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Original article

Synthesis and antimicrobial activity of new bromine-rich pyrrole derivatives related to monodeoxypyoluteorin

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Abstract

The synthesis and antimicrobial activity of new pyrrole derivatives structurally related to monodeoxypyoluteorin are described. The insertion of a keto or methylene spacer between the phenol group and the pyrroloyl moiety of brominated 2-(2′-hydroxybenzoyl)pyrroles leads to a decrease of the antibacterial activity.

Keywords: Antimicrobics; Bromopyrroles; Pyoluteorin analogs

1. Introduction

In a previous paper [1] the results of the bromination of 2-(2′-hydroxybenzoyl)pyrrole were reported giving a series of derivatives, related to monodeoxypyoluteorin **1** (Fig. 1), whose antimicrobial activity against *Staphylococcus aureus* increased with the degree of halogenation. The most active member of this series, the pentabromo compound **2**, exhibited an MIC (minimum inhibitory concentration) against *S. aureus* ATCC 25923 of 0.005 μg/ml and promising activity against preformed *Staphylococcus epidermidis* and *S. aureus* biofilms [2].

Compound **2** presents particular structural analogies with halogenated pyrroles from natural sources, such as pentabromopseudilin **3**. This compound, produced by marine bacterium *Pseudoalteromonas luteoviolaceus*, shows a good antibacterial effect against *Bacillus subtilis* and *Escherichia coli* and an interesting antifungal activity against *Candida albicans* and *Mucor miehei* [3].

Comparison of the antimicrobial activity of **2** with that of pentabromopseudilin **3**, implies that the insertion of a carbon atom between the aromatic rings, yielding benzoylpyrrole **2**, leads to a decrease in the antifungal activity but an improvement of the antibacterial activity. Laatsch et al. [4] suggest that separation of the phenol and pyrrole systems, increasing the coplanarity of the two rings, enhances the formation of an intermolecular hydrogen bond with the hypothetical receptor. The strength of this hydrogen bond is indicative of antibacterial activity.

On the basis of such considerations, we have investigated the antibacterial activity of 2-(2′-hydroxybenzoyl)pyrrole bromine derivatives with a keto or methylene spacer used to separate the phenol and pyrroloyl moiety of the original compound.

In this paper the synthesis of the most halogenated derivatives **4a**,**b** and **5a**,**b** (Fig. 2) is described.

2. Chemistry

Our synthetic strategy for the preparation of compounds **4a**,**b** is outlined in Scheme 1. The starting material, (3,5-dibromo-2-hydroxyphenyl)acetic acid **6**, can be obtained from commercially available (2-hydroxyphenyl)acetic acid and *N*-bromosuccinimide, using the methodology previously described in Carreño et al. in the nuclear bromination of methoxybenzene [5]. The alkylation of **6** with an excess of dimethyl sulfate in the presence of anhydrous potassium carbonate [6], followed by hydrolysis with aqueous sodium hydroxide of

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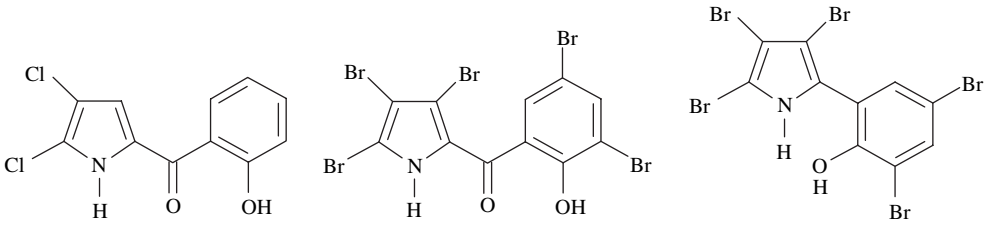


1 **2** **3**

Fig. 1. Monodeoxypyoluteorin **1**, 3,4,5,3′,5′-pentabromo-2-(2′-hydroxybenzoyl)pyrrole **2**, pentabromopseudilin **3** molecular structures.

methyl ester so obtained, provided the (3,5-dibromo-2-methoxyphenyl)acetic acid **7**. Compound **7** was reacted with thionyl chloride to give the (3,5-dibromo-2-methoxyphenyl)-acetyl chloride which, by reaction with pyrrylmagnesium bromide in anhydrous diethyl ether (see our preceding syntheses of pyrrylketones [1,7]), furnished the 2-(3,5-dibromo-2-methoxyphenyl)-1-(1*H*-pyrrol-2-yl)ethanone **8** in moderate yield (side products of the reaction were 3-isomer and variable amounts of oxidation products from unreacted pyrrole). When **8** was allowed to react with 2 molar equivalents of NBS, for 6 h at room temperature, the tetrabromoderivative **9** was obtained in excellent yield.

The further halogenation of **9** at 60 °C with 1 molar equivalent of NBS gave the pentabromo derivative **10**. The subsequent demethylation of compounds **9** and **10**, using boron tribromide in dichloromethane [8], afforded the desired 2-(3,5-dibromo-2-hydroxyphenyl)-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethanone **4a** and 2-(3,5-dibromo-2-hydroxyphenyl)-1-(3,4,5-tribromo-1*H*-pyrrol-2-yl)ethanone **4b**. Aluminium chloride was found not suitable for this demethylation.

Another pathway for the preparation of compounds **4a** and **4b** employed the methodology shown below (see Scheme 2). The reaction of the unstable 5,7-dibromo-1-benzofuran-2(3*H*)-one **11**, obtained according to the lactonization method of Johnson [9], with pyrrylmagnesium bromide in anhydrous diethyl ether, led to the formation of 2-(3,5-dibromo-2-hydroxyphenyl)-1-(1*H*-pyrrol-2-yl)ethanone **12** in moderate yield. Following the same procedure used for the bromination of **8**, compounds **4a** and **4b** were easily obtained.

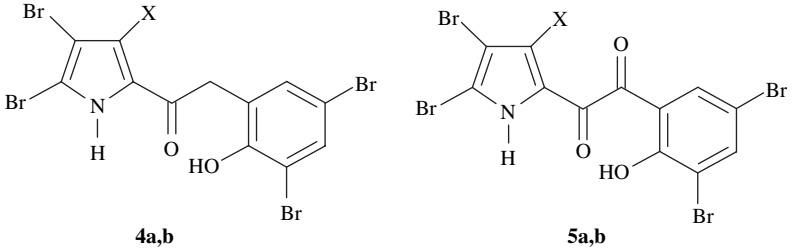
Compounds **5a** and **5b** were synthesized adopting a protecting/deprotecting strategy slightly different from the one illustrated in the preceding Scheme 1 for the preparation of **4a**,**b**. The oxidation of **9** and **10** with selenium dioxide [10], followed by demethylation of the resulting **13** and **14** derivatives with aluminium chloride in dichloromethane, gave rise to 1-(3,5-dibromo-2-hydroxyphenyl)-2-(4,5-dibromo-1*H*-pyrrol-2-yl)ethane-1,2-dione **5a** and 1-(3,5-dibromo-2-hydroxyphenyl)-2-(3,4,5-tribromo-1*H*-pyrrol-2-yl)ethane-1,2-dione **5b**, respectively (see Scheme 3).

The structures of all the newly prepared compounds were assigned according to reaction mechanisms and were confirmed by analytical and spectroscopic data.

3. Biological results and discussion

Our compounds were screened for their in vitro antimicrobial activity against two representative Gram-positive and Gram-negative bacterial strains and against a human pathogen fungal strain. The results expressed in MIC values (minimum inhibitory concentration) are reported in Table 1 along with the activity of Amikacin and Amphotericin B for comparison.

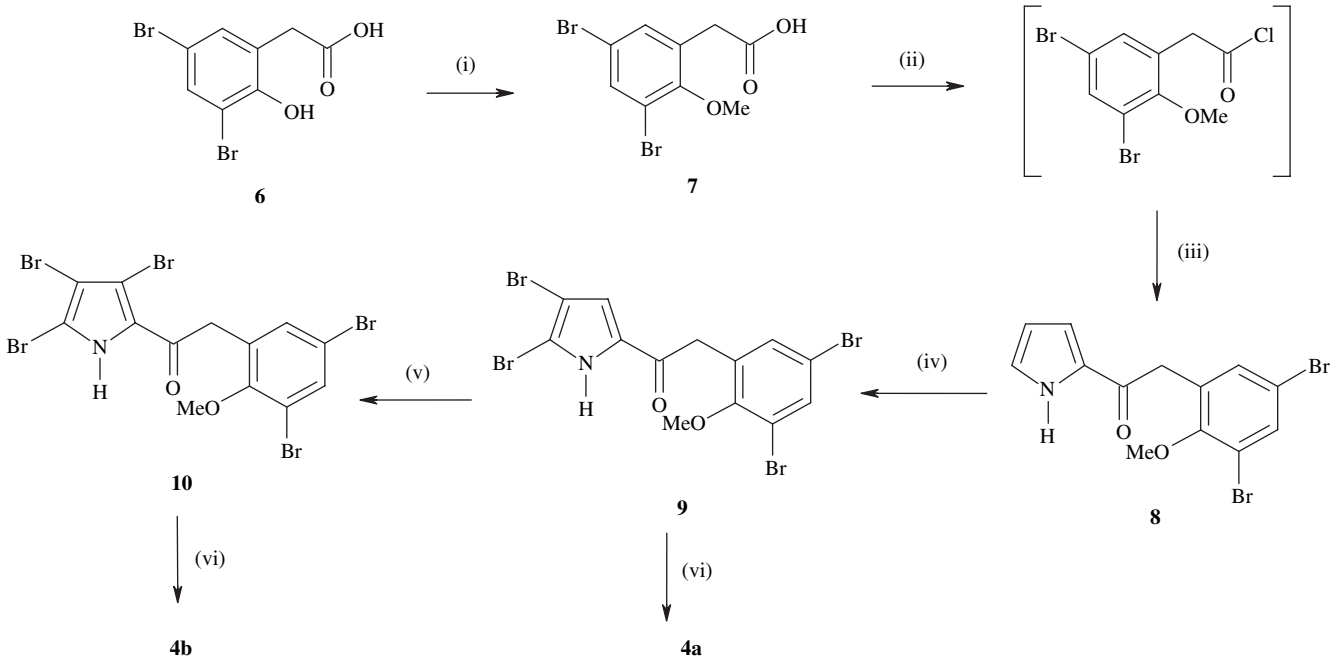
Compounds **4a**,**b** and **5a**,**b** were found to be active against *S. aureus*. In particular, ethanones **4a** and **4b** resulted the most active compounds with MIC values equal to 0.75 and 0.093 μg/ml, respectively. Ethanediones **5a** and **5b** possessed a weaker activity against *S. aureus* with MIC values of 1.5 and 0.37 μg/ml, respectively. In both cases the activity is linked to the degree of halogenation. Weak (MIC equal to



4a,b **5a,b**

a: X = H
b: X = Br

Fig. 2. 2-(3,5-Dibromo-2-hydroxyphenyl)-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethanone **4a**, 2-(3,5-dibromo-2-hydroxyphenyl)-1-(3,4,5-tribromo-1*H*-pyrrol-2-yl)ethanone **4b**, 1-(3,5-dibromo-2-hydroxyphenyl)-2-(4,5-dibromo-1*H*-pyrrol-2-yl)ethane-1,2-dione **5a** and 1-(3,5-dibromo-2-hydroxyphenyl)-2-(3,4,5-tribromo-1*H*-pyrrol-2-yl)ethane-1,2-dione **5b** molecular structures.



Scheme 1. Synthesis of compounds **4a**,**b**. Reagents: (i) $(CH_3)_2SO_4$–K_2CO_3 in acetone reflux, 24 h, then NaOH 10% at 60 °C, 12 h; (ii) $SOCl_2$ reflux, 8 h; (iii) pyrrylmagnesium bromide in anhydrous diethyl ether; (iv) *N*-bromosuccinimide (2 equiv) in acetonitrile at room temperature; (v) *N*-bromosuccinimide (1 equiv) in acetonitrile at 60 °C; (vi) BBr_3 99% in anhydrous dichloromethane.

6.25 μg/ml) anti-Gram-negative activity was only shown by **4b**. The other substances resulted inactive at the maximum tested concentration of 12.5 μg/ml. The synthesized compounds were shown to be inactive against *C. albicans* at the maximum screening concentration of 12.5 μg/ml.

From the obtained results we can conclude that further separation of the pyrrolyl moiety from the phenol group in compound **2** leads to decreased anti-Gram-positive activity. Moreover, introduction of a methylene spacer moderates the anti-Gram-positive activity to a lesser degree than the introduction of a keto spacer (compounds **4b** and **5b** are about 18 times and 74 times less active, respectively, than benzoylpyrrole **2**).

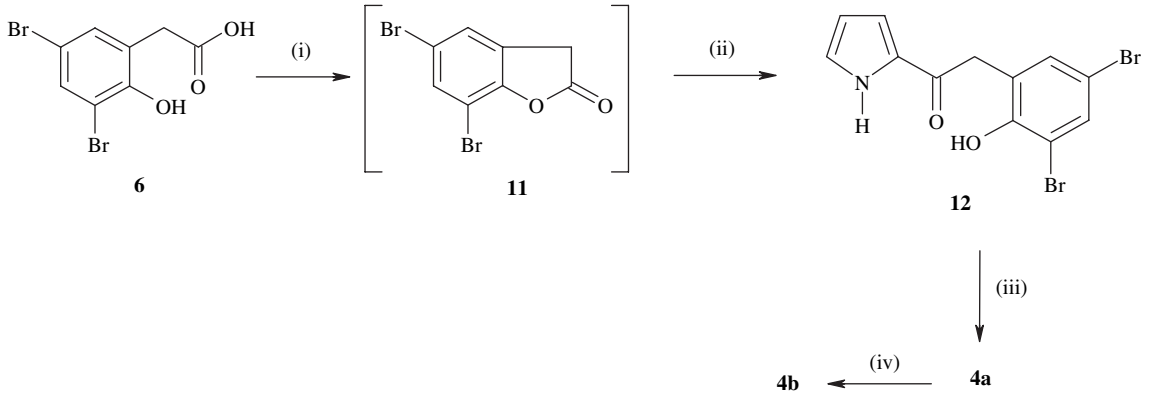
Such a decrease leads us to believe that reference compound **2** fits better with the hypothetical target than the newly synthesized compounds.

At present, our group is concerned with molecular modelling studies aimed at the synthesis of new derivatives of lead compound **2** with improved antibacterial and anti-biofilm activity. In particular our aim is the discovery of new agents with activity against bacteria in a protective physiological form such as biofilms, which are intrinsically refractory to conventional antibiotics [11].

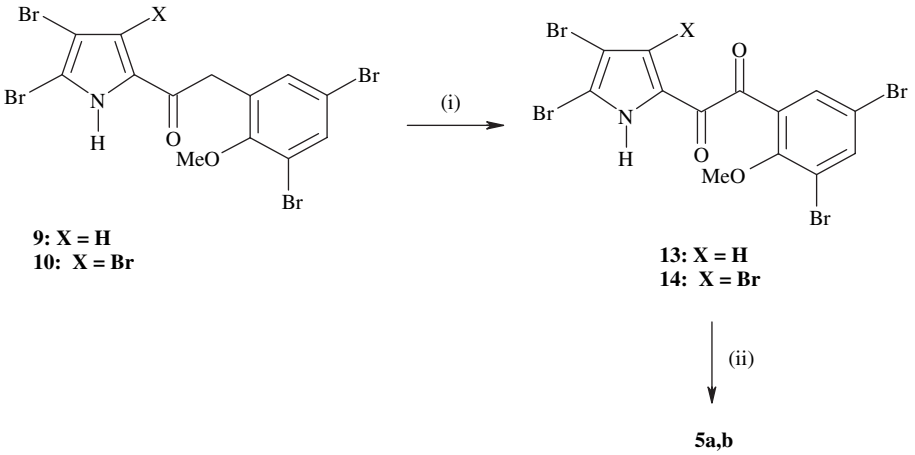
4. Experimental protocols

4.1. General methods

Melting points were determined on a Büchi–Tottoli micro melting point apparatus in open capillary tubes and are uncorrected. The IR spectra were recorded at room temperature in Nujol mulls with a Perkin Elmer Spectrum RXI FT-IR System



Scheme 2. Alternative synthesis of compounds **4a**,**b**. Reagents: (i) *p*-toluenesulfonic acid monohydrate in toluene reflux, 12 h; (ii) pyrrylmagnesium bromide in anhydrous diethyl ether; (iii) *N*-bromosuccinimide (2 equiv) in acetonitrile; (iv) *N*-bromosuccinimide (1 equiv) in acetonitrile.



Scheme 3. Synthesis of compounds **5a**,**b**. Reagents: (i) selenium dioxide in 1,4-dioxane reflux; (ii) anhydrous aluminium chloride in dry dichloromethane.

spectrometer. The ^{1}H NMR spectra were recorded at room temperature on a Bruker SF 250 spectrometer in DMSO-d_6, unless otherwise specified, using tetramethylsilane as the internal standard. Microanalyses (C, H, N) were carried out with Elemental Vario EL III apparatus and were in agreement with theoretical values ±0.4%. Chromatographic separations were carried out on columns packed with Macherey Nagel Kiesel gel 60 (70–230 mesh ASTM). All reactions were monitored by TLC on precoated aluminium sheets 20 × 20 of silica gel (0.2 mm Kiesel gel 60 G F254, Merck, Germany) and C-18 reverse phase (RP-18 F254 S, Merck, Germany) using UV light at 254 nm for visualization.

The compounds **4a**,**b** and **5a**,**b** were tested for their in vitro growth inhibitory activity against the following bacteria and yeasts: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *C. albicans* ATCC 10231. Amikacin and Amphotericin B (Sigma) were used for comparative purposes and quality control of the methods. MICs against bacterial strains were determined using the broth dilution micro-method as described in Ref. [2]. Antifungal activity was carried out using the broth dilution macro-method as described [12].

4.1.1. (3,5-Dibromo-2-hydroxyphenyl)acetic acid **6**

To a stirred solution of 6.08 g (40 mmol) of 2-hydroxyphenylacetic acid in 100 ml of acetonitrile 14.24 g (80 mmol) of *N*-bromosuccinimide was added slowly. After 24 h the reaction mixture was evaporated under vacuum. The solid residue was triturated with water, filtered, dried, then crystallized from toluene to give **6** (11.40 g, 92% yield) as white needles, m.p. 145 °C, lit. 155 °C [13]. ^{1}H NMR (δ): 3.61 (s, 2H, CH_2), 7.37 (d, 1H, $J = 2.4$ Hz, H-6), 7.61 (d, 1H, $J = 2.4$ Hz, H-4), 9.54 (br s, 1H, exchangeable with D_2O, OH); IR (ν): 3466 (OH), 1703 (CO) cm^{-1}. Anal. Calc. (Found) for $C_8H_6Br_2O_3$: C, 31.00% (31.13%); H, 1.95% (2.01%).

Table 1
Antimicrobial activity in vitro, MIC values expressed in μg/ml for all strains tested

	S. aureus ATCC 25923	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
2	0.005	6.2	>12.5
4a	0.75	>12.5	>12.5
4b	0.093	6.2	>12.5
5a	1.5	>12.5	>12.5
5b	0.37	>12.5	>12.5
Amikacin	1	10	–
Amphotericin B	–	–	0.15

4.1.2. (3,5-Dibromo-2-methoxyphenyl)acetic acid **7**

A mixture of 12.40 g (40 mmol) of **6**, 22 g (160 mmol) of anhydrous potassium carbonate and 25.20 g (200 mmol) of dimethyl sulfate was refluxed in 140 ml of acetone for 24 h. After cooling the solid was filtered out and the solution was rotavaped to dryness. The oil residue was heated at 60 °C for 12 h with 100 ml of 10% sodium hydroxide. The cold clear solution was acidified with 10% sulfuric acid. The obtained crude acid was filtered, washed with water until neutrality, dried and crystallized from ethanol to give **7** (11.27 g, 87% yield) as white crystals, m.p. 146 °C. ^{1}H NMR (δ): 3.63 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3), 7.52 (d, 1H, $J = 2.3$ Hz, H-6) 7.76 (d, 1H, $J = 2.3$ Hz, H-4); IR (ν): 1706 (CO) cm^{-1}. Anal. Calc. (Found) for $C_9H_8Br_2O_3$: C, 33.37% (33.52%); H, 2.49% (2.55%).

4.1.3. 2-(3,5-Dibromo-2-methoxyphenyl)-1-(1H-pyrrol-2-yl)ethanone **8**

6.48 g (20 mmol) of **7** and 15 ml (24.50 g, 200 mmol) of thionyl chloride were refluxed until the generation of hydrochloric acid ceased (approximately 8 h). The excess thionyl chloride was removed under vacuum and the crude (3,5-dibromo-2-methoxyphenyl)acetyl chloride, dissolved in 100 ml of anhydrous diethyl ether, was added, dropwise under nitrogen atmosphere, to a stirred suspension of pyrrylmagnesium bromide (20 mmol in 150 ml of anhydrous diethyl ether) [1]. The reaction mixture was heated at reflux for 0.5 h, cooled to room temperature, then quenched with 5% sulfuric acid (50 ml). After stirring for 1 h, the ethereal solution was separated and the aqueous layer was extracted with diethyl ether

(3 × 50 ml). The combined organic phases were washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by chromatography, eluted with 9/1 (v/v) cyclohexane–ethyl acetate mixture, to provide **8** (3.51 g, 47% yield) as white needles from ethanol, m.p. 134 °C. ^{1}H NMR (δ): 3.71 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 6.28 (m, 1H, H-4′), 7.17 (m, 2H, H-3′ and H-5′), 7.51 (d, 1H, $J = 2.4$ Hz, H-6″), 7.77 (d, 1H, $J = 2.4$ Hz, H-4″), 11.89 (s, 1H, exchangeable with D_2O, NH). IR (ν): 3289 (NH), 1642 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{13}H_{11}Br_2NO_2$: C, 41.86% (41.68%); H, 2.97% (2.80%); N, 3.75% (3.61%).

4.1.4. 2-(3,5-Dibromo-2-methoxyphenyl)-1-(4,5-dibromo-1H-pyrrol-2-yl)ethanone **9**

A solution of 3.56 g (20 mmol) of *N*-bromosuccinimide in 20 ml of acetonitrile was added dropwise to a stirred solution of 3.73 g (10 mmol) of **8** in 150 ml of acetonitrile. After 6 h, the reaction mixture was evaporated under reduced pressure. The residue was partitioned between water (100 ml) and diethyl ether (150 ml) and, subsequently, extracted twice with diethyl ether (100 ml). The combined extracts were washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was crystallized from acetonitrile to afford **9** (4.67 g, 88% yield) as white needles, m.p. 168 °C. ^{1}H NMR (δ): 3.66 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 7.40 (d, 1H, $J = 1.8$ Hz, s after exchange with D_2O, H-3′), 7.51 (d, 1H, $J = 2.5$ Hz, H-6″), 7.78 (d, 1H, $J = 2.5$ Hz, H-4″), 13.12 (br s, 1H, exchangeable with D_2O, NH); IR (ν): 3220 (NH), 1635 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{13}H_9Br_4NO_2$: C, 29.41% (29.65%); H, 1.71% (1.82%); N, 2.64% (2.60%).

4.1.5. 2-(3,5-Dibromo-2-methoxyphenyl)-1-(3,4,5-tribromo-1H-pyrrol-2-yl)ethanone **10**

To a solution of **9** (1.06 g, 2 mmol, in 150 ml of acetonitrile) heated at 60 °C, a solution of *N*-bromosuccinimide (0.36 g, 2 mmol) in 10 ml of acetonitrile was added dropwise under stirring.

After addition was complete the reaction mixture was stirred at 60 °C for an additional 2 h, then left at room temperature overnight. The white needles of **10** (0.83 g, 68% yield) so obtained were filtered out and the solution was concentrated under reduced pressure. The residue was partitioned between water (100 ml) and diethyl ether (100 ml) and extracted twice with diethyl ether (50 ml). The combined extracts were washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was crystallized from acetone to give a further 0.18 g of **10** (overall yield 83%) as white needles, m.p. 216 °C. ^{1}H NMR (δ): 3.65 (s, 3H, CH_3), 4.33 (s, 2H, CH_2), 7.53 (d, 1H, $J = 2.4$ Hz, H-6″), 7.79 (d, 1H, $J = 2.4$ Hz, H-4″), 13.51 (s, 1H, exchangeable with D_2O, NH); IR (ν): 3200 (NH), 1649 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{13}H_8Br_5NO_2$: C, 25.61% (25.80%); H, 1.32% (1.38%); N, 2.30% (2.45%).

4.1.6. General procedure for the demethylation of compounds **9** and **10**

In a typical experiment, to a solution of 1 mmol of **9** or **10** in 20 ml of anhydrous dichloromethane in an ice–salt bath, 1 ml of BBr_3 99% (10 mmol) was added. After 2 h, the solution was cautiously decomposed with ice–sulfuric acid 5% (30 ml), then 50 ml of diethyl ether was added. The mixture was stirred vigorously for 10 min, the organic layer was separated and the aqueous phase extracted with diethyl ether (2 × 30 ml). The combined extracts were washed with water until neutrality, dried over anhydrous sodium sulfate, and evaporated. The crude product was crystallized to give, respectively,

(a) 2-(3,5-Dibromo-2-hydroxyphenyl)-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethanone **4a** (0.44 g, 85% yield) as white crystals from ethanol, m.p. 178 °C. ^{1}H NMR (δ): 4.15 (s, 2H, CH_2), 7.32 (d, 1H, $J = 2.4$ Hz, s after exchange with D_2O, H-3′), 7.35 (d, 1H, $J = 2.3$ Hz, H-6″), 7.61 (d, 1H, $J = 2.3$ Hz, H-4″), 9.50 (s, 1H, exchangeable with D_2O, OH), 13.06 (s, 1H, exchangeable with D_2O, NH); IR (ν): 3495 (OH), 3229 (NH), 1640 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{12}H_7Br_4NO_2$: C, 27.89% (27.59%); H, 1.37% (1.22%); N, 2.71% (2.50%).

(b) 2-(3,5-Dibromo-2-hydroxyphenyl)-1-(3,4,5-tribromo-1*H*-pyrrol-2-yl)ethanone **4b** (0.43 g, 72% yield) as white crystals from ethanol, m.p. 198 °C. ^{1}H NMR (δ): 4.27 (s, 2H, CH_2), 7.35 (d, 1H, $J = 2.3$ Hz, H-6″), 7.62 (d, 1H, $J = 2.3$ Hz, H-4″), 9.50 (s, 1H, exchangeable with D_2O, OH), 13.43 (s, 1H, exchangeable with D_2O, NH); IR (ν): 3486 (OH), 3202 (NH), 1648 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{12}H_6Br_5NO_2$: C, 24.19% (23.93%); H, 1.02% (0.96%); N, 2.35% (2.15%).

4.1.7. 5,7-Dibromo-1-benzofuran-2(3H)-one **11**

A mixture of 6.20 g (20 mmol) of (3,5-dibromo-2-hydroxyphenyl)acetic acid **6** and 0.38 g (2 mmol) of *p*-toluenesulfonic acid monohydrate in 100 ml of toluene was refluxed under a Dean-Stark trap until the evolution of water ceased (12 h). The cooled solution was chromatographed over silica gel (100 g) and activated at 200 °C for 24 h eluting with anhydrous toluene (200 ml).

The solution of unstable compound **11** so obtained was used directly for the next reaction as described below.

4.1.8. 2-(3,5-Dibromo-2-hydroxyphenyl)-1-(1H-pyrrol-2-yl)ethanone **12**

To a stirred suspension of pyrrylmagnesium bromide (20 mmol in 100 ml of anhydrous diethyl ether freshly distilled) the previously obtained toluenic solution of **11** was added, slowly under nitrogen atmosphere. The reaction mixture was heated at reflux for 0.5 h then quenched with sulfuric acid 5% (50 ml). After stirring for 1 h, the organic solution was separated and the aqueous layer was extracted with diethyl ether (2 × 50 ml). The combined extracts were washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product, purified by

chromatography, gave **12** (2.0 g, 28% yield) as white needles from acetonitrile, m.p. 160 °C. ^{1}H NMR (δ): 4.17 (s, 2H, CH_2), 6.22 (m, 1H, H-4′), 7.13 (m, 2H, H-3′ and H-5′), 7.92 (d, 1H, $J = 2.4$ Hz, H-4″), 8.30 (d, 1H, $J = 2.4$ Hz, H-6″), 9.56 (s, 1H, exchangeable with D_2O, OH), 11.67 (s, 1H, exchangeable with D_2O, NH); IR (ν): 3314 (NH), 1622 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{12}H_9Br_2NO_2$: C, 40.15% (40.35%); H, 2.53% (2.60%); N, 3.90% (3.71%).

4.1.9. 2-(3,5-Dibromo-2-hydroxyphenyl)-1-(4,5-dibromo-1H-pyrrol-2-yl)ethanone **4a** from **12**

A solution of 0.71 g (4 mmol) of *N*-bromosuccinimide in 10 ml of acetonitrile was added dropwise to a stirred solution of 0.72 g (2 mmol) of **12** in 20 ml of acetonitrile. After 12 h, the reaction mixture was evaporated. The residue was added to 20 ml of water, then extracted with diethyl ether (3 × 30 ml). The combined extracts were washed with water, dried over anhydrous sodium sulfate, evaporated to dryness and then crystallized to give **4a** (0.38 g, 75% yield).

4.1.10. 2-(3,5-Dibromo-2-hydroxyphenyl)-1-(3,4,5-tribromo-1H-pyrrol-2-yl)ethanone **4b** from **4a**

0.089 g (0.5 mmol) of solid *N*-bromosuccinimide was added to a stirred solution of 0.26 g (0.5 mmol) of **4a** in 10 ml of acetonitrile. After 48 h, the mixture was evaporated. The residue was partitioned between water (20 ml) and diethyl ether (20 ml) and, subsequently, extracted twice with diethyl ether (30 ml). The combined extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude product was chromatographed over silica gel, using an 8/2 (v/v) mixture of cyclohexane–ethyl acetate as the eluent to give **4b** (0.14 g, 46% yield).

4.1.11. General procedure for the oxidation of **9** and **10** with selenium dioxide

A mixture of **9** (0.32 g, 0.6 mmol) or **10** (0.37 g, 0.6 mmol) in 30 ml of 1,4-dioxane and selenium dioxide (1.0 g, 9 mmol) in 20 ml of water was refluxed for 96 h. The resulting solid was filtered out and the solution, diluted with 200 ml of water and saturated with sodium chloride, was extracted with diethyl ether (3 × 50 ml). The combined organic extracts were washed with water, dried with sodium sulfate, and evaporated. The remainder was chromatographed over silica gel using as eluents the following, respectively,

(a) 85/15 cyclohexane–ethyl acetate to give 1-(3,5-dibromo-2-methoxyphenyl)-2-(4,5-dibromo-1*H*-pyrrol-2-yl)ethane-1,2-dione **13** (0.14 g, 43% yield) as pale yellow crystals from ethanol, m.p. 124 °C. ^{1}H NMR (δ): 3.64 (s, 3H, CH_3), 7.16 (s, 1H, H-3′), 7.92 (d, 1H, $J = 2.2$ Hz, H-6″), 8.30 (d, 1H, $J = 2.2$ Hz, H-4″), 13.71 (s, 1H, exchangeable with D_2O, NH); IR (ν): 3244 (NH), 1688 and 1619 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{13}H_7Br_4NO_3$: C, 28.66% (28.71%); H, 1.30% (1.21%); N, 2.57% (2.44%).

(b) 8/2 cyclohexane–ethyl acetate to give 1-(3,5-dibromo-2-methoxyphenyl)-2-(3,4,5-tribromo-1*H*-pyrrol-2-yl)ethane-1,2-dione **14** (0.27 g, 73% yield) as white crystals from acetonitrile, m.p. 180 °C. ^{1}H NMR (δ): 3.66 (s, 3H, CH_3), 8.04 (d, 1H, $J = 2.4$ Hz, H-6″), 8.35 (d, 1H, $J = 2.4$ Hz, H-4″), 14.20 (s, 1H, exchangeable with D_2O, NH); IR (ν): 3208 (NH), 1680 and 1624 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{13}H_6Br_5NO_3$: C, 25.03% (25.32%), H, 0.97% (1.09%), N, 2.25% (2.11%).

4.1.12. 1-(3,5-Dibromo-2-hydroxyphenyl)-2-(4,5-dibromo-1H-pyrrol-2-yl)ethane-1,2-dione **5a** and 1-(3,5-dibromo-2-hydroxyphenyl)-2-(3,4,5-tribromo-1H-pyrrol-2-yl)ethane-1,2-dione **5b**

To a solution of 0.27 g (0.5 mmol) of **13** or 0.31 g (0.5 mmol) of **14** in 10 ml of dry dichloromethane 0.65 g (5 mmol) of anhydrous aluminium chloride was added under stirring. After 24 h the mixture was cautiously decomposed with ice–sulfuric acid 5% (30 ml), then 50 ml of diethyl ether was added. The mixture was vigorously stirred until the solid residue was entirely dissolved. The organic layer was separated and the aqueous phase extracted with diethyl ether (2 × 30 ml). The combined extracts were washed with water until neutrality, dried over anhydrous sodium sulfate and evaporated to give

(a) **5a** (0.23 g, 85% yield) as yellow needles from ethanol, m.p. 211 °C. ^{1}H NMR ($CDCl_3$) (δ): 7.03 (d, 1H, $J = 2.5$ Hz, H-4′, s after exchange with D_2O), 7.88 (d, 1H, $J = 2.4$ Hz, H-6″), 7.95 (d, 1H, $J = 2.4$ Hz, H-4″), 9.80 (br s, 1H, exchangeable with D_2O, OH), 11.92 (s, 1H, exchangeable with D_2O, NH) [14]; IR (ν): 3232 (NH), 1621 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{12}H_5Br_4NO_3$: C, 27.15% (27.12%); H, 0.95% (1.06%); N, 2.64% (2.77%).

(b) **5b** (0.28 g, 92% yield) as yellow crystals from acetone–petroleum ether, m.p. 176 °C. ^{1}H NMR ($CDCl_3$) (δ): 7.58 (d, 1H, $J = 2.4$ Hz, H-6″), 7.96 (d, 1H, $J = 2.4$ Hz, H-4″), 10.43 (br s, 1H, exchangeable with D_2O, OH), 14.66 (s, 1H, exchangeable with D_2O, NH) [15]; IR (ν): 3208 (NH), 1625 (CO) cm^{-1}. Anal. Calc. (Found) for $C_{12}H_4Br_5NO_3$: C, 23.64% (23.92%); H, 0.66% (0.81%); N, 2.30% (2.59%).

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[14] Only very broad signals for the different protons have been shown in DMSO-d_6.
[15] The ^{1}H NMR spectrum in DMSO-d_6 seems to suggest the co-existence, in this solvent, of two potential tautomeric forms of comparable stability.