Phosphine containing oligonucleotides for the development of metallodeoxyribozymes†

Loïc Ropartz,[a] Nico J. Meeuwenoord,[c] Gijsbert A. van der Marel,[c] Piet W. N. M. van Leeuwen,[b] Alexandra M. Z. Slawin[a] and Paul C. J. Kamer*[a]

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Novel transition metal catalysts based on oligonucleotides can be easily obtained by functionalization of 5-iodouridine with phosphine ligands, resulting in good asymmetric induction in palladium catalyzed allylic nucleophilic substitution.

Catalysis plays a key role in chemical conversions by making them faster and more selective. The rates and selectivities of enzymatic catalysis are seldom equalled by man-made transition metal catalysts, which are responsible for the majority of production in the chemical industry. More and more attempts are being made to use the concepts of biology in order to obtain efficient catalysts that can compete with enzymes, such as incorporation of transition metal complexes in proteins[1] and antibodies.[2] The interest in DNA double-helix structures has nowadays shifted from its initial area of biological research to reach all domains of science.[3] Nucleic acids are at least as powerful in molecular recognition as peptides,[4,5] while their application in transition metal catalysis has hardly been explored.[6,7] Indeed, the application of oligonucleotides as catalysts[8] or as scaffolds for transition metal catalysts[6,9] is an emerging field of research. A great advantage of oligonucleotides over proteins is that the secondary structure can be easily engineered, resulting in the design of countless possibilities of 3-dimensional structures[10] and conformations of DNA.[11]

Our approach for the development of highly functionalized late transition metal catalysts that are capable of selective molecular recognition is the introduction of suitable phosphine ligands into oligonucleotides. This enables the development of transition metal deoxyribozymes with well-defined secondary structures based on Watson–Crick base pairing. These DNA based metal complexes can provide a new class of highly selective catalyst for demanding transformations. The strong molecular recognition power of oligonucleotides, combined with the catalytic properties of transition metal phosphine complexes, enables catalytic reactions for which no enzymes or ribozymes[12] are known. The high substrate specificity and affinity of aptamers[5] could allow conversion of a single substrate present, even at extremely low concentrations, in complex mixtures, like those in biological systems.

[a]*School of Chemistry, University of St. Andrews, St. Andrews, Fife, UK KY16 9ST. E-mail: pcjk@st-andrews.ac.uk; Fax: +44 (0)1334463808; Tel: +44 (0)1334467285*
[b]*Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands*
[c]*Leiden Institute of Chemistry, Gorlaeus Laboratories, University of Leiden, Einsteinweg 55, 2333 CC, Leiden, The Netherlands*
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Obviously, the phosphine ligand needs to be introduced after DNA synthesis to avoid exposure to the oxidation step. Liu *et al.* developed a method for introduction of phosphine at the 3′ and 5′ ends.[13] We decided to use (+)-5-iodo-2′-deoxyuridine (IdU) as an intermediate to enable phosphine introduction at any desired position. DNA is the biomolecule of choice because it can be synthesized more easily than RNA and it has much better hydrolytic stability.

Palladium catalyzed coupling of diphenylphosphine to the commercially available IdU leads to the desired functionalized nucleoside compound (dppdU, Fig. 1) in high yield (>95%) (Scheme 1). A catalytic amount (1–3%) of $Pd(OAc)_2$ and a slight excess of diphenylphosphine are used to obtain fast and complete conversion of IdU. The 5-iodo-3′,5′-di-*O*-acetyl-2′-deoxyuridine (AcIdU) is also easily converted to the corresponding AcdppdU. This compound is surprisingly more prone to oxidation than its counterpart dppdU and must be purified under anaerobic conditions.[14]

Although the reaction proceeds smoothly for the 5-iodo-2′-deoxyuridine, higher palladium loadings (up to stoichiometric amount) are required for the conversion of the supported oligonucleotides dAIdUdT and dCIdUdG into the corresponding dAdppdUdT and dCdppdUdG. Adsorption of the metal on the CPG support (controlled pore glass), poor mass transport and Pd coordination to the base pairs are believed to be responsible for the poor efficiency of the catalytic coupling. Indeed, faster conversion is obtained for polystyrene supported oligonucleotides although a substantial amount of palladium catalyst is still indispensable.

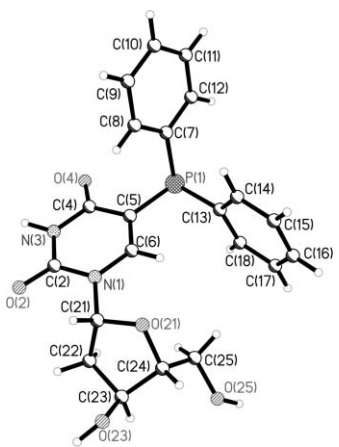
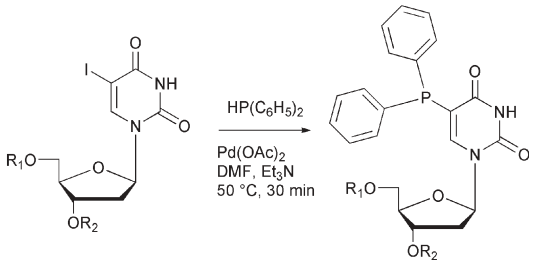


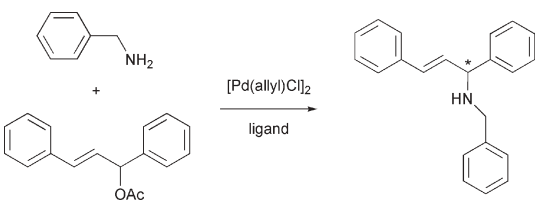
Fig. 1 Crystal structure of dppdU.



Scheme 1 Synthesis of 5-diphenylphosphine-2′-deoxyuridine. $R^1 = R^2 =$ H (dppdU); $R^1 = R^2 =$ Ac (AcdppdU); R^1 = deoxyadenosine-3-phosphate, R^2 = deoxythymidine-5-phosphate (trinucleotide dAdppdUdT).

The diphenylphosphine-containing trimers are deprotected and released from the solid support by treatment with a NH_4OH–methylamine aqueous solution for 2 h at 80 °C under an argon atmosphere. Subsequently, traces of palladium are removed by washing with acetonitrile and 1,2-bis(diphenylphosphino)ethane (dppe) in CH_2Cl_2 to prevent oxidation of the phosphine catalyzed by the metal in water. Finally the phosphine-modified oligonucleotides are purified by FPLC under aerobic conditions to provide the products in moderate yields (>40%).

The structure of dppdU (Fig. 1) confirms the successful synthesis.‡ The determined structure is chiral and shows complete conservation of the stereointegrity of the nucleoside. The P–C bond lengths are normal and the C–P–C bond angles are, as expected, approximately tetrahedral. In the crystal, O(2) is hydrogen bonded to both OH groups to give a layer structure; the NH group also H-bonds to O(25). There are channels containing the included [partial weight] water, which forms short contacts to both of the OH groups.



Scheme 2 Asymmetric allylic amination: addition of benzylamine to 1,3-diphenyl-2-propenyl acetate.

The major challenges in the development of hybrid catalysts based on oligonucleotides and transition metal complexes are in the field of important reactions like (asymmetric) catalytic carbon–carbon and carbon–heteroatom bond forming reactions. Since the most powerful catalysts for these reactions, rhodium and palladium phosphine complexes, have not been used in nucleic acid based systems, we have tested the (oligo)nucleotide based phosphine ligand systems in palladium catalyzed allylic substitution as a model reaction. Good enantiomeric excess is obtained (up to 82% for the *S* enantiomer) in asymmetric palladium catalyzed allylic amination with the monodentate ligand dppdU (Scheme 2, Tables 1 and 2). Such chiral induction is startling as the chiral centers on the molecule are situated far away from the coordinating phosphine and thus from the catalytic metal center (see Fig. 1).

Another remarkable result is the solvent effect on the reaction as it reverses the stereoselectivity of the product. Whilst the reaction in tetrahydrofuran (THF) gives 80% of the *S* enantiomer, we obtain 16% ee of the *R* form in acetonitrile–THF (Table 1). The enantioselectivity using AcdppdU is also dependent on the reaction conditions as the opposite enantiomers are obtained when switching from THF (*S* form) to dichloromethane (DCM) (*R* form). The stereoselectivity is, however, highly reduced (respectively 8% and 23%). The 5′-hydroxy moiety seems, therefore, to play an important role in the stereoselectivity of the catalytic species. Hydrogen bonding interactions, as observed in the solid-state structure, are probably influencing the structure in solution also. Importantly, good conversions are also obtained with the trinucleotides dAdppdUdT and dCdppdUdG in the allylic amination reactions, albeit at the expense of the enantioselectivity. This is a good model reaction for the use of large DNA sequences as ligands, showing that the approach of molecular recognition by DNA aptamers[5] can be used for this palladium catalyzed reaction and also demonstrating that the reaction still proceeds in an aqueous environment. Other nucleophiles like *N*-methylbenzylamine and dimethyl malonate result in good conversions with dppdU as the ligand in the allylic substitution reaction (see Table 2).

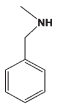
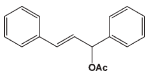
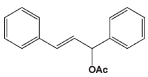
The NMR studies of dppdU with palladium and platinum complexes confirm the importance of the interaction between the nucleoside and the solvent. Platinum complexes were studied to distinguish between *cis* and *trans* coordination modes. Reaction of platinum(II) $Cl_2Pt(CH_3CN)_2$ with dppdU in THF–$CDCl_3$ leads to

Table 1 Palladium catalyzed allylic substitution of 1,3-diphenyl-2-propenyl acetate with benzylamine

Ligand (L)	Solvent	L : Pd ratio	Incubation time/h	Conversion (%)	ee (%)	Absolute configuration
dppdU[a]	THF	0.5	0.5–4	>99	62	*S*
dppdU[a]	THF	2	2	>99	72	*S*
dppdU[a]	THF	2	4–16	>99	80	*S*
dppdU[a]	THF	4	4	>99	79	*S*
dppdU[a]	CH_3CN–THF	2	4	>99	16	*R*
dppdU[a]	DMF	2	4	>99	14	*R*
AcdppdU[a]	THF	2	2	>99	8	*S*
AcdppdU[a]	DCM	2	2	>99	21	*R*
AcdppdU[a]	DCM	2	2	>99	23	*R*
dAdppdUdT[b]	THF–H_2O 3 : 1	4	8–24	83	8–12	*R*
dAdppdUdT[b]	H_2O	4	24	15	—	—
dCdppdUdG[b]	THF–H_2O 3 : 1	4	24	30	5	*R*

[a] Reaction conditions: 7.5 μmol $[Pd(allyl)Cl]_2$, benzylamine : propenyl acetate : Pd = 120 : 40 : 1, solvent 2 cm^3, 25 °C, 24 h. [b] Reaction conditions: 0.5 μmol $[Pd(allyl)Cl]_2$, benzylamine : propenyl acetate : Pd : K_2CO_3 = 120 : 40 : 1 : 120, solvent 1 cm^3, 25 °C, 36 h.

Table 2 Asymmetric allylic substitution using dppdU as ligand

Nucleophile	Allyl	Solvent	Conversion (%)	ee (%)	Absolute configuration
		THF	>99[a]	82	*S*
		THF	>99[a]	70	*S*
		THF	>99[b]	15	*S*
		THF	>99[b]	12	*S*

[a] Reaction conditions: 7.5 μmol $[Pd(allyl)Cl]_2$, amine : allyl acetate : Pd : L = 120 : 40 : 1 : 2, solvent 2 cm^3, 25 °C, 24 h. [b] Reaction conditions: 7.5 μmol $[Pd(allyl)Cl]_2$, nucleophile : BSA (*N*,*O*-bis-(trimethylsilyl)acetamide) : allyl acetate : Pd : L = 120 : 120 : 40 : 1 : 2, KOAc 1 mg, solvent 2 cm^3, 25 °C, 2 h.

the formation of $Cl_2Pt(dppdU)_2$ in a *cis* conformation, showing a singlet in the ^{31}P NMR spectrum at δ = 15.7 ppm with large platinum–phosphine coupling (satellite J_{P-Pt} = 5344 Hz). When the same complex is formed in acetonitrile-d_3–methanol (2 drops added for solubility), the ^{31}P NMR spectrum shows a signal at δ = 14.2 ppm with a considerably smaller platinum–phosphine coupling (satellite J_{P-Pt} = 2660 Hz), indicating a change to a *trans* conformation. Also, a single complex is obtained when dppdU is reacted with MeClPd(COD), showing a singlet in the ^{31}P NMR spectrum (δ = 22.3 ppm, DMSO-d_6) characteristic of a *trans* complex.

In summary, we report the Pd catalyzed synthesis of nucleosides and oligonucleotides functionalized with phosphine moieties and their use as ligands for asymmetric catalytic substitution reactions. Secondary interactions (such as hydrogen bonding) are believed to rule the transfer of chirality from the distant sugar to the phosphine as both the solvent and the substituent of the ribose modify the stereoselectivity of the reaction. Importantly, the catalytic reaction does proceed in water as solvent, which allows the use of longer DNA sequences. This opens an efficient route to introduce a phosphine ligand at any position of oligonucleotides, adding an important new possibility for the application of DNA aptamers in the field of transition metal catalysis.

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Notes and references

‡ Crystallographic data for dppdU: empirical formula $C_{21}H_{21}N_2O_5P\cdot(H_2O)_{0.25}$, formula weight = 416.87, temperature = 93(2) K, wavelength = 0.71073 Å, monoclinic, space group *C*2, a = 18.165(4) Å, b = 5.1771(9) Å, c = 22.063(5) Å, β = 108.12(3)°, V = 1972.0(7) $Å^3$, Z = 4, μ = 0.177 mm^{-1}, D_c = 1.404 Mg m^{-3}, crystal size 0.2 × 0.1 × 0.01 mm, reflections collected 6287, independent reflections 3282 [R_{int} = 0.03]. Refinement method full-matrix least-squares on F^2, data/restraints/parameters 3282/4/278, goodness-of-fit on F^2 = 1.110, $R1$ [$I > 2\sigma(I)$] = 0.0527, w$R2$ = 0.1352, absolute structure parameter = 0.02(14). CheckCIF does not reveal any serious issues. CCDC 626863. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b617871e

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14 The alternative routes, protection of the dppdU phosphoramidite compound by BH_3 during DNA synthesis or deprotection of the oligonucleotides from the support followed by a palladium catalyzed P–C coupling reaction in an aqueous medium, failed to give the expected product.

