Communication

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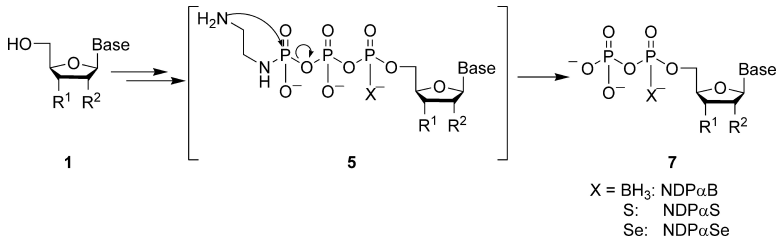

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Synthesis of α-P-Modified Nucleoside Diphosphates with Ethylenediamine

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At the forefront of antiviral therapeutics has been the design of new classes of nucleosides and nucleotides.[1] Most nucleoside analogues become active after a series of phosphorylation steps (through mono- and diphosphates) to their triphosphate forms.[1] Thus, nucleoside di- (NDP) and triphosphates (NTP) are ubiquitous biological molecules, and their analogues could have important diagnostic and therapeutic applications. For example, phosphorothioate analogues have been well studied and employed to determine enzymatic mechanisms.[2] Recently, boranophosphate analogues[3] have shown promising therapeutic applications in antiviral drug research.[4] The *Rp*-α-boranotriphosphate is the preferred isomer as a chain terminator with viral reverse transcriptase,[4a,b,5] while the viral DNA repair by pyrophosphorolysis is significantly reduced compared with its parent nucleotide.[5c−f] In addition, an increased phosphorylation activity of the human NDP kinase (NDPK) toward nucleoside *Rp*-α-boranodiphosphate (*Rp*-NDPαB) is observed.[5a−c] Although these α-P-modified nucleotide analogues have wide applications, their preparation, especially NDPαB,[6] remains a challenge.[7]

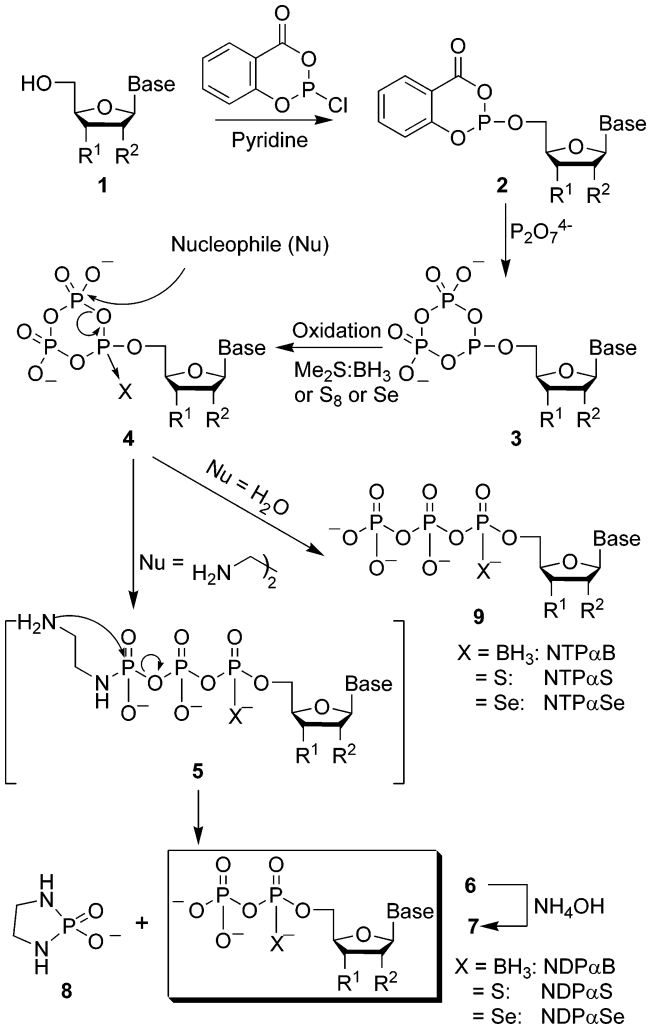
The successful preparation of various α-P-modified NTP analogues **9** by the Ludwig−Eckstein approach[8] prompted us to explore its use for the synthesis of α-P-modified NDP analogues in conjunction with ethylenediamine, which has been shown to act as a dephosphorylating reagent.[9] On the basis of these findings, we have developed a novel method for the synthesis of α-P-modified NDP analogues **7**, as outlined in Scheme 1. Since our main interest was the borane analogues, the applicability of this procedure for the synthesis of NDPαB was examined using all eight common deoxyribo- and ribonucleosides.

The opening of the cyclic structure **4** by nucleophilic attack usually occurs at the phosphate moiety rather than at the phosphorus atom bearing the borane group.[10] Therefore, treatment of intermediates **4a**−**h** with ethylenediamine resulted in NDPαB **6a**−**h** and cyclic phosphorodiamidate **8**.[11] The formation of these two products could be explained by ring opening of **4** first to **5**, followed by a second, intramolecular nucleophilic displacement. It is worth mentioning that such a two-step mechanism has suggested a new concept for designing drugs targeting Ras, a major protein responsible for the formation of human tumors.[12a] Furthermore, the use of diamine derivatives in the ring opening reaction[12b] may also suggest novel solutions for NTP derivatives with controlled stability.

We demonstrate that the same method can also be employed to synthesize NDPαS and NDPαSe[13] analogues. The final products **7a**−**j** were isolated in good yields (Table 1) along with the H-phosphonate as the major byproduct in about 15% yield, which might derive from intermediate **2**.[8c]

Modification at the α-phosphate group produces a pair of P-diastereomers, which were successfully resolved by RP-HPLC

Scheme 1. One-Pot Synthesis of NDPαB, NDPαS, and NDPαSe with Ethylenediamine[a]



[a] See Supporting Information for R1, R2, and the base protection.

as described in Table 1. The absolute configurations of NDPαB were recently determined by cocrystallization with NDPK,[5c] in which the first and second eluting diastereomers from HPLC are *Rp* and *Sp* isomers, respectively.[4d,6] Since the group priorities around α-P for NDPαS and NDPαSe are opposite to those of NDPαB, and assuming the same order of elution in HPLC, the assignment of configuration for ADPαS and ADPαSe are *Sp* and *Rp* for the first and second eluting diastereomers, respectively.

The assignment of the absolute configuration for both P-diastereomers was further confirmed by ^{1}H NMR. A difference in the chemical shift ($\Delta\delta$) for H8 of ADP analogues is observed between the two diastereomers, as shown in Table 2. The H8 signal

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Table 1. Synthesis and HPLC Profile of α-Modified NDP Analogues

compound		yield[a]	HPLC condition[b] t_R (min) [area %] MeOH %	*Rp* isomer	*Sp* isomer
dADPαB	**7a**	22	9	5.58 [45.2]	11.97 [54.8]
dGDPαB	**7b**	32	9	10.69 [49.3]	13.29 [50.7]
dCDPαB	**7c**	30	6	10.41 [48.0]	17.48 [52.0]
dTDPαB	**7d**	31	6	6.72 [48.9]	9.02 [51.1]
ADPαB	**7e**	28	8	8.53 [50.5]	17.24 [49.5]
GDPαB	**7f**	26	7	10.73 [51.5]	18.38 [48.5]
CDPαB	**7g**	29	5	10.10 [50.4]	13.62 [49.6]
UDPαB	**7h**	30	8	6.57 [59.9]	10.19 [40.1]
ADPαS	**7i**	30	9	11.11 [48.8]	7.09 [51.2]
ADPαSe	**7j**	19	8	10.34 [51.1]	5.47 [48.9]

[a] Yield calculated in percentage by UV. [b] Waters Delta-Pak C18, 15 μm, 100 Å, 3.9 × 300 mm eluted at 1 mL/min with isocratic condition of 100 mM TEAB (pH = 8.0) and MeOH.

Table 2. Chemical Shifts of α-Modified ADP Analogues in D_2O[a]

compound	H8	H2	H1′	H2′	H3′	H4′	H5′	H5″
7e, ADPαB								
I-*Rp*	8.45	8.08	5.99	4.60	4.47	4.23	4.11	4.00
II-*Sp*	8.43	8.08	5.99	4.62	4.39	4.24	4.11	4.00
7i, ADPαS								
I-*Sp*	8.51	8.03	5.94	4.61	4.47	4.22	4.15	4.05
II-*Rp*	8.44	8.03	5.94	4.61	4.47	4.22	4.15	4.05
7j, ADPαSe								
I-*Sp*	8.57	8.10	5.99	4.66	4.49	4.26	4.19	4.09
II-*Rp*	8.48	8.09	5.99	4.64	4.47	4.27	4.16	4.11

[a] Proton assignment based on $^1H-^1H$ COSY.

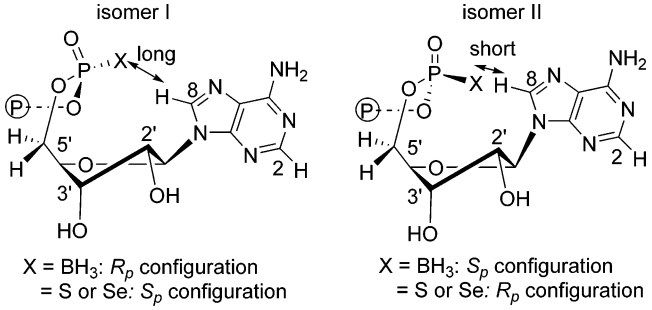

Figure 1. Conformation of P-diastereomers of ADP analogues.

of isomer II is more shielded than that of isomer I due to the influence of the vicinal X group (Figure 1) at the α-P position.[14] Calculation by Fischer indicated that the P^{α} is much further away from H8 in isomer I than in isomer II (Figure 1).[14] Thus, the $\Delta\delta$ between H8 of isomers I and II is attributed to a difference in the proximity of H8 to the negative charge on X. Because the distance between X and H8 decreases as the P−X bond length increases for P−B (1.91 Å) < P−S (1.96 Å) < P−Se (2.24 Å),[15] the $\Delta\delta$ for H8 between two diastereomers increases accordingly with **7e** < **7i** < **7j**. Moreover, the larger $\Delta\delta$ of H3′ in **7e**, but not in **7i** and **7j**, may be attributed to the existence of the B−H bond (1.25 Å) in addition to the P−B bond, which results in the close proximity of the BH_3 group and H3′ in *Sp*-ADPαB.

To better understand the biological effect of BH_3 substitution, we investigated the binding affinity of *Rp*- and *Sp*-ADPαB to rabbit muscle creatine kinase (CK) and pyruvate kinase (PK), which may be responsible for the last phosphorylation of some antiviral NDP analogues.[16] By using a fluorescence quenching technique,[17] the results indicate that both enzymes bound two NDP molecules per CK dimer and PK tetramer with strong negative cooperativity. Moreover, opposite stereospecificity toward ADPαB was observed for CK (*Sp* preferred) and PK (*Rp* preferred), and the preferred isomer bound more tightly than ADP.

In summary, we have developed an efficient and convenient protocol to synthesize α-P-modified NDP analogues that are otherwise difficult to obtain. The absolute configurations of P-diastereomers were confirmed by analysis of their ^1H NMR. Affinity studies revealed that the NDPαB is potentially useful in antiviral research. This protocol guarantees the availability of these biologically important compounds for further study.

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Supporting Information Available: Experimental procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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