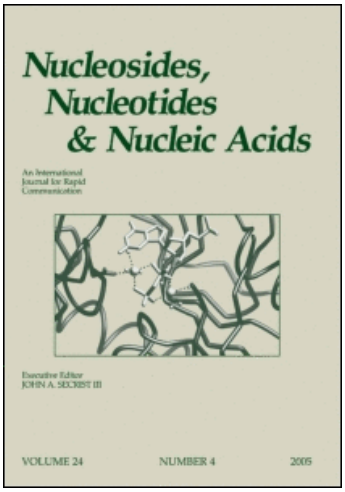

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Highly Effective Non-Explosive Activators Based on Saccharin for the Synthesis of Oligonucleotides and Phosphoramidites

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HIGHLY EFFECTIVE NON-EXPLOSIVE ACTIVATORS BASED ON SACCHARIN FOR THE SYNTHESIS OF OLIGONUCLEOTIDES AND PHOSPHORAMIDITES

Nanda D. Sinha, Patrick Foster, Satya N. Kuchimanchi, Greg Miranda, Saied Shaikh, and Dennis Michaud □ *Avecia Biotechnology, Inc. Milford, Massachusetts, USA*

□ *A new class of non-explosive activators has been developed based on heterocyclic tertiary amine salts of saccharin. These salts have been found to be highly effective in the synthesis of oligonucleotides and nucleoside phosphoramidites.*

Keywords Saccharin; oligonucleotides; nucleoside

INTRODUCTION

Tetrazole and nitrophenyltetrazole,[1] mild acidic compounds, have been used routinely for oligonucleotide synthesis. In addition to tetrazole and related compounds,[2–5] several other organic salts[6–10] also have been used for this purpose. Since tetrazole and some of its derivatives are explosive[11] and some of the acidic salts are hygroscopic, they cannot be used safely for large scale synthesis. To overcome these problems, a new class of activators generated from saccharin, saccharin salts of heterocyclic tertiary bases,[12] has been developed. These activators are non-explosive, non-hygroscopic and highly effective in oligonucleotide synthesis. Their synthesis and applications for nucleoside phosphoramidites and oligonucleotides are described below in the experimental section.

EXPERIMENTAL

a. General Method for Synthesis of Activators

Saccharin salts are prepared according to the Scheme 1. To a solution of saccharin (18.3 g, 0.1 mol) in acetonitrile (∼300 mL), was added a solution

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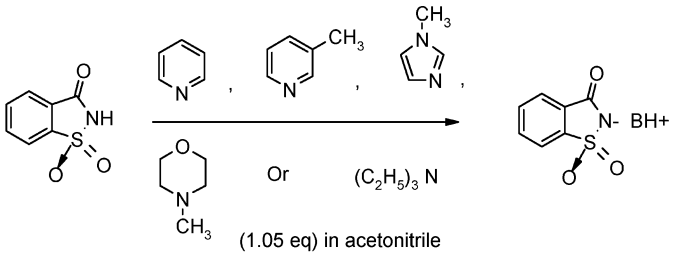


B = Pyridine, Collidine, Lutidine, Picoline, N-Methylimidazole, N-Methylmorpholine or Triethylamine

SCHEME 1 Synthesis of saccharin based activators.

of tertiary amine (0.105 mol) in acetonitrile (50 mL) with constant stirring, at ambient temperature. Initially, the saccharin suspension dissolved and in most cases the salt precipitated out during stirring for 2 hours. The solvent was removed by filtration and the product was crystallized in greater than 85% yield. These salts were characterized by ^{1}H and ^{13}C NMR [as an example: chemical shifts of saccharin-N-methylimidazole salt in DMSO; ^{1}H ppm: 13.9(1H,bs), 9.11(1H,s), 7.75(6H,m) & 3.95(3H,s) and ^{13}C ppm: 168, 146, 137, 135, 133, 132, 124, 122, 120, 119 & 36].

b. General Method for Synthesis of Nucleoside Phosphoramidites

To a suitably protected nucleoside (50 mmol) solution in acetonitrile (150 mL) under argon was added phosphitylating reagent, O-β-cyanoethyl-N,N,N′,N′-tetraisopropyl phosphorodiamidite, (18 g, 60 mmol; 1.2 eq.), the reaction mixture was stirred for 5–10 minutes and then solid saccharin-N-methylimidazole (SMI, 6.63 g; 25 mmol, 0.5 eq.) was added. Most of reactions were completed within 2–4 hours. 2′-Deoxyguanosine and 2′-O-t-BDMSilyl-guanosine derivatives required about 2 equivalents of phosphitylating reagent and longer reaction time (12–16 hours). The Purity of crude phosphoramidites were >90%. The formation of 3′-3′dimer phosphite triester, an impurity commonly seen with guanosine derivative was also reduced. Chromatographic purifications over silica gel using ethyl acetate-hexane combination afforded phosphoramidites with high purity (>99%) and high recovery (80–90%).

c. Synthesis of Oligonucleotides

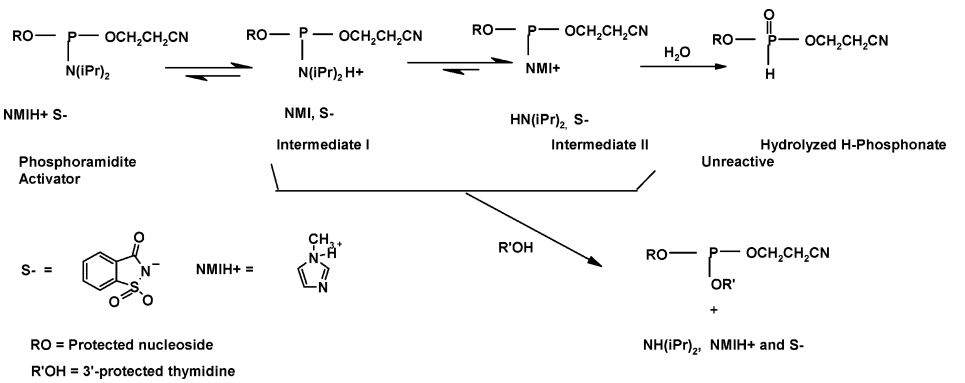
Various oligonucleotides (deoxy-, ribo- chimeric, phosphate diester, and phosphorothioate diester) were synthesized on an AKTA Oligo Pilot 100 following standard phosphoramidite chemistry. The coupling times for deoxy- and 2′-OMe nucleoside phosphoramidite were ~4–5 minutes and 10 minutes for the ribonucleoside phosphoramidite with recycling similar to tetrazole mediated synthesis. Initially, various equivalents (1–4 eq.) of

TABLE 1 Synthesis results using various activators SMI, tetrazole, and S-ethylthiotetrazole

Oligonucleotides	Solid support	Activator and no. of equivalents	%FLP by IEX-HPLC	Crude OD/umol
Phosphorothioate 18-mer	CPG	Tetrazole, 4.3 eq.	77	113
18-mer	CPG	SMI, 4 eq.	78	111
18-mer	Rigid Polystyrene	SMI, 4 eq.	76	124
18-mer	CPG	SMI, 2 eq.	78	108
18-mer	CPG	SMI, 1 eq.	76	113
Oligoribonucleotide 21-mer	CPG	SMI, 2 eq.	76	90
21-mer	CPG	SMI, 1 eq.	72	89
21-mer, same sequence	CPG	SET, 3 eq. with	65	90
28-mer Chimeric oligonucleotide	CPG	SMI, 2 eq.,	54	128
28-mer	CPG	SMI, 1 eq.	53	126
28-mer	CPG	SET, 2 eq.	45	85

SMI (saccharin-N-methylimidazole) with respect to phosphoramidite were used. It was observed that this salt was effective as an activator at 1:1 molar equivalent (Scheme 2). Compared to deoxyoligonucleotide synthesis using tetrazole, the yield, and purity results were almost identical. However, when compared with synthesis of oligoribonucleotide and chimeric oligonucleotide (sequences with 2′-F, 2′-O-Me nucleosides), using S-ethyltetrazole (SET/ETT), saccharin salt activator provided better yield and purity as shown in Table 1.

Formation of diastereomeric ratios were compared by synthesizing various di-nucleosides mono-phosphorothioate. The analyses based on RP-HPLC indicate that statistically there were no significant differences in the diastereomeric ratios formed using tetrazole and saccharin-N-methylimidazole. Overall, diastereomeric populations for phosphorothioate oligonucleotide are very similar to those derived from tetrazole.



SCHEME 2 Possible mechanism for catalytic activation of phosphoramidite and phosphite bond formation.

CONCLUSION

A new class of highly effective activators based on saccharin has been developed that are capable of activating phosphoramidite at much lower equivalent than other activators. Importantly, these salts are non-explosive and non-hygroscopic. This class of activator is equally effective for deoxy- and ribo-nucleoside phosphoramidites. Saccharin based activators also activate phosphitylating reagent O-β-cyanoethyl-N,N,N′,N′-tetraisopropyl phosphorodiamidite for phosphoramidite synthesis.

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