

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 September 2007 (27.09.2007)

PCT

(10) International Publication Number
WO 2007/107775 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/GB2007/001056

(22) International Filing Date: 23 March 2007 (23.03.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0605787.1 23 March 2006 (23.03.2006) GB

(71) Applicant (for all designated States except US): **THE QUEEN'S UNIVERSITY OF BELFAST** [GB/GB];
University Road, Belfast BT7 1NN (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **VYLE, Joseph, S.** [GB/GB]; c/o School of Chemistry and Chemical Engineering, David Keir Building, Stranmillis Road, Belfast, BT9 5AG (GB). **TUCKER, James Robert** [GB/GB]; c/o School of Chemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT (GB).

(74) Agent: **MURGITROYD & Company**; 165-169 Scotland Street, Glasgow G5 8PL (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PREPARATION OF OLIGONUCLEOTIDES WITH PHOTOSWITCHABLE PROPERTIES

(57) Abstract: Linkers which provide for the attachment of photoswitchable compounds to nucleic acid are provided. These linkers can be used to provide photo-modulatable compounds wherein the chemical and biological properties of polynucleic acid incorporating these photo-modulatable compounds can be modulated by light. Further, methods for modulation of hybridisation of polynucleic acid sequences and binding of factors, for example polymerases to polynucleic acid sequences incorporating said photo-modulatable compounds are provided.



1
2 **Preparation of oligonucleotides with photoswitchable**
3 **properties**

4
5 The present invention relates to linkers which
6 provide for the attachment of photoswitchable
7 compounds to nucleic acid to provide photo-
8 modulatable compounds wherein the chemical and
9 biological properties of polynucleic acid
10 incorporating these photo-modulatable compounds, for
11 example the hybridisation of polynucleic acid
12 sequences, including duplex formation or triplex,
13 can be modulated by light. Further, the invention
14 relates to methods for modulation of hybridisation
15 of polynucleic acid sequences and binding of
16 factors, for example polymerases to polynucleic acid
17 sequences incorporating said photo-modulatable
18 compounds.

19
20 **BACKGROUND**

21
22 Reversible optical control of DNA recognition has
23 been reported using backbone-substituted azobenzene
24 (AB)-derivatives attached via a non-nucleotidic
25 spacer.

26
27 In particular, azobenzenes tethered on D-threoninol
28 to allow incorporation of the azobenzenes into DNA
29 have been disclosed (H. Asunama, T. Ito, T. Yoshida,
30 X. Liang, M. Komiyama, **1999** *Angew. Chem. Int. Ed.*
31 *Engl.*, 38, 2393-2395).

32

1 $E \rightarrow Z$ photoisomerization of azobenzenes occurs with
2 high quantum yields at 330 - 370 nm, is not
3 particularly environment sensitive, is fatigue
4 resistant and leads to large conformational and
5 polarity changes. Modest discrimination in
6 activities between the so-called *irradiated* (mainly
7 *Z*) and more active *dark-adapted* (mainly *E*) states of
8 proteins modified by non-specific attachment of
9 azobenzenes to surface lysines was reported in 1991
10 (I. Willner, S. Rubin, A. Riklin, *J. Am. Chem. Soc.*
11 **1991**, 113, 3321-3325) and recent reports have
12 demonstrated that localization of azobenzenes at
13 strategic residues of transmembrane proteins or DNA-
14 binding oligopeptides in which the *E*-isomer is
15 inactive enables highly effective activity-
16 modulation using light (M. Banghart, K. Borges, E.
17 Isacoff, D. Trauner, R. H. Kramer, *Nat. Neurosci.*
18 **2004**, 7, 1381-1386; M. Volgraf, P Goroztiza, R.
19 Numano, R. H. Kramer, E. Y. Isacoff, D. Trauner,
20 *Nat. Chem. Biol.* **2006**, 2, 47-52 and L. Guerrero, O.
21 S. Smart, G. A. Woolley, R. K. Allemann, *J. Am.*
22 *Chem. Soc.* **2005**, 127, 15624-15629).

23

24 A single report of photoswitchable 8-17
25 deoxyribozyme-mediated RNA cleavage has been made
26 (Y. Liu, D. Sen, *J. Mol. Biol.* **2004**, 341, 887-892).
27 In this demonstration using 200-fold excess of the
28 deoxyribozyme over substrate, the incorporation of
29 two azobenzenes units for significant irradiated /
30 dark-adapted discrimination and only highly
31 attenuated activities were reported.

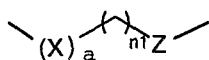
32

1 In addition, there has been reports of photo-
 2 regulation of RNA polymerase reaction by use of
 3 modified DNA carrying an azobenzene (H. Asanuma, D.
 4 Tamaru, T. Yoshida, A. Yamazawa, T. Mitsui, T.
 5 Okuni, M. Kimoto, I. Hirao, S. Yokoyama, M.
 6 Komiyama, **2001**, *Nucleic Acids Res Suppl.* (1):55-6).

8 SUMMARY OF INVENTION

10 It would be advantageous if photoswitchable
 11 properties of nucleic acids (e.g., RNA cleavage by
 12 nucleic acid catalysts) with improved properties and
 13 a greater range of targets could be achieved.

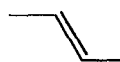
15 According to a first aspect of the present invention
 16 there is provided a linker for linking a
 17 photoswitchable compound (PS) to a base, nucleoside
 18 or a nucleotide, the linker comprising:



23 wherein X independently represents



26 (I)



26 (II)



30 (III)

or



31 (IV)

32 and a is 0 or 1;

33

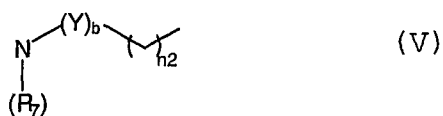
1

2

3 Z independently represents

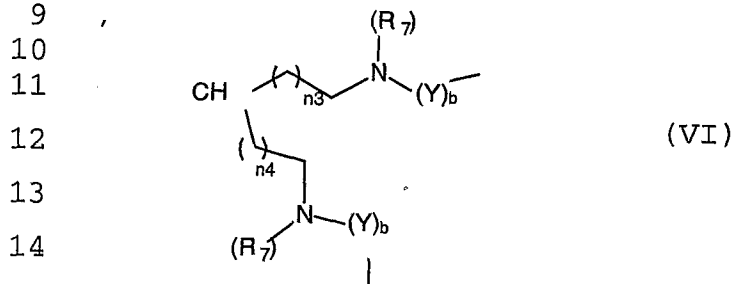
4

5



8

9



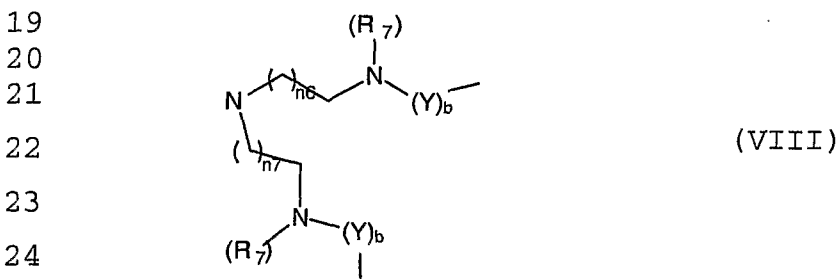
15



17

18 or

19



25

26

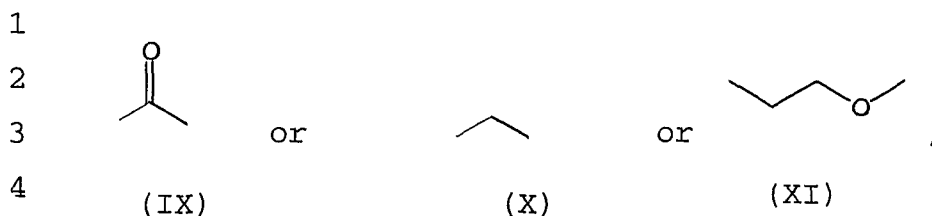
27 wherein n1, n2, n3, n4, n5, n6, and n7 are
28 independently selected from each other and each of
29 n1, n2, n3, n4, n5, n6, and n7 is 0 or an integer
30 selected from the range 1 to 12;

31

32 (R₇) represents H or CH₃;

33

34 Y independently represents



and b is independently selected from 0 or 1 or 2.

In particular embodiments, the linker may be asymmetric.

In one embodiment of a linker of the present invention X is represented by formula (I), Z is independently selected from the structures as represented by one of formula (V), (VI), (VII), or (VIII) and Y is independently selected from the structures as represented by one of formula (IX), (X) or (XI).

In an another embodiment of a linker of the present invention X is represented by formula (II), Z is independently selected from the structures as represented by one of formula (V), (VI), (VII), or (VIII) and Y is independently selected from the structures as represented by one of formula (IX), (X) or (XI).

In an another embodiment of a linker of the present invention X is represented by formula (III), Z is independently selected from the structures as represented by one of formula (V), (VI), (VII), or (VIII) and Y is independently selected from the

1 structures as represented by one of formula (IX),
2 (X) or (XI).

3

4 In an another embodiment of a linker of the present
5 invention X is represented by formula (IV), Z is
6 independently selected from the structures as
7 represented by one of formula (V), (VI), (VII), or
8 (VIII) and Y is independently selected from the
9 structures as represented by one of formula (IX),
10 (X) or (XI).

11

12 In a particular embodiment of a linker of the
13 present invention X is represented by one of the
14 structures as represented by formula (I), (II),
15 (III), or (IV), Z is independently selected from the
16 structure as represented by the formula (V), and Y
17 is independently selected from the structures as
18 represented by one of formula (IX), (X) or (XI).

19

20

21 In other particular embodiments of a linker of the
22 present invention X is represented by one of the
23 structures as represented by formula (I), (II),
24 (III), or (IV), Z is independently selected from the
25 structure as represented by the formula (VI), and Y
26 is independently selected from the structures as
27 represented by one of formula (IX), (X) or (XI).

28

29 In other particular embodiments of a linker of the
30 present invention X is represented by one of the
31 structures as represented by formula (I), (II),
32 (III), or (IV), Z is independently selected from the

1 structure as represented by the formula (VII), and Y
2 is independently selected from the structures as
3 represented by one of formula (IX), (X) or (XI).
4

5 In other particular embodiments of a linker of the
6 present invention X is represented by one of the
7 structures as represented by formula (I), (II),
8 (III), or (IV), Z is independently selected from the
9 structure as represented by the formula (VIII), and
10 Y is independently selected from the structures as
11 represented by one of formula (IX), (X) or (XI).
12

13 In alternative embodiments of a linker of the
14 present invention X is represented by one of the
15 formula (I), (II), (III), or (IV), Z is
16 independently selected from the structures as
17 represented by the formula (V), (VI), (VII), or
18 (VIII), and Y is a structure as represented by (IX).
19

20 In further alternative embodiments of a linker of
21 the present invention X is represented by one of the
22 formula (I), (II), (III), or (IV), Z is
23 independently selected from the structures as
24 represented by the formula (V), (VI), (VII), or
25 (VIII), and Y is a structure as represented by (X).
26

27 In further alternative embodiments of a linker of
28 the present invention X is represented by one of the
29 formula (I), (II), (III), or (IV), Z is
30 independently selected from the structures as
31 represented by the formula (V), (VI), (VII), or
32 (VIII), and Y is a structure as represented by (XI).

1

2 In a preferred embodiment a linker is provided
3 wherein Z is represented by formula (V) and (R7) is
4 H i.e. -NH- and Y is represented by formula (IX)
5 i.e. -C(O)-.

6

7 In embodiments of the linker wherein X is not
8 present (i.e. a = 0) linkage of the photoswitchable
9 compound to the nucleoside or nucleotide may be via
10 nucleoside or nucleotide -NH-(C-linker)-PS or
11 nucleoside or nucleotide -(C-linker)-NH-PS or
12 nucleoside or nucleotide -(C-linker)-PS.

13

14 Optionally the aliphatic groups or glycol groups of
15 the linker may suitably contain one or more amine,
16 amide, nitrile, halogen, ether, alcohol, thiol, acid
17 (such as carboxylic, sulphonic or phosphonic acid),
18 ester, aldehyde, ketone, phosphine or phosphine
19 oxide groups.

20

21 Advantageously linkers of the present invention can
22 provide for a photoswitchable compound to be
23 provided to a base, a nucleoside or a nucleotide
24 wherein the base, nucleoside or nucleotide can be
25 part of a polynucleic acid. Suitably a base is
26 selected from adenine, guanine, cytosine, uracil,
27 thymine or an analogue thereof. Suitably a
28 nucleoside can be selected from adenosine,
29 guanosine, cytidine, uridine, thymidine or an
30 analogue thereof. Suitably a nucleotide comprises a
31 nucleoside selected from adenosine, guanosine,
32 cytidine, uridine, thymidine or an analogue thereof

1 and one or more phosphate groups, in particular a
2 mono, di or tri phosphate group.

3

4 The linkers of the invention advantageously provide
5 for the attachment of photoswitchable compounds, for
6 example azobenzene moieties, to readily accessible
7 nucleoside derivatives.

8

9 Linkers of the invention interposed between a
10 nucleoside or a nucleotide and a photoswitchable
11 compound advantageously provide photo-modulatable
12 compounds.

13

14 These photo-modulatable compounds have the potential
15 to maintain essential base contacts and biological
16 activity of nucleic acids. This has been
17 demonstrated using photomodulated deoxyribozyme-
18 catalyzed RNA cleavage under multiple turnover
19 conditions.

20

21 The photo-modulatable compounds provided may be used
22 to control DNA and / or RNA function using an
23 external stimulus (light) that can be easily and
24 quantitatively provided. The photo-modulatable
25 compounds may be used, for example, in the
26 development of regulatory biocatalysts,
27 spatiotemporal control of gene expression, array-
28 based computation, information storage, biosignal
29 amplifiers or the like.

30

31 According to a second aspect of the invention there
32 is provided a nucleic acid complex comprising a

1 nucleic acid and a linker of the first aspect of the
2 invention.

3

4 The term nucleic acid comprises DNA or RNA
5 nucleosides or nucleotides. A nucleic acid complex
6 comprising a nucleic acid and a linker of the first
7 aspect of the invention can be produced
8 recombinantly, synthetically, or by any means
9 available to those in the art, including cloning
10 using standard techniques. Polynucleic acids
11 including such a nucleic acid complex may be single
12 stranded or double stranded.

13

14 Suitably a linker of the present invention can be
15 attached to the base portion of a nucleoside or
16 nucleotide. In particular embodiments, a linker is
17 attached to a 5-proparglyamino function of a
18 nucleoside.

19

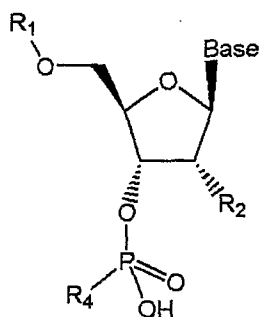
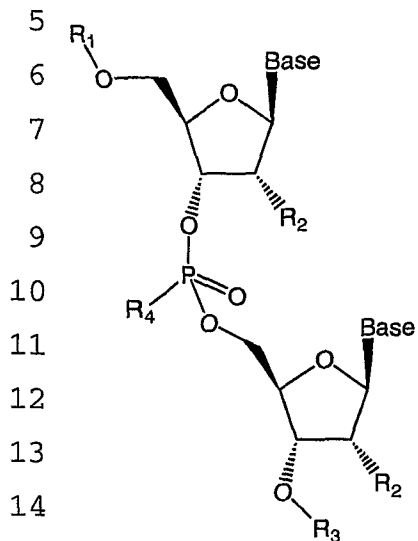
20 In other embodiments, a linker is attached to the
21 sugar portion of a nucleoside or nucleotide. In
22 particular embodiments, a linker can be attached to
23 a 2' amino function of a nucleoside.

24

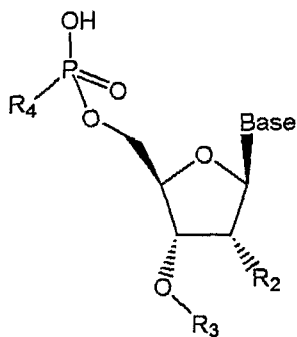
25 Advantageously a linker of the first aspect of the
26 invention allows a photoswitchable compound to be
27 conjoined to a nucleotide or nucleoside rather than
28 replace the nucleotide or nucleoside. This may be of
29 benefit as the polynucleic acid comprising such a
30 nucleotide or nucleoside including a photo-
31 switchable compound may retain properties of a
32 nucleic acid, for example base pairing.

11

1 In particular embodiments of a nucleic acid complex
2 wherein a linker of the first aspect is linked to a
3 modified phosphate diester the nucleic acid complex
4 may comprise at least one of:



17
18 or



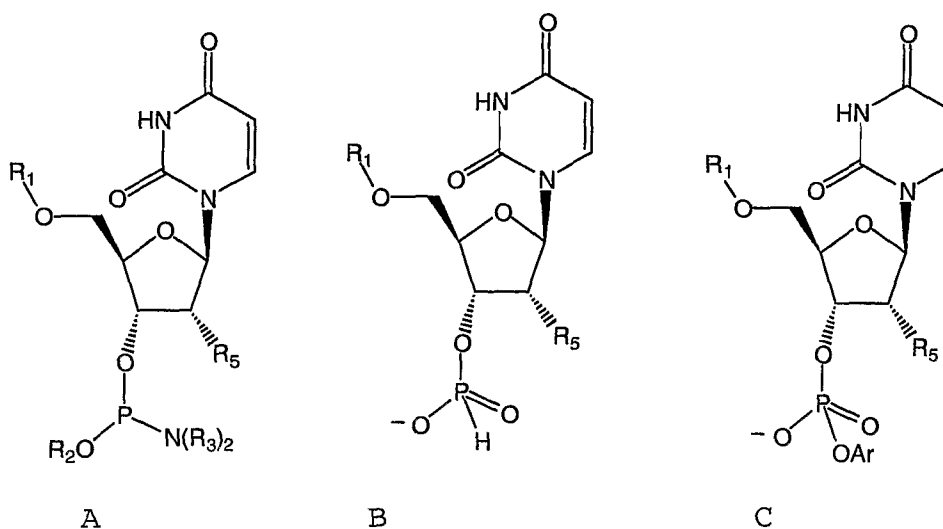
26 wherein

1 R1 is O- or is any 5' nucleic acid fragment either
 2 DNA or RNA; Base is selected from adenosine,
 3 cytosine, guanine, thymine or an analogue thereof;
 4 R2 is selected from H (DNA), OMe (2'-O-methyl RNA)
 5 or OH (RNA), R3 is O- or is any 3' nucleic acid
 6 fragment either DNA or RNA; and R4 is a linker
 7 according to a first aspect of the present
 8 invention.

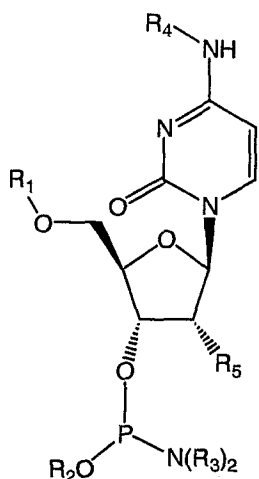
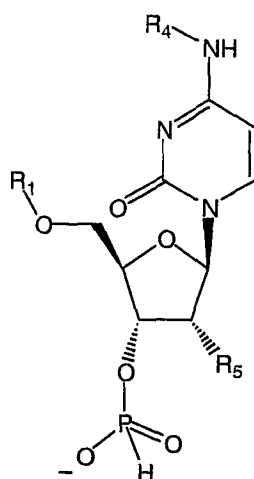
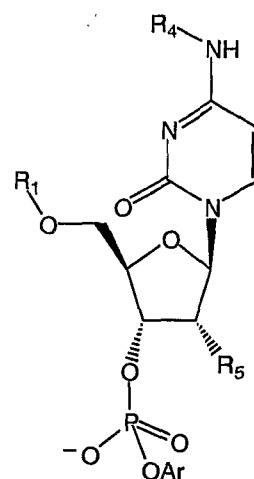
10 In embodiments of nucleic acid complex, the nucleic
 11 acid can comprise a linker of the first aspect
 12 linked to a 2' amino function of a nucleoside.

14 In such embodiments, a photoswitchable compound
 15 linked to a linker at this position will interact
 16 with the minor groove of a duplex.

18 In particular embodiments the nucleic acid complex
 19 may comprise at least one nucleotide A, B, C, D, E
 20 or F



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

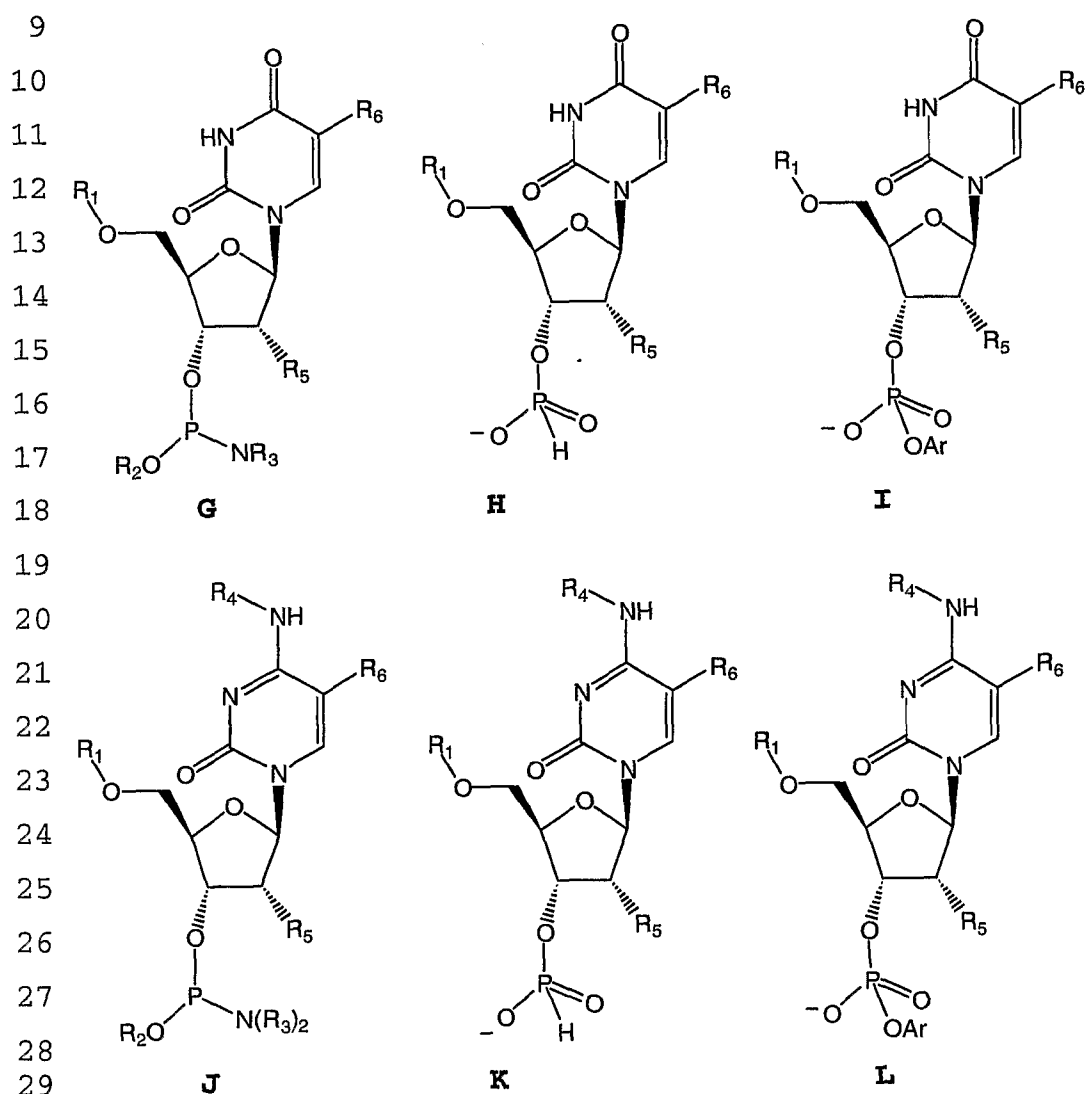
**D****E****F**

wherein

R_1 is any protecting group typical for 5';
 R_2 is any protecting group for phosphate or phosphate triesters;
 R_3 is any alkyl group for phosphoramidite;
 R_4 is any protecting group for N^4 of cytosine;
 R_5 is a linker according to a first aspect of the present invention.

Suitably R_1 can be dimethoxytrityl, pixyl, benzhydryloxy-bis(trimethylsilyloxy)silyl or levulinyl. Suitably R_2 can be 2-cyanoethyl, methyl or 3-chlorophenyl. Suitably $(R_3)_2$ can be iso-propyl (twice), methyl (twice), morpholinyl or pyrrolidinyl. Suitably R_4 can be benzoyl, N,N -dimethylformamyl or acetyl.

1 In embodiments of nucleic acid complex the nucleic
 2 acid can further comprise a photoswitchable compound
 3 linked to a 5-propargylamino function of a
 4 nucleoside via a linker of the first aspect of the
 5 invention. In such embodiments the photoswitch
 6 interacts in the major groove. In particular
 7 embodiments the nucleic acid complex may comprise at
 8 least one nucleotide G, H, I, J, K or L



30 wherein

31
 32 R1 is any protecting group typical for 5';

1 R2 is any protecting group for phosphate or
 2 phosphate triesters;
 3 R3 is any alkyl group for phosphoramidite;
 4 R4 is any protecting group for N⁴ of cytosine;
 5 R5 is selected from H (DNA), OMe (2'-O-methyl RNA),
 6 or an O-protecting group (RNA);
 7 R6 is a linker according to the first aspect of the
 8 present invention.

9
 10 Suitably R1 can be dimethoxytrityl, pixyl,
 11 benzhydryloxy-bis(trimethylsilyloxy)silyl or
 12 levulinyl. Suitably R2 can be 2-cyanoethyl, methyl
 13 or 3-chlorophenyl. Suitably (R3)₂ can be iso-propyl
 14 (twice), methyl (twice), morpholinyl or
 15 pyrrolidinyl. Suitably R4 can be benzoyl, N,N-
 16 dimethylformamyl or acetyl. Suitably R5 can be O-
 17 tert-butyldimethylsilyl, 2-fluorophenyl)-4-
 18 methoxypiperidin-4-yl, bis(2-acetoxyethoxy)methyl or
 19 triisopropylsilyloxymethyl.

20

21

22 In particular embodiments the nucleic acid complex
 23 can comprise at least one of:

24

25

26

27

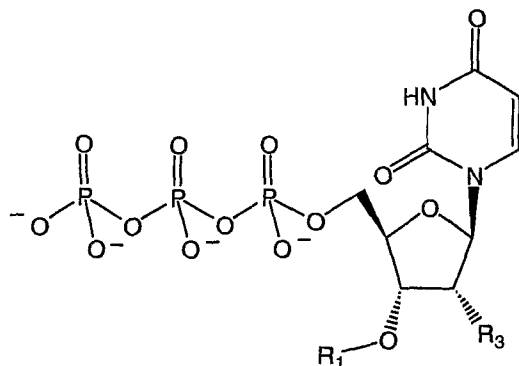
28

29

30

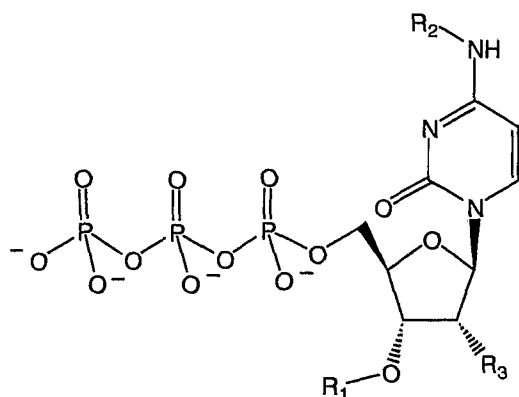
31

32



16

1 or



10 wherein

11 R1 is H or any protecting group for 3'

12 R2 is H or any protecting group for N⁴ of cytosine

13 R3 is a linker of the first aspect of the invention.

14

15 Suitably R1 can be hydrogen or acetyl.

16 Suitably R2 can be hydrogen or acetyl.

17

18 In particular embodiments the nucleic acid complex

19 can comprise at least one of:

20

21

22

23

24

25

26

27 or

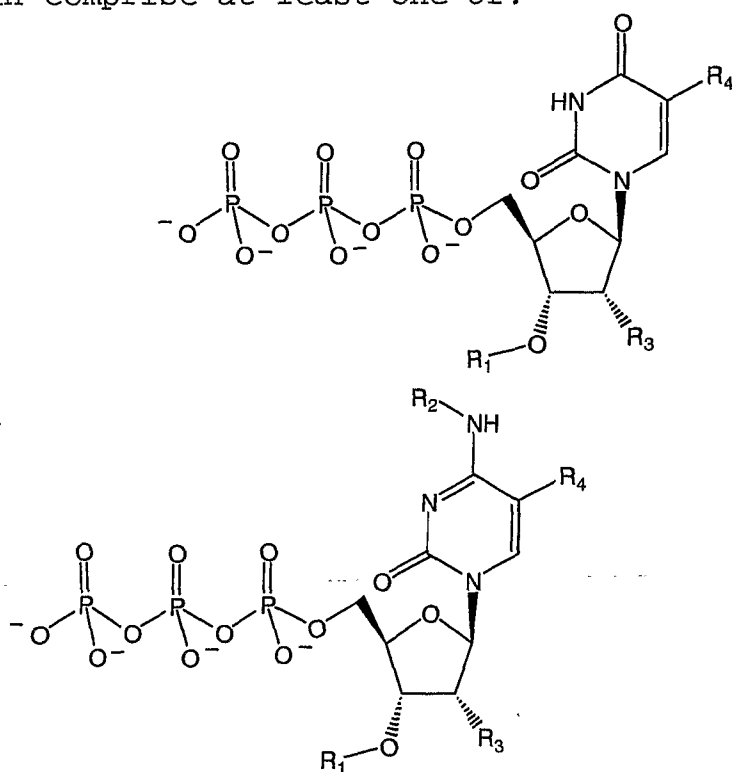
28

29

30

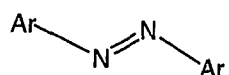
31

32

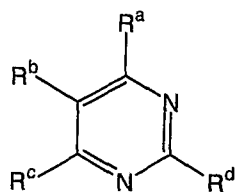
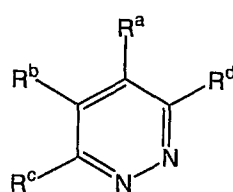
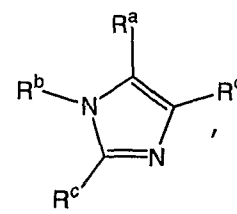
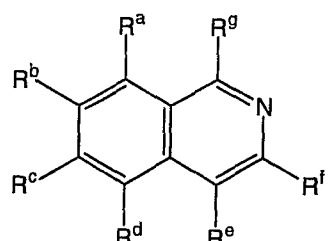
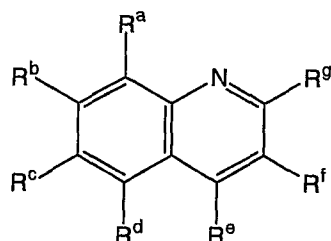
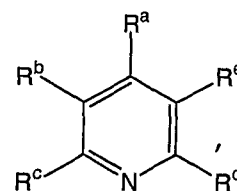
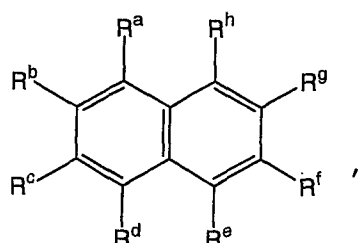
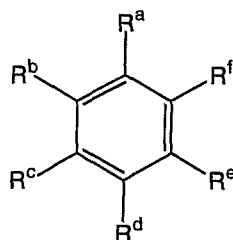
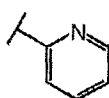
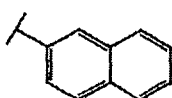
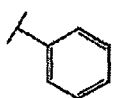


1
2
3
4 wherein
5 R1 is H or any protecting group typical for 3';
6 R2 is H or any protecting group for N⁴ of cytosine;
7 R3 is selected from H (DNA), OMe (2'-O-methyl RNA),
8 or OH (RNA);
9 R4 is a linker according to the first aspect of the
10 invention.
11
12 Suitably R1 can be hydrogen or acetyl.
13 Suitably R2 can be hydrogen or acetyl.
14
15 According to a third aspect of the present invention
16 there is provided a linker of the first aspect of
17 the present invention coupled to a photoswitchable
18 compound.
19
20 According to a fourth aspect of the invention there
21 is provided a photo-modulatable compound comprising
22 a nucleic acid complex of the second aspect of the
23 invention and a photoswitchable compound.
24
25 Any suitable photoswitchable compound can be
26 attached to the linker of the first aspect of the
27 invention.
28
29 Any suitable photoswitchable compound can be
30 attached to the linker of a nucleic acid complex of
31 the second aspect of the invention.
32

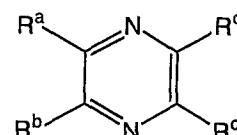
1 Suitably a photoswitchable compound is at least one
 2 compound selected from a group consisting of:
 3 azobenzenes, azonaphthalenes, azopyridines,
 4 azoimidazoles, diaryl alkene, spiropyran,
 5 spiroxazine, or azoarenes of formula



6
 7
 8 wherein AR is independently selected from the group
 9 consisting of



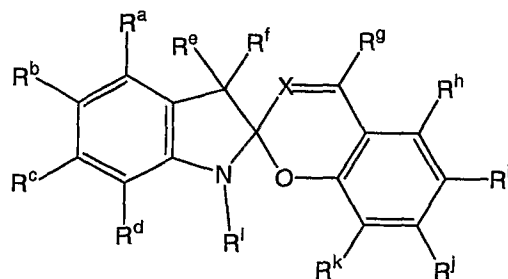
, or



24
 25
 26
 27
 28
 29 wherein each R^a may be independently selected from a
 30 C₁ to C₄₀ linear or branched alkyl or a C₃ to C₈
 31 cycloalkyl group, wherein said alkyl or cycloalkyl
 32 group may be substituted by one to three groups

1 selected from: C₁ to C₆ alkoxy, C₁ to C₆
 2 (di)alkylamino, C₆ to C₁₀ aryl, C₁ to C₃₀ aralkyl and
 3 C₁ to C₃₀ alkaryl; NO₂; CO₂R; C(O)NH₂; F;
 4 each R^b, R^c, R^d, R^e, R^f, R^g and R^h can be the same or
 5 different and are each independently selected from H
 6 or any of the defined R^a groups.

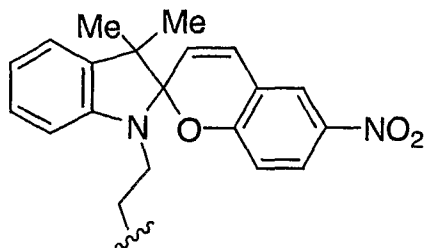
7
 8 Suitably a photoswitchable compound can be selected
 9 from the group comprising spiropyrans or
 10 spirooxazines of formula



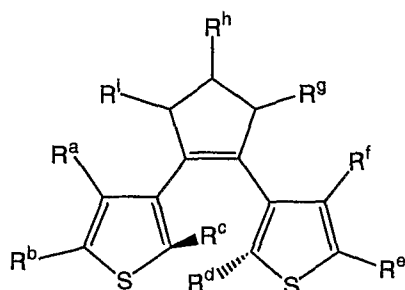
11
 12
 13
 14
 15
 16
 17
 18
 19 wherein each R^a may be independently selected from a
 20 C₁ to C₄₀ linear or branched alkyl or a C₃ to C₈
 21 cycloalkyl group, wherein said alkyl or cycloalkyl
 22 group may be substituted by one to three groups
 23 selected from: C₁ to C₆ alkoxy, C₁ to C₆
 24 (di)alkylamino, C₆ to C₁₀ aryl, C₁ to C₃₀ aralkyl and
 25 C₁ to C₃₀ alkaryl; NO₂; CO₂R; C(O)NH₂; F;
 26 each R^b, R^c, R^d, R^e, R^f, R^g, R^h, Rⁱ, R^j, R^k and R^l can
 27 be the same or different and are each independently
 28 selected from H or any of the defined R^a groups.

29
 30 Suitably a photoswitchable compound can be

31



Suitably a photoswitchable compound can be selected from the group comprising diaryl alkene of formula

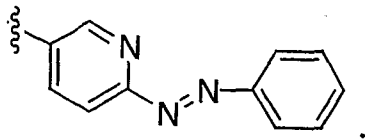


wherein each R^a may be independently selected from a C_1 to C_{40} linear or branched alkyl or a C_3 to C_8 cycloalkyl group, wherein said alkyl or cycloalkyl group may be substituted by one to three groups selected from: C_1 to C_6 alkoxy, C_1 to C_6 (di)alkylamino, C_6 to C_{10} aryl, C_1 to C_{30} aralkyl and C_1 to C_{30} alkaryl; NO_2 ; CO_2R ; $C(O)NH_2$; F ; each R^b , R^c , R^d , R^e , R^f , R^g , R^h and R^i can be the same or different and are each independently selected from H or any of the defined R^a groups.

Preferred photoswitchable compounds are those which are stable to heat, for example *meta*-azobenzenes and azopyridines.

As will be appreciated to those of skill in the art, the photoswitchable compounds may be attached to a base, a nucleoside or a nucleotide at an ortho, meta

1 or para configuration, as for example shown in
2 figure 1 in relation to the azobenzene compound
3



5
6 Preferably a 2'-deoxy-2'-aminouridine is linked to
7 the azobenzene via an amide.

8
9 According to a fifth aspect of the present invention
10 there is provided a polynucleic acid comprising a
11 photo-modulatable compound of the fourth aspect of
12 the present invention.

13
14 In one embodiment said polynucleic acid comprises
15 DNA. In another embodiment said polynucleic acid
16 comprises RNA.

17
18 In a polynucleic acid comprising a particular
19 embodiment of a photo-modulatable compound of the
20 fourth aspect of the invention wherein the
21 photoswitchable compound is phenylazobenzene,
22 irradiation of the compound at 350-360 nm converts
23 the E-azobenzene to a Z-azobenzene.

24
25 In a polynucleic acid comprising a particular
26 embodiment of a photo-modulatable compound of the
27 fourth aspect of the invention wherein the
28 photoswitchable compound is spiropyran, irradiation
29 of the compound at 350-360 nm converts the

1 spiropyran to a merocyanine zwitterion or cation
2 depending upon the pH.

3

4 The *E*-azobenzene and spiropyran isomers promote
5 nucleic acid duplex stability whilst *Z*-azobenzene
6 and merocyanine zwitterion isomers decrease nucleic
7 acid duplex stability.

8

9 In particular embodiments a polynucleic acid
10 comprising a photo-modulatable compound of the
11 fourth aspect of the present invention may further
12 comprise a fluorescent-quencher probe.

13

14 In said embodiments a fluorescent-quencher probe may
15 be used to measure distance and geometry within
16 nucleic acids, and/ or to monitor hybridisation of
17 target sequences.

18

19 According to a sixth aspect of the present invention
20 there is provided a method of modulating
21 hybridisation of at least a first polynucleic acid
22 with at least a second polynucleic acid comprising
23 the steps:

24 - providing the at least first polynucleic acid
25 with at least one photo-modulatable compound of
26 the fourth aspect of the invention;

27 - providing the at least second polynucleic
28 acid capable of hybridising to the at least
29 first polynucleic acid in conditions suitable
30 to allow hybridisation of the at least second
31 polynucleic acid to the at least first
32 polynucleic acid; and

1 - providing a wavelength of light capable of
2 inducing photoisomerization of the photo-
3 modulatable compound in said at least first
4 polynucleic acid,
5 wherein photoisomerization of the photo-modulatable
6 compound modulates hybridisation of the at least
7 first and second polynucleic acids to each other.
8

9 According to a seventh aspect of the present
10 invention there is provided a method of modulating
11 the activity of a ribozyme comprising the steps of:

12 - providing a ribozyme wherein at least one
13 nucleotide of the catalytic core of the
14 ribozyme is replaced by at least one photo-
15 modulatable compound of the fourth aspect of
16 the invention; and

17 - providing a wavelength of light capable of
18 inducing photoisomerization of the photo-
19 modulatable compound,
20 wherein the activity of the ribozyme is modulated.

21

22 Suitably the ribozyme is a deoxyribozyme.

23

24 According to an eighth aspect of the present
25 invention there is provided a method of modulating
26 the binding of a polymerase to a polynucleic acid
27 comprising the steps of:

28 - providing a polynucleic acid with at least
29 one a photo-modulatable compound of the fourth
30 aspect of the invention; and

1 - providing a wavelength of light capable of
2 inducing photoisomerization of the photo-
3 modulatable compound,
4 wherein the binding of the polymerase to the
5 polynucleic acid is modulated.

6

7 In one embodiment the polymerase is a RNA
8 polymerase.

9

10 Preferred features and embodiments of each aspect of
11 the invention are as for each of the other aspects
12 mutatis mutandis unless context demands otherwise.

13

14 Embodiments of the present invention will now be
15 described by way of example only and with reference
16 to the accompanying drawings in which:

17

18 Figure 1 illustrates a structure of azobenzene-
19 appended to uridylate analogues;

20

21 Figure 2 illustrates 10-23 deoxyribozymes as
22 prepared (FAM = 6-fluoresceinyl-), cleavage
23 site indicated by arrow.

24

25 Figure 3 illustrates photo-isomerization of
26 azobenzene unit (*irrDR* and *d-aDR* refer to
27 *irradiated* and *dark-adapted* 10-23
28 deoxyribozymes respectively);

29

30 Figure 4 illustrates reversibility of RNA (20
31 μM) cleavage by 10-23 deoxyribozymes DR_o (1
32 μM), DR_m (1 μM) and DR_p (2 μM) under multiple

1 turnover conditions. Key: ○ *d*-aDR_o; ● *irr*DR_o;
2 △ *d*-aDR_m; ▲ *irr*DR_m; □ *d*-aDR_p; ■ *irr*DR_p;

3

4 Figure 5 is an illustration of a photo-
5 modulatable compound of the fourth aspect of
6 the invention comprising a linker of the first
7 aspect of the invention interposed between a
8 photoswitchable compound (PS) and a nucleotide,
9 nucleoside or base;

10

11 Figure 6 is an illustration of a nucleic acid
12 complex of the second aspect of the invention
13 comprising a linker of the first aspect of the
14 invention linked to base, nucleotide or
15 nucleoside;

16

17 Figure 7 is an illustration of a linker of the
18 first aspect of the invention linked to a
19 photoswitchable compound (PS);

20

21 Figure 8 is an illustration of photoswitchable
22 compounds (PS) which may be bound to a linker
23 of the invention;

24

25 Figure 9 illustrates photoswitching of
26 azobenzenes (E↔Z) or spiropyran (SP) ↔
27 merocyanine (MC);

28

29 Figure 10 illustrates a methodology for
30 preparing photoswitchable 2'-amino-
31 2'-deoxyuridine derivatives, noting that the
32 photoswitch compound (PS) as illustrated in

1 (2a-d), (3a-d) and (4a-d) can be replaced by
2 any of (4a), (4b), (4c) or (4d) as shown;

3
4 Figure 11 illustrates photoswitching of
5 d(ACC4dGGTA). a) HPLC chromatograms of oligomer
6 following purification and irradiation b)
7 degradation pathway during irradiation; and

8
9 Figure 12 illustrates Arrhenius plots derived
10 from first order rate constants of the thermal
11 isomerisation of Z-ABs within Irr-d(ACC4a-
12 cGGTA) under isothermal conditions at 55°C -
13 80°C.

14
15 **Synthesis of phosphoramidites**

16
17 Dichloromethane (DCM) was dried under reflux over
18 calcium hydride, distilled and stored over activated
19 3Å molecular sieves under argon. Triethylamine and
20 n-butylamine were dried under reflux over calcium
21 hydride and distilled immediately prior to use; N,N-
22 diisopropylethylamine (DIPEA) was similarly purified
23 but stored over activated 3Å molecular sieves for
24 upto 6 weeks. DMF was obtained as anhydrous solvent
25 over molecular sieves from Fluka. Dry MeOH was
26 obtained by distillation from magnesium turnings and
27 stored over activated 3Å molecular sieves.
28 Tetrahydrofuran (THF) was distilled from
29 sodium/benzophenone, stored over activated 3Å
30 molecular sieves and used within 7 days. Acid-free
31 ethyl acetate was prepared by prewashing with
32 saturated Na₂CO₃. CDCl₃ was passed through activated

1 basic Al₂O₃ immediately prior to use and NMR's
2 obtained within 1 hour (³¹P, ¹³C) or 12 hours (¹H) of
3 the CDCl₃ solution being prepared. All other reagents
4 were purchased from commercial suppliers and used
5 without further purification. Silica gel (particle
6 size 30-60 μm) for flash column chromatography was
7 obtained from BDH and dried at 300°C immediately
8 prior to use.

9
10 UV-Visible data were obtained with a VARIAN CARY 100
11 Spectrometer. Nuclear Magnetic Resonance (NMR)
12 spectra were run on Bruker AC-250 and AMX-400
13 spectrometers.

14

15 5'-O-(4,4'-dimethoxytrityl)-2'-(N-(o-
16 phenylazo)benzamido)-2'-deoxyuridine-3'-O-(2-
17 cyanoethyl-N,N-diisopropyl-amino)-phosphoramidite.

18

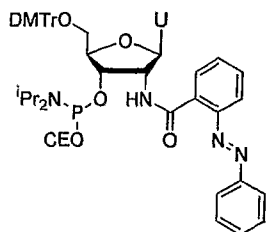
19

20

21

22

23



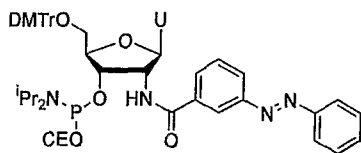
24 To a stirred solution of o-phenylazobenzoic acid-N-
25 hydroxysuccinimidyl ester (260 mg, 0.80 mmol) in
26 anhydrous DMF (5 ml) at room temperature was added
27 n-butylamine (10 μl, 0.10 mmol) and the reaction
28 mixture maintained under these conditions for a
29 further 10 min. This solution was transferred under
30 argon to a flask containing 5'-O-(4,4'-
31 dimethoxytrityl)-2'-amino-2'-deoxyuridine (250 mg,
32 0.46 mmol) and N,N-diisopropylethylamine (DIPEA: 80

1 μ l, 0.47 mmol) added. The reaction was stirred under
2 ambient conditions for a further 6 hours, a further
3 aliquot of DIPEA (100 μ l, 0.59 mmol) was added and
4 the reaction stored at 3°C overnight. Excess
5 activated ester was quenched by addition of *n*-
6 butylamine (190 μ l, 1.92 mmol) and stirring at room
7 temperature for 1 hour. The reaction mixture was
8 diluted with ethyl acetate (250 ml) and washed
9 successively with aqueous saturated NaHCO₃ (2 x 150
10 ml) and saturated NaCl (2 x 150 ml). The organics
11 were dried over anhydrous sodium sulfate, filtered
12 and solvent removed *in vacuo*. The resultant red gum
13 was purified by silica gel column chromatography,
14 eluting with a gradient of 0 - 2% (v/v) methanol in
15 DCM containing 1% (v/v) triethylamine to give the
16 title compound as an orange solid (250 mg, 72 %).

17
18 To a stirred solution of 5'-O-(4,4'-
19 dimethoxytrityl)-2'-(*N*-(*o*-phenylazo)benzamido)-2'-
20 deoxyuridine (180 mg, 0.24 mmol) and DIPEA (90 μ l,
21 0.98 mmol) in anhydrous DCM (4 ml) and THF (1 ml) at
22 room temperature was added 2-cyanoethyl-*N,N*-
23 diisopropylaminochlorophosphoramidite (86 μ l, 0.53
24 mmol) dropwise over 3 min. The reaction was
25 maintained under these conditions for a further 30
26 min and then quenched following addition of
27 anhydrous methanol (60 μ l, 1.48 mmol) and DIPEA (60
28 μ l, 0.65 mmol) and stirring for a further 10 min.
29 The reaction mixture was diluted with acid-free
30 ethyl acetate (50 ml) and washed successively with
31 aqueous saturated NaHCO₃ (2 x 50 ml), saturated NaCl
32 (1 x 50 ml). The organics were dried over anhydrous

1 sodium sulfate, filtered and solvent removed in
 2 vacuo. The resultant solid was purified by silica
 3 gel column chromatography, eluting with 2% (v/v)
 4 solutions of triethylamine in 3:1 DCM:40-60
 5 petroleum ether; pure DCM and 3:1 DCM : ethyl
 6 acetate. The title compound was isolated as a brown
 7 foam (194 mg, 0.20 mmol, 85 %).

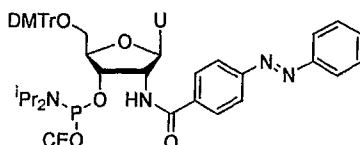
8
 9 5'-O-(4,4'-dimethoxytrityl)-2'-(N-(m-
 10 phenylazo)benzamido)-2'-deoxyuridine-3'-O-(2-
 11 cyanoethyl-N,N-diisopropylamino)-phosphoramidite.



13
 14
 15
 16
 17 To a stirred solution of *m*-phenylazobenzoic acid-*N*-
 18 hydroxysuccinimidyl ester (260 mg, 0.80 mmol) in
 19 anhydrous DMF (5 ml) at room temperature was added
 20 *n*-butylamine (10 μ l, 0.10 mmol) and the reaction
 21 mixture maintained under these conditions for a
 22 further 10 min. The reaction mixture was transferred
 23 under argon to a flask containing 5'-O-(4,4'-
 24 dimethoxytrityl)-2'-amino-2'-deoxyuridine (250 mg,
 25 0.46 mmol) and *N,N*-diisopropylethylamine (DIPEA: 80
 26 μ l, 0.47 mmol) added. The reaction was stirred under
 27 ambient conditions for a further 6 hours, a further
 28 aliquot of DIPEA (100 μ l, 0.59 mmol) was added and
 29 the reaction stored at 3°C overnight. Excess
 30 activated ester was quenched by addition of *n*-
 31 butylamine (190 μ l, 1.92 mmol) and stirring at room
 32 temperature for 1 hour. The reaction mixture was

1 diluted with ethyl acetate (250 ml) and washed
2 successively with aqueous saturated NaHCO_3 (2 x 150
3 ml) and saturated NaCl (2 x 150 ml). The organics
4 were dried over anhydrous sodium sulfate, filtered
5 and solvent removed *in vacuo*. The resultant red gum
6 was purified by silica gel column chromatography,
7 eluting with a gradient of 0 - 2% (v/v) methanol in
8 DCM containing 1% (v/v) triethylamine to give the
9 title compound as an orange solid (250 mg, 72 %).
10
11 To a stirred solution of 5'-O-(4,4'-
12 dimethoxytrityl)-2'-(N-(m-phenylazo)benzamido)-2'-
13 deoxyuridine (134 mg, 0.18 mmol) and DIPEA (67 μl ,
14 0.73 mmol) in anhydrous DCM (3 ml) and THF (0.5 ml)
15 at room temperature was added 2-cyanoethyl-N,N-
16 diisopropylaminochlorophosphoramidite (57 μl , 0.36
17 mmol) dropwise over 3 min. The reaction was
18 maintained under these conditions for a further 30
19 min and then quenched following addition of
20 anhydrous methanol (90 μl , 2.22 mmol) and DIPEA (90
21 μl 0.98 mmol) and stirring for a further 10 min. The
22 reaction mixture was diluted with acid-free ethyl
23 acetate (50 ml) and washed successively with aqueous
24 saturated NaHCO_3 (2 x 50 ml), saturated NaCl (1 x 50
25 ml). The organics were dried over anhydrous sodium
26 sulfate, filtered and solvent removed *in vacuo*. The
27 resultant solid was purified by silica gel column
28 chromatography, eluting with 2% (v/v) solutions of
29 triethylamine in 3:1 DCM:40-60 petroleum ether; pure
30 DCM and 3:1 DCM : ethyl acetate. The title compound
31 was isolated as an orange foam (160 mg, 0.17 mmol,
32 93 %).

5'-O-(4,4'-dimethoxytrityl)-2'-(N-(p-phenylazo)benzamido)-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N-diisopropylamino)-phosphoramidite.



To a stirred solution of *p*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester (260 mg, 0.80 mmol) in anhydrous DMF (5 ml) at room temperature was added *n*-butylamine (10 μ l, 0.10 mmol) and the reaction mixture maintained under these conditions for a further 10 min. The reaction mixture was transferred under argon to a flask containing 5'-O-(4,4'-dimethoxytrityl)-2'-amino-2'-deoxyuridine (250 mg, 0.46 mmol) and *N,N*-diisopropylethylamine (DIPEA: 80 μ l, 0.47 mmol) added. The reaction was stirred under ambient conditions for a further 6 hours, a further aliquot of DIPEA (100 μ l, 0.59 mmol) was added and the reaction stored at 3°C overnight. Excess activated ester was quenched by addition of *n*-butylamine (190 μ l, 1.92 mmol) and stirring at room temperature for 1 hour. The reaction mixture was diluted with ethyl acetate (250 ml) and washed successively with aqueous saturated NaHCO₃ (2 x 150 ml) and saturated NaCl (2 x 150 ml). The organics were dried over anhydrous sodium sulfate, filtered and solvent removed *in vacuo*. The resultant red gum was purified by silica gel column chromatography,

1 eluting with a gradient of 0 - 2% (v/v) methanol in
2 DCM containing 1% (v/v) triethylamine to give the
3 title compound as an orange solid (250 mg, 72 %).

4

5 To a stirred solution of 5'-O-(4,4'-
6 dimethoxytrityl)-2'-(N-(p-phenylazo)benzamido)-2'-
7 deoxyuridine (250 mg, 0.33 mmol) and DIPEA (125 µl,
8 1.36 mmol) in anhydrous DCM (5 ml) and THF (2 ml) at
9 room temperature was added 2-cyanoethyl-N,N-
10 diisopropylaminochlorophosphoramidite (106 µl, 0.66
11 mmol) dropwise over 3 min. The reaction was
12 maintained under these conditions for a further 30
13 min and then quenched following addition of
14 anhydrous methanol (60 µl, 1.48 mmol) and DIPEA (60
15 µl 0.65 mmol) and stirring for a further 10 min. The
16 reaction mixture was diluted with acid-free ethyl
17 acetate (250 ml) and washed successively with
18 aqueous saturated NaHCO₃ (2 x 250 ml), saturated NaCl
19 (1 x 150 ml). The organics were dried over anhydrous
20 sodium sulfate, filtered and solvent removed in
21 vacuo. The resultant solid was purified by silica
22 gel column chromatography, eluting with 2% (v/v)
23 solutions of triethylamine in 3:1 DCM: 40-60
24 petroleum ether; pure DCM and 3:1 DCM : ethyl
25 acetate. The title compound was isolated as an
26 orange foam (308 mg, 0.32 mmol, 97 %).

27

28 5'-O-(4,4'-Dimethoxytrityl)-2'-N-(3-[3',3'-dimethyl-
29 6-nitrospiropyro[2H-1-benzopyran-2,2'-indoline]-1-
30 yl]-propanamidyl)-2'-deoxyuridine

1 To a stirred solution of *N*-hydroxysuccinimidyl 3-
2 [3',3'-dimethyl-6-nitrospiropyro[2*H*-1-benzopyran-
3 2,2'-indoline]-1-yl propanoate (P. Zhang, J. B.
4 Meng, X. L. Li, T. Matsuura, and Y. M. Wang, *Journal*
5 *of Heterocyclic Chemistry*, 2002, 39, 179) (381 mg,
6 0.80 mmol) in anhydrous DMF (5 ml) under argon at
7 room temperature was added *n*-butylamine (10 μ l, 0.10
8 mmol) and the reaction mixture maintained under
9 these conditions for a further 10 min. The reaction
10 mixture was transferred under argon to a flask
11 containing 5'-*O*-(4,4'-dimethoxytrityl)-2'-amino-2'-
12 deoxyuridine (250 mg, 0.46 mmol) and to the stirred
13 suspension was added *N,N*-diisopropylethylamine
14 (DIPEA: 80 μ l, 0.47 mmol) following which addition a
15 clear violet solution resulted. The reaction was
16 stirred under ambient conditions for a further 4
17 hours, a further aliquot of DIPEA (190 μ l, 1.12
18 mmol) was added and the reaction stored at 3°C
19 overnight. Excess activated ester was quenched by
20 addition of *n*-butylamine (190 μ l, 1.92 mmol) and
21 stirring at room temperature for 1 hour. The
22 reaction mixture was diluted with ethyl acetate (250
23 ml) and washed successively with aqueous saturated
24 NaHCO₃ (2 x 150 ml) and saturated NaCl (150 ml). The
25 organics were dried over anhydrous sodium sulfate,
26 filtered and solvent removed *in vacuo*. The resultant
27 violet oil was purified by silica gel column
28 chromatography, packed with DCM containing 2% (v/v)
29 triethylamine and eluting with a gradient of 0 - 2%
30 (v/v) methanol in DCM to give the title compound as
31 an impure violet foam which was used directly in the

1 subsequent phosphitylation (370 mg, <0.41 mmol, <90
2 %).

3

4 5'-O-(4,4'-Dimethoxytrityl)-2'-N-(3-[3',3'-dimethyl-
5 6-nitrospiropyro[2H-1-benzopyran-2,2'-indoline]-1-
6 yl]-propanamidyl)-2'-deoxyuridine-3'-O-(2-
7 cyanoethyl-N,N-diisopropylamino)phosphoramidite

8 To a stirred red-purple solution of 5'-O-(4,4'-
9 Dimethoxytrityl)-2'-N-(3-[3,3-dimethyl-6'-
10 nitrospiropyro[2'H-chromene-2,2'-(2,3- dihydro-1H-
11 indole)]-1-yl]-propanamidyl)-2'-deoxyuridine (370
12 mg, <0.41 mmol) and DIPEA (154 µl, 1.69 mmol) in
13 anhydrous DCM (6 ml) and THF (1 ml) at room
14 temperature under argon was added 2-cyanoethyl-N,N-
15 diisopropylaminochloro-phosphoramidite (132 µl, 0.83
16 mmol) dropwise over 3 min during which time the
17 solution decoloured. The reaction was maintained
18 under these conditions for a further 30 min and then
19 quenched following addition of anhydrous methanol
20 (80 µl, 1.97 mmol) and DIPEA (80 µl, 0.88 mmol) and
21 stirring for a further 10 min. The reaction mixture
22 was diluted with acid-free ethyl acetate (50 ml) and
23 washed successively with aqueous saturated NaHCO₃ (2
24 x 50 ml), saturated NaCl (1 x 50 ml). The organics
25 were dried over anhydrous sodium sulfate, filtered
26 and solvent removed *in vacuo*. The resultant solid
27 was purified by silica gel column chromatography,
28 eluting with 2% (v/v) solutions of triethylamine in
29 3:1 DCM:40-60 petroleum ether; pure DCM and 3:1 DCM
30 : ethyl acetate.

31

1 Linking of a photoswitchable compound to phosphate

2

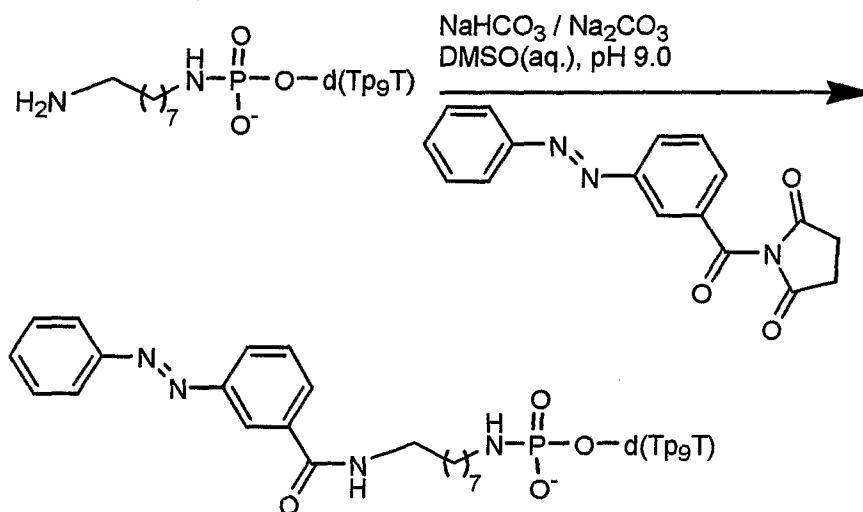
3 An example of a method of linking a photoswitchable
 4 compound to a phosphate of an oligonucleotide is
 5 described. An amino-modified oligonucleotide
 6 (typically 1 μmol) was dissolved in sodium
 7 carbonate/bicarbonate buffer at pH 9 (500 μl) and
 8 DMSO (500 μl). Solid NHS ester (typically 5 mg) was
 9 added to give a saturated solution following
 10 sonication and vortex mixing. The reaction mixture
 11 was incubated at 37°C overnight. The oligo-
 12 azobenzene conjugate was separated from salts by
 13 size exclusion on a NAP-10 column. It was then
 14 further purified by RP HPLC.

15

16

17

18



Key

p = -O-PO₂-O-

19

1

2

3

Synthesis of oligonucleotides

4 Oligosynthesis was carried out on Beckman Oligo
5 1000M and Expedite 8909 DNA/RNA synthesizers on a 1
6 μ mol scale using standard DNA and RNA
7 phosphoramidites from Link Technologies. Standard
8 nucleobase protecting groups and 2'-O-TBDMS-
9 protection were used. Benzylthiotetrazole (0.2 M)
10 was used as activating agent. Oxidation was
11 performed using 8:1:1 THF:pyridine:H₂O containing
12 either 50 mM or 20 mM I₂ for, respectively, Beckman-
13 or Expedite-run syntheses. Unmodified DNA and RNA
14 were synthesized DMT-on and deprotected and purified
15 according to standard procedures. The coupling time
16 was extended (5-6 min) for 2'-azobenzene-modified
17 amidites. Deprotection of azobenzene-modified
18 oligonucleotides was performed with 1.5 ml of an
19 anhydrous mixture of NH₃/MeOH (dry MeOH saturated
20 with gaseous ammonia at -10°C) overnight at room
21 temperature. The decanted supernatant was evaporated
22 and the crude oligonucleotides purified by RP-HPLC
23 (column: Hichrom KR100-5C18; buffer A: 0.1 M TEAAc,
24 pH 6.5, 5% (v/v) MeCN; buffer B: 0.1 M TEAAc, pH
25 6.5, 65% (v/v) MeCN) monitoring absorbance in the
26 range 200 - 400 (or 600) nm with a diode array
27 detector. After evaporation of the buffer the
28 oligonucleotides were desalted using standard SepPak
29 protocols (Waters) and concentrations determined
30 using UV absorbance at 260 nm.

32

1 **MALDI-TOF analysis of DNA-azobenzene conjugates with**
2 **reference figure 1**

3

DNA oligomer	Mass (calc.)
DRO	8438.79 (8440.46)
DRm	8434.24 (8440.46)
DRp	8434.17 (8440.46)
ACC1oGGTA	2620.29 (2617.51)
ACC1mGGTA	2619.58 (2617.51)
ACC1pGGTA	2626.46 (2617.51)

4

5 **Photoswitching of DNA-azobenzene conjugates**

6

7 For the irradiation a Medium Pressure Hg Arc-lamp
8 (100 W, Engelhard Hanovia of Canada Ltd.) was
9 utilized. A band-pass filter was used for
10 irradiation at 366 nm (4.13 W) and a cutoff filter
11 was used for irradiation >400 nm (435 nm, 3.36 W) in
12 combination with a water filter (1 cm) to prevent
13 warming of the samples during prolonged irradiation.

14

15 As the high concentrations of deoxyribozyme and
16 labeled substrate used prevented full kinetic
17 analysis, all constructs were assayed at a fixed set
18 of standard conditions (20 μ M substrate, 2 μ M

1 deoxyribozyme), and from the measured initial rates,
2 apparent first order rate constants of the Z- and E-
3 isomers were determined.

4

5 **UV-spectroscopy to determine the thermal stability**

6

7 Following irradiation to the Z-form at 366 nm for 10
8 min, UV spectra of the *ortho*, *meta* and *para* modified
9 oligonucleotides (5 μ M in buffer: 50 mM pH 7.5 tris,
10 50 mM NaCl, 25 mM MgCl₂) were measured every 30 min
11 for 6 hours. This procedure was carried out at the
12 following temperatures; 25, 37, 45, 55 and 65 °C.
13 Before the final UV measurement samples were
14 irradiated to the E-form at >400 nm for 2 min. The
15 changing absorbances at 325 nm were converted into
16 concentrations of the E-form of the photoisomerised
17 oligonucleotides. The initial rates of these
18 concentration changes were calculated and used in
19 Arrhenius plots. Analysis of these data provided the
20 activation energies.

21

22

23 **Activity assays for 10-23 deoxyribozymes in the PAGE**
24 **format**

25

26 For the measurements of RNA-cleavage activities
27 under multiple turnover conditions the deoxyribozyme
28 construct (final concentration 2 μ M or 1 μ M) was
29 added to a buffered solution containing 50 mM Tris,
30 pH 7.5, 40 mM NaCl and 25 mM MgCl₂ (all final
31 concentrations). Preirradiation of these mixtures
32 was performed at 366 nm for 10 min and at 435 nm for

1 2 min in clear Eppendorf™ tubes. The reaction was
2 initiated by addition of the deoxyribozyme solutions
3 to the substrate RNA (20 μ M), yielding a total
4 volume of 50 or 100 μ l. Incubation was performed in
5 the absence of light at 26°C, except for *irrDRp*,
6 which was incubated under continuous irradiation at
7 366 nm (temperature under these conditions was
8 determined to be 26°C). After appropriate time
9 intervals aliquots (10 μ l) were withdrawn, mixed
10 with a stop solution (6 M urea, 50 mM EDTA) and
11 frozen at -20°C. The samples were loaded on a 15%
12 (w/v) (3% cross-linked) denaturing polyacrylamide
13 sequencing gel and run at 900 V for 2 hr. The gel
14 was then removed from the plates and transferred
15 onto a gel documentation system (Versadoc, Biorad)
16 and analyzed. Quantitation of the bands was
17 performed with the accompanying software
18 (QuantityOne) such that the intensity of the product
19 bands was set in relation to the sum of the
20 intensities of the product and substrate bands which
21 resulted in relative cleavage values.

22

23 **Activity assays for 10-23 deoxyribozymes using RP-**
24 **HPLC analysis**

25

26 For the measurements of RNA-cleavage activities
27 under multiple turnover conditions the deoxyribozyme
28 construct (final concentration 2 μ M) was added to a
29 buffered solution containing 50 mM Tris, pH 7.5, 40
30 mM NaCl and 25 mM MgCl₂ (all final concentrations).
31 Preirradiation of these mixtures was performed at

1 366 nm for 10 min and at 435 nm for 2 min in clear
2 Eppendorf™ tubes. The reaction was initiated by
3 addition of the deoxyribozyme solutions to the
4 substrate RNA (20 μM), yielding a total volume of 50
5 μl. Incubation was performed in the absence of light
6 at 26°C, except for *irrDRp*, which was incubated
7 under continuous irradiation at 366 nm (temperature
8 under these conditions was determined to be 26°C).
9 After 1.5 hr an aliquot (45 μl) was removed and
10 quenched with 10 μl 250 mM EDTA. For the injection
11 40 μl 0.1 M TEAAc were added and the samples
12 analyzed on RP-HPLC (column: Hichrom KR100-5C18,
13 buffer A: 0.1 M TEAAc, pH 6.5; buffer B: 0.1 M
14 TEAAc, pH 6.5, 65% (v/v) MeCN; UV detection at 260
15 nm; gradient indicated by turquoise line).
16 Quantitation was performed by determining peak areas
17 for the two product peaks and set in relation to the
18 sum of the peak areas of the product and substrate
19 peaks which resulted in relative cleavage values.

20

21 **Chemical synthesis and analysis of nucleoside**
22 **conformation (wherein references apply to figure**
23 **10).**

24

25 Methodology for the preparation of photoswitchable
26 2'-amino-2'-deoxyuridine derivatives (**3a-d**; as
27 illustrated in figure 10) was developed initially
28 using phenyl *para*-azobenzoic acid in trial
29 reactions. Direct condensation of this acid with
30 5'-dimethoxytrityl-2'-amino-2'-deoxyuridine (**1**)
31 mediated by EEDQ or IIDQ using literature protocols

1 (D. P. C. McGee, A. Vaughnsettle, C. Vargeese, and
2 Y. S. Zhai, *Journal Of Organic Chemistry*, 1996, **61**,
3 781; B. Belleau and G. Malek, *Journal of the*
4 *American Chemical Society*, 1968, **90**, 1651 and Y.
5 Kiso, Y. Kai, and H. Yajima, *Chemical and*
6 *Pharmaceutical Bulletins*, 1973, **21**, 2507) yielded
7 high (upto 30%) levels of side-products (in
8 particular, from esterification of the 3'-hydroxyl)
9 which were difficult to resolve from the desired
10 product except following saponification.

11
12 Alternatively, reaction of **1** with the N-
13 hydroxysuccinimidyl activated ester (**2a**) was found
14 to overcome these side-reactions under conditions in
15 which residual DCC and anhydrides were quenched
16 prior to addition of the aminonucleoside. Quenching
17 of these contaminants was achieved by treatment of
18 **2a** with sub-stoichiometric quantities of *n*-
19 butylamine prior to addition of the aminonucleoside.
20 This methodology was therefore adopted for the
21 preparation of the photoswitchable nucleoside
22 analogues **3b** - **3d**.

23
24 The large polarity difference between *E*-**3a** and *Z*-**3a**
25 enabled facile resolution of these isomers using
26 silica gel chromatography. However, attempted
27 phosphitylation of pure *Z*-**3a** was not successful.
28 Both *E*-**3a** and *Z*-**3a** exhibited DNA-like C2'-endo ring
29 puckers characterised by strong coupling between H1'
30 and H2' ($J = 6.5 - 7.0$ Hz) and weak H3'-H4'
31 interactions (<1Hz). The C2'-endo conformation of
32 the furanoside of both azobenzene isomers was

1 maintained following detritylation of *E*-**3a** and
2 subsequent irradiation of the unprotected nucleoside
3 at 300-350 nm; this gave a mixture of *E*-**3a** and *Z*-**3a**
4 (ca. 4/5) and revealed no significant change in the
5 ring conformation. These observations contrast with
6 recent descriptions of RNA-like C3'-endo sugar
7 conformations observed for related deoxynucleotide
8 2'-amine and 2'-amide analogues⁴³ and also for 2'-O-
9 *para*-phenylazobenzyl uridine.³⁷

10

11 Methodology for the phosphitylation of *E*-/*Z*-**3a-c** and
12 their subsequent application to the solid-phase
13 synthesis of oligodeoxyribonucleotides incorporating
14 the corresponding nucleotide analogues (**4a-c**) has
15 been described in S. Keiper and J. S. Vyle,
16 *Angewandte Chemie-International Edition*, 2006, **45**,
17 3306. Synthesis of the previously unreported
18 spiropyran-nucleoside derivative **3d** was performed by
19 adapting related protocols developed in the
20 laboratory of Meng (P. Zhang, J. B. Meng, T.
21 Matsuura, and Y. M. Wang, *Chinese Chemical Letters*,
22 2002, **13**, 299 and D. P. C. McGee, A. Vaughnsettle,
23 C. Vargeese, and Y. S. Zhai, *Journal Of Organic*
24 *Chemistry*, 1996, **61**, 781). Oligodeoxyribonucleotides
25 incorporating the corresponding nucleotide analogue
26 (**4d**) were synthesised using an RNA coupling protocol
27 for the coupling of the modified phosphoramidite.

28

29 The synthesis of the oligoribonucleotides
30 incorporating **4a-c** was performed trityl-on using 2'-
31 tBDMS-protected monomers. Deprotection was thus
32 effected following treatment with anhydrous

1 saturated methanolic ammonia overnight at ambient
2 temperatures followed by a 33% (v/v) solution of
3 triethylamine trihydrofluoride in 1-methyl-2-
4 pyrrolidinone:triethylamine (2:1) at 65 °C.
5 Significant loss of the photoswitch from oligomers
6 (both DNA and RNA) was observed upon initial
7 treatment with either aqueous ammonia at room
8 temperature or methylamine solutions under heating.
9 The deprotected oligomers were then purified by
10 reversed-phase HPLC, detritylated and desalted.

11

12 Example 1 - with reference to figures 1 to 4

13

14 Deoxyribozymes were prepared in which a single
15 nucleotide (T₈) within the catalytic core was
16 replaced by a 2'-deoxyuridylate analogue. *Ortho*-,
17 *meta*- or *para*-phenylazobenzoyl moieties were
18 appended to this residue via a 2'-amido linkage.
19 This site was chosen as deletion of T₈ or
20 substitution by 2'-O-methyluridine has previously
21 been shown to only minimally perturb deoxyribozyme
22 activities.

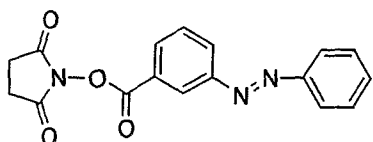
23

24 The deoxyribozyme constructs were engineered with
25 short binding arms to promote product-release under
26 multiple turnover conditions.

27

28 Modified nucleoside precursors were prepared by
29 reaction of the protected 2'-aminonucleoside with
30 the *N*-hydroxysuccinimidyl ester of the appropriate
31 azobenzene, for example, but not limited to,

32



1
2 and subsequently phosphitylated under standard
3 conditions. These were incorporated into model 8-
4 mers (ACC**1**GGTA) and also 10-23 deoxyribozyme
5 sequences using automated solid-phase synthesis with
6 extended reaction times for introduction of the
7 modified residue. Coupling yields as monitored by
8 trityl release were greater than 98%. Following
9 deprotection under anhydrous conditions, the
10 oligonucleotides were purified by reversed-phase
11 HPLC and their identities confirmed by MALDI-TOF
12 analysis.

13
14 The photoisomerization of oligodeoxynucleotide-
15 appended azobenzenes under irradiation at 366 nm was
16 investigated by UV/Vis spectroscopy of the model 8-
17 mers. When the photoswitches were in the thermally
18 stable *E*-configuration, the spectra showed local
19 maxima at 330 (**1o**), 321 (**1m**), 329 (**1p**) nm typical
20 for azobenzenes π - π^* transitions.

21
22 Irradiation at 366 nm led to loss of these
23 absorption maxima with photostationary states being
24 achieved within eight minutes. These results were
25 reproduced with azobenzene-appended deoxyribozymes
26 and the *E* $\rightarrow$ *Z* conversion yields determined
27 immediately by HPLC. Peak quantification at 260 nm
28 was used to determine the level of *E*- and *Z*-isomers
29 either directly for DR**m** and DR**p** or by inference
30 using the model 8-mer sequence containing **1o**.
31 Following irradiation, *E* $\rightarrow$ *Z* conversion yields of
32 86% for irrDR**o**, 75% for irrDR**m** and 61% for irrDR**p**

1 were thus determined. At 26°C the *ortho*- and *meta*-
2 photoswitches within both the model sequences and
3 also deoxyribozymes were found to be stable towards
4 $Z \rightarrow E$ thermal back-isomerization. In all cases Z
5 $\rightarrow E$ photoisomerization was complete following
6 irradiation at 435 nm for two minutes.

7
8 Both azobenzene-modified and unmodified (wild type;
9 DR~~wt~~), deoxyribozymes catalyzed the site-specific
10 cleavage of a 13-mer oligoribonucleotide substrate
11 labelled at its 3'-terminus with a fluoresceinyl
12 compound (FAM) to yield a labelled 6-mer and a
13 2',3'-cyclic phosphate terminated 7-mer. The
14 cleavage reactions were thus resolved, visualized
15 and quantified in a PAGE assay. Deoxyribozyme
16 solutions were exposed to light at 366 nm for ten
17 minutes or at 435 nm for two minutes and reactions
18 initiated by addition of substrate RNA and incubated
19 at 26°C in the absence of light. To assess the
20 activity of the more thermally labile *irrDR~~p~~*,
21 continuous irradiation at 366 nm was performed
22 during the assay.

23
24 Under multiple turnover conditions, *irradiated*
25 azobenzene-deoxyribozymes maintained essentially
26 wild-type activities; thus *irrDR~~o~~* showed 100%,
27 *irrDR~~m~~* 84% and *irrDR~~p~~* 50% activity. In contrast, RNA
28 cleavage rates by *dark-adapted* (*d-a*) deoxyribozymes
29 were considerably attenuated. k_{irr}/k_{d-a} ratios in this
30 assay were determined to be 9 for DR~~o~~ and 8 for DR~~m~~
31 and DR~~p~~). Photocontrol of RNA-cleavage by DR-
32 azobenzene conjugates was also demonstrated using an

1 unlabeled RNA substrate and reversed-phase HPLC
2 analysis. The effects observed in these assays
3 compare well with the results from the PAGE
4 analyses. Thus, the relative cleavage activities of
5 *irrDR_o* and *irrDR_m* are both the same as for the
6 unmodified deoxyribozyme *DR_{wt}*, and *irrDR_p* shows 44%
7 of the wild-type activity. *Dark-adapted*
8 deoxyribozymes give significantly less conversion
9 than the irradiated constructs; k_{irr}/k_{d-a}
10 discrimination factors of six for *DR_o* and five for
11 *DR_m* and *DR_p* constructs were observed.

12
13 The effect of photoswitching upon catalysis by the
14 azobenzene-conjugated deoxyribozymes was also
15 demonstrated to be reversible under multiple
16 turnover conditions with either 5 mol% (*DR_o* or *DR_m*)
17 or 10 mol% (*DR_p*) deoxyribozyme. Following initial
18 irradiation of deoxyribozyme solutions at 366 nm,
19 the reactions were initiated by addition of
20 substrate and RNA-cleavage activities comparable
21 with those previously described were observed. After
22 120 minutes the reactions were irradiated at 435 nm
23 for two minutes to afford the *dark-adapted*
24 deoxyribozymes which gave rise to significantly
25 retarded reaction rates although these were higher
26 than previously observed using purified *E*-DRs. This
27 observation might be accounted for by perturbation
28 of the $Z \rightarrow E$ photoisomerization process in the
29 presence of substrate RNA or kinetic folding traps,
30 which do not respond to this isomerization.
31 However, initial cleavage rates were recovered
32 following irradiation at 366 nm.

1

2 Preliminary NMR investigations indicate that both *E*
3 and *Z* isomers of **1p** reside in the C2'-*endo*
4 furanoside pucker typical of 2'-amido
5 deoxyribonucleoside analogues and their unmodified
6 congeners and so the active conformation of the
7 catalytic core containing the photoswitch may be
8 modulated in some other fashion.

9

10 Example 2

11

12 **Photoswitching efficiencies and thermal stabilities**
13 **of irradiated states**

14 Spiropyran derivatives of indolene exhibit both
15 thermochromism and photochromism arising from the
16 heat- or UV-induced isomerisation to the
17 corresponding highly coloured merocyanine (MC)
18 isomers. This was also observed for CPG-supported
19 oligonucleotides which upon irradiation at 366 nm
20 for 130 s gave a deep purple colouration. However,
21 in contrast to the stability observed for the
22 *irradiated* AB-modified oligonucleotides, irradiation
23 of SP-modified DNA at 366nm over extended periods of
24 time (as illustrated in figure 11) led to both the
25 expected photoisomerisation product (R_t 17.6 min)
26 but also significant decomposition of the
27 photoswitchable moiety (R_t 14.2 min.

28

29 The decomposition product was isolated by HPLC (R_t
30 14.2 min) and characterised by MALDI mass
31 spectrometry. The degradation process was thereby

1 attributed to a reteroaldol reaction of the
2 merocyanine to the corresponding methyleneindole-
3 derivative. Such reactivity has previously been
4 described for the preparation of substituted
5 salicaldehydes from the corresponding spiropyrans
6 under heating. Thermal degradation of the spiropyran
7 modification via this route was also observed in
8 aqueous solution.

9
10 The photochemical and thermal isomerisations of
11 model octadeoxynucleotides bearing photoswitchable
12 moieties at an internal site - d(ACC**4a-d**GGTA) - were
13 investigated by UV / Vis spectroscopy and reversed-
14 phase HPLC.

15
16 *Irradiated* photostationary states of the azobenzene-
17 appended oligomers (containing **4a-c**) were achieved
18 following exposure to near-UV radiation from a
19 medium pressure Hg Arc-lamp at 366 nm. At this
20 wavelength, excitation of the $\pi - \pi^*$ transition
21 induced $E \rightarrow Z$ photoisomerisation of the azobenzene
22 which was monitored by decrease in absorption at 325
23 nm. *Irr* states were thus induced within six minutes
24 and the E/Z ratio determined after 10 minutes
25 following analysis by RP-HPLC; oligomers bearing the
26 more hydrophobic E -AB's were resolved from those
27 incorporating the corresponding Z -isomer

28
29 *Dark-adapted* photostationary states were induced
30 much more rapidly following excitation of the $n - \pi^*$
31 transition at 436 nm. Thus, within two minutes full
32 restoration of the original E - $\pi - \pi^*$ absorbances was

1 observed and this was found to be independent of the
2 nature of the AB.

3

4 The mole fractions of Z-AB-appended 8-mers (χ_Z) in
5 both photostationary states (Table 1) were
6 quantified at 26°C and 40°C either directly by
7 monitoring the HPLC output at the isosbestic points
8 of the modified oligomers or indirectly from the
9 apparent χ_Z values at 260nm. High $E \rightarrow Z$
10 photoswitching yields (>90%) are typical of the *Irr*-
11 states of azobenzenes belonging to the "azobenzene
12 type" due to large separation of the $\pi - \pi^*$
13 transitions in the different isomers ($\lambda_{\max}$ ~325nm for
14 *E*-AB's and ~260nm for *Z*-AB's). However, attenuated
15 yields of *Z*-para-AB in particular were observed
16 following irradiation at 366nm at 26°C arising from
17 the large positive structural volume change
18 associated with the light-induced helix-coil
19 transition at this temperature. This is illustrated
20 in Table 1.

21

22

23

24

25

26

27

28

29

30

31

32

1 **Table 1** Mole fractions (χ_z) of oligomers containing
2 Z-4a, Z-4b or Z-4c (wherein Z-4a is the para, Z-4b
3 is the meta and Z-4c the ortho azobenzene photo-
4 modulatable compounds as illustrated in figure 10)
5 in irradiated and dark-adapted photostationary
6 states at 26°C and 40°C.

7

Oligo- nucleotide	χ_z	
	irradiated	dark- adapted
ACC 4a GGTA	0.93	0.17
ACC 4b GGTA	0.79	0.25
ACC 4c GGTA	0.69	0.05

8

9

10 The stabilities of the Irr-oligonucleotides towards
11 thermal $Z \rightarrow E$ isomerisation were also investigated.
12 Thus, the absorbances of Irr-d(ACC**4a-c**GGTA) were
13 monitored at 325 nm over six hours at temperatures
14 between 55°C and 80°C. First order rate constants
15 were derived for each experiment and Arrhenius and
16 Eyring plots of these data were made as illustrated
17 in Figure 12.

18

19 The kinetic parameters derived from the plots for
20 oligonucleotides containing Z-**4a** and Z-**4c** (Table 2)
21 are typical for the "azobenzene" class described by
22 Rau. In contrast, thermal isomerisation of the *meta*
23 isomer (Z-**4a**) displayed an elevated activation
24 energy (high $\Delta H^\ddagger$) and fast pre-exponential factor

(positive $\Delta S^\ddagger$) (e.g., $E_a = 129 \text{ KJmol}^{-1}$; $A = 1.71 \times 10^{15} \text{ s}^{-1}$). Such values have previously only rarely been described for azobenzenes in which rotation around the NN-C bond is inhibited by the presence of tetra-alkyl substituents *ortho*-to the azo bridge, by short alkyl tethers within azobenzenophanes, or azobenzene-linked crown ethers embedded within cast PMMA.

Table 2 Kinetic parameters describing the thermal Z - E isomerisation (wherein Z-4a comprises a para azobenzene photoswitchable compound, z-4b comprises a meta azobenzene photoswitchable compound and z-4c comprises an ortho azobenzene photoswitchable compound as illustrated in figure 10).

Oligonucleotide	E_{act} (KJmol^{-1})	A (s^{-1})	$\Delta H^\ddagger$ (KJmol^{-1})	$\Delta S^\ddagger$ ($\text{Jmol}^{-1} \text{K}^{-1}$)	$T_{1/2}$ at 37°C (hr)
ACC 4a GGTA	100.6±	6.64x 10 ¹¹ ±	97.8±	- 28.X±	26.2
ACC 4b GGTA	132.0±	5.03x 10 ¹⁵ ±	130±	46.3±	669
ACC 4c GGTA	101.4.0±	4.16x 10 ¹¹ ±	94.6±	- 42.8±	55.7

1 **Duplex stability T_m -measurements**2 **Self-complementary 8-mers Sequence context**

3

4 To investigate the influence of the position and
 5 conformation of the nucleotide analogues 1a-c (as
 6 illustrated in figure 1) on the stability of the
 7 duplex as measured by melting temperatures a
 8 octanucleotide sequence was designed. The
 9 modification of an internal position, as shown in
 10 Table 1, led to an overall destabilisation of the
 11 duplexes (Table 3), whereas modification near the
 12 end was considerably less perturbing (Table 4).

13

14 **Table 3** Melting temperatures of oligonucleotides
 15 (wherein Z-4a comprises a para azobenzene
 16 photoswitchable compound, z-4b comprises a meta
 17 azobenzene photoswitchable compound and z-4c
 18 comprises an ortho azobenzene photoswitchable
 19 compound as illustrated in figure 10).

20

Target	Probe	T_M / °C		ΔT_M / °C
		DA	Irr	
5'-d(TACCAGGT)	5'-d(ACCTGGTA)	31.7		-
	5'-d(ACC 4c GGTA)	<20	<20	ND
	5'-d(ACC 4b GGTA)	<20	<20	ND
	5'-d(ACC 4a GGTA)	32.0	<15	>17
	5'-d(ACCTGG 4ca)	29.0	28.2	0.8
	5'-d(ACCTGG 4ba)	30.0	27.5	2.5
	5'-d(ACCTGG 4aa)	29.0	31.3	-2.3
5'-r(UACCAGGU)	5'-d(ACCTGGTA)	35.0		-
	5'-d(ACC 4c GGTA)	27.5	24.5	3.0
	5'-d(ACC 4b GGTA)	27.0	23.1	3.9
	5'-d(ACC 4a GGTA)	28.5	31.0	-2.5
	5'-d(ACCTGG 4ca)	32.5	33.1	-0.6
	5'-d(ACCTGG 4ba)	33.0	32.8	0.2

	5'-d(ACCTGG 4aA)	35.5	33.2	2.3
	5'-d(TCGT 4aG AC) 5'-d(GTC 4aA ACGA)	31.6	28.0	3.6
	5'-d(TCGTCGAC) 5'-d(GTC 4aA ACGA)	29.2	29.3	-0.1
	5'-d(TCGTTGAC) 5'-d(GTC 4aA ACGA)	22.3	22.0	0.3
	5'-d(TCGTAGAC) 5'-d(GTC 4aA ACGA)	<20	<20	ND
	5'-d(TCGTGGAC) 5'-d(GTC 4aA ACGA)	<20	<20	ND
	5'-d(ACC 4aG GTA)	30.5	<15	>15.5
	5'-d(ACG 4aC GTA)	37.2	31.5	5.7
	5'-d(ACC 4aG GTA)	32.0	27.1	4.9
	5'-d(CGT 4aA ACGA)	34.5	27.7	7.8
	5'-d(ACC 4a4aG GTA)	31.5	29.1	2.4
	5'-d(GATATC 4aG ATATC) SEQ ID NO 1	41.8	39.9	1.9

1

2 Table 4

3 (wherein Z-4a comprises a para azobenzene

4 photoswitchable compound, z-4b comprises a meta

5 azobenzene photoswitchable compound and z-4c

6 comprises an ortho azobenzene photoswitchable

7 compound as illustrated in figure 10)

Target	Probe	T _M / °C		ΔT _M / °C
		DA	Irr	
5'-d(TACCAGGT)	5'-r(ACCTGGTA)	32.5		-
	5'-r(ACC 4aG GTA)	30.5	<20	>10.5
	5'-r(ACC 4bG GTA)	32.0	<20	>12.0
	5'-r(ACC 4cG GTA)	25.0	28.1	-3.1
	5'-r(ACCTGG 4aA)	27.9	28.5	-0.6
	5'-r(ACCTGG 4bA)	28.5	28.7	-0.2
	5'-r(ACCTGG 4cA)	<20	<20	ND
5'-r(UACCAGGU)	5'-r(ACCTGGTA)	48.1		-
	5'-r(ACC 4aG GTA)	33.0	31.7	1.3
	5'-r(ACC 4bG GTA)	33.0	34.1	-0.9
	5'-r(ACC 4cG GTA)	32.5	32.6	-0.1
	5'-r(ACCTGG 4aA)	48.0	45.3	2.7
	5'-r(ACCTGG 4bA)	45.0	45.0	0.0
	5'-r(ACCTGG 4cA)	44.5	46.5	-2.0

8

9 Only minor effects of the conformation of the

10 azobenzene moieties were observed in most cases.

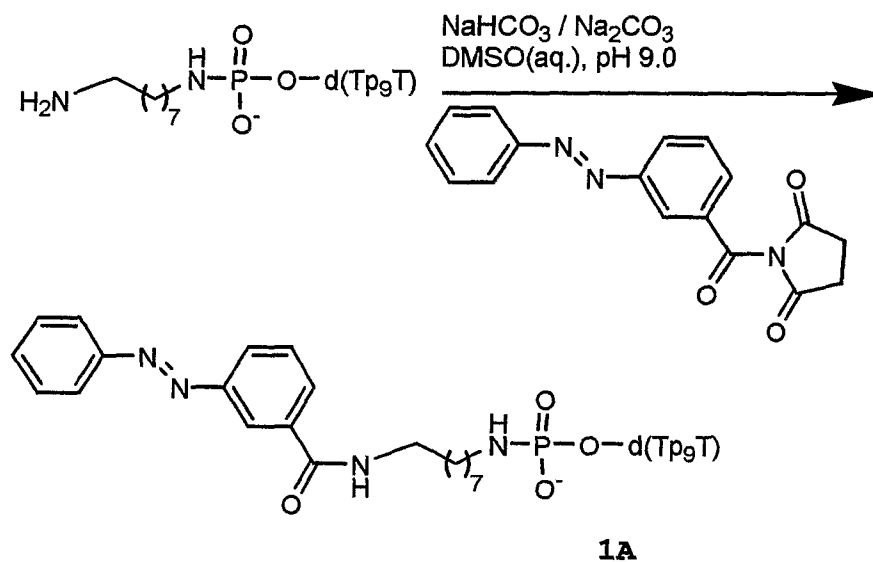
11 Incorporation of 1a leads to a strong

1 destabilisation of the DNA duplex when switched to
 2 the Z-conformation.

3

4

5



Key

p = -O-PO₂-O-

6

7

8

9 Surprisingly, in the presence of an RNA counter
 10 strand both the irradiated and dark-adapted
 11 sequences demonstrated similar melting behaviours.

12

13 Upon further investigation, the strong
 14 destabilisation of the DNA duplex modified with **1a**
 15 was found to result from the self-complementary
 16 nature of the oligonucleotide sequence. When the
 17 experiment was performed in the absence of the
 18 counter strand a similar destabilisation was
 19 measured (Table 2).

1

2 A further set of duplex stability measurements were
3 then performed designed to probe the generality of
4 this phenomenon. Thus, for the melting behaviour of
5 three related self-complementary octanucleotides
6 with a single internal AB modification in which the
7 heptanucleotide duplex region is flanked by 5'-
8 overhangs, the magnitude of the effect is sequence-
9 dependent.

10

11 The lack of difference of duplex stability observed
12 for the double-substituted sequence following
13 photoswitching was rationalised by the low
14 efficiency encountered for this process
15 which would result in the presence of over 50% of
16 irradiated sequences containing an E-AB (and also
17 the well precendented diminishing efficiency of
18 photoisomerisation of proximal AB's)

19

20 Oligo d(CnGn) sequences are known to adopt A-form
21 DNA and in order to probe the potential for
22 conformational switching, 1a was inserted into a
23 central position of a 13-mer oligonucleotide flanked
24 by two EcoRV restriction sites. A → B switching was
25 observed upon irradiation at 366 nm.

26

27 From these data the inventors conclude that a strong
28 effect (i.e. >6°C) is only found for symmetrical
29 sequences that bear the photoswitch opposite each
30 other in both strands and 1 nt overhangs on either
31 end for the particular modification under
32 investigation. The inventors have also determined

1 that for unmodified counterstrands (as is the case
2 in potential applications) a cytidine residue
3 opposite the modified base gives the most stable
4 duplex.

5

6 The above demonstrates light-modulated self-
7 recognition by DNA and describes conformational
8 photoswitching of a DNA duplex.

9

10 Various modifications may be made to the invention
11 herein described without departing from the scope
12 thereof.

13

14

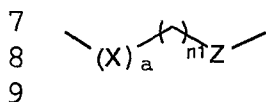
15

1 **Claims**

2

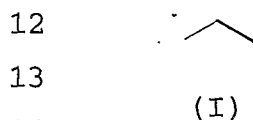
3 **1.** A linker for linking a photoswitchable compound
 4 (PS) to a base, nucleoside or a nucleotide, the
 5 linker comprising:

6



10 wherein X independently represents

11



14

15

16

17

18

19

20

21 Z independently represents

22

23

24

25

26

27

28

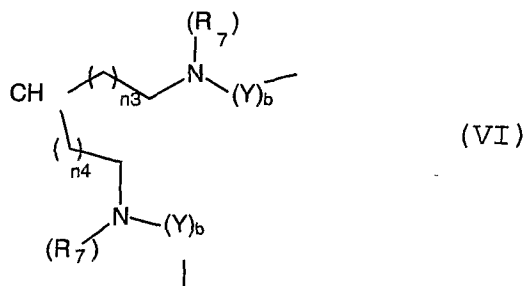
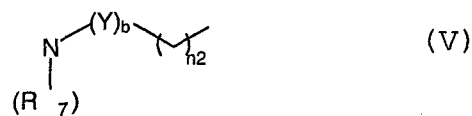
29

30

31

32

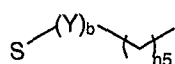
33



1

2

3



(VII)

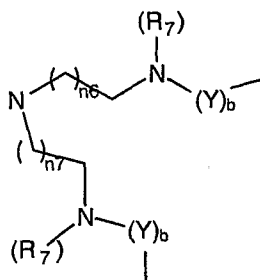
4

5 or

6

7

8



(VIII)

9

10

11

12

13

14 wherein n_1 , n_2 , n_3 , n_4 , n_5 , n_6 , and n_7 are
 15 independently selected from each other and each of
 16 n_1 , n_2 , n_3 , n_4 , n_5 , n_6 , and n_7 is 0 or an integer
 17 selected from the range 1 to 12;

18

19 (R_7) represents H or CH_3 ;

20

21 Y independently represents

22

23



(IX)

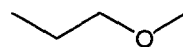
24

;



(X)

or



(XI)

25

26

27 and b is 0 or 1 or 2.

28

29 2. A linker as claimed in claim 1 wherein X is
 30 represented by formula (I), Z is independently
 31 selected from the structures as represented by one
 32 of formula (V), (VI), (VII), or (VIII) and Y is

1 independently selected from the structures as
2 represented by one of formula (IX), (X) or (XI).

3

4 3. A linker as claimed in claim 1 wherein X is
5 represented by formula (II), Z is independently
6 selected from the structures as represented by one
7 of formula (V), (VI), (VII), or (VIII) and Y is
8 independently selected from the structures as
9 represented by one of formula (IX), (X) or (XI).

10

11 4. A linker as claimed in claim 1 wherein X is
12 represented by formula (III), Z is independently
13 selected from the structures as represented by one
14 of formula (V), (VI), (VII), or (VIII) and Y is
15 independently selected from the structures as
16 represented by one of formula (IX), (X) or (XI).

17

18 5. A linker as claimed in claim 1 wherein X is
19 represented by formula (IV), Z is independently
20 selected from the structures as represented by one
21 of formula (V), (VI), (VII), or (VIII) and Y is
22 independently selected from the structures as
23 represented by one of formula (IX), (X) or (XI).

24

25 6. A linker as claimed in claim 1 wherein X is
26 represented by one of the structures as represented
27 by formula (I), (II), (III), or (IV), Z is
28 independently selected from the structure as
29 represented by the formula (V), and Y is
30 independently selected from the structures as
31 represented by of formula (IX), (X) or (XI).

32

1 7. A linker as claimed in claim 1 wherein X is
2 represented by one of the structures as represented
3 by formula (I), (II), (III), or (IV), Z is
4 independently selected from the structure as
5 represented by the formula (VI), and Y is
6 independently selected from the structures as
7 represented by one of formula (IX), (X) or (XI).

8

9 8. A linker as claimed in claim 1 wherein X is
10 represented by one of the structures as represented
11 by formula (I), (II), (III), or (IV), Z is
12 independently selected from the structure as
13 represented by the formula (VII), and Y is
14 independently selected from the structures as
15 represented by one of formula (IX), (X) or (XI).

16

17 9. A linker as claimed in claim 1 wherein X is
18 represented by one of the structures as represented
19 by formula (I), (II), (III), or (IV), Z is
20 independently selected from the structure as
21 represented by the formula (VIII), and Y is
22 independently selected from the structures as
23 represented by one of formula (IX), (X) or (XI).

24

25 10. A linker as claimed in claim 1 wherein X is
26 represented by one of the formula (I), (II), (III),
27 or (IV), Z is independently selected from the
28 structures as represented by the formula (V), (VI),
29 (VII), or (VIII), and Y is a structure as
30 represented by (IX).

31

- 1 11. A linker as claimed in claim 1 wherein X is
2 represented by one of the formula (I), (II), (III),
3 or (IV), Z is independently selected from the
4 structures as represented by the formula (V), (VI),
5 (VII), or (VIII), and Y is a structure as
6 represented by (X).
7
- 8 12. A linker as claimed in claim 1 wherein X is
9 represented by one of the formula (I), (II), (III),
10 or (IV), Z is independently selected from the
11 structures as represented by the formula (V), (VI),
12 (VII), or (VIII), and Y is a structure as
13 represented by (XI).
14
- 15 13. A nucleic acid complex comprising a nucleic acid
16 and a linker as claimed in any one of claims 1 to
17 12.
18
- 19 14. A nucleic acid complex as claimed in claim 13
20 wherein the nucleic acid comprises a DNA or RNA
21 nucleoside or nucleotide.
22
- 23 15. A nucleic acid complex as claimed in claim 13 or
24 claim 14 wherein the linker is attached to a base of
25 a nucleoside or nucleotide.
26
- 27 16. A nucleic acid complex as claimed in any claim
28 13 or claim 14 wherein a linker is attached to the
29 sugar portion of a nucleoside or nucleotide.
30

1 17. A nucleic acid complex as claimed in claim 13
2 or claim 14 wherein the linker is attached to a 5-
3 propargylamino function of a nucleoside.

4

5 18. A nucleic acid complex as claimed in claim 13 or
6 claim 14 wherein the linker is attached to a 2'
7 amino function of a nucleoside.

8

9 19. A nucleic acid complex as claimed in claim 13 or
10 claim 14 wherein the linker is attached to a
11 modified phosphate diester such that the nucleic
12 acid complex is selected from at least one of:

13

14

15

16

17

18

19

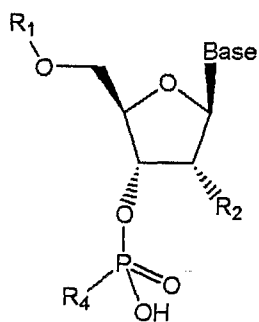
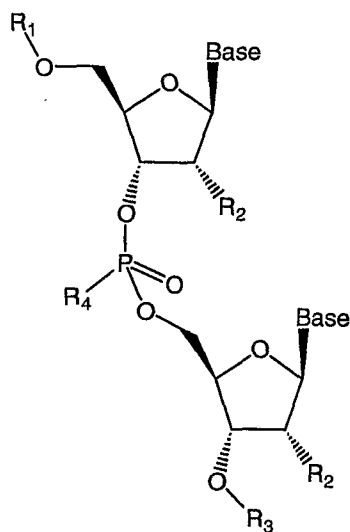
20

21

22

23

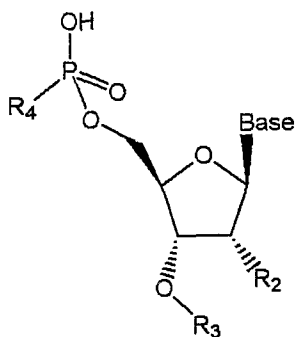
24



25

26

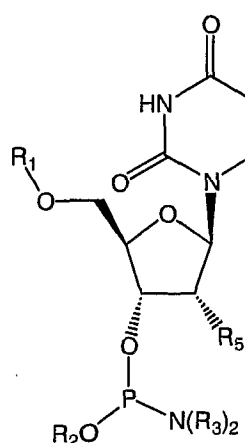
1 or



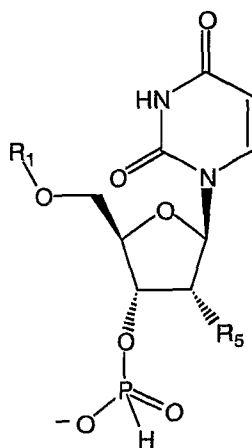
9 wherein

10 R1 is O- or is any 5' nucleic acid fragment either
 11 DNA or RNA; Base is selected from adenosine,
 12 cytosine, guanine, thymine or an analogue thereof;
 13 R2 is selected from H (DNA), OMe (2'-O-methyl RNA)
 14 or OH (RNA), R3 is O- or is any 3' nucleic acid
 15 fragment either DNA or RNA; and R4 is the linker.

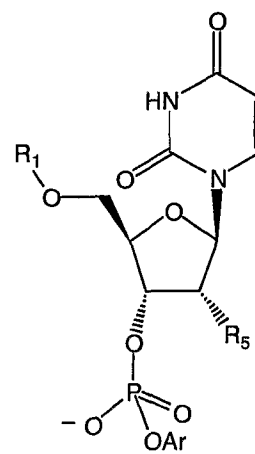
16
 17 20. A nucleic acid complex as claimed in claim 13 or
 18 claim 14 wherein said nucleic acid complex is
 19 selected from A, B, C, D, E or F



31 **A**

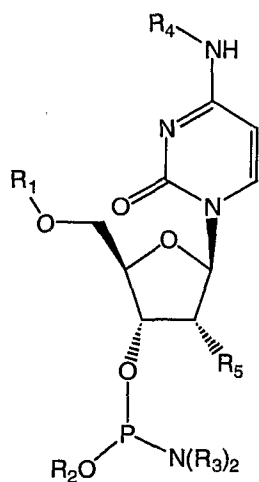
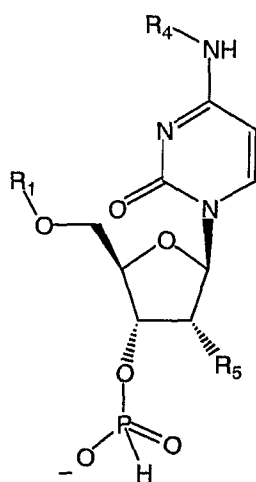
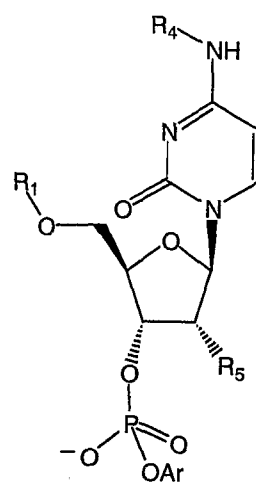


B



C

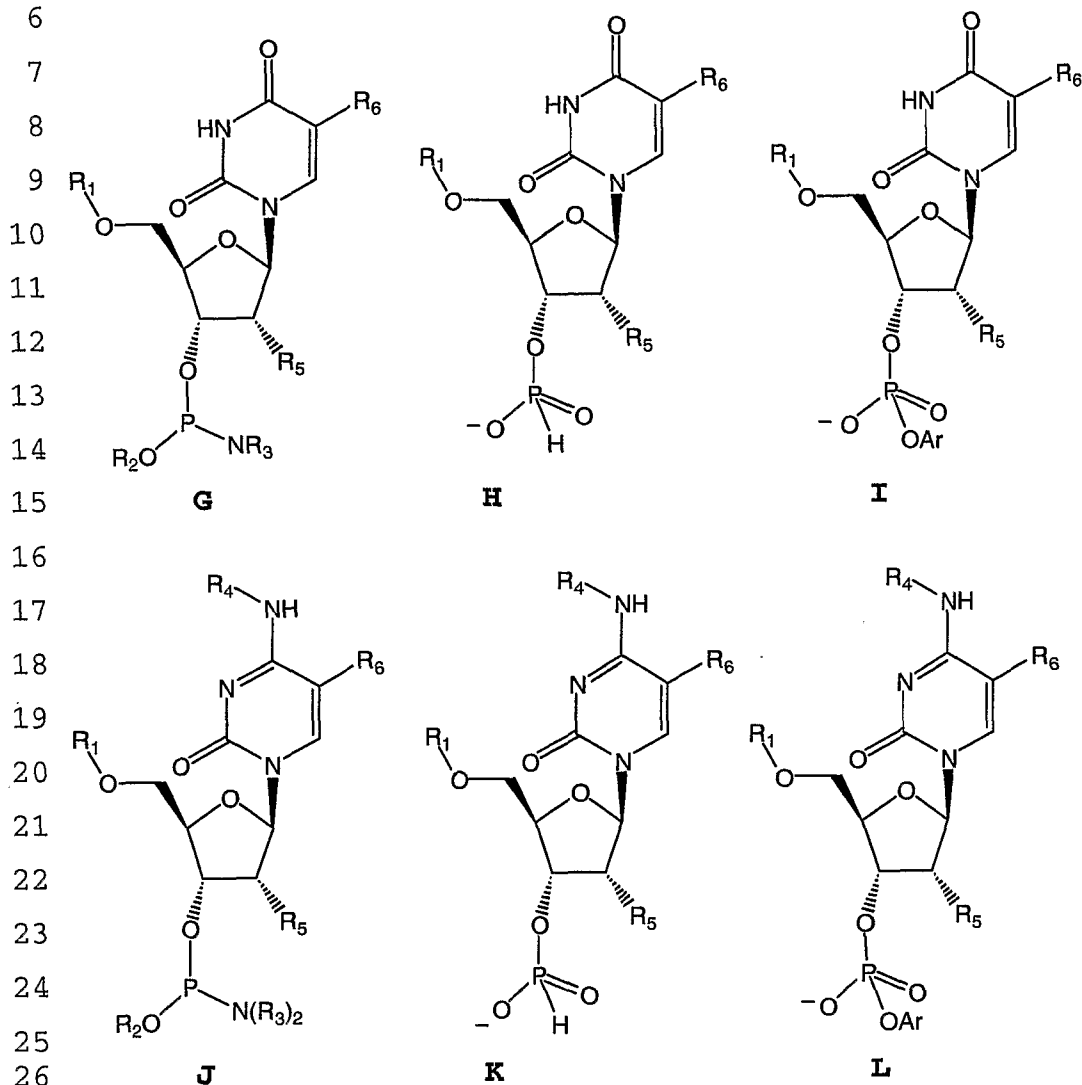
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

**D****E****F**

wherein

R1 is any protecting group typical for 5';
R2 is any protecting group for phosphate or
phosphate triesters;
R3 is any alkyl group for phosphoramidite;
R4 is any protecting group for N⁴ of cytosine; and
R5 is the linker.

1 21. A nucleic acid complex as claimed in claim 13 or
2 claim 14 wherein said nucleic acid complex is
3 selected from G, H, I, J, K or L



27 wherein

29 R1 is any protecting group typical for 5';

30 R2 is any protecting group for phosphate or
31 phosphate triesters;

32 R3 is any alkyl group for phosphoramidite;

- 1 R4 is any protecting group for N⁴ of cytosine;
 2 R5 is selected from H (DNA), OMe (2'-O-methyl RNA),
 3 or an O-protecting group (RNA);
 4 R6 is the linker.

5

- 6 22. A nucleic acid complex as claimed in claim 13 or
 7 claim 14 wherein said nucleic acid complex is
 8 selected from:

9

10

11

12

13

14

15

16

17

18 or

19

20

21

22

23

24

25

26

27 wherein

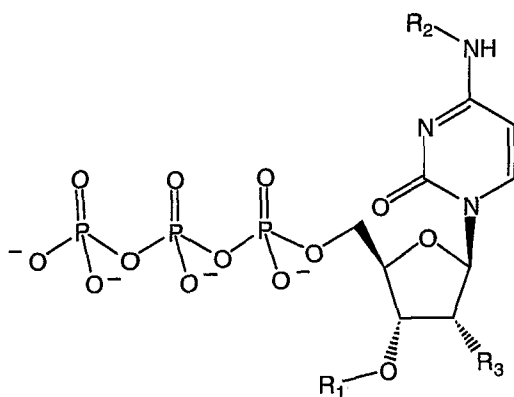
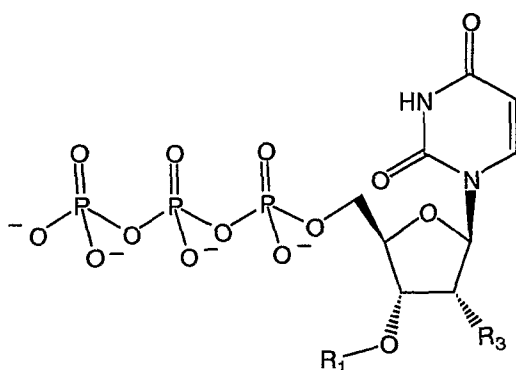
28 R1 is H or any protecting group for 3'

29 R2 is H or any protecting group for N⁴ of cytosine

30 R3 is the linker.

31

32



1 23. A nucleic acid complex as claimed in claim 13 or
 2 claim 14 wherein said nucleic acid complex is
 3 selected from:

4

5

6

7

8

9

10

11

12

13

14

15

16 or

17

18

19

20

21

22

23 wherein

24 R1 is H or any protecting group typical for 3';

25 R2 is H or any protecting group for N⁴ of cytosine;

26 R3 is selected from H (DNA), OMe (2'-O-methyl RNA),

27 or OH (RNA);

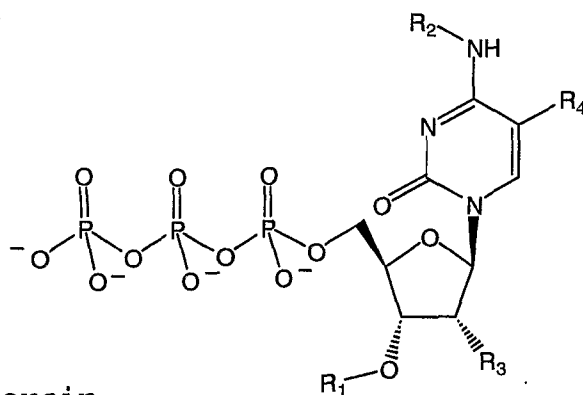
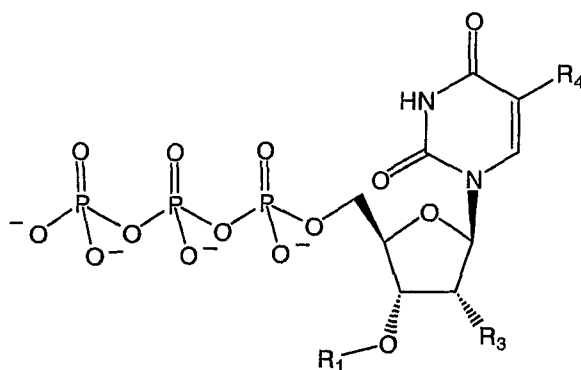
28 R4 is the linker.

29

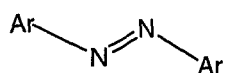
30 24. A photo-modulatable compound comprising a

31 nucleic acid complex as claimed in any one of claims

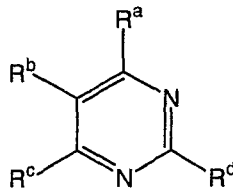
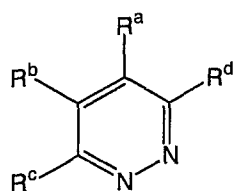
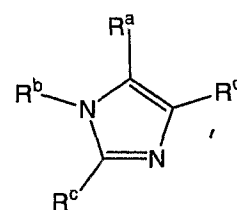
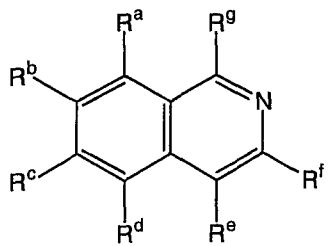
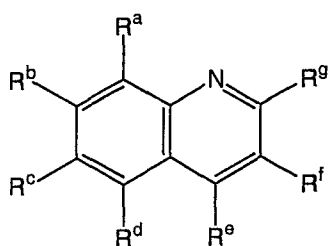
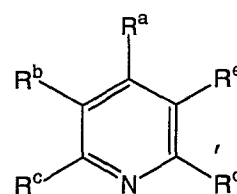
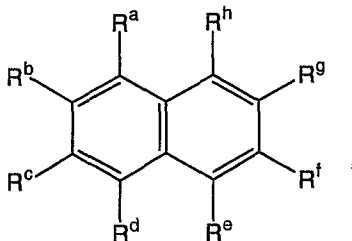
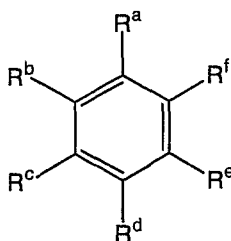
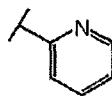
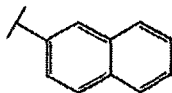
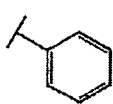
32 13 to 23 and a photoswitchable compound.



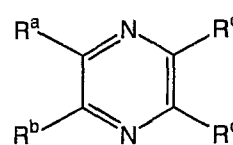
25. A photo-modulatable compound as claimed in claim 24 wherein the photoswitchable compound is at least one compound selected from a group consisting of: azobenzene, azonaphthalene, azopyridine, azoimidazole, diaryl alkene, spiropyran, spiroxazine, or azoarene of formula



wherein AR is independently selected from the group consisting of



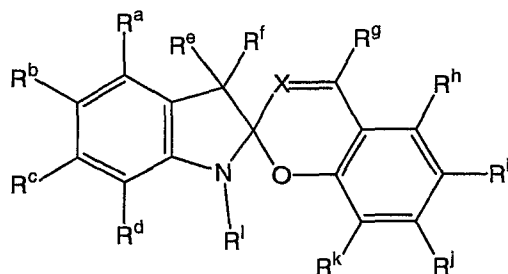
, or



wherein each R^a may be independently selected from a C₁ to C₄₀ linear or branched alkyl or a C₃ to C₈

1 cycloalkyl group, wherein said alkyl or cycloalkyl
 2 group may be substituted by one to three groups
 3 selected from: C₁ to C₆ alkoxy, C₁ to C₆
 4 (di)alkylamino, C₆ to C₁₀ aryl, C₁ to C₃₀ aralkyl and
 5 C₁ to C₃₀ alkaryl; NO₂; CO₂R; C(O)NH₂; F;
 6 each R^b, R^c, R^d, R^e, R^f, R^g and R^h can be the same or
 7 different and are each independently selected from H
 8 or any of the defined R^a groups.

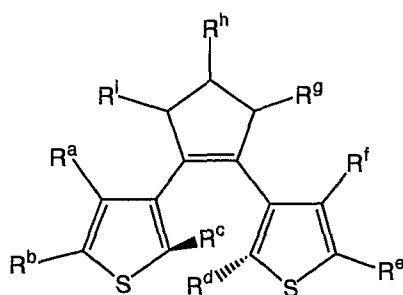
9
 10 26. A photo-modulatable compound as claimed in claim
 11 24 or claim 25 wherein the spiropyran wherein X is
 12 CH or the spirooxazine wherein X is N is of the
 13 formula



14
 15
 16
 17
 18
 19
 20
 21
 22 wherein each R^a may be independently selected from a
 23 C₁ to C₄₀ linear or branched alkyl or a C₃ to C₈
 24 cycloalkyl group, wherein said alkyl or cycloalkyl
 25 group may be substituted by one to three groups
 26 selected from: C₁ to C₆ alkoxy, C₁ to C₆
 27 (di)alkylamino, C₆ to C₁₀ aryl, C₁ to C₃₀ aralkyl and
 28 C₁ to C₃₀ alkaryl; NO₂; CO₂R; C(O)NH₂; F;
 29 each R^b, R^c, R^d, R^e, R^f, R^g, R^h, Rⁱ, R^j, R^k and R^l can
 30 be the same or different and are each independently
 31 selected from H or any of the defined R^a groups.

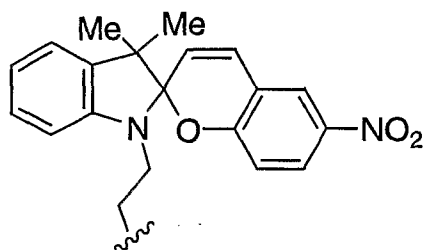
32

27. A photo-modulatable compound as claimed in claim 24 or claim 25 wherein the diaryl alkene is of the formula



wherein each R^a may be independently selected from a C_1 to C_{40} linear or branched alkyl or a C_3 to C_8 cycloalkyl group, wherein said alkyl or cycloalkyl group may be substituted by one to three groups selected from: C_1 to C_6 alkoxy, C_1 to C_6 (di)alkylamino, C_6 to C_{10} aryl, C_1 to C_{30} aralkyl and C_1 to C_{30} alkaryl; NO_2 ; CO_2R ; $C(O)NH_2$; F ; each R^b , R^c , R^d , R^e , R^f , R^g , R^h and R^i can be the same or different and are each independently selected from H or any of the defined R^a groups.

28. A photo-modulatable compound as claimed in claim 24 or claim 25 wherein the photoswitchable compound is



29. A polynucleic acid comprising a photo-modulatable compound of any one of claims 24 to 28.

1

2 30. A polynucleic acid as claimed in claim 29
3 wherein said polynucleic acid comprises DNA.

4

5 31. A polynucleic acid as claimed in claim 29
6 wherein said polynucleic acid comprises RNA.

7

8 32. A polynucleic acid as claimed in any one of
9 claims 29 to 31 wherein the photo-modulatable
10 compound comprises the photoswitchable compound
11 phenylazobenzene wherein irradiation of the compound
12 at 350-360 nm converts the E-azobenzene to a Z-
13 azobenzene.

14

15 33. A polynucleic acid as claimed in any one of
16 claims 29 to 31 wherein the photo-modulatable
17 compound comprises the photoswitchable compound
18 spiropyran wherein irradiation of the compound at
19 350-360 nm converts the spiropyran to a merocyanine
20 zwitterion or cation depending upon the pH.

21

22 34. A linker of any one of claims 1 to 12 further
23 comprising a photoswitchable compound.

24

25 35. A method of modulating hybridisation of at least
26 a first polynucleic acid with at least a second
27 polynucleic acid comprising the steps:

28 - providing the at least first polynucleic acid
29 with at least one photo-modulatable compound of
30 any one of claims 24 to 28;

31 - providing the at least second polynucleic
32 acid capable of hybridising to the at least

1 first polynucleic acid in conditions suitable
2 to allow hybridisation of the at least second
3 polynucleic acid to the at least first
4 polynucleic acid; and
5 - providing a wavelength of light capable of
6 inducing photoisomerization of the photo-
7 modulatable compound in said at least first
8 polynucleic acid,
9 wherein photoisomerization of the photo-modulatable
10 compound modulates hybridisation of the at least
11 first and second polynucleic acids to each other.

12
13 36. A method of modulating the activity of
14 deoxyribozyme comprising the steps of:
15 - providing a deoxyribozyme wherein at least
16 one nucleotide of the catalytic core of the
17 deoxyribozyme is replaced by at least one
18 photo-modulatable compound of any one of claims
19 24 to 28; and
20 - providing a wavelength of light capable of
21 inducing photoisomerization of the photo-
22 modulatable compound,
23 wherein the activity of the deoxyribozyme is
24 modulated.

25
26 37. A method of modulating the binding of a
27 polymerase to a polynucleic acid comprising the
28 steps of:
29 - providing a polynucleic acid with at least
30 one a photo-modulatable compound of any one of
31 claims 24 to 28; and

1 - providing a wavelength of light capable of
2 inducing photoisomerization of the photo-
3 modulatable compound,
4 wherein the binding of the polymerase to the
5 polynucleic acid is modulated.

6

7

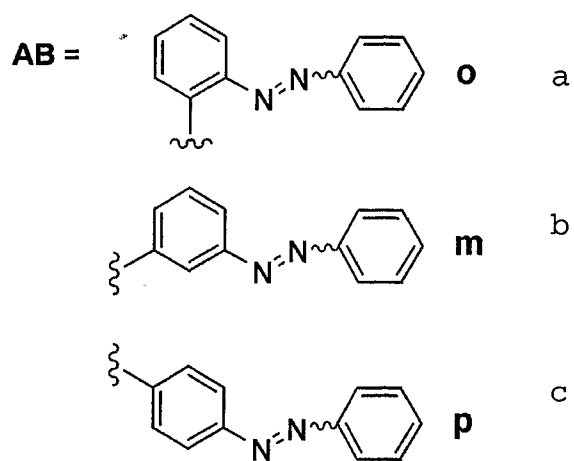
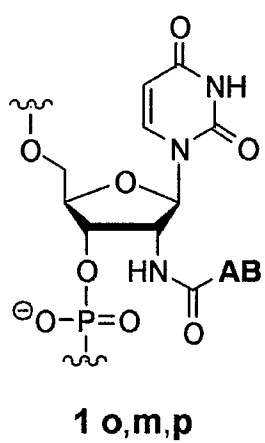


Figure 1

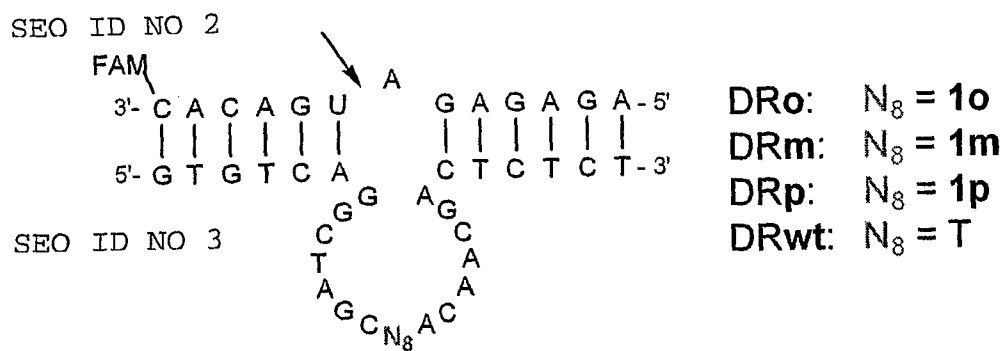


Figure 2

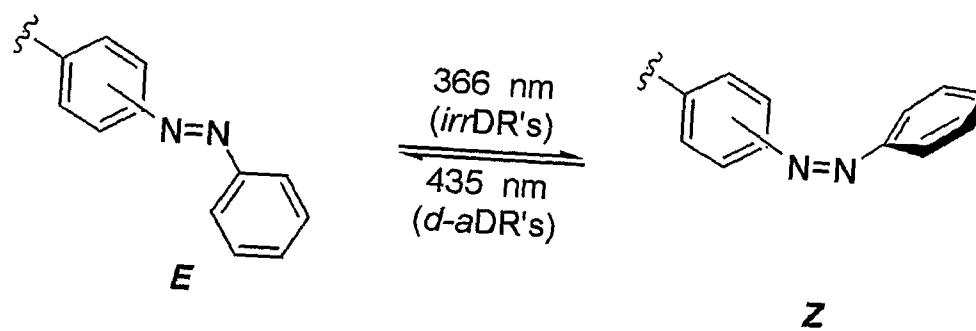


Figure 3

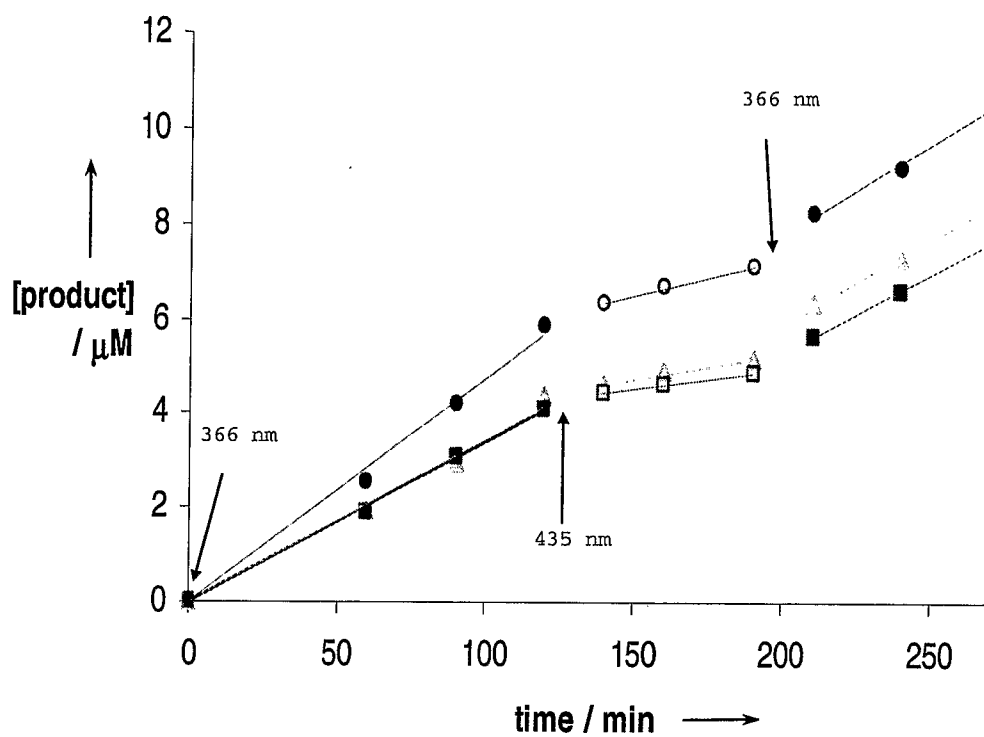


Figure 4

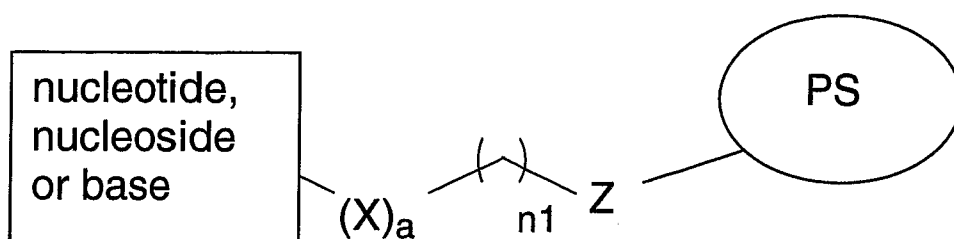


Figure 5

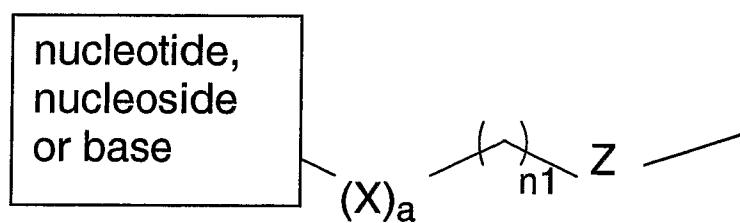
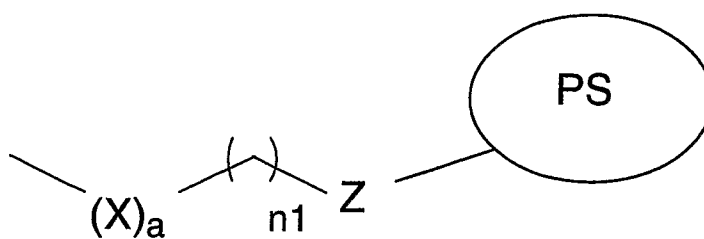


Figure 6

Figure 7



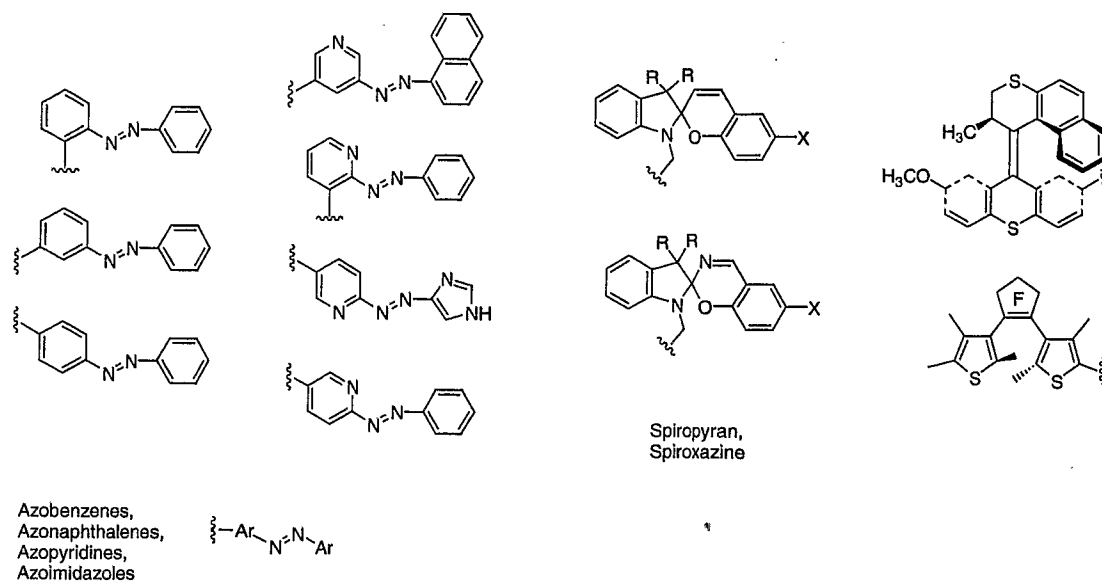


Figure 8

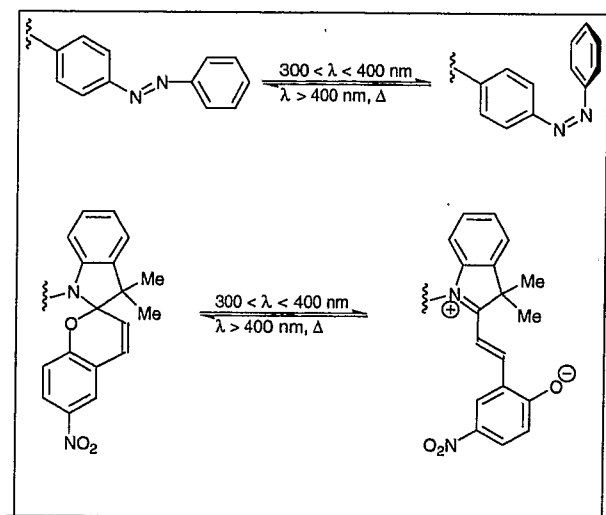


Figure 9

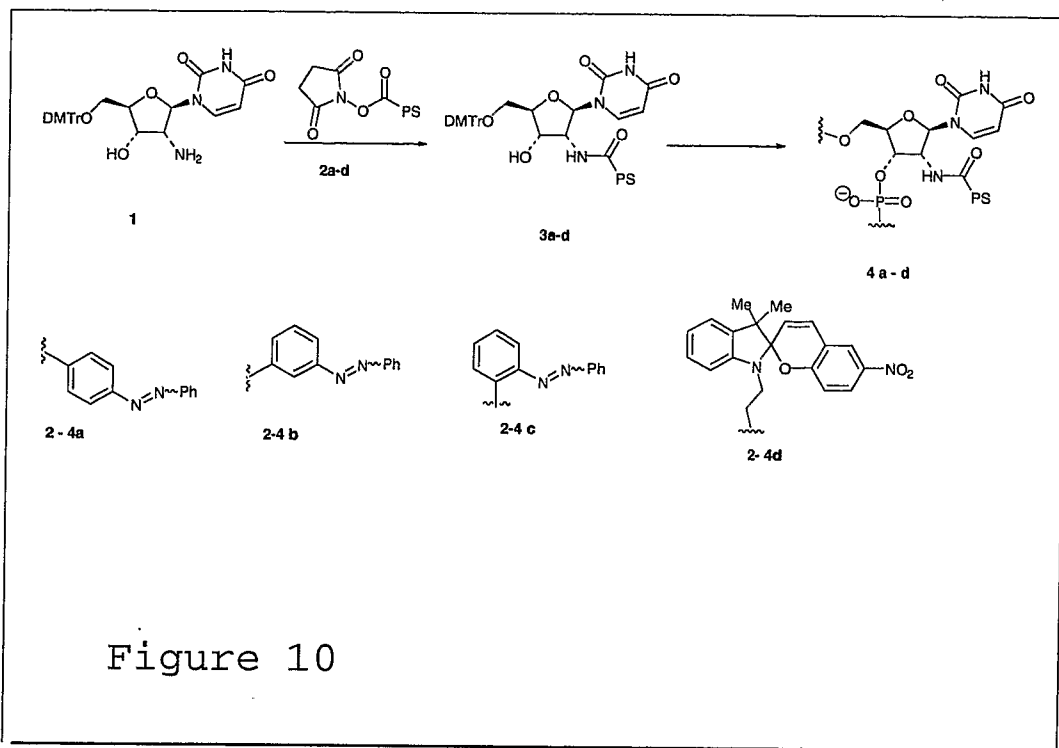


Figure 11

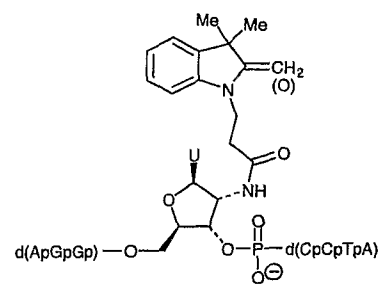
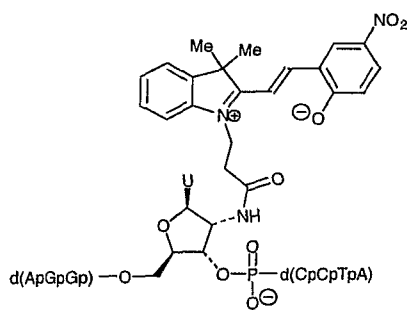
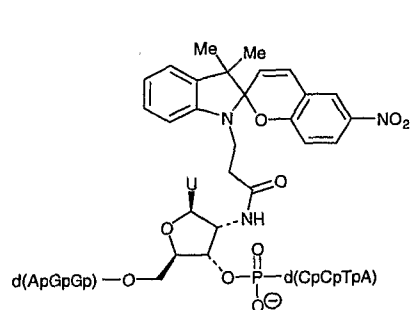
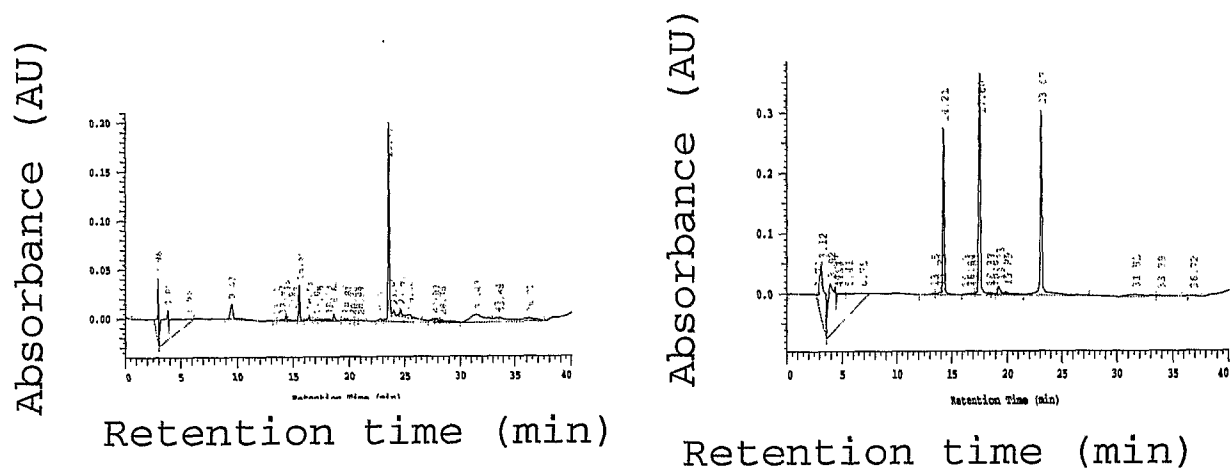


Figure 12

