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# Addressing subphthalocyanines and subnaphthalocyanines features relevant to fluorescence imaging

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ABSTRACT

A series of new synthetic subphthalocyanines bear structural features aimed at allowing either fluorescence activation or a bathochromic shift of the absorption band towards the near-infrared window, relevant to optical imaging. X-ray diffraction studies of four subphthalocyanines are reported. Spectrofluorimetric studies on subnaphthalocyanines and activatable subphthalocyanine pro-fluorophores are reported.



## 1. Introduction

Subphthalocyanines (SubPcs) are tris-isoindolic phthalocyanine analogs that were reported as synthons for ring enlargement to afford A3B phthalocyanines.[1] Their fluorescence properties place them properly in the Lavis and Raines diagram.[2] Their concave shape and the axial substituent are useful to prevent aggregation, which made SubPc interesting fluorophores for biological studies.[3] We recently reported pH-sensitive SubPc absorbing in the 570 nm window.[3] Herein, we present in the first section of the study the synthesis of SubPc species with features relevant to fluorescence imaging studies (Fig. 1): a) range of substituents for future turn-ON fluorescence at various biological pH (probes **1**–**4**), b) substituents responsible for a bathochromic shift up to the near-infrared window, i.e. the "optical window" where biological tissues are most transparent (probes **5**–**7**).[4] A hanging function X is appended to the probe for subsequent conjugation to a biomolecule or another contrast agent or therapeutic agent (bi-modality/theranostic). Subsequent characterization and examination of the optical properties (spectrofluorimetry studies) are presented in the last two sections of the study.

## 2. Results and discussion

### 2.1. Syntheses of subphthalocyanine (SubPc)

#### 2.1.1. Activatable N-Alkylaminophenoxy subphthalocyanine (pro-fluorophores)

They were shown to be pH-sensitive and be of interest for fluorescence turn-ON at biological pH. The newer versions presented therein may afford pKa fine-tuning by adjusting the nature of the apical aniline as mentioned on other fluorescent dialkylated aminoplatforms[5] as follows: a) secondary and tertiary anilines have higher pKa than primary anilines, hence they will be more suitable for biology; b) *para* vs *meta* orientation may affect the outcome, not only because of the electronic effect of the O donor atom, but also because the meta orientation may get the donor Nitrogen atom closer to the fluorescent platform, hence facilitating the transfer; c) the lenght of the alkyl chains grafted on the aniline may also address the pKa. Herein, *N*-alkylaminophenoxy groups were introduced in apical position of the subphtalocyanine ring. Alkyl groups are either methyl or ethyl groups, leading to organosoluble SubPcs. Future studies in aqueous media will necessitate subsequent incorporation in liposomes. Two synthetic routes were examined to afford *N*-alkylaminophenoxy-substituted subphthalocyanines: direct alkylation of SubPc-$NH_2$ **8** or apical substitution of SubPc-Cl **9** with alkyl amine bearing phenol groups. The

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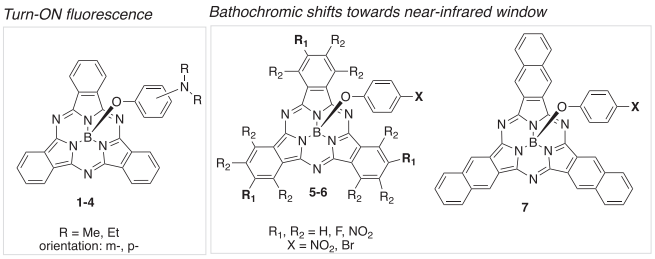


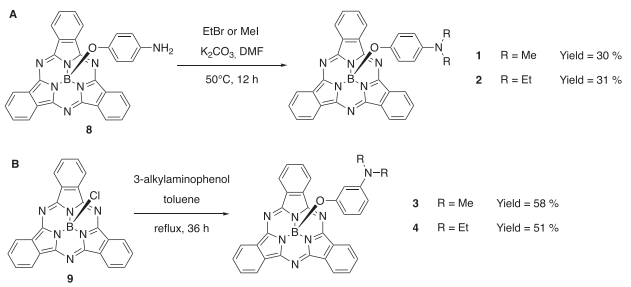

**Fig. 1.** Three structural modifications to adapt Subphthalocyanines to fluorescence imaging studies in biology by addressing: a) SubPc pKa and turn-ON fluorescence at various biological pHs, b) bathochromic shift to address the (near-infrared) "optical window", c) introduction of apical function **X** to future conjugation of other contrast agent or biomolecule.

first strategy allowed to get alkylated species **1** et **2** using two equiv. methyl iodide or ethylbromide in the presence of potassium carbonate in DMF (Scheme 1A). Low yields were obtained because the reactions also led to mono-alkylated products and more polar trialkylated/quaternized cationic, that were eliminated upon purification of silica column chromatography. In the second synthetic route no alkylation was achieved, commercially available *meta*-substituted phenols were used instead (Scheme 1B). The apical chlorine atom in compound **9** could be substituted with phenols using classical operating conditions. Such a reaction with meta-substituted phenols appeared to be quite long (48 h), and led to the SubPcs **3** and **4** in 58 and 51% yield, respectively.

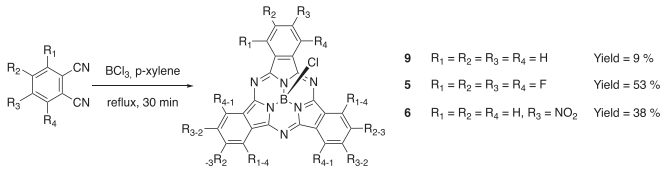
### 2.1.2. Bathochromically shifted subphthalocyanines

We recently studied subphthalocyanines bearing twelve H atoms (H12) at the peripheral isoindolic moieties, which absorb in the 570 nm window.[3] Although they fit well in the Lavis and Raynes diagram,[2] they still lie below the so-called "optical window".[4] Herein, a few examples report the appending of a series of electron-withdrawing nitro groups or fluorine atoms, or the extension of the conjugation on the SubPc ring to one more phenyl (Subnaphthalocyanine), which results in a *bathochromic shift* that becomes an asset for optical imaging in deeper tissues.

*2.1.2.1. SubPc with peripheral EW groups.* ***Cyclotrimerization*** reactions were achieved following a general procedure consisting of the stochiometric condensation of Boron trichloride (1 M in p-xylene) and dry phthalonitrile, according to a procedure reported by Claessens et al. (Scheme 2).[6] The reaction mixture was heated under refluxing conditions for 30 min under inert atmosphere. Solvent and excess $BCl_3$ were subsequently removed by evaporation, and the resulting residue was subjected to chromatography on silica. Three phthalonitriles were selected to examine this reaction:




**Scheme 1.** A: Methylation and ethylation of SubPc-$NH_2$ **8** to afford targets **1** and **2**. B: Syntheses of targets **3** and **4** upon substitution of the apical chlorine atom in compound **9**.




**Scheme 2.** Syntheses of subphthalocyanines bearing an apical chlorine atom, and various atoms/groups at the periphery: 12 Hydrogen atoms (**9**), twelve fluorine atomes (**5**), three nitro groups and nine Hydrogen atoms (**6**).

when tetrafluorophtalonitrile and 4-nitrophtalonitrile were used, subphtalocyanines **5** and **6** were obtained in satisfactory yields.[1,6] However, with non-substituted phthalonitrile, isolated yields in **9** was not greater than 9%, unlike the 82% reported yields.[6] We hypothesize that solubility issues may possibly explain such low yields: either the poor solubility of the starting phtalonitrile may not favor the reaction, or the poor solubility of subphtalocyanine **9** in common organic solvents (such as dichloromethane) may explain why its purification on column is so tricky and leads to severe loss of compound. Such a phenomenon is less an issue in the case of substituted subphtalocyanines **5** and **6**, that are much more soluble. Isolation of the subphthalocyanine by precipitation as reported in the literature, was not satisfactory in our hands.[6] Although the amount of isolated product is significantly higher, $^1$H NMR analysis indicates mild purity even after numerous washings. A careful analysis of $^1$H NMR and $^{19}$F NMR spectra was achieved to characterize compounds **5** and **6**, showing that they are free of starting phthalonitrile.

***Apical substitution*** of the chlorine atom could be achieved on compounds **9** and **5**, upon condensation of p-substituted phenol in excess (3.3 equiv., 4-bromophenol with **9**, 4-nitrophenol with **5**) under refluxing conditions in toluene until complete consumption of the starting phthalonitrile (CCM monitoring) (Scheme 3AB). Reaction times vary and appeared to be a function of both the electron density of the SubPc aromatic platform and the nature of the phenol. The yield of apical substitution was found to be 75% (**10**) and 71% (**11**).

***One pot cyclotrimerization + apical substitution*** Another and more straightforward method consists in the cyclo-trimerization of the subphthalocyanine ring immediately followed by evaporation of both solvents (*p*-xylene) and liquid reagents ($BCl_3$) in the reaction mixture without isolation of the chlorinated subphthalocyanine **9**. Both dry toluene and phenol reagent were subsequently added to the residue, and the resulting suspension was heated under reflux (Scheme 3B). The subphthalocyanine was subsequently purified by a) filtration on alumina plug (short column) that allowed the elimination of excess phenol, b) followed by chromatography column on silica, c) and recrystallization if necessary. The overall yield in compound **10** vary depending on the protocol: from 7% (two-steps procedure) to 26% (one-pot procedure). However, the overall yield droped significantly as the amount of starting phthalonitrile was raised, which hampered the larger-scale synthesis of subphthalocyanine synthons.

*2.1.2.2. Extended conjugation: subnaphthalocyanine (SubNc).* The « one-pot » general procedure described for synthons **10**–**11** was adapted to the synthesis of apical nitrophenoxy group bearing *naphthalocyanine* by replacing phthalonitrile for naphthalonitrile (Scheme 4). TLC analysis of the reaction mixture shows four blue spots, which were isolated by column chromatography on silica. Mass spectrometry analysis (MALDI-TOF) showed that one of these fractions corresponds to subnaphthalocyanine **7** that was obtained in a low 1.7% isolated yield (Note that the other two isolated