EFFECT OF A HYDROGEN BONDING CARBOXAMIDE GROUP ON UNIVERSAL BASES

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Dedicated to Professor Antonín Holý, on the occasion of his 70th birthday, in recognition of his outstanding contributions to the area of nucleic acid chemistry.

A number of aromatic universal base analogues have been described in the literature, but most are non-hydrogen bonding. We have examined the effect of introducing hydrogen bonding carboxamide groups onto the pyrrole ring of 5-nitroindole. The modified analogues retain universal base features, but there are no overall effects on duplex stability. This leads to the suggestion that the nitro group is within the hydrogen bonding face of the duplex, and the hydrogen bonding carboxamide group is in the duplex major groove.

Keywords: Nucleosides; Universal nucleobases; 5-Nitroindole; Indoles; Hydrogen bonding; Stacking energies; Duplex stability; Oligonucleotides; DNA duplexes.

Universal bases have the ability to pair with each of the natural bases found in DNA or RNA without distinguishing between them to a greater or lesser degree. Whilst there have been many such analogues described, they each have a defined set of features, namely that they are aromatic, planar, hydrophobic and non-hydrogen bonding[1,2]. As yet there are no universal bases described that function through hydrogen bonding to the natural bases and still retain the property of not showing any preference to any particular nucleobase, though Seela et al. have described an 8-aza-7-deaza-adenine derivative that may meet some of these requirements[2]. A number of analogues, such as azolecarboxamides, have been shown to pair with the natural DNA bases by hydrogen bonding. These analogues exhibit two alternative hydrogen bonding faces by rotation about the amide bond. In so doing, azolecarboxamides present base pairing properties that, in principle, allow them to hydrogen bond to all four DNA bases. However, in practice these analogues always show base pairing preferences[3,4].

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As universal bases do not possess hydrogen bonding features, they stabilise duplexes with DNA (or RNA) by enhanced stacking interactions. The stability of the base pair with a natural nucleobase in a duplex is related to the surface area of the universal base, larger surface areas resulting in greater stability. The most widely used universal base analogues are 3-nitropyrrole and 5-nitroindole, with the larger 5-nitroindole forming more stable duplexes when incorporated into oligodeoxynucleotides[5–7]. A recent report by Klewer et al. discussed the solution structure of a duplex containing 3-nitropyrrole showing that the 3-nitropyrrole base had reduced π–π stacking interactions with its adjacent nucleobases. The lower stacking interaction of the 3-nitropyrrole group was due to its reduced size compared to a natural DNA base. Interestingly, it was shown that the nitro group protrudes into the major groove in order to adopt an orientation reducing crowding within the duplex structure[8,9].

Universal base analogues have shown value in a number of applications involving their hybridisation properties. Thus the principle uses of these analogues are in probes and primers. However, despite a number of different analogues having been assayed in polymerase-based reactions, none have yet been shown to be good substrates for any DNA polymerase. In general they act as blocking lesions either when present in the template or when incorporated as a 5′-triphosphate. When they are present in a template they generally direct the incorporation of dAMP, which is presumably more a function of the polymerase extendase activity than of any property of the universal base.

In this work we wanted to assess the action of incorporating a hydrogen bonding functional group onto a universal base. We have previously examined the effect of indole nucleosides, which had hydrogen bonding potential[10]. Reduction of 5-nitroindole-5′-triphosphate gave the 5-aminoindole derivative **1**, which was also converted to its formamido derivative **2**. The incorporation properties of these were examined, where it was found that the formamidoindole **2** behaved essentially as a universal base, though like 5-nitroindole **3** it acted as a chain terminator. However, the 5-aminoindole derivative **1** was no longer a universal base, incorporating preferentially opposite adenine, but under these conditions the primer could be chain extended. To further examine universal base analogues with hydrogen bonding functionality we chose to synthesise analogues of 5-nitroindole bearing a carboxamide group at the 3-position (**4** and **5**) (Chart 1). If the carboxamide group of such an analogue were to be directed towards the hydrogen bonding face in a duplex then it might be expected that its universal base properties would be altered. It might be anticipated that such an analogue

would still possess stabilising stacking interactions, but the added hydrogen bonding functionality may alter its universal base properties. This might, in turn, make such an analogue a better substrate for polymerase recognition.

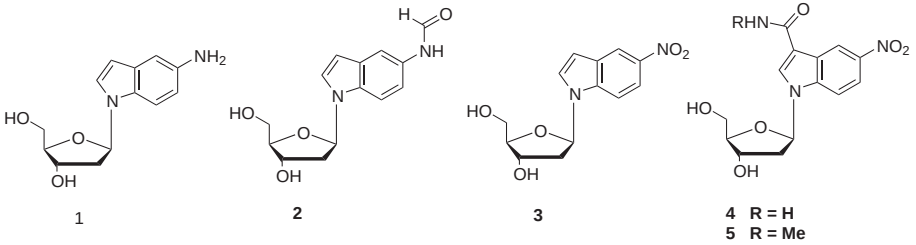


CHART 1

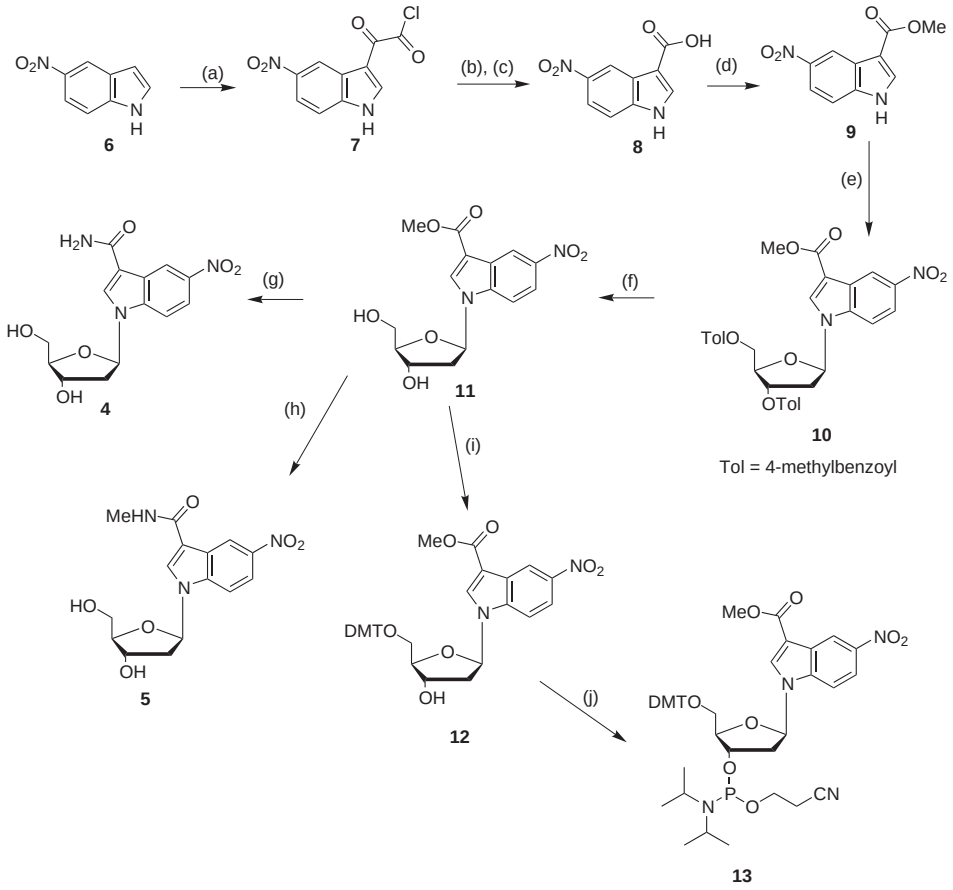
RESULTS

C3-substituted 5-nitroindole was prepared by action of 5-nitroindole **6** with oxalyl chloride yielding the glyoxalyl chloride derivative **7**. This was converted to the C3-substituted carboxylic acid derivative **8** by first alkaline hydrolysis followed by an oxidative decarboxylation with hydrogen peroxide[11]. The resultant 3-carboxylic acid was esterified by refluxing in methanol in the presence of sulfuric acid. Glycosylation using the same method used for the synthesis of 5-nitroindole nucleoside **10** proceeded in greater than 70% yield, giving only the desired β-anomer of the methyl ester. Deprotection to the free nucleoside **11** was carried out by refluxing in methanol, in the presence of triethylamine. For characterisation, and to study the conversion to the corresponding amide, portions of the ester **11** were treated with concentrated ammonia and 40% aqueous methylamine at 50 °C to generate the amide **4** and the *N*-methylamide **5**, respectively (Scheme 1). It was observed that these reactions required longer than 24 h for complete conversion to the corresponding amide, and therefore for oligonucleotide synthesis a 48-h deprotection would be required. Finally, conversion to the phosphoramidite derivative **13** was carried out in the usual manner.

The phosphoramidite was incorporated into oligodeoxynucleotides using standard phosphoramidite DNA synthesis chemistry. Conversion of the methyl 5-nitroindole-3-carboxylate containing oligonucleotides to the corresponding carboxamide **4** was carried out in ammonia, and to the *N*-methylcarboxamide **5** using 40% aqueous methylamine. Oligonucleotides (ODNs) were then purified by HPLC and analysed by MALDI-TOF mass spectrometry. It was observed in mass spectrometry that, as with 5-nitro-

indole **3** containing ODNs, the molecular ion often showed loss of oxygen from the nitro group.

In order to determine whether the 3-carboxamide group substitution onto 5-nitroindole had any effect on duplex stability, thermal melting (T_m) studies were conducted. Duplexes were prepared containing 5-nitroindole **3**, the 3-carboxamide **4** and the 3-*(N*-methylcarboxamide) **5** derivatives.



Reaction conditions: (a) $(COCl)_2$, Et_2O, 0 °C, 24 h, 88 %. (b) KOH, H_2O, 90 °C, 1 h. (c) H_2O_2 (30 % wt in H_2O), reflux, 64 % from **7**. (d) H_2SO_4, MeOH, reflux, 6 h, 67 %. (e) NaH, 3,5-di-*O*-*p*-toluoyl-2-deoxy-α-ribofuranosyl chloride, MeCN, RT, 2 h, 73 %. (f) MeOH, NEt_3, reflux, 16 h, 90 %. (g) aq. NH_3 (d = 0.88), 50 °C, 16 h, 46 %. (h) $MeNH_2$ (40 % aq. solution), 50 °C, 16 h, 48 %. (i) Dimethoxytrityl chloride, pyridine, RT, 87 %. (j) 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-Diisopropylethylamine, DCM, RT, 1 h, 72 %.

SCHEME 1

The T_ms of these duplexes were measured (Table I) and from these the stacking energy (ΔH) calculated[5]. It can be seen that each analogue behaved as a universal base as expected, though the effect of incorporation of a hydrogen bonding group was negligible. The 3-carboxamide group **4** actually had a slight destabilising effect compared to 5-nitroindole **3**, and the 3-(*N*-methylcarboxamide) **5** derivative caused a further slight destabilisation.

Analysis of the stacking energies of the duplexes showed greater variation. 5-Nitroindole showed a higher stacking energy opposed to adenine and lowest against thymine. This could be explained by the fact that adenine has a larger surface area than thymine. However, the carboxamide derivatives showed higher stacking energies when opposed to pyrimidines than purines in contrast to that of the parent 5-nitroindole.

TABLE I
Melting temperatures and stacking energy of duplexes containing 5-nitroindole **3** and its 3-carboxamide **4** and 3-(*N*-methylcarboxamide) **5** derivatives

	T_m, °C	$-\Delta H$, kJ/mol
GTAAAACGA**3**GGCCAGT		
CATTTTGCT**G**CCGGTCA	64	311.87
---------**A**-------	64	329.32
---------**C**-------	65	318.34
---------**T**-------	64	304.39
GTAAAACGA**4**GGCCAGT		
CATTTTGCT**G**CCGGTCA	64	315.35
---------**A**-------	63	303.17
---------**C**-------	63	327.35
---------**T**-------	62	334.38
GTAAAACGA**5**GGCCAGT		
CATTTTGCT**G**CCGGTCA	63	302.87
---------**A**-------	62.5	309.38
---------**C**-------	62.5	317.81
---------**T**-------	62	315.44
CGCGAA**X**TTCGCG		
GCGCTT**X**AAGCGC		
X = 5-nitroindole	62	nd[a]
X = 5-nitroindole-3-carboxamide	63.5	nd[a]

[a] nd = not determined.

From this initial data it appears that the presence of the hydrogen bonding carboxamide group has a slight destabilising effect on duplex stability compared to 5-nitroindole **3**. In order to examine the effect of self-pair **3** and its carboxamide derivative **4**, each was incorporated into a tridecamer at the centre of an otherwise self-complementary duplex (Table I). Under these conditions it was observed that the 5-nitroindolecarboxamide self-pair was slightly more stable than the 5-nitroindole self-pair[12].

A further feature of universal bases that we had observed is that contiguous substitutions maintain duplex stability[12], and the decrease in stability due to the loss of further hydrogen bonding is off-set by enhanced stacking interactions. This was also observed for the 3-carboxamide derivative **4** (Table II) using a different sequence context. However, the 3-(*N*-methylcarboxamide) **5** showed a slight decrease in duplex stability when two contiguous substitutions are made, and this is reflected in a lowering of its stacking energy.

TABLE II
Effect of contiguous substitutions on duplex stability

	T_m, °C	$-\Delta H$, kJ/mol
CATTTTGCTGCCGGTCA		
GT3AAACGACGGCCAGT	72	326.64
--**33**----------	72	339.07
--**4**----------	71	318.20
--**44**----------	70	323.15
--**5**----------	71	330.00
--**55**----------	69	314.16

The final sequence contexts that we examined are near-end effects. When each of the 5-nitroindole analogues was incorporated at the end of the duplex, they still acted as a universal base with the 3-(*N*-methylcarboxamide) derivative **5** again showing the lowest stability. Stacking energy of these duplexes was not so clear, though the 3-carboxamide derivative **4** showed greatest stacking energy with cytosine again, and the 3-(*N*-methylcarboxamide) derivative **5** with adenine and cytosine (Table III).

Kool et al. have studied the effect of dangling bases and shown that a single additional nucleotide at the end of a duplex aids stabilisation, described as acting as an end-cap[13]. We studied the effect of adding the 5-nitroindole

derivatives as a dangling base and the 3-carboxamide derivative showed the highest stabilisation, and this is reflected in the highest stacking energy (Table IV).

TABLE III
Near-end effects on duplex stability

	T_m, °C	$-\Delta H$, kJ/mol
GTAAAACGACGGCCAG**3**		
CATTTTGCTGCCGGTC**A**	75	375.32
----------------**T**	74.5	408.17
----------------**C**	75	447.49
----------------**G**	75.5	450.40
GTAAAACGACGGCCAG**4**		
CATTTTGCTGCCGGTC**A**	75	406.54
----------------**T**	76.5	378.54
----------------**C**	75	439.34
----------------**G**	76.5	413.54
GTAAAACGACGGCCAG**5**		
CATTTTGCTGCCGGTC**A**	74	446.56
----------------**T**	74	373.19
----------------**C**	72.5	403.06
----------------**G**	74	320.78

TABLE IV
Effect of dangling bases on duplex stability

	T_m, °C	$-\Delta H$, kJ/mol
CATTTTGCTGCCGGTC		
GTAAAACGACGGCCAG	69.5	435.13
----------------**3**	71.5	400.75
----------------**4**	72.5	491.22
----------------**5**	71	399.59

DISCUSSION

Generally, non-hydrogen bonding universal bases cause some duplex instability, though larger residues (e.g. pyrene) can raise the T_m above that observed for a fully native DNA duplex[13]. The instability caused by such universal bases is due to a number of factors, the major one being the loss of hydrogen bonding stabilisation, but other stabilising factors, such as solvation, are involved[13]. However, these losses are in part made up for by enhanced stacking interactions, and this is generally proportional to the surface area of the universal base. We were interested to know what would be the effect of introducing a hydrogen bonding group onto a universal base, to know whether it could lead to further duplex stability. We chose the carboxamide group as there have been many azolecarboxamides studied as potential universal bases[14]. The presence of the carboxamide group can, in theory, present two alternative hydrogen-bonding faces allowing base pairing to each of the native DNA/RNA nucleosides.

In the present study we decided to introduce a carboxamide group onto the pyrrole ring of 5-nitroindole. Klewer et al. have shown by NMR that the nitro group of the universal base 3-nitropyrrole protrudes into the major groove, away from the hydrogen bonding face[8]. If the 5-nitroindole residue adopts a similar conformation, then the nitro group would protrude into the major groove leaving the new carboxamide group in the duplex hydrogen bonding face. In such a conformation the carboxamide group might give additional stability by forming hydrogen bonds to the native base in the complementary strand. The alternative conformation would direct the carboxamide group into the major groove leaving the nitro group within the hydrogen bonding region. Due to the increased size of the indole derivatives, compared to native bases, we assume that the indole bases would intercalate into the opposing strand. The *N*-methylcarboxamide derivative **5** was prepared as the methyl group may alter this new interaction by introducing steric bulk which might interfere with any hydrogen bonding interaction. It was anticipated that the experiments described in this paper might lead us to infer which conformation the indole base adopts within a duplex.

Melting temperature studies of the two 5-nitroindole-3-carboxamide derivatives demonstrated that they still retained universal base hybridisation features. Compared to 5-nitroindole itself, however, they were slightly destabilising in most of the sequence contexts examined. The *N*-methylcarboxamide group in **5** showed lower stability than the carboxamide group in **4**, and this may then be due to steric effects rather than reducing

hydrogen bonding capability of the analogue. Analysis of the melting data provides further information about stacking interactions. As noted above, 5-nitroindole exhibits greatest stacking energy when opposed to adenine, presumably as a result of enhanced stacking interactions with it. However, the two carboxamide derivatives show enhanced stacking interactions when opposed to a pyrimidine. This suggests that the carboxamide group is not involved in the hydrogen bonding face and may be protruding into the major groove of the duplex, with the nitro group directed towards the complementary strand, and end-cap experiments, where intercalation cannot be involved, supports this conclusion.

One of the requirements for a universal base is that it should be able to form "base pairs" with each of the natural bases in polymerase reactions. This would allow the synthesis of libraries of oligonucleotides from a single universal base-containing oligonucleotide[12]. 5-Nitroindole can behave as a universal base by adding, as its 5′-triphosphate, opposite each of the natural template bases, and can direct the incorporation of all four natural dNTPs when in a template. However, polymerase reactions involving 5-nitroindole are very inefficient, and it usually acts as a terminator once incorporation has occurred. Work is underway to determine whether the 3-carboxamide derivative is a better polymerase substrate, and will be reported in due course.

EXPERIMENTAL

^{1}H and ^{31}P NMR spectra were obtained on a Bruker DRX 300 spectrometer, and spectra were obtained in DMSO-d_6, unless otherwise stated. ^{31}P NMR spectra are externally referenced to phosphoric acid. Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. Ultraviolet spectra (λ in nm) were recorded on a Perkin Elmer Lambda 2 or Lambda 40 spectrophotometer dissolved in 10% aqueous methanol unless otherwise stated. Melting points were measured with a Stuart Scientific SMP3 melting point apparatus and are uncorrected. Mass spectra were recorded on a Bruker FTICR Bioapex II. TLC was carried out on pre-coated F_{254} silica plates and column chromatography with Merck kieselgel 60. Unless otherwise stated, reactions were worked up as follows: the solvent was removed by evaporation and the product dissolved in chloroform and washed with saturated aqueous sodium hydrogencarbonate. The chloroform fractions were dried over anhydrous sodium sulfate, filtered and evaporated to dryness. Oligonucleotides were synthesised on an Applied Biosystem 380B synthesiser with the normal synthesis cycle. 5-Nitroindole phosphoramidite was purchased from Glen Research. 5-Nitroindole-3-carboxamide nucleotides were prepared by deprotecting with ammonia (d = 0.91) at 80 °C for 2 days. *N*-Methylcarboxamide oligonucleotides were prepared by deprotection with 40% aqueous methylamine at 80 °C for 2 days. Oligonucleotides were purified by HPLC on a Dionex Nucleopac PA100 ion exchange column using a gradient of 1 to 400 mM sodium perchlorate containing 0.02 M Tris (pH 6.8) and 25% aqueous formamide, followed by dialysis and concentration. MALDI-TOF mass

spectroscopy was carried out on a Hewlett–Packard G205A spectrometer using negative polarity, in a matrix of 2-cyano-3-(4-hydroxyphenyl)propenoic acid in acetonitrile/water (1:1) with 3% trifluoroacetic acid.

5-Nitroindole-3-carboxylic Acid (**8**)[11]

To a solution of 5-nitroindole (3 g, 18.5 mmol) in ether (100 cm^3) at 0 °C, oxalyl chloride (8 cm^3, 92 mmol) was added dropwise, and stirring continued at 0 °C for 24 h. The solid was filtered and dried. Yield 4.1 g (88%). The acid chloride **7** was suspended in water (100 cm^3) and potassium hydroxide (1.1 g, 19.6 mmol) added and the solution heated at 90 °C for 1 h. The solution was cooled, acidified and the yellow solid filtered. The solid was resuspended in hydrogen peroxide solution (30%, 50 cm^3) and the solution heated at reflux for 3 h. After cooling the solid was filtered, dried and recrystallised from aqueous ethanol to give a greenish-yellow solid. Yield 2.45 g (64%), m.p. 285–287 °C. ^{1}H NMR: 7.64 (1 H, d, J = 9, H-7), 8.07 (1 H, dd, J_1 = 9, J_2 = 2.2, H-6), 8.27 (1 H, s, H-2), 8.88 (1 H, d, J = 2.2, H-4), 12.44 (2 H, br s, NH, CO_2H).

Methyl-5-nitroindole-3-carboxylate (**9**)

To a solution of 5-nitroindole-3-carboxylic acid (2.45 g, 12 mmol) in methanol (50 cm^3), sulfuric acid (1 cm^3) was added and the solution stirred at reflux for 6 h. The solution was allowed to cool and then poured onto ice-water, neutralised with sodium hydrogencarbonate and the product filtered to give a yellow solid, which was recrystallised from methanol as an off-white solid. Yield 1.76 g (67%), m.p. 282–284 °C. ^{1}H NMR: 3.85 (3 H, s, OCH_3), 7.65 (1 H, d, J = 9, H-7), 8.08 (1 H, dd, J_1 = 9, J_2 = 2.3, H-6), 8.35 (1 H, s, H-2), 8.83 (1 H, d, J = 2.3, H-4), 8.5 (1 H, s, NH). UV: λ_{max} 320 (15 000), 251 (32 450), λ_{min} 284, 213; pH 12 λ_{max} 366 (13 100), 271 (31 500), 213 (48 100). MS, *m/z*: 243.1 $(M + Na)^+$. Accurate mass measurement on $(M + Na)^+$ $C_{10}H_8N_2NaO_4$ 243.0396, deviation –5.73 ppm.

Methyl 1-[2-Deoxy-3,5-bis(4-methylbenzoyl)-β-D-ribofuranosyl]-5-nitroindole-3-carboxylate (**10**)

To a solution of methyl 5-nitroindole-3-carboxylate (1.22 g, 5.5 mmol) in acetonitrile (50 cm^3), sodium hydride (60%, 0.27 g, 7 mmol) was added and the solution stirred at room temperature for 30 min. To this, 3,5-di-*O*-*p*-toluoyl-2-deoxy-α-ribofuranosyl chloride (2.6 g, 6.7 mmol) was then added and stirring continued for 2 h. The solvent was evaporated and the product worked up to give a brown foam, which was chromatographed (CH_2Cl_2/0–2% MeOH) to give a pale yellow foam. Yield 2.25 g (73%). ^{1}H NMR: 2.36 (3 H, s, toluoyl-CH_3), 2.40 (3 H, s, toluoyl-CH_3), 2.80–2.87 (1 H, m, H-2′), 2.99–3.08 (1 H, m, H-2″), 3.82 (3 H, s, OCH_3), 4.44–4.67 (3 H, m, H-4′, H-5′, H-5″), 5.71–5.73 (1 H, m, H-3′), 6.72 (1 H, t, J = 6.7, H-1′), 7.26–7.39 (4 H, m, toluoyl-CH), 7.78–8.03 (6 H, m, 4 × toluoyl-CH, H-7, H-6), 8.57 (1 H, s, H-2), 8.81 (1 H, d, J = 1.9, H-4). UV: λ_{max} 316 (9600), 246 (32 300), λ_{min} 302, 222; pH 1 λ_{max} 274 (28 400), 253 (29 800), λ_{min} 268, 225; pH 12 λ_{max} 316 (13 700), 229 (34 900), λ_{min} 306, 232, 209. MS, *m/z*: 595.1 $(M + Na)^+$. Accurate mass measurement on $C_{31}H_{28}N_2NaO_9$ 595.16750, deviation –2.99 ppm.

Methyl 1-(2-Deoxy-β-D-ribofuranosyl)-5-nitroindole-3-carboxylate (**11**)

A solution of methyl 1-(2-deoxy-3,5-bis(4-methylbenzoyl)-β-D-ribofuranosyl)-5-nitroindole-3-carboxylate (2.3 g, 4 mmol) in methanol (100 cm^3) containing triethylamine (5 cm^3) was heated at reflux overnight. The solution was evaporated and the product chromatographed (CH_2Cl_2/0–5% MeOH) to give a pale yellow solid, which recrystallised from ethanol to give off-white needles. Yield 1.22 g (90%), m.p. 162–164 °C. For $C_{15}H_{16}N_2O_7$ (336.1) calculated: 53.57% C, 4.80% H, 8.33% N; found: 53.41% C, 4.83% H, 8.27% N. ^{1}H NMR: 2.31–2.39 (1 H, m, H-2′), 2.48–2.56 (1 H, m, H-2″), 3.51–3.63 (2 H, m, H-5′, H-5″), 3.30–3.38 (1 H, m, H-4′), 3.33 (3 H, s, CH_3), 4.35–4.40 (1 H, m, H-3′), 5.04 (1 H, t, J = 5.1, 5′-OH), 5.36 (1 H, d, J = 4.1, 3′-OH), 6.50 (1 H, t, J = 6.2, H-1′), 7.96 (1 H, d, J = 9.2, H-7), 8.13 (1 H, dd, J_1 = 9.1, J_2 = 2.3, H-6), 8.62 (1 H, s, H-2), 8.84 (1 H, d, J = 2.2, H-4). ^{13}C NMR: 163.7, 142.9, 138.8, 135.5, 125.7, 118.1, 117.0, 112.5, 108.7, 87.9, 85.8, 70.4, 61.3, 51.4, 40.3. UV: λ_{max} 318 (9100), 266 (24 300), λ_{min} 290, 216. MS, *m/z*: 359.1 $(M + Na)^+$. Accurate mass measurement on $C_{15}H_{16}N_2NaO_7$ 359.08480, deviation –2.09 ppm.

1-(2-Deoxy-β-D-ribofuranosyl)-5-nitroindole-3-carboxamide (**4**)

A solution of the above ester (300 mg, 0.89 mmol) in ammonia solution (d = 0.91; 10 cm^3) was stirred at 50 °C overnight. The solution was evaporated and the product crystallised from ethanol to give a pale green solid. Yield 133 mg (46%), m.p. 220–222 °C. For $C_{15}H_{16}N_3O_7$ (350.3) calculated: 52.34% C, 4.71% H, 13.08% N; found: 52.15% C, 4.75% H, 12.87% N. ^{1}H NMR: 2.34–2.49 (2 H, m, H-2′, H-2″), 3.46–3.59 (2 H, m, H-5′, H-5″), 3.85–3.89 (1 H, m, H-4′), 4.39 (1 H, br s, H-3′), 4.89 (1 H, t, J = 5.3, 5′-OH), 5.38 (1 H, d, J = 4.2, 3′-OH), 6.47 (1 H, t, H-1′), 7.16, 7.70 (2 × br s, $CONH_2$), 7.86 (1 H, d, J = 9, H-7), 8.08 (1 H, dd, J_1 = 9, J_2 = 2, H-6), 8.48 (1 H, s, H-2), 9.06 (1 H, d, J = 2, H-4). ^{13}C NMR: 165.2, 142.3, 139.0, 131.2, 126.5, 118.1, 117.7, 112.8, 111.7, 87.7, 85.0, 70.5, 61.8, 40.2. UV: λ_{max} 320 (9700), 267 (24 600), λ_{min} 291, 223. ε_{260} (μmol/l) = 22.7. MS, *m/z*: 344.1 $(M + Na)^+$. Accurate mass measurement on $C_{14}H_{15}N_3NaO_6$ 344.08470, deviation –3.45 ppm.

1-(2-Deoxy-β-D-ribofuranosyl)-*N*-methyl-5-nitroindole-3-carboxamide (**5**)

A solution of methyl ester (200 mg, 0.59 mmol) in 40% aqueous methylamine solution (10 cm^3) was stirred at 50 °C overnight. The solution was evaporated and the product crystallised from ethanol to give a yellow solid. Yield 96 mg (48%), m.p. 248–250 °C. For $C_{15}H_{17}N_3O_7 \cdot 0.5H_2O$ (359.3) calculated: 52.32% C, 5.26% H, 12.20% N; found: 52.51% C, 5.07% H, 12.03% N. ^{1}H NMR: 2.33–2.46 (2 H, m, H-2′, H-2″), 2.79 (3 H, d, J = 4.4, $NHCH_3$), 3.46–3.57 (2 H, m, H-5′, H-5″), 3.85–3.89 (1 H, m, H-4′), 4.39 (1 H, br s, H-3′), 4.91 (1 H, br s, 5′-OH), 5.39 (1 H, d, J = 3, 3′-OH), 6.47 (1 H, t, J = 6.5, H-1′), 7.86 (1 H, d, J = 9, H-7), 8.08 (1 H, dd, J_1 = 9.1, J_2 = 2.2, H-6), 8.19 (1 H, br, NH), 8.41 (1 H, s, H-2), 9.06 (1 H, d, J = 2, H-4). ^{13}C NMR: 163.7, 142.3, 138.8, 130.3, 126.3, 118.0, 117.7, 113.1, 111.7, 87.7, 85.0, 70.5, 61.8, 40.2, 25.6. UV: λ_{max} 321 (8300), 267 (20 700), 203 (25 800), λ_{min} 291, 222. ε_{260} (μmol/l) = 19.2. MS, *m/z*: 358.1 $(M + Na)^+$. Accurate mass measurement on $C_{15}H_{17}N_3NaO_6$ 358.10120, deviation –0.94 ppm.

Methyl 1-[2-Deoxy-5-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-5-nitroindole-3-carboxylate (**12**)

To a solution of the above ester (0.86 g, 2.55 mmol) in pyridine (25 cm^3), 4,4′-dimethoxytrityl chloride (0.95 g, 2.8 mmol) was added and the solution stirred at room temperature overnight. Further portions of dimethoxytrityl chloride were then added until the reaction was complete. The solution was evaporated and the reaction worked up and chromatographed (CH_2Cl_2/1% MeOH) to give a yellow foam. Yield 1.42 g (87%). ^{1}H NMR: 2.43–2.49 (1 H, m, H-2′), 2.73–2.77 (1 H, m, H-2″), 3.31 (2 H, br s, H-5′, H-5″), 3.69 (6 H, s, 2 × OCH_3), 3.80 (3 H, s, CO_2CH_3), 3.99–4.01 (1 H, m, H-4′), 4.47–4.51 (1 H, m, H-3′), 5.43 (1 H, d, J = 4.6, 3′-OH), 6.53 (1 H, t, J = 5.9, H-1′), 6.73–6.84, 7.13–7.26 (13 H, m, Ar-H), 7.97 (1 H, d, J = 9.1, H-7), 8.07 (1 H, dd, J_1 = 9.1, J_2 = 2, H-6), 8.49 (1 H, s, H-2), 8.85 (1 H, d, J = 1.9, H-4). UV: λ_{max} 318 (6600), 265 (22 800), 237 (34 400), λ_{min} 298, 228. MS, *m/z*: 661.2 $(M + Na)^+$. Accurate mass measurement on $C_{36}H_{34}N_2NaO_9$ 661.21560, deviation –0.95 ppm.

Methyl-1-(3-*O*-{[(2-cyanoethoxy)(diisopropylamino)phosphonyl]oxy}-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl)-5-nitroindole-3-carboxylate (**13**)

To a solution of the dimethoxytritylated nucleoside (0.6 g, 0.9 mmol) in dichloromethane (10 cm^3), ethyl(diisopropyl)amine (0.49 cm^3, 2.8 mmol) followed by 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.31 cm^3, 1.39 mmol) was added, and the solution stirred at room temperature for 1 h. The reaction was quenched with methanol (100 μl), and the solution diluted with ethyl acetate (100 cm^3) and then washed (5% sodium carbonate solution (2 × 25 cm^3) followed by saturated brine (2 × 25 cm^3)), dried and evaporated to a gum. This was chromatographed (ethyl acetate/hexane/triethylamine 10:10:1) to give an off-white foam which was then precipitated from an ice-cold solution of hexane. Yield 0.57 g (72%). ^{31}P NMR: 146.95, 147.59.

Thermal Dissociation (T_m) Measurements

Ultraviolet spectra were recorded on a Perkin Elmer Lambda 2 or Lambda 40 spectrophotometer fitted with a Peltier cell. Melting transitions were measured at 260 nm in 6 × SSC (0.9 M sodium chloride, 0.09 M sodium citrate, pH 7) at an oligomer strand concentration of approximately 2 μmol/l. Absorbance versus temperature for each duplex was obtained at a heating and cooling rate of 0.5 °C/min, and melting temperatures (T_m) were determined as the maxima, or minima, of the differential curves, with an error of ±0.5 °C.

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