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Albumin-controlled stereoselective reduction of 1,3-diketones to *anti*-diols†

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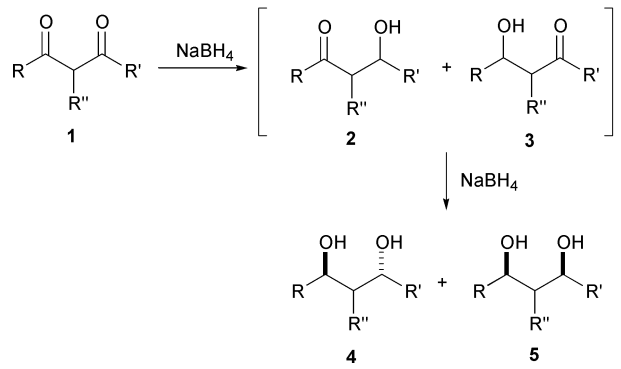
An unprecedented combination of high chemo- and stereoselectivity in the $NaBH_4$ reduction of 1:1 complexes between albumin and aromatic 1,3-diketones results in the formation of *anti* 1,3-diols with de up to 96%.

Albumins are well known for their ability to bind a wide range of small organic molecules.[1] While this property is inherent to their biological role as transport proteins, it can also be exploited to control the reactivity of bound species. Thus, the hydrophobic binding site and the presence of a general base in proximity of the bound substrate are at the basis of albumin's ability to behave as enzyme-like catalysts in reactions such as the Kemp elimination[2] and the decomposition of Meisenheimer complexes.[3] The asymmetric environment surrounding the bound substrate, on the other hand, allows albumins to be used as chiral auxiliaries and catalysts.[4]

We report here that albumins induce high levels of stereoselectivity in the borohydride reduction of β-diketones **1** and β-hydroxyketones **2** (Scheme 1, Table 1), leading to the formation of the corresponding *anti*-diols **4** with de up to 96%.‡



Scheme 1

The reduction of the dicarbonyl compounds **1** (Table 1) with sodium borohydride in 9:1 water–acetonitrile was followed by HPLC. In the absence of albumins the first reduction is not chemoselective and at 50% conversion the hydroxyketones **2** and **3** are present in nearly equimolar amounts (Table 1). Little or no diastereoselectivity is observed in the overall reduction to the *anti* and *syn* diols **4** and **5**.

When the reduction of diketones **1** is carried out in the presence of an equimolar amount of BSA, high levels of chemo- and stereoselectivity are observed (Table 1).§ With aryl-alkyl diketones **1a–g** the first reduction takes place preferentially at the aliphatic carbonyl giving β-hydroxyketones **2**, that are almost the sole monoreduction products observed at any conversion. Further reduction of **2** then yields racemic *anti* diols **4** with dr up to 98:2. Large aromatic groups such as *p*-iodophenyl (**1d**) or naphthyl (**1e**) lead to a decrease in selectivity (*anti*:*syn* ratio of 75:25 and 80:20, respectively). The selectivity also decreases when a bulky aliphatic group is present, as in **1g**; the presence of BSA, however, results in a shift of selectivity from 66:33 in favour of the *syn* diol **5g** to 55:45 in favour of the *anti* isomer **4g**. A stereoselective reduction is also observed in the symmetric diketone **1h** and in the branched diketone **1i**.

The presence of an aromatic ring is an essential requirement for the BSA-directed *anti* reduction of 1,3-diketones. This is clearly demonstrated by the reduction of pentane-1,3-dione **1l** which is unaffected by albumin giving a 1:1 mixture of *syn* and *anti* diols.

The regioisomeric hydroxyketones **2b** and **3b** (Table 1) were independently synthesized and reduced with $NaBH_4$ in the presence of BSA. Reduction of racemic **2b** proceeds with high diastereoselectivity giving the *anti*/*syn* pair of diols **4b** and **5b**, as racemic mixtures§ in a ratio (95:5) comparable with that obtained in the direct reduction of the diketone **1b**. Under the same conditions, enantiomerically pure (+)-(*S*)-**2b**, obtained by baker's yeast reduction of diketone **1b**,[5] gave (Scheme 1) the

† Electronic supplementary information (ESI) available: Scatchard and Lineweaver–Burk plots. See http://www.rsc.org/suppdata/cc/b2/b200474g/

Table 1 Albumin-directed reduction of 1,3-diketones and 3-hydroxyketones

				2:**3**[a]		*anti* (**4**):*syn* (**5**)[b]	
Substrate	R	R′	R″	No albumin	BSA	No albumin	BSA
1a	Ph	Me	H	60:40	>98:2	47:53	93:7
1b	*p*-MeC_6H_4	Me	H	66:33	>98:2	45:55	98:2
1c	*p*-MeC_6H_4	$(CH_2)_3OH$	H	60:40	>95:5	40:60	>95:5
1d	*p*-IC_6H_4	Me	H	65:35	80:20	43:57	75:25
1e	2-Naphthyl	Me	H	65:35	90:10	45:55	80:20
1f	Ferrocenyl	Me	H	60:40	90:10	60:40	94:6
1g	Ph	*t*-Bu	H	40:60	55:45	33:66	56:44
1h	Ph	Ph	H	—	—	52:40	96:4
1i	Ph	Me	Me	nd	nd	33:27:15:25[c]	5:45:5:45[c]
1l	Me	Me	H	—	—	50:50	50:50
(±)-**2b**	*p*-MeC_6H_4	Me	H	—	—	45:55	95:5
(+)-S-**2b**	*p*-MeC_6H_4	Me	H	—	—	45:55	94:6[d]
3b	*p*-MeC_6H_4	Me	H	—	—	50:50	50:50

[a] At 50% conversion. [b] At 100% conversion. [c] Reduction gives four diols that have been separated by HPLC but not identified. [d] (+)-(1*R*,3*S*)-**4b**: ee > 97%.

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anti diol (+)-(1*R*,3*S*)-**4b** with the same 95 : 5 dr observed for the racemic mixture and an enantiomeric excess greater than 97%. This clearly indicates that BSA does not discriminate between enantiomeric hydroxyketones **2** and that hydride addition takes place on both enantiomers with the same *anti*-stereoselectivity.

The reduction of hydroxyketone **3b**, on the contrary, is unaffected by BSA, leading to a 1 : 1 mixture of *syn* and *anti* diols (Table 1). This is consistent with the observation that a carbonyl adjacent to an aromatic ring is an essential feature for recognition by the protein and stereoselective reduction.

In the presence of BSA, a marked change is observed in the UV spectrum of diketone **1b**, the maximum at 316 nm, due to the enol form, being shifted to 325 nm with a corresponding increase in absorbance (Fig. 1a). This strong absorption is also observed in the CD spectrum (Fig. 1b): the absence of asymmetric centres in the substrate and the non-linear dependence from the substrate concentration (Fig. 1b) indicate that this band is due to a conjugated species bound to the protein. A Scatchard analysis† of the CD data reveals a single binding site for **1b** with an association constant of 2.4×10^4 l mol^{-1}.

In order to investigate the nature of the bound species, diketone **1b** and BSA were incubated in $H_2{}^{18}O$ for 5 days at room temperature, after which time no ^{18}O incorporation in the diketone was detected by ES-MS. Reaction of the diketone with the lysine residue present in the IIA binding site of BSA (Lys 199)[6] can thus be excluded as the reversible formation of an enaminone should lead to a fast isotope exchange in the diketone.[7] The characteristic UV and CD spectra of Fig. 1 can thus be attributed to the enol form of diketone **4b** non-covalently bound to albumin. The value of the binding constant obtained by the Scatchard analysis is consistent with literature data on the formation of non-covalent complexes between albumin and small aromatic molecules.[6]

Binding of hydroxyketones **2** and **3** to albumins can not be studied directly, since no spectral changes are observed. However, recognition of diketone **1b** by BSA is competitively and reversibly inhibited by the hydroxyketone **2b**. The inhibition effect is rather large, indicating that the binding constants of **4b** and **2b** are similar, and a value of 9×10^3 l mol^{-1} for the association constant of this hydroxyketone with BSA was obtained by a preliminary Lineweaver–Burk analysis of the competition experiments.† Hydroxyketone **3b** does not inhibit binding of the diketone **1b**, thus indicating that this substrate is not recognized by BSA, at least in the same site. Similarly, neither the *anti* nor the *syn* diols **4b** and **5b** inhibit diketone binding.

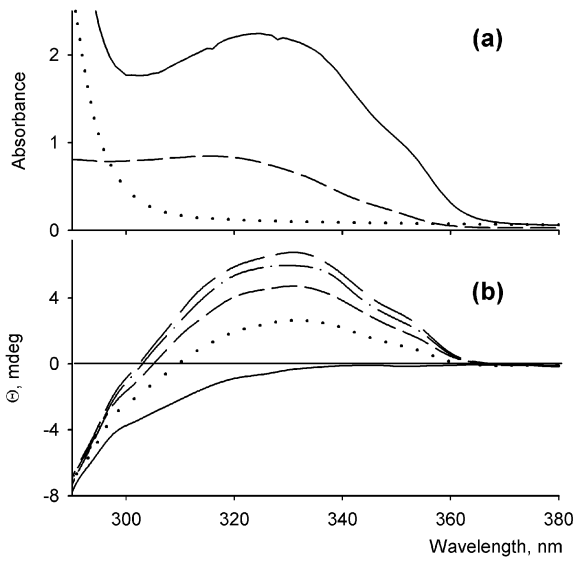

Fig. 1 (a) UV spectra of BSA (●●●●●), diketone **1b** (---), and a 1 : 1 mixture of BSA and **1b** (——). All substrates are 160 μM in water. (b) CD spectra of 60 μM BSA (——) and of BSA/**1b** mixtures. [BSA] = 60 μM; [**1b**] = 30μM (●●●●●), 60 μM (---), 90 μM (-●-●-) and 120 μM (— —).

From these findings a mechanistic hypothesis can be proposed. In the presence of albumin the first reduction of diketones **4** is highly regioselective favouring the formation of hydroxyketones **2** (Scheme 1). The UV and CD experiments indicate that the substrate is converted into enol upon binding, but do not exclude that a fraction of substrate is bound as the more reactive diketone tautomer (UV-transparent). While it is not possible at this stage to identify the reactive species, the control of chemo-selectivity by binding to albumins is, to our knowledge, unprecedented. Following the first reduction, both enantiomers of **2** are recognized by BSA in such a way that the second addition of hydride is directed toward the formation of the *anti* diol. Hydroxyketones **3** are not recognized by the protein and their reduction takes place without stereoselection. The *anti*-stereoselectivity in the reduction of diketones to diols thus originates from a combination of chemo-selectivity in the first step and diastereoselectivity in the second.

Work is in progress to verify the proposed mechanism and the structure of the complexes. The synthetic utility of the albumin-directed reduction is also being explored under catalytic conditions.

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Notes and references

‡ The stereochemistry of the diols was assigned from the ^{13}C shifts of the corresponding acetonides: S. D. Rychnovsky and D. J. Skalitzky, *Tetrahedron Lett.*, 1990, **31**, 945.

§ In a typical experiment, 6 eq. of $NaBH_4$ were added to a solution of BSA (100 mg, 1.5 μmol) and the diketone in 2 ml of H_2O–CH_3CN 9 : 1. The reaction mixture was sampled by drawing 200 μl aliquots to which 200 μl of ethanol and 5 μl TFA were added. The denaturated protein was removed by centrifugation and the solution was analyzed by HPLC with an Alltech Alltima C18 column. The enantiomeric excess of diol **4b** was determined by HPLC using a Polytech CHIRAL-PS1 column. The diol was obtained as a racemic mixture (ee ≤ 5%), except in the reduction of (+)-**2b** giving (+)-**4b** (ee > 97%). Hydroxyketone **2a** and diols **4a** and **4b** were also obtained from a 100 mg scale reduction of **1a** and **1b**, respectively. After extraction of the reaction mixture with ether and purification by preparative TLC, they resulted optically inactive.

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