Tetrahedron Letters 52 (2011) 434–437

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Reactivity of 3*H*-1,2,4-dithiazole-3-thiones and 3*H*-1,2-dithiole-3-thiones as sulfurizing agents for oligonucleotide synthesis

Andrei P. Guzaev *

AM Chemicals LLC, 4065 Oceanside Blvd., Suite M, Oceanside, CA 92056-5824, USA

ARTICLE INFO

Article history:
Received 26 October 2010
Revised 13 November 2010
Accepted 15 November 2010
Available online 19 November 2010

Keywords:
Oligonucleotide synthesis
Phosphorothioate
Sulfurization
Phosphoramidite synthesis

ABSTRACT

The reactivity of 5-amino-3*H*-1,2,4-dithiazole-3-thiones substituted at their amino group and 5-amino-3*H*-1,2-dithiole-3-thiones substituted at their amino group and C4 toward compounds containing P(III) atoms has been studied. *N*,*N*-Disubstituted-*N*′-(3-thioxo-3*H*-1,2,4-dithiazol-5-yl)methanimidamides were selected as novel efficient sulfur transfer reagents suitable for DNA and RNA synthesis.

1. Introduction

Oligonucleotides that contain unnatural internucleoside linkages where one of the non-bridging oxygen atoms of the phosphate group is replaced by a sulfur atom are referred to as oligonucleotide phosphorothioates. Due to their enhanced nucleolytic stability, improved biodistribution, and pharmacokinetic properties, oligonucleotide phosphorothioates are among the most commonly used analogs. Their widespread use has led to an increasing demand for more practical, inexpensive, and efficient methods and reagents for their preparation.

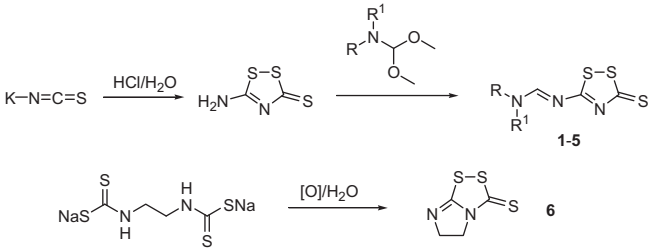
Examples of such agents include 3*H*-1,2-benzodithiol-3-one-l,l-dioxide, or the Beaucage reagent,[1] tetraethylthiuram disulfide,[2] phenylacetyl disulfide,[3] dibenzoyl tetrasulfide,[4] bis-(*O*,*O*-diisopropoxyphosphinothioyl) disulfide,[5] benzyltriethylammonium tetrathiomolybate,[6] bis-(*p*-toluenesulfonyl) disulfide,[7] 3-ethoxy-l,2,4-dithiazoline-5-one (EDITH) and 1,2,4-dithiazolidine-3,5-dione,[8] 3-amino-1,2,4-dithiazole-5-thione,[9] 3-methyl-1,2,4-dithiazolin-5-one,[8a,10] and 3-phenyl-1,2,4-dithiazoline-5-one.[11] However, when this study began, only the Beaucage reagent[1] and tetraethylthiuram disulfide[2] were commercially available. The widely used Beaucage reagent displays a rather low hydrolytic stability, and the reaction kinetics of tetraethylthiuram disulfide with solid support-bound phosphite triesters is somewhat slow, which makes it less useful in high-throughput applications. Consequently, more efficient sulfurizing agents are needed.

2. Results and discussion

2.1. Synthesis of 3*H*-1,2,4-dithiazole-3-thiones 1–5 and 3*H*-1,2-dithiole-3-thiones 6–15

Compounds **1**–**15** with the potential of becoming sulfurizing agents were synthesized starting from simple precursors as outlined in Schemes 1 and 2. As shown in Scheme 1, xanthane hydride was synthesized by acid-catalyzed trimerization of thiocyanic acid and was treated with the dimethylacetals of the corresponding commercial dialkylformamides to give compounds **1**–**5** in 80–90% yield.[12] Endodane **6** was prepared as reported previously[13](Scheme 1).

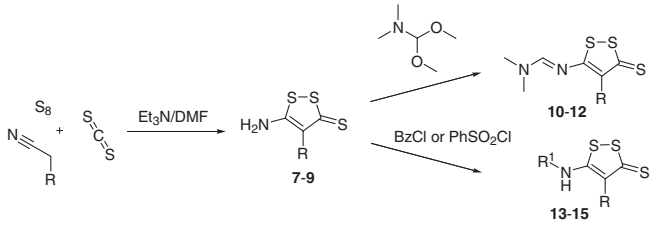
Compounds **7**–**9** were synthesized by the previously reported method[14] (Scheme 2) and were then converted to the *N*-(*N*′,*N*′-dimethylaminomethylene)-protected derivatives **10**–**12** by reaction with dimethylformamide dimethylacetal as described for compound **1**.[12] Additionally, compound **7** was converted to its



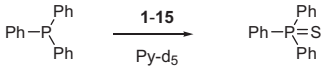
Scheme 1. Synthesis of 3*H*-1,2,4-dithiazole-3-thiones; **1**: R = R^1 = Me; **2**: R = R^1 = Bu; **3**: R + R^1 = (CH)$_4$; **4**: R + R^1 = (CH)$_5$; **5**: R + R^1 = –(CH$_2$)$_2$O(CH$_2$)$_2$–.

* Tel./fax: +1 760 631 0330.
E-mail address: aguzaev@amchemicals.com

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.11.086



Scheme 2. Synthesis of 3*H*-1,2-dithiole-3-thiones; **7**, **10**, **13**: R = CO_2Et; **8**, **11**, **14**: R = CN; **9**, **12**, **15**: R = SO_2Ph; **13**: R^1 = SO_2Ph; **14**: R^1 = Bz; **15**: R^1 = Bz.



Scheme 3. Sulfurization of PPh_3 with compounds **1**–**15** in solution.

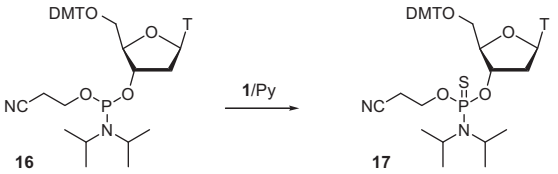
phenylsulfonyl derivative **13** (Scheme 2), and compounds **8** and **9** were *N*-benzoylated to give compounds **14** and **15**, respectively.

2.2. Testing of compounds 1–15 as sulfurizing agents in solution

The efficiency of compounds **1**–**15** as sulfur transfer reagents was first evaluated for their ability to convert PPh_3 into $Ph_3P{=}S$ in solution. Equimolecular amounts of **1**–**15** were mixed with triphenylphosphine in Py-d_5 and the progress of the reaction was monitored by ^{31}P NMR (Scheme 3). Compounds **1**–**5** and **7**–**12** reacted quantitatively with the substrate in less than 5 min to give triphenylphosphine sulfide in more than 99.9% yield along with a minor amount of triphenylphosphine oxide (<0.1%). In contrast, compounds **6** and **13**–**15** failed to produce good yields of $Ph_3P{=}S$ over a period of 5 min. The results obtained thus warranted continued testing of compounds **1**–**5** and **7**–**12** as sulfur transfer reagents in solid-phase oligonucleotide synthesis while compounds **6** and **13**–**15** were excluded from further study.

In a more detailed investigation, the stoichiometry of the sulfur transfer was ascertained by reacting aliquoted amounts of *N*,*N*-dimethyl-*N*′-(3-thioxo-3*H*-1,2,4-dithiazol-5-yl)methanimidamide (DDTT, **1**) with triphenylphosphine (1–5 equiv) and determining the ratio of $Ph_3P/Ph_3P{=}S$ by ^{31}P NMR. It was found that **1** transferred sulfur to PPh_3 in pyridine with a stoichiometric ratio of **1** to PPh_3 of 1:2.

In a similar manner, compound **1** was reacted with an equimolecular amount of 5′-*O*-(4,4′-dimethoxytrityl)thymidine 2-cyanoethyl 3′-*O*-(*N*,*N*-diisopropyl)phosphoramidite **16** (Scheme 4) to



Scheme 4. Sulfurization of **16** using compound **1**.

give thionophosphoramidate **17** as a mixture of diastereomers in more than 99.9% yield as judged by ^{31}P NMR.

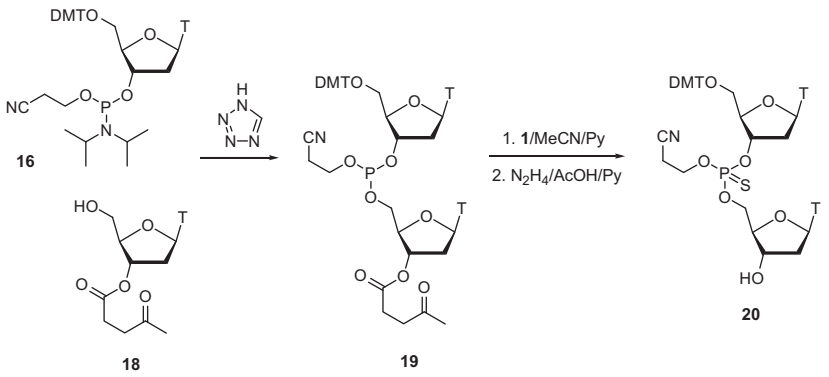
To further evaluate the usefulness of **1** as a sulfur transfer reagent, the protected dithymidylyl monophosphorothioate **20** was synthesized by reacting the phosphoramidite **16** with 3′-*O*-levulinylthymidine **18**[15] in the presence of 1*H*-tetrazole (Scheme 5).[16] Without isolation, the protected phosphite triester **19** was treated with a 1.2 molar excess of **1**, and the 3′-*O*-levulinyl protecting group was removed by treatment with hydrazinium acetate in a mixture of pyridine and AcOH to give compound **20** as a mixture of R_p and S_p diastereomers. Upon aqueous work-up, the crude **20** was analyzed by ^{31}P NMR and reverse-phase HPLC to show a 99.3% efficiency of the sulfur transfer. In order to characterize the compound, a portion of **20** was separated using reverse-phase HPLC to give the individual diastereomers **20a** and **20b**.[16] The latter compounds were decyanoethylated with a mixture of aqueous ammonium hydroxide and pyridine (1:5) followed by removal of the DMT protection with 80% aqueous AcOH resulting in the individual R_p and S_p diastereomers of thiothymidylyl-(3′ → 5′)-thymidine (**21a**[17] and **21b**,[18] ^{31}P NMR δ 56.65 and 56.28, respectively). Based on the reported data,[19] the R_p configuration was assigned to **21a** having a larger chemical shift for its P-atom.

Titration of compounds **16** and **19** with DDTT (**1**) showed that the stoichiometric ratio of **1** to a phosphite in these reactions was 2:3 (^{31}P NMR).

2.3. Testing of compounds 1–15 as sulfurizing agents in oligonucleotide synthesis on solid-phase

To test compounds **1**–**5** and **7**–**12** as sulfurizing agents in oligonucleotide synthesis, the oligonucleotide DMT-T_{10} phosphorothioate (**22**) was assembled on a 1 μmol scale using the standard protocol of the chain assembly, 0.05 M solutions of **1**–**5** and **7**–**12** in pyridine, and 4 min sulfurization time in each synthetic cycle.

After the solid-phase-bound material was released and deprotected with concentrated aqueous ammonium hydroxide, the crude oligonucleotide phosphorothioates were analyzed by re-



Scheme 5. Solution-phase synthesis of 2-cyanoethyl 5′-*O*-(4,4′-dimethoxytrityl)thiothymidylyl-(3′ → 5′)-thymidine **20**.