Synthesis of Acyclic Nitroazole Nucleosides and Their Incorporation into Oligonucleotides, and Their Duplex and Triplex Formation

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Acyclic nucleosides of 4-nitro-1*H*-imidazole and 4-nitropyrazole have been synthesized by nucleophilic addition of the appropriate 4-nitroazole to (−)-(*S*)-(hydroxymethyl)oxirane in the presence of a catalytic amount of potassium carbonate. (+)-(*R*)-3-(4-nitro-1*H*-imidazol-1-yl)propane-1,2-diol and (+)-(*R*)-3-(2-methyl-4-nitro-1*H*-imidazol-1-yl)propane-1,2-diol were also obtained in an independent reaction starting from appropriate 1,4-dinitro-1*H*-imidazole and (+)-(*R*)-3-aminopropane-1,2-diol. (+)-(*R*)-3-(4-Nitropyrazol-1-yl)-propane-1,2-diol was also obtained by direct noncatalyzed addition of 4-nitropyrazole to (−)-(*S*)-(hydroxymethyl)oxirane, whereas the (*S*)-enantiomer was obtained by reaction of 4-nitropyrazole with (+)-(*S*)-1,2-*O*-isopropylideneglycerol under *Mitsunobu* reaction conditions, followed by a cleavage of the isopropylidene group with 80% AcOH. Racemization during any of these syntheses has not been observed. 3-(4-Nitroazol-1-yl)propane-1,2-diols were incorporated into a 26-mer oligonucleotide. UV Thermal melting studies of duplexes of the oligonucleotides with 4-nitropyrazole or 4-nitro-1*H*-imidazole paired with four natural bases showed moderately decreased stabilities of the duplexes. A narrow range of melting temperatures, typically being within 2° for each acyclic nucleoside, fulfill one of the requirements of using acyclic 4-nitroazoles as general bases. Single incorporation of 4-nitroazoles into a 14-mer triplex forming oligonucleotide resulted in considerably decreased triplex stabilities.

1. Introduction. – Universal bases, non-discriminatory analogues of naturally-occurring nucleobases, have been the subject of a number of recent studies [1] [2]. The major requirement among others for a universal base is to form pairs with all four DNA/RNA bases with equal affinity [3]. At present, a broad range of compounds that fullfill these criteria is known. The naturally occurring hypoxanthine as its ribo- or 2′-deoxyribonucleoside form such base pairs with the other natural DNA/RNA bases [4]. Similar behavior exhibit 2′-deoxyinosine analogues: 7-deaza-2′-deoxyinosine [5] and 2-aza-2′-deoxyinosine [6]. Melting studies of oligonucleotides containing the deoxyribonucleotides of 3-nitro-1*H*-pyrrole [7–9], 4-, 5-, or 6-nitro-1*H*-indole [10, 11] or 4-nitro-1*H*-imidazole [12] have demonstrated that the nitroazoles show less discrimination in their base pairs with the four natural bases than other types of analogues investigated as universal base candidates. To study base-pairing properties azolecarboxamides were also investigated [13–16]. Another approach that could be followed is to combine base modification with sugar alterations of the nucleoside. Because of the conformational

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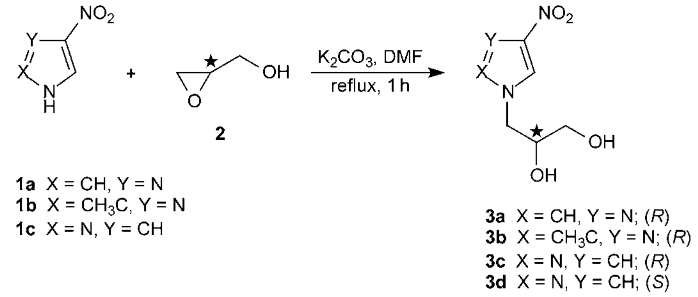
flexibility of acyclic nucleosides, it could be expected that the base-pairing properties of such analogues are different from those with classical sugar backbones. The replacement of the sugar moiety by an acyclic structure has been used to increase the enzymatic stability of oligonucleotides. Indeed, acyclic hypoxanthine and 5-nitro-1*H*-indazole analogues were designed as good candidates for universal bases [17]. Acyclic nucleosides possessing a C_3, C_4, or C_5 chain have been incorporated into oligonucleotides [17–24].

As an attempt to improve the potential of acyclic oligonucleotide analogues with respect to base-pairing affinity, we describe here the synthesis and incorporation of four acyclic nitroazole nucleosides into an oligonucleotide chain. The target 3-(4-nitroazol-1-yl)propane-1,2-diols are considered as alkyl derivatives of azoles. The most-convenient synthesis of such compounds involves the alkylation of azoles with a suitable alkylating agent (an alkyl derivative with a potential nucleofuge such as a halide atom, a tosylate group *etc.*) [25–27] or addition to an activated multiple bond [28][29]. In both cases, the azoles have to be transformed into an active form, commonly an anion. Usually, K_2CO_3 or tertiary amines are sufficiently deprotonating agents [30][31]. More recently, 1,8-diazabicyclo[5.4.0]undec-7-enium salts were introduced [26][27].

2. Results and Discussion. – (+)-(*R*)-3-(4-Nitroazol-1-yl)propane-1,2-diols **3a**–**3c** were obtained from the nucleophilic addition of appropriate nitroazole **1a**–**1c** to the (−)-(*S*)-2-(hydroxymethyl)oxirane (**2**) in the presence of catalytic amounts of anhydrous K_2CO_3 (*Method A*) in satisfactory yields (*Scheme 1* and *Table 1*). The same procedure was applied when (+)-(*R*)-2-(hydroxymethyl)oxirane is used in the synthesis of the (−)-(*S*)-3-(4-nitro-1*H*-pyrazol-1-yl)propane-1,2-diol (**3d**). In the synthesis of (+)-(*R*)-3-(4-nitro-1*H*-imidazol-1-yl)propane-1,2-diol (**3a**), its regioisomeric 5-NO_2 derivative (not shown in *Scheme 1*) was also isolated in low yield. The assignment of these regioisomers was based on comparison of their long-range H,H-coupling constants. For the 1,4-disubstituted 1*H*-imidazole derivative, the *J*(2,5) value was 1.5 Hz, while, for the 5-NO_2 isomer, the ring H-atoms appear as *singlets* in ^{1}H-NMR spectrum, in agreement with the literature reporting the larger coupling constants for 1,4-disubstituted imidazoles (including *N*-alkyl-nitroimidazoles) (typically >1.0 Hz) when compared with the 1,5-disubstituted regioisomers [25][32][33]. In the reaction of

Scheme 1



1a X = CH, Y = N
1b X = CH_3C, Y = N
1c X = N, Y = CH

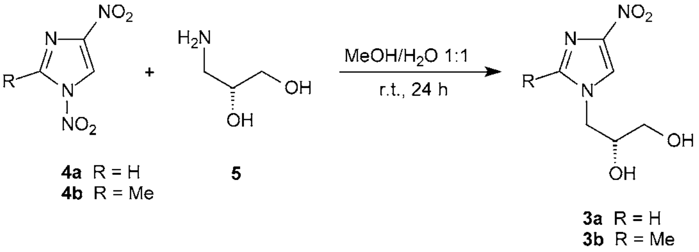
3a X = CH, Y = N; (*R*)
3b X = CH_3C, Y = N; (*R*)
3c X = N, Y = CH; (*R*)
3d X = N, Y = CH; (*S*)

2-methyl-4-nitro-1*H*-imidazole (**1b**) with **2**, only the formation of the 4-NO_2 isomer was observed. To confirm this assignment, compounds **3a** and **3b** were obtained in the reaction of 1,4-dinitro-1*H*-imidazole **4a** and its 2-Me derivative **4b**, respectively, with (+)-(*R*)-3-aminopropane-1,2-diol according to a recently reported method (*Method B*; *Scheme 2*) [32][33]. In this reaction, the so-called degenerate imidazole-ring transformation occurs after nucleophilic attack at C(5) of the imidazole ring by the amino compound. The ring transformation is then followed by elimination of a nitro amide. As a result of this regioselective reaction, only 4-nitro-1*H*-imidazole derivatives were formed with retention of configuration [32][33]. The spectroscopic and optical-rotation data confirm that identical compounds were obtained by these two independent routes. These results also confirm that racemization does not occur with the 2-(hydroxymethyl)oxirane as the alkylating reagent in *Method A*.

Table 1. *Yields and Properties of Chiral Azole Derivatives* **3**

Compound	Configuration of the sterogenic center	*Method*	Yield [%]	$[\alpha]_D$ ($c = 1$, MeOH)
3a	(*R*)	*A*	48	+39.0
		B	59	+39.9
3b	(*R*)	*A*	55	+34.0
		B	84	+31.8
3c	(*R*)	*A*	82	+37.1
		C	70	+36.7
3d	(*S*)	*A*	79	−36.7
		D	49	−36.0

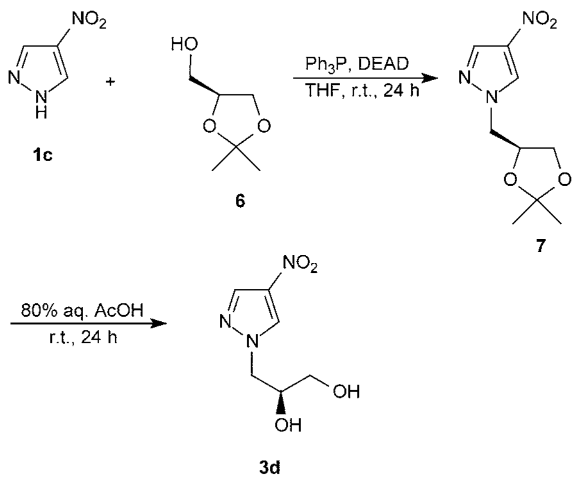
Scheme 2



Only one mono-alkylated product is possible in *Method A* on *N*-alkylation of 4-nitro-1*H*-pyrazole with 2-(hydroxymethyl)oxirane (**2**). With the appropriate pure enantiomers of 2-(hydroxymethyl)oxirane (**2**), the desired (+)-(*R*)-3-(4-nitro-1*H*-pyrazol-1-yl)propane-1,2-diol (**3c**) was obtained in 82% yield and the enantiomeric (−)-(*S*)-3-(4-nitro-1*H*-pyrazol-1-yl)propane-1,2-diol (**3d**) in 79% yield. Two other methods were also considered. Thus, **3c** (with (*R*)-configuration) was obtained by addition of 4-nitro-1*H*-pyrazole (**1c**) to (−)-(*S*)-2-(hydroxymethyl)oxirane (**2**) in refluxing EtOH (*Method C*). In this way, the reaction takes longer time (6 h) than when it is performed in the presence of K_2CO_3 to give the same product with the same optical rotation within the experimental uncertainty. The (*S*)-enantiomer **3d** was also obtained in a reaction of (+)-(*S*)-1,2-isopropylideneglycerol (**6**) with 4-nitro-1*H*-

pyrazole under *Mitsunobu* conditions [34][35], followed by cleavage of the isopropylidene protecting group with 80% AcOH (*Method D*, *Scheme 3*). From optical rotations from different synthetic methods, it is concluded that the alkylation reaction of 4-nitroazoles with 2-(hydroxymethyl)oxiranes proceeds with retention of configuration (*Table 1*).

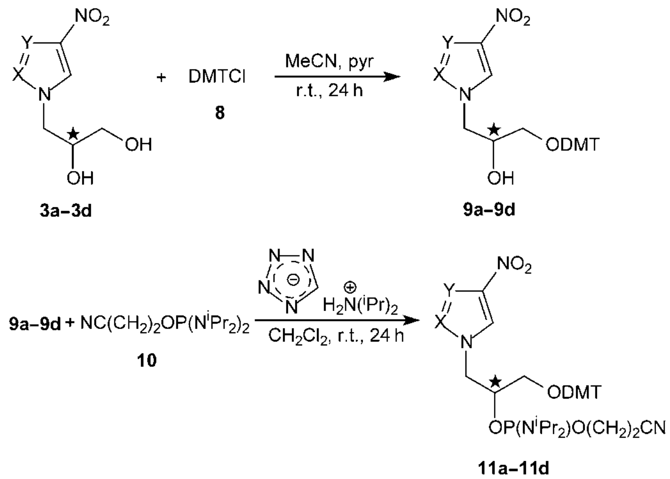
Scheme 3



DEAD = Diethyl azodicarboxylate

For incorporation into oligonucleotides, the 3-(4-nitroazol-1-yl)propane-1,2-diols **3a**–**3d** were converted to 3′-*O*-C(4,4′-dimethoxytrityl) (DMT)-protected 2′-phosphoramidites **11a**–**11d** as outlined in *Scheme 4*. The modifications were incorporated with neighboring Ts in the middle of a 26-mer DNA. Stepwise yields, evaluated by

Scheme 4



spectrophotometric monitoring of DMT release at each cycle of synthesis, were equivalent for the modifications to those obtained with phosphoramidites of natural bases. Melting temperatures with the four natural complementary sequences were determined (*Table 2*). Whereas the acyclic 4-nitro-1*H*-imidazole **3a** gave a range of 4°, the 2-methyl-4-nitro-1*H*-imidazole **3b**, and 4-nitro-1*H*-pyrazole derivatives **3c** and **3d** each gave a very narrow range of 2° in the thermal melting temperatures when they were paired with all natural bases. Melting temperatures of duplexes, however, are lower when compared with those containing natural matching base pairs. It is noteworthy that, in the case of acyclic nucleosides **3c** and **3d**, the configuration of the stereogenic C-atom has no significant influence on the duplex stability.

Table 2. *Synthesised Sequences and Duplex Thermal Melting Temperature* (T_m) *with a 26-mer DNA*

`3'- TGTCAA TTCTTCTT`**`X`**`TTTCT AACTCG -5'`
`5'- ACAGTT AAGAAGAA`**`Y`**`AAAGA TTGAGC -3'`

X,**Y**	A,T	T,A	C,G	G,C
T_m [°]	59	60	60	62
Y	T_m [°]			
	X = **3a**	**X** = **3b**	**X** = **3c**	**X** = **3d**
A	54	54	53	54
C	52	52	55	52
G	53	52	53	53
T	50	52	53	52
MS calc.	7797	7783	7783	7783
MS found	7795	7784	7785	7786

It is interesting to observe that the data reported in *Table 2* for acyclic nitroazoles are in agreement with previously reported data for 1-(2′-deoxy-*β*-D-ribofuranosyl)-3-nitro-1*H*-pyrrole. When acting as a universal base, the T_m varied only 3° when paired with the four natural bases [8]. The duplexes from oligonucleotides with the sugar 3-nitropyrrole in the middle of a T region was reported to melt by 11–14° lower than the corresponding sequences containing an A–T base pair, whereas we observed for the 26-mer only a 5–10° lower melting for all the investigated acyclic azoles **3a**–**3d** in a T region when compared with the A–T base pair. In fact, the acyclic pyrazole derivative with (*R*)-configuration lowered the thermal melting by only 5–7°. This is even lower than what has previously been observed for the sugar 3-nitropyrrole when placed between two C–G base pairs where the modification lowered the thermal melting by 8–11°. However, the shorter 18-mer DNA sequence (*Table 3*) was more sensitive to azole modifications **3a**–**3c**, and the melting was lowered by 10–13° in a T region when compared with the A–T base pair. Concerning the use of **3a**–**3c** as a universal base, it should be noted that T_m also for the shorter sequence varied only by 3° when paired with the four natural bases, which is in the same range as observed for the longer 26-mer DNA shown in *Table 2*. For these reasons, we believe it will be an advantage to use acyclic nitroazoles as universal bases. Another advantage of their use in this context is their simple and straightforward synthesis.

Table 3. *Synthesised Sequences and Duplex Thermal Melting Temperature* (T_m) *with an 18-mer DNA*

3′- TGTCAA TTCTTCTT**X**TTTCT AACTCG -5′
5′- AAGAAGAA**Y**AAAGA TTGA -3′

X,**Y**	A,T	T,A	C,G	G,C
T_m [°]	47	48	49	53

Y	T_m [°]		
	X = **3a**	**X** = **3b**	**X** = **3c**
A	36	36	37
C	34	35	35
G	36	36	35
T	34	34	35

For triplex studies, a 26-mer oligonucleotide duplex and a 14-mer oligonucleotide containing a nitroazole in the middle were used (*Table 4*). Single incorporation of 4-nitroazole units into 14-mer oligonucleotides resulted in decreased triplex stabilities, which were even lower than those observed for natural non-matching nucleosides in the triplex forming oligonucleotide. When a single base pair was inverted within the duplex, the acyclic azoles, also in all cases, afforded lower triplex stabilities than a natural base at the same position in the triplex-forming oligonucleotide. This is in agreement with a previous investigation of the sugar 3-nitropyrrole in the same sequences where substantial lowering of the triplex melting temperatures were observed [36].

Table 4. *Synthesised Sequences and Their Thermal Melting Temperatures* (T_m) *in DNA-DNA-DNA Triplexes*

3′- TGTCAA TTCTTCTT**X**TTTCT AACTCG -5′
5′- ACAGTT AAGAAGAA**Y**AAAGA TTGAGC -3′
5′- TTCTTCTT**Z**TTTCT -3′

	X-Y				Masses of 14-mers	
	T-A	A-T	G-C	C-G	MS	
Z	T_m [°]	T_m [°]	T_m [°]	T_m [°]	calc.	found
A	18	17	16	29		
C	13	15	22	44		
G	< 5	30	20	22		
T	37	14	27	26		
3a	11	12	14	15	4108	4110
3b	< 5	11	15	13	4094	4095
3c	< 5	11	13	20	4094	4094
3d	11	< 5	14	< 5	4094	4095

Experimental Part

General. 4(5)-Nitro-1*H*-imidazole **1a** [37], 4-nitro-1*H*-pyrazole (**1c**) [38], 1,4-dinitro-1*H*-imidazole (**4a**), and 2-methyl-1,4-dinitro-1*H*-imidazole (**4b**) [39] were obtained according to literature procedures. TLC: TLC plates *60 F_{254}* (*Merck*), visualization by UV light (254 nm) or with a 5% soln. of H_2SO_4 in MeOH containing

0.3% of vanillin. Column chromatography (CC) was carried out on silica-gel-packed column (silica gel *60*, 0.040–0.063 mm, *Merck*). NMR Spectra: *Varian Gemini-2000* spectrometer (^{1}H: 300 MHz, ^{13}C: 75.5 MHz, ^{31}P: 121.5 MHz); δ values in ppm relative to Me_4Si as internal standard (^{1}H-NMR); for ^{13}C-NMR: $CDCl_3$ (δ 77.0), CD_3CN (δ 118.3), (D_6)DMSO (δ 39.9); 85% H_3PO_4 as an external standard for ^{31}P-NMR spectra. EI-MS: *Finnigan MAT SSQ 701* mass spectrometer, MALDI experiments: *FTICR MS* ion spectrometer.

Elemental analyses were performed by the microanalytical laboratory at Copenhagen University.

1. *3-(4-Nitroazol-1-yl)propane-1,2-dioles* **3a**–**3d** (*Method A*). To a soln. of the appropriate nitroazole **1a**–**1c** (5 mmol) in DMF (7 ml) was added anh. K_2CO_3 (10 mg, 7.2×10^{-3} mmol), and the soln. was heated to reflux. The soln. of *2-(hydroxymethyl)oxirane* (**2**; (*R*) or (*S*); 5 mmol) in DMF (2 ml) was added dropwise. Refluxing was continued for 1 h, and the soln. was cooled to r.t. H_2O (2 ml) was added, followed by addition of a few drops of an aq. HCl soln. (5%) to pH 7. The soln. was evaporated under reduced pressure. The oily residue was purified by CC (AcOEt/cyclohexane 1:1 (*v*/*v*)). The products were recrystallized from MeOH.

*Data of (+)-(*R*)-3-(4-Nitro-1*H*-imidazol-1-yl)propane-1,2-diol* (**3a**): yield 0.45 g (48%). M.p. 136–137°. ^{1}H-NMR ((D_6)DMSO): 8.31 (*d*, *J* = 1.5, H–C(5′)); 7.80 (*d*, *J* = 1.5, H–C(2)); 5.20 (*d*, *J* = 4.8, OH–C(2′)); 4.88 (br. *s*, OH–C(1)); 4.22 (*dd*, *J* = 3.0, 13.8, H_a–C(3)); 4.01 (*dd*, *J* = 7.5, 13.8, H_b–C(3)); 3.77 (*m*, H–C(2)); 3.43–3.39 (*m*, 2 H–C(1)). ^{13}C-NMR ((D_6)DMSO): 146.66 (C(4′)); 137.96 (C(2′)); 122.25 (C5′)); 70.06 (C(2)); 62.84 (C(1)); 50.64 (C(3)). EI-MS: 187 (10, M^+), 157 (20), 127 (100), 114 (80), 61 (55), 43 (65). Anal. calc. for $C_6H_9N_3O_4$ (187.16): C 38.51, H 4.85, N 22.45; found: C 38.32, H 4.74, N 23.03.

*Data of (*R*)-3-(5-Nitro-1*H*-imidazol-1-yl)propane-1,2-diol* (Regioisomer of **3a**): yield 0.10 g (12%), semisolid. ^{1}H-NMR ((D_6)DMSO): 8.07 (*s*, H–C(4′)); 7.98 (*s*, H–C(2′)); 5.09 (*d*, *J* = 5.7, OH–C(2)); 4.84 (*t*, *J* = 5.8, OH–C(1)); 5.58 (*dd*, *J* = 3.0, 13.7, H_a–C(3)); 4.16 (*dd*, *J* = 9.0, 13.7, H_b–C(3); 3.75–3.70 (*m*, H–C(2)); 3.44–3.29 (*m*, 2 H–C(1)). ^{13}C-NMR ((D_6)DMSO): 144.27 (C(2′)); 139.42 (C(5′)); 133.65 (C(4′)); 70.01 (C(2)); 63.89 (C(1)); 50.86 (C(3)). EI-MS: 188 (10, $[M+1]^+$), 187 (9, M^+), 141 (50), 140 (100), 127 (84), 110 (38), 98 (90), 97 (64), 81 (35), 67 (38), 61 (39), 53 (76).

*Data of (+)-(*R*)-3-(2-Methyl-4-nitro-1*H*-imidazol-1-yl)propane-1,2-diol* (**3b**): yield 0.56 g (55%). M.p. 130–131°. ^{1}H-NMR ((D_6)DMSO): 8.20 (*s*, H–C(5′)); 5.19 (*d*, *J* = 5.4, OH–C(2)); 4.89 (*t*, *J* = 4.8, OH–C(1)); 4.16 (*dd*, *J* = 3.3, 14.1, H_a–C(3)); 3.91 (*dd*, *J* = 7.8, 14.1, H_b–C(3)); 3.76 (*m*, H–C(2)); 3.46–3.36 (*m*, 2 H–C(1)); 2.38 (*s*, Me). ^{13}C-NMR ((D_6)DMSO): 145.62 (C(4′)); 145.12 (C(2′)); 122.83 (C(5)); 70.24 (C(2)); 62.93 (C(1)); 49.64 (C(3)); 12.83 (Me). EI-MS: 201 (16, M^+), 171 (16), 141 (32), 128 (38), 69 (16), 61 (17), 43 (100). Anal. calc. for $C_7H_{11}N_3O_4$ (201.18): C 41.79, H 5.51, N 20.89; found: C 41.77, H 5.56, N 20.87.

*Data of (+)-(*R*)-3-(4-Nitro-1*H*-pyrazol-1-yl)propane-1,2-diol* (**3c**): yield 0.77 g (82%). M.p. 108–109°. ^{1}H-NMR ((D_6)DMSO): 8.76 (*s*, H–C(3′)); 8.26 (*s*, H–C(5′)); 5.11 (br. *s*, OH–C(2)); 4.83 (br. *s*, OH–C(1)); 4.31 (*dd*, *J* = 3.4, 14.1, H_a–C(3)); 4.09 (*dd*, *J* = 8.1, 14.1, H_b–C(3)); 3.86 (*m*, H–C(2)); 3.43 (*dd*, *J* = 4.6, 10.8, H_a–C(1)); 3.35 (*dd*, *J* = 6.0, 10.8, H_b–(C(1)). ^{13}C-NMR ((D_6)DMSO): 135.88 (C(5′)); 135.73 (C(4′)); 131.25 (C(3′)); 70.33 (C(2)); 63.35 (C(1)); 56.64 (C(3)). EI-MS: 188 (100, M^+), 170 (10), 156 (36), 126 (12), 114 (4). Anal. calc. for $C_6H_9N_3O_4$ (187.16): C 38.51, H 4.85, N 22.45; found: C 38.29, H 4.77, N 22.33.

*Data of (−)-(*S*)-3-(4-Nitro-1*H*-pyrazol-1-yl)propane-1,2-diol* (**3d**): yield 0.74 g (79%). M.p. 111–112°. ^{1}H-NMR ((D_6)DMSO): 8.76 (*s*, H–C(3′)); 8.27 (*s*, H–C(5′)); 5.12 (*d*, *J* = 5.1, OH–C(2)); 4.84 (*t*, *J* = 5.4, OH–C(1)); 4.30 (*dd*, *J* = 3.6, 13.8, H_a–C(3)); 4.08 (*dd*, *J* = 8.4, 13.8, H_b–C(3)); 3.85 (*m*, H–C(2)); 3.39–3.30 (*m*, 2 H–C(1)). ^{13}C-NMR ((D_6)DMSO): 135.88 (C(5′)); 135.73 (C(4′)); 131.25 (C(3′)); 70.33 (C(2)); 63.35 (C(1)); 56.64 (C(3)). EI-MS: 188 (100, M^+), 170 (10), 156 (36), 126 (12), 114 (4). Anal. calc. for $C_6H_9N_3O_4$ (187.16): C 38.51, H 4.85, N 22.45; found: C 38.41, H 4.81, N 22.38.

2. *Propane-1,2-diols* **3a** *and* **3b** (*Method B*). To the soln. of *(+)-(*R*)-3-aminopropane-1,2-diol* (**5**; 0.46 g, 5 mmol) in aq. MeOH 1:1 (10 ml) was added 1,4-dinitro-1*H*-imidazole (5 mmol; 0.79 g of **4a** or 0.86 g of **4b**) in two portions while stirring. The resulting yellow-reddish soln. was stirred overnight and then concentrated under reduced pressure. The oily residue was purified on a silica-gel column with $MeOH/CHCl_3$ 1:9. **3a**: 0.55 g (59%). M.p. 134–135°; **3b**: 0.85 g (84%). M.p. 129–130°.

3. *Compound* **3c** (*Method C*). To the soln. of (−)-(*S*)-**2** (0.37 g, 5 mmol) in anh. EtOH (20 ml) was added **1c** (0.56 g, 5 mmol). The mixture was refluxed for 6 h, evaporated to dryness, and purified by CC (AcOEt/cyclohexane 1:1). **3c**: 0.66 g (70%). M.p. 109–110°.

4. *Compound* **3d** (*Method D*). To a soln. of **1c** (0.56 g, 5 mmol) and 1,2-*O*-isopropylidene-D-glycerol (**6**; 0.66 g, 5 mmol) in anh. THF (20 ml) was added Ph_3P (1.97 g, 7.5 mmol), followed by dropwise addition of diethyl azodicarboxylate (DEAD; 1.31 g, 7.5 mmol) while stirring. The soln. was stirred for 24 h and after evaporation purified on a silica-gel column (cyclohexane/AcOEt 1:1). The product *1-[(2,2-dimethyl[1,3]dioxolan-4-yl)methyl]-4-nitro-1*H*-pyrazole* (**7**) was obtained as colorless oil in 69% yield (0.79 g). ^{1}H-NMR ($CDCl_3$): 8.29 (*s*, H–C(3)); 8.09 (*s*, H–C(5)); 4.50 (*m*, H–C(4′)); 4.36 (*dd*, *J* = 3.8, 14.2, 1 H, CH_2); 4.23

(*dd*, *J* = 6.8, 14.2, 1 H, CH_2); 4.15 (*dd*, *J* = 6.5, 8.7, $H_a-C(5')$); 3.80 (*dd*, *J* = 5.7, 8.7, $H_b-C(5')$); 1.48, 1.36 (2*s*, 2 Me). ^{13}C-NMR ($CDCl_3$): 135.71 (C(5)); 129.75 (C(3)); 121.55 (C(4)); 110.20 (C(2′)); 73.61 (C(4′)); 66.12 (C(5′)); 55.50 (CH_2); 26.60, 24.97 (2 Me). EI-MS: 228 (22, $[M+1]^+$), 212 (100), 170 (20), 152 (10).

Compound **7** (0.79 g, 3.5 mmol) was dissolved in 80% AcOH (2 ml) and stirred 24 h at r.t. TLC (AcOEt/cyclohexane, 1 : 1) indicated complete consumption of the starting material. The soln. was evaporated to dryness under reduced pressure and purified by silica-gel CC with the same eluent as in *Method A*. **3d**: 0.46 g (71%). M.p. 110 – 111°.

4. *1-[Bis(4-methoxyphenyl)(phenyl)methoxy]-3-(4-nitro-1*H*-imidazol-1-yl)propan-2-ol* (**9a**). To a suspension of **3a** (0.25 g, 1.34 mmol) in anh. MeCN (15 ml) containing anh. pyridine (0.4 ml, 5 mmol) was added 4,4′-dimethoxytrityl chloride (DMTCl; 0.5 g, 1.48 mmol) while stirring. The suspension dissolved immediately, and, after 24 h, the reaction was terminated by addition of MeOH (2 ml). The solvent was removed by evaporation under reduced pressure, and the residue was dissolved in CH_2Cl_2 (20 ml), washed with a sat. aq. soln. of $NaHCO_3$ (2 × 5 ml), dried (Na_2SO_4), and evaporated to dryness. Purification by silica-gel CC (cyclohexane/AcOEt/Et_3N 49 : 49 : 2) gave **9a** (0.55 g, 85%). Pale yellow solid. M.p. 127 – 128°. ^{1}H-NMR ($CDCl_3$): 7.69 (*d*, *J* = 1.5, H−C(5′)); 7.40 (*d*, *J* = 1.5, H−C(2′)); 7.32 – 7.28 (*m*, Ph, C_6H_4); 8.84 (2*d*, *J* = 9.0, C_6H_4); 4.17 – 4.04 (*m*, 2 H−C(3), H−C(2)); 3.78 (*s*, 2 MeO); 3.42 (br. *s*, OH); 3.25 (*dd*, *J* = 4.6, 9.6, $H_a-C(1)$); 3.06 (*dd*, *J* = 5.7, 9.6, $H_b-C(1)$). ^{13}C-NMR ($CDCl_3$): 158.64, 147.44, 144.25, 136.81, 135.32, 135.24, 129.84, 127.99, 127.92, 127.80, 127.05, 120.30, 113.28 (arom. C); 86.65 (Ar_3C), 69.27 (C(2)); 63.93 (C(1)); 55.20 (2 MeO); 51.31 (C(3)). EI-MS: 489 (2, M^+), 303 ($(MeOC_6H_4)_2CPh^+$, 100), 135 (16), 127 (20), 114 (16), 77 (10).

6. *1-[Bis(4-methoxyphenyl)(phenyl)methoxy]-3-(2-methyl-4-nitro-1*H*-imidazol-1-yl)propan-2-ol* (**9b**). This compound was prepared similarly to **9a** with **3b** (0.2 g, 1 mmol), MeCN (10 ml), pyridine (0.16 ml, 2 mmol), and DMTCl (0.38 g, 1.1 mmol). Yield 0.39 g (78%). M.p. 117 – 118°. ^{1}H-NMR ($CDCl_3$): 7.64 (*s*, H−C(5′)); 7.44 – 7.29 (*m*, Ph, C_6H_4); 6.85 (*dd*, 4 H, *J* = 9.0, C_6H_4); 4.13 – 4.06 (*m*, $H_a-C(3)$, H−C(2)); 3.93 (*dd*, *J* = 6.6, 9.8, $H_b-C(3)$); 3.80 (*s*, 2 MeO); 3.41 (br. *s*, OH); 3.26 (*dd*, *J* = 5.4, 9.8, $H_a-C(1)$); 3.18 (*dd*, *J* = 5.4, 9.8, $H_b-C(1)$); 2.37 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 145.45 (C(4′)); 144.29 (C(2′)); 120.58 (C(5′)); 158.70, 135.32, 135.29, 129.88, 128.02, 127.85, 127.10, 113.31 (arom. C); 86.75 (Ar_3C), 69.72 (C(2)); 64.35 (C(1)); 55.23 (MeO); 50.08 (C(3)); 13.18 (Me). EI-MS: 503 (2, M^+), 303 (100, DMT^+), 288 (10), 215 (20), 152 (15), 128 (15), 77 (10). Anal. calc. for $C_{28}H_{29}N_3O_6$ (503.56): C 66.79, H 5.81, N 8.34; found: C 67.05, H 6.04, N 8.21.

7. *(*R*)- and (*S*)-1-[Bis(4-methoxyphenyl)(phenyl)methoxy]-3-(4-nitro-1*H*-pyrazol-1-yl)propan-2-ols* (**9c** and **9d**, resp.). Compound **3c** or **3d** (0.37 g, 2 mmol) was dissolved in anh. MeCN containing anh. pyridine (0.4 ml). DMTCl (0.75 g, 2.2 mmol) was added, and the mixture was stirred for 12 h. The reaction was terminated by addition of MeOH (2 ml), and the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (20 ml), washed with sat. aq. $NaHCO_3$ (2 × 5 ml), dried (Na_2SO_4), and evaporated to dryness. Purification by CC (cyclohexane/AcOEt/Et_3N 49 : 49 : 2) gave **9c** or **9d** as pale yellow solids.

Data of **9c**: yield 0.84 g (86%). M.p. 77 – 78°. ^{1}H-NMR ($CDCl_3$): 8.10 (*s*, H−C(3′)); 7.96 (*s*, H−C(5′)); 7.42 – 7.21 (*m*, Ph, C_6H_4); 6.83 (*d*, *J* = 8.7, C_6H_4); 4.28 (*dd*, *J* = 6.3, 14.2, $H_a-C(3)$); 4.17 (*m*, H−C(2)); 4.10 (*dd*, *J* = 7.1, 14.2, $H_b-C(3)$); 3.77 (*s*, 2 MeO); 3.16 (*dd*, *J* = 5.3, 9.7, $H_a-C(1)$); 3.09 (*dd*, *J* = 5.4, 9.7, $H_b-C(1)$). ^{13}C-NMR ($CDCl_3$): 158.62, 144.26, 135.77, 135.21, 135.29, 129.86, 127.95, 127.86, 127.05, 113.22 (arom. C), 86.53 (Ar_3C), 69.39 (C(2)); 63.87 (C(1)); 55.81 (C(3)); 55.20 (MeO); 55.19 (MeO). EI-MS: 489 (10, M^+), 303 (100, DMT^+). Anal. calc. for $C_{27}H_{27}N_3O_6$ (489.53): C 66.25, H 5.56, N 8.58; found: C 66.43, H 5.71, N 8.34.

Data of **9d**: yield 0.66 g (68%).

8. *1-{[3-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-[(2-cyanoethoxy)(diisopropylamino)phosphinoxy]-propyl}-4-nitro-1*H*-azoles* **11a – 11d**. *General Procedure.* Compounds **9a – 9d** (1 equiv.) was dissolved in anh. and acid-free CH_2Cl_2 (10 ml), 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (1 equiv.) was added, followed by addition of diisopropylammonium tetrazole salt (1.5 êquiv.). The mixture was stirred at r.t. for 12 h, diluted with CH_2Cl_2 (20 ml), and washed with sat. aq. $NaHCO_3$ (2 × 5 ml) and brine (2 × 5 ml). The org. layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by CC (cyclohexane/AcOEt/Et_3N 8 : 2 : 1). In all cases, the product was obtained as a diastereoisomeric mixture in the form of an amorphous colorless solid.

*Data of 1-{3-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-[(2-cyanoethoxy)(diisopropylamino)phosphinoxy]-propyl}-4-nitro-1*H*-imidazole* (**11a**): yield 0.30 g (70%), starting from 0.27 g (0.55 mmol) of **9a**. ^{1}H-NMR ($CDCl_3$): 7.75 (*s*, H−C(5)); 7.72 (*s*, H−C(5)); 7.38 – 7.08 (*m*, 2 H−C(2), 2 Ph, 4 C_6H_4); 6.77 – 6.72 (*m*, 2 C_6H_4); 4.23 – 4.16 (*m*, 2 H−C(2′)); 3.65 (*s*, 2 MeO); 3.64 (*s*, 2 MeO); 3.62 – 3.37 (*m*, 2 H−C(3′), 4 (CH)N, 2 CH_2O); 3.14 (*dd*, *J* = 3.6, 9.6, $H_a-C(1')$); 3.06 (*dd*, *J* = 3.6, 9.6, $H_b-C(1')$); 2.85 (*dd*, *J* = 6.4, 9.6, $H_a-C(1')$); 2.69 (*dd*, *J* = 6.4, 9.6, $H_b-C(1')$); 2.50 (*t*, *J* = 6, CH_2CN); 2.33 (*t*, *J* = 6.0, CH_2CN); 1.08 – 0.90 (*m*, 4 Me_2C). ^{31}P-NMR ($CDCl_3$): 150.40; 149.73.

*Data of 1-{3-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-[(2-cyanoethoxy)(isopropylamino)phosphinoxy]propyl}-2-methyl-4-nitro-1*H*-imidazole* (**11b**): yield 0.19 g (66%), starting from 0.21 g (0.42 mmol) of **9b**. ^{1}H-NMR (CDCl$_3$): 7.74 (*s*, H–C(5)); 7.67 (*s*, H–C(5)); 7.43–7.39 (*m*, 8 H, Ar); 7.32–7.23 (*m*, 12 H, Ar); 6.85–6.80 (*m*, 6 H, Ar); 4.24–4.15 (*m*, 2 H–C(2′)); 4.14–4.07 (*dd*, $J = 7.3$, 14.3, 4 H–C(3′)); 3.78 (*s*, 2 MeO); 3.77 (*s*, 2 MeO); 3.74–3.25 (*m*, 4 (CH)N, 2 CH$_2$O, 2 H–C(1′)); 2.54–2.58 (*m*, 2 CH$_2$CN); 2.41 (*s*, Me); 2.39 (*s*, Me); 1.28–1.04 (*m*, 4 Me$_2$C). ^{31}P-NMR (CDCl$_3$): 151.13; 150.50.

*Data of 1-{3-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-[(2-cyanoethoxy)(diisopropylamino)phosphinoxy]propyl}-4-nitro-1*H*-pyrazole* (**11c**): yield 0.24 g (74%), starting from 0.23 g (0.47 mmol) of **9c**. ^{1}H-NMR (CD$_3$CN): 8.21 (*s*, 1 H–C(3)); 8.18 (*s*, 1 H–C(3)); 7.92 (*s*, 1 H–C(5)); 7.90 (*s*, 1 H–C(5)); 7.36–7.11 (*m*, 18 H, Ar); 6.77–6.72 (*m*, 8 H, Ar); 4.38–4.16 (*m*, 2 H–C(2′)); 3.65 (*s*, 2 MeO); 3.64 (*s*, 2 MeO); 3.61–3.58 (*m*, 4 (CH)N, 2 CH$_2$O, 2 H$_a$–C(3′); 2 H$_b$–C(3′), 2 H$_a$–C(1′), 2 H$_b$–C(1′)); 2.49 (*t*, $J = 6.1$, CH$_2$CN); 2.33 (*t*, $J = 6.1$, CH$_2$CN); 1.02–0.91 (*m*, 4 Me$_2$C). ^{31}P-NMR (CD$_3$CN): 150.28; 150.12.

*Data of 1-{3[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-[(2-cyanoethoxy)(diisopropylamino)phosphinoxy]propyl}-4-nitro-1*H*-pyrazole* (**11d**): yield 0.49 g (70%), starting from 0.49 g (1.0 mmol) of **9d**. ^{31}P-NMR (CD$_3$CN): 150.25; 150.09.

9. *ODN Synthesis, Purification, and Measurement of Melting Temperatures.* The ODN synthesis was carried out on an *Expedite*™ nucleic acid synthesis system model *8909* from *Applied Biosystems*. The appropriate nitroimidazole/pyrazole amidites **11a**–**11d** were dissolved in dry MeCN, as a 0.075M soln. and inserted into the growing oligonucleotides chain under the same conditions as for normal nucleotide couplings (2-min coupling time): the coupling efficiency of the modified nucleotide was > 99%. The ODNs were synthesized with DMT on and purified on a *Waters Prep LC 4000 HPLC* with a *Waters Prep LC* controller and a *Waters 2487 Dual λ* absorbance detector on a *Waters Xterra*™ *MS C$_{18}$* column. Buffer *A:* 950 ml of 0.1M NH$_4$HCO$_3$ and 50 ml of MeCN (pH 9.0), and buffer *B:* 250 ml of 0.1M NH$_4$HCO$_3$ and 750 ml of MeCN (pH 9.0). Gradients: 5 min 100% *A*, linear gradient to 70% *B* in 30 min, 2 min with 70% *B*, linear gradient to 100% *B* in 8 min and then 100% *A* in 15 min (product peak at *ca.* 35 min). The ODNs were DMT-deprotected in 100 μl of 80% AcOH. After 20 min, 100 μl of H$_2$O, 50 μl of a 3M AcONa, and 600 μl of abs. EtOH were added. The ODNs were kept at −20° for 1/2 h or until precipitation occurred. They were then centrifuged for 15 min upon cooling. The soln. was decanted off, and the ODNs were dried in a vacuum centrifuge. All modified ODNs were analyzed by MALDI-TOF-MS on a *Voyager Elite Bio* spectrometry research station from *Perceptive Biosystems*.

Melting-temp. measurements were performed on a *Perkin-Elmer* UV/VIS spectrometer fitted with a *PTP-6* temp. programmer. Melting-temp. (T_m) measurements for triplex studies were performed in a 20 mM sodium cacodylate, 100 mM NaCl, 10 mM MgCl$_2$ buffer at pH 6.0 for the 26-mer strands. The triplexes were formed by first mixing the two 26-mer strands, each at a concentration of 1.0 μM, and then adding 1.5 μM of the 14-mer strand. Melting-temp. measurements for duplex studies were conducted in a 1 mM EDTA, 10 mM Na$_2$HPO$_4$ · 2 H$_2$O, 140 mM NaCl buffer at pH 7.0 for 1.0 μM of each strand. The melting temp. was determined as the maximum of the first derivative plots of the melting curve and are with an uncertainty ± 1.0°.

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