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Synthesis and Hybridization Studies of 2′-Amino-α-L-LNA and Tetracyclic "Locked LNA"†

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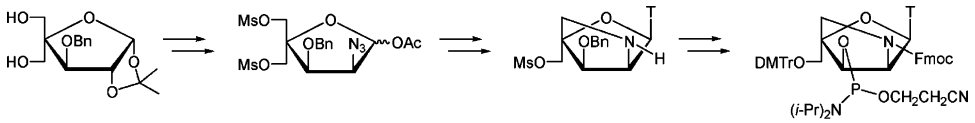

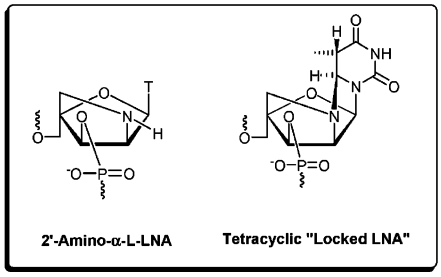

A convergent route to a new class of locked nucleic acids, i.e., 2′-amino-α-L-LNA, has been developed. The optimized synthetic route to the corresponding phosphoramidite building block of thymine proceeds in 4% overall yield over 15 steps from the starting diol. Crucial synthetic steps include (a) introduction of a C2-azido group prior to nucleobase coupling, (b) Vorbrüggen glycosylation primarily affording the desired α-anomer, (c) separation of α-L-*ribo*- and *β*-L-*ribo*-configured bicyclic nucleosides, and (d) selection of a suitable protecting group to avoid intramolecular Michael addition of the C2′-amino group onto the C6-position. Incorporation of a 2′-amino-α-L-LNA monomer into oligodeoxyribonucleotides results in modest changes in thermal stability with complementary DNA, whereas significant increases in thermal stability are observed with RNA complements along with excellent Watson−Crick discrimination. These results, along with the flexibility of the synthetic strategy allowing chemoselective N2′-functionalization at a late stage, render 2′-amino-α-L-LNA a promising building block for nucleic acid based nanobiotechnology and therapeutics. A slight modification in strategy facilitated the synthesis of the corresponding phosphoramidite building blocks of Michael adducts, which due to their tetracyclic skeletons exhibit a conformationally restricted furanose ring and glycosidic torsion angle (*anti*-range). Incorporation of such a "locked LNA" monomer into oligodeoxyribonucleotides results in large decreases in thermal affinity toward DNA/RNA complements.

Introduction

In the past 10−15 years significant efforts have been directed toward the use of oligonucleotides as potential antisense or antigene therapeutics.[1−3] More recently, the use of small interfering RNA (siRNA) or ribozymes/DNAzymes has emerged as promising alternatives for gene regulation.[1,4] Common to these therapeutic strategies is the necessity of using chemically modified oligonucleotides to improve the target affinity and to confer protection from enzymatic degradation.[2,3,5,6] Incorporation of nucleosides with conformationally restricted carbohydrate

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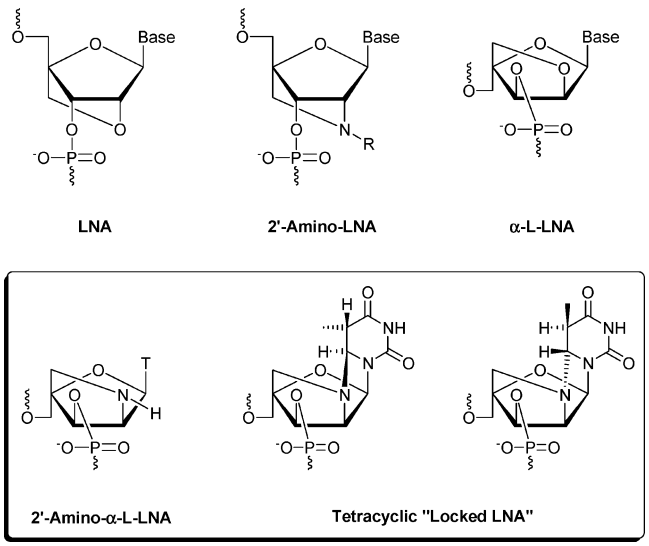

FIGURE 1. Structures of LNA, 2′-amino-LNA, and α-L-LNA, along with structures of the novel derivatives described herein, i.e., 2′-amino-α-L-LNA and tetracyclic "locked LNAs". T = thymin-1-yl.

rings has been an especially popular approach to realize this.[7,8] LNA (locked nucleic acid, *β*-D-*ribo* configuration)[9−11] and α-L-LNA (α-L-*ribo* configuration, a diastereomer of LNA),[9,12] are two prominent examples hereof (Figure 1). The dioxabicyclo-[2.2.1]heptane skeleton efficiently locks the furanose ring of both nucleosides in a North-type conformation.[13] Incorporation of LNA or α-L-LNA building blocks in oligodeoxyribonucleotides (ONs) results in significant increases in thermal affinity against DNA/RNA complements of up to +10.0 °C per modification relative to that of unmodified ONs and confers improved stability against exo- and endonucleases, which has rendered LNA[14] and α-L-LNA[15] with significant therapeutic and diagnostic potential.

It is widely accepted that the ability of ONs to modulate gene expression in vivo is rather variable due to inadequate target accessibility and poor cellular uptake and incorrect intramolecular localization of the probe.[3,16] Consequently, it would be highly desirable to develop a next generation of modified nucleotides that addresses these issues in addition to having excellent target affinity and enzymatic stability. This, along with the potential of functional nucleic acid architectures[17] as tools in nucleic acid based nanobiotechnology,[18] has spawned our interest in amino-modified nucleotides, in which an amino group serves as a conjugation site for attachment of hydrophilic, hydrophobic, basic, or fluorescent groups.[8c,19] Special attention has been devoted to derivatives of the amino analog of LNA, i.e., 2′-amino-LNA[9,20] (Figure 1). N2′-Functionalization of 2′-amino-LNAs allows precise positioning of chemical functionalities in the minor groove of nucleic acid duplexes without compromising their thermal stability.[21] This has facilitated the development of (a) nucleic acid architectures that autosignal their self-assembly by fluorescence,[22,23] (b) probes that generate brightly fluorescent duplexes upon hybridization to complementary DNA/RNA,[24] and (c) probes that allow optimized targeting of single-stranded DNA.[25] In contrast, the secondary amino group of a 2′-amino-α-L-LNA[9] (Figure 1) would be

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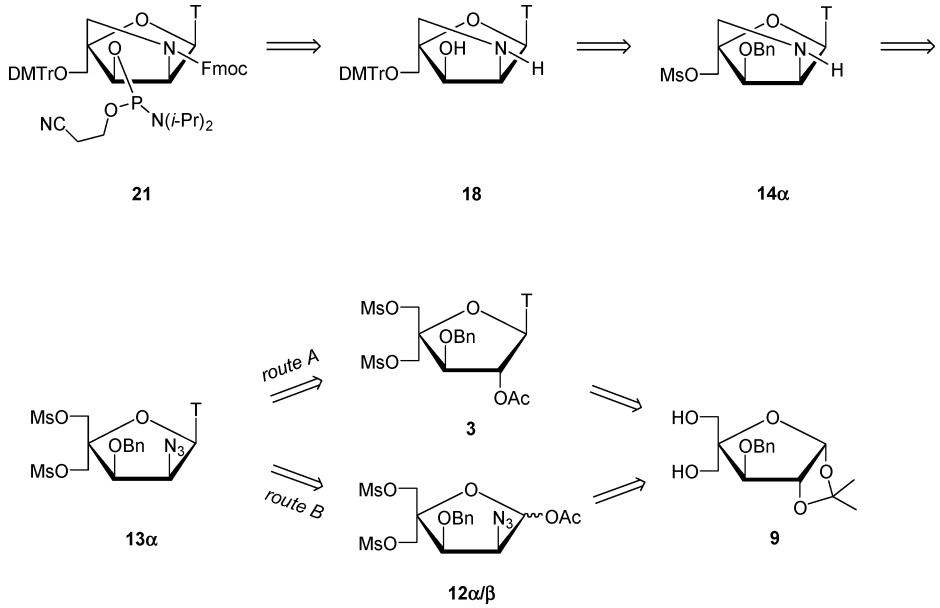
SCHEME 1. Retrosynthetic Analysis of 2′-Amino-α-L-LNA Phosphoramidite 21



anticipated to position functional groups in the major groove or the core of nucleic acid duplexes.

Herein are presented full experimental details[26] of the synthesis of the 2′-amino-α-L-LNA phosphoramidite **21** (Scheme 5), its incorporation into ONs, and initial thermal denaturation studies.

En route toward the desired 2′-amino-α-L-LNA phosphoramidite **21**, formation of two tetracyclic nucleosides containing seven chiral centers was observed. The tetracyclic skeletons of these nucleosides render them with (1) a conformationally locked furanose ring, (2) a conformationally restricted glycosidic torsion angle (*anti*-range), and (3) a nonaromatic nucleobase moiety (*vide infra*). Oligonucleotides with incorporations of nucleotides possessing *all* these features have, to our knowledge, never been reported, which stimulated us to synthesize the corresponding tetracyclic "locked LNA" phosphoramidites **27** and **31** (Schemes 7 and 8), to study their incorporation into ONs and to evaluate their thermal affinity toward DNA/RNA complements (Figure 1).

Results and Discussion

Retrosynthetic Analysis. We set out to synthesize the 2′-amino-α-L-LNA thymine phosphoramidite building block **21** since thymine derivatives typically alleviate synthesis by not needing nucleobase protection groups. Hence, O5′-tritylated amino alcohol **18** was identified as an intermediate and, moreover, as a suitable divergence point for future N2′-functionalizations (Scheme 1). Amino alcohol **18** could conceivably be derived from bicyclic α-L-*ribo*-configured nucleoside **14α** after protecting group manipulations. Retrosynthetic scission of the azamethylene bridge of nucleoside **14α**, as inspired by the recently improved synthesis of 2′-amino-β-D-LNA,[27] identified C2′(α)-azido-nucleoside **13α** as a potential precursor, as it was expected that azide reduction of **13α** would result in a subsequent intramolecular ring-closing nucleophilic substitution. At least two synthetic pathways leading to key intermediate **13α** can be envisioned. The C2′(α)-azido group can be introduced on the nucleoside level, which identifies known thymidine derivative **3**[28] as a suitable precursor (route A). It is important to notice that anchimeric assistance of the O2-acetyl group during glycosylation of the corresponding coupling sugar is known to exclusively afford 1′,2′-*trans* nucleoside **3** in high yield.[28] Introduction of substituents such as azido groups in the C2′-"up" position of pyrimidine nucleosides by intermolecular nucleophilic substitution is, however, recognized to be problematic due to the ease of intramolecular attack by the C2-carbonyl group of the nucleobase moiety on the activated C2′-position.[29] Nonetheless, it has been demonstrated that deactivation of thymine moieties with protecting groups that reduce the nucleophilicity of the C2-carbonyl group may facilitate intermolecular S_N2 reactions to occur at the C2′-position.[30]

As an alternative, installation of the C2′(α)-azido group may be carried out prior to glycosylation, whereby glycosyl donor **12α/*β*** is identified as a precursor to key intermediate **13α** (route B). The major concern associated with this strategy is that the lack of a group at the C2-position to provide anchimeric assistance during glycosylation could lead to unfavorable anomeric mixtures of azido-nucleoside **13α** and the corresponding β-anomer.

Inspired by the recently improved synthetic route to LNA,[31] we decided to install methanesulfonate groups at the C5/C5′-positions prior to glycosylation. Thus, it was realized that both potential substrates (**3** and **12**) for generation of key intermediate **13α** could be derived from known pentofuranose **9**.[32]

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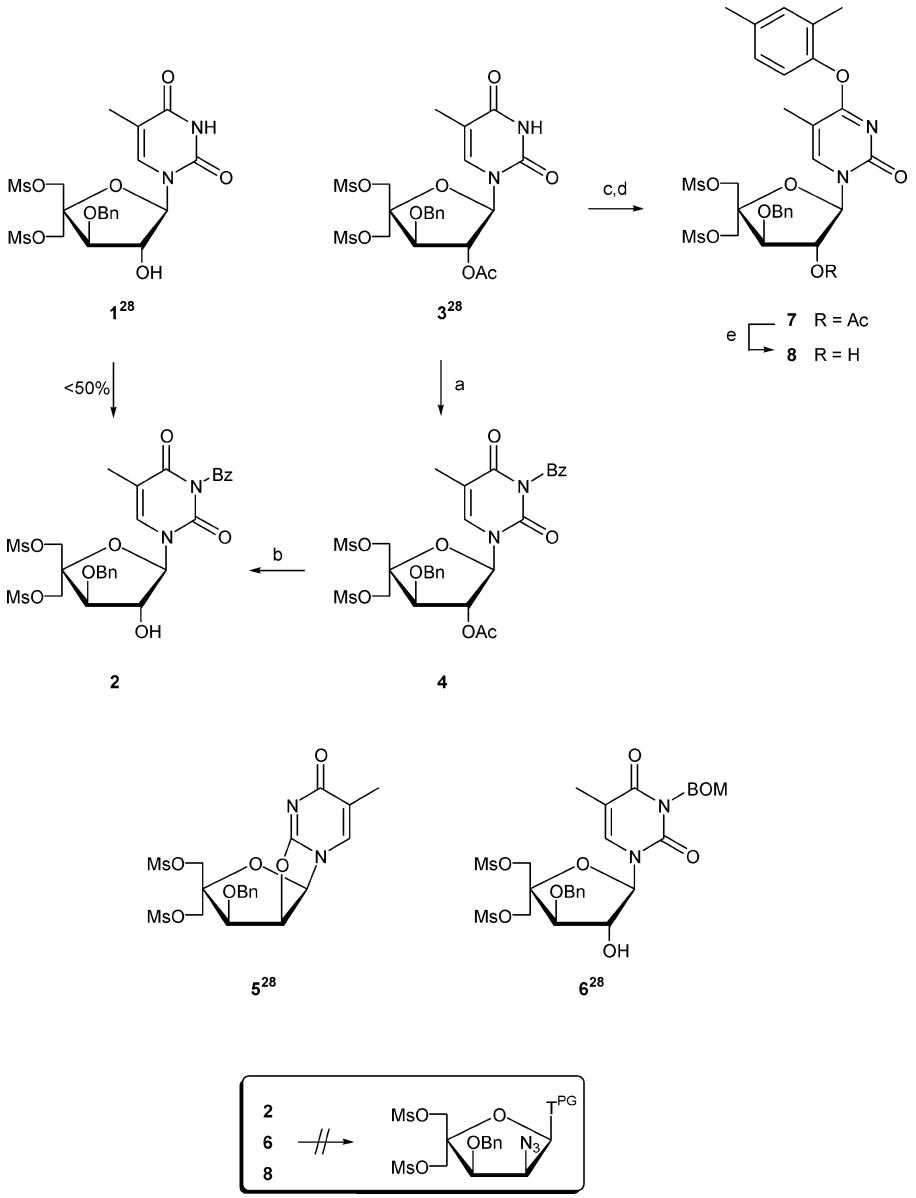
SCHEME 2[a]



[a] Reagents and conditions: (a) BzCl, DMAP, pyridine, 0−40 °C, 70%; (b) guanidinium nitrate, NaOMe, MeOH, CH_2Cl_2, room temperature, 96%; (c) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, CH_3CN, room temperature; (d) 2,4-dimethylphenol, DABCO, Et_3N, room temperature, 74% over two steps; (e) NH_3/MeOH, CH_2Cl_2, 0 °C to room temperature, 71%. PG = protecting group.

Attempted Synthesis of Key Intermediate 13α via Route A. N3-Benzoylated nucleoside **2** was identified as a possible substrate for introduction of a C2′(α)-azido group via route A (Scheme 2). Chemoselective N3-benzoylation of known O2′-unprotected nucleoside **1**,[28] using benzoyl chloride and triethylamine,[30a] phase transfer conditions[33] or transient protection protocols[34] either furnished substantial amounts of the undesired O2′-benzoylated or O2′,N3-dibenzoylated products whereby nucleoside **2** only was obtained in unsatisfying and inconsistent yields (0−50%, data not shown). On the contrary, N3-benzoylation of known nucleoside **3**,[28] affording fully protected nucleoside **4** in 70% yield, followed by chemoselective O2′-deacylation using a mixture of guanidine and guanidinium nitrate in methanol and dichloromethane,[35] furnished N3-benzoylated nucleoside **2** in excellent and reproducible yields (96%). It is noteworthy that ring-closed O2′,C4′-linked products were not observed. Other synthetic protocols that are ordinarily used to realize selective deacylations in nucleoside chemistry, e.g., cold dilute methanolic ammonia or aqueous sodium hydroxide in pyridine,[36] were unsuccessful. These observations suggest that the guanidine/guanidinium nitrate reagent mixture may be very useful to accomplish challenging chemoselective deacylations in nucleoside chemistry.[37]

Next, numerous attempts to convert N3-benzoylated nucleoside **2** into key intermediate **13α** were made (Scheme 2). Conversion of **2** into a reactive O2′-sulfonyl ester unit ($CF_3SO_2^-$,

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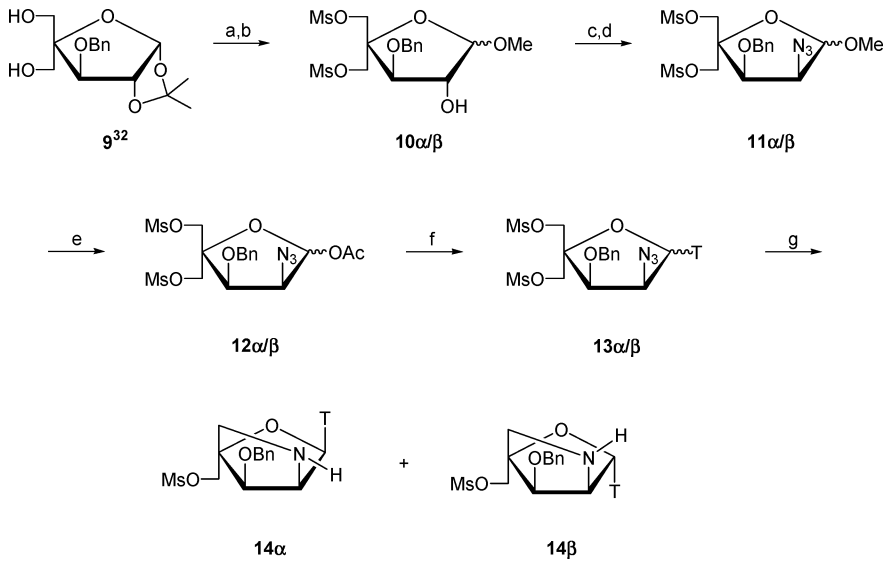
SCHEME 3[a]



[a] Reagents and conditions: (a) MsCl, pyridine, 0 °C to room temperature; (b) CH_3COCl, MeOH, 0 °C to room temperature, quantitative yield, two steps; (c) Tf_2O, pyridine, CH_2Cl_2, −15 °C to room temperature; (d) NaN_3, 15-crown-5, DMF, 50 °C; (e) concentrated H_2SO_4, Ac_2O, AcOH, 0 °C to room temperature, 80%, three steps; (f) thymine, BSA, TMSOTf, $ClCH_2CH_2Cl$, reflux, 81%, $\alpha:\beta \sim 55:45$; (g) PMe_3, aqueous NaOH, THF, room temperature, 58% combined.

$CF_3CH_2SO_2^-$, or $ClCH_2SO_2^-$) followed by treatment with sodium azide and 15-crown-5 in DMF or dichloromethane either resulted in no reaction or in predominant formation of known O2,O2′-anhydro nucleoside **5**.[28] Neither subjection of **2** to modified Mitsunobu conditions[38] using PMe_3/DIAD/diphenylphosphoryl azide (DPPA)[39] nor treatment of the bis(2,4-dichlorophenyl) phosphate activated species with sodium azide and DMAP[40] succeeded in installing the azido group. The observed lack of reactivity most likely reflects the steric hindrance at the C2′(α)-position as imposed by the 1,3-*cis* arrangement of the thymine moiety and the C3′-benzyloxy group.

Similar observations[41,42] were made with the known N3-benzyloxymethyl-protected nucleoside **6**[28] or O4-phenyl ether[46] protected nucleoside **8**, which was obtained from **3** in three steps. First the O4-position of nucleoside **3** was activated with 2,4,6-triisopropylbenzenesulfonyl chloride,[33] which was followed by treatment with 2,4-dimethyl phenol in the presence of base to afford nucleoside **7** (74% yield). Finally O2′-deacylation of **7** furnished nucleoside **8** in 71% yield.

Successful Synthesis of Key Intermediate 13α via Route B. The difficulties encountered while pursuing route A toward key intermediate **13α** prompted us to investigate the alternative route B in greater detail. Dimesylation of known diol **9**[32,47] followed by treatment with 2.5% hydrogen chloride in methanol, generated *in situ* by the addition of acetyl chloride to methanol, afforded an anomeric mixture of methyl furanoside **10** in quantitative yield over two steps (Scheme 3). Methyl furanoside **10**, reacted as an anomeric mixture, was O2-triflated, and the product was subsequently treated with sodium azide and 15-crown-5 in DMF at 50 °C. Gratifyingly, both the *β*-anomer of the crude O2-triflated pentofuranose as well as the sterically more encumbered α-anomer (i.e., C1-methoxy group pointing up) smoothly underwent selective nucleophilic displacement to afford 2-azido-2-deoxy-L-*erythro*-pentofuranose **11**. IR spectroscopy verified the presence of the azido group (sharp band at 2114 cm^{-1}) and provided, along with NMR, MALDI-HRMS, and elemental analysis data, evidence for the proposed structure of pentofuranose **11**.[48] The capability of both anomers of methyl furanoside **10** to efficiently undergo nucleophilic displacement is in noticeable contrast to attempted nucleophilic displacements of O2-triflates in *β*-configured methyl ribofuranosides[49] or O2-tosylates in α-configured methyl arabinofuranosides,[50] where elimination reactions are observed as a consequence of the steric hindrance at the C2-position. Acetolysis of the anomeric mixture of pentofuranose **11** concluded the three-step sequence to give glycosyl donor **12** in excellent yield (80% from **10**) without purification of intermediates. Notably, this three-step sequence has been performed routinely on a 50 g scale without observable

(37) These conditions have also been successfully applied for selective O2′-deacetylation of the corresponding 6-*N*-benzoyladenine or O5′/O5″-dibenzoylated thymine analog of nucleoside **3** (Kumar, T. S.; Andersen, N. K.; Madsen, A. S.; Wengel, J.; Hrdlicka, P. J., 2004, Unpublished results).

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(42) In addition, subjection of nucleoside **8** to modified Mitsunobu conditions using Ph_3P/DIAD/zinc azide-bis-pyridine complex (ref 43), Ph_3P/DDQ/NaN_3/15-crown-5 (ref 44), or Ph_3P/2,4,4,6-tetrabromo-2,5-cyclohexadienone/zinc azide-bis-pyridine complex (ref 45) all failed to yield the desired product.

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(47) Known diol **9** can conveniently be obtained in 64% yield over four steps (+50 g scale) from inexpensive diacetone-α-D-glucose without purification of intermediates: Hrdlicka, P. J.; Andersen, N. K.; Jepsen, J. S.; Hansen, F. G.; Haselmann, K. F.; Nielsen, C.; Wengel, J. *Bioorg. Med. Chem*. **2005**, *77*, 2597−2621.

(48) Although the configuration at C2 of pentofuranoses **10** or **11** could not be established at this stage, it was indirectly proven upon elucidating the structures of nucleosides **14α** and **15** (*vide infra*).

(49) Su, T.-L.; Klein, R. S.; Fox, J. J. *J. Org. Chem.* **1981**, *46*, 1790−1792.

(50) Mikhailopulo, I. A.; Poopeiko, N. E.; Pricota, T. I.; Sivets, G. G.; Kvasyuk, E. I.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1991**, *34*, 2195−2202.

decreases in yield. Optimization of the subsequent glycosylation step revealed that the most favorable conditions for generation of the desired α-anomer of C2′-azido-nucleoside **13** (**13α**/**13*β***, 55:45, 81% combined yield, inseparable mixture) involves treatment of the anomeric mixture of glycosyl donor **12** with persilylated thymine and trimethylsilyl triflate as Lewis acid[51] in refluxing 1,2-dichloroethane for 26 h.[52] Interestingly, prolonged heating leads to decreased yields of nucleoside **13α** due to decomposition, while heating the reaction mixture at lower temperature (50 °C), only affords nucleoside **13*β*** suggesting this to be the kinetic product.

The anomeric mixture of azido nucleoside **13** was subsequently reacted in a one-pot tandem Staudinger/intramolecular nucleophilic substitution reaction[27] to afford an easily separable mixture of bicyclic nucleosides **14α** and **14*β*** in moderate yield (58% combined yield). Efforts to improve the yield by changing the stoichiometry of reagents, the nature of the hydrolytic environment (H_2O, aqueous NaOH, or aqueous NH_3), by performing this conversion as a classical two-step Staudinger reaction[53] (PMe_3/THF followed by separate hydrolysis), or by using other reducing conditions (ammonium formate and Pd/C or H_2S/pyridine/Et_3N) were unsuccessful. Regrettably, anomerization of bicyclic nucleoside **14*β*** using catalytic trifluroacetic anhydride and concentrated sulfuric acid[54] afforded the desired N2′-trifluoroacetamido analog of **14α** in only 17% yield (results not shown).

Structural Verification of Bicyclic Nucleosides 14α and 14*β*. Conclusive evidence for the proposed 2-oxa-5-azabicyclo-[2.2.1]heptane skeletons of nucleosides **14α** and **14*β*** was obtained by NMR experiments. The ^{1}H NMR signals of H1′, H2′, and H3′ of **14α** and **14*β*** appear either as singlets or narrow doublets (J < 2 Hz), which is in agreement with ^{1}H NMR signals of α-L-LNA nucleosides.[12e] This is a consequence of the torsion angles ψ (H1′−C1′−C2′−H2′) and ψ' (H2′−C2′−C3′−H3′) which are fixed in +*gauche* and −*gauche* conformations, respectively, by the locked furanose moiety. Furthermore, a characteristic upfield shift of the ^{1}H NMR signals from the H5″ protons of **14α** and **14*β*** is observed in comparison with nucleoside **13α**/***β***.

The configuration at the anomeric center of nucleosides **14α** and **14*β*** was ascertained by NOE difference spectroscopy. Key NOE contacts between H1′/H2′ (7%), H1′/H3′ (4%), and H2′/H3′ (2%) were observed for bicyclic nucleoside **14α** indicating a *cis* relationship between these protons (Figure 2).[55] Since the stereochemistry of C3′ is defined by the choice of starting material and remains unchanged throughout synthesis, H1′ must be "down", confirming the nucleobase as "up" and hence establishing the α-L-*ribo* configuration of **14α**. This conclusion is further substantiated by the strong signal enhancement between H5″$_A$/H6 (8%) and H5″$_B$/H6 (2%) (H5″$_A$ is tentatively assigned as the H5″ proton closest to H6), indicating a *cis* relationship between the nucleobase and the aza-methylene ring. Single-crystal X-ray diffraction studies of nucleoside **15** (Scheme 4, Figure S1 in the Supporting Information)[56] unequivocally established the proposed structure of 2′-amino-α-L-LNA nucleoside **14α**. The *β*-L-*ribo* configuration of bicyclic nucleoside **14*β*** was indicated by a weaker signal enhancement between H1′/H3′ (1%) than for **14α**, which suggests a *trans* relationship between these protons and therefore "down" positioning of the nucleobase (Figure 2).[55] Also, a very strong enhancement between H6/H3′ (11%), absent in nucleoside **14α**, was observed for nucleoside **14*β***. Enhancement of H5″$_A$/H1′ (2%) and H5″$_B$/H1′ (5%) indicates a *cis* relationship between the anomeric proton and the aza-methylene ring and thereby "up" configuration of the ring. Moreover, the ^{1}H NMR and ^{13}C NMR data are identical to those reported data for the corresponding enantiomer (*β*-D-*ribo* configuration),[27] thereby unequivocally establishing the structure of 2′-amino-*β*-L-LNA nucleoside **14*β***.

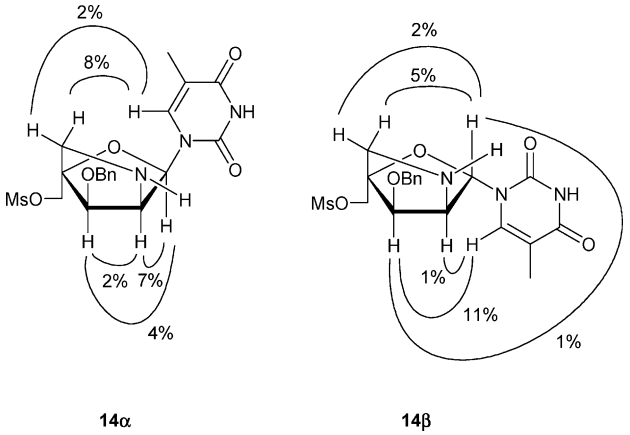


FIGURE 2. Key NOE contacts in bicyclic nucleosides **14α** and **14*β***.

Synthesis of 2′-Amino-α-L-LNA Phosphoramidite Building Block 21. Protection of the secondary amino group of bicyclic nucleoside **14α** as its trifluoroacetamide was necessary to avoid an intramolecular aza-Michael-type conjugate addition (*vide infra*). Subsequent nucleophilic substitution of the C5′-mesylate group and O5′-deacylation with saturated methanolic ammonia afforded amide **15** as a mixture of rotamers in 64% yield over three steps (Scheme 4). The trifluoroacetamido group of **15** was remarkably stable to nucleophilic and/or alkaline conditions (saturated methanolic ammonia, concentrated aqueous ammonia 80 °C, concentrated aqueous methylamine 55 °C, or 0.4 M NaOH in MeOH/H_2O) but was readily cleaved by treatment with sodium borohydride to afford amino alcohol **16** in 83% yield. Attempts to directly obtain amino alcohol **16** from nucleoside **14α** via demesylation using methylmagnesium bromide[57] were not satisfactory as the desired product was only obtained in low yields (<20%, results not shown).

Protection of the O5′-hydroxyl group of amino alcohol **16** as the 4,4′-dimethoxytrityl (DMTr) ether furnished protected nucleoside **17** in 73% yield. Debenzylation of **17** via transfer hydrogenolysis using ammonium formate and $Pd(OH)_2$/C[58] afforded amino alcohol **18** in yields up to 86% when carried

(51) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234−1255.

(52) ^{13}C NMR data of one of the anomers in the obtained mixture of 2′-azido nucleoside **13** was identical with previously reported data for the corresponding *β*-D-*ribo*-configured analog of **13** (ref 27), showing this anomer to be **13*β*** [*β*-L-*ribo* configuration]. The identity of the α-L-*ribo*-enantiomer **13α** could not be established at this stage but was indirectly proven upon elucidating the structures of nucleosides **14α** and **15** (*vide infra*).

(53) (a) Gololobov, Y. G.; Zhmurova, I. N.; Kasukhin, L. F. *Tetrahedron* **1981**, *37*, 437−472. (b) Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353−1406.

(54) Ward, D. I.; Jeffs, S. M.; Coe, P. L.; Walker, R. T. *Tetrahedron Lett.* **1993**, *34*, 6779−6782.

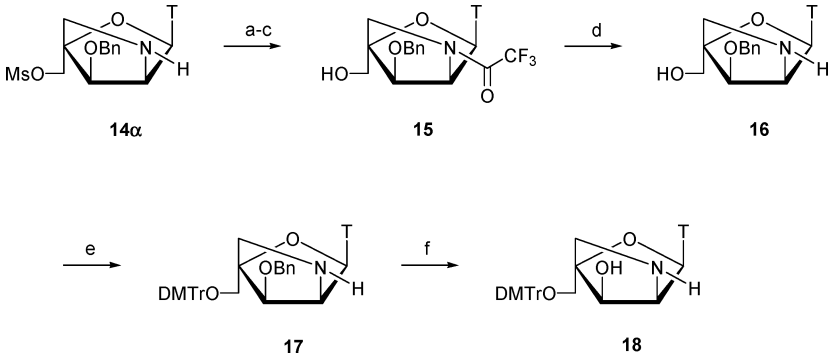
(55) The reported NOE enhancements are an average of the two values observed upon individual irridation of the protons in question.

(56) See the Supporting Information.

(57) Cossy, J.; Ranaivosata, J.-L.; Bellosta, V.; Wietzke, R. *Synth. Commun.* **1995**, *25*, 3109−3112.

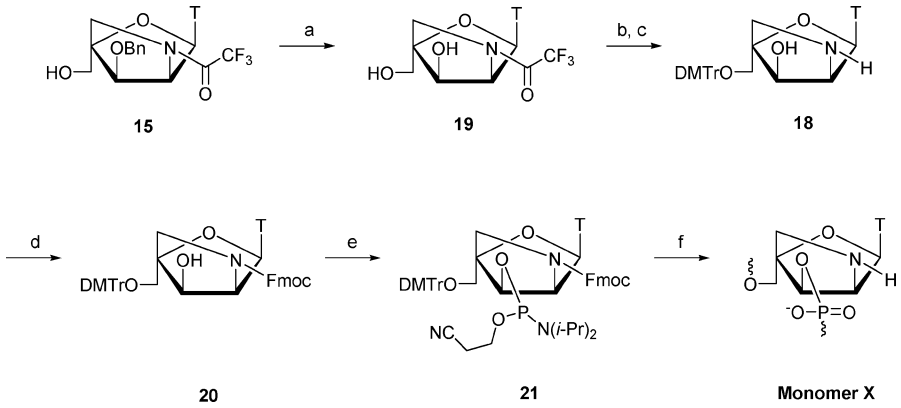
(58) Bieg, T.; Szeja, W. *Synthesis* **1985**, *1*, 76−77.

SCHEME 4[a]



[a] Reagents and conditions: (a) $(CF_3CO)_2O$, pyridine, CH_2Cl_2, 0 °C to room temperature; (b) KOAc, 18-crown-6, 1,4-dioxane, 80 °C; (c) saturated NH_3/MeOH, room temperature, 64%, three steps; (d) $NaBH_4$, absolute EtOH, room temperature, 83%; (e) DMTrCl, DMAP, pyridine, 70 °C, 73%; (f) HCO_2NH_4, $Pd(OH)_2$/C, EtOAc, reflux, 40−86%. DMTr = 4,4′-dimethoxytrityl.

SCHEME 5[a]



[a] Reagents and conditions: (a) BCl_3 in hexanes, CH_2Cl_2, −70 °C to room temperature, 77%; (b) DMTrCl, DMAP, pyridine, room temperature; (c) 2 M aqueous NaOH, absolute EtOH, pyridine, 0 °C to room temperature, 81%, two steps; (d) FmocCl, saturated aqueous $NaHCO_3$, 1,4-dioxane, 0 °C to room temperature, 81%; (e) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, room temperature, 56%; (f) DNA synthesizer. Fmoc = 9′-fluorenylmethoxycarbonyl.

out on a small scale (∼0.15 mmol). However, when the reaction was performed on a larger scale (∼3 mmol), significant amounts of the corresponding product with a reduced thymine moiety were formed (results not shown), whereby the yield of key intermediate **18** dropped to ∼40%. This most likely reflects the tendency of free amines[59] and/or nucleobases[60] to inhibit hydrogenolysis of benzyl ethers.

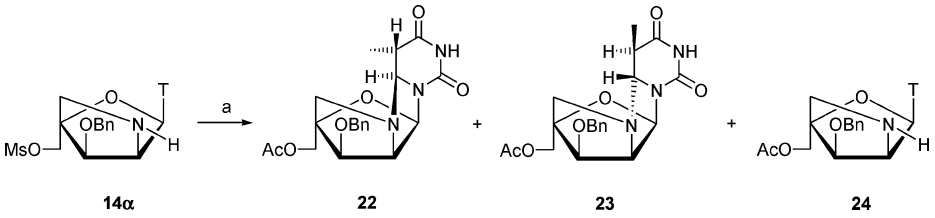
To circumvent this predicament, nucleoside **15** was instead debenzylated using boron trichloride to afford diol **19** in 77% yield (Scheme 5), which was followed by selective O5′-tritylation and removal of the trifluoroacetamido group under basic conditions furnishing amino alcohol **18** in a reliable and high yield (81%, two steps). Thus, the optimized synthetic route (**9** → → **12** → → **14α** → → **19** → **18**) to key intermediate **18** proceeds in 8.3% overall yield over 13 steps from diol **9**, requires only 7 chromatographic purification steps, and is reliable also on a large scale (∼50 g of diol **9**).

Amino alcohol **18**, which recently has been shown to serve as a suitable intermediate for chemoselective N2′-functionalization,[61] was carbamoylated with 9′-fluorenylmethyl chloroformate (FmocCl) under Schotten−Baumann conditions to afford alcohol **20** in 81% yield. The disappearance of the ^1H NMR signals of the exchangeable 3′-OH protons upon D_2O addition ascertained the N2′-functionalized constitution of nucleoside **20**, which subsequently was converted to the corresponding phosphoramidite **21** using standard procedures in 56% yield.

Synthesis of Tetracyclic Phosphoramidite Building Blocks 27 and 31. To our surprise, exposure of 2′-amino-α-L-LNA nucleoside **14α** to conditions that typically facilitate nucleophilic displacement of a O5′-mesylate group (potassium acetate/18-crown-6/1,4-dioxane)[31] resulted in the formation of an easily separable mixture of nonaromatic and non-UV active tetracyclic dihydro-thymine derivatives **22** (18%) and **23** (32%) along with the expected bicyclic nucleoside **24** in 26% yield (Scheme 6).[62] Tetracyclic nucleosides **22** and **23** are formed as a result of a diastereoselective conjugate 1,4-addition of the secondary C2′-(α)-amino group to the C6-position of the thymine moiety of nucleoside **14α**, i.e., an intramolecular aza-Michael reaction[63] where the chiral sugar moiety is the main stereochemical controller. Validation of the proposed structures of **22** and **23**, which have a conformationally locked furanose ring, a conformationally restricted glycosidic torsion angle, and a nonaromatic

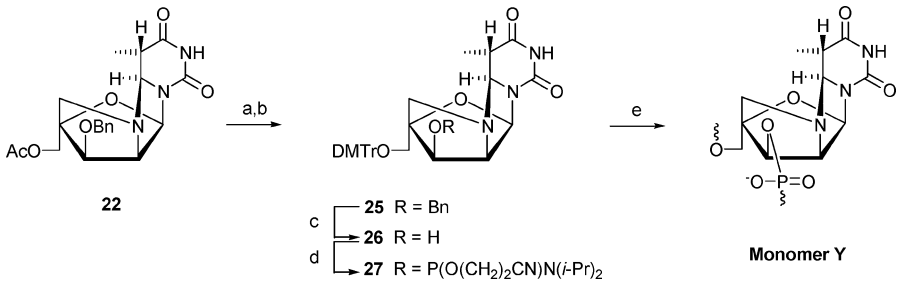
(59) Sajiki, H. *Tetrahedron Lett.* **1995**, *36*, 3465−3468.
(60) Johnson, D. C.; Widlanski, T. S. *Org. Lett.* **2004**, *6*, 4643−4646.
(61) (a) Hrdlicka, P. J.; Kumar, T. S.; Wengel, J. *Chem. Commun.* **2005**, 4279−4281. (b) Kumar, T. S.; Madsen, A. S.; Wengel, J.; Hrdlicka, P. J. Manuscript in preparation.
(62) Similar results were obtained upon subjecting nucleoside **14α** to sodium benzoate and 15-crown-5.
(63) For a recent review on aza-Michael reactions see: Xu, L.-W.; Xia, C.-G. *Eur. J. Org. Chem.* **2005**, 633−639.

SCHEME 6[a]



[a] Reagents and conditions: (a) KOAc, 18-crown-6, 1,4-dioxane, reflux, 18%, 32%, and 26% for **22**, **23**, and **24**, respectively.

SCHEME 7[a]



[a] Reagents and conditions: (a) Saturated NH_3/MeOH, room temperature; (b) DMTrCl, DMAP, pyridine, room temperature, 83%, two steps; (c) $HCOONH_4$, 20% $Pd(OH)_2$/C, EtOAc, 50 °C, 72%; (d) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, *N*,*N*-diisopropylethylamine, CH_2Cl_2, room temperature, 55%; (e) DNA synthesizer.

nucleobase moiety, is presented in a subsequent section. Intramolecular Michael additions of oxygen[64] or nitrogen nucleophiles[65] on the C6-position of pyrimidines have been previously reported. In comparison to the extensive work on nucleosides with conformationally restricted furanose rings, remarkably few nucleosides with conformationally restricted glycosidic torsion angles have been incorporated into oligonucleotides,[66] even though such modifications could provide insight on the influence of the glycosidic torsion angle on nucleic acid duplex stability. Similarly, very few reports have emerged on oligonucleotides containing modified nucleotides that have nonaromatic nucleobase moieties with hydrogen-bonding acceptors or donors,[66a,b,67] whereas much more focus has been devoted on the synthesis and evaluation of nucleotides having nonpolar, non-hydrogen-bonding aromatic nucleobases.[68] These studies have demonstrated that neither hydrogen bonding nor large hydrophobic surfaces *de facto* are needed for duplex stability.

(5*S*,6*R*)-Configured tetracyclic nucleoside **22** was deacylated using saturated methanolic ammonia, and the liberated C5′-hydroxyl group was protected as a 4,4′-dimethoxytrityl ether using standard conditions to furnish fully protected nucleoside **25** in 83% yield over two steps (Scheme 7). Subsequent catalytic transfer hydrogenation using ammonium formate and $Pd(OH)_2$/C afforded alcohol **26** in 72% yield, which upon standard phosphitylation provided phosphoramidite **27** in 55% yield as a suitable building block for oligonucleotide synthesis.

(5*R*,6*S*)-Configured tetracyclic nucleoside phosphoramidite **31** was obtained using a similar synthetic route as for phosphoramidite **27** (Scheme 8). However, it is noteworthy that deacylation of nucleoside **23** afforded the expected tetracyclic nucleoside **28** (60%) along with small amounts of the retro-aza-Michael product **16** (19%). Alcohol **28** was O5′-tritylated to afford fully protected derivative **29** in 82% yield, which upon debenzylation gave alcohol **30** in 66% yield. Subsequent standard phosphitylation afforded phosphoramidite **31** in 73% yield.

Structural Verification of Tetracyclic Nucleosides 22 and 23. As mentioned above, tetracyclic nucleosides **22** and **23** are formed as a result of an intramolecular aza-Michael reaction of bicyclic nucleoside **14α**. Molecular models indeed suggest that the secondary amino group of **14α** is conformationally preorganized for intramolecular attack onto the C6-position as a consequence of the *cis* relationship between the aza-methylene bridge and the nucleobase. To further validate this hypothesis, bicyclic nucleoside **14*β***, i.e., the C1′-anomer of **14α**, was subjected to identical conditions as used for conversion of **14α** to **22** and **23**. The *trans* relationship between the aza-methylene bridge and thymine moiety of **14*β*** is anticipated to prevent intramolecular aza-Michael reaction to occur. As expected, only formation of the C1′-anomer of **24** [*β*-L-*ribo* configuration] was observed (results not shown).

(64) (a) Boesen, T.; Madsen, C.; Pedersen, D. S.; Nielsen, B. M.; Petersen, A. B.; Petersen, M. Å.; Munck, M.; Henriksen, U.; Nielsen, C.; Dahl, O. *Org. Biomol. Chem.* **2004**, *2*, 1245−1254. (b) Dahl, O.; Jensen, J.; Petersen, M. Å.; Henriksen, U. *Org. Biomol. Chem.* **2005**, *3*, 1964−1970.

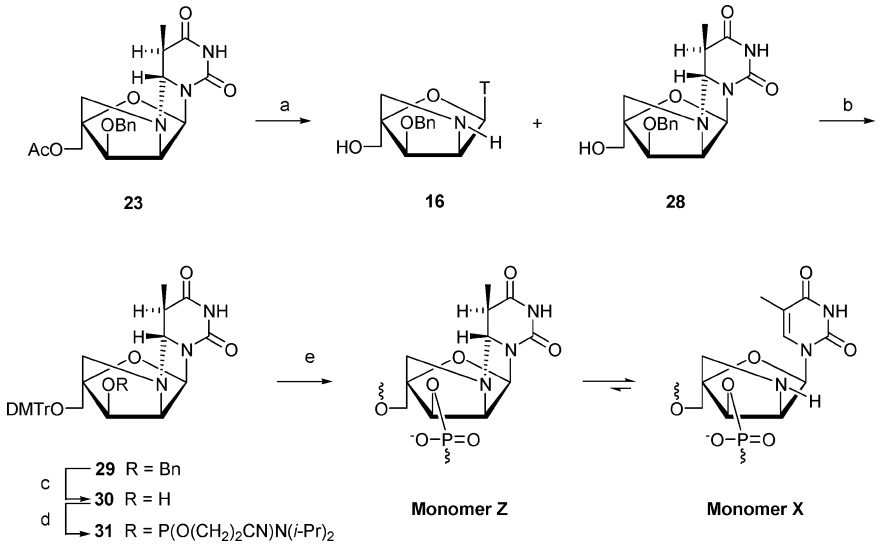
(65) (a) Minamoto, K.; Azuma, K.; Hoshino, Y.; Eguchi, S. *Chem. Lett.* **1989**, 825−828. (b) Tronchet, J. M. J.; Benhamza, R.; Bernardinelli, G. *Nucleosides Nucleotides* **1993**, *12*, 55−71. (c) Chatgilialoglu, C.; Ferreri, C.; Gimisis, T.; Roberti, M.; Balzarini, J.; De Clerq, E. *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 1565−1581.

(66) (a) Muller, E.; Gasparutto, D.; Jaquinod, M.; Romieu, A.; Cadet, J. *Tetrahedron* **2000**, *56*, 8689−8701. (b) Muller, E.; Gasparutto, D.; Cadet, J. *ChemBioChem* **2002**, *3*, 534−542. (c) Urata, H.; Miyagoshi, H.; Kumashiro, T.; Yumoto, T.; Mori, K.; Shoji, K.; Gohda, K.; Akagi, M. *Org. Biomol. Chem.* **2004**, *2*, 183−189.

(67) Gao, J.; Liu, H.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 11826−11831.

(68) (a) Kool, E. T.; Morales, J. C.; Guckian, K. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 990−1009. (b) Babu, B. R.; Prasad, A. K.; Trikha, S.; Thorup, N.; Parmar, V. S.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2509−2519. (c) Raunak; Babu, B. R.; Sørensen, M. D.; Parmar, V. S.; Harrit, N. H.; Wengel, J. *Org. Biomol. Chem.* **2004**, *2*, 80−89. (d) Lai, J. S.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 3040−3041. (e) Henry, A. A.; Olsen, A. G.; Matsuda, S.; Yu, C.; Geierstanger, B. H.; Romesberg, F. E. *J. Am. Chem. Soc.* **2004**, *126*, 6923−6931. (f) Matsuda, S.; Romesberg, F. E. *J. Am. Chem. Soc.* **2004**, *126*, 14419−14427. (g) Kim, T. W.; Kool, E. T. *J. Org. Chem.* **2005**, *70*, 2048−2053. (h) Brotschi, C.; Mathis, G.; Leumann, C. J. *Chem. Eur. J.* **2005**, *11*, 1911−1923.

SCHEME 8[a]



[a] Reagents and conditions: (a) Saturated NH_3/MeOH, room temperature, 19% for **16**, 60% for **28**; (b) DMTrCl, DMAP, pyridine, room temperature, 82%; (c) $HCOONH_4$, 20% $Pd(OH)_2$/C, EtOAc, 50 °C, 66%; (d) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, *N*,*N*-diisopropylethylamine, CH_2Cl_2, room temperature, 73%; (e) DNA synthesizer.

The proposed constitution of tetracyclic nucleosides **22** and **23** was confirmed by MALDI-HRMS, 1D-NMR, and 2D-NMR. The characteristics of the NMR signals of tetracyclic nucleosides **22** and **23**, compared to those of the expected bicyclic nucleoside **24** (Scheme 6), are (a) ~1.0 ppm upfield shift of the ^{1}H NMR signal of the exchangeable NH proton in the nucleobase, (b) ~3.0 ppm upfield shift of the ^{1}H NMR signal of H6, which appears as a doublet ($J \sim 12$ Hz), (c) ^{1}H NMR signal of H5 appearing between 2.5−3.0 ppm as a double doublet or multiplet, which is coupled to H6 and CH_3−C5 according to ^{1}H−^{1}H COSY NMR, (d) ~0.7 ppm upfield shift of the ^{1}H NMR signal of CH_3−C5 and its appearance as a doublet with a relatively large coupling constant ($J \sim 7$ Hz), and (e) ^{13}C NMR signals of C5 and C6 shifted upfield to 38−39 ppm and 77−83 ppm, respectively. These observations are in agreement with previous reports on C6,N2′-cyclopyrimidines.[65a,b] Furthermore, the appearance of the H1′ signals (^{1}H NMR) as narrow doublets ($J \sim 2$ Hz) and the observation that the AB systems of H5′ and H5″ appear at significantly different chemical shift values strongly suggest[12e] that the integrity of the 2′-amino-α-L-LNA subskeleton of nucleosides **22** and **23** is preserved.

Inspection of molecular models of bicyclic nucleoside **14α** indicates that intramolecular aza-Michael-type conjugate additions only can occur when the chiral nitrogen of the C2′-(α)-amino group is in an *R*-configuration implying that only tetracyclic nucleosides in which the chiral nitrogen atom adopts an *S*-configuration can be formed. The assignment of stereochemistry at the C5- and C6-positions of **22** and **23** was achieved by combining force-field calculations, coupling constant analysis, and NOE difference spectroscopy. In principle, four diastereomers may be formed during the aza-Michael reaction (5*S*,6*S*; 5*S*,6*R*; 5*R*,6*S*; 5*R*,6*R*). All of these were built and subjected to Monte Carlo based conformational searches using the AMBER force-field[69] and generalized Born/surface area solvation model as implemented in the MacroModel V7.2 suite.[70] The torsion angle ϕ (H5−C5−C6−H6) in the lowest energy structure of each diastereomer was measured, and the theoretical coupling constant J_{H5-H6} was assessed by using the Diez−Altona−Donders equation as implemented within the MestRe J program.[71] The H5 and H6 protons are orientated −*gauche* and +*gauche* (−46° and +51°, respectively) with respect to each other in two of the four diastereomers (5*S*,6*S*; 5*R*,6*R*), which corresponds to a predicted coupling constant J_{H5-H6} of ~5 Hz. The H5/H6 protons in the two other diastereomers, i.e., (5*S*,6*R*) or (5*R*,6*S*), are orientated *anti* (176° and 183°, respectively) with respect to each other, which corresponds to a predicted coupling constant J_{H5-H6} of ~11 Hz. The experimentally observed coupling constants J_{H5-H6} for **22** and **23** are 11.7 and 12.8 Hz, respectively, which strongly suggests an *anti* orientation of H5 and H6.

NOE difference experiments of the diastereomer with higher mobility (nucleoside **22**, *vide infra*) displayed significant NOE contacts between H1′/H2′ (6%) and H1′/H3′ (3%)[55] indicating a *cis* relationship between these protons, and since the stereochemistry of C3′ is known, this corresponds to a "down" positioning of these protons (Figure 3). More importantly, an NOE enhancement between H6 and CH_3−C5 (4%) was observed, indicating a *cis* relationship between these groups, which along with the strong NOE contacts between $H5''_B$/H6 (10%) and H5/H2′ (6%) strongly supports the assignment of tetracyclic nucleoside **22** as the (5*S*,6*R*)-diastereomer. The diastereomer with lower mobility (nucleoside **23**, *vide infra*) also exhibited NOE enhancements between H1′/H2′ (5%), H1′/H3′ (2%), and H6/CH_3−C5 (4%) indicating a *cis* relationship between these protons (Figure 3).[55] Furthermore, a strong NOE enhancement between H6/H2′ (5%) was observed, which, along with the absence of an NOE enhancement between H5″/H6, strongly suggests a 6*S*-configuration and supports the assignment of tetracyclic nucleoside **23** as the (5*R*,6*S*)-diastereomer. It was

(69) (a) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765−784. (b) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230−252.

(70) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(71) Program available from http://www.mestrec.com.

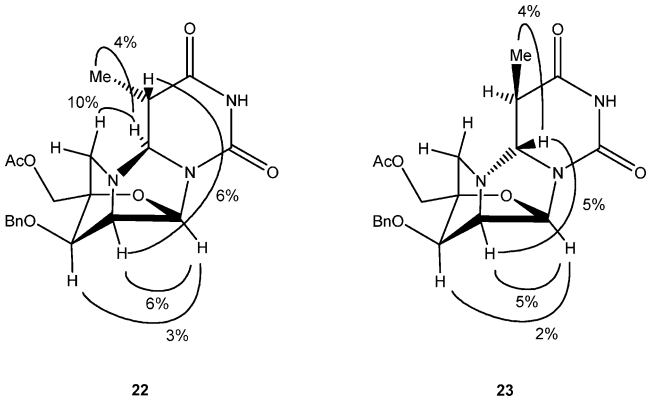


FIGURE 3. NOE contacts in tetracyclic "locked LNA" nucleosides **22** and **23**.

not possible to observe the expected NOE contacts between H5/H5″ due to spectral overlap of these signals.

The glycosidic torsion angle χ (O4′−C1′−N1−C2) of the tetracyclic nucleosides **22** and **23** is conformationally constrained as a consequence of the tetracyclic skeleton, and the lowest energy structures exhibit values of $\chi = -116°$ and $-87°$ for **22** and **23**, respectively. Nucleosides **22** and **23** would after incorporation into ONs yield monomers **Y** and **Z** (Schemes 7 and 8), respectively, which due to their high degree of rigidity would be expected to exhibit similar glycosidic torsion angles to those observed at the nucleoside level.

It is noteworthy that only formation of *syn*-addition products **22** and **23** was observed during the aza-Michael reaction, i.e., the nitrogen nucleophile and H5 are both added from either above (**22**) or below (**23**) the plane of the aromatic ring. Interestingly, the lowest energy structures reveal that the CH_3−C5 group occupies a pseudoequatorial position in both tetracyclic nucleosides **22** and **23**, whereby it is projected away from the bulky furanose ring.[56] In contrast, the CH_3−C5 group in the (5*R*,6*R*)- and (5*S*,6*S*)-diastereomers occupies a pseudoaxial position, whereby it may interfere with the H2′- and H5″-protons, respectively, which may account for the stereoselectivity of the aza-Michael reaction.[56]

Synthesis of ONs. Incorporation of 2′-amino-α-L-LNA phosphoramidite **21** or "locked LNA" phosphoramidites **27** and **31** (see Schemes 5, 7, and 8 for structures) was performed on a 0.2 μmol scale using an automated DNA synthesizer. Preliminary attempts to incorporate monomer **X** into ONs using phosphoramidite **21** failed due to partial decomposition of the ONs during deprotection conditions used for DNA synthesis (32% aqueous ammonia, for either 12 h at 55 °C or 4 h at room temperature). The use of *tert*-butylphenoxyacetyl-protected (TAC-protected) dA, dC, and dG phosphoramidites[72] along with phosphoramidite **21** (12 min coupling time, 1*H*-tetrazole as catalyst, ∼99% stepwise coupling yield), however, allowed removal of the protecting groups and cleavage from solid support using ultramild deprotection conditions (0.05 M K_2CO_3 in MeOH, room temperature, 2 h),[73] and successfully afforded the desired sequences **ON3**−**ON5** (Table 1).

The observed base-induced retro-aza-Michael reaction of tetracyclic nucleoside **23** leading to the formation of 2′-amino-α-L-LNA nucleoside **16** (Scheme 8) indicated that precautions during deprotection of ONs containing monomers **Y** and **Z** would be necessary.[74] Thus, TAC chemistry was applied as described above resulting in ∼94% stepwise coupling yield of phosphoramidites **27** and **31**. The ONs were subsequently cleaved from solid support and deprotected using 32% aqueous NH_3 at room temperature for 2 h.

TABLE 1. Thermal Denaturation Studies[a]

		T_m (ΔT_m)/°C	
		DNA	RNA
ON1	5′-GTG ATA TGC	28.5[b,c]	26.5[d]
ON2	5′-GCA TAT CAC	28.5[b]	24.5
ON3	5′-GTG A**X**A TGC	29.0 (+0.5)[e]	28.5 (+2.0)[f]
ON4	5′-GCA **X**AT CAC	31.0 (+2.5)	29.0 (+4.5)
ON5	5′-GCA TA**X** CAC	28.0 (−0.5)	27.5 (+3.0)
ON6	5′-GTG A**Y**A TGC	<10	<10
ON7	5′-GCA TA**Y** CAC	<10	<10

[a] Thermal denaturation temperatures of **ON1**−**ON7** toward DNA or RNA complements [T_m values/°C (ΔT_m = change in T_m value calculated relative to that of the DNA/DNA or DNA/RNA reference duplex)] measured as the maximum of the first derivative of the melting curve (A_{260} vs temperature) recorded in medium salt buffer ([Na^+] = 110 mM, [Cl^-] = 100 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)), using 1.0 μM concentrations of the two complementary strands. T_m values are averages of at least two measurements; A = adenin-9-yl DNA monomer, C = cytosin-1-yl DNA monomer, G = guanin-9-yl DNA monomer, T = thymin-1-yl DNA monomer; see Schemes 5 and 7 for the structures of monomers **X** and **Y**. [b] **ON1** and **ON2** are complementary. [c] T_m values (°C) toward DNA strands containing a single mismatch in the central position C/G/T: 12.0/19.0/11.5. [d] T_m values (°C) toward RNA strands containing a single mismatch in the central position C/G/U: <10.0/22.0/<10.0. [e] T_m values (°C) toward DNA strands containing a single mismatch in the central position C/G/T: <10.0/17.5/<10.0. [f] T_m values (°C) toward RNA strands containing a single mismatch in the central position C/G/U: <10.0/22.5/<10.0.

After purification,[56] the composition and purities (>75%) of **ON3**−**ON10** were investigated by MALDI-MS (Table S1) and ion-exchange HPLC, respectively. Despite the very mild conditions used for deprotection of ONs with expected incorporations of (5*R*,6*S*)-configured "locked LNA" monomer **Z** (**ON8**−**ON10**),[56] a series of observations (including thermal denaturation temperatures, ion-exchange HPLC profiles and ^{1}H NMR studies) demonstrated that monomer **Z** was unstable and converted into 2′-amino-α-L-LNA monomer **X** (Scheme 8). However, we cannot exclude the possibility of an equilibrium between monomers **X** and **Z** during the applied experimental conditions, as has been observed for other 13-oxa-2,4,8-triazatetracyclo[8.2.1.0.^{2,7}0^{8,12}]tridecanes.[65a] The experimental observations suggest, however, that if such an equilibrium exists it is strongly shifted toward 2′-amino-α-L-LNA monomer **X**. The reader is invited to consult the Supporting Information for a more elaborate discussion. Intriguingly, the (5*S*,6*R*)-configured "locked LNA" monomer **Y** was stable to the applied deprotection conditions. Examination of lowest energy structures of the precursors **22** and **23** does not suggest an adequate explanation for the difference in alkaline stability between monomers **Y** and **Z**.

Preliminary Thermal Denaturation Studies. The effect on duplex stability upon incorporation of 2′-amino-α-L-LNA monomer **X** or locked LNA monomer **Y** into non-self-complementary 9-mer ONs (**ON3**−**ON7**) was evaluated by UV−thermal denaturation experiments using medium salt buffer ([Na^+] = 110 mM) (Table 1). The UV−thermal denaturation curves of the duplexes between 2′-amino-α-L-LNAs **ON3**−

(72) Sinha, N. D.; Davis, P.; Usman, N.; Perez, J.; Hodge, R.; Kremsky, J.; Casale, R. *Biochimie* **1993**, *75*, 13−23.

(73) Zhu, Q.; Delaney, M. O.; Greenberg, M. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1105−1107.

(74) Intriguingly, a retro aza-Michael reaction of nucleoside **22** under identical conditions was not observed.

ON5 and DNA/RNA complements displayed smooth sigmoidal monophasic transitions, which were similar to those recorded for the unmodified reference duplexes (Figure S2 in the Supporting Information).[56] Incorporation of a single monomer **X** in ONs results in slightly decreased to moderately increased thermal affinities toward complementary DNA compared to the unmodified reference ONs (see data for **ON3**−**ON5**, Table 1). In contrast, prominent stabilization is observed with RNA complements (ΔT_m = +2.0 to +4.5 °C). Comparison with previously published thermal denaturation data for α-L-LNA[12c] suggests that 2′-amino-α-L-LNA exhibit slightly less favorable thermal affinities than their 2′-oxy counterparts. It may be speculated, that this is a consequence of small amounts of potentially destabilizing monomer **Z** present at equilibrium (Scheme 8). Thermal denaturation studies of 2′-amino-α-L-LNA **ON3** and DNA/RNA strands containing a centrally placed single mismatch demonstrate that the increased thermal affinity of monomer **X** is accompanied by a Watson−Crick selectivity, which is superior to that of the corresponding unmodified ON (Table 1, footnotes c−f). These are highly desirable characteristics for nucleic acid probes intended for medicinal and biotechnological applications.[75]

Incorporation of a single (5*S*,6*R*)-configured "locked LNA" monomer **Y** resulted in dramatic decreases in thermal affinity toward both complementary DNA and RNA (see data for **ON6**−**ON7**, Table 1). A similar trend has been observed for ONs modified with 5,6-dihydropyrimidines.[66a,b] It is noteworthy, that incorporation of monomer **Y** into an ON is more destabilizing than a mismatched DNA/DNA or DNA/RNA base pair (compare data for **ON6** with mismatch data for **ON1**). In fact, monomer **Y** destabilizes duplexes as much as incorporation of an abasic site (a THF analog) at this position.[61b] Hence, monomer **Y** not only does not contribute to stabilization of duplexes but actively destabilizes neighboring base pairs. We speculate that this may be a consequence of the loss of aromaticity, and the thereby increased steric bulk of the nucleobase moiety (from planar to tetrahedral geometry at the C5/C6-positions). This may lead to energetically unfavorable intrastrand interactions with the neighboring nucleobases. Alternatively, nucleobase atoms normally participating in hydrogen bonding may be positioned suboptimally as a consequence of the constrained glycosidic torsion angle; however, structural studies would be required to determine this.

To conclude, a viable synthetic route toward a 2′-amino-α-L-LNA phosphoramidite has been developed. Introduction of a C2′-azido group prior to Vorbrüggen glycosylation proved to be crucial for the success of the route. The secondary amino group of the aza-methylene bridge of 2′-amino-α-L-LNA nucleosides is conformationally preorganized for intramolecular Michael addition to the C6-position. Under certain conditions formation of tetracyclic "locked LNAs", in which the furanose ring and the glycosidic torsion angle are conformationally restricted, is observed. Incorporation of one such "locked LNA" monomer into ONs has a detrimental effect on thermal affinity toward DNA/RNA complements. In contrast, incorporation of 2′-amino-α-L-LNA monomers into ONs leads to modest changes in thermal affinity toward DNA complements relative to unmodified reference ONs but to prominent increases toward RNA complements. The flexible synthetic strategy allows chemoselective N2′-functionalization of amino alcohol **18**, which enables the introduction of functional groups in the major groove or core of nucleic acid duplexes.[61] The potential of N2′-functionalized 2′-amino-α-L-LNA monomers within nucleic acid based nanobiotechnology and therapeutics has very recently been emphasized by the ability of double-stranded pyrene-functionalized 2′-amino-α-L-LNA to target short double-stranded DNA.[61a] Extensions of this work will be reported in due course.

Experimental Section

Methyl-3-*O*-benzyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-1-*O*-methyl-α,*β*-L-*threo*-pentofuranoside (10α/*β*). Known diol **9**[32] (100.0 g, 0.32 mol) was coevaporated with anhydrous pyridine (300 mL) and redissolved in anhydrous pyridine (500 mL), whereupon methanesulfonyl chloride (62.3 mL, 0.81 mol) was added over 10 min at 0 °C. After warming the reaction mixture to room temperature, it was stirred for 17 h. Then, crushed ice (400 mL) was added, and the mixture was extracted with EtOAc (2 × 400 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$ (2 × 200 mL) and evaporated to dryness to afford the known crude bis-sulfonic ester[12e] (∼152 g) [R_f = 0.7 (70% EtOAc in petroleum ether), MALDI-HRMS *m*/*z* 489.0841 ([M + Na]$^+$, $C_{18}H_{26}O_{10}S_2{\cdot}Na^+$, Calcd 489.0860)] as a colorless oil, which was used in the next step without purification. To an ice-cold solution of the bis-sulfonic ester in MeOH (1.6 L) was added acetyl chloride (85 mL, 1.20 mol). The green-colored reaction mixture was warmed to room temperature and stirred for 39 h, whereupon it was poured into crushed ice (500 mL). The solution was neutralized by addition of $NaHCO_3$(s) until evolution of CO_2 ceased. The mixture was concentrated, and solids were filtered off. The resulting aqueous solution was extracted with EtOAc (4 × 400 mL) and the combined organic phase evaporated to dryness. The resulting residue was purified by silica gel column chromatography (EtOAc) to afford an anomeric mixture (∼2:3 by ^{1}H NMR) of methylfuranoside **10α/*β*** (142 g, quantitative yield over two steps) as colorless oil. A couple of fractions of pure anomers were obtained facilitating their individual characterization. Physical data for anomer A: R_f = 0.4 (1% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m*/*z* 463.0697 ([M + Na]$^+$, $C_{16}H_{24}O_{10}S_2{\cdot}Na^+$, Calcd 463.0703); ^{1}H NMR (DMSO-d_6) δ 7.27−7.39 (m, 5H, Ph), 5.44 (d, 1H, ex, J = 7.3 Hz, 2-OH), 4.76 (d, 1H, J = 4.4 Hz, H1), 4.69−4.74 (d, 1H, J = 11.7 Hz, CH_2Ph),[76] 4.63−4.68 (d, 1H, J = 11.7 Hz, CH_2Ph), 4.22−4.31 (m, 3H, 2 × H5, H5′), 4.15−4.21 (m, 1H, H2), 4.08−4.13 (d, 1H, J = 10.3 Hz, H5′), 4.03−4.07 (d, 1H, J = 8.1 Hz, H3), 3.33 (s, 3H, CH_3O), 3.24 (s, 3H, CH_3SO_2), 3.14 (s, 3H, CH_3SO_2); ^{13}C NMR (DMSO-d_6) δ 137.9, 128.1 (Ph), 127.5 (Ph), 101.5 (C1), 84.3 (C3), 79.4, 75.7 (C2), 72.4 (CH_2Ph), 70.2 (C5′), 68.7 (C5), 54.7 (CH_3O), 36.62 (CH_3SO_2), 36.57 (CH_3SO_2). Physical data for anomer B: R_f = 0.2 (1% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m*/*z* 463.0695 ([M + Na]$^+$, $C_{16}H_{24}O_{10}S_2{\cdot}Na^+$, Calcd 463.0703); ^{1}H NMR (DMSO-d_6) 7.29−7.38 (m, 5H, Ph), 5.80 (d, 1H, ex, J = 4.4 Hz, 2-OH); 4.81 (d, 1H, J = 1.5 Hz, H1), 4.65−4.71 (d, 1H, J = 11.7 Hz, CH_2Ph),[76] 4.53−4.59 (d, 1H, J = 11.7 Hz, CH_2Ph), 4.27−4.32 (m, 3H, 2 × H5, H5′), 4.15−4.22 (m, 2H, H2, H5′), 3.94 (d, 1H, J = 2.9 Hz, H3), 3.30 (s, 3H, CH_3O), 3.21 (s, 3H, CH_3SO_2), 3.19 (s, 3H, CH_3SO_2); ^{13}C NMR (DMSO-d_6) 137.6, 128.2 (Ph), 127.5 (Ph), 127.4 (Ph), 109.4 (C1), 85.5 (C3), 83.2, 78.4 (C2), 71.8 (CH_2Ph), 68.7 (C5′), 68.6 (C5), 54.9 (CH_3O), 36.64 (CH_3SO_2), 36.55 (CH_3SO_2). A trace impurity of anomer A was identified. Physical data for anomeric mixture: Anal. Calcd for $C_{16}H_{24}O_{10}S_2$: C, 43.63; H, 5.49. Found: C, 43.61; H, 5.55.

Methyl-2-azido-3-*O*-benzyl-2-deoxy-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-1-*O*-methyl-α,*β*-L-*erythro*-pento-

(75) Demidov, V. V.; Frank-Kamenetskii, M. D. *Trends Biochem. Sci.* **2004**, *29*, 62−71.

(76) Assignments of ^{1}H NMR signals of CH_2Ph, H5, and H5′ (H5′/H5″ for nucleosides), and of the corresponding ^{13}C NMR signals, may in principle be interchanged.

furanoside (11α/β). An anomeric mixture of methyl furanoside **10α/β** (49.22 g, 0.11 mol) was coevaporated with anhydrous pyridine (2 × 125 mL) and dissolved in a mixture of anhydrous pyridine (110 mL) and anhydrous CH_2Cl_2 (440 mL). The solution was cooled to −15 °C, and triflic anhydride (Tf_2O, 24.3 mL, 0.14 mol) was added over a period of 10 min. The reaction mixture was warmed to room temperature and stirred for 90 min. The slightly orange reaction mixture was then cooled to −15 °C, and saturated aqueous $NaHCO_3$ (100 mL) was added. The phases were separated, and the organic phase was washed with additional saturated aqueous $NaHCO_3$ (100 mL). The organic phase was evaporated to dryness, coevaporated with toluene (3 × 100 mL), and the resulting anomeric mixture of crude triflate was used immediately in the next step without further purification. To a solution of the crude triflate in anhydrous DMF (250 mL) was added NaN_3 (10.89 g, 0.17 mol) and 15-crown-5 (22.2 mL, 0.11 mol). After stirring for 21 h at 50 °C, the reaction mixture was cooled to room temperature and diluted with EtOAc (600 mL). The organic phase was washed sequentially with saturated aqueous $NaHCO_3$ (200 mL) and brine (2 × 200 mL) and evaporated to dryness to afford a slightly yellow oil (∼65 g), which was used in the next step without purification. An aliquot was purified by silica gel column chromatography (0−1.75% MeOH in CH_2Cl_2, v/v) to afford an anomeric mixture (∼1:2 by ^{1}H NMR) of azido methylfuranoside **11α/β**. Physical data for the anomeric mixture: R_f = 0.5 (70% EtOAc in petroleum ether, v/v); selected IR signal, ν_{max} 2114 cm^{-1} (azido group); MALDI-HRMS *m/z* 488.0749 ([M + Na]$^+$, $C_{16}H_{23}N_3O_9S_2$·Na$^+$, Calcd 488.0768); ^{13}C NMR (DMSO-d_6) 137.03, 137.01, 128.32, 128.26, 128.2, 127.92, 127.90, 127.7, 104.9, 104.3, 83.4, 81.1, 80.6, 79.6, 74.4, 72.9, 69.4, 68.9, 68.8, 68.7, 64.1, 59.7, 55.3, 54.7, 36.68, 36.64. Anal. Calcd for $C_{16}H_{23}N_3O_9S_2$: C, 41.28; H, 4.98; N, 9.03; S, 13.78. Found: C, 41.58; H, 4.89; N, 8.69; S, 13.66.

1-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-5-*O*-methanesulfonyl-4-*C*-methanesulfonoxymethyl-α,β-L-*erythro*-pentofuranose (12α/β). To an ice-cold solution of the anomeric mixture of crude azido methylfuranoside **11α/β** (∼65 g) in acetic acid (170 mL) was added acetic anhydride (42.3 mL, 0.45 mol) and concentrated H_2SO_4 (21.5 mL). After stirring at room temperature for 41 h, the reaction mixture was cooled to 0 °C and neutralized with aqueous NaOH (4.0 M, ∼130 mL). The resulting solution was further diluted with H_2O (50 mL), and the aqueous phase was extracted with EtOAc (4 × 500 mL). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (10−60% EtOAc in petroleum ether, v/v) affording an anomeric mixture (∼1:6 by ^{1}H NMR) of the glycosyl donor **12α/β** (44.10 g, 80%, over three steps) as a colorless oil. Physical data for anomeric mixture: R_f = 0.5 (70% EtOAc in petroleum ether, v/v); selected IR signal, ν_{max} 2117 cm^{-1} (azido group); MALDI-HRMS *m/z* 516.0724 ([M + Na]$^+$, $C_{17}H_{23}N_3O_{10}S_2$·Na$^+$, Calcd 516.0712); ^{13}C NMR (DMSO-d_6) δ 169.1, 168.7, 137.1, 137.0, 128.33, 128.29, 127.92, 127.85, 127.81, 127.7, 97.1, 95.5, 85.2, 82.2, 79.4, 79.0, 74.1, 73.0, 68.9, 68.7, 68.6, 68.4, 63.8, 59.6, 36.9, 36.69, 36.67, 20.9, 20.7. Anal. Calcd for $C_{17}H_{23}N_3O_{10}S_2$: C, 41.37; H, 4.70; N, 8.51. Found: C, 41.80; H, 4.55; N, 7.99. Calcd with $^3/_{16}$ EtOAc: C, 42.05; H, 4.87; N, 8.29.

1-[2-Azido-3-*O*-benzyl-2-deoxy-5-*O*-methanesulfonyl-4-*C*-methanesulfonoxymethyl-α,β-L-*erythro*-pentofuranosyl]thymine (13α/β). Thymine (18.80 g, 0.15 mol) was coevaporated with anhydrous 1,2-dichloroethane (60 mL) and resuspended in anhydrous 1,2-dichloroethane (190 mL). To this was added *N*,*O*-bis-(trimethylsilyl)acetamide[77] (BSA) 56.0 mL, 0.23 mol), and the solution was refluxed until becoming homogeneous (40 min). After cooling to room temperature, glycosyl donor **12α/β** (36.67 g, 74.3 mmol) was dissolved in anhydrous 1,2-dichloroethane (190 mL), and trimethylsilyl triflate (TMSOTf, 34.0 mL, 0.18 mol) was added. The reaction mixture was refluxed for 26 h whereupon it was cooled to room temperature and poured into saturated aqueous $NaHCO_3$ (200 mL) and diluted with brine (200 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (4 × 200 mL). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (0−70% EtOAc in petroleum ether, v/v) to afford an anomeric mixture (α:β ∼ 55:45 by ^{1}H NMR) of the azido nucleoside **13α/β** (33.60 g, 81%) as a white solid material. A few fractions of the pure β-L-*ribo*-configured anomer were obtained. Physical data for anomeric mixture: R_f = 0.6 (80% acetone in petroleum ether, v/v), selected IR signal, ν_{max} 2118 cm^{-1} (azido group); MALDI-HRMS *m/z* 582.0942 ([M + Na]$^+$, $C_{20}H_{25}N_5O_{10}S_2$·Na$^+$, Calcd 582.0935); ^{13}C NMR (DMSO-d_6) δ 163.5, 163.4, 150.3, 150.0, 137.0, 136.9, 135.9, 135.7, 128.3, 127.9, 127.8, 110.3, 108.5, 86.4, 83.1, 83.0, 81.0, 79.4, 78.7, 73.9, 73.0, 68.2, 68.0, 67.5, 62.4, 61.3, 37.0, 36.8, 36.7, 36.6, 12.0, 11.9. Physical data for β-L-*ribo*-configured anomer **13β**: ^{13}C NMR ($CDCl_3$) δ 163.6, 150.2, 137.0, 136.0, 128.7, 128.5, 112.0, 90.7, 83.8, 78.9, 75.0, 68.2, 67.6, 63.5, 37.5, 12.2. ^{13}C NMR ($CDCl_3$) data are identical with previously reported data for the corresponding β-D-*ribo*-enantiomer.[27]

(1*S*,3*R*,4*S*,7*R*)-7-Benzyloxy-1-methanesulfonoxymethyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (14α) and (1*S*,3*S*,4*S*,7*R*)-7-Benzyloxy-1-methanesulfonoxymethyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (14β). To a solution of an anomeric mixture of nucleoside **13** (17.66 g, 31.6 mmol) in THF (215 mL) and aqueous NaOH (2.0 M, 24 mL, 48.0 mmol) was added PMe_3 (1.0 M in THF, 47.4 mL, 47.4 mmol). After stirring at room temperature for 20 h the phases were separated, and the aqueous phase was extracted with 8% MeOH in $CHCl_3$ (8 × 100 mL, v/v). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (0−85% acetone in petroleum ether, v/v) to afford α-L-*ribo*-configured nucleoside **14α** (4.11 g) and β-L-*ribo*-configured nucleoside **14β** (3.92 g, 58% combined), both as white solid materials. Physical data for **14α**: R_f = 0.5 (80% acetone in petroleum ether, v/v), MALDI-HRMS *m/z* 460.1128 ([M + Na]$^+$, $C_{19}H_{23}N_3O_7S$·Na$^+$, Calcd 460.1149); ^{1}H NMR (DMSO-d_6) δ 11.31 (s, 1H, ex, NH), 7.58 (s, 1H, H6), 7.28−7.43 (m, 5H, Ph), 5.87 (d, 1H, *J* = 1.8 Hz, H1′), 4.66−4.70 (d, 1H, *J* = 11.7 Hz, CH_2Ph),[78] 4.58−4.61 (d, 1H, *J* = 11.7 Hz, CH_2Ph), 4.53 (s, 2H, H5′), 4.26 (br s, 1H, H3′), 3.71 (br s, 1H, H2′), 3.22 (s, 3H, CH_3SO_2), 3.13−3.19 (d, 1H, *J* = 10.4 Hz, H5″), 2.93−2.99 (d, 1H, *J* = 10.4 Hz, H5″), 1.82 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.8, 150.2, 137.8, 136.6 (C6), 128.2 (Ph), 127.53 (Ph), 127.47 (Ph), 107.1, 87.3, 86.1 (C1′), 79.8 (C3′), 70.8 (CH_2Ph), 66.6 (C5′), 58.9 (C2′), 50.6 (C5″), 36.8 (CH_3SO_2), 12.2 (CH_3). Anal. Calcd for $C_{19}H_{23}N_3O_7S$: C, 52.16; H, 5.30; N, 9.61; S, 7.33. Found: C, 52.25; H, 5.36; N, 9.18; S, 7.01. Calcd with $^1/_8$ EtOAc: C, 52.46; H, 5.42; N, 9.41; S, 7.18. Physical data for **14β**: R_f = 0.2 (80% acetone in petroleum ether, v/v), MALDI-HRMS *m/z* 460.1126 ([M + Na]$^+$, $C_{19}H_{23}N_3O_7S$·Na$^+$, Calcd 460.1149); ^{1}H NMR (DMSO-d_6) δ 11.38 (s, 1H, ex, NH), 7.46 (d, 1H, *J* = 0.9 Hz, H6), 7.26−7.34 (m, 5H, Ph), 5.44 (s, 1H, H1′), 4.49−4.70 (m, 4H, CH_2Ph, H5′), 3.84 (s, 1H, H3′), 3.65 (s, 1H, H2′), 3.26 (s, 3H, CH_3SO_2), 3.04−3.09 (d, 1H, *J* = 9.9 Hz, H5″), 2.76−2.81 (d, 1H, *J* = 9.9 Hz, H5″), 1.77 (d, 1H, *J* = 0.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.8, 149.9, 137.7, 134.5 (C6), 128.1 (Ph), 127.7 (Ph), 127.5 (Ph), 108.1, 88.2 (C1′), 85.5, 76.2 (C3′), 70.7 (CH_2Ph),[78] 66.4 (C5′), 59.2 (C2′), 49.9 (C5″), 36.7 (CH_3SO_2), 12.2 (CH_3). Anal. Calcd for $C_{19}H_{23}N_3O_7S$: C, 52.16; H, 5.30; N, 9.61; S, 7.33. Found: C, 52.12; H, 5.27; N, 9.08; S, 7.11. Calcd with $^1/_8$ EtOAc: C, 52.46; H, 5.42; N, 9.41; S, 7.18. ^{13}C NMR (DMSO-d_6) data are identical with previously reported data for the corresponding β-D-*ribo*-enantiomer.[27]

(1*S*,3*R*,4*S*,7*R*)-7-Benzyloxy-1-hydroxymethyl-3-(thymin-1-yl)-5-trifluroacetyl-2-oxa-5-azabicyclo[2.2.1]heptane (15). Nucleo-

(77) El-Khawaga, A. M.; Hoffmann, H. M. R. *J. Prakt. Chem.* **1995**, *337*, 332−334.

(78) Assignments of ^{1}H NMR signals of CH_2Ph and H5′, and of the corresponding ^{13}C signals, may in principle be interchanged.

side **14α** (10.61 g, 24.3 mmol) was coevaporated with anhydrous pyridine (2 × 75 mL) and dissolved in a mixture of anhydrous CH_2Cl_2 (240 mL) and anhydrous pyridine (8 mL, 98.9 mmol). Trifluoroacetic anhydride (6.8 mL, 48.9 mmol) was added dropwise over 5 min at 0 °C. The reaction mixture was warmed to room temperature and stirred for 100 min, whereupon crushed ice (50 mL) was added. The reaction mixture was diluted with CH_2Cl_2 (150 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 70 mL). The organic phase was evaporated to dryness and coevaporated with toluene to give the crude trifluoroacetamide-protected nucleoside [R_f = 0.5 (10% *i*-PrOH in $CHCl_3$, v/v), MALDI-HRMS *m*/*z* 556.0960 ([M + Na]$^+$, $C_{21}H_{22}F_3N_3O_8S{\cdot}Na^+$, Calcd 556.0972)] as a white powder, which was used in the next step without further purification. To a solution of the crude trifluoroacetamide-protected nucleoside in 1,4-dioxane (240 mL) were added potassium acetate (7.13 g, 72.7 mmol) and 18-crown-6 (6.40 g, 24.2 mmol), and the reaction mixture was stirred at 80 °C for 13 h. The reaction mixture was cooled to room temperature, concentrated to 1/5 volume, and diluted with H_2O (50 mL) and EtOAc (350 mL). The phases were separated, and the organic phase was washed with brine (2 × 100 mL) and evaporated to dryness to afford the crude acylated product [R_f = 0.6 (10% *i*-PrOH in $CHCl_3$, v/v), MALDI-HRMS *m*/*z* 520.1293 ([M + Na]$^+$, $C_{22}H_{22}F_3N_3O_7{\cdot}Na^+$, Calcd 520.1302)] as a white solid material, which was used in the next step without further purification. The crude acylated nucleoside was dissolved in saturated NH_3/MeOH (250 mL) and stirred at room temperature for 15 h, whereupon the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0−5% *i*-PrOH in CH_2Cl_2, v/v) to give a rotameric mixture (∼1:1.3 by ^{1}H NMR) of nucleoside **15** (7.12 g, 64% over three steps) as a white solid material. Physical data for the mixture of rotamers: R_f = 0.3 (10% *i*-PrOH in $CHCl_3$, v/v); MALDI-HRMS *m*/*z* 478.1174 ([M + Na]$^+$, $C_{20}H_{20}F_3N_3O_6{\cdot}Na^+$, Calcd 478.1196); ^{1}H NMR (DMSO-d_6)[79] δ 11.40 (s, 2.3H, ex, NH_{A+B}), 7.60 (d, 1.3H, J = 0.8 Hz, $H6_A$), 7.47 (d, 1H, J = 0.8 Hz, $H6_B$), 7.25−7.40 (m, 11.5H, Ph_{A+B}), 6.08 (d, 1H, J = 1.8 Hz, $H1'_B$), 6.04 (d, 1.3H, J = 1.5 Hz, $H1'_A$), 5.21 (ap t, 2.3H, ex, J = 5.9 Hz, 5′-OH_{A+B}), 4.92 (br s, 1.3H, $H2'_A$), 4.84 (br s, 1H, $H2'_B$), 3.61−4.74 (m, 16.1H, $H3'_{A+B}$, $H5'_{A+B}$, $H5''_{A+B}$, CH_2Ph_{A+B}), 1.78 (d, 3H, J = 0.8 Hz, $CH_{3\text{-}B}$), 1.76 (d, 3.9H, J = 0.8 Hz, $CH_{3\text{-}A}$); ^{13}C NMR (DMSO-d_6) δ 163.6, 163.5, 155.2 (q, J = 36.6 Hz, $COCF_3$), 155.0 (q, J = 36.6 Hz, $COCF_3$), 150.1, 149.9, 137.5 (C6), 137.3 (C6), 134.0, 133.9, 128.2, 127.6, 127.2, 115.6 (q, J = 289 Hz, CF_3), 115.5 (q, J = 288 Hz, CF_3), 108.7, 108.4, 89.7, 88.3, 85.1 ($C1'_A$), 84.5 ($C1'_B$), 78.8, 76.9, 71.3, 62.1 ($C2'_B$), 60.6 ($C2'_A$), 57.1, 56.9, 53.1, 52.9, 11.9, 11.7. Anal. Calcd for $C_{20}H_{20}F_3N_3O_6$: C, 52.75; H, 4.43; N, 9.23. Found: C, 53.17; H, 4.19; N, 9.07. Calcd with $^1/_{16}$ *i*-PrOH: C, 52.81; H, 4.50; N, 9.15.

(1*S*,3*R*,4*S*,7*R*)-7-Hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-5-trifluroacetyl-2-oxa-5-azabicyclo[2.2.1]heptane (19). Nucleoside **15** (8.28 g, 18.2 mmol) was coevaporated with anhydrous 1,2-dichloroethane (2 × 50 mL), suspended in anhydrous CH_2Cl_2 (230 mL), and cooled to −70 °C. To this was added BCl_3 (1.0 M in hexanes, 91 mL, 91.0 mmol) over 20 min. The reaction mixture was warmed to room temperature and stirred for 20 h, whereupon it was evaporated to dryness and coevaporated with toluene (3 × 100 mL). The resulting residue was purified by silica gel column chromatography (0−8% MeOH in CH_2Cl_2, v/v) to give a rotameric mixture (∼1:1.7 by ^{1}H NMR) of diol **19** (5.14 g, 77%) as a white solid material. Physical data for the mixture of rotamers: R_f = 0.4 (20% MeOH in $CHCl_3$, v/v); MALDI-HRMS *m*/*z* 388.0727 ([M + Na]$^+$, $C_{13}H_{14}F_3N_3O_6{\cdot}Na^+$, Calcd 388.0711); ^{1}H NMR (DMSO-d_6)[79] δ 11.34 (br s, 2.7H, ex, NH_{A+B}), 7.56 (d, 1.7H, J = 0.9 Hz, $H6_A$), 7.44 (d, 1H, J = 0.9 Hz, $H6_B$), 6.28 (d, 1H, ex, J = 4.0 Hz, 3′-OH_B), 6.25 (d, 1.7H, ex, J = 4.0 Hz, 3′-OH_A), 6.06 (d, 1H, J = 1.5 Hz, $H1'_B$), 6.04 (d, 1.7H, J = 1.5 Hz, $H1'_A$), 5.08 (ap t, 2.7H, ex, J = 5.9 Hz, 5′-OH_{A+B}), 4.67 (br s, 1.7H, $H2'_A$), 4.53 (m, 1H, $H3'_B$), 4.45−4.48 (m, 2.7H, $H2'_B$, $H3'_A$), 4.04−4.10 (d, 1.7H, J = 11.0 Hz, $H5''_A$), 3.66−3.83 (m, 8.1H, 2 × $H5'_{A+B}$, $H5''_A$, $H5''_B$), 3.57 (d, 1H, J = 12.4 Hz, $H5''_B$), 1.78 (d, 3H, J = 0.9 Hz, $CH_{3\text{-}B}$), 1.76 (d, 5.1H, J = 0.9 Hz, $CH_{3\text{-}A}$); ^{13}C NMR (DMSO-d_6) δ 163.7, 163.6, 154.4−155.8 (m, 2 × $COCF_3$), 150.1, 149.9, 134.05, 133.98, 115.7 (q, J = 289 Hz, CF_3), 115.6 (q, J = 289 Hz, CF_3), 108.6, 108.3, 90.6, 89.2, 85.1, 84.4, 72.0, 70.0, 64.6, 62.3, 57.4, 57.3, 52.7, 52.6, 12.0, 11.7.

(1*S*,3*R*,4*S*,7*R*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (18). Method A from **17**: see the Supporting Information. Method B from **19**: diol **19** (5.14 g, 14.1 mmol) was coevaporated with anhydrous pyridine (2 × 50 mL) and redissolved in anhydrous pyridine (200 mL). To this was added 4,4′-dimethoxytrityl chloride (DMTrCl, 7.15 g, 21.10 mmol) and DMAP (0.87 g, 7.12 mmol), and the reaction mixture was stirred at room temperature for 20 h, whereupon it was evaporated to dryness. The resulting residue was taken up in EtOAc (200 mL), and the organic phase was washed sequentially with saturated aqueous $NaHCO_3$ (75 mL) and brine (2 × 75 mL). The organic phase was evaporated to dryness and coevaporated with toluene (2 × 50 mL) to give the crude O5′-tritylated alcohol (∼12 g) [R_f = 0.5 (10% MeOH in CH_2Cl_2, v/v), MALDI-HRMS *m*/*z* 690.2034 ([M + Na]$^+$, $C_{34}H_{32}F_3N_3O_8S{\cdot}Na^+$, Calcd 690.2008)] as a white powder, which was used in the next step without further purification. Aqueous NaOH (2.0 M, 22 mL, 44.0 mmol) was added to an ice-cold solution of the crude O5′-tritylated alcohol (∼12 g) in a mixture of absolute EtOH and pyridine (240 mL, 3:1, v/v). The reaction mixture was warmed to room temperature and stirred for 4 h, whereupon it was evaporated to dryness and coevaporated with toluene (2 × 75 mL). The resulting residue was purified by silica gel column chromatography[80] (0−5% MeOH in CH_2Cl_2, v/v) to afford amino alcohol **18** (6.50 g, 81% over two steps) as a white solid material. R_f = 0.3 (10% MeOH in CH_2Cl_2, v/v), MALDI-HRMS *m*/*z* 594.2211 ([M + Na]$^+$, $C_{32}H_{33}N_3O_7{\cdot}Na^+$, Calcd 594.2192); ^{1}H NMR (DMSO-d_6) δ 11.28 (s, 1H, ex, NH), 7.57 (s, 1H, H6), 7.21−7.46 (m, 9H, Ar), 6.88−6.93 (d, 4H, J = 9.2 Hz, Ar), 5.90 (d, 1H, J = 1.5 Hz, H1′), 5.58 (d, 1H, ex, J = 4.6 Hz, 3′-OH), 4.26 (d, 1H, J = 4.6 Hz, H3′), 3.74 (s, 6H, 2 × CH_3O), 3.34 (d, 1H, H2′ overlapping with H_2O), 3.25−3.29 (d, 1H, J = 10.8 Hz, H5′),[81] 3.19−3.24 (d, 1H, J = 10.8 Hz, H5′), 3.03−3.08 (d, 1H, J = 10.3 Hz, H5″), 2.94−2.99 (d, 1H, J = 10.3 Hz, H5″), 1.84 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.8, 158.0, 150.3, 144.8, 136.5 (C6), 135.4 (Ar), 135.3 (Ar), 129.7 (Ar), 127.7 (Ar), 127.6 (Ar), 126.6 (Ar), 113.1 (Ar), 106.9, 89.5, 85.8 (C1′), 85.1, 73.4 (C3′), 61.5 (C2′), 60.7 (C5′), 54.9 (CH_3O), 50.6 (C5″), 12.4 (CH_3). Anal. Calcd for $C_{32}H_{33}N_3O_7{\cdot}^{10}/_{16}H_2O$: C, 65.94; H, 5.92; N, 7.21. Found: C, 66.33; H, 5.66; N, 6.81.

(1*S*,3*R*,4*S*,7*R*)-1-(4,4′-Dimethoxytrityloxymethyl)-5-(9′-fluorenylmethoxycarbonyl)-7-hydroxy-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]-heptane (20). To an ice-cold suspension of amino alcohol **18** (0.25 g, 0.44 mmol) in saturated aqueous $NaHCO_3$ (2 mL) and 1,4-dioxane (2 mL) was added 9′-fluorenylmethyl chloroformate (FmocCl, 125 mg, 0.48 mmol). After stirring for 3 h at room temperature, the reaction mixture was diluted with H_2O (10 mL) and EtOAc (35 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (3 × 35 mL). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (20−80% EtOAc in petroleum ether, v/v)[80] to afford a rotameric mixture (∼1:1.2 by ^{1}H NMR) of nucleoside **20** (0.28 g, 81%) as a white solid material. Physical data of rotamers: R_f = 0.6 (5% MeOH in CH_2Cl_2, v/v),

(79) The integral of the H1′-signal of the least predominant rotamer (termed B) is set to 1.0.

(80) During purification of this compound the silica gel chromatography column was initially packed using an eluent containing either Et_3N or pyridine (1%, v/v).

(81) Assignments of ^{1}H NMR signals of H5′ and H5″, and of the corresponding ^{13}C signals, may in principle be interchanged.

MALDI-HRMS *m*/*z* 816.2903 ([M + Na]$^+$, $C_{47}H_{43}N_3O_9$·Na$^+$, Calcd 816.2892); selected signals ^{1}H NMR (DMSO-d_6)[79] δ 11.54 (s, 1H, ex, NH_B), 11.31 (s, 1.2H, ex, NH_A), 6.21 (d, 1H, ex, J = 4.2 Hz, 3′-OH_B), 6.14−6.17 (m, 2.2H, 1.2ex, 3′-OH_A, H1′$_B$), 6.08 (s, 1.2H, H1′$_A$), 4.59 (d, 1H, J = 4.2 Hz, H3′$_B$), 4.53 (d, 1.2H, J = 3.7 Hz, H3′$_A$); ^{13}C NMR (DMSO-d_6) δ 163.8, 163.7, 158.0, 154.8, 154.5, 150.2, 150.0, 144.7, 144.6, 143.7, 143.53, 143.46, 143.0, 140.6, 140.5, 140.4, 135.2, 135.1, 134.6, 134.1, 129.7, 127.8, 127.74, 127.65, 127.56, 127.2, 126.7, 125.2, 125.0, 124.9, 120.1, 113.2, 108.2, 107.8, 89.1, 88.6, 86.0, 85.7, 85.3, 72.2, 71.4, 67.3, 66.9, 63.0, 62.2, 60.5, 60.3, 60.0, 54.9, 52.4, 52.1, 46.3, 46.2, 12.3, 12.0. Anal. Calcd for $C_{47}H_{43}N_3O_9$·$^3/_8H_2O$: C, 70.51; H, 5.51; N, 5.25. Found: C, 70.13; H, 5.40; N, 5.07.

(1*S*,3*R*,4*S*,7*R*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-5-(9′-fluorenylmethoxycarbonyl)-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (21). Nucleoside **20** (140 mg, 0.176 mmol) was coevaporated with anhydrous 1,2-dichloroethane (2 × 8 mL) and dissolved in a mixture of anhydrous EtN(*i*-Pr)$_2$ in CH_2Cl_2 (2 mL, 20%, v/v). To this was added 2-cyanoethyl *N*,*N*′-(diisopropyl)-phosphoramidochloridite (47 μL, 0.21 mmol), and the reaction mixture was stirred at room temperature for 1 h, whereupon the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ (10 mL), and the aqueous phase was back-extracted with CH_2Cl_2 (20 mL). The combined organic phase was evaporated to dryness, and the resulting crude residue was purified by silica gel column chromatography (0−50% EtOAc in petroleum ether, v/v)[80] to afford amidite **21** (98 mg, 56%) as a white solid material. R_f = 0.6 (90% EtOAc in petroleum ether, v/v); MALDI HRMS *m*/*z* 1016.3944 ([M + Na]$^+$, $C_{56}H_{60}N_5O_{10}P$·Na$^+$, Calcd 1016.3970); ^{31}P NMR (CH_3CN + DMSO-d_6) δ 150.4, 150.3, 149.8, 149.5.

(1*R*,6*S*,7*R*,8*S*,10*S*,11*R*,12*S*)-10-Acetoxymethyl-11-benzyloxy-6-methyl-13-oxa-2,4,8-triazatetracyclo[8.2.1.0^{2,7}.0^{8,12}]tridecan-3,5-dione (22), (1*R*,6*R*,7*S*,8*S*,10*S*,11*R*,12*S*)-10-Acetoxymethyl-11-benzyloxy-6-methyl-13-oxa-2,4,8-triazatetracyclo[8.2.1.0^{2,7}.0^{8,12}]-tridecan-3,5-dione (23), and (1*S*,3*R*,4*S*,7*R*)-1-Acetyloxymethyl-7-benzyloxy-3-(thymin-1-yl)-2-oxa-5-aza-bicyclo[2.2.1]heptane (24). Nucleoside **14α** (2.20 g, 5.03 mmol) was dried by coevaporation with anhydrous toluene (2 × 25 mL) and dissolved in anhydrous 1,4-dioxane (160 mL). To this were added KOAc (1.98 g, 20.1 mmol) and 18-crown-6 (2.63 g, 10.1 mmol), and the reaction mixture was refluxed for 52 h, whereupon it was concentrated to 1/3 volume and diluted with H_2O (25 mL) and EtOAc (75 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (3 × 75 mL). The combined organic phase was evaporated to dryness, and the resulting crude residue purified by silica gel column chromatography (0−100% EtOAc in petroleum ether, v/v) to afford tetracyclic nucleosides **22** (365 mg, 18%), **23** (650 mg, 32%), and bicyclic 2′-amino-α-L-LNA nucleoside **24** (520 mg, 26%) as white solid materials. Physical data of nucleoside **22**: R_f = 0.4 (EtOAc); MALDI-HRMS *m*/*z* 424.1471 ([M + Na]$^+$, $C_{20}H_{23}N_3O_6$·Na$^+$, Calcd 424.1479); ^{1}H NMR (DMSO-d_6) δ 10.28 (s, 1H, ex, NH), 7.28−7.41 (m, 5H, Ph), 5.55 (d, 1H, J = 1.8 Hz, H1′), 4.61−4.66 (d, 1H, J = 12.1 Hz, CH_2Ph),[78] 4.56−4.60 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.49 (d, 1H, J = 11.7 Hz, H6), 4.25−4.31 (d, 1H, J = 12.5 Hz, H5′), 4.15−4.20 (d, 1H, J = 12.5 Hz, H5′), 4.06 (s, 1H, H3′), 3.83 (br s, 1H, H2′), 3.57 (d, 1H, J = 11.4 Hz, H5″), 2.79 (d, 1H, J = 11.4 Hz, H5″), 2.49−2.58 (m, 1H, H5 partial overlap with solvent signal), 2.01 (s, 3H, CH_3CO), 1.11 (d, 3H, J = 6.5 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 172.4, 169.9, 150.8, 137.7, 128.2 (Ph), 127.6 (Ph), 127.4 (Ph), 86.4 (C1′), 83.0 (C6), 81.3, 80.1 (C3′), 71.2 (CH_2Ph), 64.6 (C2′), 62.8 (C5″), 60.1 (C5′), 39.0 (C5), 20.4 (CH_3CO), 10.8 (CH_3). Physical data of compound **23**: R_f = 0.3 (EtOAc); MALDI-HRMS *m*/*z* 424.1460 ([M + Na]$^+$, $C_{20}H_{23}N_3O_6$·Na$^+$, Calcd 424.1479); ^{1}H NMR (DMSO-d_6) δ 10.32 (s, 1H, ex, NH), 7.26−7.41 (m, 5H, Ph), 5.56 (d, 1H, J = 1.8 Hz, H1′), 4.66−4.71 (d, 1H, J = 12.1 Hz, CH_2Ph),[78] 4.56−4.61 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.37 (d, 1H, J = 12.5 Hz, H6), 4.27−4.33 (d, 1H, J = 12.6 Hz, H5′), 4.11−4.17 (d, 1H, J = 12.6 Hz, H5′), 3.97 (s, 1H, H3′), 3.85 (br s, 1H, H2′), 2.94−3.12 (m, 3H, H5″, H5), 2.01 (s, 3H, CH_3CO), 1.11 (d, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 172.1, 169.9, 149.3, 137.7, 128.2 (Ph), 127.6 (Ph), 127.4 (Ph), 83.8 (C1′), 81.5, 79.7 (C3′), 77.3 (C6), 71.1 (CH_2Ph), 67.7 (C2′), 59.9 (C5′), 50.5 (C5″), 37.9 (C5), 20.4 (CH_3CO), 10.3 (CH_3). Physical data of compound **24**: R_f = 0.2 (EtOAc); MALDI-HRMS *m*/*z* 424.1470 ([M + Na]$^+$, $C_{20}H_{23}N_3O_6$·Na$^+$, Calcd 424.1479); ^{1}H NMR (DMSO-d_6) δ 11.29 (s, 1H, ex, NH), 7.54 (br s, 1H, H6), 7.26−7.41 (m, 5H, Ph), 5.84 (d, 1H, J = 1.5 Hz, H1′), 4.64−4.70 (d, 1H, J = 12.1 Hz, CH_2Ph),[78] 4.53−4.59 (d, 1H, J = 12.1 Hz, CH_2Ph), 4.39−4.45 (d, 1H, J = 12.6 Hz, H5′), 4.21−4.27 (d, 1H, J = 12.6 Hz, H5′), 4.18 (s, 1H, H3′), 3.68 (br s, 1H, H2′), 3.10−3.16 (d, 1H, J = 10.4 Hz, H5″), 2.89−2.94 (d, 1H, J = 10.4 Hz, H5″), 2.03 (s, 3H, CH_3CO), 1.82 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 169.9, 163.7, 150.2, 137.9, 136.6 (C6), 128.1 (Ph), 127.5 (Ph), 127.4 (Ph), 106.9, 87.5, 86.0 (C1′), 79.9 (C3′), 70.8 (CH_2Ph), 60.1 (C5′), 58.9 (C2′), 50.8 (C5″), 20.5 (CH_3CO), 12.3 (CH_3).

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Supporting Information Available: General Experimental Section, experimental procedures for preparation of nucleosides **2**, **4**, **7**, **8**, **16**−**18**, and **25**−**31**, description of the preparation and purification of oligonucleotides **ON3**−**ON10**, protocol for thermal denaturation and molecular modeling studies, X-ray structure of nucleoside **15** (Figure S1), MALDI-MS data of synthesized ONs (Table S1), representative thermal denaturation curves of **ON1** and **ON3** (Figure S2), additional discussion regarding the instability of ONs with incorporations of (5*R*,6*S*)-configured tetracyclic "locked LNA" phosphoramidite **31**, copies of ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, ^{1}H−^{1}H COSY, and/or ^{1}H−^{13}C HETCOR spectra of nucleosides **2**−**31**, PDB files of the lowest energy structures of tetracyclic nucleosides **22** and **23**, and the corresponding (5*S*,6*S*)- and (5*R*,6*R*)-diastereomers. This material is available free of charge via the Internet at http://pubs.acs.org.

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