Baker's yeast-mediated enantioselective reduction of substituted fluorenones

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Received (in Cambridge, UK) 21st November 2005, Accepted 6th January 2006
First published as an Advance Article on the web 13th January 2006
DOI: 10.1039/b516475c

In the presence of DMSO as co-solvent and under vigorous agitation, baker's yeast in water was found to reduce substituted fluorenones to the corresponding fluorenols in good to excellent enantioselectivities.

Fluorenol and substituted fluorenols are very important structural units in many biologically active compounds,[1] and are also widely used as synthetic intermediates,[2] transition metal ligands[3] and analytical reagents.[4] However, enantiomerically pure substituted fluorenols have been scarcely applied due to the lack of efficient preparation methods to obtain them. Very recently, enantiomerically pure substituted fluorenols, which were obtained by chiral HPLC resolution of racemates, were used as key intermediates for the synthesis of unique ferroelectric SmC* liquid crystals and exhibited their attractive potential as organic synthons.[5]

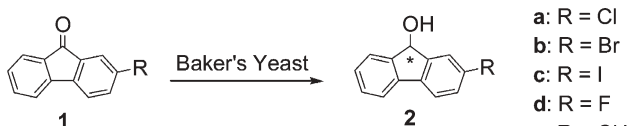
Although the enantioselective reduction of prochiral ketones is the most important and powerful method for the preparation of enantiomerically pure alcohols, the reduction of fluorenones by this means is still extremely challenging.[6] It may be due to the following two reasons: (1) high steric hindrance caused by the rigid structure of substituted fluorenone prevents the approach of the chiral reductor; (2) the prochiral center is, moreover, linked to two coplanar aromatic rings, which due to strong conjugation are highly electronically similar and thus it becomes extremely difficult to distinguish between them. Therefore, the development of a novel and efficient method for the enantioselective reduction of substituted fluorenones is not only of considerable synthetical significance but is also methodologically crucial.

In the field of organic synthesis, enzyme-catalysed reactions have become increasingly important, especially for the preparation of enantiomerically pure compounds difficult to obtain by conventional chemical means. For biocatalytic asymmetric reduction of various carbonyl compounds, the baker's yeast whole-cell system is often the reagent of choice.[7] Unfortunately, it has already been demonstrated and widely accepted that baker's yeast is inefficient for reducing highly sterically hindered ketones[7,8] In addition, baker's yeast-catalysed reductions are often hampered by low enantioselectivities, which can be traced to the presence of multiple enzymes with divergent enantiomeric preferences. As part of our continued interest in enzyme chemistry,[9] we herein describe our effort towards enantioselective reduction of substituted fluorenones using baker's yeast (Scheme 1).

Initially, 2-chloro-fluorenone **1a** was chosen as a model to explore the feasibility of the reaction. In a typical experiment,† **1a** was added to a dry baker's yeast suspension and the reaction mixtures were incubated in an orbital shaker. Even though the reaction was carried out for 7 days, no desired product was detected by HPLC (Table 1, entry 1). Because the substrate **1a** has poor solubility in water, improved solid-to-liquid mass-transfer was desired. Hence a magnetic stirrer was used as an alternative agitation method. As expected, the desired alcohol **2a** was detected, but the conversion was only about 3% after stirring for 7 days (entry 2). To further improve mass-transfer, the magnetic stirrer was replaced by a mechanical one, which provided a higher agitation speed and larger impeller area. In this way and when the agitation speed was maintained at 600 rpm, the conversion was improved to 23% after 4 days. More importantly, the enantioselectivity of product **2a** was 80% ee (entry 3).

To further facilitate the mass-transfer progress, the substrate was dissolved in a 10% v/v water-miscible co-solvent before it was added into the baker's yeast suspension. As shown in Table 1, various co-solvents markedly improved the conversion. With acetonitrile as an exception (entry 6), the co-solvents enhanced the enantioselectivity in the reactions. When DMSO was used, not only was the conversion dramatically improved to 65%, but surprisingly the enantioselectivity was enhanced to >99% ee (entry 10).

In order to explore the reaction scope, a series of 2-substituted fluorenones were examined under the optimized reaction conditions (Table 1, entry 10). The results are summarized in Table 2.‡ Reduction of 2-bromo-fluorenone **1b** and 2-iodo-fluorenone **1c** gave **2b** and **2c** with >99% ee in 52% conversion and 100% ee in 41% conversion, respectively (entries 2 and 3). However, reduction of 2-fluoro-fluorenone **1d** afforded **2d** with 65% ee, and the reaction conversion was up to 97% after 3 days (entry 4). Obviously, the very small difference in effective volume between a fluorine and a hydrogen atom makes it more difficult for the carbonyl reductases present in baker's yeast to distinguish between the two aromatic rings attached to the prochiral center in **1d**. The high reactivity of this compound may be attributed to the fact that



Scheme 1

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Table 1 Enantioselective reduction of **1a** using baker's yeast[a]

Entry	Agitation	Co-solvent	Time/d	Conversion[b] (%)	ee[c,d] (%)
1	Orbital shaker	None	7	0	—
2	Magnetic stirrer	None	7	3	—
3	Mechanic stirrer	None	4	23	80
4	Mechanic stirrer	Ethanol	4	47	85
5	Mechanic stirrer	THF	4	44	88
6	Mechanic stirrer	Acetonitrile	4	51	70
7	Mechanic stirrer	1,4-Dioxane	4	37	91
8	Mechanic stirrer	1,2-Dimethoxyethane	4	53	87
9	Mechanic stirrer	DMF	4	46	88
10	Mechanic stirrer	DMSO	4	65	>99

[a] Reaction conditions: substrate (100 mg), water (100 ml), co-solvent (10 ml), dry baker's yeast (10.0 g), sucrose (2.5 g), at 30 °C. [b] The conversion was determined by the ^{1}H NMR spectrum of the crude product. [c] The absolute configuration was determined to be *R* by comparing its optical rotation with the literature data. [d] The ee was determined by HPLC using a chiralcel OD-H column.

Table 2 Enantioselective reduction of **1a–e** using baker's yeast[a]

Entry	Substrate	Time/d	Conversion[b] (%)	Yield[c] (%)	ee[d] (%)	$[\alpha]_D^{20}$[e]
1	**1a**	4	65	49	>99 (*R*)	+12.7
2	**1b**	4	52	40	>99	+22.7
3	**1c**	4	41	31	100	+13.0
4	**1d**	3	98	78	65	+0.27
5	**1e**	4	43	32	52	+7.74

[a] Reaction conditions: substrate (100 mg), water (100 ml), DMSO (10 ml), dry baker's yeast (10.0 g), sucrose (2.5 g), at 30 °C. [b] The conversion was determined by the ^{1}H NMR spectrum of the crude product. [c] Isolated yield. [d] The ee was determined by HPLC using a chiralcel OD-H column. [e] *c* 0.33–0.40, $CHCl_3$, 20 °C.

the strong electronegativity of a fluorine substituent gives rise to an electron deficient carbonyl group, which facilitates attack by hydride. Furthermore, substituted fluorenone **1e** bearing an electron-donating group was also successfully reduced to the corresponding alcohol. Despite the sterical similarity between methyl and chloro groups, the reduction of **1e** gave **2e** with 52% ee in 43% conversion, indicating that electronic effects also play an important role for the enantioselectivity (entry 5).

The absolute configuration of **2a** was determined to be *R* by comparing its optical rotation with the literature data.[10] It indicates that the baker's yeast-catalysed reduction of **1a** did not follow Prelog's rule and the hydrogen transfer took place preferentially from the prochiral *Si*-face. The enantiomerically pure **2b–e** were first obtained and their absolute configurations will be determined by X-ray crystallography as soon as well diffracting crystals have been obtained.

The experimental results clearly revealed that when mass-transfer limitations are overcome, baker's yeast is able to reduce even highly sterically hindered ketones, such as substituted fluorenones. Agitation efficiency, which has been almost neglected in enzyme-catalysed reactions, was proven to be crucial to whether or not the reaction could proceed. It is not clear why DMSO leads to significant enhancements in enantioselectivity. At present, we suggest that DMSO has different effects on the activities of the *R*-and *S*-reductases in baker's yeast, obviously with increased enantioselectivity as a consequence. The experimental results also demonstrate the unique advantage of using a biocatalyst instead of a chemical one. The former can better distinguish two sterically and electronically similar aromatic rings.

In summary, we have demonstrated the first example of a highly enantioselective baker's yeast-reduction of substituted fluorenones. Notably, the research expands the substrate spectrum of baker's yeast-catalysed reactions.

We are grateful to the National Key Project for Basic Research (2003CB114400), the National Natural Science Foundation of China, and the Ministry of Science and Technology Foundation of Shanghai (04DZ05605) for financial support of this research.

Notes and references

† Experimental procedure: dry baker's yeast (10.0 g) was suspended in water (100 ml) containing sucrose (2.5 g) and stirred at 30 °C for 0.5 h, then the substrate (100 mg) dissolved in organic solvent (10 ml) was added. The reaction mixture was vigorously stirred at 30 °C and the progress of the reaction was monitored by HPLC. After completion of the reaction, ethyl acetate (500 ml) was added to the reaction mixture. The separated organic phase was filtered through a celite pad and dried over anhydrous $MgSO_4$. Then, after filtration and removal of organic solvent under reduced pressure, the crude mixture was purified by flash chromatography (silica gel, *n*-hexane : ethyl acetate =10 : 1, v/v) to afford the pure substituted fluorenols.

‡ Spectral data for the compounds **2a–e**. 2-Chloro-fluorenol (**2a**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81–7.78 (m, 2H), 7.60–7.58 (m, 2H), 7.45–7.32 (m, 3H), 5.50 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.0, 146.6, 138.2, 138.3, 131.8, 128.5, 128.3, 127.7, 125,1, 125.0, 121.4, 120.1, 73.3; IR (neat, cm^{-1}) 3298.6, 1027.4; HRMS-EI (70 eV) *m/z* calcd for $C_{13}H_9ClO$ $[M - H]^-$ 215.0264, found 215.0260; mp 141.0–142.0 °C; $[\alpha]_D^{20}$ + 12.7 (*c* 0.35, $CHCl_3$); HPLC analysis was performed by chiral column (Chiralcel OD-H), *n*-hexane : isopropyl alcohol = 98.5 : 1.5, UV detection at 254 nm, flow 1.0 mL min^{-1}, retention time: t_R = 39.21 min, t_S = 41.80 min. 2-Bromo-fluorenol (**2b**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (d, *J* = 7.6 Hz, 1H), 7.76–7.72 (m, 2H), 7.59–7.56 (m, 2H), 7.41–7.33 (m, 2H), 5.50 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.2, 146.4, 138.6, 138.2, 131.1, 128.5, 127.9, 127.8, 125.0, 121.8, 120.3, 120.1, 73.3; IR (neat, cm^{-1}) 3420.7, 1028.3; HRMS-EI (70 eV) *m/z* calcd for $C_{13}H_9BrO$ $[M - H]^-$ 258.9759, found 258.9749; mp 135.0–136.0 °C; $[\alpha]_D^{20}$ + 22.7 (*c* 0.33, $CHCl_3$); HPLC analysis was performed by chiral column (Chiralcel OD-H), *n*-hexane : isopropyl alcohol = 99.0 : 1.0, UV detection at 254 nm, flow 1.0 mL min^{-1}, retention time: $t_{(+)}$ = 38.34 min, $t_{(-)}$ = 40.09 min. 2-Iodo-fluorenol (**2c**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (s, 1H), 7.77–7.73 (m, 2H), 7.61–7.59 (m, 2H), 7.39–7.32 (m, 3H), 5.49 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.5, 146.4, 139.3, 138.7,137.3, 134.1, 128.8, 128.3, 125.3, 122.3, 120.4, 93.3, 73.5; IR (neat, cm^{-1}) 3274.6, 1026.6; HRMS-EI (70 eV) *m/z* calcd for $C_{13}H_9IO$ $[M - H]^-$ 306.9620, found 306.9623; mp 122.1–123.0 °C; $[\alpha]_D^{20}$ + 13.0 (*c* 0.38, $CHCl_3$); HPLC analysis was performed by chiral column (Chiralcel OD-H); *n*-hexane : isopropyl alcohol = 95.5 : 4.5, UV detection at 254 nm, flow 1.0 mL min^{-1}, retention time: $t_{(-)}$ = 17.18 min, $t_{(+)}$ = 18.25 min. 2-Fluoro-fluorenol (**2d**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82–7.75 (m, 2H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.38 (m, 2H), 7.30 (dd, *J* = 7.6, 7.2 Hz, 1H), 7.21 (t, *J* = 10.0 Hz, 1H), 5.50 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8–161.3 (d), 149.7, 146.9, 138.9, 136.1, 129.1, 127.7, 125.5, 121.9–121.8 (d), 120.2, 115.9–115.6 (d), 112.9–112.6 (d), 73.6; IR (neat, cm^{-1}) 3308.8, 1024.4; mp 141.4–142.0 °C; $[\alpha]_D^{20}$ + 0.27 (*c* 0.40, $CHCl_3$); HRMS-EI (70 eV) *m/z* calcd for $C_{13}H_9FO$ 200.0637, found 200.0636; HPLC analysis was performed by chiral column

(Chiralcel OD-H), *n*-hexane : isopropyl alcohol = 98.95 : 1.05, UV detection at 254 nm, flow 1.0 mL min^{-1}, retention time: $t_{(-)}$ = 60.18 min, $t_{(+)}$ = 65.59 min. 2-Methyl-fluorenol (**2e**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 (d, 7.6, 1H), 7.64 (d, 7.6, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.40 (s, 1H), 7.36 (dd, J = 7.2, 7.6 Hz, 1H), 7.28 (dd, J = 7.6, 7.2 Hz, 1H), 7.19 (d, J = 7.6 Hz, 1H), 5.44 (s, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.2, 146.8, 139.6, 137.0, 136.9, 129.1, 128.4, 127.1, 125.8, 125.1, 119.8, 119.6, 73.5, 21.3; IR (neat, cm^{-1}) 3328.6, 1027.0; HRMS-EI (70 eV) *m/z* calcd for $C_{14}H_{12}O$ 196.0888, found 196.0885; mp 143.5–144.5 °C; $[\alpha]_D^{20}$ + 7.74 (*c* 0.37, $CHCl_3$); HPLC analysis was performed by chiral column (Chiralcel OD-H), *n*-hexane : isopropyl alcohol = 95.0 : 5.0, UV detection at 254 nm, flow 1.0 mL min^{-1}, retention time: $t_{(-)}$ = 16.35 min, $t_{(+)}$ = 19.29 min.

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