MECHANISM OF FORMATION OF FLUORESCENT ADENOSINE ANALOGUES

Richard F. de Boer, Daniëlle G.I. Petra, Martin J. Wanner, Aldo Boesaart, and Gerrit-Jan Koomen*

Laboratory of Organic Chemistry, University of Amsterdam,
Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands

Abstract - During studies towards the synthesis of a transition state inhibitor of the enzyme adenylosuccinate lyase, two different pyrimido[2,1-*i*]purines were found, depending on the reaction conditions. It was shown that one of the products, formed under kinetically controlled reaction conditions can be converted in the thermodynamically more stable one *via* a Dimroth type rearrangement. The latter isomer shows strong fluorescent properties in the uv/vis region, which makes it potentially useful as a fluorescent probe in biological studies.

INTRODUCTION

Adenylosuccinate lyase is an essential enzyme catalyzing two steps of the biosynthesis of adenosine nucleotides.[1] In the conversion of IMP to AMP it facilitates the elimination of fumaric acid from adenylosuccinate (**1**) (Figure 1).[2] The activity of the enzyme in human mammacarcinoma is significantly enhanced as compared to benign mammary tumors and can therefore be used as a tumor marker.[3] Based on these results, we reasoned that selective inhibition of the enzyme could lead to a specific anti-cancer effect, since the malignant cells might be more sensitive to this inhibition.

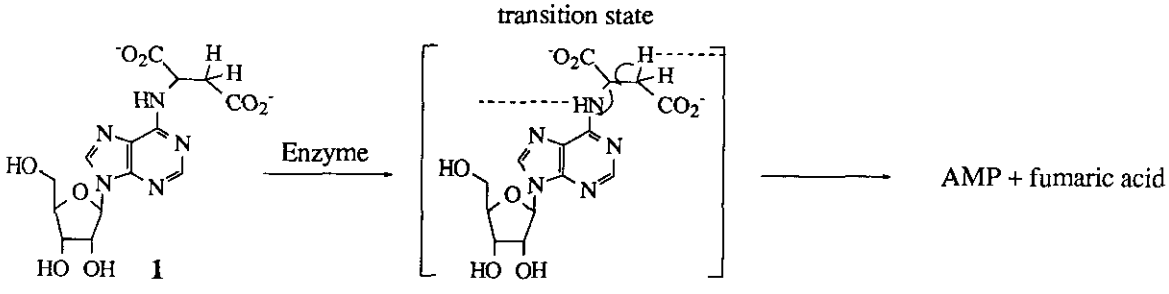


Figure 1

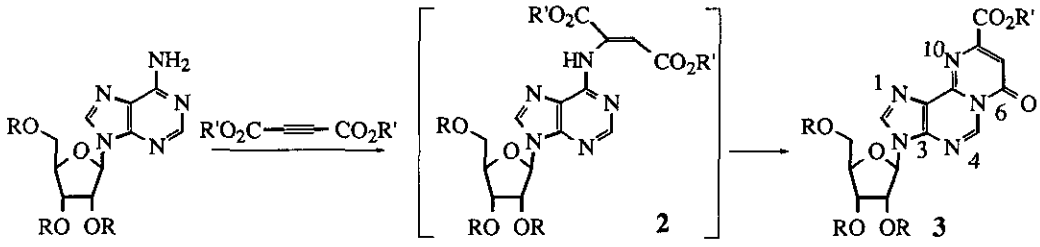
Earlier work in our group on inhibitors of the adenylosuccinate synthase/adenylosuccinate lyase system consisted of the synthesis of substituted analogues of adenylosuccinate in which the elimination of fumaric acid is prevented *via* the introduction of methyl groups and fluorine atoms at the position of the proton which has to be removed by the enzyme (Figure 1).[2] Another approach towards diminishing the rate of fumaric acid elimination consisted of the replacement of the NH-function by a methylene group.[4] More recently we reported on the synthesis and properties of a suicide inhibitor of the enzyme, capable of forming a very reactive ketene within the active site of adenylosuccinate lyase.[5]

In this paper we describe our efforts to prepare a transition state inhibitor of adenylosuccinate lyase. The transition state of the enzymic reaction is closely approached by derivatives of structure (**2**) (Figure 2; R = R' = H). For the synthesis of this compound the most straightforward approach consists of a Michael addition of adenosine derivatives to acetylenedicarboxylic esters. Addition reactions of adenosine to acetylenic esters have been studied before by Roques *et al.*, who reported the formation of two isomeric tricyclic adenosine products, depending on the reaction conditions.[6]

Although simple product formation was not expected, we decided to carry out a variety of addition experiments, both in polar and nonpolar media. Due to these experiments we could explain the mechanism of these cyclization reactions. Furthermore we were interested in the role of proton donors in these reactions which might be essential to stabilize the Michael addition product initially formed.

RESULTS AND DISCUSSION

In nonpolar solvents the use of protective groups on the hydroxyl functions was required, due to the insolubility of adenosine. Thus 2',3',5'-tri-*O*-acetyladenosine was used in the reaction with dimethyl acetylenedicarboxylate in dichloroethane. Without addition of acid complex reaction mixtures were obtained, but upon acidification with 5% acetic acid, pyrimido[2,1-*i*]purine (**3a**) was obtained in 78% yield. Apparently formation of **2a** was followed immediately by cyclization to **3a**, *via* nucleophilic attack of N-1 onto the carbonyl fuction. As described before, this compound has a characteristic blue fluorescence.[6]



	R	R'
a	Ac	Me
b	H	Me
c	Ac	Bn
d	Ac	*t*-Bu
e	Ac	H

Figure 2

Reaction with dibenzyl and di-*t*-butyl acetylenedicarboxylate followed the same course, leading to **3c** and

3d, respectively. Even the steric bulk of the *t*-butyl group was insufficient to prevent cyclization under these conditions. Application of silyl protected adenosine in this mixture of solvents gave identical results.

To compare the results of the studies in polar solvents, **3b** was obtained *via* deprotection of **3a** with sodium methoxide. Debenzylation of **3c** with hydrogen/palladium produced the free acid (**3e**) without loss of fluorescence.

In order to study the addition/cyclization reaction in polar solvents, for solubility reasons, unprotected adenosine was used. Initially the reaction was carried out in ethanol/water containing 5% of acetic acid, producing the isomeric pyrimido[2,1-*i*]purine (**5b**), which crystallizes from the reaction mixture in 78% yield, completely in agreement with the results reported by Roques *et al.* (Figure 3).[6] The improvement of the yield is probably due to lowering of the pH, which facilitates trapping of the anion, initially formed.

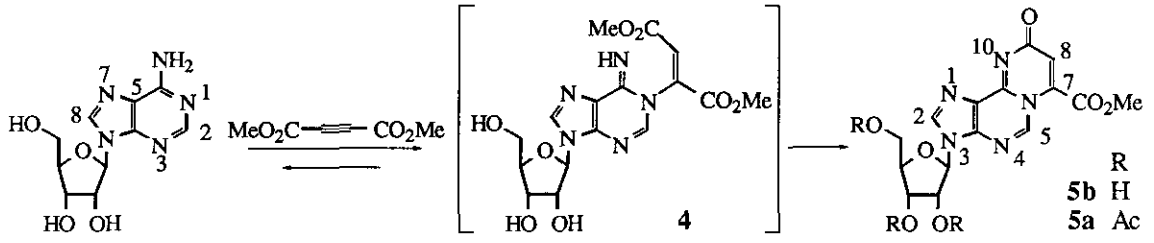


Figure 3

The same product was obtained when dimethyl acetylenedicarboxylate was added to a suspension of adenosine in CH_2Cl_2.

Both isomers (**3** and **5**) have been characterized by their physical and spectral properties. Generally, fused pyrimidones like isomers (**3**) have a lower melting point[7] and a higher ir absorption of the lactam CO band[8] than the corresponding isomers of general structure (**5**). For compound (**3b**) the melting point is 185-188 °C and the ir absorption of the lactam CO is 1700 cm^{-1} and for **5b**, respectively, 228-229 °C (lit.,[6] 239 °C) and 1640 cm^{-1}. Furthermore, the structures of both compounds were established *via* ^1H nmr. The H_5 signal of compound (**3b**) is strongly deshielded (9.61 ppm) as compared to the signal of **5b** (9.28 ppm). Although in both isomers the methyl ester is quite far away from H_5 and H_8 to exhibit strong NOE effects, irradiation of the OMe gave for isomer (**3b**) no effect on H_5 and 0.5 % effect on H_8, and for isomer (**5b**) 0.5 % effect on H_5 and 0.5 % effect on H_8. Irradiation of H_5 gave no effect for isomer (**3b**) and for isomer (**5b**) 2.5 % effect on the OMe and 1.5 % effect on H_8.

Fused pyrimido[2,1-*i*]purines and pyrido[2,1-*i*]purines[9] show fluorescent properties in the uv/vis region (**3a**: $\lambda_{em.}$= 420 nm, $\lambda_{abs.}$= 370 nm, quantum yield = ± 50 %), and might be interesting for biological studies. The fluorescence data for isomer (**5b**) could not be obtained, due to low traces of isomer (**3b**), which fluoresces much stronger. Attempts to purify **5b** by recrystallization failed, due to attack of the solvents at carbon atom C-5, resulting in formation of isomer (**3b**) (see further and Figure 4).

Apparently solubility of the products formed determines whether products of general structure (**3**) or (**5**) are obtained. Due to the low solubility of the unprotected isomers they precipitate under the reaction conditions and therefore further reactions are prevented. The primary attack is performed by the ring

nitrogen atom N-1 of adenosine leading to intermediate (**4**),[7] followed by cyclization leading to **5b** as the kinetically favoured product (Figure 3). This also explains the results of Roques who found formation of adducts of type (**5**) in the reaction of adenosine with methyl chlorotetrolate in DMF, conditions under which the product precipitated.[6] When they carry out the same reaction with AMP, ADP and ATP in water/ethanol, conditions under which the products stay in solution, adducts of type (**3**) are obtained.[10]
The formation of kinetically and thermodynamically controlled products suggests an equilibrium between the adducts (**3**) and (**5**), as observed by Roques for comparable systems. The presence of such an equilibrium was revealed to us during acetylation reactions. Isomer (**5b**) was acetylated to compound (**5a**) with acetic anhydride in pyridine. Under these conditions 40% of the product was converted into the other isomer (**3a**) probably due to ring opening by nucleophilic attack of either pyridine or the acetate ion, followed by ring closure due to the abstraction of a proton by pyridine, facilitated by the leaving group properties of the acetate ion (Figure 4).

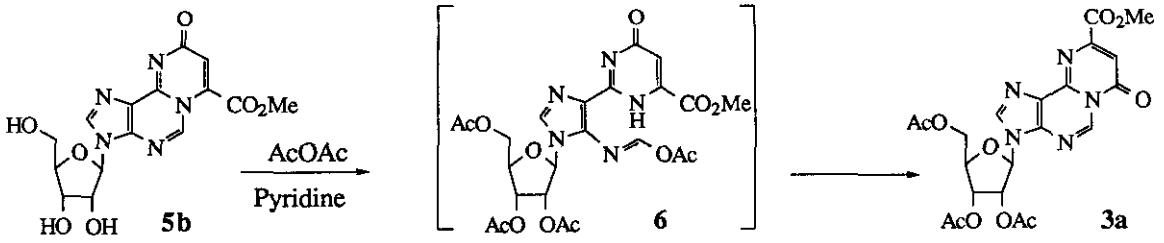


Figure 4

Previously we described the reaction of pyrido[2,1-*i*]purine systems with nucleophiles.[11] Attack at the C-5 position leads to ring opening. The reaction of **5b** with nucleophiles is an example of this reaction in the pyrimido[2,1-*i*]purine series. In order to be able to isolate the intermediate, thus establishing the course of the reaction, **3b** and **5b** were treated with nucleophiles with poorer leaving group abilities.
Thus, both **3b** and **5b** were treated with dimethylamine leading to the same open product (**7**), which could be isolated by carrying out the reaction at 0 °C / room temperature. Since this reaction occurs with loss of fluorescence, it is easily monitored. Compound (**7**) can readily be recyclized under acidic conditions leading completely to the thermodynamically favoured fluorescent product (**3b**) (Figure 5).

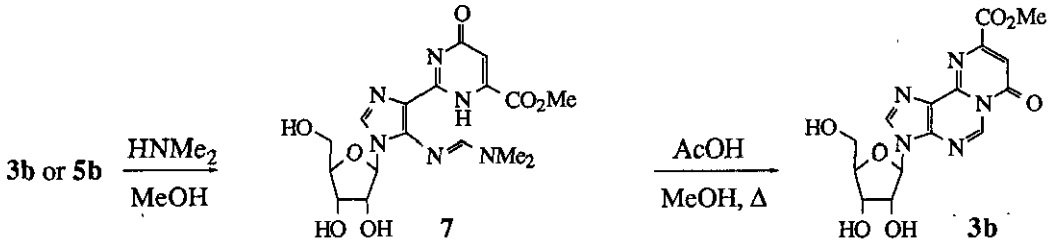


Figure 5

These results are in agreement with the observations of Roques *et al.* in the reaction of a comparable system with benzyl mercaptan and NaOD. The presence of two nucleophiles makes this reaction more

complex and leads to an equilibrium mixture of 2:1. The intermediate was not isolated under these conditions.[6]

Under hydrolytic conditions, carbon atom 5 is often lost completely in these ring opening reactions, due to instability of the amidine system under these conditions[6,11,12] (Figure 6). For instance when compound (**5b**) is heated under reflux in H_2O, compound (**8**) is formed. Reaction of **3a** with NH_3 in MeOH produces **9**, *via* nucleophilic attack and ester aminolysis.

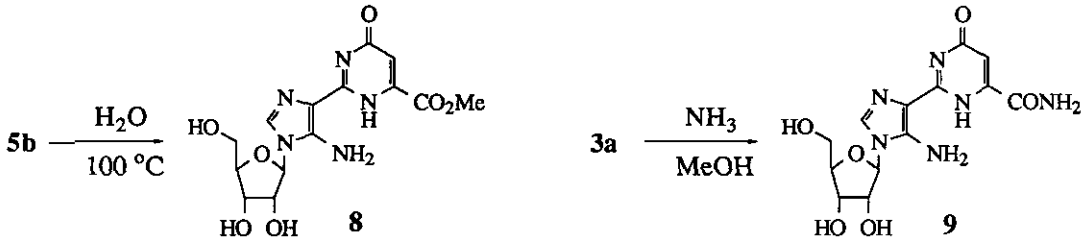


Figure 6

CONCLUSIONS

The Michael addition of adenosine to dimethyl acetylenedicarboxylate is complex, due to the different equilibria present in the reaction mixture. The primary addition product (**4**) is in equilibrium with the starting materials and formation of the product may be strongly dependent on the presence of proton donors in the reaction medium. The equilibrium of **4** with the starting material can convert all of the addition product in the Z-form, which is required for cyclization. The rate of cyclization is dependent on the leaving group of the ester function. In some cases Roques *et al.* used a *p*-nitrophenyl ester, which will quickly cyclize to the kinetically favoured products of general structure (**5**), which precipitates.[6]

The thermodynamically stable derivatives of general structure (**3**) are only formed when the products and intermediates are fully soluble in the reaction medium, thus giving rise to the next equilibrium **5** → **6** → **3**, the thermodynamically favoured products. The solubility of the starting material and the products in nonpolar solvents was increased by using protected adenosine derivatives.

This equilibrium was established unequivocally by isolating **7** and converting it completely into one isomer (**3b**). Due to the low solubility of the free riboside derivatives (**3b**) and (**5b**) in the used alcohol / water mixtures as solvents, they crystallise from the reaction medium. Under these crystallization conditions only the kinetic product (**5b**) will be formed.

Compounds of general structure (**3**) show strong blue fluorescence (λ_{em} = 420 nm), which might be useful for biological applications. Although described in literature,[6] there was no fluorescence observed for derivatives with general structure (**5**), the weak fluorescence observed can probably be attributed to the presence of traces of derivative (**3**).

EXPERIMENTAL SECTION

All melting points are uncorrected. Ir spectra were recorded on a Perkin Elmer 1310 spectrophotometer. The absorptions are given in cm^{-1}. Nmr spectra were run on a Bruker ARX 400 MHz for ^1H and 100 MHz for ^{13}C. Unless stated otherwise, ir and nmr spectra were taken in $CHCl_3$ and $CDCl_3$, respectively. Fast Atom Bombardment (FAB) mass spectrometry was carried out using a V.G. Micromass ZAB-HFqQ mass spectrometer, coupled to a V.G. 11/250 data system. Flash chromatography was performed on silica gel 60 (230-400 mesh). Thin layer chromatography was carried out with F 254 plates. Electronic absorption spectra were recorded on a Cary 3 (Varian) spectrophotometer. Emission and excitation spectra were recorded on a Spex Fluorolog II emission spectrometer, employing a RCA-C31034 GaAs photomultiplier as a detector.

3-[2',3',5'-Tri-*O*-acetyl-(β-D-ribofuranosyl)]-7*H*-7-oxo-9-methoxycarbonylpyrimido[2,1-*i*]purine (**3a**)

To a solution of 50 mg (0.13 mmol) of 2',3',5'-tri-*O*-acetyladenosine in 2 ml of dichloroethane/5% AcOH was added dimethyl acetylenedicarboxylate (37 mg, 0.26 mmol). The mixture was refluxed during 1 h, concentrated in vacuo followed by flash chromatography with EtOAc yielding 50 mg (78%) of yellow crystals. The product was recrystallized from MeOH. mp 104-105 °C R_f (EtOAc) = 0.21. ^1H Nmr: 2.08, 2.12 and 2.15 (3xs, 3x OAc), 3.99 (s; OMe), 4.42 (m, 2x $H_{5'}$), 4.43 (m, $H_{4'}$), 5.58 (dd, J = 4.8 and 5.2 Hz, $H_{3'}$), 5.86 (dd, J = 5.2 and 5.4 Hz, $H_{2'}$), 6.27 (d, J = 5.4 Hz, $H_{1'}$), 7.18 (s, H_8), 8.27 (s, H_2), 9.63 (s, H_5). ^{13}C Nmr: 20.23, 20.40 and 20.65 (3x Me), 53.23 (OMe), 62.79 ($C_{5'}$), 70.41 ($C_{3'}$), 73.53 ($C_{2'}$), 77.63 ($C_{4'}$), 86.87 ($C_{1'}$), 108.31 (C_8), 126.76, 139.16 (C_5), 141.27 (C_2), 144.43, 146.49, 152.09, 158.22, 164.45, 169.15, 169.42, 170.09. Ir: 3030 (w), 2990 (w), 2950 (w), 1750 (s), 1720 (s, CO lactam), 1610 (m), 1490 (w). $[\alpha]_D$= -14.0 (*c* , 0.020, $CHCl_3$). HRms: M+1: found: 504.1301, calcd for $C_{21}H_{21}N_5O_{10}$ + H: 504.1367. Anal. Calcd for $C_{21}H_{21}N_5O_{10}$•MeOH: C, 49.35; H, 4.71; N, 13.08. Found: C, 49.3; H, 4.5; N, 13.4.

When using THF / 5% AcOH as a solvent in the reaction described above, the product was obtained in 68% after 4 days at room temperature.

3-β-D-Ribofuranosyl-7*H*-7-oxo-9-methoxycarbonylpyrimido[2,1-*i*]purine (**3b**)

To a solution of 150 mg (0.30 mmol) of **3a** in 2 ml of dry methanol was added 3.6 mg (0.15 mmol) of NaH under nitrogen. After 2 h stirring at 0 °C, AcOH was added to decrease the pH and the reaction mixture was stirred for another hour at room temperature. The white crystals were collected and washed with methanol and acetone. (91.7 mg, 81%). R_f (CH_2Cl_2/10% MeOH) = 0.25. The product was recrystallized from H_2O. mp 185-188 °C. ^1H Nmr (DMSO): 3.67 (m, $H_{5'}$), 3.95 (s, OMe), 4.02 (d, J = 3.8 Hz, $H_{4'}$), 4.20 (m, $H_{3'}$), 4.58 (m, $H_{2'}$), 5.15 (m, OH-5'), 5.37 (m, OH-3'), 5.69 (m, OH-2'), 6.10 (d, J = 5.5 Hz, $H_{1'}$), 6.98 (s, H_8), 8.86 (s, H_2), 9.61 (s, H_5); Irradiation of OMe gave a 0.50 % NOE for H_8; Irradiation of H_5 gave no effect. ^{13}C Nmr: 52.90 (OMe), 60.89 ($C_{5'}$), 70.01 ($C_{3'}$), 74.40 ($C_{2'}$), 85.72 ($C_{4'}$), 87.56 ($C_{1'}$), 106.68 (C_8), 125.53, 139.14 (C_5), 142.16 (C_2), 144.89, 146.49, 152.01, 158.10, 164.51. Ir (KBr): 3380 (m), 2910 (w), 1740 (m, CO ester), 1700 (s, CO lactam), 1610 (s), 1490 (m).

HRms: M+1: found: 378.1019, calcd for $C_{15}H_{15}N_5O_7$ + H: 378.1050. Anal. Calcd for $C_{15}H_{15}N_5O_7 \cdot H_2O$: C, 45.57; H, 4.33; N, 17.72. Found: C, 45.4; H, 4.4; N, 17.4.

3-[2',3',5'-Tri-*O*-acetyl-(β-D-ribofuranosyl)]-7*H*-7-oxo-9-benzyloxycarbonylpyrimido[2,1-*i*]purine (**3c**)

To a solution of 530 mg (1.35 mmol) of 2',3',5' tri-*O*-acetyladenosine in 20 ml of dichloroethane/5% AcOH was added dibenzyl acetylenedicarboxylate (780 mg, 2.65 mmol). The mixture was refluxed during 4 h and concentrated in vacuo followed by flash chromatography with EtOAc yielding 400 mg (51%) as an yellow oil. R_f (EtOAc) = 0.45. ^{1}H Nmr: 2.09, 2.15 and 2.17 (3x s, 3x OAc), 4.43 (m, 2x $H_{5'}$), 4.51 (m, $H_{4'}$), 5.45 (s, CH_2), 5.59 (dd, J = 4.8 and 5.4 Hz, $H_{3'}$), 5.87 (dd, J = 5.3 and 5.4 Hz, $H_{2'}$), 6.28 (d, J = 5.3 Hz, $H_{1'}$), 7.21 (s, H_8), 7.39 (m, Ph), 8.28 (s, H_2), 9.65 (s, H_5). Ir : 2990 (w), 2950 (w), 1745 (s), 1710 (s, CO lactam), 1610 (m), 1490 (w). $[\alpha]_D$= -15.6 (*c* , 0.011, $CHCl_3$). HRms: M+1: found: 580.1608, calcd for $C_{27}H_{25}N_5O_{10}$ + H: 580.1680. Anal. Calcd for $C_{27}H_{25}N_5O_{10}$: C, 55.96; H, 4.35; N, 12.08. Found: C, 55.8; H, 4.4; N, 12.0.

3-[2',3',5'-Tri-*O*-acetyl-(β-D-ribofuranosyl)]-7*H*-7-oxo-9-*t*-butoxycarbonylpyrimido[2,1-*i*]purine (**3d**)

To a solution of 265 mg (0.67 mmol) of 2',3',5'-tri-*O*-acetyladenosine in 10 ml of dichloroethane/5% AcOH was added di-*t*-butyl acetylenedicarboxylate (390 mg, 1.33 mmol). The mixture was refluxed during 4 h and concentrated in vacuo followed by flash chromatography with EtOAc yielding 311 mg (85%) as an yellow oil. ^{1}H Nmr: 1.63 (s, *t*-Bu), 2.09, 2.15 and 2.17 (3x s, 3x OAc), 4.43 (m, 2x $H_{5'}$), 4.50 (m, $H_{4'}$), 5.58 (dd, J = 4.7 and 5.2 Hz, $H_{3'}$), 5.87 (dd, J = 5.3 and 5.4 Hz, $H_{2'}$), 6.27 (d, J = 5.3 Hz, $H_{1'}$), 7.12 (s, H_8), 7.39 (m, Ph), 8.25 (s, H_2), 9.63 (s, H_5). Ir : 2990 (w), 2950 (w), 1745 (s), 1710 (s, CO lactam), 1615 (s), 1495 (m). HRms: M+1: found: 546.1812, calcd for $C_{24}H_{27}N_5O_{10}$ + H: 546.1836. Anal. Calcd for $C_{24}H_{27}N_5O_{10}$: C, 52.84; H, 4.99; N, 12.84. Found: C, 52.9; H, 4.8; N, 12.7.

3-[2',3',5'-Tri-*O*-acetyl(β–D-ribofuranosyl)]-7*H*-7-oxo-9-carboxypyrimido[2,1-*i*]purine (**3e**)

To a solution of 35.5 mg (0.06 mmol) of **3c** in 1 ml of dichloromethane was added 4.2 mg of Pd/C catalyst (10%). The reaction mixture was stirred under H_2-pressure (1 atm.) for 5h and filtered over Hyflow. The filtrate was concentrated in vacuo yielding 32 mg (100%) as an yellow oil. R_f (CH_2Cl_2/MeOH 4:1) = 0.57. ^{1}H Nmr: 2.10, 2.16 and 2.18 (3xs, 3x OAc), 4.44 (m, $H_{5'}$), 4.53 (m, $H_{4'}$), 5.58 (dd, J = 4.8 and 5.4 Hz, $H_{3'}$), 5.87 (dd, J = 5.2 and 5.4 Hz, $H_{2'}$), 6.30 (d, J = 5.3 Hz, $H_{1'}$), 7.29 (s, H_8), 8.34 (s, H_2), 9.67 (s, H_5). Ir : 2990 (w), 2950 (w), 1750 (s), 1710 (s, CO lactam), 1615 (s), 1490 (m). HRms: M+1: found: 490.1183, calcd for $C_{20}H_{19}N_5O_{10}$+ H: 490.1210. Anal. Calcd for $C_{20}H_{19}N_5O_{10}$: C, 49.08; H, 3.91; N, 14.31. Found: C, 49.0; H, 4.1; N, 14.2.

3-β-D-Ribofuranosyl-9*H*-9-oxo-7-methoxycarbonylpyrimido[2,1-*i*]purine (**5b**)

To a solution of 500 mg (1.34 mmol) of adenosine in 5 ml of H_2O was added a solution of dimethyl acetylenedicarboxylate (500 mg, 3.6 mmol) in 5 ml of EtOH/5% AcOH. The mixture was kept at room temperature and after 7 h a second portion of 250 mg of diester was added. After 18 h the precipitate was

collected and washed successively with 2 ml of water, 2 ml of ethanol and 2 ml of acetone yielding 394 mg (78%) of pale yellow crystals. mp 228-229 °C (lit.,[6] 239 °C). R_f (CH_2Cl_2/10% MeOH) = 0.25. ^{1}H Nmr (DMSO): 3.65 (m, 2H, $H_{5'}$), 4.00 (s, 4H, $H_{4'}$ and OMe), 4.19 (m, $H_{3'}$), 4.55 (m, $H_{2'}$), 5.12 (dd, J = 5.3 Hz, 1H, OH-5'), 5.33 (d, J = 5.0 Hz, OH-3'), 5.64 (d, J = 6.0 Hz, OH-2'), 6.00 (d, J = 5.5 Hz, $H_{1'}$), 6.90 (s, H_8), 8.69 (s, H_2), 9.28 (s, H_5); Irradiation of OMe gave a 0.50 % NOE for H_8 and a 0.45 % NOE for H_5; Irradiation of H_5 gave a 2.5% NOE for OMe and a 1.5% NOE for H_8. ^{13}C Nmr: 53.91 (OMe), 60.98 ($C_{5'}$), 70.07 ($C_{3'}$), 74.31 ($C_{2'}$), 85.72 ($C_{4'}$), 87.48 ($C_{1'}$), 117.74 (C_8), 123.03, 137.55, 141.36, 141.87, 142.91, 147.91, 161.13, 164.51. Ir (KBr): 3360 (m), 2910 (w), 1750 (s, CO ester), 1640 (s, CO lactam), 1590 (s), 1495 (m). HRms: M+1: found: 378.0994, calcd for $C_{15}H_{15}N_5O_7$+ H: 378.1050.

The same compound was obtained when adenosine (100 mg, 0.27 mmol) was suspended in 4 ml of dichloroethane/ 5% AcOH and treated with 100 mg of dimethyl acetylenedicarboxylate. The reaction mixture was refluxed for 6 h and the precipitate was collected and washed with methanol and acetone (78 mg, 55%).

3-[2',3',5'-Tri-*O*-acetyl-(β-D-ribofuranosyl)]-9*H*-9-oxo-7-methoxycarbonylpyrimido[2,1-*i*]purine (**5a**)

To a suspension of 47 mg (0.13 mmol) of **5b** in 3 ml of pyridine was added 0.22 ml (2.2 mmol) of acetic anhydride at 0 °C. After 1 h a second portion of acetic anhydride was added. The reaction mixture was stirred for 3 h at room temperature, 1 h at 55 °C and then concentrated in vacuo after 5 ml of EtOH was added. Repeating this for 8 more times gave 58 mg (92%) of a mixture of **5a** and **3a** (6:4) as a yellow solid. R_f (EtOAc) = 0.21. ^{1}H Nmr: 2.10, 2.13 and 2.15 (3xs, 3x OAc), 4.05 (s, OMe), 4.41 (m, $H_{4'}$ and 2x $H_{5'}$), 5.56 (m, $H_{3'}$), 5.87 (m, $H_{2'}$), 6.15 (d, J = 5.3 Hz, $H_{1'}$), 7.18 (s, H_8), 8.13 (s, H_2), 9.48 (s, H_5). Ir: 3000 (w), 1750 (s), 1630 (s, CO lactam), 1600 (s), 1500 (m). HRms: M+1: found: 504.1386, calcd for $C_{21}H_{21}N_5O_{10}$ + H: 504.1367.

6-Methoxycarbonyl-2-[(1'-β-D-ribofuranosyl-5'-dimethylaminoimino)-4-imidazolyl]-4*H*-4-oxopyrimidine (**7**)

To a suspension of 25 mg (0.08 mmol) of **3b** (or **5b**) in 2.5 ml of MeOH was added 9 µl (0.08 mmol) of dimethylamine at 0 °C. After 1.5 h another equivalent of dimethylamine was added and after 3 h the temperature was raised to room temperature. A third equivalent dimethylamine was added after 5h and the reaction mixture was stirred over the weekend. The orange crystals were collected and washed with methanol and acetone yielding 21 mg (75%) of orange crystals. The product was recrystallized from H_2O. mp 189-190 °C. R_f (CH_2Cl_2/MeOH 4:1) = 0.36. ^{1}H Nmr (DMSO): 3.05 and 3.17 (2xs, 2x NMe), 3.61 (m, $H_{5'}$), 3.87 (m, 4H, $H_{4'}$ and OMe), 4.08 (m, $H_{3'}$), 4.25 (m, $H_{2'}$), 5.06 (br s, OH-5'), 5.16 (br s, OH-3'), 5.41 (br s, OH-2'), 5.77 (d, J = 4.8, $H_{1'}$), 6.61 (s, H_5), 7.94 (s, H_8), 9.14 (s, H_2), 11.16 (s, NH). Ir (KBr): 3400, 3210 (m), 2920 (w), 1740 (m), 1655 (s), 1620 (s), 1520 (m). HRms: M+1: found: 423.1553, calcd for $C_{17}H_{22}N_6O_7$ +H: 423.1628. Anal. Calcd for $C_{17}H_{22}N_6O_7$•H_2O: C, 46.36; H, 5.49; N, 19.08. Found: C, 46.3; H, 5.4; N, 19.0.

3-β-D-Ribofuranosyl-7*H*-7-oxo-9-methoxycarbonyl-pyrimido[2,1-*i*]purine (**3b**)

0.5 ml of AcOH was added to a suspension of 9 mg (0.02 mmol) of **7** in 3 ml of MeOH. The reaction mixture was refluxed for half an hour and after cooling down, the mixture gave 6 mg of a white precipitate which was filtered and washed with methanol and acetone yielding 4.1 mg (50%) of compound (**3b**).

6-Methoxycarbonyl-2-(1'-β-D-ribofuranosyl-5'-amino-4'-imidazolyl)-4*H*-4-oxopyrimidine (**8**)

20.4 mg (0.054 mmol) of compound(**5b**)was refluxed for 3 h in 3 ml of H_2O, the reaction mixture was concentrated in vacuo yielding 19.8 mg (100%) **8** as an yellow oil. No reaction was observed at 60 °C. ^1H Nmr (DMSO): 3.63 (m, 2x $H_{5'}$), 3.86 (s, OMe), 3.97 (m, $H_{4'}$), 4.09 (m, $H_{3'}$), 4.36 (m, $H_{2'}$), 5.44-5.22 (2x br s, 3x OH), 5.58 (d, J = 6.7 Hz, $H_{1'}$), 6.42 (s, H_5), 7.06 (s, NH_2), 7.57 (s, CH imidazole), 11.64 (s, 1H, NH). Anal. Calcd for $C_{14}H_{17}N_5O_7$: C, 45.78; H, 4.66; N, 19.07. Found: C, 45.6; H, 4.5; N, 19.2.

6-Amido-2-(1-β-D-ribofuranosyl-5-amino-4-imidazolyl)-4*H*-4-oxopyrimidine (**9**)

A solution of **3a** (20 mg, 0.04 mmol) in 2 ml of dry methanol containing 8 M ammonia was stirred at 0 °C for 15 h and concentrated in vacuo (bath temperature below 30°C). Pale yellow crystals were collected and washed with methanol and acetone. (12 mg, 85%). The product was recrystallized from H_2O. mp 238-240 °C. R_f (MeOH/EtOAc 2:1) = 0.54. ^1H Nmr (DMSO): 3.63 (m, 2x $H_{5'}$), 3.97 (m, $H_{4'}$), 4.09 (m, $H_{3'}$), 4.35 (m, $H_{2'}$), 5.39 (s, 3H, 3x OH), 5.61 (d, J = 6.7 Hz, $H_{1'}$), 6.43 (s, H_5), 6.68 (m, NH_2), 7.56 (s, 1H, CH imidazole), 7.70 (s, 1H, NH_2 amide), 8.27 (s, 1H, NH_2 amide), 10.55 (s, 1H, NH). Anal. Calcd for $C_{13}H_{16}N_6O_6 \cdot H_2O$: C, 42.16; H, 4.90; N, 22.69. Found: C, 42.3; H, 4.8; N, 22.6.

REFERENCES

1. J. S. Gotts and E. G. Gollub, *Proc. Nat. Acad. Sci. U.S.A.*, 1957, **43**, 826.
2. E. G. E. Jahngen and E. F. Rossomando, *Arch. Biochem. Biophys.*, 1984, **229**, 145; P. J. Casey and M. Lowenstein, *J. Biochem.*, 1987, **246**, 263.
3. V. L. Reed, D. O. Mack, and L. D. Smith, *Clin. Biochem.*, 1987, **20**, 349.
4. M. J. Wanner, J. J. M. Hageman, G.-J. Koomen, and U. K. Pandit, *Rec. Trav. Chim.*, 1978, **97**, 211; M. J. Wanner, E. M. van Wijk, G.-J. Koomen, and U. K. Pandit, *Rec. Trav. Chim.*, 1980, **99**, 20; G.-J. Koomen, *Rec. Trav. Chim.*, 1993, **112**, 51.
5. W. A. Hartgers, R. F. de Boer, M. J. Wanner, and G.-J. Koomen, *Nucleosides and Nucleotides*, 1992, **11**, 1325.
6. P. Roques, J. Y. Le Gall, L. Lacombe, and M. Olomucki, *J. Org. Chem.*, 1992, **57**, 1579.
7. H. Reimlinger and M. A. Peiren, *Chem. Ber.*, 1972, **105**, 103; H. Reimlinger and M. A. Peiren, *Chem. Ber.*, 1972, **105**, 794; R. Adams and I. J. Pachter, *J. Am. Chem. Soc.*, 1952, **74**, 5491.
8. D. W. Dunwell and S. Evans, *J. Chem. Soc. C*, 1971, 2094; H. Reimlinger and M. A. Peiren, *Chem. Ber.*, 1970, **103**, 3266; C. F. H. Allen, H. R. Beilfuss, D. M. Burness, G. A. Reynolds,

J. F. Tinker, and J. A. VanAllan, *J. Org. Chem.*, 1959, **24**, 779.

9. W. M. Odijk and G.-J. Koomen, *Tetrahedron*, 1985, **41**, 1893; W. M. Odijk and G.-J. Koomen, *J. Chem. Soc., Trans. II*, 1986, 1561; W. M. Odijk and G.-J. Koomen, *J. Histochem. Cytochem.*, 1987, **35**, 1161.
10. F. Thomé, F. Blois, A. Olomucki, and M. Olomucki, *Eur. J. Biochem.*, 1987, **162**, 433.
11. W. M. Odijk and G.-J. Koomen, *J. Chem. Soc., Perkin Trans. II*, 1987, 733.
12. T. Fujii, T. Saito, I. Inoue, Y. Kumazawa, and K. Tamura, *Chem. Pharm. Bull.*, 1988, **36**, 107.

Received, 12th December, 1994