Structure-Activity Relationships in Distamycin A Analogues: Effect of Alkyl Groups on the Pyrrole Nitrogen at the Non-amidine End of the Molecule Combined with Methyl Elimination in the Following Ring

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Distamycin A analogues $5a$–f (R=C_nH_{2n+1}, n=0–5) were synthesized using our previous strategy with some improved modifications and screened for their effects on herpes simplex virus (HSV-1). Virus yield assays show that $5a$–$5d$ were potent antiviral agents whereas $5e$ and $5f$ had lower activity. Considerable cellular toxicity was however observed for $5a$–$5c$. Thus $5d$ combining significant antiviral activity with moderate cellular toxicity seems to be the most promising derivative in this series.
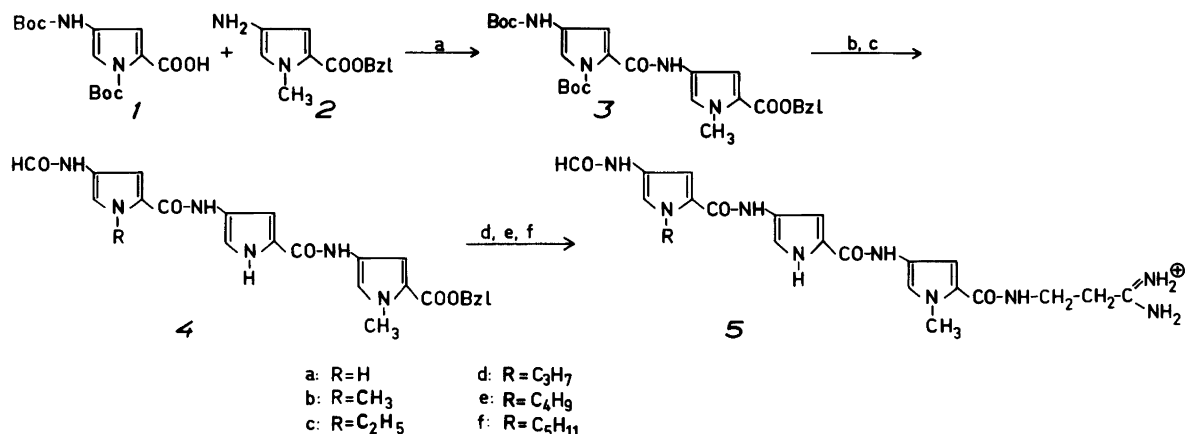
Introduction

The antiviral antibiotic distamycin A is a basic polyamide containing three 4-amino-1-methyl-pyrrole-2-carboxylate residues. Many aspects of its chemistry and biochemistry have hitherto been studied and some of the more pertinent results in this field have been well covered in a recent review.[1]

We have previously shown that some distamycin analogues lacking methyl substituents on one pyrrole nitrogen possess enhanced antiviral activity toward herpes simplex virus type 1 (HSV-1) in comparison with the parent compound.[2] The minor groove in the DNA helix is the main target site for this drug.[3] Since it is assumed that the distamycin-DNA complex is stabilized by hydrogen bonds, we had reason to expect analogues such as $5a$ (Scheme 1) lacking two pyrrole nitrogen substituents to be even more potent. However, elimination of these methyl groups also appeared to

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render these analogues more toxic to the host cells. On the other hand, our toxicity data for some less active analogues carrying ethyl substituents on pyrrole nitrogens or with methyl groups in the amidine function, indicated that the cellular toxicity might be reduced by introducing more hydrophobic groups into the molecule.[2] Conceivably, such a structural change would, at least to some extent, alter the ability to penetrate cell membranes. In order to combine high antiviral activity with low cellular toxicity, one fruitful approach might be to design analogues containing one pyrrole ring substituted with alkyl groups of variable lengths together with a pyrrole moiety lacking the nitrogen substituent. In order to facilitate the synthetic efforts we chose to incorporate the variable alkyl-bearing pyrrole fragment in the "non-amidine" end in a late step. It also seemed reasonable to avoid an unsubstituted pyrrole function as the C-terminal element since such an arrangement would imply synthetic difficulties if this pyrrole nitrogen was left unprotected. Thus, to test our hypothesis, we prepared

Scheme 1. Reagents: (a) DCC, DMAP, CH₂Cl₂. (b) TFA, CH₂Cl₂. (c) 1-alkyl-4-formylaminopyrrole-2-carboxylic acid, EDC, DMF. (d) H₂(Pd), DMF. (e) HOSu, DCC. DMF. (f) β-aminopropionamidine · 2 HBr, NaHCO₃, aqueous dioxane.

the new distamycin analogues *5a, 5c–5f* and assayed them for activity toward HSV-1 as well as for toxicity to the host cells.

Chemistry

The preparations of the distamycin analogues *5a–5f* (Scheme 1) were carried out essentially according to our earlier strategy and the main features of the synthesis are outlined in Scheme 1[2,4]. Earlier experiences indicated that protection of the pyrrole nitrogen is desirable during the coupling of substrates lacking 1-substituents.[2] For our purpose, *tert*-butyloxycarbonyl (Boc) proved suitable in that respect. Thus, benzyl 4-Boc-aminopyrrole-2-carboxylate was smoothly converted to the corresponding 1,4-di-Boc analogue by Boc₂O/4-dimethylaminopyridine (DMAP) in good yield.[5] The removal of the benzyl group from this fully protected derivative was conveniently accomplished by catalytic transfer hydrogenation with 1,4-cyclohexadiene in the presence of a palladium catalyst to give *1* in excellent yield.[6] Conventional hydrogenation (H₂,Pd/ C,400 kPa, room temperature, 4 h) gave saturation of the pyrrole ring. This is, however, not surprising since the presence of acyl functions in the 1-position is known to facilitate this reaction.[7] The subsequent coupling of *1* with the amine *2* was readily achieved with dicyclohexylcarbodiimide (DCC) in the presence of catalytic amounts

of DMAP.[8] The dimeric fragment *3* was thereby obtained in high yield with only traces of by-products present in the crude mixture. The corresponding coupling without protection of the pyrrole nitrogen only afforded a mixture from which the desired product could be isolated in low yield by chromatography.[2] The removal of both Boc groups from *3* with trifluoroacetic acid (TFA) offered no difficulties and the amino analogue *3x* (see Experimental section) was obtained essentially pure in good yield. The assembly of the trimeric building blocks *4a–4f* (Scheme 1) was effected by coupling *3x* with the appropriate 1-alkyl-4-formylaminopyrrole-2-carboxylic acid (for preparation see Experimental section) using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) as condensing agent. The yields in this step were generally satisfactory except in the case of *4a*. The very low yield in this case is due to the lack of protection of the pyrrole nitrogen of the acid component. Preliminary experiments aiming at protection of this site have shown that benzyl 4-formylaminopyrrole-2-carboxylate readily reacted with two equivalents of Boc₂O under standard conditions to give benzyl 1-Boc-4-formyl(Boc)aminopyrrole-2-carboxylate whereas one equivalent of acylating agent furnished a mixture of products. However, attempts to remove the benzyl(Bzl) group by catalytic hydrogenation in methanol as described for preparation of *1* gave in fact largely *1* together

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with various by-products. Obviously, the formyl group is labilized by the introduction of a second acyl function on the amide nitrogen and is therefore lost during the reaction.

The deprotection of *4a–4f* by catalytic hydrogenation gave the corresponding acids in satisfactory yields. Activation of the carboxyl group with DCC/*N*-hydroxysuccinimide (HOSu) as usual provided the succinimidyl esters which were allowed to react with β-aminopropionamidine as in our standard procedure to give the desired distamycin analogues *5c–5f* in acceptable yields. The poor yield of *5a* is probably due to losses during the chromatographic workup.

Biology

The antiviral effects of the distamycin analogues *5a–5f* were measured in a HSV-1 virus yield assay according to a standard procedure (see Experimental). Attempts at a more quantitative estimation of the antiherpes activity by conventional plaque reduction test gave rather inconclusive results. As evident from Table 1, compounds *5a–5c* exhibit increased antiviral activity in comparison with the parent antibiotic.[2] The propyl analogue *5d* was only slightly less potent whereas *5e* and especially *5f* were largely inactive. No antiviral effects were observed when virus was incubated with the drugs before infection of the cells and omitting the drugs during further incubation. Obviously, the alkyl substituents in the *5e* and *5f* derivatives are too bulky to permit of sufficient interaction with viral DNA.

The enhanced virus inhibition displayed by *5a*, *5b* and *5c* was, however, accompanied by considerable cellular toxicity. Conceivably, the high antiviral responses in these cases might, at least partly, be due to cellular toxicity. Interestingly enough, a significant drop in the inhibition of cellular growth was observed for *5d* and therefore, the relatively high value of HSV-1 inhibition by *5d* could more safely be interpreted as a true antiviral effect in this case. In conclusion, it appears that the propyl analogue *5d*, being the least toxic while retaining antiviral effect, is the most promising derivative in this series. Future investigations following this new lead might give rise to distamycin analogues with more satisfactory therapeutic indices. Furthermore, extended structure-activity relationship studies pursuing these guidelines will probably offer a more complete understanding of the basic mechanisms by which these compounds exert their antiviral effect.

Experimental

1-(tert-Butyloxycarbonyl)-4-[(tert-butyloxycarbonyl)amino]-pyrrole-2-carboxylic acid (1). To a solution of benzyl 1-(*tert*-butyloxycarbonyl)-4-[(*tert*-butyloxycarbonyl)amino]pyrrole-2-carboxylate[5] (1.45 g, 3.48 mmol) in ethanol (35 ml) under nitrogen was added a Pd catalyst (5 % on carbon, 0.7 g) followed by 1,4-cyclohexadiene (1.75 ml, 18.4 mmol) and the mixture was stirred under nitrogen at room temperature. After 3 h, TLC (CH_2Cl_2:acetone:HOAc = 40:10:1) indicated that all starting material had been con-

Table 1. Virus inhibition and cellular toxicity of distamycin analogues *5a–f*.

Compound No.	R	Reduction in HSV-1 yield/%		Cellular toxicity/% inhibition of cellular growth	
		Conc = 10 µM	Conc = 100 µM (1 % DMSO)	Conc = 10 µM	Conc = 50 µM
5a	H	59	92	45	100
5b	CH_3	80	94	50	100
5c	C_2H_5	83	93	50	100
5d	C_3H_7	59	80	–	30
5e	C_4H_9	–	51	35	70
5f	C_5H_{11}	–	No effect	50	70
Distamycin[2]		27	–	–	69 (100 µM)

Table 2. Physical properties of 4-R^1-1-R^2-pyrrole-2-$COOR^3$ used as synthetic intermediates.

Compound No.	R^1	R^2	R^3	M.p.(B.p.)/C°	Solvent for recrystallisation	^{1}H NMR, δ/ppm[a]
6	H	C_4H_9	C_2H_5	(120–120.5/10 mm Hg)	[b]	6.95 (q, 1H), 6.82 (t, 1H), 6.10 (q, 1H) 4.31 (t, 2H), 4.27 (q, 2H), 1.71 (m, 2H 1.34 (t, 3H), 1.30 (m, 2H), 0.93 (t, 3H)
7	H	C_5H_{11}	C_2H_5	(94–96/2 mm Hg) [b]		6.95 (q, 1H), 6.82 (t, 1H), 6.10 (q, 1H) 4.29 (t, 2H), 4.27 (q, 2H), 1.76 (m, 2H 1.34 (t, 3H), 1.26 (m, 4H), 0.89 (t, 3H)
8	NO_2	C_4H_9	C_2H_5	60.5–61.0	EtOH	8.05 (d, 1H), 7.34 (d, 1H), 4.47 (t, 2H) 4.32 (q, 2H), 1.78 (m, 2H), 1.35 (m+t 5H), 0.94 (t, 3H)[f]
9	NO_2	C_5H_{11}	C_2H_5	44–44.5	EtOH	8.06 (d, 1H), 7.34 (d, 1H), 4.47 (t, 2H) 4.32 (q, 2H), 1.83 (m, 2H), 1.36 (m+t, 7H), 0.89 (t, 3H)[f]
10	NO_2	C_4H_9	H	165–166	Toluene, EtOH-H_2O	8.06 (d, 1H), 7.37 (d, 1H), 4.48 (t, 2H) 1.82 (m, 2H), 1.35 (m, 2H), 0.94 (t, 3H)[f]
11	NO_2	C_5H_{11}	H	138–138.5	EtOH-H_2O	8.07 (d, 1H), 7.38 (d, 1H), 4.48 (t, 2H) 1.84 (m, 2H), 1.35 (m, 4H), 0.89 (t, 3H)[f]
12	HCO-NH	C_3H_7	H	157.5–158.5[d]	[c]	8.12 (s, 1H), 7.35 (d, 1H), 6.83 (d, 1H) 4.25 (t, 2H), 1.76 (m, 2H), 0.88 (t, 3H)[i]
13	HCO-NH	C_4H_9	H	164–165[d]	EtOH-H_2O	8.12 (s, 1H), 7.35 (d, 1H), 6.82 (d, 1H) 4.29 (t, 2H), 1.72 (m, 2H), 1.28 (m, 2H), 0.93 (t, 3H)[g]
14	HCO-NH	C_5H_{11}	H	163–164[d]	EtOH-H_2O	9.18 (broad s, 1H), 8.24 (s, 1H), 7.47 (d, 1H), 6.85 (d, 1H), 4.33 (t, 2H), 1.75 (m, 2H), 1.31 (m, 4H), 0.88 (t, 3H)[f]

[a]At 90 MHz. [b]Liquids at room temperature. [c]Purified by chromatography. [d]Decomposition. [e]Chloroform-d_1. [f]Acetone-d_6. [g]Methanol-d_4.

sumed. The catalyst was then removed by filtration and the colourless filtrate evaporated to dryness at room temperature. The semisolid residue was partitioned between ether (150 ml) and 1 M $KHSO_4$ (50 ml) and the organic extract was washed with 1 M $KHSO_4$ and saturated sodium chloride (3×50 ml of each). After drying over $MgSO_4$, the solvent was removed *in vacuo* at room temperature and the white crispy residue was thoroughly triturated with petroleum ether. After brief cooling, the insoluble solid was collected, rinsed with small portions of cold petroleum ether and dried *in vacuo* over paraffin. The yield of crude, chromatographically pure product obtained as a white powder was 1.15 g (100 %). m.p. ~ 150 °C (dec.). ^{1}H NMR (CDCl$_3$): δ = 7.63 (m, 1H), 7.16 (d, 1H), 6.57 (broad s, ≈1H), 1.65 (s, 9H), 1.51 (s, 9H).

Benzyl 4-[[[1-(tert-butyloxycarbonyl)-4-[(tert-butyloxycarbonyl)amino]-pyrrole-2-yl]carbonyl] amino]-1-methylpyrrole-2-carboxylate (3). A solution of *1* (1.114 g, 3.42 mmol), *2* (786 mg, 3.42 mmol) and DMAP (42 mg, 0.34 mmol) in dry dichloromethane (10 ml) was treated at room temperature dropwise with DCC (774 mg, 3.76 mmol) dissolved in dry dichloromethane (5 ml) with vigorous stirring. Within a few minutes a white precipitate appeared and after 2 h TLC (system above) indicated that the reaction was completed. The white solid was filtered off, rinsed with small portions of dichloromethane and the filtrate was taken to dryness at room temperature. The residual sticky mass was partitioned between ether (90 ml) and 1 M KHSO₄ (30 ml) and the organic phase was washed in turn with 1 M KHSO$_4$, 1 M NaHCO$_3$ and saturated sodium chloride (3×30 ml of each). After drying over MgSO$_4$, the extract was evaporated at room temperature leaving the desired product as an almost colourless crispy foam. The yield of crude *3*, essentially pure by TLC and suitable for further synthesis, was 1.717 g (93%). An analytical specimen was obtained by chromatography on a silica gel column with dichloromethane:acetone = 9:1 as eluent.

The chromatographed material was dissolved in dry ether (5 ml/g) and upon tenfold dilution with petroleum ether, the product precipitated as a white powder which was thoroughly dried *in vacuo*, m.p. 132–134 °C (dec.). ^{1}H NMR (CDCl$_3$): δ = 7.52 (d, 1H), 7.38 (pert. sign., 7H), 6.95 (d, 1H), 6.85 (d, 1H), 6.51 (broad s, 1H),

Table 3. Physical properties of benzyl esters *4a, 4c–f.*

Compound No.	R	Yield/%[a]	M.p./°C	^{1}H NMR, δ/ppm[b]
4a	H	16[c]	[d]	11.40 (br.s, 2H), 10.14 (br.s, 1H), 10.09 (br.s, 1H), 10.05 (br.s, 1H), 8.14 (s, 1H), 7.49 (pert. sign., 1H), 7.41 (s, 5H), 7.24 (pert. sign., 1H), 7.13 (pert. sign., 1H), 7.07 (pert. sign., 1H), 6.99 (d, 2H), 5.26 (s, 2H), 3.87 (s, 3H)
4c	C$_2$H$_5$	80	252–253 (dec.)	11.27 (br.s, 1H), 10.07 (br.s, 1H), 9.94 (br.s, 2H), 8.15 (s, 1H), 7.48 (pert. sign., 1H), 7.41 (s, 5H), 7.26 (pert. sign., 1H), 7.17 (pert. sign., 2H), 6.99 (pert. sign., 1H), 6.91 (pert. sign., 1H), 5.27 (s, 2H), 4.34 (q, 2H), 3.87 (s, 3H), 1.29 (t, 3H)
4d	C$_3$H$_7$	82	198–200 (dec.)	11.27 (br.s, 1H), 10.07 (br.s, 1H), 9.95 (br.s, 2H), 8.15 (s, 1H), 7.41 (pert. sign., 6H), 7.25 (pert. sign., 1H), 7.16 (pert. sign., 2H), 6.98 (pert. sign., 1H), 6.90 (pert. sign., 1H), 5.26 (s, 2H), 4.28 (t, 2H), 3.87 (s, 3H), 1.69 (m, 2H), 0.81 (t, 3H)
4e	C$_4$H$_9$	85	232-233 (dec.)	11.28 (br.s, 1H), 10.08 (br.s, 1H), 9.96 (br.s, 1H), 9.93 (br.s, 1H), 8.14 (pert. sign., 1H), 7.48 (d, 1H), 7.41 (s, 5H), 7.25 (d, 1H), 7.14 (pert. sign., 2H), 6.98 (d, 1H), 6.89 (d, 1H), 5.26 (s, 2H), 4.30 (t, 2H), 3.87 (s, 3H), 1.66 (m, 2H), 1.22 (m, 2H), 0.87 (t, 3H)
4f	C$_5$H$_{11}$	90	213–214 (dec.)	11.28 (br.s, 1H), 10.08 (br.s, 1H), 9.96 (br.s, 1H), 9.93 (br.s, 1H), 8.15 (pert. sign., 1H), 7.49 (d, 1H), 7.41 (s, 5H), 7.25 (pert d, 1H), 7.16 (pert. m, 2H), 6.98 (d, 1H), 6.89 (d, 1H), 5.26 (s, 2H), 4.30 (pert.t, 2H), 3.87 (s, 3H), 1.66 (m, 2H), 1.22 (pert.m, 4H), 0.84 (t, 3H)

[a]Crude. [b]At 90 MHz in DMSO-*d$_6$*. [c]After chromatography. [d]Not obtained completely pure (TLC).

Table 4. Physical properties of distamycin analogues *5a, 5c–f.*

Compound No.	R	Yield/%[a]	^{1}H NMR, δ/ppm[b]	Elemental analyses
5a	H	11	8.15 (s, 1H), 7.29 (d, 1H), 7.26 (d, 1H), 7.18 (d, 1H), 7.06 (d, 1H), 6.97 (d, 1H), 6.92 (d, 1H), 3.88 (s, 3H), 3.66 (t, 2H), 2.72 (t, 2H)	$C_{20}H_{23}N_9O_4 \cdot$ HBr $\cdot$ 2H$_2$O (C[a]HN[f])
5c	C_2H_5	24	8.14 (s, 1H), 7.30 (d, 1H), 7.26 (d, 1H), 7.20 (d, 1H), 7.05 (d, 1H), 6.93 (d, 1H), 6.88 (d, 1H), 4.37 (q, 2H), 3.87 (s, 3H), 3.66 (t, 2H), 2.72 (t, 2H), 1.37 (t, 3H)[c]	$C_{22}H_{27}N_9O_4 \cdot$ HBr $\cdot$ 0.5 H$_2$O (C H N[g])
5d	C_3H_7	50	8.14 (s, 1H), 7.30 (d, 1H), 7.25 (d, 1H), 7.20 (d, 1H), 7.05 (d, 1H), 6.93 (d, 1H), 6.88 (d, 1H), 4.29 (t, 2H), 3.87 (s, 3H), 3.66 (t, 2H), 2.73 (t, 2H), 1.77 (m, 2H), 0.88 (t, 3H)[c]	$C_{23}H_{29}N_9O_4 \cdot$ HBr $\cdot$ 0.5 H$_2$O (C H N)
5e	C_4H_9	51	8.14 (s, 1H), 7.30 (d, 1H), 7.25 (d, 1H), 7.20 (d, 1H), 7.06 (d, 1H), 6.93 (d, 1H), 6.87 (d, 1H), 4.33 (t, 2H), 3.87 (s, 3H), 3.65 (t, 2H), 2.73 (t, 2H), 1.72 (m, 2H), 1.29 (m, 2H), 0.91 (t, 3H)[d]	$C_{24}H_{31}N_9O_4 \cdot$ HBr $\cdot$ 2.5 H$_2$O (C H[h]N[i])
5f	C_5H_{11}	40	8.14 (s, 1H), 7.29 (d, 1H), 7.25 (d, 1H), 7.20 (d, 1H), 7.06 (d, 1H), 6.93 (d, 1H), 6.87 (d, 1H), 4.32 (t, 2H), 3.87 (s, 3H), 3.66 (t, 2H), 2.73 (t, 2H), 1.75 (m, 2H), 1.29 (m, 4H), 0.88 (t, 3H)[d]	$C_{25}H_{33}N_9O_4 \cdot$ HBr $\cdot$ H$_2$O (C H N[j])

[a]Yield as calculated from the acid after LH-20 chromatography and precipitation from methanol-ether. [b]At 90 MHz in methanol-d_4. [c]The analytical specimen was obtained by recrystallization from water (20 ml/g). Attempts to crystallize *5a* were not successful (too high solubility). *5e* and *5f* gave a very fine grained precipitate which passed the filter. [d]H NMR revealed the presence of traces (1 %) of *N*-hydroxysuccinimide (δ = 2.67, singlet). [e]Calc. 40.8. Found 40.3. [f]Calc. 21.4. Found 18.5. [g]Calc. 22.1. Found 21.2. [h]Calc. 5.9. Found 5.3. [i]Calc. 19.8. Found 19.1 [j]Calc. 20.3. Found 19.6.

5.27 (s, 2H), 3.90 (s, 3H), 1.60 (s, 9H), 1.50 (s, 9H). Anal. $C_{28}H_{34}N_4O_7$ C, H, N.

Benzyl 4-[[(4-aminopyrrole-2-yl)carbonyl]amino-]-1-methylpyrrole-2-carboxylate (3x). *Improved procedure from 3.* Crude *3* (770 mg, 1.43 mmol) dissolved in dry dichloromethane (15 ml) was flushed with nitrogen and TFA (7 ml, purged with nitrogen) was slowly added with gentle agitation at room temperature. The brown yellow solution was left under nitrogen for 1 h and then quenched in a mixture of deaerated 30 % K$_2$CO$_3$ (100 ml) and dichloromethane (25 ml). The yellow organic phase was separated and the aqueous layer was further extracted with dichloromethane (2×25 ml). The combined extracts were washed with deaerated saturated sodium chloride (3×25

ml) and dried over Na$_2$SO$_4$. Removal of the solvent at room temperature left a yellowish solid residue which was thoroughly triturated with dry ether (5 ml). The fine-grained insoluble light-yellow solid was collected, rinsed with dry ether (3×1 ml) and dried *in vacuo.* This crude amine, essentially pure by TLC (chloroform:ethanol:water = 100:50:4), was immediately used for the next step. The yield of dried product was 433 mg (90 %).

Preparation of 1-alkyl-4-formylaminopyrrole-2-carboxylic acids (12–14). 4-Formylamino-1-propylpyrrole-2-carboxylic acid (*12*) was synthesized from the known 4-nitro-1-propylpyrrole-2-carboxylic acid[9] according to our previous procedure. The corresponding butyl and pentyl analo-

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gues (*13* and *14*) were available by a multistep sequence from ethyl pyrrole-2-carboxylate by analogy with the synthesis of 1-ethyl-4-formylaminopyrrole-2-carboxylic acid and the yields in the separate steps were in the usual range.[2] Physical data for intermediates and end products not previously described have been collected in Table 2.

Synthesis of trimeric analogues (a–f). Fresh, thoroughly dried *3x* as obtained above was coupled to the appropriate 1-alkyl-4-formylaminopyrrole-2-carboxylic acid using EDC in DMF, essentially according to the procedure earlier described for *4b*.[2] Crude *4c–4f* were suitable for synthetic work without further purification but crude *4a* required chromatographic purification before use. Analytical samples of all compounds were obtained as earlier. Yields and selected physical data for *4a, c–f* are compiled in Table 3.

Distamycin analogues (5a–f). Trimeric benzyl esters *4a–f* were hydrogenolyzed in DMF over a Pd catalyst affording the corresponding acids in 80–90 % yields. Activation of the carboxyl group was accomplished with DCC-HOSu in DMF as usual. The resulting *N*-succinimidyl esters were used without chromatographic purification in the subsequent coupling to β-aminopropionamidine in aqueous dioxane. Workup and purification of *5a–f* were carried out as before to give chromatographically pure material.[2] The high purity of these compounds was further confirmed by HPLC.[10] Yields and selected data of *5a, c–f* are summarized in Table 4.

Biological assays. The extent of virus multiplication was measured by yield reduction tests using monolayers of Vero cells infected with 1 pfu per cell of HSV-1, strain C-42 as described earlier.[11] Inhibition of cellular growth was measured as previously described using human embryo cells.[12]

References

1. Bialer, M. in *Antiviral Drugs and Interferon: The Molecular Basis of Their Activity,* Becker, Y. (ed.). Martinus Nijhoff Publishing, Boston 1984, p. 142.
2. Grehn, L., Ragnarsson, U., Eriksson, B. and Öberg, B. *J. Med. Chem. 26* (1983) 1042.
3. Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H. and Waring, M. J. The Molecular Basis of Antibiotic Action, 2nd ed., Wiley, New York 1981, p. 345.
4. Grehn, L. and Ragnarsson, U. *J. Org. Chem. 46* (1981) 3492.
5. Grehn, L. and Ragnarsson, U. *Angew. Chem. 96* (1984) 291; *Angew. Chem. Int. ed. Engl. 23* (1984) 296.
6. Felix, A. M., Heimer, E. P., Lambros, T. J., Tzougraki, C. and Meienhofer, J. *J. Org. Chem. 43* (1978) 4194.
7. For a brief discussion see Jones, R. A. and Bean, G. P. *The Chemistry of Pyrroles,* Academic Press 1977, p. 241.
8. Hassner, A. and Alexanian, V. *Tetrahedron Lett. 46* (1978) 4475.
9. Grokhovskii, S. L., Zhuse, A. L. and Gottikh, B. P. *Bioorg. Khim. 1* (1975) 1616.
10. Fransson, B., Grehn, L. and Ragnarsson, U. *J. Chromatogr. 268* (1983) 347.
11. Schnürer, J. and Öberg, B. *Arch. Virol. 68* (1981) 203.
12. Stenberg, K. *Biochem. Pharmacol. 30* (1981) 1005.