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DNA-Based Phosphane Ligands

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Abstract: In order to expand the repertoire of DNA sequences specifically interacting with transition metals, we report here the first examples of DNA sequences carrying mono- and bidentate phosphane ligands as well as P,N-ligands. Aminoalkyl-modified oligonucleotides have been reacted at predetermined internal sites with carboxylate derivatives of pyrphos, BINAP and phosphinooxazoline (PHOX) **2b–d**. Carbodiimide coupling in the presence of *N*-hydroxysuccinimide provided the DNA–ligand conjugates in 38–78% yield. Phosphane-containing oligonucleotides and their phosphane sulfide analogues were characterized by mass spectrometry (MALDI-TOF and FT-ICR-ESI) and their stability after purification and isolation was systematically investigated. While DNA-appended pyrphos ligand was quickly oxidized, BINAP and PHOX conjugates showed high stabilities, making them useful precursors for incorporation of transition metals into DNA.

Keywords: DNA · nucleic acids · oxazoline · phosphane ligands

Introduction

The incorporation of transition-metal complexes into DNA and RNA is an important objective for the development of functional biomolecules with potential applications as therapeutics,[1] artificial nucleases,[2–4] and as nanotechnology construction material.[5,6] Inspired by the pioneering work of Whitesides,[7] who showed that asymmetric catalytic hydrogenations could be performed by anchoring an achiral Rh^{I} complex in a chiral cavity of the protein avidin,[8,9] we aim at embedding transition-metal complexes in nucleic acids folds in order to generate metalloribozymes and metallodeoxyribozymes.

The potential of nucleic acids in asymmetric catalysis is thus far practically unexplored. To date, there are only two documented examples of nucleic acid-based asymmetric catalysis. Our lab has discovered RNA enzymes that catalyze Diels–Alder cycloadditions of anthracene and maleimide derivatives with fast multiple turnover and high enantioselectivity.[10,11] Structural and mechanistic data, however, indicate that in this system, metal ions play no catalytic role.[12,13] Feringa and co-workers recently demonstrated that double-helical DNA could be employed as chirality source in Lewis acid catalyzed Diels–Alder reactions using Cu^{II} complexes tethered to an intercalator[14] or able to interact directly with double stranded DNA.[15] In these systems, however, the exact position of the metal complexes within the DNA is not defined, making a thorough understanding of DNA's role difficult. Towards this end, a well-defined positioning of the metal complex and a precise control of the coordination environment are essential.

The most common approach to introduce metal complexes into DNA is the post-synthetic derivatization of oligonucleotides that contain tethered primary amino groups with activated esters, affording nucleic acids that carry metal-based cleavage reagents, luminescent probes, photooxidants and redox-active species.[16–22] Alternatively, solid-phase strategies have utilized ligand-tethered[2–4] or metallated[23–25] nucleoside analogues or metal-coordinating nucleoside mimics (ligandosides).[26–30] However, the major drawback of these solid-phase strategies is the requirement for stable ligands that can survive the conditions used in DNA automated synthesis (capping, oxidation), deprotection and isolation. Therefore the known repertoire of metal-binding

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author: Experimental procedure for the synthesis of amino-modified oligonucleotides **ODN1–3**, MALDI TOF analysis, reversed-phase HPLC gradients and retention times for all DNA conjugates **ODN1–6**, and original electrospray mass spectrum of **ODN4b**.

functionalities is rather scarce and consists mainly of nitrogen- and oxygen-donor ligands.

Phosphanes are among the most efficient and extensively used ligands in transition-metal catalysis.[31,32] Attracted by the broad applicability of this class of ligands we report here the first examples of DNA conjugates carrying bisphosphane and phosphinooxazoline ligands. We envision the post-synthetic modification of oligonucleotides as the most suitable and versatile approach for the preparation of such conjugates. The synthesis of DNA precursors containing a primary alkylamino functionality that can be selectively addressed,[33–38] followed by coupling of phosphane ligands equipped with a carboxyl group allows their attachment at defined predetermined internal sites.

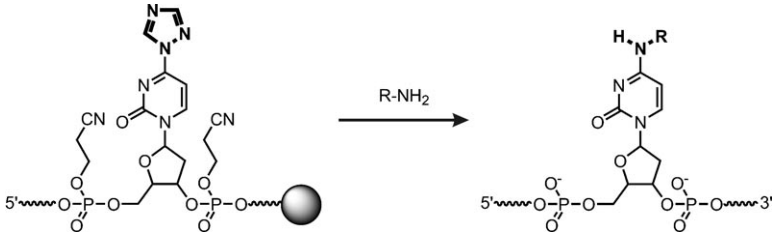
Results and Discussion

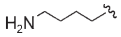
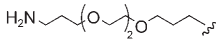
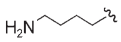
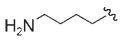
For the preparation of amino-modified oligonucleotides (ODN), the "convertible nucleoside approach"[39,40] was adapted and optimized. Together with base-labile TAC (*tert*-butylphenoxyacetyl) protecting groups, this allows high-yielding syntheses of long ODNs with the convertible nucleotide at varying internal positions and also in combination with other non-standard phosphoramidite building blocks (e.g., a decaethyleneglycol spacer molecule **1** as for **ODN3**). After the synthesis, the ODNs were treated with diamines (Table 1), affording conversion of the 4-triazolyl-dU to different 4-alkylamino-dC derivatives that can base-pair like a normal cytidine nucleotide.[41] At the same time, the ODN is cleaved from the support and deprotected. Compared with reported methods, this mild one-pot conversion, deprotection and cleavage procedure gives consistently high yields of amino-modified DNA sequences in short reaction times (4 h). Furthermore, it allows the parallel synthesis of various conjugates differing in length and structure of the spacer, which may be of particular relevance in determining the interaction between the transition metal complex and the biopolymer.[14]

Amide bond formation between **ODN1a** and commercially available 4-(diphenylphosphano)-benzoic acid (**2a**)[42] was chosen as the model reaction to investigate the coupling of phosphane-based ligands (Scheme 1). Phosphane **2a** was first activated by using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) in the presence of *N*-hydroxysuccinimide (NHS), and the in situ generated active ester was directly added to the **ODN1a** solution.

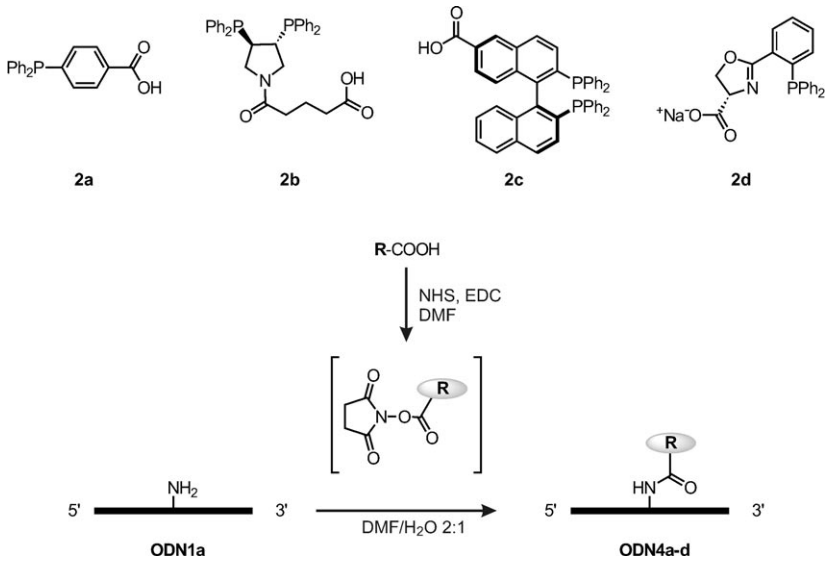
The coupling reaction was analyzed by reversed-phase HPLC and proceeded to completion, affording 60% of the desired DNA–phosphane conjugate **ODN4a**. Not surprisingly, a fraction ($\leq$20%) of **ODN4a** was oxidized to the corresponding phosphane oxide **ODN4a(O)**, presumably during

Table 1. Preparation of amino-modified **ODN1–3**.[a]



ODN	Sequence	R	Yield[b] [%]
ODN1a			35
ODN1b	5'-GC AGT GAA GGC^{R} TGA GCT CC-3'		42
ODN1c			40
ODN2	5'-GC AGT GAA GGC TGA GCT CCT AC^{R}C-3'		32
ODN3[c]	5'-GC AGT GAA GGC TGA GCT CC**1** C^{R}C-3'		30

[a] Reaction conditions: 5 M aqueous solution of 1,4-diaminobutane or ethylenediamine, RT, 4 h or neat 1,13-diamino-4,7,10-trioxatridecane, RT, 4 h (followed by additional treatment with water, 5 h). [b] Isolated yields after solid phase synthesis (1 μmol), conversion and purification. [c] A decaethylene glycol unit **1** was incorporated during solid phase synthesis.



Scheme 1. Post-synthetic functionalization of amino-modified ODN with phosphanes **2a–c** and phosphinooxazoline **2d**.

workup. Small amounts of byproducts were observed that did not carry a phosphane moiety, suggesting slight degradation of the starting material (Figure 1).

The desired phosphane-containing oligonucleotide could

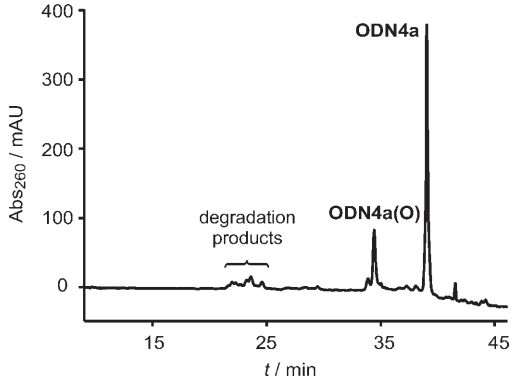


Figure 1. HPLC chromatogram of **ODN4a** pre-purified by chloroform extraction and ethanol precipitation to remove the excess of coupling reagents. Trace amounts of degradation products elute between 20 and 24 min, similarly to the starting material **ODN1a** ($t_R = 21.9$ min).

easily be isolated by HPLC (Figure 1) and was stable under the purification conditions.[43] MALDI-TOF mass spectrometry of the HPLC purified **ODN4a**, however, gave only the mass of the oxidized product **ODN4a(O)** (Table 2). To

Table 2. Isolated yields and MALDI-TOF analysis of **ODN4a**, **ODN5** and **ODN6**.[a]

Entry	Conversion [%]	Isolated yield [%]	ODN(O)[b]		ODN(S)[c]	
			$[M-H]^-$			
			calcd	obsd	calcd	obsd
ODN4a	>99	60	6228	6234	6244	6249
ODN5	96	65	7424	7430	7440	7447
ODN6	>99	68	7328	7332	7344	7350

[a] **ODN5** and **ODN6** are the coupling products of **2a** with **ODN2** and **ODN3**, respectively. [b] **ODN(O)**: DNA–phosphane oxide. [c] **ODN(S)**: DNA–phosphane sulfide.

prove the identitity of the oligonucleotide eluting with $t_R =$ 39 min as phosphane–DNA conjugate **ODN4a**, the HPLC eluate was treated with sulfur to yield the air-stable phosphane sulfide analogue **ODN4a(S)**. The MALDI mass spectrum clearly confirmed that the isolated species was the pure phosphane–DNA **ODN4a** and no trace of the oxide **ODN4a(O)** was detected.

Amino-modified oligonucleotides **ODN2** and **ODN3** were also reacted with phosphane **2a**. The coupling reactions proceeded consistently well, affording 65 and 68% of **ODN5** and **ODN6**, respectively (Table 2).

Having established the optimal conditions for coupling the monophosphane derivative **2a** to DNA, we then studied the reaction of **2b–d** with **ODN1a** (Scheme 1). Bisphosphanes **2b**[44] and **2c**[45] are derivatives of the well-known ligands pyrphos and BINAP, respectively, extensively used in organometallic catalysis,[46–50] while **2d** belongs to the family of PHOX ligands (phosphinooxazolines) with applications in allylic substitution, hydrogenation and asymmetric Heck reactions.[51,52] Compound **2d** was synthesized starting from commercially available 2-(diphenylphosphano)-benzoic acid (**3**) and L-serine methyl ester hydrochloride (**4**) (HCl·H-L-Ser-OMe) (Scheme 2).

The coupling reactions of **2b–d** to **ODN1a** were monitored by reversed-phase HPLC and proceeded with 98 and 95% conversion for **2b** and **2d**, respectively. The amounts of oxidized species (mono- and bisoxide for **ODN4b** and monoxide for **ODN4d**) were below 10%. In case of **2c**, the observed conversion was lower (55%), most probably due to the limited solubility of the BINAP derivative in the aqueous reaction mixture (Table 3). Oxidation products

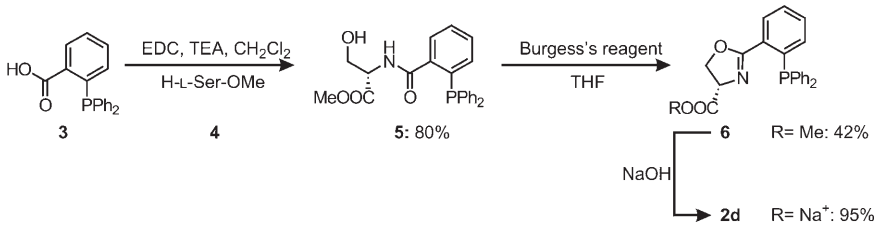
Table 3. Isolated yields and MALDI-TOF MS analysis of **ODN4b–d**.

Entry	Isolated yield [%]	$ODN(O)_n$[a]		ODN(O)(S)		$ODN(S)_n$[a]	
		m/z[b]					
		calcd	obsd	calcd	obsd	calcd	obsd
ODN4b	74	6489	6491	6508	6510	6524	6528
ODN4c	38	6605	6610	6621	6626	6637	6637
ODN4d	78	6297	6296	–	–	6313	6314

[a] $n = 2$ for **2b** and **2c**, and $n = 1$ for **2d**. [b] **ODN4b** and **ODN4d** detected in negative mode ($[M-H]^-$), **ODN4c** in positive mode ($[M+H]^+$).

(mono- and bisoxide of **ODN4c**) were found to be formed in <7% yield. Conjugates **ODN4b–d** were purified and isolated by reversed-phased HPLC. Figure 2a illustrates a typical HPLC chromatogram obtained for the coupling of **2d**. The isolated products were analyzed by mass spectrometry (Table 3) in the form of the corresponding phosphane sulfide analogues. All other byproducts generated by full or partial oxidation were also isolated and characterized: phosphane bisoxides (for **2b** and **2c**) and phosphane monoxides (for **2d**; and for **2b** and **2c** characterized as monoxide–monosulfide).

While MALDI mass spectrometry was found unsuitable for the direct detection of phosphane conjugates, ESI-MS gave the main peak corresponding to the non-oxidized phosphane in one case (**ODN4b**), indicating that this technique



Scheme 2. Synthesis of phosphinooxazoline **2d**.

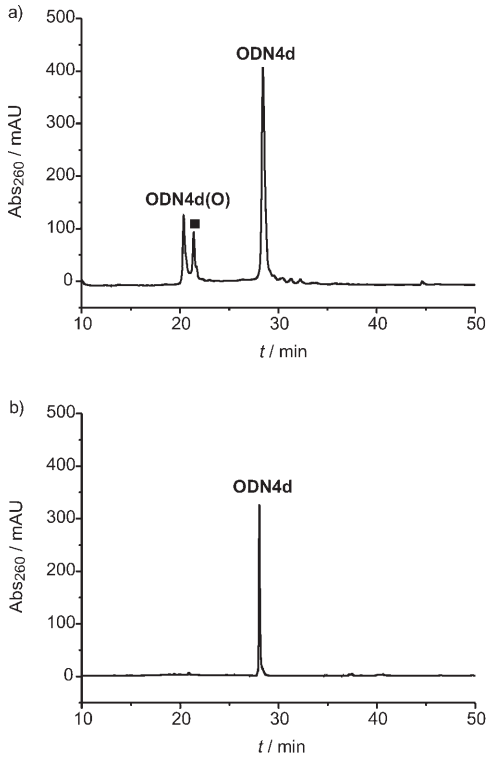


Figure 2. HPLC chromatograms of the phosphinoxazoline derivatized DNA conjugate **ODN4d**. a) crude product after chloroform extraction and ethanol precipitation. ■ Trace impurity from the starting material **ODN1a**. b) HPLC purified product, after 1 h at RT.

might be suitable for the characterization of phosphane–DNA species without the need of sulfur treatment (Figure 3).

DNA–phosphane conjugates **ODN4a–d**, **ODN5** and **ODN6** are generally air sensitive and must be manipulated under oxygen-free conditions, as commonly done with phosphane ligands. Nevertheless, the observed rates of oxidation are notably different, depending on the attached ligand. The relative stabilities and the conditions under which these conjugates could be handled were investigated by an HPLC assay. Oligonucleotides **ODN4a–d** were isolated by HPLC, the eluates stored at room temperature for 1 h under argon, and then re-analyzed by HPLC. This allowed to measure the extent of oxidation caused by oxygen dissolved in the HPLC solvents from the very moment after their isolation. Oligomers **ODN4a** and **ODN4b** showed disappointingly low stabilities, yielding large amounts of fully oxidized species (60 and 90%, respectively). In contrast, **ODN4c** and **ODN4d** were found to be stable under these conditions, giving $<10\%$ of oxidized product in case of **ODN4c** and no detectable amount for **ODN4d** (Figure 2b). These results demonstrate that the stability of **ODN4c** and **ODN4d** is high enough to allow manipulations of such conjugates even under suboptimal conditions, for example, outside a glove box.

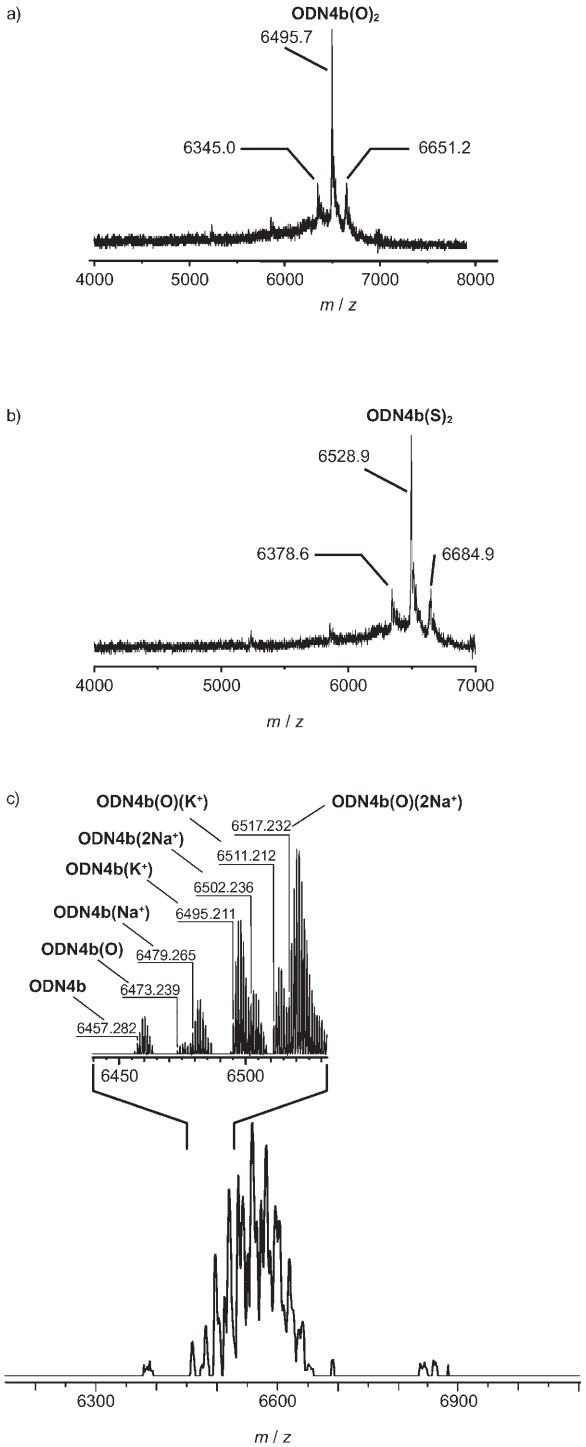


Figure 3. Mass spectrometry analysis of **ODN4b**. a) MALDI-TOF spectrum of **ODN4b(O)$_2$** (HPLC isolated **ODN4b**). b) MALDI-TOF spectrum of **ODN4b(S)$_2$**. c) ESI MS spectrum (part of the deconvoluted spectrum) of **ODN4b** (measured: 6457.28, calculated: 6457.27).

Conclusion

We have established an efficient post-synthetic strategy for the site-specific incorporation of phosphane ligands into DNA sequences. Bisphosphane- and phosphinooxazoline-

containing DNA conjugates **ODN4b–d** are easily formed by coupling amino-modified oligonucleotides with compounds **2a–d** and are stable under HPLC purification conditions. Moreover, the stability of **ODN4c** and **ODN4d** is reasonably high after isolation and storage in solution, which makes them attractive precursors for the development of metal-containing oligonucleotides.

The approach described herein provides new chelating functionalities for introducing metal centers at well-defined positions in DNA or RNA sequences, affording a unique collection of DNA–phosphane ligands which could be used in catalysis. In particular, the combination with in vitro selection techniques is expected to generate metal-DNAzymes and -ribozymes, allowing new research at the interface between the fields of transition-metal catalysis and biocatalysis.[53,54] Current work in our laboratory is focusing on the use of oligonucleotides **ODN4b–d** as ligands in transition-metal catalyzed reactions.

Experimental Section

General: Solid-phase DNA synthesis was performed on an Expedite 8909 automated synthesizer using dC (*tert*-butyl-phenoxyacetyl, TAC) controlled pore glass support (40 μmol g^{-1}, 500 Å) and TAC-protected phosphoramidites (Proligo). 4-Triazolyl-deoxyuridine phosphoramidite was purchased from Glen Research. All reagents were purchased from Aldrich, Fluka, Acros Organics or Proligo (for oligonucleotide synthesis) and used without further purification. DMF and THF were purchased from Fluka (dry solvents over molecular sieves). HPLC analyses were performed on an Agilent 1100 Series HPLC system equipped with a diode array detector using a Phenomenex Luna 5 μm C18 column (4.6× 250 mm) and eluting with a gradient of 100 mM triethylammonium acetate (TEAA) pH 7.0 (buffer A) and 100 mM TEAA in 80% acetonitrile (buffer B) at 1 mL min^{-1} flow-rate.

Degassing of reaction mixtures containing O_2-sensitive phosphanes was achieved through a minimum of three successive freeze–pump–thaw cycles. All subsequent operations were performed under argon using standard Schlenk techniques.

Compounds **2b**[44,46] and **2c**[45,55] were prepared according to literature procedures. Derivative **2b** was synthesized starting from (*R*,*R*)-3,4-bis(diphenylphosphano)pyrrolidine. For **2c** preparation, the commercially available (*S*)-2,2′-dihydroxy-1,1′-binaphthalene was used. TLC analyses were carried out using silica gel plates Polygram Sil G/UV_{254} (40× 80 mm) from Macherey–Nagel. Flash chromatography was carried out on silica gel 40 μm from J.T. Baker. NMR spectra were recorded on Mercury Plus 300, Varian VNMR S 500, Bruker AC-300, or DRX-300 spectrometers. ^1H and $^{13}C\{^1H\}$ NMR spectra were calibrated to TMS on the basis of the relative chemical shift of the solvent as an internal standard. $^{31}P\{^1H\}$ NMR spectra were calibrated to an external standard (85% H_3PO_4). Abbreviations used are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, brs = broad singlet, brd = broad doublet. FAB and EI mass spectra were recorded on a JEOL JMS-700 sector field mass spectrometer. MALDI-TOF mass spectra were recorded on a Bruker BIFLEX III spectrometer. ESI MS analysis for small compounds was performed on a Finnigan MAT TSQ 700 spectrometer.

Conditions for MALDI-TOF MS analysis: Oligonucleotides were dissolved in water to a final concentration of 10 μM. The samples for analysis were prepared using the dried droplet method with the following matrix solutions: 1) 6-aza-2-thiothymine/diammonium hydrogen citrate in 1:2 water/acetonitrile (detection in negative mode); 2) 3-hydroxy-picolinic acid/diammonium hydrogen citrate in 1.2:1 water/acetonitrile (detection in positive mode).

Conditions for ESI MS analysis of ODN4b: ESI mass spectra were recorded in the negative mode on a Bruker APEX IV Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer with a 7.05 T magnet and an Apollo electrospray (ESI) ion source equipped with an off-axis 70° stainless steel spray needle. Typically, 50 μM analyte solutions (CH_3CN/H_2O 1:1) were introduced into the ion source with a syringe pump (Cole–Parmers Instruments, Series 74900) at flow rates of 3 to 4 μL min^{-1}. Ion transfer into the first of three differential pump stages in the ion source occurred through a glass capillary with 0.5 mm inner diameter and nickel coatings at both ends. Ionization parameters were adjusted as follows: capillary voltage: 4.1 kV; end plate voltage 3.6 kV; capexit voltage: −280 V; skimmer voltages: −5 to −7.5 V; temperature of drying gas: 40 C. Nitrogen was used as nebulizing (25 psi) and drying gas (5 psi). The ions were accumulated in the instruments hexapole for 1–1.5 s, introduced into the FT-ICR cell which was operated at pressures below 10^{-10} mbar, and detected by a standard excitation and detection sequence. For each measurement, up to 128 scans were averaged to improve the signal-to-noise ratio.

General procedure for the functionalization of amino-ODN with phosphane ligands: The phosphane derivatives **2a–d** (1.0 equiv) were converted to the corresponding activated esters in degassed DMF in 45–60 min at room temperature by reaction with NHS (1.0 equiv) in the presence of EDC (1.2 equiv). In parallel, **ODN1a**, **ODN2** and **ODN3** were dissolved in $NaHCO_3$ (0.1 M, pH 8.3) and the resulting solutions were degassed. The coupling reactions were performed by combining the solutions of the in situ generated NHS-ester (200–500 equiv) and the amino-modified DNA (final DMF/H_2O ratio 2:1) to achieve final **ODN1a**, **ODN2** and **ODN3** concentration of 115, 103, and 104 μM, respectively, for coupling with **2a** (22 mM), and final **ODN1a** concentration of 35, 45, and 45 μM, respectively, for coupling with **2b–d** (17 mM in all cases). After stirring overnight, at room temperature, the reaction mixtures were diluted with water, extracted with chloroform (3×2 mL), and the crude products isolated by ethanol precipitation. The phosphane–DNA conjugates **ODN4–6** were purified by reversed-phase HPLC, lyophilized and redissolved in degassed water. Conversions (Table 2) were estimated by comparing the amount of conjugated oligonucleotide to the amount of unreacted **ODN1–3** as shown in the chromatograms and isolated yields (Tables 2, 3) were calculated based on UV measurements. ε_{260} for **ODN4–6** were approximated to the ones of the corresponding starting materials (Supporting Information).

(4,4′-Dimethoxytrityl)decaethylene glycol-*O*-2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidite (1): (4,4′-Dimethoxytrityl)decaethylene glycol[56] (0.42 g, 0.55 mmol) was dried by coevaporation with toluene (3×5 mL), dissolved in acid-free CH_2Cl_2 (2 mL) under argon, and *N*,*N*-diisopropylethylamine was added (0.29 mL, 1.7 mmol, 3.0 equiv). The mixture was cooled to 0°C before addition of 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.15 mL, 0.66 mmol, 1.2 equiv). Stirring was continued for 20 min during which the temperature was slowly raised to RT. The reaction mixture was then directly loaded on a silica gel column. Purification by flash chromatography (ethyl acetate/Et_3N 95:5) afforded the product as pale yellow oil (0.44 g, 0.46 mmol, 83%). ^1H NMR (300 MHz, [D_6]DMSO): δ = 7.52–7.47 (m, 2H), 7.39–7.27 (m, 6H), 7.24–7.18 (m, 1H), 6.91–6.85 (m, 4H), 3.90–3.54 (m, 50H), 3.18 (t, J = 5.0 Hz, 2H), 1.19 (dd, J = 6.8, 1.1 Hz, 12H); ^{31}P NMR (121.5 MHz, [D_6]DMSO): δ = 148.81; FAB MS: m/z: calcd for $C_{50}H_{77}N_2O_{14}P+H$: 961.52; found: 961.6 $[M+H]^+$, 983.7 $[M+Na]^+$.

(*S*)-*N*-(2-Hydroxy-1-carboxymethyl-ethyl)-2-(diphenylphosphano)-benzamide (5): Et_3N (0.68 mL, 4.9 mmol, 1.1 equiv) and EDC (0.94 g, 4.9 mmol, 1.1 equiv) were added to a stirred solution of 2-(diphenylphosphane)benzoic acid **3** (1.5 g, 4.9 mmol, 1.1 equiv) and HCl·H-Ser-OMe **4** (0.693 g, 4.45 mmol) in CH_2Cl_2 (40 mL). The reaction mixture was stirred 4 h at RT, until the starting material was consumed according to TLC (ethyl acetate/hexanes 1:1). The mixture was diluted with CH_2Cl_2 (100 mL), washed with 5% $NaHCO_3$ (50 mL), 1 M HCl (50 mL) and brine (2×50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by chromatography on silica gel eluting with ethyl acetate/hexanes 1:1 to yield amide **5** (1.6 g, 0.65 mmol, 80%) as a white, amorphous solid. ^1H NMR (500 MHz,

$CDCl_3$): δ = 7.65 (dd, J=7.4, 3.7 Hz, 1H), 7.42–7.29 (m, 12H), 7.01 (dd, J=7.6, 4.3 Hz, 1H), 6.87 (br d, J=7.0, 1H), 4.71 (m, 1H), 3.88 (m, 2H), 3.74 (s, 3H), 2.82 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.62, 168.88, 140.79 (d, $J_{C,P}$=25.3 Hz), 136.38 (d, $J_{C,P}$=18.9 Hz), 136.31 (d, $J_{C,P}$=19.1 Hz), 135.58 (d, $J_{C,P}$=18.3 Hz), 134.23, 133.98, 133.82, 133.66, 130.52, 129.14, 129.03, 128.97, 128.79, 128.74, 128.66, 128.61, 127.87, 127.83, 62.86, 55.35, 52.73; ^{31}P NMR (202 MHz, $CDCl_3$): δ = −10.51; FAB MS: m/z: calcd for $C_{23}H_{22}NO_4P$: 408.13; found: 408.1 $[M]^+$.

(*S*)-Methyl 2-(2-diphenylphosphanophenyl)-4,5-dihydrooxazolo-4-carboxylate (6): (Methoxycarbonylsulfamoyl)triethylammonium hydroxide, inner salt (Burgess's reagent, 0.703 g, 2.95 mmol, 1.2 equiv) was added to a stirred solution of **5** (1.0 g, 2.5 mmol) in dry THF (20 mL).[57] After the solution was heated under reflux for 4 h (TLC control: ethyl acetate/hexanes 3:7), the reaction mixture was allowed to cool down to room temperature and diluted with ethyl acetate (200 mL). The resulting solution was washed with water (2×100 mL) and brine (100 mL), and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded the crude product as brownish oil. Purification by flash chromatography (elution with ethyl acetate/hexanes 3:7, column preconditioned with the eluent containing 1% Et_3N) gave phosphinooxazoline **6** as colorless oil (0.401 g, 1.13 mmol, 42%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.92 (ddd, J=7.5, 3.5, 1.5 Hz, 1H), 7.36–7.30 (m, 12H), 6.91 (ddd, J=7.6, 4.3, 1.0 Hz, 1H), 4.69 (dd, J=10.6, 8.2 Hz, 1H), 4.38 (t, J=8.4 Hz, 1H), 4.26 (dd, J=10.5, 8.6 Hz, 1H), 3.68 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.08, 166.45, 139.30 (d, $J_{C,P}$=25.9 Hz), 137.69 (d, $J_{C,P}$=11.9 Hz), 137.50 (d, $J_{C,P}$=10.4 Hz), 134.28, 134.11, 134.02, 133.85, 133.67 (d, $J_{C,P}$=1.9 Hz), 130.95 (d, $J_{C,P}$=19.0 Hz), 130.94, 130.36 (d, $J_{C,P}$=2.8 Hz), 128.74, 128.61, 128.52, 128.46, 128.41, 128.35, 128.00, 69.10, 68.45, 52.50; ^{31}P NMR (202 MHz, $CDCl_3$): δ = −4.80; EI MS: m/z: calcd for $C_{23}H_{20}NO_3P$: 389.12; found: 389.0 $[M]^+$.

(*S*)-2-(2-Diphenylphosphanophenyl)-4,5-dihydrooxazolo-4-carboxylic acid sodium salt (2d): Compound **6** (0.35 g, 0.90 mmol) was stirred in a 0.5 M solution of NaOH (3 mL) 6 h at RT. The reaction mixture was diluted with water (3 mL) and the product was precipitated by slow addition of acetone. After filtration and drying under vacuum, the sodium salt **2d** (0.34 g)[58] was recovered (0.85 mmol, 95%, white solid). ^{1}H NMR (500 MHz, D_2O): δ = 7.66 (dd, J=7.4, 3.0 Hz, 1H), 7.10 (t, J=7.5 Hz, 1H), 6.98–6.78 (m, 10H), 6.70 (t, J=7.5 Hz, 1H), 6.55–6.50 (m, 1H), 4.17–4.11 (m, 1H), 3.97 (t, J=8.3 Hz, 1H), 3.88–3.81 (m, 1H); ^{13}C NMR (126 MHz, D_2O): δ = 178.16, 166.89, 137.24 (d, $J_{C,P}$=19.7 Hz), 136.11 (d, $J_{C,P}$=7.9 Hz), 135.87 (d, $J_{C,P}$=7.6 Hz), 133.88, 133.75, 133.72, 133.59, 133.15, 131.73, 131.39, 131.23, 130.91, 130.24, 129.06, 128.73, 128.61, 128.59, 128.56, 71.20, 69.56; ^{31}P NMR (202 MHz, D_2O): δ = −6.57; ESI MS: m/z: calcd for $C_{22}H_{17}NO_3P-Na$: 397.08; found: 374.2 $[M-Na]^-$.

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[1] C. X. Zhang, S. J. Lippard, *Curr. Opin. Chem. Biol.* **2003**, *7*, 481–489.
[2] G. B. Dreyer, P. B. Dervan, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 968–972.
[3] D. E. Bergstrom, N. P. Gerry, *J. Am. Chem. Soc.* **1994**, *116*, 12067–12068.
[4] J. K. Bashkin, E. I. Frolova, U. Sampath, *J. Am. Chem. Soc.* **1994**, *116*, 5981–5982.
[5] K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shionoya, *Science* **2003**, *299*, 1212–1213.
[6] G. A. Burley, J. Gierlich, M. R. Mofid, H. Nir, S. Tal, Y. Eichen, T. Carell, *J. Am. Chem. Soc.* **2006**, *128*, 1398–1399.
[7] M. E. Wilson, G. M. Whitesides, *J. Am. Chem. Soc.* **1978**, *100*, 306–307.
[8] J. Collot, J. Gradinaru, N. Humbert, M. Skander, A. Zocchi, T. R. Ward, *J. Am. Chem. Soc.* **2003**, *125*, 9030–9031.
[9] C.-C. Lin, C.-W. Lin, A. S. C. Chan, *Tetrahedron: Asymmetry* **1999**, *10*, 1887–1893.
[10] B. Seelig, A. Jäschke, *Chem. Biol.* **1999**, *6*, 167–176.
[11] B. Seelig, S. Keiper, F. Stuhlmann, A. Jäschke, *Angew. Chem.* **2000**, *112*, 4764–4768; *Angew. Chem. Int. Ed.* **2000**, *39*, 4576–4579.
[12] F. Stuhlmann, A. Jäschke, *J. Am. Chem. Soc.* **2002**, *124*, 3238–3244.
[13] A. Serganov, S. Keiper, L. Malinina, V. Tereshko, E. Skripkin, C. Höbartner, A. Polonskaia, T. Phan Anh, R. Wombacher, R. Micura, Z. Dauter, A. Jäschke, D. J. Patel, *Nat. Struct. Mol. Biol.* **2005**, *12*, 218–224.
[14] G. Roelfes, B. L. Feringa, *Angew. Chem.* **2005**, *117*, 3294–3296; *Angew. Chem. Int. Ed.* **2005**, *44*, 3230–3232.
[15] G. Roelfes, A. J. Boersma, B. L. Feringa, *Chem. Commun.* **2006**, 635–637.
[16] J. Telser, K. A. Cruickshank, K. S. Schanze, T. L. Netzel, *J. Am. Chem. Soc.* **1989**, *111*, 7221–7226.
[17] T. Ihara, M. Nakayama, M. Murata, K. Nakano, M. Maeda, *Chem. Commun.* **1997**, 1609–1610.
[18] R. E. Holmlin, P. J. Dandliker, J. K. Barton, *Bioconjugate Chem.* **1999**, *10*, 1122–1130.
[19] I. Ortmans, S. Content, N. Boutonnet, A. Kirsch-De Mesmaeker, W. Bannwarth, J. F. Constant, E. Defrancq, J. Lhomme, *Chem. Eur. J.* **1999**, *5*, 2712–2721.
[20] S. I. Khan, A. E. Beilstein, M. Sykora, G. D. Smith, X. Hu, M. W. Grinstaff, *Inorg. Chem.* **1999**, *38*, 3922–3925.
[21] D. Ossipov, S. Gohil, J. Chattopadhyaya, *J. Am. Chem. Soc.* **2002**, *124*, 13416–13433.
[22] T. T. Williams, C. Dohno, E. D. A. Stemp, J. K. Barton, *J. Am. Chem. Soc.* **2004**, *126*, 8148–8158.
[23] S. I. Khan, M. W. Grinstaff, *J. Am. Chem. Soc.* **1999**, *121*, 4704–4705.
[24] J. J. Rack, E. S. Krider, T. J. Meade, *J. Am. Chem. Soc.* **2000**, *122*, 6287–6288.
[25] D. J. Hurley, Y. Tor, *J. Am. Chem. Soc.* **2002**, *124*, 3749–3762.
[26] E. Meggers, P. L. Holland, W. B. Tolman, F. E. Romesberg, P. G. Schultz, *J. Am. Chem. Soc.* **2000**, *122*, 10714–10715.
[27] H. Weizman, Y. Tor, *J. Am. Chem. Soc.* **2001**, *123*, 3375–3376.
[28] J. L. Czlapinski, T. L. Sheppard, *J. Am. Chem. Soc.* **2001**, *123*, 8618–8619.
[29] M. Shionoya, K. Tanaka, *Curr. Opin. Chem. Biol.* **2004**, *8*, 592–597.
[30] G. H. Clever, K. Polborn, T. Carell, *Angew. Chem.* **2005**, *117*, 7370–7374; *Angew. Chem. Int. Ed.* **2005**, *44*, 7204–7208.
[31] For a review on phosphanes applications in catalysis see: *Comprehensive Asymmetric Catalysis, Vol. 1–3* (Eds.: E. N. Jacobsen, A. Pfaltz, H. Yamamoto), Springer, Berlin, **1999**.
[32] T. Hayashi, *Acc. Chem. Res.* **2000**, *33*, 354–362.
[33] J. Telser, K. A. Cruickshank, L. E. Morrison, T. L. Netzel, *J. Am. Chem. Soc.* **1989**, *111*, 6966–6976.
[34] N. Haginoya, A. Ono, Y. Nomura, Y. Ueno, A. Matsuda, *Bioconjugate Chem.* **1997**, *8*, 271–280.
[35] K. G. Rajeev, V. R. Jadhav, K. N. Ganesh, *Nucleic Acids Res.* **1997**, *25*, 4187–4193.
[36] O. Gorchs, M. Hernandez, L. Garriga, E. Pedroso, A. Grandas, J. Farras, *Org. Lett.* **2002**, *4*, 1827–1830.
[37] R. Narukulla, D. E. G. Shuker, Y.-Z. Xu, *Nucleic Acids Res.* **2005**, *33*, 1767–1778.
[38] S. Jin, C. V. Miduturu, D. C. McKinney, S. K. Silverman, *J. Org. Chem.* **2005**, *70*, 4284–4299.
[39] A. M. MacMillan, G. L. Verdine, *J. Org. Chem.* **1990**, *55*, 5931–5933.
[40] Y. Z. Xu, Q. Zheng, P. F. Swann, *J. Org. Chem.* **1992**, *57*, 3839–3845.
[41] A. M. MacMillan, G. L. Verdine, *Tetrahedron* **1991**, *47*, 2603–2616.
[42] Liu and co-workers tethered the same phosphine derivative **2a** to a 3′-amino terminated DNA sequence and used the resulting conju-

gate in a DNA-templated Wittig olefination. See: Z. J. Gartner, M. W. Kanan, D. R. Liu, *J. Am. Chem. Soc.* **2002**, *124*, 10304–10306.
[43] While HPLC purification as described herein does not cause oxidation of the phosphine–DNA, sample preparation, collection and manipulation should be performed in oxygen free conditions.
[44] U. Nagel, E. Kinzel, *Chem. Ber.* **1986**, *119*, 3326–3343.
[45] Y. Otomaru, T. Senda, T. Hayashi, *Org. Lett.* **2004**, *6*, 3357–3359.
[46] G. D. Engel, L. H. Gade, *Chem. Eur. J.* **2002**, *8*, 4319–4329.
[47] T. Belser, M. Stoehr, A. Pfaltz, *J. Am. Chem. Soc.* **2005**, *127*, 8720–8731.
[48] Y. Ribourdouille, G. D. Engel, M. Richard-Plouet, L. H. Gade, *Chem. Commun.* **2003**, 1228–1229.
[49] R. Noyori, H. Takaya, *Acc. Chem. Res.* **1990**, *23*, 345–350.
[50] Y. Takaya, M. Ogasawara, T. Hayashi, M. Sakai, N. Miyaura, *J. Am. Chem. Soc.* **1998**, *120*, 5579–5580.
[51] G. Helmchen, A. Pfaltz, *Acc. Chem. Res.* **2000**, *33*, 336–345.
[52] O. Loiseleur, M. Hayashi, M. Keenan, N. Schmees, A. Pfaltz, *J. Organomet. Chem.* **1999**, *576*, 16–22.
[53] L. A. Gugliotti, D. L. Feldheim, B. E. Eaton, *Science* **2004**, *304*, 850–853.
[54] T. M. Tarasow, S. L. Tarasow, B. E. Eaton, *Nature* **1997**, *389*, 54–57.
[55] H. Hocke, Y. Uozumi, *Tetrahedron* **2003**, *59*, 619–630.
[56] R. Fiammengo, K. Musilek, A. Jäschke, *J. Am. Chem. Soc.* **2005**, *127*, 9271–9276.
[57] J. Hu, M. J. Miller, *J. Am. Chem. Soc.* **1997**, *119*, 3462–3468.
[58] Acidifying results in oxazoline ring opening. See: A. Laaziri, J. Uziel, S. Juge, *Tetrahedron: Asymmetry* **1998**, *9*, 437–447.

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