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Novel synthesis and pharmacological evaluation as α_2-adrenoceptor ligands of *O*-phenylisouronium salts

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ABSTRACT

The synthesis of nine new mono- and bis-*O*-phenylisouronium compounds (**2**, **6b**–**10b** and **12b**–**14b**) and their Boc-protected isourea precursors (**2a**, **6a**–**10a** and **12a**–**14a**) is described. The carbodiimide **4**, which was formed, had been suggested as the reactive intermediate species and driving force of the reaction. All final substrates were tested as potential α_2-ARs ligands in human brain tissue by means of radioligand binding experiments and were compared to the potential antidepressant **1**, as well as other related guanidine containing derivatives.

1. Introduction

α_2-Adrenoceptors (α_2-ARs) play an important role in neurotransmitter release[1] and are implicated in a variety of pharmacological functions.[2] Thus, α_2-AR agonists have applications as analgesics,[3] anaesthetics,[4] and as antihypertensive[5] or antiglaucoma agents,[6] whereas α_2-AR antagonists can act as antidepressants.[7] In this context, the number of people affected by depression has increased significantly in recent years and by 2020 the disease is expected to be the second largest health burden as stated by the World Health Organisation.[8] The pathophysiological origin of depression remains unknown, however, the monoaminergic hypothesis[7] assumes that it is a consequence of a low concentration of noradrenaline (NA) or serotonin in the brain.

It is well established that central noradrenergic transmission is regulated by inhibitory presynaptic noradrenergic receptors (α_2-ARs) and that the activation of these autoreceptors induces an inhibition of NA release. It has been proposed that depression is associated with a selective increase in the high-affinity conformation of the α_2-ARs in the human brain,[9] enhancing their activity and resulting in the deficit in NA transmission described in the etiology of depression. Chronic treatment with antidepressants produces an in vivo desensitization of the α_2-ARs regulating the local release of NA.[10] Bearing this in mind, the development of new selective α_2-AR antagonists can be considered an effective therapeutical approach for the treatment of depressive disorders, since it has been demonstrated that administration of different α_2-AR antagonists, both locally in the locus coeruleus or systemically, increases the release of NA in the prefrontal cortex.[11,12] In fact, mirtazapine (Fig. 1), one of the most recently developed antidepressants, displays its activity by blocking the α_2-ARs.[13]

Interested in the development of new α_2-AR antagonists, and therefore new potential antidepressants, we recently started a program consisting of the synthesis of a library of guanidine and 2-aminoimidazoline derivatives and their biological evaluation in human prefrontal cortex (PFC).[14] Among the new α_2-AR antagonists found, compound **1** (Fig. 1) showed an affinity towards the α_2-ARs similar to that of Idazoxan[14b] and was subject to in vivo microdialysis experiments in rats. The levels of NA in the rat brains increased remarkably after local and systemic administration of **1**, confirming its ability to cross the blood–brain barrier (BBB) and its potential as antidepressant.[14b]

Encouraged by the excellent results displayed by the lead compound **1**, we decided to perform some chemical modifications in order to explore the affinity towards the α_2-ARs and try to identify the functional groups required for a better binding and antagonist

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† This article is dedicated to the memory of Áine Goonan, who started this project during her fourth year project in TCD and has prematurely died at the age of 23.

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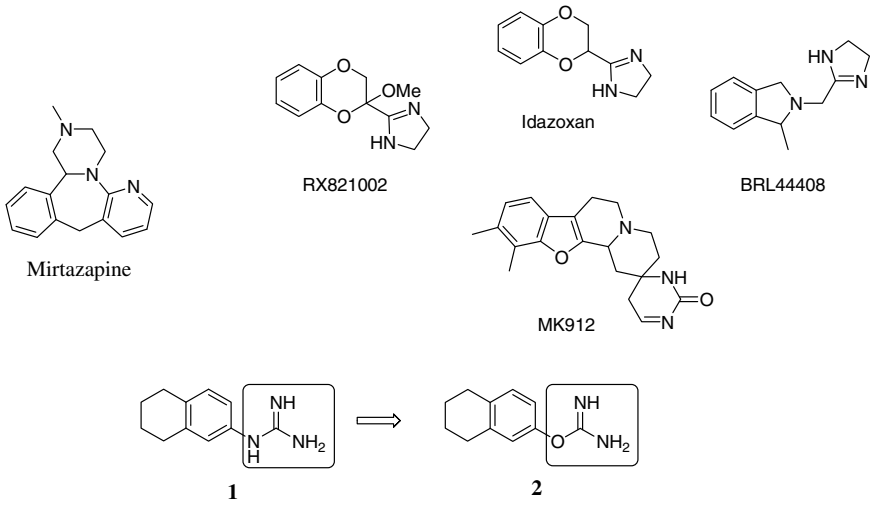


Figure 1. Structures of a known α_2-adrenoceptor targeting antidepressant (mirtazapine), known α_2-AR antagonists with an 'amidinium' moiety, a new α_2-AR antagonist previously described in our group (**1**) and a proposed molecule (**2**).

activity. The guanidinium cation seems to be the most important group in the binding to the receptor, thus modifications in that moiety were pursued. The presence of several hydrogen bond (HB) donors, such as amino groups, seems to be responsible of the binding; yet, the introduction of at least one HB acceptor, such as an O atom, could potentially improve the interaction with the binding pocket of the α_2-ARs. Several antagonists with only an imidazolinium or a urea group are already known (see Fig. 1), thus, we could assume that only the 'amidinium' moiety is needed for an efficient binding. Moreover, Serine residues have been proposed to play an important role in the interaction in the binding pocket.[15] Hence, considering that the OH of Ser is a very good HB donor the introduction of a HB acceptor could reinforce the binding to the receptor. Taking all this into account, the guanidinium cation was substituted by the isouronium one (as in compound **2**, Fig. 1) and a set of *O*-phenylisouronium salts were synthesised.

In this article, we present a novel methodology for the preparation of nine new (bis)*O*-phenylisouronium chlorides and their Boc-protected *O*-phenylisourea precursors, together with an in vitro pharmacological study of their affinity towards the α_2-ARs in human brain. Human PFC was chosen because there is an important density of α_2-ARs in this tissue[16] and many studies have reported changes in PFC activity in the brain of patients with depression.[17] Moreover, the final goal of our research is the synthesis of antidepressants. Hence, the use of human brain tissue to pharmacologically characterize the new compounds will be relevant from a therapeutic point of view.

2. Results and discussion

2.1. Chemistry

A number of *O*-alkylisouronium salts have been recently described in the literature as intermediates in the synthesis of diazirines to perform mechanistic fragmentation studies in alkoxyhalocarbenes.[18] In all cases, the synthesis of these substrates relies on the reaction of the corresponding alcohol with cyanamide in the presence of either methane- or trifluoromethanesulfonic acid.[19] The same strategy can be applied to convert phenols into *O*-phenylisouronium salts.[20] Even though this is the main and most straightforward way for the preparation of isouroniums from alcohols, purification of the products could prove to be problematic. In addition, starting materials with acid labile groups are not suitable for the method, since they require milder conditions.

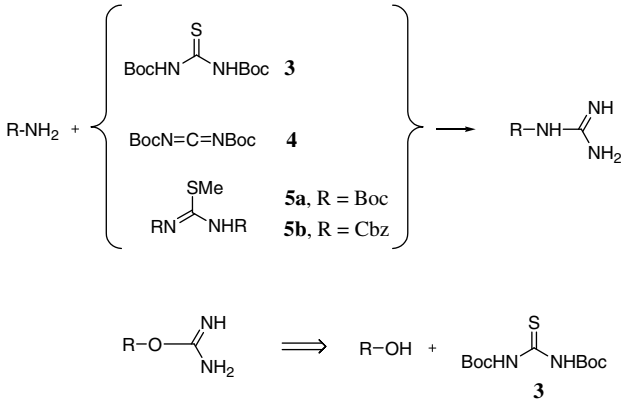
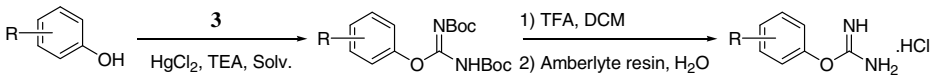


Figure 2. Typical preparation of guanidines from primary amines and Boc- or Cbz-protected thiourea, possible carbodiimide intermediate or *S*-methylisourea and proposed synthesis of isourea derivatives.

On the other hand, the use of carbodiimides in the reaction with aliphatic alcohols and phenols to provide *O*-alkyl and *O*-phenylisoureas, respectively, is well documented.[21] In a similar context, the reaction between *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea **3** (Fig. 2) and deactivated amines assisted by mercury (II) chloride to provide Boc-protected guanidines[22] has been suggested to proceed via formation of *N*,*N*′-bis(*tert*-butoxycarbonyl)carbodiimide **4** (Fig. 2).[22a] Further standard deprotection of the Boc groups leads to guanidinium containing derivatives in moderate to good overall yields under mild conditions.[14,23]

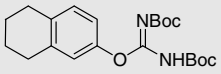
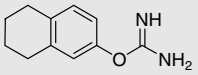
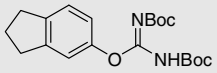
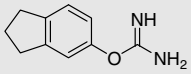
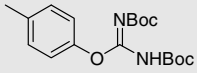
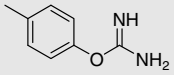
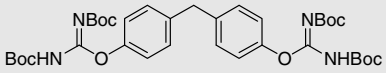
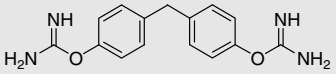
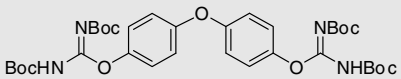
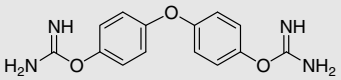
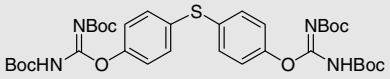
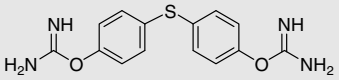
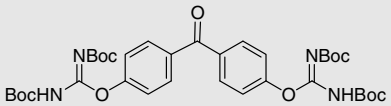
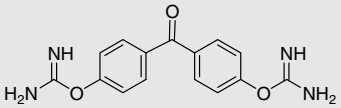
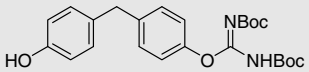
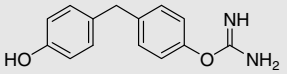
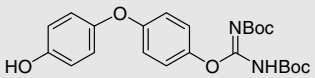
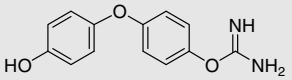
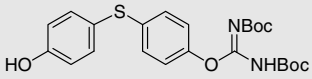
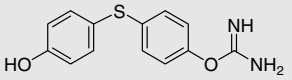
Based on the assumption that intermediate **4** is the reactive species in this reaction and considering the reactivity of carbodiimides with alcohols,[21] exchange of the starting deactivated amines by phenols appears to be a rational approach to the synthesis of aromatic isouronium salts (Fig. 2). In addition, the treatment of a group of aromatic amino-alcohols with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-pseudothiourea **5a** and 1,3-bis(benzyloxycarbonyl)-2-methyl-2-pseudothiourea **5b** (Fig. 2) in the presence of mercury (II) chloride had been reported by Sharma et al.[24] As expected, the Boc- and Cbz-protected guanidines were the main products with yields ranging from 78% to 81%. Interestingly, Boc- and Cbz-protected isoureas were also formed as side products (yield < 4%).

With that in mind, and following the general synthetic pathway described in Scheme 1, the synthesis of compounds **2**, **6b** and **7b** (Table 1) was attempted. Thus, reaction of one equivalent of each



Scheme 1.

Table 1
Overall, first and second stage yields (in%) obtained for the new compounds prepared

Compound	Structure	1st stage	Compound	Structure	2nd stage	Overall
2a		63	**2**		95	60
6a		66	**6b**		94	62
7a		73	**7b**		93	68
8a		58	**8b**		92	53
9a		67	**9b**		91	61
10a		61	**10b**		92	56
11a		–	**11b**		–	–
12a		74	**12b**		91	67
13a		77	**13b**		93	72
14a		78	**14b**		92	72

alcohol with one equivalent of **3** in the presence of a slight excess mercury (II) chloride and an excess of triethylamine in dichloromethane at room temperature gave the Boc-protected *O*-phenylisoureas **2a**, **6a** and **7a** (Table 1) after column chromatography. Deprotection of the Boc groups with trifluoroacetic acid in dichloromethane followed by treatment with Amberlyte resin in water led to the desired *O*-phenylisouronium chloride salts.

Then, we proceeded to the preparation of the bis-isouronium salts **8b**–**11b** by reacting in the first stage one equivalent of the corresponding diol with two equivalents of **3**, a slight excess of mercury (II) chloride and an excess of triethylamine. The reaction was carried out at room temperature in dichloromethane for **8a**–**10a** and in dimethylformamide for **11a**.

As can be seen in Table 1, the bis Boc-protected isoureas **8a**–**10a** were obtained in similar yields after silica gel column chromatography. In all three cases, the mono Boc-protected isoureas **12a**, **13a** and **14a** were isolated as side products. The carbonyl group proved to be too deactivating and the synthesis of **11a** could not be accomplished. Boc deprotection of **8a**–**10a** afforded the final isouronium chlorides **8b**–**10b** after anion exchange in moderate overall yields (Table 1).

Single crystals of **8a**[25] and **9a**[26] were grown by slow evaporation of a solution in a concentrate chloroform/ethyl acetate mixture and in hexane, respectively. As can be seen in Figure 3, both have very similar structures. The phenyl rings are almost perpendicular to each other in order to minimize steric interactions between the aromatic protons in *ortho* with the bridge group. Interestingly, this geometry disrupts the symmetry observed in solution and two enantiomers are possible for each substrate. In Figure 3 only one enantiomer per compound is presented, however, in the unit cell, the two mirror images can be found for both, **8a** and **9a**.

After isolation of the monocations **12a**, **13a** and **14a** as side products in the synthesis of **8a**, **9a** and **10a**, different approaches to improve their yields were attempted. Comparison of **12b**, **13b**

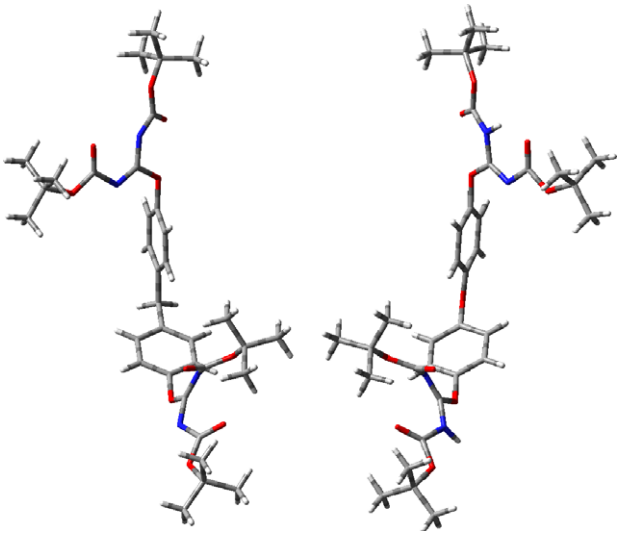


Figure 3. X-ray diffraction structures of **8a**, and **9a**, respectively.

and **14b** with their dicationic counterparts **8b**, **9b** and **10b** could provide some interesting Structure–Activity Relationships (SAR) in the study of the affinity towards the α_2-ARs. Thus, reaction of an excess of three equivalents of the diols with only one equivalent of **3**, one equivalent of mercury (II) chloride and an excess of triethylamine afforded the mono-Boc-protected isoureas **12a**–**14a**, which were purified by silica gel column chromatography. Compounds **8a**–**10a** are the side products under the new conditions (yield lower than 6% in all cases) and the unreacted starting diols were recovered from the column eluting with a more polar solvent system. Usual Boc deprotection with trifluoroacetic acid and further Amberlyte resin treatment in aqueous solution afforded **12b**–**14b** in good overall yields (Table 1). All the chemicals used for the syntheses described in Scheme 1 are commercially available either from Aldrich or Fluka.

The results obtained suggest that this two-step method is a valid option for the preparation of *O*-phenylisouronium salts when the starting phenols have either alkyl or electro-donor substituents. Moreover, in a long multiple-step synthesis, this strategy would allow us to introduce the isouronium precursor at any stage and transform it when necessary. However, the presence of electro-withdrawing substituents such as the carbonyl group is still a shortcoming, since the starting phenols are not sufficiently reactive. This methodology is yet to be tested with aliphatic alcohols.

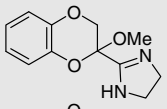
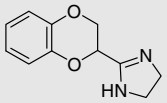
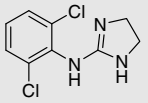
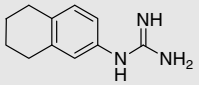
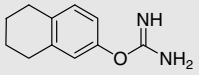
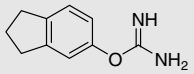
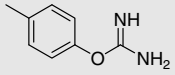
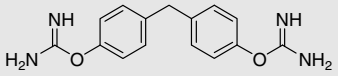
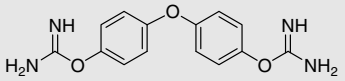
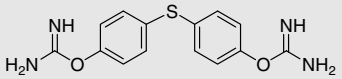
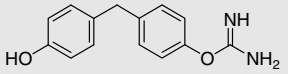
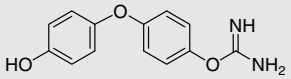
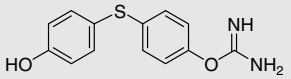
2.2. Pharmacology

The affinity towards the α_2-ARs in human PFC tissue of all compounds prepared was measured by competition with the α_2-AR selective radioligand [^{3}H]RX821002 (2-methoxy-idazoxan), which was used at a constant concentration of 1 nM. Idazoxan and Clonidine, two well-known α_2-AR ligands, were also used as references.

2.2.1. Affinity towards the α_2-ARs

Given the chemical structure of the lead compound **1**, the synthesis and pharmacological evaluation of the isouronium analogue **2** seems a rational step in the search of some relationship between structure and activity. Furthermore, as in our previous work,[14] we prepared a number of structurally related analogues to try to find some useful information from the behaviour of these molecules in the α_2-ARs. It is worth mentioning that to the best of our knowledge, this is the first group of *O*-phenylisouronium compounds that have been biologically tested. The affinities obtained, expressed as pK_i, are displayed in Table 2.

Table 2
Affinity values (expressed as pK_i) obtained for all the compounds studied

Compound	Structure	pK_i
RX821002		9.04
Idazoxan		7.29
Clonidine		7.68
1[a]		7.11
2		5.35
6b		5.27
7b		5.09
8b		5.40
9b		4.72
10b		5.10
12b		4.98
13b		4.49
14b		4.94

[a] α_2-AR antagonist previously prepared by us.[14b]

Replacement of the guanidinium cation (**1**) by the isouronium (**2**) led to a loss in affinity of nearly two logarithmic units (Table 2). In the mono *O*-isouronium series, compound **2** displays an affinity slightly higher than that of **7b** and very similar to that of **6b**. Although the differences are not very significant, it seems that the substrates with longer aliphatic chains tend to bind better to the receptors (Table 2).

Amongst the dicationic molecules, the methylene derivative **8b** presented the highest affinity (5.40, Table 2), with the ether analogue **9b** showing the lowest pK_i of its series. Regarding the hydroxybenzene *O*-isouronium salts **12b**–**14b**, the same order in

affinity as for their dicationic counterparts can be identified (CH_2 > S > O). The ether derivative **13b** presented the poorest affinity of the whole series (4.49, Table 2). In all cases, the bis-*O*-isouronium salts displayed better affinities than their monocationic analogues.

Comparing the data obtained for **6b**–**10b** with that of the corresponding guanidinium derivatives previously described by us,[14] as was found for **1** and **2**, all new isouronium compounds presented lower affinities. The pK_i difference was higher than one logarithmic unit in every case except for **8b** ($\Delta pK_i = 0.98$).

All compounds prepared have a low affinity towards the α_2-ARs. This result highlights the different behaviour of the guanidinium and isouronium cations in the binding site of the receptors. Thus, the replacement of an amino group for the isosteric oxygen atom, leads to remarkable changes in the affinity and/or activity of the compounds. The substitution of a HB donor (NH group) by a HB acceptor (O group) results in a dramatic loss of affinity. Hence, it seems that the presence of three HB donors (NH type groups) in the cation is required and plays an important role in the affinity.

3. Conclusions

In this paper we have reported the synthesis of nine new *O*-phenylisouronium compounds (**2**, **6b**–**10b** and **12b**–**14b**) and their Boc-protected precursors (**2a**, **6a**–**10a** and **12a**–**14a**) in moderate to good overall yields. Starting from phenols, Kim and Qian's strategy for the synthesis of protected guanidines was followed.[14] The X-ray diffraction structure of the intermediates **8a** and **9a** is presented. Compounds **8b**, **9b** and **10b** are dicationic compounds, whereas the rest of final products are monocationic species.

All final substrates were evaluated as potential α_2-ARs ligands in human PFC. The dicationic molecules **8b**–**10b** displayed better affinities than their hydroxy-monocationic analogues. Unfortunately, all of the isouroniums (**2**, **6b**–**10b**) showed lower pK_i values than their guanidinium counterparts.[14]

Nevertheless, negative results sometimes can be as important as positive ones since they provide with important information on what molecular features are essential for a good interaction with the receptor. Our initial hypothesis was that the substitution of one of the NH group of the guanidinium cation (HB donor) by an isosteric O atom (HB acceptor) while keeping the other two NH_2 groups (HB donors) could improve the interactions of the compounds in the antagonist binding pocket of the α_2-ARs. As the isouronium containing products poorly bind the receptors showing a loss of affinity, we can conclude that in our series of compounds, the interaction with the residues involved in the antagonist binding pocket requires the three HB donor groups oriented as in the guanidinium cation.

4. Experimental protocol

4.1. Pharmacology: materials and methods

4.1.1. Preparation of membranes

Neural membranes (P_2 fractions) were prepared from the PFC of human brains obtained at autopsy in the Instituto Vasco de Medicina Legal, Bilbao, Spain. Postmortem human brain samples of each subject (1 g) were homogenized using a Teflon-glass grinder (10 up-and-down strokes at 1500 rpm) in 30 volumes of homogenization buffer (1 mM $MgCl_2$, and 5 mM Tris–HCl, pH 7.4) supplemented with 0.25 M sucrose. The crude homogenate was centrifuged for 5 min at 1000g (4 °C) and the supernatant was centrifuged again for 10 min at 40,000g (4 °C). The resultant pellet was washed twice in 20 volumes of homogenization buffer and recentrifuged in similar conditions. Aliquots of 1 mg protein were stored at −70 °C until use in the assay. Protein content was measured according to the method Bradford using BSA as standard, and was similar in the different brain samples.

4.1.2. [^{3}H]RX821002 binding assays

Specific [^{3}H]RX821002 binding was measured in 0.55 mL-aliquots (50 mM Tris–HCl, pH 7.5) of the neural membranes which were incubated with [^{3}H]RX821002 (1 nM) for 30 min at 25 °C in the absence or presence of the competing compounds (10^{-12}–10^{-3} M, 10 concentrations). Incubations were terminated by diluting the samples with 5 mL of ice-cold Tris incubation buffer (4 °C). Membrane bound [^{3}H]RX821002 was separated by vacuum filtration through Whatman GF/C glass fibre filters. Then, the filters were rinsed twice with 5 mL of incubation buffer and transferred to minivials containing 3 mL of OptiPhase 'HiSafe' II cocktail and counted for radioactivity by liquid scintillation spectrometry. Specific binding was determined and plotted as a function of the compound concentration. Non-specific binding was determined in the presence of adrenaline (10^{-5} M).

4.1.3. Analysis of binding data

Analysis of competition experiments to determine the inhibition constant (K_i) were performed by nonlinear regression using the GraphPad Prism program. All experiments were analysed assuming a one-site model of radioligand binding. K_i values were normalized to pK_i values.

4.1.4. Drugs

[^{3}H]RX821002 (specific activity 59 Ci/mmol) was obtained from Amersham International, UK. Idazoxan HCl was synthesised by Dr. F. Geijo at S.A. Lasa Laboratories, Barcelona, Spain. Clonidine HCl and RX821002 HCl were purchased from Sigma (St. Louis, USA). All other chemicals were of the highest purity commercially available.

4.2. Chemistry

All the commercial chemicals were obtained from Sigma–Aldrich or Fluka and were 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 Silica gel 60 (230–400 mesh ASTM) or Aluminium Oxide (activated, Neutral Brockman I STD grade 150 mesh). Solvents for synthesis purposes were used at GPR grade. Analytical TLC was performed using Merck Kieselgel 60 F_{254} silica gel plates or Polygram Alox N/UV_{254} aluminium oxide plates. Visualisation was by UV light (254 nm). NMR spectra were recorded in a Bruker DPX-400 Avance spectrometer, operating at 400.13 MHz and 600.1 MHz for ^{1}H NMR and 100.6 MHz and 150.9 MHz for ^{13}C NMR. Shifts are referenced to the internal solvent signals. NMR data were processed using Bruker Win-NMR 5.0 software. Electrospray mass spectra were recorded on a Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector with methanol, water or ethanol as carrier solvents. Melting points were determined using an Electrothermal IA9000 digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrometer equipped with a Gateway 2000 4DX2-66 workstation and on a Perkin Elmer Spectrum One FT-IR Spectrometer equipped with Universal ATR sampling accessory. Elemental analysis was carried out at the Microanalysis Laboratory, School of Chemistry and Chemical Biology, University College Dublin.

4.2.1. General procedure for the synthesis of (bis)Boc-protected isoureas: Method A

Each of the corresponding phenols (or diphenols) was treated with 1.1 equivalents (or 2.2 for the diols) of mercury (II) chloride,

1.0 equivalents (or 2.0 for the diols) of *N,N′*-di(*tert*-butoxycarbonyl)thiourea **3** and 3.1 equivalents (or 7.2 for the diols) of TEA in dry DCM at 0 °C and under argon. The resulting mixture was stirred at 0 °C for 1 h and for the appropriate duration at room temperature. Then, the reaction mixture was diluted with EtOAc and filtered through a pad of Celite to get rid of the mercury sulfide precipitate formed. The filter cake was rinsed with EtOAc. The organic phase was extracted with water (2× 30 mL), washed with brine (1× 30 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum to give a residue that was purified by silica gel column chromatography, eluting with the appropriate hexane:EtOAc mixture.

4.2.2. 1,3-Di(*tert*-butoxycarbonyl)-2-(5,6,7,8-tetrahydro-naphtalene-2-yl)isourea (2a): Method A

$HgCl_2$ (1.1 mmol, 299 mg) was added over a solution of 149 mg (1.0 mmol) of 5,6,7,8-tetrahydro-naphthalen-2-ol, 277 mg (1.0 mmol) of **3** and 0.43 mL (3.1 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 16 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (5:2) afforded **2a** as a colourless oil (248 mg, 63% yield); IR (nujol) ν 3261 (NH), 1770, 1611 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H, $(CH_3)_3$), 1.48 (s, 9H, $(CH_3)_3$), 1.71–1.85 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.69–2.82 (m, 4H, $2PhCH_2$), 6.85 (s, 1H, Ar.), 6.91 (d, 1H, J = 8.2 Hz, Ar.), 7.02 (d, 1H, J = 8.2 Hz, Ar.), 10.59 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 22.7, 23.0 ($CH_2CH_2CH_2CH_2$, 27.8 ($2(CH_3)_3$), 28.7, 29.2 ($PhCH_2$), 80.7, 82.5 ($C(CH_3)_3$), 118.7, 121.4, 129.6, 134.5, 138.1, 148.2 (Ar.), 148.6, 158.3, 161.9 (CO, CN).

4.2.3. 1,3-Di(*tert*-butoxycarbonyl)-2-indan-5-yl-isourea (6a): Method A

$HgCl_2$ (1.1 mmol, 299 mg) was added over a solution of 135 mg (1.0 mmol) of indan-5-ol, 277 mg (1.0 mmol) of **3** and 0.43 mL (3.1 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 16 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (5:2) afforded **6a** as a colourless oil (251 mg, 66% yield); IR (nujol) ν 3261 (NH), 1774, 1629 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H, $(CH_3)_3$), 1.54 (s, 9H, $(CH_3)_3$), 1.99–2.14 (m, 2H, $CH_2CH_2CH_2$), 2.83–2.97 (m, 4H, $2PhCH_2$), 6.91 (d, 1H, J = 8.0 Hz, Ar.), 7.02 (s, 1H, Ar.), 7.17 (d, 1H, J = 8.0 Hz, Ar.), 10.63 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 25.6 ($CH_2CH_2CH_2$, 27.9 ($2(CH_3)_3$), 32.2, 32.9 ($PhCH_2$), 80.1, 82.7 ($C(CH_3)_3$), 117.5, 119.2, 124.5, 141.7, 145.4, 149.6 (Ar.), 148.3, 158.6, 162.1 (CO, CN).

4.2.4. 1,3-Di(*tert*-butoxycarbonyl)-2-*p*-tolyl-isourea (7a): Method A

$HgCl_2$ (1.1 mmol, 299 mg) was added over a solution of 109 mg (1.0 mmol) of 4-methyl-phenol, 277 mg (1.0 mmol) of **3** and 0.43 mL (3.1 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 23 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (3:2) afforded **7a** as a white solid (259 mg, 73% yield); mp 90–92 °C; IR (nujol) ν 3253 (NH), 1769, 1622 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H, $(CH_3)_3$), 1.53 (s, 9H, $(CH_3)_3$), 2.32 (s, 3H, CH_3), 7.05 (d, 2H, J = 8.0 Hz, Ar.), 7.14 (d, 2H, J = 8.0 Hz, Ar.), 10.64 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 20.8 (CH_3), 27.9 ($2(CH_3)_3$), 80.8, 82.7 ($C(CH_3)_3$), 121.2, 129.7, 135.4, 148.2 (Ar.), 148.8, 158.4, 162.0 (CO, CN); MS (ESI^+) m/z 373.1706 $[M+Na]^+$.

4.2.5. 4,4′-Bis[*N,N′*-di(*tert*-butoxycarbonyl)-isoureido]-diphenyl methane (8a): Method A

$HgCl_2$ (1.1 mmol, 299 mg) was added over a solution of 101 mg (0.5 mmol) of 4,4′-methylenediphenol, 277 mg (1.0 mmol) of **3** and 0.5 mL (3.6 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 21 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (3:1) afforded **8a** as a white solid (202 mg, 58% yield). Thirty-two milligrams (14% yield) of **12a** was also obtained; mp 144–146 °C; IR (neat) ν 3178 (NH), 1767, 1655, 1625 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H, $(CH_3)_3$), 1.54 (s, 18H, $(CH_3)_3$), 3.98 (s, 2H, CH_2), 7.12 (d, 4H, J = 8.5 Hz, Ar.), 7.17 (d, 4H, J = 8.5 Hz, Ar.), 10.69 (br s, 2H, NH); ^{13}C NMR ($CDCl_3$) δ 27.9 ($2(CH_3)_3$), 40.5 (CH_2), 81.0, 82.8 ($C(CH_3)_3$), 121.6, 129.8, 138.3 (Ar.), 148.2 (CO), 149.5 (Ar.), 158.4, 162.0 (CO, CN).

4.2.6. 4,4′-Bis[*N,N′*-di(*tert*-butoxycarbonyl)-isoureido]-diphenyl ether (9a): Method A

$HgCl_2$ (1.1 mmol, 299 mg) was added over a solution of 102 mg (0.5 mmol) of 4,4′-dihydroxyphenyl ether, 277 mg (1.0 mmol) of **3** and 0.5 mL (3.6 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 20 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (3:1) afforded **9a** as a white solid (233 mg, 67% yield). Nine milligrams (4% yield) of **13a** was also obtained; mp 142–144 °C; IR (neat) ν 3171 (NH), 1766, 1655, 1623 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 18H, $(CH_3)_3$), 1.56 (s, 18H, $(CH_3)_3$), 7.02 (d, 4H, J = 9.0 Hz, Ar.), 7.18 (d, 4H, J = 9.0 Hz, Ar.), 10.70 (br s, 2H, NH); ^{13}C NMR ($CDCl_3$) δ 27.5 ($2(CH_3)_3$), 80.7, 82.5 ($C(CH_3)_3$), 119.0, 122.5, 146.2 (Ar.), 147.8 (CO), 154.4 (Ar.), 158.1, 161.2 (CO, CN).

4.2.7. 4,4′-Bis[*N,N′*-di(*tert*-butoxycarbonyl)-isoureido]-diphenyl thioether (10a): Method A

$HgCl_2$ (1.1 mmol, 299 mg) was added over a solution of 110 mg (0.5 mmol) of 4,4′-thiodiphenol, 277 mg (1.0 mmol) of **3** and 0.5 mL (3.6 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 21 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (5:1) afforded **10a** as a white solid (218 mg, 61% yield). Forty-eight milligrams (20% yield) of **14a** was also obtained; mp 136–138 °C; IR (neat) ν 3171 (NH), 1769, 1657, 1624 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (s, 18H, $(CH_3)_3$), 1.53 (s, 18H, $(CH_3)_3$), 7.15 (d, 4H, J = 8.8 Hz, Ar.), 7.34 (d, 4H, J = 8.8 Hz, Ar.), 10.69 (br s, 2H, NH); ^{13}C NMR ($CDCl_3$) δ 27.9 ($2(CH_3)_3$), 81.2, 83.0 ($C(CH_3)_3$), 122.6, 131.9, 133.0 (Ar.), 148.1 (CO), 150.2 (Ar.), 158.1, 161.8 (CO, CN).

4.2.8. General procedure for the synthesis of hydroxybenzene Boc-protected isourea derivatives: Method B

$HgCl_2$ (1.1 equivalents) was added over a solution containing an excess (3.0 equivalents) of each of the corresponding diols, 1.0 equivalent of *N,N′*-di(*tert*-butoxycarbonyl)thiourea **3** and 3.1 equivalents of TEA in dry DCM (5 mL) at 0 °C under argon. The resulting mixture was stirred at 0 °C for 1 h and for the appropriate duration at room temperature. Then, the reaction mixture was diluted with EtOAc and filtered through a pad of Celite. The filter cake was rinsed with EtOAc. The organic phase was extracted with water (2× 30 mL), washed with brine (1× 30 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum to give a residue that was purified by silica gel column chromatography, eluting with the appropriate hexane:EtOAc system to yield the desired mono-Boc-protected isoureas as white solids. Formation of the disubstituted compound was also observed, and the starting material diol can be recovered from the column eluting with a more polar hexane:EtOAc mixture.

4.2.9. 1,3-Di(*tert*-butoxycarbonyl)-2-[4-(4-hydroxy-benzyl)-phenyl]-isourea (12a): Method B

$HgCl_2$ (3.3 mmol, 896 mg) was added over a solution of 1803 mg (9.0 mmol) of 4,4′-methylenediphenol, 830 mg

(3.0 mmol) of **3** and 1.3 mL (9.3 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 16 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (2:1) afforded **12a** as a white solid (987 mg, 74% yield). (Less than 6% of the disubstituted compound was observed.) Mp 141–143 °C; IR (neat) ν 3345, 3261 (OH, NH), 1762, 1667, 1614 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (s, 9H, $(CH_3)_3$), 1.54 (s, 9H, $(CH_3)_3$), 3.80 (s, 2H, CH_2), 6.46 (br s, 1H, OH), 6.61 (d, 2H, J = 8.5 Hz, Ar.), 6.88 (d, 2H, J = 8.0 Hz, Ar.), 7.03 (d, 2H, J = 8.5 Hz, Ar.), 7.07 (d, 2H, J = 8.0 Hz, Ar.), 10.72 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.8 ($2(CH_3)_3$), 40.2 (CH_2), 81.3, 83.1 ($C(CH_3)_3$), 115.3, 121.3, 129.6, 129.7, 131.7, 139.6, 148.3, 149.0 (Ar.), 154.5, 158.7 (CO), 161.6 (CN); MS (ESI^+) m/z 465.2025 $[M+Na]^+$.

4.2.10. 1,3-Di(*tert*-butoxycarbonyl)-2-[4-(4-hydroxy-phenoxy)-phenyl]-isourea (13a): Method B

$HgCl_2$ (1.5 mmol, 419 mg) was added over a solution of 852 mg (4.2 mmol) of 4,4′-dihydroxydiphenyl ether, 387 mg (1.4 mmol) of **3** and 0.6 mL (4.3 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 15 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (2:1) afforded **13a** as a white solid (481 mg, 77% yield). (Less than 5% of the disubstituted compound was observed.) Mp 134–136 °C; IR (neat) ν 3346, 3283 (OH, NH), 1763, 1667, 1615 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 (s, 9H, $(CH_3)_3$), 1.53 (s, 9H, $(CH_3)_3$), 6.63 (d, 2H, J = 8.8 Hz, Ar.), 6.74 (d, 2H, J = 8.8 Hz, Ar.), 6.77–6.86 (m, 3H, OH + Ar.), 7.03 (d, 2H, J = 9.3 Hz, Ar.), 10.74 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.7, 27.9 ($(CH_3)_3$), 81.5, 83.2 ($C(CH_3)_3$), 116.2, 117.8, 120.7, 122.4, 145.3, 148.2, 148.9, 152.8 (Ar.), 156.6, 159.1 (CO), 161.6 (CN); MS (ESI^+) m/z 467.1799 $[M+Na]^+$.

4.2.11. 1,3-Di(*tert*-butoxycarbonyl)-2-[4-(4-hydroxy-phenyl-sulfanyl)-phenyl]-isourea (14a): Method B

$HgCl_2$ (3.3 mmol, 896 mg) was added over a solution of 1965 mg (9.0 mmol) of 4,4′-thiodiphenol, 830 mg (3.0 mmol) of **3** and 1.3 mL (9.3 mmol) of TEA in dry DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and 16 h more at room temperature. Usual work up followed by silica gel column chromatography, eluting with hexane/EtOAc (2:1) afforded **14a** as a white solid (1082 mg, 78% yield). (Less than 5% of the disubstituted compound was observed.) Mp 153–155 °C; IR (neat) ν 3332, 3239 (OH, NH), 1761, 1667, 1611 (CO, CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (s, 9H, $(CH_3)_3$), 1.54 (s, 9H, $(CH_3)_3$), 6.64 (d, 2H, J = 8.0 Hz, Ar.), 6.93–7.00 (m, 4H, Ar.), 7.02 (br s, 1H, OH), 7.21 (d, 2H, J = 8.0 Hz, Ar.), 10.76 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.7, 27.9 ($(CH_3)_3$), 81.7, 83.4 ($C(CH_3)_3$), 116.6, 121.6, 121.9, 127.9, 135.8, 137.3, 148.2, 148.4 (Ar.), 157.3, 159.0 (CO), 161.4 (CN).

4.2.12. General procedure for the synthesis of the hydrochloride salts: Method C

Each of the corresponding Boc-protected precursors (0.5 mmol) was treated with 14 mL of a 50% solution of trifluoroacetic acid in DCM for 3 h. After that time, the solvent was eliminated under vacuum to generate the trifluoroacetate salt. This salt was redissolved in 20 mL of water and treated for 24 h with IRA400 Amberlyte resin in its Cl^- form. Then, the resin was removed by filtration and the aqueous solution washed with DCM (2× 10 mL). Evaporation of the water afforded the pure hydrochloride salt. Absence of the trifluoroacetate salt was checked by ^{19}F NMR.

4.2.13. 2-(5,6,7,8-Tetrahydro-naphthalen-2-yl)-isouronium chloride (2): Method C

White solid (95%); mp 105–107 °C; ^1H NMR (D_2O) δ 1.65–1.78 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.68–2.80 (m, 4H, $2PhCH_2$), 6.94–7.07 (m, 2H, Ar.), 7.22 (d, 1H, J = 8.0 Hz, Ar.); ^{13}C NMR (D_2O) δ 21.6, 21.9 ($CH_2CH_2CH_2CH_2$, 27.8, 28.3 ($PhCH_2$), 117.3, 120.6, 130.6, 137.3, 139.8, 146.2 (Ar.), 161.5 (CN); MS (ESI^+) m/z 191.2918 $[M+H]^+$. Anal. ($C_{11}H_{15}ClN_2O{\cdot}1.6H_2O$) Calcd: C, 51.70; H, 7.18; N, 10.96. Found: C, 51.38; H, 6.97; N, 10.74.

4.2.14. 2-Indan-5-yl-isouronium chloride (6b): Method C

White solid (93%); mp 151–153 °C; ^1H NMR (D_2O) δ 2.01–2.17 (m, 2H, $CH_2CH_2CH_2$), 2.75–2.93 (m, 4H, $2PhCH_2$), 7.03 (d, 1H, J = 8.0 Hz, Ar.), 7.15 (s, 1H, Ar.), 7.36 (d, 1H, J = 8.0 Hz, Ar.); ^{13}C NMR (D_2O) δ 25.0 ($CH_2CH_2CH_2$, 31.3, 31.9 ($PhCH_2$), 116.5, 118.0, 125.4, 144.3, 147.0, 147.1 (Ar.), 161.6 (CN); MS (ESI^+) m/z 177.1009 $[M+H]^+$. Anal. ($C_{10}H_{13}ClN_2O{\cdot}0.9H_2O$) Calcd: C, 52.48; H, 6.52; N, 12.24. Found: C, 52.52; H, 6.16; N, 12.07.

4.2.15. 2-*p*-Tolyl-isouronium chloride (7b): Method C

White solid (94%); mp decomposes over 160 °C; ^1H NMR (D_2O) δ 2.44 (s, 3H, CH_3), 7.28 (d, 2H, J = 8.2 Hz, Ar.), 7.44 (d, 2H, J = 8.2 Hz, Ar.); ^{13}C NMR (D_2O) δ 19.9 (CH_3), 120.7, 131.0, 138.7, 146.9 (Ar.), 161.9 (CN); MS (ESI^+) m/z 151.2527 $[M+H]^+$. Anal. ($C_8H_{11}ClN_2O{\cdot}0.2H_2O$) Calcd: C, 50.51; H, 6.04; N, 14.72. Found: C, 50.40; H, 5.84; N, 14.76.

4.2.16. 4,4′-Diisouronium diphenylmethane dichloride (8b): Method C

White solid (92%); mp decomposes over 256 °C; ^1H NMR (D_2O) δ 4.09 (s, 2H, CH_2), 7.25 (d, 4H, J = 8.0 Hz, Ar.), 7.42 (d, 4H, J = 8.0 Hz, Ar.); ^{13}C NMR (D_2O) δ 39.3 (CH_2), 120.8, 130.5, 141.0, 147.2 (Ar.), 161.4 (CN); MS (ESI^+) m/z 285.1338 $[M+H]^+$. Anal. ($C_{15}H_{18}Cl_2N_4O_2{\cdot}1.8H_2O$) Calcd: C, 46.24; H, 5.59; N, 14.38. Found: C, 46.05; H, 5.30; N, 14.28.

4.2.17. 4,4′-Diisouronium diphenylether dichloride (9b): Method C

White solid (91%); mp decomposes over 264 °C; ^1H NMR (D_2O) δ 7.19 (d, 4H, J = 9.5 Hz, Ar.), 7.34 (d, 4H, J = 9.0 Hz, Ar.); ^{13}C NMR (D_2O) δ 120.3, 122.4, 144.5, 155.4 (Ar.), 161.4 (CN); MS (ESI^+) m/z 287.1132 $[M+H]^+$. Anal. ($C_{14}H_{16}Cl_2N_4O_3{\cdot}1.5H_2O$) Calcd: C, 43.54; H, 4.96; N, 14.51. Found: C, 43.42; H, 4.65; N, 14.56.

4.2.18. 4,4′-Diisouronium diphenylthioether dichloride (10b): Method C

White solid (92%); mp decomposes over 247 °C; ^1H NMR (D_2O) δ 7.30 (d, 4H, J = 8.5 Hz, Ar.), 7.53 (d, 4H, J = 8.5 Hz, Ar.); ^{13}C NMR (D_2O) δ 121.8, 132.8, 134.6, 148.2 (Ar.), 161.1 (CN); MS (ESI^+) m/z 303.0922 $[M+H]^+$. Anal. ($C_{14}H_{16}Cl_2N_4O_2S{\cdot}1.5H_2O$) Calcd: C, 41.80; H, 4.76; N, 13.93. Found: C, 41.90; H, 4.43; N, 13.59.

4.2.19. 2-[4-(4-Hydroxy-benzyl)-phenyl]-isouronium chloride (12b): Method C

White solid (91%); mp decomposes over 208 °C; ^1H NMR (D_2O) δ 3.90 (s, 2H, CH_2), 6.80 (d, 2H, J = 7.0 Hz, Ar.), 7.09–7.19 (m, 4H, Ar.), 7.35 (d, 2H, J = 7.5 Hz, Ar.); ^{13}C NMR (D_2O) δ 39.1 (CH_2), 115.0, 120.7, 129.5, 130.2, 132.8, 142.0, 146.9, 153.3 (Ar.), 161.4 (CN); MS (ESI^+) m/z 243.1123 $[M+H]^+$. Anal. ($C_{14}H_{15}ClN_2O_2{\cdot}1.2H_2O$) Calcd: C, 55.98; H, 5.84; N, 9.33. Found: C, 56.09; H, 5.48; N, 9.26.

4.2.20. 2-[4-(4-Hydroxy-phenoxy)-phenyl]-isouronium chloride (13b): Method C

White solid (93%); mp decomposes over 190 °C; ^1H NMR (D_2O) δ 6.89 (d, 2H, J = 8.0 Hz, Ar.), 7.99 (d, 2H, J = 8.0 Hz, Ar.), 7.04 (d, 2H, J = 8.6 Hz, Ar.), 7.24 (d, 2H, J = 8.6 Hz, Ar.); ^{13}C NMR (D_2O) δ 116.0, 118.5, 120.8, 122.1, 143.5, 148.4, 151.8, 157.1 (Ar.), 161.5 (CN); MS (ESI^+) m/z 245.0933 $[M+H]^+$. Anal. ($C_{13}H_{13}ClN_2O_3{\cdot}2.0H_2O$) Calcd: C, 49.30; H, 5.41; N, 8.84. Found: C, 49.18; H, 5.25; N, 8.71.

4.2.21. 2-[4-(4-Hydroxy-phenylsulfanyl)-phenyl]-isouronium chloride (14b): Method C

White solid (92%); mp decomposes over 210 °C; ^{1}H NMR (D_2O) δ 6.92 (d, 2H, J = 8.6 Hz, Ar.), 7.18 (d, 2H, J = 8.5 Hz, Ar.), 7.29 (d, 2H, J = 8.6 Hz, Ar.), 7.43 (d, 2H, J = 8.5 Hz, Ar.); ^{13}C NMR (D_2O) δ 116.8, 121.8, 122.2, 129.8, 136.1, 138.7, 147.3, 156.6 (Ar.), 161.7 (CN); MS (ESI^+) m/z 261.0686 $[M+H]^+$. Anal. ($C_{13}H_{13}ClN_2O_2S{\cdot}2.5H_2O$) Calcd: C, 45.68; H, 5.31; N, 8.20. Found: C, 45.99; H, 5.25; N, 8.44.

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25. Crystal data for **8a**: $C_{140}H_{192}N_{16}O_{40}$, M_w = 2739.10, colourless prism of 0.20 × 0.20 × 0.20 mm, T = 125 K, Monoclinic, space group $P2_1/c$, Z = 1, a = 20.063(8), b = 10.081(4), c = 20.371(8) Å, V = 3699(8) Å^3, D_{calcd} = 1.230 g cm^{-3}, $F(000)$ = 1464, λ = 0.71070 Å (Mo Kα), μ = 0.090 mm^{-1}, Rigaku Saturn 724 CCD diffractometer, θ range 2.31–25.00°, 43,678 collected reflections, 6484 unique, full-matrix least-squares (SHELXL-97[a]), R_1 = 0.1026, wR_2 = 0.1666 (R_1 = 0.1145, wR_2 = 0.1713 all data), goodness-of-fit = 1.382, residual electron density between 0.311 and −0.277 e Å^{-3}. Hydrogen atoms were located using a Riding model. Sheldrick, G.M. SHELXL-97: program for the refinement of crystal structures. University of Göttingen, Germany, 1997. Cambridge Crystallographic Data Centre Deposition Number CCDC 686281.
26. Crystal data for **9a**: $C_{68}H_{92}N_8O_{22}$, M_w = 1373.50, colourless plate of 0.20 × 0.10 × 0.06 mm, T = 121 K, triclinic, space group $P1$, Z = 1, a = 10.005(2), b = 10.496(3), c = 19.230(5) Å, V = 1857.1(8) Å^3, D_{calcd} = 1.228 g cm^{-3}, $F(000)$ = 732, λ = 0.71070 Å (Mo Kα), μ = 0.092 mm^{-1}, Rigaku Saturn 724 CCD diffractometer, θ range 2.21–25.00°, 28,696 collected reflections, 6521 unique, full-matrix least-squares (SHELXL-97[a]), R_1 = 0.0583, wR_2 = 0.1601 (R_1 = 0.0699, wR_2 = 0.1687 all data), goodness-of-fit = 1.122, residual electron density between 0.272 and −0.267 e Å^{-3}. Hydrogen atoms were located using a Riding model. Sheldrick, G.M. SHELXL-97: program for the refinement of crystal structures. University of Göttingen, Germany, 1997. Cambridge Crystallographic Data Centre Deposition Number CCDC 686280.