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Purification of synthetic oligomers

Shiyue Fang
Michigan Technological University, shifang@mtu.edu

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(12) **United States Patent**
Fang

(10) **Patent No.: US 7,850,949 B2**
(45) **Date of Patent: Dec. 14, 2010**

(54) **PURIFICATION OF SYNTHETIC OLIGOMERS**

(75) Inventor: **Shiyue Fang**, Houghton, MI (US)

(73) Assignee: **Michigan Technological University**, Houghton, MI (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 117 days.

(21) Appl. No.: **11/865,499**

(22) Filed: **Oct. 1, 2007**

(65) **Prior Publication Data**

US 2008/0081902 A1 Apr. 3, 2008

Related U.S. Application Data

(60) Provisional application No. 60/827,592, filed on Sep. 29, 2006.

(51) **Int. Cl.**
A61K 8/55 (2006.01)

(52) **U.S. Cl.** **424/57**

(58) **Field of Classification Search** .... None
See application file for complete search history.

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WO	WO-2004/002995	*	1/2004
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Primary Examiner—Cecilia Tsang
Assistant Examiner—Satyanarayana R Gudibande
(74) *Attorney, Agent, or Firm*—Michael Best & Friedrich LLP

(57) **ABSTRACT**

This invention provides a novel method for purifying synthetic oligomers comprising capping, polymerizing and separating any failure sequences produced during oligomer synthesis. Either the failure sequence or the full-length oligomer may be polymerized. Optionally, small molecule impurities may also be incorporated into the polymerized material. The invention provides novel capping agents having a polymerizable functional group. The invention also provides kits comprising at least one composition of the present invention.

1 Claim, 16 Drawing Sheets

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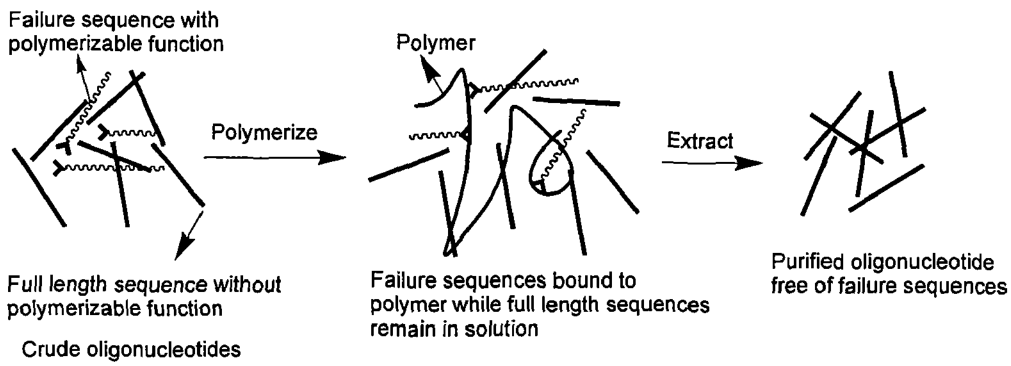

Failure sequence with polymerizable function
Polymerize
Polymer
Extract
Full length sequence without polymerizable function
Crude oligonucleotides
Failure sequences bound to polymer while full length sequences remain in solution
Purified oligonucleotide free of failure sequences

FIG. 1

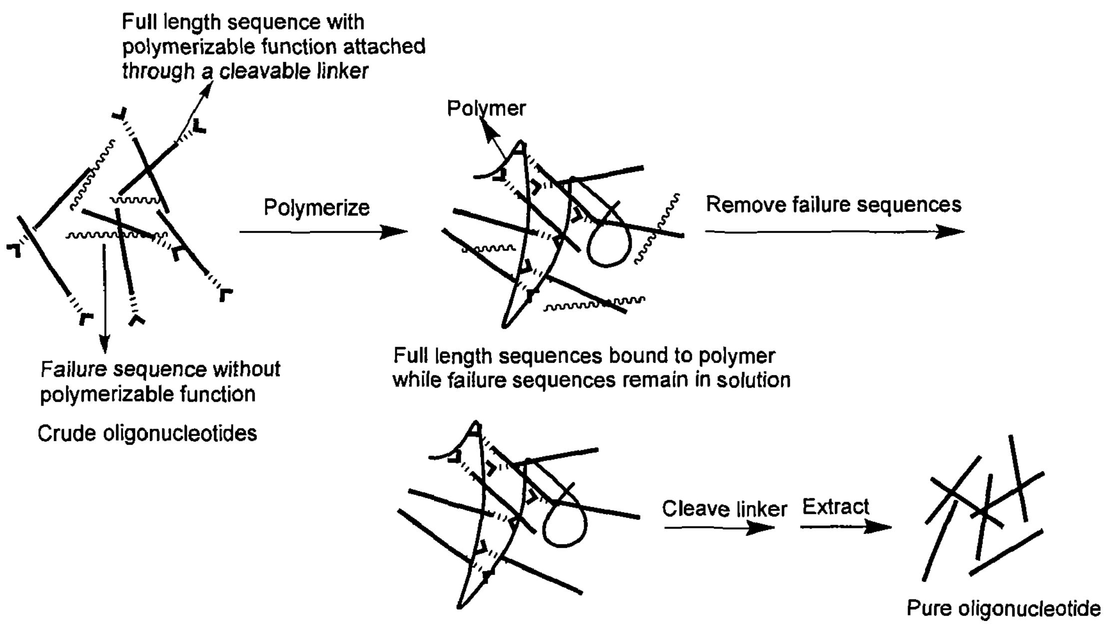
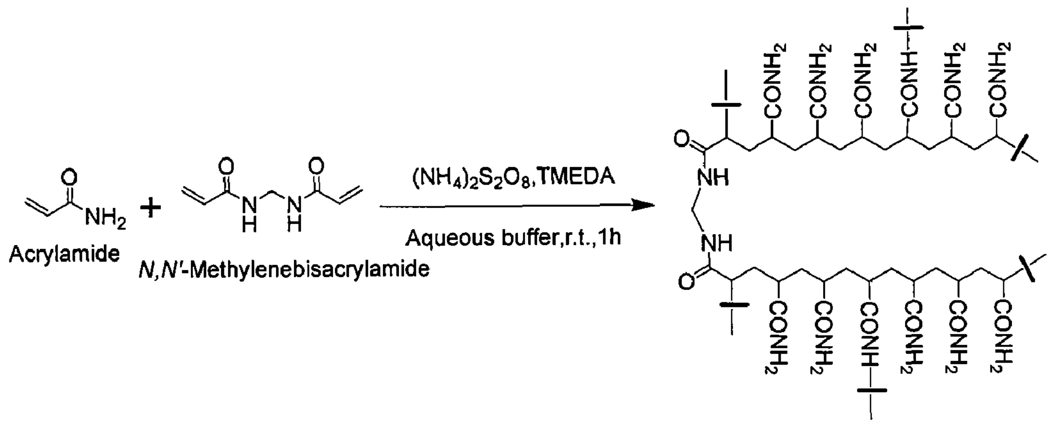
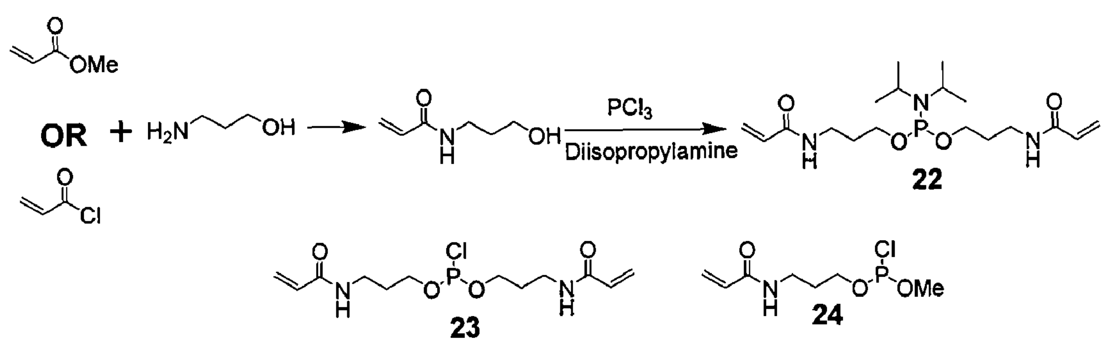

Full length sequence with polymerizable function attached through a cleavable linker
Polymerize
Polymer
Remove failure sequences
Failure sequence without polymerizable function
Crude oligonucleotides
Full length sequences bound to polymer while failure sequences remain in solution
Cleave linker
Extract
Pure oligonucleotide

FIG. 2



Acrylamide
N,N'-Methylenebisacrylamide
(NH4)2S2O8,TMEDA
Aqueous buffer,r.t.,1h
CONH2
CONH
NH

FIG. 3



OMe
OR
H2N
OH
Cl
PCl3
Diisopropylamine
22
23
24

FIG. 4

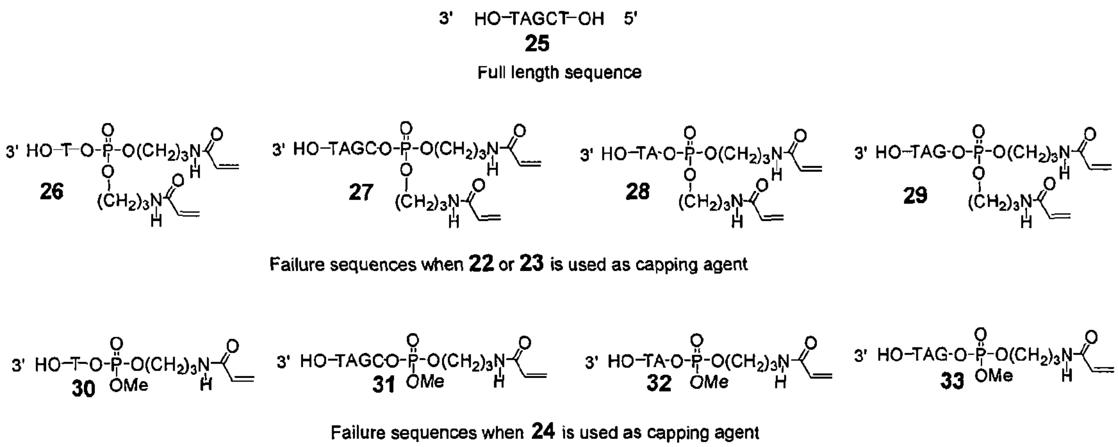


FIG. 5

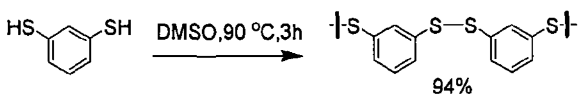


FIG. 6

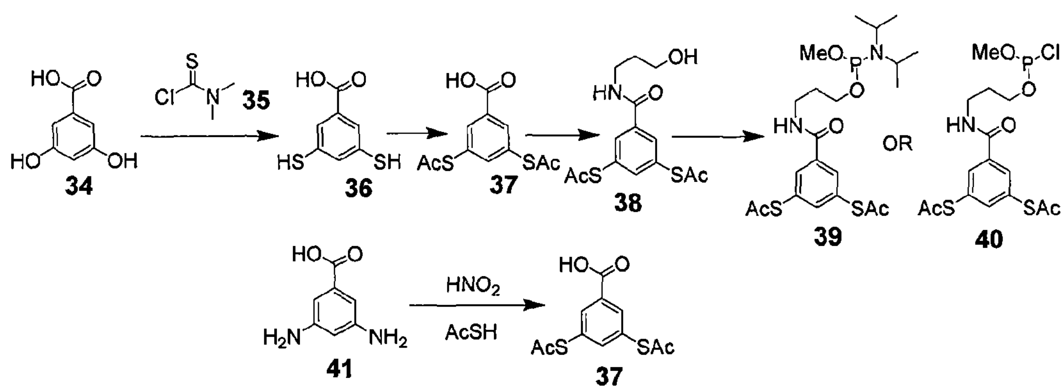
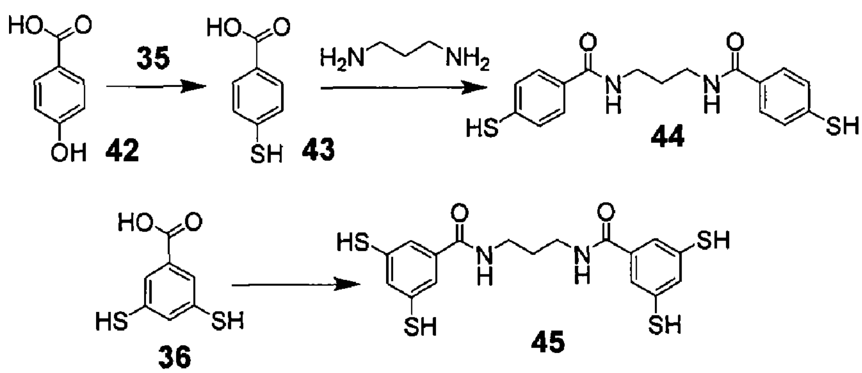
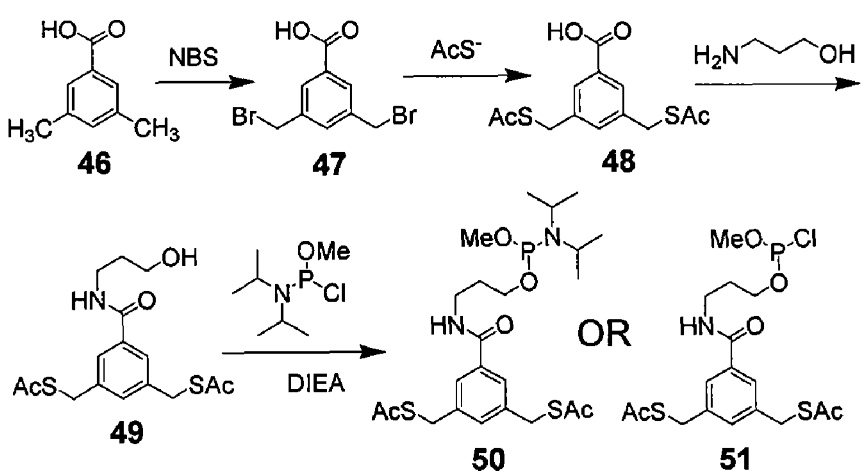


FIG. 7



HO O
35
HO O
H_2N NH_2
O
N H
O
N H
HS
SH
OH 42
SH 43
44
HO O
HS
SH
HS
SH
SH
SH
36
45

FIG. 8



HO O
NBS
HO O
AcS⁻
HO O
H_2N OH
H_3C
CH_3
Br
Br
AcS
SAc
46
47
48
OH
HN O
AcS
SAc
49
OMe
N P Cl
DIEA
MeO P N
O
HN O
AcS
SAc
50
OR
MeO P Cl
O
HN O
AcS
SAc
51

FIG. 9

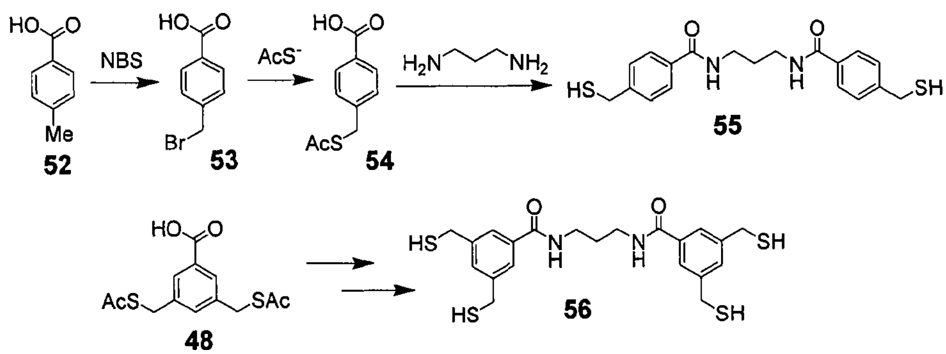


FIG. 10

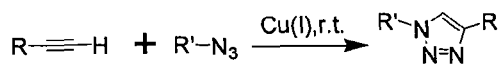


FIG. 11

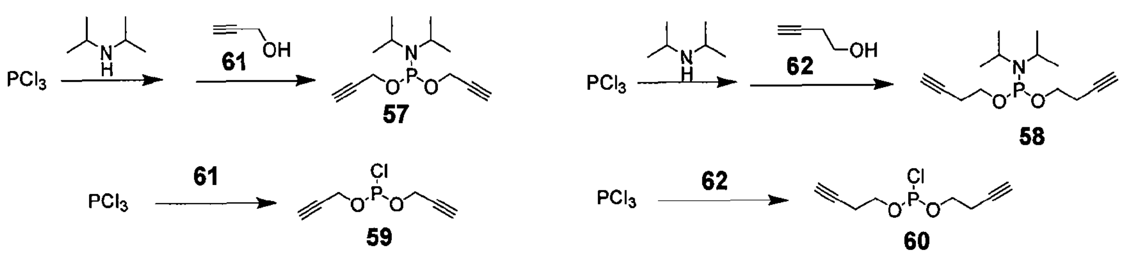


FIG. 12

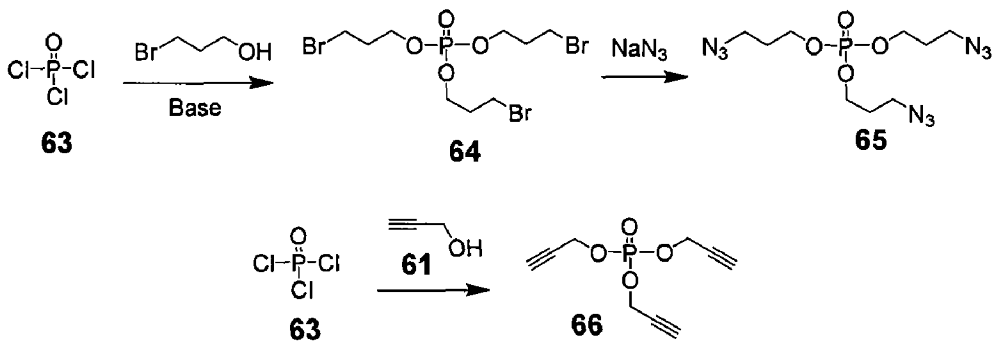


FIG. 13

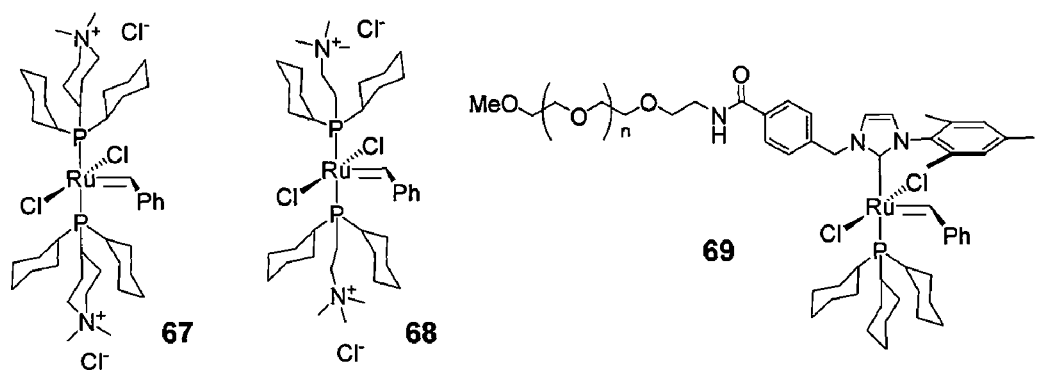


FIG. 14

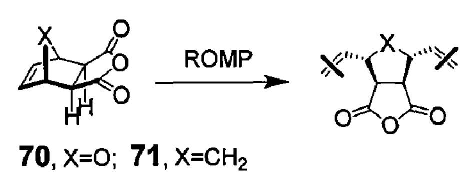


FIG. 15

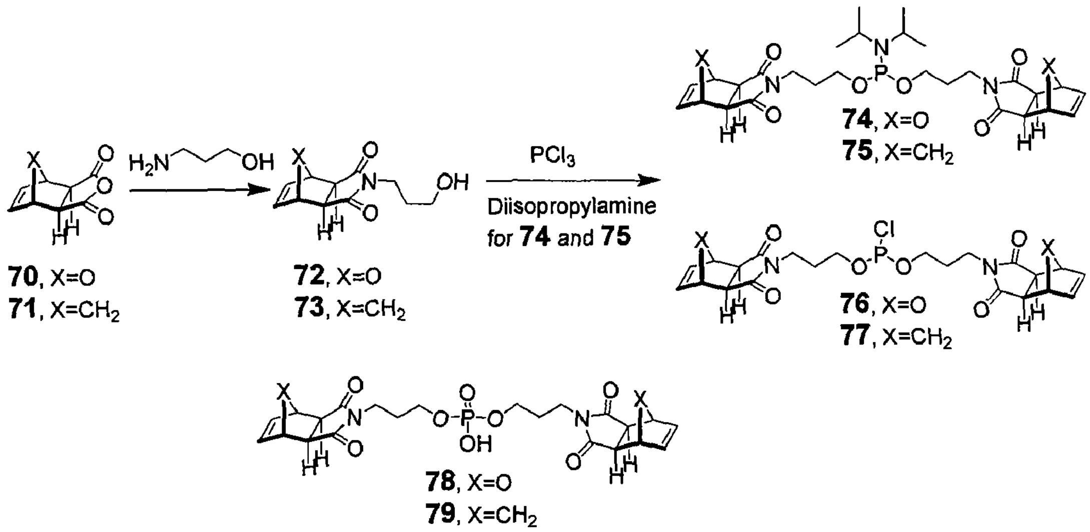

H2N OH
PCl3
Diisopropylamine
for 74 and 75
70, X=O
71, X=CH2
72, X=O
73, X=CH2
74, X=O
75, X=CH2
76, X=O
77, X=CH2
78, X=O
79, X=CH2

FIG. 16

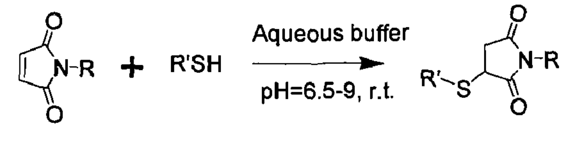
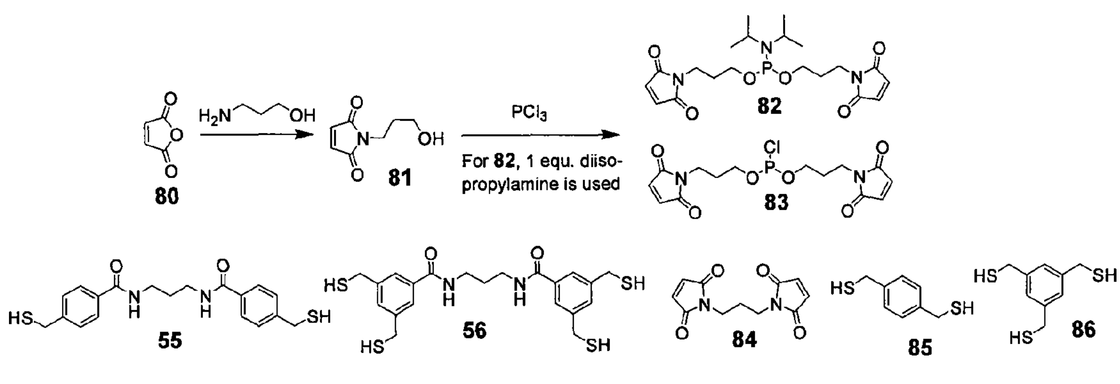
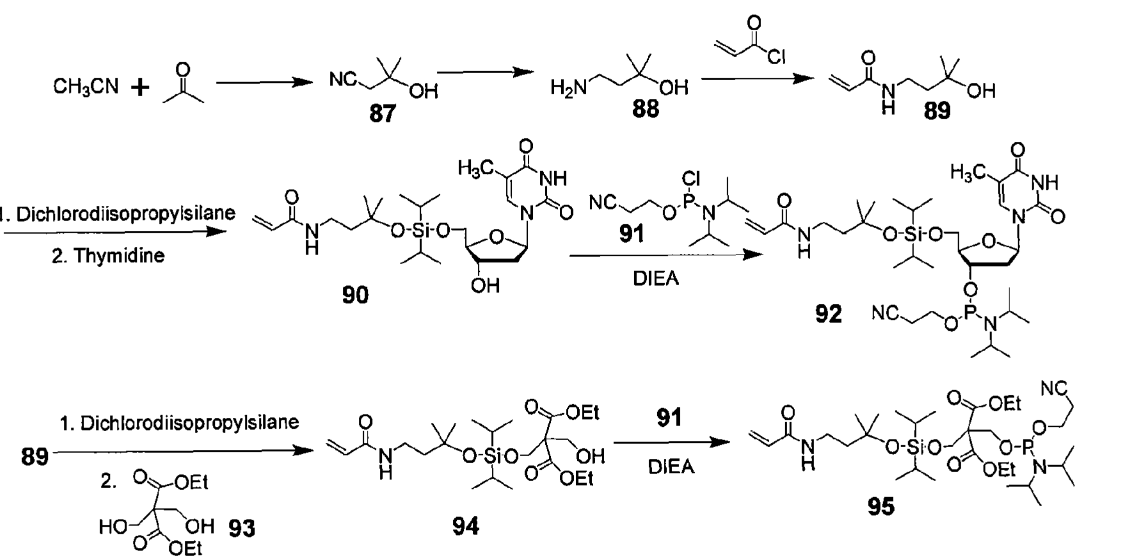

N-R
+
R'SH
Aqueous buffer
pH=6.5-9, r.t.
R'
S
N-R

FIG. 17



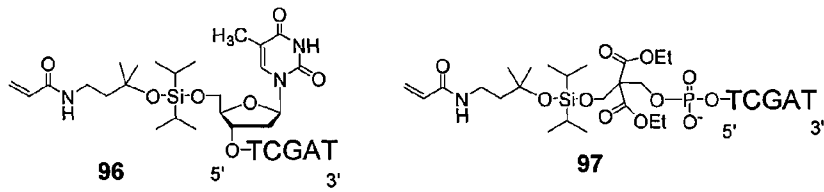
H2N OH
PCl3
For 82, 1 equ. diisopropylamine is used
80
81
82
83
55
56
84
85
86

FIG. 18



CH3CN +
87
88
89
1. Dichlorodiisopropylsilane
2. Thymidine
90
91
DIEA
92
1. Dichlorodiisopropylsilane
2.
93
94
95

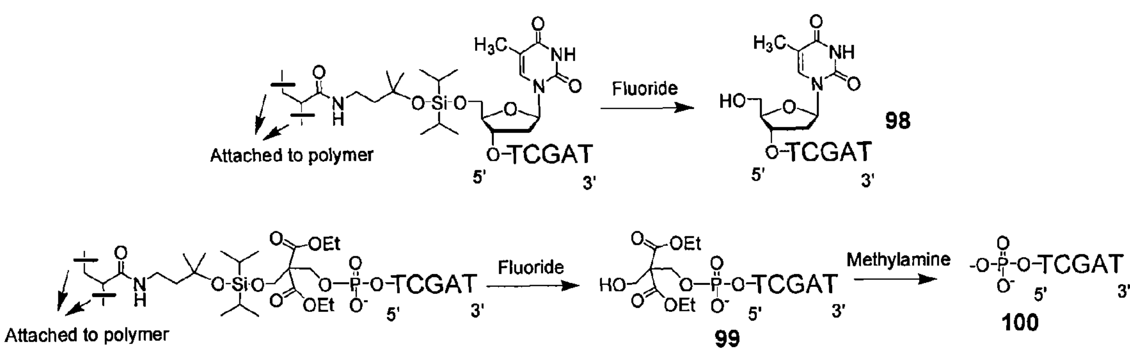
FIG. 19



96

97

FIG. 20



98

99

100

FIG. 21

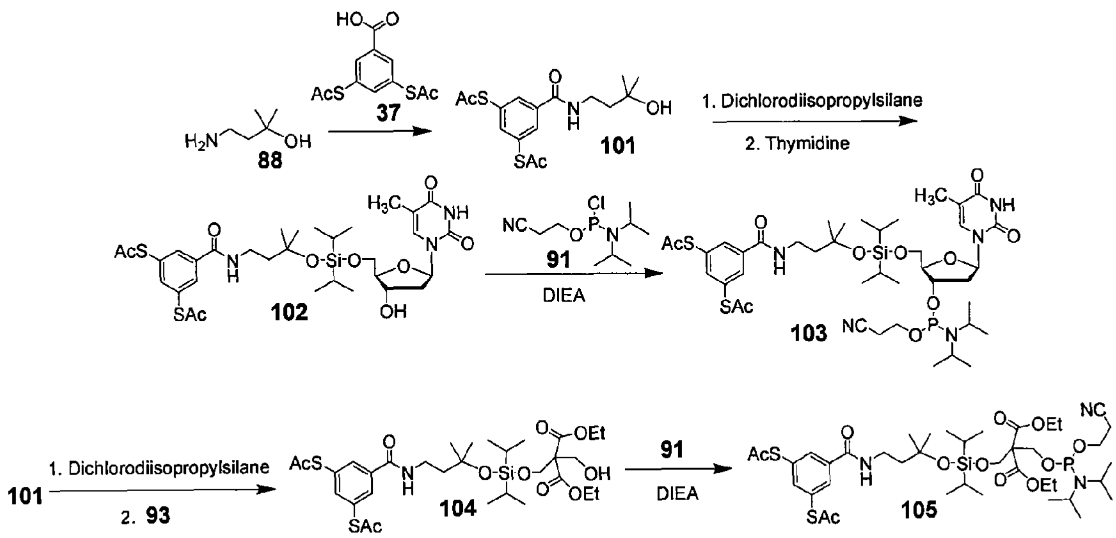

HO O
AcS SAc
37
H₂N OH
88
AcS O N H OH
101
SAc
1. Dichlorodiisopropylsilane
2. Thymidine
H₃C O NH N O
AcS O N H O-Si-O O
SAc
102
OH
NC O-P Cl N
91
DIEA
AcS
SAc
103
NC O P N
1. Dichlorodiisopropylsilane
101
2. 93
O OEt
OH
O OEt
104
SAc
91
DIEA
O OEt NC
O OEt
105
SAc

FIG. 22

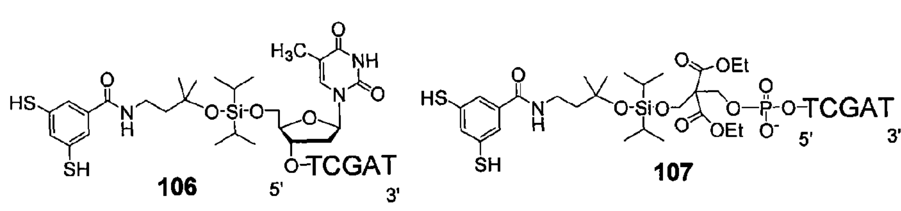

HS
SH
106
O-TCGAT
5' 3'
O OEt
O OEt
O-P-O-TCGAT
O⁻
5' 3'
107

FIG. 23

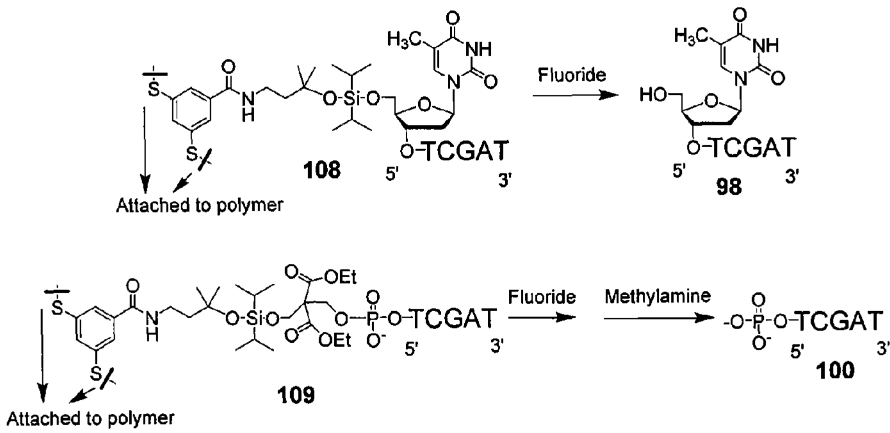

Fluoride
108
98
5'
3'
O-TCGAT
HO
Attached to polymer
Fluoride
Methylamine
109
100
OEt
Attached to polymer

FIG. 24

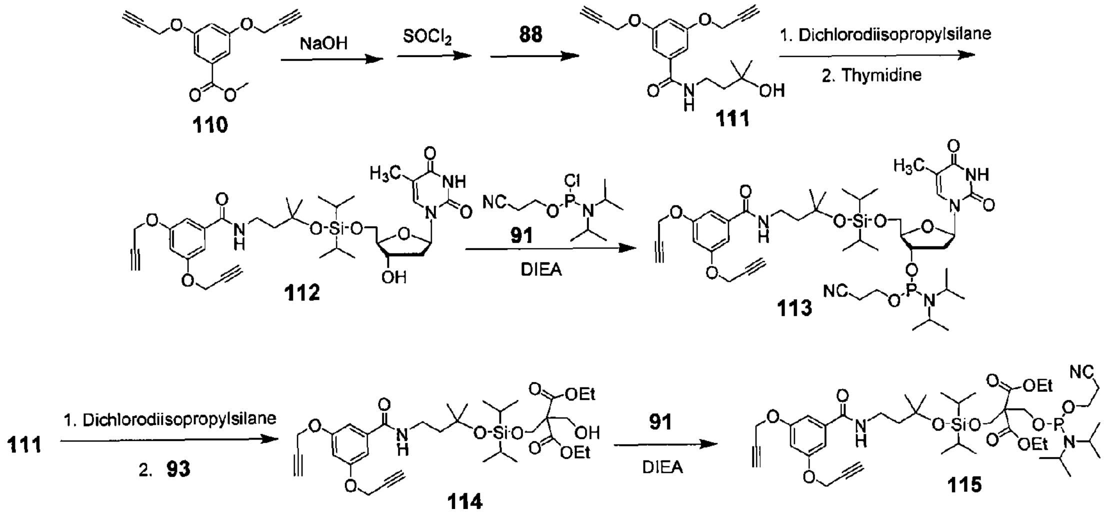
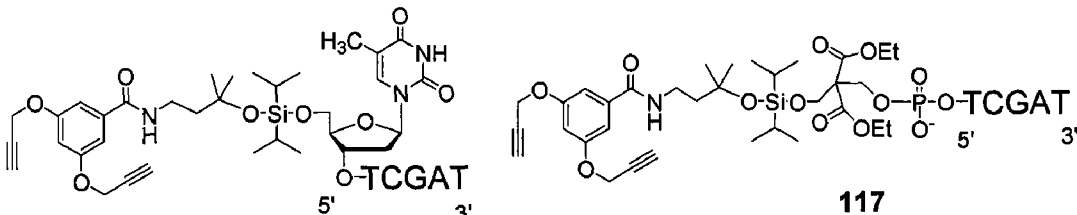

NaOH
$SOCl_2$
88
110
111
1. Dichlorodiisopropylsilane
2. Thymidine
112
91
DIEA
113
111
1. Dichlorodiisopropylsilane
2. 93
114
91
DIEA
115

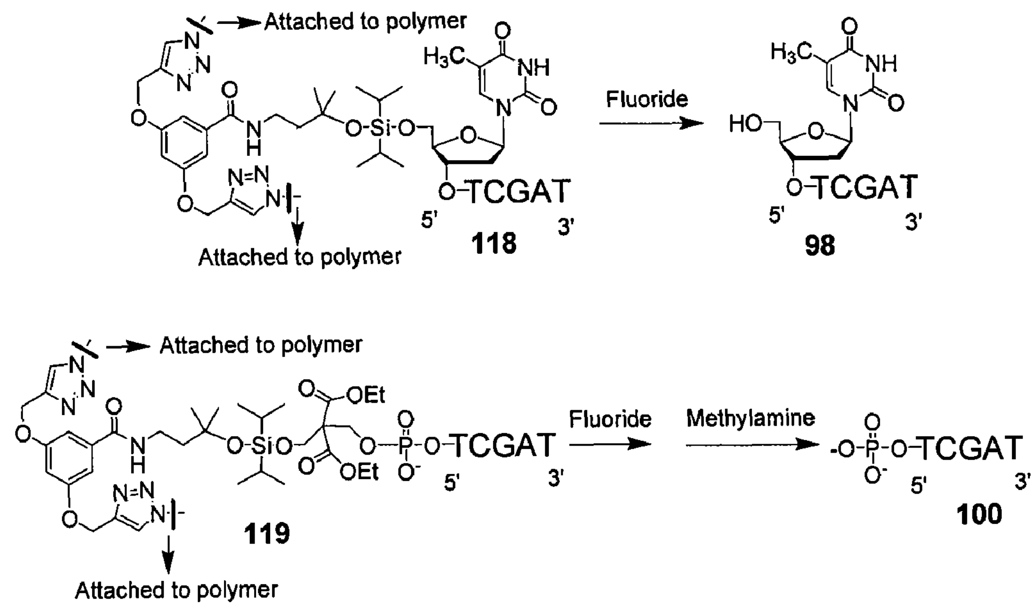
FIG. 25



116

117

FIG. 26



118

98

119

100

FIG. 27

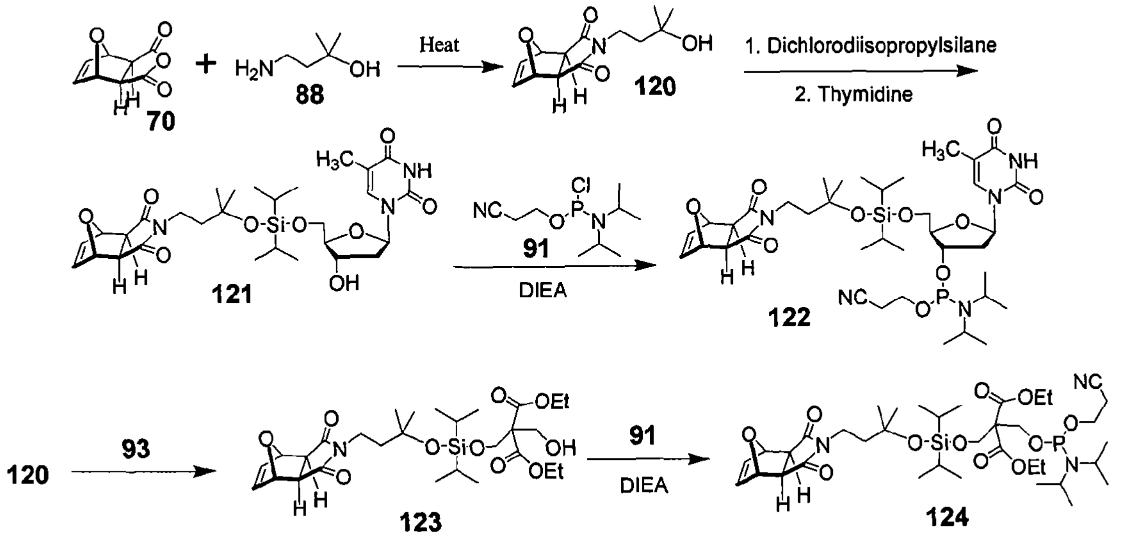

70
88
Heat
120
1. Dichlorodiisopropylsilane
2. Thymidine
121
91
DIEA
122
120
93
123
91
DIEA
124

FIG. 28

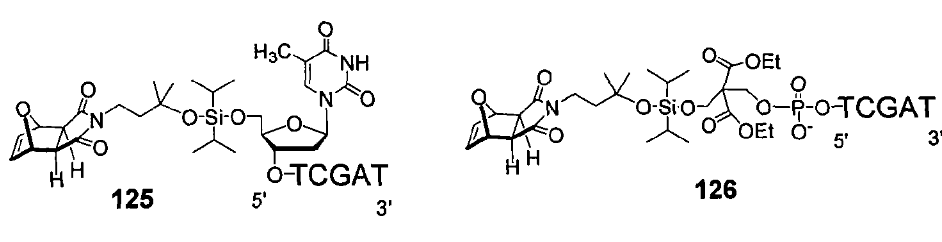

O-TCGAT
5'
3'
125
O-P-O-TCGAT
5'
3'
126

FIG. 29

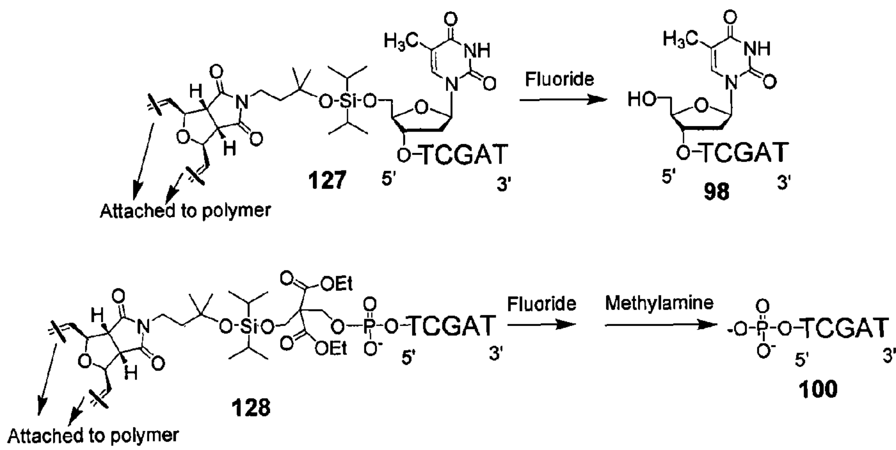

Fluoride
127
98
Attached to polymer
Methylamine
128
100

FIG. 30

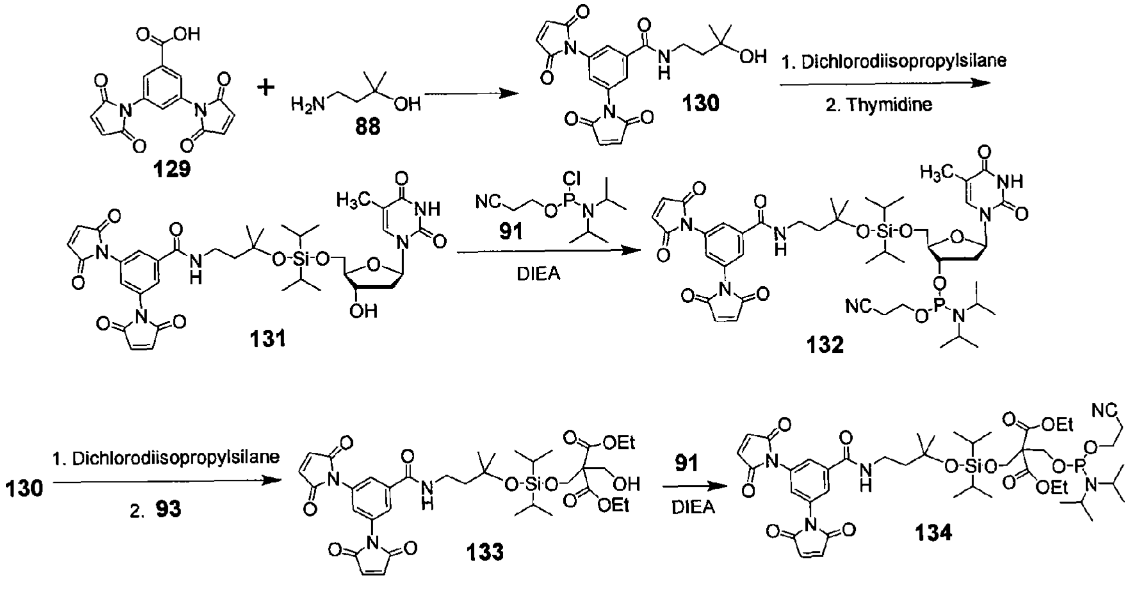
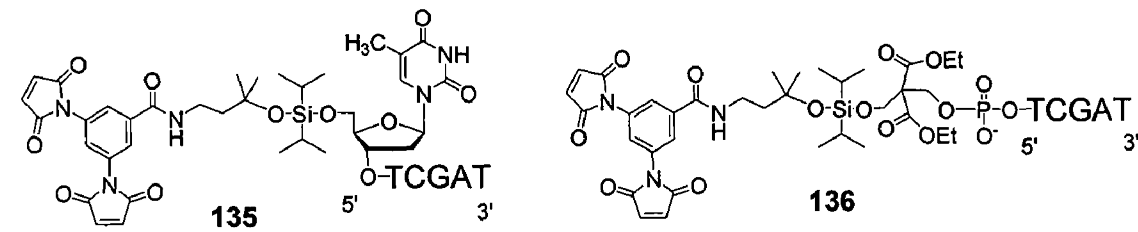
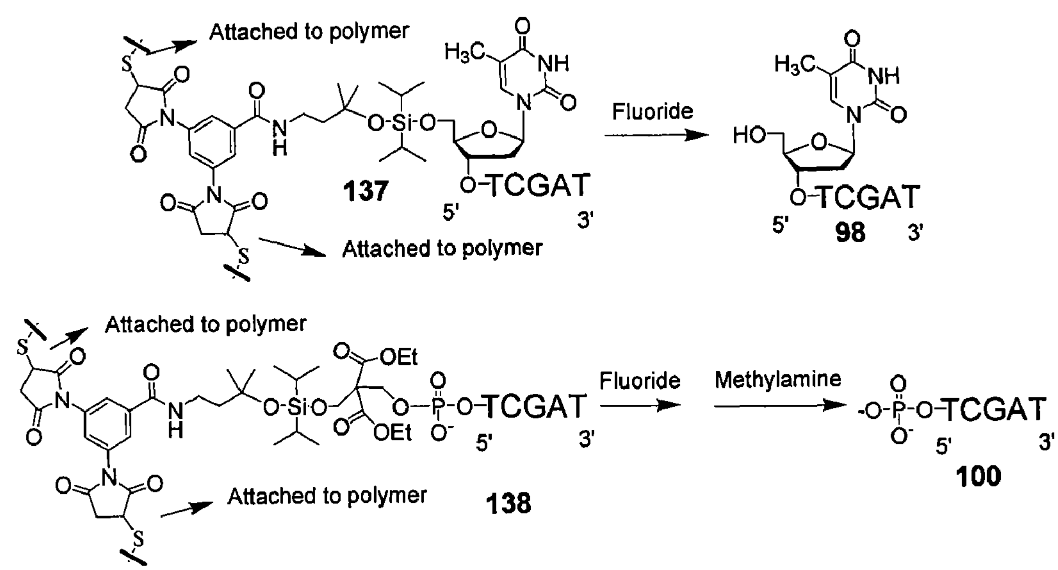

129
88
130
1. Dichlorodiisopropylsilane
2. Thymidine
131
91
DIEA
132
1. Dichlorodiisopropylsilane
2. 93
133
134

FIG. 31



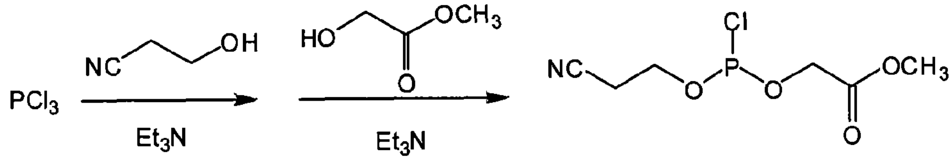
135
136

FIG. 32



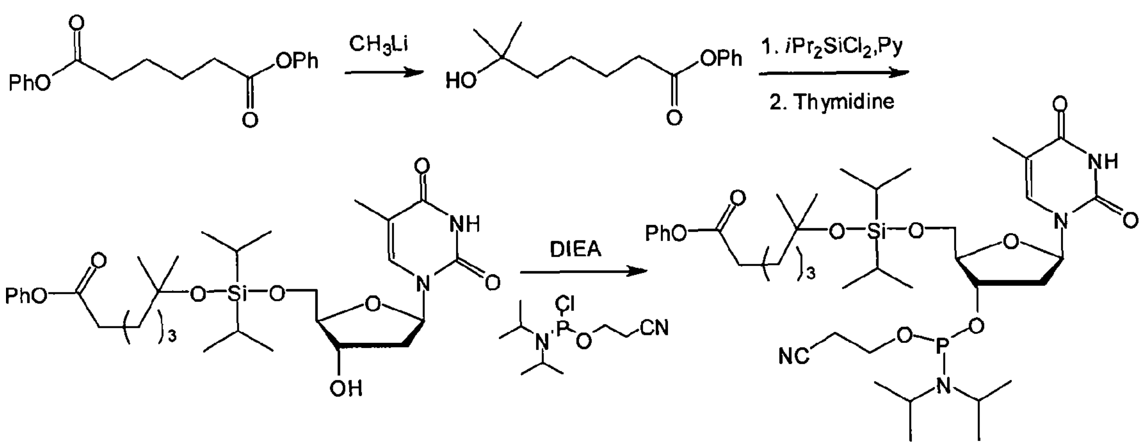
Attached to polymer
Fluoride
137
98
Attached to polymer
Attached to polymer
Fluoride
Methylamine
100
Attached to polymer
138

FIG. 33



141

FIG. 34



150

FIG. 35

PURIFICATION OF SYNTHETIC OLIGOMERS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 60/827,592 filed Sep. 29, 2006, which is hereby incorporated by reference.

INTRODUCTION

Synthetic oligonucleotides have wide applications in biology and medicine. With one oligonucleotide drug on the market and more than 40 others in various stages of clinical trials, the interest in using oligonucleotides as therapeutic agents continues to grow. This growing demand requires large quantities of oligonucleotides. For many purposes, including use as therapeutic agents to cure human diseases, these crude oligonucleotides must be purified to remove the failure sequences generated in the coupling steps in the synthesis. Currently used purification methods include gel electrophoresis, HPLC and others—all of which are expensive, labor intensive and unsuitable for large scale purification. The most frequently used purification methods such as gel electrophoresis are not suitable for large scale purification. Reverse phase and ion exchange HPLC have been adapted to large scale purification, but there are high costs associated with instrumentation, eluents (including their evaporation) and columns. Other known purification methods are also not ideal. Consequently, the development of highly efficient and low cost methods for large scale production of oligonucleotides is desired.

SUMMARY OF THE INVENTION

The present invention provides a method of purifying oligomers comprising capping any failure sequences produced during synthesis with a capping agent having a polymerizable functional group, polymerizing the capped failure sequences; and separating the polymerized material from the full-length oligomer.

The present invention also provides a method of purifying an oligomer comprising attaching a polymerizable functional group to an end of a full length oligomer, polymerizing the full length oligomers, removing the failure sequences from the polymerized full length oligomers, and recovering the full length oligomers.

In addition, the present invention provides capping agents comprising polymerizable functional groups and its for purifying oligomers.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates one method for oligonucleotide purification. Unwanted failure sequences contain polymerizable function, while the desired full length sequences do not. After polymerization, failure sequences are incorporated into a polymer, and full length sequences are isolated by simple extraction.

FIG. **2** illustrates an additional method for oligonucleotide purification. Desired full length sequences contain polymerizable function, while unwanted sequences do not. After polymerization, full length sequences are incorporated into a polymer, failure sequences are removed by washing, and full length sequences are cleaved from the polymer and extracted with a buffer.

FIG. **3** represents the chemical reaction for formation of polyacrylamide gel.

FIG. **4** represents the chemical reaction for synthesis of the capping agents **22-24**.

FIG. **5** illustrated the structures of full length sequence and failure sequences when using **22-24** as capping agents.

FIG. **6** represents the chemical reaction for formation of disulfide polymer.

FIG. **7** represents the chemical reaction for synthesis of capping agents **39** and **40**.

FIG. **8** represents the chemical reaction for synthesis of copolymerization agents **44** and **45**.

FIG. **9** represents the chemical reaction for synthesis of capping agents **50** and **51**.

FIG. **10** represents the chemical reaction for synthesis of copolymerization agents **55** and **56**.

FIG. **11** represents the Sharpless "click" reaction.

FIG. **12** represents the chemical reaction for synthesis of capping agents **57-60**.

FIG. **13** represents the chemical reaction for synthesis of copolymerization agents **65** and **66**.

FIG. **14** represents the water soluble Grubbs' ROMP catalysts.

FIG. **15** represents the Grubbs' ROMP reaction.

FIG. **16** represents the chemical reaction for synthesis of capping agents **74-77**, and the copolymerization agents **78** and **79**.

FIG. **17** represents the conjugate addition reaction between maleimide and thiol.

FIG. **18** represents the chemical reaction for synthesis of capping agents **82** and **83**, and the copolymerization agents **55** and **56**, and **84**, **85** and **86**.

FIG. **19** represents the chemical reaction for synthesis of phosphoramidites **92** and **95**.

FIG. **20** represents the full length oligonucleotides after deprotection and cleavage using phosphoramidites **92** and **95**.

FIG. **21** represents the chemical reaction of cleavage of DNAs **98** and **100** from polymer.

FIG. **22** represents the chemical reaction for synthesis of phosphoramidites **103** and **105**.

FIG. **23** represents the structures of the oligonucleotides after deprotection and cleavage, using **103** or **105** as the phosphoramidite in the last synthetic cycle in oligonucleotide synthesis.

FIG. **24** represents the chemical reaction for the cleavage of DNAs **98** and **100** from polymer.

FIG. **25** represents the chemical reaction for synthesis of phosphoramidites **113** and **115**.

FIG. **26** are the structures of the oligonucleotides after deprotection and cleavage, using **113** or **115** as phosphoramidite in the last synthetic cycle in oligonucleotide synthesis.

FIG. **27** represents the chemical reaction for cleavage of DNAs **98** and **100** from polymer.

FIG. **28** represents the chemical reaction for synthesis of phosphoramidites **122** and **124**.

FIG. **29** represents the structures of the oligonucleotides after deprotection and cleavage, using **122** or **124** as phosphoramidite in the last synthetic cycle in oligonucleotide synthesis.

FIG. **30** represents the chemical reaction for cleavage of DNAs **98** and **100** from polymer.

FIG. **31** represents the chemical reaction for synthesis of phosphoramidites **132** and **134**.

FIG. **32** represents the chemical structures for the oligonucleotides after deprotection and cleavage, using **132** or **134** as phosphoramidite in the last synthetic cycle in oligonucleotide synthesis.

FIG. **33** represents the chemical reaction for the cleavage of DNAs **98** and **100** from polymer.

FIG. **34** represents the chemical reaction for the synthesis of capping agent **141**.

FIG. **35** represents the chemical reaction for the synthesis of phosphoramidites **150**.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes a method of purifying synthetic oligomers via a polymerization technique, capping agents having a polymerizable functional group, and kits for purifying oligomers comprising a capping agent having polyerizable functional group. Synthesis of oligomers generally proceeds in a step-wise manner with each monomer being added in sequence to the ends of a plurality growing oligomer. After synthesis and deprotection/cleavage, crude oligomers normally contains the following impurities:

(i) Truncated failure sequences. These impurities result from the coupling steps of the synthesis. For a successful 20-mer synthesis, these impurities comprise about 30% of the oligonucleotide content of the crude mixture. They have similar physical properties as the desired full length sequences, and so are difficult to remove. They are usually capped with acetic anhydride in the synthesis. As a result, if the 5'-OH DMTr (4,4'-dimethoxytrityl) protecting groups in the last synthesis cycle are not removed, then after basic deprotection and cleavage, the full length sequences have the hydrophobic DMTr group on their 5'-end while the failure sequences do not (acyl groups on the failure sequences are removed under deprotection conditions). This is the basis of DMTr-on reverse phase HPLC purification. Although this is the most widely used oligonucleotide purification method, it is very costly for large scale production.

(ii) Small organic impurities. These result from the phosphate and exo-amino protecting groups which include acrylonitrile, benzamide, acetamide and isobutyramide and others depending on which protecting groups are used. Because of their very different physical properties from oligonucleotides, they can be removed by precipitation from aqueous buffer with ethanol or 2-propanol.

(iii) Other oligonucleotide impurities. These are very difficult to remove; fortunately, only very limited quantities are typically present. Two examples are N+1 and N+2 sequences, which result from double coupling due to the mild acidity of activating agents that causes premature detritylation in the coupling step. They can be troublesome to remove even on small scale. When the DMTr-on reverse phase HPLC strategy is used, they also contain a 5'-DMTr group. Ion exchange HPLC cannot resolve a single nucleotide difference for a typical 20-mer. Gel electrophoresis can do the job but can only on a very small scale. Additional impurities are acrylonitrile-oligonucleotide adducts. In addition, for oligophosphorothioate synthesis, because of incomplete sulfurization, impurities such as $(P{=}O)_1$ and $(P{=}O)_2$ mers exist, they can be kept to a minimum amount by using a more efficient sulfurization agent.

These impurities need to be separated from the desired full-length oligomer when synthesis is complete. The present invention contemplates the purification of oligomers through the use of capping agents containing polymerizable functional groups. Both failure sequences and small organic impurities can be removed using the method of the present invention.

As used herein, "oligomer" includes oligonucleotides, modified oligonucleotides, polynucleotides, modified polynucleotides, oligosaccharides, modified oligosaccharides, polysaccharides, modified polysaccarides, peptides, modified peptides, polypeptides, modified polypeptides, and any conjugates of two or more of these different types of oligomers such as peptide nucleic acid conjugates and glycopeptides. Modified oligomers include peptide nucleic acids, locked DNA, phosphotihioate oligonucleotides, beta-peptides and quaternary peptides. Modifications to oligosaccharides include alterations of ring size, ring atoms and various substitutions on the sugar rings. One of ordinary skill in the art can envision other modified oligomers which fall within the scope of the present invention. Oligomers may contain both natural and unnatural monomers. For example, both D- and L-amino acids can be used. The term "oligomer" is not intended to be limited to any specific number of monomers. Instead, it is meant to encompass an oligomer (or polymer) of any length that can be made by a step-wise process.

As used herein, "failure sequence" means an oligomer to which the next monomer did not attach during synthesis. Thus, a failure sequence of any given step in the synthesis contains all monomers except for the most recently added monomer.

As used herein, "lower alkyl" means an alkyl group of 1 to 4 carbon atoms, that may be branched, such as methyl, ethyl, propyl, isopropyl and butyl.

The oligomer purification methods of the present invention are suitable for both large and small scale purification. Oligomers purified by the methods of the present invention are substantially free of failure sequences and have similar or better quality than those purified by DMTr-on reverse phase HPLC method. The purified oligomer may be of greater than about 90% purity, or greater than about 95%, or greater than about 97%, or greater than about 99% or greater than about 99.5%. By "substantially free", it is meant that the oligomer contains less than about 5% by weight of failure sequences, or less than about 3%, or less than about 1% or less than about 0.5%.

In one embodiment of the present invention, simple phosphorous compounds that contain functional groups capable of polymerizing in the presence of an initiator and/or a polymerization partner are used as capping agents (in place of the commonly used acetic anhydride) during oligomer synthesis to block failure sequences. Thus, all unwanted failure sequences contain the polymerizable functions, while the desired full length sequences do not. After synthesis, failure sequences are incorporated into a polymerized material, and the full length oligomers remain in solution or in the polymer matrix and are separated from the polymerized material using any technique known to one of ordinary skill in the art including extraction with buffer and filtration. If the oligomer is synthesized on a solid support, the oligomer can be cleaved from the solid support prior to polymerization. (See FIG. **1**).

In another embodiment of the present invention, polymerizable functional groups are incorporated onto the end of the full length sequences in the last step of oligomer synthesis. Because failure sequences are capped with a standard capping agent, such as acetic anhydride, dimethyl-formanide, diethylene glycol monoethyl ether phosphoramidite, or bis(1,1,1,3,3,3-hexafluoro-2-propyl)-2-propyl phosphate, in each synthetic cycle, only the full length sequence contains the polymerizable functional group. Once the full length sequences are incorporated into a polymerized material, the failure sequences are removed by simple washing because they do not contain a polymerizable functional group, and then, full length sequences are recovered from the polymerized material using a cleavage reagent. (See FIG. **2**). If the synthesis occurs on a solid support, the oligomer can be cleaved from the solid support before or after polymerization.

Using either of these methods, the small molecules resulted from deprotection of nucleobases and phosphate groups can also be removed if suitable polymerizable functions are incorporated into the protecting groups. When the first method is used, the small molecules resulted from deprotection will be incorporated into the polymerized material in the same fashion as the failure sequences are incorporated. When the second method is used, the small molecules resulted from deprotection will also be incorporated into the polymerized material. However, the full length sequence can be cleaved from the polymerized material and the small molecule impurities cannot because there is no cleavable linker in them.

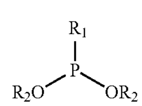
Polymerization reactions suitable for the methods of the present invention have one or more of the following characteristics: (i) the polymerization reaction is highly efficient once initiated; (ii) the reaction tolerates moisture and air and, suitably, can be performed in aqueous buffer; (iii) oligomers are stable under the polymerization conditions; and (iv) the polymerizable functional groups are easily accessible and are stable under oligomer synthesis conditions.

Reactions that are suitable for oligonucleotide purification include, but are not limited to, the radical acrylamide polymerization reaction, the thiol oxidation to disulfide reaction, the Sharpless "click" reaction, the Grubbs aqueous ROMP reaction, the conjugate addition reaction between maleimide and thiol, and the amide bond formation reaction between carboxylic acid esters and alkyl amines. Reactions that are suitable for oligosaccharide and peptide purification include, but are not limited to, the Sharpless "click" reaction.

Radical Acrylamide Polymerization

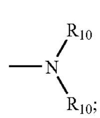
The general acrylamide polymerization reaction is shown in FIG. **3**. The materials for this reaction are inexpensive, and the reaction is highly efficient and can be performed in aqueous buffer open to air at room temperature. In addition, acrylamide functionalities are stable under oligonucleotide synthesis and deprotection/cleavage conditions using phosphoramidite chemistry. Appropriate polymerization conditions can be readily determined by one of ordinary skill in the art. Suitable conditions include $(NH_4)_2S_2O_8$/TMEDA/water at room temperature for about 1 hour. For example, for a 1 mmol oligonucleotide synthesis, optionally about 10 mmol to about 100 mmol acrylamide, optionally about 0.2 mmol to about 2.0 mmol N,N'-methylene-bisacrylamide, about 1 μmol to about 10 tμmol $(NH_4)_2S_2O_8$, and about 1 μmol to about 10 μmol TMDEA may be used.

Suitable capping agents for purification using radical acrylamide polymerization include compounds of formula (I):

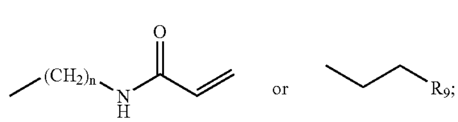


(I)

wherein R_1 is halogen, such as Cl, Br, or F or a secondary amine group, such as



R_2 is independently selected from lower alkyl,

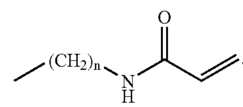


n is an integer from 1 to 5;

R_9 is an electron withdrawing group such as cyano, $COOR_{10}$, SO_2Ph and NO_2;

R_{10} is lower alkyl; and

at least one R_2 is



Suitably, the capping agent contains more than one polymerizable functional group. It is hypothesized that additional polymerizable functional groups on the capping agent increase the likelihood that capped polymers will be incorporated into the polymer.

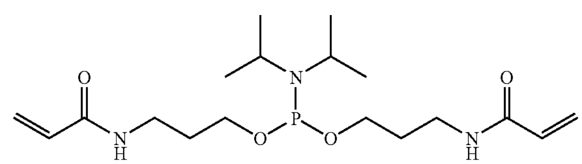
However, capping agents containing only one polymerizable functional group have the advantage of more stable under basic deprotection/cleavage conditions when the other R_2 is a removable group such as 2-cyanoethyl group.

When R_1 is a secondary amino group, the capping agents for radical acrylamide polymerization require the use of an activating agent. The most commonly used activating agent is 1H-tetrazole. Other suitable activating agents include, but are not limited to 4,5-dicyanoimidazole, 5-(4-nitrophenyl)-1H-tetrazole, 5-methylthio-1H-tetrazole, 5-ethylthio-1H-tetrazole, ethylthiotetrazole, and 5-benzylmereapto-1H-tetrazole.

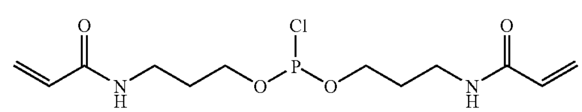
Optionally, when R_1 is a halogen atom, a base may be added to neutralize acid produced during the capping reaction. The base may be an amine base such as trimethylamine, pyridine, diazobicyclo base, or 5-methoxybenzimidazole.

Syntheses of suitable phosphorous capping agents **22-24** (shown below) are shown in FIG. **4**.

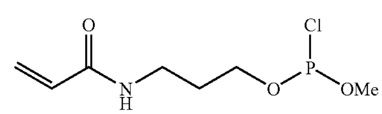
22



23



24



Compounds **22** and **23** contain two polymerizable functional groups, which can increase the likelihood that failure sequences will be incorporated into the polymer. Compound **24** contains only one polymerizable functional group. When compound **22** is used for capping, an activator is required. If **23** or **24** is used, no activating agent is required, but an amine base, such as trimethylamine, pyridine, diazobicyclo base, or 5-methoxybenzimidazole, must be used to neutralize the acid generated.

Because there is less concern about premature detritylation in capping steps than in coupling steps, other capping agents such as ammonium salts developed by Beaucage, Caruthers and Wada and co-workers can be used.

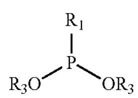
For purification of oligomers using polyacrylamide formation reaction, an alternative procedure is to use a fluoride cleavable linker to attach the growing oligomers to the solid support (instead of using the more common base cleavable linkers; besides fluoride cleavable linkers, other non-base cleavable linker such as photo cleavable linkers can also be used) for oligomer synthesis. After synthesis, the oligomers are cleaved from the solid support by treating with fluoride. The crude un-deprotected oligomers are subjected to polymerization reaction conditions. The failure sequences are incorporated into a polymerized material and the full length sequences remain in solution or in polymer matrix. After removal of failure sequences by filtration and extraction of the full length sequences from polymer matrix, the full length sequences are subjected to base de-protection conditions and further purified by recrystallization to remove small molecules resulted from de-deprotection. This alternative purification procedure avoids the possibility of adding nucleophiles to acrylamide functionalities in the base de-protection steps.

Formation of Disulfide Polymer

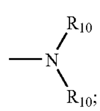
Thiols may be oxidized to form polymers. One of ordinary skill in the art can determine polymerization conditions that are suitable for such polymerization. For example, for a 1 mmol oligonucleotide synthesis, about 10 mmol to about 100 mmol of a copolymerization agent and about 0.2 mmol to about 2 mmol of a cross-linking copolymerization agent that contains more than 2 thiols in one molecule such as **45** and **56** can be used. Suitably, the mixture is heated in dimethylsulfoxide, for example, to about 90° C. If dimethylsulfoxide is used as a solvent, no additional oxidizing agent is necessary. If another solvent is used, then an oxidizing agent, such as oxygen or others known to those skilled in the art should be added.

Suitable capping agents for purification using formation of a disulfide polymer include compounds of formula (I):

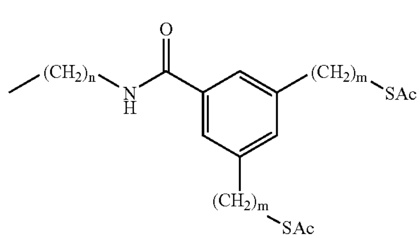
(II)



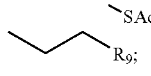
wherein R_1 is halogen, such as Cl, Br or F or a secondary amine, such as



R_3 is independently selected from lower alkyl or



or



n is an integer from 1 to 5;

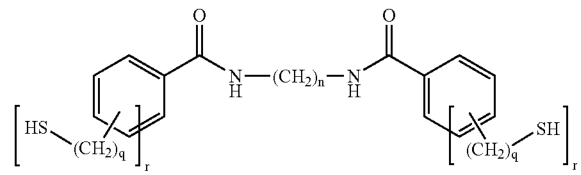
m is an integer from 0 to 4;

R_9 is an electron withdrawing group such as cyano, $COOR_{10}$, SO_2Ph and NO_2; and

R_{10} is lower alkyl.

Suitable copolymerization agents include compounds of formula (III):

(III)



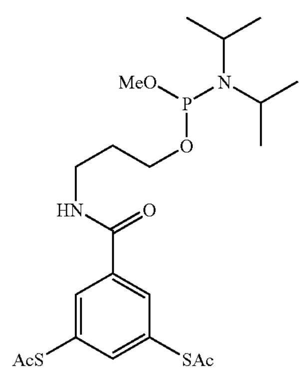
wherein n is an integer from 1 to 5;

q is independently an integer from 0 to 3; and

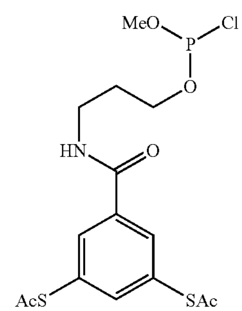
r is independently an integer from 1 to 2. Suitably, if r is 1 then the thiol group is at the para-position; if r is 2 then the thiol groups are at the meta-positions.

Thiols **39**, **40**, **50** and **51** (shown below) are exemplary capping agents for this reaction.

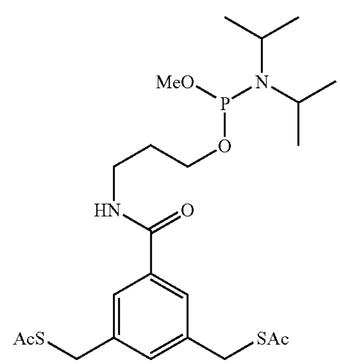
39



40



50



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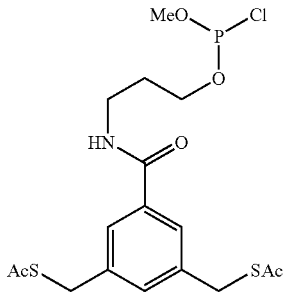
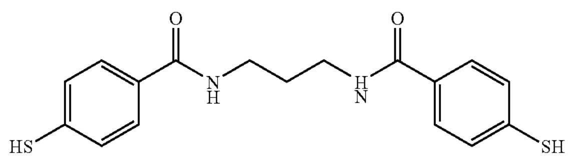
51

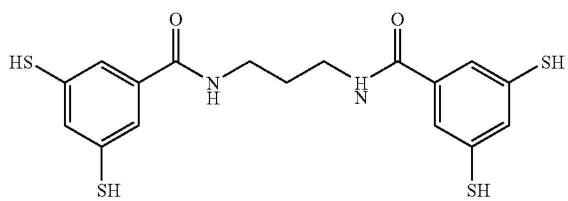


Thiols **44**, **45**, **55** and **56** (shown below) are exemplary copolymerization agents for this reaction.

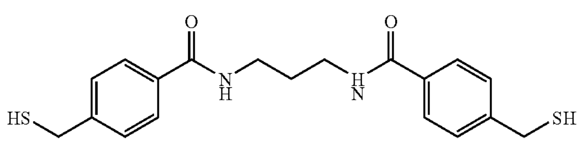
44



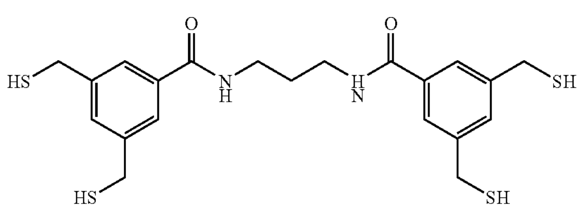
45



55



56

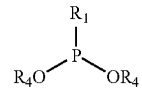


The Sharpless "Click" Reaction

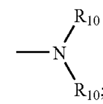
The Sharpless "click" reaction is a highly reliable reaction (FIG. **11**) and can be performed under aqueous mild conditions. The reaction partners—the terminal alkyne and the alkylazide—are readily accessible stable functional groups. The mild reaction conditions are compatible with many functional groups including those in oligonucleotides. One of ordinary skill in the art can readily determine suitable reaction conditions. For example, for a 1 mmol oligonucleotide synthesis, one can use about 10 mmol to about 100 mmol of each of the copolymerization agents, about 0.05 mmol to about 5.0 mmol $CuSO_4$ and about 0.05 mmol to about 5.0 mmol sodium ascorbate.

Suitable capping agents for purification using the Sharpless "click" reaction include compounds of formula (IV)

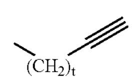
(IV)



wherein R_1 is a halogen, such as Cl, Br or F or a secondary amine, such as



R_4 is

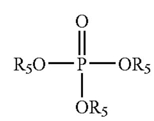


t is an integer from 1 to 3

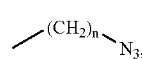
R_{10} is lower alkyl.

Copolymerization agents are suitably of formula (V):

(V)



wherein R_5 is

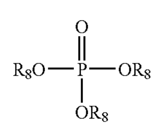


and

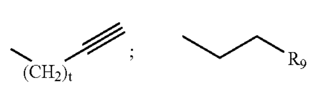
n is independently an integer from 1 to 5.

A second copolymerization agent is suitably of formula (VI):

(VI)



wherein R_8 is



or

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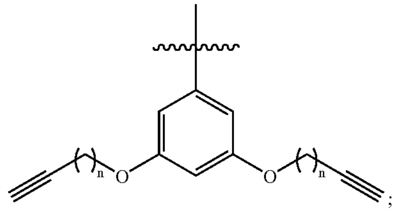


n is independently an integer from 1 to 5,

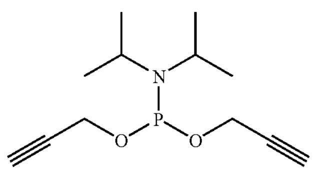
t is an integer from 1 to 3;

R_9 is an electron withdrawing group such as cyano, $COOR_{10}$, SO_2Ph and NO_2;

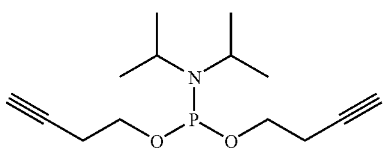
R_{10} is lower alkyl

Alkynes **57-60** (shown below) are exemplary capping agents for this reaction.

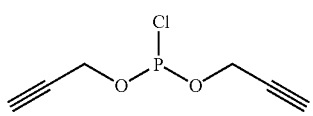
57



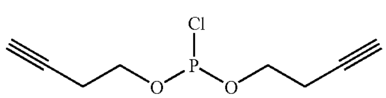
58



59

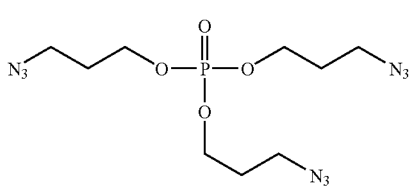


60



Compounds **65** and **66** are exemplary copolymerization agents.

65



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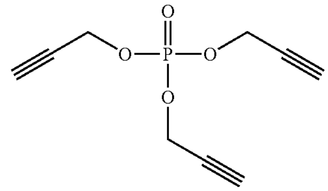
66



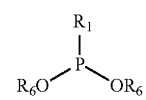
Grubbs' ROMP Reaction

The ring opening metathesis polymerization (ROMP) reaction is also useful for oligonucleotide purification. The polymerization reaction can be performed in aqueous solution using water soluble catalysts (**67-69**, FIG. **14**), and the reaction tolerates a wide range of functional groups. One of ordinary skill in the art can readily determine suitable polymerization conditions. For example, for a 1 mmol oligonucleotide synthesis, one can use about 10 mmol to about 100 mmol of a copolymerization agent, about 0.02 mmol to about 2.0 mmol cross-linking copolymerization agent that contains more than one alkene function such as **78** and **79**, and about 0.01 mmol to about 1.0 mmol metathesis catalyst.

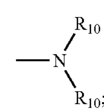
The substrates for polymerization can be those such as **70** and **71** as shown in FIG. **15**. Suitable catalysts for the ROMP purification include, but are not limited to, catalyst **69**. After polymerization, the catalyst may be removed by extraction with organic solvents such as CH_2Cl_2.

The capping agent is suitably a compound of formula (VII):

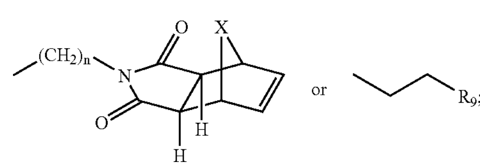
(VII)



wherein R_1 is halogen, such as Cl, Br or F or a secondary amine, such as



R_6 is



X is independently O or CH_2;

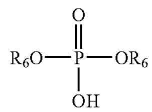
R_9 is an electron withdrawing group such as cyano, $COOR_{10}$, SO_2Ph and NO_2;

R_{10} is lower alkyl; and

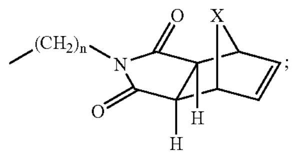
n is independently an integer from 1 to 5.

A suitable copolymerization agent is a compound of formula (VII)

(VIII)



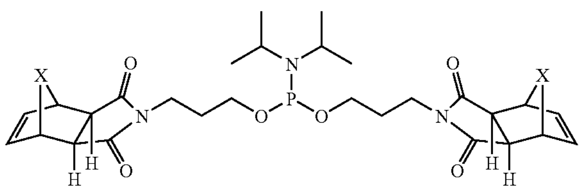
wherein R_6 is



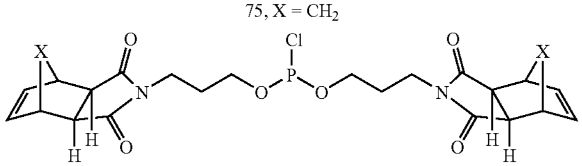
X is independently O or CH_2; and

n is independently an integer from 1 to 5.

Compounds **74-77** are exemplary capping agents.

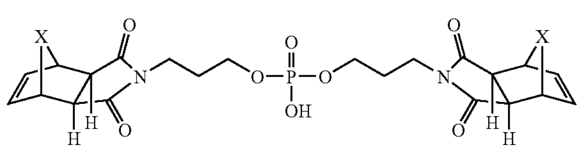


74, X = O
75, X = CH_2

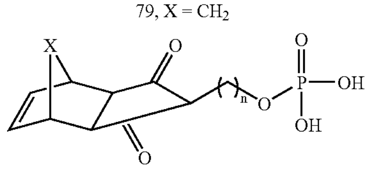


76, X = O
77, X = CH_2

Compounds **78**, **79**, **139**, and **140** are exemplary copolymerization agents.



78, X = O
79, X = CH_2

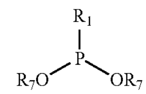


139, X = O, n = 1-5
140, X = CH_2, n = 1-5

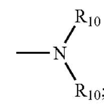
Conjugate Addition Reaction Between Maleimide and Thiol

The conjugate addition reaction between maleimide and thiol (FIG. **25**) is widely used in bioconjugate chemistry. This reaction can be performed in different aqueous buffers in a relatively wide range of pH values (6.5-9) at room temperature, and the reaction is known to be compatible with oligonucleotides. One of ordinary skill in the art can readily determine suitable reaction conditions. For example, for a 1 mmol oligonucleotide synthesis, one can use about 10 mmol to about 100 mmol of a bis-thiol copolymerization agent, about 10 mmol to about 100 mmol of a bis-maleimide copolymerization agent and about 0.2 mmol to about 2.0 mmol of a cross-linking thiol that contains more than 2 thiols in one molecule such as **56** and **86** in a suitable buffer such as phosphate buffered saline buffers and at a suitable temperature such as room temperature and 60° C. Suitable capping reagents are compounds of formula (IX)

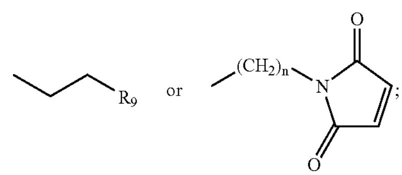
(IX)



wherein R_1 is halogen, such as Cl, Br or F or a secondary amine, such as



R_7 is



R_9 is an electron withdrawing group such as cyano, $COOR_{10}$, SO_2Ph and NO_2;

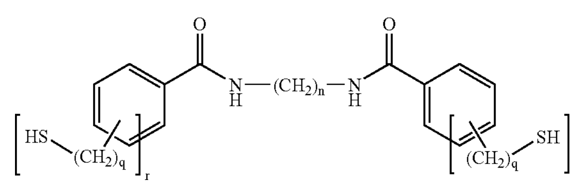
R_{10} is lower alkyl; and

n is independently an integer from 1 to 5.

One of the R_7 may also be any functionality that contains 2 or more α,β-unsaturated carbonyl functions

Suitable copolymerization agents include compounds of formula (III):

(III)



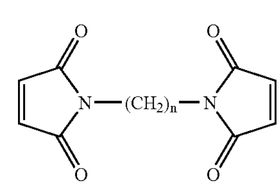
wherein n is an integer from 1 to 5;

q is independently an integer from 0 to 3; and

r is independently an integer from 1 to 2. Suitably, if r is 1 then the thiol group is at the para-position; if r is 2 then the thiol groups are at the meta-positions.

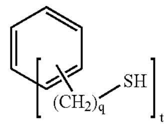
or a compound of formula (X):

(X)



wherein n is an integer from 1 to 5 or a compound of formula (XI):

(XI)

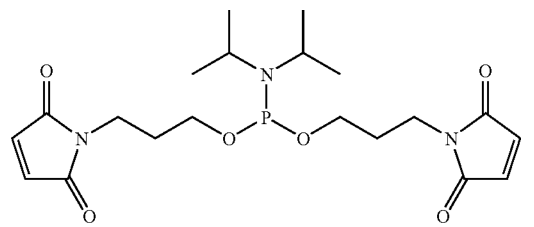


wherein q is an integer from 0 to 3; and

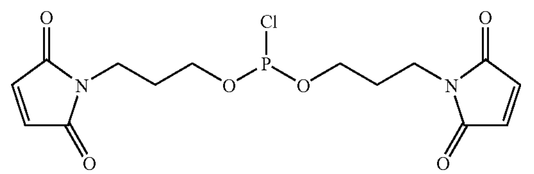
t is independently an integer from 1 to 3. Suitably, if t is 2 then the thiol groups are para to each other; if t is 3 then the thiol groups are in the meta-positions.

Compounds **82** and **83** are exemplary capping agents.

82

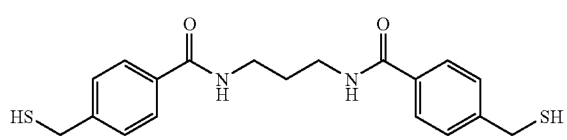


83

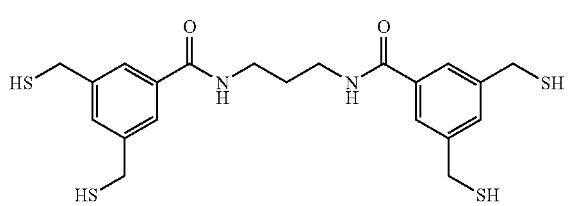


Compounds **55**, **56**, **84**, **85** and **86** are exemplary copolymerization agents.

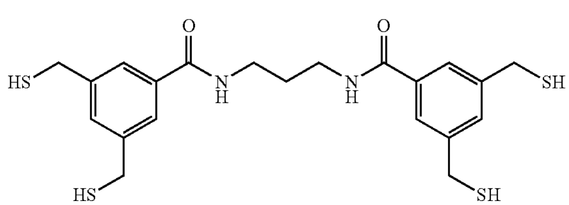
55



56



56



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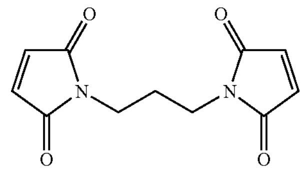
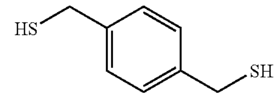
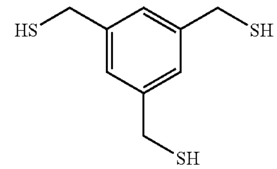
84



85

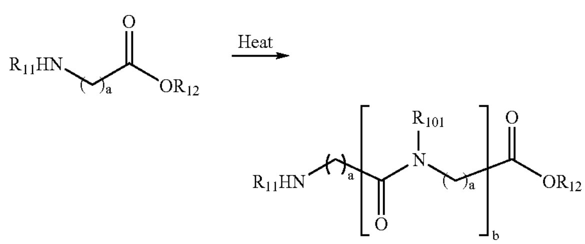


86



Amide Formation Between an Ester and an Amine

Amines can react with carboxylic acid derivatives to form amides. This reaction can also be used as the polymerization reaction for purification of oligomers that are synthesized step-wise. One possible such polymerization reaction is shown here:



wherein R_{12} is a suitable leaving group, such as CH_3, CH_2CH_3, $CH_2CH_2OCH_3$, CH_2CH_2OPh, or OPh;

R_{11} is selected from hydrogen or lower alkyl$_3$;

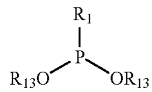
a is an integer from 1 to 6; and

b is an undetermined integer.

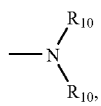
When using such polymerization reactions to purify oligomers, a suitable condition for a 1 mmol oligomer synthesis, is: about 10 mmol to about 100 mmol of a copolymerization agent and about 0.2 mmol to about 2 mmol of a cross-linking copolymerization agent. The oligonucleotides do not need to be deprotected/cleaved prior to polymerization. The oligonucleotides on solid support can be treated directly under these polymerization conditions. The oligonucleotides will be deprotected/cleaved by the amino group in the copolymerization monomer. The advantages of this method include incorporation of both failure sequences and small molecules resulted from deprotection such as acetyl amide into the polymer and no need for separate deprotection and cleavage step.

Suitable capping agents for purification using the amide bond formation reaction for polymerization includes compounds of formula (XII):

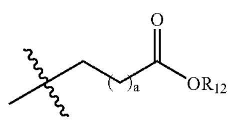
(XII)



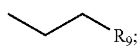
wherein R_1 is halogen, such as Cl, Br or F or a secondary amine such as



R_{13} may be



or



wherein R_9 is an electron withdrawing group such as cyano, $COOR_{10}$, SO_2Ph and NO_2;

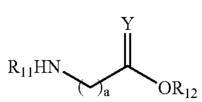
R_{10} is lower alkyl;

R_{12} is a suitable leaving group, such as CH_3, CH_2CH_3, $CH_2CH_2OCH_3$, CH_2CH_2OPh, or OPh; and

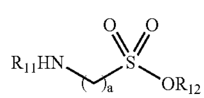
a is an integer from 1 to 6.

One of the R_{12} groups may also be any functionality that contains 2 or more ester groups.

Suitable copolymerization reagents include but are not limited to



or



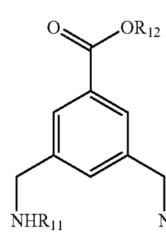
wherein R_{12} is a suitable leaving group, such as CH_3, CH_2CH_3, $CH_2CH_2OCH_3$, CH_2CH_2OPh, or OPh;

R_{11} is selected from the group consisting of hydrogen or lower alkyl;

Y═O or S; and

a is an integer from 1 to 6.

Suitable cross-linking copolymerization agents include but are not limited to:

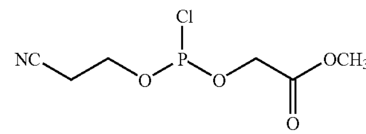


wherein R_{12} is a suitable leaving group, such as CH_3, CH_2CH_3, $CH_2CH_2OCH_3$, CH_2CH_2OPH, or OPh;

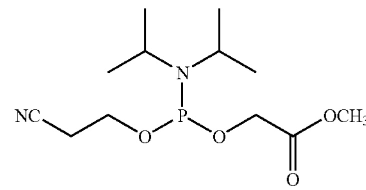
R_{11} is selected from the group consisting of hydrogen and lower alkyl;

Compounds **141**, **142**, **143**, and **144** are exemplary capping agents for this reaction:

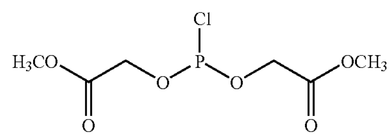
141



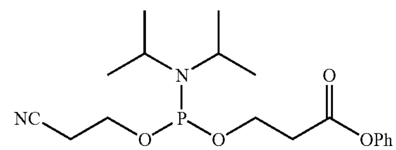
142



143

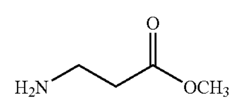


144

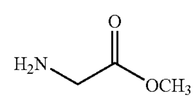


Compounds **145**, **146**, **147**, **148** are exemplary copolymerization agents for this reaction:

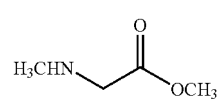
145



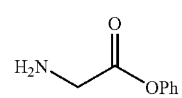
146



147

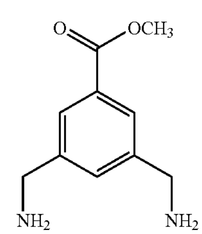


148



Compound **149** is an exemplary cross-linking copolymerization agent for this reaction:

149

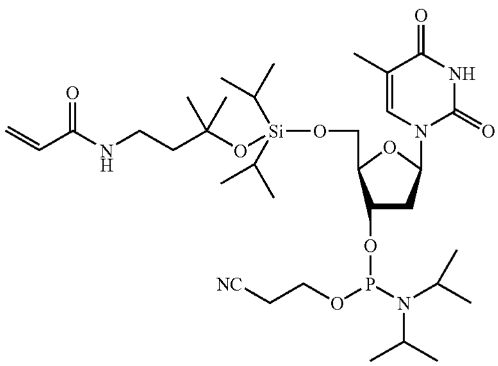


Polymerization of Full Length Oligomer

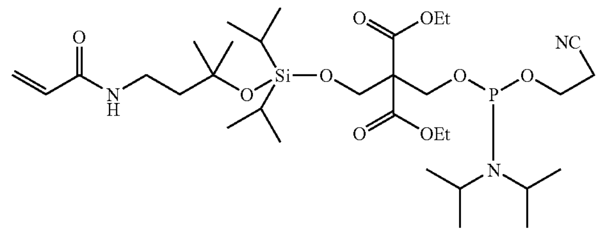
In another embodiment of the present invention, the polymerization reactions can be used to polymerize the desired full-length oligomer sequence. In this embodiment, the capping agent is the normal acetic anhydride or any other suitable capping agent, but at the end of solid phase synthesis, a phosphoramidite that contains a suitable polymerizable functional group is coupled to the end of the oligomer through a cleavable linker. Because failure sequences are all capped with acetic anhydride in each synthetic cycle, only the full length sequence contains the polymerizable functional group. After synthesis, deprotection and cleavage, the crude oligomer is subjected to polymerization; the full length sequence is incorporated into the polymerized material while failure sequences and other impurities remain in solution, which can be removed by filtration or extraction with a buffer. The pure full length sequences are then cleaved from the polymerized material and extracted with a buffer.

Suitable phosphoramidites include compounds **92**, **95**, **103**, **105**, **113**, **115**, **122**, **124**, **132**, **134**, **150**, **151**:

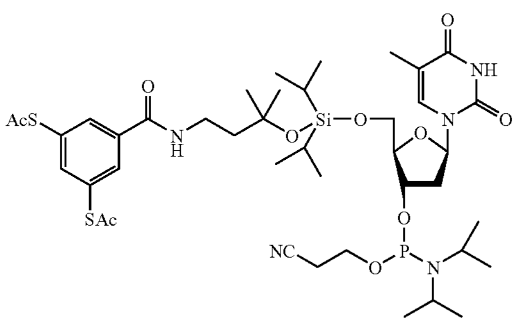
92



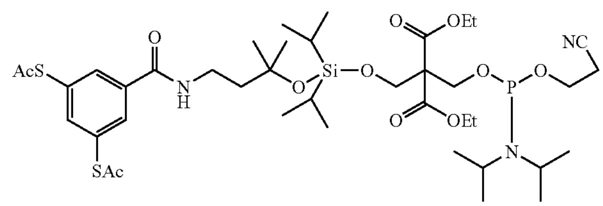
95



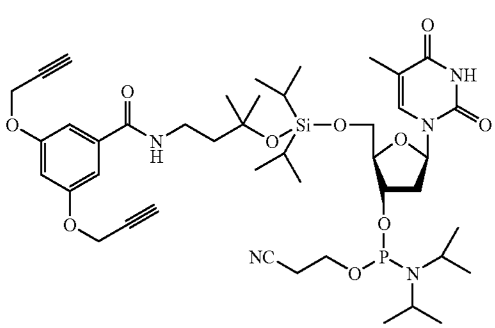
103



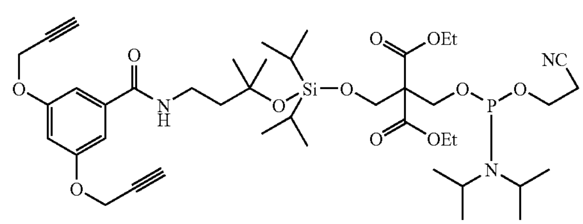
105



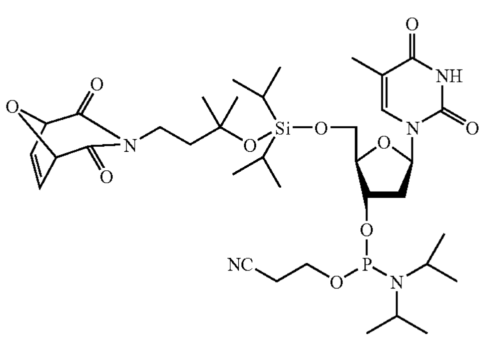
113



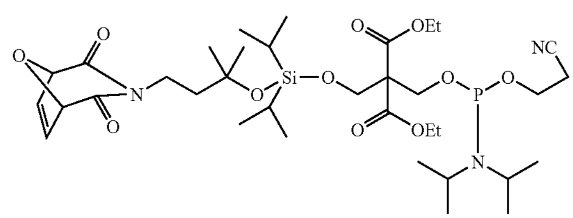
115



122



124



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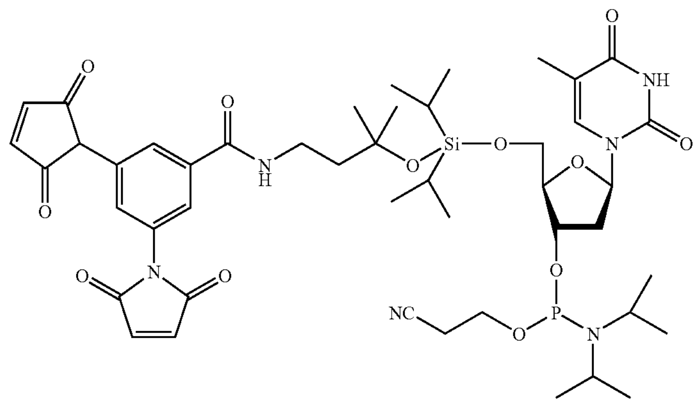
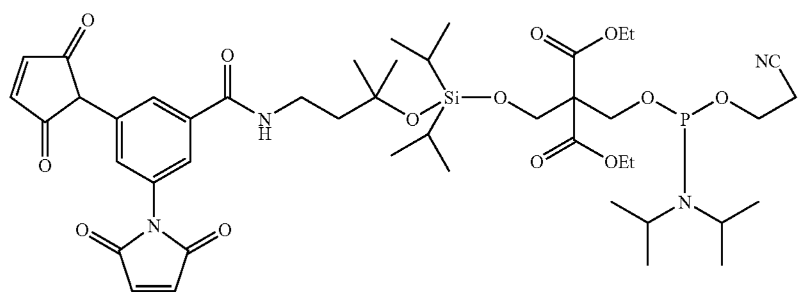
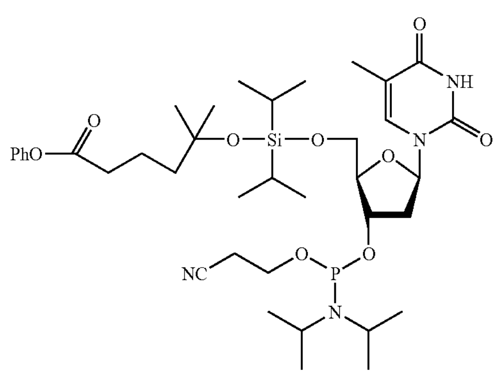
132



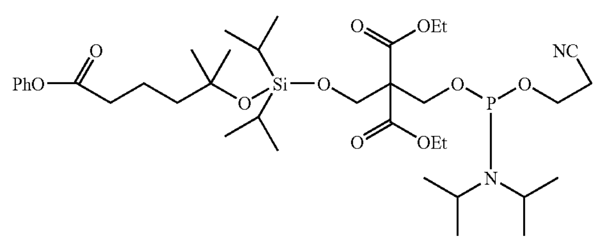
134



150



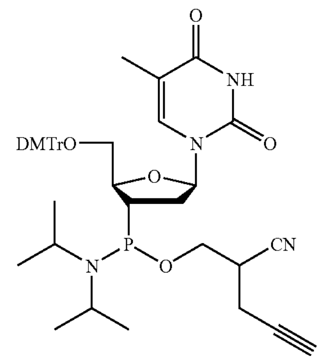
151



Polymerization of Small Molecule Impurities

In yet another embodiment of the present invention, small molecule impurities can be incorporated into the polymerized material and removed along with the failure sequences. In this embodiment, the protecting groups contain a polymerizable functional group, and are also incorporated into the polymerized material. Here, the Sharpless "click" reaction is used as example.

The oligomer is synthesized under standard conditions. However the protecting groups for the nucleobases and the phosphate groups contain a polymerizable functional group. The following phosphoramidite monomers are exemplary:



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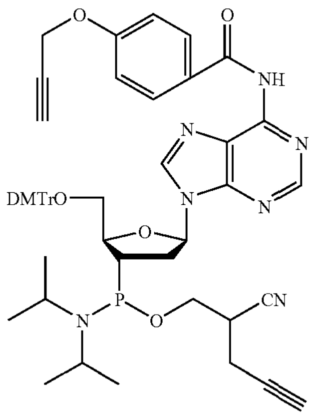
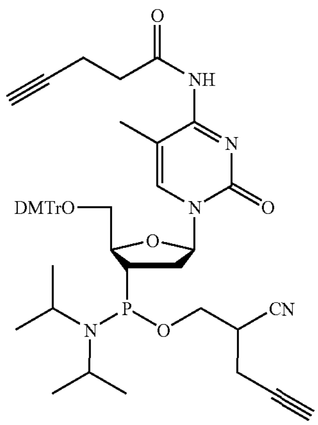
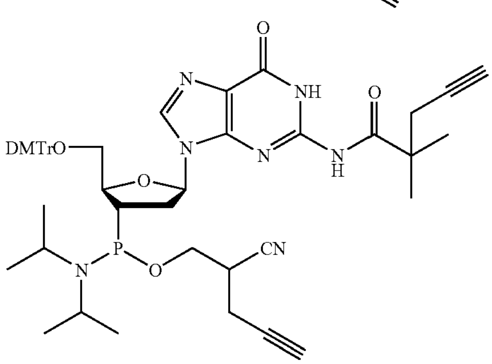
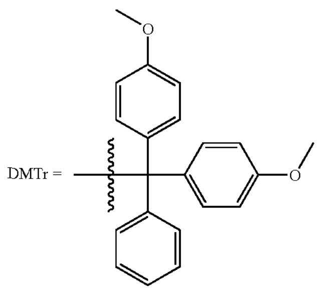
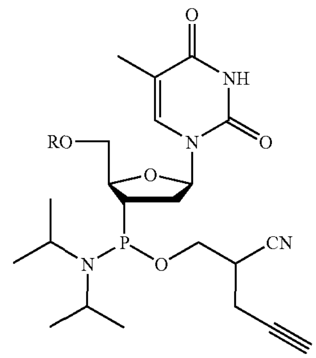


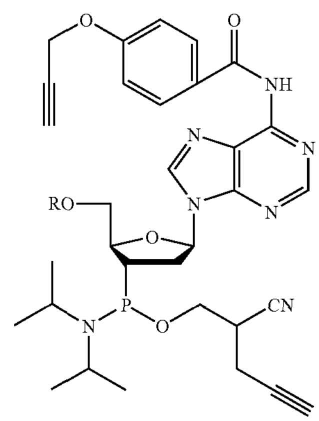


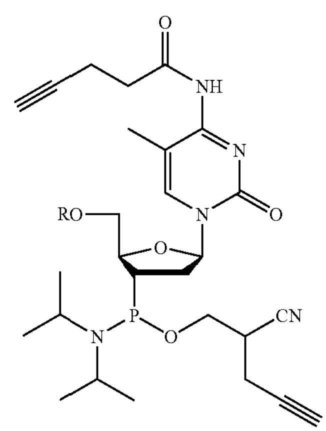


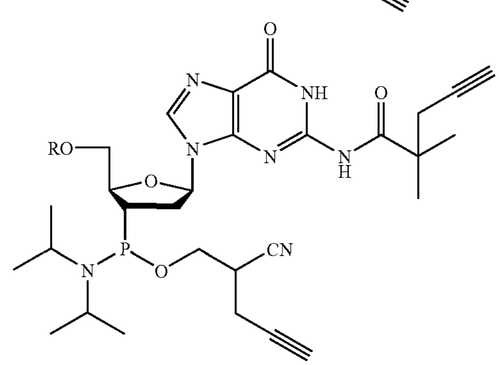


If failure sequences are capped with agents such as **57-60**, after cleavage/deprotection, the failure sequence and the small molecules resulted from the protecting groups will all be incorporated into the polymerized material. The full length sequence can be obtained by filtration and extraction. Alternatively, the failure sequences can be capped with a traditional capping agent, such as acetic anhydride and one of the phosphoramidites shown below can be used in the last synthetic cycle.









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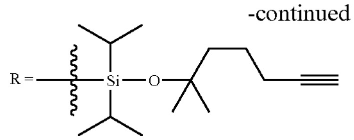


After synthesis and cleavage/deprotection, the full length sequence and the small molecule impurities resulting from protecting groups are incorporated into the polymerized material upon initiation of the polymerization reaction. The failure sequences can be removed by washing. The full length sequences (not small molecules resulted from protecting groups) are then cleaved from the polymer and extracted with buffer.

Kits for Purifying Oligomers

A further embodiment of the present invention is a kit comprising either a capping agent having a polymerizable functional group or a compound having a polymerizable functional group for attaching to the end of the full length oligomer. Kits can further comprise monomers, coupling reagents, polymerization reagents, buffers, cleavage agents, and other components necessary to synthesize and purify an oligomer in accordance with the present invention.

As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise. All publications, patents and patent applications referenced in this specification are indicative of the level of ordinary skill in the art to which this invention pertains. All publications, patents and patent applications are herein expressly incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated by reference. In case of conflict between the present disclosure and the incorporated patents, publications and references, the present disclosure should control.

It also is specifically understood that any numerical range recited herein includes all values from the lower value to the upper value, i.e., all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification.

The following examples are provided to assist in a further understanding of the invention. The particular materials, methods and conditions employed are intended to be illustrative of the invention and are not limiting upon the scope of the invention.

EXAMPLES

Example 1

Purification Via Radical Acrylamide Polymerization of Failure Sequences

After synthesis, the oligonucleotide **25** is cleaved and deprotected under standard basic conditions (for example, K_2CO_3, anhydrous methanol, room temperature, 24 hours and concentrated ammonium hydroxide, 60° C.). The crude oligonucleotides are subjected to polymerization conditions, e.g. $(NH_4)_2S_2O_8$/TMEDA/water/r.t./1 h, to incorporate the failure sequences into polymer. The full length sequence **25** (FIG. **5**) remains in solution or in the polymer matrix because it does not contain an acrylamide function. Collection of the full length sequence can be achieved by filtration and/or extraction from the polymer gel using a buffer. Impurities resulting from the protecting groups in the synthesis such as acrylonitrile benzamide, acetamide and isobutyramide can be removed by first dissolving the material in sodium acetate buffer and then precipitating the oligonucleotide with 2-propanol (for less than 15-mer) or ethanol (for more than 15-mer) at −10° C. The small organic impurities remain in solution. Alternatively, protecting groups containing acrylamide functions can be used so that all small organic impurities can be removed by polymerization.

Example 2

Synthesis of Thiol Capping Agents and Purification Using Disulfide Formation of Failure Sequences

As shown in FIG. **7**, **34** is converted to **36** using **35**, and acetylated to give **37**. Then **37** is converted to **39** or **40** using similar procedures for preparing **22-24**. Alternatively, **37** can be synthesized from **41** in one pot as shown in FIG. **7**. The thiols **44** and **45**, which are required for copolymerization, can also be readily synthesized (FIG. **8**).

After synthesis and deprotection/cleavage, the crude oligonucleotide is subjected to polymerization conditions in the presence of **44** and **45** mentioned above. Full length oligonucleotides are collected by filtration and/or extraction. Small organic impurities can be removed by precipitation.

Alternatively, thiols **50** and **51** can be used for capping failure sequences, and **55** and **56** can be used as copolymerization agents. Their syntheses are illustrated in FIGS. **9** and **10**. Compound **48** is prepared from **46** and NBS followed by reacting with AcS^- under basic conditions (FIG. **9**), which is then converted to the capping agents **50** and **51** using the above described conditions. The copolymerization agents **55** and **56** are readily available as shown in FIG. **10** from compounds **52** and **48**. The oligonucleotide synthesis, capping failure sequences with **39**, **40**, **50** or **51**, deprotection/cleavage, polymerization in the presence with **44** and **45** or **55** and **56**, purification and analysis of purification results are the same as previously described.

Example 3

Synthesis of Capping Agents and Purification via Sharpless "Click" Polymerization of Failure Sequences

The capping agents that contain terminal alkynes (**57-60**) can be easily synthesized according to FIG. **12** from **61** and **62**. The polymerization partner **65** is prepared by reacting **63** with 3-bromo-1-propanol to give **64**, followed by treating with sodium azide (FIG. **13**). The other copolymerization partner **66** is prepared similarly from propargyl alcohol **61** and **63** (FIG. **13**). The oligonucleotide synthesis, capping failure sequences with **57-60**, deprotection/cleavage, polymerization in the presence of **65** and **66**, purification and analysis of purification results are the same as above.

Example 4

Synthesis of Capping Agents for Purification Via Grubb's Ring Opening Metathasis Polymerization of Failure Sequences

The synthesis of the capping agents **74-77** is shown in FIG. **16**. Compounds **72** and **73** can be synthesized from **70** (this compound can be prepared readily by stirring the solution of maleic anhydride and furan in ether at room temperature for 12 hours; the exo isomer is formed exclusively as a white precipitate) and **71** (both are commercially available and can be prepared using known procedures) by heating with 3-amino-1-propanol under vacuum in high yields. These two compounds are then converted to the capping agents **74-77** under conditions described above. Suitably, the exo isomers of these compounds are used. The water soluble copolymerization monomers **78** and **79** can be synthesized similarly (FIG. **16**). The oligonucleotide synthesis, capping failure sequences with **74-77**, deprotection/cleavage, polymerization in the presence of **78** or **79**, purification and analysis of purification results are the same as described.

Example 5

Synthesis of Capping Agents and Purification Via Conjugate Addition of Maleimide and Thiol of Failure Sequences

The synthesis of the capping agents **82** and **83** is shown in FIG. **18**. Commercially available maleic anhydride (**80**) is condensed with 1-amino-3-propanol to give **81**, which will be converted to the capping agents **82** and **83** under conditions described earlier. The copolymerization agents **55** and **56** are described earlier; **84-86** can be synthesized easily by one of ordinary skill in the art. After synthesizing oligonucleotide on solid support using standard phosphoramidite chemistry and capping failure sequences with **82** (with activator such as 1H-tetrazole) or **83** (with base such as Et_3N), the crude oligonucleotide will be mixed with **55**, **56** and **84** to incorporate the failure sequences into polymer using reported conjugate addition conditions. Alternatively, **84-86** may be used for copolymerization.

Example 6

Synthesis of Capping Agents and Purification Via Amide Bond Formation Reaction to Incorporate Failure Sequences Into Polymer

The synthesis of the capping agent **141** is shown in FIG. **34**. After synthesizing oligonucleotide on solid support using standard phosphoramidite chemistry and capping failure sequences with **141** the crude oligonucleotide is not cleaved or deprotected. The solid support is then directly treated with copolymerization agent **145** at 60° C. for 12 hours. Then cross-linking copolymerization agent **149** is added and the mixture is then heated to about 90° C. for 6 hours. The failure sequences and small molecules that resulted from deprotection are now incorporated into polymer, while the full length sequence remains in solution or in polymer matrix. Filtration and extraction provide pure full-length sequence.

Example 7

Synthesis of Phosphoramidites **92** and **95** and Purification Via Radical Acrylamide Polymerization of Full-Length Oligomers

As shown in FIG. **19**, the reaction between acetonitrile and acetone produces **87**, which is reduced to the amine **88**. Compound **88** reacts with acryloyl chloride to produce **89** in the presence of a base. The tertiary hydroxyl group in **89** is silyated with 1 equivalent dichlorodiisopropylsilane followed by addition of thymidine (suitably protected other nucleobases can also be used, but are not described in this proposal), and this affords **90**. The tertiary hydroxyl groups in **89** have two advantages: they stabilize the diisopropylsilyl acetal linkage in **90**, and they prevent dimer formation when **89** is mixed with dichlorodiisopropylsilane. Compound **90** is phosphinylated with **91** to produce the target phosphoramidite **92** under standard conditions. 5'-OH oligonucleotide can be produced using **92** for purification. 5'-phosphate oligonucleotides can be purified using the phosphoramidite **95**. Phosphoramidite **95** can be synthesized by reacting **89** with 1 equivalent dichlorodiisopropylsilane followed by addition of the commercially available **93** to afford **94**, which is phosphinylated to produce **95** under standard conditions (FIG. **19**).

Oligonucleotides can be synthesized using a solid phase synthesizer under standard conditions using phosphoramidite methodology. At the end of synthesis, phosphoramidite **92** or **95** is coupled to the 5'-end of oligonucleotide on the synthesizer.

After deprotection and cleavage, oligonucleotide **96** (when **92** is used) or **97** (when **95** is used) is produced, along with failure sequences and small organic impurities. The failure sequences and impurities do not contain the acrylamide functionality. The crude oligonucleotide is then subjected to polymerization conditions, such as acrylamide, N,N'-methylenebisacrylamide, $(NH_4)_2S_2O_8$, TMEDA, r.t., 1 h. The full length sequence **96** or **97** is incorporated into a polymer, and the failure sequences and small organic impurities remain in solution.

The failure sequences and small organic impurities can be removed by filtration or extraction. The gel that contains the full length sequence is washed with water, DMF and THF. This is followed by treatment with fluoride ion (TBAF or HF/pyridine, r.t. FIG. **21**) resulting in oligonucleotide **98** or **99**. Oligonucleotide **98** is the target full length sequence with 5'-OH group. Oligonucleotide **99** can be treated with concentrated methylamine to give oligonucleotide **100**.

Example 8

Synthesis of Phosphoramidites **103** and **105** and Purification Via Disulfide Formation of Full-Length Oligomers

Phosphoramidites **103** and **105** are synthesized according to FIG. **22**. The amino alcohol **88** is reacted with carboxylic acid **37** (**48** can also be used to give **101**, which is coupled with thymidine through the diisopropylsilyl acetal linker, and phosphinylated to give phosphoramidite **103**. For 5'-phosphate oligonucleotide synthesis, **101** is coupled with **93** using the diisopropylsilyl acetal linker, and phosphinylated to afford phosphoramidite **105**. Using **103** or **105** as phosphoramidite in the last synthetic cycle in oligonucleotide synthesis, after deprotection/cleavage, the oligonucleotide has a structure as shown by **106** or **107**. Polymerization in the presence of **44** and **45** incorporates **106** or **107** into polymer (see **108** and **109**, FIG. **24**). After removing failure sequences and other impurities by filtration or extraction, oligonucleotides **98** or **100** are collected as described above (FIG. **24**).

Example 9

Synthesis of Phosphoramidites **113** and **115** and Purification Via Sharpless "Click" Polymerization of Full-Length Oligomers

Phosphoramidites **113** and **115** are synthesized according to FIG. **25**. The known alkyne **110** (can be synthesized from inexpensive materials) is hydrolyzed, and coupled with **88** to give **111**, which is converted to the thymidine analog **112** and phosphinylated to afford the phosphoramidite **113**. The phosphoramidite **115** is synthesized from **111** and **93** in the same way as described for the synthesis of **105**. Using **113** or **115** as phosphoramidite in the last synthetic cycle in oligonucleotide synthesis, after deprotection and cleavage, the oligonucleotide has a structure as shown by **116** or **117**. Polymerization in the presence of **65** and **66** incorporates **113** or **115** into polymer (see **118** and **119**, FIG. **27**). After removing failure sequences and other impurities by washing, oligonucleotides **98** or **100** is collected as described above.

Example 10

Synthesis of Phosphoramidites **122** and **124** and Purification Using Via Grubb's Ring Opening Metathasis Polymerization of Full-Length Oligomers

The required phosphoramidites **122** and **124** are synthesized according to FIG. **28**. Compound **70** is reacted with **88** to give **120**, which is converted to the thymidine analog **121** and phosphinylated to afford the phosphoramidite **122**. The phosphoramidite **124** is synthesized from **120** and **93** in the same way as described for the synthesis of **105**. Using **122** or **124** as phosphoramidite in the last synthetic cycle in oligonucleotide synthesis, after deprotection and cleavage, the oligonucleotide has a structure as shown by **125** or **126**. Polymerization in the presence of **78** incorporates **125** or **126** into polymer (see **127** and **128**, FIG. **30**). After removing failure sequences and other impurities, oligonucleotides **98** or **100** are collected as described above.

Example 11

Synthesis of Phosphoramidites **132** and **134** and Purification Via Conjugate Addition of Maleimide and Thiol Full-Length Oligomers

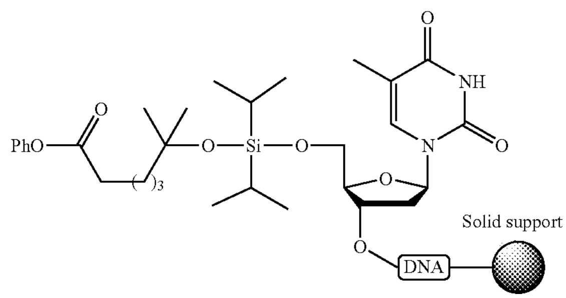
The required phosphoramidites **132** and **134** are synthesized according to FIG. **31**. Compound **129** (which can be prepared from 3,5-diaminobenzoic acid and maleic anhydride) is reacted with **88** to give **130**, which is converted to the thymidine analog **131** and phosphinylated to afford the phosphoramidite **132**. The phosphoramidite **134** is synthesized from **130** and **93** in the same way as described for the synthesis of **105**. Using **132** or **134** as phosphoramidite in the last synthetic cycle in oligonucleotide synthesis, after deprotection and cleavage, the oligonucleotide has a structure as shown by **135** or **136** (FIG. **32**). Polymerization in the presence of **84**, **85** and **86** incorporates **135** or **136** into polymer (see **137** and **138**, FIG. **33**). After removing failure sequences and other impurities, oligonucleotides **98** or **100** are collected as described above.

Example 12

Synthesis of Phosphoramidites and Purification Via Incorporation of Full Length Sequence into Polymer using the Amide Bond Formation Reaction

Phosphoramidite **150** is synthesized according to FIG. **35**. Using phosphoramidite **150** in the last synthetic cycle, the oligonucleotide synthesized on the solid support has structure **152**:

152



Structure **152** is then treated with **146** at about 60° C. for about 6 hours, without standard basic deprotection or cleavage. During the reaction, the phenoxide in the full length sequence is displaced by the amino group in **146**. Next, the cross-linking copolymerization reagent **149** is added and the mixture heated to 90° C. for about 6 hours. During this reaction the oligonucleotides are cleaved from the solid support, all protecting groups are removed, and the full length sequence and the small molecules resulting from the protecting groups are incorporated into a polymerized material. Pure full length sequence will be collected by treating with fluoride as described above.

All patents, publications and references cited herein are hereby fully incorporated by reference. In case of conflict between the present disclosure and incorporated patents, publications and references, the present disclosure should control.

The invention claimed is:

1. A method of purifying an oligomer comprising:

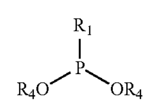
capping any failure sequences produced during synthesis with a capping agent haying a polymerizable functional group;

polymerizing the capped failure sequences; and

separating the polymerized material from the full-length oligomer;

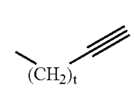
wherein the capping agent is a compound of formula (IV):

(IV)



wherein R_1 is a halogen or a secondary amine;

R_4 is



; and

t is an integer from 1 to 3.

* * * * *