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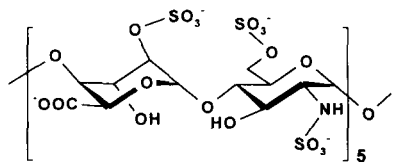
A SYNTHETIC HEPARIN/HEPARAN SULFATE-LIKE DECASACCHARIDE RELEASES LIPASE ACTIVITY *IN VIVO*. CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITY.

M. Petitou*, P. Duchaussoy, A. Bernat, P. Hoffmann and J.M. Herbert

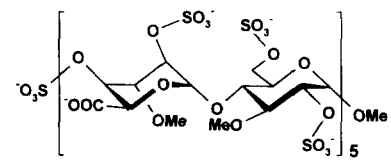
Sanofi Recherche, Haemobiology Research Department, 195, Route d'Espagne, 31036 Toulouse, France

Abstract. A decasaccharide mimic of the heparan sulfate fragment reported to have high affinity for lipoprotein lipase has been synthesised, and shown to release lipase activity *in vivo* in the rat. © 1997 Elsevier Science Ltd.

Lipoprotein lipase (LpL), a key enzyme in lipoprotein metabolism, catalyses the hydrolysis of triacylglycerols in circulating lipoproteins[1]. It is located at the luminal surface of the vascular endothelium, non-covalently bound to cell membrane heparan sulfates[2], and may be displaced and released in the circulation by heparin. Heparin not only displaces the extrahepatic LpL, but also hepatic lipase, from their endothelial binding sites, this indeed constitutes a side effect of heparin treatment[3]. The lipase releasing activity of heparinoids has been correlated to the molecular weight[4] and the charge density[5] of the polysaccharide chains. We recently observed that a synthetic pentasaccharide, representing the antithrombin binding sequence of heparin, was unable to release lipase activity into the plasma[6], its short size most probably made it unable to interact with the enzyme. Recently, Parthasarathy *et al.* isolated[7], from endothelial cells, a heparan sulfate decasaccharide sequence **1** , that was the shortest sequence able to strongly bind LpL. Our interest in this field prompted us to investigate whether a well defined synthetic mimic of **1** could actually release the enzyme from its endothelium compartment.



1



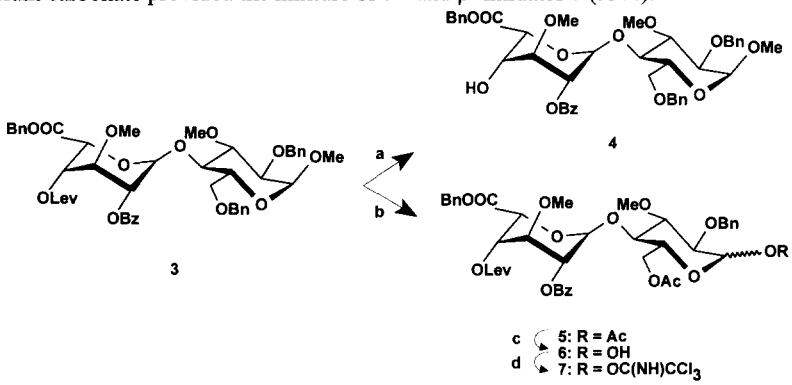
2

The decasaccharide **1**, characterized by the above authors [*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1 → 4)-*O*-(2-deoxy-2-*N*-sulfo-6-*O*-sulfo-α-D-glucosaminyl)-(1 → 4)]$_5$ possesses a complex structure, the synthesis of which, according to our own experience, would be long and delicate. However, since the role of the carbohydrate backbone in such a compound is most probably to precisely locate the charged sulfate and

* e-mail: maurice.petitou@tls1.elfsanofi.fr ; fax: +33 5 61 16 22 86

carboxylate groups in space, simpler analogues can be designed, made up of monosaccharides adopting the same conformation in solution[8,9]. Thus, a mimic of this compound should be obtained by replacing *N*-sulfo by *O*-sulfo groups, and by methylating the hydroxyl groups. We report here on the synthesis and the biological activity in the rat of such a decasaccharide mimic (similar compounds were prepared using polymer-supported solution synthesis, C.A.A. van Boeckel, personal communication).

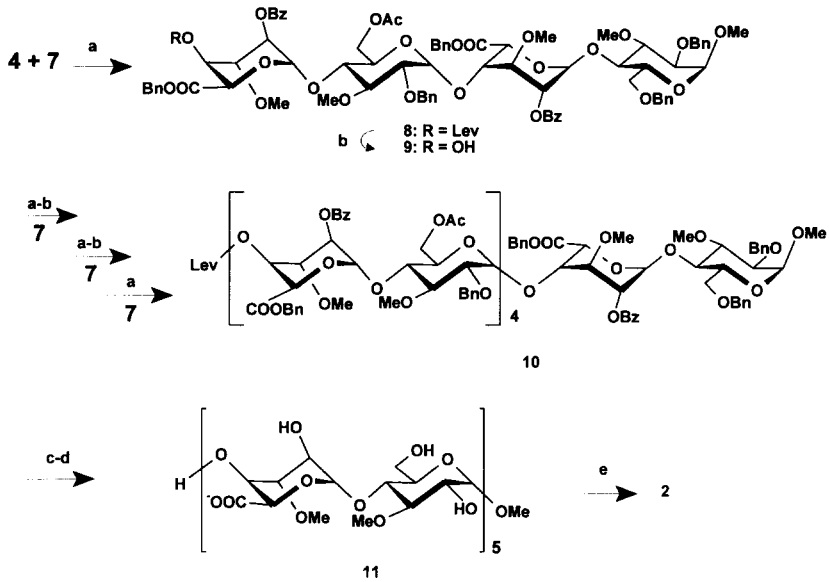
Compound **2** was obtained using a strategy where **10**, a fully protected equivalent of the target decasaccharide, was first prepared and then functionalized. The synthesis of **10** (scheme 2) required the preparation of two synthons, **4** and **7**, both of them deriving from **3** (scheme 1). We have already reported the preparation of **3** and **4**[10]. The glycosyl donor **7**[11] was obtained from **3** in three steps: acetolysis in a mixture of sulfuric acid and acetic anhydride gave **5** (α/β mixture); selective removal of the anomeric acetate by action of benzylamine in ether gave **6** (60% from **3**); finally, treatment of this hemiacetal with trichloroacetonitrile in the presence of cesium carbonate provided the mixture of α– and β–imidates **7** (92%).



Scheme 1. a) NH_2NH_2, AcOH, pyridine, 0 °C, 1.5 h (90%); b) H_2SO_4, Ac_2O, -20 °C, 15 min; c) $BnNH_2$, THF, r.t., 4 h (60% from **3**); d) CCl_3CN, Cs_2CO_3, CH_2Cl_2, RT, 45 min (92%).

Condensation of **4** and **7** (using 1.1 molar equivalent of the latter) in dichloromethane at -20 °C, in the presence of trimethylsilyl triflate yielded the tetrasaccharide **8**. The α configuration of the new interglycosidic bond was confirmed by the small coupling constant (3.5 Hz) observed between H-1 and H-2. Removal of the levulinyl group furnished the glycosyl acceptor **9**. Repetition of this sequence successively led to the hexasaccharide (60% over delevulinylation and glycosylation), the octasaccharide (34% over the two steps), and finally ended up with the decasaccharide **10** (29%), the fully protected equivalent of **2**. In spite of using a larger excess of the glycosyl donor **7** (up to 2 molar equivalent) the yield of the glycosylation reaction relative to the acceptor alcohol decreased when the size of the latter increased. Similar observations were reported in glycosylation reactions[12]. Hydrogenolysis of the benzyl groups of **10** in dimethylformamide, in the presence of 10% Pd/C, was followed by saponification (using aqueous sodium hydroxide in methanol) of the acetate and benzoate protecting groups. The saponified **11** was lyophilized after gel permeation using water. High field ^{1}H-NMR analysis confirmed the complete removal of the protective groups. Sulfation using pyridine/sulfur trioxide complex in DMF gave pure **2** after one day heating at 55 °C. The sodium salt was obtained (79% from **10**) as a

white fluffy material after gel permeation (Sephadex G 25 first in 0.2 M NaCl then in water) and lyophilization[13].



Scheme 2. a) TMSOTf, MS 4Å, CH_2Cl_2, -20 °C, 30 min; b) NH_2NH_2, AcOH, pyridine, 0 °C, 45 min (70% from **4**); c) 10% Pd/C, DMF, H_2 ; d) 5M NaOH, MeOH; e) Pyridine-SO_3, DMF, 55 °C, 24 h, (79% from **10**).

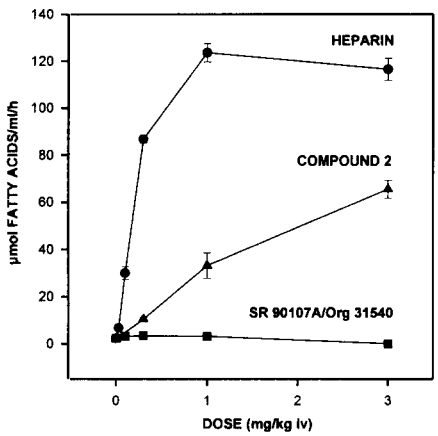


Figure 1. Dose response curves for evoked glycerol trioleate activity in plasma by **2**, the synthetic pentasaccharide representing the binding site of heparin to antithrombin III SR 90107A/ORG 31540, and a standard heparin preparation (mean ± SEM; n = 5).

Determination of triglyceride lipase activity was carried out in rats[14] after iv injection. The results (Figure 1) indicate that **2** induced the release of lipase activity in a dose dependent manner into rat plasma. Since **1** was not available we used heparin and a heparin pentasaccharide[6] as positive and negative controls, respectively. The decasaccharide **2** released lipase activity, with lower potency and efficacy than heparin. This is in agreement with Merchant *et al.*[4] who reported that a decasaccharide fraction derived from heparin was about 30% as active as heparin itself. Several authors correlated the lipase releasing activity of heparin and heparan sulfate to

molecular weight[4] and charge density[5], however, the impossibility to elucidate the structure of the heparin fragments used in these studies made it impossible to precise the structural bases (length, role of individual sulfates) of lipase releasing activity. The present approach provides a way to do so if we assume that the mimetic described here exhibits a similar conformation as the corresponding "natural" heparin fragments. Such an assumption is supported by previous work on the highly specific interaction of heparin and antithrombin III, which strongly depends on the conformation of the oligosaccharide, and where mimetics were shown to display similar conformational and binding properties as their natural counterparts[8,9].

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13. Analytical data for **2** : $[\alpha]_D$ +18° (*c* 1, water). ESI MS, negative mode : monoisotopic mass = 3603.53, average mass = 3606.28, experimental mass = 3606.18 ± 0.81 a.m.u. ^{1}H-NMR (D_2O) δ: 3.57, 3.56, 3.55, 3.54, 3.53, 3.44 (s, 33H, 11 OMe); anomeric protons from non reducing-end (NR) to reducing-end (R) unit: 5.13 (NR, $J_{1,3}$ observed); 5.36 (NR-1, $J_{1,2}$ 3-4 Hz); 5.11 (NR-2, no $J_{1,3}$ observed); 5.34 (NR-3, $J_{1,2}$ 3-4 Hz); 5.11 (NR-4, no $J_{1,3}$ observed); 5.34 (R-4, $J_{1,2}$ 3-4 Hz); 5.11 (R-3, no $J_{1,3}$ observed); 5.34 (R-2, $J_{1,2}$ 3-4 Hz); 5.09 (R-1, no $J_{1,3}$ observed); 5.05 (R, $J_{1,2}$ 3.5 Hz).
14. The experimental protocol has been approved by the Animal Care and Use Committee of Sanofi Recherche. The products were injected iv, into pentobarbital (48 mg/kg ip) anesthetized male Sprague-Dawley rats, 10 min prior to blood withdrawal. Blood was collected from the abdominal aorta into a 3.8% sodium citrate solution. Plasma was separated by centrifugation (100 x g for 20 min at 4 °C) and stored at -20 °C. Conditions for the enzyme assay were chosen according to Baginsky (Baginsky, M. L., In *Methods in enzymology*; Lowenstein, J. M., Ed.; Academic Press: New York, 1981; Vol. 72, pp 325-338). Stock solutions of substrate were prepared by mixing tri[1-^{14}C]oleylglycerol with unlabeled trioleylglycerol (both in hexane) at the day of the experiment. Hexane was evaporated under a stream of nitrogen and the substrate was incorporated into 5% gum arabic by sonication. The incubation medium contained 20% of such substrate emulsion, 10% rat serum, 100 mM NaCl, 2% BSA and 40 mM Tris-HCl buffer pH 8.4. Incubation was carried out for 1 h at 28 °C. The hydrolyzed fatty acids were extracted by a mixture of methanol/chloroform/heptane 1.4/1.25/1 v/v/v and counted for radioactivity in a liquid scintillation counter. Enzyme activity was expressed as fatty acids μmol released/ml/h.

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