Molecular tools for light-navigated therapy

Reeßing, Friederike

DOI:
10.33612/diss.128516808

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 18-10-2023
Chapter 2:

PASSERINI multi-component reaction for the synthesis of visible-light cleavable scaffolds

Photoactivated targeted pharmacotherapy holds great promise for improving the effectiveness and safety of drug treatment. However, its application is still largely limited by the requirement of UV light for the activation, which is strongly absorbed by biological tissue and may have toxic effects. In order to tackle this challenge, we describe here our efforts to develop a general methodology for the synthesis of visible light responsive molecular structures that could be used for targeted photoactivated therapy.

In collaboration with Dr. Kaja Sitkowska
Introduction

Light-activated pharmacotherapy, used for example for the treatment of cancer or microbial infections, has gathered increasing interest over the last few years. Most of the published examples are based on known, biologically active molecules of which the activity is caged by the attachment of a photocleavable protecting group. Irradiation of this type of constructs leads to selective release of the active compound with high spatiotemporal resolution. Compared to the use of photoswitches, this approach has the advantage that photo-activation restores the original structure, affording significant differences in activity before and after light exposure. In contrast, incorporation of a photoswitch often requires modulation of the pharmacophore which may go along with loss in activity or less pronounced differences in activity upon irradiation. However, the applicability of the photocaging strategy is still limited as well, for example due to the fact that in most cases UV or blue light is needed for activation which is strongly absorbed in biological tissue and may even have cytotoxic effects. Therefore, new red- or near-infrared (NIR) light cleavable groups are needed in order to advance the development and clinical implementation of photoactivated pharmacotherapy.

Chapter 1 gave an overview of photoactivatable cytotoxic agents for cancer chemotherapy developed to date. The illustrated cases are mostly based on ortho-nitrophenyl-derived groups, which can be cleaved with maximum $\lambda = 400$ nm light. Others are based on coumarin photocages, responsive to light of higher wavelength, but still not within the desired optical window between $\lambda = 600 – 900$ nm, which would be suitable for biological applications. Agents that can be activated with light within this wavelength range were prepared by coupling to upconverting nanoparticles (UCNP), allowing the conversion from incoming, lower energy (higher wavelength) light to light of higher energy (lower wavelength) which in turn can activate the cytotoxic agents. This approach enables the use of red- or NIR light, but its application is still limited as it requires the administration of an additional moiety (UCNP) that needs to reach the target. Moreover, very intense irradiation is needed due to low efficiency of the upconversion. Similarly, implementation of two-photon excitation is hindered by the same limitation. Two-photon induced photorelease relies on the simultaneous absorption of two photons of longer wavelength generating higher energy excited states that result in photocleavage. Even though this technique allows the use of NIR light, the high light intensities needed and the fact that only few molecular structures display a sufficient two-photon uncaging cross section restricts the application.
Fig. 2.1: Design strategies used for increasing the photocleavage quantum yield and shifting
the activation wavelength to red- or NIR part of the spectrum.

In order to tackle this challenge and to develop light-responsive photocleavable groups
independent of nanoparticles, different molecular structures have been investigated as
photocaging groups, among them the boron-dipyrromethene (BODIPY) scaffold.
BODIPYs are well established as (fluorescent) dyes and widely used in e.g. microscopy
and molecular biology. More recently, this class of compounds has also been
explored for the application as photocleavable group. Strategic modifications of the
core structure allowed to increase the quantum yield of photodeprotection and shift
the activation wavelength to red light. Those modifications include (i) 2,6-
dihalogenation, (ii) boron-alkylation, and (iii) extension of the delocalized π-electron
system (Fig. 2.1). It has to be noted, however, that these modifications may significantly
decrease the stability as well as solubility of the respective molecules. To date, examples
of carboxylic acids, phenols and amines, but also other small molecules (e.g. carbon
monoxide, methanol, chloride), caged with different BODIPY-based groups, have been
reported, showing the versatility of this photocage and its high potential for the use in
photoactivated pharmacotherapy.

Inspired by those promising results, our goal was to broaden the applicability of this
photoprotecting group and establish a modular photocaging strategy based on a multi-
component reaction. This modular approach would permit the conjugation of a
biologically active compound with e.g. a targeting group via a photocleavable linker
(Fig. 2.2c). Multi-component reactions (MCR), such as the Passerini MCR, enable the
synthesis of such a structure in just one step (Fig. 2.2a-b), improving atom-economy
and saving time and resources normally spent in the several synthetic steps and
purification of intermediates. The Passerini MCR is particularly privileged as it
provides an ester linkage which affords a good leaving group for light-induced uncaging.
PASSERINI MULTI-COMPONENT REACTION FOR THE SYNTHESIS OF VISIBLE-LIGHT CLEAVABLE SCAFFOLDS

Fig. 2.2: Schematic comparison of a multi-step synthesis and a one-step multi-component reaction; a) a multi-step synthesis requires several independent reaction step with isolation and purification of intermediates; b) a multi-component reaction allows the conjugation of multiple different substrates in one step; c) general approach for the synthesis of light-activatable bioactive compounds (blue), conjugated to a targeting group (green/black) via a photocleavable linker (red).

The Passerini MCR has been proven useful for various purposes ranging from the synthesis of functional polymers or providing quick access to a library of compounds, for instance for drug discovery.26,27 Also in our group, it has been successfully applied to synthesize different photocages of carboxylic acids, as this functionality is very abundant in biologically active compounds, oftentimes as part of the pharmacophore.28 For this purpose the aldehydes of different photo-reactive groups, such as ortho-nitrophenyl or coumarines, were reacted with different carboxylic acids and isocyanides affording the corresponding photocaged products. Furthermore, Passerini MCRs incorporating a BODIPY scaffold were reported by the group of Vendrell.29 However, those conjugates were designed as fluorescent probes and do not undergo photolysis. Encouraged by this previous study, our aim was to establish a synthetic strategy that allows the conjugation of two different structures via a visible-light-responsive photoprotecting group.

Fig. 2.3: Proposed reaction mechanisms of the Passerini MCR. a) concerted mechanism; b) ionic mechanism.
In the Passerini MCR, the three components – isocyanide, aldehyde (or ketone) and carboxylic acid – react to form α-acyloxyamides following a mechanism which is still not fully elucidated. It is generally accepted that the first step is the activation of the carbonyl group from the aldehyde or ketone via the formation of a hydrogen-bonded adduct with the carboxylic acid (Fig. 2.3) promoting the nucleophilic attack of the isocyanide in the next step. This reaction step is assumed to proceed via a concerted mechanism, in which the isocyanide, while functioning as a nucleophile, also acts as an electrophile, as it is simultaneously attacked by the carbonyl oxygen of the carboxylic acid (Fig. 2.3a). Alternatively, it was suggested that in more polar solvents the reaction proceeds via an ionic step, as shown in Fig. 2.3b. Finally, an intramolecular Mumm type rearrangement affords the final acyloxyamide. The Passerini MCR stands out due to the high functional group tolerance, which stems from the selective reactivity of the reagents involved. Another advantage is that the reaction generally does not require dry conditions, or special experimental setups and reagents.

The aim of the research reported in the following was to establish a Passerini MCR as a reaction of choice for the one step synthesis of photolabile conjugates bearing a targeting moiety and releasing an active substance upon irradiation. This chapter describes our efforts to test the scope and limitations of this approach.

RESULTS AND DISCUSSION

In the first step, the BODIPY core structure was synthesized following an adapted literature procedure and the afforded ester was subsequently hydrolyzed to the alcohol (Fig. 2.4). In order to incorporate the BODIPY structure in a Passerini MCR analogous to the reported examples, it was necessary to install an aldehyde functionality. Hence, alcohol 2 was oxidized to aldehyde 3 using Dess-Martin periodinane (DMP). The corresponding synthetic procedure is based on literature reports and was optimized in our group as previously reported. Additionally, with the aim to obtain red-light activatable structures, two more aldehydes were synthesized as reported previously: iodinated BODIPY 4 and BODIPY 5 with an extended chromophoric system (Fig. 2.5).

![Fig. 2.4: Synthesis of BODIPY aldehyde 3](image-url)
Consequently, we proceeded with the synthesis of a library of possible Passerini reaction products, exploring the possibilities to connect different carboxylic acids with various isocyanides. The reaction was performed in dichloromethane or chloroform at 40 °C for several days.

![Molecular structures of aldehydes 3, 4 and 5 and the schematic representation of corresponding reaction products.](image)

Fig. 2.5: Molecular structures of aldehydes 3, 4 and 5 and the schematic representation of corresponding reaction products.

The molecular structures of all the derivatives is depicted in Fig. 2.6. In total five different derivatives of aldehyde 3 were synthesized, isolated and characterized. Reaction with different aromatic, aliphatic and even sterically hindered isocyanides and different carboxylic acids successfully afforded the final products 6 – 10, albeit in low yields ranging from 10% to 38%, whereas no conversion to the desired products 11 and 12 was observed in the reactions with 2-isocyano-N-(prop-2-yn-1-yl)acetamide (Table 2.1). Possibly, the electron-withdrawing effect of the amide functionality in the α-position reduces the nucleophilicity of the isocyanide leading to decreased reactivity. However, as reported in following chapters (chapter 4 and 5) this specific isocyanide generally does react with another aldehyde and different carboxylic acids in a Passerini MCR.\(^{37}\)

After the successful synthesis of compounds 6 – 10, we further explored the reactivity of the iodinated analogue (compound 4) of aldehyde 3. We were pleased to see that the reaction proceeded similarly to the reaction with the non-iodinated aldehyde, leading to product 13 in 10% yield. In contrast, the MCR with aldehyde 5, carrying an extended π-electron system afforded only trace amounts of desired product 14.
As shown in Table 2.1, the isolated yields of the novel compounds account for only up to 48%. It has to be noted, that all reactions were performed on a small scale (15-20 mg of aldehyde) and thus losses during purification are relatively high and the isolated yields are not representative for the conversion. The discrepancy between observed conversion and isolated yield was particularly pronounced in the synthesis of compound 8. Hence, with the aim to minimize this problem and possibly increase the isolated yield, we attempted to upscale it twice. Unfortunately, a clear drop in conversion to the desired product was observed consequently. Presumably, this outcome stems from difficulties to provide identical reaction conditions on a larger scale. It is particularly challenging to obtain the exact same substrate concentrations in the reaction mixture as evaporation of the solvent (dichloromethane/chloroform) cannot be completely prevented, especially on a smaller scale, resulting in a more concentrated reaction mixture. This effect may play a substantial role regarding the irreproducibility of the
reaction since it is known that the substrate concentration may significantly influence the reaction outcome of MCRs. \(^{38}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Isolated yield</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>(\varepsilon) ((\times 10^3 , \text{M}^{-1} , \text{cm}^{-1}))</th>
<th>Half-life (min)*</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>22%</td>
<td>523</td>
<td>53</td>
<td>97.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>24%</td>
<td>523</td>
<td>61</td>
<td>66.6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>19%</td>
<td>520</td>
<td>36</td>
<td>61.4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10%</td>
<td>520</td>
<td>-</td>
<td>-</td>
<td>aggregation</td>
</tr>
<tr>
<td>10</td>
<td>38%</td>
<td>521</td>
<td>54</td>
<td>60.3</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>48%</td>
<td>553</td>
<td>54</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>33%</td>
<td>665</td>
<td>-</td>
<td>64.0**</td>
<td>unstable</td>
</tr>
</tbody>
</table>

Table 2.1: Isolated yields and photochemical properties of compounds 6 – 14. *Half-life of the respective compound upon irradiation with \(\lambda = 532\) nm (compounds 6-8, 10, 13) or \(\lambda = 650\) nm (compound 14). **Due to the instability of compound 14 under the experimental conditions, decrease in absorption may partially stem from light-independent decomposition.

For further investigation of the parameters affecting the product formation in this reaction, we set up two test reactions for the synthesis of product 8, with 3 mg of aldehyde 3, 2 eq. of acetic acid and 2 eq. tert-butyl isocyanide: one reaction with dichloromethane as a solvent and one reaction under neat conditions. In addition, we added 1,3,5-trichlorobenzene as an internal standard to be able to draw a direct comparison of the reaction outcomes, as illustrated in Fig. 2.7. After two days, no conversion to the desired product was observed in the reaction performed under neat conditions, whereas clear product signals (amongst others: 7.97 ppm, 2.92 ppm, 2.83 ppm) were detected when adding dichloromethane as solvent. Comparison of the product and substrate signals indicates ca. 70% conversion to product versus the 19% isolated yield from earlier experiments. Overall, both factors, namely relatively high losses during product purification on small scale and difficulties in repeating and reproducing reaction conditions on larger scale, probably lead to low isolated yields.
Fig. 2.7: $^1$H NMR spectrum of two reaction mixtures for the synthesis of compound 8 with 0.37 eq. of 1,3,5 trichlorobenzene as internal standard (7.31 ppm). The signals of aldehyde 3 are highlighted in orange and the signals of product 8 in green. a) reaction under neat conditions; b) reaction with deuterated dichloromethane as solvent.

With the purified conjugates in hand, we consequently investigated their photochemical properties. Towards this end, we acquired the UV-Vis absorbance spectra at different concentrations for the determination of the extinction coefficients of all derivatives. Furthermore, after confirming the stability of the compounds under the experimental conditions (25% acetonitrile in phosphate buffer pH 7.4), we irradiated the respective samples with $\lambda = 532$ nm (compound 14: $\lambda = 650$ nm) light and followed the photocleavage by UV-Vis spectrometry. Table 2.1 summarizes the results of the photochemical analysis of all synthesized conjugates and Fig. 2.8 illustrates the results exemplified for the evaluation of compound 6. As anticipated, all analyzed derivatives absorb visible light with absorbance maxima above 520 nm (Fig. 2.9). Moreover, the findings confirm that 2,6-di-halogenation and extension of the conjugated $\pi$-electron system indeed shift the absorbance maxima to higher wavelengths.
PASSERINI MULTI-COMPONENT REACTION FOR THE SYNTHESIS OF VISIBLE-LIGHT CLEAVABLE SCAFFOLDS

Fig. 2.8: Photochemical analysis of compound 6 (25% acetonitrile in phosphate buffer, pH 7.5): a) UV-Vis absorption spectrum at different concentrations; b) absorbance at $\lambda_{\text{max}}$ (523 nm) at different concentrations; c) collection of UV-Vis absorption spectra recorded over 9 min in the dark; d) absorbance at $\lambda_{\text{max}}$ (523 nm) over 9 min in the dark; e) collection of UV-Vis absorption spectra upon irradiation with $\lambda = 532$ nm for the indicated times; f) absorbance at $\lambda_{\text{max}}$ (523 nm) upon irradiation with $\lambda = 532$ nm for the indicated times.

Kinetic analysis of the decrease in absorbance at the corresponding absorption maxima allowed the determination of the half-life upon irradiation with 532 nm light. Unfortunately, the tested solution of compound 9 was not stable and showed aggregation under the experimental conditions. This behavior is not unexpected, since
BODIPY scaffolds tend to aggregate due to π-π stacking.\textsuperscript{39,40} In addition, the long alkyl chain of 9 enhances the lipophilicity of the molecule decreasing the water solubility and hence the stability in aqueous medium. Moreover, compound 14 carrying an extended chromophoric system was not stable either. Therefore, it was not possible to accurately determine the extinction coefficient and the half-life upon light-induced cleavage.

![UV-Vis absorbance spectra for all synthesized derivatives at 20 µM concentration in a mixture of acetonitrile and phosphate buffer (pH 7.4). The irradiation wavelengths λ = 532 nm (green) and λ = 650 nm (red) are highlighted.](image)

Generally, the photocleavage proceeded considerably slower than anticipated with half-lives longer than 1 h. Only compound 13 stood out as it shows much faster light-induced cleavage (Table 2.1). In fact, 13 cleaves ca. 7.5 times faster than its non-halogenated analogue 8. This result is in agreement with published reports describing the influence of halogenation of the BODIPY core on the photoreactivity.\textsuperscript{17} Taking into account that 2,6-dihalogenation, and in particular 2,6-diiodination, substantially increases intersystem crossing efficiency,\textsuperscript{41} it is assumed that the triplet excited state plays a critical role in the light-induced cleavage of BODIPY derivatives and therefore accounts for the higher photoreactivity of iodo-BODIPYs. In addition, it has to be noted that compound 13 is characterized by a 1.5 times higher extinction coefficient and absorbance at 532 nm (Fig. 2.9), which indicates a possible explanation for the higher photoactivation efficiency.

**CONCLUSIONS**

In summary, we used a Passerini MCR to successfully synthesize various photocleavable conjugates responsive to visible light irradiation. Towards this end, we used a BODIPY scaffold as a light-responsive core structure. Employing a Passerini MCR allowed to connect two different molecular structures, one bearing a carboxylic acid and another one bearing an isocyanide group, in one step, saving time and resources. To our delight,
PASSERINI MULTI-COMPONENT REACTION FOR THE SYNTHESIS OF VISIBLE-LIGHT CLEAVABLE SCAFFOLDS

the synthesized compounds cleaved upon irradiation with visible light, even though photocleavage was slower than expected. Among all the conjugates, iodinated compound 13 emerged as most promising photocage, since it is clearly the most photoreactive structure among all the examined derivatives.

Even though the scalability of the reaction remains problematic and needs to be optimized for future applications, we were able to demonstrate the use of MCRs for the synthesis of visible-light responsive conjugates. By further extension of the substrate scope, the presented strategy can be used for the efficient synthesis of photocaged bioactive compounds connected to targeting moieties or anchoring groups in a one-step procedure. In conclusion, this approach provides a valuable step towards the development of new agents for photoactivated and targeted drug therapy.

AUTHOR CONTRIBUTIONS

F. Reeßing: Design; synthesis, purification and characterization of compounds 1-3 and 6-13; photochemical analysis of all conjugates

Dr. K. Sitkowska: Design; synthesis, purification and characterization of compounds 1-5 and 14; photochemical analysis of 6

EXPERIMENTAL SECTION

GENERAL INFORMATION

Starting materials, reagents and solvents were purchased from Sigma–Aldrich, Acros and Combi-Blocks and were used without any additional purification. Anhydrous solvents for the reactions were purified by passage through solvent purification columns (MBraun SPS-800). The reaction progress was monitored by Thin Layer Chromatography (TLC). TLC analyses were performed on commercial Kieselgel 60, F254 silica gel plates with fluorescence-indicator UV254 (Merck, TLC silica gel 60 F254). For detection of components, UV light at λ = 254 nm or λ = 365 nm was used. Flash column chromatography was performed with Silicagel, pore size 60 Å, 40-63 μm particle size.

Nuclear Magnetic Resonance spectra were measured with an Agilent Technologies 400-MR (400/S4 Premium Shielded) spectrometer (400 MHz). All spectra were measured at room temperature (22–24 °C). The multiplicities of the signals are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signal). All 13C-NMR spectra are 1H-broadband decoupled. High-resolution mass spectrometric measurements were performed using a Thermo Fisher scientific LTQ OrbitrapXL spectrometer with ESI ionization. The ions are given in m/z-units. Melting points were recorded using a Stuart analogue capillary melting point SMP11 apparatus. For spectroscopic measurements, solutions were measured in a 10 mm
quartz cuvette. UV/Vis absorption spectra were recorded on an Agilent 8453 UV-Vis spectrophotometer with diode array detection. Temperature-control was exerted through a Peltier based temperature controlled cuvette holder (QuantumNorthwest).

Irradiation experiments were performed with a $\lambda = 530$ nm (3x LXML PM01, optical power 810 mW, $\lambda_{\text{max}} = 526$ nm, FWHM 35.1 nm) and $\lambda = 650$ nm (3x LXML PD01, optical power 1200 mW, $\lambda_{\text{max}} = 652$ nm, FWHM 26.4 nm) LED system (Sahlmann Photochemical Solutions).

SYNTHETIC PROCEDURES AND COMPOUND CHARACTERIZATION

1: (5,5-Difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)methyl acetate

Compound 1 was prepared by a modification of literature procedures.34,35 2-Chloro-2-oxoethyl acetate (0.60 mL, 5.6 mmol) was added to a solution of 2,4-dimethylpyrrole (1.0 mL, 9.3 mmol) in dry DCM (40 mL) under a nitrogen atmosphere. The reaction mixture was stirred in the dark at room temperature for 24 h. Subsequently, the flask was opened and triethylamine (3.2 mL, 28 mmol) was added. The resulting mixture was allowed to stir for 15 min. Then, the flask was again put under nitrogen atmosphere and boron trifluoride diethyl etherate (5.2 mL, 42 mmol) was added. After one hour, another portion of triethylamine (3.2 mL, 28 mmol) and boron trifluoride diethyl etherate (5.2 mL, 42 mmol) was added. Then, silica was added to the flask and the solvents were evaporated. Compound 1 was purified by flash column chromatography (pentane/Et$_2$O, 2:1 v/v). The product was obtained as red-gold crystals (440 mg, 25% yield). $R_f = 0.7$ (DCM); Mol. 184-187 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 2.13 (s, 3H), 2.36 (s, 6H), 2.53 (s, 6H), 5.30 (s, 2H), 6.08 (s, 2H); $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -146.43 (dd, $J = 65.1, 32.5$ Hz); HRMS (ESI+) calcd. for [M+H]$^+$ (C$_{16}$H$_{20}$BF$_2$N$_2$O$_2$): 321.1580, found: 321.1585. $^1$H spectrum in agreement with published data.18
PASSERINI MULTI-COMPONENT REACTION FOR THE SYNTHESIS OF VISIBLE-LIGHT CLEAVABLE SCAFFOLDS

2: (5,5-Difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-y)methanol

The compound was prepared by modification of a literature procedure. A mixture of aq. NaOH solution (1.9 mL, 0.10 M) and MeOH (9 mL) was stirred for 10 min and then added to a solution of compound 1 (150 mg, 0.47 mmol) in DCM (4.5 mL). The reaction mixture was stirred for 4 h in the dark at room temperature. Next, H2O was added to the reaction mixture and the product extracted with DCM (3x). The combined organic layers were washed with 1 N aq. HCl and brine, and dried with MgSO4. The product was obtained as a red precipitate (101 mg, 78% yield). Rf = 0.3 (DCM); Mp. 247-249 °C; 1H NMR (400 MHz, CDCl3): δ 2.51 (s, 6H), 2.52 (s, 6H), 4.91 (s, 2H), 6.08 (s, 2H); 19F NMR (376 MHz, CDCl3): δ -146.52 (dd, J = 65.3, 32.4 Hz). HRMS (ESI+) calc. for [M+H]+ (C14H18BF2N2O): 279.1475, found: 279.1488. 1H NMR spectrum in agreement with published data.

3: 5,5-Difluoro-13,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-carbaldehyde

To a suspension of Dess–Martin periodinane (135 mg, 0.36 mmol) in dry THF (12.5 mL), a solution of compound 2 (50 mg, 0.18 mmol) in dry THF (12.5 mL) was slowly added at 0 °C. After 10 min, the reaction mixture was allowed to warm to room temperature and was left to stir overnight. Then, AcOEt was added, the mixture was washed with brine and dried with MgSO4. The crude mixture was purified by flash column chromatography (DCM). Compound 3 was obtained as a violet-green solid (30 mg, 60% yield). Rf = 0.8 (DCM); 1H NMR (400 MHz, CDCl3): δ 2.12 (s, 6H), 2.54 (s, 6H), 6.07 (s, 2H), 10.56 (s, 1H); 19F NMR (376 MHz, CDCl3): δ -146.15 (dd, J = 64.6, 32.3 Hz), 13C NMR (101 MHz, CDCl3) δ 15.4, 121.8, 128.8, 136.0, 141.5, 158.5, 193.0; HRMS (ESI+) calc. for [M+H]+ (C14H16BF2N2O): 277.1218, found 277.1338. 1H NMR spectrum in agreement with published data.
6: 2-(Benzylamino)-1-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-2-oxoethyl 4-fluorobenzoate

Compound 3 (10 mg, 0.04 mmol), 4-fluorobenzoic acid (8.5 mg, 0.07 mmol) and benzylisocyanide (8.4 mg, 0.08 mmol) were stirred at 40 °C in DCM (1 mL) for 4 d. The solvents were evaporated in vacuo and the crude mixture was purified by flash column chromatography (pentane/Et₂O, 4:1-1:4 v/v). The product was obtained as a red solid (4.1 mg, 22%).

1H NMR (400 MHz, CDCl₃): δ 2.33 (s, 3H), 2.54 (s, 6H), 2.58 (s, 3H), 4.42 (dd, J = 14.7, 5.7 Hz, 1H), 4.56 (dd, J = 14.7, 6.3 Hz, 1H), 6.11 (s, 1H), 6.14 (s, 1H), 6.26 (t, J = 6.3 Hz, 1H), 7.09-7.14 (m, 2H), 7.21-7.23 (m, 1H), 7.29-7.31 (m, 3H), 7.43 (s, 1H), 8.01 (m, 2H);

19F NMR (376 MHz, CDCl₃): δ -103.93 (tt, J = 8.4, 5.4 Hz);

13C NMR (151 MHz, CDCl₃) δ 15.0, 15.8, 17.3, 29.9, 44.3, 68.2, 77.2, 115.9 (d, J = 22.2 Hz), 123.2, 124.0, 125.1, 125.2, 128.0, 128.1, 131.4, 132.7 (d, J = 9.5 Hz), 132.8, 137.4, 142.0, 142.8, 157.3, 158.2, 164.8, 166.1, 166.2 (d, J = 249.8 Hz); HRMS (ESI+) calc. for [M+H]+ (C₂⁹H₂₈BF₃N₃O₃): 532.2014, found 532.2006.

7: 2-((2-(((tert-Butoxycarbonyl)amino)ethyl)amino)-1-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-2-oxoethyl 4-fluorobenzoate.

Compound 3 (15 mg, 0.05 mmol), 4-fluorobenzoic acid (11.35 mg, 0.08 mmol) and tert-butyl (2-isocyanoethyl)carbamate (13.78 mg, 0.08 mmol) were stirred at 35 °C in DCM (1 mL) for 1 d. Evaporation of the volatiles and purification by flash column chromatography (pentane/diethyl ether 4:1-1:4 v/v) yielded the product as a red solid (8.9 mg, 24%). Rf = 0.1 (pentane/AcOEt, 3:1 v/v); 1H NMR (400 MHz, CDCl₃): δ 1.30 (s, 9H), 2.43 (s, 3H), 2.52 (s, 3H), 2.54 (s, 3H), 2.62 (s, 3H), 3.17-3.33 (m, 2H), 3.33 – 3.53 (m, 2H), 4.84 (s, 1H), 6.09 (s, 1H), 6.15 (s, 1H), 7.18 – 7.06 (m, 2H), 7.40 (s, 1H), 7.96 – 8.15 (m, 2H); 19F NMR (376 MHz, CDCl₃): δ -104.17 (m), -142.04 – -148.31 (m); 13C NMR (101 MHz, CDCl₃) δ 14.9, 14.9, 15.9, 17.3, 28.3, 40.4, 42.2, 68.3, 80.2, 116.0 (d, J = 22.2 Hz), 123.2, 123.9, 125.3, 125.3, 132.9 (d, J = 9.5 Hz), 133.8, 141.9, 142.6, 156.4, 157.3, 158.0, 164.8, 165.0, 166.30 (d, J = 255.3 Hz), 166.7; HRMS (ESI-): calc. for [M-H]⁻ (C₂⁹H₂₈BF₃N₄O₅): 585.2514, found 585.2514.
8: 2-(tert-Butylamino)-1-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrrolo[1,2-c:2', 1'-f][1,3,2]diazaborinin-10-yl)-2-oxoethyl acetate.

Compound 3 (15 mg, 0.05 mmol), acetic acid (4.6 µL, 0.08 mmol) and tert-butyl isocyanide (9.2 µL, 0.08 mmol) were stirred at 35 °C in DCM (1 mL) for 2 d. Evaporation of the volatiles and purification by flash column chromatography (pentane/Et2O 4:1-1:4 v/v) yielded the product as a red solid (4.3 mg, 19%). Rf = 0.5 (pentane/AcOEt, 3:1 v/v); 1H NMR (400 MHz, CDCl3): δ 1.33 (s, 9H), 2.17 (s, 3H), 2.34 (s, 3H), 2.54 (s, 9H), 5.56 (s, 1H), 6.10 (s, 1H), 6.14 (s, 1H), 7.06 (s, 1H); 19F NMR (376 MHz, CDCl3): δ -145.26 – -146.88 (m); 13C NMR (101 MHz, CDCl3): δ 14.9, 14.9, 16.1, 17.1, 20.9, 28.8, 52.6, 67.9, 123.1, 123.7, 133.8, 141.8, 143.2, 146.2, 151.6, 157.1, 157.5, 164.8, 169.6; HRMS (ESI-) calc. for [M-H]-(C21H27BF2N3O3): 418.2119, found 418.2108.

9: 2-(Benzylamino)-1-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-2-oxoethyl dodecanoate.

Compound 3 (15 mg, 0.05 mmol), lauric acid (16.2 mg, 0.08 mmol) and benzyl isocyanide (9.9 µL, 0.08 mmol) were stirred at 35 °C in DCM (1 mL) for 2 d. Evaporation of the volatiles and purification by flash column chromatography (pentane/Et2O 4:1-1:4 v/v) yielded the product as a red solid (3.2 mg, 10%). Rf = 0.8 (pentane/AcOEt, 3:1 v/v); 1H NMR (400 MHz, CDCl3): δ 0.83 – 0.92 (m, 3H), 1.20 – 1.34 (m, 16H), 1.57 – 1.69 (m, 2H), 2.24 (s, 3H), 2.31 – 2.50 (m, 2H), 2.52 (s, 9H), 4.38 (dd, J = 14.7, 6.0 Hz, 1H), 4.54 (dd, J = 14.7, 6.0 Hz, 1H), 6.07 (s, 1H), 6.12 (s, 1H), 6.17 (t, J = 6.0 Hz, 1H), 7.17 – 7.23 (m, 2H), 7.22 (s, 1H), 7.25 – 7.34 (m, 3H); 19F NMR (376 MHz, CDCl3): δ -145.68 – -146.21 (m); 13C NMR (101 MHz, CDCl3): δ 14.3, 14.9, 14.9, 16.2, 17.2, 22.8, 24.8, 29.1, 29.4, 29.5, 29.7, 32.1, 34.1, 44.1, 67.3, 128.0, 128.0, 128.9, 133.3, 137.4, 166.1, 172.6; HRMS (ESI-) calc. for [M-H]- (C34H45BF2N3O3): 592.3528, found 592.3517.

10: 2-(Benzylamino)-1-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-2-oxoethyl acetate.

Compound 3 (15 mg, 0.05 mmol), acetic acid (4.6 µg, 0.08 mmol) and benzyl isocyanide (9.9 µL, 0.08 mmol) were stirred at 35 °C in DCM (1 mL) for 2 d. Evaporation of the volatiles and purification by flash column chromatography (pentane/Et2O 4:1-1:4 v/v) yielded the product as a red solid (9.3 mg, 38%). Rf = 0.3 (pentane/AcOEt, 3:1 v/v); 1H NMR (400 MHz, CDCl3): δ 2.18 (s, 3H), 2.23 (s, 3H), 2.53 (s, 9H), 4.39 (dd, J = 14.7, 6.0 Hz, 1H), 4.52 (dd, J = 14.7, 6.0 Hz, 1H), 6.07 (s, 1H), 6.12 (s, 1H), 6.18 (t, J = 6.0 Hz, 1H), 7.18 – 7.22 (m, 3H), 7.27 – 7.32 (m, 3H); 19F NMR (376 MHz, CDCl3): δ -145.49 – -146.95 (m); 13C NMR (101 MHz, CDCl3): δ 14.9, 14.9, 16.1, 17.2, 20.9, 44.2, 67.5, 123.2, 123.9, 124.4, 125.7, 128.0, 128.0, 128.9, 133.1, 137.4, 142.0, 143.3, 157.3, 157.8, 166.0, 169.6; HRMS (ESI-) calc. for [M-H]- (C24H23BF2N3O3): 452.1963, found 452.1952.
13: 2-(tert-Butylamino)-1-(5,5-difluoro-2,8-diiodo-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-y1)-2-oxoethyl acetate

Compound 4 (20 mg, 0.04 mmol), acetic acid (4.3 µL, 0.08 mmol) and tert-butyl isocyanide (8.6 µL, 0.08 mmol) were stirred at 40 °C in a mixture of DCM and chloroform (1:1 v/v, 1 mL) for two days. The volatiles were evaporated and the product purified by flash column chromatography (pentane/Et₂O, 9:1 v/v). The product was then dissolved in acetonitrile and the solution was washed with heptane (3x) to obtain a red solid (13 mg, 48%) after evaporation of the volatiles. R_f = 0.7 (pentane/EtOAc, 3:1 v/v); ^1H NMR (400 MHz, CDCl₃): δ 1.36 (s, 9H), 2.37 (s, 3H), 2.57 (s, 3H), 2.62 – 2.66 (m, 9H), 5.62 (s, 1H), 7.10 (s, 1H); ^19F NMR (376 MHz, CDCl₃): δ ~ -145.36 (ddd, J = 62.3, 31.6, 8.0 Hz); ^13C NMR (101 MHz, CDCl₃): δ 20.9, 52.8, 52.8, 68.6, 133.9, 137.7, 144.5, 158.1, 164.4, 168.5, 169.5; HRMS (ESI-) calc for [M-H]⁻ (C₂₁H₂₅BF₂I₂N₃O₃): 670.0052, found 670.0041.

14: 2-(tert-Butylamino)-1-(5,5-difluoro-3,7-bis((E)-4-methoxystyryl)-1,9-dimethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-y1)-2-oxoethyl acetate.

Compound 9 (15 mg, 29.3 µmol), acetic acid (4.3 µL, 75.2 µmol) and tert-butyl isocyanide (8.6 µL, 76.0 µmol) were stirred at 45 °C in DCM (1 mL) in a sealed tube for two days. The crude reaction mixture was purified by flash column chromatography (DCM – DCM/MeOH, 99:1 v/v). The product was obtained as a green solid (6.4 mg, 33%). R_f = 0.2 (DCM); ^1H NMR (400 MHz, CDCl₃): δ 1.35 (s, 9H), 2.19 (s, 3H), 2.41 (s, 3H), 2.61 (s, 3H), 3.86 (s, 6H), 5.61 (s, 1H), 6.74 (s, 1H), 6.78 (s, 1H), 6.94 (d, J = 8.4 Hz, 4H), 7.13 (s, 1H), 7.22-7.32 (m, 2H), 7.54-7.64 (m, 6H); ^19F NMR (376 MHz, CDCl₃): δ ~ -138.49 – -138.04 (m).
REFERENCES