Manchester, UK), modified by addition of two pairs of collision gas cells and a low-temperature CI source equipped with a thoriated filament. Typical CI source conditions were as follows: source temperature 100 °C; repeller voltage 0.0 V; ion extraction voltage 8 kV; source pressure 0.05–0.1 Torr. The CAD spectra were recorded with helium as collision gas, admitted into the cell to such a pressure as to reduce the beam intensity by 30 %. The NR experiments were performed in the first pair of collision cells positioned between the magnet and the second electrostatic analyzer, reionization being achieved with O_2 as the collider. Any ions leaving the first collision cell were removed using a high-voltage (1 kV) deflector whose efficiency was checked by control experiments. The NR spectra were averaged over 20 to 50 acquisitions to achieve a satisfactory signal-to-noise ratio. The gases, research-grade products from commercial sources with a stated purity exceeding 99.95 mol %, were used as such. Ozone was prepared from dry O_2 in a commercial ozonizer, collected in a silica trap at 77 K and recovered by controlled warming. Some of the initially pure O_3 desorbed from the trap undergoes dissociation into O_2 in the transfer line and/or in the ion source. Furthermore, trace impurities such as H_2O and CO_2 are produced by unspecified reactions of O_3 with the transfer line and the source materials. $^{15}N_2$ (98 atom %) was obtained from Aldrich Chemical Co., Inc.

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Preorganization of the Bioactive Conformation of Sialyl Lewis^x Analogues Correlates with Their Affinity to E-Selectin

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Excessive infiltration of leukocytes from blood vessels into surrounding tissues can cause acute or chronic reactions as observed in reperfusion injuries, stroke, psoriasis, rheumatoid

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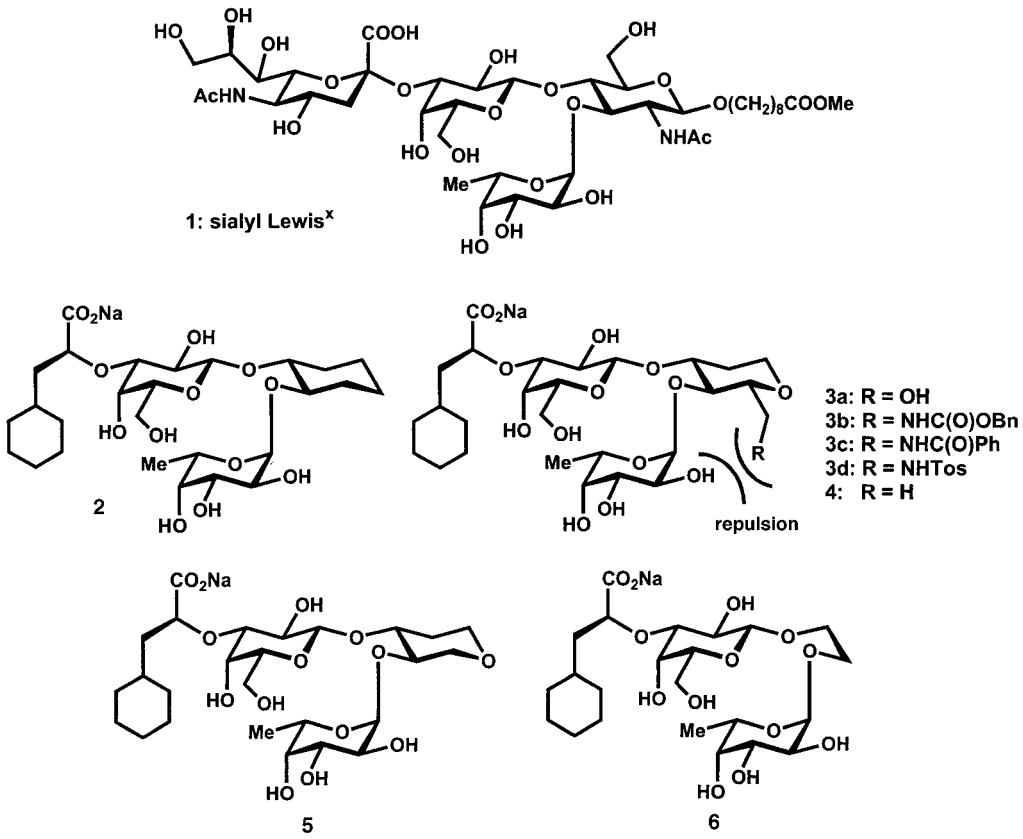
arthritis, or respiratory diseases.[1] An early step in the inflammatory cascade that finally leads to leukocyte extravasation is mediated by selectin–carbohydrate interactions.[2] With both selectin-knockout mice[3a–c] and leukocyte adhesion deficiency type 2 patients,[3d] it could be demonstrated that the selectin–carbohydrate interaction is a prerequisite for the inflammatory cascade to take place.

The tetrasaccharide sialyl Lewis^x (sLe^x, **1**, Scheme 1) is the carbohydrate epitope recognized by E-selectin[4] and, therefore, became the lead structure for the design of selectin antagonists.[5] The pharmacophores required for E-selectin recognition are the carboxylic acid function, all three hydroxyl groups of the fucose, and the 4- and the 6-hydroxyl groups of the galactose.[6] By replacing *N*-acetylglucosamine (GlcNAc) with (*R*,*R*)-cyclohexane-1,2-diol and by introducing lactic acid derivatives instead of neuraminic acid, we discovered simplified sLe^x analogues with improved affinities (for example, **2** in Figure 1).[7] By incorporating a glucal-derived replacement for GlcNAc we obtained the antagonist **3a**[8] with 30-fold improved activity compared to sLe^x.[9] Further modifications of the R group in order to explore a potential additional binding site of E-selectin did not affect the biological activity (for example, **3b–d** are similarly active to **3a**; Table 1).[10] Thus, additional interactions of the R group with E-selectin are unlikely.



Scheme 1. Sialyl Lewis^x and analogues. Bn = benzyl, Tos = toluene-4-sulfonyl.

Here we show that the cause for the improved activity of all glucal-derived derivatives **3** is the improved preorganization of the bioactive conformation due to beneficial steric constraints imposed by the equatorial substituent CH_2R. For this purpose we designed compound **4** (Scheme 1) which lacks the R group but still provides an equatorial substituent adjacent to the fucose that could preorganize the bioactive conformation of the core structure. Compound **5** was prepared as a control to investigate the role of the additional ring oxygen atom which could affect both the flexibility and the solvation of the molecule. Finally, compound **6** was designed to still provide all the essential pharmacophores, but with a flexible linker between fucose and galactose to substantially reduce steric constraints.

Selectin antagonist **4**[11] was prepared from the galactose derivative **7**[12] using the glucal-derived building block **8**[13] and the fucosyl donor **9**[14] (Scheme 2). The thioglycoside **7** was transformed into the galactosyl donor **10** by tin-mediated selective alkylation with triflate **11**, followed by benzoylation. Treatment of a mixture of **8** and **10** with DMTST followed by acid-catalyzed removal of the acetal gave a diol intermediate, which was then regioselectively tritylated to furnish **12**. Fucosylation with **9** and subsequent cleavage of the trityl group yielded **13**. The deoxygenation of alcohol **13** was performed by treatment with *O*-pentafluorophenyl chlorothioformate, followed by radical reduction with tri-*n*-butyltin hydride.[15] Advanced intermediate **14** was deprotected and the final product **4** isolated as a sodium salt.

Control compound **5** was obtained from xylal **15**[16] (Scheme 3). Regioselective β-galactosylation with trichloroacetimidate **16**,[17] followed by hydrogenation, gave **17**. Fucosylation applying thioglycoside **9** (→**18**) and removal of the benzoate groups furnished **19**, which was selectively alkylated in the 3-position using triflate **20**. Subsequent deprotection of intermediate **21** gave **5**.[11] Compound **6**[11] was prepared from galactose derivative **22** and ethyleneglycol **23** as shown in Scheme 4.

Compounds **4**, **5**, and **6** were tested in a static, cell-free ligand binding assay that measures E-selectin inhibition under equilibrium conditions[18] and the results were compared with those for antagonists **2** and **3** (Table 1). Inhibitor **4** is equally as potent as **3a–d** and showed a threefold improvement in activity relative to **2**. The control compound **5** is less active than **2**. Compound **6** with a flexible linker between galactose and fucose showed no E-selectin inhibition up to 10 mM. Antag-

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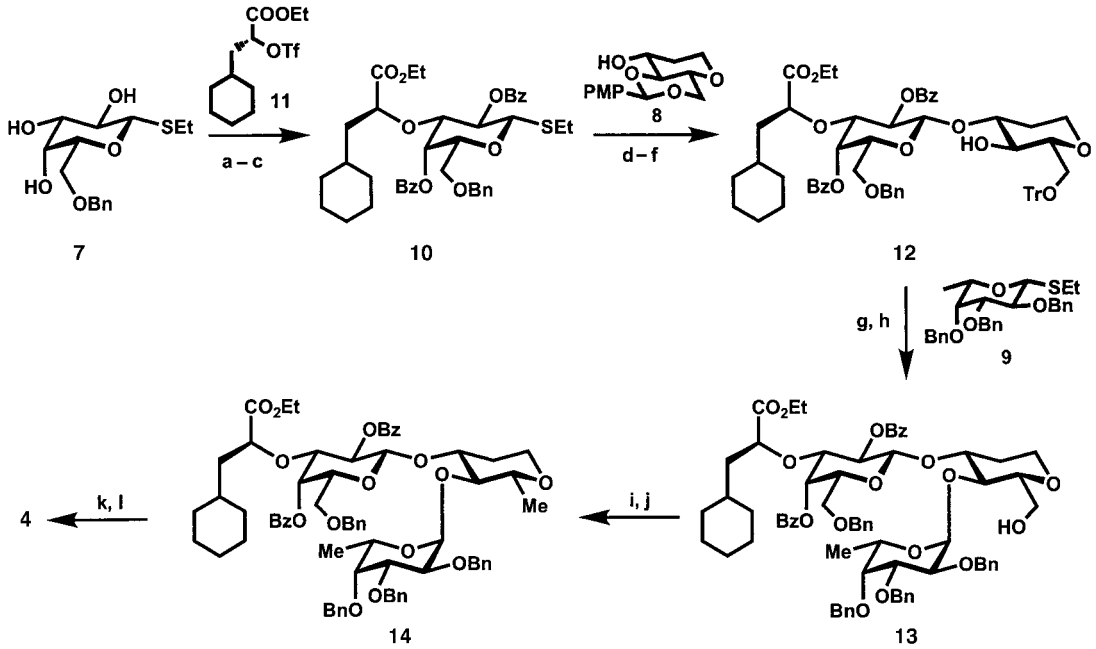
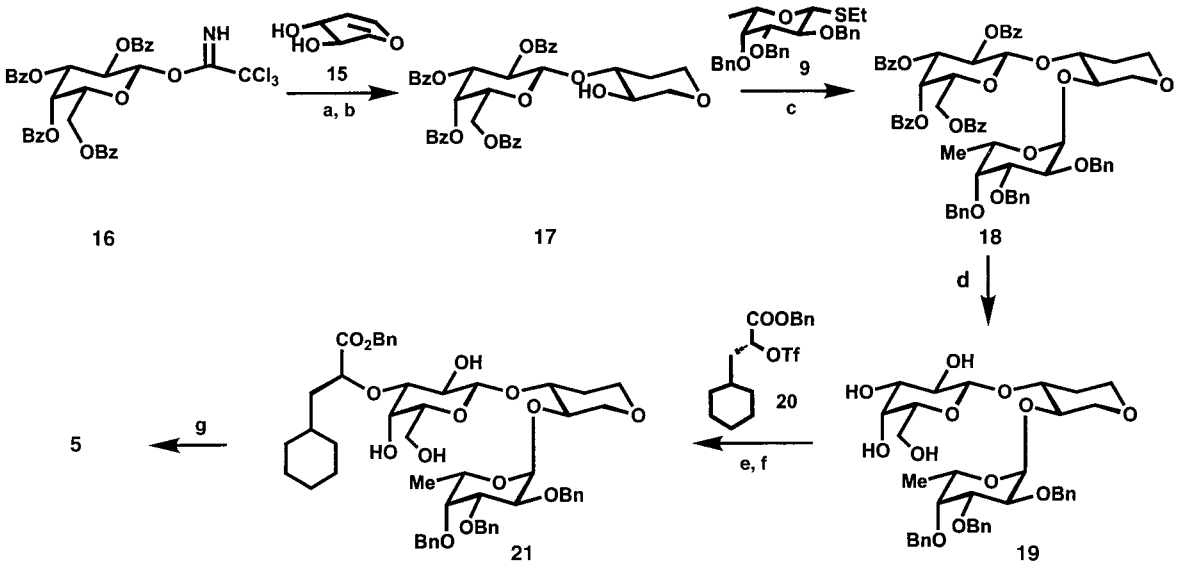


Scheme 2. a) Bu_2SnO, CH_3OH, reflux; b) ethyl (*R*)-3-cyclohexyl-2-trifluoromethanesulfonyloxypropionate (**11**), CsF, DME, 45% over 2 steps; c) BzCl, pyr, 90%; d) **8**, DMTST, CH_2Cl_2; e) CSA, CH_2Cl_2, MeOH, 63% over 2 steps; f) Ph_3CCl, pyr, 70 °C, 93%; g) ethyl tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**9**), Br_2, Et_4NBr, DMF/CH_2Cl_2 (3/2); h) HCOOH, Et_2O, 52% over 2 steps; i) $C_6F_5OC(S)Cl$, C_6H_6, *N*-hydroxysuccinimide, 80 °C, 85%; j) Bu_3SnH, AIBN, C_6H_6, 80 °C, 48%; k) NaOH, MeOH, dioxane, 90%; l) H_2, $Pd(OH)_2$/C, MeOH, AcOH, Dowex 50 (Na^+ form), 83%. AIBN = azobisisobutyronitrile, Bz = benzoyl, CSA = camphorsulfonic acid, DME = 1,2-dimethoxyethane, DMF = *N*,*N*-dimethylformamide, DMTST = (dimethylthio)methylsulfonium trifluoromethanesulfonate, PMP = 4-methoxyphenyl, pyr = pyridine, Tf = triflate = trifluoromethanesulfonyl, Tr = triphenylmethyl.



Scheme 3. a) Xylal (**15**), TESOTf, CH_3CN, −40 °C, 66%; b) H_2, PtO, MeOH, 87%; c) ethyl tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**9**), Br_2, Et_4NBr, DMF/CH_2Cl_2 (3/2), 56%; d) NaOMe, MeOH, 83%; e) Bu_2SnO, MeOH, reflux; f) benzyl (*R*)-3-cyclohexyl-2-trifluoromethanesulfonyloxypropionate (**20**), CsF, DME, 50%; g) H_2, Pd/C, dioxane/H_2O (3/1), AcOH, Dowex 50 (Na^+ form), 78%. TES = triethylsilyl.

onists **2**, **3**, and **4** were also tested in a dynamic in vitro assay that allows the E-selectin-dependent rolling of neutrophils on activated endothelial cells to be monitored and, hence, mimics the nonequilibrium in vivo conditions (Table 1).[19] As observed under static conditions, antagonist **4** was equally as active as **3a** and **3b**, but superior to **2**. Interestingly, the improved activity of **3** and **4** relative to **2** is more pronounced in the dynamic flow assay (sixfold improvement) than in the static equilibrium assay (threefold improvement).

In order to investigate conformational effects, the solution conformations of compounds **2**, **4**, **5**, and **6** were studied by NMR spectroscopy[20] and compared to the bioactive conformation of **2**.[7b, 21] Three interglycosidic NOE effects ($H2^G$ – $H5^F$, $H2^G$ – $H6^F$, and $H6^G$ – $H5^F$) were used to characterize the distance between the galactose and fucose moieties (Table 2). In solution, the NOE values suggest the closest proximity is in **4**, followed by **2**, **5**, and **6**. In the bound conformation of **2**, the distance between galactose and fucose is even shorter.[22]

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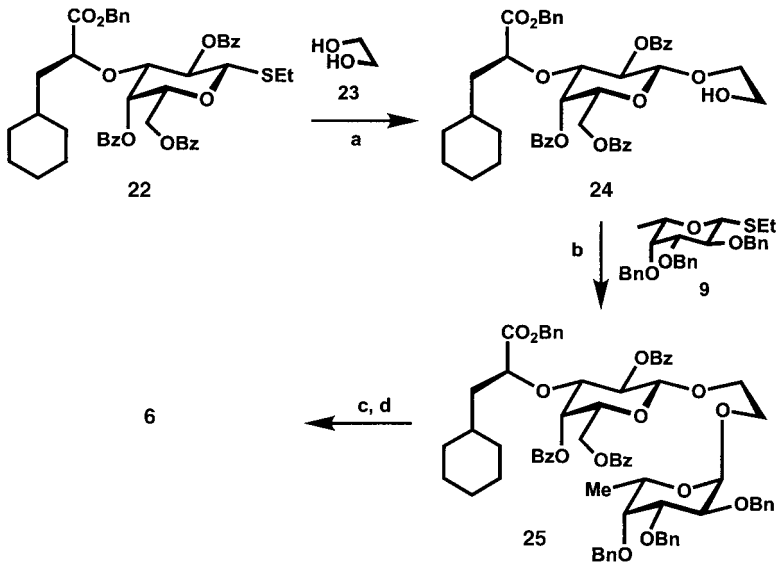

Scheme 4. a) Ethyleneglycol (**23**), DMTST, CH_2Cl_2, 70%; b) ethyl tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**9**), DMTST, DMF/CH_2Cl_2, 80%; c) H_2, Pd/C, MeOH, AcOH, 80%; d) LiOH, MeOH/H_2O (3/1), Dowex 50 (Na^+ form), 75%.

Table 1. E-selectin inhibition in static and dynamic assays.

Compound	IC_{50} values [μM]	
	Static cell-free assay	Dynamic cell assay
1 (sLe^x)	1100[a]	> 1000
2	110[a]	150 – 200
3a	36[a]	30 – 40
3b	33[a]	20 – 30
3c	37[b]	n.d.[d]
3d	42[b]	74% reduction at 50 μM
4	39[a]	20 – 30
5	230[b]	n.d.[d]
6	> 10000[c]	n.d.[d]

[a] Mean value from three assay runs. [b] Mean value from two assay runs. [c] Value from one assay. [d] n.d. = not determined.

Table 2. Normalized NOE values and selected chemical shifts [ppm] for **2**, **4**, **5**, and **6** in solution and for **2** bound to E-selectin.

NOE	**6** (soln)	**5** (soln)	**2** (soln)	**4** (soln)	**2** (bound)
$H2^G - H5^F$	0.003	0.043	0.094	0.108	0.081
$H6^G - H5^F$	< 0.001	0.008	0.013	0.022	0.088
$H2^G - H6^F$	0.006	0.062	0.070	0.157	0.172
$\delta(H5^F)$	4.12	4.42	4.60	4.77	n.d.

Therefore, according to these interglycosidic NOE values the bioactive conformation of **2** is best mimicked by the solution conformation of compound **4**, followed by **2**, **5**, and **6**. Their in vitro activity increases with decreasing distance between galactose and fucose, most probably due to the decreasing entropic cost of adopting the bioactive conformation in the binding process.[23]

Based on calculations using the experimental $H2^G - H6^F$ NOE values (Table 2), the weighted mean distance between $H2^G$ and $H6^F$ is 4.1 Å for **2** and 3.55 Å for **4**. The weighted mean distance in **4** is thus very close to the distance in the bioactive conformation of **2** (3.5 Å). If a two-site jump model with an equilibrium of the ligand in the bioactive conformation and the ligand in an extended conformation (when there is no $H2^G - H6^F$ NOE) is considered, the bioactive conformation is populated by 40% in **2**, and by 90% in **4** according to the ratio of the corresponding NOE values.

The reduced distance between galactose and fucose in higher-affinity ligands is also reflected by the chemical shifts of protons that directly face the opposing carbohydrate. As an example, the chemical shift of $H5^F$ changes from δ=4.12 for the inactive compound **6** to $\delta = 4.77$ for inhibitor **4** (Table 2). This pronounced downfield shift is caused by the decreasing distance between $H5^F$ and the galactose and is in line with the binding affinities of the compounds.

In conclusion, we have demonstrated that improved preorganization of the bioactive conformation in solution contributes substantially to the affinity of E-selectin antagonists. The effects can be detected in a static equilibrium assay but are even more pronounced in a nonequilibrium flow assay. Our results suggest that NOE intensities or NMR spectroscopic chemical shifts can be used as surrogate markers to predict biological activities. Further examples are currently under investigation.

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[11] Compound **4**: ^{1}H NMR (600 MHz, D_2O, 25 °C, DSS): $\delta = 5.03$ (H1^F), 3.78 (H2^F), 3.88 (H3^F), 3.80 (H4^F), 4.77 (H5^F), 1.19 (H6^F), 4.49 (H1^G), 3.60 (H2^G), 3.38 (H3^G), 3.89 (H4^G), 3.60 (H5^G), 3.72 (H6^G), 3.47 (H1a^S), 3.94 (H1e^S), 1.62 (H2a^S), 2.21 (H2e^S), 3.98 (H3^S), 3.27 (H4^S), 3.43 (H5^S), 1.35 (H6^S). Compound **5**: ^{1}H NMR (600 MHz, D_2O, 25 °C, DSS): $\delta = 4.96$ (H1^F), 3.74 (H2^F), 3.88 (H3^F), 3.79 (H4^F), 4.42 (H5^F), 1.19 (H6^F), 4.49 (H1^G), 3.58 (H2^G), 3.38 (H3^G), 3.90 (H4^G), 3.62 (H5^G), 3.72 (H6^G), 3.55 (H1a^S), 3.90 (H1e^S), 1.67 (H2a^S), 2.15 (H2e^S), 4.01 (H3^S), 3.66 (H4^S), 3.46 (H5a^S), 3.99 (H5e^S). Compound **6**: ^{1}H NMR (600 MHz, D_2O, 25 °C, DSS): $\delta = 4.91$ (H1^F), 3.76 (H2^F), 3.87 (H3^F), 3.78 (H4^F), 4.12 (H5^F), 1.20 (H6^F), 4.44 (H1^G), 3.59 (H2^G), 3.40 (H3^G), 3.91 (H4^G), 3.66 (H5^G), 3.75 (H6^G), 3.88, 3.70 (H1^S), 4.08, 3.86 (H2^S). F = fucose, G = galactose, S = spacer between F and G.

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[20] NMR spectroscopic measurements were carried out on a Varian UnityPlus 600 spectrometer operating at a frequency of 600 MHz. All experiments were carried out at 25 °C with compound concentrations of approximately 10 mM in D_2O solutions. ROESY experiments were carried out with mixing times of 150 ms. For some compounds, ROESY spectra were recorded using 50 ms and 100 ms mixing times, and the results were indistinguishable. Typical acquisition times in the direct and indirect dimensions were 340 ms and 64 ms, respectively. The data were zero-filled to yield a digital resolution of 1.46 Hz and 2.92 Hz, respectively. For bound **2**, NOE values were extracted from transfer NOE spectra (see ref. [7b]). Signal integration was performed using Varian VNMR software. All NOEs were normalized using intraglycosidic NOE enhancements such as H1^G–H3^G and H4^F–H5^F. The distances between these hydrogens are given by the chair conformations of fucose and galactose and should be very similar for compounds **2**, **4**, **5**, and **6**. It should be noted that the measured NOE values do not refer to a distinct conformation of the molecule but represent the weighted mean distance over several coexisting low energy conformations.

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[22] The H2^G–H5^F NOE value decreases in the bound state, not only for antagonist **2**, but also for sLe^x.[21] This effect can be readily interpreted as a lateral translation of fucose relative to galactose in the direction of more negative (ϕ,ψ) values, which accompanies the relative approach of fucose and galactose upon binding.

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Benzene-Free Synthesis of Phenol**

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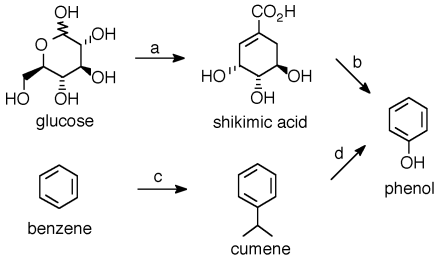
Although phenol has been synthesized by a succession of processes, the Hock oxidation of benzene-derived cumene (Scheme 1) is currently the predominant method used in the



Scheme 1. Synthesis of phenol. Conditions: a) *E. coli* SP1.1PTS$^-$/pSC6.090B; b) 1) H_2O, 350 °C, 2) Cu^0, H_2O, 350 °C; c) propene, $AlCl_3$; d) 1. O_2, 100 °C, 2. △, H_2SO_4.

production of phenol, which amounts to 5×10^9 kg annually.[1] Most of the past and currently employed phenol syntheses use benzene,[1] a volatile organic carcinogen, as the starting

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