Potential carcinostatics V[1]. Synthesis and properties of potential inhibitors of the adenylosuccinate synthetase and adenylosuccinate lyase system

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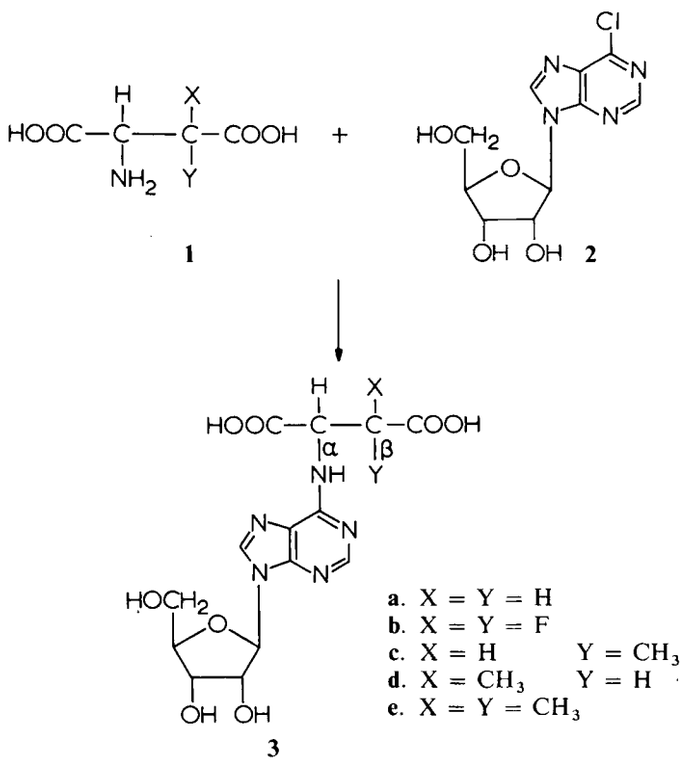
(Received July 4th, 1979)

Abstract. β,β-Dimethylaspartic acid was obtained by the aluminium-amalgam reduction of the *O*-methyloxime of diethyl 2-oxo-3,3-dimethyl-1,4-butanedioate and subsequent hydrolysis. The (latter) diester was conveniently prepared by the condensation of ethyl 2-bromo-2-methylpropionate with diethyl oxalate, under the influence of magnesium. β,β-Difluoroaspartic acid, *threo* β-methylaspartic acid and β,β-dimethylaspartic acid (as racemates) were coupled with 6-chloropurin-9-yl riboside to yield the corresponding nucleosides. The bases methyl 7-oxo-7*H*-pyrido[2.1-*i*]purine-9-carboxylate and 6-[2,3-bis(methoxycarbonyl)propyl]purine were converted into the corresponding nucleosides by coupling with O^2,O^3,O^5-tribenzoylribofuranosyl chloride and removal of the protecting groups. The biological test results on leukemia P-388 are presented.

The salient role of adenylosuccinate (**3a**-monophosphate Scheme 1) in the biosynthesis of purine nucleotides, outlined in the previous communication[2], has prompted us to investigate the analogues of adenylosuccinate, in which the critical fumarate-elimination step is either suppressed or rendered unfeasible as the result of specific structural modifications. With this objective the development of two general types of analogues was undertaken. In one class of analogues the replacement of the β-hydrogens of the succinate moiety, in **3a**, by fluorine atoms or methyl group(s) was envisaged, while in the second, it was projected to replace the *exo*-cyclic nitrogen of N^6-succinyladenosine (**3a**) by a carbon atom. The present communication describes the synthesis and the preliminary biological evaluation of the *ribosides* (nucleosides, **3b-e**, Scheme 1 and **10b** and **11b** (Scheme 4) corresponding to the adenosinosuccinate analogues).



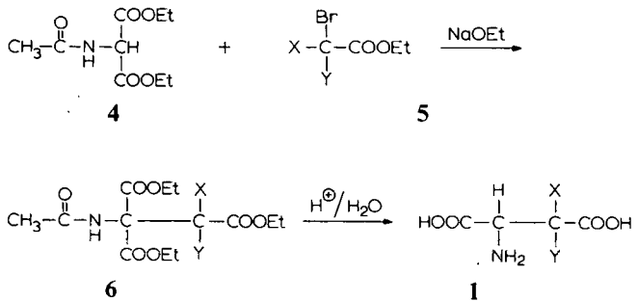
Scheme 1

Preparation of β (mono)- and β,β (di)-substituted N^6-succinyl adenosines

Following an orientation study on the synthesis of nucleosides **3b-e** and after discarding several proposed routes, the approach involving a direct coupling of the aspartate moiety to a suitable purin-9-yl riboside derivative was chosen. The synthesis of the required β,β-difluoroaspartic acid (**1b**) has been reported earlier from this laboratory[3]. Racemic *erythro* and *threo* β-methylaspartic acids (**1c** and **1d**, respectively) were prepared according to literature procedures[4,5,6]. In agreement with the experience of the earlier workers[5], only the *threo* compound **1d** could be isolated without contamination of the *erythro* isomer. The latter could not be obtained free from the *threo* isomer. The synthesis of the disubstituted aspartic acid **1e** has been reported by *Burrows* et al.[4], via the sequence of reactions described in Scheme 2. Although the scheme works very well for the preparation of amino acids **1c** and **1d**, attempts to react bromide **5e** with acetamidomalonate (**4**) failed to give the desired triester **6e**. The sole product obtained after acid hydrolysis of the reaction mixture was identified (IR, PMR) as γ-methylglutamic acid. The formation of the latter is readily understood in terms of base-catalyzed dehydrobromination of **5e** to ethyl methacrylate, which serves as a Michael acceptor towards the anion of **4**; subsequent hydrolysis and decarboxylation leads to the observed methylglutamic acid. In the light of these results and the expected resistance of **5e** towards nucleophilic substitution, it is difficult to explain the observations of *Burrows* and coworkers[4], who according to the melting point of the reported product appeared to have obtained **1e**. The synthesis of β,β-dimethylaspartic acid (**1e**) was finally achieved via the sequence of reactions described in Scheme 3[7,8].

[1] Part IV: *M. J. Wanner, J. J. M. Hageman, G. J. Koomen* and *U. K. Pandit*, J. Med. Chem. (accepted for publication).
[2] *M. J. Wanner, J. J. M. Hageman, G. J. Koomen* and *U. K. Pandit*, Recl. Trav. Chim. Pays-Bas **97**, 211 (1978).
[3] *J. J. M. Hageman, M. J. Wanner, G. J. Koomen* and *U. K. Pandit*, J. Med. Chem. **20**, 1677 (1977).
[4] *I. E. Burrows, G. Shaw* and *D. V. Wilson*, J. Chem. Soc. C 40 (1968).
[5] *H. A. Barker* and *E. J. Wawszkiewicz*, Biochem. Prep. **8**, 93 (1961).
[6] *M. Bodanszky* and *G. G. Marconi*, J. Antibiot. **23**, 238 (1970).
[7] *E. Vogel* and *H. Schinz*, Helv. Chim. Acta **33**, 116 (1950).
[8] *A. J. Jönsson*, Sven. Kem. Tidskr. **67**, 188 (1955).

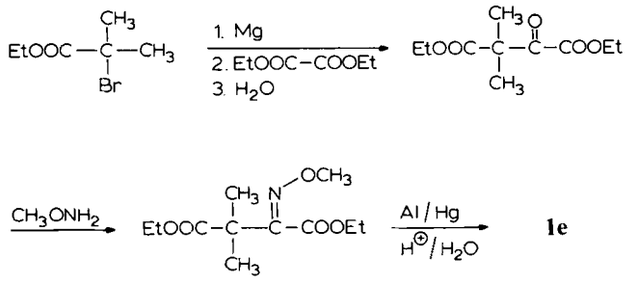
The intermediate dimethyloxaloacetate was prepared according to the procedure of *Vogel* and *Schinz*[7]. The last step of the sequence, namely the reduction of the *O*-methyl oxime function to the amino group, was achieved according to procedures described by us earlier[3].



c. X = H Y = CH_3
d. X = CH_3 Y = H
e. X = Y = CH_3

Scheme 2

The acids **1b,d,e** (as racemates) were coupled with 6-chloropurin-9-yl riboside (**2**) in water, at pH 9.5, in a manner analogous to that described for the preparation of **3a**[8,10].

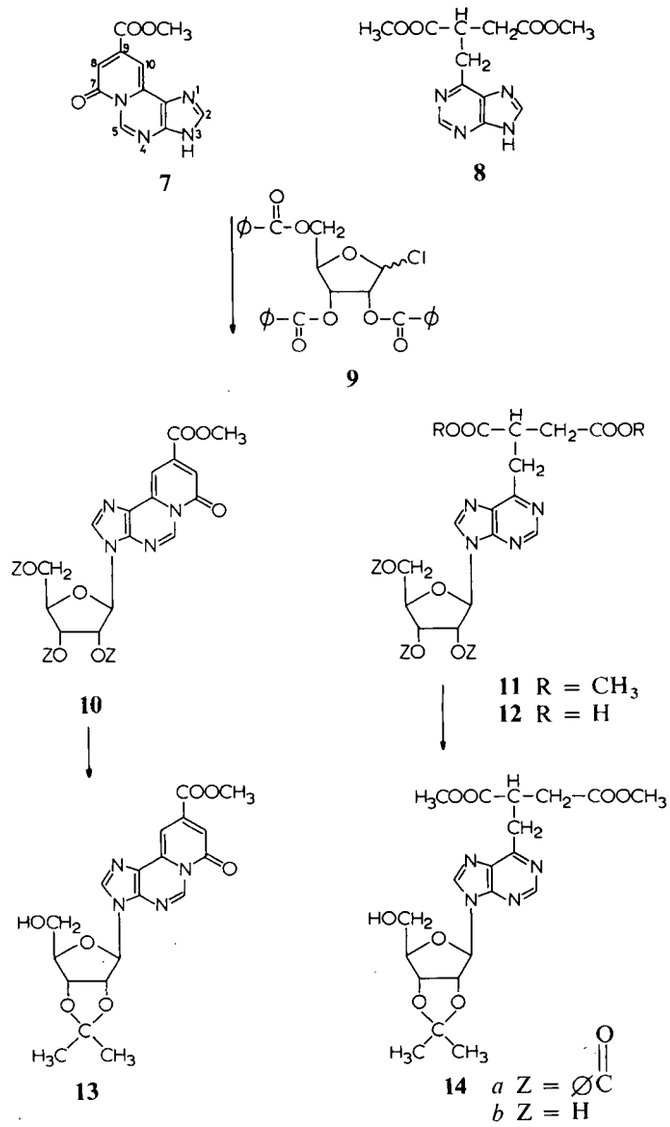


Scheme 3

While diastereomeric coupling products are expected from these reactions, efforts to identify such isomers, were, except in the case of **3b**, unsuccessful[11]. The ^{19}F NMR of **3b** is accountable on the basis of a 1 : 1 mixture of diastereomers (vide experimental). The stereomeric nucleosides **3b**, **3d** and **3e** were obtained in good to modest yields.

Synthesis of pyridopurine- and 6-(2,3-dicarboxypropyl)-purinenucleosides (10, 11b, 12b)

The nucleobases **7** and **8**, described earlier[2], were converted into the corresponding mercury salts by treatment with sodium methoxide and mercuric chloride[12]. These salts could be conveniently coupled with D-O^2,O^3,O^5-tribenzoyl-ribofuranosyl chloride **9** by refluxing in xylene (Scheme 4). In the case of **7**, the coupling reaction proceeded to give the riboside **10a** in 89% yield. Removal of the benzoate protecting groups (sodium methoxide and methanol) gave the fluorescent nucleoside **10b** as a crystalline product, m.p. 175–176°. In an analogous manner the mercury salt of **8** yielded the nucleoside analogue **11b**, via **11a**, in high yield. In order to obtain better soluble products and for structural identification, both nucleoside analogues were converted into their corresponding isopropylidene derivatives **13** and **14**. The observed difference of 0.22 and 0.27 ppm respectively between the chemical shifts of the isopropylidene methyl groups attested to the expected β-configuration[13] of the nucleosides. Hydrolysis of the ester groups of **11b** finally afforded **12b** which could be obtained in pure form as its barium-salt.



Scheme 4

Biological results

For studying interactions of the nucleoside analogues described with isolated adenylosuccinate synthetase and adenylosuccinate lyase *in vitro*, presence of a 5'-phosphate is of vital importance. This is however not the case for in vivo anti-leukemina screening in mice, since it is generally accepted that nucleotides do not enter the cell as such. The lipophilic character of the cell-membrane prevents the free passage of the very polar phosphates. Phosphorylation has thus to take place within the cell. The nucleoside analogues described in this communication were therefore tested without further transformation for their antileukemic

[9] *G. B. Chheda*, Nucl. Acid Res. **4**, 739 (1977).
[10] *P. Narayanan, H. M. Berman* and *R. Rousseau*, J. Am. Chem. Soc. **98**, 8472 (1976).
[11] This was also observed by *Burrows* et al.[4] for the coupling products of D and L *threo* β-methylaspartic acid with methyl 5-amino-1-(O^2,O^3-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate. In seven different solvent systems, both in paper and thin-layer chromatography the products exhibited one spot.
[12] *B. R. Baker, K. Hewson, H. J. Thomas* and *J. A. Johnson*, J. Org. Chem. **22**, 957, 958 (1957).
[13] *J. L. Imbach*, Ann. N.Y. Acad. Sci. **255**, 177 (1975).

properties against leukemia P-388 in mice. Cytostatic activity was determined by comparing median survival times of leukemic mice, treated with the compounds with that of untreated control animals. The results are summarized in Table I.

Table I Antileukemia tests in leukemia P-388.

compound	NSC no.	dosage (mg/kg)	*T/C* %
3b	294195	400, 200, 100	99, 100, 97
3d	294196	400, 200, 100	98, 100, 98
3e	294197	400, 200, 100	toxic, 106, 100
10b	294194	400, 200, 100	100, 100, 98
11b	294198	400, 200, 100	99, 99, 100

As can be seen from the Table, none of the compounds exhibited biological activity within the experimental error. Also hardly any toxicity is observed. Lack of biological activity can be due to a variety of reasons. To obtain a better insight into this, study of the compounds as their corresponding 5'-monophosphates in *in vitro* enzyme systems will be necessary. This work is currently in progress.

Experimental

All melting points are uncorrected. Analyses were carried out by Mr. H. Pieters of the Microanalytical Department of this laboratory. IR spectra were recorded on a Unicam SP 200 or on a Perkin Elmer 125 spectrometer. PMR spectra were scanned on Varian Associates Model A-60 D and HA-100 instruments, using TMS as an internal standard. Chemical shifts are given in δ relative to TMS.

Attempted synthesis of dimethylaspartic acid **1e**

To a solution of 26 g of diethyl acetamidomalonate **4** in 65 ml of ethanol a solution of 3.2 g sodium in 60 ml of ethanol was added. To the sodium salt thus obtained, a solution of 32 g of ethyl 2-bromo-2-methylpropionate **5e** was added and the mixture was refluxed during seven h. The alcohol was evaporated, the residue taken up in 40 ml of water and extracted with ether. The combined ether layers were concentrated and the residue refluxed with 40 ml of water and 120 ml of concentrated hydrochloric acid. After evaporation, the residue was taken up in ethanol and treated with aniline to pH 4. Cooling the solution produced 6.2 g (32%) of γ-methylglutamic acid (m.p. 159–160°C).
PMR: (D_2O, Na_2CO_3) 1.25 1.25 [m, 3H, CH_3], 2.1 [m, 2H, CH_2], 2.6 [m, 1H, $CH(CH_3)$], 3.8 [m, 1H, $CH(NH_2)$–COOH].
Calc. for $C_6H_{11}NO_4$ (161.16): C, 44.71, H, 6.88, N, 8.6%. Found: C, 44.8, H, 6.85, N, 8.6%.

β,β-*Dimethylaspartic acid* **1e**

A solution of 4.3 g of diethyl dimethyloxaloacetate and 6.8 g of *O*-methylhydroxylamine hydrochloride (4 eq.) in 50 ml of ethanol was stirred at room temperature during five days. The excess of reagent was precipitated with ether. Concentrating the filtrate produced 4.2 g (85%) of the crude *O*-methyloxime. IR ($CHCl_3$): 1730 (C=O), 1625 (weak) (C=N–), P ⟨R ($CDCl_3$): 1.2 [d × t, 6H, $COOCH_2CH_3$], 1.4 [s, 6H, C–CH_3], 3.85 [s, 3H, $NOCH_3$], 4.2 [d × q, 4H, $COOCH_2CH_3$]. The oxime was reduced with aluminiumamalgam in a mixture of ether and water during 18 h[3]. The reaction mixture was filtered and the filtrate evaporated to dryness. The residue was refluxed with 100 ml of hydrochloric acid (10%) during 8 h. After evaporation, the residue was taken up in ethanol and treated with aniline to pH 4. Cooling the solution produced 1.5 g (56%) of β,β-dimethylaspartic acid **1e**. The product was purified by recrystallisation from water, m.p. 267–272° (dec.).
PMR: (D_2O, Na_2CO_3), 1.25 [d, 6H, CH_3], 3.8 [s, 1H, –$CH(NH_2)$].
Calc. for $C_6H_{11}NO_4$, H_2O (178.16): C, 40.44, H, 7.30, N, 7.87%. Found: C, 40.3, H, 7.4, N, 7.9%.

Coupling reactions of substituted aspartic acids **1b**, **1d** *and* **1e** *with 6-chloro-9-(β-D-ribofuranosyl)purine* **2**[9]

To a solution of the substituted aspartic acids in water at pH 9.5 (KOH) a solution of 0.33 eq. of **2** in water was added. The mixture was refluxed during 3 h. After cooling, the reaction mixture was diluted with water, acidified to pH 2.5 (HCl) and stirred with activated charcoal (amount: six times the quantity of starting aspartic acids). The suspension was filtered (hy-flow) and the charcoal washed with water. The products were obtained by eluting the charcoal with water/ethanol/concentrated ammonia, 50/48/2. The solvents were evaporated and the residue taken up in a small amount of water. Products **3b**, **3d** and **3e** were obtained by addition of acetone to the solution.

N-*(9-β-D-ribofuranosylpurin-9-yl)*-β,β-*difluoroaspartic acid* **3b**

Yield: 53%. M.p. 150° (dec.), PMR (D_2O), 3.85 [m, 2H, CH_2OH], 4–4.50 [m, 3H, $H_{2'}$, $H_{3'}$, $H_{4'}$], 5.50 [t, (broadened), *J* 14, 1H, CH–CF_2], 6.02 [d, *J* 5, 1H, O–CN–N], 8.38 [s, 2H, purine protons], ^{19}FMR. (D_2O, internal standard $CFCl_3$); diastereomer I, δ −108.03, −110.11. [AB part of ABX system, J_{FF} 248, J_{HF} 13, J_{HF} 15]; diastereomer II, δ −108.06, −110.12, [AB part of ABX system, J_{FF} 248, J_{HF} 13, J_{HF} 15].
Calc. for $C_{14}H_{15}F_2N_5O_8$, 2.5 H_2O (464): C, 36.21, H, 4.34, F, 8.18%. Found: C, 36.2, H, 4.0, F, 8.8.

N-*(9-β-D-Ribofuranosylpurin-9-yl)*-threo-β-*methylaspartic acid* **3d**

Yield: 58%. M.p. (diammonium salt) 157°C (dec.). PMR: (D_2O), 1.35 [d, 3H, CH_3], 3.3 [m, 1H, CH–CH_3], 3.65 [m, 1H, $CHNH_2$], 3.95 [m, 2H, CH_2OH], 4.2, 4.35, 4.5 [3m, 3H, ribose protons], 6.15 [d, 1H, O–CH–N], 8.35 [2s, 2H, purine protons].
Calc. for $C_{15}H_{25}N_7O_8$ (431): C, 41.76, H, 5.80, N, 22.74%. Found: C, 41.9, H, 5.6, N, 22.5%.

N-*(9-β-D-ribofuranosylpurin-9-yl)*-β,β-*dimethylaspartic acid* **3e**

Yield: 60%. M.p. (diammonium salt): 117°C (dec.). PMR (D_2O), 1.3 [2s, 6H, CH_3], 3.6 [m, 1H, $CHNH_2$], 3.8 [m, 2H, CH_2OH], 4–4.5 [m, 3H, ribose protons], 6.05 [d, 1H, O–CH–N], 8.3 [2s, 2H, purine protons].
Calc. for $C_{16}H_{27}N_7O_8$ (445): C, 43.15, H, 6.07, N, 22.02%. Found: C, 43.3, H, 5.9, N, irreproducable.

*Methyl 7-oxo-7*H-*pyrido[2,1-i]purine-9-carboxylate* **7**

This compound was prepared from its corresponding 3-pyranyl-ether[2] by refluxing the protected derivative in methanol, containing a catalytic amount of *p*-toluenesulphonic acid. The product precipitated after 16 h of reaction. Yield: 85%. M.p.: 285–290° (dec.).

6-[2,3-bis(methoxycarbonyl)propylpurine **8**

Removing the pyranyl group from the protected base[2] was carried out by refluxing in methanol, containing a catalytic amount of *p*-toluenesulphonic acid during 16 h. The reaction mixture was diluted with water and repeatedly extracted with chloroform. Evaporation and recrystallisation from ethyl acetate/hexane produced pure **8** in 80% yield. M.p.: 108–110°C.

Methyl 3-(O^2,O^3,O^5-*tribenzoylribofuranosyl)-7-oxo-7*H-*pyrido-[2,1-i]purine-9-carboxylate* **10a**

To 4 ml of methanol 0.10 g sodium hydride was added. The solution of methoxide in methanol was added to a suspension of 0.4 g of **7** in methanol. After stirring for 10 minutes the resulting suspension was added to a solution of 0.55 g mercuric dichloride in aqueous methanol[12]. After stirring at room temperature during 1 h the suspension was filtered (hy-flow). To the mixture of the mercury salt and hy-flow in 60 ml refluxing xylene, a solution of O^2,O^3,O^5-tribenzoylribofuranosyl chloride was added (obtained from 1.0 g O^1-acetyl-O^2,O^3,O^5-tribenzoylribofuranose and dry hydrogen chloride[12]).
After refluxing during 1.5 h, the reaction mixture was filtered, the solvents removed and the residue purified by column chromatography (silica, ethyl acetate). **10a** was obtained as a yellow solid (1.0 g = 89%). M.p.: 100–110°. IR ($CHCl_3$): 1730 (ester C=O), 1690 (amide C=O), 1610. PMR ($CDCl_3$): 3.97 [s, 3H, $COOCH_3$], 4.6–5.0 [m, 3H, $H_{4'}$, $H_{5'}$], 6.2–6.5 [m, 3H, $H_{1'}$, $H_{2'}$, $H_{3'}$], 7.13 [d, 1H, H_8], 7.3–8.14 [m, 17H, PhCO + H_{10}], 8.16 [s, 1H, H_2], 9.47 [s, 1H, H_5].

*Methyl 3-(ribofuranosyl)-7-oxo-7*H-*pyrido[2,1-i]purine-9-carboxylate* **10b**

To a solution of 0.5 g **10a** in 5 ml of dimethoxyethane a solution of 20 mg of sodium methoxide in 10 ml of methanol was added.

After stirring during $3\frac{1}{2}$ h at room temperature, the mixture was neutralized with acetic acid and the solvents evaporated. The residue was purified by column chromatography (silica, ethyl acetate/methanol 10 : 1). Yellow coloured fractions were concentrated. Recrystallising the residue from methanol afforded **10b** as yellow needles (0.16 g, 59%). M.p.: 175–176°, IR (KBr): 3200–3500 (OH), 1740 (ester C=O), 1690 (amide C=O).
Calc. for $C_{16}H_{16}N_4O_7$ (376.32): C, 51.06, H, 4.29, N, 14.92%. Found: C, 50.8, H. 4.4, N, 14.7%.

Methyl 3-(O^2,O^3-isopropylideneribofuranosyl)-7-oxo-7H-pyrido-[2,1-i]purine-9-carboxylate **13**

A mixture of 0.15 g **10b**, 0.19 *p*-toluenesulphonic acid and 0.4 ml of dimethoxypropane in 5 ml of dry acetone was stirred at room temperature. After 45 minutes the starting nucleoside had dissolved and another 30 minutes later all **10b** according to TLC had been converted. The reaction mixture was poured into a solution of 0.1 g of sodiumcarbonate in 25 ml of water. Extraction with chloroform, drying (Na_2SO_4) and evaporating the solvents produced **13**, which could be purified by recrystallisation from methanol/ethyl acetate. Yield: 0.08 g (50%). M.p.: 237–238°C. IR ($CHCl_3$): 3370 (OH), 1730 (C=O), 1690, 1600. PMR (d_6-DMSO): 1.33, 1.55 [2s, 6H, isopropylidene CH_3], 3.58 [d, *J* 5, 2H, CH_2OH], 4.29 [m, 1H, H(4')], 5.02 [d × d, *J* 6, *J* 3, 1H, H(3')], 5.39 [d × d, *J* 6, *J* 2.5, 1H, H(2')], 6.23 [d, *J* 2.5, 1H, H(1')], 6.76 [d, *J* 1.5, 1H, H(10)], 7.19 [m, 1H, H(8)], 8.64 [s, 1H, H(2)], 9.52 [s, 1H, H(5)].
Calc. for $C_{19}H_{20}N_4O_7$ (416.38): C, 54.80, H, 4.84, N, 13.46%. Found: C, 54.9, H, 4.8, N, 13.3%.

9-Ribofuranosyl-6-[2,3-bis(methoxycarbonyl)propyl]purine **11b**

The protected nucleoside **11a** was prepared in 65% yield (0.59 g) from 0.33 g **8**, 0.35 g mercuric chloride and 0.5 g O^1-acetyl-O^2,O^3,O^5-tribenzoyl-β-D-ribofuranose following the same procedure as described for **10a**. Treatment of 2.7 g **11a** dissolved in 5 ml of dimethoxyethane with a solution of 0.07 g sodium methoxide in 25 ml of methanol as for **10a**, produced after chromatography (silica, ethyl acetate gradient to ethyl acetate/methanol 10 : 1) 1.29 g (85%) **11b** as a thick oil. IR ($CHCl_3$): 3200–3500 (OH), 1735 (C=O), 1600.

9-(O^2,O^3-isopropylideneribofuranosyl)-6-[2,3-bis(methoxycarbonyl)propyl]purine **14**

A solution of 0.17 g **11b**, 0.09 g *p*-toluenesulphonic acid and 0.35 ml of dimethoxyethane in 10 ml of dry acetone was kept at room temperature during 1.5 h. After neutralizing with sodiumcarbonate and evaporation of the solvent, the residue was purified by column chromatography (silica, ethyl acetate gradient to ethyl acetate/methanol 20 : 1. Yield: 0.18 g **14** (oil) (96%). IR ($CHCl_3$): 3300 (OH), 1735 (C=O), 1600, PMR ($CDCl_3$): 1.31, 1.58 [2s, 6H isopropylidene $-CH_3$], 2.65 [2H, AB part of ABX system, purine $-CH_2$], 3.3–4.1 [m, 5H, CH$-CH_2-$COOR + H(5')], 3.60, 3.62 [2s, 6H, $2COOCH_3$], 4.45 [m, 1H, H(4')], 5.05 [d, 1H, H(3')], 5.12 [d × d, 1H, H(2')], 5.89 [d, 1H, H(1')], 8.09 [s, 1H, H(8)], 8.81 [s, 1H, H(2)].

9-Ribofuranosyl-6-(2,3-dicarboxypropyl)purine **12b**

The methyl esters of **10b** were hydrolysed in 1M potassium hydroxide at 0°C. The solution was acidified (pH 3.5) with Dowex-50 ($H^{\oplus}$ form.). Since the product could not be obtained in a pure form, barium hydroxyde was added to a solution of **12b** in water, until pH reached 7.5. Addition of ethanol produced **12b** as its barium salt.
Calc. for $C_{15}H_{16}BaN_4O_8$, 4 H_2O (589): C, 30.56, H, 4.07, N, 9.51%. Found: C, 30.64, H, 4.12, N, 9.55%.

Acknowledgement

The authors wish to express their thanks to Dr. *G. Atassi*, Free University of Brussels for the in vivo antileukemia screening.

Chemistry of 2*H*-thiopyran and derivatives IV[1]. Deprotonation and methylation of 2*H*-thieno[2,3-*b*]thiopyran and some derivatives

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(Received May 2nd, 1979)

Abstract. Metallation of 2*H*-thieno[2,3-*b*]thiopyran **1** by strong bases under relatively apolar conditions proceeds exclusively in the 2-position of the thiophene ring. In polar media a 2-proton of the thiopyran ring is removed to give the anion **1b**. By addition of hexamethylphosphoric triamide (HMPT) to the lithiated compound **1a** conversion into **1b** takes place. The anions **1a** and **1b** react with methyl iodide to give the compounds **2** and **3**, respectively. Compound **2** can be further methylated by reaction with BuLi in tetrahydrofuran (THF)/HMPT mixtures or sodamide in liquid NH_3, followed by addition of methyl iodide. The resulting compound **4** can be converted into the compounds **5**, **6** or **7**, depending on the base-solvent system: Compound **5** is produced by interaction between **4** and KNH_2 in liquid NH_3 at $-33°$, followed by methylation at $-60°$. If the reaction with KNH_2 is carried out at $-60°$, however, methylation gives **6**, this result is also obtained from **4**, BuLi in THF/HMPT and CH_3I. Omission of HMPT during the metallation gives rise to vinylic proton abstraction, and subsequent addition of CH_3I leads to **7**.

Introduction

In the first part of the series on thiopyran chemistry we showed that it is possible to abstract the protons in the 2- and 6-positions of 2*H*-thiopyran, the 2-proton under thermodynamic, the 6-proton under kinetic conditions. The anions (or organolithium derivatives) reacted *inter alia* with alkyl halides to give mixtures of 2-alkyl-2*H*-thiopyrans and 4-alkyl-4*H*-thiopyrans or 6-alkyl-2*H*-thiopyrans, respectively.

Although no pK_a values of the various protons in thiophene, 2*H*-thiopyran and related systems are known, a rough estimation is possible on the basis of experimental data.

[1] Part I, *R. Gräfing* and *L. Brandsma*, Recl. Trav. Chim. **97**, 208 (1978);
Part II, *R. Gräfing* and *L. Brandsma*, Synthesis **1978**, 578;
Part III. *R. Gräfing*, *H. D. Verkruijsse* and *L. Brandsma*, J. Chem. Soc. Chem. Commun. **1978**, 596.