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Chemical synthesis of bioactive siRNA in solution phase by using 2-(azidomethyl)benzoyl as 3′-hydroxyl group protecting group

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ABSTRACT

A protecting group AZMB was introduced to ribonucleosides 3′-hydroxyl group to facilitate solution phase synthesis of siRNA. The protection and cleavage reaction were carried out in mild conditions, that is protection by acyl chloride and cleavage by triphenylphosphine. The synthesized siRNA showed good biological activity to suppress targeted superoxide dismutase gene expression.

RNA interference (RNAi) has showed great potential that could be utilized for the treatment of infection,[1] cancer,[2] and many other diseases.[3] More than a dozen of potential drugs based on RNAi are on different stages of clinical trials.[4] Though current research mostly focuses on off-target effects[5] and drug delivery systems,[6] there is still a need for progress in chemical synthesis of siRNA both in scale and purity for therapeutic applications. The phosphoramidite approach[7–10] for RNA synthesis, relies on stepwise addition of phosphoramidite monomers on solid support, has been very successful in the past years. As an alternative choice, performing the synthesis procedure in solution phase through 'block' condensation strategy[11,12] is suitable for large scale synthesis of a specific sequence. And this is likely to happen if a systemic siRNA drug is to be approved to enter the market.[11] Reese and Yan[13] have reported synthesis of the first antisense deoxyoligonucleotide drug Vitravene in solution phase, through levulinyl (Lev) protected 3′-hydroxyl group in their synthesis of oligodeoxynucleotide trimer building blocks by H-phosphonate approach.[14]

In the synthesis of oligo-RNA, the additional 2′-hydroxyl group of ribonucleosides brings in much complication, suitable protecting group is pivotal in the synthesis of oligo-RNA. Various kinds of protecting groups for 2′-hydroxyl group in solid phase synthesis have been reported.[15–28] The TBDMS protecting group for 2′-hydroxyl, combined with the DMTr protecting group for 5′-hydroxyl group is the most common protection strategy, not only for their convenience and solubility, but also for cost consideration, which is important especially for scale-up synthesis. When the Lev group is cleaved by hydrazine in pyridine-acetic acid solution, a migration of TBDMS group from 2′ to 3′ position may occur.[12] Sekine and co-workers[29] reported 2-(azidomethyl)benzoyl (AZMB) as a novel protecting group in deoxynucleosides. The AZMB group could be cleaved in neutral condition by mild reducing agent, such as triphenylphosphine. Azhayev and co-workers[30] applied AZMB in the synthesis of trimer deoxynucleotide phosphoroamidite. Here we introduced the AZMB group into the synthesis of oligo-RNA in solution phase for protection of the 3′-hydroxyl group. With the purpose of scale-up synthesis of biological active siRNA, we synthesized a pair of siRNA targeting human superoxide dismutase gene for cellular activity assay.

As shown in Scheme 1, 2-(azidomethyl)benzoyl chloride **4** was prepared from *o*-methyl methyl benzoate and introduced to the 3′-hydroxyl group position of four types of ribonucleosides. *o*-Methyl methyl benzoate was brominated by NBS, and then substituted by the azido group. After hydrolization and recrystallization, pure white product **3** was obtained in 71% yield. The 2-(azidomethyl)benzoic acid was characterized by ^{1}H NMR and element analysis before being chlorinated to 2-(azidomethyl)benzoyl chloride **4**. For neutralization of its strong acidity, **4** was firstly added into methyl imidazole in dichloromethane solution, resulted in pH 7–8. Then the nucleoside **5** in dichloromethane was added dropwise. The reaction was incubated overnight at 0 °C to give **6**, and treated by acid for removing DMTr to give 5′-hydroxyl group free **7** (HO-component) as white foam. The yields for four types of nucleoside were usually higher than 80%.

The protection strategy with AZMB protecting 3′-hydroxyl group and TBDMS protecting 2′-hydroxyl group was tested for the synthesis of siRNA in solution phase. Moreover, the phospho-

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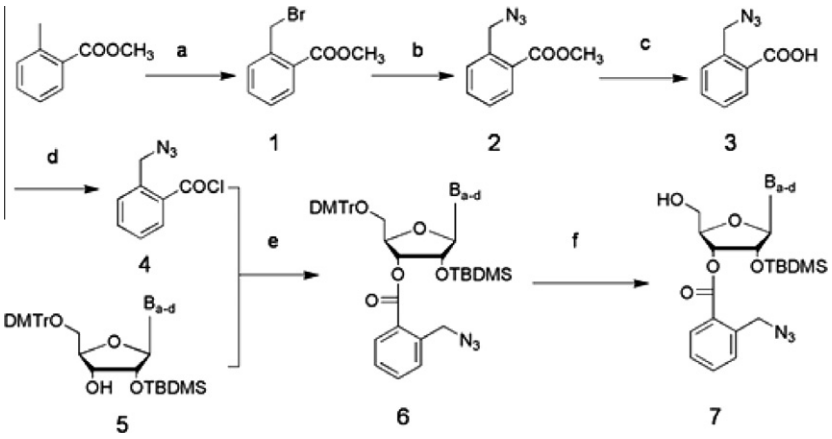
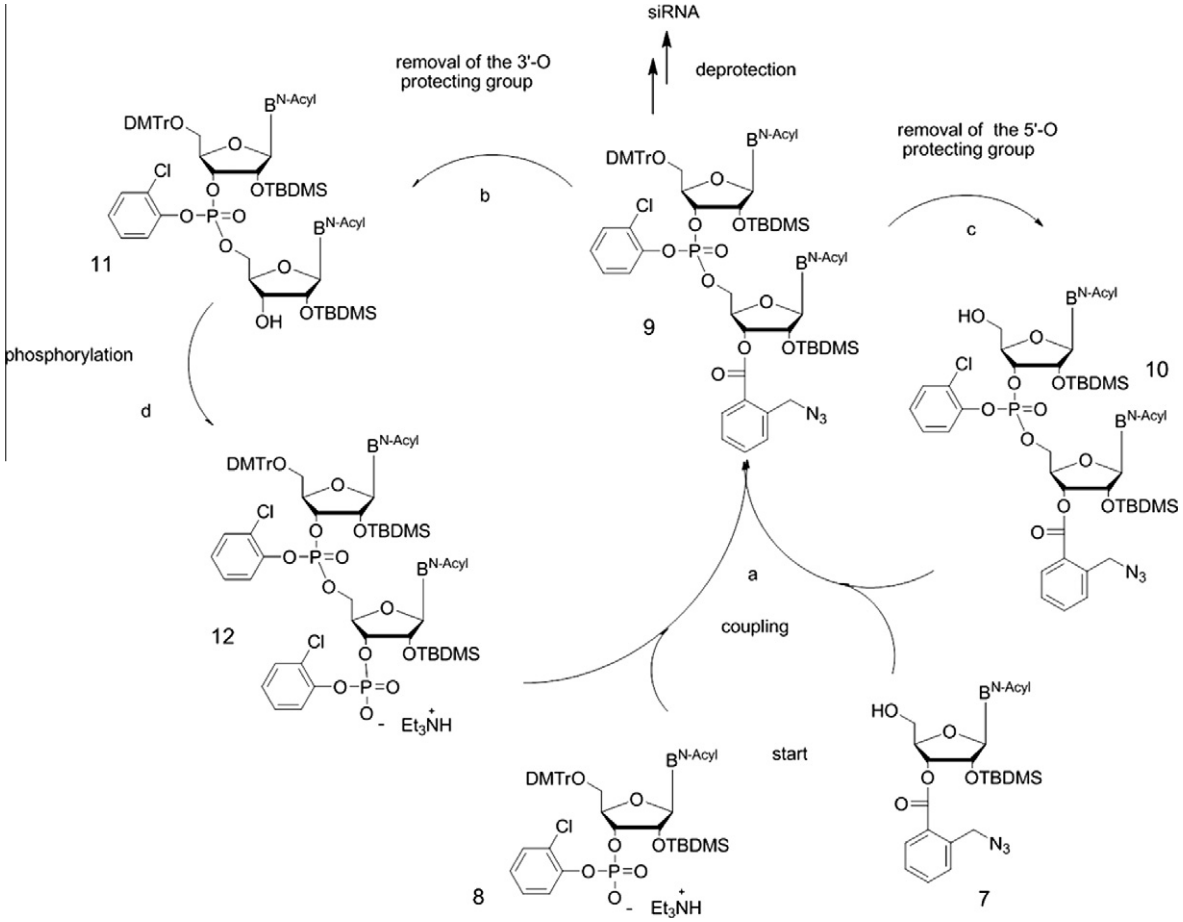


Scheme 1. Reagents and conditions: (a) NBS (1.1 equiv), $(BzO)_2$ (0.02 equiv), CCl_4, reflux, 1.5 h; (b) NaN_3 (1.5 equiv), EtOH, overnight; (c) 2 M NaOH–CH_3OH (1:1, v:v), rt, 30 min; (d) $SOCl_2$ (2 equiv), reflux, 2 h; (e) AZMBCl (1.8 equiv), MI (2.5 equiv), CH_2Cl_2, 0 °C, overnight; (f) 2% TsOH (10 equiv), CH_2Cl_2/CH_3OH (7:3, v:v), 0 °C, 10 min.



Scheme 2. The reaction cycle of synthesis, the first cycle conditions: (a) MSNT (2.8 equiv), C_5H_5N, rt, 2 h; (b) Ph_3P (4 equiv), dioxane/H_2O (9:1, v:v), rt, overnight; (c) 2% TsOH (10 equiv), CH_2Cl_2/CH_3OH (7:3, v:v), 0 °C, 10 min; (d) 2-chlorophenyldichlorphospate (1.6 equiv), triazole (4 equiv), C_5H_5N (8 equiv), CH_2Cl_2, 0 °C, 2 h; then wash by TEAB (1 M).

triester approach[31,32] which condenses a P-component (triethylammonium salt of nucleotide phosphate) and a HO-component (5′-hydroxyl group free nucleoside) in ambient temperature without high sensitivity to moisture was our choice. As shown in Scheme 2, the P-component **8** and HO-component **7** were coupled by condensation reagent MSNT[33] in pyridine at room temperature, to form a fully-protected dimer **9**. 5′-O-DMTr of the dimer could be removed to get a dimer HO-component **10**. For cleavage of the 3′-O-AZMB, fully-protected nucleotide was dissolved in 1,4-dioxane/water (v:v = 9:1), stirred with 4 equiv of triphenylphosphine at room temperature overnight. The azido group was reduced to amino, and subsequent intramolecular O→N acyl migration generated isoindolin-1-one, and the 3′-hydroxyl group was released. Cleavage of the 3′-O-AZMB of **9** followed by phosphorylation afforded a dimer P-component **12**.

The newly generated P-component or HO-component could be further coupled as the reaction cycle displayed by Scheme 2. We synthesized different 2–4mer building blocks by adding monomers