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Development of Activity-Based Probes for Imaging Human α-L-Fucosidases in Cells

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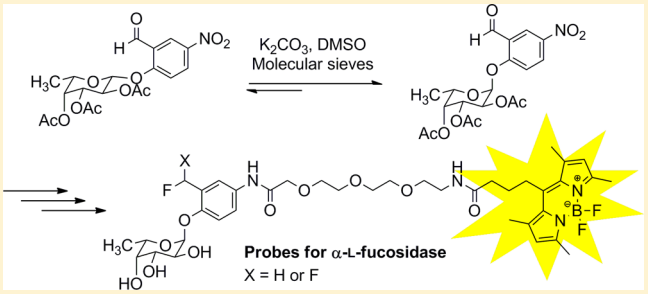
Supporting Information

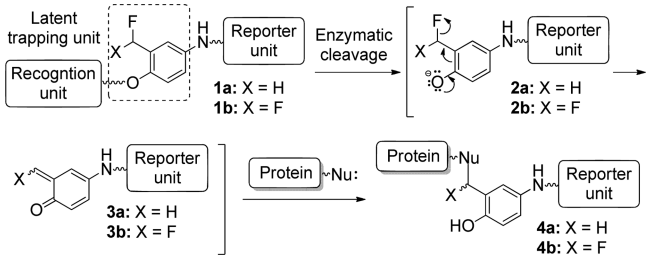
ABSTRACT: We have established a concise synthetic route relying on a key base-promoted epimerization step to synthesize two series of activity-based probes carrying a BODIPY fluorophore for α-L-fucosidase. The resulting probes were evaluated for labeling performance. The one utilizing an *o*-fluoromethylphenol derivative as the latent trapping unit was successfully applied for the first time to visualize and locate lysosomal α-L-fucosidase activity in human cells.



α-L-Fucosidase, a member of the family of glycoside hydrolases (GHs), catalyzes the hydrolytic removal of L-fucose residues from glycoconjugates. Its clinical importance is linked to the abnormal level of enzyme activity in several pathological conditions, such as genetic disorder, inflammation,[1] carcinoma of stomach, ovary[2] and liver,[3] cystic fibrosis[4] and the infection of *Helicobacter pylori*.[5] For instance, fucosidosis is a lysosomal storage disorder caused by the deficient α-L-fucosidase activity owing to genetic defect. As a consequence, the growing number of clinical findings makes it necessary to study the details of the role and activity of α-L-fucosidases in disease conditions. To date, intracellular detection of fucosidase relies heavily on antibodies raised against the target enzyme.[6,7] However, this immunological approach could only reveal the amount of proteins instead of their catalytic activity. We therefore envisioned that the development of an activity-based probe that is capable of indicating the intracellular α-L-fucosidase activity would be a valuable diagnostic tool.

To date, activity-based probes exploiting quinone methide chemistry have been developed for a wide variety of hydrolases, including tyrosine phosphatase,[8−10] sulfatase,[11,12] and numerous glycosidases (glycoside hydrolases, GHs; EC 3.2.1).[13−17] Generally, the probes (**1a** and **1b**) comprise three structural components, namely, a recognition unit, a latent trapping unit, and a reporter unit (Scheme 1). The latent trapping unit, of which *o*-fluoromethylphenol (**2a**) and *o*-difluoromethylphenol (**2b**) derivatives are two important representatives, plays a key role in forming a covalent linkage with the target. The covalent bond-forming feature of activity-based probes has been extensively explored for versatile applications, ranging from

Scheme 1. General Structure of Activity-Based Probes 1a and 1b, and the Proposed Labeling Mechanism



protein profiling[18] to the capture of influenza viruses.[19] More recently, we have seen a growing trend to developing probes for intracellular imaging applications.[12,14,20] However, most activity-based probes for GHs reported to date are limited to those that have 1,2-*trans* configuration on the pyranoside ring. Probes for α-L-fucosidase, with their 1,2-*cis* configuration, represent a highly challenging task. We herein report a concise synthesis and the efficacy of two activity-based probes **5a** and **5b** carrying a BODIPY fluorescent tag, as shown in Figure 1, for the detection of α-L-fucosidase in human cells.

Recent progress on glycosylation reactions indicated that a high degree of control of the anomeric configuration could be achieved through manipulations of the participating glycosyl donor, protecting group, solvent, and promoter.[21−25] However,

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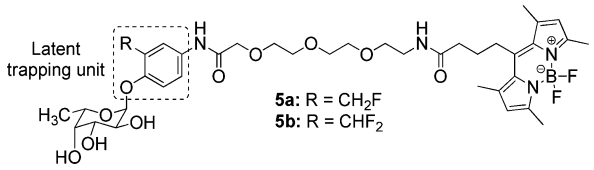
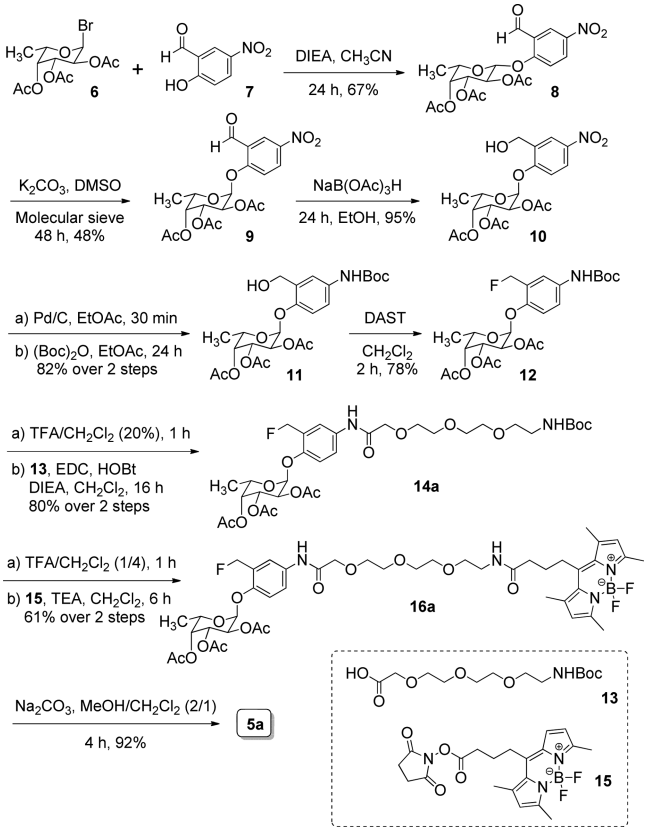


Figure 1. Structure of activity-based probes **5a** and **5b** for α-L-fucosidase.

by adopting these strategies, it also implies that a longer synthetic route would be inevitable which in turn would result in the final products being less accessible for practical applications. We herein employ a straightforward approach to develop a concise synthetic route for probes **5a** and **5b** beginning with the easily accessible peracetylated fucosyl bromide **6**[15] and 2-hydroxy-5-nitrobenzaldehyde **7**. It is interesting to note that peracetylated fucopyranoside could couple with a phenol derivative under BF_3-mediated condition to give mainly the 1,2-*cis* product in a moderate yield.[15,26] However, we failed to observe the formation of any desired glycosylated product when compound **7** was subjected to this condition. In contrast, **7** reacted smoothly with fucosyl bromide **6** under classical Koenigs–Knorr condition[27] to afford glycosylated product **8** in 67% yield (Scheme 2). Although

Scheme 2. Synthesis of Probe 5a

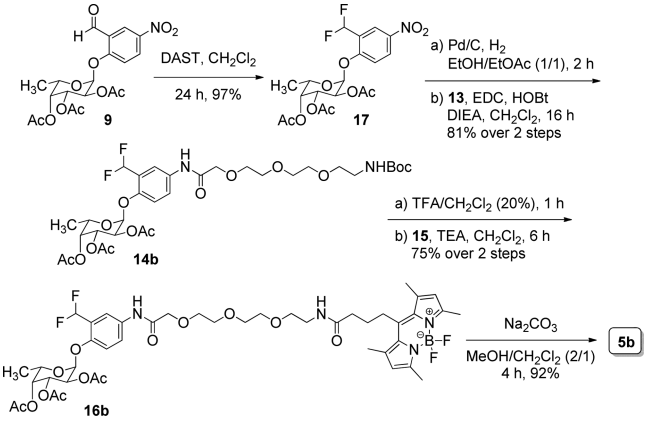


compound **8** is a 1,2-*trans* product, we discovered that it could undergo base-promoted epimerization[28] on treatment with K_2CO_3 in DMSO in the presence of molecular sieves. The reaction mixture consisted of anomers **8** and **9** with the latter being the dominant form as determined by NMR spectroscopy (**8**/**9** = 29/71). More importantly, the two isomers could be separated by silica gel column chromatography. Compound **9** was thus obtained in 48% yield with concomitant recovery of compound **8** (24%). This was the most critical step in the preparations of probes **5a** and **5b** as compound **9** is the key intermediate that stands at a branch point of the synthetic route for both probes.

For the synthesis of probe **5a**, the formyl group of compound **9** was first reduced with $NaBH(OAc)_3$ to give benzylic alcohol **10**. The nitro group was then reduced by catalytic hydrogenation. Without further purification, the newly generated arylamine was Boc-protected by treatment with $(Boc)_2O$ to give compound **11**. The benzylic alcohol of compound **11** was converted to a benzylic fluoride in the presence of DAST in CH_2Cl_2 to afford compound **12** in 78% yield. Subsequent treatment with TFA to remove the Boc-protecting group followed by coupling with carboxylic acid **13**[29] under a standard carbodiimide condition (EDC/HOBt) afforded compound **14a**. Compound **14a** was deprotected by TFA to remove the Boc group and coupled with the BODIPY fluorophore (BODIPY-OSu, **15**)[30,31] to give the protected probe precursor **16a**. Final deprotection was achieved by treatment with Na_2CO_3 in MeOH to remove all the acetyl groups which provided probe **5a** in high yield (92%).

For the synthesis of probe **5b**, as shown in Scheme 3, the aldehyde of compound **9** was directly converted to a

Scheme 3. Synthesis of Probe 5b



difluoromethyl group under the same fluorination condition as that for compound **11**. The nitro group of compound **17** was then reduced by catalytic hydrogenation, followed by amide formation with carboxylic acid **13** under the carbodiimide method to give compound **14b**. Compound **14b** was then consecutively subjected to the Boc deprotection, amide formation with BODIPY-OSu **15**, and peracetate deprotection as those for probe **5a** to smoothly afford probe **5b**. Particularly because the difluoromethyl group of compound **17** could survive the catalytic hydrogenation condition, we were able to perform fluorination prior to the hydrogenation. Owing to this advantage, the synthetic route of probe **5b** was two steps shorter than that of probe **5a**.

To investigate and compare the labeling efficacy of probes **5a** and **5b** after enzymatic cleavage, as depicted in the proposed mechanism (Scheme 1), we conducted a simple slot blot analysis[11] on a protein extract containing α-L-fucosidase. In brief, a protein extract (40 μg) from *Escherichia coli* expressing *Thermotoga maritima* fucosidase (TmFA) was incubated with probes **5a** and **5b**, respectively, at four different concentrations (5, 10, 20, and 50 μM). After incubation for 1 h at room

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temperature in the dark, the reaction mixtures were blotted onto PVDF membrane and the resulting membrane was washed three times. The retained fluorescent signals were then visualized using Fujifilm LAS-4000 Image acquisition system (λ_{ex} = 497 nm, λ_{em} = 520 nm). As shown in Figure 2, probe **5a**

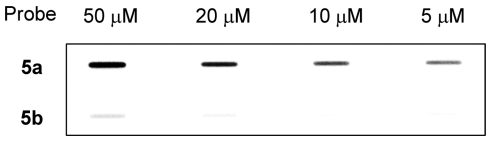

Figure 2. Comparative slot blot analysis of probes **5a** and **5b** on protein extracts containing *T. maritima* α-L-fucosidase. The fluorescence signals were visualized with Fujifilm LAS-4000 Image acquisition system (λ_{ex} = 497 nm, λ_{em} = 520 nm).

gave clear signals at all four concentrations, displaying an increasing trend in labeling intensity with concentrations. By contrast, probe **5b** resulted in negligible signals, producing only a relatively weak signal even at the highest concentration (50 μM). Interestingly, the different performance of chemical probes caused by the latent trapping unit derived from either a fluoromethylaryl or a difluoromethylaryl group has been observed in other systems as well.[12,17,32] Probe **5a** was shown to detect the active form of TmFA as low as 120 nM *in vitro* and was able to distinguish between active enzyme and inactive one (prepared by heating the enzyme at 90 °C for 5 min) (Figure S1a). In addition, the labeling intensity toward TmFA displayed good linear response up to 480 nM (Figure S1b). The slot blot assay of TmFA using **5a** also gave comparable results to the conventional fucosidase assay (Figure S2).

To show the permeability and specificity of **5a** in complex systems, we performed confocal imaging in **5a**-treated human gastric adenocarcinoma epithelial cells (AGS) and HEK-293T cells. AGS and HEK-293T cells were first incubated with **5a** (10 μM). After extensive washes to remove the unreacted probe, the images of confocal fluorescence microscopy were acquired to indicate the probe was cell permeable (Figure 3a, upper panel). To validate the observed signals in cells to be α-L-fucosidase-specific, 5 μM of 1-aminomethyl-1-deoxy-fuconojirimycin (FNJ, a previously reported potent inhibitor)[33,34] was added to the cultured AGS or HEK-293T cells prior to the

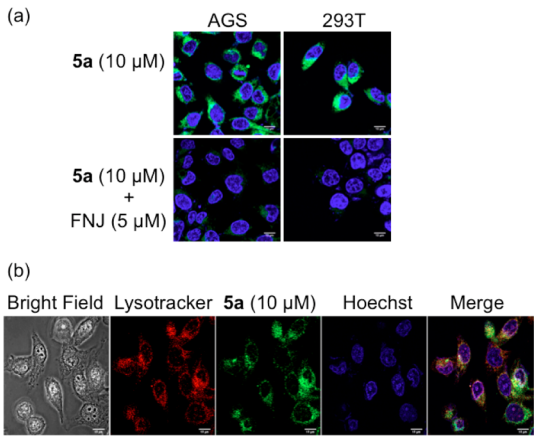

Figure 3. Specificity of probe **5a** in cells: (a) confocal imaging of AGS and HEK-293T cells that were treated with **5a** (10 μM) in the presence or absence of FNJ (5 μM); (b) co-localization of **5a**-treated AGS cells with Lysotracker. Hoechst was used to stain the nucleus.

labeling step. The presence of FNJ was able to significantly abolish the signal of **5a** (Figure 3a, lower panel), supporting the probe efficacy. Furthermore, to find the subcellular localization of the enzyme, we treated AGS cells with **5a** (10 μM), and then with counterstains of a lysosome marker (lysotracker) and a nucleus marker (Hoechst). The probe signal was found to majorly overlap with that of lysotracker (Figures 3b), indicating that the labeling of **5a** was likely corresponding to the lysosomal α-L-fucosidase.

In conclusion, we have developed a concise synthetic route to synthesize two series of activity-based probes for detection of α-L-fucosidase, one with a fluoromethylaryl group and the other with a difluoromethylaryl group. The former probe was successfully applied for the first time to visualize and locate lysosomal α-L-fucosidase activity in human cells. It should also be emphasized that the key base-promoted epimerization step exploited in this study could be further extended to the preparation of probes for other glycosidases, such as α-D-glucosidase and α-D-galactosidase, that require 1,2-*cis* configuration in the glycosylated products.

EXPERIMENTAL SECTION

General Methods. All reagents and starting materials were obtained from commercial suppliers and were used without further purification. Pyridine, dichloromethane, and acetonitrile were distilled from calcium hydride. Analytical TLC (silica gel, 60 F254) was visualized under UV light or stained with phosphomolybdic acid-ethanol and 5% sulfuric acid in ethanol. Column chromatography was performed with silica gel (230−400 mesh). ^{1}H, ^{19}F, and ^{13}C NMR were recorded in the designated deuterated solvents ($CDCl_3$ or CD_3OD). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. Melting points were recorded without correction.

(2-Formyl-4-nitrophenyl)-tetra-O-acetyl-β-L-fucopyranoside (**8**). To an ice-cooled solution of the glycosyl bromides **6** (941.2 mg, 2.67 mmol) and 2-hydroxy-5-nitrobenzaldehyde **7** (490.8 mg, 2.94 mmol) in 5 mL of anhydrous MeCN was slowly added DIEA (1.3 mL, 8.02 mmol). The reaction mixture was stirred for 24 h under N_2 at room temperature, and then it was concentrated under reduced pressure. The residue obtained was dissolved in EtOAc (50 mL) and the solution was washed with 5% citric acid (25 mL × 3), 5% $NaHCO_3$ (25 mL × 3), H_2O (25 mL × 3) and brine (25 mL). The organic phase was then dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by silica gel column chromatography (eluent: 70% hexane/EtOAc) provided the desired glycosidation products as white solids in 67% yield. R_f = 0.38 (hexane/EtOAc = 1/1), mp 156−157 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.33 (s, 1 H), 8.68 (d, *J* = 2.8 Hz, 1 H), 8.41 (dd, *J* = 9.1, 2.8 Hz, 1 H), 7.25 (d, *J* = 9.1 Hz, 1 H), 5.59 (dd, *J* = 10.5, 7.9 Hz, 1 H), 5.36 (d, *J* = 3.2 Hz, 1 H), 5.29 (d, *J* = 7.9 Hz, 1 H), 5.18 (dd, *J* = 10.5, 3.2 Hz, 1 H), 4.10 (q, *J* = 6.3 Hz, 1 H), 2.21 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.30 (d, *J* = 6.3 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 187.2 (Al), 170.4 (C═O), 170.1 (C═O), 169.4 (C═O), 162.2 (C), 143.1 (C), 130.2 (CH), 125.6 (C), 124.3 (CH), 115.6 (CH), 98.7 (CH), 70.6 (CH), 70.3 (CH), 69.5 (CH), 68.0 (CH), 20.7 (CH_3), 20.6 (CH_3), 20.6 (CH_3), 16.0 (CH_3). IR (KBr): 3340, 1740, 1684, 1530, 1383, 1341, 1236, 1061 cm^{-1}. FAB MS *m*/*z* (%) 273.1 (100), 462.2 (M + Na^+, 6). HRMS (FAB) calcd for $C_{19}H_{21}NNaO_{11}$ $(M + Na)^+$ 462.1012, found 462.0992.

(2-Formyl-4-nitrophenyl)-tetra-O-acetyl-α-L-fucopyranoside (**9**). To a mixture of the glycosidation product **8** (1.0 g, 2.3 mmol), trace amount of 3 Å molecular sieve, and K_2CO_3 (3.1 g, 22 mmol) was added dropwise 8 mL of anhydrous DMSO. The reaction mixture was stirred for 48 h at room temperature, diluted with 50 mL of $CHCl_3$, and washed with 5% $NaHCO_3$ (25 mL × 3). The aqueous layers were collected and re-extracted with 25 mL $CHCl_3$, and then the organic phase was combined with previous $CHCl_3$ layer, washed with 5% citric

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acid (25 mL × 2) and brine (25 mL). The organic phase was then dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by silica gel column chromatography (eluent: 70% hexane/EtOAc) provided the desired product **9** as pale yellow solid in 48% yield and the recovery yield of compound **8** was 24%. R_f = 0.45 (hexane/EtOAc = 1/1), mp 143−145 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.51 (s, 1 H), 8.71 (d, J = 2.8 Hz, 1 H), 8.41 (dd, J = 9.2, 2.8 Hz, 1 H), 7.41 (d, J = 9.2 Hz, 1 H), 5.92 (d, J = 3.5 Hz, 1 H), 5.51 (dd, J = 11.0, 3.0 Hz, 1 H), 5.40 (d, J = 3.0 Hz, 1 H), 5.36 (dd, J = 11.0, 3.5 Hz, 1 H), 4.25 (q, J = 6.3 Hz, 1 H), 2.20 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.18 (d, J = 6.3 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 186.7 (Al), 170.3 (C═O), 170.2 (C═O), 169.9 (C═O), 162.6 (C), 142.9 (C), 130.6 (CH), 125.6 (C), 124.4 (CH), 116.1 (CH), 96.3 (CH), 70.2 (CH), 67.5 (CH), 67.3 (CH), 66.8 (CH), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 15.8 (CH_3). IR (KBr): 2919, 2846, 1746, 1699, 1613, 1587, 1540, 1487, 1381, 1348, 1229, 1076, 1036, 977, 910, 738 cm^{-1}. FAB MS m/z (%) 273.1 (100), 462.2 (M + Na$^+$, 11). HRMS (FAB) calcd for $C_{19}H_{21}NNaO_{11}$ (M + Na)$^+$ 462.1012, found 462.0992.

*(2S,3S,4R,5R,6S)-2-(2-(Hydroxymethyl)-4-nitrophenoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl Triacetate (**10**).* To a solution of compound **9** (100 mg, 0.228 mmol) in 2 mL of EtOH was added $NaBH(OAc)_3$ (168.9 mg, 0.797 mmol). The reaction mixture was stirred for 24 h, diluted with 50 mL of EtOAc, and washed with H_2O (25 mL × 3) and brine (25 mL). The organic phase was then dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by silica gel column chromatography (eluent: 70% hexane/EtOAc) provided desired product **10** as a colorless solid in 95% yield. R_f = 0.38 (hexane/EtOAc = 50/50), mp 174−175 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, J = 2.7 Hz, 1 H), 8.05 (dd, J = 9.0, 2.7 Hz, 1 H), 7.16 (d, J = 9.0 Hz, 1 H), 5.75 (d, J = 3.6 Hz, 1 H), 5.45 (dd, J = 10.9, 3.2 Hz, 1 H), 5.32 (d, J = 3.2 Hz, 1 H), 5.31 (dd, J = 10.9, 3.6 Hz, 1 H), 4.80 (d, J = 14.0 Hz, 1 H), 4.66 (d, J = 14.0 Hz, 1 H), 4.20 (q, J = 6.4 Hz, 1 H), 3.06 (s, 1 H), 2.15 (s, 3 H), 2.02 (s, 3 H), 1.97 (s, 3 H), 1.11 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (C═O), 170.1 (C═O), 170.0 (C═O), 158.7 (C), 142.6 (C), 131.6 (C), 124.7 (CH), 123.6 (CH), 113.7 (CH), 95.6 (CH), 70.4 (CH), 67.7 (CH), 67.3 (CH), 66.2 (CH), 59.9 (CH_2), 20.5 (CH_3), 20.5 (CH_3), 20.4 (CH_3), 15.7 (CH_3). IR (KBr): 3555, 1749, 1519, 1348, 1232 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{21}H_{26}NO_{13}$ (M + AcOH-H)$^+$ 500.1404, found 500.1414.

*(2S,3S,4R,5R,6S)-2-(4-(tert-Butoxycarbonylamino)-2-(hydroxymethyl)phenoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl Triacetate (**11**).* To a solution of compound **10** (224 mg, 0.509 mmol) in 5 mL EtOAc was added 20% Pd/C catalyst (44.9 mg). The reaction mixture was purged and filled with H_2, stirred for 30 min at room temperature. Thereafter, $(Boc)_2O$ (49.9 μL, 0.233 mmol) was added to the reactor and and the solution was stirred for another 24 h. It was then filtered through a fritted funnel dry packed with Celite and concentrated under reduced pressure. The residue was diluted with 50 mL of EtOAc, and washed with H_2O (25 mL × 3) and brine (25 mL). The organic phase was then dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by silica gel column chromatography (eluent: 70% hexane/EtOAc) provided desired product **11** as a colorless solid in 82% yield. R_f = 0.41 (hexane/EtOAc = 4/6), mp 68−70 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (s, 1 H), 7.21 (d, J = 8.8 Hz, 1 H), 7.03 (d, J = 8.8 Hz, 1 H), 6.63 (s, 1 H, NH), 5.54 (d, J = 3.7 Hz, 1 H), 5.45 (dd, J = 10.9, 3.3 Hz, 1 H), 5.33 (d, J = 3.3 Hz, 1 H), 5.31 (dd, J = 10.9, 3.7 Hz, 1 H), 4.76 (d, J = 13.1 Hz, 1 H), 4.47 (d, J = 13.1 Hz, 1 H), 4.28 (q, J = 6.5 Hz, 1 H), 2.92 (s, 1 H), 2.15 (s, 3 H), 2.02 (s, 3 H), 1.97 (s, 3 H), 1.45 (s, 9 H), 1.12 (d, J = 6.5 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5 (C), 170.1 (C), 169.9 (C), 153.0 (C), 150.7 (C), 133.5 (C), 130.9 (C), 119.7 (CH), 119.3 (CH), 115.4 (CH), 96.2 (CH), 80.4 (C), 70.7 (CH), 68.0 (CH), 67.6 (CH), 65.5 (CH), 61.4 (CH_2), 28.2 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 15.8 (CH_3). IR (KBr): 3368, 2978, 2931, 1748, 1534, 1500, 1369, 1224, 1161, 1051 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{24}H_{33}NNaO_{11}$ (M + Na)$^+$ 534.1951, found 534.1940.

*(2S,3S,4R,5R,6S)-2-(4-(tert-Butoxycarbonylamino)-2-(fluoromethyl)phenoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl Triacetate (**12**).* To an ice-cooled solution of compound **11** (47.0 mg, 0.0922 mmol) in 0.5 mL of anhydrous CH_2Cl_2 was added a solution of DAST (17 μL, 0.138 mmol) in 0.5 mL of anhydrous CH_2Cl_2. The reaction mixture was allowed to warm to room temperature, and stirred for 2 h under N_2. It was then quenched by adding small amount of silica gel and 1 drop of MeOH, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (eluent: 70% hexane/EtOAc) provided the desired product **12** as a pale yellow oil in 78% yield. R_f = 0.50 (hexane/EtOAc = 4/6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 (s, 1 H), 7.26 (d, J = 8.7 Hz, 1 H), 7.04 (d, J = 8.4 Hz, 1 H), 6.60 (s, 1 H), 5.57 (d, J = 3.6 Hz, 1 H), 5.51−5.26 (m, 4 H), 5.23 (dd, J = 10.9, 3.7 Hz, 1 H), 4.26 (q, J = 6.5 Hz, 1 H), 2.15 (s, 3 H), 2.01 (s, 3 H), 1.98 (s, 3 H), 1.46 (s, 9 H), 1.11 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5 (C), 170.4 (C), 170.0 (C), 152.9 (C), 150.3 (d, J = 4.3 Hz, C), 133.5 (C), 126.4 (d, J = 16.7 Hz, C), 120.6 (CH), 120.2 (CH), 115.9 (CH), 96.1 (CH), 80.5 (C), 79.7 (d, J = 166.0 Hz, CH_2F), 70.8 (CH), 67.9 (CH), 67.7 (CH), 65.4 (CH), 28.2 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 15.8 (CH_3). ^{19}F NMR (376 MHz, $CDCl_3$): δ −213.3 (t). IR (neat): 3368, 2978, 2931, 1748, 1534, 1500, 1369, 1224, 1161, 1051 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{24}H_{32}FNNaO_{10}$ (M + Na)$^+$ 536.1908, found 536.1919.

*(2S,3S,4R,5R,6S)-2-(4-(2,2-Dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azahexadecanamido)-2-(fluoromethyl)phenoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl Triacetate (**14a**).* Compound **12** (135 mg, 0.26 mmol) was dissolved in 0.7 mL of 20% TFA/CH_2Cl_2. The solution was stirred at rt for 1 h. The volatiles were concentrated under reduced pressure and then kept under high vacuum for 3 h to remove the residual TFA. The residue obtained was dissolved in 1.8 mL of CH_2Cl_2 and the solution was added compound **13** (89.8 mg, 0.266 mmol), HOBt (17.8 mg, 0.132 mmol), DIEA (109 μL, 0.658 mmol) and EDC (100 mg, 0.527 mmol). The reaction mixture was stirred for 24 h, diluted with 50 mL of EtOAc, and washed with 5% citric acid (25 mL × 3), 5% $NaHCO_3$ (25 mL × 3), H_2O (25 mL × 3) and brine (25 mL). The organic phase was then dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by silica gel column chromatography (eluent: 99% $CHCl_3$/MeOH) provided desired product **14a** as a pale yellow oil in 80% yield over two steps. R_f = 0.43 ($CHCl_3$/MeOH = 95/5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1 H), 7.58 (d, J = 8.9 Hz, 1 H), 7.52 (s, 1 H), 7.08 (d, J = 8.8 Hz, 1 H, aromatic), 5.58 (d, J = 3.5 Hz, 1 H), 5.51−5.29 (m, 4 H), 5.22 (dd, J = 10.9, 3.5 Hz, 1 H), 4.99 (s, 1 H), 4.24 (q, J = 6.5 Hz, 1 H), 4.07 (s, 2 H), 3.72−3.65 (m, 6 H), 3.59 (m, 2 H), 3.44 (m, 2 H), 3.20 (m, 2 H), 2.13 (s, 3 H), 2.00 (s, 3 H), 1.97 (s, 3 H), 1.36 (s, 9 H), 1.10 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (C), 170.3 (C), 170.0 (C), 168.1 (C), 155.8 (C), 151.1 (d, J = 4.4 Hz, C), 132.5 (C), 126.2 (d, J = 16.9 Hz, C), 122.0 (CH), 121.3 (d, J = 7.2 Hz, CH), 115.5 (CH), 96.0 (CH), 79.6 (d, J = 165.9 Hz, CH_2F), 79.1 (C), 71.0 (CH_2), 70.7 (CH), 70.3 (CH_2), 70.3 (CH_2), 70.0 (CH_2), 69.9 (CH_2), 69.8 (CH_2), 67.8 (CH), 67.6 (CH), 65.4 (CH), 40.1 (CH_2), 28.3 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 15.8 (CH_3). ^{19}F-NMR (376 MHz, $CDCl_3$) δ −214.2 (t, J = 47.8 Hz). IR (neat): 3339, 2918, 2361, 1748, 1708, 1536, 1502, 1369, 1223, 1074 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{32}H_{47}FN_2O_{14}Na$ for (M + Na)$^+$ 725.2909, found 725.2914.

*10-(4-((2,5-Dioxopyrrolidin-1-yl)oxy)-4-oxobutyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-4-ium-5-uide (**15**).* To a solution of 4-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-yl)-butyric acid (135 mg, 0.404 mmol) in 4 mL DMF was added *N*-hydroxysuccinimide (60 mg, 0.52 mmol), DIEA (67 μL, 0.404 mmol), and EDC (103 mg, 0.54 mmol). The reaction mixture was stirred for 16 h. It was then quenched with 0.2 mL 5% citric acid and the mixture stirred for another 10 min. The solvent was then removed under high vacuum. The residual oil was dissolved in 50 mL EtOAc and the solution was washed with 5% citric acid (20 mL × 2), 5% $NaHCO_3$ (20 mL × 2), and brine (20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The desired product **15** was obtained as a dark orange solid in 83% yield after crystallization at 4 °C from $CHCl_3$/hexane. R_f = 0.40 (hexane/EtOAc = 4/6), mp 160 °C (dec). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.04 (s, 2 H), 3.13−3.03 (m, 2 H), 2.83 (s, 4 H),

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2.78 (t, J = 7.1 Hz, 2 H), 2.49 (s, 6 H), 2.39 (s, 6 H), 2.12−1.97 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9 (C), 167.8 (C), 154.4 (C), 144.0 (C), 140.4 (C), 131.4 (C), 121.9 (CH), 30.9 (CH_2), 27.0 (CH_2), 26.2 (CH_2), 25.6 (CH_2), 16.4 (CH_3), 14.5 (CH_3). ^{19}F NMR (376 MHz, $CDCl_3$): δ −147.0 (m). IR (KBr): 3454, 2082, 1814, 1784, 1738, 1634, 1551, 1510, 1473, 1409, 1370, 1200, 1159, 1068, 984, 647 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{21}H_{24}BF_2N_3NaO_4$ (M + Na)$^+$ 454.1726, found 454.1735.

General Procedure for the Preparation of Compound 16. The corresponding **14** (138 mg, 0.196 mmol) was dissolved in 2 mL of 20% TFA/CH_2Cl_2. The solution was stirred for 1 h. The volatiles were concentrated under reduced pressure and the residual was kept under high vacuum for 3 h. To a solution of the residue in 2 mL of CH_2Cl_2 was added BODIPY-OSu **15** (88.9 mg, 0.206 mmol) and TEA (164 μL, 1.18 mmol). The reaction mixture was stirred for 6 h at room temperature, diluted with 50 mL of EtOAc, and washed with 5% citric acid (25 mL × 3), 5% $NaHCO_3$ (25 mL × 3), H_2O (25 mL × 3) and brine (25 mL). The organic phase was then dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by silica gel column chromatography (eluent: 98% $CHCl_3$/MeOH) provided the desired product **16** as orange solids in 61−75% yield over two steps.

5,5-Difluoro-10-(1-((3-(fluoromethyl)-4-(((2S,3S,4R,5R,6S)-3,4,5-triacetoxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)phenyl)amino)-1,13-dioxo-3,6,9-trioxa-12-azahexadecan-16-yl)-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-4-ium-5-uide (**16a**). Orange solid, 61% yield. R_f = 0.63 ($CHCl_3$/MeOH = 9/1), mp 89−91 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (s, 1 H), 7.58 (d, J = 8.9 Hz, 1 H), 7.51 (s, 1 H), 7.08 (d, J = 8.8 Hz, 1 H), 6.37 (m, 1 H), 5.98 (s, 2 H), 5.59 (d, J = 3.6 Hz, 1 H), 5.51−5.28 (m, 4 H), 5.23 (dd, J = 10.9, 3.6 Hz, 1 H), 4.23 (q, J = 6.3 Hz, 1 H), 4.07 (s, 2 H), 3.80−3.47 (m, 8 H), 3.46 (m, 2 H), 3.33 (m, 2 H), 2.90 (m, 2 H), 2.43 (s, 6 H), 2.33 (s, 6 H), 2.25 (m, 2 H), 2.14 (s, 3 H), 2.00 (s, 3 H), 1.97 (s, 3 H), 1.85 (m, 2 H), 1.10 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7 (C), 170.4 (C), 170.3 (C), 170.0 (C), 168.2 (C), 153.8 (C), 151.1 (d, J = 4.3 Hz, C), 145.4 (C), 140.5 (C), 132.4 (C), 131.3 (C), 126.3 (d, J = 16.7 Hz, C), 121.9 (d, J = 3.0 Hz, CH), 121.6 (CH), 121.1 (d, J = 7.6 Hz, CH), 115.6 (CH), 96.0 (CH), 79.6 (d, J = 165.7 Hz, CH_2F), 70.7 (CH_2), 70.6 (CH), 70.4 (CH_2), 70.2 (CH_2), 70.2 (CH_2), 69.8 (CH_2), 69.7 (CH_2), 67.8 (CH), 67.6 (CH), 65.5 (CH), 39.1 (CH_2), 35.9 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 16.1 (CH_3), 15.7 (CH_3), 14.3 (CH_3). ^{19}F NMR (376 MHz, $CDCl_3$): δ −147.3 (m), −214.1 (t, J = 47.6 Hz). IR (KBr): 3327, 2923, 1748, 1665, 1550, 1509, 1203 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{44}H_{58}BF_3N_4NaO_{13}$ (M + Na)$^+$ 941.3943, found 941.3967.

10-(1-((3-(Difluoromethyl)-4-(((2S,3S,4R,5R,6S)-3,4,5-triacetoxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)phenyl)amino)-1,13-dioxo-3,6,9-trioxa-12-azahexadecan-16-yl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-4-ium-5-uide (**16b**). Orange solid, 75% yield. R_f = 0.45 ($CHCl_3$/MeOH = 9/1), mp 102−104 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1 H), 7.84 (dd, J = 9.0, 2.2 Hz, 1 H), 7.60 (d, J = 2.2 Hz, 1 H), 7.17 (d, J = 9.0 Hz, 1 Hc), 6.96 (t, J = 55.4 Hz, 1 H), 6.24 (t, J = 5.4 Hz, 1 H), 6.00 (s, 2 H), 5.62 (d, J = 3.7 Hz, 1 H), 5.45 (dd, J = 10.9, 3.3 Hz, 1 H), 5.35 (m, 1 H), 5.24 (dd, J = 10.9, 3.7 Hz, 1 H), 4.25 (q, J = 6.5 Hz, 1 H), 4.10 (s, 2 H), 3.72−3.59 (m, 8 H), 3.49 (m, 2 H), 3.36 (m, 2 H), 2.95 (m, 2 H), 2.47 (s, 6 H), 2.37 (s, 6 H), 2.30 (m, 2 H), 2.16 (s, 3 H) 2.02 (s, 3 H), 2.00 (s, 3 H), 1.91 (m, 2 H), 1.13 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7 (C), 170.4 (C), 170.4 (C), 170.0 (C), 168.4 (C), 153.9 (C), 151.3 (C), 145.4 (C), 140.5 (C), 132.7 (C), 131.4 (C), 124.2 (C), 124.0 (CH), 121.7 (CH), 117.9 (CH), 116.3 (CH), 109.8 (t, J = 118.7 Hz, CH), 96.5 (CH), 70.9 (CH_2), 70.6 (CH), 70.4 (CH_2), 70.3 (CH_2), 70.3 (CH_2), 69.8 (CH_2), 69.8 (CH_2), 67.8 (CH), 67.7 (CH), 65.8 (CH), 39.2 (CH_2), 36.0 (CH_2), 27.5 (CH_2), 27.4 (CH_2), 20.6 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 16.3 (CH_3), 15.8 (CH_3), 14.4 (CH_3). ^{19}F-NMR (376 MHz, $CDCl_3$): δ −114.0 (dd, J = 300.7, 55.5 Hz), −117.6 (dd, J = 300.8, 55.4 Hz), −147.5 (m). IR (KBr): 3341, 2926, 1749, 1550, 1509, 1224 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{44}H_{57}BF_4N_4NaO_{13}$ (M + Na)$^+$ 959.3849, found 959.3841.

General Procedure for the Preparation of Compound 5. To a solution of the corresponding **16** (90.0 mg, 0.0980 mmol) in 1 mL of MeOH/CH_2Cl_2 (2/1) was added Na_2CO_3 (41.5 mg, 0.392 mmol). The reaction mixture was stirred for 4 h, diluted with 50 mL of EtOAc, and washed with 5% citric acid (25 mL × 3), H_2O (25 mL × 2) and brine (25 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was triturated with hexane, and recrystallized in ether/MeOH to provide the desired products **5a** and **5b**.

5,5-Difluoro-10-(1-((3-(fluoromethyl)-4-(((2S,3S,4R,5S,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)phenyl)amino)-1,13-dioxo-3,6,9-trioxa-12-azahexadecan-16-yl)-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-4-ium-5-uide (**5a**). Orange solid, 92% yield. R_f = 0.40 ($CHCl_3$/MeOH = 85/15), mp 103−104 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.61 (s, 1 H), 7.54 (d, J = 8.7 Hz, 1 H), 7.15 (d, J = 8.7 Hz, 1 H), 6.06 (s, 2 H), 5.55 (dd, J = 48.0, 11.3 Hz, 1 H), 5.45 (d, J = 2.1 Hz, 1 H), 5.42 (dd, J = 47.8, 11.3 Hz, 1 H), 4.06 (s, 2 H), 3.99 (q, J = 6.7 Hz, 1 H), 3.93 (m, 2 H), 3.71 (s, 1 H), 3.66−3.60 (m, 8 H), 3.50 (m, 2 H), 3.34 (m, 2 H), 2.87 (m, 2 H), 2.42 (s, 6 H), 2.33 (s, 6 H), 2.30 (m, 2 H), 1.80 (m, 2 H), 1.15 (d, J = 6.6 Hz, 3 H). ^{13}C NMR (100 MHz, CD_3OD): δ 174.8 (C), 170.7 (C), 155.0 (C), 152.9 (d, J = 4.3 Hz, C), 147.1 (C), 142.4 (C), 133.2 (C), 132.5 (C), 127.9 (d, J = 16.6 Hz, C), 123.3 (d, J = 2.6 Hz, CH), 122.7 (CH), 122.5 (d, J = 8.1 Hz, CH), 116.5 (CH), 99.7 (CH), 81.2 (d, J = 163.8 Hz, CH_2F), 73.4 (CH), 71.9 (CH_2), 71.6 (CH_2), 71.5 (CH), 71.4 (CH_2), 71.4 (CH_2), 71.1 (CH_2), 70.4 (CH_2), 69.6 (CH), 68.8 (CH), 40.4 (CH_2), 36.9 (CH_2), 28.9 (CH_2), 28.5 (CH_2), 16.7 (CH_3), 16.5 (CH_3), 14.5 (CH_3). ^{19}F−NMR (CD_3OD, 376 MHz): δ −147.6 (m), − 216.7 (t, J = 47.7 Hz). IR (KBr): 3450, 1658, 1549, 1502 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{38}H_{52}BF_3N_4NaO_{10}$ (M + Na)$^+$ 815.3626, found 815.3621.

10-(1-((3-(Difluoromethyl)-4-(((2S,3S,4R,5S,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)phenyl)amino)-1,13-dioxo-3,6,9-trioxa-12-azahexadecan-16-yl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-4-ium-5-uide (**5b**). Orange solid, 92% yield. R_f = 0.19 ($CHCl_3$/MeOH = 9/1), melting point 97−99 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 8.08 (s, 1 H), 7.82 (s, 1 H), 7.67 (d, J = 8.6 Hz, 1 H), 7.24 (d, J = 8.9 Hz, 1 H), 7.16 (t, J = 55.4 Hz, 1 H), 6.08 (s, 2 H), 5.48 (s, 1 H), 4.08 (s, 2 H), 3.98 (q, J = 6.6 Hz, 1 H), 3.94 (m, 2 H), 3.73 (s, 1 H), 3.67−3.63 (m, 8 H), 3.52 (m, 2 H), 3.34 (m, 2 H), 2.94 (m, 2 H), 2.42 (s, 6 H), 2.38 (s, 6 H), 2.32 (m, 2 H), 1.86 (m, 2 H), 1.17 (d, J = 6.3 Hz, 3 H). ^{13}C NMR (100 MHz, CD_3OD): δ 175.0 (d, J = 8.6 Hz, C), 170.9 (C), 155.1 (C), 153.0 (d, J = 4.3 Hz, C), 147.1 (C), 142.4 (C), 133.4 (C), 132.6 (C), 125.5 (C), 125.3 (CH), 122.7 (CH), 119.5 (CH), 117.0 (CH), 111.7 (t, J = 117.8 Hz, CH), 100.0 (CH), 73.3 (CH), 71.9 (CH_2), 71.6 (CH_2), 71.4 (CH_2), 71.4 (CH), 71.1 (CH_2), 70.4 (CH_2), 69.5 (CH), 69.0 (CH), 40.6 (CH_2), 40.4 (CH_2), 37.0 (CH_2), 29.1 (CH_2), 28.5 (CH_2), 16.6 (CH_3), 16.5 (CH_3), 14.5 (CH_3). ^{19}F NMR (376 MHz, CD_3OD): δ −112.3 (dd, J = 300.3, 55.8 Hz), −122.9 (dd, J = 300.3, 54.9 Hz), −147.9 (m). IR (KBr): 3315, 2922, 1662, 1550, 1501, 1202, 1084 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{38}H_{51}BF_4N_4NaO_{10}$ (M + Na)$^+$ 833.3532, found 833.3533.

(2-Difluoromethyl-4-nitro-phenyl)-tetra-O-acetyl-α-L-fucopyranoside (**17**). To an ice-cooled solution of compound **9** (100 mg, 0.228 mmol) in 1 mL of anhydrous CH_2Cl_2 was added a solution of DAST (0.42 mL, 0.342 mmol) in 1 mL of anhydrous CH_2Cl_2. The reaction mixture was allowed to warm to room temperature, and stirred for another 24 h under N_2. It was quenched by adding a small amount of silica gel and 1 mL MeOH, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (eluent: 80% hexane/EtOAc) provided the desired fluorinated product **17** as a colorless solid in 97% yield. R_f = 0.50 (hexane/EtOAc = 1/1), mp 185−187 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, J = 2.7 Hz, 1 H), 8.32 (dd, J = 9.2, 2.7 Hz, 1 H), 7.34 (d, J = 9.2 Hz, 1 H), 6.95 (t, J = 54.8 Hz, 1 H), 5.85 (d, J = 3.7 Hz, 1 H), 5.45 (dd, J = 10.9, 3.2 Hz, 1 H), 5.38 (d, J = 3.2 Hz, 1 H), 5.28 (dd, J = 10.9, 3.7 Hz, 1 H), 4.19 (q, J = 6.5 Hz, 1 H), 2.19 (s, 3 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 1.14 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (C═O), 170.3 (C═O), 169.9 (C═O), 158.9 (C), 142.6 (C), 128.0 (CH), 124.6 (t, J = 23.5 Hz, C), 122.7 (CH), 115.1 (CH), 110.1 (t, J = 237.5 Hz,

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-CHF_2), 96.0 (CH), 70.3 (CH), 67.6 (CH), 67.3 (CH), 66.6 (CH), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 15.8 (CH_3). ^{19}F NMR (376 MHz, $CDCl_3$): δ −116.9 (dd, J = 302.5, 54.9 Hz), −118.5 (dd, J = 324.0, 60.0 Hz). IR (KBr): 1747, 1614, 1516, 1474, 1369, 1348, 1214, 1074, 1032 cm^{-1}. FAB MS m/z (%) 273.1 (100), 484.2 (M + Na^+, 22). HRMS (ESI-quadrupole) calcd for $C_{20}H_{22}F_2NO_{12}$ (M + FA − H)$^-$ 506.1110, found 506.1083.

*(2S,3S,4R,5R,6S)-2-(2-(Difluoromethyl)-4-(2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azahexadecanamido)phenoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl Triacetate (**14b**).* To a solution of compound **17** (93.5 mg, 0.203 mmol) in 2 mL of EtOAc/EtOH (1/1) was added 20% Pd/C catalyst (20 mg). The reaction mixture was purged and filled with H_2, then stirred for 2 h at rt. It was then filtered through a fritted funnel dry packed with Celite and concentrated under reduced pressure. The residue obtained was dissolved in 1 mL of CH_2Cl_2 and the solution was added compound **13** (71.8 mg, 0.213 mmol), HOBt (13.7 mg, 0.101 mmol), DIEA (84 μL, 0.51 mmol) and EDC (77.7 mg, 0.405 mmol). The reaction mixture was stirred for 16 h, diluted with 50 mL of EtOAc, and washed with 5% citric acid (25 mL × 3), 5% $NaHCO_3$ (25 mL × 3), H_2O (25 mL × 3) and brine (25 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by silica gel column chromatography (eluent: 98% $CHCl_3$/MeOH) provided desired product **14b** as a pale yellow oil in 81% yield over two steps. R_f = 0.50 ($CHCl_3$/MeOH = 95/5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91 (s, 1 H), 7.83 (dd, J = 9.0, 2.2 Hz, 1 H), 7.61 (d, J = 2.2 Hz, 1 H), 7.16 (d, J = 9.0 Hz, 1 H), 6.95 (t, J = 55.4 Hz, 1 H), 5.62 (d, J = 3.6 Hz, 1 H), 5.45 (dd, J = 10.9, 3.3 Hz, 1 H), 5.35 (m, 1 H), 5.24 (dd, J = 10.9, 3.7 Hz, 1 H), 4.96 (s, 1 H), 4.27 (q, J = 6.5 Hz, 1 H), 4.09 (s, 2 H), 3.74−3.60 (m, 8 H), 3.47 (m, 2 H), 3.22 (m, 2 H), 2.16 (s, 3 H), 2.02 (s, 3 H), 1.99 (s, 3 H), 1.39 (s, 9 H), 1.14 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (C), 170.3 (C), 169.9 (C), 168.3 (C), 155.8 (C), 151.1 (C), 132.8 (C), 124.1 (t, J = 22.6 Hz, C), 124.1 (CH), 118.1 (CH), 116.1 (CH), 111.0 (t, J = 236.7 Hz, CH), 96.3 (CH), 79.1 (C), 71.1 (CH_2), 70.6 (CH), 70.3 (CH_2), 70.3 (CH_2), 70.1 (CH_2), 69.9 (CH_2), 69.8 (CH_2), 67.8 (CH), 67.6 (CH), 65.7 (CH), 40.2 (CH_2), 28.3 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 15.8 (CH_3). ^{19}F NMR (376 MHz, $CDCl_3$): δ −114.2 (dd, J = 301.1, 55.6 Hz), −117.6 (dd, J = 301.1, 55.3 Hz). IR (neat): 3341, 2932, 1750, 1712, 1538, 1504, 1370, 1247 cm^{-1}. HRMS (ESI-quadrupole) calcd for $C_{32}H_{45}F_2N_2O_{14}$ (M − H)$^-$ 719.2839, found 719.2850.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01204.

^{1}H/^{13}C NMR spectra for compounds **5**, **8**−**17**; conditions and data for the slot blot and confocal imaging experiments (PDF)

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Notes

The authors declare no competing financial interest.

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