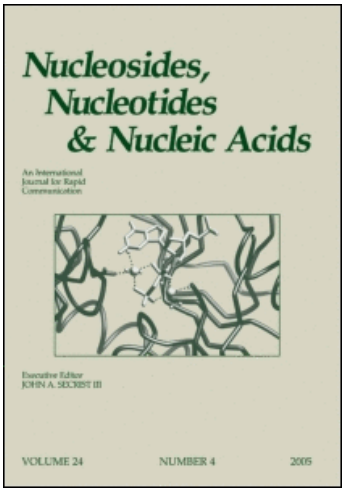

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INCORPORATION OF 2'-DEOXYSANGIVAMYCIN IN DNA DUPLEXES: THE CONVERSION OF A PYRROLO[2,3-*d*]PYRIMIDINE NITRILE TO A CARBOXAMIDE UPON OLIGONUCLEOTIDE DEPROTECTION

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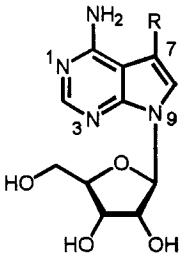
ABSTRACT: Oligonucleotides containing 2'-deoxysangivamycin are described. The phosphoramidite of 2'-deoxytoyocamycin was prepared and used in solid-phase synthesis. Upon deprotection the pyrrolo[2,3-*d*]pyrimidine nitrile residues were converted to carboxamides. According to the T_m-measurements the 7-carboxamido group of the 7-deazaadenine moiety stabilizes the DNA duplex significantly.

Introduction

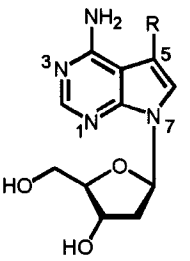
The 7-deazapurine (pyrrolo[2,3-*d*]pyrimidine) nucleosides (purine numbering is used throughout the general section) are a class of compounds with diversified biological activity.[1-4] They include the nucleoside antibiotics tubercidin (**1a**), toyocamycin (**1b**) and sangivamycin (**1c**) as well as the 2'-deoxyribonucleosides **2a-d.**[5-7] The chemical syntheses of the 7-deazapurine ß-D-ribonucleosides as well as of the 2'-deoxyribonucleosides are well established.[8-22]

Earlier, 7-substituted 7-deazapurine nucleosides have been incorporated into oligonucleotides.[23-29] The 7-halogeno substituents, as well as the 7-alkyl or 7-alkynyl groups have been found to enhance the DNA-duplex stability compared to the unmodified counterparts.[23-29] This effect has been attributed to increased stacking interactions of the nucleobase as well as to the hydrophobicity of the substituents. As a 7-cyano group is expected to have the same favorable influence on the oligonucleotide duplex structure[27,29] the incorporation of 2'-deoxytoyocamycin (**2c**) into oligonucleotides was



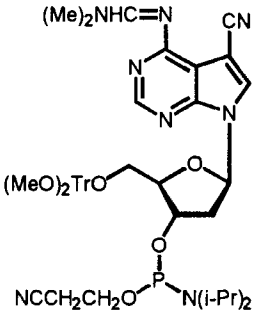
1a R = H
b R = CN
c R = $CONH_2$

(purine numbering)



2a R = H
b R = I
c R = CN
d R = $CONH_2$

(systematic numbering)



3

performed. The phosphoramidite **3** was prepared as building block for the solid-phase synthesis.

Results and discussion

1. Monomers.- The already reported synthesis of 2'-deoxytoyocamycin (**2c**) is laborious as the nitrile function was introduced in an early step of the synthetic route which started with a pyrrole derivative.[4,9,10] Therefore, the palladium(0)-catalyzed reaction of 7-iodo-7-deaza-2'-deoxyadenosine (**2b**) ($c^7I^7A_d$)[30] with tri-*n*-butyltincyanide was employed. The reaction which was performed in DMF resulted in the formation of 7-cyano-7-deaza-2'-deoxyadenosine (2'-deoxytoyocamycin) (**2c**) (21% yield) together with the formation of 7-deaza-2'-deoxyadenosine (2'-deoxytubercidin) (**2a**) (41% yield). The formation of **2a** is the result of a Pd-catalyzed reduction of the iodo compound **2b**. Because of the low yield of compound **2c**, another reaction protocol was employed.

Previously, 6-cyanopurine nucleosides have been synthesized from 6-iodo compounds using copper cyanide in pyridine.[31] This reaction had already been employed on the 7-iodinated 7-deaza-2'-deoxyguanosine.[20] It was now performed on compound

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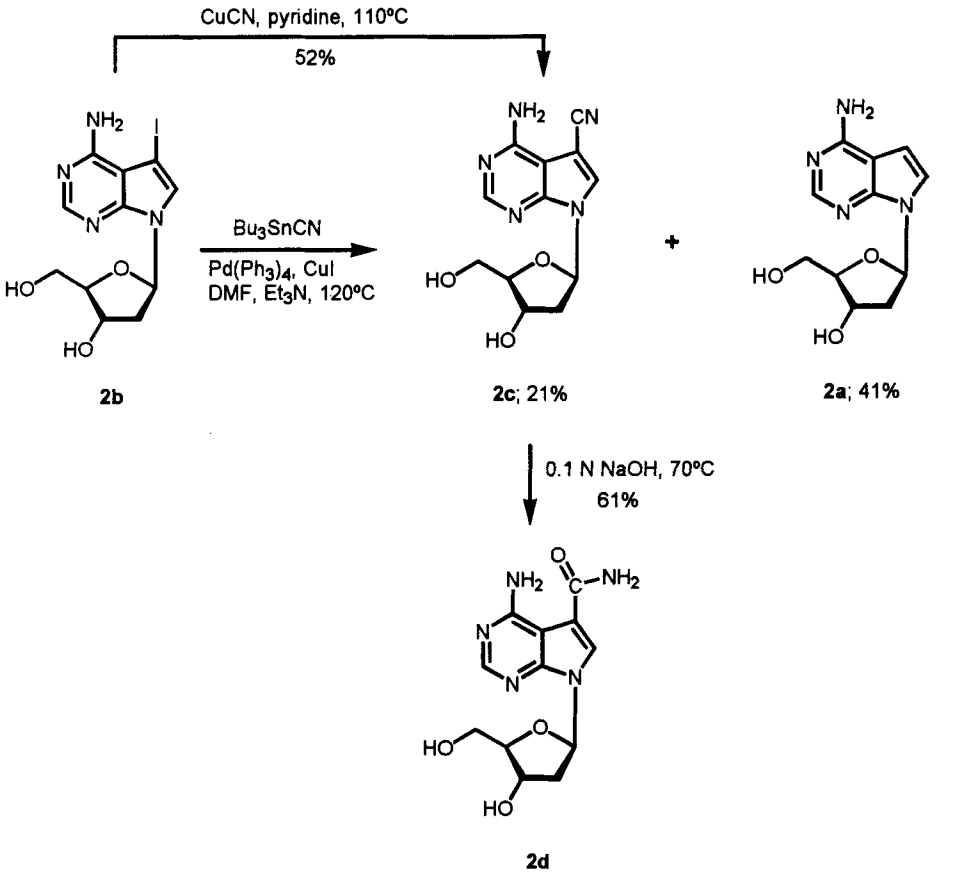
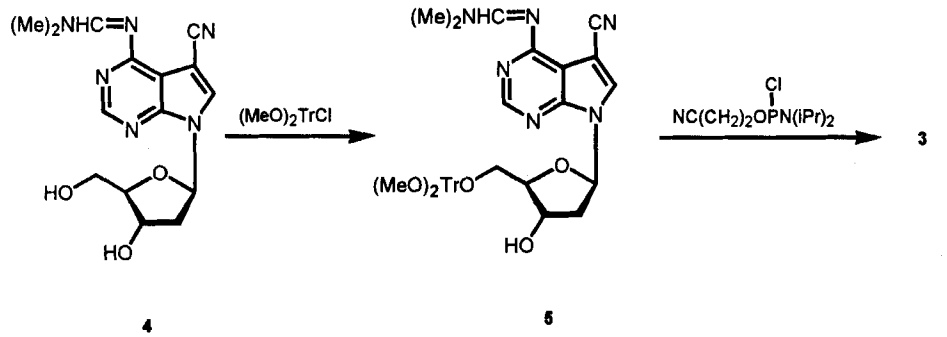
2'-Deoxysangivamycin (**2d**) was prepared from **2c** upon treatment with 0.1 N NaOH (61% yield).



2b **2c**; 21% **2a**; 41%

2d

Previously, 6-cyanopurine nucleosides have been synthesized from 6-iodo compounds using copper cyanide in pyridine.[31] This reaction had already been employed on the 7-iodinated 7-deaza-2'-deoxyguanosine.[20] It was now performed on compound 2'-Deoxysangivamycin (**2d**) was prepared from **2c** upon treatment with 0.1 N NaOH (61% yield). Next, the oligonucleotide building block **3** was synthesized. The dimethylamino-methylidene residue was chosen as the amino protecting group to give compound **4**. The half-life for deprotection of this derivative was determined UV-spectrophotometrically (320 nm) at 40°C in 25% aq. ammonia. The $t_{1/2}$ value of 42 min indicates the suitability of this protecting group for further transformations. Subsequent tritylation with 4,4'-dimethoxytriphenylmethyl chloride yielded the 5'-protected nucleoside **5**. Phosphitylation with chloro(2-cyanoethoxy)-N,N-diisopropylamino-phosphine in THF furnished the phosphoramidite **3**.



4 5

All compounds were characterized by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra (Table 1 and Exp. Part) as well as by mass spectra. On the basis of the ^{1}H-NMR- and $^{1}H/^{13}C$-NMR correlation spectra, an unambiguous assignment for the protons H-2 and H-6 of **2c,d** was made, which was found to be different from already reported assignments (see Experimental Section).[8-10] For this purpose gated-decoupled ^{13}C-NMR- and hetero-nuclear correlation spectra were recorded for the derivatives **2c,d** and **4,5**. The exchange of the 7-iodo substituent by the cyano residue (→ **2c**) leads to a downfield shift of carbon C-6 (31 ppm). Conversion to the carboxamido group (→ **2d**) resulted once more in a downfield shift (18 ppm). The amidine protecting group (→ **4**) has an influence on the electronic properties of the base which results in a significant shift of the C-4 signal. The 5'-OH-tritylation (→ **5**) is indicated by an upfield shift of C-4' and a downfield shift of C-5'.

2. Oligonucleotide Synthesis.- Earlier, the 7-halogenated 7-deaza-2'-deoxyadenosines have been incorporated in oligonucleotides and their physicochemical properties have been studied.[23,24] In order to investigate the influence of the 7-cyano group of **2c** on the oligonucleotide duplex structure and stability, the oligonucleotides shown in Table 3 were prepared using the phosphoramidite **3**. The oligonucleotides were recovered, deprotected and purified using oligonucleotide purification cartridges.[32] Their homogeneity was checked by reverse phase HPLC. The nucleoside composition was determined by *MALDI-TOF* mass spectra (Table 2) and by enzymatic hydrolysis (Fig. 1b,c).

Recently, the synthesis of oligonucleotides containing 2-amino-7-cyano-7-deaza-adenines was reported.[27,29] However, the mass spectra as well as the enzymatic digestion

TABLE 1. ^{13}C-NMR Chemical Shifts of 7-Cyano-7-deazaadenine 2'-Deoxyribofuranosides; Measured in $(CD_3)_2SO$ at 25°C.

a	C(2)	C(6)	C(5)	C(7)	C(8)	C(4)	C≡N	C(1')	C(3')	C(4')	C(5')
b	C(2)	C(4)	C(4a)	C(5)	C(6)	C(7a)					
2a	151.6	157.5	102.9	99.6	121.6	149.6		83.3	71.1	87.3	62.1
2b[30]	152.0	157.3	103.2	51.9	126.9	149.8		83.0	71.0	87.5	62.0
2c	153.5	157.0	101.2	82.9	132.1	149.7	115.4	83.8	70.6	87.8	61.6
2d	152.8	158.0	100.9	110.9	125.0	150.5	166.4[c]	82.9	70.8	87.5	62.1
4	153.0	160.4	109.3	84.7	133.0	150.6	115.4	83.7	70.6	87.8	61.6
5	153.0	160.3	109.4	84.8	132.9	150.8	115.3	83.4	70.2	85.4	63.8

	OMe	$(Me)_2$	HC=N
4		34.5, *d*	156.8
5	54.9	34.5, 38.2	156.8

[a] Purine numbering. [b] Systematic numbering. [c] C≡N is replaced by $CONH_2$.

[d] Superimposed by DMSO; C(2') is superimposed by DMSO.

TABLE 2. Molecular Weights of Oligonucleotides Containing 2'-Deoxysangivamycin (**2d**); Determined by *MALDI-TOF* Mass Spectra.

	M^+ (calcd) [Da]	M^+ (found) [Da]
5'-d(GTAG(**2d**)$_2$TTCTAC) (**7**)	3728.5	3728.3
5'-d(T**2d**GGTCAAT**2d**CT) (**11**)	3728.5	3722.9
3'-d(ATCC**2d**GTT**2d**TGA) (**12**)	3728.5	3727.4

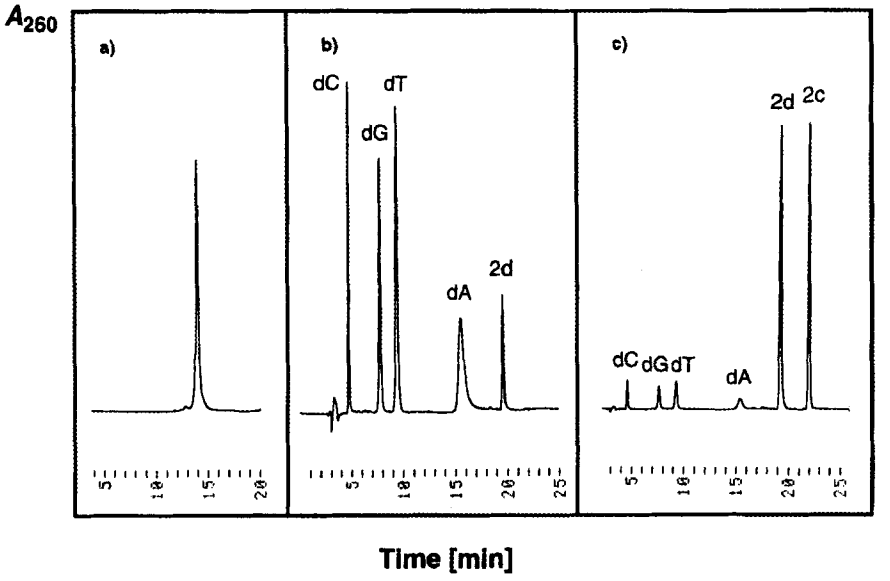


FIG. 1. HPLC-Profiles of the oligonucleotide **11,** (a) after oligonucleotide synthesis and work-up, (b) after enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 1M Tris-HCl buffer (pH 8.3), (c) enzymatic digestion in the presence of **2c** and **2d** (c); for a) gradient *I*, for b) and c) gradient *II*; details see Exp. Part.

pattern of the oligomers obtained from **3** indicated, that the cyano group was no longer present in the molecule. The new peak arising in the composition analysis was identified as 2'-deoxysangivamycin (**2d**). This was confirmed by the addition of 2'-deoxytoyocamycin (**2c**) and 2'-deoxysangivamycin (**2d**) to the enzymatic digest (Fig. 1c).

A complete conversion of the nucleoside 2'-deoxytoyocamycin (**2c**) in 2'-deoxysangivamycin (**2d**) was observed under the conditions of the oligonucleotide deprotection (33% aq. ammonia, 60°C) within 6 h. The determination of the protecting group stability is accompanied by a 30% conversion of the nucleoside within 2 h. The reactions were monitored by HPLC.

3. Oligonucleotide Properties.- The influence of the carboxamido function on the duplex structure and stability was studied. The self-complementary oligonucleotide 5'-d(GTAGAATTCTAC) (**6**) containing the recognition site of the endodeoxyribonuclease Eco RI[31] was modified with **2d**. The two central dA-residues were replaced by

TABLE 3. T_m-Values and Thermodynamic Data of Oligonucleotides Containing 2'-Deoxysangivamycin (**2d**).

	T_m [°C][a] ( )[b]	ΔH [kcal/mol]	ΔS [cal/mol.K]	ΔG^0 [kcal/mol]
5'-d[(GTAGAATTCTAC)]$_2$ (**6•6**)	46(43)	-79(-84)	-226(-225)	-9.2(-8.6)
5'-d[(GTAG(**2d**)$_2$TTCTAC)]$_2$ (**7•7**)	51	-68	-187	-9.8
5'-d[(GTAG(c^{7}I^{7}A)$_2$TTCTAC)]$_2$ (**8•8**)	52	-78	-215	-10.8
5'-d(TAGGTCAATACT) (**9**) 3'-d(ATCCAGTTATGA) (**10**)	50(47)	-90(-82)	-252(-230)	-11.8(-10.4)
5'-d(T**2d**GGTCAAT**2d**CT) (**11**) 3'-d(ATCC**2d**GTT**2d**TGA) (**12**)	55(52)	-75(-73)	-205(-201)	-11.7(-11.1)
5'-d(Tc^{7}I^{7}AGGTCAATc^{7}I^{7}ACT) (**13**) 3'-d(ATTCCc^{7}I^{7}AGTTc^{7}I^{7}ATGA) (**14**)	57(52)	-94(-82)	-261(-228)	-13.4(-11.5)
5'-d(TAGGTCAATACT) (**9**) 3'-d(ATCC**2d**GTT**2d**TGA) (**12**)	52(48)	-85(-81)	-236(-225)	-11.7(-10.7)
5'-d(TAGGTCAATACT) (**9**) 3'-d(ATCCc^{7}I^{7}AGTTc^{7}I^{7}ATGA) (**14**)	54	-85	-236	-12.3
5'-d(T**2d**GGTCAAT**2d**CT) (**11**) 3'-d(ATCCAGTTATGA) (**10**)	53	-90	-249	-12.4
5'-d(Tc^{7}I^{7}AGGTCAATc^{7}I^{7}ACT) (**13**) 3'-d(ATCCAGTTATGA) (**10**)	53	-86	-238	-12.1

[a] Measured at 270 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH = 7.0) with 5 μM single strand concentration. [b] Measured at 270 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH = 7.0) with 5 μM single strand concentration. **2d** = 2'-Deoxysangivamycin.

2'-deoxysangivamycin (**2d**). This results in an enhanced duplex stability compared to the parent hybrid **6•6** (Table 3).

Also non-self-complementary duplexes derived from 5'-d(TAGGTCAATACT) (**9**) and 3'-d(ATCCAGTTATGA) (**10**) were studied. When 2'-deoxysangivamycin (**2d**) was replacing 2'-deoxyadenosine residues the stability of these duplexes was also increased

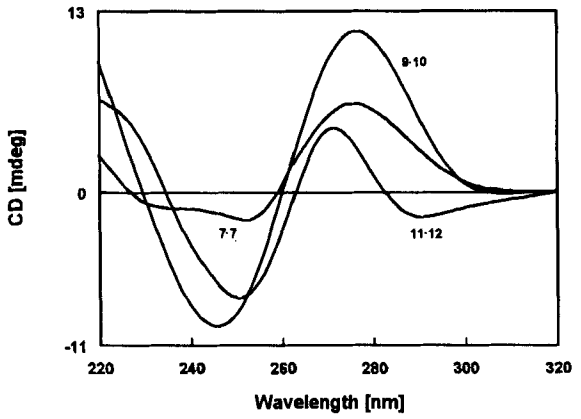


FIG. 2. CD spectra of the heteroduplexes **7•7**, **9•10** and **11•12**, measured at 10°C in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH = 7.0) with 10 μM oligomer concentration; for sequences see Table 3.

(Table 3). The enhanced thermal stability amounts to 1-2°C per one 7-carboxamido residue which derives from a more favorable entropy induced by the modified base. These effects are in the range caused by a modification with 7-iodo-7-deazaadenine residues (Table 3).[24]

According to the CD-spectra (Figure 2) the base-modified duplexes form a B-type DNA structure.

Conclusion

Attempts to incorporate 2'-deoxytoyocamycin (**2c**) instead of 2'-deoxyadenosine into oligonucleotides failed. Instead of this, oligonucleotides were formed which contain 2'-deoxysangivamycin (**2d**). The conversion of the pyrrolo[2,3-*d*]pyrimidine cyano group to a carboxamide group (→ **2d**) is occurring on the oligonucleotide level during the deprotection with ammonia. This reaction can be used for the post-modification of DNA.[34] Ammonia has to be replaced by diaminoalkanes. The new primary amino group is ready to accept reporter groups with an activated side chain. Furthermore, the 2'-deoxy-sangivamycin (**2d**) residue was found to stabilize DNA duplexes significantly in a similar manner as 7-iodo-7-deaza-2'-deoxyadenosine (**2b**).[24]

Experimental

Monomers. Chemicals were supplied by Aldrich, Sigma or Fluka. Solvents were of laboratory grade, except those used for the HPLC which were of HPLC grade. FAB mass spectra were provided by Dr. M. Sauer, University of Heidelberg. NMR Spectra were measured on Avance DPX or AMX 500 spectrometers (Bruker, Germany) operating at proton resonance frequencies of 250.13 MHz (125.13 MHz for ^{13}C and 101.3 MHz for ^{31}P, respectively). Chemical shifts are in ppm relative to TMS as internal standard or external 85% H_3PO_4. UV-spectra were recorded on a U 3200 spectrometer (Hitachi, Japan). Thin-layer chromatography (TLC) was performed on aluminium sheets, silica gel 60 F_{254}, 0.2 mm layer (Merck, Germany), and column chromatography (flash chromatography: FC) on silica gel 60 (Merck, Germany) at 0.4 bar (4×10^4 Pa) using the following solvent systems: (A) CH_2Cl_2-MeOH (9:1, v/v), (B) petroleum ether (boiling range 40-60°C)-acetone (1:1, v/v). Samples were collected with an UltroRac II fractions collector (LKB Instruments, Sweden).

Oligonucleotides. Oligonucleotide synthesis was performed on a ABI 392-08 DNA synthesizer (Applied Biosystems, Weiterstadt, Germany) using a standard protocol. The oligonucleotides were recovered, deprotected (33% aq. ammonia, 60°C, 24 h) and purified using oligonucleotide purification cartridges.[32] *MALDI-TOF* mass spectra were provided by Dr. J. Gross (University of Heidelberg, Germany). The enzymatic hydrolysis of the oligomers was performed as described in ref. [35]. Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleoside (ε_{260} values: dA 15400, dC 7300, dG 11400, dT 8800, $c^7CONH_2{}^7A_d$ 8000). Snake-venom phophodiesterase (EC 3.1.15.1, *Crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1, *E. coli*) were generous gifts of Roche Diagnostics (Mannheim, Germany). RP-18 HPLC: 250 x 4 mm RP-18 column; Merck-Hitachi HPLC; gradients of 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5 (*A*) and MeCN (*B*); gradient *I*: 50 min 0-50% *B* in *A*, flow rate 1 mL/min; gradient *II*: 25 min 0-20% *B* in *A*, flow rate 0.7 mL/min.

Determination of T_m-values and thermodynamic data. Absorbance *vs* temperature profiles were measured on a Cary-1/1E UV/VIS spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The T_m-values were measured in the reference cell with a Pt-100 resistor and the thermodynamic data (ΔH, ΔS, ΔG^0) were

calculated using the program "MeltWin 3.0"[36]. Circular dichroism spectra: The CD-spectra were recorded with a Jasco-600 (Jasco, Japan) spectropolarimeter with thermostatically (Lauda-RCS-6 bath) controlled 1 cm cuvettes.

4-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (2'-Deoxytubercidin) (2a)[21] and 4-amino-5-cyano-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (2'-Deoxytoyocamycin) (2e)[8]. *Method 1*: To a solution of **2b**[30] (1.0 g, 2.66 mmol) in DMF (70 mL) were added tetrakis(triphenylphosphine)palladium(0) (700 mg, 0.61 mmol) and tri-*n*-butyltincyanide (840 mg, 2.66 mmol). The mixture was stirred at 120°C for 24 h under argon. After adding a second portion of tetrakis(triphenyl)palladium(0) (700 mg, 0.61 mmol) and tri-*n*-butyltincyanide (420 mg, 1.33 mmol) stirring was continued for 32 h at 120°C (argon). The solvent was evaporated, and the residue was purified on a silica gel column [15 x 4 cm, solvent (A)]. From the faster migrating zone, compound **2c** was isolated, the lower migrating zone afforded **2a**. Both compounds were crystallised from MeOH to give **2a** (273 mg, 41%) and **2c** (153 mg, 21%) as colorless needles.

Method 2: To hot pyridine (90°C) CuCN (2 g, 22.3 mmol) and **2b**[30] (300 mg, 0.80 mmol) were added. The solution was heated at 110°C for 10 h. The mixture was cooled to r.t. and diluted with hot MeOH. After filtration and evaporation the residue was purified by FC [column 12 x 3 cm, solvent (A)]. Recrystallisation from MeOH furnished **2c** as colorless crystals (114 mg, 52%).

Compound **2a**: Mp 214-215°C (Lit.[21] 216°C). R_f 0.22 (A). ^{1}H-NMR (DMSO-d_6) δ: 2.13 (m, 1 H, H_α-2'), 2.39 (m, 1 H, H_β-2'), 3.53 (m, 2 H, $H_{\alpha,\beta}$-5'), 3.80 (m, 1 H, H-4'), 4.33 (m, 1 H, H-3'), 5.15 (t, J = 5.6 Hz, 1 H, OH-5'), 5.24 (d, J = 3.9 Hz, 1 H, OH-3'), 6.47 (dd, J = 8.0 and 6.1 Hz, 1 H, H-1'), 6.56 (d, J = 3.5 Hz, 1 H, H-5), 7.02 (s, 2 H, NH_2), 7.33 (d, J = 3.5 Hz, 1 H, H-6), 8.03 (s, 1 H, H-2). Anal. Calcd for $C_{11}H_{14}N_4O_3$ (250.3): C 52.79, H 5.64, N 22.39; Found C 52.61, H 5.61, N 22.35.

Compound **2c**: Mp 206-207°C (Lit.[8] 208-209°C). R_f 0.42 (A). ^{1}H-NMR (DMSO-d_6) δ: 2.26 (m, 1 H, H_α-2'), 2.38 (m, 1 H, H_β-2'), 3.53 (m, 2 H, $H_{\alpha,\beta}$-5'), 3.84 (m, 1 H, H-4'), 4.35 (m, 1 H, H-3'), 5.05 (t, J = 5.4 Hz, 1 H, 5'-OH), 5.30 (d, J = 4.0 Hz, 1 H, 3'-OH), 6.48 ('t', J = 6.7 Hz, 1 H, H-1'), 6.86 (br s, 2 H, NH_2), 8.21 (s, 1 H, H-2), 8.39 (s, 1 H,

H-6). Anal. Calcd for $C_{12}H_{13}N_5O_3$ (275.3): C 52.36, H 4.76, N 25.44; Found C 52.26, H 4.89, N 25.30.

4-Amino-5-carboxamido-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine (2'-Deoxysangivamycin) (2d)[8]. Compound **2c** (100 mg, 0.36 mmol) was dissolved in MeOH/0.1 N NaOH (20 mL, 1:1) and heated at 70°C for 5 h. The solution was evaporated and the residue applied to a silica gel column [10 x 2 cm, solvent (A)]. Crystallisation from MeOH afforded **2d** (65 mg, 61%) as colorless crystals. R_f 0.23 (A). λ_{max}(MeOH)/nm 231 (ε/dm^3 mol^{-1} cm^{-1} 10500) and 280 (15200) [Lit.[8] λ_{max}(MeOH)/ nm 231 (ε/dm^3 mol^{-1} cm^{-1} 10200) and 279 (14800)]. ^{1}H-NMR (DMSO-d_6) δ: 2.26 (m, 1 H, H_α-2'), 2.40 (m, 1 H, H_β-2'), 3.55 (m, 2 H, $H_{\alpha,\beta}$-5'), 3.84 (m, 1 H, H-4'), 4.37 (m, 1 H, H-3'), 4.95 (t, J = 4.7 Hz, 1 H, OH-5'), 5.30 (d, J = 3.8 Hz, 1 H, OH-3'), 6.51 ('t', J = 6.8 Hz, 1 H, H-1'), 7.33 (br s, 2 H, NH_2), 7.90 (br s, 2 H, $CONH_2$), 8.08 (s, 1 H, H-2), 8.13 (s, 1 H, H-6). Anal. Calcd for $C_{12}H_{15}N_5O_4$ (293.3): C 49.14, H 5.15, N 23.88; Found C 49.05, H 5.26, N 23.81.

5-Cyano-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-{[(dimethylamino)-methyl-idene]amino}-7*H*-pyrrolo[2,3-*d*]pyrimidine (4). A solution of 2'-deoxytoyoca-mycin (**2c**) (130 mg, 0.47 mmol) and N,N-dimethylformamide dimethylacetal (1.0 g, 8.4 mmol) in MeOH (10 mL) was stirred for 2 h at 40°C. After evaporation, the residue was applied to FC [column 12 x 3 cm, solvent (A)]. Compound **4** was isolated as colorless foam (119 mg, 76%). R_f 0.40 (A). λ_{max}(MeOH)/nm 230 (ε/dm^3 mol^{-1} cm^{-1} 14000) and 320 (22900). ^{1}H-NMR (DMSO-d_6) δ: 2.29 (m, 1 H, H_α-2'), 2.51 (m, 1 H, H_β-2', super-imposed by DMSO), 3.18 and 3.21 (2 s, 6 H, Me_2N), 3.46 (m, 2 H, $H_{\alpha,\beta}$-5'), 3.85 (m, 1 H, H-4'), 4.36 (m, 1 H, H-3'), 5.08 (t, J = 5.5 Hz, 1 H, OH-5'), 5.34 (d, 1 H, J = 4.1 Hz, OH-3'), 6.53 ('t', J = 6.3 Hz, 1 H, H-1'), 8.43 (s, 1 H, H-2), 8.45 (s, 1 H, H-6), 8.91 (s, 1 H, N=CH). FAB-MS: 331.2 $[M+H]^+$; $C_{15}H_{18}N_6O_3$ requires $[M^+]$ 330.3.

5-Cyano-7-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-*erythro*-pento-furanosyl]-4-{[(dimethylamino)methylidene]amino}-7*H*-pyrrolo[2,3-*d*]pyrimidine (5). To a solution of compound **4** (100 mg, 0.30 mmol) in dry pyridine (0.5 mL) 4,4'-di-methoxytriphenylmethyl chloride (130 mg, 0.38 mmol) was added. After stirring for 1 h at 50°C the mixture was poured into an ice-cold 3% aq. $NaHCO_3$ soln. (10 mL) and

extracted with CH_2Cl_2 (2 x 100 mL). The combined org. layers were dried over Na_2SO_4, filtered and evaporated. The residue was applied to FC [column 15 x 3 cm, solvent (A)]. Compound **5** was isolated as colorless foam (136 mg, 71%). R_f 0.61 (A). λ_{max}(MeOH)/ nm 232 (ε/dm^3 mol^{-1} cm^{-1} 36400), 274 (9000) and 320 (27100). ^{1}H-NMR (DMSO-d_6) δ: 2.38 (m, 1 H, 2'-H$_\alpha$), 2.70 (m, 1 H, 2'-H$_\beta$), 3.16 (m, 2 H, 5'-H$_{\alpha,\beta}$), 3.17 and 3.21 (2 s, 6 H, Me_2N), 3.72 (s, 6 H, 2 MeO), 3.95 (m, 1 H, 4'-H), 4.45 (m, 1 H, 3'-H), 5.39 (d, J = 4.7 Hz, 1 H, 3'-OH), 6.55 ('t', J = 6.2 Hz, 1 H, 1'-H), 6.82 (m, 4 H, ArH), 7.18-7.34 (m, 9 H, ArH), 8.33 (s, 1 H, 6-H), 8.43 (s, 1 H, 2-H), 8.90 (s, 1 H, N=CH). FAB-MS: 633.4 [M+H]$^+$; $C_{36}H_{36}N_6O_5$ requires [M$^+$] 632.7.

5-Cyano-1-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4-{[(dimethylamino)methylidene]amino}-7*H*-pyrrolo[2,3-*d*]pyrimidine-3'-[(2-cyanoethyl)-N,N-diisopropyl-phosphoramidite] (3). To a stirred solution of the dry nucleoside **5** (120 mg, 0.19 mmol) and anh. N,N-diisopropylethylamine (73 mg, 0.57 mmol) in dry THF (1 mL) chloro-(2-cyano-ethoxy)-N,N-diisopropylaminophosphine (55 mg, 0.23 mmol) was added under an Ar atmosphere. The stirring was continued for 30 min and the solution was filtered. The filtrate was diluted with ethyl acetate (50 mL) and extracted (twice) with an ice-cold aq. 3% $NaHCO_3$ solution (2 x 10 mL) and H_2O (10 mL). The org. layer was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was applied to FC [column 12 x 2 cm, solvent (B)]. Compound **3** was isolated as a colorless foam (109 mg, 69%). R_f 0.4 and 0.5 (B). ^{31}P-NMR [101 MHz; $CDCl_3$] δ: 149.9 and 150.0.

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REFERENCES

1. Suhadolnik, R. J. *Nucleoside Antibiotics*; Wiley: New York, **1970**.
2. Tolman, R. L.; Robins, R. K.; Townsend, L. B. *J. Am. Chem. Soc.*, **1969** *91*, 2102-2108.
3. Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Baird-Lambert, J. A.; Jamieson, D. D. *Aust. J. Chem.*, **1983** *36*, 165-170.
4. Revankar, G. R.; Robins, R. K. *Nucleosides Nucleotides*, **1989** *8*, 709-724.

5. Cottam, B. H.; Wasson, D. B.; Shih, H. C.; Raychaudhuri, A.; Di Pasquale, G.; Carson, D. A. *J. Med. Chem.*, **1993** *36*, 3424-3430.
6. Cook, A. F.; Holman, M. J. *Nucleosides Nucleotides*, **1984** *3*, 401-411.
7. Seela, F.; Zulauf, M.; Chen, S.-F. unpublished results.
8. Robins, M. J.; Wilson, J. S.; Hanske, F. *J. Am. Chem. Soc.*, **1983** *105*, 4059-4065.
9. Maruyama, T.; Wotring, L. L.; Townsend, L. B. *J. Med. Chem.*, **1983** *26*, 25-29.
10. Ramasamy, K.; Robins, R. K.; Revankar, G. R. *Tetrahedron*, **1986** *42*, 5869-5878.
11. Kazimierczuk, Z.; Revankar, G. R.; Robins, R. K. *Nucleic Acids Res.*, **1984** *12*, 1179-1192.
12. Robins, M. J.; Muhs, W. H. *J. Chem. Soc., Chem. Commun.*, **1976**, 269.
13. Watanabe, S.; Ueda, T. *Nucleosides Nucleotides*, **1982** *1*, 191-203.
14. Watanabe, S.; Ueda, T. *Nucleosides Nucleotides*, **1983** *2*, 113-125.
15. Cottam, H. B.; Kazimierczuk, Z.; Geary, S.; McKernan, P. A.; Revankar, G. R.; Robins, R. K. *J. Med. Chem.*, **1985** *28*, 1461-1467.
16. Cheng, C. S.; Hoops, G. C.; Earl, R. A.; Towensend, L. B. *Nucleosides Nucleotides*, **1997** *16*, 347-364.
17. Seela, F.; Kehne, A. *Tetrahedron*, **1985** *41*, 5387-5392.
18. Seela, F.; Thomas, H. *Helv. Chim. Acta*, **1994** *77*, 897-903.
19. Ramzaeva, N.; Seela, F. *Helv. Chim. Acta*, **1995** *78*, 1083-1090.
20. Ramzaeva, N.; Becher, G.; Seela, F. *Synthesis*, **1998**, 1327-1330.
21. Seela, F.; Kehne, A. *Liebigs Ann.*, **1983**, 876-884.
22. Kazimierczuk, Z.; Cottam, H.; Revankar, G. R.; Robins, R. K. *J. Am. Chem.Soc.*, **1984** *106*, 6379-6382.
23. Seela, F.; Thomas, H. *Helv. Chim. Acta*, **1995** *78*, 94-108.
24. Seela, F.; Zulauf, M. *Chem. Eur. J.*, **1998** *4*, 1781-1790.
25. Ramzaeva, N.; Mittelbach, C.; Seela, F. *Helv. Chim. Acta*, **1997** *80*, 1809-1822.
26. Ramzaeva, N.; Seela, F. *Helv. Chim. Acta*, **1996** *79*, 1549-1558.
27. Balow, G.; Brugger, J.; Lesnik, E. A.; Acevedo, O. L. *Nucleosides Nucleotides*, **1997** *16*, 941-944.
28. Buhr, C. A.; Wagner, R. W.; Grant, D.; Froehler, B. C. *Nucleic Acids Res.*, **1996** *24*, 2974-2980.
29. Balow, G.; Mohan, V.; Lesnik, E. A.; Johnston, J. F.; Monia, B. P.; Acevedo, O. L. *Nucleic Acids Res.*, **1998** *26*, 3350-3357.
30. Seela, F.; Zulauf, M. *Synthesis*, **1996**, 726-730.
31. Westhover, J. D.; Revankar, G. R.; Robins, R. K.; Madson, R. D.; Ogden, J. R.; North, J. A.; Mancuso, R. W.; Rousseau, R. J.; Stephen, E. L. *J. Med. Chem.*, **1981** *24*, 941-946.
32. Applied Biosystems, Oligonucleotide Purification Cartridges.
33. Seela, F.; Kehne, A. *Biochemistry*, **1987** *26*, 2232-2238.
34. Haginoya, N.; Ono, A.; Nomura, Y.; Ueno, Y.; Matsuda, A. *Bioconjugate Chem.*, **1997** *8*, 271-280.
35. Seela, F.; Lampe, S.; *Helv. Chim. Acta*, **1991** *74*, 1790-1800.
36. McDowell, J. A.; Turner, D. H. *Biochemistry*, **1996** *35*, 14077-14089.

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