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A New Approach for Preparing Methyl 6-azido-2,3,4-tri-O-benzyl-6-deoxy-alpha-D-glucopyranoside

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Abstract:

Azide sugars are key intermediates in the synthesis of aminglycosides, which themselves are useful for the synthesis of glycopeptides. A new method has been achieved by using DPPA and DBU in dry DMF with high temperature for replacement of the azide group from alcohol (glucoside) on the position 6. The results of this method are very important for preparing 6-azido sugar without needing to prepare and separate glucosyl halide completely. The proposed method was successfully applied with direct, simple, and time saving. In addition, excellent yield of 95% was obtained from azide, which could be used to obtain pharmaceutical active compounds.

Keywords: Methyl 6-azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranoside, DPPA, DBU, azides.

1. Introduction

Recently, the synthesis of glycosyl azides and their applications has been one of the most important areas of research in chemistry and biochemistry. Moreover, azides are known as starting materials or a key intermediate in preparation of a number of biologically active compounds and aminglycosides, which can be used as glycosyl donors for the synthesis of glycopeptides and construction of heterocycles (Biswajit *et al.,* 2008; de Oliveira *et al.*, 2006; Wilkinson *et al.*, 2006 & Rokhum and Bez, 2012). Due to their easily performed reduction to amines and their stability with reaction conditions, they can maintain amines against variety of carbohydrate synthesis such as 'click chemistry' (Lutz, 2007). Moreover, glycoproteins have many important functions like carriers, hormones, enzymes, lectins, antibodies, and receptors (Karlsson *et al.*, 1993).

A common way for the preparation of azido sugar is by nucleophilic substitution of the corresponding halides or sulfonates under classical homogeneous conditions by azide ion at the anomeric center (Alvarez and Alvarez, 1997). While elevated temperatures are often required in case of homogeneous one-phase reactions in DMF. The phase-transfer catalysis at milder conditions may cause instability of acetylated halides, which is considered as a major limitation of this method. These methodologies have been developed to avoid the preparation of glycosyl halides completely (Beckmann and Wittmann, 2010). Consequently, the direct method is used to convert acetylated sugars into acetylated 1-azide using trimethylsilyl azide in the presence of lewis acid catalysis. This method is a useful one, but it has some limitations. For example, the change of the position of halide, this is commonly axial with the pyranose ring to β-glycosyl halide.

In addition, some spodric and limited direct methods for the said transformation are available in literature. However, few modifications have been reported by the activation of only acyclic organic alcohol using diphenyl phosphorazidate (DPPA) and 1,8-Diazabicyclo [5.4.0]undec-7-ene (DBU) (Lal, 1977) and the use of bis(*p*-nitrophenyl) phosphorazidate (Mizuno and Shioiri, 1997). Along this line, method based on the work of Danishefsky group (Chan *et al.*, 2005) is widely applied. They successfully employed DPPA with other reagents to displace a benzyl alcohol in high yield. DPPA undergoes pseudohalogen replacement of the azide group by treatment with nucleophilic reagents, such as: butanol, water, ammonia, and various amines. Herein DPPA is used as an azide source in preparing some organic azide compounds. In addition, DBU is used in organic synthesis as a catalyst, non-nucleophilic base and also as complexing ligand.

Researchers at Merck Research Laboratories developed a one-pot method to react a secondary alcohol with DPPA and DBU and that did not involve Mitsunobu conditions (Thompson *et al.*, 1993), where triphenylphosphine is used as an activator for hydroxyl group and diethyl azadicarboxylate as azide source. But

hydrazoic acid is health hazard. These methods valid only for a secondary organic alcohol and didn't apply for primary sugar. Furthermore, similar reaction was performed by Mizuno and Shioiri using bis(*p*-nitrophenyl) phosphorazidate instead of DPPA (Mizuno and Shioiri, 1997 & Akhlaghinia and Samiei, 2007).

There is a need for continuous new process that is capable of providing glycosyl azides with the azide group at position 6 directly from alcohol with high yield, short time and avoiding the formation of glycosyl halide completely. Therefore, the starting materials utilized by such a method should be very stable.

The object of this work is to provide such a process wherein the use of a novel and cheap reagent for efficient synthesis of Methyl 6-Azido-2,3,4-tri-O-benzyl-α-D-glucopyranoside from methyl-2,3,4-tri-O-benzyl-α-D-glucopyranoside using DPPA and DBU in dry DMF with high temperature. This new approach of synthesis gave an advantage of short time and less preparation steps. The method could be used for preparing pharmaceutical active compounds.

2. Experimental

2.1 General method and measurements

The starting material and chemicals were purchased from Sigma-Aldrich chemical company (Germany) and used without further purification. All solvents were dried according to standard procedures (Becker *et al.*, 1974). The reaction was performed under Argon atmosphere and was monitored by thin-layer chromatography using TLC sheets coated with silica gel 60 F254 (Merck). The sugar spots were visualized with UV light at a wavelength of 254 nm, followed by spraying with a solution of thymol-sulfuric acid solution (0.5 g of thymol and 5 ml of conc. H_2SO_4 in 95 ml of ethanol) (Göllner *et al.*, 2009) heating to 130 °C for 10 minutes, the sugar can be detected as pink spots. Azide was purified by column chromatography on silica gel 60.

The ^{1}H NMR and H-H-Cosy spectra were recorded on a Bruker DRX-500 (500 MHz) spectrometer using deuterated chloroform solvent and TMS as internal standard. Chemical shifts are reported as δ-values in ppm relative to δ_H =7.24 ppm for $CDCl_3$. The mass spectrum (MS) was recorded off-line with nano-ESI method. All the above analyses were carried out at the Department of Medicinal Chemistry, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

2.2 Synthesis of glucosyl azide from alcohol

In a clean, dry one neck flask fitted with magnetic stirring bar. Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (200 mg, 0.43 mmol) was dissolved in 5 ml dry DMF, with Ar and stirred for 5 minutes. Then 1.5 equivalent of DBU was added and allowed to stir at room temperature for 10 minutes. After that DPPA (0.118 g, 0.43 mmol, 1 equivalent) was added to the reaction mixture and heated to 127 °C. The same amounts of DBU and DPPA were added to reaction mixture after two hours to get excellent yield. Once completion by TLC (Chloroform / Ethyl acetate 9:1), the reaction mixture was co-evaporated with toluene on a rotary evaporator. The residue was purified by column chromatography to yield azide as high-viscous, colorless oil (250 mg, 95 %). There was another method for separating the product where ethyl acetate was added to the mixture, washed two times with 30 ml H_2O. Then extracted three times with 30 ml ethyl acetate and the combined ethyl acetate phases were washed with water and brine, then dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography to give the desired product (242 mg, 92

^{1}H NMR (500 MHz, $CDCl_3$):

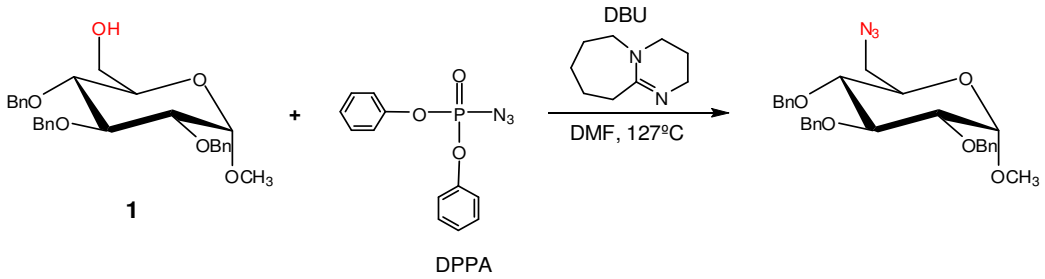
δ 7.38 – 7.24 (m, 15H, **H**-Ph), 4.99 (d, *J* = 10.9 Hz, 1H, -C***H*****$_2$**-Ph), 4.90 (d, *J* = 11.1 Hz, 1H, -C***H*****$_2$**-Ph), 4.80 (dd, 2H, -C***H*****$_2$**-Ph), 4.67 (d, *J* = 12.1 Hz, 1H, -C***H*****$_2$**-Ph), 4.62 (d, *J* = 3.5 Hz, 1H, ***H***-1α), 4.58 (d, *J* = 11.1 Hz, 1H, -C***H*****$_2$**-Ph), 3.98 (t, *J* = 9.2 Hz, 1H, ***H***-3), 3.78 (ddd, *J* = 9.7, 5.7, 2.3 Hz, 1H, ***H***-5), 3.54 (dd, *J* = 9.6, 3.6 Hz, 1H, ***H***-2), 3.47 – 3.44 (m, 1H, ***H***-6), 3.47 – 3.41 (m, 1H, ***H***-4), 3.40 (s, 3H, -OC**H**$_3$), 3.33 (dd, *J* = 13.1, 5.7 Hz, 1H, ***H***-6').

ESI-MS: m/z calculated for $[C_{28}H_{31}N_3O_5+Na]^+$: 512.2161, observed: 512.30

3. Results and Discussion

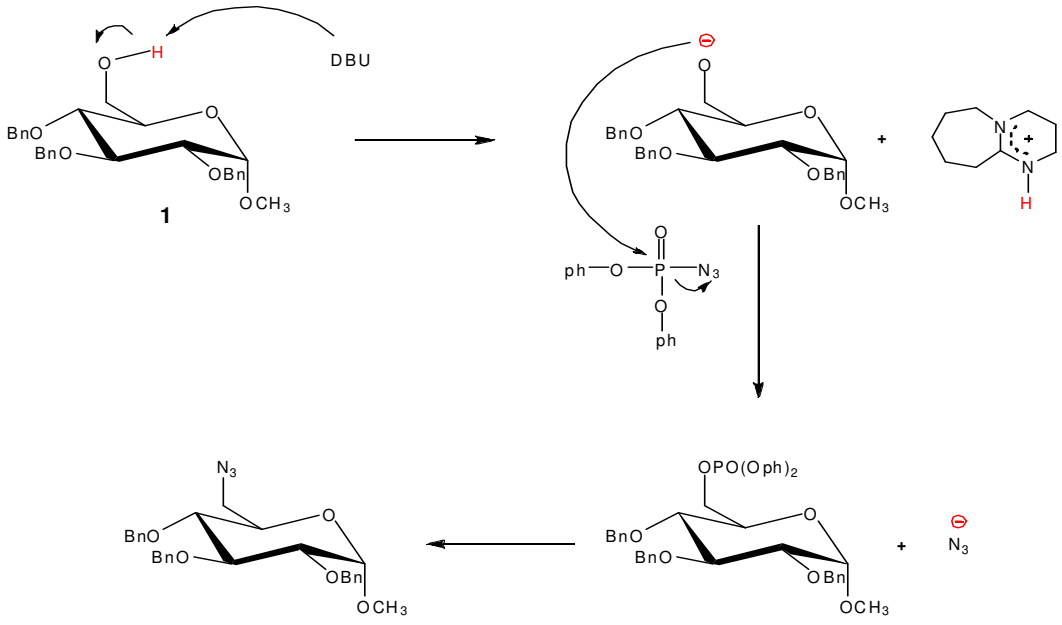
The proposed research was conducted to explore the generalities of the reaction on carbohydrate system based primary alcohols, while designing a procedure that allowed for excellent conversion of the hydroxyl sugar to the azide product. This is new method for converting benzylated sugar with hydroxyl group at position C6 into azide directly without going through to the classical SN_2 conditions, which substitutes primary hydroxyl group by a good leaving group like halides or sulfonates. The second step is converting it to azide. In this work, primary alcohol can be efficiently and smoothly converted into an azide using DPPA under mild basic conditions using DBU in DMF as a polar aprotic solvent with heating to 127ºC.

These novel conditions were used for preparing glucosyl azide from commercially available and inexpensive methyl-2,3,4-tri-O-benzyl-α-D-glucopyranoside **1** directly with high yield 95% and short time. The general reaction conditions are shown in Scheme 1.



Scheme 1: General reaction for synthesis Methyl 6-Azido-2,3,4-tri-O-benzyl-α-D-glucopyranoside

The reaction focuses on use of DBU, which acts as base in the reaction, to activate primary hydroxyl group at C6 and DPPA as azide source for the SN_2 displacement. Use of DPPA allows the in situ generation of N_3^- nucleophile via displacement from phosphate group upon alcohol attack. Then, glucosyl azide is prepared by nucleophilic substitution of the corresponding phosphate by azide ion. Proposed mechanism for the new azidation step is shown in Scheme 2.



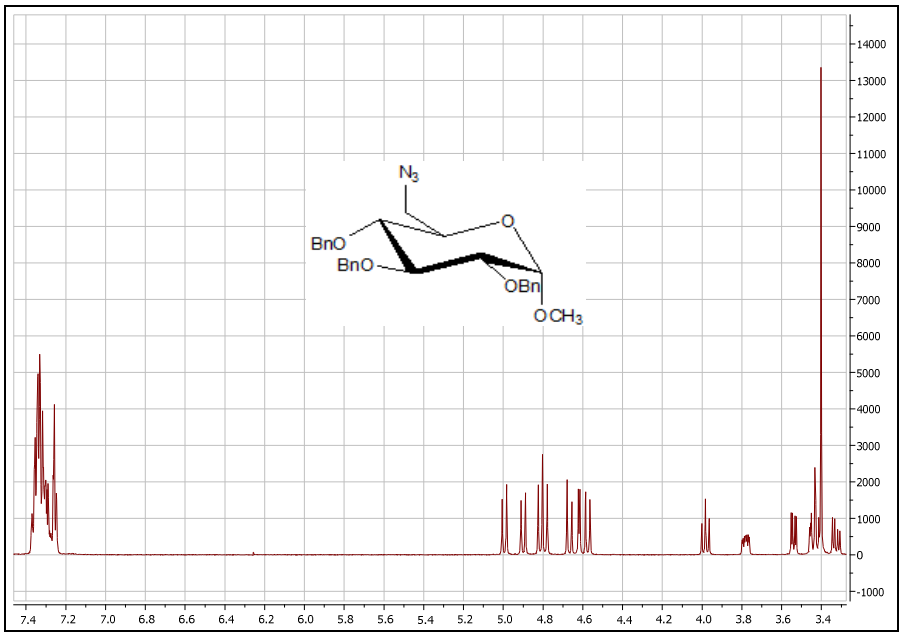
Scheme 2: Proposed mechanism for synthesis glucosyl azide

In order to optimize the reaction conditions, at first the temperature was increased gradually from -7 to 10 ºC in dry DMF. There was only a minimal amount of azide produced, as observed via TLC. The benzene rings in **1** and azide allow the easy detection of sugars with UV-lamp.

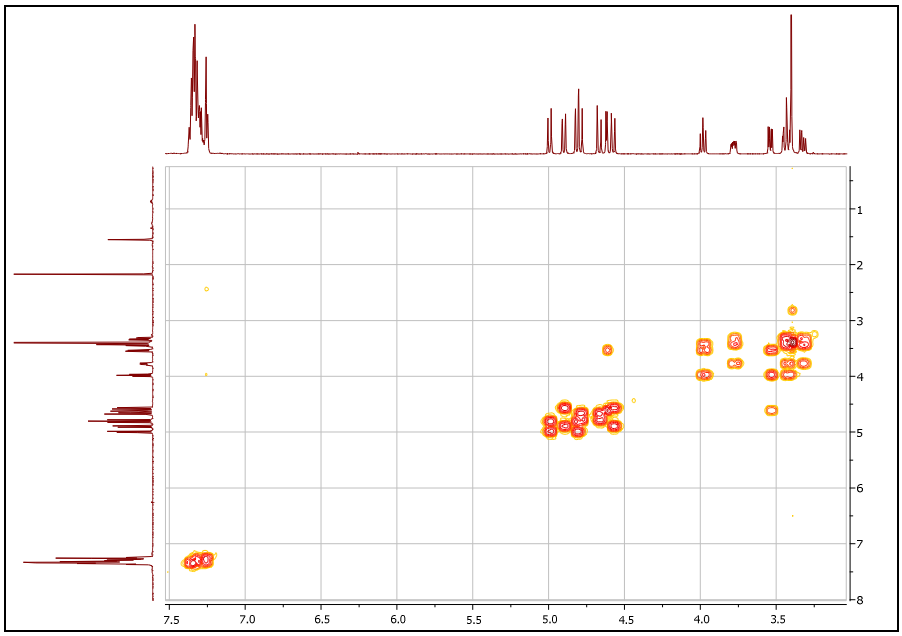
TLC plates were efficient for determining crude reaction completeness by spraying with a solution of thymol-sulfuric acid solution. This reagent causes any sugar to appear as pink spots against a red background upon heating. Percentage of conversion was increased with temperature and TLC shows a good product at 127ºC. The temperature would still be far enough below the boiling point of DMF to avoid any decomposition in the reaction. These tests showed that DPPA and DBU amounts did not seem to be enough to afford complete conversion to azide. So that, the same amount of reagents were added again to the reaction mixture after 2 hours with 127ºC to get reaction completion.

There are two procedures for workup first one by co-evaporated the reaction mixture with toluene on a rotary evaporator, which then purified by column chromatography. This gave with the best yield 95% of azide as colorless oil. The second method was the extraction of the product by ethyl acetate and washed with water, and brine. Subsequent purification steps led to losses in overall yield to 92%.

The HNMR spectra (Scheme 3 and 4) were taken for the azide product after column purification. The spectra gave a clear indication that the azide had been formed in the reaction. The ^{1}HNMR and H-H-Cosy spectra were processed with MestReNova software (V6.0.2-5475). NMR clearly indicated a double beak at 4.62 ppm that α-H is present in the product and further indication that all peaks of azide product are present in both NMRs. So that, reaction worked excellently as evident from its NMR for isolated yield and mass spectrometry. It confirmed azide production by detecting an m/z of 512.30, where the calculated m/z is 512.216. Interestingly, HNMR spectra for azide are in excellent agreement with those reported in the literature (Hansen and Jensen, 2009).



Scheme 3: ^{1}HNMR spectrum of azide product



Scheme 4: H-H-Cosy spectrum of azide product

4. Conclusion

In summary, we achieved a new strategy with a simple and efficient protocol for direct conversion of benzylated glucoside into its azide on position 6 avoiding preparing and separating glucosyl halide completely. While the reaction produced azide and confirmed by NMR and MS. There were no side products that might be formed during heating. This study clearly showed the ability to prepare the desired product without changing the stereochemistry of the sugar. This reaction conditions proposed could, in fact, show excellent selectivity for conversion of primary glucoside to its corresponding azide in the presence of DPPA and DBU in DMF as solvent with excellent yield and high purity.

Future work will focus on applying these new conditions for conversion of a wide variety of protected and unprotected sugars with different positions and organic alcohols to their corresponding azides. Another area of research is to test the ability of the prepared azide to be intercalated in layered hydroxide for further study on controlled drug release which is currently underway.

5. Acknowledgement

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