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3′-Amino-5′-carboxymethyl-3′,5′-dideoxy nucleosides for the synthesis of fully amide-linked RNA mimics

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ABSTRACT

A convenient protocol is developed for the synthesis of 3′-[*N*-(fluorenylmethoxycarbonyl)-amino]-5′-carboxymethyl derivatives of all four natural ribonucleosides from cheap chiral pool compound glucose. Synthesis of fully amide-linked RNA analogues of small oligonucleotides containing, for the first time, all four nucleoside amino acids using standard solid phase Fmoc-chemistry is described.

1. Introduction

Since the discovery of RNA interference (RNAi) in 1998,[1] it has remained one of the most thoroughly investigated class of molecules for developing effective antisense therapeutic strategies.[2,3] An ideal antisense oligonucleotide should possess high binding affinity to the target RNA, high nuclease resistance, binding selectivity to transport proteins, and cell permeability in vivo.[4] But the inherent properties of unmodified oligonucleotides having polyanionic backbone with enzymatic susceptibilities lead to poor cellular uptake, limited tissue distribution, and rapid clearance, restricting their therapeutic applications. Chemical modifications[5] can not only address these shortcomings, but can also be fine tuned to reduce toxicity and other side-effects of siRNAs. Various types of chemically modified RNA analogues have been prepared by many groups, and their properties have been studied in detail.[6] Replacement of the phosphodiester linkages with amide bonds has been extensively studied for potential therapeutic applications involving antisense strategy.[7] The advantages of this approach are that it not only facilitates the assembly of such substrates using standard solid peptide synthesis but would also help to enhance the physiological stabilities of these analogues over the native phosphodiester linkages. However, the success of this strategy depends on the easy availability of the monomeric building blocks.[8] Recently, some reports have shown that amides are remarkably good mimics of phosphate linkages in RNA.[9] We herein plan for the synthesis of RNA mimics with two carbon extension on the 5′-terminal using amide as a linker (Fig. 1).

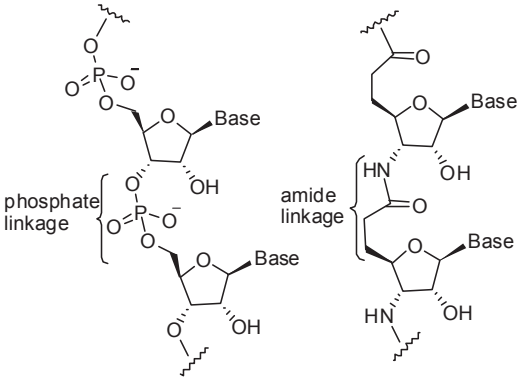

Fig. 1. RNA backbones with natural phosphate and mimicking amide linkages.

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Our initial efforts for the solid phase synthesis of RNA mimics using Boc-protected nucleoside esters on different resins did not yield the desired results.[8e] This prompted us to change to Fmoc-strategy, for which efficient syntheses of Fmoc protected monomeric building blocks were warranted. In the present paper, we report the synthesis of all the four monomeric building blocks (**1**–**4**) with *N*-Fmoc protection and free acid group (Fig. 2) and the solid phase synthesis of two small oligonucleotides **ON1** and **ON2**, which were randomly selected as representative examples to showcase the practical applicability of our synthetic protocol. To the best of our knowledge, this is the first report where all four nucleoside amino acids have been used by us to build the fully amide-linked RNA mimics.

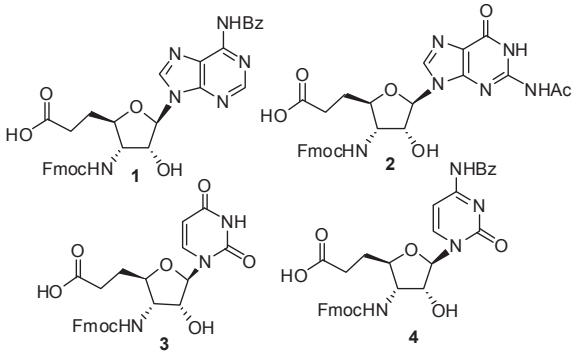
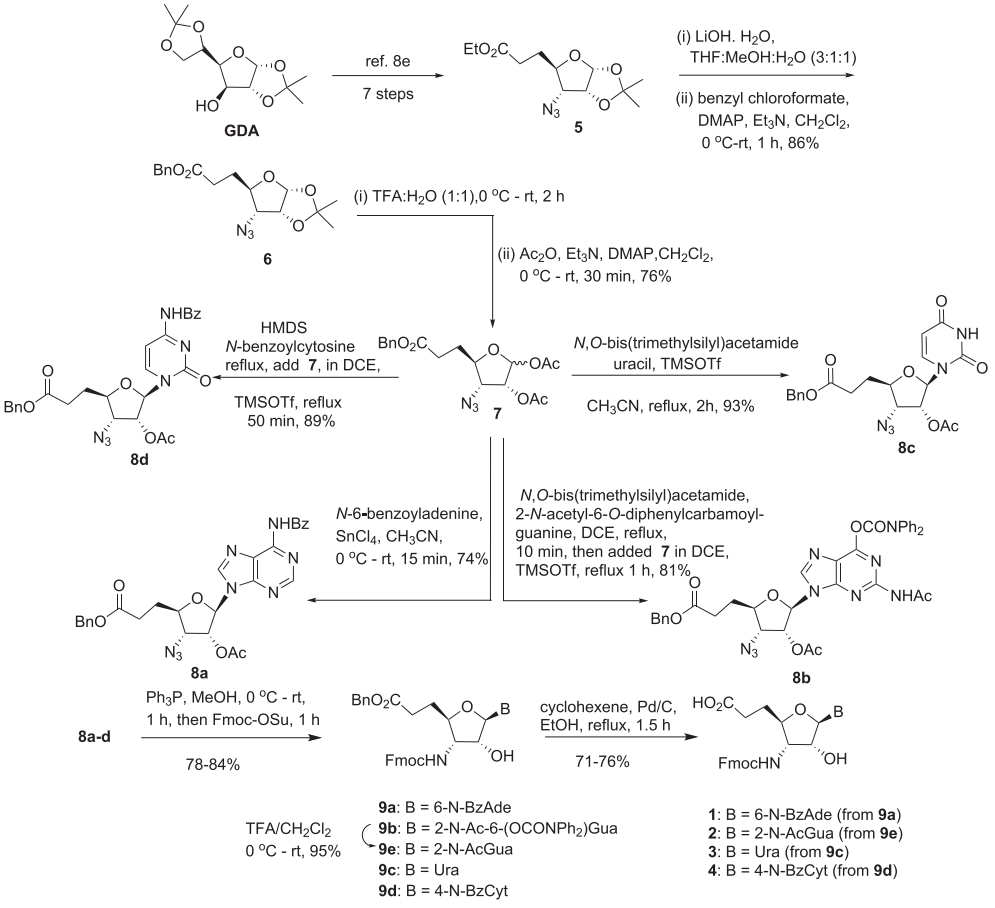


Fig. 2. Structures of Fmoc-protected ribonucleoside amino acid **1**–**4**.

2. Results and discussion

The required Fmoc protected monomeric building blocks **1**–**4** were synthesized starting from 3-azido-5-(ethoxycarbonyl)-methyl derivative **5** (Scheme 1), which was prepared from commercially available glucose diacetonide (GDA) as described earlier.[8e] To avoid the saponification of the ester group, which could jeopardize the side chain protections of the bases, we opted for benzyl protection, which could be removed under neutral conditions like hydrogenolysis. So, compound **5** was hydrolyzed with $LiOH\cdot H_2O$ in THF/MeOH/H_2O, followed by benzyl protection with benzyl chloroformate, Et_3N, and DMAP in CH_2Cl_2 to give **6** in 86% over two steps.[10] Acetonide deprotection of **6** with 50% aqueous TFA, followed by acetolysis with Ac_2O, Et_3N, and DMAP in CH_2Cl_2 gave a common glycosyl donor **7**, in 76% yield over two steps,[8e] which was coupled with the suitably functionalized bases to give all four modified nucleosides. The adenosine derivative **8a** was synthesized in 74% yield from **7** in the presence of $SnCl_4$ with *N*-benzoyladenine in CH_3CN.[8a,e] The regioselective synthesis of guanosine derivative **8b** was achieved (in 81% yield) from **7** by using Zou and Robins method.[8e,11] Compound **7** was coupled with bis(-trimethylsilyl) heterocycles in the presence of TMSOTf for the synthesis of **8c**[12] and **8d**[8b,e] in 93% and 89% yields, respectively. Reduction of the azide functionality in **8a**–**d** using PPh_3 in methanol carried out the unexpected concurrent methanolysis also to afford the corresponding amino hydroxyl compound, which on in situ Fmoc protection furnished **9a**–**d**, respectively. In fact, we did not re-protect the hydroxyl group to its acetate suspecting an acetate migration in subsequent steps as was encountered also by



Scheme 1. Synthesis of monomeric building blocks **1**–**4**.