

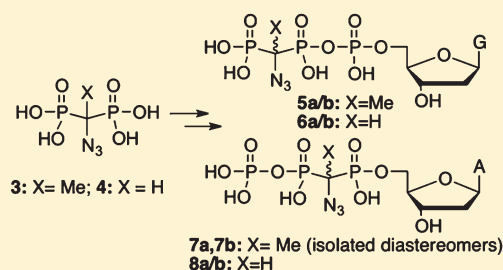
α -Azido Bisphosphonates: Synthesis and Nucleotide Analogues

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Supporting Information

ABSTRACT: The first examples of α -azido bisphosphonates [(RO)₂P(O)]₂CXN₃ (**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 -CXN₃ dGTP (**5–6**) and α,β -CXN₃ dATP (**7–8**) analogues are described. The individual diastereomers of **7** (**7a/b**) 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 -CXY⁵ 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 CXN₃ 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 = H, methyl or halide derivatives prepared thus far.^{3,5} These considerations, combined with the prospect of 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

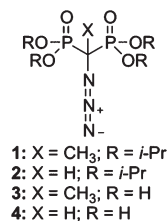
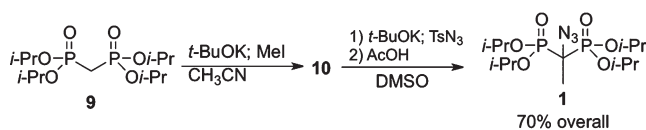


Figure 1. Structures of α -azido bisphosphonate esters and acids.

Scheme 1. Synthesis of **1**



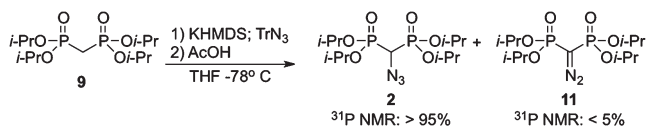
and electron-donating substituents, such as 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide; TrN₃), favor azido transfer^{8,9} whereas those containing strongly electron-withdrawing moieties, for example trifluoromethylsulfonyl azide (triflyl azide; TfN₃), favor diazo transfer.^{9,10}

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; TsN₃) in DMSO at room temperature to give the azido

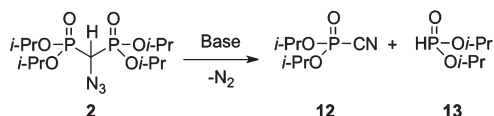
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Scheme 2. Synthesis of 2



Scheme 3. Base-Induced Decomposition of 2



ester **1** in 70% yield after purification by column chromatography (Scheme 1).

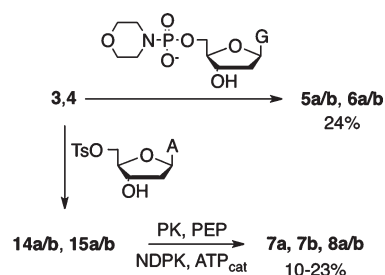
Attempts to synthesize **2** from **9** using tosyl azide led to the unwanted diazo transfer product. Reaction of a carbanion derived from an activated methylene group with a sulfonyl azide is a known route to tetraalkyl diazomethylenebis(phosphonates) and trialkyl α -diazo phosphonoacetates.¹¹ However, use of trisyl azide with careful optimization of conditions (THF, -78 °C, potassium counterion, 5-fold azide reagent excess, immediate quenching by AcOH) generated **2** from the carbanion of **9** in greater than 95% yield by ³¹P 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 hypochlorite¹² in wet ethyl acetate¹³ prior to chromatography.

Care must be taken during workup 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 derivative¹⁴ 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 N₂ with formation of diisopropyl phosphorocyanidate (**12**)¹⁵ and diisopropyl phosphite (**13**) (Scheme 3). The base-induced elimination of nitrogen from α -azido esters,¹⁶ malonates,¹⁴ and sulfones¹⁷ 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 N₂,¹⁴ **2** loses N₂ 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 CH₃ triplet at δ 1.60 ppm (*J*_{HP} 15 Hz) and that of **2** has an α -H triplet at δ 3.53 ppm (*J*_{HP} 21 Hz). The ¹³C NMR spectrum of **1** has resonances at δ 17.6 (CH₃) 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 ³¹P chemical shifts of **1** (δ 17.0 ppm) and **2** (δ 13.3 ppm) are consistent

Scheme 4. Synthesis of Nucleotide Azidomethylene Analogues



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 ³¹P shifts, $\Delta\delta$ + (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 ¹H NMR spectra include resonances at δ 1.43 ppm (C(CH₃)N₃, *t*, *J*_{HP} 13 Hz) for **3** and δ 3.26 ppm (CHN₃, *t*, *J*_{HP} 17 Hz) for **4**. The ¹³C NMR displays resonances at δ 23.5 (CH₃) and δ 68.4 ppm (*C* _{ω} , *t*, *J*_{CP} 129 Hz) for **3**, and at δ 65.1 ppm (*C* _{ω} , *t*, *J*_{CP} 124 Hz) for **4**. The ³¹P 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 reagent⁹ 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 Just²⁵ produced triethyl diazophosphonoacetate, characterized by ¹H, ³¹P 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 -CXN₃ dGTP analogues **5** and **6** were prepared by coupling^{3,4} the Bu₃NH⁺ salts of **3** and **4** with dGMP-morpholidate.²⁹ α,β -CXN₃ analogues of dATP (**7, 8**) were synthesized by reaction of the Bu₄N⁺ salt of **3** or **4** with dA-5'-tosylate followed by enzymatic phosphorylation of the resulting α,β -CXN₃ dADP intermediates (**14, 15**) by ATP-phosphoenolpyruvate (PEP) catalyzed by pyruvate kinase (PK) and nucleoside diphosphate kinase (NDPK) in HEPES buffer, pH 7.5⁵ (Scheme 4). The final compounds were purified by dual-pass (strong-anion exchange, SAX, followed by reverse phase, RP) HPLC to $\geq 97\%$ as determined by ³¹P NMR and analytical HPLC and were characterized by NMR (¹H, ³¹P) and HRMS.

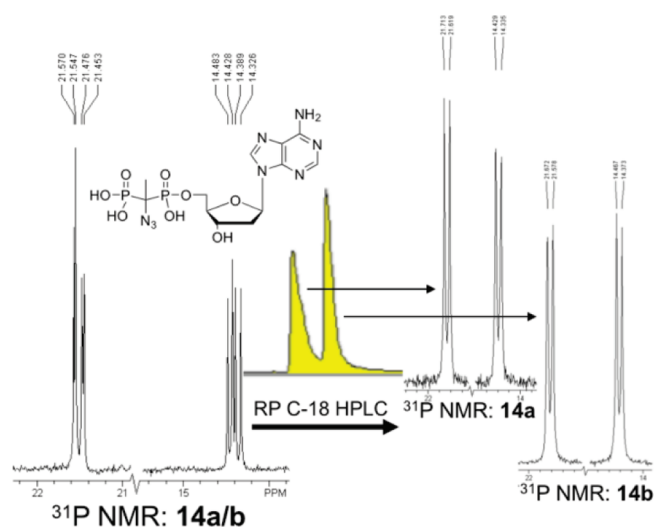


Figure 2. Separation of **14** diastereomers by RP preparative HPLC. (Left) ^{31}P NMR spectrum of **14a/b** as synthesized displays two pairs of doublets for both P_α and P_β . (Right) ^{31}P NMR (**14a** and **14b**) spectra of separated diastereomers show single pairs of doublets for P_α and P_β .

Nucleotide triphosphate α,β -CXY or β,γ -bisphosphonate analogues with an asymmetrically substituted bridging carbon ($X \neq Y$) are obtained as mixtures of diastereomers by existing synthetic procedures starting from 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 ^1H NMR of **5a/b** shows a pair of doublets centered at δ 1.58–1.59 ppm ($\text{C}(\text{CH}_3)_2\text{N}_3$) 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** are doublets at δ –9.45 and 14.5 ppm respectively, two resolvable ($\Delta\delta$ 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/b** 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 $\text{P}_\beta\gamma$ CXN_3 chiral center invites further investigation.

In the preparation of the α,β - $\text{C}(\text{CH}_3)_2\text{N}_3$ dATP analogues **7a** and **7b**, we found that the corresponding dADP bisphosphonate 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 $\text{C}(\text{CH}_3)_2\text{N}_3$ 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 - $\text{C}(\text{CH}_3)_2\text{N}_3$ dADP (**14a**, **14b**) and corresponding dATP analogues (**7a**, **7b**) 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 SECTION

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 -CXY⁵ dNTP analogues. Analytical HPLC analysis was conducted on a Varian PureGel SAX 10 mm \times 100 mm 7 μL column eluted with an 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 of *t*-BuOK and 1.2 equiv of 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 of DMSO and stirred with 0.689 g (6 mmol) of *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, 2086 cm^{-1} (N_3). δ_{H} (399.8 MHz; CDCl_3): 1.33–1.37 (24 H, m, $4 \times \text{OCH}(\text{CH}_3)_2$), 1.60 (3H, t, $J_{\text{HP}} 15.2$ Hz, $\text{PCCH}_3\text{N}_3\text{P}$), 4.83 (4 H, m, $4 \times \text{OCH}(\text{CH}_3)_2$); δ_{C} (100.5 MHz; CDCl_3): 17.6 (t, $J_{\text{CP}} 3.9$ Hz, $\text{PCCH}_3\text{N}_3\text{P}$), 23.7–23.8, 24.3–24.4 ($\text{OCH}(\text{CH}_3)_3$), 59.8 (t, $J_{\text{CP}} 150.3$ Hz, PCP), 72.6–72.8 ($(\text{OCH}(\text{CH}_3)_3)$; δ_{P} (161.9 MHz; CDCl_3 ; ext. 85% H_3PO_4): 17.0 ($\{^1\text{H}\}$ s; coupled $J_{\text{PH}} 15.2$ Hz (qm), $J_{\text{PC}} 150$ 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 of 1 M 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 by 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 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, $4 \times \text{OCH}(\text{CH}_3)_2$), 3.53 (1H, t, $J_{\text{HP}} 20.8$ Hz, PCHN_3P), 4.79 (4 H, m, $4 \times \text{OCH}(\text{CH}_3)_2$); δ_{C} (100.5 MHz; CDCl_3): 23.7–23.8, 24.1–24.2 ($\text{OCH}(\text{CH}_3)_3$), 55.0 (t, $J_{\text{CP}} 144.8$ Hz, PCP), 72.7, 72.9 (2t, $J_{\text{CP}} 3.5$ Hz

OCH(CH₃)₃); δ_P (161.9 MHz; CDCl₃; ext. 85% H₃PO₄): 13.3 (¹H) s; coupled J_{PH} 20.5 Hz (dm), J_{PC} 145 Hz (satellites)).

Base-Induced Decomposition of 2. Five mg (13 μ mol) of **2** was dissolved in 1.5 mL of anhydrous THF and cooled to -78 °C. *t*-BuOK (1.5 equiv) was added at once, causing rapid evolution of gas. Identical results were obtained with BuLi and KHMDs. NMR: δ_P (202.5 MHz; CDCl₃; ext. 85% H₃PO₄): 22.3 (diisopropyl phosphorocyanidate),¹⁵ 4.8 (diisopropyl 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 ³¹P NMR). From **1**: δ_P (202.5 MHz; CH₃CN, ext. 85% H₃PO₄) 0.9. From **2**: δ_P -3.2 (s). Hydrolysis with ~ 1 mL of EtOH/H₂O (1:1) and removal of volatiles by prolonged evaporation at low pressure provided the acids as hygroscopic white solids (>98% overall yield). The acids were further dried under vacuum in a desiccator containing P₂O₅.

3: IR: ν (KBr): 2085 (m), 2126 cm⁻¹ (N₃). NMR: δ_H (399.8 MHz; D₂O; pH 10.88): 1.43 (t, J_{HP} 13.2 Hz, PCCH₃N₃P); δ_C (100.5 MHz; D₂O; ext. pH 10.88): 23.5 (s, PCCH₃P), 68.4 (t, J_{CP} 129 Hz, PCP); δ_P (161.9 MHz; D₂O; 85% ext. H₃PO₄; pH 10.88): 18.3 (¹H) s; coupled, J_{PH} 13.2 Hz (q); J_{PC} 129 Hz (satellites)). HRMS (ESI/APCI) [M - H]⁻: calcd for C₂H₆N₃O₆P₂: 229.9737. Found: 229.9734.

4: IR: ν (KBr): 2113 cm⁻¹ (N₃). δ_H (399.8 MHz; D₂O; pH 10.88): 3.26 (t, J_{HP} 16.8 Hz, PCHN₃P); δ_C (100.5 MHz; D₂O; pH 10.88): 65.1 (t, J_{CP} 124 Hz, PCP); δ_P (161.9 MHz; D₂O; 85% ext. H₃PO₄; pH 10.88): 13.5 (¹H) s; coupled, J_{PH} 16.5 Hz (d); J_{PC} 122 Hz (satellites)); HRMS (ESI/APCI) [M - H]⁻: calcd for CH₄N₃O₆P₂: 215.9581. Found: 215.9584.

Ethyl diazo(diethoxyphosphoryl)acetate from triflyl azide and ethyl(diethylphosphoryl)acetate. Following the procedure of Hakimelahi and Just,²⁵ 72 mg of NaN₃ (1.1 mmol) was suspended in 20 mL of anhydrous DMF and 106 μ L (1.0 mmol) of CF₃SO₂Cl was added under N₂. After several min, a solution of 222 mg (1.0 mmol) of ethyl (diethylphosphoryl)acetate and 101 mg (1.0 mmol) of Et₃N in 5 mL anhydrous DMF was added dropwise. After 40 min, a mixture of compounds were detected by ³¹P NMR with δ (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 δ_P 10.4 (40%) and 20.8 (60%). Ether (30 mL) was added and the reaction mixture was washed 5 \times with 5 mL water. The organic layer was dried over MgSO₄ 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; CDCl₃): 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, CDCl₃; 85% H₃PO₄) 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 -C(CH₃)N₃, 5a/b. Compound **3** (2 equiv of the 1.5 tributylammonium salt) was coupled to 100 mg (0.242 mmol) of 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; D₂O; pH 10.88): 1.58 (dd, PCCH₃N₃P) 2.48–2.54, 2.72–2.79, 4.11–4.21, 4.12, 6.32 8.05. δ_P (161.9 MHz; D₂O; 85% H₃PO₄; 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 C₁₂H₁₈N₈O₁₂P₃: 559.0263. Found: 559.0266.

2'-Deoxyguanosine 5'-Triphosphate β,γ -CHN₃, 6a/b. Compound **4** (2 equiv of the 1.5 tributylammonium salt) was coupled to 100 mg (0.242 mmol) of 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; D₂O; pH 10.88): 2.48–2.53, 2.71–2.76, 3.53 (ddd, PCHN₃P), 4.10–4.20, 4.25, 6.31 (t, J 6.0 Hz) 8.03, 8.03; δ_P (161.9 MHz; D₂O; ext. 85% H₃PO₄; 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) 10.41 (d, $J_{\beta\gamma}$ 12.9 Hz, P γ). HRMS (ESI/APCI) [M - H]⁻: calcd for C₁₁H₁₆N₈O₁₂P₃: 545.0106. Found: 545.0121.

2'-Deoxyadenosine 5'-Diphosphate, α,β -C(CH₃)N₃, 14a/b.

The tris(tetrabutylammonium) salt from 60 mg (0.260 mmol) of **3** was allowed to react with 115 mg (~ 1.1 equiv) of dA-5'-Ts in anhydrous acetonitrile⁵ to yield 65.1 mg of the nucleoside diphosphonate diastereomers in a ratio of 2:3 (54% crude conversion), which could be separated by RP HPLC. **14a:** NMR: δ_H (399.8 MHz; D₂O; pH 10.88): 1.50 (dd, J_{HP} 12.4, J_{HP} 15.2 Hz, PCCH₃N₃P), 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; D₂O; 85% H₃PO₄; 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; D₂O; pH 10.88): 1.50 (dd, J_{HP} 12.8, 15.2 Hz, PCCH₃N₃P), 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; D₂O; ext. 85% H₃PO₄; 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(CH₃)N₃, 7a/b.

Enzymatic phosphorylation⁵ of **14a** and **14b** followed by HPLC purification of each product gave 17.3 mg (yield: 12.2% overall from starting acid) of **7a** and 14.9 mg (10.5%) of **7b**. **7a:** Analytical HPLC: retention time = 9.5 min; purity = 98%. NMR: δ_H (499.8 MHz; D₂O; pH 10.88): 1.58 (t, J_{HP} 15.0 Hz, PCCH₃N₃P), 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; D₂O; 85% H₃PO₄; 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 C₁₂H₁₈N₈O₁₁P₃: 543.0313. Found: 543.0333. **7b:** Analytical HPLC: retention time = 9.5 min; purity = 96%. NMR: δ_H (499.8 MHz; D₂O; pH 10.88): 1.59 (t, J_{HP} 15 Hz, PCCH₃N₃P), 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; D₂O; ext. 85% H₃PO₄; 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 C₁₂H₁₈N₈O₁₁P₃: 543.0315. Found: 543.0315.

2'-Deoxyadenosine 5'-Diphosphate, α,β -CHN₃, 15a/b. The tris(tetrabutylammonium) salt was prepared from 60 mg (0.28 mmol) of **4** and allowed to react with 120 mg (~ 1.1 equiv) of dAdo-5'-Ts in anhydrous acetonitrile⁵ to yield a white solid, 32 mg (26%). NMR: δ_P (242.8; D₂O; pH 10.88): 9.9 (P β); 17.2 (P α).

2'-Deoxyadenosine 5'-Triphosphate α,β -CHN₃, 8a/b. Enzymatic phosphorylation⁵ of **15a/b** above 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; D₂O; pH 10.88): 2.57–2.63, 2.82–2.90, 3.86 (dt, PCHN₃P), 4.11–4.25, 4.29, 6.50 (t, J 6.5 Hz), 8.24, 8.528, 8.532; δ_P (202.3 MHz; D₂O; ext. 85% H₃PO₄; 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 C₁₁H₁₆N₈O₁₁P₃: 529.0157. Found: 529.0147.

ASSOCIATED CONTENT

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

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- (28) The IR band at 2100 cm^{-1} for the putative azido product²⁵ 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 ¹H 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-Br³⁴ 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 peak $[M + 1]^+$ at m/z 251. Finally, the authors supported their structure by reducing their product to the aminophosphonoacetate with H₂ (Pd/C) in EtOH. *t*-Butyl diazo(diethoxyphosphoryl)acetate is converted to a cognate PCH-(NH₂)C product under these conditions,³⁵ demonstrating that the reduction would not necessarily distinguish the azido compound from triethyl diazophosphonoacetate.
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