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A Readily Available, Highly Potent E-Selectin Antagonist

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Excessive infiltration of leukocytes from blood vessels into surrounding tissues can cause acute or chronic inflammatory disorders such as reperfusion injuries, stroke, psoriasis, rheumatoid arthritis, or respiratory diseases.[1] An early step in the cascade of events which finally leads to leukocyte extravasation—their rolling on activated endothelial cells—is mediated by selectin–carbohydrate interactions.[2] The adverse effects could thus be prevented by selectin blockade. The tetrasaccharide sialyl Lewis^x (**1**, Scheme 1) is a weak[3] ligand for E-, P- and L-selectin[4] and became a lead to discover more potent inhibitors.[5] To assess our E-selectin antagonists we used a static, cell-free ligand-binding assay which measures inhibition under equilibrium conditions.[6] Sialyl Lewis^x ($IC_{50} = 1000 - 2000$ μM) was tested on each plate to allow direct comparison of data from different test plates. Thus, we obtained relative IC_{50} values (see Table 1). To further profile our compounds we developed a dynamic in vitro assay which allows to monitor E-selectin-dependent rolling of neutrophils on activated endothelial cells under shear stress and, hence, mimics the nonequilibrium in vivo conditions[7] (see Table 1). Recently we described the promising E-selectin inhibitor **2** (Scheme 1), which showed good activities in both the static (rel. $IC_{50} = 0.030$) and the dynamic ($IC_{50} = 10$ μM) assay.[8] Here we report on our search for simplified analogues of **2** which led to the discovery of **3** (Scheme 1) being the most potent small-molecule E-selectin antagonist to date ($IC_{50} = 1 - 2$ μM in the dynamic flow assay).

To simplify **2** we designed compound **4** (Scheme 1) with a glucal-derived moiety instead of the glucosamine residue. Furthermore, the benzamide in **2** was replaced by the corresponding anilide. We expected compound **4** to be readily available from the earlier described advanced intermediate **5**, which can be assembled from building blocks **6**–**9** in eight steps in an overall yield of >30% (Scheme 2).[9] The primary hydroxyl group of **5** was oxidized to yield the carboxylic acid **10**, which was then coupled with 3,4-dimethoxyaniline to give **11** (Scheme 2). Hydrogenolytic removal of the benzyl protecting groups proceeded smoothly (**12**), but subsequent cleavage of all three benzoyl groups to obtain **4** failed. Transesterification using NaOMe (1.1 equiv) gave clean removal of the gal-4 and the gal-6 benzoyl groups leading to partly protected compound **13**[10] (Scheme 2). The gal-2 benzoate remained untouched. Harsher conditions (aqueous NaOH; 50 °C) resulted in decomposition, most probably

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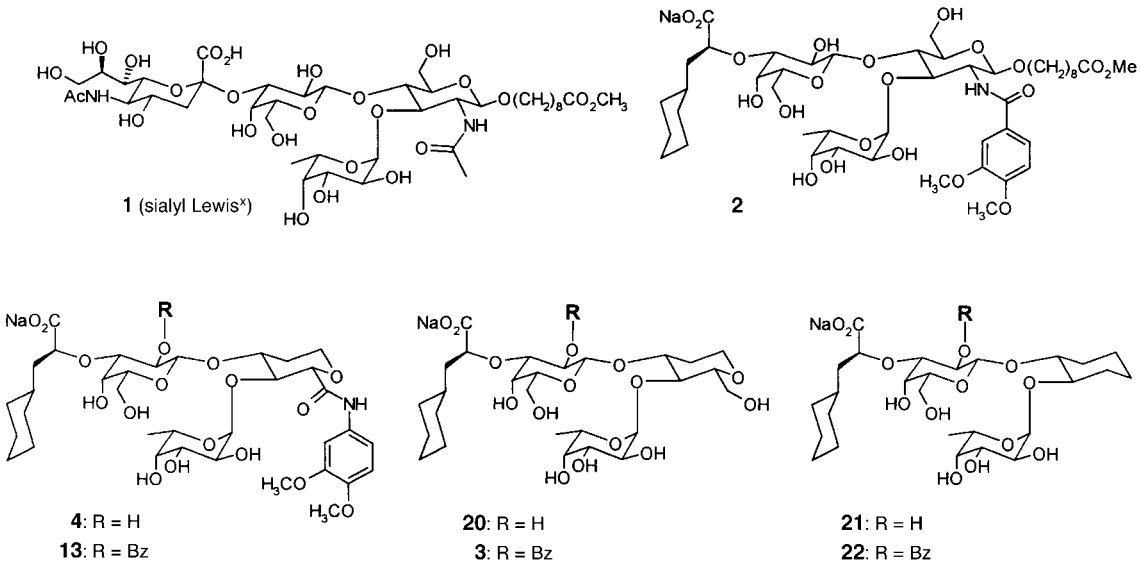
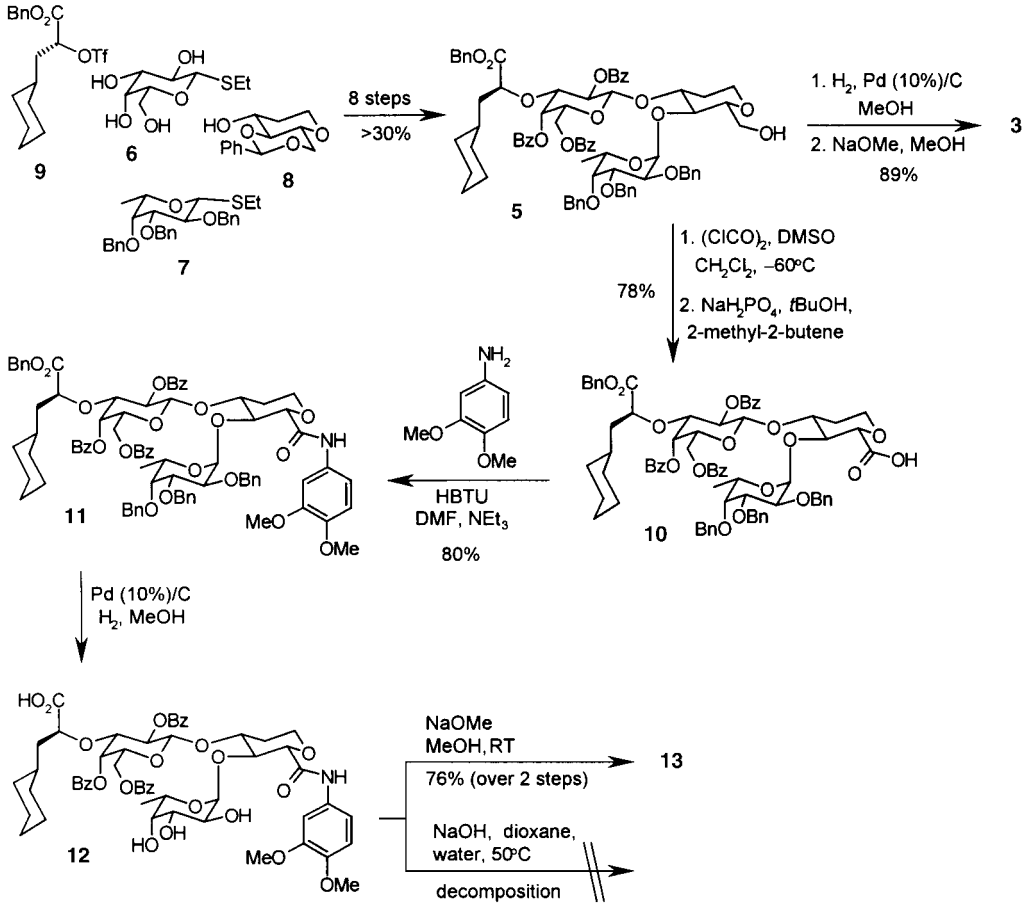


Scheme 1. Structures of selected E-selectin inhibitors. Bz = benzoyl.



Scheme 2. Synthesis of **13**. Bn = benzyl, DMSO = dimethylsulfoxide, HBTU = benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.

initiated by deprotonation at the 5-position of the glucal-derived moiety.[11] Compound **13** was tested in the static assay and, to our surprise, showed >100-fold improved potency compared to sLe^x (rel. $IC_{50} = 0.009$). In the flow assay we determined an IC_{50} value of 1-2 μM which is remarkable for a small-molecule lectin ligand[3] (see Table 1). Sialyl Lewis^x did not show any inhibition in this assay at concentrations up to 1000 μM.

To elucidate the effect of the 2-benzoate on the bioactivity we decided to prepare the originally designed compound **4** using a different route (Scheme 3). Disaccharide **14** was assembled in three steps from benzoyl-protected thiogalacto-

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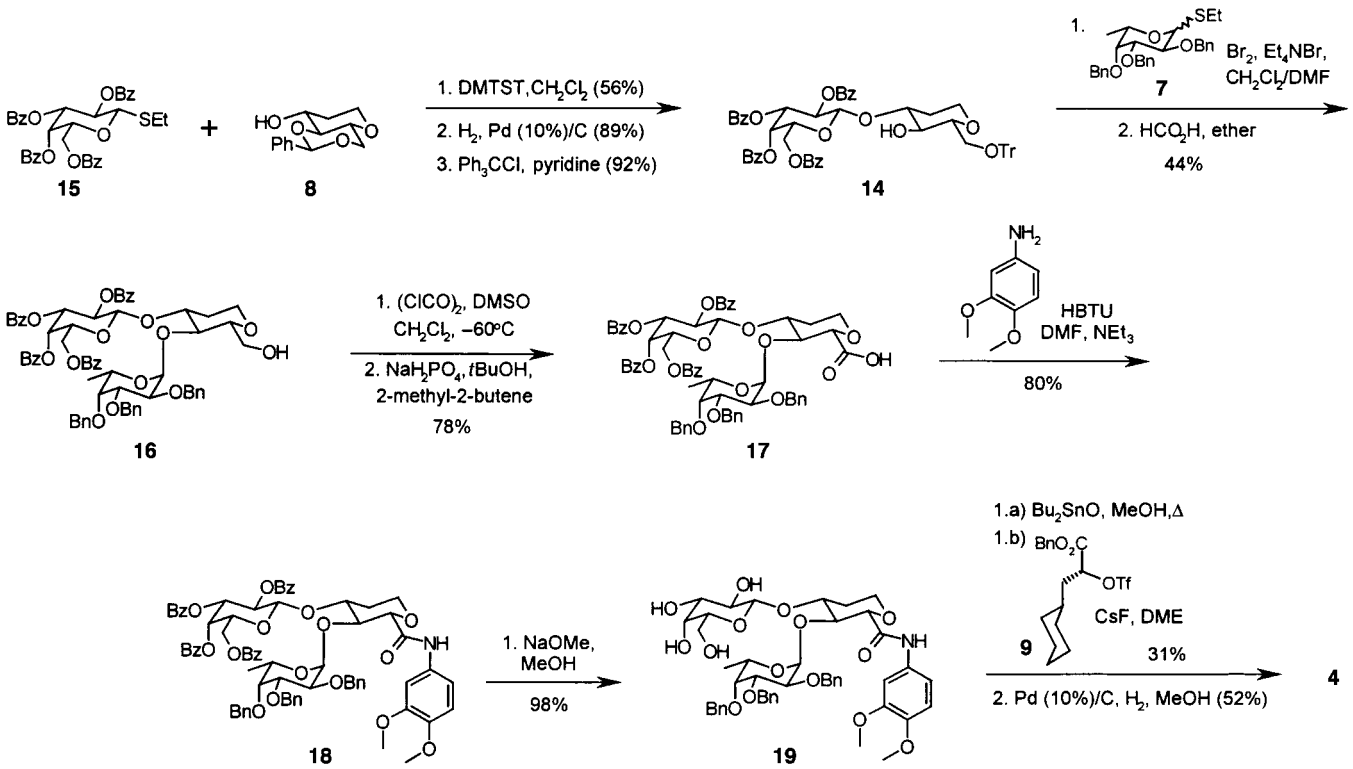

Scheme 3. Synthesis of **4**. DMTST = (dimethylsulfanyl)methylsulfonium trifluoromethanesulfonate, DMF = *N*,*N*-dimethylformamide, Tf = trifluoromethanesulfonyl.

side **15** and the glucal-derived building block **8**.[12] Fucosylation of **14** using **7** followed by removal of the trityl protecting group gave **16**. A two-step oxidation of the free hydroxyl group yielded the carboxylic acid **17** which was transformed into the anilide **18**. Transesterification using catalytic quantities of NaOMe led to complete removal of all four benzoyl groups giving the tetraol **19**. Tin-mediated selective alkylation of the 3-OH group using the triflate **9**[12] followed by hydrogenation afforded target molecule **4**.[10] It was found to be highly active in the static assay (rel. $IC_{50} = 0.025$) but significantly less potent than **13** (rel. $IC_{50} = 0.009$). Obviously, the gal-2 benzoyl group substantially affects binding to E-selectin.

Recently, we have reported on the readily available E-selectin inhibitor **20** (see Scheme 1; ten linear steps, 18% overall yield, rel. $IC_{50} = 0.031$) which is related to **4** and similarly potent.[12] We decided to prepare compound **3**, the gal-2 benzoyl derivative of **20**.[11] It was obtained in 89% yield over two steps (see Scheme 2) from **5** by hydrogenation followed by selective transesterification. Compound **3**[10] was found to be as potent as **13** in both the static (rel. $IC_{50} = 0.010$) and the dynamic assay ($IC_{50} = 1-2$ μM).

The beneficial effect of the gal-2 benzoate on E-selectin inhibition is not general. Introduction of a benzoate into our first-generation E-selectin antagonist **21**[13] (rel. $IC_{50} = 0.100$) led to **22**[10, 14] (see Scheme 1) which is slightly less potent (rel. $IC_{50} = 0.140$). Thus, it is unlikely that the benzoate group binds to the protein.[15] Both sLe^x and our inhibitors exhibit decreased fuc–gal distances in the bound form compared to their solution conformations.[16] Earlier we have demonstrated that the improved bioactivity of **20** compared to **21** is due to an improved preorganization of the bioactive conformation, caused by intramolecular steric interactions between the CH_2OH substituent of the glucal-derived moiety and the fucose residue, resulting in a decreased fuc–gal distance.[17] We believe that the gal-2 benzoate and the CH_2OH substituent in **3** (the anilide in **13**) act in concert decreasing the distance between galactose and fucose even more. This is supported by strong NOE's between galactose and fucose observed for **3**. In the static assay compound **3** is 3-fold more potent than our previously described inhibitor **20**. The effect is much more pronounced in the more relevant dynamic flow assay (20-fold) pointing out the importance of the solution conformation of compounds that interfere with dynamic, nonequilibrium processes such as cell–cell recognition

Table 1. In vitro activities of E-selectin antagonists (n.d. = not determined).

	Static rel. IC_{50}[a]	Flow[b] 200 μM	50 μM	10 μM	2 μM	IC_{50} [μM]
sLe^x	1.000	no inhibition at 1000 μM				n.d.
2[8]	0.030	97%	93%	54%	n.d.	10
3	0.010	n.d.	92%	77%	53%	1–2
4	0.025	n.d.	n.d.	n.d.	n.d.	n.d.
13	0.009	n.d.	96%	81%	56%	1–2
20[12]	0.031	94%	60%	10%[c]	n.d.	30–40
21[13]	0.100	70%	20%[c]	n.d.	n.d.	n.d.
22	0.140	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Relative IC_{50} values are defined as rel. $IC_{50} = IC_{50}$(test compound)/$IC_{50}(sLe^x)$; compounds were tested at least twice (mean value). [b] Reduction of the number of interacting cells given as percentage. [c] Statistically not significant.

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events. The results of all the inhibition tests are found in Table 1.

In conclusion, by a mix of accident, serendipity and rational design, we have discovered compound **3** which is the most potent small-molecule E-selectin inhibitor to date. Compound **3** can be prepared in ten steps in $>25\,\%$ overall yield.

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[10] **3:** MS (70 eV): *m*/*z* (%): 713(100) $[M-H]^-$; ^{1}H NMR (400 MHz, D_2O, 25 °C; selected signals): $\delta = 1.24$ (d, ^3J(H,H) = 6.5 Hz, 3H, H-6 Fuc), 4.76 (q (br), ^3J(H,H) = 6.5 Hz, 1H, H-5 Fuc), 4.84 (d, ^3J(H,H) = 4.0 Hz, 1H, H-1 Fuc), 4.84 (d, ^3J(H,H) = 8.0 Hz, 1H, H-1 Gal), 5.24 (dd, ^3J(H,H) = 9.5/8.0 Hz, 1H, H-2 Gal), 7.50 (t, ^3J(H,H) = 7.5 Hz, 2H, Ar-H), 7.65 (t, ^3J(H,H) = 7.5 Hz, 1H, Ar-H), 8.07 (d, ^3J(H,H) = 7.5 Hz, 2H, Ar-H); **4:** MS (70 eV): *m*/*z* (%): 758(100) $[M-H]^-$; ^{1}H NMR (400 MHz, D_2O, 25 °C; selected signals): $\delta = 1.10$ (d, ^3J(H,H) = 6.5 Hz, 3H, H-6 Fuc), 3.77 (s, 6H, 2 × OCH_3), 4.43 (d, ^3J(H,H) = 8.0 Hz, 1H, H-1 Gal), 4.62 (q (br), ^3J(H,H) = 6.5 Hz, 1H, H-5 Fuc), 4.78 (d, ^3J(H,H) = 4.0 Hz, 1H, H-1 Fuc), 6.95 (s, 2H, Ar-H), 7.08 (s, 1H, Ar-H); **13:** MS (70 eV): *m*/*z* (%): 862(100) $[M-H]^-$; ^{1}H NMR (400 MHz, D_2O, 25 °C; selected signals): $\delta = 1.18$ (d, ^3J(H,H) = 6.5 Hz, 3H, H-6 Fuc), 3.71 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.50 (q (br), ^3J(H,H) = 6.5 Hz, 1H, H-5 Fuc), 4.81 (d, ^3J(H,H) = 8.0 Hz, 1H, H-1 Gal), 4.82 (d, ^3J(H,H) = 4.0 Hz, 1H, H-1 Fuc), 5.17 (t (br), ^3J(H,H) = 9.0 Hz, 1H, H-2 Gal), 6.86 (m, 2H, Ar-H), 6.96 (s, 1H, Ar-H), 7.36 (t, ^3J(H,H) = 7.5 Hz, 2H, Ar-H), 7.52 (t, ^3J(H,H) = 7.5 Hz, 1H, Ar-H), 7.87 (d, ^3J(H,H) = 7.5 Hz, 2H, Ar-H); **22:** MS (70 eV): *m*/*z* (%): 681(100) $[M-H]^-$; ^{1}H NMR (500 MHz, D_2O, 25 °C; selected signals): $\delta = 1.16$ (d, ^3J(H,H) = 6.5 Hz, 3H, H-6 Fuc), 4.55 (q (br), ^3J(H,H) = 6.5 Hz, 1H, H-5 Fuc), 4.84 (d, ^3J(H,H) = 8.0 Hz, 1H, H-1 Gal), 4.89 (d, ^3J(H,H) = 4.0 Hz, 1H, H-1 Fuc), 5.16 (dd, ^3J(H,H) = 9.5/8.0 Hz, 1H, H-2 Gal), 7.52 (t, ^3J(H,H) = 7.5 Hz, 2H, Ar-H), 7.65 (t, ^3J(H,H) = 7.5 Hz, 1H, Ar-H), 8.09 (d, ^3J(H,H) = 7.5 Hz, 2H, Ar-H).

[11] Contrary to **12** compound **5**, lacking the electron-withdrawing amide substituent, could be completely debenzoylated to give **20** in $>90\,\%$ yield applying aqueous NaOH/dioxane at 50 °C.

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Acids and Bases in One Pot while Avoiding Their Mutual Destruction**

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We have demonstrated recently that separate entrapment in sol–gel matrices of a catalyst and of a reagent which poisons it enables the simultaneous use, in one pot, of these chemicals, which otherwise must be used in consecutive steps.[1] Here we extend the methodology of changing "chemical hostility" into "chemical friendliness" and compatibility, to the classical family of opposing reagents namely acids and bases.

We routinely teach at elementary-level chemistry that since acids and bases annihilate each other when brought together, one needs to separate acidic steps from basic ones in reaction sequences. We show here that sol–gel entrapment[2] solves this problem, and makes it possible to place in one-pot acids and bases without their mutual destruction, while still allowing these reagents to activate or participate in desired reactions. In numerous studies it has been shown that molecules entrapped within sol–gel matrices retain their chemical and physical properties[3] and that external substrate molecules can enter the pore network, react with the dopant, and emerge from the pores as products. Thus, the only

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