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FLUORESCENT NUCLEOSIDE ANALOGUES

Synthesis of fluorescent pyrido[2,1-i]purines, and their corresponding ribosides

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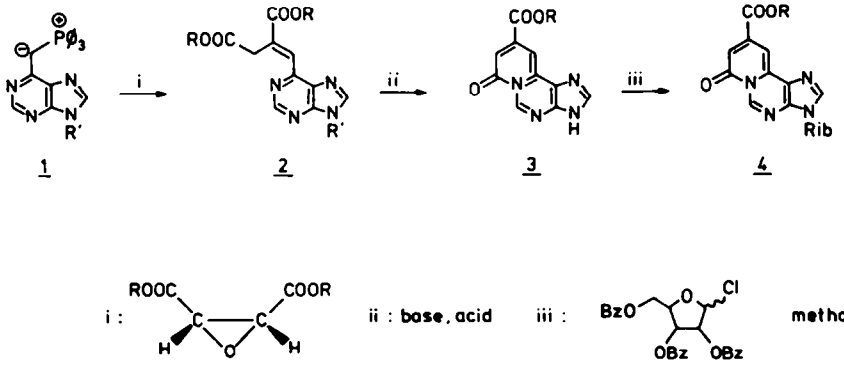
Abstract - Fluorescent pyrido[2,1-i]purines can in principle be obtained via Michael-addition of a suitable anion of a purine derivative to an acetylenic ester, followed by based-catalyzed cyclization, as depicted in Scheme II. 6-Substituted purine-derivatives are obtained via nucleophilic substitution of 6-chloro- and 6-methylsulfonylpurine (8a and 8b). In the presence of methyl propiolate and sodium methoxide, before cyclization, two consecutive Michael-additions take place, leading to 13 and 14. With substituted acetylenic esters, cyclization occurs after one Michael-addition. Michael-additions with ethylenic esters did not lead to expected cyclization products, except in cases where oxidation took place. -For the conversion of the pyrido[2,1-i]purines into the corresponding ribosides protection against nucleophilic attack was necessary.

Introduction

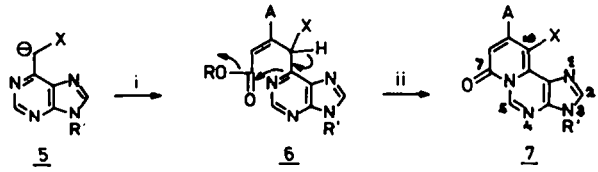
In connection with a program aimed at the synthesis of inhibitors of the enzyms adenylosuccinate synthetase and -lyase, we prepared the pyrido[2,1-i]purine 3, and its corresponding riboside 4; Scheme I[1,2].

These novel purine analogues exhibited strong fluorescence in the visible region, λ_{em} being 478 and 503 nm. (Absorption at 425(log ε = 4.1) and 450 (log ε = 4.1)nm.). Fluorescent nucleosides are of interest as substrates or inhibitors of enzymes, since they can be easily



Scheme I

monitored in the biological system of choice[3-5]. The synthesis of a series of pyrido[2,1-i]-purines, and a study of their physical, chemical and biological properties, was, consequently undertaken. The preparation of these compounds is presented in this communication.
In contrast with Scheme I, the most straight-forward approach for the preparation of pyrido-[2,1-i]purines is that described in Scheme II, i.e. the reaction sequence 5 → 6 → 7.
The synthesis involves a Michael-addition of the anions of suitably protected, 6-substituted purines to electron poor acetylenic esters, followed by base catalyzed cyclization.



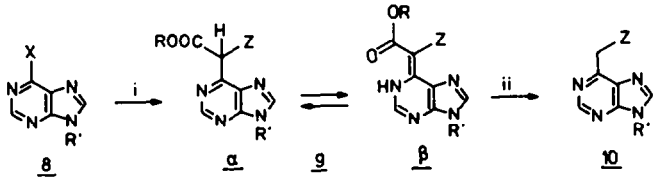
5 6 7

i : A-C≡C-COOR , ii : base

Scheme II

6-Substituted purine-derivatives

Substituted purine-derivatives 9a-c can be obtained, in principle, via nucleophilic substitution reactions at the C(6)-position of 6-chloropurine 8a or the more reactive 6-methylsulphonylpurine 8b; Scheme III[6-8].
Reaction of 8a with the anion of dimethyl malonate in dry DMSO, produced 9a in high yield. According to its NMR- and UV-spectra 9a exists in two tautomeric forms: In aprotic solvents, like DMSO or $CHCl_3$, there is a shift to the longer wavelength (336 nm., β-form) whereas in



8 α 9 β 10

a : X = Cl
b : X = SO_2CH_3

a . Z = COOR
b . Z = CN
c : Z = CHCHCOOR

i : $ROOC\overset{\ominus}{C}HZ$, ii : methoxide / methanol, reflux
R = CH_3, R' = tetrahydropyranyl

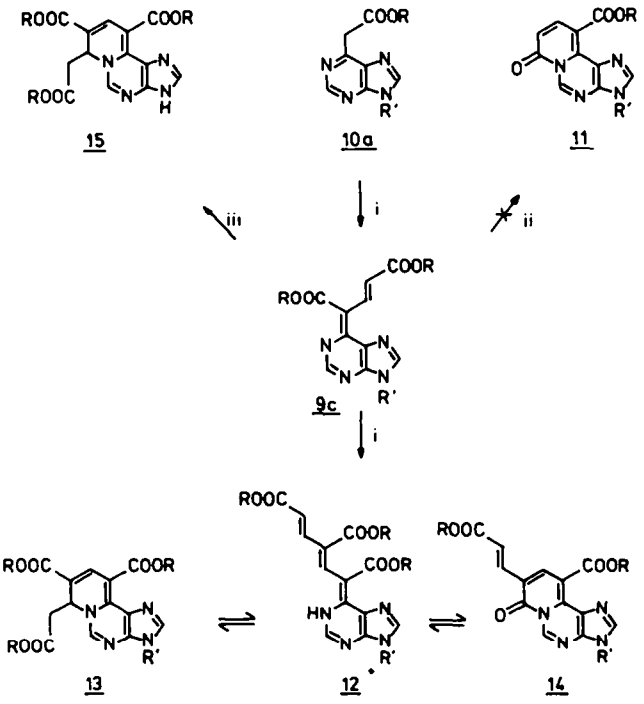
Scheme III

CH_3OH or H_2O the absorption at 256 nm. dominates (α-form). Obviously, hydrogen bonding and conjugation are the factors, that rule the tautomeric equilibrium.
Reaction of 8a with methyl cyanoacetate produced 9b in high yield too. This compound exists in only one tautomeric form (β) under all conditions; λ_{max} = 341 nm.
As a starting material for the study of the cyclization reactions, 9c was prepared by reacting 8a with the anion of dimethyl glutaconate[9]; in this case also one tautomeric form (β) was observed, though the existence of a tautomeric equilibrium could be attested by

D-exchange of one of the vinylic protons; λ_{max} = 380 nm.
Treatment of 9a with sodium methoxide in methanol produced 10a in one step. The anion of 10a is apparently stabilized to such an extent, that attack of methoxide ion on the carbomethoxy-function of 9a leads to C-C bond cleavage with formation of dimethyl carbonate. With 9b and 9c this reaction did not take place; most likely, deprotonation in these cases is favoured over nucleophilic attack. Also, attempts to remove the ester-function under non-basic, Krapcho conditions[10] were unsuccessfull. Long reaction times led to loss of the protecting tetrahydropyranyl-ether, followed by further decomposition.

Michael reactions with methyl propiolate

In an attempt to prepare pyrido[2,1-i] purine11 (Scheme IV), 9a was converted in situ, into 10a, by treatment with sodium methoxide in refluxing methanol, and the refluxing reaction mixture was subsequently treated, with more than two equivalents of methyl propiolate. The next day a crystalline fluorescent compound could be filtered off (70%), to which, on the basis of its spectral data, structure 14 has been assigned. Another product of the reaction, isolated by column chromatography, was the triester 13. The expected cyclization product 11 could not be detected. The formation of the products can be explained in terms of a double Michael addition, leading to 12, from which 14 can be formed



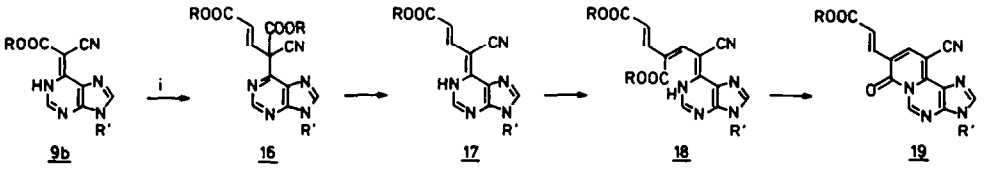
Scheme IV

by attack of nitrogen on the ester function, while 13 is the result of an intramolecular Michael addition to the unsaturated ester moiety. These processes are reversible since the isolated product 13 could be converted into 14 with methoxide in refluxing methanol. There is no obvious reason, why 11, a potential cyclization product of 9c, cannot be formed

under the reaction conditions employed. There is, however, some precedence for these results in the literature. Acheson and Woollard[11] observed that reaction of methyl 2-pyridylacetate with methyl propiolate resulted in cyclization to a quinolizinone derivative only after two consecutive Michael reactions. Attempts to cyclize 9c by reactions with different bases and employing various solvents were unsuccessfull in our hands. However, in the presence of extra methyl propiolate, also 9c could be converted into 13 and 14.

In view of the aforementioned results, efforts were made to convert 9c into 11 under influence of acid. These reactions resulted in the formation of 15, in which the protecting tetrahydropyranyl-group had been lost. The structure of 15 could be established on the basis of its NMR- and mass-spectral data; NMR data are similar to those of 13 and the mass-spectrum exhibited a strong peak (100%), corresponding to $(M\text{-}CH_2COOR)^+$. The quantum-yield of emission at 520 nm. in $CHCl_3$ is 20 times larger than in water.

It has been mentioned, that it was not possible to decarbomethoxylate 9b, but its reaction with methyl propiolate and methoxide, in refluxing methanol, led to the formation of 19. The formation of this product can be understood by the reaction sequence 9b → 16 → 17 → 18 → 19. The first Michael addition replaces the acidic proton, which prevented attack of methoxide on the ester function, by an acrylic ester. Reaction with methoxide now produces 17, which undergoes a second Michael addition (18), followed by cyclization (19); Scheme V.



i : methoxide/methanol , methyl propiolate , reflux

R = CH_3 , R' = tetrahydopropyranyl

Scheme V

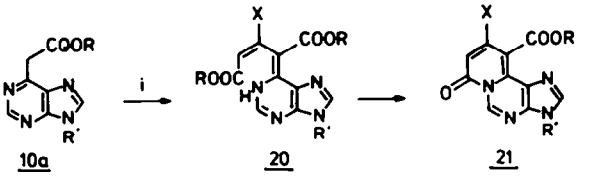
Michael addition reactions with substituted acetylenic esters

When a solution of 10a in methanol, obtained in situ, was treated with sodium methoxide and dimethyl acetylenedicarboxylate, a yellow precipitate was formed, to which structure 21a was assigned (vide experimental); Scheme VI.

In the reaction mixture, products resulting from the addition of methanol to the acetylenic ester[12] were observed, but there was no indication, that double Michael reactions had occured. An analogous reaction with methyl phenylpropiolate did not lead to 21b as a precipitate. Using methanol/cyclohexane as the solvent 21b could be filtered off in a high yield. Apparently formation of a pyrido[2,1-i]purine is completed by its precipitation from the equilibrium mixture. The influence of substituent X on the course of the reaction is probably twofold:

a) It lowers the rate of a second Michael-addition step by steric hindrance.

b) It influences the rotamer distribution of the side chain of the primary adduct 20 in such a way, that cyclization to pyrido[2,1-i]purines is facilitated.

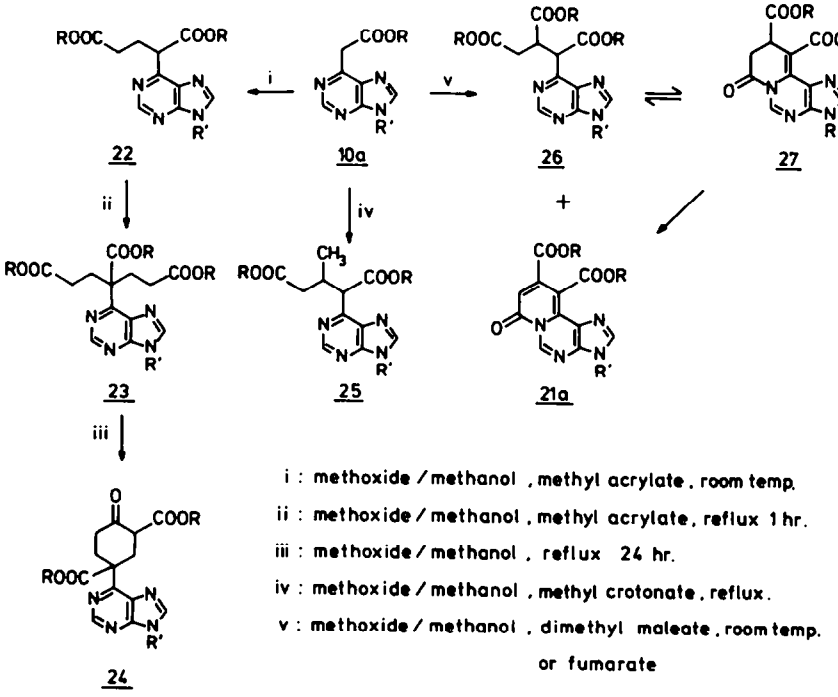


i : methoxide / methanol (cyclohexane) a : X = COOR
XC≡CCOOR , room temp b : X = ∅

R = CH_3 , R' = tetrahydropyranyl

Scheme VI

In the reaction with methyl propiolate, this extra substituent is introduced by the second Michael addition; after this, cyclization takes place.
With 9b and 9c addition reactions with substituted acetylenic esters were not observed, probably due to unfavourable steric interactions, caused by an extra substituent in both the starting materials.



i : methoxide / methanol , methyl acrylate , room temp.
ii : methoxide / methanol , methyl acrylate, reflux 1 hr.
iii : methoxide / methanol , reflux 24 hr.
iv : methoxide / methanol , methyl crotonate , reflux.
v : methoxide / methanol , dimethyl maleate , room temp. or fumarate

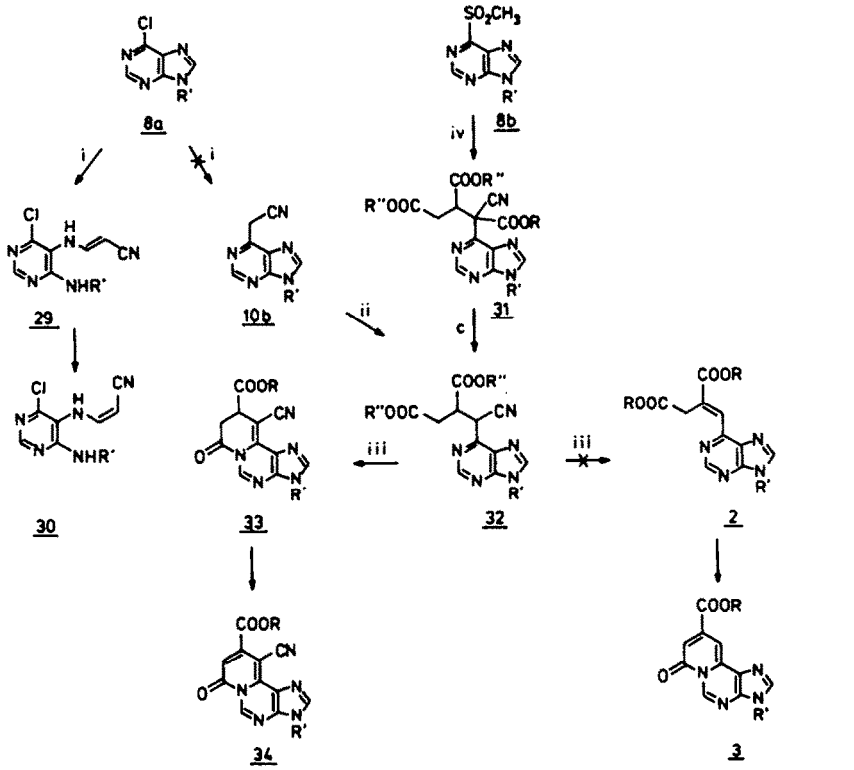
R = CH_3 , R' = tetrahydropyranyl

Scheme VII

Michael reactions with ethylenic esters

Reaction of 10a with one equivalent of methyl acrylate in the presence of sodium methoxide in methanol, at room temperature, resulted in the mono-Michael adduct 22 in high yield (Scheme VII). There was no trace of any saturated pyrido[2,1-i]purine. However, upon refluxing, a double Michael addition product was formed, and after longer periods the Dieckmann cyclization product 24 could be isolated. The observed products indicate, that a retro-Michael reaction is involved. When the reaction was repeated with an excess of

methyl acrylate in a controlled fashion, all the products could be isolated in high yields. With methyl crotonate the diester 25 could be isolated in a low yield, but only after refluxing for a longer period of time. No double Michael adduct was detected, presumably due to steric hindrance. Once again, no cyclization product was formed.
With methyl fumarate or maleate two products were isolated, namely 26 and 21a. Obviously,



Scheme VIII

cyclization occurs and the intermediate 27 is oxidized under the reaction conditions. Carrying out the reaction in a nitrogen atmosphere prevents the formation of 21a. In case of 26 the extra ester function, which is absent in 22 and 25, probably assists oxidation to the aromatic pyrido[2,1-i]purine 21a, which is less susceptible to attack of methoxide at the amide function.

Attempted synthesis of pyrido[2,1-i]purine 3

The products, obtained via Scheme II, are all substituted at C_{10}. Using above mentioned procedures, we wished to prepare pyrido[2,1-i]purine 3, following the reaction sequence 8a → 10b → 32 → 2 → 3; (Scheme VIII).
The nitrile 10b could not be formed by decarbomethoxylation of 9b; consequently a direct introduction of the cyanomethylene-group by reaction of the anion of acetonitrile with 8a was attempted. However, instead of substitution of the chlorine atom, it was found that attack at the C_8 of the purine system, leading to 29, had occured. Structure 29 was as-

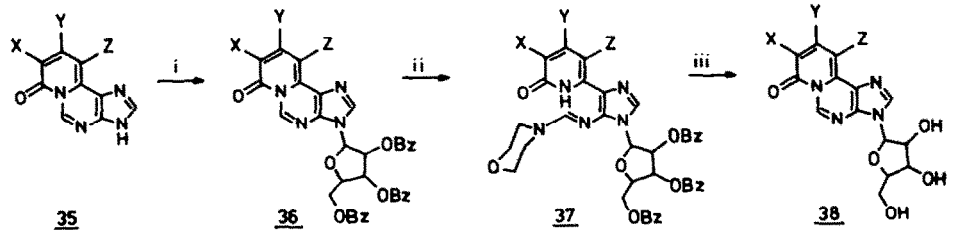
signed on the basis of mass- and NMR-spectral data; the coupling of 9 Hz between the N-H and the C_2-H (tetrahydropyranyl function) being particularly revealing. In DMSO-solution, isomerization of 29 from E- to Z-isomer was observed; the coupling of the vinyl protons decreased from 14 to 9 Hz.

In a different approach towards the synthesis of 3, triester 28 was prepared via addition of the anion of methyl cyanoacetate to diethyl fumarate. Reactions of 28 with 8a failed in our hands, but with the more reactive purine derivative 8b a mixture of 31 and its decarbomethoxylation product 32 was obtained. Treatment of this mixture with methoxide in methanol to induce elimination, resulted in the formation of 34. Under the conditions of the reaction, after ester interchange, cyclization, followed by oxidation of 33, is favoured over elimination of HCN. Exlusion of oxygen did prevent the formation of 33, but did not lead to elimination.

Ribosidation of pyridopurines

Two of the pyridopurines obtained in this study were converted into the corresponding ribosides, via the reactions depicted in Scheme IX.

As reported earlier[2] reaction of the mercury-derivative of 3 with chloride 39a was quite effective for its ribosidation. With 35a,b, however, better results were obtained with the acetate 39b, in acetonitrile, in the presence of $SnCl_4$ as Lewis acid catalyst[13]. From the resulting products 36a,b the protecting benzoate groups can, in principle, be removed via nucleophilic attack by ammonia or methoxide. As will be described in a following paper, pyrido[2,1-i]purines with two electron withdrawing groups are themselves very susceptible to nucleophilic attack at position 5. In contrast to the reaction with alkoxides, the analogous reaction with amines is reversible. Thus 36a,b reacted with morpholine to produce 37a,b. Reaction of the latter compounds with methoxide led to removal of the protecting benzoates, after which the pyrido[2,1-i] purine ring was reconstructed by acidification.



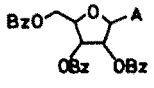
i : $SnCl_4$. 39b . CH_3CN . roomtemp.

ii : morpholine . methanol . roomtemp.

iii : methoxide/methanol . roomtemp. followed by acid

a : X = H, Y = Z = COOR

b : X = CHCHCOOR, Y = H, Z = COOR



39

a : A = Cl (α,β)

b : A = OAc (β)

R = CH_3 , Bz = benzoyl , Ac = acetyl

Scheme IX

In this way, ribosides 38a,b were obtained in reasonable yields.

Fluorescent properties of the different pyrido[2,1-i]purines and reactions with a variety of nucleophiles are described in a following paper, whereas the application of these molecules in biological systems will be published elsewhere.

Experimental

All m.ps are uncorrected. IR spectra were recorded on a Perkin-Elmer 257 spectrometer. The absorptions are given in cm^{-1} and all spectra are taken in $CHCl_3$. PMR spectra were run on a Varian Associates Model A-60-D and XL-100 or Bruker WM 250 instruments, using TMS as an internal standard. Mass-spectra were obtained with a Varian Mat-711 spectrometer. Absorption and Emission spectra were recorded on Cary 17 D respectively Spex Fluorolog instruments.

6-methylsulphonyl-9-(tetrahydropyran-2-yl)purine 8b

According to literature[6] procedures, 6-methyl mercaptopurine was protected with a tetrahydropyranyl ether. To 20 mmol of this compound was added two equiv. of m-chloroperbenzoïc acid in $CHCl_3$. After completion two equiv. of NH_3 aq. were added, followed by extraction with $CHCl_3$. Column chromatography (silica, EtOAc) and recrystallization afforded 5.1 gram (90%) 8b: m.p. 109°. IR: 1590, 1565 (purine), 1330, 1140 (sulfonyl). PMR(d_6-DMSO): 1.5-2.5 (m,6H,pyranyl), 3.60(s,3H,-CH_3), 3.78(m,1H,pyranyl C(6)-H), 4.07(m,1H,pyranyl C(6)-H), 5.89(m,1H,N-CH-O), 9.13(s,1H,H-8), 9.18(s,1H,H-2).

Dimethyl[9-(tetrahydropyran-2-yl)-purin-6-yl]-malonate 9a

To a suspension of 40 mmol NaH in dry DMSO was added 40 mmol dimethyl malonate at room temp. After the evolution of H_2 had ceased, 20 mmol of 8a was added. After 4 hr. of reaction at 80°, water was added and the mixture extracted with EtOAc. The organic layer was washed twice with water (pH=10) and the collected water layers acidified and extracted with $CHCl_3$. The chloroform layer was extracted again with water and dried on $MgSO_4$. Evaporating the solvent afforded crude 9a. Recrystallization from EtOAc/P.A. 60-80 yielded 5.68 gram (85%) 9a: m.p.:160-161°. IR: 1740 (ester), 1625 (hydrogen bonded ester), 1600, 1570 (purine). PMR($CDCl_3$): 3.87(s,6H,-OCH_3), 5.60(m,0.4H,N-CH-O), 5.63(s,0.6H,O=C-CH-C=O), 5.83(m,0.6H,N-CH-O), 8.03(s,0.4H,H-8), 8.06(d,0.4H,J=3,H-2), 8.40(s,0.6H,H-8), 9.07(s,0.6H,H-2). Abs.($CHCl_3$): 336 nm. (CH_3OH): 256 nm.

Methyl 2-cyano-2-[9-(tetrahydropyran-2-yl)purin-6-yl]-acetate 9b

Identical procedure as for 9a with methyl cyanoacetate. Yield 5.6 gram (93%) 9b: m.p. dec. above 200°. IR: 2230 (CN), 1630 (hydrogen bonded ester), 1570 (purine). PMR($CDCl_3$): 3.90(s,3H,-OCH_3),5.68(m,1H,N-CH-O), 8.14(s,1H,H-8), 8.20(d,1H,J=3,H-2). Abs.($CHCl_3$ or CH_3OH): 341 nm.

Dimethyl 4-[9-(tetrahydropyran-2-yl)-purin-6-yl]-pent-2-enedioate 9c

To 40 mmol of NaH in dry THF was added 40 mmol dimethyl glutaconate at room temp. After the evolution of H_2 had ceased, 20 mmol of 8a was added. The mixture was stirred at room temp. overnight. Water was added, neutralized with 5% HCl and after extraction with EtOAc, the product was purified by column chromatography (silica, EtOAc). Yield 4.3 gram (60%). 9c m.p.: 134-136°. IR: 1690 (ester), 1625 (hydrogen bonded ester), 1595, 1555 (purine). PMR ($CDCl_3$): 3.67(s,3H,-OCH_3), 3.85(s,3H,-OCH_3), 5.70(m,1H,N-CH-O), 6.47(d,1H, J=16,-C=CH-C=O), 8.15(d,1H,J=2,H-2), 8.18(s,1H,H-8), 9.70(d,1H,J/16,-CH=C-C=O), (d-DMSO): 6.26(d,1H,J=16,-C=CH-C=O), 9.58(d,1H,J=16,-CH=C-C=O), 14.2(N-H). Addition of D_2O: peaks at 6.26 and 14.2 disappear, 9.58 s. Abs. ($CHCl_3$ or CH_3OH): 380 nm.

Methyl[9-tetrahydropyran-2-yl)-purin-6-yl]-acetate 10a

To a suspension of 5 mmol of 9a in dry methanol was added a catalytic amount of NaH. After refluxing for 1 hr. the solvent was evaporated and the resulting oil was dissolved in EtOAc. The solution was washed twice with water, and dried with $MgSO_4$. Evaporating the solvent yielded 1.35 gram (98%) of 10a as a slightly yellow oil. 10a: IR: 1740 (ester), 1600 (purine). PMR($CDCl_3$): 3.78(s,3H,-OCH_3), 4.32(s,2H,-CH_2-C=O), 5.88(m,1H, N-CH-O), 8.38(s,1H,H-8), 9.02(s,1H,H-2). Abs($CHCl_3$): 265 nm.

Reaction of 10a with methyl propiolate

5 mMol of 9a was converted to 10a as above and without isolation 3 equiv. of methyl propiolate were added. After refluxing overnight 1.45 gram (70%) of 14 was obtained as yel-

low needles, showing green fluorescence in solution. The motherliquor contained 13, which could be isolated by column chromatography (silica, EtOAc); yield 420 mgram (19%) as a glass. 13: IR: 1730 (ester), 1675 (enamine ester), 1610, 1590 (aromate).PMR($CDCl_3$): 2.34 (dxd,1H,J=17x3,CH_2CO), 3.10(dxd,1H,J=17x10,-CH_2-C=O), 3.60(s,3H,-OCH_3), 3.78(s,3H,-OCH_3), 3.90(s,3H,-OCH_3), 5.75(m,1H,N-CH-O), 5.80(dxd,1H,J=10x3,H-7), 7.94(s,1H,H-2), 8.05(s,1H, H-9), 8.22(s,1H,H-5). Abs.($CHCl_3$): 417 nm. 14: m.p.: 200° (dec.) IR: 1690(br., ester, amide), 1605, 1585 (aromate). PMR(d-DMSO): 3.73(s,3H,-OCH_3), 3.87(s,3H,-OCH_3), 5.84(dxd, 1H,J=11x2,N-CH-O), 7.0(d,1H,J=17,-C=CH-C=O), 7.72(d,1H,J=17,-CH=C-C=O), 8.38(s,1H,H-2), 8.73(s,1H,H-9), 9.78(s,1H,H-5). Abs.($CHCl_3$): 441,425 nm. Em: 490,466 nm. MS(FD): 412(M^+). Mass spectrum: m/e= 328.0829(M^+-tetrahydropyranyl). (Calc. for $C_{15}H_{12}N_4O_5$: 328.0807). Found: C, 58.3; H, 4.9; N, 13.7; $C_{15}H_{12}N_4O_5$ requires: C, 58.25; H, 4.89; N, 13.59.

8,10-di-(methoxycarbonyl)-7-methoxycarbonylmethyl-7H-pyrido[2,1-i]purine 15

1 mMol 9c was suspended in 5 ml. toluene. A catalytic amount of p-toluene sulphonic acid was added and after a short period of reflux the starting material had dissolved. Chromatography over a short column(silica,EtOAc) afforded 15 as a glass. Yield 140 mgram (38%). 15: IR: 3220 (N-H), 1725(ester), 1660 (enamine ester), 1600 (aromate). PMR(D-DMSO): 2.48 (dxd,1H,J=15x5,-CH_2-C=O), 2.78(dxd,1H,J=15x9,-CH_2-C=O), 3.51(s,3H,-OCH_3), 3.75(s,3H, -OCH_3), 3.83(s,3H,-OCH_3), 5.91(dxd,1H,J=9x5,H-7), 7.81(s,1H,H-9), 8.30(s,1H,H-2), 8.58 (s,1H,H-5). Abs.($CHCl_3$): 423 nm. Em: 520 nm. MS(EI): 360.2(M^+,6.71%), 287.1(M^+-CH_2COOCH_3, 100%). M/e = 360.1051 (Calc. for $C_{16}H_{16}N_4O_6$: 360.1033). Found: C, 53.1; H, 4.7; N, 15.5; $C_{16}H_6N_4O_6$ requires: C, 53.33; H, 4.48; N, 15.55.

Methyl[10-cyano-7-oxo-3-(tetrahydropyran-2-yl)-7H-pyrido[2,1-i]purin-8-yl]-acrylate 19

15 mMol of methyl propiolate was added to 5 mmol of 9b in methanol with a catalytic amount of NaH. After refluxing overnight 1.35 gram (71%) of yellow needles, showing green fluorescence in solution, was filtered off. 19: m.p.: dec. above 210°. IR: 2220 (CN) 1710 (ester), 1650 (amide), 1615, 1585 (aromate). PMR ($CDCl_3$): 3.83(s,3H,-OCH_3), 5.80 (m,1H,N-CH-O), 7.04(d,1H,J=16,-C=CH-C=O), 7.69(d,1H,J=16,CH=C-C=O), 8.06(s,1H,H-2), 8.37(s,1H,H-9), 9.88(s,1H,H-5). Abs.($CHCl_3$): 441,419 nm. EM: 473,445 nm. MS(FD): 379 (M^+). Mass spectrum: m/e 295.0714(M^+-tetrahydropyranyl) (Calc. for $C_{14}H_9N_5O_3$: 295.0705). Found: C, 60.0; H, 4.5; N, 18.6; $C_{14}H_9N_5O_3$ requires: C, 60.15, H, 4.52; N, 18. 46.

9,10-di-(methoxycarbonyl)-7-oxo-3-(tetrahydropyran-2-yl)-7H-pyrido[2,1-i]purine 21a

At room temp. two equiv. of dimethyl acetylenedicarboxylate were added to 15 mmol of 10a (prepared in situ). After stirring overnight 3.75 gram (65%) of yellow (fluorescent) crystals were obtained. 21a: m.p.: 196-197°. IR: 1735 (ester), 1685 (amide), 1600 (aromate). PMR($CDCl_3$) 3.96(s,3H,-OCH_3), 4.04(s,3H,-OCH_3), 5.78(m,1H,N-CH-O), 7.07(s,1H,H-8), 8.15 (s,1H,H-2), 9.72(s,1H,H-5). Abs.($CHCl_3$): 456,432,353,335 nm. EM: 499,474 nm. MS(FD): 386(M^+). Mass spectrum: m/e=302.0649(M^+-tetrahydropyranyl)(Calc. for $C_{13}H_{10}N_4O_5$: 302.0651). Found: C, 55.5; H, 4.8; N, 14.1; $C_{18}H_{19}N_4O_6$ requires: C, 55.96; H 4.50; N, 14.50.

9-phenyl-10-methoxycarbonyl-7-oxo-3-(tetra-hydropyran-2-yl)-7H-pyrido[2,1-i]purine 21b

Two equiv. of ethyl phenylpropiolate was added to 1 mmol of 10a in 2 ml. methanol and 8 ml. cyclohexane with a catalytic amount ofNaH. After 24 hr. of reflux 320 mg (79%) of 21b was filtered off as yellow (weakly fluorescent) crystals. 21b: m.p.: 218-219°. IR: 1730 (ester), 1680 (amide), 1600 (aromate). PMR(d-DMSO): 3.82(s,3H,-OCH_3), 5.80(m,1H, N-CH-O), 6.52(s,1H,H-8), 7.48(s,5H,Ø), 8.16(s,1H,H-2), 9.75(s,1H,H-5). Abs.($CHCl_3$): 415, 397,346,334(sh) nm. EM: 461 nm. MS(FD): 404(M^+). Mass spectrum: m/e=320.0893(M^+-tetrahydropyranyl) (Calc. for $C_{17}H_{12}N_4O_3$: 320.0909).

Reaction of 10a with methyl acrylate

At room temp. 1.1 equiv. of methyl acrylate was added to 2 mmol of 10a (prepared in situ) in methanol. After the starting material had disappeared, the solvent was removed by evaporation. The resulting oil was dissolved in EtOAc and washed twice with water. After drying on $MgSO_4$ 580 mg (80%) of a colourless oil resulted.22: IR: 1740 (ester), 1600 (purine). PMR($CDCl_3$): 2.2-2.7(m,4H,-CH_2-CH_2-), 3.61(s,3H,-OCH_3), 3.67(s,3H,-OCH_3), 4.61

(t,1H,J=7, purine-CH-), 5.82(m,1H,N-CH-O), 8.31(s,1H,H-8), 8.92(s,1H,H-2). MS(FD): 362(M^+).

Using more than 2 equiv. of methyl acrylate and refluxing for one hr yielded 23 as a solid, following the same procedure as above. Recrystallization from EtOCa/P.A. 60-80. Yield 654 mg (73%). 23: m.p.: 93°. IR: 1730 (ester), 1580 (purine). PMR($CDCl_3$):2.1-2.4 (m,4H,-CH_2-), 2.5-2.8 (m,4H,-CH_2-), 3.62(s,6H,-OCH_3), 3.72(s,3H,-OCH_3), 5.81(m,1H,N-CH-O), 8.23(s,1H,H-8), 8.92(s,1H,H-2). MS(FD): 448(M^+).

Refluxing the mixture mentioned above for one night resulted in 690 mg (83%) of 24 as an oil. 24: IR: 1730 (ester), 1660 (ketone), 1620 (C=C), 1580 (purine). PMR($CDCl_3$): 2.2-3.0 (m,6H,-CH_2-), 3.72(s,3H,-OCH_3), 3.78(s,3H,-OCH_3), 5.88(m,1H,N-CH-O), 7.99(s,1H,H-8), 8.64 (s,1H,H-2), 12.2(s,1H,O=C-CH-C=O). MS(FD): 416(M^+).

Dimethyl-2-methyl-3-[(tetrahydropyran-2-yl)purin-6-yl]pentanedioate 25

10 Equiv. of methyl crotonate were added to 1 mmol of 10a (prepared in situ). After 7 days of reflux the solvent (methanol) was evaporated, the resulting oil dissolved in EtOAc and washed twice with water. Column chromatography (silica,EtOAc) gave 40 mg (11%) of 25 as an oil: IR: 1735 (ester), 1590 (purine). PMR($CDCl_3$): 0.92 and 1.18(2xd,3H,J=7,-CH_3), 1.85-3.0 (m,3H,-CH-CH_2-), 3.61(s,3H,-OCH_3), 3.70(s,3H,-OCH_3), 4.57(d,1H,J=8,purine-CH-), 5.83(m,1H, N-CH-O), 8.33(s,1H,H-8), 8.99(s,1H,H-2). MS(FD): 376(M^+).

Dimethyl 2-methoxycarbonyl-3-[(tetrahydropyran-2-yl)purin-6-yl]pentanedioate 26

At room temp. 1.1 equiv. of dimethyl fumarate (or maleate) was added to 2 mmol of 10a (prepared in situ). The next day the solvent was removed by evaporation. The resulting oil was dissolved in EtOAc and washed twice with water. Column chromatography (silica, EtOAc) afforded 500 mg (60%) of 26 as an oil and 62 mg (8%) of 21a. 26: IR: 1740 (ester), 1600 (purine). PMR($CDCl_3$): 2.5-3.3(m,3H,-CH-CH_2-), 3.55-3.8(m,9H,-OCH_3), 5.15(m,1H,purine--CH-), 5.85(m,1H,N-CH-O), 8.33(s,1H,H-8), 8.92(s,1H,H-2). MS(FD): 420(M^+).

Methyl ethyl 2-cyano-3-methoxycarbonylpentanedioate 28

200 mMol methyl cyanoacetate was added to 200 mmol sodiumethoxide in methanol. After a short time the solvent was evaporated. The resulting salt was suspended in dry THF, to which an extra equiv. methyl cyanoacetate was added. 200 mMol diethyl maleate was added dropwise. Destillation gave 37.9 gram (70%) of 28 as a colourless oil: b.p. 124-128° (0.02mm). IR: 2270 (CN), 1730 (ester). PMR($CDCl_3$): 1.32(t,6H,J=7,-CH_3), 2.95(m,2H,-CH_2--C=O), 3.6(m,1H,-CH-C=O), 3.9(s,3H,-OCH_3), 4.2(m,1H,-CH-CN); 4.27(k,4H,J=7,-OCH_2-).

Reaction of 8a with methylcyanide

At -60° 4 mmol of CH_3CN was added slowly to a suspension of 4 mmol NaH in THF. When the evolution of H_2 had ceased and the temp. became -40°, 2 mmol of 8a was added. After a short time the reaction was completed and water was added at room temp. Neutralizing and extraction with EtOAc, followed by recrystallization from EtOAc, afforded 515 mg (92%) of 29: m.p.: 192-194°. IR: 3410,3330 (N-H), 2210 (CN), 1635 (C=C), 1565 (pyrimidine). PMR(d-DMSO): 3.90(d,1H,J=14,-C=CH-CN), 5.30(dxdxd,1H,J=9x9x2,N-CH-O), 7.23(dxd,1H,J=7x14, -CH=C-CN), 7.68(d,1H,J=9,NH-4), 8.27(s,1H,H-2), 8.50(d,1H,J=7,NH-5). Abs.($CHCl_3$): 235nm. Mass spectrum: m/e=279.0852 (Calc. for $C_{12}H_{14}ClN_5O$: 279.0887).

Found: C, 51.5; H, 5.0; N, 25.1; $C_{12}H_{14}ClN_5O$ requires: C, 51.53; H, 5.04; N, 25.04. Next day in DMSO-solution 29 was partly contaminated with its Z-isomer 30: PMR(d-DMSO): 4.13(d,J=9,-C=CH-CN), 6.67(dxd,J=9x9,-CH=C-CN), 7.75(d,J=9,NH-4), 8.24(s,1H,H-2), 8.43 (d,J=9,NH-5).

Reaction of 8b with triester 28

4mMol of 28 was added to a suspension of 4 mmol of NaH in dry DMSO. After the evolution of H_2 had ceased, 2 mmol of 8b was added and the mixture was heated at 80°. After completion of the reaction the solution was acidified, more water was added and extraction with $CHCl_3$, followed by column chromatography (silica,EtOAc), yielded two oily substances: 31, 510 mg (54%). IR: 2250 (CN,weak), 1735 (ester), 1600, 1580 (purine). PMR($CDCl_3$): 1.23(t, 3H,J=7,-CH_3), 1.27(t,3H,J=7,-CH_3), 2.9(m,2H, -CH_2-C=O), 3.87(s,3H,-OCH_3), 4.19(q,2H,J=7, -OCH_2-),4.33(q,2H,J=7,-OCH_2-), 5.1(m,1H,-CH-C=O), 5.93(m,1H,N-CH-O), 8.52(s,1H,H-8), 9.11

(s,1H,H-2). 32: 180 mg (22%). IR: 2250 (CN,weak), 1745 (ester), 1585 (purine). PMR($CDCl_3$): 1.30(t,6H,J=7,-CH_3), 3.0(m,2H,-CH_2-C=O), 4.23(q,4H,J=7,-OCH_2-,m,1H,-CH-C=O), 5.3(m,1H, -CH-CN), 5.95(m,1H,N-CH-O), 8.48(s,1H,H-8), 9.10(s,1H,H-2).

10-cyano-9-methoxycarbonyl-7-oxo-3(tetrahydropyran-2-yl)-7H-pyrido[2,1-i]purine 34

A catalytic amount of NaH was added to a mixture of 31 (300 mg) and 32 (100 mg) in methanol. After 20 hr. of reflux 180 mg (59%) of 34 could be filtered off as a yellow solid, showing green fluorescence in solution. 34: m.p. dec. above 200°. IR: 2220 (CN,strong), 1740 (ester), 1695 (amide), 1600 (aromate). PMR($CDCl_3$): 4.06(s,3H,-OCH_3), 5.84(m,1H, N-CH-O), 7.01(s,1H,H-8), 8.37(s,1H,H-2), 9.78(s,1H,H-5). Abs.($CHCl_3$): 415nm. EM: 466nm. MS(FD): 353(M^+). Mass spectrum: m/e=269.0524(M^+-tetrahydropyranyl)(Calc. for $C_{12}H_7N_5O_3$: 269.0499).

Conversion of 35a,b in the corresponding ribosides

The tetrahydropyranyl protected pyridopurines 14 and 21a were quantitatively converted to the free bases by the action of catalytic pTsOH in refluxing methanol. After 1 hr. the pyridopurines 35a,b could be filtered off. Of each pyridopurine 2 mmol was suspended in dry CH_3CN together with 2.1 mmol of 39b. Two equiv. of $SnCl_4$ were added and immediately the mixture became clear. After the reaction was completed the solution was slowly added to icecold saturated $NaHCO_3$. Extraction with EtOAc, followed by column chromatography (silica,EtOAc/P.A.60-80 4:1) gave the protected ribosides as glassy solids. 36a: 806 mg (54%, 90% on recovered free base by eluting the column with CH_3OH). 36b: 1.16 gram (75%). 0.5 mMol of 36a,b was dissolved in CH_3OH and 1.1 equiv. of morpholine was added. After the fluorescence had disappeard, a catalytic amount of NaH was added. Next day the solution was acidified and the solvent was removed. The resulting solids were purified over a short column (silica,EtOAc/CH_3OH 8:1) and the products recrystallized from CH_3OH. 38a: yield 130 mg (60%). m.p.: 140°. IR: 3400 (OH), 1740 (ester), 1695 (amide), 1600 (aromate). PMR(d-DMSO): 3.65(symm.m,2H,H-5'), 3.85(s,3H, -OCH_3), 3.87(s,3H,-OCH_3), 4.00(dxdxd,1H,J=5x5x5,H-4'), 4.18(dxdxd,1H,J=5x5x5,H-3'), 4.53 (dxdxd,1H,J=5x5x6,H-2'), 5.09(dxd,1H,J=5x6,OH-5'), 5.28(d,1H,J=5,OH-3'), 5.63(d,1H,J=6, OH-2'), 6.05(d,1H,J=5,H-1'), 6.76(s,1H,H-8), 8.71(s,1H,H-2), 9.62(s,1H,H-5). 38b: yield 100 mg (43%). m.p.: 198°. IR: 3400 (OH), 1690(br.,ester,amide), 1600 (aromate). PMR(d-DMSO): 3.67(symm.m,2H,H-5'), 3.73(s,3H,-OCH_3), 3.89(s,3H,-OCH_3), 4.03(dxdxd,1H,J=5x5x5, H-4'), 4.22(dxdxd,1H,J=5x5x5,H-3'), 4.57(dxdxd,1H,J=5x5x6,H-2'), 5.12(dxd,1H,J=5x6,OH-5'), 5.33(d,1H,J=5,OH-3'), 5.67(d,1H,J=6,OH-2'), 6.09(d,1H,J=5,H-1'), 7.05(d,1H,J=16,C=CH-C=O), 7.78(d,1H,J=16, CH=C-C=O), 8.43(s,1H,H-9), 8.80(s,1H,H-2), 9.80(s,1H,H-5).

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