Published in final edited form as:
J Org Chem. 2011 June 17; 76(12): 5132–5136. doi:10.1021/jo200045a.

α-Azido bisphosphonates: synthesis and nucleotide analogues

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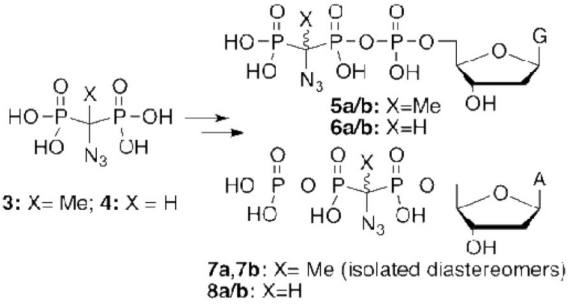
Abstract



The first examples of α-azido bisphosphonates $[(RO)_2P(O)]_2CX(N_3)$ (**1**, R = *i*-Pr, X = Me; **2**, R = *i*-Pr, X = H; **3**, R = H, X = Me; **4**, R = H, X = H) and corresponding β,γ-$CX(N_3)$ dGTP (**5–6**) and α,β-$CX(N_3)$ dATP (**7–8**) analogues are described. The individual diastereomers of **7** (**7a, 7b**) were obtained by HPLC separation of the dADP synthetic precursor (**14a/b**).

Triphosphate analogues in which a bridging oxygen is replaced by a functionalized carbon atom have been of interest as enzymatic probes since the early 1980s.[1,2] Recently, we described the preparation of a series of highly purified and well characterized β,γ-CXY[3,4] and α,β-CXY[5] dNTP derivatives and applied them as probes of structure, function, and fidelity in DNA polymerase β (pol β),[3–5] a base excision repair (BER) enzyme that is overexpressed in some cancers. By appropriate substitution on the CXY carbon (X, Y= H, Me, F, Cl, Br) a range of stereoelectronic properties is made available to probe binding and catalytic interactions with the enzyme. Linear free-energy relationship (LFER) plots of DNA pol β-catalyzed dGMP incorporation rates into nascent DNA from β,γ-CXY dGTPs have revealed a base match- dependent leaving group effect,[4] and X-ray crystallographic data have provided evidence for stereospecific binding of analogues containing a CXF group.[3,6]

To extend these studies, we wished to construct β,γ (**5**, **6**) and α,β (**7**, **8**) dNTPs that incorporate a bridging $CX(N_3)$ group. As a pseudohalide[7] the azido functionality could usefully extend the tunability of the analogue series, providing more information on molecular interactions at the active site arising from the stereochemistry at the bridging carbon. Introduction of the azido group might also facilitate separation of individual CXY diastereomers, which has not been accomplished with the various X,Y = methyl and halide derivatives prepared thus far.[3,5] These considerations, combined with the prospect of

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Supporting Information Available: IR (**1–4**), ^{1}H NMR (**1–8**, **14**, **15**), gCOSY (**1**, **2**), ^{13}C NMR (**1–4**), ^{31}P NMR (**1–8**, **14**, **15**), HRMS (**3–8**), UV/VIS and CD spectra (**7a**, **7b**), HPLC data for **5–8**, ^{31}P NMR spectrum for conversion of **2** to **12** and **13**, ^{31}P NMR of **5a/b** at two field strengths, ^{1}H and ^{31}P NMR and MS data for product from reaction of phosphonoacetate carbanion with trifyl azide. Available free of charge via the Internet at http://pubs.acs.org.

developing a new and potentially useful bisphosphonate synthon, motivated an investigation of α-azido bisphosphonic esters and acids, a previously unknown class of compounds (Figure 1).

Electrophilic azido transfer was explored as a methodology offering a direct route to **1** and **2** from readily available starting materials. In this approach, a sulfonyl azide reacts with a carbanion species to generate a triazene intermediate that can fragment into either diazo or azido products.[8] Various factors influence the fragmentation pathway, but the nature of the sulfonyl azide is particularly important. Sulfonyl azides with sterically bulky and electron-donating substituents, such as 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide; TrN_3), favor azido transfer[8,9] while those containing strongly electron-withdrawing moieties, e.g. trifluoromethylsulfonyl azide (triflyl azide; TfN_3), favor diazo transfer.[9,10]

Although the methylene group of a bisphosphonate ester such as tetraisopropyl methylenebis(phosphonate) **9** is exposed in principle to either pathway, replacement of one α-H by a methyl group, as in tetraisopropyl ethylidenebis(phosphonate) **10**, will enforce azido transfer. In the synthesis of **1** from **10**, methylation of **9** with potassium tert-butoxide (*t*-BuOK) followed by methyl iodide gave a mixture of 78% **10** and 20% α,α-dimethyl side product. Fortunately, this crude **10** preparation could be directly treated with *t*-BuOK and then *p*-toluenesulfonyl azide (tosyl azide; TsN_3) in DMSO at room temperature to give the azido ester **1** in 70% yield after purification by column chromatography (Scheme 1).

Attempts to synthesize **2** from **9** using tosyl azide led to the unwanted diazo transfer product. Reaction of a carbanion derived from an activated methylene group with a sulfonyl azide is a known route to tetraalkyl diazomethylenebis(phosphonates) and trialkyl α-diazo phosphonoacetates.[11] However, use of trisyl azide with careful optimization of conditions (THF, −78° C, potassium counterion, fivefold azide reagent excess, immediate quenching by AcOH) generated **2** from the carbanion of **9** in greater than 95% yield by ^{31}P NMR (Scheme 2). Despite the efficient conversion, purification of **2** proved to be problematic initially due to difficulty in removing the large excess of trisyl azide and a minor side product, tetraisopropyl diazomethylenebis(phosphonate) (**11**), by preparative chromatography on silica gel. Pure **2** (50% yield) was eventually obtained by crystallization of remaining trisyl azide, filtration, and selective oxidation of **11** with *t*-butyl hypochlorite[12] in wet ethyl acetate[13] prior to chromatography.

Care must be taken during work-up of **2**, which retains a relatively acidic α-H, because it decomposes slowly on silica gel, more quickly in aqueous solution at pH 6, and rapidly at pH 8. This contrasts with the reported stability of the malonate derivative[14] and presumably reflects the resonance stabilization available in the enolate form of the latter compound.

Exposure of **2** to bases such as BuLi, NaH, KHMDS, and *t*-BuOK results in the release of N_2 with formation of diisopropyl cyanophosphate (**12**)[15] and diispropyl phosphite (**13**) (Scheme 3). The base-induced elimination of nitrogen from α-azido esters,[16] malonates,[14] and sulfones[17] has been previously reported and transition metal-catalyzed versions of this reaction have found synthetic utility.[18,19] In contrast to diethyl azidomalonate, in which the carbanion was further derivatized at −15 °C without the loss of N_2,[14] **2** loses N_2 rapidly upon exposure to a variety of bases in organic solvents at −78 °C and does not give appreciable amounts of adducts in the presence of a large excess of N-chlorosuccinimide.

The structures of **1–4** are supported by their suite of IR, NMR, and MS data. Esters **1** and **2** are both colorless oils with strong IR absorbances in the azido region (**1**: 2123, 2086 cm^{-1}; **2**: 2104, sh. at 2134 cm^{-1}). The ^1H NMR spectrum of **1** includes a CH_3 triplet at δ 1.60 ppm (t, J_{HP} 15 Hz) and that of **2** has an α-H triplet at δ 3.53 ppm (J_{HP} 21 Hz). The ^{13}C NMR spectrum of **1** has resonances at δ 17.6 (CH_3) and δ 59.8 ppm (C_α t, J_{CP} 150 Hz). The C_α of

2 resonates at δ 55.0 ppm (t, J_{CP} 145 Hz). The ^{31}P chemical shifts of **1** (δ 17.0 ppm) and **2** (δ 13.3 ppm) are consistent with a previously observed 3–4 ppm downfield effect of α-methylation in other bisphosphonate esters.[20,21]

Reaction of **1** and **2** with bromotrimethylsilane (BTMS)[22] in acetonitrile at room temperature quantitatively gave the tetrakis(trimethylsilyl) esters, which display the expected upfield ^{31}P shifts, Δδ +16–17 ppm. Desilylation with aqueous ethanol produced the acids **3** and **4** (> 98% overall) as hygroscopic white solids. These acids are stable in dilute aqueous solution pH 2–12 for at least several days at room temperature. They have IR bands at 2126, 2085 (sh) cm^{-1} (**3**) and 2113 cm^{-1} (**4**) attributed to the azido group. The ^{1}H NMR spectra include resonances at δ 1.43 ppm (C(CH_3)N_3, t, J_{HP} 13 Hz) for **3** and δ 3.26 ppm (CHN_3, t, J_{HP} 17 Hz) for **4** (CHN_3). The ^{13}C NMR displays resonances at δ 23.5 (CH_3) and δ 68.4 ppm ($C_α$, t, J_{CP} 129 Hz) for **3**, and at δ 65.1 ppm (t, J_{CP} 124 Hz) ($C_α$) for **4**. The ^{31}P chemical shifts are in the expected regions, δ 18.3 ppm (**3**) and δ 13.5 ppm (**4**). The HRMS m/z values for the [M−H]$^-$ anion of **3** and **4** match calculated values within error, confirming the assigned structures.

The present study and our previous interest in diazo transfer chemistry[23,24] prompted us to reinvestigate a reported azido transfer to triethyl phosphonoacetate using triflyl azide in the presence of triethyl amine.[25] Triflyl azide is known to be a powerful diazo transfer reagent[9] and attempts by other authors to extend the procedure to benzocyclic β-keto esters were unsuccessful;[8] nevertheless, the original reaction has been cited as a viable azido transfer reaction in recent reviews.[26,27] In our hands, the procedure of Hakimelahi and Just[25] produced triethyl diazophosphonoacetate, characterized by ^{1}H, ^{13}C, ^{31}P NMR and MS (ESI and APCI) and generated no detectable amounts of azido transfer product. In light of these results and analysis of the literature,[28] we propose that the reaction of triflyl azide with triethylphosphonoacetate is consistent with known diazo transfer chemistry and is not an anomalous case of azido transfer.

With acids **3** and **4** in hand, we then proceeded to the synthesis of β,γ (**5**, **6**) and α,β (**7**, **8**) azido methylene nucleotide triphosphate analogues. The β,γ-CXN_3 dGTP analogues **5** and **6** were prepared by coupling[3,4] the Bu_3NH^+ salts of **3** and **4** with the dGMP-morpholidate.[29] α,β-CXN_3 analogues of dATP (**7, 8**) were synthesized by reaction of the Bu_4N^+ salt of **3** or **4** with dA-5'-tosylate followed by enzymatic phosphorylation of the resulting α,β-C(XN_3) dADP intermediates (**14, 15**) by ATP-phosphoenolpyruvate (PEP) catalyzed by pyruvate kinase (PK) and nucleoside diphosphate kinase (NDPK) in HEPES buffer, pH 7.5[5] (Scheme 4). The final compounds were purified by dual-pass (strong-anion exchange 'SAX' followed by reverse phase 'RP') HPLC to ≥ 97% as determined by ^{31}P NMR and analytical HPLC, and were characterized by NMR (^{1}H, ^{31}P) and HRMS.

Nucleotide triphosphate α,β-CXY or β,γ-bisphosphonate analogues with an asymmetrically substituted bridging carbon (X ≠ Y) are obtained as mixtures of diastereomers by existing synthetic procedures starting from a prochiral bisphosphonate precursors, and thus far none have been separated chromatographically.[3, 5, 6] Diastereomeric β,γ-fluoromethylene dNTPs can be individually observed in the synthetic mixture by ^{19}F NMR.[3,6] In addition to the expected nucleoside resonances for dGuo, the ^{1}H NMR of **5a/b** shows a pair of doublets centered at δ 1.58–1.59 ppm (C(CH_3)N_3) and that of **6a/b** an apparent triplet at δ 3.40 ppm (CHN_3). However, although the proton-decoupled $P_α$ and $P_γ$ ^{31}P NMR signals of **5a/b** at 202 MHz are doublets at δ −9.45 and 14.5 ppm respectively, two resolvable (Δδ 0.03 ppm) diastereomeric pairs of doublets (J = 34, 19 Hz) are observed for $P_β$. The δ vs. J assignments were confirmed by comparing ^{31}P spectral data obtained at 162 and 202 MHz.

A similar pattern is seen with **6a**/**6b** except that resolvable diastereomeric multiplets are observed for both P_α (δ −9.78, −9.81 ppm) and P_β (δ 9.04, 9.06 ppm), but not for P_γ (10.4 ppm). This difference between **5a**,**b** and **6a**,**b** with respect to the sensitivity of the P_α chemical shift to the $P_{\beta\gamma}$ CXN_3 chiral center invites further investigation (we speculate that it might implicate an H-bonding effect involving the relatively acidic CHN_3 hydrogen, or alternatively reflects a more asymmetric shielding effect of the CHN_3 group on the P_α nucleus).

In the preparation of the α,β-$C(CH_3)N_3$ ATP analogues **7a** and **7b,** we found that the corresponding diphosphate intermediates (**14a/b**) could be isolated individually by isocratic RP HPLC (Figure 2). Subsequent enzymatic phosphorylation as described above then generated the individual diastereomers **7a** and **7b**. CD spectra measured for **7a** and **7b** showed prominent features of opposite polarity centered near 260 nm (**7a**, (−); **7b** (+)). Curiously, the α,β-CHN_3 dADP intermediate (**15a/b**) did not separate under similar conditions and its phosphorylation yielded the triphosphates **8a/b** as a mixture of diastereomers which could not be distinguished by ^{31}P NMR.

In conclusion, we have described the first examples of α-azido bisphosphonate esters and acids. The latter compounds can be used to synthesize novel nucleotide analogues containing a CHN_3 or $CMeN_3$ at either the α,β or β,γ bridging position. The ability to obtain for the first time the individual diastereomers of the α,β-CH_3N_3 dADP (**14a**, **14b**) and corresponding dATP analogues (**5a**, **5b**) provides more refined probes for stereochemical interactions of these compounds with appropriate enzymes. A further use of these new bisphosphonates may be to prepare derivatives made more conveniently or uniquely accessible by the presence of the synthetically versatile azido function.

Experimental

General Experimental Methods

The nucleotide triphosphate analogues were prepared by adapting our previously published methods for β,γ-CXY[3,4,6] and α,β-CXY[5] dNTP analogues. Analytical HPLC analysis was conducted on a Varian PureGel SAX 10 mm × 100 mm 7 μL column eluted with A: H_2O B: 0.5 M (0–50%) LiCl gradient over 30 min at a 4 mL/min flow rate. Products were detected at 259 nm for adenosine derivatives and at 253 nm for guanosine derivatives. All dNTP derivatives were prepared as triethylammonium salts.[3–6] CD spectra were obtained on a JASCO J-815 spectropolarimeter.

Tetraisopropyl (1-azidoethane-1,1-diyl)bis(phosphonate), 1

Tetraisopropyl methylenebis(phosphonate) (1.75 g, 5 mmol) was treated with 1.2 equiv. *t*-BuOK and 1.2 equiv. MeI in anhydrous acetonitrile overnight at rt. Volatiles were removed at reduced pressure and the residue was dissolved in water, extracted into DCM and dried over $MgSO_4$. Evaporation of the solvent left an oil (1.84 g) that contained ~20% dimethylated product, ~2% unmethylated material, and 78% monomethylated product. This mixture (~4 mmol monomethylated product) was dissolved in 20 mL DMSO and stirred with 0.689 g (6 mmol) *t*-BuOK. TsN_3 1.05 g (5 mmol) was added dropwise. The reaction mixture was stirred for 30 s, quenched with 1.5 mL of AcOH, and then allowed to stir for several hours at rt. The reaction mixture was extracted with hexanes and the organic phase was dried over $MgSO_4$. Following concentration by evaporation, the product was purified on silica gel using ACE:DCM (1:3), R_f = 0.69, giving **1** as a colorless oil. Yield: 1.45 g (70% overall). IR: ν (film) 2123 cm^{-1}, 2086 (N_3). δ_H (399.8 MHz; $CDCl_3$): 1.33-1.37 (24 H, m, 4 × OCH(CH_3)$_2$), 1.60 (3H, t, J_{HP} 15.2 Hz, PCCH_3N_3P), 4.83 (4 H, m, 4 × OC*H*(CH_3)$_2$); δ_C (100.5 MHz; $CDCl_3$): 17.6 (t, J_{CP} 3.9 Hz, PC*C*H_3N_3P), 23.7-23.8, 24.3-24.4 (OCH(*C*H_3)$_3$, 59.8 (t, J_{CP} 150.3 Hz, P*C*P), 72.6-72.8 ((O*C*H(CH_3)$_3$; δ_P (161.9

MHz; $CDCl_3$; 85% H_3PO_4): 17.0 ({^{1}H} s; coupled J_{PH} 15.2 Hz (qm), J_{PC} 150 Hz (satellites)).

Tetraisopropyl (azidomethanediyl)bis(phosphonate), 2

In a 500 mL RB flask under N_2, ~6 g (19 mmol) of TrN_3 were dissolved in 30 mL of anhydrous THF and stirred vigorously at −78 °C. A solution of 1.517 g (4.41 mmol) of tetraisopropyl methylenebis(phosphonate) and 4.41 mL 1M KHMDS in 10 mL THF was mixed at rt, cooled to −78 °C and added at once to the TrN_3 solution by syringe. The solution was stirred for 10 s and then quenched with 2.5 mL AcOH. The flask was moved to a −20 °C freezer and allowed to stand for 48 h, or until the triazene had decomposed as determined by ^{31}P NMR. The precipitate was filtered and volatiles removed at reduced pressure. The reaction mixture was then seeded with solid TrN_3 and allowed to sit overnight at 0 °C. The resulting precipitate was washed with H_2O and filtered off. After removing water with rotovap, the residue was dissolved in 20 mL wet EtOAc. Ten drops of *t*-BuOCl were added and stirred at rt until evolution of N_2 ceased. EtOAc was washed with a saturated aqueous NH_4Cl solution, dried and concentrated. The residue (95% by ^{31}P NMR) was purified on silica gel eluted with ACE:DCM (1:9), R_f = 0.36. Contaminating phosphite was removed by rotary evaporation (oil pump) with gentle warming to afford 0.83 g of **2** as a colorless oil (yield: 50% overall). IR: ν (film) 2134 (m), 2104 cm^{-1} (N_3). NMR: δ_H (399.8 MHz; $CDCl_3$): 1.31-1.34 (24 H, m, 4 × OCH(C*H*$_3$)$_2$), 3.53 (1H, t, J_{HP} 20.8 Hz, PC*H*N_3P), 4.79 (4 H, m, 4 × OC*H*$(CH_3)_2$); δ_C (100.5 MHz; $CDCl_3$): 23.7-23.8, 24.1-24.2 **(OCH(*C*$H_3)_3$, 55.0 (t, J_{CP} 144.8 Hz, P*C*P), 72.7, 72.9 (2t, J_{CP} 3.5 Hz O*C*H$(CH_3)_3$); δ_P (161.9 MHz; $CDCl_3$; 85% H_3PO_4): 13.3 ({^{1}H} coupled, s; J_{PH} 20.5 Hz (dm), J_{PC} 145 Hz).

Base-induced decomposition of 2

5 mg (13 μmol) of **2** was dissolved in 1.5 mL anhydrous THF and cooled to −78 °C. 1.5 equiv. *t*-BuOK was added at once, causing rapid evolution of gas. Identical results were obtained with BuLi and KHMDS. NMR: δ_P (202.5 MHz; $CDCl_3$; 85% H_3PO_4): 22.29 (diisopropyl cyanophosphate),[15] 4.8 (diispropyl phosphite) with integration 1:1.

(1-Azidoethane-1,1-diyl)bis(phosphonic acid), 3 and (azidomethanediyl)bis(phosphonic acid), 4

The appropriate bisphosphonate ester was dissolved in 2 mL anhydrous acetonitrile and 6 equiv. of BTMS were added by syringe. Stirring overnight gave the tetrakis(trimethylsilyl) esters (100% by ^{31}P NMR). From **1**: δ_P (202.5 MHz; CHCN, ext. 85% H_3PO_4): 0.9; from **2**: δ_P −3.2 (s). Hydrolysis with ~1 mL of EtOH:H_2O (1:1) and removal of volatiles by prolonged evaporation at low pressure provided the acids as hygroscopic white solids (>98% overall yield). Acid is further dried under vacuum in desiccator containing P_2O_5.

3: white solid, IR: ν (KBr): 2085 (m), 2126 cm^{-1} (N_3). NMR: δ_H (399.8 MHz; D_2O; pH 10.88): 1.43 (t, J_{HP} 13.2 Hz, PCC*H*$_3$$N_3$P); δ_C (100.5 MHz; D_2O; ext. pH 10.88): 23.5 (s, PC*C*H_3P), 68.4 (t, J_{CP} 129, P*C*P); δ_P (161.9 MHz; D_2O; 85% ext. H_3PO_4; pH 10.88): 18.3 ({^{1}H} s; coupled, J_{PH} 13.2 Hz (q); J_{PC} 129 Hz (satellites)). HRMS (ESI/APCi) [M−H]$^-$: calcd for $C_2H_6N_3O_6P_2$: 229.9737. Found: 229.9734.

4: IR: ν (KBr): 2113 cm^{-1} (N_3). δ_H (399.8 MHz; D_2O; pH 10.88): 3.26 (t, J_{HP} 16.8 Hz, PC*H*N_3P); δ_C (100.5 MHz; D_2O; pH 10.88): 65.1 (t, J_{CP} 124 Hz, P*C*P); δ_P (161.9 MHz; D_2O; 85% ext. H_3PO_4; pH 10.88): 13.5 ({^{1}H} s; coupled, J_{PH} 16.5 Hz (d); J_{PC} 122 Hz (satellites)); HRMS (ESI/APCi) [M−H]$^-$: calcd for $CH_4N_3O_6P_2$, 215.9581. Found: 215.9584.

Ethyl diazo(diethoxyphosphoryl)acetate

from trifyl azide and ethyl(diethylphosphoryl)acetate. According to the procedure of Hakimelahi and Just,[25] 72 mg NaN_3 (1.1 mmol) was suspended in 20 mL anhydrous DMF and 106 μL (1.0 mmol) CF_3SO_2Cl was added under N_2. After several min, a solution of 222 mg (1.0 mmol) of ethyl(diethylphosphoryl)acetate and 101 mg (1.0 mmol) Et_3N in 5 mL anhydrous DMF was added dropwise. After 40 min, a mixture of compound was detected by ^{31}P NMR with δp (DMF) 20.8, 18.3, 10.4 in a ratio of 5:4:1. Gentle heating for an additional 30 min gave a binary mixture of the compounds at 10.4 (40%) and 20.8 (60%). Ether was added and the reaction mixture was washed 5 × with water. The organic layer was dried over $MgSO_4$ and evaporated. A portion of the residue was purified by preparative TLC on silica gel eluted with ACE:DCM (1:9) giving a single mobile band (UV), $R_f = 0.65$ which was identified as ethyl diazo(diethoxyphosphoryl)acetate. NMR: δ_H (400.2 MHz; $CDCl_3$): 1.32 (3H, t, J_{HH} 7 Hz), 1.38 (6H, dt, J_{HH} 7 Hz, J_{HP} 0.8 Hz), 4.22 (4H, m), 4.29 (2H, q, J_{HH} 7 Hz); δ_P (202.5 MHz, $CDCl_3$; 85% H_3PO_4) 10.3 (s). MS (APCi, m/z), 251 $(M+1)^+$; an MS/MS analysis of the m/z 251 species, gave a major fragment at m/z 223.[11]

2'-Deoxyguanosine 5'-triphosphate β,γ CCH_3N_3, 5a/b

Compound **3** (2 equiv. as the 1.5 tributylammonium salt) was coupled to 100 mg (0.242 mmol) dGuo-morpholidate in anhydrous DMSO to yield a white solid, 32.5 mg (23.8%) after dual pass (SAX then RP) HPLC purification.[4] Analytical HPLC: retention time = 9.6 min; purity = 98%. NMR: δ_H (399.8 MHz; D_2O; pH 10.88): 1.58 (dd, PCC*H*$_3$N$_3$P) 2.48-2.54, 2.72-2.79, 4.11-4.21, 4.12, 6.32 8.05. δ_P (161.9 MHz; D_2O; 85% H_3PO_4; pH 10.88): −9.5 (d, $J_{\alpha\beta}$ 33.7 Hz, P_α), 13.28 (dd, $J_{\alpha\beta}$ 33.7 Hz, $J_{\beta\gamma}$ 19.4 Hz, P_β), 13.31 (dd, $J_{\alpha\beta}$ 33.7 Hz, $J_{\beta\gamma}$ 19.4 Hz, P_β) 14.5 (d, $J_{\beta\gamma}$ 19.4 Hz, P_γ). HRMS (ESI/APCi) $[M-H]^-$: calcd for $C_{12}H_{18}N_8O_{12}P_3$, 559.0263. Found: 559.0266.

2'-Deoxyguanosine 5'-triphosphate β,γ CHN_3, 6a/b

Compound **4** (2 equiv. as the 1.5 tributylammonium salt) was coupled to 100 mg (0.242 mmol) dGuo-morpholidate in anhydrous DMSO to yield a white solid, 31.1 mg (23.5%) **6a/b** after dual pass (SAX then RP) HPLC purification.[4] Analytical HPLC: retention time = 10.3 min, purity = 98%. NMR: δ_H (499.8 MHz; D_2O; pH 10.88): 2.48-2.53, 2.71-2.76, 3.53 (ddd, PC*H*N$_3$P), 4.10-4.20, 4.25, 6.31 (t, *J* 6.0 Hz) 8.03, 8.03; δ_P (161.9 MHz; D_2O; 85% H_3PO_4; pH 10.88): −9.78 (d, $J_{\alpha\beta}$ 27.8 Hz, P_α), −9.81 (d, *J* 27.8 hz P_α), 9.04 (dd, $J_{\alpha\beta}$ 27.8 Hz, $J_{\beta\gamma}$ 12.9 Hz, P_β), 9.06 (dd, $J_{\alpha\beta}$ 27.8 Hz, $J_{\beta\gamma}$ 12.9 Hz, P_β) 10.414 (d, $J_{\beta\gamma}$ 12.9 Hz, P_γ). HRMS (ESI/APCi) $[M-H]^-$: calcd for $C_{11}H_{16}N_8O_{12}P_3$, 545.0106. Found: 545.0121.

2'-Deoxyguanosine 5'-diphosphate, α,β $C(CH_3)N_3$, 14a/b

The tris(tetrabutylammonium) salt from 60 mg (0.260 mmol) of **3** was allowed to react with 115 mg (~1.1 equiv.) dA-5'-Ts in anhydrous acetonitrile[5] to yield 65.1 mg of the nucleoside diphosphonate diasteromers in a ratio of 2:3; (54% crude conversion), which could be separated by RP HPLC. **14a**: NMR: δ_H (399.8 MHz; D_2O; pH 10.88): 1.50 (dd, J_{HP} 12.4, J_{HP} 15.2 Hz, PCC*H*3N3P), 2.57-2.63, 2.82-2.91, 4.10-4.16, 4.22-4.27, 6.48 (t, 6.4 Hz), 8.25, 8.52; δ_P (161.9 MHz; D_2O; 85% H_3PO_4; pH 10.88) 14.5 (d, *J* 15 Hz, P_β); 21.7 (d, *J* 15 Hz, P_α). **14b**: NMR: δ_H (399.8 MHz; D_2O; pH 10.88): 1.50 (dd, J_{HP} J_{HP} 12.8, 15.2 Hz, PCC*H*3N3P), 2.57-2.63, 2.82-2.88, 4.10-4.16, 4.22-4.27, 6.47 (t, 6.4 Hz), 8.22, 8.50; δ_P (161.9 MHz; D_2O; 85% H_3PO_4; pH 10.88) 14.4 (d, *J* 15 Hz, P_β); 21.6 (d, *J* 15 Hz, P_α).

2'-Deoxyadenosine 5'-triphosphate α,β $C(CH_3)N_3$, 7a/b

Enzymatic phosphorylation[5] of **14a** and **14b** followed by HPLC purification of each product gave 17.3 mg (yield: 12.2% overall from starting acid) **7a** and 14.9 mg (10.5%) **7b**. **7a:** Analytical HPLC: retention time = 9.5 min; purity = 98%. NMR: δ_H (499.8 MHz; D_2O; pH

10.88): 1.58 (t, J_{HP} 15.0 Hz, PCC*H*$_3$N$_3$P), 2.58-2.63, 2.83-2.89, 4.21-4.29, 6.50 (t, 6.5 Hz), 8.25, 8.54; δ_P (202.5 MHz; D_2O; 85% H_3PO_4; pH 10.88) −4.5 (d, $J_{\beta\gamma}$ 31.9 Hz, P_γ); 7.0 (dd, P_β); 18.51 (d, $J_{\alpha\beta}$ 19.0 Hz, P_α). HRMS (ESI/APCi) $[M-H]^-$: calcd for $C_{12}H_{18}N_8O_{11}P_3$, 543.0313. Found: 543.0333. **7b:** Analytical HPLC: retention time = 9.5 min; purity = 96%. NMR: δ_H (499.8 MHz; D_2O; pH 10.88): 1.59 (t, J_{HP} 15 Hz, PCC*H*$_3$N$_3$P), 2.58-2.63, 2.85-2.90, 4.17-4.22, 6.51 (t, 6.5 Hz), 8.26, 8.56; δ_P (202.3 MHz; D_2O; 85% H_3PO_4; pH 10.88) −4.4 (d, $J_{\beta\gamma}$ 30.1 Hz, P_γ); 8.0 (dd, P_β); 18.47 (d, $J_{\alpha\beta}$ 18.2 Hz, P_α). HRMS (ESI/APCi) $[M-H]^-$: calcd for $C_{12}H_{18}N_8O_{11}P_3$, 543.0315. Found: 543.0315.

2'-Deoxyguanosine 5'-diphosphate, α,β CHN_3, 15a/b

The tris(tetrabutylammonium) salt was prepared from 60 mg (0.276 mmol) of **4** and allowed to react with 120 mg (~1.1 equiv.) dAdo-5'-Ts in anhydrous acetonitrile[5] to yield a white solid, 32 mg (26%). NMR: δ_p (242.8; D_2O; pH 10.88): 9.9 (s, P_β); 17.2 (s, P_α).

2'-deoxyadenosine 5'-triphosphate α,β CHN_3, 8a/b

Enzymatic phosphorylation[5] followed by HPLC purification gave 33.2 mg (22.7%) **8a/b**. Analytical HPLC: retention time = 10.4 min; purity = 98%. NMR: δ_H (400.2 MHz; D_2O; pH 10.88): 2.57-2.63, 2.82-2.90, 3.86 (dt, PC*H*N$_3$P), 4.11-4.25, 4.29, 6.50 (t, *J* 6.5 Hz), 8.24, 8.528, 8.532; δ_P (202.3 MHz; D_2O; 85% H_3PO_4; pH 10.88) −5.1 (d, $J_{\beta\gamma}$ 25.1 Hz, P_γ); 2.8 (dd, P_β); 14.3 (d, $J_{\alpha\beta}$ 13.8 Hz, P_α). HRMS (ESI/APCi) $[M-H]^-$: calcd for $C_{11}H_{16}N_8O_{11}P_3$, 529.0157. Found: 529.0147.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by NIH grant CA105010. We thank Ms. Inah Kang for assistance in preparing the manuscript and Dr. Ron New (UC Riverside Mass Spec Facility) for obtaining the HRMS data.

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28. The IR band at 2100 cm^{-1} for the putative azido product[25] is within the range of literature values[30, 31] for triethyl diazophosphonoacetate including a sample prepared by a methodology that could not generate an azido product.[32] The ^{1}H NMR data do not positively identify a PC(HX)C hydrogen. The α-proton is distinguishable in analogous H-F,[33] H-Cl, and H-Br[34] triethyl phosphonoacetates and should be apparent in authentic trialkyl azidophosphonoacetate. The C.I. MS data in the original report identifies a m/z 223 peak which was assigned to a $[M-N_3]^+$ species. However, this peak also corresponds to a prominent fragment which we observe in the MS/MS of the triethyl diazophosphonoacetate parent peake $[M+1]^+$ at m/z 251. Finally, the authors supported their structure by reducing their product to the aminophosphonoacetate with H_2 Pd/C in EtOH. *t*-Butyl diazo(diethoxyphosphoryl)acetate is converted to a cognate $PC(HNH_2)C$ product under these conditions,[35] demonstrating that the reduction would not necessarily distinguish the azido compound from triethyl diazophosphonoacetate.
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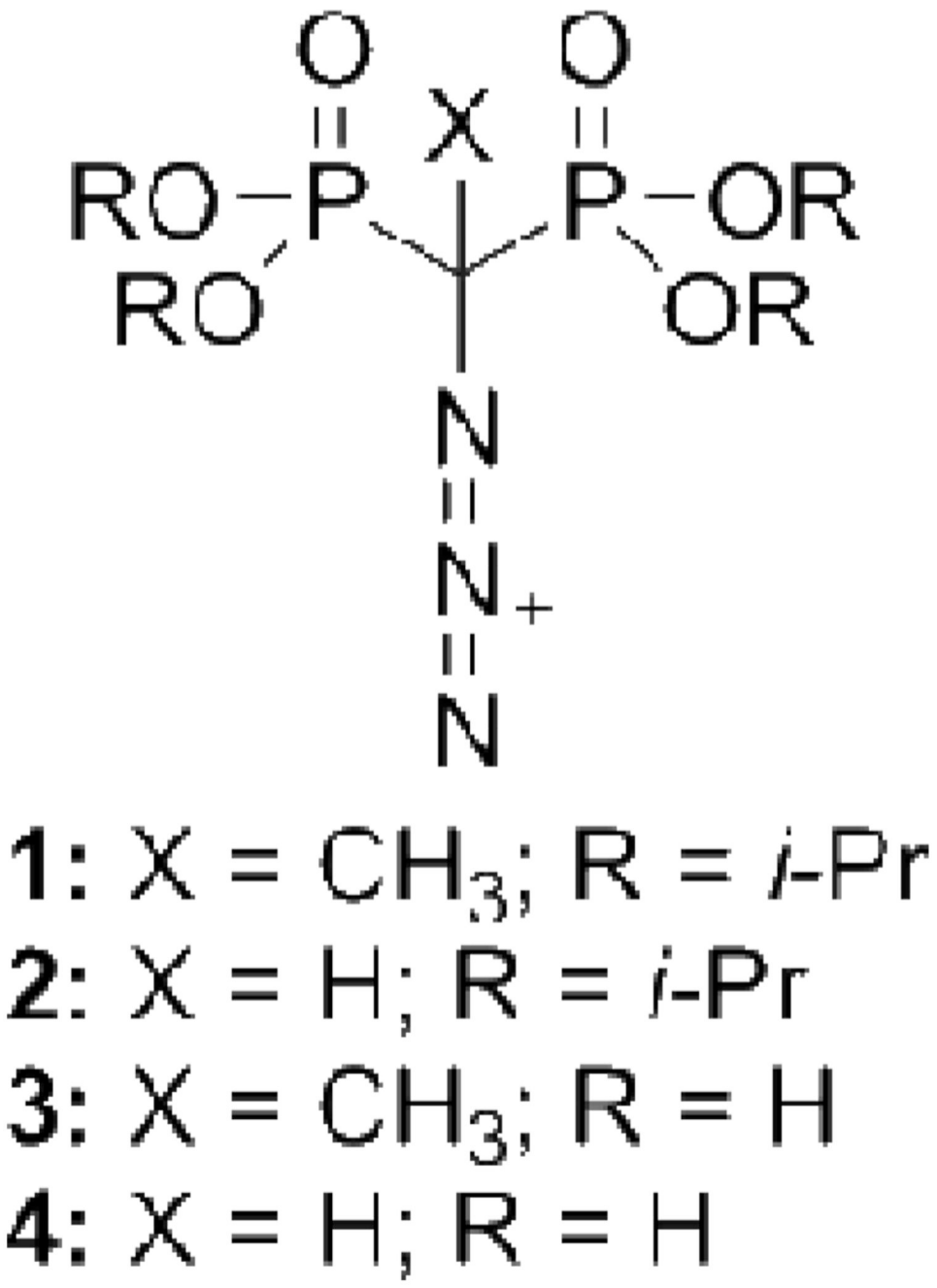


FIGURE 1.
Structures of α-azido bisphosphonate esters and acids.

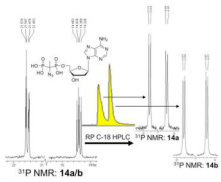
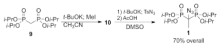
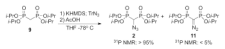


FIGURE 2.

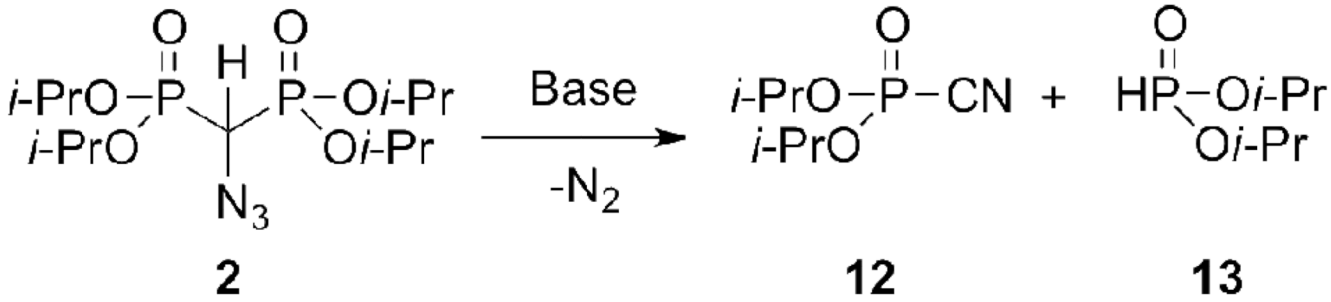
Separation of **14** diastereomers by RP preparative HPLC. Left: ^{31}P NMR spectrum of **14a/b** as synthesized displays two pairs of doublets for both P_α and P_β. Right: ^{31}P NMR (**14a** and **14b**) spectra of separated diastereomers show single pairs of doublets for P_α and P_β.



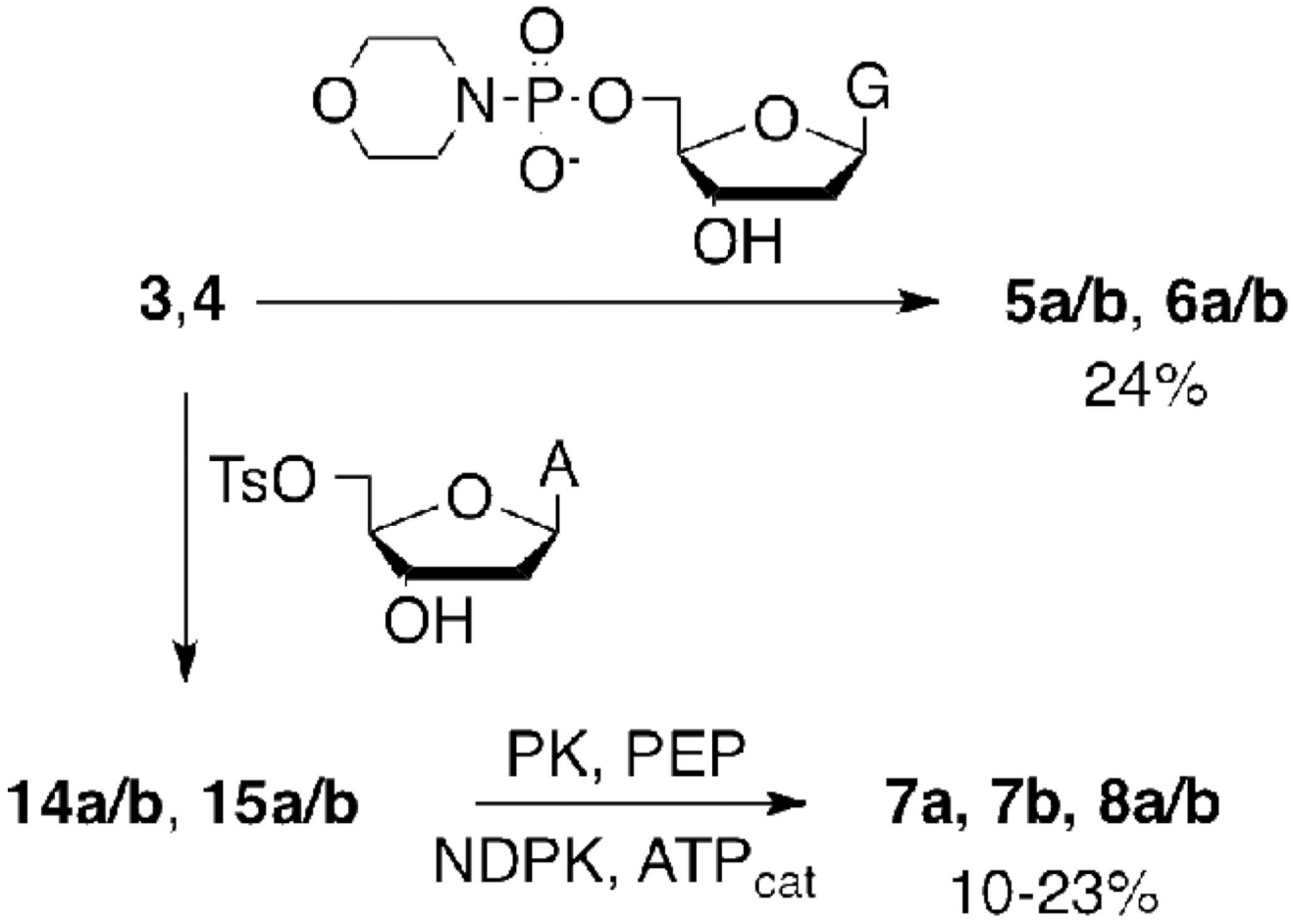
SCHEME 1.
Synthesis of **1**.



SCHEME 2.
Synthesis of **2**.



SCHEME 3.
Base-induced decomposition of **2**.



SCHEME 4.
Synthesis of nucleotide azidomethylene analogues.