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Synthesis of Homo- and Heterofunctionalized Glycoclusters and Binding to *Pseudomonas aeruginosa* Lectins PA-IL and PA-IIL

Béatrice Gerland,[†] Alice Goudot,[‡] Gwladys Pourceau,[†] Albert Meyer,[†] Sébastien Vidal,[§] Eliane Souteyrand,[‡] Jean-Jacques Vasseur,[†] Yann Chevolot,[*,‡] and François Morvan[*,†]

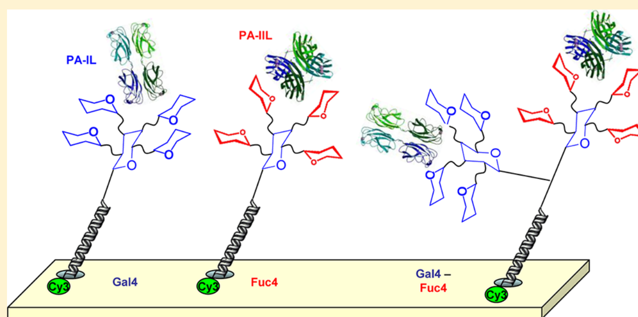
[†]Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS - Université Montpellier 1, Université Montpellier 2, Place Eugène Bataillon, CC1704, 34095 Montpellier Cedex 5, France

[‡]Institut des Nanotechnologies de Lyon (INL), Université de Lyon, UMR 5270 CNRS Ecole Centrale de Lyon, 36 Avenue Guy de Collongue, 69134 Ecully Cedex, France

[§]Institut de Chimie et Biochimie Moléculaires et Supramoléculaires (ICBMS), Laboratoire de Chimie Organique 2 - Glycochimie, UMR 5246 CNRS, Université Claude Bernard Lyon 1, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne, France

Supporting Information

ABSTRACT: Homo- and heterofunctionalized glycoclusters with galactose and/or fucose residues targeting both PA-IL and PA-IIL lectins of *Pseudomonas aeruginosa* were synthesized using "Click" chemistry and DNA chemistry. Their binding to lectins (separately or in a mixture) was studied using a DNA Directed Immobilization carbohydrate microarray. Homoglycoclusters bind selectively to their lectin while the heteroglycocluster binds simultaneously both lectins with a slight lower affinity.



Biological processes such as cell−cell interactions, innate response, and bacterial or viral infection involve carbohydrate−lectin interactions.[1,2] As the lectin avidity is relatively low for monovalent interactions, Nature uses multivalency creating a cooperative effect known as the "cluster effect".[3,4] The possibility of inhibiting or promoting carbohydrate−lectin interactions with synthetic multivalent glycoconjugates has been widely investigated to identify synthetic molecules displaying high affinity for lectins.[5]

Pseudomonas aeruginosa (PA) is an opportunistic Gram-negative bacterium involved in nosocomial infections and is the major cause of morbidity and mortality for cystic fibrosis patients. PA has two soluble lectins, PA-IL (LecA) and PA-IIL (LecB), that bind selectively to galactose and fucose, respectively. Several multivalent galactosylated or fucosylated glycoclusters have been reported in the literature as potential high affinity ligands of these lectins for applications as antiadhesive drugs.[6−14]

While many homoglycoclusters have been synthesized,[5] there are only few examples of heteroglycoconjugates exhibiting different combinations of carbohydrates. Among them, the following combinations of carbohydrates have been reported with D-galactose and L-fucose,[15] mannose and galactose,[16−19] mannose and lactose,[20,21] mannose and glucose,[22,23,20] glucose and galactose,[24] or mannose, glucose, and galactose.[25,26]

We have recently described the synthesis of carbohydrate-centered glycoclusters obtained by combination of DNA phosphoramidite chemistry[27] and copper(I)-catalyzed azide−alkyne Huisgen 1,3-dipolar cycloaddition (CuAAC)[28,29] between carbohydrate-centered alkynes and carbohydrate azides leading to tetra- or hexadeca-galactosylated mimics.[30]

We present herein the design, synthesis, and biological properties of an original heteroglycocluster combining two mannose-centered cores, one bearing four galactose and the other four fucose moieties (**20-Gal$_4$-Fuc$_4$**, Scheme 2) in comparison with two mannose-centered homoglycoclusters containing either four galactose (**9-Gal$_4$**) or four fucose (**10-Fuc$_4$**) moieties (Scheme1). The synthesis of the heteroglycocluster was performed by combining four CuAAC reactions with phosphoramidite chemistry to introduce selectively the alkyne functions sequentially. Most of the reactions were performed on solid support giving a relative rapid access to such elaborate structures. Each glycocluster was tagged with an oligonucleotide for its subsequent DNA directed immobilization (DDI) on a DNA microarray[31,32] to evaluate its binding to PA-IL or/and PA-IIL. The galactosylated and the fucosylated homoglycoclusters are anticipated to bind selectively to PA-IL and PA-IIL, respectively,[33] while the heteroglycocluster should bind both lectins. As a result, the heteroglycocluster would create a stronger and probably more specific interaction with the whole bacterium in comparison to the homoglycoclusters.

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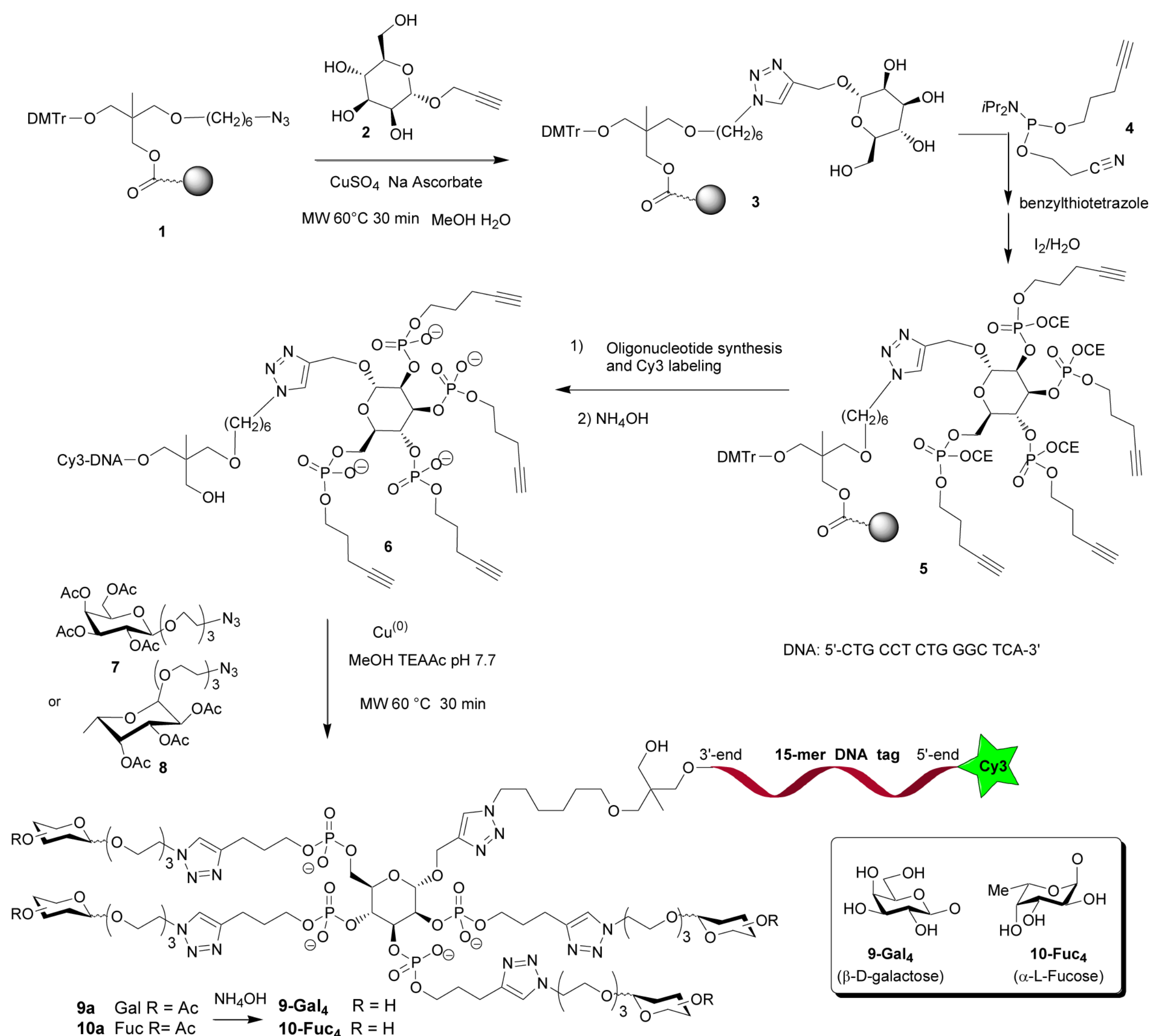
Scheme 1. Synthesis of Mannosyl-Centered Homo-Glycoclusters Bearing Either Four β-D-Galactose or Four α-L-Fucose Residues



Synthesis of Glycocluster Oligonucleotide Conjugates. The homoglycoclusters exhibiting either four β-D-galactose or four α-L-fucose moieties were synthesized according to the strategy recently reported,[30] starting from an azide solid support **1**[34] on which a 1-*O*-propargyl-α-D-mannopyranoside **2** was immobilized by CuAAC with copper sulfate and sodium ascorbate under microwave assistance (MW) (Scheme 1). The key step involved the phosphorylation of the mannoside hydroxyls with a pent-4-ynyl phosphoramidite **4** performed by oligonucleotide chemistry, using a DNA synthesizer, affording the tetra-alkyne intermediate **5** (Scheme 1). Since the alkyne function is orthogonal with the other functions of an oligonucleotide, the elongation of an oligonucleotide and its labeling with cyanine 3 (Cy3) were directly performed on a DNA synthesizer. Then, the expected tetra-alkyne mannose **6** tagged with a 5′-fluorescent oligonucleotide was obtained in solution by an ammonia treatment. Crude compound **6** was converted into homoglycocluster by CuAAC reaction using either 1-azido-3,6-dioxaoct-8-yl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **7** or 8-azido-3,6-dioxapent-1-yl 2,3,4-tri-*O*-acetyl-α-L-fucopyranoside **8** in presence of Cu(0) in triethylammonium acetate buffer (0.1 M, pH 7.7) and methanol for 30 min at 60 °C with microwave assistance. After centrifugation, the supernatant was desalted by size-exclusion chromatography (SEC) to give the expected acetylated tetraglycoclusters. To facilitate the purification by reversed-phase HPLC, we took advantage of the greater lipophilicity of acetylated glycoclusters, as the polar impurities were eluted first. A final treatment with concentrated ammonia afforded the expected mannosyl-centered tetra-β-D-galactoses **9-Gal$_4$** and tetra-α-L-fucoses **10-Fuc$_4$** labeled with the 5′-Cy3-DNA sequence which were characterized by MALDI-TOF MS.

For the synthesis of the heteroglycocluster **20-Gal$_4$-Fuc$_4$**, we used another strategy. The mannose cores were successively introduced by CuAAC using an azide derivative **12** first on the alkyne solid support **11** and second on an alkyne function introduced by means of the phosphoramidite derivative **16**. This strategy is more straightforward than the one using the propargyl mannose **2** requiring the introduction of an azide function into the oligonucleotide. Because of the Staudinger reaction, azide phosphoramidite derivatives are unstable;,[35−37] hence, azide functions should be introduced either by means of an azide *H*-phosphonate derivative[35,38] using *H*-phosphonate chemistry with specific reagents or by means of bromoalkyl

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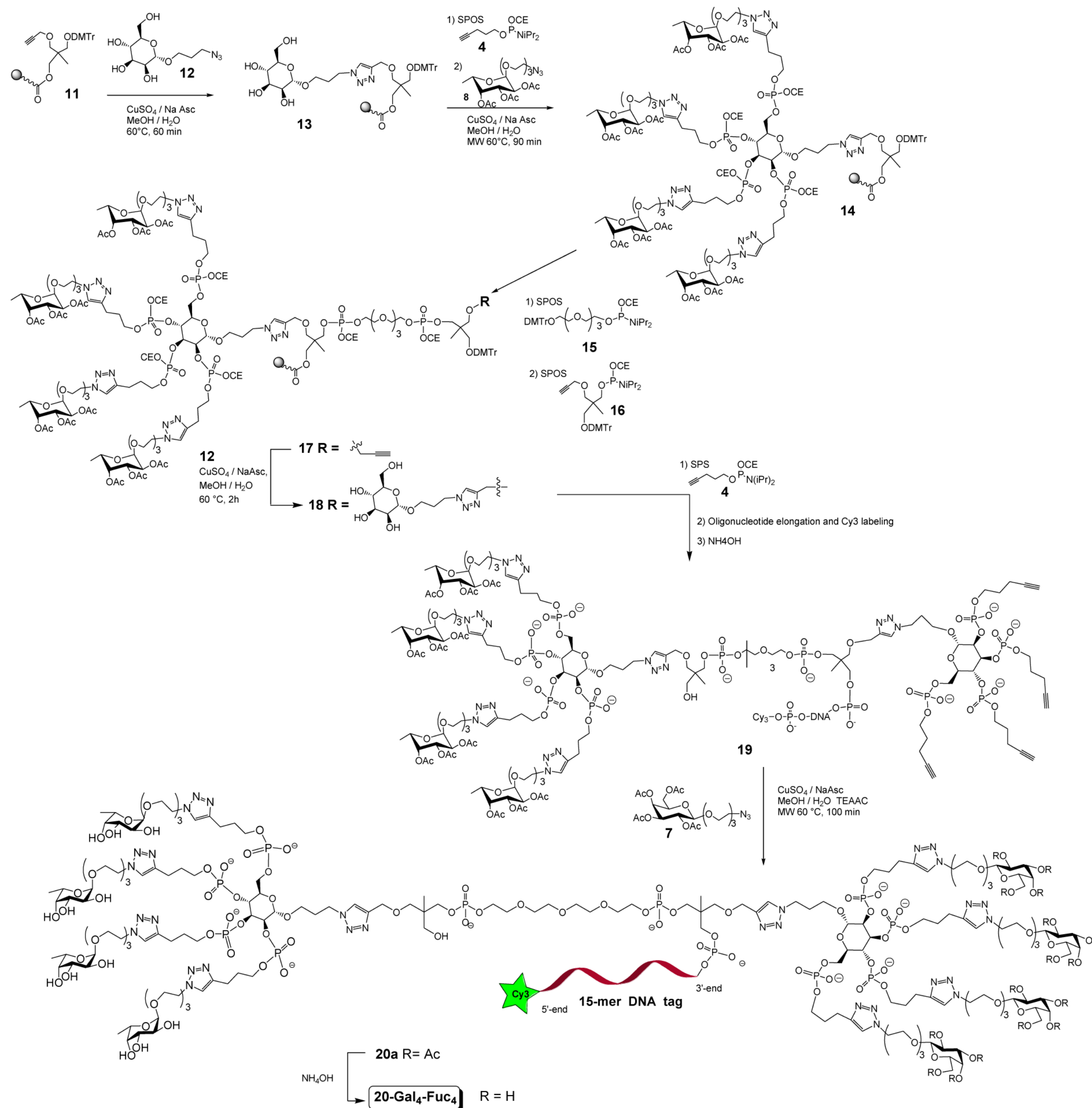
Scheme 2. Synthesis of Mannosyl-Centered Hetero-Glycocluster Bearing Four α-L-Fucoses and Four β-D-Galactoses



phosphoramidite followed by an azidation carried out manually.[19] Both methods are not convenient since they require extra steps.

The introduction of α-L-fucose and β-D-galactose moieties was performed sequentially (Scheme 2). From a first mannose core immobilized by CuAAC on an alkyne solid support **11** using 3-azidopropyl mannoside **12**, the four pent-4-ynyl chains were coupled. A second CuAAC, done on solid support, with 8-azido-3,6-dioxapent-1-yl 2,3,4-tri-*O*-acetyl-α-L-fucopyranoside **8** afforded the tetra-α-L-tetraacetylfucosylated solid-support **14**. To minimize possible steric hindrance between both lectins that would bind the heteroglycocluster, a tetraethylene glycol linker **15** was introduced to bring some space between the two different glycoclusters and an alkyne function was introduced using a monoalkynyl phosphoramidite **16**. A second 3-azidopropyl mannoside **12** was added to **17** by a third CuAAC to give **18**. The end of the synthesis was straightforward. Using a DNA synthesizer, four pent-4-ynyl chains were coupled on the second mannose core, and the oligonucleotide was elongated and labeled with a Cy3 dye. After a standard ammonia treatment, the fluorescent oligonucleotide **19** exhibiting four α-L-fucoses and a mannose core with four pentynyl chains was released in solution. Compound **19** was treated with 8-azido-3,6-dioxaoct-1-yl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **7**, copper sulfate, and sodium ascorbate for 45 min at 60 °C under microwave assistance affording the expected heteroglycocluster exhibiting four α-L-fucoses and four tetra-acetyl-β-D-galactoses conjugated to the fluorescent oligonucleotide. This partially protected conjugate was purified by HPLC, and a final aqueous ammonia treatment gave the desired conjugated heteroglycocluster **20-Gal$_4$-Fuc$_4$** which was characterized by MALDI-TOF MS (see the Supporting Information).

Interactions with PA-IL and PA-IIL Using a DNA-Based Carbohydrate Microarray. The three glycoclusters **9-Gal$_4$**, **10-Fuc$_4$**, and **20-Gal$_4$-Fuc$_4$** were immobilized on a DNA

microarray by DDI.[31,32] The Cy3 dye attached at the 5′-end of each glycocluster allowed the assessment of the immobilization efficacy by a fluorescence scanning at 532 nm. The glycoclusters were homogeneously immobilized on the slide with less than 40% deviation (Figure 1). The Alexa 647 labeled-

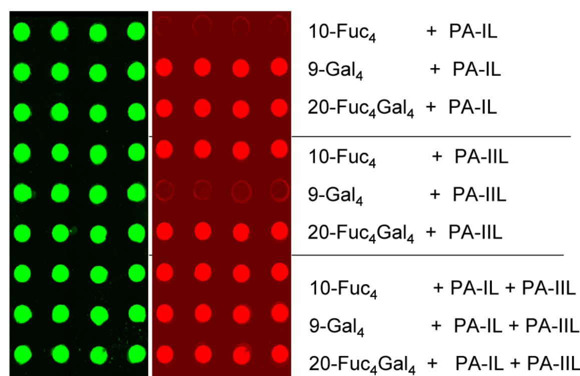


Figure 1. Fluorescence scanning for the interactions of the homo- and heteroglycoclusters with PA-IL, PA-IIL, or both lectins in a mixture (Cy3, 532 nm, left and Alexa Fluor 647, 635 nm, right).

lectins PA-IL or PA-IIL were then incubated on the slide, and their binding to the glycoclusters was visualized by fluorescence signal at 635 nm (Figure 1). The Alexa 647 signal was normalized based on the Cy3 fluorescence signal. Incubation of PA-IL with **9-Gal$_4$** and PA-IIL with **10-Fuc$_4$** gave a significant fluorescence signal intensity of roughly 30000 au, whereas the signal was at background level (below 500 au) for incubation of PA-IIL with **9-Gal$_4$** and PA-IL with **10-Fuc$_4$** (Figure 2). These

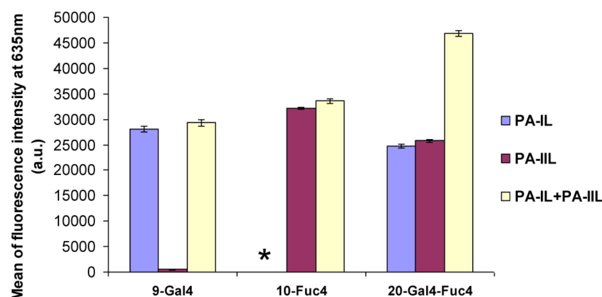


Figure 2. Fluorescence signal measured for the interaction of PA-IL and/or PA-IIL incubated with **9-Gal$_4$**, **10-Fuc$_4$**, or **20-Gal$_4$-Fuc$_4$**. *85 au not visible on the histogram.

results confirmed the high ligand specificity of each lectin and the low level of nonspecific interactions or adsorption of the proteins on the microarray. In another experiment, incubation of an equimolar mixture of PA-IL and PA-IIL with either **9-Gal$_4$** or **10-Fuc$_4$** gave a very slight increase of the signal intensities (~4%) to the signals observed upon incubation with a single lectin (PA-IL or PA-IIL). These results demonstrated that the presence of the specific lectin did not induce a significant nonspecific adsorption of the other lectin.

PA-IL or PA-IIL were then incubated separately with the heteroglycocluster **20-Gal$_4$-Fuc$_4$**, and the signal intensities were at roughly 25 000 au corresponding to a slight decrease (12% and 20%) in comparison with the incubation with the homoglycoclusters **9-Gal$_4$** and **10-Fuc$_4$**. This results suggested that the second glycocluster induced a slight steric hindrance despite the tetraethyleneglycol linker between both glycoclusters.

An equimolar mixture of PA-IL and PA-IIL was incubated with the heteroglycococluster **20-Gal$_4$-Fuc$_4$** providing a fluorescence signal of ca. 47000 au which is 7% lower than the sum of the individual lectins' fluorescence signal. These data suggested that both lectins interact with the heteroglycocluster, and the presence of a lectin had a limited effect on the other. Finally, we determined the IC$_{50}$ values of each glycocluster,using the DDI carbohydrate microarray.[10,39] Thus, **9-Gal$_4$** and **20-Gal$_4$-Fuc$_4$** were incubated with PA-IL and an increasing concentration of lactose as inhibitor, and **10-Fuc$_4$** and **20-Gal$_4$-Fuc$_4$** were incubated with PA-IIL and an increasing concentration of L-fucose as inhibitor. The IC$_{50}$ value corresponds to the concentration of inhibitor (lactose or L-fucose) to displace 50% of the lectin bound to the glycocluster. Hence, a higher value corresponds to a higher binding of the glycocluster. The calculated IC$_{50}$ values of lactose for **9-Gal$_4$** and **20-Gal$_4$-Fuc$_4$** were 29 and 15 μM respectively and those of L-fucose for **10-Fuc$_4$**, and **20-Gal$_4$-Fuc$_4$** were 11 and 6 μM, respectively, corresponding to 48% and 45% decrease of binding, respectively. Note that IC$_{50}$ values are determined by a log scale of concentration so a 50% of difference is not really significant.

These results showed that there is a slight decrease in binding of the lectins to the heteroglycocluster in comparison with the homoglycoclusters. The same trend was previoulsy observed by Deguise et al. with a heterotetragalactose-tetrafucose synthesized on another scaffold.[15]

In conclusion, two homoglycoclusters incorporating a mannosyl-centered core with either four galactose or four fucose residues and a heteroglycocluster displaying a mannosyl-centered conjugated with both galactose and fucose residues were synthesized using a combination of DNA phosphoramidite supported chemistry and up to four successive microwave-assisted CuAAC conjugations. Comparison of their binding properties toward PA-IL and PA-IIL was achieved using a DNA-based carbohydrate microarray by direct fluorecence scanning and determination of IC$_{50}$ values. The specific binding of each lectin was monitored and verified with the homoglycoclusters, while the heteroglycocluster was found to interact with both soluble lectins of *Pseudomonas aeruginosa* although with a slight lower affinity in comparison with the homoglycoclusters. The present results obtained are a general proof of concept for the dual binding to both soluble bacterial lectins of *Pseudomonas aeruginosa*.

■ EXPERIMENTAL PROCEDURES

The solid supports **1**[30] and **11**,[40] the phosphoramidite derivatives **4**,[34] **15**,[41] and **16**,[42] and the carbohydrate derivatives **2**,[43] **7**,[44] **8**,[45] and **12**[16] were synthesized according to reported protocols.

Immobilization on Azide Solid Support 1 of 1-*O*-Propargyl-α-D-mannopyranoside 2 by Cu(I)-Catalyzed 1,3-Dipolar Cycloaddition. An aqueous solution of 1-*O*-propargylmannose **2** (100 mM, 50 μL), freshly prepared aqueous solutions of $CuSO_4$ (100 mM, 4 μL), and sodium ascorbate (500 mM, 4 μL), water (42 μL), and MeOH (100 μL) were added to 1 μmol of azide solid support **1**. The resulting mixture was treated in a sealed tube with microwave synthesizer at 60 °C for 30 min (premixing time: 30 s). The temperature was monitored with an internal infrared probe. The solution was removed and CPG beads were washed with H_2O (3 × 2 mL) and MeOH (3 × 2 mL) and dried.

Immobilization of 3-azidopropyl-α-D-mannopyranoside 12 by Cu(I)-Catalyzed 1,3-Dipolar Cycloaddition. *On Propargyl Solid Support 11.* A solution of 3-Azidopropyl-α-D-mannopyranoside

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Table 1. CuCAAC Conditions

CuAAC reaction compd	scale (nmol)	carbohydrate derivative	MeOH volume (μL)	water volume (μL)	TEAAc buffer (μL)	activation	time (min)
6→**9a**	100	12 equiv of **7**, 12 μL	113	100	25	MW, 60 °C	30
6→**10a**	100	12 equiv of **8**, 12 μL	113	0	25	MW, 60 °C	30
→**14**	1000	12 equiv of **8**, 120 μL	5	100	0	oil bath, 60 °C	90
19→**20a**	70	12 equiv of **7**, 8.4 μL	118	85	25	MW, 60 °C	100

12 (100 mM in MeOH, 300 μL), freshly prepared aqueous solution of $CuSO_4$ (100 mM, 24 μL), and sodium ascorbate (500 mM, 24 μL), water (552 μL), and MeOH (300 μL) were added to 6 μmol of solid support **11**. The tube containing the resulting suspension was sealed and placed in an oil bath at 60 °C for 1 h under gentle magnetic stirring. After the reaction, the solution was removed, and the CPG beads were washed with H_2O (3 × 2 mL), MeOH (3 × 2 mL), and CH_3CN (3 × 2 mL) and dried.

On Derivative 17. A solution of 3-azidopropyl-α-D-mannopyranoside **12** (100 mM in MeOH, 50 μL), freshly prepared aqueous solutions of $CuSO_4$ (80 mM, 5 μL) and sodium ascorbate (100 mM, 20 μL), water (100 μL), and MeOH (75 μL) were added to 1 μmol of alkyne solid support **17**. The tube containing the resulting mixture was sealed and placed in an oil bath at 60 °C for 2 h. The solution was removed, and CPG beads were washed with H_2O (3 × 2 mL), MeOH (3 × 2 mL), and CH_3CN (3 × 2 mL) and dried. Some CPG beads (<1 mg) were treated with aqueous ammonia (1 mL) to deprotect and release the glycocluster **18 dep** in order to control the efficiency of last three steps of the synthesis since **14**. After evaporation to dryness, the resulting crude glycocluster **18 dep** was suspended in water for subsequent analysis.

General Procedure for Introduction of Pentynyl Chains on Mannose Hydroxyls. Solid-supported mannose derivatives **3**, **13**, and **18** were treated by nucleoside phosphoramidite chemistry, on a DNA synthesizer, with pentyn-4-yl phosphoramidite **4**. Only coupling and oxidation steps were performed. For the coupling step, benzylthiotetrazole was used as activator (0.3 M in anhydrous CH_3CN), and **4** (0.2 M in anhydrous CH_3CN) was introduced three times (120 μmol) with a 180 s coupling time. Oxidation was performed with commercial solutions of iodide (0.1 M I_2, THF/pyridine/water 90:5:5) for 15 s.

General Procedure for Solid-Phase Oligonucleotide Synthesis (SPOS). The oligonucleotide sequences were synthesized at the 1 μmol scale on a DNA synthesizer by standard phosphoramidite chemistry. For the coupling step, benzylthiotetrazole was used as activator (0.3 M in anhydrous CH_3CN), commercially available nucleoside phosphoramidites (0.09 M in anhydrous CH_3CN) were introduced with a 20 s coupling time, phosphoramidites **15** and **16** (0.09 M in anhydrous CH_3CN) with a 40 s coupling time and Cy3 amidite (0.06 M in anhydrous CH_3CN) with a 180 s coupling time. The capping step was performed with acetic anhydride using commercial solution (Cap A: Ac_2O/pyridine/THF, 10:10:80 and Cap B: 10% *N*-methylimidazole in THF) for 15 s. Each oxidation was performed for 15 s. Detritylation was performed with 2.5% TCA in CH_2Cl_2 for 35 s.

General Procedure for Deprotection of Solid-Supported Oligonucleotides. CPG beads were treated with concentrated aqueous ammonia (1.5 mL) for 15 h at room temperature and warmed to 55 °C for 2 h to give modified oligonucleotides **6** and **19**. The supernatants were withdrawn and evaporated to dryness. Residues were dissolved in water.

General Procedure for Introduction of L-fucose or D-Galactose Residues. *Using Cu(0).* To the 5′-fluorescent-3′-alkyne oligonucleotide glycoconjugate **6** (100 mM) were added galactosyl **7** or fucosyl **8** derivatives (100 mM in MeOH), ~1 mg of Cu(0) nanopowder, triethylammonium acetate buffer 0.1 M, pH 7.7 (25 μL), water, and MeOH to obtain a final volume of 250 μL. The resulting preparations were treated by microwave irradiation in a microwave synthesizer Initiator from Biotage 60 °C for 30 min affording **9a** or **10a**.

Using $CuSO_4$ Sodium Ascorbate. To 1 μmol of solid-supported glycocluster were added a fucosyl derivative **8** (100 mM in MeOH, 120 μL), freshly prepared aqueous solutions of $CuSO_4$ (80 mM, 5 μL) and sodium ascorbate (100 mM, 20 μL), water (100 μL) and MeOH (5 μL). The tube containing the resulting mixture was sealed and placed in an oil bath at 60 °C for 90 min. The solution was removed and CPG beads were washed with H_2O (3 × 2 mL), MeOH (3 × 2 mL) and CH_3CN (3 × 2 mL) and dried to afford **14**.

To oligonucleotide **19** (70 nmol) were added galactosyl derivative **7** (100 mM in MeOH, 8.4 μL), freshly prepared aqueous solution of $CuSO_4$ (1 equiv/alkyne, 40 mM, 7 μL) and sodium ascorbate (5 equiv/$CuSO_4$, 200 mM, 7 μL), triethylammonium acetate buffer 0.1 M, pH 7.7 (25 μL), water, and MeOH to obtain a final volume of 250 μL. The resulting preparation was treated by microwave irradiation in a microwave synthesizer Initiator from Biotage affording **20a**.

EDTA (aqueous saturated solution, 400 μL) was added to the mixtures containing **9a**, **10a** and **19** to complex copper salt. After centrifugation, the supernatants were withdrawn to eliminate Cu(0) and solutions were desalted on NAP10 and evaporated. Acetylated glycoclusters (**9a**, **10a**) were dissolved in water and purified by reversed-phase preparative HPLC. Pure compounds (**9a** and **10a**) and partially deprotected **20a** were treated with concentrated aqueous ammonia (3 mL) for 2 h at room temperature to remove acetyl groups, and evaporated to dryness. Compounds **9-Gal$_4$**, **10-Fuc$_4$**, and **20-Gal$_4$-Fuc$_4$** were finally purified again by reversed-phase preparative HPLC. Residues were dissolved in water for subsequent analyses.

General Remarks on HPLC Analysis, Purification, and MS Characterization. For analyses, a reversed-phase C18 Nucleosil (5 μm) column (150 × 4.6 mm) was used at a flow rate of 1 mL min^{-1} using a linear gradient of acetonitrile 6% to 75% in 0.05 M aqueous triethylammonium acetate (TEAAc) pH 7 for 20 min. For purifications, a reversed-phase C_{18} Delta Pak (15 μm) column (7.8× 300 mm) was used at a flow rate of 2 mL min^{-1} using a linear gradient of acetonitrile in 0.05 M TEAAc pH 7 for 20 min as described in Tables 1 and 2.

Table 2. HPLC Conditions for Purification

compd	analysis gradient (% of acetonitrile in 0.05 M TEAAc, pH 7)	purification gradient (% of acetonitrile in 0.05 M TEAAc, pH 7)
9a and **10a**	6−75	24−48
9-Gal$_4$ and **10-Fuc$_4$**	6−75	6−60
19 (crude)	8−32	
20-Gal$_4$-Fuc$_4$	8−32	8−32

MALDI-TOF mass spectra were recorded on a mass spectrometer equipped with a nitrogen laser. MALDI conditions were: accelerating voltage 24000 V; guide wire 0.05% of the accelerating voltage; grid voltage 94% of the accelerating voltage; delay extraction time 500 ns. One μL of sample was mixed with 5 μL of a saturated solution of HPA in acetonitrile/water (1:1, v/v) containing 10% of ammonium citrate and few beads of DOWEX 50W-X8 ammonium sulfonic acid resin were added. Then, 1 μL of the mixture was placed on a plate and dried at room temperature and pressure.

Analyses and Characterization of Oligonucleotide Glycoclusters. Compound **6** (crude): HPLC t_R = 6.70 min; MS MALDI-TOF$^-$ m/z calcd for $C_{213}H_{283}N_{56}O_{118}P_{20}$ [M-H]$^-$: 6135.31 found 6134.92; n= 972 nmol calculated by UV measurement at 550 nm.

Compound **9-Gal$_4$**: HPLC t_R = 7.07 min; MS MALDI-TOF$^-$ m/z calcd for $C_{261}H_{375}N_{68}O_{150}P_{20}$ [M − H]$^-$ 7484.61 found 7483.97; n = 28 nmol calculated by UV measurement at 550 nm.

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Compound **10-Fuc$_4$**: HPLC t_R = 7.69 min; MS MALDI-TOF$^-$ m/z calcd for $C_{261}H_{375}N_{68}O_{146}P_{20}$ $[M - H]^-$ 7420.62 found 7420.58; n = 11 nmol calculated by UV measurement at 550 nm.

Compound **19** (crude): HPLC t_R = 12.4 min; MS MALDI-TOF$^-$ m/z calcd for $C_{303}H_{445}N_{71}O_{176}P_{26}$ $[M - H]^-$ 8702.46 found 8701.36; n = 229 nmol calculated by UV measurement at 550 nm.

Compound **20-Gal$_4$-Fuc$_4$**: HPLC t_R = 12.7 min; MS MALDI-TOF m/z calcd for $C_{303}H_{445}N_{71}O_{176}P_{26}$ $[M - H]^-$ 10051.76 found 10052.90; n = 7 nmol calculated by UV measurement at 550 nm.

Fabrication of Glycoarrays. The fabrication of the DNA microarray is detailed in ref 32. Microstructured borosilicate glass slides were used.[46,47] NHS ester surface modified glass slides were obtained using the protocol of Dugas et al.[48] We have used the "on-chip" approach, which means that lectin/carbohydrate binding is performed on solid support after hybridization of the glycoconjugates by DDI method (DNA Direct Immobilization method).

*Direct Immobilization of Glycoconjugates **9-Gal$_4$**, **10-Fuc$_4$**, and **20-Gal$_4$-Fuc$_4$**.* First, DNA strands 3′-amino-modified oligonucleotide purchased from Eurogentec were immobilized using the same protocol as Moni et al.[10] One microliter of a DNA strands solution at 25 μM in PBS 10X, pH 8.5 was incubated overnight at room temperature at the bottom of each well. The water was then allowed to completely evaporate. The slide was washed with SDS 0.1% at 70 °C for 30 min then rinsed with deionized water, then run dry by centrifugation. Blocking was performed with a BSA 4% solution in PBS 1X, pH 7.4 at 37 °C for 2 h. Washing steps were 3 × 3 min cycles: in PBS 1X-Tween$_{20}$ 0.05% and in PBS 1X before rapidly rinsing with deionized water and centrifugation. Glycoconjugates **9-Gal$_4$**, **10-Fuc$_4$**, or **20-Gal$_4$-Fuc$_4$** bearing DNA tag were incubated overnight at 37 °C at 1 μM in PBS 1X, pH 7.4, in the corresponding wells (Figure 1). The resulting slide was washed in SSC 2 × 0.1% SDS at 51 °C for 1 min followed by SSC 2X at room temperature for 5 min and then with deionized water and centrifuged. A second blocking step was performed after hybridization with the same protocol as described above but with only 1 h incubation at 37 °C. Before lectin recognition and IC$_{50}$ assay, hybridization control was performed scanning the slide at 532 nm, pmt 400 with the Microarray scanner, GenePix 4100A (Figure 1).

On-Chip Biological Recognition. PA-IL and PA-IIL were labeled according to the manufacturer protocol (Alexa Fluor 647 microscale protein labeling kit). Concentration and degree of labeling were controlled by optical density. Lectins were diluted (final concentration 0.12 μM) in a PBS 1X, pH 7.4, solution with $CaCl_2$ (final concentration 5 μM) and BSA (2%). One microliter of the desired lectin solution was deposited in each corresponding well. The recognition reaction was performed during 3 h at 37 °C. The slide was washed for 5 min at 4 °C in PBS 1X-Tween$_{20}$ 0.02% and then with deionized water and centrifuged. Slide was scanned at 635 nm. The fluorescence signal of each lectin, bound with their corresponding glycoconjugate, was determined as the average of the mean fluorescence signal of the four spots per row.

IC$_{50}$ Assays. Fucose or lactose was added to the solution of labeled PA-IL or labeled PA-IIL at different final concentrations (from 0 μM to 50000 μM maximum of fucose, and from 0 μM to 50000 μM for lactose). Each solution (1 μL) was placed at the bottom of each well and incubated at 37 °C in a water vapor saturated chamber for 3 h. The slides were then washed in PBS-Tween 20 (0.02%) 5 min at 4 °C and finally rinsed with deionized water before being dried by centrifugation.

■ ASSOCIATED CONTENT

Supporting Information

HPLC profiles and MALDI-TOF spectra of **6**, **9-Gal$_4$**, **10-Fuc$_4$**, **19**, and **20-Gal$_4$-Fuc$_4$**, and IC$_{50}$ curves. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(F.M.) Tel: +33/467 144 961. Fax: +33/467 042 029. E-mail: morvan@univ-montp2.fr. (Y.C.) Tel: +33/472 186 240. Fax: +33/4 78 43 35 93. E-mail: yann.chevolot@ec-lyon.fr.

Notes

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