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# A novel structural class of photoswitchable oligonucleotide

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**Abstract**—Directed Michaelis–Arbuzov reactions of support-bound internucleotide *O*-benzyl- or *O*-methyl-phosphite triesters with *meta*-phenylazobenzylamine or alkane-/glycol-linked α,ω-diamines were effected in the presence of iodine. The corresponding tritylated phosphoramidate-linked 11-mers were fully deprotected and released from the support under standard conditions and the *fast*- and *slow*-diastereoisomers of both the *E*- and the *Z*-*meta*-phenylazobenzyl-appended oligomers were readily resolved by RP-HPLC. The primary amine-functionalised oligonucleotides were either purified, detritylated and then finally treated with *N*-hydroxysuccinimidyl carboxylic acid ester derivatives of photoswitchable moieties (Route A) or first derivatised and then subsequently purified and detritylated (Route B). This latter route enabled resolution of *fast*- and *slow*-isomers of the trityl-on oligomers bearing novel photoswitchable azopyridine or 9-alkoxyanthracene moieties using RP-HPLC, following which the pure diastereoisomers were detritylated and characterised by MALDI-MS.


Photon-driven DNA or RNA activation both in vivo and in vitro using biocompatible wavelengths typically follows irreversible removal of masking groups from strategic functionalities of the 'caged' nucleic acid and an overall 'OFF'→'ON' state transition.[1–3] These masks can be positioned on the nucleobase,[4–6] the sugar[7,8] or the phosphate diester[9,10] and their removal can be highly controlled in both space and time thereby facilitating the fabrication of DNA arrays[11] or in vivo studies of developmental cues.[12]

In contrast, *reversible* optical control of nucleic acid conformations using oligonucleotides appended with bistable photoswitches such as azobenzenes or anthracene dimers (Fig. 1) has received less attention. This is despite considerable success with reversible photoregulation of transmembrane protein function.[13] Currently, three structural classes of oligonucleotides containing such photoswitches have been described: (i) sugar-appended,[14–16] (ii) nucleobase-analogues,[17] and (iii) the single largest class comprising non-nucleotide derivatives inserted internally[18,19] or at the 5′-terminus.[20,21]

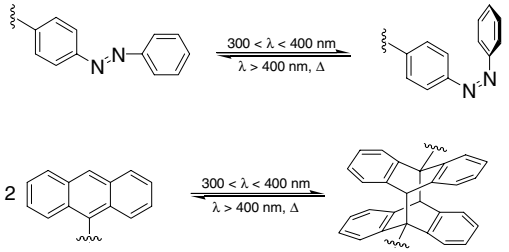


**Figure 1.** Photoswitchable moieties previously incorporated into oligonucleotides.

Although internucleotide phosphate diester analogues are one of the most extensively studied structural classes of modified oligonucleotides in particular, in the context of therapeutic sequences,[22] to the authors' knowledge there has been no description of photo*switchable* (in contrast to caged)[23] analogues belonging to this class. Herein, we describe a divergent strategy for the preparation of novel internucleotide phosphoramidate-linked photoswitchable moieties using commercially-available phosphoramidites.

Solution-phase methodologies from the laboratories of Caruthers (Route A: Scheme 1)[24] or Debart (Route

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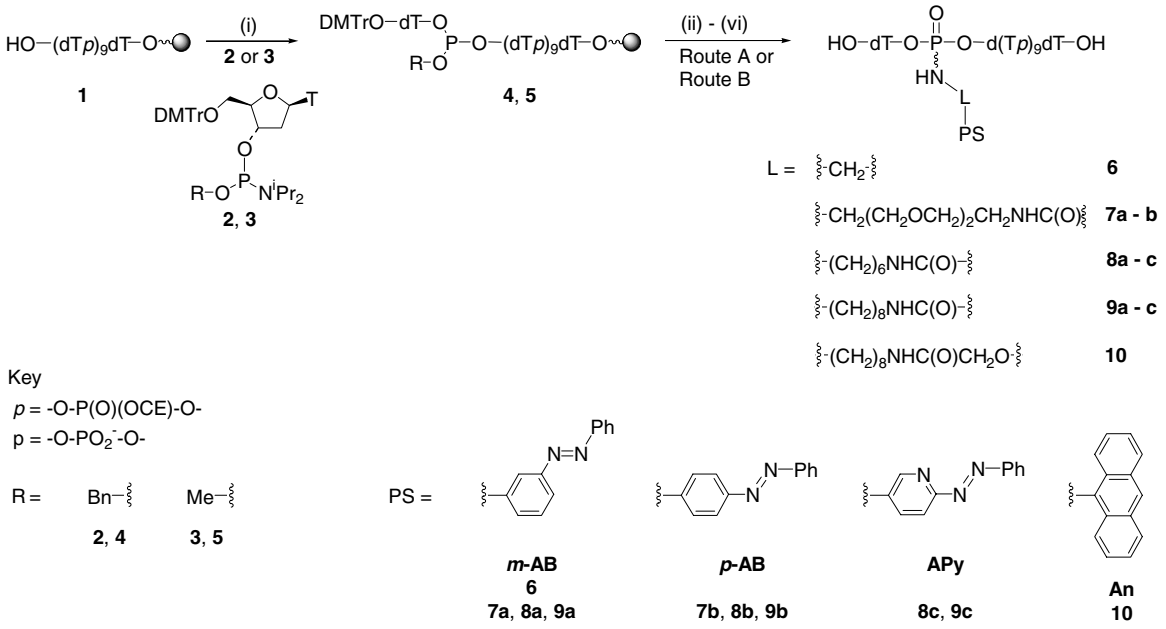


**Scheme 1.** Reagents and conditions. (i) 0.1 M **1a** or **1b**, 0.2 M 5-*S*-benzylthiotetrazole, MeCN, 4.2 min; Route A (R = Bn): (ii) 0.45 M *meta*-45 M *meta*-phenylazobenzylamine, 45 mM DIPEA, 0.1 M $I_2$, THF, rt, 7 min or 0.45 M α,ω-diamine, 0.1 M $I_2$, THF (**6**, **7**) or DCM (**8**, **9**), rt, 8 min; (iii) 40% $MeNH_2$ (aq), 65 °C, 30 min; (iv) purification of trityl-on oligonucleotides by RP-HPLC—see Table 1; (v) 80% AcOH (aq), rt, 1 h; (vi) satd $R^2$–NHS,[17] 1:1 DMSO:100 mM $Na_2CO_3$/$NaHCO_3$ aq (pH 9–9.5), 37 °C, 18 h; or Route B (R = Bn): (ii) 0.45 M α,ω-diamine, 0.1 M $I_2$, 0.1 M $Bu_4NI$, DCM, rt, 8 min; (iii) 40% $MeNH_2$ (aq), 65 °C, 30 min; (iv) satd $R^2$–NHS,[25] 1:1 DMSO:100 mM $Na_2CO_3$/$NaHCO_3$ aq (pH 9–9.5), 37 °C, 18 h; (v) purification and resolution of *fast*- and *slow*-diastereoisomers by RP-HPLC; (vi) 80% AcOH (aq), rt, 1 h.

B)[23] were adapted for the installation of internucleoside phosphoramidates on solid-supported substrates. Thus, following Route A, the 5′-hydroxyl of CPG-supported $T_{10}$ (**1**) was coupled with *O*-benzyl-phosphoramidite **2** under standard conditions to afford the putative phosphite triester intermediate **4**. The support was immediately washed with anhydrous acetonitrile under argon and further treated with 0.1 M $I_2$ in the presence of 0.45 M *meta*-phenylazobenzylamine,[26] 2,2′-(ethylenedioxy)diethylamine, 1,6-diaminohexane or 1,8-diaminooctane in anhydrous THF (0.5 ml). The crude, tritylated 11-mers thus formed were liberated from the support and β-cyanoethyl moieties concomitantly removed upon exposure to aqueous methylamine under heating. Greater than 80% reaction to phosphoramidate derivatives (which in the case of α,ω-diamine also included cross-linked species) was observed following analysis of the crude reaction mixtures by C-18 RP-HPLC (e.g., **6**; Fig. 2a). Following detritylation, the *fast*- and *slow*-phosphoramidate diastereoisomers of both *Z*-**6** and *E*-**6** were readily purified in this manner (e.g., *fast*-*E*-**6**; Fig. 2b) and individually characterised by MALDI-MS (Table 1). Irradiation of a solution of *fast*-*E*-**6** at 365 nm for 10 min induced $E \rightarrow Z$ photoswitching and the resultant *irradiated* (*irr*) photostationary state was examined by HPLC; the mole fraction of *Z*-isomer ($\chi_Z$) in *irr*-*fast*-**6** was thereby shown to be 87% (Fig. 2c).

In contrast, oligonucleotides bearing the amine-terminated glycol-, hexyl- or octyl- linkers (L; Scheme) were isolated as mixtures of isomers following purification of the tritylated sequences and subsequent deprotection (see Supplementary data). Solution-phase transformations of the amine moieties into the corresponding *meta*-(**7a**, **8a**, **9a**—***m*-AB**) or *para*-(**7b**, **8b**, **9b**—***p*-AB**) phenylazobenzoic acid amides were effected in good yield following reaction with the appropriate *N*-hydroxysuccinimidyl esters under standard conditions.[27] Attempted preparation of the *ortho*-isomer in this fashion was unsuccessful.

Although *O*-benzyl-phosphite triesters typically demonstrate high yielding directed-M-A reactions[28] as described above, in our hands phosphoroamidite coupling efficiencies using commercial **2** (with DMTr or MMTr protection) were highly variable (0–99%). In contrast, reproducibly high coupling yields of the *O*-methyl phosphoramidite **3** were accompanied by moderate (typically 40–60%) conversions of the putative phosphite triester intermediate **5** to the corresponding phosphoramidates (Route B). Tetrabutylammonium iodide (0.1 M) was added to iodine/amine mixtures following a recent report of the utility of [BMIM][Cl] in promoting M-A reactions.[29] We also observed reduced levels of side-products, especially of the phosphate diester, in the presence of halide anions.

Post-synthetic derivatisation of oligonucleotide-appended amine-functionalities introduced in this manner was performed upon the crude tritylated oligomers following the work by Kojima et al. describing enhanced reactivity of such amines in the presence of proximal aryl groups.[30] Oligonucleotides bearing two novel photoswitchable moieties, phenylazopyridine (**8c**, **9c**) and 9-alkoxyanthracene (**10**) were thereby prepared as pure diastereoisomers. $E \rightarrow Z$ Photoswitching efficiency

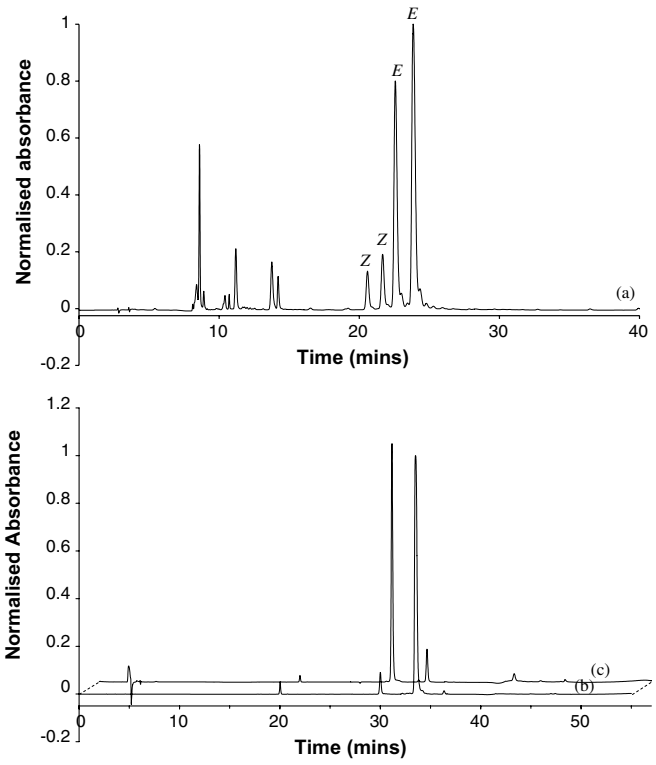


**Figure 2.** RP-HPLC profiles of: (a) crude 6; (b) purified *fast*-*E*-**6**; (c) *irr*-*fast*-**6**.

**Table 1.** RP-HPLC characterisation of phosphoramidate-linked oligonucleotides–photoswitch conjugates

| Product | Linker | Photoswitch | C18-HPLC rt/min[a] (conditions)[b] | | MALDI-MS | |
|---|---|---|---|---|---|---|
| | | | | | *m*/*z* | Theory |
| *Fast*-**6** | –$CH_2$– | ***m*-AB** | 22.56 | (I) | 3477.0 | *3475.7* |
| *Slow*-**6** | | | 23.84 | (I) | 3478.4 | |
| **7a** | –$CH_2(CH_2OCH_2)_2CH_2NHCO$– | ***m*-AB** | 35.36 | (II) | 3627.6 | *3620.7* |
| **7b** | –$CH_2(CH_2OCH_2)_2CH_2NHCO$– | ***p*-AB** | 35.23 | (II) | 3624.4 | *3620.7* |
| **8a** | –$(CH_2)_6NHCO$– | ***m*-AB** | 38.91 | (II) | 3595.7 | *3588.7* |
| **8b** | –$(CH_2)_6NHCO$– | ***p*-AB** | 39.04 | (II) | 3590.7 | *3588.7* |
| *Fast*-**8c** | –$(CH_2)_6NHCO$– | **APy** | 17.36 | (III) | 3593.6 | *3589.7* |
| *Slow*-**8c** | | | 17.39 | | 3593.9 | |
| **9a** | –$(CH_2)_8NHCO$– | ***m*-AB** | 40.64 | (II) | 3619.8 | *3616.8* |
| **9b** | –$(CH_2)_8NHCO$– | ***p*-AB** | 40.99 | (II) | 3621.6 | *3616.8* |
| *Fast*-**9c** | –$(CH_2)_8NHCO$– | **APy** | 19.84 | (III) | 3620.8 | *3617.8* |
| *Slow*-**9c** | | | 21.47 | | 3621.9 | |
| *Fast*-**10** | –$(CH_2)_8NHCOCH_2O$– | **An** | 24.91 | (III) | 3644.7 | *3642.8* |
| *Slow*-**10** | | | 25.44 | | 3647.5 | |

[a] Times given for *E*-isomer only.

[b] Monitoring at 260 nm. Flow rate: 1 ml $min^{-1}$. Mobile phases: A: 0.1 M TEAA, 5% (v/v) MeCN, pH 6.5; B: 0.1 M TEAA, 65% (v/v) MeCN, pH 6.5; C: MeCN—gradients use varying proportions of A and B (I/II) or A and C (III). Stationary phases: RP-C18 Column. (I/II): 5 μm, 250 × 4.6 mm; (III): 5 μm, 150 × 4.6 mm. Gradient I (% B): 0–5 min, 0%; 38 min, 40%; 40 min 100%. Gradient II (% B): 0–5 min, 0%; 35 min, 47%; 38–43 min, 100%; 50–55 min, 0%. Gradient III (% C): 0–5 min, 0%; 45 min, 20%; 50 min, 50%; 55 min 100%.

of the novel photoswitch was found to be comparable to that of the *irradiated* photostationary state of *para*-azobenzenes;[14] $\chi_Z$ for the *irr*-*slow*-**9c** (75%).

In conclusion, divergent methodology for the preparation of a novel structural class of photoswitchable oligonucleotides has been described in which both prochiral non-bridging oxygen atoms within an internucleotide phosphate diester are substituted by a nitrogen atom to which is appended a photoswitchable moiety through linkers of variable length. By choosing a suitable deprotection and labelling strategy the diastereoisomeric phosphoramidates can be resolved, further increasing the diversity of photoswitchable conformation-space which can be accessed via this route and in addition offers the potential for programmable metal–ion coordination by azopyridine–oligonucleotide conjugates.[31] We envisage that this methodology should therefore

significantly enhance the accessiblility of this class of oligonucleotides with properties, which are both 'light-programmable' and also susceptible to the acidic endosomal pH enabling highly focused delivery of therapeutic oligonucleotides.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.07.028.

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