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Synthesis of a Novel Series of Arylmethylisothiuronium Derivatives

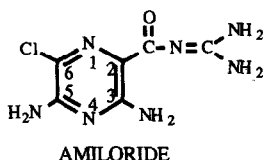
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Abstract: The bromination by N-bromosuccinimide of various mesitylene derivatives, to produce arylmethyl bromides or bromoarylmethyl bromides was compared in different reaction conditions. These compounds are intermediates in the synthesis of arylmethylisothiuronium bromide salts, achieved by the quantitative addition of thiourea to the methyl bromide derivatives. The final products are potent inhibitors of trans-membrane cation transport, and are therefore of potential interest for pharmacological properties. The incorporation of a radioactive carbon, important for the use as affinity labels and stoichiometric studies, is demonstrated.

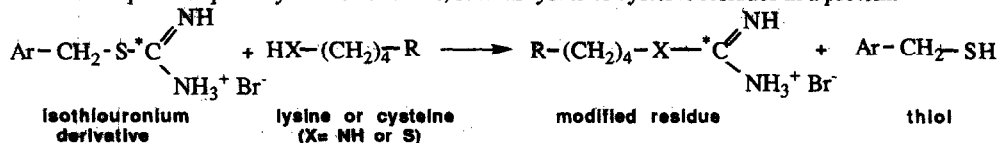
INTRODUCTION

Amiloride [1-(3,5-diamino-6-chloropyrazinoyl)guanidine] (1) is a K^+ -sparing diuretic agent used in the treatment of fluid retention and hypertension. It blocks the epithelial Na^+ channel and other plasma membrane Na^+ -transport systems¹.



Analogues of amiloride with appropriate modification in the structure, exhibit increased affinity and specificity for particular transport systems¹. Amiloride and derivatives contain a guanidinium group, which is necessary for the inhibitory action on the Na^+ channel, Na^+/H^+ exchanger, Na^+/Ca^{2+} exchanger and Na^+/K^+ -ATPase.

We have synthesized a number of guanidinium derivatives as possible precursors of affinity labels for sodium binding sites on Na^+/K^+ -ATPase, and have found them to be relatively high affinity antagonists of Na^+ ions in occlusion sites² ($K_D \sim 5\text{--}10\ \mu\text{M}$). An effective cation analog should have an affinity in the order of the site concentration (in practice $0.1\text{--}1\ \mu\text{M}$). Based on experience of the guanidinium derivatives and amiloride analogs³, we have now designed and synthesized a new family of aryl methyl isothiuronium derivatives, in the expectation that these will bind more tightly to Na^+/K^+ -ATPase. An additional benefit is that this moiety can react at alkaline pH with primary amines or thiols, such as lysine or cysteine residues in a protein.



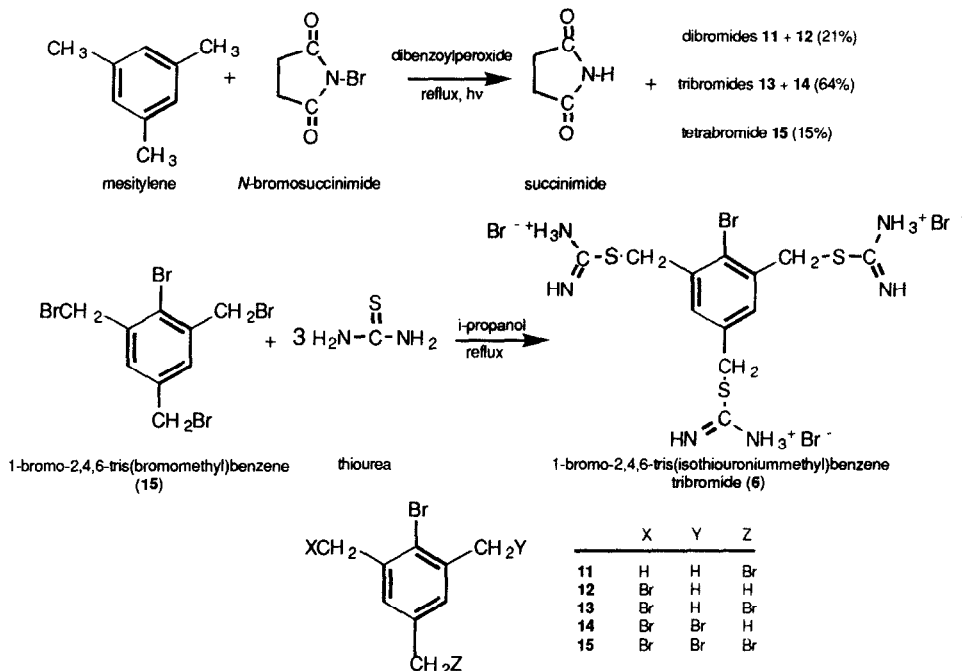
Thus isothiuronium derivatives might act as competitors at neutral pH, but they could chemically modify the protein at pH 9, provided a reactive residue is located near the site⁴. The present article outlines the synthesis of a series of novel phenylalkylisothiuronium derivatives (TITU compounds) with the predicted effects on Na^+/K^+ -ATPase ($K_D \sim 0.3\ \mu\text{M}$)⁴, and in addition one TITU derivative blocks the epithelial Na^+ channel with high affinity ($K_D \sim 100\ \text{nM}$)⁵. Furthermore, an example of the introduction of a radioactive label into the TITU compounds is described (the asterisk in the reaction shown above, denotes the location of the ^{14}C -carbon atom). The significance of this family of compounds resides in its potential for development of pharmacological

agents in disorders of cation transport⁶.

RESULTS AND DISCUSSION

The strategy for synthesis of TITU compounds was designed in two steps: a) bromination of the corresponding polymethylated hydrocarbon, and b) reaction of the isolated arylmethyl bromide with thiourea to yield the isothiurea derivative. For example, Br-TITU (6) was prepared from mesitylene according to scheme I:

Scheme I:



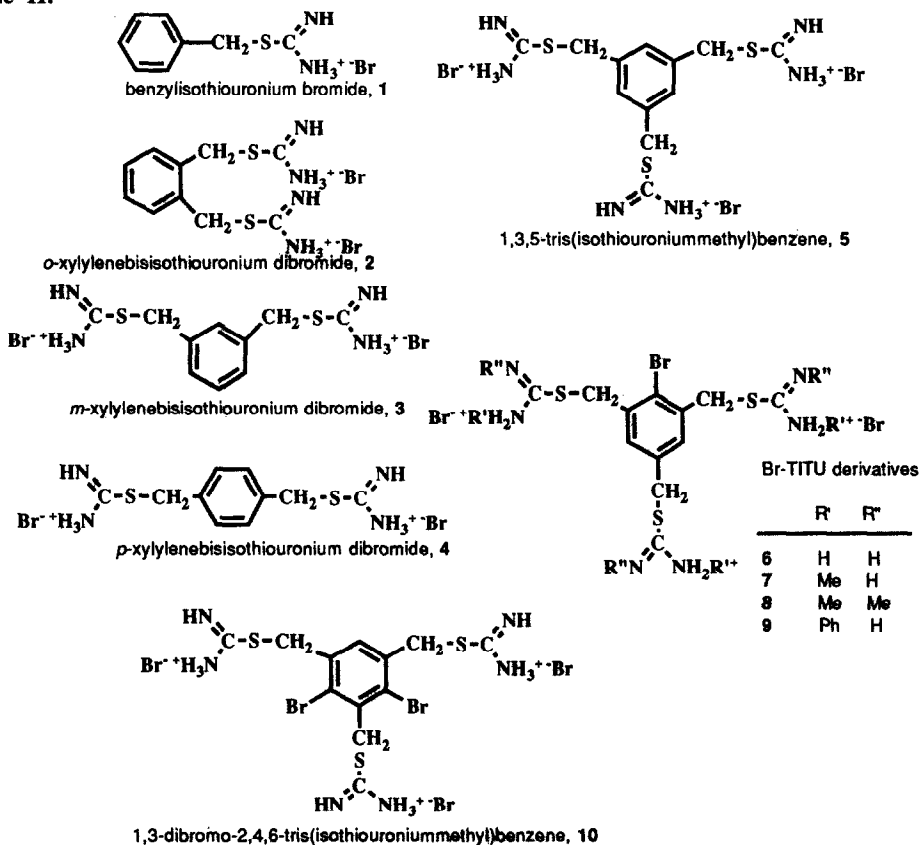
N-BROMOSUCCINIMIDE BROMINATION OF MESITYLENE DERIVATIVES⁶.

The reaction of choice for the preparation of arylmethyl bromide derivatives is side chain bromination by *N*-bromosuccinimide in tetrachloromethane⁷. Serendipitously, this reaction introduced an additional bromine atom in the nucleus, leading to products which are far more potent inhibitors than the intended ones⁴. In the example above, the reaction products were separated into a mixture of two dibromides, a mixture of two tribromides and the desired tetrabromide 15. A careful analysis of each of the mixtures by GC-MS and NMR, allowed structure determination of the components⁸. In every case, the products were the result of a competition between side-chain and nuclear brominations. There was always a bromine substitution in the ring and in no case could a product of only methyl bromination be found. Mechanistically, this could be interpreted by assuming nuclear reaction to be the fastest. Although the bromination was performed in conditions favoring radical side-chain halogenation, the mechanism of reaction seems to be a combination of electrophilic and free-radical mechanisms competing between them⁹. In the electrophilic mechanism a benzenonium ion is formed with a proton and a bromine atoms attached to the same carbon. This benzenonium ion can lose a proton and lead to nuclear substitution, or undergo a rearrangement in a concerted process involving an intramolecular migration of the halogen from the ring to the adjacent methyl⁹. Different degrees of aliphatic bromination were found in the methyl groups bound to the aromatic nucleus, but only a single bromine was introduced in the ring, since this first halogen largely decreases the rate of substitution of a second hydrogen atom in the benzene ring, especially in the meta (only available) position. The dibromides 11 and 12 were obtained in a 2.6:1 ratio, discrimination

attributable to steric hindrance of the nuclear bromine. The same was true for the tribromides **13** and **14**, with a ratio of 6:1 respectively. It should be pointed out that the bromination reaction reached an equilibrium, irrespective of the excess of NBS used, or in how many portions it was added. However, when one of the incompletely brominated fractions isolated by flash chromatography (2 and/or 3), was submitted to the same treatment, a mixture of products similar to the original was obtained (data not shown). One possible explanation is that one of the products formed (most probably, succinimide) inhibited the reaction and only when excluded by chromatography, could the reaction continue. The presence of side products is inevitable due to the relatively low ratio of rate constants for most substrates¹⁰.

It was reported that the selectivity of bromine attack can be modified by the solvent employed¹¹ and that the use of methyl formate for the NBS-bromination of mesitylene would double the yield, as compared to the reaction in CCl₄. In the present work, it was found that the yields and composition of products in both solvents were analogous, and led to a bromine atom directly attached to the ring. These discrepancies indicate the incidence of adventitious catalysis. In this regard, the claim that volatile impurities in NBS are responsible for the nuclear bromination¹² was investigated. Indeed, when a fresh batch of NBS was purified by high vacuum over P₂O₅ as reported¹², the appearance of a product lacking nuclear bromination (**16**) was observed in NMR and a mixture enriched on it (78%) was obtained by HPLC.

Scheme II:



Catalysis of photobromination with ferric halide was tried for increasing the nuclear substitution. E.g. a mixture of **15** + **16** (35 and 65%, respectively) treated with Fe filings and Br₂ at 0°C, changed the product distribution to 80% and 20% respectively. Furthermore, previous addition of one crystal of I₂ to the reaction medium, induced a difficult second nuclear substitution, yielding 19% of **17** (data not shown). When the reaction started

from an already brominated nucleus, the NBS-bromination occurred solely on the side chains and the yield increased (from 15% to 26% for compound **15**).

ARYLMETHYLISOTHIOURONIUM DERIVATIVES:

Thiourea reacts with alkyl bromides in refluxing alcohols, fast and nearly quantitatively to form an addition product, alkylisothiuronium bromide salt. This reaction was employed to obtain compounds **1** to **10** (Scheme II). Although **1** and **2** are known compounds (C.A. Registry numbers, 621-85-2 and 103386-74-9, respectively), the melting points of the bromide salts was unavailable in the literature.

Due to the highly polarized charges, it is difficult to find an analytical tool to examine their absolute purity. These compounds do not elute from the columns in normal phase HPLC, in reverse phase HPLC they elute in the void volume (typical behavior for strong salts) and ion exchange chromatography failed as well. Therefore, completion of the reaction was followed by TLC (SiO₂, 50% ethanol/hexane, by disappearance of starting materials and the presence of a new spot at R_f ≈ 0.0), and purity of the obtained product was confirmed by NMR analysis. In the case of the N-phenyl derivative **9**, HPLC analysis (both in normal and reverse phase) was possible because of charge masking by the phenyl substituent. By the use of N-mono- or N,N'-disubstituted thioureas, the respective N-substituted isothiuronium compounds were obtained (**7**, **8**, **9**). Finally, an introduction of radioactive carbon atom was shown in the preparation of [¹⁴C]**6**, where a microsynthesis in a flame-sealed ampoule was developed. The same procedure could be used for the synthesis of other radioactive TITU compounds. [¹⁴C]-**6** is presently being used for investigation of the cationic binding domain in Na⁺/K⁺-ATPase.

EXPERIMENTAL

All chemicals used herein were of analytical quality and were obtained from commercial sources. [¹⁴C]-thiourea had a specific activity of 53.0 mCi/mmol and was purchased from NEN, Dupont. HPLC was performed in a Waters system composed of a model 600E multisolvent delivery pump and a model 991 photodiode array detector controlled from a PC-386 computer. The HPLC solvents were HPLC grade and were filtered through 0.22 μm filters. Melting points were measured with a Fisher-Johns apparatus and are uncorrected. ¹H NMR spectra were recorded on a Bruker AMX-400 at 400 MHz, a Bruker WH-270 at 270 MHz and a Varian FT-80A spectrometer at 80 MHz. In all NMR spectra chemical shifts are expressed in δ (ppm) values. Spectra determined in CDCl₃ used tetramethylsilane as internal standard (0.00 ppm ¹H), the others are related to the solvent peak. Gas chromatography-mass spectrometry (GC-MS) was measured in a Finnigan 4500 instrument, using a capillary Supelco SPB-5 column. The Fourier transformed-infrared (FT-IR) spectra were determined in a Nicolet 510 instrument.

General Procedure for NBS-Bromination of Mesitylene Derivatives¹¹.

Whenever the arylmethyl bromide was not commercially available, it was prepared by the following procedure. A mixture of the mesitylene derivative, N-bromosuccinimide, a catalytic amount of dibenzoyl peroxide and methyl formate (100 ml) was stirred and refluxed (~34°C) in a water bath at 50°C under illumination (300 W, incandescent bulb, 10 cm distance, no filter) for a total of 5-20 h. The resulting yellowish mixture was extracted twice with 100 ml of a saturated solution of sodium carbonate and twice with 100 ml of water. The combined aqueous layer was extracted with 100 ml of methyl formate and both organic phases were united and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was dried in vacuum to yield a mixture of brominated products from which the desired compound was separated.

General Procedure for Addition of Thiourea to Bromomethyls.

A heterogeneous mixture of thiourea and the respective bromomethylbenzene derivative in isopropanol was heated under reflux and stirred for one hour. Initially, the compounds dissolved up on heating, but after 2-10 min. a precipitate appeared. The progress of the reaction was followed by TLC (silica, 50% ethanol/hexane). The mixture was left to cool to room temperature and overnight at 4 °C. A white crystalline powder was separated, washed with ice-cold isopropanol, and dried under high vacuum to yield the isothioureia

hydrobromide in nearly quantitative yield.

BENZYLISOTHIOURONIUM BROMIDE (BITU) (1):

Benzyl bromide (1.71 g, 10 mmol) and one equivalent of thiourea (0.76 g, 10 mmol) were reacted following the general procedure. No precipitate was obtained. Thus, the solvents were evaporated to render a pure product. mp: 150-152°C. ¹H NMR (80 MHz) in D₂O δ 7.56 ppm (broad s, 5H, aromatic) and 4.52 ppm (s, 2H, CH₂).

***o*-XYLYLENEBISISOTHIOURONIUM DIBROMIDE (OITU) (2):**

This compound was prepared from *o*-xylylene dibromide (2.65 g, 10 mmol) and thiourea (1.52 g, 20 mmol) by the general procedure to yield 3.55 g (85%) of 2. mp: 239-241°C. ¹H NMR (80 MHz) in D₂O δ 7.55 ppm (d, J=1.5 Hz, 4H, aromatic) and 4.63 ppm (s, 4H, CH₂).

***m*-XYLYLENEBISISOTHIOURONIUM DIBROMIDE (MITU) (3):**

m-Xylylene dibromide (2.65 g, 10 mmol) by the same procedure yielded 3.52 g (84.5%) of the title product 3. mp: 215-217°C. ¹H NMR (80 MHz) in D₂O δ 7.52 ppm (s, 1H, C2-H), 7.46 (broad s, 3H, C4,5,6-H) and 4.43 (s, 4H, CH₂).

***p*-XYLYLENEBISISOTHIOURONIUM DIBROMIDE (PITU) (4):**

p-Xylylene dibromide (2.65 g, 10 mmol) and thiourea (1.52 g, 20 mmol) were reacted in 250 ml of isopropanol as described, to yield 3.93 g (94%) of the title product 4. mp: 253-254°C. ¹H NMR (80 MHz) in D₂O using sodium 3-(trimethylsilyl)-*d*₄-propionate as reference, δ 7.52 ppm (s, 4H, aromatic) and 4.45 ppm (s, 4H, CH₂).

1,3,5-TRIS(ISOTHIOURONIUMMETHYL)BENZENE TRIBROMIDE (TITU) (5):

A) 1,3,5-TRIS(BROMOMETHYL)BENZENE (16):

Mesitylene (3 g, 25 mmol) was brominated with *N*-bromosuccinimide (previously purified by high vacuum over P₂O₅ for 16 h, to remove volatile impurities¹², 18 g, 101 mmol) as described above. The organic solvent was concentrated to 5 ml and the precipitate was filtered off. This mixture was separated by flash chromatography on silica (Kieselgel 60, particle size 0.040-0.063 mm, Merck, 600 ml) using hexane as eluent. Three main fractions were collected. The last one, containing the desired product was further fractionated in HPLC with a silica column (LiChrospher Si-60, 5 μm, 250 x 4 mm, Merck) with hexane flowing at 2 ml/min. Two fractions were collected, the first one eluting at 32 min. was a mixture of 1-bromo-2,4,6-tris(bromomethyl)benzene (15) (64%), 1,3-bis(bromomethyl)-5-dibromomethylbenzene (18%) and 1,3,5-tris(bromomethyl)benzene (16) (18%). The second fraction (35 min.) was composed of 78% 1,3,5-tris(bromomethyl)benzene (16) [¹H NMR in CDCl₃ (270 MHz): δ 7.36 ppm (s, 3H, aromatic) and 4.46 ppm (s, 6H, CH₂)] plus 22% of 1-bromo-2,4,6-tris(bromomethyl)benzene (15).

B) ISOTHIOURONIUM BROMIDE FORMATION:

The second HPLC fraction from the previous bromination (15 + 16) was reacted with thiourea as follows: after drying the NMR sample (1-2 mg), a solution of thiourea (1.75 mg, 23 μmol, ~4 equivalents excess) in isopropanol (300 μl) was added in the same NMR tube and heated to reflux for 60 min. A white precipitate was formed. The mixture was cooled to 4°C and centrifuged, the precipitate was washed with 300 μl of cold isopropanol, spun again and dried under high-vacuum to yield 1.0 mg of a mixture of products 5 (78%) and 6 (22%).

1-BROMO-2,4,6-TRIS(ISOTHIOURONIUMMETHYL)BENZENE TRIBROMIDE (Br-TITU) 6:

A) 1-BROMO-2,4,6-TRIS(BROMOMETHYL)BENZENE (15):

Mesitylene (3 g, 25 mmol) was brominated with *N*-bromosuccinimide (16 g, 90 mmol, not previously purified) to give 8.64 g of a mixture, which was crystallized from chloroform/hexane. The crystals were identified by ¹H NMR as succinimide. The products in solution were flash chromatographed on silica with hexane as eluent. Three main fractions were collected with yield distribution of 21%, 64% and 15% respectively. Fraction 1 was identified by ¹H NMR and GC-MS to be a mixture of two dibromides in a ratio 1:2.6. The more abundant dibromide is 1-bromo-4-bromomethyl-2,6-dimethylbenzene (11) [¹H NMR in

CDCl_3 (270 MHz): δ 7.10 ppm (s, 2H, aromatic), 4.39 (s, 2H, CH_2Br) and 2.40 (s, 6H, CH_3) and the less abundant is 1-bromo-2-bromomethyl-4,6-dimethylbenzene (**12**) [^1H NMR in CDCl_3 (270 MHz): δ 7.27 ppm (s, 1H, C3-H), 7.00 (s, 1H, C5-H), 4.61 (s, 2H, CH_2Br), 2.43 and 2.39 (2s, 3H + 3H, 2 CH_3)]. Fraction 2 was a mixture of two tribromides: 1-bromo-2,4-dibromomethyl-6-methylbenzene (**13**) [^1H NMR in CDCl_3 (270 MHz) δ 7.31 ppm (d, $J=2$ Hz, 1H, C3-H), 7.22 (d, $J=2$ Hz, 1 H, C5-H), 4.62 (s, 2H, $\text{C2-CH}_2\text{Br}$), 4.40 (s, 2H, $\text{C4-CH}_2\text{Br}$) and 2.43 (s, 3H, CH_3)] and 1-bromo-2,6-dibromomethyl-4-methylbenzene (**14**) [^1H NMR in CDCl_3 (270 MHz): δ 7.22 ppm (s, 2H, aromatic), 4.60 (s, 4H, CH_2Br) and 2.31 (s, 3H, CH_3)] in a ratio 6:1. Fraction 3 is 1-bromo-2,4,6-tris(bromomethyl)benzene (**15**). mp: 97-99°C. ^1H NMR in CDCl_3 (270 MHz): δ 7.44 ppm (s, 2H, C3+C5-H), 4.62 (s, 4H, $\text{C2+C6-CH}_2\text{Br}$) and 4.41 (s, 2H, $\text{C4-CH}_2\text{Br}$).

A reaction done in CCl_4 instead of methyl formate gave similar results.

B) ISOTHIOURONIUM FORMATION:

15 (320 mg, 0.48 mmol) and thiourea (204 mg, 2.69 mmol) were mixed with 35 ml of isopropanol and treated as described to yield 450 mg of product **6**. The filtrate and washings were united and concentrated for a second crop, 45 mg. Total yield 94.5% (theoretical weight 524 mg). mp and ^1H NMR spectrum identical to those of **6** in the subsequent preparation.

1-BROMO-2,4,6-TRIS(ISOTHIOURONIUMMETHYL)BENZENE TRIBROMIDE (Br-TITU) (6) ALTERNATIVE PROCEDURE:

A) 1-BROMO-2,4,6-TRIS(BROMOMETHYL)BENZENE (15):

An alternative and preferred way to prepare **15** is by NBS-bromination of 2-bromomesitylene. A mixture of 2-bromomesitylene (25 mmol, 4.98 g), N-bromosuccinimide (82.5 mmol, 14.685 g), dibenzoyl peroxide (about 75 mg) and methyl formate (100 ml) was reacted as described before. On evaporation 10.01 g of crude were obtained. 5.0 g of the resulting mixture were purified by flash-chromatography (column = 52 mm ID x 650 mm height) filled with 720 ml silica gel 60 (230-400 mesh) using n-hexane as eluent at 2 inches/min. of flow, to render 1,264 mg (26%) of pure 1-bromo-2,4,6-tris(bromomethyl)benzene (**15**). R_f = 0.07 in TLC (SiO_2 , hexane). mp: 98-100°C. ^1H NMR in CDCl_3 (270 MHz): δ 7.44 ppm (s, 2H, C3+C5-H), 4.62 (s, 4H, $\text{C2+C6-CH}_2\text{Br}$) and 4.42 (s, 2H, $\text{C4-CH}_2\text{Br}$).

B) ISOTHIOURONIUM PREPARATION:

15 (2.89 mmol, 1.26 g), thiourea (16.2 mmol, 1.23 g) and 200 ml of isopropanol were reacted to yield 1,824 mg (95%) of pure 1-bromo-2,4,6-tris(isothiuroniummethyl)benzene tribromide (**6**). We have not been able to recrystallize it. Mp (of the powder): 250-251°C. ^1H NMR in D_2O (270 MHz): δ 7.56 ppm (s, 2H, C3+C5-H), 4.56 (s, 4H, $\text{C2+C6-CH}_2\text{-S-}$) and 4.37 (s, 2H, $\text{C4-CH}_2\text{-S-}$).

1-BROMO-2,4,6-TRIS(N-METHYL ISOTHIOURONIUMMETHYL)BENZENE TRIBROMIDE (MeBr-TITU) (7):

15 (400 mg, 0.918 mmol) and 1-methyl-2-thiourea (248 mg, 2.75 mmol, Aldrich) were mixed with 25 ml of isopropanol. The mixture was stirred and refluxed for 140 min. A clear solution was obtained after 6 min. and no precipitate was observed. This solution was cooled down and crystallized at -18°C. The crystals (**7**) were then filtered, but they melted at room temperature (or by being hygroscopic, absorbed water from the ambient). ^1H NMR in $\text{DMSO}-d_6$ (270 MHz): δ 9.44 ppm (broad, 9H, -NH), 7.58 (s, 2H, C3+C5-H), 4.64 (s, 4H, $\text{C2+C6-CH}_2\text{-S-}$), 4.55 (s, 2H, $\text{C4-CH}_2\text{-S-}$), 2.93 (s, 6H, N- CH_3 at C2+C6) and 2.90 (s, 3H, N- CH_3 at C4).

1-BROMO-2,4,6-TRIS(N,N'-DIMETHYL ISOTHIOURONIUMMETHYL)BENZENE TRIBROMIDE (Me₂Br-TITU) (8):

15 (400 mg, 0.918 mmol) and 1,3-dimethyl-2-thiourea (286.9 mg, 2.754 mmol, Aldrich) were stirred with 25 ml of isopropanol and reacted as described to yield 583 mg of the title compound **8** (84.9% yield). mp: 226-229°C. ^1H NMR in $\text{DMSO}-d_6$ (270 MHz): δ 9.31 ppm (broad, 6H, -NH), 7.64 (s, 2H, C3+C5-H), 4.66 (s, 4H, $\text{C2+C6-CH}_2\text{-S-}$), 4.58 (s, 2H, $\text{C4-CH}_2\text{-S-}$), 2.94 (s, 12H, N- CH_3 at C2+C6) and 2.93 (s, 6H, N- CH_3 at C4).

1-BROMO-2,4,6-TRIS(N-PHENYL ISOTHIOURONIUMMETHYL)BENZENE TRIBROMIDE (P-TITU) (9):

15 (50 mg, 115 μ moles), 1-phenyl-2-thiourea (55 mg, 361 μ moles, Aldrich) and 3 ml of isopropanol were stirred and refluxed. In 1 min. the components solubilized; a TLC analysis (on SiO₂, with 50% ethanol/hexane) after 1 h reflux showed a major product (R_f 0.56) and traces of an intermediate product (R_f 0.69) and the starting material (R_f 0.77, phenylthiourea). HPLC analysis in normal phase: 20 μ l of the reaction mixture were injected in a RESOLVE RadialPak silica column (100 x 8 mm, 10 μ m, Waters) and eluted with a linear gradient from 20% to 80% isopropanol in hexane in 20 min. Three main peaks were observed, the first one ($k' = 1.23$) coeluting with phenylthiourea (3.6%, integrated at 254 nm), the second at $k' = 2.54$ (6.1%) and the major one at $k' = 6.32$ (90.3%). A preparative HPLC separation was performed in a normal phase column (ModCol-Bakerbond silica, 150 Å pore size, 10 μ m particle size, 25 x 2.54 cm) eluted with a gradient of 20 to 50% isopropanol in hexane in 20 min. at a flow rate of 20 ml/min. Three main peaks were collected at $k' = 1.16$, 1.84 and 6.03. Crystals obtained from the 3rd fraction were found to be 97% pure in reverse phase HPLC (column: RadialPak NovaPakC18, 100 x 5 mm, 4 μ m, Waters; gradient: 30 to 0% methanol in water in 20 min. + 10 min. at 0%, at 1 ml/min.). mp: slow melting with decomposition between 155-205°C. ¹H NMR (400 MHz) in DMSO-*d*₆, aided by the use of irradiation techniques: δ 7.680 ppm (s, 2H, C3+C5-H), 7.515 (t, J = 7 Hz, 4H, *m*- ϕ at C2+C6), 7.500 (t, J = 7.0 Hz, 2H, *m*- ϕ at C4), 7.414 (d, J = 6.0 Hz, 2H, *p*- ϕ at C2+C6), 7.397 (d, J = 6.0 Hz, 1H, *p*- ϕ at C4), 7.304 (d, J = 6.3 Hz, 4H, *o*- ϕ at C2+C6), 7.256 (d, J = 7.1 Hz, 2H, *o*- ϕ at C4), 4.730 (s, 4H, C2+C6-CH₂-S-) and 4.620 (s, 2H, C4-CH₂-S-). The -NH resonances appeared as wide flat peaks over 9 ppm. FT-IR (film) 3072, 1636, 1595, 1574, 1493, 1451, 1430, 1382, 1243, 1028, 760, 741 and 691 cm⁻¹.

1,3-DIBROMO-2,4,6-TRIS(ISOTHIOURONIUMMETHYL)BENZENE TRIBROMIDE (Br₂-TITU) (10):**A) 1,3-DIBROMO-2,4,6-TRIS(BROMOMETHYL)BENZENE, (17):**

A mixture of 2,4-dibromomesitylene (10 mmoles, 2.78 g), N-bromosuccinimide (33 mmoles, 5.87 g), dibenzoyl peroxide (about 30 mg) and methyl formate (40 ml) was reacted as before to yield 4.34 g of a mixture containing the title compound (as observed in NMR). This mixture was flash chromatographed in a 52 mm ID x 650 mm height column filled with 660 ml of silica gel 60 (230 - 400 mesh) and eluted with n-hexane at 2 inches/min. The last pool was 530 mg (12%) ($R_f = 0.08$) of pure 1,3-dibromo-2,4,6-tris(bromomethyl)benzene (17). mp: 140-143°C. ¹H NMR (270 MHz) in CDCl₃: δ 7.61 ppm (s, 1H, C5-H), 4.93 (s, 2H, C2-CH₂-Br) and 4.58 (s, 4H, C4 + C6-CH₂-Br).

B) ISOTHIOURONIUM PREPARATION:

17 (1.02 mmoles, 525 mg) and thiourea (5.71 mmoles, 435 mg) were reacted in 60 ml of isopropanol as described before, and 720 mg (95% yield) of pure 1,3-dibromo-2,4,6-tris(isothiuroniummethyl)benzene tribromide (10) was obtained. mp: 222-224°C. ¹H NMR in D₂O (270 MHz): δ 7.65 ppm (s, 1H, C5-H), 4.89 (s, 2H, C2-CH₂-S-) and 4.53 (s, 4H, C4+C6-CH₂-S-).

1-BROMO-2,4,6-TRIS([¹⁴C]ISOTHIOURONIUMMETHYL)BENZENE TRIBROMIDE ([¹⁴C]Br-TITU) ([¹⁴C]6):

Due to the difficulties of a radioactive synthesis and the handling of the small volumes involved, this procedure was preceded by a series of preliminary experiments using non-radioactive materials to find the optimal conditions. The preferred vessel for the reaction was found to be a flame sealed glass ampoule with the following dimensions: 7 mm external diameter, 1 mm glass thickness and 100 mm length with a narrow neck at 40 mm from the opening. This ampoule was loaded by means of a syringe, with 200 μ l of a solution of 1-bromo-2,4,6-tris(bromomethyl)benzene, 15 in methylene chloride (627.5 μ g, 1.44 μ moles) and 200 μ l of a solution of [¹⁴C]-thiourea in methylene chloride (250 μ Ci, ca. 4.7 μ moles). The solvent was evaporated under a very gentle stream of nitrogen gas. The original bottle of [¹⁴C]-thiourea was sequentially washed several times with 900 μ l of methylene chloride, acetone and water, until the amount of radioactivity found in the washing was negligible. Each time the washings were loaded into the ampoule and the solvents were

evaporated in the same way. Before the last evaporation, two aliquots of 5 μ l were taken (out of 900 μ l) and the counts were measured in a minivial with 3 ml of scintillation fluid. The total radioactivity was calculated to be 450×10^6 cpm. 80 μ l of isopropanol were added and the neck of the ampoule was flame-sealed after freezing the mixture in liquid nitrogen. When the suspension has thawed, the bottom part of the ampoule was immersed in a 90°C water bath for 1 h. At first the reaction materials dissolved, then the solution became cloudy and on cooling a precipitate was formed and the solution became clear. The isopropanol was evaporated with a stream of nitrogen and the residue was dried in vacuum and stored at -18°C.

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