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Genomic salmon testes DNA as a catalyst for Michael reactions in water

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ABSTRACT

The DNA molecule, recognized as the carrier of genetic information in vivo, can function as an efficient organocatalyst for Michael additions of 1,3-dicarbonyl compounds to activated alkenes in aqueous media. The procedure described here is environmentally benign and offers several advantages in terms of simplicity, generality and efficiency. We have used fluorescence spectroscopy to evaluate the catalytic activity of a molecule of DNA.

1. Introduction

The main component of all living organisms is water and it is the solvent of choice for nature to perform syntheses.[1] For a long time the use of water as a reaction medium in organic synthetic procedures has been rejected. The scant solubility of the reagents and the incompatibility between water molecules and some functional groups present in the reactants themselves were the main reasons. The pioneer studies of Breslow,[2] Grieco,[3] and Sharpless,[4] have triggered a more widespread interest in water highlighting its unique physical and chemical properties and currently the list of organic processes performed efficiently in water includes a notable number of different types of reactions.[5] The last years have witnessed a significant interest in an interdisciplinary research aimed at the evolution of new bio-inspired catalysts in an attempt to mimic nature's way of making new chemical bonds and to found catalysts with a synergy between the attractive properties of biocatalysis and chemocatalysis. In this context a biopolymer such as DNA shows a great potential since it is chemically stable, commercially available in large quantities with costs comparable to those of small molecule catalysts. In the literature, two strategies have proven to be very successful: DNA-templated synthesis (DTS)[6] and DNA-based asymmetric catalysis[7–10] but to our knowledge, only two other papers describe the use of unmodified double-stranded DNA as a 'catalyst' for the Henry reaction[11] and the aldol reaction.[12]

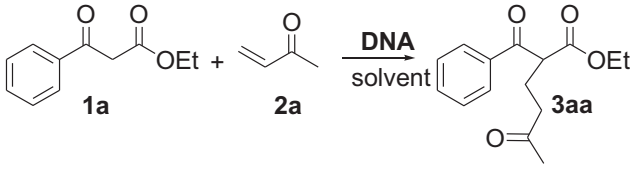
From these observations we evaluated the possibility of extending the catalytic repertoire of the genomic DNA by examining whether the two most important molecules in the life, water and DNA can be used to perform the Michael addition, an important C–C bond forming reaction.

The 1,4-conjugate additions play an important role in organic synthesis leading to functionalized adducts of high synthetic value and recently examples of Michael reactions that use water as a solvent have been reported.[13–18]

Herein, we describe a new, easy and general procedure of Michael reaction in water catalyzed by genomic salmon testes DNA (st-DNA).

2. Results and discussion

We used the reaction of ethyl-3-oxo-3-phenylpropanoate (**1a**) and methyl vinyl ketone (**2a**) to investigate the reaction conditions (Scheme 1).



Scheme 1. Michael addition of ethyl-3-oxo-3-phenylpropanoate to methyl vinyl ketone.

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The effects of reaction temperature, pH value, DNA loading, reaction time and reaction mixture molarity were investigated (Table 1).

Table 1
Screening studies on the reaction conditions[a]

Entry	DNA (mg)	Solvent (mL)	pH[b]	T (°C)	Time (h)	Conv. (%)[c]
1	10	H_2O(1 mL)	6.80	rt	24	—
2	10	H_2O(1 mL)	6.80	40	24	28
3	10	H_2O(1 mL)	6.80	70	24	50
4	—	H_2O(1 mL)	6.80	70	24	7
5	10	MOPS(1 mL)	6.50	70	24	20
6	10	MES(1 mL)	5.40	70	24	8
7	20	H_2O(1 mL)	6.80	70	24	65
8	30	H_2O(1 mL)	6.80	70	24	66
9	20	H_2O(1 mL)	6.80	70	48	64
10	20	H_2O(2 mL)	6.80	70	24	80
11	20	H_2O(3 mL)	6.80	70	24	90
12	20[d]	H_2O(3 mL)	6.80	70	24	90

[a] All reactions were performed on 0.5 mmol of **1a** and 0.7 mmol of **2a**. All experiments were carried out with salmon testes DNA.
[b] The pH values refer to the values of the solutions without DNA and reagents.
[c] Conversion values were determined by ^{1}H NMR analysis and are the average of duplicate experiments (standard deviation: 3%).
[d] The third recycling experiment. See experimental section.

We initially investigated the reaction of aqueous **1a** and **2a** at various temperatures in the presence of 10 mg of genomic salmon testes DNA, which is readily available and inexpensive (entries 1–3, Table 1). The range of the reaction temperatures was chosen on the basis of the results of the melting curve of genomic salmon testes DNA in aqueous solution. The values of T_m for DNA were determined by CD spectroscopy by monitoring the absorbance of DNA in aqueous solution at 260 nm by varying the temperature. The value of T_m for genomic salmon testes DNA was 87.5 °C (Fig. 1).

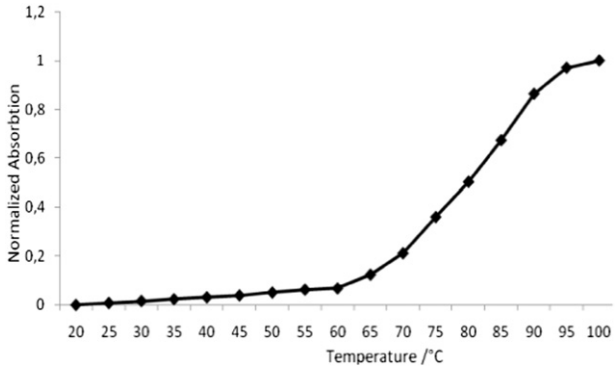


Fig. 1. Melting curve of genomic salmon testes DNA. The absorbance was measured in aqueous solution with excitation at 260 nm. The concentration of DNA was of 6.0×10^{-7} M.

At room temperature no conversion was observed (entry 1, Table 1), but when the reaction temperature was raised to 40 °C the process appeared to benefit from it (entry 2, Table 1), and with a further rise in temperature to 70 °C, the conversion reached 50% (entry 3, Table 1).

Although the conversion was not very high (50% entry 3, Table 1) the reaction was accelerated in the presence of genomic salmon testes DNA, specifically, when the reaction was run without st-DNA (entry 4, Table 1) the conversion dropped to 7%. An important observation was that the reaction proceeded very cleanly and the Michael adduct **3aa** was formed as the only product. By-products, such as polycondensation or cyclized products were not observed.

To investigate whether it was possible to increase the efficiency of the reaction, we examined other parameters of the reaction system. Experimental results about the influence of the pH values on reaction efficiency (entries 5–6, Table 1) indicated that at lower pH values the conversion was decreased.

By exploring the influence of DNA quantity on the reaction efficiency (entries 7–8, Table 1), we found that the conversion was enhanced with 20 mg of st-DNA but increasing the amount of st-DNA to 30 mg did not significantly improve conversion. Therefore, 20 mg of st-DNA was the best catalyst loading. Extending the reaction time to 48 h (entry 9, Table 1) resulted in a conversion comparable to that achieved after 24 h.

Then, we studied the influence of reaction mixture molarity on reaction efficiency (entries 10–11, Table 1) finding that the conversion reached 90% when the reaction was run in 3 mL of water. At last, the recycling experiments of the catalyst (entry 12, Table 1) showed that the DNA could be easily recovered and used in subsequent reactions with comparable activity.

Thus, 0.5 mmol of **1a** and 0.7 mmol of **2a** in 3 mL of pure water with 20 mg of st-DNA at 70 °C for 24 h (entry 11, Table 1) were established to be the optimal reaction conditions.

In order to exclude any artifacts and to confirm the catalytic activity of the genomic salmon testes DNA, a number of control experiments were performed (Table 2).

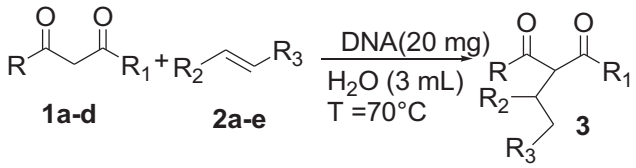
Table 2
Control experiments[a]

Entry	Catalyst (mg)	Solvent (mL)	T (°C)	Time (h)	Conv. (%)[b]
1	DNA(20)[c]	H_2O(3 mL)	70	24	90
2	Adenine(20)	H_2O(3 mL)	70	24	18
3	Cytosine(20)	H_2O(3 mL)	70	24	26
4	Guanine(20)	H_2O(3 mL)	70	24	26
5	Tymine(20)	H_2O(3 mL)	70	24	20
6	NaCl(20)	H_2O(3 mL)	70	24	0
7	$MgCl_2$(20)	H_2O(3 mL)	70	24	0
8	H_3PO_4(20)[d]	H_2O(3 mL)	70	24	30

[a] All reactions were performed on 0.5 mmol of **1a** and 0.7 mmol of **2a**.
[b] Conversion values were determined by ^{1}H NMR analysis and are the average of duplicate experiments (standard deviation: 3%).
[c] DNA was extensively dialyzed against deionized water (pH 6.80).
[d] The pH of solution was buffered to 6.80 by 1 M NaOH aqueous solution.

When the reaction was carried out using dialyzed st-DNA, the reaction efficiency was not changed (compare entry 1, Table 2 with entry 11, Table 1). The use of nucleobases as general base catalysts in the reaction was screened (entries 2–5, Table 2) finding that they were very poor catalysts (18–26%). Subsequently, we ran the reaction in the presence of various salts excluding a salt effect (entries 6–8, Table 2). Finally, given the helicity of DNA, we performed experiments to test whether its chirality was transferred to the catalytic reaction. No ee value was observed indicating that the double helix of DNA, under our conditions, did not provide enantioselectivity.

With these results in hand, we then investigated the general scope of this method (Scheme 2) with different donors (Fig. 2) and acceptors (Fig. 3). The results are summarized in Table 3.



Scheme 2. Michael addition of various donors and acceptors.

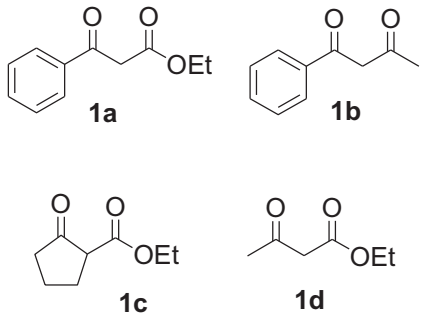


Fig. 2. Michael donors **1a**–**d** used in conjugate addition.

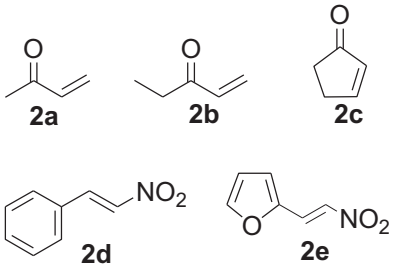


Fig. 3. Olefins **2a**–**e** used as Michael acceptors.

Table 3
Michael additions of β-dicarbonyl compounds in water catalyzed by DNA[a]

Entry	Donor	Acceptor	Product	Conv. (%)[b]
1	1a	2a	**3aa**	90
2	1b	2a	**3ba**	91
3	1c	2a	**3ca**	99
4	1d	2a	**3da**	72
5	1a	2b	**3ab**	64
6	1b	2b	**3bb**	53
7	1c	2b	**3cb**	72
8	1d	2b	**3db**	74
9	1a	2c	**3ac**	80[c]
10	1a	2d	**3ad**	60[c]
11	1a	2e	**3ae**	51[c]

[a] All reactions were performed on 0.5 mmol of the donor and 0.7 mmol of the acceptor. All experiments were carried out with salmon testes.
[b] Conversion values were determined by ^{1}H NMR analysis and are the average of duplicate experiments (standard deviation: 3%).
[c] Obtained as a 1/1 mixture of two diastereomers.

The 1,3-dicarbonyl compounds **1a**–**d** were treated with Michael acceptor **2a** in the presence of DNA under the optimized conditions (entries 1–4, Table 3). We found that the reaction had a wide substrate scope with respect to 1,3-dicarbonyl compounds affording the desired Michael adducts in yields ranging from 72% to 99% (entries 1–4, Table 3). In addition to **2a**, ethyl vinyl ketone **2b** was also used (entries 5–8, Table 3) and the reactions proceeded smoothly though with moderate conversion: part of the starting material remained unconverted, even after prolonged reaction times. Surprisingly, when the cyclic enone **2c** (entry 9, Table 3) reacted with **1a** the conversion was 80%. When the reaction of **1a** was carried out using the two nitroolefins **2d** and **2e** as the acceptors, the catalyst was able to promote the reaction but the conversion was lower (entries 10–11, Table 3).

Based on the outcome of our experimental results, and in an effort to obtain more information on the present reaction, further investigations were performed. Many methods have been used to study the interaction of small molecules with DNA[19] and among these methods fluorometric techniques are the most popular because of their sensitivity and high speed. We envisaged that information on the substrate-DNA interaction could be obtained by fluorescence spectrometry.

We first examined the interaction of the acceptor **2a** and the donor **1a** with DNA at room temperature and at the reaction temperature (70 °C). The fluorescence emission spectrum of **2a** with and without st-DNA is shown in Fig. 4.

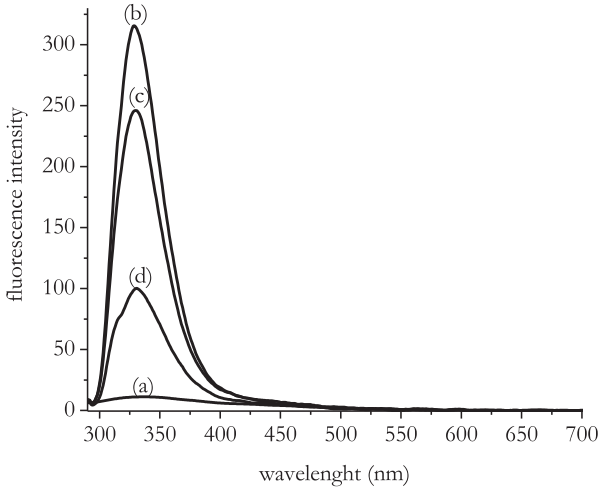


Fig. 4. Fluorescence spectra of: (a) DNA. (b) methyl vinyl ketone (**2a**). (c) **2a**-DNA at r t. (d) **2a**-DNA at 70 °C. All spectra were measured in water with excitation at 280 nm. The concentrations of DNA and methyl vinyl ketone were 90 μM and 1.4 mM, respectively.

The acceptor **2a** has an emission band located at 329 nm with an excitation peak at 280 nm as shown in Fig. 4. As a control, the fluorescence emission of free st-DNA at 280 nm was tested to establish whether the presence of the DNA would interfere with the signal of the molecule under investigation. A very weak fluorescence emission band was seen under the experimental conditions tested (Fig. 4).[20] The addition of st-DNA quenched the fluorescence intensity of **2a** at room temperature (22%) and the extent of fluorescence quenching significantly increased with increasing temperature (68% at 70 °C), together with a bathochromic shift of 2 nm. These results indicate that there was an interaction between **2a** and DNA at room temperature and that this interaction increased at higher temperatures.

The fluorescence emission spectrum of **1a** is shown in Fig. 5. The fluorescence emission maximum for **1a** was obtained at 397 nm upon excitation at 280 nm. Addition of DNA at room temperature did not quench fluorescence to any significant extent (6%), while

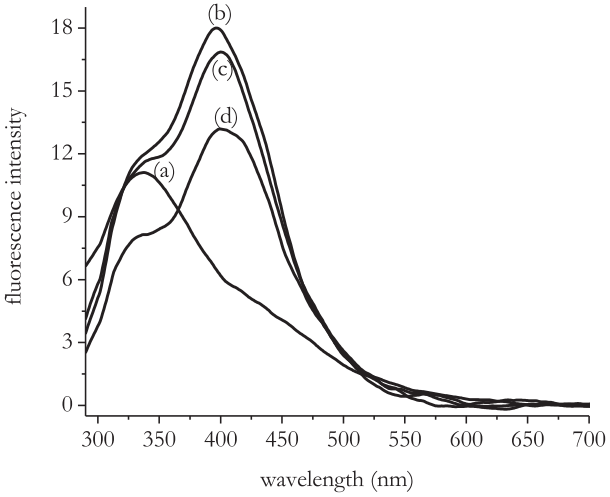


Fig. 5. Fluorescence spectra (a) DNA. (b) ethyl-3-oxo-3-phenylpropanoate (**1a**). (c) **1a**-DNA at rt (d) **1a**-DNA at 70 °C. All spectra were measured in water with excitation at 280 nm. The concentrations of DNA and ethyl-3-oxo-3-phenylpropanoate were 90 μM and 1 mM, respectively.

increasing the temperature to 70 °C resulted in a fluorescence quenching of 27%, with a concomitant bathochromic shift of 3 nm.

Therefore, the experiments suggest that both **2a** and **1a** interact with the DNA and this interaction is enhanced at 70 °C (reaction temperature). On the basis of fluorescence data, and in order to determine whether there was a relationship between substrate/DNA interaction efficiency and reaction efficiency, we carried out the model reaction at room temperature. We observed a conversion of 16% (compare with entry 11, Table 1). These results seem to confirm a relationship between the reaction efficiency and the fluorescence data. In fact, as the reaction temperature increases from room temperature to 70 °C, a higher quenching efficiency is observed for both substrates with a corresponding increase in the reaction yield.

We then studied the fluorescence emission spectra of the other donors under study. The fluorescence emission spectra of the Michael donor **1c** in the absence and presence of DNA upon excitation at 280 nm are shown in Fig. 6.

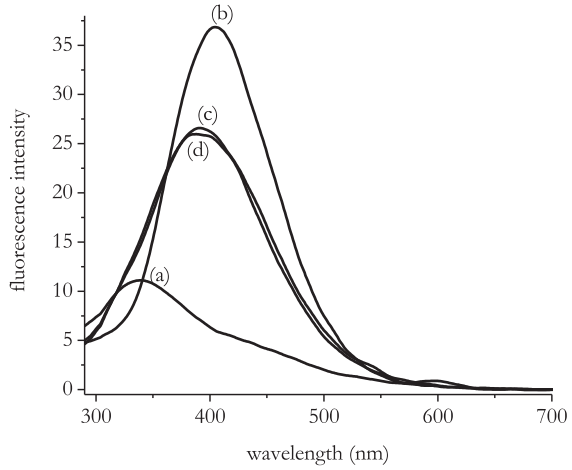
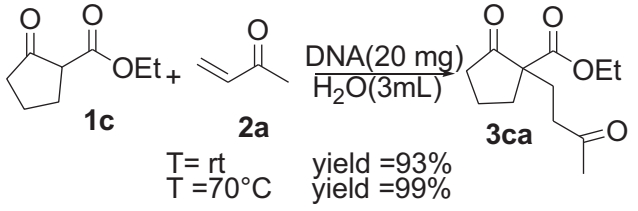


Fig. 6. Fluorescence spectra (a) DNA. (b) ethyl-2-oxocyclopentanecarboxylate (**1c**). (c) **1c**-DNA at rt (d) **1c**-DNA at 70 °C. All spectra were measured in water with excitation at 280 nm. The concentrations of DNA and ethyl-2-oxocyclopentanecarboxylate were 90 μM and 1 mM, respectively.

The addition of DNA significantly quenched the fluorescence intensity of **1c** at room temperature, with a hypsochromic shift of 14 nm and, to our surprise, the fluorescence quenching did not change with increasing temperature.

If our hypothesis is correct, namely that the interaction between the target molecule and DNA results in a change in the substrate fluorescence emission spectrum, and that the magnitude of the interaction also affects the efficiency of the reaction, our data suggest that the reaction between **1c** and **2a** should run at room temperature with a conversion, that is, similar to the reaction carried out at 70 °C. Indeed, when we performed the reaction of **1c** with **2a** at room temperature it proceeded efficiently with an excellent conversion (93%) (Scheme 3). This value closely resembles the conversion found at 70 °C (99%,Table 2, entry 3).



Scheme 3. Michael addition of **1c** to methyl vinyl ketone at room temperature and at 70 °C.

Finally, we registered the fluorescence emission spectrum of the Michael donor **1d** with and without DNA upon excitation at 250 nm (Fig. 7).

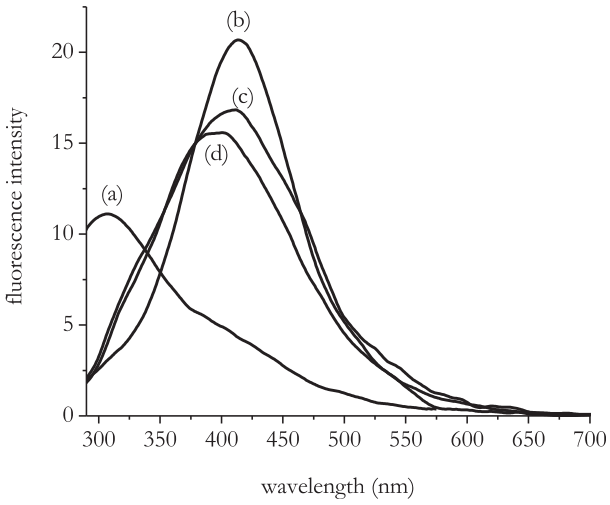
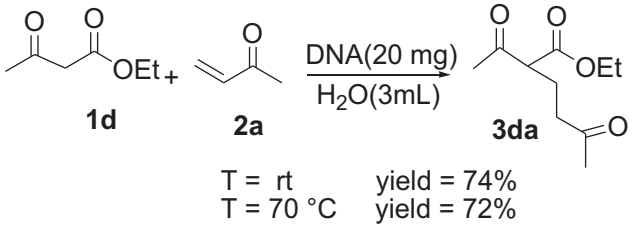


Fig. 7. Fluorescence spectra (a) DNA. (b) ethyl-3-oxobutanoate (**1d**). (c) **1d**-DNA at rt (d) **1d**-DNA at 70 °C. All spectra were measured in water with excitation at 250 nm. The concentrations of DNA and ethyl-3-oxobutanoate were 90 μM and 1 mM, respectively.

When DNA was added, the fluorescence intensity of **1d** was reduced even at room temperature. Specifically, a fluorescence quenching of 20% was registered with a small hypsochromic shift of 2 nm. The heating of the system to 70 °C (reaction temperature) resulted in a further small enhancement of the fluorescence quenching (25%) with a higher hypsochromic shift of 18 nm. Again, the fluorescence data seemed to indicate the possibility of performing the reaction at room temperature with an efficiency comparable to that of higher temperatures. Accordingly, it was found that the reaction of **1d** with 3-buten-2-one **2a** proceeded at room temperature with a conversion (74%) (Scheme 4) comparable to that reported at 70 °C (72%).



Scheme 4. Michael addition of **1d** to methyl vinyl ketone at room temperature and at 70 °C.

To further test our hypothesis, we selected the acceptor **2e** because it displayed a significant fluorescence emission spectrum and it was a nitro-acceptor (Fig. 8). In this case, a slight fluorescence quenching (5%) was observed when DNA was added at room temperature, whereas, a further 37% quenching with a bathochromic shift of 2 nm was registered when monitoring the fluorescence emission of **2e**-DNA at 70 °C. This result is consistent with the fact that no reaction between **1a** and **2e** was observed when the reaction was performed at room temperature.

This fluorescence trend closely resembles what we observed when following the fluorescence intensity of ethyl-3-oxo-3-phenylpropanoate in the presence of st-DNA (Fig. 5). Upon DNA

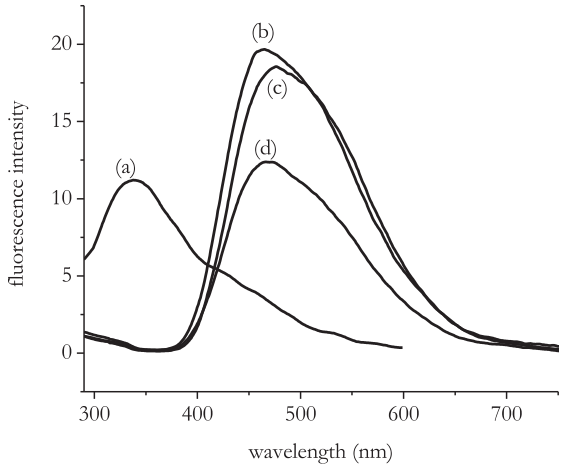


Fig. 8. Fluorescence spectra (a) DNA. (b) 2-(*E*)-2 -nitrovinyl-furan (**2e**). (c) **2e**-DNA at rt (d) **2e**-DNA at 70 °C. All spectra were measured in water with excitation at 280 nm. The concentrations of DNA and **2e** were 90 μM and 1.4 mM, respectively.

addition to the sample solution the fluorescence emission of all the molecules tested was quenched. The intensity of the fluorescence quenching was related to the efficiency of the Michael reactions performed experimentally.

3. Conclusion

In conclusion, the results described here prove that genomic salmon testes DNA can be efficiently used as an alternative catalyst for the aqueous Michael additions of 1,3-dicarbonyl compounds to activated alkenes. The procedure reported is operationally simple, general and affords moderate to excellent yields of the Michael adducts, in addition, DNA can be reused at least three times without loss of activity. The synthetic work was supplemented by fluorescence studies allowing us to obtain more preliminary information about the reaction. To our knowledge, this is the first time that fluorescence spectroscopy has been used to evaluate the catalytic activity of a genomic DNA and to gain insights into an organic reaction.

4. Experimental section

4.1. General remarks

All purchased chemicals were used without further purification. Salmon testes DNA and fish sperm DNA were obtained from Sigma and DNA solutions were prepared in super-pure water. The purity of DNA was checked by monitoring the ratio of absorbance at 260 nm to that at 280 nm (1.8–1.9). Concentrations in base pairs of st-DNA solutions were determined by absorption spectroscopy by using the extinction of 12,800 M^{-1} cm^{-1} at 260 nm.[20] The pH value of solutions was recorded on a CRISON micropH2001 precision acidity metre.

Elemental analyses were performed on the FlashEA 1112 Series with Thermal Conductivity Detector (Thermo Electron Corporation). ^1H and ^{13}C NMR spectra were recorded on a Bruker AM 250 (250.13 MHz for ^1H, 62.89 MHz for ^{13}C), Bruker DRX 300 (300 MHz for ^1H; 75 MHz for ^{13}C) and Bruker DRX 400 (400 MHz for ^1H; 100 MHz for ^{13}C). *J* values are given in Hertz. The ^1H chemical shifts were referenced to the solvent peak: $CDCl_3$ (7.26 ppm), and the ^{13}C chemical shifts were referenced to the solvent peak: $CDCl_3$ (77.0 ppm). Mass spectra were recorded on a Micromass Quattro micro API mass spectrometer (EI, 70 eV). IR spectra were recorded on an FTIR instrument (Bruker Vector 22). Thin-layer chromatography was performed on Merck Kiesegel 60 (0.25 mm) in appropriate solvent. Column chromatography was carried out using silica gel 60 (70–230 mesh ASTM, Merck).

4.2. Fluorescence measurements

Absorption spectra were recorded on a Cary-50 Spectrophotometer with a slit width equivalent to a bandwidth of 5 nm. Fluorescence spectra were measured on a Cary Eclipse Spectrophotometer with excitation wavelength of 280 nm or 250 nm (as specified in the figure caption) and emission slit width of 5 nm. A 10×10 mm^2 airtight quartz cuvette (Hellma Benelux bv, Rijswijk, Netherlands) was used for all the measurements. Experiments were performed in super-pure water. For the experiments at 70 °C, the cuvette was filled with the sample solution, the temperature was set by using a digital thermostat and checked prior to each measurement.

4.3. General procedure for DNA-catalyzed Michael reaction

After dissolution of salmon testes DNA (6.6 mg mL^{-1}) in 3 mL of super-pure water at room temperature, the solution was stirred for 15 min before adding donor (0.5 mmol) and acceptor (0.7 mmol). The reaction temperature was raised to 70 °C and the whole was stirred for 24 h at the same temperature. After cooling to room temperature the reaction mixture was diluted with ethyl acetate and poured into a separating funnel. The organic material was extracted (2×3 mL). The combined ethyl acetate fractions were then dried over $MgSO_4$ and after removal of the solvent the mixture was purified by column chromatography (Hexane/AcOEt as eluent) to give pure products.

The spectral data of compound **3aa**,[20] **3ba**,[20] **3ca**,[21] **3da**,[22] **3db**,[20] **3ac**,[22] **3ad**,[20] **3ae**,[23] matched with those reported in literature.

4.4. Procedure for catalyst recycling

The recovered DNA aqueous solution after reaction work-up was used as the reaction medium for the next run.

To this recovered aqueous solution, 0.5 mmol of donor and 0.7 mmol of acceptor were added. After the mixture was heated at 70 °C for 24 h, the mixture was extracted three times with ethyl acetate. Repeating the procedure described above, the corresponding products were obtained.

*4.4.1. Ethyl-2-benzoyl-5-oxo-heptanoate (**3ab**).* Colourless oil; [Found: C, 69.51; H, 7.27. $C_{16}H_{20}O_4$ requires C, 69.54; H, 7.30%]; R_f (20% hexane/AcOEt) 0.40; ν_{max} (neat) 2979, 1738, 1714, 1687, 1448, 1186 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.02–8.00 (2H, m, Ph), 7.58–7.53 (1H,m, Ph), 7.48–7.42 (2H, m, Ph), 4.43 (1H,dd, *J* 7.9, 6.5 Hz, COC*H*CO), 4.11 (2H, q, *J* 7.2 Hz, O$CH_2$$CH_3$), 2.57–2.50 (2H, m, CO$CH_2$$CH_2$), 2.37 (2H, q, *J* 7.3 Hz, CO$CH_2$$CH_3$), 2.28–2.16 (2H, m, COCH$_2$$CH_2$), 1.13 (3H, t, *J* 7.2 Hz, OCH$_2$$CH_3$), 1.01 (t, *J*=7.3 Hz, 3H); δ_C (62.89 MHz, $CDCl_3$) 210.5, 195.2, 169.7, 135.8, 133.5, 128.7 (x2), 128.6 (x2), 61.3, 52.6, 39.0, 35.8, 22.7, 13.9, 7.7; *m*/*z* 299 ($M+Na^+$).

*4.4.2. 3-Benzoyl-octane-2,6-dione (**3bb**).* Pale yellow oil; [Found: C, 73.12; H, 7.39. $C_{15}H_{18}O_3$ requires C, 73.15; H, 7.37%]; R_f (20% hexane/AcOEt) 0.35; ν_{max}(neat) 2976, 2938, 1719, 1707, 1596, 1580, 1449, 1358 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 8.03–7.99 (2H, m, Ph), 7.60–7.44 (3H, m, Ph), 4.56 (1H, t, *J* 6.9 Hz, COC*H*CO), 2.60–2.12 (6H, m), 2.12 (3H, s, COCH_3), 1.02 (3H, t, *J* 7.3 Hz, CH_2CH_3); δ_C (62.89 MHz, $CDCl_3$) 210.6, 204.0, 196.6, 136.2, 133.8, 128.9 (x2), 128.7 (x2), 61.2, 39.1, 35.9, 28.5, 22.5, 7.7; *m*/*z* 246 (M^+).

*4.4.3. 2-Ethoxycarbonyl-2-(3-oxopentyl)cyclopentanone (**3cb**).* Colourless oil; [Found: C, 65.01; H, 8.42. $C_{13}H_{20}O_4$ requires C, 64.98; H, 8.39%]; R_f (20% hexane/AcOEt) 0.45; ν_{max} (neat) 2978, 1742, 1714,

1448, 1370, 1163 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.10 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 2.64–2.56 (1H, m, CH_2CH_2), 2.42–2.21 (5H, m, CH_2CH_2), 2.09–1.79 (6H, m, CH_2CH_2), 1.18 (3H,t, *J*=7.1 Hz, OCH_2CH_3), 0.97 (3H, t, *J* 7.4 Hz, $OCCH_2CH_3$). δ_C (100 MHz, $CDCl_3$) 214.8, 210.4, 171.3, 61.3, 59.0, 37.9, 37.4, 35.8, 34.2, 26.9, 19.5, 14.0, 7.7; *m*/*z* 240 (M^+).

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