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Synthesis of Mono- and Dimannoside Phosphoramidite Derivatives for Solid-Phase Conjugation to Oligonucleotides.

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Abstract: Mannose and dimannose derivatives have been prepared with an anomerically-linked hydrophobic spacer, and converted to phosphoramidite derivatives suitable for conjugation to oligoribodeoxynucleotides on a DNA/RNA synthesizer. Synthesis of a monomannoside-linked 15-mer oligoribodeoxynucleotide is reported.

Targeting oligodeoxyribonucleotides to complementary base sequences in mRNA (antisense and ribozyme strategies), or to duplex DNA (antigene strategy) offers a potentially powerful avenue for selective therapeutic actions.[1] The use of antisense and ribozyme oligonucleotides as potential therapeutic agents in anti-HIV therapy is currently undergoing pre-clinical and clinical evaluation.[2] The successful development of antisense technologies as therapeutic agents will require the design of delivery strategies that can improve the cellular targeting and uptake of these agents to diseased cells.[3] Since macrophages represent an important target for HIV infection, we have been interested in developing macrophage-specific targeting strategies. Macrophages express high levels of cell-surface mannose-binding proteins (lectins) which facilitate the rapid and efficient uptake of mannosylated compounds such as glycoproteins and several therapeutic agents.[4] For enhanced receptor-mediated delivery of drug molecules to macrophages, mannose residues have usually been coupled to therapeutic agents, including oligonucleotides, *via* bulky, high molecular weight, protein carriers, such as bovine serum albumin.[5] In an attempt to obviate the need for large protein carriers and to automate the conjugation chemistry, we report for the first time synthesis of mannoside phosphoramidite derivatives that can be used to couple mannose residues directly onto oligonucleotides using automated DNA synthesis.

Although it is likely that multiple mannose residues will be required for optimal binding by the mannose lectin,[6] as a first step, we targeted preparation of mono- and dimannose derivatives and their coupling to oligoribodeoxynucleotides. This would establish the necessary synthetic protocols which should be generally applicable to subsequent targets (designed structures with a defined array of mannose residues). Pre-requisites for synthetic simplicity included identification of saccharide protecting groups labile to conventional post-synthesis oligonucleotide deprotection procedures. Thus, the synthesis aimed to utilize only ester protections. Our first objective was to prepare monomannosides bearing a suitable hydrophobic spacer group with a terminal hydroxyl that could be converted to a phosphoramidite. The latter would then be suitable for automated synthesis of 5'-end mannose-linked oligoribodeoxynucleotides, as shown schematically in Figure 1.

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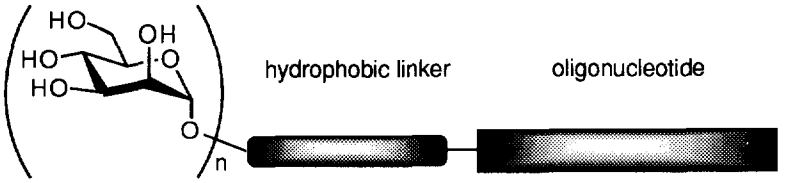
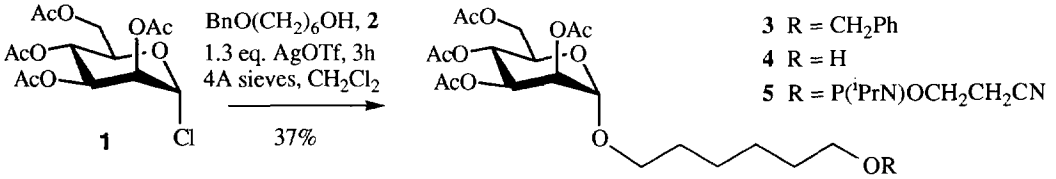
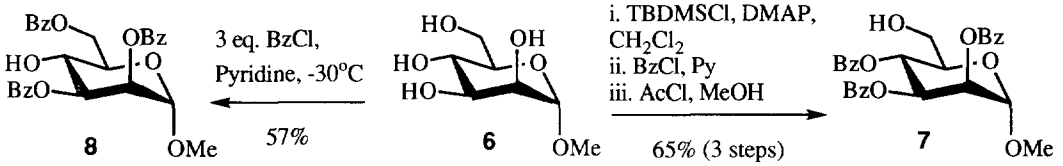
Figure 1: Schematic of mannose-linker-oligonucleotide conjugate.



The monomannoside was prepared by glycosylation of α-chlorotetraacetylmannose, **1**, with 6-benzyloxyhexan-1-ol, **2**,[7a] affording the α-glycoside, **3**,[7b] in 37% yield. During glycosylation, some accompanying 2-deacylation was observed, which was recycled to the tetraacetylglycoside with acetic anhydride/pyridine.[8] Debenzylation afforded the purified alcohol, **4**, in 97% yield which was then converted with (2-cyanoethyl-N,N-diisopropyl)phosphoramidite to the phosphoramidite derivative, **5**, in high yield.

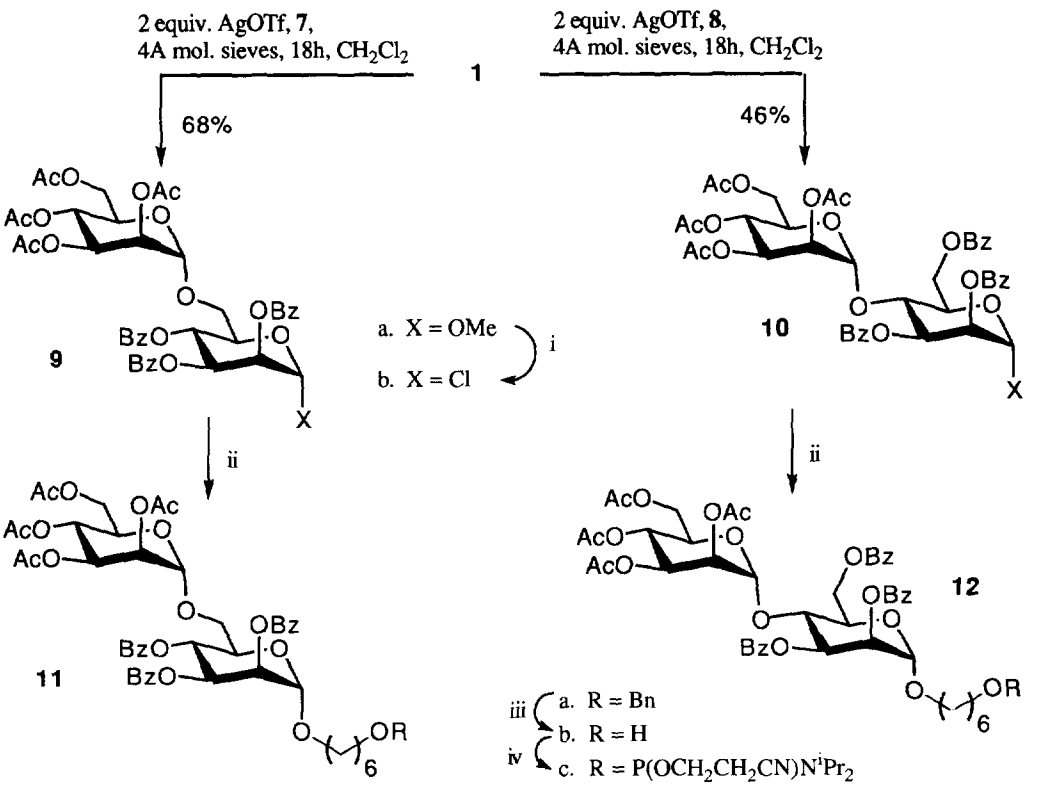


In the disaccharide series, synthesis of the isomeric α1→6 and α1→4 dimannosides from **1** was targeted, requiring the methylmannoside derivatives, **7** and **8**. Methyl-tri-*O*-benzoyl-2,3,4-α-D-mannopyranoside, **7**, was prepared in 3 steps from methyl-α-D-mannopyranoside, **6**, in good yield *via* temporary 6-OH silylation, benzoylation, and silyl ether deprotection with methanolic HCl generated *in situ*. Methyl-tri-*O*-benzoyl-2,3,6-α-D-mannopyranoside, **8**, was prepared directly by kinetic tribenzoylation of methyl-α-D-mannopyranoside, **6**.[9]



Both α1→6 and α1→4 linked dimannosides were prepared from 1-α-chloro-tetraacetylmannose, **1**, by AgOTf-promoted glycosylation with the monosaccharides **7** and **8** respectively, the α1→6 isomer, **9a**, obtained in 68% yield and the α1→4 isomer, **10a**, in 46% yield. Conversion of the disaccharides' anomeric methoxy groups to chloro was effected in reasonable yields using dichloromethylmethyl ether and zinc chloride. Glycosylation with linker, **2**, afforded, after isolation and subsequent catalytic hydrogenolysis, the linker-bearing disaccharides, **11b** and **12b**. Both disaccharides were then converted to their phosphoramidites, **11c** and **12c**, using (2-cyanoethyl-N,N-diisopropyl)phosphoramidite, in 77% and 83% yields, respectively.[10]

The monomannoside phosphoramidite, **5**, was coupled to the 5'-end of a mono-T nucleoside (whilst attached to a controlled-pore glass column (Cruachem)) on an automated DNA synthesiser (Model 392-02; Applied Biosystems) to evaluate conditions for this coupling. Conjugation of **5**, to a 15-mer (5'-CCA GGG GCA AGC CAT-3') oligonucleotide was then carried out to give, after deprotection, **13**.



i. Cl_2CHOMe-$CHCl_3$, 60 mol% $ZnCl_2$ ii. 1.3 equiv. AgOTf, 4A mol. sieves, 18h, CH_2Cl_2
iii. H_2 (1 atm), Pd-C, EtOH, 93-98% iv. $^iPr_2EtN$, CH_2Cl_2, $(^iPr_2N)_2POCH_2CH_2CN$, 77-83%

Evidence for this conjugation was provided by gel mobility assays (Figure 2). The electrophoretic gel mobility of a monomannoside-linked 15-mer sequence was significantly retarded (Figure 2, Lane C) compared to the unconjugated 15-mer oligonucleotide of the identical sequence (Lane D). In both native and denaturing[11] (7M Urea) polyacrylamide gels, the conjugate (Lane C) migrated at the level of a 16-mer oligonucleotide (Lane B), which contained the same sequence as the 15-mer except for an additional guanine at the 5'-end, suggesting that covalent coupling consistent with structure **13** had been achieved. Preliminary studies in murine macrophage cell lines (RAW264.7 and J774) have shown 2-4 fold increases in uptake for the conjugated systems when compared to the unconjugated oligonucleotides. In an attempt to further improve binding to mannose lectins, synthesis and evaluation of multivalent carriers in automated synthesis is currently in progress.

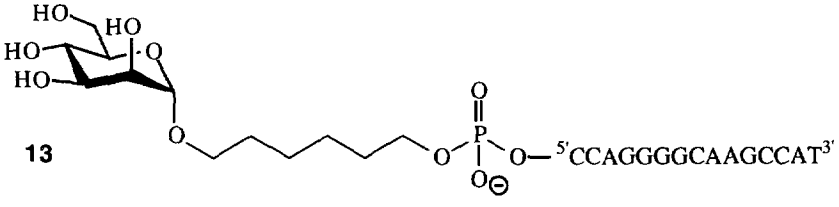
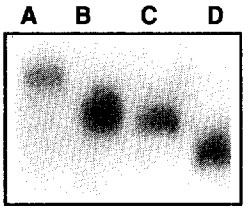


Figure 2. Characterization of mannose-conjugated 15-mer oligonucleotide, **13**, by electrophoretic gel mobility assay. Electrophoretic mobilities of control oligonucleotides and the conjugate are compared on a 20% polyacrylamide/7M urea gel. Lane A is a 20-mer oligonucleotide (5'-ATG CCC CTC AAC GTT AGC-3'), Lane B is a 16-mer (5'-G CCA GGA GCA AGC CAT-3'), Lane C is the mannose-C_6-linked-oligonucleotide (15-mer) conjugate, Lane D is the unconjugated 15-mer (5'-CCA GGA GCA AGC CAT-3').



In summary, efficient routes to a protected monomannoside, and two isomeric dimannosides, bearing a C_6-hydrophobic glycosyl-linker have been developed, and these have been converted to their phosphoramidites, suitable for 5'-conjugation to solid-supported oligodeoxyribonucleotides. The choice of saccharide protecting groups ensures full carbohydrate deprotection occurs concomitantly with standard oligonucleotide deprotection.

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