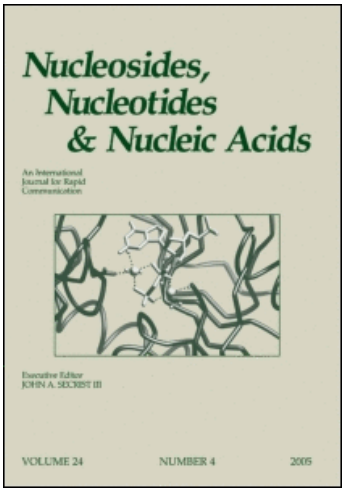

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Evaluation of Sulfur-Transfer Reagents for Large Scale Synthesis of Oligonucleotide Phosphorothioate Analogues

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VI. CHEMICAL DEVELOPMENT AND ANALYTICAL CHEMISTRY

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1585-1588 (1997)

EVALUATION OF SULFUR-TRANSFER REAGENTS FOR LARGE SCALE SYNTHESIS OF OLIGONUCLEOTIDE PHOSPHOROTHIOATE ANALOGUES

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ABSTRACT: Several sulfur-transfer reagents have been evaluated for large scale synthesis of oligonucleotide phosphorothioate analogues, in which 3-ethoxy-1,2-dithiazoline-5-one (EDITH, **5**) shows potential as an alternative to Beaucage reagent.

Oligonucleotide phosphorothioates are of considerable interest in nucleic acid research and among the most promising analogues tested as "oligonucleotide therapeutics".[1] In order to support the increasing demand for preclinical and clinical trials and future commercialization, a series of large scale syntheses have been developed up to 100 mmol,[2] that have dramatically reduced costs. Based on superior coupling efficiency as well as the capability to control the state of each linkage in a site-specific manner, the phosphoramidite approach appears to be the method of choice. It is imperative that an efficient sulfur transfer reagent is required for the synthesis of oligonucleotide phosphorothioates via the phosphoramidite approach. A number of sulfur-transfer reagents have also been reported, which include phenylacetyl disulfide[3], 3H-1,2-benzodithiol-3-one-1,1-dioxide (Beaucage reagent)[4], tetraethylthiuram disulfide (TETD)[5], dibenzoyl tetrasulfide[6], bis(O,O-diisopropoxyphosphinothioyl) disulfide (S-Tetra)[7], benzyltriethyl-ammonium tetrathiomolybate (BTTM)[8], bis(p-toluenesulfonyl) disulfide[9] and 3-ethoxy-1,2,4-dithiazoline-5-one (EDITH)[10]. Of these compounds, Beaucage reagent has been widely used and applied successfully in large scale synthesis, however, its synthetic accessibility and stability in solution are not optimal. Beaucage reagent is also very costly in large scale production. We are investigating on an

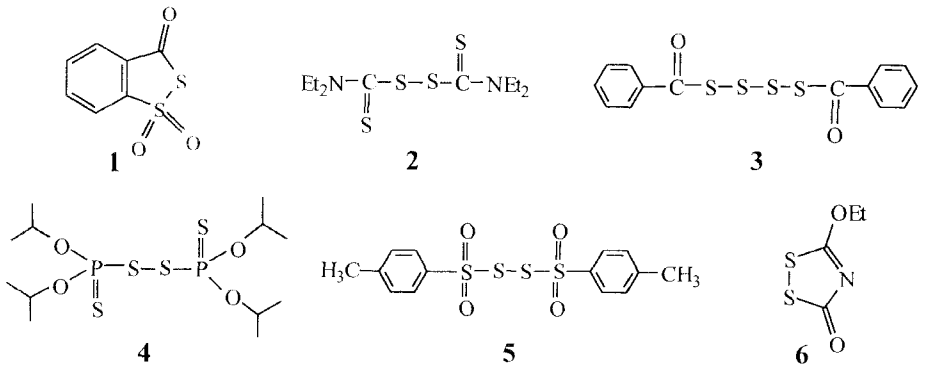

FIG. 1. Sulfur-Transfer Reagents.

alternative sulfurizing reagent to reduce costs. Herein we report our studies on some known sulfur-transfer reagents.

The efficiency of sulfurizing reagents **1-6**[11] (FIG. 1) was investigated by solid-phase syntheses of dinucleotide and oligonucleotide phosphorothioates. Synthesis of dinucleotide phosphorothioate d(TsT) was performed at the 1.0 μmol scale using an automated synthesizer, 8909 Expedite™ (Millipore, Bedford, MA). Solutions of the sulfurizing reagents (except **5**) were dried over activated 4 Å molecular sieves overnight before use. The synthesis protocol "THIO 1 μmol" (Expedite™ software version 1.01) was used with the following modifications: 1. Capping was performed after the sulfurization step; 2. Delivery time of sulfurization reagents and acetonitrile wash step following sulfurization was extended in some cases as indicated. After cleavage and deprotection, the unpurified dimers were analyzed by reverse-phase HPLC. The results are given in TABLE 1. TABLE 1 shows that (a) the sulfurization yield was improved by increasing reaction time for **2-5,** however, the prolonged reaction time may lower the sulfurization yield for **1** and **6**; (b) in our hands, the compounds **2-5** were much less effective compared to Beaucage reagent (**1**) even at high concentration; (c) EDITH (**6**) was more efficient than Beaucage reagent (**1**).

To further evaluate the usefulness of **6** as an sulfurizing reagent for large scale synthesis, a 25mer (5'-CTCTCGCACCCATCTCTCTCCTTCT-3') oligonucleotide

TABLE 1. Sulfur-transfer efficiency for synthesis of the dimer 5'-d(TsT)-3'.

Sulfurizing Reagents	Concentration (M)	Molar equivalent	Solvent	Reaction Time (min)	P=O* (%)	P=S* (%)
1	0.06	11	CH_3CN	1	0.68	99.32
				5	0.92	99.08
	0.10	18		1	0.69	99.31
2	0.50	90	CH_3CN	1	4.55	95.45
				5	3.00	97.00
3	0.40	72	THF	1	4.42	95.58
				5	2.21	97.79
4	0.20	37	Pyridine	1	1.55	98.45
				4	0.71	99.29
	Saturated	37	CH_3CN	1	9.49	90.51
				5	8.18	91.82
5	0.20	37	DCM:Py(9:1)	1	2.07	97.93
6	0.02	4	CH_3CN	1	0.41	99.59
				4	0.60	99.40

* P=O indicates the phosphodiester linkage; P=S indicates the phosphorothioate linkage.

TABLE 2. Synthesis of 25mer phosphorothioate Using the Sulfurizing Reagents **1** and **6**.

Sulfurizing Reagents	Scale (μmol)	Molar equivalent	Ion-Exchange (%) DMT-ON	PO*	CE (%) n	n-1
1	1.0	18.3	87.5	0.53	83.3	3.3
	214	7.0	70.8	0.32	66.2	3.9
6	1.0	4.0	88.4	0.41	85.5	1.9
	310	2.5	71.0	0.38	64.0	3.0

*PO indicates the phosphodiester linkage.

phosphorothioate was synthesized on the 1.0 and 310 μmol using automated synthesizers, 8909 Expedite™ and OligoPilot II™ (Pharmacia Biotech, Uppsala, Sweden), respectively. The syntheses were carried out using both the reagents **1** and **6**. After ammonolytic release from CPG and deprotection, the unpurified oligonucleotide phosphorothioate was analyzed by ion exchange-HPLC and CE. The results are shown in TABLE 2.

The results show that in the large scale synthesis (310 μmol) more than 99.5% sulfur transfer efficiency was achieved at each step using as low as 2.5 equivalents of **6**.

In conclusion, by comparing several sulfur transfer reagents we find EDITH (**6**) is a highly efficient reagent. It is also reported that this reagent is relatively easy and inexpensive to prepare, and stable in solution.[10] Due to its high efficiency and favorable properties, compound **6** can be considered an advantageous alternative to Beaucage reagent, especially in large-scale preparation of oligonucleotide phosphorothioates. Further works on the optimization of reaction condition and scale are actively underway.

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11. Beaucage reagent (**1**) was purchased from R. I. Chemical (Orange, CA); TETD (**2**) was from Applied Biosystems (Foster City, CA); dibenzoyl tetrasulfide (**3**)[6] and bis(O,O-diisopropoxyphosphinothioyl) disulfide (S-Tetra, **4**)[7] were prepared by literature's procedure; benzyltriethyl-ammonium tetrathiomolybate (BTTM, **5**) was provided by Aronex Pharmaceuticals (The Woodlands, TX); EDITH (**6**) was provided by PerSeptive Biosystems (Framingham, MA).