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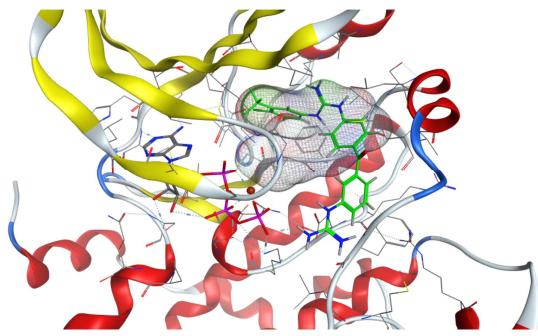
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**GRAPHICAL ABSTRACT**

# Guanidinium-based derivatives: searching for new kinase inhibitors

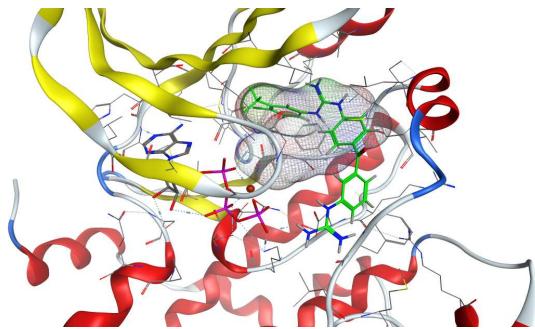
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**GRAPHICAL ABSTRACT****HIGHLIGHTS**

- New families of diaromatic 3,4'-(substituted)guanidinium derivatives were prepared
- Good cytotoxicity and apoptosis effects in HL-60 cancer cell line were observed
- Some compounds show high percentage inhibition on the RAF-1/MEK-1 pathway
- Docking studies on RAF-1 and MEK-1 structures suggest MEK-1 allosteric inhibition

**KEYWORDS**

Guanidine, sorafenib, protein kinases, RAF-1, MEK-1, docking

**ABBREVIATIONS LIST**

EGFR: Epidermal growth factor receptor; ERK: extracellular signal-regulated kinase; HB: hydrogen bond; MAPK: Mitogen-activated protein kinase; MEK: Mitogen-activated protein/extracellular signal-regulated kinase kinase; PDGFR: Platelet-derived growth factor receptor; PI3K: Phosphatidylinositol-3 kinase; PK: Protein kinase; FRET: Fluorescence Resonance Energy Transfer; B-RAF: rapidly accelerated fibrosarcoma isoform B; C-RAF or RAF-1: rapidly accelerated fibrosarcoma-1; VEGFR: Vascular endothelial growth factor; SEM: Standard error of the mean.

**ABSTRACT**

Considering the structural similarities between the kinase inhibitor sorafenib and 4,4'-*bis*-guanidinium derivatives previously prepared by Rozas and co., which display interesting cytotoxicity in cancer cells, we have studied whether this activity could result from kinase inhibition. Five new families have been prepared consisting of unsubstituted and aryl-substituted 3,4'-*bis*-guanidiniums, 3,4'-*bis*-2-aminoimidazolinium and 3-acetamide-4'-(4-chloro-3-trifluoromethylphenyl)guanidinium derivatives. Cytotoxicity (measuring the IC<sub>50</sub> values) and apoptosis studies in human HL-60 promyelocytic leukemia cells were carried out for these compounds. Additionally, their potential inhibitory effect was explored on a panel of kinases known to be involved in apoptotic pathways. The previously prepared cytotoxic 4,4'-*bis*-guanidiniums did not inhibit any of these kinases; however, some of the novel 3,4'-substituted derivatives showed a high percentage inhibition of RAF-1/MEK-1, for which the potential mode of binding was evaluated by docking studies. The interesting antitumor properties showed by these compounds open up new exciting lines of investigation for kinase inhibitors as anticancer agents and also highlights the relevance of the guanidinium moiety for protein kinase inhibitors chemical design.

## 1. INTRODUCTION

During the last 12 years, we have worked on the preparation, biophysical and biochemical evaluation of a number of symmetric and asymmetric 4,4'-*bis*-guanidine-like diaromatic derivatives as DNA minor groove binders (see, for example, compound **1** in Figure 1) [1,2,3,4]. These compounds consist in two phenyl rings connected by a linker (for example O, S, CH<sub>2</sub>, NH, CO, CONH) and guanidine-like groups in the 4,4' positions. Curiously, some of these compounds, which showed poor DNA binding, displayed interesting cytotoxic and pro-apoptotic activity in cancer cells [5], which drove us to consider a different mechanism of action.

Targeting systems only deregulated in cancer cells can help to achieve selective therapies and good examples of this include members of the protein kinase family [6]. The activity of these enzymes is tightly regulated in normal cells; however, they are often hyper-activated or mutated in cancer cells, leading to malfunctioning of signaling networks [7]. Several compounds have been developed to inhibit tyrosine (TK) kinases (i.e. imatinib -Gleevec®- or gefitinib -Iressa®- [2]) or serine/threonine (Ser/Thr) kinases (i.e. vemurafenib -Zelboraf®- [8]). Among the most important Ser/Thr kinases are the mitogen-activated protein (MAP) kinases such as rapidly accelerated fibrosarcoma (RAF), mitogen-activated protein kinase (MEK), extracellular signal-regulated kinase (ERK), or p38 mitogen-activated protein kinase (p38-MAPK) [9], which are inhibited by sorafenib (Nexavar®, Figure 1) a known anticancer agent used for the treatment of advanced renal and hepatocellular carcinomas [10].

When sorafenib inhibits the RAF/MEK/ERK pathway in tumor cells, proliferation and survival are blocked, inducing apoptosis [11]. Furthermore, in the tumor vasculature, where sorafenib can additionally inhibit receptor tyrosine kinases (RTK) such as c-Kit, vascular

endothelial growth factor receptor (VEGFR) or platelet-derived growth factor receptor (PDGFR- $\beta$ ), angiogenesis is inhibited [6]. Additionally, it has been found that sorafenib can modulate p38-MAPK signaling in macrophages and that it partially inhibits glycogen synthase kinase-3 (GSK3- $\beta$ ) phosphorylation [12].

We noticed a number of structural similarities between our 4,4'-*bis*-guanidinium-like diaromatic derivatives and sorafenib, i.e. an aryl ring carrying an amidine-like group (Figure 1, red balloon), a phenyl ring bound to a urea/guanidine functionality (Figure 1, green balloon) and a heteroatomic linker between both aryl systems (Figure 1, blue balloon); hence, taking this into account, kinase inhibition could be the mechanism of action responsible for the observed cytotoxicity of those guanidinium-like compounds. Thus, we have prepared five different families of compounds (Figure 1) in a synthetic-evolution process. By changing one feature at a time, our compounds will become more sorafenib-like with a view to exploring the utility of our established guanidinium moieties and linker variations over the established scaffold. The synthetic and biochemical results obtained are presented herein.

A difference between sorafenib and our proposed compounds is the presence of a pyridine ring which forms a hydrogen bond (HB) and a  $\pi$ - $\pi$  interaction with amino acids of the hinge region of the kinases (RAF-1 in particular) [13]. In our proposed compounds a phenyl ring occupies that position instead, which could form the same  $\pi$ - $\pi$  interaction as the pyridine ring. Regarding the HB formed by the pyridine N atom, we propose that the guanidinium moiety attached to that phenyl ring could form similar interactions. Guanidinium-like moieties, are known to form electrostatic interactions with negatively charged residues and also  $\pi$ -cation interactions with aromatic amino acids, as we have shown in a number of theoretical studies [14,15,16,17]. Additionally, the guanidine functionality has been considered a ‘urea-

equivalent' in the development of different drugs [18]. More importantly, we cannot discard the possibility that our compounds could interact with the kinases in a different way (i.e. allosterically).

About here Figure 1

In the first two families prepared, the 3,4'-substitution pattern (as present in sorafenib) was introduced to produce 3,4'-*bis*-guanidinium (**2a-d**) and 3,4'-*bis*-(2-aminoimidazolinium) (**3a-c**) derivatives. The next change proposed was the addition of an aromatic moiety to the guanidine in the 4'-position (phenyl in compounds **4a-d** and 4-chloro-3-trifluoromethylphenyl in compounds **5a-d**, Figure 1) as present in sorafenib. Also, these two aryl groups were introduced on the guanidine in the 3-position (compounds **6a,b** and **7a,b**, respectively; Figure 1), opposite to the pattern observed in sorafenib. Finally, in compounds **8a,b** (Figure 1), an acetamide group was introduced in the 3-position instead of the *N*-methylcarbamoyl found in sorafenib. All of these modifications would generate enough structure activity relationship (SAR) information to understand the cytotoxic activity of the guanidine-like derivatives.

## 2. RESULTS AND DISCUSSION

### 2.1. CHEMISTRY

The preparation of all the compounds was performed following established guanidylation and 2-aminoimidolidylation procedures [19,20], starting from the corresponding aromatic amines. Hence, the preparation of all of the starting 3,4'-diamines, with the exception of the commercially available 3-(4-aminophenoxy)aniline (**9a**), was required, and even though these

diamines had been previously described [21,22,23], we attempted their preparation by different and more efficient methods which are fully described in the Supporting information.

Compounds **2a-d** and **3a-c** were prepared by the guanidylation or 2-aminoimidazolidylation of the diamines **9a-d**, which was carried out by coupling these with *N,N'*-bis-(*tert*-butoxycarbonyl)-S-methylisothiourea or *N,N'*-bis-(*tert*-butoxycarbonyl) imidazolin-2-thione, respectively, in the presence of HgCl<sub>2</sub> and triethylamine, affording the Boc-protected guanidine (**10a-d**) and 2-aminoimidazoline (**11a-c**) derivatives in moderate to good yield (30-80%). The removal of the Boc protecting groups with TFA, followed by anion exchange, afforded the hydrochloride salts **2a-d** and **3a-c** (Scheme 1).

#### About here Scheme 1

For the synthesis of the *mono*-aryl substituted *bis*-guanidine derivatives (compounds **4a-d**, **5a-d**, **6a,c** and **7a,c**), the corresponding aryl substituted thioureas **12** and **13** (see structures in Scheme 2) had to be prepared from thiourea as described in the Supporting information. Then, the preparation of the final derivatives followed different protocols depending on the conjugative and inductive nature of the linker of the starting amines as explained in Scheme 2. In the case of electron donating linkers such as O or NH the first guanidylation was carried out with the aryl substituted thioureas. Subsequently, the Boc protected thiourea was used in the second guanidylation. The reverse trend was followed in the case of the electron withdrawing (CO) and the inductive electron donating (CH<sub>2</sub>) linkers.

#### About here Scheme 2

In all cases, the guanidylating reaction is promoted by  $\text{HgCl}_2$  and  $\text{NEt}_3$ , as previously described [19], affording in the first step the *mono*-guanidylated phenyl derivatives (**14a,d**), 4-chloro-3-trifluoromethylphenyl derivatives (**15a,d**) and Boc-protected *mono*-guanidine derivatives (**16b,c** and **17a,c**) as shown in Scheme 2, which after the second guanidylation afforded the Boc-protected intermediates (**18a-d**, **19a-d**, **20a,c** and **21a,c**). The yields of these two steps were moderate to excellent depending on the influence of the X linker on the nucleophilicity of the amines. Finally, the Boc groups were removed using HCl in dioxane (4M, 55 °C, 6-8 h) to afford the final hydrochloride salts (**4a-d**, **5a-d**, **6a,c** and **7a,c**, Scheme 2) in excellent yields.

Derivatives **8a,d** were synthesized from compounds **15a,d** by acetylation of the remaining primary amine using acetic anhydride, affording Boc-protected derivatives **22a,d** in good yields. Boc deprotection was carried out using HCl in dioxane 4M diluted in a 1:1 IPA: $\text{CH}_2\text{Cl}_2$  solution until reaching a final concentration of 0.2 M, affording the final products **8a,d**, (Scheme 3).

About here Scheme 3

## 2.2. BIOLOGICAL RESULTS

### 2.2.1. Cell viability and apoptosis studies

First, the cytotoxicity of all the compounds prepared was assessed in the human caucasian promyelocytic leukemia (HL-60) cell line using an AlamarBlue assay to determine their effects on cell viability and proliferation [24], and the results obtained ( $\text{IC}_{50}$  values) are presented in Table 1. Derivatives **2a-d** have moderate activity in this cancer cell line; both the

O and CH<sub>2</sub> linker derivatives (**2a** and **2c**) show the highest cytotoxicity, followed by **2d** (X=NH) with nearly half of their activity and, finally, **2b** (X= CO), indicating that either the electron withdrawing effect or the geometry of the CO linker are not favourable for cytotoxicity in these compounds. All of the 2-aminoimidazoline derivatives (compounds **3a-c**) exhibited IC<sub>50</sub> values >100 μM indicating poor cytotoxicity (Table 1). Additionally, the introduction of a phenyl group into the guanidinium functionality in 3- or in 4'- positions (compounds **4a-d** and **6a,c**, respectively) reduced even further the activity of the unsubstituted guanidines (Table 1).

However, when a 4-chloro-3-trifluoromethylphenyl group was introduced either in the 4'- or in the 3-guanidine (compounds **5a-d** and **7a,c**, respectively), the cytotoxic activity sharply increased showing even better IC<sub>50</sub> values than those for compounds **2a-d** (Table 1), probably due to the possible further interactions caused by the lipophilic nature of the CF<sub>3</sub> and Cl groups in the aryl moiety. The different X linkers do not seem to highly influence the cytotoxic activity of these compounds. When the 4-Cl-3-CF<sub>3</sub>-phenyl moiety is incorporated in the 3-guanidine instead of the 4'-guanidine, the activity of the O-linked derivative (**7a**) is enhanced with a decrease in the IC<sub>50</sub> values from 24.5 (**5a**) to 9.72 (**7a**) μM. Conversely, in the case of the CH<sub>2</sub>-linked derivative (**7c**), no significant difference in the cytotoxicity between the two analogues **5c** and **7c** was observed (Table 1).

Compounds **8a,d** showed an increase in activity (Table 1) with even more satisfactory IC<sub>50</sub> values. The replacement of the 3-guanidinium group by an acetamide one (-NHCOCH<sub>3</sub>) seems to be relevant for the HL-60 cytotoxicity of these compounds even though a different mechanism of action cannot be ruled out.

## About here Table 1

In order to confirm and assess the extent of apoptosis being induced by our guanidine derivatives, cell morphology and flow cytometry studies were carried out in HL-60 cells using one of our most potent and structurally relevant compounds, **7a**. In Figure 2, an obvious change in the morphology of the HL-60 cell line is shown indicating the cytotoxic effect of this compound. The control cells are full and round as they proliferate (Figure 2, left); however, the treated cells display cell shrinkage, nuclear fragmentation and cell membrane blebbing, typical indicators of apoptosis (Figure 2, right).

## About here Figure 2

Flow cytometry allows the evaluation of cell populations in different stages of the cell cycle and furthermore the amount of cells undergoing apoptosis (pre-G1 peak). Thus, compound **7a** was chosen to determine the DNA content analyzed by flow cytometry on the HL-60 cell line and the results are presented in Table 2. Different time points (48 and 72 h) and a range of concentrations of **7a** (1, 10, 25, 50  $\mu$ M) were examined to obtain a clear profile of the effect of the compound on the different cell cycle phases. The results are presented in Table 2 as mean  $\pm$ S.E.M. values; increasing concentrations of the compound induced G<sub>0</sub>/G<sub>1</sub> cell arrest (from 1 to 50  $\mu$ M) with a concomitant decrease in the rest of the cell cycle phases prior to the appearance of apoptosis at 48 h treatment which increases up to 25.1% by 50  $\mu$ M at 72 h.

## About here Table 2

### **2.2.2. Inhibition of kinase activity**

Considering the promising cytotoxic and apoptotic results obtained in HL-60 cells with both the 4,4'-*bis*-guanidinium compounds previously prepared by us and the five 3,4'-disubstituted new families, we next carried out a number of preliminary cell-free assays to assess if these effects are the consequence of kinase inhibition.

It is well known that Casein Kinases 1 (CK-1) play an essential role in diverse physiological processes, such as DNA repair, cell cycle proliferation, cytokinesis, differentiation and apoptosis [25,26]. In particular, CK-1 $\delta$  are key regulators of proteins such as p53 and  $\beta$ -catenin, which act as signal integration molecules in apoptosis [25]. Additionally, the GSK-3 family also plays an important role in signal transduction systems (*i.e.* Wnt/wingless and PI-3 kinases) which influence proliferation and cell survival [27]. Therefore, we first chose to investigate these two Ser/Thr kinases since the study of their inhibition could reveal whether or not the apoptosis previously observed (by both 4,4'-*bis*-guanidinium derivatives and the new 3,4'-compounds) could involve their blockade.

Thus, Kinase-Glo® luminescent kinase assays were used to determine the CK-1 $\delta$  and GSK-3 activity. This assay is used to determinate the activity of kinases by quantifying the amount of ATP remaining in solution following a kinase reaction. The assay involves the addition of a single reagent directly to a completed kinase reaction. This addition results in the generation of a luminescent signal correlated with the amount of ATP present and inversely proportional to the amount of kinase activity. The results obtained with these two kinases showed IC<sub>50</sub> values >100  $\mu$ M for all the new compounds as well as the previously prepared 4,4'-*bis*-guanidine derivatives with NH, O (compound **1**), CH<sub>2</sub> and CO linkers [8,20]. Given that our most potent new compounds (**5a-d**, **7a,c** and **8a,d**) caused effective growth inhibition at

lower concentrations in cell models in vitro, their specific targeting of CK-1 and GSK3 can be discarded.

Additionally, we have explored if our molecules show inhibitory activity towards different kinase pathways known to be targeted by sorafenib. To investigate the potential inhibition of RAF kinases, we evaluated if our new compounds selectively bind to B-RAF by performing a LanthaScreen® Eu Kinase Binding Assay [28]. This assay is based on the binding/displacement of a fluorophore (Alexa Fluor® 647-labeled) in an ATP-competitive kinase inhibitor scaffold (tracer) to/from the kinase of interest (B-RAF). Binding of the tracer to B-RAF was detected using a Eu-labelled anti-tag antibody; thus, the simultaneous binding of both the tracer and the antibody results in a high degree of fluorescence resonance energy transfer (FRET) from the Eu donor to the fluorophore on the tracer. However, when a kinase inhibitor competes for binding to the ATP-site of the kinase-tracer complex a displacement of the tracer results, with a loss of FRET (no emission detected). Firstly, the evaluation of sorafenib binding to B-RAF was performed in order to verify the suitability of the assay. Thus, three independent experiments were carried out showing, as expected, that sorafenib competitively binds to B-RAF with an  $IC_{50}$  value of  $136 \pm 23$  nM, very similar to that reported in the literature (120 nM; [29]). Subsequently, the binding assay was performed on those compounds with the best  $IC_{50}$  values in the HL-60 assays. Therefore, compounds **5a-d**, **7a,c** and **8a,d** were tested but none of them displayed dose-response competitive binding to B-RAF at concentrations ranging from 100 nM to 100  $\mu$ M, as no decrease in FRET emission was observed.

In addition, we commissioned a commercial battery screening [30] involving the measurement of the percentage inhibition of the enzymatic activity of the ERK 1/2, p38

MAPK, VEGFR and (C-RAF or RAF-1)/MEK-1 pathway. The description of the different assays is presented in the Experimental section. A compound representative of the different substituents and substitution patterns was evaluated including one of the 4,4'-*bis*-guanidines previously prepared by Rozas and co. [8], which had shown cytotoxic activity but an uncertain mode of action (compound **1**) and the results obtained are shown in Table 3.

The results achieved for the previously prepared compound **1** indicate no kinases inhibition (Table 3) suggesting that this 4,4'-*bis*-guanidine does not mediate cytotoxicity by targeting this particular set of protein kinases. Similar lack of kinase inhibition was observed for the 3,4'-*bis*-2-aminoimidazoline **4a**, in agreement with the poor cytotoxicity observed in the HL-60 cell line.

Acetamido derivatives **8a** and **8d** show poor inhibition of the RAF-1/MEK-1 pathway and, additionally, **8d** poorly inhibits p38 and ERK1/2 (Table 3). While the good HL-60 cytotoxicity observed for these compounds may be a cumulative effect of modest impact on the individual kinases, other mechanisms should be explored for these acetamido derivatives.

#### About here Table 3

Better results were obtained for compounds **2a** (X= O) and **2d** (X= NH), which selectively inhibit the RAF-1/MEK-1 pathway (Table 3) showing no inhibition of the other kinases tested. In particular, compound **2d** shows 86% inhibition of this pathway in comparison to a moderate 22% inhibition in the case of **2a**, hinting that the NH linker could induce more selectivity for this pathway than the O linker. Considering the IC<sub>50</sub> values obtained with HL-60 cells for **2a** (36.2 µM; Table 1) and **2d** (67.7 µM; Table 1), it can be proposed that

effective targeting of the RAF-1/MEK-1 pathway and its subsequent downstream signaling may be a less important target and that additional kinases must be involved in the growth inhibitory effects.

Best results were obtained with derivatives **5a-d**, which showed good HL-60 cytotoxicity and also inhibited more than one of the kinases examined, with the most relevant inhibition being that of the RAF-1/MEK-1 pathway (Table 3). This seems to indicate the need for the 4-chloro-3-trifluoromethylphenyl moiety for the inhibition of these particular kinases. The percentage inhibition values obtained for the RAF-1/MEK-1 pathway range from 74% for **5d** to 96%, 96% and 99% for **5a**, **5b** and **5c**, respectively. This supports the idea that the nature of the linker X in the diaromatic moiety is not critical for the inhibition of the RAF-1/MEK-1 pathway. Compound **5c** (the most potent growth inhibitor in HL-60 of its family) shows the best profile of inhibition towards VEGFR, ERK1/2 (both moderately) and RAF-1/MEK-1. Compounds **5a**, **5b** and **5d** (X= O, CO and NH) also displayed a modest inhibition of p38 MAPK and **5b** additionally for ERK1/2. Therefore, these guanidine derivatives are the most promising kinase inhibitors of all the compounds tested.

### 2.3. MOLECULAR DOCKING

With the aim of designing next generation compounds and rationalizing the promising anti-proliferative activity obtained for the compounds tested in the HL-60 cell line, as well as the FRET and kinase panel inhibition results, a molecular docking study was undertaken using the MOE 2011.10 modelling software (Molecular Operating Environment, [31]). The only known crystal structure of Sorafenib complexed with a RAF kinase is that with the B-RAF kinase [32]; thus, we carried out our first docking experiments using this crystallographic

structure (1UWH, wild-type B-RAF) as template; however, in agreement with the FRET study that showed that our new derivatives do not competitively bind to the ATP binding site of B-RAF, no coherent or positive results were achieved.

Since our commercial kinase panel results indicate good percentage inhibition of the RAF-1/MEK-1 pathway, crystal structures of RAF-1 (also known as C-RAF) and MEK-1 were used as models to investigate the interactions between our compounds and these kinases. Only crystal structures of the RAF-1 kinase in complex with ATP-competitive ligands are known, including 3OMV [33] where RAF-1 is complexed with SM5 ((1*E*)-5-(1-piperidin-4-yl-3-pyridin-4-yl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-inden-1-one oxime, Figure 3). This structure also reveals that there is little space for allosteric binding near the ATP site; hence, our ligands were docked into the ATP site of RAF-1. Additionally, as the crystallized MEK-1 kinase in the 3PP1 crystal structure [34] is complexed with an allosteric ligand (IZG: 3-[(2*R*)-2,3-dihydroxypropyl]-6-fluoro-5-[(2-fluoro-4-iodophenyl)amino]-8-methylpyrido[2,3-d]pyrimidine-4,7(3*H*,8*H*)-dione, Figure 3), ATP and a Mg<sup>2+</sup> ion, the ligands were docked into this kinase both in the presence and absence of ATP and Mg<sup>2+</sup>. In their absence, both the ATP and allosteric binding sites were made available to ligands. The ligand IZG establishes a HB between its hydroxyl proton (see structure in Figure 3, right) and the phosphate group attached to the ribose 5'-oxygen of ATP. Given the nature of our ligands, an ionic interaction between the guanidinium cations and a phosphate group of ATP is highly likely, so it is expected that our compounds also bind to the kinase in such an allosteric manner.

About here Figure 3

Docked ligands were ranked based on the London dG scores of generated poses after a molecular mechanics refinement in which the kinase amino acids were free to move [31]. The generalized Born implicit solvent model was included during the calculation of interaction energies to give the E-refine values reported in Table 4.

The optimized complexes and the docking scores obtained indicate that our derivatives (**2a-d**, **3a-c**, **4a-d**, **5a-d**, **6a,c**, **7a,c** and **8a,d**) are not suited to the ATP binding site available in the RAF-1 kinase. This is understandable, given the evident structural and electronic differences between our ligands and both ATP and SM5 (the ligand in 3OMV, Figure 3). The binding energies for all ligands fall within a similar energy range and there is little consistency in their orientation within the binding site. Given the high percentage inhibition of the RAF-1/MEK-1 pathway displayed for our ligands (Table 3), these docking results indicate that it is unlikely their target is RAF-1.

On the other hand, the docking scores obtained for our compounds bound to MEK-1 correlate well with the kinase assays, both in the presence and absence of ATP/Mg<sup>2+</sup>. Compounds follow similar trends and the differences in interaction energies for both systems are small (Table 4). Some of our ligands are well suited to interact with the allosteric binding site, particularly those with the hydrophobic 4-chloro-3-trifluoromethylphenyl ring which occupies the same hydrophobic region of the site as the iodofluoroaniline ring of IZG (the 3PP1 ligand, Figure 3) in the top-ranked poses. When ATP is present, all complexes show a guanidinium-phosphate electrostatic interaction after docking. The most favorable poses also orient the 4-chloro-3-trifluoromethylphenyl ring in the hydrophobic pocket of the kinase.

About here Table 4

Considering our docking results indicate that the preferred mode of binding is that in the allosteric site in the presence of ATP and Mg<sup>2+</sup>, the ligands can be divided into four general classes. Firstly, compounds **2a-d** and **3a-c** show poor kinase binding probably due to the placement of a cationic group in the hydrophobic pocket. Even though the 2-aminoimidazolinium groups of compounds **3a-c** are slightly more lipophilic than the guanidinium ones of compounds **2a-d** their MEK-1 binding is still poor. Secondly, phenylguanidiniums **4a-d** and **6a,c** have higher affinity for the MEK-1 allosteric site due to placement of the phenyl ring in the hydrophobic pocket, and maintaining a guanidinium-phosphate electrostatic interaction. Thirdly, the top-ranked poses of the highest affinity group of ligands (compounds **5a-d** and **7a,c**) place the hydrophobic 4-chloro-3-trifluoromethylphenyl ring in the hydrophobic pocket and the unsubstituted guanidinium in contact with a phosphate group of ATP. Finally, compounds **8a,d** have poor affinity for the MEK-1 allosteric binding site. Two repeating poses were observed; one which places the 4-chloro-3-trifluoromethylphenyl ring in the hydrophobic pocket and includes a weaker HB between the amide NH and a phosphate of ATP, and another which places this amide in the hydrophobic pocket to allow for a guanidinium-phosphate electrostatic interaction. Neither of these poses is expected to be highly favoured.

Despite the small variance amongst the docking scores obtained for RAF-1 and MEK-1 kinases, the most noteworthy difference is that found for compounds **5a-d** and **7a-c**, which show significantly higher affinity for MEK-1 kinase than for RAF-1. These compounds were the highest ranked for MEK-1 binding, and display docking scores which are as much as 5.9 kcal mol<sup>-1</sup> better than those for RAF-1. The top-ranked poses for these compounds are

consistent with our proposed mode of binding to the MEK-1 kinase (Figure 4), whilst their orientation in the RAF-1 kinase was more variable.

About here Figure 4

Furthermore, these observations correlate well with our biological testing, which indicated that compounds **5a-d** and **7a-c** were amongst the best performers in both cell and kinase assays. This leads us to propose that their more likely molecular target is the MEK-1 kinase.

### 3. CONCLUSIONS

To understand the cytotoxicity obtained in previously prepared 4,4'-*bis*-guanidinium derivatives (i.e. **1**), different families of compounds were prepared: **2a-d** and **3a-c** (3,4'-*bis*-guanidinium and 3,4'-*bis*-(2-aminoimidazolinium) derivatives, respectively); **4a-d** and **6a,c** (with a phenyl group in the 4'- and 3-guanidinium, respectively); **5a-d** and **7a-c** (with a 4-chloro-3-trifluoromethylphenyl group in the 4'- and 3-guanidinium, respectively); and **8a,d** (with an acetamide group at the 3-position). Their preparation involved guanidylation of aromatic amines by reaction with the appropriate Boc-protected thioureas in the presence of mercury(II) chloride and triethylamine followed by Boc deprotection to yield the corresponding hydrochloride salts [8-12,18,20].

The effect on cell viability of all the compounds prepared was measured (as IC<sub>50</sub> values) using the AlamarBlue assay in the HL-60 cell line. Additionally, flow cytometry studies in HL-60 were carried out with one of the most potent compounds (**7a**), showing that these derivatives induce apoptosis.

The inhibitory effect of these compounds on a number of kinases was explored. No inhibitory effect was observed on the enzymatic activity of the CK-1 $\delta$  and GSK-3 kinases as well as in the FRET study of the B-RAF kinase. This B-RAF comparison highlights a differentiation point for our guanidine-like derivatives from sorafenib, and implies greater specificity for these new scaffolds as kinase inhibitors.

Percentage inhibitory activity tested in a commercial kinase panel (including ERK 1/2, p38 MAPK, VEGFR, and the [C-RAF or RAF-1]/MEK-1 pathway) showed that previously prepared compound **1** and the acetamido derivatives **8a** and **8d** are poor or null inhibitors of all these kinases, hence, their favourable HL-60 cytotoxicity should result from other mechanisms of action. Compounds **2a** and **2d** selectively inhibited the RAF-1/MEK-1 pathway showing no inhibition of the other kinases tested.

Compounds from the most cytotoxic series (**5a-d**) show the largest inhibitory effect, especially in the RAF-1/MEK-1 pathway, indicating the need for the 4-chloro-3-trifluoromethylphenyl moiety for the inhibition of these kinases. Considering the kinase inhibition and cytotoxicity exhibit by **5a-d** and **7a-c** and the results of the docking study, it seems that these compounds are potential inhibitors of the RAF-1/MEK-1 pathway. Moreover, based on the favourable allosteric binding observed in the docking studies with both kinases, we suggest that MEK-1 is the likely molecular target of these compounds.,

In summary, we have demonstrated that the cytotoxicity observed in the previously prepared 4,4'-*bis*-guanidinium derivatives (i.e. **1**) is not due to kinase inhibition and we have identified a new series of 4-chloro-3-trifluoromethylphenyl substituted 3,4'-*bis*-guanidinium derivatives

(**5a-d** and **7a,c**) with potential as allosteric MEK-1 inhibitors. Further research should be carried out to clearly identify their targets and understand their pro-apoptotic activity, which is commonly absent in current therapies. Moreover, this work illustrates the importance of the guanidinium moiety for targeting protein kinases in an allosteric manner by electrostatic bonding to ATP.

## 4. EXPERIMENTAL

### 4.1. CHEMISTRY

All commercially available chemicals were obtained from Sigma-Aldrich or Fluka and used without further purification. Deuterated solvents for NMR use were purchased from Apollo. Dry solvents were prepared using standard procedures, according to Vogel, with distillation prior to use. Chromatographic columns were run using a Biotage SP4 flash purification system with Biotage SNAP silica cartridges. Solvents for synthesis purposes were used at GPR grade. Analytical TLC was performed using Merck Kieselgel 60 F254 silica gel plates or Polygram Alox N/UV254 aluminium oxide plates. Visualisation was by UV light (254 nm). NMR spectra were recorded on Bruker DPX-400 Avance spectrometers, operating at 400.13 MHz and 600.1 MHz for <sup>1</sup>H NMR; 100.6 MHz and 150.9 MHz for <sup>13</sup>C NMR. Shifts are referenced to the internal solvent signals. NMR data were processed using Bruker TOPSPIN software. HRMS spectra were measured on a Micromass LCT electrospray TOF instrument with a WATERS 2690 autosampler and methanol/acetonitrile as carrier solvent. Melting points were determined using a Stuart Scientific Melting Point SMP1 apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR Spectrometer equipped with a Universal ATR sampling accessory.

HPLC purity analysis was carried out using a Varian ProStar system equipped with a Varian Prostar 335 diode array detector and a manual injector (20 µL). For purity assessment, UV detection was performed at 245 nm and peak purity was confirmed using a purity channel. The stationary phase consisted of an ACE 5 C18-AR column (150 × 4.6 mm), and the mobile phase used the following gradient system, eluting at 1 mL min<sup>-1</sup>: aqueous formate buffer (30 mM, pH 3.0) for 10 min, linear ramp to 85% methanol buffered with the same system over 25 min, hold at 85% buffered methanol for 10 min. Minimum requirement for purity was set at 95.0%.

#### **4.1.1. Method A: General method for the deprotection of *N*-Boc-protected guanidines using trifluoroacetic acid**

A solution of the corresponding Boc-protected precursors (0.5 mmol) in 20 mL of a 50% v/v solution of TFA and CH<sub>2</sub>Cl<sub>2</sub> was stirred at room temperature for 3 hours. After this, the solvent was eliminated under vacuum to generate the trifluoroacetate salt. The residue was dissolved in water (20 mL) and 1000 mg of IRA400 Amberlite resin in its Cl<sup>-</sup> form was added, allowing the reaction to proceed overnight. The resin was then removed by filtration and the aqueous solution washed with CH<sub>2</sub>Cl<sub>2</sub> (2 × 10 mL). Purification was carried out by reverse phase chromatography using C-8 silica, typical elution gradient: 100% water to 85:15 water:acetonitrile, respectively. The purified fractions were evaporated until dry to afford the pure hydrochloride salt. Absence of the trifluoroacetate salt was confirmed by <sup>19</sup>F NMR.

#### **4.1.2. Method B: General method for the deprotection of *N*-Boc-protected guanidines using hydrochloric acid solutions.**

To a solution of the corresponding Boc-protected guanidine (1 eq.) in dioxane, 4M HCl in dioxane (6 eq. per Boc group) was added to reach a final concentration of 0.2M. The mixture

was stirred at 55 °C until the reaction was complete (typically 8 h, adjudged by TLC). At the reaction endpoint, solvent and HCl were evaporated under vacuum. The aqueous phase was purified by reverse phase chromatography using C-8 silica, typical elution gradient: 100% water to 85:15 water:acetonitrile, respectively. The purified fractions were evaporated until dry to afford the pure hydrochloride salt.

#### **4.1.3. Method C: General method for the deprotection of *N*-Boc-protected acetylated guanidines using hydrochloric acid solutions.**

A solution of the corresponding Boc-protected acetylated guanidine precursors (1 eq.) in 1:1 CH<sub>2</sub>Cl<sub>2</sub>:IPA was treated with 4M HCl in dioxane (6 eq. per Boc group) to reach a final concentration of 0.2M. The mixture was stirred at 30 °C until the reaction was complete (typically 6 h, determined by TLC). At the reaction endpoint, solvents and HCl were evaporated under vacuum, followed by dilution in water and extraction with CH<sub>2</sub>Cl<sub>2</sub> (2 × 10 mL) and then purified by using reverse phase chromatography using C-8 silica, typical elution gradient: 100% water to 85:15 water:acetonitrile, respectively. The purified fractions were evaporated until dry to afford the pure hydrochloride salt.

#### **4.1.3. 3-[4-(Guanidino)phenoxy]phenylguanidine dihydrochloride (2a)**

Following method A, using **10a** (151 mg, 0.22 mmol) as starting material, the hydrochloride salt **2a** was obtained as a yellow solid (60 mg, 76%). Mp: 159-160 °C. δ<sub>H</sub> (600 MHz, D<sub>2</sub>O): 6.90 (t, 1H, *J* = 2.1 Hz, Ar), 7.00 (dd, 1H, *J* = 8.3, 2.1 Hz, Ar), 7.06 (dd, 1H, *J* = 8.3, 2.1 Hz, Ar), 7.08 (d, 2H, *J* = 8.8 Hz, Ar), 7.27 (d, 2H, *J* = 8.8 Hz, Ar), 7.43 (t, 1H, *J* = 8.3 Hz, Ar). δ<sub>C</sub> (150 MHz, D<sub>2</sub>O): 115.7 (CH, Ar), 117.9 (CH, Ar), 120.2 (2 CH, Ar), 120.7 (CH, Ar), 127.9 (2 CH, Ar), 129.6 (q), 131.3 (CH, Ar), 135.5 (q), 155.5 (q), 155.9 (q), 156.3 (q), 157.4

(q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3311 (NH), 3134 (NH), 1665 (CN), 1580, 1505, 1487, 1214, 1154, 1016, 962, 843. HRMS (*m/z* - ES): 284.1394 (M<sup>+</sup> + H). HPLC: 98.6% (*tR*: 19.8 min).

#### **4.1.4. 3-[4-(Guanidino)benzoyl]phenylguanidine dihydrochloride (2b)**

Following method A, using **10b** (116 mg, 0.55 mmol) as starting material, the dihydrochloride salt **2b** was obtained as a yellow solid (32 mg, 53%). Mp: 177-179 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 7.44 (d, 2H, *J* = 8.6 Hz, Ar), 7.60 (d, 1H, *J* = 7.5 Hz, Ar), 7.63 (t, 1H, *J* = 7.5 Hz, Ar), 7.69 (s, 1H, Ar), 7.73 (d, 1H, *J* = 7.5 Hz, Ar), 7.86 (d, 2H, *J* = 8.6 Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 124.2 (2 CH, Ar), 127.1 (CH, Ar), 129.6 (CH, Ar), 130.3 (CH, Ar), 130.6 (CH, Ar), 132.1 (2 CH, Ar), 134.6 (q), 134.7 (q), 138.3 (q), 139.3 (q), 155.8 (q), 155.9 (q), 198.2 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3302 (NH), 3130 (NH), 2901, 1644 (CN), 1589, 1485, 1382, 1285, 1083, 1019, 933, 844. HRMS (*m/z* - ES): 297.1471 (M<sup>+</sup> + H). HPLC: 97.3% (*tR*: 19.6 min).

#### **4.1.5. 3-[4-(Guanidino)benzyl]phenylguanidine dihydrochloride (2c)**

Following method A, using **10c** (44 mg, 0.065 mmol) as starting material, the hydrochloride salt **2c** was obtained as a brown solid (16 mg, 70%). Mp: 97-99 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 4.01 (s, 2H, Ar), 7.14 (d, 1H, *J* = 7.8 Hz, Ar), 7.17 (s, 1H, Ar), 7.22 (d, 2H, *J* = 8.3 Hz, Ar), 7.27 (d, 1H, *J* = 7.8 Hz, Ar), 7.34 (d, 2H, *J* = 8.3 Hz, Ar), 7.38 (t, 1H, *J* = 7.8 Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 40.1 (CH<sub>2</sub>), 123.6 (CH, Ar), 125.9 (CH, Ar), 126.2 (CH, Ar), 128.2 (CH, Ar), 130.1 (CH, Ar), 130.1 (CH, Ar), 132.1 (q), 134.2 (q), 140.9 (q), 143.2 (q), 156.2 (q), 156.3 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3159 (NH), 2981 (NH), 2904, 2475, 1642 (CN), 1585, 1514, 1414, 1487, 1417, 1382, 1288, 1200, 1084, 1019, 839. HRMS (*m/z* - ES): 283.1673 (M<sup>+</sup> + H). HPLC: 95.1% (*tR*: 20.4 min).

#### **4.1.6. 3-[4-(Guanidino)phenylamino]phenylguanidine dihydrochloride (2d)**

Following method A, using **10d** (50 mg, 0.07 mmol) as starting material, the dihydrochloride salt **2d** was obtained as a yellow-brown solid (16 mg, 62%). Mp: decomp. above 229 °C.  $\delta_H$  (600 MHz, D<sub>2</sub>O): 6.80 (d, 1H, *J* = 8.0 Hz, Ar), 7.01 (d, 1H, *J* = 8.0 Hz, Ar), 7.09 (d, 1H, *J* = 8.0 Hz, Ar), 7.17 (d, 2H, *J* = 8.8 Hz, Ar), 7.21 (d, 2H, *J* = 8.8 Hz, Ar), 7.36 (t, 1H, *J* = 8.0 Hz, Ar).  $\delta_C$  (150 MHz, D<sub>2</sub>O): 114.5 (CH, Ar), 117.1 (CH, Ar), 118.1 (CH, Ar), 118.8 (2 CH, Ar), 126.8 (q), 127.7 (2 CH, Ar), 130.7 (CH, Ar), 135.1 (q), 142.6 (q), 144.4 (q), 156.2 (q), 156.6 (q).  $\nu_{max}$  (ATR)/cm<sup>-1</sup>: 3289 (NH), 3127 (NH), 1659 (CN), 1582, 1508, 1505, 1331, 1238, 1167, 880. HRMS (*m/z* - ES): 284.1621 (M<sup>+</sup> + H). HPLC: 96.05% (*tR*: 21.99 min).

#### **4.1.7. 3-[4-N-(Amino-2-imidazolinyl)phenoxy]phenylamino-2-imidazoline dihydrochloride (3a)**

Following method A, using **11a** (78 mg, 0.191 mmol) as a starting material, the dihydrochloride salt **3a** was obtained as a white solid (60 mg, 76%). Mp: 146-150 °C.  $\delta_H$  (400 MHz, D<sub>2</sub>O): 3.65 (s, 4H, CH<sub>2</sub>), 3.65 (s, 4H, CH<sub>2</sub>), 6.87 (t, 1H, *J* = 2.0 Hz, Ar), 6.92 (dd, 1H, *J* = 8.0, 2.0 Hz, Ar), 6.99 (dd, 1H, *J* = 8.0, 2.0 Hz, Ar), 7.05 (d, 2H, *J* = 8.8 Hz, Ar), 7.22 (d, 2H, *J* = 8.8 Hz, Ar), 7.36 (t, 1H, *J* = 8.0 Hz, Ar).  $\delta_C$  (100 MHz, D<sub>2</sub>O): 42.7 (2 CH<sub>2</sub>), 42.7 (2 CH<sub>2</sub>), 114.5 (CH, Ar), 117.4 (CH, Ar), 119.4 (CH, Ar), 120.2 (CH, Ar), 126.7 (CH, Ar), 130.8 (q), 131.2 (CH, Ar), 136.5 (q), 155.8 (q), 157.5 (q), 158.6 (q), 159.1 (q).  $\nu_{max}$  (film)/cm<sup>-1</sup>: 3155 (NH), 2978, 2903, 1647 (CN), 1588, 1505, 1484, 1384, 1269, 1212, 1150, 1086, 1014, 956, 847. HRMS (*m/z* - ES): 337.1763 (M<sup>+</sup> + H). HPLC: 98.03% (*tR*: 20.79 min).

**4.1.8. 3-[4-N-(Amino-2-imidazolinyl)benzoyl]phenylamino-2-imidazoline dihydrochloride (3b)**

Following method A, using **11b** (144 mg, 0.07 mmol) as starting material, the hydrochloride salt **3b** was obtained as a brown solid (44 mg, 55%). Mp: 155-158 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 3.72 (s, 4H, 2 CH<sub>2</sub>), 3.77 (s, 4H, 2 CH<sub>2</sub>), 7.33 (d, 2H, *J* = 8.6 Hz, Ar), 7.52 (s, 1H, Ar), 7.53 (d, 1H, *J* = 7.6 Hz, Ar), 7.56 (t, 1H, *J* = 7.6 Hz, Ar), 7.60 (d, 1H, *J* = 7.6 Hz, Ar), 7.77 (d, 2H, *J* = 8.6 Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 42.6 (4 CH<sub>2</sub>), 122.1 (2 CH, Ar), 125.0 (CH, Ar), 128.6 (CH, Ar), 128.8 (CH, Ar), 130.1 (CH, Ar), 132.0 (2 CH, Ar), 133.7 (q), 135.3 (q), 137.9 (q), 140.1 (q), 157.7 (q), 158.4 (q), 197.5 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3299 (NH), 3122 (NH), 1627 (CO), 1593, 1559 (CN), 1453, 1292, 1257, 1088, 968, 845, 808, 759. HRMS (*m/z* - ES): 349.1781 (M<sup>+</sup> + H). HPLC: 95.9% (*t*R: 20.6 min).

**4.1.9. 3-[4-N-(Amino-2-imidazolinyl)benzyl]phenylamino-2-imidazoline dihydrochloride (3c)**

Following method A, using **11c** (400 mg, 0.55 mmol) as starting material, the hydrochloride salt **3c** was obtained as a yellow solid (153 mg, 70%). Mp: 94-96 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 3.69 (s, 8H, 4 CH<sub>2</sub>), 3.99 (s, 2H, Ar), 7.11 (d, 1H, *J* = 7.8 Hz, Ar), 7.13 (s, 1H, Ar), 7.19 (d, 2H, *J* = 8.3 Hz, Ar), 7.22 (d, 1H, *J* = 7.8 Hz, Ar), 7.31 (d, 2H, *J* = 8.3 Hz, Ar), 7.36 (t, 1H, *J* = 7.8 Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 40.1 (CH<sub>2</sub> linker), 42.6 (4 CH<sub>2</sub>), 122.1 (CH, Ar), 124.5 (CH, Ar), 124.7 (CH, Ar), 127.7 (CH, Ar), 129.9 (CH, Ar), 130.0 (2 CH Ar), 133.1 (q), 135.2 (q), 140.4 (q), 143.1 (q), 158.7 (q), 158.8 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3138 (NH), 2898, 1644 (CN), 1585, 1513, 1435, 1381, 1281, 1282, 1201, 1083, 1018, 983. HRMS (*m/z* - ES): 335.1984 (M<sup>+</sup> + H). HPLC: 98.8% (*t*R: 21.1 min).

**4.1.10. 3-[4'-(Phenylguanidino)phenoxy]phenylguanidine dihydrochloride (4a)**

Following Method B, **18a** (66 mg, 0.10 mmol) was dissolved in 4M HCl in dioxane (0.452 mL, 1.81 mmol) and in additional dioxane (0.05 mL) until a final concentration of 0.2M was reached. After 8 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as an white solid (38 mg, 88%). Mp: decomp. above 140 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 6.96 (app. t, 1H, *J* = 2.1 Hz, Ar), 7.03 (dd, 1H, *J* 8.0, 2.1 Hz, Ar), 7.08 (dd, 1H, *J* 8.0, 2.1 Hz, Ar), 7.13 (d, 2H, *J* = 8.9 Hz, Ar), 7.31-7.36 (m, 4H, Ar), 7.38 (app. t, *J* = 7.5 Hz, Ar), 7.43-7.47 (m, 3H, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 116.0 (CH, Ar), 118.0 (CH, Ar), 120.3 (2 CH, Ar), 121.0 (CH, Ar), 125.9 (2 CH, Ar), 127.9 (CH, Ar), 128.0 (2 CH, Ar), 129.7 (q), 129.8 (2 CH, Ar), 131.2 (CH, Ar), 134.0 (q), 135.6 (q), 155.1 (q), 155.6 (q), 156.1 (q) 157.5 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3268 (NH), 3105 (NH), 2953, 2050, 1655, 1619, 1578 (CN), 1486 (CN), 1415, 1211, 1154, 1104, 1016, 963, 840, 755, 691. HRMS (*m/z* - ES): 361.1775 (M<sup>+</sup> + H). HPLC: 99.1 (*t*R: 22.8 min).

**4.1.11. 3-[4'-(Phenylguanidino)benzoyl]phenylguanidine dihydrochloride (4b)**

Following Method B, **12b** (228 mg, 0.34 mmol) was dissolved in 4M HCl in dioxane (1.53 mL, 6.12 mmol) and in additional dioxane (0.17 mL) until reach a final concentration of 0.2M. After 8 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic off-white solid (92 mg, 61%). Mp: decomp. above 161 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 7.33 (d, 2H, *J* = 7.5 Hz, Ar), 7.36 (d, 1H, *J* = 7.5 Hz, Ar), 7.43-7.48 (m, 4H, Ar), 7.59 (dd, 1H, *J* 7.9, 1.8 Hz, Ar), 7.62 (t, 1H, *J* = 7.9 Hz, Ar), 7.65 (app. t, 1H, *J* = 1.8 Hz, Ar), 7.73 (d, 1H, *J* = 7.5, 1.8 Hz, Ar), 7.83 (d, 2H, *J* = 8.6 Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 123.7 (2 CH, Ar), 125.3 (2 CH, Ar), 127.1 (CH, Ar), 127.8 (CH, Ar), 129.4 (CH, Ar),

129.8 (2 CH, Ar), 130.1 (CH, Ar), 130.5 (CH, Ar), 131.9 (2 CH, Ar), 134.2 (q), 134.3 (q), 134.5 (q), 138.2 (q), 140.1 (q), 154.3 (q), 156.3 (q), 198.0 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3143 (NH), 2970, 2287, 1738, 1560 (CN), 1490, 1448, 1228, 1076, 1020, 753, 694. HRMS (*m/z* - ES): 373.1769 (M<sup>+</sup> + H). HPLC: 99.8% (*t*R: 21.7 min).

#### **4.1.12. 3-[4'-(Phenylguanidino)benzyl]phenylguanidine dihydrochloride (4c)**

Following Method B, **18c** (50 mg, 0.076 mmol) was dissolved in 4M HCl in dioxane (0.345 mL, 1.38 mmol) and in additional dioxane (0.04 mL) until reach a final concentration of 0.2M. After 6 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic off-white crystalline solid (31 mg, 94%). Mp: decomp. above 132 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 4.00 (s, 2H, CH<sub>2</sub>), 7.14 (d, 1H, *J* = 8.0 Hz, Ar), 7.15 (s, 1H, Ar), 7.25-7.26 (m, 3H, Ar), 7.30-7.34 (m, 4H, Ar), 7.36-7.39 (m, 2H, Ar), 7.44 (t, 2H, *J* = 8.0 Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 40.2 (CH<sub>2</sub>), 123.6 (CH, Ar), 125.6 (2 CH, Ar), 125.9 (CH, Ar), 126.2 (2 CH, Ar), 127.9 (CH, Ar), 128.3 (CH, Ar), 129.8 (2 CH, Ar), 130.1 (CH, Ar), 130.2 (2 CH, Ar), 132.2 (q), 134.0 (q), 134.3 (q), 141.0 (q), 143.2 (q), 154.9 (q), 156.3 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3143 (NH), 2970, 2287, 1738, 1560 (CN), 1490, 1448, 1228, 1076, 1020, 753, 694. HRMS (*m/z* - ES): 359.1981 (M<sup>+</sup> + H). HPLC: 98.15% (*t*R: 23.5 min).

#### **4.1.13. 3-[4'-(Phenylguanidino)phenylamino]phenylguanidine dihydrochloride (4d)**

Following Method B, **18d** (168 mg, 0.255 mmol) was dissolved in 4M HCl in dioxane (1.15 mL, 4.59 mmol) and in additional dioxane (0.125 mL) until reach a final concentration of 0.2M. After 8 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic light dark solid (64 mg, 58%). Mp: decomp. above 160

°C.  $\delta_H$  (600 MHz, D<sub>2</sub>O): 6.83 (d, 1H, *J* = 7.6 Hz, Ar), 6.96 (s, 1H, Ar), 7.07 (d, 1H, *J* = 7.6 Hz, Ar), 7.15 (d, 2H, *J* = 6.9 Hz, Ar), 7.22 (d, 2H, *J* = 6.9 Hz, Ar), 7.31-7.38 (m, 4H, Ar), 7.45 (app. t, 2H, *J* = 7.1, 6.5 Hz, Ar).  $\delta_C$  (150 MHz, D<sub>2</sub>O): 114.3 (CH, Ar), 117.0 (CH, Ar), 118.0 (CH, Ar), 188.8 (2 CH, Ar), 125.9 (2 CH, Ar), 126.6 (q), 127.6 (2 CH, Ar), 127.9 (CH, Ar), 129.8 (2 CH, Ar), 130.8 (CH, Ar), 133.9 (q), 135.1 (q), 142.6 (q), 144.4 (q), 155.2 (q), 156.1 (q).  $\nu_{max}$  (ATR)/cm<sup>-1</sup>: 3259 (NH), 3129 (NH), 2162, 1619, 1578 (CN), 1509, 1492 (CN), 1331, 1226, 1195, 1167, 1112, 1077, 1022, 997, 819, 756, 691. HRMS (*m/z* - ES): 360.1932 (M<sup>+</sup> + H). HPLC: 99.47% (*tR*: 22.51 min).

#### **4.1.14. 3-{4'-[{(4-Chloro-3-trifluoromethylphenyl)guanidino]phenoxy}phenylguanidine dihydrochloride (5a)}**

Following Method B, **19a** (117 mg, 0.154 mmol) was dissolved in 4M HCl in dioxane (0.69 mL, 2.77 mmol) and in additional dioxane (0.076 mL) until a final concentration of 0.2M was reached. After 6 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic off-white solid (72 mg, 88%). Mp: decomp. above 160 °C.  $\delta_H$  (600 MHz, D<sub>2</sub>O): 6.93 (app. t, 1H, *J* = 2.05 Hz, Ar), 6.98 (dd, 1H, *J* = 8.5, 1.7 Hz, Ar), 7.07 (d, 2H, *J* 8.9 Hz, Ar), 7.08 (dd, 1H, *J* 8.5, 1.7 Hz, Ar), 7.27 (d, 2H, *J* = 8.9 Hz, Ar), 7.44-7.47 (m, 2H, Ar), 7.61 (d, 1H, *J* = 8.5 Hz, Ar), 7.67 (dd, 1H, *J* 1.9 Hz, Ar).  $\delta_C$  (150 MHz, D<sub>2</sub>O): 116.0 (CH, Ar), 118.0 (CH, Ar), 120.0 (2 CH, Ar), 121.1 (CH, Ar), 123.3 (c, q, *J* = 273 Hz, CF<sub>3</sub>), 124.6 (CH, Ar), 127.4 (2 CH, Ar), 128.4 (d, q, *J* = 30 Hz), 129.6 (q), 129.9 (CH, Ar), 130.0 (q), 131.2 (CH, Ar), 132.7 (CH, Ar), 133.5 (q), 135.5 (q), 154.7 (q), 155.5 (q), 156.1 (q), 157.3 (q).  $\nu_{max}$  (ATR)/cm<sup>-1</sup>: 3323 (NH), 1656, 1626, 1579 (CN), 1484, 1505, 1484, 1322, 1259, 1214, 1174, 1130, 1113, 1035, 832, 664. HRMS (*m/z* - ES): 463.1269 (M<sup>+</sup> + H). HPLC: 99.7% (*tR*: 27.4 min).

**4.1.15. 3-{4'-[**(4-Chloro-3-trifluoromethylphenyl)guanidino]benzoyl}phenylguanidine dihydrochloride (5b)****

Following Method B, **19b** (159 mg, 0.21 mmol) was dissolved in 4M HCl in dioxane (0.925 mL, 3.7 mmol) and in additional dioxane (0.105 mL) until reach a final concentration of 0.2M. After 6 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic off-white crystalline solid (62 mg, 54%). Mp: decomp. above 170 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 7.36 (d, 2H, *J* = 8.6 Hz, Ar), 7.45 (dd, 1H, *J* = 8.5, 2.3 Hz, Ar), 7.56-7.60 (m, 3H, Ar), 7.63 (t, 1H, *J* = 7.7 Hz, Ar), 7.64 (s, 1H, Ar), 7.69 (ddd, 1H, Ar, *J* = 7.7, 1.6, 1.3 Hz, Ar), 7.75 (d, 2H, *J* = 8.6 Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 123.0 (c, q, *J* = 273 Hz, CF<sub>3</sub>), 123.5 (2 CH, Ar), 123.9 (CH, Ar), 127.1 (CH, Ar), 128.3 (d, q, *J* = 30 Hz), 129.2 (CH, Ar), 129.4 (CH, Ar), 129.6 (q), 130.2 (CH, Ar), 130.7 (CH, Ar), 131.8 (2 CH, Ar), 132.6 (CH, Ar), 133.9 (q), 134.1 (q), 134.4 (q), 138.1 (q), 139.7 (q), 154.0 (q), 156.3 (q), 197.9 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3285, 3143 (NH), 2324, 1648, 1561 (CN), 1482, 1421, 1315, 1258, 1176, 1129, 1035, 969, 834, 759, 665. HRMS (*m/z* - ES): 475.1246 (M<sup>+</sup> + H). HPLC: 97.5% (*t*R: 26.1 min).

**4.1.16. 3-{4'-[**(4-Chloro-3-trifluoromethylphenyl)guanidino]benzyl}phenylguanidine dihydrochloride (5c)****

Following Method B, **19c** (107 mg, 0.14 mmol) was dissolved in 4 M HCl in dioxane (0.635 mL, 2.54 mmol) and in additional dioxane (0.071 mL) until reach a final concentration of 0.2M. After 6 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic white crystalline solid (69 mg, 92%). Mp: decomp. above

140 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 3.97 (s, 2H, CH<sub>2</sub>), 7.12-7.13 (m, 2H, Ar), 7.18 (d, 2H, *J* = 8.0 Hz, Ar), 7.22 (d, 1H, *J* = 7.7 Hz, Ar), 7.27 (d, 2H, *J* = 8.0 Hz, Ar), 7.38 (t, 1H, *J* = 7.7 Hz, Ar), 7.42 (d, 1H, *J* = 8.3 Hz, Ar), 7.56 (d, 1H, *J* = 8.3 Hz, Ar), 7.64 (s, 1H, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 40.1 (CH<sub>2</sub>), 123.1 (c, q, *J* = 273 Hz, CF<sub>3</sub>), 123.6 (CH, Ar), 124.4 (CH, Ar), 125.6 (2 CH, Ar), 125.9 (CH, Ar), 128.2 (CH, Ar), 128.4 (d, q, *J* = 30 Hz), 129.6 (CH, Ar,), 130.0 (2 CH, Ar), 130.1 (CH, Ar), 132.5 (q), 132.6 (CH, Ar), 134.1 (q), 134.2 (q), 140.6 (q), 143.2 (q), 154.5 (q), 156.2 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3025 (NH), 2970, 2949, 2162, 1738, 1655, 1625, 1571 (CN), 1511, 1433, 1366, 1322, 1257, 1229, 1217, 1128, 1034, 891, 830. HRMS (*m/z* - ES): 461.1468 (M<sup>+</sup> + H). HPLC: 97.4 9 (*t*R: 27.6 min).

#### **4.1.17. 3-{4'-[**(4-Chloro-3-trifluoromethylphenyl)guanidino]phenylamino} phenylguanidine dihydrochloride (5d)****

Following Method B, **19d** (272 mg, 0.357 mmol) was dissolved in 4M HCl in dioxane (1.6 mL, 6.43 mmol) and in additional dioxane (0.18 mL) until reach a final concentration of 0.2M. After 8 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic light dark solid (112 mg, 59%). Mp: decomp. above 205 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 6.83 (d, 1H, *J* = 8.1 Hz, Ar), 6.93 (s, 1H, Ar), 7.02 (d, 1H, *J* = 8.1 Hz, Ar), 7.10 (d, 2H, *J* = 8.6 Hz, Ar), 7.15 (d, 2H, *J* = 8.6 Hz, Ar), 7.34 (t, 1H, *J* = 8.1 Hz, Ar), 7.44 (dd, 1H, *J* = 8.3, 2.05 Hz, Ar), 7.58 (d, 1H, *J* = 8.3 Hz, Ar), 7.63 (s, 1H, Ar).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 114.1 (CH, Ar), 117.0 (CH, Ar), 118.0 (CH, Ar), 188.8 (2 CH, Ar), 122.2 (c, q, *J* = 273 Hz, CF<sub>3</sub>), 124.5 (c, *J* = 5.2 Hz, CH, Ar), 126.8 (q), 127.0 (2 CH, Ar), 128.4 (d, q, *J* = 30 Hz), 129.8 (CH, Ar), 130.8 (CH, Ar), 132.6 (CH, Ar), 133.6 (q), 135.0 (q), 142.3 (q), 144.4 (q), 154.6 (q), 156.2 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3260 (NH), 3133 (NH), 2163, 1619,

1578 (CN), 1509, 1493, 1332, 1226, 1167, 1112, 1077, 1021, 997, 819, 757, 691. HRMS (*m/z* - ES): 462.1417 (M<sup>+</sup> + H). HPLC: 99.95% (*tR*: 26.75 min).

#### **4.1.18. 4-[3'-(Phenylguanidino)phenoxy]phenylguanidine dihydrochloride (6a)**

Following Method B, **20a** (108 mg, 0.164 mmol) was dissolved in 4M HCl in dioxane (0.74 mL, 2.94 mmol) and in additional dioxane (0.084 mL) until a final concentration of 0.2M was reached. After 7 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic off-white solid (50 mg, 70%). Mp: decomp. above 289 °C. δ<sub>H</sub> (600 MHz, D<sub>2</sub>O): 7.02-7.03 (m, 2H, Ar), 7.11-7.12 (m, 3H, Ar), 7.27-7.29 (m, 4H, Ar), 7.37 (t, 1H, *J* = 7.4 Hz, Ar), 7.43-7.46 (m, 3H, Ar). δ<sub>C</sub> (150 MHz, D<sub>2</sub>O): 115.6 (CH, Ar), 117.8 (CH, Ar), 120.2 (2 CH, Ar), 120.6 (CH, Ar), 125.5 (2 CH, Ar), 127.9 (CH, Ar), 128.0 (2 CH, Ar), 129.7 (q), 129.8 (2 CH, Ar), 131.2 (CH, Ar), 134.0 (q), 135.8 (q), 154.7 (q), 155.6 (q), 156.4 (q), 157.4 (q). ν<sub>max</sub> (ATR)/cm<sup>-1</sup>: 3159 (NH), 3058 (NH), 2289, 1560 (CN), 1504, 1484, 1449, 1215, 1166, 1150, 1102, 1076, 1014, 1002, 964, 842, 788, 752, 690 HRMS (*m/z* - ES): Found: 361.1772 (M<sup>+</sup> + H. C<sub>20</sub>H<sub>21</sub>N<sub>6</sub>O Requires: 361.1777). HPLC: 99.5% (*tR*: 23.13 min).

#### **4.1.19. 4-[3'-(Phenyl)guanidinobenzyl]phenylguanidine dihydrochloride (6c)**

Following Method B, **20c** (164 mg, 0.25 mmol) was dissolved in 4M HCl in dioxane (1.122 mL, 4.49 mmol) and in additional dioxane (0.125 mL) until reach a final concentration of 0.2M. After 6 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a white crystalline solid (83 mg, 80%). Mp: decomp. above 170 °C. δ<sub>H</sub> (600 MHz, D<sub>2</sub>O): 4.00 (s, 2H, CH<sub>2</sub>), 7.15-7.20 (m, 4H, Ar), 7.25 (d, 1H, *J* = 8.0 Hz, Ar), 7.27

(d, 2H,  $J = 7.7$  Hz, Ar), 7.32 (d, 2H,  $J = 8.2$  Hz, Ar), 7.34-7.39 (m, 2H, Ar), 7.43 (t, 2H,  $J = 7.7$  Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 40.1 (CH<sub>2</sub>), 123.4 (CH, Ar), 125.6 (2 CH, Ar), 125.7 (CH, Ar), 126.1 (2 CH, Ar), 127.8 (CH, Ar), 128.1 (CH, Ar), 129.8 (2 CH, Ar), 130.1 (CH, Ar), 130.1 (2 CH, Ar), 132.1 (q), 134.0 (q), 134.4 (q), 140.9 (q), 143.2 (q), 154.8 (q), 156.2 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3029 (NH), 2970, 2162, 1738, 1571 (CN), 1492, 1440, 1366, 1230, 1217, 756, 692. HRMS (*m/z* - ES): Found: 369.1980 (M<sup>+</sup> + H. C<sub>21</sub>H<sub>23</sub>N<sub>6</sub> Requires: 359.1984). HPLC: 98.5% (*t*R: 23.6 min).

#### **4.1.20. 4-[3'-(4-Chloro-3-trifluoromethylphenyl)guanidinophenoxy]phenylguanidine**

##### **dihydrochloride (7a)**

Following Method B, **21a** (150 mg, 0.197 mmol) was dissolved in 4M HCl in dioxane (0.89 mL, 3.54 mmol) and in additional dioxane (0.1 mL) until a final concentration of 0.2M was reached. After 6 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic off-white solid (103 mg, 98%). Mp: decomp. above 125 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 6.95 (app. t, 1H,  $J = 2.2$  Hz, Ar), 6.98 (dd, 1H,  $J = 8.5$  Hz, 2.2, Ar), 7.06-7.09 (m, 3H, Ar), 7.24 (d, 2H,  $J = 8.7$  Hz, Ar), 7.41 (t, 1H,  $J = 8.5$  Hz, Ar), 7.44 (dd, 1H,  $J = 8.5$  Hz, 2.2, Ar), 7.60 (d, 1H,  $J = 8.5$  Hz, Ar), 7.64 (d, 1H,  $J = 2.2$  Hz, H-12).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 115.0 (CH, Ar), 117.6 (CH, Ar), 120.1 (CH, Ar), 121.2 (2 CH, Ar), 123.0 (c, q,  $J = 273$  Hz, CF<sub>3</sub>), 124.1 (c,  $J = 6.5$  Hz, CH, Ar), 127.8 (2 CH, Ar), 128.5 (c, q,  $J = 30$  Hz), 129.4 (CH, Ar), 129.7 (q), 129.8 (q), 131.2 (CH, Ar), 132.7 (CH, Ar), 133.9 (q), 135.8 (q), 154.4 (q), 155.5 (q), 156.4 (q), 157.4 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3294 (NH), 3117 (NH), 1981, 1663, 1583 (CN), 1505, 1482, 1321, 1258, 1215, 1173, 1130, 1034, 878, 829, 665. HRMS (*m/z* - ES): Found: 463.1253 (M<sup>+</sup> + H. C<sub>21</sub>H<sub>19</sub>N<sub>6</sub>OF<sub>3</sub>Cl Requires: 463.1261). HPLC: 99.3% (*t*R: 27.7 min).

**4.1.21. 4-[3'-(4-Chloro-3-trifluoromethylphenyl)guanidinobenzyl]phenylguanidine dihydrochloride (7c)**

Following Method B, **21c** (110 mg, 0.145 mmol) was dissolved in 4M HCl in dioxane (0.651 mL, 2.6 mmol) and in additional dioxane (0.072 mL) until reach a final concentration of 0.2M. After 6 h stirring at 55 °C, the reaction was adjudged complete (TLC), solvents were evaporated and the residue was purified by reverse phase chromatography to afford the pure hydrochloride salt as a hygroscopic white crystalline solid (62 mg, 80%). Mp: 135-137 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 3.91 (s, 2H, CH<sub>2</sub>), 7.05-7.10 (m, 4H, Ar), 7.15 (d, 1H, J = 7.8 Hz, Ar), 7.23 (d, 2H, J = 8.5 Hz, Ar), 7.29 (d, 1H, J = 7.8 Hz, Ar), 7.34 (dd, 1H, J = 8.5 Hz, 2.5, Ar), 7.51 (d, 1H, J = 8.5 Hz, Ar), 7.57 (d, 1H, J = 2.5 Hz, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 40.0 (CH<sub>2</sub>), 122.8 (CH, Ar), 123.5 (c, q, J = 273 Hz, CF<sub>3</sub>), 124.1 (c, J = 5.2 Hz, CH, Ar), 125.2 (CH, Ar), 125.8 (2 CH, Ar), 127.9 (CH, Ar), 128.4 (c, q, J = 30 Hz, Ar), 129.4 (CH, Ar), 129.8 (q), 130.0 (CH, Ar), 130.2 (2 CH, Ar), 132.1 (q), 132.7 (CH, Ar), 133.7 (q), 134.4 (q), 140.8 (q), 143.1 (q), 154.5 (q), 156.1 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3118 (NH), 2970, 2163, 1655, 1577 (CN), 1505, 1483, 1318, 1258, 1214, 1172, 1146, 1035, 949, 833, 753, 689. HRMS (*m/z* - ES): Found: 461.1473 (M<sup>+</sup> + H. C<sub>22</sub>H<sub>21</sub>N<sub>6</sub>F<sub>3</sub>Cl Requires: 461.1468). HPLC: 97.7% (tR: 27.41 min).

**4.1.22. N-(3-{4-[N'-(4-Chloro-3-trifluoromethylphenyl)guanidino]phenoxy}phenyl)acetamide hydrochloride (8a)**

Following Method C, a solution of **22a** (376 mg, 0.67 mmol) in 1:1 CH<sub>2</sub>Cl<sub>2</sub>:IPA (2.35 mL) was treated with 4 M HCl in dioxane (1 mL). After 6 h. stirring at 30 °C, the reaction was adjudged to completion by TLC. Solvents were evaporated and the residue was extracted with CH<sub>2</sub>Cl<sub>2</sub> and purified by reverse phase chromatography to afford the pure hydrochloride salt as a white solid (174 mg, 52%) Mp: 140-142 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 2.09 (s, 3H, CH<sub>3</sub>),

6.81 (dd, 1H,  $J = 8.1, 1.76$  Hz, Ar), 7.05 (d, 2H,  $J = 8.9$  Hz, Ar), 7.09 (dd, 1H,  $J = 8.1, 1.76$  Hz, Ar), 7.17 (app. t, 1H, Ar), 7.27 (d, 2H,  $J = 8.9$  Hz, Ar), 7.36 (t, 1H,  $J = 8.1$  Hz, Ar), 7.47 (d, 1H,  $J = 8.5$  Hz, Ar), 7.61 (d, 1H,  $J = 8.5$  Hz, Ar), 7.68 (app. d, 1H, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 22.6 (CH<sub>3</sub>), 112.4 (CH, Ar), 115.5 (CH, Ar), 117.1 (CH, Ar), 119.8 (2 CH, Ar), 123.0 (c, q,  $J = 273$  Hz, CF<sub>3</sub>), 124.6 (c,  $J = 5.2$  Hz, CH, Ar,), 127.5 (2 CH, Ar), 128.5 (d, q,  $J = 30$  Hz), 129.3 (q), 129.9 (CH, Ar), 130.0 (q), 130.3 (CH, Ar), 132.7 (CH, Ar), 133.5 (q), 138.3 (q), 154.7 (q), 156.0 (q), 156.6 (q), 172.8 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3126 (NH), 2971, 2878, 2035, 1659 (CO), 1596 (CN), 1544, 1505, 1483, 1418, 1257, 1216, 1036, 967, 921, 878, 832, 785, 713, 668. HRMS (*m/z* - ES): 463.1162 (M<sup>+</sup> + H). HPLC: 96.1% (*t*R: 30.51 min).

#### **4.1.23. N-(3-[4-[N'-(4-Chloro-3-trifluoromethylphenyl)guanidino]phenylamino]phenyl)acetamide hydrochloride (8d)**

Following Method C, a solution of **22d** (238 mg, 0.42 mmol) in 1:1 CH<sub>2</sub>Cl<sub>2</sub>:IPA (1.48 mL) was treated with 4 M HCl in dioxane (0.63 mL). After 6 h. stirring at 30 °C, the reaction was adjudged to completion by TLC. Solvents were evaporated and the residue was extracted with CH<sub>2</sub>Cl<sub>2</sub> and purified by reverse phase chromatography to afford the pure hydrochloride salt as a white solid (83 mg, 43%). Mp: 155-157 °C.  $\delta_{\text{H}}$  (600 MHz, D<sub>2</sub>O): 2.11 (s, 3H, CH<sub>3</sub>), 6.87-6.91 (m, 2H, Ar), 7.11 (d, 2H,  $J = 8.7$  Hz, Ar), 7.17 (dd, 1H,  $J = 8.7$  Hz, Ar), 7.23 (s, 1H, Ar), 7.28 (t, 1H,  $J = 8.0$  Hz, Ar), 7.47 (app. dd, 1H, Ar), 7.61 (d, 1H,  $J = 8.1$  Hz, Ar), 7.67 (s, 1H, Ar).  $\delta_{\text{C}}$  (150 MHz, D<sub>2</sub>O): 22.6 (CH<sub>3</sub>), 111.3 (CH, Ar,), 114.7 (CH, Ar), 115.1 (CH, Ar), 118.6 (2 CH, Ar), 123.5 (c, q,  $J = 273$  Hz, CF<sub>3</sub>), 124.7 (c,  $J = 5.2$  Hz, CH, Ar), 127.2 (2 CH, Ar), 128.5 (d, q,  $J = 30$  Hz), 129.3 (q), 129.9 (CH, Ar), 130.0 (CH, Ar), 130.3 (q), 132.7 (CH, Ar), 133.5 (q), 137.8 (q), 154.7 (q), 156.0 (q), 156.6 (q), 172.9 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3073 (NH), 3016, 2971, 2360, 2036, 1982, 1915, 1739, 1588, 1547 (CN), 1509,

1484, 1460, 1416, 1366, 1290, 1229, 1217, 1170, 1140, 1037, 856, 767, 728, 687. HRMS (*m/z* - ES): 460.1155 (M<sup>+</sup> - H). HPLC: 95.8% (*t*R: 29.92 min).

## 4.2. BIOCHEMISTRY

### 4.2.1. Preparation of Stock Solutions

Stock solutions (10 mM) of the compounds were prepared in sterile distilled water (ddH<sub>2</sub>O) and were then sterile filtered (0.2 µM filters). Required concentration ranges (10–0.1 mM) of each drug were prepared in sterile ddH<sub>2</sub>O and stored at -20 °C until required. Sorafenib was purchased from LC laboratories.

### 4.2.2. HL-60 Cell Line

The HL-60 (human caucasian promyelocytic leukemia) cell line was obtained from European Collection of Cell Cultures (Porton Down, Wiltshire, U.K.). They were maintained between 200,000 – 2,000,000 cells/mL in Roswell Park Memorial Institute (RPMI) 1640 medium with stable glutamate (GlutaMax I) supplemented with 10% (v/v) foetal bovine serum (FBS) and 50 µg mL<sup>-1</sup> penicillin/streptomycin (pen-strep). The growth medium was stored in the fridge at 4 °C and heated to 37 °C prior to culture work. Cells were grown at 37 °C in a humidified environment maintained at 95% O<sub>2</sub> and 5% CO<sub>2</sub> and passaged at least twice weekly depending on their levels of confluence. When required for sub-culturing, cells were transferred to a sterile tube and centrifuged at 300 × g for 5 min. The supernatant was discarded and the cell pellet was resuspended in 10 mL of fresh medium. Cells were then counted using a haemocytometer slide and seeded at the required density (200,000 cells mL<sup>-1</sup>).

#### **4.2.3. Cell Viability (*AlamarBlue Assay*)**

AlamarBlue, which is soluble and non-toxic dye, is commonly used to assess cell viability in response to toxic agents. It is used to measure cell proliferation by monitoring the reducing environment of the cell, as the internal environment of the proliferating cell is more reduced than that of non-proliferating cells. As AlamarBlue accepts electrons from reducing compounds such as NADH and FADH, it changes colour from the oxidized indigo blue non-fluorescent state to the reduced fluorescent pink state. A spectrofluorometric microtiter well plate reader measures this change in fluorescence. HL-60 cells in the log phase of growth were seeded in 96-well plates at a density of 200,000 cells/mL (200 µL/well or 40,000 cells/well) in complete RPMI medium. The cells were then treated with a 1:100 dilution of stock concentrations of drugs or ddH<sub>2</sub>O as vehicle control in triplicate. Three blank wells containing 200 µL RPMI with no cells plus 20 µL AlamarBlue were also set-up as blanks. After a 72 h incubation, 20 µL of AlamarBlue was added to each well. The plates were incubated in darkness at 37 °C for 5 h. Using a Molecular Devices microplate reader, the fluorescence (F) was then read at an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Cell viability was then determined by subtracting the mean blank fluorescence (F<sub>b</sub>) from the treated sample fluorescence (F<sub>s</sub>) and expressing this as a percentage of the fluorescence of the blanked vehicle control (F<sub>c</sub>). This is demonstrated in the equation below. The results were then plotted as nonlinear regression, sigmoidal dose-response curves on Prism, from which the IC<sub>50</sub> value for each drug was determined.

$$\frac{(F_s - F_b)}{(F_c - F_b)} \times 100 = \% \text{ Cell Viability}$$

#### **4.2.4. HL-60 Cell Morphology Studies**

Cells were seeded at 200,000/mL in T25 flasks and treated with an appropriate volume of compound of particular concentration or with sterile ddH<sub>2</sub>O for vehicle control. At 72 h, cells

were centrifuged at  $625 \times g$  for 10 min. Old media was discarded and cell pellets resuspended in 2 mL fresh media. Pellets were then resuspended in 2 mL of fresh media. 200  $\mu L$  of this was then cytocentrifuged (Shandon Cytospin) onto appropriate glass slides at  $187 \times g$  for 10 min. Slides were then air-dried for 10 min and cells stained using the RapiDiff kit according to manufacturer's instructions. Images were taken at a magnification of  $40\times$  using an Olympus DP71 1X81 microscope coupled to a CCD camera.

#### **4.2.5. Flow Cytometry (determination of DNA content)**

HL-60 cells were seeded at 200,000 cells  $mL^{-1}$  in 6-well plates and treated with appropriate drug concentrations and ddH<sub>2</sub>O as vehicle control (20  $\mu L$  of drug or ddH<sub>2</sub>O in 2 mL of cells) for 48 h and 72 h time points. Cells were harvested at each time-point by centrifugation at  $550 \times g$  for 8 min. Media was removed and the pellets resuspended in 200  $\mu L$  non-sterile PBS. The cells were then fixed by a drop-wise addition of 2 mL of ice-cold 70% EtOH/PBS while vortexing. Following overnight fixation at -20 °C, 10  $\mu L$  of non-sterile FBS was added to the cells and the solutions centrifuged at  $550 \times g$  for 10 min. The ethanol was then removed and the cell pellet resuspended in 0.4 mL PBS. Then, 25  $\mu L$  10 mg  $mL^{-1}$  RNase A (which destroys RNA so that only DNA can be observed) was added. Also, 75  $\mu L$ , 1 mg  $mL^{-1}$  propidium iodide (PI) (a DNA binding dye which fluoresces when activated and thus allows the amount of DNA present to be determined) was added to each sample. Cells were vortexed and incubated in the dark at 37 °C for 30 min. The PI fluorescence was measured on a linear scale using a FACs Calibur flow cytometer (Becton Dickinson, San Jose, CA). Data collections (10,000 events per sample) were gated to exclude cell debris and cell aggregates. The amount of fluorescence is proportional to the amount of DNA present and hence the population of cells in each phase of the cell cycle can be determined. Cells are gated as follows: M1= pre-G<sub>1</sub> (< 2N DNA), M2= G<sub>0</sub>/G<sub>1</sub> (2N DNA), M3= S (2N – 4N DNA), M4=

G<sub>2</sub>/M (4N DNA), M5= G<sub>n</sub> (> 4N DNA). Apoptotic cells are hypoploid (< 2N DNA). Therefore, apoptosis was determined from the peak in M1. All data was recorded and analysed using the CellQuest software (Becton Dickinson, San Jose, CA).

#### **4.2.6. CK1 $\delta$ assay protocol**

Human recombinant Casein Kinase 1 $\delta$  was purchased from Millipore (Millipore Ibérica S.A.U.) Casein solution from bovine milk, 5%, was purchased from Sigma-Aldrich (St. Louis, MO). Kinase-Glo Luminescent Kinase Assay was obtained from Promega (Promega Biotech Ibérica, SL). ATP and all other reagents were from Sigma-Aldrich (St. Louis, MO). Assay buffer contained 50 mM HEPES, pH 7.5; 0.01% Brij-35; 10 mM Cl<sub>2</sub>Mg; 1 mM EGTA and 0.01 % NaN<sub>3</sub>. The “Kinase-Glo” Kit from Promega was used to screen compounds for activity against CK1 $\delta$ . Kinase-Glo assays were performed in assay buffer using black 96-well plates. In a typical assay, 10  $\mu$ L of test compound (dissolved in dimethyl sulfoxide [DMSO] at 1 mM concentration and diluted in advance in assay buffer to the desired concentration) and 10  $\mu$ L (16 ng) of enzyme were added to each well followed by 20  $\mu$ L of assay buffer containing 0.1 % casein as substrate and 4  $\mu$ M ATP. The final DMSO concentration in the reaction mixture did not exceed 1%. After a 60 min incubation at 30 °C the enzymatic reaction was stopped with 40  $\mu$ L of Kinase-Glo reagent. Glow-type luminescence was recorded after 10 min using a FLUOstar Optima multimode reader (BMG Labtechnologies GmbH, Offenburg, Germany). The activity is proportional to the difference of the total and consumed ATP. The inhibitory activities were calculated on the basis of maximal activities measured in the absence of inhibitor. The IC<sub>50</sub> was defined as the concentration of each compound that reduces the enzymatic activity by 50% with respect to that without inhibitors.

#### 4.2.7. Inhibition of GSK-3 (*luminescent assay*)

Human recombinant GSK-3 $\beta$  was purchased from Millipore (Millipore Iberica S.A.U.) The prephosphorylated polypeptide substrate was purchased from Millipore (Millipore Iberica S.A.U.). Kinase-Glo Luminescent Kinase Assay was obtained from Promega (Promega Biotech Ibérica, SL). ATP and all other reagents were from Sigma-Aldrich (St. Louis, MO). Assay buffer contained 50 mM HEPES (pH 7.5), 1 mM EDTA, 1 mM EGTA, and 15 mM magnesium acetate. The method of Baki et al.<sup>35</sup> was followed for the inhibition of GSK-3 $\beta$ . Kinase-Glo assays were performed in assay buffer using black 96-well plates. In a typical assay, 10  $\mu$ L (10  $\mu$ M) of test compound (dissolved in DMSO at 1 mM concentration and diluted in advance in assay buffer to the desired concentration) and 10  $\mu$ L (20 ng) of enzyme were added to each well followed by 20  $\mu$ L of assay buffer containing 50  $\mu$ M substrate and 2  $\mu$ M ATP. The final DMSO concentration in the reaction mixture did not exceed 1%. After 30 min incubation at 30 °C the enzymatic reaction was stopped with 40  $\mu$ L of Kinase-Glo reagent. Glow-type luminescence was recorded after 10 min using a FLUOstar Optima (BMG Labtechnologies GmbH, Offenburg, Germany) multimode reader. The activity is proportional to the difference of the total and consumed ATP. The inhibitory activities were calculated on the basis of maximal activities measured in the absence of inhibitor. The IC<sub>50</sub> is defined as the concentration of each compound that reduces to 50% the enzymatic activity with respect to that without inhibitors.

#### 4.2.8. LanthaScreen® Eu Kinase Binding Assay for B-RAF

Control (sorafenib) and test compounds 100 $\times$  concentrations were prepared. Sorafenib solution was made in DMSO, **7a** and **8d** in EtOH and the rest of the compound solutions were made in ddH<sub>2</sub>O. Intermediate dilutions were prepared (3 $\times$ ) by transferring fixed volumes of 100 $\times$  compound into fixed volumes of kinase buffer A (DMSO/EtOH

concentration ratio was 3%). Additionally, tracer solution (Kinase Tracer 178) was prepared at 60 nM (3× final) in kinase buffer A. Finally, 15 nM of B-RAF kinase and 6 nM antibody (3× final), kinase/antibody solution in kinase buffer A (on ice) was prepared. Prior to kinase/antibody solution preparation, antibody was centrifuged at 10000 × g for 10 min and solution aspirated from the top of the vial to minimise the potential effect of spurious donor emission spikes in assay. Once all the reagents were prepared 5 µL of each concentration of serial diluted compound (test or control) was added in a 384-well plate in triplicate wells. Then, 5 µL of kinase/antibody solution followed by 5 µL of tracer solution was then added to each well. Each plate also contains a blank, a vehicle control and a positive control. The blank contains kinase buffer A only, the vehicle control contains the solvent used for the drug solutions preparation which was DMSO, EtOH and ddH<sub>2</sub>O and the positive control contains varying concentrations of sorafenib. The plate was incubated at room temperature for 60-120 min and was read in the plate reader (BMG LABTECH PHERAstar) where the general instrument settings for excitation wavelength was 340 nm, the kinase trace emission was at 665 nm and the LanthaScreen® Eu-anti-Tag antibody emission was at 615 nm.

The data analysis consists of division of the acceptor or tracer emission (665 nm) by the antibody or donor emission (615 nm) to calculate the emission ratio. Then, the results were plotted in a graph where test/control compound concentration (M) in the x-axis versus emission ratio in the y-axis was performed. Where applicable, data was fitted using a sigmoidal dose-response curve (variable slope) using Prism GraphPad 5.0 and the IC<sub>50</sub> was determined.

#### **4.2.9. General kinase screening**

These specific tests were performed by the screening company Cerep [30] following, in each case, the protocols described below.

##### **4.2.9.1. RAF-1/MEK-1 Kinase Assay**

The test compound, reference compound or water (control) were preincubated for 5 min at room temperature with RAF-1 (2 ng) in a buffer containing 50 mM Hepes/NaOH (pH 7.4), 5 mM MgCl<sub>2</sub>, 1 mM DTT, 40 µM Na<sub>3</sub>VO<sub>4</sub> and 0.005% Tween 20. Thereafter, the reaction was initiated by adding 0.2 µM of inactive MEK-1, 100 nM of inactive ERK-2 and 15 µM ATP, and the mixture was incubated for 15 min at room temperature. For control basal measurements, RAF-1 was omitted from the reaction mixture. Following incubation, the reaction was stopped by adding 33 mM EDTA. The fluorescence acceptor (XL665-labelled anti-GST antibody) and the fluorescence donor (anti-phospho-ERK2 antibody labelled with europium cryptate) were then added. After 180 min, the fluorescence transfer was measured at  $\lambda_{\text{ex}}=337$  nm,  $\lambda_{\text{em}}=620$  and  $\lambda_{\text{em}}=665$  nm using a microplate reader (Rubystar, BMG). The enzyme activity is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results were expressed as a percent inhibition of the control enzyme activity. The standard inhibitory reference compound was staurosporine.

##### **4.2.9.2. ERK 1/2 Kinase (Inhibitory Effect)**

Confluent A431 cells (epidermoid carcinoma cell line) were suspended in HBSS buffer (Invitrogen) complemented with 0.1% BSA, then distributed in microplates at a density of 4.104 cells/well and preincubated for 5 min at 22 °C in the presence of either HBSS (basal and stimulated control) or the test compounds. Thereafter, 0.1 nM EGF is added and the mixture was incubated for 10 min at 22 °C. For control basal measurements, EGF is omitted

from the reaction mixture. Thereafter, cells were incubated during 15 min at room temperature with a lysis buffer. The fluorescence acceptor (alphalisa protein A coupled-beads) and the fluorescence donor (streptavidin coupled-beads) were then added. Following 120 min incubation at 22 °C, the signal is measured at  $\lambda_{\text{ex}} = 680$  nm and  $\lambda_{\text{em}} = 500$  and 600 nm using a microplate reader (EnVision, Perkin Elmer). The results were expressed as a percent inhibition of the control response to 0.1 nM EGF. The standard reference inhibitor was AG1478.

#### **4.2.9.3. P38 MAPKinase Assay (Inhibitory Effect)**

A431 (epidermoid carcinoma cell line) are grown to confluence for 48 h at 37 °C (CO<sub>2</sub> incubator). Then, cells are preincubated for 5 min at 22 °C in the presence of either of HBSS (basal and stimulated control) or the test compounds. Thereafter, 1 nM TNFα is added and the mixture is incubated for 10 min at 22 °C. For control basal measurements, TNFα is omitted from the reaction mixture. Thereafter, cells are lysed during 15 min at room temperature by adding the lyse buffer. To the cell lysat samples, the fluorescence acceptor (alphalisa protein A coupled-beads) is then added. Following 120 min incubation at 22°C, fluorescence donor (streptavidin coupled-beads) is added. Following 60 min incubation at 22 °C, the signal is measured at  $\lambda_{\text{ex}} = 680$  nm and  $\lambda_{\text{em}} = 500$  and 600 nm using a microplate reader (EnVision, Perkin Elmer). The results are expressed as a percent inhibition of the control response to 1 nM TNFα. The standard reference inhibitor was SB202190.

#### **4.2.9.4. VEGFR Assay (Inhibitory Effect)**

HUV-EC-C cells (Human Umbilical Vein Endothelial Cell Culture are primary cells used to measure angiogenesis activity) were seeded onto 96-well plate at 5×10<sup>4</sup> cells/well and allowed to grow overnight in standard growth media containing 76% F12K, 15% inactivated

FCS, 5% inactivated human serum, 1% antibiotics, 1% ECGS, 1% glutamine and 1% heparin under standard culture conditions. Growth media was exchanged to HBSS buffer + 20 mM HEPES (Invitrogen) and cells are allowed to equilibrate for 30 min at 28 °C before the start of the experiment. Plates were placed onto the system and measurements were made at a temperature of 28 °C. HBSS (basal and stimulated controls) and the test compounds were preincubated for 20 min before the addition of HBSS (basal control) or the reference stimulant VEGF at 0.1 nM (EC<sub>80</sub>). Impedance measurements are monitored for 10 min. The standard reference inhibitor was VEGF receptor tyrosine kinase inhibitor II.

#### **4.3. MOLECULAR DOCKING METHODOLOGY**

##### ***4.3.1. Preparation of initial structures for docking***

Compounds were docked into representative crystal structures of both RAF-1 and MEK-1: the 3OMV crystal structure of RAF-1 in complex with ligand was used to represent RAF-1 and the 3PP1 crystal structure of the human mitogen-activated protein kinase kinase 1 (MEK-1) in complex with ligand, ATP and Mg<sup>2+</sup> ion was used for MEK-1. The structures were imported into the MOE2011.10 modelling software (Molecular Operating Environment) and all crystallographic water molecules and other small molecules were removed as they were located away from the ligand-binding regions. The binding site was defined using the ligand from the crystal structures, while a second site was also defined for MEK-1 which included the space occupied by ATP and the Mg<sup>2+</sup> ion.

The ligands were built in MOE and minimized using the MMFF94x force field with a 0.05 gradient. A full conformational search was conducted using the LowModeMD [36] method

with a RMS gradient of 0.005, a strain cutoff of 10 kcal mol<sup>-1</sup> and a minimum of 100 iterations, to generate approximately 60 conformations per ligand.

#### 4.3.2. Docking protocol

The generated conformers were docked into the relevant kinase in MOE using the triangle matcher placement method and the London dG scoring function [36]. A post placement molecular mechanics forcefield refinement was carried out on the top 30 poses in which side chains of the protein were free to move and a non-interacting cutoff of 12 Å was applied. The final docking energy (E-refine) was calculated using the GB/VI solvent model [37].

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## 6. SUPPORTING INFORMATION

Supporting Information is available for the preparation and spectroscopic data of all starting amines (**9b-d**), aryl substituted thioureas (**12** and **13**) and Boc-protected intermediates (**10a-d**, **11a-c**, **14a,d**, **15a,d**, **16b,c**, **17a,c**, **18a-d**, **19a-d**, **20a,c**, **21a,c**, and **22a,d**) as well as for the spectroscopic and HPLC data of final hydrochloride salts (**2a-d**, **3a-c**, **4a-d**, **5a-d**, **6a,c**, **7a,c**, and **8a,d**). This material is available free of charge via the Internet.

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## Figures & Schemes captions

**Figure 1.** General approach to the different families of compounds synthesized.

**Figure 2.** Cytospin images of vehicle control of HL-60 cell line (left) and cells treated with **7a** at 50  $\mu$ M for 72 h (right).

**Figure 3.** Ligands from 3OMV (SM5, *left*) and 3PP1 (IZG, *right*) crystal structures.

**Figure 4.** Top ranked pose of compound **5b** (green) in: (a) RAF-1, (b) MEK-1 with ATP/Mg<sup>2+</sup> and (c) MEK-1 without ATP/Mg<sup>2+</sup>. Compound **5b** is representative of what was observed for compounds **5a-d** and **7a-c**. Mesh surfaces indicate the binding site space occupied by the crystallographic ligands.

**Scheme 1.** Preparation of 3,4'-*bis*-guanidine (**2a-d**) and 3,4'-*bis*-(2-aminoimidazoline) (**3a-c**) derivatives, Families 1 and 2, respectively.

**Scheme 2.** Synthesis of aryl substituted *bis*-guanidines **4a-d**, **5a-d**, **6a,c** and **7a,c**.

**Scheme 3.** Synthesis of acetyl *mono*-guanidine derivatives **8a,d**.

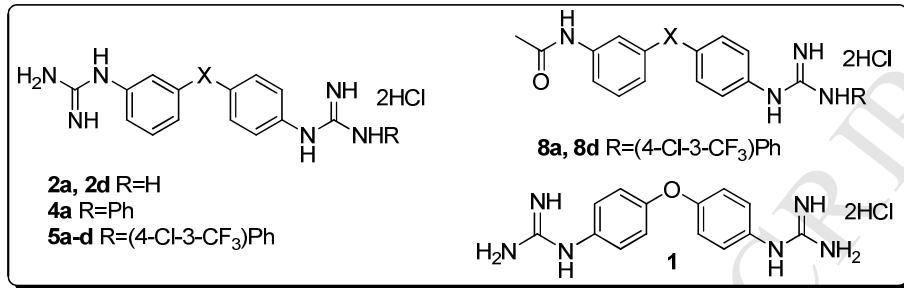
**Tables**

**Table 1.** IC<sub>50</sub> values ( $\mu\text{M}$ ) in HL-60 cells obtained for the previously prepared compound **1**, for the new compounds **2a-d**, **3a-c**, **4a-d**, **5a,c**, **6a-d**, **7a,c** and **8a,d** (Figure 1), and for sorafenib. Values are mean  $\pm$ S.E.M. representatives of at least three independent experiments performed in triplicate.

Compound	X	IC <sub>50</sub> $\pm$ SEM ( $\mu\text{M}$ )
<b>1</b>	O	88.4 $\pm$ 1.2
<b>2a</b>	O	36.2 $\pm$ 1.1
<b>2b</b>	CO	141.4 $\pm$ 2.5
<b>2c</b>	CH <sub>2</sub>	35.1 $\pm$ 1.2
<b>2d</b>	NH	67.7 $\pm$ 1.8
<b>3a</b>	O	>100
<b>3b</b>	CO	>100
<b>3c</b>	CH <sub>2</sub>	>100
<b>4a</b>	O	>100
<b>4b</b>	CO	>100
<b>4c</b>	CH <sub>2</sub>	>100
<b>4d</b>	NH	>100
<b>6a</b>	O	>100
<b>6c</b>	CH <sub>2</sub>	>100
<b>5a</b>	O	24.5 $\pm$ 1.2
<b>5b</b>	CO	22.3 $\pm$ 0.6
<b>5c</b>	CH <sub>2</sub>	15.9 $\pm$ 0.5
<b>5d</b>	NH	36.6 $\pm$ 2.1
<b>7a</b>	O	9.72 $\pm$ 0.9
<b>7c</b>	CH <sub>2</sub>	16.6 $\pm$ 1.6
<b>8a</b>	O	9.11 $\pm$ 0.1
<b>8d</b>	NH	3.96 $\pm$ 0.2
<b>Sorafenib</b>		1.68 $\pm$ 0.2

**Table 2.** DNA content of cells (mean  $\pm$ S.E.M.) as analyzed by flow cytometry after treatment with **7a** for 48 and 72 h. Results are from at least three independent experiments.

Conc. <b>20a</b> ( $\mu$ M)	<b>M1</b> (pre-G <sub>1</sub> )	<b>M2</b> (G <sub>0</sub> /G <sub>1</sub> )	<b>M3</b> (S)	<b>M4</b> (G <sub>2</sub> /M)	<b>M5</b> (G <sub>n</sub> )
<b>48 h</b>					
Vehicle	0.17 $\pm$ 0.0	57.0 $\pm$ 1.0	21.5 $\pm$ 1.0	18.4 $\pm$ 1.3	2.49 $\pm$ 0.4
1	0.13 $\pm$ 0.0	56.6 $\pm$ 0.2	20.6 $\pm$ 0.3	20.4 $\pm$ 0.1	2.17 $\pm$ 0.1
10	0.32 $\pm$ 0.0	68.9 $\pm$ 0.1	14.7 $\pm$ 0.1	14.6 $\pm$ 0.1	2.17 $\pm$ 0.1
25	1.75 $\pm$ 0.4	69.2 $\pm$ 0.4	7.15 $\pm$ 0.0	20.2 $\pm$ 0.4	2.17 $\pm$ 0.1
50	12.5 $\pm$ 0.3	44.6 $\pm$ 0.1	15.7 $\pm$ 0.2	18.6 $\pm$ 0.2	3.14 $\pm$ 0.2
<b>72 h</b>					
Vehicle	0.14 $\pm$ 0.0	65.7 $\pm$ 0.1	16.4 $\pm$ 0.8	14.8 $\pm$ 0.7	2.49 $\pm$ 0.3
1	0.13 $\pm$ 0.0	61.7 $\pm$ 0.9	17.1 $\pm$ 0.4	16.7 $\pm$ 0.1	3.23 $\pm$ 0.0
10	0.23 $\pm$ 0.0	61.3 $\pm$ 0.6	14.1 $\pm$ 0.4	18.9 $\pm$ 0.3	3.36 $\pm$ 0.1
25	2.22 $\pm$ 0.1	73.4 $\pm$ 1.7	4.19 $\pm$ 0.1	12.9 $\pm$ 0.3	2.56 $\pm$ 0.1
50	25.1 $\pm$ 0.9	43.7 $\pm$ 0.7	14.4 $\pm$ 0.2	13.6 $\pm$ 0.4	1.75 $\pm$ 0.2

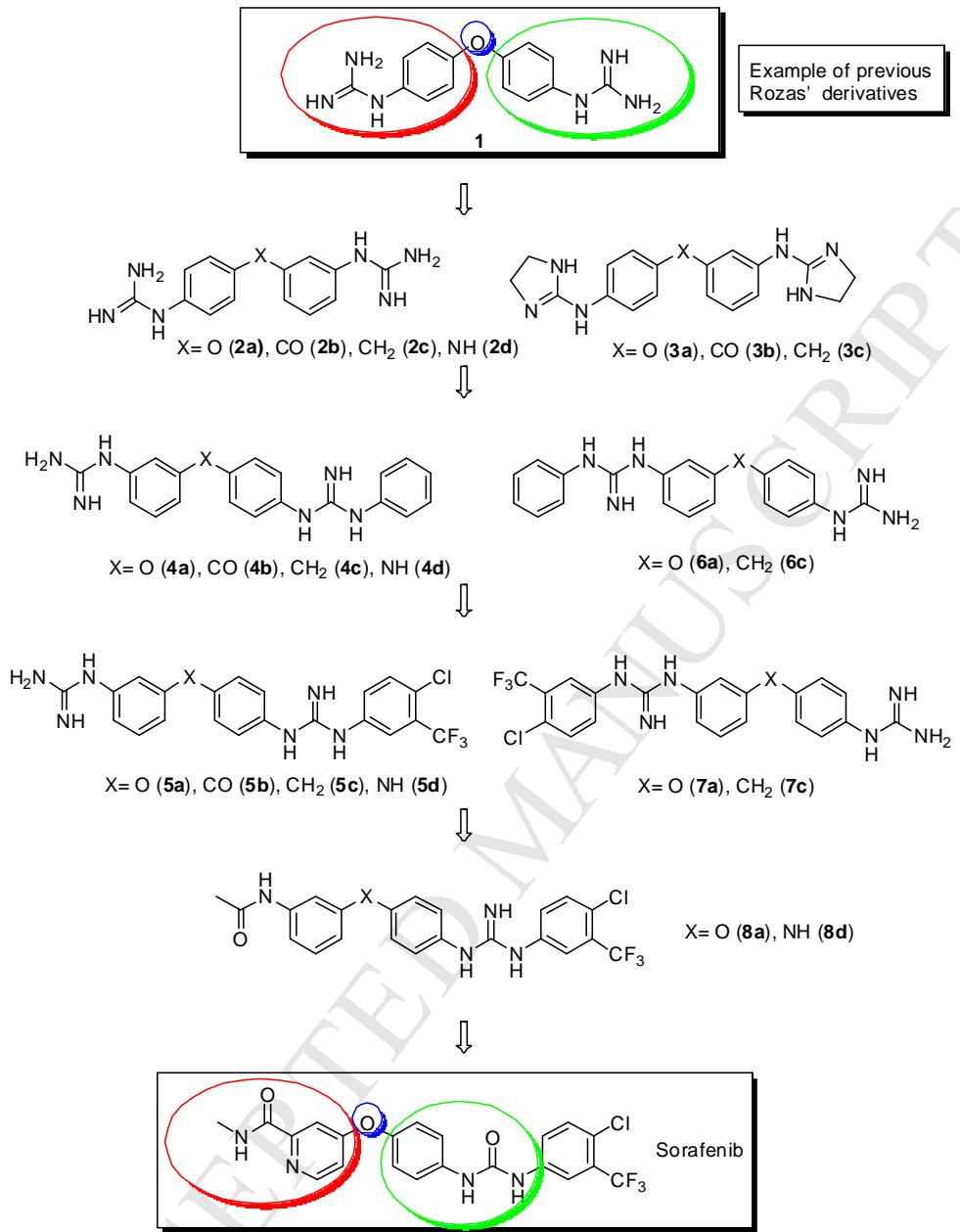
**Table 3.**- Inhibition of the kinase activity (%) of ERK1/2, p38 MAPK, VEGFR and RAF-1/MEK-1 systems by compounds **1**, **2a,d**, **4a**, **5a-d** and **8a,d** at 10 µM.

Compound	X	% Inh ERK1/2	% Inh p38 MAPK	% Inh VEGFR	% Inh RAF-1/ MEK-1
<b>1</b>	O	6	-1	7	2
<b>2a</b>	O	-4	-1	-1	22
<b>2d</b>	NH	-37	4	-6	86
<b>4a</b>	O	-15	1	-1	4
<b>5a</b>	O	-7	21	0	96
<b>5b</b>	CO	21	20	0	96
<b>5c</b>	CH <sub>2</sub>	17	-7	17	99
<b>5d</b>	NH	-27	12	-3	74
<b>8a</b>	O	8	0	-15	35
<b>8d</b>	NH	12	12	1	38

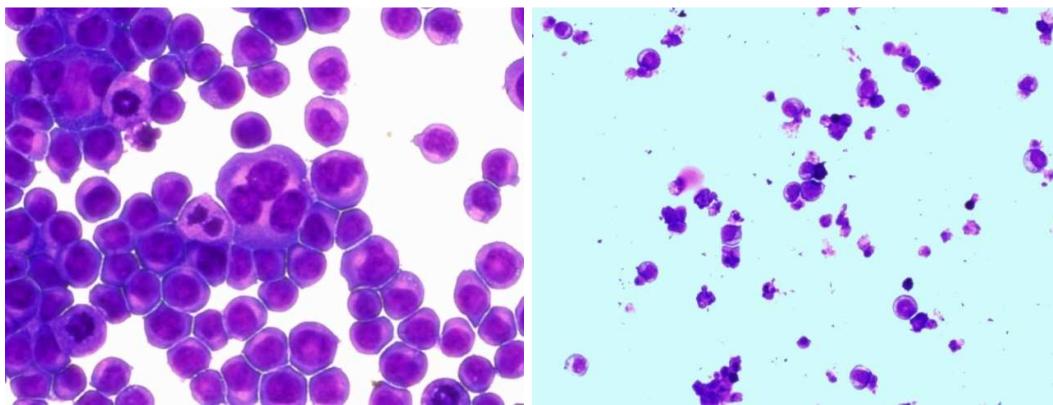
Note: Low to moderate negative values have no real meaning and are attributable to variability of the signal around the control level.

**Table 4.** E-refine docking scores (kcal mol<sup>-1</sup>) calculated for ligands in RAF-1 and in MEK-1 with and without ATP/Mg<sup>2+</sup>.

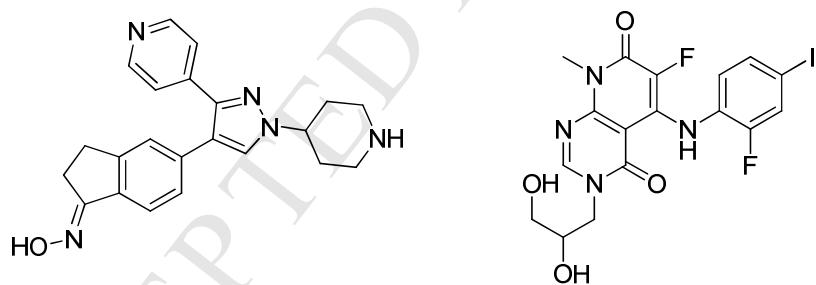
Ligand	Linker	RAF-1	MEK-1 with ATP/Mg <sup>2+</sup>	MEK-1 without ATP/Mg <sup>2+</sup>
<b>2a</b>	O	-22.1	-27.5	-27.9
<b>2b</b>	CO	-26.1	-27.8	-25.2
<b>2c</b>	CH <sub>2</sub>	-24.3	-26.3	-25.6
<b>2d</b>	NH	-24.7	-27.9	-24.9
<b>3a</b>	O	-25.1	-29.7	-27.3
<b>3b</b>	CO	-27.2	-29.3	-30.0
<b>3c</b>	CH <sub>2</sub>	-26.3	-30.7	-29.9
<b>4a</b>	O	-26.4	-28.7	-30.3
<b>4b</b>	CO	-29.3	-29.1	-28.4
<b>4c</b>	CH <sub>2</sub>	-27.2	-28.5	-29.1
<b>4d</b>	NH	-29.4	-28.4	-29.2
<b>6a</b>	O	-28.1	-29.0	-29.5
<b>6c</b>	CH <sub>2</sub>	-29.1	-30.0	-30.0
<b>5a</b>	O	-28.6	-32.6	-29.8
<b>5b</b>	CO	-28.2	-34.1	-33.0
<b>5c</b>	CH <sub>2</sub>	-29.2	-31.0	-30.4
<b>5d</b>	NH	-28.5	-34.3	-34.8
<b>7a</b>	O	-28.8	-34.6	-34.0
<b>7c</b>	CH <sub>2</sub>	-26.6	-31.8	-33.5
<b>8a</b>	O	-27.3	-28.8	-28.5
<b>8d</b>	NH	-26.6	-26.1	-28.4



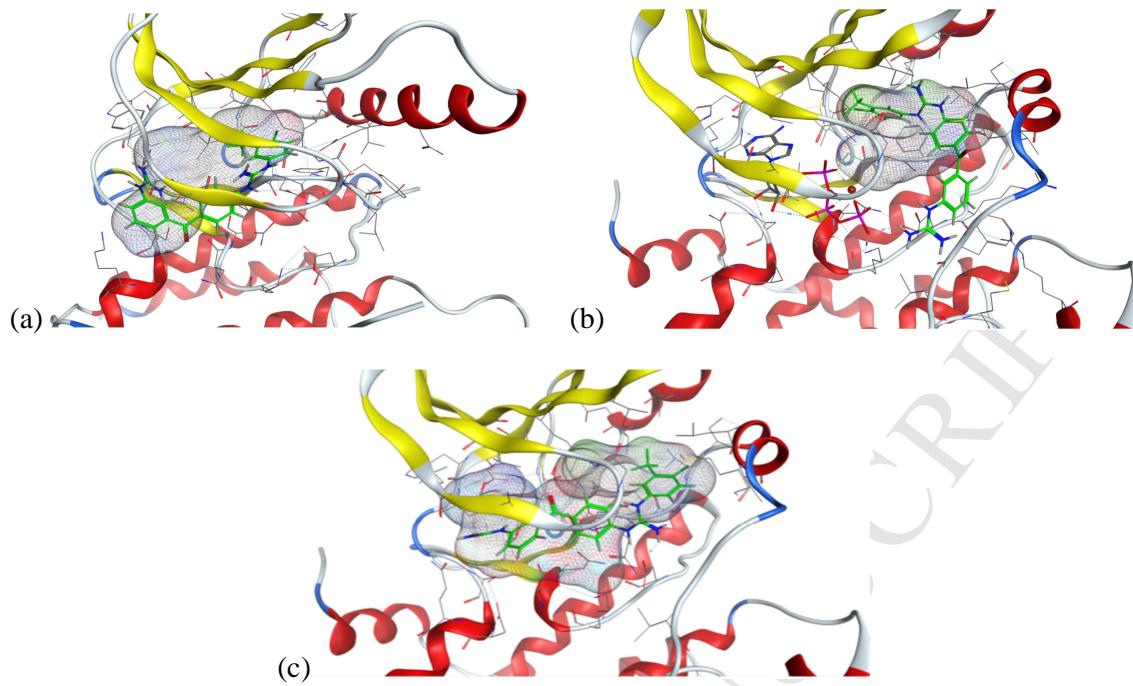
**Figure 1.** General approach to the different families of compounds synthesized.



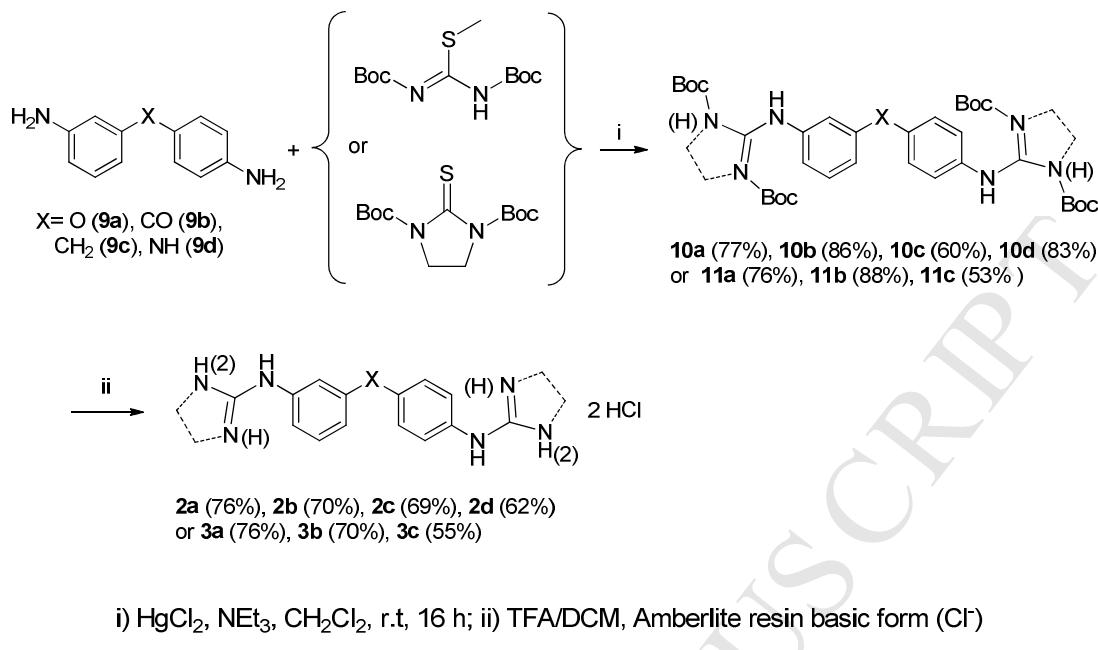
**Figure 2.** Cytospin images of vehicle control of HL-60 cell line (left) and cells treated with **7a** at 50  $\mu$ M for 72 h (right).



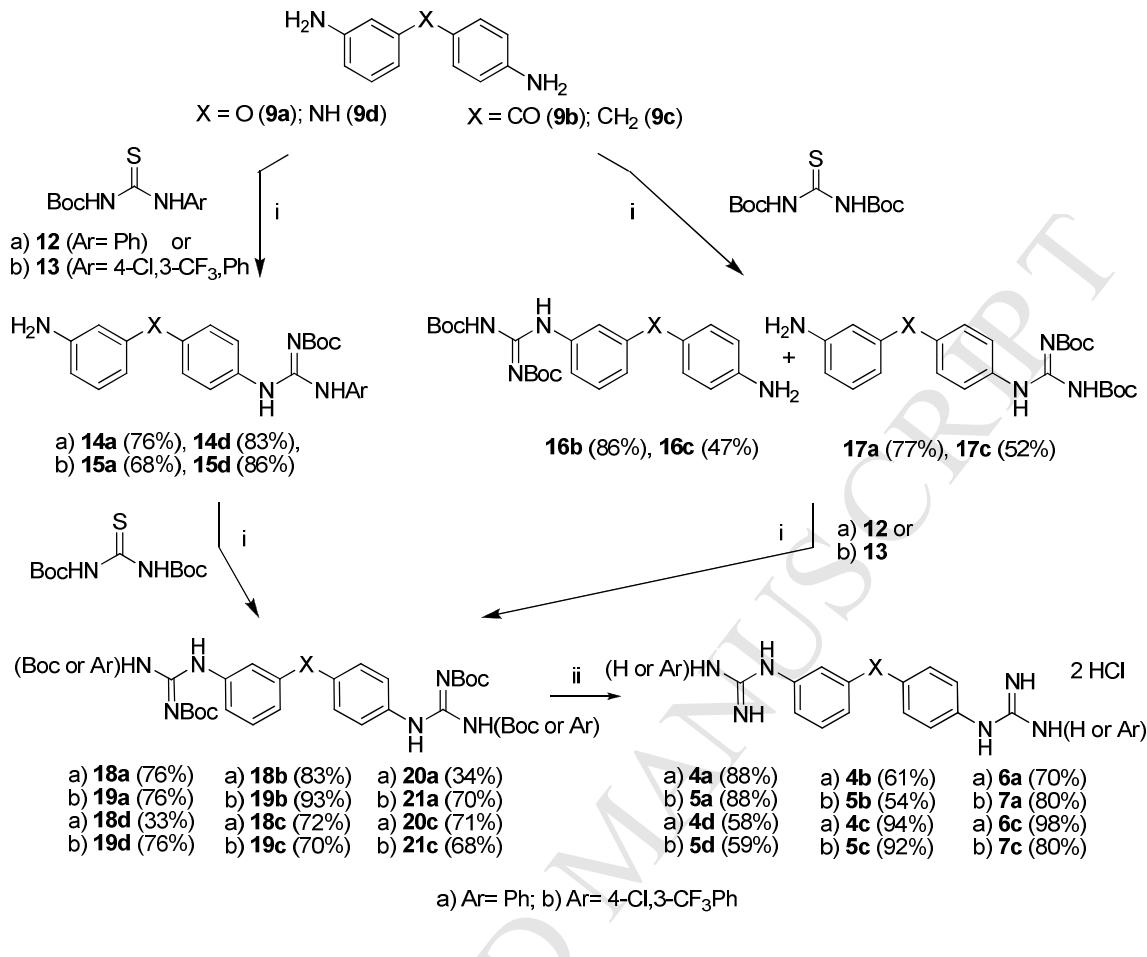
**Figure 3.** Ligands from 3OMV (SM5, *left*) and 3PP1 (IZG, *right*) crystal structures.



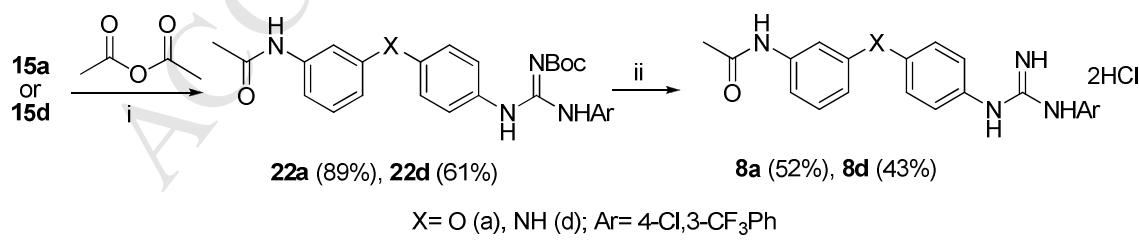
**Figure 4.** Top ranked pose of compound **5b** (green) in: (a) RAF-1, (b) MEK-1 with ATP/Mg<sup>2+</sup> and (c) MEK-1 without ATP/Mg<sup>2+</sup>. Compound **5b** is representative of what was observed for compounds **5a-d** and **7a-c**. Mesh surfaces indicate the binding site space occupied by the crystallographic ligands.



**Scheme 1.** Preparation of 3,4'-*bis*-guanidine (**2a-d**) and 3,4'-*bis*-(2-aminoimidazoline) (**3a-c**) derivatives, Families 1 and 2, respectively.



**Scheme 2.** Synthesis of aryl substituted *bis*-guanidines **4a-d**, **5a-d**, **6a,c** and **7a,c**.



i) NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, r.t, 6 h; ii) HCl/dioxane 4M, 1:1 IPA:CH<sub>2</sub>Cl<sub>2</sub>, 30 °C, 6 h.

**Scheme 3.** Synthesis of acetyl *mono*-guanidine derivatives **8a,d**.

**HIGHLIGHTS**

- New families of diaromatic 3,4'-(substituted)guanidinium derivatives were prepared
- Good cytotoxicity and apoptosis effects in HL-60 cancer cell line were observed
- Some compounds show high percentage inhibition on the RAF-1/MEK-1 pathway
- Docking studies on RAF-1 and MEK-1 structures suggest MEK-1 allosteric inhibition

## *Supporting Information*

### **Diaromatic guanidinium-like derivatives: synthesis, cytotoxicity and kinase inhibitory activity**

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## 1. General methods

### Method A: General procedure for the synthesis of the *bis*-Boc-protected guanidine and *bis* -Boc-protected 2-aminoimidazoline derivatives.

Mercury (II) chloride (2.2 eq.) was added over a 0.25 M solution of the corresponding diamine (1 eq.), 1,3-*bis*-(*tert*-butoxycarbonyl)-2-methylisothiourea (2.2 eq) in case of the guanidinium derivatives, or *N,N'*-di(*tert*-butoxycarbonyl)imidazolidine-2-thione (2.2 eq.) in case of the 2-aminoimidazolinium derivatives, and triethylamine (9 eq.) in DCM for guanidinium or DMF for 2-aminoimidazolinium derivatives at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then overnight at room temperature. Next, the reaction mixture was diluted with EtOAc (100 mL) and filtered through a pad of Celite® in order to remove the mercury sulfide precipitate formed. The filter cake was rinsed with EtOAc (2 × 25 mL). The organic phase was extracted with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over anhydrous MgSO<sub>4</sub>, and concentrated under vacuum to give a residue that was purified by flash chromatography on silica gel for the guanidines and on alumina for the 2-aminoimidazoline, as specified.

### Method B: General procedure for the synthesis of the *mono*-Boc-protected guanidine derivatives.

Mercury (II) chloride (1.2 eq.) was added over a 0.19 M solution of the corresponding diamine (3 eq.), the corresponding thiourea (1 eq.) and triethylamine (3.1 eq.) in DCM at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then at room temperature for further 2 - 4 hours or overnight depends on the reactivity of the substrates and possible generation of side products (reaction progress adjudged by TLC). Then, it was diluted with EtOAc (100 mL) and filtered through a pad of Celite® in order to remove the mercury sulfide precipitate formed. The filter cake was rinsed with EtOAc (2 × 25 mL). The organic phase was extracted

with water ( $2 \times 30$  mL), washed with brine ( $1 \times 30$  mL), dried over anhydrous MgSO<sub>4</sub>, and concentrated under vacuum to give a residue that was purified by flash chromatography on silica gel (eluting with a gradient of hexane:EtOAc) as specified.

**Method C: General procedure for the synthesis of the asymmetric 3,4'-Boc-protected guanidine derivatives.**

To a 0.193 M solution of the corresponding *mono*-Boc-protected guanidine (1 eq.), the corresponding thiourea (1.2 eq.) and triethylamine (3.1 eq.) in DCM and additional isopropanol in some cases to increase solubility, mercury (II) chloride (1.2 eq.) was added. The resulting mixture was stirred at room temperature for 3 - 6 hours depending on the reactivity of the substrates (reaction progress adjudged by TLC). Then, it was diluted with EtOAc (100 mL) and filtered through a pad of Celite® in order to remove the mercury sulfide precipitate formed. The filter cake was rinsed with EtOAc ( $2 \times 25$  mL). The organic phase was extracted with water ( $2 \times 30$  mL), washed with brine ( $1 \times 30$  mL), dried over anhydrous MgSO<sub>4</sub>, and concentrated under vacuum to give a residue that was purified by flash chromatography on silica gel (eluting with a gradient of hexane:EtOAc) as specified.

**Method D: Generic base mediated acetylation of the *mono*-Boc-protected guanidine derivatives.**

To a 0.2 M solution of the corresponding *mono*-Boc-protected guanidine derivative (1 eq.) and acetic anhydride (2 eq.) in DCM, triethylamine (3 eq.) was added. The reaction was stirred at room temperature for 6 hours. Afterwards, it was diluted with EtOAc, extracted with water ( $2 \times 30$  mL) and washed with brine ( $1 \times 30$  mL). The organic layer was dried over anhydrous MgSO<sub>4</sub> and concentrated under vacuum to give a residue that was purified by

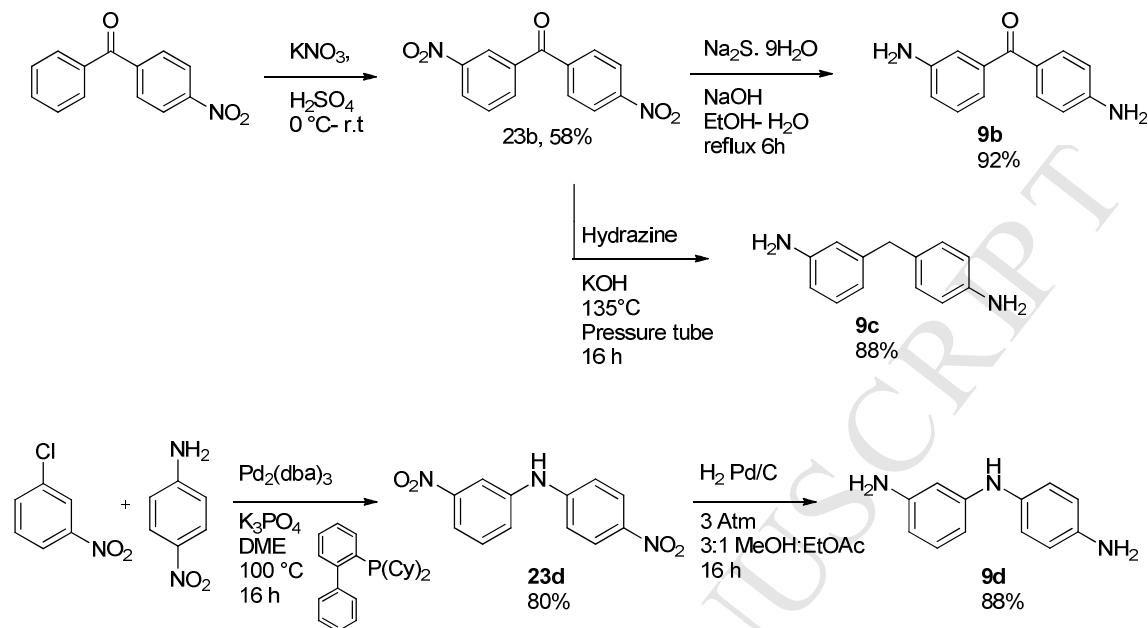
flash column chromatography on silica gel (eluting with a gradient of hexane:EtOAc) as specified.

## 2. Preparation of starting amines

Synthesis of 3-(4-aminobenzoyl)aniline (**9b**) was achieved by nitration of 4-nitrobenzophenone using  $H_2SO_4$  and  $KNO_3$  at 0 °C affording 3,4'-dinitrobenzophenone (**23b**). This was followed by reduction of the nitro groups using  $Na_2S \cdot 9H_2O$  (Scheme S1).

The synthesis of 3-(4-aminobenzyl)aniline (**9c**) was achieved, in an 88% yield, following a protocol developed in our laboratory. It consists of the simultaneous one-step reduction of the carbonyl and nitro groups of 3,4'-dinitrobenzophenone (**23b**) using hydrazine, KOH and a pressure tube.<sup>1</sup>

In the case of 3-(4-aminoanilino)aniline (**9d**), a Pd-catalysed C-N cross coupling reaction was performed and several conditions explored. Coupling of 1-chloro-3-nitrobenzene and 1,4-aminoaniline was carried out by using 3% of  $Pd_2(dbu)_3$ , (2-diphenyl)dicyclohexyl phosphine as a ligand,  $K_3PO_4$  and DME as a solvent. In that way, 3-nitro-*N*-(4-nitrophenyl)aniline (**23d**) was obtained in 80% yield. This was followed by the reduction of the nitro groups to amines using catalytic hydrogenation with  $H_2$  and Pd/C to generate **9d** in 88% yield (Scheme 1).

**Scheme S1.** Synthetic pathways for the preparation of the non-commercial starting amines.

### 3,4'-Dinitrobenzophenone (**23b**)

4-Nitrobenzophenone (2273 mg, 10.0 mmol) was added slowly over 15 mL of  $\text{H}_2\text{SO}_4$  at  $0^\circ\text{C}$ .  $\text{KNO}_3$  (1010 mg, 10.0 mmol) was then added to the suspension over 10 min maintaining a constant temperature. The mixture was stirred at  $0^\circ\text{C}$  for 30 min and a further 1.5 h at room temperature. Afterwards, the mixture was poured onto 50 g of ice and the precipitate that formed filtered, washed and dried until acid free and recrystallised from acetone to yield **23b** as a white solid (1576 mg, 58%). Mp: 162-164 °C. (lit. 162-167 °C).<sup>2</sup>  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ): 7.79 (t, 1H,  $J = 8.0$  Hz, Ar), 7.98 (d, 2H,  $J = 8.8$  Hz, Ar), 8.18 (dd, 1H,  $J = 8.0, 2.0$  Hz, Ar), 8.42 (d, 2H,  $J = 8.8$ , Ar), 8.54 (dd, 1H,  $J = 8.0, 2.0$  Hz, Ar), 8.57 (t, 1H,  $J = 2.0$  Hz, Ar).

### 3,4'-Diaminobenzophenone (**9b**)

An aqueous solution (50 mL) of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  (2800 mg, 12 mmol) and  $\text{NaOH}$  (1100 mg, 28 mmol) was added to a stirred suspension of **23b** (685 mg, 2.5 mmol) in ethanol (40 mL). The mixture was refluxed for 6 h and allowed to stand overnight. Ethanol was removed by rotary

evaporation and the resulting precipitate was collected and washed with water and dried to yield **1b** as an orange-yellow solid (493 mg, 92%). Mp: 122-124 °C. (lit. 123-124 °C).<sup>1</sup> δ<sub>H</sub> (400 MHz, DMSO): 5.30 (bs, 2H, NH<sub>2</sub>), 6.10 (bs, 2H, NH<sub>2</sub>), 6.60 (d, 2H, *J* = 8.5 Hz, Ar), 6.70 (d, 1H, *J* = 8.0 Hz, Ar), 6.73 (d, 1H, *J* = 8.0 Hz, Ar), 6.80 (s, 1H, Ar), 7.12 (t, 1H, *J* = 8.0 Hz, Ar), 7.51 (d, 2H, *J* = 8.5 Hz, Ar).

### **3-(4-Aminobenzyl)aniline (9c)**

A solution of **23b** (272 mg, 1.0 mmol) in 2 mL of distilled hydrazine hydrate was stirred for 3 h in a sealed pressure tube placed in an oil bath at 135 °C. The reaction was then allowed to reach room temperature and KOH (280 mg) was added. The mixture was allowed to react overnight at 135 °C. It was then extracted with a mixture 80:20 of DCM:IPA and washed with water (3 × 30 mL) and the organic solvent was evaporated to give a residue that was purified by silica gel column chromatography, eluting with hexane:EtOAc (1:1) to yield the desired product as a brown solid (239 mg, 88%). Mp: 87-89 °C. (lit. 89-90 °C).<sup>3</sup> δ<sub>H</sub> (600 MHz, DMSO): 3.58 (s, 2H, CH<sub>2</sub>), 4.85 (bs, 2H, NH<sub>2</sub>), 4.93 (bs, 2H, NH<sub>2</sub>), 6.34 (1H, *J* = 7.4 Hz, Ar), 6.36 (m, 2H, Ar), 6.49 (d, 2H, *J* = 8.0, Ar), 6.84 (d, 2H, *J* = 8.0 Hz, Ar), 6.89 (t, 1H, *J* = 7.4 Hz, Ar).

### **3-Nitro-N-(4-nitrophenyl)aniline (23d)**

An oven-dried Schlenk flask was evacuated and backfilled with argon. Afterwards, it was charged with Pd<sub>2</sub>(dba)<sub>3</sub> (27.5 mg, 3.0 mol %), (2-diphenyl)dicyclohexylphosphine (10.5 mg, 3.0 mol %) and 1,2-dimethoxyethane (DME) (2 mL/mmol of halide). The mixture was left to react for 5 min without heating. Then, K<sub>3</sub>PO<sub>4</sub> (297.2 mg, 1.4 mmol), 1-chloro-3-nitrobenzene (157.5 mg, 1.0 mmol) and 1,4-aminoaniline (138.12 mg, 1.0 mmol) were added as solids maintaining the flow of argon. The mixture was stirred at 100 °C for 24 h. It was then cooled

to room temperature, diluted with 1:1 ether:EtOAc (40 mL), filtered through celite, and concentrated under vacuum. The crude material was purified by chromatography on silica gel using 1:1 (EtOAc:hexane) solvent system to yield an orange solid **23d** (207 mg, 80%). Mp: 217-218 °C. (lit. 214-218 °C).<sup>4</sup> δ<sub>H</sub> (600 MHz, DMSO): 7.23 (d, 2H, *J* = 8.6 Hz, Ar), 7.65 (t, 1H, *J* = 8.0 Hz, Ar), 7.7 (d, 1H, *J* = 8.0 Hz, Ar), 7.85 (d, 1H, *J* = 8.0 Hz, Ar), 8.00 (s, 1H, Ar), 8.17 (d, 2H, *J* = 8.6 Hz, Ar), 9.67 (bs, 1H, NH).

### **3-(4-Aminoanilino)aniline (9d)**

To a hydrogenator flask, **23d** (200 mg, 0.77 mmol), 10% Pd/C mixture (40 mg, 0.04 mmol, 5 mol %) and 2:1 EtOH:EtOAc (20:10 mL) were added. The mixture was vigorously stirred at room temperature under 3 atm of H<sub>2</sub> pressure overnight. The reaction mixture was filtered and the solvent was removed under vacuum to afford **9d** (135 mg, 88% yield) as a brown-purple solid. Mp: 99 °C. (lit. 97 °C).<sup>5</sup> δ<sub>H</sub> (600 MHz, DMSO): 4.71 (bs, 2H, NH<sub>2</sub>), 4.76 (bs, 2H, NH<sub>2</sub>), 5.87 (dd, 1H, *J* = 7.9, 2.0 Hz, Ar), 5.98 (dd, 1H, *J* = 7.9, 2.0 Hz, Ar), 6.05 (t, 1H, *J* = 2.0, Ar), 6.50 (d, 2H, *J* = 8.6 Hz, Ar), 6.74 (t, 1H, *J* = 7.9 Hz, Ar), 6.79 (d, 2H, *J* = 8.6 Hz, Ar), 7.13 (bs, 1H, NH).

### 3. Preparation of diaromatic 3,4'-*bis*-2,3-di(*tert*-butoxycarbonyl)guanidino derivatives

#### 3,4'-*Bis*[2,3-di(*tert*-butoxycarbonyl)guanidino]diphenylether (**10a**)

Following method A, using **9a** (455 mg, 0.66 mmol) as starting material and after purification with silica gel chromatography, eluting with a gradient of hexane:EtOAc, **10a** was obtained as a white solid (397 mg, 87%). Mp: 120-121 °C.  $\delta_{\text{H}}$  (400 MHz, CDCl<sub>3</sub>): 1.51 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.55 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 6.75 (d, 1H, *J* = 8.3 Hz, Ar), 7.03 (d, 2H, *J* = 9.0 Hz, Ar), 7.27 (t, 1H, *J* = 8.3 Hz, Ar), 7.30 (app. d, 1H, *J* = 8.3 Hz, Ar), 7.32 (s, 1H, Ar), 7.60 (d, 2H, *J* = 9.0 Hz, Ar), 10.3 (bs, 2H, NHBoc), 11.6 (bs, 1H, NH), 11.7 (bs, 1H, NH).  $\delta_{\text{C}}$  (100 MHz, CDCl<sub>3</sub>): 26.9 (2(CH<sub>3</sub>)<sub>3</sub>), 28.1 (2(CH<sub>3</sub>)<sub>3</sub>), 79.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 79.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.7 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.8 (q, C(CH<sub>3</sub>)<sub>3</sub>), 112.4 (CH, Ar), 114.5 (CH, Ar), 116.8 (CH, Ar), 119.8 (2 CH, Ar), 123.6 (2 CH, Ar), 129.7 (CH Ar), 132.4 (q, Ar), 138.1 (q), 153.2 (q), 153.3 (q), 153.4 (2q), 153.5 (q), 153.6 (q), 150.7 (q), 158.0 (q), 163.6 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3260 (NH), 3160 (NH), 2979, 2933, 1718 (CO), 1628 (CN), 1598, 1567, 1506, 1478, 1450, 1407, 1367, 1336, 1306, 1290, 1233, 1213, 1143, 1106, 1055, 1027, 879, 830, 804, 769, 686. HRMS (*m/z* - ES): 685.3560 (M<sup>+</sup> + H)

#### 3,4'-*Bis*[2,3-di(*tert*-butoxycarbonyl)guanidino]benzophenone (**10b**)

Following method A, using **9b** (696 mg, 1.0 mmol) as starting material and after purification with silica gel chromatography, eluting with a gradient of hexane:EtOAc, **10b** was obtained as a pale orange solid (418 mg, 60%). Mp: 184-185 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.50 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.54 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.55 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 7.45 (t, 1H, *J* = 8.0 Hz, Ar), 7.53 (d, 1H, *J* = 8.0 Hz, Ar), 7.79 (d, 2H, *J* = 8.5 Hz, Ar), 7.85 (d, 2H, *J* = 8.5 Hz, Ar), 7.87 (s, 1H, Ar), 7.96 (d, 1H, *J* = 8.0 Hz, Ar), 10.45 (bs, 1H, NHBoc), 10.64 (bs, 1H, NHBoc), 11.66 (bs, 1H, NH), 11.68 (bs, 1H, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 28.1

((CH<sub>3</sub>)<sub>3</sub>), 28.1 ((CH<sub>3</sub>)<sub>3</sub>), 28.2 ((CH<sub>3</sub>)<sub>3</sub>), 79.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 79.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.7 (q, C(CH<sub>3</sub>)<sub>3</sub>), 121.1 (2 CH, Ar), 123.6 (CH, Ar), 128.2 (CH, Ar), 128.3 (CH, Ar), 128.7 (CH, Ar), 131.4 (2 CH, Ar), 130.8 (q), 133.0 (q), 138.5 (q), 140.9 (q), 153.2 (q), 153.3 (q), 153.7 (q), 153.8 (q), 163.2 (q), 163.3 (q), 194.9 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3259 (NH), 3135 (NH), 2979, 2933, 1719 (CO), 1633 (CN), 1599, 1552, 1407, 1368, 1336, 1297, 1230, 1148, 1099, 1056, 1028, 941, 881, 845, 805, 757. HRMS (*m/z* - ES): 697.3564 (M<sup>+</sup> + H).

### **3,4'-Bis[2,3-di(*tert*-butoxycarbonyl)guanidino]benzylbenzene (10c)**

Following method A, using **9c** (1.03 g, 0.0015 mol) as a starting material and after purification with silica gel chromatography, eluting with a gradient of hexane:EtOAc, **10c** was obtained as a white solid (885 mg, 86%). Mp: 160-162 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.53 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.55 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.57 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.94 (s, 2H, Ar), 6.93 (d, 1H, *J* = 8.0 Hz, Ar), 7.16 (d, 2H, *J* = 8.3 Hz, Ar), 7.26 (t, 1H, *J* = 8.0 Hz, Ar), 7.32 (s, H, Ar), 7.53 (d, 2H, *J* = 8.3 Hz, Ar), 7.58 (d, 1H, *J* = 8.0 Hz, Ar), 10.3 (bs, 2H, NHBoc), 11.6 (bs, 2H, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 (2(CH<sub>3</sub>)<sub>3</sub>), 28.1 (2(CH<sub>3</sub>)<sub>3</sub>), 41.1 (CH<sub>2</sub>), 79.4 (q, 2 C(CH<sub>3</sub>)<sub>3</sub>), 83.4 (q, 2 C(CH<sub>3</sub>)<sub>3</sub>), 120.1 (CH, Ar), 122.1 (2 CH, Ar), 122.5 (CH, Ar), 125.3 (CH Ar), 128.8 (CH, Ar), 129.2 (2 CH, Ar), 134.8 (q), 136.7 (q), 137.1 (q), 141.6 (q), 153.2 (q), 153.2 (q), 153.3 (q), 153.4 (q), 163.4 (q), 163.4 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3287 (NH), 3188 (NH), 2980, 2933, 1796 (CO), 1628 (CN), 1600, 1560, 1477, 1454, 1407, 1367, 1338, 1300, 1232, 1145, 1106, 1056, 1028, 804, 766, 693. HRMS (*m/z* - ES): 683.3762 (M<sup>+</sup> + H).

### **3,4'-Bis[2,3-di(*tert*-butoxycarbonyl)guanidino]-*N*-phenylaniline (10d)**

Following method A, using **9d** (62 mg, 0.30 mmol) as starting material and after purification with silica gel chromatography, eluting with a gradient of hexane:EtOAc, **10d** was obtained

as a pale solid (170 mg, 83%). Mp: 117-120 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.54 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.57 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 5.78 (bs, 1H, NH linker), 6.80 (d, 1H, *J* = 8.0 Hz, Ar), 7.01 (d, 1H, *J* = 8.0 Hz, Ar), 7.09 (d, 2H, *J* = 8.6 Hz, Ar), 7.20 (t, 1H, *J* = 8.0 Hz, Ar), 7.45 (s, 1H, Ar), 7.51 (d, 2H, *J* = 8.6 Hz, Ar), 10.2 (bs, 1H, NHBoc), 10.3 (bs, 1H, NHBoc), 11.6 (bs, 1H, NH), 11.7 (bs, 1H, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 ((CH<sub>3</sub>)<sub>3</sub>), 27.9 ((CH<sub>3</sub>)<sub>3</sub>), 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 28.1 ((CH<sub>3</sub>)<sub>3</sub>), 79.3 (q, C(CH<sub>3</sub>)<sub>3</sub>), 79.4 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.4 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 110.7 (CH, Ar), 113.1 (CH, Ar), 114.1 (CH, Ar), 119.0 (CH, Ar), 123.4 (CH, Ar), 129.4 (CH, Ar), 130.3 (q), 137.6 (q), 139.4 (q), 143.8 (q), 153.1 (q), 153.2 (q), 153.3 (q), 153.4 (q), 163.4 (q), 163.5 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3283 (NH), 1720 (CO), 1625 (CN), 1587, 1570, 1500, 1510, 1473, 1336, 1288, 1211, 1140, 1117, 1055, 879, 823, 767, 685. HRMS (*m/z* - ES): 684.3719 (M<sup>+</sup> + H).

#### 4. Preparation of diaromatic 3,4'-bis[2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidino] derivatives

##### **3,4'-Bis[2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]diphenylether (11a)**

Following method A, **9a** (113 mg, 0.565 mmol) and after purification with neutral alumina chromatography, eluting with a gradient of hexane:EtOAc, **11a** was obtained as a white solid (283 mg, 68%). Mp: 88-89 °C.  $\delta_H$  (400 MHz, CDCl<sub>3</sub>): 1.37 (s, 18H, 2(CH<sub>3</sub>)<sub>3</sub>), 1.38 (s, 18H, 2(CH<sub>3</sub>)<sub>3</sub>), 3.81 (s, 4H, 2 CH<sub>2</sub>), 3.84 (s, 4H, 2 CH<sub>2</sub>), 6.52 (dd, 1H, *J* = 8.3, 2.0 Hz, Ar), 6.60 (t, 1H, *J* = 2 Hz, Ar), 6.72 (dd, 1H, *J* = 8.3, 2.0 Hz, Ar), 6.91 (d, 2H, *J* = 8.7 Hz, Ar), 6.98 (d, 2H, *J* = 8.7 Hz, Ar), 7.13 (t, 1H, *J* = 8.3 Hz, Ar).  $\delta_C$  (100 MHz, CDCl<sub>3</sub>): 27.9 (2(CH<sub>3</sub>)<sub>3</sub>), 28.0 (2(CH<sub>3</sub>)<sub>3</sub>), 43.1 (2 CH<sub>2</sub>), 43.2 (2 CH<sub>2</sub>), 82.8 (q, 2 C(CH<sub>3</sub>)<sub>3</sub>), 82.9 (q, 2 C(CH<sub>3</sub>)<sub>3</sub>), 110.9 (CH, Ar), 111.8 (CH, Ar), 116.3 (CH, Ar), 120.1 (CH, Ar), 122.6 (CH, Ar), 129.5 (CH, Ar), 139.2 (q), 139.5 (q), 144.2 (q), 149.8 (q), 150.2 (q), 150.3 (q), 150.3 (q), 150.7 (q), 152.1 (q), 158.8 (q).  $\nu_{max}$  (ATR)/cm<sup>-1</sup>: 2977, 1756 (CO), 1700 (CN), 1594, 1499, 1477, 1366, 1298, 1248, 1217, 1144, 976, 848, 766, 699. HRMS (*m/z* - ES): 736.3802 (M<sup>+</sup> + H).

##### **3,4'-Bis[2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]benzophenone (11b)**

Following method A, using **9b** (212 mg, 1.0 mmol) as starting material and after purification with neutral alumina chromatography, eluting with a gradient of hexane: EtOAc **11b** was obtained as a pale orange solid (397 mg, 53%). Mp: 84-85 °C.  $\delta_H$  (600 MHz, CDCl<sub>3</sub>): 1.29 (s, 18H, (CH<sub>3</sub>)<sub>3</sub>), 1.30 (s, 18H, (CH<sub>3</sub>)<sub>3</sub>), 3.76 (s, 4H, 2 CH<sub>2</sub>), 3.80 (s, 4H, 2 CH<sub>2</sub>), 6.95 (d, 2H, *J* = 8.5 Hz, Ar), 7.15 (d, 1H, *J* = 7.5 Hz, Ar), 7.22 (s, 1H, Ar), 7.26 (m, 1H, Ar), 7.28 (t, 1H, *J* = 7.5 Hz, Ar), 7.69 (d, 2H, *J* = 8.5 Hz, Ar).  $\delta_C$  (150 MHz, CDCl<sub>3</sub>): 27.7 (4(CH<sub>3</sub>)<sub>3</sub>), 43.0 (2 CH<sub>2</sub>), 43.1 (2 CH<sub>2</sub>), 82.6 (q, 2 C(CH<sub>3</sub>)<sub>3</sub>), 82.9 (q, 2 C(CH<sub>3</sub>)<sub>3</sub>), 120.6 (2 CH, Ar), 121.6 (CH, Ar), 123.5 (CH, Ar), 125.4 (CH, Ar), 128.2 (CH, Ar), 131.2 (q), 131.3 (2 CH, Ar), 138.6 (q), 139.8 (q), 139.9 (q), 148.4 (q), 149.7 (q), 150.1 (q), 152.7 (q), 196.9 (q).  $\nu_{max}$  (ATR)/cm<sup>-1</sup>:

2980, 2932, 1757, 1702 (CO), 1648 (CN), 1588, 1477, 1457, 1366, 1291, 1251, 1144, 974, 848, 766. HRMS (*m/z* - ES): 749.3871 (M<sup>+</sup> + H).

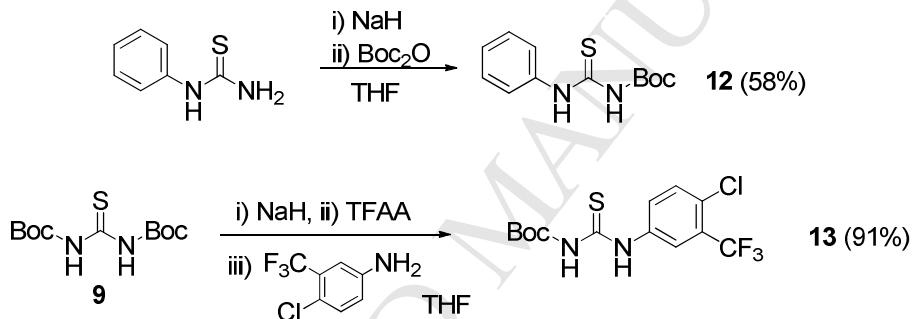
**3,4'-Bis[2,3-di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]benzylbenzene (11c)**

Following method A, using **9c** (1105 mg, 1.5 mmol) as starting material and after purification with neutral alumina chromatography, eluting with a gradient of hexane: EtOAc, **11c** was obtained as a white solid (973 mg, 88%). Mp: 90-92 °C. δ<sub>H</sub> (600 MHz, CDCl<sub>3</sub>): 1.33 (s, 36H, 4(CH<sub>3</sub>)<sub>3</sub>), 3.83 (s, 8H, 4 CH<sub>2</sub>), 3.84 (s, 2H, Ar), 6.73 (d, 1H, *J* = 7.8 Hz, Ar), 6.82 (d, 1H, *J* = 7.8 Hz, Ar), 6.83 (s, 1H, Ar), 6.91 (d, 2H, *J* = 8.3 Hz, Ar), 7.06 (d, 2H, *J* = 8.3 Hz, Ar), 7.09 (t, 1H, *J* = 8.3 Hz, Ar). δ<sub>C</sub> (150 MHz, CDCl<sub>3</sub>): 27.7 (4(CH<sub>3</sub>)<sub>3</sub>), 41.2(CH<sub>2</sub> linker), 42.9 (4 CH<sub>2</sub>), 82.5 (q, 2 C(CH<sub>3</sub>)<sub>3</sub>), 82.6 (q, 2 C(CH<sub>3</sub>)<sub>3</sub>), 118.9 (CH, Ar), 121.2 (CH, Ar), 122.9 (CH, Ar), 123.2 (CH, Ar), 128.4 (CH, Ar), 129.2 (CH, Ar), 135.2(q), 138.6 (q), 138.7 (q), 141.8 (q), 146.(q), 148.1 (q), 150.2 (q), 150.3 (q). ν<sub>max</sub> (film)/cm<sup>-1</sup>: 2977, 2929, 1754 (CO), 1695 (CN), 1596, 1477, 1457, 1366, 1297, 1247, 1143, 974, 848, 765, 695. HRMS (*m/z* Maldi): 757.3864 (M<sup>+</sup> + Na).

## 5. Preparation of substituted thioureas

The synthesis of **12** (Scheme S2) involves the deprotonation of the phenylthiourea with sodium hydride (NaH), followed by reaction with di-*tert*-butyl dicarbonate (Boc<sub>2</sub>O) in tetrahydrofuran (THF) to obtain the desired product in 58% yield. For the synthesis of **13** (Scheme S2), *N,N'*-di-Boc-protected thiourea was deprotonated by NaH and then reacted with trifluoroacetic anhydride (TFAA) in THF, followed by the nucleophilic attack of the 4-chloro-3-(trifluoromethyl)aniline, yielding the product in 91%.

**Scheme S2.** Preparation of aryl substituted thioureas **12** and **13**.



### *N*-*tert*-Butoxycarbonyl-*N'*-phenylthiourea (**12**)

To a solution of *N*-phenylthiourea (500 mg, 3.29 mmol) in dry tetrahydrofuran (25 mL) under argon at 0 °C, sodium hydride as a 60% suspension in mineral oil (290 mg, 7.24 mmol) were added. The reaction was stirred at 0 °C for 1 h and then 3 h at room temperature to complete formation of the anion. The reaction is cooled again to 0 °C prior to addition of di-*tert*-butyl dicarbonate (862 g, 3.95 mmol). The reaction was stirred for 1 h at 0 °C and then it was brought to room temperature and stirred overnight. The reaction was cooled again to 0 °C and dropwise H<sub>2</sub>O (20 mL) was added to quench the reaction, followed by extraction with EtOAc (3 × 20 mL). The combined organic phases were washed with 80% brine (30 mL) and dried using anhydrous MgSO<sub>4</sub>, followed by removal of solvents under vacuum. Purification

proceeded by silica gel chromatography using gradient elution of hexane:EtOAc, followed recrystallisation from boiling hexane to afford the product **12** as white needles (483 mg, 58%). Mp: 104 °C. (lit. 106 °C).<sup>6</sup> δ<sub>H</sub> (600 MHz, CDCl<sub>3</sub>): 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 7.28 (t, 1H, *J* = 7.92 Hz, Ar), 7.42 (t, 2H, *J* = 7.92 Hz, Ar), 7.66 (d, 2H, *J* = 7.92 Hz, Ar), 7.96 (bs, 1H, NHPh), 11.52 (bs, 1H, NHBoc). HRMS (*m/z* - ES): 275.0830 (M<sup>+</sup> + Na)

### **N-(4-Chloro-3-trifluoromethylphenyl)-N'-(*tert*-butoxycarbonyl)thiourea (13)**

To a solution of *N,N'*-bis(*tert*-butoxycarbonyl)thiourea (2 g, 7.25 mmol) in dry tetrahydrofuran (58.5 mL) under argon at 0 °C, 1.5 equivalents of sodium hydride as a 60% suspension in mineral oil (435 mg, 10.9 mmol) were added. The reaction mixture was stirred at the same temperature for 1 h and 20 min, then 1.1 equivalents of trifluoroacetic anhydride (1.11 mL, 8.0 mmol), and the stirring continue for an additional 30 min. Then, 1.15 equivalents of the 4-Chloro-3-(trifluoromethyl)aniline (1.63 g, 8.34 mmol) were added and the reaction was stirred at 0 °C for 5 h. The reaction was cooled again to 0 °C and dropwise H<sub>2</sub>O (20 mL) was added to quench the reaction, followed by extraction with EtOAc (3 × 20 mL). The combined organic phases were washed with 80% brine (30 mL) and dried using anhydrous MgSO<sub>4</sub>, followed by removal of solvents under vacuum. Purification proceeded by silica gel chromatography using gradient elution (hexane:EtOAc), followed by removal of solvents under vacuum. Recrystallisation from boiling hexane afforded the product as white needles (1680 mg, 92%). Mp: 140-142 °C. δ<sub>H</sub> (600 MHz, CDCl<sub>3</sub>): 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 7.53 (d, 1H, *J* = 8.5 Hz, Ar), 7.94 (d, 1H, *J* = 8.5 Hz, Ar), 7.99 (bs, 1H, NHPh), 8.02 (s, 1H, Ar), 11.7 (bs, 1H, NHBoc). δ<sub>C</sub> (150 MHz, CDCl<sub>3</sub>): 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 84.9 (q, CCH<sub>3</sub>), 123.2 (c, *J* = 5.2 Hz, CH, Ar), 123.3 (c, q, *J* = 274 Hz, CF<sub>3</sub>), 128.2 (CH, Ar), 128.8 (d, q, *J* = 30 Hz), 129.5 (q), 131.7 (CH, Ar), 136.5 (q), 152.0 (q), 178.0 (q). v<sub>max</sub> (ATR)/cm<sup>-1</sup>: 3172, 2979,

2900, 1727, 1710, 1589, 1527 (CO), 1478, 1425, 1324, 1258, 1239, 1128 (CS), 1113, 1033, 1015, 972, 838, 820, 775, 691, 663. HRMS (*m/z* - ES): 353.0332 (M<sup>-</sup> - H).

## 6. Preparation of diaromatic 3-amino-4'-[2-(*tert*-butoxycarbonyl)-3-arylguanidino] derivatives

Due to the presence of E/Z isomerism and rotamers, the NMR spectra of compounds of this type were amorphous and poorly resolved making full assignments of peaks impossible. This phenomenon was studied by low temperature NMR and theoretical means.<sup>6</sup> After removal of the Boc groups this is no longer an issue.

### **3-Amino-4'-[2-(*tert*-butoxycarbonyl)-3-phenylguanidino]diphenylether (14a).**

Following Method B, HgCl<sub>2</sub> (407 mg, 1.5 mmol) was added over a solution of **9a** (751 mg, 3.75 mmol), **12** (315 mg, 1.25 mmol) and triethylamine (539 μL, 3.87 mmol) in DCM (6.5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and further 3 h at room temperature. Usual work-up, followed by purification of the *meta* and *para* mono-guanidylated products by silica gel chromatography eluting with a gradient of 100% hexane to 80:20 hexane:EtOAc respectively to afford the title product as a light brown hygroscopic solid (395 mg, 76%). Mp: 70-72 °C. δ<sub>H</sub> (600 MHz, CDCl<sub>3</sub>): 1.51 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.70 (bs, 2H, NH<sub>2</sub>), 6.32 (Ar), 6.36-6.50 (m, Ar), 6.72 (bs, NH), 6.84-7.20 (m, Ar), 7.30-7.41 (m, Ar), 7.70 (Ar), 9.56 (bs, NH). δ<sub>C</sub> (150 MHz, CDCl<sub>3</sub>): 28.1 ((CH<sub>3</sub>)<sub>3</sub>), 83.2 (q, C(CH<sub>3</sub>)<sub>3</sub>), 104.8 (CH, Ar), 108.2 (CH, Ar), 109.7 (CH, Ar), 120.0 (CH, Ar), 120.9 (CH, Ar), 121.4 (CH, Ar), 122.6 (CH, Ar), 123.6 (CH, Ar), 128.8 (CH, Ar), 129.6 (CH, Ar), 130.3 (CH, Ar), 131 – 150 (Quaternary signals in this range could not be assigned as they were highly amorphous and there was not enough resolution), 140.3 (q),

147.9 (q), 152.9 (q), 159.2 (q).  $\nu_{\text{max}}$  (film)/cm<sup>-1</sup>: 3339, 2976, 2928, 1719, 1660, 1590, 1551, 1487, 1460, 1388, 1366, 1327, 1284, 1207, 1143, 1089, 960, 837, 804, 750, 687. HRMS (*m/z* - ES): 419.2071 (M<sup>+</sup> + H).

### **3-Amino-4’-[2-(*tert*-butoxycarbonyl)-3-phenylguanidino]-N-phenylaniline (14d)**

Following Method B, HgCl<sub>2</sub> (308 mg, 1.13 mmol) was added over a solution of **9d** (564 mg, 2.83 mmol), **12** (238 mg, 0.944 mmol) and triethylamine (407 μL, 2.93 mmol) in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and further 3 h at room temperature. Usual work-up, followed by purification using silica gel chromatography eluting with a gradient of hexane:EtOAc to afford the title product as a light dark solid (328 mg, 83%). Mp: 69-71 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.53 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.74 (bs, 2H, NH<sub>2</sub>), 5.71 (bs, NH), 6.26 (d, 1H, *J* = 7.9 Hz, Ar), 6.36 (t, 1H, *J* = 1.98, Ar), 6.43 (d, 1H, *J* = 7.9 Hz, Ar), 6.59-6.67 (m, Ar), 6.98-7.36 (m, Ar and NH), 9.52 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 28.2 ((CH<sub>3</sub>)<sub>3</sub>), 82.9 (q, C(CH<sub>3</sub>)<sub>3</sub>), 103.6 (CH, Ar), 108.0 (CH, Ar), 116.1 (CH, Ar), 118.3 – 127.2 (Aromatic signals in this range could not be assigned as they were broad and highly amorphous), 129.3 (CH, Ar), 130.2 (CH, Ar), 133.3 – 163.7 (Quaternary signals in this range could not be assigned as they were broad and highly amorphous), 147.7 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3341 (NH), 3320 (NH), 2977, 2930, 1981, 1719, 1589 (CO), 1509, 1494, 1366, 1336, 1289, 1237, 1151, 1090, 1044, 966, 901, 828, 803, 750, 690. HRMS (*m/z* - ES): 418.2255 (M<sup>+</sup> + H).

### **3-Amino-4’-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethyl)guanidino]**

#### **diphenylether (15a)**

Following Method B, HgCl<sub>2</sub> (92 mg, 0.34 mmol) was added over a solution of **9a** (170 mg, 0.85 mmol), **13** (100 mg, 0.282 mmol) and triethylamine (121.7 μL, 0.874 mmol) in

DCM (1.5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and further 2 h at room temperature. Usual work-up, followed by purification by silica gel chromatography eluting with a gradient of 100% hexane to 80:20 hexane:EtOAc respectively to afford the title product as a light dark solid (100 mg, 68%). Mp: 48-49 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.71 (bs, 2H, NH<sub>2</sub>), 6.29-6.48 (m, 3H, Ar), 6.69-7.70 (m, 7H, Ar), 6.76 (bs, 1H, NH), 7.90-8.11 (m, 1H, Ar), 9.47-9.94 (m, 1H, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 28.1 ((CH<sub>3</sub>)<sub>3</sub>), 83.8 (q, C(CH<sub>3</sub>)<sub>3</sub>), 105.0 (CH, Ar), 108.4 (CH, Ar), 109.9 (CH, Ar), 118.6 (CH, Ar), 119.9 (CH, Ar), 120.9 (CH, Ar), 121.9 (CH, Ar), 123.3 (CH, Ar), 123.5 (CH, Ar), 126.7 (CH, Ar), 130.5 (2 CH, Ar), 131.6 (CH, Ar), (132.4 – 145.6 (Quaternary signals in this range could not be assigned as they were broad and highly amorphous), 147.9 (q), 153.1 (q).  $\nu_{\text{max}}$  (film)/cm<sup>-1</sup>: 3357 (NH), 3202 (NH), 2980, 2934, 1718 (CO), 1657 (CN), 1594, 1480, 1461, 1410, 1317, 1207, 1138, 1030, 991, 960, 834, 771, 685. HRMS (*m/z* - ES): 521.1560 (M<sup>+</sup> + H).

### **3-Amino-4’-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenylguanidino]-N-phenylaniline (15d)**

Following Method B, HgCl<sub>2</sub> (220 mg, 0.81 mmol) was added over a solution of **9d** (404 mg, 2.03 mmol), **13** (239 mg, 0.676 mmol) and triethylamine (291.7 μL, 2.10 mmol) in DCM (3.5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and further 3 h at room temperature. Usual work-up, followed by purification using silica gel chromatography eluting with a gradient of hexane:EtOAc to afford the title product as a dark solid (263 mg, 86%). Mp: 78-80 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.72 (bs, 2H, NH<sub>2</sub>), 5.44-5.73 (bs, NH), 6.27 (d, 1H, *J* = 7.2 Hz, Ar), 6.39 (s, 1H, Ar), 6.43 (d, 1H, *J* = 7.2 Hz, Ar), 6.55-6.73 (m, 1H, Ar), 6.76-7.47 (m, 9H, Ar and 1NH), 7.89-8.13 (m, 1H, Ar), 9.40-10.00 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 83.5

(q,  $\underline{C}(\text{CH}_3)_3$ , 103.2 (CH, Ar), 107.6 (CH, Ar), 108.1 (CH, Ar), 116.0 (CH, Ar), 120.7 (CH, Ar), 121.8 (c, q,  $J = 274$  Hz,  $\text{CF}_3$ ) 123.0 (CH, Ar), 123.6 (CH, Ar), 124.6 (d, q,  $J = 30$  Hz), 126.6 (CH, Ar), 130.2 (CH, Ar), 131.5 (CH, Ar), 132.2 (CH, Ar), 134.4 – 160.0 (Quaternary signals in this range could not be assigned as they were broad and highly amorphous), 147.5 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3340 (NH), 3333 (NH), 2978, 2933, 2162, 1720, 1591 (CO), 1556, 1509, 1479, 1368, 1318, 1240, 1135, 1111, 1080, 1031, 991, 831, 771, 688, 666. HRMS (*m/z* - ES): 520.1727 ( $\text{M}^+ + \text{H}$ ).

## 7. Preparation of diaromatic 4-amino-{3'-[2,3-di(*tert*-butoxycarbonyl)]guanidino} derivatives

### **4-Amino-{3'-[2,3-di(*tert*-butoxycarbonyl)]guanidine}benzophenone (16b)**

Following Method B,  $\text{HgCl}_2$  (231 mg, 0.85 mmol) was added over a solution of **9b** (451 mg, 2.13 mmol), *N,N'*-bis(*tert*-butoxycarbonyl)-S-methylisothiourea (184 mg, 0.71 mmol) and triethylamine (306.5  $\mu\text{L}$ , 2.2 mmol) in DCM (4 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and further 1 h and 30 min at room temperature. Usual work-up, followed by purification using silica gel chromatography eluting with a gradient of hexane:EtOAc to afford the title product as a brown-orange solid (263 mg, 86%). Mp: 92-94 °C.  $\delta_{\text{H}}$  (600 MHz,  $\text{CDCl}_3$ ): 1.53 (s, 9H,  $(\text{CH}_3)_3$ ), 1.56 (s, 9H,  $(\text{CH}_3)_3$ ), 4.15 (bs, 2H,  $\text{NH}_2$ ), 6.70 (d, 2H,  $J = 8.6$  Hz, Ar), 7.45 (t, 1H,  $J = 7.8$  Hz, Ar), 7.50 (d, 1H,  $J = 7.8$  Hz, Ar), 7.76 (d, 2H,  $J = 8.6$  Hz, Ar), 7.81 (s, 1H, Ar), 7.97 (d, 1H,  $J = 7.8$  Hz, Ar), 10.4 (bs, 1H, NH), 11.6 (bs, 1H, NHBoc).  $\delta_{\text{C}}$  (150 MHz,  $\text{CDCl}_3$ ): 28.1 (( $\text{CH}_3$ )<sub>3</sub>), 28.2 (( $\text{CH}_3$ )<sub>3</sub>), 79.7 (q,  $\underline{C}(\text{CH}_3)_3$ ), 84.0 (q,  $\underline{C}(\text{CH}_3)_3$ ), 113.7 (2 CH, Ar), 123.3 (CH, Ar), 125.6 (CH, Ar), 130.0 (CH, Ar), 127.3 (q), 128.7 (CH, Ar), 133.0 (2 CH, Ar), 136.7 (q), 139.4 (q), 150.9 (q), 153.3 (q), 153.6 (q), 153.7 (q), 163.5 (q), 194.5 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3357, 3235,

2983, 2928, 1718 (CO), 1627 (CN), 1587, 1407, 1368, 1291, 1235, 1149, 1105, 1057, 841, 803, 759, 690. HRMS (*m/z* - ES): 477.2127 (M<sup>+</sup> + Na).

#### **4-Amino-{3’-[2,3-di(*tert*-butoxycarbonyl)]guanidine}benzylbenzene (16c)**

Following Method B, HgCl<sub>2</sub> (154 mg, 0.6 mmol) was added over a solution of **9c** (282 mg, 1.42 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiourea (138 mg, 0.5 mmol) and triethylamine (204.3 μL, 1.5 mmol) in DCM (2.5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then overnight at room temperature. Usual work-up, followed by purification using silica gel chromatography eluting with a gradient of 100% hexane to 80:20 hexane:EtOAc respectively to afford the title product as a brown solid (103 mg, 47%). Mp: decomp. over 145 °C. δ<sub>H</sub> (600 MHz, CDCl<sub>3</sub>): 1.53 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.55 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.88 (s, 2H, CH<sub>2</sub>), 6.65 (d, 2H, *J* = 8.0 Hz, Ar), 6.94 (d, 1H, *J* = 7.8 Hz, Ar), 7.00 (d, 2H, *J* = 8.0 Hz, Ar), 7.25 (t, 1H, *J* = 7.7 Hz, Ar), 7.30 (s, 1H, Ar), 7.58 (d, 1H, *J* = 7.8 Hz, Ar), 10.2 (bs, 1H, NHBoc), 11.6 (bs, 1H, NH). δ<sub>C</sub> (150 MHz, CDCl<sub>3</sub>): 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 28.1 ((CH<sub>3</sub>)<sub>3</sub>), 41.0 (CH<sub>2</sub>), 79.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 115.6 (2 CH, Ar), 120.2 (CH, Ar), 122.4 (CH, Ar), 125.4 (CH, Ar), 128.9 (CH, Ar), 129.7 (2 CH Ar), 130.7 (q), 136.3 (q), 142.1 (q), 143.5 (q), 153.2 (q), 153.4 (q), 163.5 (q). ν<sub>max</sub> (ATR)/cm<sup>-1</sup>: 3370, 3260, 3026, 2921, 2851, 2163, 1722 (CO), 1640 (CN), 1602, 1493, 1451, 1366, 1240, 1155, 1111, 1064, 1029, 905, 803, 753, 645. HRMS (*m/z* - ES): 441.2502 (M<sup>+</sup> + H).

#### **3-Amino-{4’-[2,3-di(*tert*-butoxycarbonyl)]guanidino}diphenylether (17a)**

Following Method B, HgCl<sub>2</sub> (561 mg, 2.1 mmol) was added over a solution of **9a** (1500 mg, 5.16 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiourea (500 mg, 1.72 mmol) and triethylamine (744.3 μL, 5.34 mmol) in DCM (8.2 mL) at 0 °C. The resulting

mixture was stirred at 0 °C for 1 h and then overnight at room temperature. Usual work-up, followed by purification of the *meta* and *para* mono-guanidylated products by silica gel chromatography eluting with a gradient of 100% hexane to 80:20 hexane:EtOAc respectively to afford the title product as a white solid (586 mg, 77%). Mp: 78-79 °C.  $\delta_H$  (600 MHz, DMSO): 1.52 (s, 9H,  $(CH_3)_3$ ), 1.56 (s, 9H,  $(CH_3)_3$ ), 3.71 (bs, 2H, NH<sub>2</sub>), 6.33 (app. t, 1H,  $J$  = 2.1 Hz, Ar), 6.42 (m, 2H, Ar), 7.01 (d, 2H,  $J$  = 8.8 Hz, Ar), 7.10 (t, 1H,  $J$  = 8.0 Hz, Ar), 7.56 (d, 2H,  $J$  = 8.8 Hz, Ar), 10.3 (bs, 1H, NHBoc), 11.6 (bs, 1H, NH).  $\delta_C$  (150 MHz, DMSO): 28.0 ( $(CH_3)_3$ ), 28.3 ( $(CH_3)_3$ ), 79.3 (q,  $C(CH_3)_3$ ), 83.7 (q,  $C(CH_3)_3$ ), 104.0 (CH, Ar), 106.0 (CH, Ar), 109.6 (CH, Ar), 119.1 (2 CH, Ar), 125.1 (2 CH, Ar), 130.4 (CH, Ar), 132.0 (q, Ar), 148.0 (q, Ar), 152.0 (q), 153.0 (q), 154.0 (q), 158.0 (q), 163.0 (q).  $\nu_{max}$  (ATR)/cm<sup>-1</sup>: 3474 (NH), 3381 (NH), 3264 (NH), 2976, 2930, 1724 (CO), 1617 (CN), 1492, 1414, 1367, 1289, 1305, 1217, 1145, 1112, 958, 802, 753. HRMS (*m/z* - ES): 443.2290 (M<sup>+</sup> + H)

### **3-Amino-{4'-[2,3-di(*tert*-butoxycarbonyl)]guanidino}benzylbenzene (17c)**

Following Method B, HgCl<sub>2</sub> (154 mg, 0.6 mmol) was added over a solution of **9c** (282 mg, 1.42 mmol), *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (138 mg, 0.5 mmol) and triethylamine (204.3 μL, 1.5 mmol) in DCM (2.5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then overnight at room temperature. Usual work-up, followed by purification of the *meta* and *para* mono-guanidylated products by silica gel chromatography eluting with a gradient of 100% hexane to 80:20 hexane:EtOAc respectively to afford the title product as a brownish solid (107 mg, 52%). Mp: 118-119 °C.  $\delta_H$  (600 MHz, CDCl<sub>3</sub>): 1.51 (s, 9H,  $(CH_3)_3$ ), 1.56 (s, 9H,  $(CH_3)_3$ ), 1.92 (bs, 2H, NH<sub>2</sub>), 3.89 (s, 2H, CH<sub>2</sub>), 6.59 (s, 1H, Ar), 6.64 (d, 1H,  $J$  = 7.7 Hz, Ar), 6.68 (d, 1H,  $J$  = 7.7 Hz, Ar), 7.11 (t, 1H,  $J$  = 7.7 Hz, Ar), 7.16 (d, 2H,  $J$  = 8.1 Hz, Ar), 7.51 (d, 2H,  $J$  = 8.1 Hz, Ar), 10.3

(bs, 1H, NHBoc), 11.6 (bs, 1H, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 ((CH<sub>3</sub>)<sub>3</sub>), 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 41.1 (CH<sub>2</sub>), 79.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 113.2 (CH, Ar), 116.0 (CH, Ar), 119.7 (CH, Ar), 122.2 (2 CH, Ar), 129.2 (CH, Ar), 129.3 (2 CH, Ar), 134.6 (q), 137.5 (q), 142.2 (q), 145.5 (q), 153.2 (q), 153.4 (q), 165.1 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3377(NH), 3261(NH), 2984, 2965, 2924, 1722 (CO), 1643 (CN), 1603, 1559, 1477, 1405, 1367, 1294, 1242, 1226, 1153, 1112, 1058, 1029, 855, 803, 768, 695. HRMS (*m/z* - ES): 441.2488 (M<sup>+</sup> + H)

## 8. Preparation of diaromatic [2,3-di(*tert*-butoxycarbonyl)guanidino]-[2-(*tert*-butoxycarbonyl)-3-arylguanidino] derivatives

### **3-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4'-[2-(*tert*-butoxycarbonyl)-3-(phenylguanidino)]diphenylether (18a)**

Following Method C, to a solution of **14a** (395 mg, 0.944 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-thiourea (313 mg, 1.13 mmol) and triethylamine (407  $\mu$ l, 2.93 mmol) in DCM (5 mL), HgCl<sub>2</sub> (307 mg, 1.13 mmol) was added. The reaction mixture was stirred at room temperature for 5 h and 30 min. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as transparent hydroscopic solid (471 mg, 76%). Mp: 90-91 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.55 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 6.71 (bs, NH), 6.67-6.79 (m, Ar), 6.86-7.15 (m, Ar), 7.21-7.43 (m, Ar), 7.53 (d, *J* = 7.7 Hz, Ar), 7.65-7.80 (m, Ar), 9.57 (bs, NH), 9.87 (bs, NH), 10.33 (bs, NH), 11.6 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 ((CH<sub>3</sub>)<sub>3</sub>), 28.0 (2(CH<sub>3</sub>)<sub>3</sub>), 79.7 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.3 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.7 (q, C(CH<sub>3</sub>)<sub>3</sub>), 112.0 (CH, Ar), 114.0 (CH, Ar), 116.5 (CH, Ar), 119.8 (2 CH, Ar), 120.0 (CH, Ar), 122.5 (CH, Ar), 124.0 (CH, Ar), 128.8 (2 CH, Ar), 129.6 (CH, Ar), 130 – 150 (Quaternary signals in this range could not be assigned as they were highly

amorphous and there was not enough resolution), 137.8 (q), 153.1 (q), 153.2 (q), 162.9 (q), 163.2 (q).  $\nu_{\text{max}}$  (film)/cm<sup>-1</sup>: 3407, 3285, 2978, 2932, 1718 (CO), 1596 (CN), 1555, 1490, 1408, 1367, 1331, 1289, 1233, 1143, 1105, 1056, 946, 804, 752, 690. HRMS (*m/z* - ES): 661.3339 (M<sup>+</sup> + H).

**3-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(phenyl)-guanidino]benzophenone (18b)**

Following Method C, to a solution of **16b** (186 mg, 0.41 mmol), **12** (124 mg, 0.49 mmol) and triethylamine (177 µl, 1.27 mmol) in DCM (2.1 mL), HgCl<sub>2</sub> (133 mg, 0.49 mmol) was added. The reaction mixture was stirred at room temperature for 5 h. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as light brown solid (228 mg, 83%). Mp: 119-121 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 6.75 (bs, NH), 6.97 (Ar), 7.12 (Ar), 7.32-7.42 (m, Ar), 7.47 (Ar), 7.78-8.04 (m, Ar), 9.56-10.0 (bs, NH), 10.4-10.5 (bs, NH), 11.6 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 ((CH<sub>3</sub>)<sub>3</sub>), 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 79.6 (q,  $\underline{\text{C}}(\text{CH}_3)_3$ ), 83.5 (q,  $\underline{\text{C}}(\text{CH}_3)_3$ ), 83.7 (q,  $\underline{\text{C}}(\text{CH}_3)_3$ ), 118.6 (CH, Ar), 122.2 (CH, Ar), 123.1 (CH, Ar), 123.5 (CH, Ar), 123.6 (CH, Ar), 126.0 (CH, Ar), 126.1 (CH, Ar), 128.8 (CH, Ar), 129.7 (CH, Ar), 130.9 (CH, Ar), 131.6 (CH, Ar), 132.2 (CH, Ar), 136.6 (q), 136.7 (q), 138.5 (q), 138.7 (q), 139.4 (q), 143.3 (q), 146.1 (q), 152.9 (q), 153.2 (q), 153.6 (q), 153.6 (q), 163.4 (q), 194.7 (q), 195.0 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3407, 3256, 2978, 2932, 1717 (CO), 1637 (CN), 1584, 1546, 1461, 1409, 1368, 1293, 1233, 1148, 1106, 1057, 941, 848, 804, 758, 691. HRMS (*m/z* - ES): 673.3339 (M<sup>+</sup> + H).

**3-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(phenyl)-guanidino]benzylbenzene (18c)**

Following Method C, to a solution of **16c** (30 mg, 0.07 mmol), **12** (21 mg, 0.084 mmol) and triethylamine (30 µl, 0.22 mmol) in DCM (0.36 mL), HgCl<sub>2</sub> (23 mg, 0.084 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as off-white solid (50 mg, 72%). Mp: 92-94 °C. δ<sub>H</sub> (600 MHz, CDCl<sub>3</sub>): 1.50 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.53 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.95 (s, 2H, CH<sub>2</sub>), 6.71 (bs, NH), 6.80 – 7.44 (Aromatic signals in this range could not be assigned as they were broad and highly amorphous), 7.56 (d, 2H, J = 7.8 Hz, Ar) 7.60-7.77 (m, Ar) 9.54 (bs, NH), 10.30 (bs, NH), 11.60 (bs, NH). δ<sub>C</sub> (150 MHz, CDCl<sub>3</sub>): 28.0 (2 CH<sub>3</sub>)<sub>3</sub>, 28.2 ((CH<sub>3</sub>)<sub>3</sub>), 41.2 (CH<sub>2</sub>), 79.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.0 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 119.8 – 129.8 (Aromatic signals in this range could not be assigned as they were broad and highly amorphous) 122.6 (2 CH, Ar), 129.8 (2 CH, Ar), 128.9 (CH, Ar), 135 – 150 (Quaternary signals in this range could not be assigned as they were highly amorphous and there was not enough resolution), 136.8 (q), 152.9 (q) 153.3 (q), 153.5 (q), 163.5 (q). ν<sub>max</sub> (film)/cm<sup>-1</sup>: 3408, 3266, 2983, 2939, 1720 (CO), 1646 (CN), 1597, 1568, 1480, 1466, 1331, 1300, 1238, 1207, 1147, 1098, 1070, 838, 802, 765, 678. HRMS (m/z - ES): 659.3540 (M<sup>+</sup> + H).

### **3-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(phenyl)**

### **guanidino]-N-phenylaniline (18d)**

Following Method C, to a solution of **14d** (328 mg, 0.78 mmol), *N,N'*-bis-(*tert*-butoxycarbonyl)-thiourea (260 mg, 0.94 mmol) and triethylamine (337 µl, 2.42 mmol) in DCM (4 mL), HgCl<sub>2</sub> (254 mg, 0.94 mmol) was added. The reaction mixture was stirred at room temperature for 5 h. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as light dark solid (168 mg, 33%). Mp: 95-98 °C. δ<sub>H</sub> (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 18H, 2(CH<sub>3</sub>)<sub>3</sub>), 1.56 (s, 9H,

(CH<sub>3</sub>)<sub>3</sub>), 5.82 (bs, NH), 6.03 (bs, NH), 6.68-7.73 (m, 13H, Ar), 9.46 (bs, NH), 9.56 (bs, NH), 10.30 (bs, 1H, NH), 11.50 (bs, 1H, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 (2 ((CH<sub>3</sub>)<sub>3</sub>), 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 78.8 (q, C(CH<sub>3</sub>)<sub>3</sub>), 79.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 82.9 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 110.3 (CH, Ar), 111.8 (CH, Ar), 112.6 (CH, Ar), 113.6 (CH, Ar), 113.9 (CH, Ar), 114.8 (CH, Ar), 118.4 (CH, Ar), 119.7 (CH, Ar), 119.9 (CH, Ar), 120.7 (CH, Ar), 120.7 (CH, Ar), 122.6 (CH, Ar), 123.1 (CH, Ar), 124.9 (CH, Ar), 126.1 (CH, Ar), 128.6 (CH, Ar), 129.0 (CH, Ar), 129.4 (2 CH, Ar), 133.2 (q), 136.9 (q), 137.6 (q), 139.0 (q), 140.1 (q), 140.7 (q), 141.2 (q), 143.2 (q), 144.6 (q), 147.0 (q), 152.8 (q), 153.1 (q), 153.5 (q), 156.6 (q), 163.0 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3406 (NH), 3299 (NH), 2978, 2930, 2167, 1718 (CO), 1593 (CN), 1478, 1497, 1459, 1406, 1367, 1326, 1233, 1146, 1105, 1057, 903, 804, 771, 750, 690. HRMS (*m/z* - ES): 660.3527 (M<sup>+</sup> + H).

### 3-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4'-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]diphenylether (19a)

Following Method C, to a solution of **15a** (60 mg, 0.12 mmol), *N,N'*-bis(*tert*-butoxycarbonyl)-thiourea (36 mg, 0.14 mmol) and triethylamine (50.2  $\mu$ l, 0.36 mmol) in DCM (0.6 mL), HgCl<sub>2</sub> (38 mg, 0.14 mmol) was added. The reaction mixture was stirred at room temperature for 5 h and 30 min. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as off-white hygroscopic solid (67 mg, 76%). Mp: decomp. above 114 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.54 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.55 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 6.75 (Ar), 6.92 (Ar), 6.99-7.14 (m, Ar), 7.22-7.48 (m, Ar), 7.60 (Ar), 7.92-8.07 (m, Ar), 9.64 (bs, NH), 9.84 (bs, NH), 10.30 (bs, NH), 11.60 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 ((CH<sub>3</sub>)<sub>3</sub>), 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 28.1 ((CH<sub>3</sub>)<sub>3</sub>), 79.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.8 (q, C(CH<sub>3</sub>)<sub>3</sub>), 112.2 (CH, Ar), 114.3 (CH, Ar), 116.7 (CH, Ar), 118.7 (CH, Ar), 119.8 (CH, Ar), 120.9 (CH, Ar), 122.0 (CH, Ar), 123.4 (CH, Ar), 123.6 (c, q, *J* = 274

Hz, CF<sub>3</sub>), 124.7 (q), 126.7 (Ar), 128.4 (d, q, *J* = 30 Hz), 129.7 (CH, Ar), 131.6 (CH, Ar), 132.4 (CH, Ar), 137.9 (q), 138.1 (q), 140.1 (q), 141.8 (q), 152.5 (q), 153.0 (q), 153.2 (q), 153.4 (q), 158.1 (q), 163.4 (q).  $\nu_{\text{max}}$  (film)/cm<sup>-1</sup>: 3290, 3119, 2966, 1654, 1561, 1482, 1468, 1405, 1316, 1258, 1176, 1136, 1035, 960, 834, 808, 681. HRMS (*m/z* - ES): 763.2825 (M<sup>+</sup> + H).

**3-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4'-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]benzophenone (19b)**

Following Method C, to a solution of **16b** (100 mg, 0.22 mmol), **13** (94 mg, 0.264 mmol) and triethylamine (95  $\mu$ L, 0.68 mmol) in DCM (1.1 mL), HgCl<sub>2</sub> (72 mg, 0.264 mmol) was added. The reaction mixture was stirred at room temperature for 5 h. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as light brown solid (159 mg, 94%). Mp: 88-90 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.53 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 6.49 (bs, NH), 6.66 (bs, NH), 7.03-7.05 (bs, 1H, Ar) 7.28 (app. s, 2H, Ar), 7.43-7.58 (m, 3H, Ar), 7.81-8.03 (m, 5H, Ar), 9.95 (bs, NH), 10.0 (bs, NH), 10.4 (bs, NH), 10.5 (bs, NH), 11.6 (bs, 1H, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 28.1 ((CH<sub>3</sub>)<sub>3</sub>), 79.8 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.9 (q, C(CH<sub>3</sub>)<sub>3</sub>), 84.2 (q, C(CH<sub>3</sub>)<sub>3</sub>), 118.9 (2 CH, Ar), 121.9 (CH, Ar), 122.1 (CH, Ar), 122.7 (c, q, *J* = 274 Hz, CF<sub>3</sub>), 123.8 (3 CH, Ar), 126.1 (2 CH, Ar), 126.2 (2 CH, Ar), 126.4 (CH, Ar), 128.9 (CH, Ar), 131.4 (2 CH, Ar), 132.4 (2 CH, Ar), 132.7 (CH, Ar), 136.8 – 145.6 (Quaternary signals in this range could not be assigned as they were broad and highly amorphous), 152.9 (q), 153.2 (q), 153.7 (q), 163.4 (q), 195.0 (q).  $\nu_{\text{max}}$  (ART)/cm<sup>-1</sup>: 3328, 2958, 2927, 2858, 2163, 1979, 1721 (CO), 1662 (CN), 1596, 1547, 1482, 1420, 1321, 1260, 1173, 1111, 1072, 1033, 896, 827, 797, 742, 703, 664. HRMS (*m/z* - ES): 775.2837 (M<sup>+</sup> + H).

**3-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4'-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]benzylbenzene (19c)**

Following Method C, to a solution of **16c** (75 mg, 0.17 mmol), **13** (72.64 mg, 0.21 mmol) and triethylamine (73.9  $\mu$ L, 0.53 mmol) in DCM (1 mL) and isopropanol (0.3 mL),  $HgCl_2$  (56 mg, 0.21 mmol) was added. The reaction mixture was stirred at room temperature for 5 h and 30 min. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as off-white solid (91 mg, 70%). Mp: 92-94 °C.

$\delta_H$  (600 MHz,  $CDCl_3$ ): 1.53 (s, 9H,  $(CH_3)_3$ ), 1.56 (s, 9H,  $(CH_3)_3$ ), 3.95 (s, 2H,  $CH_2$ ), 6.79 (bs, NH), 6.86 (Ar), 6.95 (Ar), 6.99 (Ar), 7.17 (Ar), 7.22 (Ar), 7.28 (Ar), 7.36 (CH Ar), 7.42 (Ar), 7.57 (Ar), 7.58 (Ar), 7.92-8.09 (m, Ar), 9.46-9.94 (m, bs, NH), 10.3 (bs, NH), 11.7 (bs, NH).  $\delta_C$  (150 MHz,  $CDCl_3$ ): 27.9 (2  $CH_3)_3$ ), 28.0 ( $(CH_3)_3$ ), 41.1 ( $CH_2$ ), 79.4 (q,  $\underline{C}(CH_3)_3$ ), 83.5 (q,  $\underline{C}(CH_3)_3$ ), 118.5 (CH, Ar), 120.2 (CH, Ar), 122.1 (CH, Ar), 122.4 (CH, Ar), 122.5 (CH, Ar), 123.5 (CH, Ar), 125.2 (CH, Ar), 126.6 (q), 128.9 (CH, Ar), 129.3 (CH, Ar), 130.1 (CH, Ar), 131.5 (CH, Ar), 132 – 151 (Quaternary signals in this range could not be assigned as they were highly amorphous and there was not enough resolution), 136.7 (q), 153.2 (q), 153.4 (q), 163.1 (q), 163.4 (q).  $\nu_{max}$  (film)/cm<sup>-1</sup>: 2978, 2929, 2162, 1719 (CO), 1593 (CN), 1554, 1464, 1409, 1368, 1318, 1240, 1147, 1107, 1057, 1030, 770, 694. HRMS (*m/z* - ES): 761.3055 ( $M^+ + H$ ).

**3-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4'-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]-*N*-phenylaniline (19d)**

Following Method C, to a solution of **15d** (203 mg, 0.39 mmol), *N,N'*-*bis*-(*tert*-butoxycarbonyl)-thiourea (130 mg, 0.47 mmol) and triethylamine (168.7  $\mu$ L, 1.21 mmol) in DCM (2.0 mL),  $HgCl_2$  (128 mg, 0.47 mmol) was added. The reaction mixture was stirred at room temperature for 5 h. Usual work-up followed by silica gel chromatography (eluting with

a gradient of hexane:EtOAc) to afford the desired product as light dark solid (272 mg, 76%).

Mp: 108-110 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.49 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.51 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.55 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 5.94 (bs, 1H, NH), 6.76 (app. d, 1H, *J* = 6.5 Hz, Ar), 6.85 (bs, 1H, Ar), 6.94 (app. d, 1H, *J* = 6.5 Hz, Ar), 7.00-7.23 (m, 4H, Ar), 7.36-7.50 (m, 4H, Ar), 7.90-8.08 (m, 1H, Ar), 9.82 (bs, NH), 9.56 (bs, NH), 10.2 (bs, NH), 10.3 (bs, NH), 11.7 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 (2(CH<sub>3</sub>)<sub>3</sub>), 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 79.3 (q, CCH<sub>3</sub>), 79.4 (q, CCH<sub>3</sub>), 83.5 (q, CCH<sub>3</sub>), 110.7 (CH, Ar), 112.7 (CH, Ar), 113.9 (CH, Ar), 118.8 (CH, Ar), 119.2 (CH, Ar), 120.2 (CH, Ar), 121.7 (q, c, *J* 274, CF<sub>3</sub>), 122.8 (CH, Ar), 123.5 (CH, Ar), 123.6 (CH, Ar), 124.4 (CH, Ar), 125.3 (CH, Ar), 126.6 (CH, Ar), 129.3 (CH, Ar), 131.4 (CH, Ar), 132.0 (CH, Ar), 137.5 (q), 137.9 (q), 138.3 (q), 139.6 (q), 144 – 149 (Quaternary signals in this range could not be assigned as they were broad and highly amorphous).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3264 (NH), 2978, 2929, 2049, 1719 (CO), 1596 (CN), 1560, 1509, 1479, 1368, 1320, 1234, 1146, 1106, 1057, 1029, 806, 771, 688, 665. HRMS (*m/z* - ES): 762.2999 (M<sup>+</sup> + H).

#### **4'-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-3-[2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidino]diphenylether (20a)**

Following Method C, to a solution of **17a** (212 mg, 0.48 mmol), **12** (145 mg, 0.576 mmol) and triethylamine (207 µl, 1.49 mmol) in DCM (2.5 mL), HgCl<sub>2</sub> (156 mg, 0.576 mmol) was added. The reaction mixture was stirred at room temperature for 6 h. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as off-white hydroscopic solid (108 mg, 34%). Mp: 78-80 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.53 (s, 18H, 2 (CH<sub>3</sub>)<sub>3</sub>), 1.57 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 6.63 (bs, NH), 6.61-7.68 (m, 13H, Ar), 9.60 (bs, NH), 10.3 (bs, NH), 11.6 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 28.1 ((CH<sub>3</sub>)<sub>3</sub>), 28.2 (2 (CH<sub>3</sub>)<sub>3</sub>), 79.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.3 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.7 (q, C(CH<sub>3</sub>)<sub>3</sub>), 110.6 (CH, Ar), 112.7 (CH, Ar), 112.8 (CH, Ar), 114.7 (CH, Ar), 117.3 (CH, Ar), 119.3 (CH, Ar), 119.7 (2 CH, Ar), 120.0

(CH, Ar), 122.5 (CH, Ar), 122.8 (CH, Ar), 123.7 (2 CH, Ar), 128.7 (CH, Ar), 129.5 (CH, Ar), 129.6 (2 CH, Ar), 130.6 (CH, Ar), 132.0 (q), 132.5 (q), 138.8 (q), 139.8 (q), 140.2 (q), 140.5 (q), 146.7 (q), 148.5 (q), 152.9 (q), 153.3 (q), 153.5 (q), 163.0 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3411, 3264, 3158, 2978, 2932, 1666, 1718, 1637, 1590, 1559, 1481, 1461, 1408, 1367, 1337, 1305, 1230, 1211, 1145, 1095, 1056, 963, 944, 835, 806, 771, 750, 690. HRMS (*m/z* - ES): 661.3364 (M<sup>+</sup> + H).

**4'-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-3-[2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidino]benzylbenzene (20c)**

Following Method C, to a solution of **17c** (131 mg, 0.29 mmol), **12** (90 mg, 0.36 mmol) and triethylamine (153.7  $\mu$ L, 1.104 mmol) in DCM (1.54 mL), HgCl<sub>2</sub> (97 mg, 0.36 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as off-white solid (164 mg, 71%). Mp: 99-100 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 18H, 2 (CH<sub>3</sub>)<sub>3</sub>), 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.93 (s, 2H, CH<sub>2</sub>), 6.73-7.32 (Aromatic signals in this range could not be assigned as they were broad and highly amorphous), 7.55 (d, 2H, J = 7.4 Hz, Ar), 7.70 (bs, Ar), 9.54 (bs, NH), 10.3 (bs, NH), 11.6 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 28.1 (2 (CH<sub>3</sub>)<sub>3</sub>), 28.2 ((CH<sub>3</sub>)<sub>3</sub>), 41.3 (CH<sub>2</sub>), 79.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 120.0 – 130 (Aromatic signals in this range could not be assigned as they were broad and highly amorphous) 122.3 (2 CH, Ar), 129.2 (2 CH, Ar), 135 – 150 (Quaternary signals in this range could not be assigned as they were highly amorphous and there was not enough resolution), 152.9 (q), 153.3 (q), 153.5 (q), 163.6 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3411(NH), 3264, 2978, 2932, 1718 (CO), 1636 (CN), 1590, 1559, 1481, 1461, 1408, 1337, 1305, 1230, 1211, 1145, 1095, 1056, 835, 806, 750, 690. HRMS (*m/z* - ES): 659.3544 (M<sup>+</sup> + H).

**4'-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-3-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]diphenylether (21a)**

Following Method C, to a solution of **17a** (263 mg, 0.60 mmol), **13** (253 mg, 0.715 mmol) and triethylamine (257.2  $\mu$ L, 1.85 mmol) in DCM (3.1 mL), HgCl<sub>2</sub> (194 mg, 0.715 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as off-white solid (91 mg, 70%). Mp: 85-87 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 1.57 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 6.57 (bs, Ar), 6.65 (bs, Ar), 6.69-6.80 (m, Ar and NH), 7.25 (bs, Ar), 7.43 (bs, Ar), 7.60 (bs, Ar), 7.89-8.03 (m, Ar), 9.60-9.90 (m, NH), 10.3 (bs, NH), 11.6 (bs, NH).  $\delta_{\text{C}}$  (150 MHz, CDCl<sub>3</sub>): 27.9 (2 (CH<sub>3</sub>)<sub>3</sub>), 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 79.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 111.9 (CH, Ar), 113.2 (CH, Ar), 116.7 (CH, Ar), 118.7 (CH, Ar), 119.7 (CH, Ar), 123.6 (CH, Ar), 130.7 (CH, Ar), 131.5 (CH, Ar), 132.4 (CH, Ar), 139.7 (q), 140 – 151 (Quaternary signals in this range could not be assigned as they were highly amorphous and there was not enough resolution) 145.0 (q), 153.2 (q), 153.4 (q), 163.3 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3411 (NH), 3258, 2981, 2932, 2037, 1720 (CO), 1639 (CN), 1593, 1556, 1506, 1479, 1462, 1408, 1368, 1305, 1232, 1145, 1111, 1029, 957, 834, 805, 771, 686. HRMS (*m/z* - ES): 763.2825 (M<sup>+</sup> + H)

**4'-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-3-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]benzylbenzene (21c)**

Following Method C, to a solution of **17c** (107 mg, 0.24 mmol), **13** (104 mg, 0.29 mmol) and triethylamine (105.3  $\mu$ L, 0.76 mmol) in DCM (1.3 mL) and isopropanol (0.3 mL), HgCl<sub>2</sub> (80 mg, 0.29 mmol) was added. The reaction mixture was stirred at room temperature for 5 h and 30 min. Usual work-up followed by silica gel chromatography (eluting with a gradient of hexane:EtOAc) to afford the desired product as off-white solid (110 mg, 68%). Mp: 98-100 °C.  $\delta_{\text{H}}$  (600 MHz, CDCl<sub>3</sub>): 1.52 (s, 18H, 2 (CH<sub>3</sub>)<sub>3</sub>), 1.56 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 3.95 (s, 2H, CH<sub>2</sub>),

6.77-6.80 (m, Ar and NH), 6.94 (bs, Ar), 7.09-7.33 (m, Ar), 7.38-7.44 (m, Ar), 7.51-7.58 (m, Ar, CF<sub>3</sub> ring), 7.95 (bs, Ar), 8.01 (bs, Ar), 9.40-9.85 (m, bs, NH), 10.3 (bs, NH), 11.6 (bs, NH). δ<sub>C</sub> (150 MHz, CDCl<sub>3</sub>): 27.9 (2 CH<sub>3</sub>)<sub>3</sub>, 28.0 ((CH<sub>3</sub>)<sub>3</sub>), 41.1 (CH<sub>2</sub>), 41.2 (CH<sub>2</sub>), 79.4 (q, C(CH<sub>3</sub>)<sub>3</sub>), 83.5 (q, C(CH<sub>3</sub>)<sub>3</sub>), 118.5 (CH, Ar), 119.6 (CH, Ar), 122.1 (CH, Ar), 122.2 (CH, Ar), 122.7 (c, q, *J* = 274 Hz, CF<sub>3</sub>), 123.6 (CH, Ar), 123.8 (CH, Ar), 126.6 (CH, Ar), 129.6 (CH, Ar), 128.1 (c, q, *J* = 30 Hz, Ar), 131.5 (CH, Ar), 132.2 (CH, Ar), 134.8 (q), 137.1 (q), 137.8 (q), 139.5 (q), 142.9 (q), 146.1 (q), 152.9 (q), 153.2 (q), 153.3 (q), 163.4 (q). ν<sub>max</sub> (ATR)/cm<sup>-1</sup>: 3408 (NH), 3261, 3156, 2981, 2933, 2162, 1718 (CO), 1632 (CN), 1602, 1559, 1480, 1463, 1409, 1368, 1319, 1303, 1235, 1146, 1116, 1057, 957, 804, 770, 687. HRMS (*m/z* - ES): 761.3030 (M<sup>+</sup> + H).

## 9. Preparation of diaromatic *N*-{3-[4-(*N'*-Phenyl-*N''*-*tert*-butoxycarbonylguanidino) acetamide derivatives

### *N*-(3-{4-[*N'*-(4-Chloro-3-trifluoromethylphenyl)-*N''*-*tert*-butoxycarbonylguanidino] phenoxy}phenyl)acetamide (22a)

Following method D, to a solution of **15a** (391 mg, 0.75 mmol), and acetic anhydride (153 mg, 1.5 mmol) in DCM (3.75 mL), triethylamine (228 mg, 2.25 mmol) was added. The reaction was stirred at room temperature for 6 h. Workup as described, followed by silica gel chromatography, eluting with a gradient of hexane:EtOAc, afforded the title compound as a red solid (376 mg, 89%). Mp: 59-61 °C. δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>): 1.52 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 2.13 (s, 3H, CH<sub>3</sub>), 6.50-8.00 (Aromatic signals in this range could not be assigned as they were broad and highly amorphous), 6.78 (bs, NH), 7.70 (bs, NH), 8.44-9.95 (m, bs, NH). δ<sub>C</sub> (100 MHz, CDCl<sub>3</sub>): 24.4 (CH<sub>3</sub>), 27.9 ((CH<sub>3</sub>)<sub>3</sub>), 83.7 (q, C(CH<sub>3</sub>)<sub>3</sub>), 109.3 (CH, Ar), 109.7 (CH, Ar), 113.8

(CH, Ar), 114.1 (CH, Ar), 118.7 (CH, Ar), 119.8 (CH, Ar), 120.7 (CH, Ar), 122.1 (CH, Ar), 123.4 (CH, Ar), 123.5 (c, q,  $J = 273$  Hz, CF<sub>3</sub>), 126.7 (CH, Ar), 128.4 (d, q,  $J = 30$  Hz), 129.3 (q), 129.9 (CH, Ar), 131.5 (CH, Ar), 132.4 (CH, Ar), 137.8 (q), 139.3 (q), 140.1 (q), 141.8 (q), 152.4 (q), 152.9 (q), 158.2 (q), 168.3 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3264 (NH), 2980, 2933, 2163, 1722 (CO), 1655 (CN), 1597, 1547, 1502, 1481, 1438, 1316, 1246, 1208, 1089, 1031, 1015, 967, 920, 836, 772, 687, 666. HRMS (*m/z* - ES): 561.1514 (M<sup>+</sup> - H).

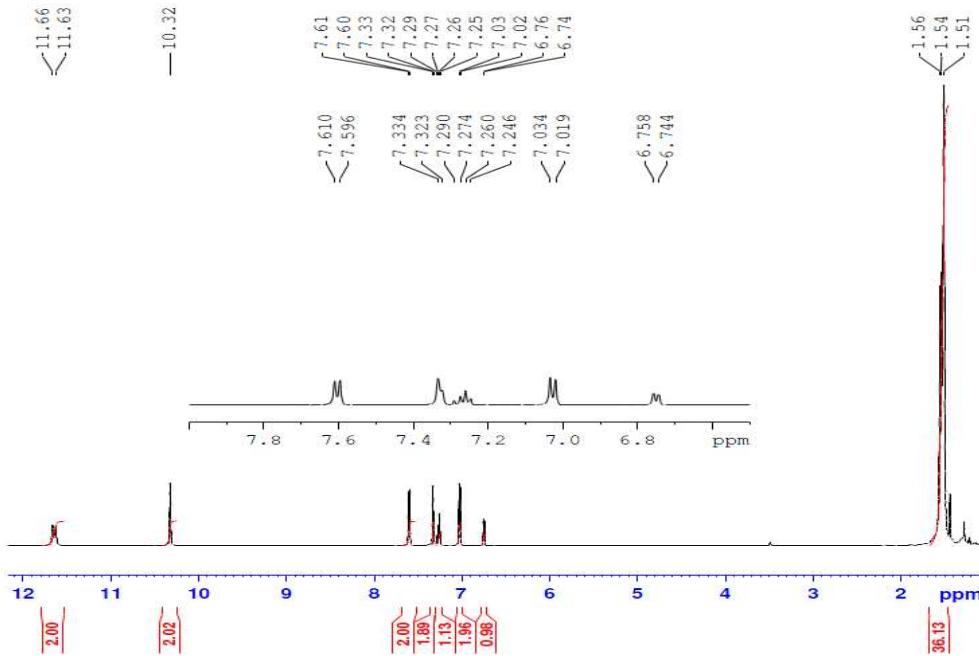
**N-(3-{4-[N'-(4-Chloro-3-trifluoromethyl-phenyl)-N''-*tert*-butoxycarbonyl-guanidino]-phenylamino}-phenyl)-acetamide (22d)**

Following method D, to a solution of **15d** (442 mg, 0.85 mmol), and acetic anhydride (174 mg, 1.70 mmol) in DCM (4.2 mL), triethylamine (258 mg, 2.55 mmol) was added. The reaction was stirred at room temperature for 6 h. Workup as described, followed by silica gel chromatography, eluting with hexane:EtOAc, afforded the title compound as a red solid (291 mg, 61%). Mp: 100-102 °C.  $\delta_{\text{H}}$  (400 MHz, CDCl<sub>3</sub>): 1.51 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>), 2.12 (s, 3H, CH<sub>3</sub>), 5.82 (bs, NH), 5.84 (bs, NH), 6.50-8.50 (Aromatic signals in this range could not be assigned as they were broad and highly amorphous), 7.90 (bs, NH), 9.30-10.0 (bs, NH).  $\delta_{\text{C}}$  (100 MHz, CDCl<sub>3</sub>): 24.4 (CH<sub>3</sub>), 28.03 ((CH<sub>3</sub>)<sub>3</sub>), 83.6 (q, C(CH<sub>3</sub>)<sub>3</sub>), 108.1 (CH, Ar), 112.1 (CH, Ar), 118.7 (CH, Ar), 119.6 (CH, Ar), 120.7 (CH, Ar), 121.7 (CH, Ar), 122.0 (CH, Ar), 123.0 (CH, Ar), 123.5 (c, q,  $J = 273$  Hz, CF<sub>3</sub>), 123.6 (CH, Ar), 126.9 (CH, Ar), 129.6 (CH, Ar), 131.6 (CH, Ar), 132.2 (CH, Ar), 137.9 (q), 139.1 (q), 140.1 (q), 142 – 150 (Quaternary signals in this range could not be assigned as they were broad and highly amorphous), 153 (q), 168.7 (q).  $\nu_{\text{max}}$  (ATR)/cm<sup>-1</sup>: 3312 (NH), 2982, 2936, 2080, 1724 (CO), 1650 (CN), 1598, 1509, 1479, 1425, 1370, 1315, 1239, 1138, 1090, 1043, 1033, 994, 938, 725, 690, 666. HRMS (*m/z* - ES): 560.1696 (M<sup>+</sup> - H).

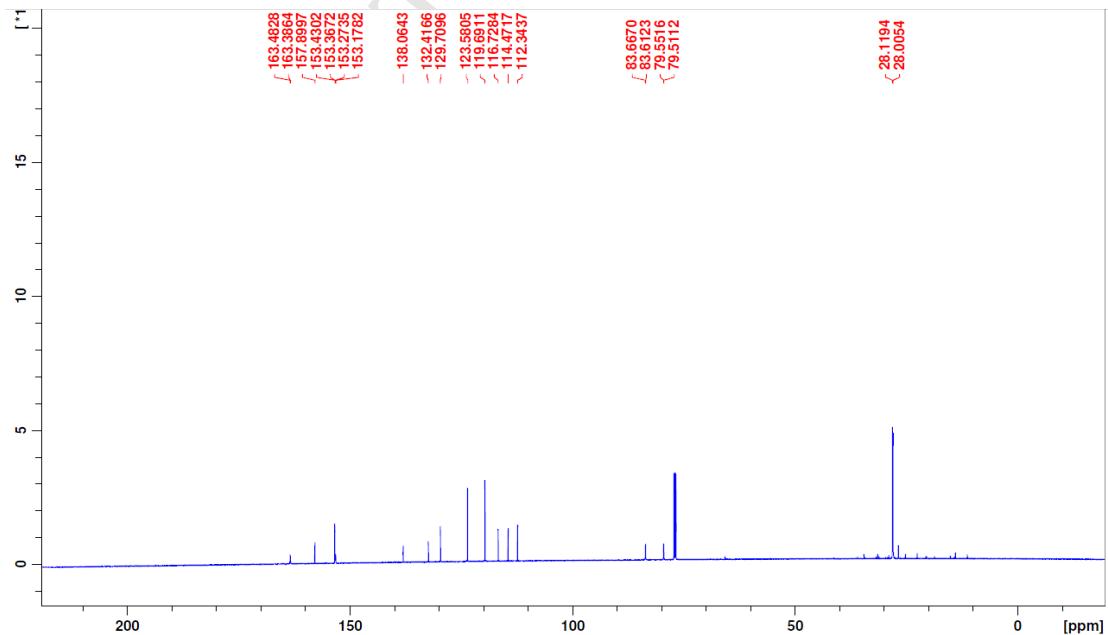
**10. Spectroscopic and purity data of Boc-protected derivatives and final hydrochloride salts**

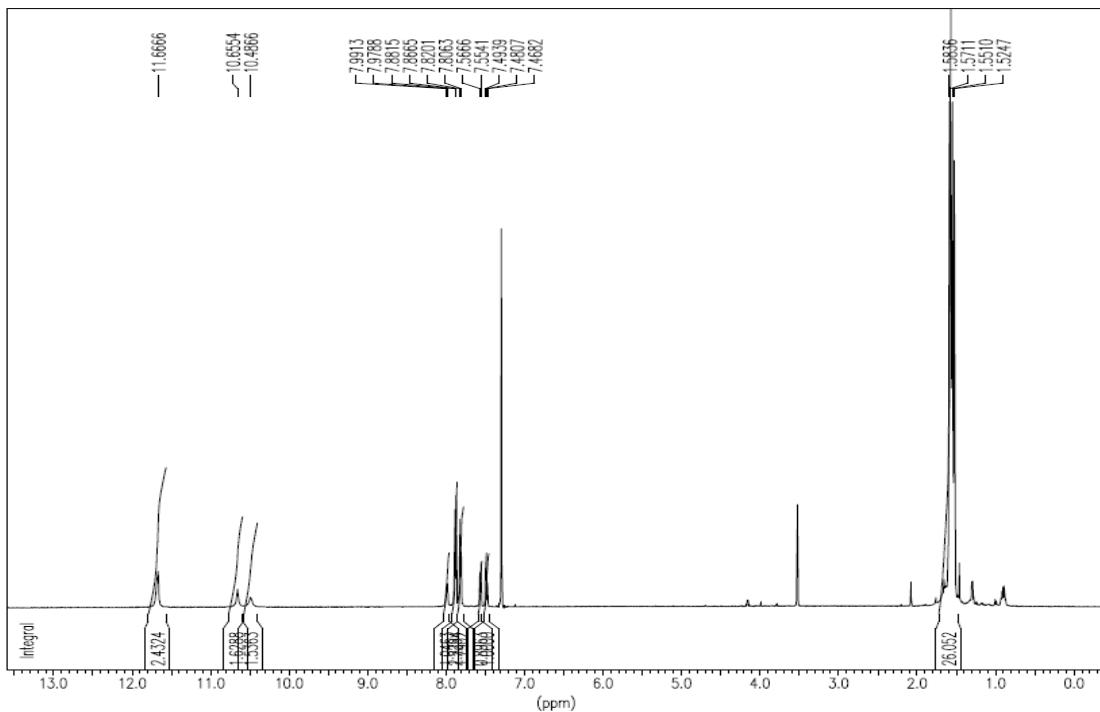
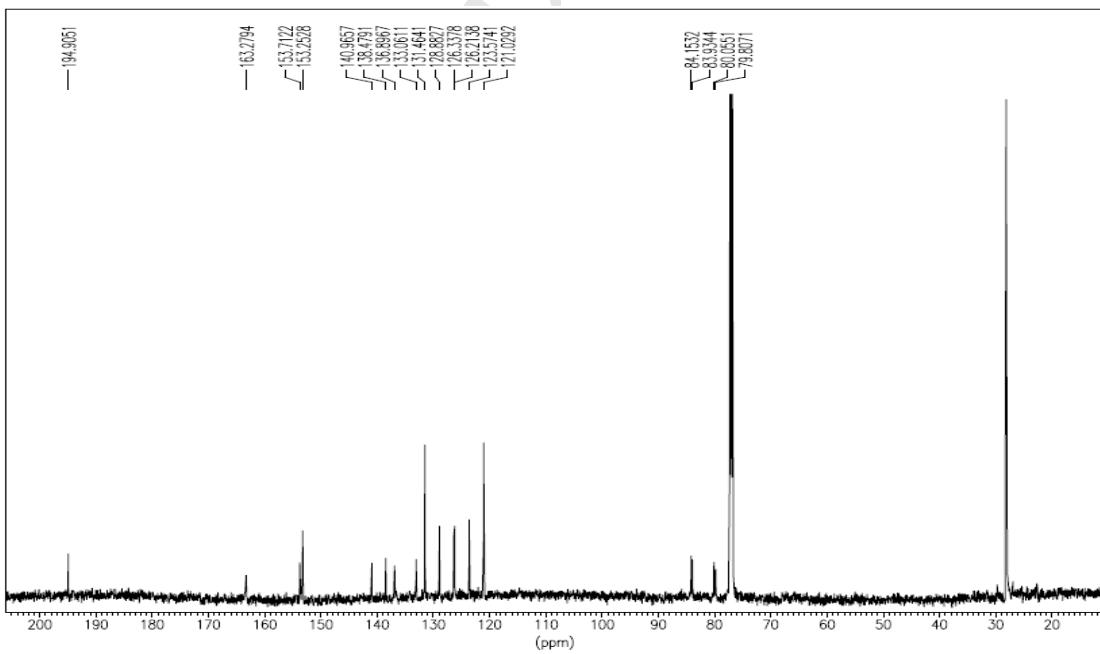
**3,4'-Bis-[2,3-di-(*tert*-butoxycarbonyl)-guanidine]-diphenylether (10a)**

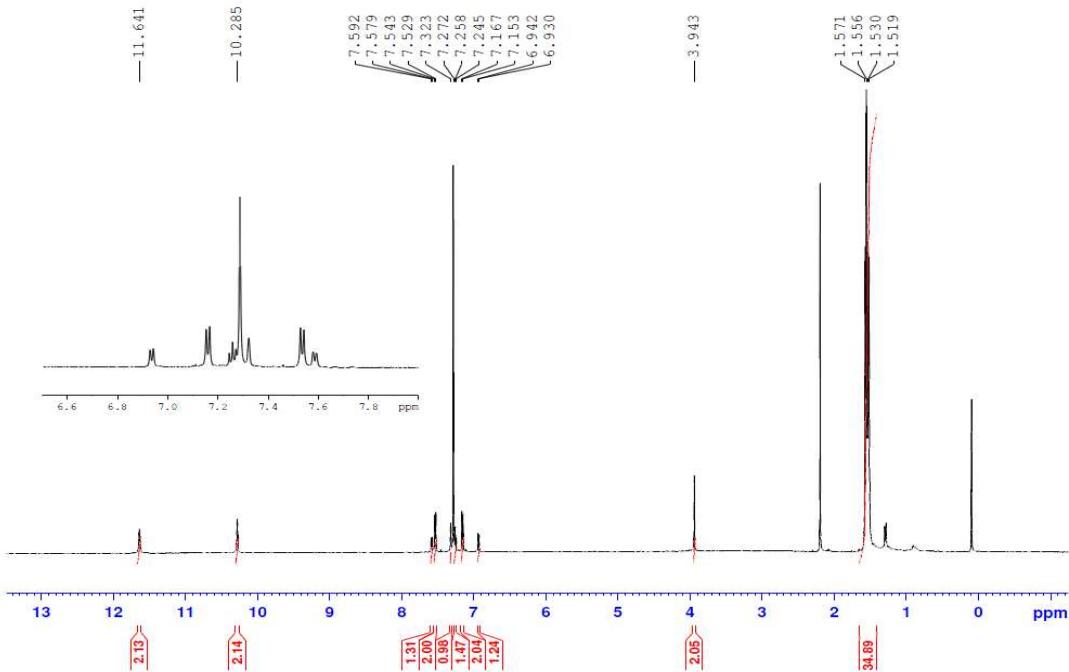
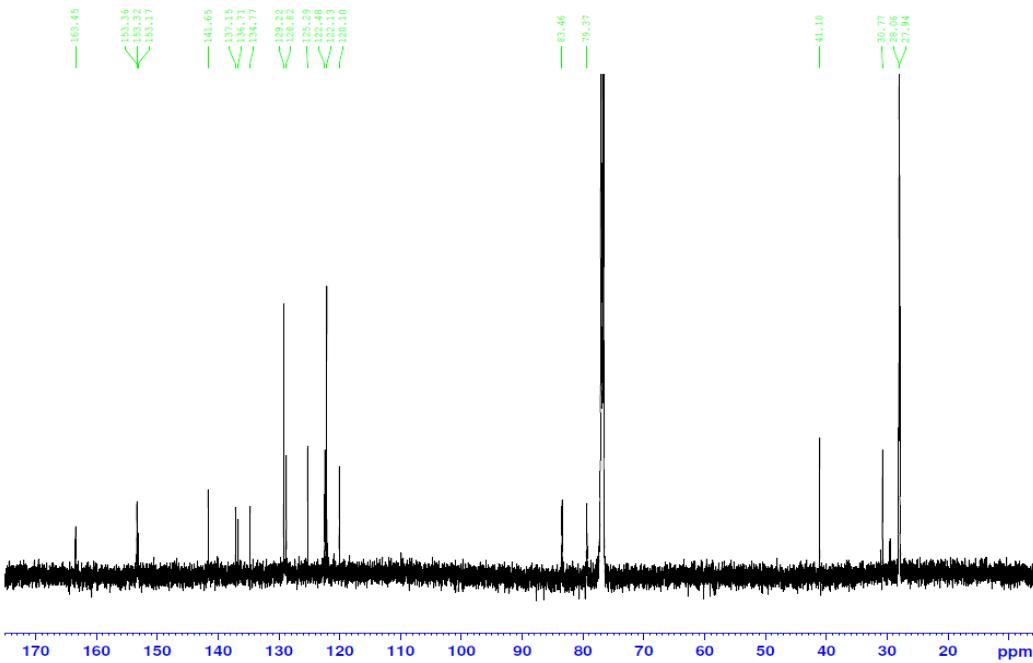
**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**



**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

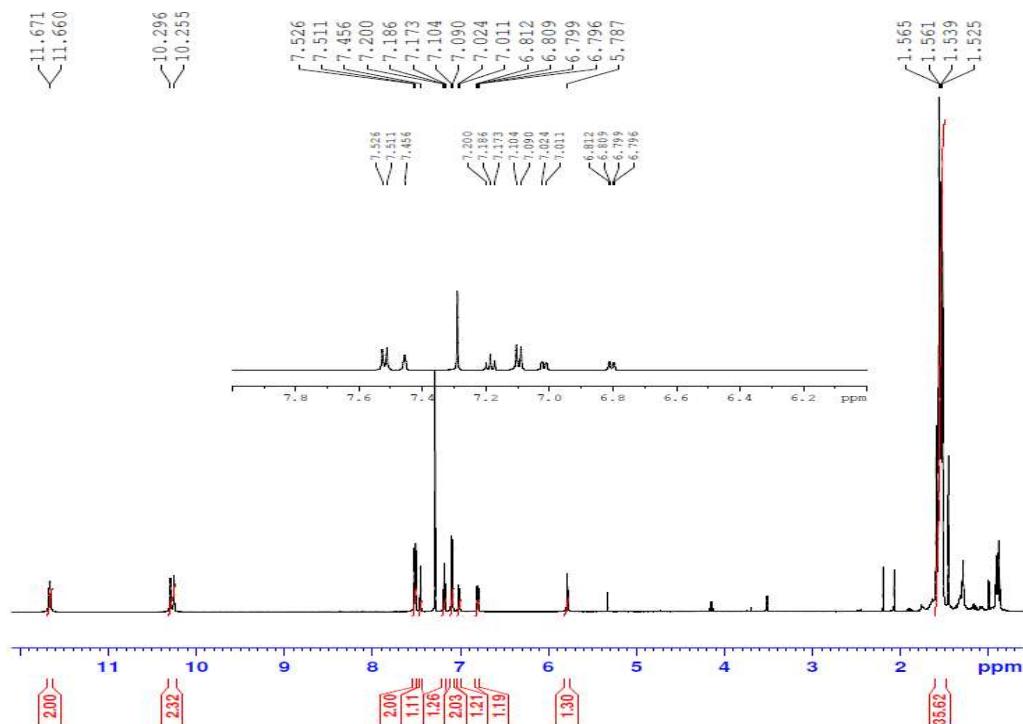


**3,4'-Bis-[2,3-di(*tert*-butoxycarbonyl)-guanidino]-benzophenone (10b)** **$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )** **$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

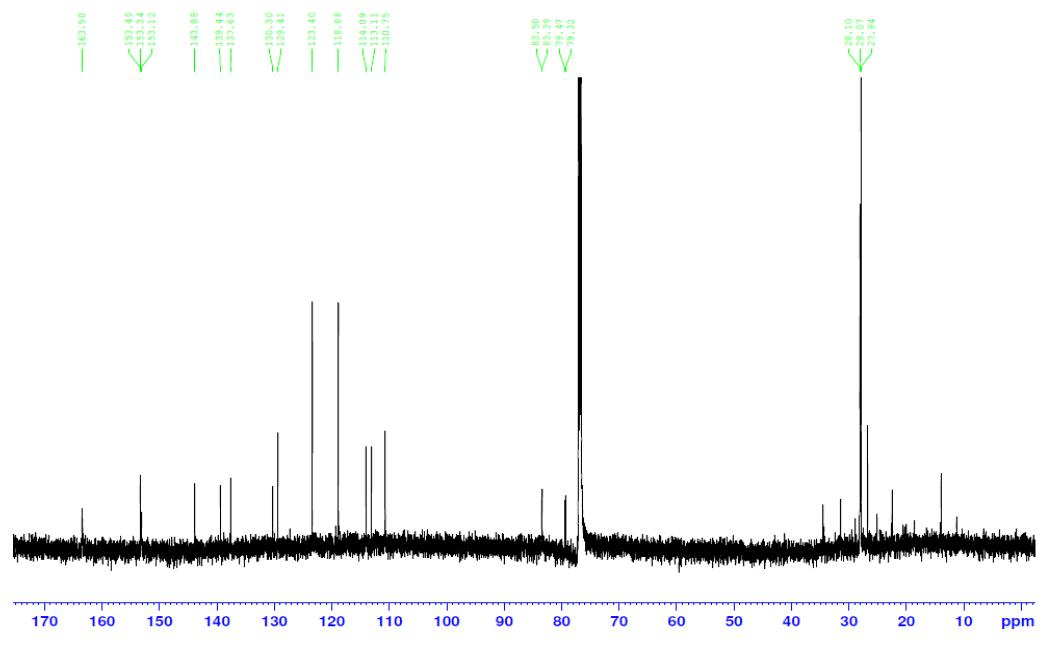
**3,4'-Bis-[2,3-di-(*tert*-butoxycarbonyl)-guanidino]-benzylbenzene (10c)** **$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )** **$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

### 3,4'-Bis-[2,3-di-(tert-butoxycarbonyl)-guanidino]-N-phenylaniline (10d)

### **<sup>1</sup>H-NMR (CDCl<sub>3</sub>)**

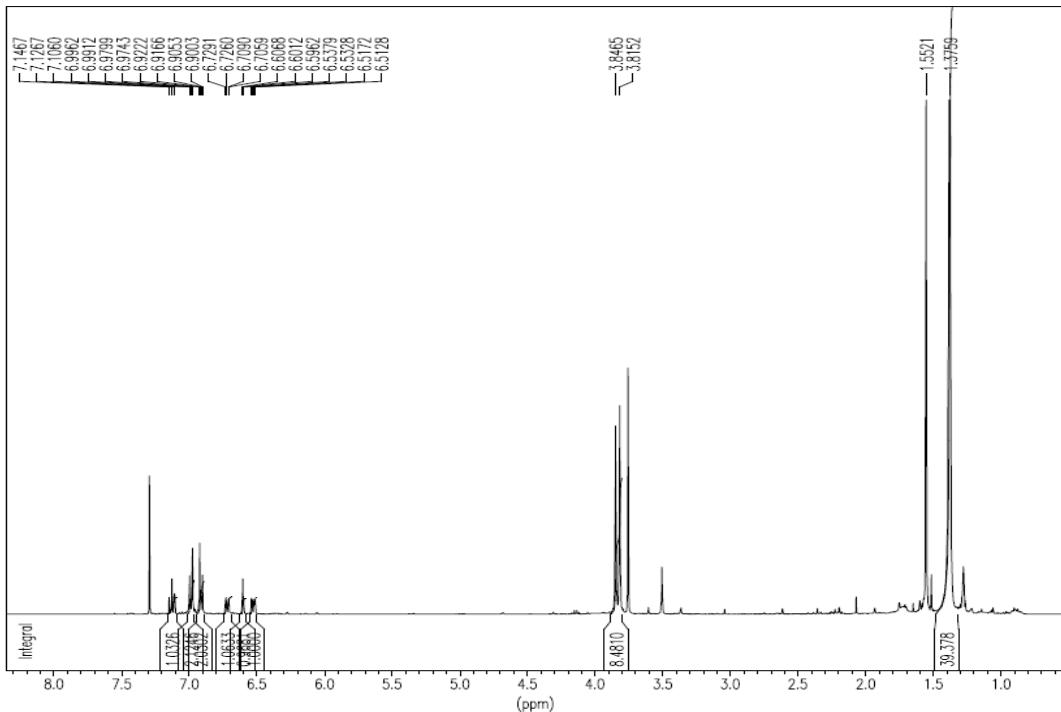


<sup>13</sup>C-NMR ( $\text{CDCl}_3$ )

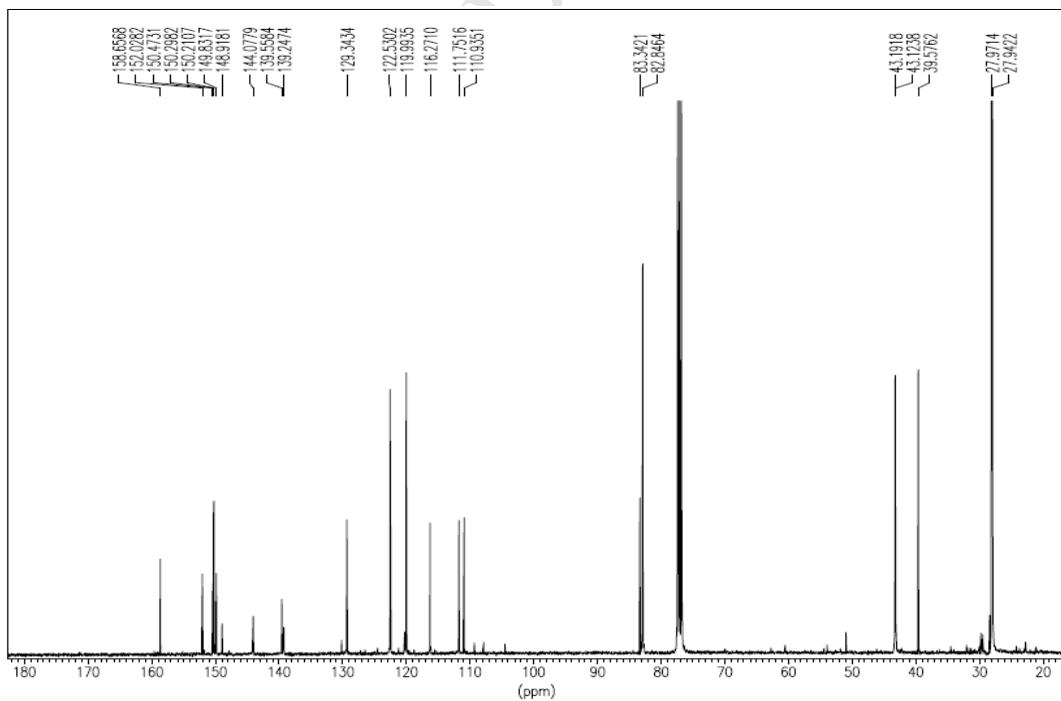


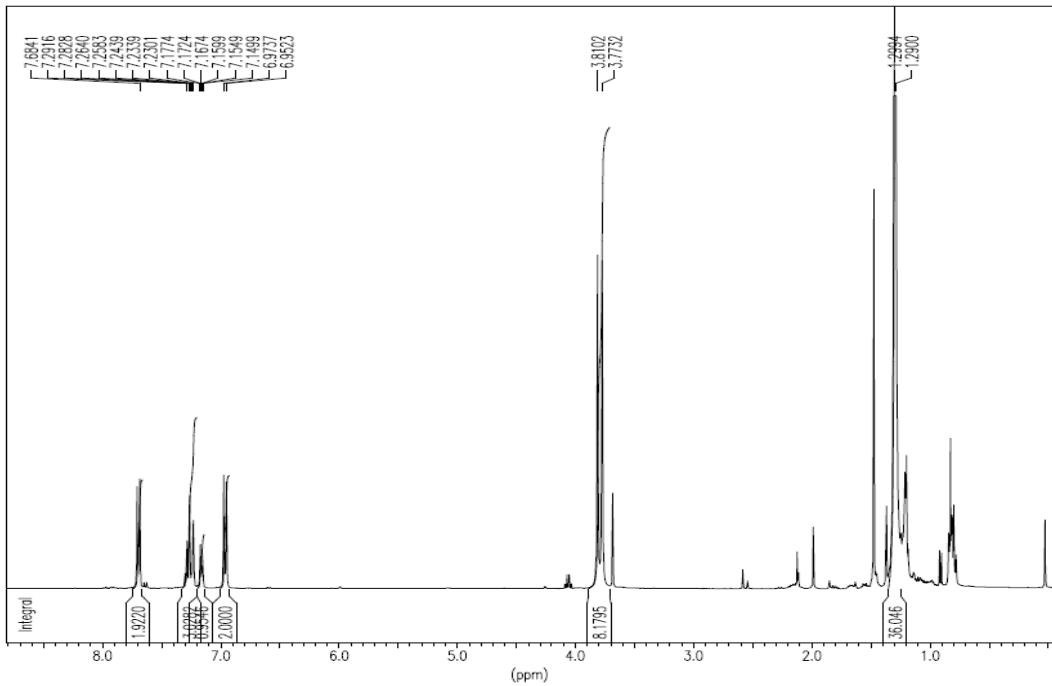
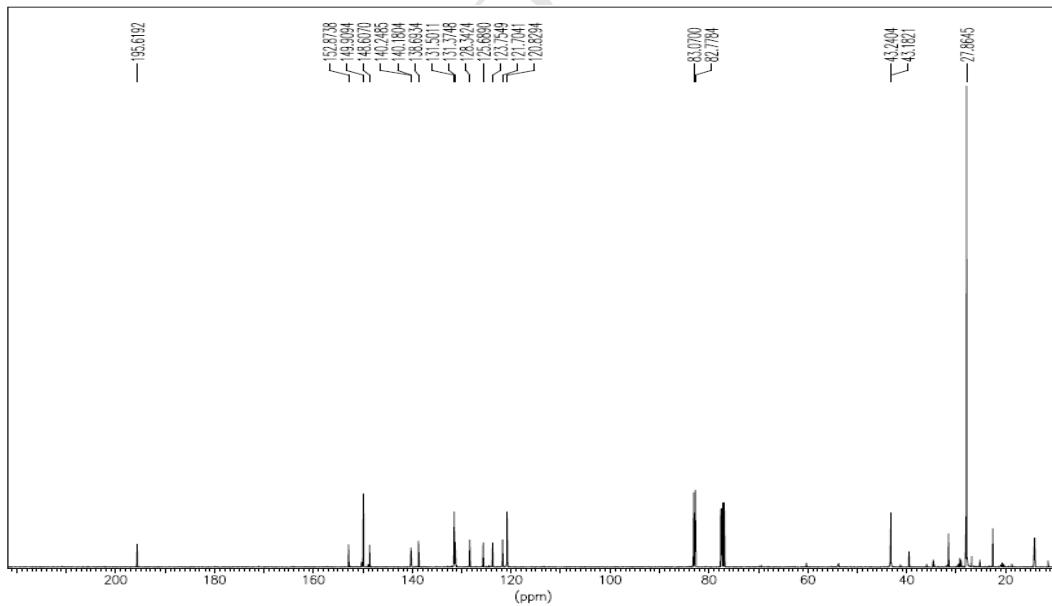
### 3,4'-Bis-[2,3-di-(*tert*-butoxycarbonyl)-N-2-iminoimidazolidinyl]-diphenylether (11a)

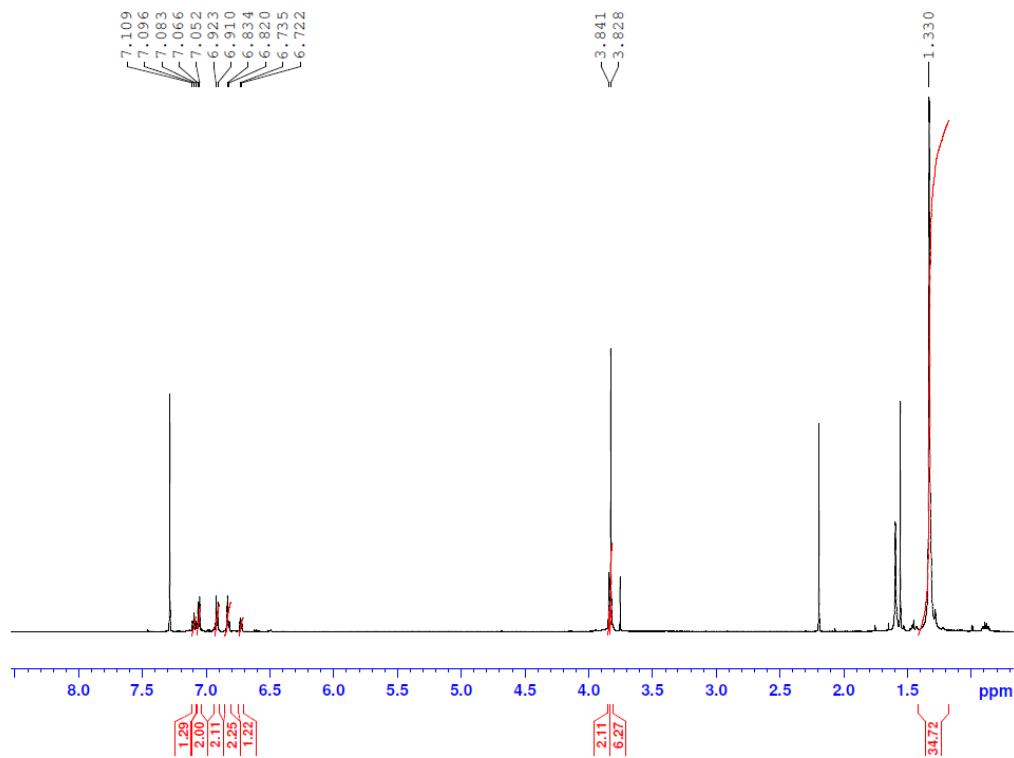
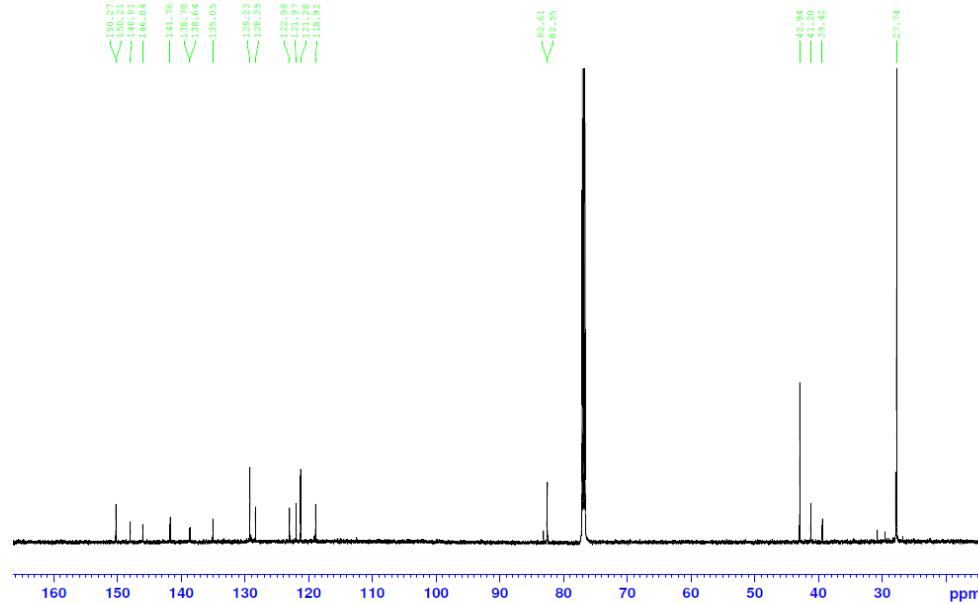
**<sup>1</sup>H-NMR (CDCl<sub>3</sub>)**

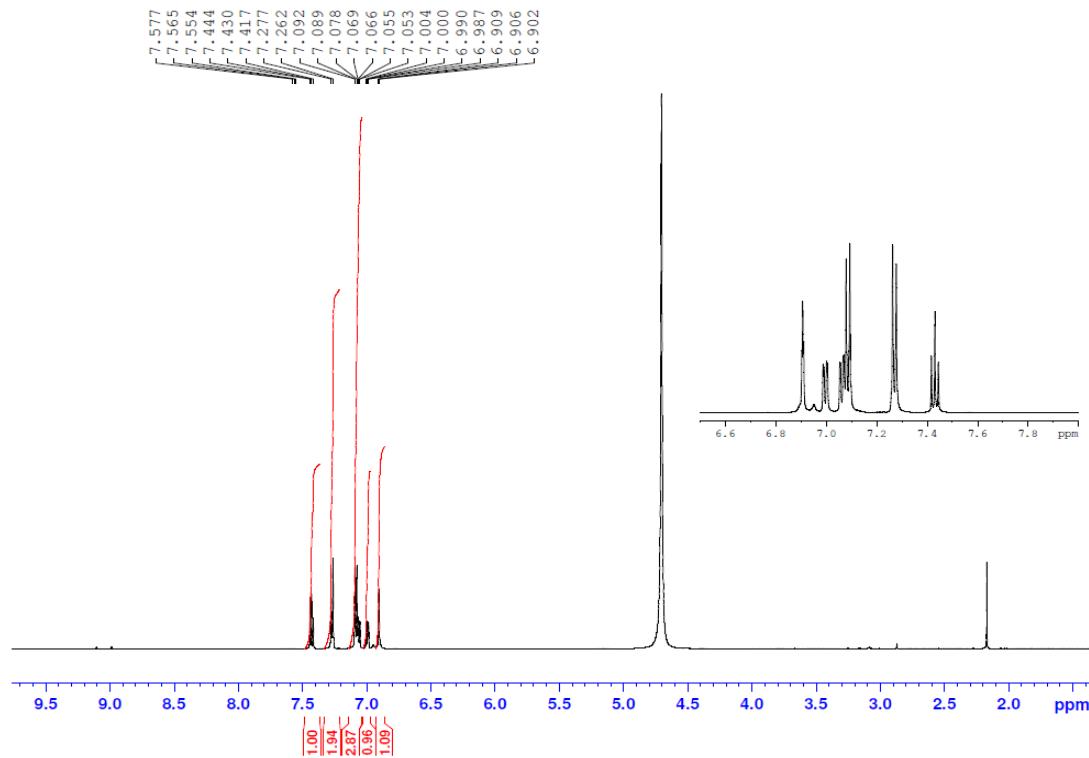
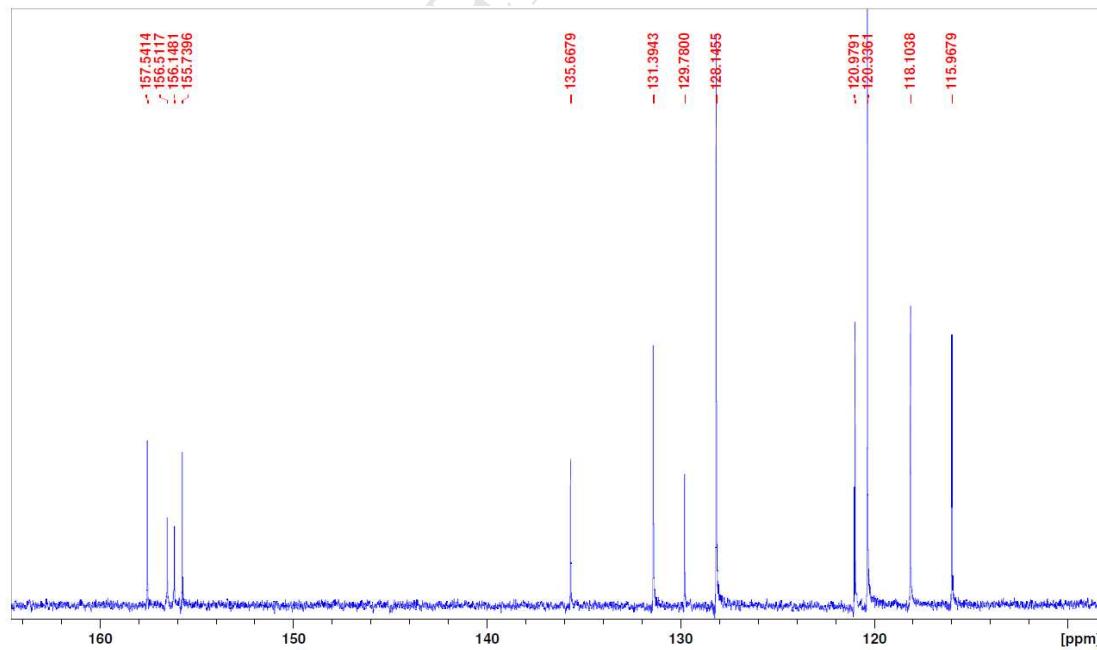


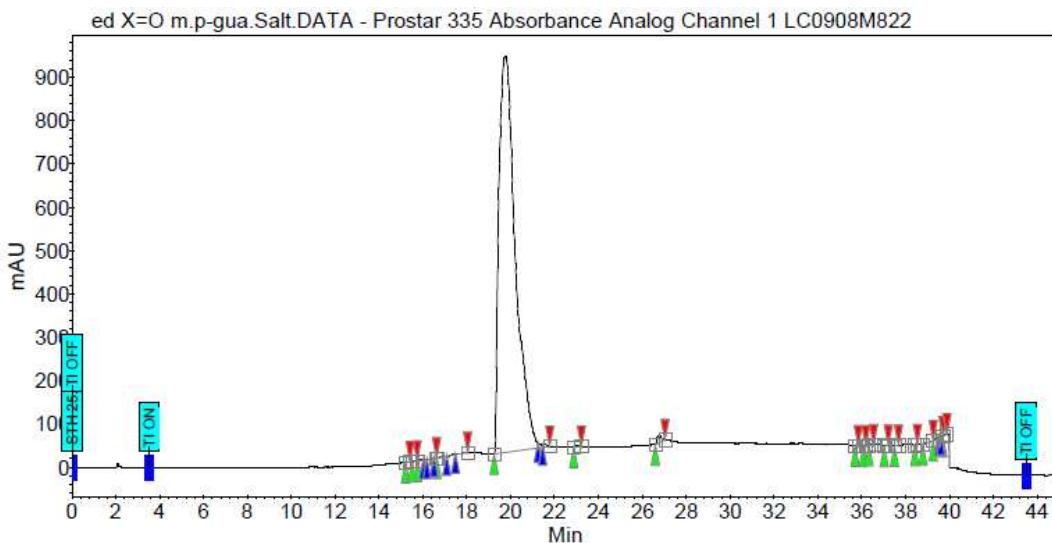
### **<sup>13</sup>C-NMR (CDCl<sub>3</sub>)**



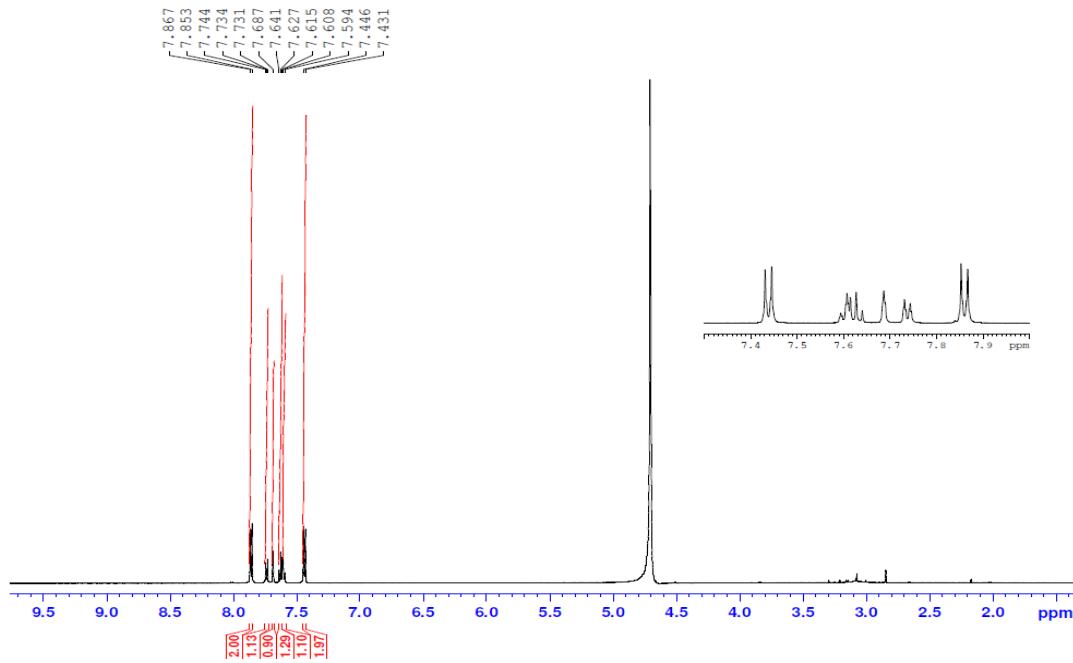
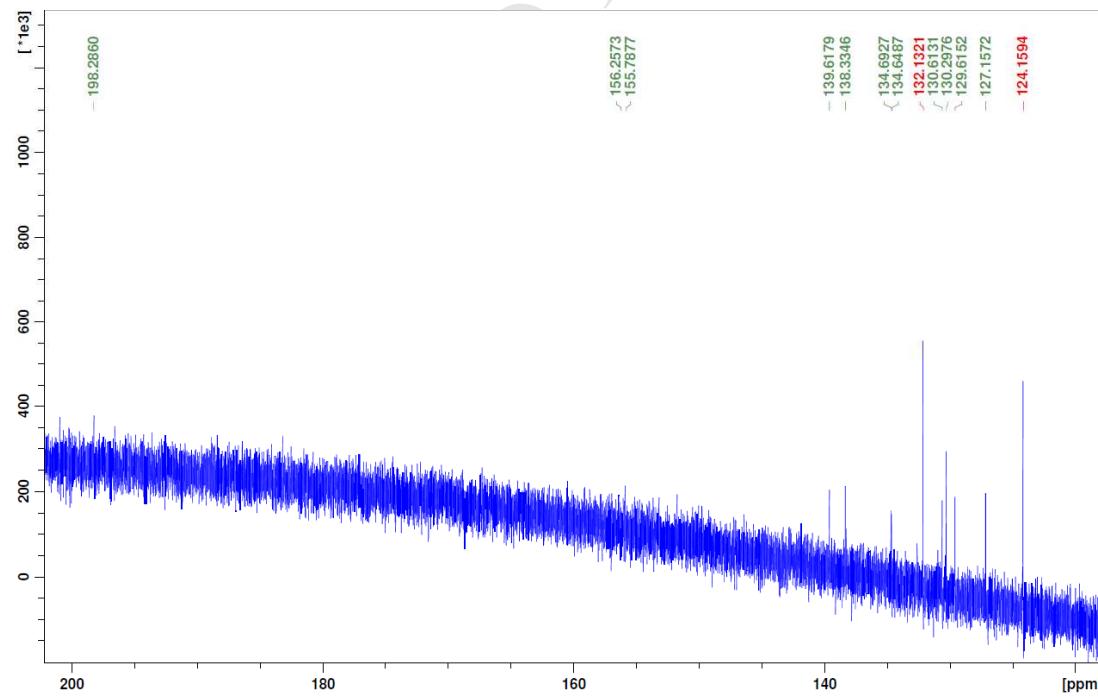
**3,4'-Bis-[2,3-di-(*tert*-butoxycarbonyl)-N-2-iminoimidazolidinyl]-benzophenone (11b)** **$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )** **$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

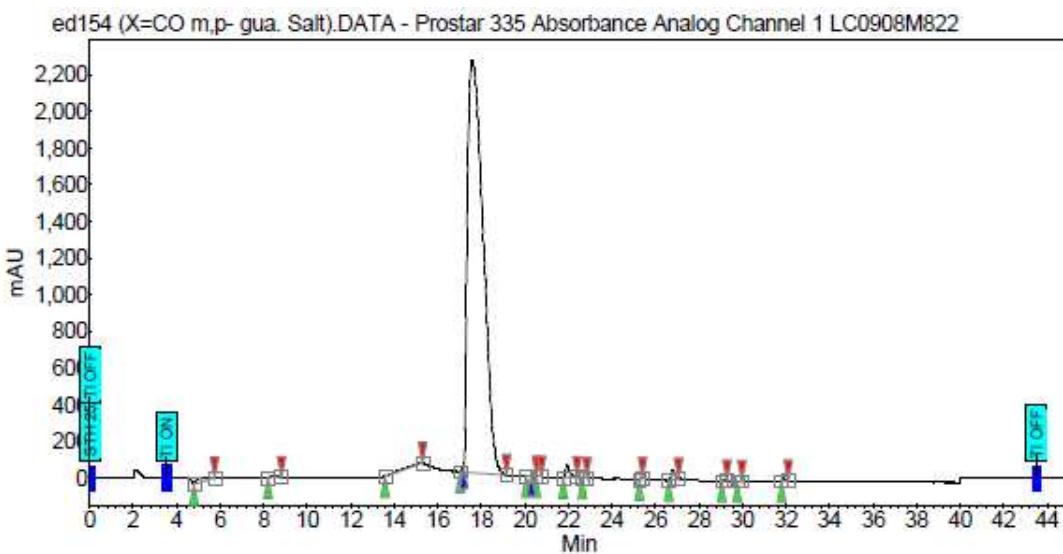
**3,4'-Bis-[2,3-di-(*tert*-butoxycarbonyl)-N-2-iminoimidazolidinyl]-benzylbenzene (11c)** **$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )** **$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

**3-[4-(Guanidino)-phenoxy]-phenylguanidine di-hydrochloride (2a)** **$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )** **$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

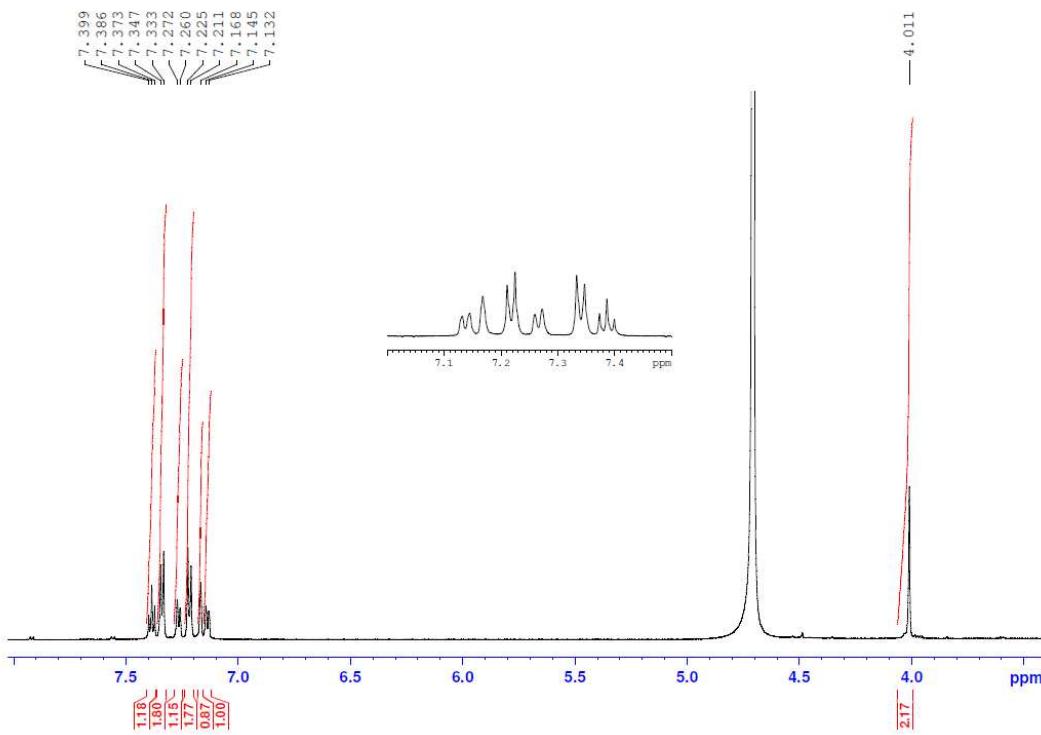
**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	15.29	0.01	0.8	0.1	0.012
2	UNKNOWN	15.61	0.02	1.1	0.1	0.016
3	UNKNOWN	15.89	0.03	2.0	0.3	0.033
4	UNKNOWN	16.11	0.02	1.1	0.1	0.015
5	UNKNOWN	16.36	0.04	1.5	0.3	0.042
6	UNKNOWN	16.51	0.01	1.1	0.1	0.008
7	UNKNOWN	16.81	0.05	1.4	0.4	0.052
8	UNKNOWN	17.40	0.04	1.6	0.4	0.045
9	UNKNOWN	17.91	0.07	1.6	0.6	0.069
10	UNKNOWN	19.76	98.60	914.1	793.7	98.598
11	UNKNOWN	21.31	0.17	9.9	1.4	0.173
12	UNKNOWN	21.56	0.15	7.3	1.2	0.151
13	UNKNOWN	23.04	0.08	3.8	0.6	0.078
14	UNKNOWN	26.76	0.44	17.1	3.5	0.440
15	UNKNOWN	35.76	0.00	0.5	0.0	0.004
16	UNKNOWN	36.13	0.00	0.4	0.0	0.003
17	UNKNOWN	36.43	0.01	1.0	0.1	0.012
18	UNKNOWN	37.13	0.01	0.7	0.1	0.011
19	UNKNOWN	37.55	0.01	0.7	0.1	0.009
20	UNKNOWN	38.47	0.00	0.6	0.0	0.004
21	UNKNOWN	39.19	0.04	1.4	0.3	0.038
22	UNKNOWN	39.47	0.08	4.5	0.7	0.085
23	UNKNOWN	39.56	0.05	4.1	0.4	0.048
24	UNKNOWN	39.61	0.02	2.5	0.1	0.018
25	UNKNOWN	39.76	0.03	2.3	0.3	0.035
Total			100.00	983.2	805.0	100.000

**3-[4-(Guanidino)-benzoyl]-phenylguanidine di-hydrochloride (2b)** **$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )** **$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

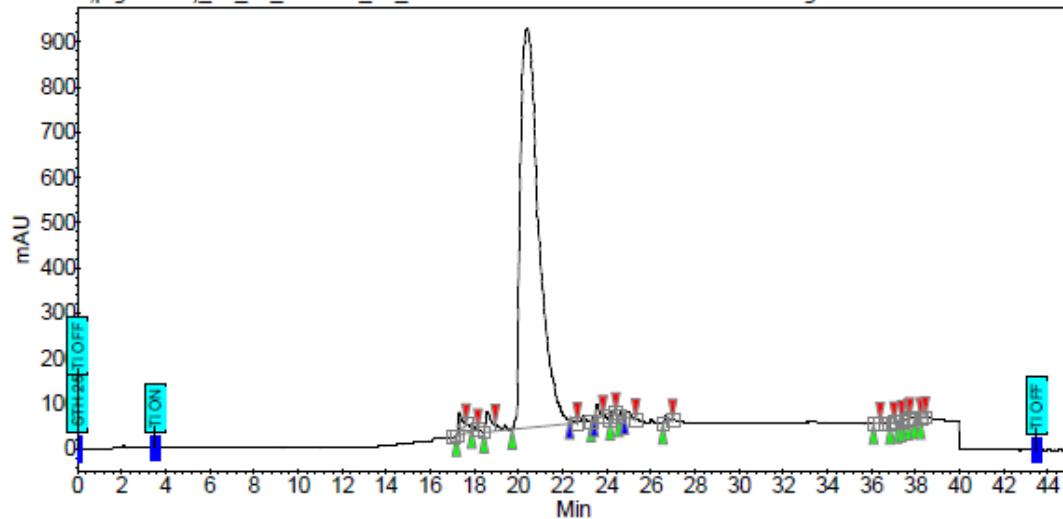
**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU·Min]	Area % [%]
1	UNKNOWN	5.04	0.65	22.4	12.4	0.649
2	UNKNOWN	8.51	0.22	13.2	4.1	0.215
3	UNKNOWN	14.19	0.35	5.8	6.6	0.347
4	UNKNOWN	17.15	0.10	19.4	1.8	0.096
5	UNKNOWN	17.50	97.29	2247.5	1861.2	97.290
6	UNKNOWN	20.13	0.02	4.3	0.5	0.024
7	UNKNOWN	20.37	0.02	3.7	0.4	0.022
8	UNKNOWN	20.83	0.03	4.2	0.5	0.028
9	UNKNOWN	21.93	0.69	68.3	13.3	0.695
10	UNKNOWN	22.71	0.02	3.4	0.4	0.019
11	UNKNOWN	25.32	0.02	3.8	0.3	0.015
12	UNKNOWN	26.77	0.47	51.2	8.9	0.466
13	UNKNOWN	29.16	0.02	3.9	0.4	0.023
14	UNKNOWN	29.85	0.02	3.8	0.4	0.023
15	UNKNOWN	31.91	0.09	12.4	1.7	0.087
Total			100.00	2487.1	1913.1	100.000

**3-[4-(Guanidino)-benzyl]-phenylguanidine di-hydrochloride (2c)** **$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**

**HPLC**

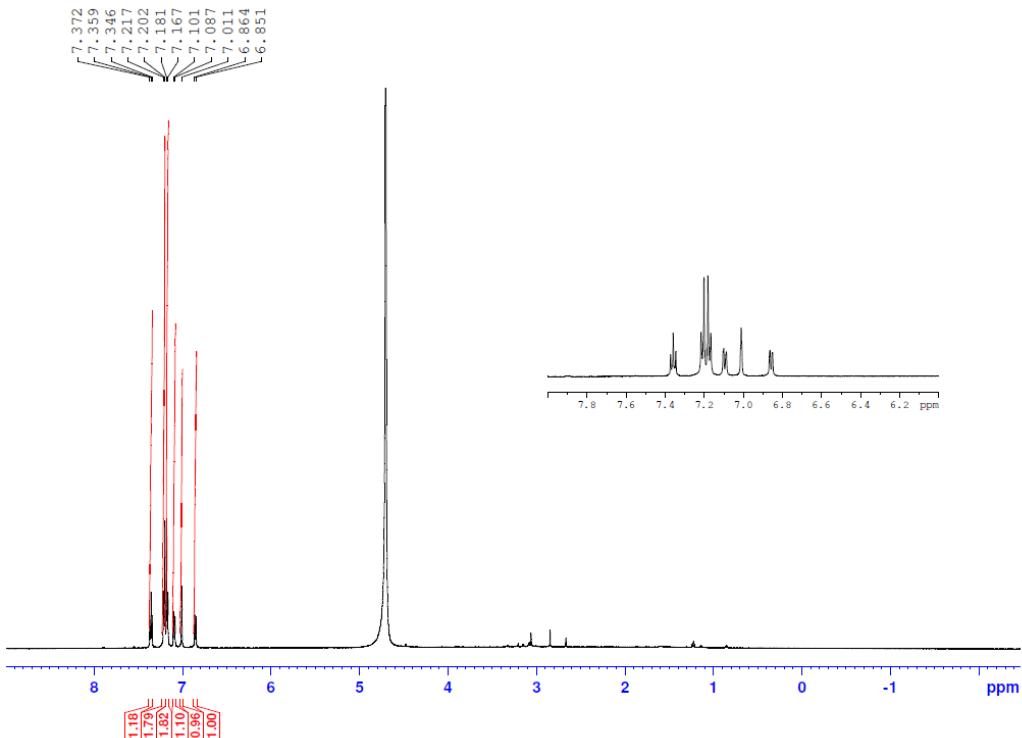
ed(X=CH2 m,p-gua.Salt)\_17\_10\_2011 16\_37\_41.DATA - Prostar 335 Absorbance Analog Channel 1 LC0908M822

**Peak results :**

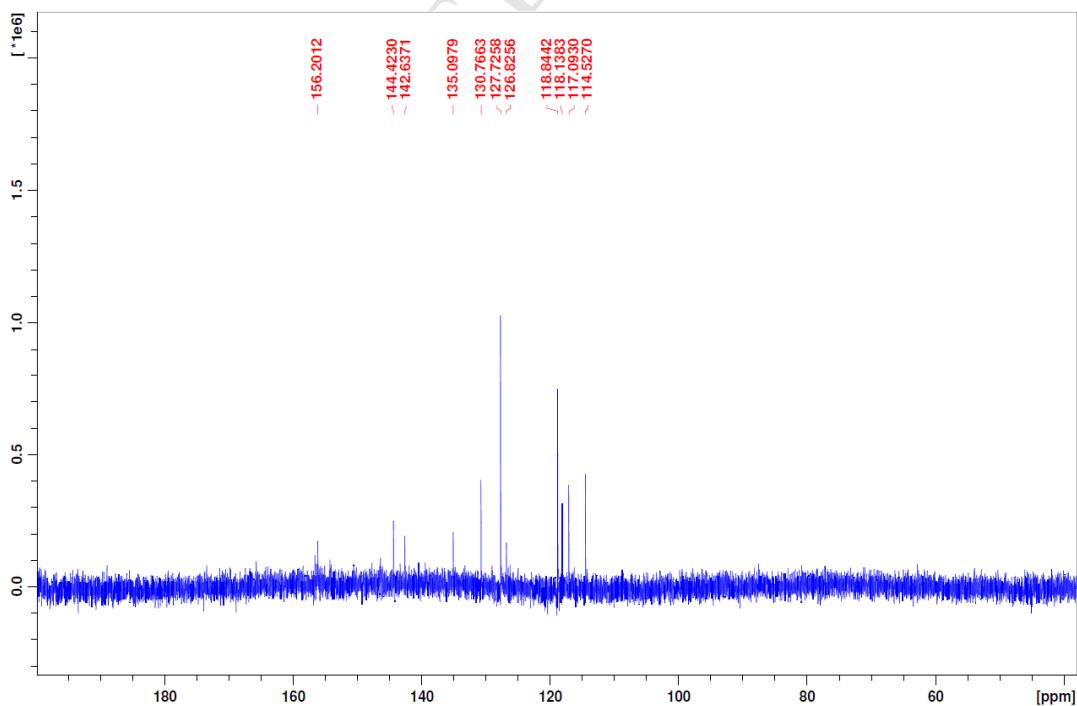
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
18	UNKNOWN	17.31	1.07	45.3	9.5	1.087
1	UNKNOWN	17.92	0.16	9.2	1.4	0.162
2	UNKNOWN	18.59	1.46	44.7	12.9	1.456
3	UNKNOWN	20.41	95.07	882.5	843.2	95.070
4	UNKNOWN	22.37	0.21	9.2	1.9	0.212
5	UNKNOWN	23.37	0.06	6.5	0.6	0.064
6	UNKNOWN	23.53	0.85	31.3	5.7	0.846
7	UNKNOWN	24.24	0.21	14.8	1.8	0.207
8	UNKNOWN	24.67	0.19	10.5	1.7	0.193
9	UNKNOWN	24.89	0.44	14.4	3.9	0.444
10	UNKNOWN	26.69	0.33	14.4	2.9	0.326
11	UNKNOWN	36.32	0.03	1.2	0.2	0.028
12	UNKNOWN	36.91	0.01	0.8	0.1	0.010
13	UNKNOWN	37.25	0.01	1.0	0.1	0.014
14	UNKNOWN	37.52	0.02	1.6	0.2	0.020
15	UNKNOWN	37.71	0.00	0.7	0.0	0.004
16	UNKNOWN	38.08	0.05	3.3	0.5	0.053
17	UNKNOWN	38.35	0.02	1.8	0.2	0.023
Total			100.00	1093.1	886.9	100.000

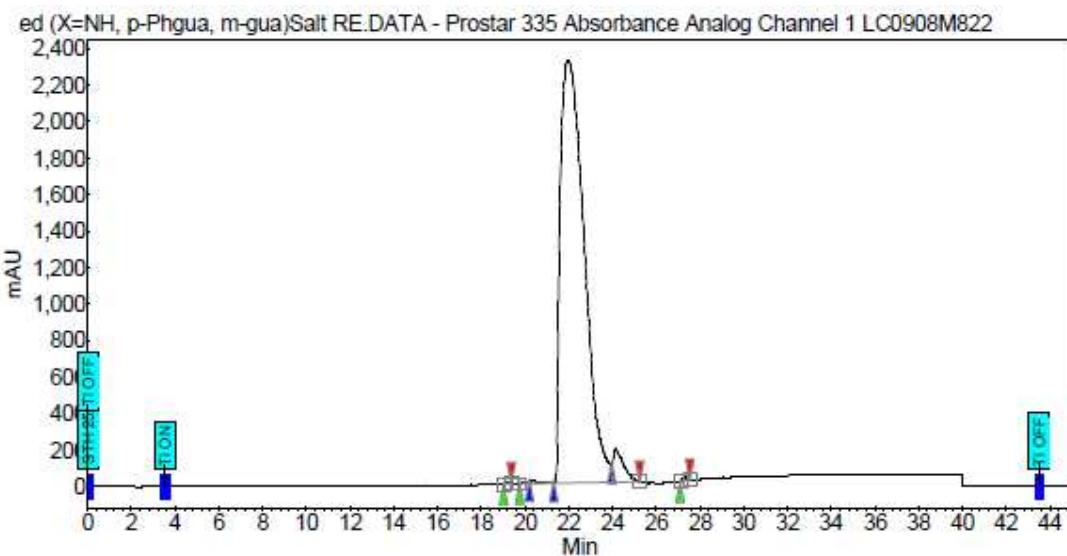
**3-[4-(Guanidino)-phenylamino]-phenylguanidine di-hydrochloride (2d)**

**$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**



**$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**



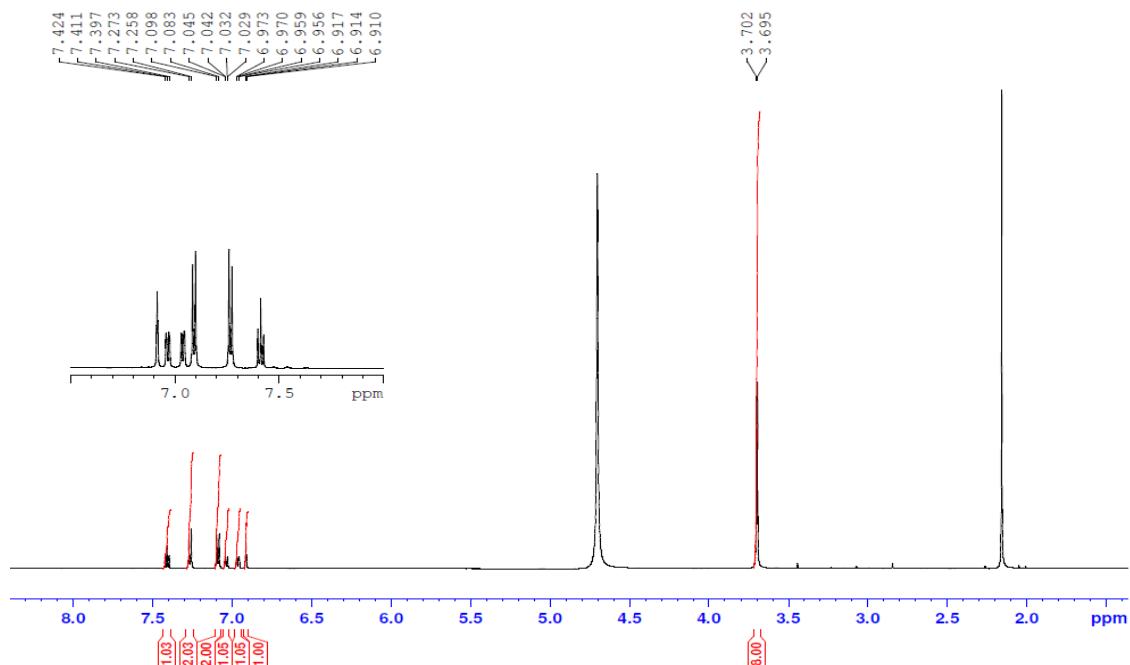
**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	19.16	0.06	10.3	1.9	0.061
2	UNKNOWN	20.01	0.11	10.3	3.4	0.113
3	UNKNOWN	20.36	0.25	15.2	7.7	0.254
4	UNKNOWN	21.99	96.05	2312.2	2912.1	96.050
5	UNKNOWN	24.13	3.37	182.6	102.0	3.366
6	UNKNOWN	27.28	0.18	23.5	4.7	0.156
Total			100.00	2554.2	3031.9	100.000

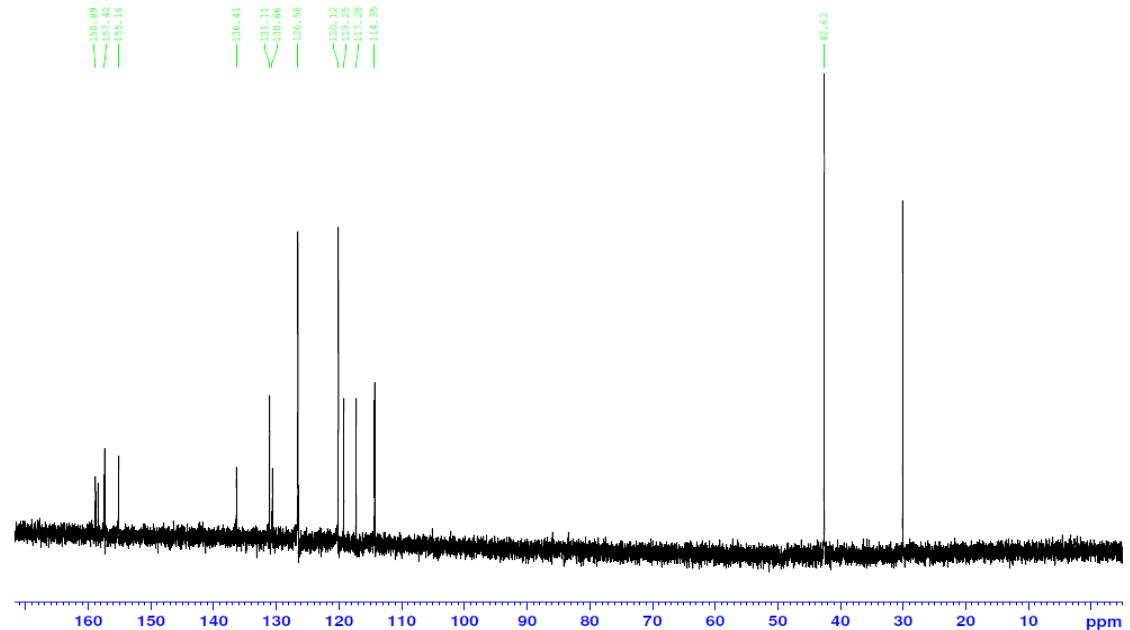
**3-[4-N-(Amino-2-imidazolinyl)phenoxy]-phenylamino-2-imidazoline di-hydrochloride**

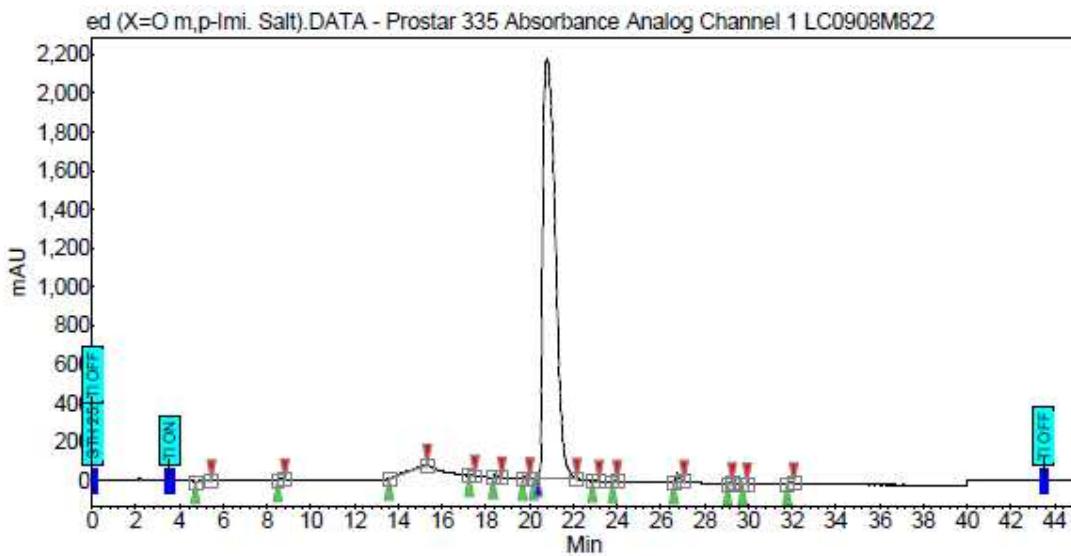
**(3a)**

**$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**

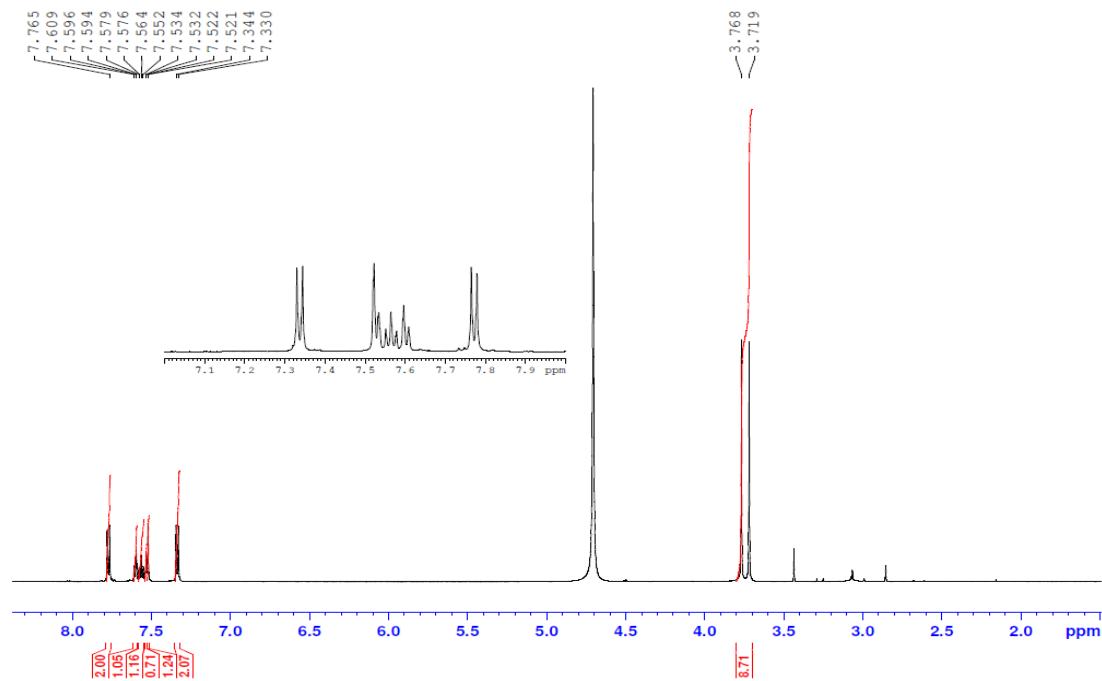
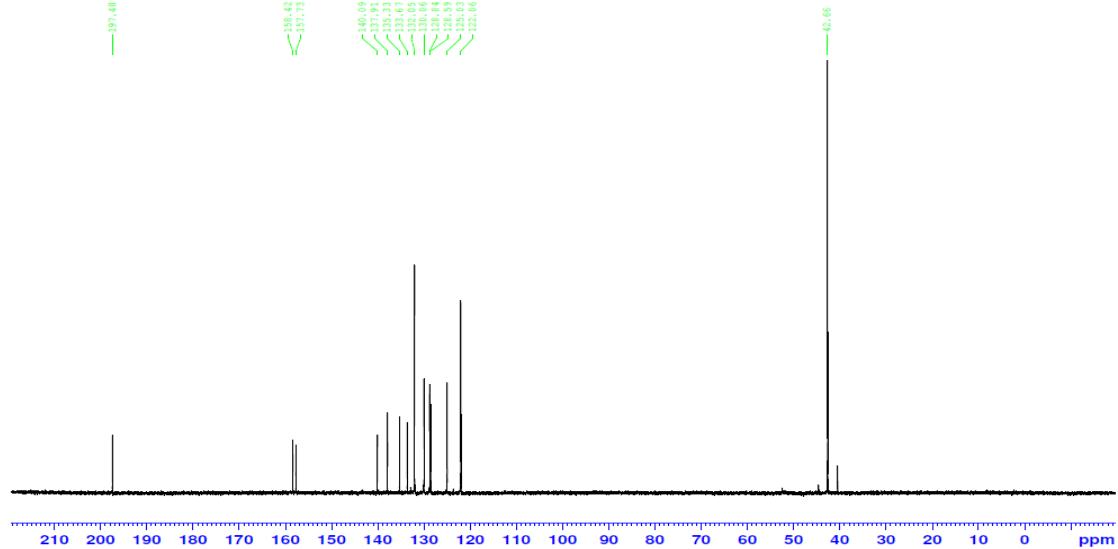


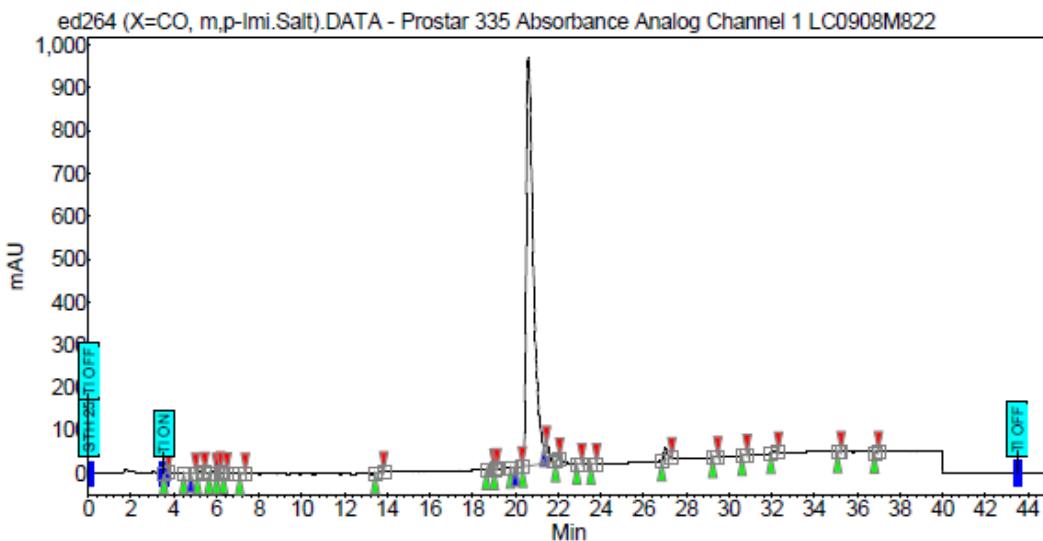
**$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**



**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	4.95	0.15	4.8	2.1	0.146
2	UNKNOWN	8.64	0.07	6.1	1.1	0.073
3	UNKNOWN	14.47	0.46	5.8	6.7	0.485
4	UNKNOWN	17.33	0.06	7.7	0.9	0.063
5	UNKNOWN	18.45	0.24	23.3	3.5	0.241
6	UNKNOWN	19.79	0.15	16.9	2.1	0.148
7	UNKNOWN	20.35	0.08	11.2	1.2	0.081
8	UNKNOWN	20.79	98.03	2167.6	1421.6	98.028
9	UNKNOWN	23.00	0.05	5.5	0.8	0.052
10	UNKNOWN	23.91	0.03	3.7	0.4	0.030
11	UNKNOWN	26.76	0.53	44.2	7.7	0.529
12	UNKNOWN	29.15	0.02	3.2	0.3	0.022
13	UNKNOWN	29.83	0.03	3.3	0.4	0.026
14	UNKNOWN	31.89	0.10	10.9	1.4	0.097
Total			100.00	2314.2	1450.2	100.000

**3-[4-N-(Amino-2-imidazolinyl)-benzoyl]-phenylamino-2-imidazoline di-hydrochloride****(3b)** **$^1\text{H}$ -NMR ( $\text{D}_2\text{O}$ )** **$^{13}\text{C}$ -NMR ( $\text{D}_2\text{O}$ )**

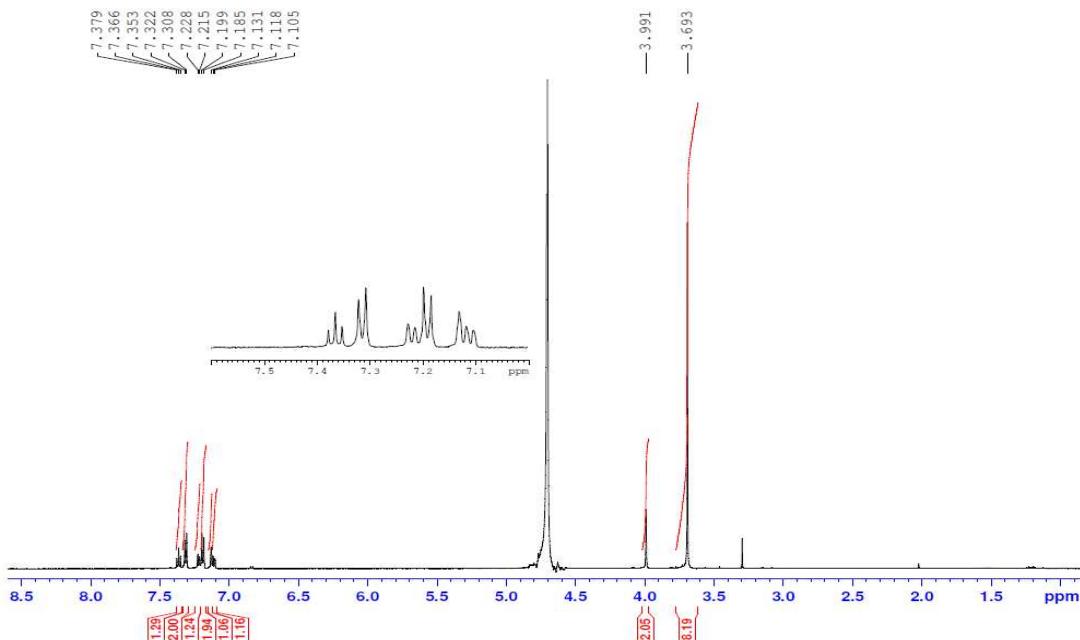
**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	3.61	0.03	0.9	0.1	0.030
2	UNKNOWN	4.64	0.11	2.2	0.4	0.112
3	UNKNOWN	4.88	0.06	1.8	0.2	0.056
4	UNKNOWN	5.28	0.20	4.4	0.8	0.200
5	UNKNOWN	5.76	0.17	4.4	0.7	0.175
6	UNKNOWN	6.08	0.05	2.1	0.2	0.052
7	UNKNOWN	6.40	0.02	1.0	0.1	0.024
8	UNKNOWN	7.19	0.04	1.2	0.2	0.041
9	UNKNOWN	13.64	0.09	1.6	0.4	0.094
10	UNKNOWN	18.80	0.08	1.6	0.3	0.077
11	UNKNOWN	19.05	0.01	0.8	0.0	0.012
12	UNKNOWN	19.84	0.03	0.8	0.1	0.029
13	UNKNOWN	20.07	0.07	1.5	0.3	0.073
14	UNKNOWN	20.59	95.92	951.7	370.9	95.917
15	UNKNOWN	21.40	1.25	42.3	4.8	1.248
16	UNKNOWN	21.98	0.08	2.9	0.3	0.077
17	UNKNOWN	22.99	0.04	1.4	0.2	0.041
18	UNKNOWN	23.64	0.07	1.7	0.3	0.065
19	UNKNOWN	27.01	1.31	27.9	5.1	1.315
20	UNKNOWN	29.37	0.06	1.9	0.2	0.058
21	UNKNOWN	30.73	0.04	1.5	0.2	0.044
22	UNKNOWN	32.12	0.21	4.9	0.8	0.210
23	UNKNOWN	35.17	0.01	0.6	0.1	0.014
24	UNKNOWN	36.88	0.04	1.2	0.1	0.035
Total			100.00	1062.4	386.7	100.000

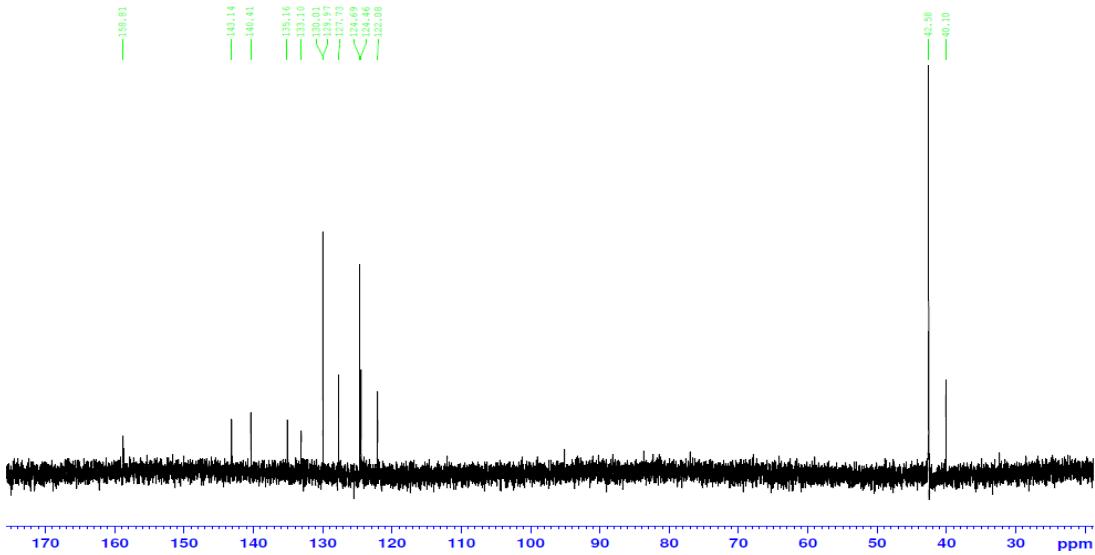
**3-[4-N-(Amino-2-imidazolinyl)-benzyl]-phenylamino-2-imidazoline di-hydrochloride**

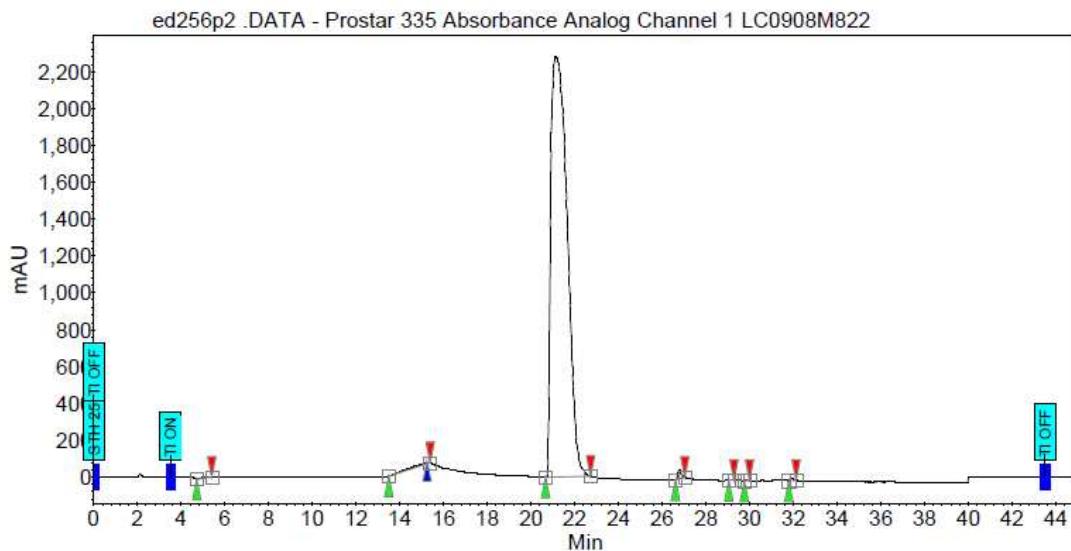
**(3c)**

**$^1\text{H}$ -NMR ( $\text{D}_2\text{O}$ )**

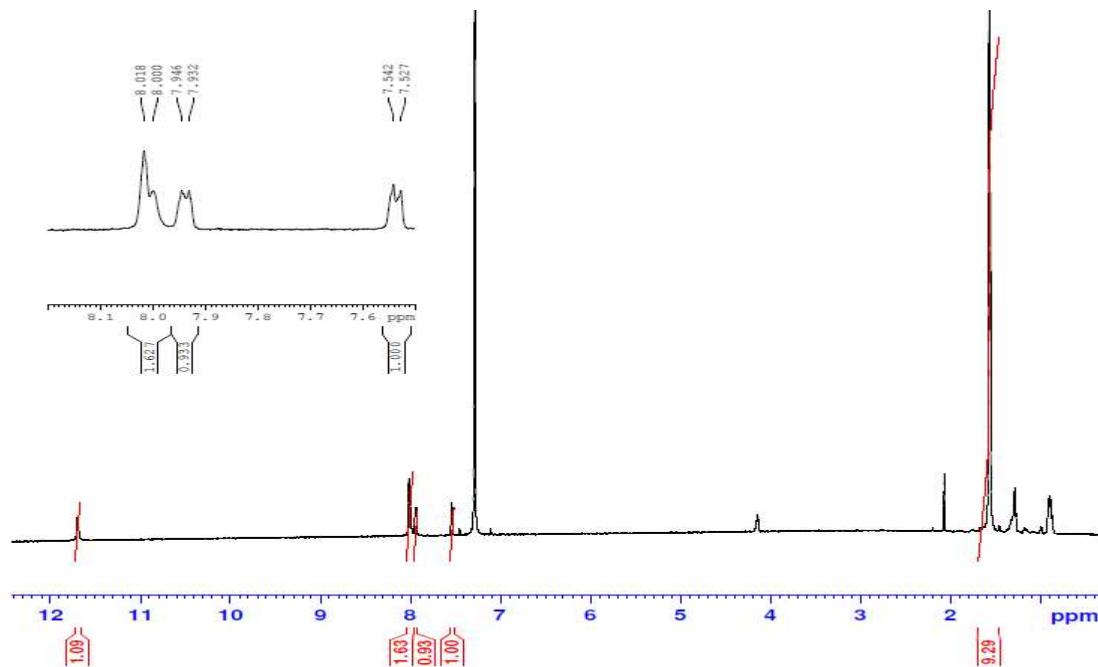
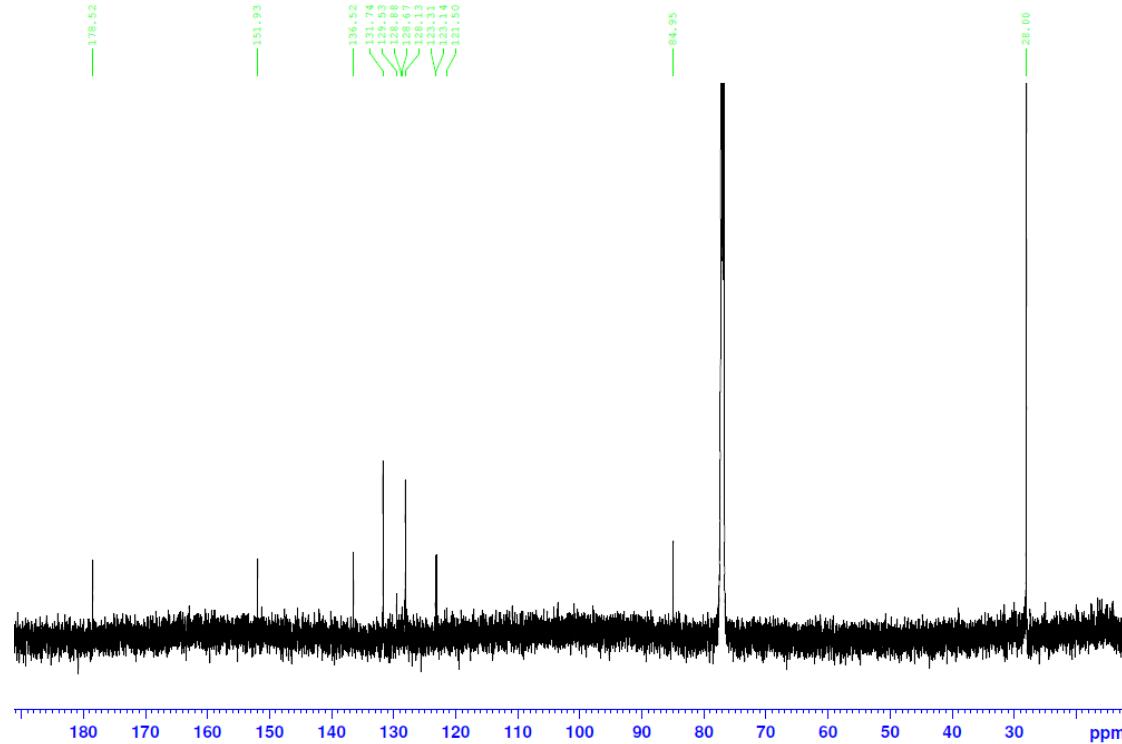


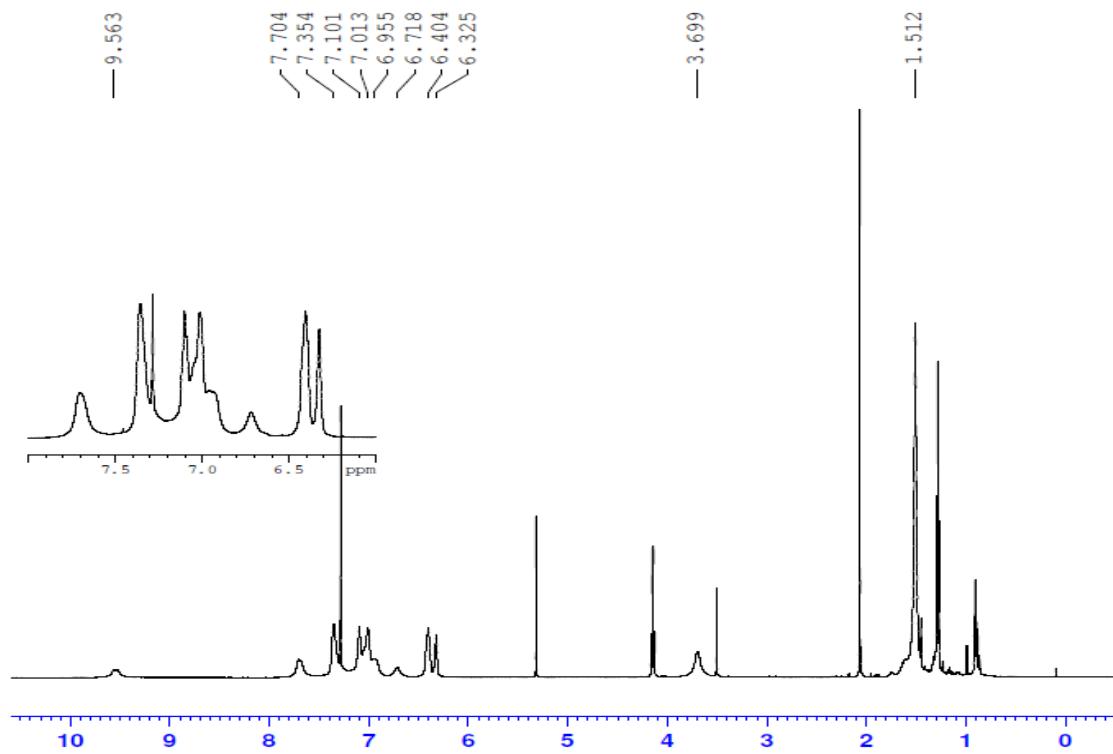
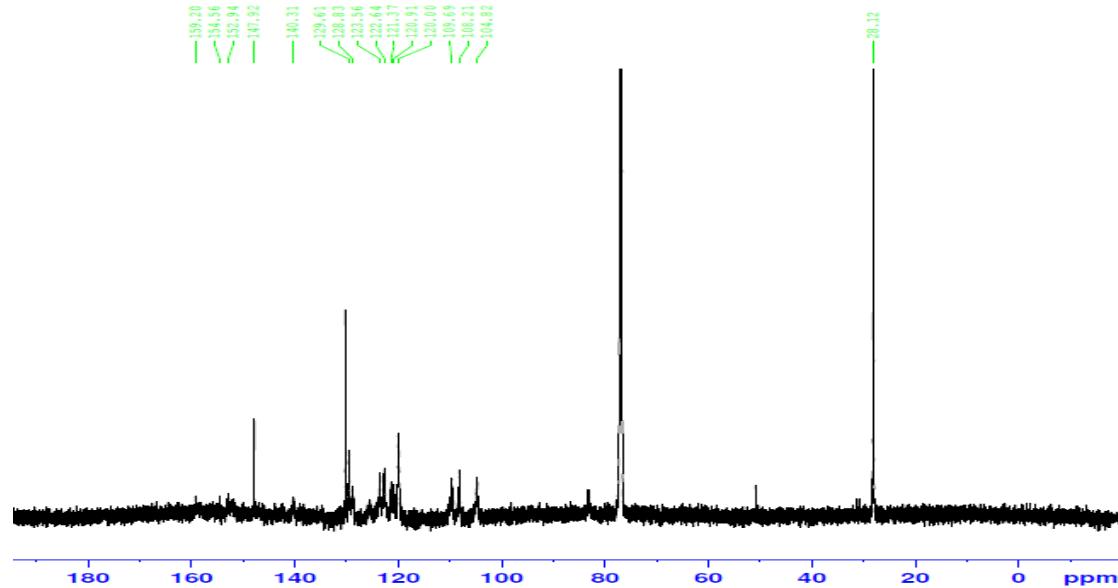
**$^{13}\text{C}$ -NMR ( $\text{D}_2\text{O}$ )**

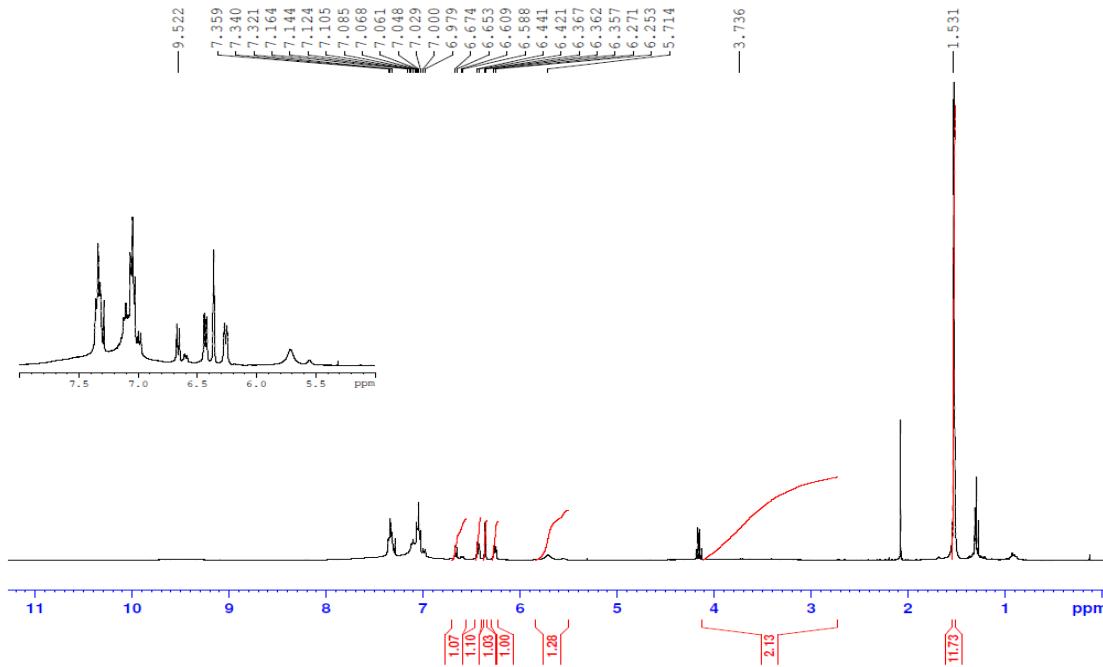
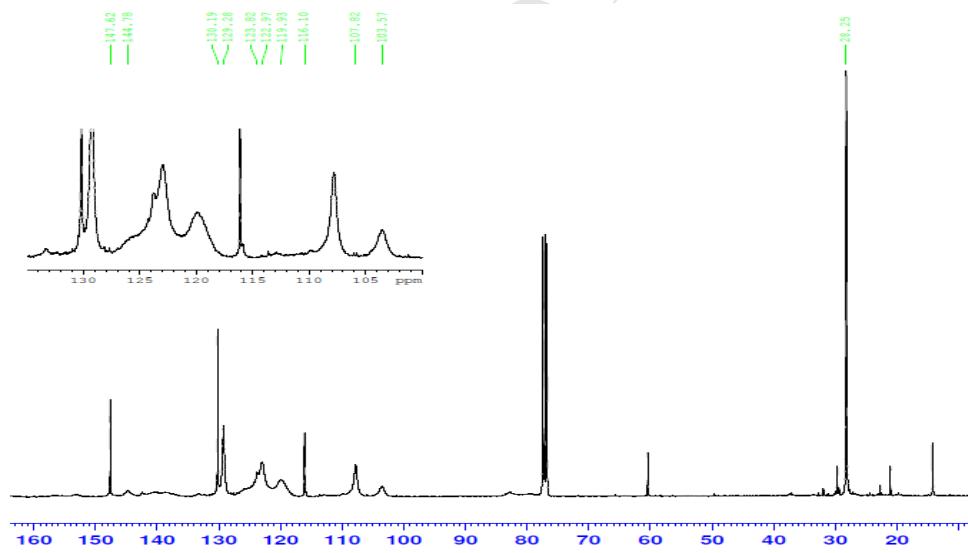


**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area	
					[mAU.Min]	Area % [%]
1	UNKNOWN	4.95	0.11	5.6	2.3	0.115
2	UNKNOWN	15.13	0.53	9.5	10.5	0.529
3	UNKNOWN	15.25	0.03	7.8	0.6	0.029
4	UNKNOWN	21.13	98.78	2277.5	1968.4	98.778
5	UNKNOWN	26.77	0.42	49.4	8.3	0.417
6	UNKNOWN	29.17	0.02	4.0	0.4	0.022
7	UNKNOWN	29.87	0.02	3.6	0.4	0.023
8	UNKNOWN	31.92	0.09	12.9	1.7	0.087
Total			100.00	2370.2	1992.8	100.000

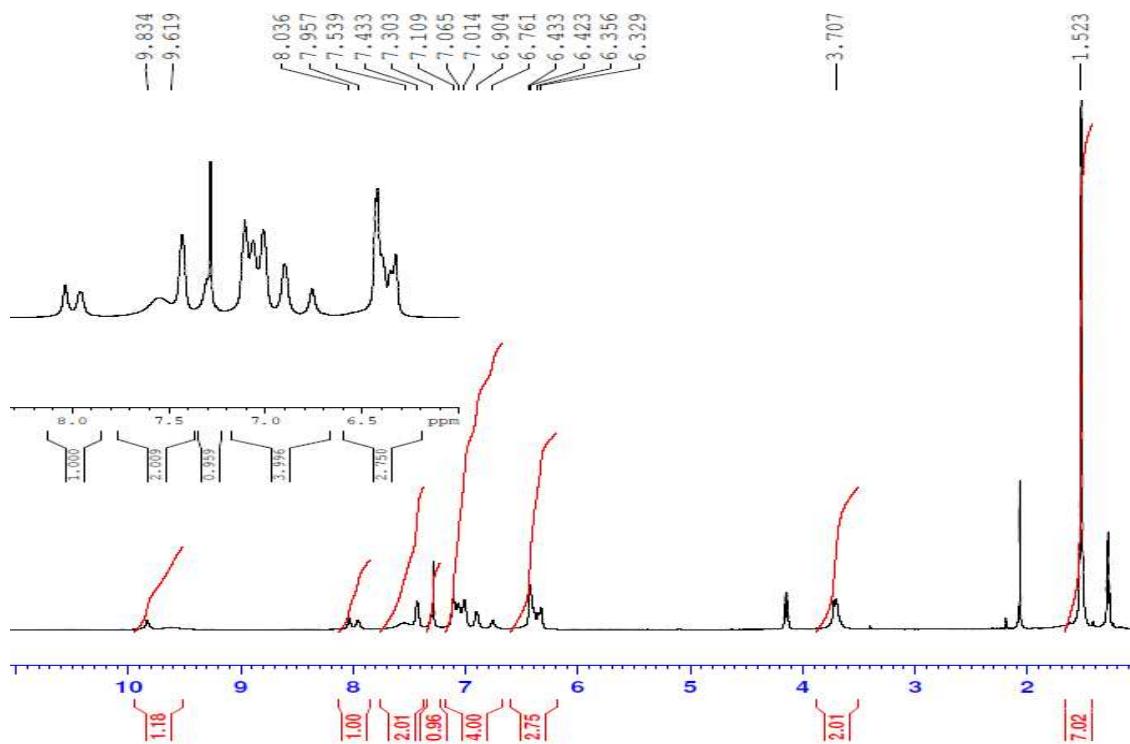
***N*-(4-Chloro-3-trifluoromethylphenyl)-*N'*-(*tert*-butoxycarbonyl)-thiourea (13)** **$^1\text{H}$ -NMR ( $\text{CDCl}_3$ )** **$^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )**

**3-Amino-4'-[2-(*tert*-butoxycarbonyl)-3-phenylguanidino]diphenylether (**14a**).**<sup>1</sup>H-NMR (CDCl<sub>3</sub>)<sup>13</sup>C-NMR (CDCl<sub>3</sub>)

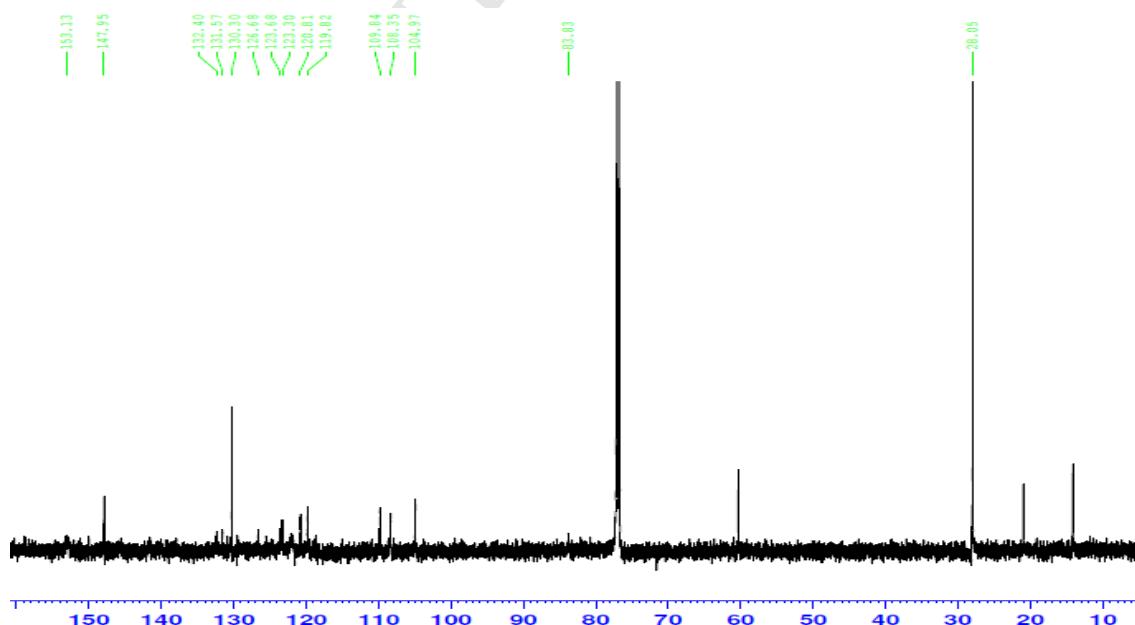
**3-Amino-4'-[2-(*tert*-butoxycarbonyl)-3-phenylguanidino]-N-phenylaniline (14d)** **$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )** **$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

**3-Amino-4'-(2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethyl)-guanidino]-diphenylether (15a)**

**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**

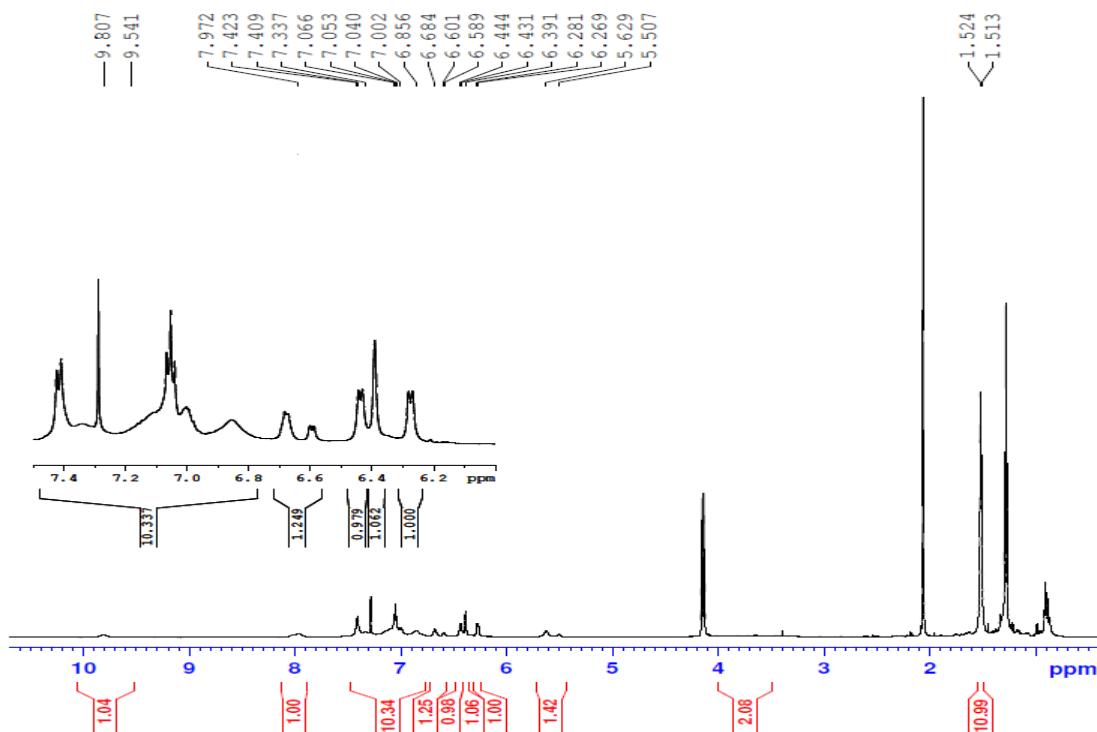


**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

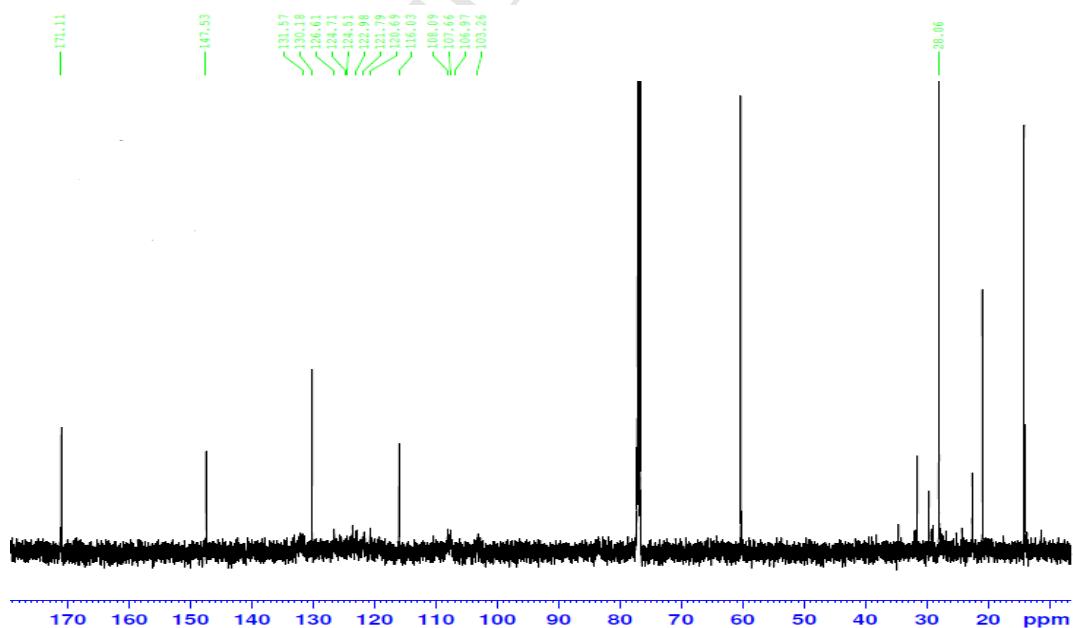


**3-Amino-4'-(2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenylguanidino)-*N*-phenylaniline (15d)**

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)

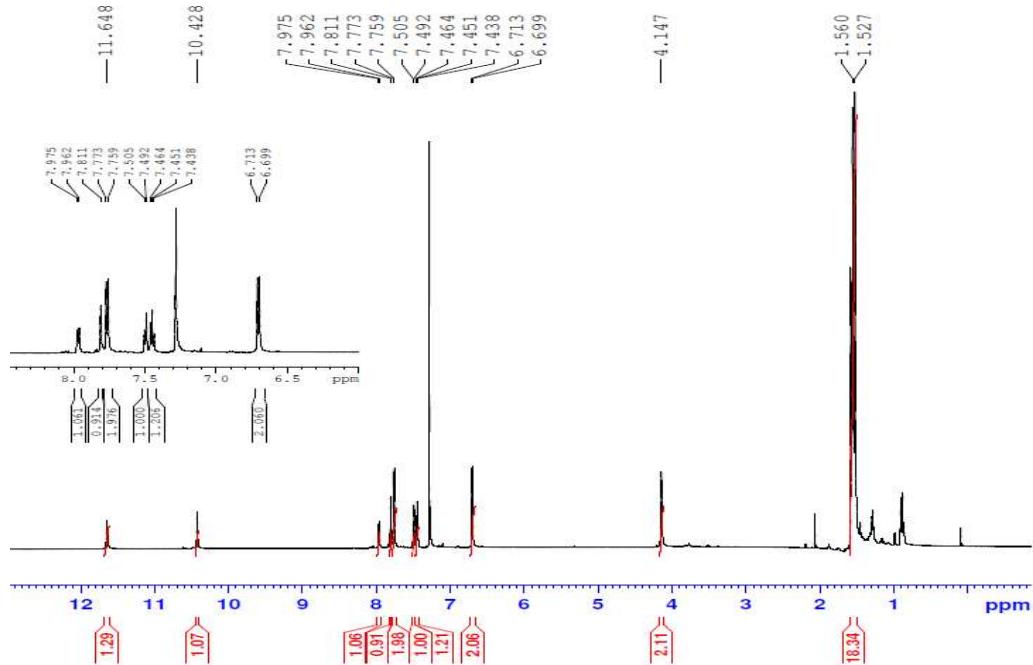


<sup>13</sup>C-NMR (CDCl<sub>3</sub>)

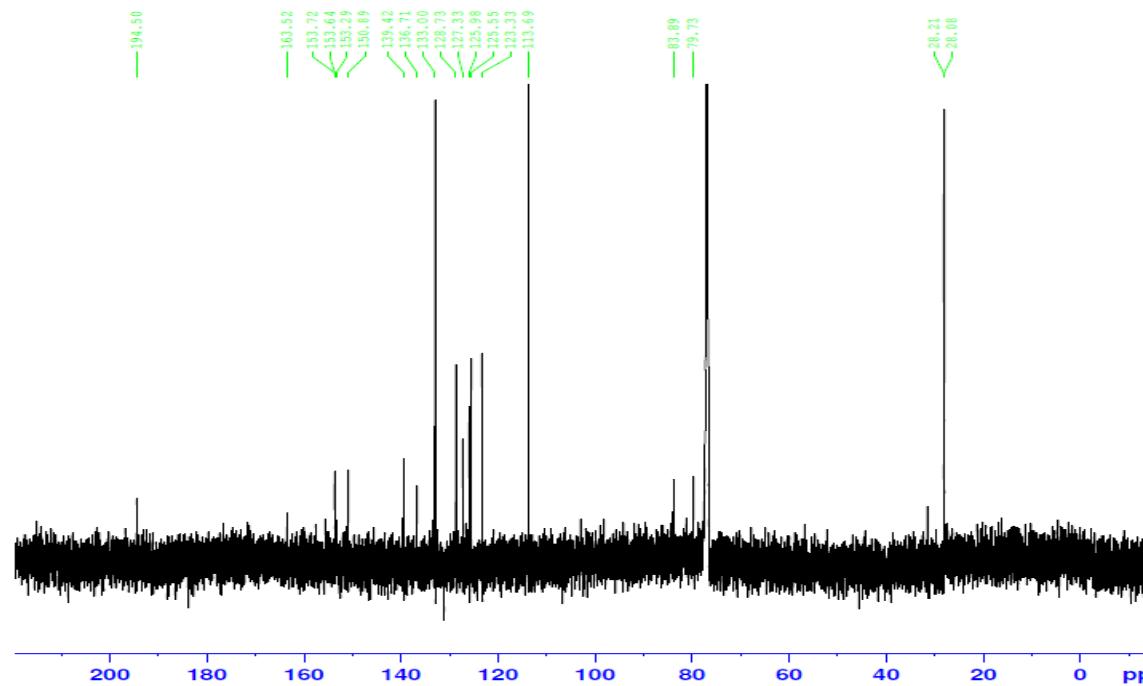


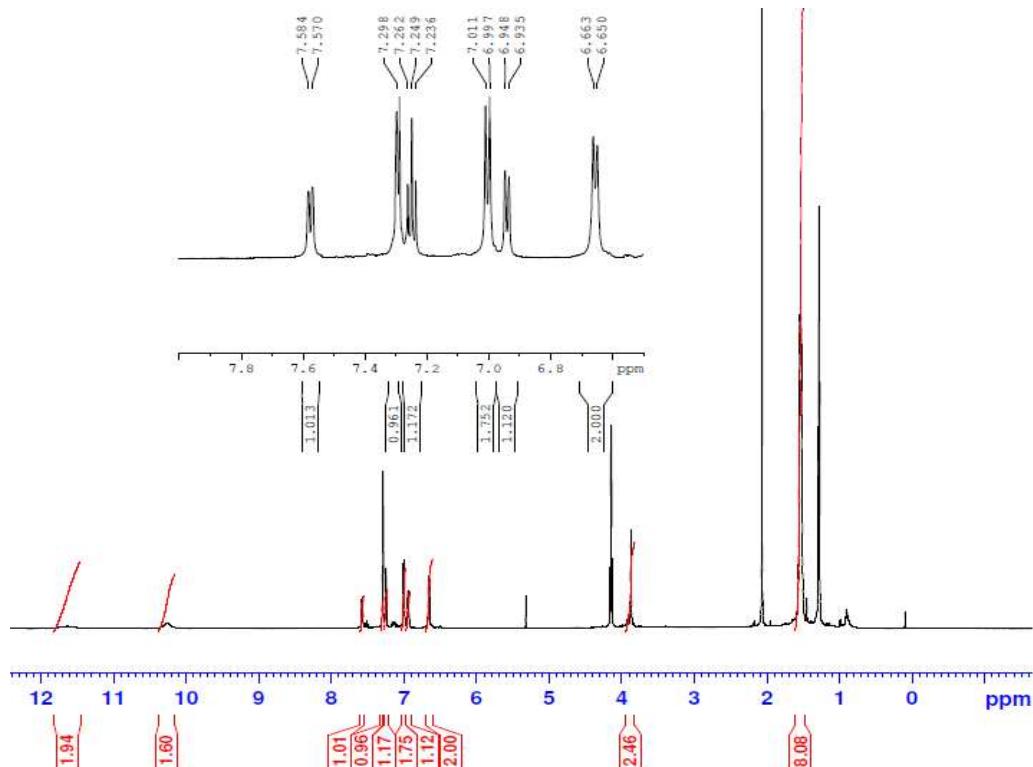
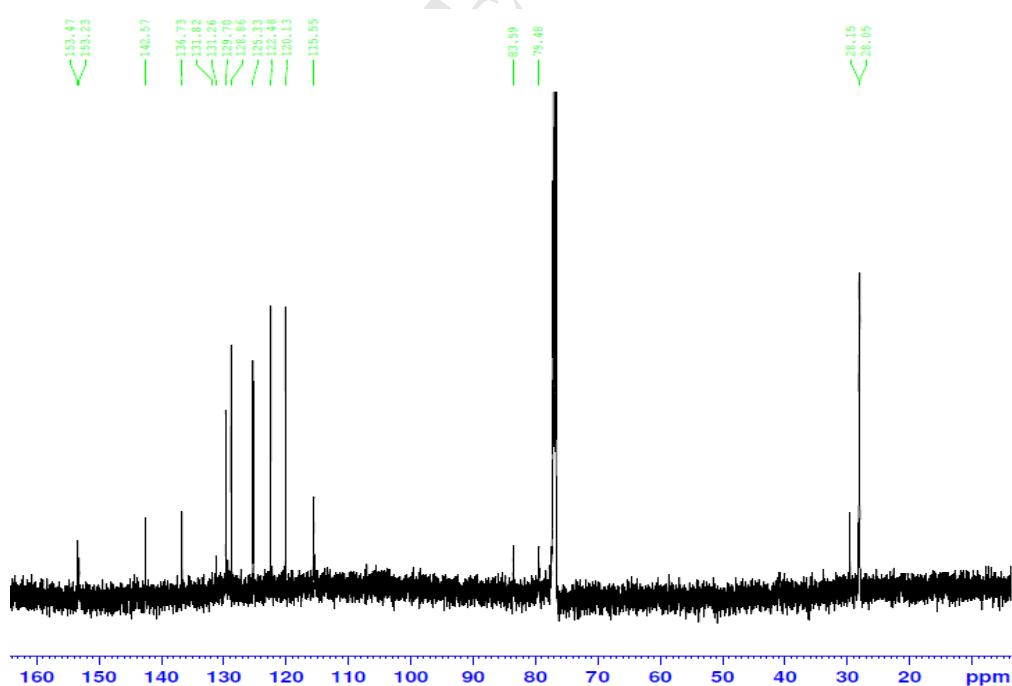
#### 4-Amino-[3'-(2,3-di-*tert*-butoxycarbonyl)guanidino]benzophenone (16b)

**<sup>1</sup>H-NMR (CDCl<sub>3</sub>)**



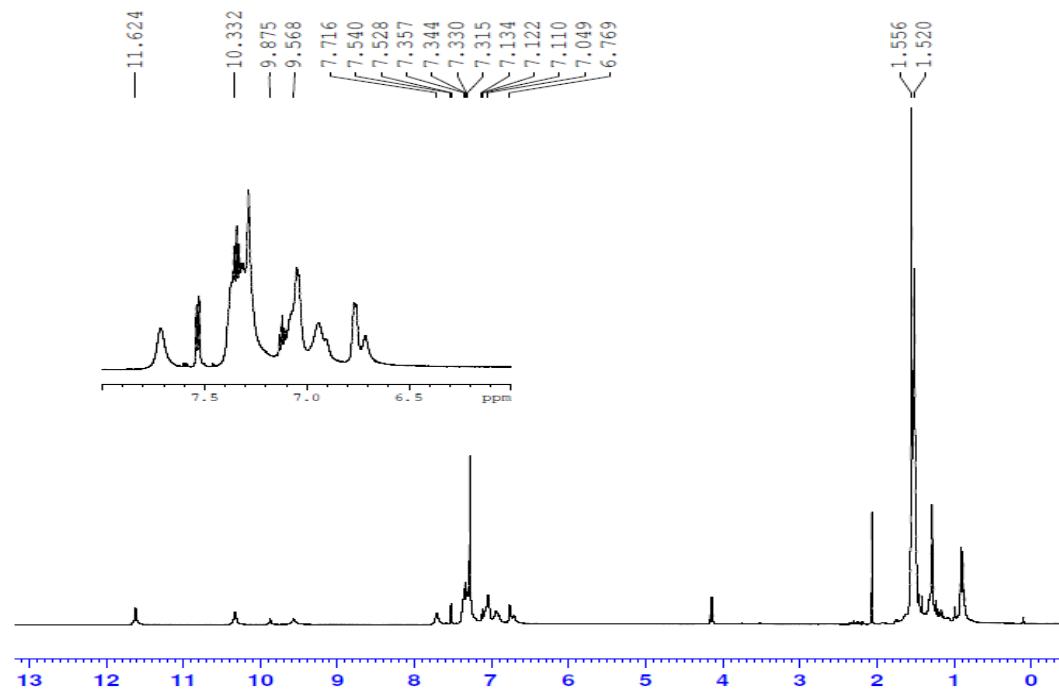
### **<sup>13</sup>C-NMR (CDCl<sub>3</sub>)**



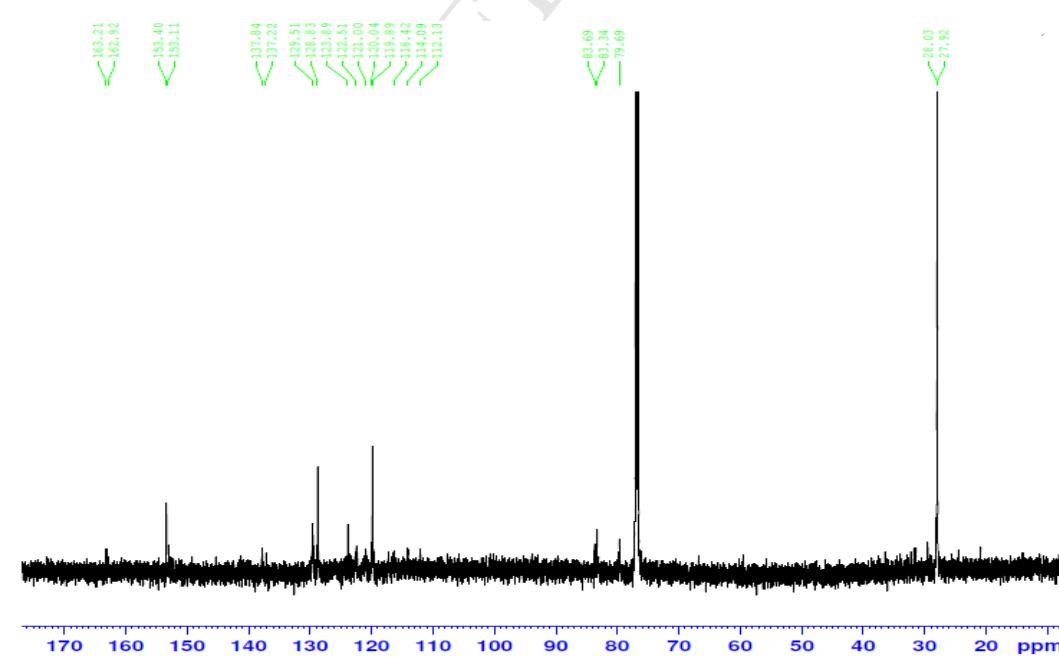
**4-Amino[3'-(2,3-di-*tert*-butoxycarbonyl)guanidino]benzylbenzene (16c)** **$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )** **$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

**3-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidino)diphenylether (**18a**)**

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)

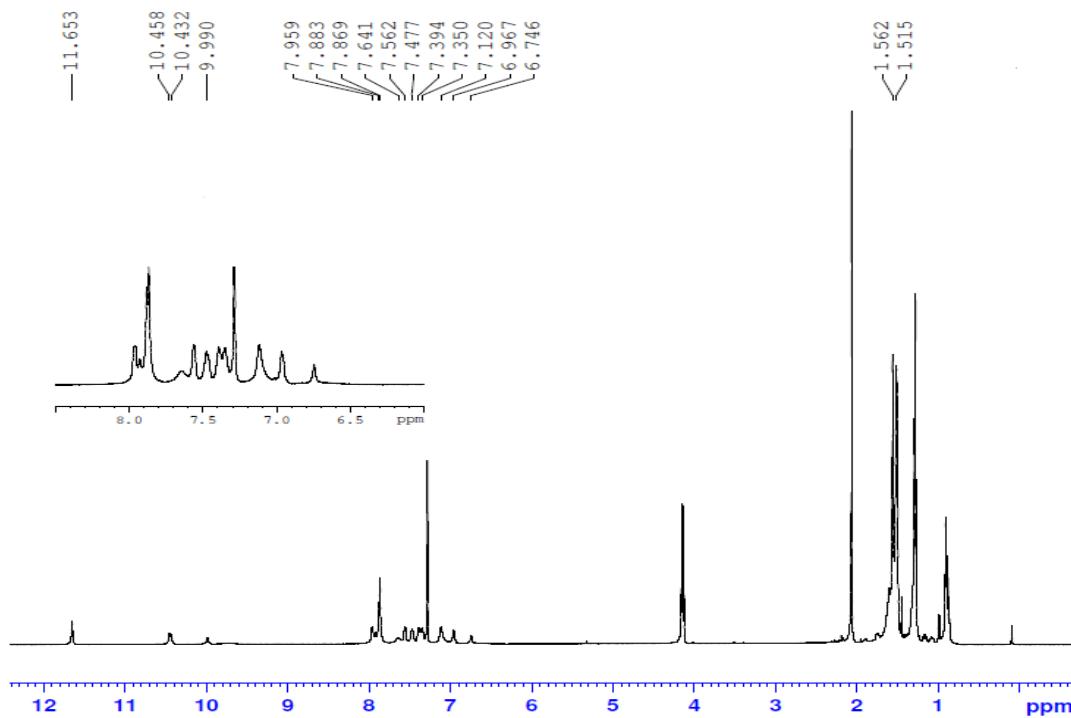


<sup>13</sup>C-NMR (CDCl<sub>3</sub>)

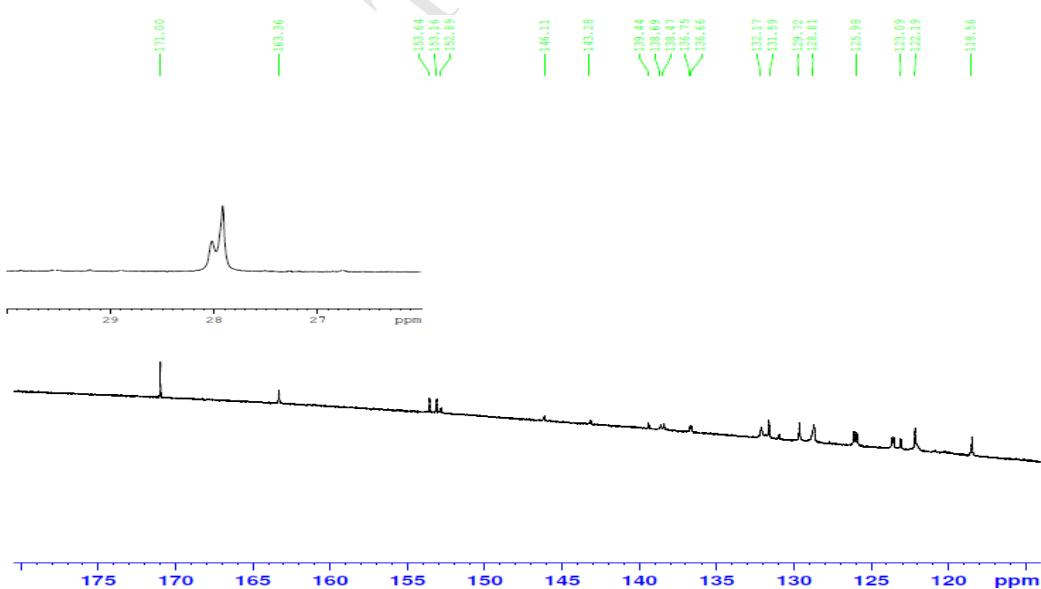


**3-[2,3-Di-(*tert*-butoxycarbonyl)-guanidino]-4'-[2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidino]benzophenone (18b)**

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)

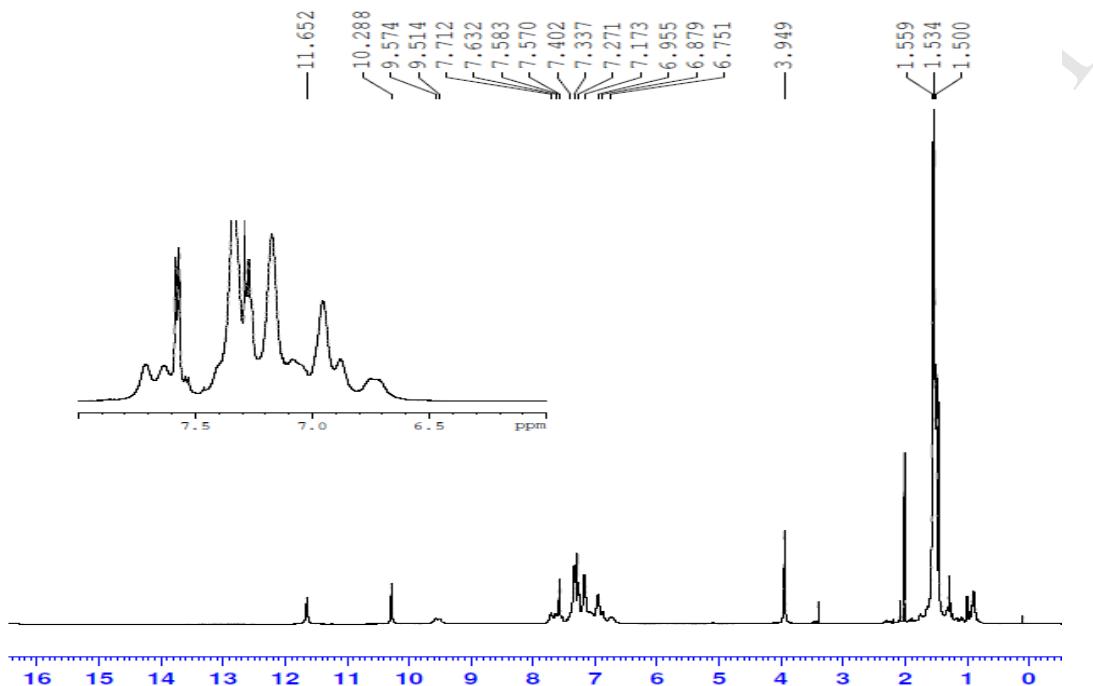


<sup>13</sup>C-NMR (CDCl<sub>3</sub>)

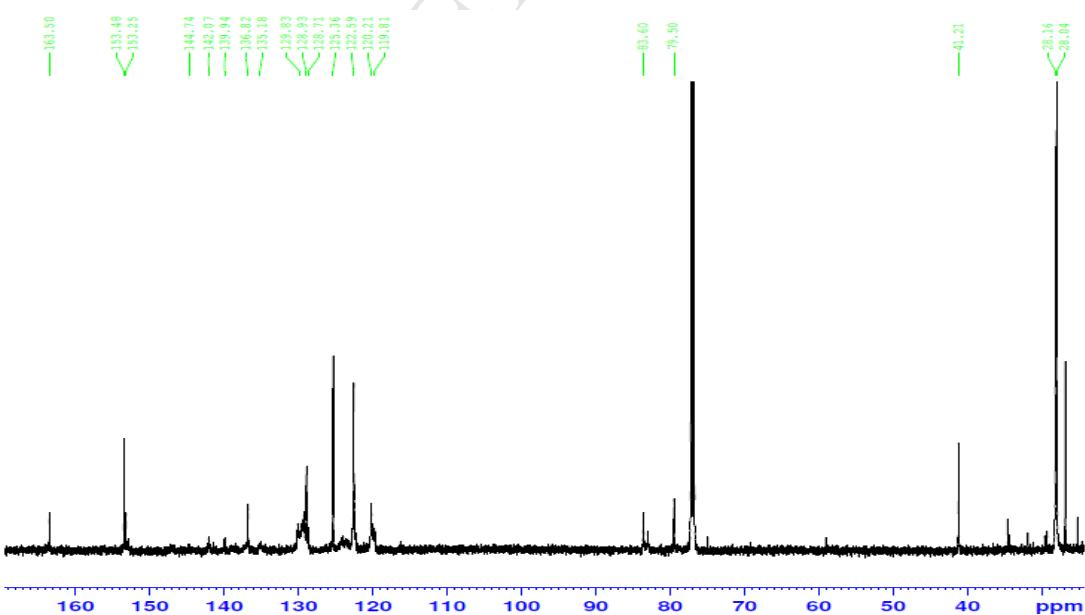


**3-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidino]benzylbenzene (18c)**

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)

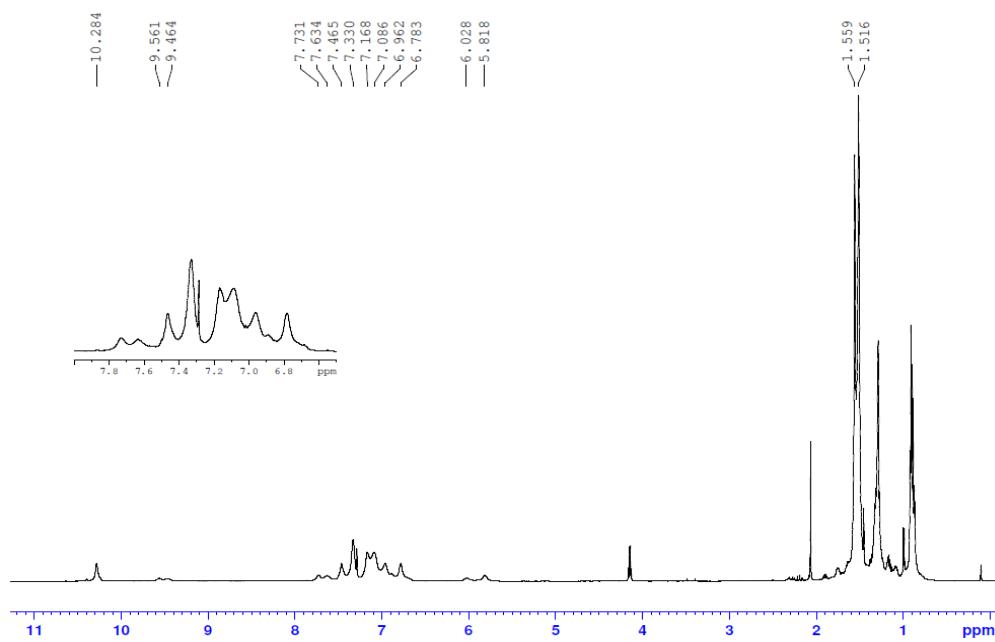


<sup>13</sup>C-NMR (CDCl<sub>3</sub>)

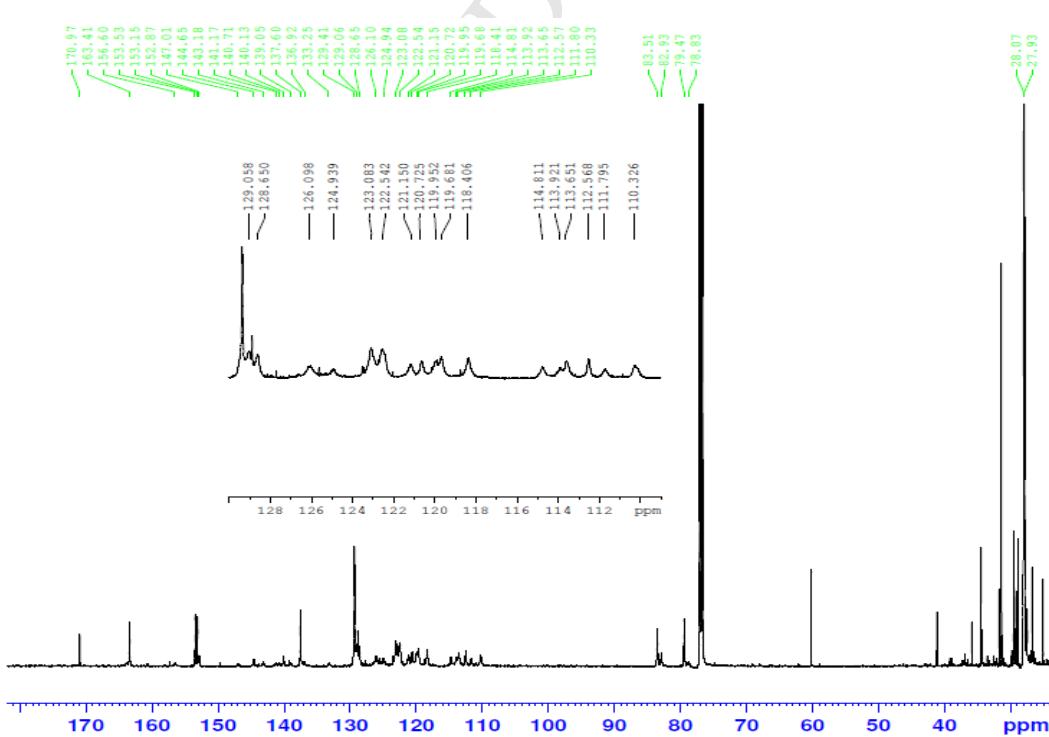


**3-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidino]-*N*-phenylaniline (18d)**

**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**

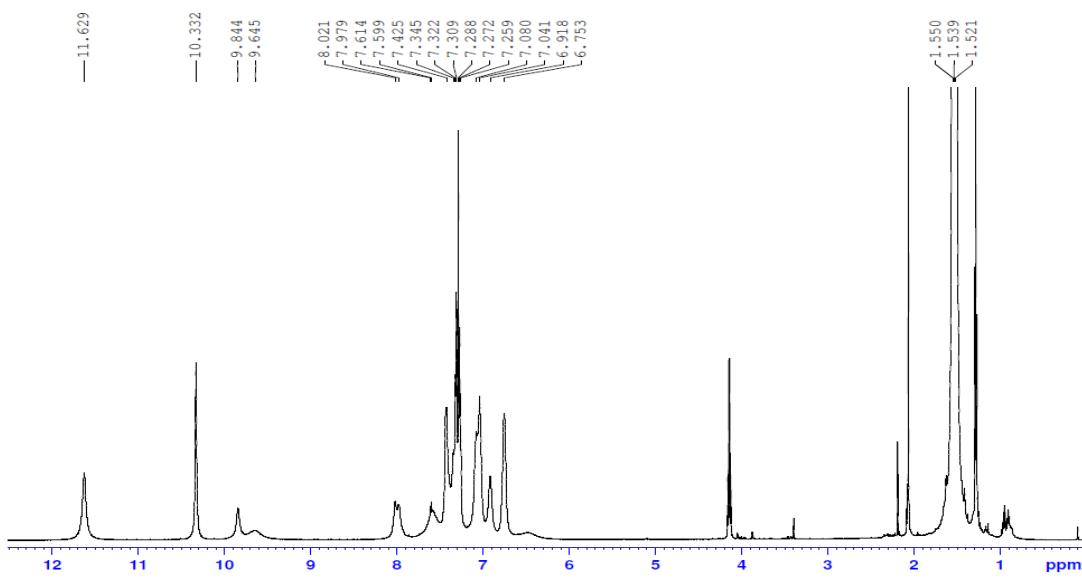


**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

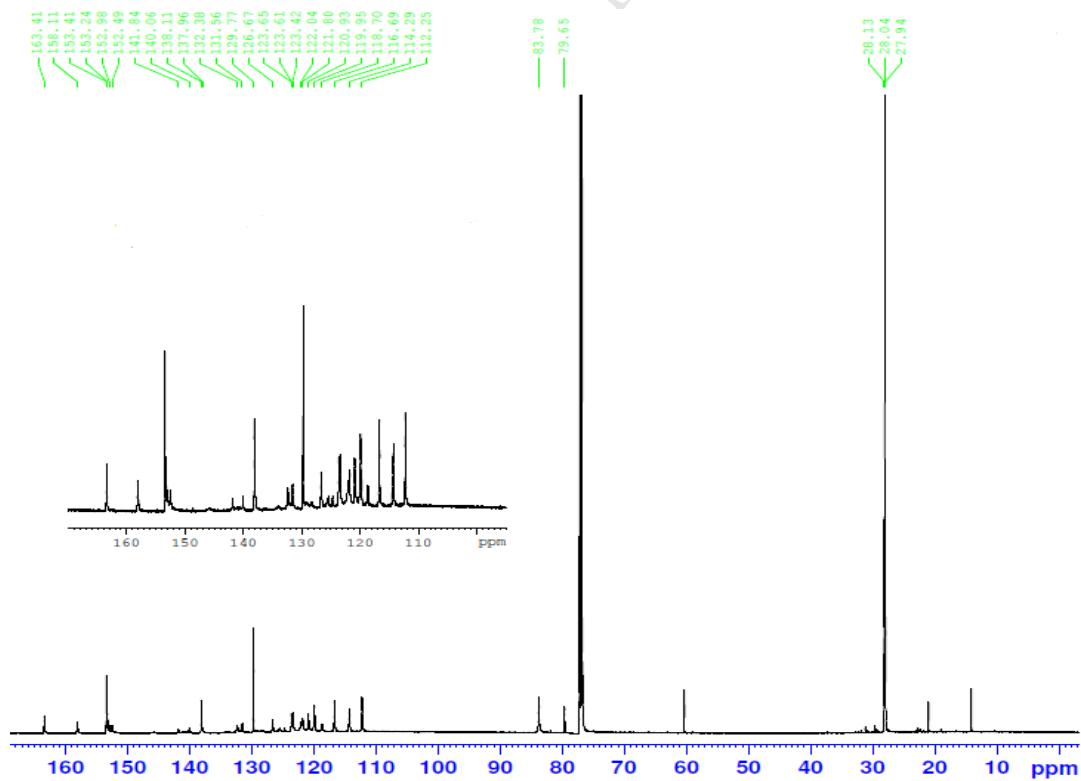


**3-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino)diphenylether (19a)**

**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**

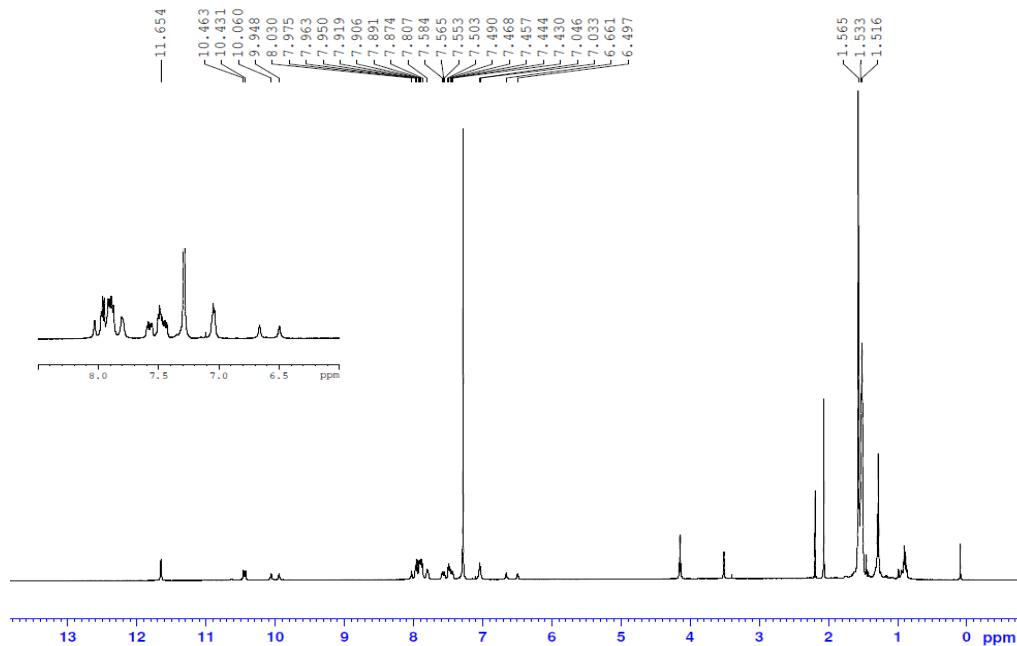


**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

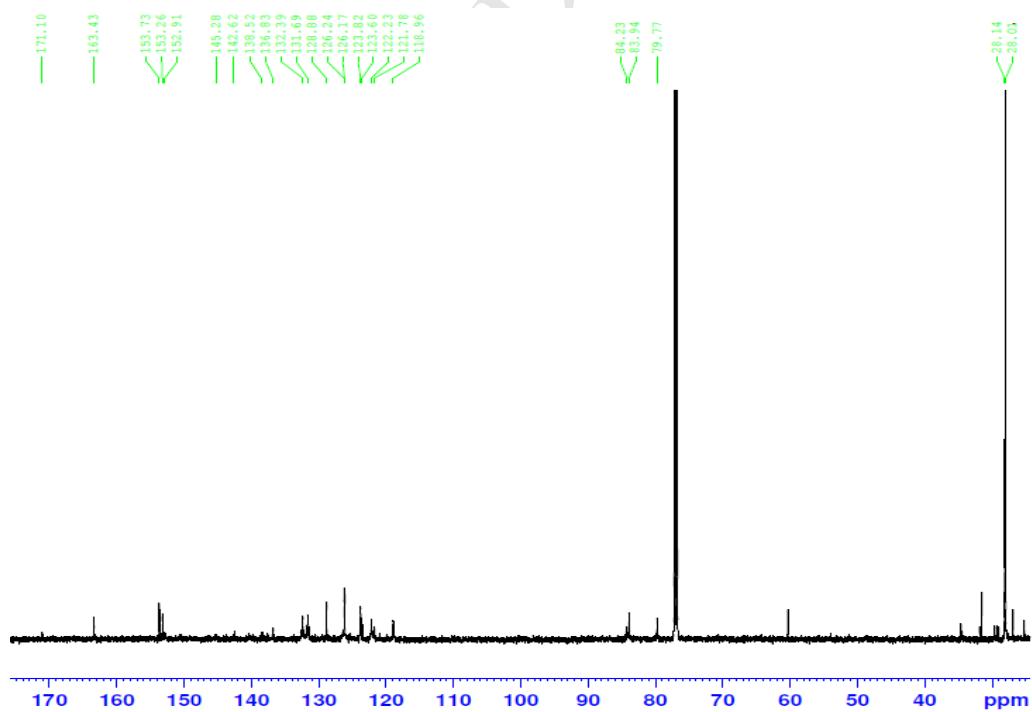


**3-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]benzophenone (19b)**

**<sup>1</sup>H-NMR (CDCl<sub>3</sub>)**

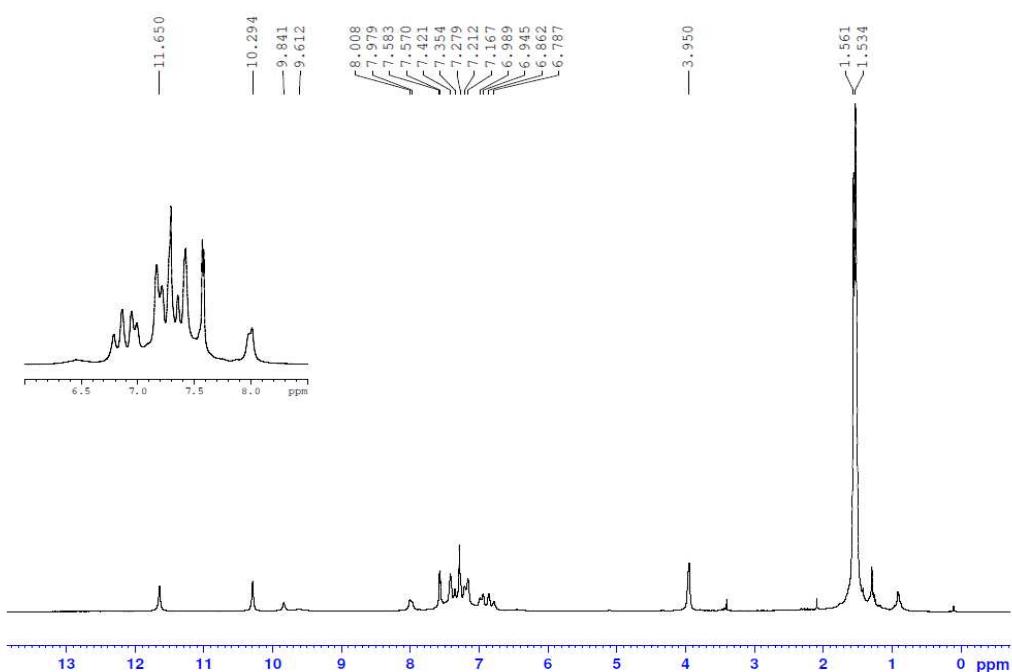


**<sup>13</sup>C-NMR (CDCl<sub>3</sub>)**

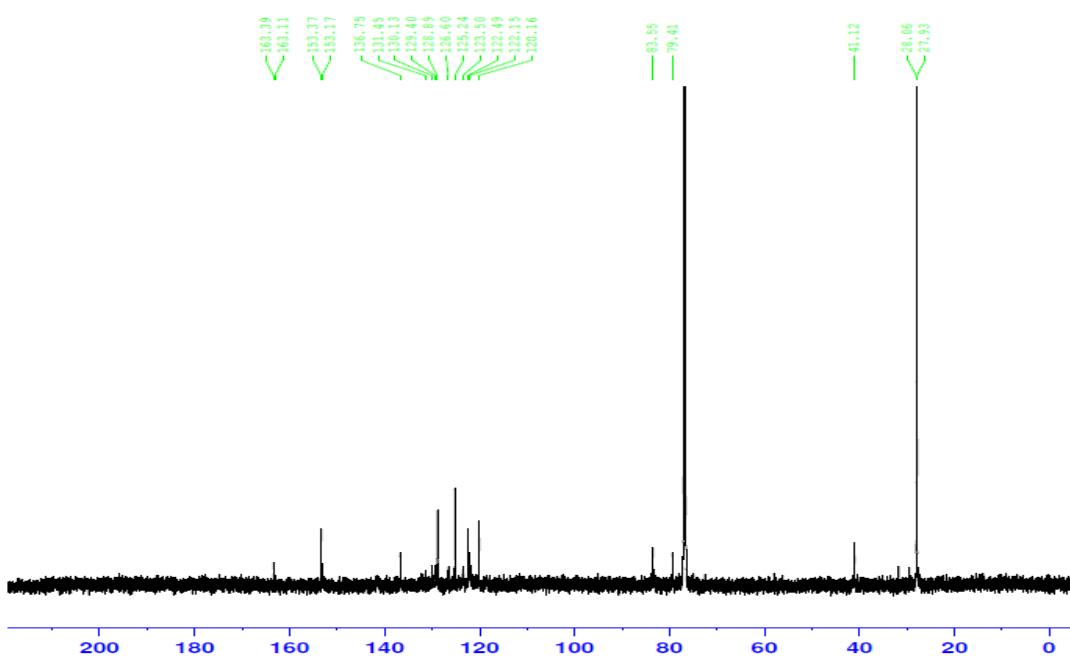


**3-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]benzylbenzene (19c)**

**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**

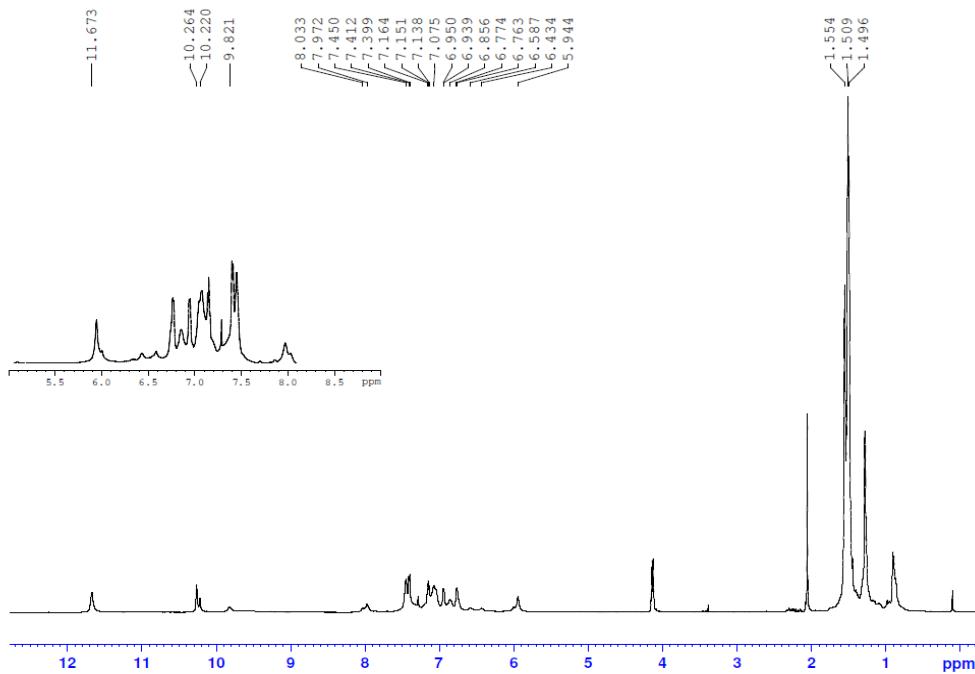


**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

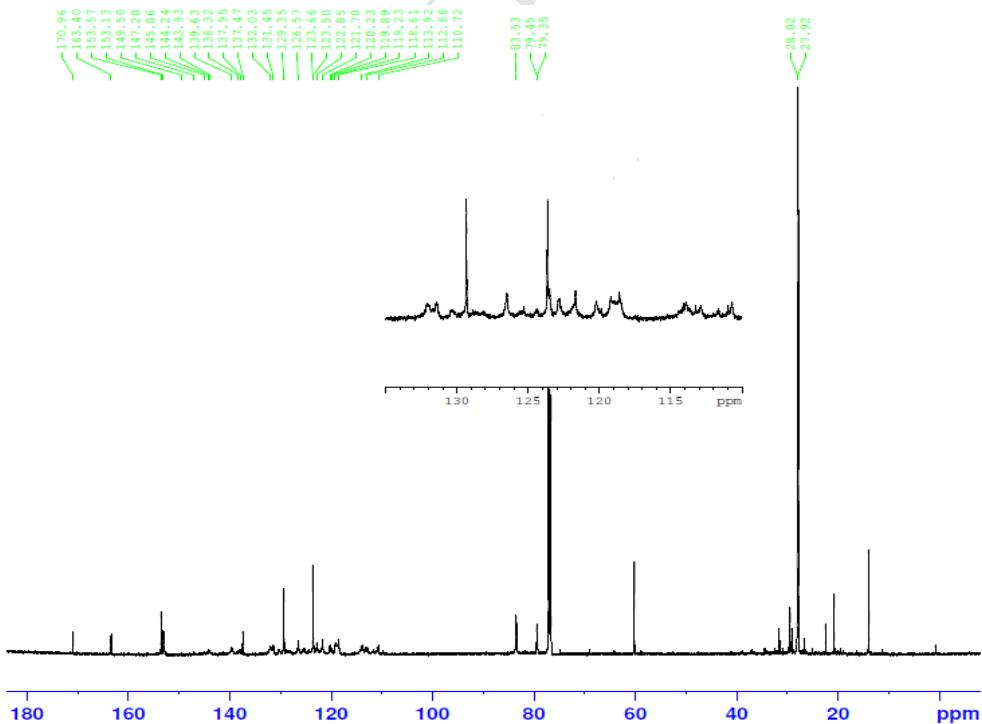


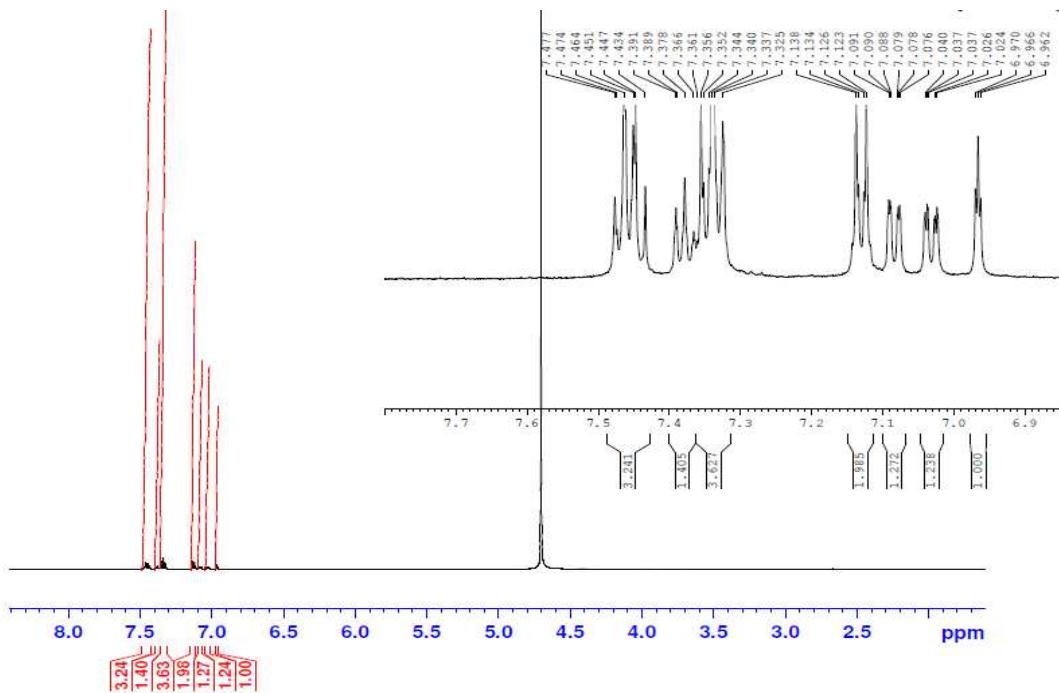
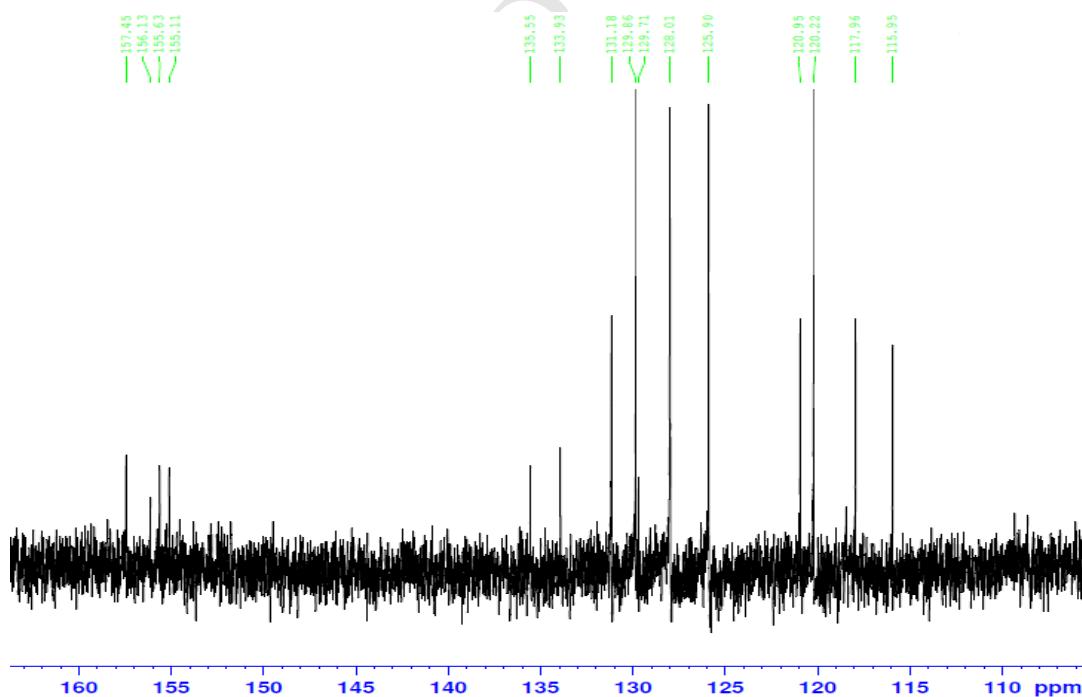
**3-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-4'-(2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethyl)phenylguanidino]-N-phenylaniline (19d)**

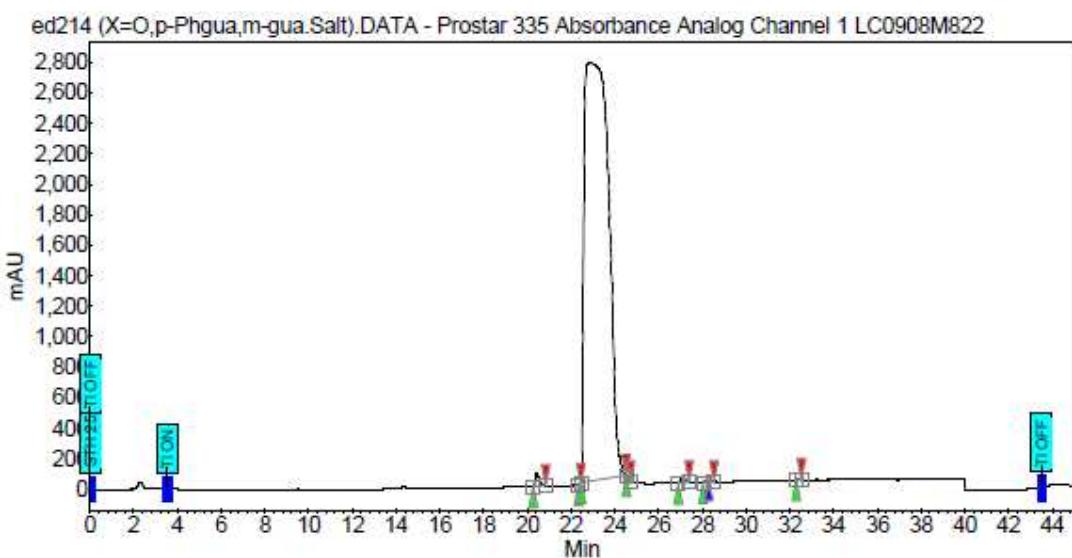
**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**



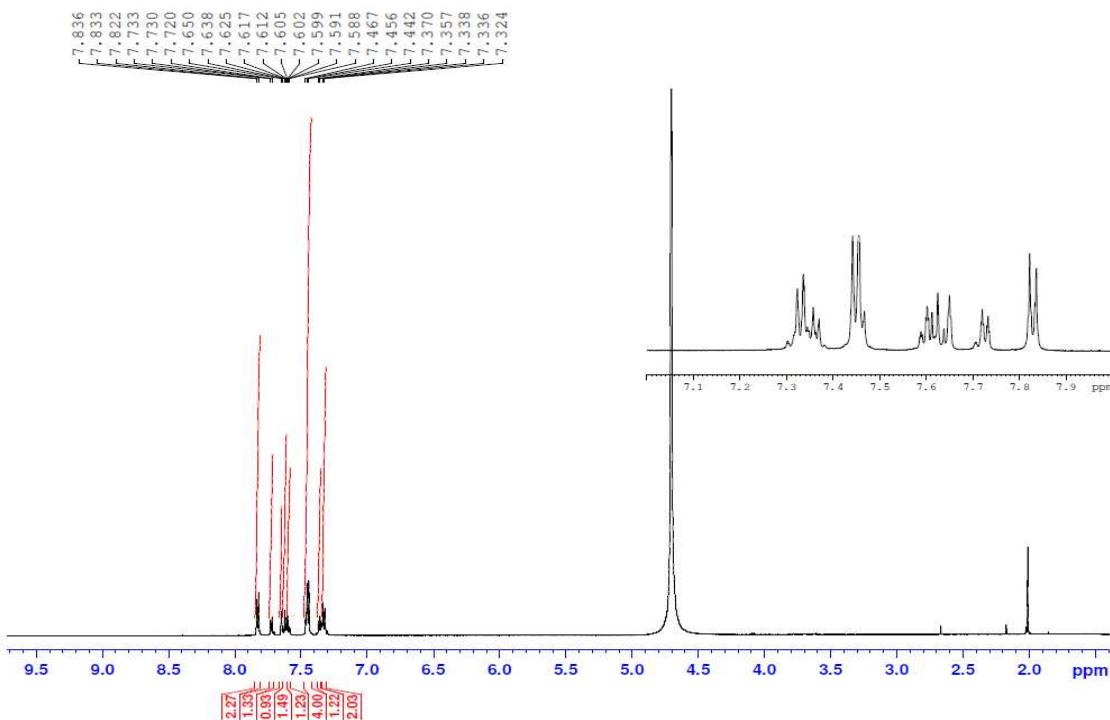
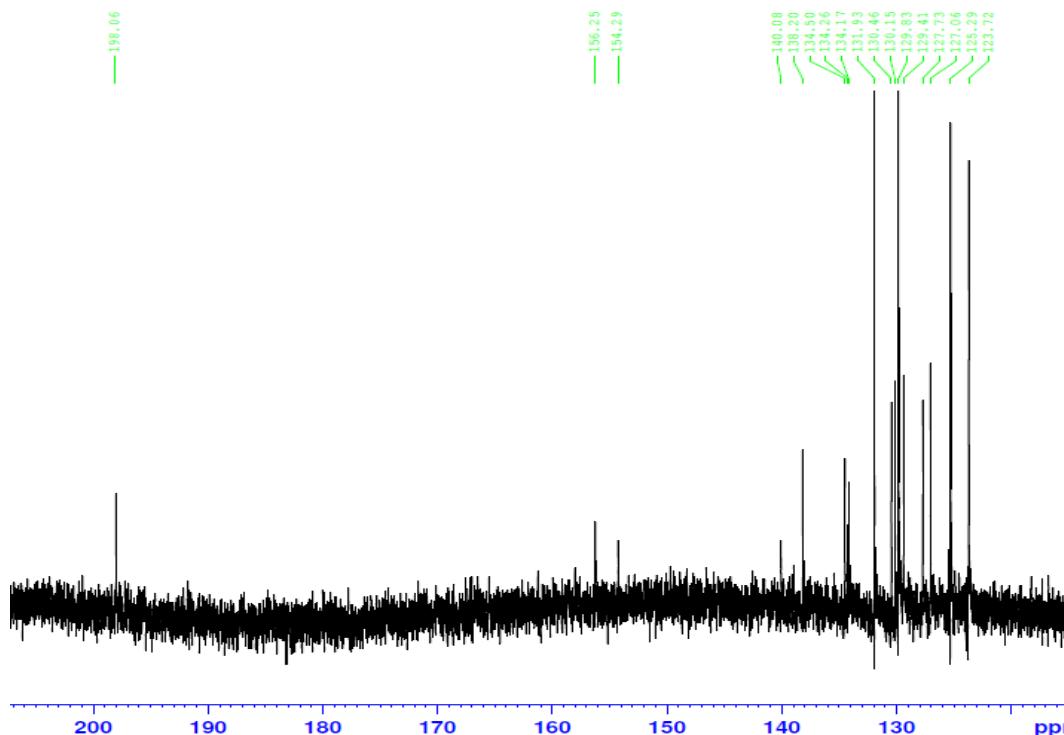
**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

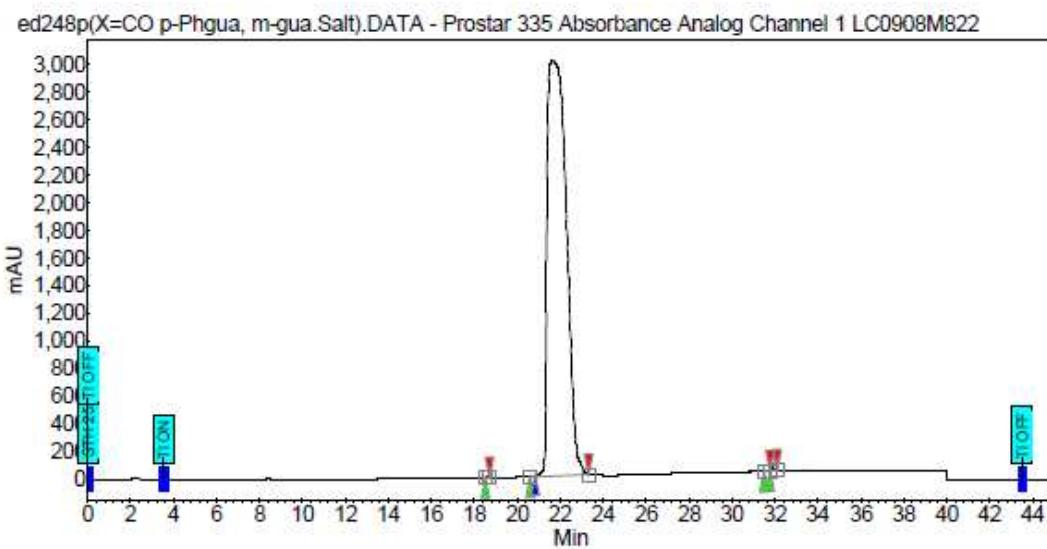


**3-[4'-(Phenylguanidino)phenoxy]phenylguanidine di-hydrochloride (**4a**)** **$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )** **$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

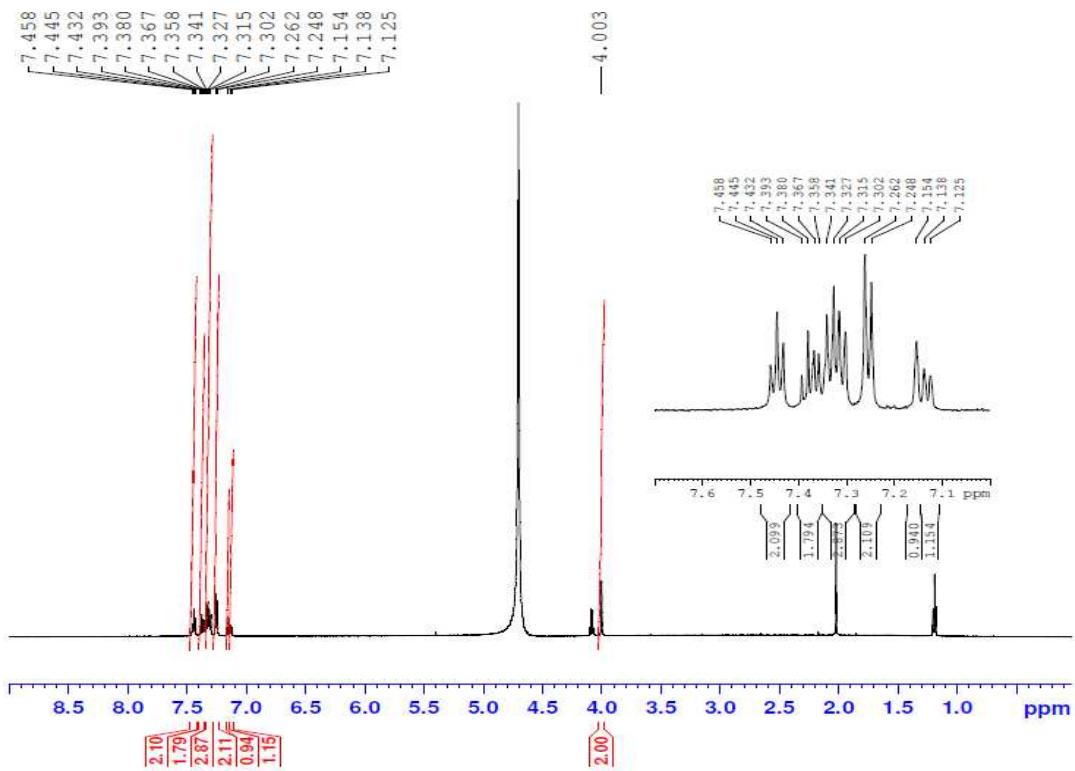
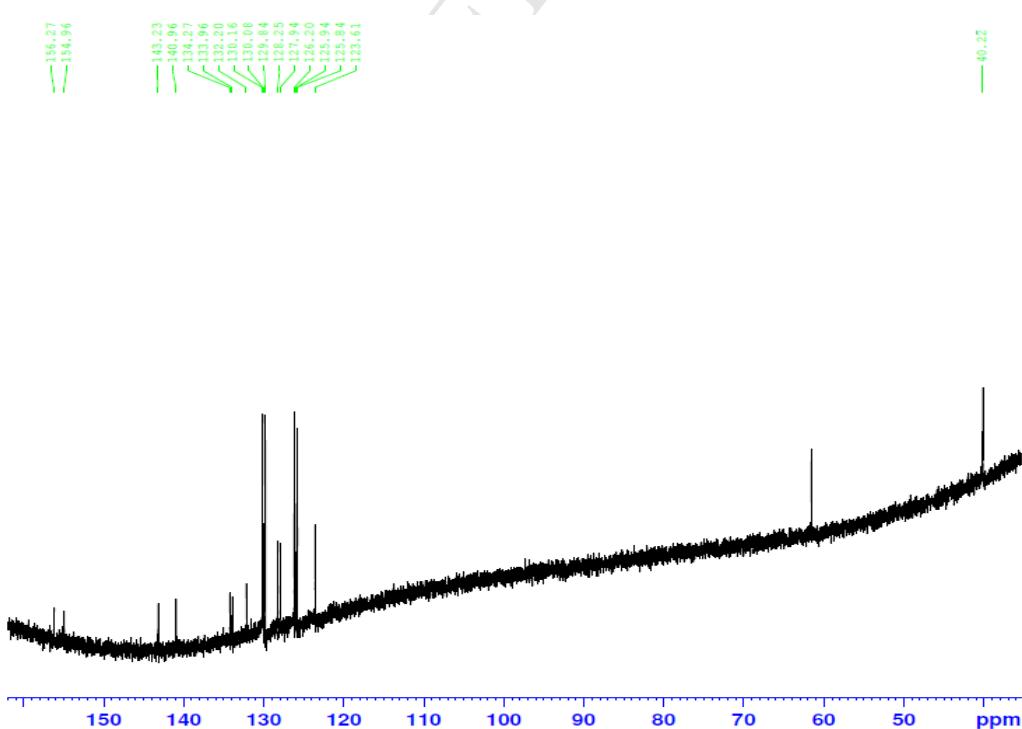
**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU·Min]	Area % [%]
1	UNKNOWN	20.41	0.53	89.3	18.3	0.526
2	UNKNOWN	22.39	0.00	1.6	0.1	0.003
7	UNKNOWN	22.80	99.14	2740.6	3486.2	99.139
8	UNKNOWN	24.61	0.02	4.8	0.6	0.016
3	UNKNOWN	27.11	0.23	43.2	8.1	0.233
4	UNKNOWN	28.19	0.04	8.4	1.3	0.038
5	UNKNOWN	28.36	0.03	7.3	1.1	0.031
6	UNKNOWN	32.40	0.01	3.5	0.4	0.013
Total			100.00	2898.6	3486.2	100.000

**3-[4'-(Phenylguanidino)benzoyl]phenylguanidine di-hydrochloride (4b)** **$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )** **$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

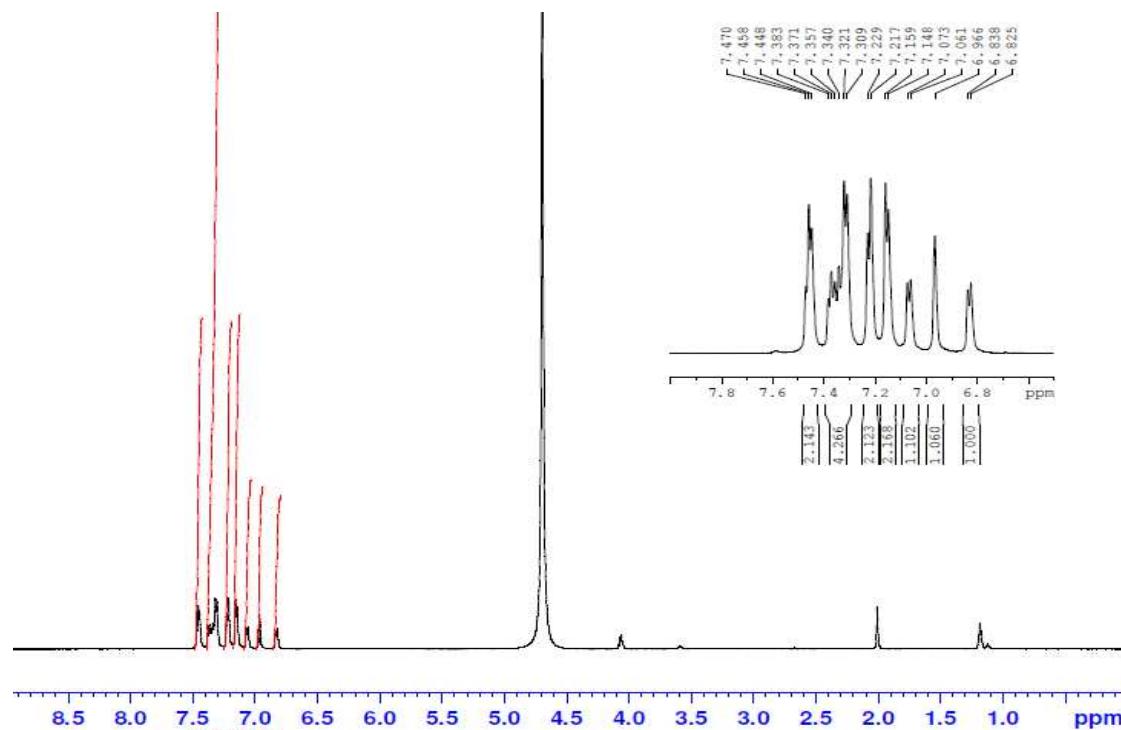
**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	18.60	0.01	4.8	0.4	0.014
2	UNKNOWN	20.72	0.02	3.9	0.5	0.018
3	UNKNOWN	21.69	99.85	3005.2	3053.8	99.853
4	UNKNOWN	31.57	0.03	7.3	1.0	0.031
5	UNKNOWN	31.91	0.08	18.4	2.5	0.083
Total			100.00	3039.5	3058.3	100.000

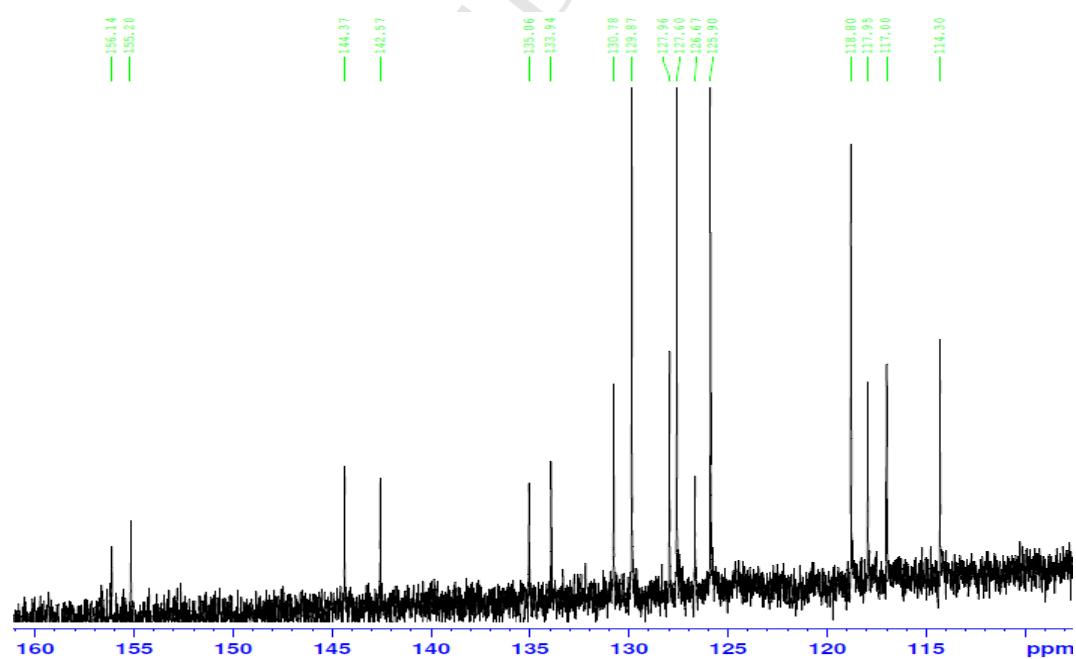
**3-[4'-(3-Phenylguanidino)benzyl]phenylguanidine di-hydrochloride (4c)** **$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )** **$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

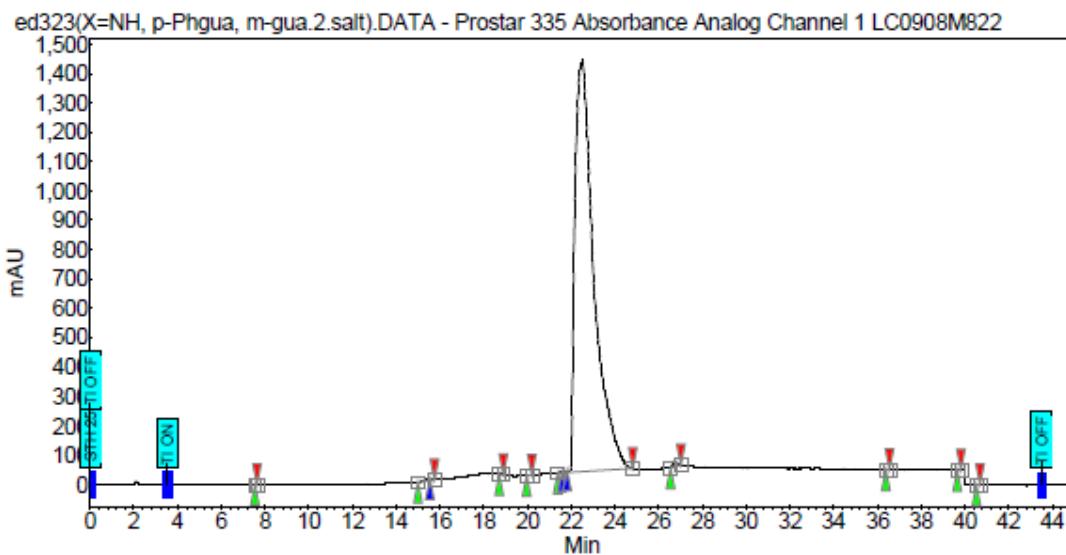
### 3-[4-(Phenylguanidino)phenylamino]phenylguanidine di-hydrochloride (4d)

### **<sup>1</sup>H-NMR (D<sub>2</sub>O)**



<sup>13</sup>C-NMR (D<sub>2</sub>O)

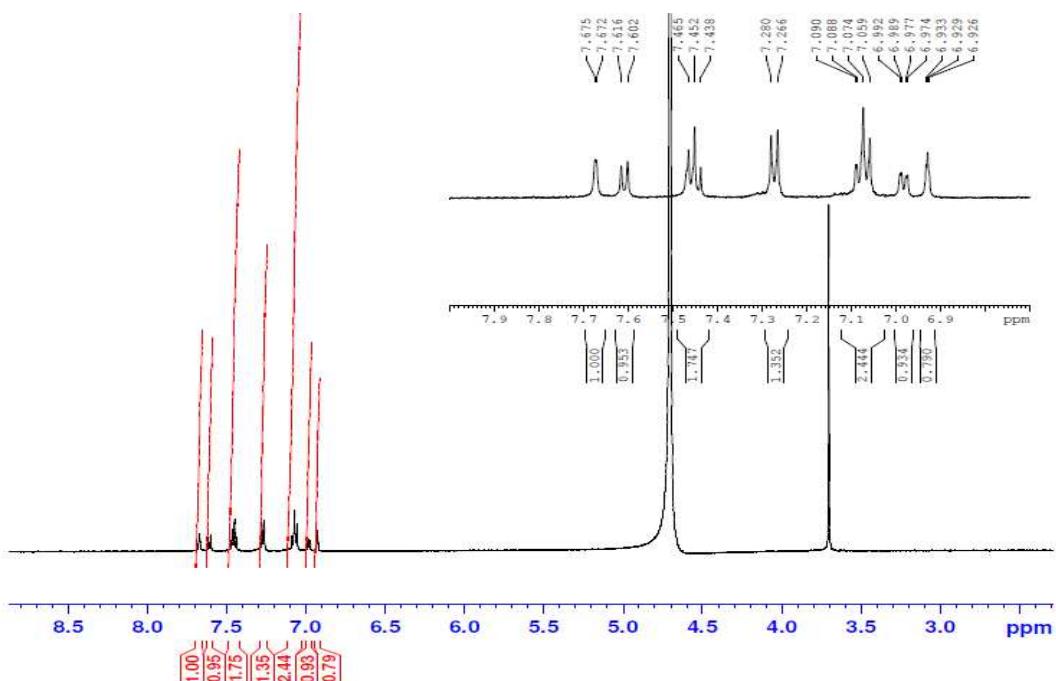


**HPLC****Peak results :**

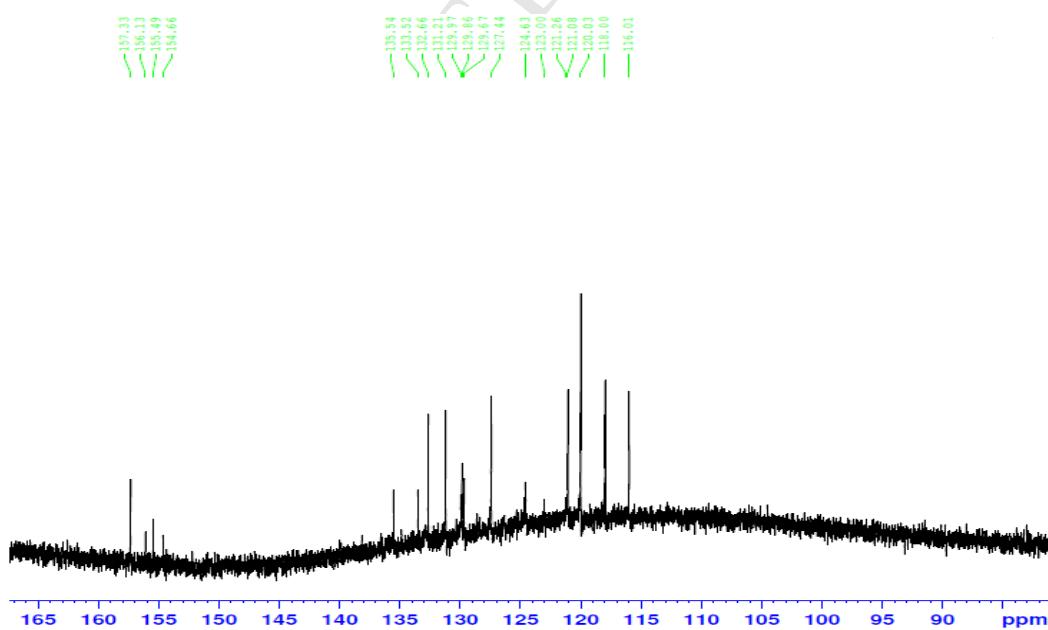
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	7.56	0.00	1.8	0.0	0.003
2	UNKNOWN	15.43	0.09	3.7	1.2	0.087
3	UNKNOWN	15.64	0.02	2.1	0.3	0.018
4	UNKNOWN	18.79	0.01	2.1	0.2	0.014
5	UNKNOWN	20.09	0.02	2.3	0.3	0.019
6	UNKNOWN	21.49	0.02	1.8	0.2	0.016
7	UNKNOWN	21.89	0.05	4.4	0.7	0.052
8	UNKNOWN	22.51	99.47	1402.1	1397.4	99.473
9	UNKNOWN	26.72	0.30	20.7	4.2	0.300
10	UNKNOWN	36.48	0.01	1.0	0.1	0.006
11	UNKNOWN	39.72	0.01	1.1	0.1	0.008
12	UNKNOWN	40.59	0.01	0.7	0.1	0.005
Total			100.00	1443.9	1404.8	100.000

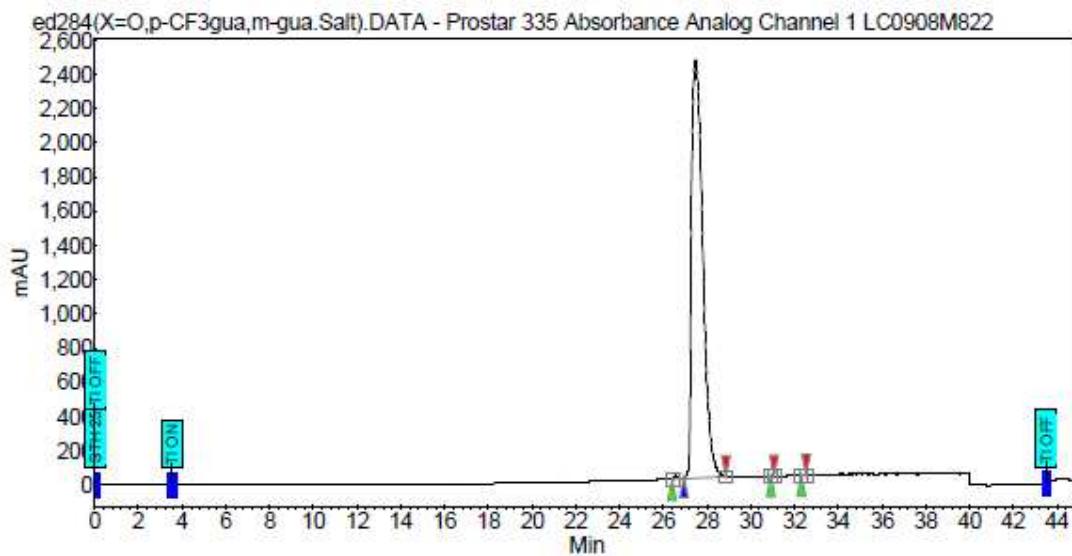
**3-[4'-(4-Chloro-3-trifluoromethylphenyl)guanidinophenoxy]phenylguanidine dihydrochloride (5a)**

### **<sup>1</sup>H-NMR (D<sub>2</sub>O)**



### <sup>13</sup>C-NMR (D<sub>2</sub>O)

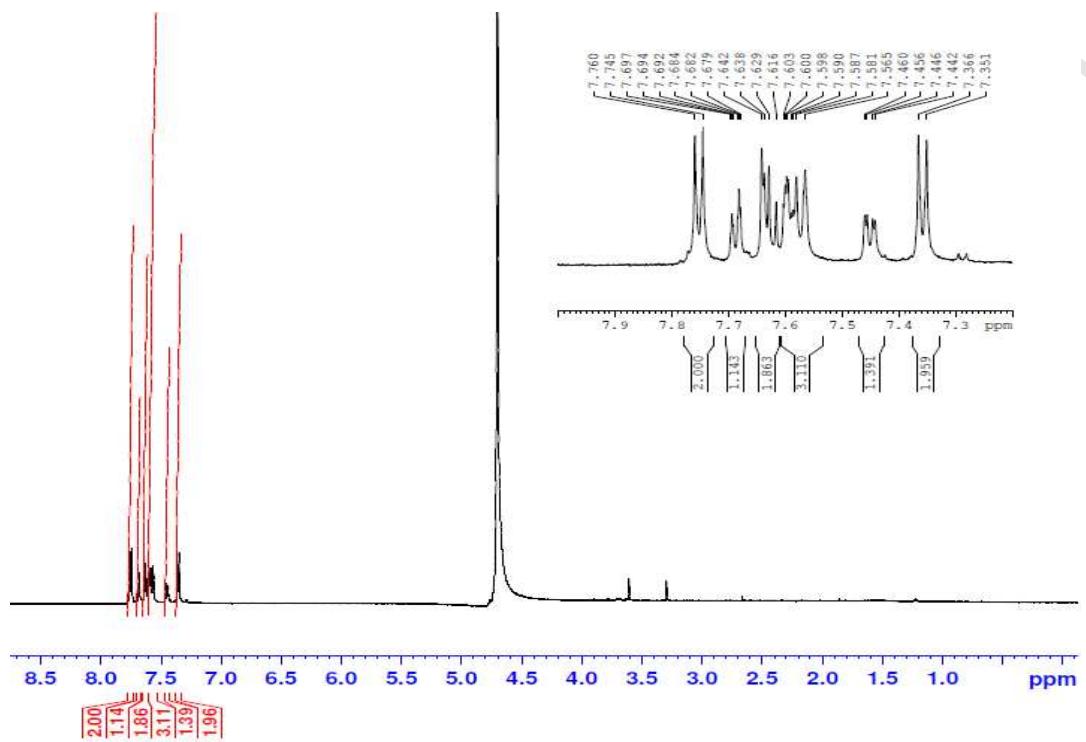


**HPLC****Peak results :**

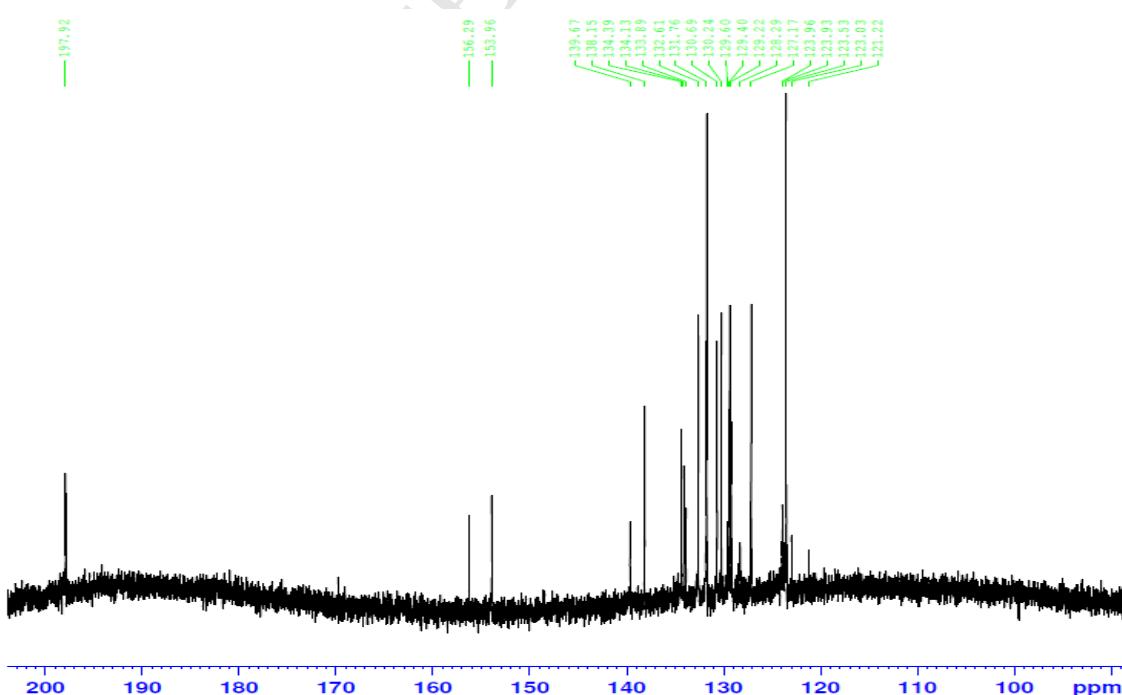
Index	Name	Time [Min]	Quantity (% Area)	Height [mAU]	Area [mAU·Min]	Area % [%]
1	UNKNOWN	26.58	0.29	21.5	4.2	0.289
2	UNKNOWN	27.45	99.69	2448.5	1456.5	99.677
3	UNKNOWN	30.97	0.01	1.6	0.1	0.009
4	UNKNOWN	32.44	0.02	3.4	0.4	0.025
Total			100.00	2475.0	1461.3	100.000

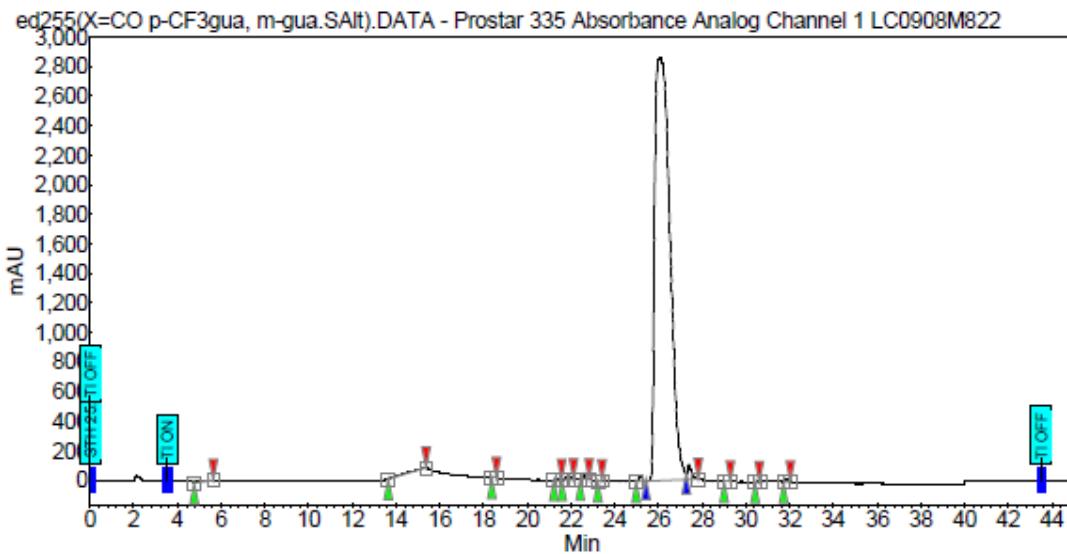
**3-[4'-3-(4-Chloro-3-trifluoromethylphenyl)guanidinobenzoyl]phenylguanidine dihydrochloride (5b)**

## **<sup>1</sup>H-NMR (D<sub>2</sub>O)**



### **<sup>13</sup>C-NMR (D<sub>2</sub>O)**

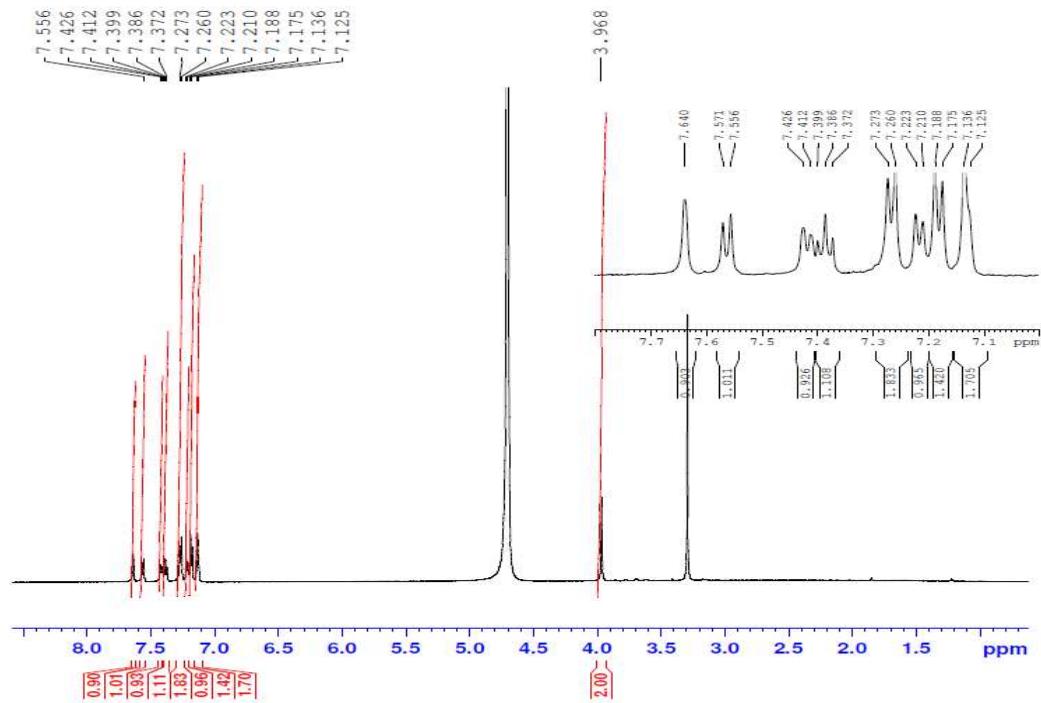


**HPLC****Peak results :**

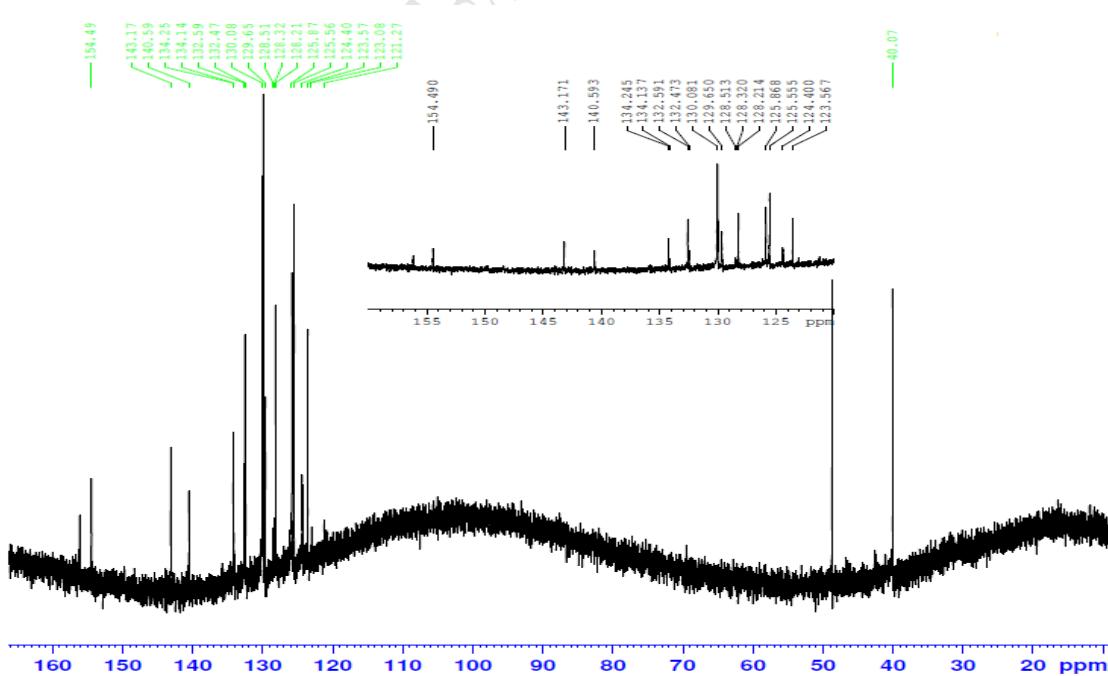
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	5.00	0.27	12.5	6.4	0.273
2	UNKNOWN	14.43	0.31	6.6	7.3	0.312
3	UNKNOWN	18.45	0.03	6.2	0.7	0.028
4	UNKNOWN	21.31	0.05	11.1	1.3	0.055
5	UNKNOWN	21.89	0.22	21.4	5.2	0.221
6	UNKNOWN	22.55	0.32	47.0	7.4	0.315
7	UNKNOWN	23.32	0.02	3.8	0.4	0.015
8	UNKNOWN	25.16	0.23	30.6	5.3	0.226
9	UNKNOWN	26.09	97.50	2860.1	2288.8	97.498
10	UNKNOWN	27.40	0.92	100.6	21.5	0.916
11	UNKNOWN	29.13	0.04	7.4	0.9	0.040
12	UNKNOWN	30.52	0.01	2.9	0.3	0.011
13	UNKNOWN	31.83	0.09	16.2	2.1	0.090
Total			100.00	3126.3	2347.5	100.000

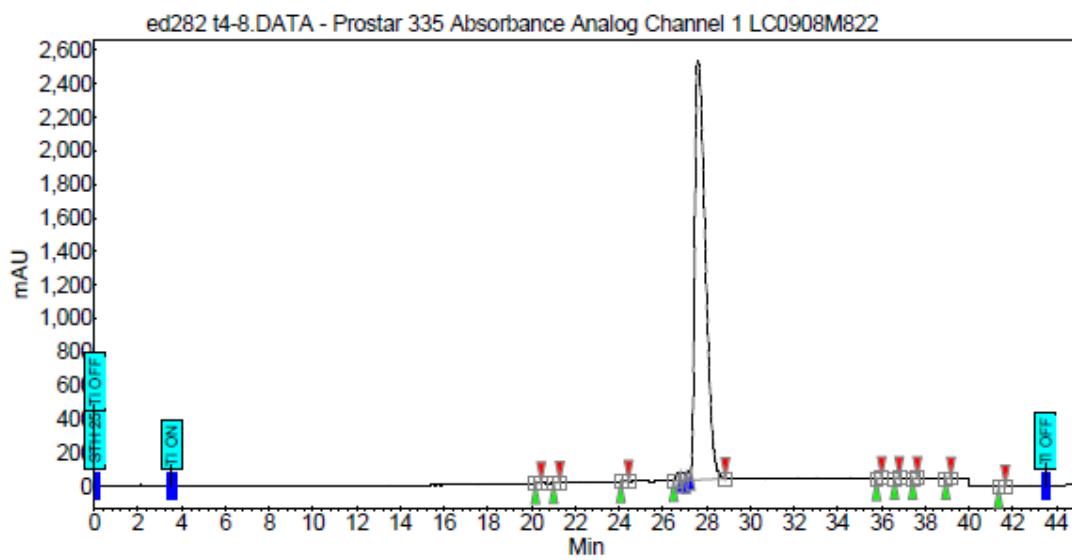
**3-[4'-3-(4-Chloro-3-trifluoromethylphenyl)guanidinobenzyl]phenylguanidine di-hydrochloride (5c)**

**$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**



**$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

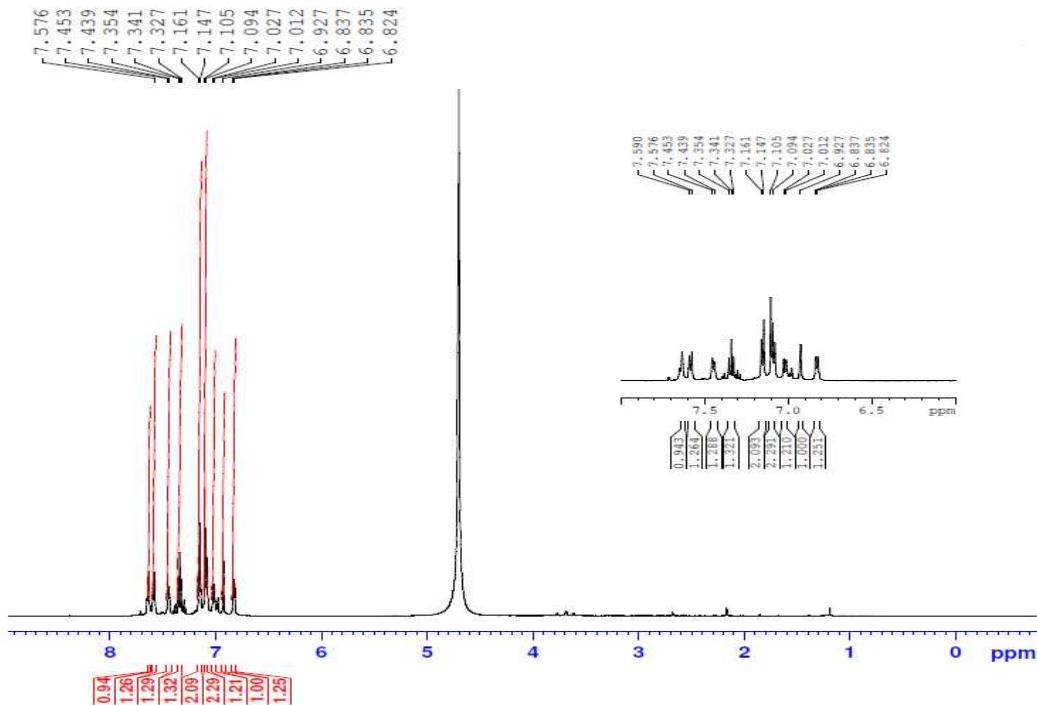


**HPLC****Peak results :**

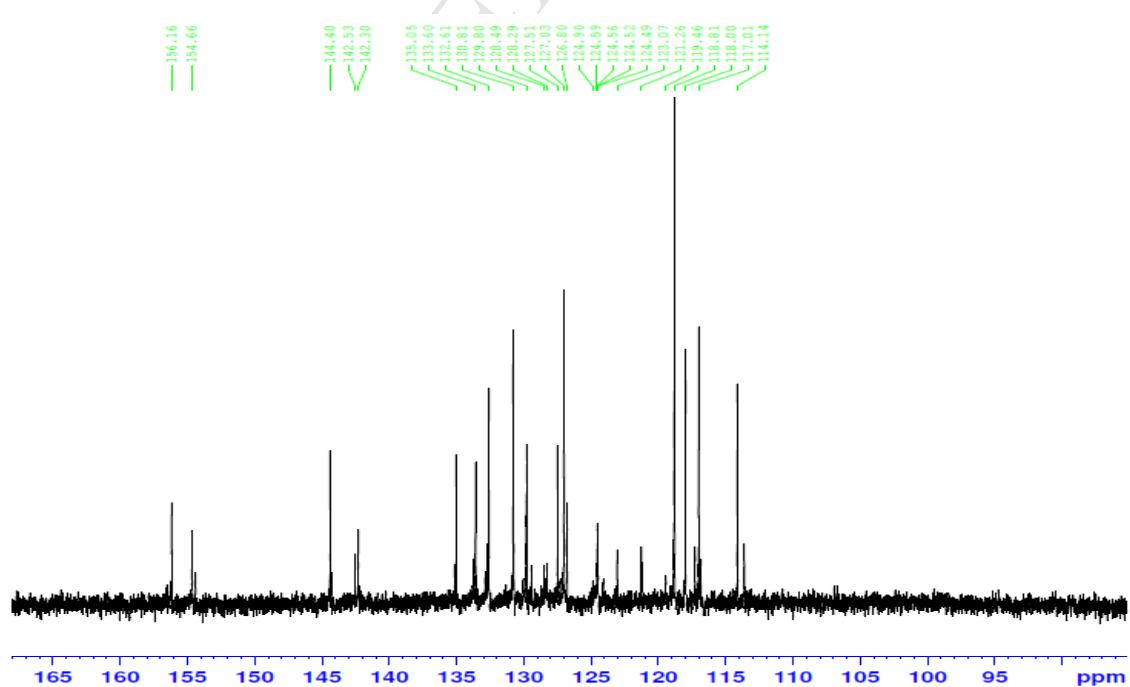
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	20.28	0.04	3.4	0.5	0.038
2	UNKNOWN	21.11	0.05	4.0	0.7	0.047
3	UNKNOWN	24.24	0.10	7.6	1.5	0.103
4	UNKNOWN	26.72	0.62	48.5	8.9	0.623
5	UNKNOWN	26.95	0.76	46.8	10.8	0.763
6	UNKNOWN	27.23	0.81	59.2	11.5	0.807
7	UNKNOWN	27.60	97.43	2496.3	1385.9	97.429
8	UNKNOWN	35.89	0.05	5.2	0.8	0.054
9	UNKNOWN	36.71	0.02	2.7	0.3	0.025
10	UNKNOWN	37.53	0.02	2.7	0.4	0.025
11	UNKNOWN	39.09	0.03	3.1	0.4	0.031
12	UNKNOWN	41.49	0.06	4.1	0.8	0.056
Total			100.00	2683.5	1422.5	100.000

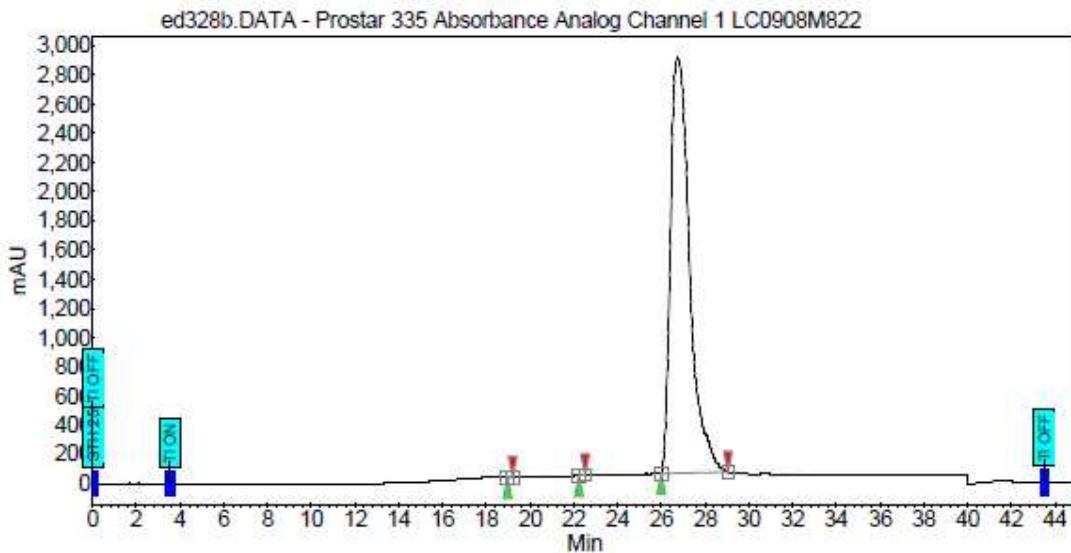
**3-[4'-(4-Chloro-3-trifluoromethyl)phenylguanidino]phenylamino]phenylguanidine di-hydrochloride (5d)**

**$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**

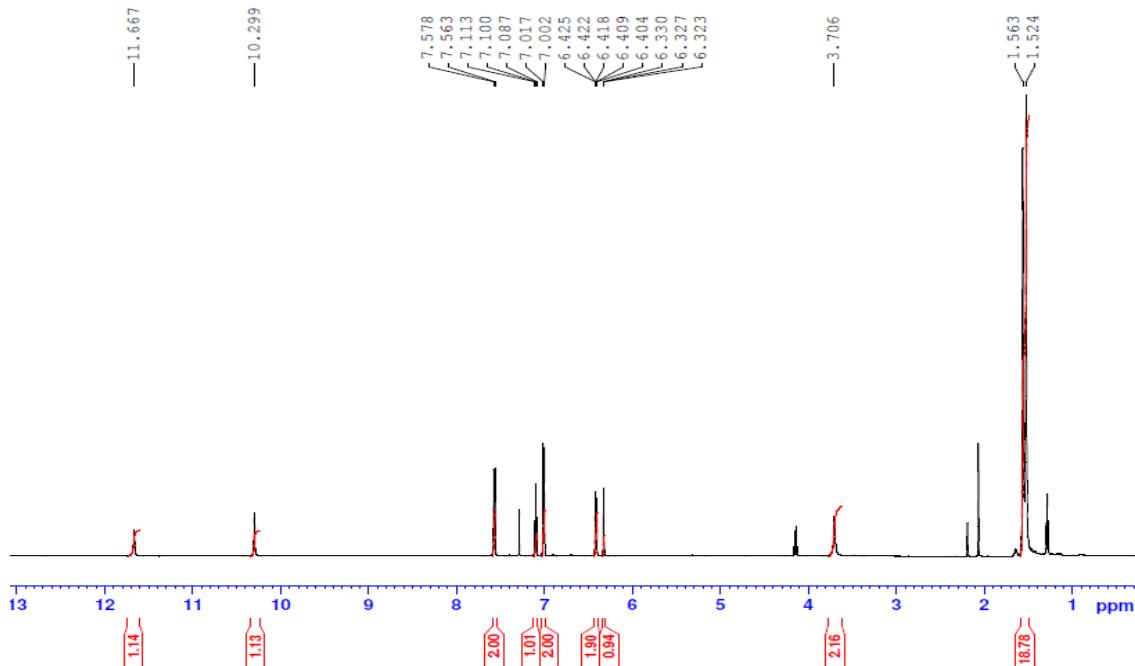
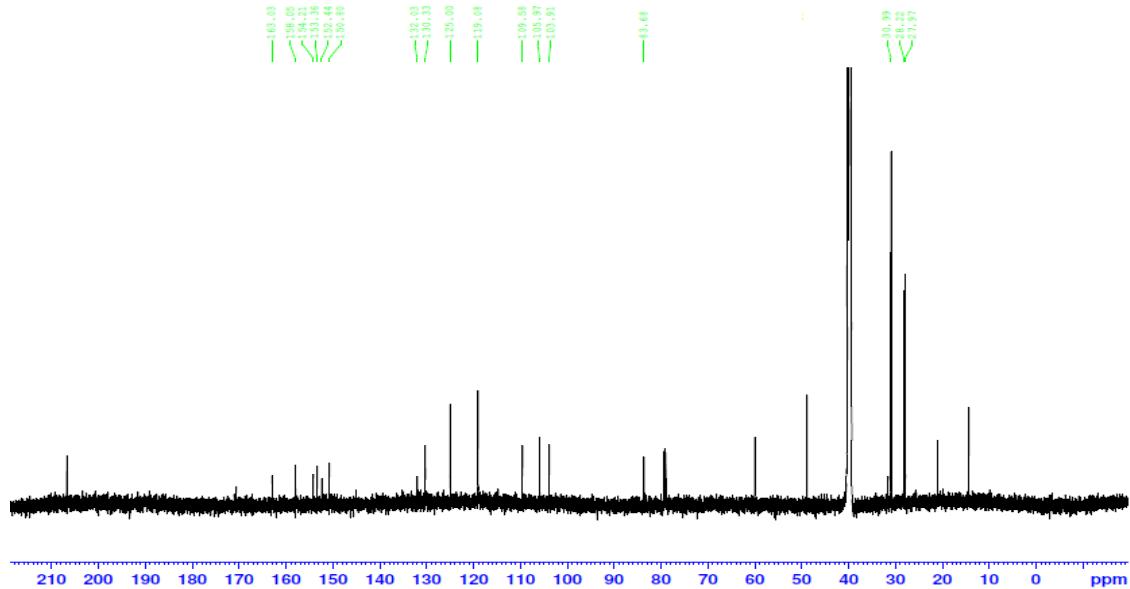


**$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**



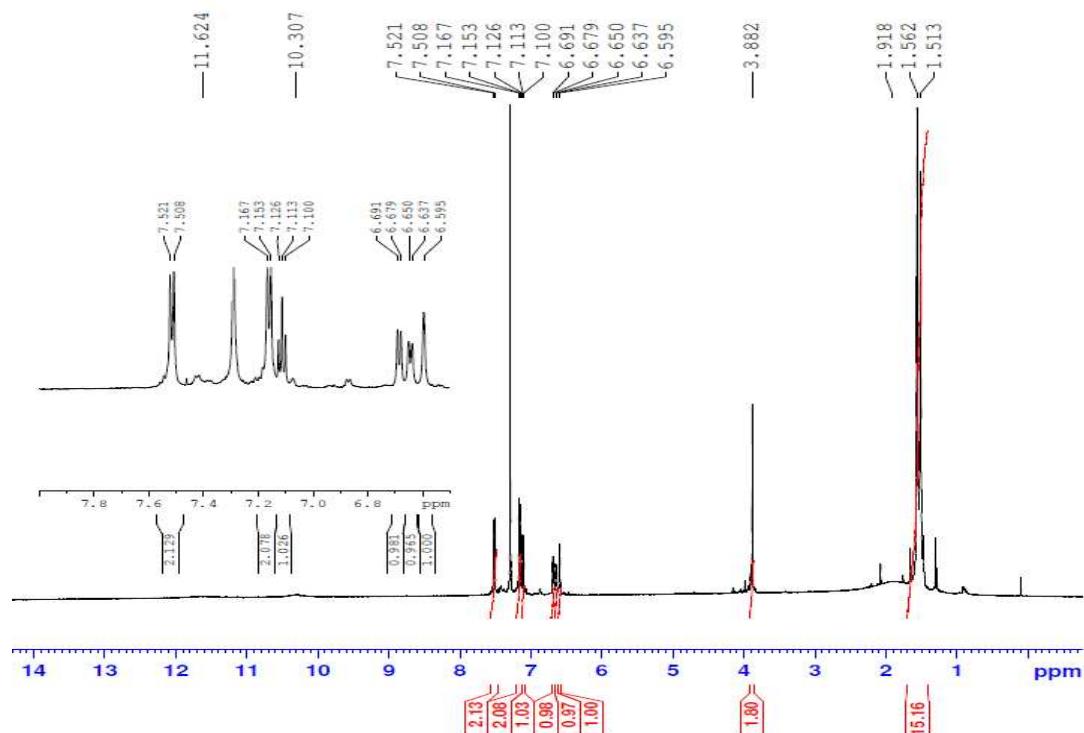
**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	19.04	0.01	3.3	0.4	0.015
2	UNKNOWN	22.35	0.03	6.3	1.0	0.034
3	UNKNOWN	26.75	99.95	2849.5	2887.1	99.951
Total			100.00	2850.1	2887.1	100.000

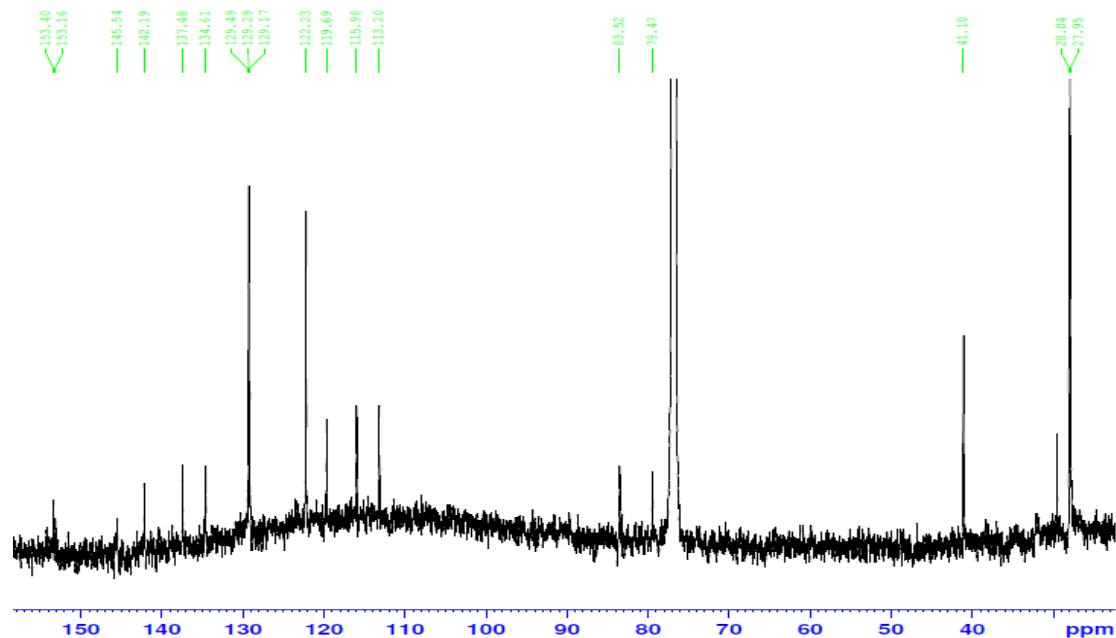
**3-Amino-{4'-[2,3-di(*tert*-butoxycarbonyl)]guanidino}diphenylether (**17a**)** **$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )** **$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

### 3-Amino-{4'-[2,3-di(*tert*-butoxycarbonyl)]guanidino}benzylbenzene (17c)

### **<sup>1</sup>H-NMR (CDCl<sub>3</sub>)**

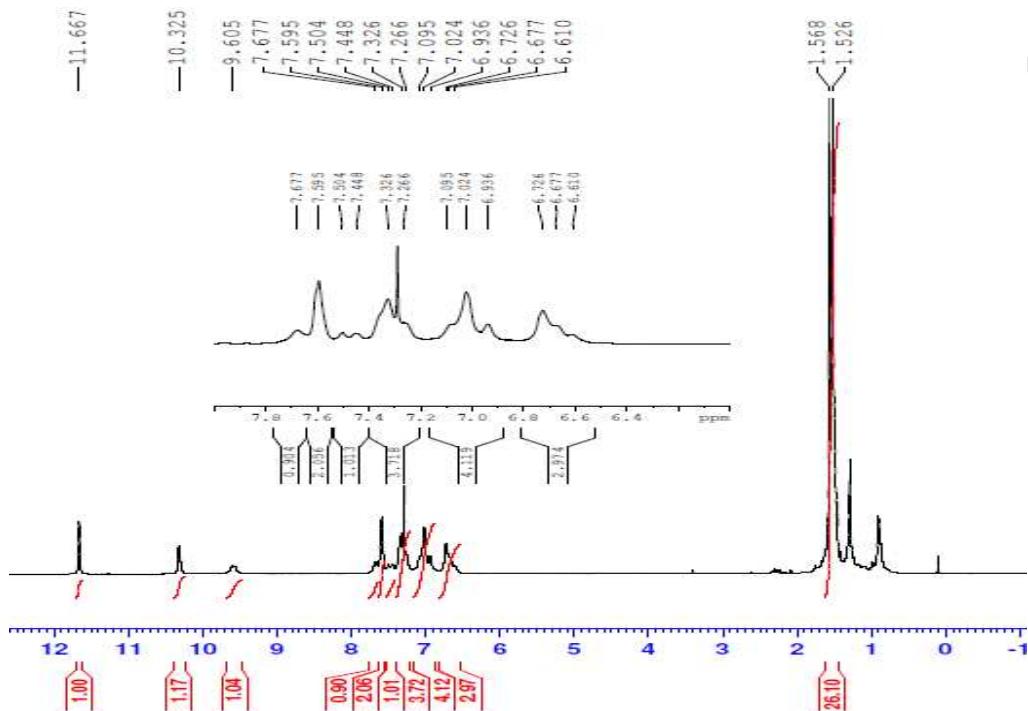


### **<sup>13</sup>C-NMR ( $\text{CDCl}_3$ )**

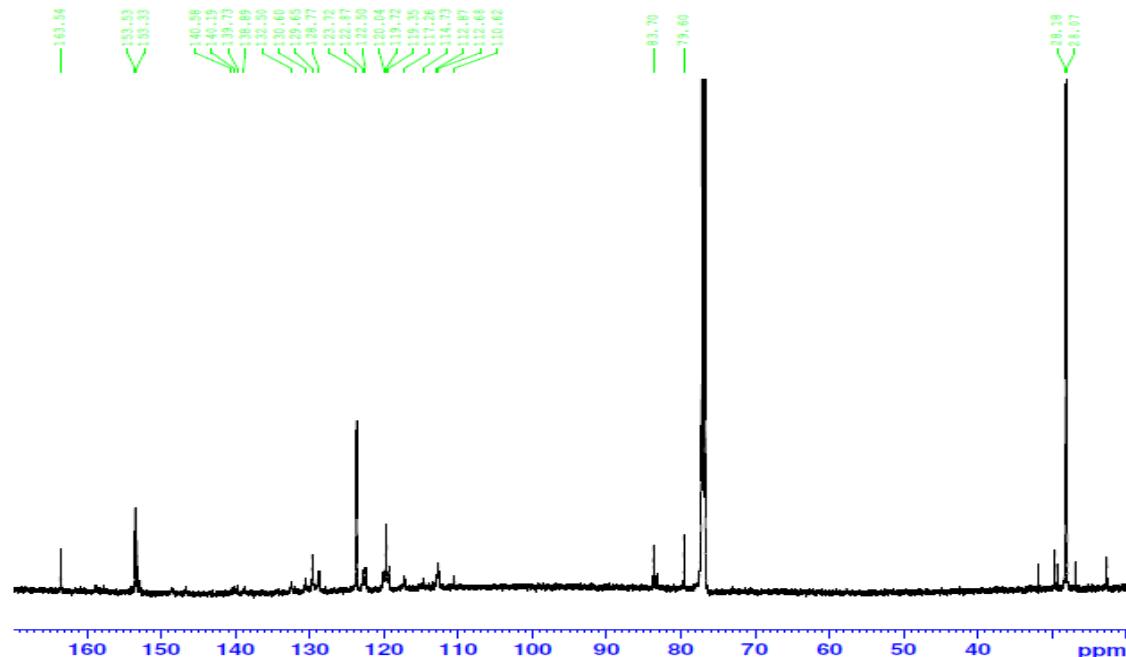


**4'-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-3-[2-(*tert*-butoxycarbonyl)-3-(phenyl)guanidino]diphenylether (20a)**

**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**

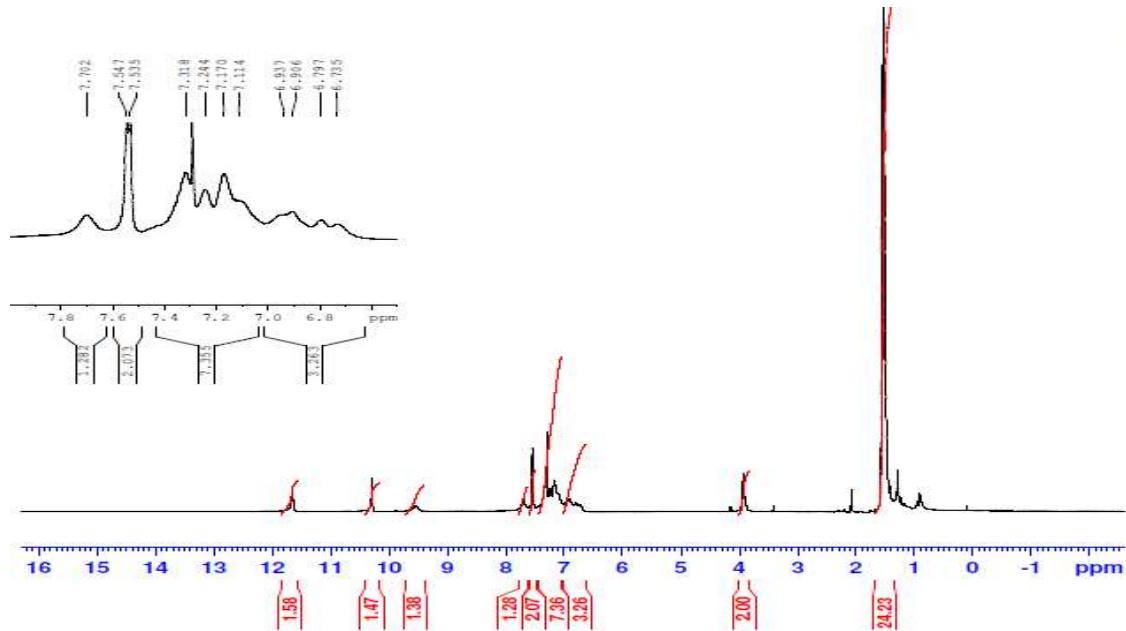


**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

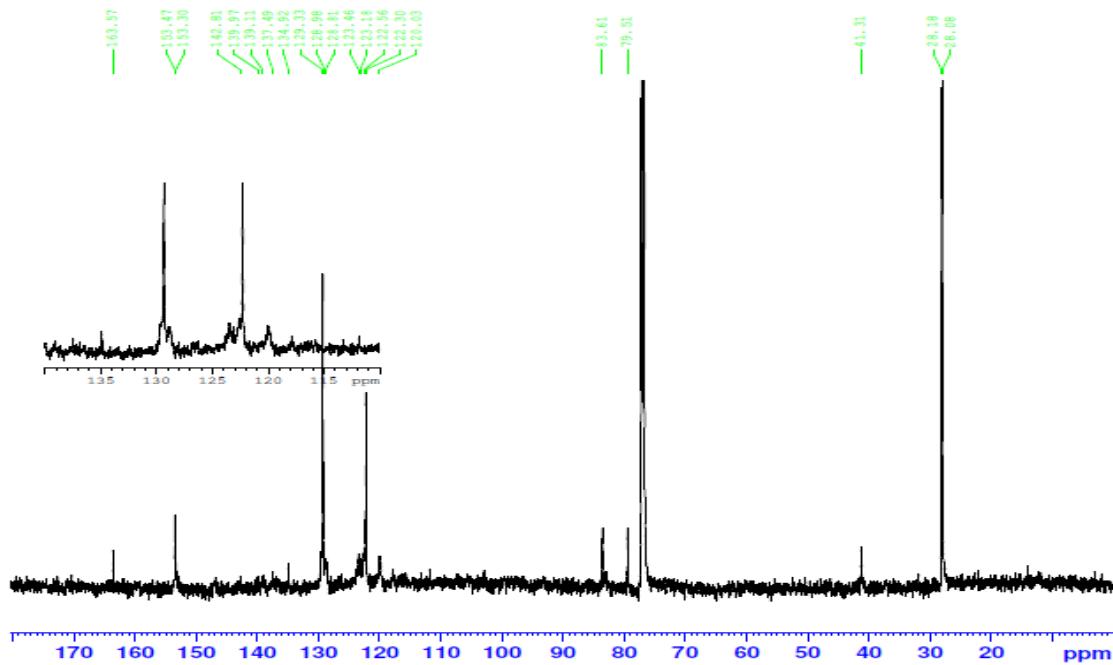


**4'-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-3-[*tert*-butoxycarbonyl]-3-(phenyl)guanidino]benzylbenzene (20c)**

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)

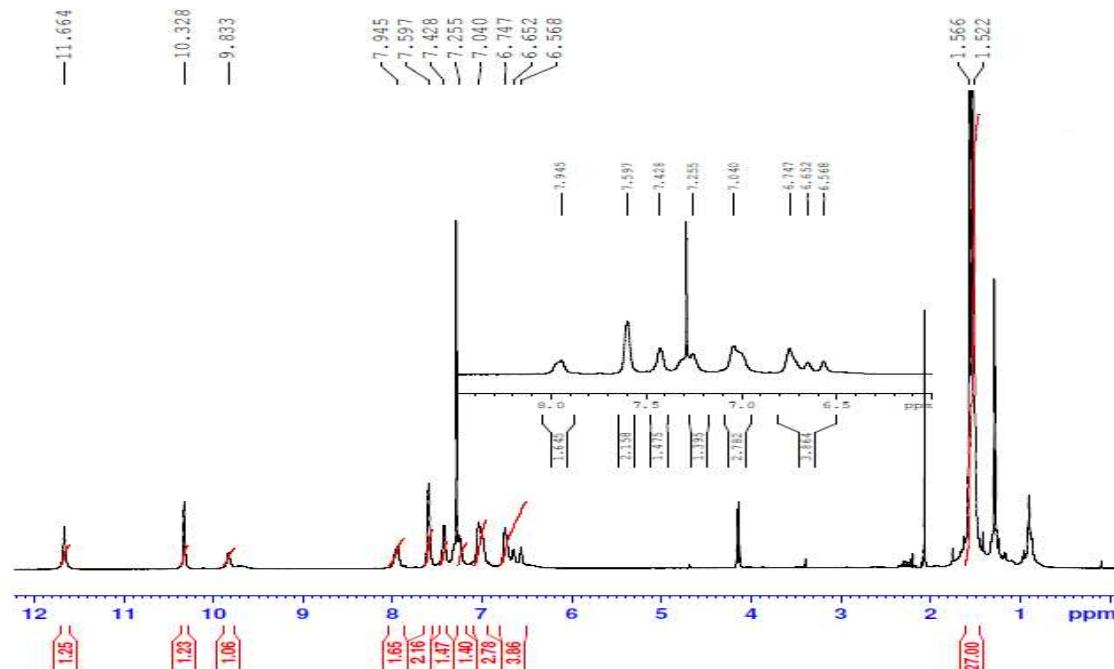


<sup>13</sup>C-NMR (CDCl<sub>3</sub>)

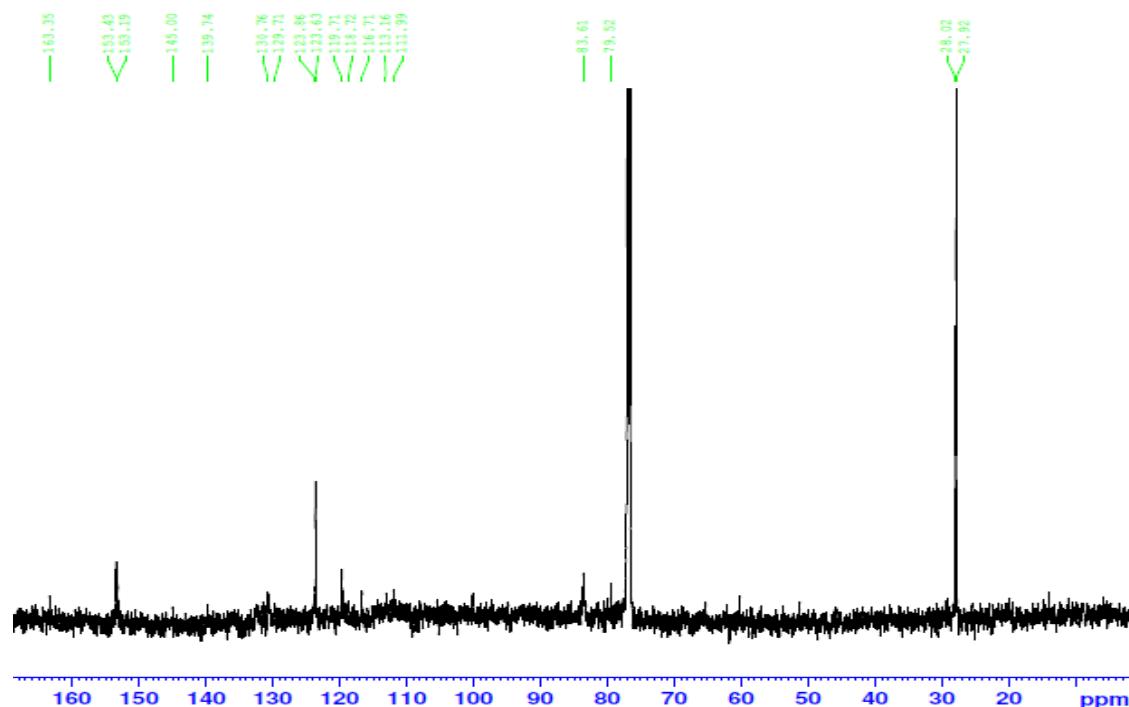


**4'-[2,3-Di-(*tert*-butoxycarbonyl)guanidino]-3-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]diphenylether (21a)**

### **<sup>1</sup>H-NMR (CDCl<sub>3</sub>)**

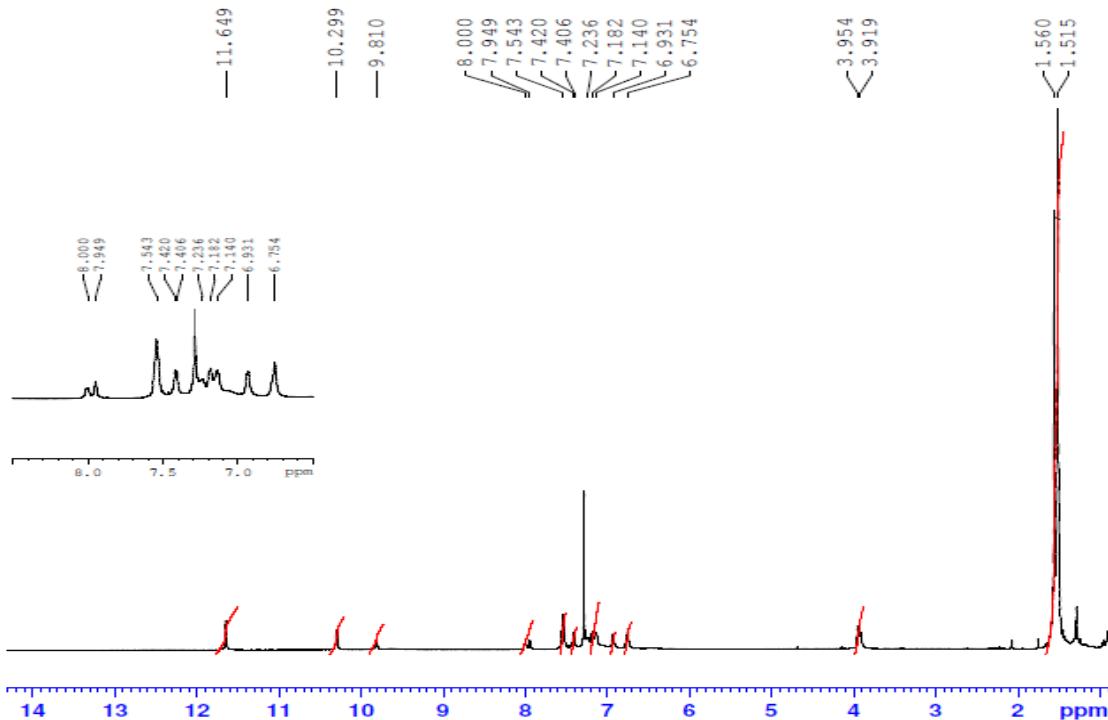


### <sup>13</sup>C-NMR ( $\text{CDCl}_3$ )

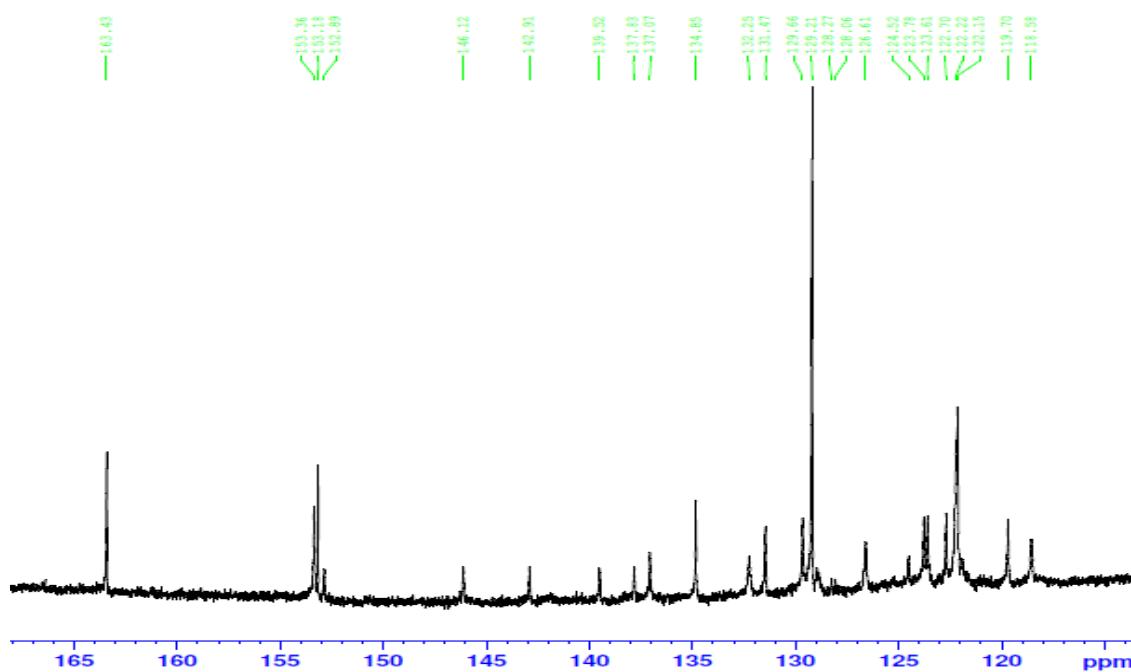


**4'-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-3-[2-(*tert*-butoxycarbonyl)-3-(4-chloro-3-trifluoromethylphenyl)guanidino]benzylbenzene (21c)**

**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**

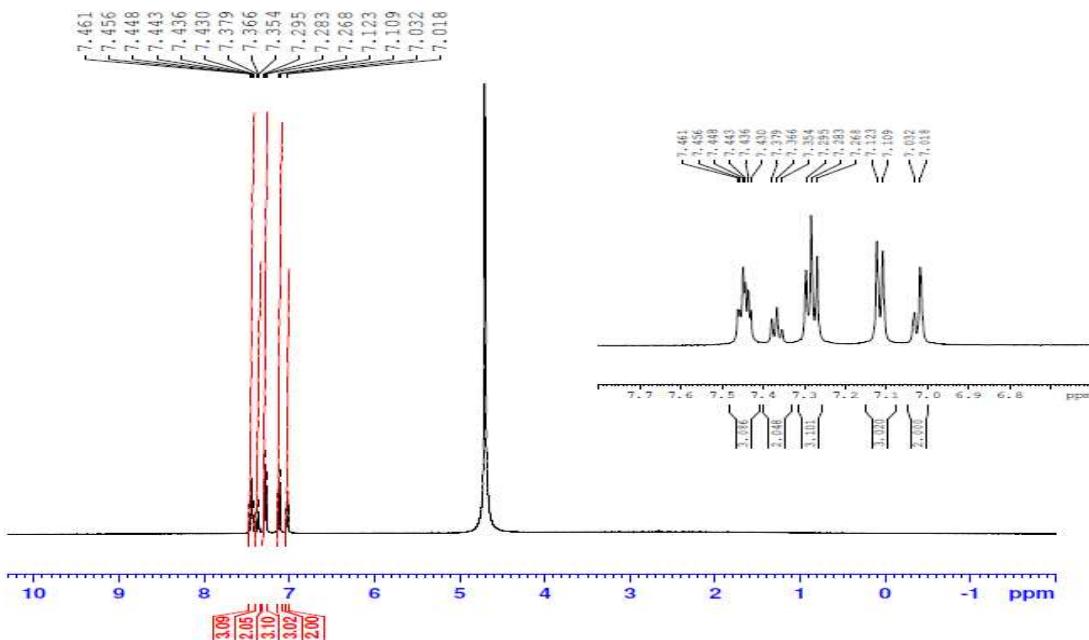


**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

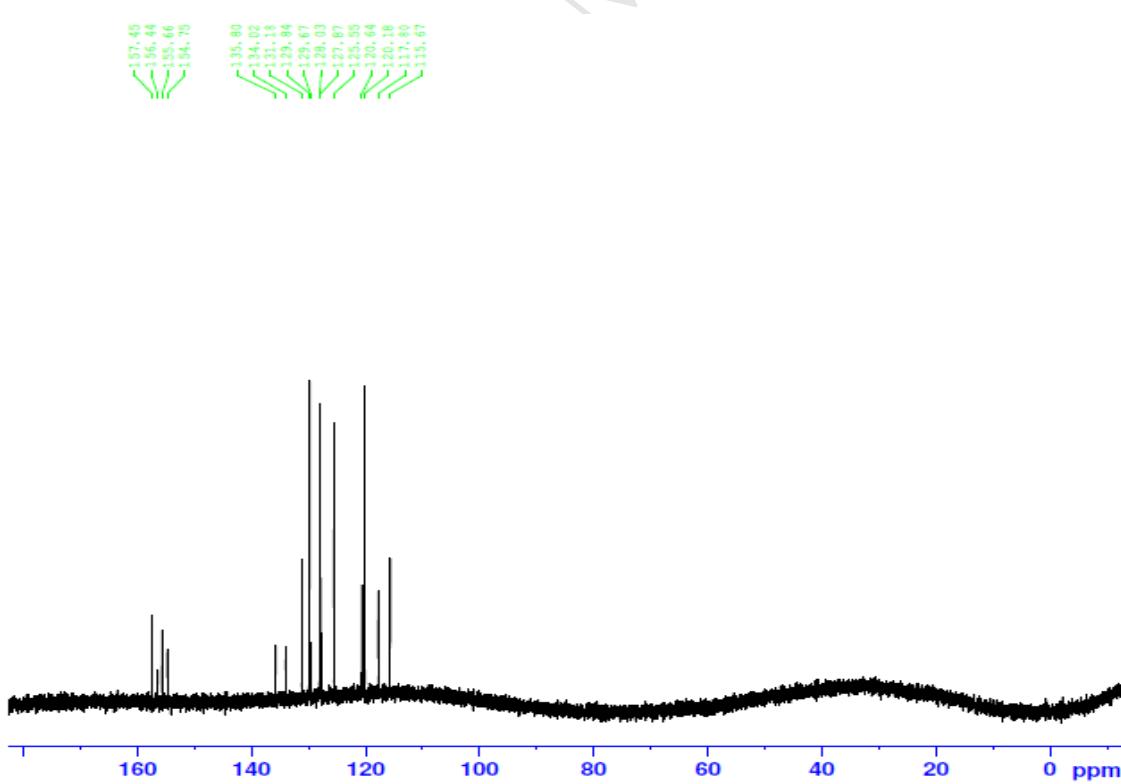


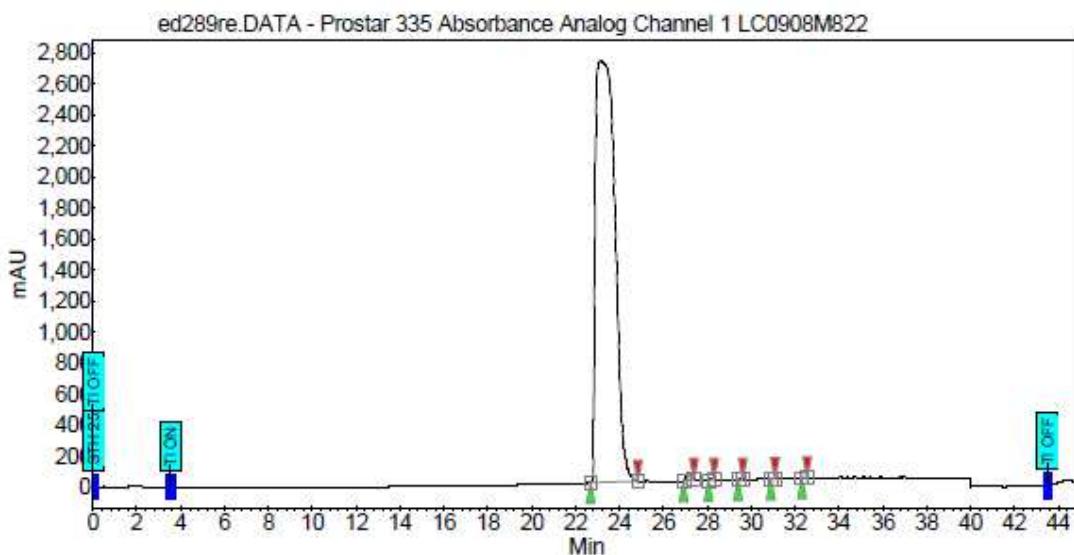
**4-[3'-(Phenylguanidino)phenoxy]phenylguanidine dihydrochloride (6a)**

**$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**

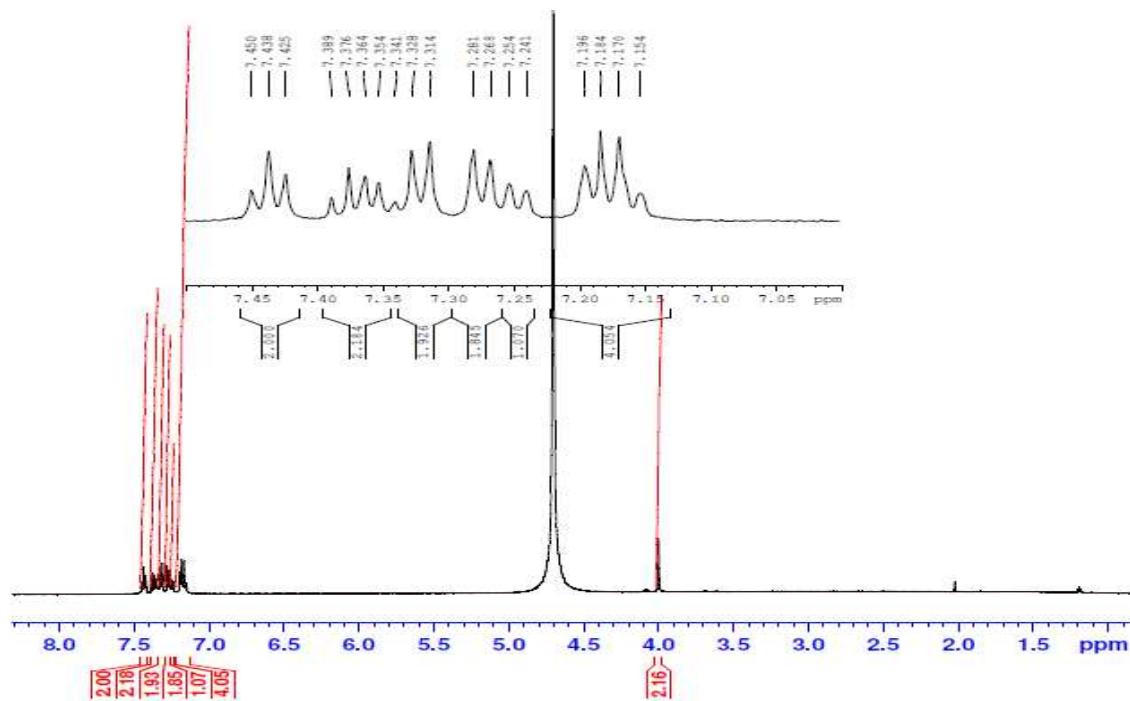
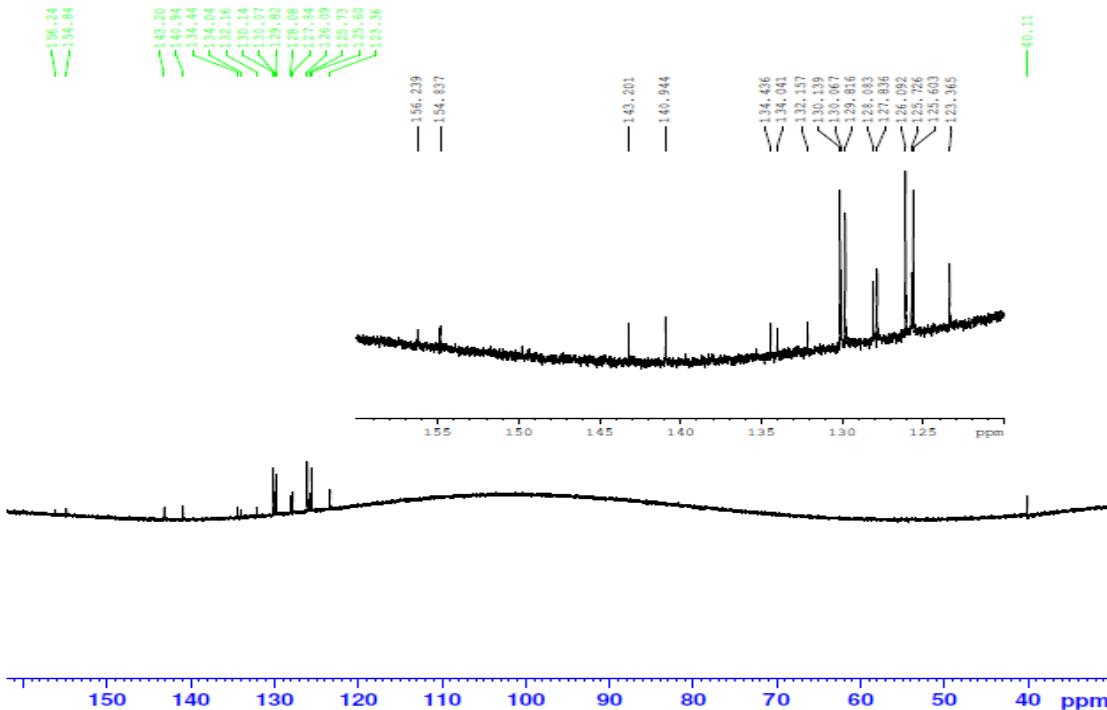


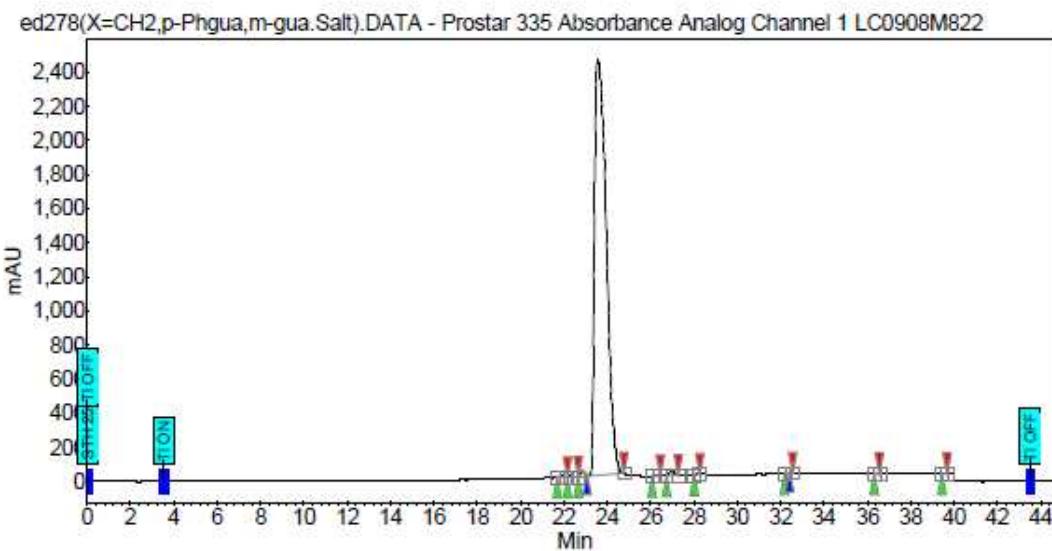
**$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**



**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU·Min]	Area % [%]
1	UNKNOWN	23.13	99.53	2716.7	2748.5	99.529
2	UNKNOWN	27.09	0.39	58.9	10.9	0.393
3	UNKNOWN	28.17	0.04	8.5	1.2	0.045
4	UNKNOWN	29.51	0.01	3.1	0.3	0.011
5	UNKNOWN	30.99	0.01	2.4	0.2	0.008
6	UNKNOWN	32.45	0.01	3.6	0.4	0.014
Total		100.00	2781.2	2781.5	100.000	

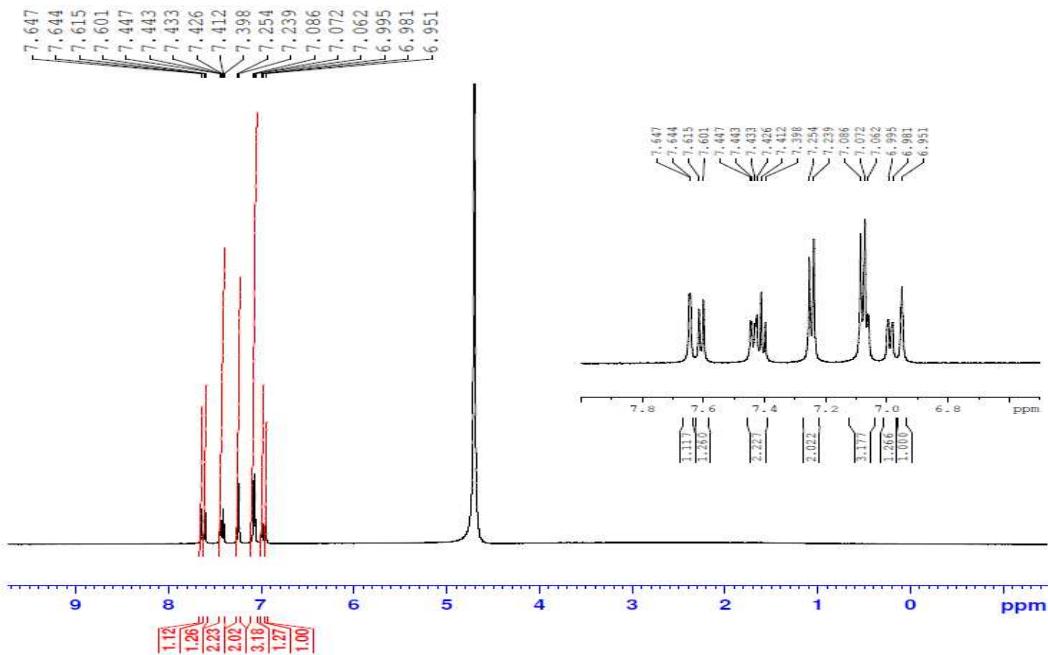
**4-[3'-(Phenyl)guanidinobenzyl]phenylguanidine dihydrochloride (6c)** **$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )** **$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

**HPLC****Peak results :**

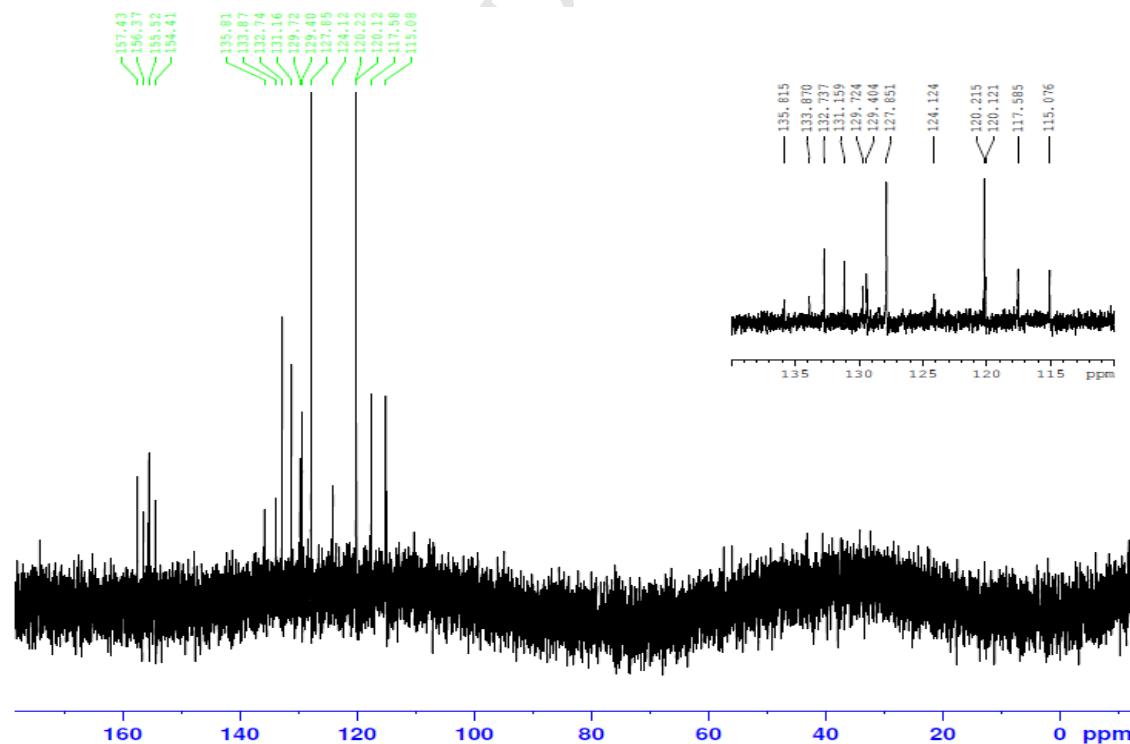
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU Min]	Area % [%]
1	UNKNOWN	21.88	0.13	11.3	2.1	0.134
2	UNKNOWN	22.31	0.13	10.9	2.0	0.127
3	UNKNOWN	22.84	0.46	36.4	7.2	0.460
4	UNKNOWN	23.58	98.54	2433.9	1536.4	98.538
5	UNKNOWN	26.27	0.11	8.8	1.7	0.107
6	UNKNOWN	26.97	0.51	32.7	8.0	0.512
7	UNKNOWN	28.15	0.03	3.2	0.5	0.030
8	UNKNOWN	32.36	0.04	4.1	0.8	0.037
9	UNKNOWN	32.40	0.02	3.8	0.3	0.021
10	UNKNOWN	36.44	0.02	2.7	0.3	0.022
11	UNKNOWN	39.53	0.02	1.6	0.2	0.015
Total			100.00	2549.5	1550.2	100.000

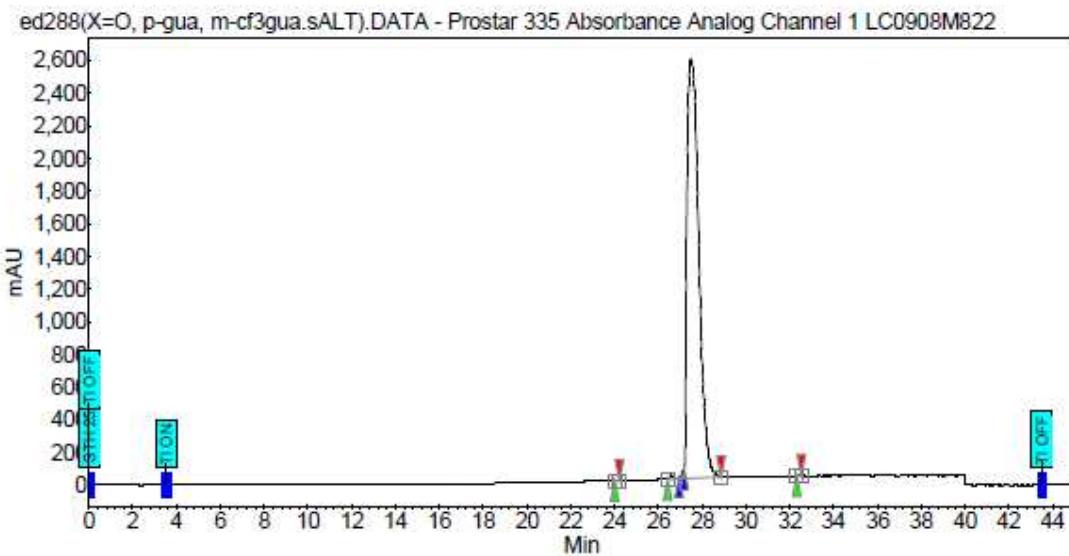
**4-[3'-(4-Chloro-3-trifluoromethylphenyl)guanidinophenoxy]phenylguanidine  
dihydrochloride (7a)**

**$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**



**$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

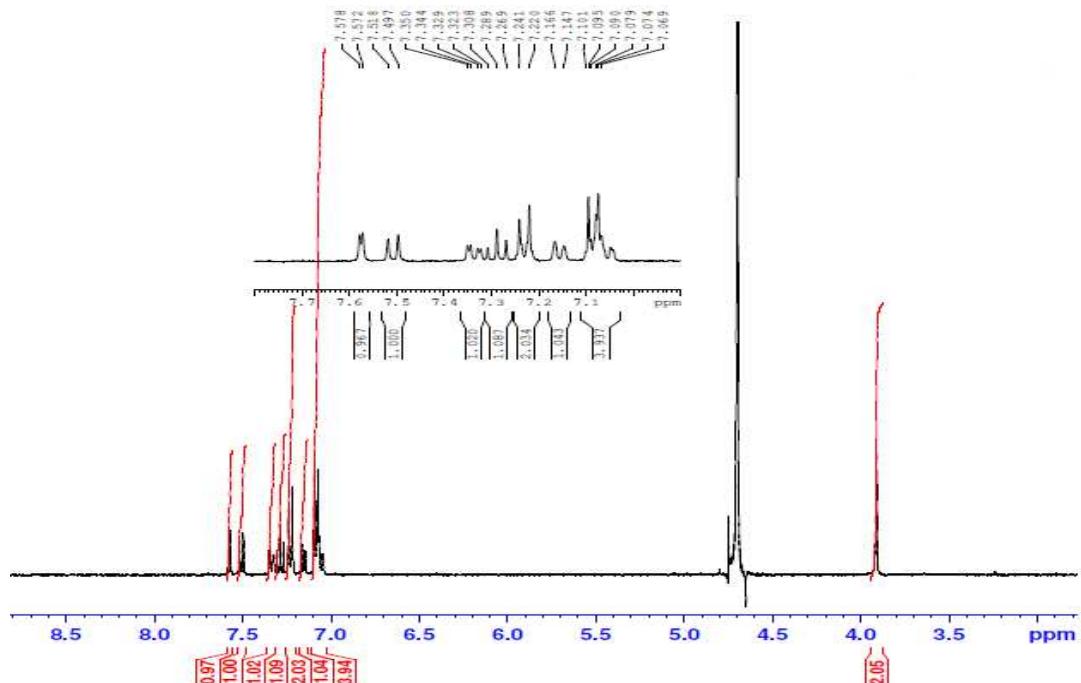


**HPLC****Peak results :**

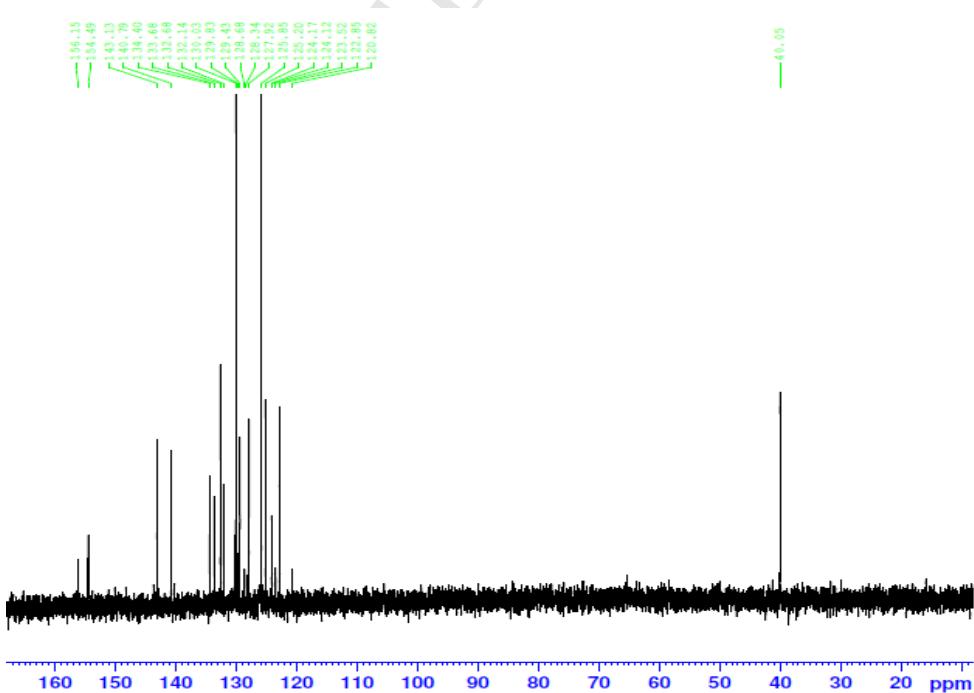
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU·Min]	Area.% [%]
1	UNKNOWN	24.12	0.02	2.8	0.3	0.019
2	UNKNOWN	26.59	0.29	25.2	4.6	0.287
3	UNKNOWN	27.13	0.40	54.8	6.4	0.388
4	UNKNOWN	27.48	99.27	2567.0	1591.4	99.270
5	UNKNOWN	32.44	0.03	3.8	0.4	0.025
Total			100.00	2653.7	1603.1	100.000

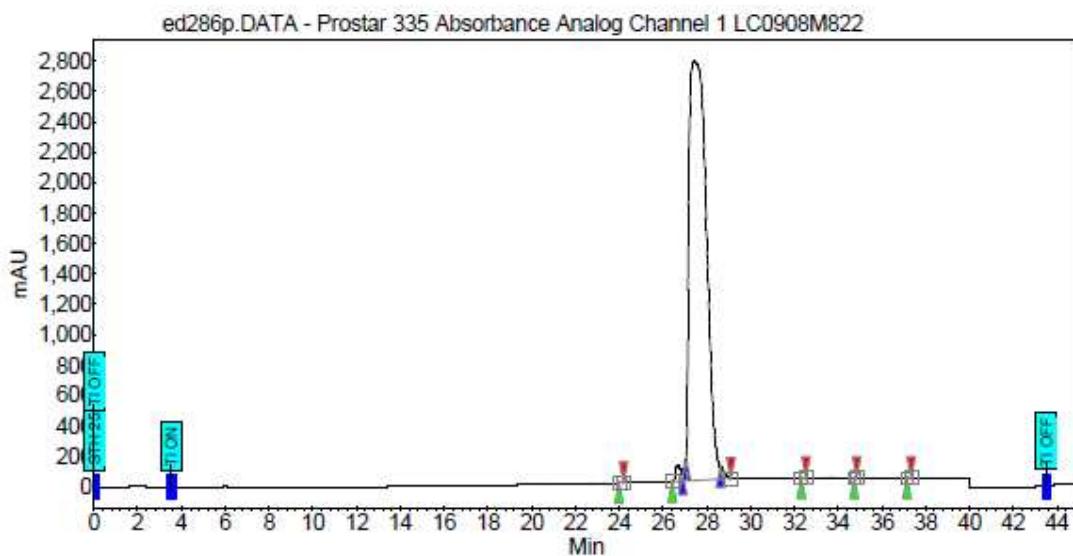
**4-[3'-(4-Chloro-3-trifluoromethylphenyl)guanidinobenzyl]phenylguanidine  
dihydrochloride (7c)**

**$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**



**$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**

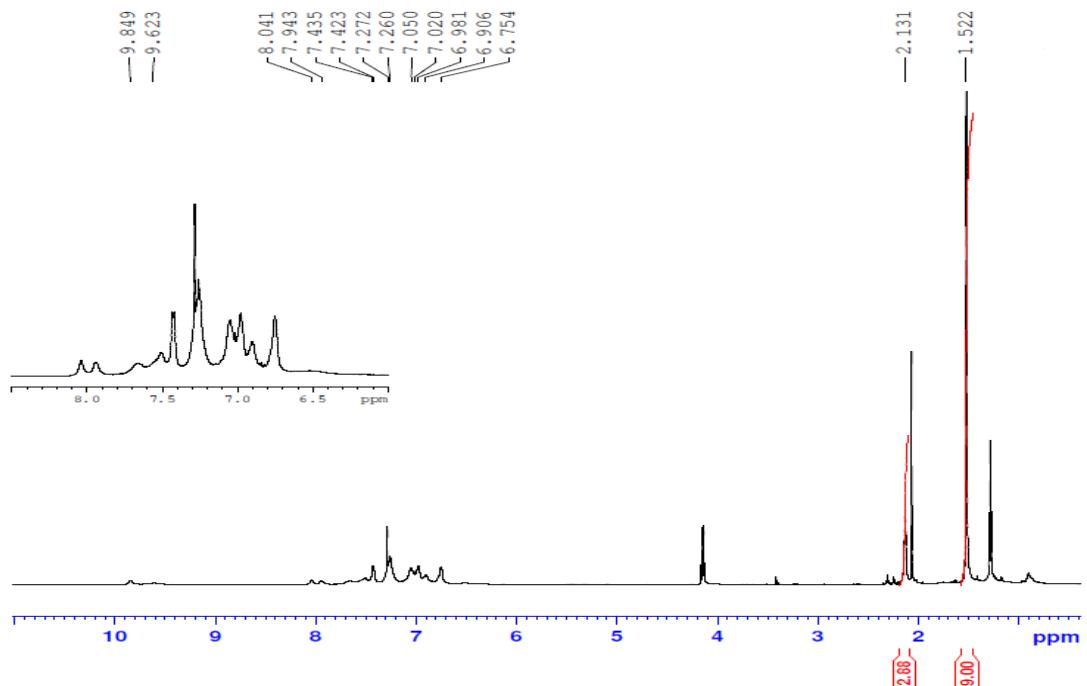


**HPLC****Peak results :**

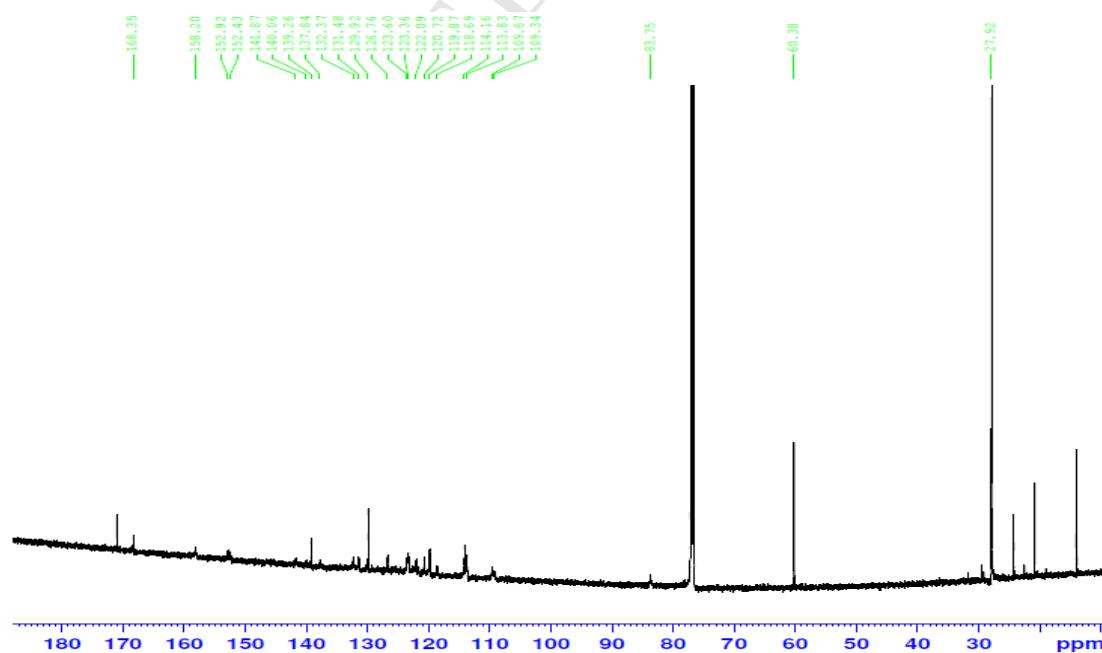
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU·Min]	Area % [%]
1	UNKNOWN	24.08	0.02	4.2	0.5	0.018
2	UNKNOWN	26.67	1.09	107.1	28.0	1.089
3	UNKNOWN	27.00	0.62	135.4	15.9	0.617
4	UNKNOWN	27.41	97.66	2759.4	2510.4	97.662
5	UNKNOWN	28.67	0.59	73.6	15.1	0.588
6	UNKNOWN	32.44	0.01	2.9	0.3	0.013
7	UNKNOWN	34.77	0.00	1.7	0.1	0.004
8	UNKNOWN	37.25	0.01	1.7	0.2	0.008
Total			100.00	3086.1	2570.5	100.000

***N*-(3-{4-[*N'*-(4-Chloro-3-trifluoromethylphenyl)-*N''*-*tert*-butoxycarbonylguanidino]phenoxy}phenyl)acetamide (22a)**

**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**

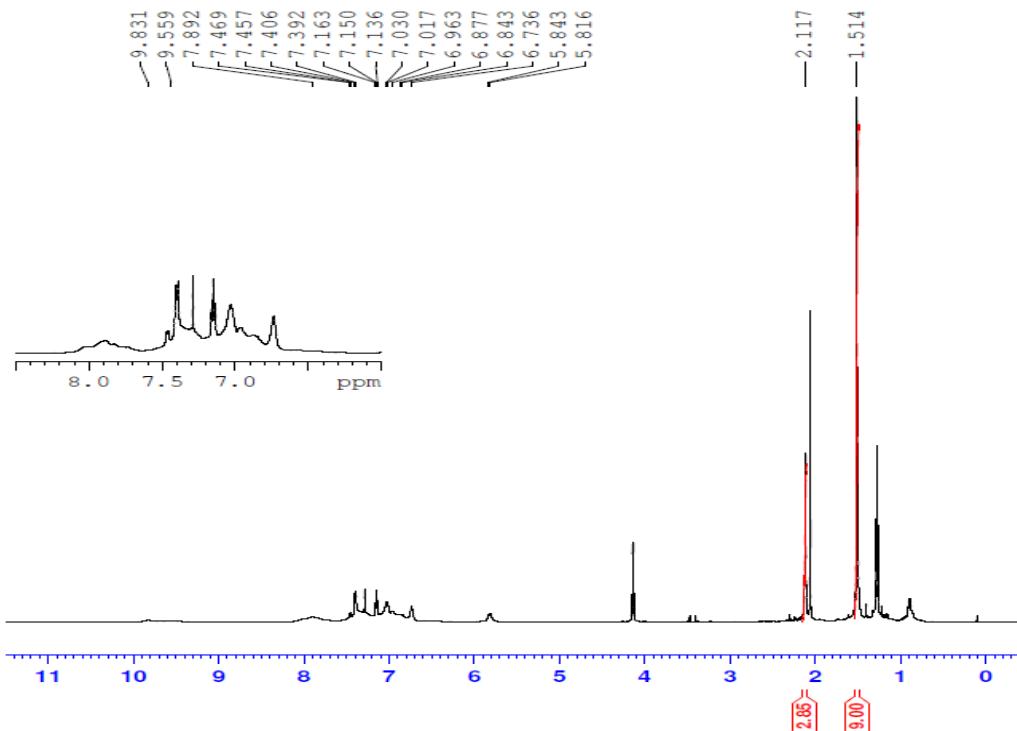


**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

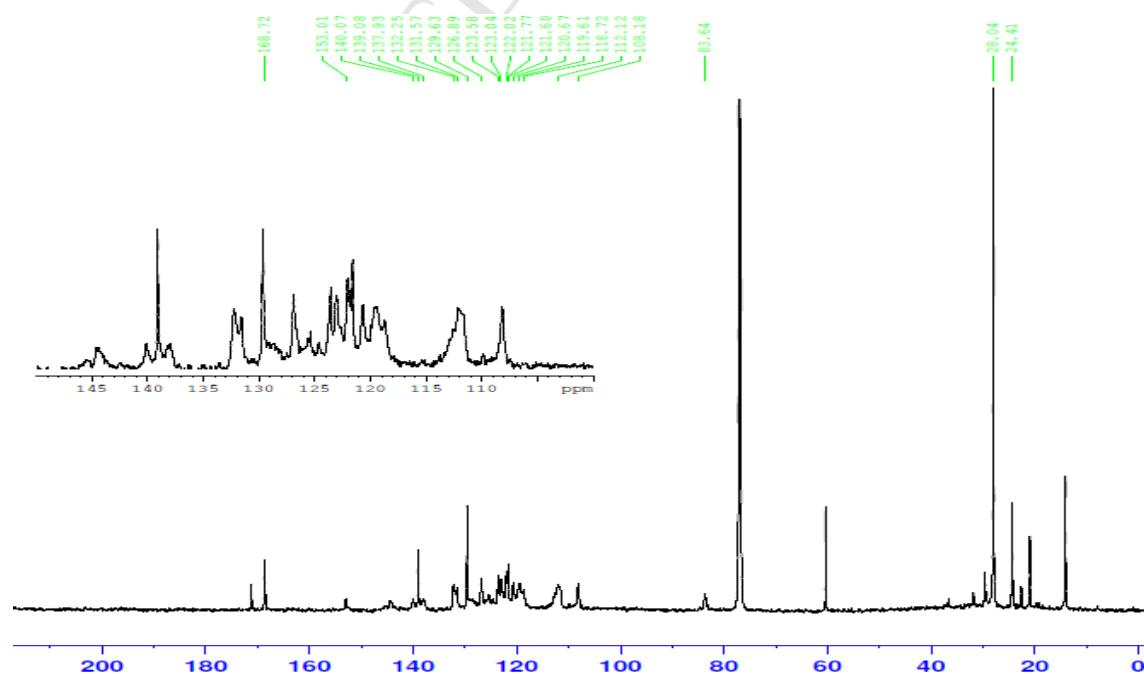


***N*-(3-{4-[*N'*-(4-Chloro-3-trifluoromethylphenyl)-*N''*-tert-butoxycarbonylguanidino]phenylamino}phenyl)acetamide (22d)**

**$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )**

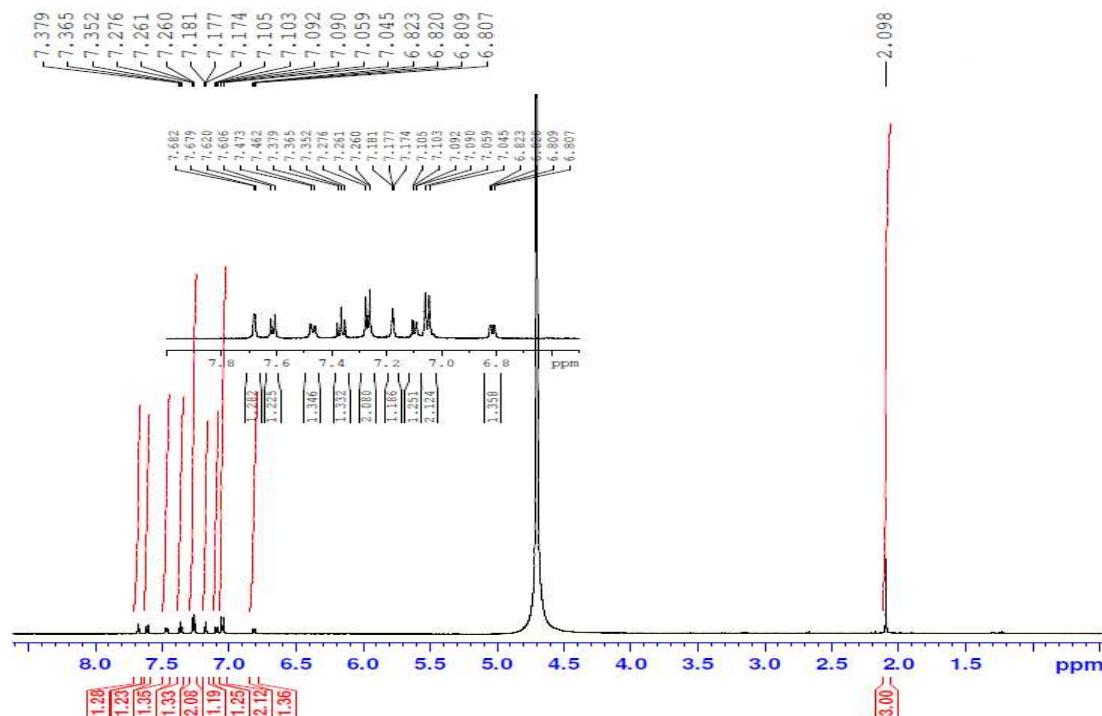


**$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )**

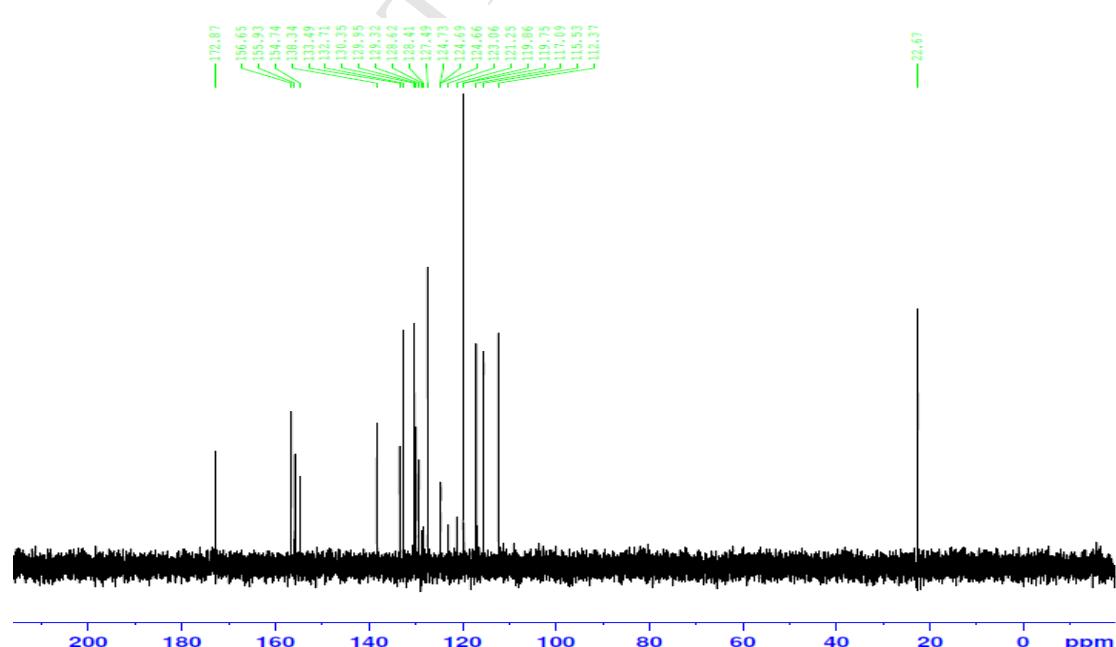


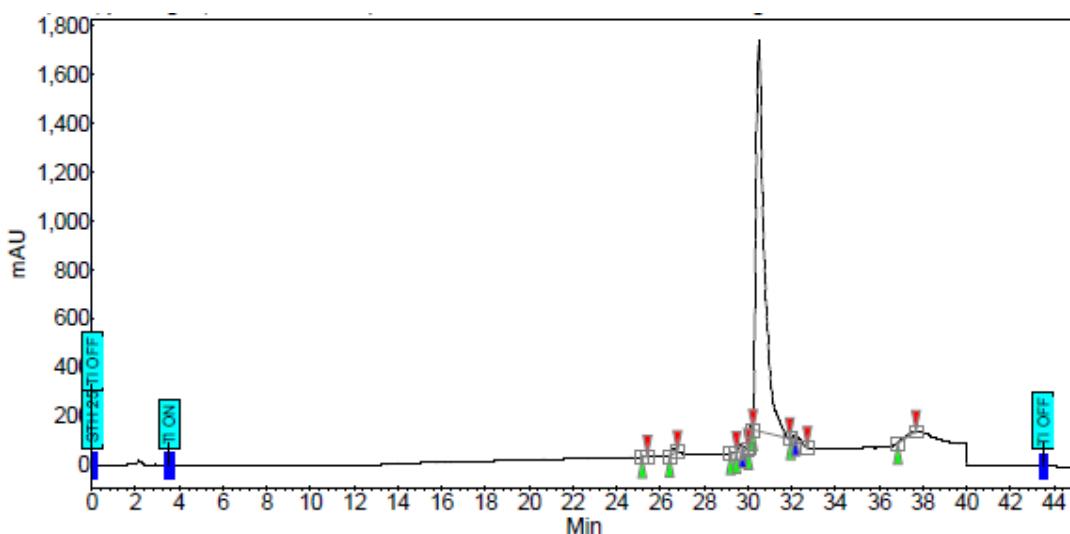
**N-(3-{4-[N'-(4-Chloro-3-trifluoromethylphenyl)guanidino]phenoxy}phenyl)acetamide hydrochloride (8a)**

### **<sup>1</sup>H-NMR (D<sub>2</sub>O)**



### **<sup>13</sup>C-NMR (D<sub>2</sub>O)**

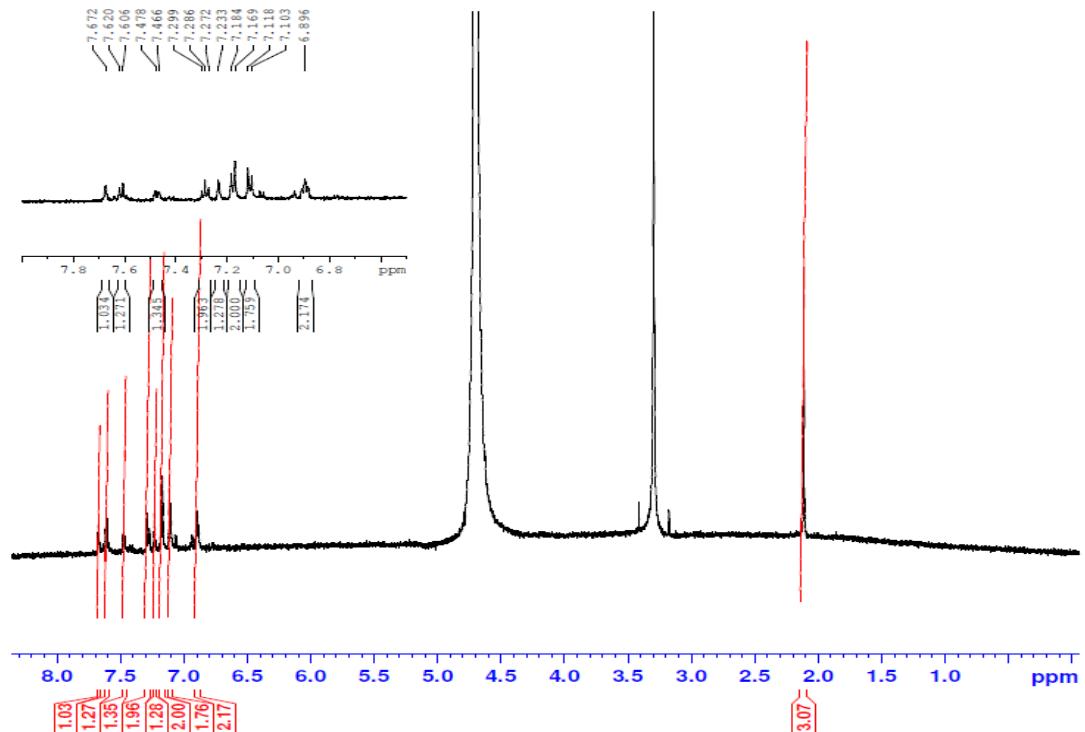


**HPLC****Peak results :**

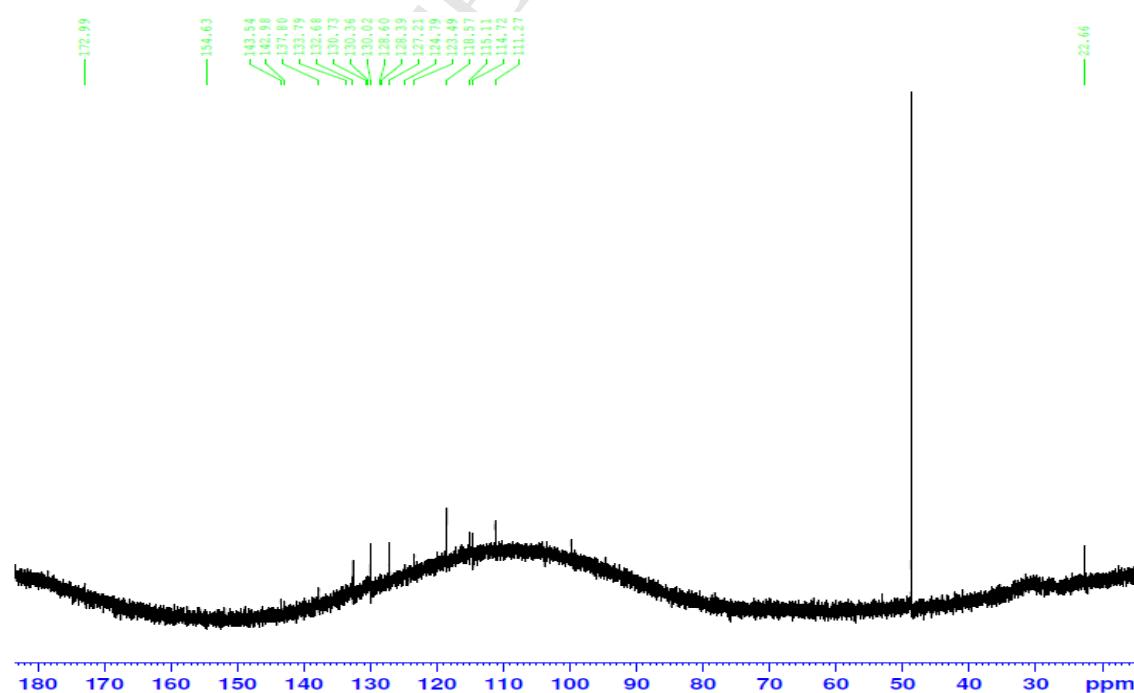
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	25.28	0.05	3.3	0.4	0.052
2	UNKNOWN	26.59	0.50	23.9	3.7	0.502
3	UNKNOWN	29.35	0.03	1.7	0.2	0.030
4	UNKNOWN	29.60	0.68	34.0	5.0	0.681
5	UNKNOWN	29.83	0.29	14.8	2.1	0.286
9	UNKNOWN	30.13	0.81	52.6	6.0	0.814
10	UNKNOWN	30.51	96.01	1602.6	704.1	96.006
6	UNKNOWN	32.12	0.31	18.8	2.3	0.309
7	UNKNOWN	32.21	0.78	23.2	5.7	0.776
8	UNKNOWN	37.37	0.54	9.0	4.0	0.543
Total			100.00	1783.8	733.3	100.000

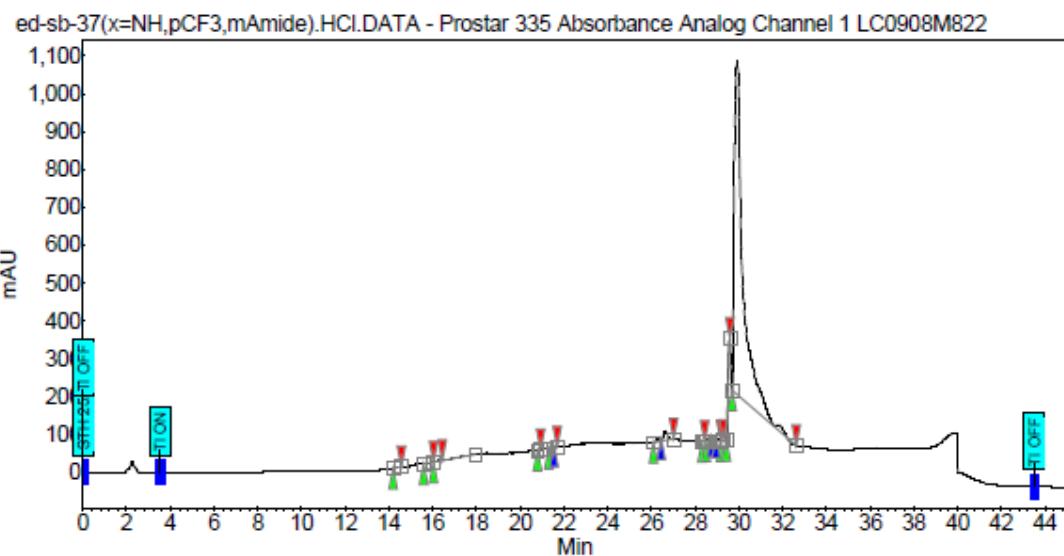
***N-(3-[4-[N'-(4-Chloro-3-trifluoromethylphenyl)guanidino]phenylamino]phenyl)acetamide hydrochloride (8d)***

**$^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )**



**$^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )**



**HPLC****Peak results :**

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	14.35	0.04	0.8	0.2	0.038
2	UNKNOWN	15.89	0.08	1.5	0.4	0.085
3	UNKNOWN	16.36	0.08	1.4	0.3	0.082
4	UNKNOWN	20.85	0.02	1.2	0.1	0.020
5	UNKNOWN	21.44	0.04	1.1	0.1	0.035
6	UNKNOWN	21.61	0.02	1.2	0.1	0.025
7	UNKNOWN	26.27	0.20	4.7	0.9	0.203
8	UNKNOWN	26.63	1.29	26.4	5.5	1.289
9	UNKNOWN	28.37	0.02	1.0	0.1	0.015
10	UNKNOWN	28.65	0.38	11.4	1.6	0.385
11	UNKNOWN	28.81	0.55	15.6	2.3	0.547
12	UNKNOWN	29.00	0.17	5.7	0.7	0.174
13	UNKNOWN	29.23	0.00	0.3	0.0	0.004
15	UNKNOWN	29.55	1.35	98.0	5.7	1.348
14	UNKNOWN	29.92	95.75	1051.7	406.2	95.750
Total			100.00	1051.7	424.2	100.000

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5. Wang, Z.; Fu, H.; Jiang, Y.; Zhao, Y. *Synlett* **2008**, *16*, 2540-2546
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