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Bioorganic & Medicinal Chemistry Letters 16 (2006) 1665–1667

Bioorganic & Medicinal Chemistry Letters

Convenient methods for the synthesis of P^1-farnesyl-P^2-indicator diphosphates

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Received 10 November 2005; accepted 5 December 2005
Available online 10 January 2006

Abstract—To access P^1-farnesyl-P^2-indicator diphosphates, more efficient methods for the synthesis of farnesyl-phosphate and diphosphates were developed. The procedures reported here provide more flexible conditions than the conventional imidazolide and morpholidate coupling methods. Milder conditions for the synthesis of sensitive allylic diphosphates and greatly improved reaction efficiencies provide access to novel reagents for analysis of diphosphate-based enzymatic reactions.

Plants, fungi, and other organisms synthesize terpenoid natural products from allylic diphosphate starting materials.[1] Sensitive assays for production of terpenoids could link consumption of the allylic diphosphate starting material with turnover of an indicator dye. To develop novel substrates for monitoring such reactions, we report efficient syntheses of farnesyl diphosphates attached to indicator moieties as shown in Figure 1. The improved syntheses described here are applicable to a wide range of biosynthetic substrates.

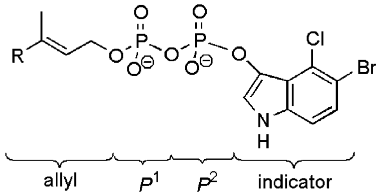

Figure 1.

Allylic diphosphates are unstable, especially in the presence of acids[2,3] or strong nucleophiles, which complicates their synthesis and manipulation. Furthermore, such amphiphilic diphosphates often form emulsions, preventing the removal of inorganic impurities by extraction.

Typically, P^1-terpenoid-P^2-substituted diphosphates are formed in the following three steps. First, an allylic phosphate is synthesized via Cramer's method (activation of the alcohol with trichloro-acetonitrile and reaction with bis(triethylammonium) phosphate), which usually results in a mixture of mono- and diphosphates.[3] Second, the monophosphate is converted to a P^1-allyl-P^2-diphenyl diphosphate, which is finally coupled to a second phosphate with the diphenyl phosphate moiety functioning as a leaving group.[4,5] In our hands, this method, especially the second step, proved unsatisfactory due to instability of the resultant diphenyl diphosphate. Other coupling methods have also been reported,[6] but, in general, the procedures are unsatisfactory, due to their slow reaction rates and poor yields (e.g., 2% overall yield for P^1-(tetra-*O*-acetyl-α-D-galactopyranosyl) P^2-farnesyl diphosphate from farnesol).[4]

Recently, Ryu and Scott reported an efficient method for coupling commercially available diphosphates to allyl chlorides or homoallyl tosylates.[7] The yields achieved through this method are very good, but require availability of the mono-substituted diphosphate. In most cases, however, the diphosphate must be synthesized first, which is often non-trivial. For example, the synthesis of 5-bromo-4-chloro-3-indolyl diphosphate from the commercially available monophosphate is quite time consuming and provides only low yields of the diphosphate. Therefore, we focused on the development of a different method for the synthesis of farnesyl diphosphate derivatives that would access a wide variety of compounds from readily available starting materials.

Keywords: Disubstituted diphosphate; Farnesyl derivatives; Enzyme pseudosubstrates; Indoxyl derivative.

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doi:10.1016/j.bmcl.2005.12.016

The reactivity of a phosphate or diphosphate anion is strongly influenced by the associated counter ion. Solubility, stability, and nucleophilicity of the respective phosphate can be tuned by the choice of counter ion. Davisson et al. have demonstrated that the tetra-butylammonium counter ion greatly improves the yield of terpenoid diphosphate formation from allylic chlorides or bromides.[8] Therefore, we also used a tetra-butylammonium salt for the formation of farnesyl monophosphate.

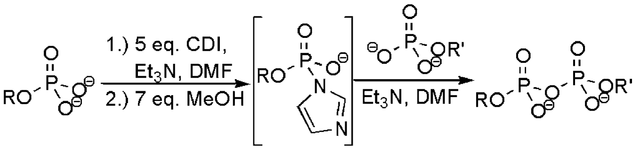
Accordingly, 85% phosphoric acid was titrated with 40% tetra-butylammonium hydroxide to pH 9.8. Lyophilization yielded tacky, highly hygroscopic bis(tetra-*n*-butylammonium) hydrogen phosphate (**2**). This salt was reacted with freshly prepared farnesyl bromide in DMF for 18 h.[9] The crude product was submitted to a column of Dowex 50WX8 (NH_4^+-form). Inorganic phosphate was removed through precipitation with organic solvents and the resulting product was extracted with ether to remove traces of unreacted farnesyl bromide (**1**). The farnesyl monophosphate (**3**) obtained by this method was used for subsequent reactions without further purification (Scheme 1).

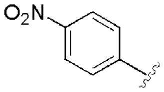
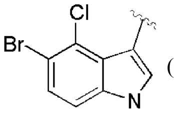
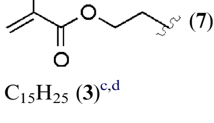
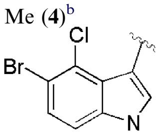
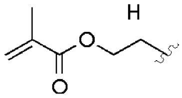
Two different methods for coupling farnesyl phosphate to a series of different phosphates were investigated. Cramer, Schaller, and Staab introduced 1,1′-carbonyldiimidazole (CDI) as a useful coupling reagent for phosphates,[10] but Ryu and Scott reported very slow coupling of geranyl phosphate with the imidazolides of adenosine and guanosine monophosphates, including reaction times greater than a month.[7] By optimizing the reaction conditions, we were able to decrease the reaction time to 3–6 days and obtain good yields for the resulting diphosphates.

Phosphates **4**–**6** were converted to bis(triethylammonium) salts and were then treated with five equivalents of CDI. Imidazolide formation was complete after two hours, as shown by an upfield shift of the ^{31}P NMR signals. Excess CDI was quenched with a stoichiometric amount of methanol. Subsequently, farnesyl phosphate **3** was added and the reaction was monitored by ^{31}P NMR. After a few hours, two new signals were detected that marked formation of the diphosphate. After three to six days, only traces of starting material remained and no further product formation could be detected. The crude compounds were submitted to preparative LC–MS under basic conditions (Table 1). This procedure has worked well with a wide range of starting materials, ill suited to previously reported conditions.

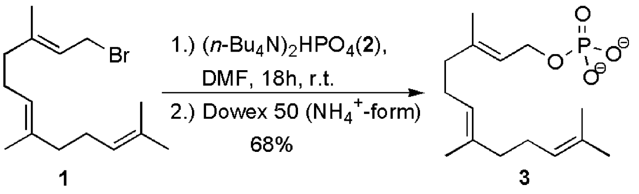
The presence of CDI and imidazole in the reaction mixture compromises the robustness of the reaction. For example, reaction of **7** with CDI led to the formation of three different products as determined by ^{31}P NMR. Most likely, this mixture resulted from hydrolysis of the ester by imidazole formed during the reaction. Similarly, in the reaction with the indigo dye precursor, 5-bromo-4-chloro-3-indolyl phosphate **6**, the indolyl nitrogen reacted with CDI, and the methyl carbamate formed. This was removed by stirring the crude product in aqueous methanol with K_2CO_3 at room temperature for 1–3 days, though more efficient reaction conditions could be envisioned.

Table 1. Coupling of phosphates with 1,1′-carbonyldiimidazole



R	R′	Time	Yield[a]
Me (**4**)[b]	$C_{15}H_{25}$ (**3**)[c,d]	87 h	16% (**8**)
 (**5**)[e]	$C_{15}H_{25}$ (**3**)[c,d]	87 h	16% (**9**)
 (**6**)[f]	$C_{15}H_{25}$ (**3**)[c,d]	145 h	14% (**10**)[g]
 (**7**)[b]		n.r.[h]	
$C_{15}H_{25}$ (**3**)[c,d]	Me (**4**)[b]	87 h	12% (**8**)
$C_{15}H_{25}$ (**3**)[c,d]	 (**6**)[f]	145 h	41% (**10**)
$C_{15}H_{25}$ (**3**)[c,d]	 (**7**)[b]	87 h	3% (**11**)

[a] Based upon the initial monophosphate ($ROPO_3^{2-}$).
[b] Free acid.
[c] Bisammonium salt.
[d] $C_{15}H_{25}$ = farnesyl.
[e] Bis(cyclo-hexylammonium) salt.
[f] Free acid or *p*-toluidine salt.
[g] After removal of methylcarbamate moiety.
[h] Initial monophosphate ($ROPO_3^{2-}$) decomposed upon reaction with CDI.



Scheme 1.

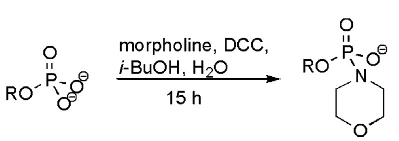
To improve the synthesis of diphosphates **10** and **11**, the reverse reaction order was explored. Farnesyl phosphate **3** was first converted to the imidazolide and then reacted with phosphates **4**, **6**, and **7**, respectively. Through this route, diphosphate **10** was obtained in good yield. The ester-based phosphate **11** could also be synthesized by this strategy, though yields remained low.

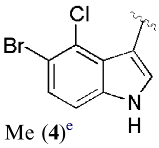
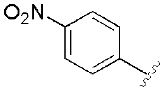
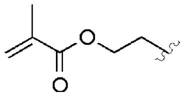
Due to the difficulties encountered in the synthesis of diphosphates **10** and **11**, other coupling methods were evaluated. The tetrazole-catalyzed coupling of phospho-morpholidates to other phosphates,[11,12] for example, was attempted. Unfortunately, the Moffatt and

Khorana procedure for morpholidate synthesis caused decomposition of **6**, and no product could be detected. However, a simple change of the solvent system from H_2O/*t*-butanol to H_2O/*i*-butanol led to quantitative formation of the desired morpholidate. This new solvent system led to better yields and cleaner products in the morpholidate formation for phosphates **4–6** (Table 2). This improvement is likely due to the fact that *i*-butanol and water are only slightly miscible. Therefore, the phosphate likely remains mainly in the aqueous phase and the morpholine in the organic phase. This separation diminishes hydrolysis of the phosphate by the strongly basic morpholine. Morpholidate formation of phosphates **3** and **7** could not be achieved using either method. Therefore, diphosphate **11** remains inaccessible through the morpholidate method.

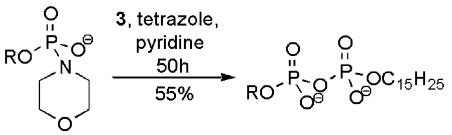
The synthesis of diphosphate **10** demonstrates the usefulness of the tetrazole-catalyzed coupling reaction for allylic diphosphates. After two days reaction time, the solvent was evaporated, and diphosphate **10** was isolated via preparative LC–MS in 55% yield (Scheme 2).

Table 2. Synthesis of phosphomorpholidates by improved conditions



R	Yield[a] (H_2O/*t*-BuOH)	Yield[a] (H_2O/*i*-BuOH)
 (**6**)[b]	n.r.[c]	99% (**12**)
Me (**4**)[e]	n.a.[d]	84% (**13**)
 (**5**)[e]	39% (**14**)	100% (**14**)
$C_{15}H_{25}$ (**3**)[f]	n.r.[c]	n.r.[c]
 (**7**)[e]	n.r.[c]	n.r.[c]

[a] 4-Morpholine-*N*,*N′*-dicyclohexylcarboxamidinium salt of morpholidate.
[b] *p*-Toluidine salt.
[c] No reaction.
[d] Not available.
[e] Free acid.
[f] Ammonium salt.



12 **10**

R = 5-bromo-4-chloro-indolyl

Scheme 2.

In summary, we present a significantly faster method for the synthesis of farnesyl monophosphate, which can easily be extended to the synthesis of other allyl monophosphates. We also evaluated and optimized two different monophosphate coupling methods for the formation of P^1-farnesyl-P^2-substituted diphosphates. Though the use of morpholidate intermediate compounds can result in better yields for diphosphates with imidazole- or CDI-sensitive functionalities, it is less convenient than the CDI-catalyzed coupling reactions, requiring two separate reaction steps in place of one. Furthermore, as demonstrated here, a broader variety of diphosphates are accessible via CDI coupling, including compounds inaccessible via the morpholidate route, such as diphosphate **11**. The reactions presented in this publication demonstrate that the CDI-based reaction conditions can be used in the presence of esters, Michael acceptors, and unprotected amines.

Supporting information

Experimental procedures, NMR, and mass spectral data for compounds **1–3** and **8–14** are available.

Acknowledgments

We gratefully acknowledge support by the ACS Petroleum Research Fund and the Arnold and Mabel Beckman Foundation. We also thank Brett Howard for preliminary experiments and Dr. John Greaves for the acquisition of high resolution mass spectral data.

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