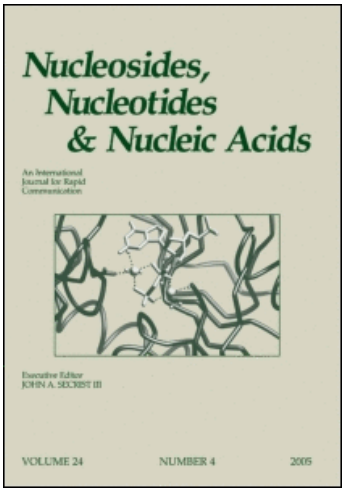

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Improved Synthesis of 2′-Amino-LNA

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ABSTRACT

2′-Amino-LNA phosphoramidite (**10**) was synthesised by means of a new strategy, which is convergent with the synthesis of 2′-oxy-LNA up until a late stage intermediate (**1**).

Key Words: 2′-Amino-LNA; Convergent strategy; Gram-scale synthesis.

LNA (Locked Nucleic Acid) was introduced in 1998[1–3] as a novel class of conformationally restricted oligonucleotide analogues: The first LNA monomer was based on the 2′-OCH_2-4′ bicyclic structure (LNA/2′-oxy-LNA). Later similar high affinity/specificity LNA monomers such as 2′-$NHCH_2$-4′, 2′-$N(CH_3)CH_2$-4′ (2′-amino-LNA)[4,5] and 2′SCH_2-4′(2′-thio-LNA)[4,6] were synthesised.

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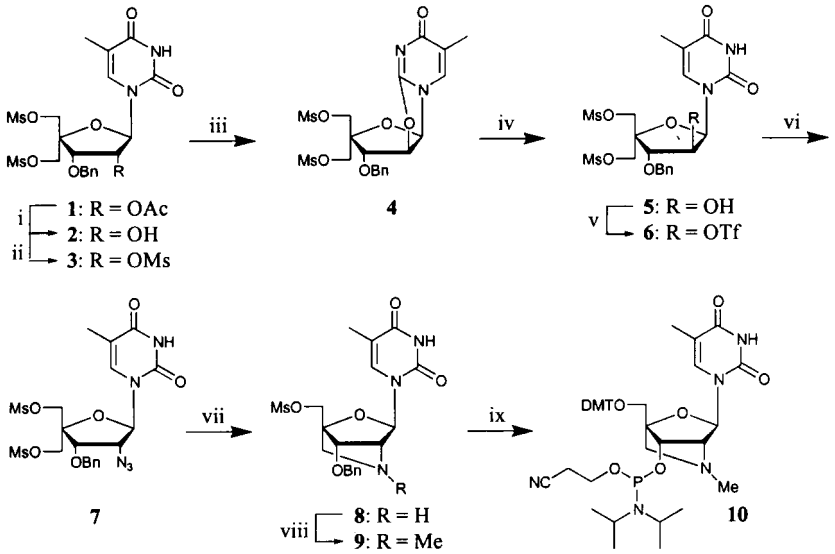

Scheme 1. i) half sat. NH_3 in MeOH (97%); ii) MsCl, pyridine (93%); iii) DBU, MeCN (91%); iv) acetone, 0.1M H_2SO_4 (98%); v) Tf_2O, DMAP, pyridine, CH_2Cl_2 (80%); vi) NaN_3, 15-crown-5, DMF (91%); vii) PMe_3, NaOH(aq), THF (93%); viii) CH_2O, HCO_2H (90%); ix) a) NaOBz, b) MeONa, DMF (98%), c) 20% $Pd(OH)_2/C$, H_2, AcOH (97%), d) DMTCl, pyridine (92%), e) $NC(CH_2)_2OP(N(iPr)_2)_2$, 4,5-dicyanoimidazole, MeCN, CH_2Cl_2 (98%).

Scale up of the original synthesis of 2′-amino-LNA proved to be difficult in our hands, and we therefore developed a new synthetic approach, which converges the syntheses of 2′-oxy-LNA and 2′-amino-LNA. The 2′-*O*-acetyl dimesylated nucleoside **1** (Sch. 1), used in the improved and recently published synthesis of 2′-oxy-LNA nucleosides,[7] was deacetylated using half-saturated methanolic ammonia to afford nucleoside **2** in quantitative yield. Subsequent reaction with mesyl chloride in pyridine gave the trimesylate **3** in 96% on a 20 g scale. The 2,2′-anhydro intermediate **4** was synthesised quantitatively by treatment of **3** with 1.1 equivalents of DBU in anhydrous acetonitrile. Opening of the 2,2′-anhydro intermediate by refluxing it in a mixture of aqueous sulfuric acid (0.1M) and acetone (1:1, v/v) resulted in a clean reaction giving the *threo*-configured nucleoside **5** in 91%. Treatment of **5** with trifluoromethanesulfonic anhydride, pyridine and DMAP in anhydrous dichloromethane at 0°C gave the desired triflate **6** in 80% after chromatography. Subsequent treatment with sodium azide afforded the 2′-azido-2′-deoxynucleoside **7** in 91%. Reduction of the azide using trimethylphosphine and aqueous NaOH in THF gave the nucleoside **8** with the desired 2-oxa-5-azabicyclo [2.2.1] heptane skeleton in 93%. Methylation using Eschweiler-Clarke conditions gave the 2′-*N*-methyl derivative **9** in 90% yield. Nucleophilic replacement of the mesylate on C5′ with benzoate followed by transesterification with methoxide afforded the 5′-hydroxy nucleoside in 98% yield. Reductive debenzylation with hydrogen and 20% $Pd(OH)_2/C$ in acetic acid yielded the deprotected nucleoside in 97%. 4,4′-Dimethoxytritylation

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of the 5′-hydroxygroup and phosphitylation of the 3′-hydroxy group gave the amidite **10** in 90% yield ready for the automated incorporation into oligonucleotides.

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