Organic co-solvents in aqueous DNA-based asymmetric catalysis†

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Water-miscible organic co-solvents can be used in DNA-based catalytic asymmetric reactions at appreciable concentration without a negative effect on enantioselectivity. While the rate of the copper(II) Diels–Alder reaction is affected negatively by the presence of organic co-solvents, the copper(II) catalyzed Michael addition and Friedel–Crafts alkylation reaction are significantly faster. Additionally, the presence of organic co-solvents allows for reaction temperatures <0 °C, which results in higher ee's. This is used to perform enantioselective Michael additions and Friedel–Crafts alkylations at gram scale, using catalyst loadings as low as 0.75 mol%. These results are an important step towards application of the DNA-based catalysis concept in organic synthesis

Introduction

Hybrid catalysis is a new approach to catalysis that aims to merge homogeneous and bio-catalysis.[1,2] A special subset of this field is DNA-based asymmetric catalysis, in which the chirality of DNA is used to induce enantioselectivity in a metal-catalyzed reaction (Fig. 1). This is achieved by non-covalent binding of a copper complex of an achiral ligand to DNA. Thus, the catalytically active copper center is brought into close proximity of the DNA helix, which provides the chiral second coordination sphere and directs the catalyzed reaction towards one of the enantiomers of the product (Fig. 1). This concept has been demonstrated successfully in several of the archetypical C–C bond forming reactions, such as the copper(II) catalyzed Diels–Alder,[3–7] Michael addition[8] and Friedel–Crafts alkylation reactions.[9] For all these reactions multiple examples with >90% ee were found, which represent the highest ee values found for these reactions in water, to date. Additionally, enantioselective fluorination reactions[10] and hydrolytic kinetic resolution of epoxides[11] have been achieved using this concept.

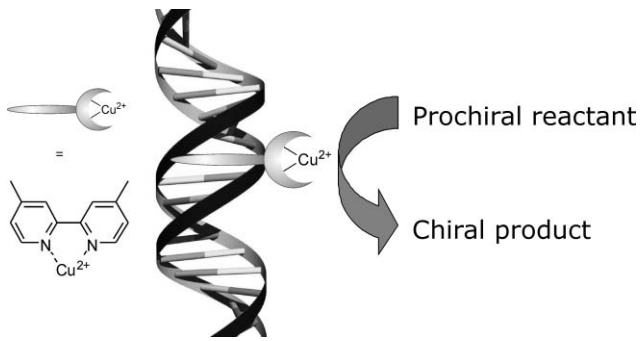

Fig. 1 Schematic representation of the concept of DNA-based asymmetric catalysis.

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Recently, an alternative approach to DNA-based catalysis, which involves covalent attachment of the catalyst to one of the DNA-strands, has been developed. Several examples of this approach have been reported, including some that involve enantioselective catalysis.[12–18]

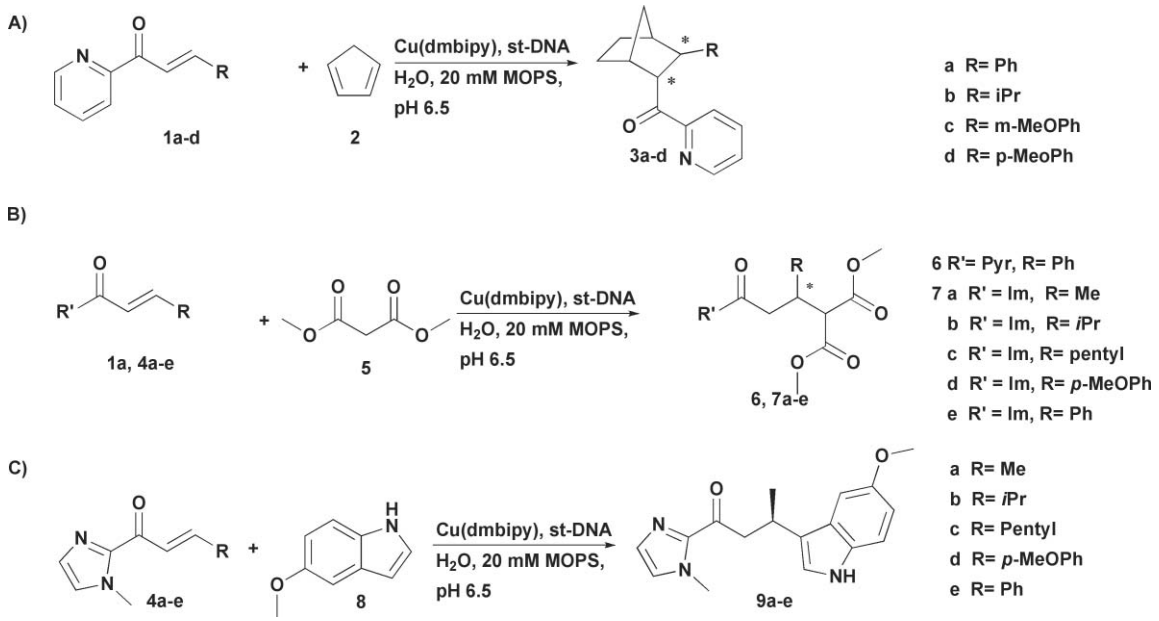
A common feature in these DNA-based catalysis approaches is that they inherently require water as the reaction medium. Aqueous phase catalysis is an area of considerable interest due to the potential advantages of replacing organic solvents with water and the special properties of water as a reaction medium.[19–21] For example, water has been shown to be beneficial for the rate and enantioselectivity of catalyzed reactions.[21] An obvious complication is the limited solubility in water of many organic substrates and reagents, which may hamper applications of this concept in organic synthesis. However, this does not necessarily pose a problem as is illustrated by the recently developed on water protocols,[22,23] which involve insoluble reagents. Efficient conversions can sometimes also be obtained in partially heterogeneous reaction mixtures.[8] Nevertheless, for many DNA-based catalytic reactions organic co-solvents will be required to achieve chemical transformations at synthetically relevant scales. The challenge herein lies in the presence of DNA, which might precipitate and/or undergo a structural change.[24]

Previously, it has been shown by Liu *et al.* that up to 99% organic solvents can be used in DNA templated synthesis.[25,26] Furthermore, the use of organic solvents in combination with DNA has also been demonstrated in the DNA mediated aldol and Henry reactions, albeit that the organic solvents had a negative effect on the yields of the latter reaction.[27,28]

Here, we present the results of a study on the effect of organic co-solvents on DNA-based asymmetric catalysis. The goal of this study was two-fold: first of all to establish the effect that organic co-solvents have on the rate and enantioselectivity of DNA-based catalytic reactions and secondly, to enable the application of the DNA-based asymmetric catalysis concept in organic synthesis.

Results and discussion

In this study we have focused on the Diels–Alder reaction, the Michael addition and the Friedel–Crafts alkylation



Scheme 1 Cu-dmbipy/DNA catalyzed Diels–Alder reaction (A), Michael reaction (B) and Friedel–Crafts alkylation (C). General conditions: 0.15 mM Cu-dmbipy, 1 mM st-DNA in basepairs, 1 mM enone substrate, 20 mM MOPS pH 6.5. Pyr = 2-pyridyl, Im. = 1-methylimidazol-2-yl.

(Scheme 1), catalyzed by [Cu^{2+}(4,4′-dimethyl-2,2′-bipyridine)-(NO_3)$_2$](Cu-dmbipy)/salmon testes-DNA (st-DNA) (15 mol% in copper), which is the most enantioselective catalyst to date for these reactions.[4,8,9] The substrates azachalcone (**1a**) and the α,β-unsaturated 2-acyl imidazole **4a** provide a bidentate coordination, which is generally required in copper(II) catalyzed reactions of this type.[29]

Solvent scope

Initially the effect of organic solvents on enantioselectivity and conversion in the Diels–Alder reaction of azachalcone (**1a**) with cyclopentadiene (**2**), the Michael addition of dimethyl malonate (**4**) to azachalcone (**1a**) and the Friedel–Crafts alkylation of 5-methoxyindole (**7**) with α,β-unsaturated 2-acylimidazole (**4a**) (Scheme 1), after a fixed reaction time were investigated. A wide variety of organic solvents was screened (Table 1). Water-miscible solvents such as MeCN, alcohols, DMSO and DMF were tolerated well in the Diels–Alder reaction, the Michael addition and the Friedel–Crafts alkylation (Table 1). However when either THF or CH_2Cl_2, which are not or only partially water-miscible, was used, a strong decrease in conversion and enantioselectivity was observed. This can be ascribed to the partial DNA precipitation that was observed. The Diels–Alder reaction has the highest tolerance towards organic solvents as up to 33% v/v of water-miscible organic solvent can be applied without a decrease in ee compared to the reactions in water. Further increase of the amount of organic co-solvent resulted in DNA precipitation during the reaction (Table S1, ESI†).

For the Michael addition and the Friedel–Crafts alkylation a similar trend was observed; with up to 10% v/v of co-solvent the same ee's were obtained compared to water alone. Further increase of the fraction of organic solvents led to a slow decrease in enantioselectivity (Tables S2,3, ESI†).

Table 1 Solvent scope of DNA-Based Catalysis

	Diels–Alder reaction [a] of **1a**		Michael addition [b] of **1a**		Friedel–Crafts alkylation [c] of **4a**	
Solvent	Fraction (% v/v)	ee (conversion %)	Fraction (% v/v)	ee (conversion %)	Fraction (% v/v)	ee (conversion %)
H_2O	—	99 (Full)	—	96 (Full)	—	83 (Full)
MeCN	33	99 (Full)	10	96 (Full)	10	83 (Full)
DMF	33	99 (Full)	10	95 (Full)	10	82 (Full)
THF	33	99 (34)[d]	10	90 (Full)[d]	10	81 (Full)
CH_2Cl_2	10–33	n.d[d]	10	94 (8)[d]	10	75 (78)[d]
EtOH	33	99 (Full)	10	96 (Full)	10	82 (Full)
MeOH	33	99 (Full)	10	96 (Full)	10	83 (Full)
DMSO	33	99 (Full)	10	95 (Full)	10	83 (Full)
1,4-Dioxane	33	99 (Full)	10	93 (Full)	10	83 (Full)
2-Propanol	33	99 (Full)	10	96 (Full)	10	83 (Full)

General conditions: 0.15 mM Cu(dmbipy), 1 mM st-DNA in basepairs, 1 mM substrate, 20 mM MOPS pH 6.5.[a] 15 mM Cyclopentadiene, **3d**. [b] 100 mM Dimethyl malonate, **1d**. [c] 5 mM 5-Methoxyindole, **1d**. [d] DNA precipitation was observed.

Table 2 Binding constant of Cu(dmbipy)$(NO_3)_2$ to DNA

	$K_b/10^4$ M^{-1}
H_2O	1.18 ± 0.01
10% v/v MeCN	1.18 ± 0.01
25% v/v MeCN	0.55 ± 0.06
10% v/v DMSO	1.08 ± 0.02
10% v/v EtOH	—
25% v/v EtOH	0.31 ± 0.02

The decrease in enantioselectivity at higher fractions of organic co-solvents could theoretically be related to a change in DNA structure; it has been reported that the rate acceleration and enantioselectivity in DNA based catalysis are DNA sequence, and hence, structure dependent.[6,7,9] However, no differences were observed in the circular dichroism spectra upon addition of organic solvents to a DNA solution (Fig. S4, ESI†).

Alternatively, the decrease in enantioselectivity at higher organic solvent content could be the result of a decrease in binding affinity of the copper complex to the DNA, perhaps as result of a weakening of the interactions between the catalyst and the DNA. This would result in more unbound copper complex being present, which will catalyze the reaction in a racemic fashion. Indeed, the binding constant (K_b) of the copper complex to DNA for different solvents decreased when >10% v/v of organic co-solvent was employed, albeit that this decrease was only up to 3-fold (Table 2).

Yet, this implies that the fraction of copper complexes bound to DNA decreases from 92% to 84% and 75% for 25% v/v CH_3CN and EtOH, respectively. The fact that this significant decrease in fraction of complexes bound to DNA is not translated into a similar drop in ee is the result of the significant rate acceleration induced by DNA in these reactions.[6,9] In the presence 10% v/v of ethanol no reliable data could be obtained. The reason for this is not yet understood. Possibly, it relates to a change in binding geometry of the copper complex at this solvent composition.

Influence on reaction rate

The effect of organic solvents on the reaction rate was studied using MeCN as benchmark solvent. The reactions were monitored by UV/Vis spectroscopy, following the decrease of the absorption of the enone substrate. In the case of the Diels–Alder reaction a significant deceleration was observed (Fig. 2A). This is not surprising since it is well-established that the reaction is water accelerated,[30,31] which is due to the hydrophobic effect;[32] addition of acetonitrile disrupts these favourable interactions (Fig. 2A).[33]

In contrast, in the Friedel–Crafts alkylation and Michael reaction, both conjugate additions, the reaction is significantly faster when the content of MeCN is increased. (Fig. 2B, Fig. S7, ESI†). These data were verified by HPLC analysis of samples taken from the reaction at different time intervals (Fig. S8,9, ESI†). For example, the conversion after 3.5 h with 10% v/v MeCN increased from 53% to 90% and 72% to 85% in the Friedel–Crafts alkylation and Michael addition, respectively.

To verify these results the apparent second-order rate constant (k_{app}) for the Diels–Alder and Friedel–Crafts alkylation were

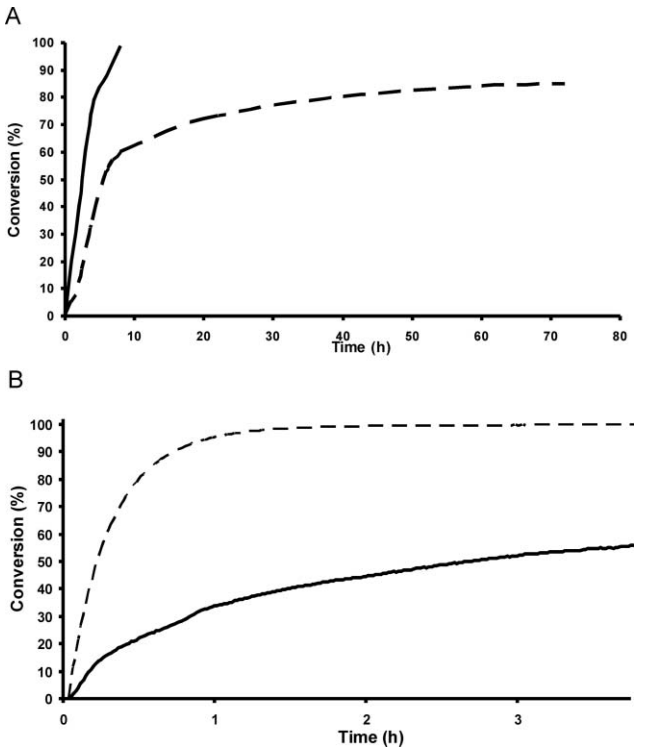


Fig. 2 Temporal conversion curve of A; Cu(dmbipy)/DNA catalyzed Diels–Alder reaction. 0.15 mM Cu(dmbipy), 1 mM st-DNA in basepairs, 1 mM **1a**, 15 mM **2**, 20 mM MOPS pH 6.5, 5 °C; — 0%, --- 33% MeCN. B; Temporal conversion curve of the Friedel–Crafts alkylation, 0.15 mM Cu(dmbipy), 1 mM st-DNA in basepairs, 1 mM **4a**, 5 mM **8**, 20 mM MOPS pH 6.5, 5 °C — 0%, --- 10% v/v MeCN.

determined using the methods developed by Engberts *et al.*[6,7,34],‡ In this model, the overall rate is determined by the equilibrium constant for the reversible binding (K_a) of the enone substrate to the Cu^{2+} complex, the rate of the reaction of the reactant, that is, cyclopentadiene or methoxyindole, with the Cu^{2+} bound enone substrate (k_{cat}) and the reversible dissociation of the product from the Cu^{2+} complex (K_d). In accordance with the generally accepted approach, the kinetic experiments were performed using a large excess of Cu(dmbipy) with respect to the substrates, assuming that the contribution of the K_d to the overall rate (k_{app}) is negligible (Scheme S5,6, ESI†).[34]

In the case of the Diels–Alder reaction, comparison of the k_{app} values showed the same trend as could be seen in the temporal conversion curve (Table 3). A decrease in the k_{app} of 2 orders of magnitude was found upon increasing the acetonitrile content to 30% v/v.

Surprisingly, in the Friedel–Crafts alkylation an almost 10-fold decrease in k_{app} was found in the presence of 15% v/v MeCN. This represents the opposite trend as observed in the experiments under turnover conditions, that is, with an excess of enone substrate with respect to the catalyst. This suggests that in these reactions the dissociation step, and not the actual conjugate addition reaction, is rate limiting, and that it is this step in which the favourable effect of organic co-solvents is found. This proposed acceleration of the

‡ Determination of the kinetic parameters for the Michael addition is complicated by the additional enolisation equilibrium of dimethyl malonate that is involved.

Table 3 k_{app} of the Diels–Alder reaction and Friedel–Crafts alkylation catalyzed by Cu(dmbipy) with DNA in various mixtures H_2O–MeCN

Diels–Alder reaction[a]		Friedel–Crafts alkylation[b]	
% v/v MeCN	k_{app} ($\times 10^{-2}$ M^{-1} s^{-1})	% v/v MeCN	k_{app} ($\times 10^{-2}$ M^{-1} s^{-1})
0	39.0 ± 0.6	0	52.6 ± 2.5
10	15.6 ± 2.1	5	31.6 ± 4.3
20	2.18 ± 0.17	10	20.1 ± 1.4
30	0.81 ± 0.01	15	6.31 ± 1.0

[a] k_{app} Determined for reaction of **1a** (6 μM) with **2** (0.5–2.0 mM) with 0.15 mM Cu(dmbipy) and 1 mM DNA in basepairs in 20 mM mops pH 6.5 at 18 °C, 226 nm. [b] k_{app} Determined for reaction of **4a** (14 μM) with **8** (0.5–2.0 mM) with 0.15 mM Cu(dmbipy) and 1 mM DNA in basepairs in 20 mM mops pH 6.5 at 18 °C, 265 nm.

dissociation step is only to a minor extent reflected in the position of the dissociation equilibrium. The K_d of **9a** was determined to be $2.8 \pm 0.1 \times 10^{-4}$ M^1 in water, while the K_d was $5.55 \pm 0.04 \times 10^{-4}$ M^1 in 10% v/v MeCN.

The observations presented here show that, although the kinetic model provides valuable information about the initial steps of the catalytic reaction, care should be exercised in extrapolating this data to the overall reaction. The assumptions underlying this kinetic model, that is the negligibility of the dissociation step, should always be verified by experiments under turnover conditions.

Substrate scope

Using MeCN as benchmark solvent, the substrate scope of the DNA-based catalytic reactions was explored. In all cases the ee found in the presence of 10% v/v MeCN was similar to that obtained in water (Table 4, Table S11, ESI†) Unsurprisingly, in the Diels–Alder reaction, always higher conversions were observed in water compared to 10% v/v MeCN, even though with enones **1c** and **1d** the reaction was partly heterogeneous due to precipitation of these substrates (Fig. S10†).

For the Michael addition higher conversions were observed when the substituent at the enone [R] was Me (**4a**) and p-methoxyphenyl (**4d**). However, enones **4b** and **4c**, which contain a large alkyl substituent at the enone, surprisingly gave rise to lower conversions in 10% v/v MeCN compared to the reaction in water (Fig. 3A).

A similar trend was observed for the Friedel–Crafts alkylation (Fig. 3B); in case of R being an aryl group, that is, with substrates

Table 4 Substrate scope of DNA-based catalytic Michael addition and Friedel–Crafts alkylation reactions with and without organic co-solvent

Enone	Nucleophile	% ee (H_2O)	% ee (10% v/v MeCN)
4a	**5**	56	56
4b	**5**	8	10
4c	**5**	13	15
4d	**5**	93	94
4b	**8**	70	70
4c	**8**	57	56
4d	**8**	77	78
4e	**8**	69	69

General condition: 0.15 mM Cu(dmbipy), 1 mM st-DNA in basepairs, 1 mM enone, 20 mM MOPS pH 6.5, 2 days.

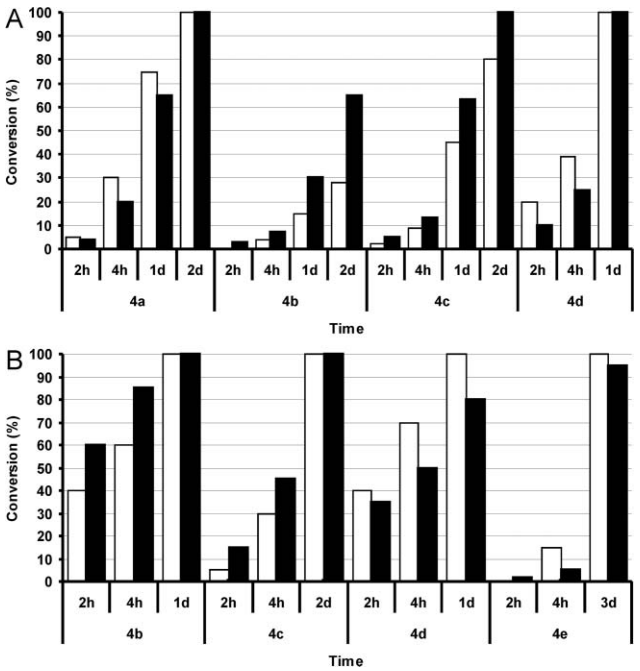


Fig. 3 Temporal conversion of the Michael addition (A) and the Friedel–Crafts alkylation (B). 0.15 mM Cu-dmbipy, 1 mM st-DNA in basepairs, 1 mM enone substrate, 20 mM MOPS pH 6.5, □ = Water ■ = 10% MeCN.

4d and **4e**, higher conversions were obtained in the presence of 10% v/v MeCN. But also here, the reactions with **4b** and **4c**, were more efficient in water alone. The reason why **4b** and **4c** undergo conjugate addition more efficiently in water compared to 10% v/v MeCN is at present unknown. It can be speculated that for these substrates, which carry large alkyl substituents at the enone moiety, hydrophobic effects contribute favorably to the reaction in water. Analogous to the Diels–Alder reaction, this favorable interaction is disturbed by the presence of an organic co-solvent, resulting in lower conversion.

DNA based catalysis at lower temperature

Lowering of the reaction temperature is an often used approach to increase the enantioselectivity of catalytic asymmetric reactions. When using water as the solvent, obviously the temperature cannot be lowered much. However, in the presence of organic co-solvents the freezing temperature is decreased significantly and this allows for DNA-based asymmetric catalysis at temperatures below 0 °C. The Friedel–Crafts reaction was investigated at −18 °C. At least 25% v/v of co-solvent was required to keep the solutions liquid. At this temperatures the enantioselectivity increased from 83% to 90% using MeOH and EtOH, with 90% conversion after 1.5 h (Table 5). Using DMSO a comparable reactivity was found, albeit that the enantioselectivity was slightly lower. DMF and 1,4-dioxane however, did not improve the efficiency of the reaction and full conversion was not reached within 3 days.

Increasing the scale

The effect of increasing substrate concentration was investigated and the results were compared to those obtained with water alone. The azachalcone (**1a**) concentration could be increased to 5 mM

Table 5 Friedel–Crafts alkylation of **8** with **4a** at −18 °C

		4 °C		−18 °C	
Co-solvent	% v/v	Conv. (%)	ee (%)	Conv (%)	ee (%)
MeOH	25	Full	82	90	90
	30	Full	82	90	90
EtOH	25	Full	82	—[a]	—
	30	Full	82	90	90
DMF	25	Full	83	90[b]	84
	30	Full	81	70[b]	83
DMSO	25	Full	83	90	89
	30	Full	82	75	89
1,4-dioxane	25	Full	80	—[a]	—
	30	Full	78	73[b]	76

General condition: 0.15 mM Cu(dmbipy), 0.67 mg ml^{-1} st-DNA, 1 mM **4a**, 5 mM **8**, 20 mM MOPS pH 6.5, 1.5 h. [a] Reaction mixture freezes. [b] After 3 d.

in 33% v/v MeCN for the Diels–Alder reaction (71% conversion; 99% ee). However, in water full conversion was found in the same time (Table S9, ESI†). This was not unexpected in view of the well-established acceleration of the Diels–Alder reaction in water and the results presented above.[30,31] It has to be noted that in the latter case the concentrations used are above the solubility limit and, hence, the reaction mixture is partly heterogeneous. Still the reaction is more efficient than in the presence of MeCN, even though the reaction mixture is homogeneous in this case.

Since both the Michael addition and the Friedel–Crafts alkylation are accelerated in the presence of organic co-solvents, these reactions were performed on a synthetically relevant scale.

The Michael addition of dimethyl malonate to the α,β-unsaturated 2-acylimidazole (**4e**) was investigated since the N-methylimidazole auxiliary can be displaced readily afterwards (Table 6).[35–37] Using a final concentration of 14.5 mM of **4e**, full conversion was obtained in 3d using only 1 mol% of catalyst. A slightly higher isolated yield (85%) was obtained from the reaction in 10% v/v MeCN compared to water (72%) (Table 6), whereas the ee was 94 and 95%, respectively.

The Friedel–Crafts alkylation of indole **8** with **4a** and **4d** was carried out at an enone concentration of 20 mM on 1.0 g scale and 300 mg scale, respectively. In water and water/10% v/v MeCN, at a reaction temperature of 4 °C, full conversion was reached overnight in the case of **4a**, while 6 days were needed for the reaction of **4d** to reach full conversion in 10% v/v MeCN. In this latter case only 40% conversion was reached in water in the same time. It should be noted that the catalyst loading was reduced to 0.75 mol% compared to 15 mol% in the small scale experiments. The yields were higher when organic co-solvents were used, albeit that in these cases slightly lower ee were observed compared to the reactions at small scale. When this reaction was carried out with 30% v/v MeOH at −18 °C, **9a** was obtained in an excellent isolated yield of 85% with 93% ee, which is significantly higher than what was obtained for this reaction with Cu(dmbipy)/st-DNA to date.[9]

Conclusions

In the present work, we have shown that water-miscible organic co-solvents can be used in DNA-based asymmetric catalytic Diels–Alder, Michael addition and Friedel–Crafts alkylation reactions at appreciable concentrations without negatively affecting the ee. Whereas in the Diels–Alder reaction organic co-solvents are tolerated, but at the expense of the reaction rate, in the Michael addition and Friedel–Crafts alkylation generally a positive effect on reactivity was observed. It was found that this is not the result of the actual conjugate addition reaction going faster, but is most likely the result of a faster dissociation of the product. An exception was found for enones containing a large alkyl moiety. In these cases higher conversions were obtained in the absence of co-solvents.

Furthermore, by using organic co-solvents these DNA-based catalytic reactions can be performed at synthetically relevant scales, that is, gram scale in 333 ml solvent and also at lower temperatures and low catalysts loadings, resulting in the products being obtained in good isolated yields and excellent ee's. The fact that the costs of salmon testes DNA used in these experiments are comparable to that of commonly used chiral ligands and that protective atmospheres are not required, a bright future for application of the DNA-based asymmetric catalysis concept in organic synthesis is envisioned.

Experimental section

General remarks

Salmon testes DNA was obtained from Sigma. Indoles were obtained from Aldrich. Copper complexes,[4] Azachalcone (**1a–d**),[21] 2-acyl imidazole **4a–d**[36] and **4e**.[37] were synthesized according to literature procedures. Cyclopentadiene was prepared freshly from its dimer ^{1}H-NMR and ^{13}C-NMR were recorded on a Varian 400 (400 MHz). Chemical shifts (δ) are quoted in ppm using residual solvent as internal standard (δ_H 7.26 and δ_C 77.0 for $CDCl_3$). CD-spectra were measured on a JASCO J-715 spectropolarimeter, with a temperature control attachment. The

Table 6 Large scale Michael addition and Friedel–Crafts alkylation reaction

	Michael addition[a]		Friedel–Crafts alkylation			
			4a		**4d**	
	Yield	ee	Yield[b]	ee[b]	Yield[c]	ee[c]
Water, 4 °C	1.06 g (72%)	94% (R)	1.21 g (66%)	81% (+)	0.153 g (32%)	57% (+)
10% MeCN, 4 °C	1.25 g (85%)	95% (R)	1.45 g (79%)	82% (+)	0.401 g (83%)	68% (+)
30% MeOH, −18 °C	—	—	1.56 g (85%)	93% (+)	—	—

Conditions: 0.15 mM Cu(dmbipy), 1 mM st-DNA in basepairs, 20 mM MOPS pH 6.5, 3 days, 333 ml. [a] 40 eq. Dimethyl malonate, 1 g **4d**, 1 day. [b] 5 eq. 5-Methoxyindole, 1 g **4a**, 1 day. [c] 5 eq. 5-Methoxyindole, 300 mg **4d** in total volume of 100 ml, 6 days.

UV-VIS spectra were measured on a JASCO v-560 or a JASCO v-570 with a temperature control attachment. Enantiomeric excess determination was performed by HPLC analysis on a Shimadzu 10AD-VP system.

DNA based catalysis, representative procedure[4,8,9]

A buffered solution (20 mM Mops, pH 6.5) of DNA bound catalyst (1 mM salmon testes DNA in basepairs and 0.15 mM [Cu(dmbipy)(NO_3)$_2$]) was prepared by mixing a solution of salmon testes DNA (5 ml of a 2 mg ml^{-1} solution in 30 mM MOPS, prepared 24 h in advance) with an aqueous solution of catalyst (5 ml of a 0.45 mM solution of [Cu(dmbipy)(NO_3)$_2$] in 30 mM MOPS pH 6.5) and adding water and/or organic solvent to a total volume of 15 ml. 15 μmol of substrate in 10 μL MeCN was added and the mixture was cooled to <5 °C. The reaction was started by addition of the appropriate amount of reactant (Diels–Alder 15 eq. cyclopentadiene; Michael addition 100 eq. dimethylmalonate; Friedel–Crafts reaction 5 eq. 5-methoxyindole) and mixed by continuous inversion for the indicated time, followed by extraction of the product with Et_2O, drying (Na_2SO_4) and removal of the solvent. The crude product was analyzed by ^{1}H-NMR and HPLC.

Large scale reaction, representative procedure

A buffered solution (20 mM MOPS, pH 6.5) of DNA bound catalyst (1 mM st-DNA salmon testes DNA in basepairs and 0.15 mM [Cu(dmbipy)(NO_3)$_2$]) was prepared by mixing a solution of salmon testes DNA (111 ml of a 2 mg ml^{-1} solution in 30 mM MOPS pH 6.5, prepared 48 h in advance) with an aqueous solution of catalyst (111 ml of a 0.45 mM solution of [Cu(dmbipy)(NO_3)$_2$] in 30 mM MOPS) and adding water and/or organic solvent up to a total volume of 333 ml. To this was added 1 g of enone (**4a** or **d**). After addition of reactant (40 eq. **5** for Michael addition; 5 eq. **8** for Friedel–Crafts alkylation) at <5 °C, the reaction was mixed for 1 days by continuous inversion at 5 °C. The product was isolated by extraction with Et_2O. After drying (Na_2SO_4) and removal of the solvent the crude product was further purified by column chromatography (SiO_2, EtOAc–pentane 2 : 3) and analyzed by NMR and HPLC.

HPLC conditions (Michael addition): Daicel chiralcel-AD, heptane/iPrOH 90 : 10, 0.5 ml min^{-1}. Retention times: 29.8 and 34.1 min. (**7d**), 24.0 and 29.3 min.(**9d**)

HPLC conditions (Friedel–Crafts alkylation): Daicel chiralcel-AD, heptane/iPrOH 90 : 10, 0.5 ml min^{-1}. Retention times: 32.8 and 39.4 min.

Determination of binding constant (K_b)

Equilibrium binding constants to salmon testes DNA were determined by UV/Vis titration, following the procedure of Meehan.[38] After dissolution of salmon testes DNA (2 mg ml^{-1}), the stock solution was dialyzed extensively against MOPS buffer (20 mM pH 6.5) prior to use. The concentration in base pairs was determined spectrophometrically, using $\varepsilon_{260} = 12800$ M^{-1} cm^{-1}. The absorbance ratio of $\lambda_{260}/\lambda_{280}$ was 1.8–1.9, indicating the DNA was sufficiently free of protein. The K_b was determined by titration of DNA to a solution of copper complex in buffered solution. Concentrations of copper complex was 30 μM. Under conditions where the ratio of bound complex : DNA base pairs approaches zero, the K_b can determined using:

$$\frac{D}{\Delta\varepsilon_{ap}} = \frac{1}{\Delta\varepsilon}D + \frac{1}{\Delta\varepsilon K_b}$$

where $\Delta\varepsilon_{ap} = |\varepsilon_a - \varepsilon_f|$, $\Delta\varepsilon = |\varepsilon_b - \varepsilon_f|$, ε_a, ε_f and ε_b are the apparent, free and bound extinction coefficients for the complex, respectively, and D is the DNA concentration in base pairs. In a plot of $D/\Delta\varepsilon_{ap}$ *vs.* D, K_b is given by the ratio of the slope to the *y* intercept.

Determination of k_{app} (Diels–Alder reaction)

The procedures to determine k_{app} were adapted from Engberts *et al.*[34] A 2.0 μL portion of a fresh solution of azachalcone **1a** (1.0 mg mL^{-1} in MeCN) was added to a 0.15 mM [Cu(dmbipy)(NO_3)$_2$], 1.0 mM of salmon testes DNA in base pairs in buffer (20 mM MOPS, pH 6.5) in a quartz cuvette. After the absorption stabilized, 1–10 μL of a freshly prepared cyclopentadiene solution in MeCN was added, resulting in a final concentration of 0.5–2.0 mM. The cuvette was closed immediately and sealed tightly to prevent evaporation of cyclopentadiene. The reaction was monitored at 326 nm, at the appropriate temperature, on a JASCO V-560 or a JASCO V-570 spectrophotometer. The decrease in absorption of **1a** was followed for the first 15% of the reaction, and the following expression was used to calculate k_{app}:

$$k_{app} = \frac{dA_1}{dt} \cdot \frac{1}{d \cdot (\varepsilon_1 - \varepsilon_3) \cdot [\mathbf{1}]_0 \cdot [\mathbf{2}]_0}$$

in which ε_1 and ε_3 are the extinction coefficients of **1a** and **3a**, respectively, and *d* is the path length of the cuvette. The observed rate constants were determined at different concentrations of **2**, after which the k_{app} was extracted from the slope of the resulting plot. Thus, reactions other than the reaction of **1a** with **2** were excluded.

Determination of k_{app} (Friedel–Crafts reaction)

The measurements were performed as described earlier.[6] The samples contained 0.15 mM of [Cu(dmbipy)(NO_3)$_2$], 1.0 mM of salmon testes DNA in base pairs, 14 μM of **4a**, and 0.5–2.0 mM of 5-methoxy indole, with a total volume of 1.0 mL in 20 mM MOPS-buffered water at pH 6.5. The decrease of absorption at 265 nm was followed in time until the reaction was complete. Pseudo-first-order rate constants were obtained using a fitting program. The rate constants were plotted *versus* the concentration indole, and the k_{app} was subsequently determined from the slope of this graph.

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