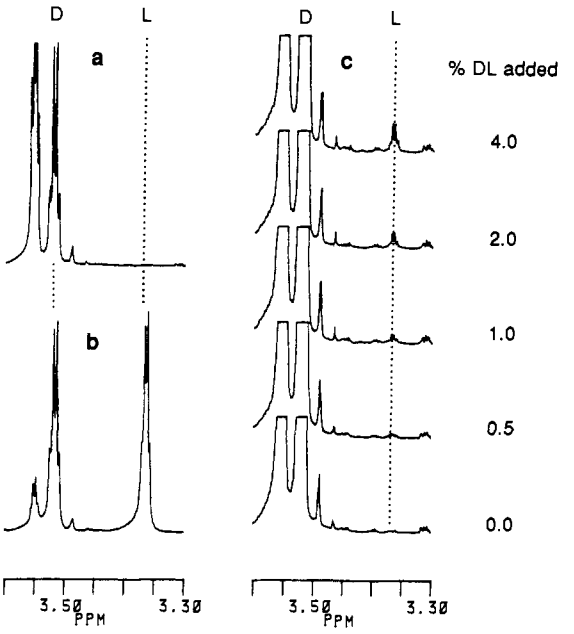

Figure 10. ^{1}H NMR spectra (300 MHz) at 3.30-3.60 ppm of the methoxy ether peaks of the (*R*)-(+)-MTPA derivatives of (a) methyl D-α-*cis*-crotylglycinate, (b) methyl DL-α-*cis*-crotylglycinate, and (c) a + 0.0-4.0% of b (base-line detail).

solution of **44-CA**, pH 7.5, and stirred gently under nitrogen. After the reaction had leveled off at 50% conversion, the dialysis tubing was removed and dialyzed against distilled, degassed water to remove products. The same enzyme was used subsequently to hydrolyze **43-CA** and **42-CA**.

Determination of the Enantiomeric Purities of Amino Acids. The enantiomeric purities of amino acids were determined by ^{1}H NMR analysis of the (+)-MTPA amides[60] of the amino acid methyl esters. Methyl esters were prepared without racemization with methanol/thionyl chloride[79] or diazomethane/methanol. The (+)-MTPA methoxy groups of L- and D-amino acid derivatives appeared as narrow quartets (J_{HF} = 1.0-1.5 Hz) at approximately δ 3.35 and 3.50, respectively. The α-methyl singlets of α-methyl amino acid derivatives (δ 1.69 and 1.65 for D- and L-amino acids, respectively) were also distinguishable. Calibration by the addition of known amounts of the (+)-MTPA derivative of the appropriate racemic amino acid showed that a 0.25% diastereomeric impurity (99.5% ee for the amino acid) could be detected (Figures 9 and 10).

Conversion of D- and L-α-Aminobutyric Acid to (*S*)- and (*R*)-Butene Oxide. Resolved α-aminobutyric acid was converted in three steps to enantiomerically enriched (96% ee) butene oxide as described previously.[80]

Iodolactonization of 7-A, 10-A, and 11-A. Allylglycine derivatives **7-A**, **10-A**, and **11-A** were iodolactonized under kinetic-controlled conditions.[64] Analysis by ^{1}H NMR and NOED spectroscopy indicated that **57**, **58**, and **59** were formed with cis to trans ratios of 8:1, 8:1, and 7:1, respectively.

Acknowledgment. We thank Jaesang Kim for preparing **10-A** and for performing some of the direct chloroacetylations and the resolution of **3-A**.

Supplementary Material Available: Calibration of the variable colorimetric response of amino acids with ninhydrin and tables of spectroscopic and analytical data for potential substrates of acylase I and compounds **57-59** (25 pages). Ordering information is given on any current masthead page.

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Probing of the Combining Site of the PapG Adhesin of Uropathogenic *Escherichia coli* Bacteria by Synthetic Analogues of Galabiose

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Abstract: The binding between uropathogenic *Escherichia coli* bacteria and human P erythrocytes was investigated by use of the hemagglutination inhibitors methyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (methyl β-D-galabioside, **1**), the monodeoxy (**2**, **3**, **7**, **10**, **11**, **13**, **16**), some *O*-methyl (**6**, **9**, **12**), deoxyfluoro (**8**, **14**, **17**), *C*-methyl (**4**), and *C*-ethyl (**5**) derivatives and various glycosides (**18-26**). The β-galabiose portion of the glycolipids present on P erythrocytes was found to bind to the pilus-located adhesin of the bacteria by hydrogen bonds directed toward five oxygen atoms situated on an edge of the disaccharide and by interactions between nonpolar surfaces in the sugar and the protein. One of the two remaining hydroxyl groups of galabiose seems to be in contact with a nonpolar cavity of the adhesin and the other with the surrounding aqueous medium.

Bacterial adhesion is considered to be important for growth and probably for expression of pathogenicity.[1,2] Adhesion is often mediated via proteinaceous appendages termed pili or fimbriae.[3,4] The adhesive bacteria identified so far show specificity toward a limited number of sugars, which was suggested to depend on the fact that most investigations have only been performed with readily available mono- and oligosaccharides for inhibition experiments.[2] Recently, various glycosides of galabiose (α-D-Gal*p*-(1-4)-β-D-Gal*p*) have been synthesized[5] and used as in-

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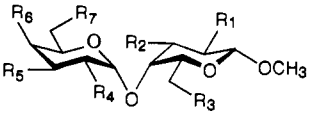
Table I. Inhibition of the Hemagglutination of *E. coli* (HB101/pPAP5) by Methyl β-D-Galabioside (**1**) and Various Analogues



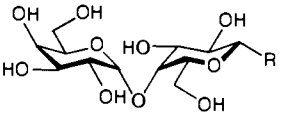
cmpd	R_1	R_2	R_3	R_4	R_5	R_6	R_7	IC_{50},[a] mM	inhib power,[b] %	$\Delta\Delta G$,[c] kJ mol^{-1}
1	OH	OH	OH	OH	OH	OH	OH	0.18	100	0.0
2	H	OH	OH	OH	OH	OH	OH	0.30	61	1.1
3	OH	H	OH	OH	OH	OH	OH	0.98	19	3.9
4	OH	Me	OH	OH	OH	OH	OH	0.37	50	1.6
5	OH	Et	OH	OH	OH	OH	OH	3.4	5.4	6.7
6	OH	OMe	OH	OH	OH	OH	OH	1.6	11	5.1
7	OH	OH	H	OH	OH	OH	OH	4.2	4.4	7.2
8	OH	OH	F	OH	OH	OH	OH	9.2	1.9	9.1
9	OH	OH	OMe	OH	OH	OH	OH	>25	<1.0	>11
10	OH	OH	OH	H	OH	OH	OH	2.3	7.6	5.9
11	OH	OH	OH	OH	H	OH	OH	6.4	2.7	8.3
12	OH	OH	OH	OH	OMe	OH	OH	0.082	200	−1.6
13	OH	OH	OH	OH	OH	H	OH	10	1.9	9.1
14	OH	OH	OH	OH	OH	F	OH	3.3	6.2	6.4
15	OH	OH	OH	OH	OH	epi[d]	OH	5.0	3.8	7.5
16	OH	OH	OH	OH	OH	OH	H	3.6	5.4	6.7
17	OH	OH	OH	OH	OH	OH	F	0.33	50	1.6

[a] 95% confidence limits: [0.930 × IC_{50}; 1.07 × IC_{50}] mM. [b] 95% confidence limits: [0.904 × Inhib power; 1.11 × Inhib power]%. [c] 95% confidence limits: $\Delta\Delta G \pm 0.25$ kJ mol^{-1}. [d] α-D-Glc*p*-(1-4)-β-D-Gal*p*-OMe.

hibitors[3] in the study of bacterial adhesion, thus revealing affinity differences. A similar investigation was recently performed with various mannosides.[6] However, the present investigation seems to be the first systematic probing of bacterial binding using a large set of synthetic receptor analogues, obtained by manipulation of each hydroxyl group of the parent disaccharide. Binding specificities in protein–sugar interactions have been determined on the molecular level in other systems including antibodies and plant lectins,[7,9] transport proteins,[10,11] and enzymes.[12,13]

The ability of *Escherichia coli* to attach to receptors in the urinary tract containing galabiose is thought to be an important event in pyelonephritis. The bacterial structures that are important in mediating this adherence process are known as P pili. The original observations[14,15] were based on binding (agglutination) of bacterial isolates from human urine to human erythrocytes of the P blood-group system and to urinary sediment cells. Recently, an investigation of the binding specificity of uropathogenic *E. coli* toward naturally occurring glycolipids clearly showed that galabiose is the main binding part of these glycolipids. The position of the galabiose structure in the glycolipids (terminal, internal, or next to ceramide) only had a marginal influence on the binding strength.[16] In addition, galabiose-containing glycolipids are specifically bound by Shiga toxin[17] and verotoxin[18] and are tumor-associated antigens on Burkitt lymphoma cells.[19]

Table II. Inhibition of the Hemagglutination of *E. coli* (HB101/pPAP5) by Methyl β-D-Galabioside (**1**) and Various Glycosides



cmpd	R	IC_{50},[a] mM	inhib power,[b] %	$\Delta\Delta G$,[c] kJ mol^{-1}
1	OCH_3	0.18	100	0.00
18	OCH_2CH_3	0.13	140	−0.8
19	$OCH_2CH(CH_3)_2$	0.082	200	−1.6
20	$OCH_2CH_2Si(CH_3)_3$	0.046	400	−3.2
21	$OCH_2CH(OH)CH_2OH$	0.12	150	−0.93
22	O-4Glcβ$OCH_2CH_2Si(CH_3)_3$	0.26	71	0.8
23	αOMe	2.1	8.8	5.6
24	αβOH	1.3	12	4.8
25	SCH_2CH_3[d]		ca 200[d]	−1.7
26	Galβ(1-4)GlcβOCH_2CH_3[e]	>25	<1.0	>11

[a] 95% confidence limits: [0.930 × IC_{50}; 1.07 × IC_{50}] mM. [b] 95% confidence limits: [0.904 × Inhib power; 1.11 × Inhib power]%. [c] 95% confidence limits: $\Delta\Delta G \pm 0.25$ kJ mol^{-1}. [d] Compound **25** was compared with **1** and **18** by using a separate batch of HB101/pPAP5 bacteria and P erythrocytes. [e] Ethyl β-lactoside as negative control.

Genetic studies on P pili have revealed that the bulk of the pilus consists of repeating subunits of PapA and that the galabiose-binding properties of the pilus reside in the PapG molecule located exclusively at the pilus tip.[4] The amino acid sequence of the adhesin has been determined.[20] The expression of galabiose-binding P pili by *E. coli* strain HB101 is conferred by the presence of pPAP5 and this strain has been used in the present investigation as a standardized model of the wild strain.

The PapG adhesin has been purified by affinity chromatography on galabiose-derivatized Sepharose.[21] The adhesin was eluted from the column by the methyl β-galabioside (**1**) and more efficiently by the ethyl and TMSET glycosides **18** and **20**. The 6-deoxyfluoro (**8**), 6-*O*-methyl (**9**), 2′-deoxy (**10**), and 3′-deoxy (**11**) derivatives, which were found to be inefficient inhibitors in the hemagglutination studies presented here, were also found to be unable to elute the adhesin.[21] These results strongly suggest that the PapG adhesin possesses its galabiose-binding properties

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both when free and when present at the pilus tip and that the adhesin *is* the galabiose-binding part of the bacteria, as indicated by previous studies based on recombinant DNA techniques.[4]

In the present work, a series of galabioside analogues were used as inhibitors of the agglutination of human red cells by the recombinant bacteria mentioned above. The relative inhibitory power[22] was determined for all the monodeoxy analogues of methyl β-galabioside (**1**), as well as for other derivatives where hydroxyl groups were substituted by fluoro, methoxy, methyl, and ethyl groups (cf. Table I). Furthermore, several different glycosides of galabiose were investigated (cf. Table II). The analysis of the importance of individual hydroxyl groups in **1** for binding to the adhesin rests in part on arguments put forward in other studies on protein–ligand interactions.[7,11,12,23]

In a comparative study with receptor-active carbohydrate analogues it is important that these have similar sets of low-energy conformations when bound by the receptor protein. Only if this is the case can the observed inhibitory powers be correlated with the individual functional groups of the analogue. Lemieux[24] has suggested "that carbohydrate ligands will generally be bound in a conformation that is near that which is energetically most favourable in aqueous solution." This seems to be a valid assumption for the majority of oligosaccharides when the overall conformation is concerned. It is in line with the observations by Kishi et al.[25] that the Φ/Ψ angles of oligosaccharides and their *C*-glycoside analogues are determined mainly by hard-sphere steric interactions and therefore should be rather insensitive to the polarity of the medium. However, local conformational changes such as rotations of hydroxyl and hydroxymethyl groups should be sensitive to the polarity of the surrounding medium in that a nonpolar medium should induce the formation of intramolecular hydrogen bonds, which may even result in changes in the Φ/Ψ angles. The overall conformations (Φ/Ψ angles) of **1–17** were determined in aqueous solution by NMR methods[26] and also by calculations using various computer programs.[26–28] The different compounds showed only small differences in the calculated Φ/Ψ angles and the results were in full agreement with the NMR data. In methyl sulfoxide solution, however, an intramolecular HO-2′···HO-6 hydrogen bond was observed and the corresponding overall conformation was not calculated with the computer program.[26] Furthermore, the Φ/Ψ angles in the crystal structure of galabiose[29] differed substantially ($\Delta\Phi \sim 20°$; $\Delta\Psi \sim 50°$) from the conformation determined by NMR and calculational methods and an intramolecular HO-3···*O*-5′ hydrogen bond was observed. The interior of a galabiose crystal as well as the receptor site of the PapG adhesin might be less polar than water and formation of intramolecular hydrogen bonds might therefore be favored. Consequently, the binding of the PapG adhesin to the galabiosides **1–25** might force them into a conformation that is different from that found in aqueous solution (but possibly similar to that found in the crystal or in methyl sulfoxide solution). This aspect can only be clarified by the crystal structure of a PapG adhesin–galabioside complex. In view of the results described above, crystal coordinates of di- and oligosaccharides should be used with caution in calculations of solution conformations of larger oligosaccharides.

The subject of the present work is to provide experimental evidence for a receptor model that takes into account fine molecular details of the PapG adhesin–galabiose interaction. The model encompasses specific inter- and intramolecular hydrogen bonds and van der Waals (vdW) interactions between protein and sugar. The latter was found to possess a polar edge (HO-6, -2′, -3′, -4′, -6′) involved in hydrogen bonding to the adhesin close to less polar areas made up from the α,α′- and β′-surfaces of galabiose. In addition, hydrophobic substituents on *O*-1 increased the inhibitory power, probably due to unspecific hydrophobic interactions.

Experimental Section

Synthetic Analogues of Galabiose (1–25). The inhibitors used in the present study, except **5**, **19**, **21**, **22**, and **25** (see below), were synthesized as described: methyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside[30] (**1**), methyl 2-deoxy-4-*O*-α-D-galactopyranosyl-β-D-*lyxo*-hexopyranoside[27] (**2**), methyl 3-deoxy-4-*O*-α-D-galactopyranosyl-β-D-*xylo*-hexopyranoside[31] (**3**), methyl 3-deoxy-4-*O*-α-D-galactopyranosyl-3-*C*-methyl-β-D-galactopyranoside[31] (**4**), methyl 4-*O*-α-D-galactopyranosyl-3-*O*-methyl-β-D-galactopyranoside[31] (**6**), methyl 4-*O*-α-D-galactopyranosyl-β-D-fucopyranoside[27] (**7**), methyl 6-deoxy-6-fluoro-4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside[27] (**8**), methyl 4-*O*-α-D-galactopyranosyl-6-*O*-methyl-β-D-galactopyranoside[27] (**9**), methyl 4-*O*-(2-deoxy-α-D-*lyxo*-hexopyranosyl)-β-D-galactopyranoside[28] (**10**), methyl 4-*O*-(3-deoxy-α-D-*xylo*-hexopyranosyl)-β-D-galactopyranoside[28] (**11**), methyl 4-*O*-(3-*O*-methyl-α-D-galactopyranosyl)-β-D-galactopyranoside[28] (**12**), methyl 4-*O*-(4-deoxy-α-D-*xylo*-hexopyranosyl)-β-D-galactopyranoside[28] (**13**), methyl 4-*O*-(4-deoxy-4-fluoro-α-D-galactopyranosyl)-β-D-galactopyranoside[28] (**14**), methyl 4-*O*-α-D-glucopyranosyl-β-D-galactopyranoside[28] (**15**), methyl 4-*O*-α-D-fucopyranosyl-β-D-galactopyranoside[28] (**16**), methyl 4-*O*-(6-deoxy-6-fluoro-α-D-galactopyranosyl)-β-D-galactopyranoside[28] (**17**), ethyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside[32] (**18**), 2-(trimethylsilyl)ethyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside[33] (**20**), methyl 4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside[34] (**23**), 4-*O*-α-D-galactopyranosyl-D-galactose[34] (**24**).

Methyl 3-deoxy-3-*C*-ethyl-4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (5) was synthesized essentially as described for the 3-*C*-methyl analgoue[31] **4** and 2-(trimethylsilyl)ethyl 3-deoxy-3-*C*-ethyl-4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside.[33] Compound **5**: $[\alpha]^{25}_D$ +122° (*c* 0.7, H_2O) and ^1H and ^{13}C NMR data as reported.[26]

Isobutyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (19) was synthesized by catalytic hydrogenation of the corresponding 3-bromo-2-(bromomethyl)propyl glycoside[35] essentially as described for the synthesis of **18**.[32] Compound **19**: $[\alpha]^{25}_D$ +95° (*c* 0.9, H_2O); ^1H NMR (D_2O) δ 4.94 (d, 1 H, *J* = 3.8 Hz, H-1′), 4.42 (d, 1 H, *J* = 7.7 Hz, H-1), 4.37 (br t, 1 H, *J* = 7.0 Hz, H-5′), 0.89 [d, 6 H, *J* = 6.7 Hz, $CH(CH_3)_2$].

1-Glyceryl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (21) was synthesized by silver trifluoromethanesulfonate promoted glycosylation of 1,2-di-*O*-isopropylideneglycerol with 2,3,6-tri-*O*-acetyl-**4**-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranosyl bromide[36,37] followed by removal of protecting groups. **21**: $[\alpha]^{21}_D$ +92° (*c* 0.7, H_2O); ^1H NMR (DMSO-d_6 + D_2O, 50 °C, Me_4Si) δ 4.83 (d, 1 H, *J* = 3.5 Hz, H-1′), 4.14 and 4.13 (2 d, 1 H, *J* = 7.0 Hz, H-1); ^{13}C NMR (D_2O) δ 106.2 and 106.0 (2 d, *J* = 164 Hz, C-1), 103.1 (d, *J* = 173 Hz, C-1′).

2-(Trimethylsilyl)ethyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (22) was synthesized by NaOMe/MeOH-mediated deprotection of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzoyl-4-*O*-[6-*O*-acetyl-2,3-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside.[33] **22**: $[\alpha]^{25}_D$ +47° (*c* 1.2, D_2O); ^1H NMR (D_2O) δ 4.90 (d, 1 H, *J* = 3.4 Hz, H-1″), 4.46 and 4.45 (2 d, 1 H each, *J* = 7.8, 8.3 Hz, H-1 and H-1′), 4.31 (t, 1 H, *J* = 6.1 Hz, H-5″); ^{13}C NMR (D_2O) δ 107.0, 105.1, 104.1 (C-1, 1′, 1″).

Ethyl 1-thio-4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (25) was synthesized by treatment of 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α/β-D-galactopyranose[34] and ethyl mercaptan with boron trifluoride etherate in dichloromethane followed

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by chromatographic purification and deacetylation with methanolic sodium methoxide to give **25**: $[\alpha]^{29}_D$ +67° (*c* 1, D_2O); ^1H NMR (D_2O) δ 4.93 (d, 1 H, *J* = 3.7 Hz, H-1′), 4.57 (d, 1 H, *J* = 9.7 Hz, H-1), 4.01 (d, 1 H, *J* = 3.1 Hz, H-4), 3.74 (dd, 1 H, *J* = 9.7, 3.1 Hz, H-3), 3.54 (t, 1 H, *J* = 9.7 Hz, H-2), 1.26 (t, 3 H, *J* = 7.4 Hz, CH_3).

Bacterial Strain HB101/pPAP5. *E. coli* strain HB101[38] is nonpiliated and does not bind to human erythrocytes or to other surfaces that carry galabiose-containing glycolipids. Introduction of the *pap* gene cluster, contained on the plasmid pPAP5,[39] into HB01 gave a clone (HB101/pPAP5) that produces galabiose-binding pili.[40] The galabiose-binding property resides in the PapG protein which is uniquely present at the tip of the pilus as a minor component.[20,41]

Hemagglutination (HA) Reactions. The HA titer was determined for agar-grown *E. coli* (HB101/pPAP5) bacteria suspended in phosphate-buffered saline (PBS) to a Klett reading of 490 (ca. 1×10^9 bacteria/mL). A sample of the bacterial suspension (25 μL) was serially diluted in microtiter plate wells containing 25 μL of PBS in each well. An equal volume of a 1% suspension of human P erythrocytes in PBS was added, and after mixing, the plates were incubated at 4 °C for 18 h. The HA end point was defined as the last dilution well, before erythrocyte buttons were formed. The HA titer was expressed as the reciprocal of the end-point dilution.

HA Inhibition. The HA titer of the bacterial strain to be tested was determined as described above and the bacterial suspension was diluted to give a titer of 64. The inhibitors **1–26** were diluted in PBS to give 100 mM solutions, and 25 μL of each inhibitor solution was serially diluted in microtiter plate wells containing 25 μL of PBS. The bacterial suspension (25 μL; HA titer = 64) was then added to each well. After incubation for ~15 min at room temperature, human P erythrocytes (1% suspension, 25 μL) were added and the plates were shaken gently and incubated at 4 °C for 16 h. The end point was defined as the greatest dilution of the inhibitor (minimal concentration) that gave a 50% inhibition (incomplete erythrocyte button formation) of the HA (IC_{50}). The IC_{50} values for the different compounds in Tables I and II are mean values from two or three consecutive runs.

With **1** as the reference inhibitor, the relative equilibrium constant (K_{rel}) was obtained as the ratio of the IC_{50} value of **1** and each of the inhibitors **2–26**; the inhibitory powers ($100K_{rel}$) are presented in Tables I and II. The K_{rel} values were used for the calculation of the difference free energies ($\Delta\Delta G$), using the expression $\Delta\Delta G = -RT \ln K_{rel}$. The theoretical background has been reported.[22] The natural logarithms of the IC_{50} values and inhibitory powers (logarithmation was performed in order to obtain Normal-distributed sets of data), together with the $\Delta\Delta G$ values (which were already Normal-distributed), were subjected to analyses of variance with two sources of variation (inhibitor and run). The resulting mean values and 95% confidence limits for the IC_{50} values, the inhibitory powers, and the $\Delta\Delta G$ values for compounds **1–26** are presented in Tables I and II.

Results and Discussion

Inhibition data for the compounds **1–26** are listed in Tables I and II. The data were obtained by using a hemagglutination inhibition assay where a standardized bacterial suspension was incubated with a dilution series of each inhibitor followed by addition of human P erythrocytes in PBS buffer. The sugar concentration that inhibited the agglutination to a degree of 50% (IC_{50}) was determined for each compound. Methyl β-D-galabioside (**1**) was used as the reference inhibitor in the determination of the inhibitory powers and $\Delta\Delta G$ values for **2–26** (cf. Experimental Section). The IC_{50} of **1** was 0.18 mM, which is in good agreement with the results obtained by others both for inhibition of hemagglutination[14] and attachment to urinary sediment cells[15] by wild-type strains of *E. coli*. It should be stressed that the conclusions made here about the nature of the receptor are based on the assumption that all of the inhibitors have similar overall conformations oriented in a similar way in the receptor site and that only one type of galabiose-specific receptor is present in the adhesin. Another assumption is that metal ions present in the buffers used in the agglutination assay did not have a marked influence on the protein–sugar binding, in analogy with the findings of Lemieux et al.[42]

It is widely acknowledged that the importance of individual hydroxyl groups in the binding of biological ligands can be evaluated by using deoxy and deoxyfluoro analogues. Our results also show that additional substituents can be used to reveal further fine details of the binding characteristics, and several examples are given below.

The $\Delta\Delta G$ values (Tables I and II) for the inhibitors **2–26** fit into four categories: (1) $\Delta\Delta G < 0$; more potent inhibitors than the reference compound **1**. (2) $\Delta\Delta G = 0$ to +1.6 kJ mol^{-1}; the structural change had only a marginal effect on the potency. (3) $\Delta\Delta G$ = +3.9 to +9.1 kJ mol^{-1}; the structural change had a strong effect on the potency, indicating either deletion of an intermolecular hydrogen bond, the creation of a steric repulsion, or loss of vdW bonding. (4) $\Delta\Delta G >$ +11 kJ mol^{-1}; the structural change resulted in a noninhibitor. Fersht et al. found that deletion (by site-specific mutagenesis) of a hydrogen-bonding residue in tyrosyl-tRNA synthetase resulted in decreased binding strength of the ligand by 2.1–6.3 kJ mol^{-1} if both polar groups were uncharged.[43]

The inhibitory power of the 2-deoxy derivative **2** ($\Delta\Delta G$ = +1.1 kJ mol^{-1}) was only marginally reduced as compared to **1**, and HO-2 is therefore considered not to be involved in hydrogen bonding to the adhesin but rather to stay in contact with the surrounding aqueous medium or perhaps to be in vdW contact (intramolecularly hydrogen bonded to O-1 or O-3) with the adhesin.

Replacement of HO-3 by hydrogen to form the 3-deoxy derivative **3** caused the inhibitory power to drop to $^1/_5$ of that of **1** ($\Delta\Delta G$ = +3.9 kJ mol^{-1}). However, the 3-*C*-methyl derivative **4** had only lost half of the inhibitory power of **1** ($\Delta\Delta G$ = +1.6 kJ mol^{-1}), strongly indicating that HO-3 is accepted by the adhesin intramolecularly hydrogen bonded to O-5′ (as in the galabiose crystal[29]) and that the size of the substituent in position 3 is important for effective vdW interaction to take place. In addition, the similarity in size between water and hydroxyl and methyl groups means that in **1** and **4** the intermolecular space between sugar and protein is effectively filled, whereas with the 3-deoxy compound **3**, a pocket is formed that might be forced to accommodate a water molecule. The evidence for precise intermolecular fit in the 3-position is supported by the decrease in inhibitory power of the 3-*C*-ethyl ($\Delta\Delta G$ = +6.7 kJ mol^{-1}) and 3-*O*-methyl ($\Delta\Delta G$ = +5.1 kJ mol^{-1}) derivatives **5** and **6**, which are both involved in steric interaction with the protein due to their larger sizes. It is noteworthy that the increased inhibitory power of the *C*-methyl derivative **4** over the deoxy derivative **3** is not a reflection of unspecific hydrophobic interaction due to the increased hydrophobic surface area since the derivative **5** with its large hydrophobic ethyl group is almost 10 times less efficient an inhibitor than **4**. A HO-3···O-5′ hydrogen bond was found in the crystal of galabiose[29] (**24**) and in a low-energy conformation of **1** that was calculated by the molecular mechanics force-field MM2-85,[44] supporting the view that HO-3 is intramolecularly hydrogen bonded to O-5′ and therefore occupies a nonpolar site in the adhesin.

The 6- and 2′-deoxy derivatives **7** and **10** had low inhibitory powers ($\Delta\Delta G$ = +7.2 and +5.9 kJ mol^{-1}), suggesting that both HO-6 and HO-2′ of **1** are involved in hydrogen bonding to the adhesin. The 6-fluoro derivative **8** ($\Delta\Delta G$ = +9.1 kJ mol^{-1}) was even less efficient than **7**, and the 6-*O*-methyl derivative **9** ($\Delta\Delta G$ = >11 kJ mol^{-1}) was the only analogue that was inactive. This indicates that HO-6 of **1** only donates a proton to the adhesin. Furthermore, HO-6 should be bound in a narrow site in the adhesin because of the severe steric interaction with the 6-*O*-methyl group of **9** leading to a complete loss of inhibitory power. It is possible that HO-6 accepts a proton from HO-2′, since such

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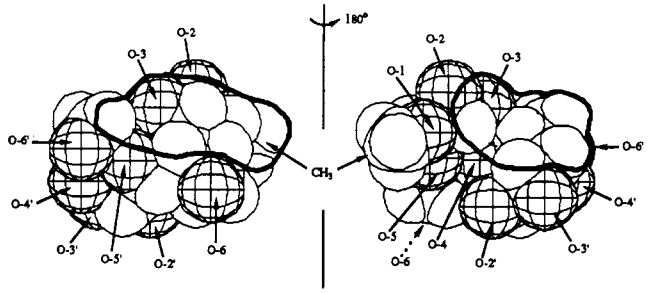

Figure 1. Molecular model of methyl β-D-galabioside (**1**). Hydroxyl group hydrogens are not shown. HO-6, HO-2′, O-3′, HO-4′, and HO-6′ are involved in hydrogen bonding with the PapG adhesin. Encircled areas are suggested to be in contact with nonpolar surfaces of the adhesin. See Results and Discussion and Figure 2 for a detailed description of the molecular recognition.

intramolecular hydrogen bonding was indicated in the calculated low-energy conformation of **1** mentioned above and an intramolecular hydrogen bond between HO-6 and HO-2′ was observed in the ^{1}H NMR spectrum of **1** in methyl sulfoxide.[26] This solvent is less polar than water and could well emulate the dielectric environment of the receptor site, thereby enforcing the formation of an intramolecular hydrogen bond. In summary, HO-6 is considered to be a proton donor and HO-2′ a proton acceptor in hydrogen bonding to the adhesin. Quiocho[10,11] pointed out that more than 60% of the hydrogen bonds in crystal complexes between protein and sugar involve planar, polar amino acid side chains (e.g., a carboxyl, amido, or guanidino group) and vicinal hydroxyl group pairs. Due to the close positioning of HO-6 and HO-2′ in low-energy conformers of **1**, the geometric requirements are fulfilled for this type of protein–sugar interaction, despite the fact that HO-6 and HO-2′ are not vicinally situated.

The 3′-deoxy analogue **11** had a low inhibitory power ($\Delta\Delta G$ = +8.3 kJ mol^{-1}), whereas the 3′-methoxy compound **12** ($\Delta\Delta G$ = −1.6 kJ mol^{-1}) was superior to the reference compound **1**. Therefore, HO-3′ of **1** is considered to be a proton acceptor in the hydrogen bonding with the adhesin. Compound **12** is more than twice as good an inhibitor as **1**. This indicates that a hydrophobic amino acid side chain of the adhesin interacts with the 3′-*O*-methyl group on the nonpolar α′,β-side of **1**. This is augmented by the finding that globotetrasoyl ceramide (which carries a terminal β-D-GalNAc unit in the 3′-position of the galabiose residue) was a stronger inhibitor than various galabiosides.[3] The terminal β-D-GalNAc unit was calculated[45,16] to be oriented with its nonpolar α-side toward the α′,β-side of the galabiose unit. Hydrophobic extensions in the 3′-position of galabiose would then be a logical way to create ligands with enhanced inhibitory power.

The 4′- and 6′-deoxy derivatives **13** and **16** were inefficient inhibitors ($\Delta\Delta G$ = +9.1 and +6.7 kJ mol^{-1}). The 4′-deoxyfluoro derivative **14** and methyl α-D-glucopyranosyl-β-D-galactopyranoside (**15**) also had low inhibitory powers ($\Delta\Delta G$ = +6.4 and +7.5 kJ mol^{-1}), whereas the 6′-deoxyfluoro derivative **17** ($\Delta\Delta G$ = +1.6 kJ mol^{-1}) retained half the inhibitory power of **1**. This indicates that HO-4′ and HO-6′ of **1** are both involved in hydrogen bonding with the adhesin, the former as proton donor and the latter as an acceptor. The low activity of the glucose derivative **15** indicated high steric demands on the hydrogen-bonding network. Energy minimization of **1**, using the MM2-85 program, revealed an intramolecular hydrogen bond between HO-6′ (proton donor) and O-4′. Such bonding would increase the further hydrogen-bonding (proton-acceptance) capacity of HO-6′ due to the cooperative effect.[46,47] The situation is similar to that described by Lemieux et al.[48] for the binding of galactosides by antibodies.

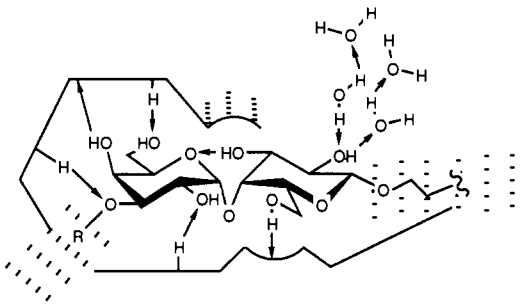

Figure 2. Schematic model of the galabiose-specific binding site of the PapG adhesin of *E. coli*.

As mentioned above for the hydroxyl group pair HO-6/HO-2′, the pair HO-4′/HO-6′ also fulfills the geometric requirements for interaction with planar, polar amino acid side chains.

Increased hydrophobicity of the aglycon in the glycoside series **1**, **18**, **19**, and **20** increased the inhibitory power of these compounds ($\Delta\Delta G$ = 0.0, −0.8, −1.6, and −3.2 kJ mol^{-1}, respectively), whereas the less hydrophobic aglycon (trimethylsilyl)ethyl glucoside in **22** ($\Delta\Delta G$ = +0.8 kJ mol^{-1}) gave a somewhat less efficient inhibitor. It should be noted that the hydrophobic (trimethylsilyl)ethyl group of **22** was not engaged in strong hydrophobic interaction with the adhesin as was the case with **20**. The axially oriented α-methoxy group of **23** ($\Delta\Delta G$ = +5.6 kJ mol^{-1}) seems to be in repulsive vdW interaction with the adhesin. This was not unexpected since it disrupts an otherwise smooth nonpolar area of the sugar. Galabiose (**24**; $\Delta\Delta G$ = +4.8 kJ mol^{-1}) was a rather inefficient inhibitor, probably due to the lack of a hydrophobic aglycon. The β/α ratio at equilibrium for **24** was determined by NMR to be ca 7:3 in D_2O, partly ruling out the negative effect an axially oriented anomeric hydroxyl group would have on the inhibitory power.

Finally, the possible involvement of O-1 in hydrogen bonding to the adhesin was investigated in a separate experiment by comparison between the thioethyl (**25**), the ethyl (**18**), and methyl (**1**) galabiosides. The inhibitory power of **25** ($\Delta\Delta G$ = −1.7 kJ mol^{-1}) was somewhat greater than that of **18**, thus ruling out hydrogen bonding between O-1 of **18** and the adhesin since sulfur is expected to be a less efficient hydrogen bond acceptor than oxygen.[23]

Conclusions

The adhesin-mediated binding of *E. coli* to glycosides of galabiose reveals general features of protein–carbohydrate interactions. The disaccharide is provided with a cluster of five oxygen atoms (O-6, -2′, -3′, -4′, -6′) that are involved in hydrogen bonding to the adhesin. Nonpolar areas are present on the α,α′- and β′-surfaces of the galactose moieties, thus providing opportunities for vdW bonding to the adhesin. The HO-3 group is accepted by a nonpolar part of the adhesin, most likely intramolecularly hydrogen bonded to O-5′. HO-2 is probably in contact with the surrounding aqueous medium. The overall amphipathic character of galabiosides is depicted in Figure 1 and a schematic model of the PapG adhesin receptor site is depicted in Figure 2. Nonpolar regions seem to be present on the adhesin, thus contributing to the binding of the hydrophobic aglycon residues of **1**, **18**–**20**, and **25** by unspecific hydrophobic and vdW interactions. Nonpolar substituents on O-3′ increase the binding. Consequently, structural variations in the 1- and 3′-positions of galabiose provide opportunities for the design of improved bacteria-binding galabiosides of potential value for diagnosis and therapy of *E. coli* infection.

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Registry No. Galabiose, 13117-26-5.

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