Supporting information

Synthesis, Radiolabeling with Fluorine-18 and Preliminary *in vivo* Evaluation of a Heparan Sulphate Mimetic as Potent Angiogenesis and Heparanase Inhibitor for Cancer Applications

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1. General.

1.1 Chemicals.

Chemicals were purchased from Aldrich, Fluka, Sigma (France) and were used without further purification.

1.2 HPLCs.

[HPLC A]: Equipment: a 510 pump (Waters), a SPD10-AVP UV-multi-wavelength detector (Shimadzu) and a Geiger-Müller detector; column: semipreparative SiO_2 Zorbax® Rx-SIL, (250 x 9.4 mm; 5 µm, Hewlett Packard); solvents and conditions: isocratic elution with Heptane/EtOAc : 60/40 (v:v); flow rate: 6 mL/min; temperature: RT; absorbance detection at $\lambda = 254$ nm.

[HPLC B] : Equipment: a 600 Controller Gradient system (Waters), a 1100 series UV-multi-wavelength detector (Hewlett Packard) and a Flow One Scintillation Analyzer equipped with a positron-dedicated cell for radioactivity monitoring (Packard); column : analytical Venusil XBP-C18 (250 x 4.6 mm; 5 µm, Agela Technologies); solvents and conditions: solvent A : aq. 25 mM NaOAc, solvent B : 25 mM NaOAc in MeOH; gradient elution: conditions B_1 : linear 10 min from 70/30 to 50/50 (A/B, v:v) then linear 10 min from 50/50 to 15/85; conditions B_2 : linear 10 min from 60/40 to 50/50 (A/B, v:v) then linear 10 min from 50/50 to 20/80; flow rate : 1.0 mL/min; temperature: RT; absorbance detection at $\lambda = 210$ nm.

HPLC retention times for compounds **10**, **11**, **12** and **14** are summarized in Table S1.

1.3 TLCs.

TLCs were run on pre-coated plates of silica gel $60F_{254}$ (VWR). Compounds were localized when possible at 254 nm using a UV-lamp. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyser (Berthold).

1.4 Spectroscopies.

NMR spectra were recorded on a Advance 400 MHz apparatus (Bruker) using the hydrogenated residue of the deuterated solvents ($CHDCl_2$ ($\delta = 5.32$ ppm)) as internal standards for ^{1}H-NMR as well as the deuterated solvents (CD_2Cl_2 ($\delta = 54.0$ ppm)) as internal standard for ^{13}C-NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, q, q^5 and m for singlet, doublet, triplet, quadruplet,

quintuplet and multiplet, respectively). The mass spectra for non-saccharide compounds (MS) were measured on an Ion Trap LCQ Deca XP+ spectrometer (ESI+) (Thermo Electron). For polysaccharides, mass spectra were recorded on an Applied Biosystems/ABI Q-STAR® system. Samples concentration were fixed to 1mg/mL in a 1/1 mixture of A/B (A : DBA 5 mM, AcOH 8 mM in water; B : DBA 30 mM, AcOH 30 mM in ACN). Resulting samples were diluted to 1/50 with water.

Mass analyses for compounds **10**, **11**, **12** and **14** are summarized in Table S1.

1.5 Radioisotope availability and handling.

Fluorine-18, as no-carrier-added aqueous [^{18}F]fluoride ion was produced *via* the [^{18}O(p,n)^{18}F] nuclear reaction by irradiation of a 2 mL [^{18}O]water target (> 97%-enriched, CortecNet) on an Cyclone-18/9 cyclotron (18 MeV proton beam, IBA) and was transferred to the appropriate hot cell. *Target hardware* : commercial, 2-mL, two-port, stainless steel target holder equipped with a domed-end niobium cylinder insert. *Target to hot cell liquid-transfer system* : 60 m PTFE line (0.8 mm internal diameter ; 1/16 inch external diameter), 2.0 bar helium drive pressure, transfer time 3-6 min. Typical production of [^{18}F]fluoride ion at the end of bombardment for a 20 µA, 30 min (10 µA.h) irradiation: 27-30 GBq. Radiosyntheses using fluorine-18, including the HPLC purifications, were performed in a 7.5-cm-lead shielded cell using a computer assisted Zymate robot system (Zymark Corporation).

2. Chemistry.

Compound **10**.

To a solution of copper (II) sulfate (50 µL, 0.45 M), sodium L-ascorbate (50 µL, 1.5 M) in a mixture of water and *t*-BuOH (1 mL, 50/50, v:v) was added compound **8** (5 mg, 1.4 µmol) and *S*-(4-azidobutyl)thioacetate (**9**, 0.5 mg, 2.8 µmol, 2 equiv). The solution was left standing at rt for 1h. Compound **10** was purified by HPLC ([HPLC B], conditions B$_2$: *Rt* = 13.4 min). The fractions corresponding to **10** were collected, combined, concentrated and finally desalted using a BioRad G25 SEC column. After lyophilisation, 4.6 mg of **10** were recovered as a white solid (92% yield). Mass calculated for : $C_{121}H_{147}N_7O_{78}S_{13}$ = 3361.4121; ESI/MS, negative mode, *m/z* : 671.7616 [M-5H]$^{5-}$.

Compounds **11** and **12**.

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Method A. Compound **10** (1 mg, 0.269 µmol) in solution in deionized water (25 µL) was treated with a freshly prepared aq. 1 M solution of LiOH (25 µL, 25 µmol, 93 equiv). The mixture was strongly vortexed and reacted at rt for 12 h. A unique compound could be detected and collected by HPLC ([HPLC B], conditions B_2 : Rt = 14.1 min). The corresponding fractions were combined, concentrated and desalted using a BioRad G25 SEC column. After lyophilisation, 0.7 mg of a compound identified as **11** (disulphide bridged dimer) by ESI/MS were recovered as a white solid (37%yield). Mass calculated for: $C_{238}H_{288}N_{14}O_{154}S_{26}$ = 6636.7874; ESI/MS, negative mode, m/z : 1321.7002 $[M+10DBA-16H]^{6-}$, 1114.1511 $[M+9DBA-16H]^{7-}$, 1077.2263 $[M+7DBA-14H]^{7-}$, 751.2876 $[M+DBA-10H]^{9-}$.

To restore the free sulfhydryl monomer **12**, compound **11** (0.7 mg, 0.0925 µmol) in deionized water (50 µL) was treated with an excess of a 3.75 mM buffered solution of TCEP (*tris*(2-carboxyethyl)phosphine) in PBS 100 mM, pH 7.4 (700 µL). After 10 min at rt, the mass analysis of the reaction showed to complete reduction of the disulphide bridge providing compound **12** (quantitative yield).

Method B. Compound **10** (1 mg, 0.269 µmol) in solution in deionized water (25 µL) was treated with a 50 mM buffered solution of hydroxylamine in PBS 100 mM, pH 7.4 (60 µL, 3 µmol, 11.2 equiv) at rt for 45 min. After this period of time, HPLC analyses ([HPLC B], conditions B_2) showed a complete deacetylation of **10** to afford a unique peak detected by HPLC ([HPLC B], conditions B_2 : Rt = 11.8 min) identified as compound **12** by mass analysis. The crude reaction mixture was then purified by HPLC and the fractions corresponding to **12**, collected, combined, concentrated and desalted using a BioRad G25 SEC column. Compound **12** was obtained as a white solid (0.6 mg) after lyophilisation (66%yield). Mass calculated for: $C_{119}H_{145}N_7O_{77}S_{13}$ = 3319.4015; ESI/MS, negative mode, m/z : 1365.1156 $[M+6DBA-9H]^{3-}$, 1322.0239 $[M+5DA-8H]^{3-}$, 926.1738 $[M+3DBA-7H]^{4-}$, 689.0743 $[M+DBA-6H]^{5-}$, 663.4343 $[M-5H]^{5-}$.

Unfortunately, HPLC analyses showed a rapid oxidization of **12** into **11** (a 50/50 mixture could be observed after a few hours only, based on peak areas).

For the preparation of compound **14**, *S*-acetylated octasaccharide **10** will be treated according to method B. Compound **12** will not be isolated but directly reacted *in situ* with the appropriate solution of **13**.

Compound **13**.

FPyME (**13**, or 1-[3-(2-fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione) was synthesized in three steps from (3-hydroxypropyl)carbamic acid *tert*-butyl ester and 2-fluoro-3-hydroxypyridine as previously described [de Bruin, 2005]. ^{1}H-NMR (CD$_2$Cl$_2$, 298.0K) : δ: 7.69 (bd, $J = 3.0$ Hz, 1H); 7.27 (t, $J = 9.0$ Hz, 1H); 7.11 (dd, $J = 9.0$ & 3.0 Hz, 1H); 6.69 (s, 2H); 4.04 (t, $J = 6.0$ Hz, 2H); 3.72 (t, $J = 6.0$ Hz, 2H); 2.11 (q^5, $J = 6.0$ Hz, 2H). ^{13}C-NMR (CD$_2$Cl$_2$, 298.0K) : δ: 171.2 (2×C); 154.1 (d, $J^1_{F-C} = 235$ Hz, C); 142.4 (d, $J^2_{F-C} = 25$ Hz, C); 137.7 (d, $J^3_{F-C} = 13$ Hz, CH); 134.5 (2×CH); 123.2 (CH); 122.2 (CH); 67.5 (CH$_2$); 35.4 (CH$_2$); 28.4 (CH$_2$). MS (DCI/NH$_4^+$) C$_{12}$H$_{11}$F$_1$N$_2$O$_3$: 251 [M + H$^+$].
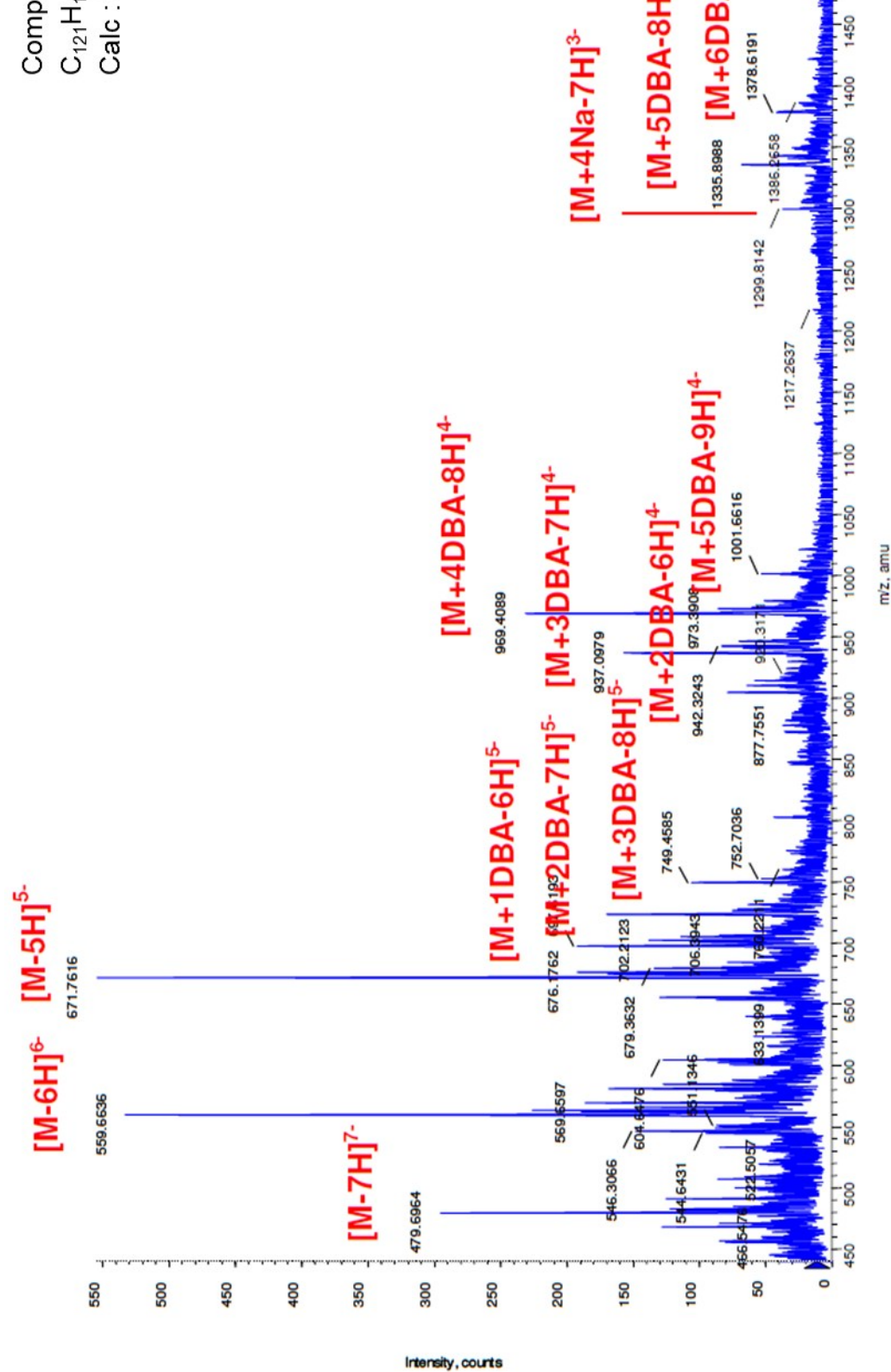
Compound **14**.

Compound **10** (1 mg, 0.269 µmol) in solution in deionized water (25 µL) was treated with a 50 mM buffered solution of hydroxylamine in PBS 100 mM, pH 7.4 (60 µL, 3 µmol, 11.2 equiv) at rt for 45 min. After this period of time, FPyME (**13**, 0.2 mg, 0.79 µmol, 3 equiv based on **10**) in solution in DMSO (10 µL) was added to the mixture and further reacted at rt for 15 min. Compound **14** was purified by HPLC ([HPLC B, Conditions B$_1$: Rt = 18.4 min) as a large peak due to the presence of 2 diastereoisomers, which cannot be separated in these HPLC conditions. After desalting using a BioRad G25 SEC column and lyophilisation, **14** (0.6 mg) could be obtained as a white solid (63%yield). Mass calculated for C$_{131}$H$_{156}$FN$_9$O$_{80}$S$_{13}$ = 3569.4769; ESI/MS, negative mode, m/z : 1448.2685 [M+6DBA-9H]$^{3-}$, 1021.0988 [M+4DBA-8H]$^{4-}$, 989.0640 [M+3DBA-7H]$^{4-}$, 713.3445 [M-5H]$^{5-}$, 594.2935 [M-6H]$^{6-}$. The retention time of compound **13** is 17.0 min using the same HPLC conditions.
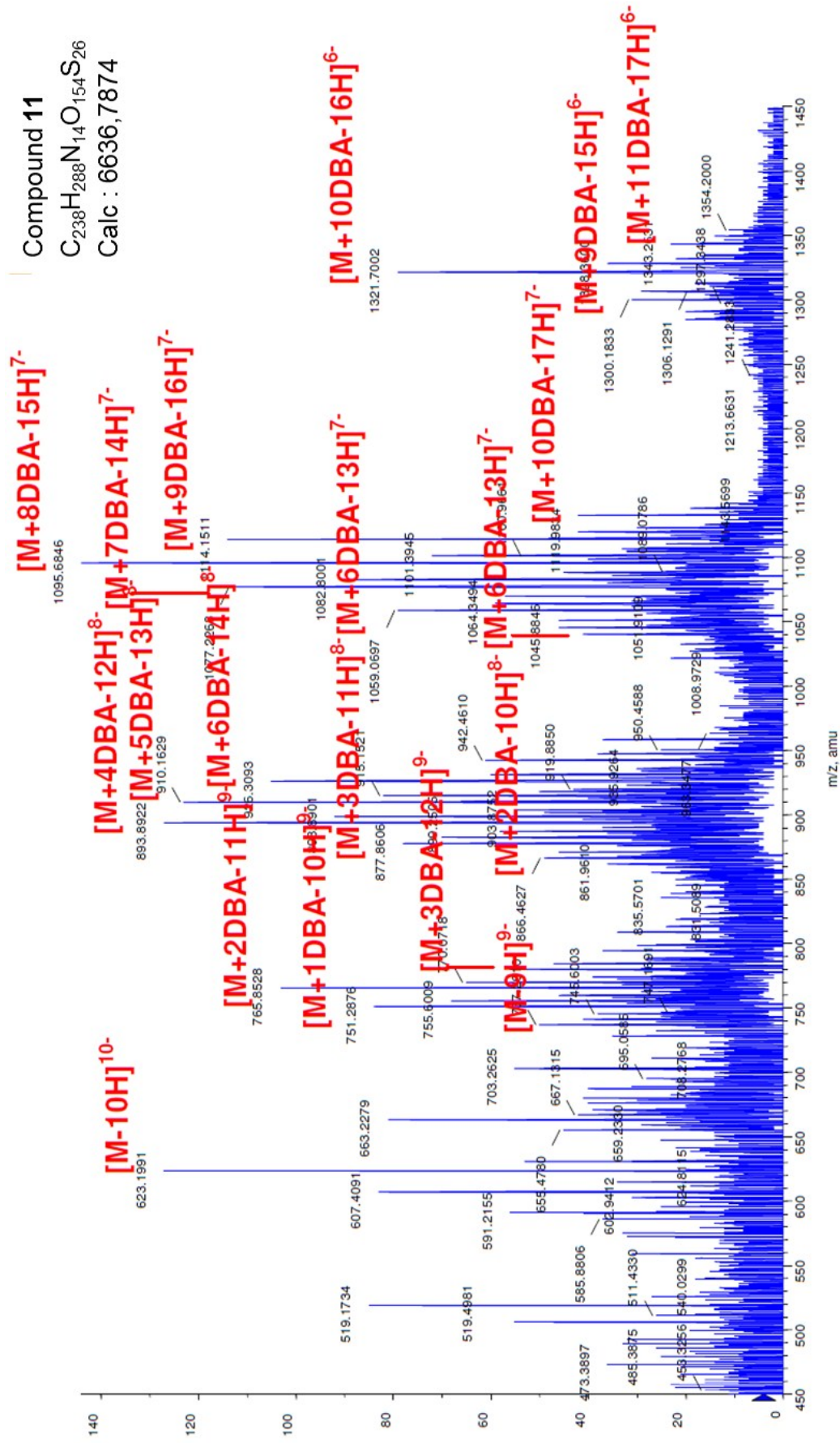
Compound **15**.

The trimethylammonium trifluoromethanesulfonate derivative **15** ([3-(3-*tert*-butoxycarbonylaminopropoxy)-pyridin-2-yl]-trimethylammonium trifluoromethanesulfonate) as precursor for fluorine-18-labeling was synthesized in two steps from commercially available (3-hydroxypropyl)carbamic acid *tert*-butyl ester and 2-dimethylamino-3-hydroxypyridine as previously described [de Bruin, 2005]. ^{1}H-NMR (CD$_2$Cl$_2$, 298K) : δ: 8.10 (bd, $J = 3.3$ Hz, 1H); 7.66 (d, $J = 8.1$ Hz, 1H); 7.60 (dd, $J = 6.1$ & 4.2 Hz, 1H); 4.31 (t, $J = 6.3$ Hz, 2H); 3.71 (s, 9H); 3.31 (q, $J = 6.3$ Hz, 2H); 2.12 (q^5, $J = 6.3$ Hz, 2H); 1.38 (s, 9H). ^{13}C-NMR (CD$_2$Cl$_2$, 298K) : δ: 156.6 [C]; 147.7 [C]; 142.6 [C]; 139.0 [CH]; 129.0 [CH]; 124.6 [CH]; 121.2 [q, $J = 319$ Hz, CF$_3$]; 79.3 [C]; 68.1 [CH$_2$]; 54.8 [3×CH$_3$]; 37.5 [CH$_2$]; 30.0 [CH$_2$]; 28.4 [3×CH$_3$].

Table S1 : analytical data (MS and HPLC) of compounds **10**, **11**, **12** and **14**.
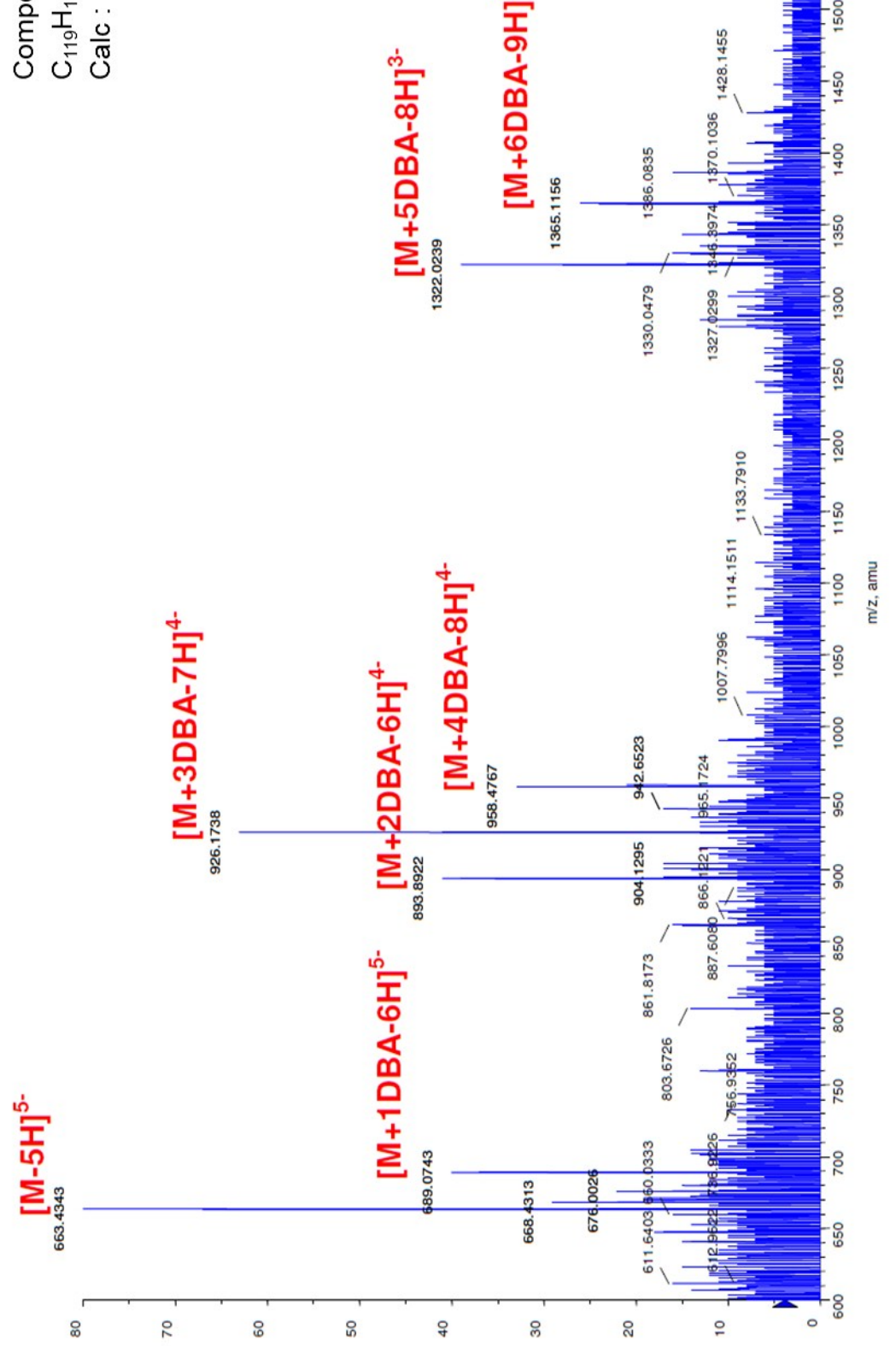
Compound #	Mass analyses		HPLC B (Rt in min)	
	Calculated	Measured	Conditions B_1	Conditions B_2
10	3361.4121 $C_{121}H_{147}N_7O_{78}S_{13}$	559.6636 [M-6H][6-], 671.7616 [M-5H][5-]	16.5	13.4
11	6636.7874 $C_{238}H_{288}N_{14}O_{154}S_{26}$	1321.7002 [M+10DBA-16H][6-], 1114.1511[M+9DBA-16H][7-], 1077.2263 [M+7DBA-14H][7-], 751.2876 [M+DBA-10H][9-]	17.2	14.1
12	3319.4015 $C_{119}H_{145}N_7O_{77}S_{13}$	1365.1156 [M+6DBA-9H][3-], 1322.0239 [M+5DA-8H][3-], 926.1738 [M+3DBA-7H][4-], 689.0743 [M+DBA-6H][5-], 663.4343 [M-5H][5-]	15.8	11.8
14	3569.4769 $C_{131}H_{156}FN_9O_{80}S_{13}$	1448.2685 [M+6DBA-9H][3-], 1021.0988 [M+4DBA-8H][4-], 989.0640 [M+3DBA-7H][4-], 713.3445 [M-5H][5-], 594.2935 [M-6H][6-]	18.4	n.d

Comp...

C₁₂₁H... → $C_{121}H$

Calc :

[M-7H]⁷⁻ → $[M-7H]^{7-}$
479.6964

[M-6H]⁶⁻ → $[M-6H]^{6-}$
559.6636

[M-5H]⁵⁻ → $[M-5H]^{5-}$
671.7616

[M+1DBA-6H]⁵⁻ → $[M+1DBA-6H]^{5-}$
676.1762

[M+2DBA-7H]⁵⁻ → $[M+2DBA-7H]^{5-}$
702.2123

[M+3DBA-8H]⁵⁻ → $[M+3DBA-8H]^{5-}$
749.4585

[M+4DBA-8H]⁴⁻ → $[M+4DBA-8H]^{4-}$
969.4089

[M+3DBA-7H]⁴⁻ → $[M+3DBA-7H]^{4-}$
937.0979

[M+2DBA-6H]⁴⁻ → $[M+2DBA-6H]^{4-}$
942.3243

[M+5DBA-9H]⁴⁻ → $[M+5DBA-9H]^{4-}$
973.3908

[M+4Na-7H]³⁻ → $[M+4Na-7H]^{3-}$
1299.8142

[M+5DBA-8H... → $[M+5DBA-8H$
1335.8988

[M+6DB... → $[M+6DB$
1378.6191
1386.2658

1217.2637

1001.6616
981.317
877.7551
752.7036
766.2211
706.3943
679.3632
633.1399
604.6476
551.1346
546.3066
544.6431
522.5057
466.5...
569.6597

Intensity, counts

m/z, amu

8

Compound **11**
$C_{238}H_{288}N_{14}O_{154}S_{26}$
Calc : 6636,7874

Compo
C$_{131}$H$_{15}$
Calc : 3

[M1+6DBA-9H]

[M1+5DBA-8H]$^{3-}$
1448.2685

1410.3852

1405.1914

1375.1998

1282.4593

1239.7625

[M1+4DBA-8H]$^{4-}$

[M1+3DBA-7H]$^{4-}$

1113.9580

1031.3662

1025.0998

1021.0988

1004.5378

993.0579

989.0640

957.7850

956.7685

902.2482

897.2759

881.7029

746.9866

739.1720

721. 413

716.5463

679.3516

[M1-5H]$^{5-}$
713.3445

[M1-6H]$^{6-}$
594.2935

640.5091

544.4042

533. 04

50 .3457

m/z, amu

3. Radiochemistry.

3.1 Preparation of the K[^{18}F]F-K$_{222}$ complex.

Fluorine-18 (half-life: 109.8 min) as [^{18}F]fluoride ion was isolated by passing the irradiated [^{18}O]water target, using helium pressure (1.5-2.0 bar), through an anion exchange resin (Sep-pak® Eight Accell™ Plus QMA cartridge (OH⁻ form, generated from the Cl⁻ form by washing with aq. 1M NaHCO$_3$ (2 mL) and rinsed with water (20 mL) and CH$_3$CN (10 mL), Waters). The [^{18}F]fluoride ion was then eluted from the resin, using an aq. K$_2$CO$_3$ solution (1.0 mL of a 4.5 mg/mL solution), into a Vacutainer® tube containing Kryptofix®222 (K$_{222}$: 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, 12.0 to 15.0 mg). The resulting solution was then gently concentrated to dryness at 145-150°C under a nitrogen stream for 10 min to give no-carrier-added K[^{18}F]F-K$_{222}$ complex as a white semi-solid residue.

11
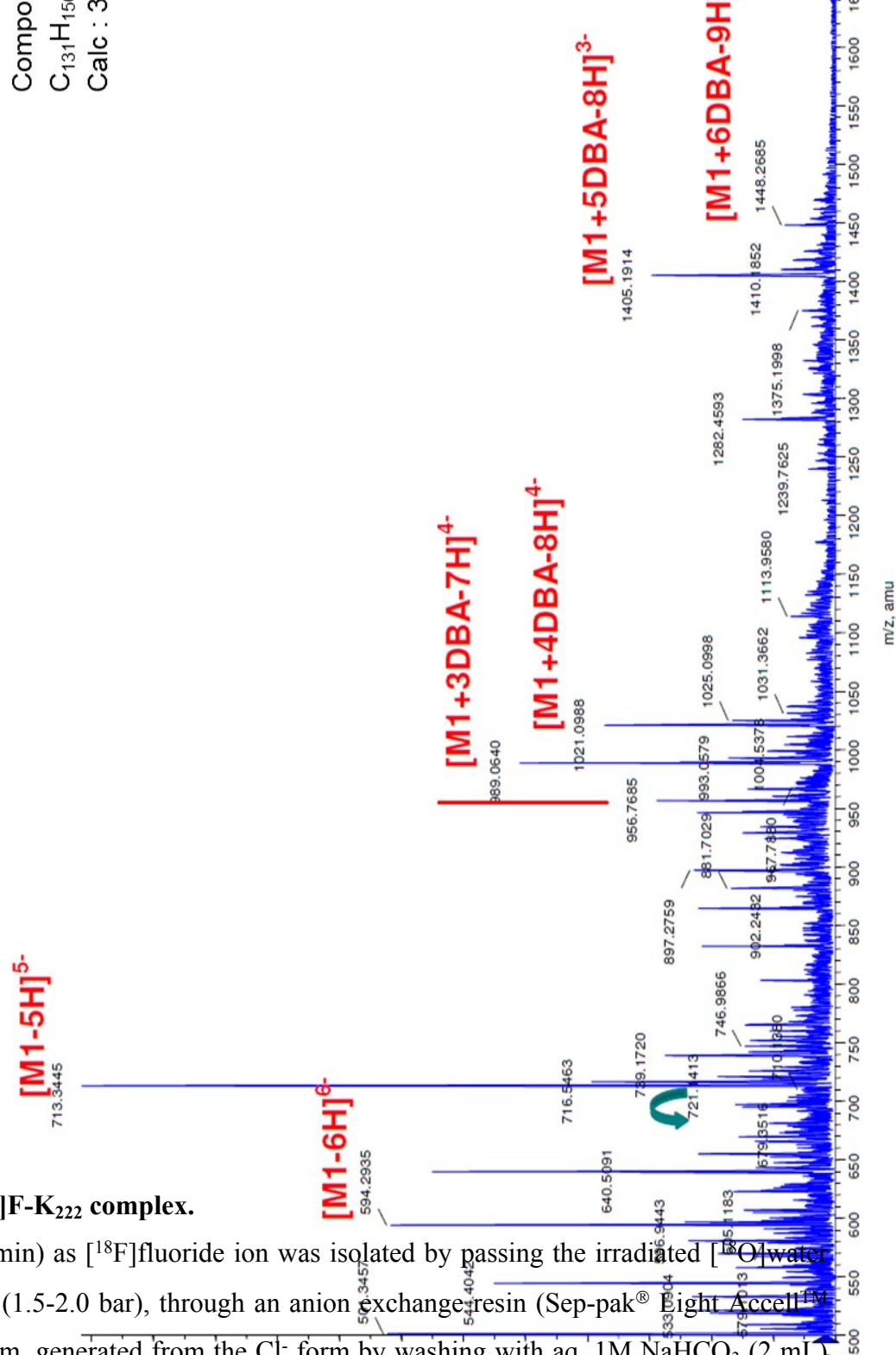
3.2 Preparation of [^{18}F]FPyME ([^{18}F]-13) (scheme S1).

DMSO (0.6 mL) containing compound **15** (4.0 mg) was directly added into the Vacutainer® tube containing the dried K[^{18}F]F-K$_{222}$ complex. The tube (not sealed) was then thoroughly vortexed for 15 s and then placed in a heating block at 145°C for 2 min without stirring the contents. The reaction vessel was then cooled using an ice-water bath. The contents was diluted with water (1 mL) and transferred on the top of a PrepSep™ R-C18 cartridge (beforehand activated with EtOH (2 mL) and then rinsed with water (10 mL), Fisher Scientific). The tube was rinsed twice with water (1 mL) which was also transferred and added to the diluted reaction mixture on top of the cartridge. After addition of another 2 mL of water, the whole was passed through the cartridge. The cartridge was washed with water (1 mL) and partially dried for 30 sec by applying a nitrogen stream. The intermediate [3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl]carbamic acid *tert*-butyl ester (**16**) was eluted from the cartridge with CH$_2$Cl$_2$ (3 mL) into a 5 mL reaction vial containing TFA (0.2 mL). Twice 1 mL of CH$_2$Cl$_2$ was used to wash the cartridge and to completely transfer the fluorine-18-labeled ester **16**. The resulting CH$_2$Cl$_2$/TFA solution (50/1, v/v) was concentrated to dryness at 65-75°C under a gentle nitrogen stream for 4-6 min giving the desired 3-(2-[^{18}F]fluoro-pyridin-3-yloxy)-propylamine (**17**). The free amine **17** was treated with a solution (0.25 mL) of *N*-methoxycarbonyl maleimide in dioxane (120 mg/mL) and diluted with an aq. saturated solution of NaHCO$_3$. The latter solution was vortexed for 1 min at rt and the reaction was then quenched by addition of a 1N aq. HCl solution (1 mL). Again, the solution was transferred on the top of a PrepSep™ R-C18 cartridge (beforehand activated with EtOH (2 mL) and then rinsed with water (10 mL), Fisher Scientific) and the reactor rinsed with water (3 x 1 mL). The whole was passed through the cartridge. The cartridge was washed with water (1 mL) and partially dried for 30 sec by applying a nitrogen stream. The desired 1-[3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione ([^{18}F]-**13**) was eluted from the cartridge with CH$_2$Cl$_2$ (3 mL) into a 5 mL reaction vial. The eluate was concentrated to a volume of 1 to 1.5 mL at 65-75°C under a gentle nitrogen stream for 4 to 6 min and purified by HPLC (HPLC A, R_t = 8.5 to 9 min) to give radiochemically pure [^{18}F]FPyME ([^{18}F]-**13**).

The overall isolated and decay-corrected radiochemical yield for the preparation of [^{18}F]FPyME ranged from 28 to 37%, based on starting [^{18}F]fluoride. Typically, 4.8 to 6.7 GBq of radiochemically pure [^{18}F]-**13** could be obtained after semi-preparative HPLC in 110 min starting from a 27 to 30 GBq cyclotron production batch of [^{18}F]fluoride.
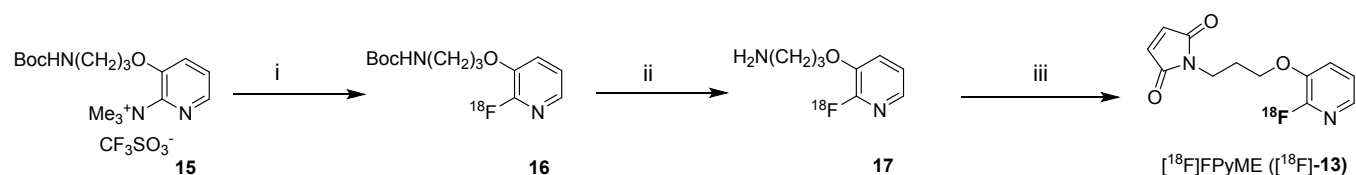
Reagents and conditions : i) K[^{18}F]F-K$_{222}$, DMSO, 145°C, 2 min; ii) TFA, CH$_2$Cl$_2$, rt, 2-5 min, iii) N-methoxycarbonyl maleimide, aq. sat. NaHCO$_3$/dioxane, rt, 10 min.

Scheme S1. Preparation of [^{18}F]FPyME ([^{18}F]-**13**).

3.3 Preparation of the [^{18}F]FPyME-conjugated oligosaccharide [^{18}F]-14.

Step a: S-acetylated octasaccharide **10** (1 mg, 0.269 µmol) in solution in PBS 100 mM, pH 7.4 (100 µL) was treated with a 50 mM buffered solution of hydroxylamine in PBS 100 mM, pH 7.4 for 45 min at rt to give the corresponding deacetylated octasaccharide **12** (HPLC B, conditions B$_2$, Rt(**12**) = 11.8 min; Rt(**10**) = 13.4 min).

Step b: The previous solution was then added to 100 µL of DMSO containing HPLC-purified [^{18}F]-**13** (HPLC-solvents having been removed by concentration to dryness at 65-75°C under a gentle nitrogen stream). The mixture was further diluted with PBS 100 mM, pH 7.4 (0.7 mL) and was left standing at rt for 15 min. The conjugation was followed by radio-TLC (Rf : Rf([^{18}F]-**14**) = 0.0 and Rf([^{18}F]-**13**) = 0.8). The crude reaction mixture (1 mL) was then subjected to SEC purification using a Sephadex NAP10™ cartridge (GE Medical Systems). The cartridge was used according to manufacturer instructions and afforded pure [^{18}F]-**14**, formulated in saline (1.5 mL). Injection into HPLC of an aliquot of formulated [^{18}F]-**14** demonstrated a radiochemical purity > 95% (HPLC B, conditions B$_1$, Rt([^{18}F]-**14**) = 18.4 min, Figure S1).
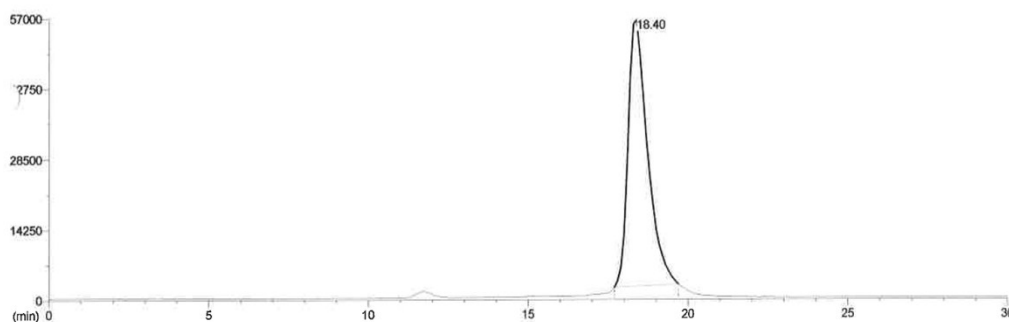
Figure S1. Quality control of [^{18}F]-**14**, HPLC B, conditions B$_1$, Rt([^{18}F]-**14**) = 18.4 min

4. Biological testing.

4.1 Screening by growth factor/heparin competition assay based on Surface Plasmon Resonance (SPR).

Heparin or low molecular weight heparin (6 kDa, Sigma) were biotinylated at the reducing end and immobilized on a BIAcore sensorchip. Different concentrations of the compounds were co-incubated at a fixed concentration of targets: FGF-2, PDGF-β, VEGF-A or SDF-1α for 30 min. The mixture was then injected onto the streptavidin control (control reference) and HP surfaces. Only free growth factor (GF) or chemokine, *i.e.* the target molecules not bound to compounds, were trapped on the heparin surface. From the binding of free targets on heparin, the percentages of inhibition were calculated and then reported in function of the compound concentrations. The plot was fitted with a four-parameter model and IC_{50} was calculated. The 0% inhibition value was obtained for the injection of the studied target in running-buffer, and the 100% inhibition value was obtained for the injection of the studied target co-incubated with 10 μM of low molecular weight heparin 6 kDa.

FGF-2/heparin competition assay. A FGF-2/heparin competition assay using the BIAcore technology was performed in the following conditions: 10 nM FGF-2, biotinylated heparin, reference streptavidin, Sensorchip C1, PBS-T 0.02%, regeneration aq. 2M NaCl.

PDGF-β /heparin competition assay. A PDGF-β/heparin competition assay using the BIAcore technology was performed using the following conditions: 10 nM PDGF-β, biotinylated heparin, reference streptavidin, Sensorchip C1, HBS-P, regeneration aq. 2M NaCl.

VEGF-A/heparin competition assay. A VEGF-A/heparin competition assay using the BIAcore technology was performed using the following conditions: 10 nM VEGF-A, biotinylated low molecular weight heparin, reference streptavidin, Sensorchip SA, HBS-P, regeneration aq. 2M NaCl.

SDF-1α/heparin competition assay. A SDF-1α/heparin competition assay using the BIAcore technology was performed using the following conditions: 96 nM SDF-1α, biotinylated low molecular weight heparin (6 kDa), reference streptavidin, Sensorchip SA, HBS-P, regeneration aq. 2M NaCl.

Effects of oligosaccharide on Growth Factor / heparin competition assay. IC$_{50}$ values were determined using the BIAcore technology for the growth factor and chemokine/heparin competition assays that contained the following proteins: VEGF-A, SDF-1α, FGF-2 and PDGF-β.

4.2 Determination of the anti-heparanase activity.

To determine the IC$_{50}$ values for the heparanase target, an assay based on the ability of heparanase to degrade fondaparinux (Sanofi patent No. 287 3377 FR) and the capacity of fondaparinux to inhibit factor Xa activity *via* AT III binding, was adapted. This assay was carried out on a STA Compact robot (Diagnostica Stago). Briefly, different concentrations of the compounds to be tested were added to a mixture containing the heparanase enzyme and fondaparinux and after a time-fixed incubation period, AT III, Factor Xa and a chromogenic substrate (CBS 31.39) were sequentially added to the reaction mix. Production of paranitroanilin resulting from the degradation of the chromogenic substrate CBS 31.39 was monitored at 405 nm. Data obtained for the different concentration points were plotted using a four-parameter fit model and IC$_{50}$ determined.

The heparanase inhibitions observed for the tested compounds were then compared with the effects of suramin (IC$_{50}$ = 922 nM), a well-known inhibitor of the enzyme.

4.3 Proliferation Assay.

The activities of the compounds were tested using a proliferation assay as already described [Ali, 2005]. A defined number of cells is seeded in each well of a culture plate. In order to stimulate cell proliferation, a growth factor is added in some wells, while others remain unstimulated (basal proliferation conditions). To monitor the effects of the tested compounds on basal and growth factor-induced cell proliferation, the octasaccharides were added at different concentrations (*i.e.* 0.1, 0.3, 1, 3, 10 or 30 μM) in presence or absence of the growth factor, respectively. After an incubation period of 24 h, the total number of cells was estimated in all samples (generally through an indirect method, such as incorporation of radioactivity into the newly synthesised DNA or colorimetric assays based on cellular enzyme activities or metabolite production). The total number of cells for the control sample in which no substance of interest nor growth factor have been added was set to 1. The total number of cells in all the other samples was compared to this value in order to obtain the relative proliferation index.

Typically, cells are seeded in 48 or 96-well plates. After 2 h (*i.e.* the time required for cells to adhere to the support) the normal culture media was replaced by a minimal essential culture media in which cells are grown for 24 h (starvation period). This step is used to reduce cell growth (cell metabolism is slowed down in order to better visualise the growth factor stimulation effect) before adding an angiogenic protein, such as a growth factor *i.e.* FGF-2 or PDGF-β. No growth factor is added in basal proliferation conditions (independently of the presence or absence of the oligosaccharide).

To evaluate the inhibition of the octasaccharides on cell proliferation, the angiogenic protein (FGF-2) was added at a fixed concentration (from 5 ng/mL) with increasing amounts of the tested compounds (0.1, 0.3, 1, 3, 10 or 30 μM), which allows the IC_{50} value to be estimated (*i.e.* the oligosaccharide concentration at which the stimulatory effect of the growth factor is inhibited by 50%).

Following addition of the angiogenic protein (growth factor) over a 24 h stimulation period, a commercial reagent containing a substrate for a cellular enzyme was added and incubated for a couple of h. The degradation of the substrate by the enzyme leads to the production of a coloured product which was titrated by absorbance measurement. Absorbencies were converted into numbers of cells using a standard curve derived from the incubation of known numbers of cells with the reagent.

5. *In vivo* imaging.

5.1 Animals.

All experiments were conducted in accordance with European Union regulations on animal research (86/609/EEC). All animal experiments were based on the same experimental setup. Male Wistar- (~250 g) were obtained from Janvier (France), housed under standard 12 h:12 h light/dark conditions (lights on from 8:00 a.m. to 8:00 p.m.) in a temperature- and humidity-controlled room. Animals have access to food and water *ad libitum*. Before using rats for experiments, they were allowed to adapt to the animal facility for one week.

5.2 PET data acquisition.

Rats were anesthetized (isoflurane in oxygen, 4% for induction and 2.5% thereafter) and the tail vein and artery catheterized. Rats were imaged using an HRRT scanner (Siemens-Healthcare, Knoxville, TS, USA), a dedicated human brain PET scanner with an isotropic spatial resolution of 2.5 mm. Rats were placed in a specific device designed to image four rats simultaneously. This device is adapted to the HRRT gantry and ensures suitable anesthetic supply to each animal. [^{18}F]-**14** (15±2 MBq) was simultaneously injected *via* the tail vein of the four rats. PET acquisition started at the time of the injection and lasted for 90 min. A dynamic series of 18 images were reconstructed, with a temporal frame duration ranging from 30 s at the beginning to 900 s at the end of the scan.

5.3 PET data analysis.

Dynamic PET images were analysed using anatomist software using manually drawn volumes of interest on the different organs. For comparison, all radioactivity concentrations were normalized to the injected dose and expressed as percentage of the injected dose per volume of tissue (% ID mL^{-1}). PET data are presented as the mean (n=4) time-activity curves (TACs) in the different organs with standard deviation (Figure S2 A and B). The area under the curve (AUC; % ID mL^{-1} min) was calculated from 0 to 90 min using a linear trapezoidal method in the organ (Figure S3).
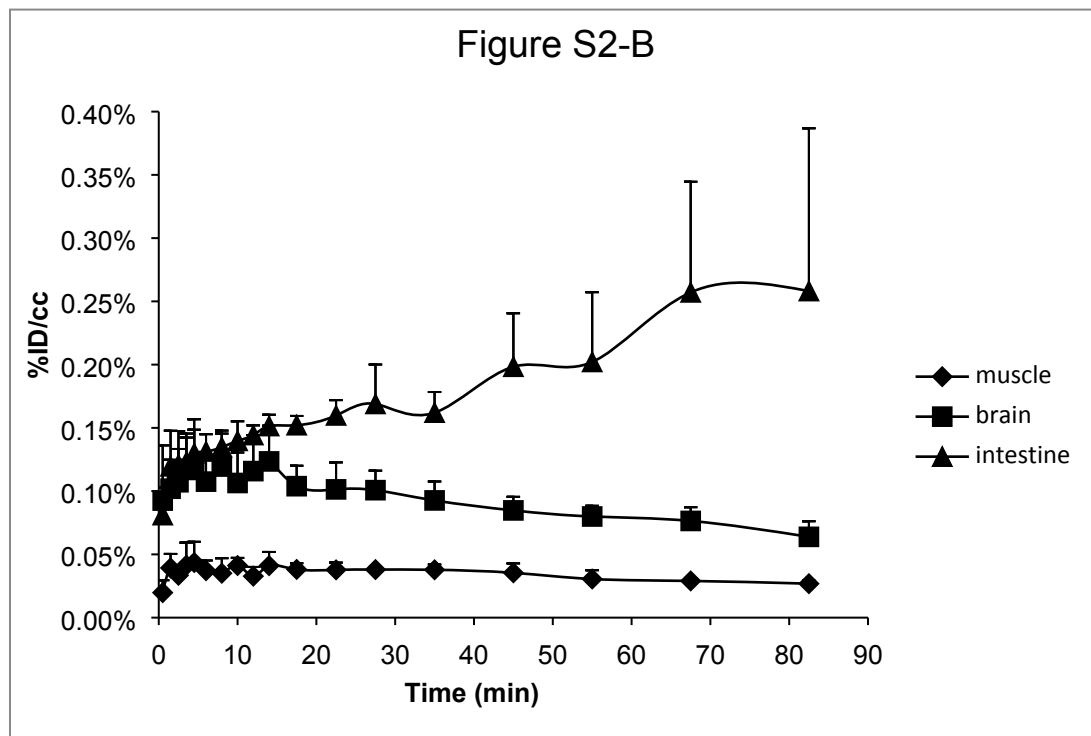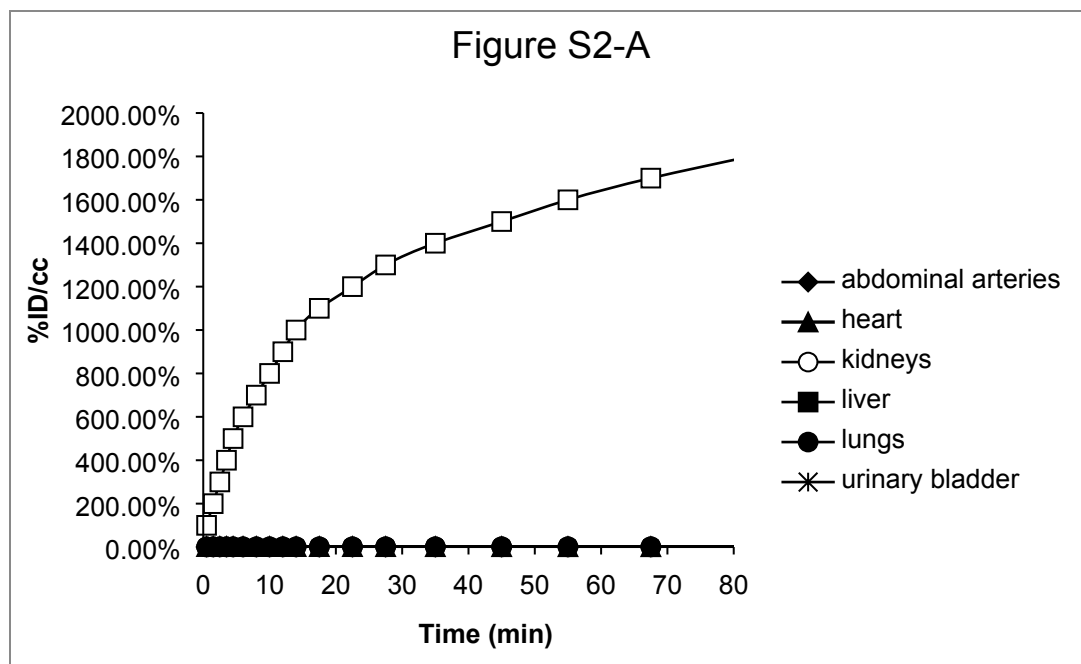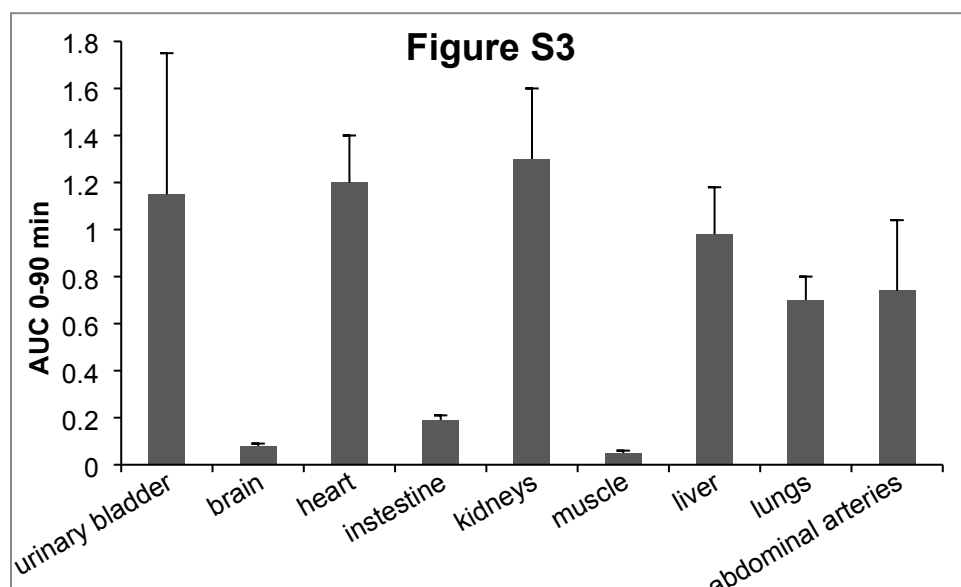
Figure S2: A and B: TACs of [^{18}F]-14 in rat (n = 4)

Figure S3: Area under curves per organ (AUC; % ID mL^{-1} min)

6. References

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