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C-Fucopeptides as Selectin Antagonists: Attachment of Lipid Moieties Enhances the Activity

Thomas J. Woltering,[a] Gabriele Weitz-Schmidt[b] and Chi-Huey Wong*,[a]

[a]Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA

[b]Preclinical Research, Sandoz Pharmaceuticals Ltd., CH-4002 Basel, Switzerland

Abstract: The biological activity of a potent selectin antagonist could be 40-fold enhanced by attachment of a lipid moiety. Also an enantioselective synthesis of β,ω-dihydroxyamino acids by Sharpless asymmetric dihydroxylation (AD-reaction) allowed general access to this important class of compounds. Copyright © 1996 Elsevier Science Ltd

The tetrasaccharide Sialyl Lewis x (SLe^x, **1**, Chart 1), a terminal unit of cell-surface glycoproteins and -lipids, triggers the recruitment of leukocytes and neutrophiles to the site of injury and inflammation by interaction with members of the selectin family in the early stages of the cell adhesion cascade.[1] This carbohydrate/protein recognition (SLe^x - selectins) leads to the leukocycte rolling on the endothelial vessel wall followed by protein/protein recognition (integrins CD11/18 - RGD-ligand ICAM-1) for firm attachment and eventually for extravasation of the blood cell. However, in the pathological case of overwhelming inflammatory response normal tissue can also be damaged by excessive adherence of neutrophiles/leukocytes.

Using the natural tetrasaccharide SLe^x as a lead structure a vast number of selectin antagonists has been developed in order to achieve antiadhesive and antiinflammatory activity.[2]

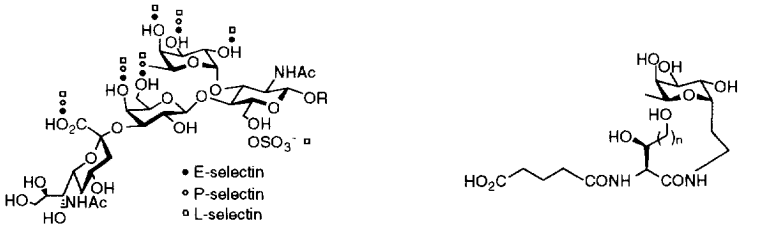
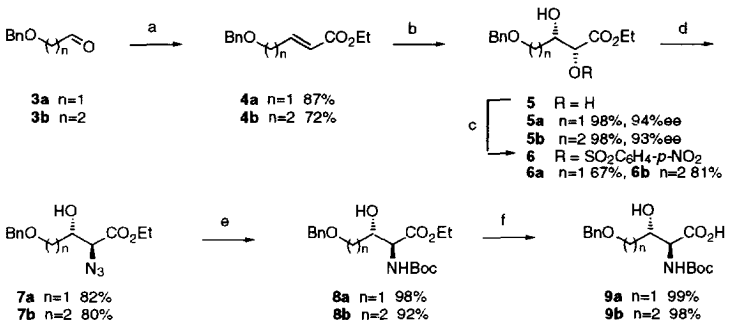


Chart 1. Sialyl Lewis^x (**1**) and its essential functional groups for binding to the selectins;[3] *C*-fucopeptides **2** and **3** as SLe^x mimetics.

Our recent series of *C*-fucopeptides as potent SLe^x mimetics successfully incorporates a β,γ-dihydroxy-L-amino acid ((2*S*, 3*R*)-2-amino-3,4-dihydroxy-butyric acid or 4-hydroxy-L-*allo*-threonine)[4] as a substitute for the essential 4- and 6-OH groups of the Gal part of SLe^x.[5] Although this particular amino acid has been convergently synthesized by L-threonine aldolase catalyzed addition of glycine to commercially available benzyloxyacetaldehyde (**3a**), the enzymatic reaction failed to produce C-3 diastereomerically pure material in the

case of higher homologes.[6]

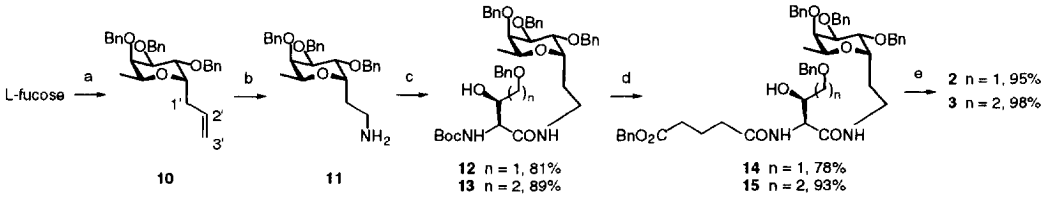
Therefore we used Sharpless asymmetric dihydroxylation (AD-reaction)[7] for a general enantioselective synthesis of the desired β,ω-dihydroxy-L-amino acids (Scheme 1).[8] The starting aldehydes **3** were converted by Horner-Emmons reaction[9] into the α,β-unsaturated esters **4**, which were dihydroxylated with high enantiomeric excess to the diols **5**.[10] Chemoselective α-(4-nitrobenzenesulfonylation)[11] of the more acidic hydroxyl group led to the compounds **6** and subsequent treatment with NaN_3 in warm DMF afforded the azides **7** by clean S_N2 displacement. Chemoselective hydrogenation with Rh/C as catalyst in the presence of Boc_2O yielded the Boc-amino esters **8**, and final saponification gave the desired *N*-Boc-ω-benzyloxy-β-hydroxy-L-amino acids **9** in 50% overall yield.



Reagents: (a) $(EtO)_2P(O)CH_2CO_2Et$, DBU, LiCl, CH_3CN; (b) AD-mix α, $MeSO_2NH_2$, *t*-BuOH/H_2O, 4°C; (c) *p*-$NO_2C_6H_4SO_2Cl$, pyr, 4°C; (d) NaN_3, DMF, 50°C; (e) H_2, Rh/C, Boc_2O, EtOAc; (f) LiOH, MeOH/H_2O, 4°C

Scheme 1. Enantioselective synthesis of the β,ω-dihydroxy-L-amino acids *via* AD-reaction.

The amine **11**, serving as the *C*-fucoside part of the SLe^x mimetics, was prepared from L-fucose as described earlier (Scheme 2).[5] Standard EDC/HOBt-mediated peptide coupling with the corresponding amino acids **9a/b** afforded the fucopeptides **12** and **13** respectively. Deprotection of the *tert.*-butyl carbamate with 50% TFA in DCM and subsequent coupling with the benzyl succinimidyl glutarate ($BnO_2C(CH_2)_3CO_2Su$)[12] gave the penta benzyl compounds **14** and **15**. Final hydrogenolysis with Pd/C in aqueous HOAc yielded the desired SLe^x mimetics **2** and **3**,[13] which were tested as inhibitors of E-selectin.



Reagents: (a) i) Ac_2O, pyr, ii) allyltrimethylsilane, BF_3•OEt_2, TMSOTf, CH_3CN, 0°C, iii) NaOMe, MeOH, iv) NaH, BnBr, TBAI, THF, 74%; (b) i) O_3, DCM/MeOH, -78°C, then $NaBH_4$, -78°C->23°C, ii) MsCl, Et_3N, DCM, 0°C, iii) NaN_3, DMF, 65°C, iv) PPh_3, H_2O, THF, 79%; (c) **9a/b**, EDC, HOBt, NMM, DMF, -20°C->23°C; (d) i) TFA, DCM, ii) $BnO_2C(CH_2)_3CO_2Su$, DMF/DCM; (e) H_2, Pd/C, HOAc-H_2O.

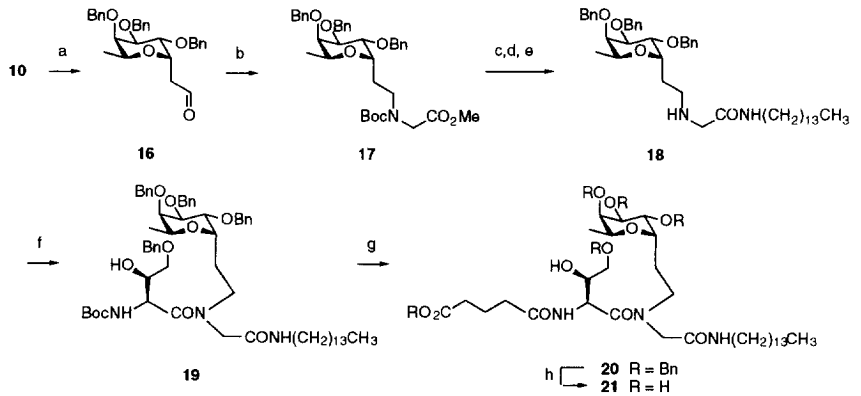
Scheme 2. Synthesis of the SLe^x mimetics **2** and **3**.

Both compounds show almost equally good inhibition with IC_{50} values of 0.3 mM (**2**) and 0.5 mM (**3**) respectively (SLe^x = 0.5 mM), and therefore served as lead structures for activity enhancing studies.

Although the interaction of a single SLe^x unit with E-selectin is relatively weak, the efficiency of the entire process is determined by the high density of SLe^x units on the cell surfaces and the corresponding multivalency of the recognition process.

Taken this enhancing effects into consideration different approaches to incorporate it into the design have been made, resulting in the synthesis of bi-, tri- up to multivalent mimetics.[14] Also monovalent lipid bearing mimetics followed by liposome generation and therefore exhibiting the multivalency of the recognition process have been synthesized.[15]

In the line of the latter approach we proceeded in attaching a lipid chain to the parent SLe^x mimetic **2** (Scheme 3). Tri-*O*-benzyl-α-*C*-allyl-L-fucose (**10**) was ozonized to the aldehyde **16**, which subsequently was condensed with glycine methyl ester.[16] The resulting imine was reduced with $NaBH_4$ in MeOH and the amine was directly protected to give *tert.*-butyl carbamate **17**. Saponification of the ester, EDC/HOBt-mediated coupling to tetradecyl amine and removal of the Boc-group afforded the secondary amine **18**. Once again, EDC/HOBt-mediated coupling to the amino acid **9a** yielded the *C*-fucopeptide **19**, which was in turn deprotected and coupled with the glutarate side chain to give the penta benzyl derivative **20**. Final hydrogenolysis removed all five benzyl groups, and the desired SLe^x mimetic **21** was obtained.[17]



Reagents: (a) O_3, DCM/MeOH, -78°C, then Me_2S, -78°C->23°C, 90%; (b) i) glycine methyl ester hydrochloride, Et_3N, $MgSO_4$, DCM, ii) $NaBH_4$, MeOH, 0°C, iii) Boc_2O, DCM, 60%; (c) LiOH, MeOH/H_2O, 4°C, 95%; (d) tetradecyl amine, EDC, NMM, $CHCl_3$, 0°C->23°C, 70%; (e) TFA, DCM, 99%; (f) **9a**, EDC, HOBt, NMM, DMF, -20°C->23°C, 62%; (g) i) TFA, DCM, ii) $BnO_2(CH_2)_3COSu$, DCM, 89%; (h) H_2, Pd/C, HOAc-H_2O, 80%.

Scheme 3. Synthesis of the lipid moiety bearing SLe^x mimetic **21**.

Testing monomeric **21** alone, it exhibits an IC_{50} of 37 μM (cell-free SLe^a-polymer/E-selectin assay),[18] which is a 8-fold increase of activity with respect to the parent compound **2**. The enhancement is even more dramatic in the HL-60/E-selectin assay, where a 40-fold increase was observed (IC_{50}: **2**, 3 mM; **21**, 69 μM). Moreover, in contrast to SLe^x at a concentration of 3 mM, mimic **21** not only inhibits E- and P-selectin to 90% and 100%, but also L-selectin (100%) almost completely! Incorporation of SLe^x mimic **21** into liposomes is in progress and further results with regard to this aspect will be forthcoming.

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13. For physical data of **2** see ref. 5. Data for **3**: ^{1}H NMR (400 MHz, D_2O) δ 1.18 (3H, d, J = 6.4, H-6), 1.61-1.72 (1H, m, H-1'a), 1.77-1.97 (3H, m, H-1'b, γ-CH_2-aa), 1.88 (2H, qui, J = 7.3, $HO_2CCH_2CH_2CH_2CH_2$), 2.37 (2H, t, J = 7.3, $CH_2CH_2CH_2CONH$), 2.39 (2H, t, J = 7.3, $HO_2CCH_2CH_2CH_2$), 3.16-3.24 (1H, m, H-2'a), 3.36 (1H, ddd, J = 13.1, 8.4, 4.5, H-2'b), 3.66-3.76 (4H, m, H-2, 3, δ-CH_2-aa), 3.87-3.95 (2H, m, H-4, β-CH-aa), 3.99-4.04 (2H, m, H-1,5), 4.30 (1H, d, J = 6.6, α-CH-aa); ^{13}C NMR (100 MHz, D_2O) δ 18.07, 23.05, 25.71, 35.75, 36.85, 37.31, 38.85, 60.48, 61.00, 69.62, 70.10, 72.25, 74.07, 75.84, 173.93, 178.47, 186.93; HRMS calcd for $C_{18}H_{33}N_2O_{10}$ (M+H) 437.2135, found 437.2149.
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17. Physical data for **21**: (NMR spectra at 23°C show rotamers, several peaks are doubled or broadened.) ^{1}H NMR (400 MHz, D_2O) δ 0.85 (3H, br s, $(CH_2)_{13}CH_3$), 1.25 (25H, br s, H-6, $(CH_2)_{11}CH_3$), 1.48 (2H, br s, $CONHCH_2CH_2$), 1.86-2.32 (8H, br m, $HO_2C(CH_2)_3CONH$, H-1'), 3.16-4.18 (15H, br m, H-1, 2, 3, 4, 5, 2', α, β, γ-H-hthr, $NCH_2CONHCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.34, 18.51, 23.70, 25.13, 29.54, 31.48, 32.02, 32.48, 34.48, 37.03, 42.06, 49.10, 52.47, 64.73, 69.72, 69.98, 72.46, 74.22, 74.32, 75.97, 172.07, 175.17, 177.81, 179.36/180.13; HRMS calcd for $CsC_{33}H_{61}N_3O_{11}$ (M+Cs) 808.3360, found 808.3368.
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