COMPOUNDS AND METHODS FOR THE SYNTHESIS OF 5-(N-PROTECTED-TRYPTAMINOCARBOXYAMIDE)-2'-DEOXYURIDINE PHOSPHORAMIDITE FOR INCORPORATION INTO A NUCLEIC ACID

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application No. 62/308,132, filed March 14, 2016, which is incorporated by reference herein in its entirety for any purpose.

FIELD

[0002] This disclosure relates to the field of modified bases, nucleotides, and phosphoramidites, including improved methods of synthesis and oligonucleotides comprising the modified nucleotides.

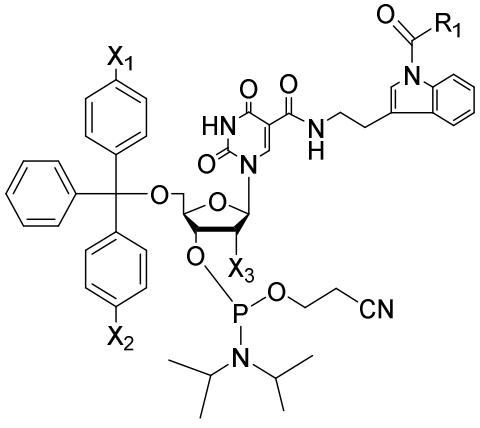
BACKGROUND

[0003] The chemically modified nucleotide, TrpdU (and its 2'-modified analogs), which bears a 3-(2-aminoethyl)-indole side chain, is useful, for example, in developing high-affinity aptamers to target analytes, such as proteins. The indole ring of TrpdU is electron-rich and polarizable, which may facilitate formation of secondary structure and complementary hydrophobic interfaces with target analytes. To date, TrpdU has not performed as well as other modified nucleotides as a phosphoramidite reagent in solid-phase oligonucleotide synthesis, and its use has therefore been limited.

[0004] There remains a need in the art for an improved TrpU phosphoramidite, and improved methods of making a TrpU phosphoramidite.

SUMMARY

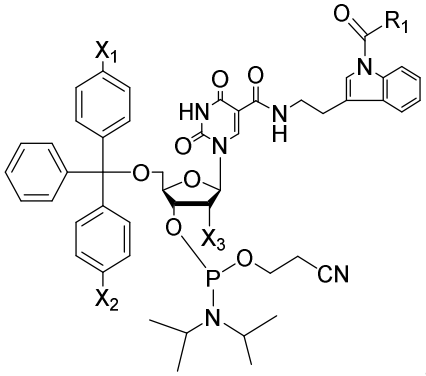
[0005] In some examples, a compound having the structure



, or a salt thereof, is provided. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-

chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, X_1 and X_2 are each independently selected from methoxy and hydrogen. In some examples, X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy.

[0005a] In one aspect, the present disclosure refers to a compound having the structure:



, or a salt thereof; wherein,

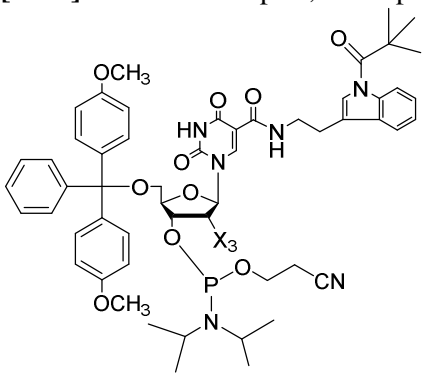
R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each independently selected from methoxy and hydrogen;

X_3 is selected from methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy.

[0006] In some examples, R_1 is *tert*-butyl. In some examples, R_1 is 1,1-dimethyl-propyl. In some examples, R_1 is 1,1-dimethyl-butyl; 2-chlorophenyl. In some examples, R_1 is 2-cyanophenyl. In some examples, R_1 is 1-methyl-cyclopentyl. In some examples, R_1 is 1-methyl-cyclohexyl. In some examples, X_1 and X_2 are methoxy. In some examples, X_3 is hydrogen. In some examples, X_3 is methoxy. In some examples, X_3 is fluoro. In some examples, X_3 is *tert*-butyldimethylsilyloxy.

[0007] In some examples, a compound provided herein is selected from:



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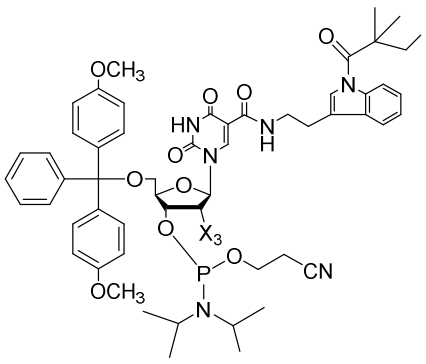

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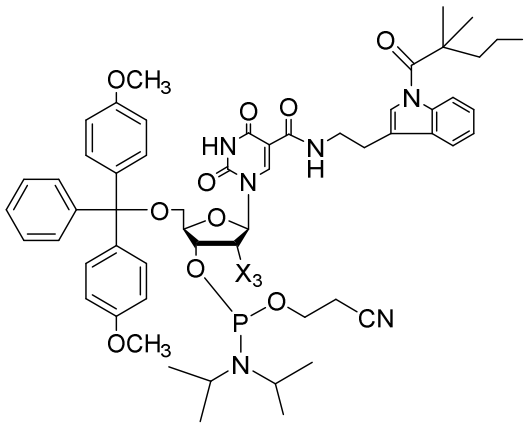
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X3
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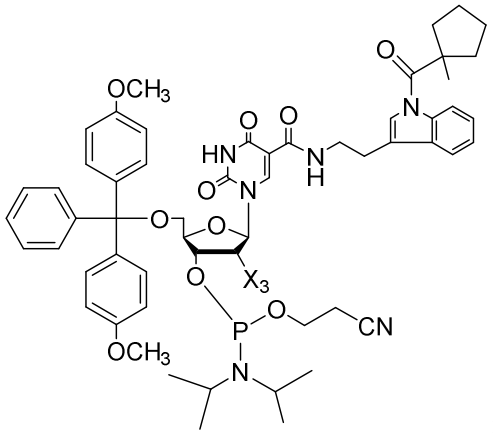
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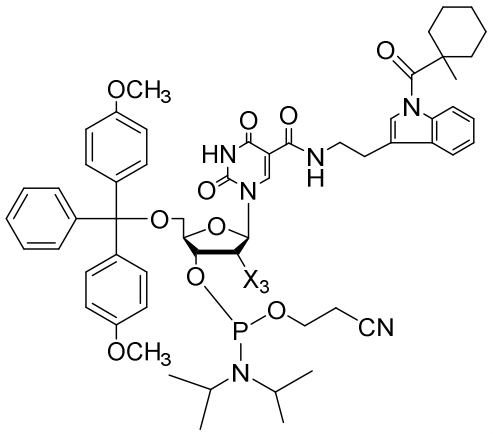
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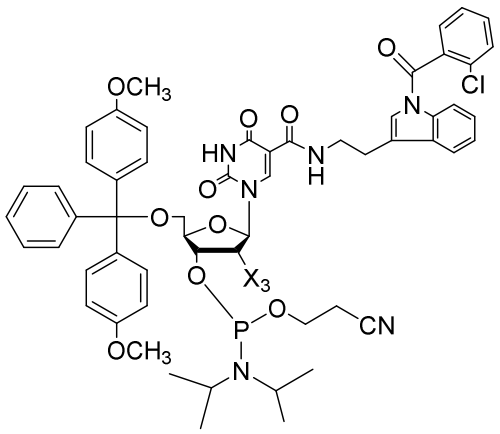
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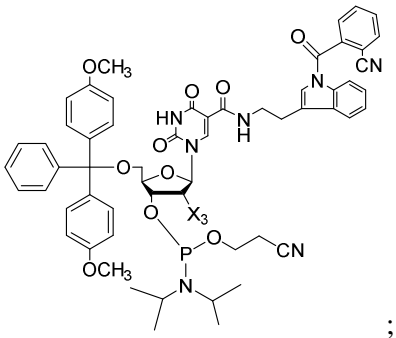
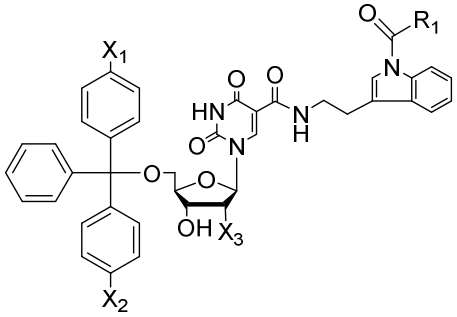


;

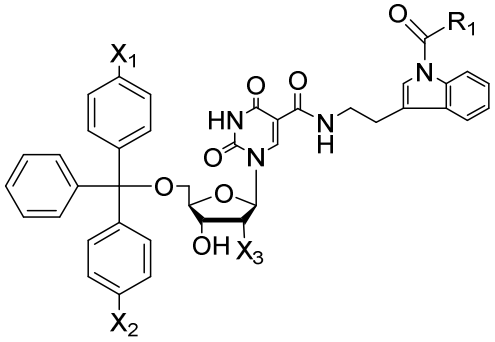
and salts thereof.

[0008] In some examples, a compound having the structure



, or a salt thereof, is provided. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, X_1 and X_2 are each independently selected from methoxy and hydrogen. In some examples, X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy.

[0008a] In another aspect, the present disclosure refers to a compound having the structure:



, or a salt thereof; wherein,

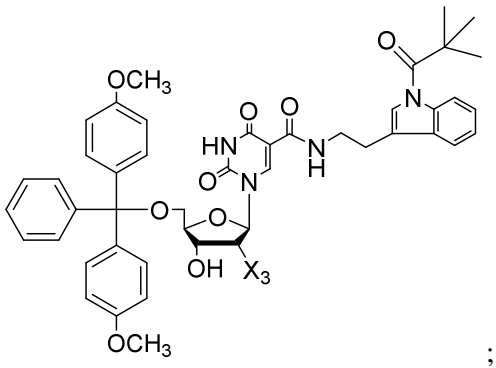
R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each independently selected from methoxy and hydrogen;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy.

[0009] In some examples, R_1 is *tert*-butyl. In some examples, R_1 is 1,1-dimethyl-propyl. In some examples, R_1 is 1,1-dimethyl-butyl; 2-chlorophenyl. In some examples, R_1 is 2-cyanophenyl. In some examples, R_1 is 1-methyl-cyclopentyl. In some examples, R_1 is 1-methyl-cyclohexyl. In some examples, X_1 and X_2 are methoxy. In some examples, X_3 is hydrogen. In some examples, X_3 is methoxy. In some examples, X_3 is fluoro. In some examples, X_3 is *tert*-butyldimethylsilyloxy.

[0010] In some examples, the compound is selected from: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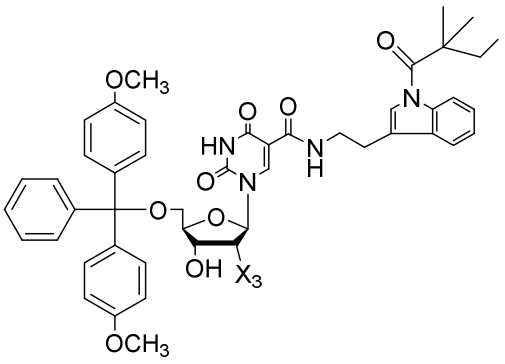
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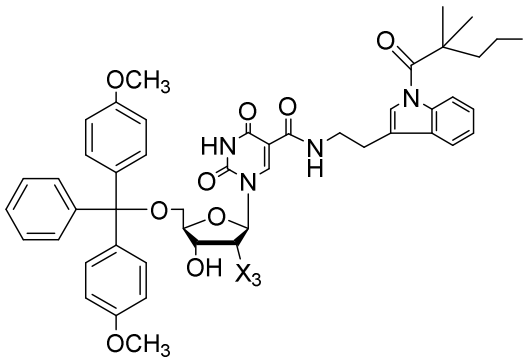
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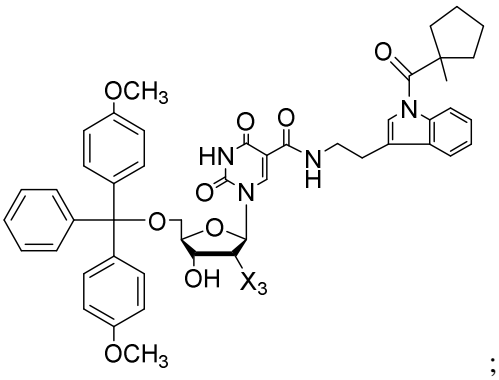
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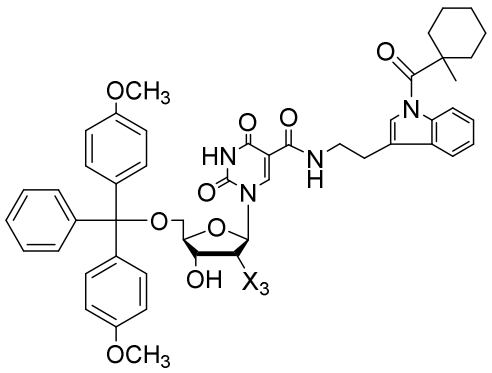
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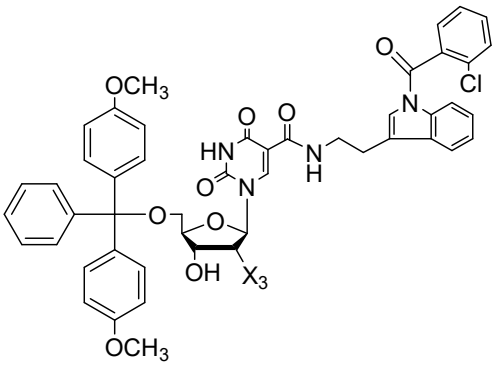
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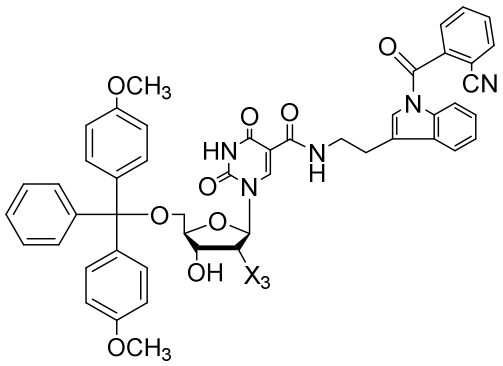
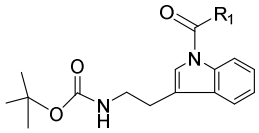
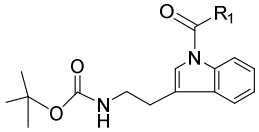

; and salts thereof.

[0011] In some examples, a compound having the structure:

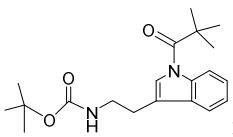

, or a salt thereof, is provided. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, R_1 is *tert*-butyl. In some examples, R_1 is 1,1-dimethyl-propyl. In some examples, R_1 is 1,1-dimethyl-butyl; 2-chlorophenyl. In some examples, R_1 is 2-cyanophenyl. In some examples, R_1 is 1-methyl-cyclopentyl. In some examples, R_1 is 1-methyl-cyclohexyl.

[0011a] In another aspect, the present disclosure refers to a compound having the structure:


, or a salt thereof; wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl.

[0012] In some examples, the compound has a structure selected from: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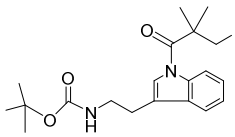
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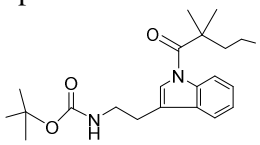
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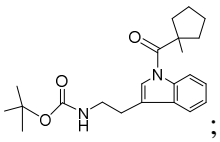
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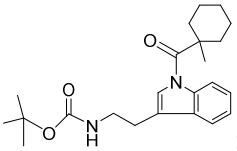
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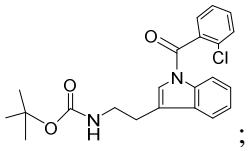
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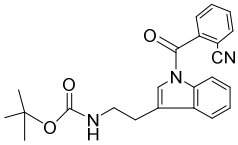
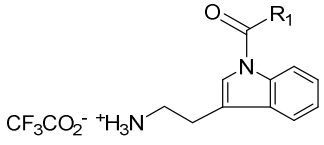
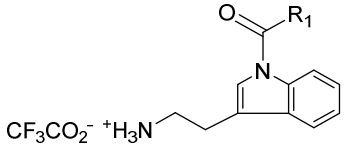

; and salts thereof.

[0013] In some examples, a compound having the structure:

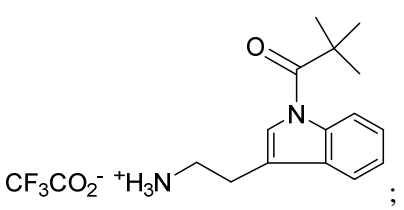

is provided. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, R_1 is *tert*-butyl. In some examples, R_1 is 1,1-dimethyl-propyl. In some examples, R_1 is 1,1-dimethyl-butyl; 2-chlorophenyl. In some examples, R_1 is 2-cyanophenyl. In some examples, R_1 is 1-methyl-cyclopentyl. In some examples, R_1 is 1-methyl-cyclohexyl.

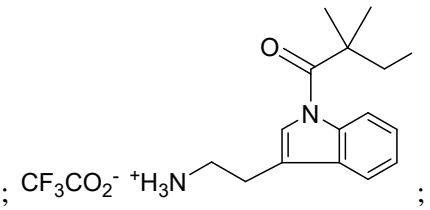
[0013a] In another aspect, the present disclosure refers to a compound having the structure:


wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl.

[0014] In some examples, a compound has a structure selected from:



; 

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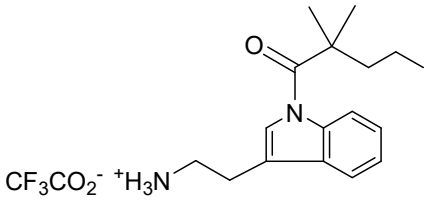
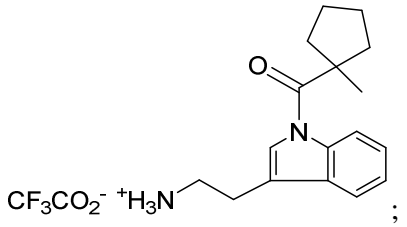


; 

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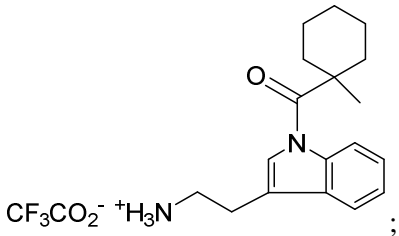
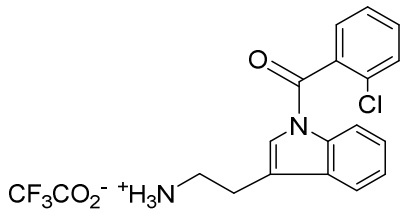
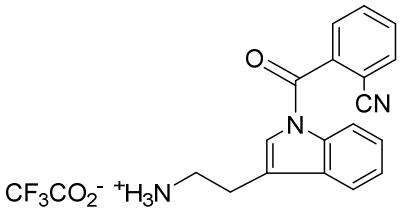


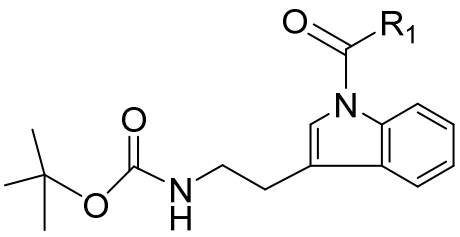
; 

; and



.

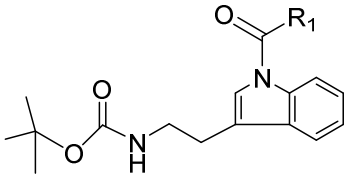
[0015] In some examples, methods of producing a compound having the structure:



, or a salt thereof, are provided. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, R_1 is *tert*-butyl. In some examples, R_1 is 1,1-dimethyl-propyl. In some examples, R_1 is 1,1-dimethyl-butyl; 2-chlorophenyl. In some examples, R_1 is 2-cyanophenyl. In some examples, R_1 is 1-methyl-cyclopentyl. In some examples, R_1 is 1-methyl-cyclohexyl. In some examples, the method comprises reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride. In some examples, R_1 is *tert*-butyl, and wherein the acid chloride is pivaloyl chloride. In some

examples, R_1 is 1,1-dimethyl-propyl and the acid chloride is 2,2-dimethylbutyroyl chloride. In some examples, R_1 is 1,1-dimethyl-butyl and the acid chloride is 2,2-dimethylvaleroyl chloride. In some examples, R_1 is 2-chlorophenyl and the acid chloride is 2-chlorobenzoyl chloride. In some examples, R_1 is 2-cyanophenyl and the acid chloride is 2-cyanobenzoyl chloride. In some examples, R_1 is 1-methyl-cyclopentyl and the acid chloride is 1-methylcyclopentane-1-carbonyl chloride. In some examples, R_1 is 1-methyl-cyclohexyl and the acid chloride is 1-methylcyclohexane-1-carbonyl chloride.

[0015a] In another aspect, the present disclosure refers to a method of producing a compound having the structure:

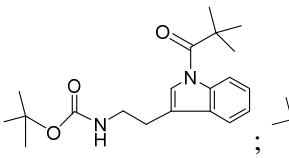


, or a salt thereof; wherein,

R1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

the method comprising reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride.

[0016] In some examples, the method produces a compound selected from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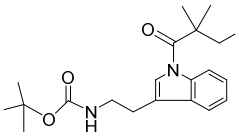
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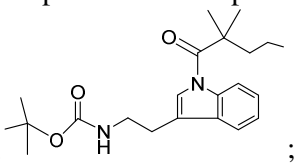
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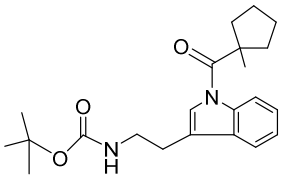
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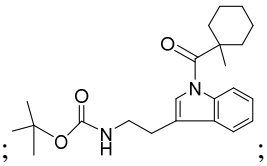
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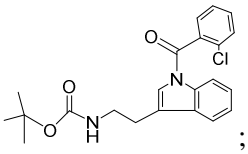
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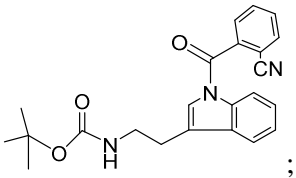
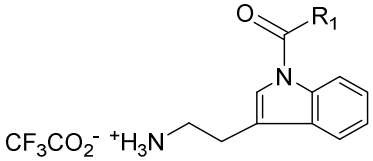
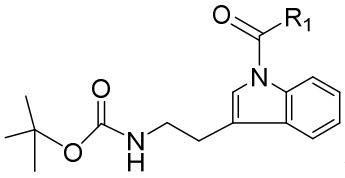

; and salts thereof.

[0017] In some examples, a method of producing a compound having the structure:

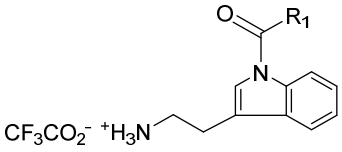


is provided. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, the method comprises reacting the compound



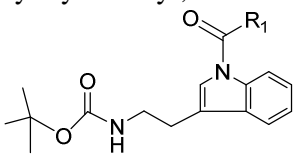
with trifluoroacetic acid.

[0017a] In another aspect, the present disclosure refers to a method of producing a compound having a structure:

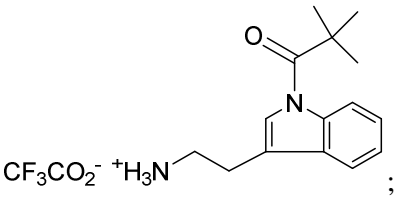


wherein,

R1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

the method comprising reacting the compound  with trifluoroacetic acid.

[0018] In some examples, the method produces a compound selected from:



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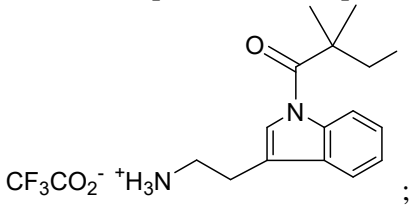

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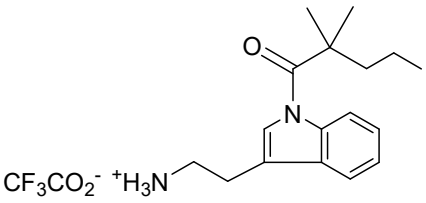

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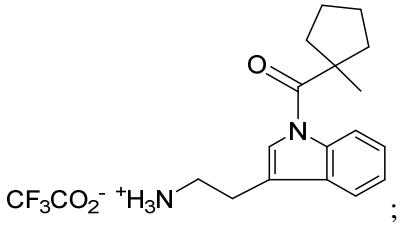

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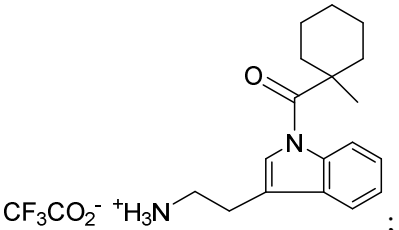

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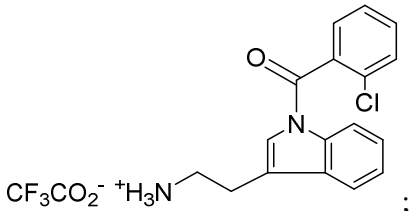
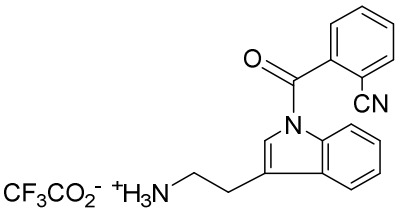
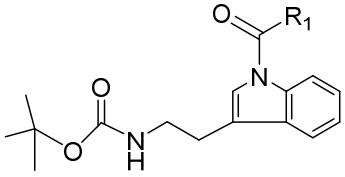


; and



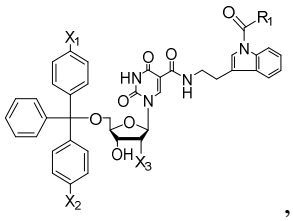
.

[0019] In some examples, the method further comprises reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound

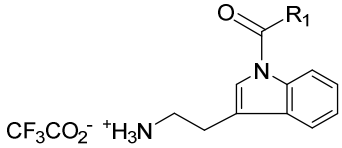


.

[0020] In some examples, a method of producing a compound having the structure:

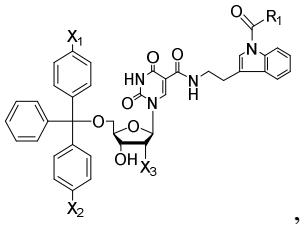


, or a salt thereof, is provided. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, X_1 and X_2 are each independently selected from methoxy and hydrogen. In some examples, X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy. In some examples, the method comprising reacting the compound



with 5'-*O*-DMT-5-(2,2,2-trifluoroethyoxy-carbonyl)-2'-deoxyuridine (TFEdU).

[0020a] In another aspect, the present disclosure refers to a method of producing a compound having the structure:

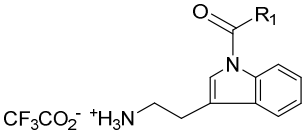


, or a salt thereof; wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

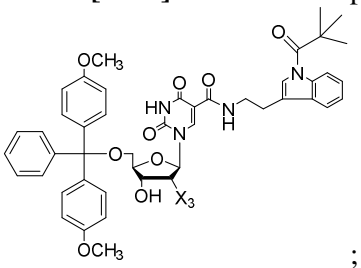
X_1 and X_2 are each independently selected from methoxy and hydrogen;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;

the method comprising reacting the compound 

with 5'-*O*-DMT-5-(2,2,2-trifluoroethyoxy-carbonyl)-2'-deoxyuridine (TFEdU).

[0021] In some examples, the method produces a compound selected from:



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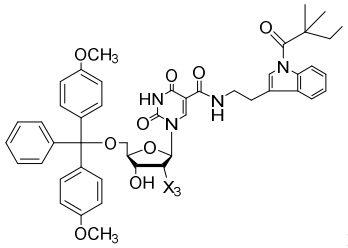

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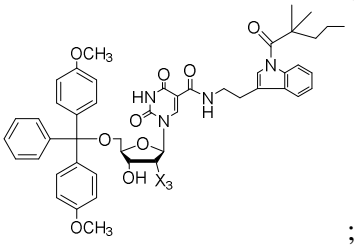
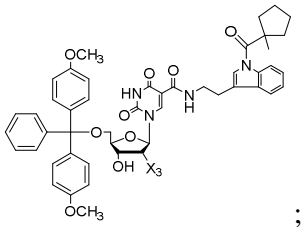


;



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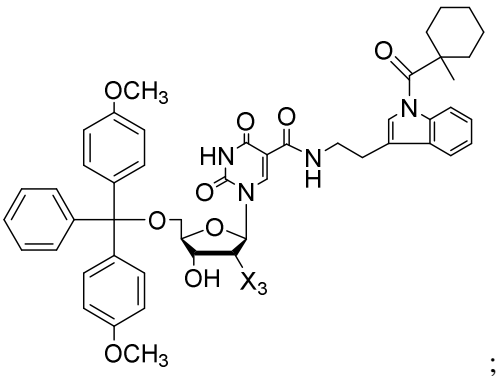

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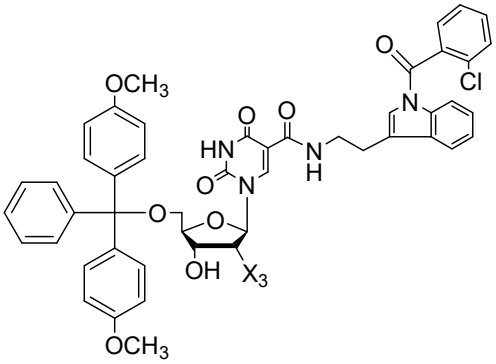

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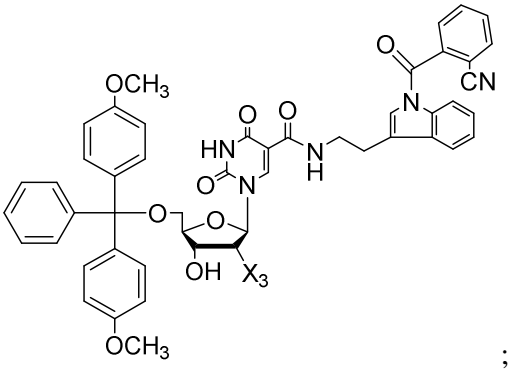
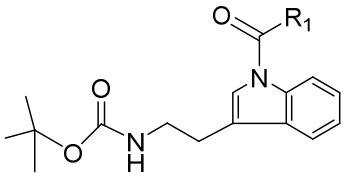
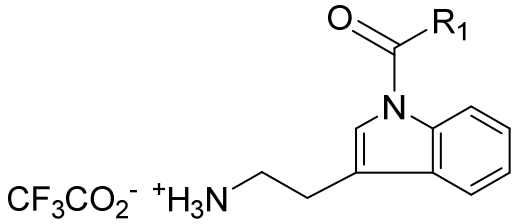


; and salts thereof.

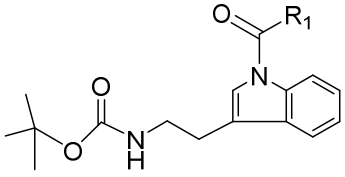
[0022] In some examples, the method further comprises reacting the compound



with trifluoroacetic acid to form the compound

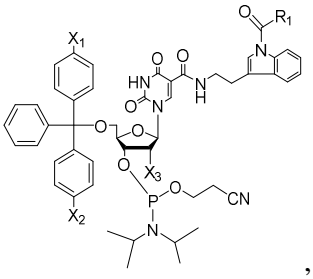


. In some examples, the method further comprises reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound

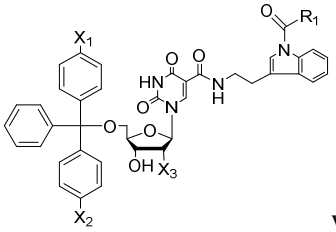


.

[0023] In some examples, a method of producing a compound having the structure:

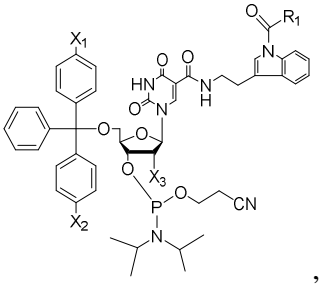


, or a salt thereof, is provided. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, X_1 and X_2 are each independently selected from methoxy and hydrogen. In some examples, X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy. In some examples, the method comprises reacting the compound



with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphor-amidite.

[0023a] In another aspect, the present disclosure refers to a method of producing a compound having the structure:

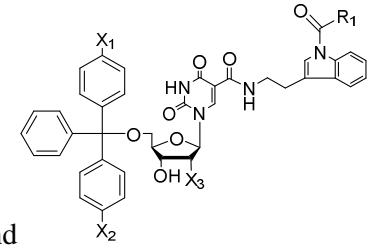


, or a salt thereof; wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

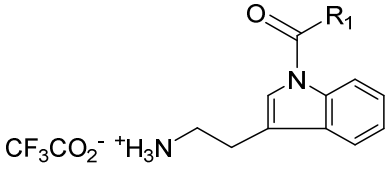
X_1 and X_2 are each independently selected from methoxy and hydrogen;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;

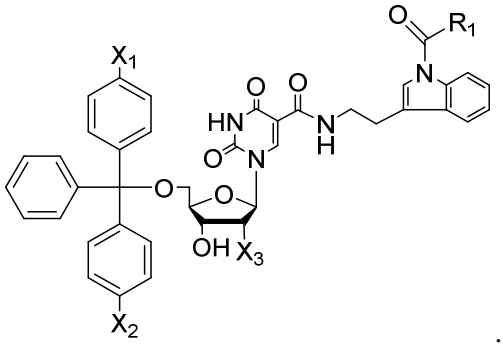


the method comprising reacting the compound with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphor-amidite.

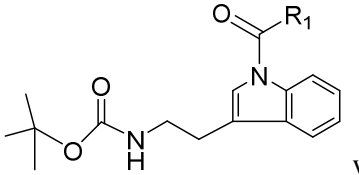
[0024] In some examples, the method comprises reacting the compound



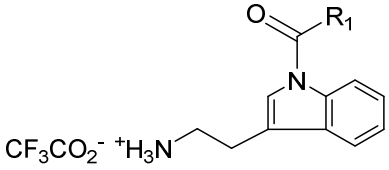
with 5'-*O*-DMT-5-(2,2,2-trifluoroethyoxy-carbonyl)-2'-deoxyuridine (TFEdU) to form the compound



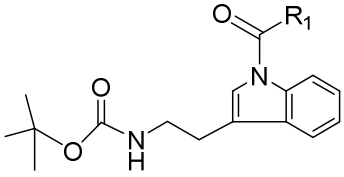
. In some examples, the method comprises reacting the compound



with trifluoroacetic acid to form the compound

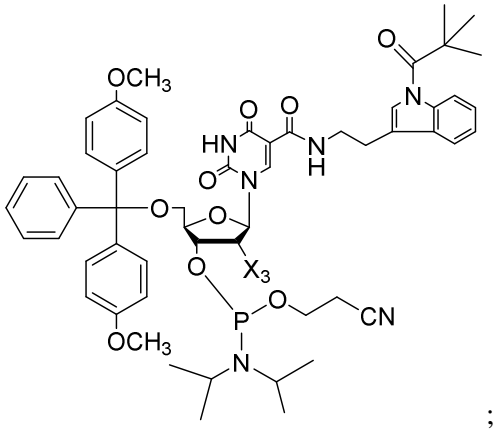


. In some examples, the method comprises reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound



.

[0025] In some examples, the method produces a compound selected from:



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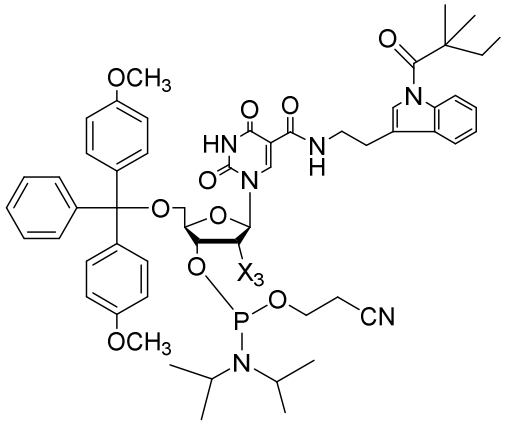

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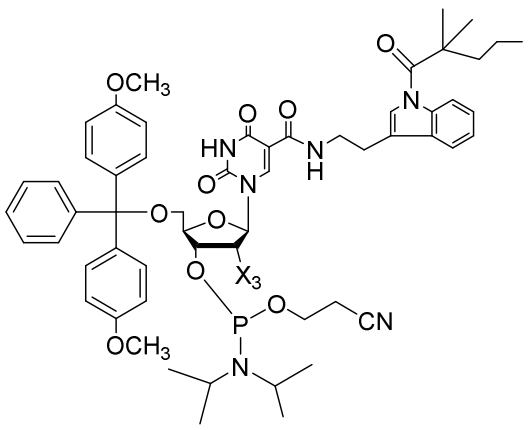
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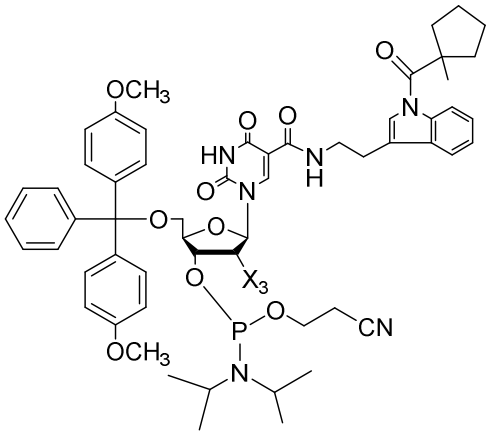
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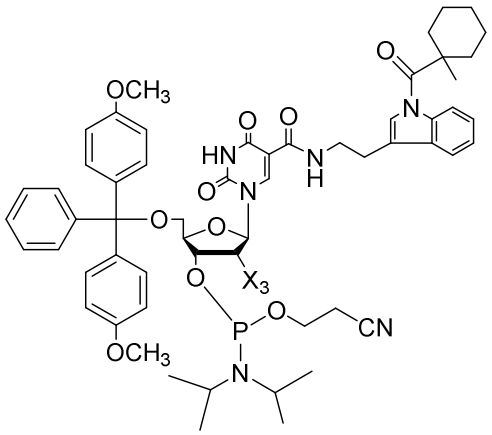
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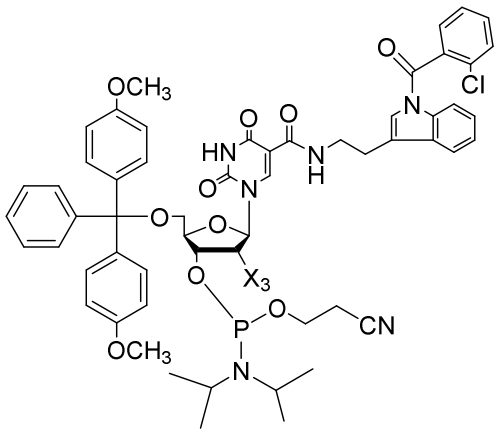
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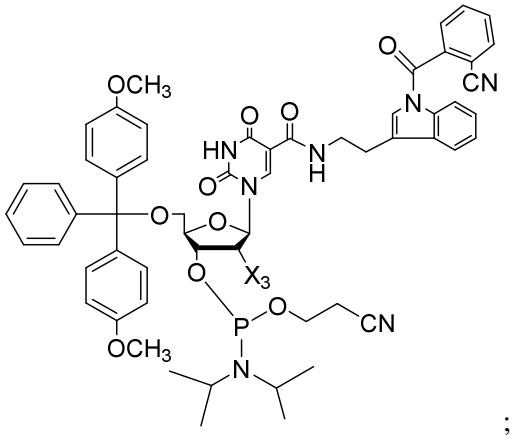
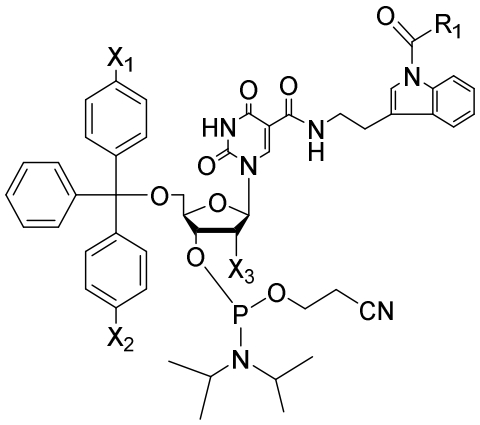

; and salts thereof.

[0026] In some examples, a method of producing a compound having the structure:



, or a salt thereof, is provided,

wherein,

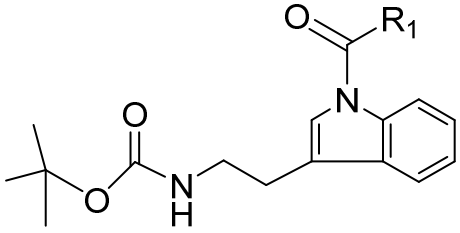
R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each independently selected from methoxy and hydrogen;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;

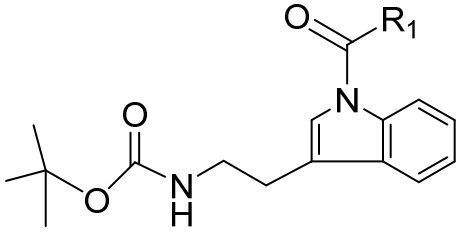
comprising the steps of:

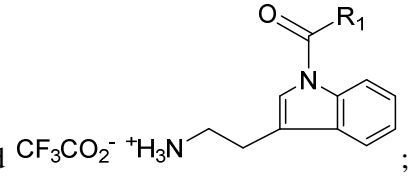
a) reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound



;

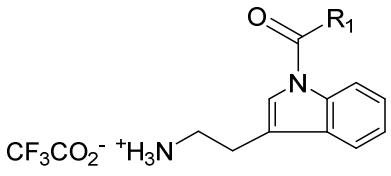
b) reacting the compound



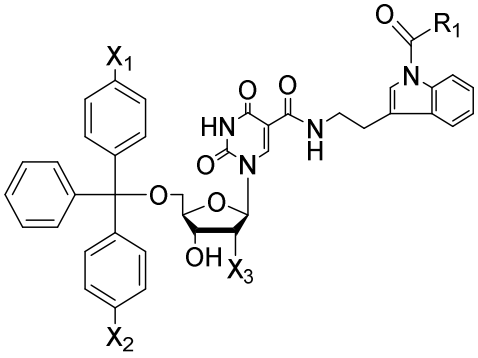
with trifluoroacetic acid to form the compound 

;

c) reacting the compound

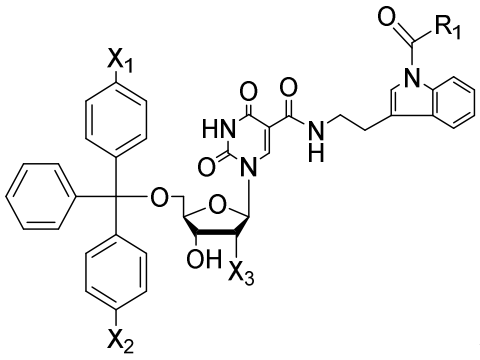


with 5'-*O*-DMT-5-(2,2,2-trifluoroethyoxy-carbonyl)-2'-deoxyuridine (TFEdU) to form the compound



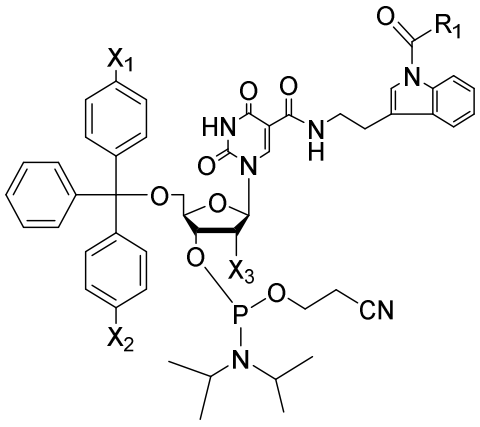
; and

d) reacting the compound



with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphor-amidite.

[0026a] In another aspect, the present disclosure refers to a method of producing a compound having the structure:



, or a salt thereof; wherein,

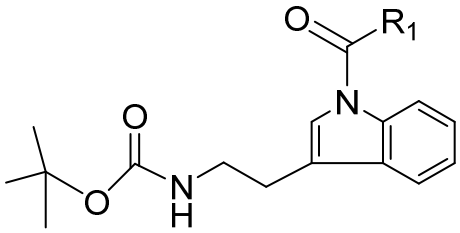
R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each independently selected from methoxy and hydrogen;

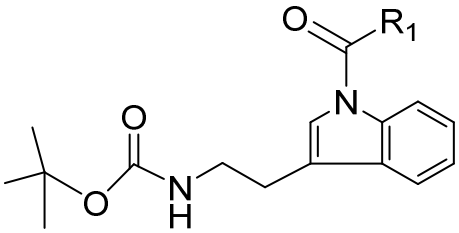
X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;

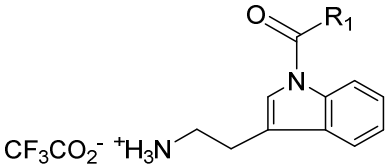
comprising the steps of:

a) reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound

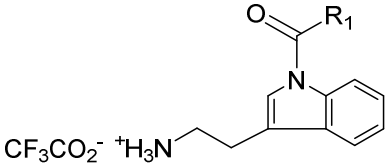


;

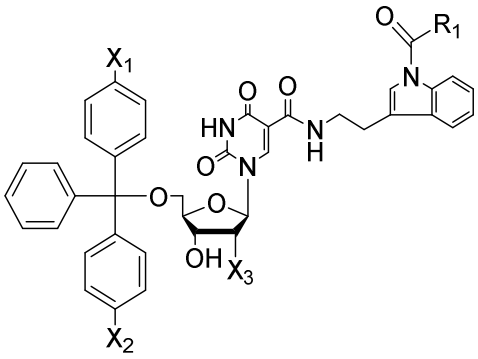
b)_reacting the compound 

with trifluoroacetic acid to form the compound 

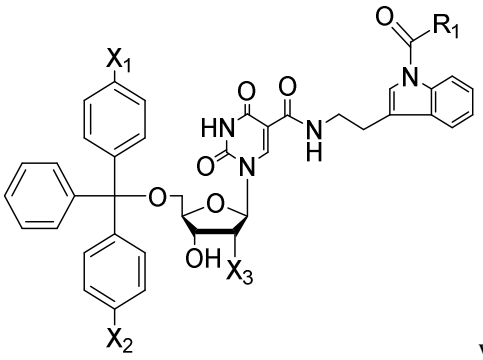
;

c) reacting the compound 

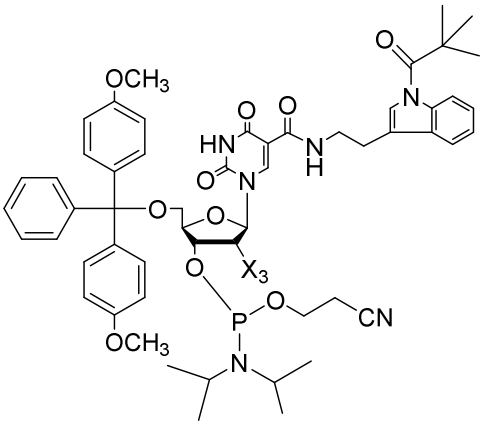
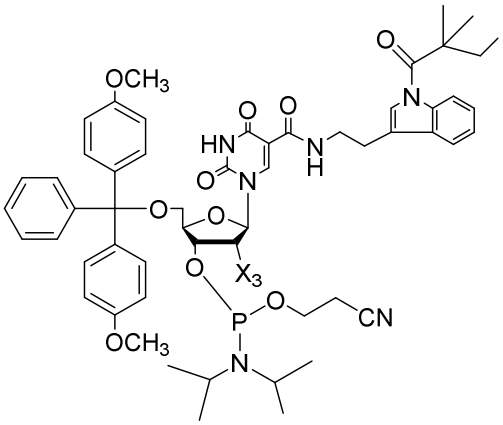
with 5'-*O*-DMT-5-(2,2,2-trifluoroethyoxy-carbonyl)-2'-deoxyuridine (TFEdU) to form the compound

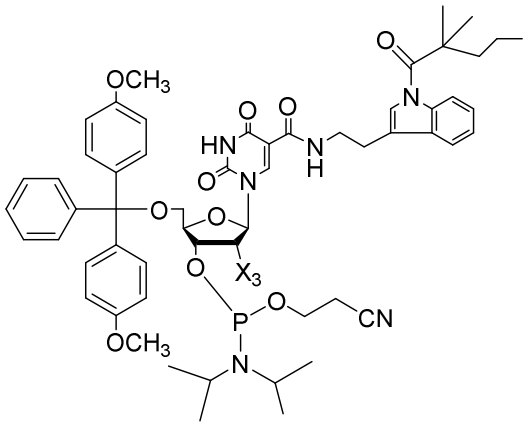


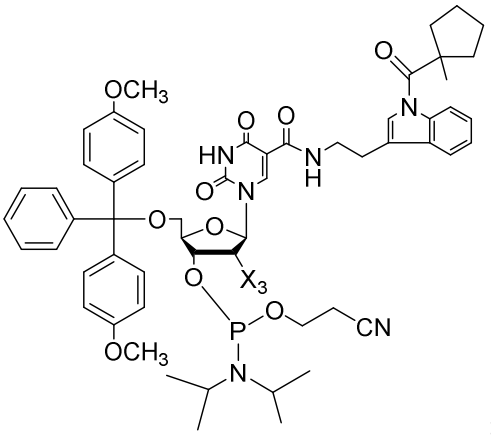
; and

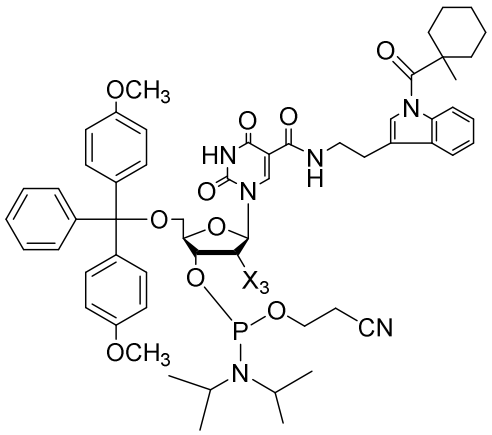
d) reacting the compound  with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphor-amidite.

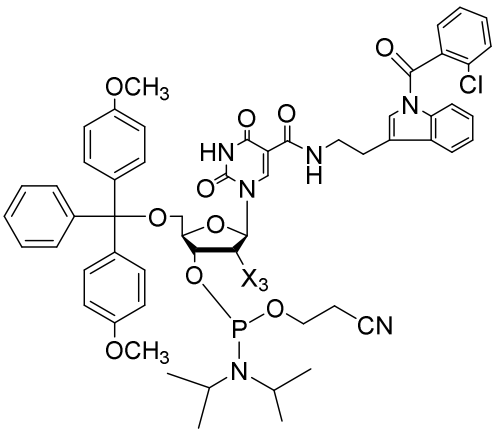
[0027] In some examples, the method produces a compound selected from:

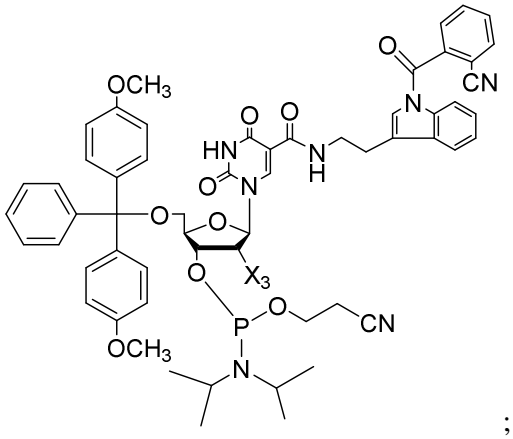
 ;  ;



;


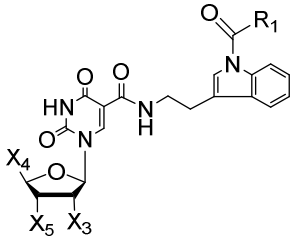
;


;


;


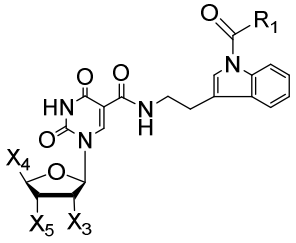
; and salts thereof.

[0028] In some examples, oligonucleotides are provided, comprising at least one protected TrpU nucleotide, wherein at least one protected TrpU nucleotide in the oligonucleotide has the structure:



. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy. In some examples, X_4 is selected from OH, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide. In some examples, X_5 is selected from –O-ss, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein ss is a solid support, Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide. In some examples, the solid support is controlled-pore glass (CPG). In some examples, Z' is S and Z" is O. In some examples, Z' and Z" are O.

[0028a] In another aspect, the present disclosure refers to an oligonucleotide comprising at least one protected TrpU nucleotide, wherein at least one protected TrpU nucleotide in the oligonucleotide has the structure:



; wherein,

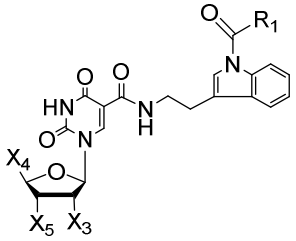
R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;

X_4 is selected from OH, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide; and

X_5 is selected from –O-ss, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein ss is a solid support, Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide.

[0029] In some examples, a method of producing an oligonucleotide comprising at least one TrpU nucleotide is provided, comprising incorporating at least one nucleotide having the structure:

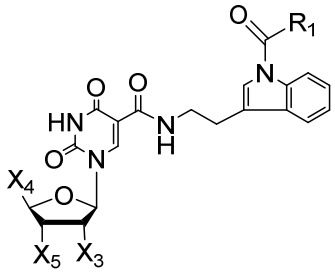


into a nucleotide sequence on a solid support; and removing the



protecting group from the at least one TrpU nucleotide incorporated into the oligonucleotide. In some examples, R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl. In some examples, X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy. In some examples, X_4 is selected from OH, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide. In some examples, X_5 is selected from –O-ss, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein ss is a solid support, Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide. In some examples, the solid support is controlled-pore glass (CPG). In some examples, Z' is S and Z" is O. In some examples, Z' and Z" are O.

[0029a] In another aspect, the present disclosure refers to a method of producing an oligonucleotide comprising at least one TrpU nucleotide, comprising incorporating at least one nucleotide having the structure:



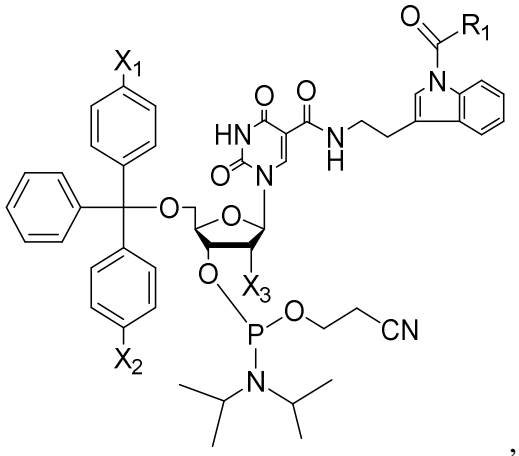
wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;
X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;
X_4 is selected from OH, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide; and
X_5 is selected from –O-ss, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein ss is a solid support, Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide;

into a nucleotide sequence on a solid support; and removing the  protecting group from the at least one TrpU nucleotide incorporated into the oligonucleotide.

We claim:

1. A compound having the structure:



, or a salt thereof;

wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each independently selected from methoxy and hydrogen;

X_3 is selected from methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy.

2. The compound of claim 1, wherein R_1 is a *tert*-butyl.

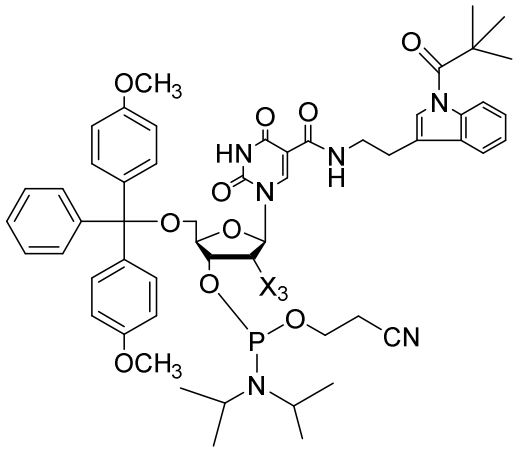
3. The compound of claim 2, wherein X_1 and X_2 are methoxy.

4. The compound of any one of claims 1 to 3, wherein X_3 is hydrogen.

5. The compound of any one of claims 1 to 3, wherein X_3 is methoxy.

6. The compound of any one of claims 1 to 3, wherein X_3 is fluoro.

7. The compound of claim 1, selected from:



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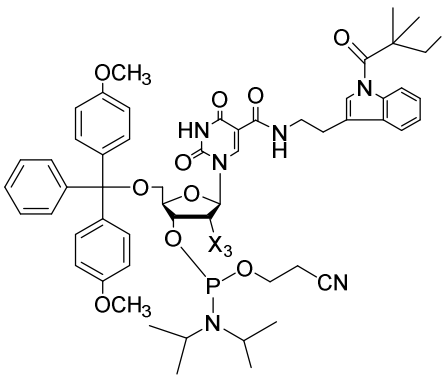

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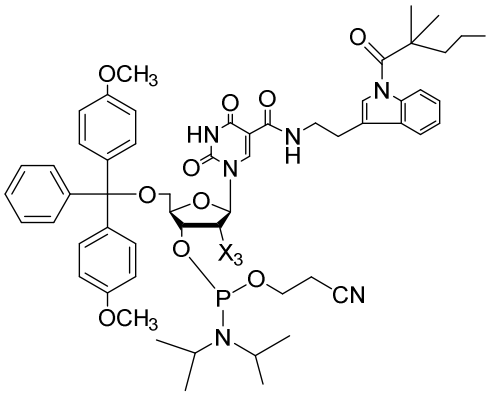

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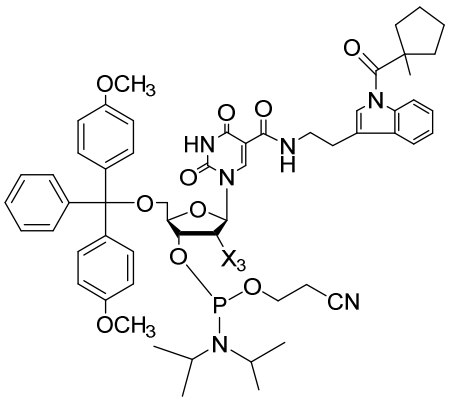

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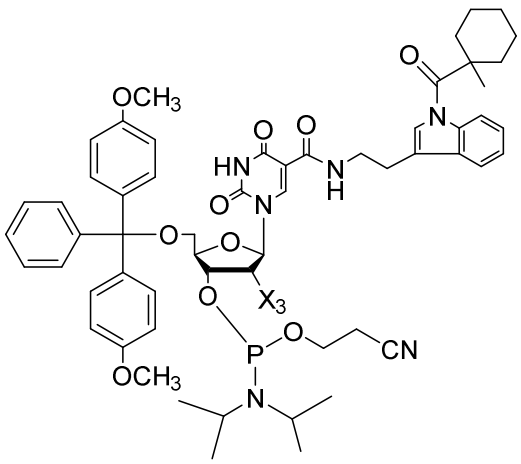

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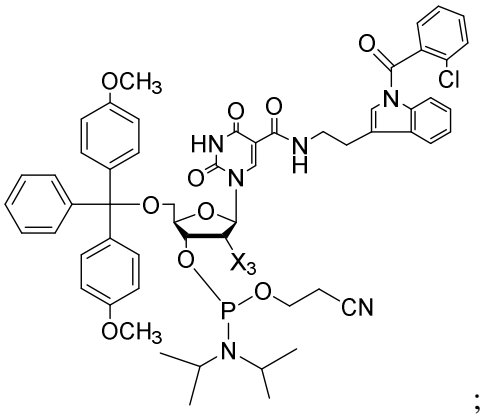

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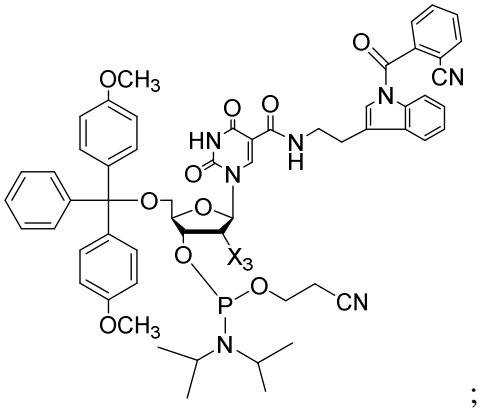
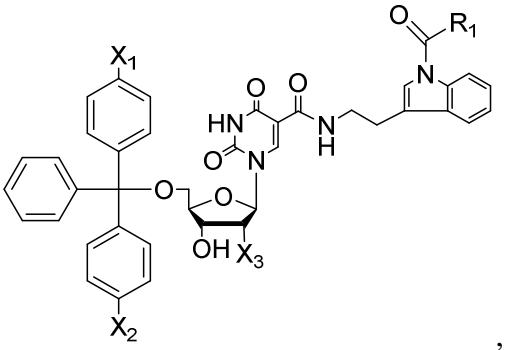


;

and salts thereof.

8. A compound having the structure:



, or a salt thereof;

wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each independently selected from methoxy and hydrogen;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy.

9. The compound of claim 8, wherein R_1 is *tert*-butyl.

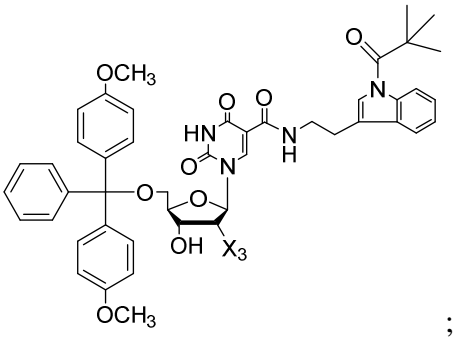
10. The compound of claim 9, wherein X_1 and X_2 are methoxy.

11. The compound of any one of claims 8 to 10, wherein X_3 is hydrogen.

12. The compound of any one of claims 8 to 10, wherein X_3 is methoxy.

13. The compound of any one of claims 8 to 10, wherein X_3 is fluoro.

14. The compound of claim 8 selected from:



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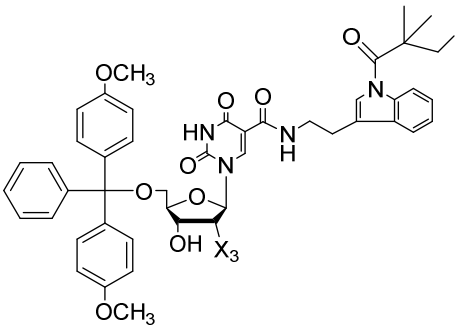

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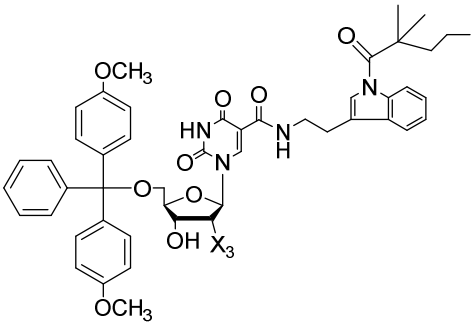

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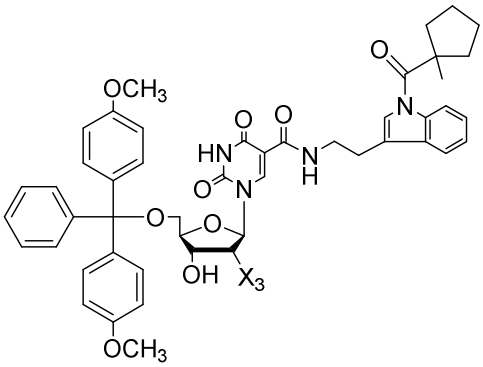
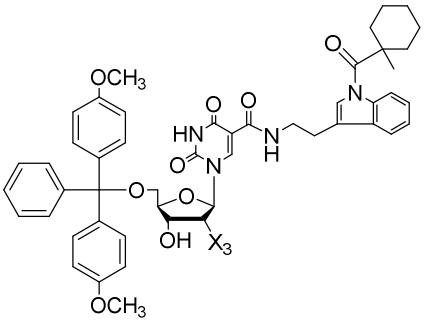


;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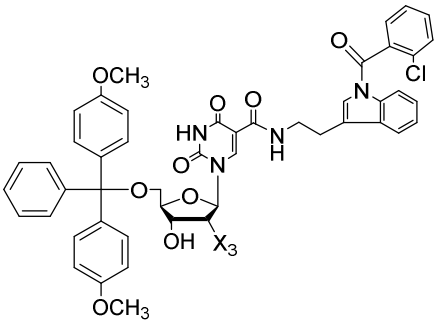
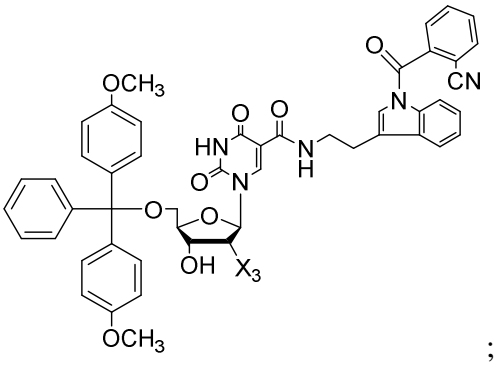
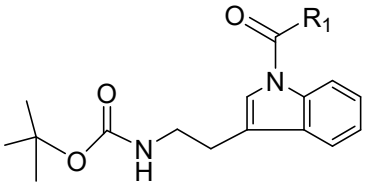

;


;

and salts thereof.

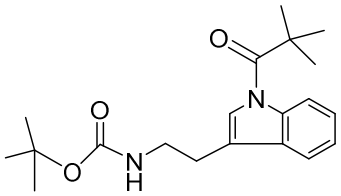
15. A compound having the structure:


, or a salt thereof;

wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl.

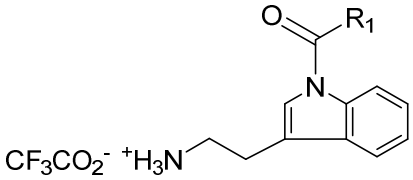
16. The compound of claim 15 having the structure:



;

or a salt thereof.

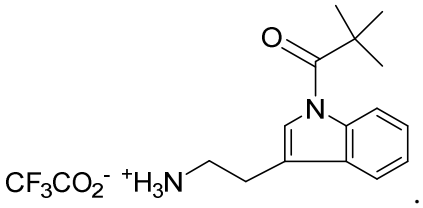
17. A compound having the structure:



wherein,

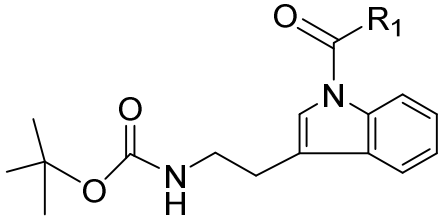
R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

18. The compound of claim 17 having the structure:



.

19. A method of producing a compound having the structure:



, or a salt thereof;

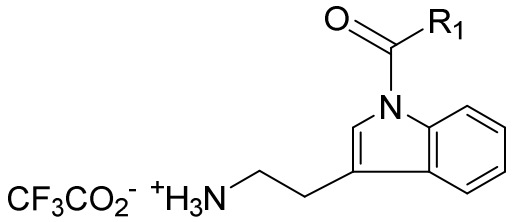
wherein,

R1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

the method comprising reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride.

20. The method of claim 19, wherein R_1 is *tert*-butyl, and wherein the acid chloride is pivaloyl chloride.

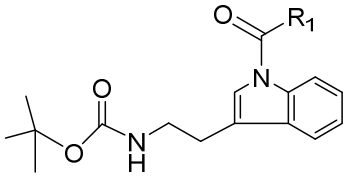
21. A method of producing a compound having the structure:



wherein,

R1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

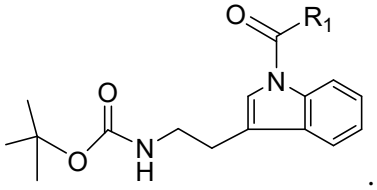
the method comprising reacting the compound



with trifluoroacetic acid.

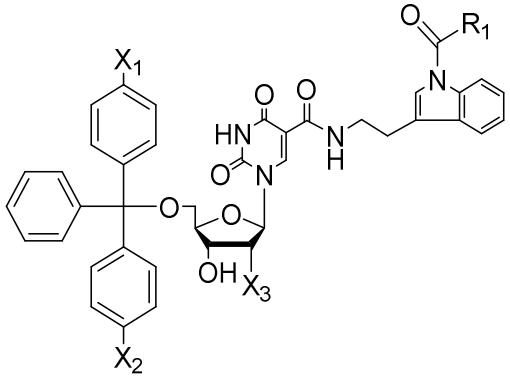
22. The method of claim 21, wherein R_1 is a *tert*-butyl.

23. The method of claim 21, wherein the method comprises reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound



.

24. A method of producing a compound having the structure:



, or a salt thereof;

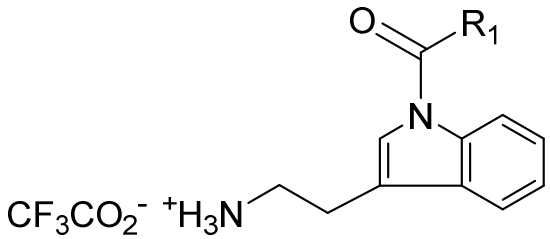
wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each methoxy;

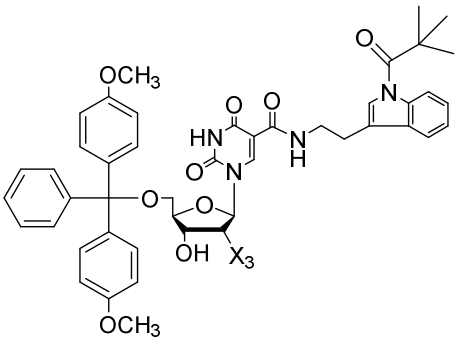
X_3 is hydrogen;

the method comprising reacting the compound

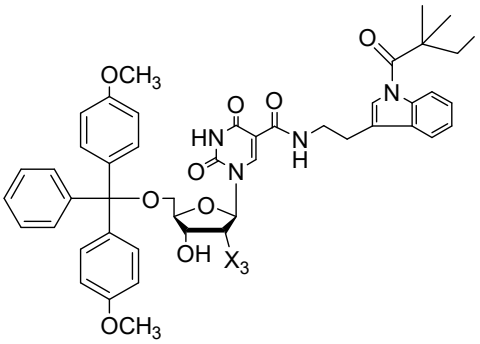


with 5'-*O*-DMT-5-(2,2,2-trifluoroethyoxy-carbonyl)-2'-deoxyuridine (TFEdU).

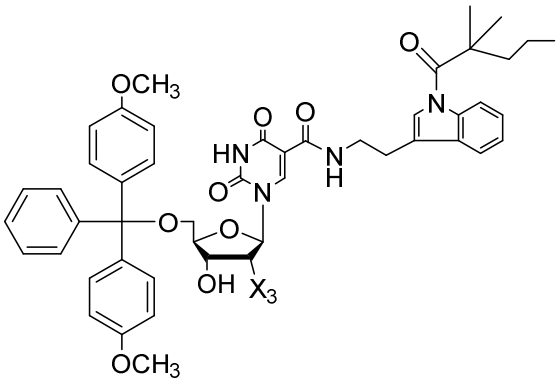
25. The method of claim 24, wherein the method produces a compound selected from:



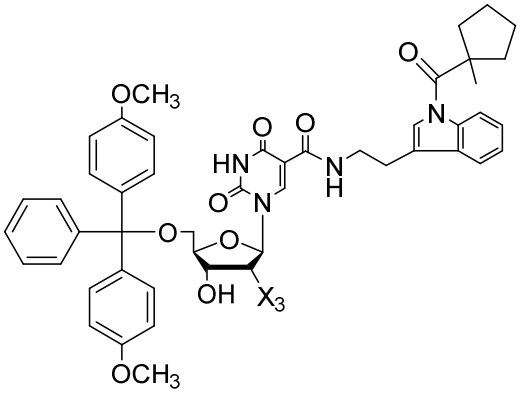
;



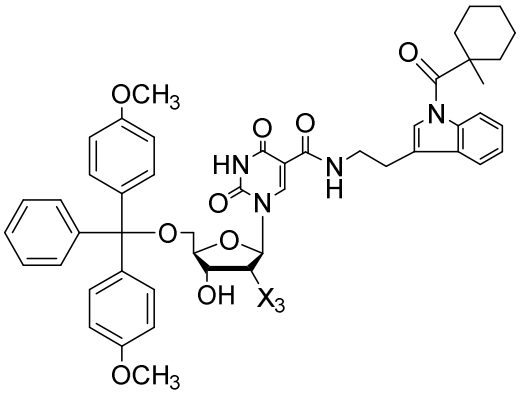
;


OCH3
O
HN
O
N
O
O
N
H
O
OH
X3
OCH3

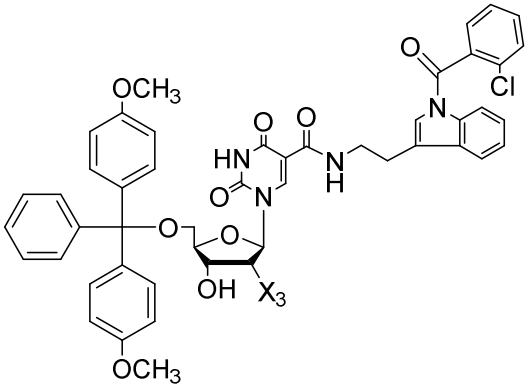
;


OCH3
O
HN
O
N
O
O
N
H
O
OH
X3
OCH3

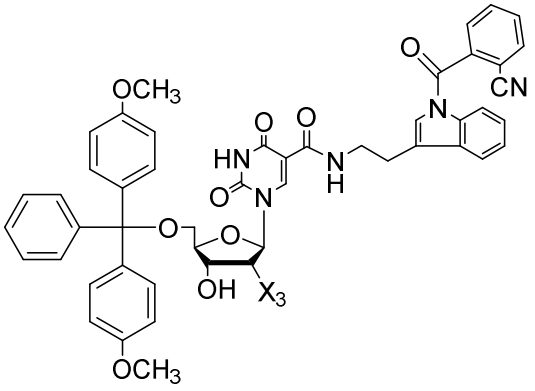
;


OCH3
O
HN
O
N
O
O
N
H
O
OH
X3
OCH3

;


OCH3
O
Cl
HN
O
N
O
O
N
H
O
OH
X3
OCH3

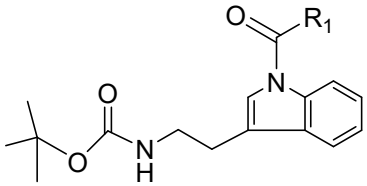
;



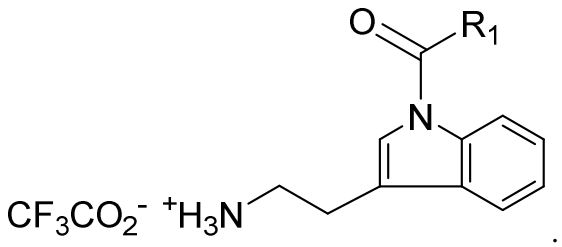
;

and salts thereof.

26. The method of claim 24 or 25, wherein the method further comprises reacting the compound



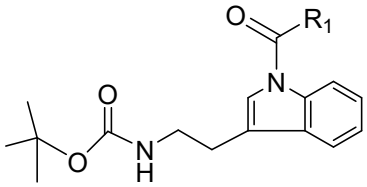
with trifluoroacetic acid to form the compound



.

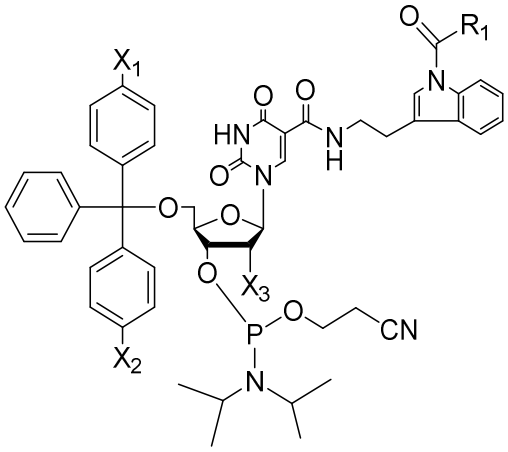
.

27. The method of claim 26, wherein the method further comprises reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound



.

28. A method of producing a compound having the structure:



, or a salt thereof;

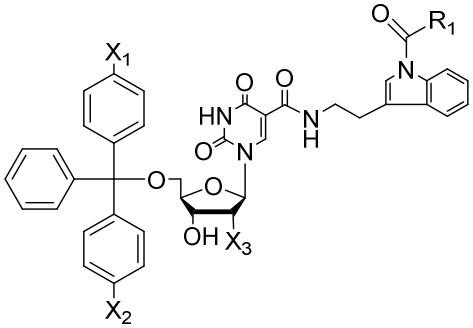
wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each independently selected from methoxy and hydrogen;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;

the method comprising reacting the compound



with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphor-amidite.

29. The method of claim 28, wherein R_1 is a *tert*-butyl.

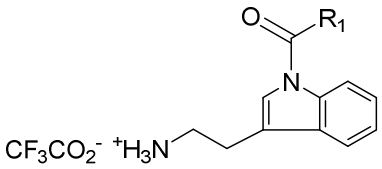
30. The method of claim 29, wherein X_1 and X_2 are methoxy.

31. The method of any one of claims 28 to 30, wherein X_3 is hydrogen.

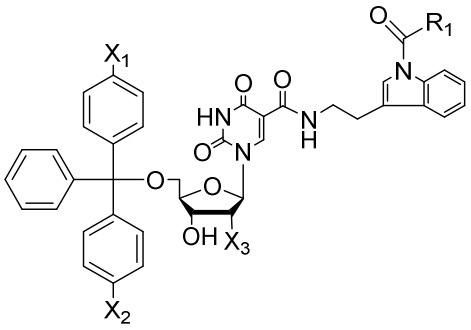
32. The method of any one of claims 28 to 30, wherein X_3 is methoxy.

33. The method of any one of claims 28 to 30, wherein X_3 is fluoro.

34. The method of any one of claims 28 to 33, wherein the method comprises reacting the compound

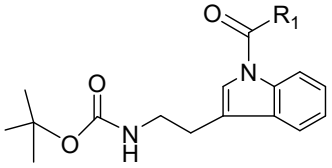


with 5'-*O*-DMT-5-(2,2,2-trifluoroethyoxy-carbonyl)-2'-deoxyuridine (TFEdU) to form the compound

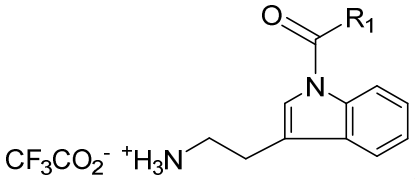


.

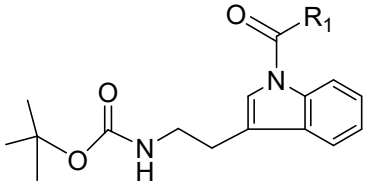
35. The method of claim 34, wherein the method comprises reacting the compound



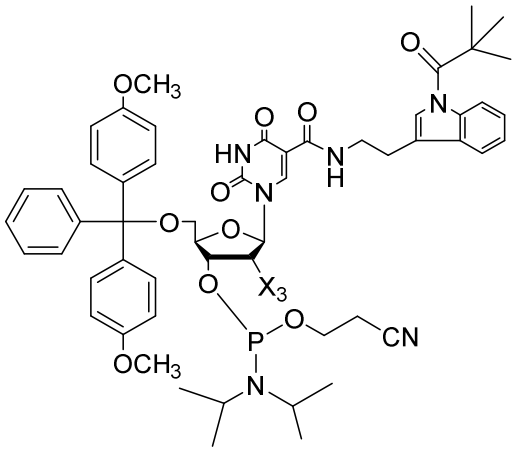
with trifluoroacetic acid to form the compound

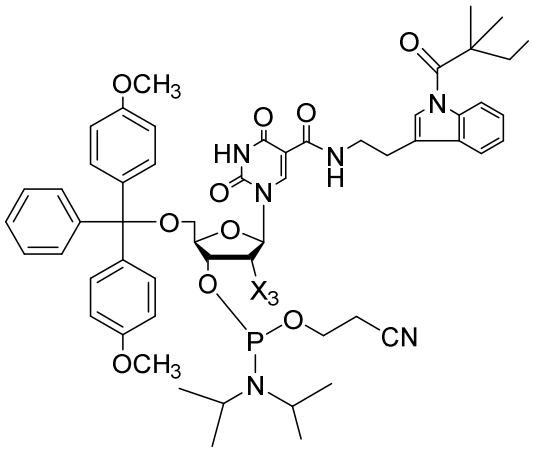


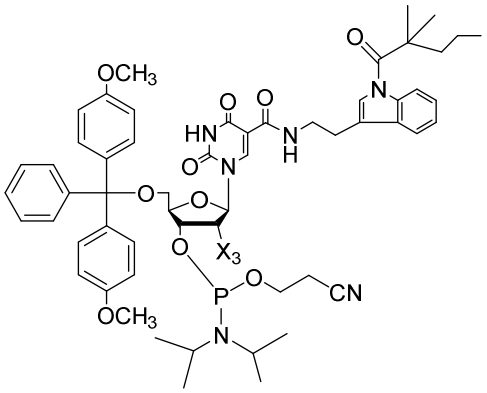
.

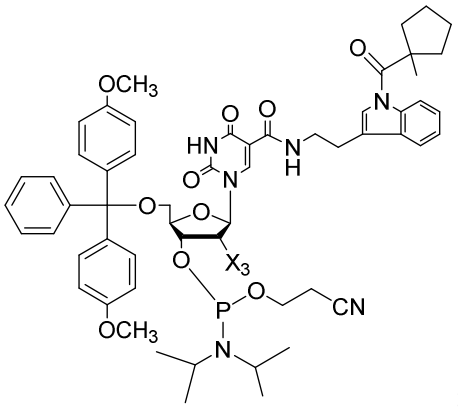
36. The method of claim 35, wherein the method comprises reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound .

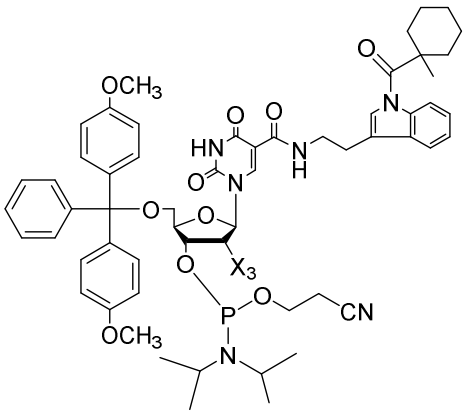
37. The method of any one of claims 28 to 36, which produces a compound selected from:

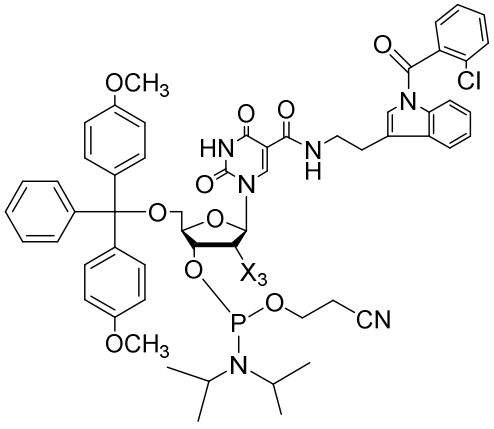

OCH3
OCH3
HN
O
N
H
X3
CN
P
N
;

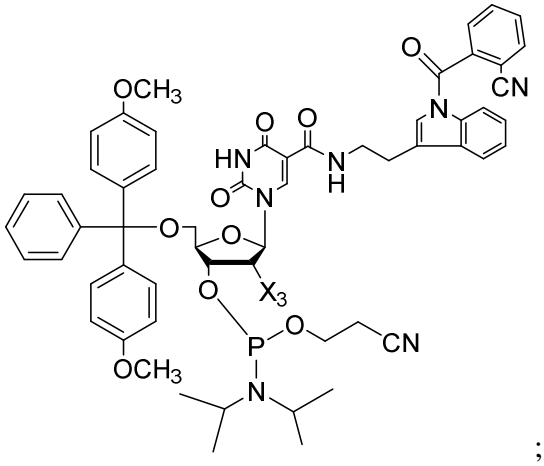

OCH3
OCH3
HN
O
N
H
X3
CN
P
N
;


OCH3
OCH3
HN
O
N
H
X3
CN
P
N
;


OCH3
O
HN
O
N
H
O
X3
CN
P
N
OCH3
;


OCH3
O
HN
O
N
H
O
X3
CN
P
N
OCH3
;

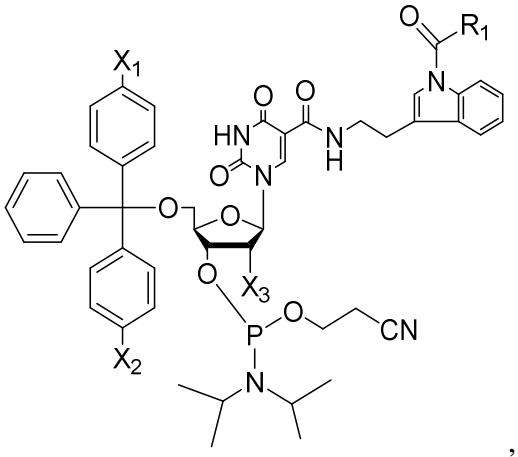

OCH3
O
Cl
HN
O
N
H
O
X3
CN
P
N
OCH3
;



;

and salts thereof.

38. A method of producing a compound having the structure:



, or a salt thereof;

wherein,

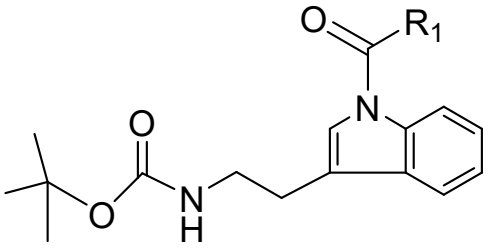
R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_1 and X_2 are each methoxy;

X_3 is hydrogen;

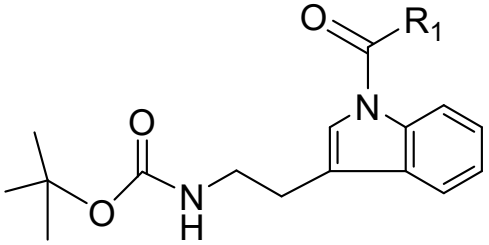
comprising the steps of:

a) reacting N-α-BOC-tryptamine with an acid chloride selected from pivaloyl chloride, 2,2-dimethylbutyroyl chloride, 2,2-dimethylvaleroyl chloride, 1-methylcyclopentane-1-carbonyl chloride, 1-methylcyclohexane-1-carbonyl chloride, 2-chlorobenzoyl chloride, and 2-cyanobenzoyl chloride, to form the compound

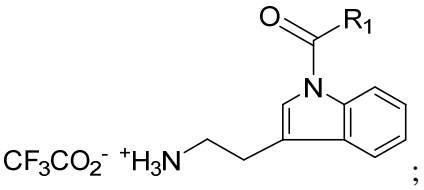


;

b) reacting the compound

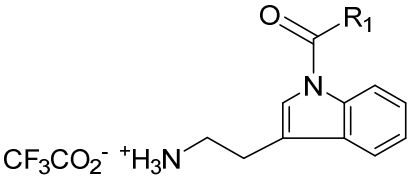


with trifluoroacetic acid to form the compound

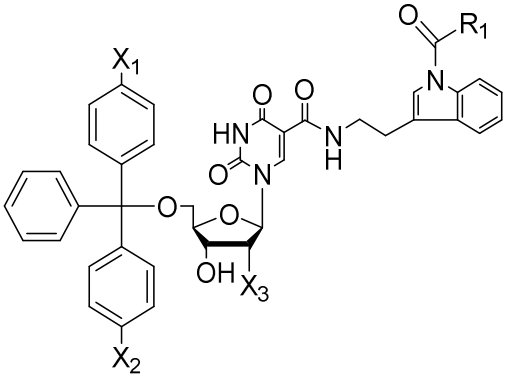


;

c) reacting the compound

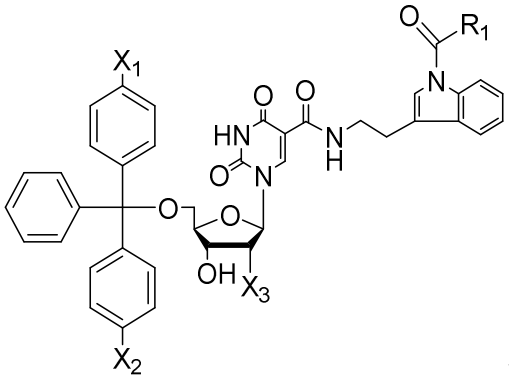


with 5'-*O*-DMT-5-(2,2,2-trifluoroethyoxy-carbonyl)-2'-deoxyuridine (TFEdU) to form the compound



; and

d) reacting the compound



with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphor-amidite.

39. The method of claim 38, wherein R_1 is a *tert*-butyl.

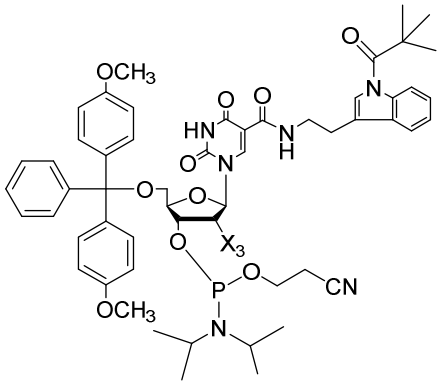
40. The method of claim 39, wherein X_1 and X_2 are methoxy.

41. The method of any one of claims 38 to 40, wherein X_3 is hydrogen.

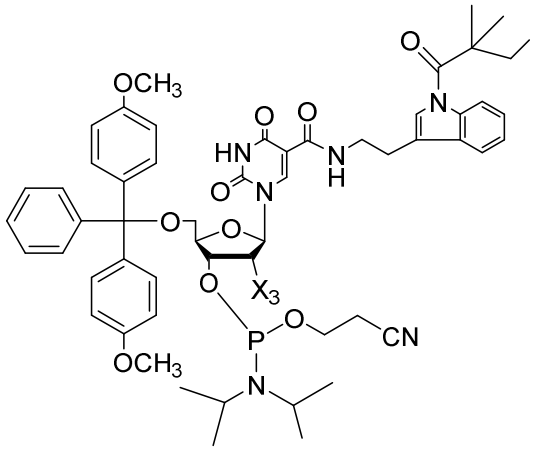
42. The method of any one of claims 38 to 40, wherein X_3 is methoxy.

43. The method of any one of claims 38 to 40, wherein X_3 is fluoro.

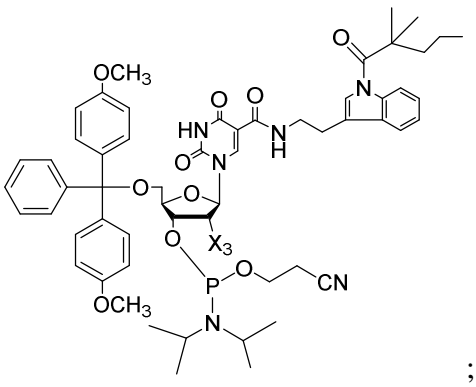
44. The method of claim 38, wherein the method produces a compound selected from:

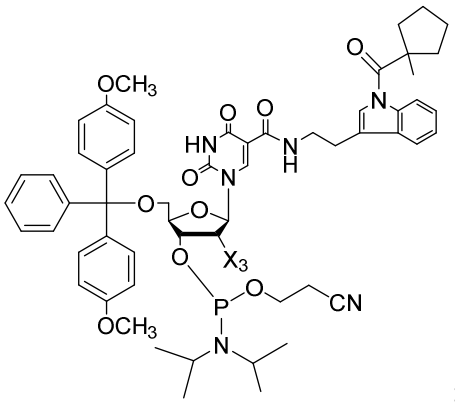


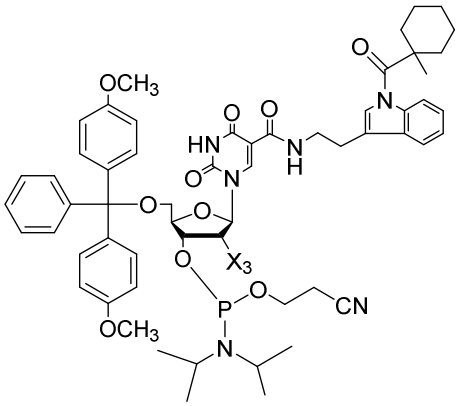
;

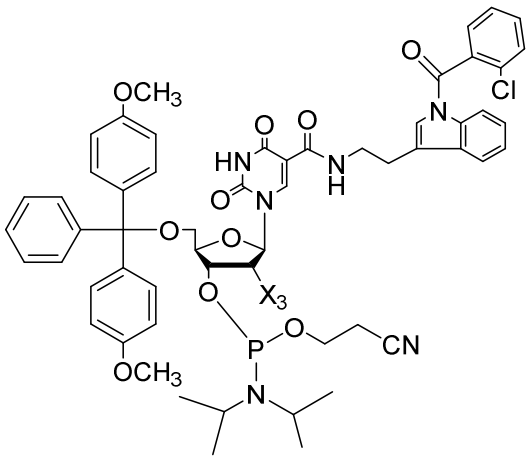


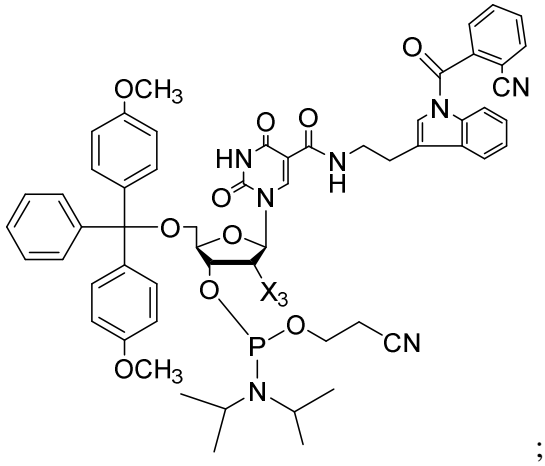
;


;


;


;

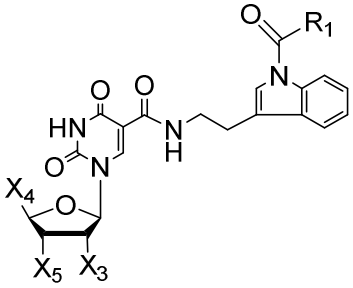

;



;

and salts thereof.

45. An oligonucleotide comprising at least one protected TrpU nucleotide, wherein at least one protected TrpU nucleotide in the oligonucleotide has the structure:



;

wherein,

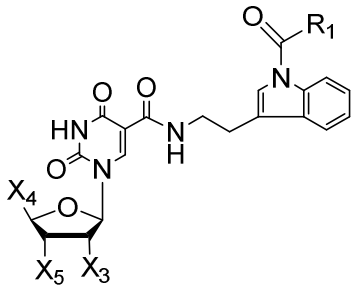
R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;

X_4 is selected from OH, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide; and

X_5 is selected from –O-ss, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein ss is a solid support, Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide.

46. A method of producing an oligonucleotide comprising at least one TrpU nucleotide, comprising incorporating at least one nucleotide having the structure:



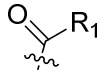
wherein,

R_1 is selected from *tert*-butyl, 1,1-dimethyl-propyl; 1,1-dimethyl-butyl; 2-chlorophenyl; 2-cyanophenyl; 1-methyl-cyclopentyl; and 1-methyl-cyclohexyl;

X_3 is selected from a methoxy, fluoro, hydrogen, and *tert*-butyldimethylsilyloxy;

X_4 is selected from OH, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide; and

X_5 is selected from –O-ss, –OR, -SR, and –Z-P(Z')(Z")O-R, wherein ss is a solid support, Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide in the oligonucleotide;

into a nucleotide sequence on a solid support; and removing the  protecting group from the at least one TrpU nucleotide incorporated into the oligonucleotide.