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Redemitting BODIPY boronic acid fluorescent sensors for detection of lactate

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ABSTRACT

Two redemitting BODIPY boronic acid pinacolate derivatives, sensors **1** and **2** were shown to act as excellent and highly selective lactate detectors at physiological pH (7.4), where the formed sensor-lactate complexes exhibited a significant emission and absorption increase. Since hyperlactataemia ([L-lac] > 6.5 mM) is a common complication in intensive care units, there is need for easy, on-line monitoring of lactate levels in patients. Semi-invasive monitoring via a lactate electrode or optic fiber would be attractive. This may beneficially replace existing lactate detection methods requiring a high degree of instrumentation. Sensors **1** and **2** can detect lactate without interference from biological important monosaccharides, such as D-glucose, D-fructose and D-mannose.

1. Introduction

L-Lactate is a biologically important α-hydroxy carboxylate, produced in anaerobic processes.[1] Despite common beliefs, L-lactate is formed at rest and also by moderate exercise ([L-lac] ≈ 1–2 mM).[2] L-Lactate is formed by the red blood cells which noteworthy lack mitochondria and have limitations from the enzyme activity occurring in muscle fibers.[3] At strenuous anaerobic exercise the L-lactate level can rise towards 20 mM,[4] and levels above 30 mM are considered lethal.[5] Hyperlactataemia ([L-lac] >6.5 mM) is a condition widely used for diagnosis and prognosis for intensive care units. This condition is associated with severe disorders, such as sepsis and trauma, but clinically reliable lactate detection relies on several determinations.[6]

Several ways of detecting L-lactate have so far been published. These rely on flow-injection analysis for analysis in food,[7] electrochemical enzymatic assays,[8] electrochemical polymeric assays,[9] and GC-methods.[10] These methods suffer from the requirement for instrumentation. Handheld lactate monitors are available on the market.[11] However, these are invasive, due to the requirement of blood samples, and monitoring relies on the enzymatic oxidation of lactate with lactate oxidase.

Semi-invasive and online detection of this compound could enable better surveillance and control.

Development of a red to near-infrared (600–900 nm) semi-invasive optical lactate sensor is thus of relevance for proper lactate monitoring through tissue. Light absorption and scattering from biological tissues over 600 nm of wavelength are usually insignificant.[12] The borondipyrromethene (BODIPY) dye is a very versatile fluorescent dye, possessing high quantum yields and narrow absorption and emission bands. Additionally, the spectroscopic properties can easily be modulated towards the red to near-infrared area.[13]

Monitoring of specific analytes through tissue can be executed by use of a specific near-infrared detector. In order to bind biologically important hydroxyl compounds, the arylboronic acid receptor is a relevant choice. Arylboronic acids are small and flexible molecules, where the reaction between 1,2-*cis*-diols and 1,3-diols in aqueous media generally affords cyclic esters rapidly and reversibly.[14] The ester formation can alter the electronic properties of the boronic acid unit significantly since boron undergoes transformation from sp^2-to sp^3-geometry upon favored arylboronate formation.[15,16]

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2. Results and discussion

The two redemitting BODIPY boronic acid pinacolate esters, **1** and **2** (Fig. 1), were prepared from the corresponding green emitting BODIPY analogues, **3** and **4** (Scheme 1), upon condensation of the two methyl groups at the 3- and 5-positions with 4-pyridylaldehyde employing piperidine as base catalyst in DMF. The precursors, i.e. the green emitting BODIPY dyes were synthesized using recently described procedures.[17,18] The overall synthesis of sensor **1** and **2** is depicted in Scheme 1.

The corresponding pinacol ester analogues of the arylboronic acids have been used throughout the binding studies due to the easier synthesis and purification procedures. It is well known from previous research that the binding efficiency of arylboronic acids and their corresponding neopentyl- and pinacol esters are similar at the concentrations utilized in fluorimetry.[19]

Sensors **1** and **2** exhibit absorption maxima at 612 nm and 613 nm respectively, and their respective absorption spectra are identical to their excitation spectra. The extinction coefficients of sensors **1** and **2** have been determined in methanol to be $\varepsilon_{1,612nm} = 75.800\ M^{-1}cm^{-1}$ and $\varepsilon_{2,613nm} = 78.500\ M^{-1}cm^{-1}$, respectively. Such high extinction coefficients are as expected for BODIPY dyes.[20]

The primary emission bands for sensor **1** and **2** are at 622 nm and 623 nm respectively. The secondary emission bands appearing around 680 nm are more intense in comparison to the secondary bands of emission around 540 nm for the non-condensed counterparts, sensor **3** and **4**. The exhibition of the more intense secondary bands might be attributed to a higher degree of excimer formation for the redemitting dipyridylsensors as compared to the green emitting sensors, **3** and **4**.

DL-Lactate and L-malate were found to influence the photo physical properties of sensor **1** and **2** significantly at physiological pH (7.4) in a 25 mM, 52.3 w/w% methanolic phosphate buffer. The intrinsic chirality of the α-hydroxy carboxylic acids is not relevant in this study, since sensors **1** and **2** are achiral. Sensor **1** and **2** exhibited a significant increase in excitation- and emission intensity upon augmenting the concentration of each of the α-hydroxy carboxylates shown in Fig. 2. This phenomenon may likely be attributed to diminished oxidative quenching of the BODIPY core upon arylboronate formation, since electrons are less likely to be accepted by the arylboronate unit, in comparison to the neutral arylboronic acid. The proposed oxidative quenching of the BODIPY core is illustrated in Scheme 2, where the emission intensity is regained upon arylboronate formation (see Figs. 3–5).

The displacement constants were determined for binding of each α-hydroxy carboxylate at pH 7.4, where 1:1 sensor:α-hydroxy carboxylate complexes were anticipated. The binding affinity of sensors **1** and **2** towards lactate was found to be around

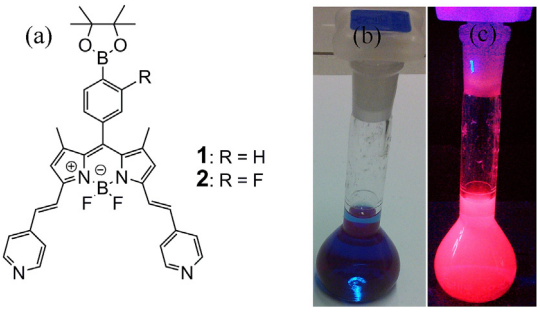
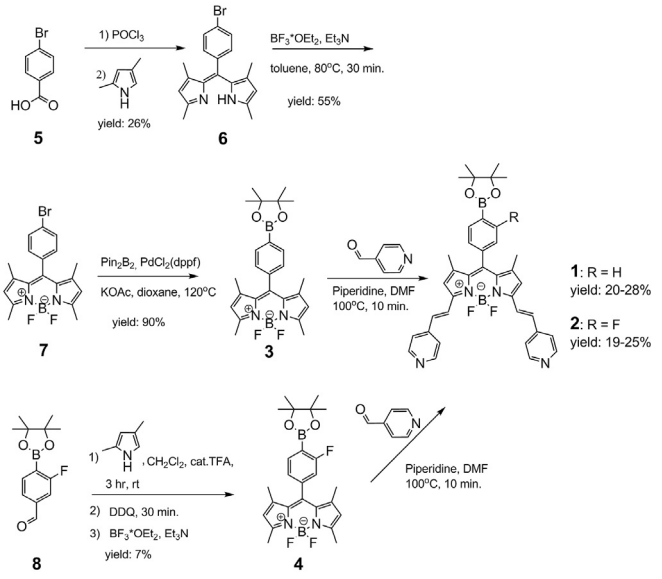


Fig. 1. (a) The two prepared redemitting BODIPY based boronic acid pinacolate dyes. (b) Sensor **2** in methanol, and (c) exposure to UV-light (366 nm).



Scheme 1. Preparation of the redemitting BODIPY boronic acid pinacolate dyes, **1** and **2**.

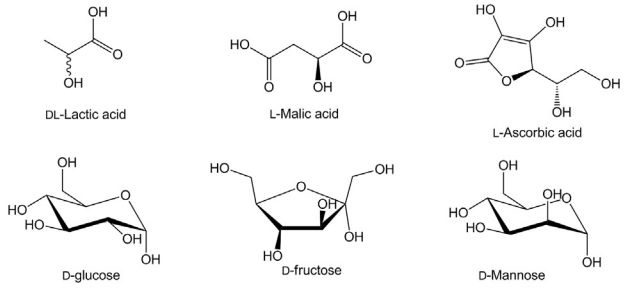


Fig. 2. Biologically occurring α-hydroxy- and polyol compounds tested for binding to the sensors, **1** and **2**.

K_d = 60 mM. DL-Lactate were bound slightly tighter to sensor **1** compared to **2**. This slight difference may be explained by the higher pK_a of sensor **1** compared to **2**. The pK_a value was determined to be 9.0 for sensor **1**, whereas the pK_a for sensor **2** was 8.2. Determination of the respective pK_a-values for sensors **1** and **2** was conducted by exploiting the slight blue shifting of the emission spectra upon boronate formation. The ratio $I_f(640\ nm)/I_f(610\ nm)$ was calculated at different pH values. At physiological pH, sensors **1** and **2** are present as the boronic acids. It is known from literature that the neutral boronic acid is more prone to bind α-hydroxy carboxylates compared to the binding ability of the boronate.[16]

L-Malate is an important metabolic product, which participates in the citric acid cycle.[21] This compound exhibited the strongest binding interaction for both sensors **1** and **2**, where the binding affinities were very similar ($K_d \approx 30$ mM). The L-malate interference is not critical for error readout in a potential lactate sensor, since L-malate exists at a very low concentration, and does not change due to physical activity.[2] The stronger binding interaction of L-malate with sensor **1** and **2** may be rationalized by the additional adjacent carboxylate moiety, which is capable of forming a hydrogen bond with the hydroxyl group in the boronate. For both α-hydroxy carboxylates the response factors were significant, i.e. up to 6.8, which means an increase in emission intensity around 700% compared to the unbound sensors. The values of the displacement constants calculated from the excitation experiments were found to