Preparation of Ureidonucleosides of the Threonine Isomers

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The hypermodified ureidonucleoside N6[((9-β-D-ribofuranosyl-9H-purine-6-yl)amino)carbonyl]-L-threonine (**5**) is a constituent of transfer ribonucleic acid (tRNA) and is secreted as a tRNA catabolite in body fluids such as blood, milk and urine. Compound **5** and the isomeric ureidonucleosides bearing D-threonine (**9**), L-allo- (**7**) and D-allo-threonine (**11**) as side chain moieties were synthesized on a preparative scale. The amido protons of **5** and **9** cause two separate ^{1}H NMR signals whereas **7** and **11** cause multiplets. The ^{13}C NMR signals of all carbon atoms of the allo-amino acid side chains (**7, 11**) are shifted downfield of the corresponding signals in compounds **5** and **9.** The chemically protected intermediate compound adenosine urethane (**3**) is potentially of interest in the analysis of amino acids because it may be converted to nucleosides of the ureido type by reaction with amino acids in biological matrices.

Introduction

The hypermodified ureidoadenine nucleoside N6[((9-β-D-ribofuranosyl-9H-purine-6-yl)amino)-carbonyl]-L-threonine (t6Ado) (**5**) belongs to the group of modified nucleosides that are 3′-adjacent to the anticodon in transfer ribonucleic acids (tRNA) and that are responsible for a correct codon-anticodon recognition in protein biosynthesis (Schweizer *et al.,* 1969; Takemura *et al.,* 1969; Parthasarathy *et al.,* 1974; Sprinzl and Gauß, 1983). In addition to this cellular function linked to biopolymers, biological activities of the modified nucleosides have been described that are related to their monomeric structure (Hong, 1973; Gehrke and Kuo, 1990a, 1990b). Like other – mainly methylated ribonucleosides – t6Ado (**5**) is one of those compounds that have been detected as products of the cellular RNA and ribonucleotide metabolism in body fluids such as, *e.g.,* urine (Gehrke and Kuo, 1990b; Chheda *et al.,* 1969; Chheda, 1969; Waalkes *et al.,* 1975; Gehrke *et al.,* 1979; Borek *et al.,* 1983; Schlimme *et al.,* 1987; Kuo *et al.,* 1987; Thomale and Nass, 1982; Schlimme *et al.,* 1986), blood (Gehrke and Kuo, 1989; Mitchell *et al.,* 1992) and milk (Raezke and Schlimme, 1990; Schlimme *et al.,* 1991; Schneehagen and Schlimme, 1992). The concentrations of a number of modified ribonucleosides including t6Ado (**5**) in urine are greater for patients with neoplastic diseases than for healthy subjects, suggesting the use of those molecules as markers in clinical diagnosis (Gehrke and Kuo, 1990b; Borek *et al.,* 1983; Schwarzenau *et al.,* 1990; Schlimme *et al.,* 1990).

Of the 4 stereoisomers of 2-amino-3-hydroxybutyric acid only L-threonine has been detected so far as a substituent in ureidoadenine nucleosides as a naturally occurring component (**5**). The synthesis of ureidopurines and their nucleosides including **5** and its isomeric component (**9**) bearing D-threonine as side chain moiety have been described (Hong and Chheda, 1973). The present paper is the first report on the preparation of the isomeric ureidoadenine nucleosides (**7, 11**) bearing L-allo- and D-allo-threonine as side chain moieties and indicates the importance of the activated ureidoadenine nucleoside **3** for the detection by liquid chromatography of amino-functional substances in biological matrices.

Materials and Methods

UV spectra: Kontron Uvikon 860 spectrophotometer, software version 8832 Kontron. ^{1}H NMR and ^{13}C NMR spectra: Bruker AC 250, Bruker AMX 500; δ-values relative to tetramethylsilane

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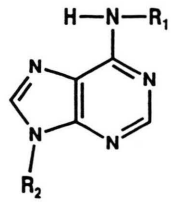

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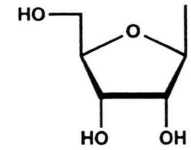
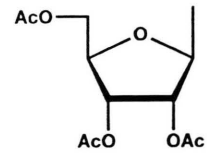
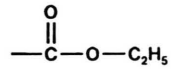
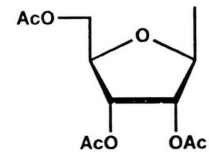
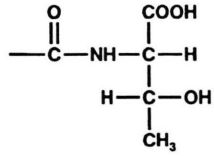
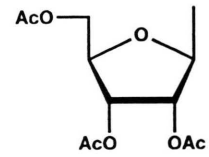
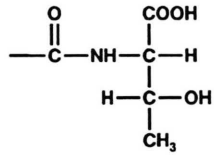
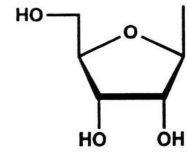
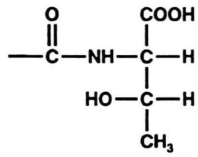
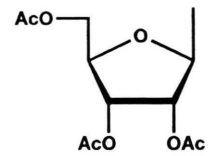
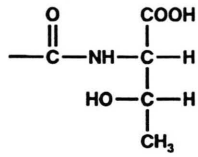
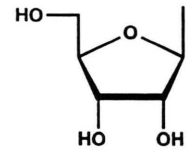
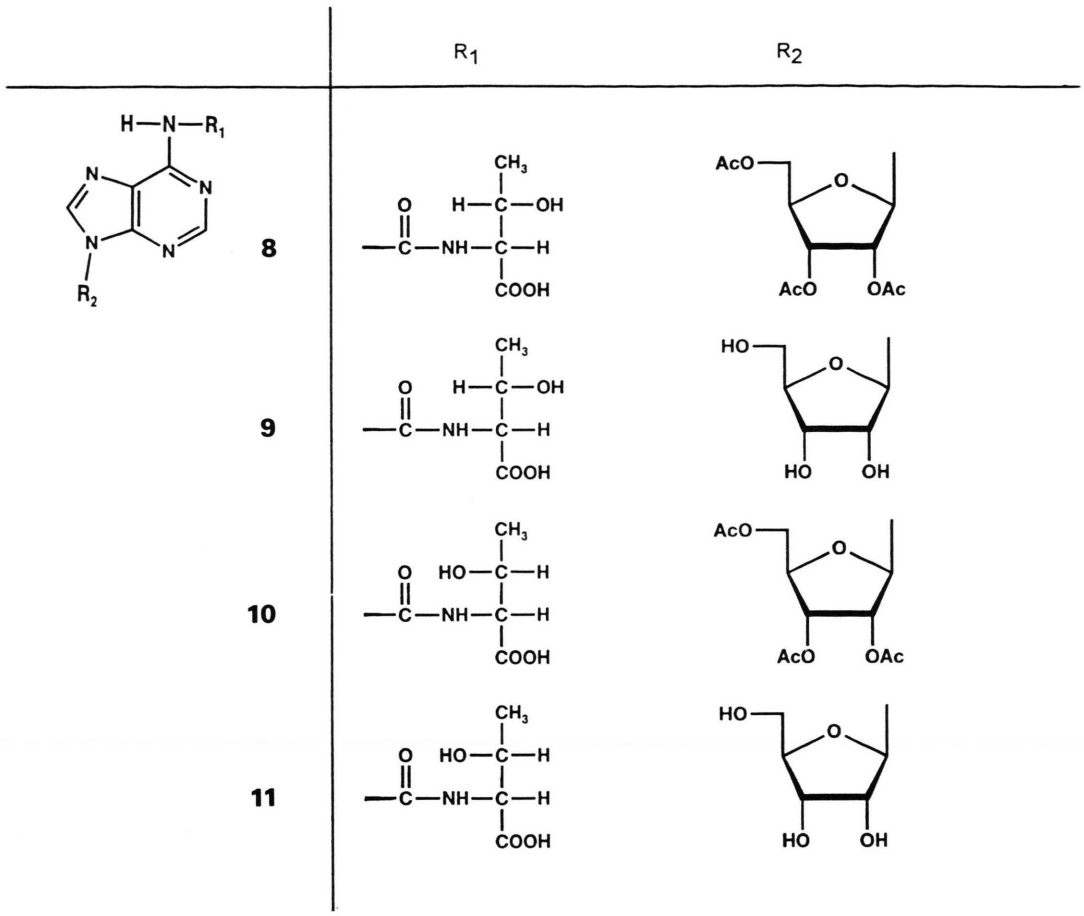
1	H	
2	H	
3		
4		
5		
6		
7		



(TMS). The number of protons was determined by off-resonance decoupling. Mass spectra: Varian MAT 311 A spectrometer, source temperature: **5:** 230 °C, **7:** 180 °C, **9:** 210 °C, **11:** 210 °C; sample inlet *via* pot; Fisons Instruments MD 800 quadrupole apparatus. GC-MS: inlet temperature: 250 °C, source temperature: **3:** 200 °C. Ionization energy of both systems: 70 eV. Melting points (uncorrected): Büchi 530 apparatus. Elementary analyses (residues and solvent inclusions, if present, are uncorrected): Microanalytical Laboratory Beller (A), Göttingen, and Microanalytical Laboratory Pascher (B), Remagen. High performance liquid chromatography (HPLC): Merck-Hitachi system with UV detection model 655 A-22; photodiode array detector 994, Waters-Millipore.

The two-column HPLC system used allows selective separation and analysis of ribonucleosides from the reaction mixture and the biological matrix and has been previously described (Raezke and Schlimme, 1990; Schlimme *et al.*, 1991; Schlimme and Boos, 1990; Boos *et al.*, 1986). Thin layer chromatography (TLC): (a) aluminium foil with silica gel 60 F_{254}, layer thickness 0.2 mm (Merck, Darmstadt). Chromatography systems (I; II) used: acetic acid ethyl ester/methanol I (96:4, v/v); dichloromethane/methanol II (6:4, v/v). Column chromatography was carried out with silica gel 60 (230–400 mesh ASTM; Merck) as stationary phase and with a dichloromethane/methanol gradient (100:0 → 0:100); for compound **3** an acetic acid ethyl ester/methanol gradient (100:0 → 96:4) was used. The fractions were collected (fraction collector LKB 2211 Superrac), and the UV absorption measured with a UV detector (LKB 2238 Uvicord S II) with

recorder (LKB 2210 Recorder 2-Channel). Chemicals used were from Merck (Darmstadt) and Sigma-Chemie (München).

Chemical syntheses

Preparation of 2',3',5'-tri-O-acetyl-β-D-ribofuranosyl adenine (**2**): 936 mg (3.50 mmol) of adenosine **1** were added to 4 ml of dry pyridine, mixed with 4 ml acetic acid anhydride and stirred for 5 h at room temperature. Subsequently, pyridine and excess acetic acid anhydride were removed by rotary evaporation (water bath temperature 55 °C). To remove pyridine traces the viscous reaction mixture was mixed with 30 ml toluene, and evaporated to dryness. This procedure was repeated. The acetylated product (**2**) was obtained as a colourless solid after crystallization from hot ethanol and subsequent drying under vacuum. Yield: 1.25 g (90.9%).

^{1}H NMR ($CDCl_3$): δ = 8.32 (s, 1H, H-8), 7.93 (s, 1H, H-2), 6.15 (d, J = 5.50 Hz, 1H, H-1'), 6.01 (s, 2H, N6-H), 5.90 (t, J = 5.50 Hz, 1H, H-2'), 5.65 (t, J = 5.50 Hz, 1H, H-3'), 4.30–4.45 (m, 3H, H-4', H-5'), 2.59 (s, 3H, 5'-CO–CH_3), 2.28 (s, 3H, 2'-CO–CH_3), 2.11 (s, 3H, 3'-CO–CH_3).

TLC (system I): R_F = 0.30

$C_{16}H_{19}N_5O_7$ (393.35 g/mol)

Calcd	C 48.86	H 4.87%,
Found (B)	C 49.50	H 4.95%.

Preparation of N6-[9-(2',3',5'-tri-O-acetyl)β-D-ribofuranosyl-9H-purine-6-yl] carbamic acid ethyl ester (**3**): 14.6 g (37.1 mmol) of triacylated adenosine (**2**) were dissolved in 200 ml of dry pyridine and cooled to −15 °C with an acetone/dry ice mixture. To the pyridine solution 10.7 ml (112.35 mmol) of chloroformic acid ethyl ester (at −15 °C) were added dropwise within 30 min. Stirring was continued at this temperature, for 1 h 10 min prior to removal of the acetone/dry ice coolant and further stirring overnight. After evaporation of pyridine *in vacuo* a brown reaction mixture was obtained. This was mixed with 50 ml toluene, which was removed by rotary evaporation (water bath temperature 40 °C). This procedure was carried out three times to remove pyridine traces. After column chromatographic purification on silica gel with an acetic acid ethyl ester/methanol gradient (100:0 → 96:4) **3** was obtained as a colourless solid. Yield: 1.22 g (7.04%). Melting point: 53 °C.

^{1}H NMR ([D_6]DMSO): δ = 10.51 (broad s, 1H, –N<u>H</u>–CO_2Et), 8.66 (s, 1H, H-8), 8.64 (s, 1H, H-2), 6.31 (d, J = 5.50 Hz, 1H, H-1'), 6.06 (t, J = 5.50 Hz, 1H, H-2'), 5.65 (t, J = 5.50 Hz, 1H, H-3'), 4.38–4.43 (m, 2H, H-5'), 4.24–4.28 (m, 1H, H-4'), 4.18 (q, J = 7.0 Hz, 2H, –O–C<u>H_2</u>–CH_3), 2.13 (s, 3H, 5'-CO–CH_3), 2.04 (s, 3H, 2'-CO–CH_3), 2.01 (s, 3H, 3'-CO–CH_3), 1.26 (t, J = 7.5 Hz, 3H, –CO–CH_2–C<u>H_3</u>).

^{13}C NMR ([D_6]DMSO]: δ = 170.08 (s, C-5'–<u>C</u>O–CH_3), 169.51 (s, C-2'–<u>C</u>O–CH_3), 169.34 (s, C-3'–<u>C</u>O–CH_3), 152.28 (s, –NH–CO_2–), 151.98 (s, C-6), 151.48 (d, C-2), 150.19 (s, C-4), 143.20 (d, C-8), 124.00 (s, C-5), 85.88 (d, C-1'), 79.63 (d, C-4'), 72.00 (d, C-3'), 70.08 (d, C-2'), 62.79 (t, –O–<u>C</u>H_2–CH_3), 61.10 (t, C-5'), 20.52 (q, C-5'–CO–<u>C</u>H_3), 20.42 (q, C-2'–CO–<u>C</u>H_3), 20.25 (q, C-3'–CO–<u>C</u>H_3), 14.41 (q, O–CH_2–<u>C</u>H_3).

MS (EI): *m*/e (%) = 465 (4.7) [M^+], 377 (4.5) [M^+ – NH-COOEt], 259 (4.6) [tri-O-acetylribosyl$^+$], 244 (4.6), 206 (100.0) [purine-6-NH-COOEt$^+$], 157 (9.3), 118 (12.0) [purine – H], 98 (7.9), 88 (67.8) [NH-COOEt$^+$].

TLC (system I): R_F = 0.50

$C_{19}H_{23}N_5O_9$ (465.42 g/mol)

Calcd	C 49.03	H 4.98%,
Calcd	C 50.25	H 5.90% (if 1.5 ethyl acetate is included),
Found (B)	C 51.09	H 5.85%.

Table I. HPLC-analytical programme for separating **5, 7, 9** and **11.** Solvent A: 0.03 mol/l ammonia formate, pH 3.4; solvent B: methanol.

Time [min]	A [%]	B [%]	Flow [ml/min]
0	100	0	1.2
4	100	0	1.2
4.1	100	0	0.5
7	100	0	0.5
7.1	100	0	1.4
14	99	1	1.4
20	99	1	1.4
20.1	99	1	1.6
24	98	2	1.6
34	97	3	1.6
37	94	6	1.6
40	92	8	1.6
44	89	11	1.6
115	66	34	1.6

Preparation of N6-[((9-β-D-ribofuranosyl-9H-purine-6-yl)amino)carbonyl]-L-threonine (**5**): 160 mg (0.33 mmol) of triacetylated adenosine urethane **3** were dissolved in 11 ml of dry pyridine, 116.4 mg (0.98 mmol) L-threonine were added and the suspension was stirred prior to heating under reflux for 5 h 15 min. Excess L-threonine was then removed by filtration and the pale-yellow solution evaporated to dryness on the rotary evaporator (water bath temperature 60 °C). To remove pyridine traces from the resulting yellow oil 20 ml toluene were added, evaporated to dryness *in vacuo*, and the procedure repeated. The almost colourless product **4** was deacetylated by dissolving it in 9.5 ml of a 4.5 mol/l solution of ammonia in methanol and stirring for 2 h at room temperature. After removal of the methanolic ammonia *in vacuo* the crude product was dissolved in methanol and **5** separated and purified by column chromatography on silica gel (27×3 cm) with a dichloromethane/methanol gradient (100:0 → 0:100). Fractions containing **5** were evaporated to dryness to give a colourless crystalline substance. Yield: 81.9 mg (60.8%). Melting point: 203–208 °C (decomposition).

^{1}H NMR ([D$_6$]DMSO): δ = 9.58 (d, *J* = 5.57 Hz, 1H, –CO–NH–CH–), 9.46 (s, 1H, –NH–CO–), 8.65 (s, 1H, 8-H), 8.52 (s, 1H, 2-H), 5.99 (d, *J* = 5.40 Hz, 1H, 1′-H), 5.80 (s, 1H, 2′-OH), 5.50 (s, 1H, 3′-OH), 5.20 (s, 1H, 5′-OH), 4.59 (s, 1H, β-OH), 4.18 (s, 1H, α-H), 3.97–4.01 (m, 3H, 2′-H, 3′-H, 4′-H), 3.55–3.71 (m, 2H, 5′-H), 3.16 (s, 1H, β-OH), 0.98 (d, *J* = 5.60 Hz, 3H, CH_3).

^{13}C NMR ([D$_6$]DMSO): δ = 173.46 (s, –COOH), 152.75 (s, –NH–CO–NH–), 151.11 (s, C-6), 150.44 (d, C-2), 150.22 (s, C-4), 142.18 (d, C-8), 120.37 (s, C-5), 87.80 (d, C-1′), 85.77 (d, C-4′), 73.95 (d, C-3′), 70.34 (d, C-2′), 66.28 (d, β-C), 61.36 (t, C-5′), 58.68 (d, α-C), 19.08 (q, CH_3).

UV data (water): λ_{max} = 276 nm (ε = 18.41 cm^2×μmol^{-1}) with 270.5 nm (sh) (pH 1.5); 269 nm (ε = 20.25 cm^2×μmol^{-1}) with 276 nm (sh) and 202 nm (ε = 18.85 cm^2×μmol^{-1}) (pH 6.6); 297 nm (ε = 1.27 cm^2×μmol^{-1}) and 269 nm (ε = 18.61 cm^2×μmol^{-1}) with 276 nm (sh) and 212 nm (ε = 14.55 cm^2×μmol^{-1}) (pH 12.0).

MS (70 eV): *m/e* (%) = 414.0 (5.7) [M$^+$+2H], 266.2 (30.3) [adenosine – H], 236.5 (54.7) [adenosine – CH_2OH], 177.5 (65.5) [purine-6-NH-$CONH_2$], 164.2 (97.9) [purine-6-NH_2–CHO^+], 134.6 (100.0) [adenine], 119.1 (38.9) [threonine or purine], 108.1 (61.1) [adenine – HCN, $C_4H_4N_4^+$], 73.0 (59.4) [CH_3-CH(OH)-$CHNH^+$], 44.2 (53.8) [CO_2], 27.5 (66.2) [CO], 17.5 (72.5) [NH_3].

TLC (system II): R_F = 0.18; HPLC: R_t = 61.6 min
$C_{15}H_{20}N_6O_8$ (412.36 g/mol)
$C_{15}H_{20}N_6O_8 \cdot 1.0\,H_2O$

Calcd	C 41.86	H 5.15%,
Found (A)	C 41.14	H 4.84%,
Found (B)	C 41.03	H 5.00%.

Preparation of N6-[((9-β-D-ribofuranosyl-9H-purine-6-yl)amino)carbonyl]-L-allo-threonine (**7**): 170 mg (0.37 mmol) of triacetylated adenosine urethane **3** were dissolved in 11 ml of dry pyridine 100 mg (0.84 mmol) of L-allo-threonine were added and the suspension stirred prior to heating under reflux for 6 h. Excess L-allo-threonine was removed by filtration and the solution evaporated on a rotary evaporator (water bath temperature 60 °C). To remove pyridine traces the resulting pale-yellow oil was diluted with 15 ml toluene, evaporated to dryness *in vacuo*, then the procedure was repeated. The light-brown product **6** was deacetylated by dissolving it in 9.5 ml of a 4.5 mol/l solution of ammonia in methanol and stirring for 2 h at room temperature. After removal of ammonia with a rotary evaporator and evaporation *in vacuo* a pale-yellow solid was obtained. The crude product was dissolved in a small amount of methanol and **7** separated and purified by column chromatography on silica gel (27×3 cm) with a dichloromethane/methanol gradient (100:0 → 0:100). Fractions containing **7** were evaporated to dryness to give a colourless crystalline substance. Yield: 87.3 mg (58.0%). Melting point: 200–211 °C (decomposition).

^{1}H NMR ([D$_6$]DMSO): δ = 9.57–9.60 (m, 2H, NH), 8.67 (s, 1H, 8-H), 8.53 (s, 1H, 2-H), 5.99 (d, *J* = 5.41 Hz, 1H, 1′-H), 5.85 (s, 1H, 2′-OH), 5.65 (s, 1H, 3′-OH), 5.24 (s, 1H, 5′-OH), 4.58 (s, weakly split, 1H, β-OH), 4.19 (s, 1H, α-H), 3.83–3.95 (m, 3H, 2′-H, 3′-H, 4′-H), 3.59–3.71 (m, 2H, 5′-H), 3.16 (s, 1H, β-H), 1.05 (d, *J* = 5.99 Hz, 3H, CH_3).

^{13}C NMR ([D$_6$]DMSO): δ = 173.62 (s, –COOH), 153.60 (s, –NH–CO–NH–), 151.10 (s, C-6), 150.38 (d, C-2), 150.26 (s, C-4), 142.23 (d, C-8), 120.39 (s, C-5), 87.82 (d, C-1′), 85.79 (d, C-4′), 74.00 (d, C-3′), 70.33 (d, C-2′), 68.84

(d, β-C), 61.36 (t, C-5′), 60.37 (d, α-C), 19.96 (q, CH_3).

UV data (water): λ_{max} = 276 nm (ε = 17.24 $cm^2 \times \mu mol^{-1}$) with 270.5 nm (sh) (pH 1.5); 269 nm (ε = 19.0 $cm^2 \times \mu mol^{-1}$) with 276 nm (sh) and 203 nm (ε = 18.15 $cm^2 \times \mu mol^{-1}$) (pH 6.6); 298 nm (ε = 1.27 $cm^2 \times \mu mol^{-1}$) and 269 nm (ε = 17.02 $cm^2 \times \mu mol^{-1}$) with 276 nm (sh) and 212 nm (ε = 13.33 $cm^2 \times \mu mol^{-1}$) (pH 12.0).

MS (70 eV): *m/e* (%) = 412.1 (2.5) [M^+], 410.8 (12.3) [M^+ – 2H), 354.3 (11.8), 282.0 (13.7) [purine-6-NH-CO-NH-threonine + 2H], 194.9 (100.0), 129.7 (22.1), 44.0 (12.7) [CO_2], 31.7 (61.8) [CH_3–OH].

TLC (system II): R_F = 0.19; HPLC: R_t = 56.0 min
$C_{15}H_{20}N_6O_8$ (412.36 g/mol)
$C_{15}H_{20}N_6O_8 \cdot 1.0\,H_2O$

Calcd	C 41.86	H 5.15%,
Found (A)	C 40.17	H 4.77%.

Preparation of N6-[((9-β-D-ribofuranosyl-9H-purine-6-yl)amino)carbonyl]-D-threonine (**9**): 140 mg (0.28 mmol) of triacetylated adenosine urethane **3** were dissolved in 10 ml of dry pyridine, 101.9 mg (0.86 mmol) D-threonine were added and the suspension heated under reflux for 5 h. Excess D-threonine was removed by filtration and the filtrate concentrated with a rotary evaporator (water bath temperature 55 °C). To remove pyridine traces the brown oil obtained was mixed with 15 ml toluene and evaporated to dryness *in vacuo;* this procedure was carried out 3 times. The crude product **8** was deacetylated by dissolving it in 8 ml of a 4.5 mol/l solution of ammonia in methanol and stirring for 2 h at room temperature. After removal of ammonia by rotary evaporation and evaporation *in vacuo* (Speedvac) a light-brown oil was obtained. The crude product was dissolved in methanol and **9** separated and purified by column chromatography on silica gel (27×3 cm) with a dichloromethane/methanol gradient (100:0 → 0:100). Fractions containing **9** were evaporated to dryness to give a fine crystalline colourless solid substance. Yield: 81.5 mg (70.6%). Melting point: 201–206.5 °C (decomposition).

^{1}H NMR ([D_6]DMSO): δ = 9.59 (d, *J* = 5.66 Hz, 1H, –CO–N<u>H</u>–CH–), 9.48 (s, 1H, –N<u>H</u>–CO–), 8.65 (s, 1H, 8-H), 8.52 (s, 1H, 2-H), 5.99 (d, *J* = 5.48 Hz, 1H, 1′-H), 5.83 (s, 1H, 2′-OH), 5.52 (s, 1H, 3′-OH), 5.21 (s, 1H, 5′-OH), 4.59 (s, split, 1H, β-OH), 4.19 (s, weakly split, 1H, α-H), 3.97–4.02 (m, 3H, 2′-H, 3′-H, 4′-H), 3.55–3.70 (m, 2H, 5′-H), 3.16 (s, 1H, β-H), 0.99 (d, *J* = 5.67 Hz, 3H, CH_3).

^{13}C NMR ([D_6]DMSO): δ = 173.53 (s, –COOH), 152.79 (s, NH–CO–NH–), 151.12 (s, C-6), 150.43 (d, C-2), 150.24 (s, C-4), 142.16 (d, C-8), 120.35 (s, C-5), 87.77 (d, C-1′), 85.78 (d, C-4′), 73.97 (d, C-3′), 70.35 (d, C-2′), 66.30 (d, β-C), 61.37 (t, C-5′), 58.72 (d, α-C), 19.15 (q, CH_3).

UV data (water): λ_{max} = 276 nm (ε = 17.89 $cm^2 \times \mu mol^{-1}$) with 270.5 nm (sh) (pH 1.5); 269 nm (ε = 19.85 $cm^2 \times \mu mol^{-1}$) with 276 nm (sh) and 204 nm (ε = 17.65 $cm^2 \times \mu mol^{-1}$) (pH 6.6); 297 nm (ε = 1.37 $cm^2 \times \mu mol^{-1}$) and 269 nm (ε = 18.97 $cm^2 \times \mu mol^{-1}$) with 276 nm (sh) and 212 nm (ε = 14.77 $cm^2 \times \mu mol^{-1}$) (pH 12.0).

MS (70 eV): *m/e* (%) = 414.4 (18.1) [M^++2H], 235.7 (36.3) [adenosine – CH_2OH], 135.0 (31.6) [adenine], 120.8 (44.4) [purine + H^+], 31.7 (71.9) [CH_3OH], 27.8 (100.0) [CO], 17.6 (97.2) [H_2O].

TLC (system II): R_F = 0.18, HPLC: R_t = 56.2 min
$C_{15}H_{20}N_6O_8$ (412.36 g/mol)
$C_{15}H_{20}N_6O_8 \cdot 1.0\,H_2O$

Calcd	C 41.86	H 5.15%,
Found (A)	C 41.51	H 4.97%,
Found (B)	C 41.71	H 5.01%.

Preparation of N6-[((9-β-D-ribofuranosyl-9H-purine-6-yl)amino)carbonyl-D-allo-threonine (**11**): 170 mg (0.37 mmol) of triacetylated adenosine urethane **3** were dissolved in 11 ml of dry pyridine. 100 mg (0.84 mmol) of D-allo-threonine were added and the suspension was stirred prior to heating under reflux for 6 h. Excess D-allo-threonine was removed by filtration and the filtrate concentrated by rotary evaporation (water bath temperature 65 °C). To remove pyridine traces the brown oil was mixed with 15 ml toluene and evaporated to dryness *in vacuo.* This procedure was repeated. The crude product **10** was deacetylated by dissolving it in 9.5 ml of a 4.5 mol/l solution of ammonia in methanol and stirring for 2 h at room temperature. The resulting light-brown solution was concentrated *in vacuo* to give a brown oil. The crude product was dissolved in methanol and **11** separated and purified by column chromatography on silica gel (27×3 cm) with a dichloromethane/methanol gradient (100:0 → 0:100). Fractions containing **11** were evaporated to dryness to give a

fine crystalline substance. Yield: 45.5 mg (30.2%). Melting point: 200–205 °C (decomposition).

^{1}H NMR ([D$_6$]DMSO): δ = 9.54–9.60 (m, 2H, NH), 8.66 (s, 1H, 8-H), 8.53 (s, 1H, 2-H), 5.99 (d, J = 5.45 Hz, 1H, 1′-H), 5.92 (s, 1H, 2′-OH), 5.62 (s, 1H, 3′-OH), 5.23 (s, 1H, 5′-OH), 4.58 (s, split, 1H, β-OH), 4.18 (s, split, 1H, α-H), 3.79–3.99 (m, 3H, 2′-H, 3′-H, 4′-H), 3.59–3.70 (m, 2H, 5′-H), 3.16 (s, 1H, β-H), 1.05 (d, J = 5.97 Hz, 3H, CH_3).

^{13}C NMR ([D$_6$]DMSO): δ = 173.74 (s, –COOH), 153.77 (–NH–CO–NH–), 151.10 (s, C-6), 150.38 (d, C-2), 150.27 (s, C-4), 142.19 (C-8), 120.38 (s, C-5), 87.77 (d, C-1′), 85.78 (d, C-4′), 74.01 (d, C-3′), 70.34 (d, C-2′), 68.88 (d, β-C), 61.37 (t, C-5′), 60.32 d, α-C), 20.03 (q, CH_3).

UV data (water): λ_{max} = 276 nm (ε = 16.22 cm^2×μmol^{-1}) with 270.5 nm (sh) (pH 1.5); 269 nm (ε = 17.25 cm^2×μmol^{-1}) with 276 nm (sh) and 203 nm (ε = 16.20 cm^2×μmol^{-1}) (pH 6.6); 299 nm (ε = 1.45 cm^2×μmol^{-1}) and 269 (ε = 16.93 cm^2×μmol^{-1}) with 276 nm (sh) and 212 nm (ε = 13.53 cm^2×μmol^{-1}) (pH 12.0).

MS (70 eV): *m/e* (%) = 456.2 (10.9) [M$^+$ + CO_2], 412.8 (5.4) [M$^+$], 366.9 (12.8) [M$^+$ – CO_2H], 306.2 (10.5), 273.7 (10.5), 134.9 (11.3) [adenine], 43.8 (100.0) [CO_2], 18.0 (11.3) [H_2O].

TLC (system II): R_F = 0.18; HPLC: R_t = 52.7 min

$C_{15}H_{20}N_6O_8$ (412.36 g/mol)

$C_{15}H_{20}N_6O_8 \cdot 1.0\,H_2O$

Calcd	C 41.86	H 5.15%,
Found (A)	C 41.19	H 5.23%.

Results and Discussion

Syntheses of the ureidoadenine nucleosides **7** and **11,** which are isomers of the naturally occurring t6Ado (**5**) have not yet been described. As expected, a comparison of the spectroscopic data of the ureido nucleosides of all 4 stereoisomers of threonine shows almost identical chemical shifts for the adenine protons 2-H and 8-H, for the ribosidic protons 1′-H, 2′-H, 3′-H, 4′-H and 5′-H, as well as for the hydroxyl protons at the 2′-, 3′- and 5′-positions, because the different stereochemical information is related to the amino acid side chain only. The C8 and C2 protons of all ureidoadenine ribonucleosides (**3, 5, 7, 9, 11**) are shifted downfield of the corresponding signals in adenosine. For all aforementioned 5 compounds 8-H is shifted by approximately 0.3 ppm, whereas the 2-H is shifted up to 0.5 ppm. After N6-*p*-chlorobenzoylation of the adenine base a similar downfield shift of the 8-H signal was observed, whereas the downfield shift of the 2-H signal was much smaller (Michels and Schlimme, 1982) than that of the ureidoadenine nucleosides described in this paper. In accordance with the ^{1}H NMR results, downfield shifts were observed in the ^{13}C NMR measurements of the ribosidic and base carbon atoms C-2, C-4, C-5, C-6 and C-8 of compounds **5, 7, 9** and **11.**

Table II presents the ^{1}H NMR signals of the ureido group protons of the compounds **5, 7, 9, 11.** In the L- and D-stereoisomers **5** and **9** the ureido protons generate 2 separate absorption signals so that the protons of the amido linkages to the adenine and to the threonyl groups can be distinguished. In both compounds the resonance H-signals of the threonyl amido groups are shifted downfield by about 0.11 ppm relative to the H-signals of the N6-amido group of the adenosine moiety. The protons of the ureido group of both allo-stereoisomers **7** and **11** appear as multiplets. The slight differences in both the chemical shift and the coupling constants of the threonyl methyl groups of all 4 ureido nucleosides allow the allo-isomers **7** and **11** to be clearly distinguished from the compounds **5** and **9.**

In the ^{13}C NMR measurements (Table III) the allo-isomeric nucleosides **7** and **11** absorb downfield of the corresponding L- and D-stereoisomers **5** and **9.** The β-carbon showed the largest difference in chemical shift (approx. 2.6 ppm). The NMR data indicate that the positions of the amido group proton signals as well as of the carbonyl carbon signals of the carbamoyl group are influenced by the different stereochemical information of the corresponding threonyl residue. This effect related to the allo-ureido nucleosides **7** and **11** may be caused by interaction (in the solvent [D$_6$]DMSO)

Table II. ^{1}H NMR signals [δ in ppm] of the ureido and methyl protons of **5, 7, 9** and **11.**

Protons	**5**	**7**	**9**	**11**
Ado-NH	9.46 s	9.57–9.60 m	9.48 s	9.54–9.60 m
Threonyl-NH	9.58 d J = 5.57 Hz		9.59 d J = 5.60 Hz	
CH_3	0.98 d J = 5.60 Hz	1.05 d J = 5.99 Hz	0.99 d J = 5.67 Hz	1.05 d J = 5.97 Hz

between the β-hydroxy group and the carbamoyl group.

In aqueous medium at pH 6.6 and 12.0, however, the UV spectra of all 4 ureido nucleosides **5, 7, 9** and **11** show an intense absorption peak at 269 nm with a shoulder at 276 nm, and at pH 1.5 an absorption maximum at 276 nm with a shoulder at 270.5 nm. The similarities of UV spectra of the 4 diastereoisomeric ureido nucleosides prove clearly that the carbamoyl-substituted adenine base chromophore is not influenced by the interaction between the allo-threonyl residue and the ureido group discussed above. Therefore interaction between the purine system and the amino acid side chain has no influence on UV absorption.

The identical UV absorption behaviour of the discussed 4 stereoisomeric ureidoadenine nucleosides confirms results published (Hong *et al.*, 1973) concerning UV-spectroscopic properties of different N6-carbamoyl adenosine compounds.

Table III. ^{13}C NMR signals [δ in ppm] in the range of the carbamoyl threonine side chain of **5, 7, 9** and **11.**

C-Atom	**5**	**7**	**9**	**11**
NH–CO–NH	152.75	153.60	152.79	153.77
α-C	58.68	60.37	58.72	60.32
β-C	66.28	68.84	66.30	68.88
CH_3	19.08	19.96	19.15	20.03

The mass spectra of **7** and **11** (electron impact ionization) show molecular peaks of weak intensity at *m/e* 412. The base peak of **11** is caused by carbon dioxide; the corresponding mass peak at *m/e* 366.9, generated by loss of carbon dioxide, and the mass peak at *m/e* 456, which results from binding of cleaved carbon dioxide, both support this finding. The $[M^+ + 2H]$ peaks are not observed for the allo-ureido nucleosides **7** and **11.**

The preparative work resulted in smaller yields of **7** and **11** than of the L- and D-compounds **5** and **9** (Table IV). 3-fold molar excesses of the appropriate amino acids were used for syntheses of **5** and **9** while 2.3-fold molar excesses were used for synthesizing **7** and **11.** It is therefore more likely that steric reasons, *e.g.*, the spatial proximity of the β-hydroxy group to the attacking nucleophilic α-amino group, cause the smaller yield of the allo-configurated ureido nucleosides rather than the slight difference in amino acid excess.

Table IV. Yield of the ureidoadenine ribonucleosides.

Nucleoside	Yield [%]
5	60.8
7	58.0
9	70.6
11	30.2

In the elementary analyses of **5, 7, 9** and **11,** the amounts of carbon found were generally smaller than expected. Liquid chromatographic analysis of the 4 ureido nucleosides detected no impurities which may influence the result of the elementary analyses. Therefore, it is likely that the crystalline substances may have incorporated water of crystallization.

Finally, it should be mentioned that triacetylated adenosine urethane **3** is not only a suitable intermediate product for the synthesis of ureidoadenine ribonucleosides, but presents itself as a "scavenger molecule" for the conversion to ureido nucleosides of substances containing amino groups in biological material – *e.g.*, with components of the so-called non-protein-nitrogen fraction of milk. Here, the *cis*-diol group of the "scavenger molecule" can be used for chemoselective separation of the ureido nucleosides formed on the pre-column while the ureidoadenine part of the structure serves as a chromophore for the UV detection of the ureido nucleosides separated on the analytical column of the two-column HPLC analyzer used (Schlimme and Boos, 1990; Boos *et al.*, 1986; Martin, 1994).

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