Organic & Biomolecular Chemistry

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ROYAL SOCIETY OF CHEMISTRY

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View Article Online
DOI: 10.1039/C6OB02320G

Journal Name

ARTICLE

Asymmetric Chemoenzymatic Synthesis of 1,3-Diols and 2,4-Disubstituted Aryloxetanes by Using Whole Cell Biocatalysts†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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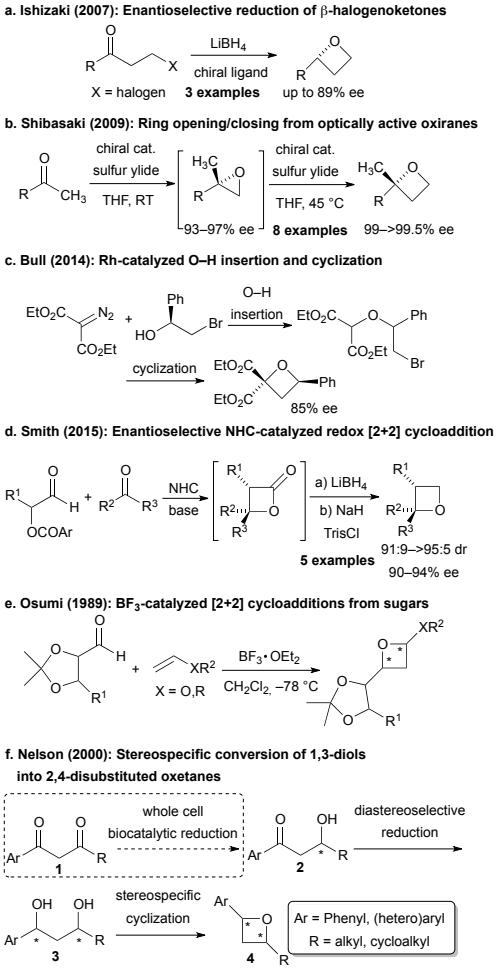
Regio- and stereoselective reduction of substituted 1,3-aryldiketones, investigated in the presence of different whole cell microorganisms, was found to afford β-hydroxyketones or 1,3-diols in very good yields (up to 95%) and enantiomeric excesses (up to 96%). The enantiomerically enriched aldols, obtained with opposite stereo-preference by baker's yeast and *Lactobacillus reuteri* DSM 20016 bioreduction, could then be diastereoselectively transformed into optically active *syn*- or *anti*-1,3-diols by a careful choice of the chemical reducing agent (diastereomeric ratio up to 98:2). The latter, in turn, were stereospecifically cyclized into the corresponding oxetanes in 43–98% yields and in up to 94% ee, thereby giving a diverse selection of stereodefined 2,4-disubstituted aryloxetanes.

Introduction

Oxetanes are an important group of four-membered heterocyclic compounds found in natural products, and widely used in synthetic organic chemistry, in the fields of polymer science and technology, and in materials science.[1] The oxetane motif has become popular within medicinal chemistry and drug discovery particularly after the pioneering studies by Carreira and co-workers that demonstrated its effectiveness for fine-tuning the physicochemical properties of organic molecules (*e.g.* improving solubility, lipophilicity, etc.) and as isosteric replacement of both the carbonyl functional group and the *gem*-dimethyl moiety.[2] In addition, oxetanes proved to be versatile templates in organic synthesis for the construction of valuable heterocyclic compounds and chiral building blocks by ring expansion, ring opening, rearrangement and desymmetrization reactions.[3] More functionalized derivatives can also be prepared by exploiting direct organolithium-mediated functionalization processes, while preserving the integrity of the oxetanyl skeleton.[4]



Scheme 1 Current available methods for the preparation of variously substituted stereodefined oxetanes.

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†Electronic Supplementary Information (ESI) available: material and methods, experimental details, additional spectroscopic data, NMR spectra and chromatograms of oxetanes. For ESI see DOI: 10.1039/x0xx00000x.

Several methods have been developed throughout the years for the preparation of diversely substituted oxetanes, however, mainly in racemic form.[5] Thus, the synthesis of stereodefined bespoke skeletons still remains a challenge in contemporary organic synthesis. The few reported methods include: (i) the asymmetric synthesis of 2-aryl-substituted oxetanes via enantioselective reduction of β-halogenoketones with $LiBH_4$ in the presence of chiral ligands (Scheme 1a);[6] (ii) ring-opening/closing from optically active oxiranes using sulfoxonium ylides to give 2,2-disubstituted oxetane derivatives (Scheme 1b);[7] (iii) the Rhodium-catalyzed O–H insertion of optically active β-bromohydrins into diazo compounds followed by C–C bond-forming cyclization *en route* to 2,2,4-trisubstituted oxetanes (one example) (Scheme 1c);[8] (iv) the enantioselective *N*-heterocyclic carbene (NHC)-catalyzed redox [2+2] cycloadditions with perfluoroketones as a means of access to 2,2,3-trisubstituted fluorinated oxetanes (Scheme 1d).[9] To our best knowledge, the only way reported for making optically active 2,4-disubstituted oxetanes is that based on a BF_3-catalyzed [2+2] cycloaddition route from sugars (Scheme 1e).[10a,b]

Whole-cell biocatalysis has emerged in the last decades as an elegant, competitive and formidable way of producing biologically active molecules of pharmaceutical interest.[11] Wild-type whole-cell biocatalysts are often preferable to isolated and purified enzymes since they are cheaper, easy to handle, with efficient internal cofactor regeneration systems, and working with high regio- and stereoselectivity under mild operational and environmentally friendly conditions.[12] In 2000, Nelson and co-workers reported the stereospecific conversion of (1*R**,3*S**)- and (1*R**,3*R**)-3-cycloxexyl-1-phenylpropane-1,3-diols **3** into the corresponding 2,4-disubstituted oxetanes **4** (*vide infra*). The former could be obtained by a diastereoselective reduction of aldols **2** (Scheme 1f).[13] Inspired by this report and building on our recent findings in using whole cell microorganisms (i.e., thermotolerant *Kluyveromyces marxianus* yeast[14] and *Lactobacillus reuteri* strain[15]) for the highly stereoselective biocatalytic reduction of arylketones to optically active 1-arylethanols, we wondered whether the synthesis of such challenging scaffolds (**4**) in an optically active form could be achieved starting directly from 1,3-aryldiketones **1** via a stereoselective whole-cell based biocatalytic reduction (Scheme 1f). Previous attempts to achieve this goal from 1,3-diphenylpropane-1,3-diol, via phosphonium ether intermediates, however, failed.[16] In this paper, we present the results of such an investigation aimed at preparing stereodefined 1,3-diols and the corresponding 2,4-disubstituted aryloxetanes by cyclization.

Results and discussion

Screening of whole-cell biocatalysts

As a bench reaction, we set out to investigate the bioreduction of 1,3-diphenyl-1,3-propandione (**1a**). This was incubated in the presence of growing cells (GC) of some previously characterized microbial biocatalysts,[14,15] from european collections (*Saccharomyces cerevisiae* CBS 7336, *Kluyveromyces marxianus* CBS 6556, *Yarrovia Lipolytica* Y16, and *Trigonopsis variabilis* DSM 70714), under the same experimental conditions (see footnote of Table 1 and Experimental Section). While baker's yeast-mediated bioreductions were run directly in tap water, the ones with *Lactobacillus reuteri* DSM 20016 resting cells (RC) were carried out with diketones (1g/L) suspended in phosphate-buffered saline (PBS) solutions.[15] The reaction progress was monitored by TLC and ^{1}H NMR, and the results are reported in Table 1.

No reduction of **1a** was noticed in the presence of *Yarrovia Lipolytica* Y16, baker's yeast (RC) and *Lactobacillus reuteri* DSM 20016 (Table 1, entries 1–3), whereas a complex reaction mixture was obtained with *Trigonopsis variabilis* DSM 70714 (GC) (Table 1, entry 4). Despite diketone **1a** is often found to be unreactive under mild conditions,[17] we were delighted to find out that *Saccharomyces cerevisiae* CBS 7336 (GC) successfully catalyzed its bioreduction, thereby allowing the isolation of the corresponding (*S*)-aldol **2a** as the main product with 50% yield and 58% enantiomeric excess (ee) after 96 h incubation at 30 °C (Table 1, entry 5). Disappointedly, under the same conditions but using *Kluyveromyces marxianus* CBS 6556 (GC) as the biocatalyst, (*S*)-aldol **2a** was formed in 12% yield and with 36% ee only. Interestingly, however, a competitive unusual reduction of both carbonyl moieties also took place[18] and *anti*-diol **3a** could be directly isolated in 76% yield as the sole diastereomer [diastereomeric ratio (dr) >98:2], albeit essentially in racemic form (8% ee) (Table 1, entry 6). Switching to RC of *Kluyveromyces marxianus* CBS 6556, did not lead to greater than 27% yield of **2a** jointly with a racemic mixture of **3a** in 55% yield (Table 1, entry 7). Hence, the formation of highly enantioenriched aldol **2a** and/or diol **3a**, *via* stereoselective reduction of β-diketone **1a**, proved to be a challenging task with all the microorganisms screened. Structural features of different 1-aryl-1,3-diones were, then, investigated *en route* to optically active 2,4-disubstituted aryloxetanes.

Reduction of 4,4,4-trifluoro-1-phenylbutane-1,3-dione (**1b**) with both GC and RC of *Kluyveromyces marxianus* afforded the corresponding aldol derivative **2b** in 91 and 74% chemical yield, respectively, while no appreciable stereoselectivity was observed after 24 or 48 h incubation at pH 7.4 (Table 1, entries 8,9). In the latter case, acetophenone (**5**) could also be isolated (10% yield), most likely as the result of a base-catalyzed retro-aldol reaction (Table 1).[19] The use of GC of *Saccaromyces cerevisiae* CBS 7336 provided aldol **2b** in 20% yield and with an ee value of 40% in favour of the *R*-enantiomer, whereas the exposure of **1b** to RC resulted in an increase in yield (60%) but a decrease in ee (8%) of **2b** after 48 h incubation at pH 7.4 (Table 1, entries 10,11). Variable amounts of **5** (10–12%) were also obtained. The different stereoselectivity observed is probably due to different ADH expression in different metabolic conditions of *Saccaromyces cerevisiae* CBS 7336.[20] Diketone **1b** was smoothly converted into aldol **2b** only after 4 h incubation in tap water at 30 °C in high chemical yield (73%) and ee (82%) when RC of baker's yeast were used (Table 1, entry 12). The apparent, unusual *anti*-Prelog *R*-

stereopreference observed in entries 10–12 is due to a change in the priority of the groups around the stereogenic center.[21]

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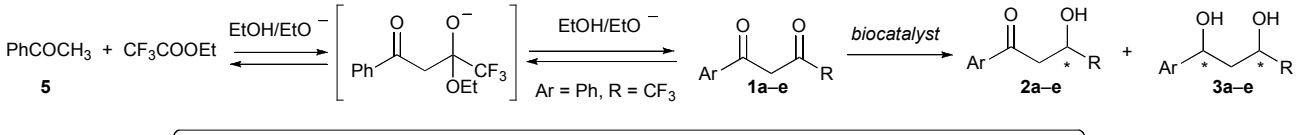
DOI: 10.1039/C6OB02320G

Table 1. Screening of different biocatalysts for the stereoselective reduction of diketones **1a–e**.[a]



a: Ar = R = Ph, **b**: Ar = Ph, R = CF_3; **c**: Ar = 2-furyl, R = CF_3; **d**: Ar = Ph, R = CH_3; **e**: Ar = 2-naphtyl, R = CF_3

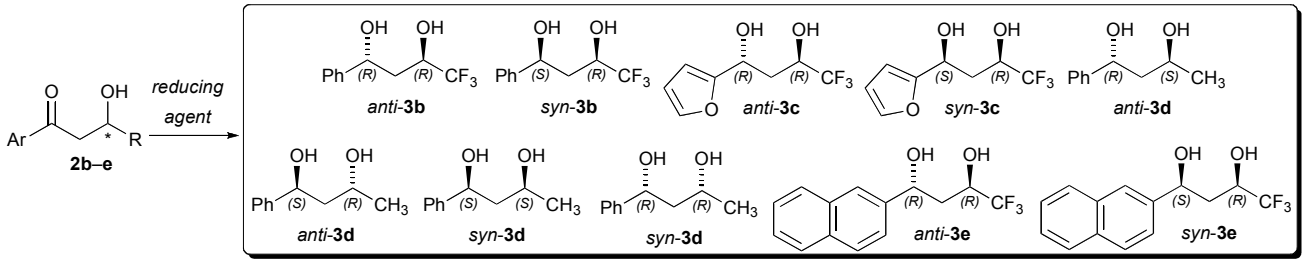
Entry	Biocatalyst	Ar	R	Compound	t (h)	Conversion (%)[b]	Product (yield %)[c]	Product (ee %, abs. conf.)[d]
1	*Yarrovia Lipolytica Y16 (GC)*	Ph	Ph	**1a**	96	NR[e]	ND[f]	–
2	*Baker's yeast (RC)*	Ph	Ph	**1a**	96	NR[e]	ND[f]	–
3	*Lactobacillus reuteri (RC)*[g,h]	Ph	Ph	**1a**	24	NR[e]	ND[f]	–
4	*Trigonopsis variabilis (GC)*[i]	Ph	Ph	**1a**	96	12	[j]	–
5	*Saccharomyces cerevisiae (GC)*[k]	Ph	Ph	**1a**	96	50	**2a** (50)	**2a** (58, *S*)
6	*Kluyveromyces marxianus (GC)*[l]	Ph	Ph	**1a**	96	90	**2a** (12) **3a** (76)[m]	**2a** (36, *S*) **3a** (8)
7	*Kluyveromyces marxianus (RC)*[l]	Ph	Ph	**1a**	96	82	**2a** (27); **3a** (55)[n]	–
8	*Kluyveromyces marxianus (GC)*[l]	Ph	CF_3	**1b**	24	95	**2b** (91)	**2b** (8, *S*)
9	*Kluyveromyces marxianus (RC)*[l]	Ph	CF_3	**1b**	48	84	**2b** (74)	**2b** (8, *S*)[o]
10	*Saccharomyces cerevisiae (GC)*[k]	Ph	CF_3	**1b**	24	30	**2b** (20)	**2b** (40, *R*)[o]
11	*Saccharomyces cerevisiae (RC)*[k]	Ph	CF_3	**1b**	48	72	**2b** (60)	**2b** (8, *R*)[o]
12	*Baker's yeast (RC)*	Ph	CF_3	**1b**	4	65	**2b** (73)	**2b** (82, *R*)
13	*Lactobacillus reuteri (RC)*[g,h]	Ph	CF_3	**1b**	4	43	**2b** (39)	**2b** (88, *R*)
14	*Lactobacillus reuteri (RC)*[g,h]	Ph	CF_3	**1b**	24	87	**2b** (80)	**2b** (84, *S*)
15	*Baker's yeast (RC)*	Furanyl	CF_3	**1c**	4	90	**2c** (90)	**2c** (64, *R*)
16	*Kluyveromyces marxianus (GC)*[l]	Furanyl	CF_3	**1c**	96	98	**2c** (78)	**2c** (38, *S*)
17	*Lactobacillus reuteri (RC)*[h]	Furanyl	CF_3	**1c**	24	36	**2c** (29)	**2c** (28, *S*)
18	*Baker's yeast (RC)*	Ph	CH_3	**1d**	24	75	**2d** (75)	**2d** (90, *S*)
19	*Lactobacillus reuteri (RC)*[g,h]	Ph	CH_3	**1d**	4	>98	**2d** (93)	**2d** (96, *R*)
20	*Lactobacillus reuteri (RC)*[g,h]	Ph	CH_3	**1d**	24	>98	**2d** (95)	**2d** (96, *R*)
21	*Saccharomyces cerevisiae (GC)*[k]	Ph	CH_3	**1d**	24	NR[e]	ND[f]	–
22	*Lactobacillus reuteri (RC)*[g]	Napht-2-yl	CF_3	**1e**	24	31	**2e** (26)	**2e** (32, *S*)
23	*Baker's yeast (RC)*	Napht-2-yl	CF_3	**1e**	24	53	**2e** (40)	**2e** (80, *R*)

[a] Typical reaction conditions: orbital incubator: 200 rpm; temperature: 30 °C; (*GC*): inoculum after 24 h growth in a sterile medium containing glucose (1%), peptone (0.5%), yeast extract (0.3%) and malt extract (0.3%) in sterile water; (*RC*): 0.5 g/L of cell wet mass in 0.1 M KH_2PO_4 buffer (pH = 7.4) enriched with 1% glucose, diketone (2 mM final concentration). [b] Calculated by ^{1}H NMR based on the diagnostic enolic protons of the unreacted diketone in the crude. [c] Isolated yield after column chromatography. [d] Enantiomeric excess (ee) determined by HPLC analysis. Absolute configuration (abs. conf.) of aldols (**2a–e**) determined by comparing optical rotation sign and retention time (HPLC analysis) with known data.[e] No reaction. [f] ND means not determined because of trace content. [g] PBS solution as reaction media (T = 37 °C). [h] DSM 20016. [i] DSM 70714. [j] Complex mixture. [k] CBS 7336. [l] CBS 6556.[m] Only the *anti*-**3a** diol (dr >98:2) was detected and isolated. [n] Racemic mixture. [o] Acetophenone (10–12% yield) was also isolated.

Similarly, aldol **2b** was recovered with high ee (88%) by using *Lactobacillus reuteri* DSM 20016 after 4 h incubation, albeit in lower yield (39%). Chemical yield, however, could be increased up to 80% by increasing the incubation time up to 24 h with only a little erosion in ee (84%) (Table 1, entries 13, 14). High conversions (90–98%) were obtained for the bioreduction of furanyl-substituted butane-1,3-dione **1c** to aldol **2c** with both RC of baker's yeast and GC of *Kluyveromyces marxianus*, although with moderate ee (38–64%) but opposite stereopreference (Table 1, entries 15,16).On the other hand, bioreduction of **1c** with *Lactobacillus reuteri* DSM 20016 provided **2c** with even lower chemical (29%) and optical (28%) yields (Table 1, entry 17). Baker's yeast and *Lactobacillus reuteri* DSM 20016 whole cells proved to be the best

biocatalysts for the conversion of 1-phenylbutane-1,3-dione (**1d**) into the two enantioenriched stereoisomeric aldols **2d**. In the former case, *S*-enantiomer was produced in a remarkable yield of 75% and ee of 90%, whereas in the latter case *R*-enantiomer was isolated in up to 95% yield and 96% ee (Table 1, entries 18–20). *Saccharomyces cerevisiae* CBS 7336 was ineffective for the reduction of **1d**, and aldol **2d** did not form even after 24 h incubation (Table 1, entry 21). Finally, upon subjecting 2-naphtyl-substituted 1-butane-1,3-dione **1e** to the action of *Lactobacillus reuteri* DSM 20016 and baker's yeast, while *S*-aldol **2e** formed both in low yield (26%) and enantioselectivity (32%), *R*-aldol **2e** could be isolated in 40% yield and in up to 80% ee (Table 1, entries 22, 23).

Table 2. Chemical reduction of aldols **2b–e** for the preparation of racemic and optically active 1,3-diols **3b–e**.



Entry	Aldol (ee %)	Reducing agent	Product (yield %)[a]	dr *anti*: *syn*[b]	Stereoisomer (ee %)[c]
1	(*R*)-**2b** (82)	$Me_4NHB(OAc)_3$	**3b** (98)	91:9[d]	*anti*-(1*R*,3*R*)-**3b** (80); *syn*-(1*S*,3*R*)-**3b** (50)
2	(*R*)-**2c** (64)	DIBAL-H	**3c** (78)	75:25[e]	*anti*-(1*R*,3*R*)-**3c** (42); *syn*-(1*S*,3*R*)-**3c** (34)
3	(*S*)-**2d** (90)	$Me_4NHB(OAc)_3$	**3d** (89)	88:12[d]	*anti*-(1*R*,3*S*)-**3d** (86); *syn*-(1*S*,3*S*)-**3d** (82)
4	(*R*)-**2d** (98)	$Et_2BOCH_3/NaBH_4$	**3d** (95)	2:98[d]	*syn*-(1*R*,3*R*)-**3d** (98)
5	(*R*)-**2d** (98)	$NaBH_4$	**3d** (93)	51:49[d]	*anti*-(1*S*,3*R*)-**3d** (98);*syn*-(1*R*,3*R*)-**3d** (96)
6	(*S*)-**2d** (90)	$NaBH_4$	**3d** (91)	57:43[d]	*anti*-(1*R*,3*S*)-**3d** (98); *syn*-(1*S*,3*S*)-**3d** (96)
7	(*R*)-**2e** (80)	$Me_4NHB(OAc)_3$	**3e** (86)	88:12[e]	*anti*-(*1R,3R*)-**3e** (60); *syn*-(*1S,3R*)-**3e** (56)
8	(*R*)-**2e** (80)	$Et_2BOCH_3/NaBH_4$	**3e** (88)	6:94[e,f]	*syn*-(*1S,3R*)-**3e** (72)

[a] Overall isolated yield in the two diastereomers. [b] Calculated by ^{1}H NMR. [c] Ee determined by HPLC analysis; abs. conf. determined by comparing optical rotation sign and retention time (HPLC analysis) of diols with known data (see Experimental); the chemical formulas refers to the major enantiomer. [d] Separable mixture of diols by column chromatography. [e] Unseparable mixture of diols. [f] Ee determination of *anti*-(1*R*,3*R*)-**3e** was impractical.

Synthesis of steredefined 1,3-diols

Having established the strain specificity and the optimal conditions for efficient biosynthesis of some representative classes of optically active aldols, we next focused on their chemical transformation into the corresponding *syn*- and/or *anti*-diols according to the reported procedures.[22] The mild reducing agent tetramethylammonium triacetoxyborohydride [$Me_4NHB(OAc)_3$] converted aldols (*R*)-**2b**, (*S*)-**2d**, and (*R*)-**2e** mainly into the corresponding *anti*-diols (1*R*,3*R*)-**3b**, (1*R*,3*S*)-**3d**, (1*R*,3*R*)-**3e** with high yield (up to 98%) and high diastereoselectivity (up to 91:9) (Table 2, entries 1,3,7). The ee of the starting aldol was mainly preserved in the final diols, with the exceptions of *syn*-(1*S*,3*R*)-**3b**, *anti*-(1*R*,3*R*)-**3e**, and *syn*-(1*S*,3*R*)-**3e** for which a slight racemization took place most probably during the acidic workup procedure (Table 2, entries 1,3,7) (see Experimental Section). As for the sensitive trifluoromethyl furanyl-substituted aldol (*R*)-**2c**, a complex mixture of diols (including products of hydrolysis and reduction of the furanyl ring), was recovered using both $Me_4NHB(OAc)_3$ and sodium borohydride ($NaBH_4$) as reducing agents, whereas diisobutylaluminum hydride (DIBAL-H) cleanly provided a mixture of the expected optically active *syn*- and *anti*-diols **3c** in good overall yield (78%), moderate diastereoselectivity (75:25), although eroded of 20–30% in their ee_s (Table 2, entry 2). Trifluoromethyl carbinol derivatives are, indeed, known to easily undergo partial racemization even under mild acidic conditions.[23] Interestingly, reduction of (*R*)-**2d** (98% ee) and (*R*)-**2e** (80% ee) with the combination diethylmethoxyborane (Et_2BOMe)/$NaBH_4$, in place of $Me_4NHB(OAc)_3$, stereospecifically afforded *syn*-(1*R*,3*R*)-**3d** and *syn*-(1*S*,3*R*)-**3e**, respectively, in excellent yield (up to 95%) and diastereoselectivity (up to 98:2), and with no or slight erosion (8%) of the enantiomeric purity (Table 2, entries 4,8). Notably, by subjecting both enantiomerically enriched aldols (*R*)- and (*S*)-**2d** to the action of $NaBH_4$, two separable mixtures of *anti*- and *syn*-diols **3d** formed in almost equimolar ratio in high chemical yield (91–93%) and high optical purity (96–98% ee) (Table 2, entries 5,6). The relative configuration of all the

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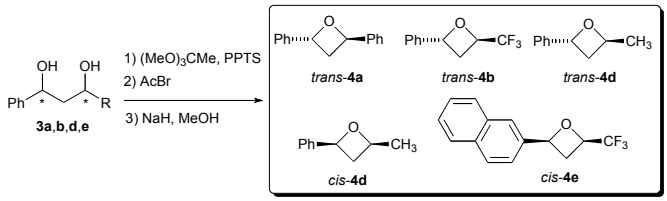
synthesized diasteromeric diols **3a–e** (Tables 1 and 2) was assigned by ^{1}H NMR analysis, particularly by comparing chemical shifts and coupling constants with those previously reported (see Experimental Section). Mohar and co-workers recently succeeded in the preparation of highly enantiomerically enriched CF_3-substituted *anti*-1,3-diols from the corresponding 1,3-diketones by exploiting an ansa-ruthenium (II)-catalyzed asymmetric transfer hydrogenation under a dynamic kinetic resolution control.[24] Complementary *syn*-1,3-diols could also be accessed from stereopure aldols, however, only by chanching the configuration of the stereocenters of the chiral catalyst. We have now shown that a variety of enantioenriched *syn*- and *anti*-1,3-diols can be easily synthesized directly from β-diketones by simply using cheap and commercially available whole cells and by selecting a chemical reducing agent.

Synthesis of stereodefined 2,4-disubstituted aryloxetanes

Sterospecific cyclization of 1,3-diols into the corresponding 2,4-disubstituted oxetanes was then investigated. We followed the two-step procedure reported by Nelson for racemic (1*R**,2*S**)- and (1*R**,3*R**)-3-cyclohexyl-1-phenylpropane-1,3-diols, which is based on a preliminary conversion of diols into orthoesters with acetyl bromide, followed by methanolysis of the putative bromoacetate intermediates and ring-closure promoted by NaH/THF. The whole transformation is known to proceed via two stereospecific inversion reactions, and thus with overall retention of configuration at the involved stereogenic centers.[13] According to such a strategy, stereospecific conversion of diastereomeric diols **3a**, **3b**, **3d**, and **3e** into the corresponding stereodefined oxetanes **4a**, **4b**, **4d**, and **4e** took place smoothly with only slight reduction of the starting ee in the case of the trifluoromethyl-substituted derivative **4b** (**3b**: 78% ee; **4b**: 60% ee) (Table 3). Formation of elimination products was also noted to compete in the cyclization of diols **3a** and **3d**. The increasing amount of *trans*-oxetane detected in the final mixture, compared to that of the starting diol, indicates a higher chemical stability of the *anti*-diastereomer under the experimental conditions used. On the other hand, cyclization of furanyl-substituted diol **3c** (Table 2) failed because of furan hydrolysis.[25]

Preparation of the corresponding racemic oxetanes, which is necessary for ee analysis, was performed as well by subjecting to cyclization mixtures of racemic *syn*- and *anti*-diols (see ESI). The relative stereochemistry of the newly synthesized oxetanes was determined by a careful analysis of both NMR chemical shifts and vicinal $^3J_{HH}$ coupling constants, and was supported for the unknown compounds by NOESY phase-sensitive experiments (see ESI).[26] The absolute stereochemistry was instead assigned based on the stereospecificity of the cyclization reaction starting from the corresponding diols.[13]

Table 3. Synthesis of stereodefined 2,4-disubstituted aryloxetanes **4a, 4b**, **4d**, and **4e**.[a]



Diol **3** (dr *anti*:*syn*,[b] ee %)	Oxetane **4** (yield %,[c] dr *trans*:*cis*,[b] ee %)
anti-**3a** (>98:2, 8)	*trans*-**4a** [75, >98:2, 6][d,e]
anti-(1*R*,3*R*)-**3b** (91:9, 78)	*trans*-(2*R*,4*R*)-**4b** [98, >98:2, 60][d]
anti-(1*R*,3*S*)-**3d** (88:12, 86)	*trans*-(2*R*,4*S*)-**4d** [51, 90:10, 88][d]
syn-(1*S*,3*S*)-**3d** (8:92, 82)	*cis*-(2*S*,4*S*)-**4d** [43, 9:91, 94][d]
syn-(1*S*,3*R*)-**3e** (6:94, 72)	*cis*-(2*S*,4*R*)-**4e** [73, 10:90, 80][f]

[a] The chemical formulas refers to the major enantiomer. [b] Calculated by ^{1}H NMR. [c] Isolated yield after column chromatography. [d] Ee determined by GC analysis on a chiral stationary phase (see ESI). [e] For this cyclization, the *anti*-**3a** diol, straightforwardly obtained by bioreduction of ketone **1a**, was used (see Table 1, entry 6). [f] Ee determined by HPLC analysis.

Conclusions

In summary, stereodefined 2,4-disubstituted aryloxetanes have, for the first time, been synthesized starting from symmetrical and unsymmetrical 1,3-diones. The key step in obtaining these challenging, still rarely present in abstracted literature, building blocks is the regio- and stereoselective bioreduction of the above diones into the corresponding aldols, which proved to be successfully catalysed by cheap and commercially available whole-cell biocatalysts such as baker's yeast and *Lactobacillus reuteri* DSM 20016. Next, diastereomerically enriched or almost equimolar mixtures of optically active *syn*- and *anti*-1,3-diols can be produced according to the nature of the reducing agent. Finally, a two-step stereospecific cyclization allowed the obtainment of the enantiomerically enriched oxetanes in good yields and with overall retention of configuration. The final dr and ee proved to be slightly affected by the relative chemical stability of the various *syn*- and *anti*-diastereomeric diols synthesized under the acidic conditions of the cyclization process. We expect the whole asymmetric methodology presented in this paper is easily and widely expanded to other oxetanyl systems, thereby enabling the preparation of target architectures for pharmaceutical exploration. Our current efforts are now focused on the preparation of stereodefined, more substituted oxetanes by exploiting lithiation-electrophilic trapping strategies starting from the valuable chiral, nonracemic oxetanes described herein.

Experimental

All the synthesized optically active aldols **2a–e** and diols **3a–e** obtained by bioreduction showed analytical and spectroscopic data identical to those previously reported,[24,27] or to the commercially available compounds. Aldols **2a–e** and diols **3a–e**

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DOI: 10.1039/C6OB02320G

were also prepared as racemic mixtures (for HPLC references) by $NaBH_4$ reduction in EtOH in 89–95% yields, according to the reported procedures,[14] excepted when otherwise specified.

Bioreduction of 1a–e by baker's yeast. General procedure.
Baker's yeast (10 g) was dispersed to give a smooth paste in tap water (50 mL). The substrate (0.1 g) was added and stirred at 30 °C in an orbital shaker (250 rpm). The reaction progress was monitored by TLC. After the time indicated in Table 1, the reaction was stopped by centrifugation, decantation and extraction by EtOAc. The extract was dried over anhyd Na_2SO_4, and the solvent evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane and EtOAc (90:10 or 60:40) as eluents to yield the desired aldols (**2a–e**) reported in Table 1.

Bioreduction of 1a–e by *Lactobacillus reuteri* resting cells. General procedure.
Lactobacillus reuteri pre-culture was inoculated in MRS[29] and incubated for 24 h (37 °C). Cells were collected after centrifugation (4000 rpm, 10 min), and washed twice with phosphate buffer saline at pH 7.4 (PBS, Sigma-Aldrich). Finally the cells were suspended in the same buffer and adjusted for cell density. To this cell suspension, 1% glucose and the desired concentration of diketone were added. To ensure the anaerobic conditions, flasks were degassed with a N_2 flux for 3 min. The reaction mixture was incubated at 37 °C, 200 rpm. After appropriate conversion, the suspension was centrifuged (4000 rpm, 10 min, 4 °C), and the aqueous phase was extracted with Et_2O (3 x 15 ml). The organic phase was dried over anhyd Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane and EtOAc (90:10 or 60:40) as eluents to yield the desired aldols (**2a–e**) reported in Table 1.

Bioreduction of 1a–c by *Kluyveromyces marxianus* growing cells (GC). General procedure.
Cells preserved on agar slants at 4 °C were used to inoculate 250 mL flasks containing 100 mL of the culture medium. The flasks were incubated aerobically at 30 °C on an orbital shaker and stirred at 250 rpm. Flasks (250 mL) containing 100 mL of the culture medium were then inoculated with 5 mL of the 24-h-old suspension and incubated under the same conditions for 24 h. Flasks (1 L) containing 400 mL of the culture medium were then inoculated with 5 mL of the latter suspension and incubated for 24 h. The optical density was checked at 620 nm for all cultures before adding aryldiketones **1a–c** (100 mg) dissolved in 1 mL of EtOH. The progress of the reactions was monitored by TLC and/or GC and stopped at the times indicated in Table 1. The content of the flask was then centrifuged and the supernatant extracted with EtOAc. All the reactions were repeated at least twice without any noticeable bias in the results. The residue was purified by silica gel column chromatography using hexane and EtOAc (90:10 or 60:40) as eluents to yield the desired aldols (**2a–c**) and the diol **3a**, as reported in Table 1.

Stereoselective reduction of aldols 2b–e with $Me_4NHB(OAc)_3$. General procedure.
Acetic acid (5 mL) was added to a stirred solution of tetramethylammonium triacetoxyborohydride (2.9 g, mmol) in dry acetonitrile (5 mL) and the reaction was stirred for 30 min. The reaction was cooled to –40 °C and a solution of the aldol (0.5 mmol) in acetonitrile (3 mL) was added. The reaction was stirred for 4 h, left overnight at –20 °C, quenched with aq. sodium potassium tartrate solution (0.5 M, 40 mL) and finally stirred for additional 30 min. Dichloromethane (100 mL) and sat. aq. sodium bicarbonate solution (100 ml) were added, the layers separated, and the aqueous fraction extracted with CH_2Cl_2 (3 × 50 mL). The combined organic fractions were finally washed with sat. aq. sodium bicarbonate solution (3 × 50 mL), dried (Na_2SO_4), filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting with 1:15 EtOAc–hexane, to give *anti*-diol.

Stereoselective reduction of aldols 2d–e with $Et_2BOCH_3/NaBH_4$. General procedure.
Diethylmethoxyborane (1.0 M in THF, 425 µL, 0.386 mmol) was added to a stirred solution of the selected 3-hydroxy-1-arylpropan-1-one (**2d**,**e**) (76 mg, 0.35 mmol) in dry THF (16 mL) and MeOH (4 mL). The reaction was stirred for 15 min at –78 °C and sodium borohydride (15 mg, 0.386 mmol) was added. The reaction mixture was stirred for additional 2 h at –78 °C, quenched with acetic acid (5 mL), and slowly warmed to room temperature overnight. The reaction mixture was diluted with EtOAc (20 mL) and washed with sat. aq. sodium bicarbonate solution (3 × 20 mL) until the vigorous evolution of CO_2 ceased. The combined organic extracts were dried over Na_2SO_4, filtered and evaporated under reduced pressure to give the crude product, which was purified by silica gel column chromatography (EtOAc/hexane 3 : 7), to give *syn*-diol in the yield reported in Table 2.

General procedure for the synthesis of oxetanes 4a–e.[13]
Trimethyl orthoacetate (132 µl, 0.86 mmol) and pyridinium toluene-*p*-sulfonate (2 mg) were added to a stirred solution of diols **3a–e** (202 mg, 0.70 mmol) in dry CH_2Cl_2 (7 mL). The reaction mixture was stirred for 10 min at room temperature, cooled to –78 °C, and acetyl bromide (156 µl, 1.78 mmol) was added. The reaction was stirred for additional 1.5 h, quenched with sat. aq. $NaHCO_3$ solution, extracted with CH_2Cl_2 (3 × 5 ml), dried (Na_2SO_4), filtered and evaporated to give a crude product. The latter was dissolved in dry THF (10 ml), and MeOH (32 µl, 0.95 mmol) and NaH (104 mg, 60% dispersion in oil, 2.13 mmol) were sequentially added. The vessel was sealed with a glass cap and the reaction stirred for 24 h at 60 °C. After this time, the reaction was quenched with water and extracted with EtOAc (3 × 15 ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated to give a crude product which was purified by flash silica gel column chromatography (10% Et_2O in petroleum ether), to give the oxetanes **4a–e** (Table 3).

Characterization data of synthesized oxetanes.

***trans*-2,4-Diphenyloxetane (4a).**[28] White solid, mp (Et_2O) 122–123 °C, 75% yield (57% overall yield; 54 mg starting from 100 mg of **1a**), dr >98:2. Er (2*S,4S*):(2*R,4R*) = 53:47 determined by HPLC, Lux Cellulose-1 column, hexane: 2-propanol = 90:10, 0.8 mL/min), t_R [major (*S,S*)-enantiomer] = 14.4 min, t_R [minor (*R,R*)-enantiomer] = 15.8 min. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52–7.50 (m, 4 H), 7.43–7.39 (m, 4 H), 7.32–7.29 (m, 2 H), 5.82 (t, *J* = 5.8 Hz, 2 H), 3.00 (t, *J* = 5.8 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.5, 128.6, 127.8, 125.3, 79.6, 38.4; GC MS (70 eV) *m/z* (%) 210 (M^+, 1), 105 (21), 104 (100), 103 (18), 89 (1), 79 (2), 78 (18), 77 (19), 63 (2), 51 (8); FT-IR (KBr): 3011, 2920, 1644, 1290, 1155, 1121, 863, 751, 699 cm^{-1}. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{15}H_{14}ONa^+$: 210.1045; Found 210.1052.

(2*R,4R*)-2-Phenyl-4-(trifluoromethyl)oxetane (4b).[26b] Colourless oil, 98% yield (58% overall yield; 56 mg starting from 100 mg of **1b**), dr >98:2. Er (2*R,4R*):(2*S,4S*) = 80:20 determined by GC-Chirasil-DEX CB capillary column, (He flow 1 mL/min, 100 °C), t_R [major (*R,R*)-enantiomer] = 18.5 min, t_R [minor (*S,S*)-enantiomer] = 17.5 min, $[\alpha]_D^{20}$ = +8.42 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.31 (m, 5 H), 5.87 (t, *J* = 7.5 Hz, 1 H), 4.93–4.86 (m, 1 H), 3.12–3.07 (m, 1 H), 2.89–2.82 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.5, 128.8, 128.5, 125.2, 124.9 (q, $^1J_{C\text{-}F}$ = 280 Hz), 81.9, 74.1 (q, $^2J_{C\text{-}F}$ = 35 Hz), 29.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ –80.4 (d, $^3J_{F\text{-}H}$ = 6.5 Hz); GC MS (70 eV) *m/z* (%) 202 (M^+, 7), 133 (1), 115 (2), 107 (4), 106 (51), 105 (100), 104 (15), 103 (10), 91 (2), 78 (14), 77 (28); FT-IR (neat): 3064, 3030, 2955, 2926, 2856, 1455, 1364, 1173, 1099, 1060, 1016, 760, 700 cm^{-1}. HRMS (EI): *m/z* Calcd for $C_{10}H_9F_3O$: 202.0605; Found: 202.0599.

(2*R,4S*)-2-Phenyl-4-methyloxetane (4d). Colourless oil, 51% yield (26% overall yield; 24 mg starting from 100 mg of **1d**), dr 90:10. Er (2*R,4S*):(2*S,4R*) = 94:6 determined by GC-Chirasil-DEX CB capillary column, (He flow 2 mL/min, 100 °C), t_R [minor (*S,R*)-enantiomer] = 12.1 min, t_R [major (*R,S*)-enantiomer] = 12.4 min), $[\alpha]_D^{20}$ = +31.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45–7.27 (m, 5 H), 5.70–5.66 (m, 1 H), 5.05–4.97 (m, 1 H), 2.71–2.67 (m, 2 H), 1.56 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (600 MHz, $CDCl_3$) δ 143.9, 128.4, 127.4, 125.1, 78.6, 75.6, 36.9, 23.8; GC MS (70 eV) *m/z* (%) 148 (8), 107 (60), 106 (10), 105 (100), 104 (88), 103 (27), 79 (11), 78 (38), 77 (53), 51 (24), 43 (11); FT-IR (neat): 2952, 2923, 2852, 1734, 1719, 1646, 1456, 1376, 1260, 1093, 1023, 873, 799, 699 cm^{-1}. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{10}H_{12}NaO^+$ 171.0786; Found 171.0783.

(2*S,4S*)-2-Phenyl-4-methyloxetane (4d). Colourless oil, 43% yield (37% overall yield; 34 mg starting from 100 mg of **1d**) (dr 91:9). Er (2*S,4S*):(2*R,4R*) = 97: 3 determined by GC-Chirasil-DEX CB capillary column, (He flow 2 mL/min, 100 °C), t_R [major (*S,S*)-enantiomer] = 11.4 min, t_R [minor (*R,R*)-enantiomer] = 11.8 min, $[\alpha]_D^{20}$ = –2.51 (*c* 0.9, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44–7.42 (m, 2 H), 7.38–7.35 (m, 2 H), 7.30–7.27 (m, 1 H), 5.70–5.65 (m, 1 H), 5.05–4.97 (m, 1 H), 3.07–3.01 (m, 1 H), 2.29–2.23 (m, 1 H), 1.47 (d, 3 H, *J* = 6.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.6, 128.4, 127.7, 125.4, 78.0, 74.4, 38.2, 24.1; GC MS (70 eV) *m/z* (%) 148 (7), 107 (60), 106 (11), 105 (100), 104 (87), 103 (27), 79 (9), 78 (40), 77 (51), 51 (25), 43 (9); FT-IR (neat): 2954, 2925, 2853, 1720, 1648, 1455, 1377, 1259, 1095, 1022, 872, 799, 699 cm^{-1}. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{10}H_{12}NaO^+$ 171.0786; Found 171.0780.

(2*S,4R*)-2-(Naphthalen-2-yl)-4-(trifluoromethyl)oxetane (4e). Colourless waxy solid, 73% yield (25% overall yield; 63 mg starting from 100 mg of **1e**), dr 90:10. Er (2*S,4R*):(2*R,4S*) = 90:10 determined by HPLC, Lux Cellulose-1 column, hexane: 2-propanol = 90:10, 0.8 mL/min), t_R [major (*S,R*)-enantiomer] = 7.6 min, t_R [minor (*R,S*)-enantiomer] = 7.0 min, $[\alpha]_D^{20}$ = –22.0 (*c* 0.35, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91–7.86 (m, 4 H), 7.58 (dd, *J* = 8.5, 1.5 Hz, 1 H), 7.54–7.50 (m, 2 H), 5.99 (t, *J* = 7.7 Hz, 1 H), 5.14–5.08 (m, 1 H), 3.18 (dt, *J* = 11.8, 7.5 Hz, 1 H), 2.90 (dt, *J* = 11.8, 7.8, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.1, 133.3, 133.1, 128.6, 128.2, 127.7, 126.4, 126.3, 124.7, 122.9, 121.9 (q, $^1J_{C\text{-}F}$ = 281.0 Hz), 80.0, 72.8 (q, $^2J_{C\text{-}F}$= 36.0 Hz), 30.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ –81.8 (d, $^3J_{F\text{-}H}$ = 6.5 Hz); GC MS (70 eV) *m/z* (%) 252 (M^+, 4), 183 (30), 156 (100), 69 (9); FT-IR (KBr): 2930, 2852, 1649, 1232, 1150, 1116, 1000, 894, 862, 820, 765 cm^{-1}. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{14}H_{11}F_3ONa^+$: 275.0660; Found 275.0654.

Acknowledgements

This work was financially supported by the University of Bari within the framework of the Project "Sviluppo di nuove metodologie di sintesi mediante l'impiego di biocatalizzatori e solventi a basso impatto ambientale" (code: Perna01333214Ricat), and by both C.I.N.M.P.I.S. and CIRCC consortia. This work was partially supported also by the "Reti di Laboratori - Produzione Integrata di Energia da Fonti Rinnovabili nel Sistema Agroindustriale Regionale " program funded by the Apulia Region Project Code 01'. (Intervento cofinanziato dall'Accordo di Programma Quadro in materia di Ricerca Scientifica – II Atto Integrativo – PO FESR 2007–2013, Asse I, Linea 1.2-PO FSE 2007–2013 Asse IV ''Investiamo nel vostro futuro''). The authors are also indebted to Mr Antonio Palermo for expert technical NMR assistance, and to Dr. Marilena D'Introno, Dr. Giovanni Clemente, Miss Rosa Giannelli, Miss Simona Summa, and Mr Roberto Capobianco for their contribution to the Experimental Section.

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