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Note

Methylation of carbohydrates by methyl trifluoromethanesulfonate in trimethyl phosphate

PETER PREHM
Max Planck-Institut für Biochemie D-8033 Martinsried (West Germany)
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The methylation of carbohydrates is an important method for the elucidation of polysaccharide structures. Current methylation procedures afford strong nucleophilic conditions for deprotonation, such as dimethylsulfinylsodium in dimethyl sulfate in the method of Hakomori[1–5]. Alkali-labile carbohydrates do not withstand these conditions[6–9]. Substitution of the carbohydrate residues by *O*-acetyl groups cannot be localized directly by this methylation procedure, but only by the methyl vinyl ether method of de Belder and Norrman[10]. Phosphate substituents may migrate under alkaline conditions *via* intramolecular cyclic esters[11]. Methylation in a less nucleophilic medium, such as in the methods of Haworth and Machemer[12] or Kuhn *et al.*[13], often yield undermethylated products and make repeated treatments necessary[14]. Methylation by diazomethane–boron trifluoride[15] or methyl trifluoromethanesulfonate[16–18] has been restricted to carbohydrates that are soluble in nonpolar organic solvents. Polar aprotic solvents, such as hexamethylphosphoric triamide, dimethyl sulfoxide, and *N*,*N*-dimethylformamide, decrease the high reactivity of methyl trifluoromethanesulfonate[19].

In order, to improve the procedure with methyl trifluoromethanesulfonate, the following solvents with less electron-donor activity[20] were tested for their ability to solubilize carbohydrates and to mediate methylation: tetramethylene sulfone, ethylene and propylene carbonate, and trimethyl phosphate. The last solvent proved to be ideal for methylation with methyl trifluoromethanesulfonate and 2,6-di-(*tert*-butyl)pyridine as proton scavenger[16]. The procedure was applied successfully to the core oligosaccharide derived from lipopolysaccharides of *Escherichia coli* B[21], *E. coli* K12[22,23], the hapten of *E. coli* O9[24], and to the triethylammonium salts of the complete lipopolysaccharides of *E. coli* O8[25] and *E. coli* B[21].

Bacterial lipopolysaccharides, which are themselves insoluble in dipolar aprotic solvents and therefore could not be methylated previously, were successfully treated by this procedure after conversion to their triethylammonium salts by electrodialysis and neutralization with triethylamine[26].

The present methylation procedure with methyl trifluoromethanesulfonate in

trimethyl phosphate is very mild and yields completely methylated carbohydrates in one step.

EXPERIMENTAL

A sample of carbohydrate (5 mg) was dried over phosphorus pentaoxide and suspended by sonication in trimethyl phosphate (1 mL, Aldrich Chemical Co., Milwaukee, WI 53233) which had been further purified by vacuum distillation in the presence of sodium carbonate[27]. To the clear solution or turbid suspension 2,6-di-(*tert*-butyl)pyridine (150 μL, ICN KL Laboratories, Plainview, NY 11803) and methyl trifluoromethanesulfonate (100 μL, Aldrich) were added and allowed to react for 2 h at 50°. The solution was then distributed between chloroform (5 mL) and water (20 mL). The chloroform layer was separated by centrifugation at 500*g* for 5 min, concentrated by evaporation, and applied to an LH-20 column. The recovered, methylated carbohydrate was treated further according to the conventional methylation analysis[4,5] (hydrolysis, reduction with sodium borohydride, and acetylation). The partially methylated alditol acetates obtained were separated by g.l.c. and identified by their retention times.

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