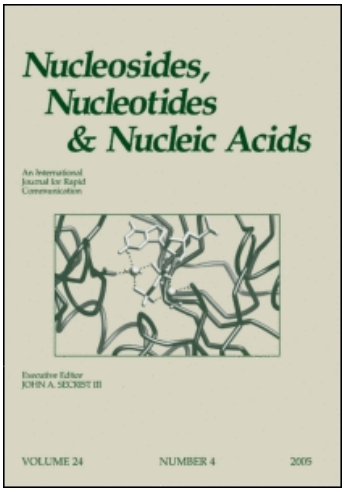

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SYNTHESIS OF 2′-AMINO-LNA PURINE NUCLEOSIDES

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□ *The first reported synthesis of 2′-amino-LNA purine nucleosides via a transnucleosidation is accomplished enabling the preparation of oligonucleotides incorporating 2′-amino-LNA with all four natural bases.*

Keywords Amino-LNA; LNA; Transnucleosidation

INTRODUCTION

The first synthesis of 2′-amino-LNA was achieved by Wengel and coworkers[1] in 1998 for the thymine nucleoside **1** (Figure 1), and five years later the synthesis of 5-methylcytosine nucleoside **2** was reported.[2] The nitrogen atom in the bicyclic carbohydrate structure of 2′-amino-LNA is located in the minor groove of a nucleic acid heteroduplex. This positioning makes the amino group well suited for conjugation purposes. An illustrative example of this is the conjugation of photochemical reporter groups, such as pyrene, that may serve as useful tools for structure elucidation and in diagnostics.[3,4] Recently, the diastereomeric 2′-amino-α-L-LNA-T nucleoside **3** has also been synthesized, giving access to the location of the reporter groups into the major groove of a nucleic acid duplex.[5,6] In vitro and in vivo data[7] on 2′-amino-LNA oligonucleotides are very limited due to the restriction that only the pyrimidines thymidine and 5-methylcytosine of amino-LNA have been available. In this article, we present for the first time the synthesis of the 2′-amino-LNA adenine and guanine nucleosides **4** and **5**. A key intermediate in the synthesis is derived from the synthesis of the 2′-amino-LNA-T **1**.[2]

RESULTS AND DISCUSSION

In our previously reported synthesis of 2′-amino-LNA thymine analogs,[2] an azide is incorporated at the 2′-position by nucleophilic

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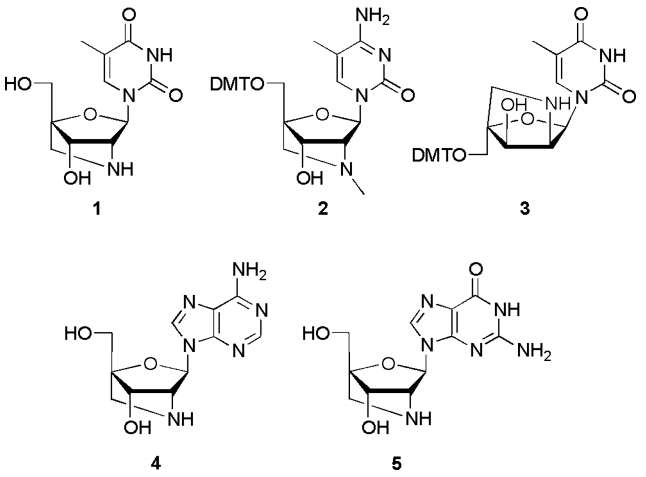
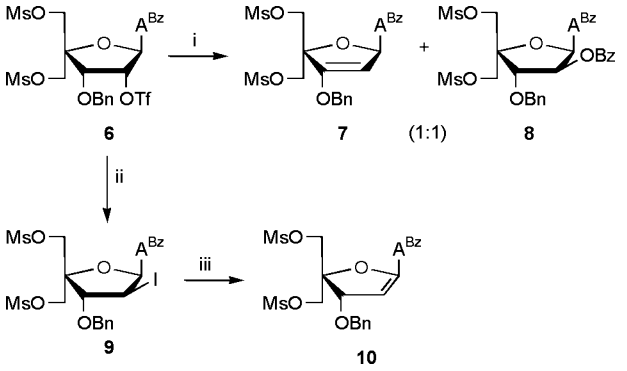


FIGURE 1 2′-amino-LNA nucleoside derivatives.

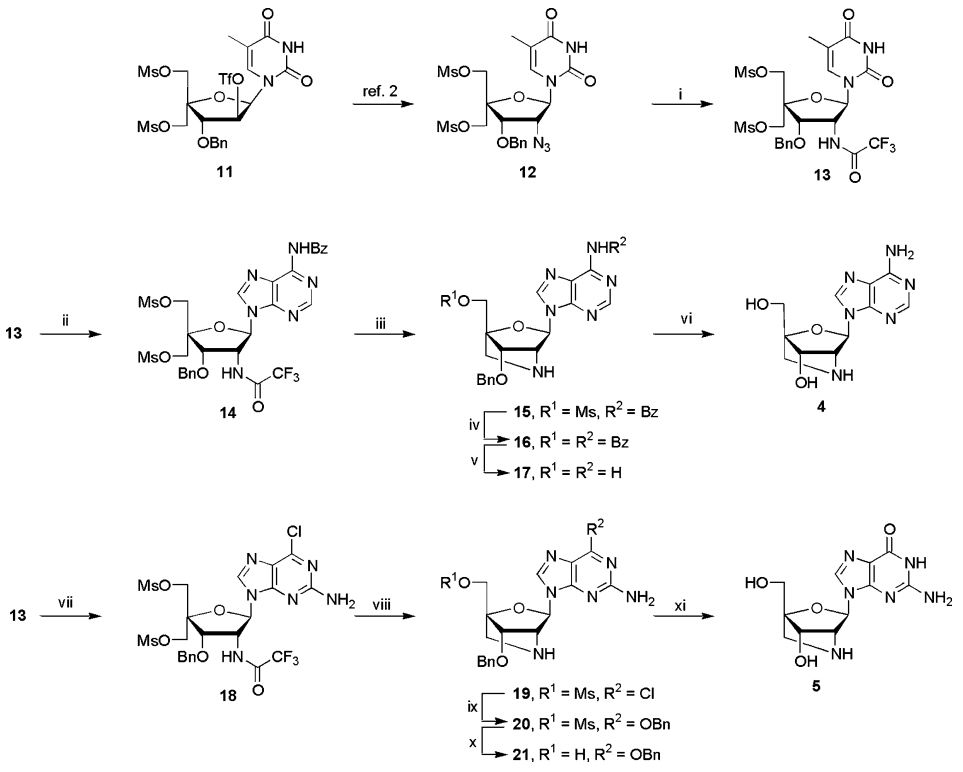
substitution of a D-*threo*–configurated OTf. The preceding inversion of configuration at C-2′ relies on the formation of a 2,2′-anhydro intermediate limited to the pyrimidine nucleobases. Inspired by this strategy, different approaches for the synthesis of D-*threo*-configurated purine nucleosides were explored (Scheme 1). However, all attempts at nucleophilic substitution with O-nucleophiles of 2′-OTf **6** also gave significant amount of the elimination product **7** like the elimantion products observed by Sharma and Nair.[8] The best conditions were reacting with NaOBz in DMSO, yielding a 1:1 mixture of the desired D-*threo*-configurated purine nucleoside **8** and the elimination product. Substitution with different halogens also resulted in elimination reactions, but refluxing **6** with LiI in CH_3CN yielded the 2′-I **9** in 90% yield. However, all attempts to displace the 2′-I with N-nucleophiles such as NaN_3, $TMSN_3$, $BnNH_2$, and phtalimide salts in different solvents either failed to react at all or gave the elimination product **10** like the elimination products obtained by Haraguchi et al.[9] Under



SCHEME 1 *Reagents and conditions:* i) NaOBz, DMSO; ii) LiI, CH_3CN, 90% iii) N-nucleophiles.

these conditions, depurination was also detected. The same strategy was attempted for other purine analogs with similar results. Oxidation of C-2′ followed by stereoselective reduction to invert the configuration[10] was also tried, but that strategy turned also out to be unsuccessful.

We finally decided to explore an approach where the purine nucleobase is incorporated *after* the 2′-azide, hence exchanging the thymine for a purine in a transnucleosidation.[11,12] In the synthesis of 2′-amino-LNA thymine analogs, the 2′-azide nucleoside **12** (Scheme 2) is a key intermediate. Initial attempts to perform the transnucleosidation on **12** were unsuccessful, which is in accordance with earlier observations.[12] Instead it was anticipated that an acylated 2′-amine would be a suitable substrate for a transnucleosidation reaction. **12** was reduced using trimethylphosphine under neutral condition, to avoid ring-closure, and the resulting 2′-amine was "trapped" by addition of trifluoroacetic anhydride directly to the reaction mixture to give **13** in 79% yield. Subjecting **13** to the



SCHEME 2 *Reagents and conditions:* i) a, PMe_3, THF; b, TFAA (79%); ii) N^6-benzoyladenine, BSA, TMSOTf, DCE (57%); iii) LiOH, THF/H_2O (99%); iv) NaOBz, DMSO (97%); v) sat. NH_3/MeOH (86%); vi) HCO_2NH_4, $Pd(OH)_2$/C, MeOH (76%); vii) 2-amino-6-chloropurine, BSA, TMSOTf, DCE (58%); viii) NaOH, THF/H_2O (93%); ix) BnOH, KOtBu, THF (62%); x) a, NaOBz, DMSO; b, LiOH, THF/H_2O (85%); xi) HCO_2NH_4, $Pd(OH)_2$/C, EtOH/H_2O (81%).

Vorbrüggen[13] conditions (BSA and TMSOTf) with an excess of either N^6-benzoyladenine or 2-animo-6-chloropurine resulted in the successful formation of the corresponding β purine nucleosides **14** and **18** in 57% and 58% yield, respectively, after chromatography. It is likely that anchimeric assistance from the trifluoroacetyl group is responsible for the preferential formation of the β-isomer in the same way as seen for 2′-*O*-acetylated glycosyl donors. Because of the nature of the reaction being a thermodynamic competition between the thymine leaving group and the silylated purine base, an excess of purine (3 eq.) was necessary to drive the reaction to completion.

Hydrolysis of the trifluoroacetyl group in **14** led to a subsequent ring closure to give the fully protected 2′-amino-LNA substituted adenosine nucleoside **15** in 99% yield. During all the preceding transformations, the 5′-OMs groups have proven extremely stable. In our experience it is actually possible to hydrolyze these mesylates by prolonged exposure to concentrated aqueous LiOH at elevated temperature, giving rise to very impure products. Instead, we usually prefer substitution of the mesylate with benzoyl using sodium benzoate to give, in this case, **16** followed by deprotection of both benzoyl groups by methanolic ammonia resulting in **17**. Finally, the fully deprotected 2′-amino-LNA adenosine **4** was obtained after debenzylation with ammonium formate and a catalytic amount of palladium hydroxide on charcoal. In a similar fashion, **18** was hydrolyzed with subsequent ring closure to give **19** in 93% yield. Substitution of the 6-chloro group with benzylalcohol gave the protected guanosine derivative **20**, which was further transformed at the 5′-position to give **21**. Finally, debenzylation of both 3′-O and 6-O resulted in the fully deprotected 2′-amino-LNA guanosine **5**. In all ^{1}H-NMR spectra for the bicyclic compounds (**15–17**, **4**, **19–21,** and **5**) the signals for 1′-H, 2′-H, and 3′-H are all singlets, confirming the nucleosides to be in a North-conformation.

In conclusion, we have developed a new practical method for the preparation of 2′-amino-LNA purine nucleosides via a transnucleosidation. The novel synthesis strategy is exemplified for the adenosine and guanosine derivatives. We are currently utilizing this method in preparing the suitably protected phosporamidate analogs to be used in an automated oligonucleotide synthesis.

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