mixture was stirred at reflux for 1.75 hr and evaporated *in vacuo* to dryness, and the residue was suspended in 25 ml of ice H_2O. The cream-colored solid was collected by filtration and washed (ice H_2O) to afford 1.8 g (90%). An analytical sample of the mono HCl salt was prepared by dissolving the free base in absolute EtOH and adding 1 equiv of HCl in absolute EtOH. The precipitate was collected and washed (absolute EtOH) to give white crystals. Physical data for the diaminoquinazolines are shown in Table I.

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References

(1) J. I. DeGraw, V. H. Brown, W. T. Colwell, and N. E. Morrison, *J. Med. Chem.*, 17, 144 (1974).
(2) R. D. Stephens and C. E. Castro, *J. Org. Chem.*, 28, 3313 (1963).
(3) M. Kerfanto and D. Raphalen, *C. R. Acad. Sci.*, 258, 6441 (1964).
(4) N. A. Lange and F. E. Sheibley, "Organic Syntheses," Collect. Vol. II, Wiley, New York, N. Y., 1943, p 79.
(5) W. T. Colwell, G. Chan, V. H. Brown, J. I. DeGraw, J. H. Peters, and N. E. Morrison, *J. Med. Chem.*, 17, 142 (1974).
(6) V. Rosenoer, *Exp. Chemother.*, 4, 9 (1966).
(7) C. C. Shepard, *J. Exp. Med.*, 112, 445 (1960).

Thymidine 5′ Variants as Inhibitors of Thymidylate Kinase

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Thymidine 5′-triphosphate, the product of the phosphorylation of thymidine 5′-monophosphate by thymidylate kinase, is essential for DNA synthesis. In a search for inhibitors of DNA synthesis *in vivo*, which would, unlike nucleotide analogs themselves, be capable of penetrating cell membranes, the synthesis of thymidine analogs not containing the phosphate moiety was undertaken. Recently the inhibition of thymidylate kinase by 5′-deoxy-5′-fluorothymidine has been demonstrated.[1] In this note the preparation of two novel series of 5′-thymidine derivatives is described and their activity against thymidylate kinase, compared with **1**, is reported.

In the first series the readily available carboxylic acid **2**[2] was converted to the esters **3a–d**. Treatment of **3a** with *d* 0.88 ammonia gave the amide **4**. Acetylation gave **5**, which was smoothly dehydrated with phosphorus oxychloride in methylene dichloride to the nitrile **6**. Deacetylation gave the nitrile **7**, which was cyclized by treatment with ammonium azide in DMF to the tetrazole **8**.

The chemical shift values of the H-4′ proton of each member of the above series lay in the expected electronegativity order for each different functional group at the 4′ position of the carbohydrate moiety. For instance, the H-4′ proton in the tetrazole **8** was at lowest field (δ 5.14) followed by that in the nitrile **7** (δ 4.65) and in the amide **4** (δ 4.20). This empirical observation was maintained in other similar series of 5′ variants of nucleosides described elsewhere.†

In the second series, 5′-azido-5′-deoxythymidine[3] (**9**) was cyclized with dimethylacetylene dicarboxylate to give the triazole **10** in high yield. This was converted by direct treatment with *d* 0.88 ammonia, *n*-propylamine, ethanolamine, and benzylamine to the bisamides **11**, **12**, **13**, and **14**, respectively. Treatment with aqueous hydrazine gave the bishydrazide **15**. Reaction of **9** with ethyl propiolate gave a major triazole isomer **16a**. The minor isomer **16b** was isolated in a very low yield by preparative tlc. The structures of the two isomers were established by comparison of the chemical shift values for the triazole ring protons with those available from a known pair of 4- and 5-phenyl-1,2,3-triazolylglucose derivatives.[4] In the case of the 4-phenyl isomer the H-5 proton occurred at δ 8.01 and for the 5-phenyl isomer the H-4 proton occurred at δ 7.70. In **16a** the ring proton was at lowest field δ 8.77 compared with δ 8.24 for the minor isomer. Thus the structure of **16a** was assigned as the 4-ethoxycarbonyl isomer and the minor isomer **16b** was considered to be 5′-(5-ethoxycarbonyl-1,2,3-triazol-1-yl)-5′-deoxythymidine. **16a** was converted directly to **17** and **18** with aqueous base and *d* 0.88 ammonia, respectively. Acetylation of **18** gave **19**, which was dehydrated with phosphorus oxychloride to the protected nitrile **20**. Deacetylation gave the nitrile **21** (Chart I).

Table I. Per Cent Inhibition of Thymidylate Kinase

Compd no.	Substrate concn, m*M*			
	1	0.5	0.25	0.1
1	95	87	83	43
2	71, 59	10		
3b	61, 40	6		
3c	41, 32	0		
3d	0, 13			
4	81, 85	47	38	16
5	68, 75	49	28	0
7	97, 95	84	80	62
8	76	58	43	23
10	14, 4			
11	1, 17			
16a	19, 5			
17	0			
18	0			
21	0			

The percentage inhibition by the analogs is given in Table I from which it can be seen that the only compound with comparable activity to 5′-deoxy-5′-fluorothymidine (**1**) is the nitrile **7**. The tetrazole **8** and the amides **4** and **5** are also active, but less so. The acid **2** has only slight activity which is diminished by esterification and the triazoles **10**, **11**, **16a**, **17**, **18**, and **21** are inactive. None of the analogs exhibited significant activity *in vivo* in the inhibition of mitosis in cultured mouse fibroblasts or in the inhibition of growth of herpes simplex type I (a DNA virus). The nitrile **7** was also tested against the virus in combination with 5-iododeoxyuridine, arabinosylcytidine, and aphidicolin[5] and no synergism was found. This lack of activity may have been due to the degradation of the analogs by intracellular nucleosidases.

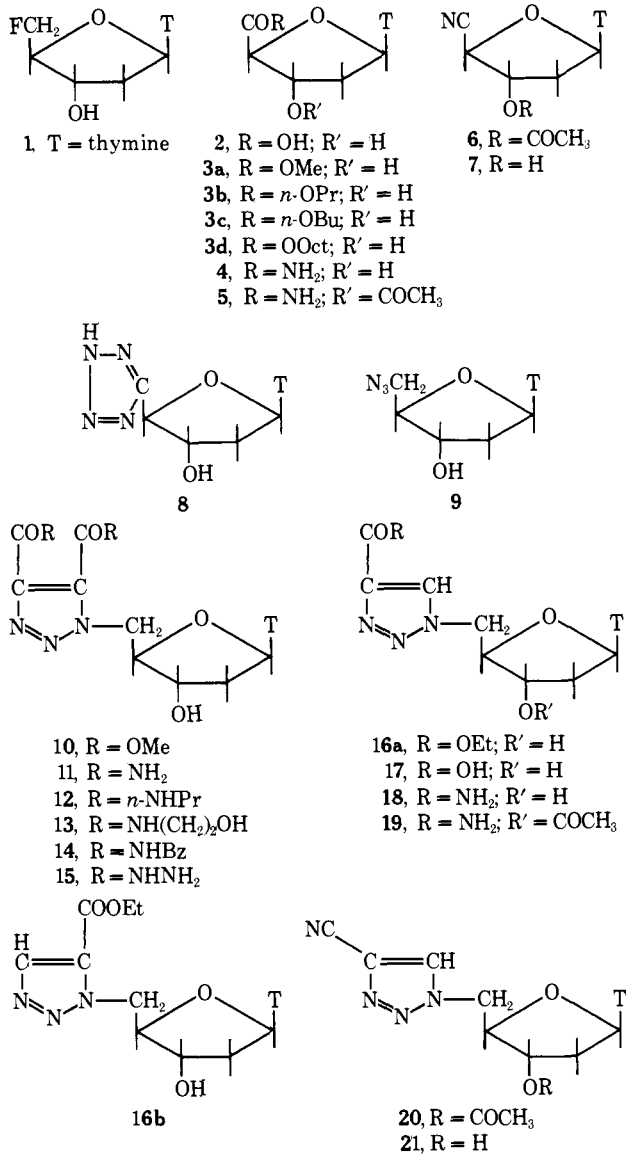
Experimental Section

Melting points were determined by use of a Büchi melting point apparatus and were uncorrected. Evaporations under reduced pressure were carried out with the aid of a Rotavapor R (Büchi, Switzerland) at 30° (24 mm) unless otherwise stated. Column chromatography was carried out on silicic acid (Kieselguhr 7734, mesh 70–200, Merck). Solvents used in the chromatographic procedures are designated as follows: solvent 1, chloroform–ethanol (19:1 v/v); solvent 2, acetonitrile–water (22:3 v/v). Nuclear magnetic resonance spectra were recorded in DMSO-d_6 by use of a Varian HA100 spectrometer. Ultraviolet spectra were recorded in spectroscopic ethanol by use of a Varian Cary 16 uv spectrometer.

General Procedure. Methyl 1′-Thymin-1-yl-2′-deoxy-β-D-ri-

†J. J. Baker, A. M. Mian, and J. R. Tittensor, manuscript in preparation.

Chart I



bofuranuronate (3a). 1′-Thymin-1-yl-2′-deoxy-β-D-ribofuranuronic acid (1 g, 3.9 mmol) was heated under reflux in anhydrous methanol (50 ml) and 98% sulfuric acid (0.1 ml) for 12 hr. The solid that deposited was collected by filtration and crystallized from ethanol to give **3a:** 0.96 g (91%); mp 251° (lit.[4] mp 247°). *Anal.* ($C_{11}H_{14}N_2O_6$) C, H, N.

Esters **3b–d** were similarly obtained. **3b:** 48%; mp 219–220°. *Anal.* ($C_{13}H_{18}N_2O_6$) C, H, N. **3c:** 67%; mp 212°. *Anal.* ($C_{14}H_{20}N_2O_6$) C, H, N. **3d:** 50%; mp 197°. *Anal.* ($C_{18}H_{28}N_2O_6$) C, H, N.

1′-Thymin-1-yl-2′-deoxy-β-D-ribofuranuronamide (4). **3a** (3.1 g, 11.5 mmol) was dissolved in *d* 0.88 ammonia (50 ml) and set aside at room temperature for 30 min. The solvent was evaporated to dryness *in vacuo* and the residue crystallized from ethanol to give **4:** 2.5 g (87%); mp 240°; uv max 266 nm (ε 9600); nmr δ 4.20 (m, 4′-H). *Anal.* ($C_{10}H_{13}N_3O_5$) C, H, N.

1′-Thymin-1-yl-3′-*O*-acetyl-2′-deoxy-β-D-ribofuranuronamide (5). **4** (600 mg, 2.4 mmol) was dissolved in dry pyridine (20 ml) and acetic anhydride (0.46 ml, 5 mmol) added. After 21 hr at room temperature, water (5 ml) was added to the mixture and the solvent evaporated to dryness *in vacuo*. The residue was crystallized from ethanol to give **5** as white needles: 556 mg (80%); mp 223°; uv max 264 nm (δ 9500); nmr δ 4.40 (d, 1, $J_{3'-4'}$ = 2 Hz, 4′-H). *Anal.* ($C_{12}H_{15}N_3O_6$) C, H, N.

1′-Thymin-1-yl-3′-*O*-acetyl-2′-deoxy-β-D-ribofuranurononitrile (6). **5** (2.7 g, 9.1 mmol) was dissolved in dry pyridine (25 ml) and the solution cooled to −5°. Phosphoryl chloride (1.2 ml, 13.5 mmol) was added and the solution maintained at −5° for 2 hr. Crushed ice was added to the mixture and the solvent evaporated to dryness *in vacuo*. The residual gum was dissolved in chloroform (5 ml) and applied to a column of silicic acid (30 × 2.5 ml). The column was eluted with solvent 1 and the major product emerged between 500 and 750 ml of eluate. The residue obtained after evaporation of the solvent *in vacuo* was crystallized from methanol to give **6:** 2 g (80%); mp 120°; uv max 263 nm (ε 9500); nmr δ 5.06 (d, 1, $J_{3'-4'}$ = 2 Hz, 4′-H). *Anal.* ($C_{12}H_{13}N_3O_5$) C, H, N.

1′-Thymin-1-yl-2′-deoxy-β-D-ribofuranurononitrile (7). **6** (100 mg, 0.42 mmol) was dissolved in 17.5% aqueous ammonia (v/v, 5 ml) and left at room temperature for 30 min. The solution was evaporated to a small volume (1 ml) and the crystals that deposited were collected by filtration and washed with methanol and ether to give **7:** 77 mg (91%); mp 235°; uv max 264 nm (ε 8900); nmr δ 4.65 (m, 1, 4′-H). *Anal.* ($C_{10}H_{11}N_3O_4$) C, H, N.

1′-Thymin-1-yl-3′-*O*-acetyl-4′(*R*)-*C*-tetrazolo-2′-deoxy-β-D-erythrofuranose (8). **7** (100 mg, 0.3 mmol) was dissolved in dry DMF (15 ml) and sodium azide (30 mg, 0.46 mmol) and ammonium chloride (25 mg, 0.48 mmol) added. The solution was stirred at 90° for 6 hr. The solvent was evaporated to dryness *in vacuo* and the residual gum purified by preparative tlc in solvent 2. Evaporation of the ethanol eluate of the combined bands with R_f 0.70 gave **8:** 92 mg (95%); mp 129–130°; uv max 266 (ε 9700); nmr δ 5.14 (d, 1, $J_{3'-4'}$ = 2 Hz, 4′-H). *Anal.* ($C_{10}H_{12}N_6O_4 \cdot 1.25H_2O$) C, H, N.

5′-[4,5-Bis(methoxycarbonyl)-1,2,3-triazol-1-yl]-5′-deoxythymidine (10). 5′-Azido-5′-deoxythymidine (4 g, 1.7 mmol) and dimethylacetylene dicarboxylate (15 ml, 0.12 mmol) were heated under reflux in 1-butanol (200 ml) overnight. The solid that deposited on cooling was collected by filtration to give **10:** 4.9 g (73%); mp 221–223° dec; uv max 263 nm (ε 9300); nmr δ 6.10 (t, 1′-H). *Anal.* ($C_{16}H_{19}N_5O_8$) C, H, N.

5′-(4,5-Dicarbamoyl-1,2,3-triazol-1-yl)-5′-deoxythymidine (11). **10** (350 mg, 0.96 mmol) was dissolved in methanol (7 ml) saturated with ammonia at 0°. The mixture was allowed to reach room temperature and set aside for 2 hr. The solid that deposited was collected by filtration to give **11:** 351 mg (93%); mp 242° dec; uv max 263 nm (ε 10,300); nmr δ 6.16 (t, 1′-H). *Anal.* ($C_{14}H_{17}N_7O_6$) C, H, N.

5′-[4,5-Bis(*n*-propylcarbamoyl)-1,2,3-triazol-1-yl]-5′-deoxythymidine (12). **10** (1 g, 2.7 mmol) was dissolved in *n*-propylamine (5 ml) and left at room temperature overnight. The solvent was evaporated to dryness *in vacuo* and the residue crystallized from methanol to give **12:** 985 mg (87%); mp 212–213°; uv max 258 nm (ε 13,900); nmr δ 6.13 (t, 1′-H). *Anal.* ($C_{20}H_{29}N_7O_6$) C, H, N.

5′-[4,5-Bis(2-hydroxyethylcarbamoyl)-1,2,3-triazol-1-yl]-5′-deoxythymidine (13). **10** (500 mg, 1.35 mmol) was dissolved in ethanolamine (5 ml) and left for 4 days at room temperature. Acetone (20 ml) was added to the mixture which was then poured with stirring onto a large volume of dry ether (500 ml) whereupon a gummy residue was obtained. The ether was decanted and the residue triturated with dry acetone. The resulting powder was crystallized from water to give **13:** 360 mg (63%); mp 198°; uv max 258 nm (ε 13,500); nmr δ 6.14 (t, 1′-H). *Anal.* ($C_{18}H_{25}N_7O_8$) C, H, N.

5′-[4,5-Bis(benzylcarbamoyl)-1,2,3-triazol-1-yl]-5′-deoxythymidine (14). **10** (1 g, 2.7 mmol) was dissolved in benzylamine (5 ml) and the mixture left at room temperature overnight. The crystals that deposited were collected by filtration to give **14:** 1.2 g (88%); mp 254–256° dec; uv max 258 nm (ε 15,000); nmr δ 6.15 (t, 1′-H). *Anal.* ($C_{28}H_{29}N_7O_6$) C, H, N.

5′-(4,5-Dicarbazoyl-1,2,3-triazol-1-yl)-5′-deoxythymidine (15). **10** (1 g, 2.7 mmol) was dissolved in ethanol (25 ml) and hydrazine hydrate (4 ml) added. The mixture was stirred at room temperature overnight. A thick white precipitate that formed was collected by filtration and recrystallized from ethanol to give **15:** 0.8 g (80%); mp 256–258° dec; uv max 265 nm (ε 11,000); nmr δ 6.13 (t, 1′-H). *Anal.* ($C_{14}H_{19}N_9O_6$) C, H, N.

5′-(4-Ethoxycarbonyl-1,2,3-triazol-1-yl)-5′-deoxythymidine (16). **9** (6.6 g, 2.8 mmol) was dissolved in 1-butanol (250 ml) and ethyl propiolate (7 ml, 0.068 mmol) added. The mixture was heated under reflux for 16 hr and left to cool. The solid that deposited was removed by filtration and crystallized from ethanol to give **16a:** 6.6 g (73%); mp 243–245°; uv max 265 nm (ε 8400); nmr δ 6.15 (t, 1′-H), 8.77 (s, 5-H). *Anal.* ($C_{15}H_{19}N_5O_6$) C, H, N.

A small amount of 5′-(5-ethoxycarbonyl-1,2,3-triazol-1-yl)-5′-deoxythymidine (**16b**, 50 mg) was isolated after preparative tlc of the liquors in solvent 2. **16b** contained a small amount of **16a** (<5%) as an impurity, which could not be removed by repeated crystallization from ethanol. **16b** has nmr δ 6.10 (t, 1′-H), 8.24 (s, 4-H).

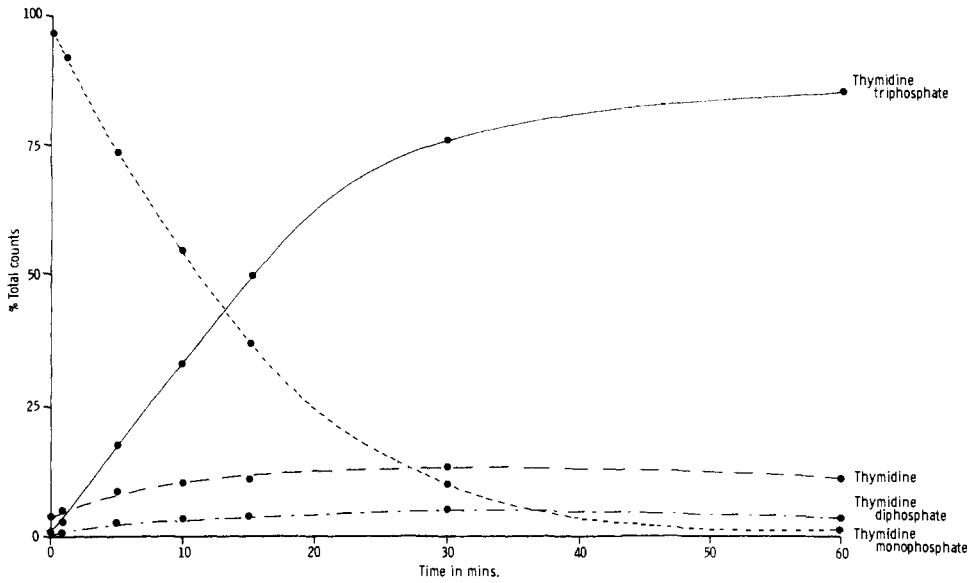


Figure 1. Thymidylate kinase time-course study (for conditions, see text).

5′-(4-Carboxy-1,2,3-triazol-1-yl)-5′-deoxythymidine (17). 16 (600 mg) was dissolved in 1 *M* sodium hydroxide (10 ml) and left at room temperature for 16 hr. The mixture was acidified with a few drops of 10 *N* hydrochloric acid. The crystals obtained on standing were collected by filtration to give **17**: 0.45 g (81%); mp 222–224° dec; uv max 265 nm (ε 8400); nmr δ 6.14 (t, 1′-H), 8.55 (s, 5-H). *Anal.* ($C_{13}H_{15}N_5O_6$) C, H, N.

5′-(4-Carbamoyl-1,2,3-triazol-1-yl)-5′-deoxythymidine (18). 16 (4 g, 1.2 mmol) was dissolved in dry methanol (200 ml) and saturated with ammonia at 0°, and the mixture was left at room temperature for 48 hr. The solid that deposited was collected by filtration to give **18**: 2.8 g (76%); mp 243–245° dec; uv max 265 nm (ε 8500); nmr δ 6.16 (t, 1′-H), 8.46 (s, 5-H). *Anal.* ($C_{13}H_{16}N_6O_5$) C, H, N.

3′-*O*-Acetyl-5′-(4-carbamoyl-1,2,3-triazol-1-yl)-5′-deoxythymidine (19). 18 (2.4 g, 1.2 mmol) was dissolved in dry pyridine (400 ml) and acetic anhydride (2.4 ml, 0.025 mmol) added. The mixture was left at room temperature for 48 hr and the pyridine removed by evaporation *in vacuo*. The residue was dissolved in chloroform and the mixture shaken with water. The chloroform layer was dried over magnesium sulfate and filtered, and the filtrate was evaporated to a solid which crystallized from ethanol to give **19**: 2.1 g (77%); mp 259–260°; nmr δ 6.14 (t, 1′-H), 8.59 (s, 5-H). *Anal.* ($C_{15}H_{18}N_6O_6$) C, H, N.

5′-(4-Cyano-1,2,3-triazol-1-yl)-5′-deoxythymidine (21). 19 (1.9 g, 0.57 mmol) was dissolved in dry pyridine (200 ml) and phosphoryl chloride (1.4 ml, 15.7 mmol) added. The red solution was stirred at −5° for 3 hr and the solvent evaporated *in vacuo* at 10°. The oily residue was dissolved in acetonitrile and fractionated on a column of silicic acid (20 × 2 cm) with a mixture of acetonitrile–water (49:1 v/v) as eluent. Fractions containing the major product were pooled and evaporated to give **20** as a white solid (1.6 g). This was treated without further purification with a solution of 17.5% aqueous ammonia (v/v, 50 ml) at room temperature for 1 hr. The solvent was evaporated *in vacuo* and the solid thus obtained was crystallized from aqueous ethanol to give **21**: 0.89 g (54%); mp 262° dec; uv max 264 nm (ε 8900); nmr δ 6.12 (t, 1′-H), 9.00 (s, 5-H). *Anal.* ($C_{13}H_{14}N_6O_4$) C, H, N.

Preparation of the Enzyme and Assay Method. Thymidylate kinase was prepared following the method of Langen and Kowollik.[1] The enzyme was isolated from Ehrlich ascites carcinoma cells‡ collected 6–10 days after passage of the tumour. The cells were sonified in 4 vol of 5.3 m*M* Tris-Cl buffer (pH 7.4) containing 0.66 m*M* $MgCl_2$ and 1 m*M* mercaptoethanol for 30 sec. The homogenate was spun at 178,000g for 40 min and the supernatant used as a crude source of thymidylate kinase.

The enzyme was assayed according to the method of Behki and Schneider.[6] The assay mixture was as follows: 10 m*M* ATP, 10 m*M* $MgCl_2$, 15.8 m*M* sodium phosphoglycerate, 80 m*M* Tris-Cl buffer (pH 7.4), 0.12 m*M* substrate (50,000 cpm of [3H]thymidine monophosphate), and 50 μl of enzyme diluted (1:1) with buffer in a total volume of 100 μl. Reactions were stopped with 10 μl of cold 32% trichloroacetic acid. The samples were washed with 3 × 1 ml of water-saturated ether and spun for 2 min in a microcentrifuge (Eppendorf). Aliquots of the samples (20 μl) were applied to PEI cellulose plates and separated using 2 *M* formic acid–0.5 *M* LiCl (1:1) as the solvent system.[7] Spots identified under uv as corresponding to thymine, TMP, TDP, and TTP were cut out and counted in 8.5 ml of toluene–butyl PBD (0.8%). A time-course study was carried out for thymidylate kinase and the results are given in Figure 1. Approximately 50% conversion of substrate was found after 15 min for thymidylate kinase (at 37°); so this incubation time was used to study the effects of the analogs.

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References

(1) P. Langen and G. Kowollik, *Eur. J. Biochem.*, **6**, 344 (1968).
(2) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, and A. R. Todd, *J. Chem. Soc.*, 1149 (1963).
(3) J. P. Horwitz, A. J. Tomson, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3045 (1962).
(4) J. P. Neenan and W. Rohde, *J. Med. Chem.*, **16**, 580 (1973).
(5) R. A. Bucknall, H. Moores, R. Simms, and B. Hesp, *Antimicrob. Ag. Chemother.*, 4, 294 (1973).
(6) R. M. Behki and W. C. Schneider, *Biochem. Biophys. Acta*, **68**, 34 (1966).
(7) K. Randerath and E. Randerath, *J. Chromatogr.*, **16**, 111 (1964).

3-Onium Derivatives of 1,4-Benzodiazepin-2-ones with Tertiary Organic Bases

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Chemistry. 1,4-Benzodiazepin-2-ones can be formed under mild conditions by cyclization of some acyclic, more hydrophilic precursors of the resulting pharmacons.[1,2] Using such hydrophilic precursors of 1,4-benzodi-