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Chemistry on Boranils: An Entry to Functionalized Fluorescent Dyes

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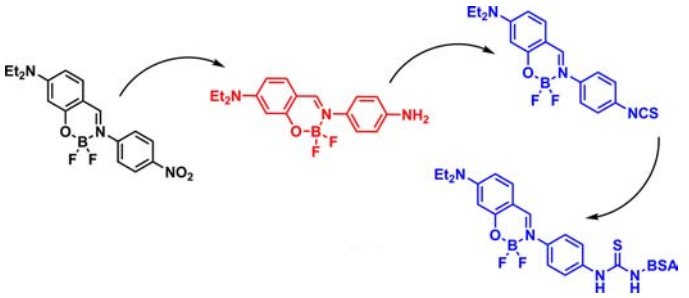
ABSTRACT



A Boranil fluorophore bearing a nitro-phenyl group has been selectively reduced to its anilino form and then successfully converted to amide, imine, urea, and thiourea derivatives which are fluorescent dyes. Its isolated isothiocyanate intermediate derivative was used in a model labeling experiment with Bovine Serum Albumin (BSA). The purified labeled-BSA exhibits strong luminescence (Φ_f = 47%) in a phosphate buffer at pH = 7.4.

There is currently a renewed interest in the field of dyes and fluorophores, propelled by a constant need for new candidates for organic optoelectronic devices (OLED, OPV),[1] molecular sensors,[2] and a parallel search for fluorophore probes for biological labeling.[3] There are currently many leading families in the fluorophore research area such as cyanines,[4] rhodamines,[5] squaraines,[6] and BODIPYs.[7] More recently, new fluorescent dyes based on borate complexes have been developed by using the boron atom as a structuring element to rigidify and flatten cyanine skeletons. Some new structures constructed from a central N−B−O[8] or N−B−N[9] pattern have generated optimism that a family of value comparable to that of BODIPY may be envisaged. We recently introduced a new family of fluorescent boron complexes, so-called Boranils, built around a N−B−O pattern and using salicylaldanilinimines.[10] These compounds are particularly appealing, due to their facile synthesis, potentially on a large scale, and the ease of their postsynthetic modifications. We describe here a particular procedure leading to a versatile amino-Boranil synthon suitable for further substitution introducing a variety of functional sites.

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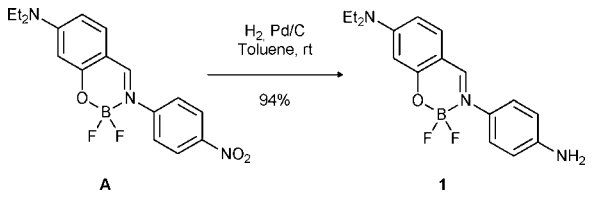
Scheme 1. Reduction of the Nitro-Boranil



Starting from the fluorescent Boranil compound **A**, we investigated its conversion into more useful derivatives. In contrast to known procedures where Boranil compounds were reduced *in situ* by borane to the corresponding aminophenol borate,[11] we were pleased to find that, with soft hydrogenation, we could obtain almost quantitatively the amino derivative **1** (Scheme 1) without reducing the imino fragment. This key synthon **1** was obtained by a standard reduction of the nitro parent under H_2 catalyzed by palladium on carbon at rt. It should be stressed that the Boranil core is stable enough to withstand these conditions. Neither the boron center nor the imine group was chemically reactive during the course of the reaction. This was shown by the retention of the characteristic imine proton multiplet at 8.36 ppm (reflecting ^{11}B coupling),[10] the triplet in the ^{11}B NMR spectrum at 0.84 ppm (J = 15.4 Hz), the presence of the chelated imine vibrational absorption at $\nu_{N=C}$ = 1620 cm^{-1}, and fluorescence properties in accordance with the other members of the Boranil family. Without formation of its boron complex, the nitrophenylsalicylaldimine present in **A** does not survive the reaction conditions, giving an intractable product mixture, illustrating the valuable protective function of B(III) against the imino and the phenol functions.

Using this versatile synthon, we were able to check the reactivity and stability of the Boranil chromophore in various types of reactions. Boranil **1** provides the amide derivatives **2** and **3** in good yields (70−85%) through peptidic coupling with both adipic acid and 3,4,5-trimethoxybenzoic acid, using DMAP and EDCI as coupling agents (Scheme 2). A diagnostic ν_{NHCO} band was found at 1664 cm^{-1} in the infrared spectra. Condensation with an aldehyde, such as dimethylaminosalicaldehyde in EtOH, afforded the corresponding salicylaldimine derivative **4** in fair yields (51%). This compound has a borate complex site and a free salicylaldimine chelating site, thus opening the way to supplementary complexation. Urea Boranil derivatives were prepared in a one-pot reaction. Initially, the amino group was transformed into an isocyanate by means of diphosgene in hot THF. No attack of the coordinated phenol was observed under these conditions providing again protection by borate complexation. Then, nucleophilic attack of an aliphatic (hexylamine) or aromatic amine (anisidine) provided the corresponding ureas **5**−**6** in fair yields (37% to 68%). Characteristic stretching vibrations were observed at ν_{NHCONH} = 1674 and 1705 cm^{-1}, respectively. Thiourea derivatives **7** can be obtained in good yield (89%) by the same procedure using thiophosgene. Significantly, the isothiocyanate intermediate **8** (ν_{NCS} = 2102 cm^{-1}) can be isolated (77% yield), thus opening the way to applications in biological materials labeling. The isolated compound **8** was used (Scheme 3) to synthethize thiourea **9** in good yield (73%), by reaction with hexylamine. All the compounds were unambiguously characterized by their ^1H and ^{13}C NMR spectra, mass spectra, and elemental analysis (see Supporting Information). Finally, we used **8** to label lysine residues of BSA (Bovine Serum Albumin) under conditions similar to those used with commercial isothiocyanate-functionalized dyes (i.e., FITC: fluorescein isothiocyanate).[12] The BSA was labeled with an excess of dye in basic aqueous DMSO and purified by use of a Sephadex G25 gel column eluted with PBS.

Table 1. Selected Optical Data Measured in Various Solvents

dye	λ_{abs} (nm)	ε ($M^{-1}\cdot cm^{-1}$)	λ_{em} (nm)	Δ_{ss} (cm^{-1})	$\varphi_f{}^a$ (%)	τ (ns)	solvent
1	405	48000	528	5800	2	0.21	DCM
2	407	56000	470	3300	7	0.22	DCM
3	405	54000	468	3300	4	0.20	DCM
5	405	46000	476	3700	4	0.20	DCM
6	404	53000	497	4600	3	0.18	DCM
7	406	58000	473	3500	8	0.27	DCM
8	414	71000	468	2800	16	0.49	DCM
9	405	56000	474	3600	7	0.37	toluene
9	403	56000	477	3900	8	0.26	THF
9	408	57000	474	3400	10	0.35	DCM

[a] Using quinine sulfate as reference Φ = 0.55 in H_2SO_4 1 N, λ_{ex} = 366 nm or Rhodamine 6G as reference, Φ = 0.88 in ethanol λ_{ex} = 488 nm.

All compounds exhibited absorption spectra typical of Boranil derivatives (Table 1), with a λ_{max} around 405−415 nm and a relatively high molar absorptivity (50000−70000 M^{-1} cm^{-1}).[10] The different substituents on the phenyl groups have negligible influence on the absorption spectra except in the case of the isothiocyanate (Figure 1) where bathochromic and hyperchromic effects were apparent, probably due to the electron-withdrawing character of this group, which would induce a stronger dipole moment and extended delocalization along the main molecular axis.

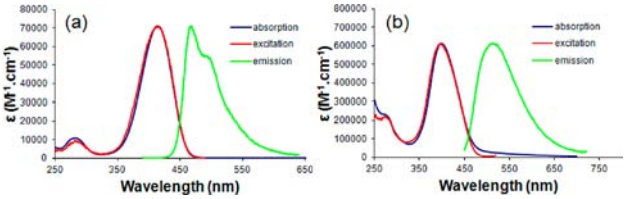


Figure 1. (a) Absorption, emission, and excitation spectra of Boranil-ITC **8** in dichloromethane at rt. (b) Absorption, emission, and excitation spectra of labeled BSA-**10** in PBS at pH = 7.4 and at rt. λ_{exc} = 380 nm.

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Scheme 2. Synthetic Transformations of Amino-Boranil **1**

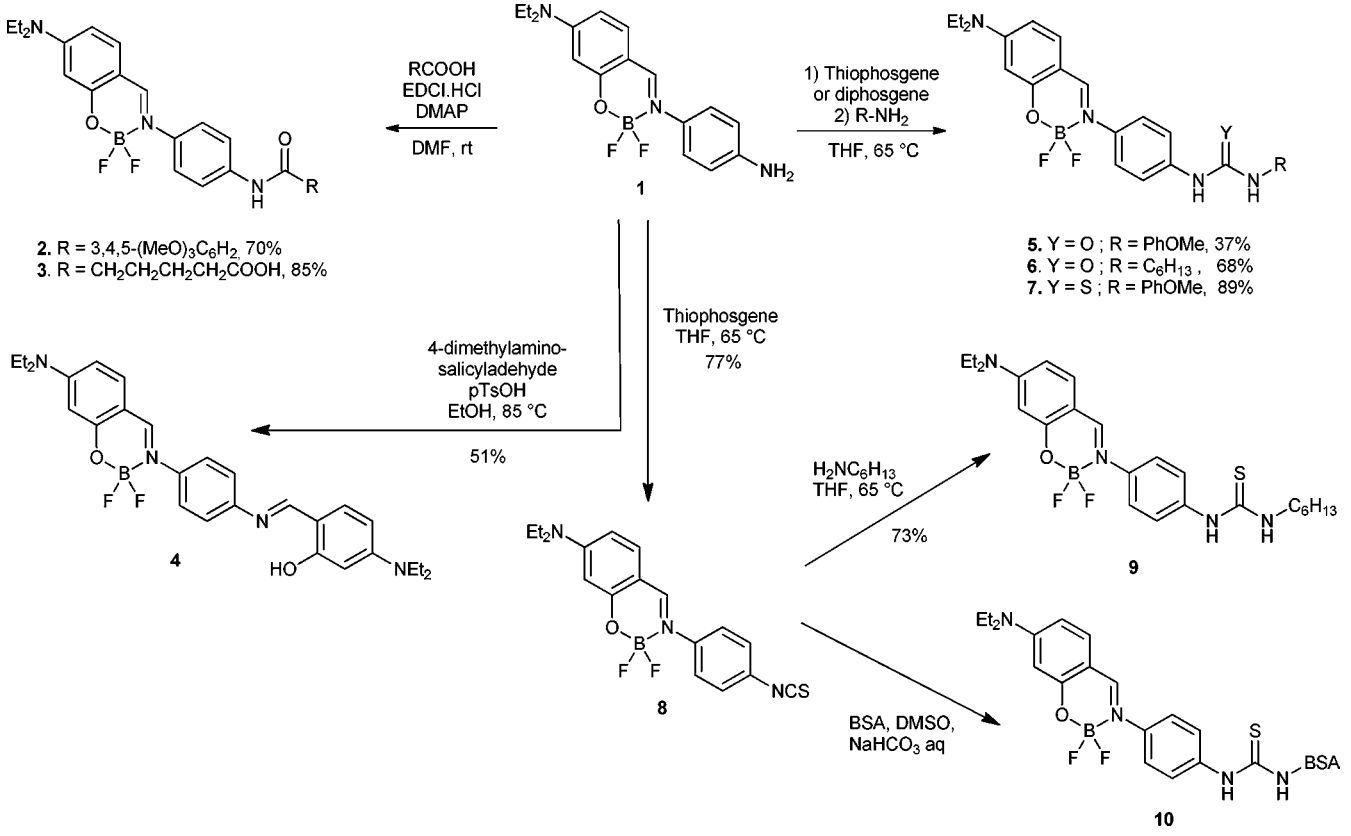

Table 2. Optical Data of Solution of **9** with Genuine BSA and of Labelled BSA-**10** in PBS Buffer at RT

dye	n_{eq} [b]	λ_{abs} (nm)	ε ($M^{-1}\cdot cm^{-1}$)	λ_{em} (nm)	Δ_{ss} (cm^{-1})	φ_f [a] (%)	τ (ns)
BSA	–	277	43000	–	–	–	–
BSA+	0.8	277	52000	–	–	–	0.91(59%)
9 (1 equiv)	0.9	399	33000	508	5400	4	3.35(41%)
BSA+	4.9	277	97000	–	–	–	0.74(75%)
9 (5 equiv)	5.0	397	181000	535	6500	2	3.12(25%)
BSA+	11.5	277	169000	–	–	–	0.68(79%)
9 (10 equiv)	10.7	397	384000	539	6600	2	3.06(21%)
10	17.0	277	230000	–	–	–	0.92(66%)
	17.1	399	615000	513	5600	47	3.14(34%)

[a] Using Rhodamine 6G as reference, $\Phi = 0.88$ in ethanol $\lambda_{ex} = 488$ nm. [b] Calculated number of *Boranil* dyes considering an average absorption coefficient for each *Boranil* of 11000 M^{-1} cm^{-1} at 277 nm and 36000 M^{-1} cm^{-1} at 397/399 nm.

All the dyes are fluorescent in dichloromethane with an emission at 470–500 nm and a large Stokes shift (ca. 3000–4000 cm^{-1}). The shape of the emission band with several shoulders on the low energy side and the nonmirror symmetry with the large absorption bands are suggestive of an intramolecular charge transfer (ICT) emissive state. However, the emission profile of Boranil **9** does not exhibit a structured emission and the emissive properties are not solvent dependent (Table 1). This suggests, as in the case for **10**, a weakly polarized excited state sensitive to intermolecular interactions. The emission lifetimes are short (ca. 200–500 ps), which confirms the high rate of nonradiative deactivation. To demonstrate the ability of boranil-isothiocyanate **8** to label biomolecules, we reacted it with BSA in aqueous DMSO containing $NaHCO_3$ (see Supporting Information). To compare the optical properties and estimate the ratio of labeling, we prepared several solutions by mixing BSA with compound **9**. Absorption measurements on these solutions provided molar absorption coefficients of 11000 M^{-1} cm^{-1} at 277 nm and 36000 M^{-1} cm^{-1} at 399 nm for **9** in PBS (Table 2, Figure 2). By subtracting the electronic absorption spectrum of BSA from that of **10**, and by comparing the ratio between the two bands, we estimated an average labeling ratio of 17 boranil cores per BSA protein. Unlabeled BSA protein has ca. 30 lysine residues.[13] An interesting comparison may be

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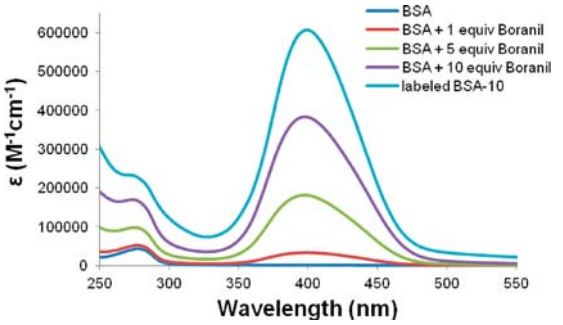

Figure 2. Absorption spectra of Bovine Serum Albumin labeled with Boranil **10**, and mixture of BSA with Boranil **9**, in PBS at pH = 7.4.

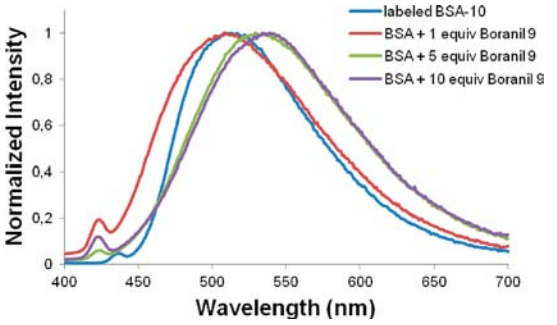

Figure 3. Emission spectra of Bovine Serum Albumin labeled with *Boranil* **10**, and mixture of BSA with *Boranil* **9**, in PBS at pH = 7.4. λ_{exc} = 370 nm for the *Boranil* **9** and λ_{exc} = 380 nm for the labeled BSA .

made of the emission spectrum of **10** with that of a simple mixture of dye **9** and BSA. For mixtures of **9** in various proportions with BSA, a weak emission is observed at 508 nm for the 1:1 mixture and at 535−539 nm for a larger amount of dye. The bathochromic shift of the emission compared to that of **9** in CH_2Cl_2 could be explained by a higher dipole moment of the solvent in the first case, concomitant with a probable surfactant action of BSA, avoiding aggregate formation. With a larger amount of dye, some emissive aggregates of **9** are probably formed, which explains the greater red shift of the emission (Figure 3) and also the presence of two decay times.

When several boranil dyes were covalently grafted onto BSA, via thiourea formation, the solution exhibited an intense fluorescence at 513 nm and a quantum yield of 47%. Notice that similar emission wavelength and excited state lifetimes are measured with respect to **9** in the presence of genuine BSA (Table 2). The bathochromic shift of 39 nm for **10** compared to **9** in organic solvents is likely due to the solvent polarity and protein environment which favors dye interaction. This clearly demonstrates that Boranil-ITC **8** is a good candidate for biological labeling. Moreover, despite its water insolubility, once grafted onto a protein, the luminescence is strongly enhanced. This is a clear indication that the Boranil dye is likely lying in hydrophobic pockets resulting from the folding of the protein after grafting.

In conclusion, we have been successful in the high yield reduction of nitrophenyl-Boranil to anilino-Boranil, thus opening an avenue to new useful derivatives. We demonstrate that imino and phenol functions coordinated to a boron center are protected against additional reactions. This strategy allowed preparation of various amide, imine, urea, and thiourea derivatives without decomplexation of a boron center. Isolation of the pivotal isothiocyanate derivative allowed us to label a model protein and to observe strong fluorescence in an aqueous solution. Further studies are currently underway to solubilize these dyes in water and extend their emission to the red part of the electromagnetic spectrum.

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Supporting Information Available. Synthetic procedures and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.