated with the binding buffer (50 mM NaCl, 10 mM imidazole, 50 mM sodium phosphate buffer, pH 8,0). First, the unbound proteins were washed out with 10 column volume of binding buffer, and then a higher imidazole concentration (25 mM) allows the elution of non-specifically bound proteins. The His_6-tagged InhA protein was eluted with an imidazole gradient from 25 mM to 300 mM over a range of 20 column volume. Fractions containing the target protein were pooled, concentrated to 2.0 mL and loaded on a HiLoad 16/60 Superdex 200 column (GE Healthcare) equilibrated with 150 mM NaCl, 30 mM PIPES, pH 6.8. Samples were analyzed using SDS-PAGE and Coomassie blue staining and then stored at 4°C for short term storage or 80°C with 20% glycerin for long-term storage.

4.3.2. InhA activity inhibition. Triclosan and NADH were obtained from Sigma-Aldrich. Stock solutions of all compounds were prepared in DMSO such that the final concentration of this co-solvent was constant at 5% v/v in a final volume of 1 mL for all kinetic reactions. Kinetic assays were performed using *trans*-2-dodecenoyl-coenzyme A (DDCoA) and wild type InhA as previously described.[38] Briefly, reactions were performed at 25 °C in an aqueous buffer (30 mM PIPES and 150 mM NaCl pH 6.8) containing additionally 250 µM cofactor (NADH), 50 µM substrate (DDCoA) and the tested compound (at 50 µM or 10 µM). Reactions were initiated by addition of InhA (100 nM final) and NADH oxidation was followed at 340 nm. The inhibitory activity of each derivative was expressed as the percentage inhibition of InhA activity (initial velocity of the reaction) with respect to the control reaction without inhibitor. Triclosan was used as a positive control. All activity assays were performed in triplicate. For the most potent compounds, IC_{50} values were determined using the 4-parameter curve-fitting software XLFit (IDBS) with at least six points.

4.3.3. MIC determination in M. tuberculosis growth inhibition. M. tuberculosis H37Rv strain, used as the reference strain, was grown at 37 °C in Middlebrook 7H9 broth (Difco), supplemented with 0.05% Tween 80, or on solid Middlebrook 7H11 medium (Difco) supplemented with oleic acid-albumin-dextrose-catalase (OADC). MICs for the new compounds were determined by means of the micro-broth dilution method. Dilutions of *M. tuberculosis* wild-type culture (about 10^5-10^6 cfu/ml) were streaked onto 7H11 solid medium containing a range of drug concentrations (0.25 µg/mL to 40 µg/mL). Plates were incubated at 37 °C for about 21 days and the growth was visually evaluated. The lowest drug dilution at which visible growth failed to occur was taken as the MIC value. Results were expressed as the average of at least three independent determinations. The MIC was also determined in the presence of efflux pumps inhibitors reserpine, verapamil and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) used at 3.0, 40.0 and 7.5 µg/mL final concentration, respectively.

4.4. *Computational chemistry***.** Molecular graphics, particularly depicted molecular surfaces [39] were performed with the UCSF Chimera package.[40] Chimera is developed by the Ressource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by the NIGMS P41-GM103311). The protein structures used in this paper were structurally aligned with structure 1BVR (chain A) set as reference (defining a reference space) and using UCSF Chimera/Matchmaker [41] program. The protein structures in the reference space were prepared (structure checks, rotamers, hydrogenation) using Accelrys (Discovery Studio Modeling Environment, Release 4.0, San Diego: Accelrys Software Inc., 2013) Discovery Studio Visualizer 4.0 (DSV) and UCSF Chimera. The new compounds were sketched using ChemAxon Marvin 5.5, (http://www.chemaxon.com). All ligands (extracted from protein structures or new) were checked (hybridization, hydrogenation, some geometry optimizations, 3D sketching) and were merged in SDF libraries using DSV.

Molecular modeling studies [42] were carried with Molegro Virtual Docker 6 software (http://www.clcbio.com) using the chain A of structure 1P44 (1P44a) as target and a search space volume of 17 Å radius centered in the binding pocket. Ligands were set flexible during the Docking. According to structural study, 23 residues were defined for flexible docking:

ARG225, TRP222, GLU219, LEU218, ILE215, ILE202, MET199, ALA198, THR196, PRO193, LYS165, MET161, TYR158, ALA157, MET155, ARG153, PHE149, SER123, MET103, GLN100, PRO99, MET98 and PHE97. Final minimization was parameterized using 10000 steps for lateral chains or binding pocket residues, other parameters (backbone) were let with default values. No water molecules were taken in account in the study. Docking process uses the PLANTS [43] function for scoring and Moldock optimizer (MVD, 6000 iteration steps, other parameters let as default) for searches. MolDock and Rerank [44] scores were calculated post-docking. Each compound was docked using 50 independent runs. For chiral compounds, both configurations *R* and *S* were taken in account in the process.

The 1050 docking poses (21 ligands including GEQ) issued from the calculations were visually checked using MVD's features and a filter was applied on these RAW results in order to give secondary data: two 'best' poses per compound. For visual inspection, the following rule was defined: *a1)* the pose was selected if the conformation of substituted fluorine group of compound was very close with the fluorine/piperazinamide/indole alignment found in crystallographic conformation of GEQ. These poses were considered to fulfill structural conformity criterion. For scores, the following rules were defined: *a2)* the pose was selected if the lowest negative values of PLANTS and MolDock and Rerank scores were found for the same pose; *a3)* if not, the pose with best scores combination (priority PLANTS > MolDock > Rerank) was retained. The PLANTS scoring scheme was used in calculations, so it was selected for the higher priority. The poses corresponding to rules *a1-a2* were called 'strong' poses because they combine best scoring results and structural conformity. Then, the rules *a2-a3* were repeated with the second ranked scores values of the set, to ensure that, at least, two poses (more conform as possible) will be available per compound. We noticed that a lot (75%) of selected poses were strong poses independently of the stereochemistry or the substitution of fluorene scaffold.

Then in order to rank ligands, another set of rules was applied on the secondary data giving a final table (See Supporting Information): *b1)* the best 'strong' pose was retained; if not found *b2)* the pose corresponding to the best score combination was retained using the priority (Rerank > MolDock) > PLANTS. Rerank score is generally used in ligand ranking, so it was selected for the higher priority. Then, in order to approach *in silico* ligand efficiencies (LE) values, we used the following metric: MolDock, PLANTS and Rerank scores were divided by the number (HA) of heavy (C, N, O) atoms, giving LE1, LE2 and LE3 descriptors [32].

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Highlights :

- Multi-step synthesis of GEQ analogues as potential inhibitors of InhA.
- One of the 25 compounds was effective against InhA and *Mtb* H37Rv strain.
- The same compound exhibited moderate antimycobacterial activity.
- Efflux pump inhibitors potentiate the activity of these inhibitors.

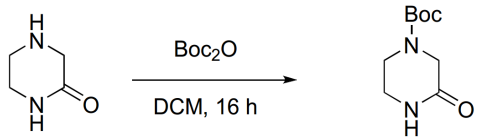
Supporting materials

Design, synthesis and evaluation of GEQ derivatives as inhibitors of InhA enzyme and *Mycobacterium tuberculosis* growth

Aurélien Chollet, Giorgia Mori, Christophe Menendez, Frédéric Rodriguez, Isabelle Fabing, Maria Rosalia Pasca, Jan Madacki, Jana Korduláková, Patricia Constant, Annaïk Quémard, Vania Bernardes-Génisson, Christian Lherbet, Michel Baltas,

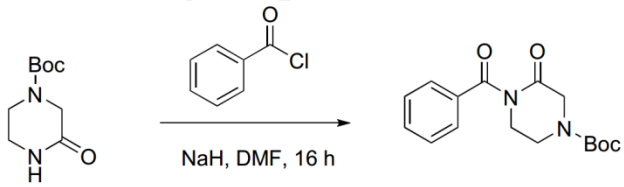
Synthesis of the intermediates

1.1. *tert*-butyl 3-oxopiperazine-1-carboxylate



Boc_2O (2.0 mmol, 436 mg, 1.0 eq) was added in portions under stirring and cooling on an ice bath to a suspension of piperazin-2-one (2.0 mmol, 200 mg, 1.0 eq) in anhydrous dichloromethane (10.0 mL). The reaction mixture was stirred overnight at room temperature, during which a homogeneous solution formed. The solvent was evaporated and the solid residue was vacuum-dried to furnish a yellow solid (300.0 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.51 (s, 9 H); 3.37-3.46 (m, 2 H); 3.66 (t, *J* = 5.2 Hz, 2 H); 4.12 (s, 2 H); 6.39 (bs, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 27.4 (CH_2); 28.3 (3 x CH_3); 41.2 (CH_2); 77.1 (CH_2); 80.9 (C); 153.9 (C); 168.0 (C). MS (DCI/NH_3) m/z: 201.1 [M+H^+]; 218.1 [M + $NH4^+$].

1.2. *tert*-butyl 4-benzoyl-3-oxopiperazine-1-carboxylate (41)

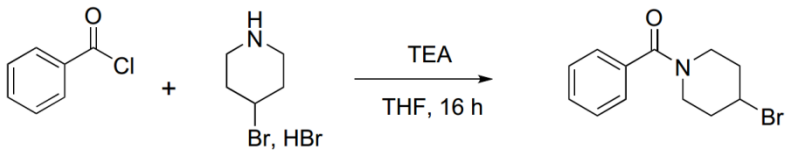


tert-butyl 4-benzoyl-3-oxopiperazine-1-carboxylate (**41**) was synthesized according to a previously reported procedure.[1]

A solution of *tert*-butyl 3-oxopiperazine-1-carboxylate (0.50 mmol, 100 mg, 1.0 eq) in anhydrous DMF (7.5 mL) was treated with sodium hydride (60% oil dispersion, 0.60 mmol, 24 mg, 1.2 eq) and benzoyl chloride (0.60 mmol, 70 µL, 1.2 eq), and stirred overnight at room temperature. The reaction mixture was concentrated under vacuum pressure and ethyl acetate (20.0 mL) was added. The organic phase was washed with brine, dried over magnesium sulfate, filtered and concentrated under vacuum pressure. The residue was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to

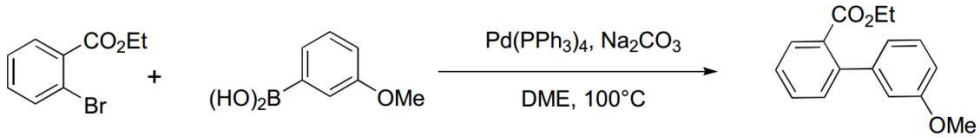
afford a colorless oil (65 mg, 43%). TLC R_f: 0.45 (PE/EtOAc 70/30). IR (cm^{-1}): 666, 684, 704, 932, 1128, 1165, 1244, 1287, 1323, 1419, 1452, 1679, 2552, 2977. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.53 (s, 9 H); 3.73-3.83 (m, 2 H); 3.92-4.01 (m, 2 H); 4.25 (s, 2 H); 7.40-7.48 (m, 2 H); 7.55 (tt, *J* = 2.4 Hz, 7.2 Hz, 2 H); 7.59 – 7.65 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 28.4 (3 x CH_3); 43.5 (CH_2); 48.4 (CH_2); 48.8 (CH_2); 81.3 (C); 128.2 (2 x CH); 128.3 (2 x CH); 132.2 (CH); 135.1 (C); 153.8 (C); 168.2 (C); 172.9 (C). MS (DCI/CH_4) m/z: 304.14 [$M+H^+$], 249.09 [M-55 (tBu)], 204.09 [M-100 (Boc)]. HRMS (DCI/CH_4): for $C_{19}H_{21}O$ [M]: calcd: 265.1592; found: 265.1599.

1.3. (4-bromopiperidin-1-yl)(phenyl)methanone (38)



4-Bromopiperidine hydrobromide (1.63 mmol, 300 mg, 1.0 eq) was dissolved in dry tetrahydrofuran (5.0 mL). After cooling the reaction mixture in an ice bath, triethylamine (3.56 mmol, 485 µL, 2.2 eq) and benzoyl chloride (1.63 mmol, 190 µL, 1.0 eq) were slowly added. After overnight stirring at room temperature, the reaction mixture was concentrated under vacuum pressure, dissolved in dichloromethane and was successively washed with water and brine. The organic phase was dried over magnesium sulfate, filtered and concentrated under vacuum pressure. The resulting crude product was purified by flash chromatography (petroleum ether/ethyl acetate 90/10 10 minutes, then gradient until 100% ethyl acetate in 10 minutes) to a colorless oil (214 mg, 98%). TLC R_f: 0.77 (DCM/MeOH 95/5). IR (cm^{-1}): 569, 638, 691, 702, 714, 787935, 996, 1139, 1209, 1263, 1270, 1335, 1343, 1367, 1431, 1623, 2874, 2928. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.70-2.42 (bs, 4 H); 3.15-4.19 (m, 4 H); 4.47 (sep, *J* = 3.6 Hz, 1 H); 7.38-7.47 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 35.5 (CH_2); 35.9 (CH_2); 40.3 (CH_2); 45.9 (CH_2); 48.9 (CH); 126.9 (2 x CH); 128.6 (2 x CH); 129.8 (C) 135.7 (C); 170.4 (C). MS (DCI/CH_4) m/z: 266.02 [M]. HRMS (DCI/CH_4): for $C_{12}H_{13}BrNO$ [M]: calcd: 266.0181 found: 266.0176.

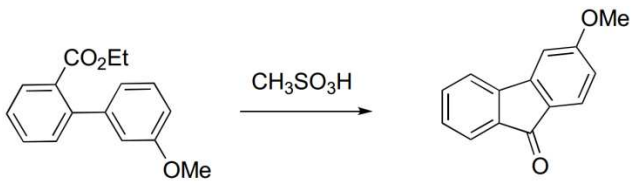
1.4. Ethyl 3'-methoxy-[1,1'-biphenyl]-2-carboxylate



Ethyl 3'-methoxy-[1,1'-biphenyl]-2-carboxylate was synthesized according to a previously reported procedure.[2]

Ethyl 2-bromobenzoate (2.2 mmol, 500 mg, 1.0 eq) and tetrakistriphenylphosphinPEalladium (0.06 mmol, 76 mg, 0.03 eq) were dissolved in 1,2-dimethoxyethane (25.0 mL). A 2 M solution of sodium carbonate (4.4 mmol, 2.2 mL of a 2 M aqueous solution, 2.0 eq) was added via a syringue. A solution of 3-methoxyphenylboronic acid (2.4 mmol, 365 mg, 1.1 eq) in 1,2-dimethoxyethane (10.0 mL)was subsequently added. The reaction mixture was heated at 100°C and allowed to stir overnight. It was then cooled, diluted with water, extracted with ethyl acetate, and washed with an aqueous saturated solution of sodium hydrogenocarbonate and brine. The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether:ethyl acetate 95:5 in 15 minutes) to afford the a colorless oil (390 mg, 70%). TLC R_f: 0.32 (PE/EtOAc 95/5). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.07 (t, *J* = 7.2 Hz, 3 H); 3.86 (s, 3H); 4.16 (q, *J* = 7.2 Hz, 2 H); 6.91-6.99 (m, 3 H); 7.29-7.38 (m, 1 H); 7.40-7.49 (m, 2 H); 7.51-7.59 (m, 1 H) ; 7.84-7.89 (m, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 13.7 (CH_3); 55.3 (CH_3); 61.0 (CH_2); 112.9 (CH); 114.0 (CH); 121.0 (CH); 127.3 (CH); 129.1 (CH); 129.6 (CH); 130.5 (CH); 131.1 (CH); 131.5 (C); 142.2 (C); 142.9 (C); 159.4 (C); 168.9 (C). MS (DCI/CH_4) m/z: 255.1 [M]. 211.1 [M-45 (OEt)]. HRMS (DCI/CH_4): for $C_{16}H_{16}O_3$ [M]: calcd: 256.1104; found: 256.1099.

1.5. 3-methoxy-9*H*-fluoren-9-one (21)



3-Methoxy-9*H*-fluoren-9-one (**21**) was synthesized according to a previously reported procedure.

A solution of ethyl 3'-methoxy-[1,1'-biphenyl]-2-carboxylate (1.5 mmol, 390 mg, 1.0 eq) in methanesulfonic acid (320 mmol, 20.7 mL, 210 eq) was stirred and heated to 110°C for 1 hour. The resulting black mixture was poured slowly into stirred ice water and then extracted with diethyl ether. The combined organic layers were washed with saturated aqueous solution of sodium hydrogenocarbonate, and water, and then dried over magnesium sulfate, filtered and concentrated under vacuum pressure. The residue was purified by flash chromatography (gradient, 100% petroleum ether to 100% ethyl acetate in 15 minutes) to afford a colorless oil (260 mg, 81%). TLC R_f: 0.38 (PE/EtOAc 90/10). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.94b (s, 3H); 6.77 (dd, *J* = 2.1 Hz, *J* = 8.1 Hz, 1 H); 7.06 (d, *J* = 2.4 Hz, 1 H); 7.29-7.36 (m, 1 H); 7.44-7.55 (m, 2 H); 7.61-7.70 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 55.8 (CH_3); 107.1 (CH); 113.0 (CH); 120.1 (CH); 123.9 (CH); 126.3 (CH); 129.3 (CH); 134.2 (CH); 136.0 (C); 143.4 (C); 147.1 (C); 165.4 (C). MS (DCI/CH_4) m/z: 211.1 [M +H^+]. HRMS (DCI/CH_4): for $C_{14}H_{11}O_2$ [M+H^+]: calcd: 211.0756; found: 211.0759

1.6. General procedure for reduction of the ketone by $NaBH_4$. The corresponding substituted 9*H*-fluoren-9-one (1.0 eq) was dissolved in methanol and cooled in an ice bath. Sodium borohydride (1.2 eq) was added and after 15 minutes stirring at room temperature, the reaction mixture became colorless. A 6 M hydrochloric acid solution was added to the reaction mixture until pH = 7. Methanol was removed under vacuum pressure and the residue was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated under vacuum to furnish the titled compound. No further purification was needed.

2-Hexyl-9*H*-fluoren-9-ol. Reagents: 2-Hexyl-9*H*-fluoren-9-one **17** (0.38 mmol, 100 mg) and sodium borohydride (0.45 mmol, 17 mg). A yellow oil was obtained (100 mg, 100%). TLC R_f: 0.20 (petroleum ether/ethyl acetate 95/5). IR (cm^{-1}): 527, 623, 735, 745, 767, 828, 1022, 1180, 1232, 1303, 1458, 1466, 2850, 2920, 3217, 3314. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.88-1.00 (m, 3 H); 1.30-1.48 (m, 6 H); 1.63-1.77 (m, 2 H); 2.70 (t, *J* = 7.5 Hz, 2 H); 5.55 (s, 1 H); 7.23 (dd, *J* = 1.5 Hz, 7.8 Hz, 1 H); 7.32 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.40 (td, *J* = 1.2 Hz, 7.8 Hz, 1 H); 7.49 (s, 1 H); 7.57 (d, *J* = 7.8 Hz, 1 H); 7.60-7.66 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 29.1 (CH_2); 31.7 (CH_2); 31.8 (CH_2); 36.1 (CH_2); 75.2 (CH); 119.7 (CH); 119.7 (CH); 125.1 (CH); 125.2 (CH); 127.3 (CH); 129.0 (CH); 129.2 (CH); 137.6 (C); 140.2 (C); 143.1 (C); 145.7 (C); 145.9 (C). MS

(DCI/CH_4) m/z: 267.17 [M+H^+]; 249.16 [M-17 (OH)]. HRMS (DCI/CH_4): for $C_{19}H_{23}O$ [M]: calcd: 267.1749; found: 267.1744.

2-Octyl-9*H*-fluoren-9-ol. Reagents: 2-Octyl-9*H*-fluoren-9-one **18** (0.34 mmol, 100 mg) and sodium borohydride (0.41 mmol, 16 mg). A white solid was obtained (99 mg, 99%). TLC R_f: 0.16 (petroleum ether/ethyl acetate 95/5); mp: 95 °C; IR (cm^{-1}): 527, 623, 735, 745, 767, 828, 1022, 1180, 1232, 1303, 1458, 1466, 2850, 2920, 3217, 3314. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.94 (t, *J* = 6.6 Hz, 3 H); 1.25 – 1.47 (m, 10 H); 1.70 (q, *J* = 7.8 Hz, 2 H); 1.93 (s, 1 H); 2.71 (t, *J* = 7.5 Hz, 2 H); 5.55 (s, 1 H); 7.23 (dd, *J* = 1.2 Hz, 8.1 Hz, 1 H); 7.32 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.40 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.49 (s, 1 H); 7.58 (d, *J* = 7.8 Hz, 1 H);-7.61-7.67 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3); 22.7 (CH_2); 29.3 (CH); 29.4 (CH_2); 29.5 (CH_2); 31.7 (CH_2); 31.9 (CH_2); 36.1 (CH_2); 75.2 (CH); 119.7 (CH); 119.8 (CH); 125.1 (CH); 125.2 (CH); 127.3 (CH); 129.0 (CH); 129.2 (CH); 137.6 (C); 140.2 (C); 143.1 (C); 145.6 (C); 145.9 (C). MS (DCI/CH_4) m/z: 295.20 [M+H^+]. HRMS (DCI/CH_4): for $C_{21}H_{27}O$ [M+H^+]: calcd: 295.2062; found: 295.2057.

2-Propoxy-9*H*-fluoren-9-ol. Reagents: 2-Propoxy-9*H*-fluorenone **20** (0.56 mmol, 134 mg) and sodium borohydride (0.67 mmol, 26 mg). A white solid was obtained (107.5 mg, 79%). TLC R_f: 0.35 (petroleum ether/ethyl acetate 80/20); mp: 122 °C; IR (cm^{-1}): 611, 743, 765, 815, 986, 1028, 1101, 1125, 1148, 1182, 1263, 1303, 1457, 1607, 2876, 2931, 2962, 3248. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.09 (t, *J* = 7.5 Hz, 3 H); 1.84 (sex, *J* = 7.2 Hz, 2 H); 2.30 (s, 1 H); 3.96 (t, *J* = 6.6 Hz, 2 H); 5.45 (s, 1 H); 6.90 (dd, *J* = 2.1 Hz, 8.1 Hz, 1 H); 7.16 (d, *J* = 2.4 Hz, 1 H); 7.25 (td, *J* = 1.2 Hz, 7.2 Hz, 1 H); 7.36 (td, *J* = 0.6 Hz, 7.5 Hz,1 H); 7.51(d, *J* = 8.4 Hz, 1 H); 7.52 – 7.60 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 10.6 (CH_3); 22.7 (CH_2); 69.8 (CH_2); 75.0 (CH); 111.2 (CH); 115.5 (CH); 119.1 (CH); 120.7 (CH); 125.0 (CH); 126.5 (CH); 127.7 (CH); 128.9 (CH); 132.5 (C); 140.1 (C); 145.4 (C); 147.6 (C); 159.5 (C). MS (DCI/CH_4) m/z: 241.12 [M+H^+]. HRMS (DCI/CH_4): for $C_{16}H_{17}O_2$ [M+H^+]: calcd: 241.1229 found: 241.1224.

3-Methoxy-9*H*-fluoren-9-ol. Reagents: 3-Methoxy-9*H*-fluorenone **21** (0.41 mmol, 80 mg) and sodium borohydride (0.46 mmol, 17 mg). A white solid was obtained (66 mg, 82%). TLC R_f: 0.32 (petroleum ether/ethyl acetate 80/20); mp: 114 °C; IR (cm^{-1}): 631, 643, 742, 768, 835, 844, 884, 942, 1010, 1019, 1096, 1111, 1167, 1210, 1236, 1277, 1303, 1441, 1454, 1488, 1608, 2835, 3345. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.10 (bs, 1 H); 3.86 (s, 3 H); 5.50 (s, 1 H); 6.83 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.14 (d, *J* = 2.4 Hz, 1 H); 7.34 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.40 (td, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.51 (d, *J* = 8.1 Hz, 1 H); 7.59 – 7.65 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 55.5 (CH_3); 74.6 (CH); 105.6 (CH); 113.3 (CH); 119.9 (CH); 125.1 (CH); 125.9 (CH); 127.9 (CH); 129.0 (CH); 137.9

(C); 139.8 (C); 141.6 (C); 146.7 (C); 160.7 (C). MS (DCI/CH_4) m/z: 212.08 [M]. HRMS (DCI/CH_4): for $C_{14}H_{12}O_2$ [M]: calcd: 212.0837 found: 212.0836.

3-(Benzyloxy)-9*H*-fluoren-9-ol. Reagents: 3-(Benzyloxy)-9*H*-fluoren-9-one **23** (0.45 mmol, 130 mg) and sodium borohydride (0.68 mmol, 26 mg). A yellow solid was obtained (128 mg, 97%). TLC R_f: 0.30 (petroleum ether/ethyl acetate 80/20); mp: 171 °C; IR (cm^{-1}): 730, 768, 791, 1020, 1236, 1289, 1383, 1446, 1450, 1583, 1624, 2853, 2919, 3021, 3060, 3385. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.96 (bs, 1 H); 5.16 (s, CH_2); 5.54 (s, 1 H); 6.95 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.28 (d, *J* = 2.4 Hz, 1 H); 7.35 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.36 – 7.53 (m, 6 H); 7.5 (d, *J* = 8.4 Hz, 1 H); 7.60 – 7.66 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 70.3 (CH_2); 74.7 (CH); 106.7 (CH); 114.2 (CH); 119.9 (CH); 125.1 (CH);126.0 (CH);127.5 (2 x CH); 128.0 (CH); 128.1 (CH); 128.7 (2 x CH); 129.0 (CH); 136.9 (C); 138.2 (C); 139.8 (C); 141.7 (C); 146.7 (C); 160.0 (C). MS (DCI/CH_4) m/z: 289.12 [M+H^+]. HRMS (DCI/CH_4): for $C_{20}H_{17}O_2$ [M+H^+]: calcd: 289.1229 found 289.1243.

3-Propoxy-9*H*-fluoren-9-ol. Reagents: 3-Propoxy-9*H*-fluorenone **24** (0.34 mmol, 80 mg) and sodium borohydride (0.40 mmol, 15 mg). A white solid was obtained (77 mg, 95%). TLC R_f: 0.48 (petroleum ether/ethyl acetate 80/20); mp: 103 °C; IR (cm^{-1}): 614, 631, 651, 738, 765, 810, 840, 980, 1011, 1019, 1097, 1170, 1182, 1206, 1303, 1449, 1490, 1582, 1609, 2875, 2964, 3312. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.10 (t, *J* = 7.5 Hz, 3 H); 1.88 (q, *J* = 7.2 Hz, 2 H); 2.05 (s, 1 H); 3.98 (t, *J* = 6.6 Hz, 2 H); 5.51 (s, 1 H); 6.83 (dd, *J* = 2.4 Hz, 8.1 Hz, 1 H); 7.15 (d, *J* = 2.1 Hz, 1 H); 7.33 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.40 (td, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.51 (d, *J* = 7.8 Hz, 1 H); 7.58-7.65 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 10.6 (CH_3); 22.6 (CH_2); 69.9 (CH_2); 74.7 (CH); 106.2 (CH); 113.9 (CH); 119.9 (CH); 125.1 (CH); 125.9 (CH); 127.9 (CH); 128.9 (CH); 137.7 (C); 139.9 (C); 141.5 (C); 146.7 (C); 160.3 (C). MS (DCI/CH_4) m/z: 241.12 [M+H^+]. HRMS (DCI/CH_4): for $C_{16}H_{17}O_2$ [M+H^+]: calcd: 241.1229 found: 241.1217.

3-(Hexyloxy)-*9H*-fluoren-9-ol. Reagents: 3-Hexyloxy-9*H*-fluorenone **25** (0.28 mmol, 80 mg) and sodium borohydride (0.34 mmol, 13 mg). A brown solid was obtained (78 mg, 97%). TLC R_f: 0.45 (petroleum ether/ethyl acetate 80/20); mp: 74 °C; IR (cm^{-1}): 648, 744, 769, 786, 1018, 1034, 1172, 1181, 1206, 1287, 139, 1450, 1622, 2868, 2929, 3484. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.97 (t, *J* = 6.9 Hz, 3 H); 1.35-1.47 (m, 4 H); 1.48-1.62 (m, 2 H); 1.85 (q, *J* = 6.9 Hz, 2 H); 2.05 (s, 1 H); 4.02 (t, *J* = 6.6 Hz, 2 H); 5.51 (s, 1 H); 6.83 (dd, *J* = 2.4 Hz, 8.1 Hz, 1 H); 7.15 (d, *J* = 2.4 Hz, 1 H); 7.33 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.40 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.51 (d, *J* = 8.1 Hz, 1 H); 7.61 (d, *J* = 3.6 Hz, 1 H); 7.63 (d, *J* = 4.5 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 25.8 (CH_2); 29.3 (CH_2); 31.7 (CH_2); 68.4 (CH_2); 74.7 (CH); 106.2 (CH); 113.9 (CH); 119.9 (CH); 125.1

(CH); 125.9 (CH); 127.9 (CH); 128.9 (CH); 137.7 (C); 139.9 (C); 141.5 (C); 146.7 (C); 160.3 (C). MS (DCI/CH_4) m/z: 283.17 [M+H^+]. HRMS (DCI/CH_4): for $C_{19}H_{23}O_2$ [M+H^+]: calcd: 283.1698 found: 283.1690.

3-Hexyl-9*H*-fluoren-9-ol. Reagents: 3-Hexyl-9*H*-fluoren-9-one **27** (0.29 mmol, 77 mg) and sodium borohydride (0.35 mmol, 13 mg). A white solid was obtained (20 mg, 26%). TLC R_f: 0.10 (petroleum ether/ethyl acetate 95/5); mp: 75 °C; IR (cm^{-1}): 630, 657, 805, 846, 1025, 1098, 1164, 1199, 1260, 1299, 1426, 1449, 1614, 2854, 2926, 2955, 3218, 3313. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.89 – 0.98 (m, 3 H); 1.24-1.48 (m, 6 H); 1.70 (quin, *J* = 7.8 Hz, 2 H); 2.72 (t, *J* = 7.5 Hz, 2 H); 5.58 (s, 1 H); 7.17 (dd, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.34 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.42 (td, J= 1.5 Hz, 7.5 Hz, 1 H); 7.50 (s, 1 H); 7.57 (d, *J* = 7.8 Hz, 1 H); 7.63 – 7.71 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 29.0 (CH_2); 31.7 (CH_2); 31.8 (CH_2); 36.2 (CH_2); 75.1 (CH); 119.9 (CH); 120.0 (CH); 124.9 (CH); 125.1 (CH); 127.7 (CH); 128.1 (CH); 129.0 (CH); 138.3 (C); 140.2 (C); 143.1 (C); 144.2 (C); 146.1 (C). MS (DCI/CH_4) m/z: 267.17 [M+H^+], 249.16 [M-OH], 181.07 [M-85]. HRMS (DCI/CH_4): for $C_{19}H_{23}O$ [M+H^+]: calcd: 267.1749 found: 267.1746.

1.7. General procedure for bromation with PBr_3. The corresponding substituted fluoren-9-ol (1.0 eq) was dissolved in anhydrous dichloromethane and cooled in an ice bath. Phosphorus tribromide (1.2 eq) was slowly added. The mixture was stayed at 0°C overnight and then saturated sodium bicarbonate aqueous solution was added under stirring until no bubble generated. Then the water extracted with dichloromethane. The combine organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuum pressure to afford the titled compound. No further purification was needed.

9-Bromo-2-hexyl-9*H*-fluorene. Reagents: 2-Hexyl-9*H*-fluoren-9-ol (0.37 mmol, 100 mg) and phosphorus tribromide (0.45 mmol, 44 µL). A yellow oil was obtained (84 mg, 68%). TLC R_f: 0.79 (petroleum ether/ethyl acetate 97/3). IR (cm^{-1}): 736, 759, 827, 994, 1057, 1117, 1136, 1210, 1456, 1607, 1716, 2854, 3924. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.88-0.98 (m, 3 H); 1.28-1.46 (m, 6 H); 1.63-1.77 (m, 2 H); 2.72 (t, *J* = 7.5 Hz, 2 H); 6.01 (s, 1 H); 7.24 (dd, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.34 (td, *J* = 1.2 Hz, 7.2 Hz, 1 H); 7.41 (td, *J* = 1.2 Hz, 7.2 Hz, 1 H); 7.51 (s, 1 H); 7.61 (d, *J* = 7.8 Hz, 1 H); 7.64-7.70 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 29.0 (CH_2); 31.6 (CH_2); 31.8 (CH_2); 36.1 (CH_2); 46.3 (CH); 119.7 (CH); 120.0 (CH); 126.3 (CH); 127.6 (CH); 129.2 (CH); 129.5 (CH); 137.4 (C); 140.0 (C); 143.0 (C); 144.1 (C); 144.3 (C). MS (DCI/CH_4) m/z: 328.08 [M]; 249.16 [M-80 (HBr)]. HRMS (DCI/CH_4): for $C_{19}H_{21}Br$ [M]: calcd: 328.0838; found: 328.0827.

9-Bromo-2-octyl-9*H*-fluorene. Reagents: 2-Octyl-9*H*-fluoren-9-ol (0.34 mmol, 100 mg) and phosphorus tribromide (0.41 mmol, 40 µL). A yellow oil was obtained (63 mg, 52%). TLC R_f: 0.90 (petroleum ether/ethyl acetate 90/10). IR (cm^{-1}): 576, 606, 658, 735, 759, 827, 994, 1057, 1136, 1210, 1456, 2852, 2923. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.95 (t, *J* = 6.3 Hz, 3 H); 1.28 – 1.45 (m, 10 H); 1.71 (q, *J* = 7.5 Hz, 2 H); 2.73 (t, *J* = 6.9 Hz, 2 H); 6.01 (s, 1 H); 7.26 (dd, *J* = 1.5 Hz, 7.8 Hz, 1 H); 7.35 (td, 1.2 Hz, 7.5 Hz, 1 H); 7.42 (td, *J* = 1.2 Hz, 7.8 Hz, 1 H); 7.52 (s, 1 H); 7.61 (d, *J* = 7.8 Hz, 1 H);-7.65-7.71 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3); 22.7 (CH_2); 29.3 (CH); 29.4 (CH_2); 29.5 (CH_2); 31.7 (CH_2); 31.9 (CH_2); 36.1 (CH_2); 46.3 (CH); 119.9 (CH); 120.0 (CH); 126.3 (CH); 126.4 (CH); 127.6 (CH); 129.2 (CH); 129.5 (CH); 137.4 (C); 140.0 (C); 142.4 (C); 144.1 (C); 144.3 (C). MS (DCI/CH_4) m/z: 356.11 [M], 277.20 [M-Br], 179.08 [M-178]. HRMS (DCI/CH_4): for $C_{21}H_{25}Br$ [M]: calcd: 356.1140 found: 356.1140.

9-Bromo-2-propoxy-9*H*-fluorene. Reagents: 2-Propoxy-9*H*-fluoren-9-ol (0.44 mmol, 107 mg) and phosphorus tribromide (0.67 mmol, 63 µL). Flash chromatography (gradient from 100% petroleum ether to 90/10 petroleum ether/ethyl acetate in 15 minutes) was performed and allowed to isolate partially a yellow oil (18 mg, 13%). TLC R_f: 0.36 (petroleum ether/ethyl acetate 90/10). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.11 (t, *J* = 7.5 Hz, 3 H); 1.88 (sex, *J* = 7.2 Hz, 2 H); 4.02 (m, 2 H); 5.97 (s, 1 H); 6.97 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.22 (d, *J* = 2.4 Hz, 1 H); 7.29 (td, *J* = 1.2 Hz, 7.5 Hz, 1 H); 7.39 (t, *J* = 7.5 Hz,1 H); 7.59 (d, *J* = 8.4 Hz, 1 H); 7.60 (d, *J* = 7.5 Hz, 1 H); 7.63 (d, *J* = 7.2 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 10.6 (CH_3); 22.6 (CH_2); 46.1 (CH); 69.9 (CH_2); 112.3 (CH); 116.0 (CH); 119.4 (CH); 121.1 (CH); 126.2 (CH); 126.8 (CH); 129.2 (CH); 132.4 (C); 140.0 (C); 143.7 (C); 145.9 (C); 159.7 (C).

9-Bromo-3-methoxy-9*H*-fluorene. Reagents: 3-Methoxy-9*H*-fluoren-9-ol (0.28 mmol, 60 mg) and phosphorus tribromide (0.34 mmol, 33 µL). A brown solid was obtained (63 mg, 81%). TLC R_f: 0.59 (petroleum ether/ethyl acetate 80/20); mp: 122 °C; IR (cm^{-1}): 644, 731, 761, 827, 841, 1030, 1124, 1175, 1211, 1240, 1278, 1304, 1312, 1439, 1454, 1488, 1609, 2833, 2937, 3367. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.89 (s, 3 H); 5.99 (s, 1 H); 6.88 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.18 (d, *J* = 2.4 Hz, 1 H); 7.34 (td, *J* = 1.2 Hz, 7.2 Hz, 1 H); 7.40 (td, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.55 (d, *J* = 8.4 Hz, 1 H); 7.61 – 7.66 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 46.3 (C); 55.6 (CH_3); 105.6 (CH); 114.0 (CH); 120.2 (CH); 126.4 (CH); 127.3 (CH); 128.2 (CH); 129.1 (CH); 136.2 (C); 139.7 (C); 141.4 (C); 145.3 (C); 161.0 (C). MS (DCI/CH_4) m/z: 275.00 [M], 195.08 [M-HBr]. HRMS (DCI/CH_4): for $C_{14}H_{12}OBr$ [M]: calcd: 275.0072; found: 275.0071.

3-(Benzyloxy)-9-bromo-9*H*-fluorene. Reagents: 3-(Benzyloxy)-9*H*-fluoren-9-ol (0.25 mmol, 71 mg) and phosphorus tribromide (0.37 mmol, 35 µL). A red powder was obtained (81 mg, 92%). TLC R_f: 0.32 (petroleum ether/ethyl acetate 90/10). The product was used in the next step without further purification. MS (DCI/CH_4) m/z: 351.04 [M+H$^+$].HRMS (DCI/CH_4): for $C_{20}H_{16}BrO$ [M+H$^+$] calcd 351.0385 found 351.0381.

9-Bromo-3-propoxy-9*H*-fluorene. Reagents: 3-Propoxy-9*H*-fluoren-9-ol (0.33 mmol, 80 mg) and phosphorus tribromide (0.40 mmol, 38 µL). A brown solid was obtained (67 mg, 67%). TLC R_f: 0.95 (petroleum ether/ethyl acetate 90/10); mp: 139 °C; IR (cm^{-1}): 625, 648, 733, 765, 980, 1185, 1203, 1235, 1273, 1450, 1489, 1578, 1610, 2874, 2933, 2963. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.13 (t, *J* = 7.5 Hz, 3 H); 1.90 (sex, *J* = 7.2 Hz, 2 H); 4.05 (t, *J* = 6.6 Hz, 2 H); 6.03 (s, 1 H); 6.92 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.23 (d, *J* = 2.4 Hz, 1 H); 7.33-7.47 (m, 2 H); 7.57 (d, *J* = 8.4 Hz, 1 H); 7.64-7.70 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 10.6 (CH_3); 22.7 (CH_2); 46.4 (CH); 69.9 (CH_2); 106.3 (CH); 114.5 (CH); 120.2 (CH); 126.4 (CH); 127.2 (CH); 128.2 (CH); 129.1 (CH); 136.0 (C); 139.8 (C); 141.4 (C); 145.3 (C); 160.5 (C). MS (DCI/CH_4) m/z: 446.22 [2M-2 Br], 302.03 [M], 223.11 [M-Br], 181.06 [M-122]. HRMS (DCI/CH_4): for $C_{16}H_{15}BrO$ [M]: calcd: 302.0306, found: 302.0301.

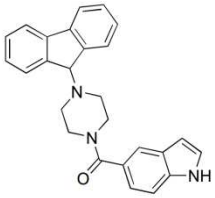
9-Bromo-3-(hexyloxy)-9*H*-fluorene. Reagents: 3-Hexyloxy-9H-fluoren-9-ol (0.25 mmol, 70 mg) and phosphorus tribromide (0.30 mmol, 29 µL). A brown oil was obtained (67 mg, 67%). TLC R_f: 0.95 (petroleum ether/ethyl acetate 90/10). IR (cm^{-1}): 649, 732, 762, 843, 940, 1024, 1137, 1184, 1207, 1238, 1285, 1303, 1450, 1467, 1489, 1580, 1609, 2856, 2927. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.93-1.03 (m, 3 H); 1.36-1.47 (m, 4 H); 1.48-1.61 (m, 2 H); 1.87 (q, *J* = 6.6 Hz, 2 H); 4.08 (t, *J* = 6.3 Hz, 2 H); 6.03 (s, 1 H); 6.91 (dd, *J* = 2.4 Hz, 8.4 Hz, 1 H); 7.22 (d, *J* = 2.4 Hz, 1 H); 7.38 (td, *J* = 1.5 Hz, 7.2 Hz, 1 H); 7.42 (td, *J* = 1.5 Hz, 7.2 Hz, 1 H); 7.57 (d, J = 8.1 Hz, 1 H); 7.64-7.69 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.7 (CH_2); 25.8 (CH_2); 29.3 (CH_2); 31.7 (CH_2); 46.4 (CH); 68.4 (CH_2); 106.2 (CH); 114.5 (CH); 120.2 (CH); 126.4 (CH); 127.2 (CH); 128.1 (CH); 129.1 (CH); 135.9 (C); 139.8 (C); 141.4 (C); 145.3 (C); 160.5 (C). MS (DCI/CH_4) m/z: 344.08 [M], 265.16 [M-Br], 181.06 [M-164]. HRMS (DCI/CH_4): for $C_{19}H_{21}BrO$ [M]: calcd: 344.0776, found: 344.0762.

9-Bromo-3-hexyl-9*H*-fluorene. Reagents: 3-Hexyloxy-9*H*-fluoren-9-ol (0.07 mmol, 20 mg) and phosphorus tribromide (0.11 mmol, 11 µL). A yellow oil was obtained (24 mg, 95%). TLC R_f: 0.92 (petroleum ether/ethyl acetate 95/5). IR (cm^{-1}): 631, 656, 736, 761, 826, 890, 1137, 1164, 1199, 1303, 1425, 1452, 1614, 2853, 2924, 2953. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.87– 0.99 (m, 3 H); 1.28-

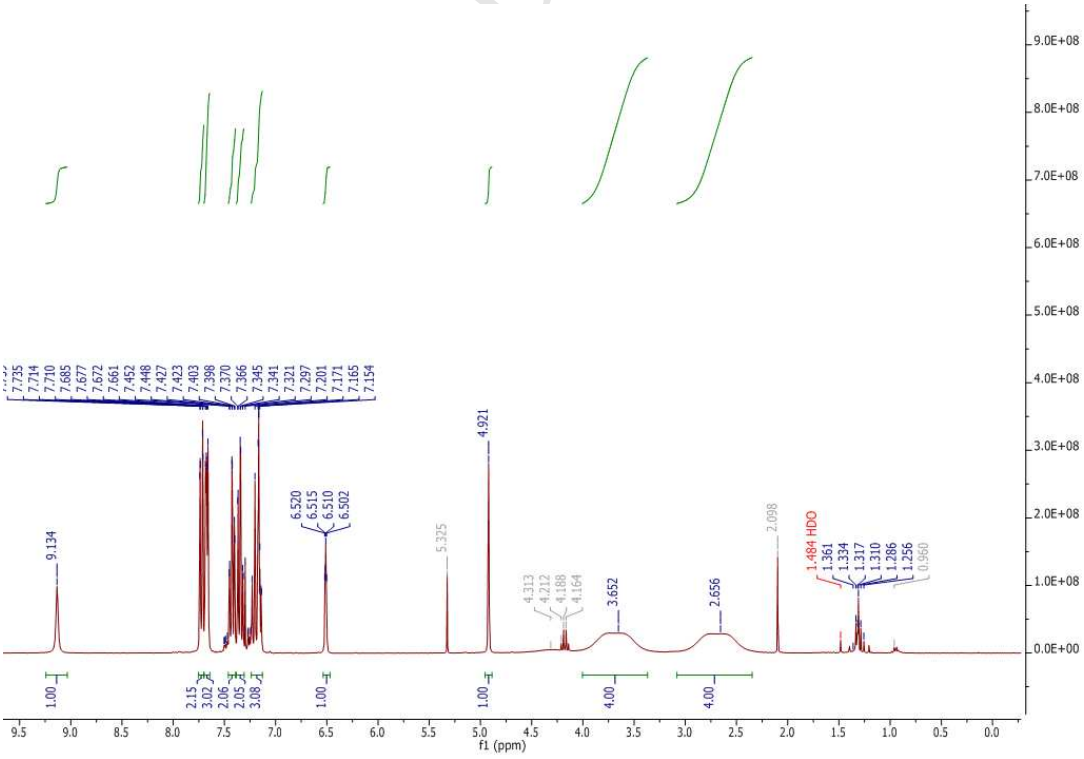
1.48 (m, 6 H); 1.64 – 1.77 (m, 2 H); 2.73 (t, *J* = 7.5 Hz, 2 H); 6.02 (s, 1 H); 7.19 (dd, *J* = 1.5 Hz, 7.5 Hz, 1 H); 7.36 (td, *J* = 1.5 Hz, 7.2 Hz, 1 H); 7.43 (td, *J*= 1.5 Hz, 7.5 Hz, 1 H); 7.51 – 7.54 (m, 1 H); 7.58 (d, *J* = 7.8 Hz, 1 H); 7.65 – 7.72 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1 (CH_3); 22.6 (CH_2); 29.0 (CH_2); 31.6 (CH_2); 31.8 (CH_2); 36.2 (CH_2); 46.2 (CH); 120.1 (CH); 120.2 (CH); 126.1 (CH); 126.4 (CH); 127.9 (CH); 128.4 (CH); 129.1 (CH); 139.9 (C); 140.0 (C); 141.5 (C); 144.5 (C); 144.6 (C). MS (DCI/CH_4) m/z: 329.09 [M], 249.16 [M-80]. HRMS (DCI/CH_4): for $C_{19}H_{22}Br$ [M]: calcd: 329.0905 found: 329.0891.

NMR spectra

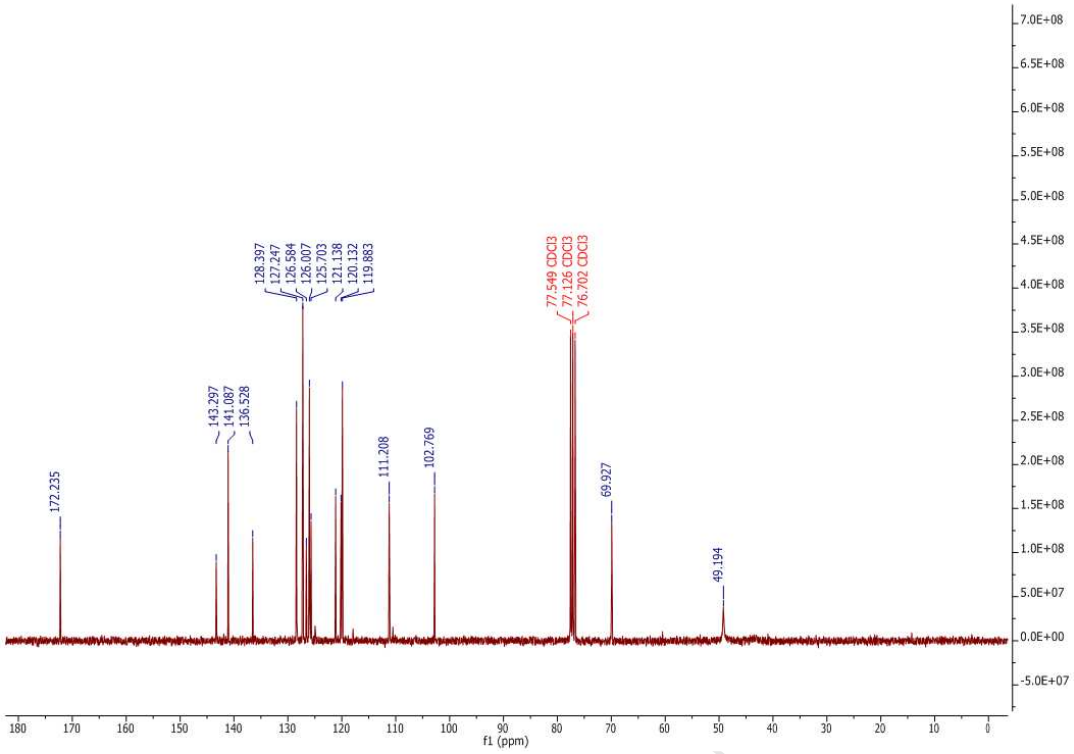
1.8. GEQ



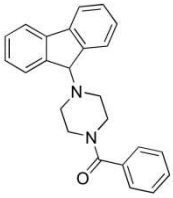
^{1}H NMR



^{13}C NMR



1.9. Compound 1



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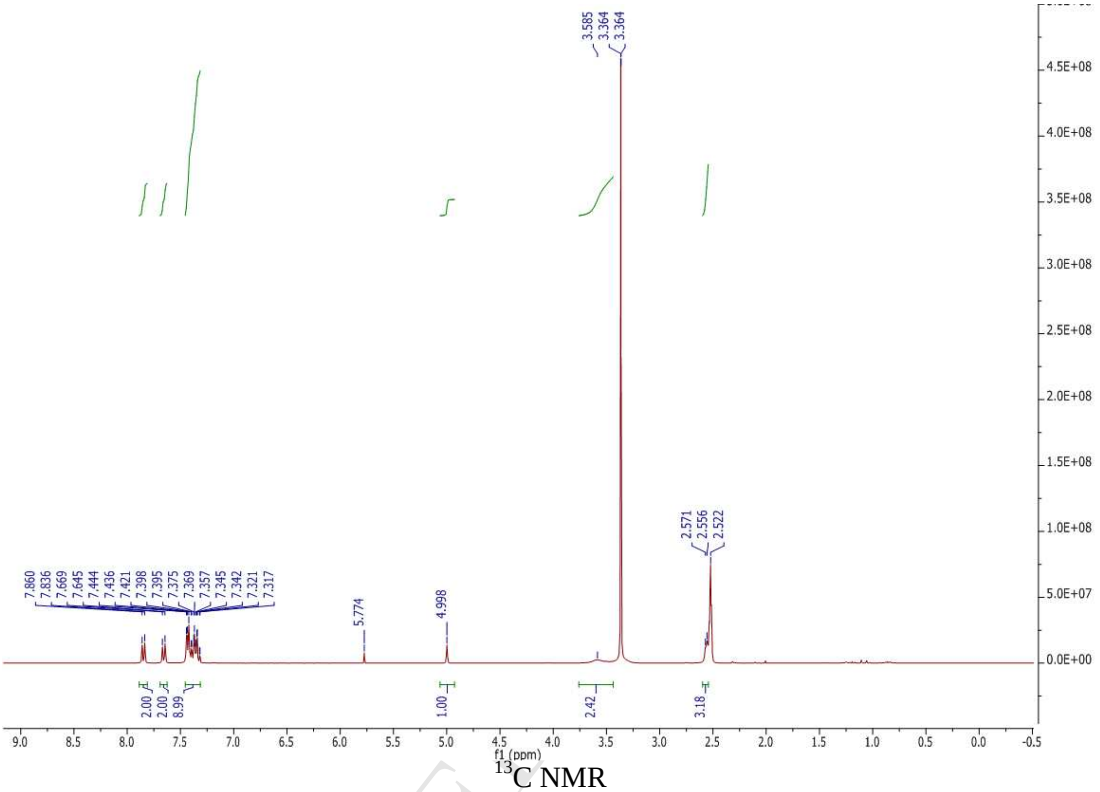

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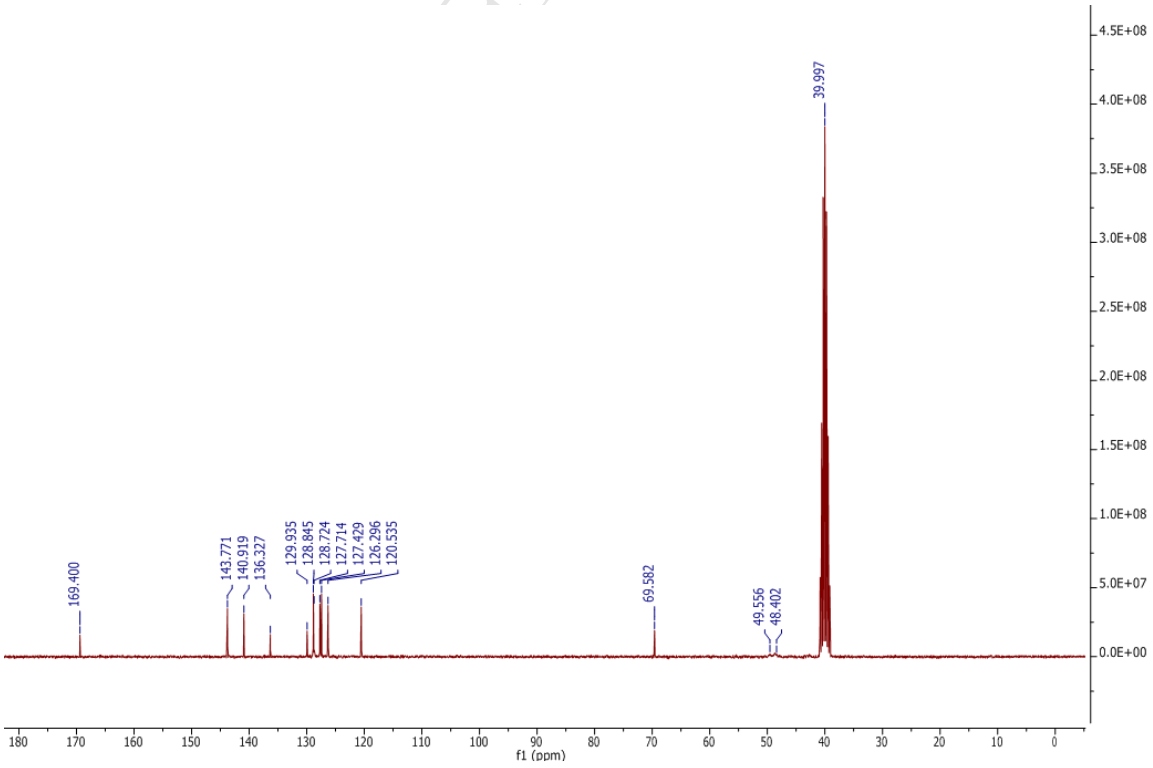
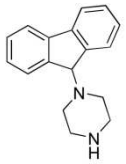


1.10. Compound 3



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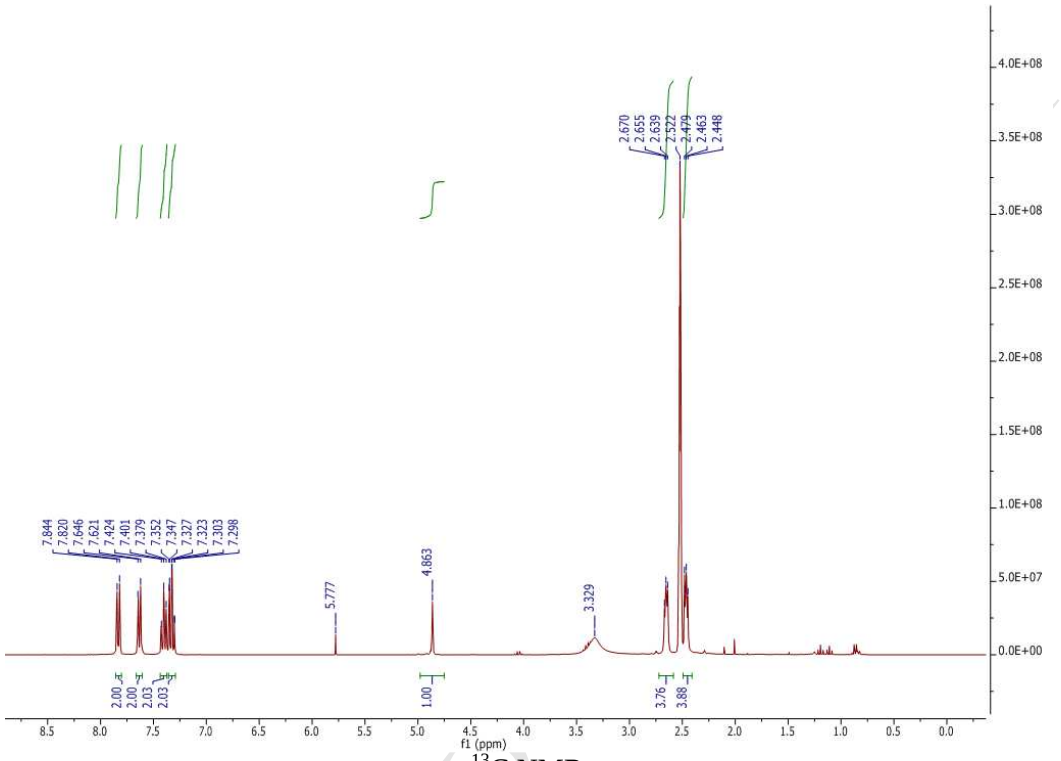
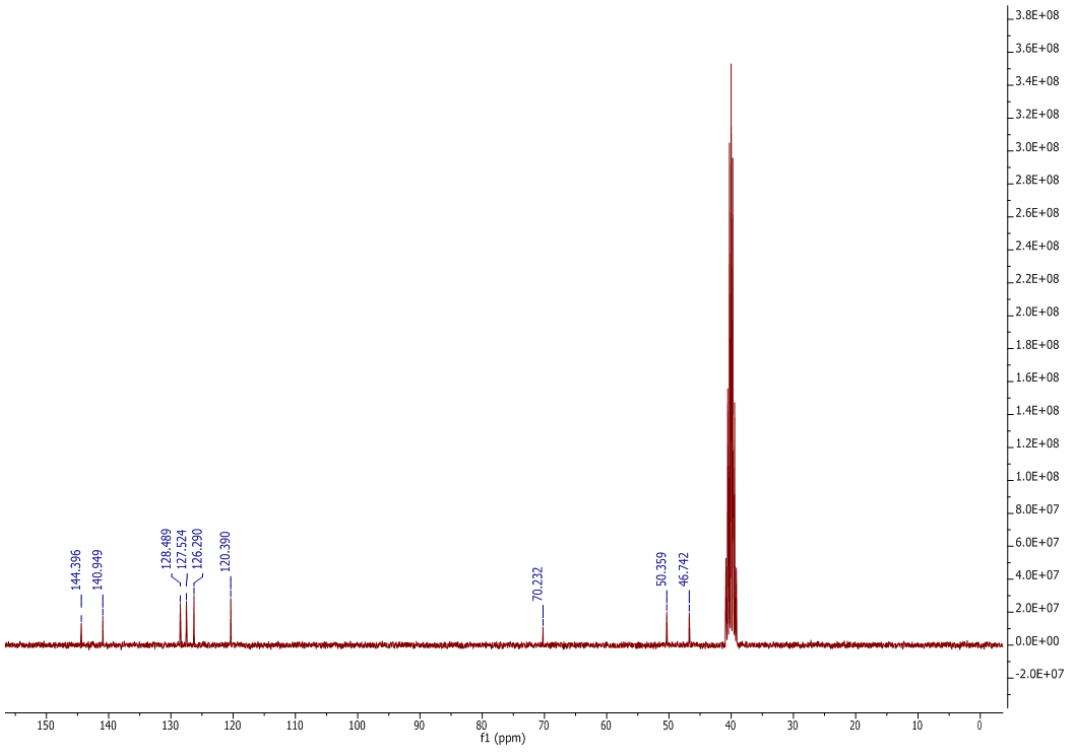


^{13}C NMR



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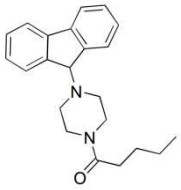

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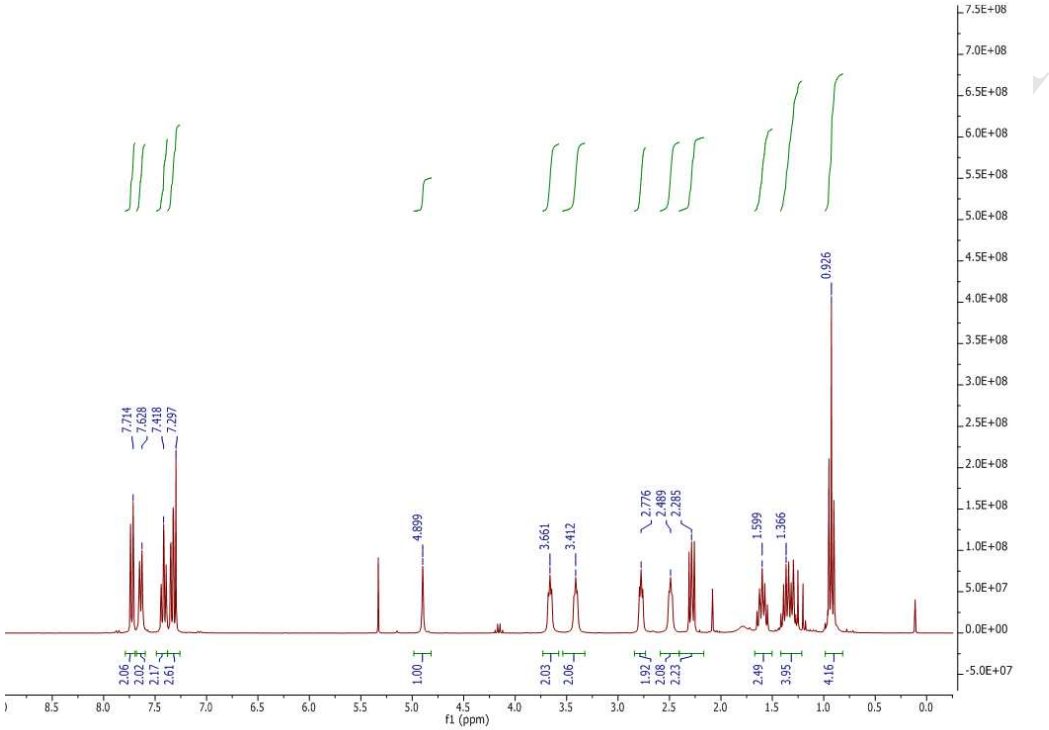

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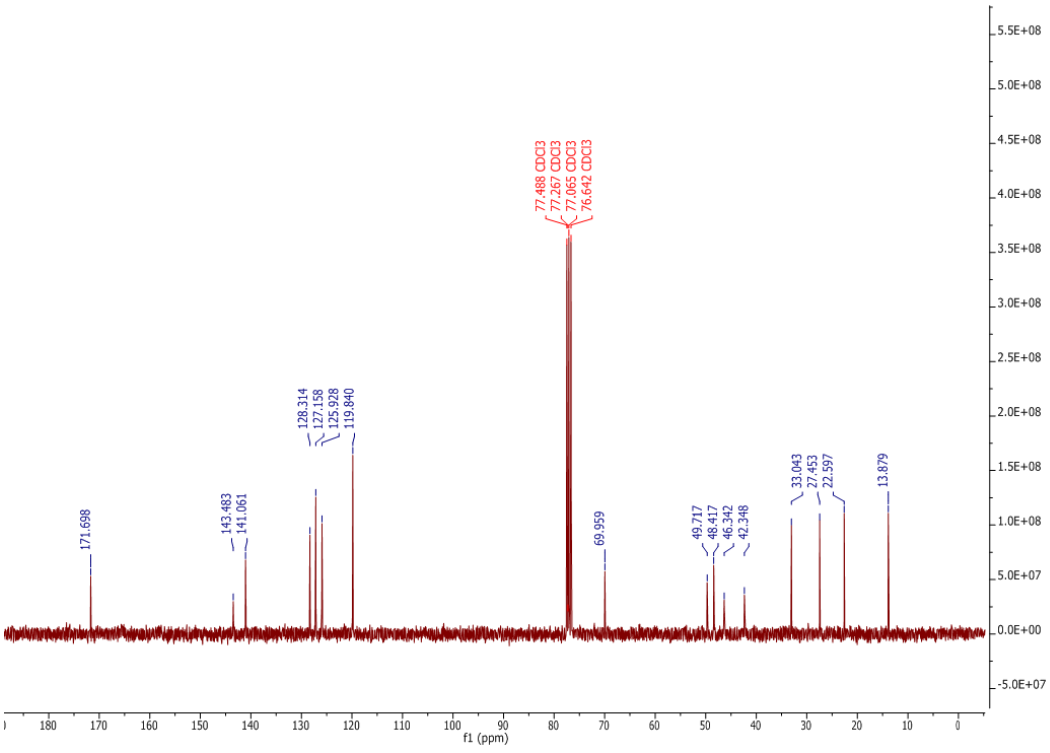
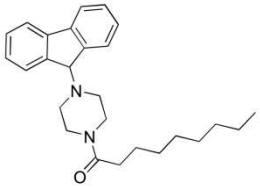


1.12. Compound 5



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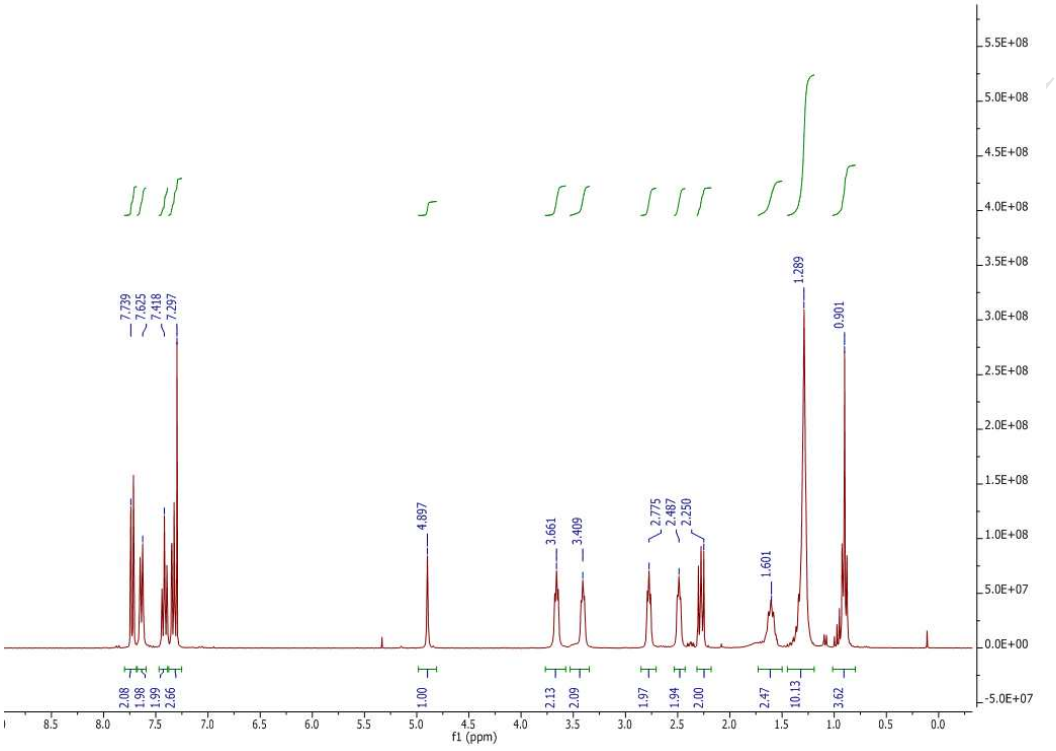

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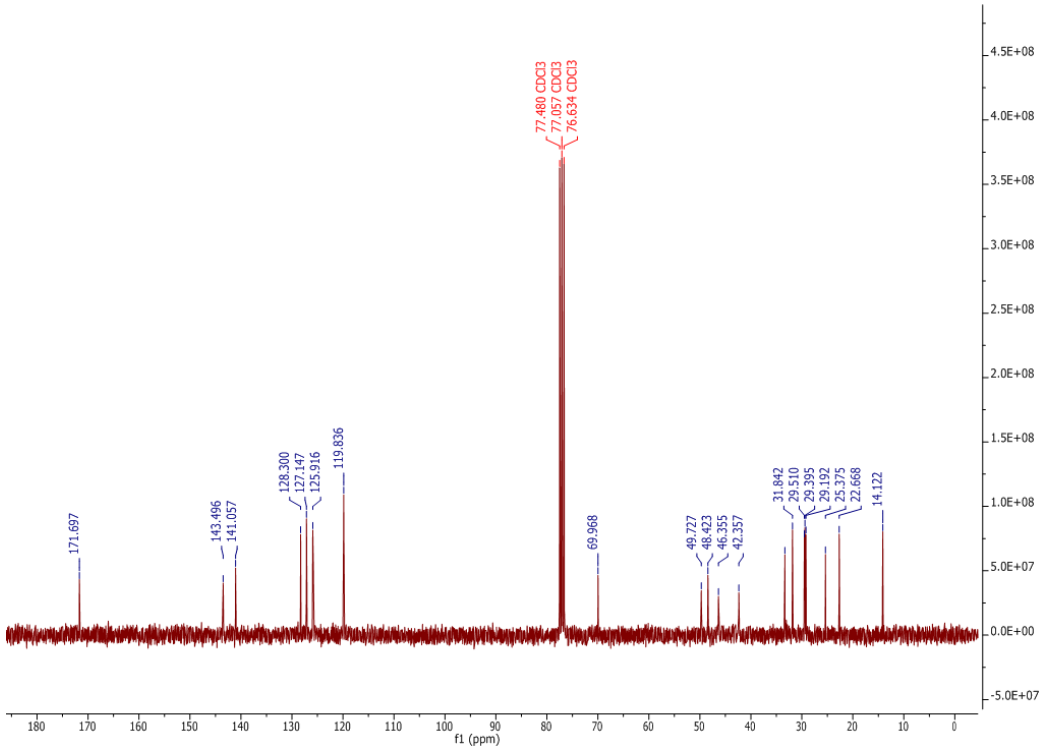
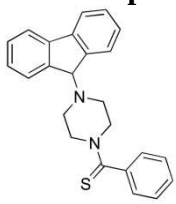


1.13. Compound 6



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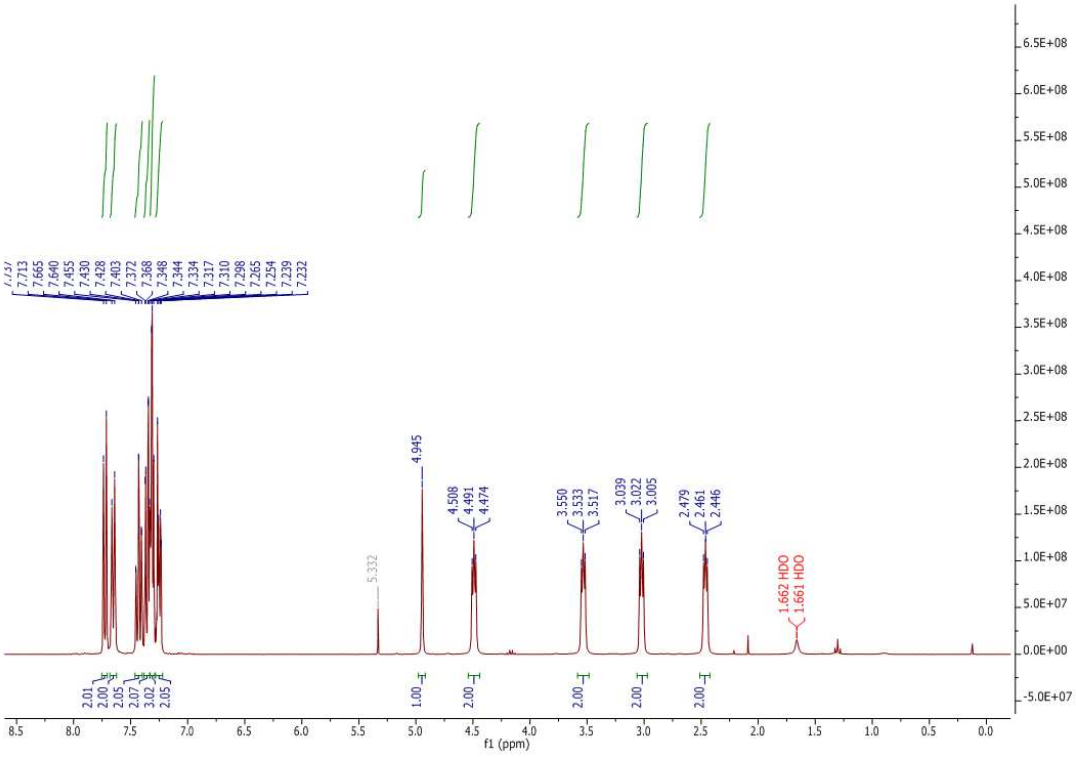

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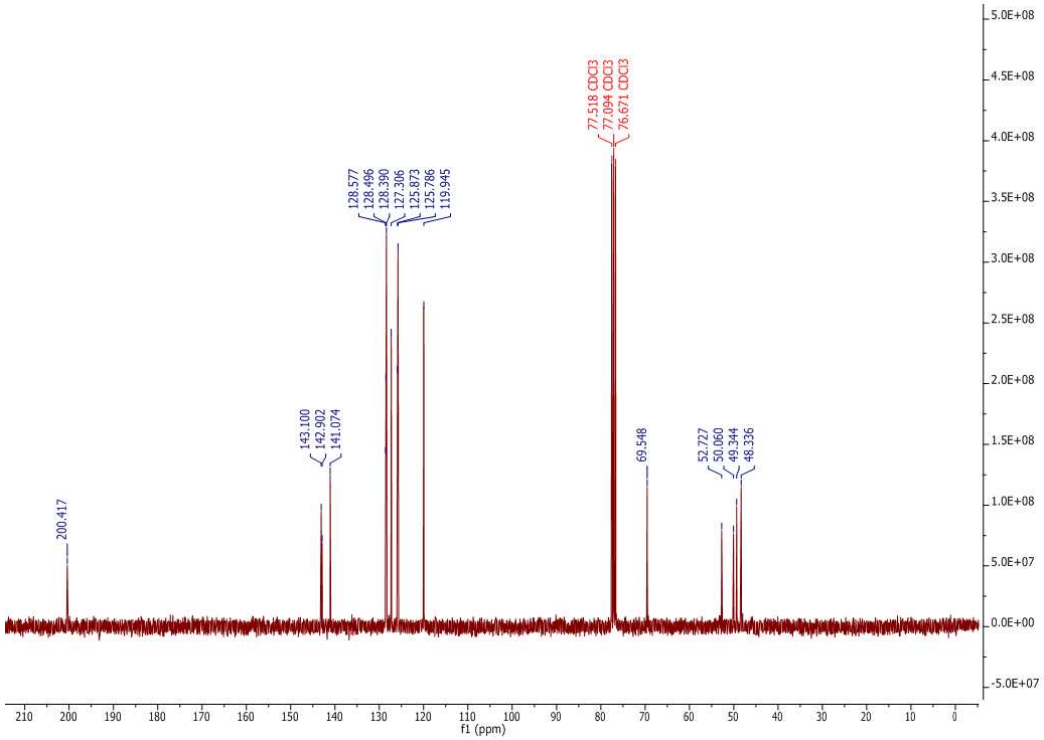

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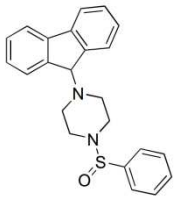

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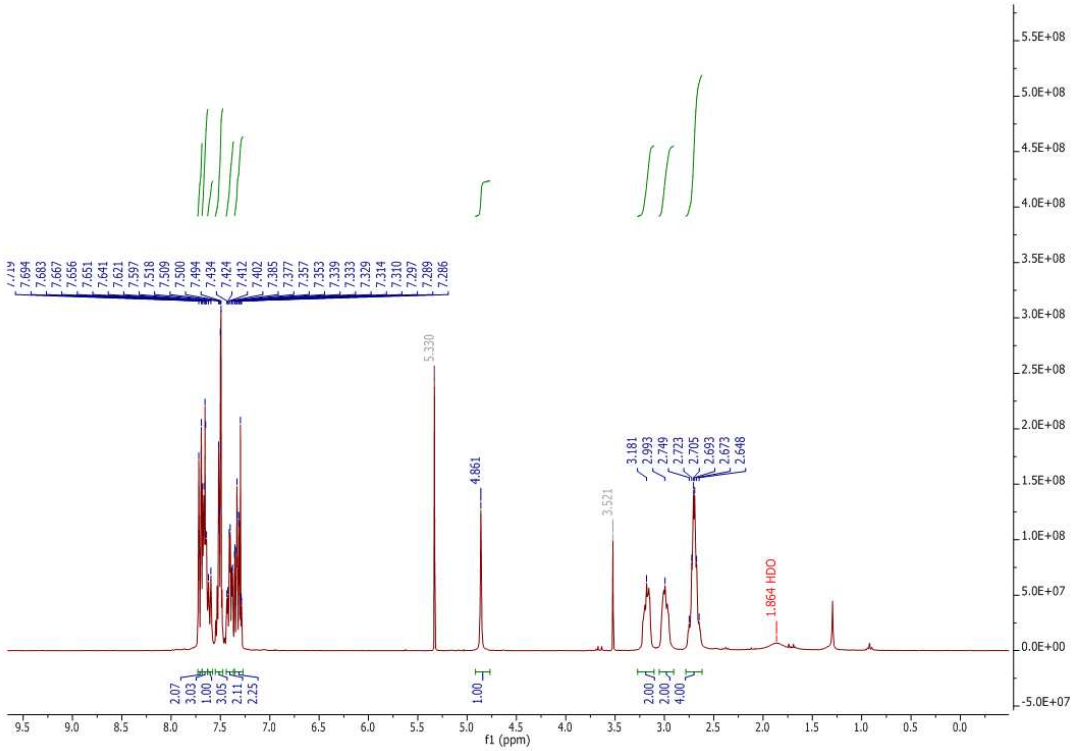

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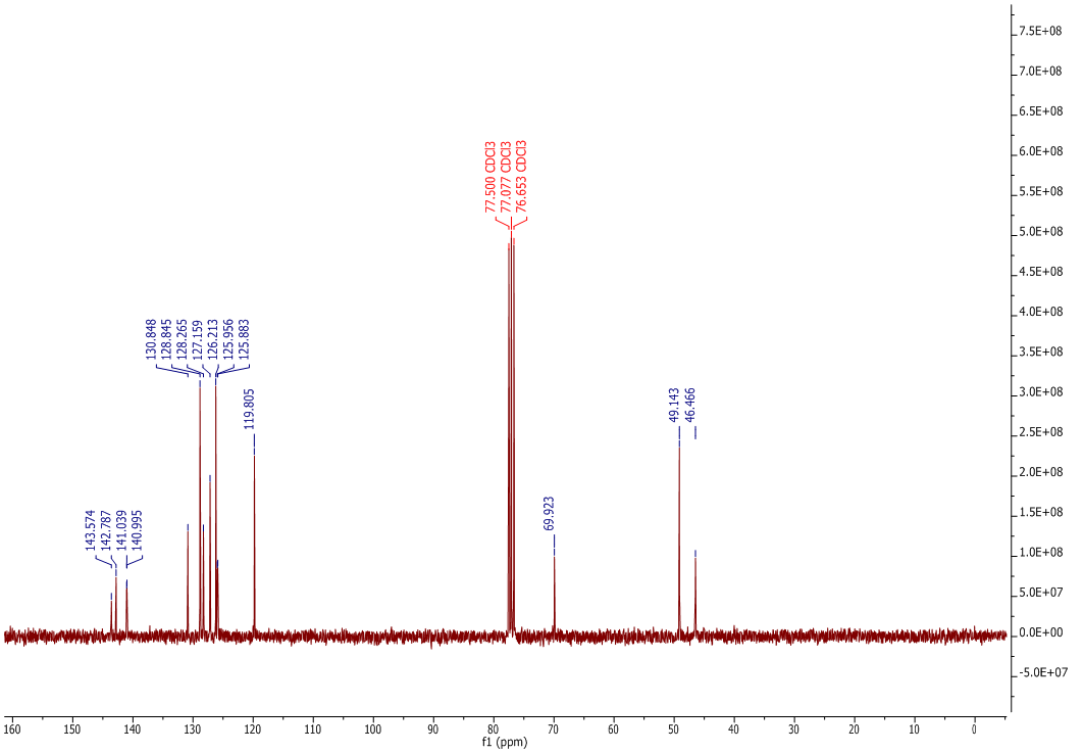
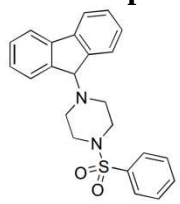
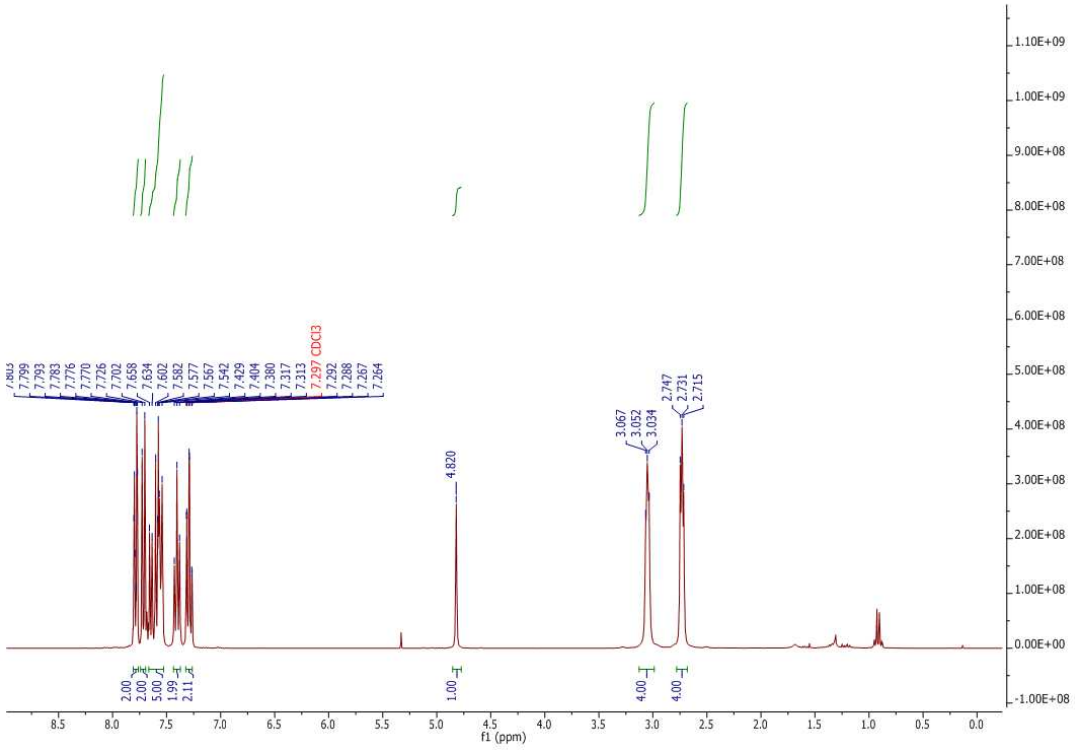


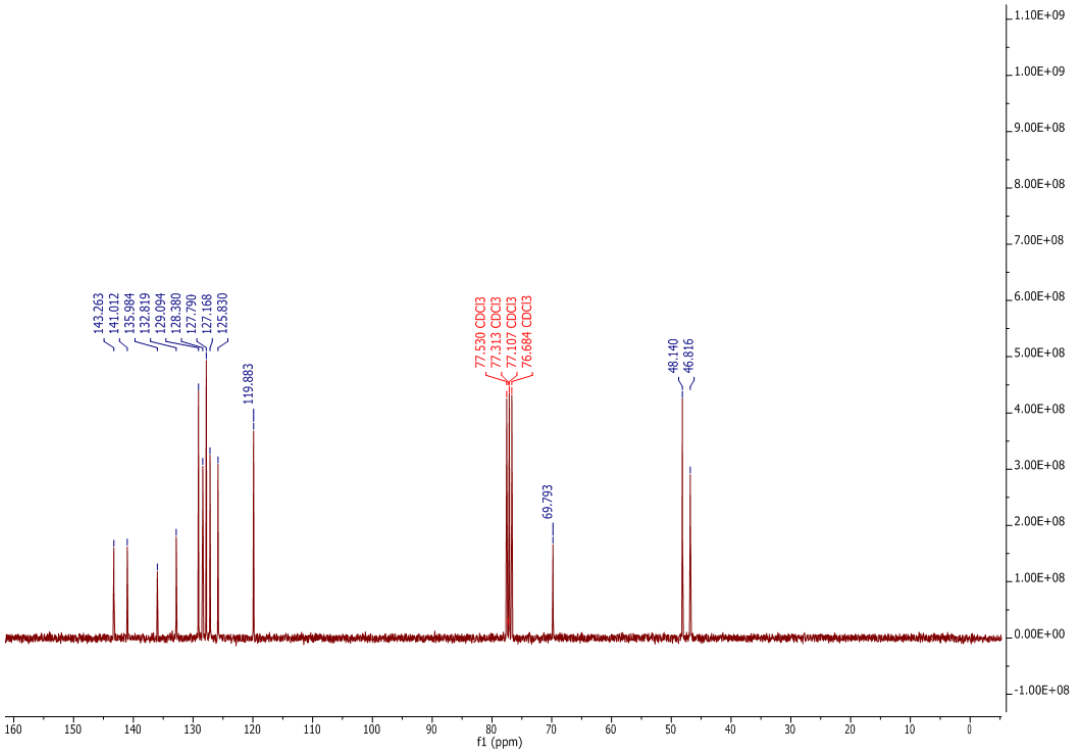
1.15. Compound 8



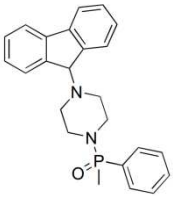
^{1}H NMR



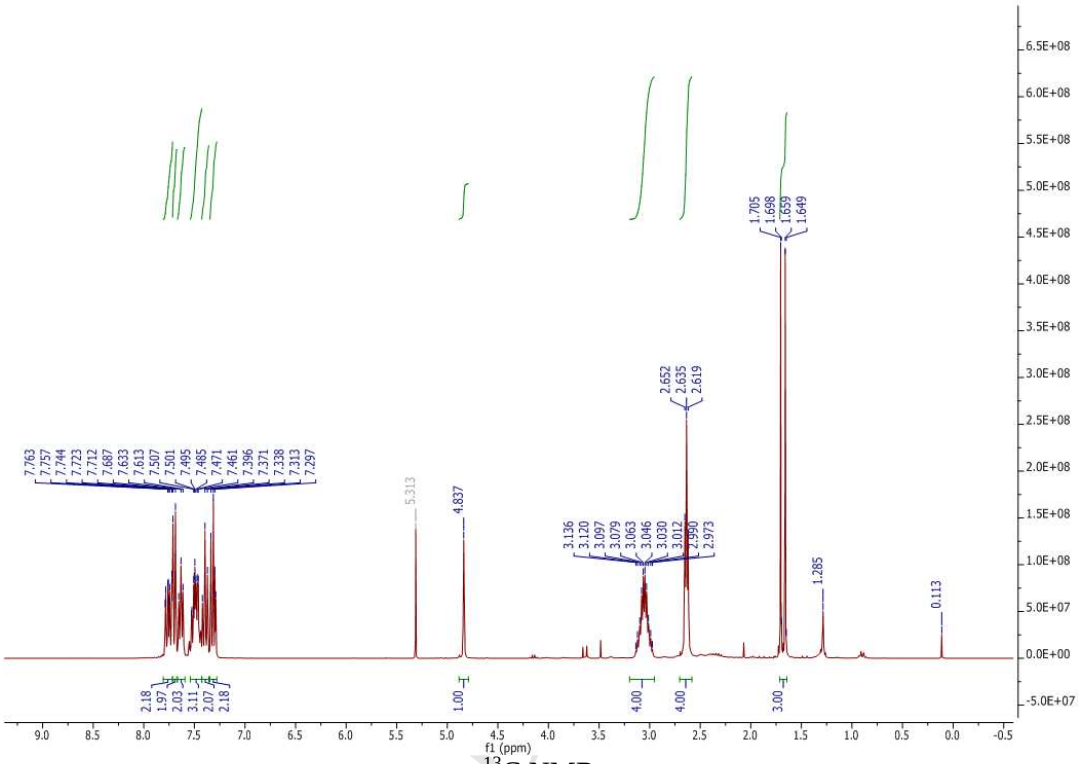
^{13}C NMR



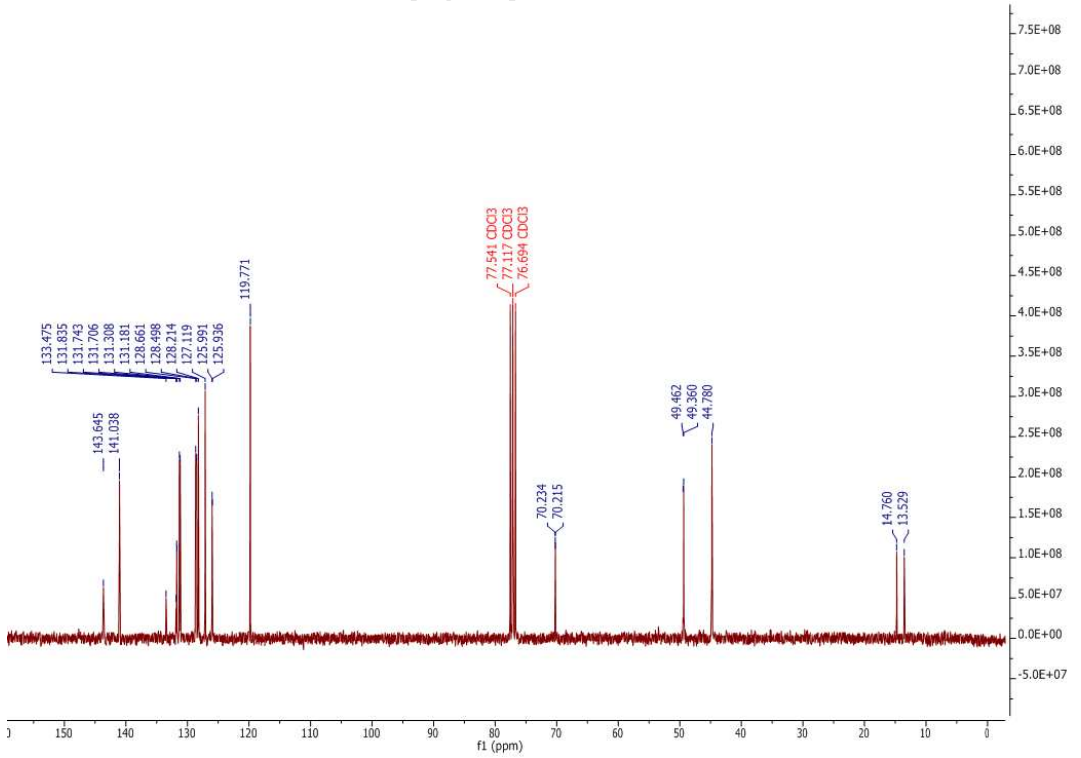
1.16. Compound 9



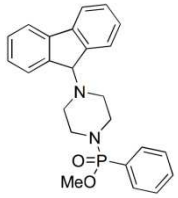
^{1}H NMR



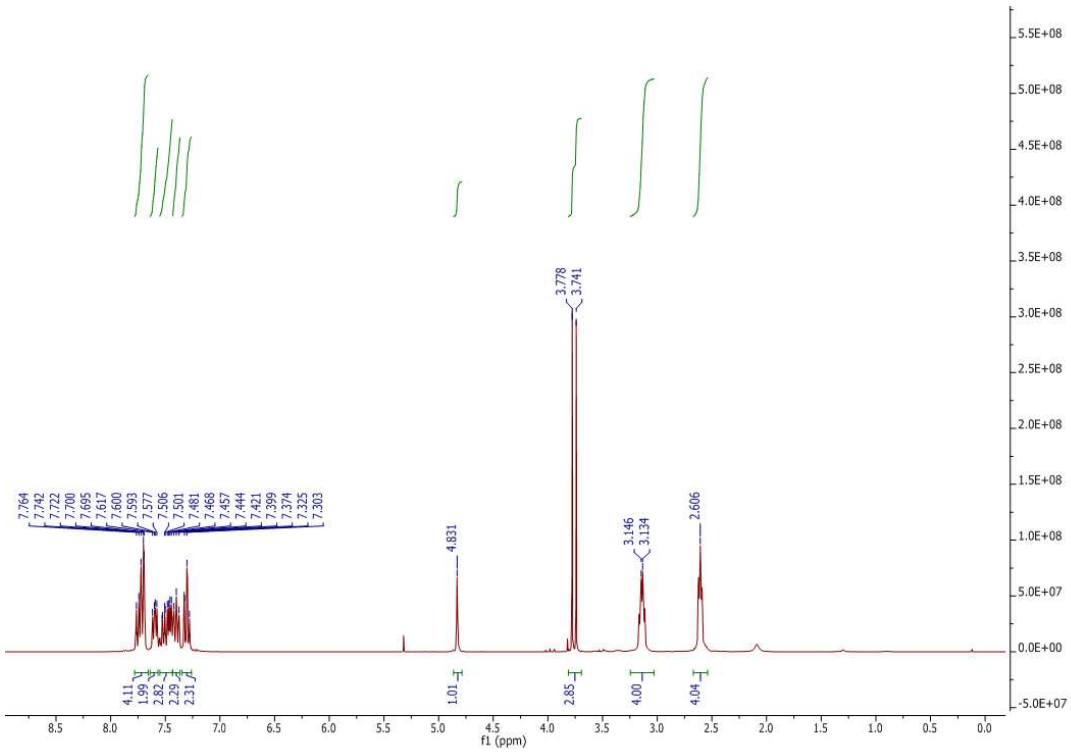
^{13}C NMR



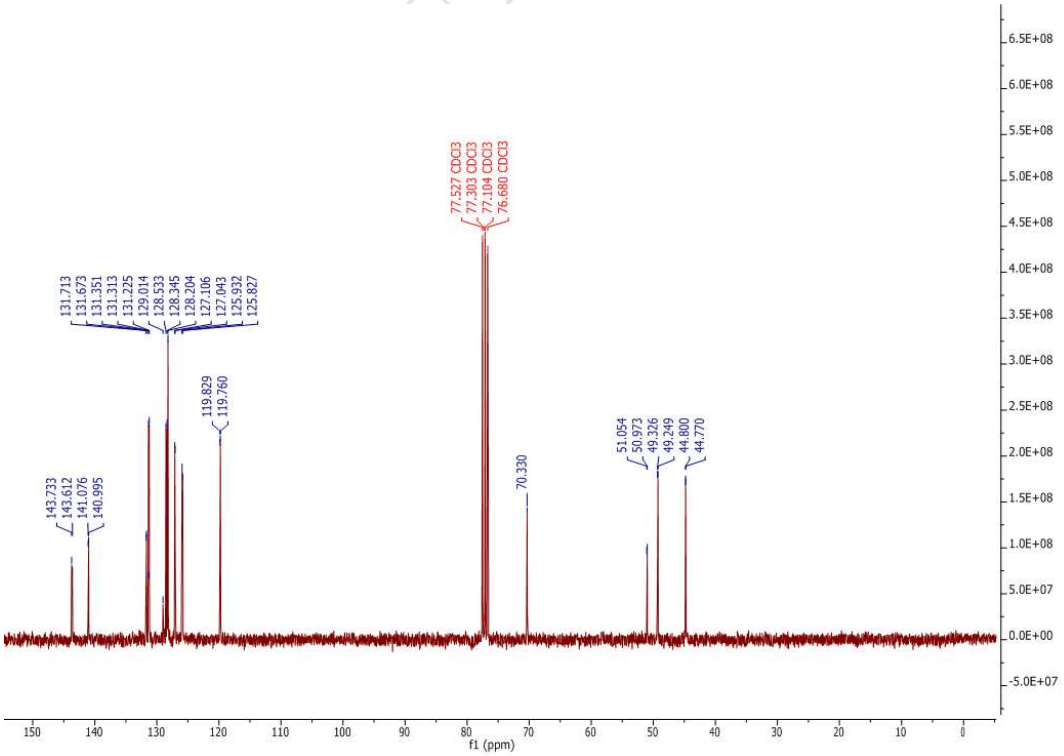
1.17. Compound 10



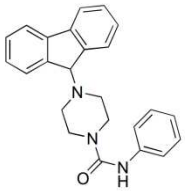
^{1}H NMR



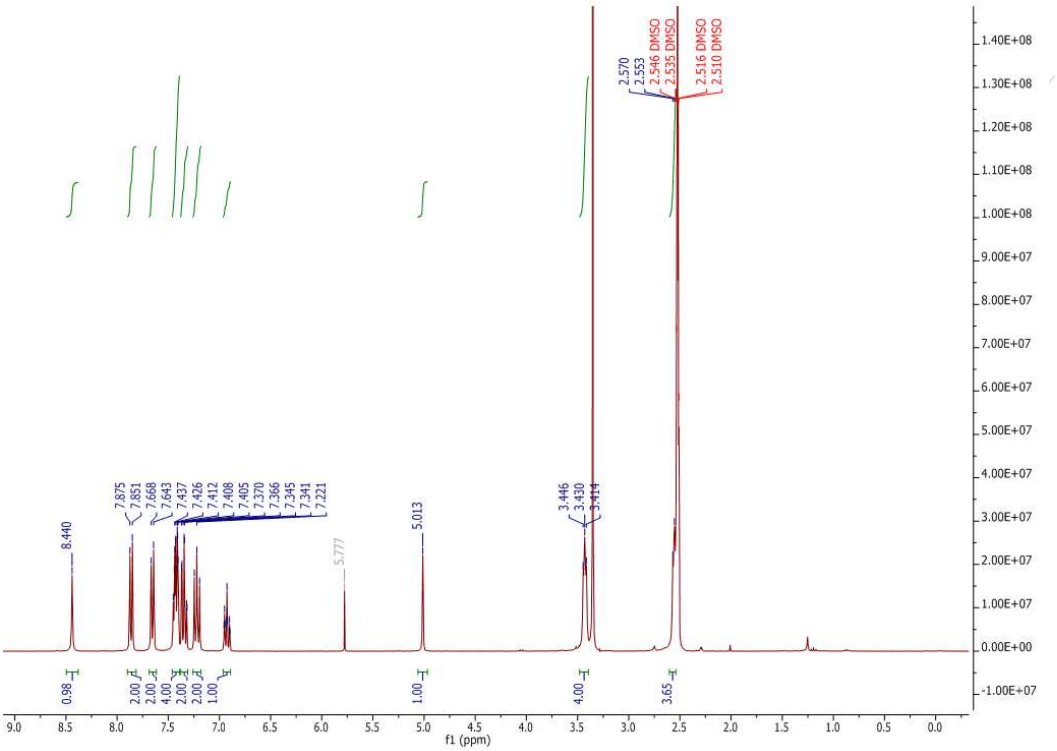
^{13}C NMR



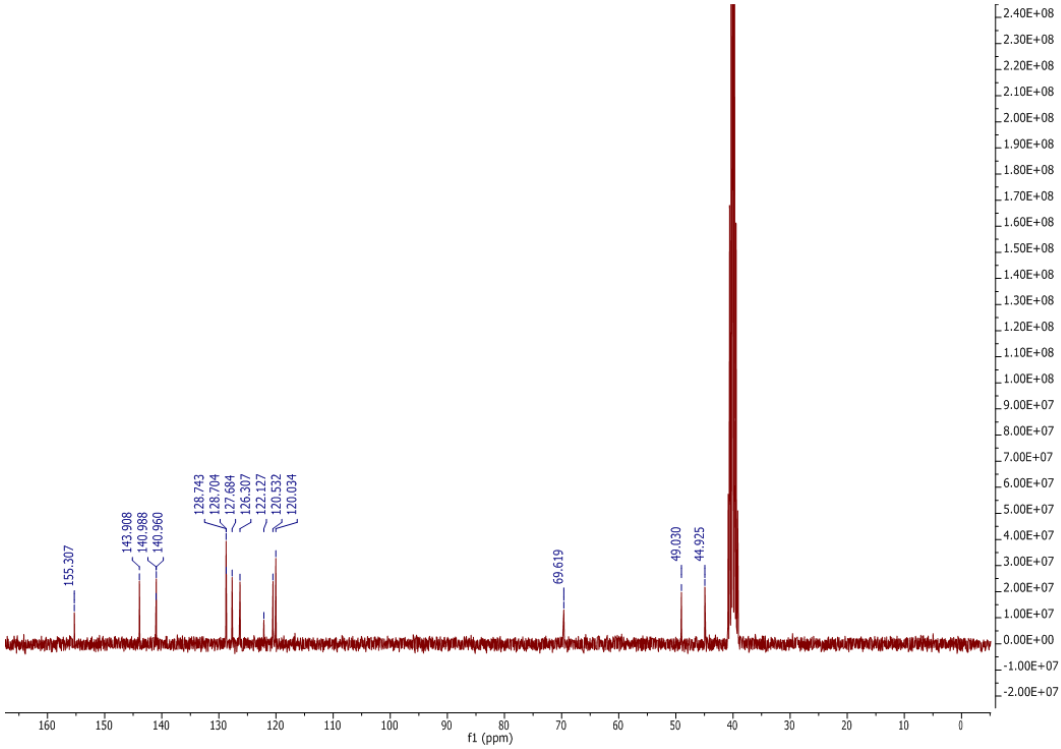
1.18. Compound 11



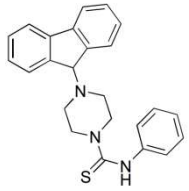
^{1}H NMR



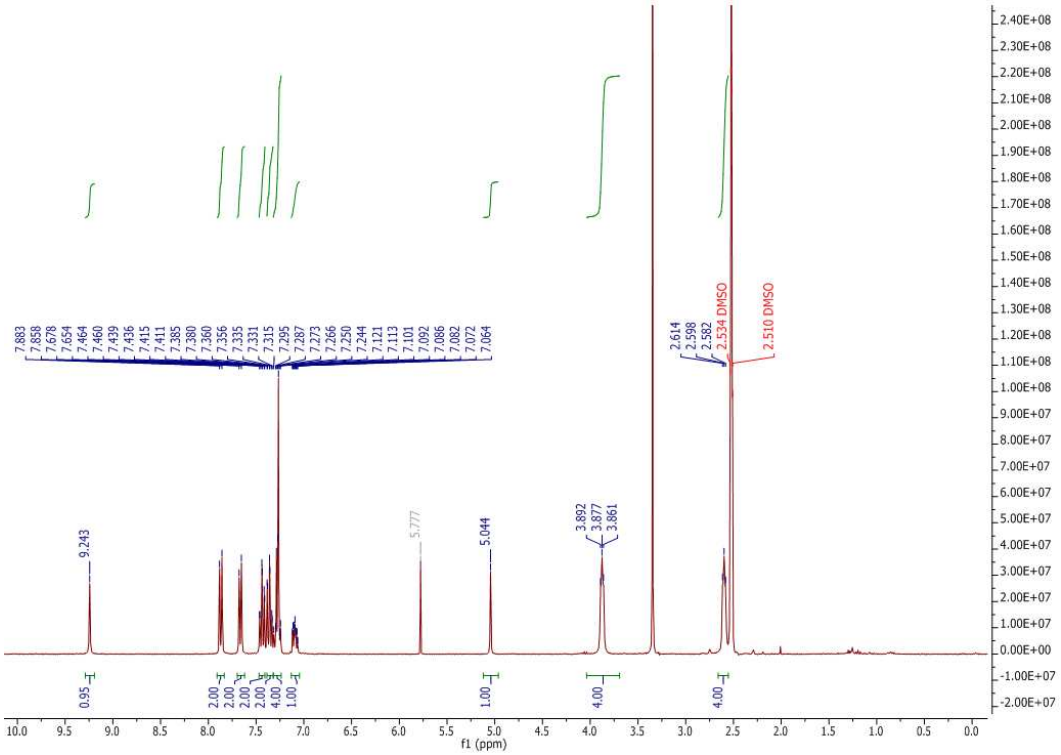
^{13}C NMR



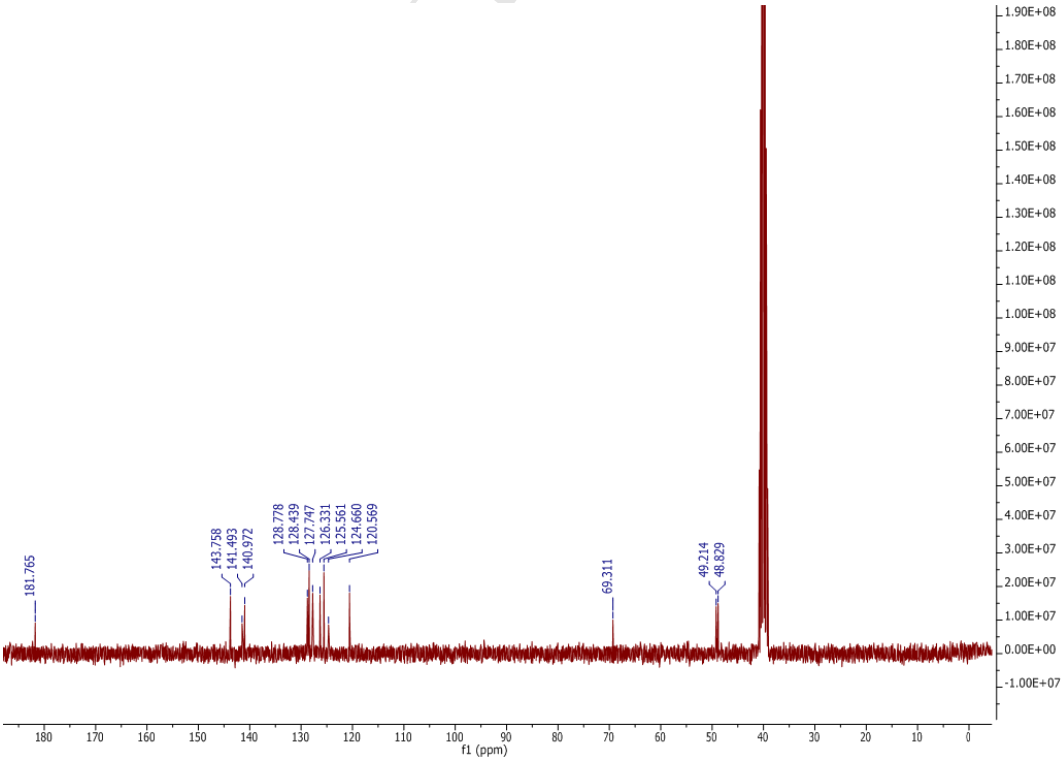
1.19. Compound 12



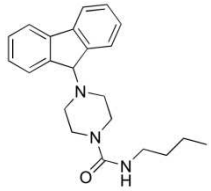
^{1}H NMR



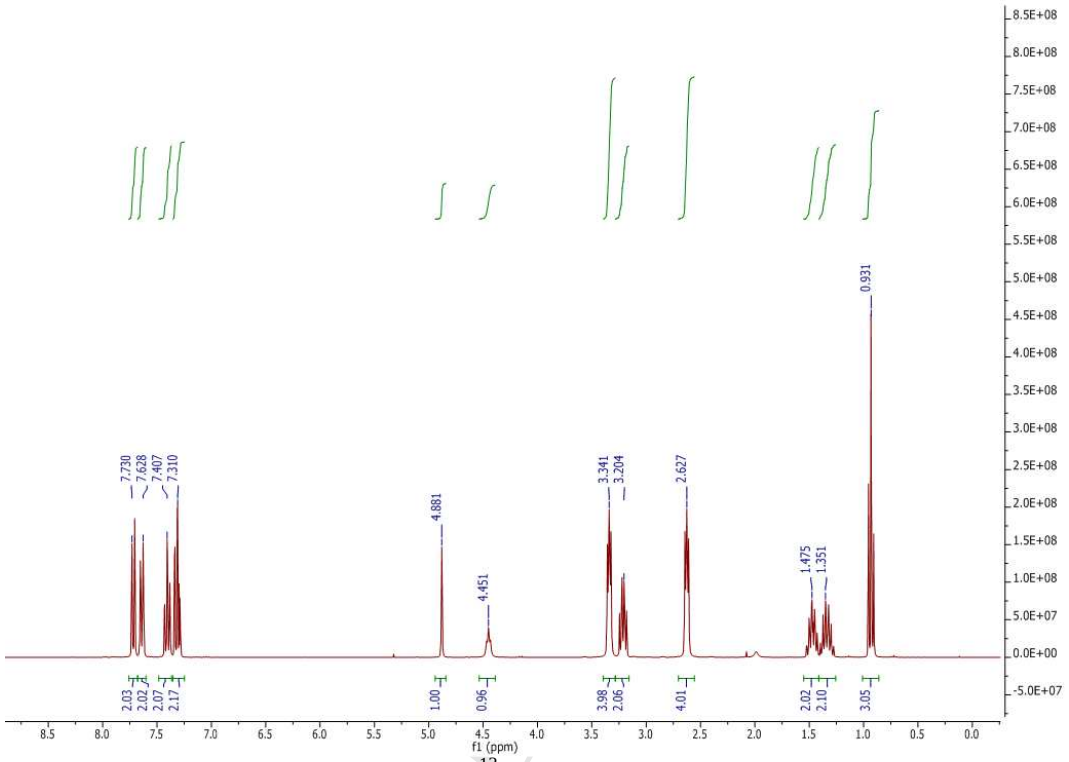
^{13}C NMR



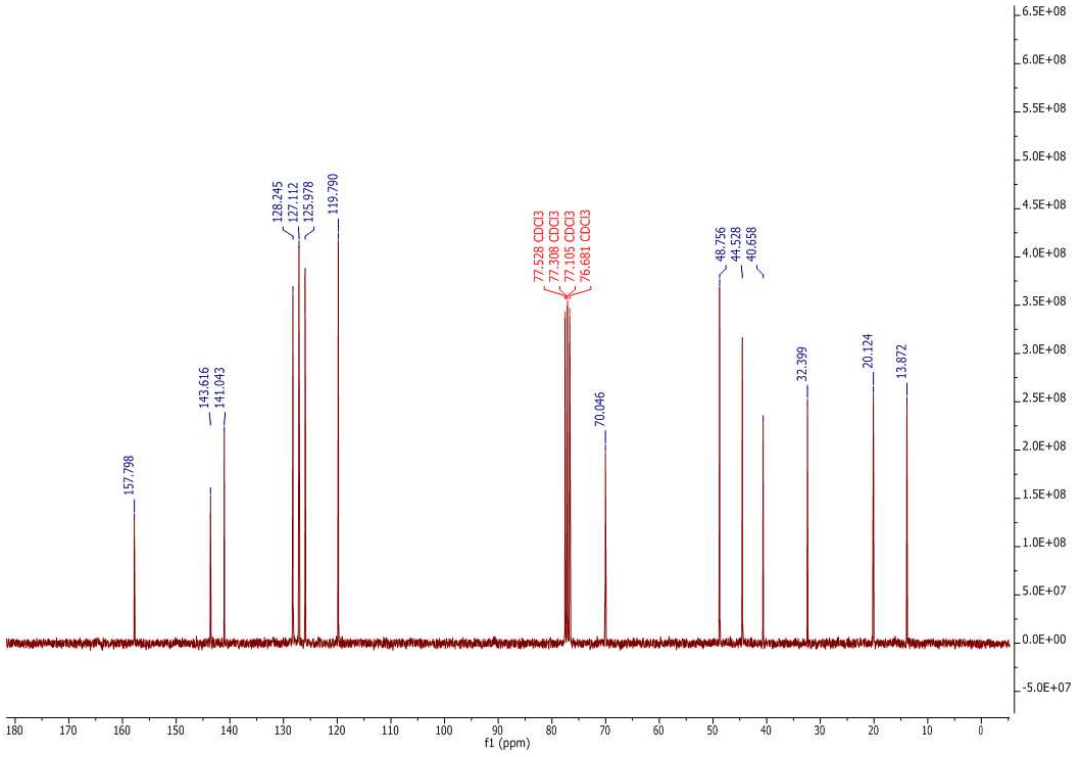
1.20. Compound 13



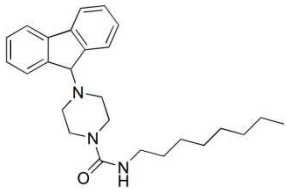
^{1}H NMR



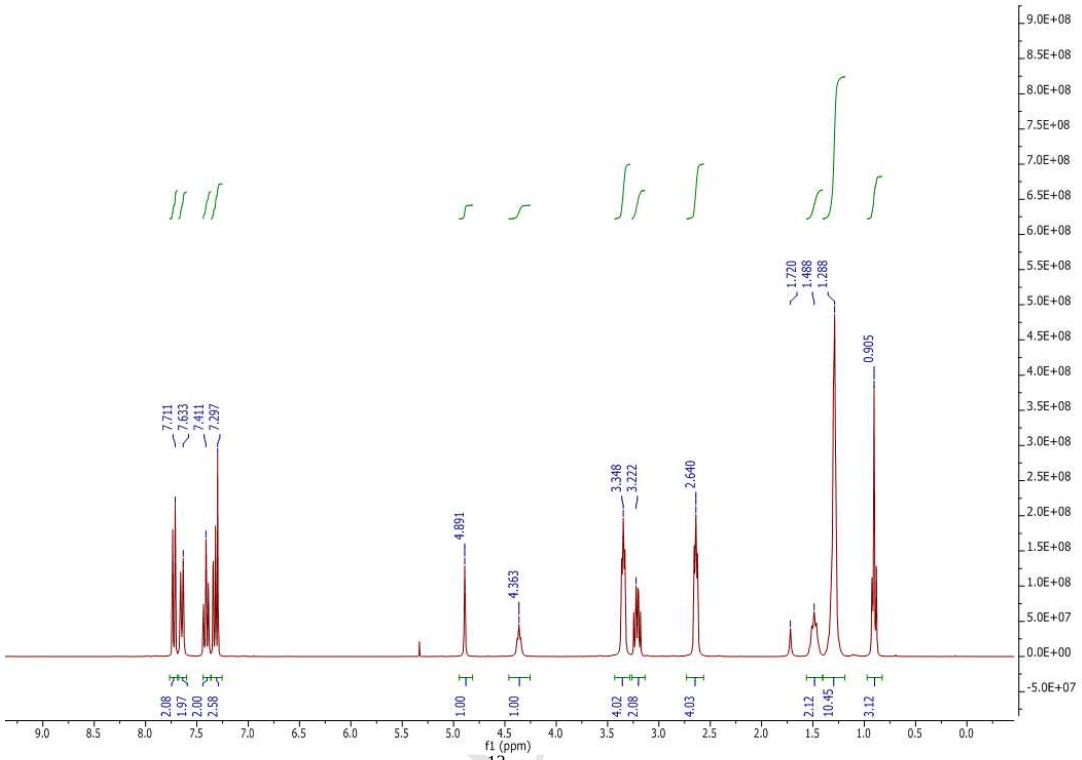
^{13}C NMR



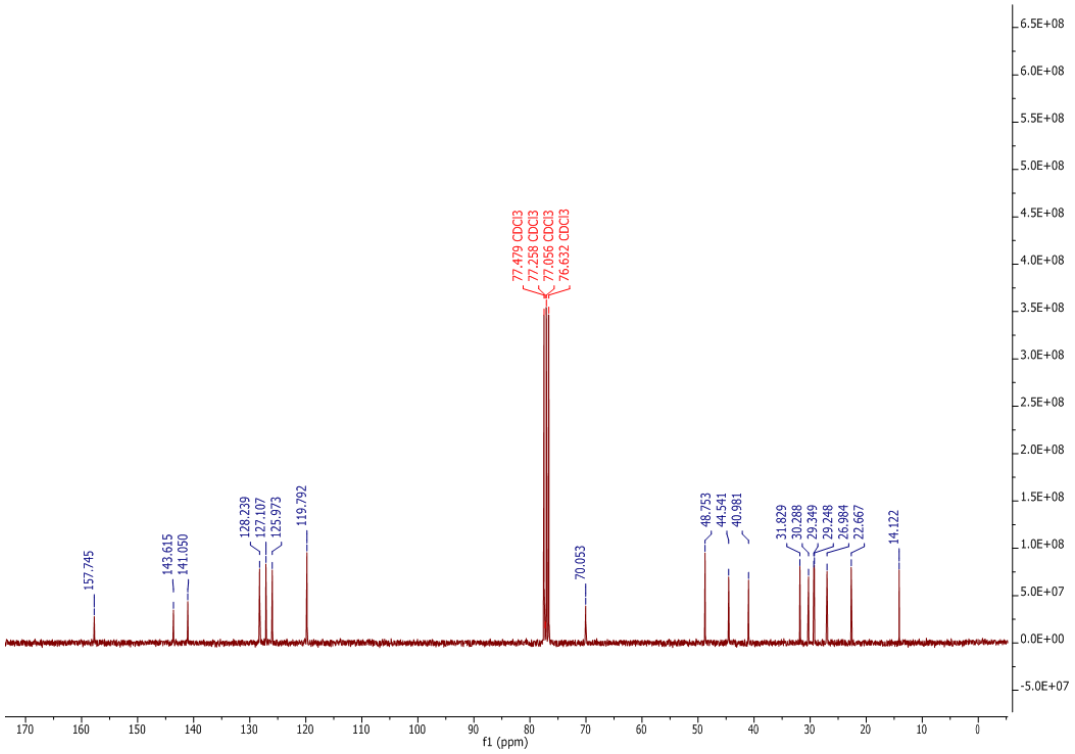
1.21. Compound 14



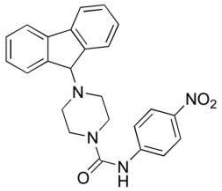
^{1}H NMR



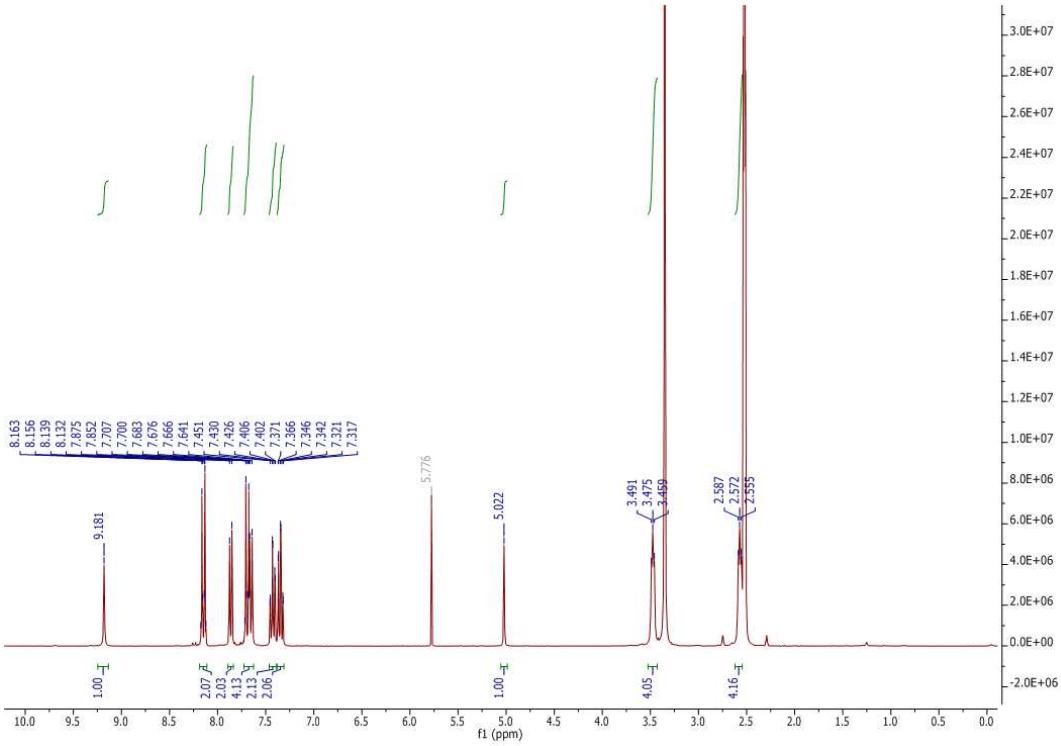
^{13}C NMR



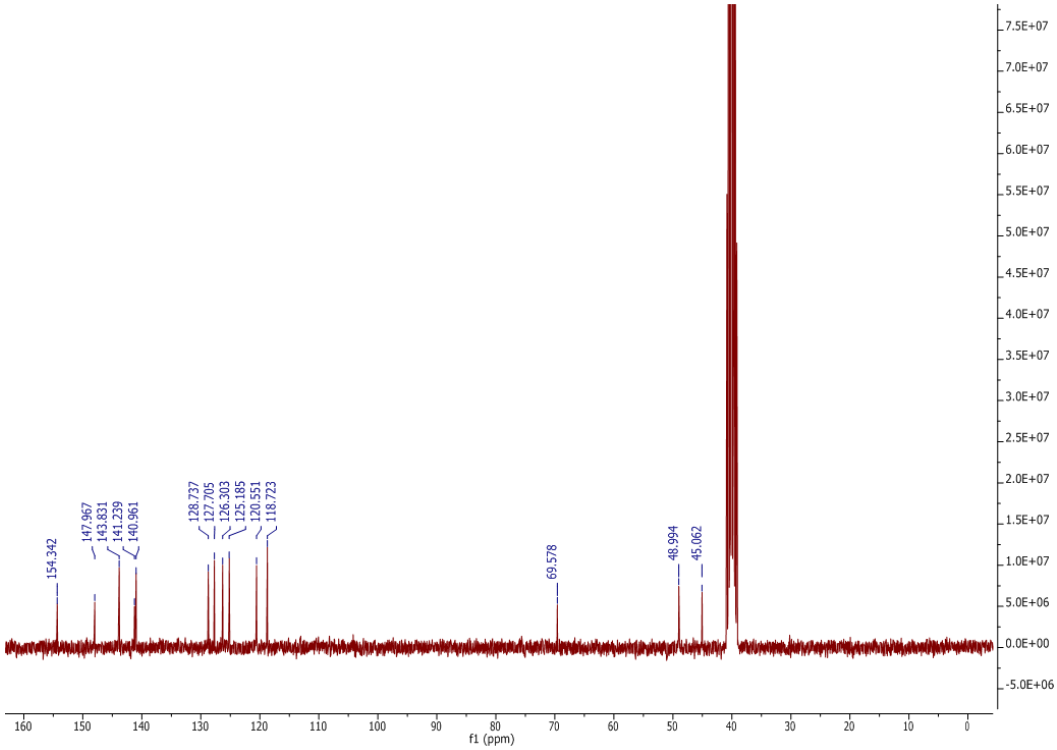
1.22. Compound 15



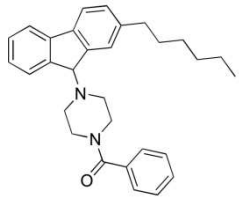
^{1}H NMR



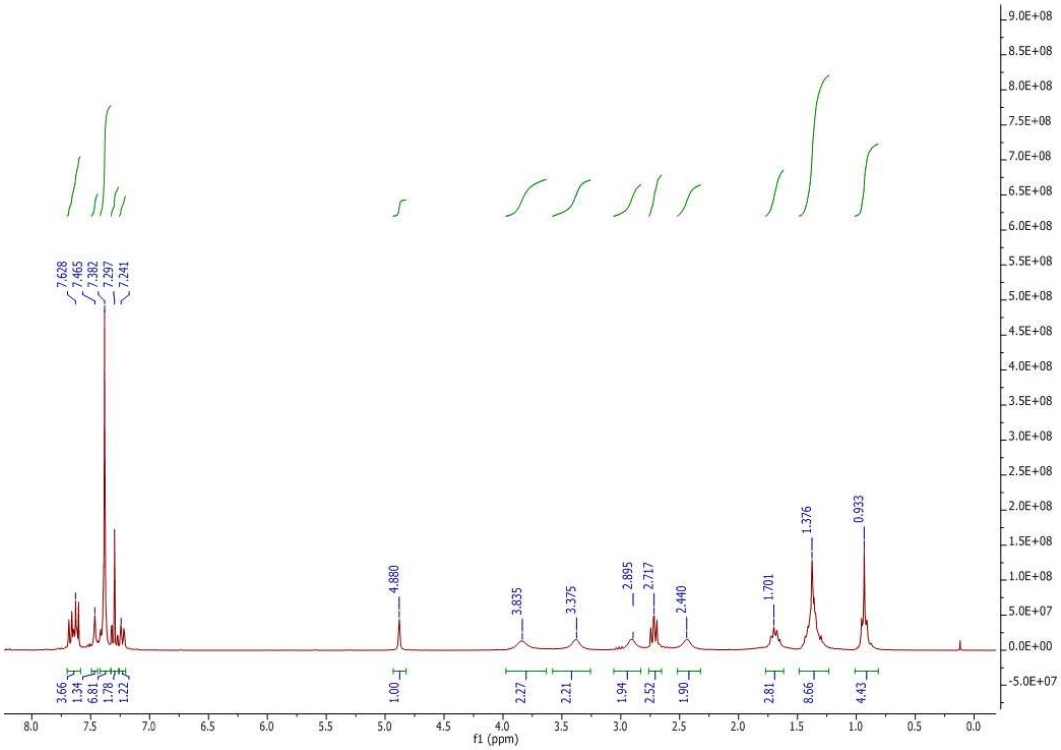
^{13}C NMR



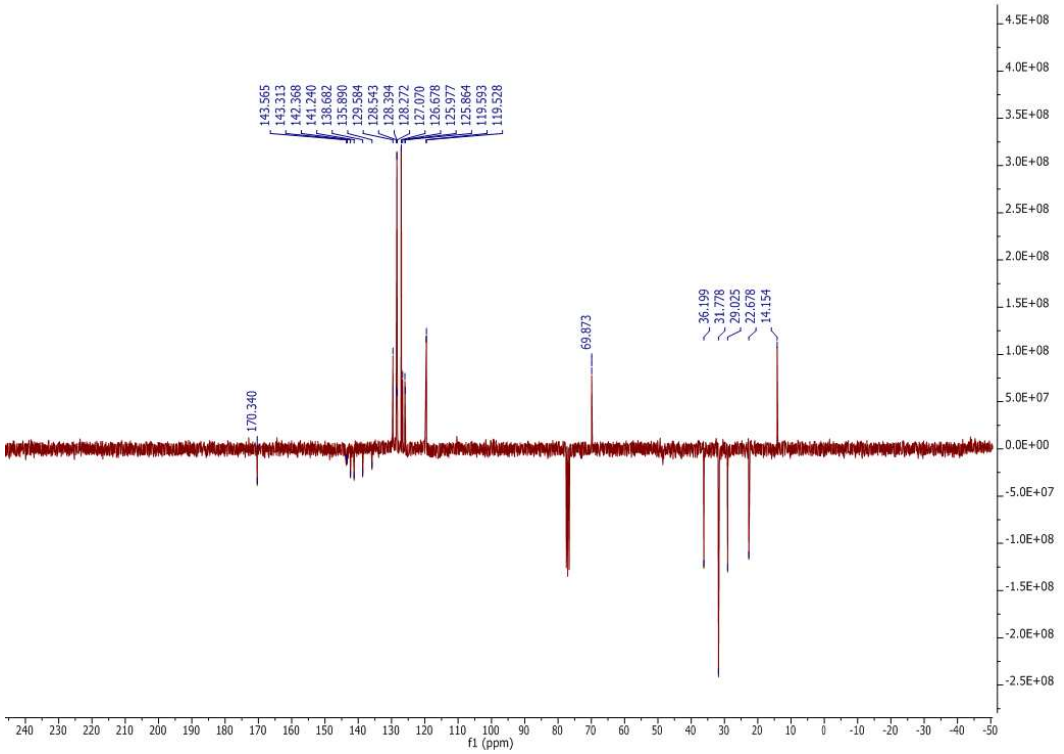
1.23. Compound 28



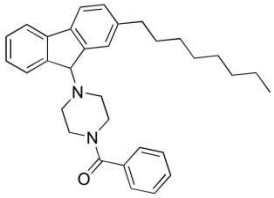
^{1}H NMR



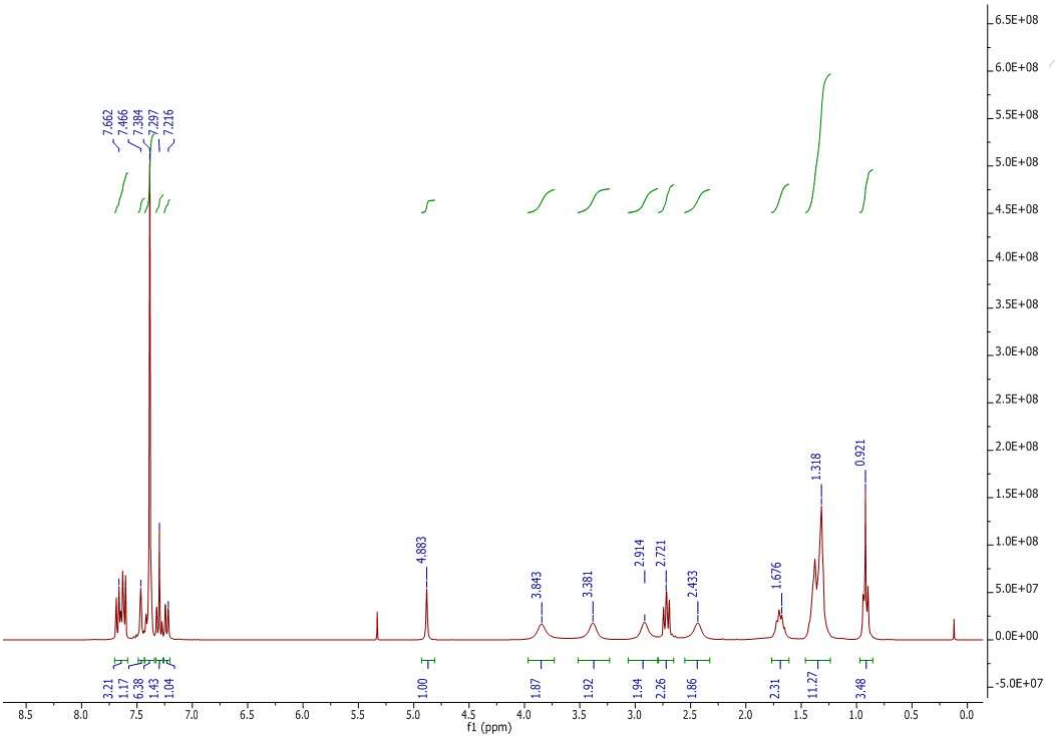
^{13}C NMR



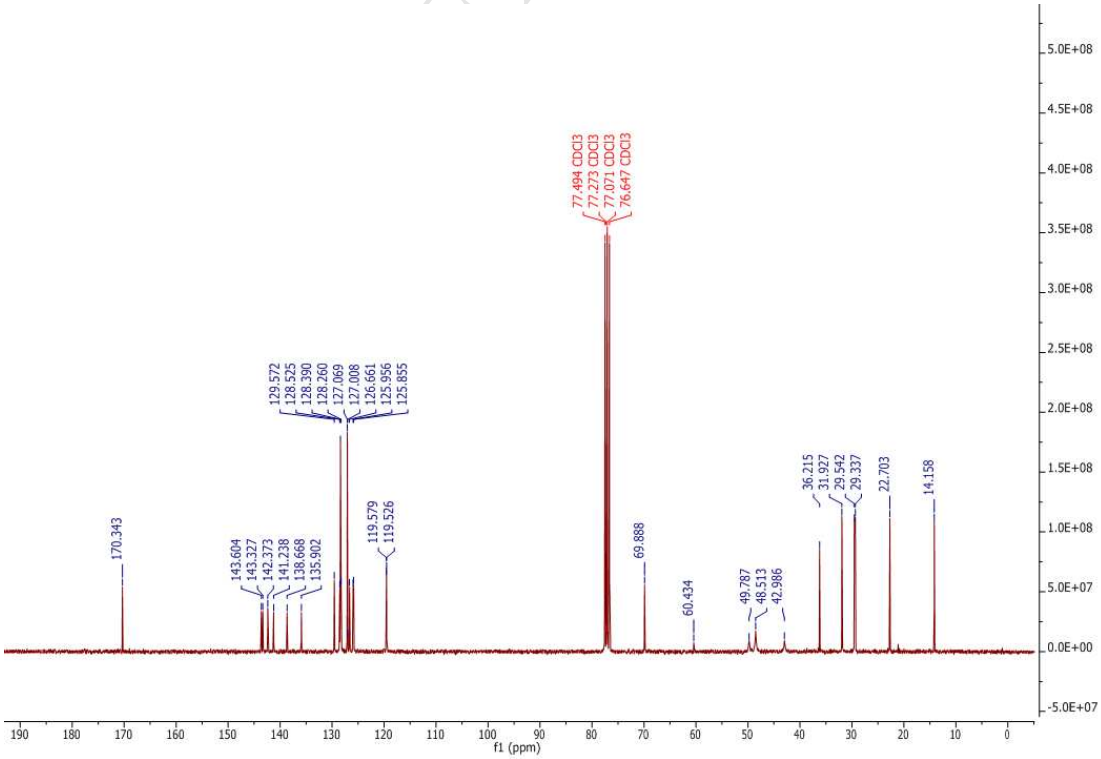
1.24. Compound 29



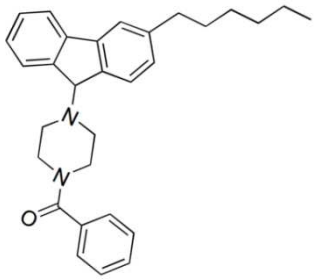
^{1}H NMR



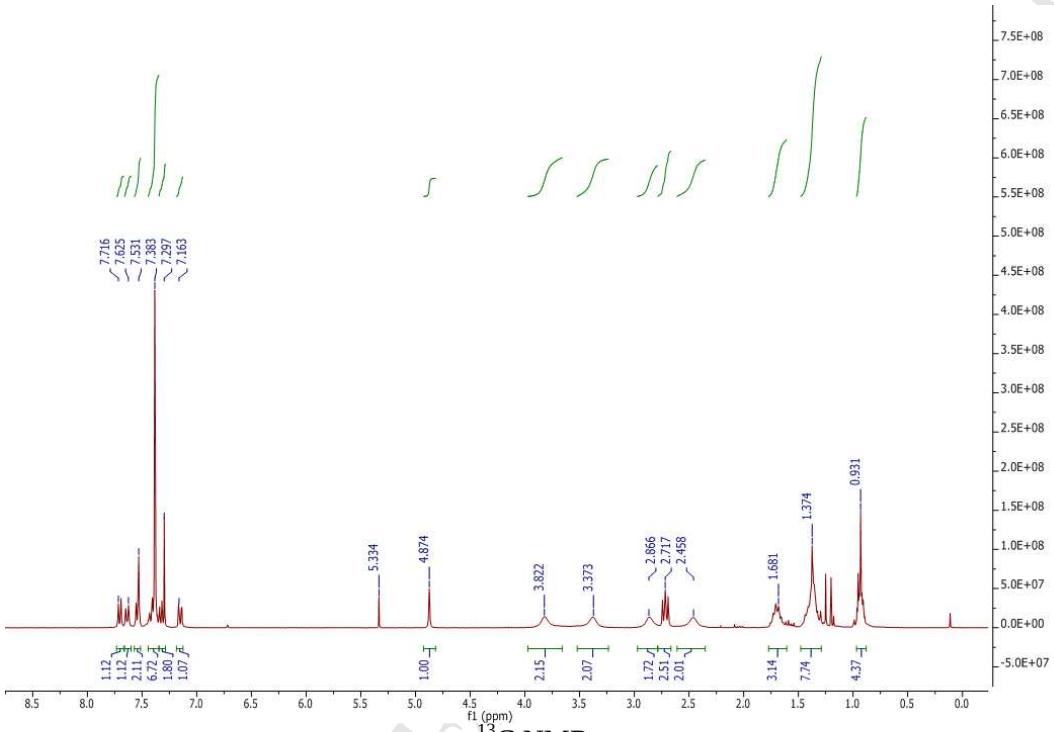
^{13}C NMR



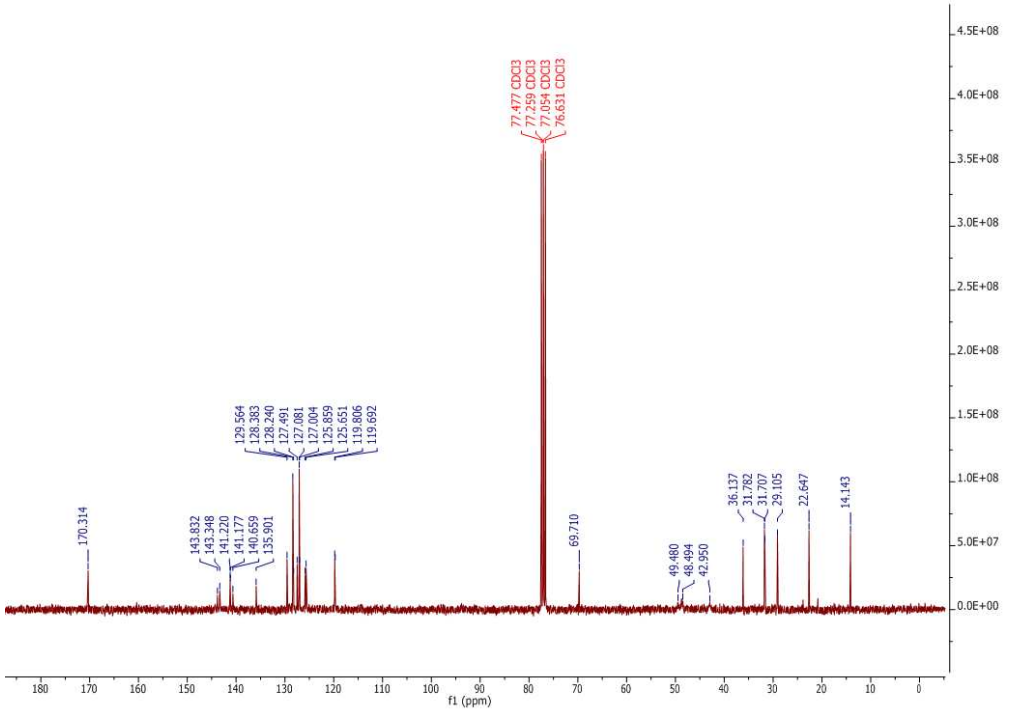
1.25. Compound 30



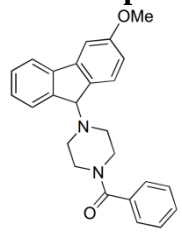
^{1}H NMR



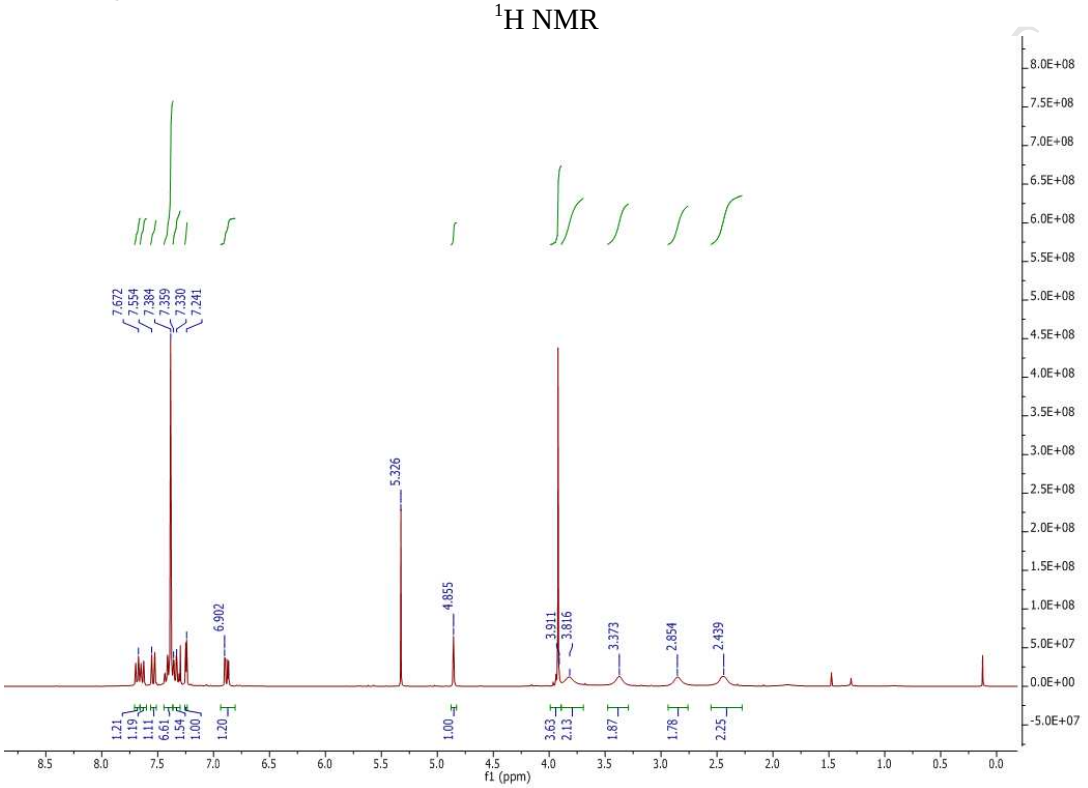
^{13}C NMR



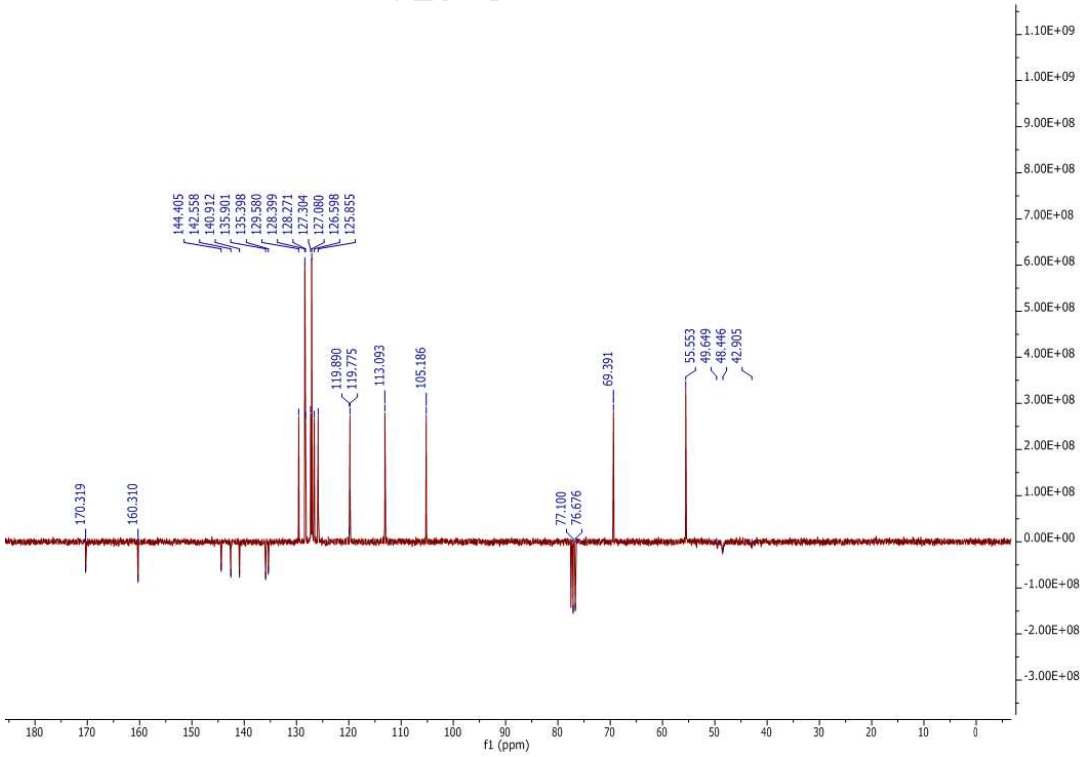
1.26. Compound 31



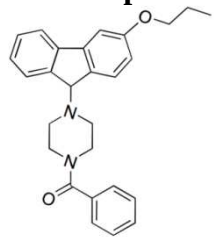
^{1}H NMR



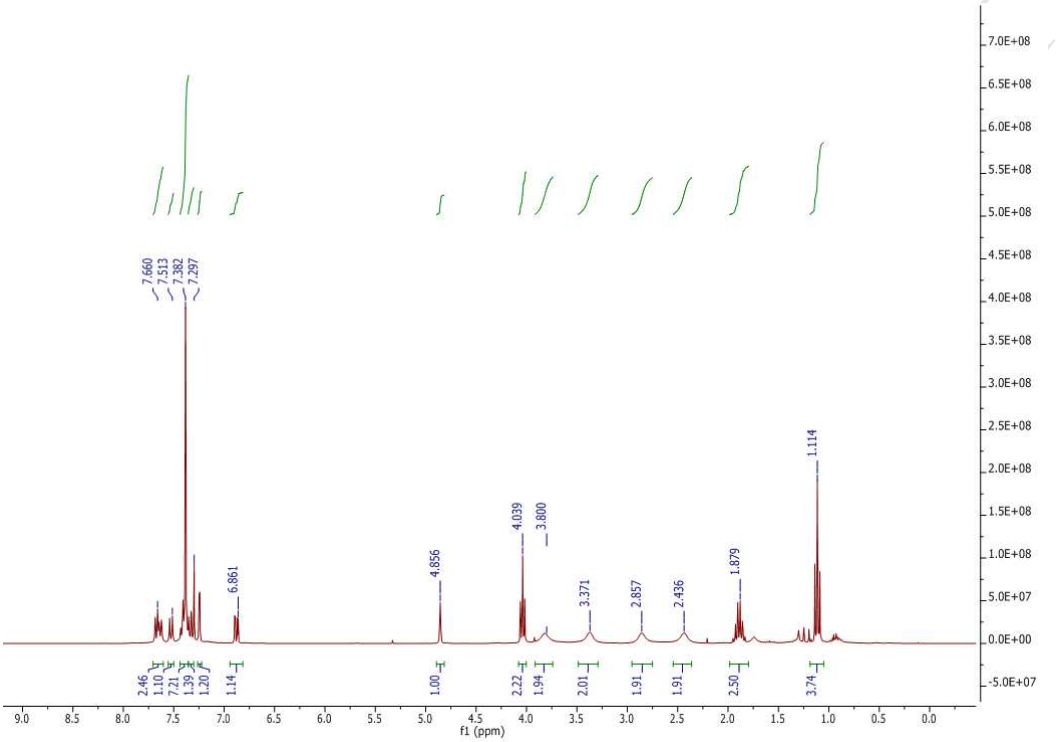
^{13}C NMR



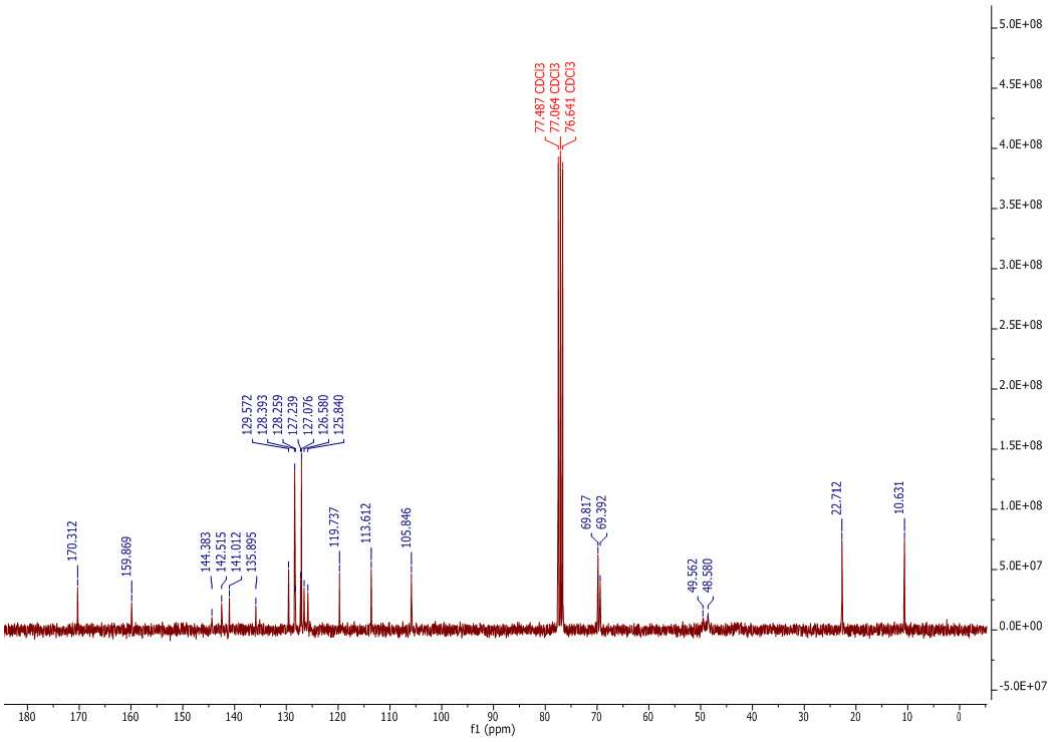
1.27. Compound 32



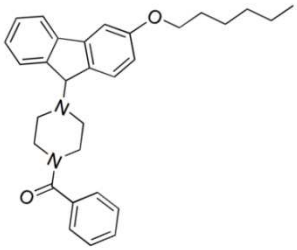
^{1}H NMR



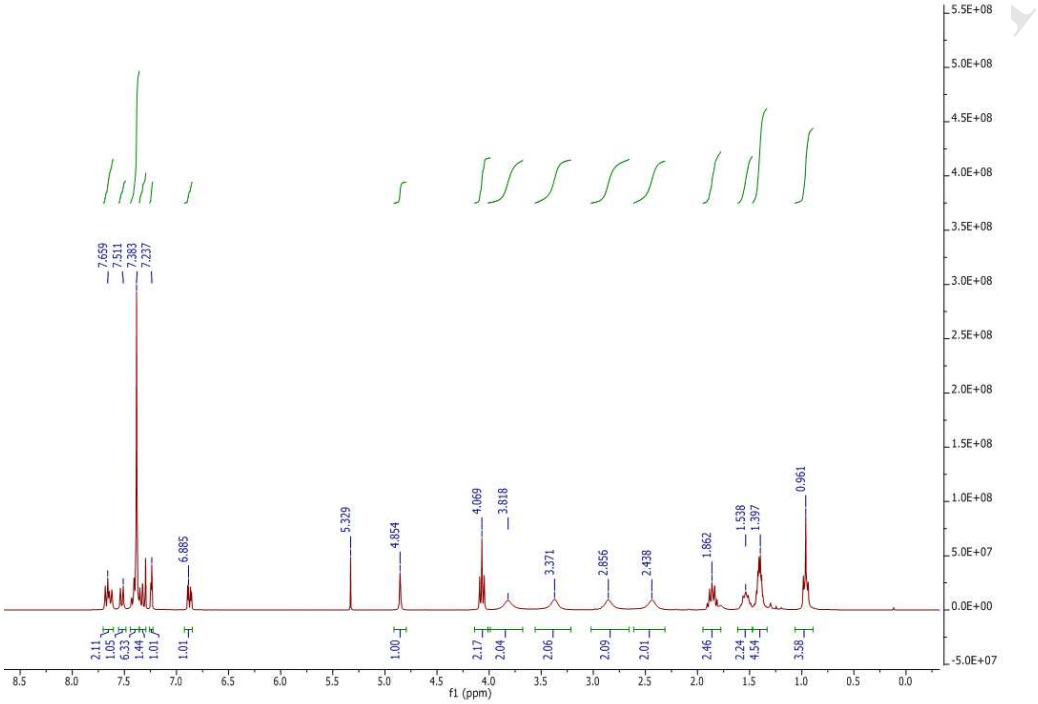
^{13}C NMR



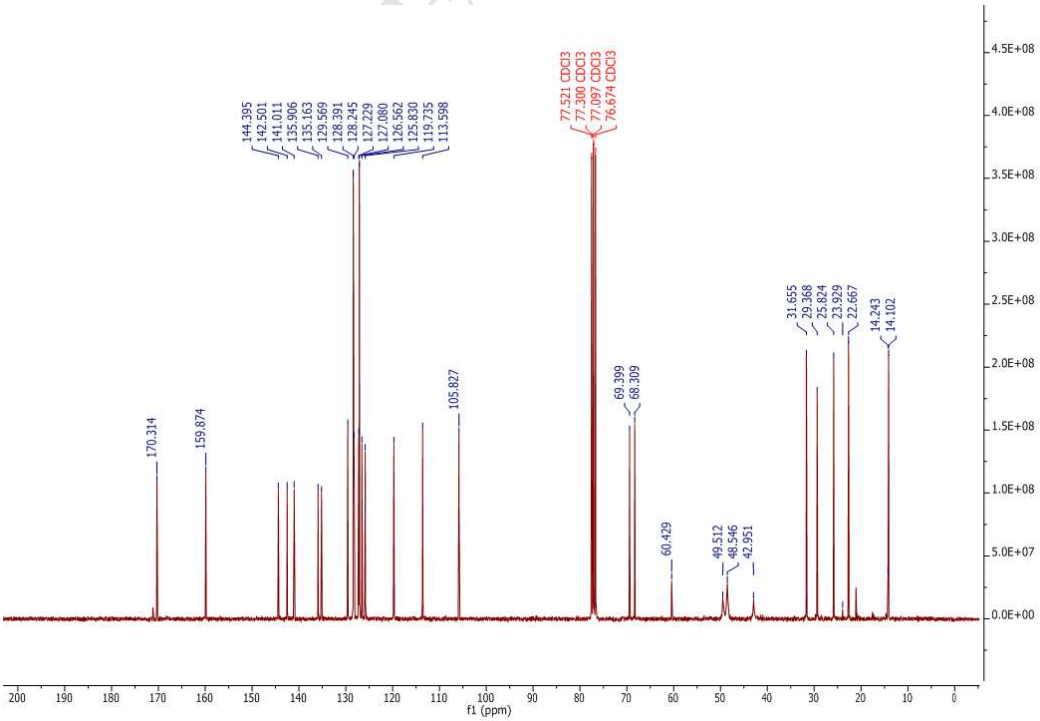
1.28. Compound 33



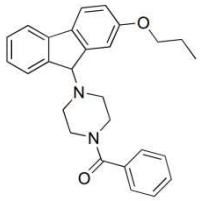
^{1}H NMR



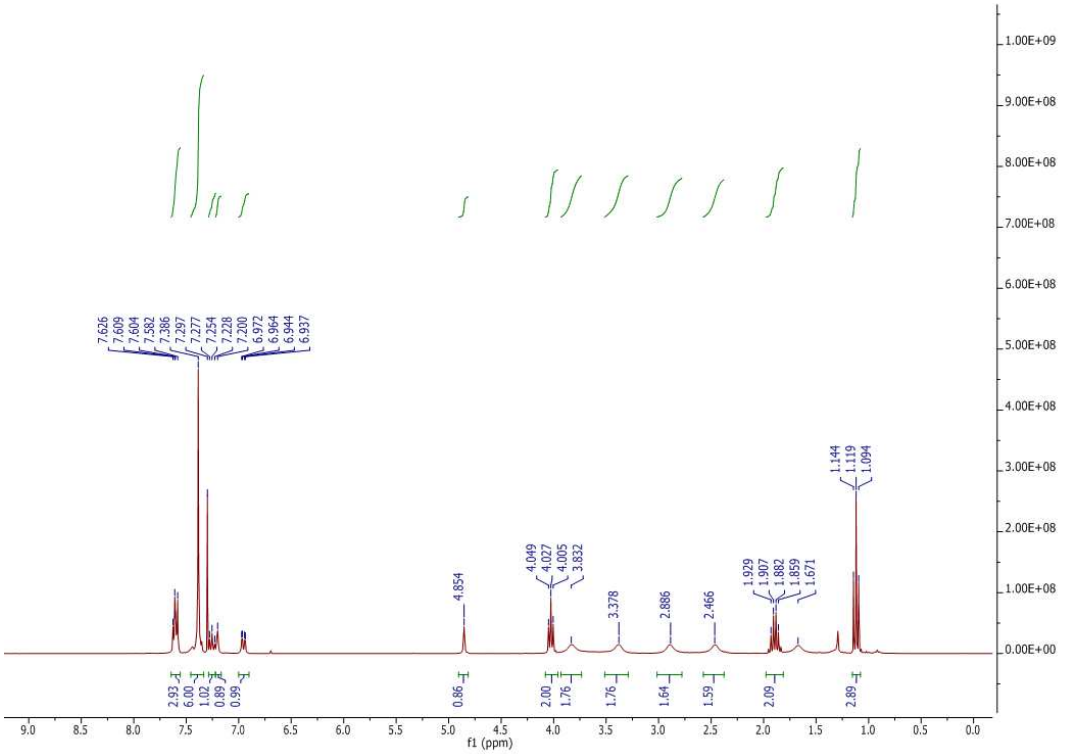
^{13}C NMR



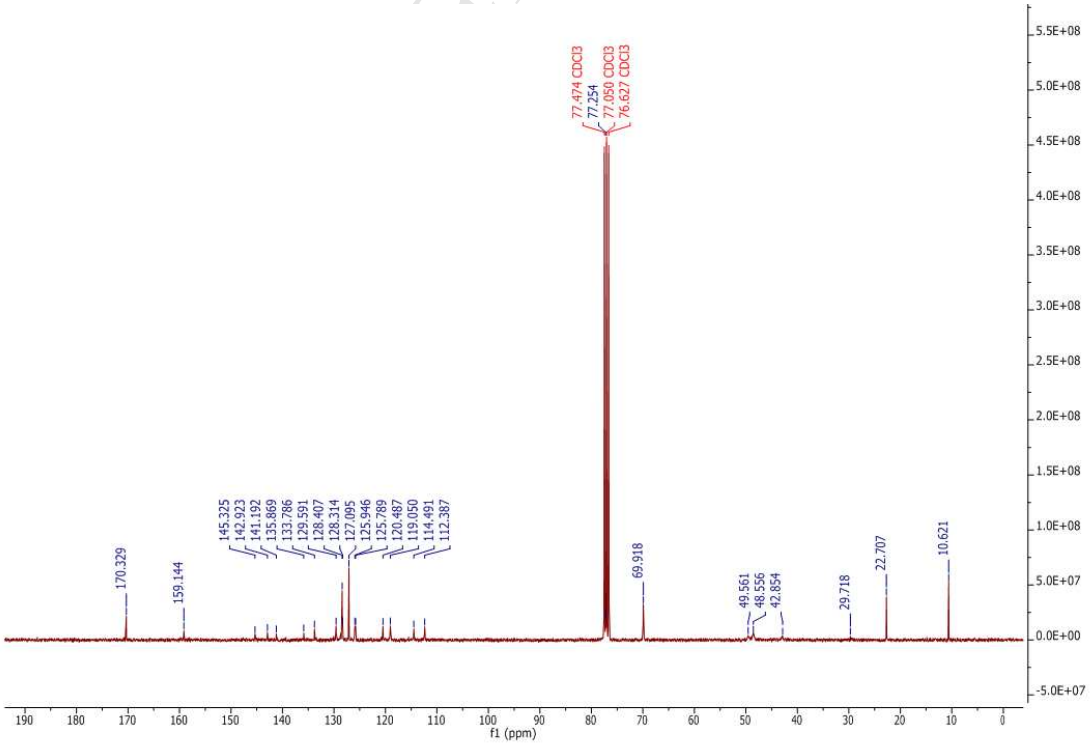
1.29. Compound 34



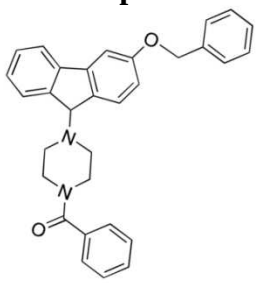
^{1}H NMR



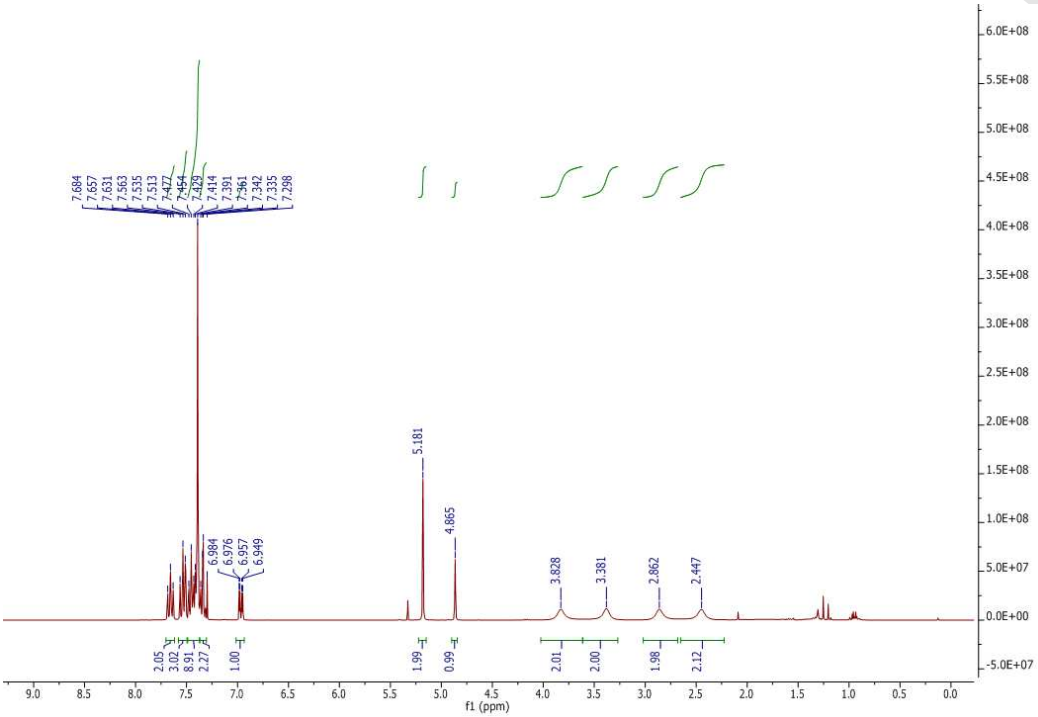
^{13}C NMR



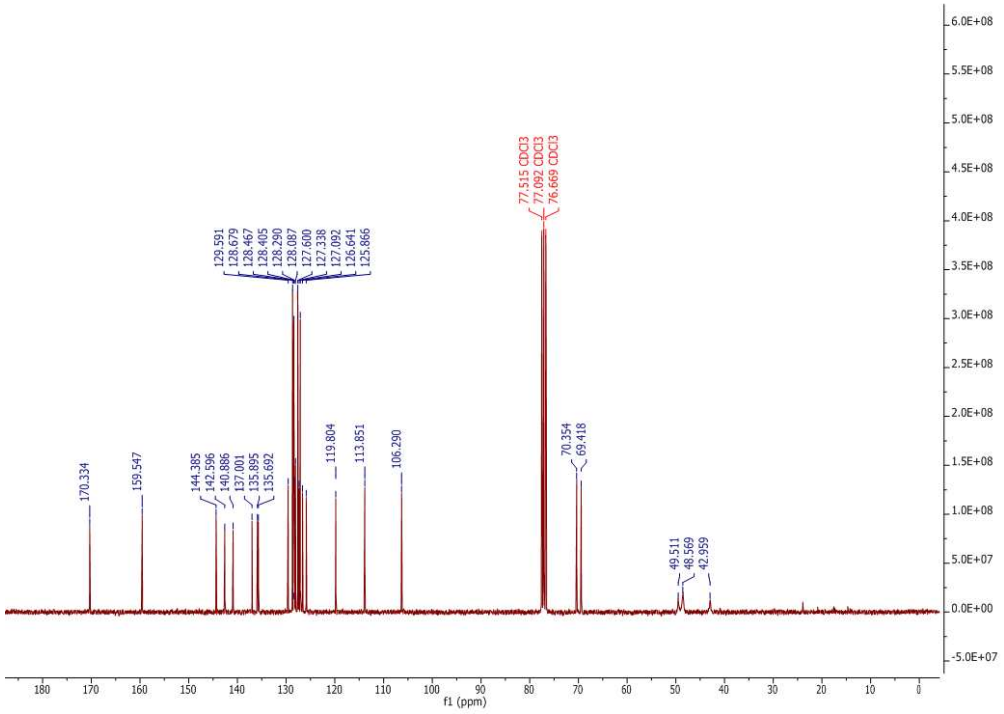
1.30. Compound 35



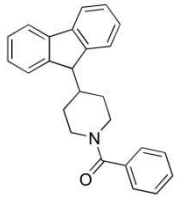
^{1}H NMR



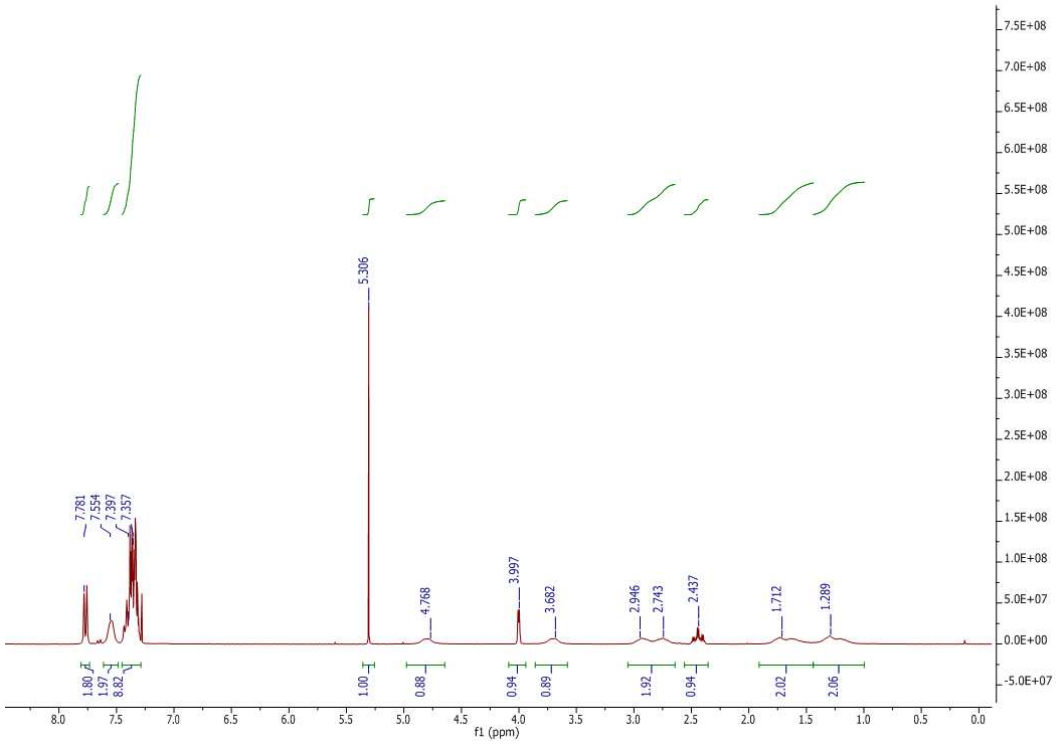
^{13}C NMR



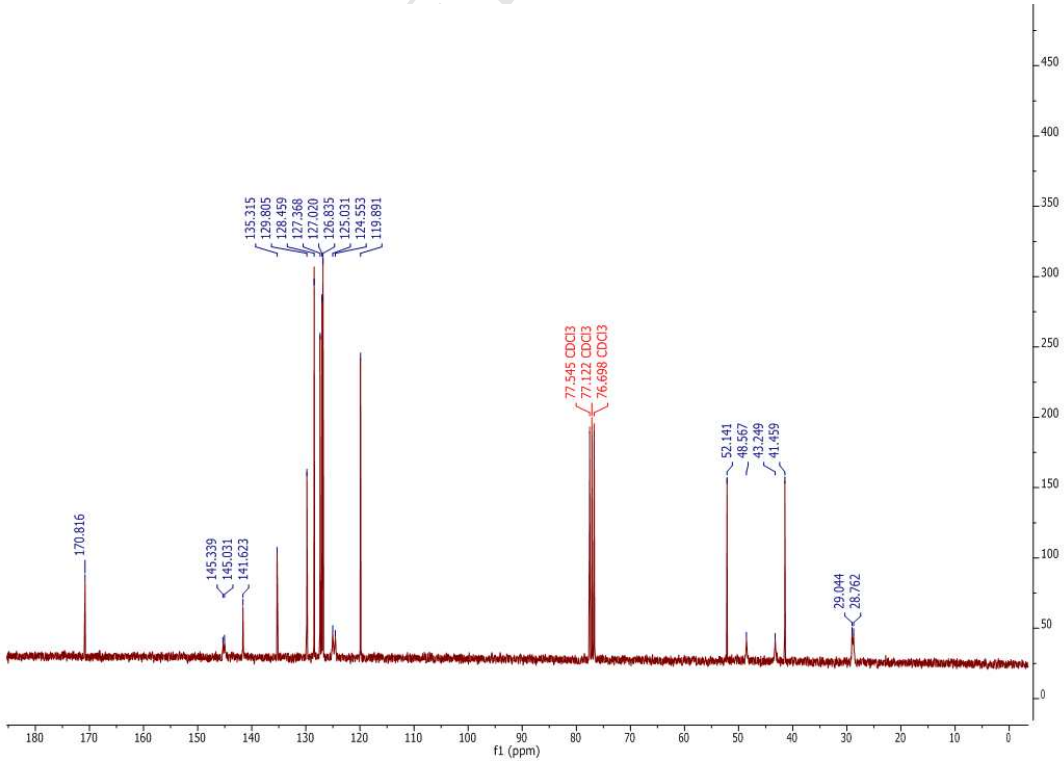
1.31. Compound 39



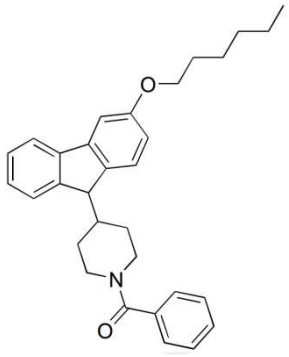
^{1}H NMR



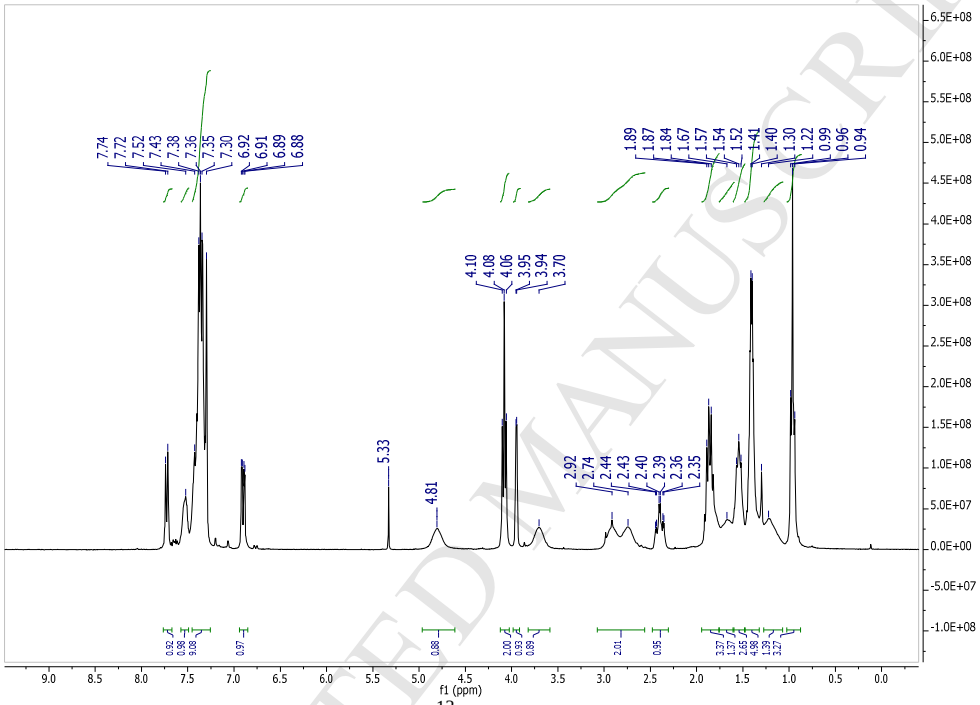
^{13}C NMR



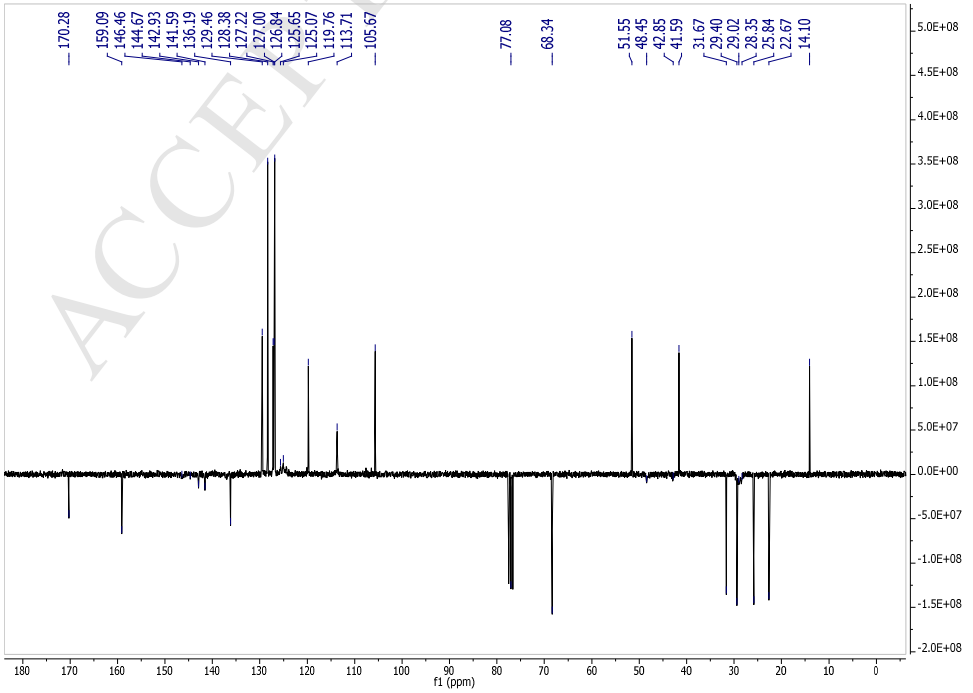
1.32. Compound 40



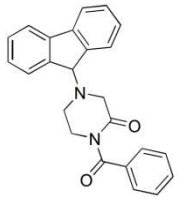
^{1}H NMR



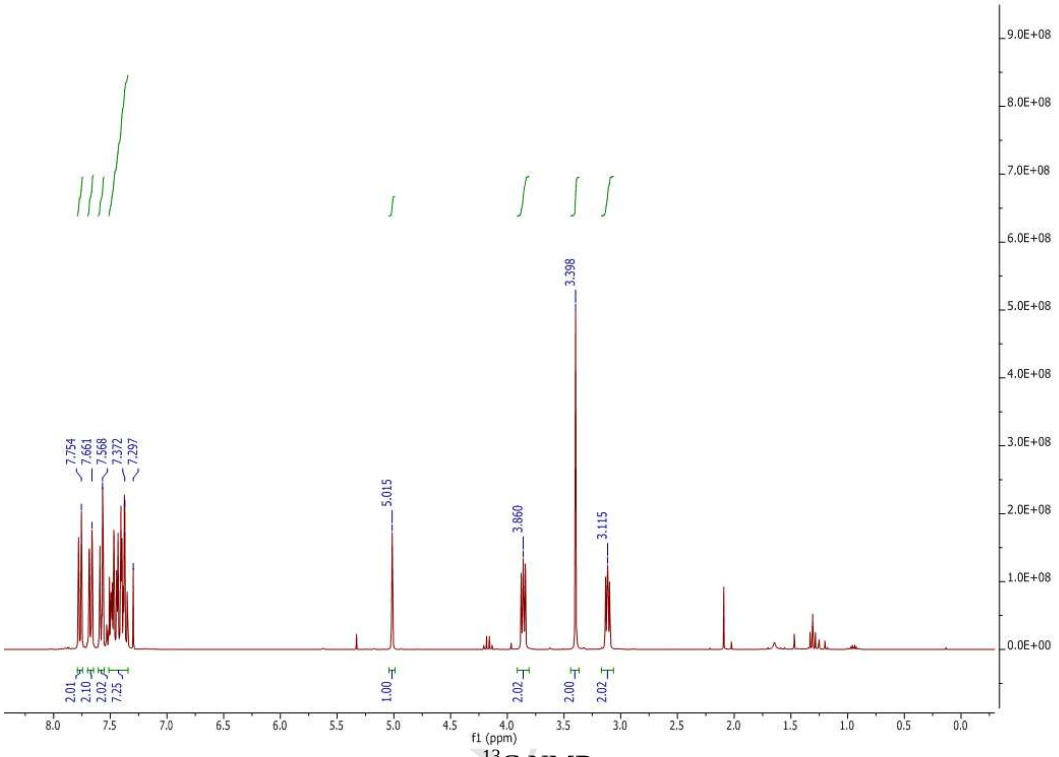
^{13}C NMR



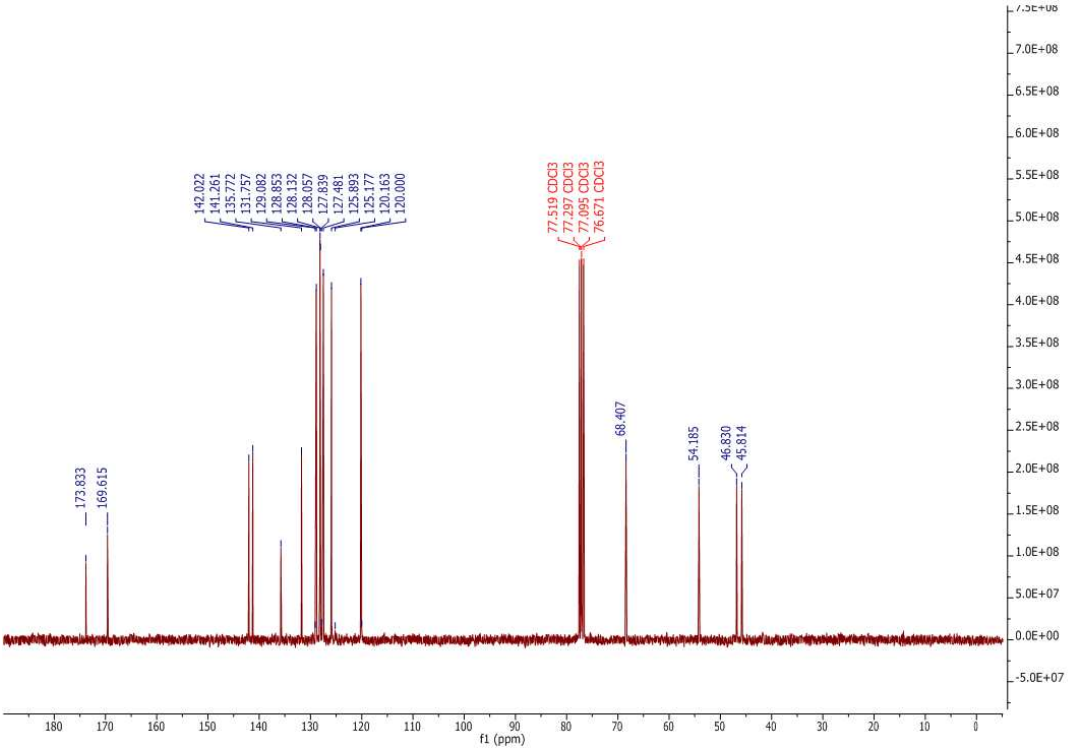
1.33. Compound 42



^{1}H NMR



^{13}C NMR

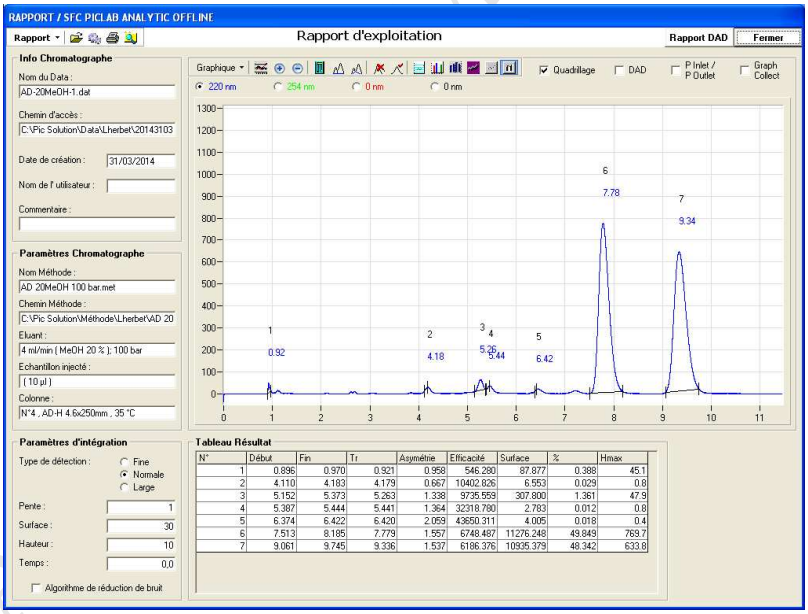


Enantiomeric separation

1.34. Enantiomeric separation of compound 31

1.34.1. Analytical chromatography of the racemic mixture

Colum	Chiralpak **AD-H** 5µm (4.6x250) mm
Flow (CO_2+co-solvant) (mL/min)	4mL/min
Co-solvant	MeOH
% Co-solvant	**20%**
Temperature (°C)	35
P_{out} (bar)	100
λ (nm)	220 nm, 254 nm
R_t (min)	7.78 and 9.34
Duration (min)	12



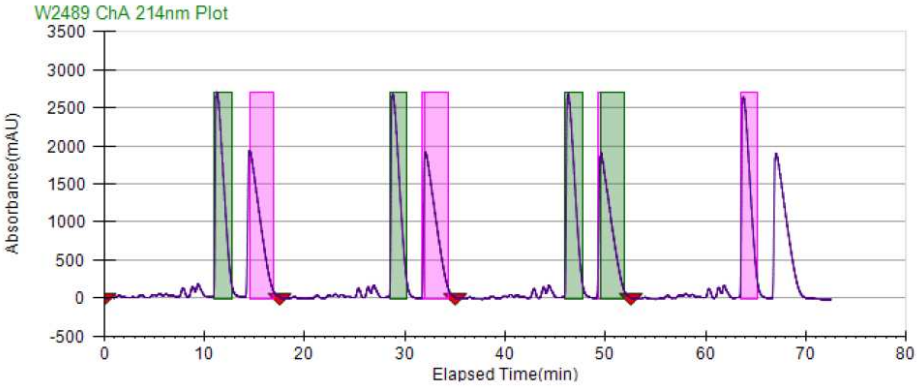
Chromatogram for the analytical chiral chromatography of the crude compound **31** at λ = 220 nm.

1.34.2. Preparative chromatography

Column	Chiralpak AD-H 5µm (10x250) mm
Flow (CO_2+co-solvant) (mL/min)	15 mL/min

Co-solvent	MeOH
% Co-solvent	20%
Temperature (°C)	40
P_{out} (bar)	100
λ (nm)	214nm
Duration (min)	25 min
Injection	2.5 mL / 4 stacking injections

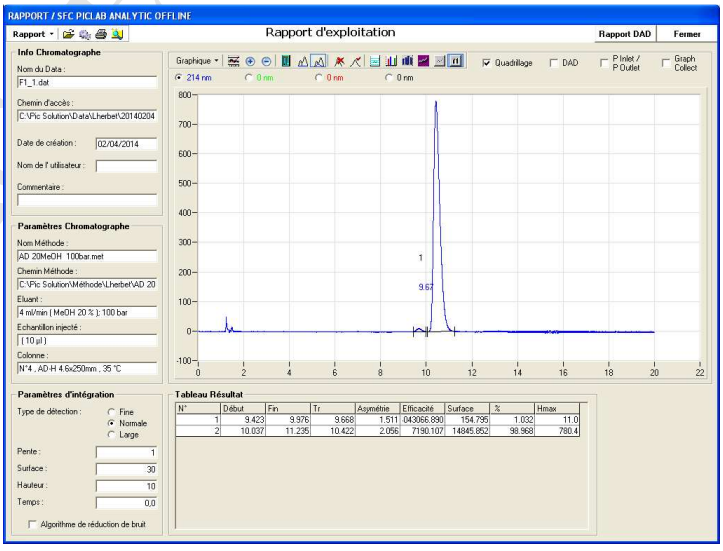
Sample: 34 mg of racemic mixture of compound **31** in 10 mL MeOH.



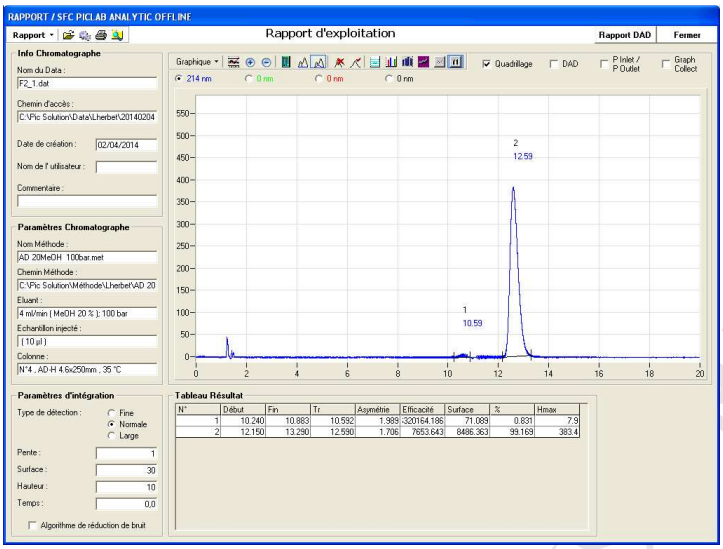
Chromatogram for the preparative chiral chromatography of compound **31**

1.34.3. *Analytical chromatography of enantiopurs compounds **31a** and **31b***

The elution fractions were respectively named **31a** and **31b**.



Chromatogram for the analytical chiral chromatography of compound **31a** at λ = 220 nm after purification.



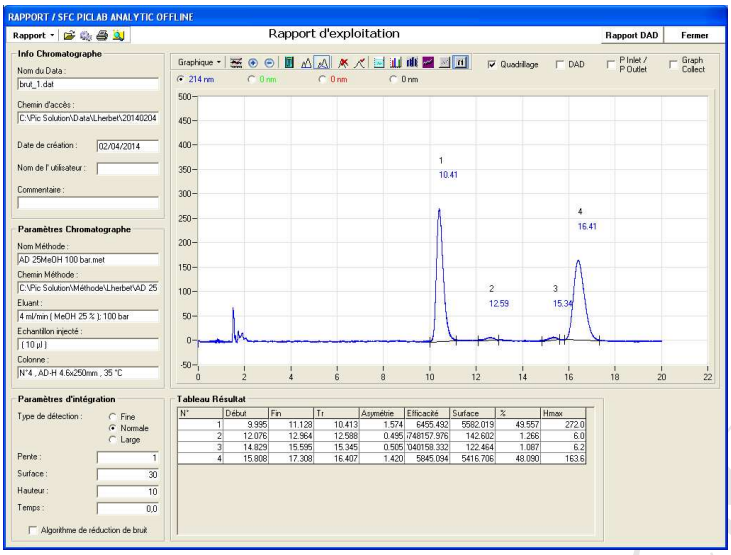
Chromatogram for the analytical chiral chromatography of compound **31b** at λ = 220 nm after purification.

Compound	Enantiomeric purity according to analytic SFC
31a	99.0%
31b	99.2%

1.35. Enantiomeric separation of compound 33

1.35.1. Analytical chromatography of the racemic mixture

Colum	Chiralpak **AD-H** 5µm (4.6x250) mm
Flow (CO_2+co-solvant) (mL/min)	4 mL/min
Co-solvant	MeOH
% Co-solvant	**25%**
Temperature (°C)	35
P_{out} (bar)	100
λ (nm)	220 nm, 254 nm
R_t (min)	6.73 and 10.62
Duration (min)	15

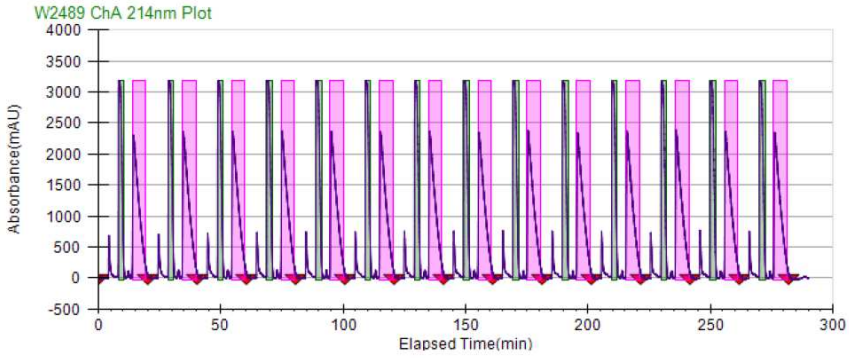


Chromatogram for the analytical chiral chromatography of the crude compound **33** at λ = 220 nm.

1.35.2. Preparative chromatography

Column	Chiralpak AD-H 5µm (10x250) mm
Flow (CO_2+co-solvant) (mL/min)	15 mL/min
Co-solvent	MeOH
% Co-solvent	25%
Temperature (°C)	40
P_{out} (bar)	100
λ (nm)	220nm
Duration (min)	30 min
Injection	1 mL / 15 stacking injections

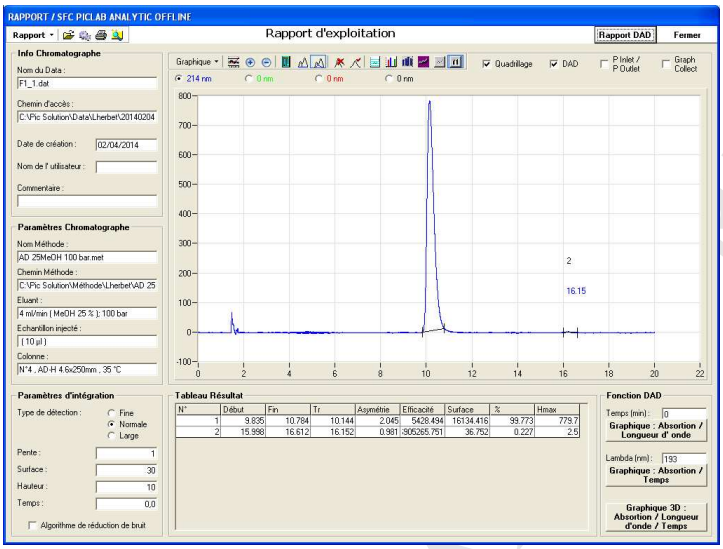
Sample: 200 mg of racemic mixture of compound **33** in 20 mL MeOH.



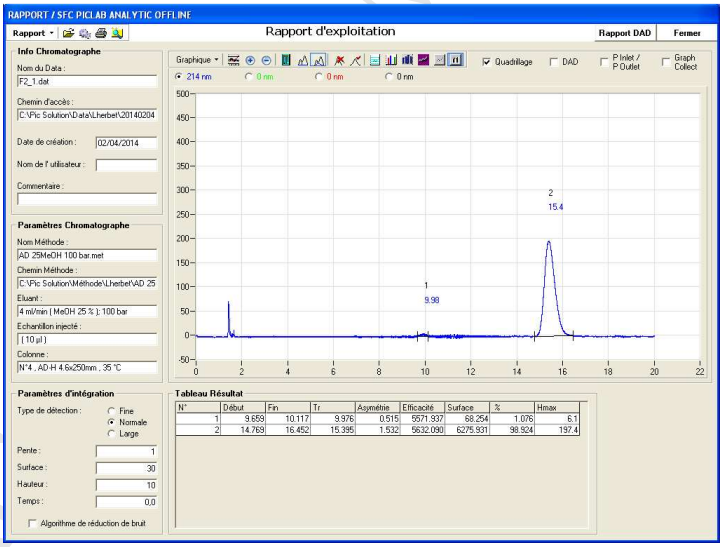
Chromatogram for the preparative chiral chromatography of compound **33**

*1.35.3. Analytical chromatography of enantiopurs compounds **33a** and **33b***

The elution fractions were respectively named **33a** and **33b**.



Chromatogram for the analytical chiral chromatography of compound **33a** at λ = 220 nm after purification.



Chromatogram for the analytical chiral chromatography of compound **33b** at λ = 220 nm after purification.

Compound	Enantiomeric purity according to analytic SFC
33a	99.8%
33b	98.9%

Computational study

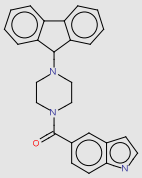
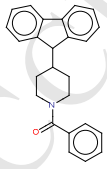
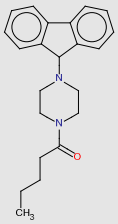
Table S1 : Docking results

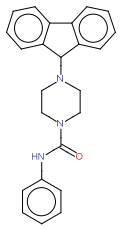
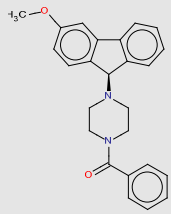
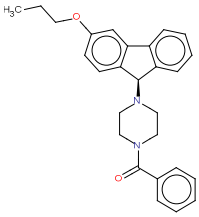
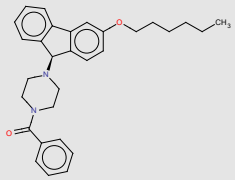
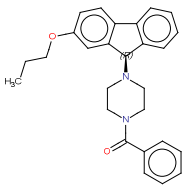
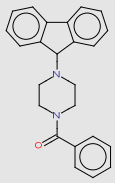
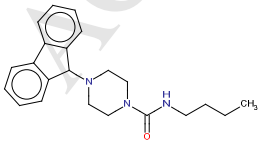
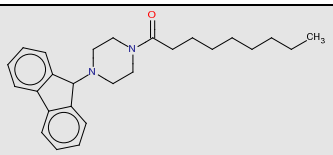
The compound identifiers are given in columns *Id* (compound) and *Ligand*. The column *Ligand* emphasizes on the corresponding stereochemistry of each compound. The value of InhA inhibition (%) at 50 µM is given in column *PI50,* racemic mixtures are marked by (*) after PI50 values.

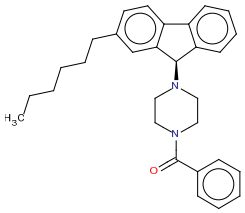
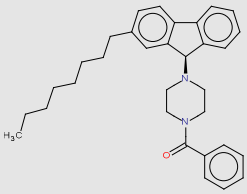
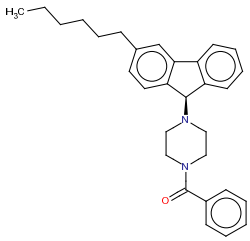
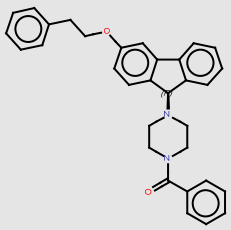
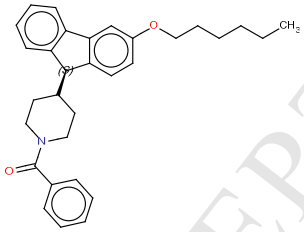
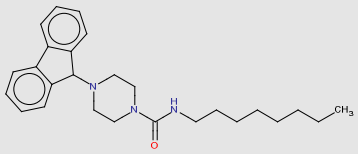
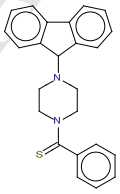
The *HA* column stands for heavy atoms (C, O, N) count for each ligand. The normalized docking scores are given in columns *LE1, LE3, LE2* for each compound (or enantiomer), these descriptors are calculated using docking scores values (MolDock and Rerank [3], PLANTS [4] scoring schemes, respectively) divided by HA values. MolDock, Rerank and PLANTS scores were calculated using Molegro Virtual Docker 6.0 software [5].

LogP values are calculated using Chemaxon Marvin log*P* calculator [6] working in default weighted mode (average of VG [7], KLOGP [8] and PHYSPROP [9] methods), molecules were sketched using Chemaxon Marvin.[6]

The *Group* column follows the compound activity class (*group1, group2, group3*) as discussed in article.

Id	*Compound*	*PI50*	*HA*	*LogP*	*Ligand*	*LE1*	*LE3*	*LE2*	*Group*
GEQ		87	30	4.13	**GEQ**	-7.52	-5.24	-4.25	1
1		94	27	4.30	**1**	-7.35	-5.24	-4.51	1
4		75	25	4.04	**4**	-7.79	-5.52	-5.08	1

Id	Compound	PI50	HA	LogP	Ligand	LE1	LE3	LE2	Group
11		84	28	4.65	**11**	-7.38	-5.06	-4.10	1
31		92 (*)	29	4.14	**31r**	-7.19	-5.16	-3.85	1
					31s	-7.11	-5.10	-3.98	
32		91 (*)	31	5.02	**32r**	-6.66	-4.79	-3.66	1
					32s	-7.14	-5.15	-3.82	
33		79 (*)	34	6.36	**33r**	-6.97	-5.02	-3.75	1
					33s	-4.88	-3.45	-3.29	
34		88 (*)	31	5.02	**34r**	-7.20	-5.24	-4.10	1
					34s	-6.47	-4.25	-3.36	
39		93	27	5.08	**39**	-7.51	-5.34	-4.52	1
13		68	26	3.66	**13**	-7.51	-5.21	-4.46	1-2
5		47	29	5.81	**5**	-7.75	-5.31	-4.32	2

Id	Compound	PI50	HA	LogP	Ligand	LE1	LE3	LE2	Group
28		48 (*)	33	7.04	**28r**	-6.85	-4.93	-4.22	2
					28s	-7.04	-5.14	-3.73	
29		54 (*)	35	7.93	**29r**	-7.12	-5.16	-4.06	2
					29s	-6.84	-4.98	-3.69	
30		60 (*)	33	7.04	**30r**	-6.87	-4.94	-3.95	2
35		63 (*)	35	5.87	**35r**	-6.37	-4.57	-3.54	2
					35s	-6.28	-3.74	-3.26	
40		64 (*)	34	7.14	**40r**	-7.01	-5.03	-3.48	2
					40s	-6.80	-4.96	-3.66	
14		34	30	5.44	**14**	-8.08	-5.63	-4.67	2-3
6		31	27	5.19	**6**	-6.83	-4.84	-4.30	3

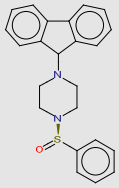
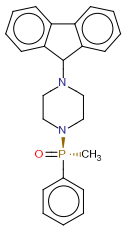
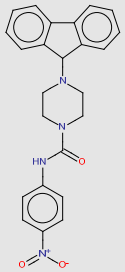
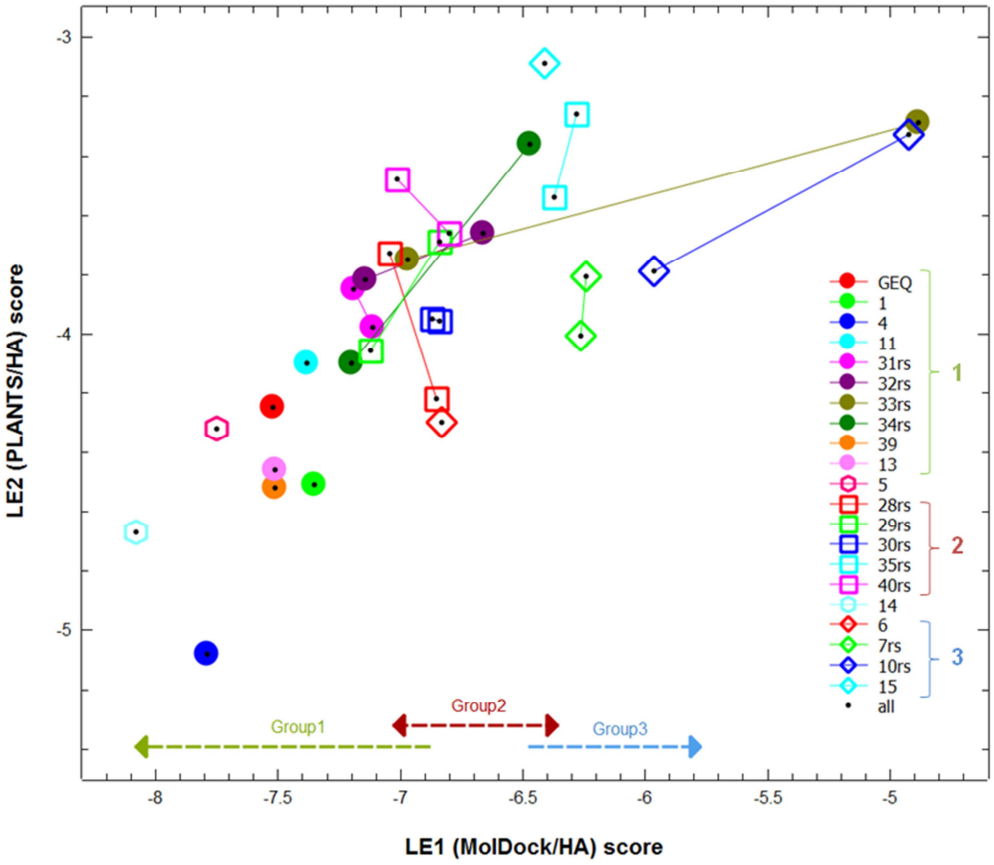
Id	Compound	PI50	HA	LogP	Ligand	LE1	LE3	LE2	Group
7		14 (*)	26	3.84	**7r**	-6.24	-4.35	-3.81	3
					7s	-6.26	-4.37	-4.01	
10		17 (*)	27	4.13	**10r**	-4.92	-3.34	-3.33	3
					10s	-5.96	-4.20	-3.79	
15		33	31	4.29	**15**	-6.41	-4.58	-3.09	3

Figure S1: Plot of LE2 vs. LE1 descriptors.

Data from **Table S1** values, when two enantiomers are found (i.e. ***10rs***) for a given compound (i.e. ***10***) the plotted score values of each enantiomer (i.e. ***10r*** and ***10s***) are connected by a continuous line.

The compounds related to group1 (PI50> 75%) are plotted using filled circles, the compounds of group2 (40-70%) are plotted using squares, and the compounds of group3 (PI50 < 30%) are plotted using diamonds symbols. Compounds ***14*** and ***5*** are plotted using hexagons. Compounds **5** and **14** seems to be difficult to classify, with good docking scores and bad activity values (*group1-group2*, *group2-group3* respectively) interestingly these compound share a structural amide function associated to an alkyl chain.

Data was plotted using SciDavis [10] software.



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