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Original Research Article

Controlled Synthesis of Mono-Dimethoxytrityl Protected Derivatives of Glycols and Diols Utilizing Chromatography-Free Purification

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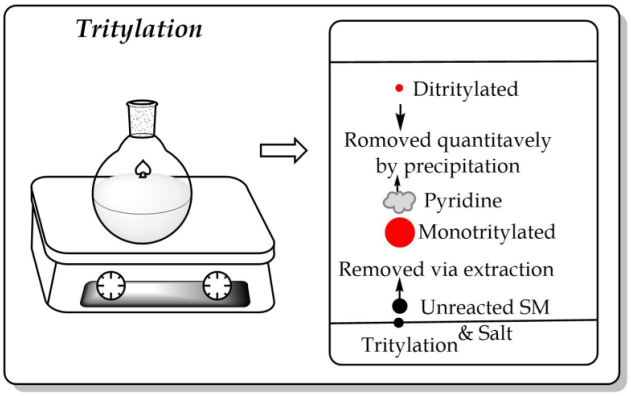
ABSTRACT

Glycols and diols, compounds characterized by the presence of two hydroxyl (-OH) groups, play a pivotal role in numerous chemical and industrial processes owing to their distinctive properties, including water solubility, pharmaceutical applications, antifreeze capabilities, and solvent properties. The principal function of a protecting group lies in temporarily concealing a reactive functional group within a molecule, thereby averting undesirable reactions while allowing other reactions to proceed unhindered. Dimethoxytrityl (DMT) stands out as a commonly employed protecting group in organic synthesis, notably in the realms of oligonucleotide and peptide synthesis. Selective DMT protection of the compounds included in the study were achieved through manipulation of temperature and limiting reagent concentration using cannula transfer in the experiments contributing to the study. The primary hindrance of traditional methodologies for synthesis of mono-DMT-protected compounds lies in the incorporation of high-cost purification of the desired products. This article outlines a chromatography-free methodology for synthesizing mono-DMT-protected derivatives of glycols and diols resulting in high yields and purity employing economically efficient purification methods such as extraction and precipitation. Characterization is achieved through thin-layer chromatography (TLC) and electrospray ionization mass spectrometry (ESI-MS). Additionally, conducted by undergraduate researchers, this methodology boasts affordability, swiftness, and operational simplicity. Given these merits, it stands as a viable option for inclusion in organic chemistry I and II laboratory projects.

GRAPHICALABSTRACT



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Introduction

Chromatography is notably a top contributor in the pharmaceutical and biomedical fields to solvent waste [1]. Improper disposal of toxic, organic solvents utilized in Flash Column Chromatography also holds the potential for large-scale chemical contamination of the environment, posing high risk to soil and groundwater in surrounding areas of its use. The use of the technique not only threatens the environment, but also human health in the event of improper disposal [2-4]. It is worth noting that failure to comply with regulations pertaining to improper toxic solvent disposal can result in costly fines and penalties for pharmaceutical and biomedical entities. These findings prompt the need for alternative routes of purification of functional products in the fields of drug and oligonucleotide synthesis. This study highlights implementation of alternative means of purification while upholding both high yield and purity of desired products using glycols and diols, compounds utilized in stepwise synthesis of biological and pharmacological compounds. Glycols and diols are attractive compounds in drug and oligonucleotide synthesis due to their chemical versatility, compatibility of the functional groups, hydrophilicity, and biological compatibility present in their structure. The hydroxyl groups present in the compounds allows for selective functionality, modification, and protection to initiate or prevent various chemical functionalities, which makes them optimal for use in stepwise synthesis. The compounds' functional compatibility stretches across a wide range of functional groups often used in relevant fields and allows for selective reactions that target other functional groups, allowing for precise modification of complex organic structures. Glycols and diols are pivotal components in the synthesis of polymers and polyurethanes. When combined with dicarboxylic acids, they undergo condensation polymerization. Alternatively, when reacted with diisocyanates, they form polyurethane polymers. [5,6]. In addition, glycols can also be used to prevent hydrate formation in gas transmission lines [7]. These properties allow for the intentional composition of compounds with specific biological and pharmacological properties [8]. The compounds also possess enhanced solubility in aqueous environments due to the presence of hydroxyl groups. This characteristic serves beneficial in drug delivery or co-delivery, as their hydrophilic nature can conduce to the solubility of drugs possessing poor water-solubility [9]. Overall, the importance of glycols and diols stems from their diverse applications in chemistry, industry, and biology, making them key components in the synthesis of materials and the development of various products.

In this study, the DMT utilization is based chiefly on its ability to selectively react with hydroxyl groups present in glycols and diols, rendering them unreactive (protected) in succeeding steps in synthesis until removal (deprotection) of DMT. This selectivity is crucial in multi-step synthesis processes where different functional groups are present [10]. DMT forms stable complexes with hydroxyl groups, providing effective protection during various chemical reactions. This stability allows the synthesis of complex molecules without premature cleavage of the protecting group [11]. DMT can be introduced and removed under relatively mild conditions that are compatible with many other functional groups. This feature is important to avoid side reactions or damage to sensitive parts of the molecule during the deprotection step [12]. DMTr can be easily deprotected when desired. This cleavage step is typically achieved using an acid, such as trifluoroacetic acid (TFA), which allows for the selective removal of the protecting group without affecting other parts of the molecule [13]. In peptide synthesis, amino acids are often

linked together through peptide bonds. DMTr is commonly used to protect the amino group of amino acids during peptide assembly. It allows for stepwise deprotection and coupling reactions, facilitating the sequential addition of amino acids to build up the peptide chain. The stability and ease of removal of DMT make it suitable for automated peptide synthesis. Automated systems can efficiently perform repetitive protection and deprotection steps, enabling the synthesis of peptides and other compounds in a high-throughput manner [14,15].

The available literature on the monotritylations of diols and glycols is rather sparse, with only a few studies dedicated explicitly to this subject. Notably, a noteworthy contribution in this domain comes from the work of Wawro *et al.* [16], while their primary research focus may differ; they have undertaken the monotritylation of glycols, initiating the process without an intermediate purification step before advancing to tosylation. Similarly, Khanal *et al.* [17] employed a comparable technique, albeit without separating the ditritylated byproduct from the main product. This method was utilized in the preparation of the trityl ether of tetramethylene glycol tosylate, a crucial monomer in the production of monodisperse polyethylene glycols. In addition, the study utilized a similar precipitation technique to purify PEG derivatives of monobenzyl derivatives of octa and dodecaethylene glycols.

In direct comparison, the current manuscript distinguishes itself by presenting a superior approach that effectively addresses the issue of impurities through an economically viable precipitation method. This distinction becomes particularly pronounced when juxtaposed with earlier methodologies that overlooked the critical purification step, potentially resulting in the presence of undesirable byproducts. The implementation of a cost-effective precipitation method in the present manuscript significantly enhances the overall purity of the synthesized compounds.

It is crucial to note that a substantial portion of the existing literature predominantly concentrates on the tritylation of the alcoholic group alone [18], lacking specific attention to monotritylation. In addition, a recurring theme in the literature centers on discussions on the tritylation of primary alcohols over secondary alcohols, emphasizing selectivity [19]. The current manuscript not only contributes to the limited literature on monotritylations, but also distinguishes itself through its explicit emphasis on purification, marking a valuable advancement in the field.

In this study, we present a meticulously controlled chromatography-free method for synthesizing mono-tritylated derivatives of glycols and diols, ensuring a high yield. The process involves the preparation of mono DMT derivatives by reacting corresponding diols or glycols with DMT-Cl in the presence of a mild base, which concurrently serves as a solvent under an inert atmosphere.

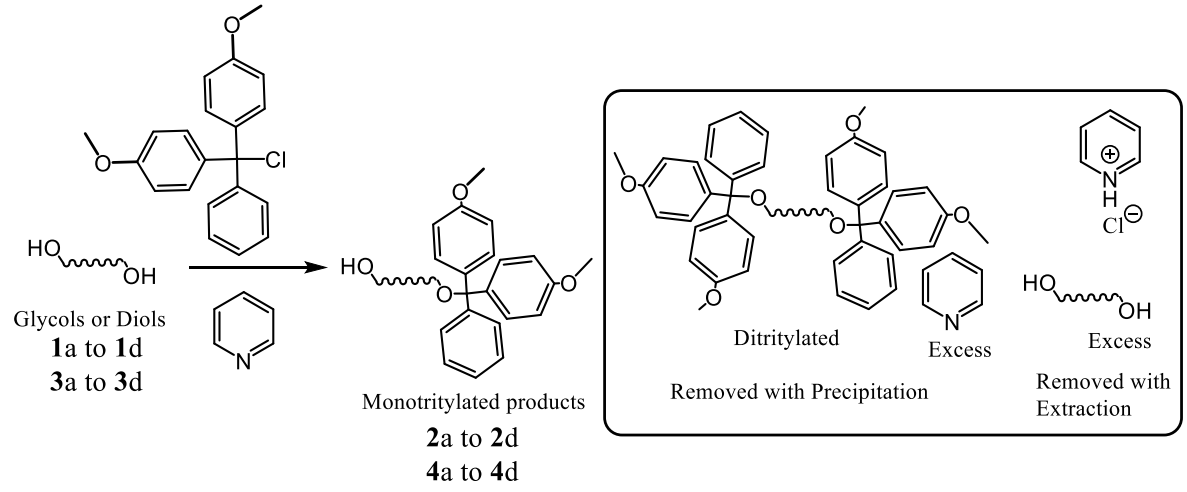
Following the completion of the reaction, the crude product undergoes purification through extraction and precipitation. The crude reaction mixture is subjected to extraction using a suitable organic solvent, followed by precipitation with diethyl ether and hexane. Subsequently, the purified product is characterized using thin-layer chromatography (TLC) and electrospray ionization mass spectrometry (ESI-MS). The entirety of the experimental procedures and characterization methods were conducted by an undergraduate researcher. This project holds significant potential as coursework for Organic Chemistry I and II. Its implementation involves assigning diverse glycols and diols to individuals or groups of students. The evaluation criteria for students encompassed their proficiency in synthesis, purification, and characterization techniques. This initiative not only offers a practical

application of theoretical knowledge, but also serves to assess and enhance students' practical skills in the synthesis, purification, and characterization of compounds within the realm of organic chemistry [20].

Materials and methods

All utilized reagents, including glycols and diols (such as ethylene glycol, diethylene glycol, triethylene glycol, tetraethylene glycol, 1,3-propanediol, 1,4-butanediol, and 1,5-pentanediol, all at 98% purity from Millipore Sigma), DMTrCl (4,4′-dimethoxytrityl chloride, 97% purity, Millipore Sigma), Pyridine (99% purity, Millipore Sigma), Triethylamine (99.5% purity, Millipore Sigma), and solvents from commercial sources, were employed as received unless specified otherwise. All reactions were conducted in oven-dried glassware under a nitrogen atmosphere. Thin-layer chromatography (TLC) utilized Sigma-Aldrich TLC plates with silica gel 60F-254 over glass support, having a thickness of 250 µm. Low-resolution mass spectrometry (LRMS) data were obtained using an Agilent 7820AGC (G4350)-5977E(G7035A) Mass Spectrometer. All glassware utilized in reactions, including two-neck and one-neck RB flask, syringes, and cannulas, were thoroughly oven-dried overnight before use in the laboratory.

To implement the project, controlled tritylation is achieved through the incorporation of three specific techniques. These consist of mploying an excess of glycols and diols to maintain a consistently low concentration of the limiting reagent, utilizing the addition of trityl chloride solution from another flask via cannula transfer over an extended period, and maintenance of low temperature throughout the reaction.Each procedure is designed to promote the formation of monotritylated products by sustaining a low concentration of trityl chloride and reducing the reaction rate. Moreover, the use of a mild base introduces a delay in the generation of reactive anions, thereby facilitating monotritylation and concurrently mitigating the risk of oxirane ring side products from glycols. The procedural roadmap is visually depicted in the accompanying Scheme 1 and Figure 1.



Scheme 1. Synthesis of monotrityl derivatives.

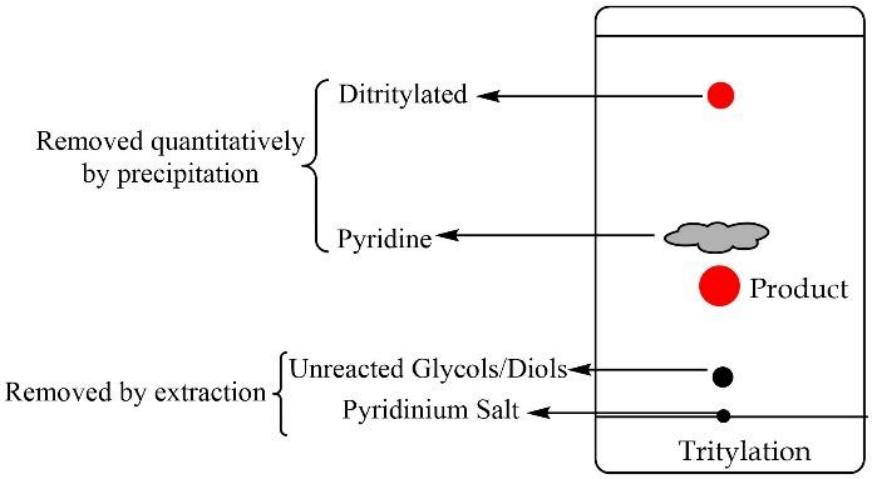


Figure 1. Theoretical roadmap in which pure product will be generated without column purification.

Experimental

General procedure to synthesize mono trityl ether of glyols and diols

Two-neck RB flasks, equipped with septa, underwent a cooling process by nitrogen flushing, followed by syringe injection under positive nitrogen pressure. This procedural step was mirrored for all the synthesized monotrityl ethers delineated in this manuscript, specifically referring to glycols (2a to 2d) and diols (4a to 4d). The two-neck RB flasks were allocated for glycols/diols, while the one-neck RB flask contained DMTCl. All reactions were maintained under positive nitrogen pressure. The flask containing DMTCl was placed at a higher level than the glycols/diols to ensure a continuous flow for cannula transfer, as illustrated in the accompanying Figure 2.

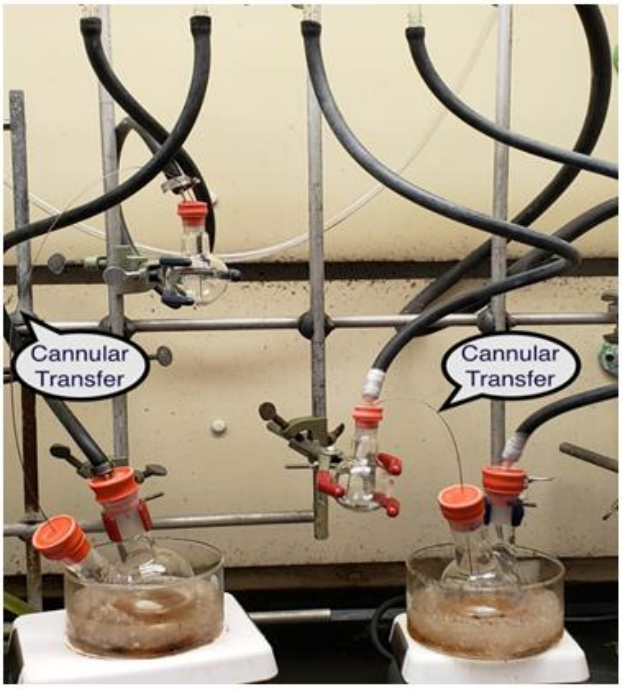


Figure 2. Reaction setup and canular transfer.

Dry pyridine was inserted into both flasks. In an ice bath, a solution of glycols and diols (1a to 1d and 3a to 3d, with 5 equivalents) received a gradual addition of DMTCl solution (1 equivalent) via cannula under a nitrogen atmosphere at room temperature, extended over a duration of 1 hour. On a 1-gram scale, 15 mL pyridine was used for glycols and diols, and 5 mL pyridine for DMTCl alone. Following cannula transfer, stirring persisted at room temperature overnight. After 24 hours, most of the pyridine was removed under reduced pressure, leaving a small amount to maintain the mixture basic. It is noteworthy that preserving some pyridine prevents the acidic pyridinium salt by-product from attacking the DMT group, thereby preventing the degradation of DMT. The residue underwent partitioning between ethyl acetate (EtOAc) and 5% sodium bicarbonate ($NaHCO_3$). The organic phase underwent four additional washes with 5% $NaHCO_3$, followed by drying over anhydrous Na_2SO_4 and filtration. The filtrate was evaporated to dryness and further desiccated under vacuum before yielding the product, a monotrityl ether of glycols and diols. At this point, the product contained unwanted byproducts. A precipitation method was employed to purify the product. The precipitation process entailed dissolving the crude product in a minimal volume of diethyl ether, followed by the addition of an excess amount of hexane. This step facilitated the dissolution of non-polar components such as excess pyridine and ditritylated byproducts into the excess hexane, while the desired monotritylated products precipitated out as white solids.

Subsequently, the solution was placed in a freezer to promote the settling of the precipitates. Once the precipitates had settled, the supernatant solution was discarded through a decantation process. This decantation process was repeated two additional times to ensure thorough removal of impurities and non-precipitated substances, thereby enhancing the purity of the final product (Figure 3).

Figure 3. Precipitation method used in purification step.

Results and discussion

In establishing chromatography-free controlled reaction conditions, we opted for DMTCl as the tritylation agent and glycol and diol (1a to 1d and 3a to 3d) as the substrates. The objective of the study was to investigate chromatography-free conditions for the selective tritylation of one of the alcoholic groups in glycols/diols. The outcomes of these experiments are outlined in Tables 1 and 2.

Monotrityl derivatives were efficiently synthesized with consistently high yields exceeding 80% across all substrates. The purity of products was assessed through thin-layer chromatography (TLC) in Figures 4 and 5. The presence of a single spot in thin-layer chromatography strongly suggests that all the products are of exceptional purity.

Table 1. Tritylation of ethylene glycol

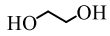
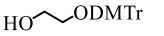
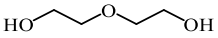
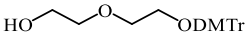
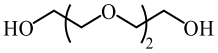
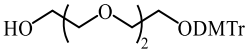
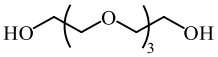
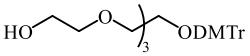
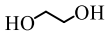
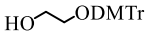
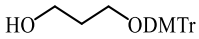
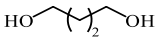
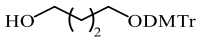
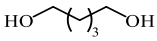
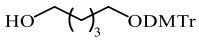
Glycols	Monotritylated	Yield %	TLC	LCMS
 **1a**	 **2a**	81	Single spot	One M^+ peak
 **1b**	 **2b**	86	Single spot	One M^+ peak
 **1c**	 **2c**	82	Single spot	One M^+ peak
 **1d**	 **2d**	89	Single spot	One M^+ peak

Table 2. Tritylation diols

Diols	Monotritylated	Yield %	TLC	LCMS
 **3a**	 **4a**	81	Single Spot	One M^+ peak
 **3b**	 **4b**	80	Single spot	One M^+ peak
 **3c**	 **4c**	85	Single spot	One M^+ peak
 **3d**	 **4d**	87	Single spot	One M^+ peak

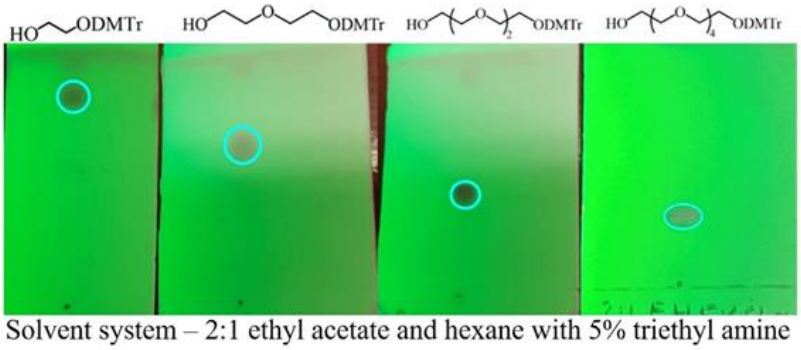


Figure 4. TLCs of monotritylated glycols after extraction and precipitation.

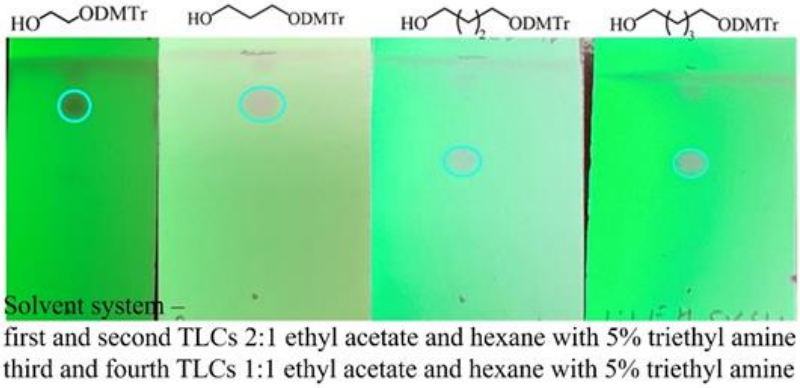


Figure 5. TLCs of monotritylated diols after extraction and precipitation.

The purity of the products underwent further validation through LCMS spectroscopy. The presence of a single molecular ion peak $[M+Na]^+$ for all products provides strong evidence of their purity, aligning with the results obtained from TLC analysis. Notably, the additional peak observed at 303 serves as robust confirmation of the DMT group falling off from the product $[DMT]^+$. This occurrence is substantiated by the stability of the DMT cation, attributed to its resonance delocalization (Figures 6-12).

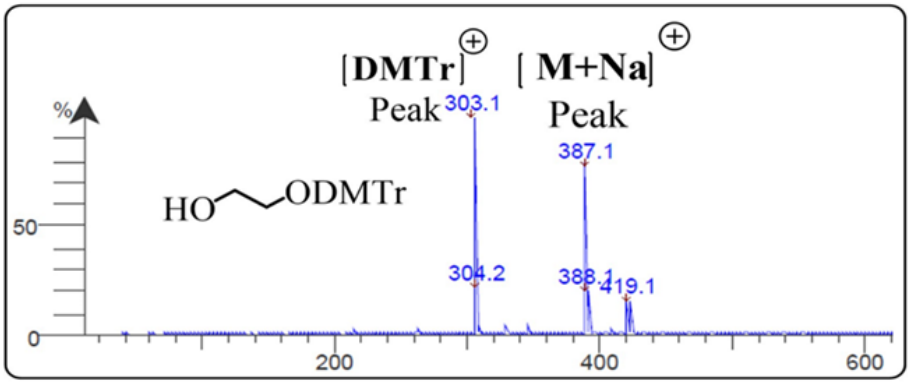


Figure 6. Mass spectra of 2a.

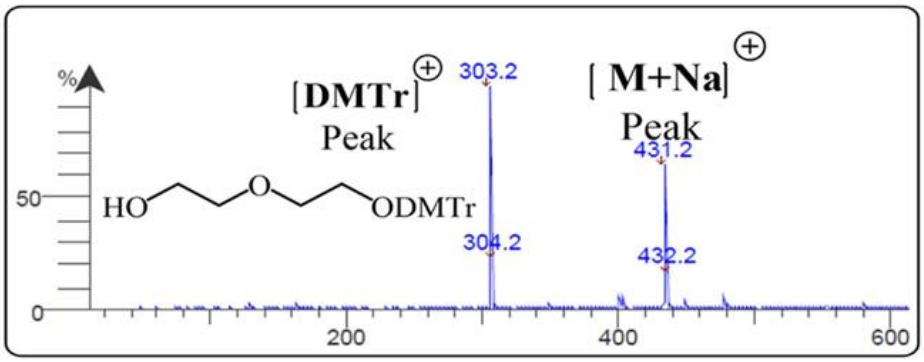


Figure 7. Mass spectra of 2b.

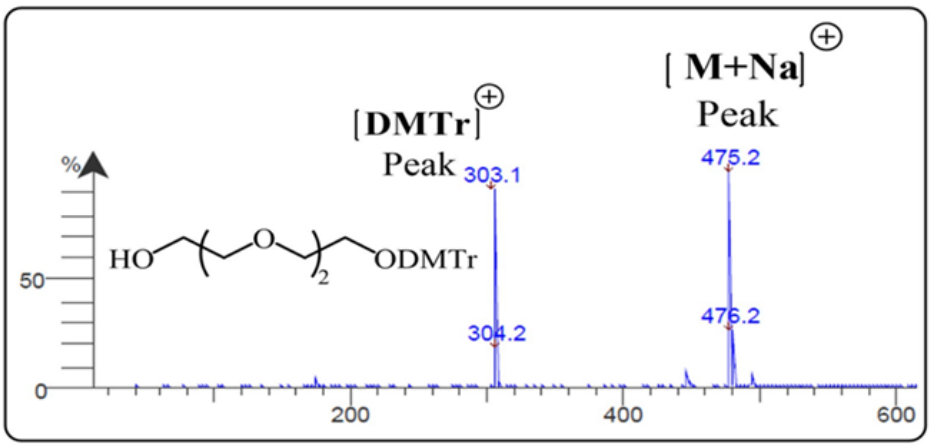


Figure 8. Mass spectra of 2c.

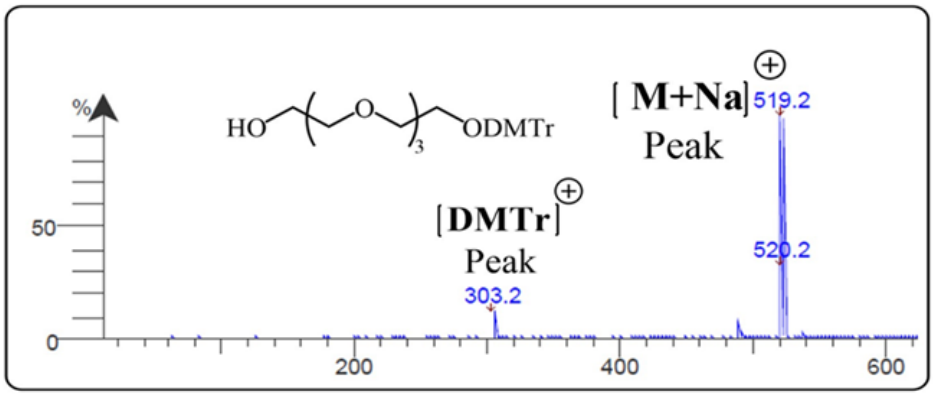


Figure 9. Mass spectra of 2d.

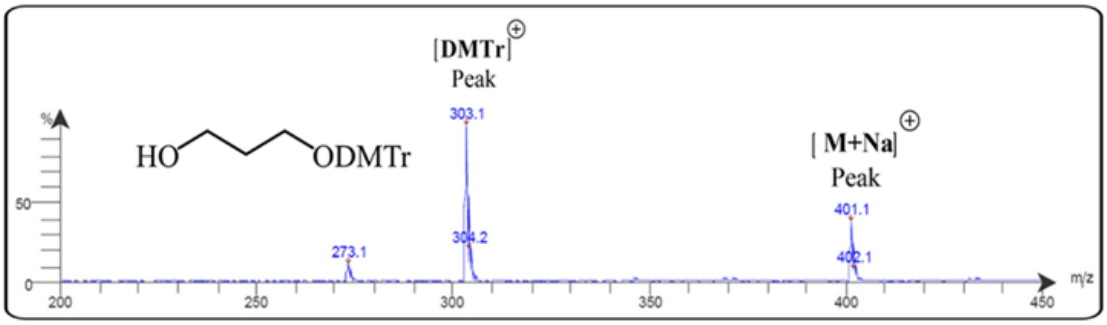


Figure 10. Mass spectra of 4b.

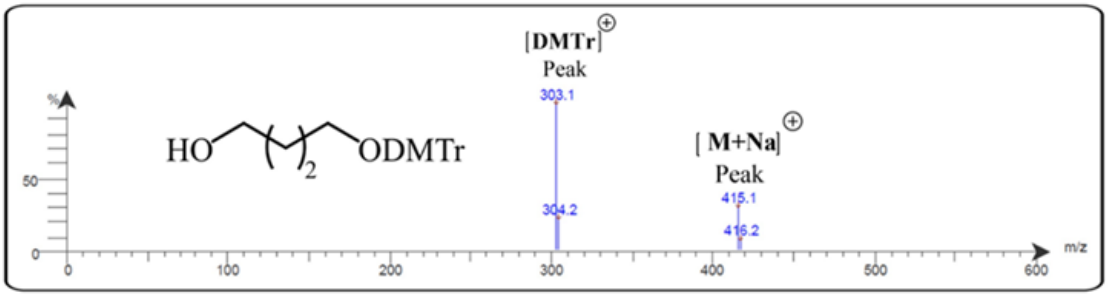


Figure 11. Mass spectra of 4c.

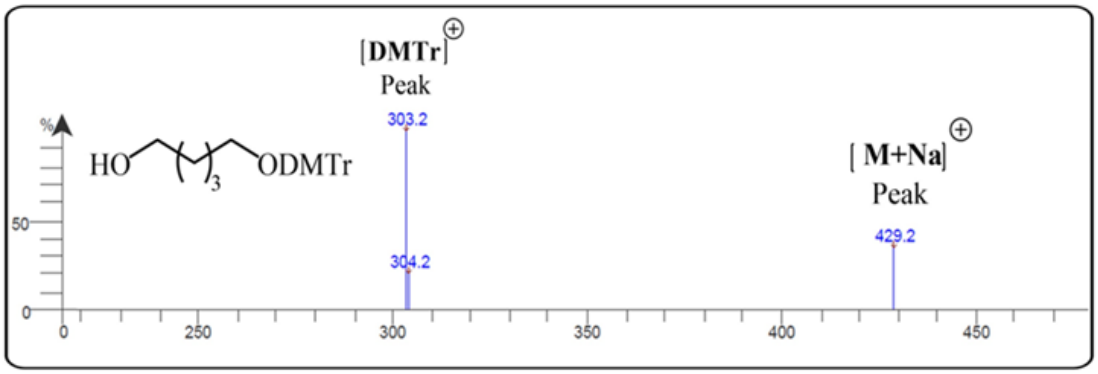


Figure 12. Mass spectra of 4d.

Conclusion

To sum up, we have successfully developed a straightforward method that is free from flash column chromatography and enables controlled monotritylation of glycols and diols. In comparison to the existing selective tritylation methods, our approach offers a more economical purification process, leading to higher yields. Significantly, this approach presents a promising solution for constructing linkers in trityl chemistry. Furthermore, its simplicity, and, efficiency make it an attractive candidate for integration into undergraduate organic chemistry labs or projects, providing a swift and accessible experimental pathway.

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Disclosure statement

No potential conflict of interest was reported by the authors in this study.

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