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Formation of anomeric phosphodiester linkages using H-phosphonate acceptors

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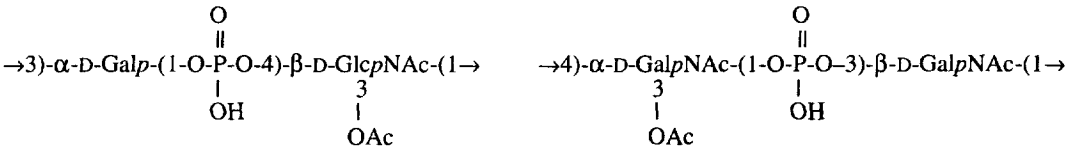
Abstract: A new way of constructing anomeric glycosyl phosphodiester linkages has been explored. Non-anomeric monosaccharide hydrogen phosphonate monoesters (**5** and **9**) have been used as acceptors in couplings using various benzylated galactosyl donors (trichloroacetimidate, thioglycoside, bromo and chloro sugars, **1–4, 7** and **8**) and promoters to give, after oxidation, good yields of phosphodiester linked "disaccharides" (**6** and **10**), products usually obtained by using an anomeric hydrogen monophosphonate, a hydroxyl acceptor and pivaloyl chloride as coupling reagent.

Keywords: Phosphorous acid and derivatives, Carbohydrates, Coupling reactions.

Glycosyl anomeric phosphodiester linkages are frequently encountered in bacterial polysaccharides. Synthetically they are in general constructed using the H-phosphonate or the amidite approach developed for oligonucleotide synthesis. To ensure the correct anomeric configuration in the product after coupling, an anomerically pure phosphonate monoester or amidite is first constructed, after which the diester formation is accomplished using standard techniques, a coupling reagent and a nucleophilic hydroxyl acceptor.[1] The yields, as compared to oligonucleotide synthesis, are usually lower due to the instability of the anomeric phosphates, which give, e.g., hydrolysis during the oxidation step.

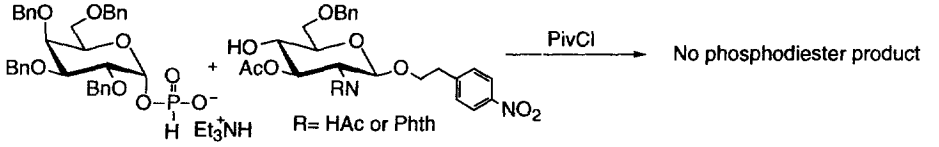
In a programme directed towards synthesis of *Haemophilus influenzae* capsular polysaccharide structures, the construction of the anomeric phosphodiester linkages present in serotype c[2] and f[3] (Fig 1) were desired.



Type c Type f

Figure 1 The repeating unit of *Haemophilus influenzae* type c and f capsular polysaccharide.

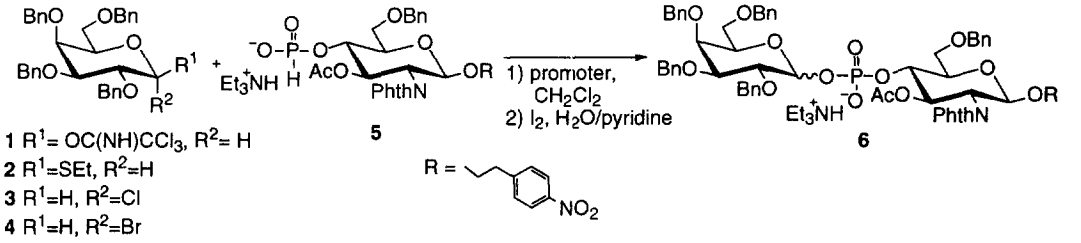
Attempts to construct the type c linkage using an anomeric galactose H-phosphonate monoester activated by pivaloyl chloride and a 4-OH glucosamine acceptor gave, however, no diester formation irrespective of whether phthalimido or acetyl was used as amino protecting group (Scheme 1).



Scheme 1

PII: S0040-4039(99)00362-7

Further experiments showed that it was possible to form an ethyl phosphonate diester with the acceptor, and also that the H-phosphonate monoester of the acceptor (**5**, Scheme 2) could be prepared.[4] This allowed, surprisingly, the diester to be formed by a "reversed" coupling between this H-phosphonate and a galactose hemiacetal derivative,[5] but also another route, in which the phosphonate is used as a nucleophile, which reacts with an electrophilically activated anomeric center of a glycosyl donor similar to a "normal" glycosidation (Scheme 2).



1 R^1= OC(NH)CCl_3, R^2= H
2 R^1=SEt, R^2=H
3 R^1=H, R^2=Cl
4 R^1=H, R^2=Br

Scheme 2

This latter approach had earlier been explored using alkyl phosphates as nucleophiles (acceptors),[6] but to our knowledge not with saccharide H-phosphonates, to construct anomeric phosphodiester linkages. To investigate the potential of this approach, several known galactosyl donors, **1**,[7] **2**,[8] **3**,[9] and **4**,[8] were prepared and reacted with the glucosamine 4-H-phosphonate **5** using various promoters (Scheme 2).[10] The results are summarized in Table 1.

Table 1

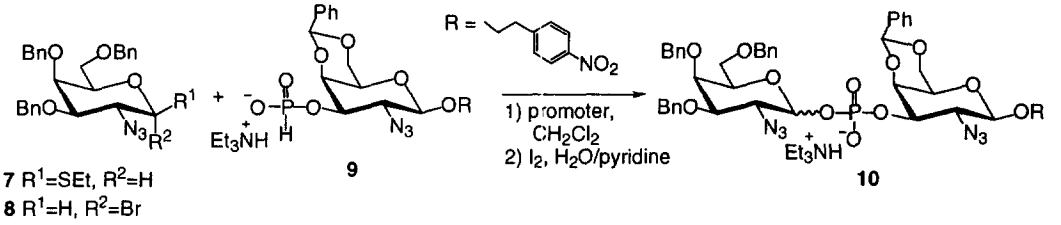
Donor/equiv	Acceptor/equiv	Promoter/equiv	Temperature[a]	α/β-ratio[b]	Yield of **6**(%)
1/1.4	**5**/1	TMSOTf/0.5	0 °C–rt	1:2	76
2/2	**5**/1	DMTST/1.8	rt	5:1	68
3/1.3	**5**/1	AgOTf/1.5	0°C	9:1	55
4/1.5	**5**/1	Et_4NBr/1.5	0 °C–rt	2:1	60

[a]Temperature in the coupling step.
[b]Determined from the relative intensities of H-1'α and H-1'β in the ^{1}H-NMR spectrum.

All donors gave good yields of products, obtained as α/β-mixtures. The chloro sugar promoted by silver triflate (AgOTf)[11] gave the best α-selectivity, but the best yield of α-anomer (57%) was obtained with a thioglycoside donor promoted by dimethyl(methylthio)sulfonium triflate (DMTST).[12] Interestingly, the two methods, which are found to be the most α-selective in ordinary glycosylation reactions, halide-assisted glycosylation[13] and β-imidates promoted by TMSOTf[14] (Entry 4 and 1, respectively), here gave low selectivity and the imidate even an excess of the β-anomer. Attempts to use diethyl ether as the solvent to improve the α-selectivity[15] had to be abandoned due to solubility problems.

In all these reactions the formation of the H-phosphonate diester appeared quantitative on TLC, however, some material is lost during the oxidation step (I_2 in H_2O/pyridine). At first, the oxidations were performed at 0

°C and yields of about 40% was then obtained. Lowering the temperature during oxidation to -40 °C increased the yields to the ones given in Table 1 (55-76%), however, there should be room for additional improvements, perhaps using other oxidants.



7 R^1=SEt, R^2=H
8 R^1=H, R^2=Br

9

10

Scheme 3

This new approach was then tested for the construction of the type f structure using donors **7**[16] and **8** and the phosphonate acceptor **9** (Figure 1 and Scheme 3). The results obtained are summarized in Table 2. Once more good yields of the phosphodiester product (**10**) were obtained with both donors. This time the DMTST-promoted reaction using a thioglycoside donor gave both the highest α/β-ratio and total yield of α-anomer (69%).

Table 2

Donor/equiv	Acceptor/equiv	Promoter/equiv	Temperature[a]	α/β-ratio[b]	Yield of **10**(%)
7/2	**9**/1	DMTST/1.8	rt	15:1	74
8/1.3	**9**/1	AgOTf/1.5	0 °C	3:1	76

[a]Temperature in the coupling step.
[b]Determined from the relative intensities of H-1'α and H-1'β in the ^{1}H-NMR spectrum.

In conclusion, a new approach to construct anomeric phosphodiester linkages using glycosyl donors and saccharide H-phosphonate acceptors has been explored. Good yields were obtained and good α-selectivity could be achieved by choosing suitable conditions, and the method is an interesting alternative to the use of anomeric H-phosphonate monoester and glycosyl acceptors. The method is presently being applied for the construction of *Haemophilus influenzae* type c and f oligomers.[17]

Acknowledgements

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10. **General procedure for the preparations of phosphodiesters:** To a stirred mixture of **5** or **9**, glycosyl donor and molecular sieves (4Å) in CH_2Cl_2 under nitrogen, promoter was added. When TLC indicated complete formation of phosphonate diester, Et_3N (2 equiv) was added and the mixture was cooled to -40 °C followed by addition of pyridine-water (95:5) and iodine (1.2 equiv). The reaction mixture was allowed to attain 0 °C, CH_2Cl_2 was then added and the mixture filtered through Celite and washed twice with cold 1M $Na_2S_2O_3$ and cold 1M TEAB (pH 8), dried by filtration through cotton and coevaporated with toluene to dryness. The residue was chromatographed on silica gel ($CHCl_3$-MeOH 30:1 + 1% Et_3N) to give **6**; NMR: ^1H , δ 5.11 (t, $J_{1,2}$=$J_{H,P}$= 8.0 Hz, H-1′β), 5.85 (bd, $J_{H,P}$= 7.1 Hz, H-1′α); ^{13}C, δ 94.7 (C-1′α), 98.8 (C-1′β); ^{31}P, δ -2.4; Anal. Calcd for $C_{71}H_{80}O_{18}N_3P$: C, 65.9; H, 6.2; N, 3.2. Found: C, 65.7; H, 6.4; N, 3.1, or **10**; NMR: ^1H, δ 5.03 (t, $J_{1,2}$=$J_{H,P}$= 8.1 Hz, H-1′β), 5.74 (bd, $J_{H,P}$= 7.7 Hz, H-1′α); ^{13}C, δ 94.3 ($J_{C,P}$= 6 Hz, C-1′α), 97.1 (C-1′β); Anal. Calcd for $C_{54}H_{65}O_{14}N_8P$: C, 60.0; H, 6.06; N, 10.4. Found: C, 59.8; H, 6.39; N, 9.87.
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