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ARTICLE TYPE

Catalytic bio-chemo and bio-bio tandem oxidation reactions for amide and carboxylic acid synthesis

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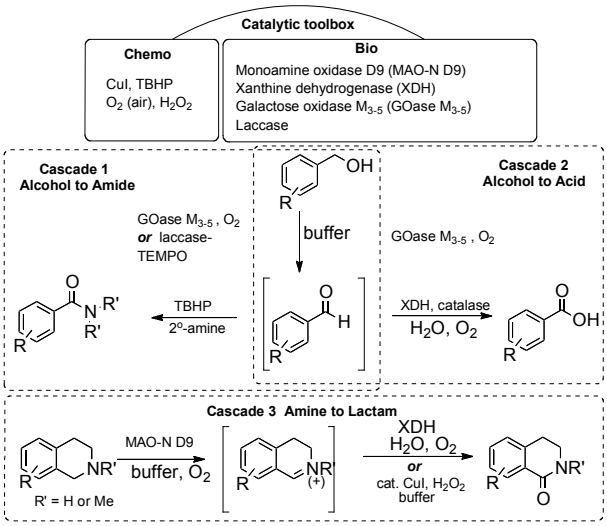
A catalytic toolbox for three different water-based one-pot cascades to convert aryl alcohols to amides and acids and cyclic amines to lactams, involving combination of oxidative enzymes (monoamine oxidase, xanthine dehydrogenase, galactose oxidase and laccase) and chemical oxidants (TBHP or CuI(cat)/H_2O_2) at mild temperatures, is presented. Mutually compatible conditions were found to afford products in good to excellent yields.

Amides, lactams and carboxylic acids are ubiquitous functional groups in organic chemistry found in natural products, pharmaceuticals and a wide range of synthetic polymers. Amide bond formation can be achieved using activated carboxylic acid derivatives or an increasingly elaborate range of coupling reagents.[1] However, these methods can be expensive and involve the use of toxic and atom inefficient reagents, increasing their environmental E factor.[2] Due to their widespread application in synthetic organic chemistry, there is a great deal of interest in new sustainable and environmentally benign alternatives for generating both carboxylic acids[3] and amides[4].

The approach described here exploits the increasing range of oxidative enzymes that can work under ambient conditions in aqueous buffer and use aerial oxygen as the electron acceptor, hence representing an ideal alternative to traditional oxidants. The ability to tune enzyme activity and substrate specificity using protein engineering and directed evolution strategies[4b,5] has resulted in the creation of biocatalysts that can be used *in vitro* in catalytic cascade pathways on unnatural substrates.[6] This approach allows combination of enzymes (bio-bio)[6a] and of enzymes with chemocatalysts (bio-chemo),[6a,7] extending the range of sustainable chemistry possible. In this paper, we demonstrate three new one-pot tandem cascade reactions using combinations of enzymes and chemocatalysts for: oxidative coupling of aryl alcohols with amines to give amides (cascade 1), conversion of aryl alcohols to carboxylic acids (cascade 2) and transformation of cyclic amines to lactams (cascade 3) (Scheme 1).



Scheme 1. Catalytic bio-chemo and bio-bio tandem oxidations.

Our objectives were realised by combination of enzymes and chemocatalysts that can work cooperatively under mild aqueous conditions, avoiding the use of organic solvents, hazardous and toxic chemicals as well as heavy metal catalysts associated with a minimization of energy and waste production.

(1) Alcohols to aldehydes to amides (Cascade 1)

Recently, there has been a great deal of interest in synthetic organic chemistry in developing catalytic oxidative amidation reactions to couple aldehydes with amines. The reactions are thought to proceed by oxidation of the imine or hemi-aminal intermediates and are catalysed by transition metals (Rh, Ru, Pd,

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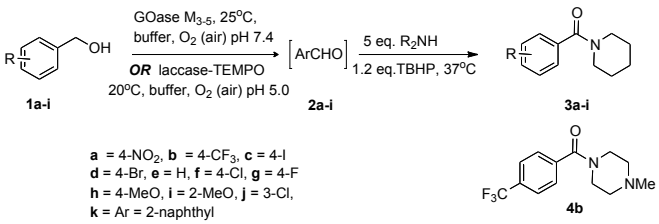
Fe),[8] *N*-heterocylic carbenes,[9] Cu,[10] Cu-Ag[11] and lanthanides.[12] Stoichiometric terminal oxidants required are *tert*-butyl hydroperoxide (TBHP) or aqueous 70 % TBHP (T-HYDRO), H_2O_2, or oxone. In some cases, it is possible to start with the alcohol, which undergoes oxidation to the aldehyde *in situ*.[4c,13,14] Yields for oxidative amidation of benzaldehydes are generally good (46-96 %), although aliphatic and heteroaryl aldehydes give lower yields and require higher reaction temperatures. Thus, mild catalytic conditions that can run at ambient temperature without the need to use amine HCl salts[10] or a large excess of the aldehyde would be an attractive tool for future chemistry.

We have previously developed variants of *F. graminearum* galactose oxidase (GOase), such as GOase M_{3-5}, that show a remarkable ability to oxidise secondary and primary benzylic alcohols to their respective ketone and aldehyde products and H_2O_2 as by-product.[15] The combination of laccase from *T. versicolor* with the redox mediator TEMPO[16] can be employed to achieve the same transformations. We now report the application of both biocatalysts in a one-pot tandem reaction with different amines (5 eq.) and TBHP (1.2 eq.) to convert benzylic alcohols to aldehydes (1st step) and subsequently to the tertiary amides (2nd step) (Table 1, Tables S1 & S2). The reactions were run sequentially as one-pot-two-step processes since the GOase M_{3-5} and laccase were found to be sensitive or inhibited by the amines or TBHP required in the second step. As GOase produces within its catalytic cycle one mole H_2O_2 per mole of alcohol substrate being oxidised, we attempted to use this natural *in situ* generated by-product in the amide formation step in place of addition of TBHP. The results, however, suggested the amount of enzymatically generated H_2O_2 to be insufficient for oxidation of the aminal intermediate to yield the desired amide products **3a-k** and **4b**.

The temperature for the one-pot amide formation was maintained at 20-37 °C. In the second step, in which the assumed aminal intermediate is oxidised to the amide, the tandem reactions worked optimally at higher initial substrate concentrations (50-80 mM) which were found to be best tolerated by the laccase-TEMPO system employed for the 1st step (aldehyde formation). Thus, the highest conversions (9-91 %) and isolated yields (22-91 %) of amides were obtained using the laccase-TEMPO combination. Different benzyl alcohol substrates exhibited a concentration-dependent effect on the efficiency of conversion to the respective amide when comparing the two biocatalytic systems. Substrates such as *para*-nitrobenzyl alcohol **1a** gave high conversion to amide **3a** at lower concentrations (10 mM) used in combination with GOase M_{3-5}. In contrast, alcohols **1c-d** and **1f-i** showed distinct variation in yields when comparing the GOase M_{3-5} (10 mM) and laccase-TEMPO system (80 mM). In general, alcohols with electron-withdrawing substituents revealed a pronounced propensity for amide formation, whereas yields declined with electron-donating groups. Strictly speaking, although the first step for aldehyde formation from benzyl alcohols was quantitative in both the GOase M_{3-5} and laccase-TEMPO systems (Tables S1 and S2), the amide forming second step was clearly identified to determine yields of amide products due to the concentration of aldehyde present. Most examples presented herein involved piperidine as a model amine, although we were pleased to find that our method can be extended to the formation of tertiary amide **4b**, a feature frequently found in drug molecules.

Table 1: Bio-Chemo tandem conversion of alcohols **1** to amides **3** and **4**.



Alcohol 1		Conversion to amide 3/4 [%]	
		GOaseM_{3-5}-TBHP[a]	Laccase-TEMPO -TBHP[b]
1a	**3a**	100	91 (91)[c]
1b	**3b**	87	89 (57)
1c	**3c**	63	90 (60)
1d	**3d**	26	86 (73)
1e	**3e**	21	32
1f	**3f**	14	75 (53)
1g	**3g**	4	69
1h	**3h**	0	26 (22)
1i	**3i**	0	9
1j	**3j**	36	87 (41)
1k	**3k**	38	45 (35)
1b	**4b**[d]	-	(40)

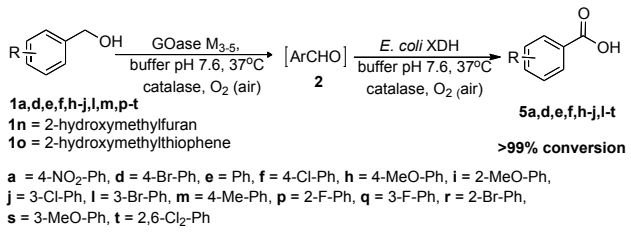
[a] Reaction conditions: GOase M_{3-5} (7.25μM), **1a-k** (10 mM) in sodium phosphate buffer (50 mM pH 7.4), 25 °C , 16 h, then piperidine (R_2NH) (5 eq.), TBHP (1.2 eq., 6.6 % *v/v*), 37 °C, 24 h. [b] Reaction conditions: Laccase (12 U), TEMPO (24 mM), **1a-k** (80 mM) in sodium citrate buffer (100 mM), 20 °C, 16 h, then piperidine (R_2NH) (5 eq.), TBHP (1.2 eq.), 37 °C, 24 h. [c] Isolated yields in parentheses; [d] R_2NH = N-methylpiperazine (5 eq.). Conversion to amides reported are based on HPLC peak areas at ƛ = 254 nm (Tables S1 and S2).

(2) Alcohols to carboxylic acids (Cascade 2)

The oxidation of alcohols to carboxylic acids very often requires a stepwise process *via* the aldehyde and typically employs catalytic ruthenium or chromium and strong oxidants such as iodate or chlorite.[17] Direct catalytic oxidation of alcohols to carboxylic acids is relatively rare.[3a,18] Biocatalytic processes using whole cells and isolated enzymes are attractive tools for synthesis of carboxylic acids due to the mild and green conditions employed.[19] However, with whole cells, products often need to be continuously removed from the reaction due to toxicity of the intermediate aldehyde or acid. Therefore, *in vitro* cascades employing isolated enzymes equally offer a very attractive alternative approach. Examples include the use of alcohol dehydrogenases and aldehyde dehydrogenases with recycling of the oxidised NAD^+ cofactor carried out by an oxygen-dependent NADH oxidase.[20] Whilst elegant, there is still the requirement for addition of cofactor and the auxiliary enzyme.

Aiming to expand the range of *in vitro* processes toward carboxylic acid synthesis, we have developed a cascade reaction using two oxidative enzymes, GOase M_{3-5} and xanthine dehydrogenase (XDH) from *E. coli*,[21] to achieve direct and clean

conversion of aryl alcohols to acids *via* the *in situ* generated aldehyde (Scheme 2). XDH belongs to a family of molybdenum-dependent enzymes[22] and uses aerial O_2 as an electron acceptor in the absence of other mediators or cofactors. This enzyme family is receiving increasing attention in the drug metabolism field[23] but has never before been exploited in synthesis. Since the substrate specificity of *E. coli* XDH has not previously been reported, we initially screened a panel of *ca.* 65 aldehydes (Table S3) using nitroblue tetrazolium (NBT), a redox active dye previously used to examine microorganisms for xanthine oxidase activity.[24] Substrate specificity of *E. coli* XDH appeared to be dictated by enzyme-substrate interactions since there were no obvious substrate electronic effects dictating reactivity. We selected the best hits from the NBT assay for more detailed analysis and were delighted to observe 81-100 % conversion in 1-5 h. While most of the aldehyde substrates were oxidised by *E. coli* XDH to >90% conversion within 1 h, aldehydes **2d**, **2i** and **2m** revealed slower turn-over, taking 5 h to reach 80 - 90% conversion. There is no structural information on *E. coli* XDH although related aldehyde oxidases are known to accept a wide range of substrates.[23] The aryl alcohols **1a, 1d-1f, 1h-j, 1l-t**, corresponding to the best aldehyde substrates **2** for *E. coli* XDH, were then selected for a one-pot-one-step GOase M_{3-5}-XDH cascade approach resulting in *quantitative* conversion of 16 benzyl and heteroaryl alcohols to the corresponding carboxylic acids over 16 h (Scheme 2, Table S4).



Scheme 2: Bio-bio cascade reaction for conversion of alcohols **1** to acids **5**. Reaction Conditions: GOase M_{3-5} (1.3 mg/mL), alcohols **1** (1 mM) in sodium phosphate buffer (50 mM, pH 7.6), catalase (0.25 mg/mL), *E. coli* XDH (0.18 mg/mL), 37 °C, 16 h.

Following optimisation, the oxidation of 3-methoxybenzyl alcohol **1s** was run at 40 mM substrate concentration (Table S5, Figure S33) showing complete conversion to the aldehyde by GOase M_{3-5} after 30 min, followed by slower conversion by *E. coli* XDH to reach 94 % conversion to the acid **5s** (81 % isolated yield) after 5 h. The addition of catalase to destroy the H_2O_2 generated by GOase M_{3-5} and delivering additional equivalents of O_2 was found to be essential for achieving high conversions. In order to facilitate increased substrate loading, current work is focussed on finding improved enzymes for both steps to increase rate and throughput.

(3) Cyclic amines to lactams (Cascade 3)

Catalytic methods for the direct α-oxidation of amines to afford lactams are receiving a great deal of attention. However, most methods require high temperatures or environmentally undesirable stoichiometric reagents such as hypervalent iodine [25] or chlorite.[26] Use of bulk gold[27] and gold nanoparticle catalysts[28] have been reported but often require temperatures up to 100 °C in organic solvents[29] or the presence of 200 mol% NaOH.[28] Recently, use of a remarkable Ru-pincer complex (150 °C, sealed tube) has been reported for the oxidation that uses water as the oxygen source and produces hydrogen.[30]

Herein, we now present our initial results on the one-pot oxidation of cyclic amines to lactams under mild (37 °C) and aqueous conditions using two novel and related approaches (Table 2). Both of these methodologies use a variant of *A. niger* monoamine oxidase (MAO-N D9) to catalyse the oxidation of the cyclic amine **6** to the imine/iminium **7** (1st step). The second step uses *either* chemocatalysis (H_2O_2/cat. CuI) or biocatalysis (xanthine dehydrogenase (XDH)/electron acceptor) to yield the desired lactam **8**.

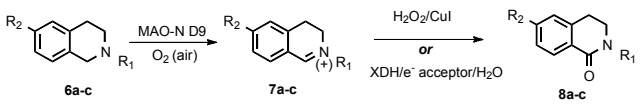
Our model substrate for initial studies on the tandem reaction was tetrahydroisoquinoline **6a** (THIQ) in view of the high activity displayed by the D9 variant of MAO-N (Table S6). Thus, the corresponding imine dihydroisoquinoline **7a** (DHIQ) generated by the MAO-N-catalysed 1st step became the substrate for subsequent investigations on the chemo or biocatalytic lactam forming 2nd step. Following the chemocatalytic approach for the 2nd step, we were able to achieve 69 % conversion of DHIQ **7a** to lactam **8a** using 10-20 equivalents of H_2O_2 with 1 mol% CuI at 37 °C.

We then searched for a biocatalytic approach making use of an enzyme that is capable of oxidising THIQ-derived imines/iminiums **7** to lactams **8**. The drug metabolism literature contains reports of molybdenum-dependent aldehyde oxidases that are capable of catalysing such oxygen-dependent conversions. However, recombinant mammalian aldehyde oxidases generally have quite low activity and have not been exploited synthetically.[31] A related bacterial enzyme, recombinant xanthine dehydrogenase (XDH) from *Rhodobacter capsulatus* can be expressed in reasonable yields and activity. Moreover, variants of *R. capsulatus* XDH were examined and showed a shift in substrate specificity from the natural substrates xanthine and hypoxanthine towards aldehyde oxidase type substrates.[31] Hence, we investigated variant XDH-E232V from *R. capsulatus* and found good activity towards DHIQ **7a**. Interestingly, the wild-type XDH from *R. capsulatus* revealed no activity against this substrate. As *R. capsulatus* XDH has in general only low reactivity with oxygen and preferentially uses other electron acceptors, we screened a range of electron acceptors (Table S8) and found that either a combination of the redox mediator DCPIP (10 mol%) and either 1 eq. $K_3Fe(CN)_6$ or *T. versicolor* laccase/aerial O_2 could drive the reaction yielding lactam **8a**. In effect, the XDH-E232V/DCPIP/laccase combination functions as an oxidase surrogate. In addition, we examined *E. coli* XDH, in which case aerial O_2 acts directly as the terminal electron acceptor. Simply adding *E. coli* XDH (0.04 mg/mL) to a solution of imine **7a** in NaPi buffer (50 mM, pH 7.4) with periodic shaking gave complete conversion to the corresponding lactam **8a** at 20 °C in 2 h. Having established chemocatalytic and biocatalytic methods for high conversion of imine **7a** to the lactam **8a** we set about combining both reaction types with the MAO-N conversion to establish the desired tandem reactions.

Bio-Chemo Tandem Catalysis

Combination of the CuI/H_2O_2 reaction conditions with the MAO-N D9 biocatalyst resulted in a one-pot conversion of **6a** to the lactam **8a** in 74 % yield, implying high conversion catalysed by MAO-N D9 and at least as good performance by the copper catalyst, if not better, in the tandem reaction (conditions A, Table 2). The MAO-N enzyme produces H_2O_2 which may provide additional equivalents for the Cu-catalysed lactam forming 2nd step, thus slightly increasing the yield and showing a mutual benefit of combining these two steps. In addition, two related tetrahydroisoquinoline derivatives **6b** and **6c** from the initial screen with MAO-N D9 also underwent the tandem reaction (Table 2). As previously described, [6b] MAO-N variant D9 has an active site which is sufficient for accommodation of bulky substrates. However, compound **6b** produced a slightly lower activity (Table S6), probably due to the position and nature of the NO_2-substitutent. Both of these substrates (**6b**, **6c**) are *N*-methylated and thus the substrate for the second step is an iminium. Although HPLC analysis of extracts indicated higher conversion, treatment of the reaction mixture with ammonia-borane prior to extraction showed the yields of **8b** and **8c** to be 47 and 42 %, respectively.

Table 2: Tandem bio-chemo and bio-bio catalysed conversion of cyclic amines **6** to lactams **8**.



a $R_1 = R_2 = H$
b R_1 = Me, $R_2 = NO_2$
c R_1 = Me, R_2 = H

Substrate	Conditions	Conversion to lactam 8a-c [%]
6a	**A** (MAO-N D9/CuI/air)	74
6a	**B** (MAO-N D9/XDH-232V/Fe(III)/DCPIP/air)	91
6a	**C** (MAO-N D9/XDH- E232V/laccase/DCPIP/	54
6a	**D** (MAO-N D9/*E. coli* XDH/catalase/air)	94
6b	**A**	47[b]
6b	**B**	-
6b	**D**	-
6c	**A**	42[b]
6c	**D**[c]	100[b]

[a] Conditions A: **6a-c** (40 mM), MAO-N D9 (0.4 mg/mL), MOPS buffer (100 mM, pH 7.5), 10 eq. H_2O_2, CuI (1 mol%), 37 °C, 16 h. Conditions B: **6a-c** (1 mM), MAO-N D9 (1 mg/mL), XDH E232V (1.7 mg/mL), potassium phosphate buffer (100 mM, 0.1 % EDTA, pH 7.6), DCPIP (10 mol%), $K_3Fe(CN)_6$ (1 eq.), 20 °C, 2 h. Conditions C: **6a** (1 mM), MAO-N D9 (1.1 mg/mL), XDH E232V (1.7 mg/mL) potassium phosphate buffer (100 mM, 0.1 % EDTA, pH 7.6), DCPIP (10 mol%), *T. versicolor* laccase (0.6 mg/mL), 20 °C, 2 h. Conditions D: **6a** (1mM), **6c** (10 mM), MAO-N D9 (1.1 mg/mL), *E. coli* XDH (0.37 mg/mL), sodium phosphate buffer (50 mM, pH 7.4), 20 °C, 2 h. [b] Addition of ammonia-borane prior to extraction and analysis; [c] Includes addition of catalase (0.1 mg/mL), reaction at pH = 8.0. Conversion to lactams reported are based on response factors obtained from NMR-HPLC correlations (SI, chapter 5.4.1.).

Bio-bio Tandem Catalysis

The conditions developed using the *R. capsulatus* XDH-E232V variant and *E. coli* XDH for the conversion of DHIQ **7a** to the lactam **8a** matched well with those required for the MAO-N D9 oxidation. A combination of MAO-N D9 and XDH-E232V with the DCPIP (10 mol%)/Fe(III) (1 eq.) acceptor system gave an excellent 91 % conversion to the lactam in 2 h (conditions B, Table 2). Moreover, laccase was also found to be applicable in place of Fe(III) in the tandem reaction (condition C). Thus the reaction uses 3 enzymes, buffer, catalytic DCPIP and aerial O_2 at 22 °C. We were also pleased to find that the *E. coli* XDH, which did not require any additional additives, could be coupled with MAO-N D9 to afford the lactam **8a** in 94 % conversion (conditions D, Table 2). Substrate **6b** gave a complex product mixture with *R. capsulatus* XDH-E232V, whereas *E. coli* XDH was not able to catalyse the conversion of the iminium **7b** to lactam **8b**. In contrast, substrate **6c** (10 mmol scale) was converted quantitatively to the lactam **8c**. The addition of catalase was found to be necessary, presumably to destroy peroxide produced by the *E. coli* XDH reaction. Interestingly, the efficiency of the *E. coli* XDH reaction on **7c** appeared to be pH dependent as at pH 7.6 the overall conversion to lactam **8c** was 36 %, whereas at pH 8.0 a quantitative conversion was achieved. In this case, we hypothesise that a greater proportion of the iminium **7c** exists in the pseudobase (hemiaminal) form at higher pH and that the latter may be the actual substrate for the enzyme.[23a]

Conclusions

We have demonstrated a toolbox of oxygen-dependent enzymes, which can be used in a simple and efficient fashion together or in combination with chemocatalysts or chemical reagents in aqueous one-pot biocatalytic tandem cascades to provide amides, carboxylic acids and lactams in good to excellent yield under very mild conditions (20-37 °C). The enzymes MAO-N D9 and GOase $M_{3.5}$ have previously been developed for deracemisation of amines and resolution of secondary alcohols. In the present work, each of these enzymes has been combined in a new way with xanthine dehydrogenases (XDHs) to create novel synthetic cascade reactions. The XDHs have been applied for the first time in preparative biocatalysis. They do not require addition of expensive cofactors and were highlighted to be ideally suited for combination with other oxidases. Evaluation of biocatalyst stability, immobilisation and recycling will facilitate scale up of these cascade processes. Work is currently ongoing to expand the range of mutually compatible and greener oxidative functional group transformations based on bio-bio and chemo-biocatalytic cascades.

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Catalytic Bio-Chemo and Bio-Bio Tandem oxidation reactions for amide and carboxylic acid synthesis

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1. General experimental information and materials

Competent *E. coli* BL21 (DE3) and BL21 Star™ (DE3) cells for expression of MAO-N variant D9 and GOase variant M_{3-5}, respectively, were purchased from Invitrogen and transformed according to the manufacturer's protocol. The empty vectors pET-16b and pET-30a used for cloning of MAO-N D9 and GOase M_{3-5} originate from Novagen. The *E. coli* TP1000 mutant strain used for XDH E232V expression is a derivative of MC4100 with a kanamycin cassette inserted in the mobAB gene region.[1] Cell lysis was performed by sonication using a Soniprep 150 (MSE UK Ltd.) and lysozyme from chicken egg white from Sigma. *Trametes versicolor* laccase, *E. coli* xanthine dehydrogenase,[2] horseradish peroxidase (HRP) and catalase were sourced from Sigma-Aldrich. Starting materials were purchased from Alfa Aesar and Sigma-Aldrich and used as received. Solvents were analytical or HPLC grade or were purchased dried over molecular sieves where necessary. Column chromatography was performed on silica gel (Sigma-Aldrich, 220-440 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400,500 or 800 without additional internal standard. Chemical shifts are reported in δ values (ppm) and are calibrated against residual solvent signal. The following abbreviations were used to define the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. HPLC analysis was performed on an Agilent system equipped with a G1379A degasser, G1312A binary pump, a G1329 autosampler unit, a G1315B diode array detector and a G1316A temperature controlled column compartment. The columns used were CHIRALPAK® IC (5 µm particle size, 4.6 mm diameter x 250 mm), CHIRALPAK® IA (5 µm particle size, 4.6 mm diameter x 250 mm) and CHIRALPAK® OJH (5 µm particle size, 4.6 mm diameter x 250 mm). Conditions are indicated separately for each compound.

2. Preparation of biocatalysts

Monoamine oxidase variant D9 (MAO-N D9)

MAO-N D9 mutant[3] was transformed into *E. coli* BL21 (DE3) cells (Invitrogen) according to the manufacturer's instructions. A single colony was used to inoculate a pre-culture (5 mL) which was grown in LB with ampicillin (100 mg/L) at 37 °C and 250 rpm until an OD_{600nm} between 0.6-1.0 was reached. 2-L-Erlenmeyer flasks containing 600 mL LB with ampicillin (100 mg/L) were inoculated with 5 mL of pre-culture and incubated at 37 °C and 250 rpm for 24 h. The cells were harvested by centrifugation at 8000 rpm and 4 °C for 20 min. The cell pellet was stored at -20°C until needed. Typically, 4 g of cells were obtained from a 600-mL culture.

Galactose oxidase variant M_{3-5} ($GOaseM_{3-5}$)

GOase mutant M_{3-5}[4] was transformed into *E. coli* BL21 Star™ (DE3) cells (Invitrogen) according to manufacturer's specifications. A single colony was picked from an overnight LB plate containing 1 µL of kanamycin of a 30 mg/mL stock solution per mL of agar and used to inoculate 5 mL LB medium supplemented with 5 µL kanamycin and grown overnight at 37 °C and 250 rpm. 500 µL of the overnight culture was used to inoculate 250 mL of an autoinduction medium (8ZY-4LAC) as described by Deacon and McPherson[5] and supplemented with 250 µL of kanamycin in a 2-L-baffled Erlenmeyer flask. The cells were grown at 26 °C and 250 rpm for 60 h. Cells were harvested by centrifugation at 6000 rpm and 4 °C for 20 min and subsequently prepared for protein purification.

Xanthine dehydrogenase variant E232V (XDH E232V)

For XDH mutant E232V expression,[6,7] the plasmid pSL207 derived from pTrcHisA (Invitrogen), containing the xdh E232V genes with a His6 tag fused to the N terminus of XDHA, was used. For heterologous expression in *E. coli*, pSL207 was transformed into *E. coli* TP1000 cells, containing a deletion in the mobAB genes responsible for Moco dinucleotide formation. The enzyme was expressed in 500-mL-cultures of TP1000 cells carrying plasmid pSL207 grown at 30 °C in LB medium supplemented with 150 µg/mL ampicillin, 1 mM molybdate, and 0.02 mM isopropyl-D-thiogalactopyranoside until the OD_{600nm} = 1. This culture was then transferred to a bottle containing 8 liters of supplemented LB medium and subsequently grown at 30 °C for 18 - 20 h. Cells were harvested by centrifugation at 5000 x *g* at 4 ^{o}C and subsequently prepared for protein purification.

Purification of MAO-N D9

5 g of frozen cell paste were thawed on ice and resuspended in 25 mL of phosphate buffer (100 mM KPi, pH 7.7; containing 1 mg/mL of lysozyme from chicken egg white) and incubated at 30 °C for 30 min. The suspension was cooled to 4 °C and cells were lysed by ultrasonication (30 s on, 30 s off; 20 cycles). Cell debris was removed by centrifugation (15000 x *g*, 40 min, 4 °C). Subsequently, the cell-free extracts were filtered through a syringe filter with a 0.22 µm pore size. The cell-free extracts, after filtration, were loaded onto a HisTrap Ni-sepharose column (1 mL, GEHealthcare) pre-equilibrated with buffer A (100 mM KPi, pH 7.7, 300 mM NaCl). The protein was eluted with a stepped gradient using an Äkta explorer system from GE Healthcare with the following profile collecting 1 mL fractions. Step 1, 10 mL buffer A; Step 2, 10 mL 80:20 buffer A : buffer B; Step 3, 30 mL 65:35 buffer A : buffer B. Buffer B contained 100 mM KPi, pH 7.7, 300 mM NaCl, 1 M imidazole. The MAO-N containing fractions (from step 3) were pooled and concentrated using a Sartorius Vivaspin 6 spin column (30 kDa mass cut-off), and the volume adjusted to 2.5 mL. The concentrated fractions were desalted using a PD-10 Sephadex column and buffer A.

Purification of GOase M_{3-5}

The cell pellet from a 250-mL-culture was resuspended in 30 mL lysis buffer containing 50 mM piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES), 25 % sucrose (w/v), 1 mg/mL lysozyme, 5 mM $MnCl_2$ and 1 % Triton X-100 (v/v). The suspension was gently shaken at 4 °C for 20 min. Afterwards, cells were mechanically disrupted *via* ultrasonication (30 s on, 30 s off; 20 cycles) followed by ultracentrifugation (20000 x *g*, 30 min, 4 °C). The cleared crude extract was transferred into a flexible tubing (30 kDa cut-off), dialysed into buffer C (50 mM NaPi buffer, 300 mM NaCl, pH 8.0) for 12 h at 4 °C and subsequently passed through a syringe filter with a 0.22 µm pore size. Protein purification was accomplished with a peristaltic tubing pump (Thermo Scientific) equipped with a 5-mL-Strep-Tag®-II column (GE Healthcare) pre-equilibrated with buffer C. After loading with crude extract, the column was washed with 5 column volumes of buffer C followed by protein elution with 70 mL of buffer D (50 mM NaPi buffer, 300 mM NaCl, 5 mM desthiobiotin, pH 8.0).

For copper-loading, GOase M_{3-5}-containing fractions were pooled and subsequently transferred into flexible dialysis tubing (30 kDa cut-off) and dialysed for 12 h into buffer E (50 mM NaPi buffer saturated with $CuSO_4$, pH 7.4) at 4 °C. Removal of excess $CuSO_4$ was attained by two cycles of dialysis into buffer E (without $CuSO_4$) for 12 h at 4 °C and protein samples concentrated to approximately 3 mg/mL using a Sartorius Vivaspin 6 spin column (30 kDa mass cut-off). The protein samples were aliquoted and aliquots were frozen in liquid nitrogen prior to storage at -80 °C.

Purification of XDH E232V

The cell pellet was resuspended in 8 volumes of 50 mM sodium phosphate, 300 mM NaCl, pH 8.0, and cell lysis was achieved by several passages through a French press. After addition of DNase I, the lysate was incubated for 30 min. After centrifugation at 17000 x *g* for 25 min, imidazole was added to the supernatant to a final concentration of 10 mM. The supernatant was mixed with 2 mL of Ni_2-nitrilotriacetic agarose (Qiagen) per liter of cell growth, and the slurry was equilibrated with gentle stirring at 4 °C for 30 min. The slurry was poured into a column, and the resin was washed with 2 column volumes of 10 mM imidazole, 50 mM sodium phosphate, 300 mM NaCl, pH 8.0, followed by a wash with 10 column volumes of the same buffer with 20 mM imidazole. His-tagged XDH E232V was eluted with 100 mM imidazole in 50 mM sodium phosphate, 300 mM NaCl, pH 8.0. Fractions containing XDH were combined and dialyzed against 50 mM Tris, 1 mM EDTA, 2.5 mM dithiothreitol, pH 7.5. The dialyzed sample was applied to a Q-Sepharose fast protein liquid chromatography column and eluted with a linear gradient of 0-250 mM NaCl. To the pool of fractions containing XDH, 15 % ammonium sulfate was added, and the protein was then applied to a phenyl-Sepharose column equilibrated with 50 mM Tris, 1 mM EDTA, 2.5 mM dithiothreitol, 15 % ammonium sulfate, pH 7.5. XDH E232V was eluted from the column with a linear gradient of 15 to 0 % ammonium sulfate. During purification, fractions were monitored using SDS-PAGE, whereas enzyme activity was measured spectrophotometrically as described earlier. The yield of protein was about 12.5 mg/L of *E. coli* culture.

3. Cascade 1

3.1 GOase M_{3-5}-catalysed oxidation of benzyl alcohols and amide formation reactions - General procedure A

For analytical scale reactions, the primary alcohol dissolved in MeCN (500 mM stock solution) and applied in 5, 7 and 10 mM final concentrations with pure GOase M_{3-5} (7.25 µM final concentration) were transferred to a solution of NaPi buffer (50 mM, pH 7.4) supplemented with HRP (75 U/mL) reaching a final volume of 500 µL in a 2-mL-Eppendorf tube. The tube was placed in a shaking incubator and incubated at 25 °C and 250 rpm. After 24 h of reaction (1st step for aldehyde formation), 5 eq. of amine with respect to the concentration of alcohol and TBHP (6 %, v/v) were directly applied to the reaction mixture followed by incubation at 37 °C and 250 rpm for 24 h (2nd step for amide formation). The reaction was monitored by HPLC and samples were prepared as follows: 500 µL DCM was added to 100 µL of sample of the reaction mixture in an1.5-mL-Eppendorf tube. After vigorous mixing by means of a vortex mixer, the sample was centrifuged at 13200 rpm for 5 min. The organic phase was collected, dried with $MgSO_4$ and analysed by normal phase HPLC.
For preparative scale reactions, primary alcohol dissolved in MeCN (500 mM stock solution) at a 10 mM final concentration and pure GOase M_{3-5} (7.25 µM final concentration) were transferred to a solution of NaPi buffer (50 mM, pH 7.4) supplemented with HRP (75 U/mL) reaching a final volume of 3 mL in a 15-mL-Falcon tube. Subsequent steps were identical to analytical scale experiments.

3.2 Laccase/TEMPO-mediated oxidation of benzyl alcohols and amide formation reactions - General procedure B

For analytical and preparative scale reactions, primary alcohol dissolved in MeCN (1 M stock solution) and used at 20, 50 and 80 mM final concentrations and a solution of *Trametes versicolor* laccase (TvL; 3.0, 7.5 and 12.0 U/mL final concentration) were transferred to a solution of sodium citrate buffer (100 mM, pH 5.0) supplemented with 6, 15 or 24 mM TEMPO reaching a final volume

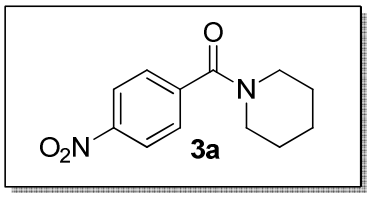
of 3 mL in a 15-mL-Falcon tube. The tube was placed in a shaking incubator and incubated at 20 °C and 250 rpm for 24 h (1st step for aldehyde formation). Afterwards, 5 eq. of amine with respect to the concentration of the primary alcohol and 1.2 eq of TBHP were directly applied to the reaction mixture followed by incubation at 37 °C and 250 rpm for 24 h (2nd step for amide formation). The reaction was monitored by HPLC and samples were prepared as described in general procedure A.

3.3 Synthesis of amide standards

The standards for the amides **3a-d**, **3f** and **3h-k**, were synthesised using general procedure B, while **3e** was purchased from Sigma Aldrich. Conversions for reactions yielding **3g** were determined by comparison of the new formed peak and the similar halogenated amides.

3.4 Analytical scale reactions according to general procedures A and B

(4-Nitrophenyl)(piperidin-1-yl)methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 10 mM *para*-nitrobenzyl alcohol (**1a**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM *para*-nitrobenzyl alcohol (**1a**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

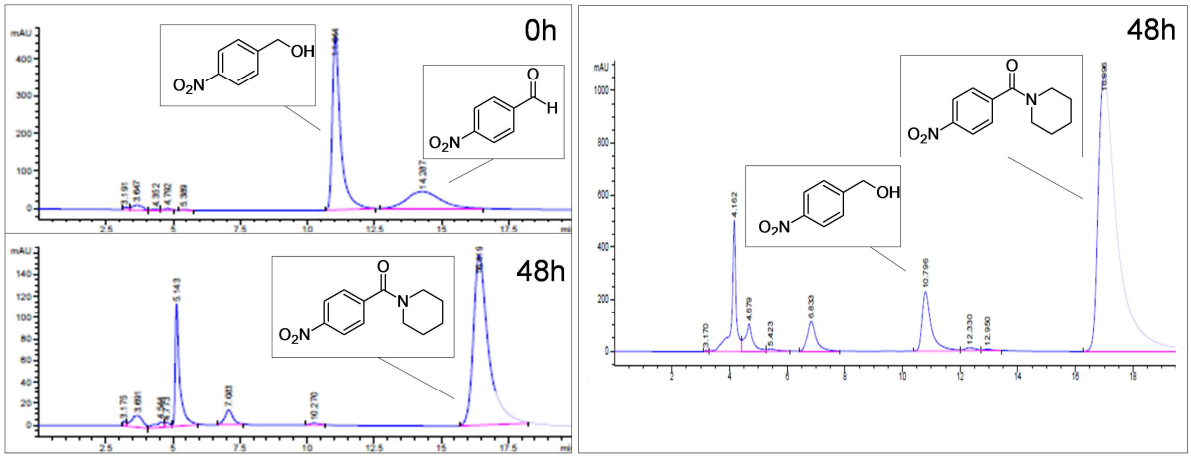
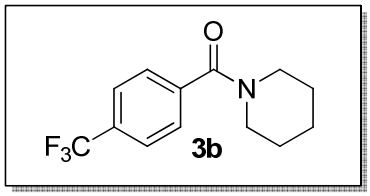


Figure S1. HPLC traces of *para*-nitrobenzyl alcohol (**1a**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding (4-nitrophenyl)(piperidin-1-yl)methanone **3a** (respective 48-h-images at the bottom). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at 5.143 (48-h-image, left) is an unknown UV-active substance derived from TBHP, whereas peak at 4.162 min (48-h-image, right) is an unknown UV-active substance derived from TEMPO.

Piperidin-1-yl[4-trifluoromethyl)phenyl]methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 10 mM *para*-trifluorobenzyl alcohol (**1b**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM *para*-trifluorobenzyl alcohol (**1b**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

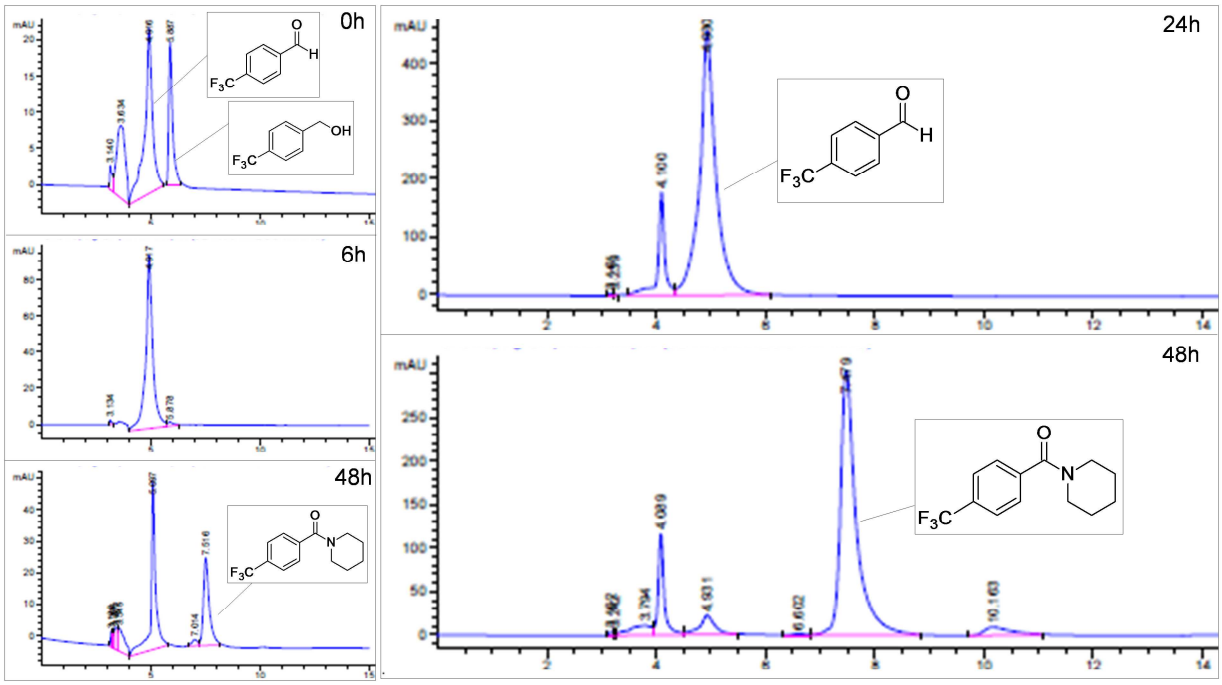
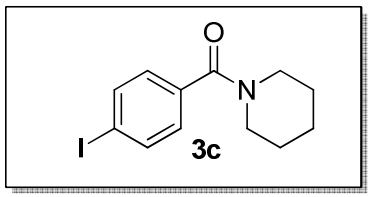


Figure S2. HPLC traces of *para*-trifluorobenzyl alcohol (**1b**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding piperidin-1-yl[4-trifluoromethyl)phenyl]methanone **3b** (respective 48-h-images at the bottom).HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at 5.097 (48-h-image, left) is an unknown UV-active substance derived from TBHP, whereas peak at 4.100/4.089 min (24- and 48-h-image, right) is an unknown UV-active substance derived from TEMPO.

(4-Iodophenyl)(piperidin-1-yl)methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 10 mM *para*-iodobenzyl alcohol (**1c**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM *para*-iodobenzyl alcohol (**1c**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

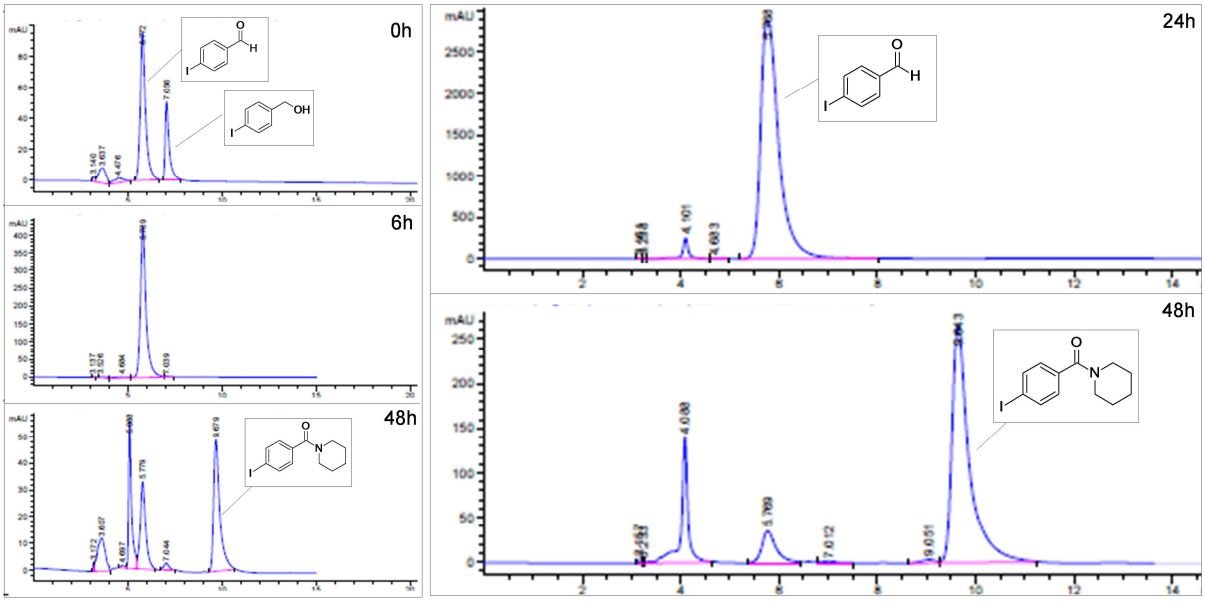
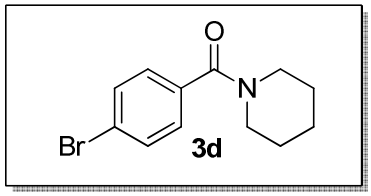


Figure S3. HPLC traces of *para*-iodobenzyl alcohol (**1c**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding (4-iodophenyl)(piperidin-1-yl)methanone **3c** (respective 48-h-images at the bottom). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at 5.008 (48-h-image, left) is an unknown UV-active substance derived from TBHP, whereas peak at 4.101/4.088 min (24- and 48-h-image, right) is an unknown UV-active substance derived from TEMPO.

(4-Bromophenyl)(piperidin-1-yl)methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 10 mM *para*-bromobenzyl alcohol (**1d**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM *para*-bromobenzyl alcohol (**1d**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

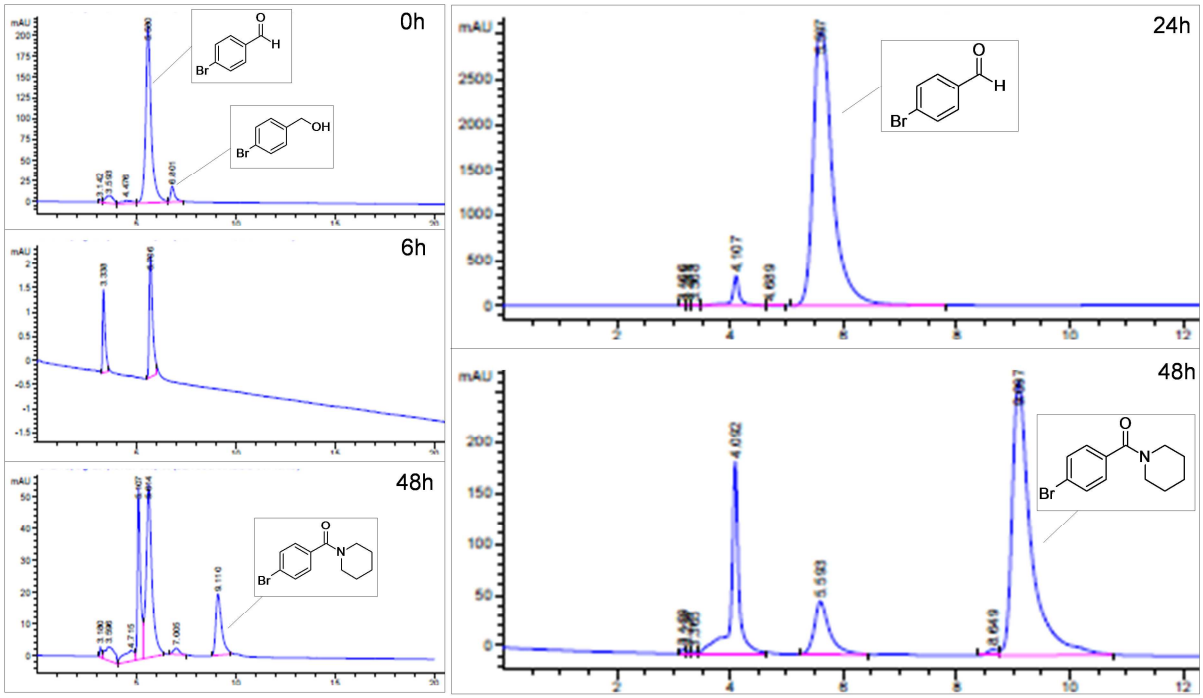
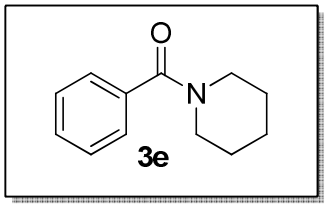


Figure S4. HPLC traces of *para*-bromobenzyl alcohol (**1d**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding (4-bromophenyl)(piperidin-1-yl)methanone **3d** (respective 48-h-images at the bottom). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at 5.107 (48-h-image, left) is an unknown UV-active substance derived from TBHP, whereas peak at 4.092 min (24- and 48-h-image, right) is an unknown UV-active substance derived from TEMPO.

Phenyl(piperidin-1-yl)methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 7 mM benzyl alcohol (**1e**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 7.5 U mL^{-1} TvL/15 mM TEMPO, 50 mM benzyl alcohol (**1e**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

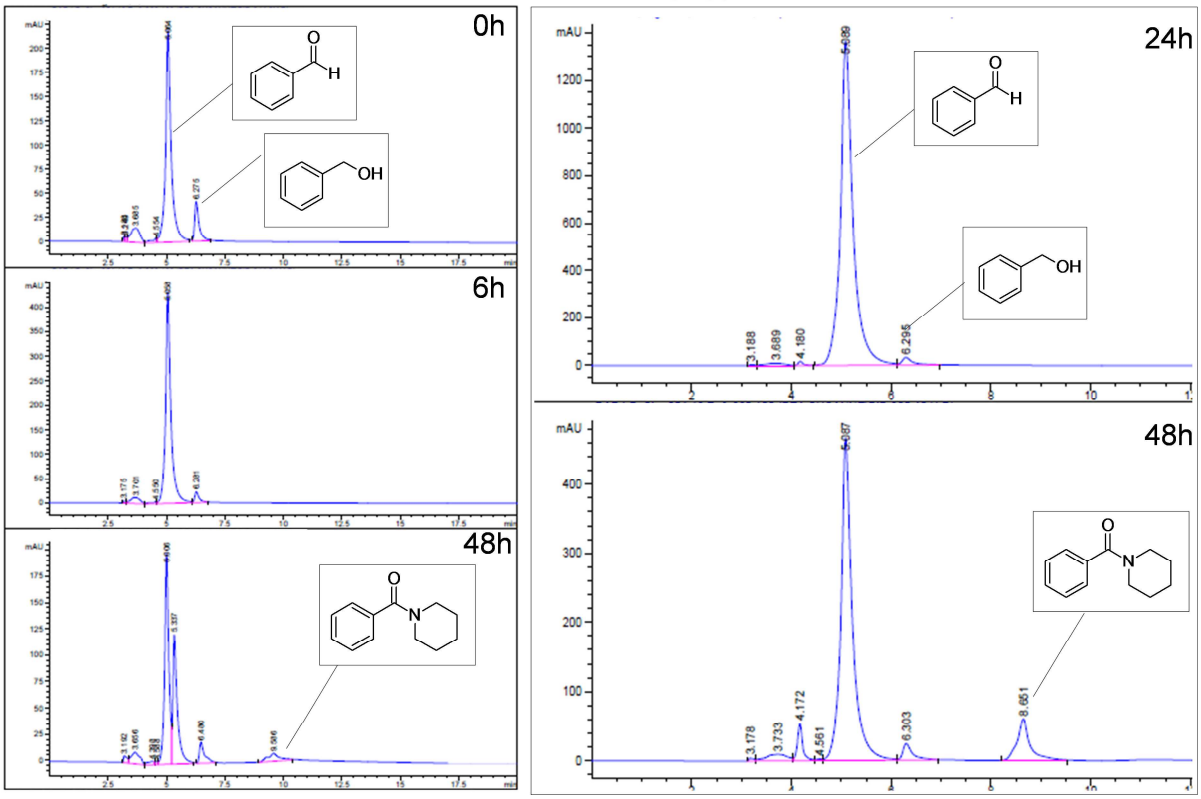
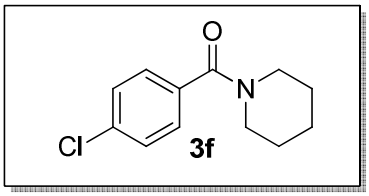


Figure S5. HPLC traces of benzyl alcohol (**1e**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding phenyl(piperidin-1-yl)methanone **3a** (respective 48-h-images at the bottom). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at 5.008 (48-h-image, left) is an unknown UV-active substance derived from TBHP, whereas peak at 4.172 min (24- and 48-h-image, right) is an unknown UV-active substance derived from TEMPO.

(4-Chlorophenyl)(piperidin-1-yl)methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 10 mM *para*-chlorobenzyl alcohol (**1f**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM *para*-chlorobenzyl alcohol (**1f**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

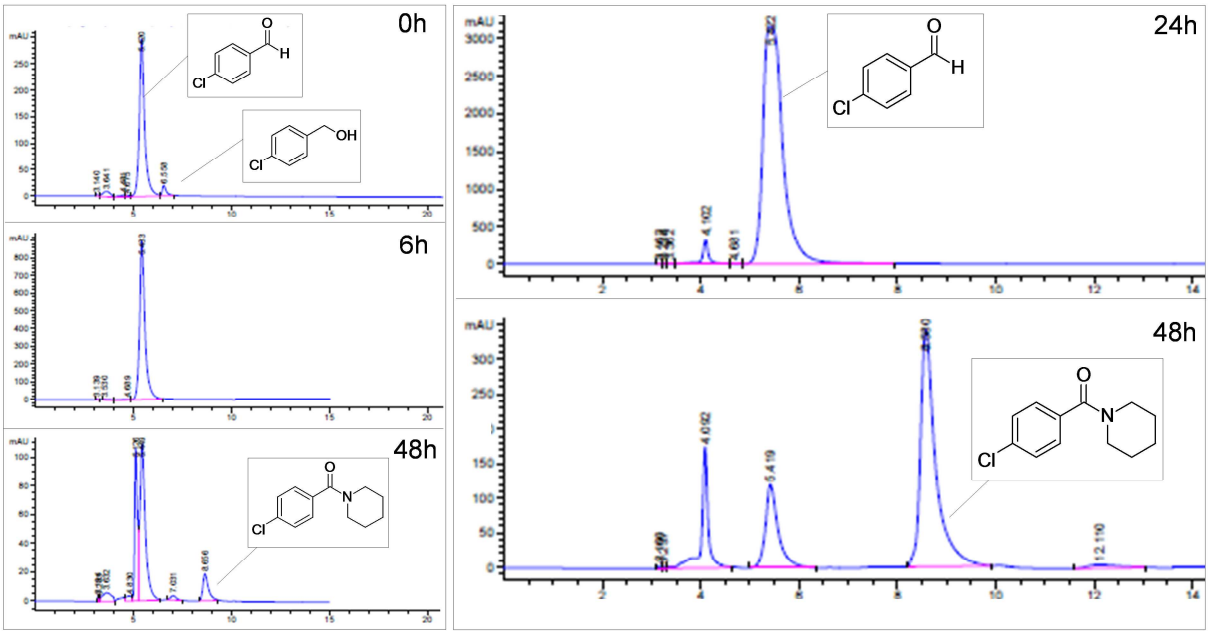
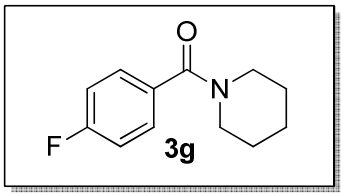


Figure S6. HPLC traces of *para*-chlorobenzyl alcohol (**1f**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding (4-chlorophenyl)(piperidin-1-yl)methanone **3f** (respective 48-h-images at the bottom). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at 5.126 (48-h-image, left) is an unknown UV-active substance derived from TBHP, whereas peak at 4.092 min (24- and 48-h-image, right) is an unknown UV-active substance derived from TEMPO.

(4-Fluorophenyl)(piperidin-1-yl)methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 10mM *para*-fluorobenzyl alcohol (**1g**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM *para*-fluorobenzyl alcohol (**1g**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

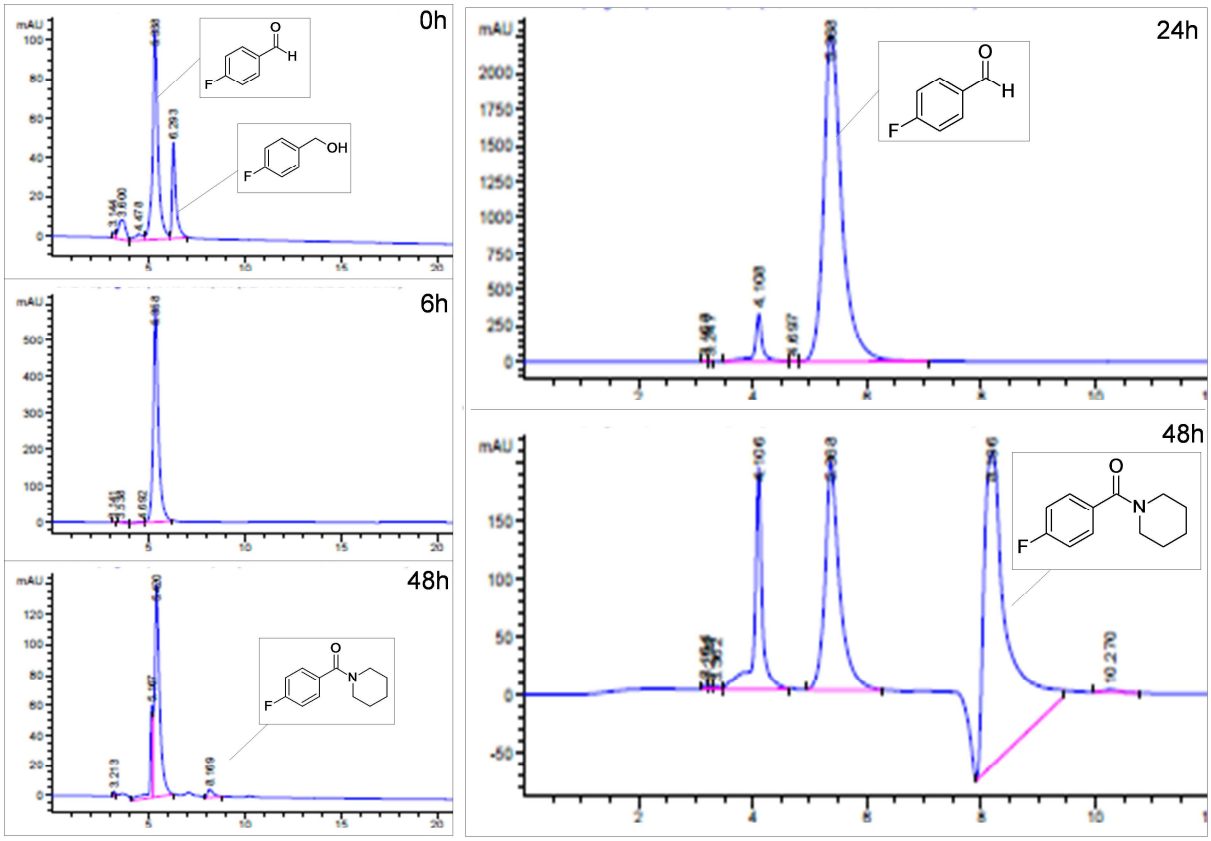
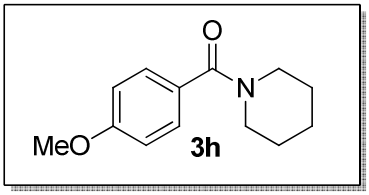


Figure S7. HPLC traces of *para*-fluorobenzyl alcohol (**1g**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding (4-fluorophenyl)(piperidin-1-yl)methanone **3g** (respective 48-h-images at the bottom). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at 5.167 (48-h-image, left) is an unknown UV-active substance derived from TBHP, whereas peak at 4.106 min (24- and 48-h-image, right) is an unknown UV-active substance derived from TEMPO.

(4-Methoxyphenyl)(piperidin-1-yl)methanone



The reaction was performed following general procedure B, using 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM 4-methoxybenzyl alcohol (**1h**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

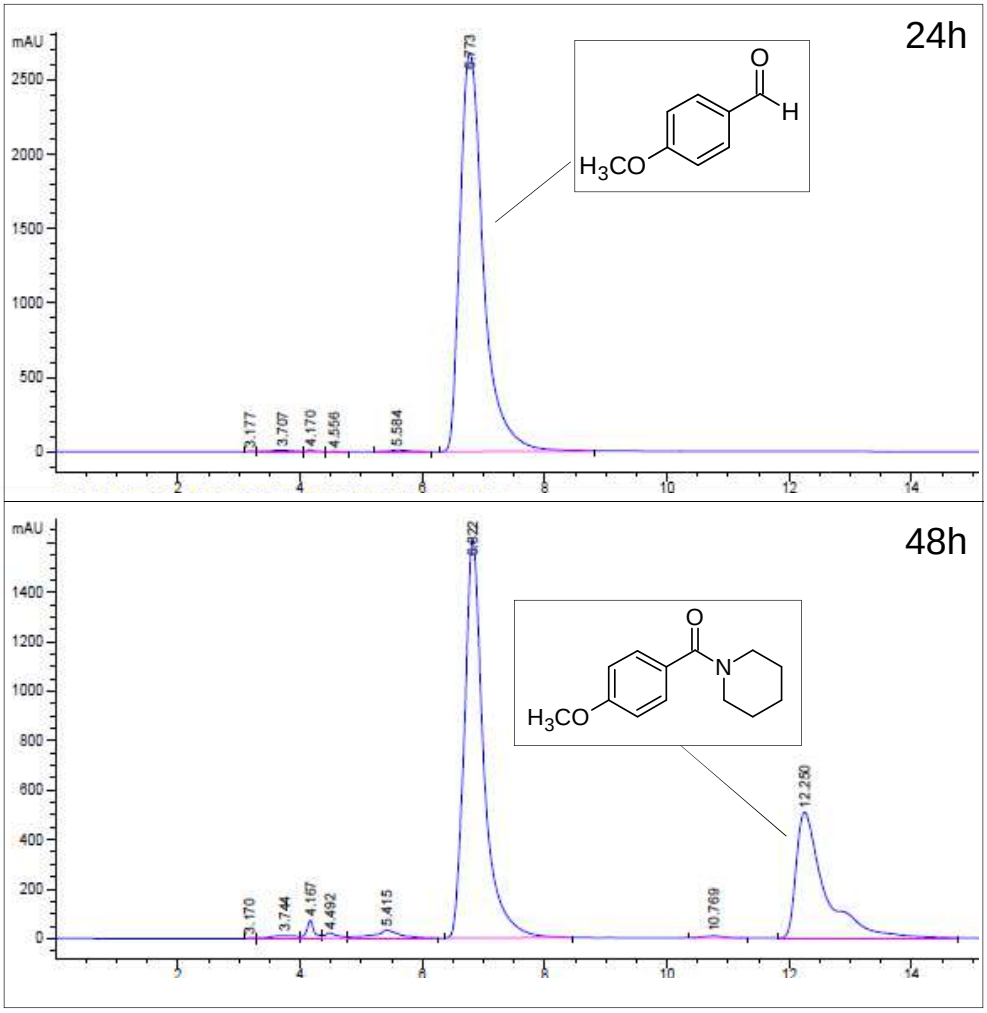
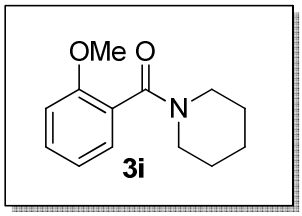


Figure S8 HPLC traces of 4-methoxybenzyl alcohol (**1h**)oxidation using TvL/TEMPO (upper image) and subsequent amide formation reaction yielding (4-methoxy phenyl)(piperidin-1-yl)methanone **3h** (lower image). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

(2-Methoxyphenyl)(piperidin-1-yl)methanone



The reaction was performed following general procedure B, using 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM 2-methoxybenzyl alcohol (**1i**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

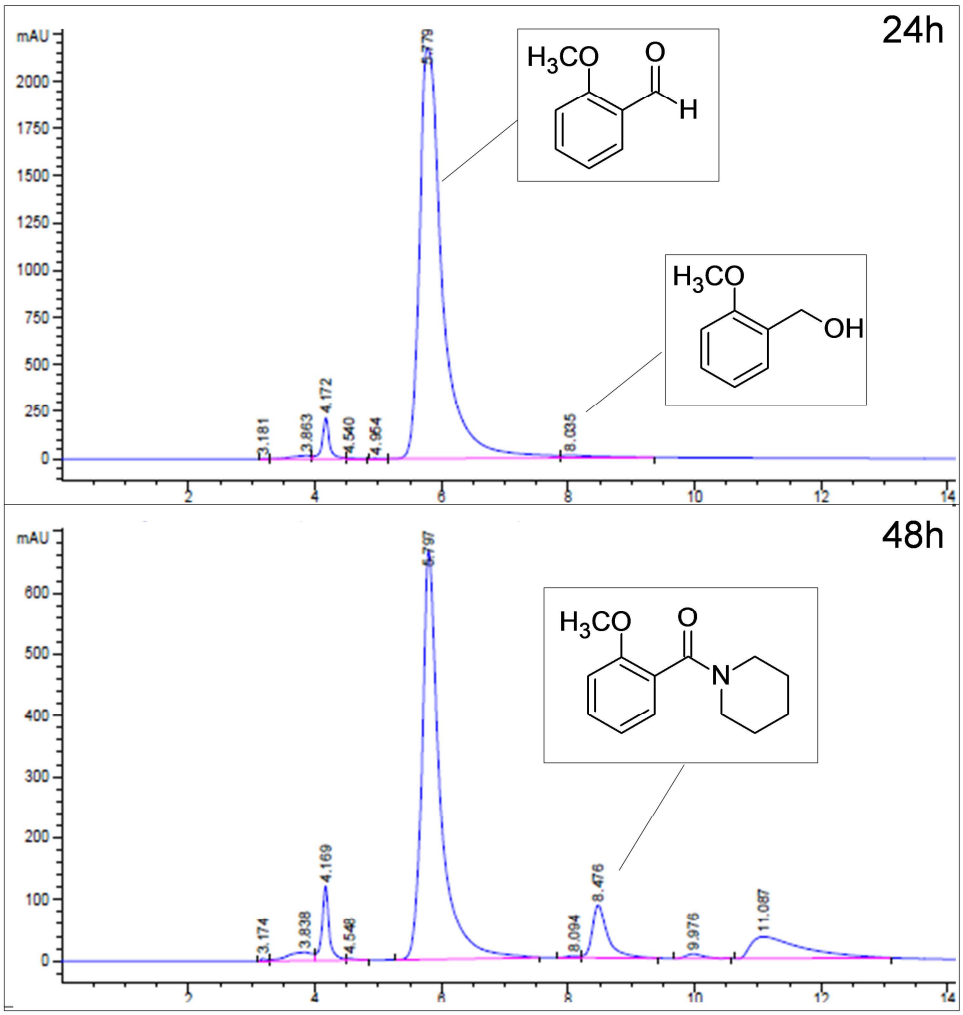
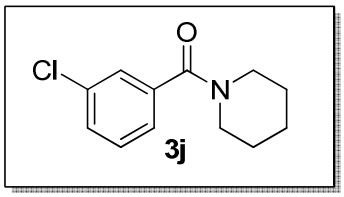


Figure S9. HPLC traces of 2-methoxybenzyl alcohol (**1i**) oxidation using TvL/TEMPO (upper image) and subsequent amide formation reaction yielding (2-methoxyphenyl)(piperidin-1-yl)methanone **3i** (lower image). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at at 4.169 min (24- and 48-h-image, right) is an unknown UV-active substance derived from TEMPO.

(3-Chlorophenyl)(piperidin-1-yl)methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 10 mM 3-chlorobenzyl alcohol (**1j**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM 3-chlorobenzyl alcohol (**1j**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

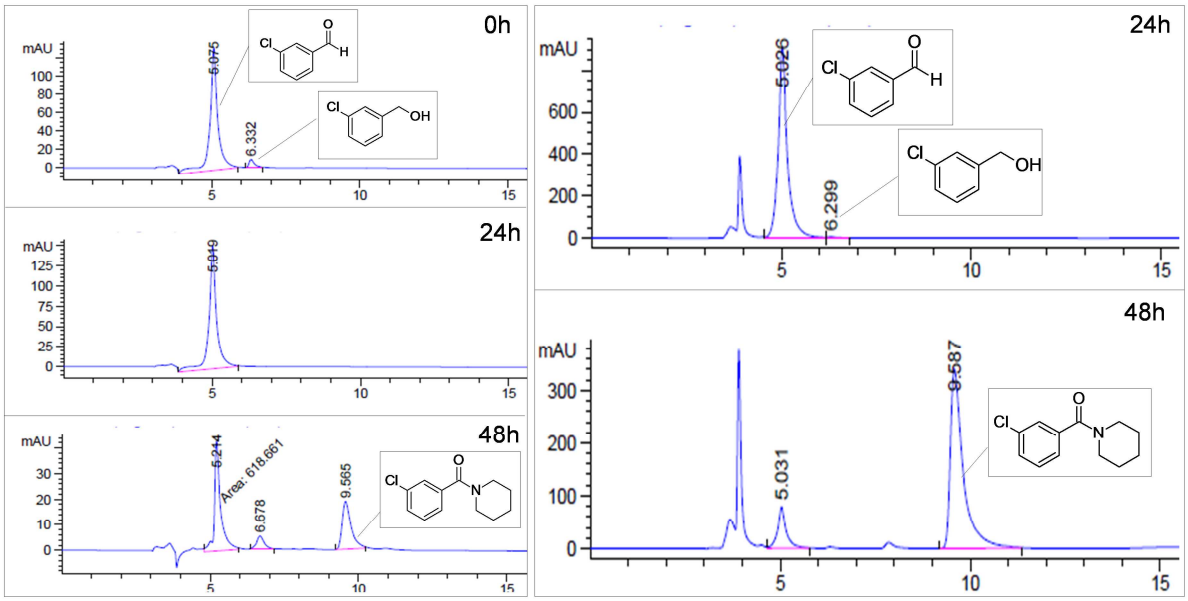
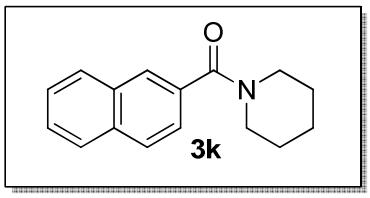


Figure S10. HPLC traces of 3-chlorobenzyl alcohol (**1j**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding (4-fluorophenyl)(piperidin-1-yl)methanone **3j** (respective 48-h-images at the bottom). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

Naphthalen-2-yl(piperidin-1-yl)methanone



The reaction was performed following general procedures A and B, respectively. Procedure A: 7.25 µM GOase M_{3-5}, 10 mM 2-naphthalenemethanol (**1k**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 6 % TBHP (v/v) and reaction for further 24 h. Procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM 2-naphthalenemethanol (**1k**), 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine and 1.2 eq TBHP and reaction for further 24 h.

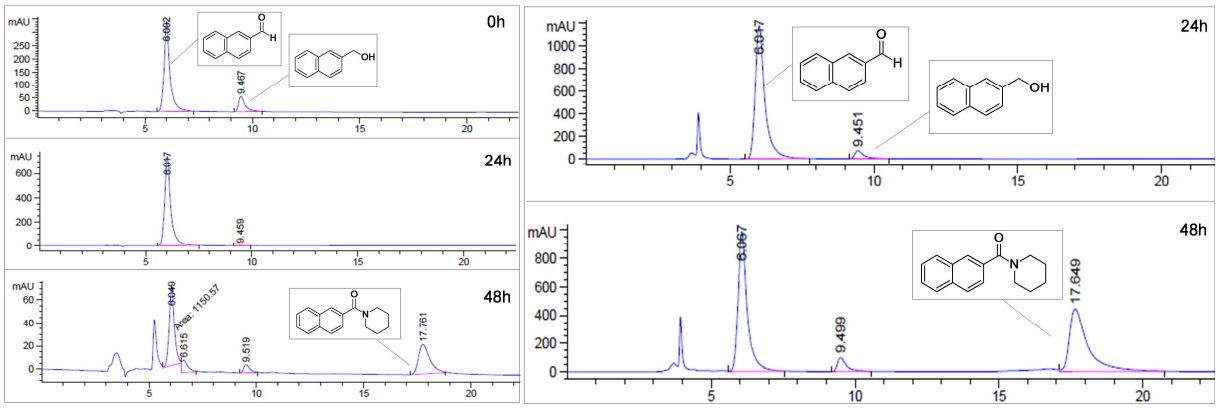


Figure S11. HPLC traces of 2-naphthalenemethanol (**1k**) oxidation using GOase M_{3-5} (left images) and TvL/TEMPO (right images) and subsequent amide formation reaction yielding naphthalen-2-yl(piperidin-1-yl)methanone **3k** (respective 48-h-images at the bottom). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10. Peak at 5.167 (48-h-image, left) is an unknown UV-active substance derived from TBHP, whereas peak at 4.106 min (24- and 48-h-image, right) is an unknown UV-active substance derived from TEMPO.

Table S1: Conversions of benzyl alcohols **1a**-**k** to respective amides **3a**-**k** in GOase-catalysed reactions after 24 h of reaction in presence of 5 eq. amine and 6 % TBHP (general procedure A; percentages reported are based on HPLC peak areas at ʎ = 254 nm).

Reaction	Concentration alcohols 1a-k [mM]	Alcohol [%]	Aldehyde [%]	Amide [%]
3a	5	0	0	100
	7.5	0	0	100
	10	0	0	100
3b	5	0	81	19
	7.5	0	14	86
	10	0	13	87
3c	5	0	85	15
	7.5	0	65	35
	10	0	37	63
3d	5	0	97	3
	7.5	0	91	9
	10	0	74	26
3e	7	0	94	6
	10	0	79	21
3f	5	0	98	2
	7.5	0	93	7
	10	0	86	14
3g	5	1	99	1
	7.5	0	98	2
	10	0	96	4
3h	5	0	100	0
	7.5	2	98	0
	10	6	94	0
3i	5	4	96	0
	7.5	5	95	0
	10	7	93	0
3j	10	8	56	36
3k	10	6	56	38

HPLC conditions: CHIRALPAK® IA column, flow rate 1.0 mL/min, UV 254 nm, eluent= hexane/iPrOH 90:10.

Table S2: Conversions of benzyl alcohols **1a-k** to respective amides **3a-k** in *T. versicolor*/TEMPO-mediated reactions after 24 h of reaction in presence of 5 eq. amine and 1.2 eq. % TBHP (general procedure B; percentages reported are based on HPLC peak areas at ʎ = 254 nm).

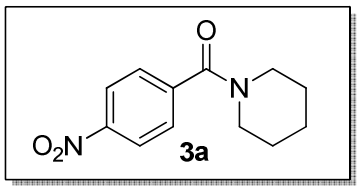
Reaction	Concentration alcohols 1a-k [mM]	Alcohol [%]	Aldehyde [%]	Amide [%]
3a	20	94	0	6
	50	38	0	62
	80	9	0	91
3b	20	0	45	47
	50	0	1	93
	80	0	6	89
3c	20	11	39	22
	50	0	7	93
	80	0	10	90
3d	20	0	94	6
	50	0	22	76
	80	0	14	86
3e	20	2	98	0
	50	4	84	12
	80	6	62	32
3f	20	1	95	4
	50	0	35	53
	80	0	23	75
3g	20	1	96	3
	50	0	61	39
	80	0	31	69
3h	20	7	94	0
	50	0	90	7
	80	0	68	26
3i	20	0	95	2
	50	0	76	9
	80	0	78	9
3j	80	0	13	87
3k	80	5	50	45

HPLC conditions: CHIRALPAK® IA column, flow rate 1.0 mL/min, UV 254 nm, eluent = hexane/iPrOH 90:10.

3.5 Preparative scale reactions according to general procedures B

All the reactions were performed following general procedure B: 12 U mL^{-1} TvL/24 mM TEMPO, 80 mM of the respective alcohol, 24 h of reaction (aldehyde formation) followed by addition of 5 eq. piperidine or methylpiperazine and 1.2 eq TBHP and then reaction for further 24 h (amide formation). The reaction mixture was extracted with CH_2Cl_2 (1 x 5 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum. Purification conditions and yields are reported for each amide.

(4-Nitrophenyl)(piperidin-1-yl)methanone



Purification with silica gel chromatography (eluent EtOAc) led to desired amide **3a**[8] (yellow solid) in 92 % yield.

HPLC analysis of purified 3a

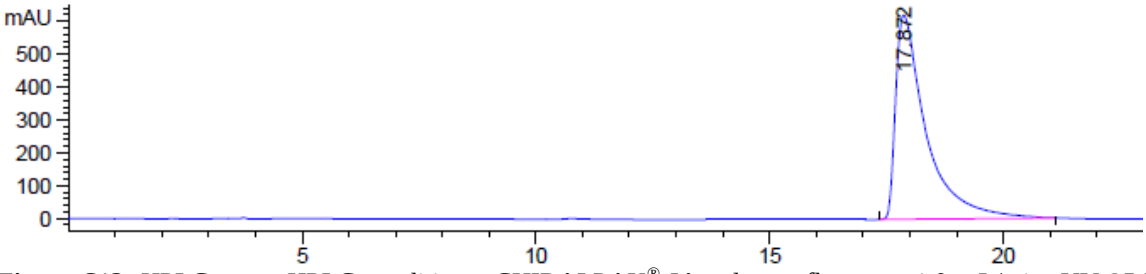


Figure S12. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

^{1}H-NMR analysis of purified 3a

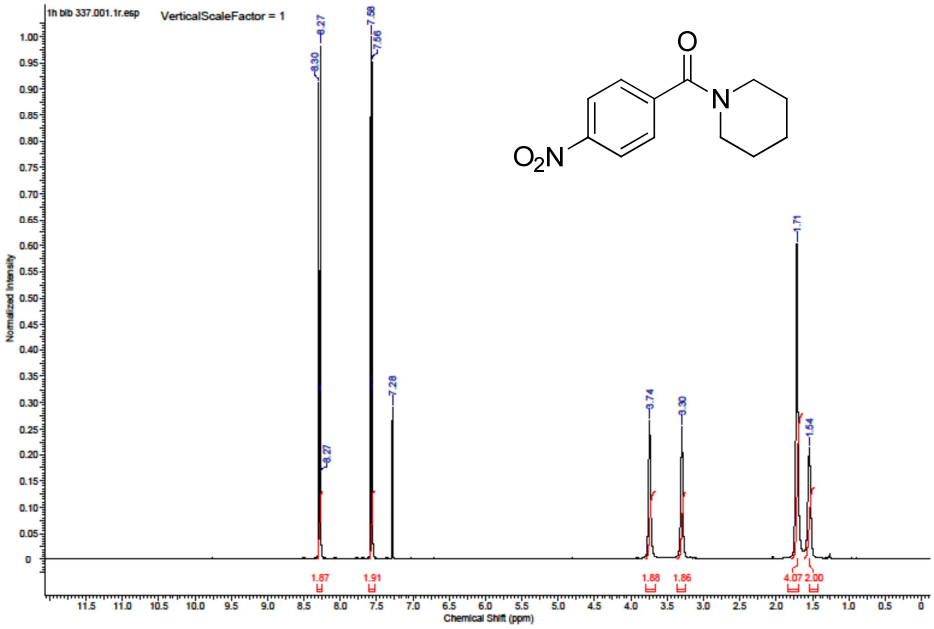
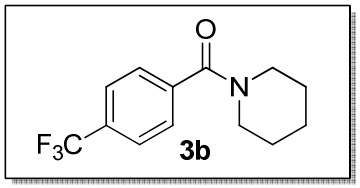


Figure S13. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **3a.**

Piperidin-1-yl[4-trifluoromethyl)phenyl]methanone



Purification with silica gel chromatography (eluent EtOAc 9:1 MeOH) led to desired amide **3b**[9] (colourless oil) in 57 % yield.

HPLC analysis of purified 3b

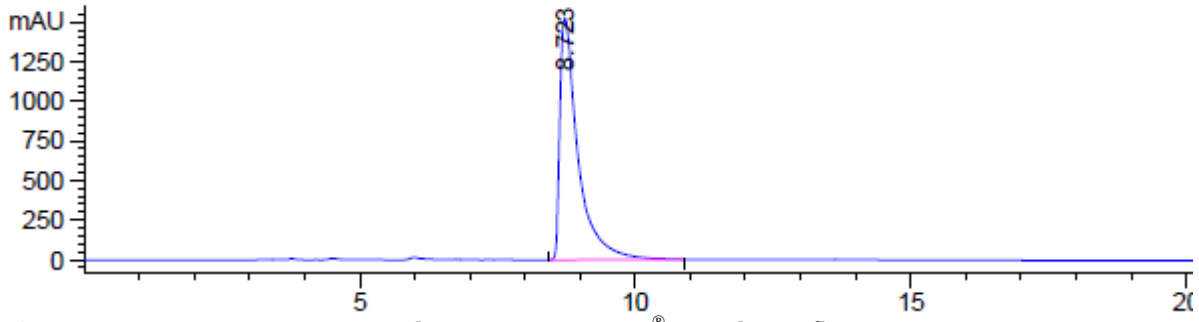


Figure S14. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

^{1}H-NMR analysis of purified 3b

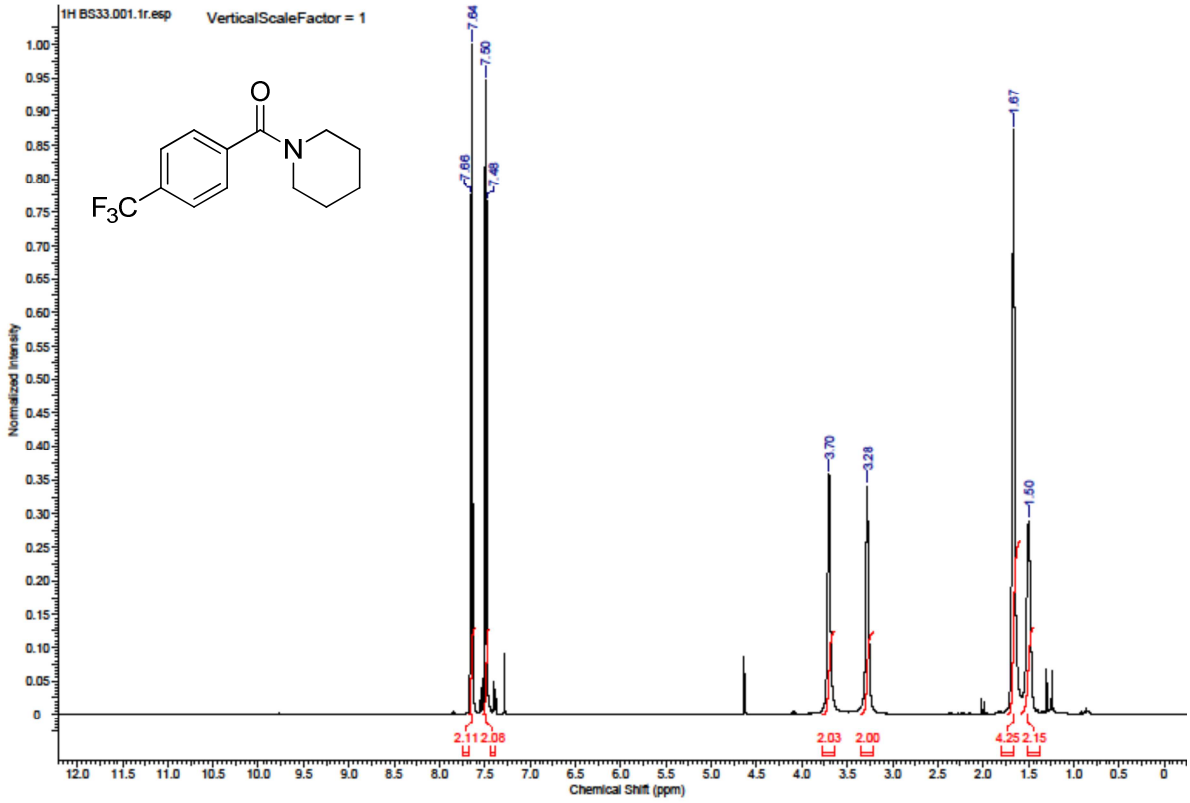
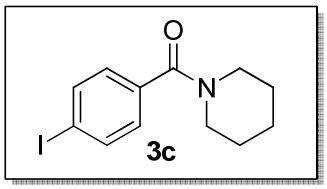


Figure S15. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **3b**.

(4-Iodophenyl)(piperidin-1-yl)methanone



Purification with silica gel chromatography (eluent EtOAc 8:2 cyclohexane) led to desired amide **3c**[10] (white solid) in 60 % yield.

HPLC analysis of purified 3c

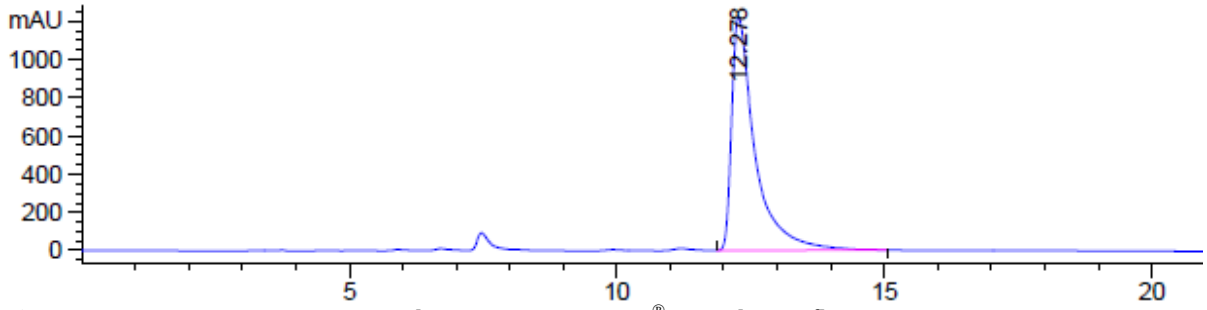


Figure S16. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

^{1}H-NMR analysis of purified 3c

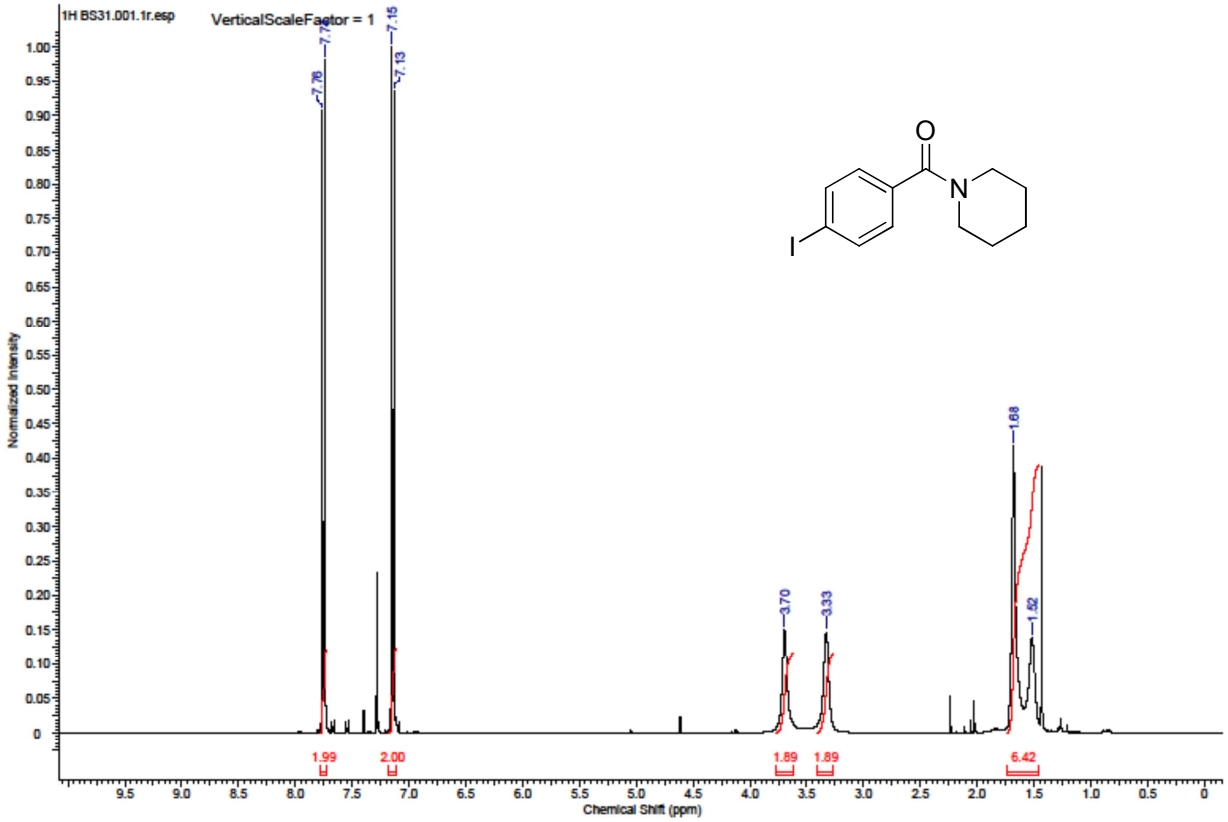
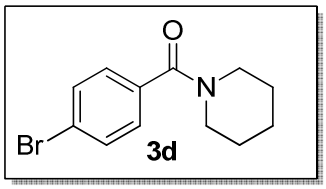


Figure S17. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **3c**.

(4-Bromophenyl)(piperidin-1-yl)methanone



Purification with silica gel chromatography (eluent EtOAc 7:3 cyclohexane) led to desired amide **3d**[11](white solid) in 73 % yield.

HPLC analysis of purified 3d

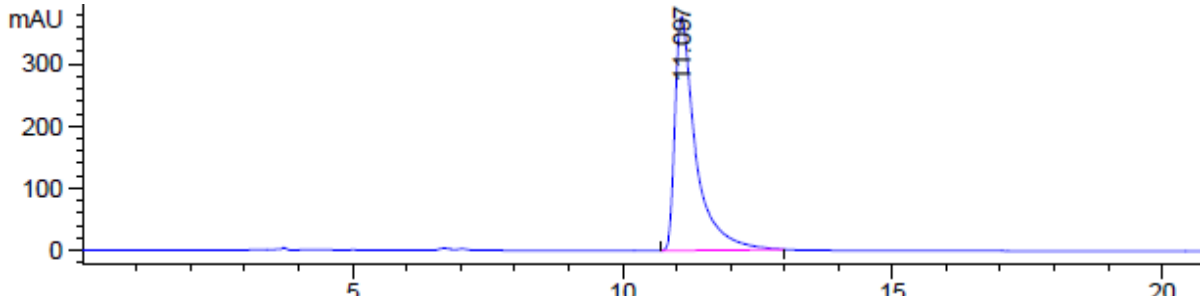


Figure S18. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

^{1}H-NMR analysis of purified 3d

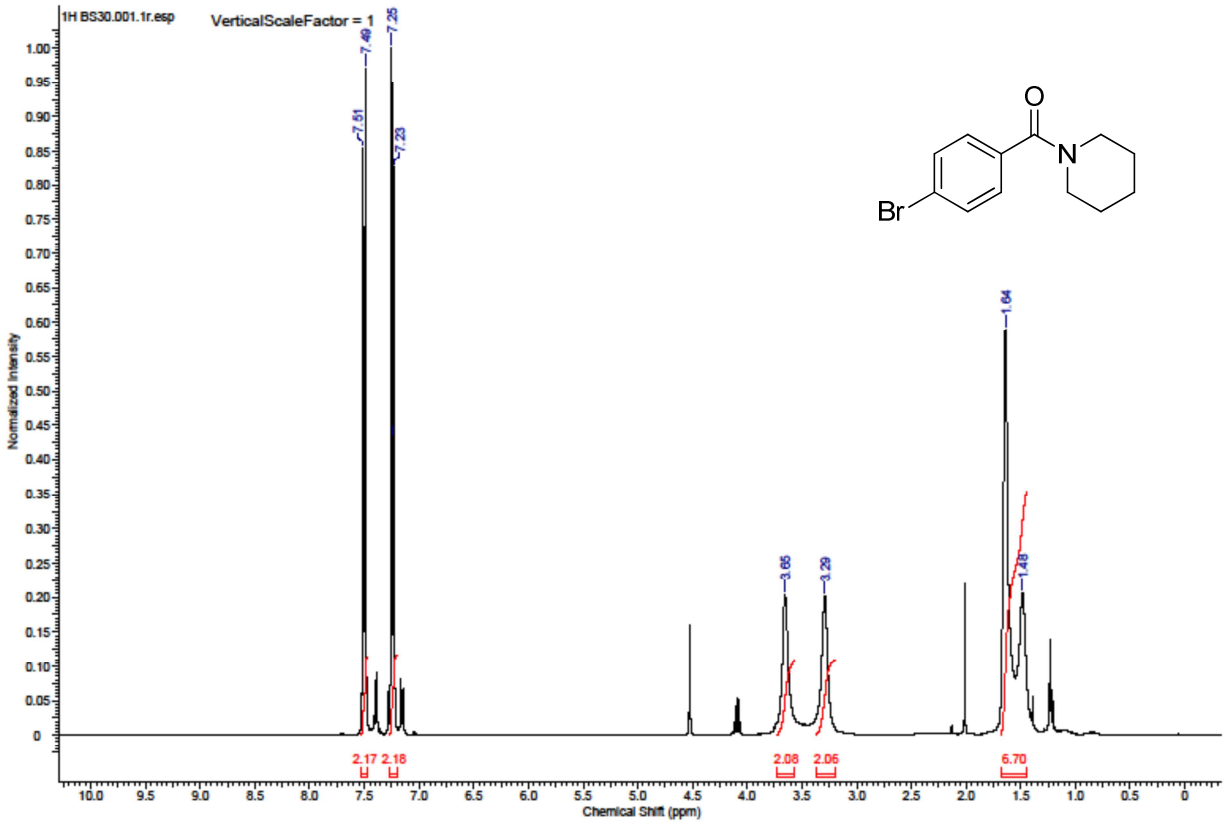
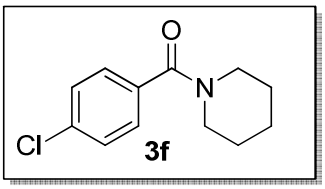


Figure S19. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **3d**.

(4-Chlorophenyl)(piperidin-1-yl)methanone



Purification with silica gel chromatography (eluent EtOAc 7:3 cyclohexane) led to desired amide **3f**[12] (colourless oil) in 53 % yield.

HPLC analysis of purified 3f

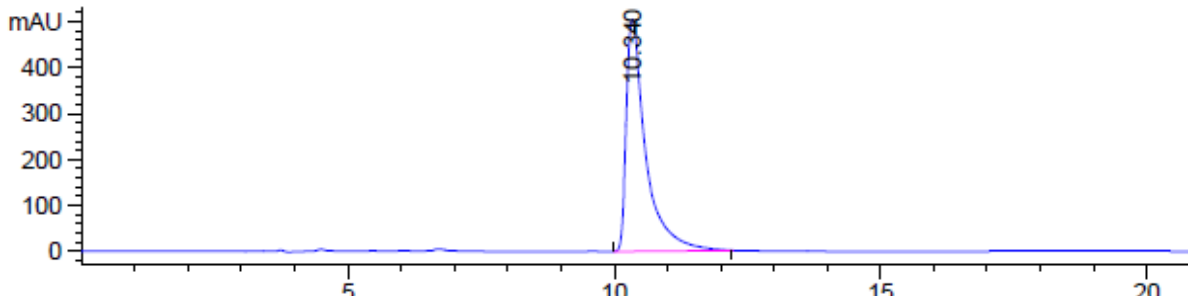


Figure S20. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

^{1}H-NMR analysis of purified 3f

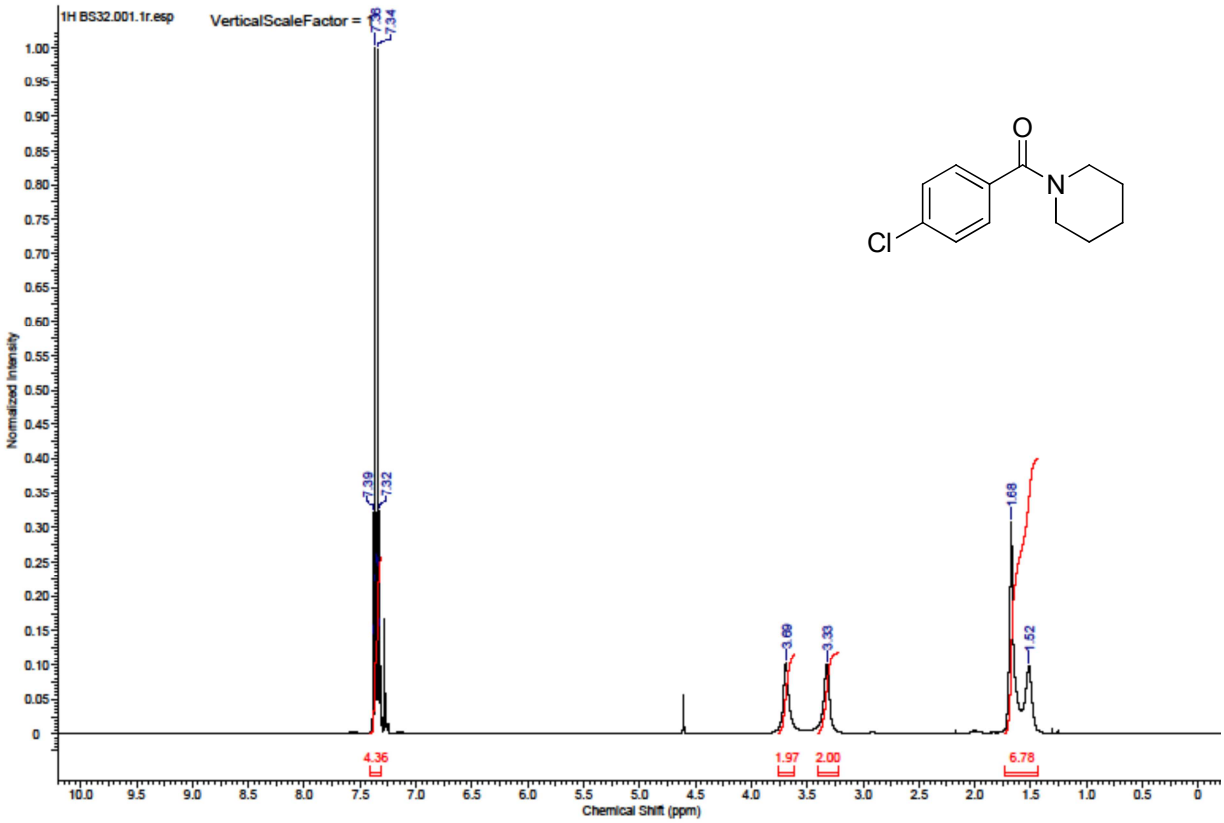
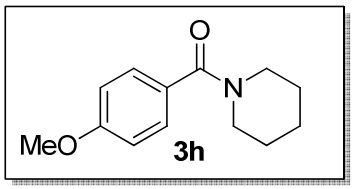


Figure S21. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **3f**.

(4-Methoxyphenyl)(piperidin-1-yl)methanone



Purification with silica gel chromatography (eluent EtOAc 5:5 cyclohexane) led to desired amide **3h**[11] (yellow oil) in 22 % yield.

HPLC analysis of purified 3h

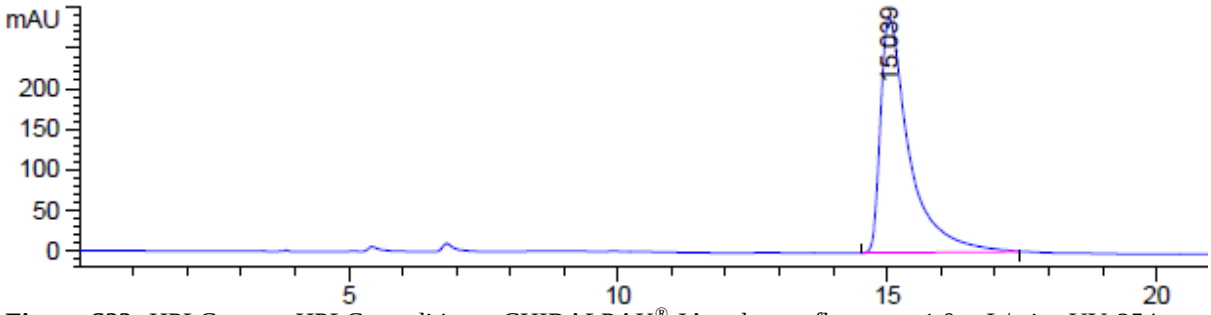


Figure S22. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

^{1}H-NMR analysis of purified 3h

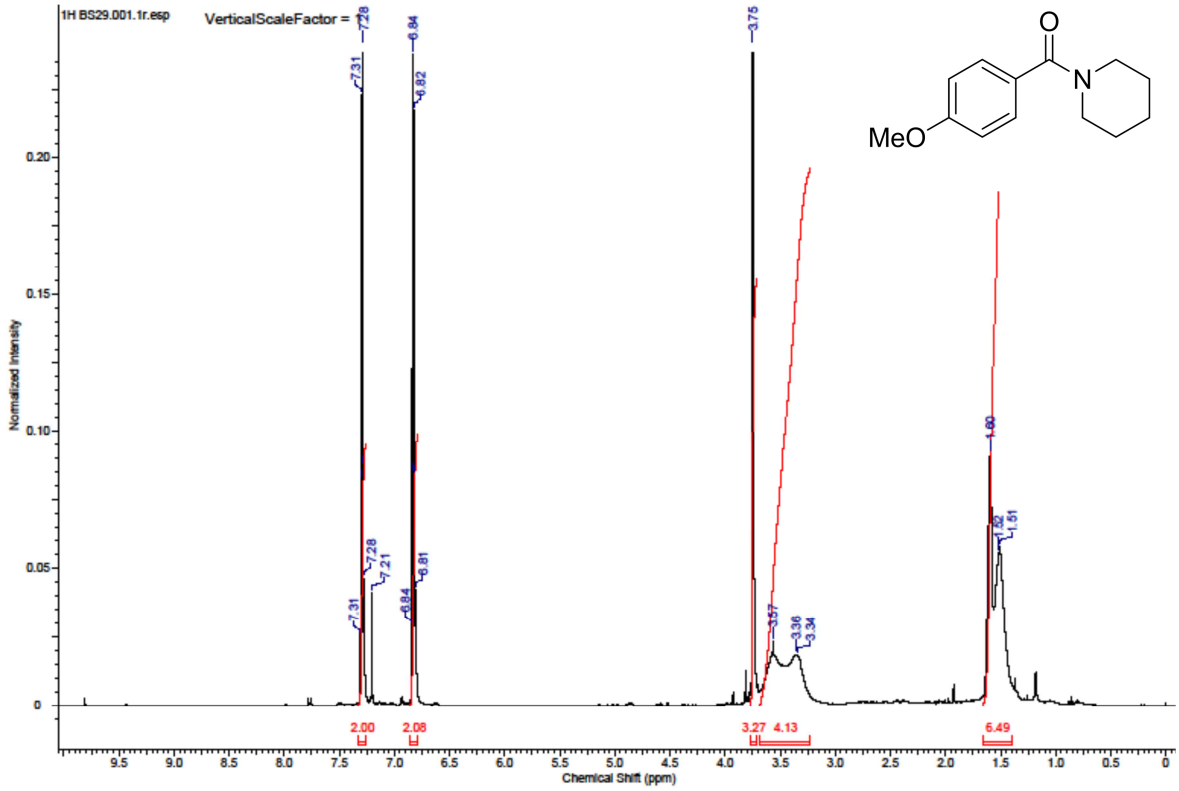
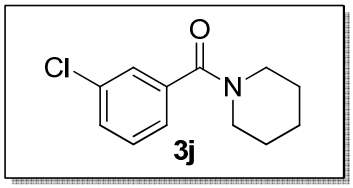


Figure S23. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **3h**.

(3-Chlorophenyl)(piperidin-1-yl)methanone



Purification with silica gel chromatography (eluent EtOAc) led to desired amide **3j**[12](yellow oil) in 41 % yield.

HPLC analysis of purified 3j

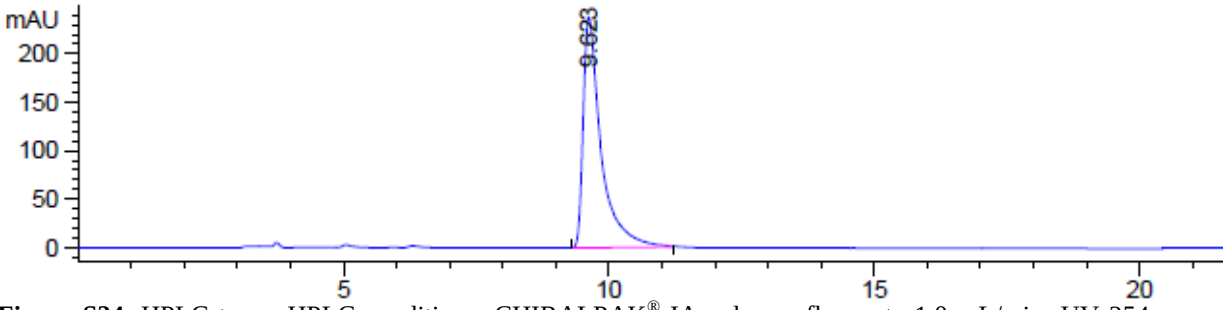


Figure S24. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

^{1}H-NMR analysis of purified 3j

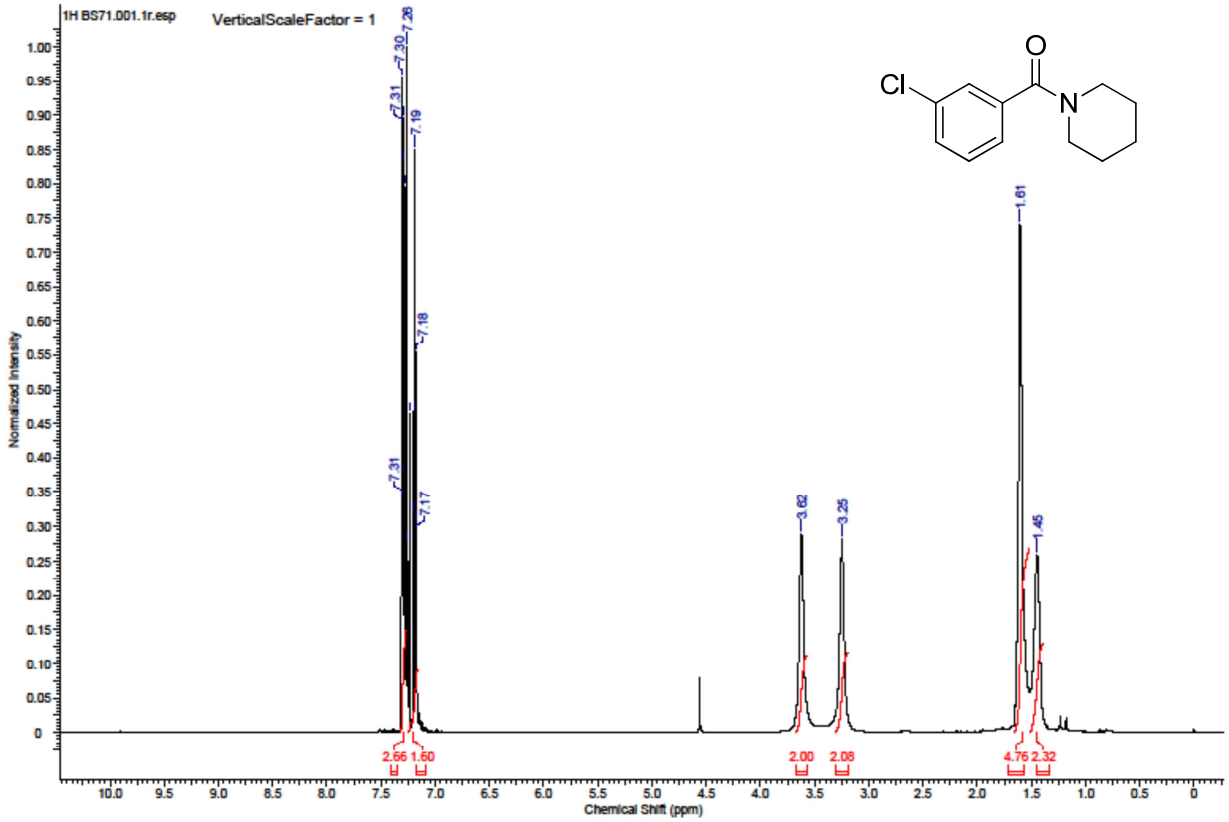
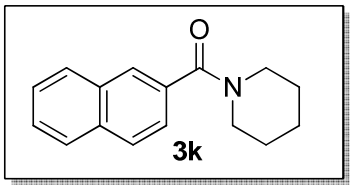


Figure S25. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **3j.**

Naphthalen-2-yl(piperidin-1-yl)methanone



Purification with silica gel chromatography (eluent EtOAc) led to desired amide **3k**[11] (white solid) in 35 % yield.

HPLC analysis of purified 3k

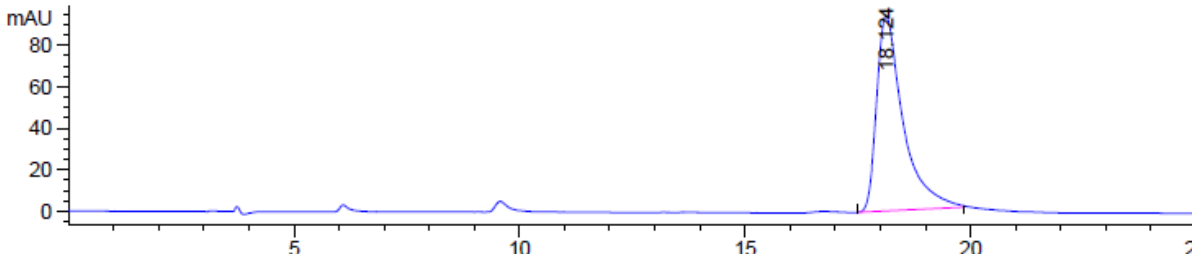


Figure S26. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

^{1}H-NMR analysis of purified 3k

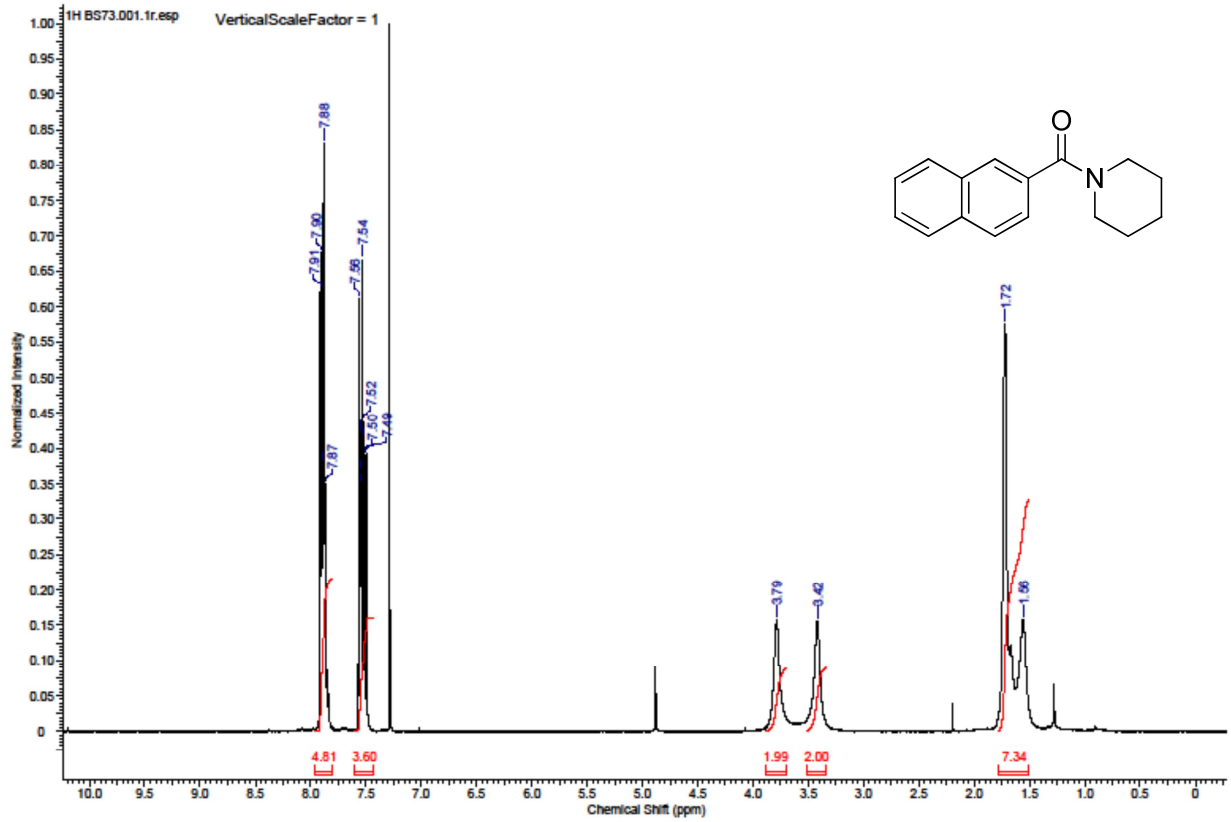
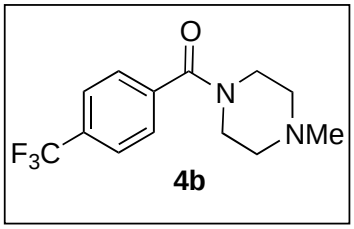


Figure S27. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **3k.**

4-Methylpiperazin-1-yl)[4-(trifluoromethyl)phenyl]methanone



Purification with silica gel chromatography (eluent EtOAc 8:2 MeOH) led to desired amide **4b** (colourless oil) in 23 % yield.). ^{1}H NMR, 800 MHz, CDCl3 δ ppm: 7.71 (d, *J* = 8.2 Hz, 2H), 7.54 (d, *J* = 8.2 Hz, 2H), 3.85 (bs, 2H), 3.43 (bs, 2H), 2.55 (bs, 2H), 2.39 (bs, 2H), 2.36 (s, 3H); ^{13}C NMR, 200 MHz, CDCl3 δ ppm: 168.8, 139.3, 131.8, 127.5, 125.6, 125.1, 124.3, 123.0, 55.1, 54.6, 47.4, 45.9, 42.0, 29.7.

^{1}H-NMR analysis of purified 4b

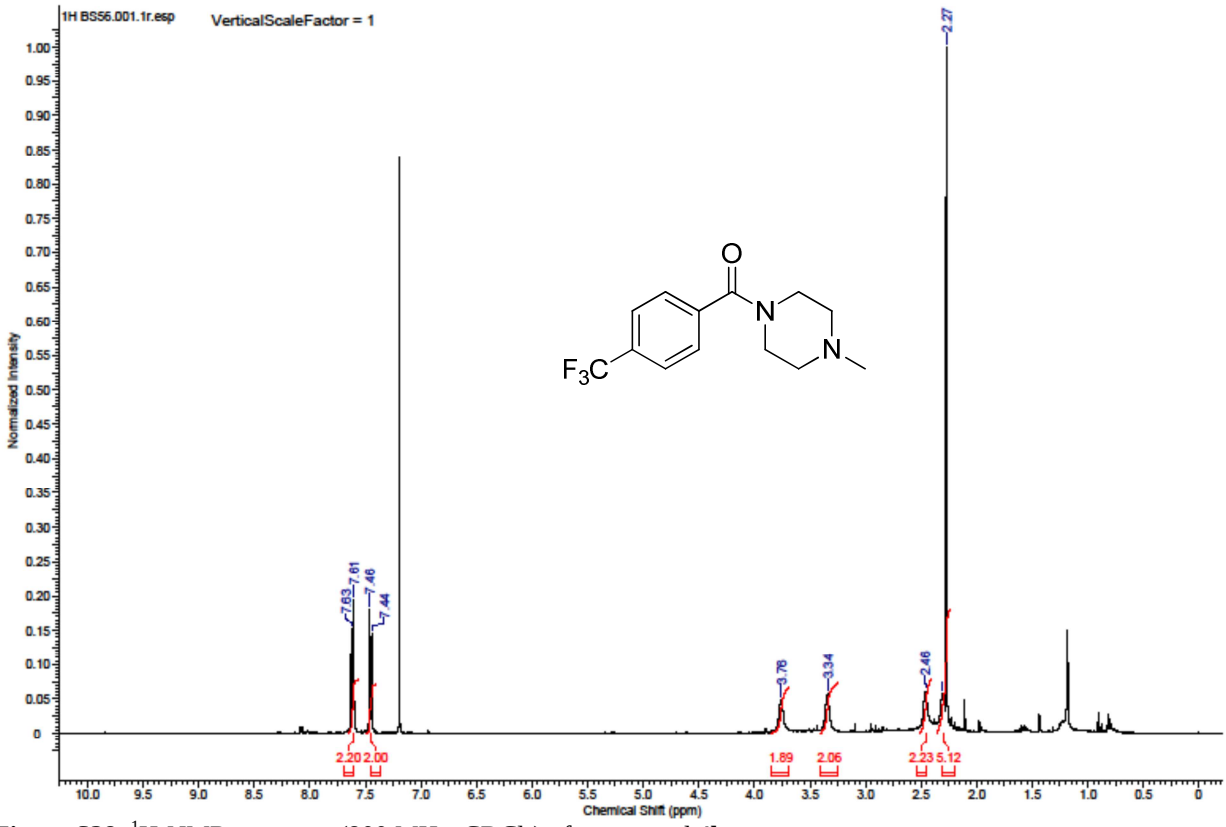


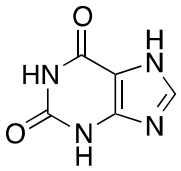
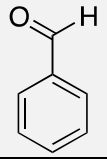
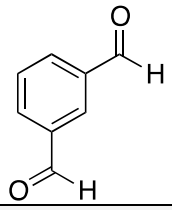
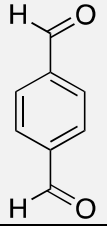
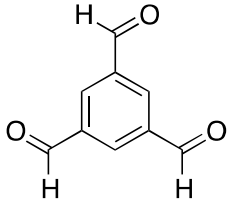
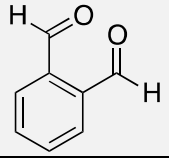
Figure S28. ^{1}H-NMR spectrum (800 MHz, $CDCl_3$) of compound **4b**.

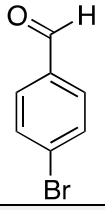
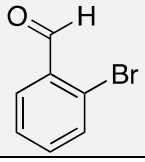
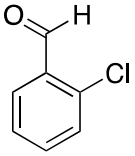
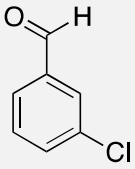
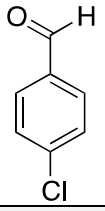
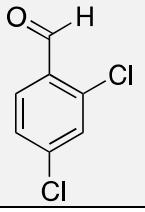
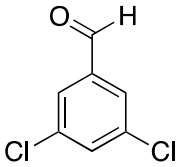
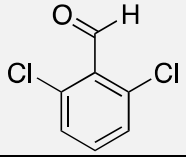
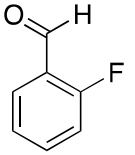
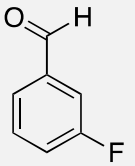
4. Cascade 2 - GOase M_{3-5}- *E.coli* XDH catalysed oxidation of benzyl alcohols to acids

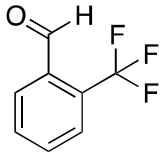
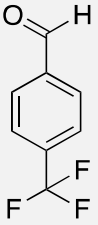
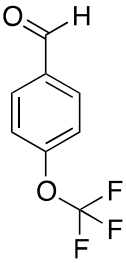
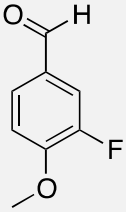
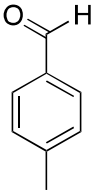
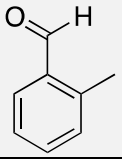
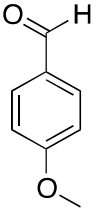
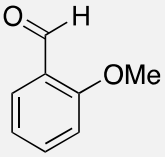
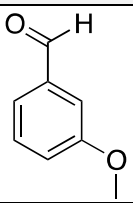
4.1 Screening of *E. coli* XDH towards a diverse set of selected aldehyde substrates using NBT

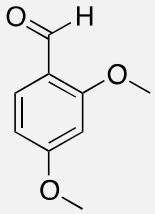
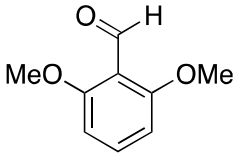
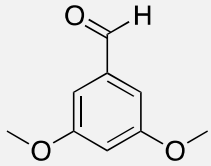
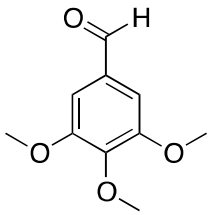
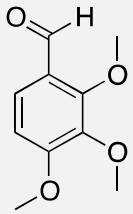
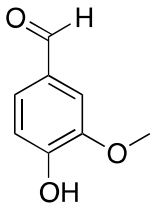
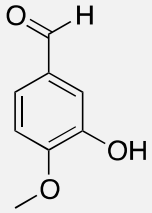
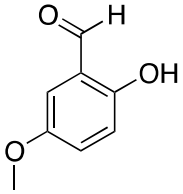
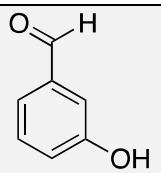
The screening of *E. coli* xanthine dehydrogenase was accomplished using a 96-well, clear, flat-bottomed polystyrene microtitre plate in a final volume of 200 µL in potassium phosphate buffer (50 mM, pH 7.6) containing per well: 1 mM of the respective substrate, 2.5 mM NBT, 20 µL XDH solution (1.1 mg/mL stock solution). The activity of *E. coli* XDH towards the compounds tested was assessed by eye based on the intensity of colour development in a defined time-frame and in relation to the activity obtained for the natural substrate xanthine, assuming xanthine = 100 %.

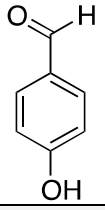
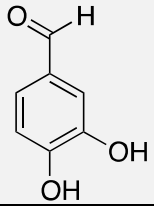
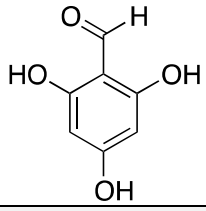
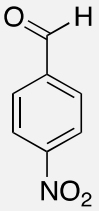
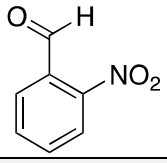
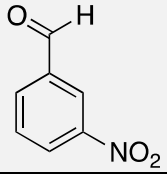
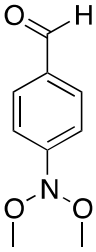
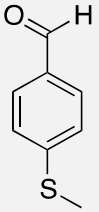
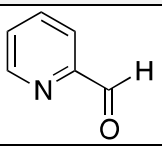
Table S3: Activity of *E. coli* xanthine dehydrogenase towards a diverse set of aldehyde substrates using the NBT assay. Activity as good as with natural substrate xanthine: +++; good activity: ++; moderate to low activity: +; no activity: -.

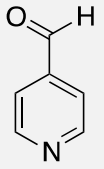
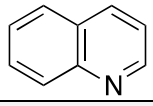
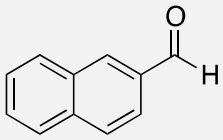
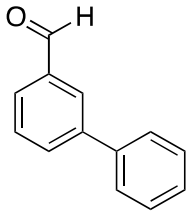
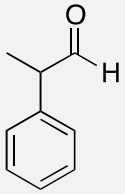
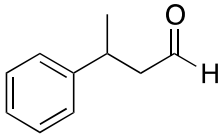
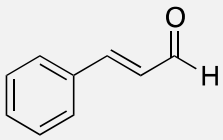
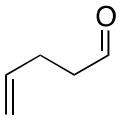
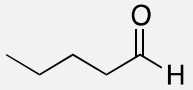
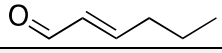
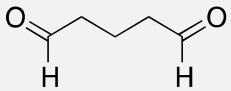
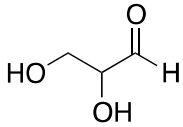
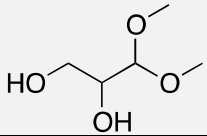
Substrate	Structure	Relative activity compared to natural substrate xanthine
Xanthine		+++
Benzaldehyde		+++
Isophthaldehyde		+
Terephthaldehyde		+
Benzene-1,3,5-tricarbaldehyde		-
Phthaldialdehyde		-

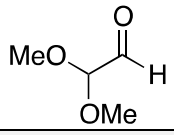
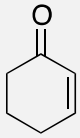
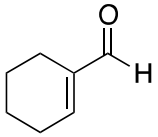
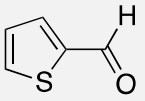
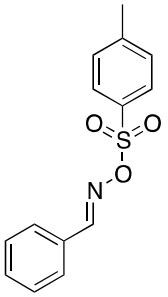
4-Bromobenzaldehyde		+
2-Bromobenzaldehyde		++
2-Chlorobenzaldehyde		+++
3-Chlorobenzaldehyde		++
4-Chlorobenzaldehyde		+
2,4-Dichlorobenzaldehyde		-
3,5-Dichlorobenzaldehyde		++
2,6-Dichlorobenzaldehyde		++
2-Fluorobenzaldehyde		++
3-Fluorobenzaldehyde		++

2-Trifluoromethylbenzaldehyde		-
4-Trifluoromethylbenzaldehyde		-
4-Trifluoromethoxybenzaldehyde		-
3-Fluoro-4-methoxybenzaldehyde		-
4-Tolualdehyde		++
2-Tolualdehyde		+
4-Methoxybenzaldhyde		+
2-Methoxybenzaldhyde		++
3-Methoxybenzaldhyde		+++

2,4-Dimethoxybenzaldehyde		-
2,6-Dimethoxybenzaldehyde		-
3,4-Dimethoxybenzaldehyde		-
3,4,5-Trimethoxybenzaldehyde		-
2,3,4-Trimethoxybenzaldehyde		-
Vanillin		++
3-Hydroxy-4-methoxy-benzaldehyde		-
2-Hydroxy-5-methoxy-benzaldehyde		+
3-Hydroxybenzaldehyde		+++

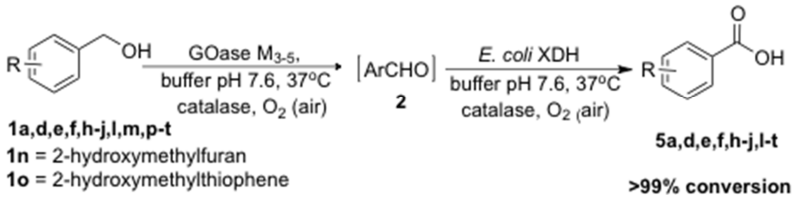
4-Hydroxybenzaldehyde		++
3,4-Dihydroxybenzaldehyde		-
2,4,6-Trihydroxybenzaldehyde		-
4-Nitrobenzaldehyde		+++
2-Nitrobenzaldehyde		-
3-Nitrobenzaldehyde		-
4-Dimethoxyaminobenzaldehyde		-
4-Methylthiobenaldehyde		-
Pyridine-2-carboxaldehyde		-

3-Pyridinecarboxaldehyde		+
Quinoline		-
2-Napthaldehyde		-
3-Phenylbenzaldehyde		-
2-Phenylpropionaldehyde		-
3-Phenylbutaraldehyde		-
trans-Cinnamaldehyde		-
4-Pentenal		-
Valeraldehyde		-
trans-2-Hexenal		-
Glutaric dialdehyde		-
DL-Glyceraldehyde		-
Glyceraldehyde dimethylacetal		-

Dimethoxyacetaldehyde		-
2-Cyclohexen-1-one		-
1-Cyclohexene-1-carboxaldehyde		-
2-Thiophencarboxaldehyde		++
Benzyl O-tosyl oxime		-

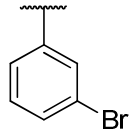
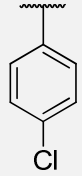
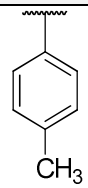
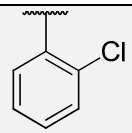
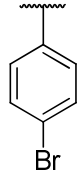
4.2. General method for bio-bio-catalytic cascade reaction for synthesis of acids 5 from alcohols 1 in a one-pot one-step approach

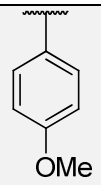
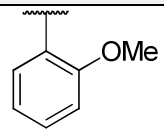
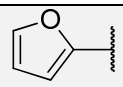
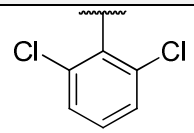
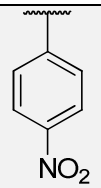
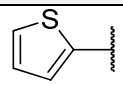
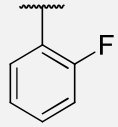
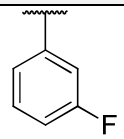
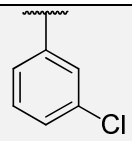
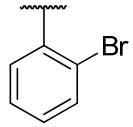
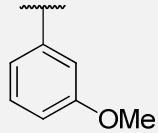
To a 1-mL-Eppendorf tube was added 69 µL of 50 mM NaPi buffer pH 7.6, 75 µL catalase (1 mg/mL), 3 µL of substrate (100 mM stock in MeCN), 50 µL of *E. coli* XDH (1.1 mg/mL) and 103 µL of GOase M $_{3\text{-}5}$ (3.7 mg/mL). The reaction was left in an incubator at 37 °C, shaken periodically and left overnight. 100 µL of the reaction mixture was acidified with 20 µL of 2 M HCl, centrifuged for 1 min at 13000 rpm and analysed by RP-HPLC.



a = 4-NO_2-Ph, **d** = 4-Br-Ph, **e** = Ph, **f** = 4-Cl-Ph, **h** = 4-MeO-Ph, **i** = 2-MeO-Ph, **j** = 3-Cl-Ph, **l** = 3-Br-Ph, **m** = 4-Me-Ph, **p** = 2-F-Ph, **q** = 3-F-Ph, **r** = 2-Br-Ph, **s** = 3-MeO-Ph, **t** = 2,6-Cl_2-Ph

Table S4: Galactose oxidase $M_{3\text{-}5}$- *E. coli* xanthine dehydrogenase cascade reactions for formation of acids from alcohols in a one-pot one-step approach. Substrates, HPLC-retention times of alcohols, aldehydes and acid and percentage of conversions after 16 h based on HPLC peak areas (ʎ = 254 nm).

Entry	Substrate	Retention time alcohol [min]	Retention time aldehyde [min]	Retention time acid [min]	Conversion Alc:Ald:Acid [10 mM]	HPLC conditions
1		6.4	14.0	10.06	0:0:100	A
2		5.4	10.94	8.9	0:0:100	A
3		3.05	5.98	4.08	0:0:100	B
4		4.7	4.4	2.1	0:100:0	A
5		4.4	9.02	6.5	0:0:100	B

6		2.5	5.2	3.4	0:0:100	A
7		2.9	5.7	2.77	0:0:100	A
8		5.1	5.9	-	0:0:100	C
9		2.73	5.4	2.37	0:0:100	E
10		3.1	5.3	4.6	0:0:100	A
11		3.1	4.5	4.9	0:0:100	D
12		2.82	5.4	4.5	0:0:100	A
13		3.1	6.0	4.6	0:0:100	A
14		3.8	7.7	5.1	0:0:100	B
15		4.0	8.9	3.5	0:0:100	B
16		4.0	8.9	3.5	0:0:100	A

HPLC conditions: ThermoFisherHypurity C-18 column, flow rate 1.0 mL/min, UV 254 nm, Method A: 25% MeCN: 75% water + 0.1% TFA; Method B: 25% MeCN: 75% water + 0.1% TFA; Method C: 1% MeCN : 99% water + 0.1% TFA; Method D: 15% MeCN : 85% water + 0.1% TFA; Method E: 40% MeCN: 60% water + 0.1% TFA.

4.3. Optimisation of the bio-biocatalytic cascade for synthesis of 3-methoxybenzoic acid 5s from 3-methoxybenzyl alcohol 1s in a one-pot one-step approach

In a 1-mL-Eppendorf was added 50 mM NaPi buffer pH 7.6, catalase (stock 1 mg/mL), 3-methoxybenzyl alcohol (10-100 mM), 50 µL of *E. coli* XDH (1.1 mg/mL) and 103 µL of GOase M_{3-5} (3.7 mg/mL). The reaction was left in a shaking incubator at 36 °C. The reaction was periodically opened to air, closed and shaken to oxygenate the buffer and put back into the incubator. After the indicated time 50 µL of the reaction mixture was acidified with 20 µL of 2 M HCl, centrifuged and analysed by RP-HPLC. Conversion reported are based on relative response factors determined *via* NMR-HPLC correlations (cf. Figures S29-32).

Table S5: Optimisation of the galactose oxidase M_{3-5}- *E. coli* XDH cascade reaction for formation of 3-methoxybenzoic acid **5s** from 3-methoxybenzyl alcohol **1s** in a one-pot one-step approach. Substrates, HPLC-retention times of alcohols, aldehydes and acid and percentage of conversions after 16 h based on HPLC peak areas (ʎ = 254 nm). 10 - 100mM scale.

Entry	Concentration 3-methoxybenzyl alcohol [mM]	Additive	µL of catalase added [1.1 mg/mL stock]	Reaction time [h]	Yield [%]
1	10	-	75	1	100[a]
2	20	-	75	2	100[a]
3	40	-	75	16	82[a]
4	40	5% IPA	75	16	69[a]
5	40	15% IPA	75	16	21[a]
6	40	-	100	5	94[a](81[b])
7	100	-	100	48	57[a](50[b])

[a]Yields calculated from peak areas of HPLC analysis using a Thermofisher Hypurity C-18 column with mobile phase 25:75 MeCN:H_2O (0.1% TFA). Yields were adjusted according to an NMR analysed 1:1:1 mix of the aldehyde:acid:alcohol. [b]Isolated yields in parenthesis.

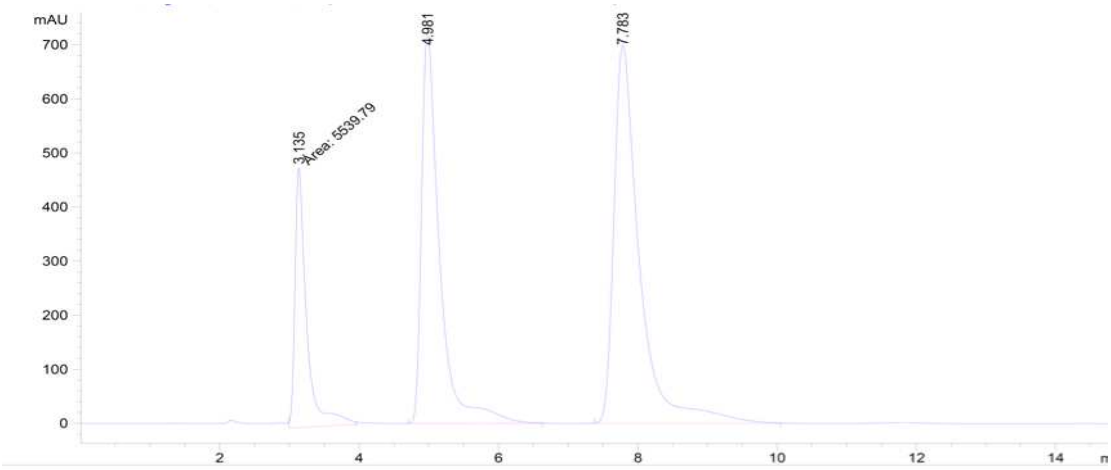


Figure S29. HPLC trace of a 1:1:1 mixture of 3-methoxybenzyl alcohol (3.14 min), 3-methoxybenzoic acid (4.98 min) and 3-methoxybenzaldehyde (7.78 min). HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

Peak	Retention time [min]	Area [%]
1	3.135	15.265
2	4.981	35.77
3	7.783	48.96

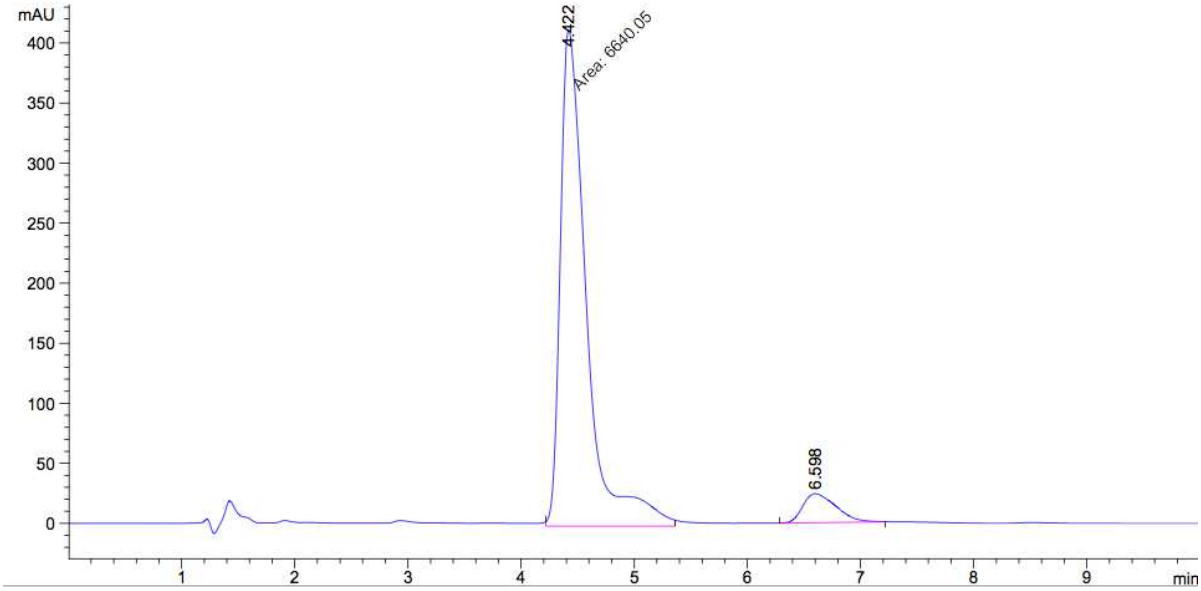


Figure S30. HPLC trace of a reaction assay with a 40 mM starting concentration of 3-methoxybenzyl alcohol. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

Peak	Retention time [min]	Area [%]
1	4.422	93.11
2	6.598	6.88

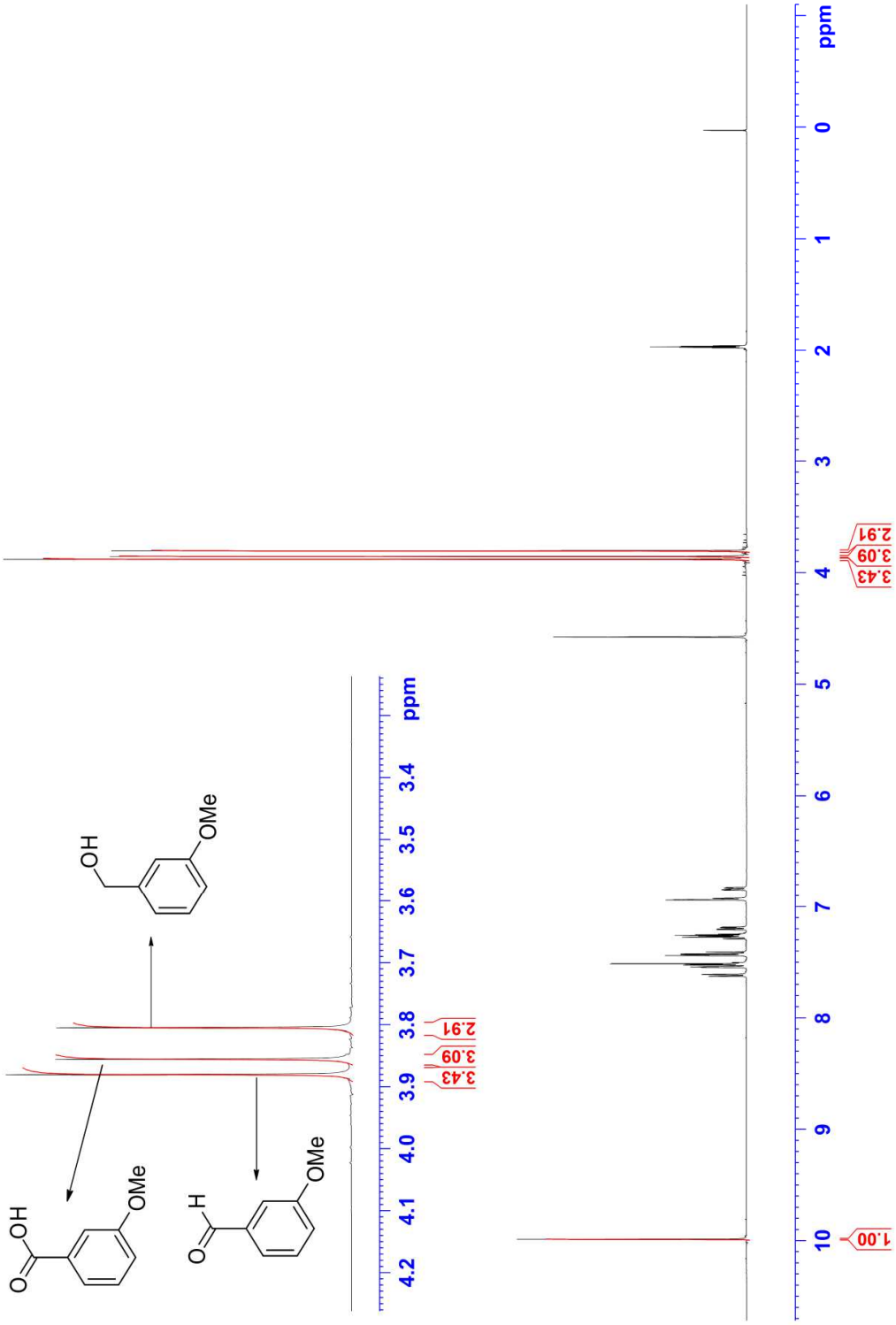


Figure S31. ^{1}H NMR spectrum (500 MHz) of a 1:1:1 mixture of 3-methoxybenzyl alcohol (3.8 ppm), 3-methoxybenzoic acid (3.86 ppm) and 3-methoxybenzaldehyde (3.89 ppm).

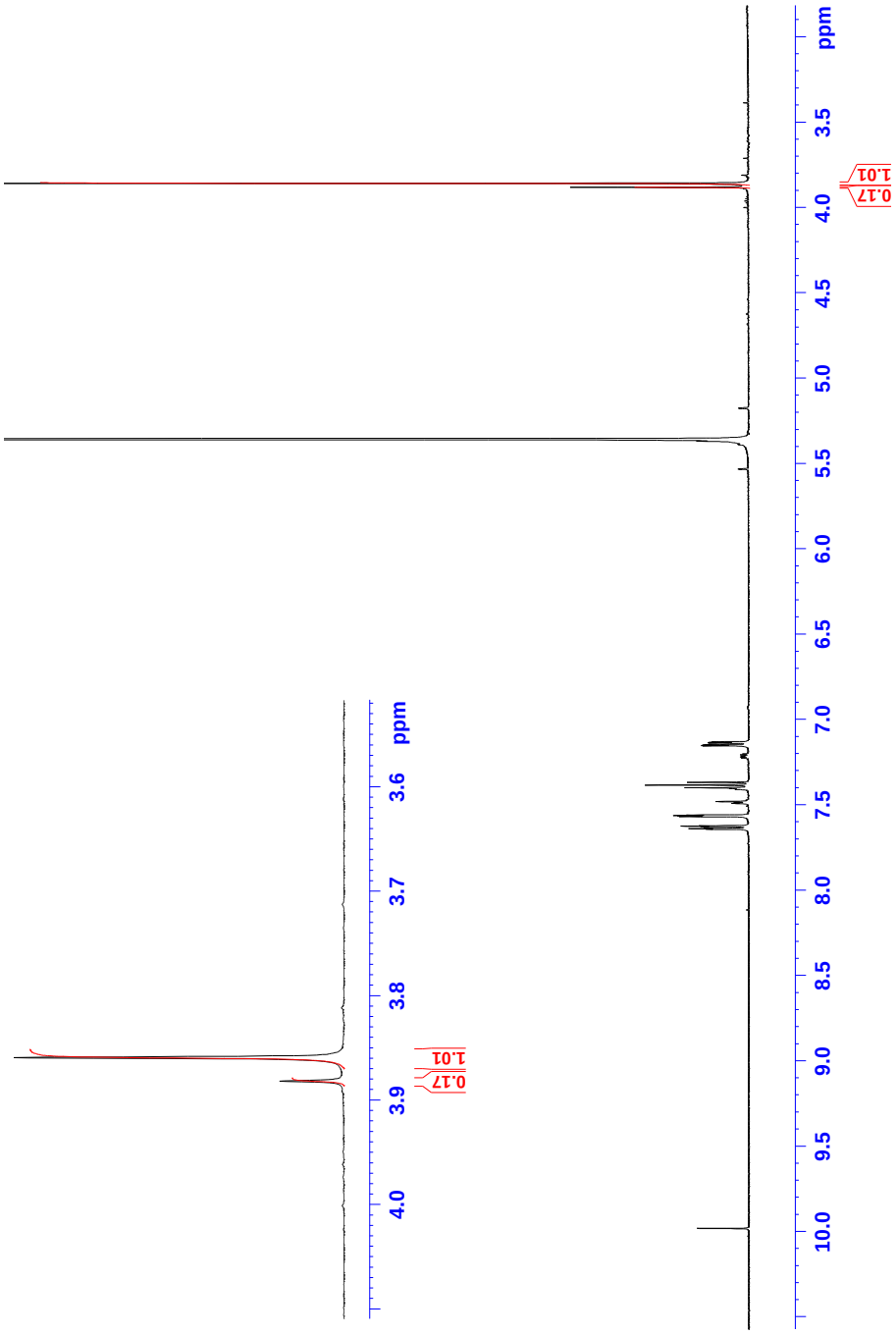


Figure S32. ^{1}H NMR spectrum (500 MHz) of a reaction assay with a 40 mM starting concentration of 3-methoxybenzyl alcohol to give 3-methoxybenzoic acid (3.86 ppm).

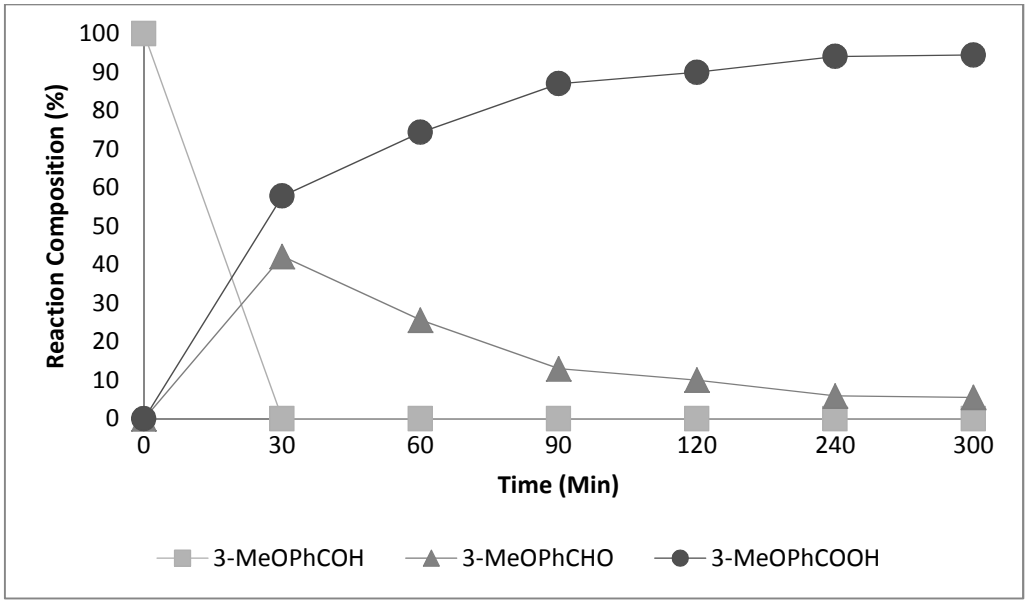


Figure S33. Time course of a reaction with a 40 mM starting concentration of 3-methoxybenzyl alcohol **1s** yielding 3-methoxybenzoic acid **5s** in a GOase M_{3-5} - *E. coli* XDH-cascade.

5. Cascade 3

5.1 Synthesis of tetrahydroisoquinolines

6-Nitro-1,2,3,4-tetrahydroisoquinoline **6b** was synthesized according to the literature.[13]

Synthesis of 2-methyl-1,2,3,4-tetrahydroisoquinoline (6c)

3,4-Dihydroisoquinoline **7a** (200 mg, 1.52 mmol) was dissolved in 2 mL of DCM. Methyl iodide (114 µL, 1.82 mmol) was added and the reaction was stirred at rt overnight. Solvent was removed under reduced pressure, and the yellow solid was dissolved in 5 mL of MeOH. $NaBH_4$ (6.08 mmol, 227 mg) was carefully added, and the reaction was stirred at rt for 4 h. H_2O (5 mL) was added, MeOH was evaporated under reduced pressure and the aqueous solution was extracted with 4 mL of DCM. The solution was dried over $MgSO_4$, filtrated and evaporated under reduced pressure to afford 2-methyl-1,2,3,4-tetrahydroisoquinoline **6c** as a colourless oil (quantitative yield). ^{1}H NMR, 400 MHz, CDCl3 δ ppm: 7.16-7.13 (m, 3H), 7.06-7.04 (m, 1H), 3.62 (s, 2H), 2.96 (t, *J* = 5.9 Hz, 2H), 2.72 (t, *J* = 5.9 Hz, 2H), 2.49 (s, 3H).^{13}C NMR, 100 MHz, CDCl3 δ ppm: 134.7, 133.8, 128.7, 126.4, 126.2, 125.6, 58.0, 52.9, 46.1, 29.2.

^{1}H-NMR analysis of purified 6c

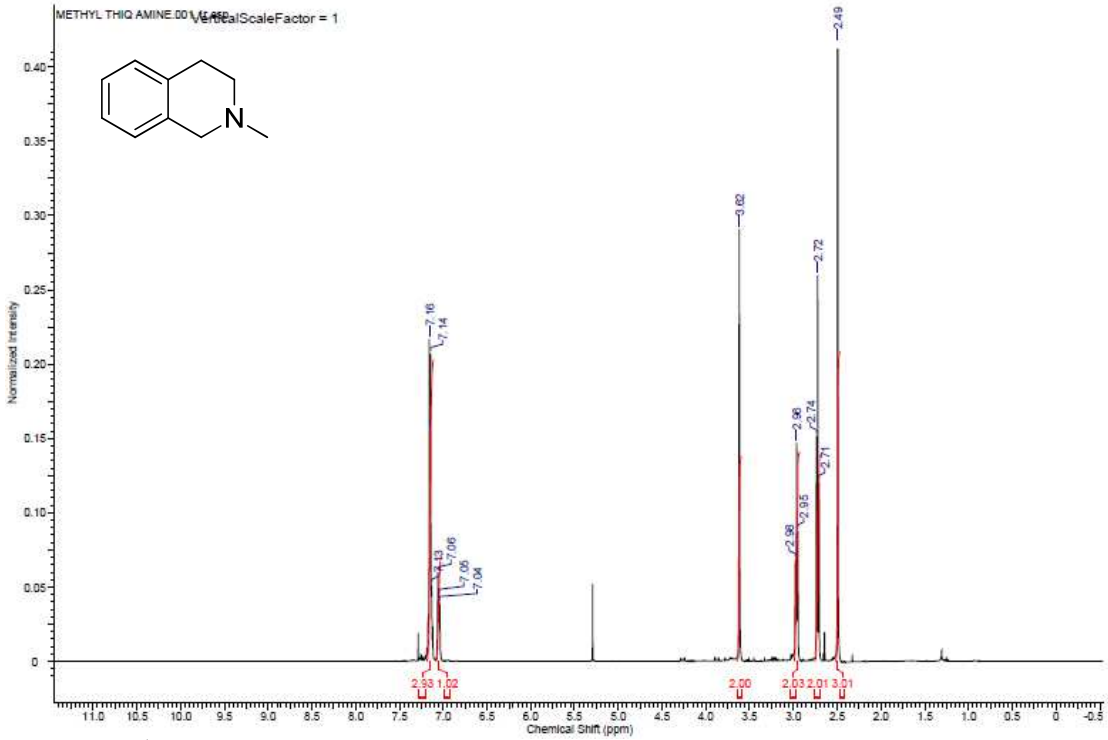


Figure S34. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **6c**.

^{13}C-NMR analysis of purified compound 6c

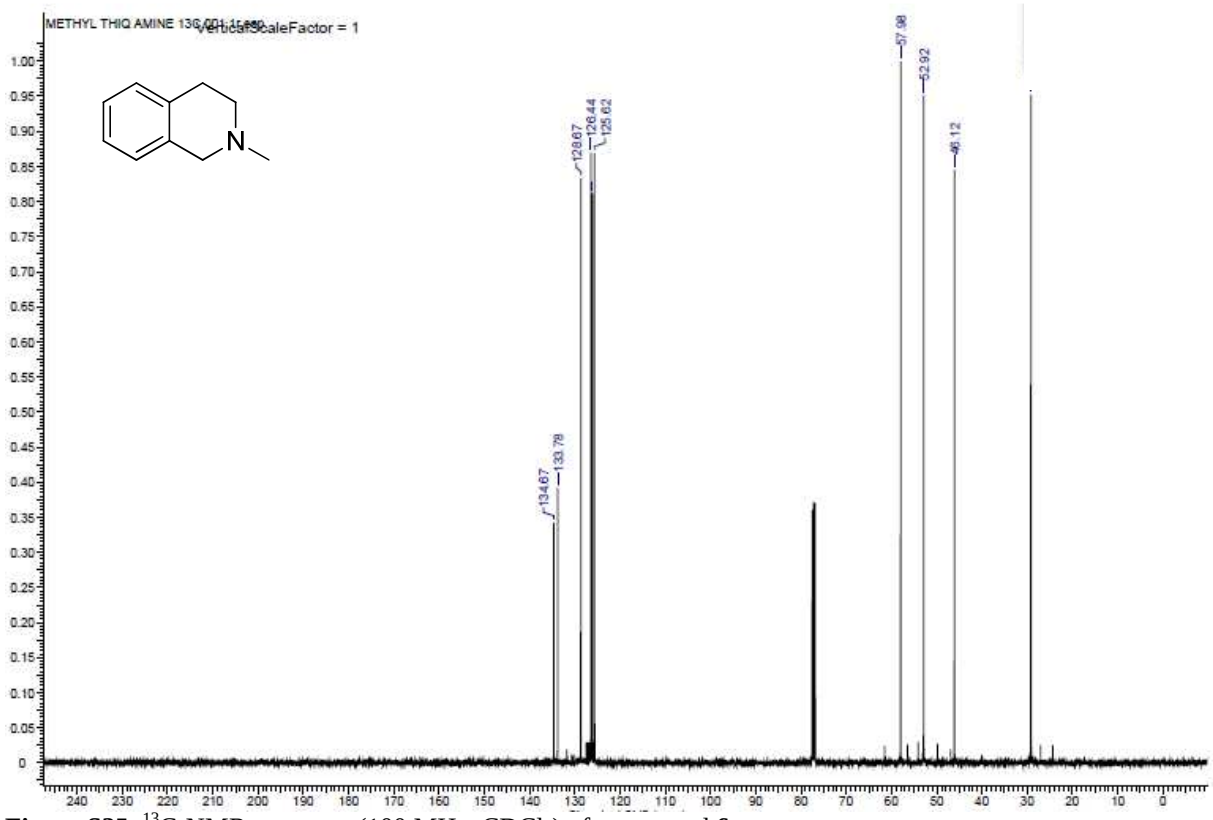
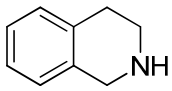
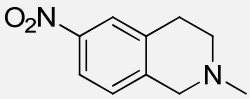
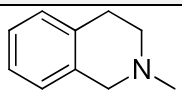


Figure S35. ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) of compound **6c**.

5.2. Liquid phase screening of MAO-N D9 for oxidation of tetrahydroisoquinolines[14]

The assay was conducted using a 96-well, clear, flat-bottomed polystyrene plate. To each well was added: 50 µL HRP solution (0.2 mg/mL solution HRP in KPi buffer 0.1 M), 50 µL dye (prepared by pre-mixing 15 µL of a 20 mg/mL solution of TBHBA in DMSO and 50 µL of a 100 mg/mL solution of 4-AAP in H_2O, and diluting this solution with 5 mL of KPi buffer 0.1 M), 50 µL of a 10 mM solution of substrate in 0.1 M KPi buffer, 50 µL of 0.2 mg/mL solution of pure enzyme in 0.1 M KPi buffer. The blank assay was run in parallel using 50 µL of 0.1 M KPi buffer instead of a 10 mM solution of substrate in 0.1 M KPi buffer. The plate was read immediately using a Tecan Infinite M200 Pro Plate reader. The formation of the red dye was monitored at 510 nm, taking the linear and early part of the graphs (V_{max}, where there is no limiting substrate). The rates for MAO-N D9 are relative to THIQ, assuming THIQ = 100 %.

Table S6: Relative rates for oxidation of substrates **6a-c** using MAO-N D9.

Entry	Substrate	MAO-N D9 Relative oxidation rate [%]
1	 **6a**	100
2	 **6b**	74
3	 **6c**	102

5.3. Optimisation of Cu(I)-catalysed imine oxidation: screening of H_2O_2 amount

3,4-Dihydroisoquinoline **7a** was chosen as model for the development and the optimization of the H_2O_2/CuI oxidation reaction.

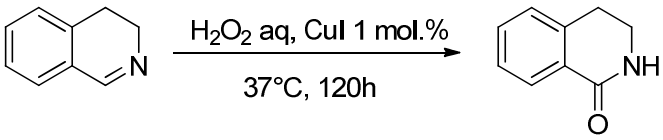


Table S7: Optimisation of peroxide concentration for Cu(I) –catalysed oxidation of **7a**.

Entry	1	2	3	4
H_2O_2	1 eq.	2 eq.	5 eq.	10 eq.
Conversion [%][a]	3	9	62	69

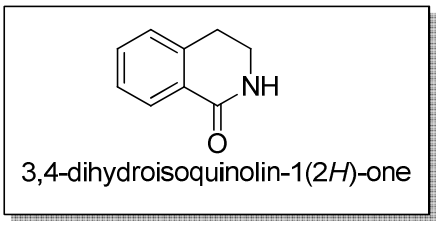
[a] Conversion to lactam, determined *via* HPLC. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

5.4. One-pot-two-step cascade reactions for the synthesis of lactams - General procedure C (Table 2 of paper "Conditions A")

5.4.1. Analytical scale method- Bio-chemo catalytic cascade reactions for synthesis of lactams 8 from cyclic amines 6

In a 15-mL-Falcon tube, cyclic amine (0.04 mmol) dissolved in 0.05 mL of DMF, 0.01 mL of a 40 mM solution of CuI in MeCN (0.0004 mmol, 1 mol %), 0.039 mL of a 35 % sol. H_2O_2 in H_2O (0.4 mmol) and pure MAO-N D9 (0.4 mg/mL final concentration) were dissolved in a solution of MOPS Buffer (0.1 M, pH = 7.8) obtaining a final volume of 1 mL. The tube was placed in a shaking incubator and shaken at 37 °C and 250 rpm. The reaction was monitored by HPLC and work up was performed when the conversion was considered maximum. HPLC samples were prepared as follows: aqueous sodium thiosulfate solution (20 µL, saturated) was added to a 100 µL sample of the reaction mixture in an Eppendorf tube, followed by 1 mL of DCM. After vigorous mixing by means of a vortex mixer the sample was centrifuged at 13200 rpm for 1 minute. The organic phase was separated, dried with MgSO4 and analysed by HPLC. When the intermediate was an iminium, the reaction mixture was allowed to react with 4 eq. of $BH_3 \cdot NH_3$ for 12 hours prior to injection in the HPLC (to allow unreacted iminium to be reduced to amine). Relative response factors were determined *via* NMR-HPLC correlations (cf. Figures S40, S41 and S60) for the conversions to lactams **8a** and **8c**. Compound **8b** was isolated and analysed *via* NMR.

3,4-Dihydroisoquinolin-1(*2H*)-one (8a)



The reaction was performed following general procedure C, using 0.4 mg/mL pure MAO-N D9 enzyme, 10 eq. of 35 % sol. H_2O_2 in H_2O and 1 mol% CuI, 24 h reaction time.

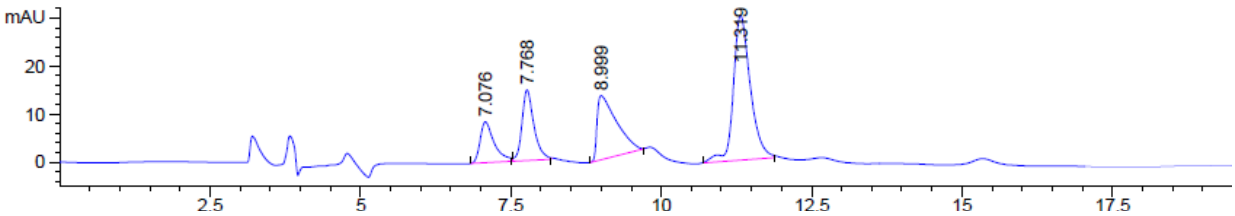


Figure S36. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

Peak	Retention time [min]	Area [%]
1 (7a)	7.076	11.22
2	7.768	16.55
3	8.999	24.17
4 (8a)	11.319	48.05

3,4-Dihydroisoquinolin-1(*2H*)-one (8a)

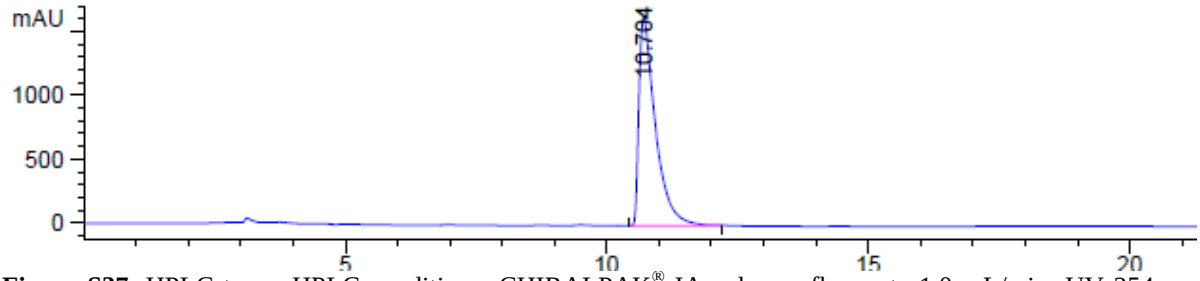


Figure S37. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

3,4-Dihydroisoquinoline (7a)

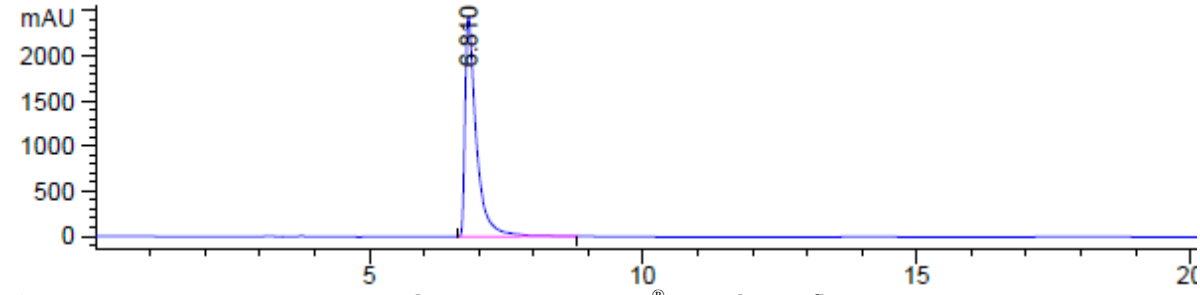


Figure S38. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

Tetrahydroisoquinoline (6a)

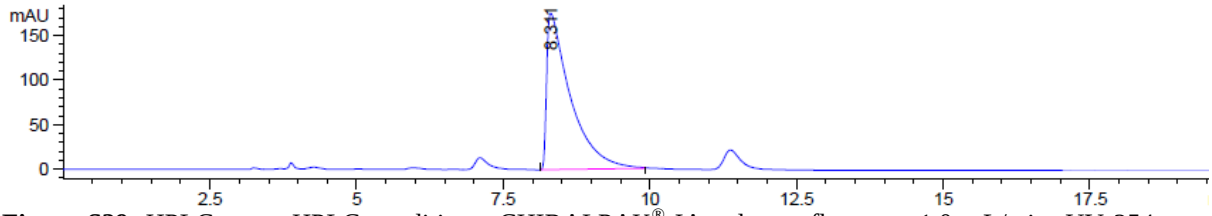


Figure S39. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

1:2 Mixture of lactam 8a and imine 7a (NMR)

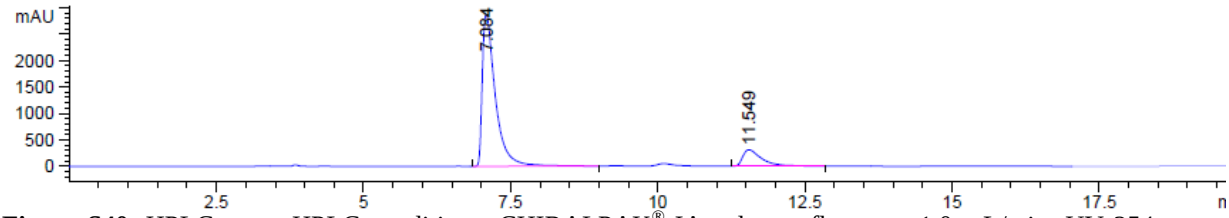


Figure S40. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

Peak	Retention time [min]	Area [%]
1 (imine 7a)	7.084	86.61
2 (lactam 8a)	11.549	13.38

Response Factor for imine**7a** = 3.3 x response for lactam **8a**.

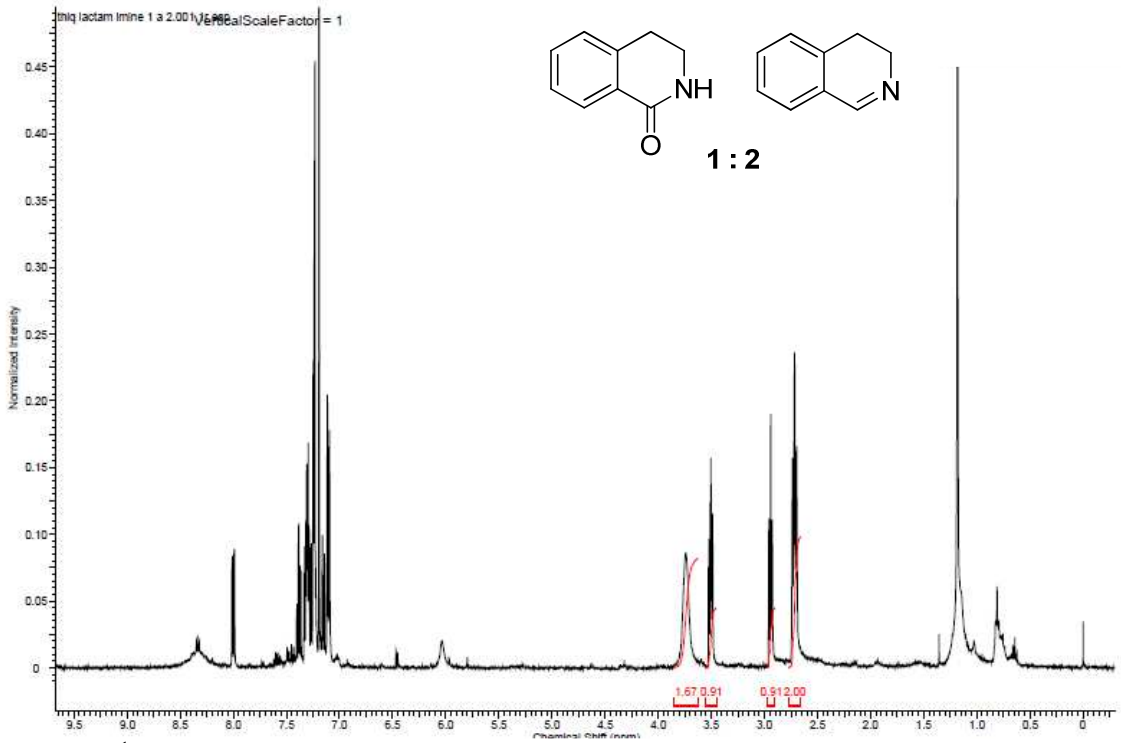
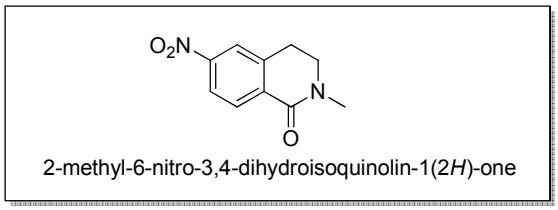


Figure S41. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of a 1:2 mixture of compounds **8a** and **7a**.

2-Methyl-6-nitro-3,4-dihydroisoquinolin-1(*2H*)-one 8b



The reaction was performed following general procedure C, using 1 mg/mL pure MAO-N D9 enzyme, 20 eq. of 35 % sol. H_2O_2 in H_2O and 2 mol% CuI, 48 h reaction time.

HPLC before addition of BH_3NH_3

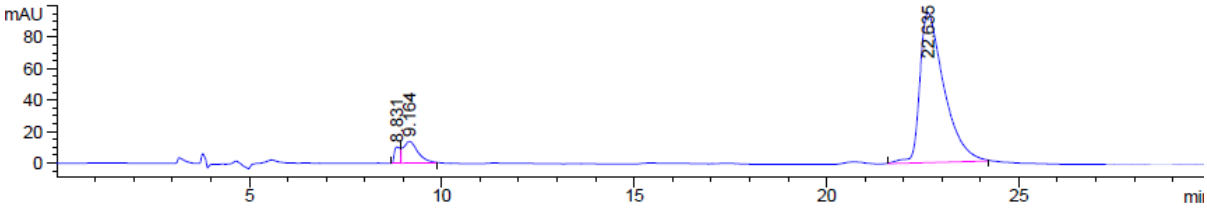


Figure S42. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

Peak	Retention time [min]	Area [%]
1	8.831	2.35
2	9.164	7.90
3	22.635	89.74

HPLC after addition of BH_3NH_3

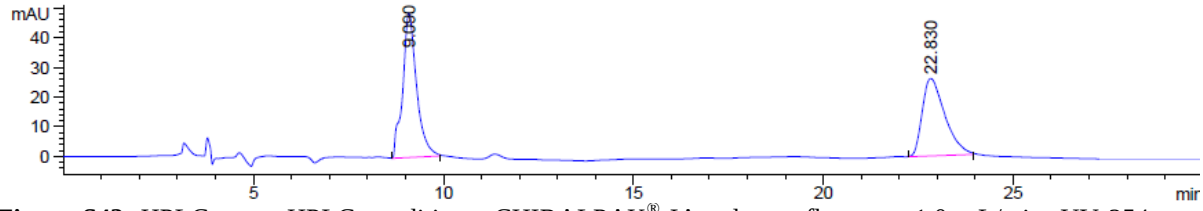
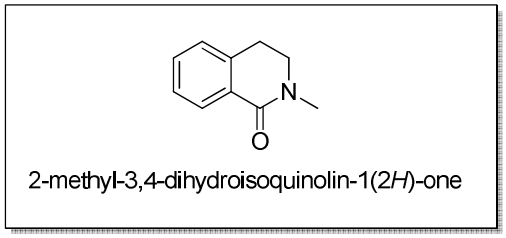


Figure S43. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

Peak	Retention time [min]	Area [%]
1	9.09	53.41
2	22.830	46.58

2-Methyl-3,4-dihydroisoquinolin-1(*2H*)-one (8c)



The reaction was performed following general procedure C, using 1 mg/mL pure MAO-N D9 enzyme, 20 eq. of 35 % sol. H_2O_2 in H_2O and 2 mol% CuI, 48 h reaction time.

HPLC before addition of BH_3NH_3

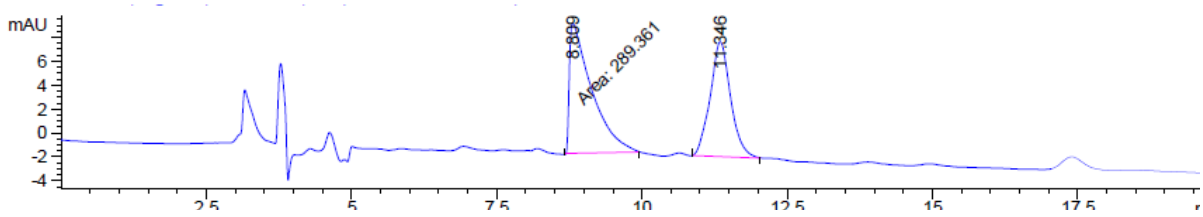


Figure S44. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

Peak	Retention time [min]	Area [%]
1	8.809	55.18
2	11.346	44.82

HPLC after addition of BH_3NH_3

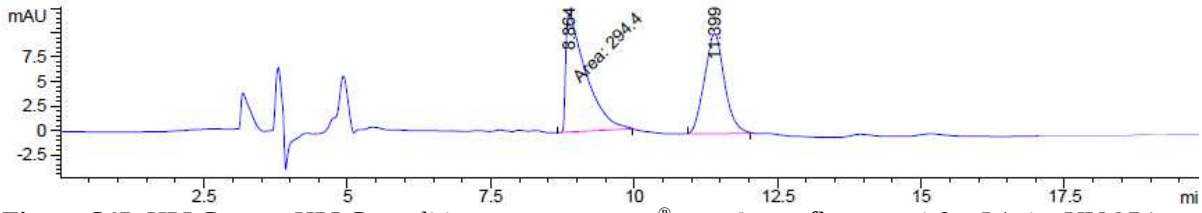


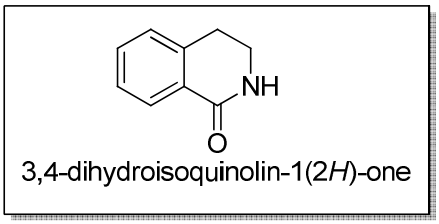
Figure S45. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent = hexane/iPrOH 90:10 + 0.1% DEA.

Peak	Retention time [min]	Area [%]
1	8.864	54.15
2	11.399	45.85

5.4.2. Preparative method - Bio-chemo catalytic cascade reactions for synthesis of lactams 8 from cyclic amines 6

In a 50-mL-Falcon tube, cyclic amine (0.2 mmol) dissolved in 0.1 mL of DMF, 0.05 mL of a 40 mM solution of CuI in MeCN (0.002 mmol, 1 mol%), 0.195 mL of a 35 % sol. H_2O_2 in H_2O (2 mmol) and pure MAO-N D9 (0.4 mg/mL final concentration) were dissolved in a solution of MOPS Buffer (0.1 M, pH = 7.8) obtaining a final volume of 5 mL. The tube was placed in a shaking incubator and shaken at 37 °C and 250 rpm. The reaction was monitored by HPLC and work up was performed when the conversion was considered maximum. HPLC samples were prepared as follows: aqueous thiosulfate solution (20 µL, saturated) was added to a 100 µL sample of the reaction mixture in an Eppendorf tube, followed by 1 mL of DCM. After vigorous mixing by means of a vortex mixer the sample was centrifuged at 13200 rpm for 1 minute. The organic phase was separated, dried with $MgSO_4$ and analysed by HPLC. Work up was performed in the following way: aqueous thiosulfate solution (1 mL, saturated) and DCM (5 mL) were added. The organic phase was separated, dried over $MgSO_4$ and analysed by HPLC. The reaction was then submitted to silica gel column chromatography.

3,4-Dihydroisoquinolin-1(*2H*)-one (8a)



The reaction was performed following general procedure C, using 0.4 mg/mL pure MAO-N D9 enzyme, 10 eq. of 35 % sol. H_2O_2 in H_2O and 1 mol% CuI, and 24 h reaction time. After work-up, purification with silica gel column cromatography using ethyl acetate as eluent gave 3,4-dihydroisoquinolin-1(*2H*)-one as a white solid. NMR data for compound **8a** are identical to those reported in the literature.[15]

^{1}H-NMR analysis of purified 8a

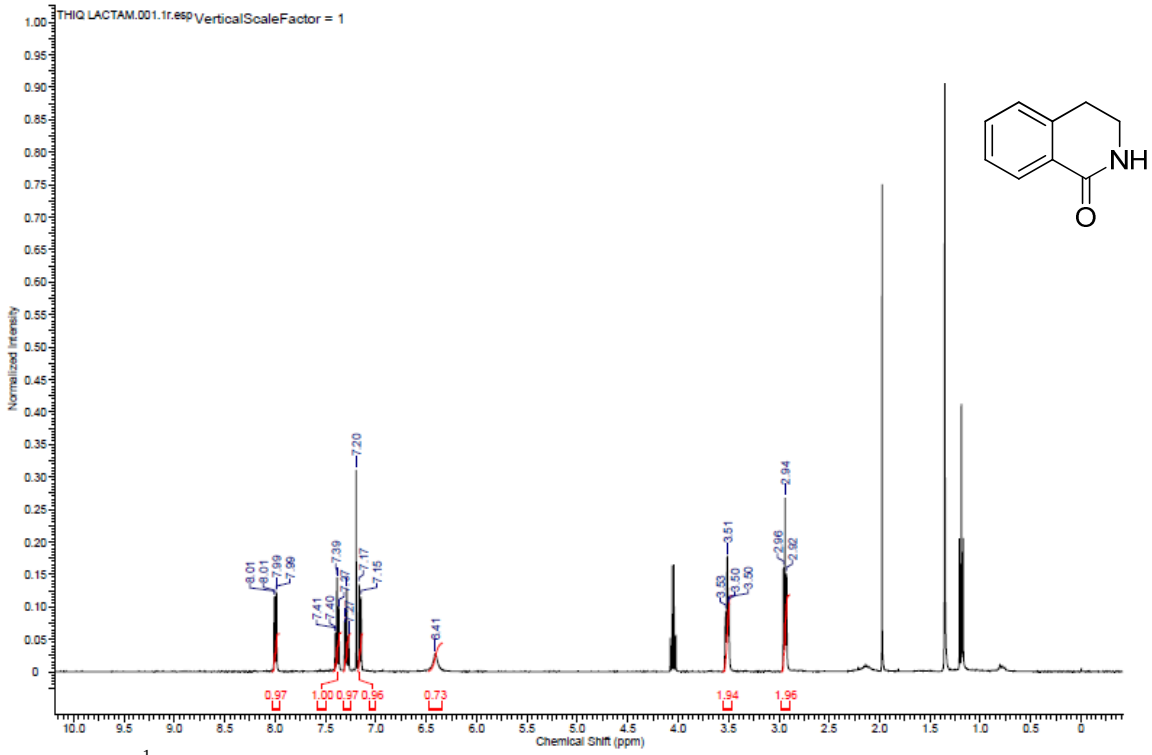
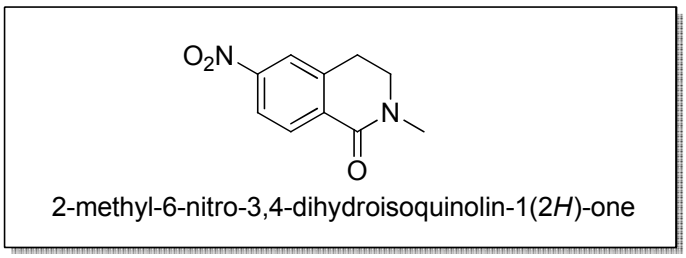


Figure S46. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **8a**.

2-Methyl-6-nitro-3,4-dihydroisoquinolin-1(*2H*)-one (8c)



The reaction was performed following general procedure C, using 1 mg/mL pure MAO-N D9 enzyme, 20 eq. of 35 % sol. H_2O_2 in H_2O and 2 mol% CuI, 48 h reaction time. After work-up, purification with silica gel column chromatography using ethyl acetate as eluent gave 2-methyl-6-nitro-3,4 dihydroisoquinolin-1(*2H*)-one as a yellow crystals. Yield: 40 %.
^{1}H NMR, 400 MHz, CDCl3 δ ppm: 8.19-8.16 (m, 1H), 8.11-8.08 (m, 1H), 8.00-7.99 (m, 1H), 3.58 (t, *J* = 6.7 Hz, 2H), 3.13 (s, 3H), 3.07 (t, *J* = 6.7 Hz, 3H).^{13}C NMR, 100 MHz, CDCl3 δ ppm: 162.7, 149.5, 139.4, 134.5, 129.6, 122.12, 122.07, 47.7, 35.4, 29.8. TOF-Ms (*m/z*) = 207.8 $[M+H]^+$.

^{1}H-NMR analysis of purified 8c

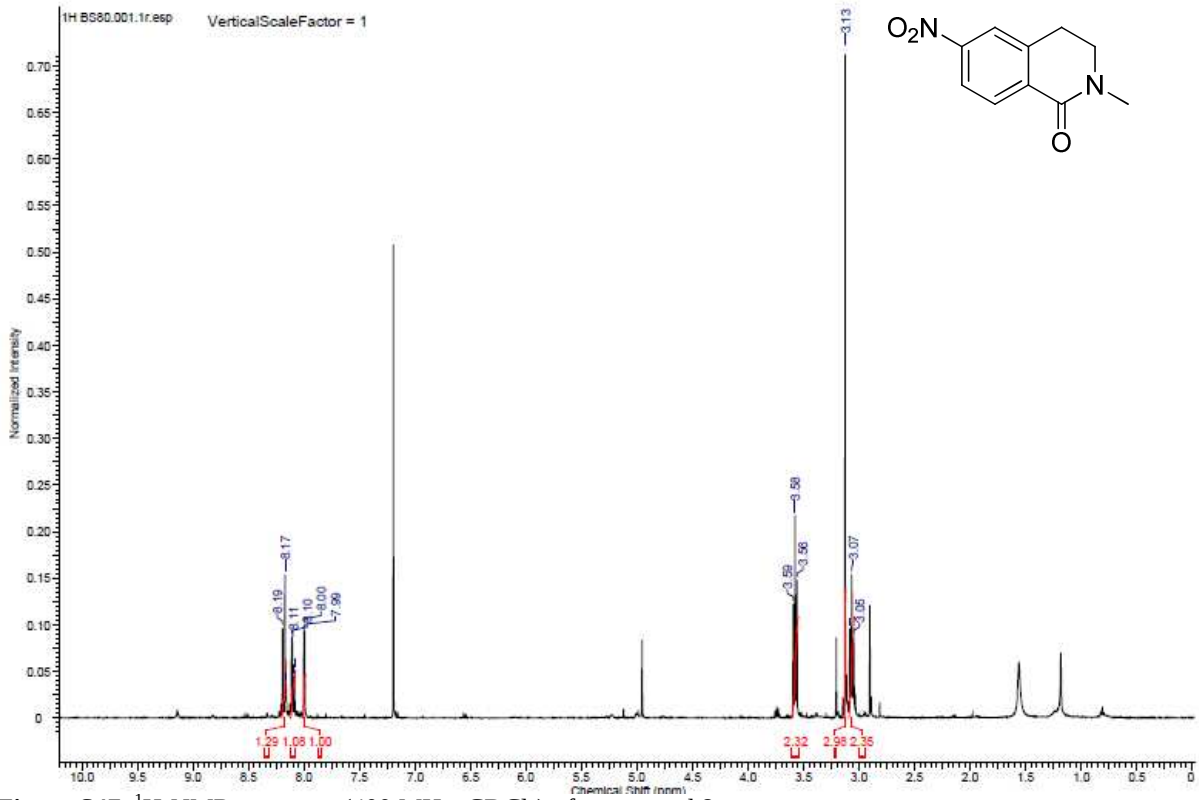


Figure S47. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **8c**.

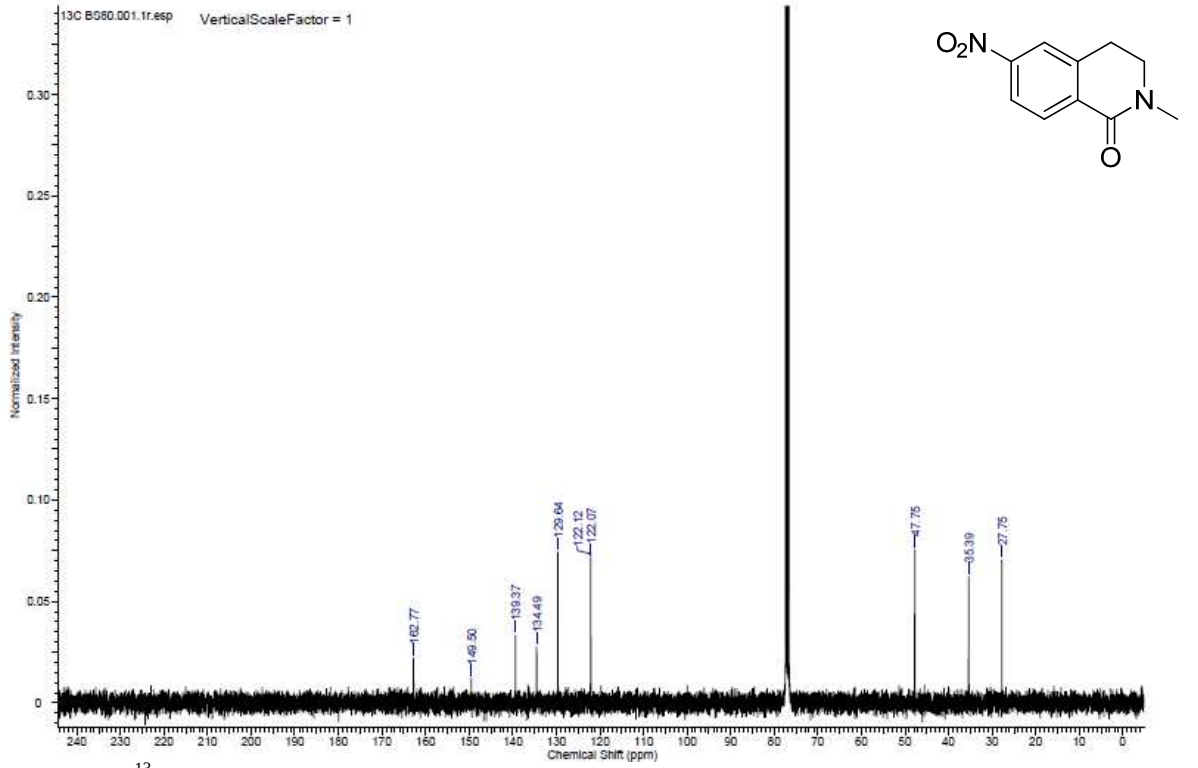


Figure S48. ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) of compound **8c**.

5.4.3. Analytical scale method - Bio-biocatalytic cascade reactions for synthesis of lactams 8 from cyclic amines 6 (Main paper Table 3, "Conditions B-D")

3,4-Dihydroisoquinolin-1(*2H*)-one (8a)

Conditions B - MAO-N D9, XDH E232V, DCPIP, $K_3Fe(CN)_6$

MAO-N D9 (30 µL of 10 mg/mL), THIQ (30 µL of 10 mM solution in KPi buffer, pH 7.6), DCPIP (30 µL of 1 mM), $K_3Fe(CN)_6$ (30 µL of 10 mM) and recombinant *R. capsulatus* XDH E232V (20 µL of 25 mg/mL in Tris buffer pH 7.6) was added to KPi buffer (100 mM, pH 7.6) (160 µL). The solution was shaken at 25 °C for 135 min. An equal volume of DCM was added, the reaction shaken and the DCM layer separated for HPLC analysis. Using the 3.3 conversion factor (vide infra) the conversion to lactam **8a** was 91 %.

HPLC analysis of a reaction according to conditions B

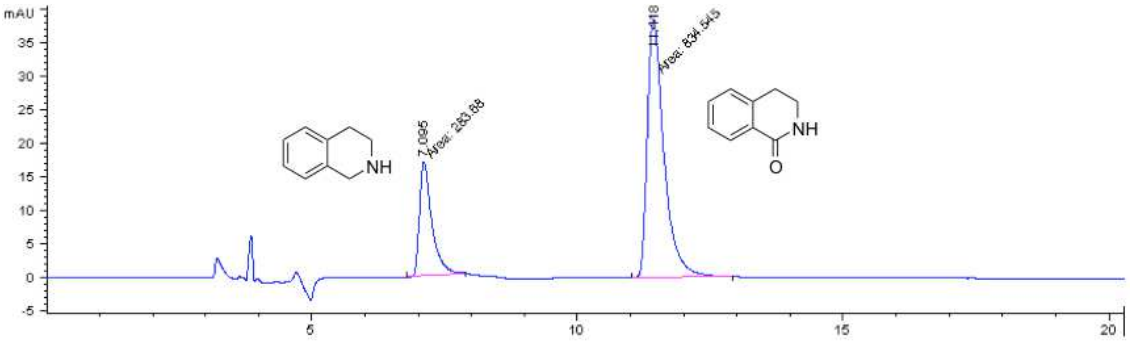


Figure S49. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

Peak	Retention time [min]	Area [%]
1 (amine 6a)	7.095	25.38
2 (lactam 8a)	11.418	74.62

After applying response factor conversion to lactam **8a** = 91 %.

Conditions C - MAO-N D9, XDH E232V, DCPIP, laccase

MAO-N D9 (30 µL of 11 mg/mL), THIQ (3 µL of 100mM solution in DMF), DCPIP (30 µL of 1 mM), *T. versicolor* laccase (30 µL of 6 mg/mL) and XDH E232V (10 µL of 33 mg/mL in Tris buffer pH 7.6) was added to KPi buffer (100 mM, pH7.6) (297 µL). The solution was shaken at 22 °C for 120 min. An equal volume of DCM was added, the reaction shaken and the DCM layer separated for HPLC analysis. Using the 3.3 conversion factor (vide infra) the conversion to lactam **8a** was 54 %.

HPLC analysis of a reaction according to condition C

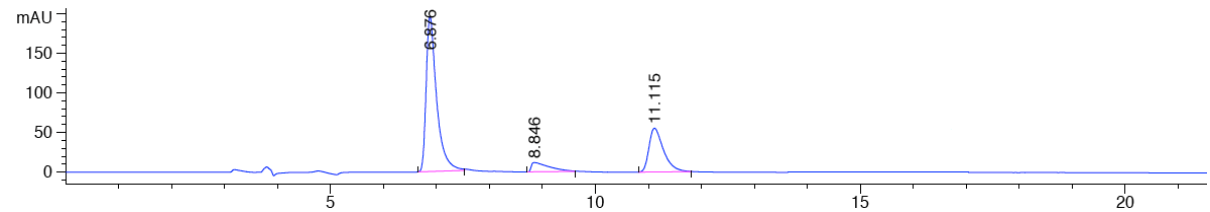


Figure S50. HPLC trace. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

Peak	Retention time [min]	Area [%]
1	6.876	66.92
2	8.846	6.88
3	11.115	26.2

After applying response factor conversion to lactam **8a** = 54 %.

Conditions D - MAO-N D9, *E. coli* XDH, catalase

MAO-N D9 (30 µL of 11 mg/mL), THIQ (3 µL of 1 M solution in DMF), catalase (100 µL of 1 mg/mL), *E. coli* XDH (100 µL, 1.1 mg/ml) and KPi buffer (67 µL, 50 mM, pH 7.6) were shaken for 7 h at 37 °C. The reaction was extracted with an equal amount of dichloromethane and analysed via HPLC.

HPLC analysis of a reaction according to condition D

Figure S51. HPLC trace. HPLC conditions: CHIRALCEL® OJ-H column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

Peak	Retention time [min]	Area [%]
1	3.014	2.81
2	5.179	2.86
3	8.832	94.33

CHIRALCEL® OJ-H column the response factor was 1:1 which is a conversion = 94%.

HPLC analysis of an authentic standard of tetrahydroisoquinoline (6a)

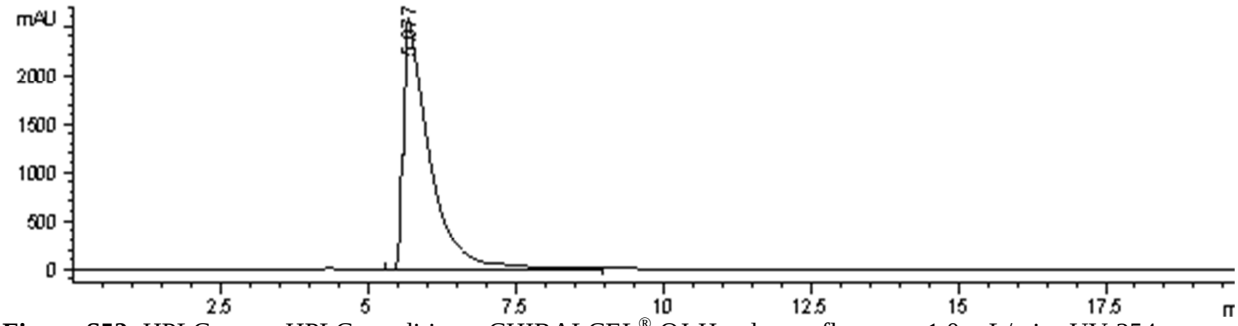


Figure S52. HPLC trace. HPLC conditions: CHIRALCEL® OJ-H column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

HPLC analysis of an authentic standard of 3,4-dihydroisoquinolin-1(*2H*)-one (8a)

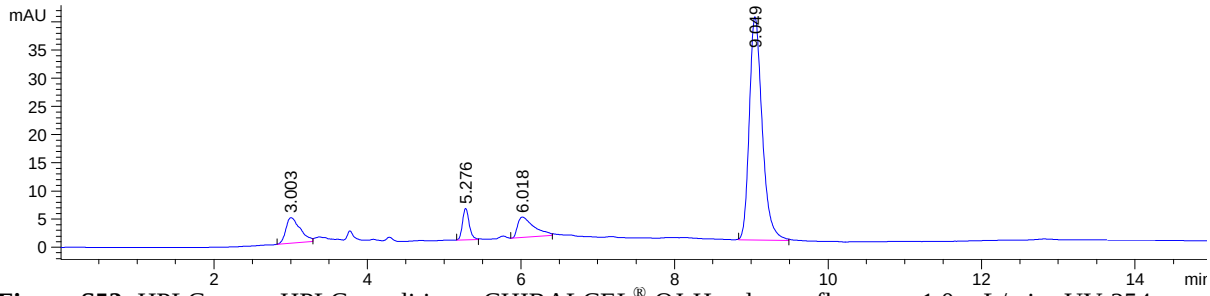


Figure S53. HPLC trace. HPLC conditions: CHIRALCEL® OJ-H column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

2-Methyl-3,4-dihydroisoquinolin-1(*2H*)-one (8c)

Conditions D

MAO-N D9 (30 µL), catalase (100 µL, 2 mg/mL of buffer pH7.6), N-Me THIQ (3 µL, 1 M in DMF) and KPi buffer (67 µL, 50 mM, pH 7.6) were added to an Eppendorf tube. The reaction was run at two pHs, pH 7.6 and pH~8.0. For the later, the solution was basified to pH 8 using dibasic potassium phosphate buffer (5 µL, 50 mM) and *E. coli* XDH (100 µL, 1.1 mg/ml) was added. After incubating for 16 h at 37 ºC with shaking, a spatula of ammonia-borane complex was added and shaken for a further 3 h prior to extraction using DCM and centrifugation

HPLC analysis of an authentic standard of 2-methyl-1,2,3,4-tetrahydroisoquinoline 6c

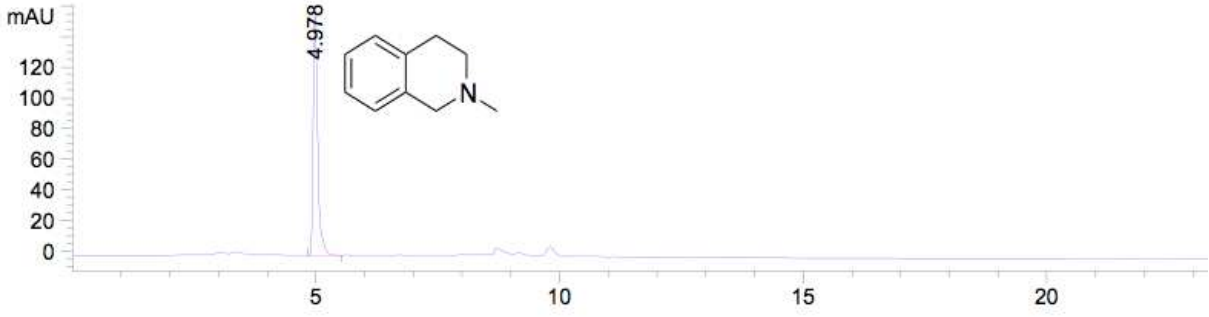


Figure S54. HPLC trace. HPLC conditions: CHIRALCEL® OJ-H column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

HPLC analysis of an authentic standard of 2-methyl-3,4-dihydroisoquinolin-1(*2H*)-one 8c

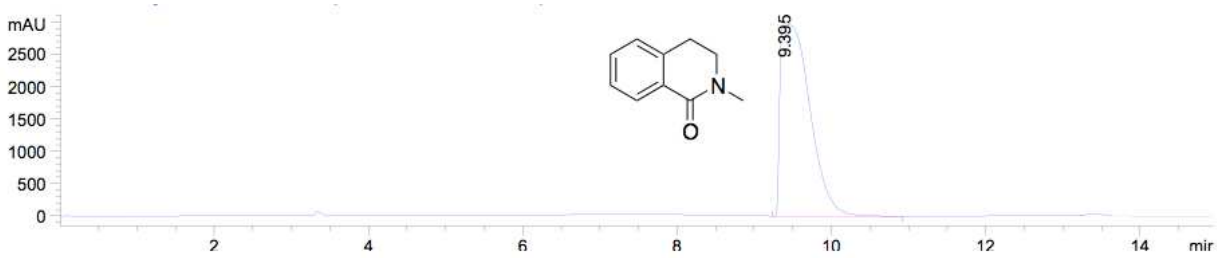


Figure S55. HPLC trace. HPLC conditions: CHIRALCEL® OJ-H column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

HPLC analysis of a reaction according to conditions D MAO-N D9/*E. coli* XDH cascade (10 mM, 50 mM KPi, pH ~8) to give 2-methyl-3,4-dihydroisoquinolin-1(*2H*)-one 8c

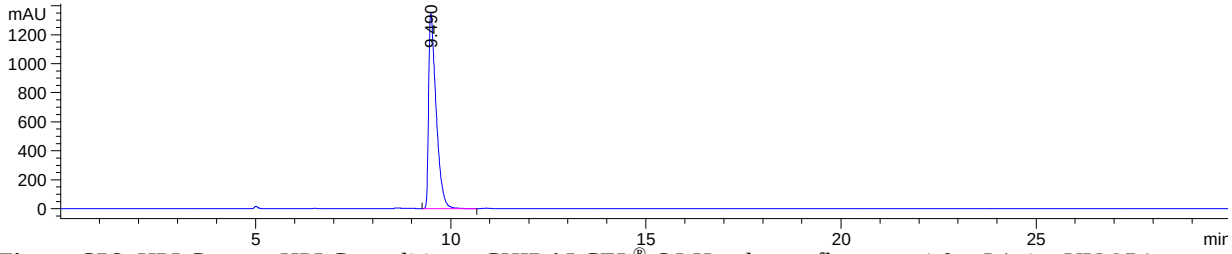


Figure S56. HPLC trace. HPLC conditions: CHIRALCEL® OJ-H column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

HPLC analysis of a reaction according to conditions D MAO-N D9/ *E. coli* XDH cascade (10 mM, 50 mM KPi, pH 7.6) giving imcomplete conversion to 8c

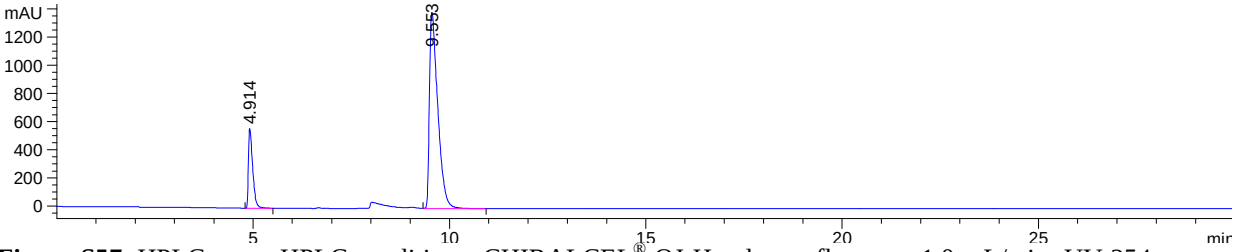


Figure S57. HPLC trace. HPLC conditions: CHIRALCEL® OJ-H column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

Peak	Retention time [min]	Area [%]
1(amine 6c)	4.914	17.15
2 (lactam 8c)	9.553	82.84

After applying HPLC response factor (see Fig. S60), the conversion to lactam **8c** in Fig. S57 is 36 %.

5.5 Synthesis of 2-methyl-3,4-dihydroisoquinolin-1(*2H*)-one 8c[16]

3,4-Dihydroisoquinoline (100 mg, 0.76 mmol) was dissolved in acetone (10 mL) and an excess of iodomethane (60 µL) was added. The mixture was left to stir overnight at room temperature, after which the solvent was evaporated *in vacuo* to give 2-methyl-3,4-dihydroisoquinolinium iodide a yellow solid (157 mg, 75 %). NMR ^{1}H (400 MHz, $CDCl_3$) δ 10.03 (1 H, s, C*H*), 8.05-8.04 (1 H, d, *J* = 4 Hz, aromatic), 7.72-7.68 (1 H, t, *J* = 8 Hz, aromatic), 7.47-7.43 (1 H, t, *J* = 8 Hz, aromatic), 7.37-7.35 (1 H, d, *J* = 8 Hz, aromatic), 4.14-4.10 (2 H, t, *J* = 8 Hz, CH_2), 4.02 (3 H, s, CH_3), 3.42-3.38 (2 H, t, *J* = 8 Hz, CH_2). ^{13}C (100 MHz, CDCl3) δ 166.59, 137.98, 135.59, 134.40, 128.62, 128.27, 124.52, 50.97, 48.72, 25.37.*m/z* 148 ([M+H]$^+$, 30). 2-Methyl-3,4-dihydroisoquinolinium iodide (100 mg, 0.4 mmol) was dissolved in DMSO (3.7 mL), to which concentrated hydrochloric acid (0.53 mL) was added. The solution was left to stir for an hour at room temperature and was worked up with distilled water and diethyl ether before being purified by column chromatography (2 % methanol/dichloromethane) to give a residue (14.8 mg, 25 %). NMR ^{1}H (400 MHz, $CDCl_3$) δ 8.09-7.16 (4H, m, Ar-*H*), 3.58-3.55 (4H, dt, *J* = 4, 8 Hz, CH_2), 3.15 (3H, s, CH_3). ^{13}C (100 MHz, CDCl3) δ 164.81, 137.94, 131.50, 129.38, 128.14, 127.00, 126.84, 48.14, 35.17, 27.92 *m/z* 162 ([M+H]$^+$, 100).

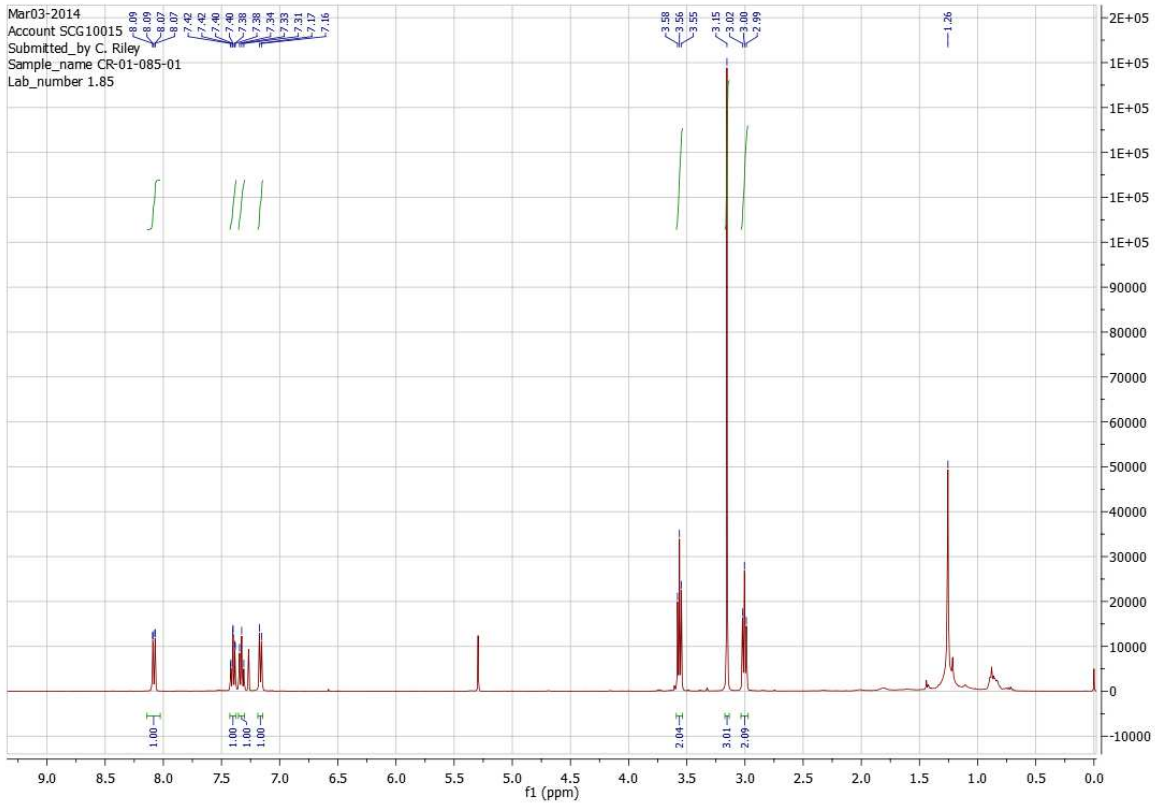


Figure S58. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **8c**.

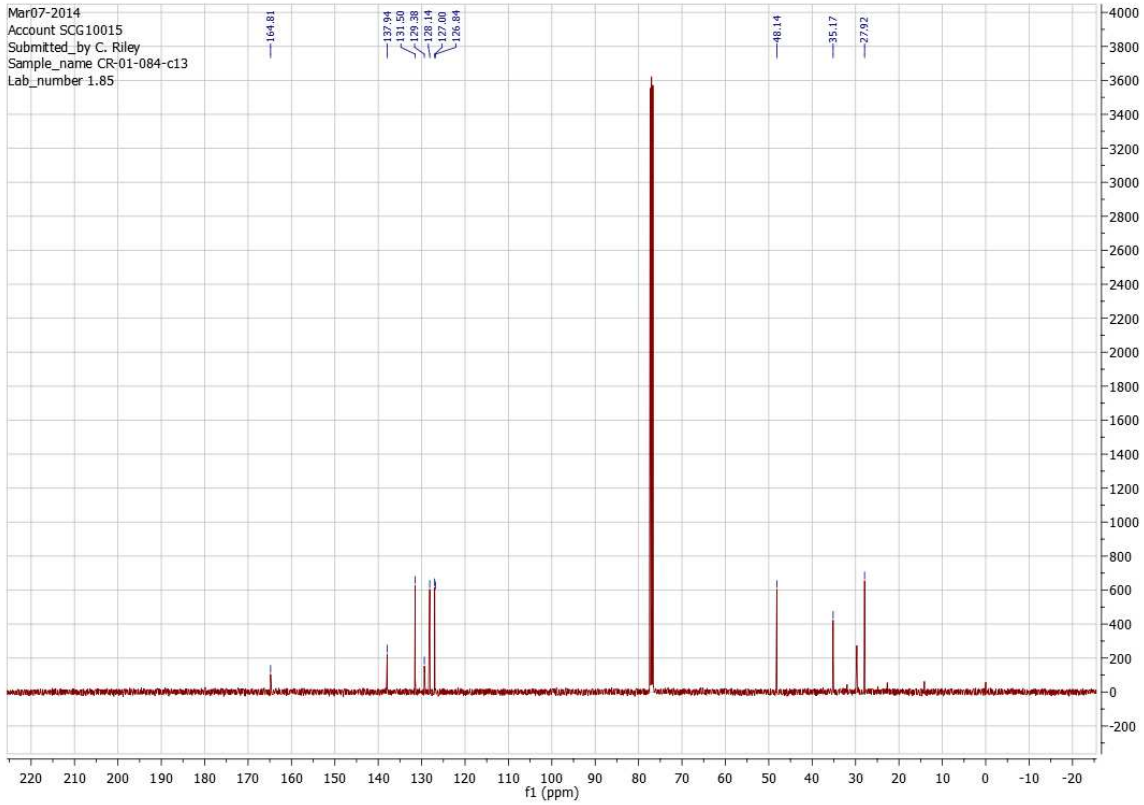
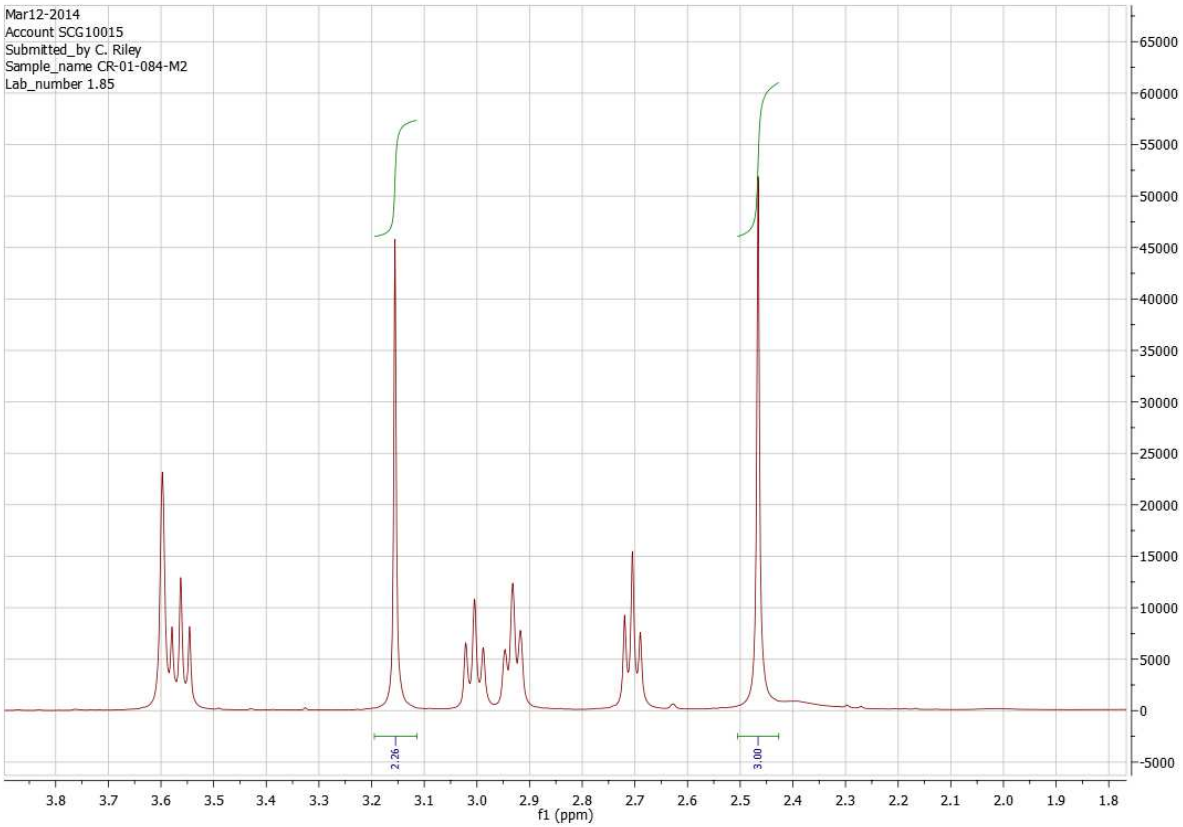
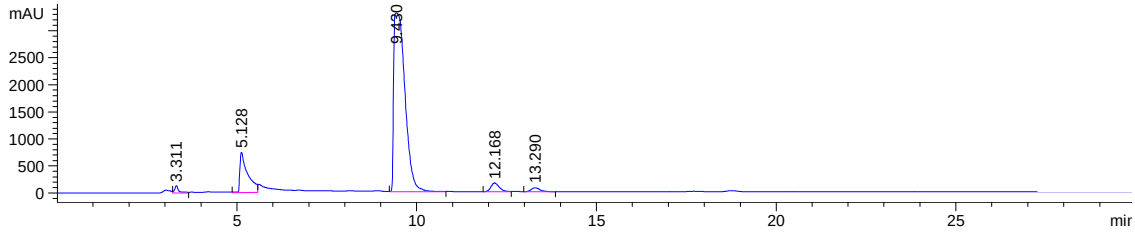


Figure S59. ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) of compound **8c**.





Peak	Retention time [min]	Area [%]
1(amine 6c)	5.128	12.78
2 (lactam 8c)	9.430	81.83

Response factor amine = lactam/8.5

Figure S60. Calibration of HPLC response for *ca*. 1:1 of **6c**:**8c** (-OCH_3 singlets) by NMR. HPLC conditions: CHIRALCEL® OJ-H column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

5.6. Screening of electron acceptors for xanthine dehydrogenases for conversion of 7a to 8a

Table S8: Screening of electron acceptors for *R. capsulatus* XDH E232V and use of *E. coli* xanthine deydrogenase for conversion of **7a** to **8a**[a] based on HPLC peak areas at λ= 254 nm.

Entry	Conditions	Time [min]	Conversion of 7a to 8a [%]
1	1 eq. DCPIP	90	22
2	15 mol% DCPIP/aeration	90	28
3	15 mol% DCPIP, SOD, aeration	90	24
4	PMS	120	0
5	10 mol% PMS, 10mol% DCPIP	120	36
6	1 eq. $K_3Fe(CN)_6$	120	21
7	**10 mol% DCPIP, 1eq. $K_3Fe(CN)_6$,**	**45**	**65**
8	**10 mol% DCPIP, *T. versicolor* laccase**	**240**	**67**
9	***E. coli* XDH**	**120**	**100**[b]

[a] Conditions: DHIQ **7a** (1 mM) in buffer (100 mM, KPi, pH 7.6), XDH E232V (20 µL, 112 µM), reaction volume 200 µL; [b] NaPi buffer (50 mM, pH 7.4), 2 h, 20 °C. HPLC conditions: CHIRALPAK® IA column; flow rate 1.0 mL/min; UV 254 nm; eluent= hexane/iPrOH 90:10.

6. References

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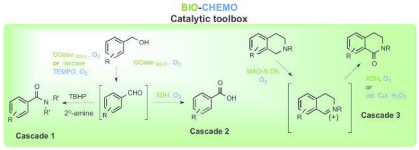
Table of contents text

Oxygen-dependent enzymes and chemocatalysts were combined to provide one-pot tandem cascade syntheses of amides and carboxylic acids.



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