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THE ASSEMBLY OF β-METHYLENE-TAD, A METABOLICALLY STABLE ANALOGUE OF THE ANTITUMOR AGENT TAD, BY THE STEPWISE ESTERIFICATION OF MONODEPROTECTED METHYLENEBIS-(PHOSPHONATE) BENZYL ESTERS UNDER MITSUNOBU CONDITIONS

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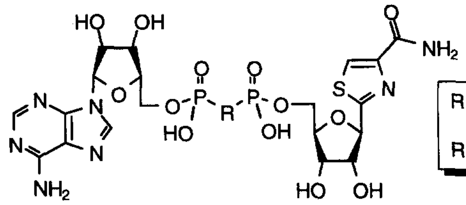
Abstract: Synthesis of the metabolically stable analogue of thiazole-4-carboxamide adenine dinucleotide (β-methylene-TAD) was achieved via the sequential monodeprotection of tetrabenzyl methylenebis(phosphonate) after two rounds of Mitsunobu esterifications with the corresponding nucleoside components, tiazofurin and adenosine. © 1999 Elsevier Science Ltd. All rights reserved.

2-β-*D*-Ribofuranosylthiazole-4-carboxamide (tiazofurin) is an oncolytic C-nucleoside with potent inhibitory activity of the cellular enzyme, inosine 5′-monophosphate dehydrogenase (IMPD).[3] Tiazofurin requires metabolic activation to the corresponding nicotinamide adenine dinucleotide (NAD) analogue, thiazole-4-carboxamide adenine dinucleotide (TAD), to compete with the natural cofactor NAD.[4] However, TAD is susceptible to cleavage by a unique phosphodiesterase present at high levels in tiazofurin-resistant tumors.[5] Since accumulation of TAD, as regulated by its synthetic and degradative enzymes, appears to be an important determinant for sensitivity to the drug, a hydrolytically resistant analogue of TAD was synthesized by replacing the middle oxygen of the pyrophosphate unit with a methylene group (β-methylene-TAD).[6] β-Methylene-TAD proved to be very stable and capable of producing the desired depletion of guanine nucleotide pools in experimental tiazofurin-resistant P388 tumor cells.[6]



R = O, Thiazole-4-carboxamide adenine dinucleotide (TAD)
R = CH_2, β-Methylene-TAD

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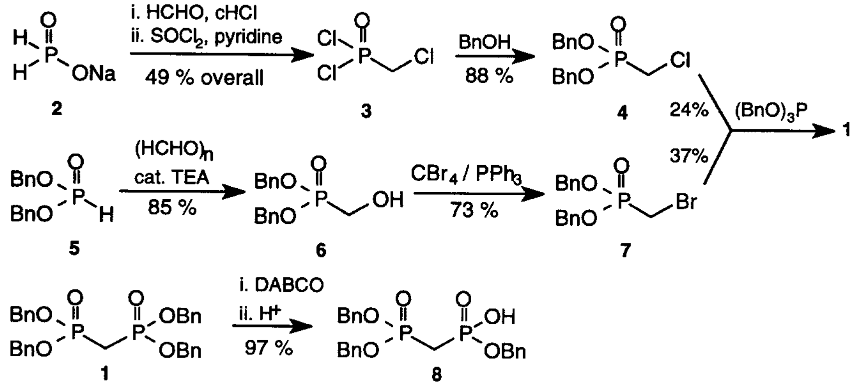
β-Methylene-TAD was originally synthesized by the dicyclohexylcarbodiimide (DCC)-catalyzed coupling of adenosine-5′-(α,β-methylene)diphosphate (protected as the 2′,3′-*O*-ethoxymethylene acetal) with tiazofurin (protected as the 2′,3′-*O*-isopropylidene ketal) in low yield.[6] This protocol and the more elegant and efficient method developed later by Pankiewicz et al.,[7] still require the purification of several key intermediates by reversed-phase HPLC, thus limiting the capacity of these methods to small scale syntheses. We now wish to report on a different approach that uses two rounds of Mitsunobu esterification starting from the partially protected [[bis(benzyloxy)phosphoryl]methyl]phosphonic acid monobenzyl ester (**8**).[8] The key elements of this approach are based on the significant work of Mioskowski et al. who described the selective monodeprotection of phosphonate benzyl esters[8] and the ensuing successful Mitsunobu esterifications of these monodeprotected substrates with conveniently protected nucleosides.[9,10]

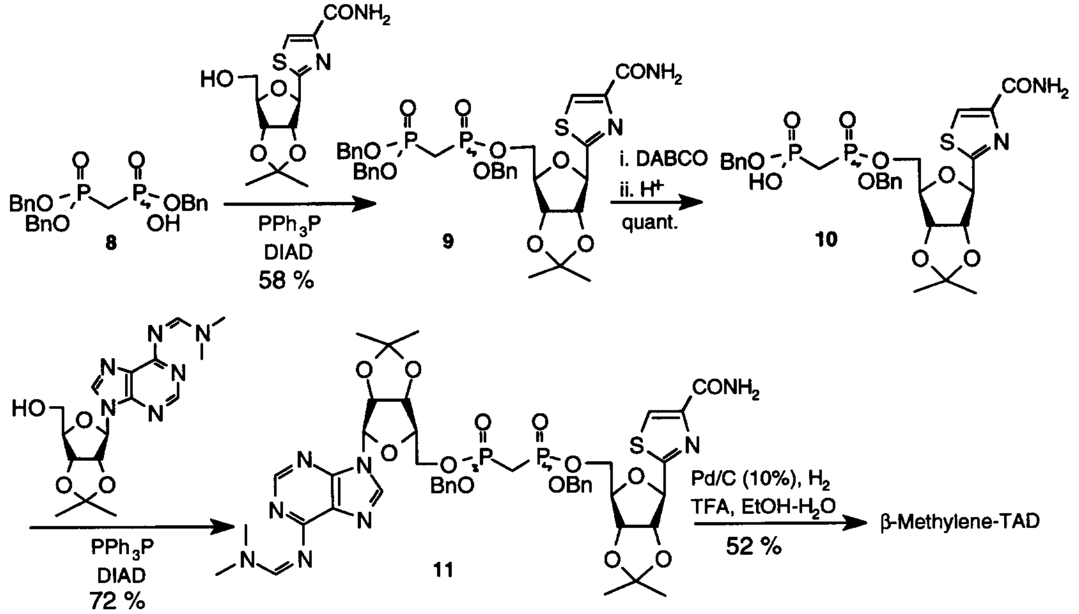
Critical to the success of this approach was the availability of adequate amounts of tetrabenzyl methylenebis(phosphonate) (**1**). This compound was first prepared according to published methods (Scheme 1).[11] Sodium hypophosphite (**2**) was treated with paraformaldehyde to give hydroxymethylphosphonic acid and immediately chlorinated with thionyl chloride in pyridine to give the corresponding chloromethyldichlorophosphonate (**3**). The latter was converted to dibenzylchloromethylphosphonate (**4**) and following a Michaelis–Arbuzov reaction with tribenzyl phosphite, compound **1** was isolated in 10% overall yield. We found a more convenient approach starting from dibenzyl bromomethylphosphonate (**7**), which was conveniently prepared by the Pudovik reaction starting with dibenzyl phosphite (**5**) and paraformaldehyde.[12] The resulting dibenzyl hydroxymethylphosphonate (**6**) was easily converted to **7** under standard bromination conditions (PPh_3/CBr_4), and following the Michaelis–Arbuzov reaction of **7** with tribenzyl phosphite, **1** was obtained in 23% overall yield. This latter method has several advantages in terms of ease of purification and mild reaction conditions.

Scheme 1



The effective assembly of β-methylene-TAD (Scheme 2) relied on the selective monodeprotection of tetrabenzyl methylenebis(phosphonate), which was accomplished in the presence of equimolar amounts of 1,4-diazabicyclo[2,2,2]octane (DABCO) in refluxing toluene to give **8**.[8] This allowed the ensuing Mitsunobu esterification of **8** with 2′,3′-*O*-isopropylidenetiazofurin.[13] Initially, the reaction in toluene gave a very poor yield of coupling product **9** (8%) due to the poor solubility of **8** in that solvent. When a mixture of toluene/THF was used, the yield of **9** increased to 58%. Due to the chirality of the proximal phosphorous to tiazofurin, **9** was obtained as a mixture of diastereoisomers. The selective deprotection methodology worked extremely well again with **9** as a substrate to give **10** quantitatively. Possibly, the steric hindrance caused by the tiazofurin moiety directed the selective debenzylation of the distal phosphonate benzyl ester. Surprisingly, the second Mitsunobu esterification with 2′,3′-*O*-isopropylidene-N^6-dimethylaminomethyleneadenosine proceeded in better yield than the first Mitsunobu coupling giving the fully protected β-methylene-TAD precursor **11** in 72% yield after column chromatography. Final hydrogenolysis of **11** over Pd/C in EtOH under acidic conditions removed simultaneously all protective groups to give the final polar product β-methylene-TAD (52%) after purification by reversed-phase HPLC.[6]

Scheme 2



Experimental

General. All chemical reagents were commercially available. Column chromatography was performed on silica gel 60, 230–400 mesh (E. Merck). TLC was performed on Analtech Uniplates silica gel GF. ^{1}H- and ^{13}C NMR were recorded on a Bruker AC 250 spectrometer and referenced to TMS. Positive-ion fast-atom bombardment mass spectra (FABMS) were obtained on a VG 7070E mass spectrometer at an accelerating voltage of 6 KV and a resolution of 2000. Glycerol was used as the sample matrix and ionization was effected by a beam of xenon atoms. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Dibenzyl (Hydroxymethyl)phosphonate (6)

A mixture of dibenzyl phosphite (**5**, 104.8 g, 0.4 mol), paraformaldehyde (12.0 g, 0.4 mol) and triethylamine (4.0 g, 0.04 mol) was heated for 5 min at 130 °C. After cooling, the crude mixture was flash-chromatographed directly on silica gel and eluted sequentially with 0 → 2% EtOH/CH_2Cl_2. The fractions containing the desired product were collected, evaporated, and repurified by flash chromatography with 0 → 1% EtOH/Et_2O to give **6** (98.8 g, 85%) as an oil; ^{1}H NMR ($CDCl_3$+D_2O) δ 7.31 (s, 10H, Ph), 5.34 (m, 4H, 2 × $PhCH_2O$), 3.88 (d, J_{HP} = 5.7 Hz, 2H, CH_2OH); ^{13}C NMR ($CDCl_3$+D_2O) δ 136.30, 136.29, 128.79, 128.71, 128.18, 68.25, 68.15, 57.4 (d, J_{CP} = 160.8 Hz).

Dibenzyl (Bromomethyl)phosphonate (7)

To a solution of **6** (58.4 g, 0.2 mol) and CBr_4 (79.6 g, 0.24 mol) in anhydrous CH_3CN (400 mL) was added triphenylphosphine (55.2 g, 0.21 mol) and stirred for 15 h at room temperature. The reaction mixture was evaporated and the residue was flash-chromatographed on silica gel (50 → 100% Et_2O/hexanes to give **7** (52.1 g, 73%) as an oil; ^{1}H NMR ($CDCl_3$) δ 7.33 (s, 10H, Ph), 5.08 (m, 4H, 2 × $PhCH_2O$), 3.19 (d, J_{HP} = 9.8 Hz, 2H, CH_2Br); ^{13}C NMR ($CDCl_3$) δ 135.75, 135.66, 128.62, 128.06, 68.84, 68.74, 18.07 (d, J_{CP} = 158.6 Hz). Anal. calcd for $C_{15}H_{16}BrO_3P$ (355.171): C, 50.73; H, 4.53. Found: C, 50.60; H, 4.60.

Tetrabenzyl Methylenebis(phosphonate) (1)

A mixture of **7** (21.3 g, 0.06 mol) and tribenzyl phosphite (21.0 g, 0.12 mol) was heated for 2 h at 140 °C under vacuum (ca. 1 mm Hg). During the reaction, one must efficiently remove the benzyl bromide by-product by distilling it off as soon as it is formed. Use of a heat gun and a heating tape to avoid condensation of the benzyl bromide back to the reaction vessel is necessary for the reaction to succeed. An additional amount of tribenzylphosphite (10.5 g, 0.06 mol) was added and heated once more under the same conditions. The crude mixture was directly applied to a silica gel column and flash-chromatographed first with 30 → 100% hexanes/EtOAc to give 1 (6.02 g, 37%) as an oil; ^{1}H NMR ($CDCl_3$) δ 7.28 (s, 20H, Ph), 5.01 (dd, J = 8.7, 2.5

Hz, 8H, 4 × $PhCH_2O$), 2.49 (t, J_{HP} = 21.1 Hz, 2H, PCH_2P). This product was identical in all respects to the material reported by Mioskowski et al.[8]

[[Bis(benzyloxy)phosphoryl]methyl]phosphonic Acid Monobenzyl Ester (8)

A stirred solution of 1 (1.07 g, 2.0 mmol) and DABCO (0.224 g, 2.0 mmol) in toluene (30 mL) was refluxed for 2 h under argon. The solvent was removed under vacuum and the residue was treated dropwise with aqueous HCl (37%, 400 μL). The mixture was extracted with EtOAc (20 mL) and the organic layer was dried (Na_2SO_4) and evaporated under vacuum to give **8** (0.89 g, 100 %) as an oil; ^1H NMR ($CDCl_3$) δ 7.40–7.20 (m, 15H, Ph), 5.05 (d, J = 12.0 Hz, 2H, $PhCH_2O$), 4.95 (d, J = 12.0 Hz, 4 H, 2 × $PhCH_2O$), 2.57 (t, J_{HP} = 21.3 Hz, 2H). This product was identical in all respects to the material reported by Mioskowski et al.[8]

Tiazofurin Coupling

Compound **8** (2.22 g, 5 mmol) was previously refluxed in benzene using a Dean–Stark trap to remove moisture. A solution of **8**, 2′,3′-*O*-isopropylidene-protected tiazofurin (2.25 g, 7.5 mmol) and triphenylphosphine (1.94 g, 7.5 mmol) in a mixture of benzene (15 mL) and THF (30 mL) was cooled to 0 °C over an ice bath and kept under argon before it was treated dropwise with diisopropyl azodicarboxylate (DIAD, 1.76 mL, 7.5 mmol). The reaction mixture was stirred for 30 min at 0 °C and for 2 h at room temperature. MeOH (10 mL) was added and the solution was stirred for 5 min. The mixture was evaporated to dryness and the residue was purified by silica gel flash chromatography (3 → 5% EtOH/CH_2Cl_2) to give **9** as a mixture of diastereoisomers (2.11 g, 58%); ^-H NMR ($CDCl_3$) δ 8.02 and 8.00 (singlets, 1H, tiazofurin H-5), 7.40–7.20 (singlets, 15H, Ph), 5.95 (br s, 2H, $CONH_2$), 5.15 (doublets, J = 3.8 Hz, 1H, H-1'), 5.10–4.85 (m, 6H, 3 × $PhCH_2O$), 4.75 (m, 1H, H-3'), 4.67 (m, 1H, H-2'), 4.30 (m, 1H, H-4'), 4.10 (m, 2H, H-5'a,b), 2.45 (triplets, 2H, PCH_2P), 1.55, 1.35, and 1.30 (singlets, 6H, 2 × CH_3). A solution of **9** (1.61 mg, 3 mmol) and DABCO (0.336 g, 3 mmol) in toluene (6 mL) was refluxed for 2 h and then reduced to dryness. The residue was dissolved in water (10 mL) and treated with 1 N HCl (ca. 180 μL, 1 equiv). The resulting mixture was extracted with EtOAc (20 mL) and the organic layer was evaporated and dried under vacuum to give **10** (1.31 g, 98%) as an amorphous powder. Anal. calcd for $C_{27}H_{32}N_2O_{10}P_2S$ (638.125): C, 50.79; H, 5.05. Found: C, 50.50; H, 5.08.

β-Methylene-TAD Assembly

A solution of **10** (1.29 g, 2 mmol), 2′,3′-*O*-isopropylidene-N^6-dimethylaminomethyleneadenosine (1.46 g, 4 mmol) and triphenylphosphine (1.06 g, 4 mmol) in THF (100 mL) was treated with diisopropyl azodicarboxylate (952 μL, 4 mmol) and stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was purified by flash chromatography (5 → 15% EtOH/CH_2Cl_2) to give **11** (1.39 g, 72%) as a yellowish powder; ^1H NMR ($CDCl_3$) δ 9.00 (singlets, adenine H-2), 8.65 (singlets, adenine H-8), 8.20 and 8.10 (singlets, 1H, tiazofurin H-5), 7.40–7.20 (singlets, 10H, Ph), 6.30 (doublets, 1H, adenosine H-1'), 5.50–4.00 (multiplets), 3.25 (singlets, 6H, $(CH_3)_2N$), 2.45 (triplets, 2H, PCH_2P), 1.65 (singlets, 6H, 2 × CH_3); FABMS *m/z* (relative intensity) 983 (MH^+, 46). Anal. calcd for $C_{43}H_{57}N_8O_{13}P_2S_2H_2O$ (1000.301): C, 50.61; H, 5.50; N, 11.00. Found: C, 50.58; H, 5.22; N, 10.91. Hydrogenolysis of **11** (49 mg, 0.05 mmol) over 10% Pd/C (ca. 20 mg) in EtOH (1 mL) and water (5 mL) under acidic conditions (TFA, 5 mL) afforded crude β-methylene-TAD. This material was further purified by reversed-phase HPLC using a 1%/min linear gradient (0 → 100%, $H_2O \rightarrow CH_3CN$, including 0.05% TFA) to give a single peak that matched an authentic sample of β-methylene-TAD (17 mg, 52% yield).

References and Notes

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2. On sabbatical leave from the University of Rhode Island.
3. Jayaram, H. N.; Dion, R. L.; Glazer, R. I.; Johns, D. G.; Robins, R. K.; Srivastava, P. C.; Cooney, D. A. *Biochem. Pharmacol.* **1982**, *31*, 2371.
4. Cooney, D. A.; Jayaram, H. N.; Glazer, R. I.; Kelley, J. A.; Marquez, V. E.; Gebeyehu, G.; Van Cott, A. C.; Zwelling, L. A.; Johns, D. G. *Adv. Enzyme Regul.* **1983**, *21*, 271.
5. Ahluwalia, G. S.; Jayaram, H. N.; Plowman, J. P.; Cooney, D. A.; Johns, D. G. *Biochem. Pharmacol.* **1984**, *33*, 1195.
6. Marquez, V. E.; Tseng, C. K. H.; Gebeyehu, G.; Cooney, D. A.; Ahluwalia, G. S.; Kelley, J. A.; Dalal, M.; Fuller, R. W.; Wilson, Y. A.; Johns, D. G. *J. Med. Chem.* **1986**, *29*, 1726.
7. Pankiewicz, K. W.; Lesiak, K.; Watanabe, K. A. *J. Am. Chem. Soc.* **1997**, *119*, 3691.
8. Saddy, M.; Lebeau, L.; Mioskowski, C. *J. Org. Chem.* **1995**, *60*, 2946.
9. Saddy, M.; Lebeau, L.; Mioskowski, C. *Synlett.* **1995**, 643.
10. Saddy, M.; Lebeau, L.; Mioskowski, C. *Tetrahedron Lett.* **1995**, *36*, 2239.
11. (a) Ivanov, B. E.; Panteeleva, A. R.; Shagidullin, R. R.; Shermergorn, I. M. *Zhur. Obsh. Khim.* **1967**, *37*, 1856. (b) Frank, A. W.; Gordon, I. *Can. J. Chem.* **1966**, *44*, 2593. (c) Saddy, M.; Lebeau, L.; Mioskowski, C. *Helv. Chim. Acta* **1995**, *78*, 670.
12. (a) Kirby, A. J.; Warren, S. G. *The Organic Chemistry of Phosphorous*; Elsevier: New York, 1967. (b) Pudovik, A. N.; Konovalova, I. V. *Synthesis* **1979**, 81.
13. Fuertes, M.; Garcia-Lopez, T.; Garcia-Munoz, G.; Sud, M. *J. Org. Chem.* **1976**, *41*, 4074.