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3′-*O*-Aminoacyl-2′-deoxyadenosines and 2′-*O*-Aminoacyl-3′-deoxyadenosines Related to Charged Transfer Ribonucleic Acid Termini†

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ABSTRACT: Aminoacyl nucleosides derived from 2′-deoxyadenosine and 3′-deoxyadenosine have been isolated as pure solids and completely characterized for the first time. Reaction of 5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**1**) with *N*-trityl blocked amino acid anhydrides (**2a–c**) (generated *in situ* from the corresponding *N*-tritylamino acid and dicyclohexylcarbodiimide) in the presence of 4-*N*,*N*-dimethylaminopyridine gave the 3′-*O*-(*N*-tritylaminoacyl)-5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosines (**3a–c**) in high yields. This coupling reaction was unsuccessful using pyridine. Analogous treatment of 5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosine (**6**) gave the corresponding 2′-*O*-(*N*-tritylaminoacyl)-5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosines (**7a–c**). The L-leucine (**a**), L-phenylalanine (**b**), and L-methionine (**c**) compounds were prepared in each series. Complete deblocking was effected using formic acid–1-butanol–toluene (1:1:1) at room temperature. Under these conditions the 3′-*O*-(L-aminoacyl)-2′-deoxyadenosines (**4a–c**) and 2′-*O*-(L-aminoacyl)-3′-deoxyadenosines (**8a–c**) were obtained in high yield with no detectable hydrolysis of either the aminoacyl ester or glycosidic bonds.

N-Formylmethionyl and *N*-acetylphenylalanyl derivatives were prepared in each series by subsequent acylation of the free aminoacyl compounds with acetic formic anhydride and *p*-nitrophenyl acetate, respectively. Biochemical rationale for the use of these compounds in the study of protein biosynthesis and initiation processes and preliminary biochemical data are discussed.

It is well established that the aminoacyl nucleoside antibiotic puromycin inhibits protein biosynthesis by simulating a charged terminus of tRNA. Acting in this role it accepts the growing peptide chain from peptidyl-tRNA and the covalently linked peptidylpuromycin dissociates from the ribosomal complex (for example, see Nathans (1964), Traut and Monro (1964), and Coutsogeorgopoulos (1967); for a recent concise review, see Suhadolnik (1970)). Various 3′(2′)-*O*-aminoacylnucleosides and nucleotide derivatives have been synthesized and evaluated as analogous peptide receptors in protein biosynthetic systems (see, for example, Rammler and Khorana, 1963; Waller *et al.*, 1966; Žemlička *et al.*, 1969; Rychlik *et al.*, 1969; Chládek *et al.*, 1970; Černá *et al.*, 1970a,b; Chládek, 1972, and other papers of the Czech group; Gottikh *et al.*, 1970; Tarusova *et al.*, 1971; Pozdnyakov *et al.* (1972), and other papers of the Russian group), and base as well as amino acid variation has been explored especially by the Czech group.

An example of such protein biosynthesis blockage by 2′,3′-bis(*O*-aminoacyl)adenosines (Černá *et al.*, 1970a) added

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interest to a hypothesis of Neumann *et al.* (1968) that protein synthesis might occur by transfer of the growing peptide to the 2′(3′)-hydroxyl of a 3′(2′)-*O*-aminoacyl charged tRNA. This step would be followed by intramolecular peptidyl transfer from the 2′(3′)-hydroxyl to the amino function of the 3′(2′)-*O*-amino acid as the actual elongation step. The significant peptide acceptor activity of 2′,3′-bis(*O*-aminoacyl)-adenosines (Černá *et al.*, 1970a), contrary to the conclusion of Pozdnyakov *et al.* (1972), does not preclude the Neumann *et al.* (1968) hypothesis since spontaneous intramolecular amino acid acylation of the adjacent amine function to give 3′(2′)-*O*-dipeptidyladenosine has been observed (Neumann *et al.*, 1968) and strong acceptor activity of such a dipeptidyl derivative demonstrated (Černá *et al.*, 1970a). The virtually complete lack of acceptor activity of 3′-*O*-(L-phenylalanyl)-2′-deoxyadenosine (Rychlik *et al.*, 1969) was compatible with the Neumann *et al.* (1968) proposal, but such a negative result neither demands the presence of a 2′-OH function nor provides any definitive support. A recent communication (Pozdnyakov *et al.*, 1972) reported the high puromycin-like activity of 3′-*O*-phenylalanyl-2′-*O*-methyladenosine. This interesting result would appear to obviate the necessity of a 2′-hydroxyl function for proper binding in the acceptor vicinity (Coutsogeorgopoulos, 1967) and for accepting activity assuming that no demethylation occurs (for such a nullifying example, see Fox *et al.* (1971)).

Although the naturally occurring aminoacyl-tRNAs are thought to occur and function as 3′ esters of the terminal adenosine (see Zachau and Feldmann (1965) and references therein), very facile and rapid 2′-O $\rightleftarrows$ 3′-O isomerization occurs in aqueous media (McLaughlin and Ingram, 1965) and, thus, 2′-*O*-aminoacyl involvement has not been precluded. Indeed, a recent communication (Sprinzl *et al.*, 1973) has reported that a modified yeast $tRNA^{Phe}$ terminating with 3′-deoxyadenosine (cordycepin) can be enzymatically aminoacylated whereas the corresponding 2′-deoxyadenosine terminated tRNA cannot. This suggests possible initial charging at the 2′-hydroxyl group of intact tRNA. The 2′-*N*-aminoacyl isomer of puromycin has been reported to be inactive as an inhibitor of protein synthesis (Nathans and Neidle, 1963). This result has been quoted (Zachau and Feldmann, 1965) as evidence for 3′-*O*-aminoacyl functionality in biological circumstances. Tarusova *et al.* (1971) have synthesized an ω-aminocaproic ester of adenosine 3′,5′-cyclic monophosphate but there appear to have been no specifically linked 2′-*O*-aminoacyl nucleosides available for evaluation previously.

Certain 5′-*N*-aminoacyl-5′-amino-5′deoxyadenosines have been shown to inhibit protein biosynthesis (Robins *et al.*, 1971). The aromatic amino acid phenylalanine was most effective in that system as has been previously observed in the puromycin reaction (Nathans and Neidle, 1963).

The initiation of protein synthesis in *Escherichia coli* (Clark and Marcker, 1966) and probably in prokaryotic systems in general (for a review, see Lucas-Lenard and Lipmann (1971)) is thought to proceed exclusively by a specific species of *N*-formylmethionyl-tRNA. Initiation by *N*-acetylphenylalanyl-tRNA in an *E. coli* system was demonstrated by Lucas-Lenard and Lipmann (1967). Eukaryotic systems, in contrast, employ other aminoacyl-tRNAs including a free methionine species. Thus, a biological species specificity at the initiation stage of protein biosynthesis apparently exists which could be of great potential significance in terms of selective inhibition.

In the present study, specific 3′-*O*-aminoacyl nucleosides containing 2′-deoxyadenosine and 2′-*O*-aminoacyl nucleosides containing 3′-deoxyadenosine (cordycepin) have been prepared and fully characterized as analytically pure solids. The amino acids L-leucine, L-phenylalanine, and L-methionine were employed as examples of aliphatic, aromatic, and control methionine types, respectively. In addition, the *N*-formylmethionyl and *N*-acetylphenylalanyl derivatives were synthesized in order to evaluate possible effects on the complex initiation process.

Experimental Section

Melting points were determined on Reichert microstage or Gallenkamp apparatus and are uncorrected. Ultraviolet (uv) spectra were determined on Cary 14 or 15 spectrophotometers. Nuclear magnetic resonance (nmr) spectra were recorded on a Varian HA-100 instrument. Optical rotations were measured using a Perkin-Elmer Model 141 polarimeter with a 10-cm 1-ml micro cell. Elemental analyses were obtained by the microanalytical laboratory of this department and Schwarzkopf Microanalytical Laboratory. Thin-layer chromatography (tlc) was effected using Eastman Kodak sheets (silica gel No. 13181) in solvent systems indicated; developed chromatograms were detected by 2537-Å light (chromophore), 20% perchloric acid spray (trityl, mono-, and di-*p*-methoxytrityl functions show yellow to red, respectively), and ninhydrin spray (slow development of yellow color upon warming, with free aminoacyl nucleosides). Column chromatography was performed on alumina (J. T. Baker No. 0537 aluminum oxide) or silica gel (Gebr. Herrmann, Kieselgel). Evaporations were effected using a Büchler rotating evaporator with a Dry Ice cooled dewar condensor under aspirator or mechanical oil pump vacuum at 30° or lower.

The preparations of 3′-deoxyadenosine (cordycepin) (Robins *et al.*, 1973), 5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**1**) (Hogenkamp and Oikawa, 1964), and *N*-trityl-L-leucine, *N*-trityl-L-phenylalanine, and *N*-trityl-L-methionine (Zervas and Theodoropoulos, 1956; Stelakatos *et al.*, 1959) followed outlined procedures. Ethyl acetate was dried by distillation from "P_2O_5" and stored over Linde molecular sieves (4A, dried at 200°). Pyridine was dried by refluxing over and then distillation from CaH_2.

5′-O-(Mono-p-methoxytrityl)-3′-deoxyadenosine (**6**). To a solution of 2.5 g (0.01 mol) of 3′-deoxyadenosine in 75 ml of dry pyridine was added 4.6 g (0.015 mol) of mono-*p*-methoxytrityl chloride and the solution was stirred for 39 hr at 13°. MeOH (150 ml) was added and stirring was continued for 1.5 hr after which the solution was poured into 400 ml of ice–water. This mixture was extracted with $CHCl_3$ and the combined extracts were evaporated to give 7.9 g of a brown gum. This material was dissolved in 10 ml of $CHCl_3$ and applied to a column (2 × 38 cm, 250 g, packed in $CHCl_3$) of alumina. The column was then washed with 2350 ml of $CHCl_3$ and the fast migrating materials (tlc, $CHCl_3$–MeOH 97:3) eluted were discarded. Solvent was changed to $CHCl_3$–MeOH (95:5) and the first 275 ml of eluate containing primarily the same fast migrating (tlc) impurities was discarded. The following 725 ml of eluate was combined and evaporated. The residue was dissolved in a minimum volume of $CHCl_3$ and dripped slowly into pentane with vigorous stirring. The precipitate was filtered and air-dried to give 3.0 g (57%) of **6**. A sample for analysis was recrystallized from MeOH to give **6**: mp 110–114°; uv (MeOH) max 259 and 233 nm (ε 16,700, 18,400), min 245 and 225 nm (ε 13,700, 16,300); nmr ($CDCl_3$, Me_4Si internal) δ 2.20 (br m, 2, $H_{3',3''}$), 3.34 (m, 2, $H_{5',5''}$),

TABLE I: Fully Blocked Aminoacyl Derivatives.[a]

Compound	Yield (%)	Mp[b] (°C)	Formula	Elemental Anal. Calcd (%) C	H	N	S	Found (%) C	H	N	S	Uv Spectra nm (ε)[c]
3a	62	119–120	$C_{56}H_{56}N_6O_6$	73.98	6.21	9.25		73.65	6.31	9.65		max 234 (32,600) sh 260 (17,300) min 225 (30,300)
3b	81	120–125	$C_{59}H_{54}N_6O_6$	75.14	5.77	8.91		74.90	5.65	8.79		max 233 (30,300) sh 260 (16,400) min 227 (29,600)
3c	90	120 softens 110–115	$C_{55}H_{54}N_6O_6S$	71.25	5.87	9.07	3.46	71.16	5.79	9.01	3.45	max 233 (31,700) sh 260 (16,700) min 225 (30,000)
7a	92	113–116	$C_{55}H_{54}N_6O_5$	75.14	6.19	9.56		74.68	6.36	9.78		max 230 (28,200) max 258 (17,000) min 226 (27,600) min 247 (15,700)
7b	88	121–123	$C_{56}H_{52}N_6O_5$	76.29	5.74	9.21		76.59	5.89	9.15		max 228 (30,300) max 259 (17,300) min 247 (16,000)
7c	84	110–114	$C_{54}H_{52}N_6O_5S$	72.30	5.84	9.37	3.58	72.38	6.22	9.10	3.61	max 231 (30,100) max 259 (17,900) min 226 (29,400) min 249 (16,500)

[a] All analytical samples were recrystallized from methanol and dried for 15 hr at room temperature over phosphorus pentoxide and paraffin wax at 0.1 mm. [b] See footnote 3 in text. [c] Determined in methanol.

3.75 (s, 3, OCH_3), 4.74 (m, 2, $H_{2'}$, $H_{4'}$), 5.94 (d, $J_{1'-2'}$ = 3.0 Hz, 1, $H_{1'}$), 6.21 (br, 2, 6-NH_2), 6.7–7.5 (m, 14, aromatic), 8.09 (s, 1, H_2), 8.30 (s, 1, H_8).

Anal. Calcd for $C_{30}H_{29}N_5O_4$: C, 68.52; H, 5.58; N, 13.38. Found: C, 68.62; H, 5.64; N, 13.45.

Preparation of 3′-O-(N-Tritylaminoacyl)-5′-O-(di-p-methoxytrityl)-2′-deoxyadenosines (**3a–c**) *and 2′-O-(N-Tritylaminoacyl)-5′-O-(mono-p-methoxytrityl)-3′-deoxyadenosines* (**7a–c**). These blocked derivatives were prepared from the appropriate *N*-tritylamino acid anhydride (**2a–c**) (generated *in situ* from the corresponding *N*-tritylamino acid by the general procedure of Rammler and Khorana (1963)) and 5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**1**) (for **3a–c**) or 5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosine (**6**) (for **7a–c**) (see Tables I and II for data) by the same general procedure as illustrated in the following.

3′-O-(N-Trityl-L-methionyl)-5′-O-(di-p-methoxytrityl)-2′-deoxyadenosine (**3c**). To a solution of 2.42 g (0.0062 mol) of *N*-trityl-L-methionine in 15.5 ml of dry EtOAc was added 1.28 g (0.0062 mol) of *N,N′*-dicyclohexylcarbodiimide (DCC). The precipitation of *N,N′*-dicyclohexylurea (DCU) began immediately and the mixture was stirred for 1 hr at room temperature. The mixture was filtered directly into a solution of 1.44 g (0.0026 mol) of dried **1** in 15.5 ml of dry EtOAc. The DCU filter cake (0.6 g) was washed with an additional 15 ml of dry EtOAc (filtrate collected into reaction solution). A 0.1-g (0.0008 mol) portion of 4-*N,N*-dimethylaminopyridine was added and the reaction was stirred for 21 hr at 15°.[1] Tlc ($CHCl_3$–Me_2CO, 80:20) showed essentially complete conversion of **1** to **3c**. An additional 0.29 g of DCU was removed by filtration and this filter cake was washed with 50 ml of EtOAc. The combined filtrate was washed with 2 × 100 ml of ice-cold saturated aqueous $NaHCO_3$ and 2 × 100 ml of H_2O and evaporated. The resulting solid foam was dissolved in 10 ml of $CHCl_3$ and precipitated into 350 ml of pentane with vigorous stirring. The white solid (3.19 g) was filtered, air-dried, dissolved in 12 ml of $CHCl_3$, and applied to a column (2.5 × 33 cm, 154 g, packed in $CHCl_3$) of alumina. The column was developed with $CHCl_3$ and fractions were evaluated by tlc ($CHCl_3$–Me_2CO, 80:20). The first 250 ml of eluate contained fast migrating material and was discarded.

[1] Several preliminary reactions were investigated using varying amounts of pyridine, without success. For example, this reaction in the presence of 2.5 ml of pyridine (but no 4-*N,N*-dimethylaminopyridine) failed to proceed observably (tlc) in 30 hr at room temperature.

TABLE II: Nmr Data on Fully Blocked Aminoacyl Derivatives.[a]

Compound	6-NH_2	$H_{1'}$	$H_{2'}$	$H_{3'}$	OCH_3	Amino Acid
3a	6.05	6.21[b]		4.82	3.76	0.91[c]
3b	5.87	5.88[b]		4.54	3.68	3.06[d]
3c	5.94	6.20[b]		4.85	3.77	2.09[e]
7a	5.89	5.63[f]	5.08		3.77	0.92[c]
7b	5.95	5.47[f]	4.87		3.72	3.12[d]
7c	5.98	5.59[f]	5.08		3.74	2.08[e]

[a] Chemical shifts in δ from Me_4Si internal in $CDCl_3$. [b] ABX "quartet", $J_{1'-2',2''} \sim 6$ and 8.5 Hz. [c] Poorly resolved doublet, $CH(CH_3)_2$. [d] Poorly resolved doublet, CH_2Ph. [e] Singlet, SCH_3. [f] Doublet, $J_{1'-2'} \sim 1.5$ Hz.

TABLE III: Aminoacyl Deoxynucleosides.[a]

Compound	Yield (%)	Mp[b] (°C)	Formula	Elemental Anal. Calcd (%) C	H	N	S	Found (%) C	H	N	S	Uv Spectra nm (ε)[c]	$[\alpha]_D$[d]
4a	90	130	$C_{16}H_{24}N_6O_4$	52.73	6.64	23.06		52.49	6.61	23.06		max 260 (14,600) min 227 (2600)	−26.5° (*c* 0.97)[f]
4b[e]	95	Slowly softens above 75	$C_{19}H_{22}N_6O_4 \cdot 0.5HCO_2H$	55.57	5.50	19.94		55.27	5.53	19.92		max 260 (14,900) min 227 (2400)	−7.58° (*c* 1.06)[f]
4c	86	136–140	$C_{15}H_{22}N_6O_4S$	47.10	5.80	21.98	8.38	47.40	5.92	21.74	8.12	max 259 (13,300) min 227 (2200)	−32.8° (*c* 0.97)[f]
8a	67	169–170	$C_{16}H_{24}N_6O_4$	52.73	6.64	23.06		52.83	6.79	22.97		max 258 (14,600) min 227 (2100)	−58.2° (*c* 0.73)[g]
8b	88	110–113	$C_{19}H_{22}N_6O_4$	57.27	5.57	21.10		56.96	5.86	20.99		max 259 (14,400) min 227 (2900)	−28.8° (*c* 1.03)[g]
8c	86	132–137	$C_{15}H_{22}N_6O_4S$	47.10	5.80	21.98	8.38	47.01	5.82	21.70	8.55	max 259 (14,400) min 227 (2800)	−60.1° (*c* 1.01)[g]

[a] All analytical samples were recrystallized from dry ethyl acetate and dried for 15 hr at 56° over potassium hydroxide pellets and paraffin wax at 0.1 mm. [b] See footnote 3 in text. [c] Determined in methanol. [d] Determined in dimethylformamide freshly distilled from phosphorus pentoxide at reduced pressure. [e] Dried at room temperature, 0.5 mole ratio of HCO_2H verified by nmr. [f] Determined at 20°. [g] Determined at 22°.

TABLE IV: Nmr Data on Aminoacyl Deoxynucleosides.[a]

Compound	6-NH_2	$H_{1'}$	$H_{2'}$	$H_{3'}$	Amino Acid
4a	7.31	6.38[b]		5.40	0.87 and 0.94[c]
4b	7.28	6.26[b]		5.30	2.90[d]
4c	7.30	6.38[b]		5.37	2.06[e]
8a	7.25	6.08[f]	5.63		0.85 and 0.92[c]
8b	7.29	6.06[f]	5.55		2.87[d]
8c	7.27	6.09[f]	5.61		2.01[e]

[a] Chemical shifts in δ from Me_4Si internal in Me_2SO-d_6. [b] ABX "quartet", $J_{1'-2',2''} \sim 6.3$ and 8.7 Hz. [c] Two doublets, $J \sim 3.0$ Hz, $CH(CH_3)_2$. [d] Doublet, $J \sim 7$ Hz, $-CH_2Ph$. [e] Singlet, SCH_3. [f] Doublet, $J_{1'-2'} \sim 2$ Hz.

The succeeding fractions containing only product were combined and evaporated. The resulting white solid foam was precipitated from 10 ml of $CHCl_3$ into 350 ml of pentane and the resulting white solid (**3c**, 2.17 g, 90%) was dried at room temperature over "P_2O_5"–paraffin wax. (See Tables I and II for data.)

Deblocking to Give the 3′-O-Aminoacyl-2′-deoxyadenosines (**4a–c**) *and 2′-O-Aminoacyl-3′-deoxyadenosines* (**8a–c**). These compounds were prepared from the blocked derivatives **3a–c** and **7a–c**, respectively, by the same general procedure as illustrated here for the following.

3′-O-(L-*Methionyl*)*-2′-deoxyadenosine* (**4c**). To 30 ml of a solution of 98% formic acid–1-butanol–toluene (1:1:1 by volume) was added 0.42 g (0.00045 mol) of **3c** and the resulting orange-red solution was allowed to stand at room temperature for 3 min.[2] An additional 100 ml of 1-butanol was added and the now colorless solution was evaporated to approximately 2 ml (vacuum pump, <20°). Two successive coevaporations with 100-ml portions of 1-butanol–toluene (1:1) gave a gum which was stirred magnetically with four successive 50-ml portions of pentane. The supernatant solutions were decanted and the final portion was filtered to give 0.16 g (86%) of **4c** (as the hemiformate). A sample for analysis was recrystallized[3] from dry EtOAc and dried at 56° (0.01 mm) over KOH pellets and paraffin wax. (See Tables III and IV for data.)

3′-O-(*N-Acetyl-*L*-phenylalanyl*)*-2′-deoxyadenosine* (**5b**). To a solution of 0.72 g (0.004 mol) of *p*-nitrophenyl acetate in 10 ml of absolute EtOH was added 0.21 g (0.0005 mol) of **4b** hemiformate. The solution was stirred for 16 hr at room temperature at which time tlc (*p*-dioxane–acetonitrile, 9:1) indicated the reaction was complete and 25 ml of H_2O was added. This mixture was extracted with 4 × 25 ml of $CHCl_3$ and the combined organic phase was evaporated. The residue was dissolved in 4 ml of Me_2CO–$CHCl_3$ (20:80) and applied to a column (2.5 × 23 cm, 35 g, packed in the same solvent mixture) of silica gel. Elution of fast migrating materials (tlc, *p*-dioxane–acetonitrile, 9:1) was effected with 250 ml of Me_2CO–$CHCl_3$ (20:80) and the solvent was changed to absolute EtOH. The first 50 ml was uv transparent and the following 225 ml contained only **5b**. These combined fractions were evaporated to give a white solid foam which was precipitated from acetone into pentane (or crystallized[3] from Et_2O–$CHCl_3$) to give 0.17 g (76%) of **5b**. A sample for analysis was recrystallized[3] from dry EtOAc–absolute EtOH–pentane and dried at 56° (0.01 mm) over KOH pellets and paraffin wax to

[2] Reaction time was 20 min for the 5′-*O*-(mono-*p*-methoxytrityl) compounds **7a–c**.

[3] These products were subjected to the usual process of recrystallization (*i.e.*, dissolved by warming in solvent and found to separate from solution upon cooling). However, most of the samples did not exhibit diffracting properties under a polarizing microscope and are probably amorphous or microcrystalline solids.

give **5b**: mp 214–215°; $[\alpha]_D^{20}$ −18.1° (*c* 0.99, DMF); uv (MeOH) max 260 nm (ε 17,100) min 227 nm (ε 3200); nmr (Me_2SO-d_6, TMS internal) δ 1.85 (s, 3, $COCH_3$), 2.22–2.62 (m, 1, $H_{2'}$), 2.74–3.14 (m, 1, $H_{2''}$), 3.01 (d, *J* = 7.5 Hz, 2, CH_2Ph), 3.57 (m, 2, $H_{5',5''}$), 3.85 (m, 1, $H_{4'}$), 4.48 (d of t, *J* = 7.5 and 7.5 Hz, 1, $-CH(NHAc)CH_2Ph$), 5.24–5.54 (m, 2, 5′-OH, $H_{3'}$), 6.27 ("q," $J_{1'-2',2''}$ = 8.4 and 5.6 Hz, 1, $H_{1'}$), 7.30 (m, 7, 6-NH_2, C_6H_5), 8.16 (s, 1, H_2), 8.34 (s, 1, H_8), 8.40 (d, *J* = 7.5 Hz, N*H*Ac).

Anal. Calcd for $C_{21}H_{24}N_6O_5$: C, 57.26; H, 5.49; N, 19.08. Found: C, 57.16; H, 5.44; N, 18.97.

3′-O-(N-Formyl-L-methionyl)-2′-deoxyadenosine (**5c**). To a solution of 0.20 g (0.0005 mol) of **4c** hemiformate in 20 ml of MeOH at 0° was added approximately 0.04 ml (0.0006 mol) of acetic formic anhydride (Krimen, 1970) and the solution was stirred for 10 min at 0°. Tlc (*p*-dioxane–acetonitrile, 9:1) indicated that reaction was complete. Addition of 30 ml of 1-butanol–toluene (1:1) was followed by evaporation of the resulting solution (vacuum pump, <20°). The residue was co-evaporated with 15 ml of absolute EtOH and then 15 ml of CH_2Cl_2 to give a white powder. This material was recrystallized[3] from CH_2Cl_2 (with a second crop obtained by adding pentane) to yield 0.15 g (73%) of **5c** (dried at room temperature (0.01 mm) over KOH pellets and paraffin wax for 15 hr): mp 165–167°; $[\alpha]_D^{20}$ −43.8° (*c* 1.07, DMF); uv (MeOH) max 260 nm (ε 17,100) min 227 nm (ε 4500); nmr (Me_2SO-d_6, TMS internal) δ 1.74–2.14 (m, 2, CH_2CH_2S), 2.05 (s, 3, SCH_3), 2.36–2.64 (m, 3, $H_{2'}$, CH_2S), 2.78–3.10 (m, 1, $H_{2''}$), 3.62 (m, 2, $H_{5',5''}$), 4.08 (m, 1, $H_{4'}$), 4.47 ("t," 1, CH(NHCOH)), 5.42 (m, 2, 5′-OH, $H_{3'}$), 6.36 ("q," $J_{1'-2',2''}$ = 8 and 6 Hz, 1, $H_{1'}$), 7.31 (s, 2, 6-NH_2), 8.14 (s, 1, $-NHCOH$), 8.16 (s, 1, H_2), 8.38 (s, 1, H_8), 8.62 (d, *J* = 8 Hz, 1, N*H*COH).

Anal. Calcd for $C_{16}H_{22}N_6O_5S$: C, 46.82; H, 5.40; N, 20.48; S, 7.81. Found: C, 46.54; H, 5.49; N, 20.52; S, 7.53.

2′-O-(N-Acetyl-L-phenylalanyl)-3′-deoxyadenosine (**9b**). Acetylation of **8b** was effected by the procedure described above for the conversion of **4b** → **5b**. The product **9b** was obtained in 80% yield and had mp 176–178°: $[\alpha]_D^{22}$ −56.7° (*c* 1.05, DMF); uv (MeOH) max 258 nm (ε 14,600) min 227 nm (ε 2500); nmr (Me_2SO-d_6, TMS internal) δ 1.80 (m, 1, $H_{3'}$), 1.82 (s, 3, $COCH_3$), 2.45 (m, 1, $H_{3''}$), 2.98 (d, *J* = 8 Hz, CH_2Ph), 3.47 and 3.56 (d and d, *J* = 3 and 3 Hz, 1 and 1, $H_{5'}$ and $H_{5''}$, observed upon D_2O exchange), 4.11 (m, 1, $H_{4'}$) 4.50 (t, *J* = 8 Hz, 1, CHNHAc, observed upon D_2O exchange), 5.08 (m, 1, 5′-OH), 5.58 (m, 1, $H_{2'}$), 6.05 (d, $J_{1'-2'}$ = 2.5 Hz, 1, $H_{1'}$), 7.25 (br s, 7, 6-$NH_{2'}$ $-C_6H_5$), 8.17 (s, 1, H_2), 8.33 (s, 1, H_8), 8.41 (d, *J* = 8 Hz, 1, CHN*H*Ac).

Anal. Calcd for $C_{21}H_{24}N_6O_5$: C, 57.26; H, 5.49; N, 19.08. Found: C, 57.12; H, 5.51; N, 19.11.

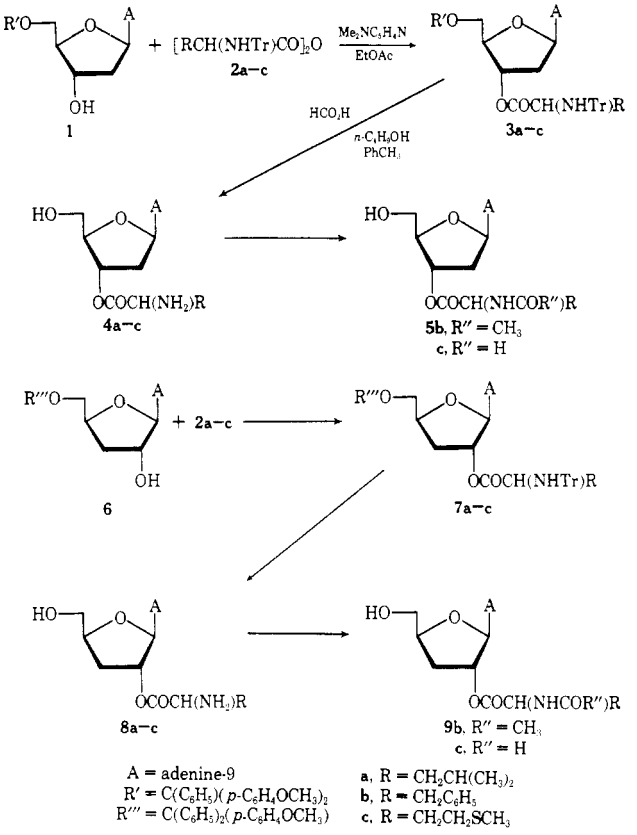
2′-O-(N-Formyl-L-methionyl)-3′-deoxyadenosine (**9c**). Formylation of **8c** was effected with acetic formic anhydride in MeOH as described above for the conversion of **4c** → **5c**. The product **9c** was obtained in 85% yield and had mp 189–191°: $[\alpha]_D^{22}$ −82.1° (*c* 0.99, DMF); uv (MeOH) max 259 nm (ε 13,700) min 227 nm (ε 2400) nmr (Me_2SO-d_6, TMS internal) δ 1.84–2.24 (m, 3, CH_2CH_2S, $H_{3'}$), 2.05 (s, 3, SCH_3), 2.4–2.6 (m, 3, CH_2S, $H_{3''}$), 3.60 (m, 2, $H_{5',5''}$), 4.20–4.60 (m, 2, CHNHCOH, $H_{4'}$), 5.10 (t, *J* = 6 Hz, 1, 5′-OH), 5.67 (m, 1, $H_{2'}$), 6.09 (d, $J_{1'-2'}$ = 2.0 Hz, 1, $H_{1'}$), 7.25 (s, 2, 6-NH_2), 8.09 (s, 1, NHCO*H*), 8.14 (s, 1, H_2), 8.32 (s, 1, H_8), 8.55 (d, *J* = 8 Hz, 1, CHN*H*COH).

Anal. Calcd for $C_{16}H_{22}N_6O_5S$: C, 46.82; H, 5.40; N, 20.48; S, 7.81. Found: C, 47.02; H, 5.61; N, 20.48; S, 7.86.

Results

The fully protected aminoacyl 2′-deoxynucleosides **3a–c** (see Reaction Scheme) were prepared by treatment of 5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (5′-*O*-di-*p*-anisylphenylmethyl-2′-deoxyadenosine) (**1**) (Hogenkamp and Oikawa, 1964) with *N*-tritylamino acid anhydrides **2a–c** in the presence of 4-*N*,*N*-dimethylaminopyridine. The anhydrides were generated *in situ* from the corresponding *N*-tritylamino acids (Zervas and Theodoropoulos, 1956; Stelakatos *et al.*, 1959) and DCC by the general procedure of Rammler and Khorana (1963). Although the coupling reactions did not proceed under the normal conditions in pyridine solution (Rammler and Khorana, 1963), the use of 4-*N*,*N*-dimethylaminopyridine obviated this difficulty and 3′-*O*-(*N*-trityl-L-leucyl)-5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**3a**), 3′-*O*-(*N*-trityl-L-phenylanyl)-5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**3b**), and 3′-*O*-(*N*-trityl-L-methionyl)-5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**3c**) were obtained in high yields (see Table I). Treatment of 3′-deoxyadenosine (cordycepin) (Robins *et al.*, 1973) with monomethoxytrityl chloride in pyridine gave 5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosine (**6**) in 57% yield. Analogous condensation reactions of **6** with **2a–c** gave high yields (see Table I) of 2′-*O*-(*N*-trityl-L-leucyl)-5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosine (**7a**), 2′-*O*-(*N*-trityl-L-phenylalanyl)-5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosine (**7b**), and 2′-*O*-(*N*-trityl-L-methionyl)-5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosine (**7c**). Deblocking of **3a–c** and **7a–c** proceeded smoothly in 98% formic acid–1-butanol–toluene (1:1:1) to give 3′-*O*-(and 2′-*O*-)L-leucyl-2′-(and 3′-)deoxyadenosines (**4a** and **8a**), 3′-*O*-(and 2′-*O*-)L-phenylalanyl-2′-(and 3′-)-deoxyadenosines (**4b** and **8b**), and 3′-*O*-(and 2′-*O*-)L-methi-

REACTION SCHEME



onyl-2′-(and 3′-)deoxyadenosines (**4c** and **8c**), respectively, in good yields (see Table III).

Treatment of **4b** and **8b** with *p*-nitrophenyl acetate in alcohol effected N acetylation of the amino acid moiety to give 3′-*O*-(and 2′-*O*-)*N*-acetyl-L-phenylalanyl-2′-(and 3′-)-deoxyadenosines (**5b** and **9b**) in 76 and 80% yields, respectively. Acetic formic anhydride in methanol smoothly N formylated the amino acid portion of **4c** and **8c** to give 3′-*O*-(and 2′-*O*-)*N*-formyl-L-methionyl-2′-(and 3′-)deoxyadenosines (**5c** and **9c**) in 73 and 85% yields, respectively.

Discussion

The wide scope of interest in the processes of protein biosynthesis in general and the puromycin reaction in particular (for example, see recent reviews by Lucas-Lenard and Lipmann (1971), Suhadolnik (1970), and the entire *Cold Spring Harbor Symp. Quant. Biol., 34* (1969)) has stimulated the preparation and study of many aminoacyl derivatives of various nucleosides, nucleotides, and oligonucleotides as noted in the introduction. Most of these studies have used the general procedure of Rammler and Khorana (1963) to produce 3′(2′) ester mixtures which were characterized by qualitative or semiquantitative spectral and chromatographic evaluation.

The ribonucleoside products are known to be quite unstable in aqueous solution with typical half-lives of 5–30 min at near neutrality and about 30° (Rammler and Khorana, 1963; Waller *et al.*, 1966; see discussion and references in Zachau and Feldmann (1965)). This instability has been attributed to hydrogen-bonding stabilization of the hydrolytic transition state by the neighboring (2′ or 3′) *cis*-hydroxyl group (Bruice and Fife, 1962; Zachau and Karau, 1960; Griffin and Reese, 1964). Removal of this vicinal hydroxyl group would be expected to confer added stability (Griffin and Reese, 1964) and make the resulting aminoacyl nucleosides more amenable to biochemical study in aqueous media.

Žemlička *et al.* (1969) reported the preparation of 3′-*O*-(L-phenylalanyl)-2′-deoxyadenosine (**4b**) using the symmetrical anhydride coupling with the carbobenzyloxy (Cbz) amino acid followed by catalytic hydrogenolysis for removal of the Cbz blocking group. Their product was characterized, however, only by qualitative chromatography and spectrophotometry and was biochemically evaluated as a "stock solution." Our initial studies employed Cbz amino acids. However, during catalytic hydrogenolysis of the Cbz group, the presence of significant quantities of free nucleoside plus amino acid were detected even before complete deblocking occurred as observed by others (Chládek *et al.*, 1970). In this regard it is probably significant to note that a nucleoside/phenylalanine ratio of 1.3 was recorded for the previously reported preparation of **4b** (Žemlička *et al.*, 1969). Conditions reported for deblocking *tert*-butyloxycarbonyl (*t*-Boc) amino acids were expected to be too severe for the deoxynucleoside glycosidic bond and so *N*-tritylamino acids (Zervas and Theodoropoulos, 1956; Stelakatos *et al.*, 1959) were chosen.

Attempted condensation of *N*-tritylamino acids with 5′-*O*-(di- and mono-*p*-methoxytrityl) deoxynucleosides **1** and **6** using the *in situ* generated symmetrical anhydride (Rammler and Khorana, 1963) with pyridine as catalyst failed completely presumably due to the steric effect of the trityl group. It had been reported (Steglich and Höfle, 1969) that 4-*N*,*N*-dimethylaminopyridine markedly expedited the acylation of hindered alcohols. Indeed, addition of catalytic quantities of this compound to the above ethyl acetate reaction solution resulted in rapid and essentially quantitative conversion (tlc) of **1** or **6** to the desired *N*-tritylaminoacyl deoxynucleosides **3a–c** or **7a–c**, respectively (see Experimental Section).[4]

Although Chládek *et al.* (1970) fully characterized blocked derivatives of aminoacyl nucleosides and obtained certain deblocked products as solids, the latter were characterized only by chromatography and spectroscopy. Indeed, it appears that previous free aminoacyl nucleosides have been studied as "stock solutions" or freeze-dried powders of not rigorously characterized constitution. This arises primarily from the inherent instability of these compounds and sometimes unsatisfactory deblocking procedures. Since the present molecules were expected to have enhanced ester stability due to the absence of a vicinal hydroxyl (Zachau and Karau, 1960; Griffin and Reese, 1964), we sought a procedure which would allow high yield isolation and complete characterization of the free aminoacyl deoxynucleosides to make them available for carefully defined biochemical studies.

It was eventually found that complete deblocking of **3a–c** and **7a–c** was affected with no observable (tlc) cleavage of aminoacyl ester or glycosidic bonds using a solution of formic acid–1-butanol–toluene (1:1:1) at room temperature (see Experimental Section). The solution was further diluted with 1-butanol and toluene to prevent N formylation during evaporations. These solvents provide favorable azeotropic properties and *n*-butyl formate and the various *n*-butyl trityl ethers are highly lipophilic, enhancing separation from product nucleoside derivatives. The final chromatographically homogeneous products **4a–c** and **8a–c** were isolated in high yields and could be recrystallized.[3] See Tables III and IV for yields, physical constants, and analytical data. It is interesting to note that oxidation problems were not encountered during the syntheses of the methionine compounds as previously observed by others (Chládek *et al.*, 1970).

Since *E. coli* and other prokaryotic systems exclusively employ a specific tRNA ($fMet\text{-}tRNA_f^{Met}$) for protein biosynthesis initiation (Lucas-Lenard and Lipmann, 1971 and references therein) and *N*-acetylphenylalanyl-tRNA can simulate this initiation (Lucas-Lenard and Lipmann, 1967), the *N*-formylmethionyl derivatives **5c** and **9c** and *N*-acetylphenylalanyl derivatives **5b** and **9b** were prepared to evaluate biochemical consequences of these initiator models. The active ester approach of Bodánszky (1955) employing *p*-nitrophenyl acetate in alcoholic medium gave smooth N acetylation of the phenylalanine amino group. Similarly, acetic formic anhydride in methanolic solution produced facile selective N formylation of the methionine amino moiety.

Compounds **4a–c**, **5b,c**, **8a–c**, and **9b,c** were evaluated in cell free protein biosynthesis systems and also against *E. coli* K-12 and *E. coli* B cell lines for antibacterial activity (M. J. Robins, C. Coutsogeorgopoulos, and A. Bloch, to be published). It is of considerable interest to note that the 3′-*O*-aminoacyl-2′-deoxy nucleosides **4a–c** and **5b,c** showed 50% inhibition of the *E. coli* lines in the order of tenfold lower concentration than puromycin (*i.e.*, in the range of 10^{-6} M *vs.* 10^{-5} M for puromycin). The *N*-formylmethionine-containing **5c** and *N*-acetylphenylalanyl-containing **5b** showed comparable inhibitory activity to the free aminoacyl products **4c** and **4b**. However, the 2′-*O*-aminoacylcordycepin deriva-

[4] The use of 4-*N*,*N*-dimethylaminopyridine dramatically facilitates other nucleoside acylations (to be published) and should be explored in peptide chemistry [*e.g.*, mixed anhydrides of bulky *N*-tritylamino acids failed to condense using triethylamine (Zervas and Theodoropoulos, 1956)].

tives **8a,b** had inhibitory levels comparable to puromycin whereas the methionine-containing **8c** was in the order of 100-fold less active and **9b,c** showed no inhibitory activity toward the two bacteria at levels tested. The recent finding by Sprinzl *et al.* (1973) that cordycepin-terminated $tRNA^{Phe}$ can be charged whereas the 2′-deoxyadenosine-terminated analog cannot enhances the interest in the differential activity of the cordycepin derivatives **8a–c** and **9b,c**.

The aminoacyl products of this study do not appear to function in the "puromycin reaction," confirming the report of Rychlik *et al.* (1969) for **4b**. Fox *et al.* (1966) have suggested an empirical proposal for the inhibition of protein biosynthesis by nucleoside antibiotics with two basic centers. However, it should be recognized that the present aminoacyl deoxynucleosides might function as antibacterial molecules by completely different mechanisms.

Details of biochemical studies on these products and the synthesis and evaluation of analogous 3′-*O*-aminoacyl-2′-*O*-methyladenosines and 2′-*O*-aminoacyl-3′-*O*-methyladenosines will be reported (M. J. Robins *et al.*, in preparation).

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