Nucleic Acids Research, 1995, Vol. 23, No. 21 **4363–4370**

An acyclic 5-nitroindazole nucleoside analogue as ambiguous nucleoside

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Received August 7, 1995; Accepted September 15, 1995

ABSTRACT

Acyclic nucleoside analogues with carboxamido- or nitro-substituted heterocyclic bases have been evaluated for their possible use as universal bases in oligodeoxynucleotides. The acyclic moiety endows the constructs with enough flexibility to allow good base stacking. The 5-nitroindazole analogue afforded the most stable duplexes among the acyclic derivatives with the least spread in T_m versus the four natural bases. In spite of the acyclic moiety, stabilities are comparable with those of duplexes incorporating the recently described 5-nitroindole nucleoside analogue, but considerably exceed those for the 3-nitropyrrole analogue.

INTRODUCTION

Efforts to isolate the gene coding for a newly identified protein are often based on the use of synthetic hybridisation probes based on a partially known amino acid sequence. However, the redundancy of the genetic code mostly leads to a multitude of possible DNA sequences. Therefore, many research efforts have been undertaken trying to devise a truly ambiguous base or 'lure' nucleoside analogue, capable of base-pairing equally well with all four natural bases. In the past 2′-deoxyinosine (dI) has been advocated as the solution for such degenerate positions (1), but dI pairs much more strongly to dC compared with the other bases, and therefore cannot be considered as the perfect ambiguous or 'universal' base.

More recently, 1-(2′-deoxy-β-D-ribofuranosyl)-3-nitropyrrole (**2**) was proposed as a valuable universal nucleoside (2) and is commercialized for these purposes. However, Loakes already reported the inefficiency of this nucleoside in fulfilling these criteria, and highly reduced T_m values were noted, when incorporating this analogue once or at several positions (3). An alternative analogue which did look very promising however, was the 5-nitroindole derivative (**3**) which even upon multiple incorporation into an oligonucleotide hybridized quite well with its complement (reduction in T_m of 22°C for incorporation of six analogues into a 17mer) (3).

Another approach which could be followed is to combine base modifications with sugar alterations of the nucleoside. Because of the conformational flexibility of acyclic nucleosides, one could expect that the base-pairing properties of such analogues could be different from the traditional Watson–Crick base-pairing. This flexibility could allow the base of the analogue to position itself and allow perfect base-pairing with all four natural base moieties. Our previous findings already indicated the acyclic hypoxanthine analogue **1** to be a good candidate as a universal base: only a small spread in T_m versus the complementary sequences was noticed when incorporated at a degenerate position (4). However, the slight destabilisation of the duplex (a drop in T_m of 5°C compared with incorporation of the normal Watson–Crick base pairs) led us to look for a base analogue which would yield analogous ambiguous pairing with all four natural bases as the counterpart, but without a drop in melting temperature. This would allow for multiple incorporation of the acyclic analogue in probes as well as in primers without the need for lowering the annealing temperature too much to allow the oligonucleotide analogue to pair with its target. A preliminary account of these results was given recently (5).

Five new acyclic nucleoside analogues with unnatural base moieties were prepared. These include 5-amino-4-imidazole-carboxamide (**6**), 4,5-imidazoledicarboxamide (**10**), 3,5-pyrazoledicarboxamide (**13**), 4-nitroimidazole (**15**) and 5-nitroindazole (**17**). Because of synthetic considerations, these compounds have a 4(*R*)-methoxy-3(*S*),5-dihydroxypentyl side chain instead of the 3(*S*),5-dihydroxypentyl group as in the already described **1**. The stereochemistry of both chiral centers is established by the choice of the starting material. The compounds **10**, **15** and **17** were converted to their dimethoxytritylated phosphoramidites and were successfully incorporated into oligonucleotides after which the hybridizing properties of these oligonucleotides were evaluated. These results are compared with those of the reference compounds **1–3**. The incorporation of **6** and **13** was impeded by troublesome synthesis of the respective phosphoramidites.

RESULTS

1-*O*-Methanesulfonyl-3,5-di-*O*-benzyl-4-*O*-methyl-2-deoxy-D-ribitol (**4**) (6) was subjected to nucleophilic substitution with the different bases. Coupling with 4-amino-5-imidazolecarboxamide was accomplished by prior deprotonation with NaH in DMF, after which the mixture was heated at 50°C to yield 75% of the protected analogue **5**. Debenzylation by transfer hydrogenation with cyclohexene (6) afforded 70% of **6** as an oil. Following

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5′-*O*-dimethoxytritylation (50% yield), phosphitylation of **21** was found to take place primarily at the base moiety yielding mono- and diphosphitylated analogues. The monophosphitylated species only contained base modified material and **26** was not detected. Protection of the base moiety thus seems mandatory to incorporate this AICAR analogue in an oligonucleotide using regular phosphoramidite chemistry. Previously, the 2′-deoxy analogue of AICAR was rather unexpectedly incorporated into oligonucleotides in moderate yield, using a protected derivative of 2-aza-2′-deoxyinosine (7). However, all protecting strategies we tried (benzoyl, *p*-nitrophenylethyloxycarbonyl, dimethylformamidine) failed, in the sense that the protecting groups chosen could not be introduced properly, and incorporation of this analogue was not pursued any further. Others likewise have had difficulties in trying to incorporate the deoxyribosyl analogue of AICAR into oligonucleotides (8).

Analogous coupling of dicyanoimidazole with **4** required a temperature of 90°C to yield 45% of the protected analogue **7**. Conversion to the bisamide **9** was accomplished with hydrogen peroxide under phase-transfer catalysed conditions in 31% yield (9), followed by transfer hydrogenation to yield 75% of **10**. This route was preferred over the alternative one which first debenzylates **7–8** in 73% with the aid of boron trichloride (10) leaving the nitrile moieties untouched and then converts the intermediate to the bisamide **10** with aqueous hydrogen peroxide.

Pyrazole-3,5-dicarboxylic acid was converted to the diethyl ester with ethylchloroformate in 90% yield. Coupling to **4** in the presence of potassium carbonate for 16 h at 60°C afforded 73% of the protected analogue **11**. Treatment with anhydrous ammonia in ethanol for 48 h at 100°C in a pressurized bottle, followed by debenzylation afforded 32% of **13**. While the 4,5-dicarboxamidoimidazole derivative **10** was converted to its phosphoramidite derivative **23** and incorporated into oligonucleotides without noticable difficulties, the pyrazole analogue, like the amino-imidazolecarboxamide, could not be functionalized to **27** to allow incorporation into oligomers. Again the base was phosphitylated in preference to the hydroxyl moiety.

4-Nitroimidazole and 5-nitroindazole were condensed with **4** in a moderate 37 and 32% yield, respectively, of the N^1-glycosylated products, with the aid of NaH. Deduction of the site of attachment of the acyclic moiety was based on NMR and UV data of analogous compounds (refs 11–12 and 13, respectively). Debenzylation to **15** and **17** was accomplished with boron trichloride 1 M in methylene chloride at –78°C (75% yield) leaving the nitro substituent untouched (10).

The analogues **10**, **15** and **17** were 5′-*O*-dimethoxytritylated and phosphitylated following standard procedures and the obtained phosphoramidites were used for incorporation of these analogues into oligonucleotides, but a 0.13 M solution was used as compared with the usual 0.1 M solution of phosphoramidites. This gave good coupling efficiency as reflected by the HPLC ion exchange profile (not shown). The target sequence was a 13mer with the modification positioned in the middle. Melting temperatures with the four complementary sequences were determined (Table 1).

Whereas the acyclic inosine analogue **1** gave a spread of 4.8°C, the 5-nitroindazole **17** and the 4,5-imidazoledicarboxamide **10** gave only 2.2 and 3.7°C, respectively. This narrow range in T_m for oligonucleotides containing **17**, warrants this analogue to be pursued further as a universal nucleoside at ambiguous sites in DNA primers (data not cited). Melting temperatures, however, are lower when compared with those containing a natural Watson–Crick base pair, but acceptable when compared with those incorporating 2′-deoxyinosine. Compared with the recently reported analogue **2** containing a 3-nitropyrrole substituent (2), our acyclic analogue **17** and the nitroindole nucleoside **3** behave much more effective. The analogue **2** is reported to be a universal nucleoside, and oligonucleotides containing **2** at several sites were reported to be useful as primers for sequencing and for PCR, while melting temperatures of these oligonucleotides in comparison with their control sequences were not given. For the sequences studied here, however, incorporation of **2** is detrimental to duplex stability. Destabilisation is of the same order as for incorporation of 1,2-dideoxy-1-phenyl-β-D-ribofuranose or the abasic counterpart 1,2-dideoxy-D-ribofuranose (14), and duplexes are less stable than those containing an A–C mismatch for the sequence reported here (4).

Table 1. T_m (°C) of the duplexes 5′-CACCGXCGGCGCC-3′
3′-GTGGCYGCCGCGG-5′
as determined under the following conditions: 0.1 M NaCl, 0.02 M potassium phosphate pH 7.5, 0.1 mM EDTA, concentration = 4 μM for each oligonucleotide, with T_m determined as the first derivative of the melting curve. The spread in T_m is indicated in the last column

Y / X	A	T	G	C	ΔT_m (°C)
10	60.8	61.9	61.3	58.2	3.7
15	57.3	55.4	61.6	53.6	8.0
17	63.4	62.9	62.3	61.2	2.2
1	65.1	62.6	67.4	65.6	4.8
2	60.4	57.7	59.7	55.3	5.1
3	62.8	61.9	61.8	62.0	1.0
dI	70.2	64.6	65.1	68.5	5.6
complementary base	70.3 (T)	70.0 (A)	73.5 (C)	72.8 (G)	–

A double and triple incorporation was done likewise with the same test sequence as can be seen in Table 2. Again, **3** and **17** are clearly superior to the nitropyrrole analogue **2**, but melting temperatures drop markedly with the third incorporation of an analogue in this test sequence, which is in contrast with the up to six incorporations reported for the nitroindole analogue **3** without noticing unsurmountable destabilization (3).

Analogously to Habener *et al.* (15) and Loakes and Brown (3), some thermodynamic calculations were carried out using the 'all or none' two state model developed by Gralla and Crothers (16). Hereto, absorbance values were sampled at a rate of 2 points/min with an increase in temperature of 0.2°C/min. The derivative at each point on the curve was determined by fitting a regression line to the point in a dynamically specified window containing 40 points (4°C). The transition enthalpy can be calculated from the equation

$$\Delta H = -18.28/(1/T_{1/2} - 1/T_{3/4})$$

Table 2. Melting temperature (T_m) and thermodynamic data for tridecadeoxynucleotide duplexes at 4 μm after a single, double or triple incorporation of the respective nucleoside analogue

Duplex	T_m	ΔH°	ΔS°	ΔG°$_{310}$
	°C	KJ/mol	KJ/mol·K	KJ/mol
3′-G T G G C T G C C G C G G-5′				
5′-C A C C G A C G G C G C C-3′	69.6	400.2	1.06	71.0
- - - - - 2 - - - - - - - -	57.7	311.5	0.84	52.4
- - - - - 2 2 - - - - - - -	47.3	181.3	0.46	38.7
- - - - - 2 2 - 2 - - - - -	15.2 (54)	–	–	–
- - - - - 3 - - - - - - - -	61.9	321.8	0.86	56.8
- - - - - 3 3 - - - - - - -	55.9	238.9	0.62	46.7
- - - - - 3 3 - 3 - - - - -	29.7	121.2	0.29	30.0
- - - - - 17 - - - - - - - -	62.5	343.7	0.92	59.2
- - - - - 17 17 - - - - - - -	57.3	282.7	0.75	50.2
- - - - - 17 17 - 17 - - - - -	41.7	93.6	0.19	34.3

as discussed in these references. The duplexes with a single mismatch versus T were included in this study for comparison purposes. For the nitropyrrole, the third mismatch destroys almost completely the duplex, but a second smaller transition (better visible on the curve for the derivative) was visible at 54°C. However, the free energy at 37°C for the duplexes with a single or double mismatch, clearly show the unfavorable effect of incorporation of **2**. While the enthalpy after three incorporations of the acyclic **17** drops tremendously, this is compensated by the lower loss in entropy compared with **3**, which makes incorporation of **17** still advantagous compared with **3** for this test sequence.

The stability of all new analogues has been verified in two other test sequences, used before by Kawase *et al.* (17) when evaluating deoxyinosine as a universal base. Table 3 uses a test sequence with the analogue in the middle of an A–T stretch, which might give a different tertiary structure (and different results) as compared with a random mixed sequence. All analogues cause about the same destabilisation, with the acyclic deoxyinosine **1** as second best after deoxyinosine itself, but with less spreading in T_m compared with the latter.

Table 3. T_m (°C) of the duplexes 5′-GGAAAA**X**AAAAGG-3′
3′-CCTTTT**Y**TTTTCC-5′
as determined under the usual conditions at 4μM, with **Y** being either dA, T, dG or dC and **X** being one of the analogues to be evaluated as complementary base to **Y**. The last column gives the spread in T_m versus all four natural bases

Y / **X**	A	T	G	C	ΔT_m (°C)
10	28.4	31.7	28.9	27.7	4.0
15	24.3	25.0	28.5	21.5	7.0
17	29.5	32.6	28.6	28.4	4.2
1	32.0	29.4	33.6	34.8	5.4
2	28.9	27.0	27.3	24.9	4.0
3	28.8	34.0	33.9	30.4	5.2
dI	36.9	31.8	32.0	40.7	8.9

Analyzing the third test sequence (Table 4) containing two mismatches in the middle next to each other, the nitroindole **3** emerges as the best hybridizing analogue with the least spread of T_ms, slightly better than the acyclic nitroindazole **17**. Both analogues are clearly superior to dI with respect for this sequence, where, unexpectedly, the duplex is strongly destabilized with a double dI–dG mismatch.

Table 4. T_m of the duplexes 5′-GGG AA **XY** TT CCC-3′ as determined at 8 μM (self-complementary sequences) and under the usual conditions

Y / **X**	A	T	G	C	ΔT_m (°C)
10	44	47	43	45	4
15	42.5	46	39	45	7
17	46	49	45	47	4
1	43.5	47	37	43.5	10
3	47	50	46.5	48	3.5
dI	45	46	18	45	

CONCLUSION

Five new acyclic nucleoside analogues were prepared for incorporation into oligomers, of which two could not be incorporated due to phosphitylation of the heterocyclic base, prior to reaction of the secondary alcohol. T_ms of the different analogues versus all four natural bases were determined for three different test sequences known from the literature, and spread of the T_m values was compared with that for other 'universal' nucleoside analogues **1–3** and dI. We can conclude that selection of the best analogue is somewhat dependent on the sequence and that the recently described 5-nitroindole nucleoside analogue **3**, and especially the new acyclic nitroindazole analogue **17**, are valuable candidates for use as universal nucleoside analogues. While T_ms in general still are somewhat lower as compared with the natural complementary bases, spread in T_ms are much smaller, which is one of the prime criterion for a universal analogue. The use of these analogues in

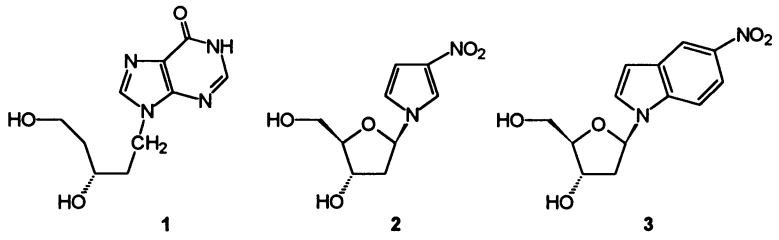

Figure 1.

hybridisation probes and in PCR experiments, is the subject for further work.

MATERIALS AND METHODS

All general methods including synthesis and purification of oligonucleotides and determination of melting temperatures were as recently described (18).

Synthesis

1-(3,5-Di-O-benzyl-4-O-methyl-2-deoxy-D-ribityl)-5-amino-4-carboxamidoimidazole ***5***. An amount of 1.34 g (8.25 mmol) of 4-amino-5-imidazolecarboxamide was treated with 725 mg (18.1 mmol) of a 60% oil dispersion of NaH for 30 min at room temperature in 50 ml DMF, after which the suspension was gently heated to 50°C. The methanesulfonyl derivative **4** (2.27 g of an oil, 5.5 mmol) was added as a 10 ml DMF solution and the mixture was left overnight at 50°C. The reaction was quenched with water, evaporated and adsorbed on silica gel. Gradient elution (CH_2Cl_2-MeOH 0–5%) afforded 1.81 g (4.12 mmol, 75%) of the title product as an oil.

UV (MeOH) λ_{max} 240 (sh), 268 nm. ^{1}H NMR ($CDCl_3$): δ (p.p.m.) 1.80–2.14 (m, 2H, H2′), 3.42 (s, 3H, OCH_3), 3.53 (t, 2H, H5′), 3.60–3.90 (m, 4H, H1′, H3′, H4′), 4.35–4.60 (m, 4H, CH_2Ar), 5.02 (br, 2H, NH_2), 6.76 (s, 1H, H2), 7.30 (s, 10H, Ar-H).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 30.0 (C2′), 39.4 (C1′), 58.0 (OCH_3), 68.1 (C5′), 71.5 and 73.1 (2× CH_2Ar), 75.1 (C3′), 80.6 (C4′), 113.5 (C4), 127–128.2 (C-Ar), 129.3 (C2), 137.4 (C_x), 142.3 (C5), 166.8 (CO). MS (EI) m/z 438 (M^+, 32), 330 (M^+-BnOH, 20), 91 ($C_7H_7^+$, 100).

1-(4-O-methyl-2-deoxy-D-ribityl)-5-amino-4-carboxamidoimidazole ***6***. The oil of the previous preparation (2.92 g, 6.66 mmol) was dissolved in 200 ml of a mixture EtOH-cyclohexene (3:1) and N_2 was bubbled through for 15 min. The catalyst $Pd(OH)_2$ 20% on carbon (1.5 g) was added and the mixture was refluxed overnight, filtered, and the residue washed thoroughly with hot ethanol. Evaporation afforded 1.41 g (5.46 mmol, 82%) as an oil. All crystallization attempts were unsuccessful.

UV (MeOH) λ_{max} 240 (sh), 267 nm. ^{1}H NMR (DMSO): δ (p.p.m.) 1.33–2.23 (m, 2H, H2′), 3.33 (s, OCH_3) and 2.95–3.56 (m, H3′, H4′, H5′) (7H), 3.86 (t, J = 7Hz, 2H, H1′), 4.47 (br, OH), 4.83 (br, OH), 5.70 (s, NH_2), 6.63 (s, NH_2), 7.06 (s, 1H, H2).

^{13}C NMR (DMSO): δ (p.p.m.) 32.5 (C2′), 38.6 (C1′), 57.8 (OCH_3), 60.0 (C5′), 66.9 (C3′), 85.3 (C4′), 112.6 (C4), 130.0 (C2), 143.0 (C5), 166.3 (CO). MS (CI, iC_4H_{10}) m/z 259 (MH^+, 100). HRMS: MH^+ calcd. for $C_{10}H_{19}N_4O_4$: 259.1406; found: 259.1430

1-(3,5-Di-O-benzyl-4-O-methyl-2-deoxy-D-ribityl)-4,5-dicyanoimidazole ***7***. Dicyanoimidazole (3.36 g, 28.5 mmol) was treated with 1.14 g (28.5 mmol) of a 60% oil dispersion of NaH in 200 ml for 1 h at 60°C, after which 7.77 g (19 mmol) of the methanesulfonate **4** was added. The mixture was heated at 90°C for 4 h, concentrated and partitioned between CH_2Cl_2 and aqueous $NaHCO_3$. Column purification (CH_2Cl_2) afforded 3.68 g (8.55 mmol, 45%) of the title product.

UV (MeOH) λ_{max} 248 nm. ^{1}H NMR ($CDCl_3$): δ (p.p.m.) 1.87–2.17 (m, 2H, H2′), 3.37 (s, 3H, OCH_3), 3.37–3.65 (m, 4H, H3′, H4′, H5′), 4.04 (t, J = 7.7Hz, 2H, H1′), 4.23–4.62 (m, 4H, CH_2Ar), 7.16 (s, 1H, H2), 7.25 (s, 10H, Ar-H).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 30.6 (C2′), 44.5 (C1′), 58.3 (OCH_3), 67.8 (C5′), 71.7 and 73.4 (2× CH_2Ar), 74.9 (C3′), 80.1 (C4′), 107.6 (C5), 111.3 and 111.8 (2× CN), 122.7 (C4), 127–129 (C-Ar), 137.4 (C_x), 141.0 (C2). MS (CI, iC_4H_{10}) m/z 431 (MH^+, 100), 339 (M-C_7H_7, 20), 119 (BH_2^+, 40), 107 ($C_7H_7O^+$, 85), 91 ($C_7H_7^+$, 80). HRMS: MH^+ calcd. for $C_{25}H_{27}N_4O_3$: 431.2083; found: 431.2069.

1-(4-O-methyl-2-deoxy-D-ribityl)-4,5-dicyanoimidazole ***8***. An amount of 330 mg (0.77 mmol) of **7** was suspended in 20 ml CH_2Cl_2, cooled to –78°C and 5 ml of a 1 M BCl_3 solution in CH_2Cl_2 was added. After 1 h TLC indicated the reaction to be complete and 50 ml of a CH_2Cl_2-MeOH mixture (1:1) was added to quench the reaction. The mixture was stirred for 30 min at room temperature, evaporated and coevaporated with water. Purification on preparative TLC plates afforded 141 mg (0.56 mmol, 73%) of the title product as a white solid.

UV (MeOH) λ_{max} 248 nm. ^{1}H NMR (DMSO): δ (p.p.m.) 1.65–2.17 (m, 2H, H2′), 3.48 (s, OCH_3) and 3.25–3.60 (m, H3′, H4′, H5′) (7H), 4.30 (t, J = 7.2Hz, 2H, H1′), 4.48 (t, J = 5Hz, 5′-OH), 4.90 (d, J=5.5Hz, 3′-OH), 8.33 (s, 1H, H2).

^{13}C NMR (DMSO): δ (p.p.m.) 32.9 (C2′), 45.6 (C1′), 58.2 (OCH_3), 60.5 (C5′), 67.2 (C3′), 85.1 (C4′), 109.1 (C5), 112.7 and 113.0 (2× CN), 121.5 (C4), 143.7 (C2). MS (CI, iC_4H_{10}) m/z 251 (MH^+, 100). HRMS: MH^+ calcd. for $C_{11}H_{15}N_4O_3$: 251.1144; found: 251.1142.

1-(3,5-Di-O-benzyl-4-O-methyl-2-deoxy-D-ribityl)-4,5-dicarboxamidoimidazole ***9***. An amount of 1.84 g (4.27 mmol) of **7** was dissolved in 3 ml CH_2Cl_2 and cooled on an ice bath. Sequentially, 4 ml of a 30% H_2O_2 solution, 440 mg (1.3 mmol) of tetrabutylammonium hydrogen sulfate and 3.2 ml of a 20% NaOH solution were added. The biphasic mixture was stirred for 1 h at room temperature, after which the mixture was partitioned between CH_2Cl_2 and saturated NaCl. Column purification on silica gel afforded 636 mg (1.37 mmol, 32%) as a foam.

^{1}H NMR (DMSO): δ (p.p.m.) 1.87–2.25 (m, 2H, H2′), 3.37 (s, OCH_3), 3.25–3.65 (m, H3′, H4′, H5′) (7H), 4.04 (t, J = 7.7Hz,

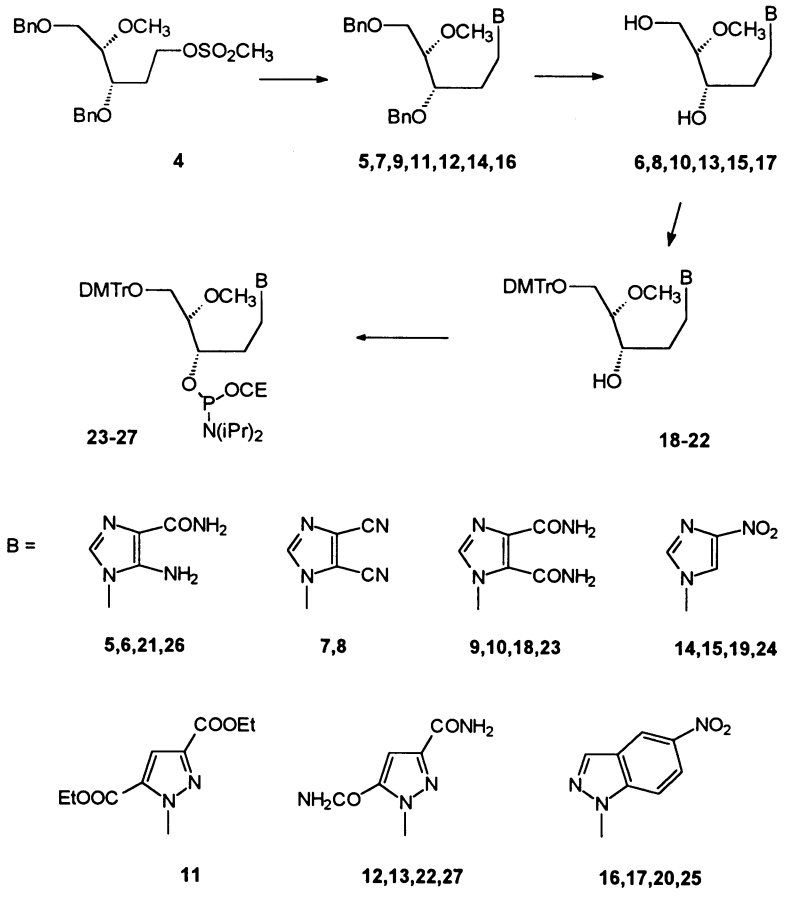

Figure 2.

2H, H1′), 4.50 (m, 4H, CH_2Ar), 7.31 (s, 10H, Ar-H), 7.58 (br, NH_2), 7.77 (s, 1H, H2), 7.94 (br, NH_2).

^{13}C NMR (DMSO): δ (p.p.m.) 31.8 (C2′), 44.6 (C1′), 58.0 (OCH_3), 69.2 (C5′), 70.9 and 72.4 (2× CH_2Ar), 76.3 (C3′), 80.6 (C4′), 126.4 and 135.3 (C4, C5), 127–129 (C-Ar), 138.3 and 138.5 (C_x), 139.8 (C2), 160.6 and 165.8 (2× CO).

1-(4-O-methyl-2-deoxy-D-ribityl)-4,5-dicarboxamidoimidazole ***10***. A: the dicyanoimidazole derivative **8** (78 mg, 0.312 mmol) was dissolved in 1 ml conc. NH_4OH and 0.5 ml of a 30% H_2O_2 solution was added. The mixture was stirred for 20 h at room temperature, concentrated, and coevaporated twice with dioxane. Purification on a preparative silica gel plate (CH_2Cl_2-MeOH 85:15) afforded 42 mg (0.15 mmol, 48%) of the title product **10**.

B: an amount of 1.14 g (2.44 mmol) of the benzylated derivative **9** was deprotected by transfer hydrogenation as described for the preparation of **7**. After filtration, the residue was adsorbed on silica gel, and column purification (CH_2Cl_2-MeOH 9:1) afforded 510 mg (1.78 mmol, 75%) of **10** as a white solid.

UV (MeOH) λ_{max} 205 (16300), 238 (min), 258 (7900) nm. ^1H NMR (DMSO): δ (p.p.m.) 1.55–1.80 (m, 1H, Ha2′), 1.82–2.05 (m, 1H, Hb2′), 2.97 (m, 1H, H3′), 3.27 (s, OCH_3) and 3.20–3.65 (m, H4′, H5′) (6H), 4.41 (m, 1H, Ha1′), 4.54 (m, 1H, Hb1′), 4.65 (br, OH), 4.90 (br, OH), 7.58, 7.79, 8.00 (exchangable NH), 7.87 (s, 1H, H2).

^{13}C NMR (DMSO): δ (p.p.m.) 34.5 (C2′), 44.9 (C1′), 57.8 (OCH_3), 60.0 (C5′), 67.0 (C3′), 85.4 (C4′), 126.5 and 135.4 (C4, C5), 140.4 (C2), 160.8 and 166.0 (2× CO). MS (CI, iC_4H_{10}) m/z 287 (MH^+, 100). HRMS: MH^+ calcd. for $C_{11}H_{19}N_4O_5$: 287.1355; found: 287.1346.

Diethyl 3,5-pyrazoledicarboxylate. 3,5-Pyrazoledicarboxylic acid monohydrate (3.5 g, 20 mmol) was dissolved in 100 ml anhydrous pyridine, 7.7 ml (80 mmol) ethylchloroformate was added and the mixture was stirred for 48 h. Concentration afforded an oil which was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$. Purification on silica gel (CH_2Cl_2 to CH_2Cl_2-MeOH 99:1) afforded 3.83 g (18.1 mmol, 90%) of the title product as a yellow oil.

^1H NMR ($CDCl_3$): δ (p.p.m.) 1.37 (t, J = 7.2Hz, 6H, CH_3), 3.93 (s, NH), 4.38 (q, J = 7Hz, 4H, CH_2), 7.32 (s, 1H, H4).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 13.8 (2× CH_3), 61.2 (2× CH_2), 111.0 (C4), 139.8 (br, C3, C5), 160.1 (2× CO). MS (CI, iC_4H_{10}) m/z 213 (MH^+, 100).

1-(3,5-Di-O-benzyl-4-O-methyl-2-deoxy-D-ribityl)-diethyl-3,5-pyrazoledicarboxylate ***11***. An amount of 1.29 g of the methanesulfonate derivative **4** was brought into reaction with 835 mg (3.94 mmol) diethyl pyrazoledicarboxylate in 50 ml DMF in the presence of 570 mg (4.1 mmol) potassium carbonate. The mixture was stirred at 60°C overnight, neutralized with acetic acid, concentrated and partitioned between CH_2Cl_2 and aqueous $NaHCO_3$. Purification on silica gel (CH_2Cl_2 to CH_2Cl_2-MeOH 99:1) afforded 1.27 g (2.42 mmol, 73%) of the title product as a yellow oil.

^1H NMR ($CDCl_3$): δ (p.p.m.) 1.20–1.45 (2t, 6H, 2× CH_3), 2.00–2.30 (m, 2H, H2′), 3.45 (s, OCH_3), 3.38–3.94 (m, H3′, H4′,

H5'), 4.20–4.90 (m, H1', 2× CH_2Ar, 2× $CH_3\underline{CH_2}O$), 7.27 (s, H4) and 7.30 (s, Ar-H) (11H).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 14.0, 14.2 (2× CH_3), 31.3 (C2'), 49.8 (C1'), 58.4 (OCH_3), 61.0, 61.2 (2× $CH_3\underline{CH_2}O$), 68.9 (C5'), 71.9 and 73.2 (2× CH_2Ar), 75.6 (C3'), 81.4 (C4'), 113.9 (C4), 127–128.8 (C-Ar), 138.0 (C_x,C5), 141.8 (C3), 158.8, 161.0 (2× CO). LSIMS (ThGly) m/z 525 (MH^+, 6), 417 (MH^+-BnOH, 5), 91 ($C_7H_7^+$, 100). HRMS: MH^+ calcd. for $C_{29}H_{37}N_2O_7$: 525.2601; found: 525.2601.

1-(3,5-Di-O-benzyl-4-O-methyl-2-deoxy-D-ribityl)-3,5-pyrazoledicarboxamide ***12***. An amount of 1.68 g (3.2 mmol) of the previous preparation was dissolved in 140 ml ethanol saturated with ammonia and reacted for 72 h in a Parr® at 100°C (140 psi). Evaporation and purification on silica gel (CH_2Cl_2-MeOH 98:2) afforded 687 mg (1.47 mmol, 46%) of the title product as a yellow oil.

^1H NMR ($CDCl_3$): δ (p.p.m.) 2.05 (m, 2H, H2'), 3.34 (s, OCH_3) and 3.30–3.40 (m, H3') (4H), 3.45–3.65 (m, 3H, H4', H5'), 4.45 (s), 4.55 (s) (2× CH_2Ar), 4.66 (t, 2H, H1'), 7.26 (s, H4) and 7.30 (s, Ar-H) (11H), 7.45 (s), 7.60 (s), 8.05 (s) (exchangable NH).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 31.3 (C2'), 48.6 (C1'), 58.0 (OCH_3), 69.1 (C5'), 71.3 and 72.4 (2× CH_2Ar), 76.3 (C3'), 80.7 (C4'), 108.6 (C4), 127–129 (C-Ar), 138.4 and 138.7 (C_x), 136.5 (C5), 144.9 (C3), 160.6, 162.7 (2× CO). LSIMS (ThGly) m/z 467 (MH^+, 8), 149 ($BnOCH_2CHO^+$, 100). HRMS: MH^+ calcd. for $C_{25}H_{31}N_4O_5$: 467.2294; found: 467.2278.

1-(4-O-methyl-2-deoxy-D-ribityl)-3,5-pyrazoledicarboxamide ***13***. Debenzylation of the acyclic analogue 12 (1.80 g, 3.86 mmol) was accomplished by transfer hydrogenation as described for the preparation of **7**. After filtration, the residue was adsorbed on silica gel, and column purification (CH_2Cl_2-MeOH 95:5–85:15) afforded 880 mg (3.07 mmol, 79%) of a white powder which crystallized from MeOH.

mp.: 155°C. UV (MeOH) λ_{max} 222 nm.

^1H NMR (DMSO): δ (p.p.m.) 1.70–1.90 (m, 1H, Ha2'), 1.92–2.12 (m, 1H, Hb2'), 3.01 (m, 1H, H3'), 3.34 (s, OCH_3) and 3.30–3.65 (m, H4', H5') (6H), 4.45–4.80 (m, H1', 3'-OH, 5'-OH), 7.25 (s, 1H, H4), 7.32 (s, 1H), 7.60 (d, 2H), 8.05 (s, 1H) (exchangable NH).

^{13}C NMR (DMSO): δ (p.p.m.) 34.0 (C2'), 49.0 (C1'), 57.7 (OCH_3), 60.0 (C5'), 67.3 (C3'), 85.3 (C4'), 108.5 (C4), 136.6 (C5), 144.8 (C3), 160.8, 162.8 (2× CO). LSIMS (ThGly) m/z 309 (M+Na^+, 100), 287 (MH^+, 14), 155 (BH_2^+, 10). HRMS: MH^+ calcd. for $C_{11}H_{19}N_4O_5$: 287.1355; found: 287.1362.

Anal. calcd. for $C_{11}H_{18}N_4O_5$: C, 46.15; H, 6.34; N, 19.57; found: C, 46.39; H, 6.35; N, 19.52.

1-(3,5-Di-O-benzyl-4-O-methyl-2-deoxy-D-ribityl)-4-nitroimidazole ***14***. 4-Nitroimidazole (1.7 g, 15 mmol) was treated with 600 mg (15 mmol) of a 60% oil dispersion of NaH in 50 ml of DMF for 1 h at 60°C, after which 4.1 g (10 mmol) of the methanesulfonate **4** dissolved in 20 ml DMF was added. The mixture was heated at 60°C for 24 h, concentrated and partitioned between CH_2Cl_2 and aqueous $NaHCO_3$. Column purification (CH_2Cl_2 to CH_2Cl_2-MeOH 99:1) afforded 2.12 g (5 mmol, 50%) of the title product as a slightly yellow oil.

UV (MeOH) λ_{max} 292 (ε = 6600) nm. ^1H NMR ($CDCl_3$): δ (p.p.m.) 2.06 (m, 2H, H2'), 3.38 (s, 3H, OCH_3), 3.55 (m, 4H, H3', H4', H5'), 4.15 (t, J = 7Hz, 2H, H1'), 4.50 (m, 4H, 2× CH_2Ar), 7.31 (s, 10H, Ar-H), 7.79 (d, J = 1.3Hz, 1H, H2), 8.37 (d, J = 1.3Hz, 1H, H5).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 30.9 (C2'), 44.6 (C1'), 58.0 (OCH_3), 69.0 (C5'), 71.0 and 72.4 (2× CH_2Ar), 75.9 (C3'), 80.3 (C4'), 121.4 (C5), 127–129 (C-Ar), 137.2 (C2), 138.3 (C_x-Ar), 147.0 (C4). LSIMS (ThGly) m/z 426 (MH^+, 100).

1-(4-O-methyl-2-deoxy-D-ribityl)-4-nitroimidazole ***15***. An amount of 4.25 g (9.9 mmol) of **14** was dissolved in 150 ml CH_2Cl_2 and cooled to –78°C. A pre-cooled 1 M BCl_3 solution in CH_2Cl_2 (45 ml) was added and the mixture was stirred for 1 h at –78°C. After addition of 200 ml of a CH_2Cl_2-MeOH mixture (1:1) to quench the reaction, stirring was continued for 30 min at room temperature, the mixture was evaporated, coevaporated with water and adsorbed on silica gel. Purification (CH_2Cl_2 to CH_2Cl_2-MeOH 95:5) afforded 1.82 g (7.42 mmol, 75%) of the title product as a slightly yellow oil.

UV (MeOH) λ_{max} 292 (ε = 6600) nm. ^1H NMR (DMSO): δ (p.p.m.) 1.65–1.90 (m, 1H, Ha2'), 1.93–2.15 (m, 1H, Hb2'), 2.98 (m, 1H, H3'), 3.36 (s, 3H, OCH_3), 3.55 (m, 3H, H4', H5'), 4.22 (t, J = 7Hz, 2H, H1'), 4.51 (t, J = 5.5Hz, 1 H, 5'-OH), 5.00 (d, J = 5.7Hz, 1H, 3'-OH), 7.87 (d, J = 1.3Hz, 1H, H2), 8.41 (d, J = 1.3Hz, 1H, H5).

^{13}C NMR (DMSO): δ (p.p.m.) 33.5 (C2'), 44.8 (C1'), 57.7 (OCH_3), 59.8 (C5'), 66.6 (C3'), 85.1 (C4'), 121.9 (C5), 137.7 (C2), 147.2 (C4). LSIMS (ThGly) m/z 246 (MH^+, 100). HRMS: MH^+ calcd. for $C_9H_{16}N_3O_5$: 246.1090; found: 246.1086.

1-(3,5-Di-O-benzyl-4-O-methyl-2-deoxy-D-ribityl)-5-nitroindazole ***16***. 5-Nitroindazole (2.45 g, 15 mmol) was treated with 600 mg (15 mmol) of a 60% oil dispersion of NaH in 50 ml DMF for 1 h at 60°C, after which 4.1 g (10 mmol) of the methanesulfonate **4** dissolved in 20 ml DMF was added. The mixture was heated at 60°C for 15 h, concentrated and partitioned between CH_2Cl_2 and aqueous $NaHCO_3$. Double column purification (CH_2Cl_2 to CH_2Cl_2-MeOH 99:1) afforded 1.52 g (3.2 mmol, 32%) of the title product as a slightly yellow oil.

UV (MeOH) λ_{max} 262 (ε = 21600), 320 (br), 244 (min), 283 (min) nm. ^1H NMR ($CDCl_3$): δ (p.p.m.) 2.00–2.25 (m, 2H, H2'), 3.33 (s, 3H, OCH_3), 3.52 (m, 4H, H3', H4', H5'), 4.31–4.69 (m, 6H, H1', 2× CH_2Ar), 7.29 (s, 10H, Ar-H), 7.72 (d, J = 9.2Hz, 1H, H7), 8.17 (dd, J = 2.2 and 9.2Hz, 1H, H6), 8.40 (d, J = 0.9Hz, 1H, H3), 8.79 (d, J = 2.2Hz, 1H, H4).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 30.1 (C2'), 45.6 (C1'), 57.9 (OCH_3), 69.0 (C5'), 71.2 and 72.3 (2× CH_2Ar), 76.2 (C3'), 80.5 (C4'), 110.3 (C7), 119.0 (C4), 120.7 (C6), 122.7 (C9), 127–128 (C-Ar), 136.1 (C3), 138.3 and 138.5 (C_x-Ar), 140.9 (C8), 141.6 (C5). LSIMS (ThGly) m/z 476 (MH^+, 65).

1-(4-O-methyl-2-deoxy-D-ribityl)-5-nitroindazole ***17***. The material **16** obtained in the previous preparation (3.21 g, 6.75 mmol) was dissolved in 100 ml CH_2Cl_2 and cooled to –78°C. A pre-cooled 1 M BCl_3 solution in CH_2Cl_2 (32 ml) was added and the mixture was stirred for 1 h at –78°C and worked-up as for the synthesis of **15**. Purification (CH_2Cl_2 to CH_2Cl_2-MeOH 95:5) afforded 1.43 g (4.86 mmol, 72%) of the title product which crystallized from MeOH.

UV (MeOH) λ_{max} 262 (ε = 21600), 320 (br), 244 (min), 283 (min) nm. ^1H NMR (DMSO): δ (p.p.m.) 1.84 (m, 1H, Ha2'), 2.08 (m, 1H, Hb2'), 3.01 (m, 1H, H3'), 3.28 (s, 3H, OCH_3), 3.36–3.57 (m, 3H, H4', H5'), 4.46 (t, 1H, 5'-OH), 4.54 (t, J = 7Hz, 2H, H1'), 4.89 (d, 1H, 3'-OH), 7.78 (d, J = 9.1Hz, 1H, H7), 8.21 (dd, J = 2.1 and 9.3Hz, 1H, H6), 8.41 (s, 1H, H3), 8.81 (d, J = 2.1Hz, 1H, H4).

^{13}C NMR (DMSO): δ (p.p.m.) 33.0 (C2′), 46.1 (C1′), 57.9 (OCH_3), 60.1 (C5′), 67.2 (C3′), 85.4 (C4′), 110.8 (C7), 119.3 (C4), 121.0 (C6), 122.8 (C9), 136.3 (C3), 141.2 (C8), 141.8 (C5). LSIMS (ThGly) m/z 296 (MH^+, 16). HRMS: MH^+ calcd. for $C_{13}H_{18}N_3O_5$: 296.1246; found: 296.1239.

1-(5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl)-4,5-dicarboxamidoimidazole ***18***. The acyclic nucleoside analogue **10** (260 mg, 0.91 mmol) was coevaporated twice with anhydrous pyridine, dissolved in 10 ml pyridine, and 370 mg (1.1 mmol) of dimethoxytrityl chloride was added. The mixture was stirred overnight at room temperature and 2 ml MeOH were added to quench the reaction. Partitioning between CH_2Cl_2 and aqueous $NaHCO_3$ and evaporation of the organics left an oil which was purified by flash chromatography on 25 g of silica gel (CH_2Cl_2 to CH_2Cl_2-MeOH 97:3, always containing 0.2% pyridine) to afford 410 mg (0.7 mmol, 77%) of **18**.

^{1}H NMR ($CDCl_3$): δ (p.p.m.) 1.60–1.85 (m, 1H, Ha2′), 1.90–2.10 (m, 1H, Hb2′), 3.15–3.50 (m, H3′, H5′), 3.37 (s, OCH_3), 3.60–3.80 (m, H4′), 3.77 (s, 2× CH_3O-trityl), 4.60 (m, 2H, H1′), 6.00 (d, J = 2Hz, 1H) and 6.12 (d, J = 4Hz, 1H) (exch NH), 6.81 (d, 4H), 7.10–7.45 (m, 9H) (H-Ar), 7.50 (s, 1H, H2), 7.61 (d, J = 4Hz, 1H) and 11.04 (d, J = 2Hz, 1H) (exch NH).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 33.9 (C2′), 44.7 (C1′), 55.1 (2× CH_3O-trityl), 58.4 (OCH_3), 62.2 (C5′), 68.5 (C3′), 82.7 (C4′), 86.3 (Ph_3C), 125.2 and 135.1 (C4, C5), 139.7 (C2), 161.1 and 165.9 (2× CO) + trityl signals. LSIMS (ThGly doped with NaCl) m/z 611 ($M+Na^+$, 5), 303 ($DMTr^+$, 100).

1-(5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl)-4-nitroimidazole ***19***. The 4-nitroimidazole analogue **15** (650 mg, 2.65 mmol) was tritylated analogously to afford after column purification with a slow gradient of MeOH (0–1%) in CH_2Cl_2-0.2% pyridine 537 mg (0.98 mmol, 38%) of **19** as a white powder.

^{1}H NMR ($CDCl_3$): δ (p.p.m.) 1.65–2.10 (m, 2H, H2′), 2.95 (m, 1H, H3′), 3.15–3.40 (m, H5′), 3.36 (s, OCH_3) (5H), 3.60–3.83 (m, H4′), 3.77 (s, 2× CH_3O-trityl) (7H), 4.10 (t, J = 7Hz, 2H, H1′), 6.82 (d, 4H), 7.12–7.40 (m, 10H), 7.75 (s, 1H) (H-Ar, H2, H5).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 33.0 (C2′), 44.8 (C1′), 55.0 (2× CH_3O-trityl), 58.2 (OCH_3), 61.7 (C5′), 68.6 (C3′), 82.1 (C4′), 86.5 (Ph_3C), 119.3 (C5), 136.1 (C2), 147.8 (C4) + trityl signals. LSIMS (ThGly doped with NaCl) m/z 570 ($M+Na^+$, 6), 303 ($DMTr^+$, 100).

1-(5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl)-5-nitroindazole ***20***. The 5-nitroindazole analogue **17** (680 mg, 2.3 mmol) was tritylated analogously to afford after a double column purification with a slow gradient of MeOH (0–1%) in CH_2Cl_2-0.2% pyridine 610 mg (1.02 mmol, 44%) of **20** as a white powder.

^{1}H NMR ($CDCl_3$): δ (p.p.m.) 1.70–1.95 (m, 1H, Ha2′), 2.05–2.20 (m, 1H, Hb2′), 2.85 (d, J = 5.5Hz, 1H, H3′), 3.34 (s, OCH_3), 3.15–3.85 (m, H4′, H5′), 3.77 (s, 2× CH_3O-trityl), 4.46–4.70 (m, H1′, 3′-OH), 6.79 (d, 4H), 7.12–7.40 (m, 9H) (H-Ar), 7.52 (d, J = 9.5Hz, 1H, H7), 8.17 (s, 1H, H3), 8.23 (dd, J = 2.2 and 9.3Hz, 1H, H6), 8.70 (d, J = 1.8Hz, 1H, H4).

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 32.4 (C2′), 45.8 (C1′), 55.1 (2× CH_3O-trityl), 58.3 (OCH_3), 62.0 (C5′), 69.0 (C3′), 82.6 (C4′), 86.5 (Ph_3C), 109.5 (C7), 118.8 (C4), 121.2 (C6), 122.7 (C9), 135.8 (C3), 141.3 (C8), 142.1 (C5) + trityl signals. LSIMS (ThGly doped with NaCl) m/z 620 ($M+Na^+$, 1), 303 ($DMTr^+$, 100).

1-(5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl)-5-amino-4-carboxamidoimidazole ***21***. The 5-amino-4-carboxamidoimidazole analogue **6** (750 mg, 2.9 mmol as an oil) was tritylated analogously to afford after a double column purification (MeOH gradient of 0–3% in CH_2Cl_2-0.2% pyridine) 820 mg (1.46 mmol, 50%) of a white foam.

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 32.5 (C2′), 39.4 (C1′), 54.8 (2× CH_3O-trityl), 58.1 (OCH_3), 62.1 (C5′), 68.0 (C3′), 82.4 (C4′), 85.9 (Ph_3C), 112.6 (C4), 129.4 (C2), 143.1 (C5), 166.5 (CO) + trityl signals. LSIMS (ThGly doped with NaCl) m/z 583 ($M+Na^+$, 6), 303 ($DMTr^+$, 100).

1-(5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl)-3,5-pyrazoledicarboxamide ***22***. The 3,5-pyrazoledicarboxamide analogue **13** (725 mg, 2.53 mmol) was tritylated analogously to afford after column purification (MeOH gradient of 0–4% in CH_2Cl_2-0.2% pyridine) 1.26 g (2.15 mmol, 85%) of a white foam.

UV (MeOH) λ_{max} 232, 274 (DMTr, small) nm. ^{1}H NMR (CD_3OD): δ (p.p.m.) 1.72–1.92 (m, 1H, Ha2′), 2.04–2.22 (m, 1H, Hb2′), 3.12–3.42 (m, H4′, H5′), 3.37 (s, OCH_3) (6H), 3.65 (m, H3′), 3.74 (s, 2 CH_3O-trityl) (7H), 4.70 (m, H1′), 6.79 (d, 4H), 7.10–7.43 (m, 11H) (H-Ar, H4) 7.78 (t), 8.50 (d) (exchangable NH).

^{13}C NMR (CD_3OD): δ (p.p.m.) 33.4 (C2′), 49.2 (C1′), 55.0 (2 CH_3O-trityl), 58.5 (OCH_3), 62.7 (C5′), 68.4 (C3′), 84.3 (C4′), 86.6 (Ph_3C), 108.9 (C4), 137.0 (C5), 144.6 (C3), 162.3, 165.1 (2× CO) + trityl signals. LSIMS (ThGly, NaOAc) m/z 611 ($M+Na^+$, 10), 303 (DMTr, 100).

1-[3-O-(P-β-Cyanoethyl-N,N-diisopropylaminophosphinyl)-5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl]-4,5-dicarboxamidoimidazole ***23***. The above dimethoxytritylated derivative **18** (353 mg, 0.6 mmol) was dissoved in 5 ml dichloromethane under argon and diisopropylethylamine (0.32 ml, 1.8 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.2 ml, 0.9 mmol) were added and the solution was stirred for 1 h. Ethanol (2 ml) was added and the solution stirred for 10 min and then partitioned between CH_2Cl_2 (50 ml) and aqueous $NaHCO_3$ (50 ml). The organic phase was washed with aqueous sodium chloride (2× 30 ml) and evaporation of the organics left an oil which was flash chromatographed (hexane:EtOAc:TEA, 43:55:2–23:75:2) to afford the product as a foam after coevaporation with dichloromethane. Precipitation in cold (–60°C) hexane gave 180 mg (0.23 mmol, 38%) of the title product **23** as a white powder.

Rf (hexane:EtOAc:TEA 23:75:2): 0.33 and 0.37 (diastereoisomers).

LSIMS (NBA) m/z 789 (MH^+, 1), 303 (DMTr, 100).

1-[3-O-(P-β-Cyanoethyl-N,N-diisopropylaminophosphinyl)-5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl]-4-nitroimidazole ***24***. The dimethoxytritylated derivative **19** (442 mg, 0.81 mmol) was phosphitylated as usual. Following flash chromatography with hexane:EtOAc:TEA, 43:55:2–23:75:2, the product was precipitated in cold hexane to afford 468 mg (0.63 mmol, 77%) of the title product as a white powder.

Rf (hexane:EtOAc:TEA 23:75:2): 0.46 and 0.52 (diastereoisomers).

LSIMS (NBA) m/z 748 (MH^+, 2), 303 (DMTr, 100).

1-[3-O-(P-β-Cyanoethyl-N,N-diisopropylaminophosphinyl)-5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl]-5-nitroindazole ***25***. The above dimethoxytritylated analogue **20** (515 mg, 0.86 mmol) was phosphitylated as usual. Following chromatography with hexane:EtOAc:TEA, 69:29:2–49:49:2, the product was precipi-

tated in cold hexane to afford 429 mg (0.62 mmol, 72%) of the title product as a white powder.

Rf (hexane:EtOAc:TEA 69:29:2): 0.21 and 0.27 (diastereoisomers).

LSIMS (NBA) m/z 798 (MH^+, 0.5), 303 (DMTr, 100).

Attempted synthesis of 1-[3-O-(P-β-Cyanoethyl-N,N-diisopropylaminophosphinyl)-5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl]-5-amino-4-carboxamidoimidazole ***26***. The dimethoxytritylated analogue **21** (675 mg, 1.2 mmol) was phosphitylated as usual. Two major products were detected by TLC (Rf 0.18 and 0.35 in hexane:EtOAc:TEA, 49:49:2) and both products were separated following flash chromatography using the same solvent system. NMR and MS analysis indicated both products to be phosphitylated on the base moiety, with the more lipophilic one carrying two phosphoramidite groups. Less polar one: 630 mg (0.65 mmol, 54%) isolated.

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 30.8 (C2′), 39.8 (C1′), 62.5 (C5′), 71.4 (d, J = 12.2Hz) and 72.0 (d, J = 14.6Hz) (C3′), 82.7 and 83.1 (C4′), 117.4 (d, C4), 129.4 (C2), 142.3 (C5), 166.8 (d, J = 13.4Hz, CO). LSIMS (THGly, NaCl) m/z 983 ($M+Na^+$, 15), 303 (DMTr, 100). More polar one: 220 mg (0.29 mmol, 24%) isolated.

^{13}C NMR ($CDCl_3$): δ (p.p.m.) 32.6 (C2′), 39.2 (C1′), 62.4 (C5′), 68.5 (C3′), 82.2 (C4′), 117.5 (d, C4) 129.7 (C2), 143.1 (C5), 166.9 (d, J = 14.6Hz, CO). LSIMS (THGly, NaCl) m/z 783 ($M+Na^+$, 20), 303 (DMTr, 100).

Attempted synthesis of 1-[3-O-(P-β-Cyanoethyl-N,N-diisopropylaminophosphinyl)-5-O-dimethoxytrityl-4-O-methyl-2-deoxy-D-ribityl]-3,5-pyrazoledicarboxamide ***27***. The dimethoxytritylated derivative **22** (1.26 g, 2.15 mmol) was phosphitylated as before. Two major products were detected by TLC (Rf 0.26 and 0.40 in hexane:EtOAc:TEA, 49:49:2) and both products were partially separated following flash chromatography using the same system as the eluting solvent. NMR and MS analysis indicated both products to be phosphitylated on the base moiety as for the attempted synthesis of **26**.

Less polar one: ^{13}C NMR ($CDCl_3$): δ (p.p.m.) 32.0 (C2′), 49.4 (C1′), 63.4 (C5′), 72.3 (d, J = 10.3Hz) and 72.6 (d, J = 7.5Hz) (C3′), 82.6 and 83.1 (C4′), 108.9 (C4), 136.2 (C5), 144.2 (C3), 160.8 (CO), 163.8 (d, J = 13.6Hz, CO). LSIMS (NBA) m/z 989 (MH^+, 10), 303 (DMTr, 100).

More polar one: ^{13}C NMR ($CDCl_3$): δ (p.p.m.) 33.6 (C2′), 49.1 (C1′), 62.5 (C5′), 68.6 (C3′), 83.1 (C4′), 109.0 (C4), 136.4 (C5), 144.8 (C3), 161.2 (CO), 163.6 (d, J = 15.1Hz, CO). LSIMS (NBA) m/z 789 (MH^+, 12), 303 (DMTr, 100).

ACKNOWLEDGEMENTS

Dr A. Van Aerschot is a research associate of the Belgian National Fund for Scientific Research. This work has been supported by a grant from the Belgian F.G.W.O. (Fonds voor Geneeskundig Wetenschappelijk Onderzoek, project 3.0076.92).

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