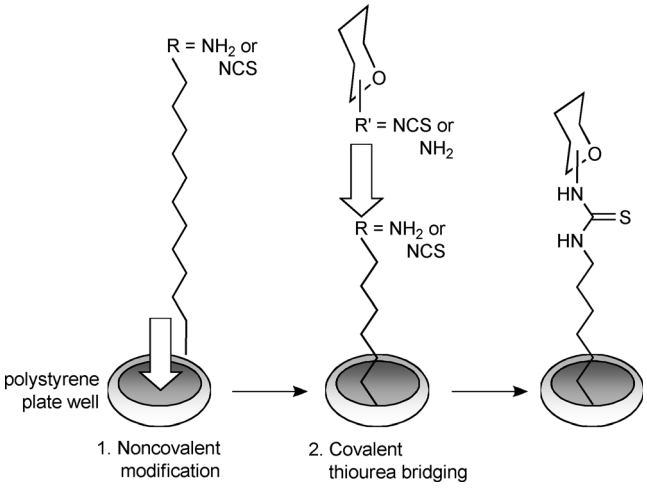
DOI: 10.1002/cbic.201200365

# Glycoarrays by a New Tandem Noncovalent–Covalent Modification of Polystyrene Microtiter Plates and their Interrogation with Live Cells

Carsten Grabosch, Katharina Kolbe, and Thisbe K. Lindhorst*[a]

Microarray-based technologies are well established in the field of genomics and proteomics and have also been developed into a powerful tool in the glycosciences (glycomics).[1] The large diversity of saccharide structures and their many different types of interactions are well suited to array-format testing.[2] In order to advance glycoarray methodologies and to investigate carbohydrate recognition on surfaces, methods for glycoarray fabrication, characterisation and interrogation are required.[3] Much effort has been expended on the fabrication of glyco-SAMS (self-assembled monolayers) on gold[4] and, for example, on the modification of glass slides with carbohydrates.[5] However, the easiest and least expensive format for both laboratory studies and high-throughput testing of carbohydrate interactions are polystyrene microtiter plates.[6] In order to immobilise relatively hydrophilic bioprobes such as carbohydrates on the hydrophobic polystyrene surface, functionalised plates have become available that allow the chemical attachment of bioprobes, for example by peptide coupling reactions. In addition, a variety of other, more chemoselective attachment strategies have been developed[7] and these have been utilised in an array format, such as thiol-maleimide ligation,[8] Diels–Alder reactions[4a,9] and "click chemistry".[10] Click chemistry ($Cu^{I}$-catalysed ligation of alkynes to azides) has also become popular for the chemoselective immobilisation of carbohydrates; however, it can be problematic in investigations with live cells, as the added copper catalyst is cytotoxic.[11,12a] Thus, many modifications have been made to this ligation strategy so that it can be performed in a copper-free fashion.[12] These methods have contributed considerably to the field, but increase the synthetic effort that is required to make the starting materials for copper-free click chemistry available.

We have recently suggested thiourea bridging as a click-type reaction for the ligation of carbohydrates on surfaces. This reaction was combined with triazole ligation in a "dual-click" approach.[13] Here, it was our goal to employ thiourea bridging for fabrication of glycoarrays on polystyrene microtiter plates, to allow assaying of carbohydrate recognition in a optimally easy and flexible way. Thiourea bridging has been used before for the synthesis of a variety of multivalent glycomimetics and for the decoration of functionalised microtiter plate surfaces.[14] Thiourea bridging requires a surface that is functionalised with either isothiocyanato or amino functions. We considered that it would be best to utilise standard noncovalent modification of polystyrene plates with long chain alkyl derivatives, and combine this with a covalent ligation step, thiourea bridging. Recently, a similar approach has utilised cyanates.[15] Carbohydrate amines and carbohydrate isothiocyanates are, however, much easier to obtain than carbohydrate cyanates.[16] Thus, noncovalent immobilisation of $NH_2$- or *N*-chlorosuccinimide (NCS)-terminated long-chain hydrocarbons and subsequent thiourea bridging with a complementary functionalised carbohydrates would provide a simple tandem strategy for glycoarray fabrication of hydrophobic surfaces (Scheme 1).




**Scheme 1.** Tandem functionalisation of microtiter plates: A combination of hydrophobic modification with long chain NCS- and $NH_2$-terminal hydrocarbons and covalent thiourea bridging with complementary carbohydrate derivatives leads to the formation of stable glycoarrays on polystyrene.

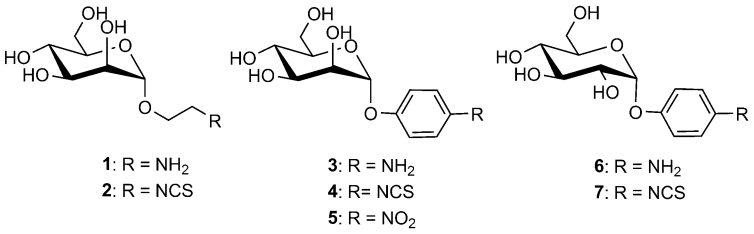
For the noncovalent modification of polystyrene microtiter plates, dodecylamine and dodecyl isothiocyanate were selected. This first immobilisation step was followed by thiourea bridging with a suitable carbohydrate derivative and subsequent carbohydrate-specific read out. For the testing system, mannose-specific bacterial adhesion was employed with green fluorescent protein (GFP)-tagged *Escherichia coli* bacteria (pPKL1162). Here, bacterial adhesion correlates with intensity of fluorescence, which can be measured by using a standard microtiter plate reader.[17] Mannose specificity of *E. coli* adhesion is mediated by type 1 fimbriae that project from the bacterial surface. These have a terminal lectin, FimH, the structure of which is known from X-ray analysis.[18] Many synthetic antagonists for FimH-mediated carbohydrate recognition have been synthesised and tested, to inhibit bacterial adhesion in anti-adhesion therapy.[19]

Thus, for our study, a selection of inhibitors of type 1 fimbriae-mediated bacterial adhesion was used. For ligation to

[a] C. Grabosch, K. Kolbe, Prof. Dr. T. K. Lindhorst
Otto Diels Institute of Organic Chemistry
Christiana Albertina University of Kiel
Otto-Hahn-Platz 3-4, 24098 Kiel (Germany)
E-mail: tklind@oc.uni-kiel.de

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.201200365.

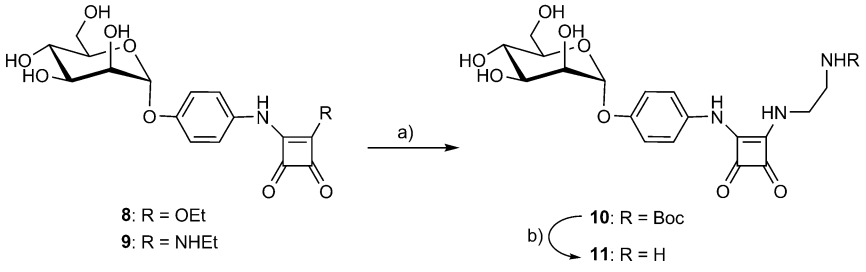
a polystyrene surface modified with either dodecylamine or dodecyl isothiocyanate, inhibitors of bacterial adhesion have to be NCS- or $NH_2$-functionalised, respectively. Hence, mannnosides **1** and **3** were used to "click" to the NCS-functionalised surface, and **2** and **4** were used to ligate to the $NH_2$-functionalised surface (Scheme 2). Mannoside **5** and glucoside **7** were




**Scheme 2.** Functionalised carbohydrate derivatives for glycoarray fabrication by thiourea bridging. The amino-functionalised glycosides **1** and **3** are suitable for the functionalisation of isothiocyanate-terminal surfaces, and the isothiocyanato-functionalised derivatives **2**, **4** and **7** can be ligated to $NH_2$-terminal surfaces.

used as control compounds. The amino-functionalised glycosides **1** and **3** were prepared as previously described.[20] The synthesis of the known isothiocyanates **4** and **7**[21] was considerably improved, thereby delivering the pure products after simple precipitation from aqueous solution in high yield and purity (see the Supporting Information). It was important to perform the synthesis of the isothiocyanates in water rather than in alcohol solution, to prevent thiocarbamate formation.[22]

Mannosides **8** and **9** have recently been described as high-affinity antagonists of FimH,[23] and thus an amino-functionalised manoside derivative was selected in this tandem approach for glycoarray fabrication, to test adhesion of mannose-specific live bacterial cells. Therefore, amine **11** was synthesised by starting from **8** (Scheme 3). To prevent possible dimer formation, mono-Boc-protected ethylene diamine was employed in




**Scheme 3.** Synthesis of the amino-functionalised squaric acid diamide **11**. a) $H_2NCH_2CH_2NHBoc$, DMF, RT, 18 h, 92%; b) TFA, $CH_2Cl_2$, RT, 3 h, 97%.

the reaction, thereby leading to the respective *N*-Boc-protected squaric acid (SA) diamide **10** in high yield. Subsequent removal of the Boc protecting group with TFA provided the target amino-functionalised SA diamide **11** in excellent yield (Scheme 3).

Having obtained a collection of NCS- and $NH_2$-functionalised glycosides, thiourea bridging on modified polystyrene surfaces was then tested. As expected, thiourea bridging in solution was a high-yielding and efficient reaction with the derivatives employed (Supporting Information). To test thiourea bridging on polystyrene microtiter plates, in the first instance commercially available dodecylamine was employed for noncovalent modification of the polystyrene surface. Then, isothiocyanates **2** and **4** were used in the covalent modification step, followed by bovine serum albumin (BSA) blocking. Type 1 fimbriated fluorescent *E. coli* bacteria (pPKL1162)[17,24] were allowed to adhere, and, after appropriate washing steps, bacterial adhesion was determined by fluorescence read out. Concentration-dependent bacterial adhesion was observed as expected (Figure 1, left). The aryl mannoside **4** clearly formed a more adhesive glycoarray than ethyl mannoside **2** on the same surface. This finding was in accordance with earlier studies on the affinity of various mannosides to type 1 fimbrial lectin.[23] *p*-Nitrophenyl mannoside (*p*NPMan, **5**) was used as control, and no significant bacterial adhesion was observed. This finding was as expected, because *p*NPMan cannot ligate to the amino-terminated surface. NCS-functionalised glucoside **7** was also used as a negative control; it underwent thiourea bridging but did not facilitate mannose-specific bacterial adhesion (see Figure S15 in the Supporting Information). Both control experiments proved that nonspecific binding does not occur when this tandem methodology is used for glycoarray preparation.

Then, a complementary approach was tested in which dodecyl isothiocyanate was used for noncovalent modification of the polystyrene plate, and the $NH_2$-functionalised glycosides **1**, **3** and the squaric acid diamide **11** were used for thiourea bridging. After blocking with BSA, mannose-specific bacterial adhesion was assessed (Figure 1, right). The glycoarrays performed as expected: concentration-dependent bacterial adhesion was observed in all cases, with decreasing adhesive ability in the order **11** > **3** > **1**. Again, control experiments with **5** confirmed absence of nonspecific adhesion. The squaric acid diamide **11** mediated bacterial adhesion at much lower concentrations than for the other prepared glycoarrays, even at 1 mM. This finding confirms the excellent quality of SA mannosides as FimH antagonists. Fabrication of glycoarrays on polystyrene plates by both complementary tandem approaches was straightforward, but thiourea bridging was faster when amino-functionalised surfaces were reacted with sugar isothiocyanates **2** and **4**. Therefore, in the next step, amino-terminated noncovalently modified polystyrene plates were employed for adhesion-inhibition studies with live cells, to test the robust-

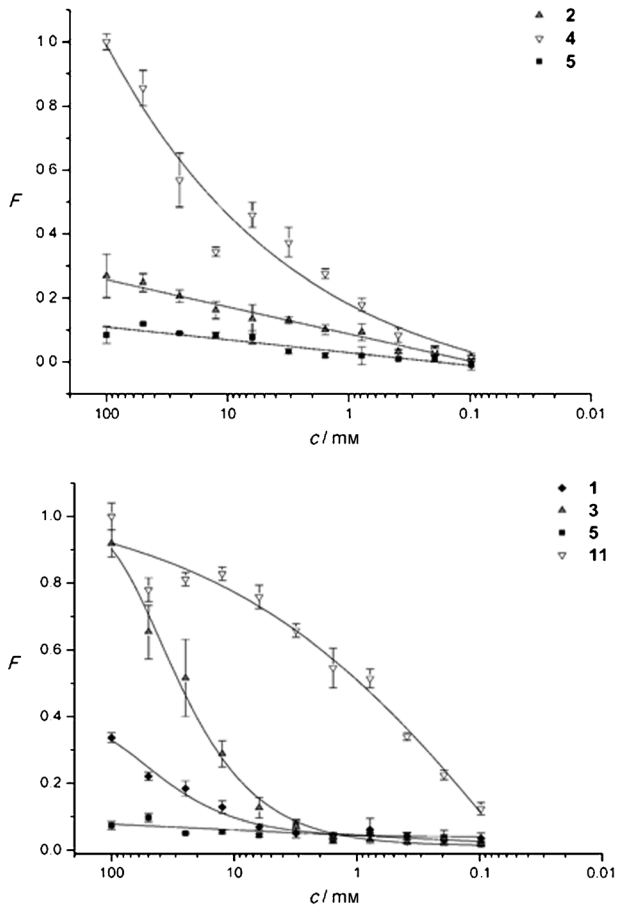



**Figure 1.** Top: Concentration-dependent mannose-specific bacterial adhesion to polystyrene plates modified by tandem functionalisation (coating with dodecylamine followed by thiourea bridging with NCS-functionalised mannosides **2** and **4**). Nitrophenyl mannoside **5** was used as negative control. Bottom: Concentration-dependent mannose-specific bacterial adhesion to polystyrene plates modified by tandem functionalisation (coating with dodecyl isothiocyanate followed by thiourea bridging with amino-functionalised mannosides **1**, **3** and **11**). Nitrophenyl mannoside **5** was used as negative control; *F*: normalised fluorescence.

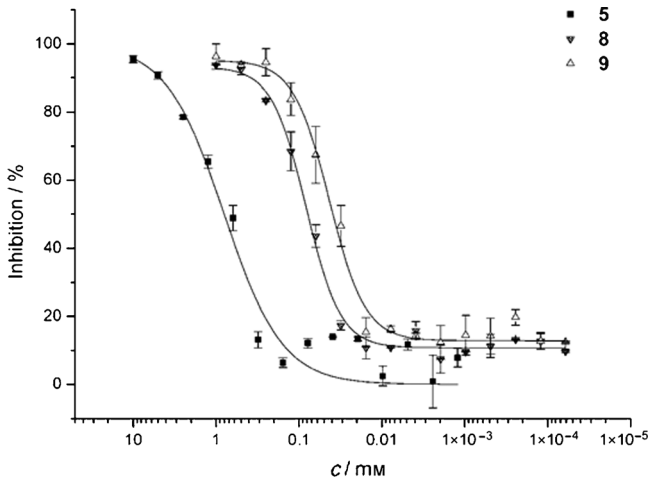



**Figure 2.** Inhibition curves obtained with mannosides **5**, **8** and **9** as inhibitors of type 1 fimbriae-mediated bacterial adhesion to a polystyrene surface modified by tandem functionalisation (dodecylamine coating followed by thiourea bridging with mannoside **4**); see Table 1.

**Table 1.** Inhibition of three inhibitors of mannose-specific bacterial adhesion on polystyrene microtiter plates.

| | **5** | **8** | **9** |
|---|---|---|---|
| $IC_{50} \pm SD$ [μM] | $811 \pm 43.7$[a] | $78 \pm 5.6$[b] | $37 \pm 2.2$[b] |
| RIP | 1 | $10 \pm 0.7$ | $22 \pm 1.2$ |

[a] Average value from duplicate results. [b] Average value from triplicate results; SD: standard deviation, RIP: relative inhibitory potency with **5** as the reference (tested on the same microtiter plate).

ness of the new glycoarrays. Thiourea bridging with isothiocyanate **4** at 100 mM led to an adhesive surface that was stable in competitive adhesion-inhibition assays. For inhibition of bacterial adhesion, serial dilutions of mannosides **5**, **8** and **9** were employed. All three resemble prominent FimH antagonists.[18,23] Sigmoidal inhibition curves were obtained (Figure 2), from which $IC_{50}$ values for each inhibitor were deduced. ($IC_{50}$ is the concentration at which a compound inhibits 50% of bacterial adhesion.) The determined $IC_{50}$ values were referenced to the inhibitory potency of *p*NPMan which was tested on the same plate. Thus, relative inhibitory potency (RIP) values can be compared even when compounds were not tested in the same experiment. We previously found that SA mannosides **8** and **9** are stronger inhibitors of bacterial adhesion to a mannan-coated plate than *p*NPMan,[23b] with RIP values of 16 and 50, respectively. $IC_{50}$ values were approximately 274 μM for **5**, 17 μM for **8** and 6 μM for **9**. For inhibition of bacterial adhesion to the fabricated surface, higher inhibitor concentrations were required (three to six times higher, Table 1). This can be explained by the nature of the immobilised FimH antagonist. Whereas, here, phenyl α-D-mannoside moieties were exposed, mannan simply consists of α-D-mannosyl residues, which have lower affinity for the bacterial lectin FimH. However, the RIP values followed a similar trend for **5**, **8** and **9** (RIPs: 1, 10, and 22, respectively, Table 1) as found earlier in the mannan-based assay (RIP values: 1, 16, and 50, respectively).

It can be concluded that tandem noncovalent–covalent modification of polystyrene microtiter plates leads to stable glycoarrays that yield valuable and valid data in adhesion-inhibition tests with live bacterial cells. Nonspecific adhesion to polystyrene is excluded in this protocol.

In addition to polystyrene plates, we tested polypropylene (PP) microtiter plates by the same approach (Supporting Information). PP plates could also be noncovalently modified with dodecylamine or dodecyl isothiocyanate, and subsequently functionalised by thiourea bridging with the appropriate carbohydrate derivatives. For testing bacterial adhesion to these fabricated surfaces, *E. coli* pPKL4 was biotinylated, and bacterial adhesion was determined by a biotin–streptavidin test.[17] This was necessary as PP plates are transparent and thus not suited for fluorescence read-out. The results of the bacterial adhesion experiments with PP microtiter plates were in good accordance with the assays on analogously modified polystyrene plates.

Hence, our approach to glycoarrays can be used on polypropylene as well as polystyrene plates. Tandem noncovalent–covalent modification of microtiter plates provides maximum flexibility in glycoarray fabrication as well as their modulation. We are currently employing this methodology in the fabrication of photoswitchable glycoarrays and more complex glycoarrays, and their testing with various cells.

## Experimental Section

**Reagents and methods:** Commercially available starting materials were used without further purification: *p*-nitrophenyl α-D-glucopyranoside, dodecylamine and thiophosgene were from Sigma–Aldrich; dodecyl isothiocyanate was from ABCR (Karlsruhe, Germany); *p*NPMan was from Senn Chemicals (Dielsdorf, Swizerland). Solvents were purified by distillation prior to use. Reaction monitoring was performed by TLC on silica gel $F_{254}$ or RP-18 plates (Merck). Detection was achieved by UV light and/or by treatment of the plates with a vanillin solution (vanillin (1.00 g) in methanol (100 mL) with glacial acetic acid (12.0 mL) and sulfuric acid (4.00 mL)) or ninhydrin solution (ninhydrin (300 mg) in butanol (100 mL) with glacial acetic acid (3.00 mL)) and subsequent heating. Flash chromatography was performed on silica gel 60 (0.040–0.063 mm; Merck) or on a reversed-phase RP-18 system (Biotage AB, Uppsala, Sweden). Preparative HPLC was performed on a VP series HPLC system with an SPD-M10A diode array detector (Shimadzu, Kyoto, Japan) and a Nucleodur 100-7 C8ec HPLC column (Macherey-Nagel). Analytical HPLC was performed on a LaChrom instrument with a D-7000 interface and L-7455 diode array detector (Hitachi) and a LiChrosorb RP-8 silica column (Merk). DRX 500 and Avance AV 600 instruments (Bruker BioSpin) were used for NMR spectroscopy. Chemical shifts ($\delta$) were calibrated relative to an internal solvent. For complete assignment the following two-dimensional NMR techniques were used: $^{1}H,^{1}H$ COSY, $^{1}H,^{13}C$ HSQC and $^{1}H,^{13}C$ HMBC. ESI-MS measurements were performed on a Mariner instrument (Applied Biosystems) and MALDI-TOF mass spectra were recorded with a Biflex III (Bruker) with 19 kV acceleration voltage and a 337 nm ionization laser (matrix: 4-chloro-α-cyanocinnamic acid). Optical rotations were measured on a model 241 polarimeter (10 cm cells, Na-D line: 589 nm; PerkinElmer) and were averaged from five measurements. An Infinite 200 PRO multifunction microplate reader (Tecan, Männedorf, Switzerland) was used for measurement of bacterial adhesion.

**Microtiter plates:** Black Polymer base 96-well optical plates (ThermoFisher Scientific) were used for the GFP-based assay.[17] For the biotin–streptavidin assay[17] transparent 96-well polypropylene microplates (Greiner Bio-one) were used.

**Tandem functionalisation of polystyrene plates:**

*Noncovalent functionalisation.* Amino-terminal and isothiocyanate-terminal surfaces: 96-well plates were treated with dodecylamine or dodecyl isothiocyanate (10 mM in ethanol, 100 μL per well), and were allowed to dry ( 24 h, room temperature, light agitation). Each desiccated well was then washed with ethanol (1×150 μL) and PBS buffer (2×150 μL).

*Covalent functionalisation by thiourea bridging.* Amino-functionalised glycosides **1**, **3** and **11** or the NCS-functionalised glycosides **2**, **4** and **7** (100 mM in DMSO with *N*,*N*-diisopropylethylamine (DIPEA, 1.01 equiv for isothiocyanates, 2.02 equiv for amines)) were applied as serial dilutions (in DMSO) to the complementary noncovalently modified plates. Thiourea bridging on the plate surface was effected by moderate shaking at 40 °C (24 h for isothiocyanates, 48 h for amines). Wells were washed with DMSO (1×150 μL) and PBS buffer (2×150 μL), then blocked with BSA (5% in PBS, 120 μL) for 2 h at 37 °C, and subsequently washed with PBS buffer (3×150 μL).

**Bioassays:**

*Media and buffer solutions:* Carbonate buffer solution: sodium carbonate (1.59 g) and sodium hydrogen carbonate (2.52 g) were dissolved in distilled, deionised water (1.00 L), and adjusted to pH 8.2 with hydrochloric acid (3 M) for biotinylation. PBS buffer solution: sodium chloride (8.00 g), potassium chloride (200 mg), sodium hydrogen phosphate dihydrate (1.44 g) and potassium dihydrogen phosphate (200 mg) were dissolved in distilled, deionised water (1.00 L), and adjusted to pH 7.2 with sodium hydroxide (0.1 M). Lysogeny broth (LB) medium: tryptone (10.0 g), sodium chloride (10.0 g) and yeast extract (5.00 g) were dissolved in distilled, deionised water (1.00 L) and autoclaved. Ampicillin (100 mg) was added, and, for *E. coli* pPKL1162 assays, chloramphenicol (50.0 mg) was added. Substrate buffer: aqueous sodium citrate dihydrate (1 M) was adjusted to pH 4.5 with aqueous citric acid (2 M). ABTS solution: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS, 10.0 mg) was dissolved in substrate buffer (10.0 mL), and treated with hydrogen peroxide (0.1%, 250 μL) prior to use.

*Cultivation of bacteria: E. coli* bacteria (strain pPKL1162 or pPKL4),[17] were grown in LB medium overnight at 37 °C in a sterilised tube. After centrifugation and washing with PBS buffer (2×2.00 mL), the tube was centrifuged again and the bacteria pellet was suspended in PBS buffer (~2.00 mg mL$^{-1}$).

**Bioassays on polystyrene plates:**

*GFP assay:* A published assay[17] was adapted and modified as follows. Each well was treated with PBS buffer (50 μL), then *E. coli* (pPKL1162) suspension was added (2.00 mg mL$^{-1}$ in PBS, 50 μL) and incubated for 1 h at 37 °C. Each well was washed with PBS buffer (2×150 μL) and filled with PBS buffer (100 μL). Fluorescence intensity was determined ($\lambda_{ex}$ = 485 nm/$\lambda_{em}$ = 535 nm).

*Adhesion inhibition assay:* Black Polystyrene microtiter plates were functionalised with dodecylamine, and thiourea bridging was performed with isothiocyanate **5** (100 mM in DMSO, 1.01 equiv DIPEA, 100 μL per well) for 24 h at 40 °C under light agitation. Each well was then washed with DMSO (1×150 μL) and PBS (2×150 μL). After BSA blocking, serial dilutions of the inhibitors **5**, **8** and **9** (starting concentrations in PBS: 10 mM for **5**, 1 mM for **8** and **9**) were pipetted into the wells, and the bacterial suspension (*E. coli* pPKL1162, 2 mg mL$^{-1}$, 50 μL per well) was added. Wells were incubated for 1 h at 37 °C, washed with PBS (2×150 μL), filled with PBS (100 μL), and the fluorescence intensity was determined ($\lambda_{ex}$ = 485 nm/$\lambda_{em}$ = 535 nm).

**Bioassays on polypropylene plates:**

*Biotinylation:*[17] An overnight culture of *E. coli* (pPKL4) was centrifuged and washed twice with carbonate buffer (pH 8.2, 2.00 mL), and after further centrifugation the pellet was suspended in carbonate buffer (~10 mg mL$^{-1}$). The solution was treated with *N*-hydroxysuccinimide (NHS)-biotin (2.50 mg mL$^{-1}$) and incubated for 2.5 h at room temperature. It was then washed with PBS buffer (3×2.00 mL), centrifuged and resuspended in PBS buffer (2.00 mg mL$^{-1}$) to give biotinylated bacteria.

*Biotin–streptavidin assay:* A published assay[17] was adapted and modified as follows. Each well was treated with PBS buffer (50 μL), then biotinylated *E. coli* (pPKL4) suspension was added (2.00 mg mL$^{-1}$ in PBS, 50 μL) and incubated for 1 h at 37 °C. Each

well was washed with PBS buffer (2×150 μL), and streptavidin–HRP (horseradish peroxidise) solution (1:2000, 500 μM in PBS buffer, 100 μL) was added to catalyse ABTS color reaction by electron transfer afterwards. After further incubation (1 h, 37 °C), each cell was washed with PBS buffer (2×150 μL) and substrate buffer (1×150 μL), then treated with ABTS solution (100 μL) and incubated (30 min, room temperature, in the dark). The enzymatic reaction was terminated by adding oxalic acid (2%, 50 μL), and the optical density (absorption, 405 nm) was determined.

*Adhesion assay:* Transparent 96-well polypropylene (PP) microplates (Greiner Bio-one) were modified as described for the polystyrene plates (vide supra). Bacterial adhesion was determined with biotinylated bacteria (Supporting Information).

***p*-[*N*-(*tert*-Butyl(2-aminoethyl)carbamoyl-2,3-dioxocyclobut-1-enyl)amino]phenyl α-D-mannopyranoside (10):** Squaric acid monoamide **8** (140 mg, 354 μmol) was dissolved in dry DMF (5 mL), then *N*-Boc-ethylenediamine (112 μL, 709 μmol, 2.0 equiv) and triethylamine (196 μL, 1.42 mmol, 4.0 equiv) were added, and the reaction mixture was stirred at room temperature for 18 h. After removing the solvent in vacuo the crude product was purified by flash chromatography (ethyl acetate/methanol, 2:1) to yield **10** (166 mg, 326 mmol, 92% yield) as a colourless lyophilisate: m.p. 201 °C; $[\alpha]_D^{20} = +25.8$ ($c = 0.35$ in DMSO); $^1$H NMR (600 MHz, [D$_6$]DMSO, 298 K): $\delta = 9.75$ (br s, 1H; NH), 7.77 (br s, 1H; NH), 7.35, 7.06 (each d, $^3J = 8.5$ Hz, each 2H; aryl-H), 6.96 (s, 1H; NH), 5.28 (d, $^3J_{1,2} = 1.3$ Hz, 1H; H-1), 5.00 (br s, 1H; OH), 4.84–4.73 (m, 2H; 2 OH), 4.46 (br s, 1H; OH), 3.82 (m$_c$, 1H; H-2), 3.66 (dd, $^3J_{2,3} = 3.1$ Hz, $^3J_{3,4} = 9.1$ Hz, 1H; H-3), 3.63–3.56 (m, 3H; H-6a, 2 H-15), 3.51–3.44 (m, 2H; H-4, H-6b), 3.42 (ddd, $^3J_{4,5} = 9.4$ Hz, $^3J_{5,6a} = 1.9$ Hz, $^3J_{5,6b} = 6.1$ Hz, 1H; H-5), 3.14 (m$_c$, 2H; 2 H-16), 1.36 ppm (s, 9H; 3 H-19); $^{13}$C NMR (150 MHz, [D$_6$]DMSO): $\delta = 183.4$ (C$_{SA}$), 180.5 (C$_{SA}$), 169.3 (C$_{SA}$), 163.3 (C$_{SA}$), 155.8 (C-17), 152.2, 133.7 (2 C$_q$, aryl-C), 119.3, 117.9 (4 CH, aryl-C), 99.5 (C-1), 77.8 (C-18), 74.9 (C-5), 70.7 (C-3), 70.1 (C-2), 66.8 (C-4), 61.0 (C-6), 43.7 (C-15), 41.0 (C-16), 28.2 ppm (3 C-19); ESI-MS: *m/z* calcd for $C_{23}H_{31}N_3O_{10}Na$: 532.1902 $[M+Na]^+$; found: 532.1880; elemental analysis calcd (%) for $C_{23}H_{31}N_3O_{16}\cdot 1.2\,H_2O$ ($M_W$ 530.81 g mol$^{-1}$): C 52.01, H 6.34, N 7.91; found: C 51.98, H 6.39, N 8.13.

***p*-[(2-Aminoethylamino)-2,3-dioxocyclobut-1-enyl)amino]phenyl α-D-mannopyranoside (11):** TFA (150 μL) was added to a suspension of *N*-Boc-protected diamide **10** (160 mg, 314 μmol) in dry $CH_2Cl_2$ (5 mL), and a clear solution was obtained. This was stirred at room temperature for 3 h, and the solvent was subsequently removed in vacuo. The crude product was purified by preparative HPLC (A = water, B = methanol; 100→70% B, 60 min, 10 mL min$^{-1}$) to obtain **11** (124 mg, 304 mmol, 97% yield) as a colourless lyophilisate: m.p. 62 °C; $[\alpha]_D^{20} = +51.6$ ($c = 0.37$ in DMSO); $^1$H NMR (600 MHz, [D$_6$]DMSO, 298 K): $\delta = 10.01$ (br s, 1H; NH), 8.08 (br s, 1H; NH), 7.95 (br s, 2H; NH$_2$), 7.40, 7.08 (each d, $^3J = 8.7$ Hz, each 2H; aryl-H), 5.29 (d, $^3J_{1,2} = 1.3$ Hz, 1H; H-1), 5.20–4.61 (br, 4H; 4OH), 3.83–3.79 (m, 3H; H-2, 2 H-15), 3.66 (dd, $^3J_{2,3} = 3.2$ Hz, $^3J_{3,4} = 9.1$ Hz, 1H; H-3), 3.61 (dd, $^3J_{5,6a} = 1.3$ Hz, $^2J_{6a,6b} = 9.1$ Hz, 1H; H-6a), 3.51–3.44 (m, 2H; H-4, H-6b), 3.42 (m$_c$, 1H; H-5), 3.08 ppm (m$_c$, 2H; 2 H-16); $^{13}$C NMR (150 MHz, [D$_6$]DMSO): $\delta = 183.5$ (C$_{SA}$), 180.8 (C$_{SA}$), 169.1 (C$_{SA}$), 164.3 (C$_{SA}$), 152.4, 133.5 (2 C$_q$, aryl-C), 119.6, 117.9 (4 CH, aryl-C), 99.5 (C-1), 74.9 (C-5), 70.7 (C-3), 70.1 (C-2), 66.8 (C-4), 61.1 (C-6), 41.2 (C-15), 40.4 ppm (C-16); ESI-MS: *m/z* calcd for $C_{18}H_{23}N_3O_8Na$: 410.1558 $[M+Na]^+$; found: 410.1556; elemental analysis calcd (%) for $C_{18}H_{23}N_3O_8\cdot 1.1\,H_2O$ ($M_W$ 428.96 g mol$^{-1}$): C 50.37, H 5.92, N 9.79; found: C 50.34, H 5.29, N 9.89.

## Acknowledgements

*This work was financed by the DFG (SFB 677). We gratefully acknowledge a stipend of the "Fonds der Chemischen Industrie" (FCI) awarded to K.K. We are grateful to Thermo Fisher Scientific for Nunc microtiter plates.*

**Keywords:** bacterial adhesion · carbohydrates · glycoarrays · high-throughput screening · thiourea bridging

[1] a) T. Feizi, W. Chai, *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 582–588; b) N. Laurent, J. Voglmeir, S. L. Flitsch, *Chem. Commun.* **2008**, 4400–4412.
[2] a) O. Blixt, S. Head, T. Mondala, C. Scanlan, M. E. Huflejt, R. Alvares, M. C. Bryan, F. Fazio, D. Calarese, J. Stevens, N. Razi, D. J. Stevens, J. J. Skehel, I. van Die, D. R. Burton, I. A. Wilson, R. Cummings, N. Bovin, C.-H. Wong, J. C. Paulson, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 17033–17038; b) V. I. Dyukova, N. V. Shilova, O. E. Galanina, A. Y. Rubina, N. V. Bovin, *Biochim. Biophys. Acta Gen. Subj.* **2006**, *1760*, 603–609.
[3] a) J. E. Turnbull, R. A. Field, *Nat. Chem. Biol.* **2007**, *3*, 74–77; b) L. L. Kiessling, R. A. Splain, *Annu. Rev. Biochem.* **2010**, *79*, 619–653.
[4] a) B. T. Houseman, M. Mrksich, *Chem. Biol.* **2002**, *9*, 443–454; b) M. Kleinert, T. Winkler, A. Terfort, T. K. Lindhorst, *Org. Biomol. Chem.* **2008**, *6*, 2118–2132; c) L. Ban, M. Mrksich, *Angew. Chem.* **2008**, *120*, 3444–3447; *Angew. Chem. Int. Ed.* **2008**, *47*, 3396–3399; d) N. Laurent, R. Haddoub, J. Voglmeir, S. C. C. Wong, S. J. Gaskell, S. L. Flitsch, *ChemBioChem* **2008**, *9*, 2592–2596.
[5] a) C. Ortiz Mellet, J. M. García Fernández, *ChemBioChem* **2002**, *3*, 819–822; b) K. Larsen, M. B. Thygesen, F. Guillaumie, W. G. T. Willats, K. J. Jensen, *Carbohydr. Res.* **2006**, *341*, 1209–1234; c) C. Haensch, S. Hoeppener, U. S. Schubert, *Chem. Soc. Rev.* **2010**, *39*, 2323–2334; d) J. Mehlich, B. J. Ravoo, *Org. Biomol. Chem.* **2011**, *9*, 4108–4115.
[6] a) M. C. Bryan, O. Plettenburg, P. Sears, D. Rabuka, S. Wacowich-Sgarbi, C.-H. Wong, *Chem. Biol.* **2002**, *9*, 713–720; b) F. Fazio, M. C. Bryan, O. Blixt, J. C. Paulson, C.-H. Wong, *J. Am. Chem. Soc.* **2002**, *124*, 14397–14402; c) T. Feizi, F. Fazio, W. Chai, C.-H. Wong, *Curr. Opin. Struct. Biol.* **2003**, *13*, 637–645; d) M. Köhn, J. M. Benito, C. Ortiz Mellet, T. K. Lindhorst, J. M. García Fernández, *ChemBioChem* **2004**, *5*, 771–777; e) M. Hartmann, P. Betz, Y. Sun, S. N. Gorb, T. K. Lindhorst, A. Krueger, *Chem. Eur. J.* **2012**, *18*, 6485–6492.
[7] a) F. Peri, F. Nicotra, *Chem. Commun.* **2004**, 623–627; b) W. R. Algar, D. E. Prasuhn, M. H. Stewart, T. L. Jennings, J. B. Blanco-Canosa, P. E. Dawson, I. L. Medintz, *Bioconjugate Chem.* **2011**, *22*, 825–858.
[8] A. Dondoni, A. Marra, *Chem. Soc. Rev.* **2012**, *41*, 573–586.
[9] a) M. L. Blackman, M. Royzen, J. M. Fox, *J. Am. Chem. Soc.* **2008**, *130*, 13518–13519; b) H. S. G. Beckmann, A. Niederwieser, M. Wiessler, V. Wittmann, *Chem. Eur. J.* **2012**, *18*, 6548–6554.
[10] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; b) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.
[11] a) M. R. Gunther, P. M. Hanna, R. P. Mason, M. S. Cohen, *Arch. Biochem. Biophys.* **1995**, *316*, 515–522; b) B. Cao, Y. Zheng, T. Xi, C. Zhang, W. Song, K. Burugapalli, H. Yang, Y. Ma, *Biomed. Microdevices* **2012**, *14*, 709–720.
[12] a) E. M. Sletten, C. R. Bertozzi, *Acc. Chem. Res.* **2011**, *44*, 666–676; b) M. F. Debets, S. S. van Berkel, J. Dommerholt, A. T. J. Dirks, F. P. J. T. Rutjes, F. L. van Delft, *Acc. Chem. Res.* **2011**, *44*, 805–815.
[13] C. Grabosch, M. Kleinert, T. K. Lindhorst, *Synthesis* **2010**, 828–836.
[14] a) T. K. Lindhorst, C. Kieburg, *Angew. Chem.* **1996**, *108*, 2083–2086; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1953–1956; b) P. Krist, L. Vannucci, M. Kuzma, P. Man, K. Sadalapure, A. Patel, K. Bezouška, M. Pospíšil, L. Petruš, T. K. Lindhorst, V. Kŕen, *ChemBioChem* **2004**, *5*, 445–452; c) J. L. Jiménez Blanco, F. Ortega-Caballero, C. Ortiz Mellet, J. M. García Fernández, *Beilstein J. Org. Chem.* **2010**, *6*, 20; d) C. Maierhofer, K. Rohmer, V. Wittmann, *Bioorg. Med. Chem.* **2007**, *15*, 7661–7676; e) C. Ortiz Mellet, J. M. García Fernández, J. M. Benito, *Chem. Soc. Rev.* **2011**, *40*, 1586–1608.

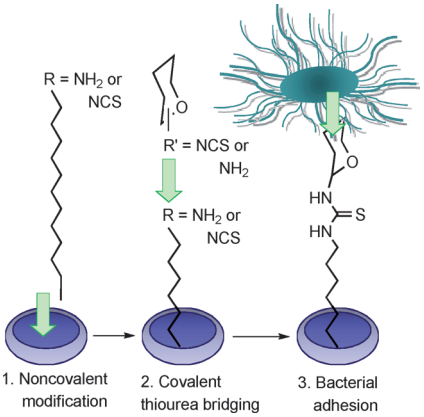
  

[15] F. Fazio, M. C. Bryan, H.-K. Lee, A. Chang, C.-H. Wong, *Tetrahedron Lett.* **2004**, *45*, 2689 – 2692.
[16] a) T. K. Lindhorst, C. Kieburg, *Synthesis* **1995**, 1228 – 1230; b) M. Walter, T. K. Lindhorst, *Monatsh. Chem.* **2002**, *133*, 473 – 483; c) M. Walter, T. K. Lindhorst, *Synthesis* **2006**, 952 – 958; d) M. Kühne, Z. Györgydeák, T. K. Lindhorst, *Synthesis* **2006**, 949 – 951; e) J. M. García Fernández, C. Ortiz Mellet, *Adv. Carbohydr. Chem. Biochem.* **2000**, *55*, 35 – 135.
[17] M. Hartmann, A. K. Horst, P. Klemm, T. K. Lindhorst, *Chem. Commun.* **2010**, *46*, 330 – 332.
[18] M. Hartmann, T. K. Lindhorst, *Eur. J. Org. Chem.* **2011**, 3583 – 3609.
[19] a) R. J. Pieters, *Med. Res. Rev.* **2007**, *27*, 796 – 816; b) I. Ofek, D. L. Hasty, N. Sharon, *FEMS Immunol. Med. Microbiol.* **2003**, *38*, 181 – 191; c) K. E. Sivick, H. L. T. Mobley, *Infect. Immun.* **2010**, *78*, 568 – 585; d) T. K. Lindhorst in *Synthesis and Biological Applications of Glycoconjugates* (Eds.: O. Renaudet, N. Spinelli), Bentham eBooks, **2011**, pp. 12 – 35.
[20] a) T. K. Lindhorst, S. Kötter, J. Kubisch, U. Krallmann-Wenzel, S. Ehlers, V. Křen, *Eur. J. Org. Chem.* **1998**, 1669 – 1674; b) T. K. Lindhorst, K. Bruegge, A. Fuchs, O. Sperling, *Beilstein J. Org. Chem.* **2010**, *6*, 801 – 809.
[21] a) C. R. McBroom, C. H. Samanen, I. J. Goldstein, *Methods Enzymol.* **1972**, *28*, 212 – 219; b) C. Kieburg, T. K. Lindhorst, *Tetrahedron Lett.* **1997**, *38*, 3885 – 3888.
[22] A. L. Martin, B. Li, E. R. Gillies, *J. Am. Chem. Soc.* **2009**, *131*, 734 – 741.
[23] a) O. Sperling, A. Fuchs, T. K. Lindhorst, *Org. Biomol. Chem.* **2006**, *4*, 3913 – 3922; b) C. Grabosch, M. Hartmann, J. Schmidt-Lassen, T. K. Lindhorst, *ChemBioChem* **2011**, *12*, 1066 – 1074; c) M. Hartmann, H. Papavlassopoulos, V. Chandrasekaran, C. Grabosch, F. Beiroth, T. K. Lindhorst, C. Röhl, *FEBS Lett.* **2012**, *586*, 1459 – 1465.
[24] A. Reisner, J. A. J. Haagensen, M. A. Schembri, E. L. Zechner, S. Molin, *Mol. Microbiol.* **2003**, *48*, 933 – 946.

Received: June 1, 2012
Published online on ■■ ■■, 0000

**Glycoarrays—easier than ever:** Glycoarrays were fabricated on polystyrene microtiter plates with great ease by using a tandem process that combined hydrophobic adsorption and thiourea bridging. They were validated by testing specific bacterial adhesion and its inhibition.




*C. Grabosch, K. Kolbe, T. K. Lindhorst**

■■–■■

**Glycoarrays by a New Tandem Noncovalent–Covalent Modification of Polystyrene Microtiter Plates and their Interrogation with Live Cells**