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Synthesis of a Targeted Library of Heparan Sulfate Hexa- to Dodecasaccharides as Inhibitors of β-Secretase: Potential Therapeutics for Alzheimer's Disease

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Abstract: Heparan sulfates (HS) are a class of sulfated polysaccharides that function as dynamic biological regulators of the functions of diverse proteins. The structural basis of these interactions, however, remains elusive, and chemical synthesis of defined structures represents a challenging but powerful approach for unravelling the structure–activity relationships of their complex sulfation patterns. HS has been shown to function as an inhibitor of the β-site cleaving enzyme β-secretase (BACE1), a protease responsible for generating the toxic Aβ peptides that accumulate in Alzheimer's disease (AD), with 6-*O*-sulfation identified as a key requirement. Here, we demonstrate a novel generic synthetic approach to HS oligosaccharides applied to production of a library of 16 hexa- to dodecasaccharides targeted at BACE1 inhibition. Screening of this library provided new insights into structure–activity relationships for optimal BACE1 inhibition, and yielded a number of potent non-anticoagulant BACE1 inhibitors with potential for development as leads for treatment of AD through lowering of Aβ peptide levels.

Keywords: Alzheimer's · carbohydrates · glycosylation · heparan sulfate · synthesis

Introduction

Heparan sulfate (HS) is a linear polysaccharide with highly diverse functionality. Through attachment to core proteins it is present as a glycoprotein ("proteoglycan") on mammalian cell surfaces and in the extracellular matrix. HS has a disaccharide repeating unit of D-glucosamine (Glc) and D-glucuronic acid (GlcA) or L-iduronic acid (IdoA), which may be variously *O*- or *N*-sulfated or *N*-acetylated, generating polyanionic structural diversity. HS regulates many important biological processes;[1] significant examples include growth factors and their receptors,[2] cytokines,[3] stem cells,[4] Wnt and bone morphogenetic proteins.[5] The diversity of cell signalling events influenced by HS is presumed to be a function of the microheterogeneity of its structure with specific sequences and/or sulfation patterns required for particular protein binding and biological activities.[1b]

In addition HS was identified as the first natural regulator of the cleavage of the amyloid precursor protein by β-secretase.[6] This cleavage is a critical step in the formation of amyloid plaques present in the brains of Alzheimer's patients and the enzyme β-secretase (BACE1) has been the focus of a number of programs searching for therapeutics to alleviate the symptoms of Alzheimer's disease (AD).[7] This neurodegenerative disease was first described by Alois Alzheimer in 1906. A key characteristic is the deposition of insoluble accumulations of the amyloid β-peptide in the brain. The amyloid β-peptide is itself derived from step-wise extracellular enzymatic cleavage of the amyloid precursor protein by BACE1, and then intramembrane cleavage by γ-secretase. The accumulation of amyloid β-peptide is a critical driving force for Alzheimer's disease pathology according to the amyloid cascade hypothesis.[8] There is also evidence that amyloid β-peptide is involved in hyperphosphorylation of the tau protein and subsequent formation of neurofibrillary tangles.[9]

Although the inhibition of BACE1 by HS and heparin is interesting, heparin, which is a highly sulfated variant of HS and a widely used anticoagulant drug, would have serious blood-thinning side effects that would likely preclude its use for the treatment of AD. However, the selective chemical modification of heparin has given rise to some homogeneous HS polymers with potent BACE1 inhibitory activity and no significant anticoagulant properties,[6,10] whereas dermatan sulfate had no effect.[6] In addition some discrete synthetic HS tetrasaccharides have shown surprising (though still moderate) potency as BACE1 inhibitors.[11] This suggests that larger synthetic oligosaccharides might have applications as high potency BACE1 inhibitors with potential as novel therapeutics for AD.

The ability to synthesise discrete HS oligomers of octasaccharide size and larger would offer potential therapeutics for Alzheimer's as well as other diseases in which HS–protein interactions play a significant role. Importantly the syn-

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thesis of such oligosaccharides would also facilitate the wider elucidation of specific HS–protein interactions—a key bottleneck in this field. For these reasons we decided to develop generic approaches for the synthesis of any desired HS oligosaccharide. In the first instance we have focused on the synthesis of a targeted library of potential BACE1 inhibitors.

Studies of chemically modified heparins have indicated that having 6-*O*-sulfate substituents on the glucosamine residues is important for potency as BACE1 inhibition, whereas *N*-acetate substituents are tolerated, and an additional 2-*O*-sulfate on the uronic acids offers some improved activity.[6,10] The synthesis and testing of discrete sulfated oligosaccharides would offer confirmation of these results and potentially provide lead single chemical entity drug compounds. We decided to make targets both with and without the uronic acid 2-*O*-sulfates, as reducing sulfation levels is likely to be beneficial for bioavailability and reducing off-target effects. In addition, we wished to explore the relative importance of glucuronic and iduronic acid residues, which has not been elucidated for this protein. Our initial synthetic targets then became hexa-, octa-, deca- and dodecasaccharides containing GlcNAc6S-UA or GlcNAc6S-UA2S disaccharides with either D-glucuronic or L-iduronic acids (Figure 1).

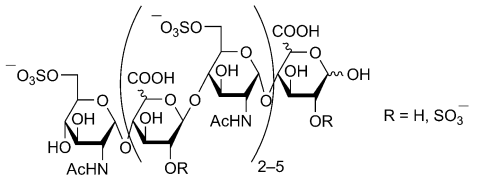


Figure 1. Synthetic targets.

Results and Discussion

Selection of building blocks for synthesis: A number of groups have reported methods for the synthesis of HS oligosaccharides.[11,12] Some are elegant without any being completely general and/or suitable for scale-up. Recently, a novel chemoenzymatic approach has used a number of HS biosynthetic enzymes to assemble some defined low-molecular-weight (LMW) heparin oligosaccharides including a heptasaccharide analogue of the heparin pentasaccharide drug, Arixtra.[13] Although this strategy clearly has a lot of promise, controlling enzyme specificity to deliver pure, as well as non-natural products has yet to be overcome. All fully synthetic approaches so far have used disaccharide building blocks as the basis for assembling their targets. The disaccharide donors can be oriented with either the uronic acid or the glucosamine moieties at the reducing end, represented by the general structures **1** and **2**, respectively (Figure 2). With donor **1**, an advantage is that the sometimes difficult-to-establish α-stereochemistry of the glucosamine is already in place. Also, the α-L- or β-D-stereochemistry of the uronic acid can be achieved by using a participating group at *O*-2

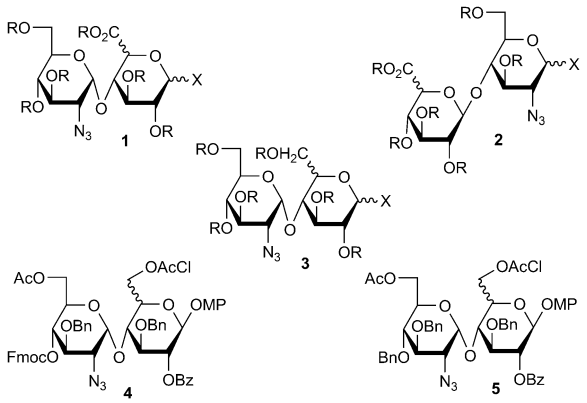
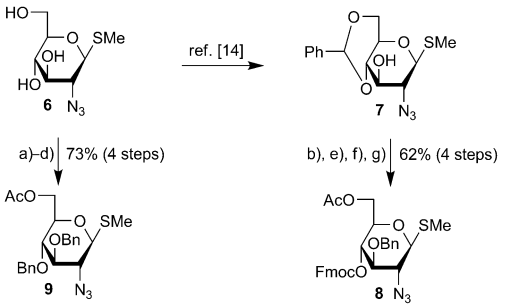


Figure 2. Possible disaccharide donors.

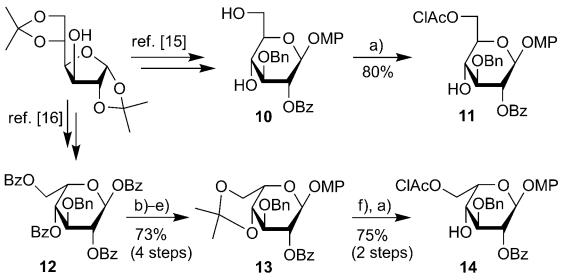
of the uronic acid. However, uronic acids make relatively poor donors and can be unreliable when assembling large oligosaccharide chains. Donor **2** has the advantage that the uronic acid glycosidic linkage is established, but it relies on achieving very good α-selectivity in glycosylations with this donor. In our hands, and those of others,[12o] the use of such 2-azido-glycosyl donors cannot be guaranteed to result in high anomeric selectivity. Subtle changes in protecting groups will sometimes exert significant effects on anomeric selectivity and we did not want to have to separate anomeric mixtures in a large oligosaccharide chain. Consequently, we have settled on the use of donors represented by **3**, which has the advantages of **1** but uses the much more reactive D-glucose or L-idose moieties as donors, which can be oxidised later to the uronic acids. Since we began this work others have reported similar strategies although with significant differences in donor activation and protecting group choices.[12a,j,l,p]

For the purposes of synthesising the BACE1 inhibitor targets outlined above, after considerable experimentation we settled on the protecting group combination in the disaccharides **4** and **5**, which offered benefits in ease of synthesis and the required selectivity. There are four orthogonal ester/carbonate protecting groups that can be selectively removed as required (vide infra). The building blocks **4** can serve as acceptors by removal of the Fmoc groups or be converted into donors by hydrolysis of the methoxyphenyl glycosides and then formation of the corresponding trichloroacetimidates.

Synthesis of disaccharide building blocks: Commercially available methyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside (**6**) was converted into the benzylidene derivative **7**,[14] followed by standard protecting group manipulation to give **8** and the alternative 4-*O*-benzyl derivative **9** was also prepared as shown (Scheme 1). The D-*gluco*- and L-*ido*- building blocks were both prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (Scheme 2), in which the latter was converted into the methoxyphenyl glycoside **10**[15] and then the acceptor **11**. Alternatively, the L-*ido* tetrabenzoate **12**[16]

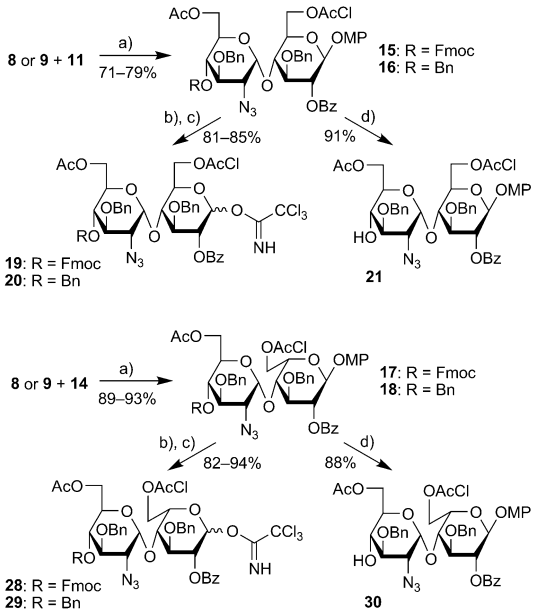


Scheme 1. Preparation of azide-glycosyl donors. a) TrCl, py; b) BnBr, NaH; c) aq. HOAc, 80 °C; d) Ac_2O, py; e) HCl, MeOH; f) AcCl, py, −78 °C to room temperature; g) FmocCl, py.



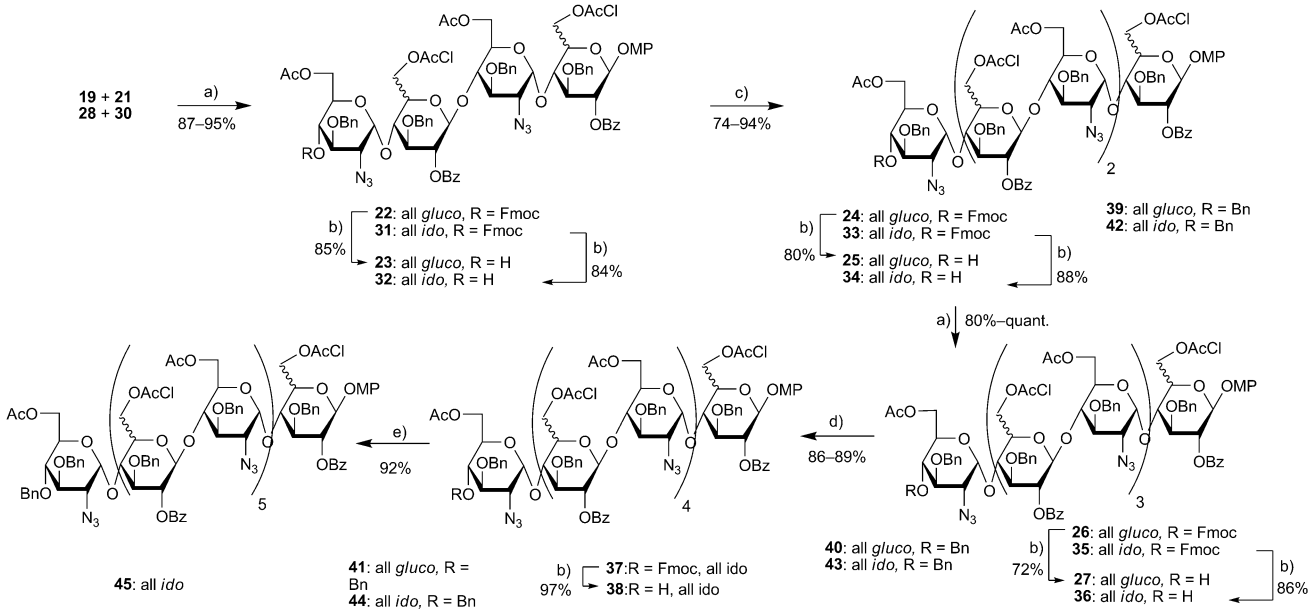
Scheme 2. Preparation of glycosyl acceptors. a) ClAcCl, py, −78 °C to room temperature; b) *p*-methoxyphenol, $BF_3{\cdot}EtO_2$; c) NaOMe; d) $Me_2C(OMe)_2$, TsOH; e) BzCl, py; f) aq. HOAc, 80 °C.

was prepared and converted via the 4,6-*O*-isopropylidene derivative **13** into the acceptor **14**. The donors **8** and **9** were



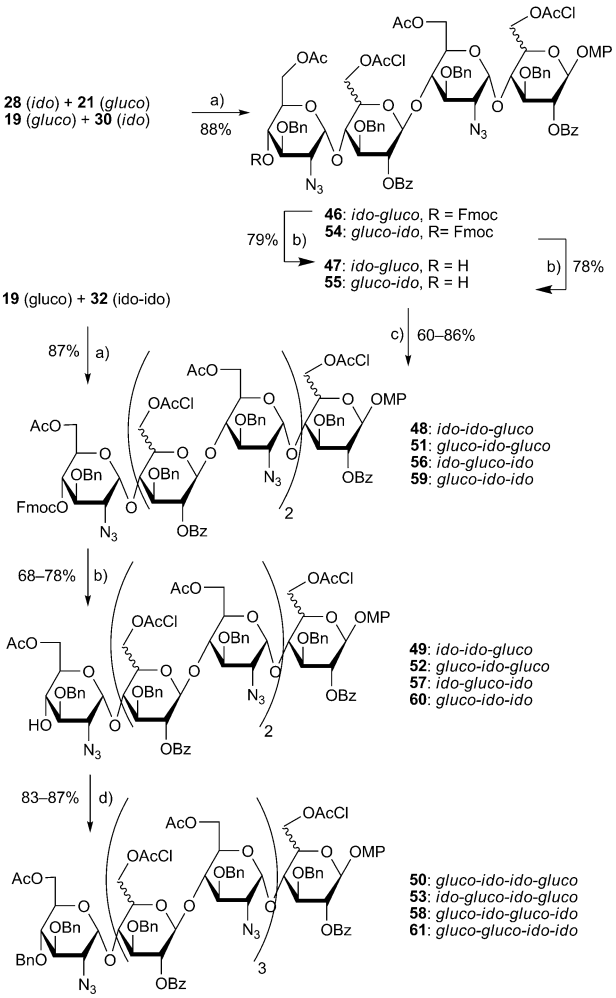
Scheme 3. Preparation of disaccharides. a) NIS, AgOTf, DCM/toluene; b) $Ce(NH_4)_2NO_3$, aq. CH_3CN; c) Cl_3CCN, NaH, DCM; d) Et_3N, DCM.

coupled with acceptors **11** and **14** in high yields, and α-selectivity to give **15**–**18** (Scheme 3). Compounds **15**–**17** are crystalline, facilitating large-scale synthesis of these building blocks.



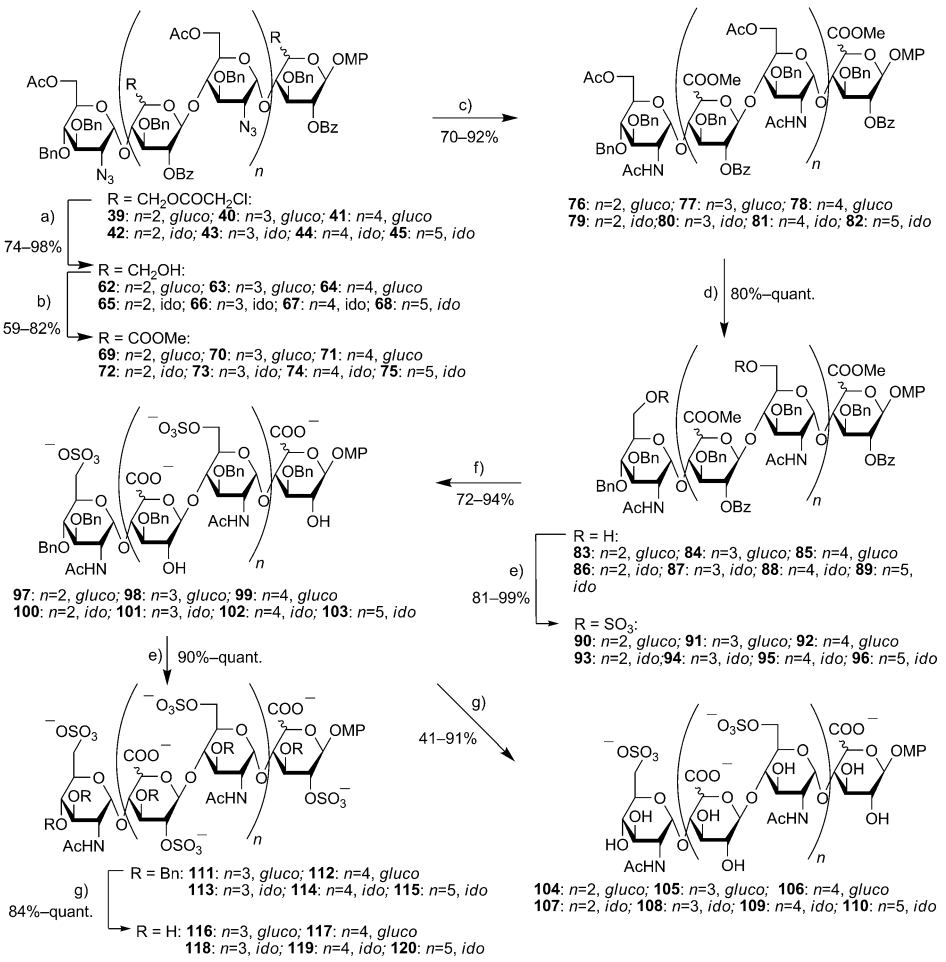
Scheme 4. Synthesis of fully protected homogenous oligosaccharides. a) TMSOTf; b) Et_3N; c) TMSOTf, **19**, **20**, **28** or **29**; d) TMSOTf, **20**, **28** or **29**; e) TMSOTf, **29**. The descriptors *ido*/*gluco* are used to describe the configuration of the carbohydrate residues representing uronic acids in the target molecule, ordered from non-reducing end to reducing end (all *gluco* for a GlcNAc-GlcA-GlcNAc-GlcA-GlcNAc-GlcA hexasaccharide).

Oligosaccharide assembly: Initially homogeneous oligosaccharides were synthesised, with either glucuronic or iduronic acid precursor components. The disaccharide **15** was converted into donor **19** by ceric ammonium nitrate promoted hydrolysis of the methoxyphenyl glycoside followed by formation of the trichloroacetimidate, and was separately converted into an acceptor **21** by selective removal of the Fmoc group with mild base (Scheme 3). Coupling of **19** and **21** afforded only the tetrasaccharide **22** in 87% yield (Scheme 4). Removal of the Fmoc gave the tetrasaccharide acceptor **23**, which was glycosylated with donor **19** to give hexasaccharide **24**, and the process was repeated to give **25** and then octasaccharides **26** and **27**. The glycosylation yields were uniformly high (>80%) and no by-products of α-anomer or orthoester formation were detected. Methoxyphenyl glycoside protection at the reducing end was retained as a useful NMR marker unlikely to influence the biological activity. This procedure was also successful in the idose series so that disaccharide **17** afforded both the donor **28** and acceptor **30** (Scheme 3). Sequential glycosylations again afforded excellent yields (>80% with no β-L anomers or orthoesters detected). In this way the oligosaccharide acceptors **32**, **34**, **36** and **38** were prepared. Then glycosylation of acceptors **23**, **25** and **27** with acetimidate **20** and of acceptors **32**, **34**, **36**, and **38** with acetimidate **29** afforded hexa-, octa-, deca- and dodecasaccharides **39**–**45** with excellent overall efficiency. Some oligosaccharides with mixed glucuronic and iduronic acid residues were also synthesised with a view to exploring the structure–activity relationship (SAR) of these compounds. Starting with the disaccharide acceptors **21** and **30** and the tetrasaccharide acceptor **32** appropriate sequential glycosylation reactions were performed as before to afford the mixed octasaccharides **50**, **53**, **58** and **61** (Scheme 5). The glycosylation efficiencies with the particular set of protecting groups used here were uniformly very high and this allowed oligosaccharide assembly on a multigram scale with relative ease.



Scheme 5. Synthesis of mixed octasaccharides. a) TMSOTf; b) Et_3N; c) TMSOTf, **19** or **28**; d) TMSOTf, **20** or **29**. The descriptors *ido*/*gluco* are used to describe the configuration of the carbohydrate residues representing uronic acids in the target molecule, ordered from non-reducing end to reducing end (*ido-gluco* for a GlcNAc-IdoA-GlcNAc-GlcA tetrasaccharide).

Conversion to sulfated oligosaccharide targets: The fully protected hexa-, octa-, deca- and dodecasaccharides **39**–**45**, **50**, **53**, **58** and **61** have three types of orthogonal ester protecting groups and the azide moieties that require processing to generate the target sulfated oligosaccharides. Firstly, the chloroacetate groups were selectively removed with mild base to expose from 3 to 6 primary hydroxyl groups without detectable loss of other ester protecting groups. These alcohols were then oxidised to the corresponding carboxylic acids in good yield using TEMPO/BAIB conditions in aqueous acetonitrile, and protection as the methyl esters was effected with diazomethane (or trimethylsilyldiazomethane). The overall yields for multiple oxidation and ester formation were not optimised but if sufficient time was allowed for complete oxidation the yields were approximately 75%. The azide groups were then converted directly to the *N*-acetates with thioacetic acid in pyridine affording **76**–**82** (Scheme 6) and **129**–**132** (Scheme 7). There remained the primary acetate and secondary benzoate groups, which needed to be differentiated to allow selective sulfation on the primary centres. Solvolysis of the acetates under mild base conditions was not sufficiently selective as some removal of benzoates occurred, especially for the longer oligosaccharides. However, acidic solvolysis gave high yields of products resulting from loss of only the acetates and without any sign of anomerization of the glycoside linkages. Subsequent sulfation of the exposed hydroxyl groups afforded **90**–**96** and **137**–**140**. Then saponification of both the benzoate and methyl esters was effected and the products were fully deprotected by hydrogenolysis to the target compounds **104**–**110** and **145**–**148**. It was necessary to add methanolic or aqueous ammonia to the hydrogenolysis solvent to prevent some loss of sulfate groups. Alternatively, the exposed secondary hydroxy groups after saponification in **97**–**103** were also sulfated, and then global deprotection of the products by hydrogenolysis gave the alternatively sulfated oligosac-



Scheme 6. Synthesis of final hexa-, octa-, deca- and dodecasaccharides. a) DABCO, MeCN/EtOH, 70 °C; b) TEMPO, BAIB, aq. MeCN, then CH_2N_2; c) py, HSAc; d) HCl, MeOH/DCM; e) SO_3·NMe_3, DMF, 60 °C; f) NaOH, aq. MeOH; g) H_2, $Pd(OH)_2$/C, aq. THF. The descriptors *ido*/*gluco* are used to describe the configuration of the carbohydrate residues representing uronic acids in the target molecule ($n=3$, *ido* for a GlcNAc-IdoA-GlcNAc-IdoA-GlcNAc-IdoA-GlcNAc-IdoA octasaccharide).

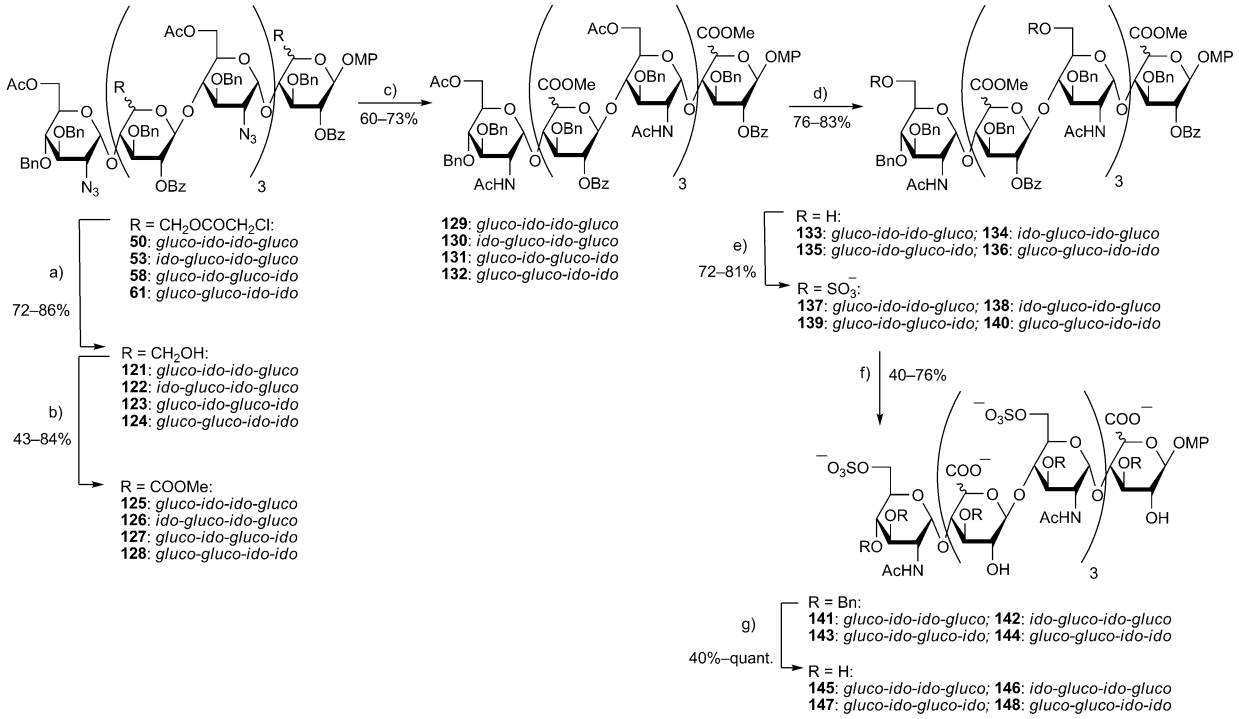
charide targets **116–120**. We found the two-step sulfation procedure to work more efficiently than removing both the acetate and benzoate groups by saponification followed by sulfation.

Compounds potently inhibit BACE1 protease and lack anticoagulant activity: The ability of the target compounds to inhibit the BACE1 protease was next investigated using fluorescence resonance energy transfer (FRET) peptide cleavage assays (see the Supporting Information). All of the compounds inhibited BACE1, with IC_{50} values varying from low potency (5–12 μM) for the hexasacharides to high potency (1–5 nM) for a number of the octa-, deca- and dodecasaccharides (Table 1). Their potency clearly increased with increasing size, within classes of compounds with similar repeating backbones; this is in agreement with previous data from heparin size fractions.[10b] However, the previous data indicated a significant reduction in activity (~tenfold) below the decasaccharide size, whereas here we found that a number of the octasaccharides were surprisingly potent, with similar activities to decasaccharides of the same basic structure (Table 1). The low potency hexasaccharides (**104**, **107**) were similar in activity to the most active tetrasaccharides described in previous studies,[11] and were approximately 15- to 20-fold less potent than equivalent octasaccharides (**105**, **108**).

Regarding the relative contribution of IdoA and GlcA residues, for the homogenous repeating unit structures in the absence of 2-*O*-sulfation, there were small differences in potency (maximum two- to threefold), suggesting that GlcA may confer a marginal improvement in activity over IdoA, at least for the hexa- and octasaccharides. The IC_{50} of the non-regular octasaccharide compounds with mixed uronate residues was similar (~100–300 nM) to those containing solely GlcA (260 nM), and only slightly more active than those containing solely IdoA (~900 nM). However, of much greater significance was the evidence for dramatically higher activity conferred by the additional presence of a 2-*O*-sulfate on the UA residues, irrespective of the GlcA or IdoA configuration. The differences were approximately 20-fold for dodecasaccharides (**120** vs. **110**), about 70- to 90-fold for decasaccharides (**117** and **119** vs. **106** and **109**) and approximately 55- to 230-fold for octasaccharides (**116** and **118** vs. **105** and **108**). Such large differences were unexpected since previous data indicated only a threefold difference in activity for heparin polysaccharides lacking 2-*O*-sulfates.[10]

Overall, these data support the view that octa- (**116** and **118**) and decasaccharides (**117** and **119**) containing either GlcNAc6S-GlcA2S or GlcNAc6S-IdoA2S units represent realistic lead compounds for the development of fully synthetic BACE1 inhibitors, with a requisite balance of activity, size and relative ease of synthesis. Only modest gains in potency were evident for deca- and dodeca- compared to octasaccharides (~two- to threefold), which are unlikely to justify the additional complexity and cost of their synthesis.

To assess the potential off-target anticoagulant activity of the saccharides, they were all tested in the dose range 0.004 to 50 $\mu g\,mL^{-1}$ in a Factor Xa assay employing cleavage of a



Scheme 7. Synthesis of final mixed octasaccharides. a) DABCO, MeCN/EtOH, 70 °C; b) TEMPO, BAIB, aq. MeCN, then CH_2N_2; c) py, HSAc; d) HCl, MeOH/DCM; e) SO_3·NMe_3, DMF, 60 °C; f) NaOH, aq. MeOH; g) H_2, $Pd(OH)_2$/C, aq. THF. The descriptors *ido*/*gluco* are used to describe the configuration of the carbohydrate residues representing uronic acids in the target molecule (*gluco-ido-ido-gluco* for a GlcNAc-GlcA-GlcNAc-IdoA-GlcNAc-IdoA-GlcNAc-GlcA octasaccharide).

peptide substrate (see the Supporting Information). None displayed any measurable ability to accelerate antithrombin-III mediated inactivation of Factor Xa, consistent with our previous data which indicated that selectively desulfated heparins with equivalent repeating structures had anticoagulant activity >1000-fold lower than parental heparin.[10b] Unlike heparin, such synthetic compounds would thus be expected to have no significant side effects related to anticoagulation. Furthermore, with regard to potential off-target inhibition of other structurally related and physiologically relevant aspartic proteases, we have previously shown that similar compounds (NAcLMWH and analogues) have markedly reduced potency for enzymes, such as renin and pepsin (>100-fold) and cathepsin D (~10–100-fold).[10b] This selectivity is encouraging with regard to the potential therapeutic applications of these compounds as BACE1 inhibitors.

Conclusion

We have described a successful synthetic approach that enables the general synthesis of specific HS oligosaccharides of a range of sizes and structures. The building blocks we developed have allowed the efficient assembly of a range of hexa- to dodecasaccharides. The targeted library of 16 sulfated oligosaccharides presented here is to our knowledge the largest of its type reported to date. We have focused on relatively low-sulfated saccharides containing NAc and 6S moieties, targeted at optimised inhibition of BACE1 with reduced off-target activities.[10] The majority were regular, homogenous repeat compounds, but the synthetic strategy also afforded irregular structures with mixed sequences containing either GlcA or IdoA residues; altered patterns of other *O*-sulfate or indeed *N*-sulfate groups could easily be introduced with usage of appropriate disaccharide building blocks. Thus, the approach has generic potential to produce any desired HS structure at scale for future chemical biology studies. Here, we have identified surprisingly potent, fully synthetic inhibitors as small as octasaccharides; this finding was unexpected based on previous studies with heterogeneous structures available by semisynthetic chemical desulfation of heparin.[10b] These compounds have significant potential as leads for the development of new drugs that inhibit BACE1, particularly as LMW heparin oligosaccharides have been demonstrated to cross the blood–brain barrier,[17] and oral availability is a tractable route for such saccharides using appropriate formulation and encapsulation strategies.[18] Successful in vivo delivery and BACE1 inhibition by these saccharides could lower brain Aβ levels and potentially provide a disease-modifying treatment for Alzheimer's disease.

Table 1. Inhibitory activities of synthetic compounds and heparin for cleavage of FRET peptide by BACE1 enzyme.

Compound	IC_{50} [nM][a]	Structure[b]	M_W
heparin	0.2	$[IdoA2S\text{-}GlcNS6S]_n$[c]	12000[d]
NAcLMWH	1.8	$[IdoA2S\text{-}GlcNAc6S]_n$[c]	4000[e]
hexasaccharides			
104	5300	GlcNAc6S-[GlcA-GlcNAc6S]$_2$-GlcA	1634
107	13000	GlcNAc6S-[IdoA-GlcNAc6S]$_2$-IdoA	1634
octasaccharides			
105	260	GlcNAc6S-[GlcA-GlcNAc6S]$_3$-GlcA	2138
108	890	GlcNAc6S-[IdoA-GlcNAc6S]$_3$-IdoA	2138
116	4.7	GlcNAc6S-[GlcA2S-GlcNAc6S]$_3$-GlcA2S	2546
118	3.9	GlcNAc6S-[IdoA2S-GlcNAc6S]$_3$-IdoA2S	2546
145	110	GlcNAc6S-GlcA-GlcNAc6S-IdoA-GlcNAc6S-IdoA-GlcNAc6S-GlcA	2138
146	310	GlcNAc6S-IdoA-GlcNAc6S-GlcA-GlcNAc6S-IdoA-GlcNAc6S-GlcA	2138
147	280	GlcNAc6S-GlcA-GlcNAc6S-IdoA-GlcNAc6S-GlcA-GlcNAc6S-IdoA	2138
148	230	GlcNAc6S-GlcA-GlcNAc6S-GlcA-GlcNAc6S-IdoA-GlcNAc6S-IdoA	2138
decasaccharides			
106	250	GlcNAc6S-[GlcA-GlcNAc6S]$_4$-GlcA	2639
109	200	GlcNAc6S-[IdoA-GlcNAc6S]$_4$-IdoA	2639
117	3.5	GlcNAc6S-[GlcA2S-GlcNAc6S]$_4$-GlcA2S	3149
119	2.2	GlcNAc6S-[IdoA2S-GlcNAc6S]$_4$-IdoA2S	3149
dodecasaccharides			
110	32	GlcNAc6S-[IdoA-GlcNAc6S]$_5$-IdoA	3142
120	1.3	GlcNAc6S-[IdoA2S-GlcNAc6S]$_5$-IdoA2S	3754

[a] For comparative IC_{50} values expressed by weight [$\mu g\,mL^{-1}$] and corresponding standard deviation values, see the Supporting Information. [b] GlcNAc, *N*-acetylated glucosamine; GlcA, glucuronic acid; IdoA, iduronic acid; 6S, 6-*O*-sulfate; 2S, 2-*O*-sulfate. NS, *N*-sulfate. The numbered synthetic compounds have a reducing end methoxyphenyl group (data not shown). [c] Predominant disaccharide repeating unit. [d] Heparin, average M_W 12 kDa. [e] *N*-Acetylated low-molecular-weight heparin.

Experimental Section

Full experimental details including characterisation and NMR spectra of new compounds are given in the Supporting Information.

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[1] a) J. R. Bishop, M. Schuksz, J. D. Esko, *Nature* **2007**, *446*, 1030–1037; b) J. Turnbull, A. Powell, S. Guimond, *Trends Cell. Biol.* **2001**, *11*, 75–82.
[2] M. Mohammadi, S. K. Olsen, O. A. Ibrahimi, *Cytokine Growth Factor Rev.* **2005**, *16*, 107–137.
[3] H. Lortat-Jacob, *Curr. Opin. Struct. Biol.* **2009**, *19*, 543–548.
[4] S. Piecewicz, S. Sengupta, *Stem Cells Dev.* **2011**, *20*, 749–758.
[5] J. Filmus, M. Capurro, J. Rast, *Genome Biol.* **2008**, *9*, 224.
[6] Z. Scholefield, E. A. Yates, G. Wayne, A. Amour, W. McDowell, J. E. Turnbull, *J. Cell Biol.* **2003**, *163*, 97–107.
[7] a) R. Silvestri, *Med. Res. Rev.* **2009**, *29*, 295–338; b) V. John, J. P. Beck, M. J. Bienkowski, S. Sinha, R. L. Heinrikson, *J. Med. Chem.* **2003**, *46*, 4625–4630; c) S. L. Cole, R. Vassar, *J. Biol. Chem.* **2008**, *283*, 29621–29625.
[8] J. Hardy, D. J. Selkoe, *Science* **2002**, *297*, 353–356.
[9] L. M. Ittner, J. Götz, *Nat. Rev. Neurosci.* **2011**, *12*, 65.
[10] a) S. J. Patey, E. A. Edwards, E. A. Yates, J. E. Turnbull, *Neurodegener. Dis.* **2008**, *5*, 197–199; b) S. J. Patey, E. A. Edwards, E. A. Yates, J. E. Turnbull, *J. Med. Chem.* **2006**, *49*, 6129–6132.
[11] S. Arungundram, K. Al Mafraji, J. Asong, F. E. Leach, I. J. Amster, A. Venot, J. E. Turnbull, G. J. Boons, *J. Am. Chem. Soc.* **2009**, *131*, 17394–17405.
[12] a) Y. P. Hu, S. Y. Lin, C. Y. Huang, M. M. Zulueta, J. Y. Liu, W. Chang, S. C. Hung, *Nat. Chem.* **2011**, *3*, 557–563; b) F. Baleux, L. Loureiro-Morais, Y. Hersant, P. Clayette, F. Arenzana-Seisdedos, D. Bonnaffé, H. A. Lortat-Jacob, *Nat. Chem. Biol.* **2009**, *5*, 743–748; c) C. L. Cole, S. U. Hansen, M. Baráth, G. Rushton, J. M. Gardiner, E. Avizienyte, G. C. Jayson, *PLoS ONE* **2010**, *5*, e11644; d) C. Noti, P. H. Seeberger, *Chem. Biol.* **2005**, *12*, 731–756; e) M. Petitou, A. Imberty, P. Duchaussoy, P. A. Driguez, M. L. Ceccato, F. Gourvenec, P. Sizun, J. P. Hérault, S. Pérez, J. M. Herbert, *Chem. Eur. J.* **2001**, *7*, 858–873; f) A. Lubineau, H. Lortat-Jacob, O. Gavard, S. Sarrazin, D. Bonnaffé, *Chem. Eur. J.* **2004**, *10*, 4265–4282; g) J. L. de Paz, M. Martín-Lomas, *Eur. J. Org. Chem.* **2005**, 1849–1858; h) Y. Zhou, F. Lin, J. Chen, B. Yu, *Carbohydr. Res.* **2006**, *341*, 1619–1629; i) C. Chen, B. Yu, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3875–3879; j) J. C. Lee, X. A. Lu, S. S. Kulkarni, Y. S. Wen, S. C. Hung, *J. Am. Chem. Soc.* **2004**, *126*, 476–477; k) T. Polat, C. H. Wong, *J. Am. Chem. Soc.* **2007**, *129*, 12795–12800; l) L. D. Lu, C. R. Shie, S. S. Kulkarni, G. R. Pan, X. A. Lu, S. C. Hung, *Org. Lett.* **2006**, *8*, 5995–5998; m) S. Sarrazin, D. Bonnaffé, A. Lubineau, H. Lortat-Jacob, *J. Biol. Chem.* **2005**, *280*, 37558–37564; n) H. A. Orgueira, A. Bartolozzi, P. Schell, R. E. J. N. Litjens, E. R. Palmacci, P. H. Seeberger, *Chem. Eur. J.* **2003**, *9*, 140–169; o) G. J. S. Lohman, P. H. Seeberger, *J. Org. Chem.* **2004**, *69*, 4081–4093; p) S. C. Hung, X. A. Lu, J. C. Lee, M. D.-T. Chang, S. I. Fang, T. C. Fan, M. M. Zulueta, Y. Q. Zhong, *Org. Biomol. Chem.* **2012**, *10*, 760–772.
[13] a) Y. Xu, S. Masuko, M. Takieddin, H. Xu, R. Liu, J. Jing, S. A. Mousa, R. J. Linhardt, J. Liu, *Science* **2011**, *334*, 498–501; b) R. Liu, Y. Xu, M. Chen, M. Weïwer, X. Zhou, A. S. Bridges, P. L. DeAngelis, Q. Zhang, R. J. Linhardt, J. Liu, *J. Biol. Chem.* **2010**, *285*, 34240–34249.
[14] P. Rajaratnam, P. Gupta, P. Katavic, K. Kuipers, N. Huyh, S. Ryan, T. Falzun, G. B. Tometzki, L. Bornaghi, G. Le Thanh, G. Abbenante, L. Liu, W. Meutermans, N. Wimmer, M. L. West, *Aust. J. Chem.* **2010**, *63*, 693–699.
[15] N. Karst, J. C. Jacquinet, *Eur. J. Org. Chem.* **2002**, 815–825.
[16] N. Barroca, J. C. Jacquinet, *Carbohydr. Res.* **2000**, *329*, 667–679.
[17] a) B. Leveugle, W. Ding, F. Laurence, M. P. Dehouck, A. Scanameo, R. Cecchelli, H. Fillit, *J. Neurochem.* **2002**, *70*, 736–744; b) Q. Ma, B. Dudas, M. Hejna, U. Cornelli, J. M. Lee, S. Lorens, R. Mervis, I. Hanin, J. Fareed, *Thromb. Res.* **2002**, *105*, 447–453.
[18] a) V. Hoffart, A. Lamprecht, P. Maincent, T. Lecompte, C. Vigneron, N. Ubrich, *J. Controlled Release* **2006**, *113*, 38–42; b) N. A. Motlekar, B. C. Youan, *J. Controlled Release* **2006**, *113*, 91–101; c) S. S. Lanke, S. G. Gayakwad, J. G. Strom, M. J. D'Souza, *J. Microencapsulation* **2009**, *26*, 493–500; d) J. W. Park, O. C. Jeon, S. K. Kim, T. A. Al-Hilal, K. M. Lim, H. T. Moon, C. Y. Kim, Y. Byun, *Thromb. Haemostasis* **2011**, *105*, 1060–1071.

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