Check for updates

Emerging Topics in Life Sciences (2021) **5** 691–697
https://doi.org/10.1042/ETLS20210169

PORTLAND PRESS

Perspective

Artificial nucleic acid backbones and their applications in therapeutics, synthetic biology and biotechnology

Sven Epple[1], **Afaf H. El-Sagheer**[1,2] **and Tom Brown**[1]

[1]Chemistry Research Laboratory, University of Oxford, Oxford OX1 3TA, U.K.; [2]Chemistry Branch, Department of Science and Mathematics, Faculty of Petroleum and Mining Engineering, Suez University, Suez 43721, Egypt

Correspondence: Tom Brown (tom.brown@chem.ox.ac.uk)

OPEN ACCESS

The modification of DNA or RNA backbones is an emerging technology for therapeutic oligonucleotides, synthetic biology and biotechnology. Despite a plethora of reported artificial backbones, their vast potential is not fully utilised. Limited synthetic accessibility remains a major bottleneck for the wider application of backbone-modified oligonucleotides. Thus, a variety of readily accessible artificial backbones and robust methods for their introduction into oligonucleotides are urgently needed to utilise their full potential in therapeutics, synthetic biology and biotechnology.

Chemical modification of DNA and RNA play myriad roles in therapeutics, diagnostics and synthetic biology. Oligonucleotides (ONs) can be modified at the nucleobase, the sugar, or the phosphodiester (PO) backbone (Figure 1) [1,2]. Backbone modifications can generate remarkably biomimetic constructs such as therapeutic oligonucleotides (TherONs) [2], xenobiotic genetic polymers [3–5], aptamers [4,6–8], ribozymes [9], single guide RNAs [10] and synthetic genes [11–13]. Many artificial backbones are known, but their applications in therapeutics and synthetic biology remain limited. This perspective focuses on artificial nucleic acid backbones and briefly describes the most common strategies for their synthesis. Selected examples demonstrate their potential and highlight the current limitations of this technology.

Therapeutic oligonucleotides

Synthesis

The preparation of TherONs, which are typically short (~20mer) chemically modified ONs [14,15], mainly relies on solid support-based oligonucleotide synthesis [16,17]. Artificial backbones are usually introduced by modified on-resin coupling of a monomer to form the unnatural backbone (monomer approach, Figure 2A), or by coupling of a dinucleoside containing the artificial linkage (dimer approach, Figure 2A). The monomer approach presents significant challenges due to the potential chemical incompatibility with standard ON chemistry and synthesis equipment. Nevertheless, on-resin formation of artificial backbones has been reported for boranophosphates (borano) [18], phosphorothioates (PS) [19], phosphorodithioates (PDS) [20], phosphoramidates (PA) [21], methylphosphonates (MP) [22,23], amides (AM) [24–27] and to a limited extend for triazole linkages (TLs) [28]. Indeed, the dimer approach is preferred for backbones that are harder to form on a solid support, such as ureas [29], squaramides (SQAM) [30], or triazoles [31–36]. The artificial linkage can be pre-formed as part of a dinucleoside that is compatible with standard oligonucleotide synthesis. However, 16 modified dinucleosides are required to cover all sequence possibilities and the construction of consecutive artificial backbones is not possible. In general, the limited availability of optimised and easy-to-use protocols for on-resin formation of artificial backbones, and the demands of the dimer approach remain major bottlenecks in research in the TherON area.

Received: 1 June 2021
Revised: 7 July 2021
Accepted: 14 July 2021

Version of Record published: 23 July 2021

© 2021 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and the Royal Society of Biology and distributed under the Creative Commons Attribution License 4.0 (CC BY).

Downloaded from http://port.silverchair.com/emergtoplifesci/article-pdf/5/5/691/924328/etls-2021-0169c.pdf by guest on 24 April 2024

Emerging Topics in Life Sciences (2021) **5** 691–697
https://doi.org/10.1042/ETLS20210169

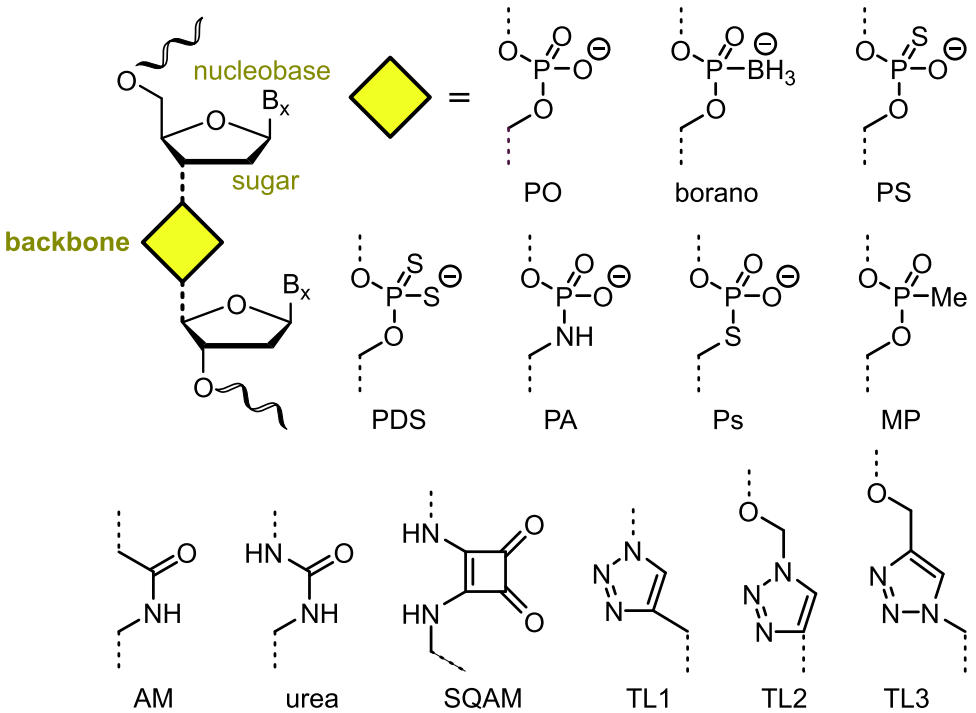

Figure 1. Selected backbone modifications.

The nucleobase (B_x), sugar and backbone are labelled in the dinucleoside structure. Backbone modifications are represented by a yellow diamond.

Antisense activity

From the plethora of artificial backbones, only a limited number of chemical modifications are found in clinically approved TherONs [37]. Synthetic accessibility, efficient target hybridisation, serum stability and the retention of antisense activity are key requirements for a successful artificial backbone (Figure 2B) [1]. As such, the PS modification is fully compatible with two of the predominant antisense approaches: (i) splice-switching to modulate mRNA maturation, or (ii) activation of RNase H to degrade an mRNA [38–40]. Moreover, advanced synthetic protocols [41] and favourable pharmacokinetics [42] of phosphorothioate oligonucleotides all contribute to its prevalence in clinically approved TherONs. However, PS backbones are linked to toxicity [43,44] and exploration of alternative modifications is urgently needed. A recent study showed that toxicity of PS-TherONs can be significantly reduced by a single MP substitution [45]. Other promising modalities of TherONs include peptide [46] and morpholino [47] nucleic acids which combine backbone and sugar modifications: The former combines amide bonds to connect acyclic subunits and the latter combines a phosphorodiamidate backbone and a morpholine ring as a sugar substitute. However, peptide nucleic acids suffer from poor solubility and inefficient cellular uptake [48,49] while morpholino oligonucleotides are associated with concerns for off-target effects [50,51]. Unfortunately, alternative backbones such as triazoles [32,33,52] or carbamates [53,54] reduce RNA target affinity which must be compensated for by additional sugar or base modifications [35,36,55,56]. Lengthy synthetic procedures limit the combination of artificial backbones with other base or sugar modifications. Nevertheless, these studies showcase the vast potential of alternative backbones in TherONs and emphasise the need for easily accessible backbone modifications for therapeutic research beyond specialised synthetic laboratories.

Synthetic biology

Nucleic acid formation

Different from short TherONs, modified long oligonucleotides for synthetic biology can be several hundreds of bases in length. This exceeds the limits of solid-phase oligonucleotide synthesis and requires different strategies.

© 2021 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and the Royal Society of Biology and distributed under the Creative Commons Attribution License 4.0 (CC BY).

Downloaded from http://port.silverchair.com/emergtoplifesci/article-pdf/5/5/691/924328/etls-2021-0169c.pdf by guest on 24 April 2024

Emerging Topics in Life Sciences (2021) **5** 691–697
https://doi.org/10.1042/ETLS20210169

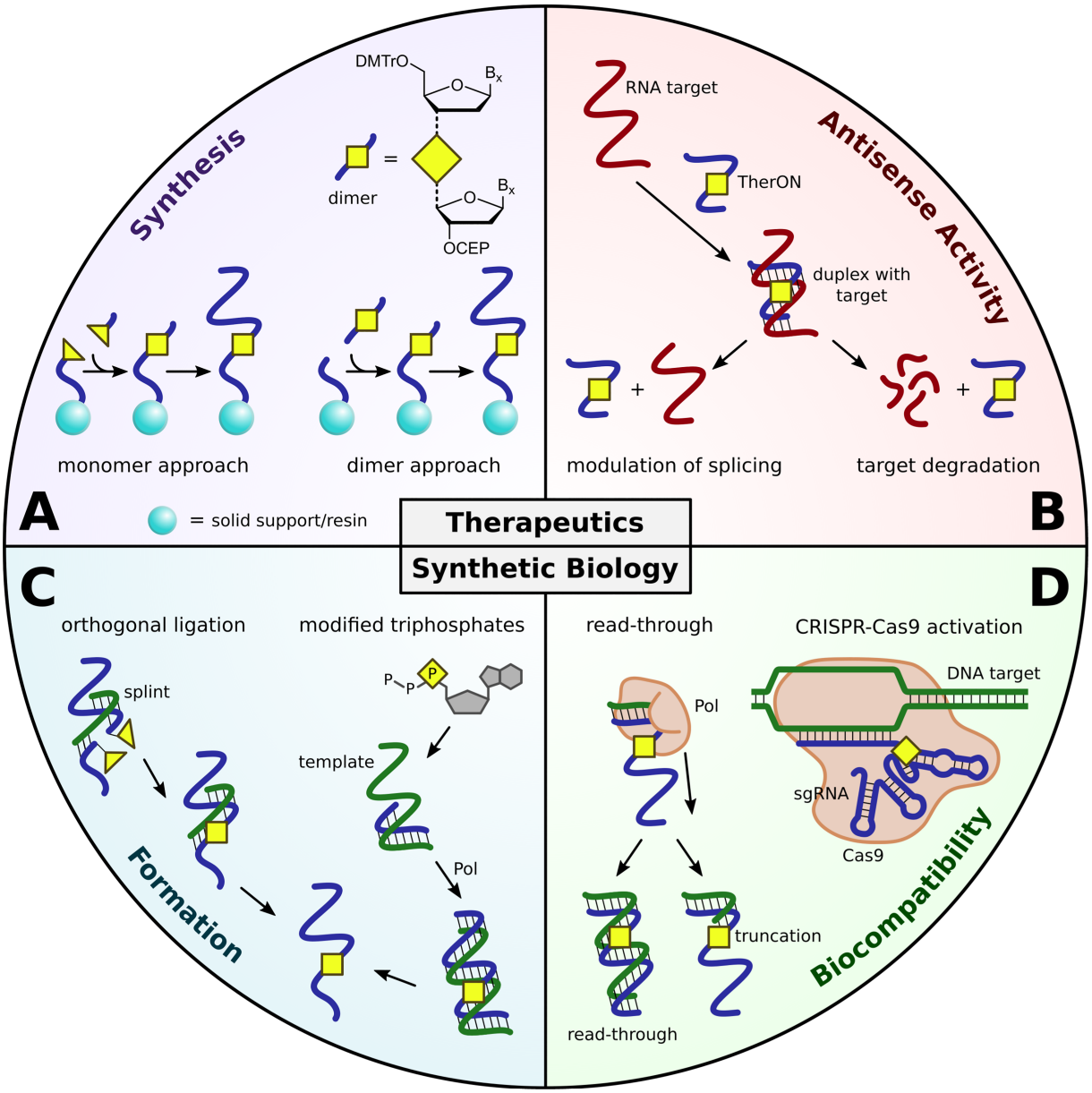

Figure 2. Modified backbones for applications in therapeutics and synthetic biology.

Applications of artificial backbones can be divided into therapeutics and synthetic biology (top and bottom half of the circle, respectively). Yellow triangles represent reactive functional groups and yellow squares represent artificial backbones. (**A**) Synthetic approaches for backbone modified TherONs. Monomer approach: Functional groups react to form the artificial backbone during ON synthesis on a solid support. Dimer approach: the artificial backbone is part of a 4,4′-dimethoxytrityl (DMTr)-protected dinucleoside cyanoethyl phosphoramidite (CEP) that can be coupled using standard ON synthesis conditions. (**B**) Antisense activity of a chemically modified TherON. Hybridisation of the TherON with a target RNA can either lead to alternative splicing or degradation of the target RNA. (**C**) Strategies to access long chemically modified ONs for applications in synthetic biology. Orthogonal ligation: Functional groups of short ONs react to form the artificial backbone. This ligation is often facilitated by a splint (template) ON. Modified triphosphates: the artificial backbone can be part of a modified triphosphate that is a substrate for a polymerase (Pol). Incorporation of the modified triphosphate leads to sites with artificial backbones. (**D**) Selected examples of long backbone modified ONs in synthetic biology. Polymerase read-through: Compatible artificial backbones in genetic templates can be read by polymerases to produce a replicon with the complementary sequence. Incompatible artificial backbones lead to truncation or mutation sites during replication. CRISPR–Cas9 activation: Backbone modified sgRNAs can direct Cas9 to sequence-specific sites in DNA to facilitate cutting of the DNA target.

Downloaded from http://port.silverchair.com/emergtoplifesci/article-pdf/5/5/691/924328/etls-2021-0169c.pdf by guest on 24 April 2024

© 2021 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and the Royal Society of Biology and distributed under the Creative Commons Attribution License 4.0 (CC BY).

Emerging Topics in Life Sciences (2021) **5** 691–697
https://doi.org/10.1042/ETLS20210169

One approach is to assemble long modified oligomers from shorter, chemically modified ONs via ligation reactions (Figure 2C). Such ligation chemistry must be orthogonal to other functional groups within the oligomers and is often facilitated by a splint/template (orthogonal ligation, Figure 2C). A combinatorial approach for the discovery of splint-templated chemical ligations has been reported to identify DNA-compatible reactions to ligate terminally functionalised ONs [57]. Moreover, the generation of artificial backbone mimics has been shown for bridging 5′-*S*-phosphorothioester linkages (Ps) [58], PA [59–62], AM [61], urea [63], SQAM [63], TL1 [64] and TL3 [61]. Indeed, copper-catalysed azide-alkyne cycloaddition (CuAAC) to form TL3 was reported for the assembly of whole genes [12,65] and long RNA [9,10] from azide and alkyne modified shorter ONs. This approach enables the precise, site-specific introduction of artificial backbones and other modifications, but is limited by the compatibility of the ligation reaction with terminally modified ONs under aqueous conditions. Another approach is the introduction of artificial backbones through enzymatic synthesis using modified nucleotide triphosphates as substrates [4,13,66–68]. This has been demonstrated by the enzymatic synthesis of a PA-modified gene in the presence of an unnatural cytidine triphosphate analogue [13]. However, the controlled introduction of artificial backbones or other modifications at specific sites is not readily achievable with this method, and engineered polymerases are often required. Whilst engineered polymerases and ligases can accept base- and sugar-modified triphosphates as substrates, such incorporations still form PO bonds [69]. The engineering of enzymes to generate unnatural internucleoside linkages is inherently harder but has been recently demonstrated for uncharged ethylphosphonates [4].

Biocompatibility of artificial backbones

Not all backbone-modified ONs have the desired biocompatibility for applications in synthetic biology. For instance, TL1 was recently described for the preparation of next-generation sequencing libraries but suffers from inefficient and inaccurate replication when used with several polymerases (polymerase read-through, Figure 2D) [34,64]. In contrast, TL3 has good read-through compatibility with DNA and RNA polymerases [70,71] and can be replicated with high fidelity [34] enabling expression of click-assembled genes in bacteria [12,59,70] and mammalian cells [11]. Similarly, phosphoramidate backbones can be read by DNA and RNA polymerases [59,61], and translated by ribosomes [60], and introduction of PA-modified genes can lead to the expression of their associated genetic information in bacteria [13]. Apart from gene synthesis, artificial backbones such as TL2, [31] urea [63] and SQAM [63] were reported as components of modified primers in PCR. In the case of SQAM, *in situ* template assembly by target-templated SQAM formation was utilised for RNA detection [63]. Other examples include the construction of a functional hammerhead ribozyme [9] or bioactive single guide RNAs (sgRNAs) for gene editing using a split-and-click strategy to form TL3 (CRISPR–Cas9 activation, Figure 2D) [10]. The versatility of artificial backbones in synthetic biology and biotechnology emphasises their vast potential. However, not all artificial backbones perform well, and the molecular requirements for biological integrity remain elusive [34]. Hence, easily accessible and structurally diverse artificial backbones are needed to fully exploit the vast potential of artificial nucleic acids in synthetic biology and biotechnology.

Conclusion

Artificial backbones only account for a fraction of ON modifications but hold great potential. Despite many trailblazing discoveries emphasising the beneficial effects of artificial backbones in therapeutics and synthetic biology, their broad application and an in-depth understanding of their molecular requirements are hampered by limited synthetic accessibility. Thus, new chemical approaches are urgently needed for the synthesis of easy-to-access modified ONs to facilitate research on artificial backbones in a broader spectrum of laboratories.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Open Access

Open access for this article was enabled by the participation of University of Oxford in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with JISC.

Author Contributions

All authors contributed to the writing and editing of the manuscript, reviewing and analysing the literature.

© 2021 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and the Royal Society of Biology and distributed under the Creative Commons Attribution License 4.0 (CC BY).

Downloaded from http://port.silverchair.com/emergtoplifesci/article-pdf/5/5/691/924328/etls-2021-0169c.pdf by guest on 24 April 2024

Acknowledgements

S.E. is grateful to the EPSRC Centre for Doctoral Training in Synthesis for Biology and Medicine (EP/L015838/1) for a studentship, generously supported by AstraZeneca, Diamond Light Source, Defence Science and Technology Laboratory, Evotec, GlaxoSmithKline, Janssen, Novartis, Pfizer, Syngenta, Takeda, UCB and Vertex. A.H.E.-S. is supported by BBSRC grant BB/R008655/1, in partnership with ATDBio Ltd.

Abbreviations

AM, amides; MP, methylphosphonates; ONs, Oligonucleotides; PA, phosphoramidates; PO, phosphodiester; PS, phosphorothioates; SQAM, squaramides; TherONs, therapeutic oligonucleotides.

References

1 Khvorova, A. and Watts, J.K. (2017) The chemical evolution of oligonucleotide therapies of clinical utility. *Nat. Biotechnol.* **35**, 238–248 https://doi.org/10.1038/nbt.3765
2 Brad Wan, B. and Seth, P.P. (2016) The medicinal chemistry of therapeutic oligonucleotides. *J. Med. Chem.* **59**, 9645–9667 https://doi.org/10.1021/acs.jmedchem.6b00551
3 Liu, C., Cozens, C., Jaziri, F., Rozenski, J., Maréchal, A., Dumbre, S. et al. (2018) Phosphonomethyl oligonucleotides as backbone-modified artificial genetic polymers. *J. Am. Chem. Soc.* **140**, 6690–6699 https://doi.org/10.1021/jacs.8b03447
4 Arangundy-Franklin, S., Taylor, A.I., Porebski, B.T., Genna, V., Peak-Chew, S., Vaisman, A. et al. (2019) A synthetic genetic polymer with an uncharged backbone chemistry based on alkyl phosphonate nucleic acids. *Nat. Chem.* **11**, 533–542 https://doi.org/10.1038/s41557-019-0255-4
5 Eremeeva, E. and Herdewijn, P. (2019) Reprint of: non canonical genetic material. *Curr. Opin. Biotechnol.* **60**, 259–267 https://doi.org/10.1016/j.copbio.2019.11.009
6 Kang, J., Lee, M.S., Copland, J.A., Luxon, B.A. and Gorenstein, D.G. (2008) Combinatorial selection of a single stranded DNA thioaptamer targeting TGF-β1 protein. *Bioorganic Med. Chem. Lett.* **18**, 1835–1839 https://doi.org/10.1016/j.bmcl.2008.02.023
7 Higashimoto, Y., Matsui, T., Nishino, Y., Taira, J., Inoue, H., Takeuchi, M. et al. (2013) Blockade by phosphorothioate aptamers of advanced glycation end products-induced damage in cultured pericytes and endothelial cells. *Microvasc. Res.* **90**, 64–70 https://doi.org/10.1016/j.mvr.2013.08.010
8 Varizhuk, A.M., Tsvetkov, V.B., Tatarinova, O.N., Kaluzhny, D.N., Florentiev, V.L., Timofeev, E.N. et al. (2013) Synthesis, characterization and in vitro activity of thrombin-binding DNA aptamers with triazole internucleotide linkages. *Eur. J. Med. Chem.* **67**, 90–97 https://doi.org/10.1016/j.ejmech.2013.06.034
9 El-Sagheer, A.H. and Brown, T. (2010) New strategy for the synthesis of chemically modified RNA constructs exemplified by hairpin and hammerhead ribozymes. *Proc. Natl Acad. Sci. U.S.A.* **107**, 15329–15334 https://doi.org/10.1073/pnas.1006447107
10 Taemaitree, L., Shivalingam, A., El-Sagheer, A.H. and Brown, T. (2019) An artificial triazole backbone linkage provides a split-and-click strategy to bioactive chemically modified CRISPR sgRNA. *Nat. Commun.* **10**, 1610 https://doi.org/10.1038/s41467-019-09600-4
11 Birts, C.N., Sanzone, A.P., El-Sagheer, A.H., Blaydes, J.P., Brown, T. and Tavassoli, A. (2014) Transcription of click-linked DNA in human cells. *Angew. Chem. Int. Ed.* **53**, 2362–2365 https://doi.org/10.1002/anie.201308691
12 Kukwikila, M., Gale, N., El-Sagheer, A.H., Brown, T. and Tavassoli, A. (2017) Assembly of a biocompatible triazole-linked gene by one-pot click-DNA ligation. *Nat. Chem.* **9**, 1089–1098 https://doi.org/10.1038/nchem.2850
13 Nguyen, H., Abramov, M., Eremeeva, E. and Herdewijn, P. (2020) In vivo expression of genetic information from phosphoramidate–DNA. *ChemBioChem* **21**, 272–278 https://doi.org/10.1002/cbic.201900712
14 Crooke, S.T. (2017) Molecular mechanisms of antisense oligonucleotides. *Nucleic Acid Ther.* **27**, 70–77 https://doi.org/10.1089/nat.2016.0656
15 Smith, C.I.E. and Zain, R. (2019) Therapeutic oligonucleotides: state of the Art. *Annu. Rev. Pharmacol. Toxicol.* **59**, 605–630 https://doi.org/10.1146/annurev-pharmtox-010818-021050
16 Beaucage, S.L. and Caruthers, M.H. (1981) Deoxynucleoside phosphoramidites-A new class of key intermediates for deoxypolynucleotide synthesis. *Tetrahedron Lett.* **22**, 1859–1862 https://doi.org/10.1016/S0040-4039(01)90461-7
17 Andrews, B.I., Antia, F.D., Brueggemeier, S.B., Diorazio, L.J., Koenig, S.G., Kopach, M.E. et al. (2021) Sustainability challenges and opportunities in oligonucleotide manufacturing. *J. Org. Chem.* **86**, 49–61 https://doi.org/10.1021/acs.joc.0c02291
18 Li, P., Sergueeva, Z.A., Dobrikov, M. and Shaw, B.R. (2007) Nucleoside and oligonucleoside boranophosphates: chemistry and properties. *Chem. Rev.* **107**, 4746–4796 https://doi.org/10.1021/cr050009p
19 Stec, W.J., Zon, G., Egan, W. and Stec, B. (1984) Automated solid-phase synthesis, separation, and stereochemistry of phosphorothioate analogues of oligodeoxyribonucleotides. *J. Am. Chem. Soc.* **106**, 6077–6079 https://doi.org/10.1021/ja00332a054
20 Brill, W.K.D., Tang, J.Y., Ma, Y.X. and Caruthers, M.H. (1989) Synthesis of oligodeoxynucleoside phosphorodithioates via thioamidites. *J. Am. Chem. Soc.* **111**, 2321–2322 https://doi.org/10.1021/ja00188a066
21 Kers, I., Stawiński, J. and Kraszewski, A. (1999) Aryl H-phosphonates. 10. synthesis of nucleoside phosphoramidate and nucleoside phosphoramidothioate analogues via H-phosphonamidate intermediates. *Tetrahedron* **55**, 11579–11588 https://doi.org/10.1016/S0040-4020(99)00656-0
22 Miller, P.S., Yano, J., Yano, E., Carroll, C., Jayaraman, K. and Ts'o, P.O.P. (1979) Nonionic nucleic acid analogues. synthesis and characterization of dideoxyribonucleoside methylphosphonates. *Biochemistry* **18**, 5134–5143 https://doi.org/10.1021/bi00590a017
23 Hogrefe, R.I., Vaghefi, M.M., Reynolds, M.A., Young, K.M. and Arnold, L.J. (1993) Deprotection of methylphosphonate oligonucleotides using a novel one-pot procedure. *Nucleic Acids Res.* **21**, 2031–2038 https://doi.org/10.1093/nar/21.9.2031
24 Peterson, M.A., Nilsson, B.L., Sarker, S., Doboszewski, B., Zhang, W. and Robins, M.J. (1999) Amide-linked ribonucleoside dimers derived from 5′-amino-5′-deoxy- and 3′-(carboxymethyl)-3′-deoxynucleoside precursors. *J. Org. Chem.* **64**, 8183–8192 https://doi.org/10.1021/jo9908647
25 Brandsen, B.M., Hesser, A.R., Castner, M.A., Chandra, M. and Silverman, S.K. (2013) DNA-catalyzed hydrolysis of esters and aromatic amides. *J. Am. Chem. Soc.* **135**, 16014–16017 https://doi.org/10.1021/ja4077233

Downloaded from http://port.silverchair.com/emergtoplifesci/article-pdf/5/5/691/924328/etls-2021-0169c.pdf by guest on 24 April 2024

© 2021 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and the Royal Society of Biology and distributed under the Creative Commons Attribution License 4.0 (CC BY).

26 Tanui, P., Kennedy, S.D., Lunstad, B.D., Haas, A., Leake, D. and Rozners, E. (2014) Synthesis, biophysical studies and RNA interference activity of RNA having three consecutive amide linkages. *Org. Biomol. Chem.* **12**, 1207–1210 https://doi.org/10.1039/c3ob42532k
27 Epple, S., Thorpe, C., Baker, Y.R., El-Sagheer, A.H. and Brown, T. (2020) Consecutive 5′- and 3′-amide linkages stabilise antisense oligonucleotides and elicit an efficient RNase H response. *Chem. Commun.* **56**, 5496–5499 https://doi.org/10.1039/d0cc00444h
28 Isobe, H. and Fujino, T. (2014) Triazole-linked analogues of DNA and RNA (TLDNA and TLRNA): synthesis and functions. *Chem. Rec.* **14**, 41–51 https://doi.org/10.1002/tcr.201300023
29 Ueno, Y., Naito, T., Kawada, K., Shibata, A., Kim, H.S., Wataya, Y. et al. (2005) Synthesis of novel siRNAs having thymidine dimers consisting of a carbamate or a urea linkage at their 3′ overhang regions and their ability to suppress human RNase L protein expression. *Biochem. Biophys. Res. Commun.* **330**, 1168–1175 https://doi.org/10.1016/j.bbrc.2005.03.100
30 Sato, K., Seio, K. and Sekine, M. (2002) Squaryl group as a new mimic of phosphate group in modified oligodeoxynucleotides: synthesis and properties of new oligodeoxynucleotide analogues containing an internucleotidic squaryldiamide linkage. *J. Am. Chem. Soc.* **124**, 12715–12724 https://doi.org/10.1021/ja027131f
31 Varizhuk, A.M., Kaluzhny, D.N., Novikov, R.A., Chizhov, A.O., Smirnov, I.P., Chuvilin, A.N. et al. (2013) Synthesis of triazole-linked oligonucleotides with high affinity to DNA complements and an analysis of their compatibility with biosystems. *J. Org. Chem.* **78**, 5964–5969 https://doi.org/10.1021/jo400651k
32 Madhuri, V. and Kumar, V.A. (2012) Design and synthesis of dephosphono DNA analogues containing 1,2,3-triazole linker and their UV-melting studies with DNA/RNA. *Nucleosides Nucleotides Nucleic Acids* **31**, 97–111 https://doi.org/10.1080/15257770.2011.644100
33 Mutisya, D., Selvam, C., Kennedy, S.D. and Rozners, E. (2011) Synthesis and properties of triazole-linked RNA. *Bioorganic Med. Chem. Lett.* **21**, 3420–3422 https://doi.org/10.1016/j.bmcl.2011.03.111
34 Shivalingam, A., Tyburn, A.E.S., El-Sagheer, A.H. and Brown, T. (2017) Molecular requirements of high-fidelity replication-competent DNA backbones for orthogonal chemical ligation. *J. Am. Chem. Soc.* **139**, 1575–1583 https://doi.org/10.1021/jacs.6b11530
35 Kumar, P., El-Sagheer, A.H., Truong, L. and Brown, T. (2017) Locked nucleic acid (LNA) enhances binding affinity of triazole-linked DNA towards RNA. *Chem. Commun.* **53**, 8910–8913 https://doi.org/10.1039/c7cc05159j
36 Kumar, P., Truong, L., Baker, Y.R., El-Sagheer, A.H. and Brown, T. (2018) Synthesis, affinity for complementary RNA and DNA, and enzymatic stability of triazole-Linked locked nucleic acids (t-LNAs). *ACS Omega* **3**, 6976–6987 https://doi.org/10.1021/acsomega.8b01086
37 Crooke, S.T., Liang, X.H., Baker, B.F. and Crooke, R.M. (2021) Antisense technology: a review. *J. Biol. Chem.* **296**, 100416 https://doi.org/10.1016/j.jbc.2021.100416
38 Iwamoto, N., Butler, D.C.D., Svrzikapa, N., Mohapatra, S., Zlatev, I., Sah, D. (2017) Control of phosphorothioate stereochemistry substantially increases the efficacy of antisense oligonucleotides. *Nat. Biotechnol.* **35**, 845–851 https://doi.org/10.1038/nbt.3948
39 Keam, S.J. (2018) Inotersen: first global approval. *Drugs* **78**, 1371–1376 https://doi.org/10.1007/s40265-018-0968-5
40 Hoy, S.M. (2017) Nusinersen: first global approval. *Drugs* **77**, 473–479 https://doi.org/10.1007/s40265-017-0711-7
41 Knouse, K.W., DeGruyter, J.N., Schmidt, M.A., Zheng, B., Vantourout, J.C., Kingston, C. et al. (2018) Unlocking P(V): reagents for chiral phosphorothioate synthesis. *Science* **361**, 1234–1238 https://doi.org/10.1126/science.aau3369
42 Eckstein, F. (2014) Phosphorothioates, essential components of therapeutic oligonucleotides. *Nucleic Acid Ther.* **24**, 374–387 https://doi.org/10.1089/nat.2014.0506
43 Liang, X.H., Sun, H., Shen, W. and Crooke, S.T. (2015) Identification and characterization of intracellular proteins that bind oligonucleotides with phosphorothioate linkages. *Nucleic Acids Res.* **43**, 2927–2945 https://doi.org/10.1093/nar/gkv143
44 Vickers, T.A., Rahdar, M., Prakash, T.P. and Crooke, S.T. (2019) Kinetic and subcellular analysis of PS-ASO/protein interactions with P54nrb and RNase H1. *Nucleic Acids Res.* **47**, 10865–10880 https://doi.org/10.1093/nar/gkz771
45 Migawa, M.T., Shen, W., Wan, W.B., Vasquez, G., Oestergaard, M.E., Low, A. et al. (2019) Site-specific replacement of phosphorothioate with alkyl phosphonate linkages enhances the therapeutic profile of gapmer ASOs by modulating interactions with cellular proteins. *Nucleic Acids Res.* **47**, 5465–5479 https://doi.org/10.1093/nar/gkz247
46 Nielsen, P.E., Egholm, M., Berg, R.H. and Buchardt, O. (1991) Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* **254**, 1497–1500 https://doi.org/10.1126/science.1962210
47 Summerton, J. and Weller, D. (1997) Morpholino antisense oligomers: design, preparation, and properties. *Antisense Nucleic Acid Drug Dev.* **7**, 187–195 https://doi.org/10.1089/oli.1.1997.7.187
48 Saarbach, J., Sabale, P.M. and Winssinger, N. (2019) Peptide nucleic acid (PNA) and its applications in chemical biology, diagnostics, and therapeutics. *Curr. Opin. Chem. Biol.* **52**, 112–124 https://doi.org/10.1016/j.cbpa.2019.06.006
49 Koppelhus, U. and Nielsen, P.E. (2003) Cellular delivery of peptide nucleic acid (PNA). *Adv. Drug Deliv. Rev.* **55**, 267–280 https://doi.org/10.1016/S0169-409X(02)00182-5
50 Gerety, S.S. and Wilkinson, D.G. (2011) Morpholino artifacts provide pitfalls and reveal a novel role for pro-apoptotic genes in hindbrain boundary development. *Dev. Biol.* **350**, 279–289 https://doi.org/10.1016/j.ydbio.2010.11.030
51 Blum, M., De Robertis, E.M., Wallingford, J.B. and Niehrs, C. (2015) Morpholinos: antisense and sensibility. *Dev. Cell* **35**, 145–149 https://doi.org/10.1016/j.devcel.2015.09.017
52 Baker, Y.R., Traoré, D., Wanat, P., Tyburn, A., El-Sagheer, A.H. and Brown, T. (2020) Searching for the ideal triazole: investigating the 1,5-triazole as a charge neutral DNA backbone mimic. *Tetrahedron* **76**, 130914 https://doi.org/10.1016/j.tet.2019.130914
53 Waldner, A., De Mesmaeker, A. and Lebreton, J. (1994) Synthesis of oligodeoxyribonucleotides containing dimers with carbamate moieties as replacement of the natural phosphodiester linkage. *Bioorganic Med. Chem. Lett.* **4**, 405–408 https://doi.org/10.1016/0960-894X(94)80005-7
54 Freier, S. and Altmann, K.-H. (1997) The ups and downs of nucleic acid duplex stability: structure-stability studies on chemically-modified DNA:RNA duplexes. *Nucleic Acids Res.* **25**, 4429–4443 https://doi.org/10.1093/nar/25.22.4429
55 El-Sagheer, A.H. and Brown, T. (2014) Combined nucleobase and backbone modifications enhance DNA duplex stability and preserve biocompatibility. *Chem. Sci.* **5**, 253–259 https://doi.org/10.1039/c3sc51753e
56 Thorpe, C., Epple, S., Woods, B., El-Sagheer, A.H. and Brown, T. (2019) Synthesis and biophysical properties of carbamate-locked nucleic acid (LNA) oligonucleotides with potential antisense applications. *Org. Biomol. Chem.* **17**, 5341–5348 https://doi.org/10.1039/c9ob00691e

Downloaded from http://port.silverchair.com/emergtopiflesci/article-pdf/5/5/691/924328/etls-2021-0169c.pdf by guest on 24 April 2024

© 2021 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and the Royal Society of Biology and distributed under the Creative Commons Attribution License 4.0 (CC BY).

57 Kanan, M.W., Rozenman, M.M., Sakurai, K., Snyder, T.M. and Liu, D.R. (2004) Reaction discovery enabled by DNA-templated synthesis and in vitro selection. *Nature* **431**, 545–549 https://doi.org/10.1038/nature02920
58 Xu, Y. and Kool, E.T. (1998) Chemical and enzymatic properties of bridging 5′-S-phosphorothioester linkages in DNA. *Nucleic Acids Res.* **26**, 3159–3164 https://doi.org/10.1093/nar/26.13.3159
59 El-Sagheer, A.H. and Brown, T. (2017) Single tube gene synthesis by phosphoramidate chemical ligation. *Chem. Commun.* **53**, 10700–10702 https://doi.org/10.1039/c7cc00858a
60 Nakamoto, K., Abe, N., Tsuji, G., Kimura, Y., Tomoike, F., Shimizu, Y. et al. (2020) Chemically synthesized circular RNAs with phosphoramidate linkages enable rolling circle translation. *Chem. Commun.* **56**, 6217–6220 https://doi.org/10.1039/d0cc02140g
61 Chen, J., Baker, Y.R., Brown, A., El-Sagheer, A.H. and Brown, T. (2018) Enzyme-free synthesis of cyclic single-stranded DNA constructs containing a single triazole, amide or phosphoramidate backbone linkage and their use as templates for rolling circle amplification and nanoflower formation. *Chem. Sci.* **9**, 8110–8120 https://doi.org/10.1039/c8sc02952k
62 Maruyama, H., Oikawa, R., Hayakawa, M., Takamori, S., Kimura, Y., Abe, N. et al. (2017) Chemical ligation of oligonucleotides using an electrophilic phosphorothioester. *Nucleic Acids Res.* **45**, 7042–7048 https://doi.org/10.1093/nar/gkx459
63 Shivalingam, A., Taemaitree, L., El-Sagheer, A.H. and Brown, T. (2020) Squaramides and ureas: a flexible approach to polymerase-compatible nucleic acid assembly. *Angew. Chem. Int. Ed.* **59**, 11416–11422 https://doi.org/10.1002/anie.202000209
64 Miura, F., Fujino, T., Kogashi, K., Shibata, Y., Miura, M., Isobe, H. et al. (2018) Triazole linking for preparation of a next-generation sequencing library from single-stranded DNA. *Nucleic Acids Res.* **46**, e95 https://doi.org/10.1093/nar/gky452
65 Manuguerra, I., Croce, S., El-Sagheer, A.H., Krissanaprasit, A., Brown, T., Gothelf, K.V. et al. (2018) Gene assembly: via one-pot chemical ligation of DNA promoted by DNA nanostructures. *Chem. Commun.* **54**, 4529–4532 https://doi.org/10.1039/c8cc00738a
66 Shaw, B.R., Dobrikov, M., Wang, X.I.N., Wan, J., He, K., Lin, J.-L. et al. (2003) Reading, writing, and modulating genetic information with boranophosphate mimics of nucleotides, DNA, and RNA. *Ann. N. Y. Acad. Sci.* **1002**, 12–29 https://doi.org/10.1196/annals.1281.004
67 Griffiths, A.D., Potter, B.V.L. and Eperon, I.C. (1987) Stereospecificity of nucleases towards phosphorothioate-substituted RNA: stereochemistry of transcription by T7 RNA polymerase. *Nucleic Acids Res.* **15**, 4145–4162 https://doi.org/10.1093/nar/15.10.4145
68 Burgers, P.M.J. and Eckstein, F. (1979) A study of the mechanism of DNA polymerase I from *Escherichia coli* with diastereomeric phosphorothioate analogs of deoxyadenosine triphosphate. *J. Biol. Chem.* **254**, 6889–6893 https://doi.org/10.1016/s0021-9258(18)50258-1
69 Guo, C., Kong, D., Lei, Y. and Hili, R. (2018) Expanding the chemical diversity of DNA. *Synlett* **29**, 1405–1414 https://doi.org/10.1055/s-0036-1591959
70 El-Sagheer, A.H., Sanzone, A.P., Gao, R., Tavassoli, A. and Brown, T. (2011) Biocompatible artificial DNA linker that is read through by DNA polymerases and is functional in *Escherichia coli*. *Proc. Natl Acad. Sci. U.S.A.* **108**, 11338–11343 https://doi.org/10.1073/pnas.1101519108
71 El-Sagheer, A.H. and Brown, T. (2011) Efficient RNA synthesis by in vitro transcription of a triazole-modified DNA template. *Chem. Commun.* **47**, 12057–12058 https://doi.org/10.1039/c1cc14316f

Downloaded from http://port.silverchair.com/emergtopiflesci/article-pdf/5/5/691/924328/etls-2021-0169c.pdf by guest on 24 April 2024

© 2021 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and the Royal Society of Biology and distributed under the Creative Commons Attribution License 4.0 (CC BY).