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Registry No. **3**, 92762-82-8; **4**, 94236-27-8; **5**, 94236-28-9; **6**, 94236-29-0; **7**, 94236-30-3; **8**, 94236-31-4; **9**, 94236-32-5; **10**, 94236-33-6; 11, 94236-34-7; **12**, 92762-84-0; **13**, 94236-35-8; **14**, 94236-36-9; **15**, 94236-37-0; **16**, 94236-38-1; 17, 92762-81-7; **18**, 94236-39-2; **19**, 94236-40-5; **20**, 94236-41-6; **21**, 94236-42-7; **22**, 94236-43-8; **23**, 94236-44-9; **24**, 94236-45-0; **25**, 94236-46-1; **26**, 92762-79-3; **27**, 94236-47-2; **28**, 94236-48-3; **29**, 94236-49-4; **30**, 94236-50-7; **31**, 94236-51-8; **32**, 94249-75-9; **33**, 94249-76-0; **34**, 88499-15-4; **35**, 32949-42-1; **36**, 94236-52-9; **37**, 94236-53-0; **38**, 94236-54-1; **39**, 94236-55-2; **40**, 94236-56-3; 41, 94236-57-4; **42**, 94236-58-5; **43**, 94236-59-6; 44, 94236-60-9; **45**, 94236-61-0; **46**, 94236-62-1; **47**, 94236-63-2; 48, 94236-64-3; **49**, 94236-65-4; **50**, 94236-66-5; **51**, 94236-67-6; **52**, 94236-68-7; **53**, 94236-69-8; **54**, 94236-70-1; **55**, 92762-83-9; **56**, 94236-71-2; **57**, 94236-72-3; **58**, 94236-73-4; **59**, 94236-74-5; BOC-Leu, 13139-15-6; Asp(Bzl)-NH_2·TFA, 92762-94-2; BOC-Trp-PNP, 15160-31-3; Trp-Leu-Asp(Bzl)-NH_2·TFA, 94236-76-7; BOC-β-Ala, 3303-84-2; gastrin, 9002-76-0.

Synthesis of New Polyoxin Derivatives and Their Activity against Chitin Synthase from *Candida albicans*

Gerhard Emmer, Neil S. Ryder, and Maximilian A. Grassberger*

Sandoz Forschungsinstitut, Brunnerstrasse 59, A-1235 Wien, Austria. *Received February 22, 1984*

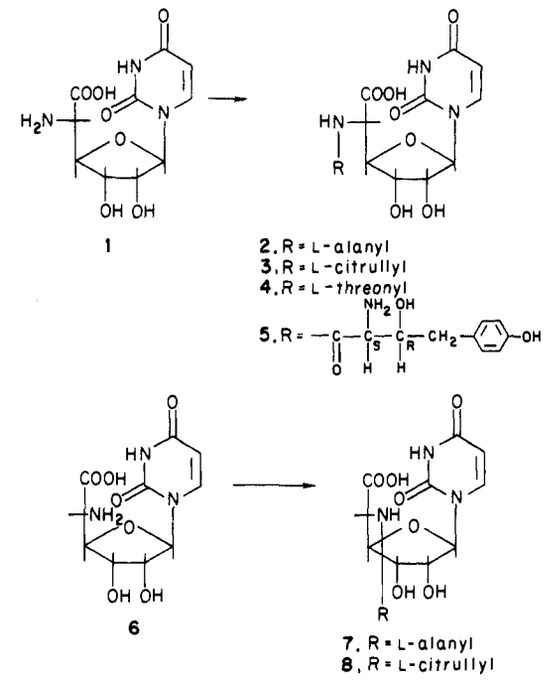
Two analogues of L-alanylpolyoxin C with a modified peptide bond were synthesized and tested for inhibition of chitin synthase in *Candida albicans*. N-Methylation of the peptide bond (compound **13**) or the replacement of it by NH_2CH_2 (compound **9**) led to loss of activity in the enzyme assay. A novel analogue (compound **5**) of nikkomycin was synthesized from uracil polyoxin C and (2*S*,3*R*)-3-hydroxyhomotyrosine, a component of echinocandin C. Despite high activity in the chitin synthase assay, **5** had no inhibitory effect on cells of *C. albicans*.

Polyoxins[1] and nikkomycins[2-4] (neopolyoxins[5-7]) are peptidyl nucleoside antibiotics with marked activity against phytopathogenic fungi. They inhibit the enzyme chitin synthase,[1,8,9] which catalyzes the final step in the biosynthesis of chitin, and bear a certain structural resemblance to uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), the natural substrate of this enzyme. By virtue of this specific mode of action against an essential fungal enzyme, they are potentially ideal agents for the treatment of human fungal diseases, in addition to their existing agricultural role.[1]

Although the chitin synthase of human pathogenic fungi, such as *Candida albicans*, is highly sensitive to polyoxins in cell-free systems (K_i values in the micromolar range[10]), the growth of intact cells is inhibited at only high concentrations (millimolar range) of the antibiotic.[11] No completely satisfactory explanation for this discrepancy has yet been advanced. The most plausible reasons include a failure to be transported into the cell, or inactivation inside the cell, for example by cleavage of the peptide bond. Recent investigations,[12] published since the completion of our studies, have shown that certain tripeptidyl polyoxins do undergo cleavage at the peptide bond when incubated with cell extracts of *Candida albicans*. Moreover, metabolic degradation by peptidases of the host must be taken into consideration as a possible drawback of application to man.

These considerations led us to prepare polyoxin analogues with a modified peptide bond and to study their biological activity. As target structures we chose a dipeptide which features a peptide bond stabilized by a

Scheme I



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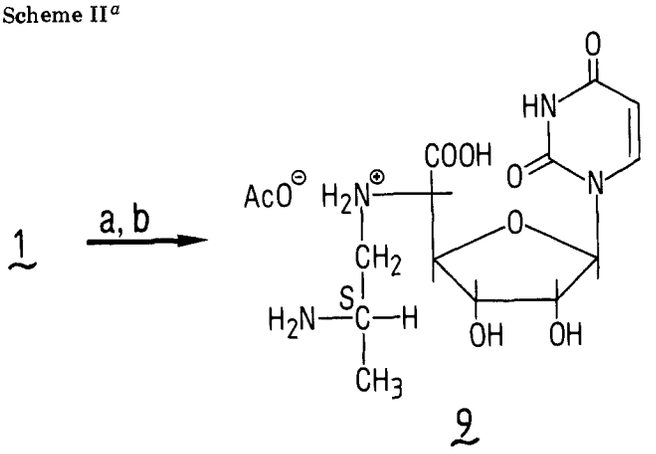
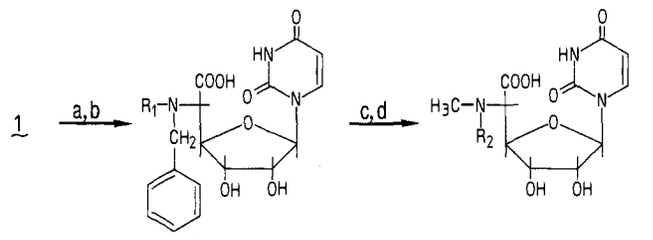
Scheme II[a]



[a] (a) Boc-L-ala-al, $NaBH_3CN$, CH_3OH. (b) Dowex 50 W (H^+).

Scheme III



[a] (a) PhCHO, $NaBH_4$. (b) HCHO, $NaBH_3CN$, CH_3OH. (c) H_2, Pd/C. (d) *N*-Cbz-L-Ala-2,4-DNP, Et_3N, DMF.

N-methyl group[13] and an analogue in which the -NHCO- linkage is replaced by $-NHCH_2-$. Nikkomycins, which have in contrast to polyoxins an aromatic hydroxyamino acid as side chain, generally show superior activity against pathogenic fungi. As this difference may be due to more effective cellular uptake of nikkomycins, we further prepared a polyoxin derivative with (2*S*,3*R*)-3-hydroxyhomotyrosine as a side chain. This amino acid is a component of echinocandin C and D, other peptide antibiotics with antifungal activity.[14,15]

Chemistry. Uracil polyoxin C, the basic nucleoside skeleton needed for the preparation of these new polyoxin derivatives, was synthesized in eight steps according to the procedure described by Moffatt et al.[16,17] This synthesis is nonstereospecific and gives a mixture of diastereomers which are separated at the fourth step by fractional crystallization. Following the literature, the synthesis was accomplished with both isomers leading to uracil polyoxin C (**1**) and its 5′ epimer **6**. For further structural proof, we prepared the L-alanyl and L-citrullyl derivatives of **1** (**2** and **3**) and of **6** (**7** and **8**) and tested their biological activities (compare biological results). Likewise **5** was obtained from **1** by reaction with pentachlorophenyl ester **14** (Scheme I). The active ester **14** was prepared from *N*,*O*-carbobenzoxy-protected (2*S*,3*R*)-3-hydroxyhomotyrosine[18] by reaction with pentachlorophenol and DCC.

Table I. Inhibition of Chitin Synthase by Polyoxin Derivatives (Mean of Triplicate Assays)

compd	percentage inhibition		
	5 μM	25 μM	100 μM
2	0	20	45
3	0	37	80
4	8	26	53
5	79	96	100
7	0	0	0
8	0	0	0
9	0	0	2
13	0	0	0

The aminoalkyl analogue **9** was prepared from **1** by reductive alkylation with *N*-Boc-L-alaninal/$NaBH_3CN$ in dry methanol (Scheme II), followed by deprotection with Dowex 50 W (H^+).

The *N*-methyl intermediate **12** was prepared from **1** in three steps (Scheme III). To avoid dimethylation, **1** was monobenzylated first with benzaldehyde/$NaBH_4$. Both reagents were consecutively applied several times in order to get complete consumption of the starting material. The benzyl derivative **10** was then treated with HCHO (methanolic solution)/$NaBH_3CN$ to give the *N*-benzyl-*N*-methyl derivative **11** in 82% yield. The benzyl group was removed by hydrogenation in the presence of Pd/$BaSO_4$ catalyst in H_2O at room temperature. The hydrogenolysis was monitored carefully by TLC, since prolonged reaction time led to concomitant reduction of the C_5–C_6 double bond. The last step, i.e., formation of the peptide bond, proved to be more difficult than with **1** or **6**, probably due to steric hindrance by the *N*-methyl group. For example, compound **12** showed no reaction with *N*-carbobenzoxyalanine pentachlorophenyl ester. The *N*-methyl dipeptide **13** could only be obtained with the more reactive *N*-carbobenzoxyalanine 2,4-dinitrophenyl ester.

Chitin Synthase Assay. *C. albicans* cells were grown in liquid shake culture in Sabouraud broth (Merck), pH 6.5. Cells were washed in 0.2 M Tris-HCl buffer, pH 7.5, and disrupted by shaking with glass beads in a Braun MSK homogenizer. Membrane preparations were made and chitin synthase assays performed as described previously.[19] The enzyme was activated with trypsin immediately before the assay.[19] Concentration of the substrate UDP-GlcNAc was 0.5 mM.

Biological Results and Discussion

Table I gives the activities of the polyoxin derivatives against chitin synthase from *Candida albicans*. L-Alanyl- and L-citrullylthymine polyoxin C are reported to be active against chitin synthase from the plant pathogenic fungus *Piricularia oryzae*.[20] In agreement with these results, compounds **2**, **3**, and **4** showed moderate activity in our test system. The L-alanyl derivative **2** could therefore serve as a standard reference for the biological evaluation of the analogues **9** and **13**. The 5′-epimeric compounds **7** and **8** proved to be completely inactive.

Modifications of the peptide bond led in our case to inactive derivatives; neither **9** or **13** showed any activity in the chitin synthase assay. The target enzyme thus appears to make very specific structural demands with respect to the peptide region of inhibitors of the polyoxin

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type. The nikkomycin analogue **5** showed a distinct activity in the chitin synthase assay, which unfortunately could not be reproduced in experiments with intact cells (MIC > 200 mg/L). In **13**, the loss of activity could conceivably be ascribed to a modified conformation at the peptide bond resulting from N-methylation.[21] The enhanced basicity of the nitrogen atom at C-5′ might contribute to a less favorable interaction of derivative **9** with the chitin synthase. A *N*-5′-acyl derivative of **9** could therefore be an interesting new target structure for future work in this field.

Experimental Section

Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter (concentration in g/100 mL). Thin-layer chromatography was performed on silica gel 60 F_{254} (Merck), on dodecylsilane-coated silica gel (reversed phase chromatography) OPTI-UP C_{12} (Antec AG), or on cellulose F (0.1 mm, Merck) plates. Unless stated otherwise, *n*-BuOH/CH_3COOH/H_2O (4:1:2) was used as the solvent system. The spots were visualized by spraying with a ninhydrin or a ceric sulfate (1%)/sulfuric acid (10%) solution. Column chromatography was conducted on silica gel 60 (0.040–0.063 mm, Merck) and LiChroprep RP 18 (0.040–0.063 mm, Merck), and pressures of 3–5 bars were applied. Cellulose chromatography was performed on Avicel (Merck) with use of *n*-BuOH/CH_3COOH/H_2O (4:1:2) as the solvent system. The purity of the products was checked by high-performance liquid chromatography (pump: Waters M 6000) on a column (250 × 4.6 mm) of LiChrosorb Diol 10 (Merck) with a pentanesulfonic acid (0.01 M) in water/acetonitrile gradient and a Schoeffel SF 770 UV detector (254 nm). ^1H NMR spectra were recorded in solutions at 60 MHz (Varian EM 360), 90 MHz (Bruker WH 90), or 250 MHz (Bruker WM 250) with $(CH_3)_4Si$ or sodium 3-(trimethylsilyl)propionate-d_4 as the internal standard. 1-(5′-Amino-5′-deoxy-β-D-allofuranosyluronic acid)uracil **1** and 1-(5′-amino-5′-deoxy-α-L-talofuranosyluronic acid)uracil **6** were prepared from uridine according to the methods of Moffatt et al.[16,17] The active esters used for the peptide coupling were prepared following literature procedures.[22-25]

Satisfactory elemental analyses for the described compounds could not be obtained. However, each compound was carefully checked by HPLC and found to be a single component.

General Procedure for the Preparation of 2–5, 7, and 8. To a solution of **1** (50 mg, 0.173 mmol) and Et_3N (0.024 mL) in a mixture of DMF (4 mL) and H_2O (4 mL) was added a solution of 1 equiv of 2,4,5-trichloro- or pentachlorophenyl ester of the N-protected amino acid in DMF (4 mL). After stirring for 2 days at room temperature, the reaction mixture was evaporated. The residue was dissolved in aqueous CH_3OH (50%) and passed through a column of Dowex 50 W (H^+). The effluent was lyophilized to yield a colorless foam. This was dissolved in aqueous CH_3OH (50%, 50 mL); a few drops of acetic acid and about 30 mg of Pd/$BaSO_4$ (5%) were added. The mixture was stirred under H_2 at atmospheric pressure until the catalyst turned black (about 1 h). The catalyst was filtered off and the filtrate was passed through a column of Dowex 50 W (H^+). After washing with aqueous methanol, the absorbed product was eluated with aqueous NH_3 (3%). Freeze-drying of the eluate gave a product which was purified by cellulose chromatography. The desired fractions were combined and evaporated to a residue, which was dissolved in water and lyophilized.

1-[5′-(L-Alanylamino)-5′-deoxy-β-D-allofuranosyluronic acid]uracil (2). According to the general procedure, reaction of **1** (45 mg, 0.156 mmol) with *N*-carbobenzoxy-L-alanine 2,4,5-trichlorophenyl ester (63 mg, 0.156 mmol) afforded **2** (26 mg, 46%) as a colorless foam, which was crystallized from C_2H_5OH/H_2O: mp 232–235 °C dec; homogeneous by HPLC; UV_{max} (H_2O) 260 nm (ε 9845 based on the calculated M_r of 358.30); ^1H NMR (D_2O) δ 7.72 (d, J = 8 Hz, H-C_6), 5.95 (d, J = 8 Hz, H-C_5), 5.90 (d, J = 4.5 Hz, H-$C_{1'}$), 3.94 (q, J = 7 Hz, H-$C_{2''}$), 1.47 (d, J = 7 Hz, CH_3).

1-[5′-(L-Citrullylamino)-5′-deoxy-β-D-allofuranosyluronic acid]uracil (3). According to the general procedure, **1** (50 mg, 0.173 mmol) was treated with *N*-carbobenzoxy-L-citrulline 2,4,5-trichlorophenyl ester (85 mg, 0.173 mmol), leading to **3** (25 mg, 32%, colorless foam, homogeneous by HPLC), which was crystallized from C_2H_5OH/H_2O: mp 225–230 °C dec; $[\alpha]^{22}_D$ 35.3° (*c* 0.66, H_2O); UV_{max} (H_2O) 258 nm (ε 9771 based on the calculated M_r of 444.39); ^1H NMR (D_2O) δ 7.72 (d, J = 8 Hz, H-C_6), 5.93 (d, J = 8 Hz, H-C_5), 5.88 (d, J = 4.5 Hz, H-$C_{1'}$), 3.82 (t, J = 5.5 Hz, H-$C_{2''}$), 3.13 (t, J = 6.5 Hz, 2H-$C_{5''}$).

1-[5′-(L-Threonylamino)-5′-deoxy-β-D-allofuranosyluronic acid]uracil (4). This was obtained in 41% yield (28 mg, colorless foam, homogeneous by HPLC) according to the general procedure by reacting **1** (50 mg, 0.173 mmol), with *N*-carbobenzoxy-L-threonine pentachlorophenyl ester (87 mg, 0.173 mmol): mp 230–234 °C dec (crystallized from C_2H_5OH/H_2O); UV_{max} (H_2O) 258.5 nm (ε 9680 based on the calculated M_r of 388.32); ^1H NMR (D_2O) δ 7.71 (d, J = 8 Hz, H-C_6), 5.92 (d, J = 8 Hz, H-C_5), 5.88 (d, J = 4.5 Hz, H-$C_{1'}$), 3.61 (d, J = 4.5 Hz, H-$C_{2''}$), 1.35 (d, J = 6.5 Hz, CH_3).

1-[5′-[[(2*S*,3*R*)-3-Hydroxyhomotyrosyl]amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil (5). Following the general procedure, a solution of **1** (14 mg, 0.049 mmol) in aqueous DMF (50%, 3 mL) was reacted with Et_3N (0.006 mL) and a solution of **14** (35 mg, 0.049 mmol) in DMF (2 mL) for 2 days. After workup and hydrogenation, **5** (16 mg, 68%, colorless foam, homogeneous by HPLC) was obtained: mp 238–242 °C dec (crystallized from C_2H_5OH/H_2O); UV_{max} (H_2O) 220.5 nm (ε 9056), 258.5 nm (9840) (ε values are based on the calculated M_r of 480.42); ^1H NMR (D_2O) δ 7.70 (d, J = 8 Hz, H-C_6), 6.80–7.30 (AA′BB′, 4 H), 5.88 (d, J = 8 Hz, H-C_5), 5.86 (d, J = 4.5 Hz, H-$C_{1'}$), 4.28 (d, J = 1.5 Hz, H-$C_{5'}$), 3.59 (d, J = 4.5 Hz, H-$C_{2''}$), 2.80 (m, 2H-$C_{4''}$).

1-[5′-(L-Alanylamino)-5′-deoxy-α-L-talofuranosyluronic acid]uracil (7). According to the general procedure, **6** (50 mg, 0.173 mmol) was reacted with *N*-carbobenzoxy-L-alanine 2,4,5-trichlorophenyl ester (70 mg, 0.173 mmol), leading to **7** (21 mg, 37%, colorless foam, homogeneous by HPLC): mp 235–237 °C dec (crystallized from C_2H_5OH/H_2O); UV_{max} (H_2O) 258.5 nm (ε 9250 based on the calculated M_r of 358.30); ^1H NMR (D_2O) δ 7.69 (d, J = 8 Hz, H-C_6), 5.90 (d, J = 8 Hz, H-C_5), 5.63 (d, J = 3.5 Hz, H-$C_{1'}$), 1.55 (d, J = 7 Hz, CH_3).

1-[5′-(L-Citrullylamino)-5′-deoxy-α-L-talofuranosyluronic acid]uracil (8). According to the general procedure, **6** (50 mg, 0.173 mmol) was reacted with *N*-carbobenzoxy-L-citrulline 2,4,5-trichlorophenyl ester (85 mg, 0.173 mmol), leading to **8** (28 mg, 36%, colorless foam, homogeneous by HPLC): mp 227–230 °C dec (crystallized from C_2H_5OH/H_2O); UV_{max} (H_2O) 259.5 nm (ε 8921 based on the calculated M_r of 444.39); $[\alpha]^{22}_D$ 5.5° (*c* 0.69, H_2O); ^1H NMR (D_2O) δ 7.69 (d, J = 8 Hz, H-C_6), 5.88 (d, J = 8 Hz, H-C_5), 5.67 (d, J = 4.5 Hz, H-$C_{1'}$), 3.10 (t, J = 6.5 Hz, H-$C_{5''}$), 1.40–2.00 (m, 4 H).

1-[5′-[[(*S*)-2-Aminopropyl]amino]-5′-deoxy-β-D-allofuranosyluronic acid]uracil Hydrogen Acetate (9). Compound **1** (21 mg, 0.073 mmol) and Boc-L-alaninal[26] (17 mg, 0.1 mmol) were dissolved in anhydrous CH_3OH (10 mL), and $NaBH_3CN$ (63 mg, 1 mmol) was added. After stirring for 16 h, the reaction was worked up by dropwise addition of 2 N HCl until a pH of 2–3 was reached. After 30 min of stirring, the solution was passed through a Dowex 50 W (H^+) column. The resin was washed with aqueous CH_3OH (50%) and afterwards the product was eluted with 3% NH_3 in aqueous CH_3OH (50%). The crude material obtained was purified by cellulose chromatography. The desired fractions were evaporated to give **9** (9 mg, 30%, slightly yellow powder, homogeneous by HPLC): mp 197–199 °C dec (crystallized from C_2H_5OH/H_2O); UV_{max} (H_2O) 259 nm (ε 9650

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based on the calculated M_r of 404.37); ^{1}H NMR (D_2O) δ 7.86 (d, J = 8 Hz, H-C_6), 5.94 (d, J = 8 Hz, H-C_5), 5.90 (d, J = 4.5 Hz, H-$C_{1'}$), 3.33 (d, J = 5.5 Hz, 2 H), 2.78 (m, 1 H), 1.93 (s, 3 H), 1.30 (d, J = 6.5 Hz, 3 H).

1-[5′-(Benzylamino)-5′-deoxy-β-D-allofuranosyluronic acid]uracil (10). To a solution of 1 (287 mg, 1 mmol) in aqueous NaOH (0.05 M, 20 mL) was added benzaldehyde (0.1 mL, 1 mmol) with stirring. When the mixture became homogeneous (after about 30 min), $NaBH_4$ (76 mg, 2 mmol) was added. To ensure nearly complete consumption of 1, monitored by thin-layer chromatography, the consecutive addition of benzaldehyde and $NaBH_4$ was repeated five times. When the reaction was finished, the solution was passed through a Dowex 50 W (H^+) column. After washing with aqueous CH_3OH (50%), the product was eluted from the resin with 3% NH_3 in aqueous CH_3OH (50%), and the desired fractions were lyophilized to give **10** (273 mg, 73%, colorless foam). An analytical sample was obtained after purification by cellulose chromatography: mp 174–175 °C (crystallized from C_2H_5OH/H_2O): $[\alpha]^{22}_D$ 29.0° (c 0.344, H_2O); ^{1}H NMR (D_2O) δ 7.68 (d, J = 8 Hz, H-C_6), 7.51 (s, 5 H), 5.91 (d, J = 8 Hz, H-C_5), 5.81 (d, J = 4.5 Hz, H-$C_{1'}$), 4.03 (d, J = 2.5 Hz, H-$C_{5'}$).

1-[5′-(Benzylmethylamino)-5′-deoxy-β-D-allofuranosyluronic acid]uracil (11). To a suspension of **10** (276 mg, 0.73 mmol) in CH_3OH (100 mL) was added a solution of HCHO in CH_3OH[27] (30%, 1.5 mL) followed by a tenfold excess of $NaBH_3CN$. The mixture became homogeneous, and after 20 min, the solution was passed through a Dowex 50 W (H^+) column. After washing of the resin with aqueous CH_3OH (50%), the product was eluted with 3% NH_3 in aqueous CH_3OH (50%). The desired fractions were lyophilized, and the crude material was purified by chromatography on cellulose to give 11 (236 mg, 82%, colorless foam): mp 170–180 °C (crystallized from C_2H_5OH/H_2O); $[\alpha]^{22}_D$ 40.6° (c 0.549, H_2O); UV_{max} (H_2O) 260 nm (ϵ 9880 based on the calculated M_r of 391.37); ^{1}H NMR (D_2O) δ 7.82 (d, J = 8 Hz, H-C_6), 7.56 (s, 5 H), 6.03 (d, J = 4.5 Hz, H-$C_{1'}$), 5.95 (d, J = 8 Hz, H-C_5), 4.16 (d, J = 1.5 Hz, H-$C_{5'}$), 2.92 (s, NCH_3).

1-[5′-(Methylamino)-5′-deoxy-β-D-allofuranosyluronic acid]uracil (12). A solution of 11 (236 mg, 0.6 mmol) in H_2O (100 mL) containing a few drops of acetic acid and 5% $Pd/BaSO_4$ (50 mg) was stirred vigorously under H_2 at atmospheric pressure until the catalyst turned black. After filtration and lyophilization of the solution, the residue obtained was purified by reversed phase chromatography (H_2O). **12** was isolated as a foam (110 mg, 60%, homogeneous by HPLC): mp 193–195 °C dec (crystallized from C_2H_5OH/H_2O); $[\alpha]^{22}_D$ 22.4° (c 0.596, H_2O); UV_{max} (H_2O) 258 nm (ϵ 9650 based on the calculated M_r of 301.25); ^{1}H NMR (D_2O) δ 7.69 (d, J = 8 Hz, H-C_6), 5.91 (d, J = 8 Hz, H-C_5), 5.90 (d, J = 4.5 Hz, H-$C_{1'}$), 5.60 (dd, J = 5.5 Hz, J' = 2.5 Hz, H-$C_{4'}$), 5.28 (dd, J = 4.5 Hz, J' = 5.5 Hz, H-$C_{2'}$), 3.98 (d, J = 2.5 Hz, H-$C_{5'}$), 2.80 (s, NCH_3).

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1-[5′-(L-Alanylmethylamino)-5′-deoxy-β-D-allofuranosyluronic acid]uracil (13). To a solution of **12** (49 mg, 0.125 mmol) in aqueous DMF (50%, 4 mL) was added Et_3N (0.03 mL) followed by a solution of *N*-carbobenzoxy-L-alanine 2,4-dinitrophenyl ester (82 mg, 0.21 mmol) in DMF (2 mL). After 2 days the reaction mixture was worked up as described in the general procedure. The obtained crude material was separated from 2,4-dinitrophenol by cellulose chromatography, affording 22 mg of the N-protected product. Hydrogenation and workup was performed according to the general procedure and **13** (7 mg, 12%) was isolated as a colorless foam: mp 208–212 °C dec (crystallized from C_2H_5OH/H_2O); homogeneous by HPLC; UV_{max} (H_2O) 258.5 nm (ϵ 9350 based on the calculated M_r of 372.33); ^{1}H NMR (D_2O) δ 7.75 (d, J = 8 Hz, H-C_6), 5.90 (d, J = 8 Hz, H-C_5), 5.88 (d, J = 4.5 Hz, H-$C_{1'}$), 3.18 (s, NCH_3), 1.55 (d, J = 6 Hz, CH_3).

(2*S*,3*R*)-*N*-Carbobenzoxy-4′-*O*-carbobenzoxy-3-hydroxyhomotyrosine Pentachlorophenyl Ester (14). Benzyl chloroformate (0.094 mL, 0.66 mmol) was added dropwise to a stirred solution of (2*S*,3*R*)-3-hydroxyhomotyrosine[18] (63 mg, 0.3 mmol) in NaOH (0.1 N, 3 mL) at 0 °C, while the pH of the solution was maintained at 9–10 by addition of 1 N NaOH. After addition was completed, stirring was continued overnight. The reaction was worked up by extraction with ethyl acetate. The organic layer was washed with 1 N HCl and brine and dried over Na_2SO_4. The crude material obtained after evaporation of the solvent was chromatographed on silica gel ($CHCl_3$–CH_3OH–H_2O = 80:20:1) to give an oil (68 mg), which was dissolved in anhydrous ethyl acetate (5 mL) and anhydrous DMF (0.5 mL). Pentachlorophenol (90 mg, 0.34 mmol) and dicyclohexylcarbodiimide (35 mg, 0.17 mmol) were added consecutively at 0 °C. The solution was stirred overnight and brought to −10 °C, and the precipitated urea was filtered off. The solution was concentrated and the resulting oil was purified by chromatography on silica (toluene–ethyl acetate = 4:1). Evaporation of the desired fractions gave **14** (35 mg, 16%, white solid): ^{1}H NMR ($CDCl_3$) δ 7.1–7.5 (14 H), 5.81 (d, J = 10 Hz, NH-Cbz), 5.28 (s, CH_2-Cbz), 5.20 (s, CH_2-Cbz), 4.5–4.9 (m, H-C_2 + H-C_3), 2.91 (m, 2H-C_4).

Registry No. 1, 24695-48-5; **2**, 93806-72-5; **2** *N*-Cbz deriv., 93806-73-6; **3**, 86632-65-7; **3** *N*-Cbz deriv., 93806-74-7; **4**, 93806-75-8; **4** *N*-Cbz deriv., 93806-76-9; **5**, 93806-77-0; **5** *N*-Cbz deriv., 93806-78-1; **6**, 34311-37-0; **7**, 93806-79-2; **7** *N*-Cbz deriv., 93806-80-5; **8**, 93806-81-6; **8** *N*-Cbz deriv., 93806-82-7; **9**, 93806-84-9; **10**, 93806-85-0; **11**, 93842-00-3; **12**, 93806-86-1; **13**, 93806-87-2; **13** *N*-Cbz deriv., 93806-88-3; **14**, 93806-89-4; HCHO, 50-00-0; Boc-L-Ala-al, 79069-50-4; *N*-carbobenzoxy-L-alanine 2,4,5-trichlorophenyl ester, 7536-54-1; *N*-carbobenzoxy-L-citrulline 2,4,5-trichlorophenyl ester, 93806-90-7; *N*-carbobenzoxy-L-threonine pentachlorophenyl ester, 18917-56-1; benzaldehyde, 100-52-7; *N*-carbobenzoxy-L-alanine 2,4-dinitrophenyl ester, 93806-91-8; (2*S*,3*R*)-3-hydroxyhomotyrosine, 74281-82-6; benzyl chloroformate, 501-53-1; *N*-carbobenzoxy-(2*S*,3*R*)-3-hydroxyhomotyrosine, 70042-19-2; chitin synthase, 9030-18-6.