Bioorganic & Medicinal Chemistry xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Fluorous-tag assisted synthesis of a glycosaminoglycan mimetic tetrasaccharide as a high-affinity FGF-2 and midkine ligand

Susana Maza, Noel Gandia-Aguado, José L. de Paz *, Pedro M. Nieto *

Glycosystems Laboratory, Instituto de Investigaciones Químicas (IIQ), cicCartuja, CSIC and Universidad de Sevilla, Americo Vespucio, 49, 41092 Sevilla, Spain

ARTICLE INFO

Article history:
Received 19 December 2017
Revised 22 January 2018
Accepted 24 January 2018
Available online xxxx

Keywords:
Fluorescence polarization
Oligosaccharide synthesis
Glycosaminoglycans
Fluorous tag
Carbohydrate-protein interactions

ABSTRACT

Here, we present the preparation of a sulfated, fully protected tetrasaccharide derivative following the glycosaminoglycan (GAG)-related sequence GlcNAc-β(1 → 4)-Glc-β(1 → 3). The tetramer was efficiently assembled via an iterative glycosylation strategy using monosaccharide building blocks. A fluorous tag was attached at position 6 of the reducing end unit enabling the purification of reaction intermediates by simple fluorous solid phase extraction. Fluorescence polarization competition experiments revealed that the synthesized tetrasaccharide strongly interacts with two heparin-binding growth factors, midkine and FGF-2 (IC_{50} of 270 nM and 2.4 μM, respectively). Our data indicate that this type of oligosaccharide derivatives, displaying sulfates, hydrophobic protecting groups and a fluorinated tail can be considered as interesting GAG mimetics for the regulation of relevant carbohydrate-protein interactions.

1. Introduction

Glycosaminoglycans (GAGs) constitute a family of linear, heterogeneous polysaccharides that include hyaluronic acid, chondroitin sulfate (CS) and heparin, among others. GAGs interact with a plethora of proteins and these molecular recognition processes regulate a broad range of biological phenomena including cell growth and differentiation, blood coagulation and inflammation.[1–5] Generally, GAGs are formed by disaccharide repeating units that are decorated with sulfate groups at different positions, giving rise to polysaccharidic chains with enormous structural diversity. It is well-known that defined GAG oligosaccharide sequences are responsible for specific protein recognition and subsequent activity.[6–9] For example, the heparin-antithrombin III binding, responsible for the anticoagulant activity of this polysaccharide, is mediated by a specific pentasaccharide structure with a well-defined sequence and sulfate group distribution.[10] On the other hand, a particular class of CS (CS-E), characterized by the disulfated sequence GalNAc(4,6-di-OSO_3)-β(1 → 4)-GlcA-β(1 → 3), has an important role in the central nervous system development and it has been demonstrated that CS-E tetrasaccharides specifically interact with several neurotrophins, controlling the survival, development and growth of neurons.[11,12]

There is a great interest in the chemical synthesis of GAG oligosaccharides[13] that specifically interact with protein receptors, paving the way for the regulation of relevant biological processes. However, the preparation of well-defined, long GAG oligosaccharides is a formidable challenge. Thus, the synthesis of more easily accessible GAG mimetics, which retain the biological properties of the natural polysaccharides, is an attractive alternative.[14] For instance, different types of multivalent scaffolds displaying short and easily prepared GAG oligomers have been proposed as promising GAG mimetics.[15–21] These multivalent systems include dendrimers and polymers functionalized with CS and heparin oligosaccharide sequences. Sulfated non-GAG oligosaccharides, such as mannose PI-88 and glucose PG545 derivatives, have also been reported as GAG mimetics with potent anticancer activity and high binding affinities to angiogenic growth factors.[22–24] Additionally, non-sugar, sulfated compounds bearing an aromatic scaffold are also potent modulators of GAG-protein binding.[25–27] The interaction of these highly hydrophobic analogues usually involves an important non-ionic contribution to binding energy. In fact, although GAG-protein binding is mainly driven by electrostatic forces between positively charged amino acid residues of the protein and anionic sulfate and carboxylate moieties of the sugar, hydrophobic interactions can also play an important role in these recognition events.[28]

In this context, several research groups have shown that the incorporation of hydrophobic scaffolds or groups improves the biological activities and the protein binding affinities of sulfated compounds.[22,23,29] In addition, we have previously discovered that

* Corresponding authors.
E-mail addresses: jlpaz@iiq.csic.es (J.L. de Paz), pedro.nieto@iiq.csic.es (P.M. Nieto).

https://doi.org/10.1016/j.bmc.2018.01.022
0968-0896/

sulfated, fully protected oligosaccharides, typical intermediates in the chemical synthesis of CS oligomers, strongly bound to certain heparin-binding proteins and could be considered as promising CS mimetics.[30] Importantly, the binding affinities of these compounds were much higher than those corresponding to the deprotected natural sequences. Therefore, our results also indicated that the presence of hydrophobic protecting groups significantly enhances the protein binding.

The preparation of these CS intermediates involved the use of expensive galactosamine (GalN) units and poorly reactive glucuronic acid (GlcA) building blocks. In order to speed up the access to these CS analogues and explore new structure-activity relationships, we decided to replace the GalN and GlcA moieties by glucosamine (GlcN) and glucose (Glc) units, respectively. Thus, we present here the synthesis of tetrasaccharide **1**, displaying the sequence GlcN(4,6-di-OSO_3)-β(1 → 4)-Glc-β(1 → 3), closely related to the CS-E structure (Scheme 1). For this purpose, we envisioned the use of a fluorous-tag assisted strategy. The interaction between **1** and two proteins, midkine and FGF-2, was then evaluated.

2. Results and discussion

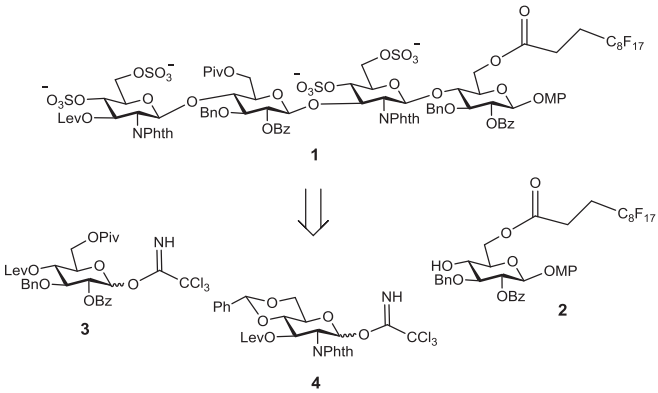
For the synthesis of tetramer **1**, we followed a 1 + 1 modular approach, using the monosaccharide building blocks shown in Scheme 1. A fluorous tag was introduced at position 6 of reducing end unit **2**. Fluorous-tag assisted approaches have been successfully applied to the synthesis of complex oligosaccharides,[31–37] including GAG oligomers.[38–40] The use of a fluorous tag reduces the number of silica gel chromatographic purifications required for the preparation of oligosaccharides since fluorinated intermediates can be easily separated from nonfluorinated side products by a simple fluorous solid-phase extraction (F-SPE).[41] Thus, the attachment of a C_8F_{17} tail to the 6 position of reducing end sugar **2** enabled the purification of growing chains by F-SPE. Moreover, the fluorous-tag assisted reactions are run in solution and can be monitored by standard TLC, NMR and mass spectrometry. Due to the homogeneous solution-phase reaction conditions, lower amounts of glycosyl donors are typically needed for the glycosylations compared to solid phase strategies.

Benzylidene acetals were chosen as temporary and orthogonal protecting groups for further installation of sulfates and benzoyl (Bz) and *N*-phthalimido (*N*-Phth) functionalities ensured the selective formation of the desired β glycosidic linkages (Scheme 1). Levulinoyl (Lev) esters were employed to protect position 4 of glucose **3** and position 3 of glucosamine **4** for subsequent chain elongation. The trichloroacetimidate method was selected for the construction of the glycosidic bonds.

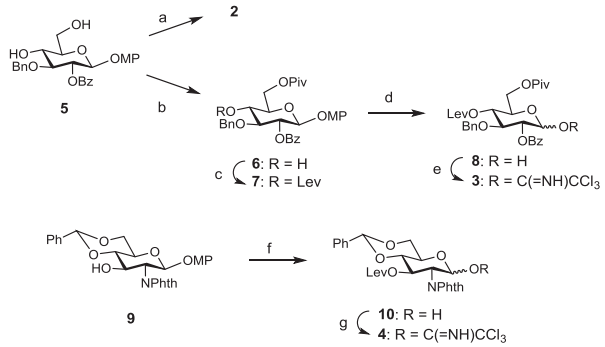
First, reducing end glycosyl acceptor **2** was prepared from known diol **5**[42] by selective acylation with heptadecafluorundecanoyl chloride at 0 °C (Scheme 2). Similar reaction conditions (acyl chloride, triethylamine, DMAP in CH_2Cl_2 at 0 °C) were used for the preparation of the 6-*O*-pivaloylated derivative **6**. Levulinoylation at position 4, followed by oxidative removal of the 4-methoxyphenyl group with cerium (IV) ammonium nitrate (CAN) afforded 1-hydroxy sugar **8** in good yield. 1-*O*-benzoylated 2-hydroxy sugar, derived from the migration of the benzoyl group to the anomeric position, was detected as a minor side product. Treatment with trichloroacetonitrile and potassium carbonate in CH_2Cl_2 gave trichloroacetimidate donor **3**. Compound **9** was converted into derivative **10** in excellent yield by treatment with levulinic anhydride and DMAP, followed by removal of the 4-methoxyphenyl group at 0 °C. Treatment with CAN at room temperature resulted in lower yields due to partial benzylidene hydrolysis. Purification of **10** by silica gel column chromatography was avoided, due to its instability and the formation of H-2/H-3 levulinate elimination side products, as previously reported.[43] To obtain the glycosyl donor **4**, crude hemiacetal **10** was treated with trichloroacetonitrile and catalytic 1,8-diazabicycloundec-7-ene (DBU).

With the appropriate building blocks in hand, fluorous-assisted synthesis of tetrasaccharide **1** was accomplished (Scheme 3). Fluorous acceptor **2** was coupled with 2 equiv of glycosyl donor **4** using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter and CH_2Cl_2 as solvent at 0 °C to give disaccharide **11** in high yield. This product was isolated by F-SPE. Non-fluorinated side products were removed by elution with MeOH/H_2O 80:20 and the desired fluorinated compound was recovered using acetone as the eluent. NMR spectroscopy and mass spectrometry confirmed the structure of disaccharide **11**. Only the β anomer was formed due to neighboring-group participation of the *N*-phthalimido group. In previous reports,[44] we observed a loss of fluorous-tagged material during F-SPE purification steps due to sample breakthrough, and fluorinated compounds were detected in the fluorophobic elution. To overcome this problem, we employed here a 9:1 DMF/H_2O mixture, instead of 100% DMF, as loading solvent.

Deprotection of the temporary levulinoyl group with hydrazine monohydrate in pyridine/acetic acid buffer smoothly afforded derivative **12** (Scheme 3). Synthesis of trisaccharide **13** proved to be a challenging task. Initial glycosylation trial between fluorous acceptor **12** and trichloroacetimidate **3**, under TMSOTf activation (5 mol% with respect to the donor) resulted in the formation of trisaccharide **13** and the corresponding orthoester side product. Trimethylsilyl etherification of **12** was also detected in the reaction mixture and, for this reason, TMSOTf was replaced by

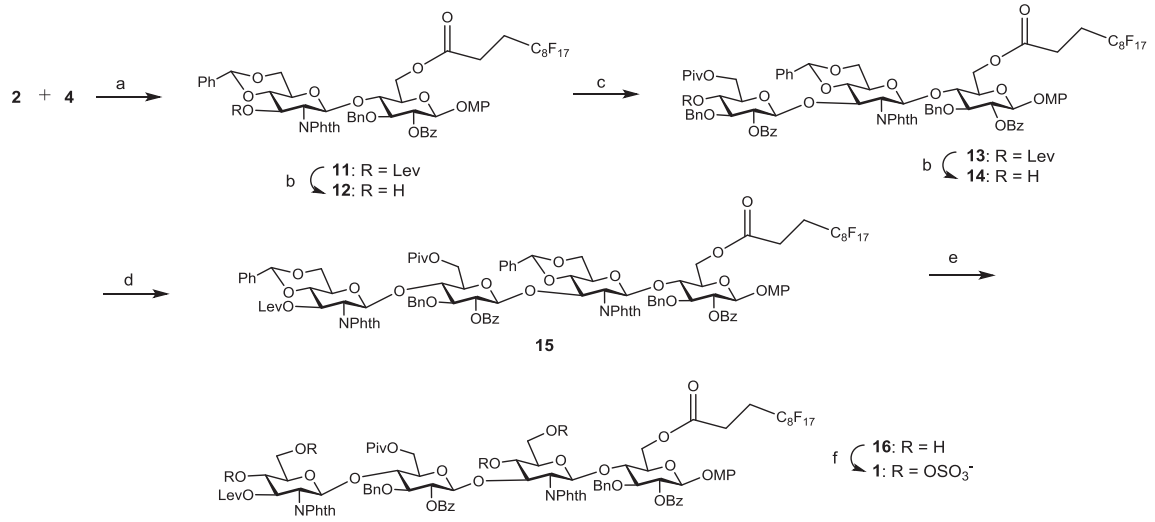


Scheme 1. Building blocks required for the synthesis of target tetrasaccharide **1**. Bn = benzyl; MP = 4-methoxyphenyl; Piv = pivaloyl; NPhth = *N*-phthalimido; Bz = benzoyl; Lev = levulinoyl.



Scheme 2. Reagents and conditions: (a) $C_8F_{17}(CH_2)_2C(=O)Cl$, Et_3N, DMAP, CH_2Cl_2, 0 °C, 81%; (b) PivCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, 82%; (c) Lev_2O, DMAP, CH_2Cl_2, 99%; (d) CAN, $CH_2Cl_2/CH_3CN/H_2O$, 81%; (e) Cl_3CCN, K_2CO_3, CH_2Cl_2, 88%; (f) Lev_2O, DMAP, CH_2Cl_2, 90%; CAN, $CH_2Cl_2/CH_3CN/H_2O$, 0 °C, 96%; (g) Cl_3CCN, DBU, CH_2Cl_2, 76%. DMAP = 4-(*N*,*N*-dimethylamino)pyridine.

Please cite this article in press as: Maza S., et al. Bioorg. Med. Chem. (2018), https://doi.org/10.1016/j.bmc.2018.01.022

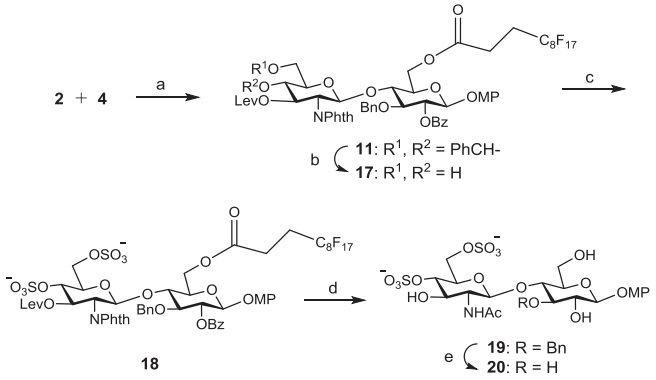


Scheme 3. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 92%; (b) $NH_2NH_2{\cdot}H_2O$, Py/AcOH, CH_2Cl_2, 91% (**12**); (c) **3**, TBSOTf, CH_2Cl_2, 0 °C; (d) **4**, TBSOTf, CH_2Cl_2, 0 °C, 52% (three steps, from **12**); (e) TFA, H_2O, CH_2Cl_2, 95%; (f) $SO_3{\cdot}Me_3N$, DMF, 100 °C, MW heating, 72%.

tert-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf).[45] When the glycosylation was run with higher amounts of Lewis acid (20 mol% of TBSOTf with respect to the donor), orthoester formation was prevented but byproducts derived from hydrolysis of the benzylidene group were observed. Therefore, careful adjustment of the amount of promoter was required to avoid both the acid-mediated cleavage of benzylidene functionalities and the formation of orthoesters. Gratifyingly, glycosylation of **12** with 2.6 equiv. of **3** using TBSOTf (10 mol% with respect to the donor) as activator at 0 °C cleanly gave trimer **13**, as confirmed by NMR and mass spectrometry analysis. After isolation by F-SPE, **13** was selectively delevulinoylated to provide glycosyl acceptor **14**. The latter compound was condensated with donor **4** (3 equiv.) using TBSOTf as the promoter at 0 °C. Pure tetramer **15** was obtained in excellent yield after F-SPE and standard silica gel column chromatography. The overall yield for the assembly of the tetrasaccharide, starting from monosaccharide **2**, was 44%, which corresponds to an 85% yield per reaction step. It is important to note that selected building blocks displayed high reactivity in all the glycosylations and just one cycle with a small excess of sugar donors (2–3 equiv.) was required to complete these reactions.

Finally, **15** was transformed into the target molecule **1** (Scheme 3). The benzylidene groups were removed using trifluoroacetic acid (TFA) in wet CH_2Cl_2. The resulting tetraol **16** was extensively sulfated, under microwave irradiation,[46,47] to provide **1** in good yield. In this case, standard silica gel chromatography was required to separate tetrasulfated sugar from partially sulfated byproducts. The structure of **1** was confirmed by NMR and mass spectroscopic analysis. NMR spectra showed the characteristic downfield shifts of the proton and carbon signals at positions bearing a sulfate group (H-4/H-6 GlcN: δ = 3.75–3.26 ppm in **16**; δ = 4.74–3.96 ppm in **1**; C-4 GlcN: δ = 70.7–70.5 ppm in **16**; δ = 77.8–75.9 ppm in **1**; C-6 GlcN: δ = 62.7–62.0 ppm in **16**; δ = 68.8–68.1 ppm in **1**).

After successful assembly of sulfated tetrasaccharide **1**, global deprotection reactions were tested at the disaccharide stage (Scheme 4). Disaccharide **17** was obtained by glycosylation between **2** and **4** under TBSOTf catalysis followed by TFA-mediated benzylidene hydrolysis. Microwave-assisted sulfation of compound **17** gave derivative **18** in excellent 93% yield. **18** was then treated with ethylene diamine in *n*-butanol at 120 °C under microwave heating. Interestingly, mass spectrometry and TLC analysis indicated the formation of a mixture of two compounds: the



Scheme 4. Reagents and conditions: (a) TBSOTf, CH_2Cl_2, 0 °C; (b) TFA, H_2O, CH_2Cl_2, 78% (two steps, from **2**); (c) $SO_3{\cdot}Me_3N$, DMF, 100 °C, MW heating, 93%; (d) ethylene diamine, *n*-BuOH, 120 °C, MW heating; NaOH, MeOH; Ac_2O, Et_3N, MeOH, 76%; (e) H_2, $Pd(OH)_2/C$, H_2O/MeOH, 75%.

desired deacylated amine derivative and a benzoylated byproduct. To completely remove the 2-*O*-benzoate group, further treatment with aqueous NaOH in MeOH was needed. Selective *N*-acetylation with acetic anhydride and triethylamine in MeOH proceeded smoothly to afford disaccharide **19** in good yield. Finally, hydrogenolysis of **19** gave disaccharide **20**, demonstrating that our protecting group design can deliver fully deprotected derivatives.

Next, we studied the interaction between midkine and the synthesized fully protected derivative **1**. Midkine is a heparin-binding growth factor involved in development, survival and migration of target cells.[48,49] This cytokine is overexpressed in most of human malignant tumors. Notably, midkine plays important roles in several diseases of the central nervous system, such as glioblastoma and multiple sclerosis. Furthermore, midkine is implicated in cell development during embryogenesis.[50] There is a great interest in the discovery of high-affinity ligands that could modulate the activity of this protein.[15,51,52] For the analysis of the tetramer-midkine interaction, we employed a fluorescence polarization competition experiment previously developed in our group.[52–54] In this experiment, we measured the ability of compound **1** to block the formation of a complex between midkine and a fluorescein labelled heparin hexasaccharide,[54] which is characterized by a high polarization value. Thus, the fluorescence polarization (FP) of samples

containing fixed amounts of protein and fluorescent probe in the presence of increasing concentrations of **1** were recorded (Fig. 1). We observed a decrease in the fluorescence polarization indicating that **1** bound to midkine and displaced the fluorescent probe from the complex. The measurements were carried out in PBS buffer containing bovine serum albumin (BSA, 0.5% w/v) to prevent non-specific binding. Compound **1** was soluble in PBS buffer at 100 μM concentration in the presence of 1% of DMSO. Mathematical curve fitting provided an IC_{50} value (Table 1, IC_{50} = 270 ± 70 nM), defined as **1** concentration required for 50% inhibition. The IC_{50} value and the error represent the average and the standard deviation from three independent experiments (see Supporting Information, Fig. S1). Interestingly, this relative binding affinity was significantly higher than that reported for a fully protected CS-like tetrasaccharide following the sequence GalNAc(4,6-di-OSO_3)-GlcA (IC_{50} = 1.3 μM).[30] The binding affinity of **1** was also much higher than that displayed by a deprotected, natural CS-E tetramer (IC_{50} = 254 μM).[30]

The interaction between midkine and disulfated dimer **18** was also evaluated (Fig. 1 and Supporting Information, Fig. S2). The FP competition assay showed that **18** strongly bound to midkine, in the high nanomolar range (Table 1, IC_{50} = 700 ± 220 nM from four independent experiments). Once again, this relative affinity was remarkably higher than those previously reported for disulfated, fully protected disaccharides lacking the perfluorinated tag (IC_{50} ranging from 15 to 20 μM).[30] Our results suggest that the presence of a fluorous tail enhances the binding of oligosaccharide precursors to midkine. On the other hand, it is well known that fully deprotected CS/GAG disaccharides exhibit weak binding affinity for proteins. In fact, compounds **19** and **20** were tested using our FP assay and no significant inhibition was detected at 250 μM concentration (Table 1, IC_{50} > 250 μM). These observations demonstrate that the display of hydrophobic protecting groups, in particular fluorinated tags, on oligosaccharide derivatives strongly increases their interactions with midkine.

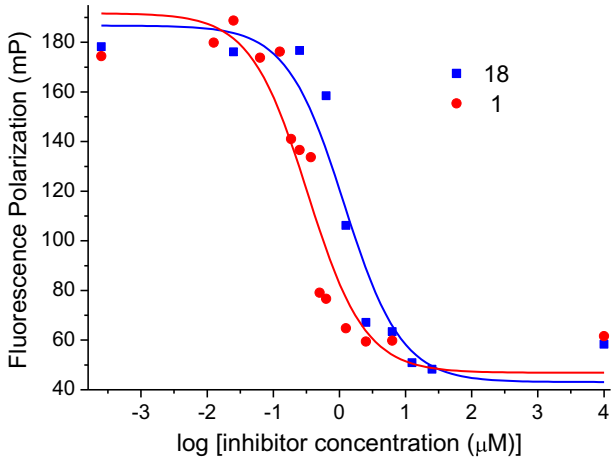


Fig. 1. Representative competition curves showing the ability of compounds **1** and **18** to inhibit the interaction between midkine and the fluorescent probe. The fluorescence polarization of samples containing midkine (63 nM), fluorescent probe (10 nM) and increasing concentrations of inhibitors were measured and the resulting curves were fitted to the equation for a one-site competitive interaction model in order to calculate the IC_{50} values. All the polarization values are the average of three replicate wells. At least three independent experiments were carried out for each IC_{50} calculation.

Table 1
IC_{50} values for compounds **1**, **18**–**20** obtained from FP midkine competition assays.

Compound	1	18	19	20
IC_{50} (μM)	0.27 ± 0.07	0.70 ± 0.22	>250	>250

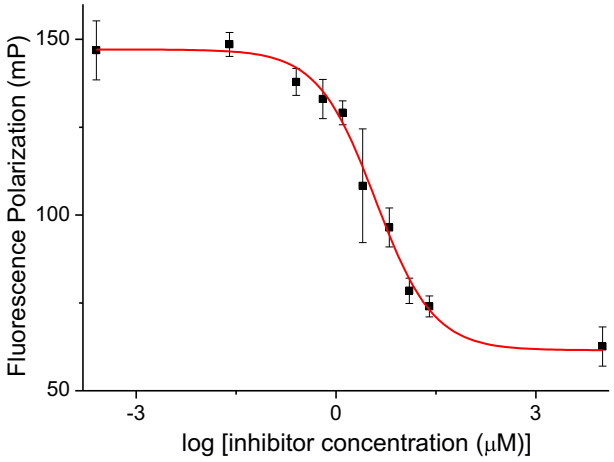


Fig. 2. Representative competition curve showing the ability of compound **1** to inhibit the interaction between FGF-2 (97 nM) and fluorescent probe (10 nM). All the FP values are the average of three replicate wells, with error bars showing the standard deviations for these measurements. The reported IC_{50} value and the error (2.4 ± 1.3 μM) represent the average and the standard deviation from three independent experiments.

In addition, we studied the interaction between tetrasaccharide **1** and a different GAG-binding protein, FGF-2 (basic fibroblast growth factor), with a key role in angiogenesis and tumor cell growth.[22,24,55] Compounds that selectively recognize one GAG-binding protein are highly demanded. The analysis of the FP competition curves afforded an IC_{50} value of 2.4 ± 1.3 μM (see Fig. 2 and Supporting Information, Fig. S3) that was again lower than the IC_{50} displayed by a CS-E persubstituted tetrasaccharide derivative without the fluorous tag (42 μM).[30] It is known that the intensity of binding of FGF-2 toward CS-E is lower than that for heparin.[56] Interestingly, our CS-E mimetic **1** displayed an FGF-2 binding affinity comparable with those shown by heparin oligosaccharides, in the low micromolar range (for example, K_D = 0.4–6 μM for heparin tetrasaccharides).[8,57] However, the FGF-2 binding affinity of **1** was clearly higher than that previously reported for a CS-E tetrasaccharide (IC_{50} = 2.4 μM against 271 μM).[30] On the other hand, although **1** strongly bound to FGF-2, our results suggest that this compound presents a slight degree of selectivity for midkine over FGF-2 (IC_{50} = 0.27 μM versus 2.4 μM).

3. Conclusions

Compounds that strongly interact with GAG-binding proteins, such as midkine and FGF-2, are highly demanded because these molecules can potentially regulate biological processes like angiogenesis and tumor cell growth. We previously found that sulfated CS-E oligosaccharides, displaying the hydroxyl, carboxylate and amine functionalities masked by different protecting groups, are excellent ligands for these proteins.[30] Based on these results, we envisaged the preparation of tetrasaccharide **1** as a CS-E mimetic. In this molecule, GalN and GlcA units, typical of CS structure, were replaced by more accessible and reactive GlcN and Glc building blocks, while maintaining the cluster of sulfate groups at positions 4 and 6 of the hexosamine moiety. For the synthesis of this CS-E analogue, we successfully employed a fluorous-supported approach. The fluorous tag at position 6 of the reducing end allowed the purification of reaction intermediates by simple and quick F-SPE, facilitating the access to these molecules. Importantly,

the designed monosaccharide building blocks showed high reactivity in all glycosylation couplings.

Our FP experiment revealed that tetramer **1**, and also disaccharide **18**, interacted with midkine in the nanomolar range (IC_{50} of 270 and 700 nM, respectively). On the other hand, tetrasaccharide **1** bound to FGF-2 in the low micromolar range (IC_{50} = 2.4 μM). All these relative binding affinities were significantly higher than those obtained for other fully protected oligosaccharide precursors with the same length, but lacking the fluorinated tag.[30] We hypothesized that the presence of the fluorous protecting group strongly enhanced the protein binding, although the influence of other structural differences cannot be ruled out. Importantly, natural deprotected CS-E di- and tetrasaccharides present very low protein affinities (in the high micromolar range) compared to fully protected oligosaccharides. Overall, our results provide valuable information for the design and synthesis of sulfated oligosaccharide derivatives as potent GAG mimetics.

4. Experimental

4.1. General synthetic procedures

Thin layer chromatography (TLC) analyses were performed on silica gel 60 F_{254} precoated on aluminium plates (Merck) and the compounds were detected by staining with sulfuric acid/ethanol (1:9), with cerium (IV) sulfate (10 g)/phosphomolybdic acid (13 g)/sulfuric acid (60 mL) solution in water (1 L), or with anisaldehyde solution [anisaldehyde (25 mL) with sulfuric acid (25 mL), ethanol (450 mL) and acetic acid (1 mL)], followed by heating at over 200 °C. Column chromatography was carried out on silica gel 60 (0.2–0.5 mm, 0.2–0.063 mm or 0.040–0.015 mm; Merck). Optical rotations were determined with a Perkin-Elmer 341 polarimeter. ^{1}H, ^{19}F and ^{13}C NMR spectra were acquired on Bruker DPX-300, Avance III-400 and DRX-500 spectrometers. Unit A refers to the reducing end monosaccharide in the NMR data. Electrospray mass spectra (ESI MS) were carried out with an Esquire 6000 ESI-Ion Trap from Bruker Daltonics. High resolution mass spectra (HR MS) were carried out by CITIUS (Universidad de Sevilla). Microwave-based sulfation reactions were performed using a Biotage Initiator Eight synthesizer in sealed reaction vessels. Compound **9** was purchased from Carbosynth. FluoroFlash silica gel was purchased from Sigma-Aldrich.

4.2. General procedure for F-SPE

FluoroFlash silica gel (5 g, Fluorous Technologies, Inc) was placed in a glass chromatography column (1.7 cm diameter). The F-SPE column was washed with DMF (2 mL) and then preconditioned with MeOH/H_2O 80:20 (15 mL). Next, the crude sample (100–300 mg) was dissolved in DMF/H_2O 9:1 (0.8 mL) and loaded on the column. The fluorophobic elution was carried out with 15 mL of MeOH/H_2O 80:20. The fluorous compounds were then eluted using 100% acetone (20 mL). To regenerate the F-SPE column, we washed with additional acetone (20 mL). DMF washing step can be omitted when reusing the F-SPE column.

4.3. 4-Methoxyphenyl 2-O-benzoyl-3-O-benzyl-6-O-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecanoyl-β-d-glucopyranoside (*2*)

A solution of DMAP (35 mg, 0.29 mmol), triethylamine (320 μL, 2.3 mmol) and diol **5** (556 mg, 1.16 mmol) in CH_2Cl_2 (8 mL) was cooled to 0 °C, and treated with heptadecafluoroundecanoyl chloride (4 mL of a 0.32 M solution in dry CH_2Cl_2). After stirring for 2 h at 0 °C, the reaction mixture was diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2-MeOH 100:0 → 99.5:0.5) to afford **2** (894 mg, 81%) as a white amorphous solid. TLC (toluene-EtOAc 3:1) Rf 0.58; $[\alpha]_D^{20}$ −11° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 3:1): δ 7.97 (m, 2H, Ar), 7.59 (m, 1H, Ar), 7.44 (m, 2H, Ar), 7.18–7.09 (m, 5H, Ar), 6.85 (m, 2H, Ar), 6.73 (m, 2H, Ar), 5.38 (t, 1H, H-2), 5.00 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.82 (d, 1H, CH_2(Bn)), 4.70 (d, 1H, CH_2(Bn)), 4.53 (dd, 1H, $J_{5,6}$ = 1.6 Hz, $J_{6,6}$ = 11.8 Hz, H-6a), 4.39 (dd, 1H, $J_{5,6}$ = 5.8 Hz, H-6b), 3.75–3.68 (m, 6H, H-3, H-4, H-5, Me (OMP)), 2.72–2.44 (m, 4H, $-CH_2-CH_2-$); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD 3:1): δ 171.3, 165.7 (2 × CO), 155.5–114.4 (Ar), 100.6 (C-1), 82.0 (C-3), 74.8 (CH_2(Bn)), 74.0 (C-4 or C-5), 73.2 (C-2), 70.3 (C-4 or C-5), 64.0 (C-6), 55.3 (Me (OMP)), 26.3 ($-CH_2-$), 25.3 ($-CH_2-$); ^{19}F NMR (376 MHz, $CDCl_3$/CD_3OD 3:1): δ −81.19 (t, 3F), −114.91 (m, 2F), −122.05 (m, 6F), −122.98 (m, 2F), −123.66 (m, 2F), −126.43 (m, 2F); HR MS: *m*/*z*: calcd for $C_{38}H_{31}F_{17}O_9Na$: 977.1589; found: 977.1577 [*M*+Na]$^+$.

4.4. 4-Methoxyphenyl 2-O-benzoyl-3-O-benzyl-6-O-pivaloyl-β-D-glucopyranoside (*6*)

A solution of DMAP (99 mg, 0.81 mmol), triethylamine (0.3 mL, 2.15 mmol) and diol **5** (516 mg, 1.07 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 °C, and treated with pivaloyl chloride (172 μL, 1.39 mmol). After stirring for 2 h at 0 °C, the reaction mixture was diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (toluene-EtOAc 4:1) to afford **6** (498 mg, 82%) as a white amorphous solid. TLC (toluene-EtOAc 4:1) Rf 0.49; $[\alpha]_D^{20}$ −1° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (m, 2H, Ar), 7.61 (m, 1H, Ar), 7.49 (m, 2H, Ar), 7.24 (m, 5H, Ar), 6.93 (m, 2H, Ar), 6.76 (m, 2H, Ar), 5.50 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 9.1 Hz, H-2), 5.01 (d, 1H, H-1), 4.76 (2d, 2H, CH_2(Bn)), 4.42 (m, 2H, H-6), 3.78–3.69 (m, 6H, H-3, H-4, H-5, Me (OMP)), 1.27 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.1, 165.2 (2 × CO), 155.6–114.4 (Ar), 101.0 (C-1), 82.0 (C-3), 74.7 (CH_2(Bn)), 74.2 (C-4 or C-5), 73.4 (C-2), 70.3 (C-4 or C-5), 63.4 (C-6), 55.6 (Me (OMP)), 39.0 ($C(CH_3)_3$), 27.2 ($C(CH_3)_3$); HR MS: *m*/*z*: calcd for $C_{32}H_{36}O_9Na$: 587.2252; found: 587.2258 [*M*+Na]$^+$.

4.5. 2-O-Benzoyl-3-O-benzyl-4-O-levulinoyl-6-O-pivaloyl-α,β-D-glucopyranose (*8*)

LevOH (1.5 mL, 14.3 mmol) was added at 0 °C to a solution of 1,3-dicyclohexylcarbodiimide (1.5 g, 7.2 mmol) in CH_2Cl_2 (20 mL). After stirring for 5 min at room temperature, the mixture was cooled (0 °C) and filtered, and the urea precipitate was washed with additional CH_2Cl_2 (4 mL). The resulting Lev_2O solution (7.2 mmol) was added at room temperature to a mixture of **6** (1.35 g, 2.39 mmol) and DMAP (44 mg, 0.36 mmol). The mixture was stirred for 1.5 h, diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was suspended in hexane/EtOAc (1:1, 19 mL) and the mixture was filtered. The solid was washed with additional hexane/EtOAc (1:1) to give **7** as a white amorphous solid (1.58 g, 99%) that was used without further purification. TLC (toluene-EtOAc 5:1) Rf 0.42; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (m, 2H, Ar), 7.60 (m, 1H, Ar), 7.46 (m, 2H, Ar), 7.16 (m, 5H, Ar), 6.91 (m, 2H, Ar), 6.73 (m, 2H, Ar), 5.54 (dd, 1H, $J_{2,3}$ = 9.1 Hz, H-2), 5.22 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 5.01 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.63 (2d, 2H, CH_2(Bn)), 4.32 (dd, 1H, H-6), 4.13 (dd, 1H, H-6), 3.94 (t, 1H, H-3), 3.81 (m, 1H, H-5), 3.74 (s, 3H, Me (OMP)), 2.76–2.49 (m, 4H, CH_2(Lev)), 2.17 (s, 3H, CH_3(Lev)), 1.23

(s, 9H, $C(CH_3)_3$); ESI MS: *m/z*: calcd for $C_{37}H_{42}O_{11}Na$: 685.3; found: 685.3 $[M+Na]^+$.

CAN (9.0 mL of a 1.06 M solution in H_2O) was added to a solution of **7** (1.58 g, 2.38 mmol) in CH_2Cl_2/MeCN (1:2; 81 mL). After stirring for 1.5 h at room temperature, the reaction mixture was diluted with EtOAc, washed with H_2O, saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (toluene-EtOAc 4:1) to afford **8** as a yellow foam (1.08 g, 81%). TLC (toluene-EtOAc 2:1) Rf 0.40 and 0.37 (α/β anomers); ^{1}H NMR (400 MHz, $CDCl_3$) (data for α anomer): δ 8.04 (m, 2H, Ar), 7.58 (m, 1H, Ar), 7.44 (m, 2H, Ar), 7.18 (m, 5H, Ar), 5.56 (t, 1H, $J_{1,2} = J_{1,OH} = 3.0$ Hz, H-1), 5.20 (t, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.08 (dd, 1H, $J_{2,3} = 9.9$ Hz, H-2), 4.74, 4.67 (2d, 2H, CH_2(Bn)), 4.26–4.18 (m, 3H, H-3, H-5, H-6a), 4.12 (dd, 1H, $J_{5,6b} = 3.7$ Hz, $J_{6a,6b} = 12.1$ Hz, H-6b), 3.75 (d, 1H, OH), 2.75–2.38 (m, 4H, CH_2(Lev)), 2.14 (s, 3H, CH_3(Lev)), 1.21 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) (data for α anomer): δ 206.5, 178.6, 171.4, 165.9 (4 × CO), 138.1–125.4 (Ar), 90.4 (C-1), 77.1 (C-3), 75.0 (CH_2(Bn)), 74.1 (C-2), 70.0 (C-4), 67.9 (C-5), 61.8 (C-6), 39.0 ($C(CH_3)_3$), 38.0 (CH_2(Lev)), 29.9 (CH_3(Lev)), 28.0 (CH_2(Lev)), 27.2 ($C(CH_3)_3$); HR MS: *m/z*: calcd for $C_{30}H_{36}O_{10}Na$: 579.2201; found: 579.2192 $[M+Na]^+$.

4.6. O-(2-O-Benzoyl-3-O-benzyl-4-O-levulinoyl-6-O-pivaloyl-α,β-D-glucopyranosyl) trichloroacetimidate (3)

Trichloroacetonitrile (2.4 mL, 26 mmol) and K_2CO_3 (214 mg, 1.55 mmol) were added to **8** (717 mg, 1.29 mmol) in dry CH_2Cl_2 (8 mL) under an argon atmosphere. After stirring at room temperature for 4 h, the mixture was filtered and concentrated in vacuo. The residue was purified by using a short silica gel column (toluene-EtOAc 3:1 + 1% Et_3N) to give **3** as a white amporphous solid (795 mg, 88%, mixture of α/β anomers). TLC (CH_2Cl_2-MeOH 60:1) Rf 0.44 and 0.38; ^{1}H NMR (400 MHz, $CDCl_3$) (data for β anomer): δ 8.61 (s, 1H, NH), 7.97 (m, 2H, Ar), 7.57 (m, 1H, Ar), 7.43 (m, 2H, Ar), 7.18 (m, 5H, Ar), 5.95 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 5.58 (dd, 1H, $J_{2,3} = 8.8$ Hz, H-2), 5.26 (t, 1H, $J_{3,4} = J_{4,5} = 9.2$ Hz, H-4), 4.64 (br s, 2H, CH_2(Bn)), 4.30–4.11 (m, 2H, H-6a, H-6b), 3.95 (t, 1H, H-3), 3.90 (ddd, 1H, $J_{5,6a} = 2.7$ Hz, $J_{5,6b} = 5.5$ Hz, H-5), 2.76–2.39 (m, 4H, CH_2(Lev)), 2.16 (s, 3H, CH_3(Lev)), 1.21 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) (data for β anomer): δ 206.2, 178.3, 171.4, 164.8 (4 × CO), 161.4 (C=NH), 137.6–125.4 (Ar), 96.0 (C-1), 90.5 (CCl_3), 79.2 (C-3), 74.0 (CH_2(Bn)), 73.3 (C-5), 71.8 (C-2), 69.7 (C-4), 61.7 (C-6), 39.0 ($C(CH_3)_3$), 38.0 (CH_2(Lev)), 29.9 (CH_3(Lev)), 28.0 (CH_2(Lev)), 27.2 ($C(CH_3)_3$); ESI MS: *m/z*: calcd for $C_{32}H_{36}Cl_3NO_{10}Na$: 722.1; found: 722.2 $[M+Na]^+$.

4.7. 4,6-O-Benzylidene-2-deoxy-3-O-levulinoyl-2-phthalimido-α,β-D-glucopyranose (10)

LevOH (4.2 mL, 41 mmol) was added at 0 °C to a solution of 1,3-dicyclohexylcarbodiimide (4.2 g, 20.5 mmol) in CH_2Cl_2 (40 mL). After stirring for 5 min at room temperature, the mixture was cooled and filtered, and the urea precipitate was washed with additional CH_2Cl_2 (5 mL). The resulting Lev_2O solution (20.5 mmol) was added at room temperature to a mixture of **9** (3.44 g, 6.83 mmol) and DMAP (125 mg, 1.0 mmol). The mixture was stirred for 1.5 h, diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was suspended in hexane/EtOAc (1:1, 30 mL) and the mixture was filtered. The solid was washed with additional hexane/EtOAc (1:1, 10 mL) to give the 3-*O*-levulinated monosaccharide as a white amorphous solid (3.70 g, 90%) that was used without further purification.

CAN (6.0 mL of a 1.27 M solution in H_2O) was added at 0 °C to a solution of the levulinated compound (1.15 g, 1.91 mmol) in CH_2Cl_2/MeCN (1:2; 54 mL). After stirring for 1.5 h at 0 °C, the reaction mixture was diluted with EtOAc, washed with H_2O, saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. EtOAc (2.5 mL) and then hexane (7.5 mL) were added to the residue at 0 °C. After stirring, the resulting suspension was filtered and the solid was washed with cold hexane to afford **10** as a yellow amorphous solid (904 mg, 96%). Analytical data were in good agreement with those described in literature previously.[43]

4.8. O-(4,6-O-Benzylidene-2-deoxy-3-O-levulinoyl-2-phthalimido-α,β-D-glucopyranosyl) trichloroacetimidate (4)

Trichloroacetonitrile (1.2 mL, 12 mmol) and catalytic DBU (18 μL, 0.12 mmol) were added to a solution of **10** (596 mg, 1.2 mmol) in dry CH_2Cl_2 (5 mL). After stirring for 3 h at room temperature, the reaction mixture was concentrated to dryness. The residue was purified by a short silica gel column (toluene-EtOAc 4:1 + 1% Et_3N) to afford **4** as a yellow foam (584 mg, 76%). Analytical data were in good agreement with those described in literature previously.[43]

4.9. 4-Methoxyphenyl O-(4,6-O-benzylidene-2-deoxy-3-O-levulinoyl-2-phthalimido-β-D-glucopyranosyl)-(1 → 4)-O-(2-O-benzoyl-3-O-benzyl-6-O-pivaloyl-β-D-glucopyranosyl)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 4)-2-O-benzoyl-3-O-benzyl-6-O-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecanoyl-β-D-glucopyranoside (15)

Donor **4** (130 mg, 0.20 mmol) and aceptor **2** (97 mg, 0.10 mmol) were dissolved in dry CH_2Cl_2 (4 mL) in the presence of activated 4 Å molecular sieves (MS, 300 mg). The reaction mixture was stirred, under an argon atmosphere, for 10 min at 0 °C and TMSOTf (200 μL of a 0.14 M solution in dry CH_2Cl_2) was added. After stirring for 1.5 h at 0 °C, the reaction mixture was quenched with triethylamine, filtered, and then concentrated under reduced pressure. The crude product was purified using a fluorous solid-phase extraction (F-SPE) column. Nonfluorous compounds were eluted with 80:20 MeOH/water and the fluorous product was eluted by 100% acetone. This acetone fraction was concentrated to give disaccharide **11** as a white amporphous solid (134 mg, 92%). TLC (hexane-EtOAc 3:2) Rf 0.42; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (m, 2H, Ar), 7.89–7.76 (m, 4H, Ar), 7.61 (m, 1H, Ar), 7.49–7.23 (m, 12H, Ar), 6.82 (m, 2H, Ar), 6.70 (m, 2H, Ar), 5.90 (t, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3′), 5.64 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1′), 5.45 (m, 2H, PhCHO, H-2), 5.03 (d, 1H, $J_{1,2} = 6.5$ Hz, H-1), 4.83 (s, 2H, CH_2(Bn)), 4.46 (br d, 1H, H-6), 4.33 (t, 1H, H-2′), 4.11 (m, 2H, H-6′, H-4), 3.90 (t, 1H, $J_{2,3} = J_{3,4} = 7.3$ Hz, H-3), 3.78–3.66 (m, 6H, H-4′, H-5, H-6, Me (OMP)), 3.59–3.46 (m, 2H, H-5′, H-6′), 2.59–2.21 (m, 8H, –CH_2–CH_2–, CH_2(Lev)), 1.88 (s, 3H, CH_3(Lev)); ^{13}C NMR (100 MHz, $CDCl_3$, selected data from HSQC experiment): δ 101.5 (PhCHO), 99.4 (C-1), 98.9 (C-1′), 80.7 (C-3), 78.7 (C-4′ or C-5), 77.1 (C-4), 73.6 (CH_2(Bn)), 72.4 (C-2), 72.2 (C-4′ or C-5), 69.4 (C-3′), 68.2 (C-6′), 65.9 (C-5′), 62.3 (C-6), 55.4 (C-2′), 55.2 (Me (OMP)); ^{19}F NMR (376 MHz, $CDCl_3$): δ −80.70 (t, 3F), −114.52 (m, 2F), −121.74 (m, 6F), −122.65 (m, 2F), −123.36 (m, 2F), −126.06 (m, 2F); ESI MS: *m/z*: calcd for $C_{64}H_{54}F_{17}NO_{17}Na$: 1454.3; found: 1454.2 $[M+Na]^+$.

Compound **11** (146 mg, 0.10 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and hydrazine monohydrate (0.41 mL of a 0.5 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 1 h, the reaction mixture was quenched with acetone (0.2 mL). The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and H_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product was purified using a fluorous solid-phase extraction (F-SPE) column. Nonfluorous compounds were eluted

with 80:20 MeOH/water and the fluorous product was eluted by 100% acetone. This acetone fraction was concentrated to give disaccharide **12** as a white amporphous solid (124 mg, 91%). TLC (hexane-EtOAc 1:1) Rf 0.61; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (m, 2H, Ar), 7.87 (m, 2H, Ar), 7.74 (m, 2H, Ar), 7.58 (m, 1H, Ar), 7.46–7.21 (m, 12H, Ar), 6.79 (m, 2H, Ar), 6.68 (m, 2H, Ar), 5.49 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1′), 5.44 (s, 1H, PhCHO), 5.42 (br t, 1H, H-2), 5.00 (d, 1H, $J_{1,2}$ = 6.8 Hz, H-1), 4.80 (s, 2H, CH_2(Bn)), 4.60 (br t, 1H, H-3′), 4.44 (br d, 1H, H-6a), 4.25 (dd, 1H, $J_{2,3}$ = 10.3 Hz, H-2′), 4.07 (m, 2H, H-6′a, H-4), 3.87 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 7.6 Hz, H-3), 3.79 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 3.71–3.64 (m, 4H, H-5, Me (OMP)), 3.52–3.43 (m, 3H, H-4′, H-5′, H-6′b), 2.41 (br d, 1H, OH), 2.24 (m, 4H, –CH_2–CH_2–); ^{13}C NMR (100 MHz, $CDCl_3$, selected data from HSQC experiment): δ 101.5 (PhCHO), 99.5 (C-1), 98.8 (C-1′), 81.7 (C-4′), 80.3 (C-3), 76.8 (C-4), 73.4 (CH_2(Bn)), 72.3 (C-2), 72.2 (C-5), 68.3 (C-6′), 68.0 (C-3′), 66.0 (C-5′), 62.4 (C-6), 56.7 (C-2′), 55.1 (Me (OMP)); ^{19}F NMR (376 MHz, $CDCl_3$): δ −80.70 (t, 3F), −114.54 (m, 2F), −121.73 (m, 6F), −122.64 (m, 2F), −123.35 (m, 2F), −126.06 (m, 2F); ESI MS: *m*/*z*: calcd for $C_{59}H_{48}F_{17}NO_{15}Na$: 1356.3; found: 1356.0 [*M*+Na]$^+$.

Donor **3** (83 mg, 0.118 mmol) and aceptor **12** (62 mg, 0.046 mmol) were dissolved in dry CH_2Cl_2 (1.4 mL) in the presence of activated 4 Å MS (105 mg). The reaction mixture was stirred, under an argon atmosphere, for 10 min at 0 °C and TBSOTf (91 μL of a 0.13 M solution in dry CH_2Cl_2) was added. After stirring for 50 min at 0 °C, the reaction mixture was quenched with triethylamine, filtered, and then concentrated under reduced pressure. The crude product was purified using a fluorous solid-phase extraction (F-SPE) column. Nonfluorous compounds were eluted with 80:20 MeOH/water and the fluorous product was eluted by 100% acetone. This acetone fraction was concentrated to give trisaccharide **13** as a white amporphous solid (85 mg). TLC (hexane-EtOAc 3:2) Rf 0.36; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (m, 2H, Ar), 7.57 (m, 1H, Ar), 7.52–7.39 (m, 12H, Ar), 7.27–7.16 (m, 9H, Ar), 7.02 (m, 3H, Ar), 6.90 (m, 2H, Ar), 6.74 (m, 2H, Ar), 6.65 (m, 2H, Ar), 5.53 (s, 1H, PhCHO), 5.37 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 7.2 Hz, H-2A), 5.29 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1B), 5.09 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4C), 5.02 (br t, 1H, H-2C), 4.93 (d, 1H, $J_{1,2}$ = 6.7 Hz, H-1A), 4.75 (m, 2H, CH_2(Bn)), 4.69 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3B), 4.63 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1C), 4.36–4.24 (m, 4H, CH_2(Bn), H-6aA, H-2B), 4.06 (m, 2H, H-6aB, H-6aC), 3.96 (dd, 1H, $J_{3,4}$ = 7.7 Hz, $J_{4,5}$ = 9.4 Hz, H-4A), 3.80 (t, 1H, H-3A), 3.77–3.70 (m, 2H, H-4B, H-6bC), 3.66 (s, 3H, Me (OMP)), 3.60 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6bA), 3.58–3.49 (m, 3H, H-3C, H-6bB, H-5A), 3.43 (td, 1H, $J_{5,6}$ = 4.5 Hz, $J_{5,6}$ = $J_{4,5}$ = 9.5 Hz, H-5B), 3.09 (m, 1H, H-5C), 2.65–2.12 (m, 8H, –CH_2–CH_2–, CH_2(Lev)), 2.09 (s, 3H, CH_3(Lev)), 1.22 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, selected data from HSQC experiment): δ 101.8 (PhCHO), 100.3 (C-1C), 99.6 (C-1A), 98.8 (C-1B), 80.7 (C-3A, C-4B), 80.4 (C-3C), 77.0 (C-4A), 75.7 (C-3B), 74.1 (CH_2(Bn)), 73.5 (C-2C, CH_2(Bn)), 72.8 (C-2A), 72.5 (C-5A), 71.9 (C-5C), 69.1 (C-4C), 68.7 (C-6B), 66.4 (C-5B), 62.6 (C-6A), 61.1 (C-6C), 55.8 (C-2B), 55.5 (Me (OMP)); ESI MS: *m*/*z*: calcd for $C_{89}H_{82}F_{17}NO_{24}Na$: 1894.5; found: 1894.1 [*M*+Na]$^+$.

Compound **13** (85 mg, 0.045 mmol) was dissolved in CH_2Cl_2 (1.7 mL) and hydrazine monohydrate (182 μL of a 0.5 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 1.5 h, the reaction mixture was quenched with acetone (0.26 mL). The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and H_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product was purified using a fluorous solid-phase extraction (F-SPE) column. Nonfluorous compounds were eluted with 80:20 MeOH/water and the fluorous product was eluted by 100% acetone. This acetone fraction was concentrated to give trisaccharide **14** as a white amporphous solid (77 mg). TLC (hexane-EtOAc 3:2) Rf 0.5; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (m, 2H, Ar), 7.57 (m, 1H, Ar), 7.51–7.38 (m, 12H, Ar), 7.27–7.18 (m, 9H, Ar), 7.05 (m, 3H, Ar), 6.95 (m, 2H, Ar), 6.74 (m, 2H, Ar), 6.65 (m, 2H, Ar), 5.53 (s, 1H, PhCHO), 5.37 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 7.2 Hz, H-2A), 5.30 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1B), 4.93 (d, 1H, $J_{1,2}$ = 6.7 Hz, H-1A), 4.92 (br t, 1H, H-2C), 4.75 (m, 2H, CH_2(Bn)), 4.68 (br dd, 1H, H-3B), 4.63 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1C), 4.48, 4.42 (2d, 2H, CH_2(Bn), 4.27 (m, 2H, H-2B, H-6aA), 4.09 (m, 2H, H-6aC, H-6aB), 3.98 (m, 2H, H-6bC, H-4A), 3.80 (t, 1H, $J_{3,4}$ = 7.7 Hz, H-3A), 3.77 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4B), 3.67 (s, 3H, Me (OMP)), 3.60 (dd, 1H, $J_{5,6b}$ = 4.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6bA), 3.56–3.50 (m, 2H, H-6bB, H-5A), 3.48–3.41 (m, 2H, H-4C, H-5B), 3.36 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3C), 2.91 (m, 1H, H-5C), 2.67 (d, 1H, $J_{4,OH}$ = 3.3 Hz, OH), 2.27–2.10 (m, 4H, –CH_2–CH_2–), 1.22 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, selected data from HSQC experiment): δ 101.8 (PhCHO), 100.3 (C-1C), 99.4 (C-1A), 98.8 (C-1B), 82.1 (C-3C), 80.8 (C-3A, C-4B), 77.0 (C-4A), 75.7 (C-3B), 74.4 (CH_2(Bn)), 74.0 (CH_2(Bn)), 73.7 (C-2C, C-5C), 72.6 (C-2A), 72.5 (C-5A), 69.7 (C-4C), 68.7 (C-6B), 66.3 (C–5B), 62.6 (C-6A), 62.3 (C-6C), 55.7 (C-2B), 55.5 (Me (OMP)); ESI MS: *m*/*z*: calcd for $C_{84}H_{76}F_{17}NO_{22}Na$: 1796.4; found: 1795.9 [*M*+Na]$^+$.

Donor **4** (82 mg, 0.128 mmol) and aceptor **14** (76 mg, 0.043 mmol) were dissolved in dry CH_2Cl_2 (1.6 mL) in the presence of activated 4 Å MS (120 mg). The reaction mixture was stirred, under an argon atmosphere, for 10 min at 0 °C and TBSOTf (140 μL of a 0.064 M solution in dry CH_2Cl_2) was added. After stirring for 35 min at 0 °C, the reaction mixture was quenched with triethylamine, filtered, and then concentrated under reduced pressure. The crude product was first purified using a fluorous solid-phase extraction (F-SPE) column. Nonfluorous compounds were eluted with 80:20 MeOH/water and the fluorous product was eluted by 100% acetone. This acetone fraction was concentrated in vacuo and then purified by a silica gel column (toluene-EtOAc 7:1) to afford **15** (54 mg, 52% from **12**; 3 steps) as a white amorphous solid. TLC (toluene-EtOAc 5:1) Rf 0.28; $[\alpha]_D^{20}$ +4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96–7.81 (m, 6H, Ar), 7.56 (m, 1H, Ar), 7.50–7.16 (m, 23H, Ar), 7.07–6.90 (m, 8H, Ar), 6.74 (m, 2H, Ar), 6.64 (m, 2H, Ar), 5.80 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3D), 5.40 (s, 1H, PhCHO), 5.39 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1D), 5.36 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 7.2 Hz, H-2A), 5.35 (s, 1H, PhCHO), 5.26 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1B), 4.92 (d, 1H, $J_{1,2}$ = 6.7 Hz, H-1A), 4.85 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.6 Hz, H-2C), 4.72 (m, 2H, CH_2(Bn)), 4.65 (d, 1H, CH_2(Bn)), 4.55 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 8.7 Hz, H-3B), 4.44 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1C), 4.35 (d, 1H, CH_2(Bn)), 4.25 (br dd, 1H, H-6aA), 4.22–4.13 (m, 3H, H-2B, H-6aC, H-2D), 4.07 (dd, 1H, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 10.7 Hz, H-6aD), 3.99 (dd, 1H, $J_{5,6a}$ = 4.6 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6aB), 3.94 (dd, 1H, $J_{3,4}$ = 8.2 Hz, $J_{4,5}$ = 9.3 Hz, H–4A), 3.88 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4C), 3.78 (t, 1H, H-3A), 3.66 (s, 3H, Me (OMP)), 3.63 (t, 1H, $J_{4,5}$ = 9.0 Hz, H-4B), 3.58 (dd, 1H, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6bA), 3.57–3.43 (m, 4H, H-4D, H-5A, H-6bB, H-5D), 3.40 (t, 1H, H-3C), 3.36 (td, 1H, $J_{5,6b}$ = 9.5 Hz, H-5B), 3.26 (t, 1H, $J_{5,6b}$ = 10.4 Hz, H-6bD), 3.00 (dd, 1H, $J_{5,6b}$ = 2.6 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6bC), 2.69 (m, 1H, H-5C), 2.56–2.06 (m, 8H, –CH_2–CH_2–, CH_2(Lev)), 1.86 (s, 3H, CH_3(Lev)), 1.24 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.7, 177.8, 172.0, 170.2, 167.7, 165.1, 164.4 (10 × CO), 155.5–114.4 (Ar), 101.8, 101.5 (2 × PhCHO), 100.2 (C-1C), 99.7 (C-1A), 98.8 (C-1B), 97.7 (C-1D), 81.0 (C-4B), 80.7 (C-3A), 80.6 (C-3C), 79.1 (C-4D), 77.1 (C-4A), 75.7 (C-3B), 75.4 (C-4C), 74.3, 74.0 (2 × CH_2(Bn)), 73.6 (C-2C), 72.7 (C-2A), 72.5 (C-5C), 72.4 (C-5A), 69.7 (C-3D), 68.5, 68.4 (C-6B, C-6D), 66.2 (C-5B), 66.0 (C-5D), 62.5 (C-6A), 60.9 (C-6C), 55.7, 55.6 (C-2B, C-2D), 55.5 (Me (OMP)), 39.0 (C(CH_3)$_3$), 37.7 (CH_2(Lev)), 29.5 (CH_3(Lev)), 27.8 (CH_2(Lev)), 27.4 (C(CH_3)$_3$), 26.2 (t, $J_{C,F}$ = 22.0 Hz, –CH_2–CF_2–), 24.7 (–CH_2–); HR MS: *m*/*z*: calcd for $C_{110}H_{99}F_{17}N_2O_{30}Na$: 2273.5903; found: 2273.5871 [*M*+Na]$^+$.

*4.10. 4-Methoxyphenyl O-(2-deoxy-3-O-levulinoyl-2-phthalimido-β-D-glucopyranosyl)-(1 → 4)-O-(2-O-benzoyl-3-O-benzyl-6-O-pivaloyl-β-D-glucopyranosyl)-(1 → 3)-O-(2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 4)-2-O-benzoyl-3-O-benzyl-6-O-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecanoyl-β-D-glucopyranoside (**16**)*

TFA (150 μL) and H_2O (10 μL) were added to a solution of **15** (55 mg, 24 μmol) in CH_2Cl_2 (1.5 mL) at 0 °C. The solution was stirred for 2 h at room temperature, then diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography (toluene/acetone 4:1) to give **16** (48 mg, 95%) as a white amorphous solid. TLC (toluene/acetone 2:1) Rf 0.37; $[\alpha]_D^{20}$ +2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93–7.70 (m, 6H, Ar), 7.56–7.37 (m, 9H, Ar), 7.19–7.06 (m, 11H, Ar), 6.97 (m, 2H, Ar), 6.72 (m, 2H, Ar), 6.63 (m, 2H, Ar), 5.67 (dd, 1H, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 8.8 Hz, H-3D), 5.38 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1D), 5.34 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 7.7 Hz, H-2A), 5.05 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.4 Hz, H-2C), 5.02 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1B), 4.84 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1A), 4.76, 4.70, 4.62, 4.40 (4d, 4H, CH_2(Bn)), 4.37 (m, 2H, H-1C, H-6aC), 4.31 (dd, 1H, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 8.2 Hz, H-3B), 4.19–4.12 (m, 3H, H-2D, H-6aA, H-2B), 3.98 (br s, 1H, OH), 3.88 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.8 Hz, H-4A), 3.82 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.8 Hz, H-4C), 3.75–3.53 (m, 10H, H-3A, H-6bC, H-6aB, H-6aD, Me (OMP), H-4D, H-3C, H-6bA), 3.51–3.26 (m, 7H, H-5C, H-5D, H-6bB, H-6bD, H-4B, H-5A, H-5B), 3.18 (br s, 1H, OH), 2.63–2.18 (m, 8H, $-CH_2-CH_2-$, CH_2(Lev)), 1.98 (s, 3H, CH_3(Lev)), 1.12 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.3, 177.8, 173.1, 170.4, 168.2, 165.2, 164.6 (10 × CO), 155.6–114.4 (Ar), 101.1 (C-1C), 100.1 (C-1A), 98.3 (C-1B), 97.5 (C-1D), 81.9 (C-3B), 80.1 (C-3A, C-3C), 76.7 (C-4A), 75.9 (C-4C), 75.7 (C-5B), 75.6 (C-5D), 74.8, 74.6 (2 × CH_2(Bn)), 73.9 (C-5C, C-3D), 73.3 (C-2C), 72.7 (C-5A), 72.6 (C-2A), 70.7 (C-4B), 70.5 (C-4D), 62.9 (C-6A), 62.7 (C-6B or C-6D), 62.3 (C-6C), 62.0 (C-6B or C-6D), 55.5 (Me (OMP)), 55.2, 54.9 (C-2B, C-2D), 38.8 ($C(CH_3)_3$), 38.3 (CH_2(Lev)), 29.6 (CH_3(Lev)), 28.1 (CH_2(Lev)), 27.0 ($C(CH_3)_3$), 26.3 (t, $J_{C,F}$ = 21.7 Hz, $-CH_2-CF_2-$), 24.9 ($-CH_2-$); HR MS: *m*/*z*: calcd for $C_{96}H_{91}F_{17}N_2O_{30}Na$: 2097.5277; found: 2097.5282 $[M+Na]^+$.

*4.11. 4-Methoxyphenyl O-(2-deoxy-3-O-levulinoyl-2-phthalimido-4,6-di-O-sulfo-β-D-glucopyranosyl)-(1 → 4)-O-(2-O-benzoyl-3-O-benzyl-6-O-pivaloyl-β-D-glucopyranosyl)-(1 → 3)-O-(2-deoxy-2-phthalimido-4,6-di-O-sulfo-β-D-glucopyranosyl)-(1 → 4)-2-O-benzoyl-3-O-benzyl-6-O-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecanoyl-β-D-glucopyranoside (**1**)*

Compound **16** (22 mg, 11 μmol) and sulfur trioxide–trimethylamine complex (59 mg, 0.42 mmol) were dissolved in dry DMF (2.0 mL) and heated at 100 °C for 30 min using microwave radiation (18 W average power). The reaction vessel was cooled and Et_3N (300 μL), MeOH (1.5 mL) and CH_2Cl_2 (1.5 mL) were added. The solution was purified by Sephadex LH 20 chromatography (CH_2Cl_2-MeOH 1:1). The residue was submitted to a second sulfation cycle with new sulfating reagent (sulfur trioxide–trimethylamine complex, 59 mg, 0.42 mmol) in dry DMF (2 mL). Then, the reaction vessel was again cooled and Et_3N (300 μL), MeOH (1.5 mL) and CH_2Cl_2 (1.5 mL) were added. The solution was purified by Sephadex LH 20 chromatography (CH_2Cl_2-MeOH 1:1) and silica gel column chromatography (EtOAc-MeOH-H_2O 32:5:3 → EtOAc-MeOH-H_2O 24:5:3). The residue was finally eluted from a Dowex 50WX2-Na^+ column (MeOH) to obtain **1** as sodium salt (19 mg, 72%, white amorphous solid). TLC (EtOAc-MeOH-H_2O 24:5:3) Rf 0.27; ^{1}H NMR (400 MHz, CD_3OD): δ 7.96–7.79 (m, 6H, Ar), 7.61–7.35 (m, 12H, Ar), 7.06–6.94 (m, 10H, Ar), 6.77 (m, 2H, Ar), 6.68 (m, 2H, Ar), 5.83 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 8.9 Hz, H-3D), 5.45 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1D), 5.37 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1B), 5.26 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 8.5 Hz, H-2A), 5.17 (d, 1H, H-1A), 4.90 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 8.9 Hz, H-2C), 4.81, 4.78 (2d, 2H, CH_2(Bn)), 4.74 (m, 2H, H-6aB, H-3B), 4.62 (d, 1H, H-1C), 4.56 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 11.4 Hz, H-6aD), 4.52, 4.49 (2d, 2H, CH_2(Bn)), 4.37 (br dd, 1H, H-6aA), 4.34 (br dd, 1H, H-6aC), 4.31 (br t, 1H, H-4B), 4.29 (br dd, 1H, H-4D), 4.21–4.08 (m, 5H, H-3A, H-2D, H-2B, H-6bB, H-6bA), 4.05–3.96 (m, 4H, H-6bD, H-4C, H-5A, H-5B), 3.95 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4A), 3.81 (m, 1H, H-5D), 3.77 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6bC), 3.65 (s, 3H, Me (OMP)), 3.56 (t, 1H, $J_{3,4}$ = 8.6 Hz, H-3C), 3.44 (m, 1H, H-5C), 2.62–2.32 (m, 8H, $-CH_2-CH_2-$, CH_2(Lev)), 1.87 (s, 3H, CH_3(Lev)), 1.21 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_3OD): δ 208.8, 179.8, 173.9, 172.2, 169.9, 169.1, 167.2, 166.9 (10 × CO), 156.7–115.4 (Ar), 100.6 (C-1C), 100.4 (C-1A), 98.3 (C-1D), 96.7 (C-1B), 81.2 (C-3C), 78.9 (C-3A), 77.8 (C-4B), 77.1 (C-4C), 76.5, 76.4 (C-3B, C-4A), 75.9 (C-4D), 75.1, 75.0 (CH_2(Bn), C-2C, C-5B, C-5D), 74.6 (C-2A), 74.4 (C-5C), 73.9 (C-5A), 73.2 (CH_2(Bn)), 71.9 (C-3D), 68.8 (C-6B), 68.1 (C-6D), 64.6 (C-6A), 64.0 (C-6C), 57.4 (C-2B), 56.4 (C-2D), 55.9 (Me (OMP)), 39.9 ($C(CH_3)_3$), 38.5 (CH_2(Lev)), 29.2 (CH_3(Lev)), 29.1 (CH_2(Lev)), 27.8 ($C(CH_3)_3$), 27.1 (t, $J_{C,F}$ = 21.5 Hz, $-CH_2-CF_2-$), 25.9 ($-CH_2-$); ^{19}F NMR (376 MHz, CD_3OD): δ −82.30 (t, 3F), −115.56 (m, 2F), −122.71 (m, 6F), −123.66 (m, 2F), −124.33 (m, 2F), −127.19 (m, 2F); ESI MS: *m*/*z*: calcd for $C_{96}H_{87}F_{17}N_2O_{42}S_4Na_2^{2-}$: 1218.2; found: 1217.6 $[M+2Na]^{2-}$.

*4.12. 4-Methoxyphenyl O-(2-deoxy-3-O-levulinoyl-2-phthalimido-β-D-glucopyranosyl)-(1 → 4)-2-O-benzoyl-3-O-benzyl-6-O-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecanoyl-β-D-glucopyranoside (**17**)*

Donor **4** (161 mg, 0.251 mmol) and acceptor **2** (120 mg, 0.126 mmol) were dissolved in dry CH_2Cl_2 (4 mL) in the presence of activated 4 Å MS (300 mg). The reaction mixture was stirred, under an argon atmosphere, for 10 min at 0 °C and TBSOTf (295 μL of a 0.085 M solution in dry CH_2Cl_2) was added. After stirring for 40 min at 0 °C, the reaction mixture was quenched with triethylamine, filtered, and then concentrated under reduced pressure. The crude product was purified using a fluorous solid-phase extraction (F-SPE) column. Nonfluorous compounds were eluted with 80:20 MeOH/water and the fluorous product was eluted by 100% acetone. This acetone fraction was concentrated to give disaccharide **11** as a white amporphous solid (175 mg).

TFA (189 μL) and H_2O (30 μL) were added to a solution of **11** (175 mg, 0.122 mmol) in CH_2Cl_2 (1.9 mL) at 0 °C. The solution was stirred for 90 min at room temperature. Then, it was diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography (toluene/EtOAc 1:1) to give **17** (132 mg, 78% from **2**; 2 steps) as a white amorphous solid. TLC (toluene/EtOAc 1:1) Rf 0.29; $[\alpha]_D^{20}$ +2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.97 (m, 2H, Ar), 7.84 (m, 2H, Ar), 7.73 (m, 2H, Ar), 7.56 (m, 1H, Ar), 7.42 (m, 2H, Ar), 7.27–7.17 (m, 5H, Ar), 6.78 (m, 2H, Ar), 6.67 (m, 2H, Ar), 5.67 (dd, 1H, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 8.8 Hz, H-3′), 5.52 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1′), 5.42 (dd, 1H, $J_{1,2}$ = 7.1 Hz, $J_{2,3}$ = 8.2 Hz, H-2), 4.94 (d, 1H, H-1), 4.85, 4.73 (2d, 2H, CH_2(Bn)), 4.40 (dd, 1H, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.24 (dd, 1H, H-2′), 4.05 (dd, 1H, $J_{3,4}$ = 8.2 Hz, $J_{4,5}$ = 9.4 Hz, H-4), 3.86 (t, 1H, H-3), 3.80 (dd, 1H, $J_{5,6b}$ = 5.2 Hz, H-6b), 3.75 (m, 1H, H-6′a), 3.71–3.68 (m, 4H, H-4′, Me (OMP)), 3.62 (ddd, 1H, H-5), 3.51–3.47 (m, 2H, H-5′, H-6′b), 3.27 (br s, 1H, OH), 2.63–2.27 (m, 8H, $-CH_2-CH_2-$, CH_2(Lev)), 1.99 (s, 3H, CH_3(Lev)); ^{13}C NMR (125 MHz, $CDCl_3$): δ 207.3, 173.1, 170.5, 168.4, 167.8, 165.3 (6 × CO), 155.7–114.5 (Ar), 100.1 (C-1), 98.1 (C-1′), 80.3 (C-3), 76.8 (C-4), 75.7 (C-5′), 74.4 (CH_2(Bn)), 73.9 (C-3′), 72.9, 72.8 (C-2, C-5), 70.3 (C-4′), 63.2 (C-6), 62.1 (C-6′), 55.5 (Me (OMP)), 55.1 (C-2′), 38.2 (CH_2(Lev)), 29.5 (CH_3(Lev)), 28.1 (CH_2(Lev)), 26.3 (t, $J_{C,F}$ = 21.7

Hz, $-CH_2-CF_2-$), 25.0 ($-CH_2-$); HR MS: *m/z*: calcd for $C_{57}H_{50}F_{17}NO_{17}Na$: 1366.2699; found: 1366.2683 $[M+Na]^+$.

4.13. 4-Methoxyphenyl O-(2-deoxy-3-O-levulinoyl-2-phthalimido-4,6-di-O-sulfo-β-D-glucopyranosyl)-(1 → 4)-2-O-benzoyl-3-O-benzyl-6-O-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecanoyl-β-D-glucopyranoside (**18**)

Compound **17** (132 mg, 0.098 mmol) and sulfur trioxide–trimethylamine complex (273 mg, 1.96 mmol) were dissolved in dry DMF (6.5 mL) and heated at 100 °C for 30 min using microwave radiation (26 W average power). The reaction vessel was cooled and Et_3N (600 μL), MeOH (2 mL) and CH_2Cl_2 (2 mL) were added. The solution was purified by Sephadex LH 20 chromatography (CH_2Cl_2-MeOH 1:1) and the residue was eluted from a Dowex 50WX2-Na^+ column (MeOH) to obtain **18** as sodium salt (141 mg, 93%, white amorphous solid). TLC (EtOAc-MeOH-H_2O 24:5:3) Rf 0.34; ^{1}H NMR (400 MHz, CD_3OD): δ 8.00 (m, 2H, Ar), 7.80–7.45 (m, 7H, Ar), 7.11–7.04 (m, 5H, Ar), 6.83 (m, 2H, Ar), 6.72 (m, 2H, Ar), 5.88 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 8.8 Hz, H-3′), 5.71 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1′), 5.35 (br t, 1H, H-2), 5.24 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.87 (d, 1H, CH_2(Bn)), 4.65–4.60 (m, 2H, H-6′a, CH_2(Bn)), 4.53 (br dd, 1H, H-6a), 4.33 (br dd, 1H, H-4′), 4.29–4.20 (m, 3H, H-3, H-2′, H-6b), 4.16–4.08 (m, 3H, H-5, H-4, H-6′b), 3.98 (m, 1H, H-5′), 3.67 (Me (OMP)), 2.65–2.33 (m, 8H, $-CH_2-CH_2-$, CH_2(Lev)), 1.87 (s, 3H, CH_3(Lev)); ^{13}C NMR (100 MHz, CD_3OD): δ 208.9, 174.0, 172.3, 169.8, 169.1, 167.0 (6 x CO), 156.7–115.4 (Ar), 100.5 (C-1), 96.9 (C-1′), 79.2 (C-3), 76.6 (C-4), 76.1 (C-4′), 74.7 (C-5′), 74.4 (C-2), 73.9 (C-5), 73.2 (CH_2(Bn)), 71.8 (C-3′), 68.3 (C-6′), 64.6 (C-6), 56.4 (C-2′), 55.8 (Me (OMP)), 38.4 (CH_2(Lev)), 29.2, 29.1 (CH_3(Lev), CH_2(Lev)), 27.1 (t, $J_{C,F}$ = 21.4 Hz, $-CH_2-CF_2-$), 25.9 ($-CH_2-$); ^{19}F NMR (376 MHz, CD_3OD): δ −82.30 (t, 3F), −115.54 (m, 2F), −122.70 (m, 6F), −123.67 (m, 2F), −124.34 (m, 2F), −127.21 (m, 2F); ESI MS: *m/z*: calcd for $C_{57}H_{48}F_{17}NO_{23}S_2Na^-$: 1524.2; found: 1524.2 $[M+Na]^-$.

4.14. 4-Methoxyphenyl O-(2-acetamido-2-deoxy-4,6-di-O-sulfo-β-D-glucopyranosyl)-(1 → 4)-3-O-benzyl-β-D-glucopyranoside (**19**)

Ethylene diamine (130 μL, 1.94 mmol) was added to a solution of **18** (20 mg, 13 μmol) in *n*-BuOH (1.5 mL) under an argon atmosphere, and the reaction mixture was subjected to microwave irradiation (30 W average power) for 90 min at 120 °C (3 cycles, 30 min each). The reaction vessel was cooled, and the mixture was concentrated to dryness. The residue was dissolved in MeOH (2.5 mL) and an aqueous solution of NaOH (4 M, 645 μL) was added. After stirring for 19 h at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give the desired amine intermediate. Triethylamine (24 μL, 0.17 mmol) and acetic anhydride (24 μL, 0.26 mmol) were added to a cooled (0 °C) solution of this amine derivative in MeOH (2.5 mL). After stirring for 2 h at room temperature, additional triethylamine (24 μL, 0.17 mmol) and acetic anhydride (24 μL, 0.26 mmol) were added at 0 °C to complete the reaction. After further stirring for 3 h at room temperature, Et_3N (300 μL) was added and the mixture was concentrated to dryness. The residue was then purified by silica gel column choromatography (EtOAc-MeOH-H_2O 40:5:3 → EtOAc-MeOH-H_2O 24:5:3) and finally eluted from a Dowex 50WX2-Na^+ column (H_2O) to obtain **19** as sodium salt (7.7 mg, 76%, white amorphous solid). TLC (EtOAc-MeOH-H_2O 24:5:3) Rf 0.26; ^{1}H NMR (400 MHz, D_2O): δ 7.56–7.39 (m, 5H, Ar), 7.12 (m, 2H, Ar), 7.00 (m, 2H, Ar), 5.03 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.99, 4.94 (2d, 2H, CH_2(Bn)), 4.66 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1′), 4.41 (dd, 1H, $J_{5,6a}$ = 1.9 Hz, $J_{6a,6b}$ = 11.4 Hz, H-6′a), 4.13 (dd, 1H, $J_{3,4}$ = 8.6 Hz, $J_{4,5}$ = 9.8 Hz, H-4′), 3.94 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4), 3.92–3.80 (m, 7H, H-6a, H-6′b, H-2′, H-3, Me (OMP)), 3.78 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-3′), 3.75–3.66 (m, 4H, H-2, H-6b, H-5, H-5′), 2.05 (s, 3H, NHAc); ^{13}C NMR (100 MHz, D_2O; selected data from HSQC experiment)): δ 100.8 (C-1), 99.8 (C-1′), 81.4 (C-3), 76.8 (C-4′), 75.1 (C-5′), 74.9 (C-4), 73.5 (CH_2(Bn)), 72.2 (C-2, C-5), 71.9 (C-3′), 67.1 (C-6′), 59.9 (C-6), 55.8 (Me (OMP)), 55.5 (C-2′), 21.8 (NHAc); ESI MS: *m/z*: calcd for $C_{28}H_{35}NO_{18}S_2Na^-$: 760.1; found: 760.0 $[M+Na]^-$.

4.15. 4-Methoxyphenyl O-(2-acetamido-2-deoxy-4,6-di-O-sulfo-β-D-glucopyranosyl)-(1→4)-β-D-glucopyranoside (**20**)

A solution of **19** (7.6 mg, 9.7 μmol, sodium salt) in H_2O/MeOH (4.5 mL/0.5 mL) was hydrogenated in the presence of 20% Pd $(OH)_2$/C (15 mg). After 24 h, the suspension was filtered over Celite and concentrated. The residue was purified by Sephadex LH 20 chromatography column which was eluted with H_2O-MeOH (9:1) to obtain **20**. This compound was then dissolved in H_2O (2 mL) and Amberlite IR-120H$^+$ resin was added (pH 3.0). The mixture was immediately filtered and treated with 0.04 M NaOH (pH 7.1) to obtain **20** as a white amorphous solid (5.1 mg, sodium salt, 75%) after lyophilization. ^{1}H NMR (400 MHz, D_2O): δ 7.12 (m, 2H, Ar), 6.99 (m, 2H, Ar), 5.06 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.67 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1′), 4.55 (dd, 1H, $J_{5,6a}$ = 2.0 Hz, $J_{6a,6b}$ = 11.4 Hz, H-6′a), 4.23 (dd, 1H, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 9.7 Hz, H-4′), 4.18 (dd, 1H, $J_{5,6b}$ = 7.9 Hz, H-6′b), 3.94 (m, 1H, H-5′), 3.92–3.81 (m, 6H, H-2′, H-6a, H-3′, Me (OMP)), 3.78 (br dd, 1H, H-3), 3.72–3.63 (m, 3H, H-5, H-6b, H-4), 3.59 (dd, 1H, $J_{2,3}$ = 9.3 Hz, H-2), 2.08 (s, 3H, NHAc); ^{13}C NMR (100 MHz, D_2O; selected data from HSQC experiment)): δ 101.4 (C-1′), 100.5 (C-1), 79.6 (C-4), 76.6 (C-4′), 74.5 (C-3, C-5), 72.3 (C-5′), 72.2 (C-2), 71.7 (C-3′), 67.2 (C-6′), 59.5 (C-6), 55.6 (Me (OMP)), 55.1 (C-2′), 21.8 (NHAc); ESI MS: *m/z*: calcd for $C_{21}H_{29}NO_{18}S_2Na^-$: 670.1; found: 669.9 $[M+Na]^-$.

4.16. Fluorescence polarization assays

Fluorescence polarization measurements were performed in 384-well microplates (black polystyrene, non-treated, Corning). The fluorescence polarization was recorded using a TRIAD multimode microplate reader (from Dynex), with excitation and emission wavelengths of 485 and 535 nm, respectively. The fluorescent probe (a fluorescein labelled heparin-like hexasaccharide previously prepared in our lab)[54] was dissolved in PBS buffer (10 mM, pH 7.4). Recombinant human midkine and FGF-2 (Peprotech) were dissolved in PBS buffer (10 mM, pH 7.4) containing 1% BSA (bovine serum albumin). Compounds **19** and **20** were dissolved in PBS buffer (10 mM, pH 7.4). 1 mM stock solutions of compounds **1** and **18** were prepared in PBS/DMSO 9:1 (v/v) and serial dilutions were then performed in PBS buffer (10 mM, pH 7.4).

For the determination of the IC_{50} values, we recorded the fluorescence polarization from wells containing 20 μL of protein solution (125 nM midkine solution or 194 nM FGF-2 solution) and 10 μL of a 40 nM probe solution in the presence of 10 μL of inhibitor solution, with concentrations ranging from 100 μM to 50 nM. The microplate was shaked in the dark for 5 min, before reading. The total sample volume in each well was 40 μL and the final buffer composition was PBS + 0.5% BSA. The final concentrations of fluorescent probe and midkine/FGF-2 in each well were 10 nM and 63/97 nM, respectively, while the final inhibitor concentration ranged from 25 μM to 12.5 nM. The average polarization values of three replicates were plotted against the logarithm of inhibitor concentration. Two control samples were included in the competition experiment. The first one only contained fluorescent probe and afforded the expected minimum polarization value for 100% inhibition; the second one contained midkine/FGF-2 and probe, in the absence of inhibitor, and gave the maximum polarization value corresponding to 0% inhibition. Blank wells contained 20 μL

of protein solution and 20 μL of a 50 μM inhibitor solution and their measurements were subtracted from all values. The curve was fitted to the equation for a one-site competition: $y = A_2 + (A_1 - A_2)/[1 + 10^{(x - \log IC_{50})}]$ where A_1 and A_2 are the maximal and minimal values of polarization, respectively, and IC_{50} is the inhibitor concentration that results in 50% inhibition. At least three independent experiments were carried out for each IC_{50} calculation.

Acknowledgments

We thank the Spanish Ministry of Economy and Competitiveness (CTQ2015-70134-P), Junta de Andalucía (P12-BIO-1938) and the European Union (ERDF) for financial support.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.01.022.

References

1. Pomin VH, Mulloy B. *Curr Opin Struct Biol*. 2015;34:17–25.
2. Meneghetti MCZ, Hughes AJ, Rudd TR, et al. *J R Soc Interface*. 2015;12:20150589.
3. Xu D, Esko JD. *Annu Rev Biochem*. 2014;83:129–157.
4. Mizumoto S, Yamada S, Sugahara K. *Curr Opin Struct Biol*. 2015;34:35–42.
5. Stern R, Asari AA, Sugahara KN. *Eur J Cell Biol*. 2006;85:699–715.
6. Hahm HS, Broecker F, Kawasaki F, et al. *Chemistry*. 2017;2:114–124.
7. Sankarayanarayanan NV, Strebel TR, Boothello RS, et al. *Angew Chem Int Ed*. 2017;56:2312–2317.
8. Zong C, Venot A, Li X, et al. *J Am Chem Soc*. 2017;139:9534–9543.
9. (a) Jayson GC, Hansen SU, Miller GJ, et al. *Chem Commun*. 2015;51:13846–13849;
(b) Copeland R, Balasubramaniam A, Tiwari V, et al. *Biochemistry*. 2008;47:5774–5783;
(c) Boothello RS, Sarkar A, Tran VM, et al. *ACS Chem Biol*. 2015;10:1485–1494;
(d) Patel NJ, Sharon C, Baranwal S, Boothello RS, Desai UR, Patel BB. *Oncotarget*. 2016;7:84608–84622;
(e) Guglier S, Hricovíni M, Raman R, et al. *Biochemistry*. 2008;47:13862–13869.
10. Petitou M, van Boeckel CAA. *Angew Chem Int Ed*. 2004;43:3118–3133.
11. Rogers CJ, Clark PM, Tully SE, et al. *PNAS*. 2011;108:9747–9752.
12. Gama CI, Tully SE, Sotogaku N, et al. *Nat Chem Biol*. 2006;2:467–473.
13. Mende M, Bednarek C, Wawryszyn M, et al. *Chem Rev*. 2016;116:8193–8255.
14. Weiss RJ, Esko JD, Tor Y. *Org Biomol Chem*. 2017;15:5656–5668.
15. Dominguez-Rodriguez P, Reina JJ, Gil-Caballero S, Nieto PM, de Paz JL, Rojo J. *Chem Eur J*. 2017;23:11338–11345.
16. Loka RS, Yu F, Sletten ET, Nguyen HM. *Chem Commun*. 2017;53:9163–9166.
17. Tyler PC, Guimond SE, Turnbull JE, Zubkova OV. *Angew Chem Int Ed*. 2015;54:2718–2723.
18. Oh YI, Sheng GJ, Chang S-K, Hsieh-Wilson LC. *Angew Chem Int Ed*. 2013;52:11796–11799.
19. Sheng GJ, Oh YI, Chang S-K, Hsieh-Wilson LC. *J Am Chem Soc*. 2013;135:10898–10901.
20. Liu P, Chen L, Toh JKC, et al. *Chem Sci*. 2015;6:450–456.
21. de Paz JL, Noti C, Bohm F, Werner S, Seeberger PH. *Chem Biol*. 2007;14:879–887.
22. Johnstone KD, Karoli T, Liu L, et al. *J Med Chem*. 2010;53:1686–1699.
23. Ferro V, Liu L, Johnstone KD, et al. *J Med Chem*. 2012;55:3804–3813.
24. Liu L, Li C, Cochran S, Jimmink S, Ferro V. *ChemMedChem*. 2012;7:1267–1275.
25. Henry BL, Connell J, Liang A, Krishnasamy C, Desai UR. *J Biol Chem*. 2009;284:20897–20908.
26. Al-Horani RA, Karuturi R, White DT, Desai UR. *Molecules*. 2015;20:608–624.
27. Raman K, Karuturi R, Swarup VP, Desai UR, Kuberan B. *Bioorg Med Chem Lett*. 2012;22:4467–4470.
28. Huang T-Y, Irene D, Zulueta MML, et al. *Angew Chem Int Ed*. 2017;56:4192–4196.
29. (a) Karuturi R, Al-Horani RA, Mehta SC, Gailani D, Desai UR. *J Med Chem*. 2013;56:2415–2428;
(b) Liu J, Pervin A, Gallo CM, Desai UR, Van Gorp CL, Linhardt RJ. *J Pharm Sci*. 1994;83:1034–1039;
(c) Ekblad M, Adamiak B, Bergstrom T, et al. *Antiviral Res*. 2010;86:196–203;
(d) Afosah DK, Al-Horani RA, Sankaranarayanan NV, Desai UR. *J Med Chem*. 2017;60:641–657.
30. de Paz JL, Nieto PM. *Org Biomol Chem*. 2016;14:3506–3509.
31. Huang W, Gao Q, Boons GJ. *Chem Eur J*. 2015;21:12920–12926.
32. Tang SL, Pohl NLB. *Org Lett*. 2015;17:2642–2645.
33. Jaipuri FA, Pohl NL. *Org Biomol Chem*. 2008;6:2686–2691.
34. Manzoni L, Castelli R. *Org Lett*. 2004;6:4195–4198.
35. Zhang F, Zhang W, Zhang Y, Curran DP, Liu G. *J Org Chem*. 2009;74:2594–2597.
36. Ko KS, Park G, Yu Y, Pohl NL. *Org Lett*. 2008;10:5381–5384.
37. Yang B, Jing YQ, Huang XF. *Eur J Org Chem*. 2010;1290–1298.
38. Zong C, Venot A, Dhamale O, Boons G-J. *Org Lett*. 2013;15:342–345.
39. Bhaduri S, Pohl NLB. *Org Lett*. 2016;18:1414–1417.
40. Cai C, Dickinson DM, Li L, et al. *Org Lett*. 2014;16:2240–2243.
41. Zhang W, Curran DP. *Tetrahedron*. 2006;62:11837–11865.
42. Karst N, Jacquinet JC. *Eur J Org Chem*. 2002;815–825.
43. Kayser MM, de Paz JL, Nieto PM. *Eur J Org Chem*. 2010;2138–2147.
44. Macchione G, de Paz JL, Nieto PM. *Carbohydr Res*. 2014;394:17–25.
45. Yu BA, Sun JS. *Chem Commun*. 2010;46:4668–4679.
46. Maza S, de Paz JL, Nieto PM. *Tetrahedron Lett*. 2011;52:441–443.
47. Maza S, Macchione G, Ojeda R, et al. *Org Biomol Chem*. 2012;10:2146–2163.
48. Muramatsu T. *Curr Pharm Des*. 2011;17:410–423.
49. Kadomatsu K, Kishida S, Tsubota S. *J Biochem*. 2013;153:511–521.
50. Lim J, Yao S, Graf M, Winkler C, Yang D. *Biochem J*. 2013;451:407–415.
51. Matsui T, Ichihara-Tanaka K, Lan C, et al. *Int Arch Med*. 2010;3:12.
52. Solera C, Macchione G, Maza S, et al. *Chem Eur J*. 2016;22:2356–2369.
53. Macchione G, Maza S, Kayser MM, de Paz JL, Nieto PM. *Eur J Org Chem*. 2014;3868–3884.
54. Maza S, Mar Kayser M, Macchione G, et al. *Org Biomol Chem*. 2013;11:3510–3525.
55. Noti C, de Paz JL, Polito L, Seeberger PH. *Chem Eur J*. 2006;12:8664–8686.
56. Deepa SS, Umehara Y, Higashiyama S, Itoh N, Sugahara K. *J Biol Chem*. 2002;277:43707–43716.
57. (a) Faham S, Hileman RE, Fromm JR, Linhardt RJ, Rees DC. *Science*. 1996;271:1116–1120;
(b) Li YC, Ho IH, Ku CC, et al. *ACS Chem Biol*. 2014;9:1712–1717.