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Synthesis of Mannose and Galactose Oligonucleotide Conjugates by Bi-click chemistry

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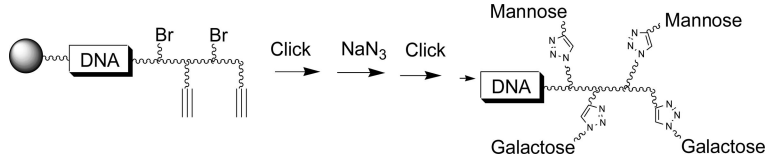

Glyco oligonucleotide conjugates, each exhibiting two mannose and two galactose residues, were efficiently synthesized by two successive 1,3-dipolar cycloadditions (click chemistry). Two phosphoramidite derivatives were used: one bearing a bromoalkyl group as a precursor to azide functionalization and another bearing a propargyl group. After a first cycloaddition with a mannosyl-azide derivative, the bromine atoms were substituted with NaN_3 and a second click reaction was performed with a 1′-*O*-propargyl galactose, affording the heteroglyco oligonucleotide conjugate.

Introduction

Carbohydrates are involved in many biological events and play crucial roles in various cellular recognition processes.[1,2] Understanding their interactions with proteins is a major issue for the development of new therapies. Glycoarrays have recently emerged as a high-throughput tool for the study of carbohydrate−lectin binding.[3] We recently designed a new glycoarray using DNA-directed immobilization[4,5] and to this end have developed very efficient strategies based on Cu(I) azide alkyne 1,3-dipolar cycloaddition,[6,7] or click chemistry, to synthesize oligonucleotide carbohydrate conjugates[5,8,9] and galactosylated[10] or fucosylated phosphodiester glycoclusters.[11] In these syntheses, the same carbohydrate (galactose, mannose, or fucose) was introduced one to ten times onto a scaffold exhibiting one to ten alkyne functions. However, because some bacteria exhibit several lectins recognizing different carbohydrate residues[12−14] and also a single lectin could recognize several carbohydrate residues,[15] it is necessary to be able to synthesize such glycomimics bearing different residues. Several strategies for coupling carbohydrates to oligonucleotides are reported in the literature,[16−21] and to our knowledge only one describes the attachment of different sugar moieties on the same scaffold.[18] Recently, a synthesis was reported for a heterobifunctional dendrimer exhibiting mannose and fucose residues.[22] Moreover, during this work,

(1) Varki, A. *Glycobiology* **1993**, *3*, 97–130.
(2) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720.
(3) Horlacher, T.; Seeberger, P. H. *Chem. Soc. Rev.* **2008**, *37*, 1414–1422.
(4) Wacker, R.; Niemeyer, C. M. *ChemBioChem* **2004**, *5*, 453–459.
(5) Chevolot, Y.; Bouillon, C.; Vidal, S.; Morvan, F.; Meyer, A.; Cloarec, J. P.; Jochum, A.; Praly, J. P.; Vasseur, J. J.; Souteyrand, E. *Angew. Chem., Int. Ed.* **2007**, *46*, 2398–2402.
(6) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
(7) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
(8) Bouillon, C.; Meyer, A.; Vidal, S.; Jochum, A.; Chevolot, Y.; Cloarec, J. P.; Praly, J. P.; Vasseur, J. J.; Morvan, F. *J. Org. Chem.* **2006**, *71*, 4700–4702.
(9) Meyer, A.; Bouillon, C.; Vidal, S.; Vasseur, J. J.; Morvan, F. *Tetrahedron Lett.* **2006**, *47*, 8867–8871.
(10) Pourceau, G.; Meyer, A.; Vasseur, J. J.; Morvan, F. *J. Org. Chem.* **2008**, *73*, 6014–6017.
(11) Morvan, F.; Meyer, A.; Jochum, A.; Sabin, C.; Chevolot, Y.; Imberty, A.; Praly, J. P.; Vasseur, J. J.; Souteyrand, E.; Vidal, S. *Bioconjugate Chem.* **2007**, *18*, 1637–1643.
(12) Mulvey, G.; Kitov, P. I.; Marcato, P.; Bundle, D. R.; Armstrong, G. D. *Biochimie* **2001**, *83*, 841–847.
(13) Bernardi, A.; Arosio, D.; Potenza, D.; Sanchez-Medina, I.; Mari, S.; Canada, F. J.; Jimenez-Barbero, J. *Chem. Eur. J.* **2004**, *10*, 4395–4406.
(14) Imberty, A.; Wimmerova, M.; Mitchell, E. P.; Gilboa-Garber, N. *Microbes Infect.* **2004**, *6*, 221–228.
(15) Sabin, C.; Mitchell, E. P.; Pokorna, M.; Gautier, C.; Utille, J. P.; Wimmerova, M.; Imberty, A. *FEBS Lett.* **2006**, *580*, 982–987.
(16) Matsuura, K.; Hibino, M.; Yamada, Y.; Kobayashi, K. *J. Am. Chem. Soc.* **2001**, *123*, 357–358.
(17) Dubber, M.; Frechet, J. M. J. *Bioconjugate Chem.* **2003**, *14*, 239–246.
(18) Katajisto, J.; Heinonen, P.; Lonnberg, H. *J. Org. Chem.* **2004**, *69*, 7609–7615.
(19) Katajisto, J.; Virta, P.; Lonnberg, H. *Bioconjugate Chem.* **2004**, *15*, 890–896.
(20) D'Onofrio, J.; de Champdore, M.; De Napoli, L.; Montesarchio, D.; Di Fabio, G. *Bioconjugate Chem.* **2005**, *16*, 1299–1309.
(21) Singh, Y.; Renaudet, O.; Defrancq, E.; Dumy, P. *Org. Lett.* **2005**, *7*, 1359–1362.
(22) Deguise, I.; Lagnoux, D.; Roy, R. *New J. Chem.* **2007**, *31*, 1321–1331.

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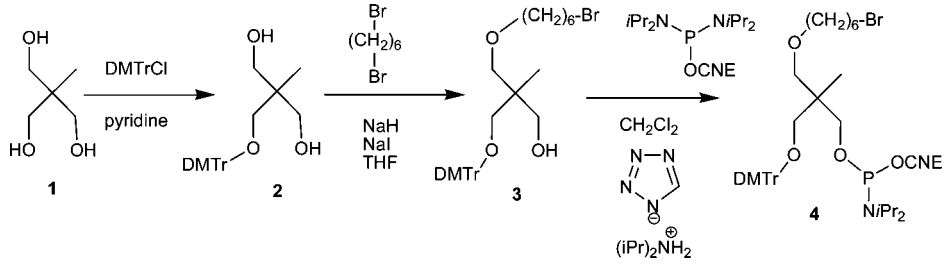
SCHEME 1. Synthesis of the Bromohexyl Phosphoramidite 4



Carell et al. developed a method[23] to functionalize DNA with one to three different labels. This strategy uses one to three successive click reactions and is based on the orthogonal protections of alkyne functions.

We present herein a new strategy for adding two different carbohydrate residues to a scaffold using successive azido- and alkyne-carbohydrate derivatives. This strategy is based on the use of a new derivative bearing a bromoalkyl group as the site for azide substitution, in combination with already-reported alkyne derivatives.[10,24] It is not possible for alkyne and azide functions to occur simultaneously on the same oligonucleotide since they would participate in an intramolecular reaction leading to cyclization.[24−27] Instead, alkyne and azide functions borne by the same oligonucleotide must be introduced or generated successively in order to react specifically with azide and alkyne derivates (respectively) by intermolecular cycloadditions. Thus, we first synthesized an oligonucleotide using an automated DNA synthesizer and introduced alkyne or bromohexyl functions at its 3′- or 5′-end using the corresponding phosphoramidite derivatives. Second, a cycloaddition was applied with an azido-carbohydrate derivative, introducing the first type of sugar on solid support. Third, the bromohexyl groups were converted into azidohexyl groups, which were finally conjugated with alkyne carbohydrate derivatives to introduce the second type of carbohydrate on solid support or in solution.

Results and Discussion

The first step in our procedure was to design a phosphoramidite bearing a bromoalkyl group and to test the efficiency of incorporating it into an oligonucleotide by phosphoramidite chemistry on a DNA synthesizer. The second step was to set up the conditions to convert the bromoalkyl group into an azidoalkyl group and finally perform a click reaction with an alkyne carbohydrate derivative.

The bromoalkyl phosphoramidite derivative was synthesized with a three-step protocol (Scheme 1) from 1,1,1-tris(hydroxymethyl)ethane **1**, which was monoprotected with a DMTr group (**2**, 56%),[24] monoalkylated with 1,6-dibromohexane and sodium hydride in the presence of sodium iodide in THF (**3**, 69%), and finally converted into the phosphoramidite derivative **4** (73%) using cyanoethyl tetraisopropylphosphorodiamidite activated with diisopropylammonium tetrazolide in dichloromethane.

To compare the efficiencies of click conjugation on solid support and in solution and to see if the presence of an oligonucleotide hampered the substitution of bromide by azide, the same trimannosylated dodecathymidine was synthesized by two different ways.

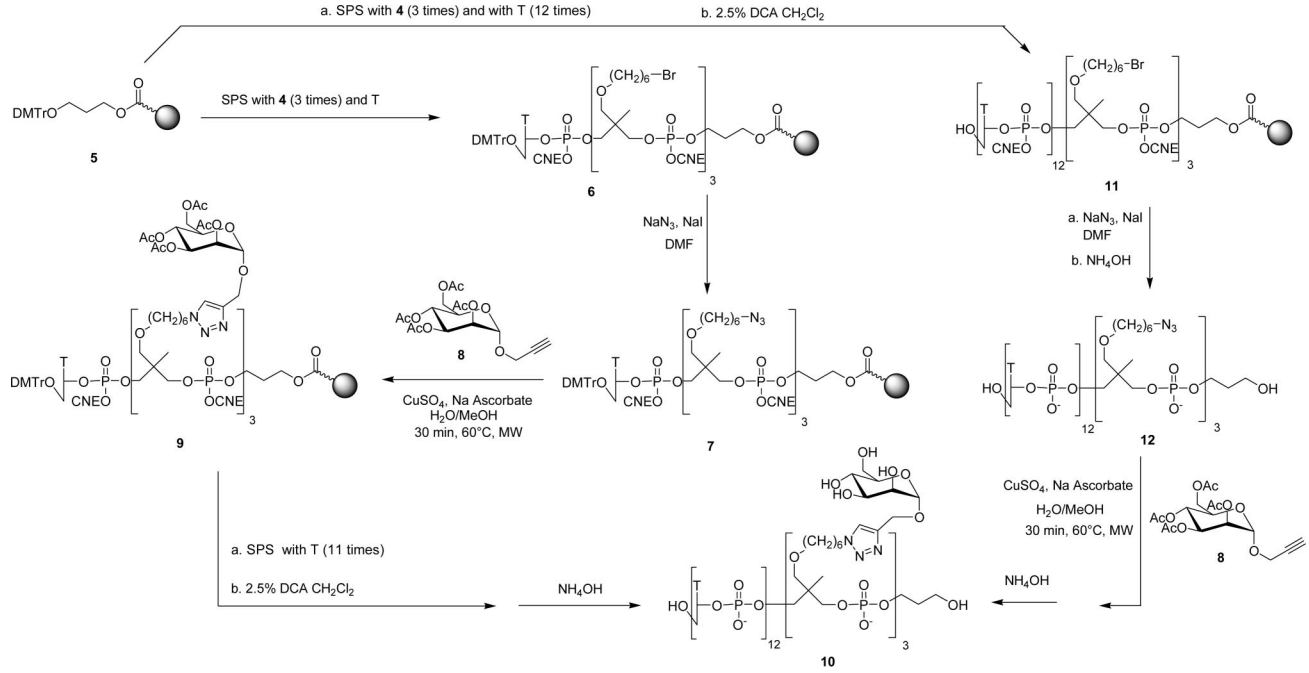
For the cycloaddition on solid support, phosphoramidite **4** was coupled three times to a universal 1,3-dipropanol solid support,[28] and then one standard thymidine phosphoramidite was added, affording **6** (Scheme 2). Treatment of **6** with a solution of sodium azide and sodium iodide in DMF at 65 °C for 1 h 30 min yielded the corresponding azido derivative **7**,[29] which was then conjugated with 1-*O*-propargyl-2,3,4,6-*O*-tetraacetyl mannose (**8**, 9 mol equiv) in a water/methanol solution of $CuSO_4$ (0.4 mol equiv) and sodium ascorbate (2 mol equiv) for 30 min at 60 °C[8] to give **9**.[29] Finally, 11 thymidines were introduced, and after treatment with ammonia, the 3′-trimannosyl-dodecathymidine **10** was obtained (recovery yield 8% from the solid support after purification).

For the click reaction in solution, all of the elongation steps were performed successively, affording **11**, which was converted into the azido derivative using the same treatment as above and deprotected by an additional ammonia treatment to give **12** (recovery yield 53% from solid support, without purification). Upon characterization of **12** by HPLC and MALDI-TOF MS, we observed a full conversion of bromoalkyl groups into azidoalkyl groups. Therefore, the presence of the 12-mer did not hamper the substitution of the bromine atom by an azido group. Intermediate **12** was conjugated with **8** (6 mol equiv) in a water/methanol solution of $CuSO_4$ (5 mol equiv) and sodium ascorbate (12.5 mol equiv) for 30 min at 60 °C to afford **10** after an additional ammonia treatment (Figure 1) (100% conversion rate determined by HPLC; recovery yield 16% from solid support after HPLC purification). Indeed, we observed (as have others[30]) that more equivalents of copper are needed to perform the click reaction in solution with unprotected oligonucleotides than on solid support with protected ones. We hypothesize that some of the copper interacts with phosphodiester linkages and hence is less available for the catalysis. However, it is interesting to note that we did not observe this phenomenon before, when a click reaction performed in solution on an oligonucleotide bearing both alkyne and azide functions afforded cyclic products.[24]

By both strategies, we obtained quite similar HPLC profiles for the final product (Figure 1), and MALDI-TOF MS gave the

(23) Gramlich, P. M. E.; Warncke, S.; Gierlich, J.; Carell, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 3442–3444.
(24) Lietard, J.; Meyer, A.; Vasseur, J. J.; Morvan, F. *J. Org. Chem.* **2008**, *73*, 191–200.
(25) Kumar, R.; El-Sagheer, A.; Tumpane, J.; Lincoln, P.; Wilhelmsson, L. M.; Brown, T. *J. Am. Chem. Soc.* **2007**, *129*, 6859–6864.
(26) Nakane, M.; Ichikawa, S.; Matsuda, A. *J. Org. Chem.* **2008**, *73*, 1842–1851.
(27) Grajkowski, A.; Cieslak, J.; Kauffman, J. S.; Duff, R. J.; Norris, S.; Freedberg, D. I.; Beaucage, S. L. *Bioconjugate Chem.* **2008**, *18*, 1696–1706.
(28) Surprisingly, we were not able to perform an efficient substitution of the bromine atom by the azide when compound **3** was directly loaded through a standard succinyl linkage to the LCAA-CPG using 500 or 1000 A CPG. It appeared that it is compulsory to have a longer spacer to obtain a complete substitution.
(29) A few CPG beads were withdrawn from the DNA column and deprotected by ammonia for HPLC and MALDI-TOF MS analyses showing a complete reaction.
(30) Alvira, M.; Eritja, R. *Chem. Biodiversity* **2007**, *4*, 2798–2809.

SCHEME 2. Synthesis of 3-Trimannosyl-dodecathymidine 10 According to Click Reactions Performed on Solid Support or in Solution[a]



[a] SPS = solid-phase synthesis: (1) 2.5% dichloroacetic acid CH_2Cl_2; (2) phosphoramidite derivative + benzylthiotetrazole; (3) Ac_2O, *N*-Me imidazole, 2,6-lutidine; (4) 0.1 M I_2 THF/H_2O/pyridine.

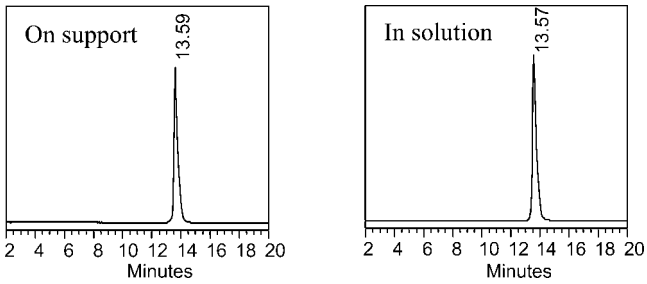


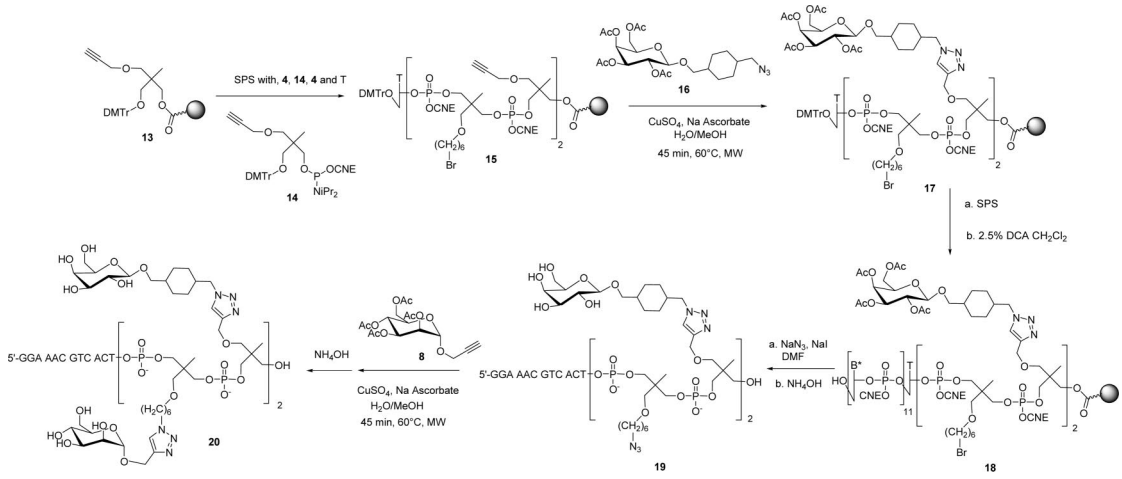
FIGURE 1. HPLC profiles of purified trimannosyl-T_{12} **10** obtained by click reactions on solid support (left) and in solution (right).

expected mass. However, the yield of the glyco oligonucleotide conjugate was 2-fold greater for the solution-phase synthesis. The main reason of this difference is that the solid-phase synthesis requires more manipulations, with two transfers of the supported material (**7** was transferred from the column to the microwave vial, and then **9** was retransferred to the DNA column). In conclusion, converting **4** into an azide derivative and using it to conjugate oligonucleotides by click chemistry is very efficient and could be applied to the synthesis of more complicated conjugates.

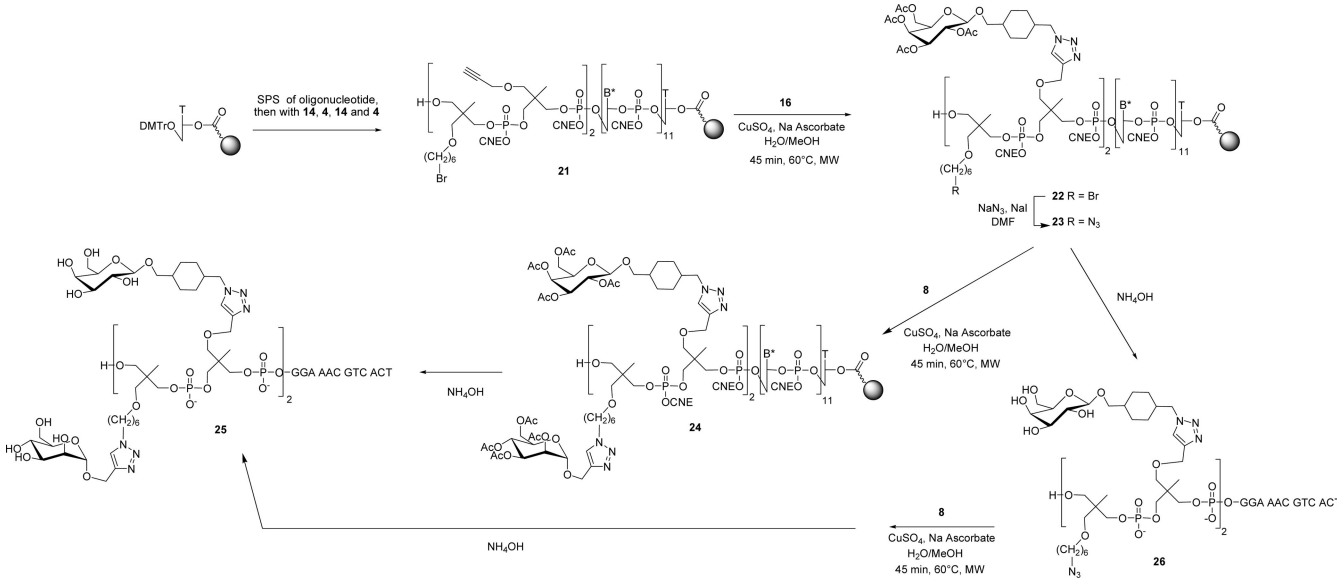
A second glyco oligonucleotide conjugate bearing bis-alternate mannose and galactose residues at the 3′-end of the sequence was synthesized using **4** and the phosphoramidite derivative **14**, which is an analog of **4** bearing a propargyl group instead of a bromohexyl group. Starting from the solid support **13**, which exhibits an alkyne function, **4**, **14**, and thymidine were successively introduced by phosphoramidite chemistry to afford **15** (Scheme 3). Then, a first click reaction was carried out on solid support under microwaves assistance (MW) for 45 min at 60 °C to introduce two copies of the galactose azide derivative **16**,[10] giving the digalactosylated species **17**. Surprisingly, with 10 mol equiv of **16**, we observed incomplete conjugation when only 0.4 mol equiv of $CuSO_4$ and 2 mol equiv of sodium ascorbate were used as previously, but total conjugation when these amounts were increased to 1 and 5 mol equiv, respectively. The oligonucleotide was elongated by introducing 11 nucleotides (5′-GGA AAC GTC AC) to yield **18**. Then, bromine atoms were displaced by azide groups by treatment with sodium azide and sodium iodide in DMF, and **19** was obtained in solution after a subsequent treatment with ammonia (recovery yield 7% from solid support after purification). A second cycloaddition was carried out in solution under MW for 45 min at 60 °C, with tetraacetyl mannose propargyl **8** (6 mol equiv), $CuSO_4$ (5 mol equiv), and sodium ascorbate (12.5 mol equiv). A final ammonia treatment removed the acetyl groups and afforded the heteroglycomimic 3′-bis(mannose-galactose) oligonucleotide conjugate **20** (100% conversion rate determined by HPLC; recovery yield 5% from solid support without purification).

Finally, we synthesized a last heteroglyco oligonucleotide conjugate in which the mannose and galactose residues were introduced at the 5′-end. For this purpose, the oligonucleotide was elongated four times by incorporating the phosphoramidites **14** and **4**, each twice and in alternation, to afford **21**, which bore two propargyl and two bromohexyl groups (Scheme 4). Compound **21** was conjugated with **16** (10 mol equiv), $CuSO_4$ (1.0 mol equiv), and sodium ascorbate (5 mol equiv), under MW assistance for 45 min at 60 °C, to afford **22** with two galactose residues, which was converted to the diazide derivative **23.** The sample was then split in two: one part was transferred into a MW vial and conjugated on solid support with tetraacetyl propargyl mannose **8** (6 mol equiv), $CuSO_4$ (0.4 mol equiv), and sodium ascorbate (2 mol equiv), under MW assistance for 45 min at 60 °C to give **24**. After removing the solution and washing the beads, **24** was treated with ammonia to afford the final heteroglyco oligonucleotide conjugate **25** (recovery yield 18% from solid support after purification).

SCHEME 3. Synthesis of the 3′-End Heteroglycomimic Oligonucleotide 20 Exhibiting Two Mannose and Two Galactose Residues



SCHEME 4. Synthesis of the 5′-End Heteroglycomimic Oligonucleotide 25 Exhibiting Two Mannose and Two Galactose Residues



The other part was deprotected with ammonia (recovery yield 28% from solid support after purification); conjugated in solution with **8** (6 mol equiv), $CuSO_4$ (5 mol equiv), and sodium ascorbate (12.5 mol equiv), under MW assistance for 45 min at 60 °C; and treated again with ammonia (to remove the acetyl groups of the mannosyl residues) to afford the expected heteroglyco oligonucleotide conjugate **25** (100% conversion rate determined by HPLC; recovery yield 26% from solid support without purification). Both methods provided **25** with the same efficiency and in similar amounts. Indeed, for the solid-phase-only synthesis, only one additional transfer was necessary and was made carefully to avoid loss of material.

Conclusion

Two phosphoramidites, one bearing a propargyl group and the other bearing a bromohexyl group, were successfully used for the synthesis of heterocarbohydrate oligonucleotide conjugates by MW-assisted click chemistry. The oligocarbohydrate could be introduced either at the 3′- or 5′-end of an oligonucleotide, and the bromine atom was easily substituted with an azide group.

We believed that this strategy could be applied for the synthesis of other heteroconjugates of oligonucleotides.

Experimental Section

1-*O*-(4,4′-Dimethoxytrityl)-2-(6-bromohexyloxymethyl)-2-methyl-1,3-propanediol 3. 2-[(4,4′-Dimethoxytrityl)oxymethyl]-2-methylpropane-1,3-diol **2**[24] (844 mg, 2 mmol), sodium iodide (30 mg, 0.2 mmol), and dibromohexane (1.54 mL, 10 mmol) were added to a solution of sodium hydride (60% in oil, 240 mg, 6 mmol) in anhydrous THF (30 mL) at 0 °C. After 19 h under magnetic stirring at room temperature, the reaction was quenched by adding 50 mL of CH_2Cl_2 and 2 mL of water. The organic layer was washed with brine (2 × 100 mL), dried over Na_2SO_4, and evaporated. The crude product was then purified by silica gel column chromatography (0 to 30% acetone in cyclohexane containing 1% triethylamine) to afford **3** (813 mg, 69%) as a colorless oil. $R_f = 0.58$ (cyclohexane/acetone 1:1 v/v). ^{1}H NMR (CD_3CN, 300 MHz): δ 0.89 (s, 3H), 1.34−1.99 (m, 15H), 2.67 (t, 1H, J= 5.7 Hz), 3.00−3.51 (m, 12H), 3.78 (s, 6H), 6.86−7.46 (m, 13H). ^{13}C NMR (CD_3CN, 300 MHz): δ 16.8, 24.7, 26.6, 27.3, 28.9, 32.0, 32.2, 33.8, 33.93, 54.5, 65.0, 66.1, 70.7, 73.8, 85.0,112.5, 126.3, 127.4, 127.7, 129.7, 136.0, 136.1, 145.2, 158.2. HRMS ESI$^+$ m/z calculated for $C_{32}H_{41}O_5Na_1Br_1$ $(M + Na)^+$ 607.2035, found 607.2083.

TABLE 1. Conditions Used for Each Click Reaction

click[a]	click reaction compound	scale (μmol)	$CuSO_4$ (mol equiv)	sodium ascorbate (mol equiv)	**8** (mol equiv)	**16** (mol equiv)	solvent volume[b] (μL)	time (min)
liquid	**12→10**	1	5	12.5	3	0	400	30
solid	**7→ 9**	1	0.4	2	3	0	200	30
solid	**15→17**	1	1	5	0	5	300	45
liquid	**19→20**	1	5	12.5	3	0	400	45
solid	**21→22**	1	1	5	0	5	300	45
solid	**23→24**	0.5	0.4	2	3	0	200	45
liquid	**26→25**	0.5	5	12.5	3	0	400	45

[a] The click reaction was performed on solid support (solid) or in solution (liquid). [b] Water/methanol 1:1, v/v, under microwave assistance (60 °C, 100 W) and magnetic stirring. The solutions of $CuSO_4$ and sodium ascorbate were prepared in water, and the carbohydrate derivatives **8** and **16** were dissolved in methanol to 100 mM concentration.

1-*O*-(4,4′-Dimethoxytrityl)-2-(6-bromohexyloxymethyl)-2-methyl-3-*O*-[(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite]-1,3-propanediol 4. 2-Cyanoethyl tetraisopropyl phophorodiamidite (457 μL, 1.4 mmol) was added to a solution of compound **3** (715 mg, 1.2 mmol) and diisopropylammonium tetrazolide (103 mg, 0.6 mmol) in anhydrous dichloromethane (15 mL). The resulting mixture was stirred at room temperature for 3 h and then diluted with CH_2Cl_2 (15 mL). The organic layer was washed with brine (2 × 50 mL), dried (Na_2SO_4), filtered, and evaporated. The residue was purified by silica gel chromatography (0 to 10% ethyl acetate in cyclohexane containing 10% of triethylamine), affording the phosphoramidite **4** (684 mg, 73%) as a colorless oil. $R_f = 0.54$ (cyclohexane/AcOEt/Et_3N 7:2:1 v/v/v). ^{1}H NMR (CD_3CN, 300 MHz): δ 0.96−0.99 (2s, 3H), 1.11−1.18 (m, 12H), 1.3−1.99 (m, 11H), 2.60 (m, 2H), 2.90−3.78 (m, 12H), 6.86−7.48 (m, 13H). ^{13}C NMR (CD_3CN, 300 MHz): δ 16.9, 19.7, 19.8, 23.5, 23.6, 23.65, 23.7, 24.8, 27.3, 28.9, 32.2, 33.9, 40.7, 40.8, 42.4, 42.5, 54.5, 57.7, 58.0, 64.6, 66.0, 66.2, 70.6, 72.6, 72.7, 85.0, 112.6, 118.13, 126.3, 127.4, 127.7, 129.7, 136.0, 145.3, 158.2. ^{31}P NMR (CD_3CN, 300 MHz): δ 147.04, 147.15. HRMS ESI$^+$ *m*/*z* calcd for $C_{41}H_{59}N_2O_6P_1Br_1$ $(M + H)^+$ 785.3294, found 785.3322.

Compounds **8**[31] and **16**[10] were synthesized according to the literature.

Synthesis of Oligonucleotides. The oligonucleotides were synthesized at a 1-μmol scale on a DNA synthesizer (ABI 394), using standard phosphoramidite chemistry, on commercially available thymidine solid support or on the supports **5** and **13**[24] using alkyne phosphoramidite **14**,[10] bromohexyl phosphoramidite **4**, and commercially available nucleoside phosphoramidites (0.075 M in anhydrous CH_3CN). Benzylmercaptotetrazole was used as the activator (0.3 M in anhydrous CH_3CN). The capping step was performed with acetic anhydride, using a 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 with a commercial solution of iodide (0.1 M I_2, THF, pyridine/water 90:5:5, v/v/v) for 13 s. Detritylation was performed with 2.5% DCA in CH_2Cl_2 for 35 s.

(31) Hasegawa, T.; Numata, M.; Okumura, S.; Kimura, T.; Sakurai, K.; Shinkai, S. *Org. Biomol. Chem.* **2007**, *5*, 2404–2412.

General Procedure for Azidation. The solid-supported bromohexyl derivative (1 μmol) was treated with a solution of NaN_3 (13 mg, 200 μmol) and NaI (30 mg, 200 μmol) in DMF (2 mL) for 1 h 30 min at 65 °C. The CPG beads were washed with DMF (2 × 1 mL) and CH_2Cl_2 (3 × 1 mL) and dried under vacuum.

General Procedure for Cu(I)-Catalyzed 1,3-Dipolar Cycloaddition. A carbohydrate derivative (**8** or **16**) dissolved in methanol and a freshly prepared solution of $CuSO_4$ and sodium ascorbate in water were added to solid-supported scaffolds or oligonucleotide derivatives in solution. The vial containing the resulting mixture was sealed and placed in a microwave synthesizer, with a 30 s premixing time, for 30−45 min at 60 °C and 100 W. The temperature was monitored with an internal infrared probe. For the click performed on solid support, the solution was removed and the CPG beads were washed with H_2O (2 mL) and MeOH (2 mL) and then dried. For the click performed in solution, the solution was desalted on NAP10 and evaporated. The residue was treated with concentrated aqueous ammonia at room temperature for 1 h to remove acetyl groups.

General Procedure for Deprotection of Solid-Supported Oligonucleotides. CPG beads were treated with concentrated aqueous ammonia (3 mL) for 1 h 30 min at room temperature to release the oligonucleotide from the solid support. The supernatant was collected, warmed to 55 °C for 6 h, and then evaporated. The residue was dissolved in water for subsequent analyses.

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Supporting Information Available: HPLC profiles and MALDI-TOF MS spectra of synthesized oligonucleotide conjugates **10**, **20**, and **25**. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR of compounds **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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