Bioorganic & Medicinal Chemistry Letters 20 (2010) 1957–1960

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Synthesis of fluorinated C-mannopeptides as sialyl Lewis$^x$ mimics for E- and P-selectin inhibition

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ARTICLE INFO

*Article history:*
Received 3 December 2009
Revised 21 January 2010
Accepted 23 January 2010
Available online 2 February 2010

*Keywords:*
Selectin inhibitors
sLe$^x$ Mimics
C-Glycosides
Fluorine

ABSTRACT

The synthesis of fluorinated C-mannopeptides and their evaluation as E- and P-selectin inhibitors is described. These molecules are difluorinated analogues of $CH_2$-glycopeptides already reported to act as sLe$^x$ mimics. The α and β anomers of these $CF_2$-glycopeptides have been prepared, as well as their 1-hydroxy analogues which were present in solution as an equilibrium mixture of α- and β-pyranose and α- and β-furanose forms. These molecules showed inhibitory activities comparable to their $CH_2$ counterparts with a moderate influence of the pseudo-anomeric center configuration.



Selectins are transmembrane glycoproteins which play an important role in the recruitment of leukocytes during the early stages of the inflammatory process.[1,2] The release of signaling cytokines by the damaged tissues stimulates the expression of these calcium-dependent, cell-adhesion molecules on the surface of endothelial cells. The weak, but effective affinity between selectins and certain glycoproteins expressed by circulating leukocytes allows them to slow down and roll along the endothelial wall. A strong adhesion of the leukocytes on the vascular wall occurs afterwards, thanks to the interaction between leukocytes integrins and cell-adhesion molecules, allowing their transendothelial migration. The elucidation of this mechanism provided a new therapeutic target since inappropriate or non-regulated recruitment of leukocytes may cause severe damage to tissues and contribute to inflammatory diseases (rheumatoid arthritis, asthma, lupus, etc.). Furthermore, it has also been demonstrated that selectins played a similar role in the metastasis of tumor cells.[3]

The carbohydrate epitope of leukocyte glycoproteins involved in the binding to selectins, known as PSGL-1 for P-selectin and ESL-1 for E-selectin, is the tetrasaccharide sialyl Lewis$^x$ (sLe$^x$).[2] The development of sLe$^x$ derivatives, analogues or mimics as selectin inhibitors has thus attracted considerable interest as it may give rise to new inflammatory drugs.[4,5] Due to the complex structure and tedious synthesis of sLe$^x$, the elaboration of smaller and easily synthesized molecules, but still ensuring a binding to selectins, has led to the discovery of many interesting inhibitors.[6] For example, Wong and Kaila rationally designed and prepared α- or β-C-mannosylpeptides as efficient inhibitors.[7] The fucose unit of sLe$^x$ could indeed be replaced by a mannose moiety, thanks to a close topology of the hydroxy groups interacting with selectins between these two sugars. The peptide moiety of these inhibitors featured the acidic function which is required for the binding to selectins and which was provided by the sialic acid unit in sLe$^x$.[2,7] Moreover, the enzymatic stability of these C-glycosidic structures was a considerable advantage compared to the poor bioavailability of O-glycosidic compounds.[8]

Our group has a long-standing interest in the synthesis of fluorinated C-glycosides, for which the replacement of the anomeric oxygen by a $CF_2$ group is expected to provide better sugar surrogates than standard C-glycosides.[9,10] Although in that case the methylene group does not act as an oxygen mimic, we wished to prepare the fluorinated analogues of Wong's and Kaila's inhibitors.[11] It would indeed allow us to assess the influence of a pseudo-anomeric $CF_2$ group on the binding to selectins and therefore bring information on the conformational and electronic changes that it may induce. With the mannosyldifluoroacetates **4**, **5** and **6** in hands, the preparation of the corresponding mannopeptides **1**, **2**, and **3** was

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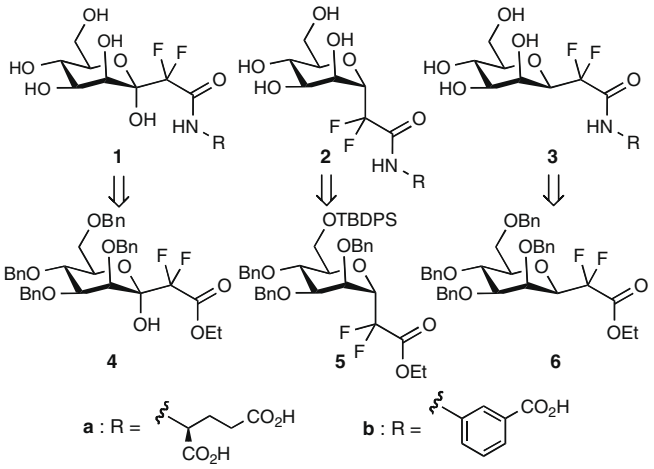
doi:10.1016/j.bmcl.2010.01.141



**Scheme 1.** Mannosyl difluoroacetates as selectin inhibitors.

straightforward (Scheme 1).[9b,d,e] Type **1** compounds feature an additional hydroxy group at C-1 allowing them to exist as an equilibrium mixture between what is supposed to be their α and β-pyranose and α and β-furanose forms. The synthesis of **1**–**3** and their evaluation as E- and P-selectin inhibitors, described herein, would thus allow us to estimate the importance of the C-1 configuration and the relevance of using structurally flexible compounds.

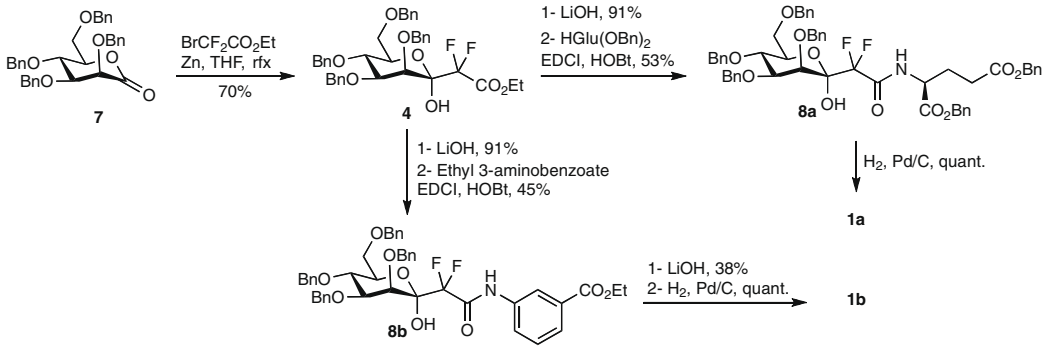
Mannoside **4** was obtained using a Reformatsky addition of ethyl bromodifluoroacetate onto lactone **7**.[9e] The reaction yielded the most stable diastereomer, that is, the one placing the difluoroacetate group in equatorial position, with a 99:1 selectivity (Scheme 2). Since lactone **7** is easily prepared from *O*-methyl-D-mannose, this straightforward preparation of a mannosyldifluoroacetate appeared as a nice entry to modified glycopeptides similar to the ones used by Wong and Kaila. The additional hydroxy group at C-1, which could not be easily reduced, did not appear to us as a drawback on first instance. The ester functionality was thus saponified and the acid was subjected to a peptidic coupling with dibenzylated glutamate or ethyl 3-aminobenzoate using classical EDC/HOBt conditions. Compounds **8a** and **8b** were obtained in moderate yield and were subsequently deprotected using a simple hydrogenolysis for **8a** and a saponification/hydrogenolysis sequence for **8b** (Scheme 2).[12]

Despite the hemi-ketal functionality, mannoside **4** and all its protected derivatives were perfectly stable and no equilibration towards their C-1 epimers was ever observed even under basic conditions in protic solvents. Debenzylated pseudo-glycopeptides **1a** and **1b** behaved differently since the NMR spectra of these compounds in $D_2O$ were much more complex than anticipated whereas mass spectrometry and elemental analysis confirmed the expected structure. The $^{19}F$ NMR spectra of these compounds indeed displayed four sets of signals corresponding to four different compounds (two doublets for each compound due to the diasterotopicity of the fluorine atoms). The description of the $^{19}F$ NMR spectrum of **1a** in $D_2O$ and its evolution (Fig. 1) might bring some explanations (a similar trend was observed for **1b**). After dissolving the product obtained from hydrogenolysis in $D_2O$, a first $^{19}F$ NMR spectrum was readily recorded which showed the presence of a major compound **I**, along with small but detectable amounts of three other compounds **II**, **III**, and **IV**. After 30 min, the composition of this mixture evolved to a 44:41:10:5 ratio for **I**:**II**:**III**:**IV** with no further variation, even after a few days. The most reasonable explanation for this was that an equilibration between the pyranose and furanose forms and their α- or β-diastereomers occurred when the deprotected mannopeptides were dissolved in a protic polar medium. Unfortunately, the complexity of the $^1H$ and $^{19}F$ NMR spectra of this mixture did not allow us to unambiguously determine the structure of each compound. However, one can reasonably postulate that the two major compounds **I** and **II** would be the β-hexopyranose and β-hexofuranose forms (i.e., the compounds for which the peptidic chain is in β position) as they should be thermodynamically favored.

This structural flexibility could of course have an influence on the binding to selectins. The preparation of mannopeptides **2** and **3**, which are locked in their α- or β-hexopyranose forms, might then provide complementary information on the structural requirements for such inhibitors. Our group recently reported a synthesis of α-mannosyldifluoroacetate **5**, via an addition of a difluoroketene acetal onto D-glucal **9**, and its conversion to **2a** and **2b** through the same sequence of reactions which was used for the synthesis of **1a** and **1b** (Scheme 3).[9b] A synthesis of β-mannosyldifluoroacetate **6** was also described by our group, involving a Reformatsky addition onto the mannose-derived ketoaldehyde **10**, and allowed the preparation of **3a** and **3b** (Scheme 3).[9d]

The targeted pseudo-mannopeptides were then tested in a competition assay for human recombinant E- and P-selectins coated to wells and using a cell-based assay (Table 1). The mannopeptides were added together with HL-60 cells expressing $sLe^x$, which therefore bind to the coated selectins as in the natural interaction between PSGL-1 and ESL-1 with P- and E-selectins.[5c,13] The ability of our compounds to displace the binding of HL-60 cells to selectins was monitored so that a percentage of inhibition and $IC_{50}$ could be assessed. Two sets of competition assays were performed. Due to their straightforward synthesis, inhibitors **1a** and **1b** were first prepared and tested. Their inhibitory activity prompted us to synthesize mannopeptides **2** and **3** which were evaluated in a second set of experiments. A control experiment was performed each time by evaluating $sLe^x$ as an inhibitor.

Compounds **1b**, **2a**, **2b**, and **3b** showed encouraging inhibitory activities on E-selectins, with $IC_{50}$ in the 2.5–5 mM range, but still inferior to $sLe^x$ ($IC_{50}$ <1 mM, entry 1). The inhibitory activities of these compounds on P-selectin were in the same range but, this time,



**Scheme 2.** Synthesis of 1-hydroxymannosides **1a** and **1b**.

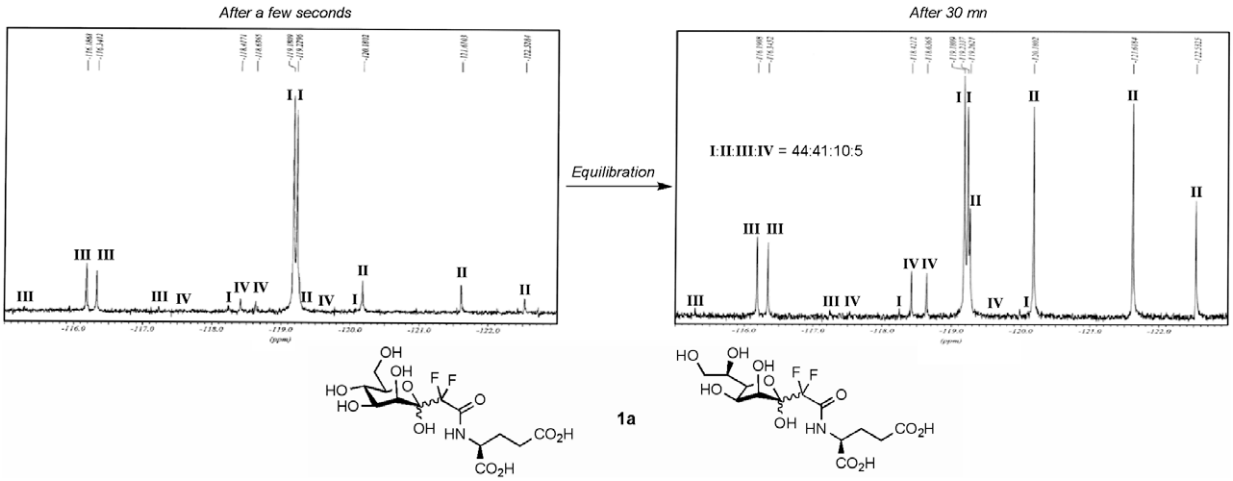
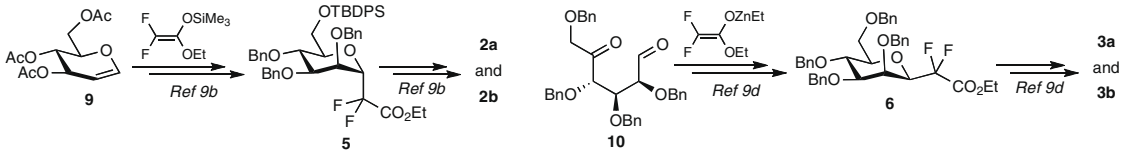



**Figure 1.** $^{19}F$ NMR spectrum of compound **1a**.




**Scheme 3.** Synthesis of α- and β-mannosides **2a**, **2b**, **3a**, and **3b**.

**Table 1**
E- and P-selectin inhibition with mannosides **1**–**3**

| Entry | Compound | E-Selectin Inhibition at 5 mM[a] (%) | E-Selectin[a] $IC_{50}$ (mM) | P-Selectin inhibition at 5 mM[a] (%) | P-Selectin[a] $IC_{50}$ (mM) |
|---|---|---|---|---|---|
| 1 | $sLe^x$ | 86 | 0.3 | 45 | >5 |
| 2 | **1a** | 13 | 11 | 19 | 11 |
| 3 | **1b** | 50 | 5 | 43 | 6.5 |
| 4 | **2a** | 61 | 3 | 59 | 3 |
| 5 | **2b** | 49 | 5 | 50 | 5 |
| 6 | **3a** | 16 | 15 | 42 | 11 |
| 7 | **3b** | 70 | 2.5 | 67 | 2.5 |

[a] All assays were performed in duplicate.

close to $sLe^x$ ($IC_{50}$ >5 mM, entry 1). The aminobenzoate residue (entries 3 and 7) appeared to be a better sialic acid surrogate than the glutamate (entries 2 and 6), but the α configuration seemed beneficial to both compounds (entries 4 and 5). More generally, α-mannosides were the most efficient compounds whereas the behaviour of the structurally flexible 1-hydroxymannosides **1** (entries 2 and 3) was highly similar to the behaviour of β-mannosides **3**. The C-1 configuration (compounds **1**, **2**, **3**) and/or the pyranose/furanose form involved in the binding (compounds **1**) appeared to have a certain influence on the inhibitory activities of the glutamate derivatives (entries 2, 4, and 6). In contrast, the 3-aminobenzoate derivatives showed similar activities for all compounds (entries 3, 5, and 7). This last consideration is in disagreement with the results reported by Kaila. Indeed, the β-mannosylglutamate prepared by this group led to an appreciable E-selectin inhibition ($IC_{50}$ = 6 mM) whereas a 3-aminobenzoate derivative displayed no activity.[7b] Finally, an analogue of compound **1a**, which featured a $OC_{16}H_{33}$ hydrophobic group on C-6 instead of the free OH group, was also prepared and tested but led to cell lysis during the competition assays. This modification, which was efficient in the case of Wong's inhibitors, was therefore not further explored.

The synthesis of the difluorinated counterparts of the C-mannopeptides described by Wong and Kaila gave rise to a new class of selectin inhibitors which showed comparable activities. Taken as a whole, the difluoromethylene group showed no striking influence on the inhibitory activities of the compounds, except for an unexpected discrepancy with Kaila's results regarding the glutamate and 3-aminobenzoate side-chains. Since the greater size of the *pseudo*-anomeric difluoromethylene group did not compromise binding to selectins, other applications for these glycomimetics are thus conceivable. A beneficial effect of the fluorine atom might indeed be expected for compounds where the $CF_2$ group acts as an oxygen mimic.

## Acknowledgments

We thank the Ministère de l'Enseignement Supérieur et de la Recherche for a Ph.D Grant to V.G.-I. and Perigene Inc. for a Ph.D Grant to F.P. and the Association pour la Recherche contre le Cancer for financial support.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.141.

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12. The experimental procedures for the synthesis of compounds **1** are similar to the preparation of **2** reported in Ref. 9b. **1a:** $[\alpha]_D$: +9.7 (*c* 0.06, $H_20$, $\lambda$ = 436 nm). $^1$H NMR (300 MHz, $D_2O$) $\delta$ 4.58 (d, 1H, *J* = 4.9 Hz), 4.37–4.30 (m, 1H), 4.22–4.15 (m, 2H), 4.05–3.98 (m, 1H), 3.95–3.55 (m, 9H), 2.41–2.28 (m, 4H), 2.20–1.85 (m, 4H). $^{19}$F NMR (282.5 MHz, $D_2O$) $\delta$ −115.7 (d, *J* = 252.4 Hz, 1F), −116.8 (d, *J* = 252.4 Hz, 1F), −117.9 (d, *J* = 248.9 Hz, 1F), −118.8 (d, *J* = 262.3 Hz, 1F), −119.1 (d, *J* = 248.9 Hz, 1F), −119.7 (d, *J* = 262.3 Hz, 1F), −119.8 (d, *J* = 258.1 Hz, 1F), −122.1 (d, *J* = 258.1 Hz, 1F). IR (neat) $\nu_{max}$ 3368, 1694, 1682 cm$^{-1}$. MS (ESI+): *m*/*z* = 425 ([M+Na]$^+$). Anal. Calcd for $C_{13}H_{19}F_2NO_{11}$: C, 38.72; H, 4.75; N 3.47. Found C, 38.54; H, 4.61; N, 3.13. **1b:** $^1$H NMR (300 MHz, $D_2O$) $\delta$ 8.20 (br s, 1H), 7.91–7.70 (m, 2H), 7.36 (br s, 1H), 3.97–3.38 (m, 6H). $^{19}$F NMR (282.5 MHz, $D_2O$) $\delta$ −115.7 (d, *J* = 253.6 Hz, 1F), −116.7 (d, *J* = 253.6 Hz, 1F), −117.8 (d, *J* = 247.6 Hz, 1F), −118.7 (d, *J* = 262.2 Hz, 1F), −118.9 (d, *J* = 247.6 Hz, 1F), −120.3 (d, *J* = 262.2 Hz, 1F), −121.0 (d, *J* = 254.7 Hz, 1F), −122.4 (d, *J* = 254.7 Hz, 1F). IR (neat) $\nu_{max}$ 3328, 1702 cm$^{-1}$. MS (FAB-): *m*/*z* = 392 ([M−H]$^-$). Anal. Calcd. for $C_{15}H_{17}F_2NO_9$: C, 45.81; H, 4.36; N 3.56. Found C, 45.55; H, 4.24; N, 3.42.
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