EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201500165

Hetero-Click Conjugation of Oligonucleotides with Glycosides Using Bifunctional Phosphoramidites

Albert Meyer,[a] Mathieu Noël,[a] Jean-Jacques Vasseur,[a] and François Morvan*[a]

Keywords: Carbohydrates / Oligonucleotides / Glycoclusters / Glycoconjugates / Click chemistry

Two phosphoramidite derivatives, **1** and **2**, each bearing two orthogonal functions: alkyne/thioacetyl or alkyne/tosyl, respectively, were synthesized and used to generate heteroglyco 5′-oligonucleotide conjugates. After coupling, the first conjugation was performed using CuAAC on solid support with an azide-bearing carbohydrate. For **1**, the second conjugation was performed in solution by a thiol "click" reaction using a bromoacetamide galactoside, whereas for **2**, the tosyl group was converted into an azide and a second CuAAC was applied on solid support. Accordingly, a deoxycholic-centered heteroglycocluster exhibiting two D-galactose and two L-fucose motifs, conjugated to an oligonucleotide, was successfully synthesized.

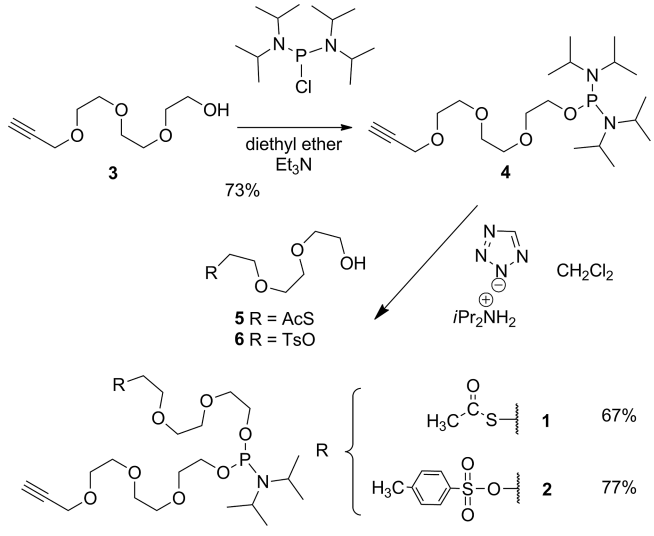
Introduction

Multivalent recognition between lectins and glycans occurs in several biological processes such as those dealing with cell–cell communication, innate immune response systems and pathogen infections.[1–3] Several synthetic multivalent glycoconjugates have been reported[4–7] in efforts to compete with natural carbohydrate ligands and to control or modulate these interactions; potential therapeutic applications are likely to result.[8,9] The resulting neoglycans are expected to display significantly enhanced binding to their targets relative to the sum of interactions of the constitutive monomers, thanks to the so-called "cluster glycoside effect".[10,11] Thus far, most synthetic glycoclusters have been synthesized as homoglycoclusters. However, natural oligosaccharides consist of a variety of carbohydrates and use this heterogeneity to achieve affinity and selectivity for specific lectins. It has been shown that the presence of non-recognized carbohydrates increases the binding affinity of those that are recognized.[12–15]

Despite earlier work, the synthesis of heteroglycoclusters remains a challenging task; Fernandez et al. have recently reviewed this area.[16] Early work relayed the use of a building block containing an orthogonal protecting group combined with protected carbohydrate phosphoramidites to generate heteroglycoclusters.[17] More recently, the synthesis of heteroglycoclusters has employed "click chemistry" reactions like Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAc),[18–23] oxime formation,[24–26] and "click thiol" reactions[27] (thiol-ene,[13,14,18] thiol Michael-type addition,[28] and thiol–chloroacetyl coupling[26,29]).

With these possibilities in mind, one option for heteroglycocluster synthesis has involved a sequential process wherein building blocks bearing the "click" reactive function are installed followed by the click reaction itself and this order of steps repeated until the desired heteroglycocluster is completed.[15,23,25] Another approach requires the combination of two orthogonal click reactions with the introduction of two distinct building blocks, each bearing an orthogonal functionality.[20,22,26,28–31]

As an alternative to these strategies, we report herein the use of two variants of a single phosphoramidite building block exhibiting two orthogonal functions that selectively react with two different, suitably functionalized carbohydrates. The main advantage of this novel strategy is that the synthesis of a single intermediate is sufficient to gener-



Scheme 1. Synthesis of bi-functional phosphoramidites **1** and **2**.

[a] Institut des Biomolécules Max Mousseron UMR 5247 CNRS-UM-ENSCM, Université de Montpellier,
Place E. Bataillon, CC1704, 34095 Montpellier cedex 5, France
E-mail: morvan@univ-montp2.fr

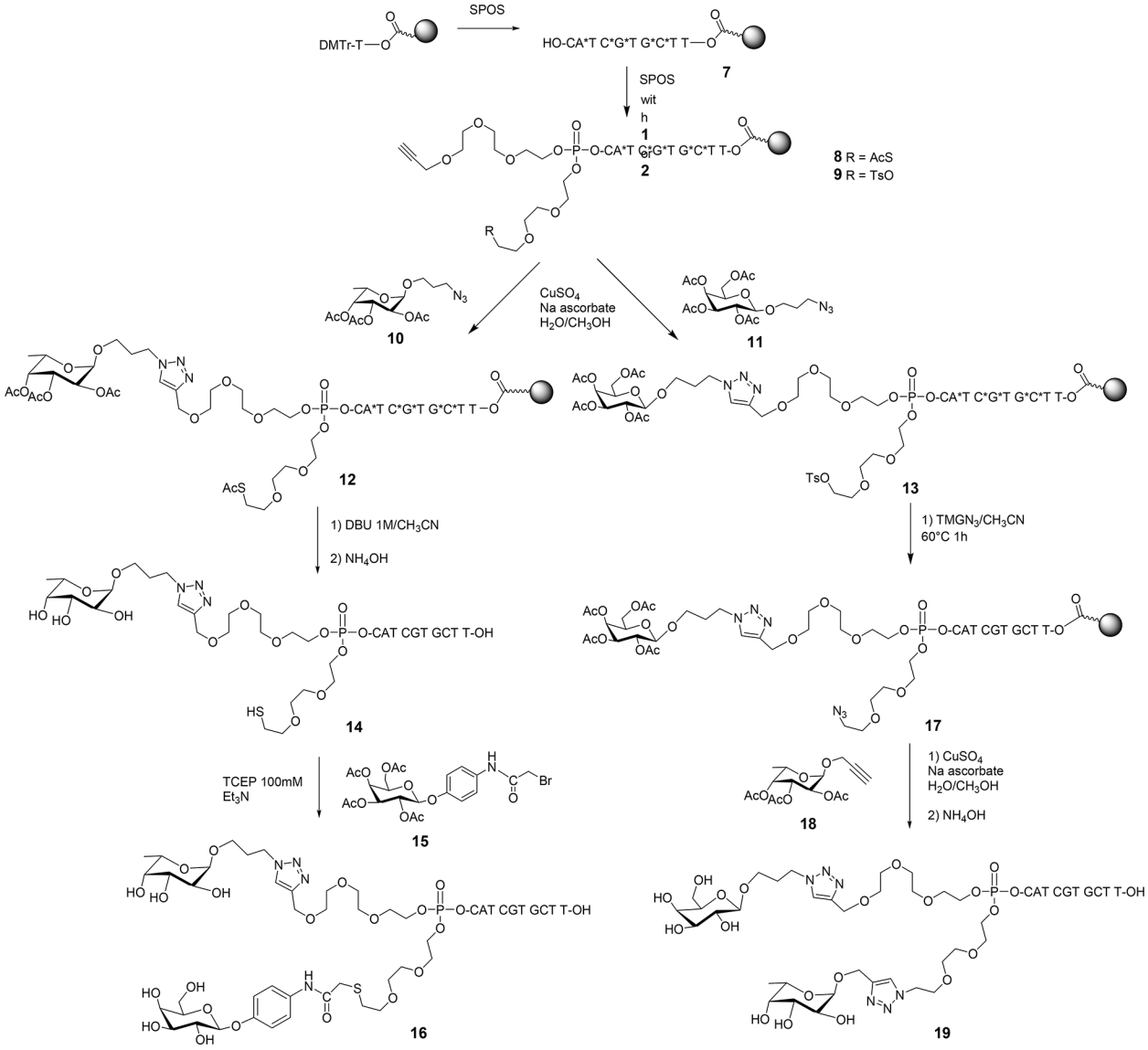
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201500165.

ate the desired heteroglycoclusters. In addition, the bifunctional building blocks described here can be introduced several times within a scaffold containing multiple hydroxy groups by using very efficient phosphoramidite chemistry. Accordingly, two new phosphoramidites **1** and **2** were synthesized and evaluated (Scheme 1). The first building block (**1**) contains one alkyne function and one *S*-acetyl function, for subsequent CuAAC reactions and thiol-bromoacetyl coupling (TBC) chemistry, respectively. The second building block (**2**) displays one alkyne moiety and one tosyl function; the latter is used as a proazide function group. Hence, after a first CuAAC reaction, the conversion of the tosyl into azide is performed followed by a second CuAAC reaction. Notably, the synthetic flexibilities embodied by **1** and **2** enable easy access to complex multivalent heteroglycoconjugates.

Results and Discussion

Phosphoramidites **1** and **2** were synthesized in two steps starting from propargyl-triethylene glycol **3** which was converted into its phosphorodiamidite **4** by reaction with bis(diisopropylamino)chlorophosphine in the presence of Et_3N (Scheme 1). Compound **4** was then activated by diisopropylammonium tetrazolide salt and reacted with either *S*-acetyl triethylene glycol **5** or tosyl-triethylene glycol **6** leading to expected phosphoramidites **1** and **2**, which were purified by silica gel chromatography and obtained in good yields (67% and 77% respectively).

The coupling efficiency of each phosphoramidite was evaluated on solid-supported oligonucleotide **7** synthesized by standard phosphoramidite chemistry (Scheme 2). After synthesis of the oligonucleotide sequence, phosphoramid-



Scheme 2. Synthesis of oligonucleotide heteroglycoclusters by using a combination of CuAAC and bromoacetamide thiolation reactions (**8–16**, left panel), or by using two sequential CuAAC reactions (**9–19**, right panel). SPOS: solid phase oligonucleotide synthesis, TCEP: tris(2-carboxyethyl)phosphine, DBU: 1,8-diazabicycloundec-7-ene. * Represents the protecting group on the nucleobases: benzoyl for A and C and isobutyryl for G.

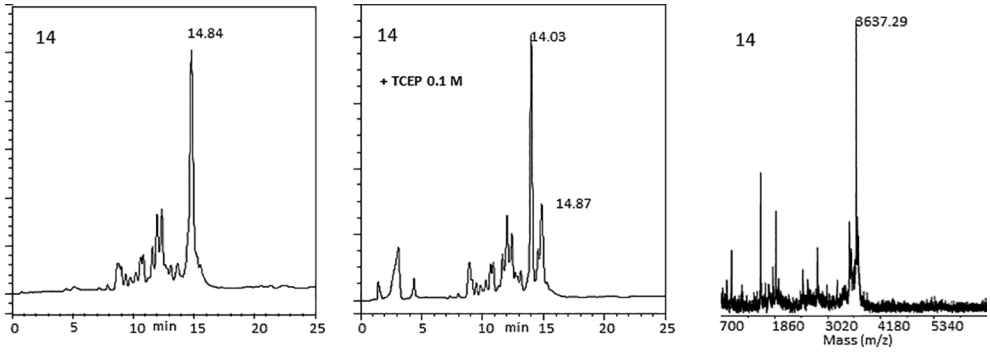


Figure 1. HPLC profiles of crude **14** [disulfide form (left)], **14** after TCEP addition, thiol form (middle) and MALDI-TOF MS with TCEP, $[M - H]^-$ calcd. 3638.62 (right).

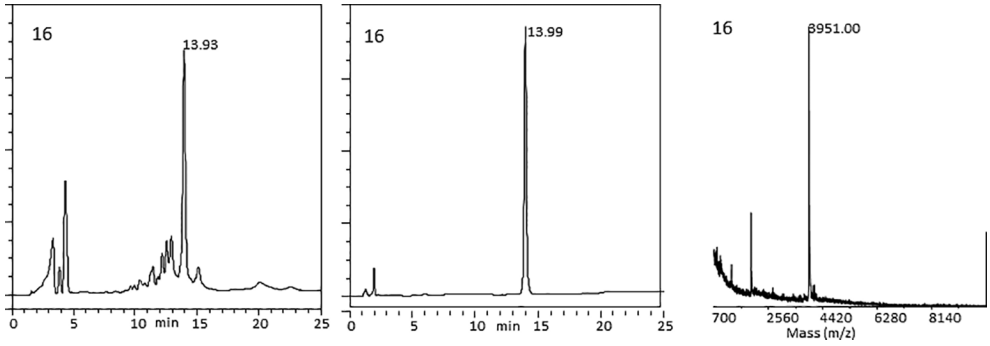


Figure 2. HPLC profiles of **16** crude (left), **16** pure (middle) and MALDI-TOF MS, $[M - H]^-$ calcd: 3949.92 (right).

ites **1** or **2** were introduced at the 5′-end of **7**, using a coupling time of 80 s to ensure completion of the coupling reaction followed by a regular oxidation step leading to solid-supported, 5′-modified oligonucleotides **8** and **9**, respectively. The first conjugation was performed, on solid support, by CuAAc using 3-azidopropyl 2,3,4-tri-*O*-acetyl-α,L-fucoside **10** for **8** and 3-azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactoside **11** for **9**, both in the presence of $CuSO_4$ and sodium ascorbate for 1 h at 60 °C with microwave assistance,[32] leading to monoconjugates **12** and **13**, respectively.

Fucosylated oligonucleotide **12**, bearing a thioacetyl function, was first treated on-support with a 1 M 1,8-diazabicycloundec-7-ene (DBU) solution in dry CH_3CN for 2 min to eliminate the phosphate-protecting cyanoethyl groups. The acrylonitrile generated during this reaction was removed by further washing of the solid support with dry CH_3CN. Indeed, acrylonitrile reacts readily with the free thiol function. Consequently, it was necessary to completely remove residual acrylonitrile prior to thiol liberation via deacetylation. Subjection of thioacetylated material to NH_4OH liberated the terminal thiol and effected oligonucleotide release from the solid support; product **14** was analyzed and characterized by HPLC and MALDI-TOF MS (Figure 1).

The formation of a disulfide bridge between two thiol-modified oligonucleotides **14** was demonstrated by treatment of a small amount of the crude product with tris(2-carboxyethyl)phosphine (TCEP) and subsequent HPLC analysis. The main peak at 14.84 min (representing dimerized **14**) underwent significant reduction in size which coincided with formation of a new peak at 14.03 min corresponding to the thiol form.

Finally, the galactose residue was introduced by thiolation of bromoacetamide **15** in the presence of Et_3N and TCEP. TCEP was required to reduce the disulfide bridge of dimerized **14**. Thiol condensation with **15** afforded hetero-glyco-oligonucleotide conjugate **16** bearing both D-galactose and L-fucose motifs, as confirmed by HPLC and MS. Notably, the acetyl groups of the galactose moiety were hydrolyzed during oligonucleotide conjugation (Figure 2).

In the orthogonal pathway involving building block **2** (Scheme 2, right panel), the solid-supported tetra-*O*-acetyl-galactosylated oligonucleotide **13**, bearing a tosyl function, was treated with tetramethylguanidinium azide ($TMGN_3$) in CH_3CN for 1 h at 60 °C to generate corresponding solid-supported azide oligonucleotide **17** (Scheme 2). The second CuAAC was then performed, still on the solid support, using propargyl 2,3,4-tri-*O*-acetyl-α,L-fucoside **18**, $CuSO_4$ and sodium ascorbate. Finally, treatment with NH_4OH led

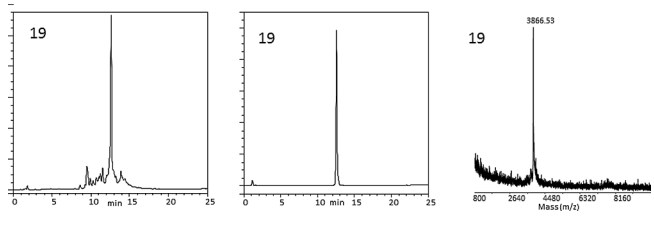
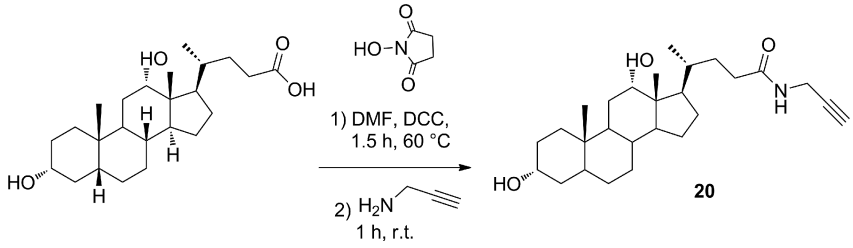


Figure 3. HPLC profiles of **19** crude (left), pure (middle) and MALDI-TOF MS, $[M - H]^-$ calcd: 3865.77 (right).

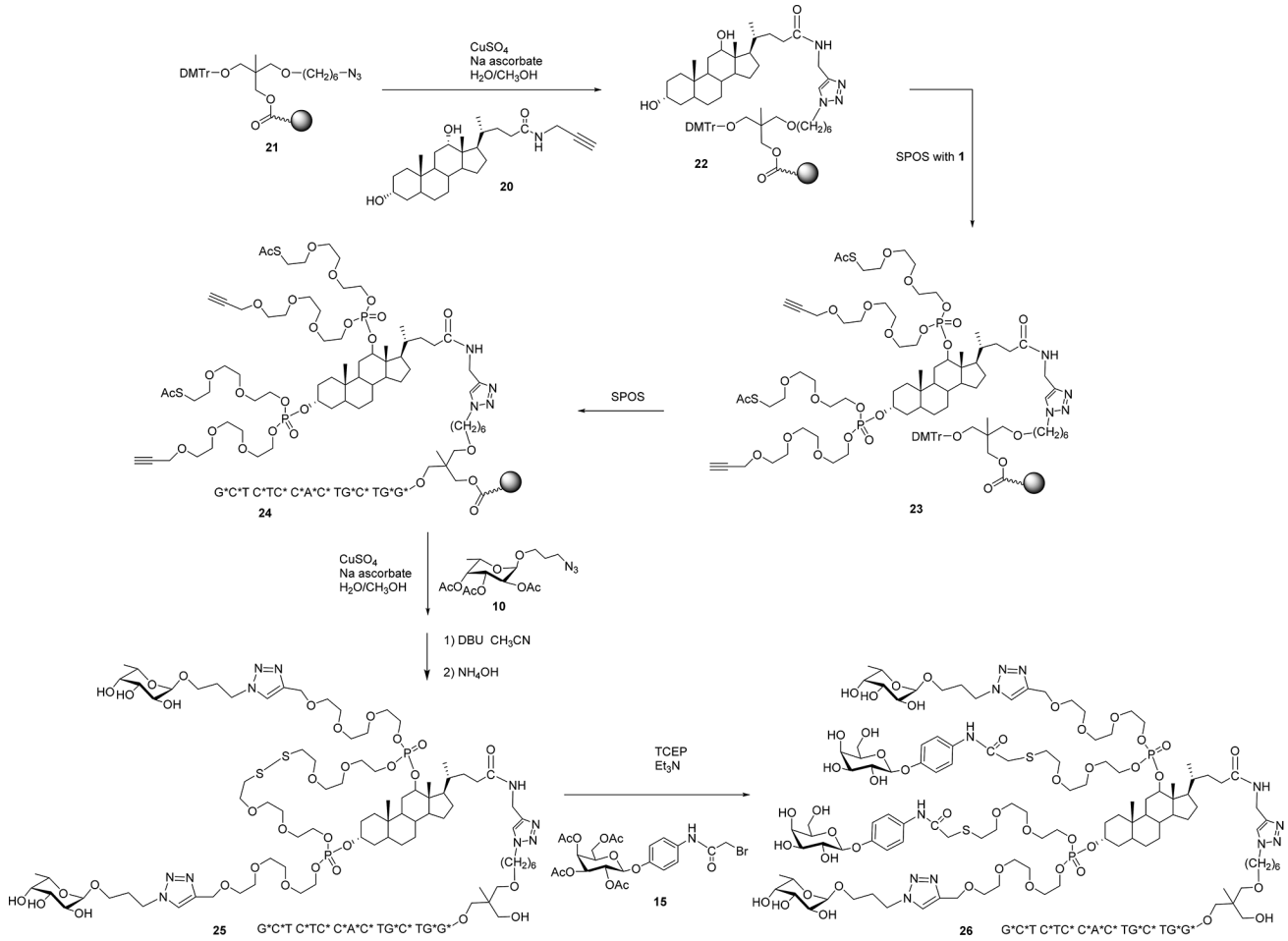
to heteroglyco-oligonucleotide conjugate **19** which was analyzed and purified by HPLC and ultimately characterized by MALDI-TOF MS (Figure 3).

We have demonstrated that both phosphoramidites **1** and **2** enable the efficient synthesis of bivalent heteroglyco-oligonucleotide conjugates bearing both D-galactose and L-fucose residues. Both reagents have certain advantages and disadvantages. An advantage of **1** over **2** is that it does not require an extra step to generate the second click functionality (tosyl→azide), as the thiol function is generated simultaneously with the deprotection of the oligonucleotide, making this approach synthetically shorter. The advantage of **2** over **1** is that the second CuAAC reaction can occur either on solid support or in solution, making the approach more versatile.

As noted before, phosphoramidites **1** or **2** could be introduced several times using a polyhydoxylated platform. Since the binding affinity of a carbohydrate to a lectin is generally low, multiple glycosylations of a scaffold usually increase the affinity of the final glycocluster in accord with the cluster glycoside effect.[11] Accordingly, we decided to introduce phosphoramidite **1** twice into a single deoxycholyl scaffold. Indeed, deoxycholic acid can be easily derivatized through the carboxylic acid moiety and the OH moieties can be used to anchor any tag using phosphoramidite chemistry. Since both hydroxyl groups are on the same face of the deoxy-



Scheme 3. Synthesis of *N*-(propargyl)deoxycholanamide **20**.



Scheme 4. Synthesis of deoxycholyl-centered tetravalent heteroglycocluster oligonucleotide conjugate exhibiting two D-galactoside and two L-fucoside motifs. SPOS: solid-phase oligonucleotide synthesis, TCEP: tris(2-carboxyethyl)phosphine, DBU: 1,8-diazabicycloundec-7-ene.

cholyl platform, carbohydrate motifs will likely be directed in the same direction. To this end, deoxycholic acid was activated as its *N*-hydroxysuccinimide ester and then reacted with propargylamine affording desired *N*-(propargyl)-deoxycholanamide[33] **20** (Scheme 3).

Alkyne **20** was then immobilized onto azide solid support **21** by CuAAC affording **22** (Scheme 4). Phosphoramidite **1** was introduced using a double coupling step with an extended time of 360 s to form bis-phosphorylated deoxycholic scaffold **23**, bearing the two bis-orthogonally functionalized tethers, as well as a 4,4-dimethoxytrityl (DMTr) protected hydroxy anchor. Following removal of the DMTr group, the full sequence oligonucleotide was synthesized by the standard protocol, affording **24**. Notably, the four click-functionalized conjugation tethers, already present on solid support **23**, were not affected by the 15 synthetic cycles needed for oligonucleotide construction. Next, azido-fucoside derivative **10** was introduced twice by CuAAC onto solid-supported **24**, as described above, and the modified oligonucleotide was deprotected (first with DBU and then with ammonia) affording bis-fucosylated oligonucleotide conjugate **25** in solution. Generation of **25** was confirmed by MALDI-TOF MS analysis (data not shown). Subsequently, bromoacetamide-containing galactoside **15** was introduced twice by TBC chemistry (as described above and in the presence of TCEP) affording oligonucleotide heteroglycocluster **26** with four pendant sugars, two fucose and two galactose motifs. Final conjugate **26** was purified by HPLC and characterized by MS (Figure 4).

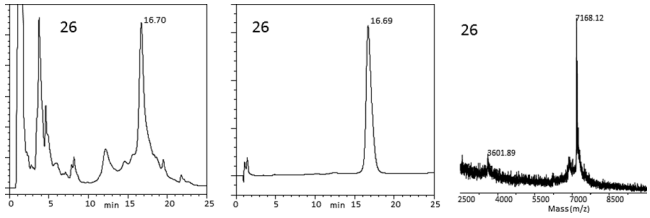


Figure 4. HPLC profiles of **26** crude (left), pure (middle) and MALDI-TOF MS [M – H]$^-$ calcd. 7169.73 (right).

Conclusions

In summary, we have synthesized two new phosphoramidite building blocks, each bearing two linkers with orthogonal functions allowing the synthesis of heteroglycooligonucleotide 5′-conjugates. These phosphoramidites can be introduced once or multiple times into a synthetic oligonucleotide. This was demonstrated with the synthesis of heteroglycocluster oligonucleotide conjugates exhibiting both D-galactose and L-fucose moieties in a monovalent or multivalent fashion. We believe that these bis-functional phosphoramidites **1** and **2** are not limited to the synthesis of heteroglycan-oligonucleotide conjugates. Phosphoramidites **1** and **2** display wide versatility for the synthesis of different monovalent or multivalent heteroconjugates of oligonucleotides carrying, for example, various motifs such as fluorescent dyes, peptides, carbohydrates or biotin, using the corresponding building blocks bearing suitable and complementary functions (i.e. azide, alkyne or bromoacetamide).

Experimental Section

***O*-(3,6,9-Trioxadodecan-11-yl)-*N*,*N*′-tetraisopropylphosphorodiamidite (4):** To a solution of 3,6,9-trioxadodecan-11-yn-1-ol[34] **3** (1.55 g, 8.25 mmol) and Et_3N (2.3 mL, 16.5 mmol) in dry Et_2O (45 mL) was added bis(diisopropylamino)chlorophosphine (2.64 g, 9.9 mmol) and the mixture stirred for 3 h at room temperature. Then, the solution was diluted with Et_2O and Et_3N (9:1, v/v, 40 mL), and the salts were removed by filtration and washed. The solution was evaporated to half and cyclohexane (20 mL) was added. Et_2O was removed by evaporation keeping cyclohexane in solution. The solution was applied to a silica gel column (50 g) and the compound was purified using cyclohexane containing 6% Et_3N (2.52, 73%). TLC: R_f = 0.70 in cyclohexane/AcOEt/Et_3N, 6:3:1, v/v/v. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.19 (dd, J = 4.0, 6.7 Hz, 12 H, isopropyl), 2.46 (t, J = 2.2 Hz, 1 H, HCC-), 3.49–3.78 (m, 16 H, -CH_2-O-P, -CH-, -O-CH_2-CH_2-O-), 4.24 (d, J = 2.4 Hz, 2 H, CC-CH_2O) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.9, 23.0, 23.6, 23.7, 24.6, 24.7, [C(CH_3)$_2$], 44.2, 44.3, 45.1, 45.2, 45.3 (NCMe$_2$), 58.4 (CC-CH_2O), 62.4, 62.5 (POCH_2), 69.1, 70.5, 70.6, 70.7 ($CH_2CH_2OCH_2CH_2$), 74.5 (CCH), 79.6 (CCH) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 127.1 ppm. HRMS (ESI/Q-TOF) *m*/*z* calcd. for $C_{21}H_{44}N_2O_4P$ [M + H]$^+$ 419.3039, found 419.3052.

***O*-(3,6-Dioxanon-8-ethylthioacetyl)-*O*′-(3,6,9-trioxadodecan-11-ynyl)-*N*,*N*-diisopropyl Phosphoroamidite (1):** Dry 3,6-dioxanon-8-ethylthioacetyl-1-ol[35] **5** (750 mg, 3.6 mmol) and dry diisopropylammonium tetrazolide (308 mg, 1.8 mmol) were dissolved in dry CH_2Cl_2 (12 mL) and *O*-(3,6,9-trioxadodecan-11-yl)-tetraisopropyl phosphorodiamidite **4** (1.26 g, 3 mmol) was added. After 3 h of stirring at room temperature, the solution was diluted with CH_2Cl_2 and washed with brine (2 × 100 mL). The organic layer was dried with Na_2SO_4 and the solvents evaporated. The crude oil was purified by chromatography (gradient 0 to 50% AcOEt in cyclohexane containing 6% Et_3N) to afford a clear oil (1.06 g, 67%). TLC: R_f = 0.45 in cyclohexane/AcOEt/Et_3N, 5:4:1; v/v/v. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.1 (d, J = 6.8 Hz, 12 H, isopropyl), 2.27 (s, 3 H, CH_3CO-S), 2.36 (t, J = 2.4 Hz, 1 H, -CCH), 3.02 (t, J = 6.4 Hz, 2 H, -CH_2S-), 3.48–3.8 (m, 24 H, -CH-, CH_2-OP, -CH_2-CH_2-, -O-CH_2-), 4.14 (d, J = 2.4 Hz, 2 H, HCC-CH_2-) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.5, 24.6 [C(CH_3)$_2$], 28.8 (CH_3CO), 30.5 (CH_2S), 42.7, 42.9 (NCMe$_2$), 58.4, (CC-CH_2O), 62.4, 62.6, (POCH_2), 69.1, 69.7, 70.3, 70.4, 70.5, 70.6, 71.2, 71.2, 71.3, 71.5, ($CH_2CH_2OCH_2CH_2$); 74.6, (CCH), 79.6 (CCH), 195.5 (C=O) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 147.6 ppm. HRMS (ESI/Q-TOF) *m*/*z* calcd. for $C_{23}H_{45}NO_8PS$ [M + H]$^+$ 526.2604 found 526.2610.

***O*-(3,6,9-Trioxadodecan-10-*p*-tosylate)-*O*′-(3,6,9-trioxadodecan-11-ynyl)-*N*,*N*-diisopropyl Phosphoroamidite (2):** Dry 3,6,9-trioxadodecan-10-*p*-tosylate-1-ol[35] **6** (1.1 g, 3.6 mmol) and dry diisopropylammonium tetrazolide (308 mg, 1.8 mmol) were dissolved in dry CH_2Cl_2 (12 mL) and 3,6,9-trioxadodecan-11-yl tetraisopropyl phosphorodiamidite **4** (1.26 g, 3 mmol) was added. After 3 h of stirring at room temperature, the solution was diluted with CH_2Cl_2, washed with brine (2 × 100 mL). The organic layer was dried with Na_2SO_4 and the solvents evaporated. The crude oil was purified by chromatography (gradient 0 to 50% AcOEt in cyclohexane containing 6% Et_3N) to afford a clear oil (1.44 g, 77%). TLC: R_f = 0.50 in cyclohexane/AcOEt/Et_3N, 5:4:1 v/v/v. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.13 (dd, J = 2.2, 6.8 Hz, 12 H, isopropyl), 2.41 (br.

s, 4 H, *H*CC, C*H*$_3$), 3.52–3.79 (m, 24 H, -CH-, CH$_2$-OP, -CH$_2$-CH$_2$-, -O-CH$_2$-), 4.11–4.17 (m, 4 H, Ts-O-C*H*$_2$-, HCC-C*H*$_2$-), 7.32 (d, J = 8.1 Hz, 2 H), 7.76 (d, J = 8.2 Hz, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6 (CH_3Ar), 24.6, 24.7 [C(*C*H$_3$)$_2$], 42.8, 42.9 (N*C*Me$_2$), 58.4 (CC-*C*H$_2$O), 62.4, 62.6 (POCH$_2$), 68.7, 69.1, 69.3, 70.4, 70.6, 70.7, 70.8, 71.2, 71.3, 71.4, (CH$_2$CH$_2$OCH$_2$CH$_2$, CH$_2$S) 74.6 (C*C*H), 79.7 (*C*CH), 128.0, 129.8, 133.1, 144.8 (Ar) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 147.5 ppm. HRMS (ESI/Q-TOF) *m*/*z* calcd. for $C_{28}H_{51}NO_{11}PS$ $[M + H_2O + H]^+$ 640.2921 found 640.2920.

Deoxycholic Solid Support 22: To 1-(6-azidohexyl)-2-[(4,4′-dimethoxytrityl)oxymethyl]-2-methyl-3-(succinic-LCAA CPG)propane-1,3-diol[36] **21** (70 mg, 3 μmol) were added *N*-(propargyl)deoxycholanamide **20**[33] (54 μL of 100 mM solution in MeOH, 5.4 μmol), a mixture of H_2O/MeOH (1.2 mL, 1:1, v/v), freshly prepared aqueous solutions of $CuSO_4$ (30 μL, 40 mM, 1.2 μmol) and sodium ascorbate (60 μL, 100 mM, 6 μmol). The vial containing the resulting mixture was sealed and gently stirred for 1 h at 60 °C. Then, the CPG beads were filtered off and washed with H_2O (5 mL), MeOH (5 mL), CH_3CN (5 mL) and dried.

General Procedure for Solid-Phase Oligonucleotide Synthesis (SPOS): The DNA sequences were synthesized starting from commercially available thymidine solid support yielding **7** or from the solid-supported deoxycholic scaffold with two thioacetylEG$_3$/propargylEG$_3$ phosphotriester moieties **23** yielding **24** at a 1 μmol scale on a DNA synthesizer (ABI 394) by standard phosphoramidite chemistry. For the coupling step, benzylmercaptotetrazole (BMT) was used as activator (0.3 M in anhydrous CH_3CN), commercially available nucleosides phosphoramidites (0.075 M in anhydrous CH_3CN, 15 equiv.) were introduced with a 20 s coupling time. The capping step was performed with acetic anhydride using commercial solution (Cap A: Ac_2O/pyridine/THF, 10:10:80 v/v/v and Cap B: 10% *N*-methylimidazole in THF) for 15 s. Oxidation was performed for 15 s using 0.1 M I_2, THF/pyridine/H_2O, 90:5:5. Detritylation was performed with 2.5% trichloroacetic acid (TCA) in CH_2Cl_2 for 35 s.

General Protocol for Phosphorylation with Phosphoramidites 1 and 2:

i) At 5′-end: Phosphoramidite **1** or **2** (0.15 M in dry CH_3CN, 30 equiv.) was coupled onto solid-supported oligonucleotide **7**, using a DNA synthesizer, with BMT as activator for 80 s, and then standard oxidative step was applied affording **8** or **9**.

ii) On Deoxycholic Scaffold 22: Phosphoramidite **1** (0.15 M in dry CH_3CN) was coupled twice (30 equiv./OH) using BMT as activator for 360 s (2 × 180 s), then standard oxidative step was applied and the capping step was extended to 180 s affording **23**.

Synthesis of Heteroglycocluster Oligonucleotide 16

CuAAC Coupling: To solid-supported alkyne oligonucleotide **8** (≈ 0.50 μmol) was added azido-functionalized galactoside[37] **10** (20 μL of 100 mM in MeOH, 2 μmol, 4 equiv.), freshly prepared $CuSO_4$ (6 μL of 40 mM in H_2O, 0.5 equiv.), sodium ascorbate (25 μL of 100 mM in H_2O, 5 equiv.), degassed H_2O (150 μL) and MeOH (150 μL). The tube containing the resulting mixture was sealed and placed in a microwave synthesizer at 60 °C for 60 min. The beads were filtered, washed with H_2O (1 mL), 0.1 M EDTA (1 mL), H_2O (1 mL), MeOH (1 mL), CH_3CN (1 mL) and dried.

Deprotection: Beads were treated with 1 M DBU in dry CH_3CN (2 mL) for 2 min and washed 5 times with dry CH_3CN (2 mL). Finally, concentrated ammonia was added and the sealed vial was heated at 55 °C for 2 h affording **14** after evaporation.

Thiol-Bromoacetyl Coupling (TBC): To a solution of crude **14** in H_2O (≈ 125 nmol, 200 μL) was added galactoside bromoacetamide derivative **15**[38] (10 μL of 100 mM in MeOH, 1.0 μmol, 8 equiv.), TCEP (25 μL of 100 mM in H_2O, 2.5 μmol, 20 equiv.), and Et_3N (200 μL, 1.5 μmol, 12 equiv.). The vial with the biphasic solution was sealed and heated for 1 h at 60 °C with stirring. Et_3N was then evaporated and the mixture was pre-purified by size exclusion chromatography (NAP 10) and purified by C_{18} HPLC affording **16** (49 nmol).

Synthesis of Heteroglycocluster Oligonucleotide 19

CuAAC Coupling: Similar protocol than above using **9** (≈ 0.50 μmol) and azidopropyl 2,3,4,6-tetra-*O*-acetyl galactoside[39] **11** (8 equiv.).

Azidation: Beads were treated with a solution of 0.3 M tetramethylguanidinium azide (TMG N3) in CH_3CN (300 μL) for 1 h at 60 °C. Beads were then washed 5 times with CH_3CN (2 mL) and dried affording **17**.

CuAAC Coupling: To compound **17** (≈ 0.5 μmol) was added propargyl 2,3,4-tri-*O*-acetyl α-L-fucoside[37] **18** (40 μL of 100 mM in MeOH, 4 μmol, 8 equiv.), freshly prepared $CuSO_4$ (12 μL of 40 mM in H_2O, 1 equiv.), sodium ascorbate (25 μL of 100 mM in H_2O, 5 equiv.), degassed H_2O 150 μL and MeOH (150 μL). The tube containing the resulting mixture was sealed and placed in a microwave synthesizer at 60 °C for 60 min. The beads were filtered, washed with water (1 mL), 0.1 M EDTA (1 mL), H_2O (1 mL), MeOH (1 mL), CH_3CN (1 mL) and dried.

Beads were heated with concentrated NH_4OH 55 °C for 2 h affording **19** after evaporation which was purified by C_{18} HPLC (220 nmol).

Synthesis of Heteroglycocluster Oligonucleotide 26: To 0.25 μmol of oligonucleotide on solid-supported deoxycholic **24** was added fucosyl azide derivative **10** (100 mM, in MeOH, 20 μL, 4 equiv./site), freshly prepared aqueous solutions of $CuSO_4$ (40 mM, 6 μL, 1 equiv.) and sodium ascorbate (100 mM, 12.5 μL), H_2O (50 μL) and MeOH (50 μL). The vial containing the resulting mixture was sealed and stirred for 1 h at 60 °C. Then, the CPG beads were filtered and washed with H_2O (5 mL), MeOH (5 mL), CH_3CN (5 mL) and dried. Then, CPG beads were treated with a solution of DBU 1 M (300 μL in 1.7 mL of CH_3CN) for 2 min, washed 5 times with 2 mL of CH_3CN. Finally, the beads were treated with concentrated NH_4OH (1 mL) for 5 h at 55 °C to give **25**.

To bis-fucosylated deoxycholic oligonucleotide **25** (30 nmol in 100 μL H_2O) was added galactoside bromoacetamide derivative **15** (100 mM in MeOH, 2.4 μL, 240 nmol), TCEP (100 mM, 6 μL, 600 nmol, 20 equiv.) and Et_3N (50 μL). The resulting preparation was sealed and stirred for 1 h at 60 °C. The mixture was desalted on NAP 10 size-exclusion cartridge and the solvents evaporated. The deacetylated glycocluster oligonucleotide was dissolved in H_2O and purified by reversed-phase HPLC to give 10 nmol **26** (33%).

Supporting Information (see footnote on the first page of this article): ^{1}H, ^{13}C and ^{31}P NMR spectra of all new compounds. Synthesis procedures for compounds **5**, **6** and **20**.

Acknowledgments

This work was financially supported by the Agence Nationale pour la Recherche (grant number ANR-12-BSV5-0020). F. M. is a member of Inserm.

[1] A. Varki, *Glycobiology* **1993**, *3*, 97–130.

[2] H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637–674.
[3] C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357–2364.
[4] A. Bernardi, J. Jimenez-Barbero, A. Casnati, C. De Castro, T. Darbre, F. Fieschi, J. Finne, H. Funken, K. E. Jaeger, M. Lahmann, T. K. Lindhorst, M. Marradi, P. Messner, A. Molinaro, P. V. Murphy, C. Nativi, S. Oscarson, S. Penades, F. Peri, R. J. Pieters, O. Renaudet, J. L. Reymond, B. Richichi, J. Rojo, F. Sansone, C. Schaffer, W. B. Turnbull, T. Velasco-Torrijos, S. Vidal, S. Vincent, T. Wennekes, H. Zuilhof, A. Imberty, *Chem. Soc. Rev.* **2013**, *42*, 4709–4727.
[5] Y. M. Chabre, R. Roy, *Chem. Soc. Rev.* **2013**, *42*, 4657–4708.
[6] J. L. Reymond, M. Bergmann, T. Darbre, *Chem. Soc. Rev.* **2013**, *42*, 4814–4822.
[7] N. Spinelli, E. Defrancq, F. Morvan, *Chem. Soc. Rev.* **2013**, *42*, 4557–4573.
[8] J. K. Nair, J. L. S. Willoughby, A. Chan, K. Charisse, M. R. Alam, Q. Wang, M. Hoekstra, P. Kandasamy, A. V. Kel'in, S. Milstein, N. Taneja, J. O'Shea, S. Shaikh, L. Zhang, R. J. van der Sluis, M. E. Jung, A. Akinc, R. Hutabarat, S. Kuchimanchi, K. Fitzgerald, T. Zimmermann, T. J. C. van Berkel, M. A. Maier, K. G. Rajeev, M. Manoharan, *J. Am. Chem. Soc.* **2014**, *136*, 16958–16961.
[9] T. P. Prakash, M. J. Graham, J. Yu, R. Carty, A. Low, A. Chappell, K. Schmidt, C. Zhao, M. Aghajan, H. F. Murray, S. Riney, S. L. Booten, S. F. Murray, H. Gaus, J. Crosby, W. F. Lima, S. Guo, B. P. Monia, E. E. Swayze, P. P. Seth, *Nucleic Acids Res.* **2014**, *42*, 8796–8807.
[10] Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321–327.
[11] J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555–578.
[12] M. Gomez-Garcia, J. M. Benito, D. Rodriguez-Lucena, J. X. Yu, K. Chmurski, C. O. Mellet, R. G. Gallego, A. Maestre, J. Defaye, J. M. G. Fernandez, *J. Am. Chem. Soc.* **2005**, *127*, 7970–7971.
[13] M. Gomez-Garcia, J. M. Benito, R. Gutierrez-Gallego, A. Maestre, C. O. Mellet, J. M. G. Fernandez, J. L. J. Blanco, *Org. Biomol. Chem.* **2010**, *8*, 1849–1860.
[14] M. Gomez-Garcia, J. M. Benito, A. P. Butera, C. O. Mellet, J. M. G. Fernandez, J. L. J. Blanco, *J. Org. Chem.* **2012**, *77*, 1273–1288.
[15] D. Ponader, P. Maffre, J. Aretz, D. Pussak, N. M. Ninnemann, S. Schmidt, P. H. Seeberger, C. Rademacher, G. U. Nienhaus, L. Hartmann, *J. Am. Chem. Soc.* **2014**, *136*, 2008–2016.
[16] J. L. J. Blanco, C. O. Mellet, J. M. G. Fernandez, *Chem. Soc. Rev.* **2013**, *42*, 4518–4531.
[17] J. Katajisto, P. Heinonen, H. Lonnberg, *J. Org. Chem.* **2004**, *69*, 7609–7615.
[18] Z. J. Witczak, *Phosphorus Sulfur Silicon Relat. Elem.* **2013**, *188*, 413–417.
[19] I. Deguise, D. Lagnoux, R. Roy, *New J. Chem.* **2007**, *31*, 1321–1331.
[20] G. Pourceau, A. Meyer, J. J. Vasseur, F. Morvan, *J. Org. Chem.* **2009**, *74*, 1218–1222.
[21] M. Ortega-Munoz, F. Perez-Balderas, J. Morales-Sanfrutos, F. Hernandez-Mateo, J. Isac-Garcia, F. Santoyo-Gonzalez, *Eur. J. Org. Chem.* **2009**, 2454–2473.
[22] A. Kiviniemi, P. Virta, M. S. Drenichev, S. N. Mikhailov, H. Lonnberg, *Bioconjugate Chem.* **2011**, *22*, 1249–1255.
[23] B. Gerland, A. Goudot, G. Pourceau, A. Meyer, S. Vidal, E. Souteyrand, J.-J. Vasseur, Y. Chevolot, F. Morvan, *J. Org. Chem.* **2012**, *77*, 7620–7626.
[24] V. Dulery, O. Renaudet, M. Wilczewski, A. Van der Heyden, P. Labbe, P. Dumy, *J. Comb. Chem.* **2008**, *10*, 368–371.
[25] M. Karskela, M. Helkearo, P. Virta, H. Lonnberg, *Bioconjugate Chem.* **2010**, *21*, 748–755.
[26] B. Thomas, M. Fiore, I. Bossu, P. Dumy, O. Renaudet, *Beilstein J. Org. Chem.* **2012**, *8*, 421–427.
[27] A. Dondoni, A. Marra, *Chem. Soc. Rev.* **2012**, *41*, 573–586.
[28] A. Meyer, J. J. Vasseur, F. Morvan, *Eur. J. Org. Chem.* **2013**, 465–473.
[29] M. Fiore, G. C. Daskhan, B. Thomas, O. Renaudet, *Beilstein J. Org. Chem.* **2014**, *10*, 1557–1563.
[30] A. Meyer, G. Pourceau, J. J. Vasseur, F. Morvan, *J. Org. Chem.* **2010**, *75*, 6689–6692.
[31] A. Meyer, N. Spinelli, P. Dumy, J. J. Vasseur, F. Morvan, E. Defrancq, *J. Org. Chem.* **2010**, *75*, 3927–3930.
[32] C. Bouillon, A. Meyer, S. Vidal, A. Jochum, Y. Chevolot, J. P. Cloarec, J. P. Praly, J. J. Vasseur, F. Morvan, *J. Org. Chem.* **2006**, *71*, 4700–4702.
[33] S. Ikonen, H. Macickova-Cahova, R. Pohl, M. Sanda, M. Hocek, *Org. Biomol. Chem.* **2010**, *8*, 1194–1201.
[34] G. L. Lu, S. Lam, K. Burgess, *Chem. Commun.* **2006**, 1652–1654.
[35] W. H. A. Kuijpers, C. A. A. Vanboeckel, *Tetrahedron* **1993**, *49*, 10931–10944.
[36] G. Pourceau, A. Meyer, Y. Chevolot, E. Souteyrand, J. J. Vasseur, F. Morvan, *Bioconjugate Chem.* **2010**, *21*, 1520–1529.
[37] B. Roy, B. Mukhopadhyay, *Tetrahedron Lett.* **2007**, *48*, 3783–3787.
[38] C. Ligeour, L. Dupin, A. Marra, G. Vergoten, A. Meyer, A. Dondoni, E. Souteyrand, J. J. Vasseur, Y. Chevolot, F. Morvan, *Eur. J. Org. Chem.* **2014**, 7621–7630.
[39] J. A. F. Joosten, V. Loimaranta, C. C. M. Appeldoorn, S. Haataja, F. A. El Maate, R. M. J. Liskamp, J. Finne, R. J. Pieters, *J. Med. Chem.* **2004**, *47*, 6499–6508.

Received: February 3, 2015
Published Online: March 17, 2015

