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Unusual reversal of regioselectivity in antibody-mediated aldol additions with unsymmetrical methyl ketones

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Dedicated to Professor R.A. Lerner (The Scripps Research Institute, La Jolla, USA)

Abstract—A catalytic regio- and enantioselective aldol reaction of various unsymmetrical methyl ketones with para-nitrobenzaldehyde has been developed using aldolase antibodies as the catalysts. It has been found that the sense and level of regioselectivity for the reactions catalysed by antibody 38C2 and 33F12 are highly dependent on the structure of both the donor and the acceptor but in contrast, antibodies 84G3 and 93F3 catalyse the exclusive formation of the linear regioisomer independent of the structure of the reactants examined. The level of enantiocontrol is very high for most reactions. Both linear aldol enantiomers could be accessed through aldol or retro-aldol reactions using the same antibody. Theoretical studies on regioisomeric α- and β-heteroatom substituted enamines derived from unsymmetrical ketones suggest that most of the linear aldol products formed in the presence of antibodies 84G3 and 93F3 must be formed from intermediate enamines which are not the thermodynamically most favourable.
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1. Introduction

The development of strategies for the preparation of enantiomerically pure aldol products remains a very important area of research.[1] An extensive number of enantioselective aldol reactions of B, Ti, Si and Zr enolates using stoichiometric amounts of chiral sources have been reported.[2] In addition, catalytic strategies involving preformed enolate equivalents have been very promising.[3] However, the development of direct catalytic asymmetric aldol reactions starting from aldehydes and unmodified ketones remains an exciting challenge. The first reports of chemical catalysts for this process from the groups of Shibasaki,[4] List[5] and Trost[6] have recently appeared. Complementing traditional synthetic strategies, many diverse aldolase enzymes[7] and antibodies[8] have also been involved in direct aldol reactions and have provided efficient routes to elaborated targets including natural products. General methods for the preparation of enantiomerically enriched aldol products derived from unsymmetrical ketones that could lead to two regioisomeric products have not been developed. The simultaneous control of the regio-, diastereo- and enantioselectivity of direct aldol reactions involving unsymmetrical ketones constitutes one of the most demanding challenges in synthetic chemistry. A few direct catalytic aldol reactions with simple unsymmetrical ketones possessing two sites of enolisation were reported in the literature but examples of high levels of regioselectivity are rare when the reaction pathways leading to the regioisomers are too close in energy. We anticipate that in more demanding cases, enzyme or antibody catalysts can accentuate the energy difference in the regioisomeric transition states through multiple interactions and possibly favour the formation of the otherwise minor regioisomer. Unfortunately, the use of natural aldolases is hindered by the fact that enzymes usually show a strict requirement for the donor substrate. Unlike natural enzymes, aldolase antibodies were found to accept a wide range of ketone donor substrates including small aliphatic ketones. We recently described aldolase antibody 84G3, as a highly efficient catalyst for the regio- and enantioselective aldol reaction between *para*-nitrobenzaldehyde and four unmodified unsymmetrical methyl ketones with the new C–C bond formed on the less substituted α-side of these ketones. We also demonstrated the use of 84G3 as an efficient catalyst for the retro-aldol reaction, allowing for the kinetic resolution of the corresponding racemic secondary aldols.[9] With antibodies 84G3, 38C2, 33F12 and 93F3, we have now addressed four issues: (a) a study of the regioselectivity with 2-pentanone and 2-hexanone in the presence of these four antibodies; (b) for antibodies 84G3 and 38C2, the effect on the regioselectivity of the presence of an α- or β-heteroatom such as oxygen, sulfur, chlorine and fluorine on the ketone; (c) the enantioselectivity for both the forward aldol and

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reverse aldol reactions as well as the determination of kinetic parameters for these reactions; (d) theoretical calculations on the tautomeric equilibrium of 'imine–enamines' of imines derived from unsymmetrical methyl ketones.

2. Results and discussion

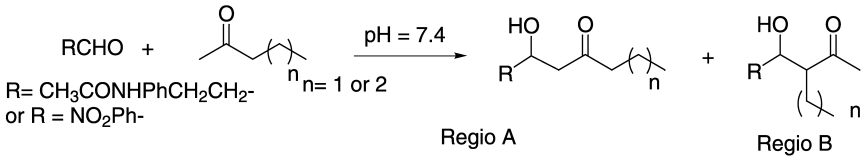
2.1. Regioselectivity of the aldol reaction of 2-pentanone and 2-hexanone with *para*-nitrobenzaldehyde or 3-(4′-acetamidophenyl)propanal (Table 1)

We set out to study the degree of regiocontrol that antibodies 38C2, 33F12, 84G3 and 93F3 could exercise on these reactions. For all experiments, the product assignment and the product distribution were proven unambiguously by comparison of the retention times with independently chemically synthesised standards using high performance liquid chromatography (HPLC). To screen for catalytic activity, we performed the reactions under the following defined conditions: 90 mM of donor, 130 μM of acceptor and 9 mol% antibody in PBS (pH=7.4) at room temperature. Control experiments revealed that, in the absence of antibody, no product formation could be detected under these conditions (entries 1, 6, 11). When higher donor and acceptor concentrations were used, the products were the *syn* and *anti* stereoisomers resulting from an addition of the more substituted carbon of 2-pentanone on the aldehyde. These results suggest that under these conditions, the reactions are under thermodynamic control. The antibody-catalysed reactions were carried out using experimental conditions under which no spontaneous reaction occurs. Under these conditions, we found that the product distribution is a function of the catalyst, the acceptor and the donor. Both antibodies 38C2 and 33F12 are moderate catalysts and afforded a mixture of regioisomeric products. The reaction of 2-pentanone with 3-(4′-acetamidophenyl)-propanal produced predominantly the branched isomer (entries 2 and 3) but in reaction with *para*-nitrobenzaldehyde, 2-pentanone led preferentially to the linear products (entries 7 and 8). The reaction of 2-hexanone with *para*-nitrobenzaldehyde did not produce any detectable amount of aldol products after 69 h in the presence of up to 25 mol% ab38C2 or ab33F12 suggesting that 2-hexanone is not a suitable donor for these two antibodies (entries 12 and 13). Previous reports in the literature revealed that in the presence of ab38C2, butanone reacts with 3-(4′-acetamidophenyl)propanal to give a 94:6 ratio of the two regioisomers with the branched aldol product as the major compound.[10] We found that in contrast to antibodies 38C2 and 33F12, antibodies 84G3 and 93F3 catalysed the exclusive formation of the linear regioisomer independent of the structure of the aldehyde or the all carbon methyl ketone. Indeed, all reactions involving 2-pentanone with *para*-nitrobenzaldehyde or 3-(4′-acetamidophenyl)propanal, and 2-hexanone with *para*-nitrobenzaldehyde afforded the corresponding aldol products resulting from a reaction on the less substituted carbon (entries 4–5, 9–10 and 14–15). In addition, antibodies 84G3 and 93F3 are more efficient catalysts as reflected by the reduced reaction times and the higher conversions. These results suggest that for the all carbon ketones that we have examined, the sense and level of regioselectivity for the reactions catalysed by antibodies 38C2 and 33F12 are highly dependent on the structure of both the donor and the acceptor. In contrast, antibodies 84G3 and 93F3 catalyse the exclusive formation of the linear regioisomer independent of the structure of the reactants examined herein (Table 1).

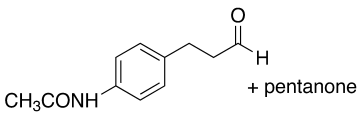
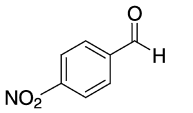
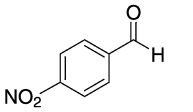
2.2. Effects of α- and β-heteroatom substituents on ketone aldolisation

Next, we focused on defining the regioselectivity in reactions involving unsymmetrical α- or β-heteroatom substituted ketones in the presence of antibodies 84G3 and 39C2. We examined first the aldol reactions between *para*-nitrobenzaldehyde and a series of unsymmetrical ketones possessing an α-heteroatom such as oxygen, sulfur, chlorine or fluorine (Table 2). The presence of the heteroatom α to the ketone greatly accelerates the spontaneous aldol additions when compared to the corresponding all carbon ketones. When performed under our standard conditions (defined as above), these reactions give preferentially the aldol products resulting from an addition at the more substituted carbon. These results suggest that under these conditions, thermodynamic control prevails but, for some of these reactions, the equilibrium constants are probably not sufficiently high to achieve a high degree of regioselectivity. The reactions leading to the products resulting from an addition of the aldehyde to the less substituted carbon of the donor are the disfavoured processes under these conditions. In order to define unambiguously the regioselectivity of these same reactions in the presence of the antibodies, it was critical to find screening conditions that suppress or at least minimize any spontaneous background reaction. This was achieved by lowering the temperature, adjusting the concentration of the different reactants and when necessary increasing the catalyst load. Under optimised conditions, it was found that, in the presence of antibody 84G3, methoxyacetone undergoes the aldol condensation with *para*-nitrobenzaldehyde to afford exclusively the linear regioisomer with a conversion of 76% after 41 h (entry 2). Similarly, the antibody-catalysed reactions of thiomethoxy- and chloroacetone are highly regioselective with the preferential formation of the otherwise disfavoured linear regioisomer resulting from an addition at the less substituted carbon (entries 5 and 8). The conversion for chloroacetone never exceeded 11% suggesting substrate or product inhibition as expected from the presence of a highly reactive electrophilic site that can chemically modify the active site lysine residue essential to the activity of the antibody. The reaction of fluoroacetone is much more efficient with 90% conversion after only 30 min in the presence of 25 mol% ab84G3 but less selective leading to a mixture of regioisomers with nevertheless preferential formation of the linear isomer (entry 11). Finally, hydroxyacetone proved to be the poorest substrate for antibody 84G3 as reflected by both the low conversion and the product distribution. After 15 h in the presence of a stoichiometric amount of ab84G3, only 10% conversion was observed with the formation of a 1/1 mixture of the two regioisomers (entry 14). The regioselectivity of these antibody-catalysed transformations contrast sharply with the results obtained for the uncatalysed reactions or in the presence of antibody 38C2. The product distribution observed for the uncatalysed reactions is in accordance with the reactivity expected from the presence

Table 1. Aldol reactions with 2-pentanone and 2-hexanone



1 n = 1 R= $CH_3CONHPhCH_2CH_2$- **2** n = 1 R= $CH_3CONHPhCH_2CH_2$-
3 n = 1 R = NO_2Ph- **4** n = 1 R = NO_2Ph-
5 n = 2 R= NO_2Ph- **6** n = 1 R = NO_2Ph-

Entry	Acceptor and donor	Conditions	Conversion (%)	Ratio A/B
1	 + pentanone	PBS, 11 days	0	0/0
2		ab38C2, 11 days	33	31/69 (lit.: 27/73)[10]
3		ab33F12, 11 days	37	29/71
4		ab84G3, 11 days	63	100/0
5		ab93F3, 11 days	79	100/0
6	 + pentanone	PBS, 135 h	0	0/0
7		ab38C2, 135 h	59	62/38
8		ab33F12, 135 h	75	60/40
9		ab84G3, 3 h	88	100/0
10		ab93F3, 2.5 h	87	100/0
11	 + hexanone	PBS, 26 h	0	0/0
12		ab38C2, 69h[a]	0	0/0
13		ab33F12, 69h[a]	0	0/0
14		ab84G3, 26 h	66	99/1
15		ab93F3, 12 h	35	100/0

[a] 25 mol% antibody for this experiment.

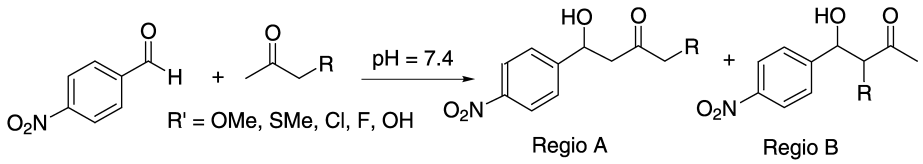
of the α-heteroatom on the donor ketone (entries 1, 4, 7, 10 and 13). Indeed, for chloroacetone and thiomethoxyacetone, it has been reported that the rates of enolisation at the methylene carbon are about 135 times faster than at the methyl carbon.[11] Aldol reactions with hydroxyacetone have also been reported to be highly regioselective providing for an easy access to the synthesis of vicinal diols. In contrast, methoxyacetone and fluoroacetone can react on both the C-1 and C-3 carbon depending on the reaction conditions.[12] Previous reports in the literature have shown that in contrast to antibody 84G3, reactions involving hydroxyacetone as the donor were the most efficient for antibody 38C2 yielding exclusively the *syn*-aldol product (entry 15).[13] Fluoroacetone is also a substrate for antibody 38C2 and combined to 3-(4′-acetamidophenyl)propanal afforded a mixture of three products, 72% of the *syn* branched isomer, 21% of the *trans*-branched isomer and only 7% of the linear regioisomer.[10] We found that ab38C2 afforded exclusively the branched isomer for the aldol reactions of *para*-nitrobenzaldehyde with methoxyacetone and with thiomethoxyacetone (entries 3 and 6). Therefore, for these two donors, it is possible to selectively access the branched and the linear regioisomer by using ab38C2 and ab84G3, respectively. Antibody 84G3 is a unique catalyst as this antibody promotes the preferential or exclusive formation of the less substituted regioisomer except for hydroxyacetone. This unusual reactivity could be regarded as an example, hitherto unknown, of the controlled generation of the less-substituted regioisomer independent of the presence of an α-heteroatom such as O, S, Cl or F under reaction conditions that would normally favour the formation of the more-substituted regioisomer.

For antibody 84G3, we also studied the regioselectivity of the reaction of *para*-nitrobenzaldehyde with three different β-heteroatom substituted ketones (Table 3). Under our standard conditions, the uncatalysed reactions proceeded slowly with the formation of the more substituted aldol product. We found that the presence of antibody 84G3 accelerated all three reactions and afforded exclusively the linear regioisomer. However, the reactions are substantially slower than the reactions involving ketones possessing an α-heteroatom with only poor conversions after extended reaction times despite the use of large amount of antibody. Nevertheless, the regioselectivity is excellent.

2.3. Enantioselectivity of the aldol and retro-aldol products and catalytic efficiency

To probe further the synthetic scope of these antibodies, we studied the enantioselectivity of several forward aldol reactions. The enantiomeric excesses of the products as determined by chiral-phase HPLC (Table 4) were all superior to 94%. All aldol products possess the (*R*)-configuration. To assign the absolute configuration unambiguously, products **5**,

Table 2. Aldol reactions of *para*-nitrobenzaldehyde and α-heteroatom substituted methyl ketones



7 R = OMe
9 R = SMe
11 R = Cl
13 R = F
15 R = OH

8 R = OMe
10 R = SMe
12 R = Cl
14 R = F
16 R = OH

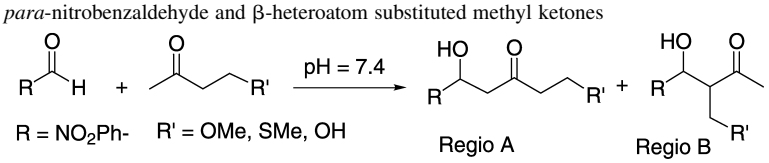
Entry	R	Conditions	Conversion (%)	Ratio A/B
1	OMe	PBS, 0 °C, 41 h	0	0/0
2	OMe	25% ab84G3, 0 °C, 41 h	76	100/0
3	OMe	25% ab38C2, 0 °C, 35 h	35	0/100
4	SMe	PBS, 0 °C, 1h40	9	10/90
5	SMe	25% ab84G3, 0 °C, 1h40	56	98/2
6	SMe	25% ab38C2, 0 °C, 29 h	53	1/99
7	Cl	PBS, 0 °C, 40 h	11	1/99
8	Cl	25% ab84G3, 0 °C, 40 h	11	95/5
10	F	PBS, 0 °C, 0.5 h.	4	30/70
11	F	25% ab84G3, 0 °C, 0.5 h	90	70/30
13	OH	PBS, rt 15 h	2	0/100
14	OH	100% ab84G3, 0 °C, 15 h	10	50/50
15	OH	ab38C2, rt	nd[a]	0/100[a]

[a] Conversion not reported, see Ref. [10].

7, **9**, **19** and **21** were prepared independently by asymmetric synthesis.[14] For compounds **3**, **13** and **17**, the absolute configuration has been attributed by analogy. We then studied the utility of antibody 84G3 in the kinetic resolution of racemic linear regioisomers. The racemic aldols were treated with ab84G3 (4 mol%) in aqueous buffer. Analysis by HPLC indicated that in each case the retro-aldolisation reactions halted at approximately 50% conversion. The catalyst was highly enantioselective and provided the recovered (*S*)-aldols with ee values typically greater than 91%. Both aldol enantiomers could, therefore, be accessed through aldol or retro-aldol reactions using the same antibody 84G3.

The results of the kinetic studies of four aldolisation reactions and four retro-aldol reactions are provided (Table 5). The kinetic parameters are reported per antibody site assuming that both sites of the antibody are active. All the forward aldol reactions with *para*-nitrobenzaldehyde and ketones performed under pseudo-first-order conditions showed Michaelis–Menten kinetics. The determination of k_{cat}/k_{uncat} was not possible because in the uncatalysed reactions, the formation of the linear regioisomer was negligible under our assay conditions. The data revealed that the introduction of a heteroatom within the ketone leads to an increase in the K_M values (entry 1 versus entries 2 and 3). Addition of an extra methylene group increases even further the K_M and results in a slower reaction rate (entry 4). The catalytic turnovers achieved by antibody 84G3 for the

Table 3. Aldol reactions of *para*-nitrobenzaldehyde and β-heteroatom substituted methyl ketones

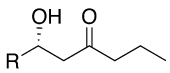
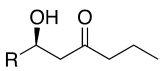
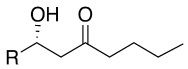
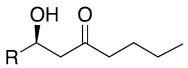
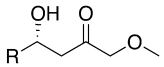
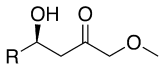
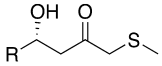
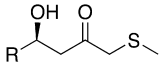
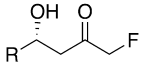
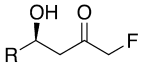
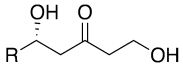
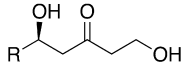
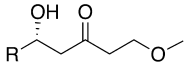
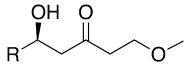
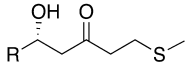
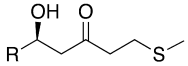


17 R = OH, R' = NO_2Ph
19 R = OMe, R' = NO_2Ph
21 R = SMe, R' = NO_2Ph

18 R = OH, R' = NO_2Ph
20 R = OMe, R' = NO_2Ph
22 R = SMe, R' = NO_2Ph

Entry	R	Conditions	Conversion (%)	Ratio A/B
1	OH	PBS, 0 °C, 77 h	7	0/100
2	OH	25% ab84G3, 0 °C, 41 h	40	100/0
3	OMe	PBS, rt, 20 h	1	0/100
4	OMe	100% ab84G3, 0 °C, 20 h	11	100/0
5	SMe	PBS, rt, 22 h	9	0/100
6	SMe	100% ab84G3, 0 °C, 22 h	41	95/5

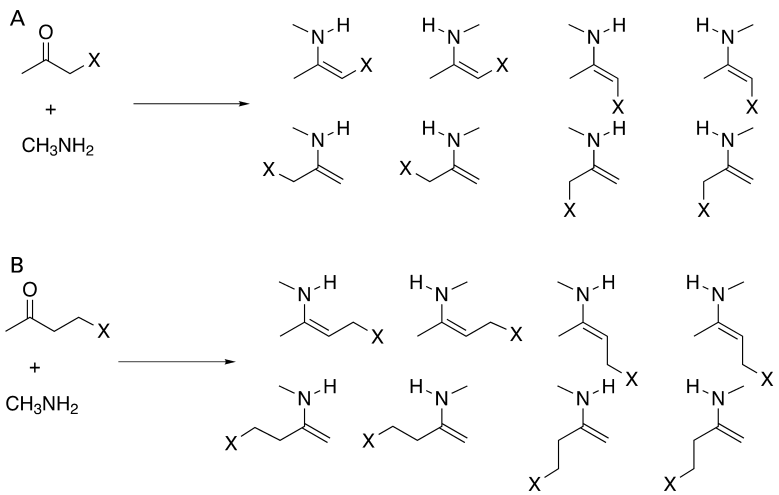
Table 4. ee Values for antibody-catalysed aldol and retro-aldol reactions with R=NO_2Ph–

Product aldol	ee (%)	Recovered product Retro-aldol	Conversion (%) ee (%)
 (*R*)-**3**	94	 (*S*)-**3**	55 97
 (*R*)-**5**	98	 (*S*)-**5**	50 94
 (*R*)-**7**	98	 (*S*)-**7**	47 97
 (*R*)-**9**	97	 (*S*)-**9**	50 96
 (*R*)-**13**	99	 (*S*)-**13**	50 94
 (*R*)-**17**	95	 (*S*)-**17**	55 91
 (*R*)-**19**	nd	 (*S*)-**19**	49 95
 (*R*)-**21**	96	 (*S*)-**21**	54 99

retro-aldol reactions were higher than those of the corresponding aldol reactions by 2.5 to 10-fold depending on the substrate (entries 5–8). The catalytic proficiency compares favourably with the efficiency of aldolase antibodies with other retro-aldol reactions.

2.4. Theoretical studies on regioisomeric α- and β-heteroatom substituted enamines derived from unsymmetrical ketones

To gain a better understanding of the unique reactivity of ab84G3, we carried out a computational study to assess the effect of various heteroatoms on the stability of regioisomeric enamines of unsymmetrical ketones. Lien et al. previously carried out similar computational studies on the substituent effects on imine–enamine tautomers of α-substituted acetaldimines $XCH_2CH{=}NH$ (X=H, BH_2, CH_3, NH_2, OH, F, Cl, CN, NO).[15] The geometries of α- and β-heteroatom substituted regioisomeric enamines derived from a series of unsymmetrical ketones (Scheme 1) were computed using hybrid density functional theory (B3LYP)[16] and the 6–31G* basis set[17] as implemented in Gaussian 98.[18] All gas phase minima were characterised by frequency analysis. Reported electronic energies include zero point energy corrections scaled by 0.9806.[19] The optimised geometries of four enamines derived from hydroxy, thiomethoxy, chloro, and fluoro acetones plus methylamine are shown in Figure 1. These include four conformations of each enamine with the substituent on the double bond, and four conformations with the substituent on the allylic carbon. The intramolecular hydrogen-bonded species are generally more stable than those lacking hydrogen-bonds, and the more substituted enamines are more stable than those with allylic substituents except for the enamine derived from fluoroacetone. The more substituted enamine is more stable by 1.1 kcal/mol for the hydroxy, 0.9 kcal/mol for the thiomethoxy and 1.1 kcal/mol for the chloro group when compared to the other regioisomer. In contrast, the allylic fluoride is more stable by only 0.2 kcal/mol. The optimised geometries of four enamines of butanone plus methylamine and five enamines derived from hydroxy butanones plus methylamine are shown in Figure 2. These include four conformations of each enamine with the substituent on the double bond, and four conformations with the substituent on the allylic carbon. The substituted enamine of butanone is more stable by 0.9 kcal/mol and the hydrogen bonded allylic enamine of hydroxy butanone is more stable by 1.5 kcal/mol when compared to the corresponding less substituted regioisomer. Antibody



Scheme 1. (A) Various conformations of regioisomeric enamines derived from the reaction of α-heteroatom substituted propanone with methyl amine. (B) Various conformations of regioisomeric enamines derived from the reaction of β-heteroatom substituted butanone with methyl amine.

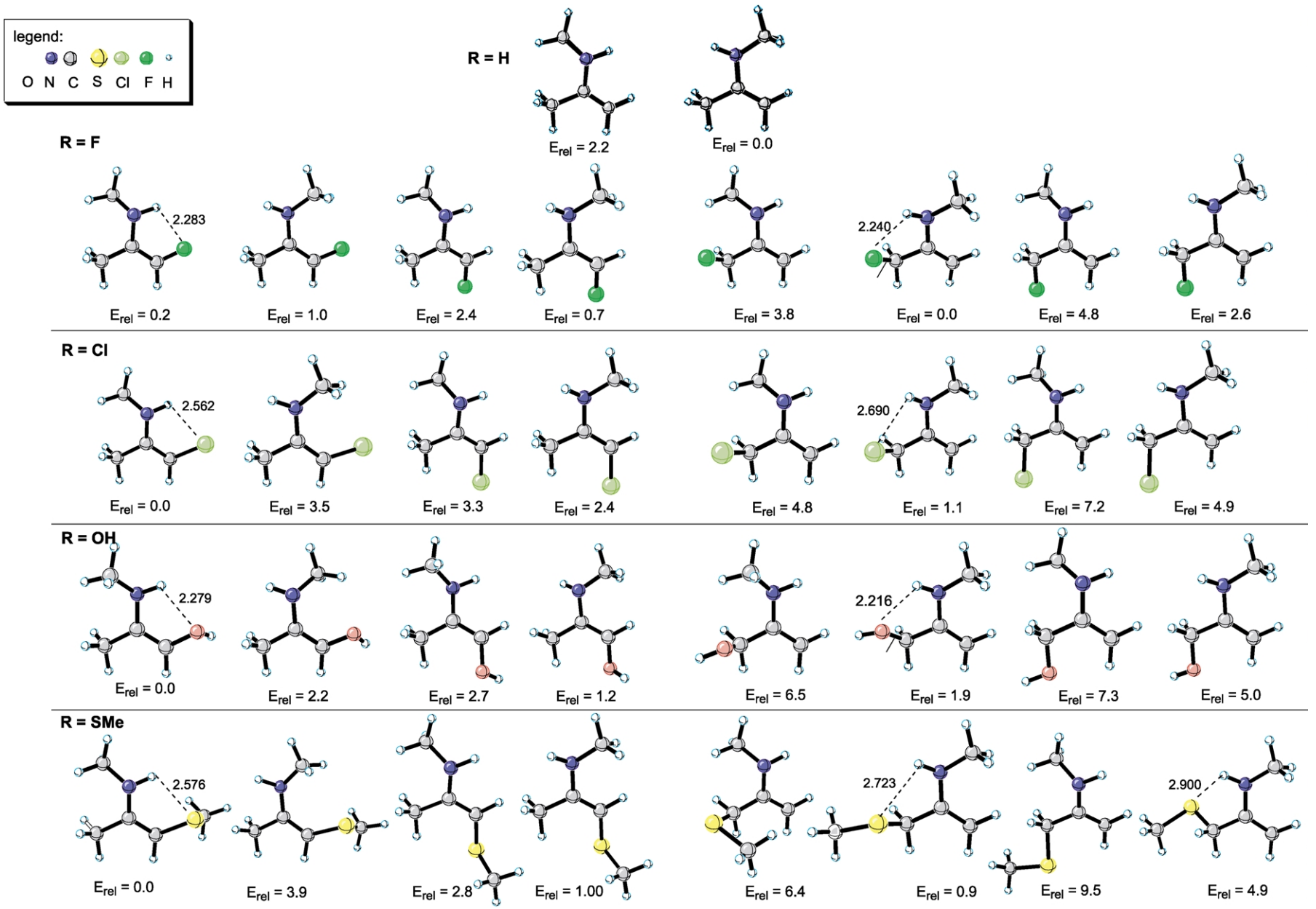


Figure 1. Optimized geometries of the various conformations of regioisomeric enamines derived from substituted methyl ketone. Relative energies are reported in kcal/mol and distances are reported in Angstroms.

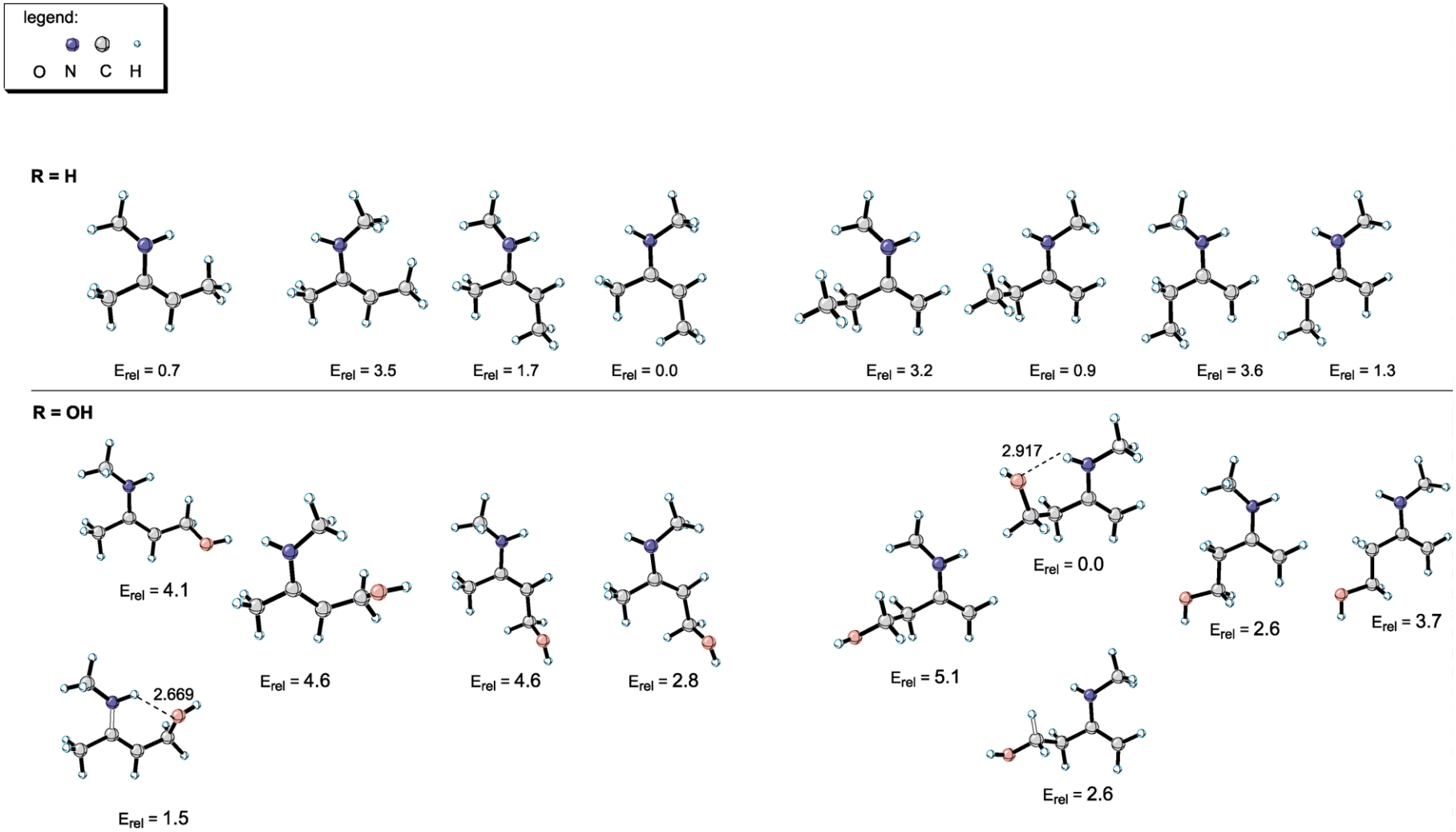


Figure 2. Optimized geometries of the various conformations of regioisomeric enamines derived from butanone and hydroxy substituted butanone. Relative energies are reported in kcal/mol and distances are reported in Angstroms.

Table 5. Kinetic parameters for selected aldol and retro-aldol reactions

Entry	Substrate	K_{cat} (min^{-1})	K_M (μM)	K_{cat}/K_{uncat}	$(K_{cat}/K_M)K_{uncat}$ (M^{-1})
1	2-Pentanone[a]	0.030	28	–	–
2	Methoxyacetone[a]	0.040	390	–	–
3	Thiomethoxyacetone[a]	0.089	217	–	–
4	4-Thiomethoxybutanone[a]	0.008	383	–	–
5	(±)-**3**	0.18	79	1.2×10^5	1.5×10^9
6	(±)-**7**	0.099	170	5.5×10^4	3.2×10^8
7	(±)-**9**	0.810	69	4.3×10^5	6.2×10^9
8	(±)-**21**	0.035	263	1.9×10^4	7.4×10^7

[a] With *p*-nitrobenzaldehyde.

84G3 is able to overcome the thermodynamic preferences shown by these calculations. That is, many of the observed products must be formed from intermediate enamines, which are not the thermodynamically most favourable.

3. Conclusion

Two major issues were under consideration. First was the critical issue as to whether aldolase antibodies 84G3 and 38C2 could differentiate the two reactive sites of unsymmetrical methyl ketones. Secondly, the possibility of controlling simultaneously the regio- and enantioselectivity of aldol reactions with a variety of unsymmetrical methyl ketones had to be determined. We have identified important differences between antibodies 38C2 and 84G3. As an overall trend, antibody 38C2 favours the formation of the branched regioisomer resulting from a reaction on the more substituted carbon whereas antibody 84G3 leads preferentially to the formation of the linear isomer. Therefore, the ab84G3-catalysed aldols and retro-aldolisations provide a rapid entry to enantioenriched linear aldol products that are difficult to access with other catalysts. Both enantiomers are accessible by using both the forward aldol and retro-aldol reactions. The retro-aldolisations are more efficient than the synthetic aldols as reflected by the higher rate constants. The unique reactivity of antibody 84G3 is quite remarkable since, to our knowledge, no known catalysts display similar level of regio- and enantiocontrol in the presence of the ketones used for this study. This unusual reactivity further demonstrates that, through a combination of immunological diversity and basic chemical principles, efficient catalysts that display new reactivity can be created. Further work is in progress to address the question of whether L-proline is capable of catalysing the aldol reactions of *para*-nitrobenzaldehyde and various unsymmetrical methyl ketones and assesses the degree of regio- and stereocontrol exercised by this catalyst. This study aims to compare the synthetic value of the antibody-with the L-proline-catalysed reactions.

4. Experimental

4.1. General

All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF) was obtained by distillation over sodium and benzophenone, dry methylene chloride (CH_2Cl_2) was obtained by distillation over calcium hydride. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Commercially available reagents were used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck aluminum foil backed sheets precoated with Kieselgel 60F-254 using UV light as visualizing agent and an ethanolic solution of potassium permanganate and heat as developing agent. Merck Silica gel C60 (40–60 μM) was used for flash column chromatography. NMR spectra were recorded on a Bruker DPX-400 or Bruker AMX-500 spectrometer and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; b, broad. The coupling constants *J* are given in hertz. IR spectra were recorded on a Perkin–Elmer Paragon 1000 FT-IR spectrometer. Mass spectra (*m*/*z*) and HRMS were recorded on Micromass GCT using Chemical Ionisation (NH_3, CI), Electronic Impact (EI+) or Field Ionization (FI). Microanalyses were performed by 'Elemental Microanalysis Limited', Devon. Melting points were determined in a capillary and are uncorrected.

4.2. Synthesis of racemic aldol products. Path A: direct cross aldolizations with LDA

To a solution of LDA, prepared by dropwise addition of *n*-butyllithium (1.1 mmol) to a solution of diisopropylamine (1.2 mmol) in dry THF (2 mL) at 0 °C, was added a solution of ketone (1 mmol) in THF (2 mL) at −78 °C. After the resulting mixture was stirred at −78 °C for 30 min, the aldehyde (1 mmol) in THF (1 mL) was added. The reaction was quenched after 3 h at −78 °C by addition of saturated NH_4Cl. The mixture was extracted three times with AcOEt, the combined organic layers were dried over $MgSO_4$ and solvents were removed in vacuo. Product ratios were determined by crude ^{1}H NMR spectroscopy before purification by flash column chromatography.

4.2.1. 1-(4′-Nitrophenyl)-1-hydroxy-3-hexanone 3. Crude ratio regio/*syn*/*anti*=78/13/9. Purification by column chromatography over silica (hexane/AcOEt: 80/20), followed by recrystallization from AcOEt yielded **3** as a white solid (38% pure and 18% in mixture with **4** (*syn* and *anti*). R_f 0.16 (hexane/AcOEt, 80/20); mp 59 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J*=8.6 Hz, 2H), 7.52 (d, *J*=8.6 Hz, 2H), 5.25 (m, 1H), 3.50 (bs, 1), 2.80 (m, 2H), 2.43 (t, *J*=7.4 Hz, 2H), 1.6 (tq, *J*=7.4, 7.4 Hz, 2H), 0.90 (t, *J*=7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.0, 150.2, 147.2, 126.4,

123.7, 69.0, 50.5, 45.4, 17.0, 13.6; IR (neat) ν_{max} 3516, 1702, 1515, 1346, 1088; Anal. calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.82; H, 6.36; N, 5.98; HRMS (EI+): M^+ 237.1001 (expected), 239.1009 (observed).

4.2.2. 1-(4′-Nitrophenyl)-1-hydroxy-3-heptanone 5. Crude ratio regio/*syn*/*anti*=78/13/9. Purification by column chromatography over silica (AcOEt/hexane: 30/70) afforded **5** as a colorless oil (26% pure and 23% in mixture with **6** (*syn* and *anti*). R_f 0.28 (AcOEt/hexane: 30/70); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J=8.8 Hz, 2H), 7.52 (d, J=8.8 Hz, 2H), 5.25 (m, 1H), 3.80 (d, J=2.4 Hz, 1H), 2.81 (m, 2H), 2.44 (t, J=7.6 Hz, 2H), 1.55 (m, 2H), 1.28 (m, 2H), 0.88 (t, J=7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.1, 150.2, 147.2, 126.4, 123.7, 69.0, 50.5, 43.3, 25.5, 22.2, 13.8; IR (neat) ν_{max} 3466, 2959, 1709, 1520, 1347, 1081; HRMS (EI+): M^+ 251.1158 (expected), 251.1162 (observed).

4.2.3. 8-(4′-Acetamidophenyl)-6-hydroxy-4-octanone 1. Crude ratio regio/*syn*/*anti*=85/8/7. Purification by column chromatography over silica (Et_2O/AcOEt: 80/20), followed by recrystallization from hexane/DCM afforded **1** as a white solid (48%); mp 96 °C. R_f 0.22 (Et_2O/AcOEt: 80/20); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (bs, 1H), 7.39 (d, J=8.0 Hz, 2H), 7.12 (d, J=8.0 Hz, 2H), 4.02 (m, 1H), 3.30 (d, J=2.8 Hz, 1H), 2.76 (m, 1H), 2.63 (m, 1H), 2.55 (m, 2H), 2.39 (t, J=7.2 Hz, 2H), 2.14 (s, 3H), 1.77 (m, 1H), 1.59 (m, 1H), 1.59 (tq, J=7.2, 7.2 Hz, 2H), 0.91 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.5, 168.5, 137.9, 135.7, 128.9, 120.0, 66.7, 48.9, 45.5, 38.0, 31.1, 24.4, 17.0, 13.6; IR (neat) ν_{max} 3304, 2962 and 2932, 1705, 1667, 1603, 1538 and 1515; HRMS (EI+): M^+ 277.1678 (expected), 277.1672 (observed).

4.2.4. 4-(4′-Nitrophenyl)-1,4-dihydroxy-2-butanone 15. 2.2 equiv. of diisopropylamine and 2.1 equivalent of *n*-BuLi were used. Purification by column chromatography over silica (DCM/AcOEt: 80/20) afforded **15** as a colourless oil (25%). R_f 0.12 (DCM/AcOEt: 80/20); ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (d, J=8.4 Hz, 2H), 7.63 (d, J=8.4 Hz, 2H), 5.28 (dd, J=8.9, 4.2 Hz, 1H), 4.26 (d, J=18.7 Hz, 1H), 4.22 (d, J=18.7 Hz, 1H), 2.88 (dd, J=15.8, 8.9 Hz, 1H), 2.75 (dd, J=15.8, 4.2 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 208.5, 152.2, 147.5, 126.8, 123.4, 68.9, 68.5, 47.7; IR (neat) ν_{max} 3413, 2913, 1703, 1512, 1348, 1035; HRMS (FI): M· 225.0637 (expected), 225.0638 (observed).

4.2.5. 4-(4′-Nitrophenyl)-4-hydroxy-1-methoxy-2-butanone 7 and 4-(4′-nitrophenyl)-4-hydroxy-3-methoxy-2-butanone 8 (*syn* and *anti*). Crude ratio regio/*syn*/*anti*=25/50/25. Purification by column chromatography over silica (AcOEt/hexane: 40/60) and (DCM/Et_2O: 90/10) afforded **7** as a white solid (8%) and *syn*-**8** and *anti*-**8** as pale yellow oils (6 and 23%, respectively).

Analytical data for **7**. R_f 0.20 (AcOEt/hexane: 40/60); mp 78 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, J=7.0 Hz, 2H), 7.55 (d, J=7.0 Hz, 2H), 5.31 (t, J=5.0 Hz, 1H), 4.05 (d, J=13.8 Hz, 1H), 4.01 (d, J=13.8 Hz, 1H), 3.48 (s, 1H), 3.42 (s, 3H), 2.90 (d, J=5.0 Hz, 2H); ^{13}C NMR (125 MHz $CDCl_3$) δ 208.5, 149.9, 147.2, 126.3, 123.7, 77.7, 68.7, 59.3, 47.2; IR (neat) ν_{max} 3436, 2936, 1726, 1519, 1348, 1088; Anal. calcd for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.86. Found: C, 55.01; H, 5.57; N, 5.83; HRMS (FI): M· 239.0794 (expected), 239.0789 (observed).

Analytical data for *syn*-**8**. R_f 0.25 (AcOEt/hexane: 40/60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, J=8.7 Hz, 2H), 7.57 (d, J=8.7 Hz, 2H), 5.06 (dd, J=6.8 and 4.1 Hz, 1H), 3.77 (d, J=4.1 Hz, 1H), 3.37 (s, 3H), 3.15 (d, J=6.8 Hz, 1H), 2.20 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.8, 147.5, 147.2, 127.1, 123.5, 89.8, 73.1, 59.6, 27.5; IR (neat) ν_{max} 3511, 1722, 1518, 1350, 1111; HRMS (FI): M· 239.0794 (expected), 239.0793 (observed).

Analytical data for *anti*-**8**. R_f 0.29 (AcOEt/hexane: 40/60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, J=8.7 Hz, 2H), 7.57 (d, J=8.7 Hz, 2H), 5.03 (dd, J=6.2, 3.7 Hz, 1H), 3.71 (d, J=6.2 Hz, 1H), 3.33 (s, 3H), 3.14 (d, J=3.7 Hz, 1H), 2.17 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.4, 147.1, 128.1, 123.9, 90.0, 73.7, 60.1, 27.9; IR (neat) ν_{max} 3398, 1708, 1517, 1347, 1108; HRMS (FI): M· 239.0794 (expected), 239.0792 (observed).

4.2.6. 4-(4′-Nitrophenyl)-4-hydroxy-1-methylsulfanyl-2-butanone 9. Crude ratio regio/*syn*/*anti*=35/20/45. Purification by column chromatography over silica (CH_2Cl_2/Et_2O: 98/2), followed by recrystallization from AcOEt afforded **9** as a pale yellow solid (10%). R_f 0.19 (DCM/Et_2O: 98/2); mp 86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J=8.6 Hz, 2H), 7.57 (d, J=8.6 Hz, 2H), 5.28 (t, J=6.4 Hz, 1H), 3.57 (s, 1H), 3.19 (s, 2H), 3.05 (d, J=6.4 Hz, 2H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.7, 149.9, 147.3, 126.5, 123.7, 69.3, 48.0, 43.4, 15.6; IR (neat) ν_{max} 3468, 2921, 1706, 1519, 1348, 1063; Anal. calcd for $C_{11}H_{13}NO_4S$: C, 51.75; H, 5.13; N, 5.49. Found: C, 51.96; H, 5.19; N, 5.49.

4.2.7. 1-(4′-Nitrophenyl)-1,5-dihydroxy-3-pentanone 17 and 4-(4′-nitrophenyl)-4-hydroxy-3-hydroxymethyl-3-butanone 18. Crude ratio regio/*syn*/*anti*=40/23/37. Purification by column chromatography over silica (AcOEt) followed by (toluene/AcOEt: 40/60) or recrystallization from AcOEt afforded **17** and **18-D2** as pale yellow solids (22 and 9%, respectively) and **18-D1** as a pale yellow oil (7%).

Analytical data for **17**: mp 63 °C. R_f 0.35 (AcOEt); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.22 (d, J=8.9 Hz, 2H), 7.55 (d, J=8.9 Hz, 2H), 5.32 (m, 1H), 3.91 (dt, J=6.0, 5.4 Hz, 2H), 3.56 (d, J=3.4 Hz, 1H), 2.89 (m, 2H), 2.74 (t, J=5.4 Hz, 2H), 2.33 (t, J=6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.6, 149.8, 147.3, 126.4, 123.8, 68.9, 57.6, 51.4, 45.4; IR (neat) ν_{max} 3388, 2897, 1710, 1605, 1518, 1348, 1056; Anal. calcd for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.86. Found: C, 55.24; H, 5.51; N, 5.82; HRMS (FI): M· 239.0794 (expected), 239.0800 (observed).

Analytical data for **18-D1**. R_f 0.31 (toluene/AcOEt: 40/60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, J=8.8 Hz, 2H), 7.64 (d, J=8.8 Hz, 2H), 4.98 (d, J=8.6 Hz, 1H), 3.62 (dd, 1H, J=10.8, 8.6 Hz), 3.38 (dd, J=10.8, 4.4 Hz, 1H), 3.07 (ddd, J=8.6, 8.6, 4.4 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (125 MHz,

$CDCl_3$) δ 211.6, 151.0, 147.9, 127.8, 123.5, 72.0, 62.3, 60.7, 30.9; IR (neat) ν_{max} 3426, 2897, 1710, 1606, 1520, 1348, 1063, 1004; HRMS (CI+): M+NH_4^+ 257.1137 (expected), 257.1132 (observed).

Analytical data for **18**-**D2**: deduced from a 13/87 mixture of D1/D2. R_f=0.21 (toluene/AcOEt: 40/60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J=8.4 Hz, 2H), 7.60 (d, J=8.4 Hz, 2H), 5.04 (d, J=7.6 Hz, 1H), 3.97 (dd, J=11.2, 7.6 Hz, 1H), 3.88 (dd, J=11.2, 4.4 Hz, 1H), 3.17 (ddd, J=7.6, 7.6, 4.4 Hz, 1H), 2.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.7, 151.1, 147.7, 127.8, 123.4, 71.6, 62.2, 60.4, 30.9; IR (neat) ν_{max} 3425, 2897, 1710, 1606, 1521, 1348, 1063, 1004; HRMS (CI+): M+NH_4^+ 257.1137 (expected), 257.1132 (observed).

4.2.8. 1-(4′-Nitrophenyl)-1-hydroxy-5-methoxy-3-pentanone 19 and 4-(4′-nitrophenyl)-4-hydroxy-3-methoxymethyl-2-butanone 20 (*syn* and *anti*). Crude ratio regio/*syn*/*anti*=34/33/33. Purification by column chromatography over silica (DCM/AcOEt: 80/20) followed by (DCM) afforded **19** as a colourless oil (20%) and **20**-*syn* and **20**-*anti* as pale yellow oils (20 and 23%, respectively).

Analytical data for **19**. R_f 0.27 (DCM/AcOEt: 80/20); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (d, J=8.8 Hz, 2H), 7.55 (d, J=8.8 Hz, 2H), 5.29 (m, 1H), 3.67 (m, 3H), 3.35 (s, 3H), 2.88 (d, J=7.2 Hz, 2H), 2.71 and 2.72 (2t, J=6.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.7, 150.4, 147.8, 126.9, 124.2, 69.4, 67.9, 59.4, 52.1, 43.9; IR (neat) ν_{max} 3470, 2857, 1710, 1516, 1345, 1106; HRMS (FI): M$^{\cdot}$ 253.0950 (expected), 253.0955 (observed).

Analytical data for *anti*-**20**. R_f 0.16 (DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J=8.6, 2H), 7.52 (d, J=8.6 Hz, 2H), 5.24 (dd, J=4.4, 3.0 Hz, 1H), 3.94 (d, J=3.0 Hz, 1H), 3.66 (dd, 1H, J=9.5, 5.2 Hz), 3.59 (dd, J=9.5, 6.2 Hz, 1H), 3.29 (s, 3H), 3.06 (m, 1H), 2.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.4, 149.1, 147.3, 126.9, 123.6, 72.6, 70.3, 59.3, 58.3, 31.2; IR (neat) ν_{max} 3424, 2928, 1711, 1521, 1343, 1111; HRMS (FI): M$^{\cdot}$ 253.0950 (expected), 253.0956 (observed).

Analytical data for *anti*-**20**: Analyses are deduced from a 10/90 mixture of *syn*/*anti*. R_f 0.16 (DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J=8.6 Hz, 2H), 7.51 (d, J=8.6 Hz, 2H), 5.12 (d, J=7.2 Hz, 1H), 3.72 (bs, 1H), 3.49 (dd, J=9.4, 4.2 Hz, 1H), 3.34 (dd, J=9.4, 6.4 Hz, 1H), 3.24 (s, 3H), 3.04 (ddd, J=7.2, 6.4, 4.2 Hz, 1H), 2.20 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.0, 149.2, 147.4, 127.1, 123.7, 71.9, 70.9, 59.2, 59.0, 31.4; IR (neat) ν_{max} 3420, 2929, 2897, 1711, 1521, 1348, 1118; HRMS (CI+): M+NH_4^+ 271.1290 (expected), 271.1294 (observed).

4.2.9. 1-(4′-Nitrophenyl)-1-hydroxy-5-methylsulfanyl-3-pentanone 21 and 4-(4′-nitrophenyl)-4-hydroxy-3-methylsulfanyl-3-butanone 22 (*syn* and *anti*). Crude ratio regio/*syn*/*anti*=74/13/13. Purification by column chromatography over silica (hexane/Et_2O: 40/60) afforded **21** as a yellow solid (40%) and **22**-*syn* and **22**-*anti* as pale yellow oils (3.4 and 5%, respectively).

Analytical data for **21**. R_f 0.21 (hexane/Et_2O: 40/60); mp 47 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, J=7.9 Hz, 2H), 7.56 (d, J=7.9 Hz, 2H), 5.31 (m, 1H), 3.48 (d, J=3.3 Hz, 1H), 2.87 (d, J=6.5 Hz, 2H), 2.77 (m, 4H), 2.12 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.7, 149.7, 147.3, 126.3, 123.7, 68.8, 51.0, 43.0, 27.6, 15.7; IR (neat) ν_{max} 3423, 2919, 1710, 1603, 1519, 1347, 1077; HRMS (FI): M$^{\cdot}$ 269.0722 (expected), 269.0719 (observed).

Analytical data for *syn*-**22**. R_f 0.29 (hexane/Et_2O: 40/60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J=8.7 Hz, 2H), 7.55 (d, J=8.7 Hz, 2H), 5.14 (d, J=4.4 Hz, 1H), 3.31 (s, 1H), 3.13 (ddd, J=9.6, 4.4, 4.4 Hz, 1H), 2.81 (dd, J=13.4, 9.6 Hz, 1H), 2.66 (dd, J=13.4, 4.4 Hz, 1H), 2.24 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.8, 148.8, 147.9, 127.5, 124.2, 73.1, 58.1, 32.9, 31.5, 16.9; IR (neat) ν_{max} 3429, 2919, 1708, 1519, 1347; HRMS (CI+): M+NH_4^+ 287.1066 (expected), 287.1064 (observed).

Analytical data for *anti*-**22**: Analyses are deduced from a 68/32 mixture of *syn*/*anti*. R_f 0.25 (hexane/Et_2O: 40/60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J=9.0 Hz, 2H), 7.53 (d, J=9.0 Hz, 2H), 5.06 (d, J=6.0 Hz, 1H), 3.45 (s, 1H), 3.14 (m, 1H), 2.72 (dd, J=13.0, 8.0 Hz, 1H), 2.63 (dd, J=13.0, 6.4 Hz, 1H), 2.17 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.3, 149.7, 148.8, 127.4, 124.3, 74.2, 58.0, 33.9, 33.2, 16.9; IR (neat) ν_{max} 3430, 2919, 1709, 1520, 1348; HRMS (CI+): M+NH_4^+ 287.1066 (expected), 287.1062 (observed).

4.3. Synthesis of racemic aldols. Path B: direct cross aldolisations with NaOH

A 1% solution of sodium hydroxide in water (5.5 mL, 1.4 mmol) was added to the ketone (132 mmol) at RT. The aldehyde (6.6 mmol) was then added. After 3 h at RT, the layers were separated and the aqueous layer was extracted three times with AcOEt, the combined organic layers were washed with saturated NH_4Cl, dried over $MgSO_4$ and solvents were removed in vacuo. Product ratios were determined by crude ^{1}H NMR spectroscopy before purification by flash column chromatography.

4.3.1. 3-[Hydroxy-(4′-nitrophenyl)-methyl]-2-pentanone 4 (*syn* and *anti*). Crude ratio regio/*syn*/*anti*=16/35/49. Purification by column chromatography over silica (hexane/AcOEt: 80/20) followed by semi-preparative HPLC (C8 column) afforded *syn*-**4** as a colourless oil and *anti*-**4** as a white solid (total yield as a mixture with *anti*-**4**, 60%).

Analytical data for *syn*-**4**. R_f 0.16 (hexane/AcOEt: 80/20); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (d, J=8.9 Hz, 2H), 7.54 (d, J=8.9 Hz, 2H), 5.11 (d, J=4.5 Hz, 1H), 3.25 (bs, 1H), 2.84 (ddd, J=9.0, 9.0, 4.5 Hz, 1H), 2.19 (s, 3H), 1.74 (m, 1H), 1.59 (m, 1H), 0.86 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 213.4, 149.4, 147.5, 127.2, 123.8, 72.6, 59.8, 32.0, 19.7, 12.4; IR (neat) ν_{max} 3409, 2967, 1702, 1521, 1348, 1044; HRMS (CI+): M+NH_4^+ 255.1345 (expected), 255.1347 (observed).

Analytical data for *anti*-**4**. R_f 0.16 (hexane/AcOEt: 80/20); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J=8.8 Hz, 2H), 7.52 (d, J=8.8 Hz, 2H), 4.93 (d, J=6.0 Hz, 1H), 3.26 (bs, 1H),

2.88 (ddd, J=8.0, 6.0, 6.0 Hz, 1H), 2.14 (s, 3H), 1.63 (m, 1H), 1.52 (m, 1H), 0.93 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 213.7, 150.1, 147.7, 127.3, 124.0, 74.3, 60.1, 32.4, 22.7, 11.7; IR (neat) ν_{max} 3401, 2922, 1702, 1522, 1347; HRMS (CI+): M+NH_4^+ 255.1345 (expected), 255.1343 (observed).

4.3.2. 3-(Hydroxy-(4′-nitrophenyl)-methyl)-2-hexanone 6 (*syn* and *anti*). Crude ratio regio/*syn*/*anti*=20/33/47. Purification by column chromatography over silica (toluene/AcOEt: 95/5) afforded *syn*-**6** and *anti*-**6** as colourless oils (22 and 49%, respectively).

Analytical data for *syn*-**6**. R_f 0.22 (toluene/AcOEt: 95/5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J=8.8 Hz, 2H), 7.52 (d, J=8.8 Hz, 2H), 5.09 (d, J=4.2 Hz, 1H), 3.32 (bs, 1H), 2.88 (ddd, J=8.8, 4.2 and 4.2, 1H), 2.17 (s, 3H), 1.70–1.60 (m, 1H), 1.51–1.42 (m, 1H), 1.34–1.20 (m, 1H), 1.17–1.04 (m, 1H), 0.82 (t, J=7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.3, 149.2, 147.3, 126.9, 123.5, 72.5, 58.1, 31.6, 28.4, 21.1, 14.2; IR (neat) ν_{max} 3456, 1703, 1521, 1348; HRMS (CI+): M+NH_4^+ 269.1501 (expected), 269.1498 (observed).

Analytical data for *anti*-**6**. R_f 0.16 (toluene/AcOEt: 95/5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J=8.7 Hz, 2H), 7.50 (d, J=8.7 Hz, 2H), 4.88 (d, J=6.8 Hz, 1H), 3.43 (bs, 1H), 2.92 (ddd, J=8.7, 6.8, 5.3 Hz, 1H), 2.13 (s, 3H), 1.56 (m, 1H), 1.28 (m, 3H), 0.86 (t, J=8.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.5, 150.0, 147.4, 127.1, 123.7, 74.4, 58.3, 32.1, 31.4, 20.4, 14.0; IR (neat) ν_{max} 3444, 1711, 1523, 1348; HRMS (FI): M$^{\cdot}$ 251.1158 (expected), 251.1164 (observed).

4.4. Synthesis of racemic aldols. Path C: direct cross aldolisations in presence of PBS pH=7.4

The ketone (1.45 mmol) was added to a solution of 4-nitrobenzaldehyde (140 mg, 0.95 mmol) in DMF (15 mL). PBS pH=7.4 (20 mL) was then added and the resulting solution was stirred at RT or at reflux for 48 h. The mixture was extracted three times with AcOEt, dried over $MgSO_4$ and solvents were removed in vacuo. Product ratios were determined, when possible, by crude ^{1}H NMR spectroscopy before purification by flash chromatography.

4.4.1. 4-(4′-Nitrophenyl)-3,4-dihydroxy-2-butanone 16 (*syn* and *anti*). Crude ratio regio/*syn*/*anti*=3/48/49. Purification by column chromatography over silica (DCM/AcOEt: 80/20) and recrystallization from AcOEt afforded a mixture of *syn*-**16** and *anti*-**16** as a yellow solid (40%). Analyses deduced from a mixture of 79% of *syn* and 21% of *anti*). R_f 0.25 (DCM/AcOEt: 80/20); ^{1}H NMR (400 MHz, CD_3OD) *Syn* δ 8.23 (d, J=8.5 Hz, 2H), 7.71 (d, J=8.5 Hz, 2H), 5.26 (d, J=2.6 Hz, 1H), 4.30 (d, J=2.6 Hz, 1H), 2.34 (s, 3H); *Anti* δ 8.22 (d, J=8.5 Hz, 2H), 7.66 (d, J=8.4 Hz, 2H), 4.94 (d, J=6.0 Hz, 1H), 4.23 (d, J=6.0 Hz, 1H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) *Syn* δ 211.9, 151.4, 149.0, 129.1, 124.5, 82.4, 74.9, 27.4; *Anti* δ 212.1, 150.8, 149.2, 129.7, 124.4, 82.3, 75.8, 28.1; IR (neat) ν_{max} 3422, 1715, 1606, 1518, 1348, 1108, 1064; HRMS of the mixture (CI+): M+NH_4^+ 243.0981 (expected), 243.0985 (observed).

4.4.2. 4-(4′-Nitrophenyl)-4-hydroxy-3-methylsulfanyl-2-butanone 10 (*syn* and *anti*). Crude ratio regio/*syn*/*anti*=5/55/40. Purification by column chromatography over silica (CH_2Cl_2/Et_2O: 98/2), followed by recrystallization from AcOEt afforded *syn*-**10** and *anti*-**10** as pale yellow solids (8 and 16%, respectively).

Analytical data for *syn*-**10**. R_f 0.35 (CH_2Cl_2/Et_2O: 98/2); mp 73 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J=8.7 Hz, 2 H), 7.58 (d, J=8.7 Hz, 2 H), 5.16 (d, J=7.8 Hz, 1 H), 3.51 (bs, 1 H), 3.45 (d, J=7.8 Hz, 1 H), 2.22 (s, 3 H), 2.06 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.8, 147.8, 147.6, 127.9, 123.5, 69.1, 59.9, 29.1, 12.3; IR (neat) ν_{max} 3478, 1701, 1520, 1348, 1064; Anal. calcd for $C_{11}H_{13}NO_4S$: C, 51.75; H, 5.13; N, 5.49. Found: C, 51.70; H, 5.13; N, 5.47.

Analytical data for *anti*-**10**. R_f 0.16 (CH_2Cl_2/Et_2O: 98/2); mp 129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J=8.8 Hz, 2 H), 7.59 (d, J=8.8 Hz, 2 H), 5.12 (d, J=9.2 Hz, 1 H), 3.42 (d, J=9.2 Hz, 1 H), 3.20 (bs, 1 H), 2.41 (s, 3 H), 1.93 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.5, 147.7, 147.6, 127.8, 123.5, 72.1, 58.7, 28.6, 13.0; IR (neat) ν_{max} 3386, 1698, 1510, 1343, 1034; Anal. calcd for $C_{11}H_{13}NO_4S$: C, 51.75; H, 5.13; N, 5.49; O, 25.07. Found: C, 51.84; H, 5.14; N, 5.36; O, 25.13.

4.4.3. 4-(4′-Nitrophenyl)-3-chloro-4-hydroxy-2-butanone 12 (*syn* and *anti*). Crude ratio regio/*syn*/*anti*=1/59/40. Purification by column chromatography over silica (DCM/Et_2O: 98/2) afforded **12** as a colourless oil (43%) in 3:2 ratio of *syn*:*anti*. Analyses deduced from a mixture of 60% of *syn* and 40% of *anti*. R_f 0.20 (DCM/Et_2O: 98/2); ^{1}H NMR (400 MHz, $CDCl_3$) *syn* δ8.25 (d, J=8.3 Hz, 2H), 7.59 (d, J=8.3 Hz, 2H), 5.45 (dd, J=4.0, 4.0 Hz, 1H), 4.44 (d, J=4.0 Hz, 1H), 3.20 (d, J=4.0 Hz, 1H), 2.40 (s, 3H); *anti* δ 8.25 (d, J=8.4 Hz, 2H), 7.61 (d, J=8.4 Hz, 2H), 5.16 (dd, J=8.0, 4.2 Hz, 1H), 4.28 (d, J=8.0 Hz, 1H), 3.35 (d, J=4.2 Hz, 1H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *syn* δ 203.3, 147.8, 146.1, 127.4, 123.6, 72.0, 67.1, 28.3; *anti* δ 203.3, 148.0, 145.8, 128.1, 123.6, 73.8, 63.4, 28.0; IR (neat) ν_{max} 3441, 1718, 1606, 1521, 1349; HRMS of the mixture (CI+): M+NH_4^+ 261.0642 (expected), 261.0638 (observed).

4.4.4. 4-(4′-Nitrophenyl)-3-fluoro-4-hydroxy-2-butanone 14 (*syn* and *anti*). Purification by column chromatography (two fold) over silica (hexane/Et_2O: 60/40, followed by pentane/Et_2O: 50/50) afforded **14** as a pale yellow solid in 3:4 ratio of *syn*:*anti* (15%). Analyses deduced from a mixture of 43% of *syn* and 57% of *anti*. R_f 0.24 (pentane/Et_2O: 50/50); mp 55 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *syn* δ8.26 (d, J=8.8 Hz, 2H), 7.61 (d, J=8.8 Hz, 2H), 5.31 (ddd, J=24.8, 6.2, 2.2 Hz, 1H), 4.86 (dd, J=48, 2.2 Hz, 1H), 2.84 (d, J=6.2 Hz, 1H), 2.34 (d, J=4.9 Hz, 3H); *anti* δ 8.24 (d, J=8.8 Hz, 2H), 7.57 (d, J=8.8 Hz, 2H), 5.18 (ddd, J=12.7, 5.4, 2.4 Hz, 1H), 4.82 (dd, J=48.9, 5.4 Hz, 1H), 3.30 (d, J=2.4 Hz, 1H), 2.23 (d, J=6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *syn* δ 207.4, 148.0, 144.7, 127.3, 123.8, 96.3 (d, J=195.0 Hz), 72.9 (d, J=18.0 Hz), 27.6; *anti* δ 207.1, 147.9, 145.8, 127.9, 123.6, 95.6 (d, J=192.0 Hz), 72.6 (d, J=21.3 Hz), 27.4; ^{19}F NMR (235 MHz, $CDCl_3$) *syn* δ −195.3 (ddq, J=48, 24.8, 4.9 Hz); *anti* δ −204.7 (ddq, J=48.9, 12.7, 6.2 Hz); IR

(neat) ν_{max} 3438, 1720, 1607, 1520, 1348; HRMS of the mixture (CI+): M+NH_4^+ 245.0938 (expected), 245.0932 (observed).

4.4.5. 6-(4′-Acetamidophenyl)-3-ethyl-4-hydroxy-2-hexanone 2 (*syn* and *anti*). A DCM solution (2 mL) of a mixture of trimethyl-(1-propyl-vinyloxy)-silane and trimethyl-(1-methyl-but-1-enyloxy)-silane (75 mg, 0.47 mmol) was added dropwise into a mixture of aldehyde (100 mg, 0.52 mmol) and $TiCl_4$ (57 μL, 0.52 mmol) in 4 mL of DCM at −78 °C. After stirring the mixture for 1.5 h at −78 °C, sat. NH_4Cl solution was added. The aqueous phase was extracted three times with AcOEt, the combined organic layers were washed with brine and dried over Na_2SO_4. Purification by column chromatography (Et_2O/AcOEt: 80/20), afforded **2** as a D1/D2: 50/50 mixture. Analytical data of the mixture. R_f 0.20 (Et_2O/AcOEt: 80/20); ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J*=8.2 Hz, 2H), 7.32 (bs, 1H), 7.13 (d, *J*=8.2 Hz, 2H), 3.80 (dt, *J*=9.2, 4 Hz, 0.5H, D1), 3.73 (ddd, *J*=8.8, 5.2, 3.6 Hz, 0.5H, D2), 2.81 (m, 1H), 2.63 (m, 1H), 2.52 (m, 1H), 2.19 (s, 1.5H, D2), 2.18 (s, 1.5H, D1), 2.16 (s, 3H,), 1.70 (m, 4H,), 0.92 (t, *J*=7.2 Hz, 1.5H, D1), 0.90 (t, *J*=7.2 Hz, 1.5H, D2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 214.7 (D2), 213.7 (D1), 168.3, 137.8 (D1), 137.7 (D2), 135.7, 128.9, 120.0, 71.2 (D2), 70.6 (D1), 59.0 (D1), 58.8 (D2), 37.8 (D2), 36.2 (D2), 31.7 (D2), 31.6 (D1), 31.5 (D1), 31.4 (D2), 24.5, 22.2 (D2), 19.8 (D1), 12.4 (D1), 11.7 (D2); IR (neat) ν_{max} 3308, 2953, 2939, 1708, 1665, 1601, 1543, 1522; HRMS of the mixture (FI): $M^{\cdot}$ 277.1678 (expected), 277.1679 (observed).

4.4.6. 4-(*tert*-Butyl-dimethyl-silanyloxy)-4-(4′-nitrophenyl)-2-butanone. Imidazole (2.9 g, 43.4 mmol) and TBSCl (3.2 g, 21.2 mmol) was added to a solution of 4-(4′-nitrophenyl)-4-hydroxy-2-butanone (1.8 g, 8.8 mmol) in 40 mL of DMF at room temperature. The reaction was stirred overnight, quenched with water, extracted with DCM and the combined organic layers were washed with brine and dried over $MgSO_4$. Removal of solvents and purification by chromatography over silica (hexane/DCM: 50/50) afforded a white solid (92%). R_f 0.26 (hexane/DCM: 50/50); mp 36 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, *J*=9.0 Hz, 2H), 7.52 (d, *J*=9.0 Hz, 2H), 5.28 (dd, *J*=8.3, 4.3 Hz, 1H), 2.94 (dd, *J*=15.8, 8.3 Hz, 1H), 2.58 (dd, *J*=15.8, 4.3 Hz, 1H), 2.16 (s, 3H), 0.86 (s, 9H), 0.04 (s, 3H), −0.14 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.0, 151.9, 147.2, 126.6, 123.7, 70.7, 53.8, 31.7, 25.6, 18.0, −4.8, −5.2; IR (neat) ν_{max} 2930, 1720, 1523, 1347, 1090; HRMS (CI+): M+H^+ 324.1631 (expected), 324.1643 (observed).

4.4.7. 1-Fluoro-4-(*tert*-butyl-dimethyl-silanyloxy)-4-(4′-nitrophenyl)-2-butanone. Trimethylsilyl triflate (436 μL, 1.9 mmol) was added to a solution of 4-(*tert*-butyl-dimethyl-silanyloxy)-4-(4′-nitrophenyl)-2-butanone (300 mg, 0.95 mmol) and lutidine (432 μL, 3.7 mmol) in DCM (3 mL) at −78 °C. The reaction mixture was stirred for 2 h at −78 °C, 30 min at 0 °C and then diluted with cold DCM and washed with cold sat. $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted three times with cold DCM. The combined organic layers were washed with cold sat. NaCl, dried over Na_2SO_4 and concentrated to give the corresponding silyl enol ether which was used in the next step without purification. To a solution of the residue in CH_3CN (3 mL) was added at RT Selectfluor (397 mg, 1.1 mmol) in dry acetonitrile (15 mL). The reaction mixture was stirred at RT for 1 h. The mixture was then diluted with EtOAc, washed with $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4. Removal of the solvents in vacuo and purification by column chromatography over silica (DCM/hexane: 60/40, followed by hexane/Et_2O: 80/20) afforded a white solid (35% overall yield). R_f 0.15 (hexane/Et_2O: 80/20); mp 64 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J*=8.6 Hz, 2H), 7.54 (d, *J*=8.6 Hz, 2H), 5.34 (dd, *J*=8.3, 4.1 Hz, 1H), 4.86 (dd, *J*=47.2, 16.0 Hz, 1H), 4.81 (dd, *J*=47.2, 16.0 Hz, 1H), 3.02 (ddd, *J*=15.8, 4.1, 2.5 Hz, 1H), 2.65 (ddd, *J*=15.8, 8.3, 2.4 Hz, 1H), 0.86 (s, 9H), 0.05 (s, 3H), −0.14 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.7 (d, *J*=19 Hz), 151.3, 147.4, 126.6, 123.8, 85.4 (d, *J*=185 Hz), 70.2, 48.6, 25.6, 18.0, −4.8, −5.3; ^{19}F NMR (235 MHz, $CDCl_3$) δ −227.0 (tt, *J*=47.2, 2.4 Hz); IR (neat) ν_{max} 2956, 1734, 1642, 1517, 1344, 1258; HRMS (CI+): M+NH_4^+ 359.1824 (expected), 359.1811 (observed).

4.4.8. 1-Fluoro-4-hydroxy-4-(4-nitrophenyl)-butan-2-one 13. To a solution of 1-fluoro-4-(*tert*-butyl-dimethyl-silanyloxy)-4-(4′-nitrophenyl)-2-butanone (69 mg, 0.2 mmol) in dry DCM (2 mL) was added at −78 °C $BF_3.Et_2O$ (56 μL, 0.44 mmol). The mixture was stirred at −78 °C for 1.5 h and at 0 °C for 5 h. The reaction was quenched by addition of water and extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed in vacuo. Purification by column chromatography over silica (hexane/EtOAc: 65:35) afforded **13** as a white solid (66%). R_f0.16 (hexane/EtOAc: 65/35); mp 76 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J*=8.4 Hz, 2H), 7.57 (d, *J*=8.4 Hz, 2H), 5.37 (dd, *J*=8.5, 3.8 Hz, 1H), 4.86 (d, *J*=47.1 Hz, 2H), 3.22 (bs, 1H), 3.02 (ddd, *J*=18.0, 8.5, 2.4 Hz, 1H), 2.98 (ddd, *J*=18.0, 3.8, 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4 (d, *J*=20 Hz), 149.6, 147.5, 126.4, 124.0, 85.0 (d, *J*=184 Hz), 68.4, 47.0; ^{19}F NMR (235 MHz, $CDCl_3$) δ −228.4 (tt, *J*=47.1 Hz, 2.4); IR (neat) ν_{max} 3423, 2921, 1733, 1642, 1520, 1343; HRMS (CI+): M+NH_4^+ 245.0938 (expected), 245.0933 (observed).

4.4.9. 1-Chloro-4-(*tert*-butyl-dimethyl-silanyloxy)-4-(4′-nitrophenyl)-butan-2-one. Trimethylsilyl triflate (336 μL, 1.9 mmol) was added to a solution of 4-(*tert*-butyl-dimethyl-silanyloxy)-4-(4′-nitrophenyl)-2-butanone (300 mg, 0.95 mmol) and lutidine (432 μL, 3.8 mmol) in DCM (3 mL) at −78 °C. The reaction mixture was stirred 2 h at −78 °C, 30 min at 0 °C and then diluted with cold DCM and washed with cold sat. $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted three times with cold DCM. The combined organic layer was washed with cold sat. NaCl, dried over Na_2SO_4 and concentrated to give the corresponding silyl enol ether which was used in the next step without purification. To a solution of the residue in CH_3CN (3 mL) was successively added at 0 °C, *N*-chlorosuccinimide (120 mg, 0.9 mmol 160 mg) and water (0.5 mL). The mixture was warmed to RT and stirred for 3 h. Then water was added and the solution was extracted with AcOEt, washed with brine and dried over Na_2SO_4. Removal of solvents and purification by chromatography over silica (hexane/Et_2O: 80/20) afforded a

white solid (150 mg, 35% for both steps). R_f 0.13 (hexane/Et_2O: 80/20); mp 54 °C; ^1H NMR (400 MHz, $CDCl_3$) δ8.22 (d, J=8.6 Hz, 2H), 7.54 (d, J=8.6 Hz, 2H) 5.30 (dd, J=8.5, 4.1 Hz, 1H), 4.15 (d, J=15.8 Hz, 1H), 4.11 (d, J=15.8 Hz, 1H), 3.06 (dd, J=15.6, 8.5 Hz, 1H), 2.70 (dd, J=15.6, 4.1 Hz, 1H), 0.86 (s, 9H), 0.04 (s, 3H), −0.15 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.5, 151.0, 147.4, 126.5, 123.8, 70.8, 50.0, 49.4, 25.6, 17.9, −4.8, −5.4; IR (neat) ν_{max} 2990, 1738, 1523, 1348, 1254, 1093; HRMS (CI+): M+H$^+$ 358.1241 (expected), 358.1235 (observed).

4.4.10. 1-Chloro-4-hydroxy-4-(4-nitrophenyl)-butan-2-one 11. To a solution of 1-chloro-4-(*tert*-butyl-dimethyl-silanyloxy)-4-(4′-nitrophenyl)-butan-2-one (79 mg, 0.22 mmol) in dry DCM (3 mL) was added at −78 °C $BF_3.Et_2O$ (61 μL, 0.48 mmol). The mixture was stirred at −78 °C for 1.5 h, at 0 °C for 1.5 h and at RT for 4 h. The reaction was quenched by addition of water and extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed in vacuo. Purification by column chromatography over silica (hexane/EtOAc: 65/35) afforded **11** as a white solid (67%). R_f 0.21 (hexane/EtOAc: 65/35); mp 94 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J=8.4 Hz, 2H), 7.57 (d, J=8.4 Hz, 2H), 5.34 (m, 1H), 4.14 (s, 2H), 3.15 (d, J=3.6 Hz, 1H), 3.03 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.1, 149.4, 147.5, 126.4, 123.9, 68.9, 48.4, 48.2; IR (neat) ν_{max} 3390, 2912, 1726, 1601, 1509, 1344; HRMS (CI+): M+H$^+$ 261.0642 (expected), 261.0645 (observed).

4.5. Antibody assays

All antibody-catalyzed reactions were performed in phosphate buffered saline (10 mM phosphate, 150 mM NaCl, pH 7.4). All antibody-catalyzed reactions and background reactions were monitored by high-pressure liquid chromatography (HPLC; Waters HPLC system (626 Pump, 600S Controller, 996 Photodiode Array Detector, Millenium^{32} Software) using a Nova-pak Waters column (C-18, 60 Å pore size, 4 μm particle size, 3.9×150 mm) and acetonitrile/water or methanol/-water mixtures (containing 0.1% trifluoroacetic acid) as eluents at a flow rate of 1.0 mL/min. For antibody assays run for significant periods of time, a control assay was performed to rule out the possibility of catalysis due to the presence of microflora.

4.6. Michaelis–Menten kinetics

Product formation or percent conversion of antibody-catalyzed reaction mixtures was monitored by HPLC. The points were determined experimentally and the best fit value of V_{max} and K_m were obtained by fitting the v_i versus $[S]_0$ data to hyperbolic saturation curves by weighted non-linear regression. All data are reported per antibody active site. An IgG antibody possesses 2 active sites per MW of ~150,000 g/mol.

4.7. Determination of enantiomeric excesses: forward aldol reaction

10 μL of a 66 mM solution of *p*-nitrobenzaldehyde in acetonitrile was added to 2 mL of antibody (32 μM in PBS). Reactions were initiated by addition of 100 μL of ketone (65 mM) in PBS (10 mM phosphate, 150 mM NaCl, pH 7.4) containing 10% of acetonitrile. The reactions were monitored by RP-HPLC and, after a minimum of 8% conversion was reached, the unreacted aldol was isolated by semi-preparative reversed-phase HPLC, Hypersil ODS column, 5 μm particle size, 7×250 mm, flow rate 2.0 mL/min. The fractions were freeze-dried, the residue was redissolved in 500 μL of dichloromethane/hexane (50/50) and the ee was determined by normal-phase HPLC using a Chiralcel OD or OJ Daicel column.

4.8. Determination of enantiomeric excesses: retro-aldol reaction

10 μL of a racemic stock solution of aldol (80 mM in acetonitrile) was added to 1 mL of antibody (32 μM in PBS, pH 7.4). The reactions were monitored by RP-HPLC. After reaching 50% conversion, the unreacted aldol was isolated by semi-preparative reverse-phase HPLC, Hypersil ODS column, 5 μm particle size, 7×250 mm, flow rate 2.0 mL/min. The fractions were freeze-dried, the residue was redissolved in 200 μL of dichloromethane/hexane (50/50) and the ee was determined by normal-phase HPLC using a Chiralcel OD or OJ Daicel column.

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