Synthesis of pyrrolomycins for the conjugation with siderophores as a strategy to fight antimicrobial resistance

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1. Aims

The emergence of multidrug-resistant bacteria represents an increasing threat to public health that requires novel and innovative therapeutical strategies[1].

Iron plays a vital role in the survival mechanism of different organisms, including bacteria. Many bacteria produce and secrete siderophores, small molecules with the ability to chelate iron from the surrounding environment.[2] Siderophores are keys for opening the door of the intracellular compartment of bacteria. By linking these moieties with an antibiotic, the conjugated molecule can act as a 'Trojan Horse' and disturb the iron supply system of the pathogenic agent.[3] Pyrrolomycin C is a natural antibiotic that have demonstrated promising activity against Gram-positive bacteria[4]. The conjugation of this natural antibiotic with siderophores has the potential to enhance its antibiotic efficacy.

In this work, I describe the synthesis of pyrrolomycin C - (4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone (**1**) as well as two related analogues (Figure 1). The retrosynthetic plan was based on the coupling of a suitable pyrrole with a methyl benzoate via organometallic chemistry. The structures of the synthesized compounds were established by spectrometric methods (FTIR, ^{1}H, and ^{13}C NMR techniques).

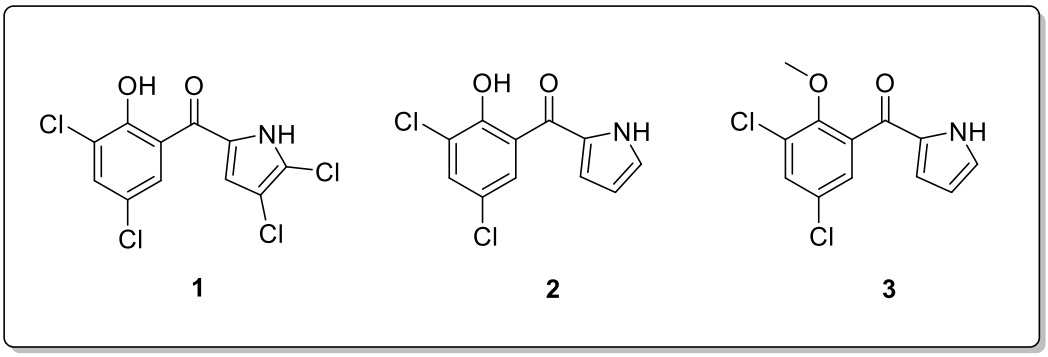
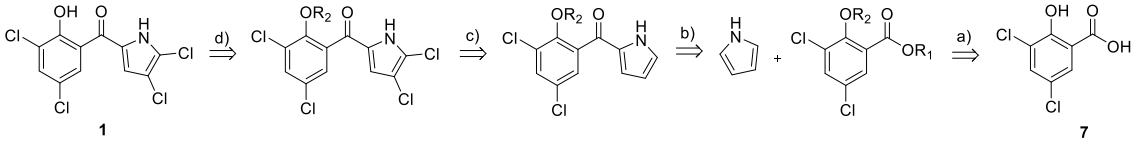


Figure 1. Pyrrolomycin C (**1**) and other two related analogues.

2. Results and discussion

2.1. Synthesis of pyrrolomycin C

For the synthesis of (4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone (1), a retrosynthetic plan with four steps was established: a) O-protection of the building block 3,5-dichlorosalicylic acid (7); b) coupling of the protected building block with pyrrole; c) chlorination; d) O-deprotection to obtain the final compound (scheme 1).



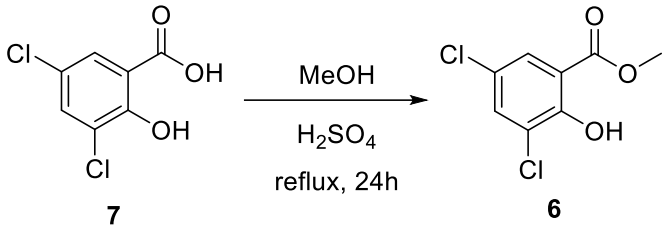
Scheme 1. Retrosynthetic plan for obtaining pyrrolomycin C. a) *O*-protection; b) coupling with pyrrole; c) chlorination; d) *O*-deprotection.

2.1.1. *O*-protection of 3,5-dichlorosalicylic acid (7)

The first step towards the obtention of (4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone (**1**) is the *O*-protection of the building block 3,5-dichlorosalicylic acid. This step is required for the subsequent coupling with pyrrole, which is incompatible with the presence of hydroxyl and carboxyl groups. Two different synthetic strategies were employed. In the first strategy, *O*-protection was carried out individually for each functional group: a Fischer esterification was followed by an etherification reaction to introduce the methoxymethyl (MOM) group. In the second strategy, *O*-protection was carried out simultaneously for both functional groups through alkylation with iodomethane.

2.1.1.1. Synthesis of methyl 3,5-dichloro-2-hydroxybenzoate (6)

3,5-Dichloro-2-hydroxybenzoate (**6**) was synthesized by the Fischer esterification of 3,5-dichlorosalicylic acid (**7**) with methanol in the presence of sulfuric acid as the acidic catalyst (Scheme 2).



7 6

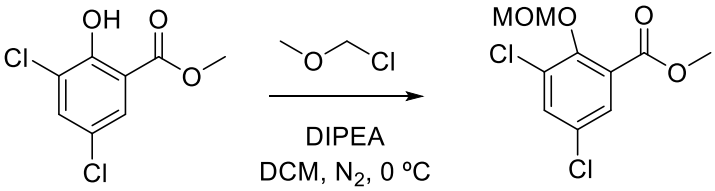
Scheme 2. Synthesis of methyl 3,5-dichloro-2-hydroxybenzoate (**6**).

Compound **6** was purified by flash column chromatography. The reaction provided the desired compound, albeit with a yield lower than initially expected. To improve the yield of the process, different strategies to shift the reaction equilibrium towards the formation of the desired product were implemented, namely: i. doubled the number of equivalents of sulfuric acid; ii. adding more 100 equivalents of methanol and iii. including molecular sieves in the set-up of the reaction. Despite these modifications, the yield remained low, between 14 and 22%.

Structural elucidation of compound **6** was performed through FTIR and ^{1}H NMR techniques.

2.1.1.2. Synthesis of methyl 3,5-dichloro-2-(methoxymethoxy)benzoate (5)

For the synthesis of methyl 3,5-dichloro-2-(methoxymethoxy)benzoate (**5**), an etherification using chloromethyl methyl ether was performed to add the MOM protective group to the compound **6** in the presence of DIPEA, as depicted in scheme 3.[5]



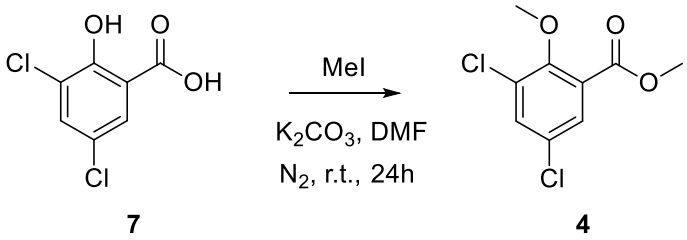
6 5

Scheme 3. Synthesis of 3,5-dichloro-2-(methoxymethoxy)benzoate (**5**).

Compound **5** was purified through flash column chromatography and obtained in 76% yield. Its structural elucidation was achieved though FTIR and ^{1}H NMR techniques.

2.1.1.3. Synthesis of methyl 3,5-dichloro-2-methoxybenzoate (4)

For the synthesis of methyl 3,5-dichloro-2-methoxybenzoate (**4**), *O*-protection of 3,5-dichlorosalicylic acid (**7**) was performed using iodomethane as the alkylating agent, as depicted in scheme 4.[6]



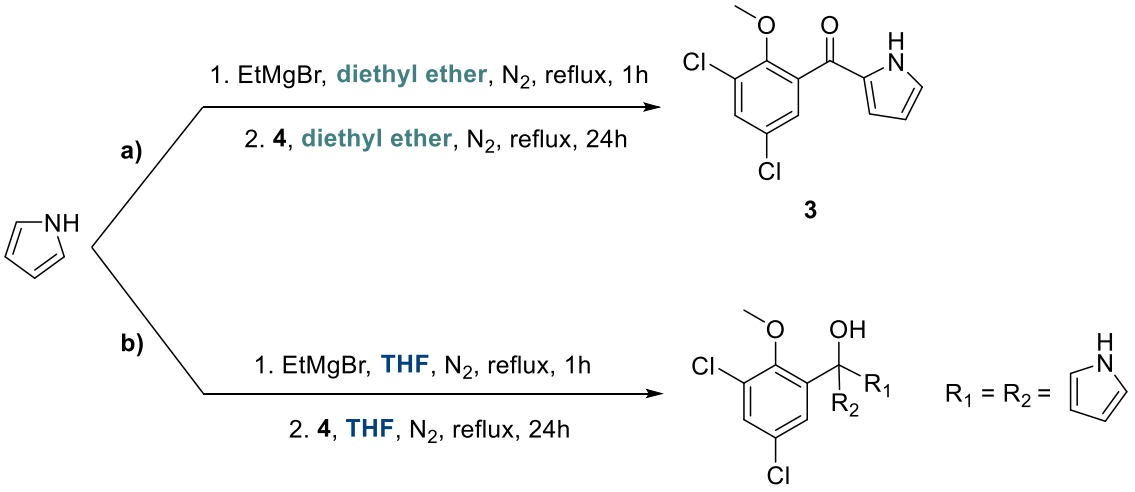
7 **4**

Scheme 4. Synthesis of methyl 3,5-dichloro-2-methoxybenzoate (**4**).

Compound **4** was purified by liquid-liquid extraction using *n*-hexane. The obtained extract was considered pure by thin layer chromatography analysis and no further purification was attained. Compound **4** was obtained in 75% yield and its structural elucidation was achieved through FTIR and ^{1}H NMR techniques.

2.1.2. Synthesis of (3,5-dichloro-2-methoxyphenyl)(1H-pyrrol-2-yl)methanone (3)

For the synthesis of (3,5-dichloro-2-methoxyphenyl)(1H-pyrrol-2-yl)methanone (**3**), a carbon-carbon coupling reaction between pyrrole and **4** was performed, using a commercial available Grignard reagent, as depicted in scheme 5 a).[6]



Scheme 5. Synthesis of (3,5-dichloro-2-methoxyphenyl)(1H-pyrrol-2-yl)methanone (**3**).

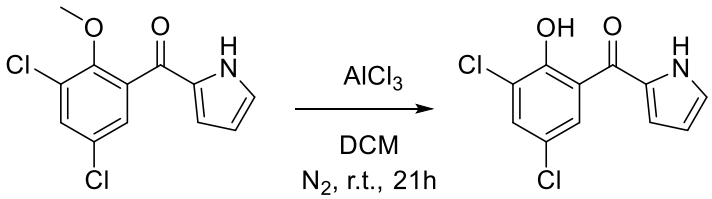
First, pyrrole was added to a suspension of ethyl magnesium bromide and refluxed for 1 hour to yield the pyrrylmagnesium bromide. Then, a solution of **4** was added to the latter and refluxed until the formation of the desired compound.

In this class of reactions, the most adequate solvents are ethereal ones, like diethyl ether or THF. Considering the moderate yield obtained, the reaction was repeated using THF instead of diethyl ether, which allowed a higher temperature of reflux, as represented in scheme 5 b). This time, the desired product was not formed. Instead, a product with a smaller value of Rf was obtained, which according to the characterization performed could correspond to a compound whose carbonyl group was further reduced to an alcohol.

Compound 3 was purified through flash column chromatography and obtained in 10% yield. Its structural elucidation was achieved through FTIR and ^{1}H NMR techniques.

2.1.3. Synthesis of (3,5-dichloro-2-hydroxyphenyl)(1H-pyrrol-2-yl)methanone (2)

For the synthesis of (3,5-dichloro-2-hydroxyphenyl)(1H-pyrrol-2-yl)methanone **(2)**, *O*-deprotection of (3,5-dichloro-2-methoxyphenyl)(1H-pyrrol-2-yl)methanone **(3)** was achieved using $AlCl_3$ as the dealkylation agent, as depicted in scheme 6.[6]



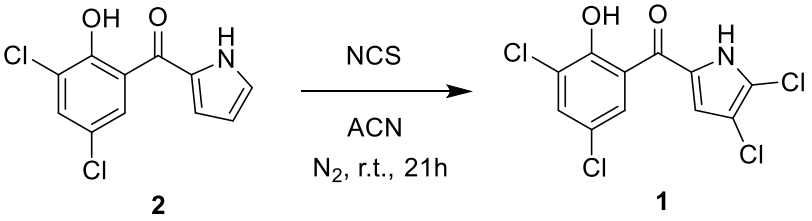
3 **2**

Scheme 6. Synthesis of (3,5-dichloro-2-hydroxyphenyl)(1H-pyrrol-2-yl)methanone (**2**).

Compound **2** was purified through flash column chromatography and obtained in 75% yield. Its structure was confirmed though FTIR and ^{1}H NMR techniques.

2.1.4. Synthesis of (4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone (1)

For the synthesis of (4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone (1), *N*-chlorosuccinimide was used as a chlorine donator, as depicted in scheme 7.[6]



2 **1**

Scheme 7. Synthesis of (4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone (**1**).

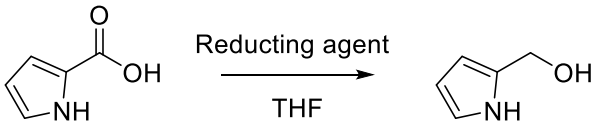
Compound **1** was purified through flash column chromatography and obtained in 65% yield. Its structure was confirmed though FTIR and ^{1}H NMR techniques.

2.2. Synthesis of other analogues

The second aim of this project was to synthesize other analogues of pyrrolomycin C. Towards this objective, reduction of pyrrole-2-carboxylic acid was performed (scheme 8). The purpose was to protect the carboxyl group, in order to obtain an analogue with this functional group in pyrrole moiety.

Therefore, $LiAlH_4$, $THF\text{-}BH_3$, $BH_3{\cdot}S(CH_3)_2$ were used as reducing agents.

However, it was not possible to gather the desired product.



Scheme 8. Reduction of pyrrole 2-carboxylic acid.

3. Conclusions

In the present work, pyrrolomycin C and two related analogues were successfully synthesized. Two different *O*-protection strategies were investigated, and it was demonstrated that the simultaneous *O*-methylation with iodomethane was more effective than the sequential *O*-methylation followed by *O*-methoxymethyl protection. The Grignard coupling step was identified as the bottleneck in the synthetic pathway, yielding only 10%. Despite attempts to enhance the yield through solvent variations, no improvement was observed in this reaction.

Until the end of the project, I will increase the mass of the produced pyrrolomycin C synthesized and I will synthesize different analogues.

4. Experimental procedures

4.1. General

All compounds were prepared in the Laboratory of Organic and Pharmaceutical Chemistry of the Faculty of Pharmacy of the University of Porto.

All the reagents were purchased from Sigma-Aldrich, Alfa Aesar, Pronalab, TCI, Acros Organics, Fisher Scientific, VWR or Honeywell Riedel-de Haën, and were used without further purification. Solvents were evaporated using rotary evaporator under reduced pressure (Buchi Waterchath B-480, BÜCHI Labortechnik AG, Flawil, Switzerland). Anhydrous solvents were either purchased from Sigma-Aldrich or dried according to published procedures[7].

Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 (GF_{254}) precoated plates (0.2 mm of thickness) with appropriate mobile phases. The chromatograms were visualized under UV light at 254 and 365 nm and using appropriate chemical stains. Synthesized compounds were purified by flash column chromatography using Merck silica gel 60 (0.040-0.063 mm, Merck, Darmstadt, Germany).

IR spectra were obtained using KBr disks in a FTIR spectrometer Nicolet iS10 from Thermo Scientific with a Smart OMNI-Transmisson accessory (Software OMNIC 8.3).

^{1}H and ^{13}C NMR spectra were performed in the Departamento de Química, Universidade de Aveiro on a Bruker Avance 300 spectrometer (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C, Bruker Biosciences Corporation, Billerica, MA, USA) or at the Centro de Materiais da Universidade do Porto (CEMUP) on a Bruker Avance III 400 spectrometer (400 MHz) and were taken in DMSO-d_6 (Deutero GmbH) or $CDCl_3$ (Deutero GmbH) at room temperature.

Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as internal reference and assignment abbreviations are the following: singlet (s), doublet 12 (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), doublet of triplets (td). ^{13}C NMR assignments were made by 2D bidimensional heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments (long-range C, H coupling constants were optimized to 7 and 1 Hz) or by comparison with the assignments of similar molecules.

4.2. Synthesis of pyrrolomycin C

4.2.1. Synthesis of methyl 3,5-dichloro-2-hydroxybenzoate (6)

In a two-necked round-bottom flask equipped with a magnetic stirring bar, 3,5-dichlorosalicylic acid **(7)** (2.50 g, 12.08 mmol) was dissolved in methanol (100 mL, 2.47 mol) followed by the dropwise addition of sulfuric acid (4 ml, 74.64 mmol). The reactional mixture was gently refluxed for 24 hours. After this time, it was cooled to room temperature and filtrated over a vacuum filtration unit, using methanol to wash the filter cake. The resulting white solid was purified by flash column chromatography (a. *n*-hexane/ethyl acetate/formic acid (8:2:0.02) as eluent; b. *n*-hexane/ethyl acetate/formic acid (9:1:0.02) as eluent). Methyl 3,5-dichloro-2-hydroxybenzoate **(6)** was isolated as a white solid (706 mg, 26%).

methyl 3,5-dichloro-2-hydroxybenzoate (6): IR ν_{max} (cm^{-1}) (KBr): 3077, 2958, 1798, 1681, 1604, 1444, 1354, 1283, 1241, 1197, 972, 911, 891, 851, 754, 717, 605, 478; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm): 11.25 (d, J = 0.5 Hz, 1H), 7.76 (d, J = 2.6 Hz, 1H), 7.55 (dd, J = 2.6, 0.6 Hz, 1H), 3.99 (s, 3H).

4.2.2. Synthesis of methyl 3,5-dichloro-2-(methoxymethoxy)benzoate (5)

In an oven-dried two-necked round-bottom flask equipped with a magnetic stirring bar, methyl 3,5-dichloro-2-hydroxybenzoate **(6)** (1.10 g, 4.98 mmol) was dissolved in anhydrous dicloromethane (16 mL) under nitrogen atmosphere. The solution was cooled to 0 ºC and chloromethyl methyl ether (1.51 mL, 19.91 mmol) was added in one portion under stirring, followed by the dropwise addition of DIPEA (3.47 mL, 19.91 mmol). The reaction mixture was left to stir for one hour at 0 ºC and then at room temperature overnight. The reaction mixture was quenched with $NaHCO_3$ solution (40 mL) and extracted with ethyl acetate (3 x 60 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash column chromatography (a. *n*-hexane/ethyl acetate (8:2) as eluent; b. *n*-hexane/ethyl acetate (9:1) as eluent). 3,5-dichloro-2-(methoxymethoxy)benzoate **(5)** was obtained as a transparent oil (1.00 g, 76%).

3,5-dichloro-2-(methoxymethoxy)benzoate (5): IR ν_{max} (cm^{-1}) (KBr): 3445, 3074, 2954, 2836, 1735, 1634, 1582, 1451, 1395, 1283, 1241, 1204, 1160, 1096, 929, 871, 836, 794, 733, 694, 586; **^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):** 7.68 (d, *J* = 2.6 Hz, 1H), 7.56 (d, *J* = 2.7 Hz, 1H), 5.12 (s, 2H), 3.92 (s, 3H), 3.61 (s, 3H).

4.2.3. Synthesis of methyl 3,5-dichloro-2-methoxybenzoate (4)

In a oven-dried two-necked round-bottom flask equipped with a magnetic stirring bar, 3,5-dichlorosalicylic acid **(7)** (2.00 g, 9.66 mmol) was dissolved in anhydrous DMF (15 mL). K_2CO_3 previously dried (3.20 g, 23.19 mmol) was added to the solution, which was then cooled to 0 ºC before the addition of iodomethane (2.40 mL, 38.65 mmol) dropwise. The reaction mixture was left to stir for 24 hours at room temperature. The reaction mixture was extracted with hexane (3 x 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Methyl 3,5-dichloro-2-methoxybenzoate (**4**) was obtained as a white solid (1.70 g, 75%).

methyl 3,5-dichloro-2-methoxybenzoate (4): IR ν_{max} (cm^{-1}) (KBr): 3437, 3093, 3062, 2955, 1734, 1556, 1469, 1237, 1204, 991, 889, 871, 836, 796; **^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):** 7.68 (d, *J* = 2.6 Hz, 1H), 7.54 (d, *J* = 2.7 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H).

4.2.4. Synthesis of (3,5-dichloro-2-methoxyphenyl)(1H-pyrrol-2-yl)methanone (3)

In an oven-dried two-necked round-bottom flask (A) equipped with a magnetic stirring bar, ethylmagnesium bromide solution 3M (2.67 mL, 8 mmol) was added under nitrogen atmosphere and cooled until 0 ºC. In another oven-dried two-necked round-bottom flask (B) equipped with a magnetic stirring bar, pyrrole (0.56 mL, 8 mmol) was dissolved in 30 mL of anhydrous diethyl ether under nitrogen atmosphere. The pyrrole solution from flask B was added to flask A dropwise. The mixture was refluxed for 1 hour and cooled to 0 ºC. In a third dried two-necked round-bottom flask (C), 3,5-dichloro-2-methoxybenzoate **(4)** (1.70 g, 7.23 mmol) was dissolved in anhydrous diethyl ether (40 mL) under nitrogen atmosphere. The solution from flask C was then added dropwise to flask B. The resulting mixture was stirred for 20h. After cooling, the mixture was quenched with H_2SO_4 10% solution (40 mL) and stirred for 1 hour. Then, the reaction was extracted with diethyl acetate (3 x 150 mL) and the organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash column chromatography (*n*-hexane/ethyl acetate (9:1) as eluent). (3,5-dichloro-2-methoxyphenyl)(1H-pyrrol-2-yl)methanone **(3)** was obtained as a pale yellow solid (210 mg, 10%).

(3,5-dichloro-2-methoxyphenyl)(1H-pyrrol-2-yl)methanone (3): IR ν_{max} (cm^{-1}) (KBr): 3289, 3073, 2975, 2943, 1558, 1396, 1253, 1047, 998, 849, 746, 573; **^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):** 9.80 (s, 1H), 7.50 (d, *J* = 2.6 Hz, 1H), 7.33 (d, *J* = 2.6 Hz, 1H), 7.17 (td, *J* = 2.7, 1.3 Hz, 1H), 6.68 (ddd, *J* = 3.8, 2.4, 1.3 Hz, 1H), 6.31 (dt, *J* = 3.9, 2.5

Hz, 1H), 3.84 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm):** 181.55, 152.75, 135.62, 131.77, 131.49, 131.46, 128.94, 127.65, 126.63, 120.94, 111.55, 62.71.

4.2.5. Synthesis of (3,5-dichloro-2-hydroxyphenyl)(1H-pyrrol-2-yl)methanone (2)

In an oven-dried two-necked round-bottom flask equipped with a magnetic stirring bar (3,5-dichloro-2-methoxyphenyl)(1H-pyrrol-2-yl)methanone **(3)** (200 mg, 0.740 mmol) and $AlCl_3$ (568 mg, 4.44 mmol) were added under nitrogen atmosphere. DCM (18 mL) was added, and the solution was stirred at room temperature for 21 hours. Then, the reaction mixture was quenched with water (15 mL) and extracted with DCM (3 x 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash column chromatography (*n*-hexane/ethyl acetate/formic acid (95:5:0.02) as eluent). (3,5-dichloro-2-hydroxyphenyl)(1H-pyrrol-2-yl)methanone **(2)** was obtained as a yellow solid (140 mg, 75%).

(3,5-dichloro-2-hydroxyphenyl)(1H-pyrrol-2-yl)methanone (2): IR ν_{max} (cm^{-1}) (KBr): 3447, 3333, 2922, 2851, 1617, 1558, 1419, 1206, 1141, 1065, 784; **^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):** 12.25 (s, 1H), 9.65 (s, 1H), 7.95 (d, *J* = 2.5 Hz, 1H), 7.56 (d, *J* = 2.5 Hz, 1H), 7.22 (ddd, *J* = 3.0, 2.5, 1.2 Hz, 1H), 7.09 (ddd, *J* = 3.9, 2.5, 1.2 Hz, 1H), 6.44 (dt, *J* = 4.0, 2.5 Hz, 1H).

4.2.6. Synthesis of (4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone (1)

In an oven-dried two-necked round-bottom flask (A) equipped with a magnetic stirring bar (3,5-dichloro-2-hydroxyphenyl)(1H-pyrrol-2-yl)methanone **(2)** was dissolved in ACN (10 mL) under nitrogen atmosphere. The solution was cooled to -10 ºC. In another oven-dried two-necked round-bottom flask (B), N-chlorosuccinimide (146 mg, 1.09 mmol) was dissolved in acetonitrile (9 mL) under nitrogen atmosphere and added dropwise to flask A. The reaction mixture was stirred at -10 ºC for 5 minutes and then at room temperature for 19 hours. After this, the mixture was concentrated under reduced pressure and purified by flash column chromatography (*n*-hexane/ethyl acetate (85:15) as eluent). (4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone **(1)** was obtained as a yellow solid (116 mg, 65%).

(4,5-dichloro-1H-pyrrol-2-yl)(3,5-dichloro-2-hydroxyphenyl)methanone (1): IR ν_{max} (cm^{-1}) (KBr): 3432, 3267, 2919, 2850, 1611, 1578, 1273, 1238, 1172, 1101, 1033, 778,

669; **^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):** 13.00 (s, 1H), 11.84 (s, 1H), 7.75 (d, *J* = 2.8 Hz, 1H), 7.57 (s, 1H), 6.90 (s, 1H).

5. References

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