Folate Analogues. 24. Syntheses of the Antitumor Agents 10-Deazaaminopterin (10-DAAM) and 10-Ethyl-10-deazaaminopterin (10-EDAAM)†

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Methods have been developed for the chemical synthesis of two recently described potent antitumor agents. They are 10-deazaaminopterin (10-DAAM) (2) and 10-ethyl-10-deazaaminopterin (10-EDAAM) (3). The methods described in this paper have general applicability for the synthesis of a variety of hitherto difficultly accessible 10-substituted-10-deazafolic acids and analogues. Reaction of methyl *p*-formylbenzoate (7) with the Wittig reagent 6 derived from *N*-(3-bromo-2-oxopropyl)phthalimide (5) and triphenylphosphine gave the common intermediate 8, which was used for the synthesis of both 2 and 3. This enone was reduced with Zn/HOAc to 15 and was used for the synthesis of 2. Alternately 8 was reacted with ethylmagnesium bromide in the presence of cuprous bromide to obtain the conjugate addition product 16. Both 15 and 16 were converted to the masked α-amino ketones 20 and 21 and reacted with 6-chloro-2,4-diamino-5-nitropyrimidine to obtain 22 and 23. These pyrimidine intermediates were subsequently elaborated to the pteroic acid analogues 26 and 27 by multistep procedures previously described from this laboratory. The target compounds 2 and 3 were prepared from 26 and 27 by standard coupling procedures involving isobutyl chloroformate and diethyl L-glutamate.

One of the most widely used anticancer drug in the antifolate series is methotrexate[1,2] (MTX) (1). It is most effective against those tumors with a high growth fraction where the demand for DNA is very high and is curative to Burkitt's lymphoma and choriocarcinoma. In contrast, MTX is not as effective in the treatment of those tumors such as lung and colon cancer which have a doubling time of ~3 months. For this reason the toxicity of MTX is more pronounced and confined to those tissues such as gastrointestinal mucosa, bone marrow, and hair follicles.[3] Several attempts in many laboratories to develop other antifolate drugs with a wider spectrum of activity and diminished toxicity eventually led to the synthesis of two close analogues. They are 10-deazaaminopterin (2) synthesized in 1974[4] and the more recently synthesized 10-ethyl-10-deazaaminopterin (10-EDAAM) (3).[5] The former compound is presently undergoing clinical trials at Memorial Sloan-Kettering Cancer Center,[6,7] and the latter has been selected for clinical trials because of its diminished toxicity and highly favorable chemotherapeutic activity in several murine tumor models.[8]

Three different[4,9,10] syntheses of 10-deazaaminopterin were reported during the past decade and have been the subject of two U.S. patents. However, only one synthesis has been reported for the 10-ethyl analogue,[5] which also appears to be the drug with a more favorable therapeutic index.[11] The present paper details a synthetic procedure which has been successfully used for the preparation of both of these drugs from a common intermediate. This synthesis also offers considerable versatility in providing a general method for the introduction of various substituents at the 10-position of the 10-deaza framework of folic acid and aminopterin (Figure 1).

Results and Discussion

In developing a strategy for the synthesis of 10-DAAM and 10-EDAAM, it was realized at the outset that the procedure should be useful not only for the syntheses of the title compounds but also for the preparation of a variety of 10-substituted derivatives of 10-DAAM such as 4 from easily available starting materials. The required synthetic fragment for this purpose was conceived to be an enone A, which on treatment with various organometallic reagents[12,13] was expected to yield a variety of β-substituted ketones (B) (Figure 2). The organometallic reagents considered for the conjugate addition reactions were various Grignard reagents or organozinc compounds[12] with nickel[12] or copper ions[13] acting as catalysts. We felt that if this conjugate addition reaction could be carried out with relative ease, it would be possible to introduce various functionalities such as the alkyl, aryl, and olefinic moieties conveniently at the 10-position of 10-deazafolic acid and 10-deazaaminopterin. To check this hypothesis the synthesis of an example of fragment A was undertaken.

A convenient high-yield synthesis of *N*-(3-bromo-2-oxopropyl)phthalimide (5) was previously reported from this laboratory.[14] Reaction of 5 with an equimolar amount of triphenylphosphine gave the corresponding triphenylphosphonium bromide which on treatment with 1 equiv of NaOMe in DMF gave the Wittig reagent 6 in excellent yield.[15] This reagent was found to be stable and reacted

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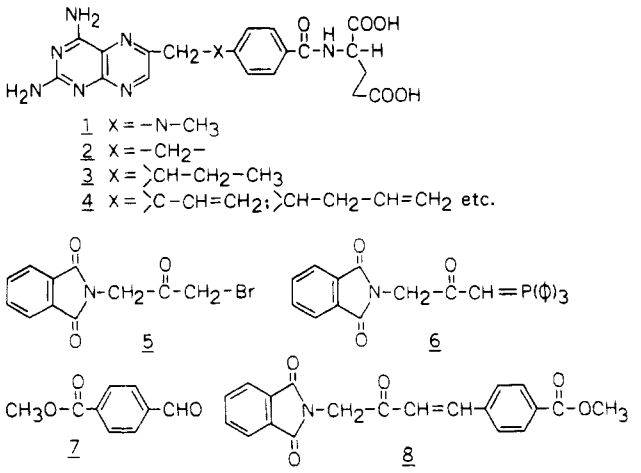

Figure 1.

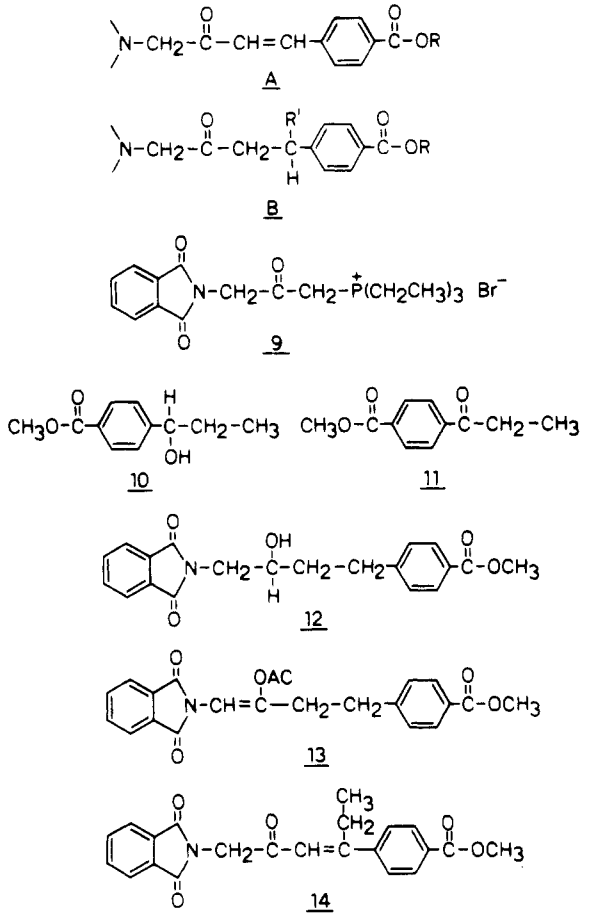

Figure 2.

with methyl *p*-formylbenzoate (7) under neutral conditions in a variety of solvents to the key enone intermediate 8 in quantitative yield. Alternately the desired enone 8 could be obtained conveniently in a one-step reaction by treating a mixture of the aldehyde and the phosphonium bromide in DMF with NaOMe under controlled conditions.

Compound 8 displayed NMR resonances due to the phthalimide and benzene ring protons in a complex pattern between 8.2 and 7.6 ppm and the methylene protons as a sharp singlet at 4.8 ppm. The olefinic protons appeared as a pair of doublets at δ 6.92 and 7.75 with a coupling constant of 18 cps, which established the stereochemistry of the double bond as trans. For the synthesis of 10-DAAM (2) the double bond of 8 had to be reduced to compound **15**, which was accomplished at room temperature with zinc and HOAc. The duration of the reduction reaction had to be carefully controlled since longer reaction time favored further reduction of **15** to the corresponding alcohol **12**. In situations where optimal reduction conditions could not be maintained a mixture of **15** and **12** were formed. However, this mixture can be cleanly converted to pure **15** by Jones oxidation[16] of the crude reaction products. Confirmation of the structure was evident from the NMR spectrum of **15**, in which the vinyl proton resonances of 8 were replaced with two overlapping triplets due to the two methylene protons at 2.95 ppm. The temperature at which this reduction is carried out was also found to be important in terms of the structure of the product obtained. For example, at 80 °C or above the product that was formed displayed NMR resonances due to the aromatic protons, the phthalimide moiety, and the carbomethoxy group at the expected positions. In addition, the reduction of the double bond was evident from the absence of the olefinic proton resonances at 6.92 and 7.75 ppm and the appearance of a four proton multiplet at 2.93 ppm. However, there was also the disappearance of the methylene singlet of 4.8 ppm, which was replaced with a one proton singlet at 6.98 and a three proton singlet at 2.1 ppm. These spectral characteristics are consistent with structure **13**.

The conjugate addition of Grignard reagents and zinc dialkyls[12] to enone 8 was next investigated. Several attempts under varying reaction conditions using nickel acetylacetonate as a catalyst for the addition of diethylzinc to 8 failed, either due to the recovery of the starting material or the formation of complex mixtures of products. However, the addition of ethylmagnesium bromide to 8 at −15 °C in THF proceeded as expected, when either cupric acetate[17] or cuprous bromide was used as a coreactant. Although the crude reaction product contained several more polar minor products, the desired β-alkyl ketone **16** was obtained in ~65% yield after column chromatography. The NMR spectrum of **16** showed the complete disappearance of the resonances due to the vinyl protons of 8 and exhibited resonances of the newly introduced ethyl group and the two methylene protons at the expected positions and splitting patterns. It should be noted that an asymmetric center has been created during this addition reaction. The separation of the enantiomers was not contemplated at this time since the target drug was not desired in an optically pure state. The separation of the individual diastereomers of 10-EDAAM and the evaluation of their biological activity is under active investigation.

Alternate strategies for the preparation of β-alkyl-substituted ketone **15** were also explored. Oxidation of the benzylic alcohol **10** with Jones reagent[16] gave the corresponding ketone **11** in excellent yield. When this ketone was reacted with the Witting reagent **6**, under a variety of conditions including fusion, not even a trace of the desired olefinic product **14** was detected by NMR spectroscopy. The failure of their reaction was initially attributed to steric factors, and to minimize the steric crowding,[18] another Wittig reagent **9**, derived from bromo ketone **5** and triethylphosphine, was employed. Again no reaction was apparent. Due to these failures, the reaction between substituted phenones and Wittig reagents to obtain compounds of general structure **14** was judged to be

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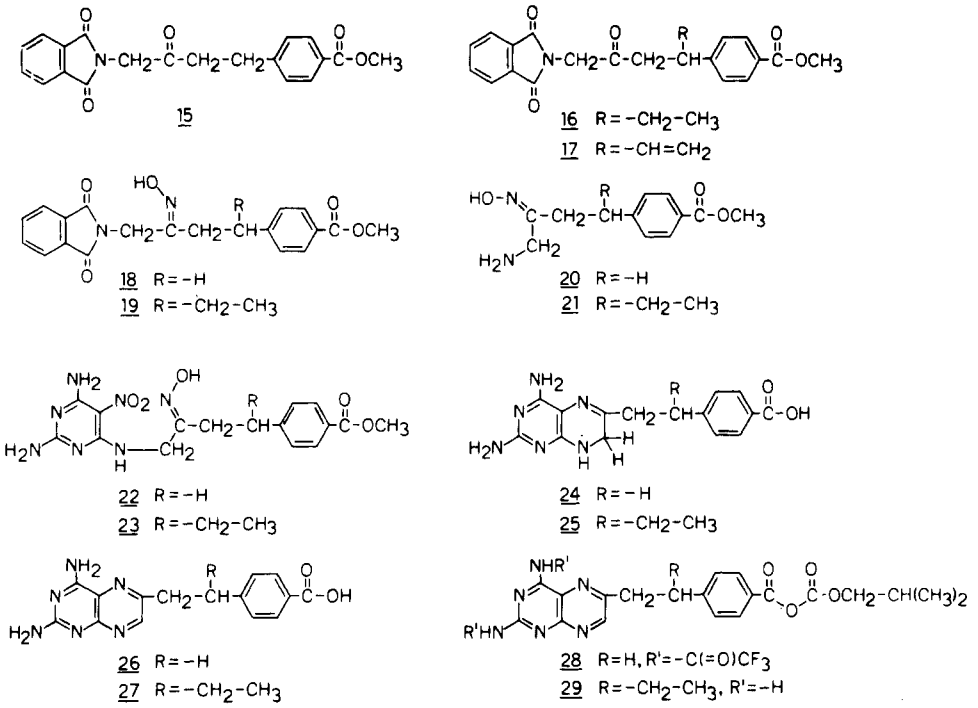

Figure 3.

unsatifactory and abandoned. However, Wittig reagent **9** reacted in the normal manner with **7** to give **8** in excellent yield.

In order to check the generality and synthetic applicability of the conjugate addition reaction of Grignard reagents to the enone, the reaction of **8** with vinyl magnesium bromide and allylmagnesium bromide was explored. These reactions were successful when carried out as described for EtMgBr and gave the desired 1,4-addition products with comparable yields. Product **17**, which had identical polarity with **8** on silica gel plates in all solvent systems examined, was easily identified as having the desired structure by NMR spectroscopy. The characteristic vinyl resonances appeared as a one proton multiplet centered at 5.90 and the vinyl methylene as a set of two proton multiplets centered at 5.1 ppm. The resonances due to the enone olefinic protons were absent in **17** and the methylene singlet was shielded and moved upfield compared to that of **8**. The use of these intermediates for the construction of 10-substituted-10-deazafolates as well as their biological activities will be the subject of future investigations in this laboratory.

Having obtained the desired key intermediates **15** and **16**, attempts were directed toward their elaboration to the target drugs **2** and **3**. Both ketones were protected as their respective oximes **18** and **19** by standard procedure, and in each case the product was a mixture of the syn and anti isomers (TLC, NMR). The isomeric mixture of the oximes **18** and **19** was subjected to hydrazinolysis.[14] In the case of **12** the hydrazinolysis product **20** was obtained as a white crystalline material. However, product **21** was a gum and resisted all attempts of crystallization. The NMR spectra of **20** and **21** were devoid of the resonances due to the phthalimide moiety, which established the cleavage of the N-protective group. Reaction of these masked α-amino ketones **20** and **21** with 6-chloro-2,4-diamino-5-nitropyrimidine gave the pyrimidine intermediates **22** and **23**, respectively. These compounds were deprotected at the carbonyl moiety[19] with a mixture of TFA and 1 N HCl to their corresponding ketones **22a** and **23a**. The nitro groups of the deprotected pyrimidine intermediates were reduced[20] to the amino groups by sodium hydrosulfite in aqueous DMF and the reduction products cyclized to the dihydropteroic acid analogues **24** and **25** with the use of 0.1 N NaOH in MeCN and oxidized to the 4-amino-4-deoxypteroic acids **26** and **27** with 5% $KMnO_4$ in 30–35% overall yield[19] starting from the oxime. Both **26** and **27** were previously reported compounds,[4,5] and their structures were established by comparison with the chemical and physical properties of authentic samples.

The carboxyl groups of the 4-amino-4-deoxypteroic acids were selectively activated as the mixed anhydride **28** and **29** with isobutyl chloroformate under controlled conditions and coupled with diethyl L-glutamate. The resulting diethyl esters were hydrolysed with 0.1 N NaOH in CH_3CN to the target compound **2** and **3**. It should be pointed out that the conversion of the dithionite reduction products of **22a** and **23a** to their respective pteroic acid analogues **26** and **27** can be carried out in a single reaction vessel without the isolation of any of the intermediates, thus offering considerable simplicity to the entire synthetic procedure.

Experimental Section

Melting points were determined on a Fisher Model 355 digital melting point analyzer. NMR spectra were run in $CDCl_3$ or CF_3COOH on a 90-MHz Perkin-Elmer spectrometer with Me_4Si as internal lock signal unless otherwise specified. Field strengths of the various proton resonances are expressed in δ (parts per million) and coupling constants in hertz. Peak multiplicity is depicted as s, singlet, d, doublet, t, triplet, q, quartet, and c, unresolved multiplet, the center of which is given. Ultraviolet spectra were determined on a Bausch & Lomb spectronic 2,000 spectrometer interfaced with a Commodore superpet computer. Elemental analyses were by Galbraith Laboratories, Inc., Knoxville, TN.

1-Phthalimido-3-(triphenylphosphoranylidene)-2-propanone (6). A solution of 21.15 g (75 mmol) of bromo ketone

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5 in 400 mL of THF was heated under reflux with 19.65 g (75 mmol) of commercial triphenylphosphine for 4 h, cooled, and concentrated. The white crystalline phosphonium bromide thus obtained was filtered, washed with ether, and dried, yield 40 g (98%), mp 292–94 °C. Anal. Calcd for $C_{29}H_{23}BrNO_3P$: C, 63.97; H, 4.23; Br, 14.70. Found: C, 63.76 H, 4.34 Br, 14.93.

To a stirred suspension of 5.44 g (10 mmol) of the above phosphonium bromide in 100 mL of DMF in N_2 was added dropwise a solution of 10 mmol of sodium methoxide (Aldrich) in 10 mL of methanol. When the addition was complete, all the solid had dissolved. After 18 h at 25 °C, DMF was removed at 60 °C under reduced pressure and the residue triturated with ice and filtered. The precipitate thus obtained was crystallized from MeOH, mp 232 °C, yield 4.21 g (91%). Anal. Calcd for $C_{29}H_{22}NO_3P$: C, 75.16; H, 4.75; O, 10.37. Found: C, 74.92; H, 4.52; O, 10.60.

4-(*p*-Carbomethoxyphenyl)-1-phthalimido-3-buten-2-one (8). To a mixture of 2.72 g (5 mmol) of the above triphenylphosphonium bromide and 820 mg (5 mmol) of methyl *p*-formylbenzoate **7** in 100 mL of DMF was added dropwise 5 mL of 1 N NaOMe in 25 mL of MeOH under nitrogen during a period of 30 min. The reaction mixture was stirred for 18 h and evaporated to dryness at 60 °C under reduced pressure. The residue was triturated with 100 g of crushed ice, filtered, and recrystallized from MeOH: yield 1.35 g (77%); mp 176–178 °C; NMR ($CDCl_3$) δ 8.05, 7.62 (d, 4 H, Ar) 7.75, 6.92 (d, 2 H, olefinic) 4.8 (s, 2 H) and 3.95 (s, 3 H, carbomethoxy). Anal. Calcd for $C_{20}H_{15}NO_5$: C, 68.77; H, 4.3; O, 22.92. Found: C, 69.1; H, 4.25; O, 23.25.

Alternately, **8** was also prepared by refluxing 2.31 g (5 mmol) of **6** with 820 mg (5 mmol) of methyl *p*-formylbenzoate in benzene for 18 h, evaporation of the solvent, trituration of the residue with ice, and recrystallization from MeOH, yield 1.68 g (96%).

(2-Oxo-3-phthalimidopropyl)triethylphosphonium Bromide (9). A solution of 5.64 g (20 mmol) of bromo ketone **5** in 100 mL of THF was stirred with 4 mL of triethylphosphine in a 300-mL round-bottomed flask for 72 h. The thick white precipitate of **9** was collected by filtration, washed with THF, and dried in vacuum, yield 7.8 g, mp 239–242 °C. When this Wittig reagent was substituted for the triphenylphosphonium bromide reagent for the preparation of **8**, the reaction proceeded as expected with the formation of the enone in comparable yields. However, when ketone **11** was substituted for the aldehyde **7**, with either of the Wittig reagents, not even a trace of the expected product **14** was detected. In all instances unreacted **11** was isolated from the reaction mixture. Anal. Calcd for $C_{17}H_{23}BrNO_3P$: C, 51.0; H, 5.75; Br, 20.0. Found: C, 49.78; H, 5.20; Br, 19.85.

1-(*p*-Carbomethoxyphenyl)-2-propanone (11). A solution of 1.94 g (10 mmol) of 1-[*p*-(carbomethoxy)phenyl]-2-propanol (**10**) in 75 mL of acetone was cooled to 0 °C by using an ice bath. To this solution was added dropwise 10 mL of Jones reagent during a period of 15 min. After it was stirred an additional 15 minutes at this temperature, the reaction mixture was evaporated to ~10 mL at 25 °C and 100 g of ice added. The white crystalline flakes of **11** thus formed were filtered, washed with water till the filtrate was colorless, and dried: yield 1.9 g (99%); mp 80–81 °C; NMR ($CDCl_3$) δ 8.1 (q, 4 H, Ar) 3.95 (s, 3 H, carbomethoxy), 3.05 (q, 2 H, ethyl), 1.25 (t, 3 H, ethyl). Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.75; H, 6.25. Found: C, 68.38; H, 6.48.

1-Phthalimido-2-acetoxy-4-(*p*-carbomethoxyphenyl)-1-butene (13). A solution of 349 mg (1 mmol) of **8** was dissolved in 20 mL in glacial HOAc under stirring in an Erlenmeyer flask at 100 °C, and 1.0 g of zinc dust was added to it portionwise (250 mg each) during a period of 30 min. The reaction mixture was filtered hot, evaporated, and triturated with 10 g of ice. The residue was separated by decantation, dried at room temperature under vacuum, and chromatographed on a column made of 15 g of silica gel. A mixture of 1:1 CH_2Cl_2 and petroleum ether eluted the product, which was obtained as a white crystalline material on evaporation of the solvent: yield 225 mg (64%), mp 104–106 °C; NMR δ 8.0, 7.3 (d, 4 H, Ar), 7.6 (c, 4 H, phthalimido), 6.98 (s, 1 H, olefenic), 3.9 (s, 3 H, carbomethoxy), 2.95 (c, 4 H, CH_2CH_2), 2.1 (s, 3 H, acetoxy). Anal. Calcd for $C_{22}H_{19}NO_6$: C, 67.17; H, 4.83; O, 24.43. Found: C, 67.38; H, 5.02; O, 24.75.

1-Phthalimido-4-(*p*-carbomethoxyphenyl)-2-butanone (15). In an Erlenmeyer flask 3.49 g (10 mmol) of enone **8** was dissolved in 250 mL of glacial HOAc by slow warming and stirring. When all **8** went in solution, it was cooled to 25 °C and 7.58 g of zinc dust was added portionwise under vigorous stirring during a period of 3 h. The reaction mixture was filtered, the Zn residue was washed 3 times with 50-mL portions of CH_2Cl_2, and the combined filtrate and washings were evaporated under reduced pressure to dryness. The white solid thus obtained was recrystallized from MeOH: yield 3.30 g (94.0%); mp 190–191 °C; NMR ($CDCl_3$) δ 8.0, 7.3 (d, 4 H, Ar), 4.5 (s, 2 H, methylene), 3.9 (s, 3 H, carbomethoxy), 2.98 (c, 4 H, CH_2CH_2). Anal. Calcd for $C_{20}H_{17}NO_5$: C, 68.37; H, 4.84; O, 22.79. Found: C, 68.56; H, 5.05; O, 23.12.

When the same reaction was carried out for a 4-h period, the product isolated by the same procedure contained a mixture of products in a 3:1 ratio (**15**/**12**). The more polar product was suspected to be the alcohol **12**, due to the presence of a one-proton multiplet at δ 4.18 in the NMR spectrum of the product mixture. When a portion of this mixture (~100 mg) was oxidized with Jones reagent (0.5 mL) in 10 mL of acetone, a single product was isolated, which was identical in all respects with authentic **15**. These observations were consistent with structure **12** for the more polar reaction product.

1-Phthalimido-4-ethyl-4-(*p*-carbomethoxyphenyl)-2-butanone (16). In a 1-L three-neck round-bottomed flask fitted with a nitrogen inlet and dropping funnel was added a mixture of 10.47 g (30 mmol) of enone **8** and 1.8 g of cupric acetate monohydrate in 300 mL of dry THF. The mixture was stirred under strictly anhydrous conditions in an atmosphere of N_2 and cooled to −15 °C with an ice/acetone mixture. To this cooled stirring mixture was added dropwise a solution of 30 mL of 2 M ethylmagnesium bromide in 200 mL of THF during a period of 1 h. After 2 h, the ice/acetone bath was removed, and the mixture was stirred at 25 °C for 18 h. A saturated solution (50 mL) of NH_4Cl was slowly added under stirring, followed by 100 mL of distilled water, and concentrated under reduced pressure at 40 °C to 200 mL. The mixture was diluted to 500 mL with distilled water and extracted with 1 L of ethyl acetate. The ethyl acetate layer was washed repeatedly with water, dried over Na_2SO_4, and evaporated. The residue was dissolved in 150 mL of CH_2Cl_2 and filtered through a bed of 250 g of silica gel suspended in CH_2Cl_2. The silica gel was washed with 500 mL of CH_2Cl_2, and the combined washing and filtrate were evaporated and evacuated. On addition of 50 mL of MeOH and overnight refrigeration crystals of **16** were formed. These crystals were filtered, washed with minimum MeOH, and dried, yield 5.4 g. An additional 1.7 g of **16** could be obtained from the mother liquor by column chromatography over silica gel with CH_2Cl_2 as the eluting solvent: total yield 7.4 g (65%); mp 126–128 °C; NMR ($CDCl_3$) δ 8.0, 7.3 (d, 4 H, Ar), 7.8 (c, 4 H, phthalimide), 4.39 (s, 2 H, methylene), 3.9 (s, carbomethoxy), 2,85 (d, 1 H, benzylic), 1.4–1.9 (c, 4 H, methylenes), 0.85 (t, 3 H, methyl). This reaction can be carried out with cuprous bromide substituting for cupric acetate as a reactant. Anal. Calcd for $C_{22}H_{21}NO_5$: C, 69.66; H, 5.54; O, 21.11. Found: C, 69.43; H, 5.23; O, 20.98.

1-Phthalimido-4-(*p*-carbomethoxyphenyl)-4-vinyl-2-butanone (17). This reaction was carried out in an identical manner as described for the preparation of **16**. A mixture of 3.49 g (10 mmol) of enone **8**, 400 mg of CuBr, and 150 mL of THF was stirred in a three-necked flask under nitrogen at −15 °C, and a solution of 12 mL of 1 N vinylmagnesium bromide in THF was added dropwise during a period of 30 min. After 2 h at −15 to −5 °C, the cooling bath was removed and stirring continued at 25 °C for 18 h. Saturated NH_4Cl (20 mL) was slowly added, the reaction mixture evaporated, then diluted to 200 mL with distilled water, and the organic products extracted in 300 mL of ethyl acetate. On evaporation of the ethyl acetate layer a viscous gum was obtained which showed two major spots on TLC, the least polar one being the desired product. The product was isolated by column chromatography over silica gel using CH_2Cl_2 as eluting solvent: mp 123–126 °C; NMR ($CDCl_3$) δ 8.05, 7.35 (d, 4 H, Ar), 7.85 (c, 4 H, phthalimide), 6.05 (c, 1 H, vinyl), 5.2 (c, 2 H, vinyl), 4.5 (s, 2 H, methylene), 4.05 (c, 1 H, benzylic), 3.95 (s, 3 H, carbomethoxy), 3.05 (d, methylene). Anal. Calcd for $C_{22}H_{19}NO_5$: C, 70.00; H, 5.04; O, 21.22. Found: C, 69.78; H, 4.84; O, 21.50.

1-Phthalimido-4-(*p*-carbomethoxyphenyl)-2-butanone Oxime (18). A mixture of 702 mg (2 mmol) of **15** and 151 mg (2.1 mmol) of hydroxylamine hydrochloride was refluxed with a solution of 80 mL of 1:1 pyridine/MeOH for 1 h and evaporated.

On addition of 20 g of crushed ice and trituration a white solid separated, which was filtered, washed with water, and dried. Examination by TLC (silica gel plates (99:1, CH_2Cl_2/MeOH) revealed that the product consisted of a mixture of two isomers in a 2:1 ratio: yield 675 mg (92%); mp 146–150 °C; NMR ($CDCl_3$) δ 7.5 (c, 6 H, phthalimide, Ar), 72 (d, Ar) 4.65, 4.25 (s, 2 H, methylene) 3.85 (s, 3 H, carbomethoxy), 2.8 (c, 4 H, CH_2CH_2). No attempts were made to separate the individual isomers. Anal. Calcd for $C_{20}H_{18}N_2O_5$: C, 65.57; H, 4.92; N, 7.65. Found: C, 65.38; H, 5.21; N, 7.90.

Preparation of Oxime 19. When this experiment was repeated with ketone **16** as the starting material under identical conditions oxime **19** was obtained in quantitative yield. Compound **19** was also a mixture of the expected syn and anti isomers, mp 93–94 °C. Anal. Calcd for $C_{22}H_{22}N_2O_5$: C, 67.00; H, 5.58; N, 7.10. Found: C, 67.35; H, 5.41; N, 6.83.

Hydrazinolysis of 18 and 19. Preparation of 1-Amino-4-(*p*-carbomethoxyphenyl)-2-butanone Oxime (20) and Its 4-Ethyl Derivative 21. In a 500-mL three-neck flask fitted with a nitrogen inlet and dropping funnel, a solution of 2.562 g (7 mmol) of **18** in 300 mL of MeOH was made by warming to ~45 °C. To this solution in a N_2 atmosphere was added a solution of 350 mg of 95% hydrazine hydrate in 10 mL of MeOH, and the mixture was stirred for 72 h. Progress of the hydrazinolysis was monitored intermittently by TLC. By 72 h all the starting material was consumed. At this stage 10 mL of 1 N HCl was added and the mixture concentrated to a small volume by rotary evaporation under reduced pressure, triturated with 100 mL of 0.1 N HCl, and filtered. The filtrate was adjusted to pH 8.5 with NH_4OH whereupon a white crystalline material (**20**) was separated. These crystals were filtered, washed with water, and dried, mp 126 °C, yield 1.23 g (74%). The NMR spectrum of **20** was identical with that of **18**, with the exception of the missing phthalimide protons. Due to the known instability of these types of compounds, **20** was used immediately in the next step without further characterization.

Compound **21** was also prepared in an identical manner. It was a noncrystalline gum and after verification of the structure by NMR it was quickly used for the next step.

Preparation of 1-[(2,4-Diamino-5-nitropyrimidin-6-yl)-amino]-4-(*p*-carbomethoxyphenyl)-2-butanone Oxime (22) and Its 4-Ethyl Analogue 23. The product **20** or **21** obtained by hydrazinolysis of 7 mmol each of **18** or **19** was refluxed with 1.4 g (~7 mmol) of 6-chloro-2,4-diamino-5-nitropyrimidine for 4 h, evaporated to dryness, and triturated with 200 g of ice. The corresponding yellow solids of **22** and **23** thus obtained were filtered and recrystallized from MeOH. Yield from a 7-mmol reaction was ~60% based on the oxime **18** or **19**, mp 216–219 °C (**22**). Compound **23** did not have a well-defined melting point. Anal. (**22**) Calcd for $C_{16}H_{19}N_7O_5$: C, 49.61; H, 4.4; N, 25.32. Found: C, 49.38; H, 4.20; N, 25.46. Anal. (**23**) Calcd for $C_{18}H_{23}N_7O_5$: C, 52.04; H, 5.06; N, 23.61. Found: C, 51.95; H, 4.83; N, 23.25.

Conversion of 22 and 23 to 4-Amino-4-deoxy-10-deazapteroic Acid (26) and 4-Amino-4-deoxy-10-deaza-10-ethylpteroic Acid (27). The following general procedure consists of several distinct steps, involving deprotection of the oxime, reduction of the 5-nitro group to the amino group, cyclization, hydrolysis of the reduction products to dihydropteroic acids **24** and **25**, and oxidation of these dihydro compounds to **26** and **27**. Due to the instability of the dithionite reduction products, and the dihydropteridines, it has been customary in the past in this laboratory to carry out the synthesis as quickly and efficiently as possible starting from the deprotected ketones to the pteroic acids directly without the isolation of any of the intervening intermediates. Typically this has been done in one reaction vessel, after obtaining the dithionite reduction product.

(a) Deprotection of Oximes 16 and 17. In a 500-mL round-bottomed flask 3.87 g (10 mmol) of **22** or 4.15 g (10 mmol) of **23** was dissolved in 100 mL of TFA, and 10 mL of 1 N HCl was added. The flask was placed in a water bath maintained between 60 and 65 °C, and during a period of 20 min an additional 90 mL of 1 N HCl was added. After the addition was complete, the clear solution was evaporated to ~15 mL under reduced pressure. After cooling, 50 of g ice was added to the flask, the contents transferred to a beaker, and the pH adjusted to 6.5 with solid $NaHCO_3$. The precipitated yellow solid was filtered, washed with water, and used for the next step, UV (0.1 N, NaOH) λ_{max} 338 nm (**22a**), 337.7 nm (**23a**). A portion of these samples was recrystallized from MeOH for analyses. Anal. Calcd for $C_{16}H_{18}N_6O_5$: C, 51.61; H, 4.30; N, 22.58. Found: C, 51.35; H, 4.03; N, 22.87. Anal. Calcd for $C_{18}H_{22}N_6O_5$: C, 54.0; H, 5.0; N, 21.0. Found: C, 54.21; H, 5.08; N, 20.85.

(b) Dithionite Reduction. The still wet deprotected compound **22a** or **23a** was dissolved in minimum amount of DMF in an Erlenmeyer flask containing a magnetic stirring bar at 60–65 °C (~75–85 mL), and an equal volume of water was added portionwise under stirring while maintaining the temperature. To this clear solution was added 20 g of solid sodium dithionite portionwise during 15 min, and 800 g of crushed ice was added. After 2 h, the precipitate was filtered, the UV spectrum of which in 0.1 N NaOH showed a λ_{max} at 325 nm indicating the reduction of the nitro group.

(c) Cyclization, Hydrolysis, and Oxidation. The dithionite reduction products were dissolved in a mixture of 500 mL of 0.1 N NaOH and 100 mL of CH_3CN and stirred at 25 °C for 18 h to ensure cyclization to the dihydropteridines and hydrolysis of the ester moiety. The pH of the reaction mixture was adjusted to 7.0 with 1 N HCl, and the mixture was concentrated by rotary evaporation under reduced pressure to ~200 mL. The oxidation was carried out by adding 30 mL of MeOH to the dihydropteridine solution followed by 10 mL of 5% $KMnO_4$ under stirring. After 30 min an aliquot was withdrawn and the UV spectrum checked for the appearance of a well defined λ_{max} between 365 and 370 nm which is indicative of the formation of a 6-substituted pteridine. Since the λ_{max} (in 0.1 N NaOH) of the oxidation products was shifted to this wavelength range after 30 min, the oxidation was judged to be complete, the solution filtered, and the filtrate acidified to pH 4.5 with glacial HOAc. A copious, bright yellow precipitate of **26** or **27** was formed in each case. These precipitates were filtered after overnight refrigeration, washed with water, and dried. The crude products were found to be >90% pure by analytical DEAE cellulose chromatography. Final purifications were done by ion exchange chromatography over DEAE cellulose in the chloride form using a linear NaCl gradient from 0 to 0.5 M. The overall yield ranged from 25% to 35% based on the oximes **18** or **19**. A lower yield was consistently observed for the 10-ethyl analogue. This was mainly due to difficulty in the recovery of the reduction products from aqueous DMF, due to enhanced solubility of the ethyl compound.

Compound **26** was found to be identical in all respects with an authentic sample suppled by Dr. Joseph I. DeGraw (SRI International, CA), and **27** was indistinguishable in all respects from a sample suppled by Dr. Francis M. Sirotnak (Memorial Sloan-Kettering Cancer Center, NY). Compounds **26** and **27** had the following spectral properties: NMR (**26**) (TFA) δ 8.25 (s, C-7H), 7.65, 6.9 (d, 4 H, Ar) 2.95 (c, 4 H, CH_2CH_2); NMR (**27**) (TFA) δ 8.1 (s, C-7H) 7.7, 7.1 (d, 4 H, Ar), 3.25 (d, 2 H, bridge methylene), 3.05 (c, 1 H, benzylic), 1.75 (c, 2 H, ethyl), 0.80 (t, 3 H, ethyl); Me_4Si was used as an external standard in both spectra; UV (**26**) (0.1 N NaOH) λ_{max} 250, 370 nm; UV (**27**) (0.1 N NaOH) λ_{max} 255.2, 369.5 nm.

Preparation of 10-Deazaaminopterin (2) and 10-Ethyl-10-deazaaminopterin (3). Although the purpose of the syntheses of **26** and **27** was their contemplated use in the preparation of the poly-γ-glutamyl metabolites of **2** and **3**, small portions of these pteroid acids were converted to the target drugs by the following general procedure.

Exactly 1 mmol of the respective pteroic acid was dissolved in a mixture of 35 mL of DMF and 5 mL of Me_2SO and cooled to 0 °C. To this cooled solution was added 1.25 mmol of freshly distilled 4-methylmorpholine followed by 1 mmol of isobutyl chloroformate. After 15 min the reaction mixture containing either **28** or **29** was removed from the ice bath and stirred at 25 °C for 30 min. during this period 2 mmol of diethyl L-glutamate hydrochloride was suspended in 10 mL of DMF and 2 mmol of 4-methylmorpholine was added. This solution was mixed with the solution of the mixed anhydride, stirred for 18 h, then heated to 80 °C, and evaporated to dryness in vacuum. The residue was triturated with 50 g of crushed ice, and after the ice had melted, the suspension was adjusted to pH 8.0 with Na_2CO_3, stirred for 15 min, and filtered to remove unreacted pteroic acid as the water-soluble sodium salt. Acidification of the filtrate with HOAc

yielded unreacted pteroic acid, which could be recycled. The precipitate of the diethyl ester was hydrolysed with 5 equiv of 0.1 N NaOH in CH_3CN at 25 °C for 18 h; the CH_3CN was removed under vacuum, acidified to pH 4.5 with glacial HOAc, and chilled. The glutamate conjugates **2** or **3** thus formed as a precipitate were filtered and further purified by ion exchange chromatography. In a typical experiment the yield of the product after purification was 67%. The physical and spectroscopic data (UV and NMR) of target drugs **2** and **3** were identical to those published earlier.[4,5]

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Registry No. **2**, 52454-37-2; **2** diethyl ester, 80577-71-5; **3**, 80576-83-6; **3** diethyl ester, 80576-82-5; **5**, 6284-26-0; **6**, 96056-26-7; **7**, 1571-08-0; **8**, 96056-27-8; **9**, 96056-28-9; **10**, 96056-29-0; **11**, 17745-40-3; **12**, 96056-30-3; **13**, 96056-31-4; **15**, 96056-32-5; **16**, 96056-33-6; **17**, 96056-34-7; (*E*)-**18**, 96095-23-7; (Z)-**18**, 96056-35-8; (E)-**19**, 96056-36-9; (Z)-**19**, 96056-37-0; **20**, 96056-38-1; **21**, 96056-39-2; **22**, 96056-40-5; **22a**, 96056-41-6; **23**, 96056-42-7; **23a**, 96056-43-8; **24**, 96056-44-9; **25**, 96056-45-0; **26**, 33047-42-6; **27**, 80576-81-4; triphenylphosphine, 603-35-0; triethylphosphine, 554-70-1; (2-oxo-3-phthalimidopropyl)(triphenyl)phosphonium bromide, 96095-24-8; ethylmagnesium, 925-90-6; vinylmagnesium bromide, 1826-67-1; hydroxylamine hydrochloride, 5470-11-1; 6-chloro-2,4-diamino-5-nitropyrimidine, 6036-64-2; diethyl L-glutamate hydrochloride, 1118-89-4.

Synthesis of Spiroimidazolidin-2-ones via Intramolecular *N*-Carbamoyliminium Ion Cyclization Reactions[1]

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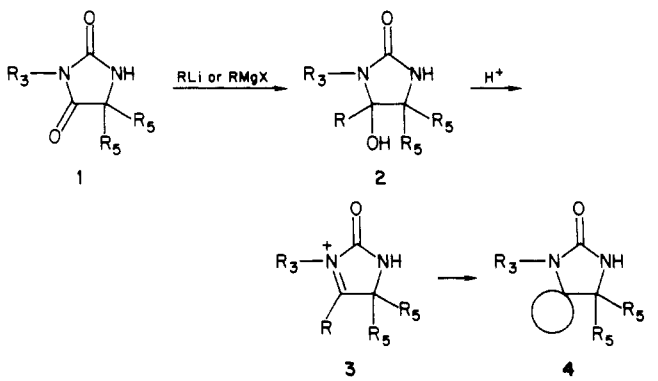
The use of *N*-carbamoyliminium ion initiated reactions for the generation of spiroimidazolidin-2-ones has been successfully exploited. Treatment of hydantoins with saturated alkyllithium and alkylmagnesium reagents gave the corresponding 4-hydroxyimidazolidin-2-one adducts. In the case of carbon-5 unsubstituted and carbon-5 monosubstituted hydantoins, dehydration of the intermediate 4-hydroxy derivatives occurred during workup to yield directly the 2-imidazolones. Addition of pent-4-enylmagnesium bromide (**13**) to the preformed magnesium salt of 3-benzyl-5,5-dimethylimidazolidine-2,4-dione (**5**) produced the expected tertiary 4-hydroxy adduct **14**. This intermediate upon treatment with formic acid underwent intramolecular cyclization to form a mixture containing two diastereomers of 2-(formyloxy)-7-benzyl-10,10-dimethyl-7,9-diazaspiro[4.5]decan-8-one (**15a,b**). The mechanism of these transformations is discussed.

N-Carbamoyliminium ions have proven to be versatile synthetic intermediates for the construction of annelated imidazolidinones and hydantoins.[1] In the cases examined the group that underwent addition to the cationic center was attached to the iminium ring nitrogen atom. We anticipated that access to the corresponding spiroimidazolidin-2-ones **4** (Scheme I) was possible by the initial treatment of 3-substituted hydantoins **1** with an excess of an organometallic reagent containing an olefinic moiety to give the 4-substituted 4-hydroxyimidazolidin-2-one derivatives **2**. These adducts **2** upon treatment with acid should lead to *N*-carbamoyliminium ion (**3**) formation followed by intramolecular olefinic cyclization to give the spiroimidazolidin-2-ones **4**. The successful implementation of this approach for the synthesis of **4** as well as the limitations of the method are the subject of this paper.

Scheme I. Proposed Synthetic Pathway Leading to the Formation of Spiroimidazolidin-2-one Derivatives



Results and Discussion

A key step in the proposed synthetic route is the formation of the tertiary hydroxy adducts **2**. We are unaware of any successful reports of the addition of organometallic reagents to hydantoin derivatives.[3] Compounds **5**–**7**[4-6] were chosen as test examples for this reaction. The hydantoins differed in the degree of substitution at carbon-5. Minor variation also existed at the N-3 position.

Treatment of 3-benzyl-5,5-dimethylhydantoin (**5**) with either *n*-BuLi, or *n*-BuMgCl, afforded 3-benzyl-4-*n*-bu-

(1) For previous papers in this series, see: (a) Kohn, H.; Liao, Z. K. *J. Org. Chem.* **1982**, *47*, 2787-2789. (b) Liao, Z. K.; Kohn, H. *Ibid.* **1984**, *49*, 3812-3819. (c) Liao, Z. K.; Kohn, H. *Ibid.* **1984**, *49*, 4745-4752.

(2) Taken, in part, from the Ph.D. dissertation of this author. Additional structure proof, discussion, and experimental and spectral data may be found in this reference.

(3) In a related example, the reaction of 3-phenyl-2-thiohydantoin with ethylmagnesium iodide in benzene led to the recovery of starting material. Shalaby, A. P. A.; Daboun, H. A. *J. Prakt. Chem.* **1971**, *313*, 1031-1038.

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