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Nucleosides, XXXVI [1]

Synthesis of 3'-Homocitrullylamino- and 3'-Lysylamino-3'-deoxyadenosine and Their Relation to *Cordyceps militaris* Derived Products [2]

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Syntheses of N^6-desmethylpuromycin (**6**), of 3'-L-homocitrullylamino-3'-deoxyadenosine (**3**) and its L-lysyl analog **4** are reported, based on a preparatively efficient procedure for the preparation of 3'-amino-3'-deoxyadenosine (**2**) from D-xylose, and on attachment of the corresponding amino acid components *via* the *N*-benzyloxycarbonyl-blocked *N*-hydroxysuccinimidyl and *p*-nitrophenyl derivatives Z,Z-Lys-OSU (**18**), Z-Phe(OMe)-OSU (**20**) and Z-*h*Cit-ONP (**23**), each characterized in crystalline form. – Synthetic **3** was identical with the product isolated previously from *Cordyceps militaris*, thus now unequivocally establishing its structure. – In biological evaluation, **3** and **4** exhibited inhibition of poly(U) directed polyphenylalanine synthesis analogous to that of puromycin (**5**), yet lower by factors of 20 and 40, respectively, whilst that of **6** was identical with **5** qualitatively and quantitatively; hence, the N^6-methyl groups in **5** are not essential for termination of ribosomal peptide chain elongation.

Nucleoside, XXXVI [1]

Synthese von 3'-Homocitrullylamino- und 3'-Lysylamino-3'-desoxyadenosin und ihre Beziehung zu Produkten aus *Cordyceps militaris* [2]

Synthesen von N^6-Desmethylpuromycin (**6**), von 3'-L-Homocitrullylamino-3'-desoxyadenosin (**3**) und seinem L-Lysyl-Analogen **4** werden beschrieben, basierend auf einem präparativ leistungsfähigen Verfahren zur Darstellung von 3'-Amino-3'-desoxyadenosin (**2**) aus D-Xylose und der Anknüpfung der entsprechenden Aminosäure-Komponenten über die *N*-benzyloxycarbonyl-geschützten, jeweils in kristallisierter Form charakterisierten *N*-Hydroxysuccinimidyl- bzw. *p*-Nitrophenyl-Derivate Z,Z-Lys-OSU (**18**), Z-Phe(OMe)-OSU (**20**) und Z-*h*Cit-ONP (**23**). – Synthetisches **3** erwies sich mit dem früher aus *Cordyceps militaris* isolierten Produkt identisch, was dessen Konstitution nunmehr beweist. – Die Untersuchung der biologischen Wirksamkeit ergab für **3** und **4** eine dem Puromycin (**5**) analoge, 20- bzw. 40fach geringere Inhibition der poly-(U)-dirigierten Polyphenylalanin-Synthese, während die Inhibitoreigenschaften von **6** qualitativ und quantitativ mit **5** übereinstimmen; folglich sind die N^6-Methyl-Gruppen in **5** kein essentielles Strukturmerkmal zur Terminierung der ribosomalen Peptidkettenverlängerung.

Of the several nucleoside components elaborated in the mycelia of *Cordyceps militaris* (Ascomycetes) 3'-deoxyadenosine (**1**) [3] ("cordycepin") of notable antineoplastic activity [4]

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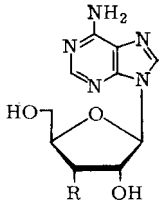
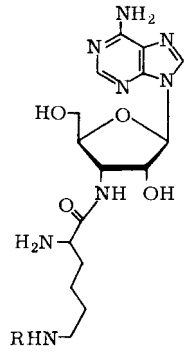
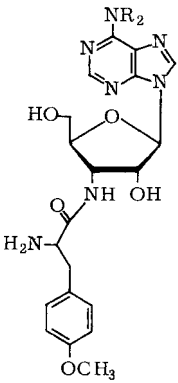
and 3'-amino-3'-deoxyadenosine (**2**)[5] with potent cytostatic properties[6] have been thoroughly investigated, both structures being sustained by syntheses[4,7-11] and a wealth of biological data[5,6]. In contrast, the two *N*-aminoacyl derivatives of **2**, with alleged structure **3**[12] and **4**[13], were isolated from *C. militaris* in very small amounts only and structural assignments were based on the chromatographic identification of hydrolysis products.



1: R = H
2: R = NH_2



3: R = H_2NCO
4: R = H



5: R = CH_3
6: R = H

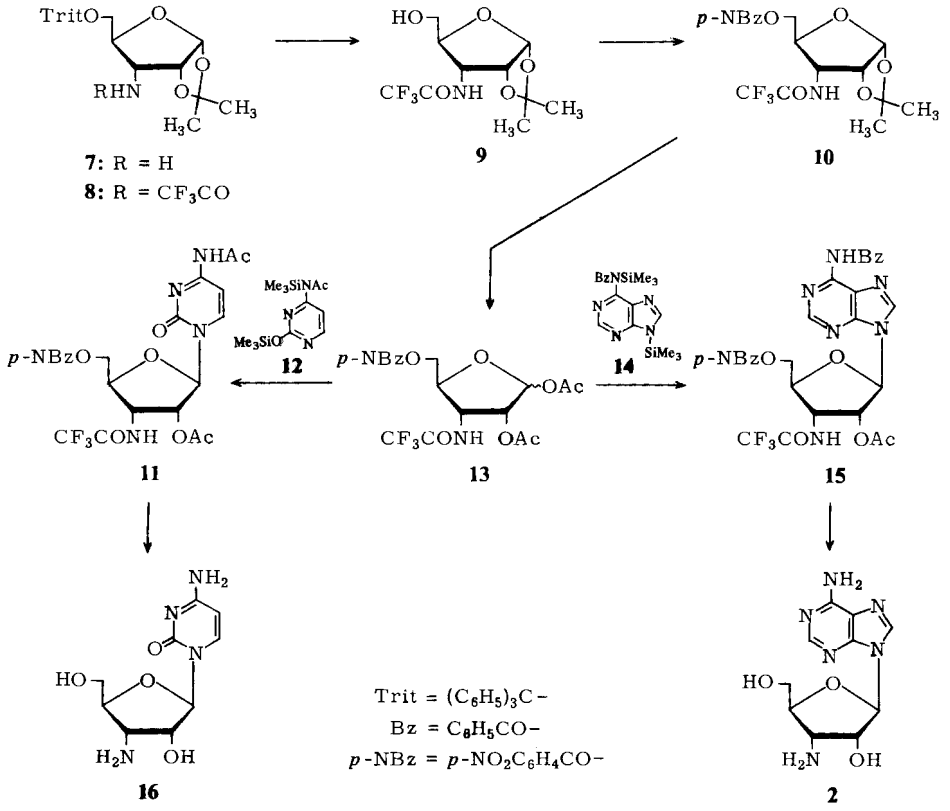
In fact, **3** and **4** may be considered as structural analogs of the broadspectrum antibiotic puromycin (**5**) in which the 3'-*N*-aminoacyl moiety is changed to a homocitrullyl and lysyl group, respectively, and in which the nucleobase is demethylated at N^6. The scarce biological data available for **3** (being approximately 2% as active as puromycin in inhibiting ribosomal protein biosynthesis[14]) indeed appear to indicate that the structural resemblance between **3**, **4** and **5** is translatable into an at least qualitatively analogous mode of interference with transpeptidation. Such a biological analogy would also imply that the N^6-methyl groups in the nucleobase portion of puromycin are not decisive for exerting the inhibitory effect on protein biosynthesis, what hitherto has only been suspected[7] on the basis of similar antitumor activities of **2** and its N^6-dimethyl derivative. However, analogy conclusions of this sort, which would have considerable bearing on the structure-activity relationships within the amino acid and nucleobase portions of puromycin, remain frail in the absence of rigid structural proof for **3** and **4**, and will do so as long as a detailed comparative biological evaluation is not available.

This lacking evidence prompted us to develop an unequivocal, efficient synthetic access to the title compounds **3** and **4**, which was apt to be of more relevance than their reisolation from the natural source which is encumbered by the minute amounts accumulating. Such syntheses of **3** and **4**, as well as of N^6-desmethylpuromycin (**6**) were completed and are the subject of this report together with the correlation of synthetic and *Cordyceps militaris* derived **3**, and their comparative biological evaluation.

Preparation of 3'-Amino-3'-deoxyadenosine

An *a priori* requirement for a synthesis of **3** and **4** were substantial amounts of 3'-amino-3'-deoxyadenosine (**2**), for which at the outset of this work only the two syntheses by *Baker* et al.[7,8] existed, requiring 18 and 19 steps, respectively, from D-xylose in overall

yields of well below 1%. For improving the accessibility of **2** it appeared advantageous to utilize the stannic chloride-catalyzed glycosylation of persilylated N^6-benzoyladenine[15] for nucleoside formation and to apply this procedure to a peracylated 3-aminoribofuranose with an acid-stable yet base-sensitive *N*-protecting group. Thus, the use of 3-acetamido-ribose derivatives, for which several preparatively useful syntheses have been advanced[16], was found inappropriate due to the relatively harsh basic conditions required[17] for regeneration of the amino function. These considerations led to the use of the trifluoro-acetyl group for *N*-protection and to 1,3,5-tri-*O*-benzoyl-3-trifluoroacetamido-3-deoxy-D-ribofuranose as the key intermediate for subsequent introduction of the nucleobase, allowing the preparation of **2** from D-xylose in a 5.9% overall yield over 12 steps[9]. A preparatively even more efficient variant was using 1,2-di-*O*-acetyl-5-*O*-(*p*-nitrobenzoyl)-3-trifluoroacetamido-3-deoxy-D-ribofuranose (**13**) as the key intermediate, which is described in the sequel entailing a 12 step access to **2** from D-xylose in an overall yield of 10.5%. In this way all steps can be performed without column separations and are well suited for large scale preparations. These particulars are of special relevance to the two quite recently advanced syntheses of **2**, which require 12 steps from adenosine in 4% overall yield[10] and 14 steps from D-glucose (5.7%)[11], each of which end up with mg amounts of **2** due to several lengthy and cumbersome column separations.



Our synthetic approach to **2** utilized the known[18] oxime of 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranos-3-ulose, accessible from D-xylose in four well elaborated steps suitable for large scale preparation. Reduction with lithium aluminium hydride to **7** followed by *in situ* treatment with trifluoroacetic anhydride in dichloromethane yielded highly crystalline **8** and also formed small amounts of the *xylo*-epimer, being readily removed by the crystallization process. In **8**, the 5-*O*-trityl function was replaced by a *p*-nitrobenzoyl group by brief heating (80°C) in acetic acid/water (4:1) for detritylation to **9** and subsequent aroylation to afford the ribofuranose derivative **10**, which had previously been obtained by another, less direct route[19]. The isopropylidene group in **10** was then selectively hydrolyzed with 90% trifluoroacetic acid to give the key intermediate **13**[20] as a crystalline 1:1 mixture of α- and β-forms upon direct acylation with acetic anhydride/pyridine, in an overall yield of 18% for the 10 steps from D-xylose. The individual anomers may be separated and characterized in pure form, which, however, is not required for subsequent conversion into nucleosides by reaction with N^6-benzoyl-N^6,9-bis(trimethylsilyl)adenine (**14**) in the presence of stannic chloride in 1,2-dichloroethane (15 h, 60°C). The resulting protected 3′-aminoadenosine **15** was not characterized but directly deblocked which – all protecting groups being alkali-labile – could be reached simply by refluxing with n-butylamine in methanol and afforded **2** in a 59% yield, based on **13**.

The protected 1-*O*-acyl-3-aminoribofuranose **13** may also be utilized for the preparation of pyrimidine nucleosides by simply placing silylated pyrimidines into the stannic chloride-catalyzed glycosylation[22]. Accordingly, when N^4-acetyl-bis(trimethylsilyl)cytosine (**12**) was reacted with **13** in the presence of $SnCl_4$/dichloroethane at room temperature, the blocked nucleoside **11** was formed smoothly; after deblocking with methanolic sodium methoxide it afforded 3′-amino-3′-deoxycytidine (**16**) in a yield of 48% (based on **13**). This adds up to a 12 step synthesis of **16** from D-xylose in an overall yield of 9%, as compared to the previous 19 step access from the same educt in overall yields well below 1%[23].

Structure and configuration of the products prepared were established by UV, NMR and analytical data (cf. experim. part); they are not discussed due to lack of particularities and the generally-established final products.

Aminoacylations

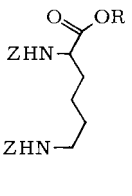
In the course of the substantial synthetic efforts following the discovery of puromycin a large number of 3′-aminosugar nucleosides and analogs thereof have been aminoacylated at the sugar amino function, utilizing the acid chloride[24], mixed anhydride[24–27], azide[24,28] and *p*-nitrophenyl ester[29] methods as well as 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[30] and dicyclohexylcarbodiimide/*N*-hydroxysuccinimide (DCC/HOSU)[26,30–32] as coupling agents for attachment of the *N*-protected amino acid. In re-evaluating their efficiency, particularly with respect to retaining the chiral integrity at the α-carbon of the *N*-protected amino acid during the coupling procedure, it seems likely that many of the early puromycin analogs prepared were partially racemized in their amino acid portion, since the conditions used – e. g. dimethylformamide/triethylamine[24,25,27,28] with additional basicity introduced into the coupling medium by the strongly basic aminosugar nucleoside – were favorable to α-epimerization of the activated amino acid.

Of these coupling methods the DCC/HOSU-procedure and the active ester method were considered most efficient in avoiding racemization, and were consequently used here for the aminoacylations of **2**, with the additional precautionary measure that the respective activated amino acids (i. e.

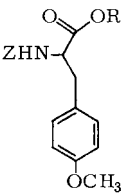
18, **20** and **23**) were not used *in situ* as usual, but were separately prepared and characterized in crystalline form before application.

The *N*-hydroxysuccinimidyl (HOSU) derivatives **18** and **20** were prepared from N^{α},N^{ε}-bis-(benzyloxycarbonyl)-L-lysine (**17**) and *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine (**19**), respectively, by reaction with *N*-hydroxysuccinimide/DCC in acetonitrile or tetrahydrofuran, and could be readily characterized in crystalline form. In the case of *N*-benzyloxycarbonyl-L-homocitrulline (**22**), however, the HOSU-derivative, although readily formed, could not as yet be induced to crystallize, and, thus, was substituted by the highly crystalline *p*-nitrophenyl ester **23** similarly prepared from **22** and *p*-nitrophenol with DCC as the coupling agent.

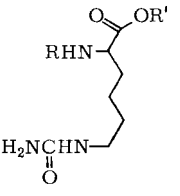
The coupling of the activated benzyloxycarbonyl-amino acids **18**, **20**, and **23** with 3'-amino-3'-deoxyadenosine (**2**) was effected in anhydrous dimethylformamide[33] at room temperature to produce the respective *N*-blocked aminoacyl nucleosides **24**, **26**, and **25**, which were carefully purified by elution from a silica gel column to assure optical purity (yields 56–60%) and could be clearly characterized by ^{1}H-NMR and analytical data. Subsequent catalytic hydrogenolysis over 10% Pd/C smoothly removed the *N*-blocking



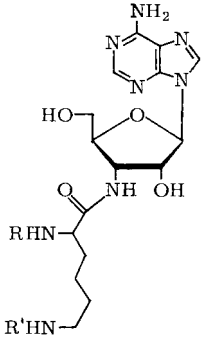
17: R = H
18: R = SU



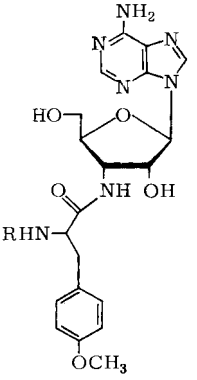
19: R = H
20: R = SU



	R	R'
21	H	H
22	Z	H
23	Z	NP



	R	R'
24	Z	Z
25	Z	H_2NCO
3	H	H_2NCO
4	H	H



26: R = Z
6: R = H

Z = $C_6H_5CH_2OCO$-

NP = O_2N-C$_6$H$_4$-

SU = 

groups and gave the final 3′-aminoacylamino-3′-deoxyadenosines **4**, **6**, and **3**, that were characterized as such, or in the case of **4**, as the dihydroacetate. Their structures and configurations are readily confirmed by analytical and ^{1}H-NMR-data.

Concerning the correlation of the synthetic products **3** and **4** with the *Cordyceps militaris* derived products, we had the inestimable good fortune of securing a small sample of the homocitrullylamino-adenosine isolated by *Kredich* and *Guarino*[12] 17 years ago[34] which sufficed to prove by TCL in several solvent systems, by IR and DC data, that natural product and synthetic **3** were identical. In addition, both samples exhibited identical inhibitory and peptide acceptor activities (*vide infra*), which provided further proof that in fact the major aminoacyl nucleoside elaborated by *Cordyceps militaris* has the structure of a 3′-L-homocitrullylamino-3′-deoxyadenosine (**3**), and also established that in synthetic **3** the chiral integrity of the amino acid has been preserved during the peptide bond formation (**23** → **25**).

A similar correlation of synthetic 3′-L-lysylamino-3′-deoxyadenosine (**4**) with the minor aminoacyl nucleoside from *Cordyceps militaris* could not be made due to lack of natural material, which had only been obtained in minute amounts[14]. However, sufficient evidence for its identity with **4** may be derived from the biological data of synthetic **4** (cf. below) and from the circumstance, that **4** is obviously a biosynthetic precursor of the homocitrullylamino nucleoside **3**.

Biological Evaluation

The ability of N^6-desmethylpuromycin (**6**), its homocitrullyl (**3**) and lysyl analogs (**4**) to interfere with ribosomal protein biosynthesis at the peptide chain elongation stage was examined in two *E. coli* derived, cell-free model systems, evaluating the inhibitory effect on poly(U) directed synthesis of poly(Phe), and their function as acceptors of AcPhe residues from AcPhe-tRNA bound in the ternary complex poly(U)/AcPhe-tRNA/70 S ribosome.

On the basis of their inhibitory effect on poly(U) directed poly(Phe) synthesis (Fig. 1), N^6-desmethylpuromycin (**6**) is as active an inhibitor as puromycin (**5**), 50% inhibition being reached at concentrations of 10^{-4} M. The 3′-homocitrullyl-3′-deoxyadenosine (**3**) was less active by a factor of about 20, i.e. a 20 fold concentration was needed to induce the same inhibition as puromycin. In comparing synthetic and *Cordyceps militaris* derived[12,34] **3**, their inhibitory curves proved to be identical. The extent of inhibition (50% at 4×10^{-3} M as compared to 10^{-4} M for puromycin) correlates well with the findings of *Guarino* et al.[14] who observed 50% inhibition of leucine incorporation with the natural endogeneous messenger in a cell-free *E. coli* system at concentrations of 2.2×10^{-6} for **5** and 1.1×10^{-4} for **3**.

The 3′-lysylamino-3′-deoxyadenosine (**4**) had a circa 40 fold lower inhibitory activity relative to puromycin (Fig. 1), which was not unexpected since the dual hydrophobic-hydrophilic nature of the positively charged lysyl side chain does not fit optimally into the obviously hydrophobic pocket on the ribosome[35,36]. Similar results (albeit in a different assay system) have been obtained for 3′-lysylamino-3′-deoxy-N^6-dimethyladenosine, the N^6-dimethyl analog of **4**, which was inferior to puromycin by a factor of 87 for inhibition of peptidyl-[^{3}H]puromycin release from *E. coli* polysomes[36].

The inhibitory behaviour of these puromycin analogs is paralleled by the acceptor activities as assayed by their ability to have an [^{3}H]AcPhe residue transferred unto their amino-acid portion from [^{3}H]AcPhe-tRNA (Fig. 2). N^6-Desmethylpuromycin (**6**) had as high an acceptor activity as puromycin which was to be anticipated, in view of similarly high activities of a 3′-*O*-analog of **6**, i.e. 2′(3′)-*O*-(phenylalanyl)-adenosine[37,38]. The homocitrullylamino-adenosine **3** was

somewhat inferior to **5** as an acceptor substrate, here too the synthetic and natural[12] product showing identical behaviour. The lysyl analog **4**, however, exhibited only comparatively low acceptor activity, which in addition, decreased at higher concentration (Fig. 2). In contrast, the ester analog of **4**, 2′(3′)-*O*-lysyladenosine showed a more differentiated behaviour towards peptidyl transfer[38], i. e. being a very efficient acceptor for AcPhe residues from AcPhe-tRNA, whereas the acceptor activity is neglegible for the transfer of oligo-lysyl peptides from $(Lys)_n$-tRNA. Whilst this behaviour must be attributed to electrostatic forces, that in addition to hydrophobic binding are obviously important in the binding of these substrates to the peptidyl transferase, the actual reasons underlying the different behaviour of **4** and its 3′-*O*-analog remain to be established.

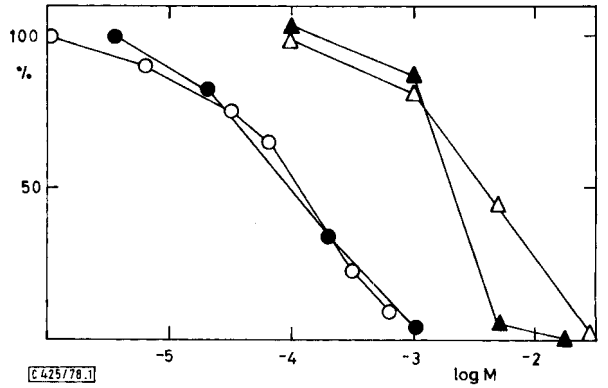


Fig. 1. Effect on poly(U) directed polyphenylalanine synthesis in an *E. coli* cell-free system by puromycin (**5**, ● —— ●), N^6-desmethylpuromycin (**6**, ○ —— ○), 3′-homocitrullylamino-3′-deoxyadenosine (**3**, ▲ —— ▲), and 3′-lysylamino-3′-deoxyadenosine (**4**, △ —— △). [Concentration of compound examined (log M) *versus* % poly(Phe) synthesized as percentage of control without inhibitor]

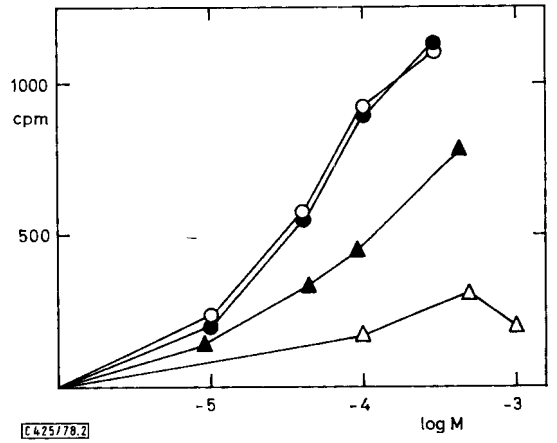


Fig. 2. AcPhe acceptor activity of puromycin (**5**, ● —— ●) and analogs **3** (▲ —— ▲), **4** (△ —— △) and **6** (○ —— ○). [Concentration of compound examined (log M) *versus* amount of Ac[^{3}H]Phe transferred (cpm)]

From these results it is clearly apparent that the interaction of N^6-desmethylpuromycin (**6**), homocitrullylamino-deoxyadenosine (**3**), and its lysyl derivative **4** with the acceptor site ("A-site")

of the peptidyl transferase centre, is analogous to that of puromycin, and in the case of **6** *defacto* identical, clearly demonstrating that the N^6-methyl groups in puromycin are not required for biological activity. By consequence, **3**, **4**, and **6** are not only structural analogs of puromycin as suggested by the similarities in their formula, but also biological analogs with respect to their interference with protein biosynthesis at the peptide chain elongation stage.

We express our appreciation with thanks to the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie* for continuous support of these investigations, and to Dr. *K. Strobel* for assistance in the preparation of some amino acid derivatives.

Experimental Part

Melting points are determined on a Bock-Monoskop and are uncorrected. Spectral measurements were effected with Perkin-Elmer 125 (IR), Perkin-Elmer 141 (rotations), Jasco J-20 (CD), Varian A-60 A and XL 100 (NMR), and Varian CH 4 B (MS) instruments. TLC was performed on Kieselgel F_{254} plastic sheets (Merck, Darmstadt) and was used to monitor the reactions and to ascertain the purity of the reaction products. Developers employed: A benzene/methanol (30:1), B benzene/methanol (4:1), C chloroform, D ethyl acetate/dichloromethane (5:1), E ethyl acetate/methanol/water (40:2:1), F ethyl acetate/methanol/water (15:2:1), G n-butanol/acetic acid/water (5:2:3). The spots were visualized by UV light or by spraying with 80% aqueous sulfuric acid and charring at 110 °C for 5 min or in the case of aminoacid derivatives, by spraying with ninhydrin. Column chromatography was carried out on Kieselgel 60 (70 – 230 mesh, Merck).

3-Deoxy-1,2-O-isopropylidene-3-trifluoroacetamido-5-O-trityl-α-D-ribofuranose (**8**): To a suspension of $LiAlH_4$ (15 g, 0.4 mol) in tetrahydrofuran (300 ml) a solution of 44.6 g (0.1 mol) of 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranos-3-ulose oxime[18] in tetrahydrofuran (200 ml) was gradually added with stirring, the temperature rising to about 60 °C with concomitant reddening towards the end of the addition. The mixture was subsequently refluxed for 4 h, cooled and the excess hydride was destroyed by dropwise addition of water (70 ml). The mixture was filtered and the residue was thoroughly washed with tetrahydrofuran. Filtrate and washings were taken to dryness *in vacuo* and the syrup was dissolved in dichloromethane (500 ml), washed with water (2 × 100 ml), dried (Na_2SO_4), and again taken to dryness *in vacuo:* 43 g of crude 7 as a yellowish syrup of $[\alpha]_D^{22} = +22°$ ($CHCl_3$), being homogeneous by TLC ($R_F = 0.42$ in A) except for a trace of a product at $R_F = 0.32$, probably the *xylo*-epimer. The syrup was dissolved in dichloromethane (150 ml) to which pyridine (8 ml) and, with cooling and stirring, a solution of trifluoroacetic anhydride (14 ml) in dichloromethane (25 ml) was added dropwise. After 2 h at ambient temperature, the mixture was diluted with dichloromethane (100 ml), washed with water (2 × 100 ml), dried (Na_2SO_4), and taken to dryness *in vacuo*, the resulting syrup crystallizing on trituration with hexane: 41.1 g (78%) of *N*-trifluoroacetyl derivative **8**; m.p. 138 – 139 °C, $[\alpha]_D^{22} = +37.4°$ ($c = 1$, $CHCl_3$), $R_F = 0.40$ in C.

^{1}H-NMR ($CDCl_3$): δ = 7.3 (broad 15 H-m, $(C_6H_5)_3C$), 6.46 (1 H-m, NH), 5.85 (3.5 Hz-d, 1 H, 1-H), 4.58 (q, 1 H, $J_{1,2} = 3.5$ and $J_{2,3} = 5.0$ Hz, 2-H), 4.32 (m, 1 H, 3-H), 3.82 (m, 1 H, 4-H), 3.30 (2 H-m, 5-H_2), 1.54 and 1.36 (two 3 H-s, isopropylidene-CH_3). – ^{13}C-NMR ($CDCl_3$): δ = 26.33 and 26.55 (isopropylidene-CH_3), 52.71 (C-3), 62.80 (C-5), 78.45 and 78.55 (C-2 and C-4), 87.10 (trityl-C), 104.34 (C-1), 112.75 (isopropyl-C), 127 – 128 and 143.5 (trityl-C_6H_5). – MS (70 eV): m/e = 527 (0.9%, M^+).

$C_{29}H_{28}F_3NO_5$ (527.5) Calc. C 66.08 H 5.35 N 2.66 Found C 65.96 H 5.38 N 2.58

3-Deoxy-1,2-O-isopropylidene-3-trifluoroacetamido-α-D-ribofuranose (**9**): A solution of trityl-compound **8** (26.5 g, 50 mmol) in a mixture of glacial acetic acid (140 ml) and water (35 ml) was kept at 80 °C for 30 min followed by filtration of tritanol separating on cooling (11.3 g, 87%). The filtrate was taken to dryness *in vacuo* followed by several co-evaporations with toluene. The

resulting syrup was dissolved in a hot mixture of isopropyl alcohol (85 ml) and n-hexane (50 ml) from which **9** crystallized: 8.9 g, m. p. 146–148 °C. Evaporation of the mother liquor afforded another 2.0 g. Total yield: 10.9 g (79%) of **9** as stapelets, m. p. 148–150 °C, and 152 °C, $[\alpha]_D^{20} = +20.7°$ (c = 1, $CHCl_3$), R_F = 0.34 in D. This product was used for all ensuing experiments, although recrystallization from isopropyl alcohol/hexane or from ether raised the m. p. to 158 °C (lit.[19] 156–158 °C).

^{1}H-NMR data corresponded with those reported previously[19]. – ^{13}C-NMR ($CDCl_3$): δ = 26.37 (isopropyl-CH_3), 51.50 (C-3), 60.66 (C-5), 78.52 (C-2), 80.07 (C-4), 104.33 (C-1).

1,2-Di-O-acetyl-3-deoxy-5-O-(p-nitrobenzoyl)-3-trifluoroacetamido-D-ribofuranose (**13**)[20]: 8.7 g (20 mmol) of 3-deoxy-1,2-*O*-isopropylidene-5-*O*-(*p*-nitrobenzoyl)-3-trifluoroacetamido-α-D-ribofuranose (**10**), prepared by *p*-nitrobenzoylation of **9** according to *Fujiwara* et al.[19], was dissolved in 100 ml trifluoroacetic acid/water (9:1) and kept at ambient temperature for 3 h and the mixture was subsequently evaporated *in vacuo* followed by several reevaporations from toluene. The colorless amorphous product obtained [8.1 g of m. p. 150–155 °C, $[\alpha]_D^{25} = +42.4$ (3 min) → +29.8° (4 h) for c = 0.7 in acetone, R_F = 0.55 in B] was dissolved in a mixture of pyridine (100 ml) and acetic anhydride (65 ml) and the clear, brown-red solution was kept at ambient temperature overnight. Concentration of the mixture *in vacuo* (2 Torr, bath temperature < 30 °C), and two re-evaporations from toluene gave a solid residue, which was dissolved in hot methanol (500 ml). After charcoal treatment and addition of water (100 ml) to the hot solution the product crystallized: 7.3 g (76%) of **13** as an approximate 1:1 mixture of α- and β-anomers [R_F = 0.77 and 0.73 in B, resp.; 1 α-H at δ = 6.35 as 4.5 Hz-d, 1 β-H as s at 6.07 in [D]$_6$DMSO], which can be directly used for nucleoside synthesis.

By fractional crystallization from methanol/water the β-anomer may be obtained in pure form in 45% yield (based on the anomeric mixture): m. p. 172 °C, $[\alpha]_D^{20} = +17.6°$ (c = 1, methanol).

^{1}H-NMR ([D$_6$]acetone): δ = 8.47 (broad m, 1 H, NH), 8.12 (4 H-m, aromat. H), 6.01 (s, 1 H, 1-H), 5.21 (4 Hz-d, 1 H, 2-H), ≈4.6 (4 H-m, 3-H, 4-H, 5-H$_2$), 2.06 and 1.93 (two 3 H-s, acetyl-CH_3).

$C_{18}H_{17}F_3N_2O_{10}$ (478.3) Calc. C 45.19 H 3.58 N 5.86 Found C 45.10 H 3.60 N 5.87

3'-Amino-3'-deoxyadenosine (**2**): To a solution of **13** (3.95 g of the crystalline mixture of α/β-anomers as obtained above) in 1,2-dichloroethane (300 ml) was added 4 g of molecular sieve[39], 5 ml of redistilled $SnCl_4$ and, after stirring for 15 min, 4.2 g of N^6-benzoyl-N^6,9-bis(trimethylsilyl)-adenine (**14**)[40], followed by heating to 60 °C for 15 h under careful exclusion of moisture. After removal of the molecular sieve by filtration and dilution of the filtrate with 1,2-dichloroethane (600 ml), the reaction mixture was washed twice with 200 ml portions of saturated $NaHCO_3$ solution[41] and water (200 ml). Filtration, drying of the filtrate (Na_2SO_4), and removal of the solvent *in vacuo* left a residue from which some N^6-benzoyladenine (R_F 0.28 in E, m. p. 241–243 °C) crystallized on trituration with acetone. The mother liquor was evaporated to dryness to give 4.2 g of a syrup, consisting of **15** and its N^6-debenzoylated derivative in an approximate 5:1 ratio (TLC in B). This mixture was deblocked by refluxing in methanol (100 ml) with n-butylamine (4 ml) for 5 h. The precipitate accumulating on standing overnight at 5 °C, was collected and thoroughly washed with methanol: 1.30 g (59%) of **2**, m. p. 264–266 °C (dec), $[\alpha]_D^{20} = -37°$ (c = 0.5, 0.1 N HCl) [lit.[7] m. p. 260–261 °C (dec) and 265–267 °C (dec); $[\alpha]_D^{25} = -40°$ (c = 0.4, DMF)].

^{1}H-NMR-data in D_2O corresponded well with those reported[42]. – ^{13}C-NMR ([D$_6$]DMSO): δ = 156.0 (C-6), 152.3 (C-2), 148.8 (C-4), 139.2 (C-8), 119.2 (C-5), 89.1 (C-1'), 85.5 (C-4'), 74.6 (C-2'), 61.0 (C-5'), 52.5 (C-3'); adenosine had nearly identical resonances except for its C-3' signal (δ = 70.8).

N^4-Acetyl-2'-O-acetyl-3'-deoxy-5'-O-(p-nitrobenzoyl)-3'-(trifluoroacetamido)cytidine (**11**): To a solution of **13** (950 mg of α/β-anomeric mixture as obtained above) in 1,2-dichloroethane (100 ml) was added molecular sieve[39] (2 g), $SnCl_4$ (1 ml, 4.2 molar equiv.) and persilylated N^4-acetyl-

cytosine (**12**)[40] (660 mg, 1.1 molar equiv.) and the mixture was kept at ambient temperature for 2 d. Subsequent dilution with dichloromethane (200 ml), washing with saturated $NaHCO_3$ solution (2 × 50 ml) and water (2 × 50 ml), filtration of the organic phase over Celite, drying (Na_2SO_4), and evaporation *in vacuo* afforded 900 mg of an amorphous solid, which was filtered upon trituration with methanol (40 ml): 720 mg (64%) of chromatographically uniform product; R_F = 0.49 (B) and 0.61 (E), m. p. 224–227 °C, $[\alpha]_D^{20}$ = +13° (*c* = 1, acetone).

^{1}H-NMR ([D_6]acetone): δ = 9.54 (broad 1 H-s, 4-NH), 8.47 (8 Hz-d, 1 H, 3'-NH), 8.09 (4 H-s, aromat. H), 7.87 and 7.09 (two 7.5 Hz-d, 1 H each, 6-H and 5-H), 5.75 (2.5 Hz-d, 1 H, 1'-H), 5.58 (q, 1 H, $J_{1',2'}$ = 2.5 and $J_{2',3'}$ = 6.5 Hz, 2'-H), 2.16 and 2.03 (two 3 H-s, 2'-OAc and N^4-Ac).

$C_{22}H_{20}F_3N_5O_{10}$ (571.4) Calc. C 46.24 H 3.53 N 12.86 Found C 45.92 H 3.48 N 12.66

3'-Amino-3'-deoxycytidine (**16**): A methanolic solution of **11** (570 mg in 20 ml) containing 1 ml of 0.1 N methanolic $NaOCH_3$ solution was refluxed for 1 h, subsequently deionized by stirring with a strongly acidic resin (Merck I) and evaporated to dryness *in vacuo*. The residue was dissolved in water (50 ml), filtered and extracted twice with ether for removal of methyl *p*-nitrobenzoate. Concentration of the aqueous phase gave an already uniform (TLC) syrup which was purified by elution from a column of weakly basic ion exchange resin (2 × 20 cm) to yield upon evaporation of the eluate 185 mg (75%) of **16**, m. p. 220–221 °C after sintering at 214 °C, $[\alpha]_D^{20}$ = +91° (*c* = 0.2, water) [lit.[23] m. p. 221–223 °C, $[\alpha]_D^{25}$ = +91.7° (*c* = 0.4, water)]. – ^{1}H-NMR ([D_6]DMSO): δ = 8.01 and 5.84 (two 8 Hz-d, 1 H each, 6-H and 5-H), 7.15 (m, 2 H, NH_2), 5.78 (1 H-s, 1'-H).

Aminoacylations

N^α,N^ε-Bis(benzyloxycarbonyl)-L-lysine N-hydroxysuccinimidester (Z,Z-Lys-OSU) (**18**): Dicyclohexylcarbodiimide (2.99 g, 14.4 mmol) was added to a solution of Z,Z-Lys-OH (**17**) (4.14 g, 10 mmol) and *N*-hydroxysuccinimide (1.40 g, 12 mmol) in acetonitrile (100 ml) and the mixture was stirred at ambient temperature for 5 h. The *N*,*N'*-dicyclohexylurea was filtered off and washed with acetonitrile, and the combined filtrate and washing were evaporated *in vacuo*. The residue was dissolved in ethyl acetate (50 ml), followed by extraction with water (3 × 15 ml), drying (Na_2SO_4) and removal of the solvent. Recrystallization of the residue from ethyl acetate gave 3.87 g (76%) of Z,Z-Lys-OSU (**18**) as needles of m. p. 115–116 °C; $[\alpha]_D^{25}$ = −19.8° (*c* = 0.5, methanol).

^{1}H-NMR ([D_6]DMSO): δ = 7.35 (10 H-s, 2 C_6H_5), 5.07 and 5.01 (two 2 H-s, 2 benzyl-CH_2), 4.48 (7 Hz-t, 1 H, α-CH), 3.0 (2 H-m, ε-CH_2), 2.80 (4 H-s, 2 SU-CH_2), 1.8 (2 H-m, β-CH_2), 1.4 (4 H-m, γ- and δ-CH_2).

$C_{26}H_{29}N_3O_8$ (511.5) Calc. C 61.05 H 5.71 N 8.21 Found C 60.96 H 5.78 N 8.15

N-Benzyloxycarbonyl-p-methoxyphenyl-L-alanine N-hydroxysuccinimidester [Z-Phe(OMe)-OSU] (**20**): To a cooled (0 °C) solution of *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[24] (**19**) (3.30 g, 10 mmol) and *N*-hydroxysuccinimide (1.38 g, 12 mmol) in tetrahydrofuran (70 ml) dicyclohexylcarbodiimide (2.80 g, 11 mmol) was added and the mixture was kept overnight in a refrigerator. Filtration and washing of the dicyclohexylurea with ethyl acetate was followed by evaporation *in vacuo* of the filtrate and washings, trituration of the residue with ethyl acetate (150 ml), and another filtration. The filtrate was then washed with water (100 ml), dried (Na_2SO_4), and evaporated *in vacuo* to a syrup, which crystallized in well-formed rosettes on standing overnight. Collection upon trituration with ether afforded 2.75 g (65%) of Z-Phe(OMe)-OSU (**20**) of m. p. 103 °C and $[\alpha]_D^{25}$ = −15.0° (*c* = 1.1, dioxane); R_F = 0.79 in A.

^{1}H-NMR ($CDCl_3$): δ = 7.31 (5 H-s, Z-C_6H_5), 7.2–6.9 (two 2 H-m, anisyl-C_6H_4), 5.2 (m, 1 H, α-CH), 5.09 (2 H-s, Z-CH_2), 3.77 (3 H-s, OCH_3), 3.21 (2 H-m, anisyl-CH_2), 2.79 (4 H-s, 2 SU-CH_2).

$C_{22}H_{22}N_2O_7$ (426.4) Calc. C 61.97 H 5.20 N 6.57 Found C 61.85 H 5.13 N 6.53

N^{α}-Benzyloxycarbonyl-L-homocitrulline (Z-hCit-OH) (**22**): To a solution of L-homocitrulline[43] (**21**) (5.7 g, 30 mmol) in 2 N NaOH (15 ml) was added by stirring and cooling 12 ml (35 mmol) of a 50% solution of benzyloxycarbonyl chloride in toluene and further 2 N NaOH (27 ml) was added gradually over 10 min. The reaction mixture was subsequently stirred at ambient temperature (30 min) and extracted with ether (4 × 100 ml). The aqueous phase was brought to pH 2 by the addition of 2 N HCl (20 ml) resulting in a milky solution which crystallized upon addition of seed crystals[44]. Filtration, thorough washing with water to the point of absence of chloride ion. and recrystallization from methanol afforded 6.6 g (68%) of **22** as stout prisms of m. p. 135 °C; $[\alpha]_D^{22} = -11°$ (c = 1.0, DMF), R_F = 0.11 in A.

^{1}H-NMR ([D$_6$]DMSO): δ = 7.32 (5 H-m, C_6H_5), 5.03 (2 H-s, benzyl-CH_2), 4.02 (1 H-m, α-CH), 3.04 (2 H-m, ε-CH_2), 1.55 (6 H-m, 3 CH_2).

$C_{15}H_{21}N_3O_5$ (323.4) Calc. C 55.71 H 6.55 N 13.00 Found C 55.63 H 6.54 N 12.94

N^{α}-Benzyloxycarbonyl-L-homocitrulline p-nitrophenyl ester (Z-hCit-ONP) (**23**): Dicyclohexylcarbodiimide (2.06 g, 10 mmol) was added to an ice-cold solution of Z-*h*Cit-OH (**22**) (3.23 g, 10 mmol) and *p*-nitrophenol (1.67 g, 12 mmol) in dimethylformamide (30 ml). The mixture was kept at 0 – 3 °C for 30 min and at ambient temperature for 3 h, after which a few drops of glacial acetic acid were added. The *N,N'*-dicyclohexylurea was removed by filtration and washed with dimethylformamide (5 ml) and to the combined filtrate and washing water (185 ml) was added. The resulting slightly yellow precipitate was collected, dried and recrystallized from methanol (80 ml): 2.90 g (66%) of Z-*h*Cit-ONP (**23**) as needles of m. p. 166 °C and $[\alpha]_D^{22} = -20.1°$ (c = 0.6, DMF); R_F = 0.46 in A.

^{1}H-NMR ([D$_6$]DMSO): δ = 8.33 (9 Hz-d, 2 H, 2 nitrophenyl 3-H), 7.96 (1 H-m, NH), 7.38 (7 H-m, Z-C_6H_5 and nitrophenyl 2-H), 5.16 (2 H-s, Z-CH_2), 4.40 (1 H-m, α-CH), 3.19 (2 H-m, ε-CH_2), 2.2 – 1.1 (broad m, 6 H, 3 CH_2).

$C_{21}H_{24}N_4O_7$ (444.4) Calc. C 56.75 H 5.44 N 12.61 Found C 56.79 H 5.56 N 12.59

3'-[N^{α},N^{ε}-Bis(benzyloxycarbonyl)-L-lysylamino]-3'-deoxyadenosine (**24**): To a stirred solution of Z,Z-Lys-OSU (**18**) (1.02 g, 2.0 mmol) in dry dimethylformamide (300 ml) 3'-amino-3'-deoxyadenosine (**2**) (450 mg, 1.7 mmol) was added in several portions over the course of 5 h. The mixture was stirred for another 12 h at room temperature and subsequently filtered. Evaporation of the filtrate to dryness *in vacuo* (2 Torr with bath temperature > 30 °C), followed by several co-evaporations with toluene left a residue which was purified by elution from a silica gel column (2.5 × 25 cm) with chloroform/methanol (10:1). Concentration of the main fraction gave a foam which crystallized on trituration with ethanol. Recrystallization from ethanol gave 570 mg (51%) of **24**, m. p. 186 – 188 °C; $[\alpha]_D^{20} = -23°$ (c = 0.9, methanol), R_F = 0.52 in F.

$C_{32}H_{38}N_8O_8$ (662.7) Calc. C 57.99 H 5.78 N 16.91 Found C 58.04 H 5.78 N 16.84

3'-L-Lysylamino-3'-deoxyadenosine dihydroacetate (**4** · 2 HOAc): A 165 mg (0.025 mmol) sample of the blocked lysyl nucleoside **24** and 100 mg 10% palladium on carbon in 15 ml methanol/acetic acid (1:1) was hydrogenated, deblocking being completed within 2 h (TLC in G). After filtration through Celite the filtrate was taken to dryness *in vacuo* and repeatedly re-evaporated from ethanol and from water to yield 120 mg (88%) of **4**-dihydroacetate as an amorphous, chromatographically uniform product which melted at 85 °C, resolidified on further raising the temperature to remelt at 135 °C with effervescence; $[\alpha]_D^{20} = -38°$ (c = 0.5, methanol); R_F = 0.17 in G; UV-data corresponded to those of adenosine.

$C_{16}H_{26}N_8O_4 \cdot 2\,CH_3CO_2H \cdot H_2O$ (532.6) Calc. C 45.10 H 6.81 N 21.04
Found C 44.94 H 6.76 N 20.70

3'-(N-Benzyloxycarbonyl-L-homocitrullylamino)-3'-deoxyadenosine (**25**): 3'-Amino-3'-deoxyadenosine (**2**) (215 mg, 0.8 mmol) was added to a solution of Z-*h*Cit-ONP (**23**) (400 mg, 0.9 mmol)

in dry dimethylformamide (90 ml) and the resulting suspension was stirred at room temperature for 2 d, after which a clear solution was obtained. Evaporation to dryness *in vacuo* (finally 2 Torr), followed by re-evaporations from n-butanol and toluene afforded a syrupy residue which was purified by elution from a silica gel column (3.5 × 20 cm) with chloroform/methanol (3:1). From the 10 ml portions collected, fractions 33–66 contained **25** and were taken to dryness *in vacuo*. The residue was filtered upon trituration with isopropyl alcohol to give 260 mg (56%, based on **2**) of **25** as an amorphous product; m. p. 166–168°C; $[\alpha]_D^{25} = -26°$ ($c = 0.5$, methanol). Extensive drying over H_2SO_4/P_4O_{10} was required to remove last traces of water and isopropyl alcohol (NMR).

^{1}H-NMR ([D$_6$]DMSO + D$_2$O): δ = 8.50 and 8.27 (two 1 H-s, 8-H and 2-H), 7.42 (5 H-s, C_6H_5), 6.08 (1 H-m, 1′-H), 5.10 (2 H-s, benzyl-CH_2), 3.0 (2 H-m, N–CH_2), 1.4 (broad 6 H-m, 3 CH_2).

$C_{25}H_{33}N_9O_7$ (571.6) Calc. C 52.52 H 5.82 N 22.05 Found C 52.41 H 5.76 N 21.92

3′-(L-Homocitrullylamino)-3′-deoxyadenosine (**3**): To a prehydrogenated suspension of 10% Pd/C (70 mg) in 10 ml of water/methanol (1:1) 140 mg (0.24 mmol) of blocked nucleoside **25** was added and the hydrogenation was continued. After 1 h, TLC indicating absence of educt, the catalyst was removed by filtration and the filtrate was evaporated to dryness leaving a solid residue which was reprecipitated from methanol: 82 mg (77%) of amorphous **3**, m. p. 177–179°C and $[\alpha]_D^{25} = -20.2°$ ($c = 0.5$, water). – CD (water): 216 ($\Delta\varepsilon = -2.3$) and 260 nm (–1.9).

^{1}H-NMR (D$_2$O): δ = 8.34 and 8.14 (two 1 H-s, 8-H and 2-H), 6.10 (1 H-m, 1′-H), 3.10 (6 Hz-t, 2 H, N–CH_2), 1.7–1.3 (6 H-m, 3 CH_2).

$C_{17}H_{27}N_9O_5 \cdot H_2O$ (455.5) Calc. C 44.83 H 6.41 N 27.68 Found C 44.98 H 6.37 N 27.63

On the basis of its IR (KBr) and CD data, of its behaviour on TLC in solvent systems F and G, and on paper chromatograms [in n-butanol/acetic acid/water (3:1:1) and water adjusted to pH 10 with aqueous ammonia] synthetic **3** and the *Cordyceps militaris*[12,34] derived product were identical. Similarly, the hydrolytic behaviour reported for the latter[12] could be reproduced with synthetic **3**: heating in N HCl at 100°C for 10 min produced (TLC) adenine and a reducing, ninhydrin-positive second component (conceivably 3-homocitrullylamino-3-deoxy-D-ribose), whilst refluxing of **3** in N methanolic sodium methoxide for 12 h produced 3′-amino-3′-deoxyadenosine (**2**) and lysine, as identified by TLC.

3′-(N-Benzyloxycarbonyl-p-methoxyphenyl-L-alanylamino)-3′-deoxyadenosine (**26**): A mixture of Z-Phe(OMe)-OSU (**20**) (450 mg, 1.05 mmol), 3′-amino-3′-deoxyadenosine (**2**) (240 mg, 0.90 mmol) and dry dimethylformamide (100 ml) was stirred at ambient temperature for 2 d and the resulting clear solution was evaporated *in vacuo* (finally 1 Torr) followed by several co-evaporations with n-butanol and toluene. Purification of the residue by elution from a silica gel column (3.5 × 20 cm) with chloroform/methanol (10:1) and concentration of the fractions containing **26** gave a solid product, which was reprecipitated from 85% aqueous methanol: 305 mg (59%, based on **2**); m. p. 241–244°C (dec.), $[\alpha]_D^{25} = -28°$ ($c = 0.5$, methanol).

^{1}H-NMR ([D$_6$]DMSO + D$_2$O): δ = 8.45 and 8.23 (two 1 H-s, 8-H and 2-H), 7.33 (5 H-s, C_6H_5), 7.25 and 6.88 (two 9 Hz-d, 2 H each, anisyl-C_6H_4), 6.00 (1 H-m, 1′-H), 5.00 (2 H-s, benzyl-CH_2), 3.76 (3 H-s, OCH_3), 3.28 (2 H-m, anisyl-CH_2).

$C_{28}H_{31}N_7O_7$ (577.6) Calc. C 58.22 H 5.41 N 16.97 Found C 58.09 H 5.38 N 16.87

3′-(p-Methoxyphenyl-L-alanylamino)-3′-deoxyadenosine ("N^6-desmethylpuromycin") (**6**): A solution of blocked nucleoside **26** (180 mg, 0.31 mmol) in dioxane (10 ml) was added to a prehydrogenated suspension of 10% Pd/C (100 mg) in 50% aqueous dioxane (5 ml) and hydrogenated for 2 h. Removal of the catalyst by filtration, evaporation of the filtrate to dryness and trituration

of the residue with ethanol afforded 84 mg (68%) of amorphous **6**, which slowly decomposed from 130 °C on; $[\alpha]_D^{25} = -14.3°$ ($c = 0.6$, 50% aqueous methanol).

$C_{20}H_{25}N_7O_5$ (443.5) Calc. C 54.16 H 5.68 N 22.11 Found C 54.08 H 5.73 N 22.04

Biological Evaluations

The cell-free ribosomes[4,5] and S-30[3,7] were prepared from *Escherichia coli B* as described earlier.

Assay for polyphenylalanine synthesis: The reaction mixture contained in a total volume of 100 µl 0.04 M tris pH 7.4, 0.16 M NH_4Cl, 0.01 M magnesium acetate, 0.4 mM GTP, 1 mM ATP, 20 µg poly(U), 2.5 pmoles [^{3}H]Phe-tRNA, 500 µg S-30, and examined components as listed in the legends to the figures. After a 20 min incubation at 35 °C samples were determined as described elsewhere[4,6]. Results see Fig. 1.

Assay for acceptor activity: The reaction mixture contained in a total volume of 100 µl 0.05 M tris pH 7.2, 0.1 M ammonium acetate, 0.01 M magnesium acetate, 350 µg ribosomes, 0.7 pmoles Ac[^{3}H]Phe-tRNA, 10 µg poly(U) and, after a 30 min incubation at 35 °C, the reaction was stopped by adding 2 ml of conc. acetic acid; the samples were cooled for 20 min in ice, centrifuged, and supernatants were transferred onto planchets, acetic acid evaporated and radioactivity was measured in a methaneflow proportional Frieseke-Höpfner counter. Results see Fig. 2.

In several experiments the reaction was terminated with 2.5% trichloroacetic acid followed by processing and counting as described earlier[3,7]. There was no significant difference between the results obtained by both methods.

Literature

[1] Part XXXV: *F. W. Lichtenthaler, Y. Sanemitsu,* and *T. Nohara,* Angew. Chem. **90**, 819 (1978); Angew. Chem., Int. Ed. Engl. **17**, 772 (1978).

[2] Presented in part at the VIII. Internat. Symp. Carbohydr. Chem., Kyoto, Aug. 1976, Abstr. 4D-9.

[3] *K. G. Cunningham, S. A. Hutchinson, W. Manson,* and *F. S. Spring,* J. Chem. Soc. **1951**, 2299; *S. Frederiksen, H. Malling,* and *H. Klenow,* Biochim. Biophys. Acta **95**, 189 (1965).

[4] *S. Frederiksen* and *H. Klenow* in Antineoplastic and Immunosuppressive Agents II, *A. C. Satorelli* and *D. G. Johns,* Ed., pp. 657–664, Springer Verlag, Heidelberg 1975.

[5] *A. J. Guarino* and *N. M. Kredich,* Biochim. Biophys. Acta **68**, 317 (1963).

[6] *R. J. Suhadolnik,* Nucleoside Antibiotics, pp. 76–86, Wiley-Interscience, New York 1970.

[7] *B. R. Baker, R. E. Schaub,* and *H. M. Kissman,* J. Am. Chem. Soc. **77**, 5911 (1955).

[8] *E. J. Reist* and *B. R. Baker,* J. Org. Chem. **23**, 1083 (1958).

[9] *F. W. Lichtenthaler, P. Voss,* and *A. Heerd,* Tetrahedron Lett. **1974**, 2141.

[10] *R. Mengel* and *H. Wiedner,* Chem. Ber. **109**, 433 (1976).

[11] *A. V. Azhayev* and *J. Smrt,* Collect. Czech. Chem. Commun. **43**, 1520 (1978).

[12] *N. M. Kredich* and *A. J. Guarino,* J. Biol. Chem. **236**, 3300 (1961).

[13] *A. J. Guarino* and *N. M. Kredich,* Federation Proc. **23**, 371 (1964), Abstract 1619.

[14] *A. J. Guarino, M. L. Ibershof,* and *R. Swain,* Biochim. Biophys. Acta **72**, 62 (1963).

[15] *F. W. Lichtenthaler, P. Voss,* and *G. Bambach,* Bull. Chem. Soc. Jpn. **47**, 2297 (1974).

[16] *A. K. M. Anisuzzaman* and *R. L. Whistler,* J. Org. Chem. **37**, 3187 (1972); *H. S. El Khadem, T. D. Audichya, E. H. El Ashry,* and *R. Sindric,* Carbohydr. Res. **41**, 318 (1975); *J. A. Montgomery, K. Hewson,* and *A. G. Laseter,* J. Med. Chem. **18**, 571 (1975).

[17] *S. Hanessian,* Methods Carbohydr. Chem. **6**, 208 (1972).

[18] *W. Sowa,* Can. J. Chem. **46**, 1586 (1968).

[19] *A. N. Fujiwara, E. M. Acton,* and *L. Goodman,* J. Heterocycl. Chem. **7**, 891 (1970).

[20] Attempts to replace the two step conversion **10** → **13** by a one step acetolysis with 17:17:1 acetic anhydride/acetic acid/sulfuric acid or with acetic anhydride/BF_3[21] gave far inferior results due to the formation of several additional products (TLC), conceivably open-chain peracetates.

[21] *F. W. Lichtenthaler, J. Breunig,* and *W. Fischer,* Tetrahedron Lett. **1971**, 2825.

[22] For scope and limitations of this reaction as applied to silylated pyrimidines, cf.: *U. Niedballa* and *H. Vorbrüggen*, Angew. Chem. **82**, 449 (1970); Angew. Chem., Int. Ed. Engl. **9**, 461 (1970); J. Org. Chem. **39**, 3654 (1974); *F. W. Lichtenthaler, A. Heerd*, and *K. Strobel*, Chem. Lett. **1974**, 449.
[23] *H. M. Kissman* and *M. J. Weiss*, J. Am. Chem. Soc. **80**, 2575 (1958).
[24] *B. R. Baker, J. P. Joseph*, and *J. H. Williams*, J. Am. Chem. Soc. **77**, 1 (1955).
[25] *A. M. Small, H. M. Kissman, J. P. Joseph*, and *M. J. Weiss*, J. Med. Chem. Pharm. **2**, 375 (1960).
[26] *L. V. Fisher, W. W. Lee*, and *L. Goodman*, J. Med. Chem. **13**, 775 (1970).
[27] *F. W. Lichtenthaler* and *H. P. Albrecht*, Angew. Chem. **80**, 440 (1968); Angew. Chem., Int. Ed. Engl. **7**, 457 (1968).
[28] *F. W. Lichtenthaler, G. Trummlitz*, and *P. Emig*, Tetrahedron Lett. **1970**, 2061.
[29] *M. J. Robins, L. N. Simon, M. G. Stout, G. A. Ivanovics, M. P. Schweitzer, R. J. Rousseau*, and *R. K. Robins*, J. Am. Chem. Soc. **93**, 1474 (1971).
[30] *R. J. Harris, J. F. B. Mercer, D. C. Skingle*, and *R. H. Symons*, Can. J. Biochem. **50**, 918 (1972).
[31] *W. W. Lee, G. L. Tong, R. W. Blackford*, and *L. Goodman*, J. Org. Chem. **35**, 3808 (1970).
[32] *R. Vince* and *S. Daluge*, J. Med. Chem. **15**, 171 (1972); **17**, 578 (1974); **20**, 930 (1977); *T. Suami, K. Tadano, M. Ayabe*, and *Y. Emori*, Bull. Chem. Soc. Jpn. **51**, 855 (1978).
[33] The choice of solvents is restricted by the low solubility of **2** in the standard peptide coupling solvents, with DMF or DMF/dioxane mixtures generally being the most useful. Also, an excess of activated amino acid was used to enhance aminoacylation of **2**, thereby reducing the basicity introduced by **2** into the reaction medium.
[34] We are grateful to Dr. *N. M. Kredich*, Duke University Medical Center, Durham, N. C., for kindly providing a *Cordyceps militaris* derived sample of **3**.
[35] *R. J. Harris* and *R. H. Symons*, Bioorg. Chem. **2**, 285 (1973).
[36] *E. F. Vanin, P. Greenwell*, and *R. H. Symons*, FEBS Lett. **40**, 124 (1974).
[37] *I. Rychlik, J. Černá, S. Chládek, J. Žemlička*, and *Z. Haladová*, J. Mol. Biol. **43**, 13 (1969).
[38] *I. Rychlik, J. Černá, S. Chládek, P. Pulkrábek*, and *J. Žemlička*, Eur. J. Biochem. **16**, 136 (1970).
[39] Grade 4 Å of 2 mm pearls (Merck, Darmstadt), freshly activated before use.
[40] *T. Nishimura* and *I. Iwai*, Chem. Pharm. Bull. **12**, 352 (1964).
[41] Phase separation is tardy and is effected by standing overnight or by filtration of the emulsion over a layer of sand/Celite followed by thorough washing of the filtering aid.
[42] *M. Morr* and *L. Ernst*, Chem. Ber. **111**, 2152 (1978).
[43] L-Homocitrulline (**21**) was prepared by carbamoylation of the copper complex of L-lysine as described by *A. C. Kurtz*, J. Biol. Chem. **180**, 1253 (1949).
[44] Seed crystals were obtained on standing of syrupy **22** over water for several days.
[45] *I. Rychlik*, Collect. Czech. Chem. Commun. **30**, 2259 (1965).
[46] *J. Jonák* and *I. Rychlik*, Biochim. Biophys. Acta **324**, 554 (1973).

[425/78]