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Ionic-tag-assisted synthesis of nucleoside triphosphates

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ABSTRACT

We describe an ionic-tag-assisted synthesis of nucleoside triphosphates that combines the advantage of one-pot triphosphate formation and liquid-phase extraction.

Phosphorylated nucleosides especially nucleoside triphosphates (NTPs and dNTPs) are heavily involved in processes such as neurotransmission, DNA replication, transcription, translation and repair, purinergic signaling, primary metabolism, signal transduction, and polysaccharide biosynthesis.[1] The ever growing need for nucleoside triphosphates and their analogues as tools for numerous biological applications has driven the development of chemical and enzymatic ways aiming at high purity and yields.[2] Unfortunately, no single chemical and/or enzymatic method reported thus far for making nucleoside triphosphates demonstrates promise and generality to extend it for the synthesis of nucleoside triphosphates of diversified nucleobases and non-nucleobases. Enzymatic ways suffer from issues like substrate specificity, scale, yield, and cost. Functionalities present in the basic structure of nucleosides itself pose a daunting challenge to the synthetic chemist to develop efficient strategies for universal chemical phosphorylation and still continue to remain as an unsolved problem. Chemical methods to synthesize nucleoside triphosphates invariably involve reactions between lipophilic substrates (protected nucleosides) and charged ionic reagents (like pyrophosphates). This situation not only limits the choice of reaction media but also complicates purification of nucleoside triphosphates as it is going to be the isolation of water soluble charged product from a mixture of hydrophilic (2′-, 3′-, and 5′-phosphates) and hydrophobic impurities.[2] Tedious purification cycles are needed to obtain nucleoside triphosphates that are free from inorganic impurities such as pyrophosphates and cyclic trimetaphosphates.[2] These inorganic impurities significantly affect and/or alter their intended molecular biology applications. The reaction yield is not just only depending on the conversion of reactants into products but also on the ability to isolate target product in its pure form from the reaction mixture. Though nucleoside triphosphate synthetic methods have evolved and improved over the past decade, the separation methods that chemists use have not changed and/or improved for a decade or more.[2] Recent attempts to simplify the purification challenges involving polymer-supported solid-phase synthesis of phosphorylated nucleosides also suffer from the use of very large excess of reagents.[2] The alternative of using soluble poly(ethylene glycol) polymer supported synthesis of phosphorylated nucleosides has been developed to alleviate the issues associated with solid-phase synthesis.[3] This method also suffers from the relative low loading of nucleosides onto the soluble polymers and experiences difficulties in the selective precipitation of nucleoside attached polymers.[3] In this line, we recently reported chromatographic purification-free synthesis of 5-[3-aminoallyl]-uridine-5′-triphosphate, AA-UTP based on fluorous solid-phase extraction (F-SPE).[4]

Ionic liquids have received considerable attention in recent years[5] as environmentally benign reaction media for organic reactions due to their lack of measurable vapor pressure and high thermal and chemical stability. Solubilities of ionic liquids can be easily tuned depending on the choice of cations and anions for a specific reaction or substrate application. One of the several possibilities that can be embraced using this liquid-phase extraction strategy is the use of ionic liquid supported substrates. Reactions can be conducted in a homogenous solution by dissolving ionic liquid supported substrates in a solvent (usually more polar). Upon reaction completion, ionic liquid supported substrates can be separated from the organic phase by the addition of less polar solvents. The

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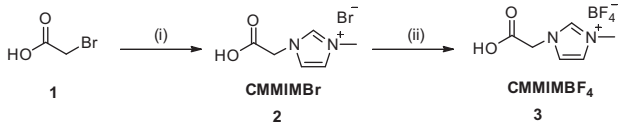
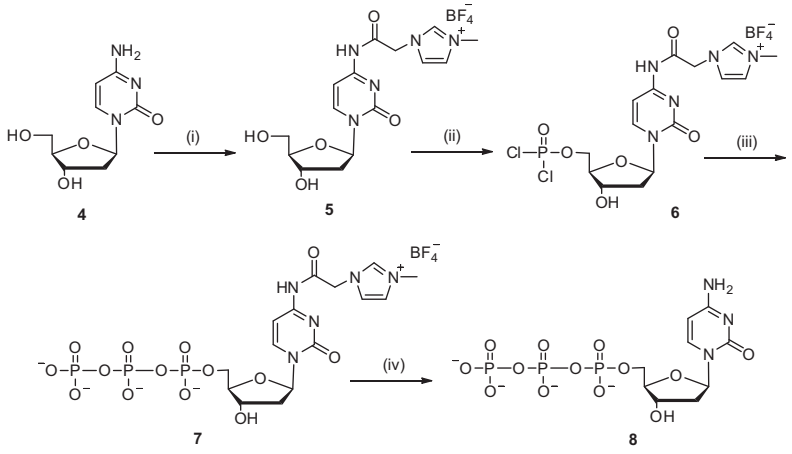
recovered ionic liquid supported substrates would then be either detached from the ionic liquid or further taken to subsequent reactions to obtain the final product before detachment. Ionic liquids are mostly recyclable and reusable. The usefulness of ionic liquids approach in carbohydrate chemical synthesis where purification is very challenging and unavoidable is well documented.[6] Although the benefits of ionic liquid supported catalysts and reagents were realized in organic synthesis, ionic liquid assisted liquid-phase extraction strategy was not explored in the synthesis of phosphorylated nucleosides. In view of these facts and in continuation of our efforts in synthesizing various phosphorylated nucleosides,[7] we are much interested to evaluate the benefits of ionic liquid assisted liquid-phase extraction to drastically ease otherwise very complex synthesis and purification of nucleoside triphosphates since there are no reports available in this context. Herein we disclose the successful use and benefits of ionic liquid assisted liquid-phase extraction for nucleoside triphosphate synthesis for the first time by synthesizing nucleoside triphosphates.

The ionic liquid 1-carboxyl-methyl-3-methylimidazolium fluoborate **3** ($CMMIMBF_4$) was synthesized (Scheme 1) according to a reported procedure.[8] Bromoacetic acid **1** was reacted with 1-methylimidazole acetone/ethanol to afford deep brown-reddish viscous liquid 1-carboxyl-methyl-3-methylimidazolium bromide **2** (CMMIMBr). Treatment of CMMIMBr **2** with potassium fluoborate in methanol afforded $CMMIMBF_4$ **3** as pale yellow viscous liquid.[9] Crauste et al[3] selectively linked PEG-(*O*-succinate)$_2$ to nucleoside through the exocyclic amino group of the nucleobase in the presence of 1-hydroxybenzotriazole (HOBT) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) as coupling reagents in a mixture of anhydrous dichloromethane and DMF. This protocol allowed anchoring regioselectively the selected nucleoside on to the polymer support without prior protection of the sugar hydroxy functions.[3] The same protocol was used to tag selectively the amino group of nucleosides with ionic liquid in this work.[10] Coupling of 2′-deoxycytidine **4** with $CMMIMBF_4$ **3** by using DCC/HOBT as a reagent in DMF/CH_2Cl_2 afforded the ionic liquid-tagged 2′-deoxycytidine **5**. The reaction mixture was well diluted with ethylacetate to aid liquid–liquid extraction by lowering the medium polarity. Ionic liquid-tagged 2′-deoxycytidine was oiled out leaving other excess organics and unreacted materials in the top organic layer. Organic layer above the oily viscous ionic liquid-tagged 2′-deoxycytidine was removed. The crude viscous ionic liquid-tagged 2′-deoxycytidine was washed two more times with ethylacetate, dried under vacuum after washing, and used as such for the next step without further purification. Improved one-pot protection-free triphosphorylation methodology[11] was used to convert thoroughly dried ionic liquid-tagged 2′-deoxycytidine **5** into ionic liquid-tagged 2′-deoxycytidine-5′-*O*-triphosphate **7** as part of our continued effort to synthesize various nucleoside triphosphates and related analogues in high yields.[7] Ionic liquid-tagged 2′-deoxycytidine **5** was monophosphorylated using $POCl_3$ as the reagent and trimethyl phosphate as the solvent at −5 °C to form dichlorophosphoridate intermediate **6**. In a one-pot fashion, without isolating the intermediate **6**, to the same flask, a prechilled cocktail containing tributylammonium pyrophosphate, tributylamine, and acetonitrile was added to the reaction mixture. The reaction mixture was quenched by the slow addition of ice-cold water and the reaction mixture was evaporated under reduced pressure to remove solvents. The residue was resuspended in acetonitrile to aid liquid–liquid extraction. Ionic liquid-tagged 2′-deoxycytidine-5′-*O*-triphosphate **7** was oiled out leaving other organics and unreacted materials in the top organic layer. Organic layer above the oily viscous product **7** was removed. The crude viscous ionic liquid-tagged 2′-deoxycytidine-5′-*O*-triphosphate **7** was washed two more times with acetonitrile and dried under vacuum after washing.[12] Ionic tag is removed by treating the ionic liquid-tagged 2′-deoxycytidine-5′-*O*-triphosphate **7** with aqueous ammonium hydroxide (33%). Upon reaction completion, solvents were stripped off under reduced pressure, reconstituted in minimal amount of 1 M triethylammonium bicarbonate (TEAB), and cooled to 4 °C to aid liquid–liquid extraction. The cleaved viscous ionic liquid stays at the bottom leaving dCTP in the top aqueous 1 M TEAB layer. The product containing aqueous TEAB layer was coevaporated twice with water. The triethylammonium salt of dCTP thus obtained was subjected to ion-exchange with sodium perchlorate in acetone for two times to afford the sodium salt of 2′-deoxycytidine-5′-*O*-triphosphate **8** in 72% yield with 97% purity (by HPLC) (Scheme 2).[13] The same reaction sequence was applied to obtain 2′-deoxyadenosine-5′-*O*-triphosphate starting from 2′-deoxyadenosine (Scheme 3) in 69% yield with 98% purity.[14]



Scheme 1. Synthesis of ionic liquid 1-carboxyl-methyl-3-methylimidazolium fluoborate ($CMMIMBF_4$). Reagents and conditions: (i) 1-methylmidazole, ethanol, acetone, −5 °C, 2 h; then rt, 8 h; (ii) potassium fluoborate, anhydrous methanol, 48 h, rt, 95%.



Scheme 2. Ionic-tag-assisted synthesis of 2′-deoxycytidine-5′-*O*-triphosphate. Reagents and conditions: (i) $CMMIMBF_4$, DCC, HOBT, CH_2Cl_2, DMF, 60 °C, 6 h; (ii) $POCl_3$, Bu_3N, $(CH_3O)_3PO$, −5 °C, 0.5 h; (iii) $(NHBu_3)_2H_2P_2O_7$, Bu_3N, CH_3CN, −5 °C, 0.5 h; (iv) NH_4OH, rt, 2 h, 72%.