Enzyme-catalyzed C–C bond formation using 2-methyltetrahydrofuran (2-MTHF) as (co)solvent: efficient and bio-based alternative to DMSO and MTBE

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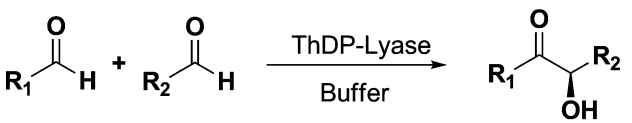
The enzymatic carboligation of aldehydes (C–C bond formation) catalyzed by benzaldehyde lyase (BAL) affords chiral α-hydroxy-ketones under mild reaction conditions in aqueous media. To enhance substrate and product availability under aqueous conditions, processes are often set-up using either DMSO as co-solvent, or MTBE as second organic phase. Although efficient, DMSO leads to difficulties in separation during downstream processing, with wastewater formation. MTBE provides a cleaner and straightforward work-up, but its petrochemical origin, together with its poor degradability, gives rise to environmental concerns. Herein it is reported that 2-methyltetrahydrofuran (2-MTHF) is a promising candidate to substitute DMSO or MTBE in lyase-catalyzed reactions. 2-MTHF can be derived from bio-based resources (*e.g.* levulinic acid), and it is abiotically degraded by air. When BAL is added to buffer-2-MTHF (5% v/v) mixtures, enzyme remains stable with a half-life of 178 ± 8 h, with productivities (benzoin synthesis) of 10 g benzoin $L^{-1}h^{-1}$. Several BAL-catalyzed aldehyde carboligations were assessed under those conditions, leading in all cases to high isolated yield (quantitative in majority), and to high enantioselectivity (up to >99%). Furthermore, preliminary results obtained with two phase systems in the BAL-catalyzed benzoin synthesis afforded 60 g benzoin L^{-1} in 24 h (*ee* > 99%). Therefore, 2-MTHF may be a valuable (co)solvent, not only to tackle environmental concerns, but also in terms of practical, efficient biocatalysis.

1. Introduction

Biocatalysis is being increasingly accepted as an alternative for the preparation of chiral building blocks. Key-factors are the high regio- and enantioselectivities often reported, together with the applied mild reaction conditions. Furthermore, once enzyme genes are cloned and overexpressed, biocatalysts can be produced "on demand" *via* cost-effective fermentative routes.[1]

Thiamine-diphosphate dependent lyases (ThDP-Lyases) are a versatile group of enzymes that enantioselectively catalyze the carboligation of aldehydes (C–C bond formation) to afford chiral α-hydroxy-ketones (Scheme 1).[2] These compounds are useful building blocks for pharmaceutical and fine chemical applications.[2a]

An outstanding example of ThDP-lyases is benzaldehyde lyase from *Pseudomonas fluorescens* (BAL, EC.4.1.2.38). BAL catalyzes the enantioselective carboligation of both aromatic and aliphatic aldehydes.[2,3] Several process-development studies concerning BAL-catalyzed synthetic systems (*e.g.* biphasic media, whole-cell approach, *etc.*) have demonstrated the potential of this enzyme at an industrial scale as well.[2,4] Apart from BAL, recently other lyase-based reactions were reported, *e.g.* enantioselective Stetter-type 1,4-additions,[5] or aldehyde-ketone and ketone-ketone carboligations to afford enatiopure/enantiomerically enriched tertiary alcohols.[6] Likewise, the cofactor of these enzymes, thiamine diphosphate, has also been the model for the development of many organocatalytic *umpolung* carboligations.[7]



R_1, R_2 = Ph, Alkyl — up to 99 % *ee*

Scheme 1 Enantioselective C–C bond formation catalyzed by thiamine-diphosphate dependent enzymes.[2–4]

Biocatalytic reactions are often conducted in aqueous media, as the more compatible milieu for enzymes (natural catalysts). Yet, that approach normally decreases synthetic productivities, since organic compounds are often poorly soluble in aqueous conditions. To overcome this, the use of either a co-solvent (*e.g.* DMSO, 2-propanol, *tert*-butanol, *etc.*), or a second organic phase (toluene, MTBE, *etc.*) is widely employed in biocatalysis.[1] For ThDP-lyases, the use of DMSO as co-solvent and outstand-

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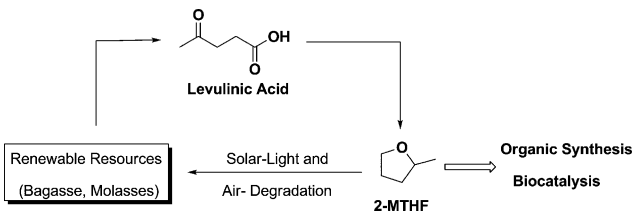
ing stabilizer of those enzymes is well-known.[8] In addition, MTBE has been reported as a proper organic solvent to set-up biphasic reactions involving ThDP-lyases, leading to high productivities (*ca.* 80–100 g L^{-1}) in short reaction times (up to 24 h).[4]

Productivities are certainly a crucial parameter when novel biocatalytic approaches are assessed. However, environmental issues must also be taken into account. In this respect, the use of DMSO leads to a considerable wastewater formation during work-up. On the other hand, the use of MTBE as a second phase provides a more straightforward extractive work-up, and may positively influence equilibrium positions.[9] However, MTBE has a petrochemical origin, with a poor biodegradability in the environment, leading to its accumulation and subsequent pollution of water reservoirs.[10]

Therefore, there is the need for novel biocatalytic strategies that can provide environmentally-friendly processes, while at the same time being economically sound for industrial needs. In this respect 2-methyltetrahydrofuran (2-MTHF) may be an alternative as (co)solvent. Albeit a profound toxicological assessment of 2-MTHF is pending – and thus 2-MTHF should not (yet) be regarded as a "green solvent" – it can be derived from biomass (Scheme 2),[11] provides a straightforward work-up (b.p. *ca.* 80 °C), and it is abiotically degraded in air.[12]



Scheme 2 Concept for the bio-based production of 2-MTHF.[11]

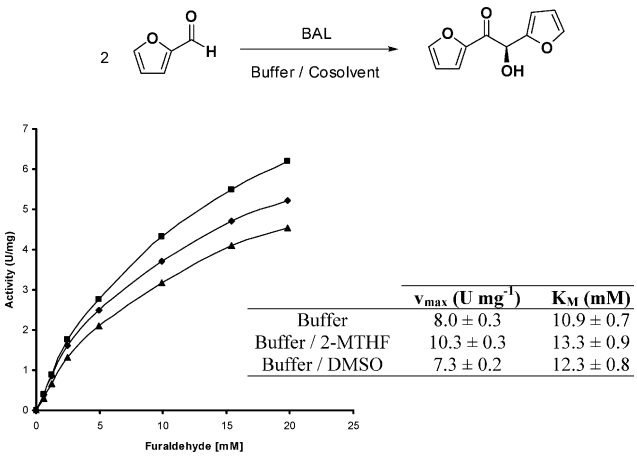
2-MTHF has found applications in the preparation of Grignard reagents, cross-coupling reactions,[12] in enantioselective 1,4-additions,[13] in classic organometallic chemistry,[14] organocatalysis,[15,16] as well as in other synthetic procedures.[17,18] In biocatalysis there is only one example dealing with lipase-catalyzed acylations in 2-MTHF as solvent,[19] with lipases as exemplary robust enzymes.[20] In general, the quest of green solvents is presently an important area of research.[21]

Herein, we report for the first time the use of 2-MTHF as (co)solvent in ThDP-lyase-catalyzed reactions. The aim of this work is to show that the combination of 2-MTHF with lyases may provide a promising scenario where enzyme stability, activity, and reaction productivity can be achieved with the use of bio-based, easily-degradable solvents.

2. Results and discussion

2.1. BAL characterization and stability in 2-MTHF

Several phosphate buffer solutions containing different amounts of 2-MTHF were prepared. In accordance with literature[12] 2-MTHF was completely miscible with the buffer in mixtures containing up to 5% v/v of 2-MTHF. At higher proportions a second phase was formed. Therefore, 2-MTHF may easily be used both as co-solvent (up to 5% v/v), as well as solvent for the set-up of a biphasic system. As first goal the activity of BAL in 2-MTHF was assessed. Traditional activity assay methods for BAL reported in the open literature (*e.g.* spectrophotometry, HPLC, or enzyme-coupled approaches),[2,8] though more or less reliable, are often time-consuming and not straightforward. Therefore, firstly a more convenient spectrophotometric protocol for BAL assays – based on the furoin formation BAL-catalyzed carboligation of 2 furfurals – was set-up. Furoin absorbance was measured at 320 nm where substrate absorbance (furfural) is negligible and product formation can be monitored directly. In this new protocol, one unit of activity was defined as the amount of BAL that catalyzes the formation of 1 µmol furoin per minute under standard conditions (30 °C, pH 8) (see experimental for details). By means of this method, a kinetic characterization of BAL was performed under three reaction conditions: pure buffer, buffer with 5% v/v of DMSO, and buffer with 5% v/v of 2-MTHF (Fig. 1).



	v_{max} (U mg^{-1})	K_M (mM)
Buffer	8.0 ± 0.3	10.9 ± 0.7
Buffer / 2-MTHF	10.3 ± 0.3	13.3 ± 0.9
Buffer / DMSO	7.3 ± 0.2	12.3 ± 0.8

Fig. 1 BAL activity as a function of 2-furaldehyde concentration. (◆) Buffer; (▲) Buffer/DMSO; (■) Buffer/2-MTHF.

BAL showed enzymatic activities in all buffer/co-solvent systems studied. Interestingly, the enzymatic performance in buffer/2-MTHF (5% v/v) displayed a slightly higher v_{max} than these observed for pure buffer or buffer/DMSO (5% v/v). Affinities of BAL (K_M) were however slightly lower than values in pure buffer. Overall, it was clear that BAL was active in the novel reaction media buffer/2-MTHF (5% v/v). Since kinetically BAL provided a promising framework (Fig. 1), later on the stability of BAL in the presence of 2-MTHF was assessed. BAL was dissolved in phosphate buffer. Stabilities were assessed in pure buffer, and using either 2-MTHF (5% v/v) or DMSO (5% v/v) as cosolvents. Samples were stored and tested for residual activity. Deactivation kinetics are depicted in Fig. 2.

In agreement with literature, BAL stability is greatly enhanced when DMSO is used as co-solvent, and thus only 10% loss of activity was observed within 250 h of incubation (Fig. 2).[8] Yet, as previously stated, using DMSO as co-solvent in enzymatic reactions is not the preferred option for practical biocatalysis, due to problems in the downstream processing. Remarkably, BAL stability in the presence of 2-MTHF is slightly better than data observed for pure buffer systems. Thus, for buffer/2-MTHF (5% v/v) system, a half-life of 178 ± 8 h was estimated, assuming first order deactivation kinetics. Parameter estimation for a

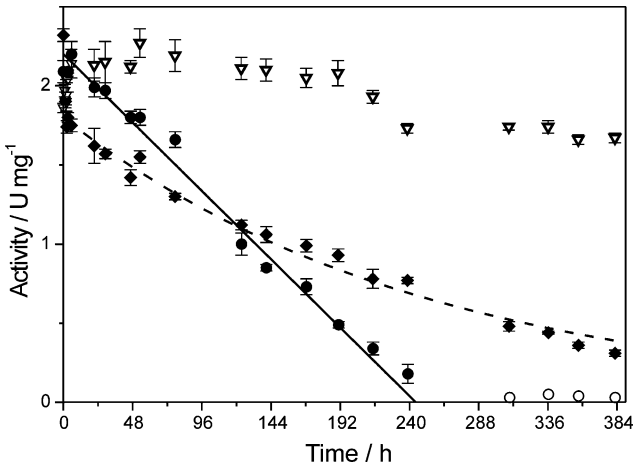
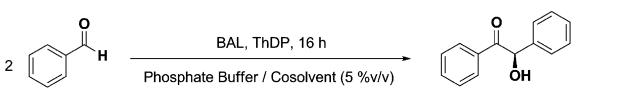


Fig. 2 Deactivation kinetics of BAL in different conditions: (●) Pure buffer (○: disregarded for estimation of half life, see text); (◆) Buffer/2-MTHF (5% v/v); (▽) Buffer/DMSO (5% v/v).

second order exponential decay, as discussed in the literature,[22] did not lead to meaningful parameter values. Interestingly, the mechanism of deactivation changes with the addition of 2-MTHF from apparently zero order in buffer. Clearly, the interaction of BAL with 2-MTHF may be subjected to further investigation. From a practical perspective 2-MTHF provides a promising framework of BAL stability from which synthetic applications could be envisaged, provided that industrial batch biocatalytic processes typically run in less than 24 h reaction time.

2.2. BAL-catalyzed carboligations using 2-MTHF as (co)solvent

As previously stated, BAL shows a broad substrate spectrum, being able to enantioselectively carboligate aromatic and aliphatic aldehydes, as well as in a cross-condensation fashion (*e.g.* aromatic and aliphatic aldehydes).[2–4,8] Therefore, to fully assess the possibilities of 2-MTHF as a (co)solvent, examples of this type of reaction were checked. First of all, benzoin formation (condensation of two benzaldehyde molecules) was studied. Again, three systems were compared: pure buffer and additions of either DMSO (5% v/v) or 2-MTHF (5% v/v) as co-solvent (Scheme 3).



No Cosolvent: 40 % Yield; > 99 % *ee*
DMSO: 99 % Yield; > 99 % *ee*
2-MTHF: 99 % Yield; > 99 % *ee*

Scheme 3 BAL-catalyzed benzoin condensations in different reaction media: Pure phosphate buffer (pH 8), and buffer with either DMSO or 2-MTHF as co-solvent (5% v/v). Conditions: benzaldehyde 95 mmol L^{-1}, 200 U BAL, room temperature.

As observed, the addition of a co-solvent to the aqueous buffer is crucial for achieving a proper performance in BAL-catalyzed reactions, in agreement with literature.[2–4,8] Importantly, both DMSO and 2-MTHF were equally useful for the enzymatic performance, leading to quantitative isolated yields and high enantioselectivities in both cases. Likewise, further experiments with the buffer/2-MTHF (5% v/v) medium showed that quantitative benzoin yields could even be obtained after 1 h reaction, with a productivity of 0.05 mol L^{-1} benzoin with 200 U BAL (Fig. 3). This gives a space-time-yield of 10 g benzoin L^{-1} h^{-1}.

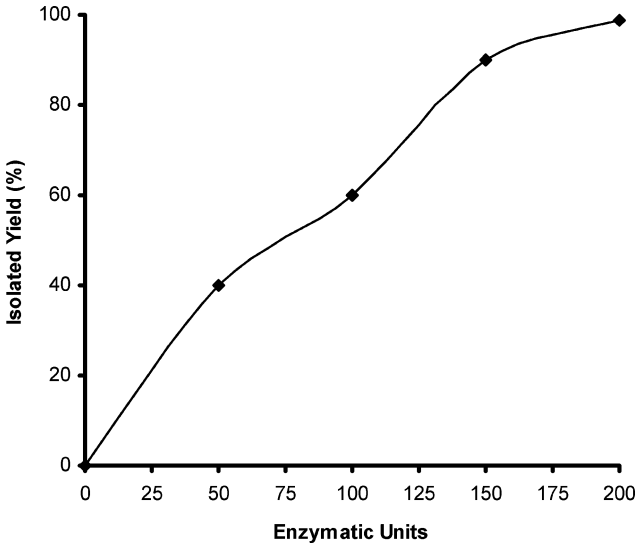


Fig. 3 Isolated yield (benzoin formation) after 1 h reaction time, as a function of the amount of enzyme employed (95 mmol L^{-1} benzaldehyde, buffer/2-MTHF 5% v/v).

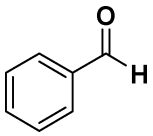
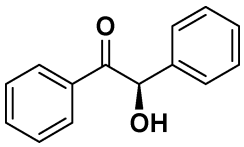
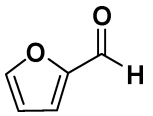
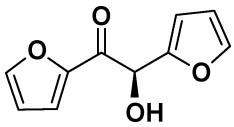
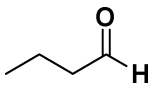
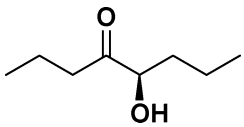
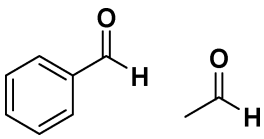
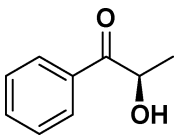
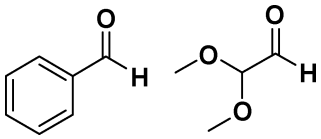
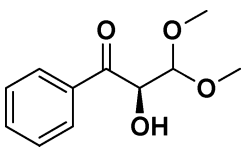
Therefore, taking those productivities together with the stability of BAL in 2-MTHF, it becomes clear that 2-MTHF is not only a bio-based alternative for organic solvents, but also an efficient reaction medium for conducting biocatalysis with high productivities. Encouraged by the promising results, the system buffer/2-MTHF (5% v/v) was extended to other aldehydes as substrates in BAL-catalyzed reactions (Table 1).

Both aliphatic and aromatic aldehydes exhibit high conversion in BAL-catalyzed reactions, when 2-MTHF (5% v/v) is used as co-solvent. Gratifyingly, cross-condensation reactions (carboligation of two different aldehydes) already showed close to quantitative conversions within 1 h as well. In agreement with literature, to obtain a pure crossed-product, a surplus of the acceptor aldehyde was added, to shift the equilibrium created by the benzoin.[2–4,8] Concerning enantioselectivities of the afforded α-hydroxy-ketones, values remained the same as those reported with DMSO or MTBE.[2–4,8] Therefore, the use of 2-MTHF did not affect the high enantioselectivity usually displayed by BAL.

Furthermore, 2-MTHF was also assessed as an extractive solvent during the work-up procedure. Taking again the benzoin synthesis as the model reaction, the work-up procedure was conducted with three different organic solvents: dichloromethane, ethyl acetate, and 2-MTHF. In all cases quantitative yields of benzoin (99%) were isolated. This may provide additional environmental benefits, since more toxic organic solvents (*e.g.* dichloromethane) can be easily replaced by other more benign bio-based derivatives (*e.g.* ethyl acetate or 2-MTHF). Likewise, fewer emulsion problems and phase separation are expected with 2-MTHF.[17]

Finally, the substrate loading was increased, taking again the benzoin synthesis as the model reaction. The aim was to evaluate

Table 1 Summary of results obtained in BAL-catalyzed carboligation of aldehydes using 2-MTHF (5% (v/v) as co-solvent in potassium phosphate buffer (50 mmol L^{-1}, pH 7.0, $MgSO_4$ (2.5 mmol L^{-1}), and ThDP (0.15 mmol L^{-1}). 16 h reaction time

Substrate(s)	Product	Isolated yield	*ee* (*R*)
		>99%	>99%
		92%	93%
		95%	52%
		99%	>99%
		95%	98%

whether practical biocatalysis could be set-up with 2-MTHF as second phase. To this end, an initial solution of 95 mmol L^{-1} benzaldeyde in buffer/2-MTHF (5% v/v) was prepared. Later on a mixture of benzaldehyde/2-MTHF was carefully dosed by using a micro syringe pump, reaching a final 2-MTHF volume of 10 mL and 1 M benzaldehyde. 250 U of BAL were added (in two portions, 100 U at the beginning and 150 U at 6 h reaction time). After 24 h work-up was performed, 60 g benzoin L^{-1} was isolated (60% yield) with high enantiomeric excess (>99%). Although reaction conditions are non-optimized (in terms of volumes, amount of substrate, dosing rate, *etc*.) achieved yields were already comparable to the outcomes of biphasic systems reported in the literature, in which yields of 80–100 g L^{-1} were provided.[4] Therefore, 2-MTHF can be successfully used for BAL-catalyzed reactions, not only as co-solvent (5% v/v), but also as a second organic phase in biphasic set-ups, if higher yields or different reactions conditions are desired.

3. Conclusions

The potential of 2-MTHF as (co)solvent in BAL-catalyzed enzymatic reactions has been assessed. Enzymatic asymmetric syntheses of α-hydroxy-ketones can be efficiently performed in buffer systems with the addition of 5% v/v of 2-MTHF (as co-solvent), leading to quantitative yields, and (in many cases) high enantioselectivity. Likewise, when 2-MTHF is applied in excess to form a second phase (as solvent), under non-optimized conditions production of 60 g benzoin L^{-1} in 24 h was achieved. Furthermore, 2-MTHF can also be used as an extractive agent during work-up, with similar performances as ethyl acetate or dichloromethane. Overall, results show that 2-MTHF may be not only a promising alternative in terms of environmental concerns – being a bio-based, abiotically-degradable derivative – but also that 2-MTHF may be an important option to set-up practical biocatalytic concepts. Research dealing with 2-MTHF in other enzymatic reactions involving organic (co)solvents may be a promising research line for the future as well.

4. Experimental

4.1. Chemicals and enzyme production

All compounds were purchased from Sigma-Aldrich, and were used directly. Benzaldehyde lyase from *Pseudomonas fluorescens* was cloned in *E. coli*, overexpressed, and produced by fermentation as described elsewhere.[2–4,8] After fermentation and purification, BAL was lyophilized and stored at –20 °C until use.

4.2. BAL characterization

Furoin absorbance was measured at 320 nm. At that range, 2-furaldehyde absorbance is negligible and therefore the analytic tool was reliable. In this new protocol, one unit of activity was defined as the amount of BAL that catalyzes the formation of

1 μmol furoin per minute under standard conditions (30 °C, pH 8). 2-Furaldehyde 5 mmol L^{-1} in phosphate buffer (pH 8) which contained 2.5 mmol L^{-1} $MgSO_4$ and 0.25 mmol L^{-1} ThDP were used as a substrate. The formation of 2,2-furoin over time was followed with spectrophotometry at 320 nm using a multiplate reader (PowerWave, BioTek Instruments) with a quartz 96 well plate (Helima).

4.3. Analytics

NMR spectra were recorded on a Bruker DPX 400, or on a Bruker AMX 300. Chemical shifts δ are reported in ppm relative to $CHCl_3$ (^{1}H: δ = 7.27) and $CDCl_3$ (^{13}C: δ = 77.0) as internal standard. Enantiomeric excesses were determined by chiral phase SFC analysis (Chiralcel IA column, UV detection at 254 nm). Benzoin: eluent: CO_2/2-propanol = 90 : 10, flow 4.0 mL min^{-1}, 40 °C. (*R*)-2-Hydroxy-phenylpropan-1-one: eluent: CO_2/2-propanol = 90 : 10, flow 4.0 mL min^{-1}, 40 °C. (*R*)-2-Hydroxy-3,3-dimethoxy-1-phenylpropan-1-one: eluent: CO_2/2-propanol = 90 : 10, flow 4.0 mL min^{-1}, 40 °C. Furoin: eluent: CO_2/2-propanol = 90 : 10, flow 3.0 mL min^{-1}, 40 °C.

4.4. Benzoin

Benzaldehyde (199 mg, 1.8 mmol) was dissolved in a mixture of 5% v/v 2-methyltetrahydrofuran and potassium phosphate buffer (20 mL, 50 mmol L^{-1}, pH 7.0, containing $MgSO_4$ (2.5 mmol L^{-1}) and ThDP (0.15 mmol L^{-1}). After addition of BAL (20 mg) the reaction mixture was gently stirred for 16 h. The reaction mixture was extracted with ethyl acetate or 2-methyltetrahydrofuran (3 × 10 mL), and the organic layer washed with water (3 times 10 mL) and brine (3 times 10 mL), and were dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by crystallization afforded (*R*)-2-hydroxy-1,2-diphenylethan-1-one as a colorless solid; yield: 198 mg (99%, (*R*)-enantiomer 99% *ee*); HPLC: (Chiralpak IA) R_t (*R*) = 5.0 min; ^{1}H NMR (400 MHz, $CDCl_3$): 7.83 (d, *J* = 7.0 Hz, 2H), 7.33–7.19 (m, 8H), 5.88 (d, *J* = 6.0 Hz, 1H), 4.58 (d, *J* = 7.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 198.9, 139.0, 133.9, 133.5, 129.2, 129.4, 129.1, 128.7, 128.6, 76.2.

4.5. Cross condensation benzaldehyde and acetaldehyde

Benzaldehyde (105 mg, 1.0 mmol) was dissolved in a mixture of 5% v/v 2-methyltetrahydrofuran and potassium phosphate buffer (20 mL, 50 mmol L^{-1}, pH 7.0, containing $MgSO_4$ (2.5 mmol L^{-1}) and ThDP (0.15 mmol L^{-1}). After addition of BAL (20 mg) the reaction mixture was gently stirred for 1 h. To this solution, acetaldehyde was added in three different intervals (1 h) with concentration of 97 mmol L^{-1} (261 mg, 6 mmol) it was allowed to stir for 16 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL) and the organic layers washed with water (3 × 10 mL) and brine (3 × 10 mL), and were dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by crystallization afforded (*R*)-2-hydroxy-1-phenylpropan-1-one as a colorless solid; yield: 149 mg (99%, (*R*)-enantiomer 99% *ee*). HPLC: (Chiralpak IA) R_t (*R*) = 2.61 min; ^{1}H NMR (400 MHz, $CDCl_3$): 7.85 (d, *J* = 8.0 Hz, 2H), 7.56–7.40 (m, 3H), 5.13 (q, *J* = 7.0 Hz, 1H), 3.75 (br. s, 1H), 1.37 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 202.3, 133.9, 133.3, 128.8, 128.7, 69.3, 22.3.

4.6. Cross condensation benzaldehyde and 2,2-dimethoxyacetaldehyde

Benzaldehyde (105 mg, 1.0 mmol) was dissolved in a mixture of 5% v/v 2-methyltetrahydrofuran and potassium phosphate buffer (35 mL, 50 mmol L^{-1}, pH 7.0, containing $MgSO_4$ (2.5 mmol L^{-1}) and ThDP (0.15 mmol L^{-1}). After addition of BAL (20 mg) the reaction mixture was gently stirred for 1 h. To this solution, dimethoxyglyoxal was added in a three different intervals (1 h) with concentrations not more than 95 mmol L^{-1} (947 mg, 6 mmol) and stirred for 16 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL) and the organic layers washed with water (3 × 10 mL) and brine (3 × 10 mL), and were dried over Na_2SO_4. Evaporation of the solvent gave yellow oil. The yield was calculated from NMR; yield: 149 mg (95%, (*R*)-enantiomer 99% *ee*). HPLC: (Chiralpak IA) R_t (*R*) = 2.1 min; R_t (*S*) = 34.5 min. NMR data consistent with literature.[4] ^{1}H-NMR (400 MHz, $CDCl_3$): 3.32 (s, 3H), 3.35 (s, 3H), 4.40 (d, *J* = 4, 1H), 5.06 (br. t, 1H), 7.38–7.42 (m, 2H), 7.51–7.56 (m, 1H), 7.89–7.91 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): 199.8, 134.9, 133.8, 129.1, 128.4, 76.7, 73.7, 56.5.

4.7. Furoin

Furaldehyde (96 mg, 1.0 mmol) was dissolved in a mixture of 5% v/v 2-methyltetrahydrofuran and potassium phosphate buffer (20 mL, 50 mmol L^{-1}, pH 7.0, containing $MgSO_4$ (2.5 mmol L^{-1}) and ThDP (0.15 mmol L^{-1}). After addition of BAL (20 mg) the reaction mixture was allowed to stir for 16 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL) and the organic layers washed with water (3 × 10 mL) and brine (3 × 10 mL), and were dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by crystallization afforded (*R*)-2-hydroxy-1,2-diphenylethan-1-one as a colorless solid; yield: 88 mg (92%, (*R*)-enantiomer 99% *ee*). HPLC (Chiralpak IA): R_t (*S*) = 3.9 min; R_t (*R*) = 4.7 min. ^{1}H NMR (400 MHz, CDCl3): 7.54 (m, 1H), 7.30 (m, 1H), 7.17 (d, *J* = 4.0 Hz, 1H), 6.46 (dd, *J* = 2.0, 4.0 Hz, 1H), 6.33–6.27 (m, 2H), 5.72 (d, *J* = 7.0 Hz, 1H), 4.12 (d, *J* = 7.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d6): 184.2, 151.2, 149.6, 147.7, 143.1, 120.2, 112.6, 110.8, 109.1, 69.3.

4.8. BAL-carboligation of butyraldehyde

Butyraldehyde (128 mg, 1.7 mmol) was dissolved in a mixture of 5% v/v 2-methyltetrahydrofuran and potassium phosphate buffer (20 mL, 50 mmol L^{-1}, pH 7.0, containing $MgSO_4$ (2.5 mmol L^{-1}) and ThDP (0.15 mmol L^{-1}). After addition of BAL (20 mg) the reaction mixture was allowed to stir for 16 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL) and the organic layers washed with water (3 × 10 mL) and brine (3 × 10 mL), and were dried over Na_2SO_4. Evaporation of the solvent gave the crude product as yellow oil. (*R*)-5-Hydroxyoctan-4-one; yield: 121 mg (95%, (*R*)-enantiomer 52% *ee*). ^{1}H NMR (400 MHz, DMSO-d_6): 5.11 (d, *J* = 5.6 Hz, 1H), 3.78 (m, 1H), 2.39 (m, 2H) 1.51–1.15 (m, 6H), 0.78 (t,

J = 7.5 Hz, 3H), 0.74 (t, J = 7.5 Hz, 3); ^{13}C-NMR (100 MHz, $CDCl_3$): 13.71, 13.84, 17.07, 18.54, 35.77, 39.69, 76.23, 212.22.

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