Investigation of DNA as a catalyst for Henry reaction in water†

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Double-stranded DNA of natural origin can be used to facilitate nitro-aldol (or Henry) reaction in aqueous solution.

DNA, with its characteristic right-handed helix structure and well-defined geometry, is a robust biopolymer that can withstand a range of pH, temperature and solvent conditions.[1] Recent years have witnessed the growth of interest in exploring DNA for the construction of catalytic systems.[2,3] For example, many studies have used DNA as a catalyst in the "DNA-templated organic synthesis" (DTS) where two small-molecule substrates are linked to two separate DNA strands that are held together on a complementary DNA template in one of several geometries.[3] The advantages of DTS include: (1) the templated reaction proceeds much faster than the uncatalyzed reaction, and (2) DTS could lead to the discovery of novel chemical transformations.[4] Recently, the Silverman group has successfully performed *in vitro* selection experiments and derived many DNA based enzymes that catalyze Diels–Alder reaction.[5] It has also been reported that DNA can mediate chemical reactions in a highly enantioselective fashion.[6] To the best of our knowledge, however, exploiting unmodified double-stranded DNA as a catalyst for organic reactions has not been reported yet.

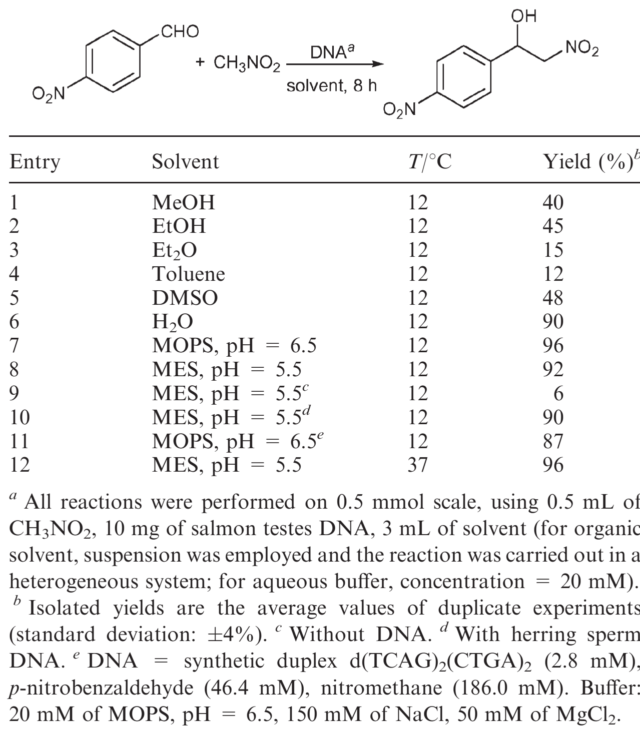
Nitro-aldol (or Henry) reaction is an important carbon–carbon bond formation reaction widely used in organic synthesis.[7] Henry reactions are usually performed in organic solvents and only a few studies have involved water as the reaction medium.[8] Herein, we describe a straightforward use of DNA as the catalyst to facilitate the Henry reaction in water without tethering the substrates to any DNA strands.

We first examined the Henry reaction between *p*-nitrobenzaldehyde and nitromethane at 12 °C under various solvent conditions (Table 1) in the presence of salmon testes DNA (entries 1–6). Product formation was observed in all these conditions. However, the yield in water (90%, entry 6) was much better than those in organic solvents, such as methanol, ethanol, ether, toluene and DMSO (entries 1–5). Similar results were obtained in different aqueous buffer solutions (entries 7 and 8). That the presence of DNA was essential was confirmed: when the DNA was omitted from the reaction mixture, the yield dropped to merely 6% (entry 9). Moreover, an excellent yield (90%) was registered when the DNA from a different source (herring sperm) was used (entry 10). We also observed that reaction yields were enhanced with an increase in the amount of the DNA (see supporting information).† As expected, a small synthetic dsDNA (the self-complementary 16-mer duplex d(TCAG)$_2$(CTGA)$_2$) also produced a similar yield (87%; entry 11), which helps to rule out the possibility that the catalysis might be caused by some unknown impurity in the DNA from natural sources. Finally, an excellent yield (96%) was obtained when the reaction was performed at 37 °C (comparing entry 12 to entry 8).

A series of experiments were then carried out from which we established the following optimal reaction conditions to be used in the remaining reactions of this work: 0.5 mmol scale, 0.5 mL of CH_3NO_2, 10 mg of salmon testes DNA, 3 mL of 20 mM MES buffer (pH = 5.5), 37 °C.‡

We next studied the influence of DNA on the reaction time and yield in reactions involving different carbonyl substrates. Referring to Table 2, all the aromatic aldehydes generated the

Table 1 Screen for different solvents and reaction temperature[a]



Entry	Solvent	*T*/°C	Yield (%)[b]
1	MeOH	12	40
2	EtOH	12	45
3	Et_2O	12	15
4	Toluene	12	12
5	DMSO	12	48
6	H_2O	12	90
7	MOPS, pH = 6.5	12	96
8	MES, pH = 5.5	12	92
9	MES, pH = 5.5[c]	12	6
10	MES, pH = 5.5[d]	12	90
11	MOPS, pH = 6.5[e]	12	87
12	MES, pH = 5.5	37	96

[a] All reactions were performed on 0.5 mmol scale, using 0.5 mL of CH_3NO_2, 10 mg of salmon testes DNA, 3 mL of solvent (for organic solvent, suspension was employed and the reaction was carried out in a heterogeneous system; for aqueous buffer, concentration = 20 mM). [b] Isolated yields are the average values of duplicate experiments (standard deviation: ±4%). [c] Without DNA. [d] With herring sperm DNA. [e] DNA = synthetic duplex d(TCAG)$_2$(CTGA)$_2$ (2.8 mM), *p*-nitrobenzaldehyde (46.4 mM), nitromethane (186.0 mM). Buffer: 20 mM of MOPS, pH = 6.5, 150 mM of NaCl, 50 mM of $MgCl_2$.

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† Electronic supplementary information (ESI) available: Procedure for catalyst recycling, determination of the pH value of the DNA aqueous solution (in super-pure water) and characterization data for the products. See DOI: 10.1039/b805767b

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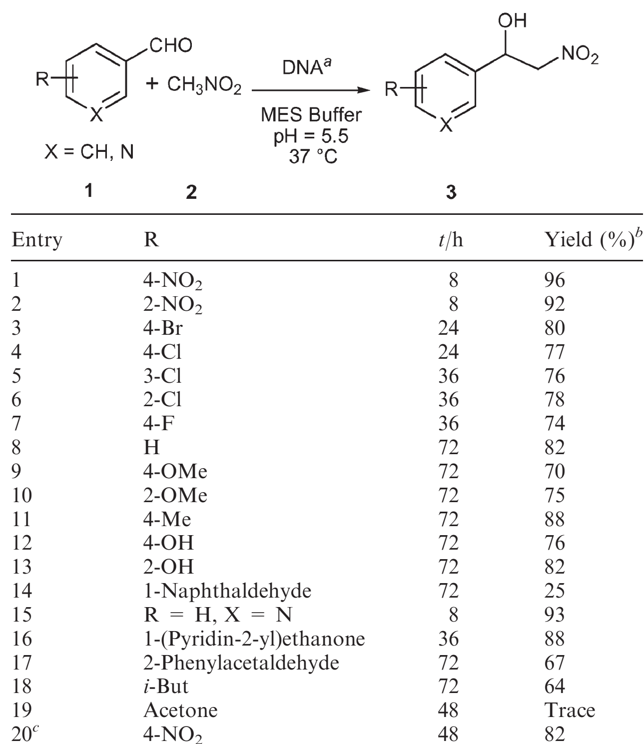
Table 2 DNA as catalyst for Henry reaction in water[a]



Entry	R	*t*/h	Yield (%)[b]
1	4-NO_2	8	96
2	2-NO_2	8	92
3	4-Br	24	80
4	4-Cl	24	77
5	3-Cl	36	76
6	2-Cl	36	78
7	4-F	36	74
8	H	72	82
9	4-OMe	72	70
10	2-OMe	72	75
11	4-Me	72	88
12	4-OH	72	76
13	2-OH	72	82
14	1-Naphthaldehyde	72	25
15	R = H, X = N	8	93
16	1-(Pyridin-2-yl)ethanone	36	88
17	2-Phenylacetaldehyde	72	67
18	*i*-But	72	64
19	Acetone	48	Trace
20[c]	4-NO_2	48	82

[a] All reactions were performed on 0.5 mmol scale, using 0.5 mL of CH_3NO_2, 10 mg of salmon testes DNA and 3 mL of solvent; for aqueous buffer, concentration = 20 mM. [b] Isolated yields are the average values of duplicate experiments (standard deviation: ±4%). [c] Reaction is performed on 10 mmol scale using 30 mg of salmon testes DNA in the optimized conditions.

corresponding Henry reaction products with good to excellent yields (entries 1–13). Generally, electron-withdrawing substituents on the phenyl ring of **1** favored the reaction (entries 1–7). More specifically, benzaldehyde produced a yield of 82% (entry 8) in a 72-h reaction. The aromatic aldehydes bearing strong electron-withdrawing substituents (entries 1 and 2) had better reactivities and gave better yields than those bearing weak electron-withdrawing substituents (entries 3–7). In contrast, the aromatic aldehydes with electron-donating substituents produced slightly lower yields than benzaldehyde (entries 9–13). In these cases, an *ortho*-substitution had a greater influence on the reaction than a *para*-substitution (entries 9, 10, 12 and 13). On the other hand, the steric hindrance of the reaction substrates also affected the reaction. For instance, 1-naphthaldehyde, with a bigger steric hindrance than benzaldehyde, gave rise to a low yield of the Henry reaction product (25%), accompanied by a dehydrated product, 2-((*E*)-2-nitrovinyl)naphthalene in 42% yield (entry 14). When an aliphatic ketone, which has more steric hindrance than aldehydes, was employed in the reaction almost no product was observed (entry 19). Notably, excellent yields were obtained for nicotinaldehyde and 1-(pyridin-2-yl)ethanone after 8 h and 36 h, respectively (entries 15 and 16). Aliphatic aldehydes, such as pivalaldehyde and 2-phenylacetaldehyde, also generated the desired Henry reaction product with good yields (64% and 67%, entries 18 and 17, respectively). It is important to note that without DNA, all the substrates produced no (or trace amounts of) Henry reaction product. We also found that the DNA-mediated Henry reaction can be performed on a large scale with a very good yield (entry 20).

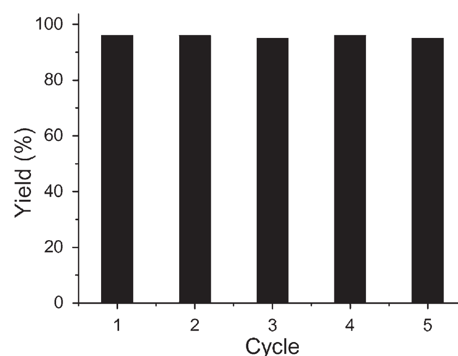


Fig. 1 Recycling experiments for the Henry reaction. The reaction is performed for 12 h using 0.5 mmol of the aldehyde, 0.5 mL of CH_3NO_2, 10 mg of salmon testes DNA, 3 mL of solvent in 20 mM MES (pH = 5.5). Isolated yields are the average values of duplicate experiments (standard deviation: ±3%).

We also investigated the recycling of the catalytic DNA. Fig. 1 shows the efficiency of the same catalyst used five times to catalyze the reaction of *p*-nitrobenzaldehyde and nitromethane under the optimized reaction conditions. Even after the fifth round, no reduction in reaction yield was observed.

Considering that DNA possesses a right-handed helix structure, the chiral information within DNA may transfer to the organic reaction products. Thus, we also performed experiments to test the chiral selectivity of the DNA-mediated Henry reaction. However, no ee value was observed, indicating that the DNA catalyst does not provide a chiral selectivity under our conditions.

In summary, we have shown that double-stranded DNA from natural sources can be used as a catalyst to facilitate the Henry reaction in aqueous solution under mild reaction conditions. Most of the substrates we tested resulted in good (~60%) to excellent (>90%) yields of the expected reaction products. More interestingly, the DNA catalyst could be recycled several times without any loss of activity. The mechanism of the DNA-catalyzed Henry reaction as well as the scope of other reactions that may be catalyzed by natural DNA will be the subjects of future investigations by us.

Double-stranded DNA is conveniently available from a variety of natural sources (including bacteria and plants). Therefore, the direct use of DNA to facilitate organic synthesis in aqueous solution may offer an excellent way to prepare useful organic compounds.

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Notes and references

‡ Representative procedure for DNA-catalyzed nitro-aldol or Henry reaction: to an aqueous MES buffer solution (20 mM, pH = 5.5) was added DNA (herring sperm or salmon testes DNA 10 mg, purchased from Sigma). After 10 min, 0.5 mmol of *p*-nitrobenzaldehyde in 0.5 mL of CH_3NO_2 was added. After the mixture was heated at 37 °C for 8 h, the mixture was extracted three times with chloroform. The combined

organic extracts were dried with anhydrous Na_2SO_4 and evaporated under reduced pressure; the residue was then purified by column chromatography over silica gel to afford the corresponding Henry product with high purity. ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 8.25 (d, J = 8.4 Hz, 2 H), 7.61 (d, J = 8.4 Hz, 2 H), 5.57–5.62 (m, 1 H), 4.57 (m, 2 H), 3.18 (br, 1 H).

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