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Syntheses and Base-Pairing Properties of Locked Nucleic Acid Nucleotides Containing Hypoxanthine, 2,6-Diaminopurine, and 2-Aminopurine Nucleobases

Alexei A. Koshkin

Department of Chemistry, Exiqon A/S, Bygstubben 9, DK-2950 Vedbæk, Denmark

koshkin@exiqon.com

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Second generation 2′-*O*,4′-*C*-methylene-linked nucleotides **1**−**3** containing hypoxanthine, 2,6-diaminopurine, and 2-aminopurine nucleobases were synthesized and incorporated into locked nucleic acid (LNA) oligonucleotides by means of the automated phosphoramidite method. The required phosphoramidite monomeric units were efficiently prepared via convergent synthesis. The glycosyl donor **4** was stereoselectively coupled with hypoxanthine and 6-chloro-2-aminopurine to give the 4′-*C*-branched nucleosides **5** and **17**. The methods for conversion of **5** and **17** into phosphoramidites **11**, **25**, and **29** were developed and described in full details for the first time. Hybridization properties of LNA octamers containing the new LNA nucleotides were assessed against perfect and singly mismatched DNA. The binding studies revealed that all LNA octamers hybridize very efficiently to DNA following Watson−Crick base-pairing rules with increased binding affinity compared to the DNA analogues. The unique properties of the nucleotides **1**−**3** make them very useful for further strengthening of the LNA technology.

Introduction

LNA (locked nucleic acid) is a novel class of recently introduced conformationally restricted oligonucleotide analogues.[1] By virtue of the fixed 2′,4′-bicyclic structure, LNA oligonucleotides were shown to possess an extremely potent hybridizing ability toward complementary single stranded nucleic acids.[1,2] Furthermore, it was found that LNA modifications of triplex-forming oligonucleotides (TFO) significantly enhance the binding affinity toward target dsDNA.[3] Along with the increased stability in vivo, these properties have established LNAs as very attractive tools for designing oligonucleotide-based antisense and/or antigene therapeutics.[4] Additionally, the remarkably high affinities of LNAs toward complementary nucleic acids in combination with generally increased selectivity paved the way for successful use of LNA in diagnostics, e.g. as capture probes for production of oligonucleotide microarrays.[5]

The widespread use of LNA oligonucleotides became possible due to the progress in LNA chemistry achieved during the past few years providing a reliable source of LNA nucleosides containing the natural nucleobases.[6] Broadening the range of available LNA nucleosides will further strengthen the LNA technology. In this context, modification of the natural nucleobases represents an obvious way to control the recognition between nucleic acids. Moreover, knowledge of specific properties of LNA nucleosides containing modified nucleobases can provide information leading to a better understanding of the mechanisms of LNA action. Thus, analogous to DNA, the modification of nucleobases in LNA can be used to study LNA biochemistry.

In the present report, the total synthesis and base-pairing properties of three new purine LNA nucleosides containing hypoxanthine (LNA-I), 2,6-diaminopurine

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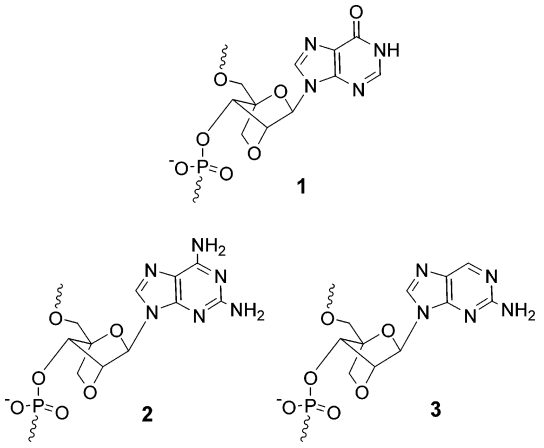


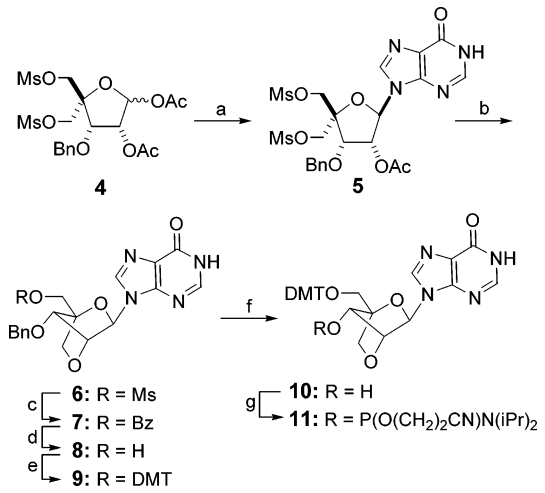
FIGURE 1. Structures of the LNA-I (**1**), LNA-D (**2**), and LNA-2AP (**3**) mononucleotides.

(LNA-D), and 2-aminopurine (LNA-2AP) nucleobases (Figure 1) are described for the first time. The ribosides containing these nucleobases are among the most intensively studied nucleoside analogues, which provided the rationale for their selection as candidates for designing second-generation LNA nucleosides.

Hypoxanthine, the base found in the nucleosides inosine and deoxyinosine, is considered as a guanine analogue in nucleic acids. These nucleosides were used for many years as universal bases in a number of applications in primers[7] and in probes for hybridization.[8]

2,6-Diaminopurine (D) is a nonnatural nucleoside analogue introduced by chemists to increase the duplex stability. Oligonucleotides containing 2,6-diaminopurine replacements for adenines are expected to bind more strongly to their complementary sequences due to the potential formation of three hydrogen bonds with thymine or uracil.[9] However, in some cases the stability of short modified double-stranded DNA turned out to be lower when compared to unmodified duplexes.[10] Importantly, much higher stabilization effects were observed when D's were involved in the formation of duplexes with a tendency to form A-type helixes (as in LNA containing duplexes[2]). This effect has previously been shown for RNA[11] and anhydrohexitol[12] duplexes. The binding properties of oligonucleotides containing D's were directly studied with a microarray, a so-called generic microchip, showing both increased stabilization and mismatch discrimination.[13] Additionally, in combination with 2-thiothymidine, the D nucleoside was successfully used for construction of selectively binding complementary (SBC)

SCHEME 1[a]



[a] Reagents and conditions: (a) hypoxanthine, BSA, trimethylsilyl triflate, 1,2-dichloromethane; 93% (mixture *N*-9/*N*-7 ≈ 4:1); (b) NaOH, THF, EtOH, H_2O; 68%; (c) NaOBz, DMSO; 76%; (d) NaOH, THF, MeOH, H_2O; 85%; (e) DMT-Cl, pyridine; 92%; (f) 10% Pd/C, HCO_2NH_4; 79%; (g) 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite, 4,5-dicyanoimidazole (DCI), CH_2Cl_2, EtOAc, 91%.

oligonucleotides.[14] Taking the extremely high stability of LNA·LNA duplexes[1d] into account, this approach seems very useful for solving the problem of LNA self-complementarity in LNA containing capture probes and antisense reagents that can arise from designing palindromic sequences.

2-Aminopurine (2AP) is a mildly fluorescent nucleobase with emission at 363 nm. The ability of photoexited 2AP to serve as a sensitive probe of DNA structural dynamics was illustrated in a diverse array of studies.[15] It is especially useful as it is capable of hybridizing with thymine in Watson−Crick geometry.

Results and Discussion

So far, two strategies have been employed for the synthesis of LNA nucleosides: a linear strategy where the natural ribonucleosides act as starting materials[1c,d,3a] and a convergnt strategy in which an appropriate glycosyl donor is coupled to a nucleobase.[1b,1d,6] Importantly, for commercially available LNA nucleosides with natural nucleobases the convergent strategy has established itself as the method of choice.[6] Taking advantage of the flexibility offered by the convergent approach, we therefore implemented this approach for the synthesis of modified LNA nucleotides **1**−**3** (Figure 1). The synthetic route to LNA-I phosphoramidite **11** is depicted in Scheme 1. The previously described 4-*C*-branched furanose **4**[6] was used as a glycosyl donor in coupling reaction with silylated hypoxanthine by the method of Vorbrüggen et

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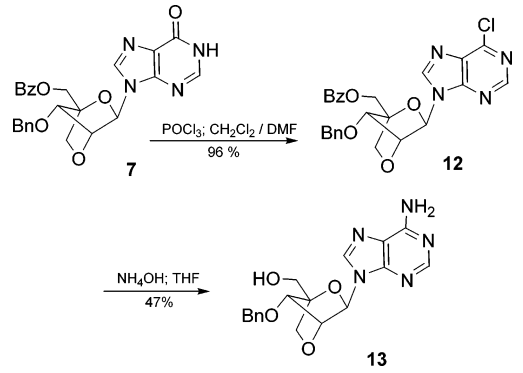
al.[16] As expected, the reaction resulted in the high yield formation of the desired β-configured nucleoside derivative **5**. However, analogous to the coupling reaction of **4** with protected guanines,[6] the formation of the undesired *N*-7 isomer (ratio of *N*-9/*N*-7 $\approx$ 4:1) was also detected. The isomers demonstrated extremely close chromatographic mobilities during silica gel TLC and reverse-phase (RP) HPLC at neutral pH (system I). Nevertheless, complete separation of the isomers was observed during RP-HPLC at elevated pH (system II; see Experimental Section and Supporting Information), apparently due to the different pK_a values of *N*-7 and *N*-9 glycosylated nucleobases. The mixture of isomers was used for the ring closure reaction without complete isolation of **5** to give the protected LNA nucleoside **6** in 68% yield after crystallization from the neutralized reaction mixture. Chemical conversion of LNA-I into LNA-A nucleoside (vide infra) later confirmed the correct structure of the isolated isomer **6**. Noteworthy, the elemental analysis of **6** showed the presence of residual amounts of water even after intensive drying of the compound for several days. A similar problem was already reported for a number of other LNA nucleosides.[3a,6] The difficulties with complete removal of residual solvents from LNA samples seems to be a generic problem for this class of compounds probably caused by a fixed rigid structure of LNA molecules.

Mild removal of the 5′-*O*-mesyl group from **6** was accomplished by a two-step procedure as reported for other LNA nucleosides.[6] First, the 5′-methylate moiety was efficiently replaced by sodium benzoate to produce nucleoside **7** in 76% yield after chromatographic purification. Then, simple saponification of **7** with aqueous sodium hydroxide afforded the 5′-hydroxy derivative **8** as a crystalline product, which crystallized from the reaction mixture in 85% yield after addition of acetic acid. The direct removal of the 3′-*O*-benzyl group from compound **8** was attempted but failed in our hands due to a solubility problem. Therefore, **8** was initially converted into the 5′-*O*-DMT-protected nucleoside **9** by treatment with DMT-chloride in hot pyridine. Catalytic hydrogenation of **9** proceeded in 77% yield without incident to give the 3′-hydroxy derivative **10**. The conventional phosphitylation[17] of **10** finally afforded the phosphoramidite monomer **11**.

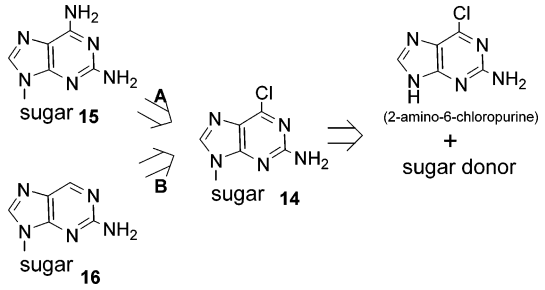
To verify the correct orientation of the glycosidic bond (*N*-9 isomer) in the synthesized LNA-I monomer, compound **7** was successfully converted into the known adenosine derivative **13**[6] (Scheme 2). Thus, phosphoryl chloride treatment[18] of **7** resulted in formation of the intermediate 6-chloropurine derivative **12** and subsequent treatment with ammonia and crystallization of the product afforded the LNA-A nucleoside **13** in moderate yield.

A number of efficient methods have been developed for conversion of the natural deoxy- and riboguanosines into the 2,6-diaminopurine (D) derivatives.[19] However, as the production of LNA-G nucleoside is a multistep synthetic procedure itself this approach for synthesizing LNA-D did not seem attractive. A shorter route based on the conventional convergent method was therefore chosen, using the universal convertible nucleobase 6-chloro-2-aminopurine that was already successfully used in the synthesis of HNA-D (anhydrohexitol nucleic acid containing D as nucleobase).[12] According to this strategy, a properly protected carbohydrate unit is conjugated with 6-chloro-2-aminopurine producing the key intermediate derivative **14** (Scheme 3), which can be converted into the desired D-nucleoside **15** (Scheme 3, path A) or 2AP-nucleoside **16** (path B).

SCHEME 2



SCHEME 3



It has previously been shown that glycosylation of 2-amino-6-chloropurine with furanose **4** resulted in a stereoselective formation of the nucleoside **17** (Scheme 4).[20] To promote ring closure, a solution of **17** in aqueous 1,4-dioxane was treated with 10-fold excess of sodium hydroxide to give the bicyclic compound **18** in 87% yield. The subsequent reaction with sodium benzoate in hot DMF led to successful substitution of the 5′-mesylate in **18**. The reaction proceeded very selectively, no side products originating from modification of the nucleobase were detected, and hence **19** could be isolated in 95% yield after chromatographic purification of the reaction mixture.

The introduction of the 6-amino group into the nucleobase moiety was achieved by synthesizing the 6-azido derivative **20** by reaction of **19** with NaN_3 followed by reduction of the azide at a later stage. In the final

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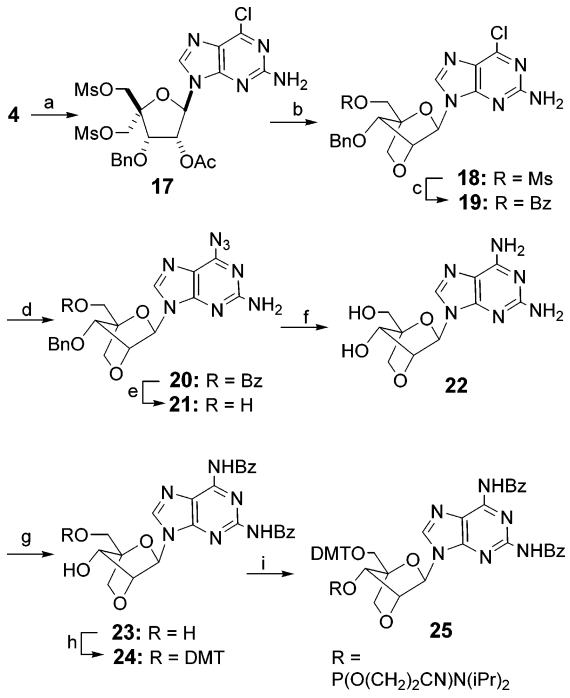
SCHEME 4[a]



[a] Reagents and conditions: (a) 2-chloro-6-aminopurine, BSA, trimethylsilyl triflate, 1,2-dichloromethane; 90%; (b) NaOH, 1,4-dioxane, H_2O; 87%; (c) NaOBz, DMSO; (d) NaN_3, DMSO; (e) NaOH, EtOH; 79% (three steps); (f) 10% Pd/C, HCO_2NH_4, MeOH, H_2O; 94%; (g) (1) BzCl, pyridine, (2) NaOH, EtOH, pyridine; 62%; (h) DMT-Cl, pyridine; 80%; (i) 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphordiamidite, 4,5-dicyanoimidazole, DMF, EtOAc, 93%.

method, the conversion of **18** into azide **20** was a one-pot procedure and sodium azide was added directly to the reaction mixture after transformation of **18** into **19** was completed. Importantly, the order of reactions could not be changed, as the azide anion could substitute both the 6-chlorine and the 5′-mesylate in the reaction conditions used (data not shown). In other words, in this particular case benzoate demonstrated remarkable ability for very selective substitution of the 5′-*O*-mesyl group. Crude **20** was precipitated from the reaction mixture by addition of water and used without any further purification in the next step. Thus**,** crude **20** was dissolved in ethanol and treated with aqueous sodium hydroxide to give **21**. The excess of alkali was then neutralized by addition of acetic acid and the nucleoside **21** was crystallized from the reaction mixture. Thus, following the above cascade of reactions, **18** was easily converted into crystalline **21** in 79% yield.

According to the data reported by Eritja[21] the nucleobase of **21** should generally exist in its tetrazolo tautomeric form **21tetr** (Figure 2). Indeed, RP-HPLC and NMR analyses detected the presence of only one form of **21**. The structure of this form (azido or tetrazolo), however, is still to be investigated. Whatever the structure of the compound may be, it released N_2 producing the 2,6-diaminopurinyl counterpart, which explains the observation that the 2,6-diaminopurinyl product was detected as the main peak in MALDI-MS spectra of **21**.

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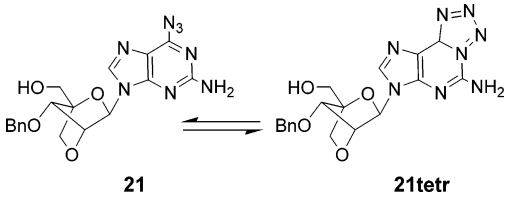
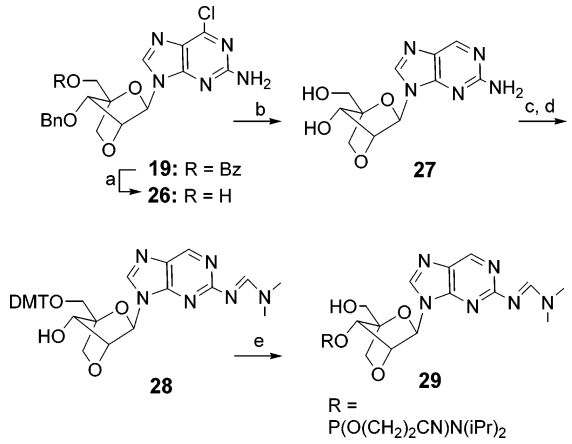


FIGURE 2. Two plausible tautomeric forms of the nucleoside **21**.

SCHEME 5[a]



[a] Reagents and conditions: (a) NaOH, 1,4-dioxane, H_2O; 72%; (b) 20% $Pd(OH)_2/C$, HCO_2NH_4, MeOH, H_2O; 88%; (c) *N,N*-dimethylformamide dimethyl acetal, DMF; (d) DMT-Cl, pyridine; 64% (two steps); (e) 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphordiamidite, 4,5-dicyanoimidazole, CH_2Cl_2, EtOAc; 82%.

Hydrogenation on palladium catalyst easily converted the 6-azido group of **21** into the corresponding amine. Thus, LNA-D diol **22** was produced from **21** in 94% yield after catalytic hydrogenation on Pd/C and following crystallization (Scheme 4). The synthetic route described for LNA-D nucleoside is comparatively short and superior to the method based on the use of LNA-G nucleoside as an intermediate.

To perform the synthesis of oligonucleotides containing the LNA-D monomer **2**, nucleoside **22** was converted into a phosphoramidite building block **25** (Scheme 4). Two benzoyl groups were selected for protection of the 2- and 6-amino groups as they were previously reported to be easily introducible and compatible with conventional oligonucleotide synthesis.[12] Thus, **22** was perbenzoylated by treatment with benzoyl chloride in anhydrous pyridine. The subsequent selective saponification of the 5′- and 3′-benzoates with aqueous sodium hydroxide afforded diol **23**, which was further DMT protected and phosphitylated by the use of standard methods.[17]

The intermediate **19** was also used to synthesize the LNA-2AP phosphoramidite **29** (Scheme 5). First, the 5′-*O*-benzoyl group of **19** was hydrolyzed by treatment with aqueous sodium hydroxide to give **26** in 72% yield after crystallization from ethyl acetate. As expected, refluxing of a methanolic solution of **26** in the presence of palladium hydroxide and ammonium formate resulted in simultaneous elimination of the 3′-*O*-benzyl and 6-chloro groups, producing the LNA-2AP nucleoside **27** in 88% yield. With the aim to simplify conversion of **19** into **27**, a one-pot hydrogenation of **19** followed by debenzoylation

TABLE 1. Melting Temperatures[a] of the Complementary DNA·DNA and LNA·DNA Duplexes Containing Modified Nucleosides[b]

entry	oligonucleotide structure	T_m (±0.5 °C) of the duplexes with complementary deoxynucleotides			
		3′-ctg**a**atcc (**44**)	3′-ctg**t**atcc (**45**)	3′-ctg**g**atcc (**46**)	3′-ctg**c**atcc (**47**)
1	5′-gac**a**tagg (**30**)	<10	**23.8**	<10	<10
2	5′-gac**t**tagg (**31**)	**22.6**	<10	<10	<10
3	5′-gac**g**tagg (**32**)	<10	<10	<10	**25.0**
4	5′-gac**d**tagg (**33**)	<10	**23.3**	<10	<10
5	5′-g**d**c**d**t**d**gg (**34**)	<10	**33.4**	<10	17.7 *(15.7)*[c]
6	5′-gac**i**tagg (**35**)	<10	<10	<10	**20.9**
7	5′-gac**x**tagg (**36**)	<10	<10	<10	<10
8	5′-GAC**A**TAGG (**37**)	38.2 *(23.4)*	**61.6**	43.4 *(18.2)*	40.6 *(21.0)*
9	5′-GAC**T**TAGG (**38**)	**60.7**	28.0 *(32.7)*	36.4 *(24.3)*	23.5 *(37.2)*
10	5′-GAC**G**TAGG (**39**)	32[d] *(40.9)*	55.0 *(15.9)*	41[d] *(29.9)*	**70.9**
11	5′-GAC**D**TAGG (**40**)	42.2 *(25.6)*	**67.8**	41.4 *(26.4)*	52.4 *(15.4)*
12	5′-G**D**C**D**T**D**GG (**41**)	55.9 *(22.4)*	**78.3**	54.7 *(23.6)*	63.8 *(14.5)*
13	5′-GAC**I**TAGG (**42**)	48.2 *(11.7)*	53.1 *(6.8)*	43.0 *(16.9)*	**59.9**
14	5′-GAC**X**TAGG (**43**)	45.5 *(15.3)*	**60.8**	44.0 *(16.8)*	53.9 *(6.9)*

[a] The melting temperatures (T_m values) were obtained as maxima of the first derivatives of the corresponding melting curves (optical density at 260 nm versus temperature). Concentration of duplexes: 2.5 μM. Buffer: 0.01 M sodium phosphate (pH 7.4) containing 0.1 M NaCl and 1 mM EDTA. Presented as the mean of three measurements. [b] LNAs are in capital letters: I, i = inosines; D, d = 2,6-diaminopurines; X, x = 2-aminopurines (2AP). [c] In parentheses is shown ΔT_m = decrease in T_m value in comparison to the most stable duplex in the row (mismatch discrimination). [d] Low cooperativity of the transition observed (accuracy ±1 °C).

of the 5′-hydroxy group with methylamine was examined. Indeed, this procedure worked and gave the desired nucleoside **27** in 57% yield. However, the rate of catalytic hydrogenation was significantly reduced in the presence of the 5′-benzoate and extended time of reaction and larger excess of the reagents were required. A similar effect was also observed for previously synthesized LNA nucleosides with the natural nucleobases.[6] Solubility problems were additionally encountered as precipitation of intermediate product(s) was observed during the reaction. Therefore, the prior removal of the 5′-*O*-benzoyl group from **19** and isolation of crystalline **26** is preferred for performing the hydrogenation step during a large-scale synthesis of **27**.

To protect the 2-amino and 5′-hydroxy groups of **27,** a convenient one-pot procedure was employed. Accordingly, a selective and quantitative protection of the nucleobase was performed taking advantage of the amidine protection group.[22] Introduction of the 5′-*O*-DMT group was then made by conventional method and nucleoside **28** was isolated in 64% yield after chromatographic purification. Subsequent phosphitylation[17] of **28** afforded the desired phosphoramidite **29** in 82% yield.

Along with the earlier described LNA phosphoramidites,[6,23] the phosphoramidite building blocks **11**, **25**, and **29** were successfully used for automated oligonucleotide synthesis[24] to produce the LNA oligonucleotides depicted in Table 1. The standard protocols for DNA synthesis (0.2 μmol scale) were applied, except for coupling (extended to 500 s) and oxidation cycles (extended to 30 s). After synthesis, the oligonucleotides were deprotected by treatment with concentrated ammonium hydroxide for 6 h at 60 °C. The oligonucleotides **40** and **41** containing LNA-D **2** were then additionally treated with aqueous methylamine for 2 h at 60 °C, which was necessary for complete removal of the benzoyl protection groups. All the synthesized oligonucleotides were purified by RP-HPLC and their structures were verified by MALDI-TOF mass spectrometry.

The hybridization properties of the oligonucleotides containing the new LNA monomers **1**−**3** were assessed against complementary DNA (Table 1). Comparative binding data from 8-mer LNA sequences are shown in Table 1 as the melting temperatures against single stranded DNA. The sequence in this study is the central part of a capture probe used for SNP detection in the GluclVS7 gene on a microarray. The thermal stabilities of reference DNA duplexes (entries 1−7, Table 1) can be directly compared with their LNA counterparts (entries 8−14). As expected, with an average T_m value about 40 °C higher, all the investigated LNA 8-mers had a much stronger affinity to the complementary DNA strand than the isosequential DNA oligonucleotides. The replacement of an internal LNA-A nucleotide by LNA-D resulted in further stabilization of the duplex (compare duplexes **37·45** and **40·45**; entries 8 and 11) by 6.2 °C. Interestingly, almost no change in the melting temperature occurred when the analogous replacement was made in the DNA octamer (compare **30·45** and **33·45**; entry 4). As already pointed out, similar effects were reported for other short DNA oligonucleotides.[10] It was also observed[25] that D-nucleosides may facilitate a B- to A-type helix transition. Apparently the A-type structure of the LNA·DNA duplex[2] is more suitable for efficient D·t pairing. Based on these findings, the mentioned stabilizing effect might be even more pronounced for LNA·RNA duplexes, which can be very useful for the design of even more powerful antisense reagents.

The introduction of three diaminopurine replacements in the same sequence (entry 5) resulted in an increased duplex stability despite the diaminopurines being used in a DNA context (compare **34·45** and **30·45**). For LNA, the additive stabilizing effect of three D monomers was even more pronounced, giving a remarkable stability of 78.3 °C for duplex **41·45** (entry 12).

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Additionally, we studied the selectivity of LNA-D (entry 11) against natural DNA nucleosides. In comparison to LNA-A (entry 8), the LNA-D nucleoside demonstrated a significantly increased mismatch discrimination against DNA-g (duplexes **40·46** and **37·46**) and slightly higher discrimination against DNA-a (duplexes **40·44** and **37·44**). At the same time the LNA-D nucleoside exhibited a comparatively high ability for pairing with DNA-c nucleoside (compare **40·47** and **37·47**). However, the selectivity of the LNA-D nucleoside in the latter case is still high (ΔT_m = 15.4 °C). These results show that incorporation of LNA-D into oligonucleotides results in an increased affinity toward complementary nucleic acids with a good mismatch discrimination thereby establishing LNA-D as an attractive tool for LNA oligonucleotides tailoring.

Oligonucleotides **42** and **43** containing LNA-I **1** and LNA-2AP **3** also formed stable duplexes with complementary DNA demonstrating the ability for effective base pairing in Watson−Crick geometry. As a part of LNA oligonucleotide content inosine **1** generally acts similar to LNA-G nucleotide with preferable binding to DNA-c (entry 13). A high T_m of the duplex **42·47** (59.9 °C) indicates the formation of the stable bidentate hydrogen bonding in the I·c base pair. At the same time LNA-I demonstrated comparatively strong binding against all other natural DNA nucleosides. However, the most pronounced discrimination (ΔT_m = 16.9 °C) detected against the DNA-g nucleoside was prohibitively high to consider LNA-I as a universal base.

Taking into account the fact that no cooperative transition was detected for the DNA·DNA duplex **36·45** (entry 7) above 10 °C, a surprisingly high T_m = 60.8 °C was measured for the duplex **43·45** containing the LNA-2AP **3** nucleotide (entry 14). Surprisingly, similar stabilities were found for all LNA·DNA base pairs expected to form double hydrogen bonds (compare T_m values for duplexes **37·45**, **38·44**, **42·47**, and **43·45**). Along with the results on different hybridization properties of D nucleoside in DNA and LNA context (vide supra), high stability of the 2AP·t base pair can be explained by a more suitable A-type structure of LNA·DNA duplexes.[2] Apparently, in this case the 2-amino groups of D and 2AP nucleosides are more accessible for effective hydrogen binding with the opposite DNA-t nucleotide. The strong binding ability of LNA-2AP makes it particularly attractive for fluorescence-based studies of LNA structural dynamics.

Conclusion

Synthetic pathways reported for the nucleotides **1**−**3** confirm the superiority of the convergent synthesis strategy for LNA synthesis in comparison to the linear route based on natural ribonucleosides as starting materials. Accordingly, the furanose **4** is a key universal intermediate for production of diverse LNA molecules.[6,26] Additionally, analogous to **4**, the dimesylated L-*theo*-pentofuranose was found to be the most suitable glycosyl donor for the synthesis of α-L-LNA nucleosides.[27]

The results from the hybridization studies of LNA oligonucleotides containing the artificial nucleobases hypoxanthine, 2,6-diaminopurine, and 2-aminopurine showed full compatibility of these nucleobases with the LNA structure. All three nucleotides demonstrated a strong binding to complementary DNA in Watson−Crick hybridization modes, which established them as very useful components in the LNA toolbox.

Experimental Section

9-(2-*O*-Acetyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-*β*-D-*erythro*-pentofuranosyl)hypoxanthine (5). To a suspension of compound **4**[6] (9.8 g, 19.2 mmol) and hypoxanthine (3.0 g, 22.1 mmol) in anhydrous 1,2-dichloroethane (150 mL) was added *N*,*O*-bis(trimethylsilyl)-acetamide (BSA) (15.0 mL, 61.3 mmol) and the mixture was stirred under refluxing for 1 h. Trimethylsilyl triflate (5.0 mL, 27.7 mmol) was added, and the mixture was refluxed for further 6 h, cooled to room temperature, and diluted with EtOAc (200 mL). The solution was washed with saturated $NaHCO_3$ (2 × 200 mL) and brine (150 mL), dried (Na_2SO_4), and concentrated under reduced pressure to a solid residue. The residue (10.5 g, 93%) was used for the next step without additional purification. An analytical sample (0.5 g) was obtained after purification by silica gel column chromatography (1.5−6% v/v MeOH/CH_2Cl_2) as a white solid material consisting of two isomers (ratio ca. 1:4 by ^{1}H NMR). RP-HPLC (system I; see Supporting Information for details) R_t = 9.58 min, $A_{290/260}$ = 0.075. RP-HPLC (system II) R_t = 7.87 min ($A_{290/260}$ = 0.109) and 8.19 min (main isomer, $A_{290/260}$ = 0.041). For compound **5** (main isomer): ^{1}H NMR (CD_3Cl) δ 12.83 (br s, 1H), 8.32 (s, 1H), 7.95 (s, 1H), 7.40−7.31 (m, 5H), 6.18 (d, J = 3.5 Hz, 1H), 6.00 (dd, J = 5.9 and 3.5 Hz, 1H), 5.03 (d, J = 6.0, 1H), 4.65 (s, 2H), 4.64 (d, J = 11.0 Hz, 1H), 4.47 (d, J = 10.6 Hz, 1H), 4.42 (d, J = 10.5 Hz, 1H), 4.39 (d, J = 11.4 Hz, 1H), 3.03 (s, 3H), 2.96 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (CD_3Cl) δ 169.5, 158.4, 148.0, 145.8, 139.6, 136.4, 128.5, 128.4, 128.3, 125.4, 87.8, 84.2, 77.6, 74.6, 73.8, 67.6, 67.4, 37.6, 37.4, 20.5. MALDI-TOF MS *m*/*z* 609.2 (M + Na^+). Anal. Calcd for $C_{22}H_{26}N_4O_{11}S_2$: C, 45.05; H, 4.47; N, 9.55. Found: C, 44.88; H, 4.38; N, 9.44.

Compounds 6 and 18. The ring closure reaction in **5** and **17** was promoted by treatment with aqueous NaOH as previously described.[6]

6: mp 200−203 °C dec. RP-HPLC (system I) R_t = 8.49 min, $A_{290/260}$ = 0.060. ^{1}H NMR (DMSO-d_6) δ 8.15 (s, 1H), 8.07 (s, 1H), 7.33−7.28 (m, 5H), 6.02 (s, 1H), 4.82 (s, 1H), 4.80 (d, J = 12.0 Hz, 1H), 4.66 (s, 1H), 4.60 (d, J = 11.9 Hz, 1H), 4.46 (s, 1H), 4.06 (d, J = 8.2 Hz, 1H), 3.93 (d, J = 8.2 Hz, 1H), 3.24 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 156.9, 147.6, 146.5, 137.6, 137.4, 128.4, 127.8, 127.7, 124.5, 85.6, 84.8, 77.7, 77.2, 71.8, 71.5, 66.0, 37.0. MALDI-TOF MS *m*/*z* 449.6 (M + H^+). Anal. Calcd for $C_{19}H_{20}N_4O_7S\cdot{}^1/_2H_2O$: C, 49.89; H, 4.63; N, 12.24. Found: C, 49.98; H, 4.39; N, 12.05.

18: RP-HPLC (system I) R_t = 10.82 min, $A_{290/260}$ = 1.67. ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H), 7.32−7.26 (m, 5H), 5.91 (s, 1H), 4.73 (s, 1H), 4.66 (d, J = 11.7 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.59 (s, 2H), 4.31 (s, 1H), 4.18 (d, J = 8.0 Hz, 2H), 3.99 (d, J = 7.9 Hz, 1H), 3.05 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 158.9, 152.2, 151.4, 139.1, 136.4, 128.4, 128.2, 127.7, 125.3, 86.5, 85.2, 77.2, 76.8, 72.4, 72.1, 64.0, 37.7. MALDI-TOF MS *m*/*z* 482.1 (M + H^+). ESI-TOF HRMS calcd for $C_{19}H_{21}ClN_5O_6S$ (M + H^+) *m*/*z* 482.0901, found *m*/*z* 482.0906.

Compounds **7** and **19** were derived from the nucleosides **6** and **18**, respectively, after standard reaction with NaOBz.[6]

7: RP-HPLC (system I) R_t = 10.59 min, $A_{290/260}$ = 0.082. ^{1}H NMR (CD_3Cl) δ 13.08 (br s, 1H), 8.23, (s, 1H), 7.98 (m, 2H), 7.89 (s, 1H), 7.60 (m, 1H), 7.46 (m, 2H), 7.25−7.23 (m, 5H),

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6.05 (s, 1H), 4.83 (s, 1H), 4.80 (d, J = 12.6 Hz, 1H), 4.68 (d, J = 11.9 Hz, 1H), 4.67 (d, J = 12.8 Hz, 1H), 4.57 (d, J = 11.7 Hz, 1H), 4.28 (d, J = 8.2 Hz, 1H), 4.27 (s, 1H), 4.10 (d, J = 7.9 Hz, 1H). ^{13}C NMR (CD_3Cl) δ 165.7, 158.8, 147.6, 145.3, 137.2, 136.4, 133.4, 129.4, 129.0, 128.5, 128.4, 128.1, 127.7, 125.1, 86.6, 85.8, 77.1, 77.0, 72.5, 72.4, 59.6. MALDI-TOF MS m/z 475.6 ($M+H^+$). Anal. Calcd for $C_{25}H_{22}N_4O_6 \cdot {}^2/_3H_2O$: C, 61.75; H, 4.80; N, 11.52. Found: C, 61.76; H, 4.58; N, 11.22.

19: RP-HPLC (system I) R_t = 12.86 min, $A_{290/260}$ = 1.31. ^{1}H NMR ($CDCl_3$) δ 7.98−7.95 (m, 2H), 7.79 (s, 1H), 7.62−7.58 (m, 1H), 7.48−7.44 (m, 2H), 7.24 (m, 5H), 5.93 (s, 1H), 4.80 (d, J = 12.6 Hz, 1H), 4.77 (s, 1H), 4.67 (d, J = 11.9 Hz, 1H), 4.65 (d, J = 12.6 Hz, 1H), 4.56 (d, J = 11.9 Hz, 1H), 4.27 (d, J = 8.0 Hz, 1H), 4.25 (s, 1H), 4.08 (d, J = 7.9 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 165.8, 159.1, 152.3, 151.6, 138.9, 136.5, 133.5, 129.6, 129.1, 128.7, 128.6, 128.3, 127.7, 125.6, 86.5, 85.9, 77.3, 76.8, 72.6, 72.5, 59.7. MALDI-TOF MS m/z 508.0 ($M + H^+$). Anal. Calcd for $C_{25}H_{22}ClN_5O_5$: C, 59.12; H, 4.37; N, 13.79. Found: C, 59.11; H, 4.36; N, 13.44.

Compounds 8 and 26. The 5′-*O*-benzoyl groups of **7** and **19** were hydrolyzed by treatment with aqueous NaOH.[6]

8: mp 302−305 °C dec. RP-HPLC (system I) R_t = 6.21 min, $A_{290/260}$ = 0.050. ^{1}H NMR (DMSO-d_6) δ 8.16 (s, 1H), 8.06 (s, 1H), 7.30−7.20 (m, 5H), 5.95 (s, 1H), 4.69 (s, 1H), 4.63 (s, 2H), 4.28 (s, 1H), 3.95 (d, J = 7.7 Hz, 1H), 3.83 (m, 3H). ^{13}C NMR (DMSO-d_6) δ 156.6, 147.3, 146.1, 137.9, 137.3, 128.3, 127.6, 127.5, 124.5, 88.2, 85.4, 77.0, 72.1, 71.3, 56.7. MALDI-TOF MS m/z 371.1 ($M + H^+$). Anal. Calcd for $C_{18}H_{18}N_4O_5 \cdot {}^5/_{12}H_2O$: C, 57.21; H, 5.02; N, 14.82. Found: C, 57.47; H, 4.95; N, 14.17.

26: mp 155−158.5 °C. RP-HPLC (system I) R_t = 8.44 min, $A_{290/260}$ = 1.72. ^{1}H NMR (DMSO-d_6) δ 8.16 (s, 1H), 7.32−7.26 (m, 5H), 7.03 (br s, 2H), 5.85 (s, 1H), 5.16 (t, J = 5.7 Hz, 1H), 4.70 (s, 1H), 4.63 (s, 2H), 4.26 (s, 1H), 3.94 (d, J = 7.9 Hz, 1H), 3.86−3.78 (m, 3H). ^{13}C NMR (DMSO-d_6) δ 159.9, 153.1, 149.6, 139.8, 138.0, 128.3, 127.7, 127.5, 123.7, 88.2, 85.3, 77.2, 76.7, 72.1, 71.3, 56.8. MALDI-TOF MS m/z 403.7 ($M + H^+$). ESI-TOF HRMS calcd for $C_{28}H_{19}ClN_5O_4$ ($M + H^+$) m/z 404.1126, found m/z 404.1138.

(1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-(4,4′-dimethoxytrityloxymethyl)-3-(hypoxanthin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (9). Compound **8** (2.2 g, 5.94 mmol) was coevaporated with anhydrous pyridine (2 × 50 mL) and suspended in anhydrous pyridine (80 mL). DMT-chloride (2.5 g, 7.42 mmol) was added and the mixture was stirred at 80 °C (oil bath) for 2 h. The solution was diluted with ethyl acetate (150 mL), washed with $NaHCO_3$ (2 × 200 mL) and brine (150 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The yellow solid residue was purified by silica gel HPLC (40−100% v/v EtOAc/hexane, containing 0.05% of pyridine) to give compound **9** (3.67 g, 92%) as a white solid material. RP-HPLC (system I) R_t = 13.79 min, $A_{290/260}$ = 0.123. ^{1}H NMR (CD_3Cl) δ 13.15 (br s, 1H), 8.23 (s, 1H), 8.15 (s, H), 7.45 (m, 2H), 7.36−7.12 (m, 12H), 6.86−6.80 (m, 4H), 6.07 (s, 1H), 4.71 (s, 1H), 4.56 (d, J 11.7 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.32 (s, 1H), 4.03 (d, J = 7.8 Hz, 1H), 3.95 (d, J = 7.8 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.58 (d, J = 10.9 Hz, 1H), 3.45 (d, J = 11.0 Hz, 1H). ^{13}C NMR (CD_3Cl) δ 159.1, 158.5, 147.6, 145.1, 144.2, 137.5, 136.7, 135.3, 135.2, 129.9, 129.8, 128.9, 128.3, 128.1, 127.9, 127.6, 126.9, 125.2, 113.2, 87.3, 86.6, 86.4, 77.2, 72.8, 72.2, 58.4, 55.1. MALDI-TOF MS m/z 694.9 ($M + Na^+$). Anal. Calcd for $C_{39}H_{36}N_4O_7 \cdot {}^2/_5H_2O$: C, 68.89; H, 5.45; N, 8.24. Found: C, 68.94; H, 5.29; N, 8.23.

Compounds 10, 22, and 27. Catalytical hydrogenation[6] of the nucleosides **9**, **21**, and **26** afforded compounds **10**, **22**, and **27**, respectively.

10: RP-HPLC (system I) R_t = 11.28 min, $A_{290/260}$ = 0.105. ^{1}H NMR (DMSO-d_6) δ 8.12, (s, 1H), 8.09 (s, 1H), 7.43−7.24 (m, 9H), 6.90 (m, 4H), 5.97 (s, 1H), 5.77 (br s, 1H), 4.44 (s, 1H), 4.33 (s, 1H), 3.97 (d, J = 7.5 Hz, 1H), 3.91(d, J = 7.7 Hz, 1H), 3.74 (s, 6H), 3.55 (d, J = 10.6 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 158.2, 156.6, 147.3, 146.1, 144.8, 137.1, 135.5, 135.3, 129.8, 127.9, 127.7, 126.8, 124.6, 113.3, 87.1, 85.6, 79.3, 71.8, 70.5, 59.9, 55.1. MALDI-TOF MS m/z 604.5 ($M + Na^+$). Anal. Calcd for $C_{32}H_{30}N_4O_7 \cdot {}^1/_2H_2O$: C, 64.97; H, 5.28; N, 9.47. Found: C, 65.15; H, 5.29; N, 8.92.

22: RP-HPLC (system III) R_t = 8.63 min, $A_{290/260}$ = 0.77. ^{1}H NMR (DMSO-d_6) δ 7.81 (s, 1H), 6.78 (br s, 2H), 5.91 (br s, 2H), 5.71 (s, 1H), 5.66 (br s, 1H), 5.04 (br s, 1H), 4.31 (s, 1H), 4.20 (s, 1H), 3.90 (d, J = 7.7 Hz, 1H), 3.77 (m, 2H), 3.73 (d, J = 7.7 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 160.5, 156.2, 150.9, 134.2, 113.4, 88.3, 85.0, 79.3, 71.5, 70.0, 56.8. MALDI-TOF MS m/z 295.0 ($M + H^+$). Anal. Calcd for $C_{11}H_{14}N_6O_4 \cdot 1.5H_2O$: C, 41.12; H, 5.33; N, 26.15. Found: C, 41.24; H, 5.19; N, 25.80.

27: mp 227.5−229 °C dec. RP-HPLC (system III) R_t = 8.88 min, $A_{290/260}$ = 4.22. ^{1}H NMR (DMSO-d_6) δ 8.60 (s, 1H), 8.15 (s, 1H), 6.64 (br s, 2H), 5.82 (s, 1H), 5.71 (br s, 1H), 5.04 (br s, 1H), 4.40 (s, 1H), 4.21 (s, 1H), 3.92 (d, J = 7.7 Hz, 1H), 3.79 (m, 2H), 3.75 (d, J = 7.7 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 160.6, 152.0, 149.4, 139.3, 127.1, 88.6, 84.8, 79.1, 71.6, 70.2, 56.8. MALDI-TOF MS m/z 826 ($M + Na^+$). Anal. Calcd for $C_{11}H_{13}N_5O_4$: C, 47.31; H, 4.69; N, 25.08. Found: C, 47.11; H, 4.66; N, 24.51.

Phosphoramidites 11, 25, and 29. The nucleoside derivatives **10**, **24**, and **28** were 3′-*O*-phosphitylated by conventional method[17,23] to give compounds **11**, **25**, and **29**, respectively.

11: RP-HPLC (system II) R_t = 14.18 min, $A_{290/260}$ = 0.12. ^{31}P NMR (DMSO-d_6) δ 148.90. MALDI-TOF MS m/z 721.2 ($M - N^iPr_2 + OH + Na^+$).

25: RP-HPLC (system II) R_t = 17.52 min, $A_{290/260}$ = 0.66. ^{31}P NMR (DMSO-d_6) δ 149.19, 148.98. MALDI-TOF MS m/z 1026.5 ($M + Na^+$).

29: RP-HPLC (system II) R_t = 14.87 and 14.97 min, $A_{290/260}$ = 1.47 (both). ^{31}P NMR (DMSO-d_6) δ 148.93, 148.85. MALDI-TOF MS m/z 753.2 ($M - N^iPr_2 + OH + H^+$).

9-(2-*O*-Acetyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-*β*-D-*erythro*-pentofuranosyl)-2-amino-6-chloropurine (17). A mixture of furanose **4** (37.0 g, 72.5 mmol) and 2-amino-6-chloropurine (14.9 g, 87.0 mmol) was suspended in anhydrous 1,2-dichloroethane (200 mL) and BSA (26.6 mL, 108.8 mmol) was added. The mixture was stirred under refluxing for 1 h to give a clear solution and cooled to room temperature. Trimethylsilyl triflate (16.4 mL, 90.6 mmol) was added, the solution was refluxed for 1.5 h then cooled to room temperature, and ice-cold saturated $NaHCO_3$ (150 mL) was added. The layers were separated and the aqueous phase was washed with CH_2Cl_2 (100 mL). The combined organic layers were washed with saturated $NaHCO_3$ (250 mL) and brine (250 mL) and subjected to silica gel column chromatography (2−4% v/v MeOH/CH_2Cl_2) to give compound **17** (40.7 g, 90%) as a slightly yellow solid material that was used for the next step without additional purification. The analytical sample (0.4 g) was further purified by silica gel HPLC (0−3% v/v MeOH/CH_2Cl_2). RP-HPLC (system I) R_t = 11.64 min, $A_{290/260}$ = 1.51. ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H), 7.40−7.32 (m, 5H), 6.00 (d, J = 3.4 Hz, 1H), 5.83 (dd, J = 5.9 and 3.3 Hz, 1H), 5.42 (br s, 2H), 5.19 (d, J = 6.0 Hz, 1H), 4.78 (d, J = 10.4 Hz, 1H), 4.67−4.61 (m, 3H), 4.40 (d, J = 11.7 Hz, 1H), 4.39 (d, J = 10.4 Hz, 1H), 3.0 (s, 3H), 3.00 (s, 3H), 2.12 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 169.5, 159.0, 152.3, 152.0, 141.4, 136.4, 128.5, 128.4, 128.2, 125.8, 88.2, 84.1, 77.8, 74.5, 73.9, 67.3, 67.1, 37.7, 37.4, 20.5. ESI-TOF HRMS. Calcd for $C_{22}H_{27}ClN_5O_{10}S_2$ ($M + H^+$) m/z 620.0888, found m/z 620.0909.

(1*S*,3*R*,4*R*,7*S*)-3-(2-amino-6-azidopurin-9-yl)-7-benzyloxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (21). A mixture of NaOBz (7.8 g, 54.2 mmol) and compound **18** (13.0 g, 27.0 mmol) was suspended in anhydrous DMSO (100 mL) and stirred for 2 h at 105 °C. NaN_3 (5.4 g, 83 mmol) was added; the mixture was stirred for 2 h at 105 °C and cooled to room temperature. H_2O (400 mL) was added and the mixture was stirred for 30 min at 0 °C (ice bath) to give a slightly yellow precipitate **20**. MALDI-TOF MS m/z 488.3 [$M - N_2 + 3H$]$^+$. The precipitate was filtered off, washed with H_2O, and dissolved in EtOH (50 mL). NaOH (2 M, 30 mL) was added; the solution was stirred for 30 min, neutralized by addition of

TABLE 2.

	MW	
oligonucleotide	calcd	found
37	2682.8	2682.9
38	2673.7	2973.8
39	2698.8	2699.9
40	2697.8	2697.8
41	2727.8	2726.2
42	2683.8	2683.0
43	2682.8	2682.2

AcOH (4 mL), and cooled in an ice bath. The crystalline precipitate was collected by filtration, washed with cold EtOH, and dried in vacuo to give compound **21** (8.75 g, 79%) as a white solid material. Mp 225−226 °C dec; starts sintering at 220 °C. RP-HPLC (system I) R_t = 8.08 min, $A_{290/260}$ = 1.27. ^{1}H NMR (DMSO-d_6) δ 8.53 (br s, 2H), 8.23 (s, 1H), 7.31−7.26 (m, 5H), 6.00 (s, 1H), 5.26 (t, J = 5.7 Hz, 1H), 4.76 (s, 1H), 4.64 (s, 1H), 4.31 (s, 1H), 3.99 (d, J = 7.9 Hz, 1H), 3.88−3.85 (m, 3H). ^{13}C NMR (DMSO-d_6) δ 146.0, 144.0, 143.8, 137.9, 137.0, 128.3, 127.7, 127.6, 112.3, 88.3, 85.6, 77.1, 77.0, 72.2, 71.4, 56.8. MALDI-TOF MS m/z 384.7 $[M - N_2 + 3H]^+$. Anal. Calcd for $C_{18}H_{18}N_8O_4$: C, 52.68; H, 4.42; N, 27.30. Found: C, 52.62; H, 4.36; N, 26.94.

(1*R*,3*R*,4*R*,7*S*)-3-(2,6-Di-(*N*-benzoylamino)purin-9-yl)-1-(4,4′-dimethoxytrityloxymethyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptane (24). Compound **22** (500 mg, 1.7 mmol) was coevaporated with anhydrous pyridine (2 × 20 mL), dissolved in anhydrous pyridine (20 mL), and cooled in an ice bath. BzCl (1.5 mL, 12.9 mmol) was added under intensive stirring. The mixture was allowed to warm to room temperature and stirred overnight. EtOH (20 mL) and 2 M NaOH (20 mL) were added and the mixture was stirred for 1 h. The solution was diluted with EtOAc (75 mL) and washed with H_2O (2 × 50 mL). The aqueous phases were combined and washed with CH_2Cl_2 (2 × 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure to give a solid residue. The residue was suspended in Et_2O (75 mL, under refluxing for 30 min) and cooled in an ice bath. The precipitate was collected by filtration, washed with cold Et_2O, and dried in vacuo to give intermediate **23** (530 mg, 62%) as a slightly yellow solid material. RP-HPLC (system I) R_t = 7.39 min, $A_{290/260}$ = 0.63. MALDI-TOF MS m/z 502.7 ($M + H^+$). The intermediate **23** (530 mg, 1.06 mmol) was coevaporated with anhydrous pyridine (2 × 20 mL) then dissolved in anhydrous pyridine (10 mL) and DMT-Cl (600 mg, 1.77 mmol) was added. The mixture was stirred overnight, diluted with EtOAc (100 mL), and washed with saturated $NaHCO_3$ (100 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel HPLC (20−100% v/v EtOAc/hexane, containing 0.1% of pyridine) gave compound **24** (670 mg, 49% from **22**) as a white solid material. ^{1}H NMR (CD_3OD) δ 8.41 (s, 1H), 8.15−8.03 (m, 4H), 7.71−7.22 (m, 15H), 6.92−6.86 (m, 4H), 6.23 (s, 1H), 4.77 (s, 1H), 4.62 (s, 1H), 4.03 (d, J = 7.9 Hz, 1H), 3.99 (d, J = 7.9 Hz, 1H), 3.79 (s, 6H), 3.67 (d, J = 10.9 Hz, 1H), 3.54 (d, J = 10.8 Hz, 1H). RP-HPLC (system I) R_t = 14.04, $A_{290/260}$ = 0.65. ^{13}C NMR (CD_3OD) δ 167.8, 160.3, 154.2, 153.3, 151.3, 146.3, 142.1, 137.2, 137,0, 135.6, 135.0, 134.1, 133.7, 131.4, 129.9, 129.8, 129.5, 129.1, 129.0, 128.0, 121.3, 114.3, 89.2, 88.0, 87.8, 81.0, 73.4, 72.3, 55.8. MALDI-TOF MS m/z 826 ($M + Na^+$). Anal. Calcd for $C_{46}H_{40}N_6O_8 \cdot H_2O$: C, 67.14; H, 5.14; N, 10.21. Found: C, 67.24; H, 4.97; N, 10.11.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-3-(2-*N*-(dimethylaminomethylidene)aminopurin-9-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptane (28). Compound **27** (0.80 g, 2.88 mmol) was coevaporated with anhydrous DMF (20 mL) and dissolved in anhydrous DMF (10 mL). *N*,*N*-Dimethylformamide dimethyl acetal (1.5 mL, 11.29 mmol) was added and the solution was stirred for 72 h. RP-HPLC (system III) showed the total consumption of the starting material **27** (R_t = 8.88 min) and formation of a single product with R_t = 10.26 min and $A_{290/260}$ = 1.58 (MALDI-TOF MS m/z 335.4 ($M + H^+$)). The solvents were removed under reduced pressure to give an oily residue. The residue was coevaporated with anhydrous pyridine (20 mL) then redissolved in anhydrous pyridine (5 mL) and DMT-Cl (1.07 g, 3.17 mmol) was added. The solution was stirred for 4 h, diluted with EtOAc (75 mL), washed with saturated $NaHCO_3$ (100 mL) and brine (75 mL), dried (Na_2SO_4), and concentrated to a yellow solid residue. Purification by silica gel HPLC (0−2% MeOH/CH_2Cl_2 v/v, containing 0.1% of pyridine) gave compound **28** (1.18 g, 64%) as a white solid material. RP-HPLC (system I) R_t = 11.95 min, $A_{290/260}$ = 1.54. ^{1}H NMR (DMSO-d_6) δ 8.86 (s, 1H), 8.67 (s, 1H), 8.33 (s, 1H), 7.44−7.41 (m, 2H), 7.34−7.24 (m, 7H), 6.92−6.88 (m, 4H), 6.05 (s, 1H), 5.78 (br s, 1H), 4.56 (s, 1H), 4.43 (br s, 1H), 4.01 (d, J = 7.9 Hz, 1H), 3.95 (d, J = 7.9 Hz, 1H), 3.74 (s, 6H), 3.57 (d, J = 10.9 Hz, 1H), XXX (d, J = 10.7 Hz, 1H), 3.12 (s, 3H), 3.02 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 162.6, 158.2, 152.0, 148.8, 144.8, 141.2, 135.5, 135.2, 129.8, 129.7, 127.9, 127.7, 126.8, 113.3, 87.0, 85.5, 85.1, 79.1, 71.8, 70.9, 60.1, 55.1, 40.4, 34.6. ESI-TOF HRMS calcd for $C_{35}H_{37}N_5O_6$ ($M + H^+$) m/z 637.2775, found m/z 637.2773.

Oligonucleotide Synthesis. All oligonucleotides were synthesized in 0.2 μmol scales on a DNA synthesizer, using the phosphoramidite method.[24] Standard conditions were used for commercial DNA amidites. For all LNA amidites coupling times and times of oxidation were extended to 500 and 30 s, respectively. After oligomerization, the solid support bound oligonucleotides were transferred into 1.5 mL reaction tubes and treated with 0.3 mL of concentrated NH_4OH for 6 h at 60 °C. Into the tubes containing **40** and **41** was added 40% aqueous $MeNH_2$ (0.3 mL) and the mixtures were heated for 2 h at 60 °C. The solvents were removed under reduced pressure and desired oligonucleotides were purified by RP-HPLC in system III. MALDI-TOF mass spectra were recorded in negative ion mode, using picolinic acid as the matrix (results shown in Table 2).

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Supporting Information Available: General procedures and experimental details for the synthesis of **6**, **7**, **8**, **10**, **11**, **13**, **18**, **19**, **22**, **25**, **26**, **27**, and **29**; copies of the ^{13}C NMR spectra of compounds **6**, **7**, **8**, **11**, **17**, **19**, **21**, **22**, **25**, **27** and **29**; and copies of the ^{1}H and ^{31}P NMR spectra of **11**, **25**, and **29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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