

OLIGONUCLEOTIDES

A P(V) platform for oligonucleotide synthesis

Yazhong Huang^{1†}, Kyle W. Knouse^{2,3†}, Shenjie Qiu^{4†}, Wei Hao^{2†}, Natalia M. Padial^{2‡§}, Julien C. Vantourout^{2†}, Bin Zheng⁴, Stephen E. Mercer¹, Javier Lopez-Ogalla^{2¶}, Rohan Narayan², Richard E. Olson¹, Donna G. Blackmond², Martin D. Eastgate^{4*}, Michael A. Schmidt^{4*}, Ivar M. McDonald^{1*}, Phil S. Baran^{2*}

The promise of gene-based therapies is being realized at an accelerated pace, with more than 155 active clinical trials and multiple U.S. Food and Drug Administration approvals for therapeutic oligonucleotides, by far most of which contain modified phosphate linkages. These unnatural linkages have desirable biological and physical properties but are often accessed with difficulty using phosphoramidite chemistry. We report a flexible and efficient [P(V)]-based platform that can install a wide variety of phosphate linkages at will into oligonucleotides. This approach uses readily accessible reagents and can install not only stereodefined or racemic thiophosphates but any combination of (*S*, *R* or *rac*)-PS with native phosphodiester (PO₂) and phosphorodithioate (PS₂) linkages into DNA and other modified nucleotide polymers. This platform easily accesses this diversity under a standardized coupling protocol with sustainably prepared, stable P(V) reagents.

In traditional small-molecule drug discovery, the features that determine target specificity and DMPK (distribution, metabolism, and pharmacokinetics) are usually inextricably linked (1). Conversely, oligonucleotide-based therapeutics have been referred to as “informational drugs” in which the pharmacophore and PK properties can, in theory, be separately optimized because the nucleoside sequence directly determines the former whereas the unifying chemistry (the phosphate linkages used to couple the nucleosides) largely affects the latter (2). Advances in organic synthesis have had a profound impact on the ability of modern medicinal chemistry to target and rapidly access increasingly complex small-molecule leads. By contrast, as the range of oligonucleotide sequences and conceivable phosphate linkages has expanded, the fundamental chemistry used to enable their synthesis has largely remained unchanged despite numerous refinements and improvements. The incorporation of varied phosphate linkages has been documented to have a profound impact on both the properties and efficacy of the resulting structures (3). The hypothetical chimeric sequence (1) illustrated in Fig. 1, adorned with four different phosphorus-based linkages and multiple sugar backbones, tests the limits of the scope

of existing methods. The impact of judiciously designing backbone chemical modifications in therapeutic oligonucleotides is only beginning to be realized (4–7). The opportunity to install broader combinations and variations at will and in any order thus presents an important step in the evolution of oligonucleotide therapeutics and requires the invention of enabling science. The commercialization of oligonucleotides and, more specifically, phosphorothioate antisense oligonucleotides (PS-ASOs) faces major challenges (8–14). The ability to easily make single-isomer species from stable species and/or fragments will aid control over product quality and has the potential to enable commercialization of these new therapeutics sustainably (i.e., through enhanced regulatory control of these complex molecules). Thus, improved methods of oligonucleotide synthesis amenable to standard methods of automation would have a near-immediate translational impact, enabling both the interrogation of greater linker permutations in drug discovery and better, more sustainable commercialization (see the supplementary materials for process mass intensity analysis) (3).

We recently demonstrated a practical approach to address the synthesis of stereopure phosphorothioates (*R*-PS and *S*-PS, mainly in the context of DNA), with the disclosure of the phosphorus sulfur incorporation (dubbed PSI or ψ) reagents. However, this initial study did not address the installation of other linkages such as phosphorodithioates and native phosphate diesters (15), other sugar chemistries [such as locked nucleic acid (LNA)], or applicability to a modern automated synthesizer protocol.

With these goals in mind, several challenges were apparent, with substantial lore and reactivity concerns to address when using a P(V)-based approach (16–20). For example, the rates associated with P(V) reagents have historically

been viewed as too sluggish to ever compete with the P(III) manifold (21–23). Such approaches also had chemoselectivity challenges in that the guanine and thymidine bases would interfere in sequential couplings, reacting with the coupling reagents (24). Using P(III)-based reagents to install phosphorodithioate (PS₂) linkages is less than ideal. For instance, Caruthers reported a protocol based on protected thiophosphoramidites that requires discreet oxidation and deprotection steps (25) and suffers from the formation of a nearly inseparable phosphorothioate by-product in ~5 to 10% yield. Despite this serious issue, this method is still commonly used because there are simply no viable alternatives (26). Finally, chimeric sequences with multiple modifications could be desired by medicinal chemists, but only certain combinations (PS/PO, PS/PS₂, PO/PS₂) are present in the literature. Even a hybrid synthetic approach that merges P(III)-based phosphoramidite chemistry with P(V) reagents is unworkable because it suffers from a lack of chemoselectivity (vide infra) as oxidation of P(III) to P(V) requires a protecting group on exposed PS/PS₂ linkages to avoid desulfurization. The use of organic oxidants was ruled out because of the extreme number of equivalents required and their extended reaction times (27).

In this disclosure, three new reagent systems are described, ψ^2 (3), *rac*- ψ (4), and ψ^O (5), which, when combined with the previously reported systems [(+)- ψ , (+)-2] and [(–)- ψ , (–)-2], provide a unified P(V) approach that entirely departs from the rubric of P(III)-based oligonucleotide synthesis and enables the at will and controlled synthesis of specific chimeric oligonucleotides (Fig. 1C). Along with these new reagents are protocols for their unified application in commercial synthesizers using a single coupling protocol that spans all coupling types. This redox-neutral platform, which is based on the native P(V) oxidation state, challenges past assumptions of sluggish reactivity and enables access to five relevant P linkages across a range of sugar backbones (DNA and LNA) and bases (A, T, G, and mC) in one oligonucleotide construct. Aside from enabling straightforward access to a wide range of chimeric oligonucleotides, the implementation of this new protocol benefits from a reduced reliance on protecting group chemistry (which eliminates the labile cyanoethyl group and therefore acrylonitrile production upon deprotection) (28, 29), bespoke additives (30), and redox fluctuations. This new P(V) platform also eliminates one full step in the standard solid-phase oligonucleotide synthesis (SPOS) protocol (namely the phosphorus oxidation).

Figure 2 outlines the synthesis of ψ^2 and ψ^O reagents for the incorporation of phosphorodithioate and native phosphodiester linkages, respectively. The development of these

¹Small Molecule Drug Discovery, Bristol Myers Squibb, Cambridge, MA 02142, USA. ²Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, USA. ³Elsie Biotechnologies, San Diego, CA 92121, USA. ⁴Chemical Process Development, Bristol Myers Squibb, New Brunswick, NJ 08903, USA.

*Corresponding author. Email: martin.eastgate@bms.com (M.D.E.); michael.schmidt@bms.com (M.A.S.); ivar.mcdonald@bms.com (I.M.M.); pbaran@scripps.edu (P.S.B.)

†These authors contributed equally to this work.

‡These authors contributed equally to this work.

§Present address: Institute of Molecular Science (ICMol), Universitat de València, Paterna 46980, Spain.

¶Present address: Clarochem Ireland Ltd., Damastown, Mulhuddart, Dublin 15, Republic of Ireland.

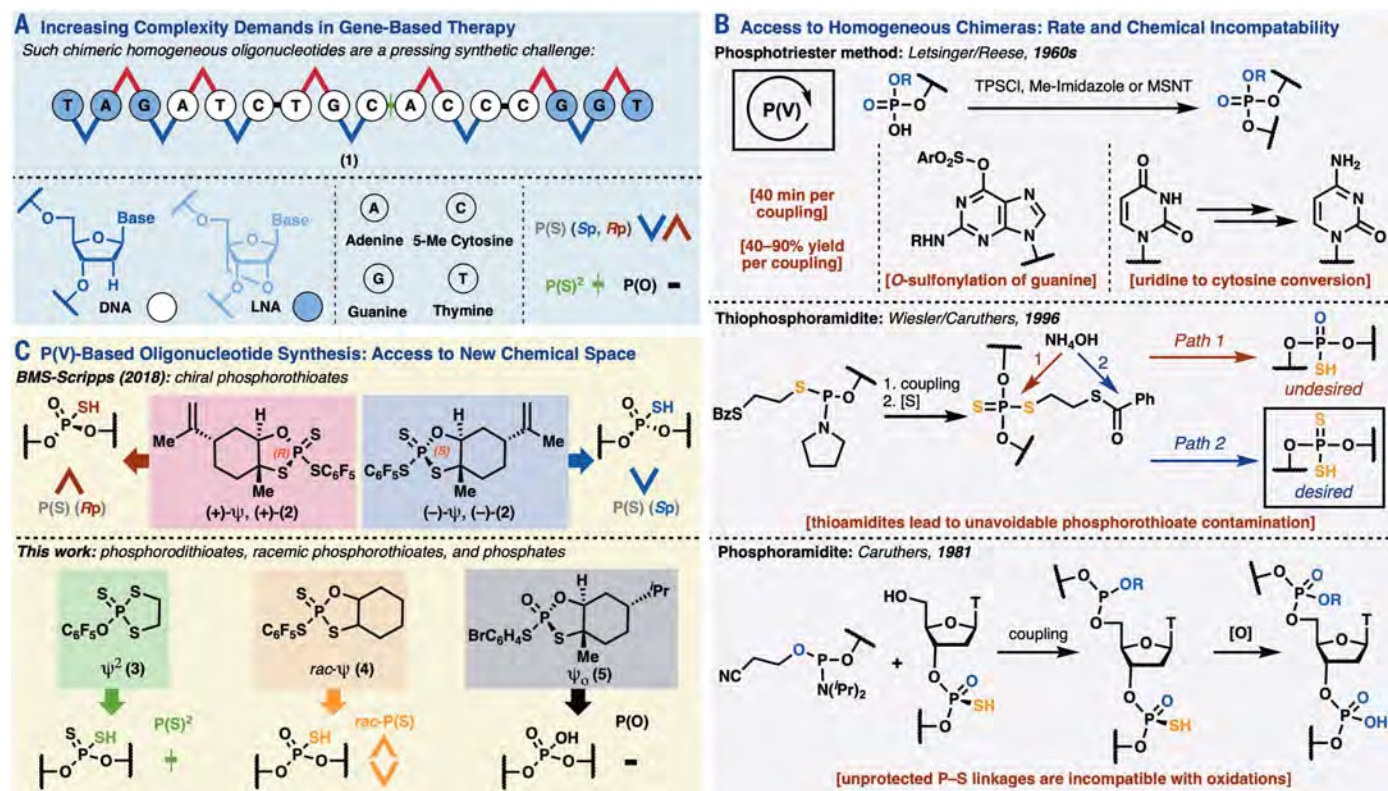


Fig. 1. Introduction and background. (A) Inspiration. (B) Challenges facing P(V)-based synthesis. (C) Recent developments and this work. 'Pr, iso-propyl; Me, methyl; Bz, benzoyl; [O], oxidation; [S], sulfuration.

reagents required extensive experimentation, whereas the synthesis of *rac*- ψ was relatively trivial and proceeded by analogy to ψ using cyclohexene oxide (see the supplementary materials). Fully sulfurized versions of diphosphate esters, known as phosphorodithioate linkages, are isopolar and isostructural analogs of phosphates that are completely stable to nucleases (31) but maintain the ability to form stable duplexes and elicit desirable mRNA cleavage by RNase H without the complexity of chirality at phosphorus (32, 33). The pioneering work of Stec and colleagues on phospholane heterocycles, which inspired the development of (+)- ψ and (–)- ψ , was an essential precedent for the present work (19). In 1995, it was disclosed that dithiaphospholanes could be installed onto nucleosides and coupled to afford dinucleotides incorporating a phosphorodithioate linkage, albeit requiring a separate oxidation step to install sulfur, and a toxic and unstable (explosive) reagent (Fig. 2A) (18). Our P(V)-centric study thus built on the lessons of these two precedents with the goals of eliminating the extraneous oxidation step and dangerous reagents. Upon identification of the optimal leaving group (21 evaluated; see the supplementary materials for Hammett correlations of leaving groups) and ring size (two evaluated), inexpensive P_2S_5 could be combined with pentafluorophenol, followed by

capping of P(V) intermediate (6) with thiirane to generate ψ^2 on large scale (>100 g). In this way, unsafe P(III) chemistry was avoided, and a stable and viable P(V) reagent was developed.

Oligonucleotides with native phosphodiester linkages have poor pharmacokinetics and are rapidly degraded by nucleases, but their use is invaluable in routine molecular biology and diagnostic settings (34). In addition, limited PO linkages are incorporated into current antisense oligonucleotides. To the best of our knowledge, there are no known P(V)-based reagents for achieving P(III)-competitive reactivity and chemoselectivity that are stable and amenable to automated synthesis (34). Although the design of such a reagent benefited from the lessons of our previous studies, it was repeatedly thwarted by the challenge of identifying both a highly reactive and stable entity upon loading to a monomer. We evaluated nearly 30 different backbones along with three different leaving groups before arriving at ψ^O (see the supplementary materials for a comprehensive summary). The backbone optimization systematically evaluated ring size, substituents, electronic effects, and stereochemistry to probe effects on loading, coupling, and overall stability, with ψ^O emerging as the only viable candidate. The synthesis outlined in Fig. 2A requires three simple, scalable steps (>50 g). In a similar fashion to ψ^2 , inexpensive

P_2S_5 was reacted with 4-bromothiophenol, yielding P(V) intermediate (7), which, when combined with hydrogenated *cis*-limonene oxide (8), yields the PS reagent (9); desulfurization with SeO_2 yields ψ^O .

With these new reagents in hand, a side-by-side comparison with state-of-the-art P(III) chemistry was conducted (Fig. 2B). For the installation of the PS_2 linkage, the three-step P(III) approach to access simple dimer (10) through P(III) adduct (12) resulted in ~7% of the PS impurity resulting from desulfurization during the deprotection event. By contrast, using ψ^2 , dimer (10) was cleanly accessed in two steps through P(V) adduct (11) in >99% purity. To field test and contrast the synthesis of mixed PO-PS backbones by the two platforms, PS dimer (13) was subjected to a standard phosphoramidite coupling with (18) to yield protected trimer (14), which, upon oxidation of P(III) to the requisite P(V), resulted in rapid desulfurization [see ratio of (15)/(16) over time]. Conversely, the redox-neutral P(V) approach using P(V) adduct (17) cleanly delivered the unprotected mixed PS-PO trimer (19) without any loss of sulfur.

The final challenge that P(V)-based reagents face is the longstanding perception that their diminished coupling rates preclude them from being used in traditional automated oligonucleotide synthesis regimes. With a full suite

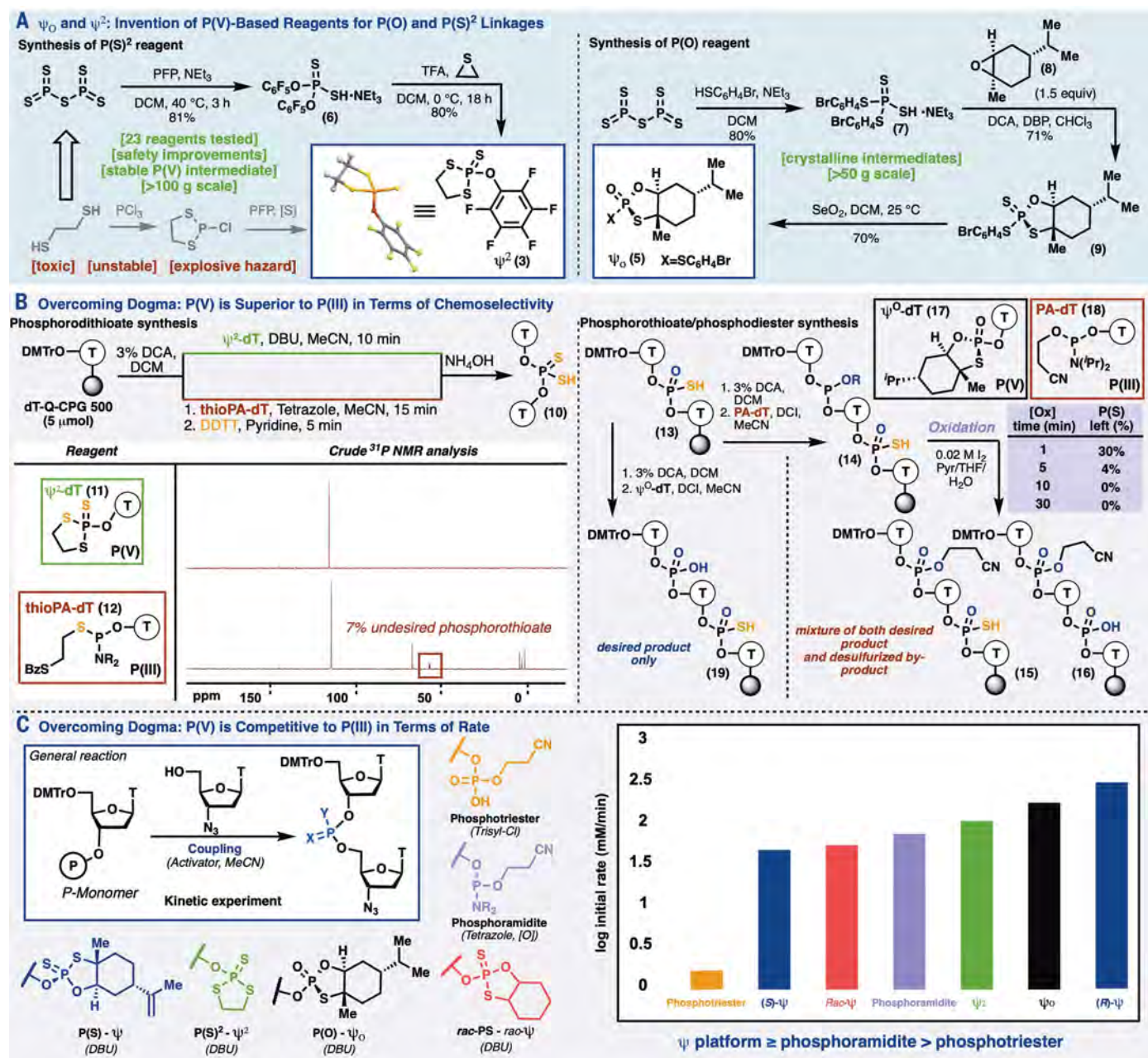


Fig. 2. Development of a fully P(V) platform for oligonucleotide synthesis. (A) Reagent development. (B) Overcoming chemoselectivity challenges. (C) Overcoming historical P(V) rate challenges. PFP, pentafluorophenol; TFA, trifluoroacetic acid; DCA, dichloroacetic acid; DBP, dibutylphosphate; DDTT, 3-[(dimethylamino-methylidene)amino]-3H-1,2,4-dithiazole-3-thione; DCl, 4,5-dicyanoimidazole; DMTr, 4,4'-dimethoxytrityl.

of reagents in hand based on P(V), we evaluated their coupling performance side by side with canonical P(III) chemistry using kinetics studies (Fig. 2C). In addition, the original P(V)-based coupling using phosphotriester chemistry was included because this was the initial benchmark. Temporal reaction progress monitoring was performed by taking aliquots of the mixture coupling P-loaded-T with AZT and monitoring product formation by high-performance liquid chromatography-mass spectrometry

using an internal standard. Full kinetic profiles are included in the supplementary materials, and here we present the main trends by considering relative rates calculated from these profiles. Consistent with the literature, the classic P(V)-based phosphotriester approach was extremely sluggish (Fig. 2C, orange bar). However, the P(V) reagent suite detailed herein performed equally well as the industry standard P(III) protocol, with all reactions reaching full conversion in under two minutes.

Extensive optimization of the SPOS methodology for phosphoramidite-based synthesis has been achieved over the course of the past 30+ years. Although some of these methods could be leveraged in this new context, there were areas in which existing solutions were not compatible with the P(V) synthesis protocol (Fig. 3A). Existing universal supports were not sufficient stable toward 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU), prompting our development of a universal support (20) with

substantially improved base stability. Guided by Stec's earlier work, Pya-protecting groups were used instead of the standard amide-protecting groups (Fig. 3B) (18). Improved results were also obtained when a Pom protection was used for thymidine (35). With all P(V) reagents in hand and the chemoselectivity and relative coupling rates established, a systematic interrogation was undertaken to test the efficiency of this redox-neutral P(V)-platform on automated SPOS (Fig. 3A). The cycle commences with the deblocking of the DMT-protecting group of a resin-bound nucleoside to afford a free 5'-alcohol that is primed to react with any P(V)-loaded nucleotide in the subsequent coupling step. This key step was carefully optimized for all reagents through systematic reactivity and hydrolysis studies using ultraviolet and ^{31}P -nuclear magnetic resonance (NMR) analyses to enable a robust double-coupling protocol for each P(V) reagent. The subsequent capping and deblocking steps complete the solid-phase cycle and set the stage for the next coupling (Fig. 3A). The utility of any new reagent system for an oligonucleotide synthesis platform is wedded to its fidelity and robustness in the context of preparing diverse sequences with a single protocol. Thus, a matrix was designed to incorporate all possible nucleobase (A, C, G, and T) and sugar (DNA and LNA) combinations templated onto a 3-10-3 DNA/LNA gapmer scaffold, the current state of the art in RNase H-activating ASOs (Fig. 3C) (3). LNA modifications that have a marked effect on binding affinity were coupled in near quantitative yields (36). A single protocol was used regardless of the P(V) monomers used to assess the generality of the method versus sequence-specific optimization. First, the general method was field tested to produce homogeneous, chiral PS-ASOs with both alternating (21, 22) and continuous stereochemical patterns (23–26). This approach is the second industrially viable platform to produce stereopure PS-ASOs and uses redox-neutral, sustainably derived P(V)-based reagents (37). With this milestone achieved, the incorporation of PO_2 linkages into these constructs was pursued. These chimeric sequences (27–30) could be accessed in high purity with no substantial loss of sulfur during synthesis. Next, sequences bearing both PS and PS_2 (31–34) linkages were prepared. Finally, constructs containing all four possible linkages were cleanly produced (35, 36). Thus, these protocols describe a convenient single platform for probing linkage SAR that could enable systematic tuning of physical and biological properties (38, 39). Given the differences in scale, chemical sequence, method of purification, and quantification, a direct comparison between yields of this P(V) platform and those of other stereopure methods is outside the scope of this report. In its present

manifestation, these homogeneous, chimeric oligonucleotides (21–38) were produced in 12 to 27% isolated yield using a sequence- and linkage-agnostic protocol. When compared directly with the stereorandom constructs (produced using state-of-the-art chemistry) that were produced in 30 to 60% yield and accounting for the ability to prepare distinctive chimeric systems, the difference in yield is a relatively small gap to fill to bring homogeneous, stereopure oligonucleotides on par with their stereorandom counterparts. From a pragmatic perspective, the observed yields even in this first disclosure are more than enough to progress a medicinal chemistry program.

Current methods to produce PS oligonucleotides generate a statistical mixture of isomers depending on the specific conditions used (40). Homogeneous, well-defined ASOs are of value, but no true P(V) oligonucleotide synthesis platform would be complete without rapid access to these stereorandom variants (41). Thus, a final need was to produce *rac-ψ* (Fig. 1), a derivative of ψ that retains high reactivity and provides mixtures of diastereomers comparable to those obtained with P(III) methodology. *rac-ψ* was loaded onto DNA cores, and the corresponding monomers were cleanly implemented (Fig. 3D) into the aforementioned workflow, thus enabling *racemic* PS oligos to be produced on this platform (37, 38).

Oligonucleotide therapeutics, so-called informational drugs, target essentially every level of the central dogma of molecular biology. Given the immense chemical space conceivable, the number of nucleic acid modifications that have been investigated to date is quite narrow, with even fewer represented in the clinic. Although P(III)-based phosphoramidites have revolutionized access to vast swaths of this space, new linkages and chemical modifications have pushed the limits of what is currently accessible. Oligonucleotide synthesis originated with P(V)-based reagents because nature only uses this oxidation state to craft its building blocks of life, but this method was soon abandoned because of perceptions of the reduced reactivity and selectivity of these reagents. This work provides a compelling justification for renewed research in this field because it can enable democratized access to a wide range of medicinally promising chimeric sequences. Finally, this new approach offers a realistic opportunity to connect the exploration of new chemical space directly to patients through a platform containing improved environmental sustainability, enhancing our ability to discover, develop, and commercialize this promising class of therapeutics.

REFERENCES AND NOTES

1. J. P. Hughes, S. Rees, S. B. Kalindjian, K. L. Philpott, *Br. J. Pharmacol.* **162**, 1239–1249 (2011).

2. A. Khvorova, J. K. Watts, *Nat. Biotechnol.* **35**, 238–248 (2017).
3. W. B. Wan, P. P. Seth, *J. Med. Chem.* **59**, 9645–9667 (2016).
4. M. Byrne et al., *Transl. Vis. Sci. Technol.* **10**, 23 (2021).
5. Y. Liu et al., *Nat. Commun.* **12**, 847 (2021).
6. K. Bleicher et al., Gapmer oligonucleotides comprising a phosphorodithioate internucleoside linkage and therapeutic use. International Patent WO/2019/122282 (2019).
7. L. Bethge, J. Hauptmann, C. Frauendorf, A. Weingartner. Nucleic acids for inhibiting expression of a target gene comprising phosphorodithioate linkages. International Patent WO/2019/092280 (2019).
8. T. Tredenick, "Oligonucleotides: Opportunities, pipeline and challenges" (Pharma Manufacturing, 2016); <https://www.pharmamanufacturing.com/articles/2016/oligonucleotides-opportunities-pipeline-and-challenges/>.
9. F. Eckstein, V. W. Armstrong, H. Sternbach, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2987–2990 (1976).
10. A. Wilk, W. J. Stec, *Nucleic Acids Res.* **23**, 530–534 (1995).
11. B. I. Andrews et al., *J. Org. Chem.* **86**, 49–61 (2021).
12. M. Shimizu, T. Wada, Asymmetric auxiliary group. U.S. Patent US/10696711 (2020).
13. N. Oka, M. Yamamoto, T. Sato, T. Wada, *J. Am. Chem. Soc.* **130**, 16031–16037 (2008).
14. R. Suchsland, B. Appel, P. Virta, S. Müller, *RSC Advances* **11**, 3892–3896 (2021).
15. K. W. Knouse et al., *Science* **361**, 1234–1238 (2018).
16. W. J. Stec, A. Grajkowski, M. Koziolkiewicz, B. Uznanski, *Nucleic Acids Res.* **19**, 5883–5888 (1991).
17. W. J. Stec et al., *J. Am. Chem. Soc.* **117**, 12019–12029 (1995).
18. A. Okruszek, A. Siercha-la, K. L. Fearon, W. J. Stec, *J. Org. Chem.* **60**, 6998–7005 (1995).
19. W. J. Stec et al., *J. Am. Chem. Soc.* **120**, 7156–7167 (1998).
20. P. Guga, W. J. Stec, *Curr. Protoc. Nucleic Acid Chem.* **14**, 4.17.11–4.17.28 (2003).
21. F. Eckstein, I. Rizk, *Angew. Chem. Int. Ed.* **6**, 695–697 (1967).
22. C. B. Reese, R. Saffhill, *Chem. Commun.* 767–768 (1968).
23. R. L. Letsinger, W. B. Lunsford, *J. Am. Chem. Soc.* **98**, 3655–3661 (1976).
24. C. B. Reese, P. A. Skone, *J. Chem. Soc., Perkin Trans. 1* 1263–1271 (1984).
25. W. T. Wiesler, M. H. Caruthers, *J. Org. Chem.* **61**, 4272–4281 (1996).
26. X. Yang, *Curr. Protoc. Nucleic Acid Chem.* **70**, 4.77.1–4.77.13 (2017).
27. E. Radzikowska, J. Baraniak, *Org. Biomol. Chem.* **13**, 269–276 (2015).
28. X. Guo, J. A. Stolee, Y. A. Fillon, L. Zou, *Org. Process Res. Dev.* **25**, 318–326 (2021).
29. D. C. Capaldi et al., *Org. Process Res. Dev.* **7**, 832–838 (2003).
30. N. Oka, T. Wada, K. Saigo, *J. Am. Chem. Soc.* **125**, 8307–8317 (2003).
31. G. M. Porritt, C. B. Reese, *Tetrahedron Lett.* **31**, 1319–1322 (1990).
32. M. H. Caruthers et al., *Nucleosides Nucleotides* **10**, 47–59 (1991).
33. W. S. Marshall, M. H. Caruthers, *Science* **259**, 1564–1570 (1993).
34. M. H. Caruthers, *J. Biol. Chem.* **288**, 1420–1427 (2013).
35. M. C. de Koning et al., *Org. Process Res. Dev.* **10**, 1238–1245 (2006).
36. S. K. Singh, A. A. Koshkin, J. Wengel, P. Nielsen, *Chem. Commun.* (4): 455–456 (1998).
37. N. Iwamoto et al., *Nat. Biotechnol.* **35**, 845–851 (2017).
38. S. T. Crooke, X. H. Liang, B. F. Baker, R. M. Crooke, *J. Biol. Chem.* **296**, 100416 (2021).
39. S. T. Crooke, B. F. Baker, R. M. Crooke, X. H. Liang, *Nat. Rev. Drug Discov.* **20**, 427–453 (2021).
40. H. Jahns et al., *Nat. Commun.* **6**, 6317 (2015).
41. M. E. Østergaard et al., *Nucleic Acids Res.* **48**, 1691–1700 (2020).

ACKNOWLEDGMENTS

We thank D.-H. Huang and L. Pasternack (Scripps Research) for assistance with NMR spectroscopy; J. Chen (Automated Synthesis Facility, Scripps Research) for purification of compounds and acquisition of HRMS data; A. L. Rheingold, C. E. Moore, and M. Gemibicky (UCSD) for x-ray analysis; and S. Mukherjee (BMS)

for calculating the process mass intensity. **Funding:** This work was supported by Bristol Myers Squibb, NIGMS (GM106210), NIH (grant number GM-118176), Marie Skłodowska-Curie Global Fellowships (749359-EnanSET, N.M.P) within the European Union research and innovation framework program (2014-2020), and the NSF SURF Program (TSRI, R.N). **Author contributions:** M.D.E., M.A.S., P.S.B., K.W.K., I.M.M., and R.E.O. conceived of this research program. K.W.K., W.H., S.Q., Y.H., B.Z., S.E.M., J.C.V., J.L.O., R.N., N.M.P., M.A.S., and I.M.M. performed the experiments. K.W.K. and P.S.B. wrote the manuscript. J.C.V., M.D.E., M.A.S., R.E.O., I.M.M., and D.G.B. assisted in editing the manuscript. **Competing**

interests: M.A.S., B.Z., K.W.K., M.D.E., P.S.B., R.E.O., and I.M.M. are listed as inventors on US Patent Application US 2019/0322694, "Novel phosphorous (V)-based reagents, processes for the preparation thereof, and their use in making stereo-defined organophosphorous (V) compounds." K.W.K. and P.S.B. are co-founders and shareholders of Elsie Biotechnologies. The remaining authors declare no competing interests. **Data availability:** The data that support the findings of this study are available in the main text or supplementary materials. Crystallographic data are available free of charge from the Cambridge Crystallographic Data Centre under reference no. CCDC 2093530.

SUPPLEMENTARY MATERIAL

<https://science.org/doi/10.1126/science.abi9727>
Materials and Methods
Figs. S1 to S7
NMR Spectra
References (42–46)

[View/request a protocol for this paper from Bio-protocol.](#)

12 April 2021; accepted 12 July 2021
10.1126/science.abi9727



A P(V) platform for oligonucleotide synthesis

Yazhong Huang, Kyle W. Knouse, Shenjie Qiu, Wei Hao, Natalia M. Padial, Julien C. Vantourout, Bin Zheng, Stephen E. Mercer, Javier Lopez-Ogalla, Rohan Narayan, Richard E. Olson, Donna G. Blackmond, Martin D. Eastgate, Michael A. Schmidt, Ivar M. McDonald, and Phil S. Baran

Science, **373** (6560), .

DOI: 10.1126/science.abi9727

Platform for the synthesis of diverse oligos

DNA is primarily viewed as a carrier of information encoded in the sequence of bases, but the chemistry of the phosphodiester backbone is crucial to oligonucleotide stability and structure. Building on previous work in synthetic P(V) phosphorothioate coupling chemistry, Huang *et al.* developed two new reagents for making phosphorodithioate- and phosphate-based linkages (see the Perspective by Virta). The authors incorporated all of these reagents into a unified P(V)-based synthesis platform capable of running at high efficiency on a commercial automated solid-phase synthesizer. They demonstrate the flexibility of this system by producing oligonucleotides with all three linkage types in specific positions. Access to such precisely constructed molecules opens new approaches to therapeutic oligonucleotide design. —MAF

View the article online

<https://www.science.org/doi/10.1126/science.abi9727>

Permissions

<https://www.science.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of service](#)

Science (ISSN) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 2021 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works