Into a round-bottomed flask was placed **12** (1.79 g, 7.90 mmol) along with mercuric trifluroacetate 3.7 g (8.65 mmol) and 30 mL of dry THF. The mixture was stirred at room temperature for 12 h, after which the reaction was made basic (pH 10–14) with aqueous 2 N sodium hydroxide (1.6 mL added) and cooled to 0 °C. A solution of 4 N sodium borohydride in 2 N sodium hydroxide (3 mL, 3 mmol) $NaBH_4$) was added slowly. During the addition the temperature was maintained around 5 °C. Deposited mercury was removed by centrifugation and the supernatant was decanted and extracted twice with 20 mL of diethyl ether. Ether layers were combined and dried with sodium sulfate, and solvent was removed to give **13**: $[\alpha]_D$ +113° (*c* 0.513, $CHCl_3$); IR (thin film) ν 1662 cm^{-1} (C=N): ^1H NMR (200 MHz, $CDCl_3$) δ 4.68 (m, 1 H, H-1), 3.85 (m, 1 H, H-5), 3.70 (d, 1 H, H-4, $J_{4,5}$ = 9 Hz), 3.38 (s, 3 H, OCH_3), 2.86 (s, 6 H, $N(CH_3)_2$) 2.15 (dd, 1 H, H-2 eq, $J_{2a,2e}$ = 15 Hz, $J_{1,2}$ = 6 Hz), 1.73 (dd, 1 H, $J_{1,2a}$ = 9 Hz), and 1.24 (m, 6 H, 3-CH_3 and CH_3 at C-5); FAB MS, *m/e* 229 (M + H), 197 (M – OCH_3).

Methyl 3-Nitro-2,3,6-trideoxy-3-*C*-methyl-α-D-*ribo*-hexopyranoside (14). A solution of MCPBA (2.16 g, 12.5 mmol) in dry dichloromethane was heated to reflux under an atmosphere of nitrogen. Amino alcohol **5** (300 mg, 1.72 mmol) in dichloromethane (7.5 mL) was added over a 20-min period with stirring. Progress of the reaction was monitored by TLC using 3:4 ethyl acetate–petroleum ether (*R* of **14** = 0.54). After a total of 40 min, the reaction mixture was cooled and successively washed with 10% aqueous sodium sulfite solution, 10% aqueous sodium carbonate solution, and water, dried (sodium sulfate), and evaporated. Purification by flash chromatography (3:4 ethyl acetate–petroleum ether) gave 150 mg (42%) of crystalline nitro alcohol: mp 97–99 °C (lit.[19] (enantiomer) mp 101.5–103 °C); $[\alpha]_D$ +183° (*c* 3.68, $CHCl_3$) lit.[19] (enantiomer) –172 (*c* 0.25, $CHCl_3$). The ^1H NMR spectrum of **14** at 200 MHz was identical with that reported[19] for its enantiomer at 360 MHz.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this work. Support from Villanova University is also acknowledged.

(19) Brimacombe, J. S.; Rahman, K. M. M. *Carbohydr. Res.* **1985**, *140*, 163–166.

Rapid and Convenient Syntheses of Polyoxin Peptides Containing *N*-Methylated Peptide Bonds

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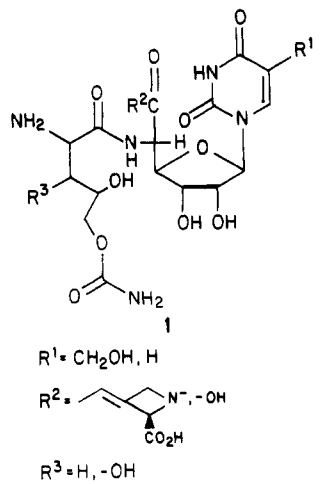
Received November 26, 1985

The N-methylated di- and tripeptide polyoxins **3** and **4** were rapidly prepared by the Ugi four component condensation (4CC) reaction and subsequent deblocking. Alternatively, entry into the dipeptide system was achieved by a short synthesis based upon the Strecker reaction. Methods for chromatographic separation of the diastereomeric intermediates were developed. The polyoxin amides **21a** and **21b** and the polyoxin nitrile **22** were also prepared.

The polyoxins **1** are pyrimdine nucleoside peptide antibiotics produced by *Streptomyces cacaoi*.[1] They have marked activity against phytophathogenic fungi but are not toxic to bacteria, plants, or animals.[2] Their biological effects apparently are due to their ability to inhibit the enzyme chitin synthase which catalyzes the final step in the biosynthesis of chitin, an essential component of the fungal cell wall structure.[3]



1

Although the chitin synthase from *Candida albicans*, a medically important human pathogen, is highly sensitive to the polyoxins in cell free systems[4] ($K_i \sim 10^{-6}$ M), the growth of intact cells can be inhibited only through selective manipulation of the growth medium.[5] This discrepancy between a high level of activity against the isolated enzyme and inactivity against whole cells could result either from failure of the polyoxin to penetrate the cell and reach the site of the chitin synthase[6] or from intracellular cleavage of the polyoxin dipeptide to the amino acid polyoxin C (**2a**) which is relatively inactive against fungal chitin synthase ($K_i \sim 10^{-3}$ M).[7]

In an attempt to prepare polyoxin analogues with activity against *C. albicans*, we focused our research on the preparation of polyoxins possessing a peptide bond that is stable to the enzymatic environment inside the fungal cell. The *N*-methylpolyoxins **3** and **4** were selected as

(1) Isomo, K.; Asahi, K.; Suzuki, S. *J. Am. Chem. Soc.* **1969**, *91*, 7490.
(2) (a) Isono, K.; Negatsu, J.; Kawashima, Y.; Suzuki, S. *Agric. Biol. Chem.* **1965**, *29*, 848. (b) Isomo, K.; Nagatsu, J.; Kobinata, K.; Sasaki, K.; Suzuki, S. *Agric. Biol. Chem.* **1967**, *31*, 190.
(3) Gooday, G. W., *Abh. Dtsch. Akad. Wiss.* DDR, *Abt. Math. Naturwiss., Tech.*, Issue 2N, **1979**, p 159.
(4) Becker, J. M.; Covert, N. L.; Shenbagamurthi, P. S.; Steinfeld, A.; Naider, F. *Antimicrob. Agents Chemother.* **1983**, *23*, 926.
(5) Mehta, R. J.; Kingsbury, W. D.; Valenta, J.; Actor, P. *Antimicrob. Agents Chemother.* **1984**, *25*, 373.
(6) Bowers, B.; Levin, G.; Cabib, E. *J. Bacteriol.* **1974**, *199*, 564.
(7) Shenbaganurthi, P.; Smith, H. A.; Becker, J. M.; Steinfeld, A.; Naider, F. *J. Med. Chem.* **1983**, *26*, 1518.

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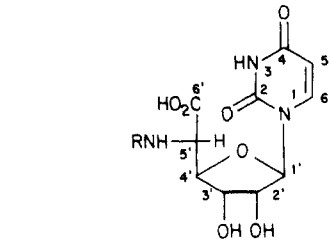
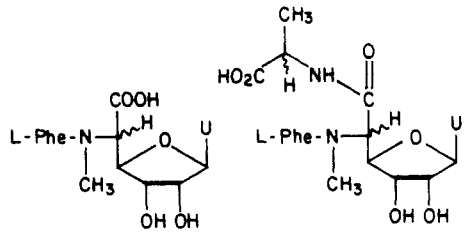
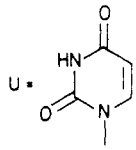


2
2a, R=H
2b, R=CH3



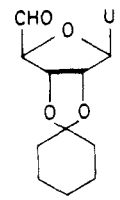
3a = LD
b = LL

4



target structures since it has been demonstrated previously that peptides in which the peptide bond nitrogen is methylated are resistant to enzymatic hydrolysis and sometimes have similar or increased affinity for target enzymes compared to the natural substrates.[8] Also, N-methylated peptides are known to be substrates for bacterial peptide transport[9] and since examples exist of an overlap between the ability of the bacterial and fungal peptide transport systems to carry peptides containing unnatural amino acids,[10] it was reasonable to expect that the N-methylated polyoxin peptides would be transported by the fungal peptide transport system.

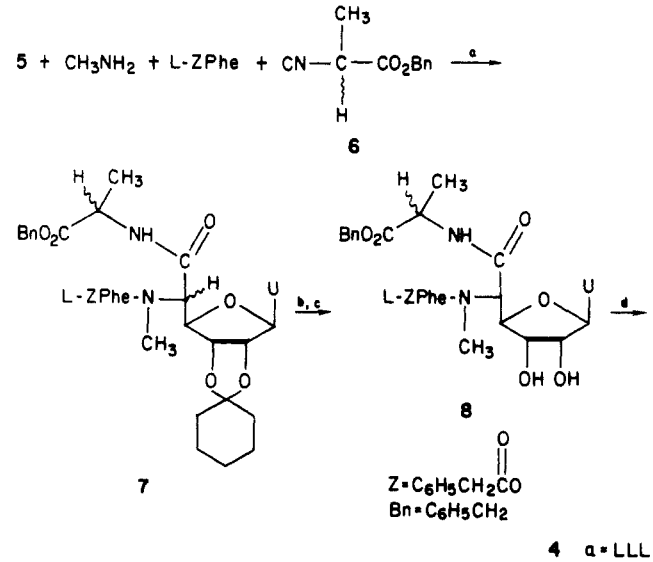
Unmethylated analogues of **3** and **4** have been synthesized by acylation of **2a**[4,7,11] obtained either by isolation and degradation of natural polyoxins,[1,11] or via Moffatt's five-step synthesis from the uridine aldehyde **5**.[12] After



5

the completion of our work, a series of methylated polyoxin peptides similar to **3** were reported.[13] These compounds

Scheme I[a]



[a] Conditions: (a) CH_3OH/H_2O; (b) $HOAc/H_2O$, Δ; (c) diastereomer separation by RP-18 HPLC; (d) Pd black, HCO_2H, CH_3OH.

were synthesized in four steps from synthetic **2a** by conversion to the *N*-methylpolyoxin **2b** in three steps followed by acylation. Some of the limitations associated with this approach include the following: the relatively large number of steps (nine) required to synthesize the N-methylated dipeptides from **5**; the necessity for a tedious separation of a diastereomeric mixture of intermediates via fractional crystallization;[12] the relative unreactivity of the methylated polyoxin C (**2b**) to standard acylation conditions; and the overall low yield.[13]

In light of reports that described the synthesis of N-alkylated peptides using the Ugi four component condensation (4CC) reaction,[14,15] as well as a reported synthesis of a protected polyoxin C analogue by this procedure,[16] it occurred to us that the entire backbone for the target peptides might be constructed in one step via the 4CC reaction. This convergent approach would have the additional advantages of eliminating the need for the difficult preparation and acylation of *N*-methylpolyoxin C. Alternatively, the N-methylated polyoxin dipeptides were expected to be accessible in six steps via a Strecker reaction[17] on the uridine aldehyde **5**, followed by nitrile hydrolysis, peptide bond formation, and deblocking.

We report here applications of both of these approaches to short syntheses of **3** and **4**. Since the approaches used are nondiastereoselective with respect to the 5′ carbon in **3** and **4** and the alanyl α carbon in **4**, mixtures of diastereomers were obtained which were separated by silica gel or reverse phase chromatography.

Results and Discussion

The diastereomeric tripeptides **4** were prepared via the 4CC reaction and subsequent deblocking (Scheme I).

(8) Spatola, A. P. Peptide Backbone Modifications: A Structure-Activity Analysis of Peptides Containing Amide Bond Surrogates. In "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins"; Weinstein, B., Ed.; Marcel Dekker: New York, 1983, Vol. 7, pp 267–357.

(9) Payne, J. W. Transport and Utilization of Peptides by Bacteria. In "Microorganisms and Nitrogen Sources"; Payne, J. W., Ed.; J. Wiley and Sons Ltd.: New York, 1980; pp 211–256.

(10) (a) Kingsbury, W. D.; Boehm, J. C.; Perry, D.; Gilvarg, C. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 4573–4576. (b) McCarthy, P. J.; Kingsbury, W. D.; Boehm, J. C.; Nisbet, L. J. *J. Bacteriol.* **1985**, *162*, 1024. (c) Kingsbury, W. D.; Boehm, J. C.; Mehta, J. J.; Grappel, S. F.; Gilvarg, C. *J. Med. Chem.* **1984**, *27*, 1447–1451.

(11) (a) Isono, K.; Suzuki, S.; Azuma, T. *Agric. Biol. Chem.* **1971**, *35*, 1986–1989. (b) Azuma, T.; Saita, T.; Isono, K. *Chem. Pharm. Bull.* **1977**, *25*, 1720–1748.

(12) Damodaran, N. P.; Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1971**, *93*, 3812–3813.

(13) Emmer, G.; Ryder, N. S.; Grassberger, M. A. *J. Med. Chem.* **1985**, *28*, 278–281.

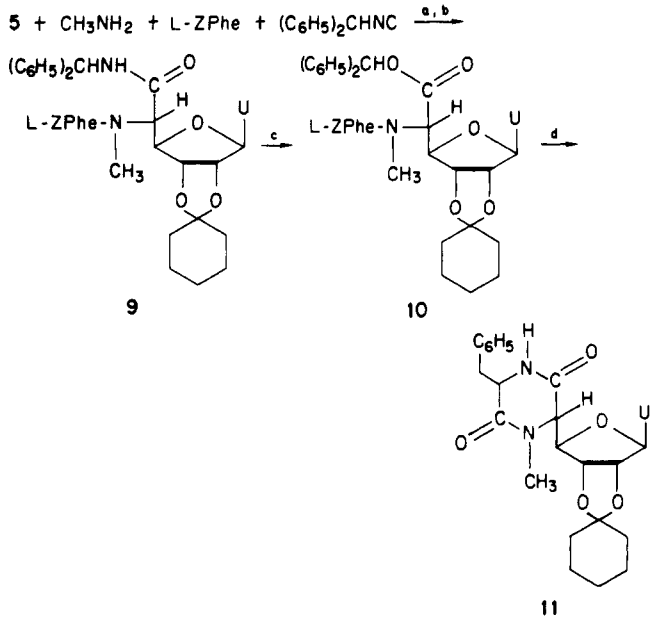
(14) (a) Ugi, I. "The Peptides"; Gross, E., Meinhofer, J., Eds.; Academic Press, New York, 1980; Vol. 2, Chapter 4, 365–381. (b) Ugi, I. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 810–819.

(15) De Laszlo, S. E.; Williard, P. G. *J. Am. Chem. Soc.* **1985**, *107*, 199–203.

(16) Tsuchida, K.; Mizuno, Y.; Ikeda, K. *Nucleic Acid Res.* **1980**, s49–s52.

(17) March, J. "Advanced Organic Chemistry", 3rd ed.; Wiley: New York, 1985; 855–856, and references therein.

Scheme II[a]

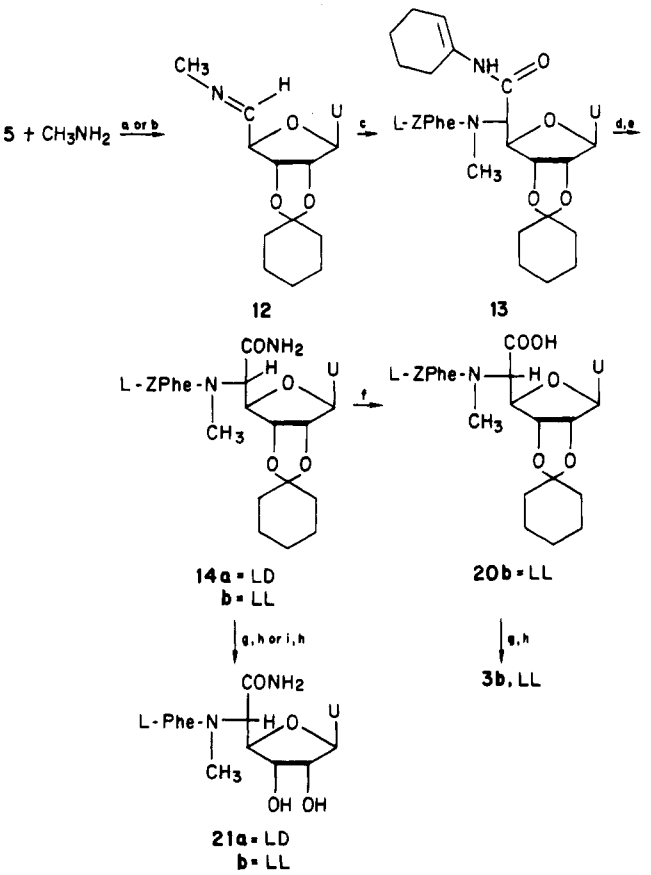


[a] Conditions: (a) CH_3OH/H_2O; (b) diastereomer separation by silica gel MPLC; (c) N_2O_4, NaOAc, CH_2Cl_2, N_2; (d) Pd black, HCO_2H, CH_3OH.

Thus, reaction of 1 equiv of aldehyde **5**,[12] aqueous methylamine, racemic alanine benzyl ester isonitrile **6**,[18-21] and L-carbobenzyloxyphenylalanine (L-ZPhe) produced **7** in 45% yield after chromatography. The presence of the four expected diastereomers was indicated by both ^1H and ^{13}C NMR. The cyclohexylidene group was then removed in 50% yield by refluxing 50% aqueous acetic acid, and the deblocked material was purified by silica gel chromatography. The resulting product, **8**, was analytically pure and was homogeneous by silica gel TLC. However, reverse phase (C18) analytical HPLC showed three peaks which were separated by reverse phase preparative HPLC. The first two of these peaks contained single diastereomers while the third to elute was an equal mixture of the remaining two diastereomers (^1H and ^{13}C NMR).

The components in the separated peaks were deblocked by using transfer hydrogenolysis with palladium black and formic acid[23] to give the diastereomeric tripeptides **4**. Stereochemistry was assigned to only one component in this series based on the relative biological activity of this isomer. Of the two pure diastereomers and the mixture of the remaining two diastereomers of **4** obtained from deblocking the separated peaks of **8** only one of the pure diasteromers was found to bind to chitin synthase. Since it has been shown that chitin synthase binds only L, di-, and tripeptides,[11a] the LLL stereochemistry[24] has been assigned to this isomer (**4a**).

Scheme III[a]



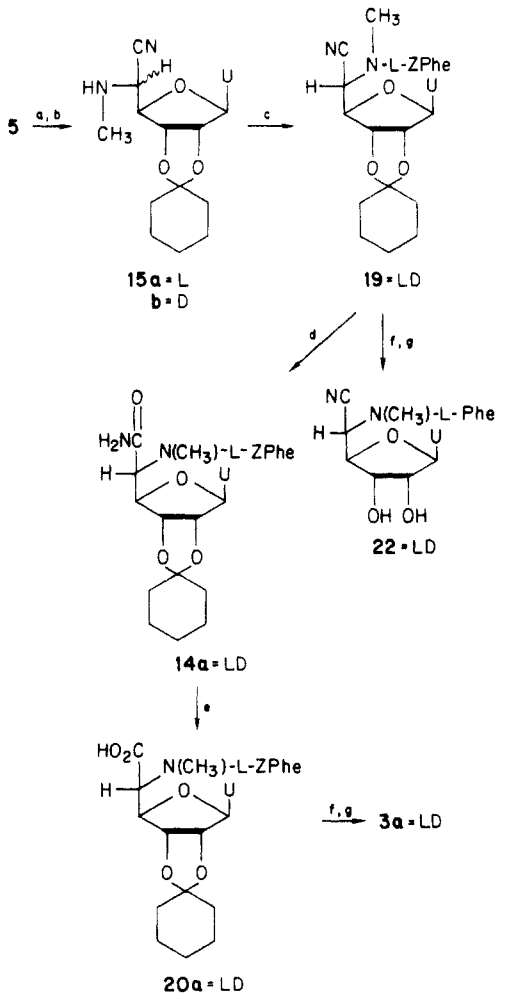
[a] Conditions: (a) THF, 3A sieves; (b) CH_3OH; (c) 1-cyclohexenyl isonitrile, L-ZPhe, CH_3OH; (d) $HOAc/H_2O$, 25 °C; (e) diastereomer separation by silica gel HPLC; (f) $NO^+HSO_4^-$, H_2O/CH_2Cl_2; (g) $HOAc/H_2O$, Δ; (h) Pd black, HCO_2H, CH_3OH; (i) dioxane, H_2O, AG50WX8(H^+), Δ.

Our initial approach to the dipeptide **3** was to use a route which included the 4CC reaction as a key step and was an adaptation of that reported by Isenring and Hofheinz for the synthesis of β-lactams (Scheme II).[25] A low yield of the mixture of diastereomers **9** was produced by the 4CC reaction of benzhydryl isonitrile, L-ZPhe, 40% aqueous methylamine, and aldehyde **5**. Following separation of the C5′-epimers by silica gel MPLC, one of the diastereomers was converted to the benzhydryl ester **10** via the *N*-nitroso amide.[25] Attempts to prepare polyoxin derivative **3** by hydrogenolytic deblocking of **10** resulted in the formation of diketopiperazine **11**. None of the deprotected dipeptide was detected.

Tsuchida and co-workers used 1-cyclohexenyl isonitrile to prepare a blocked polyoxin diamide using the 4CC reaction.[16] This approach, originally demonstrated by Ugi as a way to form acylated α-amino acid amides,[26] was applied to the synthesis of **3** as depicted in Scheme III. With the aim of obtaining an improvement in the 4CC reaction yield compared to the yields obtained in the syntheses of **7** and **9**, two different sets of conditions were tried. In the first case, imine **12** was isolated after reacting

(18) Meyer, R.; Schollkopf, U.; Madawinata, K.; Staffortst, D. *Justus Liebigs Ann. Chem.* **1978**, *12*, 1982–1989.
(19) The isonitrile could be conveniently purified by flash chromatography (1% CH_3OH/CH_2Cl_2) instead of distillation.
(20) The preparation of the alanine benzyl ester isonitrile[18] gives the racemic product. The α-carbon of α-carbalkoxy isonitriles is known to be acidic and is readily racemized both in preparation and during the 4CC reaction.[21] It should be noted, however, that at least one example is reported of the use of an optically active α-carbalkoxy isonitrile in a 4CC reaction under mild conditions giving a peptide with retention of configuration.[22]
(21) (a) Immer, H.; Nelson, W.; Robinson, W.; Groty, M. *Justus Liebigs Ann. Chem.* **1973**, 1789–1795. (b) Maia, H. L.; Ridge, B.; Ryndon, H. N. *J. Chem. Soc., Perkin Trans.* **1973**, *1*, 98–105. (c) Faili, A.; Nelson, V.; Immer, H.; Gotz, M. *Can. J. Chem.* **1973**, *51*, 2769–2775.
(22) Urban, R.; Eberle, G.; Marguarding, D.; Rehn, D.; Rehn, H.; Ugi, I. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 627–628.
(23) El Amin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442–3444.
(24) If one views polyoxin C as an α-amino acid then the natural configuration of the 5′ carbon (2a) corresponds to that of an L-α-amino acid. For the purpose of discussion in terms of binding to the fungal peptide permeases the 5′ configuration of polyoxin C is designated as either D or L.
(25) Isenring, H. P.; Hofheinz, W. *Synthesis* **1981**, 385.
(26) Ugi, I.; Rosendahl, F. K. *Liebigs Ann. Chem.* **1963**, *666*, 65–67.

Scheme IV[a]



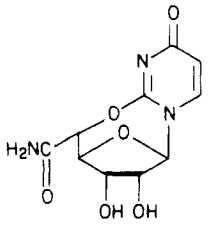
[a] Conditions: (a) CH_3NH_2·HCl, NaCN, H_2O/CH_3OH; (b) diastereomer separation by silica gel MPLC; (c) L-ZPhe–pivalic acid mixed anhydride, CH_2Cl_2, Ar, −20 °C, 18 h; (d) NaOH, H_2O_2, *n*-$Bu_4N^+HSO_4^-$, CH_2Cl_2/H_2O; (e) $NO^+HSO_4^-$, H_2O/CH_2Cl_2; (f) $HOAc/H_2O$, Δ; (g) Pd black, HCO_2H, CH_3OH.

5 with excess anhydrous methylamine. The isolated imine was immediately reacted with the isonitrile and L-ZPhe. Alternatively, 1 equiv of methylamine was prereacted with **5** in absolute methanol followed by the addition of the isonitrile and L-ZPhe. Neither of these conditions gave any improvement in yield compared to the use of aqueous methylamine, as previously described. The crude intermediate cyclohexenyl amide **13** was hydrolyzed to the primary amide **14** with 50% aqueous acetic acid at 25 °C. Following an initial flash chromatographic purification, the pure diastereomers were separated by preparative HPLC on silica gel. These isomers correspond to the diastereomeric L-Phe-D-*N*-methyl-UPOC-amide[27] (**14a**, the first to elute) and L-Phe-L-*N*-methyl-UPOC-amide (**14b**, the second to elute), respectively. The stereochemical assignments for these compounds are discussed below.

Initial attempts to convert either **14a** or **14b** to the corresponding carboxylic acids were unsuccessful due to the instability of these molecules to the usual strongly acidic or basic conditions required for amide hydrolysis. To continue our studies on the hydrolysis of these amides and since difficulties were encountered in the large scale chromatography of the complex mixtures obtained from the 4CC reaction, an alternative method for obtaining large quantities of **14a** and **14b** was sought via the Strecker reaction (Scheme IV).

Aldehyde **5** was condensed with methylamine hydrochloride and sodium cyanide to give a diastereomeric mixture of the amino nitriles **15a,b**, which were separated on silica gel by using either MPLC or, on a large scale, with a JY preparative chromatography system (see Experimental Section). The second isomer to elute (**15b**) was assigned the D configuration on the basis of its unambiguous conversion to a single dipeptide diastereomer **3a** for which the relative configuration is assigned as described at the end of this discussion section.

Various conditions were explored for hydrolyzing the nitrile function of **15a** and **15b**. Treatment of **15a** with refluxing 50% acetic acid gave the cyclized compound **16**



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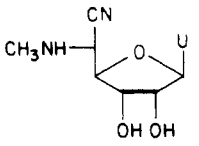
resulting from displacement of *N*-methylamine from the C5′ carbon by the oxygen of the uracil 2-carbonyl and concomitant nitrile hydrolysis to the amide. A recently reported method of hydrolyzing nitriles using titanium tetrachloride in wet acetic acid[28] failed to hydrolyze the nitrile function but did remove the cyclohexenyl blocking group to give **17**. Basic hydrogen peroxide under a variety



17

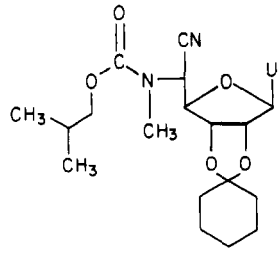
of conditions led to decomposition which included loss of uracil and possibly *N*-oxide formation.

To avoid the possibility of *N*-oxide formation during treatment with mildly basic hydrogen peroxide, investigation of methods for acylation of the *N*-methylamino group of **15a** and **15b** was undertaken. Amino nitrile **15a** was subjected to standard acylation conditions, first with a benzyloxycarbonyl-protected phenylalanine *p*-nitrophenyl ester[29] and then with L-ZPhe and DCC.[30] In both cases starting material was recovered. Although activation of L-ZPhe with isobutyl chloroformate[31] gave acylation of **15a** when allowed to proceed for an unusually long time, the resulting products were a 1:1 mixture of the desired dipeptide containing racemized phenylalanine and the (isobutyloxy)carbonyl carbamate **18**. When the procedure was carried out at lower temperatures or for shorter times, incomplete conversion to product was observed.

Pivalic acid mixed anhydrides have been extensively utilized by Wenger for acylation of unreactive N-methylated amino acids and peptides.[32] However when this procedure was applied to **15a** no reaction was observed. Surprisingly, when the reaction was attempted on

(27) **2b** = L-*N*-methyl-UPOC; the diastereomer with the reversed configuration of C5′ is abbreviated as D-*N*-methyl-UPOC.

(28) Mukaiyama, T.; Kamio, K.; Kohayashi, S.; Takei, H. *Chem. Lett.* **1973**, 357–358.
(29) Konig, W.; Geiger, R. *Chem. Ber.* **1973**, *106*, 3626–3635.
(30) Konig, W.; Geiger, R., *Chem. Ber.* **1970**, *103*, 788–798.
(31) Manger, A. B.; Stuart, O. A.; Highet, R. J.; Silverton, J. V. *J. Am. Chem. Soc.* **1982**, *104*, 174–180.
(32) Wenger, R. M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 77–85.



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the other amino nitrile diastereomer, **15b**, a rapid conversion to the desired acylated product **19** in 85% yield was observed. Although we are uncertain about the differences in reactivity of these amino nitrile diastereomers, we presume that steric factors are responsible.

As anticipated, hydrolysis of the acylated amino nitrile **19** was effected in fair yield with alkaline hydrogen peroxide using phase transfer conditions.[33] This supports the suggestion that the apparent decomposition occurring when the reaction was run on amino nitriles **15a** or **15b** was the result of *N*-oxide formation. The amide obtained was identical with the amide **14a** which eluted first from preparative HPLC of the 4CC reaction (Scheme III).

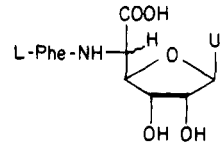
After trying a variety of conditions, it was found that amide **14a** could be conveniently hydrolyzed to acid **20a** by treatment with commercially available nitrosyl sulfuric acid.[34] Similar conditions had previously been reported to be the method of choice for hydrolyzing sterically hindered amides.[35]

Deblocking of acid **20a** gave a single polyoxin dipeptide **3a**, in 48% yield (Scheme IV). Since **15a** could not be acylated, dipeptide **3a** (from **15b**) was the only dipeptide polyoxin available via the Strecker reaction.

The amide **14b**, which could only be obtained by the 4CC reaction, was hydrolyzed and deblocked by using the methods described above for **14a** to give the second target dipeptide **3b** (Scheme III). Thus, amide hydrolysis of **14b** (the second 4CC reaction product to elute from silica gel) with nitrosyl sulfuric acid followed by deblocking via acetic acid hydrolysis and catalytic hydrogen transfer hydrogenolysis gave the new acid **3b** in 20% overall yield.

In addition to acids **3a** and **3b** the corresponding amides and nitriles were also of interest for biological testing. Amide **14b** was deblocked by refluxing aqueous acetic acid followed by hydrogenolysis to give polyoxin amide **21b** in low yield (Scheme III). The yield for the conversion of **14a** to **21a** was improved to 54% by carrying out the hydrolysis step with a sulfonic acid ion exchange resin in refluxing dioxane/water. Nitrile **19** was deblocked with refluxing acetic acid and hydrogenolysis to give polyoxin nitrile **22** (Scheme IV).

Assignment of the absolute configurations of **3a** and **3b** was based on their ability to bind to chitin synthase. The binding of L-Phe-L-UPOC (**23a**) was two orders of magnitude greater than that of L-Phe-D-UPOC (**23b**);[36] binding of the N-methylated dipeptide **3b**, although it was 20 times less than that of **23a**, was more than 10 times greater than **3a**. Therefore, we conclude that **3b** is the LL isomer. It follows that **14b**, **20b**, and **21b** are also LL isomers, and **3a**,



23a = LL
b = LD

14a, **20a**, **21a**, and **22** have the LD configuration. The amino nitriles **15a** and **15b** are L and D, respectively. Consistent with these assignments, the poor reactivity of **15a** toward acylation parallels that reported for L-*N*-methyl-UPOC (**2b**).[13]

In summary, the N-methylated di- and tripeptide polyoxins **3** and **4** were conveniently synthesized via the 4CC reaction; the tripeptides being prepared in three steps and the dipeptides in five steps from readily available starting materials. As in the previous synthesis of this class of molecules[12,13] mixtures of diastereomers were obtained. However, preparative HPLC methods were developed that separate the diastereomeric products more easily and rapidly than the earlier fractional crystallization procedure.[12]

The Strecker reaction was employed to prepare N-methylated polyoxin dipeptides in six steps. This latter approach appears to be somewhat cleaner and more amenable to scale-up than the 4CC reaction but suffers from the limitation that one of the diastereomeric amino nitriles, **15a**, is resistant to acylation. Mild conditions for the hydrolysis of the acylated polyoxin nitrile and amide were also described.

Several of the deblocked compounds exhibited modest inhibition of chitin synthase activity similar to the results reported by Grassberger and co-workers.[13]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 137 spectrophotometer; ^1H and ^{13}C NMR spectra were obtained on a JEOL FX-90 Q instrument; all values are reported in ppm (δ) downfield from $(CH_3)_4Si$. Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter and along with elemental analyses and mass spectra were performed in the Analytical, Physical, and Structural Chemistry Department of Smith Kline & French Laboratories.

Analytical TLC systems were the following: A, Merck silica gel 60 F-254 glass backed plates, 95:5 CH_2Cl_2/CH_3OH, or B, Whatman KC 18F reversed phase glass backed plates, 1:1 CH_3OH/5% NaCl in H_2O. Analytical HPLC was carried out with either a 4.6 × 250 mm Whatman Partisil 10 ODS 3 (10 μm) column or a 4.6 × 250 nm Rainin silica gel (8 μm) column with detection in each case at 254 nm. J. T. Baker, "Baker" silica gel (average particle size 40 μm) was used for flash, MPLC, and large scale silica gel chromatography. MPLC chromatography was in Altex glass columns packed with either "Baker" flash silica gel and pumped at ca. 10 mL/min or Whatman Partisil 40 ODS-3 and pumped at ca. 5 mL/min. The pressure was <80 psi.

Silica gel preparative HPLC was on a 21.4 × 500 mm Rainin Dynamax column at 20 mL/min, 800 psi. Reverse phase HPLC was on a 22 × 500 mm Whatman Magnum 20 Partisil 10 ODS 3 column, 15 mL/min, 800 psi.

Large scale (>20 g) silica gel chromatography was on a Jobin Yvon Chromatospec Prep 100 (JY) chromatography system[37] packed with ca. 1500 g of "Baker" flash silica gel and pumped at ca. 100 mL/min.

Palladium black was conveniently prepared as follows:[38] A 1.25-g portion of $PdCl_2$ was dissolved in 8 mL of concentrated HCl at ca. 90 °C, 4 mL of 98% formic acid was added, the solution was stirred for 1 min, and then 25% NaOH was added carefully

(33) Cacchi, S.; Misita, O.; LaTorre, F. *Synthesis* **1980**, 243–244.
(34) (a) Wade, L. G., Jr.; Sivey, W. B. *Org. Prep. Proced. Int.* **1982**, *14*, 357–359. (b) Nefkens, G. N. L.; Nivard, R. J. F. *Recl. Trav. Chim. Pays-Bas*, **1965**, *84*, 1315–1318.
(35) (a) Sperber, N.; Papa, D.; Schwenk, E. *J. Am. Chem. Soc.* **1948**, *70*, 3091. (b) Sarel, S.; Newman, M. S. *Ibid.* **1956**, *78*, 5416. (c) Ladenheim, H.; Bender, M. L. *Ibid.* **1960**, *82*, 1895.
(36) Dipeptides **24a** and **24b** were prepared by *p*-nitrophenyl activated ester acylation of synthetic L-**2a** and D-**2a**[11] by methods described previously.[7,11b]
(37) Krasek, F. W. *Res./Dev.* **1977**, *28*(7), 32–36.
(38) Naider, F.; Shenbagamurthi, P., personal communication.

until the mixture was basic. The resulting Pd sponge was washed with distilled H_2O until neutral and then was washed with CH_3OH for use in organic solvents.

1-[5′-(L-Carbobenzyloxyphenylalanylmethylamino)-5′-deoxy-2′,3′-cyclohexylidene-β-D-*allo*(α-L-*talo*)-furanuronosyl-DL-alanine benzyl ester]uracil (7). Aldehyde **5** (1.61 g, 5 mmol) and aqueous CH_3NH_2 (0.39 g, 5 mmol) were combined in 5 mL of CH_3OH at 0 °C and stirred for 5 min. Isocyanide 6[18-20] (0.95 g, 5 mmol) was added, stirring was continued for 5 min, L-ZPhe (1.50 g, 5 mmol) in CH_3OH (5 mL) was added, the ice bath was removed, and the reaction was stirred for 26 h. The solvent was evaporated, and the residue was dissolved in EtOAc (200 mL) and washed with 5% aqueous $NaHCO_3$ (50 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated. The crude product was chromatographed on a 25 mm × 500 mm MPLC column (silica gel, 1% CH_3OH in CH_2Cl_2). Fractions containing a mixture of the desired diastereomers of **7** in different ratios and varying state of purity (^1H, ^{13}C NMR) were obtained. The total yield was 1.87 g (45%).

The purest of the fractions (TLC system A) had the following analytical data: IR (CH_2Cl_2) 3385, 3040, 2950, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) × 1.9–1.2 (m, 13, cyclohexylidene, $CHCH_3$), 3.0 (m, 5, $CHCH_2Ph$, NCH_3), 4.2 (m, 1, $CHCH_3$), 4.95–4.4 (m, 4, H2′, 4′, 5′, $CHCH_2Ph$), 5.2–5.0 (m, 4, OCH_2Ph), 5.5 (m, 2, H1′, 3′), 5.95 (d, 1, J = 7 Hz, H5), 7.4–7.0 (m, 15, Ar), 7.5 (d, 1, J = 7 Hz, H6); ^{13}C NMR δ ($CDCl_3$) 17.2, 17.3, 17.7 (CH_3), 23.4, 23.8, 24.8 (CH_2), 31.9, 32.0 (CH_3), 34.4 (CH_2), 36.9 (CH_2), 37.5 (CH_2), 47.9 (CH), 52.5 (CH), 53.1 (CH_2), 57.0 (CH), 66.5, 66.8 (CH_2), 81.5 (CH), 83.2 (CH), 84.8 (CH), 102.4 (CH), 114.0, 115.2 (quaternary C), 126.7–129.3 (CH), 135.2, 136.1, 136.2, 136.5 (quaternary C), 143.3 (CH), 150.0, 150.2 (CO), 156.0, 156.2 (CO), 163.7, 164.0 (CO), 167.4, 167.6, 167.9 (CO), 171.9, 172.0 (CO), 173.4, 173.8 (CO); FAB mass spectrum, m/e MH^+ 824 (MH^+), 822 $(M - H)^-$. Anal. Calcd for $C_{44}H_{49}N_5O_{11}·1.5H_2O$ (850.92): C, 61.11; H, 6.15; N, 8.23. Found: C, 61.73; H, 5.97; N, 8.89.

1-[5′-(L-Carbobenzyloxyphenylalanylmethylamino)-5′-deoxy-β-D-*allo*(α-L-*talo*)-furanuronosyl-DL-alanine benzyl ester]uracil (8). A solution of **7** (780 mg 0.95 mmol) in 50% HOAc (80 mL) was refluxed for 1.5 h, poured into H_2O (200 mL), and extracted with EtOAc (3 × 150 mL). The combined extracts were washed with 5% aqueous $NaHCO_3$ (50 × 100 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated to give 0.565 g of crude **8** which was eluted from a silica gel MPLC column (25 mm × 250 mm column, stepwise gradient of 0.5 L of CH_2Cl_2, 2 L of 2% CH_3OH in CH_2Cl_2, and 1 L of 3% CH_3OH in CH_2Cl_2) to elute 0.352 g (50%) of a single spot product (TLC system A). Reverse phase HPLC (65% CH_3OH/H_2O) indicated a mixture of three components. Anal. Calcd for $C_{38}H_{41}N_5O_{11}$ (743.76): C, 61.37; H, 5.56; N, 9.42. Found: C, 61.06; H, 5.61; N. 9.15.

Preparative reverse phase HPLC (65% CH_3OH/H_2O) in four successive runs of 25 mg and 3 × 100 mg gave three purified pools.

The first to elute, **8a**, a noncrystalline solid, weighed 93 mg: ^1H NMR ($CDCl_3$) δ 1.35 (d, 3, J = 7 Hz, $CHCH_3$), 2.97 (m, 5, HCH_3, $CHCH_2Ph$), 4.8–4.2 (m, 5, 2′, 4′, 5′, $CHCH_3$, $CHCH_2Ph$), 5.3 (s, 2, OCH_2Ph), 5.0 (s, 2, OCH_2Ph), 5.8–6.4 (m, 3, H5, 3′, 1′), 7.5–6.7 (m, 19, H6, Ar, 3 NH); ^{13}C NMR ($CDCl_3$) δ 17.6 (CH_3), 32.5 (CH_3), 37.8 (CH_2), 48.2 (CH), 53.0 (CH), 57.8 (CH), 67.0 (CH_2), 71.0 (CH), 73.6 (CH), 81.6 (CH), 93.6 (CH), 102.3 (CH), 127.0, 127.5, 128.0, 128.4, 129.2 (CH), 135.1, 135.8, 136.0 (quaternary C), 150.8 (CO), 157.3 (CO), 164.5 (CO), 167.1 (CO), 172.8 (CO), 174.2 (CO).

The second isomer to elute, **8b**, a noncrystalline solid, weighed 102 mg: ^1H NMR ($CDCl_2$) δ 1.35 (d, 3, J = 7 Hz, $CHCH_3$), 3.0 (m, 5, NCH_3, $CHCH_2Ph$), 4.8–4.1 (m, 5, H2′, 4′, 5′, $CHCH_3$, $CHCH_2Ph$, 5.0 (s, 2, OCH_2Ph), 5.1 (s, 2, OCH_2Ph), 5.9–5.4 (m, 3, H5, 1′, 3′), 8.0–6.5 (m, 19, H5, Ar, 3 NH); ^{13}C NMR α ($CDCl_3$) 17.4 (CH_3), 32.7 (CH_3), 37.8 (CH_2), 48.3 (CH), 53.1 (CH), 57.8 (CH), 67.0 (CH), 71.0 (CH), 73.6 (CH), 81.4 (CH), 102.5 (CH), 127–130 (CH), 135.2, 136.0 (quaternary C), 143.0 (CH), 150.8 (CO), 157.3 (CO), 164.6 (CO), 167.1 (CO), 172.8 (CO), 174.3 (CO).

The third peak to elute, **8c,d**, weighed 117 mg. NMR analysis indicated it was a mixture of diastereomers: ^1H NMR ($CDCl_3$) δ 1.4 (m, 3, $CHCH_3$), 3.1–2.4 (m, 5, NCH_3, $CHCH_2Ph$), 4.9–4.1 (m, 5, H1′, 4′, 5′, $CHCH_3$, $CHCH_2Ph$), 5.0 (s, 2, OCH_2Ph), 5.6–5.3 (m, 2, H2′, 3′), 5.1 (s, 2, OCH_2Ph), 5.9–5.7 (m, 1, H5), 6.4–6.1 (m, 3, 3 × NH), 7.7–7.0 (m, 16, H6, Ar); ^{13}C ($CDCl_3$) δ 16.8, 17.0 (CH_3), 31.5 (CH_3), 37.0, 37.2 (CH_2), 48.3 (CH), 52.6 (CH), 58.9 (CH), 67.0 (CH_2), 79.8 (CH), 127.4–129.2 (CH), 135.1, 135.3, 135.9, 136.1 (quaternary C), 140.5 (CH), 150.6 (CO), 156.8 (CO), 163.9 (CO), 168.6, 168.9 (CO), 171.9 (CO), 174.1, 174.3 (CO).

1-[5′-(L-Phenylalanylmethylamino)-5′-deoxy-β-D-*allo*-furanuronosyl-L-alanine]uracil (4a). The first isomer of **8** which eluted, **8a** (93 mg, 0.125 mmol), HCO_2H (0.4 mL), and 0.1 g of Pd black in CH_3OH (4 mL) was stirred for 1 h. The mixture was filtered, the filtrate was concentrated, and the residue was dissolved in H_2O, refiltered, and lyophilized to give 59 mg of **4a**.

Chromatography on a 25 × 250 mm reverse phase MPLC column, 15% aqueous CH_3OH, followed by lyophilization gave 45 mg (69%) of purified **4**: $[\alpha]^{25}_D$ (1, H_2O) −14.9°; IR (KBr) 1600–2300, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (d, 3, J = 7 Hz, $CHCH_3$), 2.92 (s, 3, NCH_3), 4.85–4.0 (m, 5, H2′, 4′, 5′, $CHCH_3$, $CHCH_2Ph$), 5.19 (d, 1, J = 8 Hz, H3′), 5.73 (d, 1, J = 5 Hz, H1′), 5.81 (d, 1, J = 8 Hz, H5), 7.40–7.15 (m, 5, phenyl), 7.60 (d, 1, J = 8 Hz, H6); ^{13}C ($CDCl_3$) δ 19.0 (CH_3), 34.1 (CH_3), 37.4 (CH_2), 52.4 (CH), 53.2 (CH), 60.0 (CH), 72.2 (CH), 73.5 (CH), 81.3 (CH), 92.7 (CH), 103.7 (CH), 129.4 (CH), 130.4 (CH), 130.8 (CH), 134.5 (quaternary C), 144.4 (CH), 152.5 (CO), 167.3 (CO), 168, 5 (CO), 171.5 (CO), 180.3 (CO); FAB mass spectrum, m/e +ve, MH^+ = 520, −ve, $(M - H)^-$ = 518. Anal. Calcd for $C_{23}H_{29}N_5O_9·H_2O·HCO_2H$ (583.55): C, 49.40; H, 5.70; N, 12.00. Found: C, 49.07; H, 5.59; N, 12.36.

2′,3′-*O*-Cyclohexylideneuridine-5′-methylimine (12). Aldehyde **5** (1.9 g, 5 mmol) was mixed with dry THF (20 mL) and 3A sieves, and 1 mL of anhydrous methylamine was condensed into the mixture at −70 °C. The resulting mixture was warmed to room temperature and stirred for 2 h, filtered, and concentrated to give 1.3 g (78%) of **12** as a white foam.

FAB mass spectrum, m/e 336 (MH^+), 334 (M − H); IR (CH_2Cl_2) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.0–2.0 (m, 10, cyclohexylidene) 3.27 (s, 3, NCH_3), 4.51 (m, 1, H4′), 4.96 (m, 2, H3′, H2′) 5.47 (d, J = 7 Hz, H5), 5.64 (s, 1, H1′), 6.90 (1, J = 7 Hz, H6) 7.66 (br s, 1, (imine H); ^{13}C NMR ($CDCl_3$) δ 23.7, 24.8 34.1, 36.6 (CH_2), 30.2 (CH_3), 82.4, 84.2, 87,7, 94.9 (ribosyl CH), 102.7 (C5), 113.5 (quaternary C), 140.4 (C6), 157.6 (C2), 163.8 ($CH{=}NCH_3$), 174.8 (C4).

1-[5′-(L-Carbobenzyloxyphenylalanylmethylamino)-5′-deoxy-2′,3′-*O*-cyclohexylidene-β-D-*allo*(α-L-*talo*)-furanosyluronic acid amide]uracil (14a,b). Method A. Aldehyde **5** (1.13 g 3.5 mmol) in dry THF (12 mL) was combined at −70 °C with 3A sieves, and excess CH_3NH_2 (ca. 2 mL) was condensed into the reaction. The cooling bath and condenser were removed, and the mixture was stirred under argon at room temperature for 2 h. IR of an aliquot indicated the complete conversion to the imine, and the reaction was concentrated. To the solid obtained were added CH_3OH (25 mL), 1-cyclohexenyl isonitrile[26] (0.38 g, 3.5 mmol), and L-ZPhe (1.07 g, 3.5 mmol), and the resulting mixture was stirred at 25 °C for 18 h. The reaction was concentrated, dissolved in HOAc (22 mL) and H_2O (10 mL), stirred for 5 h, concentrated, dissolved in EtOAc (300 mL), washed with H_2O (2 × 50 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated. The crude product was flash chromatographed (2.54-cm × 25.4-cm column, eluted with 0.5 L of 1.5% and 0.5 L of 3% CH_3OH in CH_2Cl_2) to give three pools containing each of the diastereomers (TLC system A; R_f 0.4 and 0.45) plus a minor impurity which is only detectable by HPLC and elutes between these peaks. Total yield = 0.58 g (25%) of **14a,b**.

Method B. Aldehyde **5** (5.0 g, 15.5 mmol) in CH_3OH (50 mL) was combined with CH_3NH_2 (7.1 mL of 2.2 N CH_3NH_2, 15.6 mmol), and the solution was stirred at room temperature for 30 min. 1-Cyclohexenyl isonitrile[26] (1.66 g, 15.5 mmol) and L-ZPhe (4.65 g, 15.5 mmol) were added. The mixture was stirred for 36 h at room temperature and then concentrated. Hydrolysis and flash chromatography similar to that described in method A gave pools containing varying ratios of the two major products plus some impurites. Total yield = 2.31 g (23%) of **14a,b**.

Separation of 14a and 14b. A mixture of **14a** and **14b** obtained from flash chromatography and which was enriched in the slower moving isomer was run through the preparative silica HPLC column in five equal portions (ca. 71 mg/injection) (3% CH_3OH in CH_2Cl_2, 20 mL/min).

Th first isomer to elute, **14a**, a noncrystalline solid, weighed 64 mg; $[\alpha]^{25}_D$ (0.42, MeOH) +36°; IR (CH_2Cl_2) 3450, 3290, 1725, 1695, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–2.9 (m, 10, cyclohexylidene), 2.7 (m, 2, CHC*H*Ph), 3.29 (s, 3, CH_3), 4.6–5.1 (m, 4, H2′, H4′, H5′, C*H*CH_2Ph), 5.29 (br s, 1 H3′), 5.58 (br s, 1, H1′), 5.63 (d, J = 8 Hz, H5), 6.80 (d, J = 8 Hz, H6); ^{13}C NMR ($CDCl_3$) δ 23.7 (CH_2), 24.0 (CH_2), 25.0 (CH_2), 30.7 (CH_3), 82.7 (CH), 83.9 (CH), 86.6 (CH), 97.2 (CH), 103.6 (CH), 114.3 (quaternary), 127.0–129.2 (Ar CH), 136.4, 136.5 (Ar quaternary), 143.7 (CH), 151.2 (NCONH), 156.3 ($OCONH_2$), 164.4 (HNCOCH=), 169.5 (CONR), 175.6 (CONR). Anal. Calcd for $C_{34}H_{39}N_5O_9 \cdot 1^1/_2H_2O$ (688.73): C, 59.29; H, 6.15; N, 10.17. Found: C, 59.55; H, 5.80; N, 9.77.

The second isomer to elute, **14b**, a noncrystalline solid, weighed 154 mg; $[\alpha]^{25}_D$ (0.89, MeOH) −56.2°; IR (CH_2Cl_2) 3400, 3040, 2940, 1715, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–1.8 (m, 10, cyclohexyl), 2.90 (s, 3, NCH_3), 3.00 (m, 2, CHC*H*$_2$Ph), 4.49 (m, 1, C*H*CH_2Ph) 4.90 (m, 4, H2′, 3′, 4′, 5′), 5.04 (s, 2, OC*H*$_2$Ph), 5.41 (d, 1, J = 7 Hz, C*H*CH_2Ph), 5.62 (s, 1, H1′), 5.67 (d, 1, J = 5 Hz, H5), 7.1–7.4 (m, 11, Ph, H6); ^{13}C NMR ($CDCl_3$) δ 23.4 (CH_2), 23.8 (CH_2), 24.7 (CH_2), 32.2 (CH_3), 34.5 (CH_2), 36.9 (CH_2), 28.8 (CH_2), 52.2 (CH), 57.2 (CH), 66.5 (CH_2), 81.4 (CH), 82.8 (CH), 84.4 (CH), 94.8 (CH), 102.5 (CH), 115.4 (quaternary), 127.0–129.3 (Ar CH), 135.9 (quaternary C), 136.3 (quaternary), 143.0 (CH), 150.2 (NCONH), 155.8 (OCONH), 163.8 (HCOCH=C), 170.2 (RNCO), 173.4 (RNCO). Anal. Calcd for $C_{34}N_{39}N_5O_9 \cdot 1^1/_2H_2O$ (688.73): C, 59.29; H, 6.15; N, 10.17. Found: C, 59.41; H, 5.79; N, 10.02.

1-[5′-(L-Carbobenzyloxyphenylalanylmethylamino)-5′-deoxy-2′,3′-cyclohexylidene-β-D-*allo*-furanosyluronic acid]uracil (20b). To a mixture of **14b** (235 mg, 0.36 mmol) in CH_2Cl_2 (5 mL) and H_2O (4 mL) was added nitrosylsulfuric acid (ca. 2 g) in 5 portions over a period of 30 min at a rate which maintained the mixture green. The mixture was diluted with CH_2Cl_2 (100 mL), and the lower (concentrated H_2SO_4) phase was separated. The extract was washed with H_2O (10 mL) and 10 mL of saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. The residue was triturated with 10 mL of Et_2O to form a solid, and then hexane (50 mL) was added. The sample was filtered and dried to give 120 mg (51%) of **20b**: mp 134–135 °C dec; FAB mass spectrum, m/e 663 (MH^+); IR 3450–2300, 1720, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–1.9 (m, 10, cyclohexenyl), 2.95 (m, 2, CHC*H*$_2$Ph), 3.05 (s, 3, NCH_3), 4.32 (m, 1, C*H*CH_2Ph), 4.8–5.2 (m, 6, H2′, 3′, 4′, 5′, OC*H*$_2$Ph), 5.65 (s, 1, J = 2 Hz, H1′), 5.69 (d, 1, J = 6 Hz, H5), 7.0–7.5 (m, 11, (Ar, H6); ^{13}C NMR ($CD_3OD/CDCl_3$) δ 23.2 (CH_2), 38.2 (CH_2), 52.4 (CH), 58.6 (CH), 66.5 (CH_2), 81.5 (CH), 82.4 (CH), 84.6 (CH), 91.4 (CH), 102.6 (CH), 115.6 (quaternary), 126.6–129.2 (Ar CH), 135.6 (quaternary), 136.1 (quaternary), 141.3 (CH), 150.1 (NCON), 155.8 (OCONH), 164.0 (HNCOCH=CH), 170.0 ((CH_3NCO), 172.7 (CO_2H).

1-[5′-(L-Phenylalanylmethylamino)-5′-deoxy-β-D-*allo*-furanosyluronic acid]uracil (3b). A solution of **20b** (0.107 g, 0.16 mmol) and 50% aqueous HOAc (5 mL) was refluxed for 3 h and concentrated to dryness and the residue was triturated with ether and filtered to give 65 mg (70%) of the sugar deblocked, amino protected polyoxin. FAB mass spectrum (m/e) 583 (MH^+).

The above product (55 mg, 0.095 mmol) was combined with Pd black (freshly made from 0.3 g of $PdCl_2$), CH_3OH (25 mL), and HCO_2H (1 mL), stirred at room temperature for 30 min, filtered through Celite, concentrated, and lyophilized. The lyophilizate was run through a 15 × 250 mm reverse phase MPLC column with 300 mL of H_2O and then 15% CH_3OH in H_2O until product eluted. Concentration and lyophilization gave 22.3 mg (54%) (38% from **20b**) of **3b**: $[\alpha]^{25}_D$ (0.27, H_2O) +3.7°; IR (CH_2Cl_2) 3300–2200, 1695, 1650 cm^{-1}; ^1H NMR (D_2O) δ 2.8–3.5 (m, 5, NCH_3, CHC*H*$_2$Ph, 4.1–4.4 (m, 2, CHC*H*$_2$Ph, H5′), 4.5–4.8 (m, 2, H4′, H2′), 5.0 (m, 1, H3′), 7.79 (s, 1, H1′), 5.83 (d, J = 8 Hz, H5), 7.33 (s, 5, Ph), 7.66 nd, J = 8 Hz, H6); ^{13}C NMR ($CDCl_3$) δ 34.4 (CH_3), 36.9 (CH_2), 53.4 (CH), 72.2 (CH), 73.9 (CH), 83.1 (CH), 91.4 (CH), 103.7 (CH), 129.3 (CH), 130.4 (CH), 130.9 (CH), 134.6 (quaternary), 143.4 (CH), 170.8 (CO). Anal. Calcd for $C_{20}H_{24}N_4O_8 \cdot HCO_2H \cdot {}^3/_4H_2O$ (507.952): C, 49.66; H, 5.46; N, 1103. Found: C, 49.35; H, 5.41; N, 11.11.

1-[5′-(L-Phenylalanylmethylamino)-5′-deoxy-α-L-*talo*-furanosyluronic acid amide]uracil (21a). A mixture of **14a** (100 mg, 0.15 mmol) dioxane (4.5 mL), H_2O (9 mL), and 1 g of AG50WX8 (H^+) was refluxed for 16 h. The mixture was filtered, washed with THF and concentrated, and the residue was flash chromatographed (2.54 cm × 7.62 cm silica gel with the sample preabsorbed onto a 2.54 cm × 2.54 cm plug of silica gel and added to the top of the column, 5% CH_3OH in CH_2Cl_2) to give 58 mg (77%) of sugar deblocked, amino protected polyoxin amide: FAB mass spectrum, m/e 582 (MH^+).

The above product (21 mg, 0.036 mmol) and CH_3OH (20 mL), formic acid (1 mL), and Pd black (freshly prepared from 0.3 g of $PdCl_2$) were stirred for 30 min, filtered, concentrated, dissolved in H_2O, and lyophilized. The lyophilizate was chromatographed (RP-18 MPLC, 15 × 250 mm column, 30% CH_3OH in 0.02% TFA) and lyophilized to give 22 mg (70%) of **21a** (54% from **14a**): $[\alpha]^{25}_D$ (0.34, H_2O) +36.5°; IR (KBr) 3650–2400, 1710, 1675 cm^{-1}; ^1H NMR (D_2O) δ 2.86 (s, 3, CH_3), 2.9 (m, 2, CHC*H*$_2$Ph), 3.95–4.35 (m, 3, H4′, 5′, C*H*CH_2Ph), 4.55 (m, 1, H2′), 5.08 (d, 1, J = 9 Hz, H3′), 5.63 (d, 1, J = 6 Hz, H1′), 5.72 (d, 1, J = 8 Hz, H5), 6.9–7.2 (m, 5, Ph), 7.31 (d, 1, J = 8 Hz, H6); ^{13}C NMR (D_2O) δ 33.2 (CH_3), 37.2 (CH_2), 53.2 (CH), 59.9 (CH), 72.3 (CH), 73.8 (CH), 80.9 (CH), 90.5 (CH), 104.0 (CH), 129.3 (CH), 130.3 (CH), 130.8 (CH), 134.5 (quaternary C), 143.4 (CH), 152.7 (NCONH), 167.0 (NHCOC=), 171.9 (CONR), 172.5 (CONR). Anal. Calcd for $C_{20}H_{25}N_5O_7 \cdot 3^1/_4CF_3CO_2H \cdot 3H_2O$ (872.070): C, 36.50; H, 3.95; N, 8.03. Found: C, 36.19; H, 3.55; N, 8.54.

1-[5′-(L-Phenylalanylmethylamino)-5′-β-D-*allo*-furanosyluronic acid amide]uracil (21b). A solution of **14b** (0.20 g, 0.30 mmol) in 50% HOAc (10 mL) was refluxed for 2 h, concentrated, dissolved in EtOAc (25 mL), washed with H_2O (3 × 50 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (2.54 cm × 10.16 cm silica gel, 5% CH_3OH in CH_2Cl_2) gave 31 mg (18%) of sugar deblocked, amino protected product.

A 27-mg (0.46 mmol) portion of the above product was deblocked and chromatographed as described for **21a** to give 10 mg (37%) of **21b**: $[\alpha]^{25}_D$ (0.35, H_2O) +2.6°; IR (KBr) 3700–2500, 1685 cm^{-1}; ^1H NMR (D_2O) δ 2.65 (s, 3, CH_3), 3.03 (d, 2, J = 7 Hz, CHC*H*$_2$Ph), 3.9–4.8 (m, 4, H5′, H4′, H2′, C*H*CH_2Ph), 4.95 (d, 1, J = 9 Hz, H3′), 5.55 (d, 1, J = 5 Hz, H1′), 5.66 (d, 1, J = 8 Hz, H5), 6.9–7.3 (m, 5, Ph), 7.43 (d, 1, J = 8 Hz, H6); ^{13}C NMR (D_2O) δ 33.9 (CH_3), 37.7 (CH_2), 53.1 (CH), 59.8 (CH), 72.2 (CH), 73.4 (CH), 80.9 (CH), 93.0 (CH), 103.7 (CH), 129.8 (CH), 130.5 (CH), 130.8 (CH), 134.5 (quaternary), 144.2 (CH), 152.5 (NCONH), 167.3 (NHCOC=), 171.6 (CONR), 171.9 (CONR). Anal. Calcd for $C_{20}H_{25}N_5O_7 \cdot 2H_2O \cdot 2^1/_2CF_3CO_2H$ (768.737): C, 39.07; H, 4.13; N, 9.11. Found: C, 39.01% H, 3.74; N, 9.34.

1-[5′-(Methylamino)-5′-deoxy-5′-cyano-2′,3-cyclohexylidene-β-D-*allo*(α-L-*talo*)-furanosyl]uracil (15a,b). To a solution of 4.75 g (97 mmol) of NaCN, 9.89 g (145 mmol) of $CH_3NH_2 \cdot HCl$ and 195 mL of H_2O was added a hot solution of 31.2 g (97 mmol) of **5** in 195 mL of CH_3OH (heating was necessary to dissolve **5**), and the mixture was stirred for 16 h. The CH_3OH was evaporated, and aqueous phase was extracted with 5 × 150 mL of EtOAc, and the extracts were washed with 50 mL of saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to give 34.9 g of crude **15a,b**.

This sample was combined with material from a second run (91.6 mmol scale) to give a total weight of 69 g of crude product. Chromatography was conducted on the JY chromatography system with a stepwise gradient of 8 L of CH_2Cl_2, 8 L of 2% CH_3OH, 8 L of 2.5% CH_3OH, and 8 L of 3% CH_3OH in CH_2Cl_2.

The first cyano amine **15a**, eluted in 2% methanol to give as a noncrystalline solid 31.8 g (47%): $[\alpha]^{25}_D$ (0.99, $CHCl_3$) −9.7°; IR (CH_2Cl_2) 2360, 1740, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–2.0 (m, 10, cyclohexylidene), 2.57 (s, 3, NCH_3, 3.91 (d, 1, J = 7 Hz, H5′), 4.18 (dd, 1, J = 7 Hz, J = 3 Hz, H4′), 4.95 (m, 2, H3′, 2′), 5.66 (s, 1, H1′), 5.78 (d, 1, J = 8 Hz, H5), 7.35 (d, 1, J = 8 Hz, H6); ^{13}C NMR ($CDCl_3$) δ 23.2 (CH_2), 23.6 (CH_2), 33.7 (CH_3), 34.3 (CH_2), 36.6 (CH_2), 54.4 (CH), 81.8 (CH), 83.2 (CH), 86.4 (CH), 95.5 (CH), 102.8 (CH), 115.3 (quaternary C), 118.1 (CH), 143.0 (CH), 150.2 (NCONH), 163.6 (NHCO). Anal. Calcd for $C_{17}N_{22}N_4O_5 \cdot {}^3/_4H_2O$ (375.89): C, 54.32; H, 6.30; N, 14.90. Found: C, 54.17; H, 5.99; N, 14.54.

The second amino nitrile, **15b**, eluted in 2.5% methanol to give as a noncrystalline solid 25.3 g (37%): $[\alpha]^{25}_D$ (1.0, $CHCl_3$) +0.5°; IR (CH_2Cl_2) 3360, 2295, 1710, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–2.0 (m, 10, cyclohexylidene), 2.58 (s, 3, NCH_3), 4.00 (s, 1, J = 7 Hz, H5′), 4.26 (dd, 1, J = 7 Hz, J = 2 Hz, H4′), 5.07 (m, 2

H3′, 2′), 5.59 (s, 1, H1′), 5.71 (d, 1, J = 8 Hz, H5), 7.24 (d, J = 8 Hz, H6); ^{13}C NMR ($CDCl_3$) δ 23.4 (CH_2), 23.8 (CH_2), 24.8 (CH_2), 33.5 (CH_3), 34.5 (CH_2), 36.9 (CH_2), 53.7 (CH), 80.6 (CH), 83.0 (CH), 85.7 (CH), 95.7 (CH), 102.9 (CH), 115.9 (quaternary C), 117.3 (CH), 143.0 (CH), 150.1 (NCONH), 163.4 (NHCO). Anal. Calcd for $C_{17}H_{22}N_4O_5 \cdot 1H_2O$ (380.400): C, 53.68; H, 6.35; N, 14.73. Found: C, 63.65; H, 6.02; N, 14.21.

1-[5′-(L-Carbobenzyloxyphenylalanylmethylamino)-5′-deoxy-5′-cyano-2′,3′-cyclohexylidene-α-L-*talo*-furanosyl]uracil (19). A solution of L-ZPhe (20.93 g, 70 mmol) and CH_2Cl_2 (230 mL) was cooled to −20 °C under argon, then *N*-methylmorpholine (14.85 g, 147 mmol) and pivaloyl chloride (8.44 g, 70 mmol) were added, and the mixture was stirred at −20 °C for 2 h. IR indicated the formation of mixed anhydride. A solution of **15b** (25.3 g, 70 mmol) in CH_2Cl_2 (120 mL) was added dropwise with cooling at −20 °C. The resulting solution was kept at −20 °C under argon for 16 h, and then diluted with CH_2Cl_2 (0.5 L), washed with 0.5 N HCl (50 mL), H_2O (50 mL), 5% aqueous $NaHCO_3$ (2 × 50 mL), H_2O (50 mL), and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated. The crude product was chromatographed on the JY system (stepwise gradient of 6 L of CH_2Cl_2 and then 10 L of 1.5% CH_3OH in CH_2Cl_2). A total of 38.5 g (85%) of the noncrystalline solid **19** was obtained: $[\alpha]^{25}_D$ (1, MeOH) +39.8°; IR (CH_2Cl_2, 3420, 2300, 1720, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.1–2.9 (m, 10, cyclohexylidene), 2.5–3.2 (m, 5, NCH_3, $CHCH_2Ph$), 4.36 (dd, J = 9 Hz, J = 1 Hz, H4′), 4.5–5.2 (m, 4, H5′, 2′, 3′, $CHCH_2Ph$), 5.36 (s, 1, H1′), 5.65 (d, 1, J = 8 Hz, H5), 6.94 (d, 1, J = 8 Hz, H6), 6.9–7.4 (m, 10, Ph); ^{13}C NMR ($CDCl_3$) δ 23.4 (CH_2), 23.7 (CH_2), 24.7 (CH_2), 31.8 (CH_3), 33.9 (CH_2), 36.4 (CH_2), 38.6 (CH_2), 48.1 (CH), 52.4 (CH), 102.9 (CH), 114.8 (quaternary), 115.5 (CH), 126.8–129.2 (Ar CH), 135.8, 136.2 (quaternary), 143.4 (CH), 150.4 (NCONH), 155.7 (OCONH), 163.9 (NHCO), 173.4 ($CONCH_3$). Anal. Calcd for $C_{34}H_{37}N_5O_8 \cdot {}^1/_4H_2O$ (648.18): C, 63.00; H, 5.83; N, 10.80. Found: C, 63.01; H, 5.92; N, 10.45.

1-[5′-(L-Phenylalanylmethylamino)-5′-deoxy-5′-cyano-α-L-*talo*-furanosyl]uracil (22). A solution of **19** (0.238 g, 0.37 mmol) in 10 mL of 50% aqueous HOAc was refluxed for 3 h. The solvent was evaporated and the residue was dissolved in EtOAc (100 mL), washed with H_2O (2 × 50 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (1 in. × 5 in. column) eluted recovered **19** (43 mg) in 2% CH_3OH in CH_2Cl_2. The sugar deblocked, amino protected polyoxin eluted in 4% CH_3OH in CH_2Cl_2 to give **82** mg (48% based on recovered **19**): FAB mass spectrum, m/e 643 (MH^+).

A mixture of the above product (78 mg, 0.12 mmol), Pd black (freshly prepared from 200 mg of $PdCl_2$), 10 mL of CH_3OH, and 1 mL of HCO_2H was stirred for 30 min, filtered through celite, concentrated, dissolved in H_2O, and lyophilized.

The lyophilizate was run through a 15 mm × 250 mm reverse phase MPLC column, eluting with 15% CH_3OH in 0.02% TFA/H_2O to give 33 mg (64%) of **22** after lyophilization: $[\alpha]^{25}_D$ (0.19, H_2O) +55.8°; IR (KBr) 3600–2300, 2270, 1680 cm^{-1}; ^{1}H NMR (D_2O) δ 2.97 (s, 3, NCH_3), 3.13 (d, 2, J = 7 Hz, $CNCH_2Ph$), 4.34 (m, 3, H5′, 4′, $CHCH_2Ph$), 4.75 (m, 2, H2, 3′), 5.73 (s, 1, H1′), 5.82 (d, J = 8 Hz, H5), 7.26 (m, 5, Ph), 7.46 (d, 1, J = 8 Hz, H6); ^{13}C NMR (D_2O) δ 34.2 (CH_3), 37.2 (CH_2), 50.5 (CH), 53.0 (CH), 71.8 (CH), 73.7 (CH), 81.0 (CH), 92.5 (CH), 115.6 (CH), 129.3 (CH), 130.3 (CH), 130.9 (CH), 134.1 (CH), 143.8 (CH), 152.5 (NCONH), 167.2 (NHCO), 171.3 ($CONCH_3$). Anal. Calcd for $C_{20}H_{23}N_5O_6 \cdot CF_3CO_2H \cdot 1^3/_4H_2O$ (574.9821): C, 45.97; H, 4.82; N, 12.18. Found: C, 46.08; H, 4.62; N, 11.74.

1-[5′-(L-Carbobenzyloxyphenylalanylmethylamino)-5′-deoxy-2′,3′-*O*-cyclohexylidene-α-L-*talo*-furanosyluronic acid amide]uracil (14a). To **19** (10.5 g, 16.3 mmol) and CH_2Cl_2 (6 mL) at 5 °C was added 30% H_2O_2 (8 mL), 20% aqueous NaOH (6.4 mL), and tetra-*n*-butylammonium bisulfate (1.2 g, 3.5 mmol). The ice bath and removed, and the reaction exothermed to the point where the CH_2Cl_2 was refluxing. The mixture was stirred for 30 min and then poured into CH_2Cl_2 (500 mL), the aqueous phase was separated, and the organic phase was washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), concentrated, and run through a 25 mm × 500 mm MPLC column (2% CH_3OH in CH_2Cl_2) giving 4.9 g (46%) of **14a** which was chromatographically and spectroscopically identical with the first amide diastereomer **14a** to elute from the chromatography of the 4CC reaction: $[\alpha]^{25}_D$ (1, CH_3OH) +37.9°. Anal. Calcd for $C_{34}H_{39}N_5O_9 \cdot 1^1/_2H_2O$ (688.73): C, 59.29; H, 6.15; N, 10.17. Found: C, 59.14; H, 5.77; N, 9.65.

1-[5′-(L-Carbobenzyloxyphenylalanylmethylamino)-5′-deoxy-α-L-*talo*-furanosyluronic acid]uracil (20a). To **14a** (1.0 g, 1.51 mmol), CH_2Cl_2 (20 mL), and H_2O (5 mL) was added ca. 5 g of nitrosylsulfuric acid in 5 portions over 30 min. The reaction was diluted with CH_2Cl_2 (200 mL), and the lower phase (concentrated H_2SO_4) was separated. The CH_2Cl_2 was washed with H_2O (10 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated. The residue was dissolved in CH_2Cl_2 (4 mL), and crystals were obtained. Filtration and drying gave 0.39 g (39%) of **20a**. Recrystallization from 4:1 acetone/hexane gave an analytical sample: mp 160 °C; $[\alpha]^{25}_D$ (1, CH_3OH) +30.2°; IR (CH_2Cl_2) 3600–2200, 1725, 1695 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.2–2.0 (m, 10, cyclohexylidene), 2.9 (m, 2, $CHCH_2Ph$), 3.12 (s, 3, NCH_3), 4.57 (dd, 1, J = 7 Hz, J = 3 Hz, H4′), 4.7–5.4 (m, 4, H2′, 3′, 5′, $CHCH_2Ph$), 5.69 (br s, 1, H1′), 5.71 (d, J = 8 Hz, H5), 6.9–7.4 (m, 10, Ph), 7.56 (d, J = 8 Hz, H6); ^{13}C NMR (CD_3OD) β 24.7 (CH_2), 25.0 (CH_2), 26.1 (CH_2), 34.7 (CH_3), 35.7 (CH_2), 38.1 (CH_2), 39.4 (CH_2), 53.9 (CH), 60.7 (CH), 67.5 (CH_2), 83.8 (CH), 84.8 (CH), 86.2 (CH), 96.0 (CH), 103.1 (CH), 115.7 (quaternary C), 127.7, 128.6, 129.4, 130.4, 130.6 (CH), 138.3 (quaternary C), 145.4 (CH), 152.0 (NCONH), 157.8 (OCONH), 166.2 ($HNCOC{=}$), 171.7 (CO-het), 174.6 (CO-het). Anal. Calcd for $C_{34}H_{38}N_4O_{10} \cdot {}^1/_4H_2O$ (667.184): C, 62.21; H, 5.82; N, 8.40. Found: C, 61.18; H, 5.65; N, 8.31.

1-[5′-(L-Phenylalanylmethylamino)-5′-deoxy-α-L-*talo*-furanosyluronic acid]uracil (3a). A solution of **20a** (0.38 g, 0.57 mmol) in 50% aqueous HOAc (10 mL) was refluxed for 1 h and concentrated, and the residue was triturated with 30 mL of Et_2O and filtered to give 0.23 g (69%) of the amino protected, sugar deblocked product: FAB mass spectrum, m/e 583 (MH^+).

A mixture of 0.21 g (0.36 mmol) of the above product and 20 mL of CH_3OH, 1.95 mL of formic acid, and Pd black (freshly prepared from 0.4 g $PdCl_2$) were stirred for 30 min, filtered, concentrated, dissolved in H_2O, and lyophilized. The lyophilizate was chromatographed (RP-18 MPLC, 25 mm × 250 mm column, stepwise gradient of 300 mL of H_2O, 300 mL of 10% CH_3OH, and 600 mL of 20% CH_3OH) to give 0.122 g (69%) of **3a**, after lyophilization: $[\alpha]^{25}_D$ (0.52, H_2O) +43.7°; IR (KBr) 3650–2200, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.93 (s, 3, NCH_3), 3.1 (m, 2, $CHCH_2Ph$), 4.2–4.6 (m, 3, H4′, 5′, $CHCH_2Ph$), 4.7 (m, 1, H2′), 5.17 (d, J = 8 Hz, H3′), 5.80 (d, J = 4 Hz, H1′), 5.87 (d, J = 8 Hz, H5), 7.1–7.4 (m, 5, Ph), 7.42 (d, J = 8 Hz, H6); ^{13}C NMR (D_2O) δ 34.7 (CH_3), 37.4 (CH_2), 53.1 (CH), 6/96 (CH), 72.2 (CH), 73.3 (CH), 81.7 (CH), 90.2 (CH), 104.2 (CH), 129.4 (CH), 130.4 (CH), 130.9 (CH), 134.6 (quaternary), 143.4 (CH), 152.8 (NCONH), 167.0 ($NHCOC{=}$), 171.7 (CO-het), 172.1 (CD-het). Anal. Calcd for $C_{20}H_{24}N_4O_8 \cdot HCO_2H$ (494.45): C, 51.01; H, 5.30; N, 11.33. Found: C, 49.82; H, 5.42; N, 11.56.

Acknowledgment. We thank Dr. David A. Berges and Dr. George L. Dunn for helpful discussions. Thanks to Dalia R. Jakas for preparation of **23a** and to Larry D. Davis and Leon B. Hall for preparation of quantities of the immediate precursor to the cyclohexenyl isonitrile and the protected form of aldehyde **5**. Thanks to Edward McKillop and Sylvia Fagen for performing the chitin sythase binding studies.

Registry No. **3a**, 100682-75-5; **3b**, 100682-76-6; **4a**, 100682-77-7; **5**, 34311-30-3; **6**, 100762-01-4; **7** (isomer 1), 100682-78-8; **7** (isomer 2), 100762-02-5; **7** (isomer 3), 100762-03-6; **7** (isomer 4), 100762-04-7; **8** (isomer 1), 100682-79-9; **8** (isomer 2), 100762-05-8; **8** (isomer 3), 100682-80-2; **8** (isomer 4), 100762-06-9; **9** (isomer 1), 100682-81-3; **9** (isomer 2), 100700-47-8; **10**, 100682-82-4; **11**, 100682-83-5; **12**, 100682-84-6; **14a**, 100682-85-7; **14a** (sugar deblocked), 100682-99-3; **14b**, 100682-86-8; **14b** (sugar deblocked), 100683-00-9; **15a**, 100682-87-9; **15b**, 100682-88-0; **16**, 100700-62-7; **17**, 100682-89-1; **19**, 100682-90-4; **19** (sugar deblocked), 100683-01-0; **20a**, 100682-91-5; **20a** (sugar deblocked), 100683-02-1; **20b**, 100682-92-6; **20b** (sugar deblocked), 100682-98-2; **21a**, 100682-93-7; **21b**, 100682-94-8; **22**, 100682-95-9; **23a**, 100682-96-0; **23b**, 100682-97-1; L-ZPhe, 1161-13-3; CH_3NH_2, 74-89-5; $CH_3NH_2 \cdot HCl$, 593-51-1; 1-cyclohexenyl isonitrile, 1121-57-9; chitin synthase, 9030-18-6.