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A novel polymer-supported sulfur-transfer reagent for the synthesis of phosphorothioates

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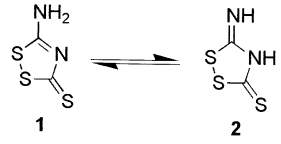
Abstract—A commercially available and inexpensive compound, 3-amino-1,2,4-dithiazole-5-thione (ADTT), has been attached onto a hydroxyl resin via a succinic acid linker. The polymer-supported ADTT is an efficient sulfur-transfer reagent for the solution-phase synthesis of phosphorothioates.

Phosphorothioate analogs are of considerable interest in nucleic acid research and are among the most promising analogs tested as oligonucleotide therapeutics.[1] The nucleotide phosphorothioate compounds and libraries have also been designed and tested as antiviral and anti-infective agents.[2] The automated solid-phase synthesis of oligonucleotide phosphorothioates has been carried out predominantly using the phosphoramidite method.[3] This approach requires stepwise sulfurization to be carried out after each coupling step. It is imperative that an efficient sulfur-transfer reagent is used in the phosphoramidite approach. On the other hand, it is believed that when higher quantities of a specific sequence of oligonucleotides or blockmers (dinucleotides or trinucleotides)[4] as well as other phosphorothioate analogs are required for commercial purposes, solution-phase synthesis may contribute further to cost reduction. Furthermore, there has been growing interest in solution-phase parallel array synthesis recently. In traditional solution-phase synthesis, the intermediates may need to be purified by chromatography, which could be a tedious process in multi-step nucleotide phosphorothioate syntheses. Thus, polymer-assisted solution-phase (PASP) synthesis has been developed to facilitate the synthesis and purification.[5] In order to further exploit PASP synthesis, it is very important to develop many new polymer-bound reagents for a variety of useful reactions.

In the synthesis of phosphorothioates, a number of sulfur-transfer reagents such as Beaucage reagent, EDITH and others have been reported in recent years.[6–16] However, it is difficult to attach these reagents to a polymer support, and no polymer-supported sulfur-transfer reagent, to the best of our knowledge, has been reported for solution-phase synthesis of phosphorothioates. A polymer-supported reagent may offer several advantages, including the possibility to drive reactions to completion with excess reagent, the easy separation of reagent and isolation of product by simple filtration and evaporation of solvents, the easy monitoring of the reaction by known analytical techniques TLC, HPLC and NMR, and a suitable approach for automation. Herein we report our studies on a novel polymer-supported sulfur-transfer reagent for the solution-phase synthesis of phosphorothioates.

The compound, 3-amino-1,2,4-dithiazole-5-thione (ADTT) or xanthane hydride, was first prepared by Wöhler as long ago as 1821.[17] There exist two main tautomeric structures as shown in Scheme 1, in which structure **1** has been reported as the predominant form based on X-ray analysis[18] as well as NMR (^{15}N and ^{13}C) and IR spectroscopies.[19] It is now commercially available from several chemical companies in bulk quantity. We have found that ADTT is an efficient sulfur-transfer reagent in solid-phase synthesis of



Scheme 1. Tautomers of ADTT.

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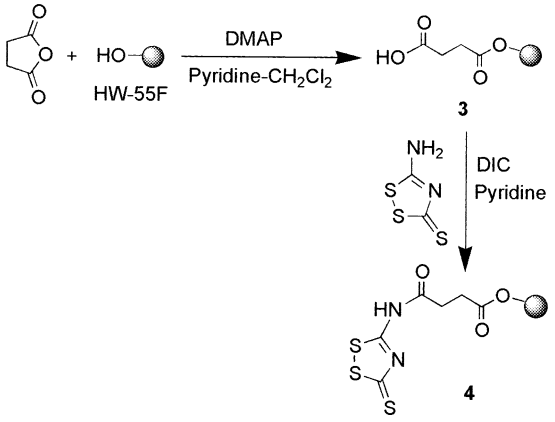
oligonucleotide phosphorothioates.[20] It also contains an amino group that provides a site for attachment on polymer supports. However, due to possible unfavorable electronic and steric interactions with highly cross-linked polymer supports, the activity of polymer-supported ADTT remained to be examined. We have chosen a hydroxyl resin, Toyopearl HW-55F (Toso-Haas, Montgomeryville, PA), as the polymer support. HW-55F is a relatively inexpensive methylacrylate and ethylene glycol copolymer resin used for size exclusion chromatography, which has also been used as a solid support in the large-scale synthesis of oligonucleotides.[21]

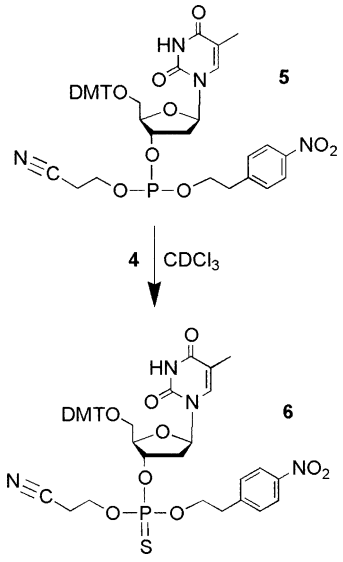
The polymer-supported ADTT **4** was prepared in two steps as revealed in Scheme 2. The succinic acid linker was attached to the hydroxyl resin (HW-55F) by treatment of the resin (12.4 g) with DMAP (0.6 g, 4.9 mmol) and succinic anhydride (2.48 g, 24.8 mmol) in a mixture of pyridine and dichloromethane (60 mL, 1:2) at room temperature overnight. The resultant linker-resin **3** was collected by filtration, washed with methanol, acetonitrile and dichloromethane, and then dried in vacuo. The support-bound succinic acid (**3**, 5.4 g) was then coupled with ADTT (1.60 g, 10.8 mmol) by use of DIC (1.69 mL, 10.8 mmol) in pyridine (30 mL) at room temperature overnight. The resultant resin **4** was collected by filtration, washed with methanol, acetonitrile and chloromethane, and then dried in vacuo.

The sulfurizing efficacy of the polymer-supported ADTT **4** was examined on a model compound **5**, 5′-*O*-dimethoxytritylthymidine 3′-*O*-((2-cyanoethyl)-4-nitrophenethyl phosphite),[12] as shown in Scheme 3. The phosphite triester **5** had been used by different research laboratories to evaluate the sulfur-transfer reagents.[11] In this experiment, **5** (30 mg) was treated with the polymer-supported ADTT **4** (0.2 g) in $CDCl_3$ at room temperature for 30 min. After the resin was removed by filtration, the $CDCl_3$ solution was examined by ^{31}P NMR.

As shown in Fig. 1, the phosphite triester **5** was completely transformed to the phosphorothioate triester **6** by reaction with **4**. The phosphodiester formed in this



Scheme 2. Synthesis of the polymer-supported sulfur-transfer reagent.



Scheme 3. Reaction of the polymer-supported sulfur-transfer reagent.

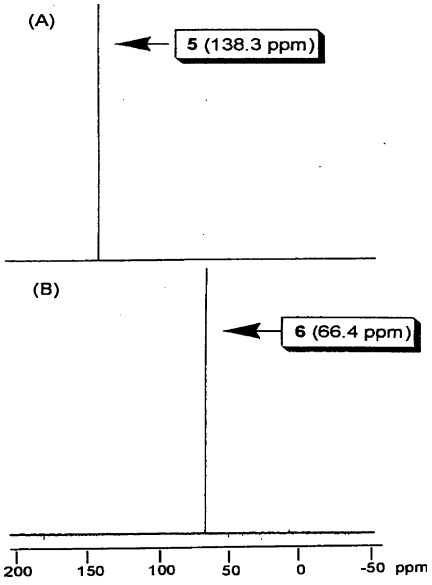


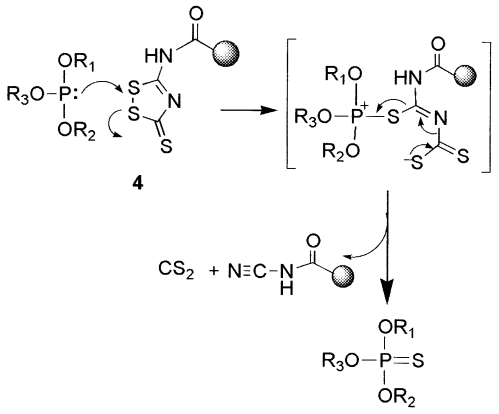
Figure 1. ^{31}P NMR: (A) Compound **5** in $CDCl_3$. (B) Compound **5** was transformed to **6** by reaction with **4**.

reaction was less than 1%. The result demonstrates that **4** is an efficient sulfur-transfer reagent in solution-phase synthesis of phosphorothioates

The proposed sulfur-transfer mechanism for polymer-supported reagent **4** is shown in Scheme 4.

To estimate the loading of **4**, we treated **4** (0.10 g) with different amounts of the phosphite triester **5** (0.1–0.3 g, 0.1–0.4 mmol) in $CDCl_3$ (1.5 mL). The suspension was agitated at room temperature overnight. After filtration, the $CDCl_3$ solution was examined by ^{31}P NMR. The loading was calculated by Formula 1.[‡] The result shows that the loading is about 1.23 mmol/g.

[‡] $M_5 = 810$; W_4 and W_5 are the weights (in grams) for resin **4** and compound **5** tested; I_5 and I_6 are the corresponding integration values of ^{31}P NMR for compounds **5** and **6**.



Scheme 4. Sulfur-transfer mechanism of **4**.

$$\text{Loading (mmol/g)} = (W_5 \times I_6 \times 1000)/[M_5 \times (I_5 + I_6) \times W_4] \quad (1)$$

In summary, a commercially available and inexpensive compound, ADTT, has been attached onto a hydroxyl resin via a succinic acid linker at a relative high loading. ADTT can be also attached to other hydroxyl resins or amino resins in similar way. The polymer-supported ADTT is an efficient sulfur-transfer reagent for the solution-phase synthesis of phosphorothioates. This reagent is easy to prepare and to use. The cost is relatively low. It is envisaged that this polymer-supported sulfur-transfer reagent will find use in the large-scale solution-phase synthesis as well as in high-throughput parallel synthesis of phosphorothioates.

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