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Imaging Agents

DMAP-BODIPY Alkynes: A Convenient Tool for Labeling Biomolecules for Bimodal PET–Optical Imaging**

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Abstract: Several new boron dipyrromethene/*N*,*N*-dimethylaminopyridine (BODIPY-DMAP) assemblies were synthesized as precursors for bimodal imaging probes (optical imaging, OI/positron emission tomography, PET). The photophysical properties of the new compounds were also studied. The first proof-of-concept was obtained with the preparation of several new BODIPY-labeled bombesins and evaluation of the affinity for bombesin receptors by using a competition binding assay. Fluorination reactions were investigated on DMAP-BODIPY precursors as well as on DMAP-BODIPY-labeled bombesins. Chemical modifications on the BODIPY core were also performed to obtain luminescent dyes emitting in the therapeutic window (650–900 nm), suitable for in vivo imaging, making these compounds promising precursors for PET/optical dual-modality imaging agents.

Introduction

Molecular imaging is a highly promising field of research and innovation with huge potential in a wide range of applications including prognostic, diagnostic, drug discovery, and development of theranostics. It enables real-time visualization of biochemical events at the cellular and molecular levels within the cells, tissues, and intact subjects.[1] In this area of research, nuclear imaging, namely positron emission tomography (PET), enables non-invasive diagnosis with high sensitivity by providing in vivo distribution of radiolabeled biovectors, thus facilitating detection of cancers. Among the radioelements available for PET imaging, ^{18}F has become the radionuclide of choice, in a similar way to ^{99m}Tc in gamma scintigraphy.[2] This is due to its optimal nuclear and chemical properties, that is, low energy, high abundance of positrons, and relatively long half-life (109.7 min) in comparison with the ^{11}C technique.[3]

Fluorescence imaging is a valuable technique for small animal imaging. The optical agents also provide the opportunity to perform histological studies based on the fluorescent signal to evidence molecular targeting. Clinical applications are unfortunately more limited owing to their poor penetrability in the tissues. Nevertheless, recent advances in fluorescence imaging instrumentation and probe developments promise new opportunities, highlighting the emerging performances of this technique, especially for fluorescence guided-surgery with targeted molecular imaging.[4]

Together, PET and fluorescence imaging presents complementary features, for example, the high penetrability of the 511 keV photons of PET and the high spatial and temporal resolution of fluorescence. These are some of the reasons why dual-modality PET/optical imaging could strongly be beneficial for preclinical and clinical applications.[5] In this context, the preparation of MonoMolecular Imaging Agents (MOMIA), which combine a fluorescent probe and a radioisotope into a single molecule, showed marked advantages compared with the conventional approach (i.e., sequential coupling of a chelator and a NIR fluorophore).[6] Notably, the modification of biomolecules on one single attachment point simplifies the dual-labeling process and minimizes the loss of affinity for the targeted receptors.[7]

Among the fluorescent dyes, BODIPY derivatives are highly suitable candidates for the design of bimodal agents because they generally provide high fluorescence quantum yields,[8] high thermal and photochemical stability, and a high degree of tunable emission into the red-NIR region from a simple variation of the molecular structure.[8b,9] Moreover, the BODIPY core bears two fluorine atoms onto its boron center (BF_2 unit), an ideal site for radiofluorination, considering the strength of the B–F bond ($>730\ kJ\,mol^{-1}$), which is one of the most thermodynamically stable covalent bonds known.[10] Beyond medical imaging, the concept of bimodality can be extended to other important areas. Indeed, promising work on applications of fluorinated BODIPY systems as bimodal XPS-fluorescence labels for the detection of amino groups on SiO_2 supports has recently been reported.[11]

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[**] *DMAP = N,N-dimethylaminopyridine; BODIPY = boron dipyrromethene; PET = positron emission tomography.*

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The high potential of ^{18}F-BODIPY dyes has recently been investigated and different methods have been developed for ^{18}F fluorination of BODIPY.[12] Indeed, rapid nucleophilic [^{18}F] radiolabeling of BODIPY dyes using a B-OH BODIPY precursor was previously reported by the group of Gabbaï and Conti.[13] B-OH BODIPY was pretreated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) as an activating/water removing agent, followed by radiofluorination.[14] This convenient approach was also exploited by the group of Weissleder and Mazitschek who employed a BF_2-BODIPY dye as starting material.[15] The group of Weissleder also performed a radiofluorination of *N*,*N*-dimethylaminopyridine (DMAP)-BODIPY precursors.[15,16] More recently, Lewis acids such as $SnCl_4$ were used to promote the ^{18}F labelling through a ^{19}F-^{18}F exchange reaction.[12,17] Additionally, in vivo stability of the B—^{18}F bond was investigated. No release of free $^{18}F^-$ was detected, proving the metabolic stability of the BF_2 core.[14–15]

Our research group has explored the possibility to expand the library of potential bimodal imaging probes based on [^{18}F]—BODIPY derivatives by using our long-standing experience in the field of BODIPY synthesis, including far-red emitting systems.[18] Moreover in our previous reports, we investigated the synthesis and the performances of BODIPY-chelators systems for bimodal single photon emission computed tomography (SPECT)/optical imaging.[19] In these systems, the radiolabeling was performed after bioconjugation, in the last synthetic step. We were thus very interested in the possibility of using a similar strategy, that is, the introduction of ^{18}F atoms during the final step.[20]

Such an approach has never been explored with [^{18}F]-BODIPY derivatives before. It requires the identification of a BODIPY probe bearing a living group that: 1) Could be stable under the bioconjugation coupling conditions; 2) Could allow ^{18}F labelling without using any additional reagent, such as a Lewis acid, considering the fragility of biomolecules. To fulfil these conditions, we used Weissleder's procedure, which replaces a DMAP group by ^{18}F on BODIPY without any other reagent (Figure 1).[15]

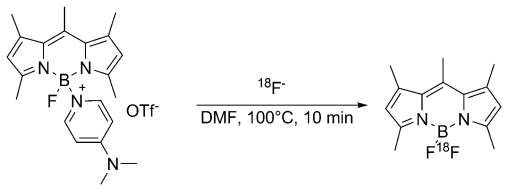
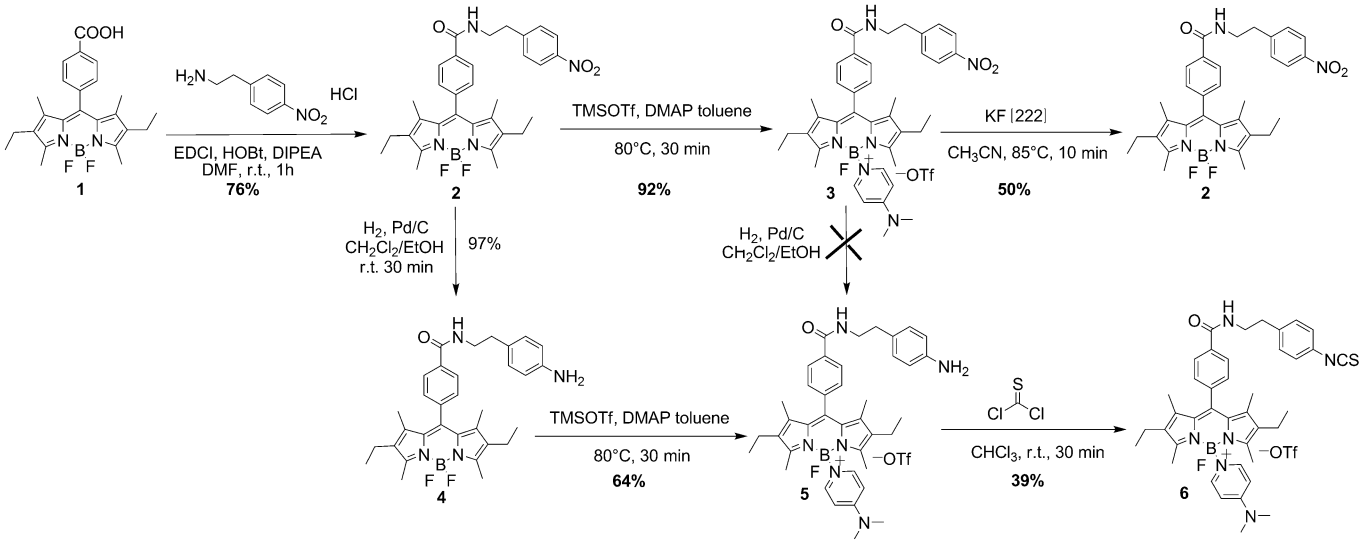


Figure 1. Synthesis of [^{18}F]-BODIPY through DMAP-activated BODIPY.

We report the synthesis and characterization of various DMAP-BODIPY precursors bearing activated functional groups for bioconjugation along with their photophysical properties. These DMAP-BODIPY assemblies have been attached to a bombesin derivative and in vitro tests towards the bombesin receptors are presented. Finally, far-red-emitting BODIPY compounds have been prepared to shift both the absorption and emission bands towards the therapeutic region for in vivo imaging.

Results and Discussion

The bifunctional target agent **6** was first prepared (Scheme 1). This molecule bears a DMAP ligand on the boron atom as a leaving group, which was previously validated as an adequate strategy for the incorporation of ^{18}F.[16] The compound is further activated using an isothiocyanate functional group for



Scheme 1. Synthesis of compound **6**.

its conjugation with amines. Compound **6** was obtained in four steps starting from the known precursor **1** (Scheme 1).[21] A nitrophenyl function was introduced by peptide coupling between **1** and 2-(4-nitrophenyl)ethanamine to obtain **2** in 76% yield. The nitro group was reduced into an amine function to yield **4** in almost quantitative yield.[18b] The next step involved the introduction of DMAP by first replacing one of the fluorine atoms by the leaving group TMSOTf and then adding DMAP in situ. Finally, the activation of the amine into an isothiocyanate was performed thus giving access to the desired compound **6**. Noteworthy, the introduction of the DMAP (i.e., formation of **3**) followed by the reduction of the nitro into an amine, as an alternative synthetic approach, was not successful because of the undesired loss of the DMAP group during the hydrogenation process.

The feasibility of a subsequent radiofluorination on the DMAP prosthetic compound was successfully verified by reaction of **3** with KF in the presence of the commonly used kryptofix 222 (KF [222]).[25] Compound **2** could then be obtained from **3** in 10 min, in 50% yield. The fluorination product can easily be monitored and characterized by using mass spectrometry and ^{11}B NMR spectroscopy. The ^{11}B NMR spectrum of the DMAP-BODIPY product exhibits the expected doublet, whereas the BF_2-BODIPY compound exhibits the typical triplet characteristic of the ^{11}B-^{19}F couplings ($I = {}^1/_2$). A typical example is shown in Figure 2.

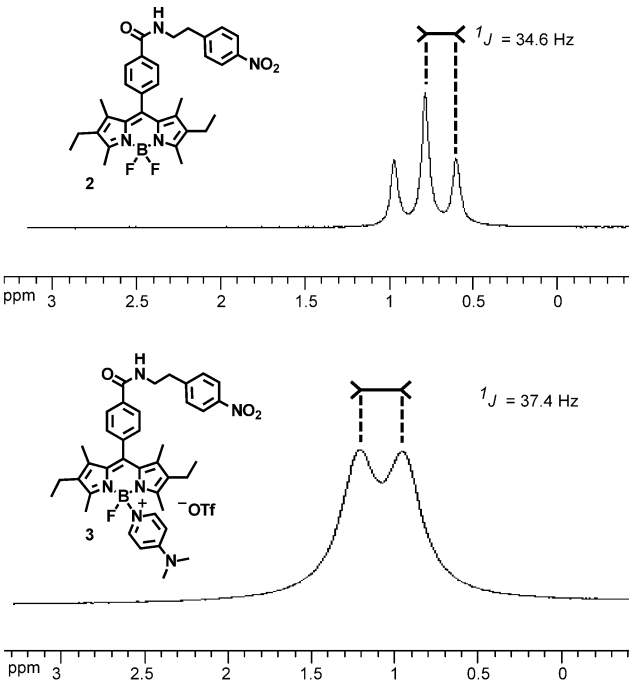
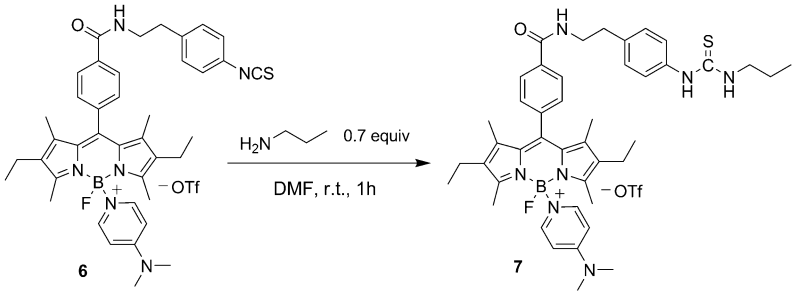


Figure 2. Comparison of the ^{11}B NMR spectra of compounds **2** and **3** (192.5 MHz, $CDCl_3$).

The feasibility of the bioconjugation step was evaluated by treating a slight excess of activated DMAP-BODIPY **6** (1.4 equiv) with propylamine, to mimic the actual conditions of bioconjugation (Scheme 2). However, the DMAP group proved to be very reactive towards amine, and yielded to some degradation products. Considering these difficulties, the substitution of the isothiocyanate by another activated function was investigated.

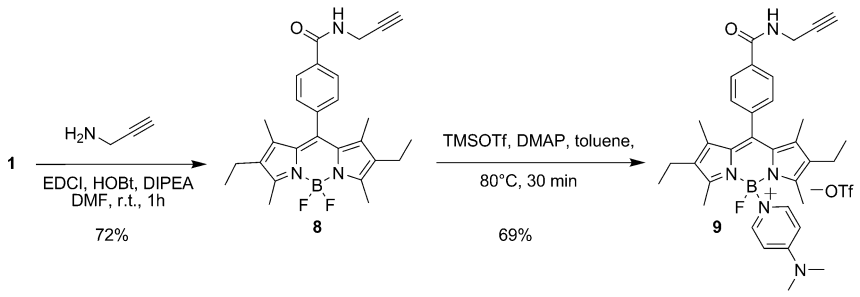


Scheme 2. Coupling test of **6** with propylamine.

Click chemistry provides a number of avenues for the design of complex structures and multicomponent functionalized systems.[22] In particular, the copper-catalyzed azide–alkyne cycloaddition has already become a powerful technique in peptide science due to its simplicity and bioorthogonality. It has been utilized in the cyclization of peptides, ligation of two or more peptide fragments, and conjugation to biomolecules, nanoparticles, polymers, and other chemical entities.[23] The impact of click chemistry on conjugation methods, especially bioconjugation, has been extensive in recent years. Indeed, azide functions can be easily introduced in peptides, and even in proteins at an adequate position. Such an approach allows for the rapid and site-selective labeling of biomolecules without modifying their binding properties to biological targets. However, implementing such a strategy requires first to functionalize the BODIPY with an alkyne function. Compound **9** was therefore synthesized in two steps, starting from **1** with, first, the introduction of a triple bond on the BODIPY using a peptidic coupling with prop-2-yne-1-amine, followed by the incorporation of the DMAP ligand (Scheme 3).

Click reaction tests were optimized starting from **9**. The coupling reaction was successful when the click conditions precluded any amine, such as diisopropylethylamine, as a base. Compound **10** was then obtained by treating **9** with one equivalent of azidomethylbenzene using $CuSO_4$ as catalyst and sodium ascorbate (Scheme 4). Noteworthy, the DMAP group desirably remained anchored on the BODIPY in these conditions. Substitution of the DMAP with one equivalent of F^- in the presence of kryptofix 222 could then be performed on **10**, yielding **11** in 42% yield.

A click reaction was also performed on a bombesin derivative, N_3-AEEAc-bombesin(7–14) (**N_3-BBN**). Bombesin is a 14-mer peptide that binds to three G-protein-coupled receptors (GRP-



Scheme 3. Synthesis pathway of **9**.

R, NMB-R, and BRS-3) overexpressed in a wide variety of tumor tissues. Bombesin and its analogues have been extensively studied as targeting agents for the detection of cancer in preclinical and clinical studies using numerous imaging modalities.[24] Here, the widely used 8-mer peptide bombesin(7–14), which contains the minimal sequence required for high affinity binding to its receptors, was chosen as model. The peptide was acylated on its N-terminal with an *N*-(2-(2-(2-azidoethoxy)ethoxy))acetyl chain (N_3-AEEAc) to introduce the azide function and to provide flexibility and water solubility to the conjugate. The coupling reaction, monitored by HPLC, provided **12** in 59% yield (Scheme 5).

The fluorination reaction was then performed by treating compound **12** with one equivalent of KF in dry acetonitrile (Scheme 6), yielding the desired product **13** in 35% yield after 10 min. This fluorination reaction was competitive with the hydrolysis of the B-DMAP bond, which yielded the side product **14**. Traces of water were probably present in the KF salt.

The fluorination should be performed in strictly anhydrous conditions to optimize the yield of formation of BF_2-BODIPY.[25] An interesting point is that the B–OH function slowly reacts with F^-, and could be radiofluorinated using acidic conditions, even if these conditions are not ideal for BODIPY.[14]

Figure 3 shows the absorption, fluorescence, and excitation spectra of compounds **12** (A) and **13** (B). Both compounds exhibit two absorption bands characteristic of the BODIPY signature, which consists of the S_0–S_1 feature placed near 520 nm, and the S_0–S_2 one located near 380 nm. Both bands are readily assigned to spin-allowed π–π* transitions. The emission bands are observed at ≈540 nm and exhibit a mirror-image relationship with the absorption, indicating that the structural perturbation in the excited state is minimal. This observation is corroborated by the fact that the excitation spectra superpose well with the absorption ones. The absorption features located in the 200–300 nm range are due to the absorption of tryptophan side chain of the bombesin derivative. Interestingly, the DMAP loss going from **12** to **13** is accompanied by the disappearance of its characteristic absorption band at 279 nm.

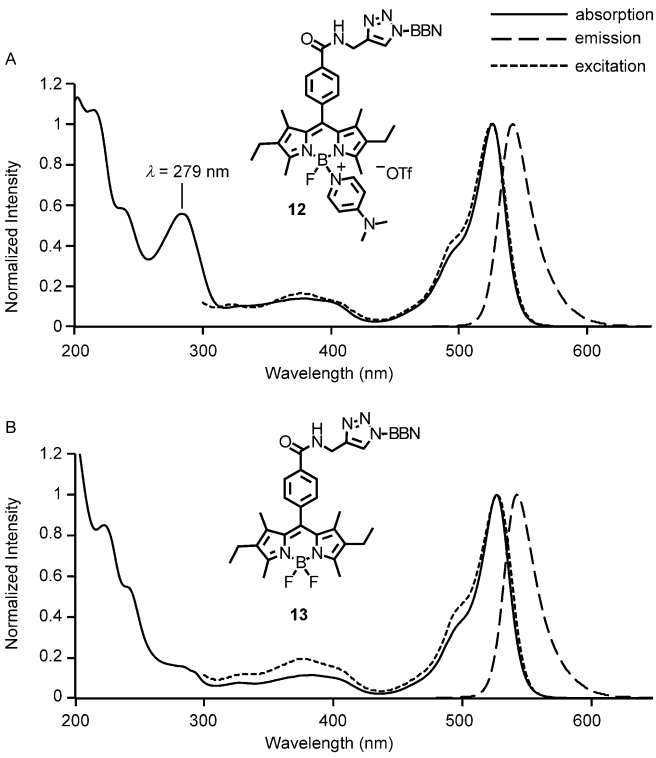
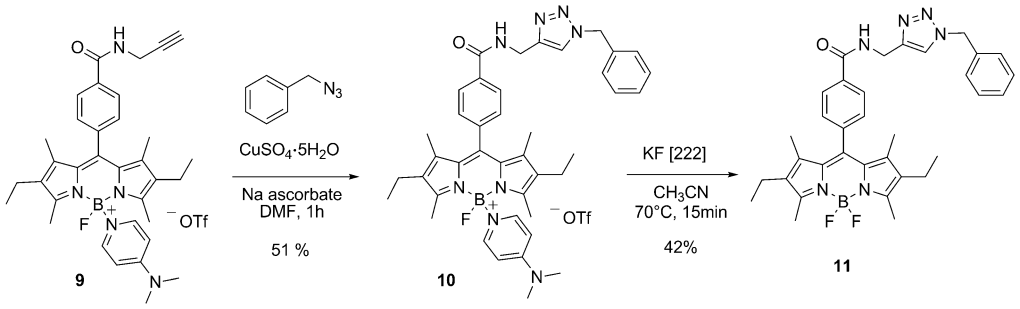
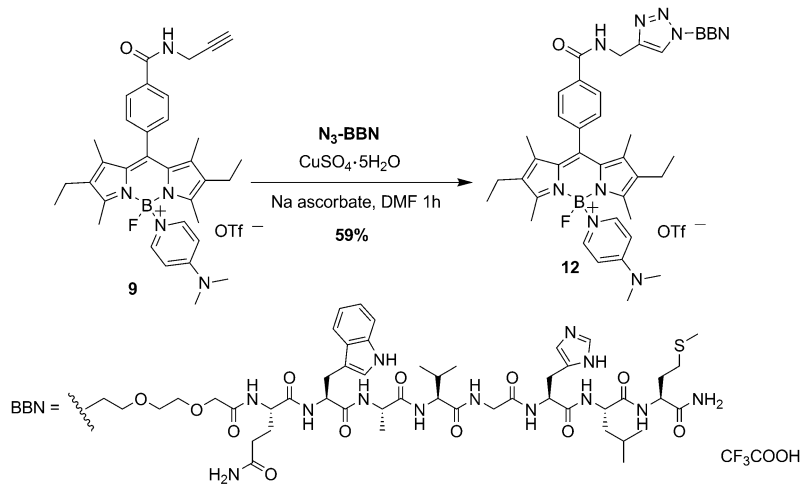


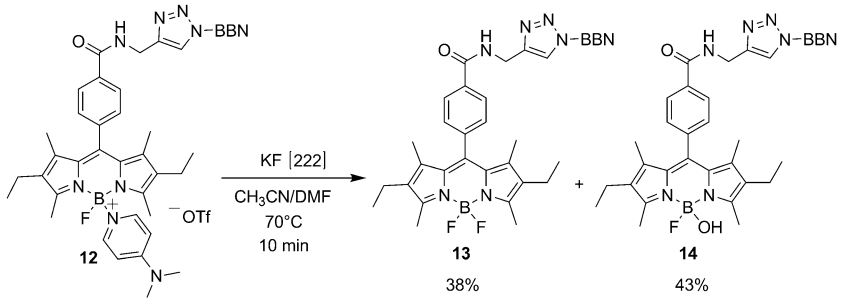
Figure 3. Absorption, emission (λ_{ex} = 470 nm) and excitation (λ_{ex} = 590 nm) spectra of **12** and **13** in 2-MeTHF.



Scheme 4. Synthesis and fluorination of compound **10**.



Scheme 5. Click reaction of **9** with **N_3—BBN**.



Scheme 6. Fluorination test on compound **12**.

Biological testing

The affinity of the bioconjugate **13** for bombesin receptors (on rat cerebral cortex membranes) was evaluated on a radioactive binding assay using [^{125}I]-[Tyr^4]bombesin(1–14) as a ligand.[26] Under these assay conditions, compound **13** was able to compete for the binding of the radioligand with a K_i of (0.33 ± 0.45) nM, whereas the unlabeled peptide **N_3-BBN** exhibited a K_i of (0.36 ± 0.19) nM (Figure 4). This result illustrates that, despite its size, conjugation of the BODIPY does not modify significantly the binding affinity of a small peptide such as BBN(7–14) for its receptors.

Towards the therapeutic region for in vivo imaging

Dyes active in the so-called therapeutic window (650–900 nm) are more adapted for in vivo applications, such as molecular imaging. Advantages include minimal interfering absorption and fluorescence from biological samples, reduced scattering, and enhanced tissue penetration depth. One simple way to extend the π-conjugation and to shift the absorption and emission bands to the NIR region is to perform a Knoevenagel condensation on the methyl groups at the α-pyrrolic positions.[27] Indeed, the target distyryl compound **15** was prepared in 79% yield by treating **8** with four equivalents of 4-methoxybenzaldehyde, in the presence of piperidine and *p*-toluenesulfonic acid (PTSA; Scheme 7). The DMAP was then introduced onto **15** and the resulting activated precursor **16** was subsequently attached to **N_3-BBN** in 53% yield.

The formation of the monostyryl derivative is also interesting because the dye exhibits an emission in the red region (at around 600 nm). In addition, it may offer the possibility of a second Knoevenagel functionalization using a selected aldehyde (bearing a grafting function for example).[28] Thus, the monofunctionalized compound **19** was synthesized by reacting **18** with 4-(prop-2-yn-1-yloxy)-benzaldehyde (Scheme 8). The low reaction yield is explained by the difficulty to control the mono- versus di-condensation and problems with the purification. As previously shown for other derivatives, the DMAP could be anchored and the resulting assembly **20** was attached to the bombesin derivative in 31% yield.

The ^{1}H NMR spectrum of compound **20** is shown in Figure 5. Each substituent in the α- and β-pyrrolic positions displays one signal. The substituents located on the side of the styryl group are the most deshielded. For instance, the two methyl groups

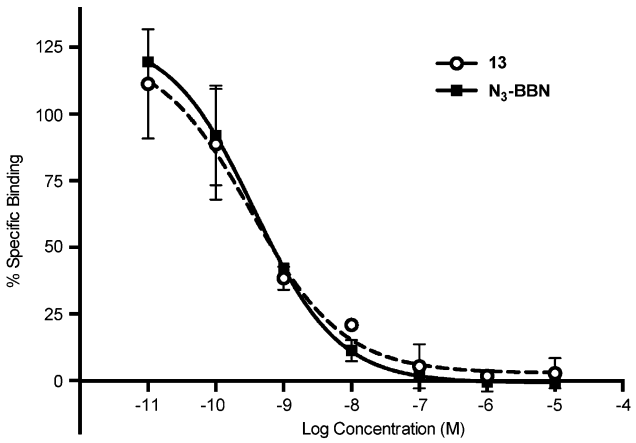
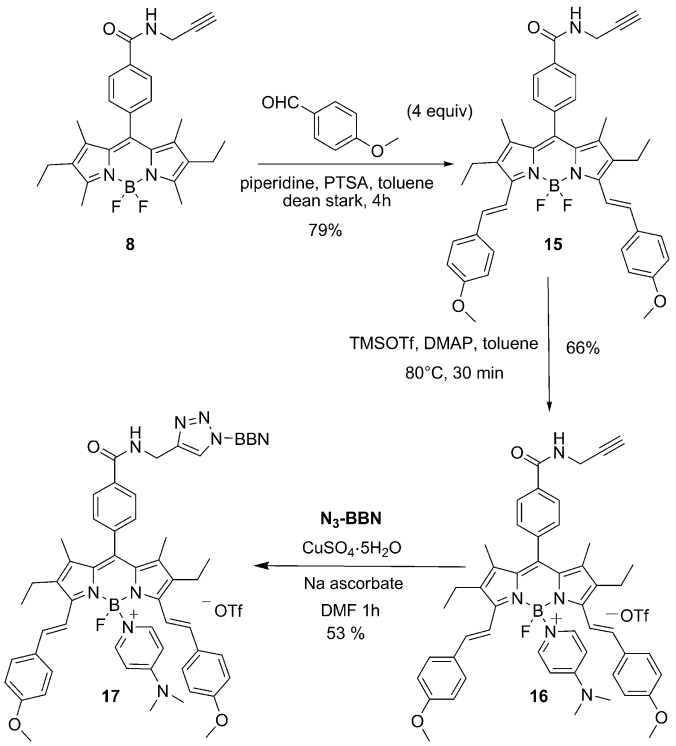
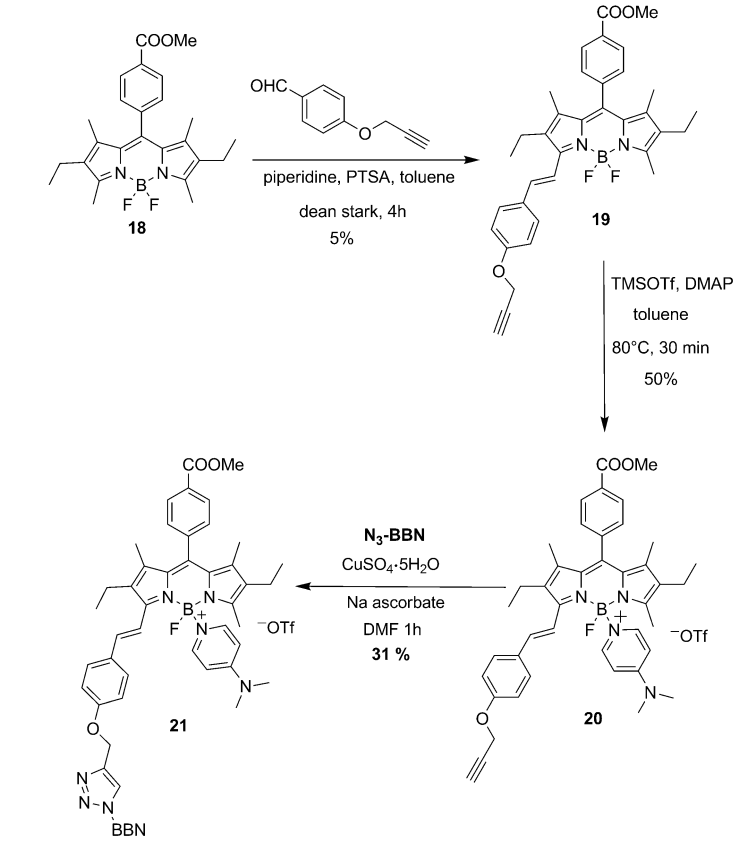


Figure 4. Displacement of radioligand [^{125}I]-[Tyr^4]bombesin(1–14) binding at rat cerebral cortex membranes by **13** and **N_3-BBN**. Non-specific binding was defined by 1 μM cold bombesin (1–14).



Scheme 7. Synthetic pathway of **17**.



Scheme 8. Synthesis pathway of **21**.

in β-pyrrolic positions display two singlets at $\delta = 1.34$ and 1.36 ppm. The tetrahedral geometry of the boron atom induces that the DMAP ligand is orthogonal to the indacene plane. Consequently, the two faces of the indacene are dissymmetric and the boron atom in BODIPY **20** is a chiral center. Therefore, protons carried by the CH_2 groups in β-pyrrolic positions are diastereotopic. Notably, protons 7 and 7′, which are on the side of the styryl group, display each a quartet ($^3J = 7.3$ Hz) at $\delta = 2.43$ and 2.45 ppm, split by a geminate coupling constant ^1J of 12.2 Hz. Additionally, as the methyl in β-pyrrolic position impedes the rotation of the aromatic ring at the *meso* position, the four protons of the aromatic ring display four signals, whereas they display two doublets for a symmetrical BF_2-BODIPY. Each proton displays a doublet of doublets ($^3J = 7.9$ Hz, $^4J = 1.5$ Hz).

Photophysical studies

Table 1 summarizes the absorption and steady-state fluorescence data of the different BODIPY-containing compounds. Noteworthy, the photophysical data of **12**, **13**, and **21** were measured using DMSO/PBS 1:1 as a solvent mixture due to lack of solubility in PBS (phosphate buffered saline). The emission lifetimes, τ_F ($3.9 < \tau_F < 6.9$ ns), and the small Stokes shifts, $\Delta\nu$, confirm the presence of fluorescence. The fluorescence quantum yields, ϕ_F, vary from 35 to 87% and are considered large enough for imaging applications.

Generally, the introduction of DMAP group onto the BODIPY chromophore induces a bathochromic shift of the absorption and emission maxima. Moreover, the Stokes shifts ($\Delta\nu$ in cm^{-1} units; a linear scale of energy) of the DMAP-containing BODIPYs are larger than in their BF_2 analogues. For instance, compounds **15** and **16** display fluorescence maxima at 681 and 703 nm, respectively, with Stokes shifts of 610 and 950 cm^{-1}. The introduction of styryl substituents induces a bathochromic shift of the absorption and fluorescence bands respectively by ≈60 and 130 nm for the mono- and distyryl-BODIPY derivatives, and does not affect their fluorescence quantum yields. Finally, compounds **12**, **13**, and **21** display high fluorescence quantum yields ($0.35 < \phi_F < 0.40$), confirming that the introduction of bombesin on dyes **8**, **9**, and **20** does not affect the intense luminescence properties of BODIPY.

Conclusion

Several new potential bimodal agents for PET–optical imaging have been synthesized and characterized. The photophysical study demonstrated that these BODIPY-containing systems exhibit high fluorescence quantum yields. Bioconjugation on the bombesin derivative **N_3-BBN** and a competitive binding assay with BF_2-BODIPY-BBN were performed evidencing that the presence of the bimodal agent does not affect the affinity of the peptide for the receptors. Thus, the use

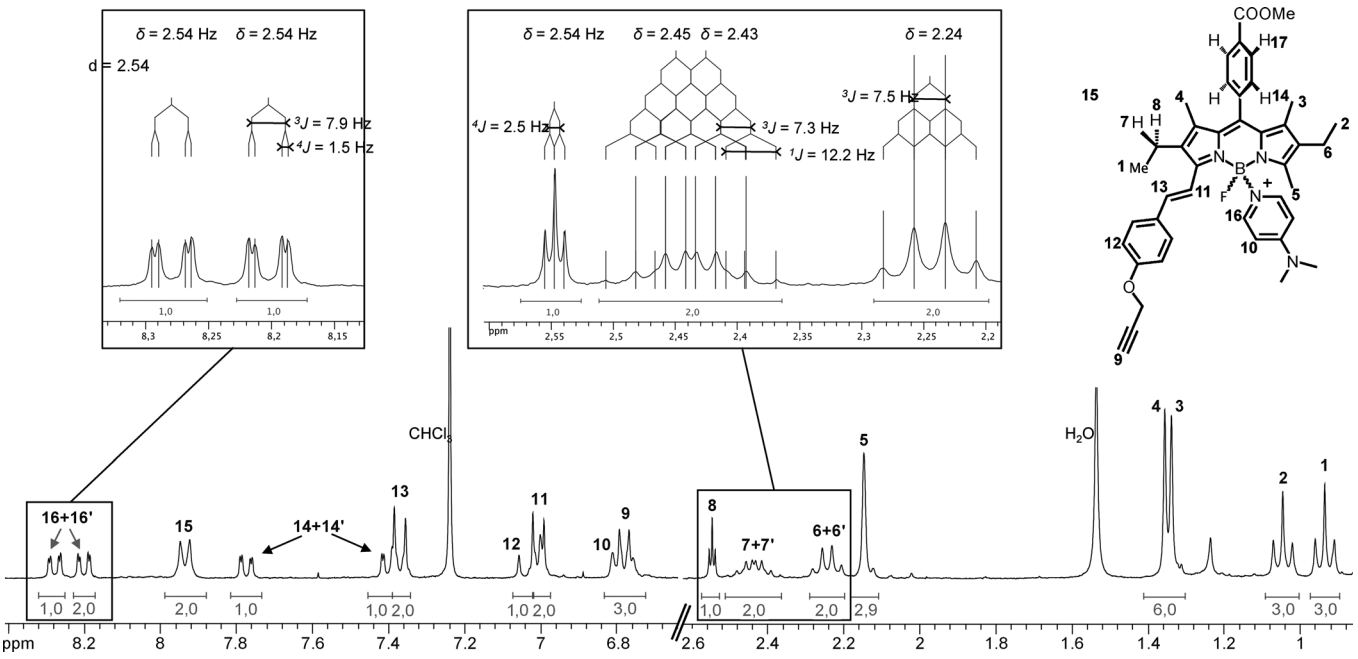


Figure 5. ^{1}H NMR spectrum and assignments of **20** ($CDCl_3$, 300 MHz, 298 K).

Table 1. Photophysical properties of selected BODIPY derivatives.[a]

Dye	λ_{abs} [nm]	λ_{em} [nm]	$\Delta\nu$ [cm^{-1}]	ϕ_F	τ_F [ns]	Solvent
2	526	540	490	0.59[a]	3.9	2-MeTHF
3	527	544	590	0.54[a]	6.2	2-MeTHF
6	528	544	560	0.37[a]	5.6	2-MeTHF
8	525	543	630	0.59[a]	4.6	2-MeTHF
9	528	543	520	0.64[a]	6.9	2-MeTHF
12	525	542	600	0.35[a]	–	DMSO/PBS 1:1
13	527	542	530	0.40[a]	–	DMSO/PBS 1:1
15	654	681	610	0.42[b]	6.3	2-MeTHF
16	659	703	950	0.51[b]	4.7	2-MeTHF
17	654	–	–	–	–	CH_3CN
19	587	603	450	0.76[c]	5.4	2-MeTHF
20	594	617	630	0.87[c]	4.7	2-MeTHF
21	592	615	630	0.51[c]	–	DMSO/PBS 1:1

[a] Stokes shift, $\Delta\nu$, was calculated using the Equation: $1/\lambda_{abs} - 1/\lambda_{em}$. The quantum yields, ϕ_F, were measured at 298 K, using rhodamine 6G ($\phi_F = 0.94$ in methanol), rhodamine 101 ($\phi_F = 1.00$ in methanol) and cresyl violet ($\phi_F = 0.54$ in methanol) as references.[29,31]

of [^{18}F]-BODIPY, instead of bulky multimodal chelation platforms, could be a relevant alternative for the labeling of small biovectors such as peptides. Additionally, the fluorination of the labeled biovectors was investigated and showed the possibility of introducing ^{18}F. Finally, several monostyryl and distyryl derivatives were synthesized, showing the high potential of these systems for future in vivo imaging, even though some issues concerning their hydrosolubility will have to be addressed.

Experimental Section

Materials

Unless otherwise noted, all chemicals and solvents were of analytical reagent grade and were used as received. Absolute dichloromethane (CH_2Cl_2) was obtained from Carlo Erba. Silica gel (Merck; 70–120 mm) was used for column chromatography. Analytical thin-layer chromatography was performed with Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick). Reactions were monitored by thin-layer chromatography, RP-HPLC, UV/Vis spectroscopy, and MALDI/TOF mass spectrometry.

Instrumentation

The ^{1}H, ^{11}B, and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance II 300 (300 MHz) or on a Bruker Avance DRX 600 (600 MHz) spectrometer at the "Welience, Pôle Chimie Moléculaire de l'Université de Bourgogne (WPCM)". Chemical shifts (^{1}H NMR spectra) are expressed in ppm relative to chloroform ($\delta = 7.26$ ppm). The UV/Visible spectra were recorded on a Varian Cary 1 spectrophotometer. The mass spectra and accurate mass measurements (HRMS) were obtained on a Bruker Daltonics Ultraflex II spectrometer in the MALDI/TOF reflectron mode using dithranol as a matrix or on a LTQ Orbitrap XL (Thermo Scientific) instrument in ESI mode. Both measurements were registered at the WPCM. The steady-state fluorescence emission and excitation spectra were obtained on a Fluorolog SPEX 1680 0.22 m double monochromator spectrometer using quartz cuvettes (1 cm, 3 mL). All fluorescence spectra were corrected for apparatus response. The fluorescence lifetimes were measured in 2-MeTHF or DMSO/PBS 1:1 using a Timemaster Model TM-3/2003 apparatus from PTI incorporating a nitrogen laser as the source and a high-resolution dye laser (full width at half maximum, fwhm = 1.4 ns). Fluorescence lifetimes were obtained from high-quality decays and deconvolution or distribution lifetime analysis. The uncertainties were 100 ps on the

basis of multiple measurements, which is also the limit of accuracy of this system.

Binding assays

Compounds **13** and **N_3-BBN** were tested by Cerep (Poitiers, France) on a radioactive binding assay. Briefly, rat cerebral cortex membranes, expressing GPR-R, NMB-R, and BRS-3 receptor subtypes, were incubated in the presence of 0.01 nM [^{125}I]-[^{Tyr}4]bombesin ligand (K_D = 0.71 nM) and a range of concentrations of tested compounds for 60 min at room temperature. Non-specific binding was determined in presence of an excess (1 μM) of cold bombesin(1–14). The results are expressed as a percent of control specific binding. The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) were determined by non-linear regression analysis of the competition curves generated with duplicate values using GraphPad Prism version 6.00 for Mac. The corresponding inhibition constants (K_i) were calculated using the Cheng–Prusoff equation.[30] Bombesin(1–14) was used as a reference compound (K_i = 0.17 nM). Further details of the methodology for the assay can be found at http://www.cerep.fr.

Quantum yield measurements

Measurements were performed in distilled 2-MeTHF or in DMSO/PBS (1:1). Quartz cuvettes of 3 mL with path length of 1 cm equipped with a septum were used, and all solutions were Ar-degassed prior to measurements. Three different measurements (i.e., different solutions) were performed for each quantum yield. The sample concentrations were chosen to obtain an absorbance of about 0.05. The fluorescence quantum yield (ϕ_F) measurements were performed with the slit width of 0.5–1.5 nm for both excitation and emission. Relative quantum efficiencies were obtained by comparing the areas under the corrected emission spectra of the sample relative to a known standard and the following Equation was used to calculate ϕ_F: $\phi_F(\text{sample}) = \phi_F(\text{standard}) \times (I(\text{sample})/I(\text{standard})) \times (A(\text{standard})/A(\text{sample})) \times (\eta(\text{sample})^2/\eta(\text{standard})^2)$, in which ϕ_F(standard) is the reported quantum yield of the standard, I is the integrated emission spectrum, A is the absorbance at the excitation wavelength and η is the refractive index of the solvents used. Rhodamine 6G (ϕ_F = 0.94 in methanol),[29a] Cresyl violet (ϕ_F = 0.94 in methanol),[29c] and Rhodamine 101 (ϕ_F = 1.00 in methanol)[31] were used as standards.[32] In all ϕ_F determinations, correction for the solvent refractive index (η) was applied (2-MeTHF: η = 1.406, DMSO/PBS 1:1: η = 1.416 methanol: η = 1.328).[33]

Purification and analysis of BODIPY-peptides

The crude products were purified by semi-preparative RP-HPLC on a Dionex Ultimate 3000 system (Thermo Scientific) equipped with a Nucleodur C18ec column (Macherey–Nagel, 250×10 mm, 5 μm) with a gradient program (solvent A is water with 0.1% TFA and solvent B is acetonitrile with 0.1% TFA) at a flow rate of 2 mL min^{-1} with UV detection at 254, 530, 590, and 650 nm. Fractions were analyzed by using RP-HPLC on a Dionex Ultimate 3000 system, with a photodiode array detector, on a Chromolith High-Resolution C18ec column (Merck, 50×4.6 mm), equipped with a guard column (5×4.6 mm), at a flow rate of 3 mL min^{-1} and the pure fractions were collected and lyophilized to yield the final compounds as highly purified (>95%) white, red, purple, or blue solids. The purity of the compounds was determined by RP-HPLC at 254 nm. The identity of the compounds was checked by low-resolution electrospray mass spectrometry (ESI-MS; Bruker) or by high-resolution electrospray mass spectrometry (HRMS; Orbitrap, Thermo Scientific).

N_3-BBN: The peptide (*N*-(2-(2-(2-azidoethoxy)ethoxy))acetyl-bombesin(7–14)) was obtained by manual solid-phase peptide synthesis (0.48 mmol scale) on a Rink Amide AM resin (Iris Biotech, 0.48 mmol g^{-1}). All amino acids, from Iris Biotech or Novabiochem, were *N*-α-terminal-protected by the fluorenylmethyloxycarbonyl group (Fmoc). 2-(2-(2-azidoethoxy)ethoxy))acetic was synthesized according to the literature.[34] Briefly, the resin (1.0 g) was swelled in DMF for 30 min and Fmoc was removed by treating the resin twice with 20% piperidine, 0.1 M HOBt in DMF (10 mL) for 10 min. After the second treatment, the resin was washed with DMF (3× 10 mL) and CH_2Cl_2 (3×10 mL). Coupling reactions were performed by adding to the resin a pre-made coupling cocktail of *N*,*N*-**diisopropylethylamine** (DIPEA, 3 equiv), 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 3 equiv) and the required amino acid or 2-(2-(2-azidoethoxy)ethoxy))acetic acid (3 equiv) in DMF (10 mL). The suspension was left shaking for 1 h, drained and washed with DMF (3×10 mL) and CH_2Cl_2 (3× 10 mL). The final peptide was cleaved from the resin (200 mg) with simultaneous removal of the side-chain protecting groups by treatment with trifluoroacetic acid/triisopropylsilane/3,6-dioxa-1,8-octanedithiol/water (TFA/TIS/DODT/H_2O, 94:2.5:2.5:1 v/v; 5 mL) at room temperature for 1.5 h. The filtrate from the cleavage mixture was concentrated, precipitated in cold Et_2O and collected by centrifugation (twice), and lyophilized to afford crude peptide.

The product was purified by semi-preparative RP-HPLC from 20 to 50% of B over 30 min to give **N_3-BBN** as a white solid (TFA salt, 25 mg, 36%, purity: >97%, t_R = 2.33 min). ESI-MS: *m/z* = 1111.6 $[M+H]^+$; 1133.55 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{49}H_{75}N_{16}O_{12}S^+$: 1111.54656; found: 1111.54399.

Compound 1: 2,4-Dimethylethylpyrrole (10.3 g, 83.9 mmol) and 4-carboxybenzaldehyde (6.30 g, 42.0 mmol) were dissolved in dichloromethane (2.60 mL). Trifluoroacetic acid (328 μL) was added and the mixture was stirred at room temperature for 48 h. A solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 9.53 g, 42.0 mmol) in dichloromethane was added and the mixture was stirred for 50 min. Triethylamine (84.0 mL, 630 mmol) and BF_3·Et_2O (85.0 mL, 672 mmol) were added and the solution turned purple. After 2 h of stirring, the reaction mixture was washed with water, dried over magnesium sulfate, and the solvent was evaporated. The residue was purified by silica gel column chromatography (AcOEt/hexane, 90:10). Recrystallization in a mixture of dichloromethane and hexane gave **1** as a red solid (8.40 g, 47%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (t, 6H, ^3J = 7.5 Hz), 1.21 (s, 6H), 2.24 (q, ^3J = 7.5 Hz, 4H), 2.47 (s, 6H), 7.39 (d, ^3J = 8.4 Hz, 2H), 8.18 ppm (d, ^3J = 8.4 Hz, 2H).

Compound 2: *N*-Hydroxybenzotriazole (640 mg, 4.70 mmol), diisopropylamine (1.15 mL, 7.10 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (900 mg, 4.70 mmol), and 4-nitrophenethylamine hydrochloride (0.49 g, 2.40 mmol) were added dropwise to a solution of **1** (1.00 g, 2.40 mmol) in dimethylformamide (50 mL) and the solution was stirred at room temperature. After total consumption of the starting material (2 h) monitored by TLC, the solvent was evaporated. The resulting solid was washed with water (2×100 mL) and extracted with dichloromethane. The organic layer was dried over magnesium sulfate and the solvent was evaporated to give a red oil. The crude product was purified by column chromatography on silica gel (ethyl acetate/hexane, 60:40) followed by recrystallization in a mixture of dichloromethane and hexane to give pure **2** as a reddish solid (1.21 g, 76% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.95 (t, ^3J = 7.5 Hz, 6H), 1.22 (s, 6H), 2.27 (q, ^3J = 7.5 Hz, 4H), 2.51 (s, 6H), 3.10 (t, ^3J_A = 7.1 Hz, 2H),

3.78 (td, $^3J_A=7.1$ Hz, $^3J_B=6.0$ Hz, 2H), 6.27 (t, $^3J_B=6.0$ Hz, 1H), 7.37 (d, $^3J=8.3$ Hz, 2H), 7.43 (d, $^3J=8.7$ Hz, 2H), 7.83 (d, $^3J=8.3$ Hz, 2H), 8.19 ppm (d, $^3J=8.7$ Hz, 2H).

Compound 3: Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 1.05 mmol, 190 μL) and dimethylaminopyridine (DMAP, 1.05 mmol, 128 mg) were added to a solution of **2** (300 mg, 0.524 mmol) in dry toluene (15 mL), and the mixture was stirred for 30 min at 80 °C. The solvent was evaporated; the residue was washed with water (3×20 mL) and dried over magnesium sulfate. The solution was concentrated to dryness and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 97:3) to give **3** as a red solid (397 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.91$ (t, $^3J=7.5$ Hz, 6H), 1.30 (s, 6H), 2.13 (s, 6H), 2.20 (q, $^3J=7.5$ Hz, 4H), 3.13 (t, $^3J_A=7.5$ Hz, 2H), 3.21 (s, 6H), 3.78 (td, $^3J_A=7.5$ Hz, $^3J_B=5.9$ Hz, 2H), 6.86 (d, $^3J=7.7$ Hz, 2H), 7.34 (dd, $^3J_C=8.0$ Hz, $^4J_D=1.8$ Hz, 1H), 7.47 (d, $^3J=8.7$ Hz, 2H), 7.55 (dd, $^3J_C=8.0$ Hz, $^4J_D=1.8$ Hz, 1H), 7.71 (t, $^3J_B=5.9$ Hz, 1H), 7.98 (d, $^3J=7.7$ Hz, 2H), 8.11 (dd, $^3J_C=8.0$ Hz, $^4J_D=1.8$ Hz, 1H), 8.12 (d, $^3J=8.7$ Hz, 2H), 8.13 ppm (dd, $^3J_C=8.0$ Hz, $^4J_D=1.8$ Hz, 1H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): $\delta=$ 12.2 (×2), 12.5 (×2), 14.0 (×2), 17.0 (×2), 29.7 (×2), 35.6, 40.1, 107.6–123.7–128.0–128.3–128.5–128.9–129.9–130.9–134.8–135.4–137.1–141.0–141.7–142.0–146.7–147.5–154.0–156.2 (×28), 166.7 ppm; ^{11}B NMR (192.5 MHz, $CDCl_3$): $\delta=1.08$ ppm (d, $^1J=37.4$ Hz); UV/Vis (THF): λ (ε): 527 (62), 498 (21), 377 (8), 285 (33), 242 nm (35×10^{-3} L mol^{-1} cm^{-1}); ESI-MS: $m/z=553.3$ $[M-C_7H_{10}N_2-OTf]^+$, 675.3 $[M-OTf]^+$. HRMS (ESI): *m/z* calcd for $C_{39}H_{45}BF_1N_6O_3^+$: 675.36315; found: 675.36018.

Fluorination of compound 3: 13,16,21,24-hexaoxa-1,10-diazabicyclo-(8,8,8)-hexacosane (23.0 mg, 61.0 μmol) and potassium fluoride (4.00 mg, 69.0 μmol) were added to a solution of 50 mg of **3** (61 μmol) in acetonitrile (2.5 mL). The solution was heated to 70 °C and the reaction was monitored by TLC. After 10 min, the solvent was removed under reduced pressure. Water (5 mL) was added and the mixture was extracted with dichloromethane (3×5 mL). The organic layer was dried over magnesium sulfate and the solvent was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/hexane, 70:30) followed by recrystallization in a mixture of dichloromethane and hexane to give pure **2** as a red solid (17.0 mg, 50%).

Compound 4: Compound **2** (300 mg, 524 μmol) was placed in a mixture of dichloromethane and ethanol (45 mL, 1:1), and 10% Pd/C (5.00 mg, 24.0 μmol) was added under H_2. After total consumption of the starting material (1 h) followed by TLC, the suspension was eliminated by filtration on Clarcel and the solvent was evaporated. The crude product was recrystallized in a mixture of dichloromethane and hexane to give **4** as a red solid (277 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.94$ (t, $^3J=7.5$ Hz, 6H), 1.21 (s, 6H), 2.26 (q, $^3J=7.5$ Hz, 4H), 2.50 (s, 6H), 2.83 (t, $^3J_A=7.1$ Hz, 2H), 3.69 (td, $^3J_A=7.1$ Hz, $^3J_B=6.0$ Hz, 2H), 6.36 (t, $^3J_B=6.0$ Hz, 1H), 6.65 (d, $^3J=8.3$ Hz, 2H), 7.02 (d, $^3J=8.3$ Hz, 2H), 7.31 (d, $^3J=8.2$ Hz, 2H), 7.81 ppm (d, $^3J=8.2$ Hz, 2H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): $\delta=12.1$ (×2), 12.8 (×2), 14.8 (×2), 17.3 (×2), 35.0, 41.7, 115.7 (×2), 127.8 (×2), 128.7 (×2), 129.0 (×2), 129.9, 130.6, 133.3, 135.2 (×2), 138.4 (×2), 139.0, 139.4 (×2), 145.2, 154.4 (×2), 166.8 ppm; ^{11}B NMR (192.5 MHz, $CDCl_3$): $\delta=0.79$ ppm (t, $^1J=33.4$ Hz); ESI-MS: $m/z=$ 523.3 $[M-F]^+$, 545.3 $[M-H-F+Na]^+$, 565.3 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{32}H_{37}BF_2N_4ONa^+$: 565.29263; found: 565.28996.

Compound 5: TMSOTf (75.0 μL, 412 μmol) and DMAP (128 mg, 412 μmol) were added to a solution of **4** (112 mg, 206 μmol) in dry toluene (7 mL), and the mixture was stirred for 30 min at 80 °C. The solvent was evaporated, the residue was washed with water (3× 20 mL) and dried over magnesium sulfate. The solution was concentrated to dryness and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 99:1) to give **5** as a red solid (105 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.91$ (t, $^3J=$ 7.5 Hz, 6H), 1.31 (s, 6H), 2.13 (s, 6H), 2.20 (q, $^3J=7.5$ Hz, 4H), 3.13 (t, $^3J_A=7.0$ Hz, 2H), 3.21 (s, 6H), 3.68 (td, $^3J_A=7.0$ Hz, $^3J_B=5.7$ Hz, 2H), 6.52 (t, $^3J_B=5.7$ Hz, 1H), 6.68 (d, $^3J=8.3$ Hz, 2H), 6.93 (d, $^3J=$ 7.6 Hz, 2H), 7.05 (d, $^3J=8.3$ Hz, 2H), 7.34 (dd, $^3J_C=8.0$ Hz, $^4J_D=$ 1.7 Hz, 1H), 7.68 (dd, $^3J_C=8.0$ Hz, $^4J_D=1.7$ Hz, 1H), 7.84 (dd, $^3J_C=$ 8.0 Hz, $^4J_D=1.7$ Hz, 1H), 8.00 (d, $^3J=7.6$ Hz, 2H), 8.01 ppm (dd, $^3J_C=8.0$ Hz, $^4J_D=1.7$ Hz, 1H); ^{11}B NMR (192.5 MHz, $CDCl_3$): $\delta=$ 1.04 ppm (d, $^1J=41.9$ Hz); ESI-MS: $m/z=523.3$ $[M-C_7H_{10}N_2-OTf]^+$, 645.4 $[M-OTf]^+$; HRMS (ESI): *m/z* calcd for $C_{39}H_{47}BFN_6O^+$: 645.38897; found: 645.38897.

Compound 6: Compound **5** (105 mg, 163 μmol) was placed in a solution of chloroform (15 mL), triethylamine (46 μL), and thiophosgene (37 μL) were added under nitrogen. The mixture was stirred 1 h at room temperature and the solvent was evaporated. The resulting solid was purified by column chromatography on silica gel (dichloromethane/methanol, 96:4) followed by recrystallization in a mixture of dichloromethane and hexane to give pure **6** (63.0 mg, 39%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.91$ (t, $^3J=7.5$ Hz, 6H), 1.32 (s, 6H), 2.13 (s, 6H), 2.21 (q, $^3J=7.5$ Hz, 4H), 3.00 (t, $^3J_A=7.4$ Hz, 2H), 3.22 (s, 6H), 3.72 (td, $^3J_A=7.4$ Hz, $^3J_B=5.7$ Hz, 2H), 6.90 (d, $^3J=$ 7.7 Hz, 2H), 7.03 (t, $^3J_B=5.7$ Hz, 1H), 7.16 (d, $^3J=8.4$ Hz, 2H), 7.28 (d, $^3J=8.4$ Hz, 2H), 7.35 (dd, $^3J_C=8.1$ Hz, $^4J_D=1.7$ Hz, 1H), 7.68 (dd, $^3J_C=8.1$ Hz, $^4J_D=1.7$ Hz, 1H), 8.01 (dd, $^3J_C=8.1$ Hz, $^4J_D=1.7$ Hz, 1H), 8.03 (d, $^3J=7.7$ Hz, 2H), 8.06 ppm (dd, $^3J_C=8.1$ Hz, $^4J_D=1.7$ Hz, 1H); ^{11}B NMR (192.5 MHz, $CDCl_3$): $\delta=1.07$ ppm (d, $^1J=37.3$ Hz); ESI-MS: $m/z=565.35$ $[M-C_7H_{10}N_2-OTf]^+$, 687.36 $[M-OTf]^+$; HRMS (ESI): *m/z* calcd for $C_{40}H_{45}BFN_6OS^+$: 687.34541; found: 687.34293; UV/Vis (THF): λ (ε): 528 (65), 498 (22), 380 (9), 285 (41), 274 (35), 243 (36), 224 nm (47×10^{-3} L mol^{-1} cm^{-1}).

Compound 7: Compound **6** (10.0 mg, 11.0 μmol) was dissolved in DMF (600 μL) and propylamine (0.70 μL, 9.00 μmol) was added. The mixture was stirred during one hour at room temperature and solvent was removed under reduced pressure. The resulting solid was purified by column chromatography on silica gel (dichloromethane/methanol, 97:3) followed by a recrystallization in a mixture of dichloromethane and hexane to give pure **7** (2.00 mg, 24%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.91$ (t, $^3J_A=7.4$ Hz, 3H), 0.91 (t, $^3J=$ 7.5 Hz, 6H), 1.31 (s, 6H), 1.65 (qt, $^3J_A=7.4$ Hz, $^3J_B=7.3$ Hz, 2H), 2.15 (s, 6H), 2.22 (q, $^3J=7.5$ Hz, 4H), 2.96 (t, $^3J_C=6.8$ Hz, 2H), 3.21 (s, 6H), 3.57 (t, $^3J_B=7.3$ Hz, 2H), 3.72 (td, $^3J_C=6.8$ Hz, $^3J_D=5.7$ Hz, 2H), 6.84 (d, $^3J=7.3$ Hz, 2H), 7.03 (t, $^3J_D=5.7$ Hz, 1H), 7.22 (d, $^3J=8.2$ Hz, 2H), 7.34 (dd, $^3J_E=8.1$ Hz, $^4J_F=1.4$ Hz, 1H), 7.39 (d, $^3J=8.2$ Hz, 2H), 7.47 (dd, $^3J_E=8.1$ Hz, $^4J_F=1.4$ Hz, 1H), 7.78 (dd, $^3J_E=8.1$ Hz, $^4J_F=$ 1.4 Hz, 1H), 7.96 (d, $^3J=7.3$ Hz, 2H), 8.09 ppm (dd, $^3J_E=8.1$ Hz, $^4J_F=$ 1.4 Hz, 1H); ^{11}B NMR (192.5 MHz, $CDCl_3$): $\delta=1.12$ (d, $^1J=36.2$ Hz); ESI-MS: $m/z=624.14$ $[M-C_7H_{10}N_2-OTf]^+$, 746.22 $[M-OTf]^+$.

Compound 8: *N*-Hydroxybenzotriazole (959 mg, 7.10 mmol), diisopropylamine (995 μL, 7.10 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.35 g, 7.10 mmol), and propargylamine (227 μL, 3.50 mmol) were successively added to a solution of compound **1** (1.53 g, 3.50 mmol) in dimethylformamide (100 mL) and the solution was stirred at room temperature. After total consumption of starting material (2 h) monitored by TLC, the solvent was evaporated. The resulting solid was washed with water (3× 100 mL) and extracted with dichloromethane. The organic layer was dried over magnesium sulfate and the solvent was evaporated to give a red oil. The crude product was purified by column chromatography on silica gel (dichloromethane/heptane, 60:40) followed by a recrystallization in a mixture of dichloromethane and hexane to give **8** as reddish solid in 72% yield (1.15 g, 2.49 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.96$ (t, $^3J=7.5$ Hz, 6H), 1.22 (s, 6H),

2.29 (q, ^3J=7.5 Hz, 4H), 2.30 (t, ^4J=2.5 Hz, 1H), 2.51 (s, 6H), 3.78 (dd, ^4J=2.5 Hz, ^3J=5.2 Hz, 2H), 6.27 (t, ^3J=5.2 Hz, 1H), 7.38 (d, ^3J=8.4 Hz, 2H), 7.91 ppm (d, ^3J=8.4 Hz, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ=11.9, 12.5, 14.6, 17.1, 30.9, 72.1, 79.3, 127.8, 128.9, 130.4, 133.1, 134.0, 138.1, 138.5, 139.7, 154.3, 166.2 ppm; ^{11}B NMR (192.5 MHz, $CDCl_3$): δ=0.78 ppm (t, ^1J=33.4 Hz).UV/Vis (THF): λ_{max}: (ε): 238 (39), 494 (27), 525 nm (90×10^{-3} L mol^{-1} cm^{-1}); MS (ESI): *m/z*=460.39 $[M+H]^+$, 484.34 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{27}H_{30}BF_2N_3ONa$: 484.23470; found: 484.23559.

Compound 9: Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 86.0 μL, 480 μmol) and dimethylaminopyridine (DMAP, 58.0 mg, 480 μmol) were added to a solution of **8** (110 mg, 238 μmol) in dry toluene (10 mL), and the mixture was stirred for 30 min at 80 °C. The solvent was evaporated, the residue was washed with water (3×20 mL) and dried over magnesium sulfate. The solution was concentrated to dryness and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 99:1) to give **9** as a red solid (118 mg, 69%). ^1H NMR (300 MHz, $CDCl_3$): δ=0.89 (t, ^3J=7.5 Hz, 6H), 1.30 (s, 6H), 2.20 (s, 6H), 2.12 (q, ^3J=7.5 Hz, 4H), 2.22 (t, ^4J_A=2.5 Hz, 1H), 3.21 (s, 6H), 4.25 (dd, ^4J_A=2.5 Hz, ^3J_B=5.4 Hz, 2H), 6.89 (d, ^3J=7.6 Hz, 2H), 7.36 (dd, ^3J_C=8.5 Hz, ^4J_D=1.8 Hz, 1H), 7.48 (t, ^3J_B=5.4 Hz, 1H), 7.61 (dd, ^3J_C=8.5 Hz, ^4J_D=1.8 Hz, 1H), 7.98 (d, ^3J=7.6 Hz, 2H), 8.11 (dd, ^3J_C=8.5 Hz, ^4J_D=1.8 Hz, 1H), 8.12 ppm (dd, ^3J_C=8.5 Hz, ^4J_D=1.8 Hz, 1H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ= 12.4 (×2), 12.8 (×2), 14.5 (×2), 17.1 (×2), 29.9, 40.2 (×2), 71.4, 80.0, 107.8–119.8–122.3–128.4–128.7–128.8–129.0–131.0–134.9–137.8–140.8–141.9–154.4–156.8 (×14), 166.5 ppm; ^{11}B NMR (192.5 MHz, $CDCl_3$): δ=1.07 ppm (d, ^1J=36.1 Hz); UV/Vis (THF): λ (ε): 528 (68), 498 (23), 381 (8), 287 (26), 244 nm (33 M^{-1} cm^{-1}); ESI-MS: *m/z*= 442.4 $[M-C_7H_{10}N_2-OTf]^+$, 564.4 $[M-OTf]^+$; HRMS (ESI): *m/z* calculated for $C_{34}H_{40}BFN_5O^+$: 564.33104; found: 564.32907.

Compound 10: Benzyl azide (11.0 μL, 84.0 μmol) and compound **8** (60.0 mg, 84.0 μmol) were solubilized in DMF (1.5 mL). $CuSO_4{\cdot}5H_2O$ (21.0 mg, 84.0 μmol) and sodium ascorbate (33.0 mg, 117 μmol) were added. The mixture was stirred under nitrogen at room temperature for 1 h and the solvent was evaporated under reduced pressure. The crude mixture was dissolved in dichloromethane and filtered over Celite. The organic layer was washed with water (3× 20 mL), dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane/MeOH, 99:1) followed by recrystallization in a mixture of dichloromethane and hexane to give **15** as a red solid (36.0 mg, 51%). ^1H NMR (300 MHz, $CDCl_3$): δ=0.91 (t, 6H, ^3J=7.5 Hz), 1.30 (s, 6H), 2.12 (s, 6H), 2.21 (q, ^3J=7.5 Hz, 4H), 3.22 (s, 6H), 4.74 (d, ^3J=5.2 Hz, 2H), 5.51 (s, 2H), 6.92 (d, ^3J=7.5 Hz, 2H), 7.29 (d, ^3J=7.2 Hz, 1H), 7.32 (m, 5H), 7.34 (t, ^3J=5.2 Hz, 1H), 7.62 (s, 1H), 7.68 (d, ^3J=7.2 Hz, 1H), 8.00 (d, ^3J=7.5 Hz, 2H), 8.01 (d, ^3J=7.2 Hz, 1H), 8.03 ppm (d, ^3J=7.2 Hz, 1H); ^{11}B NMR (192.5 MHz, $CDCl_3$): δ=1.05 ppm (d, ^1J=39.5 Hz); ESI-MS: *m/z*=575.14 $[M-C_7H_{10}N_2-OTf]^+$, 697.22 $[M-OTf]^+$; HRMS (ESI): *m/z* calcd for $C_{41}H_{47}ON_8BF^+$: 697.39515; found: 697.39581.

Compound 11: 13,16,21,24-hexaoxa-1,10-diazabicyclo-(8,8,8)-hexacosane (8.90 mg, 23.6 μmol), and potassium fluoride (1.40 mg, 23.6 μmol) were added to a solution of **10** (20.0 mg, 23.6 μmol) in acetonitrile (1.5 mL). The solution was heated at 70 °C and the reaction was monitored by TLC. After 15 min, the solvent was removed under reduced pressure. Water (3 mL) was added and the mixture was extracted with dichloromethane (3×3 mL). The organic layer was dried over magnesium sulfate and the solvent was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/hexane, 80:20) followed by recrystallization in a mixture of dichloromethane and hexane to give pure **11** as a red solid (6.00 mg, 42%). ^1H NMR (300 MHz, $CDCl_3$): δ= 0.95 (t, 6H, ^3J=7.5 Hz), 1.21 (s, 6H), 2.27 (q, ^3J=7.5 Hz, 4H), 2.51 (s, 6H), 4.70 (d, ^3J=5.4 Hz, 2H), 5.50 (s, 2H), 7.20 (t, ^3J=5.4 Hz, 1H), 7.27 (d, ^3J=8.3 Hz, 2H), 7.34 (m, 5H), 7.56 (s, 1H), 7.91 ppm (d, ^3J=8.3 Hz, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ=11.9, 12.5, 14.6, 17.0, 35.5, 54.3, 77.2, 122.3, 127.8, 128.2, 128.8, 128.9, 129.2, 130.4, 133.0, 134.4, 138.1, 138.7, 139.5, 144.7, 144.8, 154.2, 166.7 ppm; ^{11}B NMR (192.5 MHz, $CDCl_3$): δ=0.77 ppm (t, ^1J=67.0 Hz); ESI-MS: *m/z*= 595.12 $[M+H]^+$, 617.08 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{34}H_{37}BF_2N_6ONa^+$: 617.29881; found: 617.29663,.

Compound 12: 20.6 μL of a 100 mM solution of **N_3-BBN** (DMF, 2.10 μmol), 20.6 μL of a 100 mM solution of compound **8** (DMF, 2.10 μmol), 11.3 μL of a 200 mM solution of $CuSO_4{\cdot}5H_2O$ (DMF, 2.30 μmol), and sodium ascorbate (1.23 mg, 6.20 μmol) were mixed together. The mixture was stirred at room temperature for 1 h and reaction was monitored by HPLC. The crude product was purified by HPLC from 30 to 60% of B over 30 min to give **12** as a red solid (1.21 mg, 31%, purity=98.6%, t_R=2.89 min). ESI-MS: *m/z*= 1552.77 $[M-C_7H_{10}N_2-OTf-TFA]^+$, 1674.78 $[M-OTf-TFA]^+$; HRMS (ESI): *m/z* calcd for $C_{83}H_{114}BFN_{21}O_{13}S^+$: 1674.871021; found: 674.86882.

Fluorination of compound 12: 5.60 μL of a 0.03 M solution of **12** (CH_3CN/DMF (1:1), 0.17 μmol), 5.60 μL of a 0.03 M solution of 13,16,21,24-hexaoxa-1,10-diazabicyclo-(8,8,8)-hexacosane (CH_3CN, 0.17 μmol), and 0.01 mg of potassium fluoride (0.17 μmol) were mixed together. The solution was heated at 70 °C during 40 min and monitored by HPLC. The formation of compound **13** was characterized by ESI-MS and HPLC (Conversion=42% after 10 min of reaction).

Compound 13: 14.0 μL of a 100 mM solution of **N_3-BBN** (DMF, 1.40 μmol), 14.0 μL of a 100 mM solution of compound **8** (DMF, 1.40 μmol), 7.7 μL of a 200 mM solution of $CuSO_4{\cdot}5H_2O$ (DMF, 1.54 μmol), and 0.83 mg of sodium ascorbate (4.19 μmol) were mixed together. The mixture was stirred at room temperature for 1 h and reaction was monitored by HPLC. The crude product was purified by HPLC from 30 to 60% of B over 30 min to give **13** as a red solid (0.955 mg, 41%, purity=98.4%, t_R=3.23 min). ESI-MS: *m/z*: 1572.80 $[M-TFA+H]^+$, 1594.77 $[M-TFA+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{76}H_{105}BF_2N_{19}O_{13}S^+$: 1572.792111; found: 572.9795; *m/z* calcd for $C_{76}H_{104}BF_2N_{19}O_{13}SNa^+$: 1594.774711; found: 594.77718.

Compound 15: Compound **8** (500 mg, 1.08 mmol) and *p*-anisaldehyde (316 μL, 2.59 mmol) were dissolved in a mixture of dry toluene (40 mL), *p*-toluenesulfonic acid (PTSA, 40.0 mg, 0.23 mmol) and piperidine (4 mL). The mixture was heated at reflux during 2 h in a Dean–Stark apparatus and the solvent was removed in situ. Dry toluene (40 mL) and piperidine (4 mL) were added, and the mixture was heated at reflux for another 2 h. After total consumption of the starting material (monitored by UV/Vis), the solvent was evaporated. The resulting solid was washed with water (3× 100 mL) and extracted with dichloromethane. The organic layer was dried over magnesium sulfate and the solvent was evaporated to give a blue solid. The crude product was purified by column chromatography on silica gel (heptane/dichloromethane, 80:20) followed by a recrystallization in a mixture of dichloromethane and hexane to give **15** as a blue solid in 79% yield (600 mg, 0.86 mmol). ^1H NMR (300 MHz, $CDCl_3$): δ=1.14 (t, ^3J=7.5 Hz, 6H), 1.28 (s, 6H), 2.32 (t, ^4J=2.5 Hz, 1H), 2.58 (q, ^3J=7.5 Hz, 4H), 3.84 (s, 6H), 4.30 (dd, ^4J=2.5 Hz, ^3J=5.2 Hz, 2H), 6.36 (t, ^3J=5.2 Hz, 1H), 6.93 (d, ^3J=8.7 Hz, 4H), 7.20 (d, ^3J=16.7 Hz, 2H), 7.43, (d, ^3J=8.3 Hz, 2H), 7.56 (d, ^3J=8.7 Hz, 4H), 7.65 (d, ^3J=16.7 Hz, 2H), 7.93 ppm (d, ^3J=8.3 Hz, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ=11.8, 14.1, 18.4, 55.4, 72.2, 79.3, 114.3, 127.8, 128.9, 129.4, 130.3, 132.5, 133.9, 134.1, 135.8, 136.1, 138.3, 140.1, 150.9, 160.3, 166.3 ppm; ^{11}B NMR (192.5 MHz, $CDCl_3$): δ=1.23 ppm (t, ^1J=34.2 Hz); UV/Vis

(THF): λ_{max} (ε): 250 (21), 334 (31), 368 (68), 608 (37), 654 nm (93×10^{-3} L mol^{-1} cm^{-1}); MS (ESI): *m/z* = 720.35 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{43}H_{42}BF_2N_3O_2Na$: 720.31869; found: 720.32039.

Compound 16: TMSOTf (60.0 μL, 330 μmol) and DMAP (4.0 mg, 330 μmol) were added to a solution of **15** (115 mg, 165 μmol) in dry toluene (10 mL), and the mixture was stirred for 30 min at 80 °C. The solvent was evaporated, the residue was washed with water (3×20 mL) and dried over magnesium sulfate. The solution was concentrated to dryness and the residue was purified by silica gel column chromatography (dichloromethane/MeOH, 97:3) to give **16** as a red solid (103 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 (t, ^3J = 7.5 Hz, 6H), 1.36 (s, 6H), 2.29 (t, ^4J_A = 2.5 Hz, 1H), 2.46 (q, ^3J = 7.5 Hz, 4H), 3.01 (s, 6H), 3.85 (s, 6H), 4.30 (dd, ^4J_A = 2.5 Hz, ^3J_B = 5.1 Hz, 2H), 6.66 (d, ^3J = 6.4 Hz, 2H), 6.85 (d, ^3J = 16.7 Hz, 2H), 6.94 (d, ^3J = 8.6 Hz, 4H), 7.04 (t, ^3J_B = 5.1 Hz, 1H), 7.05 (d, ^3J = 16.7 Hz, 2H), 7.37 (d, ^3J = 8.6 Hz, 4H), 7.47 (d, ^3J = 7.9 Hz, 1H), 7.77 (d, ^3J = 7.0 Hz, 1H), 7.89 (d, ^3J = 6.4 Hz, 2H), 8.08 (d, ^3J = 7.0 Hz, 1H), 8.17 ppm (d, ^3J = 7.9 Hz, 1H); ^{11}B NMR (192.5 MHz, $CDCl_3$): δ = 1.45 ppm (d, ^1J = 35.5 Hz); UV/Vis (THF): λ (ε): 659 (59), 440 (14), 376 (39), 292 nm ($33\ M^{-1}cm^{-1}$); ESI-MS: *m/z* = 678.4 $[M-C_7H_{10}N_2-OTf]^+$, 800.4 $[M-OTf]^+$; HRMS (ESI): *m/z* calcd for $C_{50}H_{52}BFN_5O_3^+$: 800.41502; found: 800.41267.

Compound 17: 5.20 μL of a 100 mM solution of **N_3-BBN** (DMF, 0.52 μmol), 5.20 μL of a 100 mM solution of compound **16** (DMF, 0.52 μmol), 2.80 μL of a 200 mM solution of $CuSO_4{\cdot}5H_2O$ (DMF, 0.57 μmol), and 0.30 mg of sodium ascorbate (1.51 μmol) were mixed together. The mixture was stirred at room temperature for 1 h and the reaction was monitored by HPLC. The crude product was purified by HPLC from 40 to 70% of B over 30 min to give **17** as a red solid (conversion (HPLC) = 41%, purity = 98.4%, t_R = 3.57 min). Conversion was determined by relative integration of the chromatogram of the crude compound, at 254 nm; ESI-MS: *m/z* = 1788.79 $[M-C_7H_{10}N_2-OTf-TFA]^+$; 1910.85 $[M-OTf-TFA]^+$; HRMS (ESI): *m/z* calcd for $C_{99}H_{126}BFN_{21}O_{15}S^+$: 1910.95495; found: 1910.96003,.

Compound 18: 2,4-Dimethylethylpyrrole (7.50 g, 60.9 mmol) and methyl 4-formylbenzoate (5.00 g, 30.5 mmol) were dissolved in dichloromethane (2 L). Trifluoroacetic acid (140 μL) was added and the mixture was stirred at room temperature for 24 h. A solution of 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (6.90 g, 30.5 mmol) in dichloromethane was added to the mixture and the solution was further stirred for 50 min, triethylamine (60 mL) and $BF_3{\cdot}Et_2O$ (60 mL) were added, the reaction mixture turned purple. After stirring during 1 h, the reaction mixture was washed with water, dried over magnesium sulfate and the solvent was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/hexane 40:60) followed by recrystallization in a mixture of dichloromethane and hexane to give pure **18** as a red solid (6.00 g, 45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.90 (t, ^3J = 7.6 Hz, 6H), 1.18 (s, 6H), 2.22 (q, ^3J = 7.6 Hz, 4H), 2.46 (s, 6H), 3.90 (s, 3H), 7.33 (d, ^3J = 8.4 Hz, 2H), 8.10 ppm (d, ^3J = 8.4 Hz, 2H).

Compound 19: Compound **18** (1.10 g, 2.51 mmol) and 4-(prop-2-yn-1-yloxy)benzaldehyde (442 mg, 2.76 mmol) were dissolved in a mixture of dry toluene (50 mL), PTSA (53.0 mg, 0.31 mmol), and piperidine (3.30 mL, 33.0 mmol). The mixture was heated at reflux during 2 h in a Dean–Stark apparatus and the solvent was removed in situ. Dry toluene (50 mL) and piperidine (3 mL) were added and the mixture was heated at reflux for another 2 h. After consumption of approximately a third of starting material (monitored by UV/Vis spectroscopy), the solvent was evaporated. The resulting solid was washed with water (3×100 mL) and extracted with dichloromethane. The organic layer was dried over magnesium sulfate and the solvent was evaporated to give a blue solid. The crude product was purified by column chromatography on silica gel (dichloromethane/heptane, 40:60) followed by a recrystallization in a mixture of dichloromethane and hexane to give **19** (73.0 mg, 125 μmol) in 5% yield. Distyryl-substituted BODIPY was also obtained as subproduct in 3% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = (ppm): δ = 0.97 (t, ^3J = 7.5 Hz, 3H), 1.12 (t, ^3J = 7.5 Hz, 3H), 1.25 (s, 3H), 1.27 (s, 3H), 2.29 (q, ^3J = 7.5 Hz, 2H), 2.52 (t, ^4J = 2.4 Hz, 1H), 2.56 (s, 3H), 2.56 (q, ^3J = 7.5 Hz, 2H), 3.97 (s, 3H), 4.71 (d, ^4J = 2.4 Hz, 2H), 6.97 (d, ^3J = 8.8 Hz, 2H), 7.16 (d, ^3J = 16.8 Hz, 1H), 7.41 (d, ^3J = 8.3 Hz, 2H), 7.54 (d, ^3J = 8.8 Hz, 2H), 7.60 (d, ^3J = 16.8 Hz, 1H), 8.16 ppm (d, ^3J = 8.3 Hz, 2H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ = 11.8, 12.1, 13.0, 14.3, 14.7, 17.3, 17.5, 18.5, 52.6, 56.1, 75.9, 78.6, 115.4, 118.6, 128.8, 129.1, 130.5, 130.9, 131.3, 131.6, 133.4, 134.0, 135.1, 138.0, 141.1, 150.0, 155.8, 158.2, 166.8 ppm; UV/Vis (THF): λ_{max}: (ε) 238 (19), 319 (17), 339 (29), 389 (8), 448 (27), 587 nm (81×10^{-3} L mol^{-1} cm^{-1}); ^{11}B NMR (192.5 MHz, $CDCl_3$): δ = 0.98 ppm (t, ^1J = 33.8 Hz); MS (ESI): *m/z* = 603.4 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{35}H_{35}BF_2N_2O_3Na$: 603.26071; found: 603.26233.

Compound 20: TMSOTf (22.0 μL, 120 μmol) and DMAP (15.0 mg, 120 μmol) were added to a solution of **19** (35.0 mg, 60.0 μmol) in dry toluene (2 mL), and the mixture was stirred for 30 min at 80 °C. The solvent was evaporated, the residue was washed with water (3×10 mL) and dried over magnesium sulfate. The solution was concentrated to dryness and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 98:2) to give **20** as a purple solid (36.0 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.94 (t, ^3J = 7.5 Hz, 6H), 1.05 (t, ^3J = 7.5 Hz, 6H), 1.34 (s, 6H), 1.36 (s, 6H), 2.15 (s, 3H), 2.24 (q, ^3J = 7.5 Hz, 2H), 2.43 (qd, ^3J_A = 7.3, ^1J_B = 12.2 Hz, 1H), 2.45 (qd, ^3J_A = 7.3 Hz, ^1J_B = 12.2 Hz, 1H), 2.54 (t, ^4J = 2.5 Hz, 1H), 3.01 (s, 6H), 3.85 (s, 6H), 3.97 (s, 3H), 4.74 (d, ^4J = 2.5 Hz, 2H), 6.78 (d, ^3J = 7.6 Hz, 2H), 6.79 (d, ^3J = 16.7 Hz, 1H), 7.01 (d, ^3J = 8.9 Hz, 2H), 7.03 (d, ^3J = 16.7 Hz, 1H), 7.37 (d, ^3J = 8.9 Hz, 2H), 7.40 (dd, ^3J_C = 7.9 Hz, ^4J_D = 1.5 Hz, 1H), 7.78 (dd, ^3J_C = 7.9 Hz, ^4J_D = 1.5 Hz, 1H), 7.94 (d, ^3J = 7.6 Hz, 2H), 8.20 (dd, ^3J_C = 7.9 Hz, ^4J_D = 1.5 Hz, 1H), 8.28 ppm (dd, ^3J_C = 7.9 Hz, ^4J_D = 1.5 Hz, 1H); ^{11}B NMR (192.5 MHz, $CDCl_3$): δ = 1.18 ppm (d, ^1J = 37.9 Hz); UV/Vis (THF) λ (ε): 594 (56), 402 (9), 345 (18), 291 (25), 242 nm (20×10^{-3} M^{-1} cm^{-1}); ESI-MS: *m/z* = 561.4 $[M-C_7H_{10}N_2-OTf]^+$, 683.4 $[M-OTf]^+$; HRMS (ESI): *m/z* calcd for $C_{42}H_{45}BFN_4O_3^+$: 683.35705; found: 683.35465.

Compound 21: 5.20 μL of a 100 mM solution of **N_3-BBN** (DMF, 0.52 μmol), 5.20 μL of a 100 mM solution of compound **20** (DMF, 0.52 μmol), 2.80 μL of a 200 mM solution of $CuSO_4{\cdot}5H_2O$ (DMF, 0.57 μmol), and sodium ascorbate (0.30 mg, 1.51 μmol) were mixed together. The mixture was stirred at room temperature for 1 h and the reaction was monitored by HPLC. The crude product was purified by HPLC from 40 to 70% of B over 30 min to give **21** as a red solid (conversion (HPLC) = 59%, purity = 95.4%, t_R = 3.37 min). ESI-MS: *m/z*: 1671.78 $[M-C_7H_{10}N_2-OTf-TFA]^+$, 1793.76 $[M-OTf-TFA]^+$; HRMS (ESI): *m/z* calcd for $C_{91}H_{119}BFN_{20}O_{15}S^+$: 1793.89700; found: 1793.90512.

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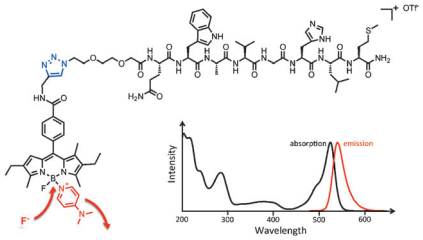
FULL PAPER

Probing further: Different boron dipyrromethene/*N*,*N*-dimethylaminopyridine (BODIPY-DMAP) compounds were synthesized as precursors for positron emission tomography (PET)-optical imaging (see figure). Biolabeling was performed on a bombesin derivative, while preserving the DMAP leaving group on the boron atom and the affinity for bombesin receptors. Extension of the conjugation on the BODIPY core was also investigated, for future in vivo imaging applications.



Imaging Agents

B. Brizet, V. Goncalves, C. Bernhard, P. D. Harvey, F. Denat, C. Goze**

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DMAP-BODIPY Alkynes: A Convenient Tool for Labeling Biomolecules for Bimodal PET–Optical Imaging