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Efficient One-Step Syntheses of Isoprenoid Conjugates of Nucleoside 5′-Diphosphates

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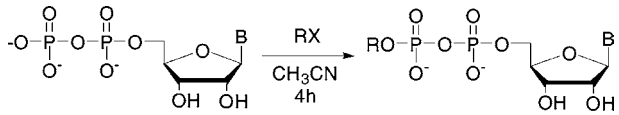
ABSTRACT



R= Isopentenyl, Geranyl or Farnesyl

B= Guanine or Adenine X= Cl or OTs

Isoprenoid conjugates of nucleoside 5′-diphosphates were efficiently synthesized by one-step nucleophilic displacement reactions of either isoprenyl chlorides or isopentenyl tosylate with nucleoside 5′-diphosphates.

Covalent lipid conjugates of nucleosides, nucleotides, and nucleic acids are of great importance because of their potential as prodrugs as well as drug and gene delivery systems.[1] Liposomal encapsulation was also proposed as a general method for selecting new nucleic acid catalysts.[2] Conventional lipids such as phospholipids have been generally used for such studies. However, it is also well-known that isoprenoids such as farnesol, geranylgeraniol, and dolichol, which are a family of lipids made up of a repeating five-carbon isoprene unit, can serve as membrane anchors of proteins and oligosaccharides.[3] Therefore, isoprenoids could potentially substitute for conventional lipids as lipophilic carriers of nucleosides, nucleotides, and nucleic acids. Consequently, we now report a highly efficient one-step synthesis of isoprenoid conjugates of nucleoside 5′-diphosphates (NDPs).

A conventional condensation of nucleotide imidazolides with isoprenyl monophosphate was initially attempted in order to prepare the pyrophosphodiesters of nucleosides and isoprenoid alcohols (Scheme 1). First, commercially available guanosine monophosphate (GMP, **1a**) and adenosine monophosphate (AMP, **1b**) were converted to their corresponding imidazolides by treatment with carbonyl diimidazole (CDI) in DMF.[4] The relatively stable imidazolide intermediates **2a** and **2b** were identified by their characteristic peaks at about δ −8.8 in the ^{31}P NMR spectrum. Isopentenyl monophosphate and geranyl monophosphate were prepared by literature procedures.[5,6] The imidazolide **2a** was then reacted with isopentenyl monophosphate in DMF, and the reaction progress was monitored by ^{31}P NMR. The reaction appeared to be extremely slow despite very little side reaction, and

(1) (a) For a review of lipids as prodrug carriers, see: Lambert, D. M. *Eur. J. Pharm. Sci.* **2000**, *11*, s15. (b) For examples of lipid conjugates of nucleic acids, see: Shea, R. G.; Marsters, J. C.; Bischofberger, N. *Nucleic Acids Res.* **1990**, *18*, 3777 and references therein. (c) For a review of liposome as a gene delivery vehicle, see: Templeton, N. S. *Curr. Med. Chem.* **2003**, *10*, 1279.

(2) Lorsch, J. R.; Szostak, J. W. *Acc. Chem. Res.* **1996**, *29*, 103.

(3) (a) For a review of prenylated proteins, see: Sinensky, M. *Biochim. Biophys. Acta* **2000**, *1484*, 93. (b) For a review of dolichol conjugates of oligosaccharides, see: Schutzbach, J. S. *Glycoconjugate J.* **1997**, *14*, 175.

(4) Zatorski, A.; Goldstein, B. M.; Colby, T. D.; Jones, J. P.; Pankiewicz, K. W. *J. Med. Chem.* **1995**, *38*, 1098.

(5) Eggerer, H.; Lynen, F. *Justus Liebigs Ann. Chem.* **1960**, *630*, 58.

(6) Bunton, C. A.; Hachey, D. L.; Leresche, J.-P. *J. Org. Chem.* **1972**, *37*, 4036.

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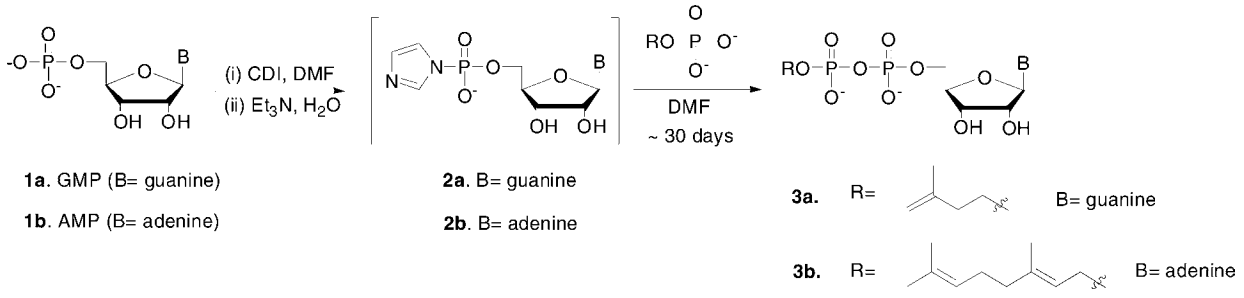
Scheme 1



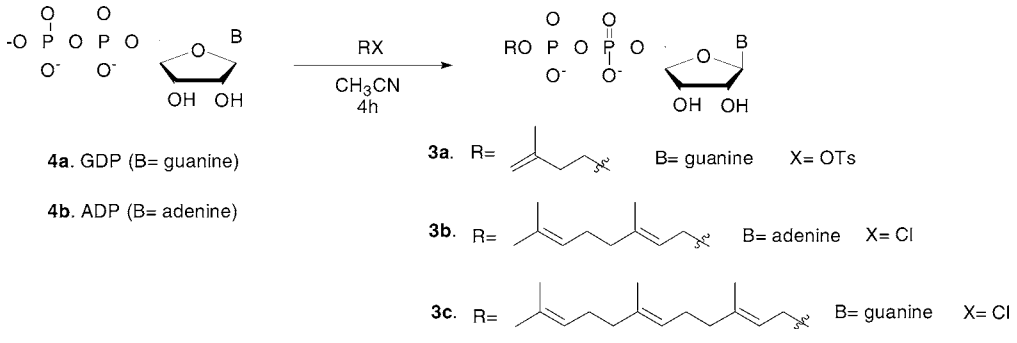
the reaction was quenched after 1 month when the ^{31}P NMR signal for imidazolide completely disappeared. Purification of the reaction mixture by HPLC gave the 5′-isopentenylpyrophosphoryl guanosine **3a** in 76% yield. Likewise, reaction of the imidazolide **2b** with geranyl monophosphate in DMF for 1 month followed by HPLC purification provided 5′-geranylpyrophosphoryl adenosine **3b** as a dibasic triethylammonium (TEA) salt in 74% yield.

Despite high selectivity and good isolated yield of products, the reaction of the imidazolides derived from nucleoside 5-monophosphates with isoprenyl monophosphates appeared to be impractical because of the extremely sluggish reaction rate. In addition, it is well-known that synthesis and purification of isoprenyl monophosphates is difficult due to their instability, especially when the isoprenyl chain becomes longer.[7] Therefore, we attempted to find a more practical route. Poulter and co-workers have reported practical syntheses of isoprenoid pyrophosphates by a direct displacement reaction of either allylic halides or homoallylic tosylates with inorganic pyrophosphate.[8] We therefore replaced inorganic pyrophosphate with commercially available NDPs and employed the same procedure to prepare the isoprenoid conjugates of NDPs.

To our surprise, despite the additional nucleophilic groups such as exocylic amino and hydroxyl groups on the nucleosides, the desired conjugates were obtained in good yield (Scheme 2).[9] Compound **3a** was synthesized from guanosine 5′-diphosphate (GDP, **4a**) and isopentenyl tosylate. GDP disodium salt was converted to its tetrabutylammonium (TBA) salt by treatment with acidic ion-exchange resin. Isopentenyl tosylate was prepared from isopentenyl alcohol according to Poulter's procedure.[8] Reaction of GDP TBA salt with isopentenyl tosylate in MeCN for 4 h followed by reversed-phase HPLC purification produced **3a** as the dibasic TEA salt in 73% yield. The compound showed two signals at δ −11.10 and −11.56 in the ^{31}P NMR spectrum. The reaction of adenosine 5′-diphosphate (ADP, **4b**) with commercially available geranyl chloride in MeCN provided compound **3b** as the dibasic TEA salt in 79% yield after HPLC purification. The ^{31}P NMR of this compound also showed two characteristic pyrophosphate resonances at δ −10.98 and −11.57. Likewise, the new conjugate, farnesyl pyrophosphoryl guanosine **3c** was synthesized from farnesyl chloride and GDP. The TBA salt of GDP was reacted with a commercially available *trans,trans*-farnesyl chloride in MeCN. HPLC purification of the reaction mixture produced the dibasic and monobasic forms of **3c** in good yields. The dibasic TEA salt of **3c** produced ^{31}P NMR signals at δ −10.95 and −11.50, whereas the monobasic TEA salt of **3c** signals were at δ −5.80 and −10.35. The downfield shift of ^{31}P signal indicates the presence of acidic phosphate.[10]

The two conjugates **3a** and **3c** derived from guanosine nucleotide could potentially serve as initiator nucleotides of T7 RNA polymerase reaction to provide isoprenoid conjugates of RNA. Preliminary results indicated that compound

Scheme 2



3a initiates in vitro transcription very well whereas compound **3c** does not.[11]

In summary, the direct displacement of either isoprenyl chloride or isopentenyl tosylate with NDPs provided the isoprenoid conjugates of NDPs in good yield. Although only purine nucleotides bearing exocylic amino groups were investigated for the conjugate formation in this report, we expect similar results with pyrimidine nucleotides as well as other nucleotide analogues.

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Supporting Information Available: Experimetal procedures and ^{1}H and ^{31}P NMR and HRMS data for compounds **3a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) The literature indicates that alkylation reagents such as alkyl halides, diakyl sulfates and trialkyl phosphates led to nonselective multi-akylation on both nucleoside bases and phosphates. For examples, see: (a) Ogilvie, K. K.; Beaucage, S. L.; Gillen, M. F.; Entwistle, D. W. *Nucleic Acids Res.* **1979**, *6*, 2261. (b) Ogilvie, K. K.; Beaucage, S. L.; Gillen, M. F.; Entwistle, D.; Quilliam, M. *Nucleic Acids Res.* **1979**, *6*, 1695.
(10) Swanson, M. G.; Robert, J. M.; Keller, R. K.; Bangalore, P. *Magn. Reson. Chem.* **1997**, *35*, 447.
(11) Unpublished result and personal communication from Dr. J. W. Szostak and Dr. B. Seelig.