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THE SYNTHESIS OF 5-CARBOXAMIDO-4-HYDROXY-3-(β-D-RIBOFURANOSYL)-THIOPHENE DERIVATIVES, ANALOGUES OF PYRAZOFURIN

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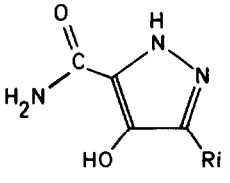
Abstract - Methyl 5-carboxamido-4-hydroxy-3(β-D-ribofuranosyl)-2-thiophene carboxylate 2a and the corresponding 2,5-thiophene dicarboxamide 2b, two new analogues of the antiviral compound pyrazofurin, were synthesized. A base mediated condensation method with dimethyl thiodiacetate and methyl 2(2,3,5,-tri-O-t-butyldimethylsilyl-β-D-ribofuranosyl)-2-oxoacetate 5 was used. The structure of these compounds and their intermediates was determined spectroscopically.

The occurrence in nature of a number of C-glycosyl nucleosides, many of which show biological activity, stimulated a lot of research on this type of compounds.[1] The natural C-nucleoside pyrazofurin 1,[2] characterized by a broad spectrum antiviral[3a,b] and antitumor activity[3c] at low concentrations, was synthesized by four groups.[4] However in vivo experiments with 1 showed significant toxicity. It was suggested[5] that this toxicity may be associated with some structural features not related to the antiviral potency of the compound. Therefore it might be possible to change the chemical structure of pyrazofurin in such a way that it looses its toxic properties and yet retains its antiviral activity. From literature data it appeared that all activity is lost on replacing the ribose moiety by a carbocyclic system,[6a] but is retained in some way if the heterocyclic base is changed;[6b] the 4-OH group might play a role in the inhibition of orotidylic acid decarboxylase by the 5'-monophosphate derivative of 1; the carboxamide group is often present in the heterocyclic position of several biologically active nucleosides. Considering these data, we performed the synthesis of 5-carboxamido-4-hydroxy-3-(β-D-ribofuranosyl)-thiophene derivatives 2a,b as valuable analogues of 1.



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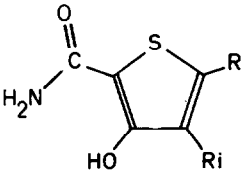


2.a : R = $COOCH_3$

b : R = $CONH_2$

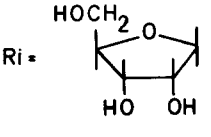
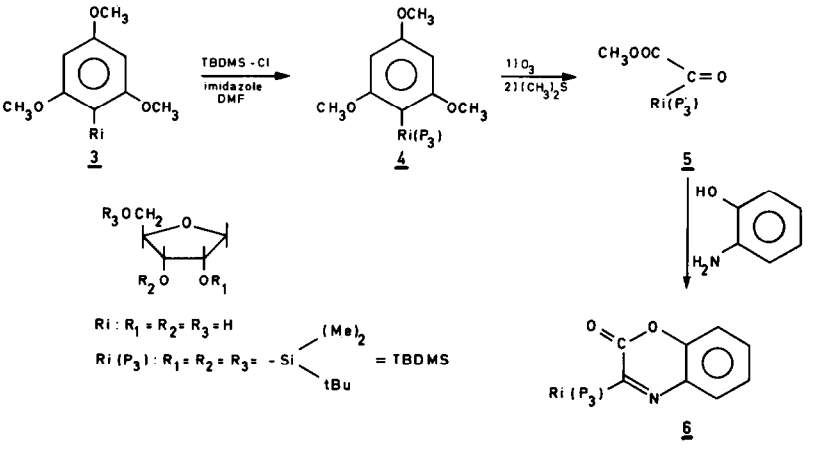


Figure 1

The synthetic procedure leading to the title compounds 2a,b makes use of a previously described method for the synthesis of a keto ester glycoside;[7] however in order to perform reactions in strongly basic conditions we introduced silyl - instead of acyl groups - as OH-protecting devices. For this purpose compound 3, obtained by both the procedure of L. Kalvoda[8] and H. Ohrui,[9] was silylated[10,11] to yield 1-(2,3,5-tri-O-t-butyldimethylsilyl-β-D-ribofuranosyl)-2,4,6-trimethoxybenzene 4.

Ozonolysis and reductive work-up gave the keto ester glycoside 5. Its mass spectral data (loss of methyl and t-butyl group in the electron impact spectrum and the signal at 563 = M^++1 in the chemical ionization spectrum) were in agreement with the proposed structure. However, because of its diffuse ^{1}H NMR data and decomposition on attempted purification, we looked for additional chemical evidence : treatment of crude compound 5 with ortho-aminophenol yielded readily the benzoxaninone 6, the structure of which was determined by spectral analysis. We assume a β-configuration for its ribosyl moiety on ground of structure determinations of compound 2b, derived from 5.



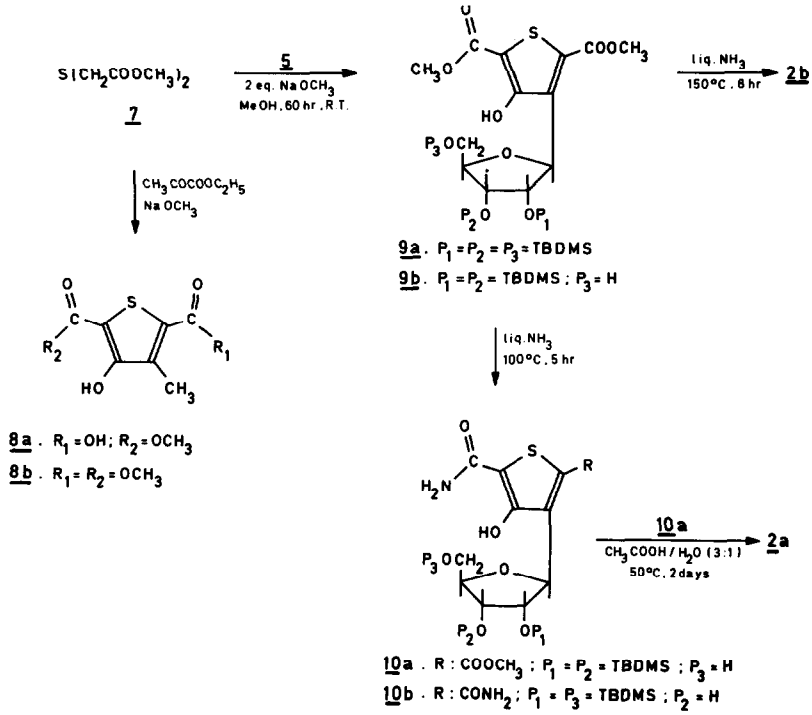
3 4 5 6

Scheme 1

Before tackling the thiophene nucleoside synthesis, we tested the ring closure concept with the ethyl pyruvate model and dimethyl thiodiacetate 7 in the presence of sodium methoxide.

In agreement with the observations of O. Hinsberg,[12] compound 8a was formed in a reaction using 3.6 equivalents of base; due to the work-up procedure the most reactive ester function in position 2 was hydrolyzed;[12] however, we found that the use of only two equivalents of sodium methoxide and careful work-up led exclusively to the dimethyl 2,5-thiophene dicarboxylate 8b. Its IR and ^{13}C NMR spectrum showed two carbonyl absorptions (1715-1685 cm^{-1} and 166.7-162.8 ppm); further evidence was provided by its ^{1}H NMR (2 x CO_2Me at δ 3.91-3.87 ppm; OH at 9.56 ppm) and mass spectrum.

A similar result was found on treatment of crude compound 5 with 7 in the presence of 2 equivalents of sodium methoxide. After neutralization with Dowex 50WX8 and chromatography of the reaction mixture, the thiophene derivative 9a (24%) and a mixture (16%) - not further purified - were isolated as syrups. The ^{1}H NMR absorptions of the dimethylbutylsilyl groups in 9a were comparable with these of compound 4 : in $CDCl_3$ three singlets (at 0.95, 0.92 and 0.77 ppm) were observed for the butyl group and four signals at 0.11(6), 0.09(6), -0.03(3) and -0.09(3) ppm for the methylsilyl groups. The upfield signals at -0.03, -0.09 and 0.77 ppm, which were comparable with values for the similarly protected adenosine[13] could be assigned to the protecting group in the 2' position whereas the absorptions at 0.95 and 0.92 correspond with the t-butyl group of the protecting moiety at C_3, and C_5,. The observed chemical shift values do not vary much on changing the solvent from $CDCl_3$ (or CD_3OD) to CD_3COCD_3, which was used by the authors of ref. 13. Taking into account these data we assigned the structure 9b to the main component of the second fraction; it showed NMR signals at 0.96(9), 0.78(9), 0.12(6), -0.07(3) and -0.41(3) ppm. The desilylation at C_5, position points to hydrolysis during the work-up procedure as the C_5,-silyl group is normally the most stable towards base, but the most sensible one to acid.[13]

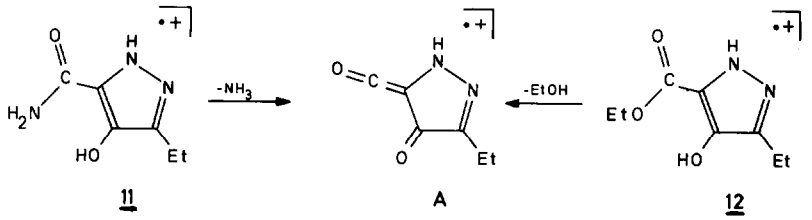


Scheme 2

Treatment of the crude mixture of compounds 9 with an excess of liquid NH_3 at 100° for 5 hours gave 54% yield of a fraction containing mainly the carbamoyl derivative 10a and 17% of the fraction with the pure 2,5 dicarbamoyl derivative 10b. The latter structure was assigned on ground of its ^1H NMR absorptions (CD_3OD) at 0.97(9), 0.81(9), 0.16(6), 0.03(3) and -0.13(3) ppm. These values are comparable with the values of 0.96, 0.84, 0.15, 0.01 and -0.08 ppm of 2'5' disilylated adenosine.[13] The main component of the other fraction was assigned structure 10a on ground of ^1H NMR absorptions ($CDCl_3$) at 0.95, 0.77, 0.11, -0.08 and -0.41 ppm. The pure compound 2a was obtained on desilylation of the latter fraction by treatment with a micture of CH_3COOH/H_2O (3:1) at 50°. After extraction with $CHCl_3$ and chromatography on silica gel plates, the pure compound could be isolated and crystallized (dihydrate) from ethanol-water.

Under more stringent conditions (longer reaction time, higher temperature) the crude mixture of compounds 9 could be directly transformed to the fully desilylated 2,5-dicarbamoyl-4-hydroxy thiophene nucleoside 2b. The crude compound could be obtained as a yellow solid (67%) by trituration with $CHCl_3$ of a methanolic solution of the reaction mixture. Further purification was realized via its 2'3'-O-isopropylidene derivatice 2b'.

Regarding the structural assignment of the thiophene nucleosides and their protected analogues, the peculiar substituent distribution in 2a and its silylated analogue 10a needs some more explanation. It is quite normal that the electrophilic reactivity of a methoxycarbonyl group is reduced when conjugated with an OH-group. This explains the selective saponification of 8b in the Hinsberg preparation of 8a. Therefore, we might expect that the 2-methoxycarbonyl function in compounds 9 would react preferentially with NH_3. In order to identify the position of the carbamoyl group in 2a and 10a their mass spectral data were compared with those of other hydroxy-thiophene analogues (Table I). Additional information on mass spectra of hydroxy pyrazole derivatives was found in the literature.[14] The pyrazoles 11 and 12 (Fig. 2) were readily decomposed in the mass spectrometer to yield fragment A by loss of ethanol or ammonia; a comparable loss of ammonia was observed in the mass spectrum of 1.[14] The thiophene analogues 8a,b showed the same fragmentation behaviour; peaks due to the loss of methanol were also present in the spectrum of 9a,b.



Mass spectral fragmentation of M^+ in hydroxy-pyrazoles 11-12

Figure 2

Table I. Electron impact mass spectral data of polysubstituted 4-hydroxythiophenes and 4-hydroxy-pyrazoles

Comp.	Basic peak : m/e, % $\xrightarrow{-ROH \text{ or } -NH_3}$	Fragment peak : m/e, %	Metastable signal
8a	216 (M^+, 38)	184 (M^+-MeOH, 100)	$184^2/216 = 156.7$
8b	230 (M^+, 42)	198 (M^+-MeOH, 100)	$198^2/230 = 170.4$
9a	633 (M^+-C_4H_9[a], 12.0)	601 (633-MeOH, 2.2)	$601^2/633 \approx 570.6$
9b	519 (M^+-C_4H_9[a], 48)	487 (519-MeOH, 13.3)	$487^2/519 \approx 457.0$
10a	504 (M^+-C_4H_9[a], 28.8)	487 (504-NH_3, 8.0)	$487^2/504 = 470.6$
10b	489 (M^+-C_4H_9[a], 15.4)	471 (489-NH_3, 4.0)	$472^2/489 = 455.6$
2a	230 (B + 30[b], 6.2)	213 (230-NH_3, 14.4)	
1[c]	156 (B + 30[b], 100)	139 (156-NH_3, 70)	
11[c]	155 (M^+, 97)	138 (155-NH_3, 100)	
12[c]	184 (M^+, 34)	138 (184-EtOH, 100)	

[a] loss of a t-butyl group is typical for TBDMS-derivatives; [b] (B+30) : heterocyclic moiety + 30 is a characteristic peak for most C-nucleosides (ref. 15); [c] information obtained from ref. 14.

Observation of a fragment due to the loss of ammonia, OR methanol in the mass spectra of 2a and 10a could solve the problem about the position of the carbamoyl function. As for most C-nucleosides,[15] a fragment (m/e 230, 6.2%), corresponding with the mass of the heterocyclic moiety +30, was observed in the electron impact mass spectrum of 2a. This fragment lost ammonia, not methanol (m/e 213, 14.4%), probably in a comparable way as observed for pyrazofurin 1. As could be expected for dimethyl-t-butyl silyl derivatives, the molecular ion of 10a lost quickly a t-butyl group (m/e 504, 28.8%); a peak at m/e 487 (8%) - due to the loss of NH_3 (not methanol) from fragment 504 - was again observed. Not only the mass spectral data but also the ^{13}C NMR data of 10a favoured the methyl 5-carboxamido 2-thiophene-carboxylate structure. The chemical shift value (δ = 115.5 ppm) for the C_5 ring atom is rather low, due to the shielding effect of the hydroxyl group. Its signal was strongly broadened in a chloroform solution ($\omega^{1/2} \geq 6.7$ Hz) by coupling with the carbamoyl function. In a CD_3OD solution this coupling is prevented and the C_5-ring atom shows a sharp signal ($\omega^{1/2} \leq 3$ Hz). Additional convincing evidence about the 5-carboxamido structure of 10a and 2a was obtained from comparison of the IR spectra of 8b, 9a and 2a,b. The 2,5 diester 8b has two carbonyl stretching frequencies at 1715 and 1685 cm^{-1}, the former being assigned to the less conjugated 2-CO_2Me group. The same carbonyl stretching frequencies in 9a are observed at 1730 and 1670 cm^{-1}. The IR absorptions of compound 2a at 1720 (CO_2Me) and 1640 cm^{-1} ($CONH_2$) compared with the broad IR absorptions at 1660 and 1670 cm^{-1} for 2b and 10b, are consistent with a 5-carboxamido structure of 2a and its precursor 10a. These results point out unambiguously that the 5-CO_2Me group in 9a,b is more reactive than the 2-CO_2Me group; the steric hindrance of the bulky silyl groups in the ribosyl moiety of these compounds could explain why the reactivity of the 2-CO_2Me group is lower than observed for 8b.

The anomeric β-configuration of 2b was determined on its 2',3'-O-isopropylidene derivative. The chemical shift difference ($\Delta\delta$) between the two methyl signals of the isopropylidene group had a value 0.23 ppm (NMR signals at δ 1.63 and 1.40 ppm respectively), which is typical of a β-configuration.[16] The ^1H NMR spectrum showed a significant coupling ($J \approx 5$ Hz) between H-3' and H-4' with the

signal for H-4' appearing as a quartet due to the approximately equal coupling with $H_{3'}$ and $H_{5'}$. This behaviour is typical of the isopropylidene derivatives of nucleosides of β-configuration.[17]

The biological activities of the compounds 2a,b were tested. It appeared from these data that the structural changes in the pyrazofurin analogues were inadequate. Neither of these compounds showed a significant inhibition of in vitro L1210 cell growth and of viral replication in any of the tested systems (HSV-1 and -2, polio-1, VSV, vaccinia, Reovirus-1, parainfluenza-3, coxsackie B4).

EXPERIMENTAL

Melting points, determined with a Leitz melting point microscope, are uncorrected. The ^1H NMR data are presented in ppm with TMS used as internal standard; the spectra were taken on a Jeol JNM-MH-100 spectrometer at 100 MHz, on a Varian EM 390 at 90 MHz and a Bruker WM 250 at 250 MHz; for the ^{13}C NMR spectra a Bruker WP 80 spectrometer was used. The EI mass spectra were recorded on a Kratos AEI-MS-902 apparatus with direct injection and an ionization energy of 70 eV. The chemical ionization mass spectra were obtained from a modified Kratos AEI-MS-12 mass spectrometer with isobutane as a reagent gas. The infrared spectra were recorded with a Perkin-Elmer 250 grating apparatus. HPLC experiments were performed on a Waters Associate HPLC system.

1-(β-D-Ribofuranosyl)-2,4,6-trimethoxybenzene (3). Compound 3 was prepared via methods described in the literature.[8,9]

1-(2,3,5-Tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-2,4,6-trimethoxybenzene (4). Compound 3 (18.3 g, 0.061 mol.), t-butyldimethylsilyl chloride (TBDMS-Cl, 36.4 g, 0.24 mol) and imidazole (32.89 g, 0.48 mol) were dissolved in 90 ml of dimethylformamide. After one night reaction at 20°, the mixture was evaporated and purified on a silica gel column with chloroform-ethyl acetate (95:5) as eluent. This yielded 37 g (95%) of compound 4, which was crystallized from methanol-water. Compound 4 had m.p.: 91°; ^1H NMR, δ (250 MHz, $CDCl_3$): 6.05 (s, aromatic, 2H), 5.38 (d, $H_{1'}$, 1H, $J_{1'2'}$ = 7Hz), 4.63 (d x d, $H_{2'}$, 1H, $J_{2'3'}$ = 5 Hz), 4.21 (d x d, $H_{3'}$, 1H, $J_{3'4'}$ = 3.2 Hz), 3.89 (m, $H_{4'}$, 1H), 3.70 (m, $H_{5'}$, 2H), 3.80 (s, OMe, 3H), 3.74 (s, OMe, 6H), 0.95, 0.90 and 0.78 (3 x s, C_4H_9, 3 x 9H), 0.13-0.11 (2 x s, 2 x 3H), 0.05-0.04 (2 x s, 2 x 3H), -0.10 and -0.30 (2 x s, 2 x 3H, SiMe); δ ((CD_3COCD_3)): 0.97, 0.92 and 0.82 (3 x s, C_4H_9, 3 x 9H), 0.18-0.15 (2 x s, 2 x 3H), 0.08 (s, 6H), -0.07 (s, 3H) and -0.25 (s, 3H): SiMe; m/z (%): 642 (M^+, 0.6), 6.27 (-Me, 0.8), 585 ($-C_4H_9$, 37) 453 (585 - TBDMSOH, 44), 73 (100); exact mass: calc. for $C_{32}H_{62}O_7Si_3$: 642.3877. Found: 642.3847 ± 0.0002. Anal. calc. for $C_{32}H_{62}O_7Si_3$: C, 59.77; H, 9.72. Found: C, 59.66; H, 9.78.

Methyl-2-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-2-oxo-acetate (5). Ozone was bubbled through a solution of compound 4 (10 g, 15 mmol) in 300 ml of ethyl acetate at -25°. The reaction was monitored by TLC; after 8 hr, when the starting compound had disappeared, dry nitrogen was introduced into the reaction mixture. An excess of dimethyl sulphide (8 ml) was then added dropwise and the mixture was kept overnight at -25° and 1 hr at 10°. After evaporation of the solvent and purification on a silica gel column (chloroform-ethyl acetate as eluent) crude α-keto ester 5 (3.5 g, 42%) could be obtained as a syrup with IR (film) cm^{-1}: 1730 (CO, CO_2Me). Due to partial decomposition on attempted purification no clear ^1H NMR spectrum could be obtained; m/z (%): 547 (M^+, -Me, 2), 505 ($-C_4H_9$, 33), 373 (505 - TBDMSOH, 15), 343 (M^+ -$COCO_2Me$, - TBDMSOH, 14), 73 (100).

3-(2,3,5-Tri-O-tert-butyldimethylsilyl)-B-D-ribofuranosyl benz[e]-oxazin(1H)-2-one (6). Compound 5 (200 mg, 0.36 mmol) and ortho aminophenol (40 mg, 0.36 mmol) was dissolved in 10 ml of dry benzene. After reflux for 2 hr, the crude mixture was purified by TLC (silica gel, chloroform-acetonitrile 95:5 as eluent) and 91 mg (41%) of an oil with the characteristics of compound 6 was obtained. IR (film), cm^{-1}: 1750 (OCO); ^1H NMR, δ (250 MHz, $CDCl_3$): 7.25-7.8 (m, aromatic, 4H), 5.16 (d, $H_{1'}$, 1H, $J_{1'2'}$ = 4.7 Hz), 4.70 (t, $H_{2'}$, 1H, $J_{2'3'}$ = 4.7 Hz), 4.33 (t, $H_{3'}$, 1H, $J_{3'4'}$ = 4.7 Hz), 4.10 (q, $H_{4'}$, 1H, $J_{4'5'}$ = 4.7 Hz), 3.68 (m, $H_{5'}$, 2H), 0.95, 0.87 and 0.80 (3 x s, C_4H_9, 3 x 9H), 0.12-0.11 (2 x s, 6H), 0.7 (s, 3H), 0.01 (s, 6H), -0.02 (s, 3H): SiMe; δ ((CD_3COCD_3)): 0.97, 0.92 and 0.83 (3 x s, C_4H_9, 3 x 9H), 0.16 (s, 6H), 0.13 (s, 3H), 0.10 (s, 3H), 0.05 (s, 3H) and 0.01 (1, 3H): SiMe; m/z (%): 621 (M^+, 3.0), 606 (-Me, 2), 564 ($-C_4H_9$, 39), 432 (564 - TBDMSOH, 11), 300 (432 - TBDMSOH, 12), 72 (100); exact mass: calc. for $C_{31}H_{55}NO_6Si_3$: 621.3322. Found: 621.3302 ± 0.0003.

Dimethyl thiodiacetate (7). Compound 7 was prepared in conventional way by mixing an aqueous solution of Na_2S. $9H_2O$ (12 g, 0.05 mol in 40 ml) with a methanolic solution of methyl chloroacetate (10.8 g, 0.1 mol in 70 ml). After reflux for 2 hr the reaction mixture was concentrated and extracted with chloroform. After usual work-up and distillation, 3.57 g (40.1%) of a fraction (bp 132-134° at 12 mm Hg) was collected. Its spectroscopic characteristics were in agreement with structure 7 (^1H NMR, δ (CCl_4): 3.7 (s, CO_2Me, 3H), 3.3 (s, CH_2, 2H) and the product was used as such.

4-Hydroxy-5-methoxycarbonyl-3-methyl-2-thiophene carboxylic acid (8a) and dimethyl-4-hydroxy-3-methyl-2,5-thiophene dicarboxylate (8b). In an experiment performed in the Hinsberg conditions,[12] compound 7 (1 g, 5.62 mmol) was mixed with ethyl pyruvate (0.655 g, 5.62 mmol) and 3.6 equivalents of sodium methoxide (1.09 g) in 8 ml of methanol. After reaction for 2 days at 20°, followed by addition of 2 ml of water and further reaction for one day, the mixture was poured into water and neutralized with hydrogen chloride. A deposit of compound 8a was obtained in this way. Crystallization from methanol-water gave 1.09 g (90%) product with the spectroscopic characteristics given below.

In another experiment the reaction of the same amount of compound 7 was performed with 2 equivalents of sodium methoxide for 2 days. The mixture was then poured in ice-water and carefully neutralized. This yielded a deposit of a compound (1.9 g, 92%) which was crystallized from methanol and identified as 8b.

Compound 8a had m.p.: 243-244°; IR (KBr) cm^{-1}: 3300 (OH), 1670 (CO); ^1H NMR, δ (90 MHz, DMSO-d_6): 10.4 (broad, OH and COOH), 3.8 (s, CO_2Me, 3H), 2.3 (s, Me, 3H); m/z (%): 216 (M^+, 38), 184 (-MeOH,

100), 156 (42); metastable: $156^2/184 = 132.2$, $184^2/216 = 156.7$; exact mass: calc. for $C_8H_8O_5S$: 216.0092. Found: 216.0093 ± 0.0002.

Compound 8b had m.p.: 97-98°; IR (KBr) cm^{-1}: 1715, 1685 (CO_2Me); ^1H NMR, δ (90 MHz, $CDCl_3$): 9.56 (s, OH, 1H), 3.91 (s, CO_2Me, 3H), 3.87 (s, CO_2Me, 3H), 2.4 (s, Me, 3H); m/z (%): 230 (M^+, 42), 198 (-MeOH, 100), 183 (15), 170 (40); metastable: $170^2/198 = 146$ and $198^2/230 = 170.4$; exact mass: calc for $C_9H_{10}O_5S$: 230.0249. Found: 230.0211 ± 0.0002.

Dimethyl 4-hydroxy-3-(2,3,5-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-2,5-thiophene dicarboxylate (9a) and its monodesilylated analogue 9b. A solution of sodium methoxide (0,61 g, 11.28 mmol) in 10 ml of methanol was added dropwise to a methanolic solution (5 ml) of the keto ester 5 (3.17 g, 5.64 mmol) and compound 7 (1 g, 5.64 mmol) kept at 0°. After 60 hr reaction at 20° the mixture was neutralized with Dowex 50 W x 8 (H^+ form). The resin was filtered off and washed with small portions of methanol. TLC analysis of the mixture showed two spots with strong fluorescence properties: R_f = 0.3 and 0.67 in chloroform-ethylacetate (9:1). Chromatography of the reaction mixture on a silica gel column using a gradient solution from chloroform to chloroform-ethylacetate (9:1), yielded two fluorescent fractions. The fastest moving compound 9a (0.926 g, 24%) showed the spectroscopic characteristics given below. The other fraction obtained as an oil (0.535 g, 16%) contained mainly a compound with the spectroscopic characteristics of structure 9b.

Compound 9a (oil) had IR (KBr) cm^{-1}: 1730, 1670 (CO_2Me); ^1H NMR, δ (90 MHz, $CDCl_3$): 9.51 (s, OH, 1H 5.80 (d, H_1', 1H, $J_{1'2'}$ = 9 Hz), 4.48 (d x d, H_2', 1H, $J_{2'3'}$ = 4.5 Hz), 4.18 (d x d, H_3', 1H, $J_{3'4'}$ 1 Hz), 3.98 (t x d, H_4', $J_{4'-5'}$ = 5 Hz), 3.85 and 3.81 (2 x s, 2 x CO_2Me, 6H), 3.7 (d, H_5', 2H), 0.95, 0.92 and 0.77 (3 x s, C_4H_9, 3 x 9H), 0.11 (br s, 6H), 0.09 (6H), -0.03 (s, 3H) and -0.09 (s, 3H): $Si(Me)_3$; m/z (%): 690 (M^+, 0.01), 675 (-Me, 0.54), 633 (-C_4H_9, 12), 601 (633-MeOH, 2.2), 501 (633 - TBDMSOH, 14.4), 73 (100); metastable signals: $501^2/633 = 396.0$, $601^2/633 = 570.6$; exact mass calc. for M^+-C_4H_9: $C_{27}H_{49}O_9S_1Si_3$: 633.242. Found: 633.240.

Compound 9b showed the following ^1H NMR, δ (250 Hz, $CDCl_3$) data: 10.25 (OH), 5.87 (d, H_1', 1H, $J_{1'2'}$ = 8.9 Hz), 4.47 (d x d, H_2', 1H, $J_{2'3'}$ = 5.0 Hz), 4.22 (d x d, H_3', 1H, $J_{3'4'}$ = 1.3 Hz), 4.04 (m, H_4', 1H), 3.93 (s, CO_2Me, 3H), 3.87 (s, CO_2Me, 3H), 3.83 (d x d, H_5', 1H, $J_{5'-5''}$ = -13 Hz, $J_{4'5'}$ = 2.5 Hz), 3.67 (d x d, H_5'', 1H, $J_{4'5''}$ = 1.8 Hz), 0.96 and 0.78 (2 x s, C_4H_9, 2 x 9H), 0.12-0.10 (2 x s, 6H), -0.07 (s, 3H) and -0.41 (s, 3H): SiMe; m/z (%): 576 (M^+, 1.3), 561 (-Me, 1.3), 519 (-C_4H_9, 48), 487 (519 -MeOH, 13.3), 387 (519 - TBDMSOH, 42.7), 73 (100); metastable signal: $487^2/519 = 456.97$; ^{13}C NMR, δ ($CDCl_3$): 166.5, 161.9, 161.8 (2 x CO_2Me, C_4), 135.3 (d, C_2), 133.9 (m, C_3), 108.25 (d, C_5), 87.8, 76.05, 75.03, 74.45 and 63.05 (C-ribosyl), 52.8, 52.7 (OMe), 26.1, 25.8 ($SiCMe_3$), 18.2, 17.9 ($SiCMe_3$), -4 → -6($SiMe_2$).

Methyl-5-carboxamido-4-hydroxy-3-(2,3 di-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-2-thiophene carboxylate (10a) and 4-hydroxy-3-(2,5 di-O-tert-butyldimethylsilyl-β,D-ribofuranosyl)-2,5-thiophene dicarboxamide (10b). A mixture of compounds 9 (0.645 g), obtained in the reaction of compounds 5 and 7, and 10 ml of liquid ammonia was heated in a pyrex tube warmed up in a bomb. After 5 hr reaction at 100°, the excess ammonia was allowed to escape slowly. The remaining syrup was taken up in methanol. Thin layer chromatography showed two main products with fluorescent properties: R_f = 0.63 and 0.57 in ethyl acetate - acetone - methanol - water (7/1/0.5/0.5). The reaction mixture was purified by column chromatography on silica gel. A gradient eluent of chloroform - methanol with increasing polarity (100% chloroform → 88%) was used. The disilylated structure 10a was assigned to the main component of the fastest moving fraction isolated as an oil (0.308 g, ± 54%). The other oily compound (0.095 g, ± 17%) showed spectroscopic characteristics in agreement with structure 10b

Compound 10a had ^1H NMR, δ (100 MHz, $DMSOd_6$): 7.8 and 7.9 (br, $CONH_2$), 5.85 (d, H_1', 1H, $J_{1'2'}$ = 9 Hz), 4.41 (d x d, H_2', 1H, $J_{2'3'}$ = 4.5 Hz), 4.22 (d, H_3', 1H), 3.95 (broad s, H_4', 1H), 3.82 (s, CO_2Me, 3H), 3.66 (m, H_5', 2H), 3.35 (OH), 0.95 and 0.77 (2 x s, C_4H_9, 2 x 9H), 0.11-0.09 (2 x s, 6H -0.08 (s, 3H) and -0.41 (s, 3H); SiMe: δ (CD_3OD): 0.92 and 0.74 (2 x 9H), 0.08-0.07 (6H), -0.12 (3H and -0.45 (3H); ^{13}C NMR, δ (CD_3OD): 164.1 (s, $CONH_2$), 161.3 (q, CO_2Me), 155.05 (d, C_4, ^3J = 5.5 Hz) 133.05 (d, C_2, ^3J = 2.5 Hz), 130.5 (d, C_3, J = 2 Hz), 115.5 (s, C_5 $\omega^{1/2} \leq 3$ Hz; $\omega^{1/2} \geq 6.7$ Hz in $CDCl_3$), 86.3, 75.6, 74.3(2) and 60.3 (C-ribosyl), 52.6 (OMe), 24.5 and 24.2 ($SiCMe_3$), 16.75 and 16.5 ($SiCMe_3$), -5 → -8 (SiMe); m/z (CI): 562 (MH^+); m/z (%) (EI): 560 (M^+ - H, 7.1), 546 (-Me, 1.3) 504 (-C_4H_9, 28.8), 487 (504 -NH_3, 8.0), 372 (504 - TBDMSOH, 27.3), 73 (100); metastable signal: $487^2/504 = 470.57$.

Compound 10b had IR (KBr) cm^{-1}: 1670 (br, $CONH_2$); ^1H NMR, δ (100 MHz, CD_3OD): 5.60 (d, H_1', 1H, $J_{1'2'}$ = 9 Hz), 4.52 (d x d, H_2', 1H, $J_{2'3'}$ = 4.5 Hz), 4.27 (d, H_3', 1H), 4.12 (t, H_4', 1H, $J_{4'5'}$ = 3 Hz), 3.82 (d, H_5', 2H), 0.97 and 0.81 (2 x s, C_4H_9, 2 x 9 H), 0.16 (s, 6H), 0.03 (s, 3H) and -0.1 (s, 3H): SiMe; ^{13}C NMR, δ (CD_3OD) 165 and 163.9 (2 x s, $CONH_2$), 155.75 (d, C_4, ^3J = 5 Hz, 137.7 (d, C_2), 128.2 (d, C_3, J = 3 Hz), 112.95 (s, C_5, $\omega^{1/2} \leq 3$ Hz), 86.8, 75.5 (2), 73.8 and 60.4 (C-ribosyl 24.4 ($SiCMe_3$), 17 and 16.9 ($SiCMe_3$), -7 → -9 (SiMe); m/z (CI): 547 (MH^+); m/z (%) (EI): 531 (M^+ -Me 1.5), 489 (M^+ -C_4H_9, 154), 472 (489 -NH_3, 4.0), 73 (100); metastable signal: $472^2/489 = 455.6$.

Methyl 5-carboxamido-4-hydroxy-3-β,D-ribofuranosyl)-2-thiophene-carboxylate (2a). The fraction containing 10a (0.270 g) of preceeding experiments was dissolved in 25 ml of a mixture of acetic water (3:1) and stirred at 50° for 2 days. The solution was then concentrated and the brownish residue was coevaporated three times with toluene. After extraction of the brownish syrup with chloroform, followed by TLC on silica gel: ethyl acetate - acetone - methanol - water (7/1/0.5/0.5), product 2a (0.096 g, ±60%) could be isolated as a light yellow solid. It was recrystallized from ethanol-water.

m.p.: 115-117°; IR (KBr) cm^{-1}: 1720 (CO_2Me), 1640 (br, $CONH_2$); ^1H NMR, δ (DMSO-d_6): 7.75 (s, $CONH_2$, 2H), 5.6 (d, H_1', 1H, $J_{1'2'}$ = 7.75 Hz), 4.23 (d x d, H_2', 1H, $J_{2'3'}$ = 5.75 Hz), 3.99 (m, H_3', 1H), 3.87 (m, H_4', 1H), 3.82 (s, OMe, 3H), 3.6 (d, H_5', 2H, $J_{4'5'}$ = 3 Hz), 3.3 (s, OH); m/z (CI): 334 (MH^+ -H_2O); m/z (%) (EI): 315 (M^+ -H_2O), 3.3), 230 (B+30, 6.2), 213 (230 -NH_3, 14.4), 57 (100). Ana calc. for $C_{12}H_{15}NO_8S.2H_2O$: C, 39.02; H, 5.19; N, 3.79. Found: C, 38.74; H, 4.85; N, 3.75.

4-Hydroxy-3(β,D)-ribofuranosyl-2,5-thiophene dicarboxamide 2b and its 2'3' isopropylidene derivative (2b'). A mixture of compounds 9 (0.710 g) was treated in the same way as described for the conversion into 10a,b but the mixture was heated at 150°. After 8 hr reaction the excess of ammonia was allowed to escape. The remaining syrup was taken up in a small amount of methanol, triturated with chloroform. The chloroform solution contained 10a and 10b. The brownish deposit was filtered off and dissolved in methanol; after decolorization with charcoal followed by concentrating, crude

compound 2b (0.235 g, ±67%) was obtained as a light yellow solid (R_f: 0.12 on silica gel with chloroform-ethyl acetate 9:1 as eluent). It was further purified via its 2'3-O-isopropylidene derivative as described below.
Crude diamide 2b (0.145 g) was dissolved in 20 ml of 2,2-dimethoxypropane (0.8 M HCl) and 4 ml of dry methanol. After stirring at room temperature for 1.5 hr, the solution was neutralized by dropwise addition of aqueous ammonia. Evaporation under reduced pressure yielded an oil which was chromatographed on 4 preparative silica plates: ethyl acetate-methanol (95:5) as eluent, twice developed. The fluorescent zone was eluted and evaporated to yield the isopropylidene derivative 2b' (90 mg, 55%) as an oil, which was homogeneous according to HPLC on μBondapack C_{18}, 20:80 (v/v) methanol-water (0.08 M ammonium acetate).
Compound 2b' had ^{1}H NMR, δ (D_2O, MeOD as ref.): 5.4 (d, H_1', 1H, $J_{1'2'}$ = 4.5 Hz), 4.94 (m, H_2' and H_3', 2H), 4.25 (q, H_4', 1H, $J_{3'4'}$ = 5 Hz), 3.83 (d, H_5', 2H), 1.63 (s, Me, 3H), 1.40 (s, Me, 3H); m/z (silylated + 4TMS): 646 (M^+, 0.5), 631 (-Me, 1), 614 (-Me, $-NH_3$, 0.2), 556 (-TMSOH, 0.3), 485 ($-TMSOCH_2$, $-Me_2CO$, 1.5), 73 (TMS^+, 100). In order to get pure compound 2b, the isopropylidene derivative 2b was dissolved in a mixture of 4 ml of H_2O and 4 ml of dioxane to which 2 g of Dowex 50 W (H^+ form) was added. After stirring for 2 hr at room temperature, the resin was filtered off and washed with water. The combined filtrate and washings were evaporated (bath temp. < 40°); yielding 43 mg of a crystalline material with m.p.: 189-191°; IR (KBr) cm^{-1} 1660 (br, $CONH_2$); ^{1}H NMR, δ, 250 MHz, DMSO-d_6): 7.7-7.8 (2 x br s, 2 x $CONH_2$), 5.1 and 5.7 (2 x br s, sec. OH), 5.15 (d, H_1', $J_{1'2'}$ = 8 Hz), 4.11 (d x d, H_2', 1H, $J_{2'3'}$ = 5.75 Hz), 4.02 (m, H_3', 1H), 3.90 (m, H_4', 1H), 3.62 (m, H_5', 2H), 3.4 (OH); m/z (CI): 319 (MH^+), 302 ($MH^+ - NH_3$), 301 ($-H_2O$); Anal. calc. for $C_{11}H_{14}O_7N_2S.2H_2O$: C, 37.29; H, 5.12; N, 7.91. Found: C, 37.30, H, 4.75; N, 7.60.

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