(s, 3 H), 6.05 (br s, 1 H), 6.14 (s, 2 H), 6.53–7.24 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 18.8 (q, 2 × CH_3), 24.6 (q, $COCH_3$), 71.7 (s, C-4), 115.2 (d, C-2′), 119.9 (d, C-4′), 128.4 (d, C-2), 129.3 (d, C-3′), 143.5 (s, C-1′), 160.9 (s, C-3), 172.2 (s, $COCH_3$), 185.7 (s, C-1); mass spectrum, *m/e* 271 (18), 270 (M, 77), 228 (M – CH_2CO, 17), 227 (18), 199 (10), 197 (11), 196 (39), 194 (13), 184 (10), 183 (41), 182 (71), 181 (10), 180 (12), 179 (21), 178 (15), 177 (M – $ArNH_2$, 25), 168 (23), 167 (32), 143 (14), 142 (10), 137 (71), 136 (97), 135 (16), 134 (10), 128 (10), 117 (11), 108 (23), 107 (15), 106 (22), 103 (16), 93 (100), 94 (63). Anal. ($C_{16}H_{18}N_2O_2$) C, H, N.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Hydrochloric acid. Dry hydrogen chloride gas was bubbled into a solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (11; 74 mg, 0.42 mmol) in ether (15 mL). A white precipitate formed readily and after 10 min the addition of the gas was stopped. The solvent was evaporated with a stream of dry nitrogen to give a crude white amorphous solid. Recrystallization from aqueous EtOH gave 3′-chloro-2′,6′-dimethyl-4′-hydroxyacetanilide (15; 79 mg, 88%) as white prisms: mp 216.5–217.5 °C; IR ν_{max} 3290 (br s, NH, OH), 1643 (s, CO), 1582 (w), 1538 (s), 1472 (m), 1432 (w), 1370 (w), 1288 (m), 1205 (w), 1160 (m), 1029 (w) cm^{-1}; ^{1}H NMR [$CDCl_3$/$(CD_3)_2SO$] δ 2.10 (s, 3 H), 2.11 (s, 3 H), 2.20 (s, 3 H), 6.66 (s, 1 H), 8.86 (s, 1 H), 9.24 (br s, 1 H); ^{13}C NMR [$CDCl_3$/$(CD_3)_2SO$] δ 14.9 (q, $ArCH_3$), 17.3 (q, $ArCH_3$), 21.6 (q, $COCH_3$), 113.9 (d, C-5′), 117.2 (s, C-3′), 126.1 (s, C-2′, C-6′), 133.7 (s, C-1′), 150.6 (s, C-4′), 168.5 (s, $COCH_3$); mass spectrum, *m/e* 215 (M + 2, 22), 213 (M, 65), 173 (59), 172 (36), 171 (M – CH_2CO, 100), 170 (46), 136 (36), 107 (15), 106 (11). Anal. ($C_{10}H_{12}ClNO_2$) C, H, N, Cl.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Sodium Dithionite. Sodium dithionite (2 g, 0.10 mol) in H_2O (10 mL) was added to a rapidly stirred solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (11; 50 mg, 0.282 mmol) in EtOAc (10 mL) at 50 °C. After 5 min the phases were separated, and the aqueous phase was extracted with EtOAc (2 × 20 mL). The organic extracts were combined, dried ($MgSO_4$), and evaporated to dryness. The residue was recrystallized from $CHCl_3$/light petroleum to yield 2′,6′-dimethyl-4′-hydroxyacetanilide (7; 50 mg, 99%), mp 183–184 °C.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Ethanethiol. Ethanethiol (0.6 mL, 7.89 mmol) was added to a solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (11; 154 mg, 0.87 mmol) in Et_2O (20 mL). A crystalline product began to form immediately and after 12 h this was filtered. Recrystallization from CH_2Cl_2/light petroleum gave 4-acetamido-3,5-dimethyl-4-(ethylthio)-2,5-cyclohexadien-1-one (16) as colorless microprisms (163 mg, 78%): mp 116.5–117.5 °C (sealed tube, with decomposition); UV λ_{max} (CH_3CN) 220 nm (ϵ 7020), 255 (10480); IR ν_{max} 3224 (s, NH), 1655 sh (s), 1650 (s, $COCH_3$), 1625 (s, CO), 1535 (m), 1439 (m), 1375 (m), 1369 (m), 1345 (w), 1315 (w), 1292 (m), 895 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.12 (t, *J* = 7.5 Hz, 3 H), 2.03 (br s, 9 H), 2.10 (q, *J* = 7.5 Hz, 2 H), 6.00 (br s, 1 H), 6.21 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 12.4 (q, SCH_2CH_3), 18.7 (q, 2 × CH_3), 22.5 (q, $COCH_3$), 23.9 (t, SCH_2CH_3), 66.3 (s, C-4), 127.5 (d, C-2), 157.4 (s, C-3), 169.3 (s, $COCH_3$), 185.2 (s, C-1); mass spectrum, *m/e* 239 (22, M), 198 (10), 197 (49, M – CH_2CO), 196 (13), 179 (45), 178 (32, M –SCH_2CH_3), 177 (34), 168 (25), 138 (13), 137 (82), 136 (100), 135 (19), 134 (13), 122 (15), 108 (27), 107 (13), 106 (17). Anal. ($C_{12}H_{17}NO_2S$) C, H, N.

Reaction of *N*-Acetyl-2,6-dimethyl-*p*-benzoquinone Imine (11) with Ethyl Alcohol. Dry EtOH (2 mL) was added to a solution of *N*-acetyl-2,6-dimethyl-*p*-benzoquinone imine (11; 130 mg, 0.73 mmol) in Et_2O (10 mL). The mixture was allowed to stand at room temperature for a week. Removal of the solvent gave a crude white solid (163 mg), mp 153–157 °C. Recrystallization from $CHCl_3$/light petroleum gave 4-acetamido-3,5-dimethyl-4-ethoxy-2,5-cyclohexadien-1-one (18; 147 mg, 89%) as white needles: mp 161.5–162.5 °C (sealed tube, dec); UV λ_{max} (CH_3CN) 219 nm (ϵ 8650), 242 (8400), 277 (2280); IR ν_{max} 3200 (br m), 1680 (s, $COCH_3$), 1650 sh (s), 1640 (s, CO), 1535 (w), 1381 (m), 1300 (w), 1280 (w), 1172 (w), 1067 (s), 969 (w), 892 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, *J* = 7.2 Hz, 3 H), 1.89 (s, 3 H), 1.90 (s, 3 H), 1.99 (s, 3 H), 3.14 (q, *J* = 7.2 Hz, 2 H), 6.26 (s, 2 H), 6.32 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.0 (q, OCH_2CH_3), 17.1 (q, 2 × CH_3), 22.9 (q, $COCH_3$), 57.7 (t, OCH_2CH_3), 82.8 (s, C-4), 130.2 (d, C-2), 154.0 (s, C-3), 167.6 (s, $COCH_3$), 185.0 (s, C-1); mass spectrum, *m/e* 223 (21, M), 195 (29), 194 (100), 182 (22), 181 (19, M – CH_2CO), 180 (11), 154 (18), 153 (42), 152 (91), 138 (12), 137 (69), 136 (86), 135 (21), 125 (25), 124 (43), 112 (53), 111 (21), 110 (34), 109 (24), 108 (66), 107 (26), 106 (22). Anal. ($C_{12}H_{17}NO_3$) C, H, N.

4′-Hydroxy-*N*-methylacetanilide (5). 4-(Methylamino)-phenol sulfate (5.0 g, 0.015 mol) was dissolved in water (65 mL) and the mixture cooled with rapid stirring to 5 °C. Ac_2O (3.4 mL) was added followed, after 1 min, by a solution of NaOAc (4.4 g), in water (15 mL). 4′-Hydroxy-*N*-methylacetanilide (5; 4.2 g, 88%) precipitated as white plates. Recrystallization from CH_3OH/$CHCl_3$ gave white prisms, mp 243–245 °C (lit.[29] 245 °C).

Acknowledgment. This work was partly supported by The Australian Research Grants Committee.

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Synthesis of Certain [6:5:6] Linear Tricyclic Nucleosides as Potential Antitumor Agents

Fung-Lung Chung, Karl H. Schram, Raymond P. Panzica, Robert A. Earl, Linda L. Wotring, and Leroy B. Townsend*

Department of Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, and Departments of Medicinal Chemistry and Chemistry, University of Utah, Salt Lake City, Utah 84112.
Received November 19, 1979

A new class of tricyclic nucleosides in which the aglycon has a linear [6:5:6] geometry has been synthesized using certain pyrrolo[2,3-*d*]pyrimidine nucleosides as the starting materials. An adenosine–adenosine analogue (12) has been prepared from 6-aminotoyocamycin using two different synthetic routes. An adenosine–guanosine analogue (4) and several adenosine-6-mercaptopurine ribonucleoside-type tricyclic nucleoside derivatives have also been synthesized. Structural assignments have been based on ^{1}H NMR spectral studies, as well as an unequivocal chemical proof of structure. An interesting chemical shift for the 2′ hydrogen of certain tricyclic nucleosides was observed and is discussed. The in vitro cytotoxicity of these nucleosides against leukemia L-1210 has been determined. The in vivo evaluation of these tricyclic nucleosides against mouse leukemia will also be discussed.

The synthesis of tricyclic nucleosides is a relatively new area of nucleoside research.[1,2] Isolation of the highly fluorescent nucleoside wybutosine,[3,4] determined to be residing at the 3′ end of the anticodon of yeast $tRNA^{Phe}$,

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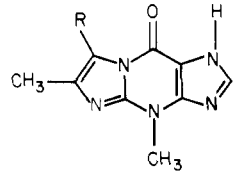
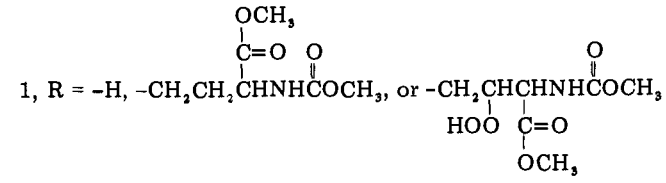
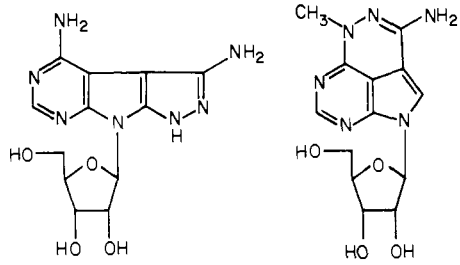
was followed by a structural elucidation of the aglycon as a unique tricyclic heterocycle (1). This assignment gen-





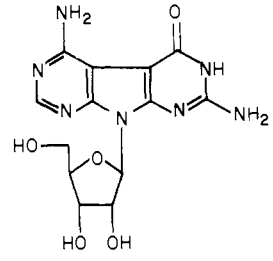
1, R = -H, $-CH_2CH_2CHNHCOCH_3$, or $-CH_2CHCHNHCOCH_3$

erated considerable interest[5-7] in the design and synthesis of other closely related tricyclic nucleosides. However, our interest in this area is related to the antitumor activity obtained[8,9] for the two synthetic tricyclic nucleosides 4,5-diamino-8-(β-D-ribofuranosyl)pyrazolo[3′,4′:5,4]pyrrolo-[2,3-*d*]pyrimidine[10] (2) and 6-amino-4-methyl-8-(β-D-ribofuranosyl)-4*H*,8*H*-pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]-pyridazine[11] (3). The tricyclic nucleosides 2 and 3 were



2

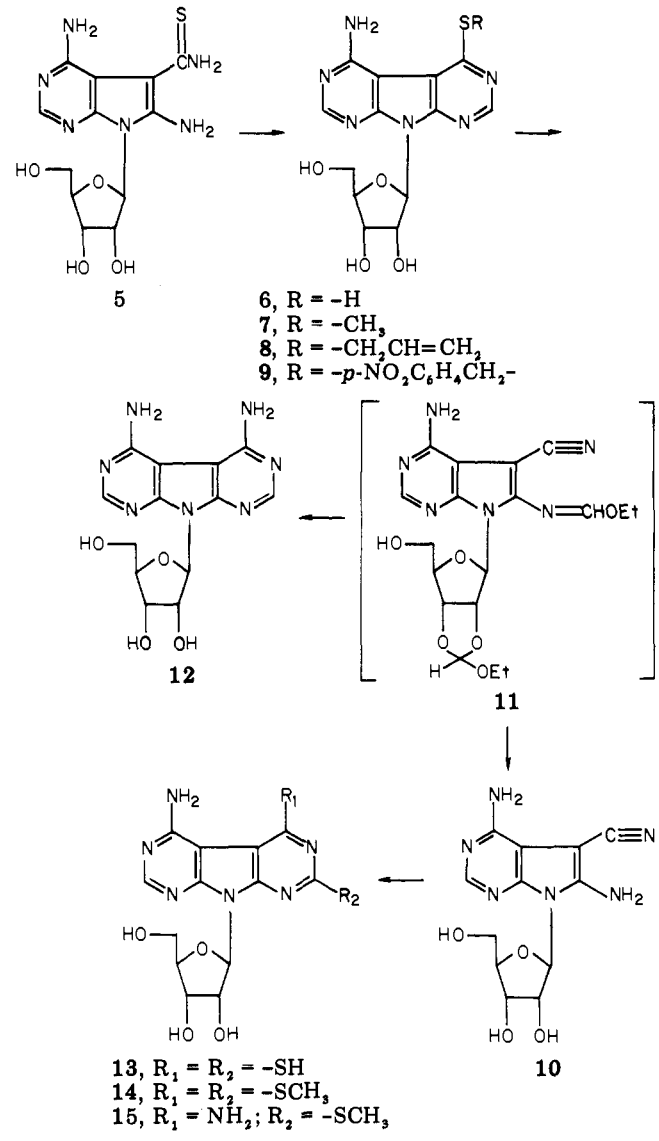
3



4

prepared in our laboratory, and, in fact, the synthesis of 2 was reported[10] prior to an assignment of the Y bases (1) as tricyclic heterocycles. We now report[12a] on the synthesis and biological evaluation of a new series of tricyclic nucleosides which possess a "linear"[12b] geometry. These nucleosides are unique, since they may be viewed as possessing the features of two completely different "purine-type" aglycons simultaneously. The tricyclic nucleoside 4 is a good example of this new type of nucleoside, since it may be viewed as an analogue of both adenosine and guanosine. The dual nature of this type of compound may produce some very interesting results in regard to the ability of the compound to act as a substrate for certain

Scheme I



5

6, R = -H
7, R = $-CH_3$
8, R = $-CH_2CH{=}CH_2$
9, R = $-p\text{-}NO_2C_6H_4CH_2-$

12

11

10

13, $R_1 = R_2 = -SH$
14, $R_1 = R_2 = -SCH_3$
15, $R_1 = NH_2$; $R_2 = -SCH_3$

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(8) Unpublished testing data from the Drug Research and Development Branch of the Division of Cancer Treatment, NCI, NIH.

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(11) Schram, K. H.; Townsend, L. B. *Tetrahedron Lett.* **1971**, 4757.

(12) (a) A preliminary account of this work has been reported: Chung, F. L.; Schram, K. H.; Panzica, R. P.; Earl, R. A.; Townsend, L. B. In "Abstracts of Papers", 157th National Meeting of the American Chemical Society, Anaheim, Calif., Mar 1978; American Chemical Society: Washington, D.C.; Abstr. MEDI 053. (b) There is a slight departure from linearity in the aglycon of the [6:5:6] tricyclic nucleosides. However, the descriptive term "linear" is still used in order to distinguish these tricyclic nucleosides from the angular or triangular tricyclic nucleosides previously prepared (see ref 1 and 2) in our laboratory.

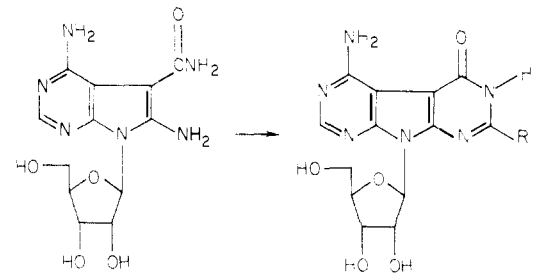
catabolic (e.g., adenosine deaminase) and/or anabolic (e.g., adenosine kinase) enzymes. In addition, it is tempting to propose that the planar geometry of the tricyclic aglycon moiety of these nucleosides may also allow the tricyclic bases to act as effective intercalating agents. The ribosyl moiety should make these agents amenable to a facilitated diffusion[13] through cell membranes which could then be followed by a possible intracellular cleavage by purine nucleoside phosphorylase[14] to release the tricyclic planar bases.

Chemistry. We elected to use 6-aminothiosangivamycin[15] (**5**) as the starting material for our initial research on the synthesis of the desired nucleosides. Ring annulation of 6-aminothiosangivamycin (**5**) with ethyl formate in ethanolic sodium ethoxide provided a good yield (65%) of nucleoside material, which was assigned structure **6** on the basis of subsequent ^{1}H NMR studies as well as an unequivocal chemical structure proof (see Discussion). This compound (**6**) can be viewed as being an analogue of adenosine and 6-mercaptopurine riboside. The tricyclic nucleoside **6** was then used as our starting material for the preparation of several 5-(alkylthio) derivatives. Thus, it was found that treatment of **6** with methyl iodide, allyl bromide, and *p*-nitrobenzyl bromide under basic conditions afforded the corresponding 5-(alkylthio) derivatives **7**, **8**, and **9**, respectively, in good yield (Scheme I).

Treatment of the 5-(methylthio) derivative **7** with ammonia (110 °C) gave an 87% yield of the symmetrical diadenosine analogue **12** via a displacement of the methylthio group by ammonia. Attempts to prepare **12** by direct ring closure of 6-aminotoyocamycin (**10**) with formamide or formamidine acetate[16] were unsuccessful. However, treatment of **10** with diethoxymethyl acetate at reflux temperature gave an intermediate which was assigned structure **11** on the basis of a ^{1}H NMR spectrum, which showed the presence of two ethoxy groups, as well as an IR spectrum, in which the presence of a nitrile group was indicated by an absorption at 2100 cm^{-1}. The intermediate was subsequently treated with saturated methanolic ammonia, followed by 25% aqueous acetic acid at room temperature to afford **12** as a major product, although the yield of **12** was lower (35%) than that obtained by the previous method.

Another route envisaged for the synthesis of **12** also used 6-aminotoyocamycin[15] (**10**) as the starting material. Treatment of **10** with a methanolic solution of sodium methylxanthate in a sealed reaction vessel at 180 °C for 3 h, followed by acidification to pH 6, afforded 4-amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine-5,7-dithione (**13**) in 80.4% yield. Methylation of **13** with methyl iodide in aqueous ammonium hydroxide gave a good yield (83%) of 4-amino-5,7-bis(methylthio)-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine (**14**). When **14** was treated with ammonia in a sealed reaction vessel at 100 °C for 11 h, a single product was isolated and shown to be 4,5-diamino-7-(methylthio)-9-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine (**15**). Finally, reductive dethiation of **15** with Raney nickel provided a product identical in all respects with the previously prepared symmetrical diadenosine analogue **12**. This could only occur if the methylthio group of **15** resided in the 7 position.

Scheme II



16

17, R = S^-Na^+
18, R = SH
19, R = $S^-NH_4^+$
20, R = SCH_3
21, R = $SO_3^-NH_4^+$
4, R = NH_2

The adenosine–guanosine analogue 4,7-diamino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidin-5-one (**4**) was synthesized via a multistep reaction sequence using 6-aminosangivamycin[15] (**16**) as our starting material (Scheme II). Treatment of **16** with carbon disulfide in methanolic sodium hydroxide at 180 °C furnished a good yield of 4-amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidin-5-one-7-thione (**17**) in the form of a sodium salt. The 7-(methylthio) derivative **20** was prepared by treating an aqueous solution of **17** with methyl iodide; however, efforts to displace the 7-(methylthio) group with ammonia were unsuccessful. The tricyclic nucleoside **17** was converted into **18**, which was subsequently converted into the ammonium salt **19**. The nucleoside **19** was then treated with hydrogen peroxide at a low temperature to obtain the ammonium sulfonate intermediate **21**, which was not isolated. Instead, the solution of **21** was saturated with ammonia at 0 °C, and the reaction mixture was sealed in a reaction vessel at 120 °C for 3 h to give **4** in 59% yield after column chromatography over silica gel. As was expected, the ammonium sulfonate group of **21** was a much better leaving group than the methylthio group at the 7 position of **20**. It was found that the use of **17** instead of **19** in the oxidation–ammonolysis sequence resulted in a lower overall yield of **4**.

The adenosine–inosine tricyclic nucleoside 4-amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidin-5-one (**22**) was easily prepared (94% yield) by treatment of 6-aminosangivamycin (**16**) with ethyl formate and ethanolic sodium ethoxide (Scheme III). The same compound (**22**) was also obtained when the tricyclic nucleoside **6** was treated with ammonium hydroxide–hydrogen peroxide or when the ammonium salt of 4-amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidin-5-one-7-thione (**19**) was dethiated using Raney nickel (94 and 40% yields, respectively).

The adenosine–xanthosine tricyclic nucleoside 4-amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine-5,7-dione (**23**) was prepared by treatment of 6-aminosangivamycin with diethyl carbonate and ethanolic sodium ethoxide (57.6% yield). The same compound could also be prepared in a 63% yield by the oxidative hydrolysis of the thione group in the 7 position of **19** using hydrogen peroxide and ammonium hydroxide.

Discussion

The annulation of diverse cyclic *o*-aminonitriles has been studied[16] extensively; however, the reactions of certain annulating reagents with 6-aminotoyocamycin (**10**) were found[17] to be unproductive. However, although 6-amino-

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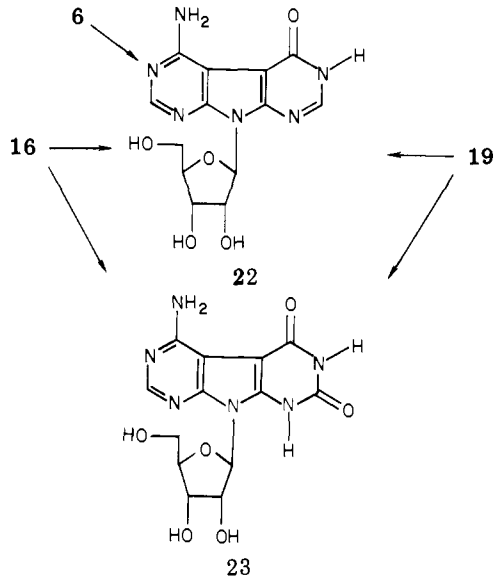
Scheme III



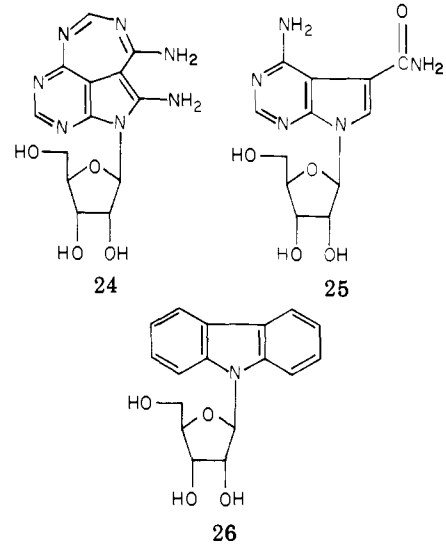
toyocamycin is rather unreactive, we have effected several successful ring closures, e.g., as discussed above, the conversion of 6-aminotoyocamycin (**10**) into the diadenosine tricyclic nucleoside analogue (**12**). We also effected a conversion[15] of the 5-cyano group of **10** into a carboxamide or a thiocarboxamide group which provided additional starting materials, such as **16** and **5**, which were also amenable toward the synthesis of certain tricyclic nucleosides.

One major problem associated with the use of **5**, **10**, and **16** as starting materials for the synthesis of tricyclic nucleosides was the question as to which direction ring closure would occur. If ring closure occurred between the 4-amino group and the 5-cyano group, instead of the 6-amino group and the 5-cyano group, then the resultant product would be a "nonlinear" type nucleoside possessing a seven-membered ring such as **24**. Strong support (vide infra) for the formation of a linear [6:5:6] tricyclic structure, rather than the "triangular" [6:7:5] structure (e.g., structure **24**), has been provided by the accumulated evidence as follows: we have noted[15] a considerable difference in the chemical shift for the signals (^{1}H NMR) observed for amino groups in the 4 and 6 positions of certain pyrrolo[2,3-*d*]pyrimidine nucleosides. The upfield signals appear to be due to the 6-amino groups, which would suggest that the 6-amino groups are more basic than the 4-amino groups. On this basis, one would assume that the 6-amino groups would enter into ring-closure reactions more readily than the 4-amino groups. We have also obtained some indirect chemical evidence which supports this assumed difference in reactivity between the 4-amino and 6-amino groups in certain pyrrolo[2,3-*d*]pyrimidine nucleosides. Attempts[17] to ring close the 4-amino and 5-carboxamido group of sangivamycin (**25**) with several ring-closing reagents, under conditions which gave ring-closed products using 6-aminosangivamycin (**16**) as the starting material, resulted only in a recovery of sangivamycin.[18]

We have also obtained some convincing spectral evidence for the linear tricyclic structure in the case of the diadenosine analogue **12**. In this example, the peak in the ^{1}H NMR spectrum which was assigned to the amino protons appeared as a broad singlet (4 protons) at δ 6.93. The peaks assigned to the aromatic ring protons also showed degeneracy and appeared as a sharp 2-proton singlet at δ 8.35. The degeneracy of these signals supports the premise that a very high degree of symmetry must exist in the tricyclic structure and this symmetry would not be observed if ring closure had occurred in the other direction to give a "triangular" tricyclic nucleoside, e.g., **24**.

Table I. Chemical Shifts (δ) of Some Linear Tricyclic Nucleosides and Their Bicyclic Nucleoside Precursors

no.	2'-H (δ)	no.	2'-H (δ)	no.	2'-H (δ)
4	5.10	**13**	4.96	**20**	5.12
6	5.14	13 (Na salt)	5.04	22	5.15
7	5.17	14	5.20	23	4.50
8	5.17	15	5.16	23 (Na salt)	5.00
9	5.16	17	5.10	27	5.26
12	5.17	18	4.61	5	4.63
				10	4.50
				16	4.68



One interesting feature in the ^{1}H NMR spectra of the linear tricyclic nucleosides prepared in this study is the observation that there is a marked downfield chemical shift in the signal observed for the 2'-H on the sugar moiety for most of the tricyclic nucleosides (ranging from $\Delta\delta$ 0.40 to 0.70) as compared to the corresponding signal for the 2'-H of their bicyclic nucleoside precursors (Table I). This downfield shift does not occur for the nonlinear tricyclic nucleosides, e.g., **3**, prepared previously in this laboratory (the chemical shift for the 2'-H of angular or triangular tricyclic nucleosides appears in the region δ 4.45–4.60 in the ^{1}H NMR spectrum). This phenomenon may be attributed to the deshielding effect that N-1 and/or N-8 of the tricyclic base exerts on the 2'-H of the sugar moiety. Aromatic ring currents of the heterocycles are most likely not responsible for this effect, since the ^{1}H NMR signal for the 2'-H of the carbazole ribonucleoside[19] (**26**) appears in the "normal" range[20] (δ 4.30–4.60). It is of some interest that the tricyclic nucleosides **18** and **23** do not show the marked downfield chemical shift for the 2'-H, whereas the

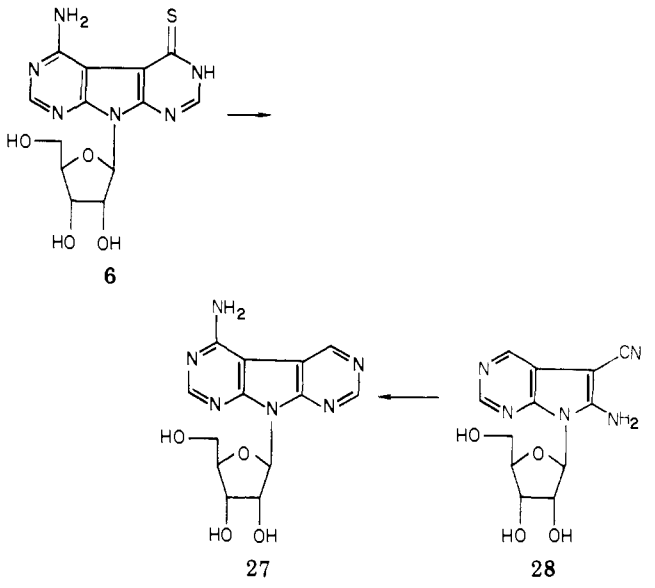
(17) Chung, F. L.; Schram, K. H.; Earl, R. A.; Townsend, L. B., unpublished results.

(18) Townsend, L. B. *Handb. Biochem. Mol. Biol., 3rd Ed.* **1975**, 1, 271–401.

(19) The isopropylidene derivative of the carbazole nucleoside (**26**) was a gift from Dr. J. L. Imbach, Montpellier, France. The deblocked nucleoside was obtained by treating the isopropylidene derivative with 75% trifluoroacetic acid at room temperature.

(20) Townsend, L. B. *Synth. Proced. Nucleic Acid Chem.* **1973**, 2, 267–398.

Scheme IV



6 27 28

sodium salt forms of these same compounds do exhibit a downfield chemical shift for the 2′-H. This would seem to indicate that the anisotropic effect of N-8 disappears when the N-8 atom of the pyrimidine ring becomes involved in a lactam structure with a proton residing on the N-8 atom. However, a downfield chemical shift is also observed for the 2′-H of **13**, but the magnitude of the shift is decreased significantly in comparison to other closely related tricyclic nucleosides of this series. This fact would suggest that **13** may exist to a significant degree in the lactam form in solution. The significance of this downfield shift lies in the observation that for purine nucleosides predominantly in the syn conformation (e.g., compounds possessing a bulky group in the 8 position), the signal for 2′-H appears downfield relative to the 2′-H for a purine nucleoside predominantly in the anti conformation.[21] The linear tricyclic nucleosides prepared in this study should always have a pyrimidine ring that is in a syn relationship to the sugar moiety; therefore, the 2′-H would be expected to experience this anisotropic effect regardless of the conformation of the heterocyclic base about the glycosidic bond (verified by space-filling CPR models).

The structural assignments (based on ^{1}H NMR spectral studies) for the linear tricyclic nucleosides discussed above have also been corroborated by an unambiguous chemical structure proof of the linear nature of the aglycon. The nucleoside 6-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine[15] (**28**) was treated with hot formamide to give the tricyclic nucleoside 4-amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d*′]dipyrimidine (**27**). This product (**27**) was found to be identical in all respects (Scheme IV) with the product obtained from a Raney nickel dethiation of **6**. This proof of structure for the tricyclic nucleoside **6** provides unequivocal assignments of the linear nature of the aglycon of the other tricyclic nucleosides prepared in this study.

Antitumor Results and Discussion

Several of these nucleosides showed moderate in vitro cytotoxicity against L-1210 (Table II). The bicyclic intermediates, 6-aminothiosangivamycin (**5**), 6-aminotoyocamycin (**10**), and 6-bromotoyocamycin,[24] had ID_{50} values of about 2×10^{-7} M. Of the tricyclic nucleosides, the most cytotoxic were compounds **4** (which resembles adenosine on one side of the molecule and guanosine on the other) and **6** (which resembles adenosine on one side and 6-mercaptopurine riboside on the other). Both **4** and **6** had ID_{50} values of about 2×10^{-6} M. Most of the other tricyclic nucleosides having oxo or thioxo substituents in the 5 position (R_1 = OH or SH) also showed cytotoxicity, but to a lesser degree (ID_{50} values $\leq 10^{-4}$ M). In contrast, most of the tricyclic nucleosides which have $R_1 = NH_2$ or a substituted thio group showed no significant cytotoxic effect (ID_{50} values $\geq \sim 10^{-4}$ M). These observations suggest that the resemblance to adenosine on one side of the molecule and to an inosine- or guanosine-type compound on the other side may be required for cytotoxic activity in this series of tricyclic nucleosides.

Certain compounds in this series have shown moderate in vivo antitumor activity against mouse leukemias (Table II). The bicyclic intermediates **5**, **10**, and **16** all had borderline activity against L-1210. The tricyclic nucleosides **6** (R_1 = SH; R_2 = H) and **22** (R_1 = OH; R_2 = H) gave ILS = 77 and 33%, respectively, against P-388. Also, the tricyclic nucleoside **14** ($R_1 = R_2 = -SCH_3$) showed some activity against L-1210 mouse leukemia, with ILS = 45%.

Preliminary data have been obtained on the formation of intraerythrocytic nucleotides from selected compounds in this group.[25] Human erythrocytes were incubated in the presence of 1 mM analogue for 4 h, and the analogue nucleotides were quantified from the nucleotide profiles obtained by high-pressure liquid chromatographic analysis of the acid-soluble fraction. The formation of analogue monophosphate was generally correlated with the L-1210 in vitro cytotoxicity (Table II). Significantly greater quantities of analogue monophosphates were formed from the cytotoxically active bicyclic intermediates, **10** and 6-bromotoyocamycin[24] (ID_{50} values $\simeq 2 \times 10^{-7}$ M), than from **16** ($ID_{50} = 1.6 \times 10^{-5}$ M). The tricyclic nucleosides **4** and **6**, which showed cytotoxicity ($ID_{50} = 2 \times 10^{-6}$ M), were also converted to monophosphate derivatives to a significant extent (5–20%), while compound **12**, which was not cytotoxic, showed less than 1% conversion in 4 h. Compound **22** was phosphorylated most extensively of the four tricyclic nucleosides tested, with 50% conversion to the monophosphate in 4 h. The in vitro cytotoxicity of **22** was slight ($ID_{50} = 6.3 \times 10^{-5}$ M), but the in vivo antitumor activity was considered to be significant with ILS = 33 when 50 mg/kg was administered in the P-388 test system (Table II). Thus, it appears that analogue nucleotide formation may be taken as an indication of potential in vitro cytotoxicity and/or in vivo antitumor activity.

Experimental Section

Antitumor Studies. The in vitro cytotoxicity against L-1210 was evaluated as described previously.[26] L-1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of various concentrations of the test compound. The ID_{50} was defined as the concentration required

(21) (a) Ikehara, M.; Uesugi, S.; Yoshida, K.; *Biochem.* **1972**, *11*, 830. (b) Jordan, F.; Hazgainiv *Biochim. Biophys. Acta* **1977**, *476*, 265.

(22) Montgomery, J. A.; Fitzgibbon, W. E., Jr. In "Nucleic Acid Chemistry; Improved and New Synthetic Procedures, Methods, and Techniques"; Townsend, L. B.; Tipson, R. S., Eds.; Wiley-Interscience: New York, 1978; Part 2, p 995.

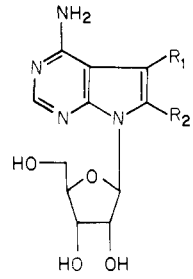
(23) Chung, F. L.; Earl, R. A.; Townsend, L. B. *Tetrahedron Lett.*, **1980**, *21*, 1599.

(24) Tolman, R. L.; Robins, R. K.; Townsend, L. B. *J. Heterocycl. Chem.*, **1971**, *8*, 703.

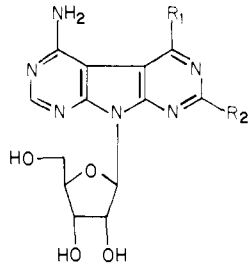
(25) Crabtree, G. W.; Parks, R. E., Jr., unpublished results.

(26) (a) Wotring, L. L.; Townsend, L. B. *Cancer Res.* **1979**, *39*, 3018. (b) Lewis, A. F.; Long, R. A.; Roti Roti, L. Wotring; Townsend, L. B. *J. Heterocycl. Chem.* **1976**, *13*, 1359.

Table II. In Vitro Cytotoxicity and In Vivo Antitumor Testing



compd	R_1	R_2	in vitro: ID_{50}, M[a]	in vivo[b] dose/injectn,[c] mg/kg	in vivo[b] ILS,[d] % of control
5	$CSNH_2$	NH_2	2.2×10^{-7}	25	37
10	CN	NH_2	1.8×10^{-7}	8	26
16	$CONH_2$	NH_2	1.6×10^{-5}	120	30
e	CN	Br	2.1×10^{-7}	600[f]	22



4	OH[g]	NH_2	2.5×10^{-6}	200	13
6	SH	H	2.0×10^{-6}	100	38
				100[i]	77
7	SCH_3	H	8.6×10^{-5}	200	9
				200[j]	5
8	$SCH_2CH=CH_2$	H	1.1×10^{-4}	200	9
9	SCH_2-C_6H_4-NO_2-*p*	H	4.5×10^{-5}	200	-2
12	NH_2	H	*h*	100	-9
l	NH_2	OH	1.2×10^{-4}	200	21
13	SH	SH	6.1×10^{-6}	400	10
l	SH	OH	1.1×10^{-5}	100	4
14	SCH_3	SCH_3	7.8×10^{-5}	200	45
l	SCH_3	OH	*h*	200	4
15	NH_2	SCH_3	*h*	400	1
17	OH	$S^- Na^+$	7.0×10^{-6}	400	14
20	OH	SCH_3	8.0×10^{-5}	200	0
22	OH	H	6.3×10^{-5}	100	20
				50[j]	33
23	$O^- Na^+$	$O^- Na^+$	$\sim 10^{-4}$ [k]	400	108[k]
27	H	H	$>10^{-4}$	200	2
l	H	OH	7.5×10^{-5}	320	10

[a] ID_{50} is the concentration required to reduce the growth rate of L-1210 cells in culture to half of the control rate. [b] Data are presented for the protocol that gave optimal activity, or for inactive compounds (ILS < 25%), the protocol using the highest nontoxic dose tested for each tumor. [c] Administered to L-1210-bearing mice once daily on days 1-5 after inoculation of the animals with tumor cells, except where otherwise noted. [d] ILS is the increase in life span for drug-treated animals as compared to control, untreated animals, expressed as a percentage of the life span of the untreated tumor-bearing animals. [e] Synthesis reported in ref 24. [f] Administered to L-1210-bearing mice once on day 2 after inoculation of the animals with tumor cells. [g] The oxo substituents are shown as hydroxy and the thione substituents as -SH for convenience in presenting the structures. [h] 10^{-4} M compound had no effect on cell growth. [i] Administered to P-388-bearing mice once daily on days 1-9 after inoculation of the animals with tumor cells. [j] Administered to P-388-bearing mice once daily on days 1-5 after inoculation of the animals with tumor cells. [k] The in vitro and in vivo results reported here were obtained using two different samples of compound 23. Subsequent in vitro evaluation of the sample used for the in vivo studies gave $ID_{50} = 3.5 \times 10^{-6}$ M. These results suggest that the latter sample may have contained trace amounts of the starting material, toyocamycin, which displays an ID_{50} of 4×10^{-9} M in the L-1210 in vitro system. [l] Synthesis reported in ref 23.

to reduce the growth rate to 50% of the control.

The in vivo antitumor data was furnished by the Division of Cancer Treatment using standard National Cancer Institute protocols for evaluation of compounds against the mouse leukemias L-1210 and P-388.[27]

Instrumentation. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained on a JEOL C60H or a Varian EM-390 spectrophotometer using dimethyl-d_6 sulfoxide (Me_2SO-d_6) or deuteriochloroform ($CDCl_3$) as solvent and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as internal standard unless otherwise noted. The chemical shifts are recorded in δ (parts per million) relative to the internal standard. Ultraviolet spectra (UV) were recorded on Bechman Acta CIII spectrophotometer. Infrared spectra (IR) were recorded on a Beckman IR 100 spectrophotometer. Optical rotations were determined with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Melting points

(27) Geran, R. I.; Greenberg, W. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1.

were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Mass spectra were obtained with a LKB 9000 S instrument and a Varian MAT 112S/SS100 c data system. Nonvolatile samples were derivatized with bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS). Samples (1–10 μg) were introduced by direct probe. The ionizing energy for the electron-ionization (EI) mass spectra was 70 eV. Characteristic nucleoside peaks from the mass spectra are given as follows (m/e/relative intensity): M, b + 2H, b + CH_2O, where b = base moiety and M = molecular ion.

Chromatography. Thin-layer chromatography (TLC) was run on glass plates coated (0.25 mm) with silica gel (SilicAR 7GF, Mallinckrodt). Solvent system E (SSE) is composed of the upper layer of ethyl acetate–1-propanol–water (4:1:2, v/v). Compounds of interest were detected using an ultraviolet lamp (mineralight, 254 nm) and by spraying with 10% sulfuric acid in water (v/v), followed by heating. Silica gel suitable for column chromatography was purchased from J. T. Baker Chemical Co. All chromatographic separations were performed using glass columns dry packed with silica gel under gravity flow unless otherwise stated. The progress of column chromatography was routinely monitored by checking individual fractions using TLC. All concentrations in vacuo were carried out at 40 °C and 1 torr unless otherwise noted.

4-Amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine-5-thione (6). The nucleoside **5** (600 mg, 1.76 mmol) and ethyl formate (2.5 g, 24.50 mmol) were added to a solution of ethanolic sodium ethoxide [sodium (1.00 g) in ethanol (30 mL)]. The mixture was heated at reflux temperature for 1.5 h and then water (30 mL) was added to the mixture (solution occurred). The pH was adjusted with 1 N HCl to approximately 5 (pH paper), and the mixture was stirred at room temperature for 30 min. The precipitate that formed was collected by filtration and then recrystallized from a minimum amount of water (120 mL) to yield 900 mg (88%) of **6**: mp 250 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 5.14 (q, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 5.0 Hz), 6.43 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.83 (br s, 1, NH_2), 8.29 (s, 1, H_2), 8.34 (s, 1, H_7), 9.58 (br s, 1, 6-NH). Anal. Calcd for $C_{13}H_{14}N_6O_4S{\cdot}0.5H_2O$: C, 43.45; H, 4.18; N, 23.39. Found: C, 43.41; H, 4.21; N, 23.29.

4-Amino-5-(methylthio)-9-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*:5,4-*d'*]dipyrimidine (7). Concentrated ammonium hydroxide (3 mL) and methyl iodide (0.38 mL, 6.13 mmol) were added to the nucleoside **6** (1.80 g, 5.14 mmol) in H_2O (100 mL), and the mixture was stirred for 1 h at room temperature. The precipitate that formed was collected by filtration and washed with cold water (2 × 25 mL) and then cold ethanol (25 mL). The filter cake was dried in vacuo (110 °C, 18 h, 0.5 torr) to afford 1.15 g (85%) of **7**: mp 267–268 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 2.82 (s, 3, SCH_3), 5.17 (t, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.56 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.52 (br s, 2, NH_2), 8.42 (s, 1, H_2), 8.83 (s, 1, H_7). Anal. Calcd for $C_{16}H_{18}N_6O_4S$: C, 46.15; H, 4.40; N, 23.08. Found: C, 46.02; H, 4.44; N, 22.86.

5-(Allylthio)-4-amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine (8). The nucleoside **6** (850 mg, 2.43 mmol) was suspended in methanol (70 mL) and then freshly prepared sodium methoxide in methanol (12.75 mL of a solution of 230 mg of sodium in 50 mL of methanol, 2.60 mmol) was added to the suspension with stirring (solution occurred). Allyl bromide (0.20 mL, 2.30 mmol) was added dropwise to the solution, and the reaction mixture was stirred at room temperature for 5 h. The solvent was removed in vacuo, and the resulting residue was recrystallized from aqueous methanol (water–methanol, 7:3, v/v) to afford 600 mg (63.4%) of **8**: mp 197 °C; $[\alpha]^{27}_D$ −12.3° (*c* 1.00, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 5.10–6.00 (m, 3, CH=CH_2), 5.17 (t, 1, $H_{2'}$), 6.53 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.60 (br s, 2, NH_2), 8.41 (s, 1, H_2), 8.82 (s, 1, H_7); MS (m/e/relative intensity, EI) M + 4TMS (678/6), b + TMS + 2H (331/66), b + TMS + CH_2O (359/100). Anal. Calcd for $C_{16}H_{18}N_6O_4S$: C, 49.23; H, 4.62; N, 21.54. Found: C, 49.18; H, 4.60; N, 21.62.

4-Amino-5-[(*p*-nitrobenzyl)thio]-9-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine (9). The nucleoside **6** (500 mg, 1.43 mmol) was suspended in methanol (50 mL), and then freshly prepared sodium methoxide in methanol (7.5 mL of a solution of 230 mg of sodium in 50 mL of methanol, 1.5 mmol) was added to the suspension to effect a clear solution. To this clear solution, *p*-nitrobenzyl bromide (296 mg, 1.37 mmol) was added, and, after 15 min of stirring at room temperature, the solvent was removed in vacuo. The reddish residue was crystallized from a mixture of water and methanol (4:6, v/v) to afford 500 mg (72.1%) of **9**: mp 180 °C; $[\alpha]^{27}_D$ −13.5° (*c* 1.00, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 4.84 (s, 2, SCH_2), 5.16 (t, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.53 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.52 (br s, 2, NH_2), 8.13 (d, 4, phenyl H), 8.38 (s, 1, H_2), 8.85 (s, 1, H_7); MS (m/e/relative intensity, EI) M + 4TMS (773/2), b + TMS + 2H (426/45), b + TMS + CH_2O (454/65). Anal. Calcd for $C_{20}H_{19}N_7O_6S$: C, 49.48; H, 3.92; N, 20.21. Found: C, 49.29; H, 3.92; N, 20.29.

4,5-Diamino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine (12). Method 1. A suspension of 6-aminotoyocamycin (**10**; 200 mg, 0.65 mmol) in an excess of diethoxymethyl acetate[22] (1.5 mL) was heated at reflux for 2 h with stirring (solution occurred shortly after refluxing was started). The excess diethoxymethyl acetate was removed in vacuo, and the resulting brown solution was dissolved in methanolic ammonia (20 mL, saturated at 0 °C). The solution was allowed to stand at 25 °C for 24 h, and the solution was then evaporated in vacuo. The resulting yellowish syrup was dissolved in chloroform (15 mL), and the undissolved solid was removed by filtration and discarded. The supernatant was evaporated in vacuo to a foam, which was dissolved in 25% acetic acid (15 mL). The solution was allowed to stand at 25 °C for 30 h and then evaporated in vacuo. Water (15 mL) was added, and the solution concentrated in vacuo. This process was repeated three times before the pH of the solution was adjusted to 6 with 1 N aqueous sodium hydroxide. This solution (pH 6) was then concentrated to a small volume (10 mL) and allowed to stand at 4 °C for 18 h. The solid which had precipitated from solution was two compounds (120 mg), as determined by TLC. The solid was dissolved in water (20 mL) and evaporated to dryness in vacuo in the presence of Baker silica gel (1.00 g). The residual solid was applied to a column (2.0 × 25 cm) of silica gel (23.00) and eluted using solvent system E, with 10-mL fractions being collected. The fractions containing the slower-moving spot on TLC were collected and evaporated in vacuo to afford 75 mg (35.2%) of **12**. Recrystallization from water yielded a sample for analysis: mp 308 °C; ^{1}H NMR (Me_2SO-d_6) δ 5.17 (t, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.50 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 6.90 (br s, 4, 4-NH_2 and 5-NH_2), 8.40 (s, 2, H_2 and H_7); MS (m/e/relative intensity, EI) M + 5TMS (698/9), b + 2TMS + 2H (346/35), b + 2TMS + CH_2O (374/7). Anal. Calcd for $C_{13}H_{15}N_7O_4$: C, 46.85; H, 4.51; N, 29.43. Found: C, 46.66; H, 4.47; N, 29.55.

Method 2. The nucleoside **7** (200 mg, 0.55 mmol) was heated with excess liquid ammonia (7 mL) in a sealed steel vessel at 110 °C for 15 h. The reaction vessel was cooled to 0 °C and the excess ammonia was allowed to evaporate. The residual solid was crystallized from water to give 160 mg (87.1%) of a product, which was shown to be identical with the product obtained by method 1 in every respect (UV, ^{1}H NMR, TLC, IR, MS, mixture melting point).

Method 3. The nucleoside **15** (100 mg, 0.25 mmol) and Raney nickel (1.50 g, wet weight) were suspended in water (15 mL). The mixture was heated and stirred at reflux temperature for 30 min. The Raney nickel was removed by filtration and washed with 10% hot aqueous ethanol (50 mL) and then hot dimethylformamide (30 mL). The combined filtrates were evaporated at 80 °C to dryness in vacuo. The white solid that remained was crystallized from water to afford 35 mg (43.7%) of **12**. This product was shown to be identical with the product obtained by methods 1 and 2 in all respects (UV, ^{1}H NMR, TLC, MS, mixture melting point).

4-Amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine-5,7-dithione (13). 6-Aminotoyocamycin (**10**; 500 mg, 1.63 mmol) was suspended in a solution of sodium hydroxide (308 mg, 7.70 mmol) in methanol (15 mL). Carbon disulfide (1 mL, 16.03 mmol) was added to the suspension, and the reaction mixture was immediately sealed in a steel vessel and heated at 180 °C for 3 h. After cooling the vessel in an ice bath, the pressure in the vessel was carefully released. The resulting yellowish suspension was allowed to stand at 5 °C for 20 h. The light yellow solid was collected by filtration to give 640 mg (91.5%) of the disodium salt of **13**. This disodium salt was then dissolved in water (20 mL), and the solution was adjusted to pH 6 with 2 N hydrochloric acid. The solid that formed was collected by filtration

and crystallized from aqueous DMF (water–DMF, 3:7, v/v) to furnish 500 mg (80.4%) of **13**: mp 230 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 4.97 (t, 1, $H_{2'}$, $J_{2',1'}$ = 5.0 Hz, $J_{2',3'}$ = 5.0 Hz), 6.29 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.0 Hz). Anal. Calcd for $C_{13}H_{14}N_6O_4S_2 \cdot H_2O$: C, 39.00; H, 4.00; N, 21.00. Found: C, 38.93; N, 3.92; N, 20.64.

4-Amino-5,7-bis(methylthio)-9-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine (14). The nucleoside **13** (1.00 g, 2.62 mmol) was suspended in water (100 mL). Ammonium hydroxide (5 mL of 28.7% solution) and methyl iodide (0.75 mL, 12.04 mmol) were added, and the solution was then stirred at room temperature for 1.5 h. The reaction mixture was evaporated in vacuo, and the residue was extracted with hot methanol (20 mL). The methanol was evaporated to dryness in vacuo, and the residual white powder was dried in vacuo over toluene to yield 900 mg (83%) of **14**: mp 267–268 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 2.54 (s, 3, SCH_3), 2.80 (s, 3, SCH_3), 5.20 (t, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.44 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.38 (br s, 2, NH_2), 8.35 (s, 1, H_2). Anal. Calcd for $C_{15}H_{18}N_6O_4S_2$: C, 43.90; H, 4.39; N, 20.47. Found: C, 44.09; H, 4.46; N, 20.68.

4,5-Diamino-7-(methylthio)-9-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine (15). The nucleoside **14** (300 mg, 0.73 mmol) was heated with an excess amount of liquid ammonia (8 mL) in a steel vessel at 100 °C for 11 h. The vessel was cooled to 0 °C in an ice bath, and the ammonia was slowly released. The residual solid was crystallized from water to furnish 186 mg (70%) of **15**: mp 175 °C; ^{1}H NMR (Me_2SO-d_6) δ 5.16 (t, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.38 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 6.80 (br s, 2, NH_2), 6.92 (br s, 2, NH_2), 8.31 (s, 1, H_2). Anal. Calcd for $C_{14}H_{17}N_7O_4S$: C, 44.33; H, 4.49; N, 25.86. Found: C, 44.16; H, 4.47; N, 25.62.

Sodium Salt of 4-Amino-9-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*:5,4-*d'*]dipyrimidin-5-one-7-thione (17). To a solution of sodium hydroxide (616 mg, 15.40 mmol) in methanol (20 mL) was added 6-aminosangivamycin (**16**; 1.00 g, 3.09 mmol) and an excess of carbon disulfide (2 mL, 33.2 mmol). The reaction mixture was heated in a sealed, steel vessel at 180 °C for 3 h. The reaction vessel was cooled to 0 °C and opened, and the excess carbon disulfide was allowed to evaporate. The yellow solid that remained was collected by filtration and washed with cold methanol (3 × 15 mL) to yield 1.20 g (100%) of **17**: ^{1}H NMR (Me_2SO-d_6) δ 5.16 (t, 1, $H_{2'}$, $J_{2',1'}$ = 7.0 Hz, $J_{2',3'}$ = 7.0 Hz), 6.33 (d, 1, $H_{1'}$, $J_{1',2'}$ = 7.0 Hz), 7.52 (br s, 2, NH_2), 8.17 (s, 1, H_2), 11.14 (br s, 1, 6-NH). Anal. Calcd for $C_{13}H_{13}N_6O_5SNa \cdot 2.0H_2O$: C, 36.79; H, 4.01; N, 19.81. Found: C, 36.49; H, 4.11; N, 19.64.

4-Amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidin-5-one-7-thione (18). The nucleoside **17** (500 mg, 1.30 mmol) was dissolved in water (20 mL), and the pH of the solution was adjusted to 4.5–5.0 by the dropwise addition of 1 N HCl. The white solid that formed was collected by filtration to afford 370 mg (77.5%) of **18**: mp 260 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 4.60 (q, 1, $H_{2'}$, $J_{2',1'}$ = 7.0 Hz, $J_{2',3'}$ = 5.0 Hz), 6.33 (d, 1, $H_{1'}$, $J_{1',2'}$ = 7.0 Hz), 7.61 (br s, 2, NH_2), 8.40 (s, 1, H_2), 12.40 (br s, 1, 6-NH); MS (*m/e*/relative intensity, EI) M + 6TMS (798/14), b + 3TMS + 2H (451/47), b + 3TMS + CH_2O (479/100).

4-Amino-7-(methylthio)-9-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*:5,4-*d'*]dipyrimidin-5-one (20). The nucleoside **17** (100 mg, 0.26 mmol) was dissolved in water (8 mL), and to this solution was added methyl iodide (0.02 mL, 0.35 mmol). The solution was stirred at room temperature, and after 4 h the solid which had precipitated out of the solution was collected by filtration. The filtrate was concentrated in vacuo to a small volume (3 mL), and some additional methyl iodide (0.01 mL, 0.13 mmol) was added. A second crop of solid was collected and combined with that previously obtained to give a total yield of 70 mg (71.3%) of **20**: mp 299 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.67 (s, 3, SCH_3), 5.12 (t, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.31 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.67 (br s, 2, NH_2), 8.27 (s, 1, H_2); MS (*m/e*/relative intensity, EI) M + 5TMS (740/7), b + 2TMS + 2H (393/50), b + 2TMS + CH_2O (421/100). Anal. Calcd for $C_{14}H_{16}N_6O_5S \cdot H_2O$: C, 42.21; H, 4.52; N, 21.10. Found: C, 42.44; H, 4.57; N, 21.40.

4,7-Diamino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidin-5-one (4). The nucleoside **18** (250 mg, 0.68 mmol) was dissolved in 1 N ammonium hydroxide (9 mL), and the solution was then evaporated to dryness in vacuo to furnish a shiny yellow solid (250 mg). Hydrogen peroxide was added, dropwise, to a suspension of the ammonia salt derivative in cold water (25 mL) at 3 °C in an ice bath (0.75 mL of 30% solution). The resulting yellowish solution was saturated with ammonia at 0 °C, and the reaction mixture was then sealed in a steel vessel and heated at 120 °C for 3 h. The reaction mixture was then evaporated in vacuo to dryness in the presence of Baker silica gel (1.00 g). This solid mixture was applied on the top of a column (2 × 25 cm) of silica gel (23 g). The column was eluted with solvent system E. Fractions containing the major product were collected and evaporated to dryness in vacuo to give a white solid which was crystallized from water to afford 101 mg (42.3%, based on **18**) of **4**: mp 310 °C; $[\alpha]^{27}_D$ −36.8° (*c* 1.00, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 5.10 (t, 1, $H_{2'}$, $J_{1',2'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.20 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 6.83 (br s, 2, 4-NH_2), 7.40 (br s, 2, 7-NH_2), 8.13 (s, 1, H_2), 11.10 (br s, 1, 6-NH); MS (*m/e*/relative intensity, EI) M + 5TMS (709/11), b + 2TMS + 2H (362/100), b + 2TMS + CH_2O (390/100). Anal. Calcd for $C_{13}H_{15}N_7O_5 \cdot H_2O$: C, 42.51; H, 4.63; N, 26.70. Found: C, 42.63; H, 4.45; N, 26.43.

4-Amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidin-5-one (22). Method 1. The nucleoside **6** (1.00 g, 2.86 mmol) was mixed with ammonium hydroxide (60 mL of a 28.7% solution) and hydrogen peroxide (7 mL of a 30% solution), and the solution was stirred at room temperature for 3 h. The reaction mixture was then allowed to stand at 5 °C for 12 h, and the pH of the solution was adjusted to 6 with 2 N HCl. The solid that precipitated was collected by filtration and dried in vacuo for 24 h at 110 °C to afford 900 mg (94%) of the product: mp 235 °C dec; $[\alpha]^{27}_D$ −40.7° (*c* 1.00, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 5.15 (t, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.36 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.80 (br s, 2, NH_2), 8.30 (s, 2, H_2 and H_7). Anal. Calcd for $C_{13}H_{14}N_6O_5$: C, 46.71; H, 4.19; N, 25.15. Found: C, 46.60; H, 4.38; N, 24.82.

Method 2. Ethyl formate (0.48 mL, 5.95 mmol) was added to a solution of 6-aminosangivamycin (**16**; 100 mg, 0.31 mmol) in ethanolic sodium ethoxide (160 mg of sodium in 10 mL of ethanol). The reaction mixture was heated at reflux temperature for 2 h, and then water (10 mL) was added to effect a clear solution. The solution was adjusted to pH 5.5–6 with 1 N HCl, and the gel-like white solid that formed was collected by filtration and purified by crystallization from 50% aqueous methanol (150 mL, v/v) to afford 60 mg (58.1%) of **22**. This product was shown to be identical with that obtained by method 1 using UV, ^{1}H NMR, and TLC.

Method 3. The ammonium salt of **18** (100 mg, 0.26 mmol) was dissolved in water (5 mL), and Raney nickel (500 mg, wet weight) was added to the solution with stirring. The reaction mixture was heated with stirring at 60 °C for 2 h and then filtered through Celite while still hot. The filter cake was washed with hot aqueous ethanol (15 mL, 1:1, v/v) and then hot dimethylformamide (10 mL). The filtrate was evaporated to dryness in vacuo at 80 °C to furnish 35 mg (40.4%) of a white solid. The product was shown to be identical with the product obtained by methods 1 and 2 by UV, ^{1}H NMR, and TLC.

4-Amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine-5,7-dione (23). Method 1. 6-Aminosangivamycin (**16**; 100 mg, 0.31 mmol) was dissolved in a freshly prepared solution of sodium (50 mg) in ethanol (20 mL). To this solution was added diethyl carbonate (0.2 mL, 1.70 mmol) while stirring. The reaction mixture was heated at reflux for 2 h. The resulting gel-like material was dissolved in water (10 mL). The solution was adjusted to pH 5.5–6.0 with 2 N hydrochloric acid and then evaporated to dryness in vacuo to afford a white solid which was extracted with hot ethanol (3 × 20 mL). The ethanol extracts were combined and evaporated to dryness in vacuo, and this residue was crystallized from aqueous ethanol (150 mL, 1:1, v/v) to afford 65 mg (57.6%) of **23**: mp 100 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 4.50 (t, 1, $H_{2'}$, $J_{2',1'}$ = 7.0 Hz, $J_{2',3'}$ = 7.0 Hz), 6.30 (d, 1, $H_{1'}$, $J_{1',2'}$ = 7.0 Hz), 7.43 (br s, 2, NH_2), 8.16 (s, 1, H_2), 11.26 (br s, 1, NH); MS (*m/e*/relative intensity, EI) M + 6TMS (782/8), b + 3TMS + 2H (435/56), b + 3TMS + CH_2O (463/100). Anal. Calcd for $C_{13}H_{14}N_6 \cdot H_2O$: C, 42.40; H, 4.35; N, 22.83. Found: C, 42.59; H, 4.35; N, 22.62.

Method 2. Hydrogen peroxide (0.5 mL of a 30% solution) was added, dropwise, with stirring to a solution of the ammonium salt of **18** (100 mg, 0.26 mmol) in ammonium hydroxide (20 mL of a 28% solution) at 25 °C. After 1.5 h, the solution was adjusted

to pH 5 with 1 N hydrochloric acid. The solid which formed was collected by filtration and purified by crystallization from 50% aqueous ethanol (150 mL, v/v) to afford 60 mg (63.0%) of the product, which was shown to be identical with that obtained by method 1 by UV, ^{1}H NMR, TLC, and MS.

4-Amino-9-(β-D-ribofuranosyl)pyrrolo[2,3-*d*:5,4-*d*']dipyrimidine (27). Method 1. Raney nickel (9.0 g, net weight) was added to a suspension of **6** (1.0 g, 2.86 mmol) in water (75 mL). The reaction mixture was heated at reflux for 2 h. The Raney nickel was removed by filtration, and the filter cake was washed with hot water (150 mL) and methanol (50 mL). The filtrate was evaporated in vacuo to furnish a white solid, which was recrystallized from water (100 mL) to give a sample for analysis: yield 300 mg (33%) of **27**; mp 300 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 5.26 (t, 1, $H_{2'}$, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 6.0 Hz), 6.54 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 7.66 (br s, 2, NH_2), 8.47 (s, 1, H_2), 9.00 (s, 1, H_5 or H_7), 9.60 (s, 1, H_5 or H_7). Anal. Calcd for $C_{13}H_{14}N_6O_4$: C, 49.06; H, 4.40; N, 26.42. Found: C, 49.01; H, 4.57; N, 26.40.

Method 2. The nucleoside **28** (500 mg, 1.68 mmol) was mixed with formamide (15 mL, 0.38 mmol), and the reaction mixture was heated at reflux temperature. After 2 h, the reaction mixture was allowed to cool to 25 °C, and the excess formamide was evaporated at 80 °C in vacuo. The resulting residual solid was dissolved in water (80 mL), and the solution was decolorized with charcoal. The charcoal was removed by filtration, and the filtrate was allowed to stand at 5 °C for 20 h. The gel-like substance which had formed was collected by filtration and triturated with hot ethanol (20 mL). The white solid that formed was collected by filtration to afford 250 mg (50%) of **27**. This product was shown to be identical by UV, ^{1}H NMR, and TLC with the product obtained by method 1.

Acknowledgment. Support for this work was provided by a research contract from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare (N01-CM-43806 and NCI-CM-77142) and Grant RO-1 CA26032 from the National Cancer Institute, National Institutes of Health.

Amino Acid and Dipeptide Derivatives of Daunorubicin. 1. Synthesis, Physicochemical Properties, and Lysosomal Digestion

Michele Masquelier, Roger Baurain,* and Andre Trouet

International Institute of Cellular and Molecular Pathology and Universite Catholique de Louvain (Laboratoire de Chimie Physiologique), B-1200 Brussels, Belgium. *Received October 31, 1979*

The synthesis of amino acid and dipeptide derivatives of daunorubicin (DNR) is described. The binding-affinity parameters for DNA of those derivatives were determined by a spectral titration method. The affinity constants of the amino acid and dipeptide derivatives are, respectively, three and ten times lower than that of DNR. The susceptibility of those derivatives toward lysosomal peptidases was studied. It was found that the Leu and the Ala-Leu derivatives are the most rapidly hydrolyzed into DNR. It is concluded that Leu-DNR and Ala-Leu-DNR could act as prodrugs of DNR, which could be activated inside or in the close vicinity of tumor cells which display a high aminopeptidase activity.

Daunorubicin (DNR), widely used in the treatment of acute leukemia,[1,2] is composed of an anthracycline aglycon linked to an amino sugar. The amino group of DNR is important for its biological activity, since it stabilizes the intercalation of the aglycon between adjacent base pairs of DNA.[3]

N-Amino acid[18] and *N*-acyl amino acid derivatives of DNR[15] have been synthesized previously in order to achieve selective biological activity of the DNR molecule on the basis of the pH difference between tumoral and normal tissues and the lower p*K* values of the derivatives formed.[15] This structure-activity approach has not taken into account the stability of the compounds toward peptidases or the tissue and subcellular distribution of the drugs and their metabolites.

We have synthesized the *N*-amino acid and dipeptide derivatives of DNR listed in Table I as prodrugs of DNR. It was hoped to achieve a selective biological activity if these latent forms of DNR could be activated more selectively inside or in the close vicinity of those tumor cells which have been shown to display high aminopeptidase activity.[4-7]

In this publication, we describe the synthesis of these derivatives, their interaction in vitro with DNA, as well as their susceptibility toward lysosomal peptidases. In the following paper in this issue, we will report their uptake, metabolism, and in vitro and in vivo antitumoral activities using the murine L1210 leukemic cells as the experimental system.

Chemistry. The synthesis of the amino acid and dipeptide derivatives of DNR was performed in such a way as to avoid drastic deprotecting steps and racemization of the amino acids. In the case of hydrophilic amino acids, the amino function was blocked by a triphenylmethyl group according to Stekalatos et al.[8] and the carboxylic function was activated by esterification with *N*-hydroxysuccinimide according to Anderson et al.[9] The bulky, hydrophobic amino acids were linked with good yields by the *N*-carboxyanhydride method of Hirshmann.[10]

The authenticity and purity of the peptide derivatives were checked by elemental analysis, thin-layer chromatography, absorption, and infrared spectroscopy. Each

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0022-2623/80/1823-1166$01.00/0