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Phosphate triester-based multifunctional handles for post-synthetic oligonucleotide functionalization

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ABSTRACT

The continued advancement of oligonucleotide-based strategies in research and therapeutics relies on expanding the repertoire of chemical modifications to overcome persistent challenges, such as improving cellular uptake and delivery. Addressing these obstacles requires innovative bioconjugation approaches that integrate seamlessly with oligonucleotide modalities. Here, we report the development of a novel phosphotriester trifunctional probe based on the H-phosphonate derivative ammonium (9H-fluoren-9-yl)methyl, introducing significant advancements in synthetic phosphate chemistry. This platform supports robust and versatile chemical transformations, enabling the incorporation of diverse functionalities, such as biotin, fluorescent markers, G4-stabilizing ligands, and azido groups, into oligonucleotide backbones. The resulting multifunctional probes are compatible with different conjugation strategies and phosphorothioate modifications, allowing late-stage functionalization in solution without requiring solid-phase synthesis. We demonstrate the utility of this approach through the synthesis of G4-ligand-conjugated oligonucleotides (GL-Os) designed to target individual G4 structures. However, the strategy's adaptability ensures compatibility with a wide range of oligonucleotide-based applications that benefit from the addition of functional probes. This flexibility broadens accessibility and applicability, facilitating the development of oligonucleotide tools for advanced chemical biology studies, including fluorescence-based imaging and pull-down experiments.

1. Introduction

Research in chemical biology and medicinal chemistry often requires the conjugation of specialized handles to bioactive molecules to provide features that advance their use as research tools to explore biology and therapeutic potential [1]. This is important to investigate cell permeability and localization using fluorescence, as well as for target identification and engagement studies. However, implementing these types of conjugations is typically challenging, requiring robust synthetic methods and a thorough understanding of the structure–activity relationships to ensure that the additional functionalization does not prevent interactions with the biological target. Furthermore, it often requires the introduction of multiple functional groups, e.g., for covalent capturing of the target and subsequent pull-down assays. The most efficient process to achieve this is to use multifunctional probes that carry both the desired functions. There are several examples of such photoaffinity labelling (PAL) probes that successfully has been used in various small-molecule medicinal chemistry projects, [2–4] and those based on amino acids that has been used in e.g. protein–protein interaction studies [5,6]. However, these strategies do not fully align with the growing diversity and utility of new modalities in medicinal chemistry and chemical biology.

This challenge is particularly evident in the rapidly growing field of oligonucleotide therapeutics and oligonucleotide-based research tools [7,8]. Interest in this area has grown rapidly over the last years as several oligonucleotide-based therapeutics have been approved [8]. Multifunctionalization remains essential for studying oligonucleotide localization, specificity, and protein interactome [9]. However, there are only a few universal strategies described for the introduction of multifunctional probes that align with oligonucleotide-based modalities [10–14]. This gap highlights the need for innovative approaches to effectively integrate multifunctional handles into oligonucleotide research.

Available methods for enabling the multifunctionality of oligonucleotides primarily involve adding new modalities one by one to the oligomer during automated solid-phase synthesis. These multifunctional

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bioconjugations are frequently based on copper-catalyzed alkyne-azide cycloaddition (CuAAC) to introduce the final functionalities [10,11], such as fluorescent labels [12] or *N-acetylgalactosamine* (GalNAc) dendrimer instalment [13]. Another stepwise approach on solid support involves CuAAC as a tool in "click cycles" to generate multiple conjugations of oligonucleotides [14]. These strategies for multiple functionalization thus often require several sequential CuAAC oligonucleotide reactions and specific protecting groups, which increase the number of reaction steps and decrease the final yields, especially when performed in solution. Additionally, it is important that the multifunctionalization strategy is compatible with the catalyst-free strain-promoted azide-alkyne cycloaddition (SPAAC), which depends on more sensitive cyclooctynyl derivatives [15].

Our need for multifunctionalized oligonucleotides in studies related to selective stabilization of individual G-quadruplex (G4) DNA structures drove us to design a multifunctional handle for incorporating multiple functionalities into the oligonucleotide in one step. G4 structures are abundant in the human genome and play central roles in gene regulation. We recently developed a strategy to selectively target individual G4 DNA structures using two recognition devices: a G4-ligand that non-selectively binds and stabilizes G4s, and a guide oligonucleotide that specifically directs the G4-ligand to the target G4 structure. We call this modality G4-Ligand conjugated Oligonucleotides (GL-Os) [16]. Further development of this strategy at the cellular level required incorporating additional functionalities (e.g. fluorescent labels, biotin) into synthetic GL-Os, which turned our focus towards late-stage multifunctionalization strategies in solution (Fig. 1).

To address the challenges with multifunctionalization of oligonucleotides, we explored a new method based on a phosphate triester handle designed to introduce two functionalities into an oligonucleotide in one synthetic step using click chemistry. This approach to multifunctionalization is modular and overcomes most of the shortcomings of the existing synthetic protocols. Specifically, the phosphotriester handle allows for (i) post-synthetic modification of oligonucleotides, which is often associated with higher reaction efficiency, (ii) adding two functionalities in one conjugation step, thus reducing the reaction time and potential side reactions, (iii) utilizing efficient and straightforward H-phosphonate chemistry [17] to form structurally diverse phosphate esters, and (iv) using both copper mediated, CuAAC, and copper-free, SPAAC, click chemistry strategies if needed.

Phosphate triester derivatives have until recently been considered too unstable for biological applications and have therefore not been considered as a possible type of modification/functionalization for nucleotides. However, recent studies suggest that these derivatives can serve as potential backbone oligonucleotide modifications for siRNA therapeutic strategies [18–21]. These studies revealed that phosphate triester modifications positively impact siRNAs, enhancing both target specificity and resistance to nucleases while retaining potent gene silencing. These promising results support our hypothesis that multifunctional probes designed for oligonucleotides could benefit from being based on phosphate chemistry to harmonize with the oligonucleotide backbone.

In this context, we here present a novel modular approach for constructing multifunctional bioconjugates based on a phosphate triester handle capable of integrating three distinct functionalities. Central to this approach is the use of ammonium (9H-fluoren-9-yl)methyl H-phosphonate as a key reagent. This H-phosphonate derivative, along with its underlying chemistry [17,22], provided a robust foundation for designing new multifunctional phosphate triester handles for further conjugation to oligonucleotides *via* click chemistry. To evaluate this strategy, the phosphate triester platform was applied to the GL-O concept and a set of five different target probes were synthesized incorporating biotin, fluorescent marker, G4-stabilizing ligands, and azido groups in different combinations. These target probes were successfully conjugated to oligonucleotides, demonstrating compatibility with multiple conjugation strategies. Preliminary evaluations of the resulting GL-O conjugates revealed that this bioconjugation strategy preserves the ability of the conjugates to bind and interfere with their targets even with the addition of multiple functionalities. This highlights the potential of this strategy and opens the door to numerous future studies by drawing on the multifunctionalization capacity of the approach. Furthermore, the method can be applied to commercial oligonucleotides as a post-synthetic modification in solution, making it independent on solid-phase synthesizers. The versatility of this approach, combined with its promising biological applicability, suggests that phosphate triester multifunctionalizations have significant potential to further advance the oligonucleotide field.

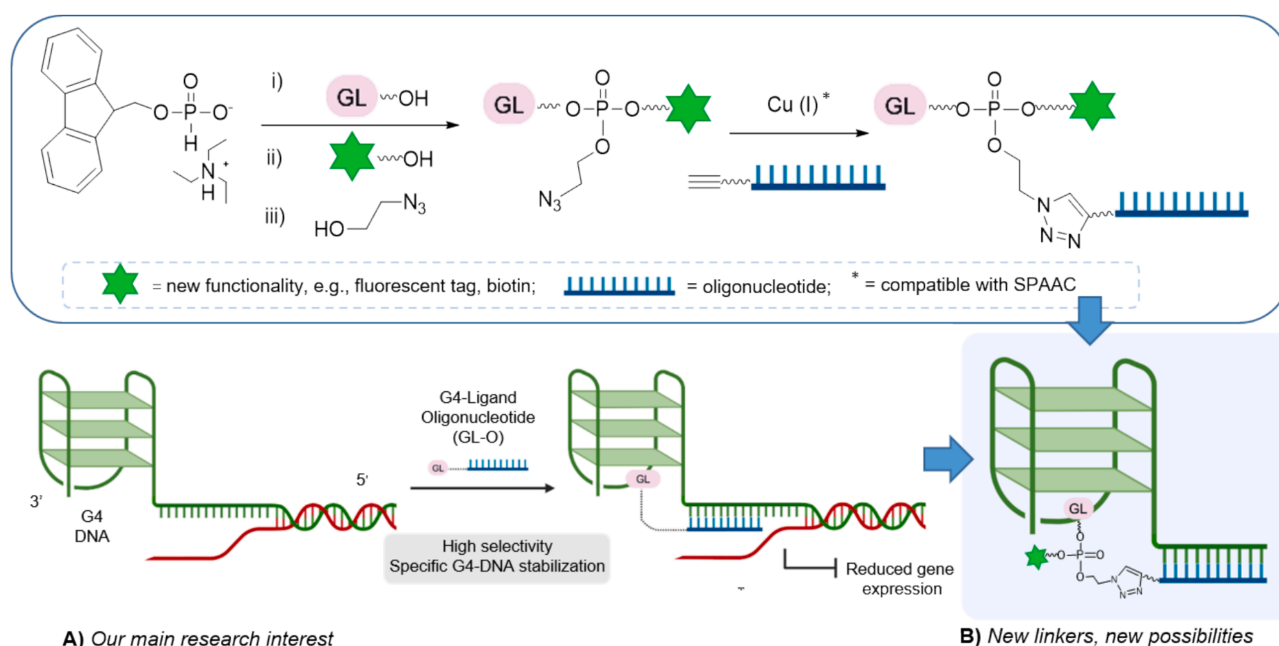


Fig. 1. Summary of the herein proposed phosphate-triester strategy and its application to specifically target G-quadruplex DNA structures.

2. Results and discussion

Ammonium (9H-fluoren-9-yl)methyl H-phosphonate is a well-known phosphonylating reagent with established applications in nucleotide chemistry, and its synthetic protocol is well-documented in the literature [23,24]. This reagent allows combining three components into a phosphate triester derivative. In our studies, we explored this capacity to design a triply functional linker that can be attached to the oligonucleotides as a post-synthetic modification.

The synthetic procedure for these triply functionalized linkers involves three main steps. First, the G4 stabilizing ligand synthesized in our laboratory [25] (**2**) was coupled with (9H-fluoren-9-yl)methyl H-phosphonate (**1**) (Scheme 1). This reaction is promoted by pivaloyl chloride, a condensing reagent that increases the electrophilic character of the H-phosphonate center [26,27], facilitating rapid and efficient nucleophilic attack at the phosphorus center. This step proceeded smoothly, achieving almost quantitative conversion into H-phosphonate diesters (**3**) as confirmed with ^{31}P NMR spectroscopy. In the second step, the H-phosphonate diesters (**3**) undergo β-elimination to remove the (9H-fluoren-9-yl)methyl group to give the H-phosphonate monoester (**4**) in 74 % yield after purification by silica gel column chromatography.

Next, the isolated compound (**4**) was subjected to a one-pot, two-step reaction where the fluorescent 7-nitrobenzofurazan derivative (**5**) was first introduced followed by the azidoethanol (**7**) to give phosphotriester (**8**). The 7-nitrobenzofurazan derivative formed an ester bond with 4 in a reaction promoted by pivaloyl chloride, resulting in the *in situ* generation of H-phosphonate diester (**6**) as confirmed by ^{31}P NMR spectroscopy. In the second step, this intermediate was oxidized by iodine [28,29] in the presence of an excess of azidoethanol (**7**), to give the key compound phosphotriester (**8**) (43 % isolated yield), which is equipped with the central azido group for subsequent copper-mediated "click" conjugation with the oligonucleotide. All the synthetic steps from **1** to **8** were followed by ^{31}P NMR spectroscopy, and the corresponding spectra are shown in Fig. 2.

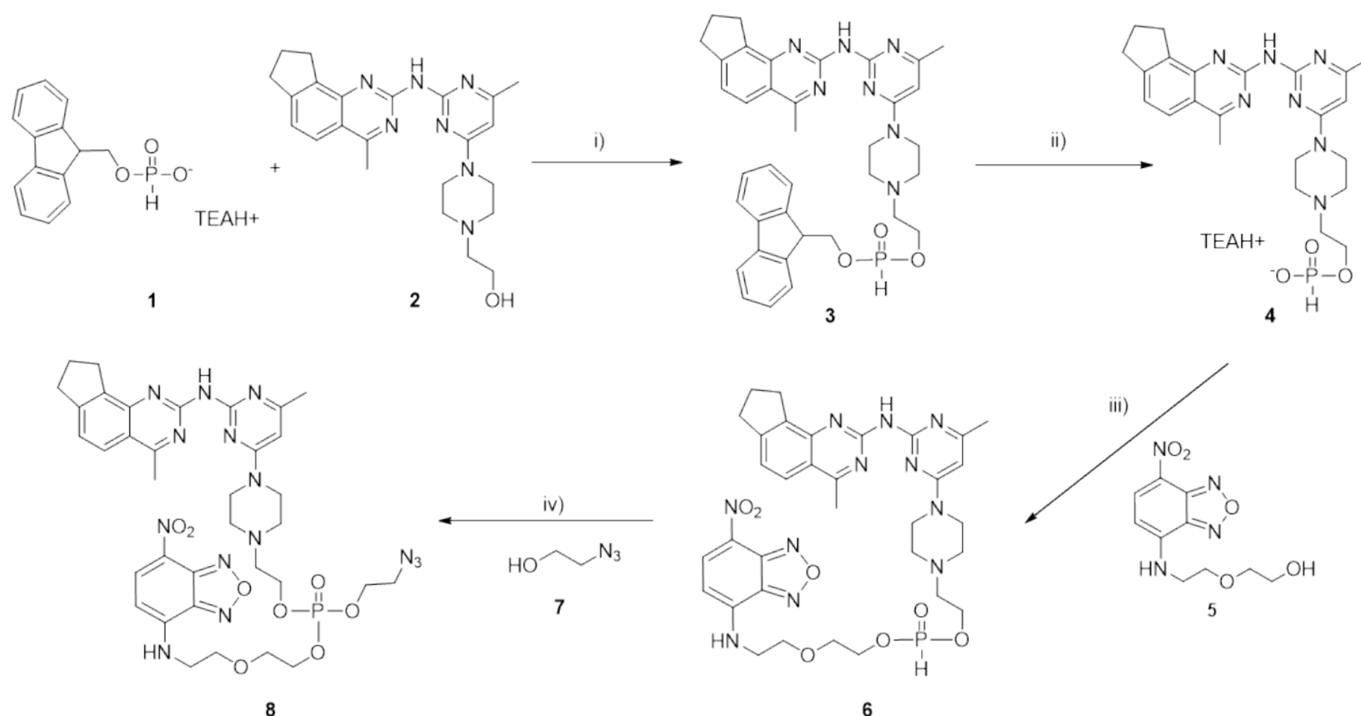
We next applied the developed synthetic route described above to prepare five different phosphate derivatives of type **8** (listed in Fig. 3) as representative examples of multifunctional handles and controls to be used in our studies. These building blocks were then incorporated into oligonucleotides using a "click" chemistry strategy, enabling their efficient and selective functionalization.

To the phosphate core of compounds **8** and **9**, we attached three functionalities: a G4-stabilizing ligand based on the quinazoline-pyrimidine structure, a fluorescent marker (an NBD-Cl derivative), and an azidoethanol moiety. These two compounds differ only in the type of linker between the quinazoline-pyrimidine G4-ligand and phosphate core, where compound **8** features a piperidine ethanol linker, while compound **9** incorporates an ethylene glycol fragment. These linkers were selected based on their frequent use in our research on G-quadruplex stabilization, providing compatibility with our model systems.

The fluorescent marker, derived from NBD-Cl, was chosen for its convenience and ease of functionalization. Using a 2-(2-aminoethoxy) ethanol linker, the fluorescent marker was efficiently attached to the phosphate core, enabling visualization in biological studies. The third functionality of the target probes was azidoethanol, a critical component to enable the final synthetic step, linking the compound to oligonucleotides via click chemistry. Together these features make compound **8** and **9** versatile biological tools, which after linking to oligonucleotides, can be used for investigating cellular processes related to G4 structures.

Compound **10** was designed in a similar way but bears a biotin derivative in place of the fluorescent marker. This substitution was motivated by the potential for pull-down experiments, allowing us to explore the specificity of these compounds in biological systems. Like compounds **8** and **9**, compound **10** retains both the G4-stabilizing ligand and azidoethanol functionalities, ensuring its applicability for studies of G4 biology.

Given our focus on the selective stabilization of G-quadruplexes, we aimed to evaluate how these newly designed handles interact with G4 structures using microscale thermophoresis (MST). To gain a comprehensive understanding of how the multifunctional probes affect their G4 interactions, we synthesized model compounds to isolate the contributions of the individual functionalities. Compound **11** includes only the



Scheme 1. Synthetic pathway of the formation of a new type of phosphate triester handles. Reaction conditions: i) DCM: Py, 9:1 (v/v), pivaloyl chloride (PvCl) 1.5 equiv., 15 min., ii) ACN: TEA, 2:1 (v/v), 30 min., iii) pivaloyl chloride (PvCl) 1.5 equiv., 15 min., iv) iodine 1.5 equiv.

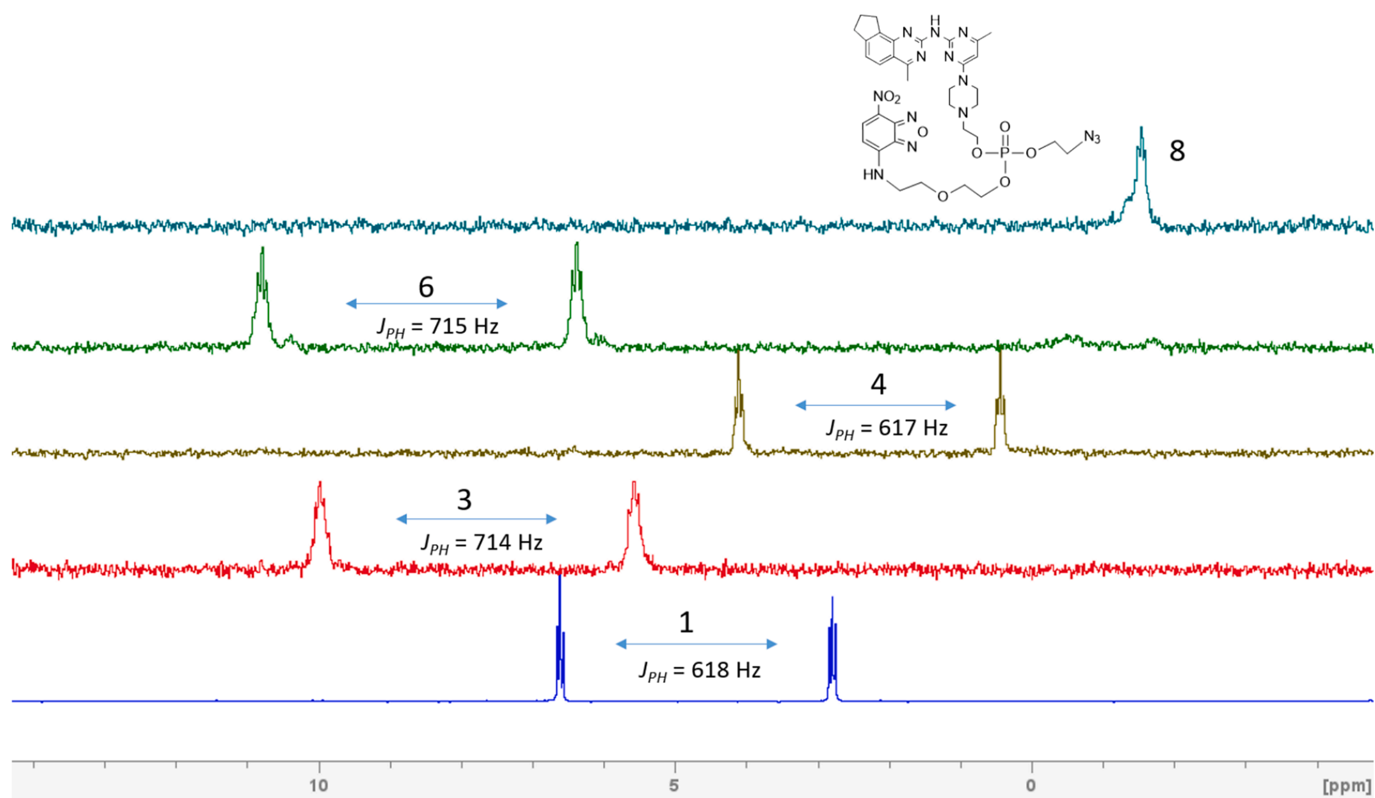


Fig. 2. ^{31}P NMR spectra illustrating the synthetic steps involved in the preparation of key intermediate **8**.

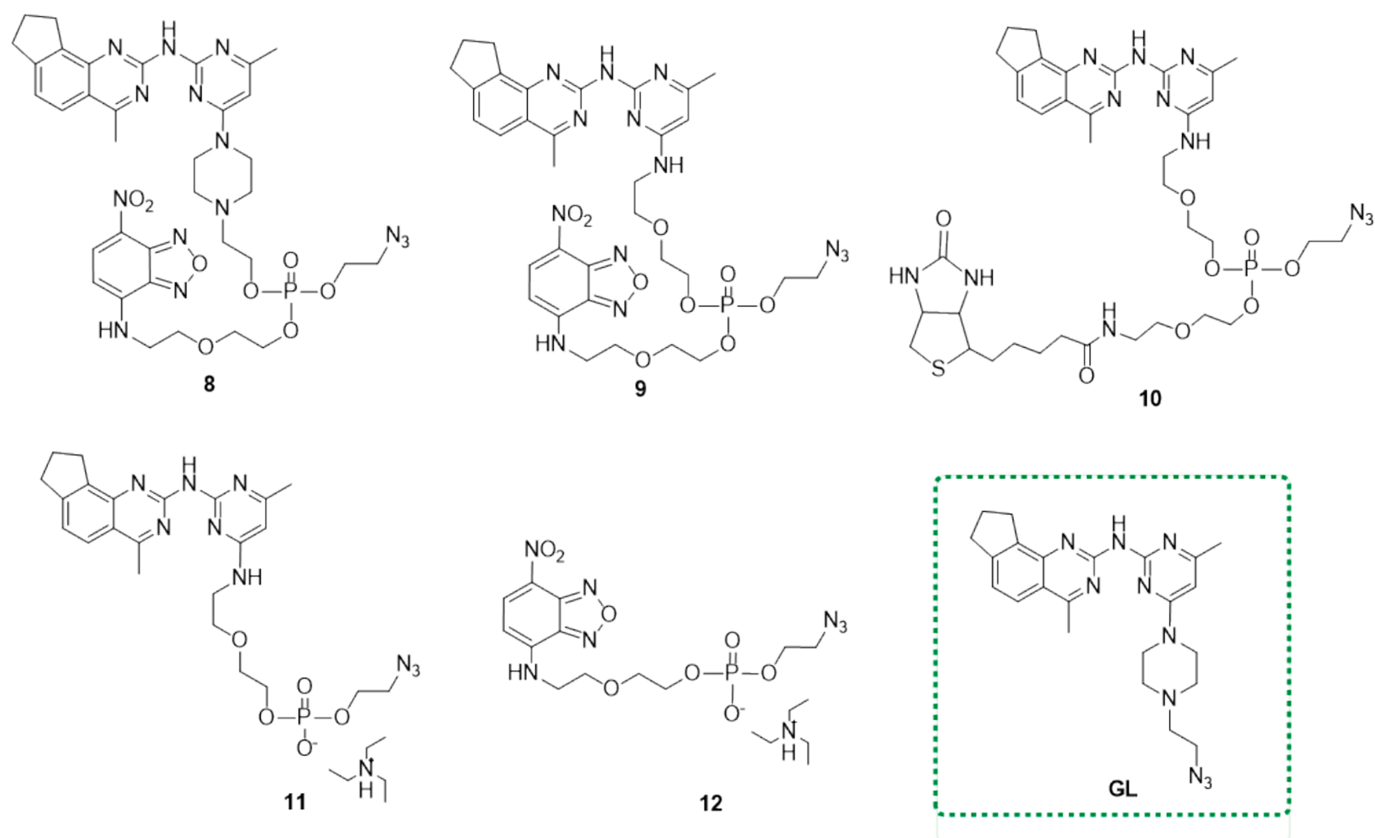


Fig. 3. The list of the synthesized phosphate handles of type **8**.

G4-stabilizing ligand, while compound **12** incorporates only the fluorescent tag, allowing us to assess the impact of each feature on G4 stabilization.

Based on these objectives, compounds **8**–**12** were designed and synthesized in our laboratory to explore the possibility of using phosphotriesters as trifunctional probes for late-stage modification of oligonucleotides to enable future studies of G4 DNA in cellular contexts.

3. Conjugation of compounds 8–12 to oligonucleotides

All the synthesized target probes **8**–**12** were attached to the model 15 nt oligonucleotide using copper-mediated click chemistry, where a copper (I) bromide dimethyl sulfide (CuBr x Me_2S) complex was used as catalyst (Scheme 2). The synthesized conjugates were isolated with moderated to good yields (25–67 %) after purification by reversed-phase HPLC and their structures were confirmed via HRMS analysis.

Additionally, we demonstrated the compatibility of an alternative, copper-free click chemistry conjugation strategy for attaching the target probes to oligonucleotides. In this experiment, we utilized a 15nt oligonucleotide with phosphorothioate (PTO) modified backbone. In the first step, the oligonucleotide was functionalized with cyclooctynyl derivative, followed by reaction with target probe **10** to give the desired conjugate in 53 % isolated yield. PTO modifications are known to enhance the stability of oligonucleotides in cellular environments [30,31] but present challenges when using copper-based click strategies [32–34]. We also showed that our approach works on already modified oligonucleotides (3́-GalNAc). This underscores the significance of demonstrating the compatibility of the copper-free click chemistry approach employed in our study (Scheme 3).

Comprehensive biological evaluations of the developed phosphate triester platform and its broader applications in chemical biology lie outside the scope of this study. However, to confirm that the introduction of new functionalities does not significantly affect the oligonucleotides' ability to bind their targets, we assessed their binding affinity as GL-Os using MST. In these experiments, we used the mutated c-MYC G4 structure, Pu24T, which is a well-established model for in vitro studies. The Pu24T G4 structure was flanked at the 5′-end by a 15 nt oligonucleotide sequence complementary to the oligonucleotide conjugated to the phosphate triester probes synthesized in this study.

First, we compared the binding affinity of the GL-O based on the phosphate diester **11**, containing a G4-ligand, with our previously published first-generation GL-Os. Gratifyingly, no differences in binding affinity were observed (Fig. 4a, c). Next, we compared the GL-O based on the phosphate diester **11** with the phosphate triester **8**, incorporating both a G4-ligand and an NBD fluorophore. Encouragingly, these constructs also displayed similar K_D values, ranging from 10 to 20 nM (Fig. 4b).

These results suggest that the phosphate triester multifunctional probes developed herein can successfully introduce additional functional groups to oligonucleotides without disrupting their binding interactions. This highlights the potential of this strategy for multifunctionalization in oligonucleotide-based applications.

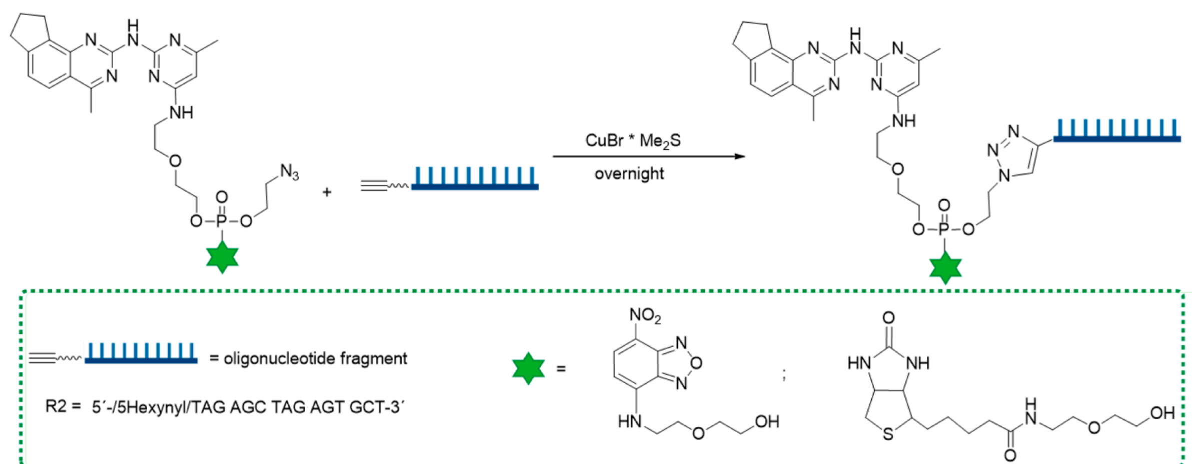
3.1. *Stability of the triester-oligonucleotides*

Due to the potential instability of the phosphate triester core, the triester-oligonnucleotide **R2-10** was selected for stability testing. The selected compound was incubated in PBS buffer (pH 7.4) at room temperature and at 37 °C while monitoring its stability by comparing the purity of chromatograms recorded over time (0 min, 30 min, 1 h, 3 h, 6 h, 24 h, and 48 h) (Fig. 5 and *SI – S8*). This demonstrated that this new type of triester-oligonucleotide conjugates are stable in buffer at physiological pH and temperature over time and may thus be suitable as potential research tools for exploring biological and therapeutic applications.

4. Conclusion

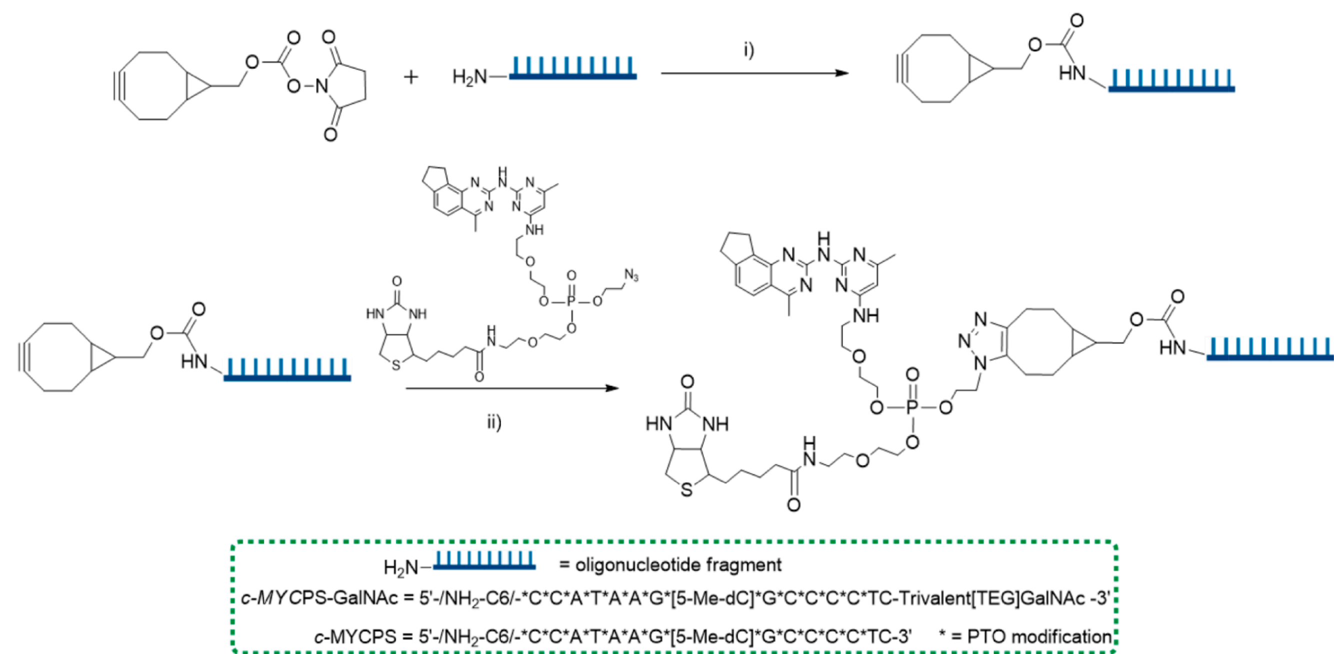
Despite recent progress, the further advancement of oligonucleotide-based strategies in both research and therapeutic applications relies on expanding the repertoire of chemical modifications to address persistent challenges, such as improving cellular uptake and delivery. An important step in overcoming these challenges is the development of innovative bioconjugation strategies that integrate seamlessly with oligonucleotide-based modalities.

In this work, we developed a novel phosphotriester trifunctional probe that introduces significant advancements in synthetic phosphate chemistry. The phosphate triester developments described herein not



Compound No.	R2-**8**	R2-**9**	R2-**10**	R2-**11**	R2-**12**	R2-**GL**
Yield [%]	33	25	58	64	67	34

Scheme 2. Conjugation of the synthesized handles **8**–**12** to oligonucleotides according to *copper-mediated click chemistry*. Reaction conditions: To the 50 μL of 1 mM oligonucleotide stock phosphate handles (compound **8**–**12**, 5 equiv. 10 mM stock in DMSO/acetonitrile, 3:7), aqueous DIPEA solution [2.5 μl, 0.25 μmol (5 equiv.), 0.043 μl], and CuBr × Me_2S solution in DMSO [7.5 μl, 0.5 μmol (10 equiv.), 0.1 mg] were added in the listed order. The reaction mixture was agitated at ambient temperature overnight.



Compound No.	*c*-MYCPS-GalNAc-**9**	*c*-MYCPS-GalNAc-**10**	*c*-MYCPS-GalNAc-**GL**	*c*-MYCPS- **10**	*c*-MYCPS- **GL**
Yield [%]	24	67	58	55	53

Scheme 3. Conjugation of the target probe **10** to oligonucleotides using the strain-promoted *copper-free click chemistry strategy*. Reaction conditions: i) 5′-Hexylamine oligonucleotide PTO was reacted to BCN-succinimidyl ester (10 equiv. solution in DMSO) in a presence of 0.1 M $NaHCO_3$ – the reaction was agitated on a vortex for 2 h then purified via precipitation in a system of CH_3COONa (3 M, pH 5.6, 10 %) and cold ethanol, ii) precipitated oligonucleotide was dissolved in water to get 1 uM stock than 2 equiv. (10 mM stock) of **9**, **10,** or **GL** was added. The reaction was agitated on a vortex overnight.

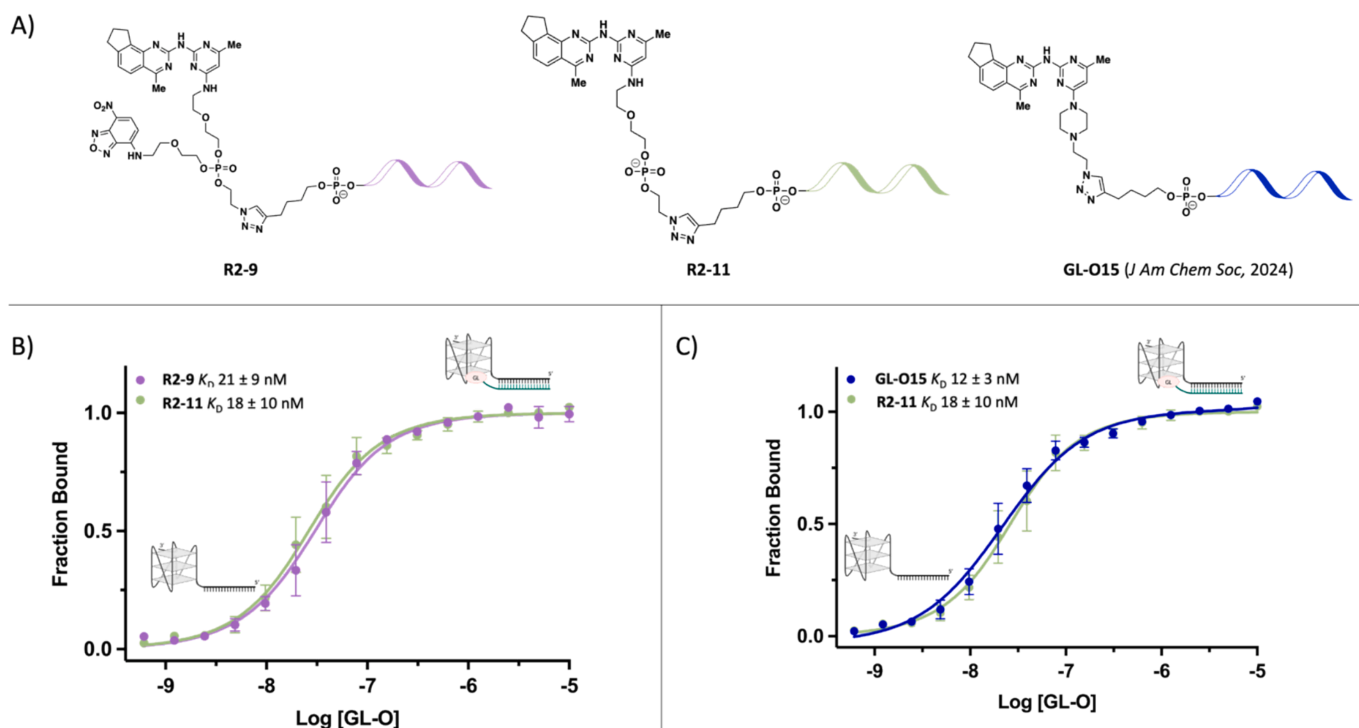


Fig. 4. Evaluating the effect of the phosphate triester multifunctional probes on their binding affinity as GL-Os. A) GL-Os evaluated for binding affinity to the G4 DNA template. Dose-response curves were obtained using MST. B) Comparison between GL-O with and without fluorescent marker NBD. C) Comparison between GL-Os containing one additional phosphate group as introduced using the herein described approach. GL-Os were titrated to c-MYC Pu24T containing the complementary flanking sequence with a 5́-labelled fluorescent tag. Binding affinity constants (K_D) values and error bars correspond to 2–3 independent measurements.

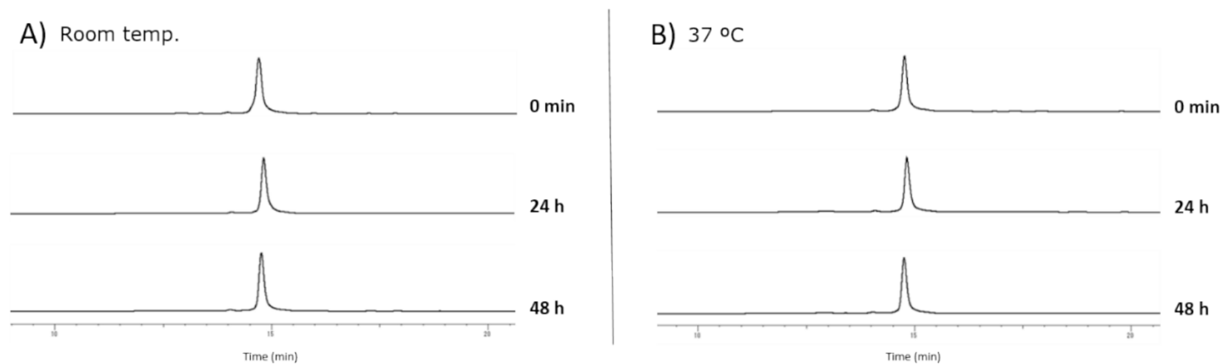


Fig. 5. Stability of **R2-10** in PBS buffer (pH 7.4) monitored over time **A**) at room temperature (reference) **B**) at 37 °C.

only demonstrate novel and robust chemical transformations but also establish a multifunctional bioconjugation platform compatible with both standard oligonucleotides and phosphorothioate-modified backbones. This development offers enhanced flexibility and functionality, enabling the incorporation of diverse features such as fluorescent tags or pull-down handles for advanced chemical biology studies.

Furthermore, the described method supports multiple conjugation strategies, including copper-free click chemistry, and facilitates late-stage functionalization of commercial oligonucleotides in solution. By eliminating the requirement for solid-phase synthesis, this approach extends its applicability to commercial oligonucleotides, making it accessible to a broader range of users. The developed approach was herein specifically designed for application in G4-ligand conjugated oligonucleotides (GL-Os) that target individual G4 structures. However, the flexibility of the approach makes it generally compatible with other oligonucleotide strategies that benefit from the incorporation of functional probes. Importantly, we demonstrate that the phosphate triester platform enables the introduction of new functionalities without disrupting the oligonucleotide's ability to bind to its target. In summary, our work presents advancements in phosphate chemistry and oligonucleotide bioconjugations that aim to address existing limitations in compatibility and pave the way for future multifunctional chemical biology studies of oligonucleotides.

5. Experimental part

5.1. General material and methods

All reagents and solvents were of analytical grade, obtained from commercial suppliers, and used without further purification. The anhydrous solvents used for the reactions were stored over molecular sieves 4 Å. TLC analyses were carried out on precoated aluminum-backed silica gel 60 F 254 (Merck) plates using a CH_2Cl_2 − MeOH 9:1 (v/v) solvent system and detected with UV light. All evaporations were carried out on rotatory evaporators under reduced pressure at 45 °C.

Purifications were performed by performing standard silica gel column chromatography using as solvent systems: CH_2Cl_2 − MeOH 9:1 (v/v) with 1 % of TEA, for compounds **11** and **12**. Flash column chromatography was performed using silica gel with an average particle diameter of 50 μm (range 40–65 μm, pore diameter 53 Å), using CH_2Cl_2 − iPrOH 9:1 (v/v) with 1 % of NH_3*H_2O for compounds **8**, **9**, **10**.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 or 600 MHz spectrometer at 298 K, calibrated by using the residual peak of the solvents as the internal standard ($CDCl_3$: δ (ppm) H = 7.26; δ (ppm) C = 77.16. DMSO-d_6: δ (ppm) H = 2.50; δ (ppm) C = 39.50). ^{31}P NMR spectra were calibrated by using H_3PO_4 as a standard determining 0 ppm of the spectrum. For compounds **8**, **9**, and **10** due to the high concentration of aliphatic protons, we indicated only diagnostic signals of the compounds. All tested compounds showed a purity of ≥ 95 % determined according to NMR analysis.

Mass spectra were obtained using an HRMS electrospray time-of-flight (ES-TOF) Agilent instrument. Oligonucleotides were eluted using an aqueous mixture of hexafluoroisopropanol and triethylamine with an increasing gradient of methanol.

Reverse phase (RP) HPLC was carried out on a Hitachi HPLC system using Clarity 5 μm Oligo RP LC, Fully Porous Organo-silica C18 (250 x 10 mm) semi-preparative column (Phenomenex) with 3 mL/min flow rate with the method: 0-1 min 95 % A, 1-15 min 95-40 % A, 15-16 min 40-5 % A, 16-19 min 5 % A, 19-20 min 5-95 % A, 20-24 min 95 % A, using detection at 260 nm at room temperature. The buffers used for RP-HPLC were as follows: (A) 50 mM triethylammonium acetate (TEAA), pH ~ 6.5; (B) 100 % acetonitrile. TEAA (1 M) buffer was prepared by dropwise addition of glacial acetic acid (57 ml) to the cooled and stirred mixture of triethylamine (139 ml) in water (800 ml). The pH was adjusted with diluted acetic acid to ~ 7 and the volume was adjusted to 1 L with water.

Mass spectra were obtained using an HRMS electrospray time-of-flight (ES-TOF) Agilent instrument, using (A) an aqueous solution of hexafluoroisopropanol and trimethylamine, and (B) 100 % methanol as buffers, with the program applying an increasing gradient of methanol.

Microscale Thermophoresis. G4 DNA was 5́-labeled with Cy5 and consisted of the G4-forming sequence and 5' flanking sequence. The G4 DNA was annealed in MST buffer (10 mM potassium phosphate, 100 mM KCl, 0.05 % Tween 20, pH 7.4) by heating at 96 °C for 5 min followed by cooling down to room temperature before storing in the fridge overnight. All MST experiments were done on a Monolith NT.115 (Nanotemper, Germany) instrument and performed in MST buffer. The G4 DNA concentration was kept constant at 10-20 nM to which GL-Os were serial diluted (1:1) with the highest concentration of 5-20 μM. MST traces and binding constants (KD) were obtained using the Monolith analysis software and plotted and visualized in GraphPad Prism 10.

5.2. Copper-mediated click chemistry procedure for conjugation compounds 8-12 to oligonucleotide.

A 50 μL of 1 mM oligonucleotide stock was transferred to an Eppendorf to which reagents were added in following order: compound **8-12** (5 equiv. 10 mM stock in DMSO/ACN, 3:7), aqueous DIPEA solution (2.5 μl, 0.25 μmol (5 equiv.), 0.043 μl), and CuBr*Me_2S solution in DMSO (7.5 μl, 0.5 μmol (10 equiv.), 0.1 mg). The reaction mixture was vortexed and subsequently agitated at ambient temperature overnight. The reaction mixture was diluted with 25 μl of a 0.5 mM EDTA solution and 200 μl water before purification by RP-HPLC.

5.3. Copper-free click chemistry procedure for conjugation compounds 9-10 to oligonucleotide

Oligonucleotide (Na + salt, 50 nmol) was dissolved in 0.1 M $NaHCO_3$ (aq) in a 1 mM solution. A freshly prepared solution of (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethyl N-succinimidyl carbonate (BCN-

NHS ester) in DMSO (5 μL, 20 equiv., 200 mM) was added to the oligo solution. The reaction mixture was vortexed and let agitate in ambient temperature overnight. The oligo was next precipitated using 10 % v/v of 3 M NaOAc and 4x volume of EtOH, incubated in the freezer for 30 min, followed by centrifugation for 15 min and removing the supernatant. The precipitation was repeated one time followed by a wash of the pellet using only EtOH. The pellet was dried using a gentle N_2 gas flow and then resuspended in 0.1 M $NaHCO_3$ (aq) (50 μL). Subsequently, the compound containing a terminal azide (25 μL, 2.5 equiv. 10 mM in DMSO) was added to the dissolved oligo. The resulting solution was vortexed and let to agitate overnight. The reaction mixture was then filtered and purified by RP-HPLC.

CRediT authorship contribution statement

Justyna Golebiewska-Pikula: Writing – original draft, Methodology, Data curation, Conceptualization. **Alva Abrahamsson:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Erik Chorell:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2025.108259.

Data availability

Data will be made available on request.

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