Direct thionation of reducing sugars

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Method Article

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Abstract

Introduction

In recent years, glycosyl thiols have become useful building blocks for the synthesis of certain glycoconjugates that may be considered to be analogues of glycopeptides and glycoproteins.[1] Herein, we present a method that allows the direct conversion of reducing sugars to the corresponding glycoyl thiols. This method uses Lawesson's reagent[2] \(LR) and was shown to be quite general and applicable for the preparation of a variety of differently protected and unprotected 1-thiosugars. A one-pot method consisting of direct thionation followed by chemoselctive ligation was developed for site-selective protein glycosylation. This procedure, which uses LR, has been shown to be fully compatible with unprotected sugars, the products of wich can be used in our selenenylsulfide-mediated protein glycosylation stratergy.[3] See figure in Figures section.

Reagents

• Lawesson's reagent \(Sigma-Aldrich, cat. no. 227439) • 1,4-dioxane purum ≥98.0% \(GC) \(Fluka, 42530) • 40-60º Petroleum spirit, glass distilled grade \(Rathburn, cat. no. RG2031) • Ethyl acetate, HPLC grade \(Rathburn, cat. no. RH1013) • Dichloromethane, HPLC grade \(Fisher Scientific, cat. no. D/1856/17) • Celite® 545 coarse \(Fluka, cat. No. 22140) • Silica gel for flash column chromatography \(BDH, cat. no. 153325P) • Thin-layer chromatography plates on aluminium backing, silica gel 60 F254 \(Merck) • Deuterated chloroform \(Sigma-Aldrich, cat. no. 151823)

Equipment

• Magnetic hotplate stirrer \(eg. IKA® RCT Basic) • Digital temperature probe • Oil bath • 10 and 25 mL two-neck round bottom flask • Water condenser \(to fit neck of flask) • Rubber septa \(to fit neck of flask) • Disposable syringes • Disposable needles • Teflon-coated magnetic stirrer bar • Balloon fitted to disposable 2.5 mL syringe barrel • Rotary evaporator \(Büchi) • Pyrex chromatographic column \(approx. diameter 3 cm) • NMR tubes IMPORTANT All glassware used during the reaction stages should be either oven-dried prior to use or, alternatively flame-dried under vacuum using a Bunsen burner.

Procedure

Protected substrates 1) Weigh out 200 mg of protected reducing carbohydrate into a 25 mL round-bottomed flask containing a Teflon-coated magnetic stirrer bar. Fit the flask with a water condenser in the main neck and also a rubber septum in the second neck. Place a rubber septum into the top of the condenser. Flush the flask with argon and maintain under a positive pressure of argon by use of an argon balloon. Place the flask into an oil bath on the hotplate stirrer and fit a digital temperature probe to the hotplate stirrer. 2) Transfer 5 mL of 1,4-dioxane into the flask using a disposable 5 mL syringe and a disposable needle. 3) Weight out Lawesson's reagent \(1.2 equivalents) in a glass vial and transfer to the

reaction vessel by rapid removal of the rubber septum and subsequent replacement following addition. PRECAUTION Lawesson’s reagent is toxic by inhalation and exudes a strong stench. It should be handled always inside a fume hood. 4) Heat the reaction vessel to 80 ºC and stir at this temperature for 3-4 hours. \(Completion of the reaction should be checked by t.l.c.) 5) Cool the reaction to room temperature by lifting the vessel out of the oil bath. 6) Filter the reaction mixture through a Celite® pad into a round-bottomed flask and wash the Celite® pad with 10 mL of 1,4-dioxane. Evaporate the solvent to dryness using a rotary evaporator. 7) Purify the desired product by flash chromatography on silica gel eluting with ethyl acetate:petroleum spirit to yield the desired glycosyl thiol. **Unprotected sugars for protein glycosylation** 1) Weigh out 50 mg of unprotected reducing carbohydrate into a 10 mL round-bottomed flask containing a Teflon-coated magnetic stirrer bar. Fit the flask with a water condenser in the main neck and also a rubber septum in the second neck. Place a rubber septum into the top of the condenser. Flush the flask with argon and maintain under a positive pressure of argon by use of an argon balloon. Place the flask into an oil bath on the hotplate stirrer and fit a digital temperature probe to the hotplate stirrer. 2) Transfer 3 mL of 1,4-dioxane into the flask using a disposable 5 mL syringe and a disposable needle. 3) Weight out Lawesson’s reagent \(1.2 equivalents) in a glass vial and transfer to the reaction vessel by rapid removal of the rubber septum and subsequent replacement following addition. PRECAUTION Lawesson’s reagent is toxic by inhalation and exudes a strong stench. It should be handled always inside a fume hood. 4) Heat the reaction vessel to 110 ºC and stir at this temperature for 48 hours. 5) Cool the reaction to room temperature by lifting the vessel out of the oil bath. 6) Filter the reaction mixture through a Celite® pad into a round-bottomed flask and wash the Celite® pad with 10 mL of 1,4-dioxane. Evaporate the solvent to dryness using a rotary evaporator. 7) Transfer 5 mL dichloromethane and 8 mL of water to the round-bottomed flask containing the resulting crude thiol and transfer the mixture into a separating funnel. Separate the layers and extract the organic layer with 4 portions of 8 mL of water. CRITICAL A few drops of methanol shall be added to the mixture to help separation. 8) Freeze dry the aqueous layer. 9) Use the crude glycosyl thiol in selenenylsulfide-mediated protein glycosylation stratergy.

Timing

Protected substrates: 24 hours Unprotected sugars for protein glycosylation: 72 hours

Critical Steps

Purification by flash column chromatography. Separation of crude thiol after reaction. Separation is slow and a few drops of methanol can be added to aid separation.

Troubleshooting

Low yield: It is critical to perform all the steps with no time intervals between completion of reaction and purification. Use of a 13 cm silica gel pad in a chromatography column with 3 cm diameter for a 200 mg

substrate scale reaction. Repeat reaction with fresh reagents and dry glassware, ensuring that all are anhydrous and that the reaction is maintained under an inert atmosphere.

Anticipated Results

Typical isolated yield of protected glycosyl thiols should be 45-85% depending on protecting group; higher yields for activated systems, such as O-benzyl protected substrates.. Typical yield of unprotected crude thiol for protein glycosylation should be 40%.

References

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