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Inhibitors

Synthesis and Biological Evaluation of Several Dephosphonated Analogues of CMP-Neu5Ac as Inhibitors of GM3-Synthase**

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Abstract: Previous studies demonstrated that reducing the GM3 content in myoblasts increased the cell resistance to hypoxic stress, suggesting that a pharmacological inhibition of the GM3 synthesis could be instrumental for the development of new treatments for ischemic diseases. Herein, the synthesis of several dephosphonated CMP-Neu5Ac congeners and their anti-GM3-synthase activity is reported. Biological activity testes revealed that some inhibitors almost completely blocked the GM3-synthase activity in vitro and reduced the GM3 content in living embryonic kidney 293A cells, eventually activating the epidermal growth factor receptor (EGFR) signaling cascade.

Introduction

Sialic acid (Neu5Ac (**1**), Figure 1) is transferred to the terminal portion of glycoconjugates through a process known as sialylation, which is mediated by sialyltransferases (STs), a class of enzymes that are localized in the Golgi apparatus and use cytidine 5′-monophospho-*N*-acetylneuraminic acid [CMP-Neu5Ac (**2**)] as the sialyl donor.[1] Of particular relevance is the sialylation of the terminal galactose residue of lactosylceramide mediated by a ST often referred to as the GM3-synthase,[2] as it leads to the formation of ganglioside GM3, a key precursor of more complex ganglioside species.[3] Gangliosides are known to be involved in the regulation of fundamental biological processes (including cell proliferation and migration, apoptosis, cancer formation, and metastasis), and efforts have been made to adjust their bioavailability by regulating their biosynthesis.[4] Along this line, we reported that sialidase NEU3 overexpression can reduce the GM3 content on the cell surface of skeletal myoblasts,[5] causing an activation of the epidermal growth factor receptor (EGFR) signaling cascade, ultimately upregulating HIF-1α and protecting cells from hypoxic stress.[6] We also showed that chemical inhibition of all ganglioside syntheses with 1-phenyl-2-palmitoyl-3-morpholino-1-propanol (PPMP) could partially mimic the effects of NEU3 overexpression.[6] Nevertheless, we speculated that chemically reducing the GM3 cell content with a specific inhibitor of its synthase could result in a more robust activation of the cell response to hypoxia. However, to the best of our knowledge, the dephosphonate derivative **3a** (Figure 1) is the only GM3-synthase inhibitor described to date, but its specificity and its activity in vitro on living cells has not been reported yet.[7] Actually, as a CMP-Neu5Ac mimetic, its specificity for GM3-synthase could be modest. Along this line, several fluorescent mimetics of CMP-Neu5Ac have been recently reported to be highly potent in inhibiting commercially available eukaryotic and bacterial sialyl transferases.[8] Actually, the use of fluorescent probes could be instrumental for an effective screening of combinatorial libraries of compounds to identify new and more specific inhibitors.

Moreover, the reported GM3-synthase inhibitor **3a** is not commercially available. Therefore, although we approached its synthesis, we developed a new synthetic route that allowed the synthesis of several congeners of compound **3a**, including

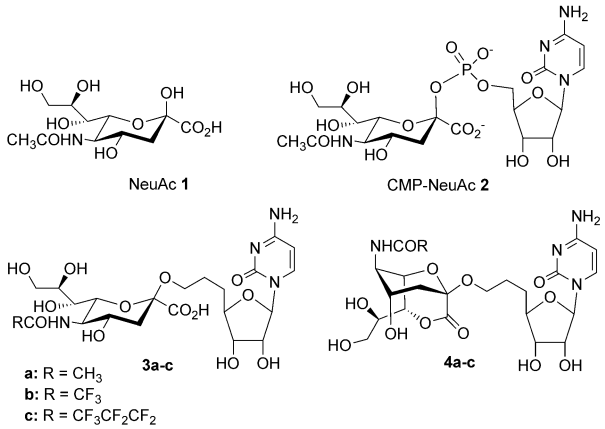

Figure 1. Sialic acid and target sialosides.

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[**] *CMP-Neu5Ac = cytidine 5′-monophospho-N-acetylneuraminic acid.*

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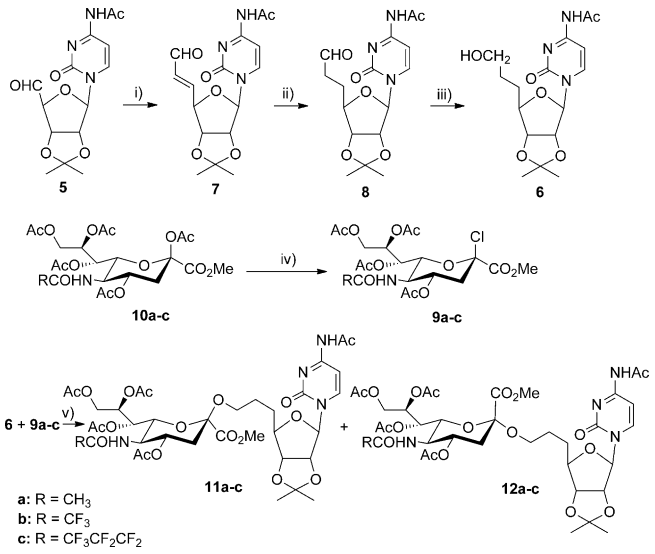
the glycosides **3b** and **3c** and the 1,7-lactones **4a**–**4c** (Figure 1). We speculated that the presence of the perfluoroacylamido groups and the inner esterification of the polar carboxylic moiety with the 7-hydroxyl group in the lactones **4a**–**4c** could modulate their lipophilicity enhancing both the inhibitory activity and the cellular uptake. Finally, the anti-GM3-synthase activity of these glycosides was tested in vitro on cell extracts and on living cultures of embryonic kidney 293A cells.

Results and Discussion

The glycosides **3a**–**3c** and their corresponding 1,7-lactones **4a**–**4c** were synthesized taking advantage of some synthetic procedures for the N-transacylation of amides[9] and the 1,7-lactonization of Neu5Ac (**1**),[10] which were developed in our laboratory. The key step of the synthesis of all inhibitors was the formation of an *O*-glycosidic linkage between the alcoholic nucleoside **6** (obtained through the elongation of the 5′ carbon chain of cytidine) and each different sialic derivative, properly protected and suitably activated at their anomeric function (Scheme 1). To this purpose, the known cytidine aldehyde **5**[11] was converted into the alcohol **6**, that is, into the aglyconic portion of the required glycosides **3a**–**3c**, with a shorter synthetic route than the one previously reported.[7]



Scheme 1. Synthesis of the sialosyl acceptor **6**, the sialosyl donors **9a**–**9c**, and the protected sialosides **11a**–**11c** and **12a**–**12c**. i) $(Ph)_3P{=}CHCHO$, CH_2Cl_2, RT, overnight; ii) H_2, Pd/C, MeOH, RT, 2 h; iii) $NaBH_4$, MeOH, −20 °C, 15 min; iv) AcCl (Ac = acetyl), MeOH, −10 °C to RT, overnight; v) AgOTf (OTf = triflate), toluene/CH_3NO_2, RT, 3 h.

Initially, the aldehyde **5** underwent a Wittig reaction followed by the catalytic hydrogenation of the formed α,β-unsaturated aldehyde **7** into the aldehyde **8**, which was then reduced to the alcohol **6** with $NaBH_4$. The direct transformation of the aldehyde **7** into the alcohol **6** was also attempted by using various chemical or catalytic methods[12] that, however, afforded unsatisfactory results due to some concurrent reactions, such as cytidine ring hydrogenation and the cleavage of acetamidic or *N*-glycosidic bonds. Moreover, the aldehydes **7** and **8** partially decomposed during the chromatographic purification step on silica. Therefore, they were both used in their crude form during the successive synthetic steps, limiting their purification to small aliquots used for their chemical characterization. Under these synthetic conditions, the alcohol **6** was isolated, in pure form, in 45% yield from the aldehyde **5**. In parallel, the 2-chlorosialo donor **9a**[13] and the new perfluorinated analogs **9b** and **9c**, which are needed for the successive glycosidation step, were synthesized by treating the appropriate peracetylated neuraminic acid methyl esters **10a**–**10c**[9b,14] with acetyl chloride/methanol (Scheme 1). Then, glycosidation of the alcohol **6** with the 2-chlorosialo donors **9a**–**9c**, under Koening–Knorr-like conditions, afforded the desired β-glycosides **11a**–**11c** in satisfactory yields (39, 36, and 27% respectively), accompanied by variable, but always minor, amounts of their 2α-epimers **12a**–**12c** (Scheme 1). The non-fluorinated β-glycoside **11a** was accompanied by trace amounts of the α-epimer **12a** (<1%). On the contrary, the fluorinated analogous were accompanied by appreciable amounts of their 2α-epimers **12b** and **12c** (20 and 24%, respectively). This confirmed that the presence of a perfluorinated acylamido group at the C-5 in the sialosyl donor increases the α-stereoselectivity of the glycosidation, as previously observed.[15] The obtained glycosides **11a**–**11c** as well as **12b** and **12c** showed physicochemical properties, which are in agreement with their structure. However, the NMR data did not allow the direct assignment of their α or β geometry that was initially attributed on the basis of the empirical rule reported for anomeric assignments in sialosides.[16] We assigned the β configuration to the anomers showing a relatively smaller coupling constant between the H-7 and H-8 sialic protons ($J = 1.6$–4.4 Hz, in contrast to $J = 8.7$–8.9 Hz in the α-anomers) and a relatively larger chemical shift difference between the two vicinal protons at the C-9 sialic carbon atom ($\Delta\delta > 0.85$ ppm) in respect to the α-anomers ($\Delta\delta < 0.2$ ppm), similarly to what was previously reported in the synthesis of compound **3a**.[7] Successively, we unequivocally confirmed the assignment by a chemical approach, showing that only those sialosides deriving from the deprotection of the sialosides **11b** and **11c**, of assigned β geometry, were able to form the corresponding 1,7-lactones. Then, after some unsuccessful attempts to improve the yields of the glycosylation reaction, we decided to experiment the preparation of the glycoside **11a**–**11c** in two steps, performing the glycosylation of the alcohol **6** with the dibromosialosyl donors **13a**–**13c**, prepared[17] by simple addition of bromine to the glycals **14a**–**14c**.[9c] We were confident that, as first reported by Goto et al.,[16a] a dibrominated sialosyl donor would preferentially give the β-anomer, as a consequence of the steric hindrance on the α side, due to the bromine atom at the 3-position. If this effect would have overbore the opposite influence of the perfluorinated acylamido groups at the C-5,[15] a more significant selectivity in favor of the β-anomers **15a**–**15c** could have been observed. These intermediates should have been converted into the desired β-glycoside **11a**–**11c** by reductive elimination with $(n\text{Bu})_3\text{SnH}$ of the remaining 3-bromine atom (Scheme 2). Actually, the glycosidation performed

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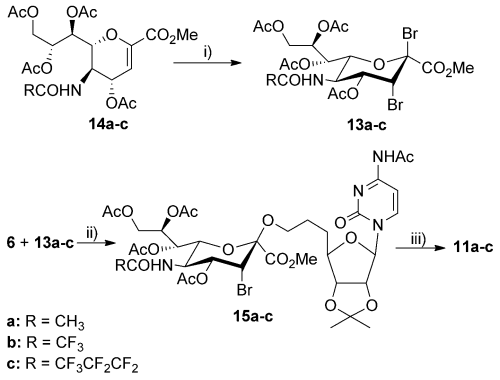
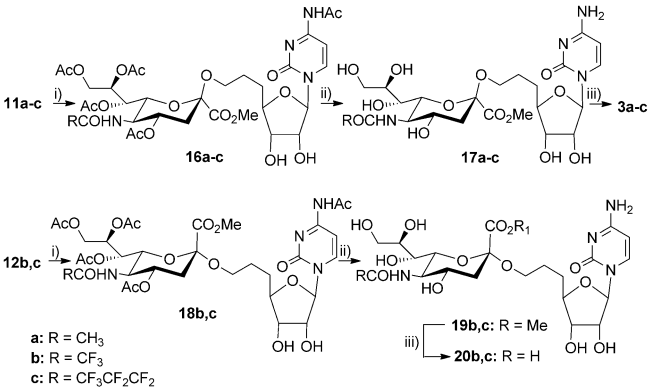
at 23 °C occurred quickly, affording exclusively the expected β-glycoside **15a–15c** that, by successive debromination, gave the glycosides **11a–11c**, showing the same physicochemical characteristics of those previously obtained. However, the overall yields of this two-step process resulted in the same range of those observed in the one-step preparation of the β-glycoside **11a–11c**. For this reason, we did not consider this second procedure as an attractive alternative and, in the successive preparations, we used the first protocol herein reported, which eventually allowed the obtainment of both α- and β-anomers useful for an NMR data comparison.



Scheme 2. Synthesis and O-sialylation of the dibromosialosyl donors **13a–13c**. i) Br_2, CH_2Cl_2, 0 °C to RT, 30 min; ii) AgOTf, toluene/CH_3NO_2, RT, 3 h; iii) $(Bu)_3SnH$, 2,2′-azobisisobutyronitrile (AIBN), THF, heating to reflux, 3 h.



Scheme 3. Synthesis of the free sialyl glycosides **3a–3c** as well as **20b** and **20c**. i) moist TFA, CH_2Cl_2, heating to reflux, 1 h; ii) NaOMe, MeOH, RT, 1 h; iii) for compound **17a**: NaOH, MeOH, RT, 40 min; for compounds **17b** and **19b**: Et_3N, MeOH/H_2O, RT, 12 h; for compounds **17c** and **19c**: K_2CO_3, H_2O/MeOH, RT, 12 h.

The obtained glycosides **11a–11c** were then sequentially deprotected to give the desired acids **3a–3c**. The acetonide group was removed by moist trifluoroacetic acid (TFA) treatment affording the diols **16a–16c** that were transformed in the esters **17a–17c** by transacetylation with MeONa in MeOH. Finally, the regeneration of the carboxyl group required the study of the appropriate conditions for selective hydrolysis of the ester function while retaining the perfluorinated amido group, known to be sensitive to basic environments. Hydrolysis of the esters **17a** and **17c** proceeded easily (although we found that $NaHCO_3$ did not work, in contrast with a previous report[7]), as compound **17a** carries an acetamido group that allowed the use of methanolic sodium hydroxide, as expected, whereas compound **17c** has a heptafluorobutyric amido group that tolerated K_2CO_3 in moist methanol.

On the contrary, the hydrolysis was critical for the ester **17b**, having a base-labile trifluoroacetyl amido group, and it could take place in a suitable way only in the presence of triethylamine in aqueous methanol under strictly controlled conditions.

The obtained target sialosides **3a–3c** showed physicochemical properties (mass spectra, NMR spectra), which are in agreement with the assigned structures (the ^1H NMR data of compound **3a** reported in the literature[7] contained some mistakes and erroneous signal attributions if compared with our data). In particular an NMR analysis with complete proton and carbon resonance assignments, achieved by combination of 1D and 2D NMR experiments, clearly supported their β-anomeric structure. In fact, a clear signal shift to relatively high fields ($\delta = 2.37–2.43$ ppm) could be observed in the ^1H NMR spectra for the sialic 3-equatorial hydrogen atoms, diagnostic for a β-sialosidic bond in the molecules.[16b] The shifts toward relatively high fields were evident when the spectra were compared with that observed for the α-glycosides **20b** and **20c** ($\delta =$ 2.68–2.76 ppm for the corresponding protons), that were obtained by regeneration of the protected functions of the α-glycosides **12b** and **12c** with the same reaction sequences set-up for the corresponding β-anomers (Scheme 3).

The correct assignment of the anomeric configurations have been confirmed by chemical evidence from the successive transformation of the β-anomers **3a–3c** into the corresponding 1,7-lactones **4a–4c**, and from the inability of the α-isomers **20b** and **20c** to undergo the same transformation.

With the appropriated hydroxyl acidic glycosides **3a–3c** in hand, we attempted their direct 1,7-lactonization by using a protocol that we recently set up for the preparation of the 1,7-lactone of Neu5Ac (**1**) (Scheme 4).[10] The reaction afforded the desired 1,7-lactones **4a–4c** with different yields, which were good for compounds **4b** and **4c** (70% yield) whereas the yield was unsatisfactory for compound **4a**. No lactone was obtained in the analogous reactions of the α-glycosides **20b** or **20c**.

Moreover, as we anticipated that the unsatisfactory yields in the lactonization reaction of **3a** were due to the lower solubility of this glycoside in the reaction solvent, we decided to attempt the reaction on the corresponding acetonide **21** (Scheme 4). In this case, the reaction afforded the protected 1,7-lactone **22** in good yields (70%).

Unfortunately, the regeneration of the glycol system of the lactone **4a**, although it was attempted under various reaction conditions with different acids, was not completely selective and difficult to be controlled. Thus, we accepted as the best conditions those when we use aqueous CF_3COOH (90%, heat-

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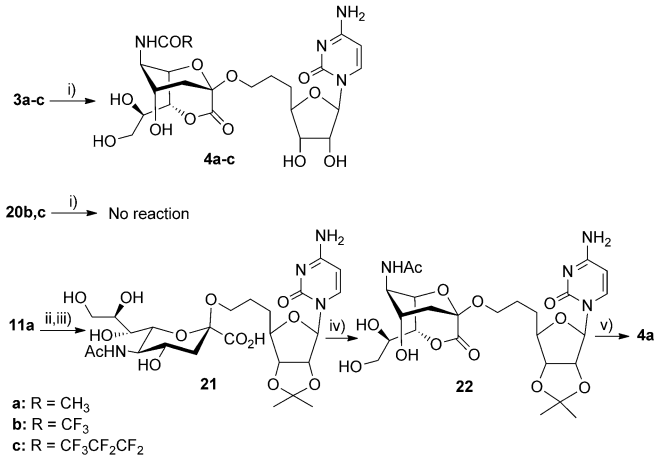
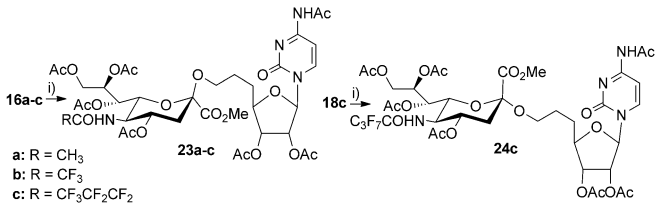


Scheme 4. Synthesis of the lactones **4a**–**4c**. i) CbzCl (Cbz = carbobenzyloxy), Et_3N, THF/DMF, 0 °C to RT, 30 min; ii) NaOMe, MeOH, RT, 1 h; iii) NaOH, MeOH, RT, 40 min; iv) moist TFA, CH_2Cl_2, heating to reflux, 1 h.

ing to reflux, RT) affording a mixture of the desired final lactone **4a** (65%) and of its parent acid **3a** (10%). Purification by reverse-phase preparative column chromatography and lyophilization afforded the pure lactone **4a**.

Successively, in order to facilitate the penetration of the highly hydrophilic compounds **3a**–**3c** through the membrane of alive cells, peracetylated derivatives of the inhibitors (i.e., compounds **23a**–**23c**) were prepared, by direct acetylation of intermediates **16a**–**16c** (Scheme 5). In fact, the possibility of administering the peracetylated sialosyl derivatives to cell cultures has been previously reported, as they become permeable to the membrane and are hydrolyzed to the corresponding free compounds once inside the cell.[18] Moreover, considering that the previous donor-based inhibitors possessed some tolerance of the ST enzymes to structural modifications at the anomeric site, we decided to test also the α-anomer **20c** and so we prepared, similarly to the corresponding β-anomer, the peracetylated derivative **22c**.



Scheme 5. Peracetylated sialosides **23a**–**23c** and **24c**. i) Ac_2O, pyridine (Py), RT, 3 h.

Inhibitory activity tests

The GM3-synthase inhibitory activity of the newly synthesized compounds **3a**–**3c**, **20c**, **4b**, and **4c** was tested in vitro on human embryonic kidney 293A cell homogenates, by using radiolabeled lactosylceramide as the substrate (Figure 2). Initially, all compounds were screened for their inhibitory activity at 1 mM concentration, revealing that compounds **3a**, **3b**, and **20c** almost completely inhibit the formation of GM3 ganglioside from lactosylceramide (Figure 2A). Then, the best inhibitors (i.e., compounds **3a**, **3b**, and **20c**) were tested at lower concentrations, revealing that the formation of GM3 could be inhibited roughly to half (IC50) at a concentration of 10 μM with all three compounds (Figure 2B). Successively, we tested their inhibitory effect on living cells. Most importantly, we wanted to test if they could penetrate the cell membrane, as this would be necessary for their potential use in vivo.

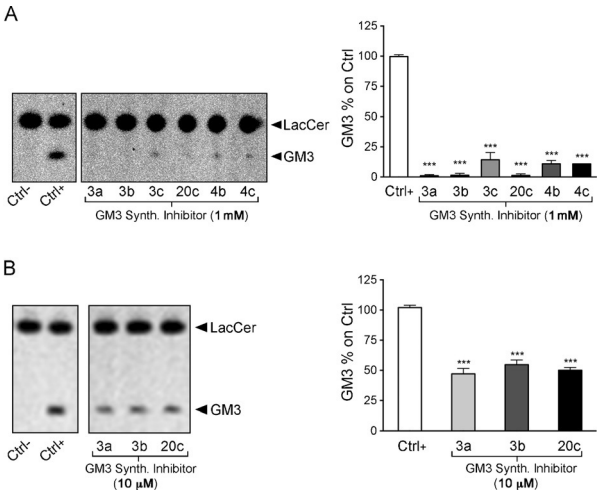


Figure 2. HPTLC and relative densitometric quantification of GM3-synthase activity with [3-^{3}H] lactosylceramide (LacCer) in the presence of A) 1 mM, or B) 10 μM solutions of the GM3-synthase inhibitors. Statistical differences were determined by one-way analysis of variance (ANOVA). ***: $p < 0.001$. Ctrl = control.

As anticipated, all inhibitors were too hydrophilic to be able to enter the cell and initial attempts were unsuccessful. On the other hand, the peracetylated inhibitors could penetrate the cell membrane. In fact, embryonic kidney 293A cells were cultured for 3 h in the presence of 10 μM GM3-synthase peracetylated inhibitors, whereas the negative controls were cultured in the presence of 0.01% DMSO, which was used to dissolve the inhibitors. Then, the cells were subjected to metabolic radiolabeling with a pulse of 2 h with [3-^{3}H]sphingosine, always in the presence of 10 μM inhibitors or DMSO. After a chase of 72 h, the sphingolipids were extracted and subjected to HPTLC, as described in the Experimental Section. Results revealed a significant decrease in the GM3 content of 18, 28, and 13% for compounds **23a**, **23b**, and **24c**, respectively (Figures 3A and B), causing a significant increase in the lactosyl ceramide and gb3 content in the organic phase (Figures 3C and D).

Finally, we tested whether treatment with GM3-synthase inhibitors would activate the EGFR signaling pathway. In fact, the GM3 ganglioside is known to bind to the EGFR and to inhibit its activation through autophosphorilation (Figure 4).[19] This pathway is crucial for cell survival, and we demonstrated that it plays an important role in myoblast survival under hypoxia.[6] In particular, treatment with GM3-synthase inhibitors could

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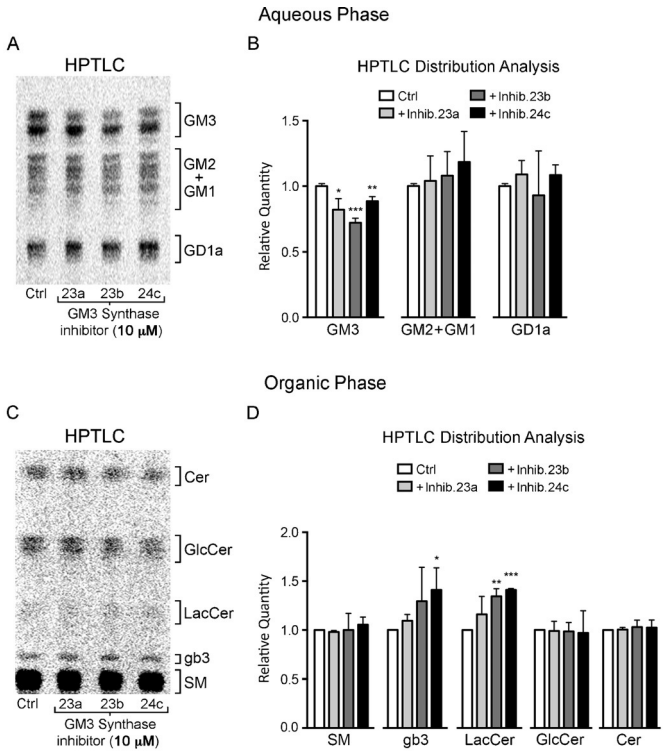

Figure 3. Effects of the GM3-synthase inhibitors on [3-^{3}H]sphingosine radiolabeled sphingolipids pattern. HEK293a cells were cultured in the presence of 10 μM inhibitors and metabolically labeled with [3-^{3}H]sphingosine. Radiochromatoscanning images of the HPTLC separation of the gangliosides contained in the aqueous phase (A) and gangliosides distribution (B). Radiochromatoscanning images of the HPTLC separation of the sphingolipids contained in the organic phase (C) and sphingolipid distribution (D). Statistical differences were determined by one-way ANOVA. *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

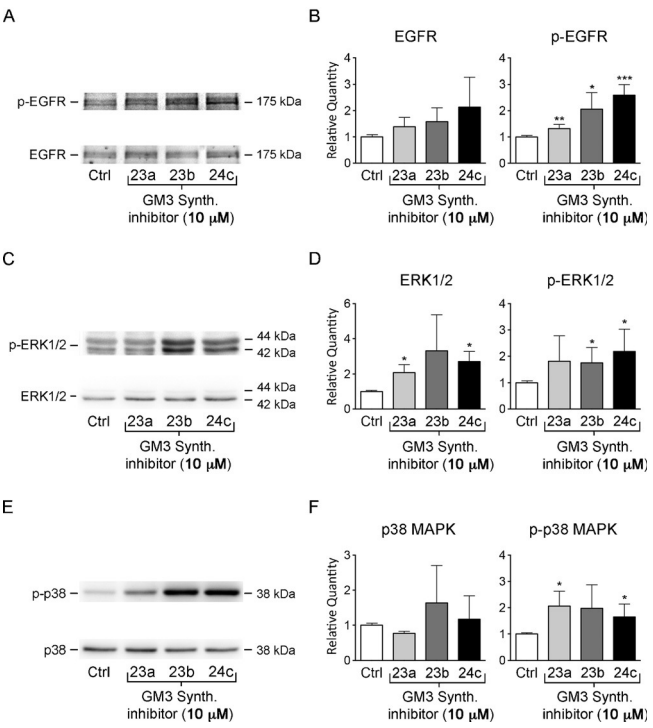

Figure 5. Western blot analysis of the EGFR pathway activation. Total proteins from Hek293 cells, cultured in the presence of 10 μM GM3-synthase inhibitors, were extracted and analyzed by a p-EGFR and total EGFR Western blot (A) and relative densitometric quantification (B), by a p-ERK1/2 and total ERK Western blot (C) and relative densitometric quantification (D), by a p-p38 and total p38 Western blot (E) and relative densitometric quantification (F). Statistical differences were determined by one-way ANOVA. *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

mimic the effects of sialidase NEU3 activation, that is, reduce the GM3 cell content, thereby activating the EGFR signaling cascade (Figure 4).

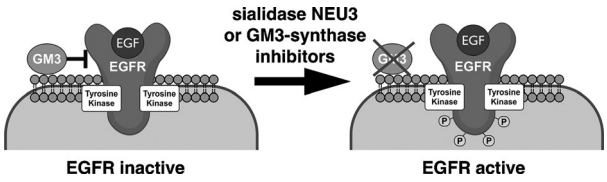

Figure 4. Representation of the EGFR signaling pathway activated by GM3-synthase inhibitors. Mechanism of the GM3-synthase inhibitors activation of the prosurvival signaling cascade, mediated by GM3 reduction and consequent phosphorilation of the EGFR.

To this purpose, Hek293A cells were incubated with 10 μM GM3 synthase peracetlyated inhibitors for 3 h and then subjected to protein extraction and Western blot analysis. Results revealed a significant increase in the EGFR and p-EGFR (Figures 5 A and B) and in its downstream targets ERK1/2, pERK1/2, p38 MAPK and p-p38 MAPK (Figures 5 C–F), confirming that the GM3-synthase inhibitors can mimic NEU3 overexpression in activating cell anti-apoptotic and prosurvival machinery.

Conclusion

These results, although preliminary, support the notion that GM3-synthase inhibitors may be used to activate the cell response to hypoxic stress, similar to what we reported for sialidase NEU3 overexpression. Although we cannot assure, at this stage, the specificity of the newly synthesized inhibitors for the GM3-synthase, the chemical approach has several advantages over gene overexpresison, as it can be finely regulated and suspended at any time. Although preliminary, these results support the feasibility of the hypothesis of chemically activating the cell defense machinery as a possible new approach for treating ischemic pathologies affecting the human heart and the nervous system. Further studies to test the effects of selected GM3-synthase inhibitors on these cell types are currently undergoing in our laboratories.

Experimental Section

General information

Chemicals: All chemicals and solvents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was prepared by filtering deionized water on a Milli-Q Simplicity 185 filtration system from Millipore (Bedford, MA, USA). Solvents were dried by using standard methods and distilled before

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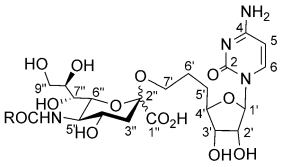
use. The progress of all reactions was monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F254) by using UV light, anisaldehyde/H_2SO_4/EtOH solution, or 0.2% ninhydrin in ethanol and heat as developing agent. All flash chromatography separations were performed with normal phase silica gel (E. Merck 230–400 mesh silica gel), following the general protocol of Still.[20] Melting points were measured on a SMP_3 m.p. apparatus (Stuart Scientific, USA) and are not corrected. Nuclear magnetic resonance spectra were recorded at 303 K on a Bruker AM-500 spectrometer equipped with a 5 mm inverse-geometry broadband probe and operating at 500.13 MHz for ^1H and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million and are referenced for ^1H spectra, to a solvent residue proton signal ($\delta = 7.26$, 3.31,and 2.50 ppm, respectively, for $CDCl_3$, CD_3OD, and $(CD_3)_3SO$) and for ^{13}C spectra, to solvent carbon signal (central line at $\delta = 77.0$, 49.05, and 39.43 ppm, respectively, for $CDCl_3$, CD_3OD, and $(CD_3)_3SO$). The chemical shifts obtained for D_2O solution are referenced to the internal $(CH_3)_3COH$ signal $\delta = 1.24$ ppm for ^1H spectra and $\delta = 30.29$ ppm for ^{13}C spectra. The ^1H and ^{13}C resonances were assigned by ^1H–^1H (COSY) and ^1H–^{13}C (HSQC and HMBC) correlation 2D experiments. The ^1H NMR data are tabulated as follows: s = singlet, d = doublet, brs = broad singlet, m = multiplet, coupling constant(s) are given in hertz ([Hz]). The carbon numeration used in the NMR spectra is given below.



Optical rotations were taken on a Perkin–Elmer 241 polarimeter equipped with a 1 dm tube. The $[\alpha]_D$ values are given in $[10^{-1}\,deg\,cm^2\,g^{-1}]$ and the concentration are given in $[g\,100\,mL^{-1}]$. Mass spectrometry was performed by using a Finnigan LCQDeca quadrupole ion-trap mass spectrometer equipped with an ESI ion source (Finnigan ThermoQuest, San Jose, CA, USA). The spectra were collected in a continuous flow mode by connecting the infusion pump directly to the ESI source. Solutions of the compounds were infused at a flow rate of 5 $mL\,min^{-1}$, the spray voltage was set at 5.0 kV in the positive and at 4.5 kV in the negative ion mode with a capillary temperature of 220 °C. Full-scan mass spectra were recorded by scanning a *m/z* range of 100–2000.

Biological: Human embryonic kidney 293A cells (Hek293A) were obtained from Invitrogen and cultured in Dulbecco's-modified Eagle's medium (DMEM) with high glucose content (4.5 $g\,L^{-1}$), (Sigma-Aldrich) supplemented with 10% (v/v) fetal bovine serum (FBS, Sigma-Aldrich), 4 mM L-glutamine (Gibco), 100 units mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin (Euroclone). All cell cultures were performed at 37 °C in a humidified incubator with 5% CO_2.

Synthetic procedure affording alcohol 6

A solution of aldehyde **5** (1.20 g, 3.71 mmol) and (triphenylphosphoranylidene)acetaldehyde (1.36 g, 4.46 mmol) in CH_2Cl_2 (60 mL) was stirred overnight at RT. Then, the orange solution was concentrated under reduced pressure to give the crude aldehyde **7**, which was used in the next step without further purification. A small amount of the crude aldehyde **7** was purified for the analytical characterization by flash chromatography (AcOEt/MeOH, 95:5, v/v). ^1H NMR (500 MHz, $CDCl_3$): $\delta = 9.56$ (d, $J_{7',6'} = 7.8$ Hz, 1H; H-7′), 8.95 (s, 1H; NH), 7.60 (d, $J_{6,5} = 7.5$ Hz, 1H; H-6), 7.43 (d, $J_{5,6} = 7.5$ Hz, 1H; H-5), 7.01 (dd, $J_{5',4'} = 6.2$, $J_{5',6'} = 15.8$, 1H; H-5′), 6.23 (ddd, $J_{6',4'} = 1.3$, $J_{6',7'} = 7.8$, $J_{6',5'} = 15.8$ Hz, 1H; H-6′), 5.57 (brs,1H; H-1′), 5.25 (brd, $J_{2',3'} = 6.3$ Hz, 1H; H-2′), 5.07 (dd, $J_{3',4'} = 3.9$, $J_{3',2'} = 6.3$ Hz, 1H; H-3′), 4.87–4.83 (m, 1H; H-4′), 2.25 (s, 3H; $NHCOCH_3$), 1.58 (s, 3H; $C(CH_3)_2$), 1.36 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 193.2$ (C-7′), 170.1 ($NHCOCH_3$), 163.3 (C-4), 154.6 (C-2), 153.0 (C-5′), 147.9 (C-6), 132.3 (C-6′), 114.3 ($C(CH_3)_2$), 98.9 (C-1′), 96.8 (C-5), 89.0 (C-4′), 85.2 (C-3′), 85.0 (C-2′), 27.0 ($C(CH_3)_2$), 25.2 ($C(CH_3)_2$), 25.0 ppm ($NHCOCH_3$); MS (ESI positive): *m/z*: 372.1 $[M+Na]^+$. The crude aldehyde **7**, dissolved in MeOH (100 mL), was hydrogenated in the presence of 10% Pd on carbon (160 mg) for 2 h. At this time, the catalyst was filtered and the solvent was removed under reduced pressure to afford the crude compound **8**, which was used in the next step without further purification. A small amount of the crude aldehyde **8** was purified for the analytical characterization by flash chromatography (AcOEt/MeOH, 95:5, v/v). M.p. 185 °C (from CH_2Cl_2/diisopropyl ether 7:3, v/v); $[\alpha]_D^{20} = +23.4$ ($c = 1$ in chloroform); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 9.75$ (brs, 1H; H-7′), 9.23 (s, 1H; NH), 7.63 (d, $J_{6,5} = 7.4$ Hz, 1H; H-6), 7.42 (d, $J_{5,6} = 7.4$ Hz, 1H; H-5), 5.60 (brs, 1H; H-1′), 5.04 (brd, $J_{2',3'} = 6.5$ Hz, 1H; H-2′), 4.71–4.67 (m, 1H; H-3′), 4.14–4.08 (m, 1H; H-4′), 2.62–2.56 (overlapping, 2H; H-6a′, H-6b′), 2.26 (s, 3H; $NHCOCH_3$), 2.19–2.03 (overlapping, 2H; H-5a′, H-5b′), 1.55 (s, 3H; $C(CH_3)_2$), 1.33 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 201.2$ (C-7′), 170.4 ($NHCOCH_3$), 163.1 (C-4), 154.5 (C-2), 146.8 (C-6), 114.5 ($C(CH_3)_2$), 96.7 (C-5), 96.0, (C-1′), 87.2 (C-4′), 84.9 (C-2′), 83.8 (C-3′), 40.0 (C-6′), 27.2 ($C(CH_3)_2$), 25.5 (C-5′), 25.3 ($C(CH_3)_2$), 25.0 ppm ($NHCOCH_3$); MS (ESI positive): *m/z*: 374.6 $[M+Na]^+$. The obtained crude aldehyde **8** was dissolved in MeOH (20 mL) and the solution was cooled at −20 °C. $NaBH_4$ (158 mg, 2.05 mmol) was added and after 15 min the reaction was stopped with the addition of acetone (4 mL). The reaction mixture was neutralized with Amberlite resin IRC-50 (H^+), filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (AcOEt/MeOH, 90:10, v/v), to afford compound **6**[7] (590 mg, 45% from compound **5**), as a white solid. M.p. 97–98 °C (from CH_2Cl_2/diisopropyl ether 7:3, v/v); $[\alpha]_D^{20} = +31.2$ ($c = 1.0$ in methanol) (lit.[7] $[\alpha]_D = +48.6$($c = 1.4$ in methanol)); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 9.76$ (s, 1H; NH), 7.73 (d, $J_{6,5} = 7.5$ Hz, 1H; H-6), 7.42 (d, $J_{5,6} = 7.5$ Hz, 1H; H-5), 5.69 (d, $J_{1',2'} = 1.6$ Hz, 1H; H-1′), 4.97 (dd, $J_{2',1'} = 1.6$, $J_{2',3'} = 6.4$ Hz, 1H; H-2′), 4.62 (dd, $J_{3',2'} = 6.4$, $J_{3',4'} = 4.5$ Hz, 1H; H-3′), 4.19–4.16 (m, 1H; H-4′), 3.70–3.64 (overlapping, 2H; H-7a′, H-7b′), 2.26 (s, 3H; $NHCOCH_3$), 1.89–1.74 (overlapping, 2H; H-5a′, H-5b′), 1.73–1.63 (overlapping, 2H; H-6a′, H-6b′), 1.56 (s, 3H; $C(CH_3)_2$), 1.33 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.9$ ($NHCOCH_3$), 163.2 (C-4), 154.8 (C-2), 146.0 (C-6), 114.3 ($C(CH_3)_2$), 96.7 (C-5), 95.3 (C-1′), 87.9 (C-4′), 85.2 (C-2′), 83.9 (C-3′), 62.2 (C-7′), 30.0 (C-5′), 28.9 (C-6′), 27.2 ($C(CH_3)_2$), 25.3 ($C(CH_3)_2$), 24.9 ppm ($NHCOCH_3$); MS (ESI positive): *m/z*: 376.2 $[M+Na]^+$, 729.1 $[2M+Na]^+$; elemental analysis calcd (%) for $C_{16}H_{23}N_3O_6$: C 54.38, H 6.56, N 11.89; found: C 54.42, H 6.60, N 11.82.

Synthesis of the chloro derivatives 9a–9c

General procedure: To a solution of the appropriated compound **10a–10c** (0.30 mmol), dissolved in acetyl chloride (5 mL) and cooled to −10 °C under an argon atmosphere, anhydrous MeOH (0.8 mL) was added. The solution was stirred overnight at RT then the solvent was removed under reduced pressure to give a syrup, which was crystallized from hexane/ethyl acetate (6:4, v/v) affording the derivatives **9a–9c**.

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Compound 9a: Starting from compound **10a**[14] (160 mg, 0.3 mmol) the chloro derivative **9a**[13](118 mg, 77%) was obtained as only stereoisomer as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.47 (dd, $J_{7,6}$ = 2.4, $J_{7,8}$ = 7.0 Hz, 1H; H-7), 5.44 (d, $J_{NH,5}$ = 10.2 Hz, 1H; NH), 5.39 (ddd, $J_{4,3a}$ = 4.8, $J_{4,5}$ = 10.4, $J_{4,3b}$ = 11.0 Hz, 1H; H-4), 5.17 (ddd, $J_{8,9a}$ = 2.7, $J_{8,9b}$ = 5.8, $J_{8,7}$ = 7.0 Hz, 1H; H-8), 4.42 (dd, $J_{9a,8}$ = 2.7, $J_{9a,9b}$ = 12.5 Hz, 1H; H-9a), 4.35 (dd, $J_{6,7}$ = 2.4, $J_{6,5}$ = 11.3 Hz, 1H; H-6), 4.20 (ddd, $J_{5,NH}$ = 10.2, $J_{5,4}$ = 10.4, $J_{5,6}$ = 11.3 Hz, 1H; H-5), 4.06 (dd, $J_{9b,8}$ = 5.8, $J_{9b,9a}$ = 12.5 Hz, 1H; H-9b), 3.87 (s, 3H; $COOCH_3$), 2.78 (dd, $J_{3a,4}$ = 4.8, $J_{3a,3b}$ = 13.9 Hz, 1H; H-3a), 2.28 (dd, $J_{3b,3a}$ = 11.2, $J_{3a,3b}$ = 13.9 Hz, 1H; H-3b), 2.12 (s, 3H; $OCOCH_3$), 2.07 (s, 3H; $OCOCH_3$), 2.04 (s, 3H; $OCOCH_3$), 2.03 (s, 3H; $OCOCH_3$), 1.91 ppm (s, 3H; $NHCOCH_3$); MS (ESI positive): *m/z*: 532.9 $[M+Na]^+$; elemental analysis calcd (%) for $C_{20}H_{28}ClNO_{12}$: C 47.11, H 5.53, N 2.75; found: C 47.18, H 5.61, N 2.81. Other physico-chemical properties were identical to those reported in literature.[13]

Compound 9b: Starting from the trifluoro derivative **10b**[9c] (176 mg, 0.30 mmol) the chloro derivative **9b** (137 mg, 81%) was obtained as only stereoisomer as a white solid. M.p. 132 °C; $[\alpha]_D^{20}$ = +25.1 (*c* = 1 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.53 (d, $J_{NH,5}$ = 10.1 Hz, 1H; NH), 5.53 (ddd, $J_{4,3a}$ = 4.9, $J_{4,5}$ = 10.2, $J_{4,3b}$ = 11.3 Hz, 1H; H-4), 5.44 (dd, $J_{7,6}$ = 2.3, $J_{7,8}$ = 7.2 Hz, 1H; H-7), 5.19 (ddd, $J_{8,9a}$ = 2.6, $J_{8,9b}$ = 5.3, $J_{8,7}$ = 7.2 Hz, 1H; H-8), 4.51 (dd, $J_{6,7}$ = 2.3, $J_{6,5}$ = 10.7 Hz, 1H; H-6), 4.40 (dd, $J_{9a,8}$ = 2.6, $J_{9a,9b}$ = 12.6 Hz, 1H; H-9a), 4.13 (ddd, $J_{5,NH}$ = 10.1, $J_{5,4}$ = 10.2, $J_{5,6}$ = 10.7 Hz, 1H; H-5), 4.08 (dd, $J_{9b,8}$ = 5.3, $J_{9b,9a}$ = 12.6 Hz, 1H; H-9b), 3.89 (s, 3H; $COOCH_3$), 2.83 (dd, $J_{3a,4}$ = 4.9, $J_{3a,3b}$ = 14.0 Hz, 1H; H-3a), 2.31 (dd, $J_{3b,3a}$ = 11.3, $J_{3a,3b}$ = 14.0 Hz, 1H; H-3b), 2.13 (s, 3H; $OCOCH_3$), 2.09 (s, 3H; $OCOCH_3$), 2.05 ppm (s, 6H; 2×$OCOCH_3$);^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.8, 170.6, 170.4, 169.5 (4×$OCOCH_3$), 165.3 (C-1), 157.6 (q, $J_{C,F}$ = 38.0 Hz, $COCF_3$), 115.3 ($J_{C,F}$ = 288 Hz, $COCF_3$), 96.0 (C-2), 73.1 (C-6), 70.7 (C-8), 68.1 (C-4), 66.9 (C-7), 62.0 (C-9), 53.7 ($COOCH_3$), 49.0 (C-5), 40.4 (C-3), 20.7, 20.5, 20.4, 20.3 ppm (4×$OCOCH_3$); MS (ESI positive): *m/z*: 586.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{20}H_{25}ClF_3NO_{12}$: C 42.60, H 4.47, N 2.48; found: C 42.52, H 4.48, N 2.63.

Compound 9c: Starting from the heptafluoro derivative **10c**[9c] (206 mg, 0.30 mmol) the chloro derivative **9c** (160 mg, 80%) was obtained as only stereoisomer as a white solid. M.p. 128 °C; $[\alpha]_D^{20}$ = +12.5 (*c* = 1 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.32 (d, $J_{NH,5}$ = 9.7 Hz, 1H; NH), 5.55 (ddd, $J_{4,3a}$ = 4.9, $J_{4,5}$ = 10.3, $J_{4,3b}$ = 11.2 Hz, 1H; H-4), 5.43 (dd, $J_{7,6}$ = 2.2, $J_{7,8}$ = 5.7 Hz, 1H; H-7), 5.15 (ddd, $J_{8,9a}$ = 2.5, $J_{8,7}$ = 5.7, $J_{8,9b}$ = 6.4 Hz, 1H; H-8), 4.57 (dd, $J_{6,7}$ = 2.2, $J_{6,5}$ = 10.7 Hz, 1H; H-6), 4.50 (dd, $J_{9a,8}$ = 2.5, $J_{9a,9b}$ = 12.5 Hz, 1H; H-9a), 4.18 (ddd, $J_{5,NH}$ = 9.7, $J_{5,4}$ = 10.3, $J_{5,6}$ = 10.7 Hz, 1H; H-5), 4.10 (dd, $J_{9b,8}$ = 6.4, $J_{9b,9a}$ = 12.5 Hz, 1H; H-9b), 3.88 (s, 3H; $COOCH_3$), 2.83 (dd, $J_{3a,4}$ = 4.9, $J_{3a,3b}$ = 13.9 Hz, 1H; H-3a), 2.24 (dd, $J_{3b,3a}$ = 11.2, $J_{3a,3b}$ = 13.9 Hz, 1H; H-3b), 2.13 (s, 3H; $OCOCH_3$), 2.10 (s, 3H; $OCOCH_3$), 2.04 (s, 3H; $OCOCH_3$), 2.03 ppm (s, 3H; $OCOCH_3$); ^{13}C NMR (125 MHz, CD_3OD): δ = 170.6, 169.7 (4×$OCOCH_3$), 165.4 (C-1), 158.1 ($J_{C,F}$ = 27 Hz, $COCF_2CF_2CF_3$), 124.0–110.0 ($COCF_2CF_2CF_3$), 95.9 (C-2), 73.1 (C-6), 70.8 (C-4), 67.8 (C-8), 67.0 (C-7), 62.0 (C-9), 53.9 ($COOCH_3$), 49.5 (C-5), 40.6 (C-3), 20.8, 20.6, 20.5, 20.4 ppm (4×CH_3COO); MS (ESI positive): *m/z*: 686.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{22}H_{25}ClF_7NO_{12}$: C 39.80, H 3.80, N 2.11; found: C 39.72, H 3.78, N 2.15.

Synthsis of the dibromo derivatives 13a–13c

General procedure: Bromine (0.80 mmol) was added to a solution of the appropriate glycal **14a–14c** (0.60 mmol) in CH_2Cl_2 (5 mL) under an argon atmosphere at 0 °C. After stirring for 30 min at RT, the solvent was removed under reduced pressure to give a syrup, which was crystallized from hexane/ethyl acetate (6:4, v/v) to afford the 2,3-dibromo derivative **13a–13c**.

Compound 13a: Starting from the glycal **14a** (284 mg, 0.60 mmol), the 2,3-dibromo derivative **13a**[17] (353 mg, 93%) was obtained as a white needles. M.p. 155–157 °C; $[\alpha]_D^{20}$ = −58.1 (*c* = 1 in chloroform) (lit.[17] m.p. 156–157 °C; $[\alpha]_D^{21}$ = −57.7 (*c* = 1 in chloroform)); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.77 (dd, $J_{4,3}$ = 3.5, $J_{4,5}$ = 10.5 Hz, 1H; H-4), 5.44 (d, $J_{NH,5}$ = 9.1 Hz, 1H; NH), 5.42 (dd, $J_{7,6}$ = 1.7, $J_{7,8}$ = 7.3 Hz, 1H; H-7), 5.26 (ddd, $J_{8,9a}$ = 2.6, $J_{8,9b}$ = 5.5, $J_{8,7}$ = 7.3 Hz, 1H; H-8), 5.05 (d, $J_{3,4}$ = 3.5 Hz, 1H; H-3), 4.54–4.42 (overlapping, 3H; H-6, H-9a, H-5), 4.14 (dd, $J_{9a,8}$ = 5.5, $J_{9a,9b}$ = 12.6 Hz, 1H; H-9b), 3.90 (s, 3H; $COOCH_3$), 2.16 (s, 3H; $OCOCH_3$), 2.12 (s, 3H; $OCOCH_3$), 2.08 (s, 3H; $OCOCH_3$), 2.06 (s, 3H; $OCOCH_3$), 1.96 ppm (s, 3H; $NHCOCH_3$). All other physico-chemical properties practically superimposable to those previously reported.[17]

Compound 13b: Starting from the glycal **14b**[9c] (316 mg, 0.60 mmol), the 2,3-dibromo derivative **13b** (391 mg, 95%) was obtained as white needles. $[\alpha]_D^{20}$ = −30.1 (*c* = 1 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.00 (d, $J_{NH,5}$ = 9.1 Hz, 1H; NH), 5.84 (dd, $J_{4,3}$ = 3.2, $J_{4,5}$ = 10.4 Hz, 1H; H-4), 5.40 (dd, $J_{7,6}$ = 1.7, $J_{7,8}$ = 6.6 Hz, 1H; H-7), 5.27–5.23 (m, 1H; H-8), 5.06 (d, $J_{3,4}$ = 3.2 Hz, 1H; H-3), 4.59 (dd, $J_{6,7}$ = 1.7, $J_{6,5}$ = 10.8 Hz, 1H; H-6), 4.52–4.45 (overlapping, 2H; H-5, H-9a), 4.19 (dd, $J_{9b,8}$ = 5.5, $J_{9b,9a}$ = 12.6 Hz, 1H; H-9b), 3.92 (s, 3H; $COOCH_3$), 2.17 (s, 3H; $OCOCH_3$), 2.11 (s, 3H; $OCOCH_3$), 2.10 (s, 3H; $OCOCH_3$), 2.05 ppm (s, 3H; $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.7, 170.6, 170.3, 170.1 (4×$OCOCH_3$), 163.8 (C-1), 157.6 ($J_{C,F}$ = 38.0 Hz, $COCF_3$), 115.3 ($J_{C,F}$ = 287.9 Hz, $COCF_3$), 91.0 (C-2), 75.3 (C-6), 70.6 (C-8), 68.1 (C-4), 66.9 (C-7), 61.9 (C-9), 54.0 ($COOCH_3$), 52.6 (C-3), 46.1 (C-5), 20.9, 20.6, 20.4 ppm (4×$OCOCH_3$); MS (ESI positive): *m/z* (%): 710.3 $[M+Na]^+$ (100), 712.7 $[M+Na]^+$ (51), 708.5 $[M+Na]^+$ (53); elemental analysis calcd (%) for $C_{20}H_{24}Br_2F_3NO_{12}$: C 34.96, H 3.52, N 2.04; found: C 34.8

Compound 13c: Starting from the glycal **14c**[9c] (376 mg, 0.60 mmol), the 2,3-dibromo derivative **13c** (439 mg, 93%) was as white needles. $[\alpha]_D^{20}$ = −18.5 (*c* = 1 in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.29 (d, $J_{NH,5}$ = 8.8 Hz, 1H; NH), 5.89 (dd, $J_{4,3}$ = 3.5, $J_{4,5}$ = 10.6 Hz, 1H; H-4), 5.37 (dd, $J_{7,6}$ = 1.8, $J_{7,8}$ = 6.3 Hz,1H; H-7), 5.25 (ddd, $J_{8,9a}$ = 2.3, $J_{8,9b}$ = 5.7, $J_{8,7}$ = 6.3 Hz, 1H; H-8), 5.08 (d, $J_{3,4}$ = 3.5 Hz, 1H; H-3), 4.64 (dd, $J_{6,7}$ = 1.8, $J_{6,5}$ = 10.8 Hz, 1H; H-6), 4.52 (dd, $J_{9a,8}$ = 2.3, $J_{9a,9b}$ = 12.6 Hz, 1H; H-9a), 4.43 (ddd, $J_{5,NH}$ = 8.8, $J_{5,4}$ = 10.6, $J_{5,6}$ = 10.8 Hz, 1H; H-5), 4.22 (dd, $J_{9b,8}$ = 5.7, $J_{9b,9a}$ = 12.6 Hz, 1H; H-9b), 3.91 (s, 3H; $COOCH_3$), 2.18 (s, 3H; $OCOCH_3$), 2.09 (s, 6H; 2×$OCOCH_3$), 2.04 ppm (s, 3H; $OCOCH_3$); ^{13}C NMR (125 MHz, $CHCl_3$): δ = 170.6, 170.5, 170.4, 169.8 (4×$OCOCH_3$), 163.8 (C-1), 158.0 ($J_{C,F}$ = 26.0 Hz, $COCF_2CF_2CF_3$), 125.0–110.0 ($COCF_2CF_2CF_3$), 90.6 (C-2), 74.7 (C-6), 70.4 (C-8), 67.5 (C-4), 67.0 (C-7), 61.8 (C-9), 54.0 ($COOCH_3$), 52.8 (C-3), 46.6 (C-5), 20.8, 20.6, 20.5, 20.3 ppm (4×$OCOCH_3$); MS (ESI positive): *m/z* (%): 810.4 $[M+Na]^+$ (100), 812.6 $[M+Na]^+$ (49), 808.7 $[M+Na]^+$ (50); elemental analysis calcd (%) for $C_{22}H_{24}Br_2F_7NO_{12}$: C 33.57, H 3.07, N 1.78; found: C 33.49, H 2.98, N 1.79.

Glycosylation with the chloro derivatives 9a–9c

General procedure: Silver trifluoromethanesulfonate (284 mg, 1.10 mmol) in anhydrous toluene-$MeNO_2$ (4 mL, 1:1, v/v) was added to a stirred mixture of acceptor **6** (265 mg, 0.75 mmol), the appropriate donor **9a–9c** (0.95 mmol), and 4 Å molecular sieves (300 mg) in anhydrous toluene/$MeNO_2$ (8 mL, 1:1, v/v), under an argon atmosphere at RT. The reaction mixture was stirred at RT for 3 h and then filtered through a pad of Celite and concentrated under reduced pressure. Column chromatography of the residue gave the β-anomer **11a–11c**, respectively, as first compound

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eluted, and then the corresponding α-anomer **12a–12c**, respectively.

Compounds 11a and 12a: Starting from compound **9a** (484 mg, 0.95 mmol), column chromatography (AcOEt/MeOH, 98:2, v/v to AcOEt/MeOH, 80:20, v/v) first afforded compound **11a** as a white solid (306 mg, 39%). $[\alpha]_D^{20}=-7.2$ ($c=1$ in methanol); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=9.19$ (s, 1H; NH at C-4), 7.86 (d, $J_{NH,5''}=9.9$ Hz, 1H; NH at C-5″), 7.58 (d, $J_{6',5'}=7.4$ Hz, 1H; H-6), 7.40 (d, $J_{5',6'}=7.4$ Hz, 1H; 5-H), 5.84 (dd, $J_{7'',8''}=1.6$, $J_{7'',6''}=3.0$ Hz, 1H; H-7″), 5.64 (dd, $J_{2',1''}=1.4$, $J_{2',3'}=6.3$ Hz, 1H; H-2′), 5.40 (d, $J_{1',2}=1.0$ Hz,1H; H-1′), 5.30 (ddd, $J_{4'',3a''}=4.9$, $J_{4'',5''}=10.5$, $J_{4'',3b''}=11.1$ Hz, 1H; H-4″), 5.07 (ddd, $J_{8'',7''}=1.6$, $J_{8'',9a''}=2.0$, $J_{8'',9b''}=9.5$ Hz, 1H; H-8″), 4.96–4.90 (overlapping, 2H; H-3′and H-9a″), 4.50 (dd, $J_{6'',5''}=10.6$, $J_{6'',7''}=3.0$ Hz, 1H; H-6″), 4.24–4.14 (overlapping, 2H; H-4′and H-5″), 4.07 (dd, $J_{9b'',9a''}=12.0$, $J_{9b'',9a''}=9.5$ Hz, 1H; H-9b″), 3.76 (s, 3H; $COOCH_3$), 3.55–3.50 (overlapping, 2H; H-7a′, H-7b′), 2.56 (dd, $J_{3a'',3b''}=12.7$, $J_{3'',4''}=4.9$ Hz, 1H; H-3a″), 2.21 (s, 3H; $NHCOCH_3$ at C-4), 2.19 (s, 3H; $OCOCH_3$), 2.01 (s, 3H; $OCOCH_3$), 1.97–1.75 (overlapping, 9H; 2×$OCOCH_3$, H-3b″, H-5a′, H-5b′, $NHCOCH_3$ at C-5″), 1.74–1.55 (overlapping, 2H; H-6a′, H-6b′), 1.53 (s, 3H; $C(CH_3)_2$), 1.38 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=173.5$ ($NHCOCH_3$ at C-5″), 172.9 ($NHCOCH_3$ at C-4), 172.4 (CH_3COO at C-9″), 172.0 (CH_3COO at C-4″ and at C-8″), 171.9 (CH_3COO at C-7″), 169.1 (C-1″), 164.7 (C-4), 157.7 (C-2), 148.6 (C-6), 115.3 ($C(CH_3)_2$), 100.0 (C-2″), 98.1 (C-5), 96.9 (C-1′), 88.8 (C-4′), 86.3 (C-2′), 85.6 (C-3′), 72.9 (C-8″), 72.4 (C-6″), 70.7 (C-4″), 70.3 (C-7″), 64.7 (C-7′), 63.7 (C-9′), 53.2 ($COOCH_3$), 50.3 (C-5″), 38.5 (C-3″), 31.5 (C-5′), 27.6 ($C(CH_3)_2$), 26.7 (C-6′), 25.6 ($C(CH_3)_2$), 24.6 ($NHCOCH_3$ at C-4), 22.9 ($NHCOCH_3$ at C-5″), 21.0, 20.8, 20.8, 20.7 ppm (4×CH_3COO); MS (ESI positive): *m/z*: 827.0 $[M+H]^+$, 849.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{36}H_{50}N_4O_{18}$: C 52.30, H 6.10, N 6.10; found: C 52.41, H 6.18, N 6.09.

Further elution afforded compound **12a** (2.3 mg, 0.3%). MS (ESI positive): *n/z*: 827.3 $[M+H]^+$, 849.0 $[M+Na]^+$.

Compounds 11b and 12b: Starting from compound **9b** (536 mg, 0.95 mmol) column chromatography (hexane/AcOEt, 70:30, v/v to AcOEt/MeOH, 95:5, v/v) first afforded compound **11b** as a white solid (301 mg, 36%). M.p. 134 °C; $[\alpha]_D^{20}=-44.5$ ($c=1$ in chloroform); $[\alpha]_D^{20}=-12.2$ ($c=1$ in methanol); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=9.16$ (d, $J_{NH,5''}=9.8$ Hz, 1H; NH at C-5″), 9.02 (s, 1H; NH at C-4), 7.58 (d, $J_{6',5'}=7.4$ Hz, 1H; H-6), 7.42 (d, $J_{5',6'}=7.4$ Hz, 1H; H-5), 5.90 (dd, $J_{7'',8''}=1.8$, $J_{7'',6''}=3.0$ Hz 1H; H-7″), 5.67 (d, $J_{2',1''}=6.1$ Hz, 1H; H-1′), 5.42–5.35 (overlapping, 2H; H-1′ and H-4″), 5.09 (ddd, $J_{8'',7''}=1.8$, $J_{8'',9a''}=2.1$, $J_{8'',9b''}=9.1$ Hz, 1H; H-8″), 4.94 (dd, $J_{9a'',8''}=2.1$, $J_{9a'',9b''}=12.1$ Hz, 1H; H-9a″), 4.91–4.89 (m, 1H; H-3′), 4.71 (dd, $J_{6'',7''}=3.0$, $J_{6'',5''}=10.5$ Hz, 1H; H-6″), 4.28–4.21 (overlapping, 2H; H-4′ and H-5″), 4.08 (dd, $J_{9b'',8''}=9.1$, $J_{9b'',9a''}=12.1$ Hz, 1H; H-9b″), 3.78 (s, 3H; $COOCH_3$), 3.58–3.52 (overlapping, 2H; H-7a′ and H-7b′), 2.65 (dd, $J_{3a'',4''}=4.9$, $J_{3a'',3b''}=12.8$ Hz, 1H; H-3a″), 2.20 (s, 6H; $NHCOCH_3$ at C-4, $OCOCH_3$), 2.17–2.12 (m, 1H; H-5a′), 2.01 (s, 3H; $OCOCH_3$), 1.95 (s, 3H; $OCOCH_3$), 1.93–1.86 (m, 1H; H-5b′), 1.82–1.75 (overlapping, 4H; $OCOCH_3$, H-3b″), 1.74–1.60 (overlapping, 2H; H-6a′, H-6b′), 1.55 (s, 3H; $C(CH_3)_2$), 1.40 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=172.9$ ($NHCOCH_3$ at C-4), 172.4, 171.9, 171.7 (4×$OCOCH_3$), 169.0 (C-1″), 164.7 (C-4), 159.3 (q, $J_{C,F}=37$ Hz, $COCF_3$), 157.7 (C-2), 148.4 (C-6), 117.0 (q, $J_{C,F}=287$ Hz, $COCF_3$), 115.4 ($C(CH_3)_2$), 100.0 (C-2″), 98.2 (C-5), 96.4 (C-1′), 88.6 (C-4′), 86.3 (C-2′), 85.5 (C-3′), 72.6 (C-8″), 71.5 (C-6″), 70.0 (C-4″, C-7″), 64.8 (C-7′), 63.5 (C-9″), 53.3 ($COOCH_3$), 51.0 (C-5″), 38.4 (C-3″), 31.4 (C-5′), 27.6 ($C(CH_3)_2$), 26.9 (C-6′), 25.6 ($C(CH_3)_2$), 24.6 ($NHCOCH_3$ at C-4), 21.0, 20.7, 20.6 ppm (4×CH_3COO); MS (ESI positive): *m/z*: 903.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{36}H_{47}F_3N_4O_{18}$: C 49.09, H 5.38, N 6.36; found: C 49.17, H 5.35, N 6.32.

Further elution afforded compound **12b** as a white solid (167 mg, 20%). M.p. 120 °C; $[\alpha]_D^{20}=-4.70$ ($c=1$ in chloroform); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=9.30$ (s, 1H; NH at C-4), 7.72 (d, $J_{6',5'}=7.5$ Hz, 1H; H-6), 7.42 (d, $J_{5',6'}=7.5$ Hz, 1H; H-5), 6.92 (d, $J_{NH,5''}=9.7$ Hz, 1H; NH at C-5″), 5.66 (br s, 1H; H-1′), 5.41 (ddd, $J_{8'',9a''}=2.9$, $J_{8'',9b''}=4.6$, $J_{8'',7''}=8.7$ Hz, 1H; H-8″), 5.33 (dd, $J_{7'',6''}=1.9$, $J_{7'',8''}=8.7$ Hz, 1H; H-7″), 5.09 (ddd, $J_{4'',3a''}=4.6$, $J_{4'',5''}=10.7$, $J_{4'',3b''}=12.0$ Hz, 1H; H-4″), 5.03 (d, $J_{2',3'}=6.1$ Hz, 1H; H-2′), 4.64 (dd, $J_{3',4'}=4.7$, $J_{3',2'}=6.1$ Hz, 1H; H-3′), 4.28 (dd, $J_{6'',7''}=1.9$, $J_{6'',5''}=10.7$ Hz, 1H; H-6″), 4.26–4.19 (overlapping, 2H; H-9a″, H-9b″), 4.16–4.04 (m, 1H; H-4′), 4.00 (ddd, $J_{5'',NH}=9.7$, $J_{5'',4''}=J_{5'',6''}=10.7$ Hz, 1H; H-5″), 3.83–3.77 (m, 1H; H-7a′), 3.75 (s, 3H; $COOCH_3$), 3.31–3.24 (m, 1H; H-7b′), 2.65 (dd, $J_{3a'',4''}=4.6$, $J_{3a'',3b''}=12.9$ Hz, 1H; H-3a″), 2.24 (s, 3H; $NHCOCH_3$ at C-4), 2.13 (s, 3H; $OCOCH_3$), 2.11 (s, 3H; $OCOCH_3$), 2.04 (s, 3H; $OCOCH_3$), 2.02 (s, 3H; $OCOCH_3$), 1.91 (dd, $J_{3b'',4''}=12.1$, $J_{3a'',3b''}=12.9$ Hz, 1H; H-3b″), 1.83–1.76 (overlapping, 2H; H-5a′, H-5b′), 1.71–1.58 (overlapping, 2H; H-6a′, H-6b′), 1.56 (s, 3H; $C(CH_3)_2$), 1.34 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=173.0$ ($NHCOCH_3$ at C-4), 172.5, 171.8, 171.6, 171.4 (4×$OCOCH_3$), 169.6 (C-1″), 164.7 (C-4), 159.3 (q, $J_{C,F}=37$ Hz, $COCF_3$), 157.7 (C-2), 147.6 (C-6), 117.2 (q, $J_{C,F}=287$ Hz, $COCF_3$), 115.4 ($C(CH_3)_2$), 100.1 (C-2″), 98.1 (C-5), 95.5 (C-1′), 88.5 (C-4′), 86.6 (C-2′), 85.3 (C-3′), 72.5 (C-6″), 70.2 (C-4″), 69.4 (C-8″), 68.4 (C-7″), 65.6 (C-7′), 63.4 (C-9″), 53.4 ($COOCH_3$), 50.8 (C-5″), 39.1 (C-3″), 31.1 (C-5′), 27.6 ($C(CH_3)_2$), 27.1 (C-6′), 25.7 ($C(CH_3)_2$), 24.6 ($NHCOCH_3$ at C-4), 21.3, 20.8, 20.7, 20.6 ppm (4×CH_3COO); MS (ESI positive): *m/z*: 903.4 $[M+Na]^+$; elemental analysis calcd (%) for $C_{36}H_{47}F_3N_4O_{18}$: C 49.09, H 5.38, N 6.36; found: C 49.15, H 5.41, N 6.30.

Compounds 11c and 12c: Starting from compound **9c** (631 mg, 0.95 mmol) column chromatography (hexane/AcOEt, 70:30, v/v to AcOEt/MeOH, 95:5, v/v) first afforded compound **11c** as a white solid (252 mg, 27%). M.p. 128–130 °C; $[\alpha]_{''}=+3.9$ ($c=1.0$ in methanol); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=9.18$ (d, $J_{NH,5''}=9.7$ Hz, 1H; NH at C-5″), 9.08 (s, 1H; NH at C-4), 7.58 (d, $J_{6',5'}=7.4$ Hz, 1H; H-6), 7.42 (d, $J_{5',6'}=7.4$ Hz, 1H; H-5), 5.96 (br s, $J_{7'',8''}=1.8$, $J_{7'',6''}=3.2$ 1H; H-7″), 5.68 (dd, $J_{2',1'}=1.2$, $J_{2',3'}=6.0$ Hz, 1H; H-2′), 5.39 (d, $J_{1',2'}=1.2$ Hz, 1H; H-1′), 5.35 (ddd, $J_{4'',3a''}=5.0$, $J_{4'',5''}=10.4$, $J_{4'',3b''}=11.0$ Hz, 1H; H-4″), 5.11 (ddd, $J_{8'',7''}=1.8$, $J_{8'',9a''}=2.4$, $J_{8'',9b''}=9.4$ Hz, 1H; H-8″), 4.96–4.90 (overlapping, 2H; H-9a″, H-3′), 4.72 (dd, $J_{6'',7''}=3.2$, $J_{6'',5''}=10.7$ Hz, 1H; H-6″), 4.32 (ddd, $J_{NH,5''}=9.7$, $J_{5'',4''}=10.4$, $J_{5'',6''}=10.7$ Hz, 1H; H-5″), 4.27–4.23 (m, 1H; H-4′), 4.07 (dd, $J_{9b'',8''}=9.4$, $J_{9b'',9a''}=12.1$ Hz, 1H; H-9b″), 3.79 (s, 3H; $COOCH_3$), 3.60–3.52 (overlapping, 2H; H-7a′, H-7b′), 2.68 (dd, $J_{3a'',4''}=5.1$, $J_{3a'',3b''}=12.8$ Hz, 1H; H-3a″), 2.22 (s, 3H; $NHCOCH_3$ at C-4), 2.21 (s, 3H; $OCOCH_3$), 2.18–2.07 (m, 1H, H-5a′), 1.99 (s, 3H; $OCOCH_3$), 1.97–1.89 (overlapping, 4H; $OCOCH_3$, H-5b′), 1.78 (s, 3H; $OCOCH_3$), 1.77–1.73 (m, 1H; H-3b″), 1.64–1.57 (overlapping, 2H; H-6a′, H-6b′), 1.55 (s, 3H; $C(CH_3)_2$), 1.40 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=172.9$ ($NHCOCH_3$ at C-4), 172.4, 172.0, 171.6, 171.5 (4×$OCOCH_3$), 168.9 (C-1″), 164.7 (C-4), 159.5 (t, $J_{C,F}=26$ Hz, $COCF_2CF_2CF_3$), 157.6 (C-2), 148.2 (C-6), 124.0–110.0 ($COCF_2CF_2CF_3$), 115.4 ($C(CH_3)_2$), 100.0 (C-2″), 98.2 (C-5), 96.0 (C-1′), 88.4 (C-4′), 86.4 (C-2′), 85.3 (C-3′), 72.6 (C-8″), 71.5 (C-6″), 70.0 (C-4″), 69.9 (C-7″), 64.8 (C-7′), 63.5 (C-9″), 53.3 ($COOCH_3$), 50.9 (C-5″), 38.4 (C-3″), 31.2 (C-5′), 27.6 ($C(CH_3)_2$), 26.9 (C-6′), 25.6 ($C(CH_3)_2$), 24.6 ($NHCOCH_3$ at C-4), 21.0, 20.7 ppm (4×CH_3COO); MS (ESI positive): *m/z* (%): 1003.0 $[M+Na]^+$ (100), 1004.1 $[M+Na]^+$ (55); elemental analysis calcd (%) for $C_{38}H_{47}F_7N_4O_{18}$: C 46.53, H 4.83, N 5.71; found: C 46.42, H 4.78, N 5.89.

Further elution afforded compound **12c** as a white solid (224 mg, 24%). M.p. 132–136 °C; $[\alpha]_D^{20}=-26.1$ ($c=1$ in methanol); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=8.77$ (s, 1H; NH at C-4), 7.71 (d, $J_{6',5'}=7.5$ Hz, 1H; H-6), 7.40 (d, $J_{5',6'}=7.5$ Hz, 1H; H-5), 6.90 (d, $J_{NH,5''}=10.0$ Hz, 1H;

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NH at C-5″), 5.66 (d, $J_{1''r2''}=1.6$ Hz, 1H; H-1′), 5.40 (ddd, $J_{8''r9a''}=2.7$, $J_{8''r9b''}=4.4$, $J_{8''r7''}=8.8$ Hz, 1H; H-8″), 5.28 (dd, $J_{7''r6''}=1.9$, $J_{7''r8''}=8.8$ Hz, 1H; H-7″), 5.15 (ddd, $J_{4''r3a''}=4.6$, $J_{4''r5''}=10.5$, $J_{4''r3b''}=12.1$ Hz, 1H; H-4″), 5.02 (dd, $J_{2''r1''}=1.6$, $J_{2''r3''}=6.5$ Hz,1H; H-2′), 4.64 (dd, $J_{3'r4'}=4.4$, $J_{3'r2'}=6.5$ Hz, 1H; H-3′), 4.32 (dd, $J_{6''r7''}=1.9$, $J_{6''r5''}=10.7$ Hz, 1H; H-6″), 4.30–4.21 (overlapping, 2H; H-9a″, H-9b″), 4.16–4.11 (m, 1H; H-4′), 3.94 (ddd, $J_{5''rNH}=10.0$, $J_{5''r4''}=10.5$, $J_{5''r6''}=10.7$ Hz, 1H; H-5″), 3.83–3.75 (overlapping, 2H; H-7a′, $COOCH_3$), 3.33–3.27 (m, 1H; H-7b′), 2.68 (dd, $J_{3a''r4''}=4.6$, $J_{3a''r3b''}=12.8$ Hz, 1H; H-3a″), 2.23 (s, 3H; $NHCOCH_3$ at C-4), 2.14 (s, 3H; $OCOCH_3$), 2.10 (s, 3H; $OCOCH_3$), 2.03 (s, 3H; $OCOCH_3$), 2.00 (s, 3H; $OCOCH_3$), 1.87 (dd, $J_{3b''r4''}=12.1$, $J_{3a''r3b''}=12.9$ Hz, 1H; H-3b″), 1.83–1.77 (overlapping, 2H; H-5a′, H-5b′), 1.73–1.63 (overlapping, 2H; H-6a′, H-6b′), 1.56 (s, 3H; $C(CH_3)_2$), 1.34 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=173.0$ ($NHCOCH_3$ at C-4), 172.5, 171.8, 171.4 ($4\times OCOCH_3$), 169.5 (C-1″), 164.7 (C-4), 159.6 (t, $J_{C'F}=27$ Hz, $COCF_2CF_2CF_3$), 157.7 (C-2), 147.6 (C-6), 124.0–110.0 ($COCF_2CF_2CF_3$), 115.4 ($C(CH_3)_2$), 100.0 (C-2″), 98.1 (C-5), 95.5 (C-1′), 88.6 (C-4′), 86.6 (C-2′), 85.3 (C-3′), 72.4 (C-6″), 70.2 (C-4″), 69.5 (C-8″), 68.5 (C-7″), 65.6 (C-7′), 63.5 (C-9″), 53.3 ($COOCH_3$), 50.7 (C-5″), 39.2 (C-3″), 31.1 (C-5′), 27.6 ($C(CH_3)_2$), 27.1 (C-6′), 25.7 ($C(CH_3)_2$), 24.6 ($NHCOCH_3$ at C-4), 21.3, 20.8, 20.7, 20.6 ppm ($4\times OCOCH_3$); MS (ESI positive): *m/z*: 1003.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{38}H_{47}F_7N_4O_{18}$: C 46.53, H 4.83, N 5.71; found: C 46.42, H 4.78, N 5.89.

Glycosylation with the dibromo derivatives 13a–13c

General procedure: Silver trifluoromethanesulfonate (308 mg, 1.20 mmol) in anhydrous toluene-$MeNO_2$ (4 mL, 1:1, v/v) was added to a stirred mixture of acceptor **6** (265 mg, 0.75 mmol), the appropriate donor **13a**–**13c** (1.10 mmol), and 4 Å molecular sieves (300 mg) in anhydrous toluene/$MeNO_2$ (8 mL, 1:1, v/v) under an argon atmosphere at RT. The reaction mixture was stirred at RT for 3 h and then filtered through a pad of Celite and concentrated under reduced pressure. The residue was purified by flash column chromatography to afford the compound **15a**–**15c**, respectively.

Compound 15a: Starting from the dibromide **13a** (697 mg, 1.10 mmol), purification by flash chromatography (AcOEt/MeOH, 98:2, v/v) afforded compound **15a** as a white solid (508 mg, 51%). M.p. 154–156 °C decomp. (from CH_2Cl_2/diisopropyl ether 8:2, v/v; $[\alpha]_D^{20}=-20.2$ ($c=0.5$ in chloroform) (lit.[7] $[\alpha]_D=-47.0$ ($c=0.5$ in methanol)); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=9.23$ (s, 1H; NH at C-4), 7.89 (d, $J_{NHr5''}=9.9$ Hz, 1H; NH at C-5″), 7.59 (d, $J_{6'r5}=7.3$ Hz, 1H; H-6), 7.42 (d, $J_{5'r6}=7.3$ Hz, 1H; H-5), 5.87 (dd, $J_{7''r8''}=1.6$, $J_{7''r6''}=2.8$ Hz, 1H; H-7″), 5.63 (d, $J_{2'r3'}=6.3$ Hz, 1H; H-2′), 5.41 (br s, 1H; H-1′), 5.25 (dd, $J_{4''r3''}=3.7$, $J_{4''r5''}=10.4$ Hz, 1H; H-4″), 5.12 (ddd, $J_{8''r7''}=1.6$, $J_{8''r9a''}=2.1$, $J_{8''r9b''}=9.5$ Hz, 1H; H-8″), 5.05 (dd, $J_{9a''r8''}=2.1$, $J_{9a''r9b''}=12.1$ Hz, 1H; H-9a″), 4.91 (dd, $J_{3'r4'}=1.8$, $J_{3'r2'}=6.3$ Hz, 1H; H-3′),4.71 (d, $J_{3''r4''}=3.7$ Hz, 1H; H-3″, 4.67 (ddd, $J_{5''rNH}=9.9$, $J_{5''r4''}=J_{5''r6''}=10.3$ Hz, 1H; H-5″), (dd, $J_{6''r5''}=10.3$, $J_{6''7''}=2.8$ Hz, 1H; H-6″), 4.25–4.20 (m, 1H; H-4′), 4.13 (dd, $J_{9b''r8''}=9.5$, $J_{9b''r9a''}=12.1$ Hz, 1H; H-9b″), 3.80 (s, 3H; $COOCH_3$), 3.63–3.53 (overlapping, 2H; H-7a′, H-7b′), 2.22 (s, 6H; $NHCOCH_3$ at C-4, $OCOCH_3$), 2.09 (s, 3H; $OCOCH_3$), 1.94 (s, 3H; $OCOCH_3$), 1.89 (s, 3H; $OCOCH_3$), 1.79 (s, 3H; $NHCOCH_3$ at C-5″), 1.70–1.58 (overlapping, 4H; H-5a′, H-5b′, H-6a′, H-6b′), 1.54 (s, 3H; $C(CH_3)_2$), 1.39 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=173.5$ ($NHCOCH_3$ at C-5″), 172.9 ($NHCOCH_3$ at C-4), 172.5, 172.0, 171.9, 171.6 ($4\times OCOCH_3$), 167.2 (C-1″), 164.7 (C-4), 157.7 (C-2), 148.9 (C-6), 115.3 ($C(CH_3)_2$), 101.5 (C-2″), 98.2 (C-5), 97.2 (C-1′), 88.8 (C-4′), 86.2 (C-2′), 85.7 (C-3′), 72.7, 72.6 (C-6″, C-8″), 70.6 (C-4″), 70.1 (C-7″), 66.3 (C-7′), 63.8 (C-9″), 53.4 ($COOCH_3$), 52.9 (C-3″), 46.4 (C-5″), 31.3 (C-5′), 27.6 ($C(CH_3)_2$), 26.6 (C-6′), 25.6 ($C(CH_3)_2$), 24.6 ($NHCOCH_3$ at C-4), 22.9 ($NHCOCH_3$ at C-5″), 21.0, 20.8, 20.7, 20.6 ppm ($4\times OCOCH_3$); MS (ESI positive): *m/z* (%): 927.1 $[^{79}BrM+Na]^+$ (100), 929.1 $[^{81}BrM+Na]^+$ (94); elemental analysis calcd (%) for $C_{36}H_{49}BrN_4O_{18}$: C 47.74, H 5.45, N 6.19; found: C 47.66, H 5.56, N 6.09.

Compound 15b: Starting from the dibromide **13b** (756 mg, 1.10 mmol), purification by flash chromatography (hexane/AcOEt, 70:30,v/v to AcOEt/MeOH, 95:5, v/v) afforded compound **15b** as a white solid (507 mg, 48%). M.p. 134–136 °C decomp. (from CH_2Cl_2/diisopropyl ether 8:2, v/v); $[\alpha]_D^{20}=+65.0$ ($c=1.0$ in CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=9.23$ (d, $J_{NHr5''}=9.1$ Hz, 1H; NH at C-5″), 9.04 (s, 1H; NH at C-4), 7.58 (d, $J_{6'r5}=7.4$ Hz, 1H; H-6), 7.43 (d, $J_{5'r6}=7.4$ Hz, 1H; H-5), 5.91 (br s, 1H; H-7″), 5.63 (dd, $J_{2'r1'}=1.3$, $J_{2'r3'}=6.3$ Hz, 1H; H-2′), 5.39 (d, $J_{1'r2'}=1.3$ Hz, 1H; H-1′), 5.31 (dd, $J_{4''r3''}=3.7$, $J_{4''r5''}=10.0$ Hz, 1H; H-4″), 5.12 (ddd, $J_{8''r7''}=2.1$, $J_{8''r9a''}=2.4$, $J_{8''r9b''}=9.5$ Hz, 1H; H-8″), 5.04 (dd, $J_{9a''r8''}=2.4$, $J_{9a''r9b''}=12.1$ Hz, 1H; H-9a″), 4.89 (dd, $J_{3'r4'}=1.8$, $J_{3'r2'}=6.3$ Hz, 1H; H-3′), 4.76 (d, $J_{3''r4''}=3.6$ Hz, 1H; H-3″), 4.75–4.67 (overlapping, 2H; H-5″, H-6″), 4.26–4.21 (m, 1H; H-4′), 4.14–4.09 (m, 1H; H-9b″), 3.82 (s, 3H; $COOCH_3$), 3.64–3.55 (overlapping, 2H; H-7a′, H-7b′), 2.23 (s, 3H; $NHCOCH_3$ at C-4), 2.21 (s, 3H; $OCOCH_3$), 2.07 (s, 3H; $OCOCH_3$), 1.94 (s, 3H; $OCOCH_3$), 1.78 (s, 3H; $OCOCH_3$), 1.77–1.55 (overlapping, 4H; H-5a′, H-5b′, H-6a′, H-6b′), 1.54 (s, 3H; $C(CH_3)_2$), 1.39 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=172.9$ ($NHCOCH_3$ at C-4), 172.5, 171.9, 171.8, 171.3 ($4\times OCOCH_3$), 167.0 (C-1″), 164.7 (C-4), 159.3 (q, $J_{C'F}=37$ Hz, $COCF_3$), 157.7 (C-2), 148.6 (C-6), 117.1 (q, $J_{C'F}=287$ Hz, $COCF_3$), 115.4 ($C(CH_3)_2$), 101.5 (C-2″), 98.2 (C-5), 96.7 (C-1′), 88.6 (C-4′), 86.2 (C-2′), 85.5 (C-3′), 72.4 (C-8″), 71.9 (C-6″), 70.0 (C-4″), 69.8 (C-7″), 66.4 (C-7′), 63.6 (C-9″), 53.5 ($COOCH_3$), 52.6 (C-3″), 47.1 (C-5″), 31.2 (C-5′), 27.6 ($C(CH_3)_2$), 26.7 (C-6′), 25.6 ($C(CH_3)_2$), 24.6 ($NHCOCH_3$ at C-4), 21.0, 20.7, 20.7, 20.5 ppm ($4\times OCOCH_3$); MS (ESI positive): *m/z* (%): 981.1 $[^{79}BrM+Na]^+$ (100), 983.1 $[^{81}BrM+Na]^+$ (94); elemental analysis calcd (%) for $C_{36}H_{46}BrF_3N_4O_{18}$: C 45.06, H 4.83, N 5.84; found: C 45.06, H 4.83, N 5.94.

Compound 15c: Starting from the dibromide **13c** (866 mg, 1.10 mmol), purification by flash chromatography (hexane/AcOEt 70:30, v/v to AcOEt/MeOH, 80:20, v/v) afforded compound **15c** as a white solid (373 mg, 32%). M.p. 138–140 °C; $[\alpha]_D^{20}=+8.5$ ($c=1$ in methanol); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=9.23$ (d, $J_{NHr5''}=8.9$ Hz, 1H; NH at C-5″), 9.04 (s, 1H; NH at C-4), 7.58 (d, $J_{6'r5}=7.4$ Hz, 1H; H-6), 7.43 (d, $J_{5'r6}=7.4$ Hz, 1H; H-5), 5.91 (dd, $J_{7''r8''}=J_{7''r6''}=1.7$ 1H; H-7″), 5.63 (dd, $J_{2'r1'}=1.1$, $J_{2'r3'}=6.3$ Hz, 1H; H-2′), 5.39 (d, $J_{1'r2'}=1.1$ Hz, 1H; H-2′), 5.32 (dd, $J_{4''r3''}=3.7$, $J_{4''r5''}=9.9$ Hz, 1H; H-4″), 5.12 (ddd, $J_{8''r7''}=1.7$, $J_{8''r9a''}=2.4$, $J_{8''r9b''}=9.4$ Hz, 1H; H-8″), 5.04 (dd, $J_{9a''r8''}=2.4$, $J_{9a''r9b''}=12.1$ Hz, 1H; H-9a″), 4.89 (dd, $J_{3'r4'}=1.7$, $J_{3'r2'}=6.3$ Hz, 1H; H-3′), 4.76 (d, $J_{3''r4''}=3.7$ Hz, 1H; H-3″), 4.75–4.67 (overlapping, 2H; H-5″ and H-6″), 4.26–4.22 (m, 1H; H-4′), 4.14–4.06 (m, 1H; H-9b″), 3.82 (s, 3H; $COOCH_3$), 3.64–3.54 (overlapping, 2H; H-7a′, H-7b′), 2.23 (s, 3H; $NHCOCH_3$ at C-4), 2.21 (s, 3H; $OCOCH_3$), 2.19–2.10 (m, 1H; H-5a′), 2.08 (s, 3H; $OCOCH_3$), 1.95 (s, 3H; $OCOCH_3$), 1.93–1.86 (m, 1H; H-5b′), 1.78 (s, 3H; $OCOCH_3$), 1.77–1.57 (overlapping, 2H; H-6a′, H-6b′), 1.54 (s, 3H; $C(CH_3)_2$), 1.39 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=173.0$ ($NHCOCH_3$ at C-4), 172.5, 172.0, 171.6, 171.2 ($4\times OCOCH_3$), 167.0 (C-1″), 164.7 (C-4), 159.6 (t, $J_{C'F}=26$ Hz, $COCF_2CF_2CF_3$), 157.7 (C-2), 148.3 (C-6), 124.0–110.0 ($COCF_2CF_2CF_3$), 115.5 ($C(CH_3)_2$), 101.5 (C-2″), 98.2 (C-5), 96.2 (C-1′), 88.3 (C-4′), 86.3 (C-2′), 85.4 (C-3′), 72.5 (C-8″), 71.8 (C-6″), 69.9 (C-4″), 69.6 (C-7″), 66.4 (C-7′), 63.5 (C-9″), 53.6 ($COOCH_3$), 52.6 (C-3″), 47.2 (C-5″), 31.1 (C-5′), 27.6 ($C(CH_3)_2$), 26.7 (C-6′), 25.6 ($C(CH_3)_2$), 24.6 ($NHCOCH_3$ at C-4), 21.0, 20.7, 20.7, 20.5 ppm ($4\times OCOCH_3$); MS (ESI positive): *m/z*: 1082.6 $[M+Na]^+$; elemental analysis calcd (%) for $C_{38}H_{46}BrF_7N_4O_{18}$: C 43.07, H 4.38, N 5.29; found: C 43.02, H 4.38, N 5.35.

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Reductive debromination

General procedure: Tri-*n*-butyltin hydride (0.05 mL, 0.20 mmol) and AIBN (10 mg, 0.061 mmol) were added to a solution of the appropriate compound **15a**–**15c** (0.10 mmol) in THF (5 mL) and the mixture was heated to reflux for 3 h. Then the reaction mixture was concentrated under reduced pressure and the residue was triturated with diisopropyl ether/hexane (8:2, v/v) to afford compound **11a**–**11c**, respectively, with all the physico-chemical properties identical to those previously reported.

Compounds 11a: Starting from compound **15a** (90 mg, 0.10 mmol) the glycoside **11a** was obtained as a white solid (67 mg, 81%).

Compounds 11b: Starting from compound **15b** (96 mg, 0.10 mmol) the glycoside **11b** was obtained as a white solid (73 mg, 83%).

Compounds 11c: Starting from compound **15c** (105 mg, 0.10 mmol) the glycoside **11c** was obtained a white solid (85 mg, 87%).

Acetonide deprotection to afford compounds 16a–16c as well as 18b and 18c: A solution of the appropriate compound **11a**–**11c** or **12b** and **12c** (0.40 mmol) in dichloromethane (5 mL) was added to CF_3COOH (45 μL) containing 5% H_2O. The mixture was heated to reflux and stirred for one hour. Then the solution was neutralized with a weak basic resin (IRA 67), filtered, and evaporated under reduced pressure to give a syrup, which was purified by flash column chromatography to afford compound **16a**–**16c** or **18b** and **18c**, respectively.

Compound 16a: Starting from compound **11a** (331 mg, 0.40 mmol), purification by flash chromatography (AcOEt/MeOH, 90:10, v/v) afforded compound **16a** as a white solid (236 mg, 75%). M.p. 118–121 °C; $[\alpha]_D^{25} = +14.2$ ($c = 1$ in methanol); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.01$ (d, $J_{6',5} = 7.5$ Hz, 1H; H-6), 7.47 (d, $J_{5',6} = 7.5$ Hz, 1H; H-5), 5.81 (d, $J_{1',2'} = 2.3$ Hz, 1H; H-1′), 5.41 (dd, $J_{7'',6''} = 2.2$, $J_{7'',8''} = 4.1$ Hz, 1H; H-7″), 5.30 (ddd, $J_{8'',9a''} = 2.5$, $J_{8'',7''} = 4.1$, $J_{8'',9b''} = 7.6$ Hz, 1H; H-8″), 5.23 (ddd, $J_{4'',3a''} = 4.9$, $J_{4'',5''} = 10.4$, $J_{4'',3b''} = 11.0$ Hz, 1H; H-4″), 4.82 (dd, $J_{9a'',8''} = 2.5$, $J_{9a'',8''} = 12.4$ Hz, 1H; H-9a″), 4.19 (dd, $J_{2',1'} = 2.3$, $J_{2',3'} = 5.2$ Hz, 1H; H-2′), 4.15—4.07 (overlapping, 2H; H-6″, H-9b″), 4.06–4.04 (m, 1H; H-4′), 3.95 (dd, $J_{5'',4''} = J_{5'',6''} = 10.4$ Hz, 1H; H-5″), 3.84 (dd, $J_{3',2'} = 5.3$, $J_{3',4'} = 7.2$ Hz, 1H; H-3′), 3.80 (s, 3H; $COOCH_3$), 3.67–3.61 (m, 1H; H-7a′), 3.48–3.42 (m, 1H; H-7b′), 2.47 (dd, $J_{3a'',4''} = 4.9$, $J_{3a'',3b''} = 13.0$ Hz, 1H; H-3a″), 2.18 (s, 3H; $NHCOCH_3$ at C-4), 2.12 (s, 3H; $OCOCH_3$), 2.04–2.02 (overlapping, 6H; 3×$OCOCH_3$), 2.00–1.76 ppm (overlapping, 11H; $NHCOCH_3$ at C-5″, $OCOCH_3$, H-5a′, H-5b′, H-6a′, H-6b′, H-3b″); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 173.5$ ($NHCOCH_3$ at C-5″), 173.0 ($NHCOCH_3$ at C-4), 172.6, 172.3, 172.0, 171.7 (4×$OCOCH_3$), 169.2 (C-1″), 164.4 (C-4), 158.0 (C-2), 146.2 (C-6), 100.0 (C-2″), 98.3 (C-5), 94.2 (C-1′), 84.4 (C-4′), 76.2 (C-2′), 75.0 (C-3′), 73.1 (C-8″), 72.4 (C-6″), 70.6 (C-4″), 70.1 (C-7″), 64.9 (C-7′), 63.7 (C-9″), 53.3 ($COOCH_3$), 50.3 (C-5″), 38.5 (C-3″), 31.3 (C-5′), 27.3 (C-6′), 24.6 ($NHCOCH_3$ at C-4), 22.8 ($NHCOCH_3$ at C-5″), 21.0, 20.8 ppm (4×$OCOCH_3$); MS (ESI positive): m/z: 809.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{33}H_{46}N_4O_{18}$: C 50.38, H 5.89, N 7.12; found: C 50.46, H 5.80, N 7.03.

Compound 16b: Starting from compound **11b** (352 mg, 0.40 mmol), purification by flash chromatography (AcOEt/MeOH, 90:10, v/v) afforded compound **16b** as white a solid (259 mg, 77%). M.p. 128–130 °C; $[\alpha]_D^{20} = +21.5$ ($c = 1$ in methanol); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.01$ (d, $J_{6',5} = 7.5$ Hz, 1H; H-6), 7.47 (d, $J_{5',6} = 7.5$ Hz, 1H; H-5), 5.81 (d, $J_{1',2'} = 2.0$ Hz, 1H; H-1′), 5.39 (dd, $J_{7'',6''} = 2.0$, $J_{7'',8''} = 4.2$ Hz, 1H; H-7″), 5.38–5.29 (overlapping, 2H; H-4″, H-8″), 4.78 (dd, $J_{9a'',8''} = 2.1$, $J_{9a'',9b''} = 12.4$ Hz, 1H; H-9a″), 4.28 (dd, $J_{6'',7''} = 2.0$, $J_{6'',5''} = 9.5$ Hz, 1H; H-6″), 4.18 (dd, $J_{2',1'} = 2.0$, $J_{2',3'} = 4.9$ Hz, 1H; H-2′), 4.12 (dd, $J_{9b'',8''} = 7.4$, $J_{9a'',9b''} = 12.4$ Hz, 1H; H-9b″), 4.05–3.96 (overlapping, 2H; H-4′, H-5″), 3.87–3.78 (overlapping, 4H; H-3′, $COOCH_3$), 3.67–3.59 (m, 1H; H-7a′), 3.48–3.42 (m, 1H; H-7b′), 2.51 (dd, $J_{3a'',4''} = 4.9$, $J_{3a'',3b''} = 12.9$ Hz, 1H; H-3a″), 2.18 (s, 3H; $NHCOCH_3$ at C-4), 2.12 (s, 3H; $OCOCH_3$), 2.03 (s, 3H; $OCOCH_3$), 2.02 (s, 3H; $OCOCH_3$), 1.97 (s, 3H; $OCOCH_3$), 1.94–1.77 ppm (overlapping, 5H; H-5a′, H-5b′, H-6a′, H-6b′, H-3b″); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 173.0$ ($NHCOCH_3$ at C-4), 172.5, 172.2, 171.7, 171.5 (4×$OCOCH_3$), 169.0 (C-1″), 164.4 (C-4), 159.3 (q, $J_{C,F} = 38$ Hz, $COCF_3$), 158.0 (C-2), 146.1 (C-6), 117.1 (q, $J_{C,F} = 287$ Hz, $COCF_3$), 100.0 (C-2″), 98.3 (C-5), 94.1 (C-1′), 84.4 (C-4′), 76.2 (C-2′), 75.0 (C-3′), 72.7 (C-8″), 71.6 (C-6″), 70.0 (C-4″), 69.9 (C-7″), 65.0 (C-7′), 63.5 (C-9″), 53.3 ($COOCH_3$), 51.0 (C-5″), 38.5 (C-3″), 31.1 (C-5′or C-6′), 27.2 (C-6′ or C-5′), 24.6 ($NHCOCH_3$ at C-4), 21.0, 20.7, 20.6 ppm (4×$OCOCH_3$); MS (ESI positive): m/z: 863.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{33}H_{43}F_3N_4O_{18}$: C 47.15, H 5.16, N 6.66; found: C 47.22, H 5.28, N 6.50.

Compound 16c: Starting from compound **11c** (392 mg, 0.40 mmol), purification by flash chromatography (AcOEt/MeOH, 90:10, v/v) afforded compound **16c** as a white solid (297 mg, 79%). M.p. 130–132 °C; $[\alpha]_D^{20} = +19.4$ ($c = 1.0$ in CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.00$ (d, $J_{6',5} = 7.5$ Hz, 1H; H-6), 7.47 (d, $J_{5',6} = 7.5$ Hz, 1H; H-5), 5.81 (d, $J_{1',2'} = 2.2$ Hz, 1H; H-1′), 5.38–5.28 (overlapping, 3H; H-7″, H-4″, H-8″), 4.77 (dd, $J_{9a'',8''} = 2.3$, $J_{9a'',8''} = 12.4$ Hz, 1H; H-9a″), 4.32 (d, $J_{6'',5''} = 10.4$ Hz, 1H; H-6″), 4.17 (dd, $J_{2',1'} = 2.2$, $J_{2',3'} = 5.2$ Hz, 1H; H-2′), 4.15–4.00 (overlapping, 3H; H-4′, H-5″ H-9b″), 3.84 (dd, $J_{3',2'} = 5.2$, $J_{3',4'} = 7.0$ Hz, 1H; H-3′), 3.81 (s, 3H; $COOCH_3$), 3.67–3.60 (m, 1H; H-7a′), 3.48–3.41 (m, 1H; H-7b′), 2.53 (dd, $J_{3a'',4''} = 5.0$, $J_{3a'',3b''} = 12.9$ Hz, 1H; H-3a″), 2.18 (s, 3H; $NHCOCH_3$ at C-4), 2.12 (s, 3H; $OCOCH_3$), 2.04 (s, 3H; $OCOCH_3$), 2.02 (s, 3H; $OCOCH_3$), 1.98–1.76 ppm (overlapping, 8H; $OCOCH_3$, H-5a′, H-5b′, H-6a′, H-6b′, H-3b″); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 173.0$ ($NHCOCH_3$ at C-4), 172.5, 172.2, 171.6, 171.4 (4×$OCOCH_3$), 169.0 (C-1″), 164.4 (C-4), 159.6 (t, $J_{C,F} = 26$ Hz,$COCF_2CF_2CF_3$), 158.0 (C-2), 146.1 (C-6), 121.0–106.0 ($COCF_2CF_2CF_3$), 99.9 (C-2″), 98.3 (C-5), 94.0 (C-1′), 84.3 (C-4′), 76.2 (C-2′), 75.0 (C-3′), 72.7 (C-8″), 71.6 (C-6″), 69.9, 69.8 (C-4″, C-7″), 65.0 (C-7′), 63.5 (C-9″), 53.3 ($COOCH_3$), 50.9 (C-5″), 38.5 (C-3″), 31.1 (C-5′), 27.2 (C-6′), 24.6 ($NHCOCH_3$ at C-4), 21.0, 20.7, 20.6 ppm (4×$OCOCH_3$); MS (ESI positive): m/z: 963.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{35}H_{43}F_7N_4O_{18}$: C 44.69, H 4.61, N 5.96; found: C 44.52, H 4.70, N 5.93.

Compound 18b: Starting from compound **12b** (352 mg, 0.40 mmol), purification by flash chromatography (AcOEt/MeOH, 85:15, v/v) afforded compound **18b** (255 mg, 76%) as a white solid. M.p. 132–134 °C; $[\alpha]_D^{20} = -24.1$ ($c = 1$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.08$ (d, $J_{6',5} = 7.4$ Hz, 1H; H-6), 7.49 (d, $J_{5',6} = 7.4$ Hz, 1H; H-5), 5.85 (d, $J_{1',2'} = 1.4$ Hz, 1H; H-1′), 5.47–5.41 (m, 1H; H-8″), 5.33 (d, $J_{7'',8''} = 8.7$ Hz, 1H; H-7″), 4.96–4.84 (m, 1H; H-4″), 4.36–4.27 (overlapping, 2H; H-6″, H-9a″), 4.18–4.15 (m, 1H; H-2′), 4.14–4.03 (overlapping, 3H; H-3′, H-4′, H-9b″), 4.02 (dd, $J_{5'',4''} = J_{5'',6''} = 10.7$ Hz, 1H; H-5″), 3.90–3.80 (overlapping, 4H; H-7a′, $COOCH_3$), 3.44–3.38 (m, 1H; H-7b′), 2.71 (dd, $J_{3a'',4''} = 4.6$, $J_{3a'',3b''} = 12.5$ Hz, 1H; H-3a″), 2.20 (s, 3H; $NHCOCH_3$), 2.17 (s, 3H; CH_3COO), 2.13 (s, 3H; CH_3COO), 2.01 (s, 3H; CH_3COO), 1.99 (s, 3H; CH_3COO), 1.94–1.72 ppm (overlapping, 5H; H-5a′, H-5b′, H-6a′, H-6b′, H-3b″); MS (ESI positive): m/z: 863.6 $[M+Na]^+$; elemental analysis calcd (%) for $C_{33}H_{43}F_3N_4O_{18}$: C 47.15, H 5.16, N 6.66; found C 47.66, H 5.20, N 6.40.

Compound 18c: Starting from compound **12c** (392 mg, 0.40 mmol), purification by flash chromatography (AcOEt/MeOH, 85:15, v/v) afforded compound **18c** (271 mg, 72%) as a white solid. M.p. 122–124 °C; $[\alpha]_D^{20} = -14.9$ ($c = 1$ in methanol); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.06$ (d, $J_{6',5} = 7.5$ Hz, 1H; H-6), 7.46 (d, $J_{5',6} =$

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7.5 Hz, 1H; H-5), 5.83 (d, $J_{1',2'}$ = 2.5 Hz, 1H; H-1′), 5.41 (ddd, $J_{8'',9a''}$ = 2.7, $J_{8'',9b''}$ = 5.2, $J_{8'',7''}$ = 8.9 Hz, 1H; H-8″), 5.29 (dd, $J_{7'',6''}$ = 2.1, $J_{7'',8''}$ = 8.9 Hz, 1H; H-7″), 4.96–4.88 (ddd, $J_{4'',3a''}$ = 4.7, $J_{4'',5''}$ = 10.4, $J_{4'',3b''}$ = 12.0 Hz, 1H; H-4″), 4.34 (dd, $J_{6'',7''}$ = 2.1, $J_{6'',5''}$ = 10.7 Hz, 1H; H-6″), 4.29 (dd, $J_{9a'',8''}$ = 2.7, $J_{9a'',8''}$ = 12.5 Hz, 1H; H-9a″), 4.15 (dd, $J_{2',1'}$ = 2.5, $J_{2',3'}$ = 5.2 Hz, 1H; H-2′), 4.09–4.00 (overlapping, 3H; H-4′, H-5″, H-9b″), 3.88–3.78 (overlapping, 5H; H-3′, H-7a′, $COOCH_3$), 3.42–3.36 (m, 1H; H-7b′), 2.70 (dd, $J_{3a'',4''}$ = 4.7, $J_{3a'',3b''}$ = 12.7 Hz, 1H; H-3a″), 2.18 (s, 3H; $NHCOCH_3$), 2.15 (s, 3H; CH_3COO), 2.11 (s, 3H; CH_3COO), 1.99 (s, 3H; CH_3COO), 1.96 (s, 3H; CH_3COO), 1.91–1.69 ppm (overlapping, 5H; H-5a′, H-5b′, H-6a′, H-6b′, H-3b); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.0 ($NHCOCH_3$ at C-4), 172.5, 171.9, 171.5, 171.4 (4×CH_3COO), 169.5 (C-1″), 164.4 (C-4), 159.6 (t, $J_{C,F}$ = 27 Hz, $COCF_2CF_2CF_3$), 158.1 (C-2), 146.1 (C-6), 124.0–110.0 ($COCF_2CF_2CF_3$), 100.1 (C-2″), 98.2 (C-5), 93.8 (C-1′), 84.7 (C-4′), 76.4 (C-2′), 75.0 (C-3′), 72.4 (C-6″), 70.2 (C-4″), 69.5 (C-8″), 68.6 (C-7″), 65.7 (C-7′), 63.5 (C-9′), 53.3 ($COOCH_3$), 50.7 (C-5″), 39.2 (C-3″), 30.7 (C-5′), 27.5 (C-6′), 24.6 ($NHCOCH_3$ at C-4), 21.3, 20.8, 20.7, 20.6 ppm (4×CH_3COO); MS (ESI positive): *m/z*: 963.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{35}H_{43}F_7N_4O_{18}$: C 44.69, H 4.61, N 5.96; found: C 44.66, H 4.73, N 6.03.

Deacetylation

General procedure: To a solution of the appropriate compound **16a**–**16c** or **18b** and **18c** (0.20 mmol) in dry methanol (5 mL), a methanolic solution of sodium methoxide (0.5 M, 1.5 mL) was added. After stirring for 1 h at RT, the solution was neutralized by addition of a weakly acid resin (Amberlite CG50), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (compounds **16b** and **16c** or **18b** and **18c**) or by preparative RP-HPLC chromatography to afford compounds **17a**–**17c** or **19b** and **19c**. In all cases, the crude residue obtained after the appropriate hydrolytic treatment was purified by preparative HPLC chromatography by using an Atlantis C-18-Preper T3 ODB (5 μm, 19×10 mm) HPLC column and starting from 100% of aqueous 0.1% (v/v) formic acid to 100% CH_3CN as eluent.

Compound 17a: Starting from compound **16a** (157 mg, 0.20 mmol), the crude product was purificated by preparative HPLC chromatography by using an Atlantis C-18-Preper T3 ODB (5 μm, 19×10 mm) HPLC column and starting from 100% of aqueous 0.1% (v/v) formic acid to 100% CH_3CN as eluent, to afford compound **17a** as white solid (104 mg, 90%). M.p. 118–120 °C; $[\alpha]_D^{25}$ = +15.1 (*c* = 1 in water); ^{1}H NMR (500 MHz, D_2O): δ = 7.71 (d, $J_{6',5}$ = 7.6 Hz, 1H; H-6), 6.12 (d, $J_{5',6}$ = 7.6 Hz, 1H; H-5), 5.88 (d, $J_{1',2'}$ = 3.9 Hz, 1H; H-1′), 4.37 (dd, $J_{2',1'}$ = 3.9, $J_{2',3'}$ = 4.9 Hz, 1H; H-2′), 4.14–4.00 (overlapping, 3H; H-4′, H-4″, H-3′), 3.96–3.92 (overlapping, 2H; H-5″, H-6″), 3.88–3.81 (overlapping, 5H; $COOCH_3$, H-8″, H-9a″), 3.80–3.69 (overlapping, 2H; H-7a′, H-9b″), 3.63 (d, $J_{7'',8''}$ = 9.3 Hz, 1H; H-7″), 3.44–3.38 (m, 1H; H-7b′), 2.47 (dd, $J_{3a'',4''}$ = 4.9, $J_{3a'',3b''}$ = 13.2 Hz, 1H; H-3a″), 2.09 (s, 3H; $NHCOCH_3$), 1.99–1.75 ppm (overlapping, 5H; H-5a′, H-5b′, H-6a′, H-6b′, H-3b″); ^{13}C NMR (125 MHz, D_2O): δ = 176.2 ($NHCOCH_3$), 171.9 (C-1″), 166.6 (C-4), 157.6 (C-2), 143.4 (C-6), 100.0 (C-2″), 97.5 (C-5), 92.0 (C-1′), 84.5 (C-4′), 75.0 (C-2′), 74.3 (C-3′), 71.9 (C-6″), 71.2 (C-8″), 69.3 (C-7″), 67.7 (C-4″), 64.7 (C-9″), 64.6 (C-7′), 54.8 ($COOCH_3$), 53.2 (C-5″), 40.7 (C-3″), 30.5 (C-5′), 26.3 (C-6′), 23.4 ppm ($NHCOCH_3$); MS (ESI positive): *m/z*: 599.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{23}H_{36}N_4O_{13}$: C 47.91, H 6.29, N 9.72; found: C 47.87, H 6.09, N 9.83.

Compound 17b: Starting from compound **16b** (168 mg, 0.20 mmol), purification of the crude product by flash chromatography (AcOEt/MeOH, 80:20, v/v) afforded compound **17b** (115 mg, 91%) as white solid. M.p. 120–121 °C; $[\alpha]_D^{20}$ = +18.1 (*c* = 1 in methanol); ^{1}H NMR (500 MHz, CD_3OD): δ = 7.60 (d, $J_{6',5}$ = 7.4 Hz, 1H; H-6), 5.93 (d, $J_{5',6}$ = 7.4 Hz, 1H; H-5), 5.77 (d, $J_{1',2'}$ = 3.2 Hz, 1H; H-1′), 4.20–4.09 (overlapping, 3H; H-2′, H-4″, H-6″), 4.00 (dd, $J_{5'',4''}$ = $J_{5'',6''}$ = 10.3 Hz, 1H; H-5″), 3.96–3.90 (m, 1H; H-4′), 3.86–3.75 (overlapping, 7H; H-3′, H-7a′, H-8″, H-9a″, $COOCH_3$), 3.67 (dd, $J_{9b'',8''}$ = 5.2, $J_{9a'',9b''}$ = 11.3 Hz, 1H; H-9b″), 3.46 (d, $J_{7'',8''}$ = 9.4 Hz, 1H; H-7″), 3.30–3.24 (m, 1H; H-7b′), 2.39 (dd, $J_{3a'',4''}$ = 4.9, $J_{3a'',3b''}$ = 12.9 Hz, 1H; H-3a″), 1.94–1.71 (overlapping, 4H; H-5a′, H-5b′, H-6a′, H-6b′), 1.66 ppm (dd, $J_{3b'',4''}$ = 11.5, $J_{3a'',3b''}$ = 12.9 Hz, 1H; H-3b″); ^{13}C NMR (125 MHz, CD_3OD): δ = 171.5 (C-1″), 167.7 (C-4), 159.7 (q, $J_{C,F}$ = 37 Hz, $COCF_3$), 158.5 (C-2), 142.8 (C-6), 117.6 (q, $J_{C,F}$ = 287 Hz, $COCF_3$), 100.1 (C-2″), 96.3 (C-5), 93.1 (C-1′), 84.3 (C-4′), 75.8 (C-2′), 75.0 (C-3′), 71.7 (C-8″), 71.4 (C-6″), 70.1 (C-7″), 67.3 (C-4″), 65.2 (C-9″), 64.4 (C-7′), 54.3 (C-5″), 53.4 ($COOCH_3$), 41.9 (C-3″), 31.1 (C-5′), 27.0 ppm (C-6′); MS (ESI positive): *m/z*: 653.0 $[M+Na]^+$; MS (ESI negative): *m/z*: 629.1 $[M-H]^-$; elemental analysis calcd (%) for $C_{23}H_{33}F_3N_4O_{13}$: C 43.81, H 5.28, N 9.04; found: C 43.62, H 5.10, N 9.30.

Compound 17c: Starting from compound **16c** (188 mg, 0.20 mmol), purification of the crude product by flash chromatography (AcOEt/MeOH, 80:20, v/v) afforded compound **17c** (130 mg, 89%) as a white solid. M.p. 130–132 °C; $[\alpha]_D^{20}$ = +5.0 (*c* = 1 in methanol); ^{1}H NMR (500 MHz, CD_3OD): δ = 7.60 (d, $J_{6',5}$ = 7.5 Hz, 1H; H-6), 5.93 (d, $J_{5',6}$ = 7.5 Hz, 1H; H-5), 5.77 (d, $J_{1',2'}$ = 3.4 Hz, 1H; H-1′), 4.20–4.02 (overlapping, 4H; H-2′, H-4″, H-5″, H-6″), 3.96–3.89 (m, 1H; H-4′), 3.86–3.74 (overlapping, 7H; H-3′, H-7a′, H-8″, H-9a″, $COOCH_3$), 3.63 (dd, $J_{9b'',8''}$ = 5.6, $J_{9a'',9b''}$ = 11.4 Hz, 1H; H-9b″), 3.44 (d, $J_{7'',8''}$ = 9.4 Hz, 1H; H-7″), 3.29–3.24 (m, 1H; H-7b′), 2.39 (dd, $J_{3a'',4''}$ = 4.8, $J_{3a'',3b''}$ = 12.9 Hz, 1H; H-3a″), 1.93–1.69 (overlapping, 4H; H-5a′, H-5b′, H-6a′, H-6b′), 1.66 ppm (dd, $J_{3b'',4''}$ = 11.2, $J_{3a'',3b''}$ = 12.9 Hz, 1H; H-3b″); ^{13}C NMR (125 MHz, CD_3OD): δ = 171.6 (C-1″), 167.7 (C-4), 159.9 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_3$), 158.4 (C-2), 142.8 (C-6), 120.9–105.0 ($COCF_2CF_2CF_3$), 100.1 (C-2″), 96.3 (C-5), 93.1 (C-1′), 84.2 (C-4′), 75.8 (C-2′), 75.0 (C-3′), 71.7 (C-8″), 71.4 (C-6″), 70.2 (C-7″), 67.3 (C-4″), 65.3 (C-9″), 64.4 (C-7′), 54.3 (C-5″), 53.5 ($COOCH_3$), 42.0 (C-3″), 31.0 (C-5′), 27.0 ppm (C-6′); MS (ESI positive): *m/z*: 753.1 $[M+Na]^+$; MS (ESI negative): *m/z*: 729.1 $[M-H]^-$; elemental analysis calcd (%) for $C_{25}H_{33}F_7N_4O_{13}$: C 41.10, H 4.55, N 7.67; found: C 41.30, H 4.40, N 7.82.

Compound 19b: Starting from compound **18b** (168 mg, 0.20 mmol), purification of the crude product by flash chromatography (AcOEt/MeOH, 80:20, v/v) afforded compound **19b** (106 mg, 84%) as a white powered. M.p. 132–134 °C; $[\alpha]_D^{20}$ = −16.2 (*c* = 1 in MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 7.61 (d, $J_{6',5}$ = 7.3 Hz, 1H; H-6), 5.95 (d, $J_{5',6}$ = 7.3 Hz, 1H; H-5), 5.79 (br d, $J_{1',2'}$ = 1.7 Hz, 1H; H-1′), 4.09 (br s, 1H; H-2′), 3.99–3.91 (overlapping, 2H; H-4′, H-5″), 3.90–3.80 (overlapping, 8H; H-3′, H-6″, H-7a′, H-8″, H-9a″, $COOCH_3$), 3.71 (ddd, $J_{4'',3a''}$ = 4.6, $J_{4'',5''}$ = 10.2, $J_{4'',3b''}$ = 12.4 Hz, 1H; H-4″), 3.64 (dd, $J_{9b'',8''}$ = 5.9, $J_{9a'',9b''}$ = 11.9 Hz, 1H; H-9b″), 3.50–3.42 (overlapping, 2H; H-7″, H-7b′), 2.68 (dd, $J_{3a'',4''}$ = 4.6, $J_{3a'',3b''}$ = 12.8 Hz, 1H; H-3a″), 1.91–1.63 ppm (overlapping, 5H; H-5a′, H-5b′, H-6a′, H-6b′, H-3b″); MS (ESI negative): *m/z*: 629.2 $[M-H]^-$; elemental analysis calcd (%) for $C_{23}H_{33}F_3N_4O_{13}$: C 43.81, H 5.28, N 8.89; found C 43.94, H 5.40, N 8.72.

Compound 19c: Starting from compound **18c** (188 mg, 0.20 mmol), purification of the crude product by flash chromatography (AcOEt/MeOH, 80:20, v/v) afforded compound **19c** (126 mg, 86%) as a white solid. M.p. 125–127 °C; $[\alpha]_D^{20}$ = −11.1 (*c* = 1 in methanol); ^{1}H NMR (500 MHz, CD_3OD): δ = 7.61 (d, $J_{6',5}$ = 7.3 Hz, 1H; H-6), 5.94 (br d, $J_{5',6}$ = 7.3 Hz, 1H; H-5), 5.78 (br s, 1H; H-1′), 4.09 (br s, 1H; H-2′), 4.03 (dd, $J_{5'',6''}$ = $J_{5'',4''}$ = 10.2 Hz, 1H; H-5″), 3.96–3.91 (m, 1H; H-4′), 3.90–3.79 (overlapping, 8H; H-3′, H-6″, H-7a′, H-8″, H-9a″, $COOCH_3$), 3.81–3.72 (ddd, $J_{4'',3a''}$ = 4.5, $J_{4'',5''}$ = 10.4, $J_{4'',3b''}$

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= 11.6 Hz, 1H; H-4″), 3.69 (dd, $J_{9b''r8''}$ = 6.3, $J_{9a''r9b''}$ = 11.6 Hz, 1H; H-9b″), 3.49–3.42 (overlapping, 2H; H-7b′, H-7″), 2.68 (dd, $J_{3a''r4''}$ = 4.5, $J_{3a''r3b''}$ = 12.7 Hz, 1H; H-3a″), 1.92–1.65 ppm (overlapping, 5H; H-5a′, H-5b′, H-6a, H-6b, H-3b″); ^{13}C NMR (125 MHz, CD_3OD): δ = 170.9 (C-1″), 167.7 (C-4), 160.0 (t, $J_{C,F}$ = 26 Hz, *C*OCF$_2$CF$_2$CF$_3$), 158.4 (C-2), 142.6 (C-6), 124.0–110.0 (CO*C*F$_2$*C*F$_2$*C*F$_3$), 100.3 (C-2″), 96.3 (C-5), 92.8 (C-1′), 84.1 (C-4′), 75.9 (C-2′), 75.0 (C-3′), 73.7 (C-6″ or C-8″), 72.8 (C-6″ or C-8″), 70.3 (C-7″), 68.3 (C-4″), 64.8 (C-7′, C-9″), 54.1 (C-5″), 53.3 (COO*C*H$_3$), 42.0 (C-3″), 30.7 (C-5′), 27.2 ppm (C-6′); MS (ESI positive): *m/z*: 753.6 [*M*+Na]$^+$; elemental analysis calcd (%) for $C_{25}H_{33}F_7N_4O_{13}$: C 41.10, H 4.55, N 7.67; found: C 41.19, H 4.61, N 7.59.

Regeneration of the carboxylic group

General procedure

For acetylamido derivate 17a: An acqueous solution of NaOH (1 M, 0.5 mL) was added to a solution of metyl ester **17a** (0.10 mmol) in MeOH (1.0 mL) and the solution was stirred for 40 min at RT. Then the reaction mixture was neutralized with weakly acid resin (Amberlite CG50), filtered, and the solvent was removed under reduced pressure.

For the trifluoroacetylamido derivatives 17b and 19b: Et_3N (0.90 mL) was added to a solution of the appropriate methyl ester (0.10 mmol) in MeOH/H_2O (1.5 mL, 2:1, v/v) and the solution was stirred for 12 h at RT. Then the solvent was removed under reduced pressure and the residue was recovered with water and lyophilized many times until complete elimination of Et_3N.

For the heptafluorobutirylamido derivatives 17c and 19c: A solution of the appropriate metyl ester (0.10 mmol) in MeOH/H_2O (1.0 mL; 2:1, v/v) saturated with K_2CO_3, was stirred for 12 h at RT. Then the reaction mixture was neutralized with weakly acid resin (Amberlite CG50), filtered, and the solvent was removed under reduced pressure.

In all cases, the crude residue obtained after the appropriate hydrolytic treatment was purified by preparative HPLC chromatography by using a Atlantis C-18-Preper T3 ODB (5 μm, 19×10 mm) HPLC column and starting from 100% of aqueous 0.1% (v/v) formic acid to 100% CH_3CN as eluent.

Compound 3a: Starting from ester **17a** (58 mg, 0.10 mmol), after hydrolysis and HPLC purification, compound **3a** (48 mg, 85%) was obtained as a white solid. $[\alpha]_D^{25}$ = +13.9 (*c* = 1 in water) (lit.[7] $[\alpha]_D$ = −26.6 (*c* = 0.1 in water)); ^{1}H NMR (500 MHz, D_2O): δ = 7.64 (d, $J_{6'r5}$ = 7.5 Hz, 1H; H-6), 6.05 (d, $J_{5'r6}$ = 7.5 Hz, 1H; H-5), 5.86 (d, $J_{1'r2'}$ = 3.9 Hz, 1H; H-1′), 4.31 (dd, $J_{2'r1'}$ = 3.9, $J_{2'r3'}$ = 5.4 Hz, 1H; H-2′), 4.12–4.04 (overlapping, 2H; H-4′, H-4″), 4.02 (dd, $J_{3'r2'}$ = 5.4 Hz, 1H; H-3′), 3.93–3.79 (overlapping, 4H; H-5″, H-6″, H-8″, H-9a″), 3.66 (dd, $J_{9b''r8''}$ = 5.3, $J_{9a''r9b''}$ = 11.7 Hz, 1H; H-9b″), 3.62–3.55 (m, 1H; H-7a′), 3.52 (d, $J_{7''r8''}$ = 9.4 Hz, 1H; H-7″), 3.37–3.30 (m, 1H; H-7b′), 2.37 (dd, $J_{3a''r4''}$ = 5.0, $J_{3a''r3b''}$ = 13.1 Hz, 1H; H-3a″), 2.04 (s, 3H; NHCOC*H*$_3$), 1.94–1.85 (m, 1H; H-5a′), 1.83–1.69 (overlapping, 3H; H-5b′, H-6a′, H-6b′), 1.63 ppm (dd, $J_{3b''r3a''}$ = 13.1 Hz, 1H; H-3b″); ^{13}C NMR (125 MHz, D_2O): δ = 176.3 (C-1″), 175.6 (NH*C*OCH$_3$), 167.0 (C-4), 158.4 (C-2), 142.4 (C-6), 100.7 (C-2″), 97.1 (C-5), 91.2 (C-1′), 84.1 (C-4′), 74.7 (C-2′), 73.8 (C-3′), 70.9 (C-6″), 70.8 (C-8″), 69.2 (C-7″), 67.9 (C-4″), 64.4 (C-9″), 63.3 (C-7′), 52.9 (C-5″), 40.8 (C-3″), 30.3 (C-5′), 25.9 (C-6′), 22.9 ppm (NHCO*C*H$_3$); MS (ESI negative): *m/z*: 561.3 [*M*−H]$^-$, 583.3 [*M*−2H+Na]$^-$; elemental analysis calcd (%) for $C_{22}H_{34}N_4O_{13}$: C 46.97, H 6.09, N 9.96; found: C 46.87, H 6.00, N 9.85.

Compound 3b: Starting from ester **17b** (63 mg, 0.10 mmol), after hydrolysis and HPLC purification, compound **3b** (51 mg, 83%) was obtained as a white solid. M.p. 129–131 °C; $[\alpha]_D^{20}$ = +18.9 (*c* = 1 in water); ^{1}H NMR (500 MHz, D_2O): δ = 7.86 (d, $J_{6'r5}$ = 7.9 Hz, 1H; H-6), 6.23 (d, $J_{5'r6}$ = 7.9 Hz, 1H; H-5), 5.83 (d, $J_{1'r2'}$ = 3.8 Hz, 1H; H-1′), 4.35 (dd, $J_{2'r1'}$ = 3.8, $J_{2'r3'}$ = 5.1 Hz, 1H; H-2′), 4.23 (ddd, $J_{4''r3a''}$ = 4.9, $J_{4''r5''}$ = 10.4, $J_{4''r3b''}$ = 11.7 Hz, 1H; H-4″), 4.13–3.97 (overlapping, 4H; H-3′, H-4′, H-5″, H-6″), 3.89–3.82 (overlapping, 2H; H-8″, H-9a″), 3.69–3.62 (overlapping, 2H; H-7a′, H-9b″), 3.53 (d app, $J_{7''r8''}$ = 9.7 Hz, 1H; H-7″), 3.42–3.36 (m, 1H; H-7b′), 2.43 (dd, $J_{3a''r4''}$ = 4.9, $J_{3a''r3b''}$ = 13.2 Hz, 1H; H-3a″), 1.96–1.87 (m, 1H; H-5a′), 1.84–1.70 ppm (overlapping, 4H; H-5b′, H-6a′, H-6b′, H-3b″); ^{13}C NMR (125 MHz, D_2O): δ = 174.4 (C-1″), 160.6–158.8 (q, $J_{C,F}$ = 37 Hz, *C*OCF$_3$, C-4), 149.0 (C-2), 144.9 (C-6), 116.4 (q, $J_{C,F}$ = 286 Hz, COC*F*$_3$), 100.0 (C-2″), 95.8 (C-5), 91.4 (C-1′), 84.6 (C-4′), 74.4 (C-2′), 73.5 (C-3′), 70.7 (C-8″), 70.2 (C-6″), 68.8 (C-7″), 66.6 (C-4″), 64.1 (C-9″), 63.6 (C-7′), 53.5 (C-5″), 40.5 (C-3″), 30.0 (C-5′), 25.8 ppm (C-6′); MS (ESI negative): *m/z*: 615.2 [*M*−H]$^-$; elemental analysis calcd (%) for $C_{22}H_{31}F_3N_4O_{13}$: C 42.86, H 5.07, N 9.09; found: C 43.01, H 5.0, N 9.12.

Compound 3c: Starting from ester **17c** (73 mg, 0.10 mmol), after hydrolysis and HPLC purification, compound **3c** (62 mg, 87%) was obtained as a white solid. M.p. 125–126 °C; $[\alpha]_D^{20}$ = + 22.1 (*c* = 1 in water); ^{1}H NMR (500 MHz, D_2O): δ = 7.64 (d, $J_{6'r5}$ = 7.6 Hz, 1H; H-6), 6.05 (d, $J_{5'r6}$ = 7.6 Hz, 1H; H-5), 5.86 (d, $J_{1'r2'}$ = 4.0 Hz, 1H; H-1′), 4.30 (dd, $J_{2'r1'}$ = 4.0, $J_{2'r3'}$ = 4.6 Hz, 1H; H-2′), 4.20 (ddd, $J_{4''r3a''}$ = 4.9, $J_{4''r5''}$ = 10.2, $J_{4''r3b''}$ = 11.2 Hz, 1H; H-4″), 4.09–4.00 (overlapping, 4H; H-3′, H-4′, H-5″, H-6″), 3.91–3.82 (overlapping, 2H; H-8″, H-9a″), 3.66–3.58 (overlapping, 2H; H-7a, H-9b″), 3.47 (d, $J_{7''r8''}$ = 9.4 Hz, 1H; H-7″), 3.37–3.31 (m, 1H; H-7b′), 2.40 (dd, $J_{3a''r4''}$ = 4.9, $J_{3a''r3b''}$ = 13.2 Hz, 1H; H-3a″), 1.95–1.69 (overlapping, 4H; H-5a′, H-5b′, H-6a′, H-6b′), 1.66 ppm (dd, $J_{3b''r4''}$ = 11.2, $J_{3a''r3b''}$ = 13.2 Hz, 1H; H-3b″); ^{13}C NMR (125 MHz, D_2O): δ = 176.1 (C-1″), 166.9 (C-4), 160.4 (t, $J_{C,F}$ = 27 Hz, *C*OCF$_2$CF$_2$CF$_3$), 158.3 (C-2), 142.2 (C-6), 120.9–107.0 (COCF$_2$CF$_2$CF$_3$), 100.6 (C-2″), 97.1 (C-5), 91.0 (C-1′), 84.0 (C-4′), 74.6 (C-2′), 73.6 (C-3′), 70.8 (C-8″), 70.0 (C-6″), 69.1 (C-7″), 67.0 (C-4″), 64.3 (C-9″), 63.2 (C-7′), 53.8 (C-5″), 41.0 (C-3″), 30.1 (C-5′), 25.9 ppm (C-6′); MS (ESI negative): *m/z:* 715.2 [*M*−H]$^-$, 737.4 [*M*−2H+Na]$^-$; elemental analysis calcd (%) for $C_{24}H_{31}F_7N_4O_{13}$: C 40.23, H 4.36, N 7.82; found: C 40.60, H 4.13, N 7.30.

Compound 20b: Starting from ester **19b** (63 mg, 0.10 mmol), after hydrolysis and HPLC purification, compound **20b** (53 mg, 86%) was obtained as a white solid. M.p. 125–128 °C; $[\alpha]_D^{25}$ = −20.5 (*c* = 1 in water); ^{1}H NMR (500 MHz, D_2O): δ = 7.56 (d, $J_{6'r5}$ = 7.4 Hz, 1H; H-6), 5.94 (d, $J_{5'r6}$ = 7.4 Hz, 1H; H-5), 5.78 (d, $J_{1'r2'}$ = 3.7 Hz, 1H; H-1′), 4.22–4.16 (m, 1H; H-2′), 4.02–3.89 (overlapping, 3H; H-3′, H-4′, H-5″), 3.86–3.70 (overlapping, 4H; H-6″, H-7a′, H-8″, H-9a″), 3.70–3.61 (m, 1H; H-4″), 3.53 (dd, $J_{9b''r8''}$ = 6.2, $J_{9a''r9b''}$ = 11.5 Hz, 1H; H-9b″), 3.49–3.39 (overlapping, 2H; H-7b′, H-7″), 2.68 (dd, $J_{3a''r4''}$ = 4.6, $J_{3a''r3b''}$ = 12.6 Hz, 1H; H-3a″), 1.80–1.54 ppm (overlapping, 5H; H-5a′, H-5b′, H-6a′, H-6b′, H-3b″); ^{13}C NMR (125 MHz, D_2O): δ = 173.6 (C-1″), 166.3 (C-4), 159.8 (q, $J_{C,F}$ = 37 Hz, *C*OCF$_3$), 157.8 (C-2), 141.5 (C-6), 120.0–110.0 (COCF$_3$), 100.8 (C-2″), 96.5 (C-5), 90.2 (C-1′), 83.4 (C-4′), 74.1 (C-2′), 73.0 (C-3′), 72.1 (C-6″ or C-8″), 71.8 (C-6″ or C-8″), 68.4 (C-7″), 68.0 (C-4″), 64.5 (C-7′), 62.7 (C-9″), 52.9 (C-5″), 40.7 (C-3″), 29.3 (C-5′), 25.5 ppm (C-6′); MS (ESI negative): *m/z*: 615.1 [*M*−H]$^-$; elemental analysis calcd (%) for $C_{23}H_{31}F_3N_4O_{13}$: C 42.86, H 5.07, N 9.09; found: C 42.79, H 5.06, N 9.06

Compound 20c: Starting from ester **19c** (73 mg, 0.10 mmol), after hydrolysis and HPLC purification, compound **20c** (60 mg, 84%) was obtained as a white solid. $[\alpha]_D^{25}$ = −11.3 (*c* = 1 in water); ^{1}H NMR (500 MHz, D_2O, MeOH as reference standard): δ = 7.63 (d, $J_{6'r5}$ = 7.5 Hz, 1H; H-6), 6.05 (brd, $J_{5'r6}$ = 7.5 Hz, 1H; H-5), 5.86 (d, $J_{1'r2'}$ = 3.9 Hz, 1H; H-1′), 4.31–4.24 (m, 1H; H-2′), 4.09–3.96 (overlapping, 3H; H-3′, H-4′, H-5″), 3.92–3.78 (overlapping, 4H; H-6″, H-7a′, H-8″, H-9a″), 3.78–3.69 (m, 1H; H-4″), 3.61 (dd, $J_{9b''r8''}$ = 6.3, $J_{9a''r9b''}$ = 11.5 Hz, 1H; H-9b″), 3.54–3.48 (overlapping, 2H; H-7b′, H-7″),

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2.76 (dd, $J_{3a''r4''}=4.8$, $J_{3a''r3b''}=12.5$ Hz, 1H; H-3a''), 1.88–1.65 ppm (overlapping, 5H; H-5a', H-5b', H-6a', H-6b', H-3b''); ^{13}C NMR (125 MHz, D_2O, MeOH as reference standard): $\delta=173.7$ (C-1''), 166.3 (C-4), 159.8 (t, $J_{C,F}=26$ Hz, $COCF_2CF_2CF_3$), 157.7 (C-2), 141.5 (C-6), 120.0–110.0 ($COCF_2CF_2CF_3$), 100.8 (C-2''), 96.5 (C-5), 90.2 (C-1'), 83.4 (C-4'), 74.0 (C-2'), 73.0 (C-3'), 72.1 (C-6'' or C-8''), 71.9 (C-6'' or C-8''), 68.4 (C-7''), 68.1 (C-4''), 64.5 (C-7'), 62.6 (C-9''), 53.0 (C-5''), 40.6 (C-3''), 29.3 (C-5'), 25.5 ppm (C-6'); MS (ESI negative): *m/z*: 715.3 $[M-H]^-$; elemental analysis calcd (%) for $C_{24}H_{31}F_7N_4O_{13}$: C 40.23, H 4.36, N 7.82; found: C 40.39, H 4.24, N 7.85.

Synthesis of compound 21: Compound **11a** (165 mg, 0.2 mmol) was treated according to the general procedures for deacetylation and for regeneration of carboxylic group, to afford compound **21** (95 mg, 79%) as a white solid. $[\alpha]_D^{20}=+39.1$ ($c=1$ in water); ^{1}H NMR (500 MHz, D_2O): $\delta=7.56$ (d, $J_{6'5}=7.5$ Hz, 1H; H-6), 5.95 (d, $J_{5'6}=7.5$ Hz, 1H; H-5), 5.74 (d, $J_{1''2'}=2.5$ Hz, 1H; H-1'), 5.01 (dd, $J_{2'1''}=2.5$, $J_{2'3'}=6.5$ Hz, 1H; H-2'), 4.73–4.69 (overlapping with H_2O, 1H; H-3'), 4.16–4.12 (m, 1H; H-4'), 4.00 (ddd, $J_{4''3a''}=4.9$, $J_{4''5''}=10.4$, $J_{4''3b''}=10.8$ Hz, 1H; H-4''), 3.84–3.70 (overlapping, 4H; H-5'', H-6'', H-8'', H-9a''), 3.66 (dd, $J_{9b''8''}=5.6$, $J_{9a''9b''}=12.1$ Hz, 1H; H-9b''), 3.42–3.51 (overlapping, 2H; H-7a', H-7''), 3.29–3.22 (m, 1H; H-7b'), 2.29 (dd, $J_{3a''4''}=4.9$, $J_{3a''3b''}=13.1$ Hz, 1H; H-3a''), 1.97 (s, 3H; $NHCOCH_3$), 1.77–1.70 (overlapping, 2H; H-5a', H-5b'), 1.66–1.51 ppm (overlapping, 6H; $C(CH_3)_2$, H-6a', H-6b'', H-3b''), 1.32 (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, D_2O): $\delta=176.1$ (C-1''), 175.4 ($NHCOCH_3$), 167.1 (C-4), 157.8 (C-2), 143.7 (C-6), 115.6 ($C(CH_3)_2$), 100.5 (C-1''), 96.7 (C-5), 93.5 (C-1'), 86.9 (C-4'), 85.0 (C-2'), 83.7 (C-3'), 70.8 (C-6''), 70.7 (C-8''), 69.0 (C-7''), 67.8 (C-4''), 64.2 (C-9''), 63.0 (C-7'), 52.8 (C-5''), 40.7 (C-3''), 30.2 (C-5'), 26.6 (C-6'), 25.7 ($C(CH_3)_2$), 25.1 ($C(CH_3)_2$), 22.8 ppm ($NHCOCH_3$); MS (ESI negative): *m/z*: 601.4 $[M-H]^-$; elemental analysis calcd (%) for $C_{25}H_{38}N_4O_{13}$: C 49.83, H 6.36, N 9.30; found: C 49.89, H 6.14, N 9.10.

1,7-Lactonization

General procedure: The appropriate starting material **3b**, **3c**, or **21** (0.10 mmol), dissolved in DMF (1.0 mL) under stirring, was cooled to 0 °C and diluted with THF (1.5 mL). Then CbzCl (0.03 mL; 0.25 mmol, 2.5 equiv) in THF (0.4 mL) was added in a single portion, followed by Et_3N (0.04 mL; 0.3 mmol) in a single addition. The mixture was then stirred for 30 min at 23 °C. At this time, methanol (0.3 mL) was added and the stirring was continued for 15 min. Evaporation of the solvent under reduced pressure (22 mmHg and then at 10^{-1} mmHg) afforded a crude residue, which, after purification by flash chromatography give the appropriate pure lactone **4b**, **4c**, or **22**.

Compound 4a: Starting from **21** (60 mg, 0.10 mmol), purification by flash chromatography (AcOEt/MeOH, 80:20, v/v) afforded compound **22** (41 mg, 70%) as a white solid. $[\alpha]_D^{20}=+23.1$ ($c=1$ in methanol); ^{1}H NMR (500 MHz, CD_3OD): $\delta=7.61$ (d, $J_{6'5}=7.5$ Hz, 1H; H-6), 5.91 (d, $J_{5'6}=7.5$ Hz, 1H; H-5), 5.77 (d, $J_{1''2'}=2.3$ Hz, 1H; H-1'), 4.95 (dd, $J_{2'1''}=2.3$, $J_{2'3'}=6.5$ Hz, 1H; H-2'), 4.63 (dd, $J_{3'4'}=4.8$, $J_{3'2'}=6.5$ Hz, 1H; H-3'), 4.58 (brs, 1H; H-6''), 4.46 (dd, $J_{7''6''}<1.0$, $J_{7'8'}=8.0$ Hz, 1H; H-7''), 4.07–4.04 (m, 1H; H-4''), 4.04–3.99 (m, 1H, H-4'), 3.98–3.95 (m, 1H, H-5''), 3.82–3.69 (overlapping, 4H; H-7a', H-8'', H-9a'', H-9b''), 3.48–3.42 (m, 1H; H-7b'), 2.12 (dd, $J_{3a''4''}=3.3$, $J_{3a''3b''}=14.1$ Hz, 1H; H-3a''), 2.06 (dd, $J_{3b''4''}=2.1$, $J_{3b''3a''}=14.1$ Hz, 1H; H-3b''), 2.02 (s, 3H; $NHCOCH_3$), 1.84–1.78 (overlapping, 2H; H-5a', H-5b'), 1.74–1.66 (overlapping, 2H; H-6a', H-6b'), 1.55 (s, 3H; $C(CH_3)_2$), 1.35 ppm (s, 3H; $C(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD): $\delta=$ 173.0 ($NHCOCH_3$), 170.3 (C-1''), 168.0 (C-4), 158.0 (C-2), 144.5 (C-6), 115.5 ($C(CH_3)_2$), 96.3 (C-5), 96.1 (C-2''), 94.7 (C-1'), 87.6 (C-4'), 86.2 (C-2'), 85.3 (C-3'), 79.8 (C-7''), 73.2 (C-8''), 72.1 (C-6''), 67.7 (C-4''), 64.7 (C-7'), 63.6 (C-9''), 52.8 (C-5''), 38.0 (C-3''), 31.0 (C-5'), 27.7 (C-6'), 27.0 ($C(CH_3)_2$), 25.7 ($C(CH_3)_2$), 22.5 ppm ($NHCOCH_3$); MS (ESI negative): *m/z:* 583.1 $[M-H]^-$; elemental analysis calcd (%) for $C_{25}H_{36}N_4O_{12}$: C 51.37, H 6.21, N 9.58; found: C 51.28, H 6.29, N 9.61.

A solution of compound **22** (50 mg, 0.08 mmol) in dichloromethane (8 mL) was added to CF_3COOH (20 μL) containing 10% of H_2O. The reaction mixture was heated to reflux and stirred for one hour. Then the solution was neutralized with a weak basic resin (IRA 67), filtered, and evaporated under reduced pressure The crude residue was purified by preparative HPLC chromatography by using an Atlantis C-18-Preper T3 ODB (5 μm, 19×10 mm) HPLC column and starting from 100% of aqueous 0.1% (v/v) formic acid to 100% CH_3CN as eluent to afford, after lyophilization, first the pure compound **4a** (30 mg, 65%) and then compound **3a** (5 mg, 10%).

Compound 4a: $[\alpha]_D^{20}=+31.0$ ($c=1$ in methanol); ^{1}H NMR (500 MHz, CD_3OD): $\delta=7.60$ (d, $J_{6'5}=7.5$ Hz, 1H; H-6), 5.91 (d, $J_{5'6}=7.5$ Hz, 1H; H-5), 5.77 (d, $J_{1''2'}=3.3$ Hz, 1H; H-1'), 4.56 (brs, 1H; H-6''), 4.44 (d, $J_{7''8''}=7.8$, 1H; H-7''), 4.11–4.08 (m, 1H; H-2'), 4.06–4.02 (m, 1H; H-4''), 3.96–3.97–3.89 (overlapping, 2H; H-4', H-5''), 3.84–3.68 (overlapping, 5H; H-3', H-7a', H-8'', H-9a'', H-9b''), 3.50–3.43 (m, 1H; H-7b'), 2.10 (dd, $J_{3a''4''}=3.4$, $J_{3a''3b''}=14.1$ Hz, 1H; H-3a''), 2.05 (dd, $J_{3b''4''}=2.1$, $J_{3b''3a''}=14.2$ Hz, 1H; H-3b''), 2.00 (s, 3H; $NHCOCH_3$), 1.92–1.66 ppm (overlapping, 4H; H-5a', H-5b', H-6a', H-6b'); MS (ESI positive): *m/z*: 567.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{22}H_{32}N_4O_{12}$: C 48.53, H 5.92, N 10.29; found: C 48.69, H 5.76, N 10.01.

All other physic-chemical properties of compound **3a** are superimposable to those previously reported for the same compound.

Compound 4b: Starting from compound **3b** (61 mg, 0.10 mmol), purification by flash chromatography (AcOEt/MeOH, 85:15, v/v) afforded compound **4b** (42 mg, 70%) as a white solid. M.p. 123–124 °C; $[\alpha]_D^{20}=+19.1$ ($c=1$ in methanol); ^{1}H NMR (500 MHz, CD_3OD): $\delta=7.62$ (d, $J_{6'5}=7.5$ Hz, 1H; H-6), 5.96 (d, $J_{5'6}=7.5$ Hz, 1H; H-5), 5.80 (d, $J_{1''2'}=3.5$ Hz, 1H; H-1'), 4.67 (brs, 1H; H-6''), 4.47 (d, $J_{7''8''}=7.9$ Hz, 1H; H-7''), 4.14–4.08 (overlapping, 2H; H-2', H-4''), 4.00 (brs, 1H; H-5''), 3.95–3.90 (m, 1H; H-4'), 3.86–3.75 (overlapping, 4H; H-3', H-7a', H-8'', H-9a''), 3.75–3.69 (m, 1H; H-9b''), 3.49–3.42 (m, 1H; H-7b'), 2.16 (dd, $J_{3a''4''}=3.4$, $J_{3a''3b''}=14.2$ Hz, 1H; H-3a''), 2.07 (dd, $J_{3b''4''}=1.5$, $J_{3b''3a''}=14.2$ Hz, 1H; H-3b''), 1.89–1.61 ppm (overlapping, 4H; H-5a', H-5b', H-6a', H-6b');^{13}C NMR (125 MHz, CD_3OD): $\delta=170.1$ (C-1''), 167.6 (C-4), 159.0 (q, $J_{C,F}=$ 38 Hz, $COCF_3$), 158.6 (C-2), 142.5 (C-6), 117.3 (q, $J_{C,F}=287$ Hz, $COCF_3$), 96.5 (C-5), 96.1 (C-2''), 92.5 (C-1'), 84.4 (C-4'), 79.7 (C-7''), 76.0 (C-2'), 74.9 (C-3'), 73.0 (C-8''), 71.3 (C-6''), 67.0 (C-4''), 65.0 (C-7'), 63.5 (C-9''), 53.8 (C-5''), 38.0 (C-3''), 30.9 (C-5'), 27.2 ppm (C-6'); MS (ESI positive): *m/z*: 621.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{22}H_{29}F_3N_4O_{12}$: C 44.15, H 4.88, N 9.36; found: C 44.71, H 4.65, N 9.27.

Compound 4c: Starting from compound **3c** (72 mg, 0.10 mmol), purification by flash chromatography (AcOEt/MeOH, 85:15, v/v) afforded compound **4c** (50 mg, 71%) as a white solid. $[\alpha]_D^{20}=-42.9$ ($c=1$ in methanol); ^{1}H NMR (500 MHz, CD_3OD): $\delta=7.62$ (d, $J_{6'5}=7.5$ Hz, 1H; H-6), 5.96 (d, $J_{5'6}=7.5$ Hz, 1H; H-5), 5.81 (d, $J_{1''2'}=$ 3.4 Hz, 1H; H-1'), 4.68 (brs, 1H; H-6''), 4.47 (d, $J_{7''8''}=7.8$ Hz, 1H; H-7''), 4.13–4.07 (overlapping, 2H; H-2', H-4''), 4.04 (brs, 1H; H-5''), 3.96–3.88 (m, 1H; H-4'), 3.87–3.76 (overlapping, 4H; H-3', H-7a', H-8'', H-9a''), 3.75–3.70 (m, 1H; H-9b''), 3.48–3.43 (m, 1H; H-7b'), 2.15 (dd, $J_{3a''4''}=3.4$, $J_{3a''3b''}=14.2$ Hz, 1H; H-3a''), 2.07 (dd, $J_{3b''4''}=2.0$, $J_{3b''3a''}=14.2$ Hz, 1H; H-3b''), 1.89–1.68 ppm (overlapping, 4H; H-5a', H-5b', H-6a', H-6b'); ^{13}C NMR (125 MHz, CD_3OD): $\delta=170.0$ (C-1''), 167.6 (C-4), 159.4 (t, $J_{C,F}=27$ Hz, $COCF_2CF_2CF_3$), 158.5 (C-2), 142.6 (C-6), 120.1–109.3 ($COCF_2CF_2CF_3$), 96.4 (C-5), 96.1 (C-2''), 92.6

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(C-1′), 84.3 (C-4′), 79.7 (C-7′′), 76.0 (C-2′), 74.9 (C-3′), 73.0 (C-8′′), 71.3 (C-6′′), 66.9 (C-4′′), 65.0 (C-7′), 63.5 (C-9′′), 54.1 (C-5′′), 38.0 (C-3′′), 30.9 (C-5′), 27.2 ppm (C-6′); MS (ESI positive): *m/z*: 721.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{24}H_{29}F_7N_4O_{12}$: C 41.27, H 4.18, N 8.02; found: C 41.11, H 4.31, N 8.13.

Acetylation affording the derivatives 23a–23c and 24c

General procedure: Acetic anhydride (0.5 mL) was added to a solution of the appropriate compound **16a–16c** or **18c** (0.20 mmol) in pyridine (1.0 mL) and the resulting solution was stirred for 3 h at RT. Then, MeOH (0.4 mL) was added and the solution was concentrated under reduced pressure. The residue was recovered with aqueous HCl (1 M) and extracted with ethyl acetate. The organic layers were washed with aqueous $NaHCO_3$ and concentrated under reduced pressure to give a crude residue, which was purified by flash chromatography.

Compound 23a: Starting from compound **16a** (157 mg, 0.20 mmol), purification by flash chromatography (AcOEt/MeOH, 99:1, v/v) afforded compound **23a** (159 mg, 91%) as a white solid. $[\alpha]_D^{20} = +32.1$ ($c = 1$ CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 9.16$ (s, 1H; NH at C-4), 7.63 (d, $J_{6',5} = 7.5$ Hz, 1H; H-6), 7.50 (d, $J_{NH,5''} = 9.9$ Hz, 1H; NH at C-5′′), 7.41 (d, $J_{5,6} = 7.5$ Hz, 1H; 5-H), 6.05 (dd, $J_{2',1'} = 4.6$, $J_{2',3'} = 5.7$ Hz, 1H; H-2′), 5.70 (dd, $J_{7'',8''} = J_{7'',6''} = 2.4$ Hz, 1H; H-7′′), 5.58 (d, $J_{1',2'} = 4.6$ Hz, 1H; H-1′), 5.36 (dd, $J_{4'',3''} = 4.6$, $J_{4'',5''} = 5.7$ Hz, 1H; H-3′), 5.23 (ddd, $J_{4'',3a''} = 5.0$, $J_{4'',5''} = 10.5$, $J_{4'',3b''} = 11.1$ Hz, 1H; H-4′′), 5.14 (ddd, $J_{8'',7''} = J_{8'',9a''} = 2.4$, $J_{8'',9b''} = 9.5$ Hz, 1H; H-8′′), 4.89 (dd, $J_{9a'',8''} = 2.3$, $J_{9a'',9b''} = 12.4$ Hz, 1H; H-9a′′), 4.38 (dd, $J_{6'',7''} = 2.4$, $J_{6'',5''} = 10.5$ Hz, 1H; H-6′′), 4.22–4.14 (overlapping, 2H; H-4′, H-5′′), 4.06 (dd, $J_{9b'',9a''} = 12.4$, $J_{9b'',8''} = 9.5$ Hz, 1H; H-9b′′), 3.76 (s, 3H; $COOCH_3$), 3.66–3.61 (overlapping, 2H; H-7a′, H-7b′), 2.52 (dd, $J_{3a'',3b''} = 12.7$, $J_{3'',4''} = 5.0$ Hz, 1H; H-3a′′), 2.22 (s, 3H; $NHCOCH_3$ at C-4), 2.18 (s, 3H; $OCOCH_3$), 2.13 (s, 3H; $OCOCH_3$), 2.09 (s, 3H; $OCOCH_3$), 2.01 (s, 3H; $OCOCH_3$), 1.96 (s, 3H; $OCOCH_3$), 189–1.85 (overlapping, 8H; $OCOCH_3$, H-5a′, H-5b′, $NHCOCH_3$ at C-5′′), 1.82–1.51 ppm (overlapping, 3H; H-3b′′, H-6a′, H-6b′); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.3$, 170.5, 170.4, 170.2, 169.5 ($NHCOCH_3$ at C-4, $NHCOCH_3$ at C-5′′, CH_3COO at C-9′′, CH_3COO at C-4′′, CH_3COO at C-8′′, CH_3COO at C-7′′, CH_3COO at C-2′, CH_3COO at C-3′), 167.2 (C-1′′), 162.6 (C-4), 155.3 (C-2), 147.7 (C-6), 98.9 (C-2′′), 96.9 (C-5), 95.3 (C-1′), 83.8 (C-4′), 74.6 (C-3′), 72.7 (C-2′), 72.4 (C-8′′), 71.5 (C-6′′), 70.6 (C-7′′), 70.0 (C-4′′), 63.6 (C-7′), 63.0 (C-9′), 52.6 ($COOCH_3$), 48.4 (C-5′′), 37.4 (C-3′′), 29.9 (C-5′), 26.1 (C-6′), 24.7 ($NHCOCH_3$ at C-4), 23.2 ($NHCOCH_3$ at C-5′′), 21.1, 20.7, 20.5 ppm ($6\times CH_3COO$); MS (ESI positive): *m/z*: 893.5 $[M+Na]^+$; elemental analysis calcd (%) for $C_{37}H_{50}F_7N_4O_{20}$: C 51.03, H 5.79, N 6.43; found C 51.23, H 5.56, N.6.32.

Compound 23b: Starting from compound **16b** (168 mg, 0.20 mmol), purification by flash chromatography (AcOEt/MeOH, 90:10, v/v) afforded compound **23b** (165 mg, 89%) as a white solid. M.p. 130–131 °C; $[\alpha]_D^{20} = +21.3$ ($c = 1$ in CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 9.00$ (s, 1H; NH at C-4), 8.94 (d, $J_{NH,5''} = 9.8$ Hz, 1H; NH at C-5′′), 7.60 (d, $J_{6',5} = 7.5$ Hz, 1H; H-6), 7.41 (d, $J_{5,6} = 7.5$ Hz, 1H; H-5), 6.15 (dd, $J_{2',1'} = J_{2',3'} = 5.5$ Hz, 1H; H-2′), 5.74 (dd, $J_{7'',8''} = J_{7'',6''} = 2.3$ Hz, 1H; H-7′′), 5.58 (d, $J_{1',2'} = 5.5$ Hz, 1H; H-1′), 5.38–5.30 (overlapping, 2H; H-3′, H-4′′), 5.15 (ddd, $J_{8'',7''} = J_{8'',9a''} = 2.3$, $J_{8'',9b''} = 9.1$ Hz, 1H; H-8′′), 4.89 (dd, $J_{9a'',8''} = 2.3$, $J_{9a'',9b''} = 12.2$ Hz, 1H; H-9a′′), 4.62 (dd, $J_{6'',7''} = 2.3$, $J_{6'',5''} = 10.5$ Hz, 1H; H-6′′), 4.22 (ddd, $J_{5'',NH} = 9.8$, $J_{5'',4''} = J_{5'',6''} = 10.5$, 1H; H-5′′), 4.19–4.14 (m, 1H; H-4′), 4.06 (dd, $J_{9b'',9a''} = 12.2$, $J_{9b'',8''} = 9.1$ Hz, 1H; H-9b′′), 3.78 (s, 3H; $COOCH_3$), 3.70–3.64 (m, 1H; H-7a′), 3.57–3.51 (m, 1H; H-7b′), 2.59 (dd, $J_{3a'',3b''} = 12.8$, $J_{3'',4''} = 5.0$ Hz, 1H; H-3a′′), 2.30–2.22 (m, 1H; H-5a′), 2.20 (s, 3H; $NHCOCH_3$ at C-4), 2.17 (s, 3H; $OCOCH_3$), 2.11 (s, 3H; $OCOCH_3$), 2.07 (s, 3H; $OCOCH_3$), 1.98 (s, 3H; $OCOCH_3$), 1.96 (s, 3H; $OCOCH_3$), 1.92–1.81 (overlapping, 5H; H-5b′, H-6a′, $OCOCH_3$), 1.79 (dd, $J_{3a'',3b''} = 12.8$, $J_{3'',4''} = 11.3$ Hz, 1H; H-3a′′), 1.54–1.46 ppm (m, 1H; H-6b′); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.9$, 170.5, 170.2, 170.2, 169.4 ($NHCOCH_3$ at C-4, CH_3COO at C-9′′, CH_3COO at C-4′′, CH_3COO at C-8′′, CH_3COO at C-7′′, CH_3COO at C-2′, CH_3COO at C-3′), 167.5 (C-1′′), 162.4 (C-4), 157.8 (q, $J_{C,F} = 37$ Hz, $COCF_3$), 155.7 (C-2), 147.8 (C-6), 116.0 (q, $J_{C,F} = 287$ Hz, $COCF_3$), 98.9 (C-2′′), 97.1 (C-5), 95.2 (C-1′), 84.0 (C-4′), 74.7 (C-3′), 72.1 (C-2′), 72.0 (C-8′′), 70.6 (2C, C-6′′, C-7′′), 69.5 (C-4′′), 63.9 (C-7′), 62.8 (C-9′), 52.6 ($COOCH_3$), 49.4 (C-5′′), 37.5 (C-3′′), 29.7 (C-5′), 26.0 (C-6′), 24.6 ($NHCOCH_3$ at C-4), 20.9, 20.7, 20.6, 20.4 ppm ($6\times CH_3COO$); MS (ESI positive): *m/z*: 947.6 $[M+Na]^+$; elemental analysis calcd (%) for $C_{37}H_{47}F_3N_4O_{20}$: C 48.05, H 5.12, N 6.06; found C 48.18, H 5.40, N 6.38.

Compound 23c: Starting from compound **16c** (188 mg, 0.20 mmol), purification by flash chromatography (AcOEt/MeOH, 90:10, v/v) afforded compound **23c** (182 mg 89%) as a white solid. $[\alpha]_D^{20} = +21.3$ ($c = 1$ in CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 9.09$ (s, 1H; NH at C-4), 8.82 (d, $J_{NH,5''} = 9.8$ Hz, 1H; NH at C-5′′), 7.65 (d, $J_{6',5} = 7.5$ Hz, 1H; H-6), 7.41 (d, $J_{5,6} = 7.5$ Hz, 1H; 5-H), 5.97 (dd, $J_{2',1'} = J_{2',3'} = 5.2$ Hz, 1H; H-2′), 5.71–5.67 (overlapping, 2H; H-7′′, H-1′), 5.35–5.27 (overlapping, 2H; H-3′, H-4′′), 5.20 (ddd, $J_{8'',7''} = J_{8'',9a''} = 2.4$, $J_{8'',9b''} = 9.0$ Hz, 1H; H-8′′), 4.88 (dd, $J_{9a'',8''} = 2.4$, $J_{9a'',9b''} = 12.1$ Hz, 1H; H-9a′′), 4.38 (dd, $J_{6'',7''} = 2.8$, $J_{6'',5''} = 10.5$ Hz, 1H; H-6′′), 4.30 (ddd, $J_{5'',NH} = 9.8$, $J_{5'',4''} = J_{5'',6''} = 10.5$, 1H; H-5′′), 4.15–4.12 (m, 1H; H-4′), 4.07 (dd, $J_{9b'',9a''} = 12.1$, $J_{9b'',8''} = 9.0$ Hz, 1H; H-9b′′), 3.78 (s, 3H; $COOCH_3$), 3.70–3.63 (m, 1H; H-7a′), 3.56–3.49 (m, 1H; H-7b′), 2.60 (dd, $J_{3a'',3b''} = 12.8$, $J_{3'',4''} = 5.0$ Hz, 1H; H-3a′′), 2.21 (s, 3H; $NHCOCH_3$ at C-4), 2.14–2.10 (overlapping, 4H; $OCOCH_3$, H-5a′), 2.10 (s, 3H; $OCOCH_3$), 2.08 (s, 3H; $OCOCH_3$), 1.97 (s, 3H; $OCOCH_3$), 1.96 (s, 3H; $OCOCH_3$), 1.91 (s, 3H; $OCOCH_3$), 1.90–1.81 (overlapping, 2H; H-5b′, H-6a′), 1.77 (dd, $J_{3a'',3b''} = 12.8$, $J_{3'',4''} = 11.4$ Hz, 1H; H-3a′′), 1.59–1.48 ppm (m, 1H; H-6b′); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.7$, 170.5, 170.3, 170.1, 169.3 ($NHCOCH_3$ at C-4, CH_3COO at C-9′′, CH_3COO at C-4′′, CH_3COO at C-8′′, CH_3COO at C-7′′, CH_3COO at C-2′, CH_3COO at C-3′), 167.5 (C-1′′), 162.4 (C-4), 158.3 (t, $J_{C,F} = 27$ Hz, $COCF_2CF_2CF_3$), 155.5 (C-2),147.2 (C-6), 125.1–109.3 ($COCF_2CF_2CF_3$), 98.8 (C-2′′), 97.1 (C-5), 94.1 (C-1′), 83.5 (C-4′), 74.2 (C-3′), 72.6 (C-2′), 72.1 (C-8′′), 70.7 (C-6′′), 70.4 (C-7′′), 69.4 (C-4′′), 63.8 (C-7′), 62.8 (C-9′), 52.7 ($COOCH_3$), 49.3 (C-5′′), 37.5 (C-3′′), 29.7 (C-5′), 25.9 (C-6′), 24.6 ($NHCOCH_3$ at C-4), 20.8, 20.7, 20.6, 20.5, 20.4, ppm ($6\times CH_3COO$); MS (ESI positive): *m/z*: 1047.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{39}H_{47}F_7N_4O_{20}$: C 45.71, H 4.62, N 5.47; found C 45.61, H 4.75, N.5.33.

Compound 24c: Starting from compound **18c** (188 mg, 0.20 mmol), purification by flash chromatography (AcOEt/hexane, 90:10, v/v) afforded compound **24c** (180 mg 88%) as a white solid. M.p. 129–132 °C; $[\alpha]_D^{20} = -18.3$ ($c = 1$ in MeOH); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 9.42$ (s, 1H; NH at C-4), 7.94 (d, $J_{NH,5''} = 9.0$ Hz, 1H; NH at C-5′′), 7.79 (d, $J_{6',5} = 7.5$ Hz, 1H; H-6), 7.49 (d, $J_{5,6} = 7.5$ Hz, 1H; 5-H), 6.05 (d, $J_{1',2'} = 4.5$, 1H; H-1′), 5.40 (ddd, $J_{8'',9a''} = 2.5$, $J_{8'',9b''} = 6.0$, $J_{8'',7''} = 8.7$ Hz, 1H; H-8′′), 5.35 (dd, $J_{7'',6''} = 2.0$, $J_{7'',8''} = 8.7$ Hz, 1H; H-7′′), 5.31 (dd, $J_{2',1'} = 4.5$, $J_{2',3'} = 5.8$ Hz, 1H; H-2′), 5.29 (ddd, $J_{4'',3a''} = 4.7$, $J_{4'',5''} = 10.5$, $J_{4'',3b''} = 12.0$ Hz, 1H; H-4′′), 5.09 (dd, $J_{3',2'} = J_{3',4'} = 5.8$ Hz, 1H; H-3′), 4.35–4.30 (overlapping, 2H; H-6′′, H-9a′′), 4.17–4.04 (overlapping, 3H; H-4′, H-5′′, H-9b′′), 3.82–3.77 (m, 1H; H-7a′), 3.67 (s, 3H; $COOCH_3$), 3.30–3.24 (m, 1H; H-7b′), 2.71 (dd, $J_{3a'',4''} = 4.7$, $J_{3a'',3b''} = 12.7$ Hz, 1H; H-3a′′), 2.25 (s, 3H; $NHCOCH_3$), 2.15 (s, 3H; CH_3COO), 2.09 (s, 3H; CH_3COO), 2.08–2.07 (overlapping, 6H; $2\times CH_3COO$), 2.02 (s, 3H; CH_3COO), 2.01 (s, 3H; CH_3COO), 1.90–1.60 ppm (overlapping, 5H; H-5a′, H-5b′, H-6a′, H-6b′, H-3b″); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.9$, 170.7, 170.6, 170.1, 170.0, 169.6, 169.4 ($NHCOCH_3$ at C-4, CH_3COO at C-9′′, CH_3COO at C-4′′,

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CH_3COO at C-8″, CH_3COO at C-7″, CH_3COO at C-2′, CH_3COO at C-3′), 168.1 (C-1″), 162.7 (C-4), 158.1 (t, $J_{C,F}=27$ Hz, $COCF_2CF_2CF_3$), 155.3 (C-2), 144.0 (C-6), 125.1–109.3 ($COCF_2CF_2CF_3$), 98.6 (C-2″), 97.2 (C-5), 88.9 (C-1′), 81.7 (C-4′), 73.9 (C-3′), 72.9 (C-2′), 71.4 (C-8″), 68.9 (C-6″), 68.5 (C-7″), 67.6 (C-4″), 64.3 (C-7′), 62.5 (C-9′), 52.6 ($COOCH_3$), 49.8 (C-5″), 38.0 (C-3″), 29.8 (C-5′), 25.7 (C-6′), 24.8 ($NHCOCH_3$ at C-4), 21.0, 20.7, 20.6, 20.5, 20.3 ppm ($6\times CH_3COO$); MS (ESI positive): *m/z*: 1047.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{39}H_{47}F_7N_4O_{20}$: C 45.71, H 4.62, N 5.47; found C 45.53, H 4.70, N.5.39.

GM3 synthase assay

Cells cultured in 100 mm dishes, as described above, were harvested by using a plastic scraper and washed two times with phosphate-buffered saline (PBS, Euroclone). Cells were resuspended in 150 mM sodium cacodylate/HCl buffer, pH 6.6 (20 mg of cell protein mL^{-1}) with protease inhibitors (AEBSF at 104 mM, aprotinin at 80 μM, bestatin at 4 mM, E-64 at 1.4 mM, leupeptin at 2 mM, and pepstatin A at 1.5 mM) (Sigma–Aldrich) and homogenized with Dounce homogenizer (10 strokes, tight). In each tube, 10 μL of Triton CF-54 1.5% in chloroform/methanol (2:1, v/v) were mixed with 0.5–50 nmol of [3-^{3}H(sphingosine)]LacCer (corresponding to 45 nCi), from a stock solution in chloroform/methanol (2:1, v/v) and dried under N_2. To this mixture, 8 μL of 750 mM sodium cacodylate/HCl buffer (pH 6.6), 4 μL of 125 mM $MgCl_2$, 4 μL of 125 mM 2-mercaptoethanol, 10 μL of 5 mM CMP-NeuAc, and 10 μL of the cell homogenate (containing 200 μg of proteins) were added in a total reaction volume of 50 μL. A negative control was performed by using heat-inactivated cell homogenate (100 °C for 3 min) whereas a positive control was performed by using an activated cell homogenate. The GM3 synthase inhibitors were tested at two different concentration, that is, 1 mM and 10 μM, in the presence of activated cell homogenate. The incubation was performed at 37 °C for three hours with continuous shaking. The reaction was stopped by adding 1.5 mL of chloroform/methanol (2:1, v/v). The reaction mixture was analyzed by HPTLC by using the solvent system chloroform/methanol/water 55:20:3 (v/v/v). Radioactive lipids were detected and quantified by radioactivity imaging as described below.

Metabolic labeling of cell sphingolipids

[3-^{3}H]Sphingosine dissolved in ethanol was transferred into a gall sterile tube and dried under a nitrogen stream. The residue was then dissolved in an appropriate volume of pre-warmed 10% FBS DMEM to obtain a final concentration of 0.25 μCi per 100 mm dish (corresponding to 2.6×10^{-9} M). Cells were pre-treated for 72 h in the presence of 10 μM GM3 synthase inhibitors, whereas the negative control were cultured for 72 h in the presence of 0.01% DMSO. Then the samples were incubated with 10% FBS DMEM containing [3-^{3}H]sphingosine and 10 μM solutions of the inhibitors or DMSO. After 2 h incubation, the medium was removed and the cells were chased for 48 h with 10% FBS DMEM always containing 10 μM inhibitors or DMSO, in order to reach the metabolic steady state. At the end of chase, the cells were washed and harvested in ice-cold phosphate-buffered saline by scraping. Cell suspensions were frozen and then lyophilized.

Extraction and analysis of the radioactive lipids

Total lipids from the lyophilized cells were extracted twice with chloroform/methanol/water (20:10:1, v/v/v). The lipids extracts were dried under a nitrogen stream, dissolved in chloroform/methanol (2:1, v/v) and subjected to a two-phase partitioning in chloroform/methanol (2:1, v/v) and 20% water. The aqueous and organic phase obtained were counted for their radioactivity and analyzed by HPTLC. [3-^{3}H]Sphingosine of the organic phase was separated by using the solvent system chloroform/methanol/water (55:20:3, v/v/v). The solvent system chloroform/methanol/0.2% aqueous solution of $CaCl_2$ (60:40:9, v/v/v) was employed to analyzed the [3-^{3}H]sphingosine of the aqueous phase. Radioactive lipids were visualized with a Beta-Imager 2000 (Biospace, Paris, France) and identified by comparison with radiolabeled standards. The radioactivity associated with the individual lipids was determined with the specific β-Vision software (Biospace, Paris, France).

Western blot analysis

Cells were cultured for 72 h with 10% FBS DMEM in the presence of 0.01% DMSO or 10 μM GM3 synthase inhibitors. After treatment, the cells were rinsed twice with cold PBS, harvested by scraping, and lysed in PBS containing aprotinin, leupeptin, and pepstatin by sonication. Protein samples corresponding to 30 μg of cell fraction were denatured by boiling for 5 min in sodium dodecyl sulfate (SDS) sample buffer and subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS/PAGE) (10% w/v gel). Subsequently, the proteins were transferred to a nitrocellulose membrane (Thermo Scientific) by electroblotting for 2 h at 100 V.

The membranes were incubated overnight in Tris-buffered saline (TBS, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl), 0.1% (v/v) Tween 20 containing 5% (w/v) dried milk (Biorad) or 5% (w/v) bovine serum albumin (BSA, Sigma–Aldrich) for the blocking buffer. Blots were incubated with a primary antibody in the appropriate blocking solution for 3 h at room temperature. The following primary antibodies were used: anti-phospho-EGFR Tyr-1148, 1:1000 dilution (cell signaling); anti-EGFR, 1:1000 dilution (cell signaling); anti-phospho-ERK1/2 Thr-202/Tyr-204, 1:1000 dilution (cell signaling); anti-ERK1/2, 1:1000 dilution (cell signaling); anti-phospho-p38 MAPK Thr-180/Tyr-182, 1:1000 dilution (cell signaling); anti-p38 MAPK, 1:1000 dilution (cell signaling); anti-calnexin, 1:10000 dilution (epitomics). The membranes were washed four times for 10 min with T-TBS and then incubated with the appropriate secondary antibody conjugated (anti-mouse and anti-rabbit HRP-conjugated, (Dako)) with horseradish peroxidase for 1 h at RT, 1:2000 dilution. After four washes in T-TBS, the protein bands were detected by using an ECL detection kit (Amersham), as described by the manufacturer.

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