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GLYCOMIMETIC SELECTIN INHIBITORS: (α-D-MANNOPYRANOSYLOXY)METHYLBIPHENYLS

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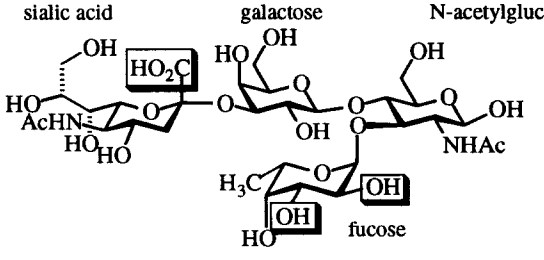
Abstract: A novel class of biphenyl-based compounds were investigated for their ability to inhibit sialyl Lewis X (sLe^x) dependent binding of HL-60 cells to E- and P-selectin fusion proteins. Compounds (**2b**) and (**2h**) demonstrated improved binding as compared to both the natural ligand sLe^x and a previously reported inhibitor TBC-265 (**1**, R = 3-CH_2CO_2H).

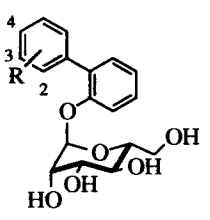
The selectins are a family of cell adhesion molecules comprised of three structurally related carbohydrate binding proteins,[1] which play a crucial role in inflammation. These proteins are expressed on the surface of vascular endothelial cells (E- and P- selectin), platelets (P-selectin), and leukocytes (L-selectin) and function in binding sialyl Lewis X (sLe^x) and related epitopes on neutrophils, monocytes, a specific subset of T lymphocytes, eosinophils, and basophils.[2] They are believed to aid in the recruitment of these cells in response to inflammatory stimuli.[1,3] Modulation of this recognition and binding process by inhibiting the selectins would potentially have clinical applications in the treatment of a number of immune system mediated disease states including reperfusion injury, septic shock, rheumatoid arthritis, psoriasis, asthma, lupus, diabetes, cancer metastasis, ARDS, and inflammatory bowel disease.[4]

sialyl Lewis^x (sLe^x)



We recently described the rational design and synthesis of a novel class of small molecule non-oligosaccharide selectin inhibitors based on a 2-(α-D-mannopyranosyloxy)biphenyl framework (**1**).[5] In this article we report an extension of this to encompass the title compounds, wherein attachment of the mannose residue to the biphenyl unit includes an additional methylene carbon (**2**). Molecular modeling indicated that alteration of the point of attachment of the mannose unit from the 2' to the 3' position would allow the same relative through-space distance and orientation between the mannose and the carboxylic acid as our initial design

(1). Previously reported designs of sLe^x mimetics[6] have typically included fragments which were intended to mimic the galactose hydroxyl groups. However, prior experience with a 2-α-D-mannopyranosyloxybiphenyl system[5] that was more potent than sLe^x indicated that omission of the galactose hydroxyls was not critical, thus this theme was maintained in the present series of compounds.



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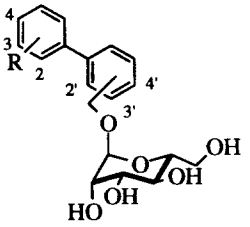

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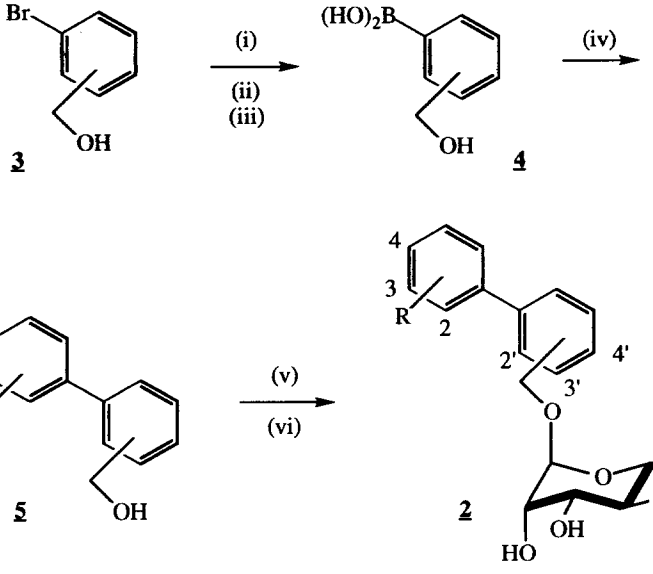
The synthesis of this class of compounds followed the general Scheme outlined below. The appropriate isomer of bromobenzyl alcohol (3) was converted to the boronic acid (4) which underwent a Suzuki palladium-catalyzed biaryl coupling[7] with the desired aryl bromide to give the hydroxymethylbiphenyl (5). Standard glycosylation followed by ester hydrolysis afforded the target compounds (2a-k).



Scheme (i) 2 equiv. BuLi; (ii) $(MeO)_3B$; (iii) H_3O^+; (iv) R'-Ar-Br, K_3PO_4, $(Ph_3P)_2PdCl_2$, DMF, Δ; (v) mannose pentaacetate, $BF_3{\bullet}Et_2O$, 1,2-$Cl_2C_2H_4$; (vi) KOH, H_2O, CH_3CN.[8,9]

The compounds were tested for their ability to inhibit sLe^x dependent binding of HL-60 cells to either E- or P-selection fusion protein,[10] and these results are summarized in the table below. There was a strong

preference for the 3'-substituted mannose attachment over the 2'- or the 4'- isomers (**2a-c**) as predicted from modeling. Activity is dependent on the distance from the biphenyl to the carboxylic acid, as seen in the series **2b**, **2d**, **2e**, and **2j** where the 3-acetic acid, **2b**, is preferred. Although the 4-substituted isomer **2f** was inactive in our assays, extending the linkage to the acid (**2g**) restored activity, and conferred selectivity for P-selectin. Restricting the tether to the carboxylic acid function by the addition of flanking methyl groups (**2h**) improved potency for both E- and P-selectin to give the most potent non-selective compound of the series. Compound **2k** was tested to verify the requirement for the carboxylic acid in these systems, and was found to rupture cell membranes as did the methyl ester **2i**.

Table. *In Vitro* Activity Data for (α-D-Mannopyranosyloxy)methylbiphenyls.

Compound	R group	(mannopyranosyl oxymethyl) attachment	E-selectin IC_{50} / IC_{50} of sLe^x *	P-selectin IC_{50} / IC_{50} of sLe^x †
2a	3-CH_2CO_2H	2'	>>14	na
2b	3-CH_2CO_2H	3'	0.4	1.2
2c	3-CH_2CO_2H	4'	>>1.5	>>2.3
2d	3-CO_2H	3'	1.7	2.5
2e	3-OCH_2CO_2H	3'	4.8	4.8
2f	4-CH_2CO_2H	3'	>14	>20
2g	4-OCH_2CO_2H	3'	4.8	1.2
2h	4-OCH_2CO_2H, 3,5-Me_2	3'	0.3	0.5
2i	3-$CH_2CO_2CH_3$	3'	nd	nd
2j	4-$C(=O)(CH_2)_2CO_2H$	3'	>6.7	>>10
2k	3-OH	3'	nd	nd

nd = not determined (the compound causes cell lysis at high concentration and prevents a determination of inhibitory activity in this assay); na = not assayed; > less than 50% inhibition at dose reported; >> no inhibition at dose reported; * sLe^x has 3 mM IC_{50} in this assay [5]; † sLe^x has 2 mM IC_{50} in this assay [5].

These results suggest that manipulation of the position of the lipophilic biphenyl portion in the carbohydrate-recognition domain of E-, and P-selectin can lead to improved binding over earlier designs. More importantly, based on these results and those reported previously,[5] it is our conclusion that while the galactose unit may be important for the binding of the native ligand (sialyl Lewis X) to the selectins, our inhibitors appear to function without a fragment which mimics the galactose hydroxyl groups. To our knowledge, the biphenyl class of compounds described here and in our previous report are the first rationally designed glycomimetics which show improved activity compared to sLe^x while maintaining only the acid and carbohydrate-recognition portions of the native tetrasaccharide ligand.

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8. Experimental for the synthesis of compound **2e**: 3-Bromophenol (9.0 g, 52.0 mM) was dissolved in DMF (105 mL), sodium hydride (2.3 g, 57.2 mM, 60% disp. in oil) was added and stirred under nitrogen for an hour. Ethyl bromoacetate (6.35 mL, 57.2 mM) was added, and stirred at rt for 18h. Water (800 mL) was added slowly with stirring at 0 °C. Filtration provided ethyl 3-bromophenoxy acetate (11.9 g, 88%). Ethyl 3-bromophenoxy acetate (1.7 g, 6.6 mM), bis(triphenylphosphine)palladium(II) chloride (0.09 g, 0.13 mM), potassium phosphate tribasic (4.2 g, 19.7 mM) and DME (30 mL) were degassed under nitrogen. 3-Hydroxymethyl benzene boronic acid (0.8 g, 5.3 mM) in DME (1 mL) was added and the mixture was heated at 80 °C overnight, then subjected to a normal work-up. Purification by flash chromatography (SiO_2, 10 : 1, hexane : ethyl acetate) gave ethyl 3-[3'-(hydroxymethyl)phenyl]phenoxy acetate (0.28 g, 19%). α-D-Mannose pentaacetate (0.5 g, 1.13 mM) was added to ethyl 3-[3'-(hydroxymethyl)phenyl]phenoxy acetate (0.28 g, 0.98 mM) in 1,2-dichloroethane (5 mL), then borontrifluoride etherate (0.42 mL, 3.2 mM) was added slowly. The mixture was stirred under nitrogen overnight at rt, then subjected to a normal work-up. The product was purified by flash chromatography (SiO_2, 10 : 1, hexane : ethyl acetate) which provided ethyl 3-[3'-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)oxymethyl]phenyl]phenoxyacetate (0.27 g, 45%) contaminated with a small amount of α-D-mannose pentaacetate, which coeluted. The ester (0.27 g, 0.44 mM) was dissolved in acetonitrile (2 mL), and treated with a solution of lithium hydroxide monohydrate (0.18 g, 4.0 mM) in water (2 mL), and stirred at rt overnight. The acetonitrile was evaporated, the residue acidified to pH 2 with concentrated hydrochloric acid, then purified by HPLC (reverse-phase, gradient elution 10-60% acetonitrile in water, 0.1% TFA, monitored at 254 nm) which gave **2e** (65 mg, 35%), m.p. 73-75 °C. ^{1}H NMR (400 MHz, D_2O): 7.66 (m, 2H), 7.54 (t, J = 7.3 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.36 (dt, J = 7.8, 1.5 Hz, 1H), 7.24 (dd, J = 2.4, 1.7 Hz, 1H), 7.02 (ddd, J = 8.3, 2.7, 0.7 Hz, 1H), 5.00 (d, J = 1.7 Hz, 1H, mannose C-1), 4.82 (d, J = 11.7, 1H), 4.74 (s, 2H), 4.67 (d, J = 11.7, 1H), 3.97 (dd, J = 3.4, 1.7, 1H, mannose C-2), 3.85-3.66 (m, 5H) ppm. IR (KBr): 3431, 2931, 1738, 1608, 1423, 1210, 1066 cm^{-1}. Analysis: C, 57.3; H, 5.6 %. $C_{21}H_{24}O_9$•0.25 TFA: requires C, 57.5; H, 5.4 %.
9. See also Kogan, T. P.; Dupré, B.; Scott, I. L.; Keller, K. M.; Dao, H.; Beck, P. J.; US patent 5,444,050, August 22, 1995.
10. Details of the assay have been reported previously: Kogan, T. P.; Revelle, B. M.; Tapp, S.; Scott, D.; Beck, P. J. *J. Biol. Chem.* **1995**, *270*, 14047, and reference 5.

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