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Aminoacyl Derivatives of 4-Thiothymidine, Cytosine, and Cytidine

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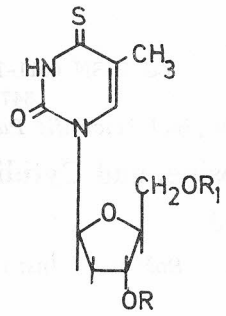
5'-*O*-Triphenylmethyl-4-thiothymidine (III) was coupled to *N*-benzyloxycarbonyl-D,L-alanine or *N*-*t*-butyloxycarbonyl-L-phenylalanine by the *N*,*N*-dicyclohexylcarbodiimide method, yielding 3'-nucleoside esters V and VII. The removal of the protecting triphenylmethyl and *t*-butyloxycarbonyl groups of compound VII afforded 3'-*O*-(L-phenylalanyl)-4-thiothymidine (IX).

Appropriately blocked glycylglycine and L-phenylalanine were also coupled to cytosine and 2',3'-*O*-*iso*propylidene-cytidine by the active ester or dicyclohexylcarbodiimide method, yielding *N*-4-acylated cytosine X and cytidine XIII. It was established that the catalytic hydrogenation of 4-*N*-(glycylglycyl)-cytosine (XI) over 5% rhodium on carbon afforded 4-*N*-(glycylglycyl)-5,6-dihydrocytosine (XII) in a quantitative yield.

Since the discovery of the direct involvement of specific 3'(2')-*O*-aminoacyl transfer-ribonucleic acids and of the possible 2'⇄3'-*O*-migration of their aminoacyl group throughout the various stages of protein biosynthesis, the isolation and synthesis of nucleoside amino acids have been the subject of considerable interest. Thus, aminoacyl and peptidyl components at positions other than at 3'-*O* of transfer-ribonucleic acids[1,2], their greater quantity firmly bound to deoxyribonucleic acids in tumors rather than in nonmalignant tissues of reference[3], as well as their possible »punctuation« role in the genetic code[4] acting as »derepressors« of structural genes[5], directed our studies towards the synthesis and properties of hitherto unknown nucleoside amino acids and peptides. This paper is concerned with suitably protected 4-thiothymidine, cytosine, and cytidine in the coupling reactions with D,L-phenylalanine and glycylglycine.

We first showed that 3'-*O*-acetyl-5'-*O*-triphenylmethyl-thymidine[6] could be thiated to give 3'-*O*-acetyl-5'-*O*-triphenylmethyl-4-thiothymidine (I), which was also obtained from 5'-*O*-triphenylmethyl-4-thiothymidine (III) by acetylation. To prepare 3'-*O*-acetyl-4-thiothymidine (II), already described in anti-conformation[7], compound I was detritylated in 80% acetic acid. Tritylation of 4-thiothymidine[8] yielded 3',5'-*O*-di-triphenylmethyl-4-thiothymidine (IV) and 5'-*O*-trityl derivative III. Compound III was also obtained by deacylation of I in concentrated ammonia — dioxane (1 : 1).



I R=Ac, $R_1=Ph_3C$

II R=Ac, $R_1=H$

III R=H, $R_1=Ph_3C$

IV $R=R_1=Ph_3C$

V R=D,L-ZHNCH(CH_2Ph)CO, $R_1=Ph_3C$

VI R=D,L-ZHNCH(CH_2Ph)CO, $R_1=H$

VII R=L-BOCHNCH(CH_2Ph)CO, $R_1=Ph_3C$

VIII R=L-BOCHNCH(CH_2Ph)CO, $R_1=H$

IX R=L-H_2NCH(CH_2Ph)CO, $R_1=H$

Z = N-benzyloxycarbonyl

BOC = N-t-butyloxycarbonyl

The 5'-*O*-protected 4-thiothymidine III was coupled to *N*-benzyloxycarbonyl-D,L-phenylalanine or *N*-*t*-butyloxycarbonyl-L-phenylalanine in the presence of dicyclohexylcarbodiimide. Thus, 3'-*O*-(*N*-benzyloxycarbonyl-D,L-phenylalanyl)-(V) and 3'-*O*-(*N*-*t*-butyloxycarbonyl-L-phenylalanyl)-(VII) 5'-*O*-triphenylmethyl-4-thiothymidines were obtained.

The detritylation of V and VII in 80% acetic acid afforded 3'-*O*-(*N*-benzyloxycarbonyl-D,L-phenylalanyl)-(VI) and 3'-*O*-(*N*-*t*-butyloxycarbonyl-L-phenylalanyl)-(VIII) 4-thiothymidines, respectively. Finally, the hydrolysis of compound VIII in 98% trifluoroacetic acid gave 3'-*O*-(L-phenylalanyl)-4-thiothymidine (IX) in an 84% yield.

To learn the stabilities of nucleoside esters in protic solvents, compound IX was exposed to water - ethanol (7 : 1) at room temperature (pH 7.72). The measurements of pH revealed a complete hydrolysis of ester linkage after 21 h (pH 7.30). At the same time chromatography on a silica gel plate registered the disappearance of nucleoside ester IX ($R_f \approx 0.6$), and the appearance of 4-thiothymidine ($R_f \approx 0.5$) and L-phenylalanine.

The uv and ir spectroscopic data of 4-thiothymidine, cytosine, and cytidine derivatives, described in this paper, confirmed the proposed structures. The NMR data for the pyrimidine and ribosyl protons of 4-thiothymidine derivatives are in accordance with those of pyrimidine ribonucleosides (see Table). Ring NH's appeared as broad signals at τ —0.10 to —0.71. The aromatic protons of triphenylmethyl, benzyloxycarbonyl, and phenyl-alanyl moieties were situated at τ 2.50—2.86 as multiplets. The methyl protons of the *t*-butyloxycarbonyl group of compounds VII and VIII appeared at τ 8.59(s) and 8.62(s), while those of acetyl group of I and II at 7.94(s) and 7.93(s), respectively. The methylene protons in phenylalanyl residues showed multiplets at τ 6.86—7.06, while those in the benzyloxycarbonyl of V and VI appeared at τ 4.94 and 4.87 as broad singlets. Nucleoside esters V and VI containing racemic phenylalanine showed undefined H-1' resonances at $\tau \approx 3.66$—3.93 or splitting at $\tau \approx 3.97$.

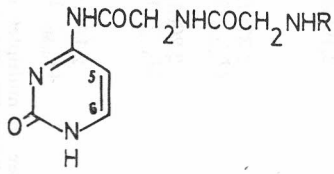
TABLE I

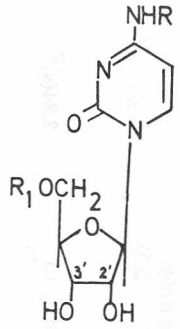
NMR spectra[a,b] *(τ values) of 4-thiothymidine derivatives*

Compound	H—1′	H_2—2′	H—3′	H—4′	H_2—5′	H—6	Me—C
I	3.60(t) ($J_{1',2'}$ 7.2)	(m)[c] 7.42——7.65	(m) 4.45——4.63	5.84(q) ($J_{4',5'}$ 2.5) ($J_{4',3'}$ 5.0)	6.52(d) ($J_{5',4'}$ 2.5)	2.34(d) ($J_{6,Me}$ 1.2)	8.39(d) ($J_{Me,6}$ 1.2)
II	3.74(t) ($J_{1',2'}$ 7.0)	7.56(q) ($J_{2',1'}$ 7.0) ($J_{2',3'}$ 4.6)	(m) 4.52——4.71	5.86(q) ($J_{4',5'}$ 2.5) ($J_{4',3'}$ 5.0)	6.07(d) ($J_{5',4'}$ 2.5)	2.35(d) ($J_{6,Me}$ 1.2)	7.93(d) ($J_{Me,6}$ 1.2)
III	3.61(t) ($J_{1',2'}$ 6.6)	(m) 7.38——7.75	(m) 5.32——5.52	5.89(q) ($J_{4',5'}$ 2.5) ($J_{4',3'}$ 5.0)	6.57(d) ($J_{5',4'}$ 2.5)	2.34(s)[d]	8.35(s)[d]
IV	3.60(t)	(m) 7.90——8.17	(m) 5.50——5.77	(m) 6.08——6.23	(m) 6.64——7.27	2.44(s)[d]	8.40(s)[d]
V	(m) 3.66——3.93 ca.	(m) 7.53——7.90	(m) 4.75——4.80	(m) 5.91——6.29 ca.	(m) 6.56——6.69	2.43(s)[d]	8.44(s)[d]
VI	ca. 3.97(t) ($J_{1',2'}$ 7.0)	(m) 7.51——7.72	(m) 4.51——4.74	(m) 5.97——6.25 ca.		2.46(s)[d]	7.91(s)[d]
VII	3.74(t) ($J_{1',2'}$ 7.0)	(m) 7.44——7.75 ca.	(m) 4.46——4.66	(m) 5.97——6.21	6.59(d) ($J_{5',4'}$ 3.0)	2.37(d) ($J_{6,Me}$ 1.2)	8.39(s)
VIII[e]	3.87(t) ($J_{1',2'}$ 7.0)	(m) 7.58——7.87	(m) 4.62——4.84	(m) 6.08——6.34		2.20(d) ($J_{6,Me}$ 1.2)	7.97(d) ($J_{Me,6}$ 1.2)
IX[e]	3.89(t) ($J_{1',2'}$ 7.0)	(m) 7.55——7.77	(m) 4.61——4.86	(m) 6.11——6.34		2.19(d) ($J_{6,Me}$ 1.2)	7.96(d) ($J_{Me,6}$ 1.2)

[a] See introduction to Experimental section. [b] Values of doublets (d) triplets (t) and quartet (q) refer to multiplet centres. Coupling constants (J) are given in Hz. [c] Unresolved multiplets. [d] Splitting ca. refers to estimated positions when resonance is obscured by those of other protons. [e] Solution in CD_3OD.

The condensation of the *N*-hydroxysuccinimide ester of *N*-triphenylmethyl--glycylglycine with cytosine proceeded to 4-*N*-(*N*-triphenylmethyl-glycylglycyl)--cytosine (X), which was then detritylated to 4-*N*-(glycylglycyl)-cytosine (XI). The catalytic hydrogenation of XI in water over 5% rhodium on carbon afforded 4-*N*-(glycylglycyl)-5,6-dihydrocytosine (XII) in a quantitative yield.





X R=Ph_3C

XI R=H

XII R=H, 5,6-dihydro

XIII R=$Ph_3C(HNCH_2CO)_2$, R_1=H

XIV R=L-BOCHNCH(CH_2Ph)CO, R_1=H; 2',3'-O-isopropylidene

The unprotected cytidine did not satisfactorily couple to 4-*N*-(*N*-triphenylmethyl-glycylglycyl)-cytidine (XIII) (yield 17%) by the active ester procedure. A modification of the dicyclohexylcarbodiimide method[9] successfully converted 2',3'-*O*-*iso*propylidene-cytidine[10] into 4-*N*-(*N*-*t*-butyloxycarbonyl-L-phenylalanyl)-2'-3'-*O*-*iso*propylidene-cytidine (XIV).

EXPERIMENTAL

The same techniques and apparatus were used as described previously[11]. In addition, optical rotations were measured for the solution in anhydrous ethanol (1 = 1 dm) with a 179707 Zeiss-Winhel apparatus.

3'-O-*Acetyl*-5'-O-*triphenylmethyl*-4-*thiothymidine* (I)

(*a*) 3'-*O*-Acetyl-5'-*O*-triphenylmethyl-thymidine[6] (780 mg, 1.5 mmol) in pyridine (8.5 ml) and water (0.04 ml) was treated with phosphorus pentasulfide (340 mg, 1.5 mmol) and refluxed for 4 h. After an oil had been separated the remaining solution was poured into cooled water (20 ml), kept for 30 min. at room temperature, evaporated to dryness and the residue partitioned in methylene chloride—water. The methylene chloride extract (100 ml) was chromatographed on a silica gel (30 g) column. Methylene chloride—acetone (1 : 1, 200 ml) eluted a fraction (230 mg, 59%) which crystallized from anhydrous ethanol as yellow needles, m. p. 158—160 °C, $[\alpha]_D^{25}$ + 60.1° (c 0.5). Acetone eluted the starting material (400 mg).

Anal. $C_{31}H_{30}N_2O_5S$ (542.63) calc'd.: C 68.61; H 5.57; N 5.16; S 5.91%
found: C 68.40; H 5.87; N 5.29; S 5.82%

Uv spectrum: λ_{max} 206, 231 (infl.), 332 nm (log ε = 4.50, 3.95, 4.20); λ_{min} 275 nm (log ε = = 3.29). Ir spectrum: ν_{max} 3534, 3279, 3077, 2933, 1730, 1701, 1626, and 701 cm^{-1}.

(b) To a solution of 5′-*O*-triphenylmethyl-4-thiothymidine (III, 300 mg, 0.6 mmol) in anhydrous pyridine (2 ml), freshly distilled acetic anhydride (1 ml) was added. The mixture was stirred at room temperature for 16 h and then poured into cooled water (160 ml). The yellow precipitate was separated (325 mg), dissolved in methylene chloride and chromatographed on a silica gel plate (developed two times with methylene chloride). The fraction, $R_f \approx 0.5$, was eluted with acetone yielding 216 mg (67%), crystallized from anhydrous ethanol, m. p. 158—160 °C, identical (ir, uv, and NMR spectra) with the compound obtained in (a).

3′-O-Acetyl-4-thiothymidine (II)

3′-*O*-Acetyl-5′-*O*-triphenylmethyl-4-thiothymidine (I, 170 mg, 0.315 mmol) was refluxed in 80% acetic acid (5 ml) for 25 min. and then evaporated to dryness. The residue was dissolved in methylene chloride and chromatographed on a silica gel plate, which was then developed two times with methylene chloride—acetone (20 : 1). The fraction, $R_f \approx 0.4$, was eluted with methanol and crystallized from acetone-*n*-hexane (75 mg, 80%), m. p. 139—141 °C, $[\alpha]_D^{19}$ + 27.4° (c 1), (Scheit[8] reported m. p. 144 °C).

Anal. $C_{12}H_{16}N_2O_5S$ (300.33) calc'd.: C 47.99; H 5.37; N 9.33; S 10.67%
found: C 48.23; H 5.50; N 9.49; S 10.85%

Uv spectrum: λ_{max} 201, 239, 334 nm (log ε = 4.09, 3.59, 4.25); λ_{min} 217, 274 nm (log ε = = 3.38, 3.25). Ir spectrum ν_{max} 3356, 3030, 2915, 1736, 1704 (br), and 1616 cm^{-1}.

5′-O-Triphenylmethyl-4-thiothymidine (III)

(a) To a solution of 4-thiothymidine[8] (1.06 g, 4.1 mmol) in anhydrous pyridine (20 ml) chlorotriphenylmethane (1.4 g, 5.03 mmol) was added. This mixture was heated and stirred at 100 °C for 30 min., cooled and then poured slowly into chilled water (300 ml). The separated yellow oil was dissolved in methylene chloride, washed with water, and then evaporated to dryness. The residue was dissolved in methylene chloride (100 ml) and chromatographed on a silica gel (60 g) column. The methylene chloride—acetone (300 ml, 10 : 1) eluted a yellow foam (1.7 g, 83%), which was purified from methylene chloride-*n*-hexane as a microcrystalline product, m. p. 109—111 °C, $[\alpha]_D^{22}$ + 65.5 °C (c 1, CH_2Cl_2).

Anal. $C_{29}H_{28}N_2O_4S$ (500.59) calc'd.: C 69.58; H 5.64; N 5.59; S 6.40%
found: C 69.83; H 5.76; N 5.86; S 6.78%

Uv spectrum: λ_{max} 208, 231 (infl.), 335 nm (log ε = 4.48, 3.96, 4.22); λ_{min} 276 nm (log ε = 3.30). Ir spectrum: ν_{max} 3448, 3077, 2941, 1695, 1634, and 704 cm^{-1}.

(b) 3′-*O*-Acetyl-5′-*O*-triphenylmethyl-4-thiothymidine (I, 105 mg, 0.2 mmol) was treated with concentrated ammonia—dioxane (6 ml, 1 : 1) at room temperature for 24 h. The solution was evaporated to dryness. Preparative TLC (developed 5 times in methylene chloride) gave a fraction, $R_f \approx 0.3$, which was eluted with methanol as a yellow foamy product (62 mg, 65%), identical (ir and NMR spectra) with the compound obtained in (a).

3′, 5′-Di-O-triphenylmethyl-4-thiothymidine (IV)

The methylene chloride eluate from the chromatography described in IIIa afforded a yellow foam (243 mg, 11.9%), $R_f \approx 0{,}6$ [TLC in methylene chloride—acetone (10 : 1)], $[\alpha]_D^{20}$ + 95.8° (c 1.8, CH_2Cl_2).

Anal. $C_{48}H_{24}N_2O_4S$ (742.90) calc'd.: C 77.60; H 5.70; N 3.77; S 4.32%
found: C 77.85; H 5.99; N 3.93; S 4.53%

Uv spectrum: λ_{max} 205, 233 (infl.), 334 nm (log ε = 4.81, 4.21, 4.27); λ_{min} 276 nm (log ε = = 3.26). Ir spectrum: ν_{max} 3460, 3226, 3049, 2907, 1701, 1621, and 703 cm^{-1}.

3'-O-(N-Benzyloxycarbonyl-D,L-phenylalanyl)-5'-O-triphenylmethyl-4--thiothymidine (V)

To a solution of 5'-*O*-triphenylmethyl-4-thiothymidine (III, 118 mg, 0.236 mmol) in anhydrous pyridine *N*-benzyloxycarbonyl-D,L-phenylalanine (71 mg, 0.236 mmol) and dicyclohexylcarbodiimide (206 mg, 1 mmol) were added and kept at room temperature for 48 h. Dicyclohexylurea was filtered off and the solution evaporated to dryness under reduced pressure. The residue was dissolved in methylene chloride and washed with 10% acetic acid. From methylene chloride an oil was separated which was chromatographed on a silica gel plate, developed in methylene chloride and then in methylene chloride—acetone (10 : 1). The acetone eluted the fraction, $R_f \approx 0.2$, as starting material (36 mg) and then a foamy product, $R_f \approx 0.5$, 117 mg (67.3%), which crystallized from chloroform-*n*-hexane, m. p. 169—170 °C, $[\alpha]_D^{22}$ + 36.7° (c 1, CH_2Cl_2).

Anal. $C_{46}H_{43}N_3O_7S$ (781.88) calc'd.: C 70.66; H 5.54; N 5.37; S 4.10%
found: C 70.80; H 5.35; N 5.23; S 3.81%

Uv spectrum: λ_{max} 207, 230 (infl.), 258, 334 nm (log ε = 4.59, 3.93, 3.54, 4.21); λ_{min} 275 nm (log ε = 3.40). Ir spectrum: ν_{max} 3484, 3077, 2933, 1706 (br), 1626, and 701 cm^{-1}.

3'-O-(N-Benzyloxycarbonyl-D,L-phenylalanyl)-4-thiothymidine (VI)

3'-*O*-(Benzyloxycarbonyl)-5'-*O*-triphenylmethyl-4-thiothymidine (V, 1.5 g, 1.9 mmol) was refluxed in 80% acetic acid (50 ml) for 15 min., and then evaporated to dryness. Chromatography on a silica gel (60 g) column and methylene chloride (300 ml), methylene chloride — acetone (50 : 1, 100 ml) separated the starting material (40 mg). The yellow foamy product was eluted with methylene chloride — acetone (50 : 2, 200 ml and 50 : 5, 300 ml) yielding a product, 750 mg (73%), which crystallized from methylene chloride — *n*-hexane, m.p. 158—160 °C, $[\alpha]_D^{22}$ + 17.6° (c 1).

Anal. $C_{27}H_{29}N_3O_7S$ (539.58) calc'd.: C 60.10; H 5.42; N 7.79; S 5.94%
found: C 60.04; H 5.57; N 8.03; S 6.15%

Uv spectrum: λ_{max} 204, 242, 333 nm (log ε = 4.36, 3.48, 4.25); λ_{min} 225, 273 nm (log ε = 3.41, 3.28). Ir spectrum: ν_{max} 3390, 3247, 3049, 2924, 1709, 1678, 1631, and 698 cm^{-1}.

3'-O-(N-t-Butyloxycarbonyl-L-phenylalanyl)-5'-O-triphenylmethyl-4--thiothymidine (VII)

5'-*O*-Triphenylmethyl-4-thymidine (III, 500 mg, 1 mmol) in anhydrous pyridine (15 ml) was treated with *N*-*t*-butyloxycarbonyl-L-phenylalanine (400 mg, 1.5 mmol) and dicyclohexylcarbodiimide (1.0 g, 5 mmol) at room temperature for 48 h and then worked up as described for compound V. From the silica gel column methylene chloride — acetone (50 : 2.5, 50 ml and 50 : 5, 100 ml) eluted a yellow foamy product (650 mg, 87%) which was purified as a yellow foam on a silica gel plate [methylene chloride — acetone (20 : 1)], $R_f \approx 0.5$, $[\alpha]_D^{22}$ + 41.9° (c 1.5).

Anal. $C_{43}H_{45}N_3O_7S$ (747.87) calc'd.: C 69.54; H 6.06; N 5.61; S 4.28%
found: C 69.58; H 6.22; N 5.59; S 4.42%

Uv spectrum: λ_{max} 203, 232 (infl.), 333 nm (log ε = 4.80, 4.06, 4.30); λ_{min} 277 nm (log ε = 3.45). Ir spectrum: ν_{max} 3483, 3077, 2941, 1745 (sh), 1706, 1634, and 703 cm^{-1}.

3'-O-(N-t-Butyloxycarbonyl-L-phenylalanyl)-4-thiothymidine (VIII)

3'-*O*-(*N*-*t*-Butyloxycarbonyl-L-phenylalanyl)-5'-*O*-triphenylmethyl-4-thiothymidine (VII, 650 mg, 0.87 mmol) was refluxed in 80% acetic acid for 30 min. and worked up as described for compound VI. From the silica gel column methylene

chloride — methanol (10 : 1) eluted a yellow foam (250 mg, 57%), which was crystallized from acetone — *n*-hexane, m.p. 177—179 °C, $[\alpha]_D^{20}$ + 15.9° (c 0.5).

Anal. $C_{24}H_{31}N_3O_7S$ (500.57) calc'd.: C 57.02; H 6.18; N 8.31; S 6.34%
found: C 57.27; H 6.39; N 8.44; S 6.23%

Uv spectrum: λ_{max} 203, 245, 333 nm (log ε = 4.30, 3.40, 4.23); λ_{min} 234, 274 nm (log ε = 3.38, 3.32). Ir spectrum: ν_{max} 3521, 3401, 3257, 2915, 1727, 1706, 1686, 1629, and 698 cm^{-1}.

3'-O-(L-Phenylalanyl)-4-thiothymidine (IX)

3'-*O*-(*N*-*t*-Butyloxycarbonyl-L-phenylalanyl)-4-thiothymidine (VIII, 134 mg, 0.265 mmol) was dissolved in 98% trifluoroacetic acid (0.8 ml) and stirred at —20 ° for 5 min. The solution was evaporated to an oil which was triturated with ether. The precipitate (110 mg) was dried and chromatographed on a silica gel plate [developed two times in methylene chloride — methanol (15 : 1 and 10 : 1)]. The fraction, $R_f \approx 0.4$, was eluted with methanol, 90 mg (84%). The analytical sample (yellow foam) was dried at 50 °C and 0.01 mmHg, $[\alpha]_D^{18}$ + 24.9° (c 0.5).

Anal. $C_{19}H_{23}N_3O_5S$ (405.45) calc'd.: C 56.28; H 5.72; N 10.36%
found: C 56.15; H 6.03; N 10.31%

Uv spectrum: λ_{max} 201, 240, 331 nm (log ε = 4.37, 3.74, 4.30); λ_{min} 224, 274 nm (log ε = 3.70, 3.56). Ir spectrum: ν_{max} 3448, 3077, 2915, 1724 (sh), 1692, 1671, and 699 cm^{-1}.

Stability of 3'-O-(L-Phenylalanyl)-4-thiothymidine (IX) *in Water*

3'-*O*-(L-Phenylalanyl)-4-thiothymidine (IX, 7 mg) was dissolved in water (7 ml) and ethanol (1 ml) and kept at room temperature (20 °C). The measurements of pH after 0, 1, 2, 3, 4, 5, and 21 h were 7.72, 7.65, 7.53, 7.45, 7.44, and 7.30. The R_f values on a silica gel plate [methylene chloride — methanol (10 : 1)] changed concurrently from ~ 0.6 (starting material) to ~ 0.5 (4-thiothymidine and zero (phenylalanine).

4-N-(N-Triphenylmethyl-glycylglycyl)-cytosine (X)

To cytosine (110 mg, 1 mmol) dissolved in dimethylformamide — dimethyl sulphoxide (1 : 1, 8 ml) the *N*-hydroxysuccinimide ester of *N*-triphenylmethyl-glycylglycine (518 mg, 1.1 mmol) in dimethylformamide (4 ml) was added. The solution was heated at 100 °C and then kept at room temperature overnight. A solid separated, yielding 286 mg (61%). From the filtrate on cooling, an additional product separated (77 mg), containing traces of the starting material ($R_f \approx 0.04$). The analytical sample recrystallized from dimethylformamide, m.p. 264—265 °C, $R_f \approx 0.4$ [silica gel, TLC in methylene chloride — methanol (8.5 : 1.5)], was detected by iodine vapour.

Anal. $C_{27}H_{25}N_5O_3 \cdot H_2O$ (458.53) calc'd.: C 66.78; H 5.61; N 14.43%
found: C 67.05; H 5.91; N 14.49%

Ir spectrum: ν_{max} 3448, 2907, 1730, 1689, 1661, 1608, 744, and 704 cm^{-1}.

4-N-(Glycylglycyl)-cytosine (XI)

The suspension of 4-*N*-(*N*-triphenylmethyl-glycylglycyl)-cytosine (243 mg, 0.5 mmol) in 50% acetic acid (5 ml) was heated on a water bath for 20 min., and then diluted with water (20 ml). The precipitate was filtered off, the filtrate evaporated to dryness, and the residue triturated and washed with ether. It yielded 120 mg (100%), recrystallized from 80% methanol, m.p. 275—278 °C (dec.) as colourless prisms, $R_f \approx 0.2$[developed in CH_2Cl_2—MeOH (8.5 : 1.5)], detected by ninhydrine.

Anal. $C_8H_{11}N_5O_3$ (225.21) calc'd.: C 42.66; H 4.92%
found: C 42.42; H 5.20%

Ir spectrum: ν_{max} 3448, 3247, 3086, 2994, and 1689 (br) cm^{-1}. NMR spectrum: (D_2O) : τ 2.50 (1H, d, 6-H; $J_{6,5} = 7.0$ Hz), 4.03 (1H, d, 5-H; $J_{5,6} = 7.0$ Hz), 5.98 (2×2H, s, 2×CH_2).

4-N-(Glycylglycyl)-5,6-dihydrocytosine (XII)

The solution of 4-*N*-(glycylglycyl)-cytosine (XI, 0.56 mg, 0.25 mmol) in water (15 ml) was quantitatively hydrogenated over 5% rhodium on carbon (25 mg) at 50 lb in^{-2} for 90 min. The product recrystallized from aqueous methanol as white needless, m.p. 295—297 °C (dec.).

Anal. $C_8H_{13}N_5O_3$ (227.22) calc'd.: C 42.29; H 5.77%
found: C 42.38; H 5.66%

Ir spectrum: ν_{max} 3215, 3040, 2924, and 1689 (br) cm^{-1}.

N-4-(N-*Triphenylmethyl-glycylglycyl)-cytidine* (XIII)

Into a solution of cytidine (61 mg. 0.25 mmol) in freshly distilled dimethylformamide (3 ml) the *N*-hydroxysuccinimide ester of *N*-triphenylmethyl-glycylglycine (130 mg, 0.276 mmol) was added. The solution was stirred and heated at 95 °C for 4 h and then kept at room temperature for 20 h. After evaporation to dryness the remaining oil was dissolved in methanol and precipitated with water (78 mg). From the filtrate cytidine was isolated (30 mg) by means of a silica gel chromatographic plate [developed in methylene chloride — methanol (10 : 1)]. The product, $R_f \approx 0.6$, was eluted with methanol, recrystallized from methanol — water (25 mg, 17%), m.p. 140—142 °C.

Anal. $C_{32}H_{33}N_5O_7$ (599.62) calc'd.: C 64.09; H 5.55; N 11.68%
found: C 64.13; H 5.55; N 11.43%

Uv spectrum: λ_{max} 246 (infl.), 296 nm (log $\varepsilon = 4.02$, 3.50); λ_{min} 273 nm (log $\varepsilon = 3.39$). Ir spectrum: ν_{max} 3401, 3096, 2941, 1754 (br), 1647, and 706 cm^{-1}.

4-N-(N-t-Butyloxycarbonyl-L-phenylalanyl)-2',3'-O-isopropylidene-cytidine (XIV)

To a solution of 2',3'-*O*-*iso*propylidene-cytidine[9] (210 mg, 0.74 mmol) in anhydrous dioxane (5 ml) *N*-*t*-butyloxycarbonyl-L-phenylalanine (236 mg, 0.89) mmol and dicyclohexylcarbodiimide (260 mg, 1.3 mmol) were added and stirred at room temperature for 48 h. The precipitate was filtered off, the filtrate evaporated to dryness, and chromatographed on silica gel plate in methylene chloride (4 ml) [three times developed in methylene chloride — methanol (20 : 1)]. The product, $R_f \approx 0.4$, was eluted with acetone (300 mg, 76%), and crystallized from acetone — *n*-hexane, m.p. 181—183 °C, $[\alpha]_D^{20}$ + 13.4° (c 0.8).

Anal. $C_{26}H_{34}N_4O_8$ (530.56) calc'd.: C 58.85; H 6.46; N 10.56%
found: C 58.58; H 6.58; N 10.34%

Uv spectrum λ_{max} 246, 298 nm (log $\varepsilon = 4.13$, 3.78); λ_{min} 225, 273 nm (log $\varepsilon = 3.74$, 3.58). Ir spectrum: ν_{max} 3448 (br), 3268, 3003, 1724, 1653, 1613, and 696 cm^{-1}. NMR spectrum: τ 2.12 (1H, d, 6-H; $J_{6,5} = 7.4$ Hz), 2.57 (1H, d, 5-H; $J_{5,6} = 7.4$ Hz), 2.79 (5H, s, aromatic protons), 4.36 (1H, d, 1'-H; $J_{1',2'} = 2$ Hz), 8.44 and 8.66 (6H, 2× s, Me_2C) and 8.66 (9H, s, Me_3C).

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REFERENCES

1. R. H. Hall, *Biochemistry* **3** (1964) 769.
2. M. P. Schweizer, G. B. Chheda, L. Baczynskyj, and R. H. Hall, *ibid.* **8** (1969) 3283.
3. J. S. Salser and M. E. Balis, *Cancer Res.* **28** (1968) 595.
4. A. Bendick and H. Rosenkranz, *Progr. Nucl. Acid Res.* **1** (1963) 219.
5. M. E. Balis, J. S. Salser, and A. Elder, *Nature* (London) **203** (1964) 1170.
6. H. Randallard and H. R. Munson in: W. W. Zorbach, R. S. Tipson (Eds.), *Synthetic Proc. Nucl. Acid Chem.* **1** (1968) 321, John Wiley Inc., New York.
7. W. Saenger and K. H. Scheit, *J. Mol. Biol.* **50** (1970) 153.
8. J. J. Fox, D. Van Praag, I. Wempen, J. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Amer. Chem. Soc.* **81** (1959) 178; K. H. Scheit, *Chem. Ber.* **101** (1968) 1141.
9. N. I. Sokolova, L. A. Boykova, M. A. Prokofiev, *Zh. Obsch. Khim.* **29** (1959) 2917; N. I. Sokolova, B. A. Bakanova, Z. A. Shabarova, and M. A. Prokofiev, *ibid.* **33** (1963) 2480.
10. A. Hampton, *J. Amer. Chem. Soc.* **83** (1961) 3640.
11. V. Škarić, V. Turjak-Zebić, and Đ. Škarić, *J. Chem. Soc. Perkin 1* (1974) 1406.

SAŽETAK

Aminoacil derivati 4-tiotimidina, citosina i citidina

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Kondenzacija 5'-*O*-trifenilmetil-4-tiotimidina (III) sa *N*-benziloksikarbonil-D,L--alaninom i *N*-*t*-butiloksikarbonil-L-alaninom uz *N*,*N*-dicikloheksilkarbodiimid daje odgovarajuće 3'-nukleozid estere V i VII. Uklanjanjem zaštitnih trifenilmetil i *t*-butiloksikarbonil grupa iz estera VII nastaje 3'-*O*-(L-fenilalanil)-4-tiotimidin (IX).

Prikladno zaštićeni glicilglicin i L-fenilalanin u kondenzacionim reakcijama sa citozinom i 2',3'-*O*-*izo*propiliden- citidinom, prelaze u odgovarajuće *N*-acilirane derivate citozina odnosno citidina. Nađeno je, također, da se 4-*N*-(glicilglicil)-citozin (XI) može kvantitativno hidrirati u 4-*N*-(glicilglicil)-5,6-dihidrocitozin (XII) uz 5% rodij na ugljenu kao katalizator.

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