

Letter

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ACS Catal., Just Accepted Manuscript • Publication Date (Web): 10 Jul 2015

Downloaded from http://pubs.acs.org on July 11, 2015

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Development of DNA Metalloenzymes Using a Rational Design Approach and Application in the Asymmetric Diels–Alder Reaction

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Received Date: (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

Abstract

We report here DNA metalloenzymes that catalyzed the asymmetric Diels–Alder reactions with high conversion, excellent *endo/exo* selectivities, and enantioselectivities up to –97% *ee.* Their catalytic-pocket architectures were organized using a rational design strategy based on the Cu(II) ion, the composition of nucleobases, and the incorporation of flexible linkers. Without using the mirror image of B-DNA, DNA metalloenzymes afforded the opposite enantiomer of the Diels–Alder product compared with those obtained using a supramolecular Cu(II)–dmbpy/st–DNA catalyst system. Furthermore, we devised DNA metalloenzymes without the incorporation of an artificial binding ligand and successfully performed Diels–Alder carbon–carbon bond-forming reactions. This study provides a new perspective on the catalytic repertoire of nucleic acids in the realm of protein-dominated metalloenzymes.

KEYWORDS

DNA, Metalloenzyme, asymmetric catalysis, Diels-Alder reaction, Cu(II) ion, ligand freedom, enantiomeric preference

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In nature, a variety of metalloenzymes catalyze biologically essential chemical reactions under mild reaction conditions with high efficiency, definitive substrate specificity, and excellent enantioselectivity, beyond the performance of synthetic catalysts. Taking inspiration from the superior characteristics of metalloenzymes, the development of artificial metalloenzymes that combine metal-assisted catalysis with a chiral biomacromolecule has received attention as an attractive research concept for developing environmentally friendly and efficient catalysts for the synthesis of enantiomerically pure compounds.¹ Among the biomolecules that are used as the chiral scaffold, DNA is a notable chiral source because of its many advantageous properties, such as its unique helical chirality and chemical stability, and the diverse three-dimensional structures created by the simple four-letter alphabet. In 2005, Feringa and Roelfes developed the novel concept of DNA-based asymmetric catalysis with a metal complex, DNA, and a binding ligand, and reported Diels-Alder reactions.² Since then, a series of DNA metalloenzymes have been developed and applied successfully to the important carbon–carbon or carbon–heteroatom bond-forming reactions, such as the Michael additions, Friedel-Crafts alkylations, syn-hydrations, and fluorination.^{3,4} Regarding the structural diversity of the DNA, G-quadruplex DNA metalloenzymes have been developed and investigated based on the relationship between the conformation of the quadruplex DNA and the catalytic reaction.⁵ In addition, remarkable approaches have been reported to control the enantiomeric preference of the DNA-based asymmetric catalysis. Roelfes and coworkers reported that the enantioselectivity of the products in the Diels-Alder and Friedel-Crafts alkylation reactions could be controlled by changing the denticity of the ligand coordinated to the Cu(II) ion.⁶ Smietana and Arsenivadis demonstrated that the left-handed DNA-based catalyst made from L-nucleic acids gave rise to the opposite enantiomeric outcome in the Cu(II)-catalyzed Friedel-Crafts alkylation and Michael addition reactions compared with the natural right-handed DNA.⁷

Very recently, we devised artificial DNA metalloenzymes with intrastrand bipyridine ligands via direct incorporation into the phosphate backbone, and performed asymmetric intramolecular Friedel–Crafts alkylation to extend our understanding of DNA-based asymmetric catalysis.⁸ In a previous study, we identified the critical factors that affect the reactivity and enantioselectivity of DNA-based asymmetric catalysis,

such as the counter base of metal complexes, neighboring bases in the active site, and disposition of the active site. Based on these observations, we established a systematic strategy for the development of DNA metalloenzymes via the rational creation of an active site. Herein, we report the development and application of copper-containing DNA metalloenzymes for asymmetric Diels–Alder reactions.

In our previous study, we found that cytosine exhibited distinct features compared with other bases (A, T, and G) in the determination of the catalytic capability of the Cu(II)-bipyridine–DNA conjugate catalyst. For instance, the DNA-based hybrid catalysts produced by the combination of an intrastrand bipyridine ligand and cytosine as a counter base afforded the best result regarding the cyclized product, with a yield of 71% and enantioselectivity of 84% *ee* in intramolecular Friedel–Crafts alkylations.⁸ Therefore, we have focused on cytosine at the active site for the creation of the coordination environment, and designed a DNA metalloenzyme via the direct incorporation of flexible linkers to control the size of the catalytic site. In this regard, we introduced a triethylene glycol linker into the DNA phosphate backbone and generated a variety of Cu(II)–DNA–triethylene glycol conjugates.⁹

Oligo(ethylene glycol)s are used widely as linkers or spacers because they are inexpensive, water soluble, and commercially available in a broad range of molecular-weight distributions. The phosphoramidites of triethylene glycol linkers were readily synthesized by phosphitylation and incorporated into the DNA oligonucleotides by automated solid-phase synthesis (see SI, Scheme S1). We generated DNA metalloenzymes based on 13-mer DNA oligonucleotides, 5'–d(GCATGXEYACGGT)– 3'/5'–d(ACCGTXCYCATGC)–3', where $\mathbf{E} =$ a triethylene glycol linker.¹⁰ The asymmetric Diels–Alder reaction between aza-chalcone **1a** and cyclopentadiene **2** was performed to prove the catalytic activity of Cu(II)–DNA–triethylene glycol conjugates. The results are summarized in Table 1. Under the reaction conditions (20 mM MOPS buffer at pH 6.5, 3 mol% of Cu(NO₃)₂, and 4 mol% of DNA), the present Cu(II)–DNA– ethylene glycol conjugates afforded the Diels–Alder adduct with significant conversion, *endo/exo* selectivity, and a promising enantioselectivity (entry 1 in Table 1). We examined the neighboring base pairs of the cytosine and the triethylene glycol linker. As

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shown in entries 1–6 in Table 1, we found that the enantioselectivities were influenced greatly by the neighboring base pairs of the cytosine and the triethylene glycol linker. For example, the DNA metalloenzyme comprising **ODN3/ODN12** (-CEC-/-GCG-) and the Cu(II) ion gave the product with -13% *ee*, whereas the DNA metalloenzyme comprising **ODN5/ODN14** (-TET-/-ACA-) and the Cu(II) ion had a markedly increased enantioselectivity, up to -81%.

To our great delight, the DNA metalloenzyme generated by the combination of cytosine and the neighboring base pair –**AET**– afforded the Diels–Alder adduct with high conversion (99%) and excellent enantioselectivity (–94% *ee*), together with very high *endo/exo* selectivity (entry 6 in Table 1). Subsequently, we replaced the triethylene glycol linker with more hydrophobic alkyl linkers. After considering the alkyl chain length, octyl and propyl linkers, such as **ODN7** and **ODN8**, were introduced into the DNA strand and investigated. As shown in entries 7 and 8, the DNA metalloenzymes that included alkyl linkers also led the completion of Diels–Alder reactions successfully and gave the desired products with high conversions and excellent *endo/exo* selectivities.

When we performed the Diels–Alder reaction using a DNA metalloenzyme with a propyl linker, the corresponding product was obtained up to -97% *ee*. The control experiment using **ODN9/ODN15** in the presence of Cu(II) ions resulted in low selectivities, which suggests that the security of the space via the introduction of ethylene glycol or alkyl linkers is important for affording high enantioselectivity (entry 9 in Table 1). When the reaction was performed using a single DNA–ethylene glycol conjugate strand, **ODN6**, and Cu(II) ions, very low enantioselectivity was obtained (see entry 10 in Table 1). The results of this experiment indicate that the double-helical structure of the Cu(II)–DNA–triethylene glycol conjugates is important for inducing enantioselectivity in the asymmetric Diels–Alder reaction.

Table 1. Asymmetric Diels–Alder reaction catalyzed by DNA metalloenzymes containing Cu(II) and a triethylene glycol (alkyl) linker.



entry ^a	DNA sequences	ee (%)	endo/exo	conversion (%)
1	5'-GCATG GEC ACGGT-3' (ODN1) 3'-CGTAC CG TGCCA-5' (ODN10)	-36	14/1	99
2	5'-GCATG EG ACGGT-3' (ODN2) 3'-CGTAC CC TGCCA-5' (ODN11)	-41	10/1	57
3	5'-GCATG <mark>CEC</mark> ACGGT-3' (ODN3) 3'-CGTAC <mark>GCG</mark> TGCCA-5' (ODN12)	-13	16/1	75
4	5'-GCATG AEA ACGGT-3' (ODN4) 3'-CGTAC TCT TGCCA-5' (ODN13)	-58	22/1	70
5	5'-GCATG TET ACGGT-3' (ODN5) 3'-CGTAC ACA TGCCA-5' (ODN14)	-81	20/1	99
6 ^{<i>b</i>}	5'-GCATG AET ACGGT-3' (ODN6) 3'-CGTAC TCA TGCCA-5' (ODN15)	-94	>40/1	99
7 ^b	5'-GCATG A[®]KT ACGGT-3' (ODN7) 3'-CGTAC T ^CA TGCCA-5' (ODN15)	-87	36/1	95
8 ^b	5'-GCATG A³KT ACGGT-3' (ODN8) 3'-CGTAC T ^CA TGCCA-5' (ODN15)	-97	>40/1	98
9	5'-GCATG AGT ACGGT-3' (ODN9) 3'-CGTAC TCA TGCCA-5' (ODN15)	-13	8/1	82
10	5'-GCATG AET ACGGT-3' (ODN6)	-10	5/1	87

^a Experiments were carried out using 3.3 mM aza-chalcone, 80 mM cyclopentadiene,

0.13 mM DNA, and 0.1 mM Cu(NO₃)₂ at 5 °C in 20 mM MOPS buffer (pH 6.5) for 3 days. The conversion and enantioselectivities were determined by chiral HPLC analysis. ^b Results represent the average value of more than two experiments. The enantioselectivities were reproducible within \pm 5%. The yields were reproducible within \pm 10%.

The enantioselectivity of the Diels–Alder products was determined by chiral HPLC based on the literature.^{2,4j,6} Interestingly, we found that the present DNA metalloenzyme afforded the opposite enantiomeric outcome compared with the previously reported Cu(II)–dmbpy/st–DNA catalytic system.^{4a,13} In addition, the circular dichroism spectra of the DNA duplex formed by **ODN6/ODN15** or **ODN8/ODN15** in the presence of the Cu(II) complex indicated a typical *right-handed* B-DNA structure (see SI). This result clearly demonstrates that the enantiomeric preference of the reaction could be changed readily via the introduction of simple linear linkers, such as an ethylene glycol linker and an alkyl chain linker, to the DNA phosphate backbone; i.e., the present study suggests a new alternative: that the enantioselectivity could be reversed completely by perturbing only the catalytic site in the DNA duplex, without an overall change in helical chirality or in the employment of special ligands.^{6,7}

To confirm the catalytic ability of the present DNA metalloenzymes for asymmetric Diels–Alder reactions, the scope of dienophiles with various functional groups was investigated under optimized reaction conditions (Table 2). Using a Cu(II)–DNA–triethylene glycol conjugate, dienophiles containing the substituted phenyl group with electron-withdrawing or -donating substituents gave the corresponding product in almost full conversion with high enantioselectivity (entries 1–3 in Table 2). In the case of a dienophile including a *p*-nitro group on the phenyl ring, a Cu(II)–DNA–propyl linker conjugate was used for the reaction, to avoid the electrostatic repulsion between the nitro group and the triethylene glycol linker.¹¹ Instead of 2-acyl pyridyl, the use of the 2-acyl imidazole group also led to a high product enantioselectivity (–88% *ee*, entry 5 in Table 2). In all cases examined, DNA metalloenzymes provided the opposite enantiomers of the Diels–Alder product compared with those obtained using a supramolecular Cu(II)–dmbpy/st–DNA catalyst system.

Table 2. Substrate scope of the asymmetric Diels-Alder reaction by DNA metalloenzymes.



3b-f (*endo*) **3b**-f (*exo*)

1b: R^1 = 2-pyridyl, R^2 = *p*-MeOC₆H₄ **1c**: R^1 = 2-pyridyl, R^2 = *o*-ClC₆H₄ **1d**: R^1 = 2-pyridyl, R^2 = *p*-BrC₆H₄ **1e**: R^1 = 2-pyridyl, R^2 = *p*-NO₂C₆H₄ **1f**: R^1 = 2-(1-methylimidazolyl), R^2 = C₆H₅

1b-f

entry ^a	substrate	ee (%)	endo/exo	conversion (%)
1	1b	-92	>40/1	99
2	1c	-97	>40/1	99
3	1d	-86	25/1	99
4^b	1e	-96	>40/1	99
5	1f	-88	21/1	99
4 ^b 5	1e 1f	-96 -88	>40/1 21/1	99 99

^a Experiments were carried out using 3.3 mM aza-chalcone, 80 mM cyclopentadiene, 0.13 mM DNA (**ODN6/ODN15**), and 0.1 mM Cu(NO₃)₂ at 5 °C in 20 mM MOPS buffer (pH 6.5) for 3 days. The conversion and enantioselectivities were determined by chiral HPLC analysis. ^b **ODN8/ODN15** was used in this experiment.

The results observed here clearly indicate that DNA metalloenzymes could perform the asymmetric Diels–Alder reaction successfully, even if they had no binding ligand for Cu(II) ions, such as bipyridine or phenanthroline. Therefore, we devised DNA metalloenzymes comprising three oligonucleotides and Cu(II) ions. To generate ample space for the active site, we excluded the counter base for cytosine. The template oligonucleotide strand (15-mer) with cytosine in the center was hybridized with two kinds of complementary oligonucleotides (7-mers) in the presence of Cu(II) ions, and gave rise to DNA metalloenzymes. The catalytic ability of these DNA metalloenzymes was investigated in the asymmetric Diels–Alder reaction. Surprisingly, as shown in Table 3, the designed DNA metalloenzymes afforded the Diels–Alder adducts in almost

full conversion with very high enantioselectivities. Regarding the neighboring base pairs of the cytosine, similar to Cu(II)–DNA–ethylene glycol (alkyl linker) conjugates, a neighboring A–T pair gave higher enantioselectivity (–64% *ee*, entry 1 in Table 3) than did a C–G pair (–17% *ee*, entry 2 in Table 3) in the present reaction. Considering the proximity to cytosine, the presence of a 3'- or 5'-end phosphate group on the short complementary oligonucleotides increased the enantioselectivity of the Diels–Alder product. As shown in entry 5 in Table 3, a DNA metalloenzyme containing 5'- and 3'-end phosphorylated complementary oligonucleotides afforded the Diels–Alder product with –83% *ee*. Furthermore, DNA metalloenzyme containing an abasic site opposite the counter base cytosine exhibited high enantioselectivity up to –96% (entry 6 in Table 3).

Li and colleagues reported enantioselective Friedel-Crafts alkylations using a human telomeric G4DNA metalloenzyme that was assembled using G4DNA and Cu(II) ions without additional ligands (up to 75% ee).^{5c} In this study, we demonstrated clearly that DNA metalloenzymes comprising a native DNA duplex and Cu(II) ions without any artificial binding ligand can function as the asymmetric catalyst for the Diels-Alder reaction to give almost full conversion, high enantioselectivity, and an excellent endo/exo ratio. At this stage the exact location of the Cu(II) ion remained distinctly unproven, however, earlier studies have reported Cu(II)-cytosine (or cytidine) binding mode based on N(3) preference.¹² Also, the examined DNA metalloenzyme libraries suggest that the combination of cytosine and copper plays an important role in the creation of the catalytic pocket to induce high enantioselectivity in the Diels-Alder reaction (Figure S2 and S3 in SI). Considering the correlation between enantioselectivity and the structure of DNA metalloenzymes, the presence of triethylene glycol (alkyl) linkers or phosphate groups on the complementary strand corresponding to the template strand containing cytosine might provide the steric conformation to induce high enantioselectivity of the product (Figure S8). Further studies are under way to explore the present DNA metalloenzymes in greater detail.

Table 3. Investigation of DNA metalloenzymes for the asymmetric Diels–Alder reaction.

N N	+ DNA (4 mol%), Cu(NO ₃) ₂ (3 mol%) MOPS buffer, 5 °C, 1 day		+	
	DNA sequences		endo/exo	conversion
entry ^a	(short strands 3' to 5'/template 5' to 3')	ee (%)		(%)
1	GTGGCAC GGTACGA CACCGTG C CCATGCT	-20	15/1	75
2^b	GTGGCAT AGTACGA CACCGTA C TCATGCT	-64	25/1	98
3 ^{<i>b,c</i>}	GTGGCAT ^P AGTACGA CACCGTA C TCATGCT	-74	33/1	95
4 ^{<i>b,c</i>}	GTGGCAT ^P AGTACGA CACCGTA C TCATGCT	-80	29/1	96
5 ^{<i>b,c</i>}	GTGGCAT ^{P P} AGTACGA CACCGTA C TCATGCT	-83	29/1	99
6 ^{<i>b,d</i>}	GTGGCAT F AGTACGA CACCGTA C TCATGCT	-96	32/1	99
7	CACCGTA C TCATGCT	-6	14/1	91

^a Experiments were carried out using 3.3 mM aza-chalcone, 80 mM cyclopentadiene, 0.13 mM DNA, and 0.1 mM Cu(NO₃)₂ at 5 °C in 20 mM MOPS buffer (pH 6.5) for 1 day. The conversion and enantioselectivities were determined by chiral HPLC analysis. ^b Results represent the average value of more than two experiments. The enantioselectivities were reproducible within \pm 5%. The yields were reproducible within \pm 10%. ^c The T^p means that the T at the 5' position contains a phosphate group. The ^pA means that the A at the 3' position contains a phosphate group. ^d5'-*O*-Dimethoxytrityl-1',2'-dideoxyribose-3'-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite was used as a dSpacer (**F**). In conclusion, we developed Cu(II)-containing DNA metalloenzymes via the direct introduction of flexible linkers and successfully applied them in the Diels–Alder carbon-carbon bond-forming reactions. The devised DNA metalloenzymes afforded Diels–Alder products with high conversion, excellent *endo/exo* selectivities, and enantioselectivities up to –97% *ee.* In addition, we established a systematic strategy for the development of *ligand-free* DNA metalloenzymes via the construction of an active site. In prebiotic world, the role of DNA has been out of the spotlight due to the lack of catalytic ability, however our finding is provocative of curiosity; DNA with a dangling base may function as a catalyst through metal binding. Further investigations of the application of DNA metalloenzymes are currently ongoing in our laboratory.

ASSOCIATED CONTENT

Supporting Information

Synthetic routes of triethylene glycol and alkyl linkers, characterization data of new compounds, HPLC data, ESI-TOF-Mass data and CD spectra of oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

Note

The authors declare no competing financial interest.

ACKNOWLEDGMENT

We express sincere thanks for a Grant-in-Aid Priority Research from Japan Society for the Promotion of Science (JSPS) and the grant from the WPI program (iCeMS, Kyoto University).

REFERENCES

[1] For reviews, see: a) B. G. Davis, *Curr. Opin. Biotechnol.* 2003, *14*, 379–386; b) C.
M. Thomas, T. R. Ward, *Chem. Soc. Rev.* 2005, *34*, 337–346; c) R. Krämer, *Angew. Chem. Int. Ed.* 2006, *45*, 858–860; d) Y. Lu, *Angew. Chem. Int. Ed.* 2006, *45*, 5588–5601; e) C. Letondor, T. R. Ward, *ChemBioChem* 2006, *7*, 1845–1852; f) M. D.
Mihovilovic, *J. Chem. Technol. Biotechnol.* 2007, *82*, 1067–1071.

[2] G. Roelfes, B. L. Feringa. Angew. Chem. Int. Ed. 2005, 44, 3230-3232.

[3] For reviews, see: a) G. Roelfes. *Mol. BioSyst.* 2007, *3*, 126–135; b) A. J. Boersma,
R. P. Megens, B. L. Feringa, G. Roelfes. *Chem. Soc. Rev*, 2010, *39*, 2083–2092; c) S. K.
Silverman. *Angew. Chem. Int. Ed.* 2010, *49*, 7180–7201; d) S. Park, H. Sugiyama. *Angew. Chem. Int. Ed.* 2010, *49*, 3870–3878; e) S. Park, H. Sugiyama. *Molecules* 2012, *17*, 12792–12803.

[4] a) G. Roelfes, A. J. Boersma, B. L. Feringa. *Chem. Commun.* 2006, 635–637; b) D.
Coquière, B. L. Feringa, G. Roelfes. *Angew. Chem. Int. Ed.* 2007, *46*, 9308–9311; c) A.
J. Boersma, B. L. Feringa, G. Roelfes. *Org. Lett.* 2007, *9*, 3647–3650; d) N. Shibata, H.
Yasui, S. Nakamura, T. Toru. T. *Syn. Lett.* 2007, 1153–1157; e) A. J. Boersma, B. L.

Feringa, G. Roelfes. Angew. Chem. Int. Ed. 2009, 48, 3346–3348; f) P. Fournier, R.
Fiammengo, A. Jäschke, Angew. Chem. Int. Ed. 2009, 48, 4426–4429; g) A. J.
Boersma, D. Coquière, D. Greerdink, F. Rosati, B. L. Feringa, G. Roelfes. Nature Chem. 2010, 2, 991–995; h) S. Park, K. Ikehata, R. Watabe, Y. Hidaka, A. Rajendran,
H. Sugiyama. Chem. Commun. 2012, 48, 10398–10400. i) J. Oelerich, G. Roelfes. Chem. Sci. 2013, 4, 2013–2017; j) S. Park, K. Ikehata, H. Sugiyama. Biomaterials Science 2013, 1, 1034–1036; k) G. P. Petrova, Z. Ke, S. Park, H. Sugiyama, K.
Morokuma. Chem. Phys. Lett. 2014, 600, 87–95; l) E. Benedetti, N. Duchemin, L.
Bethge, S. Vonhoff, A. Klussmann, J.-J. Vasseur, J. Cossy, M. Smietana, S.
Arseniyadis. Chem. Commun. 2015, 51, 6076–6079.

[5] a) S. Roe, D. J. Ritson, T. Garner, M. Searle, J. E. Moses. *Chem. Commun.* 2010, 46, 4309–4311; b) C. Wang, Y. Li, G. Jia, Y. Liu, S. Lu, C. Li. *Chem. Commun.* 2012, 48, 6232–6234; c) C. Wang, G. Jia, J. Zhou, Y. Li, Y. Liu, S. Lu, C. Li. *Angew. Chem. Int. Ed.* 2012, *51*, 9352–9355; d) M. Wilking, U. Hennecke. *Org. Biomol. Chem.* 2013, 11, 6940–6945.

[6] A. J. Boersma, B. de Bruin, B. L. Feringa, G. Roelfes. *Chem. Commun.* 2012, 48, 2394–2396.

[7] J. Wang, E. Benedetti, L. Bethge, S. Vonhoff, A. Klussmann, J.-J. Vasseur, J. Cossy, M. Smietana, S. Arseniyadis. *Angew. Chem. Int. Ed.* 2013, *52*, 11546–11549.

[8] S. Park, L. Zheng, S. Kumakiri, S. Sakashita, H. Otomo, K. Ikehata, H. Sugiyama. ACS Catalysis 2014, 4, 4070–4073.

[9] a) U. Jakobsen, K. Rohr, S. Vogel. *Nucleosides, Nucleotides, and Nucleic Acids,*2007, 26, 1419–1422; b) N. S. Oltra, G. Roelfes. *Chem. Commun.* 2008, 6039–6041; c)
L. Gjonaj, G. Roelfes. *ChemCatChem*, 2013, 5, 1718–1721.

[10] Complementary strands of **ODN6** containing natural bases (A, T, and G) were also examined to investigate the counter base effect. The DNA metalloenzymes by the combination of triethylene glycol and a counter base A, T, or G afforded lower enantioselectivities of product compared with a counter base C (entries 1-3 in Table S2).

[11] In the case of dienophile including a *p*-nitro group on the phenyl ring, a decrease in enantioselectivity was observed using a Cu(II)–DNA–triethylene glycol conjugate (99% conversion, *endo/exo* = 18/1, -59% *ee*).

[12] a) D. Tran Qui, E, Palacios. Acta Cryst. 1990, C46, 1220–1223. b) I.
Samasundaram, M. K. Kommiya, M. Palaniandavar. J. Chem. Soc., Dalton Trans. 1991, 2083–2089; c) A. García-Raso, J. J. Fiol, B. Adrover, V. Moreno, E. Molins, I. Mata. J. Chem. Soc., Dalton Trans. 1998, 1031–1036; d) A. García-Raso, J. J. Fiol, B. Adrover, A. Caubet, E. Espinosa, I. Mata. E. Molins, Polyhedron 2002, 21, 1197–1201; e) K.-H. Cho, S.-W. Joo. Bull. Korean Chem. Soc. 2008, 29, 69–75; f) D. Krilov, V, Gomzi, J. N. Herak. Spectrochimica Acta Part A. 2008, 70, 1238–1242.

[13] The control experiment using a Cu(II)-dmbpy/ODN6/ODN15 system afforded the Diels-Alder product with -87% *ee*, *endo/exo* ratio of 36:1 and 97% conversion.

