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### ► To cite this version:

Blaise Gatin-Fraudet, Mathilde Pucher, Thomas Le Saux, Gilles Doisneau, Yann Bourdreux, et al.. Hydrogen peroxide-responsive triggers based on borinic acids: molecular insights into the control of oxidative rearrangement. *Chemistry - A European Journal*, In press, 10.1002/chem.202201543 . hal-03752143

**HAL Id: hal-03752143**

**<https://hal.science/hal-03752143v1>**

Submitted on 16 Aug 2022

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# Hydrogen peroxide-responsive triggers based on borinic acids: molecular insights into the control of oxidative rearrangement.

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**Abstract:** Arylborinic acids represent new, efficient, and underexplored hydrogen peroxide-responsive triggers. In contrast to boronic acids, two concomitant oxidative rearrangements are involved in the complete oxidation of these species, which might represent a major limitation for an efficient effector (drug or fluorophore) release. Herein, we describe a comprehensive study of H<sub>2</sub>O<sub>2</sub>-mediated unsymmetrical arylborinic acid oxidation to investigate the factors that could selectively guide their oxidative rearrangement. The *o*-CF<sub>3</sub> substituent was found to be an excellent directing group since complete regioselectivity was obtained on borinic acid models. This result was successfully applied to synthesizing new borinic acid-based fluorogenic probes, which exclusively release the fluorescent moiety upon H<sub>2</sub>O<sub>2</sub> treatment. These compounds maintained their superior kinetic properties compared to boronic acids, thus further enhancing the potential of arylborinic acids as valuable new H<sub>2</sub>O<sub>2</sub>-sensitive triggers.

## Introduction

Organoborons are versatile organic chemistry reagents which are involved in a broad range of chemical transformations.<sup>[1]</sup> More specifically, borinic acids are molecules in which the boron atom is linked to two atoms of carbon and one atom of oxygen and have been less explored than boronic acids. Borinic acids have emerged as interesting new synthons in organic chemistry and have proven relevant in transition-metal-catalyzed cross-coupling.<sup>[2]</sup> They have also recently received significant attention in organic chemistry as Lewis acid catalysts.<sup>[3]</sup> For instance, borinic acids displayed a higher reactivity than the corresponding boronic acids in Oppenauer oxidation,<sup>[4]</sup> Mukaiyama aldol reaction,<sup>[5]</sup> and dipeptide synthesis<sup>[6]</sup>. In addition, borinic acids and *N,O*-chelate stabilized esters emerged as valuable catalysts to efficiently promote numerous transformations including regioselective mono-functionalization of vicinal diols using acylation, sulfonylation or alkylation reactions,<sup>[7]</sup> epoxide ring-opening,<sup>[8]</sup> selective glycosylation reaction,<sup>[9]</sup> and C-H activation.<sup>[10]</sup> In most cases, the mechanisms involve the reversible formation of a tetracoordinate boron-ate complex as a key intermediate. Borinic acids have also been used as precursors of diarylhydroborane species to promote the reduction of carbonyl species, phosphine oxides, sulfoxides, and *N*-oxide derivatives in the presence of silane.<sup>[11]</sup> Despite their broad fields of application, studies on their reactivity toward oxidants are surprisingly limited in sharp contrast to boronic acids and esters.<sup>[12]</sup> Thus, we<sup>[13]</sup> and Stains<sup>[14]</sup> showed that H<sub>2</sub>O<sub>2</sub>-mediated oxidation of arylborinic acids is dramatically faster than the commonly used boronic acid trigger, making them new powerful tools in the field of hydrogen peroxide responsive organoboron triggers.

The main straightforward route to access borinic acids relies on the addition of an organolithium or organomagnesium reagent onto boron-based electrophiles such as trialkylborates, boron halides, diborane or boronate esters.<sup>[15]</sup> However, these approaches are not versatile and require either cryogenic conditions or often suffer from a lack of compatibility with functional groups onto the aromatic halide used to prepare the organometallic agent. To circumvent these difficulties, the Pucheault group developed an elegant procedure using diisopropylaminoborane as a water and air-stable borylating reagent.<sup>[16]</sup> Thus, reaction with organomagnesium derivatives affords symmetrical or unsymmetrical borinic acids in high yield without flash chromatography purification. In 2017, Chatani *and coll.* reported an intramolecular palladium-catalyzed borylation of dihalides using diisopropylaminoborane as a boron source providing cyclic borinic acids.<sup>[17]</sup> Switching to less bulky aminoboranes such as diethylaminoborane allowed extending this double B-arylation reaction to an intermolecular

version.<sup>[18]</sup> Despite these new synthetic approaches, the synthesis of unsymmetrical arylborinic acids has not been widely reported.<sup>[15a,15c,16a,17,18]</sup>

Our group has recently been developing borinic acid-based sensors as a new powerful tool for hydrogen peroxide detection. In contrast to boronic acids, oxidation of borinic acids can trigger two different oxidative rearrangements that result in two possible pairs of compounds. A single couple of products is obtained for symmetrical borinic acids, while unsymmetrical borinic acids could give a mixture of two different couples of compounds depending on the respective properties of the two aryl moieties. As a result, the selectivity reaction needs to be controlled, and determining the factors governing the selectivity of the unsymmetrical borinic acid oxidation can improve the efficiency of the release of an effector (drug or fluorophore). To our knowledge, no study has been conducted to investigate the particular impact of this dissymmetry on reactivity.

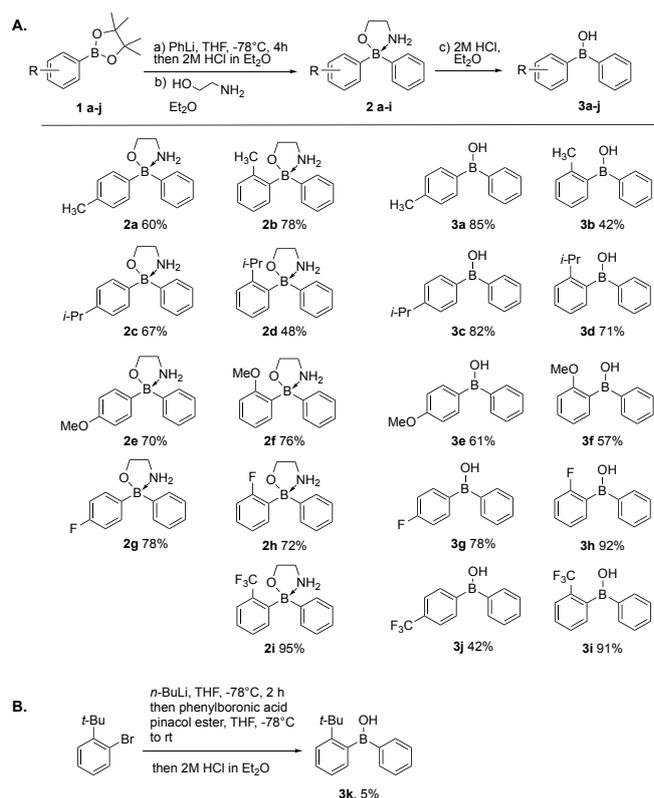
Herein, we report, for the first time, a comprehensive mechanistic study of unsymmetrical diarylborinic acids oxidation. We envisioned that regioselective oxidation could be achieved by fine-tuning electronic and/or steric effects onto the aryl unit. We describe the synthesis of various model unsymmetrical diarylborinic acids and study their oxidative rearrangement upon exposure to H<sub>2</sub>O<sub>2</sub> in an aqueous medium using <sup>1</sup>H NMR spectroscopy. This study allowed the rationalization of the impact of aryl substituents on the oxidation selectivity. These results were applied to the synthesis of coumarinborinic-based probes that were capable of releasing the fluorophore with complete control of the oxidative rearrangement upon hydrogen peroxide treatment.

## Results and Discussion

### Synthesis of unsymmetrical diarylborinic acids.

To gain information on electronic and steric effects, we prepared a collection of variously substituted arylborinic acids. The synthesis of unsymmetrical borinic acids relies on the nucleophilic addition of phenyllithium onto commercially available arylboronic acid pinacol ester.<sup>[15c]</sup> Borinic acids often proved difficult to isolate in good yield by flash column chromatography. Nevertheless, they can be isolated in a stable form by precipitation upon treatment with ethanolamine. This ligand can be easily removed in acidic conditions furnishing the expected borinic acid functionality (Scheme 1A).

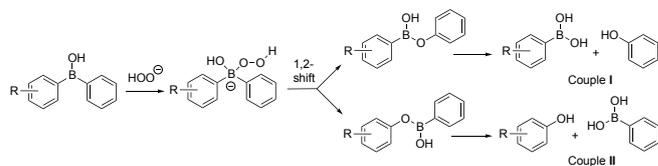
Thus, boroxazolidines **2a-2i** possessing substituents with different electronic and steric properties (alkyl, methoxy, fluoro, trifluoromethyl) and substitution patterns (*para* or *ortho* substitution) were isolated by precipitation in satisfactory to good yields ranging from 48 to 95% (Scheme 1A). Chemical shifts of boron atom at around 6 ppm using <sup>11</sup>B NMR spectroscopy are consistent with these *O,N*-chelated diarylborinates.<sup>[19]</sup> Acidic conditions using a 2M aqueous hydrochloric acid solution in diethyl ether afforded the corresponding borinic acids **3a-3i** isolated in moderate to high yield ranging from 42 to 92%. Surprisingly, compound **3j** did not require aminoethanol protection and was purified by flash column chromatography directly upon addition of phenyllithium in 42% yield (Scheme 1A). The reverse strategy involving the reaction of *tert*-butylphenyllithium with phenylboronic acid pinacol ester afforded **3k**, isolated by semi-preparative C18-reverse phase HPLC in a low yield (Scheme 1B).<sup>[20]</sup> In addition, in our hands, all attempts to obtain nitro-substituted phenylborinic acids were unsuccessful. For each borinic acid compound, <sup>11</sup>B NMR analyses showed the presence of a single peak with a resonance at 45-46 ppm, in agreement with an sp<sup>2</sup>-hybridized boron atom.<sup>[19]</sup>



**Scheme 1. Synthesis of boronic acids 3a-k.** **A.** Conditions: a) 1.9M PhLi in *n*-Bu<sub>2</sub>O (1.1 equiv), THF, -78°C, 4 h then 2M HCl in Et<sub>2</sub>O (9 equiv), rt, 1 h; b) ethanolamine (1.2 equiv), Et<sub>2</sub>O, rt, overnight; c) aq. 2M HCl (6 equiv), Et<sub>2</sub>O. **B.** Conditions: *n*-BuLi (1.1 equiv), THF, -78°C, 2 h then phenylboronic acid pinacol ester (1.1 equiv), THF, -78°C to rt, 4 h then 2M HCl in Et<sub>2</sub>O (9 equiv), rt, 1 h.

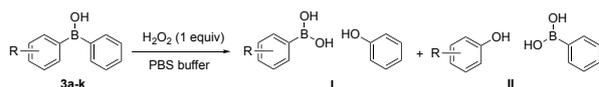
### Regioselectivity of H<sub>2</sub>O<sub>2</sub>-mediated oxidation of unsymmetrical diarylboronic acids.

With these synthesized compounds in hands, we then examined the influence of different substituents on product distribution, which is indicative of the regioselectivity of the H<sub>2</sub>O<sub>2</sub>-promoted oxidation reaction. The mechanism of oxidation is similar to that admitted for boronic acids. Upon addition of hydroperoxide anion onto the sp<sup>2</sup>-hybridized boron atom, the resulting tetracoordinate complex decomposes through a 1,2-shift aryl migration with a concomitant oxygen-oxygen bond cleavage. The resulting unstable boronic ester intermediate spontaneously hydrolyzes into a phenol/boronic acid couple. Unlike boronic acids, the oxidation of unsymmetrical boronic acids possibly affords either the phenol/substituted arylboronic acid couple **I** or the substituted phenol/phenylboronic acid couple **II** since the migration of either of the aryl groups could occur (Scheme 2).



**Scheme 2. Mechanism of H<sub>2</sub>O<sub>2</sub>-mediated oxidation of unsymmetrical boronic acids.**

The results of the H<sub>2</sub>O<sub>2</sub>-promoted oxidation reaction of the various unsymmetrical boronic acids are detailed in Table 1. The distribution of the two couples **I** and **II** was determined using <sup>1</sup>H NMR spectroscopy by integration of either aromatic or methyl protons.



**Table 1.** Selectivity of the unsymmetrical borinic acids oxidation reaction.

Entry	Starting substrate	Ratio of I <sup>[a]</sup>	Ratio of II <sup>[a]</sup>
1	<b>3a</b> : R = <i>p</i> -Me	38	62
2	<b>3b</b> : R = <i>o</i> -Me	60	40
3	<b>3c</b> : R = <i>p</i> - <i>i</i> Pr	36	64
4	<b>3d</b> : R = <i>o</i> - <i>i</i> Pr	65	35
5	<b>3e</b> : R = <i>p</i> -OMe	35	65
6	<b>3f</b> : R = <i>o</i> -OMe	54	46
7	<b>3g</b> : R = <i>p</i> -F	67	33
8	<b>3h</b> : R = <i>o</i> -F	90	10
9	<b>3j</b> : R = <i>p</i> -CF <sub>3</sub>	89	11
10	<b>3i</b> : R = <i>o</i> -CF <sub>3</sub>	100	0
11	<b>3k</b> : R = <i>o</i> - <i>t</i> Bu	100	0 <sup>[b]</sup>

[a] ratio determined by <sup>1</sup>H NMR spectroscopy within the NMR detection limit.

[b] the reaction was complete within 7 hours.

Interestingly, we found that the oxidation of all borinic acids is complete within a two-minute time-scale upon the addition of one equivalent of hydrogen peroxide, whatever the nature of the substituent (electron-donating or withdrawing), except for **3k**. In this case, a prolonged reaction time of 7 hours is required for completion. We suppose that this slower reactivity may be attributed to the steric hindrance imposed by the *ortho*-positioned *tert*-butyl group, thus hampering the approach of hydroperoxide anion onto the boron atom<sup>[21]</sup> and/or slowing down the subsequent aryl migration.

Remarkably, the selectivity of the oxidative rearrangement was highly dependent on the electronic nature of the aromatic ring substituent. Thus, the reaction involving *para*-substituted electron-donating group, i.e., methyl, *iso*-propyl, and methoxy, afforded preferentially the phenylboronic acid/substituted phenol couple **II** (entries 1, 3 and 5, Table 1) in approximately the same ratio (around 60/40 in favor of couple **II**). In contrast, *para*-electron-withdrawing groups, i.e., fluoro and trifluoromethyl, gave preferentially the opposite couple **I** (entries 7 and 9, Table 1).<sup>[22]</sup> Thus, introducing the *p*-trifluoromethyl group, the most electrophilic substituent, yields 89% of the couple **I** (entry 9, Table 1). These results reveal that the 1,2-shift migration of the most electron-poor aryl groups of *para*-substituted diarylborinic acid is unfavored during this process. Moreover, we observed a good correlation between the sigma-Hammett value of the substituents and the 1,2-shift migration of the substituted phenyl group: the higher is the sigma-Hammett value of the substituent, the lower is the 1,2-shift migration of the substituted phenyl group. In addition, we observed the same trend for *ortho*-substituted derivatives. For instance, switching from *o*-methoxy to *o*-fluoro increased the yield of the couple **I** (54 vs 90, entries 6 and 8, Table 1), indicating that the 1,2-migration of electron-poor aryl groups is disfavored.

In addition to the electronic effect, we also observed a significant steric effect with the *ortho*-substituted derivatives. Indeed, in all cases, the presence of *ortho*-substituents significantly reduced the migration of the bulky aryl group leading preferentially to the couple **I** (comparison between entries 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, Table 1). To determine the contribution of the electronic or steric effects of the substituent on the regioselectivity during oxidation of *ortho*-substituted diarylborinic acids, we compared the results for compounds **3d** and **3i**. A lower selectivity was observed for *iso*-propyl substituted borinic acid **3d** than the trifluoromethyl derivative **3i** (65/35 vs 100/0, entries 4 and 10, Table 1). This result highlights that the electronic effect contributes largely to the regioselectivity of the oxidative rearrangement process since the *iso*-propyl group is considered to be similar in bulk to the trifluoromethyl substituent.<sup>[23]</sup> Combining electronic and steric effects using an *ortho*-CF<sub>3</sub>-phenyl group allowed us to get the couple **I** exclusively (entry 10, Table 1).

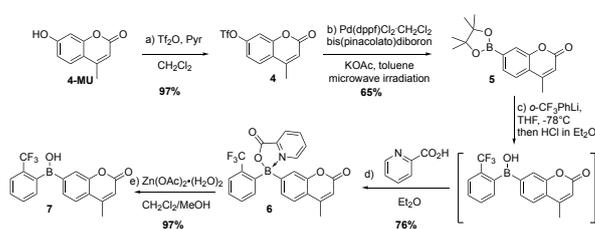
For comparison, we then investigated the *ipso*-hydroxylation reaction of electron-donating (i.e. methoxy) or withdrawing (i.e. trifluoromethyl) substituted phenylboronic acids under the same conditions (see Figure S4, *SI Appendix*). It is important to note that the reaction proceeded smoothly to afford the corresponding substituted phenols with a much slower oxidation rate than borinic acids, as we have previously observed.<sup>[13]</sup> In addition, only 10% of the *ortho*-

(trifluoromethyl)phenylboronic acid was converted to phenol within one hour, while the reaction required 35 minutes to reach completion with its *para*-isomer. These results support a slow 1,2-migration of the *o*-CF<sub>3</sub>-phenyl group from boron atom to oxygen atom, which could explain the total selectivity observed during oxidation of **3i**.

This study reveals that the presence of an *ortho*-hindered electron-withdrawing substituent on the phenyl group next to the boron atom seems crucial to fully control the selectivity of unsymmetrical borinic acid oxidation reaction. Finally, we found that the *ortho*-(trifluoromethyl)phenyl group is the most efficient group to exclusively furnish the phenol/(trifluoromethyl)phenylboronic acid couple.

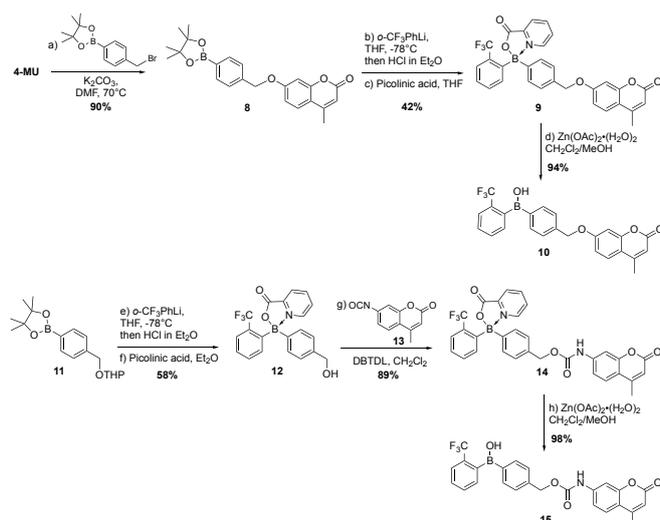
### Design and syntheses of optimized fluorogenic coumarin borinic acid-based probes.

Based on these observations, we undertook the synthesis of second-generation hydrogen peroxide sensors, in continuation of our work. Oxidation of our previous borinic acid probe, containing a phenyl moiety and a 4-methylcoumarin unit as a fluorogenic sensor, provided a mixture of two different oxidative rearrangements affording only 20% of fluorophore release. To circumvent this limitation, we therefore envisioned replacing the phenyl moiety with an *ortho*-(trifluoromethyl)phenyl group to improve the release of the fluorescent 4-methylumbelliferone for optimized H<sub>2</sub>O<sub>2</sub>-detection.<sup>[24]</sup> We first focused on the synthesis of a new probe in which the boron atom is directly linked to this motif and 4-methylcoumarin as a fluorogenic core. The synthetic pathway involving a four-step sequence started from treatment of 4-methylumbelliferone (4-MU) with trifluoromethanesulfonyl anhydride providing the corresponding triflate **4** in 97% yield (Scheme 3). This compound was then subjected to a palladium-catalyzed borylation with bis(pinacolato)diboron under microwave irradiation<sup>[25]</sup> to furnish boronic acid pinacol ester **5** in 65% yield. Addition of *ortho*-(trifluoromethyl)phenyllithium, resulting from the reaction of 2-bromobenzotrifluoride with *sec*-butyllithium, followed by acidic hydrolysis provided the borinic acid transiently. Treatment with 2-aminoethanol did not provide the corresponding *O,N*-chelated borinate in a solid form. We therefore evaluated picolinic acid ligand<sup>[26]</sup> as an alternative, a more acid-stable protecting group. We were pleased to observe that the corresponding borinate **6** was stable under flash column chromatography conditions and was isolated in 76% yield over the 2 steps. Displacement of the picolinic acid ligand was performed using zinc(II) acetate dihydrate<sup>[27]</sup> in CH<sub>2</sub>Cl<sub>2</sub>/MeOH, affording the expected compound **7** in 97% yield.



**Scheme 3. Synthesis of a new borinic acid-based sensor 7.** Conditions: a) Tf<sub>2</sub>O (1.1 equiv), Pyr (2.2 equiv), CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h, 97%; b) bis(pinacolato)diboron (1.2 equiv), KOAc (1.5 equiv), Pd(dppf)Cl<sub>2</sub>·CH<sub>2</sub>Cl<sub>2</sub> (10 mol%), toluene, microwave irradiation, 120°C, 3 h, 65%; c) *o*-CF<sub>3</sub>PhBr (1.2 equiv), *sec*-BuLi (c 1.4M in cyclohexane, 1.8 equiv), -78°C, 4 h, then HCl in Et<sub>2</sub>O (c 2M, 7 equiv), -78°C to rt; d) picolinic acid (1.2 equiv), Et<sub>2</sub>O, rt, 12 h, 76% over 2 steps; e) Zn(OAc)<sub>2</sub>·(H<sub>2</sub>O)<sub>2</sub> (1.5 equiv), CH<sub>2</sub>Cl<sub>2</sub>/MeOH, rt, overnight, 97%.

According to the above approach, the synthetic pathway should be optimized to access each sensor. Therefore, we also considered a synthetically more flexible route allowing a simplified conjugation of various fluorophores. Introducing a self-immolative spacer (SIS) between the trigger and the effector could actually represent an interesting alternative.<sup>[28]</sup> This approach combines several advantages, including the incorporation of the functionalized fluorophore in the final stages of synthesis, allowing rapid access to various fluorogenic probes, limiting side-reactions onto the reporter, thus potentially affording an easier synthetic route. Hence, we envisioned grafting 4-methylcoumarin onto a most commonly used *para*-substituted benzyl core through either an ether or a carbamate linker. The optimized syntheses of the two SIS-based probes are described in Scheme 4.

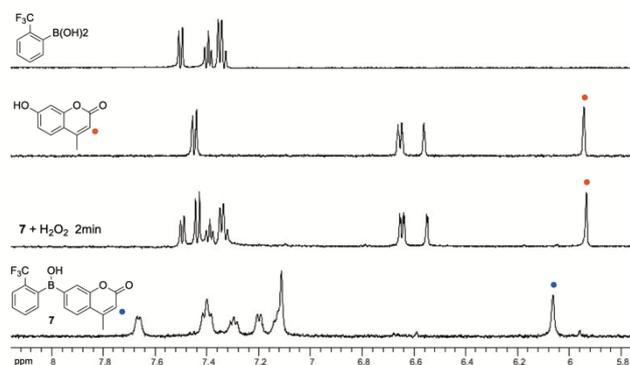


**Scheme 4. Syntheses of SIS borinic acid-based sensors.** Conditions: a) 4-bromomethylphenylboronic acid pinacol ester (1.4 equiv),  $K_2CO_3$  (1.2 equiv), DMF,  $70^\circ C$ , 3 h, 90%; b) *o*- $CF_3$ PhBr (1.4 equiv), *sec*-BuLi (c 1.4M in cyclohexane, 2.1 equiv),  $-78^\circ C$ , 4 h then HCl in  $Et_2O$  (c 2M, 7 equiv),  $-78^\circ C$  to rt; c) picolinic acid (1.2 equiv), THF, rt, 12 h, 42% over 2 steps; d)  $Zn(OAc)_2 \cdot (H_2O)_2$  (1.5 equiv),  $CH_2Cl_2/MeOH$ , rt, 94%; e) *o*- $CF_3$ PhBr (1.4 equiv), *sec*-BuLi (c 1.4M in cyclohexane, 2.1 equiv),  $-78^\circ C$ , 4 h then HCl in  $Et_2O$  (c 2M, 7 equiv),  $-78^\circ C$  to rt; f) picolinic acid (1.2 equiv), THF, rt, 12 h, 58% over 2 steps; g) **13** (1.8 equiv), DBTDL (15 mol%), MS 4Å,  $CH_2Cl_2$ , rt overnight then  $30^\circ C$  for 2 h, 89%; h)  $Zn(OAc)_2 \cdot (H_2O)_2$  (1.5 equiv),  $CH_2Cl_2/MeOH$ , rt, 5 h, 98%.

The key step of both syntheses relies on the addition of *ortho*-(trifluoromethyl)phenyllithium onto boronic acid pinacol esters **8** and **11** (see *SI Appendix* for their syntheses). A subsequent treatment with picolinic acid provided the expected *O,N*-chelated borinates **9** and **12**, which were isolated in 42% and 58% yield, respectively. Considering that the corresponding THP-protected alcohol derivative of compound **12** was isolated in 32% yield, the addition of organolithium species onto boron atom of less functionalized substrates resulted in a higher yield (90% vs 42%). The reaction of **12** with a slight excess of 4-MU-derived isocyanate **13**, in the presence of dibutyltin dilaurate (DBTDL),<sup>[29]</sup> afforded the expected carbamate derivative **14** in 89% yield. Displacement of picolinic acid ligand was carried out by reaction with zinc(II) acetate dihydrate<sup>[27]</sup> to afford the two desired fluorogenic probes **10** and **15** in nearly quantitative yield.

### *In vitro* $H_2O_2$ -mediated oxidation of borinic acid triggers.

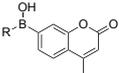
The selectivity of the oxidative rearrangement of **7** was first examined using  $^1H$  NMR spectroscopy. Upon  $H_2O_2$  exposure, the vinylic proton of coumarin (blue dot) disappeared after 2 minutes, confirming the complete consumption of the arylborinic acid **7**. The resulting spectrum showed exclusively the formation of the desired product pair, i.e., the fluorophore 4-MU and *o*-(trifluoromethyl)phenylboronic acid, as expected (Figure 1). The couple 4-methylcoumarinboronic acid and *o*-(trifluoromethyl)phenol was not observed within the  $^1H$  NMR detection limit (Figure S3, *SI Appendix*).



**Figure 1.** Regioselectivity of the oxidation of probe **7** promoted by  $H_2O_2$  (1 equiv) determined by  $^1H$  NMR spectroscopy (600 MHz) in deuterated PBS buffer (pH 7.4, 10%  $EtOH-d_6$ ).

This result confirms the efficiency of the trifluoromethylphenyl group in selectively orienting the oxidative rearrangement. The reaction rate constant was also determined by real-time measurement of the fluorescence emission of the released 4-methylumbelliferone and measured to be  $3.2 \times 10^3 \text{ M}^{-1} \cdot \text{s}^{-1}$  at 293 K (entry 3, Table 2), which is slightly lower than for the corresponding unsubstituted phenyl-core determined in our previous work (entry 2, Table 2).<sup>[13]</sup> However, the reactivity of probe **7** still remains much higher than the reactivity of 4-methylcoumarinboronic acid (comparison between entries 1 and 3, Table 2).

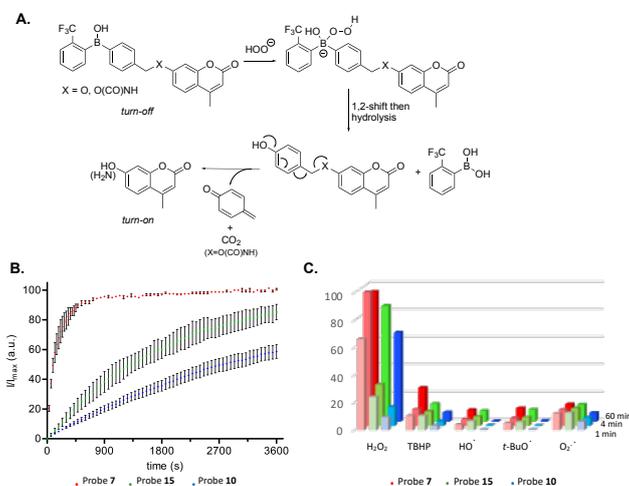
**Table 2. Features of coumarin-based probes.**

Entry	Fluorogenic probe	Regioselectivity of rearrangement		pKa (at 293 K) [a]	k (at 293 K) $\text{M}^{-1} \cdot \text{s}^{-1}$
		Ratio of I <sup>b</sup>	Ratio of II <sup>b</sup>		
1	R = OH	-	-	$7.4 \pm 0.1$ [13]	$1.8$ [13]
2	R = Ph	20	80	$5.3 \pm 0.1$ [13]	$1.9 \times 10^4$ [13]
3	R = <i>o</i> -CF <sub>3</sub> Ph	100	0	$6.0 \pm 0.1$	$3.2 \times 10^3$

[a] Determined in Britton-Robinson buffer 1X

[b] Couple I: 4-MU/*o*-CF<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>B(OH)<sub>2</sub>; Couple II: 4-methylcoumarinboronic acid/*o*-CF<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>OH

We then briefly studied the oxidation rates of the two SIS-containing fluorogenic probes **10** and **15**. From a mechanistic perspective, H<sub>2</sub>O<sub>2</sub>-urea-promoted oxidation generates a phenol, which spontaneously undergoes a fragmentation process through a 1,6-benzyl elimination resulting in the release of a quinone methide together with (in the case of probe **15**) or without (in the case of probe **10**) a decarboxylation process, to finally elicit *turn-on* fluorescence (Figure 2A).



**Figure 2. A. Mechanism of H<sub>2</sub>O<sub>2</sub>-mediated SIS-based boronic acids. B. Time-dependent fluorescence release of fluorogenic probes **7**, **10** and **15** (5  $\mu\text{M}$ ) upon addition of H<sub>2</sub>O<sub>2</sub> (100  $\mu\text{M}$ ). Data were recorded in PBS buffer 1X (pH 7.4, 0.02% DMSO) at 310 K, at  $\lambda_{\text{em}} = 450 \text{ nm}$  ( $\lambda_{\text{ex}} = 360 \text{ nm}$ ). Experiments were performed in duplicate and the results were averaged. C. Selectivity of probes **7**, **10** and **15** toward various reactive oxygen species (ROS). Fluorescence emission intensity of 5  $\mu\text{M}$  probe to 100  $\mu\text{M}$  of various ROS in PBS buffer 1X (pH 7.4) was recorded at  $\lambda_{\text{em}} = 450 \text{ nm}$  ( $\lambda_{\text{ex}} = 360 \text{ nm}$ ) at 1, 4 and 60 min at 25°C. Experiments were performed in triplicate and the results were averaged. H<sub>2</sub>O<sub>2</sub> and *tert*-butyl hydroperoxide (TBHP) were delivered from solid H<sub>2</sub>O<sub>2</sub>.urea and from 70% aqueous solution, respectively. Hydroxyl radical (HO $\cdot$ ) and *tert*-butoxy radical (*t*-BuO $\cdot$ ) were generated by the Fenton reaction of 2.5 mM Fe<sup>2+</sup> with 100  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> or 100  $\mu\text{M}$  TBHP, respectively. Superoxide (O<sub>2</sub> $\cdot^-$ ) was produced from xanthine/xanthine oxidase system.**

The different fluorescence time-courses of probes **10** and **15** oxidations were recorded at  $\lambda_{\text{em}} = 450 \text{ nm}$  ( $\lambda_{\text{ex}} = 360 \text{ nm}$ ) and compared to the directly linked fluorogenic probe **7** (Figure 2B). We observed that, upon H<sub>2</sub>O<sub>2</sub> treatment of **7**, the fluorescence increased rapidly to reach a value corresponding to a 5  $\mu\text{M}$  solution of 4-MU, therefore revealing a

quantitative release of the effector. Under similar conditions, the release of the fluorophore from the carbamate-linked probe was faster than the ether-linked analog, which is in accordance with the literature.<sup>[30]</sup> However, the fluorescence emissions for both compounds increased more slowly than the oxidation of **7**, probably due to the additional auto-immolation step.<sup>[31]</sup> With regard to these results, we did not determine accurate kinetic constants for these two last probes.

Finally, the selectivity of the three fluorogenic probes toward several reactive oxygen species (ROS) was examined (Figure 2C). In good agreement with our previously reported borinic-based probe, a selective response of the trigger toward H<sub>2</sub>O<sub>2</sub> was observed compared to various ROS such as *tert*-butyl hydroperoxide (TBHP), superoxide radical anion or hydroxyl and *tert*-butyl radical for each compound. Thus, these results confirm that arylborinic acids are valuable H<sub>2</sub>O<sub>2</sub>-sensitive triggers and that the *ortho*-trifluoromethyl substituent does not alter selectivity toward hydrogen peroxide.

## Conclusion

In summary, we demonstrated that H<sub>2</sub>O<sub>2</sub>-promoted oxidation of unsymmetrical diarylborinic acids, which was optimized on models, can be finely tuned by substitutions on the phenyl core. We show that the selectivity of the oxidative rearrangement was mainly controlled by electronic rather than steric factors. Combining these two effects, we identified that an *ortho*-trifluoromethyl substituent on the aromatic ring oriented the oxidative rearrangement with complete selectivity. We then applied this result to design a second generation of fluorogenic sensors for hydrogen peroxide, including two self-immolative-spacer-based probes. We observed that the SIS-based probes were less efficient in the release of the fluorophore at a short time scale than the directly linked probe. Since spectral properties of 4-methylumbelliferrone are not optimal for hydrogen peroxide imaging in biological samples, we envision access to various *off-on* fluorogenic probes from the same intermediate **12**, which will be reported in due course. Nevertheless, our borinic acid-based probe containing an *ortho*-electron-withdrawing sterically hindered substituent still remains more reactive than the commonly used boronic acid probes that reinforce the interest of arylborinic acids as promising new tools in the panel of H<sub>2</sub>O<sub>2</sub>-sensitive reagents. These new types of borinic acid-based probes pave the way for future developments toward applications in H<sub>2</sub>O<sub>2</sub> sensors, drugs release or theranostics.

## Experimental Section

**O,N-chelated diarylborinates synthesis:** To a solution of commercially available aryl(pinacolato)borane (1.0 equiv) in dry Et<sub>2</sub>O (c 0.15M), cooled to -78°C, was added a 1.9M solution of phenyllithium in *n*-Bu<sub>2</sub>O (1.2 equiv) dropwise. The mixture was stirred for 4 hours at -78°C before quenching with a 2M HCl solution in Et<sub>2</sub>O (9 equiv). The reaction was allowed to warm to room temperature and stirred for an additional 1 hour. The mixture was then diluted with Et<sub>2</sub>O and water. The aqueous layer was extracted twice with Et<sub>2</sub>O. All organic layers were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under vacuum. The residue was dissolved in Et<sub>2</sub>O (10.0 mL per 1.0 mmol) and 2-aminoethanol (1.2 equiv) was added. Precipitation appeared immediately and the suspension was stirred at room temperature overnight. The solid was then filtered off, washed with ice-cold pentane and dried under vacuum to afford pure borinate derivatives **2a-2i**.

**Hydrolysis of borinate derivatives:** To a solution of 2-aminoethyl borinate **2** in Et<sub>2</sub>O (c 0.35M) was added a 2M aqueous solution of HCl (3.0 mL per 1.0 mmol). The mixture was vigorously stirred at room temperature until the disappearance of the starting material. The aqueous layer was extracted with EtOAc (3x5 mL). The combined organic layers were washed with brine solution (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under vacuum to give pure borinic acid **3**.

**H<sub>2</sub>O<sub>2</sub>-promoted oxidation of unsymmetrical borinic acids:** Typically, a 4M deuterated aqueous solution of H<sub>2</sub>O<sub>2</sub>.urea (250 μL) was added to a 4M solution of borinic acids dissolved in deuterated PBS buffer containing 10% DMSO-*d*<sub>6</sub> (250 μL). The reaction progress was then monitored by <sup>1</sup>H NMR spectroscopy using a 600 MHz spectrometer. The distribution of two couples was determined using <sup>1</sup>H NMR spectroscopy by integration of either aromatic or methyl protons.

## Acknowledgements

We would like to thank IDEX Paris Saclay for a PhD grant to B. G.-F. and the Ministère de la Recherche et de l'Enseignement Supérieur for a PhD grant to M. P. The CHARMMAT program is also acknowledged for M.P. financial support. We also thank the French Agency for Research for funding (Grant ANR21-CE07-0047-03 Boriprobe).

**Keywords:** Borinic acid • Oxydation • Regioselectivity • Hydrogen peroxide • Substituent effects •

- [1] a) D. G. Hall, *Boronic Acids: Preparation and Applications in Organic Synthesis and Medicine*, Wiley-VCH, Weinheim, Germany **2005**; b) For a recent review, see: J. W. B. Fyfe, A. J. B. Watson, *Chem* **2017**, *3*, 31-55.
- [2] a) D. D. Winkle, K. M. Schaab, *Org. Process Res. Dev.* **2001**, *5*, 450-451; b) X. Chen, H. Ke, Y. Chen, C. Guan, G. Zou, *J. Org. Chem.* **2012**, *77*, 7572-7578; c) H. Ke, X. Chen, G. Zou, *J. Org. Chem.* **2014**, *79*, 7132-7140; d) X. Chen, H. Ke, G. Zou, *ACS Catal.* **2014**, *4*, 379-385; e) E. Dimitrijevic, M. Cusimano, M. S. Taylor, *Org. Biomol. Chem.* **2014**, *12*, 1391-1394; f) X. Li, G. Zou, *J. Organomet. Chem.* **2015**, *794*, 136-145; g) S. Si, C. Wang, N. Zhang, G. Zou, *J. Org. Chem.* **2016**, *81*, 4364-4370; h) C. Wang, J. Zhang, J. Tang, G. Zou, *Adv. Synth. Catal.* **2017**, *359*, 2514-2519; i) N. Zhang, C. Wang, G. Zou, J. Tang, *J. Organomet. Chem.* **2017**, *842*, 54-58; j) C. Ren, J. Zeng, G. Zou, *New J. Chem.* **2019**, *43*, 1589-1596; j) F. Wang, C. Wang, G. Sun, G. Zou, *Tetrahedron Lett.* **2020**, *61*, 151491.
- [3] K. Ishihara, H. Yamamoto, *Eur. J. Chem.* **1999**, 527-538.
- [4] K. Ishihara, H. Kurihara, H. Yamamoto, *J. Org. Chem.* **1997**, *62*, 5664-5665.
- [5] K. Ishihara, H. Kurihara, H. Yamamoto, *Synlett* **1997**, 597-599.
- [6] a) T. Mohy El Dine, J. Rouden, J. Blanchet, *Chem. Commun.* **2015**, *51*, 16084-16087; b) T. Mohy El Dine, D. Evans, J. Rouden, J. Blanchet, *Chem. Eur. J.* **2016**, *22*, 5894-5898.
- [7] a) L. Chan, M. S. Taylor, *Org. Lett.* **2011**, *13*, 3090-3093; b) D. Lee, M. S. Taylor, *J. Am. Chem. Soc.* **2011**, *133*, 3724-3727; c) D. Lee, C. L. Williamson, L. Chan, M. S. Taylor, *J. Am. Chem. Soc.* **2012**, *134*, 8260-8267; d) E. Dimitrijevic, M. S. Taylor, *Chem. Sci.* **2013**, *56*, 3298-3303; e) R.-Z. Li, H. Tang, K. R. Yang, L.-Q. Wan, X. Zhang, J. Liu, Z. Fu, D. Niu, *Angew. Chem., Int. Ed.* **2017**, *56*, 7213-7217; *Angew. Chem. Int. Ed.* **2017**, *129*, 7319-7323; f) M. Pawliczek, T. Hashimoto, K. Maruoka, *Chem. Sci.* **2018**, *9*, 1231-1235; g) V. Dimakos, M. S. Taylor, *Chem. Rev.* **2018**, *118*, 11457-11517; h) J. Alsarraf, L. Petitpoisson, A. Pichette, *Org. Lett.* **2021**, *23*, 6052-6056.
- [8] a) K. Tanveer, K. Jarrah, M. S. Taylor, *Org. Lett.* **2015**, *17*, 3482-3485; b) G. E. Garrett, K. Tanveer, M. S. Taylor, *J. Org. Chem.* **2017**, *82*, 1085-1095; c) K. Tanveer, S.-J. Kim, M. S. Taylor, *Org. Lett.* **2018**, *20*, 5327-5331; d) G. Wang, G. E. Garrett, M. S. Taylor, *Org. Lett.* **2018**, *20*, 5375-5379; e) G. Wang, M. S. Taylor, *Adv. Synth. Catal.* **2020**, *362*, 398-403.
- [9] a) C. Gouliaras, D. Lee, L. Chan, M. S. Taylor, *J. Am. Chem. Soc.* **2011**, *133*, 13926-13929; b) T. M. Beale, M. S. Taylor, *Org. Lett.* **2013**, *15*, 1358-1361; c) S. O. Bajaj, E. U. Sharif, N. G. Akhmedov, G. A. O'Doherty, *Chem. Sci.* **2014**, *5*, 2230-2234; d) M. Tanaka, J. Nashida, D. Takahashi, K. Toshima, *Org. Lett.* **2016**, *18*, 2288-2291; e) M. Tanaka, D. Takahashi, K. Toshima, *Org. Lett.* **2016**, *18*, 5030-5033; f) K. A. D'Angelo, M. S. Taylor, *J. Am. Chem. Soc.* **2016**, *138*, 11058-11066; g) K. A. D'Angelo, M. S. Taylor, *Chem. Commun.* **2017**, *53*, 5978-5980; h) J. Nashida, N. Nishi, Y. Takahashi, C. Hayashi, M. Igarashi, D. Takahashi, K. Toshima, *J. Org. Chem.* **2018**, *83*, 7281-7289; i) S. Izumi, Y. Kobayashi, Y. Takemoto, *Angew. Chem.* **2020**, *132*, 14158-14163; *Angew. Chem. Int. Ed.* **2020**, *59*, 14054-14059; j) Y. Kobayashi, Y. Takemoto, *Tetrahedron* **2020**, *76*, 131328.
- [10] a) V. Dimakos, H. Y. Su, G. E. Garrett, M. S. Taylor, *J. Am. Chem. Soc.* **2019**, *141*, 5149-5153; b) K. Sakai, K. Oisaki, M. Kanai, *Synthesis* **2020**, *52*, 2171-2189.
- [11] a) A. Chardon, O. Maubert, J. Rouden, J. Blanchet, *ChemCatChem* **2017**, *9*, 4460-4464; b) A. Chardon, T. Mohy El Dine, R. Legay, M. De Paolis, J. Rouden, J. Blanchet, *Chem. Eur. J.* **2017**, *23*, 2005-2009; c) A. Chardon, J. Rouden, J. Blanchet, *Eur. J. Org. Chem.* **2019**, 995-998.
- [12] For boronate oxidation, see a recent review: A. Sikora, J. Zielonka, K. Debowska, R. Michalski, R. Smulik-Izydorzyczyk, J. Pieta, R. Podsiadly, A. Artelska, K. Pierzcchala, B. Kalyanaraman, *Front. Chem.* **2020**, *8*, 580899.
- [13] B. Gatin-Fraudet, R. Ottenwelter, T. Le Saux, S. Norsikian, M. Pucher, T. Lombès, A. Baron, P. Durand, G. Doisneau, Y. Bourdreux, B. Iorga, M. Erard, L. Jullien, D. Guianvarc'h, D. Urban, B. Vauzeilles, *Proc. Natl. Acad. Sci. U.S.A.*, **2021**, *118*, e2107503118.
- [14] X. Zhou, L. Lesiak, R. Lai, J. R. Beck, J. Zhao, C. G. Elowsky, H. Li, C. I. Stains, *Angew. Chem.* **2017**, *129*, 4261-4264; *Angew. Chem. Int. Ed.* **2017**, *56*, 4197-4200.
- [15] a) R. L. Letsinger, N. Remes, *J. Am. Chem. Soc.* **1955**, *77*, 2489-2491; b) R. L. Letsinger, I. Skoog, *J. Am. Chem. Soc.* **1955**, *77*, 2491-2494; c) A. Hofer, G. Kovacs, A. Zappatini, M. Leuenberger, M. A. Hediger, M. Lochner, *Bioorg. Med. Chem.* **2013**, *21*, 3202-3213; d) H. C. Brown, M. Srebnik, T. E. Cole, *Organometallics* **1986**, *5*, 2300-2303; e) C. Guan, L. Huang, C. Ren, G. Zou, *Org. Process Res. Dev.* **2018**, *22*, 824-828.
- [16] a) L. Marciasini, B. Cacciuttolo, M. Vaultier, M. Pucheault, *Org. Lett.* **2015**, *17*, 3532-3535; b) J. Richard, M. Birepinte, J. B. Charbonnier, V. Liautard, S. Pinet, M. Pucheault, *Synthesis* **2017**, *49*, 736-744.
- [17] T. Igarashi, M. Tobisu, N. Chatani, *Angew. Chem.* **2017**, *129*, 2101-2105; *Angew. Chem. Int. Ed.* **2017**, *56*, 2069-2073.
- [18] R. Shimazumi, T. Igarashi, M. Tobisu, *Chem. Lett.* **2020**, *49*, 760-763.
- [19] a) B. Wrackmeyer, *Ann. Rep. NMR Spectrosc.* **1988**, *20*, 61-203; b) A. R. Siedle, *Ann. Rep. NMR Spectrosc.* **1988**, *20*, 205-314.
- [20] Protection with various ligands such as aminoethanol or picolinic acid was unsuccessful, probably due to the steric hindrance of *t*-butyl group.
- [21] This is supported by the unsuccessful protection of borinic acid with ethanolamine or picolinic acid.
- [22] Oxidation spectra of some borinic acid models are exemplified in the *SI Appendix*.
- [23] a) V. P. Reddy in *Organofluorine Compounds in Biology and Medicine* Elsevier, **2015**; b) G. Bott, L. D. Field, S. Sternhell, *J. Am. Chem. Soc.* **1980**, *102*, 5618-5626.
- [24] The synthesis of symmetrical bis-4-methylcoumarinborinic acid, which should furnish only the release of 4-methylumbelliferone upon treatment with hydrogen peroxide, was unsuccessful.
- [25] a) L. Du, M. Li, S. Zheng, B. Wang, *Tetrahedron Lett.* **2008**, *49*, 3045-3048; b) T. Ishiyama, T. Y. Itoh, T. Kitano, N. Miyaura, *Tetrahedron Lett.* **1997**, *38*, 3447-3450.
- [26] S. J. Baker, T. Akama, Y.-K. Zhang, V. Sauro, C. Pandit, R. Singh, M. Kully, J. Khan, J. J. Plattner, S. J. Benkovic, V. Lee, K. R. Maples, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5963-5967.
- [27] J. Y. Baek, Y.-J. Shin, H. B. Jeon, K. S. Kim *Tetrahedron Lett.* **2005**, *46*, 5143-5147.
- [28] For a recent review, see D.-J. Zheng, Y.-S. Yang, H.-L. Zhu, *Trends Anal. Chem.* **2019**, *118*, 625-651.
- [29] I. A. Müller, F. Kratz, M. Jung, A. Warnecke, *Tetrahedron Lett.* **2010**, *51*, 4371-4374
- [30] A. Alouane, R. Labruère, T. Le Saux, F. Schmidt, L. Jullien, *Angew. Chem.* **2015**, *127*, 7600-7619; *Angew. Chem. Int. Ed.* **2015**, *54*, 7492-7509.
- [31] A. Alouane, R. Labruère, T. Le Saux, I. Aujard, S. Dubrulle, F. Schmidt, L. Jullien, *Chem. Eur. J.* **2013**, *19*, 11717-11724.