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Structure-Based Design and Synthesis of HIV-1 Protease Inhibitors Employing β-D-Mannopyranoside Scaffolds

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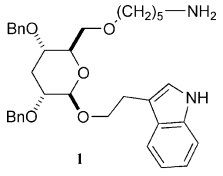
Abstract—A preliminary account on the structure-based design, synthesis and evaluation of peptidomimetic inhibitors of HIV-1 protease containing β-D-mannopyranoside scaffolds is given. The compounds prepared had IC_{50} values in the micromolar range. The results provide a platform for the development of more potent carbohydrate-based inhibitors of HIV-1 and other aspartic proteases. © 2002 Published by Elsevier Science Ltd.

Several aspartic acid proteases are of interest as therapeutic targets. Examples include renin, HIV-1 protease and memapsin 2 (β-secretase) which are respectively involved in hypertension,[1] viral infection and Alzheimer's disease.[2] Only in the case of the HIV-1 protease have inhibitors of this class of enzymes attained clinical importance.[3] This is due mostly to the lack of bioavailability of the potent inhibitors that have been developed. Also, despite the successes of using protease inhibitors for treatment of HIV, there remain a number of problems with current therapies such as overlapping resistance patterns and long-term side effects.[4] Traditional approaches for development of inhibitors have involved natural product screening or replacing the scissile bond of a peptide substrate with a non-cleavable isostere. More recently, peptidomimetic research, which has the ultimate goal of developing inhibitors with improved pharmacokinetic properties, has gained importance.[5]

In 1980, Farmer proposed the attachment of side chains to a cyclohexane ring,[6] but no experiments were carried out to assess the validity of this concept. Six years later, Bélanger and DuFresne[7] successfully explored the bicyclooctane scaffold to generate an opiate ligand; to our knowledge, the concept was not explored further. During the early 1990s, a series of publications from the University of Pennsylvania introduced the use of β-D-glucose (e.g., **1**), its enantiomers and a diastereomer (β-D-mannose) as scaffolds for the design and synthesis of ligands for the somatostatin (SRIF), the substance P (SP), and the β_2-adrenergic receptors.



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Concurrent with the early Penn studies, Olson and coworkers at Hoffmann LaRoche reported a conceptually similar approach with the synthesis of a thyrotropin-releasing hormone mimic, which employed a cyclohexane scaffold.[8] Although initially the affinities of the glucose mimics were modest (15 μM range), more potent ligands have been designed, synthesized and reported subsequently.[9] The Penn group recently obtained a glucoside with IC_{50} of 60 nM for the hSST4 receptor, and a congener revealed an IC_{50} of 8 nM at the NK-1 receptor.[10] Validation of this concept led other groups to utilize sugar scaffolds in peptidomimetic research.[11] Herein we describe the design and synthesis of first generation peptidomimetic inhibitors of HIV-1 protease that incorporate a β-D-mannopyranose scaffold.

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The structure based design work (Fig. 1) used the crystal structure of inhibitor **2** bound to HIV-1 protease;[12] this compound contains the hydroxyethylene isostere common to many aspartic acid protease inhibitors.[13] The de novo software Growmol[14] was initially used as a tool to aid in the structure based design. This software generated modified cyclohexane derivative **3** in the active site.[15] Superimposition of **2** on **3** and structural modifications using crystal structures of other ligand HIV-1 protease complexes led us to propose **5**[16] and related compounds as preliminary targets for synthesis and evaluation.[17]

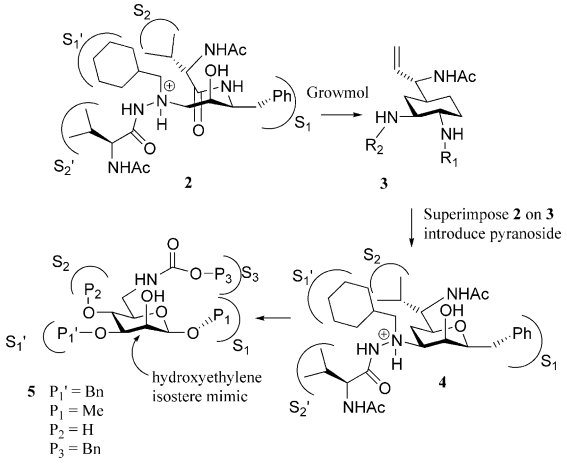


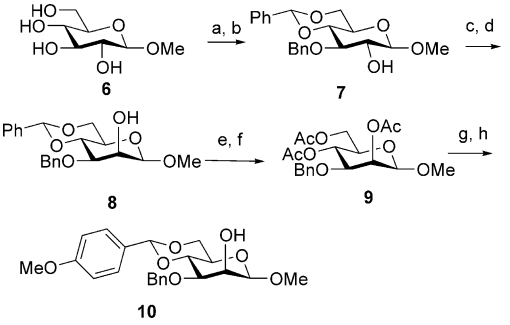
Figure 1. Design of β-D-mannopyranoside-based HIV-protease inhibitors. Inhibitor **2** is shown in its enzyme bound conformation.

From the design work, it was apparent that the β-D-mannopyranoside ring has good overlap with the peptide-like backbone and hydroxyethylene isostere of the inhibitor and could be a replacement for at least part of peptide secondary structures that bind to enzymes. It was also obvious that monosaccharides could be strategically used to orient binding groups into protease subsites and given that there are considerable similarities in the way that substrates and inhibitors generally bind to aspartic acid proteases[18] the monosaccharides and related structures could ultimately have general application as inhibitors of these enzymes.

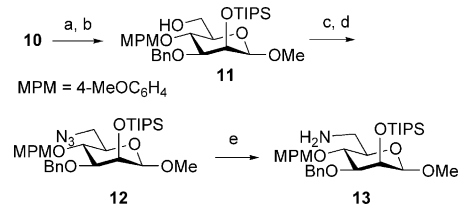
The synthesis of **5** and related compounds **15** and **16** began with commercially available methyl β-D-glucopyranoside **6**. This compound was converted to **7** by introducing a benzylidene group onto the 4- and 6-OH groups followed by regioselective benzylation using dibutyltin oxide, benzyl bromide and tetrabutylammonium iodide in benzene.[11x] Inversion of configuration at C-2 to give **8** was achieved by oxidation followed by reduction of the resulting ketone using sodium borohydride.[19] The β-D-mannopyranoside **8** was converted to **9** by removing the benzylidene protecting group using TFA in dichloromethane and treating of the crude reaction mixture with excess acetic anhydride and pyridine to give **9**.[20] The acetates were then removed from **9** using catalytic sodium methoxide in methanol and the methoxybenzylidene derivative **10** obtained by reaction of the appropriate dimethoxyacetal with camphorsulfonic acid in acetonitrile (Scheme 1).

The 2-hydroxyl was protected with the TIPS group and regiospecific cleavage of the methoxybenzylidene group proceeded smoothly to give **11**. Tosylation and substitution with azide gave **12**. Reduction of the azide to **13** was carried out using Lindlars catalyst and hydrogen in ethanol (Scheme 2). The amine **13** was converted into carbamate **14** by reaction with benzylchloroformate. Removal of the TIPS group from **14** gave **15**. Reaction of **15** with DDQ gave **5**. Amine **13** was also used to prepare **16** by first of all acetylation and subsequent removal of the TIPS group (Scheme 3).[21]

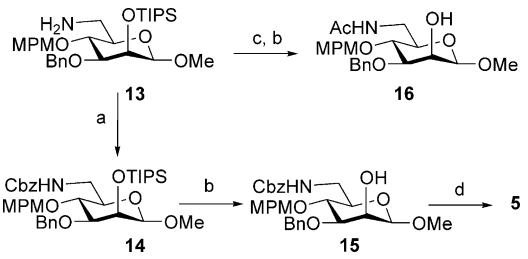
Compounds **5**, **8** and **15**–**18** were evaluated as inhibitors of HIV protease and data is summarized in Table 1.[22] Some of the compounds were moderate inhibitors (IC_{50}, 4.48–9.95 μM) whereas **17** was inactive. The activity shown by **8** is also encouraging as the synthetic route to this compound is relatively short and should facilitate preparation of a range of analogues as will be required to generate compounds with higher binding affinities. Molecular modelling using the complex of HIV-1 protease with **2** did indicate that the methoxyphenyl group in **15** and **16** and the phenyl group in **8** could have steric interactions with residues in the S_2 subsite, assuming that the compounds bind as predicted. However, large conformational changes have been observed in HIV-1 protease in response to large groups on inhibitors and it has been suggested before that attempts can be made to probe protein flexibility during inhibitor development.[13,23] Further modifications to the carbohydrate scaffold such as preparation of phenyl glycosides (see Fig. 2) should increase binding. The inhibitory activity



Scheme 1. Reagents and conditions: (a) $PhCH(OMe)_2$, CSA, MeCN, 80%; (b) Bu_2SnO, BnBr, Bu_4NI; (c) DMSO, Ac_2O (1:2), rt, 40% two steps; (d) $NaBH_4$, CH_2Cl_2, silica gel, rt, 90% (mannose/glucose ratio, 7:1); (e) TFA, CH_2Cl_2, rt; (f) Ac_2O, Py, rt; (g) NaOMe, MeOH, rt; (h) 4-$MeOC_6H_4CH(OMe)_2$, CSA, MeCN.



Scheme 2. Reagents and conditions: (a) TIPSOTf, 2,6-lutidine, CH_2Cl_2; (b) DIBAL-H, CH_2Cl_2 96% for two steps; (c) TsCl, Py, 0 °C; (d) NaN_3, DMF, 80 °C, 40% for two steps; (e) Lindlar catalyst, H_2, EtOH.



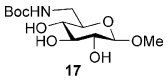
Scheme 3. Reagents and conditions: (a) BnOCOCl, Et_3N, CH_2Cl_2; (b) TBAF, THF, 0 °C; (c) Ac_2, Py, rt; (d) DDQ, CH_2Cl_2: H_2O (10:1), 0 °C.

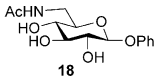
showed by **18** could suggest that this is the case and also that β-D-glucopyranosides could be useful scaffolds.

In summary, β-D-mannopyranosides have been synthesized that show inhibitory activity for HIV-1 protease. The preliminary results provide a basis for the development of more potent carbohydrate-based peptidomimetic inhibitors of aspartic proteases. Work is currently underway to optimise binding of saccharide derivatives to these enzymes.

Table 1. HIV-1 protease inhibition data for β-D-mannopyranosides[a]

Compd	Inhibition IC_{50} (μM)[b]
5	4.66 (±2.21)
8	4.85 (±1.63)
15	4.48 (±0.98)
16	7.74 (±3.19)
17	Not active
18	8.95 (±1.48)





[a]Inhibitory values for clinically used indinavir, saquinavir and nelfinavir in this assay are 0.37, 0.27 and 0.53 nM, respectively.
[b]Values are means of two experiments; standard deviation is given in parentheses.

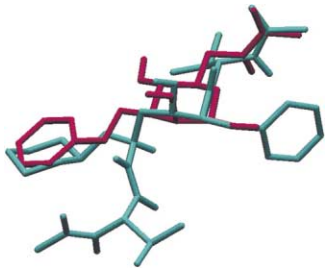


Figure 2. Overlay of **5** (red) and **2** (green). Superimposition was carried out with Macromodel 6.0. Non-polar hydrogens are omitted.

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