Synthesis of Acyl Phosphonate Analogues of Biologically Important Acyl Phosphates: *N*-(2-Amino-10-methylpteroyl)-5-amino-2-oxopentanephosphonic Acid

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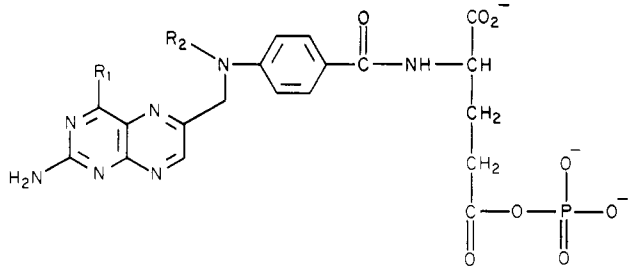
The synthesis of several β-keto phosphonates has been effected by the reaction of the carbanion derived from dimethyl methanephosphonate with appropriately substituted carboxylic acid esters. The presence of nucleophilic substituents γ to the ketone in β-keto phosphonates leads to undesired intramolecular reactions, which can be prevented by temporarily protecting the ketone as an ethylene ketal. The title compound was obtained via reaction of 2,4-diamino-6-(bromomethyl)pteridine (**12**) with a *N*-methyl-*p*-aminobenzoyl derivative of 5-amino-2-oxopentanephosphonic acid (**11b**). Stable compounds of this type are of potential use as probes of enzymes catalyzing amide bond formation via reactive acyl phosphate intermediates.

Introduction

We have been studying the biochemistry of folyloligo-γ-glutamates for several years, including their possible role as preferred coenzymes,[1,2] as well as more recent studies on the biosynthesis of these materials[3] and the related oligo-γ-glutamates of the antifolyl chemotherapeutic agents, methotrexate.[4] As a result of the biosynthetic studies using partially purified folylpolyglutamate synthetase (FPGS), we have concluded that this ATP-dependent, enzyme-catalyzed reaction involves an acyl phosphate intermediate, i.e., **1**.

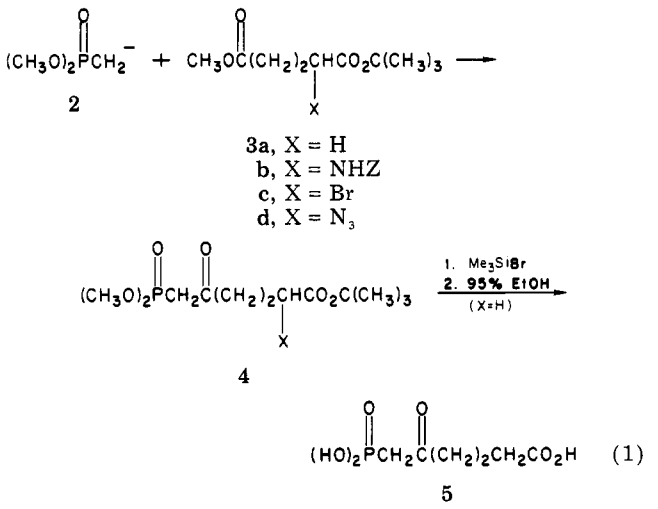


1a, R_1 = OH; R_2 = H
b, R_2 = NH_2; R_2 = CH_3

Stable analogues of acyl phosphates such as **1** are envisioned as specific FPGS inhibitors and may have utility as selective drugs in vitro and in vivo. The synthesis of β-keto phosphonate analogues of the acyl phosphates, in which a methylene group has replaced the anhydride oxygen of **1**, was chosen for investigation. This modification should convert the hydrolytically labile acyl phosphate to a much less reactive species, amenable to biochemical and biological investigations. There is already in the literature a large amount of data on the use of alkanephosphonates as stable analogues of biologically interesting alkyl phosphates, e.g., intermediates of glycolysis.[5] Unfortunately, a similar wealth of information is not available for β-keto phosphonates derived from complex, multifunctional carboxylic acids. Three β-keto phosphonate analogues of aminoacyladenylates, derived from phenylalanine, valine, and glycine, have been reported.[6,7] Molecules of this type contain the amino group α to the carbonyl and therefore are structurally distinct from the compounds of interest in this work, where the amino group is γ to the ketone. In this paper the synthesis of a β-keto phosphonate related to **1b** is described, in addition to a description of some of the unusual chemical behavior of molecules of this type.

Results

To synthesize the desired β-keto phosphonates, we investigated the reaction of the lithium salt of dimethyl methanephosphonate, **2**,[8] with appropriately protected and functionalized carboxylic acid esters, as shown in eq 1.



2

3a, X = H
b, X = NHZ
c, X = Br
d, X = N_3

4

5 (1)

Reaction of **2** with **3b**[1] failed to give the desired product **4b** but rather gave a crude product apparently arising from intramolecular cyclization to a pyroglutamate derivative. This conclusion was substantiated by the facile formation of **4a**, which was readily converted to the free phosphonic acid **5**. Considering the problems encountered using glutamyl derivatives such as **3b** in the reaction with carbanion **2** (eq 1), we sought to use amine precursors that lacked a $C(=O)NH_2$ moiety in order to preclude the facile cyclization to pyroglutamate derivatives described above.

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(1) Coward, J. K.; Parameswaran, K. N.; Cashmore, A. R.; Bertino, J. R. *Biochemistry* **1974**, *13*, 3899.
(2) Coward, J. K.; Chello, P. L.; Cashmore, A. R.; Parameswaran, K. N.; DeAngelis, L. M.; Bertino, J. R. *Biochemistry* **1975**, *14*, 1548.
(3) McGuire, J. J.; Hsieh, P.; Coward, J. K.; Bertino, J. R. *J. Biol. Chem.* **1980**, *255*, 5776.
(4) McGuire, J. J.; Hsieh, P.; Coward, J. K.; Bertino, J. R. "Folyl and Antifolylpolyglutamates"; Goldman, I. D., et al., Eds.; Plenum: New York, 1983; p 199.
(5) Engel, R. *Chem. Rev.* **1977**, *77*, 349.
(6) Goring, G.; Cramer, F. *Chem. Ber.* **1973**, *106*, 2460.
(7) Southgate, C. C. B.; Dixon, H. B. F. *Biochem. J.* **1978**, *175*, 461.
(8) Corey, E. J.; Kwiatkowski, G. T. *J. Am. Chem. Soc.* **1966**, *88*, 5654.

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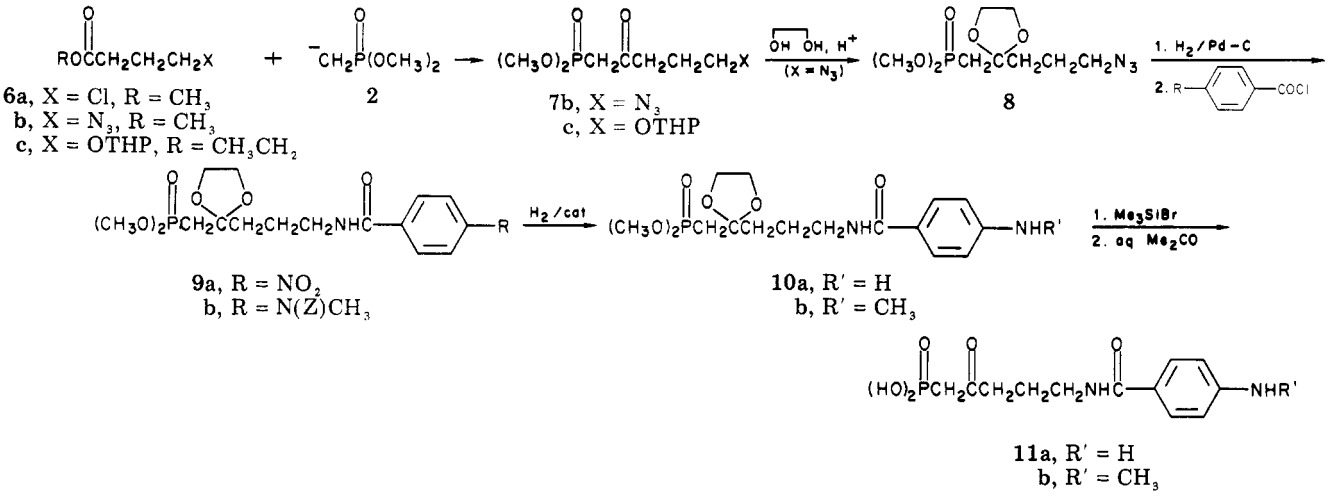
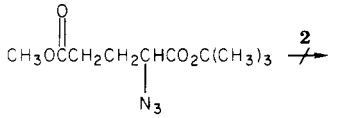
Scheme I



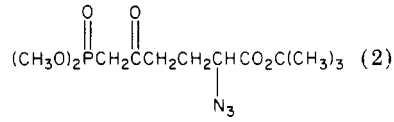
Therefore, the synthesis of **3d** was undertaken. Monomethyl glutarate was converted to the α-monobromo derivative, which was immediately esterified by use of isobutylene with acid catalysis to give **3c**. Displacement of bromide with azide ion gave **3d**, a distillable glutamate precursor for use in the reaction with the lithium salt of dimethyl methanephosphonate (**2**, eq 1).

More readily available alternatives to **3d** were desired in order to establish reaction conditions for the synthesis of the corresponding β-keto phosphonates in the presence of appropriate amine blocking groups. Therefore, the α-carboxyl group was absent in a series of model reactions with γ-aminobutyric acid (GABA) precursors, i.e., α-decarboxyglutamates. Our method of synthesizing this series of compounds is analogous to that shown in eq 1 and is outlined in Scheme I. Conversion of methyl 4-chlorobutyrate to the corresponding 4-azidobutyrate (**6b**) was readily effected. This N-protected GABA ester was allowed to react with **2** under the standard conditions; the azido product, **7b**, could be obtained in pure form by distillation, albeit in low (~20%) yield. Hydrogenation of **7b** using H_2/Pd–C failed to give the desired 5-amino-2-oxopentanephosphonate. The methylene doublet (δ 3.1, $J_{P-H} \simeq 20$ Hz) characteristic of the β-keto phosphonate was absent in the ^1H NMR spectrum of the isolated product; instead a broad singlet at δ 3.23–3.63 was observed. Control experiments using a homologue of **7** (X = NHBoc), dimethyl *N*-(*tert*-butoxycarbonyl)-4-amino-2-oxobutanephosphonate, prepared by reaction of *N*-Boc-β-alanine methyl ester with **2**, showed that the carbonyl group of this β-keto phosphonate was not reduced under these conditions; nearly quantitative recovery of starting material was observed.[9] When **6c** was allowed to react with **2**, the β-keto phosphonate, **7c** was obtained in reasonable yield. However, attempts to remove the THP groups with TsOH/MeOH again resulted in a product that lacked the characteristic two-proton doublet referred to above. As a result of these experiments, the keto group was first protected as an ethylene ketal.[10] We were then able to reduce the azide and isolated the free amine. Coupling of the free amine to para-substituted benzoyl chloride yielded the benzamide derivatives **9**. These could be converted to the *p*-aminobenzamido keto phosphonates **10**. The *N*-methyl derivative **10b** was converted to the bis(trimethylsilyl) ester with trimethylsilyl bromide (Me_3SiBr),[11] which was then hydrolyzed to the free phosphonic acid, isolated as the lithium salt **11b**.

This chemistry was then extended to the glutamyl series. Unfortunately, when the glutamyl precursor **3d** was allowed to react with carbanion **2** (eq 2), the desired β-keto



3d



4d (2)

phosphonate **4d** could not be isolated. Instead gas evolution was observed, and a dark brown reaction solution formed, in contrast to light straw-colored reaction solutions obtained in other cases (eq 1, Scheme I). The complete absence of the methylene doublet at δ 3.1 also indicated that the reaction of **2** and **3d** did not proceed as desired.

The main goal of this work is to synthesize β-keto phosphonate analogues of **1** as potential multisubstrate adduct inhibitors[12] of folylpolyglutamate synthetase. Since it is known that the antifolate methotrexate is a substrate for the isolated enzyme,[4] we sought to develop a method for the synthesis of a β-keto phosphonate analogue of **1b**, the intermediate acyl phosphate presumably involved in the enzyme-catalyzed formation of methotrexate polyglutamates in mammalian cells.[13] A new method for the synthesis of methotrexate and γ-peptidyl derivatives has been described by two groups of investigators.[14,15] We

(9) Similar results indicating the stability of a β-keto phosphonate under hydrogenation conditions have been reported: Nicolaou, K. C.; Pavia, M. R.; Seitz, S. P. *J. Am. Chem. Soc.* **1982**, *104*, 2027.

(10) Nair, M. G.; Chen, S-Y.; Kisliuk, R.-L.; Gaumont, Y.; Strumpf, D. *J. Med. Chem.* **1980**, *23*, 899.

(11) McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M.-C. *Tetrahedron* **1977**, 155. McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc. Chem. Commun.* **1979**, 739.

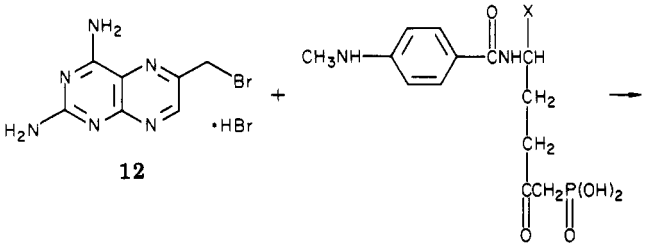
(12) Heller, J. S.; Canellakis, E. S.; Bussolotti, D. L.; Coward, J. K. *Biochim. Biophys. Acta* **1975**, *403*, 197. Anderson, G. L.; Bussolotti, D. L.; Coward, J. K. *J. Med. Chem.* **1981**, *24*, 1271. Tang, K.-C.; Mariuzza, R.; Coward, J. K. *Ibid.* **1981**, *24*, 1277.

(13) Balinska, M.; Galivan, J.; Coward, J. K. *Cancer Res.* **1981**, *41*, 2751.

(14) Piper, J. R.; Montgomery, J. A. *J. Heterocycl. Chem.* **1974**, *11*, 279; *J. Org. Chem.* **1977**, *42*, 208. Piper, J. R.; Montgomery, J. A.; Sirotnak, F. M.; Chello, P. L. *J. Med. Chem.* **1982**, *25*, 182. Piper, J. R.; McCaleb, G. S.; Montgomery, J. A. *Ibid.* **1983**, *26*, 291.

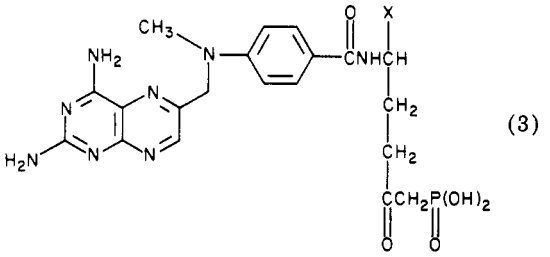
(15) Suster, D. C.; Tarnauceanu, E.; Ionescu, D.; Dobre, V.; Niculescu-Duvaz, I. *J. Med. Chem.* **1978**, *21*, 1162.

have used this method to investigate the synthesis of **13**, as shown in eq 3. Coupling of **11b** and **12** in aqueous K_2CO_3, followed by purification by DEAE-cellulose ion-exchange chromatography, afforded **13a** as a light yellow solid.



12

11b, X = H



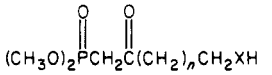
(3)

13a, X = H
b, X = COOH

Discussion

In our research, the most effective synthetic method for the preparation of β-keto phosphonates has involved the coupling of the anion of dimethyl methanephosphonate (**2**), with an appropriate carboxylic acid ester (eq 1). The apparent formation of a pyroglutamate derivative during the attempted coupling reaction of **2** with appropriately substituted glutamates (e.g., **3b**) mandated the use of alternative synthetic strategies. In order to ascertain which amine precursors might be stable to both purification by vacuum distillation and the coupling reaction with **2**, a series of α-decarboxyglutamates, i.e., γ-aminobutyrates, was investigated. In addition, an α-decarboxyaspartate, i.e., a β-alanine derivative (X = NHBoc), was studied. The coupling reaction between **2** and methyl esters **6b,c** proceeded as expected. Distillation of the crude product yielded pure keto phosphonates **7b,c**.

Reduction of the γ-azido group of **7b** in the presence of H_2/Pd–C gave rise to a product in which the characteristic ^{1}H NMR signal attributed to the $P(O)CH_2C(O)$, δ 3.1 (d, $J_{P-H} \simeq 20$ Hz), was not observed. Similarly, deblocking the γ-OTHP derivative **7c** in TsOH/CH_3OH led to a product that did not exhibit the expected methylene doublet at δ 3.1. In contrast, butanephosphonate derivatives containing a β-amino or a β-hydroxy function showed the characteristic δ 3.1 doublet in the ^{1}H NMR (data not shown). These results can be summarized by the generalized structure **14**. The isolated butanephosphonates (**14**,



14a, X = NH
b, X = O

n = 1) exhibit the expected ^{1}H NMR spectral properties, whereas the pentanephosphonates (n = 2) do not. We interpret these results as indicating intramolecular interactions (e.g., imine or hemiketal formation, enolization, etc.) between the γ-amino or hydroxy groups and the free carbonyl group of the β-keto phosphonate.[16] These results suggested ketalization as a means of avoiding the undesired intramolecular interactions. This proved to be a successful approach, as reaction of **7b** with ethylene glycol led to **8**, which could be purified by distillation. Reduction of the azido group, followed by acylation with *p*-nitrobenzoyl chloride, gave a crystalline *p*-nitrobenzamide, **9a**. Similarly, coupling of the freshly prepared amino ketal with *p*-[(carbobenzyloxy)methylamino]benzoyl chloride gave, following removal of the carbobenzyloxy group, the *p*-(methylamino)benzamide **9b** as a light yellow solid.

On the basis of the results with the γ-substituted pentanephosphonates (**14**, n = 2) discussed above, the synthesis of the appropriate glutamyl precursors was investigated. γ-Monomethyl α-bromoglutarate was prepared from monomethyl glutarate, via a modified Hell–Vollhard–Zelinsky reaction. The crude free acid thus obtained was converted to the corresponding α-*tert*-butyl ester. The desired α-bromo ester **3c** was obtained pure following several vacuum distillations to remove contaminating dibromo derivatives. Reaction of **3c** with NaN_3 resulted in a distillable product; **3d** could be obtained in high yield by this procedure. Our previous experience with γ-azidobutyrate **6b** suggested that the reaction of **3d** with carbanion **2** might proceed in low yield. The explanation proposed to account for the low yield of **7b** obtained from the reaction of **6b** and **2** was based on competition between the ester carbonyl and the azide for the carbanion.[17] Unfortunately, the reaction of **2** with **3d** apparently proceeded in a different and unexpected manner, since a gas was evolved and a dark-colored reaction solution was obtained. No product could be obtained that had the spectral properties expected for **4d**. We have not investigated in detail the mechanistic basis for the undesired reaction of **3d** with **2**, in contrast to the successful coupling of **6b** with **2**. However, it is probably reasonable to assume that the α-azido ester **3d** reacts rapidly with **2**, via proton transfer, to lose the acidic α-hydrogen of **3d**. This new carbanion can exist as a resonance hybrid in which some of the negative charge is delocalized into the azido group. One possible reaction of this resonance stabilized carbanion is ring closure to a *tert*-butyl 5-oxo-Δ^1-2-pyrrolidine-2-carboxylate, concomitant with evolution of N_2 and expulsion of methoxide ion. Recently, it has been shown that other α-azido carboxylic acid esters undergo oxidative nitrogen elimination in basic media to yield corresponding α-keto esters.[18]

Our approach to the synthesis of β-keto phosphonate analogues of acyl phosphate **1** was first to prepare the β-keto phosphonate analogues of γ-glutamyl phosphate. Condensation of this appropriately esterified β-keto phosphonate analogue with suitably blocked pteroic acid[1] should yield the desired β-keto phosphonate analogue of **1a**. Similarly, condensation with 4-amino-10-methylpteroic acid should yield the desired analogue of **1b**. Unfortunately, the apparent incompatibility of the free keto group and amino group of **14a** (n = 2) suggests that this synthetic route will not be successful. In addition, we have found that 4-amino-10-methylpteroic acid couples with amines, such as suitably blocked glutamate, and γ-glutamyl peptides, in only very low yield[13] (K-C. Tang and J. K. Coward,

(16) A synthesis of glutamyl-γ-methanephosphonic acid has been reported: Wedler, F. C.; Horn, B. R.; Roby, W. G. *Arch. Biochem. Biophys.* **1980**, *202*, 482. However, the ^{1}H NMR spectral data presented by the authors are not in accord with our data for related compounds (e.g., **5**) shown in the Experimental Section, especially their report of a single resonance at δ 2.4 assigned to the $P(=O)CH_2C=O$ methylene moiety.

(17) L'Abbé, G. *Ind. Chim. Belg.* **1969**, *34*, 519.

(18) DuBois, G. E.; Crosby, G. A.; McGarraugh, G. V.; Ng, S. Y.-W.; Stephenson, R. A.; Wang, P. C.; Wingard, R. E., Jr. *J. Org. Chem.* **1982**, *47*, 1319. Manis, P. A.; Rathke, M. W. *Ibid.* **1980**, *45*, 4952.

unpublished results). In the present work, the recently developed methods of Piper and Montgomery[14] and Suster et al.[15] have been used to effect the synthesis of the α-decarboxy keto phosphonate analogue of **1b**, via reaction of **11b** with 2,4-diamino-6-(bromomethyl)pteridine, **12**. With the successful synthesis of this complex β-keto phosphonate, **13a**, synthesis of other complex keto phosphonates should be feasible. This will permit the study of the interaction of this class of molecules with enzymes catalyzing reactions via acyl phosphate intermediates.

Experimental Section

General Procedures. All chemicals were of reagent quality and used without further purification with the following exceptions: pyridine, *N,N*-dimethylacetamide (DMA), and *N,N*-dimethylformamide (DMF) were dried over potassium hydroxide pellets and distilled; methanol was kept over molecular sieves, 4A; methylene chloride and chloroform were distilled over P_2O_5 and kept over molecular sieves, 4A; tetrahydrofuran (THF) and ether were distilled over LAH prior to use; benzene was distilled over Na prior to use. Melting points were taken on a Mel-Temp capillary melting point apparatus and reported values are uncorrected. Nuclear magnetic resonance (NMR) were recorded on a Varian T-60 ^1H NMR spectrophotometer unless other specified. NMR chemical shift data are given in ppm relative to Me_4Si (^1H) or H_3PO_4 (^{31}P). IR spectra were measured with a Perkin-Elmer 237-B or 298 spectrophotometer. UV spectra were measured with a Perkin-Elmer 552 spectrophotometer. Elemental analyses were performed by Baron Consulting Co., Orange, CT, and Atlantic Microlab Inc., Atlanta, GA. Thin-layer chromatography (TLC) was performed with either silica gel F-254 (EM Reagents) plate or Eastman cellulose plate with fluorescent indicator with the following solvent systems: (a) 1-BuOH–HOAc–H_2O (12:3:5) [BAW], (b) 1-BuOH–HOAc–H_2O–pyridine (30:6:24:20) [BAWP]. Preparative thin-layer chromatography was performed with use of Analtech Silica Gel uniplates. The *O*-tetrahydropyranyl ester **6c** was prepared by treatment of ethyl 4-hydroxybutyrate[19] with 2,3-dihydropyran in the presence of a catalytic amount of 12 N HCl; bp 79–82 °C (0.3 torr) (anal. $C_{11}H_{20}O_4$). *N*-Boc-β-alanine methyl ester (bp 72–74 °C (0.07 torr) (anal. $C_9H_{17}NO_4$) was prepared by reaction of di-*tert*-butyldicarbonate with β-alanine methyl ester.

Satisfactory combustion analytical data were reported for **3a**, **3c**, **3d**, **4a**, 5-DCHA, *N*-Boc-β-AlaOCH$_3$, **6b**, **6c**, **7b**, **7c**, **8**, **9a**, **10a**, and **10b**·$^4/_3H_2O$.

Methyl *tert*-Butyl Glutarate (3a). Freshly distilled monomethyl glutarate 15 g (0.1 mmol) was dissolved in 100 mL of dry methylene chloride and divided between two pressure bottles. To each bottle was added 50 mL of isobutylene, together with 0.7 mL of 18 N H_2SO_4 at −78 °C. The reaction mixtures were allowed to stir at ambient temperature for 3 days in the sealed bottles, after which time the bottles were opened at −78 °C and the mixtures allowed to warm to ambient temperature as the excess isobutylene evaporated. The residual material was poured into 100 mL of saturated $NaHCO_3$ solution and stirred at ambient temperature for 1 h. The aqueous layer was extracted with $CHCl_3$ (4 × 50 mL), and the combined organic extracts were washed with saturated $NaHCO_3$ solution (2 × 50 mL), H_2O (2 × 50 mL), and saturated NaCl solution (2 × 50 mL), and dried over $MgSO_4$. After removal of the solvent, the oily residue was vacuum distilled (68–70 °C (0.3 torr)) to give 15.7 g (76%) of pure **3a**: ^1H NMR ($CDCl_3$) δ 1.48 (9 H, s, *t*-Bu), 2.0 (2 H, m, CH_2), 2.43 (4 H, t, $CH_2C{=}O$), 3.68 (3 H, s, OCH_3); IR (thin film) $\nu_{C=O}$ 1724 cm^{-1}.

The syntheses of other *tert*-butyl esters, **3**, from the corresponding carboxylic acids were carried out as described above. Physical data are presented in Table I.

γ-Methyl α-*tert*-Butyl α-Azidoglutarate (3d). To 4 mL of dry DMF was added 1.7 g (5.16 mmol) γ-methyl α-*tert*-butyl α-bromoglutarate (**3c**), contaminated with ca. 10% the α,γ-dibromo derivative. NaN_3 (1.0 g, 15.5 mmol) was added, and the resulting solution was heated at 60 °C for 24 h. Solvent was then removed in vacuo, and the residue was partitioned between H_2O (20 mL) and $CHCl_3$ (50 mL). The aqueous layer was separated and extracted with $CHCl_3$ (2 × 20 mL). The combined $CHCl_3$ extracts were then washed with H_2O (3 × 20 mL) and saturated NaCl solution (2 × 30 mL) and dried over $MgSO_4$, and the solvent was removed in vacuo to give 1.2 g of crude product. Distillation afforded pure **3d** in 86% yield: bp 72–75 °C (0.02 torr); ^1H NMR ($CDCl_3$) δ 1.5 (9 H, s, *t*-Bu), 1.86–2.3 (2 H, m, CH_2), 2.48 (2 H, t, $CH_2C{=}O$), 3.7 (3 H, s, OCH_3), 3.86 (1 H, t, CHN_3); IR (thin film) ν_{N_3} 2105 cm^{-1}, $\nu_{C=O}$ 1738 cm^{-1}.

Table I. Physical Properties of *tert*-Butyl Esters 3

compd	X	yield, %	bp, °C/torr	formula[b]
3a	H	76	68–70/0.3	$C_{10}H_{18}O_4$
3c	Br	*c*	110/3.75	$C_{10}H_{17}BrO_4$
3d	N_3	86	72–75/0.02	$C_{10}H_{17}N_3O_4$

[a] Compounds **3a** and **3d** were synthesized from the corresponding carboxylic acid as described for **3a** in the Experimental Section. Compound **3d** was synthesized from the α-bromo ester **3c** as described for **3d** in the Experimental Section. [b] Satisfactory analytical data (±0.4% for C, H, N, or Br) were obtained for all new compounds listed in the table. [c] Monomethyl glutarate (0.1 mol) was converted to the α-bromo acid by the sequential action of freshly distilled $SOCl_2$ (0.4 mol) at reflux (2 h), followed by dropwise addition of Br_2 (0.1 mol) over 3 h, maintaining a gentle reflux. The crude α-bromo acid halide was added slowly to H_2O (50 mL) and the resulting α-bromo acid extracted into ether. The organic extract was dried, and the solvent was removed in vacuo to give crude α-bromo acid in 61% yield. This crude acid was converted to the *tert*-butyl ester, as described for **3a** in the Experimental Section. Overall yield: 22% from monomethyl glutarate.

Table II. Physical Properties of Dimethyl β-Keto Phosphonates[a]

compd	X	yield, %	bp, °C/torr	formula[b]
4a	H	28	132–134/0.03	$C_{12}H_{23}O_6P$
7b	N_3	15	100–102/0.08	$C_7H_{14}N_3O_4P$
7c	OTHP	44	oil[c]	$C_{12}H_{23}O_6P$[d]

[a] Synthesized from the corresponding carboxylic acid ester **3** as described for **4a** in the Experimental Section. [b] Satisfactory analytical data (±0.4% for C, H, and P) were obtained for all new compounds listed in the table, except as noted below. [c] Purified by chromatography on silica gel, with elution by EtOAc–Et_3N (99:1).[20] [d] Anal. Calcd: C, 48.98. Found: C, 48.17.

Dimethyl 2-Oxo-5-(carbo-*tert*-butoxy)pentanephosphonate (4a). A 1.6 M solution of *n*-butyllithium (180 mL, 288 mmol) in hexanes was transferred via syringe to 120 mL of dry THF and the solution cooled to −78 °C. Dimethyl methanephosphonate (29 mL, 33.5 g, 270 mmol) was added dropwise to the *n*-BuLi solution over a period of 0.5 h to generate the carbanion **2**. The resulting suspension was stirred for an additional 0.5 h, after which time a solution of 15.0 g (74 mmol) of methyl *tert*-butyl glutarate (**3a**) in 100 mL of dry THF was added dropwise over a period of 0.5 h. The temperature of the solution was then allowed to increase to −20 °C as stirring was continued for an additional 2 h. The reaction was then quenched with 1.2 mL of glacial acetic acid to give a white mixture (sludge, pH 5). Solvents were removed in vacuo, and the residue was partitioned between $CHCl_3$ (600 mL) and H_2O (350 mL). The organic layer was separated and washed with saturated $NaHCO_3$ solution (2 × 300 mL), H_2O (2 × 300 mL), and saturated NaCl solution (2 × 300 mL). After drying the organic layer over $MgSO_4$, the solvent was removed in vacuo. The product was purified by vacuum distillation (bp 132–134 °C (0.03 torr)) to yield 6.2 g (28%) of **4a**: ^1H NMR ($CDCl_3$) δ 1.42 (9 H, s, *t*-Bu), 1.82 (2 H, m, CH_2), 2.20 (2 H, t, $CH_2C{=}O$, J = 6 Hz), 2.66 (2 H, t, $CH_2C{=}O$, J = 6 Hz), 3.11 (2 H, d, $P(=O)CH_2C{=}O$, J = 21 Hz), 3.73 (6 H, d, OCH_3, J = 11 Hz); IR (thin film) $\nu_{C=O}$ 1721 cm^{-1}, $\nu_{P=O}$ 1250 cm^{-1}, $\nu_{P\text{-}OCH_3}$ 1025 cm^{-1}.

The syntheses of other dimethyl methanephosphonate derivatives, **4** and **7**, from the appropriate precursor carboxylic acid

(19) Brown, H. C.; Keblys, K. A. *J. Org. Chem.* **1966**, *31*, 485.

esters 3 were carried out as described above. Physical data are presented in Table II.

2-Oxo-5-carboxypentanephosphonic Acid (5). To 2 mL of dry $CHCl_3$ in a 15-mL round-bottom flask was added 2.2 g (7.48 mmol) of the β-keto phosphonate **4a**. The flask was fitted with a rubber septum after the solution was flushed several times with N_2, and then bromotrimethylsilane (3.8 mL, 5.7 g, 37.5 mmol) was added via syringe. The solution was allowed to stir overnight at ambient temperature, and the excess TMSBr was removed under high vacuum at ambient temperature. The residue was then treated with 95% EtOH (5 mL) for 1 h at ambient temperature, and the solvent was removed in vacuo. Trituration of the residue several times with Et_2O, followed by removal of the final traces of Et_2O under high vacuum, yielded 1.20 g of **5**: ^1H NMR (D_2O) δ 1.91 (2 H, m, CH_2), 2.43 (2 H, t, $CH_2C{=}O$, J = 6 Hz), 2.80 (2 H, t, $CH_2C{=}O$, J = 6 Hz), 3.28 (2 H, d, $P(=O)CH_2C{=}O$, J = 21 Hz). The free acid **5** was converted to its dicyclohexylamine salt by dissolving a portion of the acid in EtOH together with 1.1 equiv of dicyclohexylamine in EtOH. Precipitation of the salt by addition of ether, followed by recrystallization from EtOH–Et_2O, afforded an analytical sample: yield, 1.85 g (88%); mp 176–178 °C; ^{31}P NMR (D_2O) δ 13.0. Anal. $C_{18}H_{34}NO_6P$.

Methyl 4-Azidobutyrate (6b). Methyl 4-chlorobutyrate (31 g, 221 mmol) and 44 g (676 mmol) NaN_3, together with a catalytic amount of anhydrous LiI, were added to 160 mL of dry DMF, and the resulting mixture was heated at 60 °C for 24 h. The solvent was then removed in vacuo, and the residue was partitioned between H_2O (400 mL) and $CHCl_3$ (200 mL). The aqueous layer was separated and extracted with $CHCl_3$ (2 × 20 mL). The combined $CHCl_3$ extracts were then washed with H_2O (3 × 400 mL) and saturated NaCl solution (2 × 400 mL) and dried over $MgSO_4$, and the solvent was removed in vacuo. Distillation of the residual material gave 28.2 g of reasonably pure **6b** (bp 72–80 °C (9 torr)); a second distillation gave 23.8 g (75%) of pure material: bp 74–76 °C (7 torr); ^1H NMR ($CDCl_3$) δ 1.93 (2 H, quintet, CH_2, J = 6 Hz), 2.43 (2 H, t, $CH_2C(=O)$, J = 6 Hz), 3.4 (2 H, t, CH_2N_3, J = 6 Hz), 3.73 (3 H, s, OCH_3); IR (thin film) ν_{N_3} 2119 cm^{-1}, $\nu_{C=O}$ 1742 cm^{-1}. Anal. $C_5H_9N_3O_2$.

Dimethyl 5-Azido-2-oxopentanephosphonate Ethylene Ketal (8). In a 1-L round-bottom flask, a solution of 4.7 g (20 mmol) of **7b** in 30 mL (33.4 g, 538 mmol) of ethylene glycol containing 453 mg (2.4 mmol) of *p*-toluenesulfonic acid monohydrate was stirred for 10 min at 100 °C. Dry benzene (700 mL) was then added and the resulting solution was heated at reflux with stirring for 12 h, with continuous removal of H_2O (Dean–Stark apparatus). Benzene was then removed under reduced pressure, and 5.74 g (57.4 mmol) of $KHCO_3$ was added to the oily residue, followed by 500 mL of saturated $NaHCO_3$. Extraction of the organic material with $CHCl_3$ (2 × 250 mL) was followed by washing the combined $CHCl_3$ extracts with H_2O (2 × 400 mL) and saturated NaCl solution (400 mL) and drying over $MgSO_4$. Removal of the solvent in vacuo gave an oily residue, from which the desired product **8** was obtained by distillation (bp 125–127 °C (0.08 torr)): yield, 4.76 g (85%); ^1H NMR ($CDCl_3$) δ 1.48–2.23 (4 H, br s, CH_2CH_2), 2.27 (2 H, d, $P(=O)CH_2$, J = 19 Hz), 3.31 (2 H, t, CH_2N_3), 3.75 (6 H, d, OCH_3, J = 12 Hz), 4.01 (4 H, s, OCH_3); IR (thin film) ν_{N_3} 2090 cm^{-1}, $\nu_{P=O}$ 1179 cm^{-1}, $\nu_{P\text{-}OCH_3}$ 1030 cm^{-1}. Anal. $C_9H_{18}N_3O_5P$.

Dimethyl *N*-(*p*-Nitrobenzoyl)-5-amino-2-oxopentanephosphonate Ethylene Ketal (9a). A solution of 785 mg (2.8 mmol) of **8** in 50 mL of CH_3OH containing 300 mg 10% Pd–C was hydrogenated at 20 psi on a Parr apparatus for 12 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give an oily residue. The residue was triturated with petroleum ether, and the insoluble material was kept under high vacuum for several hours to remove any residual solvent: yield, 243 mg (96%) as an homogeneous oil; R_f 0.3 on silica gel in BAW; ^1H NMR ($CDCl_3$) δ 1.36–2.26 (4 H, br s, CH_2CH_2), 2.28 (2 H, d, $P(=O)CH_2$, J = 18 Hz), 2.77 (2 H, t, CH_2N), 3.76 (6 H, d, OCH_3, J = 12 Hz), 4.03 (4 H, s, OCH_2), 4.21 (2 H, br s, NH_2); IR (thin film) $\nu_{P=O}$ 1179 cm^{-1}, $\nu_{P\text{-}OCH_3}$ 1030 cm^{-1}.

The free amine obtained from the procedure described above was dissolved in 50 mL of dry EtOAc, and 395 μL (285 mg, 2.8 mmol) of Et_3N was added. To the resulting solution was added dropwise a solution of 520 mg (2.8 mmol) of recrystallized *p*-nitrobenzoyl chloride in 50 mL of dry ethyl acetate, and the reaction solution then was stirred at ambient temperature for 2 days. A white solid, presumably $Et_3N{\cdot}HCl$, was removed by filtration, and the filtrate was washed successively with H_2O, cold 1 N HCl, 0.1 N NaOH, and saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent in vacuo, followed by trituration with petroleum ether and removal of residual solvent (high vacuum) from the insoluble material, gave a white solid; yield, 506 mg (45% overall from **8**). Recrystallization from CH_3OH–H_2O gave an analytical sample: mp 83–85 °C; ^1H NMR ($CDCl_3$) δ 1.58–2.23 (4 H, br s, CH_2CH_2), 2.26 (2 H, d, $P(=O)CH_2$, J = 19 Hz), 3.23–3.73 (2 H, m, CH_2N), 3.75 (6 H, d, OCH_3, J = 11 Hz), 3.96 (4 H, s, OCH_3), 7.68 (1 H, br s, $C(=O)NH$), 8.11 (4 H, s, aromatic H); UV $\lambda_{max}^{0.1NH^+}$ 269 nm, $\lambda_{max}^{0.1NOH^-}$ 269 nm. Anal. $C_{16}H_{23}N_2O_8P$.

Dimethyl *N*-(*p*-Aminobenzoyl)-5-amino-2-oxopentanephosphonate Ethylene Ketal (10a). A solution of 402 mg (1 mmol) of **9a** in 20 mL of dry CH_3OH containing 20 mg of PtO_2 was hydrogenated at 20 psi for 12 h. The catalyst was removed by filtration with the aid of Celite, and the solvent was removed under reduced pressure. The solid residue was dried under high vacuum to give 356 mg (96%) of pure **10a**: mp 156–158 °C dec (preliminary softening at 150 °C); ^1H NMR (CD_3OD) δ 1.48–2.03 (4 H, br s, CH_2CH_2), 2.3 (2 H, d, $P(=O)CH_2$, J = 19 Hz), 3.08–3.45 (2 H, m, CH_2N), 3.63 (6 H, d, CH_3O, J = 11 Hz), 3.91 (4 H, s, CH_2O), 6.41–7.65 (4 H, AA'BB', aromatic); UV $\lambda_{max}^{0.1NH^+}$ 222 nm, $\lambda_{max}^{0.1NOH^-}$ 271 nm. Anal. $C_{16}H_{25}N_2O_6P$.

Dimethyl *N*-[*p*-(Carbobenzoxymethylamino)benzoyl]-5-amino-2-oxopentanephosphonate Ethylene Ketal (9b). *p*-(Carbobenzoxymethylamino)benzoic acid (937 mg, 3.22 mmol) was dissolved in 25 mL of dry Et_2O and converted to the acid chloride with use of PCl_5 (738 mg, 3.54 mmol) at ambient temperature for 1 h.[14] The ethereal reaction solution was then washed twice with 25 mL of ice–water, and the ethereal layer was added dropwise to an ice-cooled solution of freshly prepared amino ketal phosphonate, derived from 900 mg (3.22 mmol) of **8** (see synthesis of **9a**) and 2.20 g (26.18 mmol) of $NaHCO_3$ in a mixture of 15 mL each of H_2O and EtOAc. The resulting solution was stirred at 0 °C for 1.5 h and then allowed to warm to ambient temperature as stirring continued overnight. The reaction mixture was then diluted with 15 mL each of H_2O and EtOAc, and the organic layer was separated and washed successively with saturated $NaHCO_3$ solution, H_2O, cold 2 N HCl, H_2O, and saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent in vacuo gave 900 mg of crude product, from which 497 mg (44%) of chromatographically pure **9b** could be isolated by preparative TLC (silica gel, $CHCl_3$–CH_3OH–HOAc (9.5:0.5:0.5)): ^1H NMR ($CDCl_3$) δ 1.6–2.1 (4 H, br s, CH_2CH_2), 2.26 (2 H, d, $P(=O)CH_2\overline{COO}$, J = 19 Hz), 3.3 (3 H, s, CH_3N), 3.2–3.53 (2 H, m, CH_2N), 3.71 (6 H, d, OCH_3, J = 11 Hz), 3.96 (4 H, s, CH_2O), 5.13 (2 H, s, CH_2O), 7.03–8.1 (5 H, AA'BB', aromatic H, br s, NHC=O)), 7.26 (5 H, s, aromatic H); IR (thin film) $\nu_{C=O}$ 1701 cm^{-1}.

Dimethyl *N*-[*p*-(Methylamino)benzoyl]-5-amino-2-oxopentanephosphonate Ethylene Ketal (10b). To a solution of 271 mg (0.515 mmol) of **9b** in 20 mL of CH_3OH was added 70 mg of 10% Pd–C as a slurry in 95% EtOH, and the mixture was hydrogenated on a Parr apparatus at 20 psi for 6 h. The catalyst was then removed by filtration, and the filtrate was concentrated in vacuo to give 223 mg of crude product. This material was triturated with $CHCl_3$ to remove the $CHCl_3$-insoluble contaminants, and the $CHCl_3$ solution was concentrated in vacuo and finally triturated several times with petroleum ether. The petroleum ether insoluble residue was kept at high vacuum for several hours to remove trace amounts of solvent: yield, 164 mg (82%) of an amorphous light yellow solid; mp 120.5–123 °C; ^1H NMR ($CDCl_3$) δ 1.33–2.2 (4 H, br s, CH_2CH_2), 2.21 (2 H, d, $P(=O)CH_2\overline{COO}$, J = 19 Hz), 2.76 (3 H, s, CH_3N), 3.18–3.53 (2 H, m, CH_2N), 3.65 (6 H, d, OCH_3, J = 11 Hz), 3.93 (4 H, s, OCH_2), 4.26 (1 H, br s, NH), 6.3–7.83 (4 H, AA'BB', aromatic), 7.0 (1 H, t, NHC=O, J = 6 Hz); UV $\lambda_{max}^{0.1NH^+}$ 222 nm, $\lambda_{max}^{0.1NOH^-}$ 287 nm. Anal. $C_{17}H_{27}N_2O_6P{\cdot}{}^4/_3H_2O$.

***N*-[*p*-(Methylamino)benzoyl]-5-amino-2-oxopentanephosphonic Acid (11b).** In a 25-mL round-bottom flask were

(20) Overman, L. E.; Jessup, P. J. *J. Am. Chem. Soc.* **1978**, *100*, 5179.

placed 291 mg (0.75 mmol) of **10b** and 3 mL of dry $CHCl_3$ (previously distilled over P_2O_5). The solution was flushed with N_2 several times, and the flask then was fitted with a rubber septum. Bromotrimethylsilane (4.5 mL, 5.22 g, 34.2 mmol) was introduced via syringe, and the resulting mixture was stirred overnight at ambient temperature. The excess Me_3SiBr and $CHCl_3$ were then removed under high vacuum to give the bis-Me_3Si ester of **10b** in quantitative yield: ^1H NMR ($CDCl_3$) δ 2.36 (2 H, d, $P(=O)CH_2\overline{COO}$, J = 19 Hz) and all other peaks observed in the ^1H NMR spectra of **10b**, with the exception that a peak at δ 0.37 (27 H, s, $(CH_3)_3Si$) replaced the peak at δ 3.65 in **10b**. The Me_3Si ester was then treated with 10 mL of acetone containing a few drops of H_2O, and resulting solution was stirred at ambient temperature overnight. The solvent was then removed in vacuo, and the residue was subjected to several acetone wash-evaporation cycles before a final drying at high vacuum. The resulting amorphous keto phosphonate was sufficiently pure for subsequent transformations: ^1H NMR (D_2O) δ 1.48-1.78 (2 H, m, CH_2), 2.58 (2 H, t, $CH_2C{=}O$), 2.68 (2 H, d, $P(=O)CH_2C{=}O$, J = 21 Hz), 2.93 (3 H, s, NCH_3), 3.16 (2 H, t, CH_2N), 7.21-7.88 (4 H, AA'BB', aromatic H).

For further purification, the free β-keto phosphonic prepared above could be converted to its dilithium salt by addition of a saturated ethanolic solution of LiOH to an ethanolic solution of the free phosphonic acid, until a pH 8.0 was obtained. The precipitated Li_2 phosphonate was isolated by centrifugation, followed by successive washes of the precipitate with ethanol (three times) and ether (three times). The precipitate was then dried under high vacuum for several hours to give 166 mg (68%) of the dilithio salt of **11b**: ^1H NMR (D_2O, 5% CD_3COOD) δ 1.40-1.70 (2 H, m, CH_2), 2.31-2.70 (2 H, m, $CH_2C{=}O$), 2.60 (3 H, s, NCH_3), 2.78 (2 H, d, $P(=O)CH_2C{=}O$, J = 21 Hz), 3.08 (2 H, t, CH_2N), 6.43-7.56 (4 H, AA'BB', aromatic H).

***N*-(4-(((2,4-Diaminopteridin-6-yl)methyl)methylamino)benzoyl)-5-amino-2-oxopentanephosphonic Acid (13a).** The keto phosphonate **10b** (97 mg, 0.25 mmol) was converted to the free keto phosphonic acid **11b** as described above. The resulting amorphous product was dissolved in 1 mL of H_2O, and solid $KHCO_3$ was added to adjust the pH to ca. 7.5. 2,4-Diamino-6-(bromomethyl)pteridine-hydrobromide (**12**)[14] (110 mg, 0.3 mmol) was then added as a fine powder, and the resulting mixture was heated at 45 ± 5 °C. After ca. 24 h, 60 mL of H_2O was added to the reaction mixture, and stirring was continued at the same temperature for an additional period of ca. 24 h. Insoluble material was then removed by filtration of the reaction mixture, the filtrate pH was adjusted to ca. 3-4 with dilute HCl, and water was removed by lyophilization. The desired product was extracted from the crude solid residue by several triturations with CH_3OH. This process removed the insoluble inorganic salts, and evaporation of the solvent CH_3OH afforded crude **13a** as an oily residue. The crude product was dissolved in 500 mL of H_2O (pH adjusted to 8.2 with dilute NH_4OH) and applied to a DEAE-cellulose column (0.9 × 30 cm), previously equilibrated with 0.015 M NH_4HCO_3. The column was then washed with 200 mL of 0.015 M NH_4HCO_3, and the desired product was obtained by gradient elution with 0.015-0.30 M NH_4HCO_3 (total volume = 500 mL). The material eluting at 0.18 M NH_4HCO_3 accounted for ca. 95% of the UV absorbance and had spectral properties consistent with the structure **13a**. Lyophilization of the column effluent containing **13a** gave a hygroscopic fluffy yellow solid: ^1H NMR (D_2O, 200 MHz) δ 1.64-1.92 (2 H, m, CH_2), 2.80 (2 H, t, $CH_2C{=}O$), 2.92 (2 H, d, $P(=O)CH_2C{=}O$, J = 20 Hz), 3.16 (3 H, s, NCH_3), 3.35 (2 H, t, CH_2N), 6.80-7.73 (4 H, AA'BB', aromatic H), 8.60 (1 H, s, peteridine 7-H); the HOD signal at δ 4.50-4.90 obscured the pteridine 6-CH_2N resonance; UV $\lambda_{max}^{0.1NH^+}$ 241, 298, 331 (sh), 347 (sh) nm; $\lambda_{max}^{0.1NOH^-}$ 216, 257, 298, 370 nm. The overall yield of **13a** from **10b** was calculated as ca. 20%, based on absorbance at 298 nm in 0.1 N HCl. HPLC on Whatman SAX:[4] single peak at t_r = 10.2 min; mass spectrum (FAB), m/e 489 $(M + 1)^+$, 511 $(M + Na)^+$, 581 $(M + glycerol)^+$.

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Registry No. **3a**, 59378-98-2; **3c**, 87517-44-0; **3d**, 87517-45-1; **4a**, 87517-46-2; **5**, 87517-56-4; 5 (bis(dicyclohexylamine) salt), 87517-57-5; **6b**, 87517-47-3; **7b**, 87517-48-4; **7c**, 87517-49-5; **8**, 87517-50-8; **9a**, 87517-51-9; **9b**, 87517-52-0; **10a**, 87517-53-1; **10b**, 87517-54-2; **10b** (bis(trimethylsilyl) ester), 87517-59-7; **11b**, 87517-55-3; **11b**·2Li, 87517-60-0; **12**, 52853-40-4; **13a**, 87531-97-3; NaN_3, 26628-22-8; monomethyl glutarate, 1501-27-5; isobutylene, 115-11-7; dimethyl methanephosphonate, 756-79-6; bromotrimethylsilane, 2857-97-8; methyl 4-chlorobutyrate, 3153-37-5; ethylene glycol, 107-21-1; *p*-nitrobenzoyl chloride, 122-04-3; *p*-[[(carbobenzoxy)methyl]amino]benzoic acid, 2528-30-5; *p*-[[(carbobenzoxy)methyl]amino]benzoyl chloride, 66891-86-9; dimethyl [[2-(3-hydrazinopropyl)-1,3-dioxolan-2-yl]methyl]-phosphonate, 87517-58-6.

An Alternate Synthesis of Deethylvincadifformine

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A synthesis of 20-deethylvincadifformine from methyl nicotinylacetate is described. The reaction scheme involved a fast construction of the pentacyclic nucleus of the *Aspidosperma* alkaloids and the early incorporation of the 16-carbomethoxy group common to these bases.

Recently there was reported a simple three-reaction scheme for the facile production of the pentacyclic nucleus of the *Aspidosperma* alkaloids starting with β-acetylpyridine (Scheme I; R = H).[2] Reductive removal of the functional groups at C(5) and C(17), followed by C(2) oxidation, had served as a route to an aspidospermidine model, whose N-carbomethoxylation and subsequent

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