BODIPYs as chemically stable fluorescent tags on synthetic glycosylation strategies towards fluorescently-labeled saccharides

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Abstract: A series of fluorescent boron–dipyrromethene (BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes have been designed to participate, as aglycons, in synthetic oligosaccharide protocols. As such, they served a dual purpose: first, by being incorporated at the beginning of the process (at the reducing-end of the growing saccharide moiety), they can function as fluorescent glycosyl-tags facilitating the detection and purification of the desired glycosidic intermediates, and secondly the presence of these chromophores on the ensuing compounds warrants access to fluorescently-labeled saccharides. In this context, a sought-after feature of the fluorescent dyes has been their chemical robustness. Accordingly, some BODIPY derivatives described in this work are able to withstand the reaction conditions commonly employed in the chemical synthesis of saccharides, namely, glycosylation and protecting-group manipulations. Regarding their photophysical properties, the BODIPY-labeled saccharides obtained in this work displayed remarkable fluorescence-efficiency in water, reaching quantum yield values up to 82%, as well as notable lasing efficiencies and photostabilities.

Introduction

The chemical synthesis of oligosaccharides^[1] and the development of fluorescent probes^[2] are two well-recognized research areas with increasing relevance. In addition, the combination of fluorescent labels and carbohydrates for the generation of fluorescent glycoprobes has become an active topic of research in recent years.^[3] Remarkably, the interest on these assemblies could arise from at least two different research fields.

Focusing on the carbohydrate partner, the fluorescent labeling of glycans allows investigations on glycoconjugates and their interactions in biological systems at high sensitivity.^[4,5,6] Alternatively, from the fluorescent-probe standpoint, owing to the relevance that carbohydrate-receptor interactions have on a number of biological processes,^[7] a glycosyl moiety linked to a fluorophore could play a significant role as a targeting^[8] and internalizing^[9] agent for the probe, sometimes providing less cytotoxic entities.^[10]

On the other hand, borondipyrromethene (BODIPY) dyes, e.g. **A** (Scheme 1),^[11] have emerged as remarkable fluorophores owing to their outstanding properties that include strong UV-vis absorption profiles, high fluorescence quantum yields (ϕ) and excellent photochemical and thermal stabilities, which has led to their use on biomaterials labeling,^[12] among others.^[13]

Of particular interest to us, it was that the ligation-step of the fluorophore, e.g. **A**, and the glycoligand, e.g. **B**, leading to glycoprobes, e.g. **C** (Scheme 1, approach *i*), is generally carried out as the last synthetic event, sometimes *in situ*, prior to their submission to the different visualization or detection techniques.^[14] Based on these precedents, we decided to investigate the feasibility of a synthetic approach to fluorescently-labeled oligosaccharides in which the fluorescent probe, installed at the reducing-end of the growing saccharide from the beginning of the process, could additionally function as a tagging element, facilitating the visualization and detection of the synthetic saccharide intermediates (Scheme 1, approach *ii*).

Accordingly, in this manuscript, we disclose our investigations on an alternative approach to BODIPY-saccharide derivatives, i.e. **C** (Scheme 1), based on sequential glycosylation/deprotection/glycosylation events of a starting hydroxymethyl-BODIPY **D**, with different glycosyl donors, e.g. **E** (Scheme 1, approach *ii*).

Scheme 1. *i*) General synthetic approach to glycoprobe **C**, by ligation of a given saccharide (**B**) to a generic BODIPY dye (**A**, IUPAC numbering); and *ii*) proposed sequential glycosylation/deprotection/glycosylation strategy from hydroxymethyl-BODIPY **D** and glycosyl donors **E**, leading to BODIPY-saccharide structures **C**.

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Consequently, our studies have focused on the search for BODIPY derivatives able to endure the reaction events commonly employed in oligosaccharide synthesis, namely, glycosylation protocols^[15] and protecting-group manipulations.^[16,17]

Results and Discussion

The present studies on the potential of BODIPY-aglycons as glycosyl tags in synthetic oligosaccharide strategies originated from an ongoing project that required a reliable synthetic route to an appropriately protected 2-hydroxy-mannopyranoside bearing a fluorophore as aglycon, and able to undergo further glycosylation at O-2.

Accordingly, based on our experience with 1,2-methyl orthoesters^[18,19] (MeOEs) in glycosylation,^[20] and our recent method for the efficient, one-pot, access^[21] to hydroxymethyl-BODIPY **1**,^[22] we attempted the glycosylation of the latter with MeOE **2** (Scheme 2). The glycosyl coupling, mediated by $\text{BF}_3 \cdot \text{OEt}_2$, took place smoothly to provide BODIPY-mannopyranoside **3**, which upon treatment with NaOMe in MeOH, produced 4,4'-methoxy-BODIPY **4**.^[23,24] The latter was the result of an unwanted methoxy substitution at boron accompanying the desired de-O-benzoylation process. The use of milder saponification conditions ($\text{Et}_3\text{N}/\text{MeOH}$, reflux) also induced methoxy substitution at boron, but left the 2-O-Bz group in mannosyl-BODIPY **5**, unaffected.

Thus, even though compound **4**, displayed good fluorophoric behavior, it was found of no use to us since further glycosylation attempts on **4** with MeOE donors, mediated by $\text{BF}_3 \cdot \text{OEt}_2$, resulted in partial or total methoxy→fluor replacement at boron, thus giving rise to complex product mixtures.

Scheme 2. Glycosylation of BODIPY **1** with MeOE **2**. Attempted saponification of benzoate **3**, leading to O-BODIPYs **4** and **5**.

According to these results, and despite the fact that BODIPYs are typically described as “chemically robust” substrates,^[24b,25,26] we considered that a comprehensive screening of the compatibility of the BODIPY core with the reaction conditions employed in standard glycosylation protocols (including protecting-group manipulations) will be necessary prior to their use as fluorophoric tags in oligosaccharide synthesis.

In this context, a general survey of the scientific literature on the chemical stability of BODIPYs allowed us to identify -at least- two

structural aspects with an influence on their stability. Thus, the degree of peripheral alkyl-substitution on the BODIPY core^[27] and the presence of a 8-aryl substituent,^[28,29] have for long been known to stabilize the fluorophore. On the other hand, the substitution at boron has been also identified as a key factor in the stability of BODIPYs.^[30] In this context, Yan and coworkers recognized 4,4'-diphenyl BODIPY derivatives as more stable, under acidic and basic conditions, than the corresponding 4,4'-dimethyl, 4,4'-dimethoxy, or 4,4'-difluoro derivatives.^[31] More recently, reports from Vicente's and Bobadova-Parvanova's laboratories have identified 4,4'-dicyano-BODIPYs^[32] as the most stable derivatives, under acidic conditions, from a series of 4,4'-disubstituted BODIPYs (including -F, -CN, -Ph, -Me and -OMe derivatives).^[33]

According to these literature precedents, we selected readily available BODIPYs **6–10**^[21] to conduct our stability studies (Figure 1). Thus, derivatives **6** and **7**, and **8** and **9** differed in the substitution at boron (F versus CN). Compounds **8** and **9** possessed ethyl substituents at C-2 and C-6 (when compared to **6** and **7**) aimed at shielding the BODIPY core towards the electrophilic reagents^[34] required in the activation of some of the glycosyl donors evaluated in this work. Additionally, since our planned studies also include the stability of these BODIPYs under hydrogenolytic conditions (necessary to deprotect benzyl groups at the glycosyl units), the hydroxyethyl derivative **10**^[35] was also considered as a non-benzylic analogue of **8**.

Figure 1. BODIPY Derivatives **6–10** selected for our studies.

BODIPYs and protecting group manipulations

The polyhydroxylic nature of carbohydrates and the high reactivity of the hydroxyl groups in glycosylation reactions, is the reason why in most cases, only the hydroxyl group that is meant to be glycosylated can remain unprotected during the glycosylation reaction. Therefore, protecting group manipulations have become a key issue in oligosaccharide synthesis.^[17] In this context, most of the synthetic strategies towards oligosaccharides require the handling of at least three types of orthogonal^[16,17] protecting groups: acyl derivatives, silyl ethers and benzyl ethers. Among them, the first two are considered “temporary” protecting groups, since they can be placed and removed several times during the synthesis, whereas benzyl ethers are regarded as “permanent” protecting groups, since they are: *i*) only to be removed at the very end of the saccharide preparation, *ii*) stable to all reaction conditions used, and *iii*) removable under reaction conditions that do not tamper with the integrity of the target (oligo)saccharide.^[36] For these reasons, we have evaluated the compatibility of the reaction conditions used for the deprotection of benzoyl esters, and silyl and benzyl ethers with the structural integrity of some of the BODIPYs displayed in Figure 1.

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Thus, in order to find appropriate reaction conditions for the removal of the protecting groups, we carried out our initial model studies with *tert*-butyldiphenylsilyl BODIPYs (BODIPY-OTBDPS), and benzoyl BODIPYs (BODIPY-OBz) (Tables 1 and 2, respectively).

Table 1. De-O-silylation of BODIPY-*tert*-butyldiphenylsilyl ethers (BODIPY-OTBDPS) to regenerate the starting BODIPY-OHs (6–9).

Entry	BODIPY-OTBDPS	Reaction conditions	BODIPY-OH	Yield ^[c]
<i>i</i>	6 -OTBDPS	TBAF ^[a]	6	48%
<i>ii</i>	7 -OTBDPS	TBAF ^[a]	7	41%
<i>iii</i>	8 -OTBDPS	TBAF ^[a]	8	38%
<i>iv</i>	9 -OTBDPS	TBAF ^[a]	9	-----
<i>v</i>	6 -OTBDPS	HF/pyridine ^[b]	6	52% ^[d]
<i>vi</i>	7 -OTBDPS	HF/pyridine ^[b]	7	64% ^[e]
<i>vii</i>	8 -OTBDPS	HF/pyridine ^[b]	8	78% ^[f]
<i>viii</i>	9 -OTBDPS	HF/pyridine ^[b]	9	94%
<i>ix</i>	6 -OTBDPS	HCl/MeOH ^[c]	6	47% ^[g]
<i>x</i>	7 -OTBDPS	HCl/MeOH ^[c]	7	93%
<i>xi</i>	8 -OTBDPS	HCl/MeOH ^[c]	8	-----
<i>xii</i>	9 -OTBDPS	HCl/MeOH ^[c]	9	94%

[a] Tetra-*n*-butylammonium fluoride (TBAF) 1.5 equiv. CH₂Cl₂, 0 °C → r.t. [b] HF-pyridine (40 equiv.), THF, r.t. [c] HCl/MeOH (1.25 M), THF, microwave irradiation, 60 °C. [d] 88% corrected yield, based on recovered starting material. [e] 87% corrected yield, based on recovered starting material. [f] 92% corrected yield, based on recovered starting material. [g] Yield obtained after treatment of the crude reaction mixture with Et₃N, BF₃·OEt₂.

As representative methods for de-O-silylation we selected three sets of reaction conditions: *i*) tetra-*n*-butyl ammonium fluoride (TBAF); *ii*) HF-pyridine in THF; and *iii*) HCl/methanol in THF, under microwave irradiation (Table 1). The best results in terms of deprotection yields and substrate stabilities were obtained with

4,4'-dicyano BODIPYs (**7**-OTBDPS and **9**-OTBDPS) upon treatment with HCl/Methanol under microwave irradiation (Table 1, entries *x* and *xii*, respectively). Treatment with HF/pyridine furnished an excellent yield of 4,4'-dicyano-2,6-diethyl BODIPY-OH (**9**) (Table 1, entry *viii*), and reasonable yields of BODIPYs **7** and **8** (Table 1, entries *vi* and *vii*, respectively).

From the results in Table 1, we concluded that desilylation could be successfully carried out with HCl/methanol in derivatives arising from 4,4'-dicyano-BODIPYs **7** and **9**, and with HF/pyridine in 4,4'-dicyano-2,6-diethyl BODIPY-OH (**9**). In the case of silyl BODIPYs **6** and **8**, treatment with HCl/methanol (entries *ix* and *xi*, respectively, Table 1) caused desilylation accompanied by nitrogen-boron cleavage on the BODIPY core leading to the corresponding, non-fluorescent, dipyrromethenes.^[37] This could be confirmed in the case of **6**-OTBDPS, where treatment of the crude desilylation mixture with BF₃·OEt₂ produced BODIPY **6** (Table 1, entry *ix*, 47% yield).^[37]

Finally, in keeping with literature precedents,^[38] TBAF caused considerable decomposition of the BODIPY-core in all the derivatives (Table 1, entries *i–iv*).

Table 2. Saponification of BODIPY benzoates (BODIPY-OBz) to regenerate the starting BODIPY-OHs (6–9).

Entry	BODIPY-OBz	Reaction conditions	BODIPY-OH	Yield ^[c]
<i>i</i>	6 -OBz	NaOMe/MeOH ^[a]	6	92%
<i>ii</i>	7 -OBz	NaOMe/MeOH ^[a]	7	47%
<i>iii</i>	8 -OBz	NaOMe/MeOH ^[a]	8	92%
<i>iv</i>	9 -OBz	NaOMe/MeOH ^[a]	9	95%
<i>v</i>	6 -OBz	Et ₃ N/MeOH ^[b]	6	43% ^[c]
<i>vi</i>	7 -OBz	Et ₃ N/MeOH ^[b]	7	--- ^[d]
<i>vii</i>	8 -OBz	Et ₃ N/MeOH ^[b]	8	65% ^[e]
<i>viii</i>	9 -OBz	Et ₃ N/MeOH ^[b]	9	--- ^[f]

[a] Sodium methoxide in methanol, 4.4 equiv. r.t., 1–2 h [b] Triethylamine in methanol, reflux overnight [c] An additional 23% of the starting benzoate was recovered. [d] Complex reaction mixture containing at least five different compounds including B-(F,OMe) and B(OMe)₂-BODIPYs, with or without the C-8-*ortho* benzoyl substituent. [e] 86% corrected yield, based on recovered starting material. [f] The reaction mixture consisted on the corresponding B(F,OMe) and B(OMe)₂-BODIPYs, with and without the C-8-*ortho* benzoyl substituent.

Analogously, de-O-benzoylation of the corresponding BODIPY-OBz **6–9** derivatives was attempted under two sets of reaction conditions: *i*) NaOMe/MeOH and *ii*) Et₃N in methanol (reflux) (Table 2).

The best outcome in terms of reaction yields and substrate stabilities, was observed in the case of 4,4'-difluoro BODIPYs **6**-OBz and **8**-OBz, and 4,4'-dicyano-BODIPY **9**-OBz derivatives, upon treatment with NaOMe in MeOH (Table 2, entries *i*, *iii* and *iv*, respectively). Contrariwise, the treatment of B(CN)₂-BODIPY with Et₃N in MeOH produced complex mixtures containing B(OMe,F)- and B(OMe)₂-BODIPYs, with or without the ortho-8-benzoyl group (Table 2, entries *v-viii*).

In these debenzoylation studies in basic media, regardless of the reaction conditions employed, BF₂-BODIPYs, *i. e.* **6** and **8**, were found to be more stable than the corresponding B(CN)₂-BODIPYs (compare entry *i* versus entry *ii*, and entries *vi*, *vii*, versus entry *ix*, Table 2). Nevertheless, de-*O*-benzoylation of peralkylated B(CN)₂-BODIPY **9**-OBz proved to be successful when short reaction times and stoichiometric amounts of NaOMe were used.

BODIPYs and glycosylation reactions

We next set up to study the behavior of our hydroxymethyl-BODIPYs in glycosylation reactions. Accordingly, we have validated five types of, commonly employed, glycosyl donors: trichloroacetimidates (TCA),^[39] thioglycosides,^[40] glycosyl bromides,^[41] *n*-pentenyl orthoesters (NPOEs)^[42] and MeOEs^[18,20].

Scheme 3. Glycosylation of BODIPYs **7**, **9** and **10** with glycosyl trichloroacetimidate **11**, and glycosylation of BODIPY **9** with thioglycosyl donors **18**, **19**, **22** and **25**.

The results obtained are collected in Schemes 3 and 4.

Accordingly, trichloroacetimidate **11**, reacted smoothly with 4,4'-dicyano BODIPYs **7** and **9**, upon activation with BF₃·OEt₂ at low temperature, to give tetra-*O*-benzoyl mannopyranosides **12** and **14**, respectively, in moderate yields (Scheme 3A and 3B). These benzoyl glycosides yielded, upon saponification with NaOMe/MeOH, tetraols **13** and **15**, respectively (Scheme 3A and 3B). Likewise, glycosylation of 4,4'-difluoro-BODIPY **10** with TCA **11** at higher temperature yielded mannopyranoside **16**, which upon saponification, produced tetraol glycoside **17** (Scheme 3C). Next, the glycosylation of BODIPY **9** with phenyl thioglycosides **18**, **19**, **22** and **25**, was examined. Thus, armed and disarmed^[43] thioglycosides could be activated by treatment with NIS in the presence of Yb(OTf)₃,^[44] to yield BODIPY-glycosides **14**, **20** and **23** in moderate to good yields (Scheme 3D,E,F, respectively), the latter being isolated as a (2:1) α/β anomeric mixture. The use of the *N*-iodosuccinimide (NIS)/BF₃·OEt₂ system,^[45] as the promotor in the activation of the thioglycosides, caused partial replacement of -CN by fluoride at boron, thus yielding mixtures of the corresponding 4,4-difluoro- and 4-fluor-4'-cyano-BODIPY glycosides. The use of electrophilic NIS as promotor precluded the use of BODIPYs **6** and **7** as acceptors, owing to the nucleophilicity of the BODIPYs at positions C-2 and C-6.^[34] On the other hand, desilylation of BODIPY-glycosides **20** and **23**, leading to the hydroxyl derivatives **21** and **24** (Scheme 3E and 3F, respectively), could be efficiently effected by treatment with HCl/methanol, as seen before (Table 1, entry *xii*). Finally, activation of phenyl thioglycoside **25** with the thiophilic promotor system Ph₂SO/Tf₂O,^[46] in the presence of 4-*tert*-butyl-2,6-dimethylpyridine (TBMP), was also possible and yielded glucoside **26** in 68% yield, as an (8:1) α/β anomeric mixture with the α -anomer prevailing.

Scheme 4. NPOE **27**, MeOEs **2**, **29** and **30** and glycosyl bromide **31**, in the glycosylation of BODIPYs **7** and **9**.

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In keeping with these studies, the system NIS/BF₃·OEt₂ was found to successfully activate NPOE **27** in the glycosylation of BODIPY **9**, to furnish mannopyranosyl BODIPY **28** in 85% yield (Scheme 4A). In this case, no -CN replacement by fluoride at boron was observed, probably due to the short reaction time required for the reaction to be completed (15 min).

As seen earlier (Scheme 2), MeOEs could be efficiently activated by the action of BF₃·OEt₂ at low temperature, and accordingly glycosyl BODIPYs **28**, **14**, **12** and **20** were uneventfully obtained from MeOEs **2**, **29** and **30**, (Scheme 4B,C,D,E). The use of AgOTf to activate glycosyl bromide **31**, was also compatible with BODIPY **9**, and mannopyranosyl BODIPY **32** could be obtained, in 36% yield (45% yield based on recovered starting material, Scheme 4F). In this case, the low yield of **32** was explained by a competing acetylation of the BODIPY aglycon (24% of BODIPY-OAc **9** could be isolated).

Glycosyl BODIPYs and benzyl protecting groups

As mentioned earlier, benzyl groups play a key role on the synthesis of saccharides, since they are visualized as permanent protecting groups to be usually removed at one of the last steps of the synthetic sequence.^[47] Thus, the question of the stability of our BODIPYs, and their benzylic BODIPY-glycoside bonds under hydrogenolytic conditions became worthy of study.

In this context, previous studies had already shown that alkylated BODIPYs were stable under hydrogenolytic conditions.^[48] With these precedents in mind, we checked the stability of substituted BODIPYs **6–10**, compared to that of non-alkylated BODIPY **1** (Scheme 5A). Thus, whereas the former derivatives were recovered unmodified after prolonged treatment with H₂ [(35 psi), 7 h, Pd/C, MeOH/CH₂Cl₂], treatment of fluorescent BODIPY **1** with H₂ (balloon, Pd/C, MeOH/CH₂Cl₂, 40 min) caused complete disappearance of its fluorescence, thereby indicating that cleavage of the BODIPY core might have taken place (Scheme 5A). Next, we tested the stability of the benzylic-BODIPY bond in our BODIPY-glycosides by submitting perbenzoylated derivatives **12** and **14**, to hydrogenolytic conditions (Scheme 5B). Accordingly, treatment glycosides **12** and **14** with H₂ [(35 psi), 7 h, Pd/C, MeOH/CH₂Cl₂], left the substrates unchanged, proving the stability of the benzylic BODIPY bond (Scheme 5B).

Based on these preliminary results, the hydrogenation of tri-*O*-benzyl-mannopyranoside **28**, and tetrabenzyl mannopyranoside **34** took place uneventfully leading to triol **33** and tetraol α,β -**13**, respectively, in excellent yields (Scheme 6A, 6B). Likewise, hydrogenation of tri-*O*-benzyl mannopyranoside **35** gave access to triol **36** in almost quantitative yield (Scheme 6C). Finally, to gain further knowledge on the behavior of “non-substituted” BODIPY **1** towards hydrogenation, a green fluorescent solution of mannopyranosyl BODIPY **3** was treated with H₂ [(balloon), Pd/C, MeOH/CH₂Cl₂]. After 40 min, and in line with our previous observation (Scheme 5A), the fluorescence as well as the green color of the solution had completely disappeared. Then, acetylation of the ensuing crude reaction mixture led to the isolation of a non-fluorescent glycoside, for which we tentatively propose the structure **37**, based on its NMR and mass spectra (Scheme 6D).

From these studies, it appeared that benzyl ethers in the saccharide portion of these BODIPY glycosides could be removed chemoselectively leaving the (benzyl)BODIPY aglycon intact (e.g., Scheme 6A and Scheme 6B).

From these studies, it also seemed that the benzyl BODIPY bond between the BODIPY and the carbohydrate is more resistant to hydrogenolysis than the “standard” benzyl protecting groups, probably owing to the steric hindrance of its “*ortho*-BODIPY” substituent. Finally, alkylation around the BODIPY core in these derivatives played an important role in their stability towards hydrogenation, especially when compared to that of non-alkylated BODIPY **1**.

Accordingly, benzyl ethers could be used as protecting groups in combination with our BODIPY derivatives, the former being able to be chemoselectively removed without tampering with the BODIPY aglycon.

From the collection of results mentioned above, it became clear that some BODIPY derivatives could tolerate, without decomposition, the protection and deprotection events commonly employed in oligosaccharide synthesis.

Scheme 5. Hydrogenolysis studies on BODIPYs **1**, **6**, **7**, **8** and **10** and BODIPY glycosides **12** and **14**.

Scheme 6. Hydrogenolysis of BODIPY-glycosides **28**, **34**, **35** and **3**.

Synthesis of BODIPY trisaccharides

As proof of concept, and in order to illustrate the potential of BODIPY aglycons on the synthesis of oligosaccharides, we embarked on the synthesis of one linear and one branched BODIPY-labeled mannose trisaccharides **38** and **39**, respectively (Scheme 7A,B). Thus, unprotected mannopyranoside **15** (Scheme 3B) was glycosylated with perbenzoylated thioglycoside **18** to give disaccharide **40**, which was then regioselectively glycosylated at O-3 with NPOE **41**^[49] to yield branched trimannan **38a**. Saponification of the latter (NaOMe/MeOH) yielded

unprotected trisaccharide **38b** (Scheme 7A). On the other hand, the route to linear trimannan derivative **39**, started from 6-hydroxy mannopyranoside **21** (Scheme 3D), which upon glycosylation with thioglycoside donor **19**, mediated by NIS/Yb(OTf)₃, produced disaccharide **42a**. De-O-silylation of the latter (HCl/MeOH, MW irradiation) yielded disaccharide **42b**, in fairly good yield. Finally, glycosylation of disaccharide **42b** with phenyl thioglycoside **18** generated trisaccharide **39a**, which upon de-O-benzoylation yielded linear BODIPY-labeled trimannan **39b** (Scheme 7B).

Scheme 7. A) Synthesis of branched trisaccharides **38**, and B) synthesis of linear trisaccharides **39**, containing 4,4'-dicyano-BODIPY **9**, as the aglycon.

Photophysical studies

To impel the advanced applications of the new glycoprobes we have analyzed the photonic behavior under low (photophysical properties) and high (laser properties) irradiation regimes of some representative BODIPY-labeled saccharides. The *ortho*-glycosylation of the chromophoric C-8-benzyl group led to a rigid and compact molecular structure, owing to a higher steric hindrance imposed by the bulky saccharides (up to three units in **38a** and **38b**). In fact, the structural arrangement of the C-8-benzyl residue was nearly orthogonal (twisting dihedral angle computed in the ground state of 85–90° in analogues **15** and **17**, Figure 2) reducing the internal conversion pathways associated to its free motion. Such geometrical disposition explained the low impact of the grafted functionalization (number of saccharide units, sugar protecting groups, and chain-length between the saccharide and the 8-aryl unit) on the photophysical signatures and the recorded high fluorescence efficiencies (Table 3). The

replacement at the boron bridge of fluorine atoms (*F*-BODIPYs) by cyano groups (*CN*-BODIPYs) had low impact on the spectral properties of the BODIPYs (Figure 3), but induced an increase of the emission efficiency (see for example **17** versus **15** in Table 3), reaching values higher than 80%, and approaching the value reported for the parent 1,3,5,7,8-pentamethyl-2,6-diethylpyrromethene-difluoroborate (pyrromethene PM567) dye (Table 3).^[50] Such enhancement, upon chemical modification at the boron bridge with electron withdrawing groups, agreed with previously reported results with similar functionalization at the boron center.^[32b]

It is worth mentioning that the glycoprobes based on fully unprotected saccharides, such as **17** and **15** (with a monosaccharide) and **38b** (with a trisaccharide) displayed high water solubility. In fact, these derivatives, and particularly those based on 4,4'-dicyano BODIPYs, displayed a remarkable fluorescence efficiency in water, reaching quantum yield values up to 82% (Table 3). Nevertheless, they showed a slight trend

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toward molecular aggregation by increasing the dye concentration in water. For example, **38b** that became soluble in water at concentrations as high as 2 mM, tended to aggregate at concentrations higher than 0.1 mM, as it was revealed by the increased absorbance observed at the short-wavelength vibronic shoulder (Figure S1 in ESI). Such change in the absorption spectrum profiles can be regarded as a fingerprint of non-emissive H-aggregates, which decreased the fluorescence response and impaired an effective laser action. However, it is worth mentioning that a water-soluble and highly fluorescent BODIPY at concentrations up to 0.1 mM fulfilled the requirements normally demanded in biological microscopy studies. By corollary, from a photophysical standpoint, the ensuing BODIPY-labeled saccharides could be used as stable and fluorescent water-soluble chromophores addressing one of the current challenges in molecular imaging.

Figure 2. Computed frontier molecular orbitals (A, b3lyp/6-311g*) involved in the recorded absorption (B), and fluorescence (C, scaled by their efficiency) transitions in water for the BODIPY-labeled monosaccharide **17** (in black) and its analogue **15** (in red), where the 4,4'-fluorine atoms are replaced by cyano groups. The ground state optimized geometries in side-view (A) are also shown to highlight the orthogonal arrangement of the 8-aryl moiety linked to the BODIPY scaffold.

Lasing properties

According to their absorption properties, the lasing properties of the new dyes were studied under pumping at 532 nm. Under our experimental conditions (transversal excitation and strong focusing of the incoming pump radiation) the concentration of the dyes should be in the millimolar range, to ensure total absorption of the pump radiation within the first millimeter at most of the solution, in order to obtain an emitted beam with near-circular cross-section and optimize the lasing efficiency (ratio between the energy of the dye laser output and the pump energy incident on the sample surface). To determine the dye concentration that optimizes the laser emission of the new dyes, first the dependence of their laser emission on the corresponding dye concentrations was analyzed in methanol by varying the concentration from 0.1 to 2 mM, while keeping all the other experimental parameters constant. In all cases the dyes followed the typical behavior, with the lasing efficiency first increasing with dye concentration until a maximum value is reached. Increasing the dye concentration beyond this point results in a decrease of the lasing efficiency that can be related to

reabsorption/reemission processes, which become increasingly important as the dye concentration rises. The optimum value of the dye concentration so determined was then used to analyze the effect of the molecular structure solvent on the laser properties of the different derivatives. The lasing data recorded under these experimental conditions are collected in Table 3.

	$\lambda_{ab}^{[a]}$ (nm)	$\epsilon_{max} \cdot 10^4 [a]$ (M ⁻¹ ·cm ⁻¹)	$\lambda_{fl}^{[a]}$ (nm)	$\phi^{[a]}$	$\tau^{[a]}$ (ns)	%Eff ^[b]	I[%] ^[b]
PM567	516.0	6.9	534.0	0.81	6.10	48	17
15	523.5	7.7	535.0	0.87	7.21	68	100
	524.0	5.1	535.5	0.82	7.28		
17	523.0	5.4	536.0	0.74	6.67	44	25
	523.0	3.0	533.5	0.60	6.86		
28	525.0	6.7	535.5	0.81	7.24	65	100
40	524.5	6.2	534.0	0.82	6.71	64	100
38a	525.0	6.6	533.5	0.83	6.97	68	100
38b	524.0	6.9	535.0	0.81	7.18	66	100
	525.0	5.2	537.0	0.77	7.29		

Table 3. Photophysical^[a] and laser^[b] properties of the herein synthesized glycoprobes in a common solvent (methanol). The corresponding data for the water-soluble compounds (in H₂O) are also included (*in italics*). Additional photophysical data in other solvents are collected in Table S1 in ESI.

[a] Dye concentration: 2 μ M. Absorption (λ_{ab}) and fluorescence (λ_{fl}) wavelength, molar absorption at the maximum (ϵ_{max}), fluorescence quantum yield (ϕ) and lifetime (τ). [b] Dye concentration 0.8 mM. Laser efficiency (Eff(%)) and photostability (I(%)): intensity of the laser output after 100000 pump pulses with respect to its initial intensity I_0 ; I(%) = (I/I_0) $\times$ 100, at 10 Hz repetition rate.

All the new dyes exhibited laser action peaked at ca. 570 nm, with a pump threshold energy of 0.8 mJ, a beam divergence of 0.5 mrad, and a pulse duration of 8 ns full-width at half maximum (FWHM). The observed lasing efficiencies correlated well with their photophysical properties; a) the higher the fluorescence quantum yield the higher became the lasing efficiency; b) the replacement at the boron bridge of the fluorine atoms by cyano groups enhanced significantly the lasing efficiency, i.e. from a 48% recorded for **17** to an impressive 68% recorded for its similar cyano derivative **15**; and c) the lasing efficiency of the glycoprobes built on *F*-BODIPY as well as on *CN*-BODIPY scaffold became independent on the structure of the saccharide inserted in the final dye, (i.e. protected and unprotected mono-, di-, and tri-saccharides led to a lasing efficiency ca. 48% for *F*-BODIPYs and a 68% for the corresponding *CN*-BODIPYs). An important parameter for any practical applications of these glycoprobes is their lasing photostability under hard radiation conditions and long operation times. A reasonable evaluation of the photostability of these dyes can be obtained by irradiating a small amount of solution with exactly the same pumping energy and geometry as used in the laser experiments, and monitoring the evaluation of the laser-induced fluorescence intensity with respect to the number of pump pulses (see Experimental Section). In Table 3 are collected data on the decrease of the laser-induced fluorescence intensity under transversal excitation at 532 nm, with

5 mJ/pulse and 10 Hz repetition rate after 100 000 pump pulses, for the new glycoprobes herein synthesized. Once again, the lasing photostability of the new BODIPY-saccharides matched their photophysical properties since the lower the non-radiative rate constant, the higher became the resistance to pumping showed by the new dyes regardless of the saccharide inserted in its molecular structure. In this way, the glycoprobes derived from the CN-BODIPY skeleton behaved as highly efficient and photostable dyes since its laser emission remained at its initial level after 100 000 pump pulses enhancing that recorded with the corresponding F-BODIPY saccharides which lasing emission dropped more than 80% in the same pumping period.

Conclusions

In order to validate the potential of BODIPY-dyes as aglycons in synthetic sequences leading to saccharides, we have performed a comprehensive study of the compatibility of some BODIPY derivatives with the reaction conditions normally employed in these protocols, namely: *i*) glycosylation and *ii*) protecting-group manipulations. Regarding the glycosylation phase, we have evaluated the endurance of BODIPY derivatives in glycosylations with glycosyl trichloroacetimidates, thioglycosides, *n*-pentenyl orthoesters (NPOEs), 1,2-methyl orthoesters (MeOEs) or glycosyl bromides, as glycosyl donors. From the standpoint of protecting-group manipulations, we have identified reaction conditions, which do not tamper with the BODIPY-core and allow the removal of silyl and benzoyl substituents, as representative of "temporary" protecting groups, and of benzyl substituents as protecting groups regarded as "permanent".^[36] In general, and in keeping with literature precedents,^[33] we have found that B(CN)₂-BODIPYs are more resistant to acidic conditions than the corresponding BF₂-derivatives. The latter, however, are relatively more stable than the former under basic conditions. Also noteworthy, we have observed that the substitution pattern on the dipyrromethene-core also plays a significant role on the stability of the dye. Thus, 1,3,5,7-tetramethyl-2,6-diethyl BODIPY derivatives, i.e. **8**, **9**, proved to be more stable than their corresponding 2,6-*des*-ethyl analogues, i.e. **6**, **7**, under either acidic or basic conditions.

Accordingly, it has become apparent from these studies that BODIPY derivatives **8** or **9**, could be successfully employed as aglycons in synthetic oligosaccharide synthesis. As such, they could play a dual role, as glycosyl tags by facilitating the visualization and identification of reaction products, and as fluorescent labels in the target oligosaccharides.

Appealingly, from a synthetic standpoint, access to these BODIPYs could be carried out efficiently in gram-scale operations. Thus, BODIPY **8** is readily obtained by a one-step reaction from 2,4-dimethyl-3-ethyl pyrrole and phthalide,^[21] and B(CN)₂-BODIPY **9** is then easily available from BF₂-BODIPY **7** by reaction with TMSCN mediated by BF₃·OEt₂.^[32]

From the photophysical perspective, B(CN)₂-BODIPYs displayed better photophysical properties than the corresponding BF₂-derivatives, possibly making B(CN)₂-BODIPY **9** the aglycon of choice in BODIPY-assisted fluorescent-saccharides synthesis. In addition, we have found that the carbohydrate subunits have a

beneficial effect on the, already good, photophysical features of these BODIPY-dyes.

In summary, the incorporation of a BODIPY-tag at the reducing end of the target saccharide could prove particularly useful in glycosylations when an excess of non-fluorescent glycosyl donors will be required, since it allows the easy visualization of the fluorescent final product and the unreacted glycosyl acceptor.

Experimental Section

Representative example: synthesis of fluorescently-labeled branched trisaccharide 36b, using [B(CN)₂] BODIPY **9 as the aglycon.**

Synthesis of B(CN)₂-BODIPY **9 from BF₂-BODIPY **8**.** A solution of BF₂-BODIPY **8**^[21] (1.3 g, 3.17 mmol) in dry CH₂Cl₂ (20 mL) was cooled to 0 °C and treated with BF₃·OEt₂ (78 µL, 0.63 mmol). The mixture was stirred at r.t. for 5–10 min, then TMSCN (1.97 mL, 15.8 mmol) was added and the reaction mixture kept under these conditions for 120 min. The reaction was neutralized with saturated aqueous NaHCO₃ solution, diluted with CH₂Cl₂ (x mL) and washed with water (2 x x mL). The organic phase was dried over MgSO₄, concentrated and purified on a silica gel column (hexane-ethyl acetate; 9:1). **9** (1.17 g mg, 87%); ¹H NMR (400 MHz, CDCl₃) δ 7.70 (d, *J* = 7.7 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 7.7 Hz, 1H), 4.56 (s, 2H), 2.69 (s, 6H), 2.34 (q, *J* = 7.6 Hz, 4H), 1.31 (s, 6H), 1.00 (t, *J* = 7.6 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 154.4, 139.7, 139.2, 138.3, 134.5 (broad C), 132.6, 130.0, 128.8 (broad C), 128.6, 128.1, 62.3, 17.3, 14.6, 13.5, 11.5. ¹⁹F NMR (376 MHz, CDCl₃): no peaks; ¹¹B NMR (128 MHz, CDCl₃) δ -16.25; HRMS (ESI-TOF): calc for C₂₆H₃₀BN₄O: [M+H]⁺ 425.25118; found: 425.25088.

Mannopyranosyl BODIPY **14.** To a solution of BODIPY-acceptor **9** (424 mg, 1 mmol) and TCA **11** (1 g, 1.36 mmol) in anhydrous CH₂Cl₂ (15 mL) was added 4Å molecular sieves (100% w/w). The mixture was stirred at rt for about 15 min under an Ar atmosphere, then cooled down to -78 °C. NEW BF₃·OEt₂ (84 µL, 0.68 mmol) was then added and the reaction mixture kept at this temperature. The mixture was stirred under these conditions for 30 min, and then neutralized by addition of Et₃N (1 mL). The mixture was evaporated and chromatographed over silica gel flash column (hexane-ethyl acetate, 8:2) to give compound **14** (661 mg, 66%). Red film-like solid product, [α]_D²¹ = +15.1 (c 0.4 in CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.01–7.16 (m, 24H), 6.01 (t, *J* = 10.1 Hz, 1H), 5.63 (dd, *J* = 10.2, 3.3 Hz, 1H), 5.28 (dd, *J* = 3.3, 1.8 Hz, 1H), 4.91 (d, *J* = 1.8 Hz, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.57 (dd, *J* = 12.3, 2.7 Hz, 1H), 4.40 (d, *J* = 11.5 Hz, 1H), 4.34 (dd, *J* = 12.3, 3.4 Hz, 1H), 4.19 (dt, *J* = 10.1, 3.1 Hz, 1H), 2.60 (s, 6H), 2.38–2.19 (m, 4H), 1.36 (s, 3H), 1.25 (s, 3H), 0.91 (t, *J* = 7.5 Hz, 3H), 0.90 (t, *J* = 7.5 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 166.2, 165.5, 165.1, 156.1, 153.9, 140.4, 139.0, 138.4, 135.3, 134.7, 134.4, 134.3, 133.5, 133.4, 133.3, 133.1, 130.3, 130.1, 130.0, 129.9, 129.8, 129.7, 129.5, 129.2, 128.8, 128.6, 128.5, 128.4. ¹¹B NMR (128 MHz, CDCl₃) δ -16.16. ¹⁹F NMR (376 MHz, CDCl₃): no peaks. HRMS (ESI-TOF): calcd for C₆₀H₅₉BN₅O₁₀: [M+NH₄]⁺ 1020.43594, found: 1020.43754. calcd for C₆₀H₅₅BN₄NaO₁₀: [M+Na]⁺ 1025.39133, found: 1025.39126.

Mannopyranosyl-tetraol BODIPY **15.** A solution of BODIPY mannopyranoside **14** (572 mg, 0.57 mmol) in MeOH (10 mL) was treated with NaOMe (184 mg, 3.42 mmol). After stirring at room temperature for 3 h, the solution was neutralized with ion-exchange resin (H⁺), then filtered and concentrated. The residue was purified by column chromatography on silica gel (MeOH:CH₂Cl₂; 9:1) to provide tetraol **15** (228 mg, 70%) as a red solid. m. p. 140.1 °C (decomposition); [α]_D²¹ = +179.4 (c 0.07 in MeOH); ¹H NMR (500 MHz, CDCl₃) δ 7.61–7.44 (m, 3H), 7.21 (d, *J* = 7.2 Hz, 1H),

4.67 (s, 1H), 4.50 (d, $J = 11.0$ Hz, 1H), 4.22 (d, $J = 11.4$ Hz, 1H), 3.80 – 3.55 (m, 2H), 3.53 – 3.34 (m, 3H), 3.13 – 2.99 (m, 1H), 2.66 (m, 6H), 2.44 – 2.25 (m, 4H), 1.27 (s, 6H), 1.00 (q, $J = 7.5$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 154.5, 154.3, 140.0, 139.8, 139.1, 134.8, 134.7, 134.5, 133.8, 130.0, 129.9, 129.3, 129.0, 128.7, 128.3, 100.2, 72.4, 71.5, 70.6, 67.3, 65.8, 60.6, 17.3, 14.6, 14.5, 13.5, 11.6, 11.5. ^{11}B NMR (128 MHz, CDCl_3) δ -16.25. HRMS (ESI-TOF): calcd for $\text{C}_{32}\text{H}_{43}\text{BN}_5\text{O}_6$: $[\text{M}+\text{NH}_4]^+$ 604.33066 found: 604.33040. calcd for $\text{C}_{32}\text{H}_{39}\text{B N}_4\text{NaO}_6$: $[\text{M}+\text{Na}]^+$ 609.28605, found: 609.28555.

BODIPY-disaccharide 40. To a stirred solution of tetraol **15** (50 mg, 0.087 mmol), and thioglycoside donor **18** (139 mg, 0.22 mmol) and *N*-iodosuccinimide (NIS) (49 mg, 0.22 mmol) in CH_2Cl_2 (8 mL), $\text{Yb}(\text{OTf})_3$ (136 mg, 0.22 mmol) was added at room temperature. The reaction mixture was stirred for 15 min and then partitioned between aqueous sodium thiosulfate/ NaHCO_3 and CH_2Cl_2 . After washing with water and brine, the combined organic extracts were dried over anhydrous Na_2SO_4 , and evaporated. The residue was chromatographed over silica gel flash column (hexane–ethyl acetate, 6:4) to give disaccharide **40** (75 mg, 74%) as a red film-like solid. $[\alpha]_{\text{D}}^{21} = +392.1$ (c 0.2 in CHCl_3). ^1H NMR (500 MHz, CDCl_3) δ 8.21 – 7.69 (m, 8H), 7.67 – 7.21 (m, 16H), 6.11 (t, $J = 10.2$ Hz, 1H), 5.94 (dd, $J = 10.2$, 3.3 Hz, 1H), 5.69 (dd, $J = 3.3$, 1.8 Hz, 1H), 5.24 (d, $J = 1.8$ Hz, 1H), 4.72 – 4.64 (m, 3H), 4.58 – 4.52 (m, 1H), 4.47 (dd, $J = 12.2$, 4.1 Hz, 1H), 4.35 (d, $J = 11.3$ Hz, 1H), 3.97 (dd, $J = 11.4$, 5.5 Hz, 1H), 3.89 (dd, $J = 11.4$, 1.9 Hz, 1H), 3.70 (td, $J = 9.6$, 2.7 Hz, 1H), 3.59 – 3.52 (m, 1H), 3.31 (td, $J = 9.1$, 3.5 Hz, 1H), 3.28 – 3.21 (m, 1H), 2.70 (s, 3H), 2.68 (s, 3H), 2.39 – 2.31 (m, 4H), 1.35 (s, 3H), 1.30 (s, 3H), 1.04 – 0.97 (m, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 166.3, 165.7, 165.6, 154.2, 154.1, 140.6, 140.1, 139.9, 134.7, 134.6, 133.6, 133.5, 133.3, 133.1, 131.0, 130.1, 130.0, 129.9, 129.6, 129.4, 129.2, 128.7, 128.6, 128.5, 128.4, 100.6, 97.6, 71.9, 71.5, 70.9, 70.1, 69.9, 69.2, 69.0, 68.1, 67.1, 66.9, 63.0, 17.4, 17.3, 14.6, 13.6, 11.9, 11.5. HRMS (ESI-TOF): calcd for $\text{C}_{66}\text{H}_{69}\text{BN}_5\text{O}_{15}$: $[\text{M}+\text{NH}_4]^+$ 1182.4885 found: 1182.49303. calcd for $\text{C}_{66}\text{H}_{65}\text{BN}_4\text{NaO}_{15}$: $[\text{M}+\text{Na}]^+$ 1187.44424, found: 1187.44934.

BODIPY-trisaccharide 38a. To a stirred solution of BODIPY-disaccharide **40** (41 mg, 0.035 mmol), NPOE donor **41** (70 mg, 0.105 mmol) and *N*-iodosuccinimide (23 mg, 0.105 mmol) in CH_2Cl_2 (8 mL), BF_3OEt_2 (5 μL , 0.022 mmol) was added at -30°C . The reaction mixture was stirred for 15 min and then partitioned between aqueous sodium thiosulfate/ NaHCO_3 and CH_2Cl_2 . After washing with water and brine, the combined organic extracts were dried over anhydrous Na_2SO_4 , and evaporated. The residue was chromatographed over silica gel flash column (hexane–ethyl acetate, 6:4) to give compound **38a** as a non crystalline red film-like solid product (76 mg, 53%). $[\alpha]_{\text{D}}^{21} = -125.9$ (c 0.9 in CHCl_3). ^1H NMR (500 MHz, CDCl_3) δ 8.19 – 7.76 (m, 16H), 7.67 – 7.17 (m, 28H), 6.20 (t, $J = 10.2$ Hz, 1H), 6.14 (t, $J = 10.1$ Hz, 1H), 5.98 (dd, $J = 4.5$, 3.2 Hz, 1H), 5.96 (dd, $J = 4.4$, 3.3 Hz, 1H), 5.78 (dd, $J = 3.2$, 1.9 Hz, 1H), 5.73 (dd, $J = 3.3$, 1.8 Hz, 1H), 5.34 (d, $J = 1.8$ Hz, 1H), 5.10 (d, $J = 2.0$ Hz, 1H), 4.86 (dt, $J = 10.3$, 3.1 Hz, 1H), 4.73 (dd, $J = 12.2$, 2.5 Hz, 1H), 4.67 (d, $J = 1.7$ Hz, 1H), 4.64 (dd, $J = 12.3$, 2.6 Hz, 1H), 4.62 (d, $J = 11.2$ Hz, 1H), 4.60 – 4.53 (m, 2H), 4.50 (dd, $J = 12.2$, 3.9 Hz, 1H), 4.25 (d, $J = 11.1$ Hz, 1H), 4.15 (t, $J = 9.7$ Hz, 1H), 4.03 (dd, $J = 11.8$, 4.5 Hz, 1H), 3.89 (dd, $J = 11.8$, 1.8 Hz, 1H), 3.81 (s, 1H), 3.63 (dd, $J = 9.4$, 3.2 Hz, 1H), 3.52 (ddd, $J = 9.9$, 4.5, 1.8 Hz, 1H), 2.65 (s, 3H), 2.60 (s, 3H), 2.35 – 2.23 (m, 2H), 2.22 – 2.13 (m, 2H), 1.29 (s, 3H), 1.29 (s, 3H), 1.29 (s, 3H), 0.93 (t, $J = 7.6$ Hz, 3H), 0.75 (t, $J = 7.6$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 166.4, 166.3, 165.8, 165.6, 165.5, 154.6, 154.4, 140.0, 139.7, 139.4, 134.8, 134.6, 134.5, 133.7, 133.6, 133.5, 133.4, 133.3, 133.2, 133.1, 131.0, 130.2, 130.1, 130.0, 129.9, 129.5, 129.4, 129.3, 129.2, 129.1, 128.8, 128.7, 128.6, 128.5, 128.4, 100.7, 99.2, 97.8, 82.9, 72.1, 71.0, 70.8, 70.4, 70.1, 69.6, 69.4, 69.0, 68.5, 67.1, 66.6, 65.1, 63.0, 62.9, 17.4, 17.3, 14.6, 14.4, 13.6, 13.5, 11.8, 11.5. ^{11}B NMR (128 MHz, CDCl_3) δ -16.25. HRMS (ESI-TOF): calcd for

$\text{C}_{100}\text{H}_{95}\text{BN}_5\text{O}_{24}$: $[\text{M}+\text{NH}_4]^+$ 1760.64696 found: 1760.64620. calcd for $\text{C}_{100}\text{H}_{91}\text{BN}_4\text{NaO}_{24}$: $[\text{M}+\text{Na}]^+$ 1765.60236, found: 1765.61089.

BODIPY-trimannan derivative 38b. A solution of benzoylated trisaccharide **38a** (56 mg, 0.032 mmol) in $\text{MeOH}-\text{CH}_2\text{Cl}_2$ (V:V/2:1, 4 mL) was treated with NaOMe (14 mg, 0.26 mmol). After stirring at room temperature for 4 h, the solution was neutralized with ion-exchange resin (H^+), then filtered and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate: $\text{MeOH}:\text{H}_2\text{O}$; 10:2:1) to give **38b** (22 mg, 77%) as a red film-like solid. $[\alpha]_{\text{D}}^{21} = +960.9$ (c 0.3 in CHCl_3). ^1H NMR (500 MHz, CDCl_3) δ 7.70 (dd, $J = 7.7$, 1.4 Hz, 1H), 7.63 (td, $J = 7.6$, 1.5 Hz, 1H), 7.58 (dt, $J = 7.5$, 1.4 Hz, 1H), 7.33 (dd, $J = 7.5$, 1.4 Hz, 1H), 4.94 (d, $J = 1.8$ Hz, 1H), 4.78 (d, $J = 1.7$ Hz, 1H), 4.71 (d, $J = 1.7$ Hz, 1H), 4.60 (d, $J = 10.8$ Hz, 1H), 4.27 (d, $J = 10.9$ Hz, 1H), 3.94 (dd, $J = 3.4$, 1.8 Hz, 1H), 3.91 (dd, $J = 3.4$, 1.7 Hz, 1H), 3.83 – 3.74 (m, 6H), 3.73 – 3.65 (m, 3H), 3.64 – 3.57 (m, 4H), 3.45 – 3.40 (m, 2H), 3.14 (ddd, $J = 10.1$, 4.8, 1.9 Hz, 1H), 2.70 (s, 3H), 2.69 (s, 3H), 2.54 – 2.37 (m, 4H), 1.42 (s, 3H), 1.37 (s, 3H), 1.04 (t, $J = 7.5$ Hz, 67H). ^{13}C NMR (126 MHz, CDCl_3) δ 156.2, 155.0, 142.0, 141.4, 141.0, 136.5, 136.2, 136.1, 136.0, 135.5, 132.4, 131.2, 130.7, 130.5, 130.2, 129.7, 103.6, 101.9, 101.2, 81.2, 74.8, 74.0, 73.3, 72.6, 72.5, 72.1, 71.9, 71.0, 68.7, 68.6, 68.1, 66.5, 66.4, 62.8, 62.7, 18.2, 18.0, 14.9, 14.9, 13.8, 13.6, 12.1, 11.9. HRMS (ESI-TOF): calcd for $\text{C}_{44}\text{H}_{63}\text{BN}_5\text{O}_{16}$: $[\text{M}+\text{NH}_4]^+$ 928.43650 found: 928.43253. calcd for $\text{C}_{44}\text{H}_{59}\text{BN}_4\text{NaO}_{16}$: $[\text{M}+\text{Na}]^+$ 933.39190, found: 933.39046.

Photophysical properties. Spectroscopic properties were registered in diluted solutions (around 2×10^{-6} M), prepared by adding the corresponding solvent (spectroscopic grade) to the residue from the adequate amount of a concentrated stock solution in acetone, after vacuum evaporation of this solvent. UV-Vis absorption spectra were recorded on a Varian model CARY 4E spectrophotometer, whereas the fluorescence and excitation spectra, as well as the decay curves were registered in an Edinburgh Instruments spectrofluorimeter (model FLSP 920). Fluorescence quantum yields (ϕ) were obtained using as reference commercial cresyl violet ($\phi^r = 0.54$ in methanol), for the long-wavelength emission of the energy acceptor, and commercial PM546 ($\phi^r = 0.81$ in methanol) to account for the green, and quenched emission owing to energy transfer, of the energy donor subunit. The values were corrected by the refractive index of the solvent. Radiative decay curves were registered with the time correlated single-photon counting technique using a multichannel plate detector with picosecond time-resolution. Fluorescence emission was monitored at the maximum emission wavelength after excitation by means of a wavelength-tunable Fianium Supercontinuum laser. The fluorescence lifetime (τ) was obtained after the deconvolution of the instrumental response signal from the recorded decay curves by means of an iterative method. The decay curve was essentially the same regardless of the excited visible absorption band. The goodness of the exponential fit was controlled by statistical parameters (chi-square and the analysis of the residuals). The radiative (k_{R}) and non-radiative (k_{NR}) rate constants were calculated from the fluorescence quantum yield and lifetime; $k_{\text{R}} = \phi/\tau$ and $k_{\text{NR}} = (1-\phi)/\tau$.

Quantum mechanical calculations. Ground state geometries were optimized at the Density Functional Theory (DFT) using range-separated hybrid wB97XD method. The vertical Franck-Condon electronic transition was predicted by the Time Dependent (TD) method and the same functional. In both cases the double valence basis set with one polarization functions (6-31g*) was used owing to the large size of the computed multichromophoric molecules. The geometries were considered as energy minimum when the corresponding frequency analysis did not give any negative value. The solvent effect (cyclohexane) was considered in the conducted theoretical simulations by means of the Polarizable Continuum Model (PCM). All the calculations were performed using the Gaussian 16 software as implemented in the computational cluster "arina" of the UPV/EHU.

Laser properties. Laser efficiency was evaluated from concentrated solutions (millimolar) of dyes in ethyl acetate contained in 1-cm optical-path rectangular quartz cells carefully sealed to avoid solvent evaporation during experiments. The liquid solutions were transversely pumped with 8 mJ, 8 ns FWHM pulses from the third harmonic (355 nm) or the second harmonic (532 nm) of a Q-switched Nd:YAG laser (Lotis TII 2134) at a repetition rate of 1 Hz. The exciting pulses were line-focused onto the cell using a combination of positive and negative cylindrical lenses ($f = 15$ cm and $f = -15$ cm, respectively) perpendicularly arranged. The plane parallel oscillation cavity (2 cm length) consisted of a 90% reflectivity aluminium mirror acting as back reflector, and the lateral face of the cell acting as output coupler (4% reflectivity). The pump and output energies were detected by a GenTec powermeter. The photostability of the dyes in ethyl acetate solution was evaluated by using a pumping energy and geometry exactly equal to that of the laser experiments. We used spectroscopic quartz cuvettes with 0.1 cm optical to allow for the minimum solution volume ($V_s = 40$ μ L) to be excited. The lateral faces were grounded, whereupon no laser oscillation was obtained. Information about photostability was obtained by monitoring the decrease in laser-induced fluorescence (LIF) intensity. In order to facilitate comparisons independently of the experimental conditions and sample, the photostability figure of merit was defined as the accumulated pump energy absorbed by the system (E_{dose}), per mole of dye, before the output energy falls to a 50% its initial value. In terms of experimental parameters, this energy dose, in units of GJ mol^{-1} , can be expressed as:

$$E_{\text{dose}}^{50\%} (\text{GJ} \cdot \text{mol}^{-1}) = \frac{E_{\text{pump}} (\text{GJ}) \cdot (1 - 10^{-\epsilon CL}) \cdot \sum \frac{f}{\# \text{pulses}}}{CV_s} \quad (1)$$

where E_{pump} is the energy per pulse, C is the molar concentration, ϵ is the molar absorption coefficient in units of $\text{M}^{-1} \text{cm}^{-1}$, L is the depth of the cuvette expressed in cm, V_s is the solution volume, in litres, within the cuvette, and f is the ratio between the LIF intensity after $\#$ pulses and the LIF intensity in the first pulse. To speed up the experiment the pump repetition rate was increased up to 15 Hz. The fluorescence emission and laser spectra were monitored perpendicular to the exciting beam, collected by an optical fiber, and imaged onto a spectrometer (Acton Research corporation) and detected with a charge-coupled device (CCD) (SpectruMM:GS128B). The fluorescence emission was recorded by feeding the signal to the boxcar (Stanford Research, model 250) to be integrated before being digitized and processed by a computer. The estimated error in the energy and photostability measurements was 10%.

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Keywords: BODIPY • glycosylation • BODIPY labels • BODIPY saccharides • BODIPY tags

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Entry for the Table of Contents

Layout 2:

FULL PAPER

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BODIPYs as chemically stable fluorescent tags on synthetic glycosylation strategies towards fluorescently-labeled saccharides

Boron-dipyrromethene (BODIPY) fluorophores, conveniently substituted, are able to endure most of the reaction conditions involved in synthetic sequences leading to oligosaccharides. Thus, BODIPY dyes could be used as aglycons in synthetic routes leading to fluorescently-labeled saccharides. Accordingly, they could display a dual role: as colorful glycosyl-tags during the synthesis, and as fluorescent-labels on the ensuing glycosides. The resulting BODIPY-labeled saccharides displayed remarkable fluorescence-efficiency in water, reaching quantum yield values up to 82%, as well as notable lasing efficiencies and photostabilities.