function of the bis-mustards; cf. ref 9 and T. J. Bardos, N. Datta-Gupta, and P. Hebborn, *J. Med. Chem.*, **9**, 221 (1966).
(8) "Instruction 14, Screening Data Summary Interpretation", Drug Evaluation Branch, National Cancer Institute, Bethesda, Md., 1963, p 16.
(9) R. M. Peck, A. P. O'Connell, and H. J. Creech, *J. Med. Chem.*, **9**, 217 (1966). We have adopted their original method with the stated modifications.
(10) "Protocols for Screening Agents and Natural Products against Animal Tumors and Other Biological Systems", Drug Evaluation Branch, National Cancer Institute, Silver Spring, Md., 1971.
(11) R. E. Billingham and P. B. Medawar, *J. Exp. Biol.*, **28**, 385 (1951).
(12) R. L. Sneath, J. E. Wright, A. H. Soloway, S. M. O'Keefe, A. S. Dey, and W. D. Smolnycki, *J. Med. Chem.*, **19**, 1290 (1976).
(13) O. Grummitt et al., *Org. Prep. Proced.*, **2**, 5 (1970).
(14) A. Luettringhaus and R. Schneider, *Justus Liebigs Ann. Chem.*, **679**, 123 (1964).

Antitumor Septacidin Analogues

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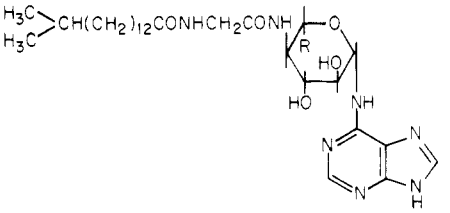
In the first approach by total synthesis to the structure of the antitumor antibiotic septacidin, analogues have been obtained which show similar inhibition of RNA–DNA synthesis in cultured leukemia L1210 cells and similar activity against transplanted leukemia P388 in mice. In these analogues, the natural aminoheptose moiety is replaced by 4-amino-4-deoxy- and 4-amino-4,6-dideoxy-L-glucose, to retain the natural configuration of the pyranose ring. Also retained is the lipophilic fatty acid–amino acid side chain attached to the 4-amino group and glycosylation at the 6-NH_2 of adenine. If the fatty acid chain was shortened from C_{16} to C_6, if the fatty chain was shifted to the glycine unit, or if the glycine unit was omitted, activity was completely lost. However, activity was retained if the C_{16} chain was shortened only to C_{12} or if the glycine unit was extended to β-alanine. Both active and inactive analogues were nonbinding to DNA and nonmutagenic to *Salmonella* strains. The synthetic approach was to start with a suitably protected sugar (L-fucose and L-galactose), construct the adenine moiety at C-1, introduce a 4-amino group, and finally attach the preformed side chain.

The antitumor antibiotic septacidin[1-3] (1) is structurally unique, combining fatty acid, glycine, amino sugar, and adenine units. The amino sugar is a heptose, 4-amino-4-deoxy-L-glycero-L-glucoheptopyranose, not encountered elsewhere. It is linked to the adenine unit by glycosylation at the 6-amino group rather than at N-9 or other ring nitrogen as in the purine nucleosides. In published data,[1,3] septacidin was cytotoxic against Earle's L cells in culture; it was active against adenocarcinoma CA 755 in mice but was inactive against Walker carcinosarcoma 256 and L1210 lymphoid leukemia, two experimental mouse tumors used extensively by the National Cancer Institute for screening new agents.[4] These data could suggest either that the septacidin structure has no potential for cancer treatment or that it has an unusual spectrum of antitumor activity.



1, R = HOCH(CH_2OH)

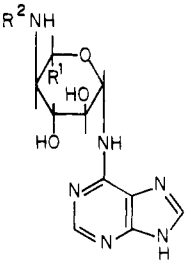
To explore the potential of 1 as a lead for cancer drug development, further studies required the flexibility of total synthesis for systematic structure variation. So far, the structure determination of septacidin rests entirely on elegant chemical degradation studies and spectral analyses.[1,5] Partial degradation permitted preparation of a number of septacidin analogues, through cleavage of the fatty acid–glycine side chain and reattachment of modified side chains and by chain shortening of the sugar unit to a hexopyranose or lengthening it to an octopyranose.[3] Activity vs. CA 755 was retained in a number of analogues, showing that certain structure changes are acceptable.

The synthesis and evaluation of any such structures have received surprisingly little attention. Only a handful of cases are reported[6-9] where a sugar is attached to the 6-amino group of adenine. Even the isomer of adenosine, 6-(β-D-ribofuranosylamino)purine, was only recently claimed.[10] Nor has 4-amino-4-deoxy-L-glucose previously been synthesized, a key degradation product in the structure proof. Comparison with the D enantiomer had to be relied upon.[5,11] This report describes the first totally synthetic approach to the septacidin structure, with the synthesis and biological evaluation of septacidin analogues **2–12** (Table I), of which all but **2** are completely new substances.

Chemistry. The general approach was to start with a sugar, construct on to it the adenine moiety, and attach the fatty acid–amino acid side chain last. In choosing the initial targets, the L-glycero-L-glucoheptose unit of **1** was replaced by hexoses (Table I, $R^1 = CH_2OH$ and CH_3) that were more accessible yet retained the L-gluco configuration of the pyranose ring. To introduce a 4-aminodeoxy function in the L-gluco configuration logically required 4-*O*-methanesulfonyl-L-galacto precursors (Scheme I, **22** → **23**). This would be as in the enantiomeric series in the synthesis of 4-aminodeoxy-D-glucose derivatives.[12,13] The most readily available L-galacto sugar as starting material was L-fucose (**14a**, 6-deoxy-L-galactose). Also, L-galactose (**14b**) was obtained by reduction of L-galactono-1,4-lactone (**13**).[14] The septacidin analogues **2–12** have been synthesized starting from these sugars.

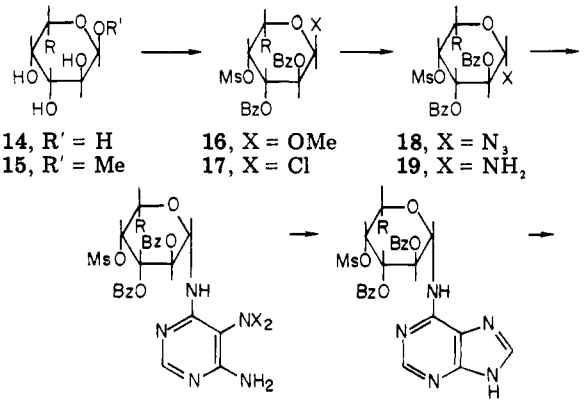
Selective functionalization of the galactopyranose ring at the axial 4 position is readily attained.[12,15,16] In the fucose series (Scheme I, series **a**) the 2,3-di-*O*-benzoate 4-*O*-mesylate **16a** is formed in high yield.[16] Treatment of **16a** with hydrogen chloride in the presence of titanium chloride in benzene solution afforded a crystalline chloro

Table I. Septacidin and Synthetic Analogues



R^2	R^1 = HOCH(CH$_2$OH)	R^1 = CH_2OH	R^1 = CH_3
$(CH_3)_2CH(CH_2)_{12}CONHCH_2CO$ isopalmitoylglycyl	**1** (septacidin)	**2** (active)	**3** (active)
$CH_3(CH_2)_{14}CONHCH_2CO$ palmitoylglycyl		**4** (active)	
$CH_3(CH_2)_{10}CONHCH_2CO$ lauroylglycyl		**5** (active)	**6** (active)
$CH_3(CH_2)_4CONHCH_2CO$ caproylglycyl			**7** (inactive)
$CH_3CH_2OCH_2CH_2CONHCH_2CO$ (ethoxypropionyl)glycyl		**8** (inactive)	**9** (inactive)
$(CH_3)_2CH(CH_2)_{12}CONHCH_2CH_2CO$ isopalmitoyl-β-alanyl		**10** (active)	
$CH_3CONHCH[CH_3(CH_2)_{15}]CO$ α-acetamidostearoyl			**11** (inactive)
$(CH_3)_2CH(CH_2)_{12}CO$ isopalmitoyl			**12** (inactive)

Scheme I



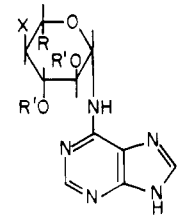
14, R′ = H; **15**, R′ = Me

16, X = OMe; **17**, X = Cl

18, X = N_3; **19**, X = NH_2

20, X = O; **21**, X = H

22

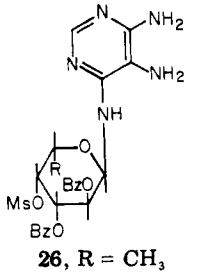


23, X = N_3; R′ = Bz
24, X = N_3; R′ = H
25, X = NH_2; R′ = H

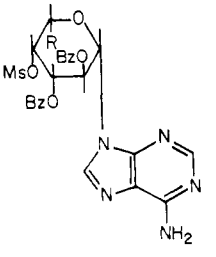
series a, R = CH_3
series b, R = CH_2OH (**14**, **15**) or CH_2OBz (**16**-**25**)

sugar as the pure α-anomer **17a**. This method,[17] though little used in the preparation of chloro sugars, is convenient in forming a relatively stable glycosyl halide directly from a methyl glycoside. The *O*-mesyl group was stable in this step and also during the subsequent construction of the adenine moiety through the glycosylamine[6,7] **19a**. The crystalline β-1-azide **18a** was obtained by selective displacement with azide ion in dimethyl sulfoxide at room temperature. Catalytic hydrogenation afforded the weakly basic glycosylamine **19a**, which was not isolated but was condensed[7] immediately with 4-amino-6-chloro-5-nitropyrimidine to give **20a**. Hydrogenation and crystallization afforded the 4,5-diaminopyrimidine **21a** as the chromatographically homogeneous β-anomer in 74% yield.

Diamine **21a** was unexpectedly accompanied by about 2% of the less soluble α-anomer **26**, identified by comparison of the circular dichroism spectrum with that of **21a**. Cotton effects at wavelengths associated with the heterocyclic chromophore showed a pseudoenantiomeric relationship, with molar ellipticities [θ] of −8520° at 291 nm for **26** and +12 000° at 285 nm for **21a**, as could be expected for a pair of α- and β-anomers. No reference compounds with measured circular dichroism were available for comparison with **26** and **21a**. Assignment of absolute configuration as α-L and β-L, respectively, was confirmed by the fact that the major isomer **21a** ultimately gave septacidin analogues (vide infra) having circular dichroism closely resembling that of septacidin, of established β-L configuration. (Anomeric pairs of adenine nucleosides, for example, have shown similar pseudoenantiomeric relationships, but of reversed sign.[18,19]) Stronger Cotton effects in **21a** and **26** at 225–230 and 239–240 nm were identical in sign and similar in intensity and were associated with the sugar benzoate chromophores. Compounds **21a** and **26** were nearly identical in infrared and mass spectra, and elemental analyses and ^1H NMR spectra clearly indicated they were isomeric. Since the

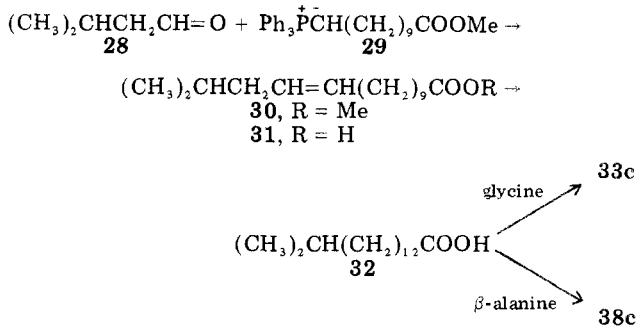


26, R = CH_3



27, R = CH_3

Scheme II



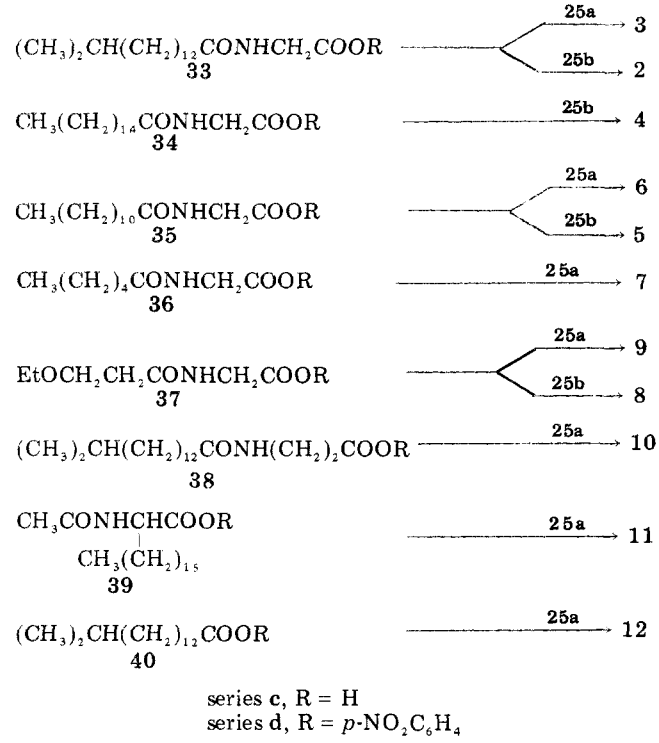
precursor **18a** was obtained as a single anomer, it must be assumed that minor anomerization occurred in a following step.

Ring closure of **21a** to form the 6-N-substituted adenine **22a** was carried out with triethyl orthoformate–acetic anhydride[6,20] to avoid or at least minimize formation of any N-9-substituted isomers, e.g., **27**. A nearly quantitative yield of **22a** was obtained, but it contained two minor impurities according to thin-layer chromatography on silica gel. One, just slightly less polar than **22a** was suspected of being the isomer **27**. This by-product showed an ultraviolet spectrum like adenosine (λ_{max} 257 nm in acid, 258 nm in base) when a sample was separated from a condensation using diethoxymethyl acetate. This reagent was tried on the likelihood[20a] that it is the true reactant when ethyl orthoformate–acetic anhydride is used. Surprisingly, **22a** was then formed with an equal amount of the by-product assumed to be **27**. Ethyl orthoformate–acetic anhydride was clearly the reagent of choice. The desired product **22a** was identified by an ultraviolet spectrum like that of septacidin.[2] A pure sample could be obtained in 25% yield, but for preparation purposes it was efficient to use this intermediate without complete purification.

Introduction of the azide group with configurational inversion at C-4 to form **23a** required 18 h of heating with sodium azide in dimethyl sulfoxide at 100 °C. Saponification of the benzoates afforded the azide **24a** as a homogeneous crystalline solid. The overall yield for eight steps from **16a** was 18%. Hydrogenation with 10% palladium on carbon gave amine **25a** as an amorphous, hygroscopic precipitate in 61% yield. According to thin-layer chromatography there was a contaminant that was negative to ninhydrin detection. A completely pure sample was not obtained by column chromatography on silica gel, and **25a** did not form a crystalline HCl salt or trifluoroacetamide. By high-pressure liquid chromatographic analysis (HPLC) the amine was 95% pure.

Acylation of **25a** to attach fatty acid–amino acid side chains yielded readily purified septacidin analogues. *N*-Acylglycine *p*-nitrophenyl esters were used as acylating agents, as was done in the modification of natural septacidin following its partial degradation.[3] Scheme III shows all the synthetic septacidin analogues formed by coupling various side chains with **25a** or with the amine **25b** when it was obtained from L-galactose. The fatty acid found in septacidin is the rare isopalmitic acid (**32**, 14-methylpentadecanoic acid) and quantities of it had to be obtained by synthesis. Isovaleraldehyde (**28**) was coupled with the Wittig reagent[21,22] **29** from methyl 11-iodoundecanoate, and the product was reduced and saponified to yield **32** (Scheme II). For the N-acylation of glycine by the mixed anhydride procedure,[23] the triethylamine salt of **32** in tetrahydrofuran was treated with ethyl chloroformate followed by aqueous sodium glycinate to yield **33c** (Scheme III). Treatment with *p*-nitrophenol and dicyclohexylcarbodiimide[24] gave the ester **33d**.

Scheme III



Acylation of amine **25a** by **33d** was carried out in dimethylformamide solution. Septacidin analogue **3** was isolated by water precipitation as a solid that could be recrystallized to chromatographic homogeneity and analytical purity. It showed the distinctive ultraviolet spectrum of septacidin,[2] λ_{max} 273 nm in acid, 264 nm at neutral pH, and 272 nm in base.

The synthesis of **25b** (Scheme I) was completely analogous to the synthesis of **25a**, except that L-galactose first had to be prepared. Subsequent intermediates to **25b**, even though enantiomeric to known sugars in the D series, were obtained in the L series for the first time. The 1-azide **18b**, like **18a**, was a readily purified crystalline solid. No attempts were made to isolate the glycosylamine **19b**, which was generated in situ and used immediately. Only small samples of intermediates **20b**, **21b**, **22b**, and **23b** were obtained completely pure. It was more efficient to accept purity of 75–80% (according to HPLC) and make use of higher yields of these intermediates. The several impurities were not isolated or identified, and it is possible that one of the impurities in **21b** might have been the α-L-anomer **26** (R = CH_2OH). Circular dichroism of the pure sample of **21b** showed a positive Cotton effect at 287 nm, like that for **21a** though weaker ([θ] +5940°), and confirmed that **21b** and **21a** had the same anomeric configuration.

Partial purification occurred during isolation of the 4-azide **24b** by ion-exchange chromatography, but the maximum purity achieved was 88%, and three impurities were still present. Reduction gave amine **25b** in nearly quantitative yield and of 85% purity. It was still most efficient to attach the side chains and complete the purification on the target septacidin analogues.

The product of coupling **25a** and **25b** with a *p*-nitrophenyl ester was in every case a solid that could be recrystallized or reprecipitated. Crude yields were excellent, but purification usually involved considerable losses in these unoptimized experiments. Each target was obtained pure by elemental analysis and homogeneous by thin-layer

Table V. Biological Test Results

Compd no.	NSC[a] no.	Vs. cultured L1210 leukemia cells[b] Inhibition of synthesis DNA, ED_{50}, μM	RNA, ED_{50}, μM	Cytotoxicity, I_{50}, μM	Vs. P388 leukemia in mice[c] Optimum dose, qd 1-9, mg/kg	Antitumor efficacy T/C, %	Binding to isolated helical DNA in soln,[b] ΔT_m, °C
1[d]	65104	0.40 (0.30)	1.0 (0.77)	>5	0.50 (0.38)	154	
2	266219	1.6	0.69	>10	0.50	149	1.7
3	268251	1.8	1.1	>20	1.0	160	0.6
4	278178	2.5	1.6	>100	0.50	136	3.6
5	267216	20	5.5	3	1.0	133	0.5
6	266218	29	18	>10	1.0	137	
7	271946	>1000	>1000	250	(0.50-16)[e]		
8	269447	>1000	>1000	>500	(3.1-50)[e]		0.4
9	269446	>1000	>1000	>500	(3.1-50)[e]		
10[f]	278179	100	93		1.6	156	1.9
11		>1000	>1000				
12	270538	>1000	>1000	>100	(0.50-8)[e]		0.5
Adriamycin[g]	123127	1.5	0.67	0.032	1.0	193	13.6

[a] Accession number of the National Cancer Institute. [b] Assay described in ref 25. [c] Assay described in ref 4, Protocol 1.200. Other doses were higher or lower by a factor of 2. Each compound had two to four active doses. Except for 7-9, compounds were of borderline solubility, and some doses were injected as suspensions. Water, water plus Tween, or saline plus Tween were used as vehicles. By definition, a compound is active if T/C ≥ 125. The T/C for 1 is the average from 13 tests; for 2, 3, 5, and 6 from two tests; for 4, 7-10, and 12 from single tests. Optimum dose is the one producing the highest T/C. [d] Purity of 1 was estimated to be 75%, based on elemental analysis for N; corrected doses assuming inactive impurities are shown in parentheses. [e] Inactive doses; for 7-9 and 12, T/C = 88-116. [f] Insoluble compound, the only one tested in suspension in vitro as well as in vivo. Cytotoxicity could not be determined for this reason. [g] Data from ref 25. The ΔT_m of 13.6 °C was obtained by the modified procedure.

chromatography. Subsequently, purity of the targets was checked by HPLC. Most were from 98.5 to 99.6% pure, but some (4, 5, 8, and 10; all from amine **25b**) were found to contain 6–12% of a single impurity that was undetectable by other means.

Compound 2 was previously reported[3] from the modification of 1, but there were no data that would permit comparison with the totally synthetic product. Ultraviolet extinctions of the synthetic septacidin analogues (Table II) were consistently much higher than those in the previous work.[3] Circular dichroism spectra (Table III) for 1–12 were qualitatively nearly identical, differing only in the magnitude of their Cotton effects near 239 nm (positive) and 267 and 273 nm (negative). This confirms that the new synthetic analogues and septacidin (1) all had the same type of attachment of the chromophore to a chiral structure and, thus, share the β-L-anomeric configuration. Table IV shows good correspondence of the ^1H NMR spectra for 1 and 2–12, with the sugar H-1 consistently at δ 5.4–5.6 as a doublet with $J_{1,2}$ = 7–8 Hz. (See paragraph at end of paper regarding supplementary material for Tables II–IV.)

The side chains were chosen first of all to duplicate the side chain of 1 (as in 2 and 3) and then to explore the effect on biological properties of an unbranched fatty acid (4), of shorter chain fatty acids (5–7), of inserting ether O in a short chain (8 and 9), of extending the natural side chain by inserting one $-CH_2-$ in the amino acid (10), of moving the fatty chain to the amino acid (11), and of omitting the amino acid (12).

Biological Tests. The septacidin analogues were tested first in cultured lymphoid leukemia L1210 cells for inhibition of nucleic acid synthesis and for cytotoxicity, by previously reported procedures.[25] A sample of natural septacidin (1) was obtained from the National Cancer Institute[26] and included for comparison. The analogues were then tested, along with 1 as parent, against lymphocytic leukemia P388 implanted in mice, under the auspices of the NCI and according to its protocols[4] which use the increased survival time of treated animals compared to controls as the measure of antitumor efficacy. Results are given in Table V. The mouse tests clearly show that synthetic analogues 2–6 and 10 retain the high antitumor potency of 1, with "confirmed activity" according to NCI criteria for 2–6 and 10 at doses of 0.5–1.6 mg/kg. In contrast, 7–9 and 12 were completely inactive at all doses tested.

The in vitro tests for inhibition of nucleic acid synthesis showed the same clean break between active and inactive compounds but gave a wider range of response for the active compounds. Analogues 2–4 showed essentially identical responses; they were nearly the same as 1 in the RNA test but not as potent as 1 in the DNA test. Analogues 5 and 6 were noticeably less potent; 10 appeared to be only weakly active, but this may be due to its insolubility such that 10 had to be tested as a suspension.

These results indicate that small changes at the terminal carbon of the sugar (CH_3 or CH_2OH, in place of $CHOH-CH_2OH$ in 1) have no effect on activity but that the nature of the fatty acid–amino acid side chain is critical. When the fatty acid was shortened from 16 carbons (2–4) to 12 carbons (5 and 6), there was decreased activity in vitro and a slight loss of efficacy in vivo. When shortened to six carbons (7) or the equivalent (8 and 9), loss of activity was total. Thus lipophilic character is important. The near identity of 2 and 4 suggests that chain branching is not required and that palmitic acid can be substituted in synthetic structures for the difficultly accessible isopalmitic. On the other hand, the lipophilic chain cannot be shifted from the *N*-acyl group to the α-carbon on the amino acid moiety as in 11 (in vitro test only), nor can the amino acid moiety simply be omitted as in 12. Apparently the side chain can be extended by inserting a carbon into the amino acid, judging from the in vivo test of 10.

Both in vivo and in vitro data show that 2–6 and 10 are comparable in potency to the antibiotic adriamycin (Table V), chosen for comparison because of the great current interest in its anticancer properties. The in vivo efficacy of adriamycin is much higher, but there are other differences. Adriamycin is highly cytotoxic, while cytotoxic effects of 2–4, 6–9, and 11 could not be observed at soluble

levels. Adriamycin binds strongly to isolated helical DNA in solution (as measured[25] by its effect on the helix–coil transition temperature of the DNA, or ΔT_m) whereas **2–5**, **8**, **10**, and **12** bind only weakly. This suggests that the potent inhibition of nucleic acid synthesis by **2–4** may be an indirect effect. Additional evidence that the active septacidin analogues do not attack nucleic acids directly is the absence of in vitro mutagenicity, observed when **2** and **5** were tested (along with the inactives **7** and **12**) in *Salmonella* strains at levels up to 1 μmol per plate.[27] Cancer drugs that are not mutagenic are of some importance, and further studies on the mechanism of action of septacidin analogues will be of considerable interest.

Experimental Section

Melting points were taken on a Fisher-Johns hot stage and are uncorrected. Organic solutions were dried over $MgSO_4$ and filtered. Evaporations or concentrations were carried out in vacuo on a spin evaporator. Stoichiometry of reactions was based on the assumption that all intermediates were completely pure, even though actual purity of some by HPLC was only 75%. Target compounds **2–12** were dried at 140 °C (1 mm).

Spectra. IR spectra were determined routinely in Nujol mull on a Beckmann IR-4 spectrometer and were consistent with chemical structure. Benzoate bands (C=O) were near 5.8 μ, NO_2 bands in **20a** and **20b** were observed at 6.6 μ, and strong N_3 bands were always at 4.71 ± 0.01 μ, whether from 4-azidodeoxy sugars or from glycosyl azides. In the methanesulfonates, OSO_2 bands were hard to distinguish from other absorption. In the pyrimidines and purines, heterocyclic absorption appeared as one or two strong bands at 6.1–6.3 μ. Spectra of **2–12** were all nearly identical, with predominant amide bands at 3.0, 6.1, and 6.45 μ. Proton magnetic resonance (^1H NMR) was determined on Varian A-60A and, where indicated, XL-100 spectrometers. Solutions in $CDCl_3$ or Me_2SO-d_6 were with Me_4Si (δ = 0.0) as internal reference; solutions in D_2O were with Me_4Si external reference except as indicated for **15b** and its β-anomer. Signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Heating to 70 °C, as noted, was often required to improve resolution, especially of H-1′. UV spectra were determined on Cary 11 or Cary 14 recording spectrophotometers. Circular dichroism was determined on a Jasco ORD/UV-5 spectropolarimeter equipped with a Sproul Scientific SS-20 CD modification and a programmed 15-Å slit-width control. Mass spectra were recorded on an LKB Model 9000 spectrometer at 12 eV.

Chromatography. Liquid column chromatography was done using Bio-Sil A 200–325 mesh silica gel or Woelm neutral alumina. TLC was done on 2 × 8 in. plates with 250-μ layers of silica gel GF (Uniplate) or aluminum oxide GF. R_f's are in solvent systems A, $CHCl_3$; B, $CHCl_3$–MeOH; and C, $ClCH_2CH_2Cl$–EtOAc (ratios of mixed solvents given in parentheses). Spots were visualized under UV light, except where otherwise noted. Analytical HPLC was done on a Chromatronix Model 3500 liquid chromatograph with a Schoeffel SF770 Spectroflow variable wavelength UV detector. System X was a Spherisorb alumina column A5Y, 23 × 0.64 cm o.d., with CH_2Cl_2–MeOH (99:1) as eluent. System Y was a μ Bondapak/carbohydrate column, 30 × 0.64 cm, supplied by Waters Associates and used with spectrograde CH_3CN–H_2O (4:1) as a reverse-phase eluent. System Y was used with targets **2–12** and with **24b**, **25a**, and **25b**, and all showed λ_{max} 264 nm for the HPLC peak; the peak for single impurities detected in **4**, **5**, and **7–10** had λ_{max} 267–269 nm. System X was used on compounds with the sugar moiety blocked.

2,3-Di-*O*-benzoyl-6-deoxy-4-*O*-methanesulfonyl-α-L-galactopyranosyl Chloride (17a). A solution of 60.0 g (0.129 mol) of methyl 2,3-di-*O*-benzoyl-6-deoxy-4-*O*-methanesulfonyl-α-L-galactopyranoside[16] (**16a**) [mp 161–163 C; 85% yield; ^1H NMR ($CDCl_3$) 5.68 (q × d, H-2 and H-3 as ABXY, H-2–H-3 = δ 5.60–5.77), 5.20 (m, H-4), 5.12 (d, H-1), 4.30 (q, H-5), 3.43 (s, OCH_3), 3.03 (s, OSO_2CH_3), 1.38 (d, CCH_3), $J_{1,2}$ = 3.3, $J_{2,3}$ = 10.7, $J_{3,4}$ = 2.3, $J_{5,6}$ = 6.5 Hz (lit.[16] mp 163–163.5 °C)] in 1.5 L of anhydrous benzene at room temperature was treated with 10 mL of titanium tetrachloride and then a slow stream of anhydrous HCl gas for 30 min. The orange-yellow solution was stored at room temperature for 18 h protected from moisture. It was washed with two 700-mL portions of ice water, 700 mL of saturated $NaHCO_3$ solution, and another 700 mL of water. The benzene solution was dried and evaporated to a syrup (~150 mL), which was dissolved in 100 mL of $ClCH_2CH_2Cl$. Petroleum ether (bp 60–110 °C) was added to the cloud point (~250 mL required), and the mixture was seeded and chilled at 3 °C (seed crystals were obtained by scratching the syrup on a watch glass with $ClCH_2CH_2Cl$–petroleum ether). After 18 h, an additional 100 mL of petroleum ether was added, and chilling was continued for 3 days. The white solid was collected by filtration to yield 50.6 g (84%): mp 124.5–126 °C; R_f 0.7 in solvent C (6:1); IR (Nujol) 5.77 (C=O), 7.33 (br), and 8.46 μ (OSO_2); ^1H NMR ($CDCl_3$) δ 6.49 (d, H-1), 5.82 (quintet, H-2 and H-3 as ABMX, H-2–H-3 = δ 5.79–5.85), 5.28 (m, H-4), 4.62 (q, H-5), 3.04 (s, OSO_2CH_3), 1.42 (d, CCH_3) ($J_{1,2}$ = 3.0, $J_{2,3}$ = 10.7, $J_{3,4}$ = 2.0, $J_{5,6}$ = 6.6 Hz). Anal. ($C_{23}H_{21}ClO_8S$) C, H.

2,3-Di-*O*-benzoyl-6-deoxy-4-*O*-methanesulfonyl-β-L-galactopyranosyl Azide (18a). The chloro sugar **17a** (54.6 g, 0.116 mol) was added to 500 mL of Me_2SO, followed by 55 g (0.85 mol) of NaN_3, and the mixture was stirred at room temperature for 18 h. The chloro sugar dissolved rapidly, and NaCl slowly precipitated. The mixture was poured into 3.2 L of water, and stirring was continued for 1 h. The white solid precipitate was collected on a filter and, while still wet, was dissolved in 2.8 L of boiling MeOH. The solution was cooled in ice for 1 h, and the white precipitate was collected to yield 42 g (76%): mp 152.5–154 °C; R_f 0.50 in solvent C (1:1); IR (Nujol) 4.69 and 4.72 (N_3), 5.75 and 5.81 μ (C=O); ^1H NMR ($CDCl_3$) δ 4.83 (d broadened, H-1, $J_{1,2}$ = 8 Hz), 3.04 (s, OSO_2CH_3), 1.45 (d, CCH_3, J = 7.5 Hz). Anal. ($C_{21}H_{21}N_3O_8S$) C, H, N.

A second crop was obtained, 10.5 g (18%), mp 138–148 °C, identical except for a minor contaminant [R_f 0.55; ^1H NMR δ 1.43 (d, CCH_3); ≤ 5%].

4-Amino-6-(2,3-di-*O*-benzoyl-6-deoxy-4-*O*-methanesulfonyl-β-L-galactopyranosylamino)-5-nitropyrimidine (20a). A solution of 10.5 g (0.022 mol) of 1-azide **18a** in 100 mL of anhydrous benzene was treated with 1.1 g of Pd black and shaken under H_2 at 2–3 atm for 7 days. The catalyst was removed by filtration and washed with 20 mL of benzene, and the combined filtrate containing amine **19a**, R_f 0.6 (ninhydrin-positive spot) in solvent C (1:2) compared to R_f 0.9 for **18a**, was used immediately without exposure to moisture. Attempts to isolate the amine gave decomposition and a ninhydrin-negative by-product.

4-Amino-6-chloro-5-nitropyrimidine (Aldrich; 4.0 g, 0.023 mol) was dissolved in 100 mL of refluxing anhydrous benzene, 10 mL of benzene was removed by azeotropic distillation to ensure dryness, and the hot solution (sometimes containing a little precipitate) was treated with the above solution of amine **19a** followed by 2.3 g (0.023 mol) of Et_3N. Another 10 mL of benzene was distilled off and the mixture protected from moisture was refluxed for 18 h. The mixture was cooled, treated with 1 mL of Et_3N and 1 mL of H_2O, and stirred overnight to convert any unreacted 6-chloropyrimidine to the less soluble 6-hydroxy analogue. The latter and Et_3N·HCl were removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in 300 mL of boiling 95% EtOH. The solution was allowed to cool very slowly to room temperature, while much of the product separated as crystals. The mixture was kept at room temperature for 2 days and filtered to yield 9.1 g (70%) that was used in the next step: mp 158–165 °C; ^1H NMR ($CDCl_3$) δ 9.58 (d, glycosyl NH, J = 8 Hz; exchanged with D_2O–CD_3COOD), 8.02 (s, pyrimidine H-2), 6.1–6.5 (m, H-1, H-2, H-3), 5.22 (br s, H-4), 4.20 (q, H-5), 3.08 (s, OSO_2CH_3), 1.39 (d, CCH_3, $J_{5,6}$ = 6.2 Hz); TLC in solvent C (1:1) revealed traces of **19a** of R_f 0.69 and 4-amino-6-chloro-5-nitropyrimidine of R_f 0.75 in addition to **20a** of R_f 0.57. A sample for analysis was recrystallized from EtOH–$ClCH_2CH_2Cl$: mp 188–189 °C. Anal. ($C_{25}H_{24}N_5O_{10}S$) C, H, N.

4,5-Diamino-6-(2,3-di-*O*-benzoyl-6-deoxy-4-*O*-methanesulfonyl-β-L-galactopyranosylamino)pyrimidine (21a) and the α-Anomer 26. A solution of 10.1 g (0.017 mol) of nitro compound **20a** in 200 mL of anhydrous pyridine was treated with 20 mL of a pyridine slurry of Raney active nickel catalyst (Grace) (prewashed with pyridine to remove water). The mixture was shaken under 2–3 atm of H_2 for 2 h and filtered. The collected

nickel was washed with 200 mL of pyridine, and the combined filtrates were evaporated to dryness. The residue was dissolved in 250 mL of $CHCl_3$. The solution was washed with 250 mL of saturated aqueous NaCl, dried, and evaporated to dryness. The solid residue was triturated with 150 mL of boiling benzene and filtered while hot. The insoluble portion was washed with 25 mL of hot benzene. The combined filtrate was chilled at 3–5 °C for 3 days to yield 7.0 g (73%) of an amorphous brown precipitate, mp 195–197 °C, that was homogeneous by TLC on alumina: R_f 0.78 in solvent B (19:1); $[\alpha]^{21}_D$ – 51.4° (*c* 1, Me_2SO); IR 3.0–3.2 (NH), 5.80 (C=O), 6.15 and 6.27 μ (heterocycle), with absence of absorption in the NO_2 region at 5.5–5.6; ^1H NMR ($CDCl_3$) δ 7.97 (s, pyrimidine H-2), 6.42 (d, H-1), 6.0–5.5 (H-2, H-3), 5.2 (br s, H-4), 4.22 (q, H-5), 3.05 (s, OSO_2CH_3), 1.40 (d, CCH_3, $J_{5,6}$ = 6.5 Hz); UV (in 95% EtOH) λ_{max} 216 nm (ϵ 44 100), 275 sh, 282 (12 400); UV (with NaOH added) λ_{max} 275 sh, 281 (12 900); UV (with HCl added) λ_{max} 226 (40 700), 284 (11 000), 312 (14 200); CD (in 95% EtOH) λ 225 nm [[θ] 52 800 (deg L)/(cm mol)], 231 (0), 239 (–52 800), 251 (0), 260 (5280), 285 (12 200); MS *m/e* 557 (M^+, 4.7), 461 (M – CH_3SO_3H, 1.8), 356 (M – CH_3SO_3H – PhCO, 2.0), 340 (M – CH_3SO_3H – $PhCO_2$, 3.6). Anal. ($C_{25}H_{27}N_5O_8S$) C, H, N.

The benzene-insoluble solid above (0.4 g, 4%) showed a second component, R_f 0.64, equal in intensity to **21** at R_f 0.78. Trituration with hot $CHCl_3$ removed **21**, leaving the insoluble α-anomer **26** (0.2 g, 2%): homogeneous on alumina, R_f 0.64 in B (19:1); $[\alpha]^{21}_D$ –191° (*c* 1, Me_2SO); IR and MS nearly identical with those of **21a**; ^1H NMR ($CDCl_3$) δ 7.98 (s, pyrimidine H-2), 6.65 (d, H-1), 6.2–5.5 (H-2, H-3), 5.1 (m H-4), 4.43 (q, H-5), 3.09 (s, OSO_2CH_3), 1.34 (d, 6-CH_3, $J_{5,6}$ = 6.5 Hz); UV (in 95% EtOH) λ_{max} 217 nm (ϵ 42 600), 275 sh, 283 (11 600); UV (with NaOH added) λ_{max} 275 sh, 281 (13 700); UV (with HCl added) λ_{max} 227 (41 200), 283 (10 300), 314 (13 000); CD (in 95% EtOH) λ 225 nm [[θ] 0 (deg L)/(cm mol)], 229 (16 200), 232 (0), 240 (–62 700), 291 (–8580); MS *m/e* 557 (M^+, 2.0), 461 (M – CH_3SO_3H, 2.7), 356 (M – CH_3SO_3H – PhCO, 2.8), 340 (M – CH_3SO_3H – $PhCO_2$, 3.6) Anal. ($C_{25}H_{27}N_5O_8S \cdot H_2O$) C, H, N.

6-(2,3-Di-*O*-benzoyl-4-*O*-methanesulfonyl-6-deoxy-β-L-galactopyranosylamino)-9*H*-purine (22a). A mixture of 138 g (0.247 mol) of diamine **21a**, 250 mL of triethyl orthoformate, and 250 mL of acetic anhydride was heated to reflux (slight exotherm). The resultant solution was stirred and refluxed for 2 h, cooled, and evaporated in vacuo. The oily residue was dissolved in 2.5 L of hot benzene, and the solution was diluted to the cloud point with cyclohexane (~300 mL), clarified by heating, allowed to cool slowly to room temperature, and stored for 24 h. The precipitate was collected by filtration to yield 105 g of dark brown solid, mp 155–167 °C. The filtrate was concentrated to 500 mL, diluted to the cloud point with more cyclohexane, chilled at 5 °C for 8 days, and filtered to yield 35 g of a second crop, mp 152–165 °C (total yield 140 g, 99%). The product, R_f 0.47 in solvent B (19:1), was acceptable for use in the next step; it was free of **21a** but showed minor impurities of R_f 0.53 (estimated 4%, perhaps the 9-isomer of **22a**, formed in the reaction) and R_f 0.93. Reprecipitation by cooling a benzene solution afforded a homogeneous sample for analysis (25% yield): mp 156–178 °C; ^1H NMR ($CDCl_3$, 100 MHz) δ 8.54 (s) and 8.18 (s) (H-2 and H-8), 6.44 (br s, H-1′), 5.9–5.6 (H-2′, H-3′), 4.31 (q, H-5′), 3.10 (s, OSO_2CH_3), 1.48 (d, CCH_3, $J_{5',6'}$ = 6.5 Hz); UV (in 95% EtOH) λ_{max} 232 nm (ϵ 28 500), 264 (19 600); UV (with HCl added) λ_{max} 231 (28 400), 278 (19 700); UV (with NaOH added) λ_{max} 278 (17 000). Anal. ($C_{26}H_{25}N_5O_8S$) C, H, N.

6-(4-Azido-2,3-di-*O*-benzoyl-4,6-dideoxy-β-L-glucopyranosylamino)-9*H*-purine (23a). A mixture of 14.0 g (24.4 mmol) of **22a** and 7.2 g (110 mmol) of NaN_3 in 72 mL of Me_2SO was stirred and heated at 100 °C for 18 h and poured into 1 L of ice and water. After 30 min of stirring, the precipitate was collected and dissolved in 200 mL of $CHCl_3$. The solution was washed with 100 mL of saturated brine, dried, and evaporated to yield 13.8 g (109%) of residual foamed glass; TLC, R_f 0.50 in solvent B (19:1), showed absence of **22a** but revealed a minor impurity of R_f 0.53 that could not be removed by preparative TLC or attempts at crystallization.

6-(4-Azido-4,6-dideoxy-β-L-glucopyranosylamino)-9*H*-purine (24a). A solution of 175 g (0.340 mol) of **23a** and 27 g (0.50 mol) of NaOMe (Aldrich, anhydrous powder) in 1.2 L of anhydrous MeOH was protected from moisture and stirred at room temperature for 2 h and then treated with ~500 g of 100–200 mesh Dowex 50 (H) to neutralize the base and absorb the purine. After 1 h more of stirring, the resin was collected, washed with 200 mL of MeOH (to remove MeOBz), and added to a chromatographic column, and the product was eluted with 8 L of ~8 N ammonium hydroxide. The eluate was concentrated with heating until a thick precipitate formed. The mixture was cooled and filtered to yield 44 g (42%). The filtrate was evaporated to dryness, and the residue was triturated with 50 mL of hot water on a steam bath. The suspension was filtered, the filtrate was chilled at 5 °C for 3 days, and the solid was collected to give an additional 11 g. The combined yield was 52% of homogeneous **24a**: mp 254–262 °C dec; R_f 0.7 in solvent B (9:1); IR 4.47 (wk) and 4.70 (str) (N_3), 6.1–6.2 μ (purine), with absence of absorption for C=O; ^1H NMR (Me_2SO-d_6, exchanged with D_2O and CD_3COOD) δ 8.38 (s) and 8.28 (s) (H-2 and H-8), 5.5 (d, H-1′, $J_{1',2'}$ = 8 ± 1 Hz), 1.25 (d, 6′-CH_3, $J_{5',6'}$ = 5.5 Hz). A sample for analysis was twice recrystallized from H_2O: mp 261–264 °C dec. Anal. ($C_{11}H_{14}N_8O_3 \cdot 0.5H_2O$) C, H, N.

6-(4-Amino-4,6-dideoxy-β-L-glucopyranosylamino)-9*H*-purine (25a). A solution of 4.1 g (0.013 mol) of azide **24a** in 40 mL of MeOH–H_2O (1:1) was treated with 0.40 g of 10% Pd/C and shaken under H_2 at 2–3 atm for 3 days. The catalyst was collected on a filter and washed with 25 mL of H_2O, and the combined filtrate was evaporated to dryness. A solution of the residue (4.0 g) in 50 mL of hot MeOH was clarified by filtration and cooled. The resultant precipitate was collected to yield 1.66 g (44%) of amorphous solid: free of IR bands for N_3; ^1H NMR (D_2O) δ 8.11 (s) and 7.97 (s) (H-2 and H-8), 5.37 (d, H-1′, $J_{1',2'}$ = 9 Hz), 1.24 (d, CCH_3, $J_{5',6'}$ = 5.6 Hz). The filtrate was poured into 500 mL of ethyl ether to precipitate an additional 2.3 g (total yield 61%) that was identical by IR and TLC, R_f 0.5 (UV absorbing and ninhydrin positive), with contaminants of R_f 0.1 and 0.9 (UV absorbing only) in solvent B (1:1). MS as the per(Me_3Si) derivative suggested there was complete pyrolysis at the glycosidic bond of **25a** in the injector at 300 °C: *m/e* 361 [dideoxy – Me_3SiNH – bis(OMe_3Si)hexosene], 279 [bis(NMe_3Si)adenine]. Purity by HPLC system Y was 95%, with three impurities (2.5 and 0.1%).

Methyl α-L-Galactopyranoside (15b). L-Galactono-1,4-lactone (**13**; Pfanstiehl) was reduced to L-galactose (**14**) with sodium amalgam by modification of previous procedures.[14] A solution of 300 g (1.68 mol) of **13** in 4 L of H_2O was chilled to 0 °C and acidified to pH 2.5–3.0 with 3.6 N H_2SO_4. With vigorous stirring, 4.1 kg of 2.5–3.0% sodium amalgam was added in 200–300-g portions during 1.5 h, while pH 2.5–3.0 was maintained by further additions of 3.6 N H_2SO_4 (2 L was required). Stirring was continued for 1 h and the temperature rose to 15 °C. The solution was decanted from the mercury, treated with decolorizing charcoal, filtered, neutralized with 10 N NaOH to pH 6.7, and evaporated. The semisolid residue was stirred with 2 L of hot MeOH, the mixture was filtered, and the filtrate was evaporated. A solution of the residue in 1 L of H_2O was added to a column (1.9 × 46 cm) of RG-501-X8 (H, OH) mixed-bed resin, 20–50 mesh. The column was eluted with H_2O (4 L) until the eluate showed little or no **14** when spotted on paper and sprayed with $AgNO_3$–NaOH. The eluate was evaporated, the residue was dissolved in 600 mL of MeOH, and the solution was chilled at 0 °C for 18 h. The solid was collected to yield 168 g (56%). The filtrate gave a second crop upon concentration to 200 mL and chilling, 32 g (total yield, 66%). The product was identified as L-galactose (**14**), containing 5–10% galactitol, by gas chromatographic comparison with D-galactose.[28]

A solution of 50 g of **14** in 500 mL of MeOH was treated with ~75 mL of Dowex 50 (H) resin, refluxed for 3 days, and filtered. The filtrate was evaporated to dryness. Processing as in an elegant study[29] of the D series is recommended over the earlier methods. The α-anomer **15b** was obtained as the monohydrate: mp 95–105 °C; ^1H NMR (D_2O, internal $Me_3SiCH_2CH_2CH_2SO_3Na$) δ 4.86 (unresolved d, H-1), 3.41 (s, OCH_3). Drying at 63 °C (1 mm) for 3 days gave the anhydrous form analytically pure: mp 90–98 °C; $[\alpha]^{20}_D$ –181° (*c* 1, H_2O). Anal. ($C_7H_{14}O_6$) C, H. For the D-monohydrate, lit.[29] mp was 90–103 °C and $[\alpha]^{23}_D$ was +178.1° (0.1 M, H_2O); ^1H NMR[30] was at δ 4.83 and 3.39. For the anhydrous D form, lit.[29] mp was 107–112 or 116–116.5 °C.

From the mother liquors, **methyl β-L-galactopyranoside** was obtained in low yield: mp 177–181 °C; ^{1}H NMR (D_2O, internal $Me_3SiCH_2CH_2CH_2SO_3Na$) δ 4.32 (q, H-1), 3.60 (s, OCH_3); $[\alpha]_D$ +0.2° (*c* 1, H_2O). Anal. ($C_7H_{14}O_6 \cdot H_2O$) C, H. For the D enantiomer, lit.[29] mp was 177–180 °C and $[\alpha]^{23}_D$ was −0.3 to −0.5° (0.1 M, H_2O); ^{1}H NMR[30] was at δ 4.30 and 3.56.

Methyl 2,3,6-Tri-*O*-benzoyl-α-L-galactopyranoside. By a procedure[15] for the D enantiomer, the yield was 27%: mp 136–138 °C; $[\alpha]^{21}_D$ −118° (*c* 1, $CHCl_3$); IR identical with that of the D form;[12] ^{1}H NMR ($CDCl_3$) δ 5.83 (s, H-2, H-3, half-width at 4 Hz), 5.28 (s, H-1, half-width at 4 Hz), 4.8–4.3 (H-4, H-5, H_2-6), 3.47 (s, OCH_3); for the D form, mp 139–140[15] and 135.5–137.0 °C,[12] $[\alpha]_D$ +123° ($CHCl_3$)[15] and +120° ($CHCl_3$).[12] Anal. ($C_{28}H_{26}O_9$) C, H.

Methyl 2,3,6-Tri-*O*-benzoyl-4-*O*-methanesulfonyl-α-L-galactopyranoside (16b). Procedures[12,15] for the D enantiomer were modified by the use of 1.7 molar equiv of methanesulfonyl chloride, keeping the temperature below 10 °C during addition. The reaction mixture was stirred overnight and then heated to 60 °C for 30 min. Hydrolysis in ice water gave a precipitate that was collected on a filter but melted at room temperature. The resultant syrup was dissolved in $CHCl_3$ (10 mL/g) with heating and processed. The product was recrystallized from benzene–petroleum ether (bp 30–60 °C) to give a 73% yield: mp 142–144 °C; $[\alpha]^{21}_D$ −100° (*c* 1, $CHCl_3$); IR identical with that of the D form;[12] ^{1}H NMR ($CDCl_3$) δ 5.9–5.65 (m, H-2 and H-3), 5.51 (m, H-4), 5.23 (d, H-1, *J* = 3 Hz), 3.44 (s, OCH_3), 3.06 (s, OSO_2CH_3); for the D form, mp 143–144[15] and 142–143 °C,[12] $[\alpha]_D$ +102° ($CHCl_3$). Anal. ($C_{29}H_{28}O_{11}S$) C, H.

2,3,6-Tri-*O*-benzoyl-4-*O*-methanesulfonyl-α-L-galactopyranosyl Chloride (17b). A stirred solution of 270 g (0.462 mol) of **16b** in 2 L of anhydrous benzene protected from moisture was treated with 30 mL of titanium tetrachloride and then a stream of anhydrous HCl gas for 1 h. After 20 h at room temperature (without stirring) the HCl was reintroduced for 30 min and again after 4 days. As measured by ^{1}H NMR, conversion to the chloro sugar was 71% complete after 3 days, but 6 days were required for 92% completion. The solution was poured into 2 L of ice water. As for **17a**, benzene extracts were washed with $NaHCO_3$ solution and water, dried, and evaporated. The residual syrup, 290 g (106% of theory), contained a little benzene: ^{1}H NMR ($CDCl_3$) δ 6.55 (d, H-1, $J_{1,2}$ = 3.2 Hz), 5.9 (rough t, H-2 and H-3), 5.58 (rough d, H-4, *J* = 2.5 Hz), 3.00 (s, OSO_2CH_3); there was no evidence of a signal for H-1 of the β-anomer (estimated ≤5%), but a weak singlet at δ 3.40 evidenced 8% of unreacted **16b**. There was no IR absorption in the OH region near 3.0 μ.

2,3,6-Tri-*O*-benzoyl-4-*O*-methanesulfonyl-β-L-galactopyranosyl Azide (18b). Using the procedure for **18a**, **17b** (285 g, 0.483 mol) was treated at room temperature with 220 g (3.38 mol) of NaN_3 in 2 L of Me_2SO. The product was precipitated with water and dissolved in 1.5 L of CH_2Cl_2. The solution was washed with 500 mL of H_2O, dried, and evaporated. The residue was crystallized from 2 L of benzene by storing the solution at room temperature for 3 days and at 10 °C for 3 h. The white solid was collected on a filter to yield 220 g (76%): mp 168–170 °C; $[\alpha]^{20}_D$ −8.7° (*c* 1, $CHCl_3$). The filtrate was evaporated and the residual syrup crystallized from 200 mL of 95% EtOH to give 30 g more, mp 168–170 °C. The total yield was 87%: homogeneous on TLC, R_f 0.45 (in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.98 (m, H-1, $J_{1,2}$ ~8 Hz), 3.10 (s, OSO_2CH_3). Anal. ($C_{28}H_{25}N_3O_{10}S$) C, H, N, S.

4-Amino-6-(2,3,6-tri-*O*-benzoyl-4-*O*-methanesulfonyl-β-L-galactopyranosylamino)-5-nitropyrimidine (20b). Hydrogenation of 1-azide **18b** was accomplished on large scale by bubbling a slow stream of H_2 through a stirred solution of 130 g (0.218 mol) of **18b** in 1 L of anhydrous benzene containing 5 g of 10% Pd on charcoal. After 2 days, 2 g more Pd on charcoal was added, and the introduction of H_2 was continued 3 days longer. According to TLC in system C (2:1) the reduction to amine **19b**, R_f 0.67 (ninhydrin-positive spot), was 90–95% complete, although a little azide **18b** was still present, R_f 0.85. The solution was purged with N_2, the catalyst was removed by filtration, and the filtrate containing **19b** was used immediately.

A hot, dried solution of 39.0 g (0.223 mol) of 4-amino-6-chloro-5-nitropyrimidine in 400 mL of benzene (after removal of ~100 mL by azeotropic distillation) was treated with the above solution of **19b**, followed by 23.0 g (0.227 mol) of Et_3N. The mixture was treated and worked up after 18 h by the procedure for **20a**. Crystallization of **20b** from 1.5 L of 95% EtOH gave 152 g (99%), mp 128–135 °C. Compared to spectra of **18b**, IR absorption for N_3 was absent from **20b**, new IR bands appeared at 2.9, 3.0 (NH), 6.2, 6.3 (pyrimidine heterocycle), and 6.6 μ (NO_2), and the ^{1}H NMR signal ($CDCl_3$) for H-1 shifted downfield where it appeared along with H-2 and H-3 as a multiplet at δ 5.8–6.2; appearance of a singlet for OSO_2CH_3 (δ 3.18), even on expansion of the scale, suggested isomeric purity. Minor impurities, R_f 0.56 and 0.78, were detected by TLC in solvent B (19:1) along with **20b** and R_f 0.67 (ninhydrin positive), but there was no amine **19b** of R_f 0.44. Purity of 75–80% was indicated by HPLC system X with five minor impurities. The impurities were not removed by attempted recrystallization from various solvents. A sample for analysis was obtained by column chromatography on silica gel in $CHCl_3$–MeOH (99:1), followed by recrystallization from MeOH by adding water until the cloud point: mp 134–139 °C. Anal. ($C_{32}H_{29}N_5O_{12}S \cdot 0.5H_2O$) C, H, N.

4,5-Diamino-6-(2,3,6-tri-*O*-benzoyl-4-*O*-methanesulfonyl-β-L-galactopyranosylamino)pyrimidine (21b). Nitro compound **20b** (152 g, 0.215 mol) in 1 L of pyridine was hydrogenated as for **21a**, using 100 mL of Raney nickel slurried in pyridine, except that on this scale H_2 was bubbled through the stirred solution in a slow stream. After 18 h, TLC in solvent B (19:1) showed that conversion of **20b** (R_f 0.52) to **21b** (R_f 0.38) was complete. The solution was purged with N_2. Processing as for **21a** afforded 145 g (99%) of residual solid, mp 136–143 °C, that was used directly in the next step. Purity was estimated at 75–80% by HPLC system X, which showed three unidentified impurities. A sample for analysis was recrystallized from benzene–cyclohexane (5% yield): mp 140–142 °C; ^{1}H NMR (Me_2SO-d_6, 100 MHz, 70 °C) δ 6.19 (uneven d, H-1′, $J_{1',2'}$ = 8.0 ± 0.3 Hz), 6.1–5.6 (H-2′, H-3′), 5.56 (d, H-4′, *J* = 2.5 ± 0.2 Hz), 4.9–4.3 (H-5′, H-6′ × 2), 3.34 (s, OSO_2CH_3); UV (in 95% EtOH) λ_{max} 218 nm (ε 50 100), 229 sh, 275 (13 000), 282 (13 100); UV (with NaOH added) λ_{max} 275 (15 200), 281 (15 600); UV (with HCl added) λ_{max} 227 (52 400), 275 sh, 282 (11 400), 311 (13 800); CD (in 95% EtOH) λ 222 nm [[θ] 24 400 (deg L)/(cm mol)], 229 (0), 239 (−49 500), 254 (0), 287 (5940). Anal. ($C_{32}H_{31}N_5O_{10}S \cdot 0.25H_2O$) C, H, N, S.

6-(2,3,6-Tri-*O*-benzoyl-4-*O*-methanesulfonyl-β-L-galactopyranosyl)-9*H*-purine (22b). A mixture of 145 g (0.214 mol) of diamine **21b**, 500 mL of triethyl orthoformate, and 510 mL of acetic anhydride was refluxed for 2 h to give, after evaporation, 137 g (93%) of black syrup that was used in the next step; by TLC on alumina in solvent B (19:1) the product, R_f 0.52, showed minor contaminants of R_f 0.0, 0.42, and 0.66 but no **21b** of R_f 0.3. Purity by HPLC system X was 80%, with four unidentified impurities. Column chromatography on alumina in $CHCl_3$ and elution with $CHCl_3$–MeOH (98:2) gave a sample of **22a** that could be reprecipitated as an amorphous solid by cooling a benzene–cyclohexane solution: 38% yield; mp 158–164 °C; 99.6% pure by HPLC; ^{1}H NMR (Me_2SO-d_6, 100 MHz, 70 °C, D_2O exchanged) δ 8.35 (s) and 8.20 (s, H-2 and H-8), 6.50 (d, H-1′, $J_{1',2'}$ = 7.0 Hz), 5.52 (m, H-4′), 3.33 (s, OSO_2CH_3).

6-(4-Azido-2,3,6-tri-*O*-benzoyl-4-deoxy-β-L-glucopyranosylamino)-9*H*-purine (23b). A stirred mixture of 137 g (0.199 mol) of **22b** and 60 g of NaN_3 in 1 L of Me_2SO was heated at 95 °C (steam bath). After 18 h, reaction was incomplete. After 3 days, the mixture was poured into 4 L of ice and water, and after 0.5 h of stirring the solution was decanted from the precipitated gum. The gum was dissolved in 1 L of hot $CHCl_3$, and the solution was washed with 1 L of brine, dried, and evaporated to yield 123 g of a foamed glass that was used in the next step. The ^{1}H NMR ($CDCl_3$) was free of any singlet for OSO_2CH_3. Purity was ~75%, with four unidentified impurities, by HPLC system X. A 2.0-g sample was purified by column chromatography on silica gel in $CHCl_3$; elution yielded 0.70 g that was crystallized from MeOH to give 0.46 g, mp 192–195 °C. Anal. ($C_{32}H_{26}N_8O_7$) C, H, N.

6-(4-Azido-4-deoxy-β-L-glucopyranosylamino)-9*H*-purine (24b). A solution of 50 g (0.93 mol) of NaOMe powder in 1 L of MeOH was added to 123 g (0.194 mol) of azide **23b**, and the solution was treated by the procedure for **24a**. The product (21 g) after elution from Dowex 50 (H) was dissolved in 20 mL of MeOH and precipitated by pouring the solution into 200 mL of ether. Filtration afforded 18 g (29%) of amorphous solid: ^{1}H

NMR (D_2O) δ 8.07 (s) and 7.98 (s, H-2 and H-8), 5.42 (d, H-1′, $J_{1',2'}$ = 8.2 Hz), δ 4.2–3.2 (m, H-2′, H-3′, H-4′, H-5′, 2 × H-6′). Purity was 88% with three unidentified impurities by HPLC system Y.

Paper chromatography on Whatman No. 1 paper in BuOH–HOAc–H_2O (4:1:5) and UV analysis of the spots suggested that two of the minor impurities possibly were the α-L-anomer (R_f 0.38; λ_{max} 264 nm, 272 in base) and the 9-substituted adenine isomer (R_f 0.28; λ_{max} 264 nm, 264 in base) of **24b** (R_f 0.57; λ_{max} 265 nm, 273 in base).

6-(4-Amino-4-deoxy-β-L-glucopyranosylamino)-9*H*-purine (25b). Using the procedure for **25a**, 13.0 g (40.3 mmol) of azide **24b** in 50 mL of H_2O was hydrogenated with 1 g of 10% Pd/C for 18 h. The solid from evaporation (12 g) was dissolved in 20 mL of hot MeOH and precipitated by pouring the solution into 50 mL of ether. Filtration afforded 11.4 g (95%) of amorphous solid that was used in the next step: no IR band for N_3 near 4.7 μ; ^1H NMR (D_2O) δ 8.03 (s) and 7.97 (s, H-2 and H-8), 5.42 (d, H-1′, $J_{1',2'}$ = 8.8 Hz). Purity was ~85%, with three unidentified impurities of comparable amounts, by HPLC system Y.

10-(Methoxycarbonyl)decyltriphenylphosphonium Iodide. 11-Bromoundecanoic acid was esterified[31] with methanol in the presence of $CaSO_4$ and Dowex 50 (H). The ester was converted[21,22] with NaI and refluxing 2-butanone to the 11-iodide and then with triphenylphosphine in C_6H_6 to the phosphonium iodide (77% yield): mp 126–129 °C (lit.[22] 128–130 °C); IR 5.78 μ (C=O).

(*Z*)-14-Methyl-11-pentadecenoic Acid (31). The phosphonium compound was treated with NaOMe in $HCONMe_2$ to give **29** and then with isovaleraldehyde (**28**; as with valeraldehyde[22]) to give **methyl (*Z*)-14-methyl-11-pentadecenoate (30**, 83% yield) as a yellow oil: ^1H NMR ($CDCl_3$) δ 5.44 m (CH=CH), 3.70 s ($COOCH_3$), 0.90 d (Me_2C, J = 6.0 Hz). The ester **31**, without distillation, still contained 5% triphenylphosphine oxide, δ 7.6. Saponification[21] afforded 78% of **31**, free of Ph_3PO. A sample was distilled: bp 134–136 °C (0.003 mm); mp 12.5 °C; homogeneous except for 2% assumed to be the *E* isomer upon GLC as the $OSiMe_3$ derivative (at 210 °C, Chromosorb bW-HP column, N_2 carrier gas); IR (film) 5.88 (C=O), 10.7 μ (COOH dimer). Anal. ($C_{16}H_{30}O_2$) C, H.

14-Methylpentadecanoic Acid (32, Isopalmitic Acid). Hydrogenation of the olefinic acid by shaking a 95% EtOH solution (4 mL/g) with 5% Pd/C under 3 atm of H_2, filtration, evaporation of the filtrate, and EtOH recrystallization of the residue gave the acid (53% yield): mp 62–63 °C; ^1H NMR ($CDCl_3$) δ 2.38 (rough t, CH_2CO), 1.30 [s, $(CH_2)_{11}$], 0.88 (d, Me_2C, J = 6 Hz). For isopalmitic acid, lit.[32] mp 61.8–62.4 °C.

Isopalmitoylglycine (33c). By the method[23] for stearoyl-β-alanine, the acid **32** and glycine yielded 48%, which was recrystallized from EtOAc: mp 101–103 °C; ^1H NMR ($CDCl_3$–Me_2SO-d_6) δ 3.92 (d, NCH_2C=O, J = 5.5 Hz), 2.25 (uneven t, CH_2C=O), 1.29 [s, $(CH_2)_{11}$], 0.89 (d, Me_2C, J = 6.0 Hz). Anal. ($C_{18}H_{35}NO_3$) C, H, N.

***p*-Nitrophenyl Isopalmitoylglycinate (33d).** A mixture of 1.67 g (5.29 mmol) of the acid **33c** and 0.75 g (5.4 mmol) of *p*-nitrophenol in 50 mL of EtOAc was stirred and cooled to 0 °C. The solution was treated[24] with 1.10 g (5.34 mmol) of dicyclohexylcarbodiimide. The suspension was stirred at 0 °C for 30 min and at room temperature for 30 min, heated to 35–40 °C for 3 h, and cooled to 5 °C. Four drops of HOAc were added, the mixture was filtered, and the filtrate was evaporated. The residue was dissolved in 50 mL of ethyl ether with heating; the solution was clarified of any dicyclohexylurea by filtration, was diluted to the cloud point with petroleum ether (bp 60–110 °C), and was chilled overnight at −10 °C. The white solid was collected by filtration to yield 2.08 g (90%): mp 87–89 °C; IR (Nujol) 3.00 (NH), 5.70 (ester C=O), 6.08 (amide C=O), 6.53 (amide II plus NO_2), 7.43 μ (NO_2); ^1H NMR ($CDCl_3$) δ 4.32 (d, NCH_2C=O, J = 6 Hz), 2.3 (uneven t, CH_2C=O), 1.23 [s, $(CH_2)_{11}$], 0.85 (d, Me_2CH, J = 6 Hz). Anal. ($C_{24}H_{38}N_2O_5$) C, H, N.

Isopalmitoyl-β-alanine (38c). β-Alanine and isopalmitic acid yielded[23] 84% crude or 59% when recrystallized from EtOAc: mp 100–102 °C; IR 3.02 (NH), 5.90 (acid C=O), 6.10 (amide C=O), 6.50 μ (amide II); ^1H NMR ($CDCl_3$–Me_2SO-d_6) δ 3.38 (q, NCH_2, J = 6 Hz), 2.42 (t, CH_2C=O of β-alanine, J = 6.5 Hz), 2.12 (t, CH_2C=O of isopalmitoyl, J = 6.5 Hz), 1.23 [s, $(CH_2)_{11}$], 0.87 (d, Me_2CH, J = 6.0 Hz).

***p*-Nitrophenyl Isopalmitoyl-β-alanate (38d).** A solution of 610 mg (1.86 mmol) of isopalmitoyl-β-alanine (**38c**) and 275 mg (1.98 mmol) of *p*-nitrophenol in 25 mL of EtOAc was treated with 410 mg (1.99 mmol) of dicyclohexylcarbodiimide. Reaction conditions and processing as for **33d** yielded 592 mg (71%): mp 84–87 °C; IR 3.02 (NH), 5.63 (ester C=O), 6.07 (amide C=O), 6.42 and 6.53 μ (amide II and NO_2); ^1H NMR ($CDCl_3$) δ 3.63 (q, $-NCH_2$, J = 6 Hz), 2.83 (t, CH_2C=O of β-alanate, J = 6 Hz), 2.20 (rough t, CH_2C=O of isopalmitoyl), 1.23 [s, $(CH_2)_{11}$], 0.87 (d, Me_2CH, J = 6 Hz).

(3-Ethoxypropionyl)glycine (37c). A solution of 16.0 g (115 mmol) of ethyl glycinate hydrochloride in 50 mL of pyridine was cooled to 5 °C and added in portions to a solution of 15.2 g (111 mol) of β-ethoxypropionyl chloride:[33] bp 74–75 °C (52 mm); IR (film) 5.53 μ ($CDCl_3$), in 10 mL of $CHCl_3$. The mixture was stirred at room temperature overnight and poured into 250 mL of ice and water, and the acylation product was extracted with two 100-mL portions of $CHCl_3$. The combined extracts were washed with 100 mL of saturated aqueous $NaHCO_3$ and 100 mL of H_2O, dried, and evaporated to yield 11.2 g (50%) of syrupy **ethyl (3-ethoxypropionyl)glycinate (37**, R = Et): IR (film) 3.05 (NH), 5.72 (ester C=O), 6.05 (amide C=O), 6.50 μ (amide II).

The ester (10.2 g, 50 mmol) was saponified with 2.5 g (62 mmol) of NaOH in 500 mL of MeOH–H_2O (80:20) solution at reflux for 1 h. The cooled solution was neutralized with ~50 mL of prewashed 100–200 mesh Dowex 50 (H) ion-exchange resin, filtered, and evaporated. The residue was recrystallized from EtOAc–petroleum ether (bp 60–110 °C) to yield 7.2 g (82%): mp 64–65 °C; ^1H NMR ($CDCl_3$) δ 11.21 (s, COOH), 7.41 (rough t, NH), 4.08 (d, CH_2C=O, J = 5.5 Hz), 3.73 (t) and 2.58 (t) ($ROCH_2CH_2C$=O, J = 6.0 Hz), 3.57 (q) and 1.21 (t) (OCH_2CH_3, J = 7.0 Hz). Anal. ($C_7H_{13}NO_4$) C, H, N.

***p*-Nitrophenyl (3-Ethoxypropionyl)glycinate (37d).** A solution of 5.48 g (31.3 mmol) of the acid **37c** in 75 mL of EtOAc at 0 °C was treated with 4.50 g (32.4 mmol) of *p*-nitrophenol, followed by 6.60 g (32.0 mmol) of dicyclohexylcarbodiimide. The mixture was heated to 35 °C for 3 h and processed by the procedure[24] for **33d** to yield 2.6 g (28%), mp 93–94 °C. Anal. ($C_{13}H_{16}N_2O_6$) C, H, N.

***p*-Nitrophenyl 2-Acetamidostearate (39d).** By the above procedure for **37d**, α-acetamidostearic acid (**39c**), mp 105.5–106.5 °C (lit.[34] 106–108 °C), yielded 62%, mp 118–120 °C.

***p*-Nitrophenyl Isopalmitate (40).** As for **33d**, isopalmitic acid (**32**) yielded 46%: mp 51–53 °C; IR (Nujol) 5.72 (C=O), 6.57 and 7.49 μ (NO_2).

6-[4-Deoxy-4-(isopalmitoylglycyl)amino-β-L-glucopyranosylamino]-9*H*-purine (2). A mixture of 1.14 g (3.85 mmol) of amine **25b** and 1.65 g (3.79 mmol) of **33d** in 25 mL of dry dimethylformamide (DMF) was stirred at 35 °C for 18 h, and the solution was poured into 500 mL of ice and water. Stirring was continued for 1 h and the precipitate was collected and washed with 100 mL of H_2O. Weak infrared bands at 5.7–5.8 μ indicated a little O-acylation. A solution of solid in 50 mL of MeOH and 1 mL of diisopropylamine was refluxed for 1 h to cleave selectively the ester bonds and was evaporated. The solid (1.50 g, 67%) was dissolved in 5 mL of DMF, and the solution was diluted with 50 mL of MeOH and chilled at −10 °C for 3 days to yield 305 mg. A second crop, 300 mg that was analytically pure, was obtained by diluting the filtrate with 100 mL of H_2O. A third crop was combined with the first crop and recrystallized from hot MeOH–H_2O (4:1) by chilling to −10 °C. The total was 625 mg (27%): mp 224–226 °C; homogeneous in solvent B (3:1), R_f 0.40.

6-[4,6-Dideoxy-4-(isopalmitoylglycyl)amino-β-L-glucopyranosylamino]-9*H*-purine (3). The crude product from 8.41 g (30.0 mmol) of **25a** and 13.2 g (30.4 mmol) of **33d** weighed 15.1 g (88%), R_f 0.40. The IR spectrum again showed weak bands at 5.7–5.8 μ, and TLC in solvent B showed minor impurities at R_f 0.0 (not **25a**, ninhydrin negative), R_f 0.50 (presumed *O*-acyl by-product), and R_f 0.80 (*p*-nitrophenol). Refluxing with 500 mL of MeOH and 5 mL of $(Me_2CH)_2NH$, followed by six recrystallizations (100 mL/g) alternately from absolute MeOH, MeOH–$CHCl_3$, and finally MeOH–H_2O, gave 8.8 g (50%): mp 229–232 °C dec; purity 99.65% by HPLC, with three contaminants (0.04, 0.23, and 0.08%). Anal. ($C_{29}H_{49}N_7O_5$·0.5H_2O).

6-[4-Deoxy-4-(palmitoylglycyl)amino-β-L-glucopyranosylamino]-9*H*-purine (4). A mixture of *p*-nitrophenyl

palmitoylglycinate[24] [**34d**, 2.25 g (5.17 mmol), mp 107–108.5 °C (lit. 108–109 °C)] and 1.50 g (5.06 mmol) of **25a** in 50 mL of dry DMF was stirred at 25 °C for 18 h and then at 40 °C for 6 h, and the solution was poured into 400 mL of ice and water. After 0.5 h, the solid was collected, washed with water, treated with 50 mL of MeOH and 5 mL of $(Me_2CH)_2NH$ at reflux, and recovered by evaporation. The residual solid (2.4 g, 80%) was 83% pure according to its UV extinction. Crystallization from 50 mL of $CHCl_3$–MeOH (4:1) and from 50 mL of MeOH–H_2O (9:1) and drying at 140 °C (1 mm) yielded 0.51 g (17%): mp 238–243 °C dec; R_f 0.45 in solvent B (3:1); purity 90% by HPLC in system Y. Anal. ($C_{29}H_{49}N_7O_6 \cdot H_2O$) C, H, N.

6-[4,6-Dideoxy-4-(lauroylglycyl)amino-β-L-glucopyranosylamino]-9*H*-purine (6). *p*-Nitrophenyl lauroylglycinate[24] [**35d**, 1.54 g (4.08 mmol), mp 98–99.5 °C (lit.[24] 96–97.5 °C)] and 1.10 g (3.92 mmol) of **25a** were treated by the procedure for **2**. The solid (1.9 g, 90%) after $(Me_2CH)_2NH$–MeOH treatment was triturated with hot EtOAc, the suspension was cooled, and the solid was collected on a filter. It was recrystallized from 40 mL of hot MeOH by adding 5 mL of H_2O and chilling to −10 °C to give 1.5 g. Another recrystallization from 15 mL of hot MeOH gave 0.63 g (30%), mp 219–223 °C dec, that was homogeneous in solvent B (3:1), R_f 0.50; purity 99.5% by HPLC. Anal. ($C_{25}H_{41}N_7O_5 \cdot 0.5H_2O$) C, H, N.

6-[4-Deoxy-4-(lauroylglycyl)amino-β-L-glucopyranosylamino]-9*H*-purine (5). A mixture of 12.5 g (0.033 mol) of **35d** and 9.2 g (0.031 mol) of **25b** in 200 mL of dry DMF similarly afforded 12.0 g (72%) after trituration with EtOAc. Four recrystallizations from MeOH (25 mL/g) by adding H_2O to the cloud point yielded 2.7 g (16%): mp 220–224 °C dec; homogeneous in solvent B (3:1), R_f 0.45; purity 94% by HPLC system Y. Anal. ($C_{25}H_{41}N_7O_6 \cdot 0.75H_2O$) C, H, N.

6-[4,6-Dideoxy-4-(hexanoylglycyl)amino-β-L-glucopyranosylamino]-9*H*-purine (7). *p*-Nitrophenyl hexanoylglycinate[24] [**36d**, 0.56 g (2.0 mmol), mp 98–99 °C (lit.[24] "caproyl" mp 102–102.5 °C)] and 0.56 g (2.0 mmol) of **25a** afforded 610 mg (70%) after methanolic diisopropylamine treatment. Trituration with boiling petroleum ether (bp 30–60 °C), filtration, and two recrystallizations from 50 mL of MeOH plus 10 mL of H_2O yielded 110 mg (13%): mp 225–228 °C dec; R_f 0.35 in solvent B; purity 99.1% by HPLC system Y. Anal. ($C_{19}H_{29}N_7O_5 \cdot 0.5H_2O$) C, H, N.

6-[4,6-Dideoxy-4-[(3-ethoxypropionyl)glycyl]amino-β-L-glucopyranosylamino]-9*H*-purine (9). A mixture of 0.90 g (3.0 mmol) of ester **37d** and 0.84 g (3.0 mmol) of **25a** in 20 mL of DMF was stirred at 35 °C for 2 days, when TLC in solvent B (3:1) revealed a large amount of unreacted **25a**, R_f 0.10, along with product **9**, R_f 0.50. An additional 0.90 g of **37d** was added, and after the third day the solution was diluted with 100 mL of Et_2O (this product could not be precipitated by adding H_2O). The crude solid was collected, treated as before with $(Me_2CH)_2NH$–MeOH, recovered, and purified on a column of 25 g of silica gel in $CHCl_3$–MeOH (3:1). Fractions (15 mL) were analyzed by TLC. Those containing one spot of R_f 0.7 were combined, evaporated, and recrystallized to yield 0.15 g (11%): mp 195–199 °C dec; purity 98.5% by HPLC system Y. Anal. ($C_{18}H_{27}N_7O_6 \cdot 0.5H_2O$) C, H, N.

6-[4-Deoxy-4-[(3-ethoxypropionyl)glycyl]amino-β-L-glucopyranosylamino]-9*H*-purine (8). Amine **25b** and **37d** were treated by the procedure for **9**, except that the reaction was continued for 4 days, and the product after $(Me_2CH)_2NH$–MeOH treatment was triturated with boiling petroleum ether (bp 30–60 °C). Three recrystallizations from 2-propanol were required to remove unreacted **25b**, R_f 0.10 in solvent B (3:1), and yield 13%: mp 192–196 °C dec; R_f 0.50; purity 92% by HPLC system Y. Anal. ($C_{18}H_{27}N_7O_7 \cdot H_2O$) C, H, N.

6-[4-Deoxy-4-(isopalmitoyl-β-alanyl)amino-β-L-glucopyranosylamino]-9*H*-purine (10). *p*-Nitrophenyl isopalmitoyl-β-alanate [**38d**, 510 mg (1.22 mmol)] and 360 mg (1.21 mmol) of **25b** by the procedure for **2** yielded, after $(Me_2CH)_2NH$–MeOH treatment, 625 mg (85%) that was 80% pure by measurement of UV extinctions. Chromatography on a column of 95 g of silica gel in $CHCl_3$–MeOH (9:1) and recrystallization of the eluted product from MeOH–H_2O (20:1) yielded in two crops 210 mg (29%): mp 231–234 °C dec; R_f 0.45 in solvent B (3:1); purity 88% by HPLC system Y. Anal. ($C_{30}H_{51}N_7O_6 \cdot 0.75H_2O$) C, H, N.

6-[4,6-Dideoxy-4-(2-acetamidostearoyl)amino-β-L-glucopyranosylamino]-9*H*-purine (11). *p*-Nitrophenyl 2-acetamidostearate [**39d**, 125 mg (0.268 mmol)] and 90 mg (0.320 mmol) of **25a** yielded, after $(Me_2CH)_2NH$–MeOH treatment, 120 mg (72%), R_f 0.45 in solvent B (3:1), containing unreacted **25a**, R_f 0.15, and two minor contaminants, R_f 0.50 and 0.80. It was recrystallized from DMF–H_2O, three times from MeOH–H_2O, and from MeOH but still showed an ash upon elemental analysis. Chromatography on a column of 25 g of silica gel in $CHCl_3$–MeOH (3:1) afforded, after 180 mL of eluate was collected, 40 mL containing **11**. Evaporation and recrystallization of the residue afforded 9 mg (6%) that was homogeneous, mp 244–249 °C dec. Anal. ($C_{31}H_{53}N_7O_5 \cdot 0.5H_2O$) C, H, N.

6-(4,6-Dideoxy-4-isopalmitoylamino-β-L-glucopyranosylamino)-9*H*-purine (12). *p*-Nitrophenyl isopalmitate [**40**, 0.80 g (2.1 mmol)] and 0.56 g (2.0 mmol) of **25a** yielded, after $(Me_2CH)_2NH$ treatment, 0.89 g (85%). It was triturated with 50 mL of boiling petroleum ether (bp 30–60 °C) and recrystallized four times from MeOH–H_2O (10:1, 60 mL/g) with chilling to −10 °C and then from MeOH to yield 0.24 g (23%): mp 232–238 °C dec; R_f 0.45 in solvent B (3:1); purity 99.5% by HPLC. Anal. ($C_{27}H_{46}N_6O_4 \cdot 0.5H_2O$) C, H, N.

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Supplementary Material Available: Tables of spectra of septacidin analogues—Table II, ultraviolet; Table III, circular dichroism; Table IV, ^1H NMR (3 pages). Ordering information is given on any current masthead page.

References and Notes

(1) R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley-Interscience, New York, N.Y., 1970, p 256.
(2) J. D. Dutcher, F. E. Pansy, and M. H. von Saltza, U.S. Patent 3 155 647 (Nov 3, 1964).
(3) A. Aszalos, P. Lemanski, B. Berk, and J. D. Dutcher, *Antimicrob. Agents Chemother., 1965*, 845 (1966).
(4) R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3** (no. 2), 9 (1972).
(5) H. Agahigian, G. D. Vickers, M. H. von Saltza, J. Reid, A. I. Cohen, and H. Gauthier, *J. Org. Chem.*, **30**, 1085 (1965).
(6) S. Fukatsu, A. Sawa, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, **47**, 917 (1974).
(7) M. Ikehara and I. Tada, *Chem. Pharm. Bull.*, **11**, 1102 (1963).
(8) P. C. Jain and N. Anand, *Indian J. Chem.*, **6**, 616 (1968).
(9) J. A. Montgomery, S. D. Clayton, and H. J. Thomas, *J. Org. Chem.*, **40**, 1923 (1975).
(10) T. Fujishima, K. Uchida, and H. Yoshiro, Japanese Patent 75 34 040 (May 11, 1975); *Chem. Abstr.*, **84**, 106007g (1976).
(11) M. H. von Saltza, J. D. Dutcher, and J. Reid, Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1964, p 15Q.
(12) E. J. Reist, R. R. Spencer, D. F. Calkins, B. R. Baker, and L. Goodman, *J. Org. Chem.*, **30**, 2312 (1965).
(13) C. L. Stevens, P. Blumbergs, F. A. Daniher, D. H. Otterbach, and K. G. Taylor, *J. Org. Chem.*, **31**, 2822 (1966).
(14) (a) N. Sperber, H. E. Zaugg, and W. M. Sandstrom, *J. Am. Chem. Soc.*, **69**, 915 (1947); (b) R. A. Pizzarello and W. Freudenberg, *ibid.*, **61**, 611 (1939); (c) H. L. Frush and H. S. Isbell, *Methods Carbohydr. Chem.*, **1**, 127 (1962).
(15) J. M. Williams and A. C. Richardson, *Tetrahedron*, **23**, 1369 (1967).
(16) A. C. Richardson and J. M. Williams, *Tetrahedron*, **23**, 1641 (1967).
(17) L. J. Haynes and F. H. Newth, *Adv. Carbohydr. Chem.*, **10**, 222 (1955).
(18) J. S. Ingwall, *J. Am. Chem. Soc.*, **94**, 5487 (1972).
(19) C. A. Bush, *J. Am. Chem. Soc.*, **95**, 214 (1973).
(20) (a) R. K. Robins in "Heterocyclic Compounds", Vol. 8, R. C. Elderfield, Ed., Wiley, New York, N.Y., 1967, pp 220–221; (b) J. H. Lister, "Fused Pyrimidines", Part II, D. J. Brown, Ed., Wiley-Interscience, New York, N.Y., 1971, p 33.
(21) L. D. Bergelson, V. A. Vaver, V. Y. Kovtun, L. B. Senyavina, and M. M. Shemyakin, *Zh. Obshch. Khim.*, **32**, 1802 (1962).

(22) N. Petragnani and G. Schill, *Chem. Ber.*, **97**, 3293 (1964).
(23) M. Fieser, L. Fieser, E. Toromanoff, Y. Hirata, H. Heymann, M. Tefft, and S. Bhattacharya, *J. Am. Chem. Soc.*, **78**, 2825 (1956).
(24) A. Aszalos, P. Lemanski, and B. Berk, *J. Chem. Eng. Data*, 11, 429 (1966).
(25) G. Tong, W. W. Lee, D. R. Black, and D. W. Henry, *J. Med. Chem.*, **19**, 395 (1976). Modified procedures for measuring ΔT_m used 0.010 M phosphate buffer containing 10^{-5} M EDTA at pH 7.0 plus 5% Me_2SO to solubilize the compound. Modified procedures for measuring DNA–RNA inhibition added 1% Me_2SO for improved solubility. The in vitro tests were done at SRI by Dorris Taylor, Charlotte Elder, Nancy Charbeneau, and Keith Hohlfeldt.
(26) We are indebted to Dr. Harry B. Wood, Jr., Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI, and to Mr. S. J. Lucania, E. R. Squibb & Sons, Inc., for supplying samples of 1.

(27) B. N. Ames, W. E. Durston, E. Yamasaki, and F. D. Lee, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2281 (1973); B. N. Ames, J. McCann, and E. Yamasaki, *Mutat. Res.*, **31**, 347 (1975). We are indebted to Dr. V. F. Simmon of SRI for these determinations.
(28) C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).
(29) J. L. Frahn and J. A. Mills, *Aust. J. Chem.*, **18**, 1303 (1965).
(30) E. B. Rathbone, A. M. Stephen, and K. G. R. Pachler, *Carbohydr. Res.*, **20**, 357 (1971).
(31) G. F. Vesley and V. I. Stenberg, *J. Org. Chem.*, **36**, 2548 (1971).
(32) C. R. Fordyce and J. R. Johnson, *J. Am. Chem. Soc.*, **55**, 3368 (1933).
(33) R. E. Leslie and H. R. Henze, *J. Am. Chem. Soc.*, **71**, 3480 (1949).
(34) J. Andrako, J. D. Smith, and W. H. Hartung, *J. Pharm. Sci.*, **50**, 337 (1961).

Biologically Active Polycycloalkanes. 4.[1] Phosphoric Esters of Trimethylenenorbornyl Alcohols

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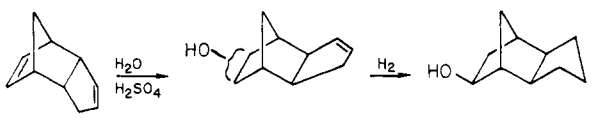
Secondary (**6**) and tertiary (**8**) phosphates of *exo*-5,6-trimethylenenorborn-*exo*-2-yl alcohol (*exo*-tricyclo-[5.2.1.$0^{2,6}$]dec-*exo*-8-yl alcohol, **3**) and a secondary ester (**9**) of a mixture of *exo*-tricyclo[5.2.1.$0^{2,6}$]dec-3-en-8- and -9-yl alcohol (**2**) were prepared. The most convenient route to **6** was direct esterification of phosphoric acid with **3**. **9** was obtainable by the addition of phosphoric acid to *endo*-dicyclopentadiene (**1**). These phosphates were tested in vitro for antiviral activity against Newcastle disease virus using a monolayer culture of chick embryo fibroblasts. **6** was found ca. twice more potent than, while **8** was as active as, amantadine hydrochloride under the present test conditions.

endo-Dicyclopentadiene (*endo*-tricyclo[5.2.1.$0^{2,6}$]deca-3,8-diene, **1**) is one of the most easily accessible tricyclic hydrocarbons. The compound comes out in the C_5 fraction in petrochemical processes and may be regarded as a by-product of isoprene production because of its limited industrial use. Sulfuric acid catalyzed hydration, accompanied by Wagner–Meerwein rearrangement, of **1** has been known[2] to proceed with high yield, giving a mixture of monohydric alcohols, *exo*-tricyclo[5.2.1.$0^{2,6}$]dec-3-en-*exo*-8- and -9-yl alcohol (**2**). The alcohols were catalytically hydrogenated to an identical tricyclodecanol, *exo*-5,6-trimethylenenorborn-*exo*-2-yl alcohol (*exo*-tricyclo-[5.2.1.$0^{2,6}$]dec-*exo*-8-yl alcohol, **3**).[2] We prepared new phosphates of these tricyclic alcohols and tested them for antiviral activity, in the hope of discovering some other utility of dicyclopentadiene.



1 2 3

Synthesis. Bis(*exo*-5,6-trimethylenenorborn-*exo*-2-yl) hydrogen phosphate (**6**) was prepared[3] by an established method (route A, Scheme I) which consists of hydrolysis[4] of the corresponding ditricyclodecyl chlorophosphate (**5**). The chlorophosphate **5** was obtained through chlorination by chlorine gas[5] of the ditricyclodecyl hydrogen phosphite (**4**) which in turn was synthesized by the reaction[6] of the tricyclodecanol **3** with phosphorus trichloride. The secondary phosphate **6** was also obtainable either by direct esterification of phosphoric acid with the tricyclodecanol **3** at 200 °C for 8 h (route B)[3] or by reaction[7c] of the sodium alcoholate (**7**) of **3** and excess free alcohol **3** with phosphorus oxychloride in xylene at 160 °C for 3.5 h (route C).[8] However, yield and purity of **6** obtained by these methods (B and C) were somewhat inferior to those obtained via route A. It is to be noted that practically no tertiary phosphate [tris(*exo*-5,6-trimethylenenorborn-*exo*-2-yl) phosphate, **8**] was formed in either of these reactions.

Direct esterification of phosphoric acid by aliphatic alcohols has never been found as any practical application to the preparation of phosphates, as the reaction usually gives rise to a complex mixture comprising primary, secondary, and tertiary phosphates.[7a] Prolonged heating at elevated temperature in order to complete the esterification eventually leads to decomposition of alcohols as well as phosphates to form olefins.[7d] The tricyclodecanol **3** and the product phosphate **6** should be resistant to β-elimination, in which a considerably strained norborn-2-ene structure is to be resulted. Therefore, esterification of **3** could be effected under more drastic conditions than are applicable to ordinary aliphatic alcohols, and these reaction conditions are considered to have allowed the esterification to proceed definitely. Another intriguing aspect of this esterification is halt of the reaction at the stage of the secondary ester. A possible cause for this effect will be discussed later in this paper, in connection with addition of phosphoric acid to *endo*-dicyclopentadiene.

Reaction of the alkoxide **7** in the presence of excess alcohol **3** with phosphorus oxychloride at room temperature for 12 h in dimethylformamide (route D) gave mainly the tertiary ester **8**, together with a small amount of the