A novel structural class of photoswitchable oligonucleotide


Published in:
Tetrahedron Letters

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Open Access
This research has been made openly available by Queen's academics and its Open Research team. We would love to hear how access to this research benefits you. – Share your feedback with us: http://go.qub.ac.uk/oa-feedback
This article was published in an Elsevier journal. The attached copy is furnished to the author for non-commercial research and education use, including for instruction at the author’s institution, sharing with colleagues and providing to institution administration.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier’s archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright
A novel structural class of photoswitchable oligonucleotide

Emma E. Smith, Jennifer N. McClean, Leonie A. Cooke, Jean-Louis Duprey, Maighréad McCourt, Martin M. Fabani, James H. R. Tucker, and Joseph S. Vyle

School of Chemistry and Chemical Engineering, Queen’s University Belfast, David Keir Building, Stranmillis Road, Belfast BT9 5AG, UK
School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Received 11 May 2007; revised 26 June 2007; accepted 5 July 2007
Available online 2 August 2007

Abstract—Directed Michaelis–Arbuzov reactions of support-bound internucleotide O-benzyl- or O-methyl-phosphate 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.© 2007 Elsevier Ltd. All rights reserved.

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 sugar7,8 or the phosphate diester9,10 and their removal can be highly controlled in both space and time thereby facilitating the fabrication of DNA arrays11 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-appended14–16 (ii) nucleobase-analogues17 and (iii) the single largest class comprising non-nucleotide derivatives inserted internally18,19 or at the 5′-terminus.20,21

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 photoswitchable (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
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. Oligonucleotides bearing two novel photoswitchable moieties, phenylazopyridine (8c, 9c) and 9-alkoxyanthracene (10) were thereby prepared as pure diastereoisomers. $E\rightarrow Z$ Photoswitching efficiency
of the novel photoswitch was found to be comparable to that of the irradiated photostationary state of para-azonobenzenes.\textsuperscript{14} \( Z \) for the \textit{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.\textsuperscript{31} We envisage that this methodology should therefore

---

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Linker</th>
<th>Photoswitch</th>
<th>C18-HPLC rt/min(^a) (conditions)(^b)</th>
<th>MALDI-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast-6</td>
<td>–CH(_2)–</td>
<td>( m\text{-AB} )</td>
<td>22.56 (I)</td>
<td>3477.0 3475.7</td>
</tr>
<tr>
<td>Slow-6</td>
<td>–CH(_2)(CH(_3)OCH(_2))(_2)CH(_2)NHCO–</td>
<td>( p\text{-AB} )</td>
<td>23.84 (I)</td>
<td>3478.4</td>
</tr>
<tr>
<td>7a</td>
<td>–CH(_2)(CH(_3)OCH(_2))(_2)CH(_2)NHCO–</td>
<td>( m\text{-AB} )</td>
<td>35.36 (II)</td>
<td>3627.6 3620.7</td>
</tr>
<tr>
<td>7b</td>
<td>–CH(_2)(CH(_3)OCH(_2))(_2)CH(_2)NHCO–</td>
<td>( p\text{-AB} )</td>
<td>35.23 (II)</td>
<td>3624.4 3620.7</td>
</tr>
<tr>
<td>8a</td>
<td>–(CH(_3))(_2)NHCO–</td>
<td>( m\text{-AB} )</td>
<td>39.04 (II)</td>
<td>3590.7 3588.7</td>
</tr>
<tr>
<td>8b</td>
<td>–(CH(_3))(_2)NHCO–</td>
<td>( p\text{-AB} )</td>
<td>38.91 (II)</td>
<td>3595.7 3588.7</td>
</tr>
<tr>
<td>Fast-8c</td>
<td>–(CH(_3))(_2)NHCO–</td>
<td>( APy )</td>
<td>17.36 (III)</td>
<td>3593.6 3589.7</td>
</tr>
<tr>
<td>Slow-8c</td>
<td>–(CH(_3))(_2)NHCO–</td>
<td></td>
<td>17.39</td>
<td>3593.9</td>
</tr>
<tr>
<td>9a</td>
<td>–(CH(_3))(_2)NHCO–</td>
<td>( m\text{-AB} )</td>
<td>40.64 (II)</td>
<td>3619.8 3616.8</td>
</tr>
<tr>
<td>9b</td>
<td>–(CH(_3))(_2)NHCO–</td>
<td>( p\text{-AB} )</td>
<td>40.99 (II)</td>
<td>3621.6 3616.8</td>
</tr>
<tr>
<td>Fast-9c</td>
<td>–(CH(_3))(_2)NHCO–</td>
<td>( APy )</td>
<td>19.84 (III)</td>
<td>3620.8 3617.8</td>
</tr>
<tr>
<td>Slow-9c</td>
<td>–(CH(_3))(_2)NHCO–</td>
<td></td>
<td>21.47</td>
<td>3621.9</td>
</tr>
<tr>
<td>Fast-10</td>
<td>–(CH(_3))(_2)NHCOCH(_2)O–</td>
<td>( An )</td>
<td>24.91 (III)</td>
<td>3644.7 3642.8</td>
</tr>
<tr>
<td>Slow-10</td>
<td>–(CH(_3))(_2)NHCOCH(_2)O–</td>
<td></td>
<td>25.44</td>
<td>3647.5</td>
</tr>
</tbody>
</table>

\( a \) Times given for \( E \)-isomer only.

\( b \) Monitoring at 260 nm. Flow rate: 1 ml min\(^{-1}\). Mobile phases: A: 0.1 M TEAAA, 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 \( l m, 250 \times 4.6 \text{ mm}; \) (III): 5 \( l m, 150 \times 4.6 \text{ mm}. \) 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\%.
significantly enhance the accessibility 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.

Acknowledgements

This work was supported by the EPSRC (EP/C00776X/1 to EES) QUESTOR (CAST award to JNMcC), the EU (ESF to LAC), the School of Chemistry, University of Birmingham (to JLD) and the School of Chemistry and Chemical Engineering, QUB. We thank Jerry Davies, AP de Silva and especially Nicholas Fletcher (all of QUB) for helpful discussions.

Supplementary data

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

References and notes

26. Prepared following transformation of the corresponding alcohol into the azide (Silberman, H.; Silberman-Mar