92.4%) of crude **2**: mp 162–164°. This was dissolved in 180 ml of hot acetone, treated with 1.16 g of Darco G-60, filtered, and concentrated to a volume of 100 ml. While maintained at 55°, 100 ml of 1 *N* NH_4OH was added dropwise over 10 min. The NH_4OH serves as a base for any regenerated 1 and retains it in solution as the anion. After standing for 30 min at 25° and 30 min at 0° the resulting crystalline yellow solid was filtered, washed with 50 ml of 1:1 1 *N* NH_4OH–acetone and 300 ml of H_2O, and dried at 50° (0.1 mm) to afford 10.30 g (0.0412 mol, 89%, 82.4% based on **3a**) of **2**: mp 166.5–167.5°. *Anal.* ($C_{11}H_{10}FN_3O_3$) C, H, N, F. This material was identical in every respect with that previously reported.[5]

3-(2-Acetoxyethyl)-2-(4-fluorophenyl)-1-methoxymethyl-4-nitroimidazolium Tetrafluoroborate (4). The reagents were prepared and combined in the quantities indicated in the previous experiment and the reaction mixture was refluxed for 40 hr. The resulting precipitate was filtered, washed with 50 ml of CH_2Cl_2, slurried with 100 ml of H_2O, refiltered, washed with 25 ml of H_2O, and dried at 50° (0.1 mm) to afford 17.3 g (81.6%) of imidazolium salt **4**: mp 134–136°. *Anal.* ($C_{15}H_{17}BF_3N_3O_5$) C, H, N.

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Synthesis and Biological Properties of 5-Aryl-4*H*-1,2,4-thiadiazine 1,1-Dioxides†

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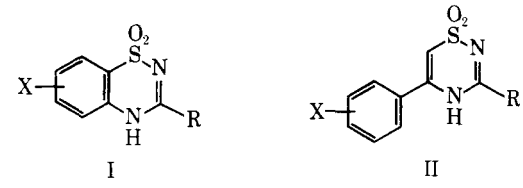
1,2,4-Benzothiadiazine 1,1-dioxides (I) are well-known diuretic and/or antihypertensive agents.[1-3] However, examples of nonfused, fully unsaturated 1,2,4-thiadiazine 1,1-dioxides have not been reported.[4] We have now synthesized 5-aryl-4*H*-1,2,4-thiadiazine 1,1-dioxides of type II and tested them for antihypertensive and diuretic activity.

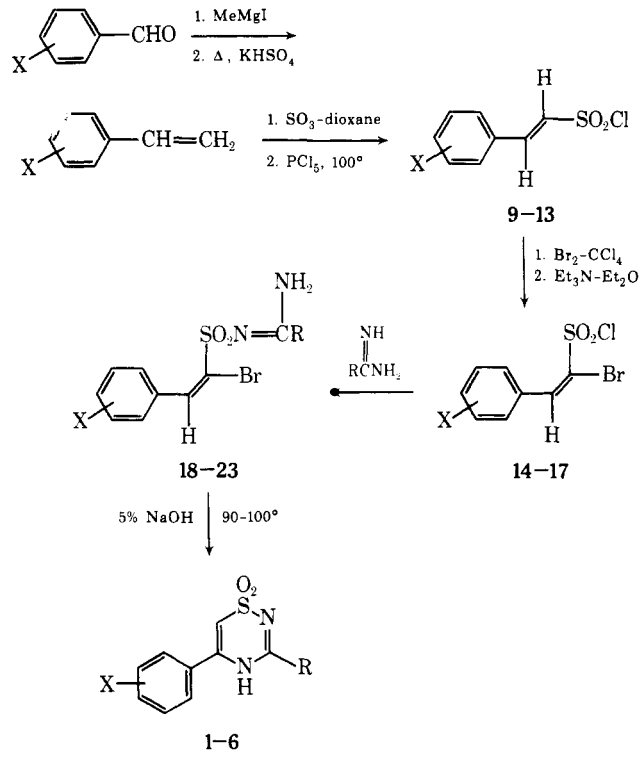
Chemistry. The 5-aryl-4*H*-1,2,4-thiadiazine 1,1-dioxides in Table I were prepared by the route shown in Scheme I. Styrene is available commercially, but chlorinated styrenes were obtained from the corresponding benzaldehydes by the method of Brooks.[5] The styrenes were converted to *trans*-styrylsulfonyl chlorides (Table II) by the method of Bordwell, *et al.*[6] The nitro derivatives **12** and **13** were readily prepared by nitration of the unsubstituted compound **9** according to the procedure of Bordwell, *et al.*[7] We assign the trans configuration to these compounds on the basis of the coupling constants of their vinyl protons in the nmr spectra ($J \sim 15.5$ Hz).



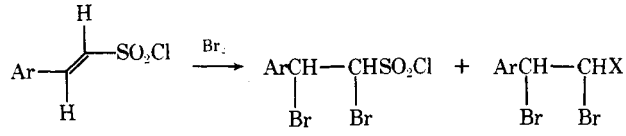
I II

chlorothiazide: R = H; X = 6-Cl, 7-SO_2NH_2
diazoxide: R = Me; X = 7-Cl

Scheme I



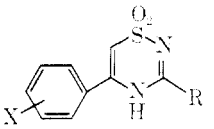
Compounds **14–17** in Table II were prepared by bromination followed by dehydrobromination of the corresponding styrylsulfonyl chlorides as described by Rondestvedt.[8] The bromination reaction is often complicated by the formation of products which have lost the sulfonyl group. These products appear to be III based on the nmr and mass spectra of the product mixtures and are probably formed *via* a free-radical process. The 4-nitro derivative **17** was obtained by nitration of **14**. These α-bromostyrylsulfonyl chlorides probably have the *E* configuration, as suggested by Rondestvedt,[8] since the parent compound could not be dehydrobrominated to the acetylenic sulfonyl chloride and, under forcing conditions, lost the sulfonyl group.



III, X = Br or Cl

†Presented in part at the Fourth International Congress of Heterocyclic Chemistry, Salt Lake City, Utah, July 8, 1973.

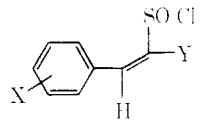
Table I. 5-Aryl-4*H*-1,2,4-thiadiazine 1,1-Dioxides (II)



Compd no.	X	R	Mp,[a] °C	Crystn solvent	Yield, %	Formula
1	H	Me	270.5–273.5	MeCN–*i*-Pr_2O	66	$C_{10}H_{10}N_2O_2S$
2	4-Cl	Me	289.5–291.5	Me_2CO	71	$C_{10}H_9ClN_2O_2S$
3	3,4-Cl_2	Me	253.5–254.5	Me_2CO–*i*-Pr_2O	66	$C_{10}H_8Cl_2N_2O_2S$
4	3,4-Cl_2	Ph	306.5–308	Me_2CO	86	$C_{15}H_{10}Cl_2N_2O_2S$
5	H	Ph	258.5–260.5	Me_2CO–*i*-Pr_2O	87	$C_{15}H_{12}N_2O_2S$
6	4-NO_2	Me	>320	MeCN	64	$C_{10}H_9N_3O_4S$
7	4-Cl-3-$ClSO_2$	Me	220–225	*i*-PrOAc	53	$C_{10}H_8Cl_2N_2O_4S_2$
8	4-Cl-3-NH_2SO_2	Me	312.5–316.5	MeOH–*i*-Pr_2O[b]	75	$C_{10}H_{10}ClN_3O_4S_2$

[a]Corrected melting points. [b]Crystallization solvent system for the diethylammonium salt. See Experimental Section. [c]Overall yield for two steps from α-bromo-4-nitrostyrylsulfonyl chloride (**17**).

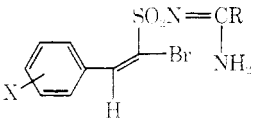
Table II. Styrylsulfonyl Chlorides



Compd no.	X	Y	Mp, °C	Crystn solvent	Yield, %	Formula
9	H	H	86–89[a]	CCl_4	95	
10	4-Cl	H	133–135	CCl_4	88	$C_8H_6Cl_2O_2S$
11	3,4-Cl_2	H	95–98	CCl_4	92	$C_8H_5Cl_3O_2S$
12	4-NO_2	H	171–174[b]	PhH	43	
13	2-NO_2	H	102–105[c]	*i*-Pr_2O	17	
14	H	Br	51–53[d]	Pet. ether	84	
15	4-Cl	Br	93–94	Pet. ether	86	$C_8H_5BrCl_2O_2S$
16	3,4-Cl_2	Br	67–69	Pet. ether	62	$C_8H_4BrCl_3O_2S$
17	4-NO_2	Br	123–124	CCl	46	$C_8H_5BrClNO_4S$

[a]Reference 6 reported melting point 86–89°. [b]Reference 7 reported melting point 172–174°. Reference 7 reported melting point 103–105°. [d]Reference 8 reported melting point 46–48°

Table III. *N*-(α-Bromostyrylsulfonyl)amidines

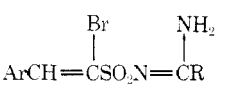


Compd no.	X	R	Mp, °C	Crystn solvent	Yield, %	Formula
18	H	Me	141–143.5[a]	*i*-PrOAc	76	$C_{10}H_{11}BrN_2O_2S$
19	H	Ph	169–170	*i*-PrOAc	92	
20	3,4-Cl_2	Me	187.5–189[a]	Me_2CO–*i*-Pr_2O	90	$C_{10}H_9BrCl_2N_2O_2S$
21	3,4-Cl_2	Ph	140–144		92	
22	4-Cl	Me	156–160	$CHCl_3$	81	$C_{10}H_{10}BrClN_2O_2S$
23	4-NO_2	Me	184–187	MeCN	8[b]	$C_{10}H_{10}BrN_3O_4S$

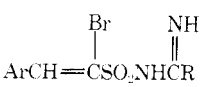
[a]Corrected melting point. [b]Isolated material. Residual sulfonylamide was cyclized to **6** in 64% yield.

The *E* configuration would also be expected for trans addition of Br_2 to the trans olefin followed by trans elimination of HBr. Attempts to confirm this structural assignment by the nmr method of Pascual, *et al.*,[9] were inconclusive.

N-(α-Bromostyrylsulfonyl)amidines (Table III) were obtained in high yields by the reaction of the appropriate styrylsulfonyl chloride with excess amidine for 10 min by a method similar to that of Northey, *et al.*[10] These compounds are drawn in the "amino form" IV, rather than in the tautomeric "imino form" V since they show strong absorption in their infrared spectra at 1630–1660 (NH_2 deformation) and 1525–1565 cm^{-1} (C=N). These values are consistent with the frequencies reported for related compounds.[11-14]



IV



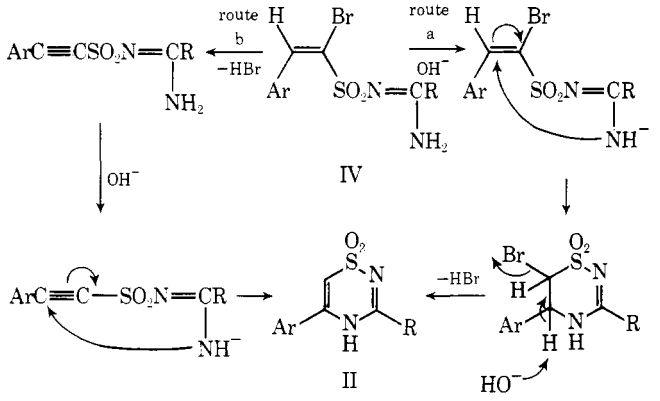
V

If the reaction of a styrylsulfonyl chloride with excess amidine is allowed to continue for a prolonged period, cyclization of the initially formed sulfonylamidine occurs to produce a 5-aryl-4*H*-1,2,4-thiadiazine 1,1-dioxide directly. The styrylsulfonyl chloride **14** afforded the thiadiazine **1** in 11% yield after 30 min and in 43% yield after 64 hr. Electron-withdrawing substituents in the aromatic ring enhance the tendency to cyclize, and α-bromo-4-nitrostyrylsulfonyl chloride (**17**) gave a mixture of sulfonylamidine **23** and thiadiazine **6** after only 5 min at 5–10°. The *N*-(α-

bromostyrylsulfonyl)amidines were conveniently cyclized to thiadiazines (Table I) in 5% NaOH solution at 90–100° for 0.5–1 hr. Surprisingly, these thiadiazines are quite stable to hot NaOH whereas 1,2,4-benzothiadiazine 1,1-dioxides are rapidly hydrolyzed under these conditions.[15] Compound 8 was prepared by chlorosulfonylation of the 4-chloro derivative 2, followed by ammonolysis. In agreement with the preferred structure for 1,2,4-benzothiadiazine 1,1-dioxides,[15,16] we have drawn the nonfused thiadiazines as 4H rather than 2H tautomers.

Two possible mechanisms for the conversion of *N*-(α-bromostyrylsulfonyl)amidines to thiadiazines are outlined in Scheme II.

Scheme II



The cyclization may proceed (route a) by intramolecular Michael addition of the sulfonylamidine anion to the activated olefinic bond affording a 6-bromo-5,6-dihydrothiadiazine, followed by dehydrobromination. Alternatively, dehydrobromination may occur first (route b) to afford an acetylenic sulfonylamidine, followed by cyclization. At present we favor the 6-bromo-5,6-dihydrothiadiazine intermediate since formation of an acetylene from IV would have to occur by an unfavorable cis elimination of HBr.

Biology. The thiadiazines in Table I were evaluated for diuretic activity in conscious rats by the procedure described by Gomoll, *et al.*[17] With exception of the unsubstituted compound 1, no diuretic activity was displayed, even at the maximum dose level of 24 mg/kg, by any other compound in Table I. The mininum effective dose of compound 1 in the conscious rat was greater than 8 mg/kg. At 24 mg/kg this compound increased volume excretion by 50% compared to controls and elevated the urinary Na/K ratio by 18%. Under the same test conditions chlorothiazide increased volume excretion by 50% and the Na/K ratio by 72%, whereas diazoxide decreased volume excretion by 40% and the Na/K ratio by 37%.

The cardiovascular effects of the thiadiazines, each given intravenously to three normotensive anesthetized dogs, were compared with the effects of intravenous diazoxide. Compounds 1, 3, and 8, at 5–10 mg/kg iv, elicited moderate hypotensive responses and reflexly augmented myocardial contractile force and heart rate. At these doses compound 6 had little effect on blood pressure but produced slight inotropic and chronotropic responses. In contrast, diazoxide effectively lowered blood pressure in this dog model at 1.0–2.5 mg/kg iv. However, intraduodenal administration of 20 mg/kg of 1 to two normotensive anesthetized dogs lowered mean arterial blood pressure by an average of 28 mm at 105 min (maximum) with a 3-min onset, compared to an average of 14–18 mm maximum with a 10-min onset for diazoxide at this dose. Compound 1 also decreased the heart rate parallel to the blood pressure effects, with an average peak lowering of 40 beats per min. Compound 8 (20 mg/kg) produced a slight hypotensive response with reflex tachycardia and the onset occurred at 60 min after intraduodenal administration; other members of this series are essentially inactive under these conditions. An explanation for the greater effectiveness of 1 compared to diazoxide by id administration but not iv is not apparent.

Although compound 1 is slightly active when administered orally to DOCA hypertensive rats,‡ 1 and 8 show considerably less blood pressure lowering and tachycardia than diazoxide at 25 mg/kg.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected except as indicated. The structures of all compounds are supported by their ir and nmr spectra. Spectra were recorded on a Beckman IR 9 and a Varian A-60 spectrometer; microanalytical and spectral data were supplied by the Physical Analytical Department of Mead Johnson and Co. Analyses are indicated by the formula of the compound and the analytical results obtained for C, H, and N are within ±0.4% of the theoretical value.

α-Bromo-4-nitrostyrylsulfonyl Chloride (17). α-Bromostyrylsulfonyl chloride (14.1 g) was added in portions over 30 min to a stirred mixture of concentrated HNO_3 (25 ml) and concentrated H_2SO_4 (25 ml) at 15–20°. The mixture was stirred for 1 hr at 25° and poured onto ice. Water (100 ml) was added and the product was extracted into $CHCl_3$, dried ($MgSO_4$), and isolated by evaporation of the solvent. The crude oily product crystallized from CCl_4 to afford 8.0 g (50%) of **17** as a yellow solid, mp 117–122°. A recrystallization from CCl_4 gave 5.2 g of analytically pure **17**, mp 123–124°.

***N*-(α-Bromostyrylsulfonyl)acetamidine (18).** A mixture of acetamidine hydrochloride (14.2 g, 0.15 mol) and 50% aqueous NaOH (12.0 g, 0.15 mol) in acetone (150 ml) was stirred vigorously for 10 min and cooled to 10–15°. A solution of α-bromostyrylsulfonyl chloride (10.1 g, 0.05 mol) in acetone (50 ml) was then added dropwise at 10–15° with vigorous stirring. The mixture was stirred for an additional 10 min and then concentrated under reduced pressure and diluted with water (200 ml). The mixture was acidified with 3 *N* HCl; insoluble solid was collected by filtration, dissolved in $CHCl_3$ (150 ml), and dried ($MgSO_4$). Evaporation of the solvent gave 5.8 g (76%) of **18** as a white solid, mp 140–142°, after recrystallization from *i*-PrOAc: nmr ($CDCl_3$) δ 8.10 (s, 1, =CH), 8.03 (br s, 1, NH), 7.9–7.6 (m, 2, Ar H), 7.6–7.1 (m, 3, Ar H), 6.55 (br s, 1, NH), 2.20 (s, 3, CH_3); ir (KBr) 3415, 3325, 3240 (NH), 1640 (NH_2), 1545 (C=N), 1280, and 1130 cm^{-1} (SO_2).

Compounds **19–23** in Table III were obtained by similar procedures.

5-Phenyl-3-methyl-4*H*-1,2,4-thiadiazine 1,1-Dioxide (1). A suspension of *N*-(α-bromostyrylsulfonyl)acetamidine (6.02 g, 0.02 mol) in 5% aqueous NaOH (25 ml) was stirred and heated on a steam bath for 1 hr. The clear solution was cooled, filtered, and acidified with 3 *N* HCl to precipitate a white solid, which was washed with water and dried in a vacuum oven at 80°. One crystallization from MeCN-*i*-Pr_2O afforded 2.9 g (66%) of analytically pure **1**: mp 270.5–273.5° cor; nmr (DMSO-d_6) δ 10.93 (br s, 1, NH), 7.61 (m, 5, Ar H), 6.63 (d, J = 2 Hz, 1, =CH), 2.30 (s, 3, CH_3); ir (KBr) 3280, 3210 (NH), 3090 (aromatic), 1645, 1625 (C=C andC=N), 1285, 1150 (SO_2), and 1245 cm^{-1}; uv max (EtOH) 247 nm (ε 5390) and 269 (2930); uv max (EtOH + NaOH) 254 nm (ε 6050) and 304 (1995).

Compounds **2–6** in Table I were prepared by similar procedures.

5-(4-Chloro-3-chlorosulfonylphenyl)-3-methyl-4*H*-1,2,4-thiadiazine 1,1-Dioxide (7). A solution of 5-(4-chlorophenyl)-3-methyl-4*H*-1,2,4-thiadiazine 1,1-dioxide (**2**, 12.8 g, 0.05 mol) in $ClSO_3H$ (100 ml) was heated at 130° for 1.5 hr. Excess $ClSO_3H$ was then removed by distillation and the residue was cooled and added dropwise to ice. The product was extracted into EtOAc, washed with water, and dried ($MgSO_4$). After evaporation of the solvent, the gummy residue was triturated with EtOAc (25 ml) to afford 7.9 g of **7**, mp 220–227°. Concentration of the filtrate gave

‡The tests for antihypertensive activity in the DOCA rat were conducted at Pharmakon Laboratories, Scranton, Pa. 18510, under the supervision of R. J. Matthews.

another 1.5 g of solid, mp 217–220°. The total yield of product was 53%. It crystallized from *i*-PrOAc, mp 220–225° dec.

5-(4-Chloro-3-sulfamoylphenyl)-3-methyl-4*H*-1,2,4-thiadiazine 1,1-Dioxide (8). NH_3 was bubbled through a solution of the chlorosulfonyl compound **7** (7.1 g, 0.02 mol) in acetone (100 ml) for 10 min. After evaporation of the solvent, the residue was dissolved in hot 5% aqueous NaOH solution, filtered, and acidified with 3 *N* HCl to precipitate a cream-colored solid. It was collected by filtration, washed with H_2O, and triturated with Me_2CO to afford 5.8 g (86.5%) of the title compound **8**, mp 303–310° dec. The crude product was dissolved in hot MeOH (50 ml) and excess Et_2NH. Addition of *i*-Pr_2O and cooling gave 3.6 g of the diethylammonium salt of **8**, mp 300–305° dec. This salt was dissolved in hot 5% aqueous NaOH, filtered, acidified with 3 *N* HCl, and cooled to afford analytically pure **8**.

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Synthesis and Antifolate Activity of 10-Deazaminopterin

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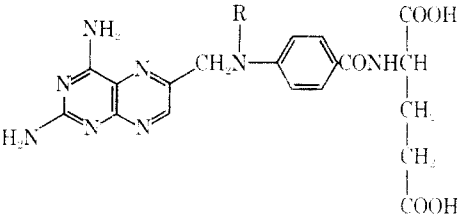
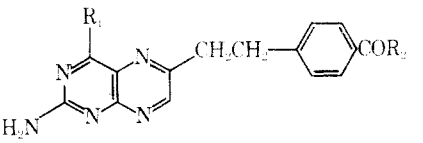
The antimicrobial and antitumor activities of the powerful dihydrofolic reductase inhibitors aminopterin (I) and its N^{10}-methyl derivative, methotrexate (MTX, II), are well known. Numerous analogs have been made to further improve the potency, cell penetration, and toxicity properties of these compounds. As part of a continuing program to investigate structure–activity relationships in folic acid analogs, we were interested in the effects of replacement of the nitrogen atom in the side chain of aminopterin. The synthesis and biological activity of 10-deazaminopterin are reported in this paper.



I. R = H
II. R = CH_3



III. $R_1 = R_2 = OH$
IV. $R_1 = OH$; R_2 = glutamyl
V. $R_1 = NH_2$; $R_2 = OH$
VI. $R_1 = NH_2$; R_2 = glutamyl

In a previous communication[1] we reported the synthesis of 10-deazapteroic acid (III) and noted the potent growth inhibitory action of III and its tetrahydro derivative against *Streptococcus faecium*, a folate dependent bacteria. Struck, *et al.*,[2] confirmed the activity of III and also reported the activity of the glutamate conjugate, 10-deazafolic acid (IV). Encouraged by the activity of the 10-deaza compounds we subsequently prepared[3] 2,4-diamino-6-*p*-carboxyphenethylpteridine (V). The compound possessed one-half the potency of aminopterin and $\frac{1}{14}$ that of amethopterin against *S. faecium*. A sixfold loss of activity was observed after reduction to the tetrahydro form. Only moderate activity was noted against *Lactobacillus casei*.

Coupling of V with glutamic acid to form 10-deazaminopterin (VI) has resulted in an analog with powerful antifolate activity. In Table I it can be seen that VI and its dihydro (VI-H_2) and tetrahydro (VI-H_4) derivatives are strong inhibitors of bacterial growth in *S. faecium* and *L. casei*. In *S. faecium* the minimum inhibitory concentrations (MIC) are similar to those for MTX and aminopterin and their corresponding reduced forms. However, VI-H_2 was the most potent antifolate we have seen for the inhibition of *L. casei*. Half maximal inhibition occurred at 5×10^{-12} *M* at a ratio of antimetabolite to folate of 1:500. It is also of interest that this compound has some activity against MTX resistant strains of *L. casei* and *S. faecium*. The tetrahydro-VI was also very potent and was about nine times more active than the respective tetrahydro MTX and tetrahydroaminopterin against *L. casei*. Strong activity against *Pediococcus cerevisiae* was also observed for VI-H_4.

The inhibition of *L. casei* dihydrofolate reductase by 10-deazaminopterin and its reduced forms is appreciable (Table II) but is not correlated with growth inhibition because the latter is 100, 1000, and 5000 times greater than reductase inhibition with the unreduced, dihydro, and tetrahydro compounds, respectively. Inhibition of thymidylate synthetase by VI and derivatives is not outstanding but is detectable with the reduced forms.

Standard procedures for the coupling of V, *via* its bis(trifluoroacetyl) derivative, and diethyl glutamate were not undertaken due to the possibility of deamination of the 4 position during hydrolysis of blocking groups. Instead, the carboxyl group of unblocked V was activated