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Stereoselective Friedel–Crafts alkylation catalyzed by squalene hopene cyclases

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ABSTRACT

In organic synthesis the Friedel–Crafts alkylation is of eminent importance, as it is a key reaction in many synthetic routes. A general access to enzymatic Friedel–Crafts alkylations would be very beneficial due to the high selectivity of biocatalysts. We used designed polyprenyl phenyl ethers to specifically address this reaction by using squalene hopene cyclases as catalysts. Polycyclic products with aromatic rings constituting important biological active compounds were obtained. Our results demonstrate that squalene hopene cyclases can be utilized for Friedel–Crafts alkylations and reveal the potential of these enzymes for chiral Brønsted acid catalysis.

1. Introduction

The formation of C–C bonds to generate carbon scaffolds of organic molecules is often the key step in synthesis. Due to their frequently high chemo-, regio-, and stereoselectivity, enzymes are promising catalysts for C–C bond formations.[1] However, the number of C–C bond forming reactions applied in biocatalysis is limited. This is mainly due to the poor availability of enzymes for such reactions. Beside the well-established aldol reaction (aldolases), benzoin-like condensation (thiamine diphosphate-dependent enzymes), and stereoselective HCN addition to carbonyls (hydroxynitrile lyases), several novel C–C bond formations have recently been applied in biocatalysis.[2] These include the catalysis of oxidative C–C bond formation,[3] Pictet–Spengler[4] as well as Stetter[5] reactions, the enantioselective ring opening of epoxides,[6] and the alkylation of enolates with crotonases.[7] The Friedel–Crafts alkylation is an important reaction in organic chemistry, however, a general approach to address this reaction in biocatalysis is somewhat unexplored. Friedel–Crafts alkylation involves the generation of an electrophile, which is then attacked by an aromatic π-system. The recently published enzymatic approach utilizing methyltransferases for methylation, allylation, propargylation, and benzylation of aromatic compounds is a magnificent progress.[8] However, these transferases depend on a challenging synthetic cofactor for alkyl group activation. Another class of enzymes, which is known to catalyze Friedel–Crafts alkylations are prenyltransferases.[9] Yet, their alkyl group activation is also challenging since it is based on diphosphate as a leaving group. In contrast to these approaches, a simple way of activation is the protonation of different functional groups like alkenes, carbonyls or epoxides. Since there exist enzymes, which use protonation for catalysis, we hypothesized that they can be utilized for Friedel–Crafts alkylation. Such an approach is highly interesting, due to the chiral environment of the active site, which leads to selective C–C bond formation between the electrophile and the aromatic moiety.

One of the most prominent examples of enzymes, which use a relative strong Brønsted acid, are squalene hopene cyclases (SHCs).[10–17] SHCs catalyze the regio- and stereoselective polycyclization of squalene **1** to form a 6/6/6/6/5-fused pentacyclic ring system with nine stereocenters (Fig. 1).

The reaction is initiated by protonation of the terminal, non-activated double bond of squalene, proceeds through carbocationic intermediates and is terminated by deprotonation or reaction with a water molecule. Initial studies using a SHC from *Alicyclobacillus acidocaldarius* (*Aac*SHC) revealed a high flexibility concerning their substrate scope. Besides truncated C_{15}–C_{25} squalene analogs,[18] elongated C_{35} polyprenoids[19,20] could also be converted in a selective manner. Furthermore, it has been shown that alternative nucleophiles, like hydroxyl groups, are accepted by these enzymes. Representative examples include the conversion of homofarnesol **5** to the important fragrance ambroxan **6**, as well as the conversion of **7** to products, which contain the scaffold of a class of cyclic meroterpenoids **8** (Fig. 2).[21,22]

In this context, we have recently published a series of reactions using a novel SHC from *Zymomonas mobilis* (*Zmo*SHC1).[23,24] Remarkably, this cyclase exhibits a higher activity for the truncated

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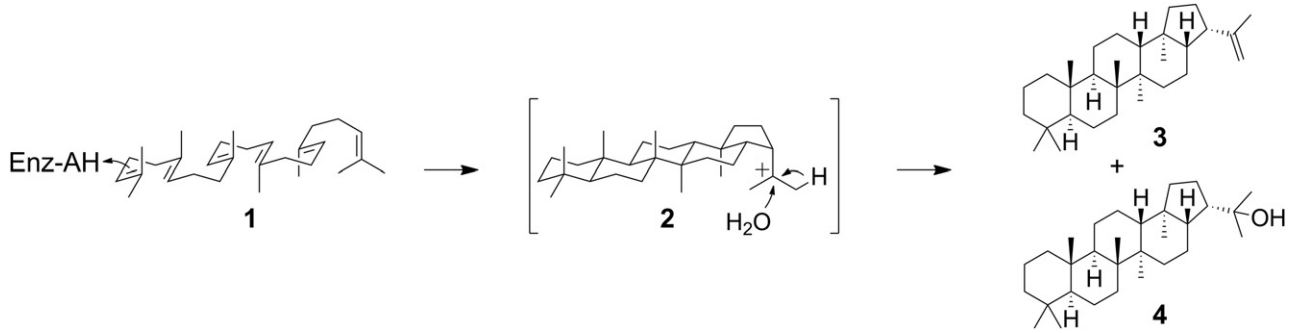

Fig. 1. Enzymatic conversion of squalene. The reaction is initiated via a Brønsted acid, which protonates the non-activated terminal C=C bond of squalene **1** whereupon a highly reactive carbocation is formed. The chiral environment of the active site enables the stereospecific attack of an adjacent C=C bond. The conversion proceeds via the final carbocation intermediate **2**. Deprotonation or reaction with water leads to products hopene **3** and hopanol **4**.

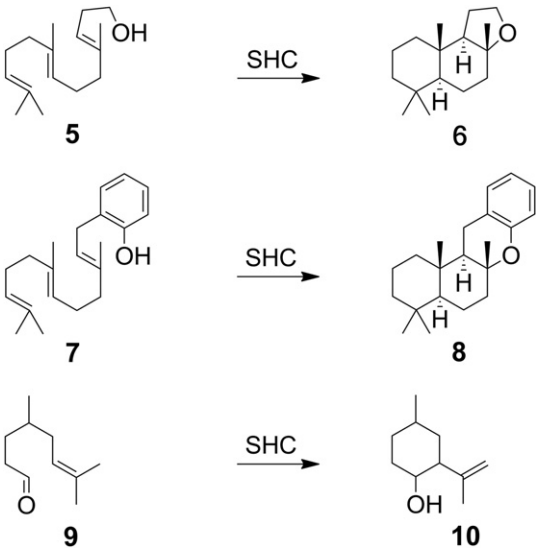

Fig. 2. Conversions of alternative substrates with SHCs.

substrate homofarnesol **5** compared to the well-known *Aac*SHC. Furthermore, *Zmo*SHC1 shows a significant acceleration of the selective conversion of citronellal **9** to compound **10**, compared to its buffer catalyzed background reaction. These results indicate that in addition to C=C bond protonation, the catalytic machinery of SHCs can be further utilized to activate C=O bonds. This catalytic flexibility is in agreement with the much higher basicity of C=O compared to C=C bonds. As a consequence, we assume that SHCs can be used in a general approach for Brønsted acid catalysis in a chiral environment. To further verify this hypothesis, we rationally designed and synthesized different substrates to address the Friedel–Crafts alkylation. We used truncated squalene analogs, which were linked with sterically challenging benzene moieties. These compounds were chosen, due to their similarity with the natural substrate squalene. We show that these compounds were accepted by *Zmo*SHC1 and demonstrate that the stereoselective Friedel–Crafts alkylation is part of the cyclization reaction. Important scaffolds of a variety of biological active compounds were formed for which a classical chemical synthesis is challenging.

2. Results and discussion

In the present paper, we extend the reaction diversity of SHCs to the Friedel–Crafts alkylation via cyclization of the different polyprenyl phenyl ethers **11**–**14**. Fig. 3 summarizes the results of these alkylation reactions with *Zmo*SHC1. Four different polyprenyl phenyl ethers, namely prenyl phenyl ether **11**, geranyl phenyl ether **12**, farnesyl phenyl ether **13**, and geranylgeranyl phenyl ether **14** were used as model substrates. These compounds were synthesized via Williamson ether synthesis to specifically address Friedel–Crafts alkylation. Biotransformations of these compounds with semi-purified *Zmo*SHC1 followed by GC analysis revealed the formation of compounds corresponding to cyclization products for most of the tested substrates. Control experiments performed in buffer alone and preparations obtained from *Escherichia coli* (*E. coli*) cells expressing empty pET-22b(+) vector did not exhibit any new peaks. To determine the structure of these products, we conducted conversions in the range of 150–300 mg. After extraction, detergent molecules were removed by passing through a small silica column and the different products were separated using preparative HPLC. The structures of the isolated compounds were solved by ^{1}H and ^{13}C NMR spectroscopy, including DEPT, $^{1}H-^{1}H$ COSY, HSQC, HMBC, and NOESY as well as IR spectra (see Supplementary data for details). The molecular formulas were determined by high-resolution EIMS (HREIMS). For the sake of clarity, herein, we only discuss the structures of the main products. Structures of side compounds can be found in Supplementary data. Compounds **15** and **17** were identified as main products for the conversion of **12** and **13** (Figs 3 and 4).

These are promising results, since they demonstrate that the Friedel–Crafts alkylation is catalyzed by SHCs. Moreover, the chiral environment of the active site directed the cyclization, where up to four stereocenters were formed. Such polycyclic compounds containing aromatic moieties are scaffolds of a variety of important biological active compounds like (+)-nimbidiol, (+)-totarol, incanone, and others.[25–29] The determined yields were between 1.3 and 5.4%, respectively, after 20 h reaction time (Fig. 3). Interestingly, these low conversions are in the same range as for the natural substrate squalene under these in vitro conditions (data not shown).

The smallest ether **11** was not transformed into the corresponding polycyclic product. However, this is in accordance with other studies, which suggested that SHCs are not, or just barely, active toward molecules smaller than C15 atoms.[18,21] The reaction of *Zmo*SHC1 with **12** led to the formation of a single product, while the transformation of **13** generated three (two main and one side product) and the reaction of **14** afforded six products (two main and four side products). The generation of several products can be attributed to the fact that the carbocation intermediates are highly reactive species. Even for the natural substrate squalene **1** several side products have been identified.[30] A high amount of a single product implies exclusion of alternative reaction pathways.[31] Hence, the number of side products should correspond to the number of cyclization steps. Therefore, it is not surprising that we see multiple products formed from a single non-natural substrate,

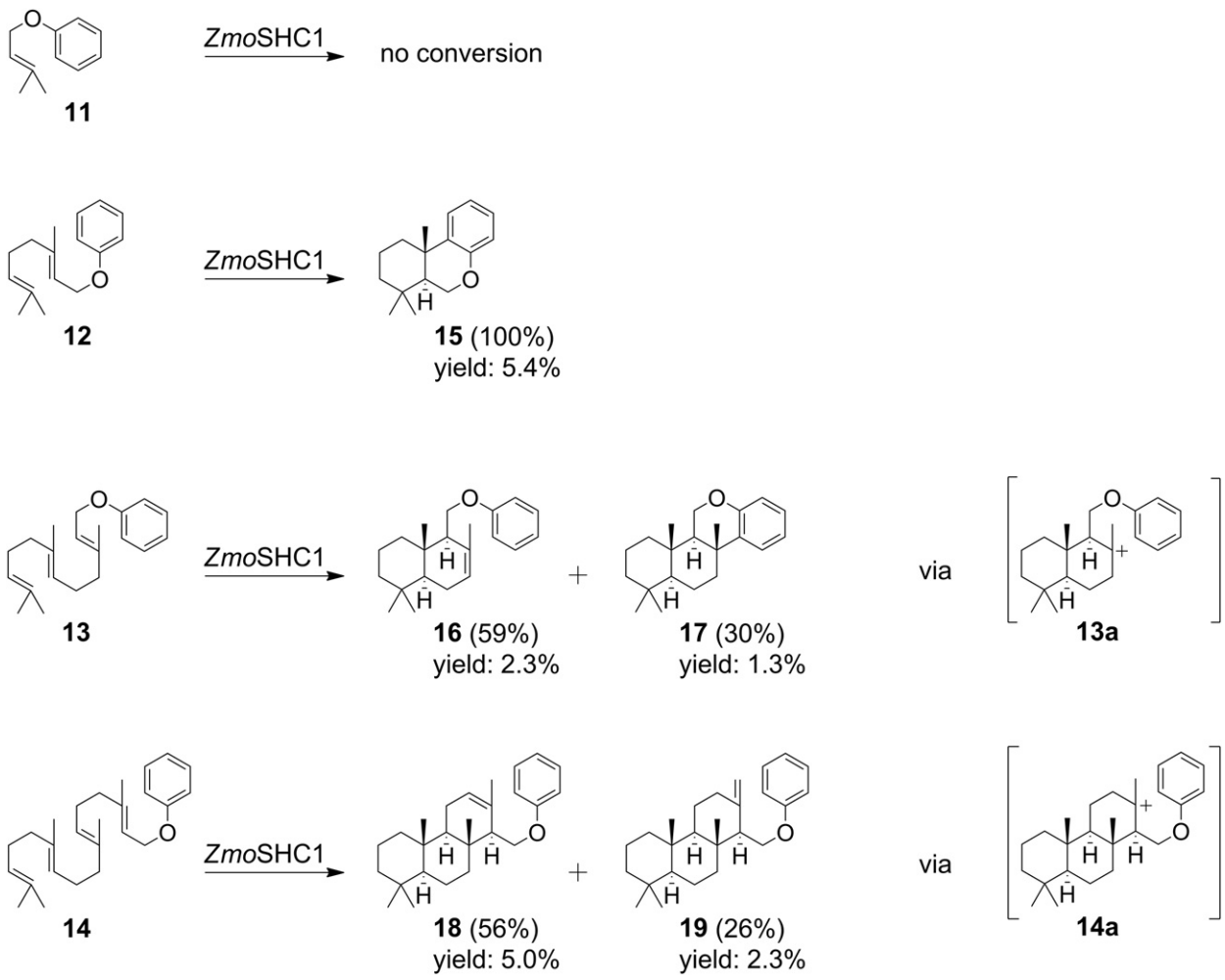

Fig. 3. Conversions of polyprenyl phenyl ethers catalyzed by *Zmo*SHC1 after 20 h reaction time. The values in brackets describe the percentage of the products formed. Differences to 100% correspond to the amount of side products.

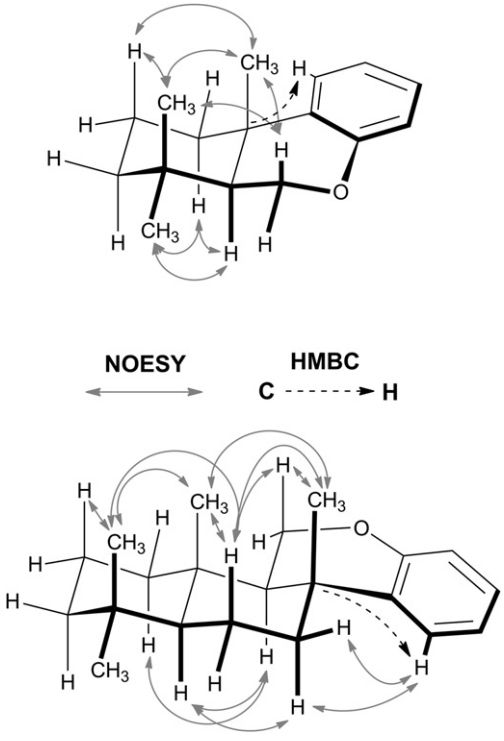

Fig. 4. Important NOE and HMBC signals for **15** and **17**.

especially for the larger compounds. While the conversion of **12** yielded a single product, several compounds were generated for **13** and **14**. The cyclization of **13** resulted in an alkene **16** as second major compound along with the Friedel–Crafts alkylation product **17**. This alkene **16** most likely originated from the carbocationic intermediate **13a**. This indicates that the attack of the aromatic moiety to **13a** is partially hindered. Since the aromatic moiety cannot attack within a specific time, a nearby base (e.g., a water molecule) deprotonates **13a** in a regioselective manner. This final attack (the Friedel–Crafts alkylation) was not observed for substrate **14**. The main products **18** and **19** most likely originated from the deprotonation of **14a**. We further isolated three additional side products for this reaction. However, we could not identify the Friedel–Crafts alkylation product for this substrate. The relative high number of cyclization products for **14** reveals the existence of a variety of alternative reaction pathways, which finally compete with the Friedel–Crafts alkylation.

Recent studies by Tanaka et al. demonstrated that *Aac*SHC was not applicable for the C–C bond formation of substrates comprising aromatic moieties.[32,33] However, in contrast to our study, only large substrates with four isoprene units similar to **14** were used. We used *Aac*SHC to determine the ability of a second SHC for Friedel–Crafts alkylation with our set of substrates. Interestingly, the same substrate acceptance and similar product patterns were found. The most obvious difference was the presence of one further product for the cyclization of **12** and **13** with *Aac*SHC (see Supplementary data for

details). A related oxidosqualene cyclase from *Arabidopsis thaliana* has been shown to catalyze Friedel–Crafts alkylation as well.[34] The aromatic alkylation product of this reaction has been observed as a minor product. These results, together with our findings, demonstrate that this class of enzymes can be in general harnessed for Friedel–Crafts alkylations. This is of great interest, especially in combination with Brønsted acid activation of different functional groups. Although the C–C bond formation of substrates comprising an aromatic moiety was not described for SHCs before, this activity is not surprising due to the intrinsic high chemical reactivity of carbocations. The surprise lies in the fact that substrates like **12** can bind to the active site without putting water molecules into a reactive position in order to quench carbocationic intermediates. Hence, the enzyme active site excludes water molecules mimicking an inert solvent even for such sterically challenging substrates.

In conclusion, we extended the reaction diversity of SHCs toward the Friedel–Crafts alkylation using challenging aromatic substrates. Several polyprenyl phenyl ethers were synthesized and studied in biotransformations using our set of SHCs. Interestingly, both *Zmo*SHC1 and *Aac*SHC showed a similar product profile. For two of four substrates the corresponding Friedel–Crafts alkylation product was obtained and characterized. This demonstrates that the polycyclization machinery of these enzymes can be utilized for 'novel' C–C bond forming reactions. These results are of great interest, particularly given that the number of enzymes for C–C bond formation is limited.

3. Experiments

3.1. General procedures

All chemicals and solvents were purchased from Sigma–Aldrich (Steinheim, Germany), Alfa Aesar (Karlsruhe, Germany) or Carl-Roth (Karlsruhe, Germany). The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.15 and 125.76 MHz, respectively. All spectra were recorded at room temperature in $CDCl_3$. Chemical shifts are expressed in parts per million (ppm, δ) and referenced to tetramethylsilane (TMS, δ=0 ppm). The correct assignment of the chemical shifts was confirmed by application of two-dimensional correlation measurements, including correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond coherence (HMBC), and nuclear Overhauser enhancement spectroscopy (NOESY). IR spectra were measured on a Bruker Vector 22 FT-IR spectrometer in an ATR mode. Mass spectra were measured using electron impact ionization on a Finnigan MAT 95. GC analyses were performed on a Shimadzu GC-2010 equipped with a flame ionization detector using a DB-5 capillary column (Agilent, 0.25 mm×30 m) and H_2 as carrier gas (linear velocity 30 cm/s). Product yields were quantified using 1-decanol as internal standard. The purified main products were used for external calibration via GC (triplicates). The products of the enzymatic cyclizations were dissolved in acetonitrile and separated via reversed phase HPLC on an Agilent HPLC 1200 Series equipped with a diode array detector using a Reprosil 100-5 C18 column (5 μm, 250×20 mm column, Trentec Analysentechnik, Rutesheim, Germany, acetonitrile/water gradient). Fractions were manually collected according to absorption at 200 nm and were concentrated in vacuo.

3.2. Preparation of geranylgeranyl bromide

The preparation of geranylgeranyl bromide followed a procedure previously described.[35] Under a nitrogen atmosphere, phosphorus tribromide (40 μL, 0.43 mmol, 0.65 equiv) was added to a solution of geranylgeraniol (192 mg, 0.66 mmol, 1.0 equiv) in 3 mL THF at 0 °C and stirred for 1 h. Saturated $NaHCO_3$ (5 mL) was added and the mixture was extracted with *n*-hexane (3×15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The geranylgeranyl bromide was used without further purification or characterization.

3.3. General procedure for the preparation of polyprenyl aryl ethers 11–14

Polyprenyl aryl ethers were prepared by Williamson ether synthesis altering a procedure previously described.[36] Under a nitrogen atmosphere in dry THF dissolved phenol (1.0 equiv) was added dropwise to a suspension of sodium hydride (1.1 equiv) in dry THF and stirred for 2 h. Polyprenyl halide (1.0 equiv, for **11**–**13** chloride, for **14** bromide) dissolved in DMSO (ca. 15% solution) was added and the mixture was stirred for 22 h. The mixture was poured onto ice-water and extracted with diethyl ether. The combined organic layers were dried over Na_2SO_4, concentrated in vacuo and purified by flash column chromatography on silica gel 60 (0.040–0.063 mm) to give polyprenyl aryl ethers **11**–**14** (45–75%). Analytical data of the ethers are given below.

*3.3.1. Prenyl phenyl ether **11**.* Pale yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.74 (s, 3H, CH_3), 1.80 (s, 3H, CH_3), 4.51 (d, J=7 Hz, 2H, O–CH_2), 5.46–5.55 (m, 1H, =CH), 6.90–6.97 (m, 3H, $Ar_{o/p}$–H), 7.23–7.33 (m, 2H, Ar_m–H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 18.2, 25.9, 64.6, 114.6 (2C), 119.7, 120.6, 129.4 (2C), 138.2, 158.9.[37]

*3.3.2. Geranyl phenyl ether **12**.* Pale yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.60 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 2.02–2.20 (m, 4H, 2×CH_2), 4.54 (d, J=6.6 Hz, 2H, O–CH_2), 5.04–5.15 (m, 1H, =CH), 5.44–5.55 (m, 1H, =CH); 6.87–6.99 (m, 3H, $Ar_{o/p}$–H), 7.22–7.34 (m, 2H, Ar_m–H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 16.7, 17.7, 25.7, 26.3, 39.6, 64.8, 114.7 (2C), 119.6, 120.6, 123.8, 129.4 (2C), 131.8, 141.1, 158.9.[36]

*3.3.3. Farnesyl phenyl ether **13**.* Pale yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.53 (s, 6H, 2×CH_3), 1.61 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.83–2.14 (m, 8H, 4×CH_2), 4.46 (d, J=6.5 Hz, 2H, O–CH_2), 4.95–5.09 (m, 2H, 2×=CH), 5.37–5.48 (m, 1H, =CH), 6.79–6.91 (m, 3H, $Ar_{o/p}$–H), 7.14–7.26 (m, 2H, Ar_m–H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 16.0, 16.7, 17.7, 25.7, 26.2, 26.7, 39.6, 39.7, 64.7, 114.7 (2C), 119.6, 120.6, 123.7, 124.3, 129.4, 131.4 (2C), 135.4, 141.2, 158.9.[38]

*3.3.4. Geranylgeranyl phenyl ether **14**.* Pale yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.58–1.62 (m, 9H, 3×CH_3), 1.68 (s, 3H, CH_3), 1.74 (s, 3H, CH_3), 1.94–2.01 (m, 4H, 2×CH_2), 2.02–2.18 (m, 8H, 4×CH_2), 4.53 (d, J=6.5 Hz, 2H, O–CH_2), 5.06–5.14 (m, 3H, 3×=CH), 5.47–5.52 (m, 1H, =CH), 6.88–6.96 (m, 3H, $Ar_{o/p}$–H), 7.24–7.30 (m, 2H, Ar_m–H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.02, 16.04, 16.7, 17.7, 25.7, 26.2, 26.6, 26.8, 39.6, 39.70, 39.73, 39.74, 114.7 (2C), 119.6, 120.6, 123.7, 124.2, 124.4, 129.4 (2C), 131.3, 135.0, 135.5, 141.1, 158.9.

3.4. Vector construction

Genes encoding for cyclases SHC1 from *Z. mobilis* (*Zmo*SHC1, NCBI no. YP_163283.1) and SHC from *A. acidocaldarius* (*Aac*SHC, NCBI no. BAA25185.1) were gratefully obtained from Michael Breuer (BASF SE, Ludwigshafen, Germany). The genes were amplified via PCR and cloned into a pET-22b(+)-vector system (Merck, Darmstadt, Germany). The plasmids were transformed into *Escherichia coli* DH5α and successful cloning was verified by sequencing (GATC Biotech, Konstanz, Germany).

3.5. Expression conditions

For protein expression each vector construct was transformed in *E. coli* BL21(DE3) (Agilent, Böblingen, Germany) and 100 μg/mL ampicillin was added to all growth media. A glycerol stock was used to inoculate a 5 mL LB medium preculture, which was incubated for approximately 8 h at 37 °C and 180 rpm. This preculture was used to inoculate 50 mL LB medium cultures in 250 mL Erlenmeyer flasks, which were incubated at same conditions (180 rpm, 37 °C) over night. These cultures were used to inoculate 500 mL TB medium in 2 L Erlenmeyer flasks (with baffles) with an optical density of OD_{600}=0.05 and were incubated as described above (180 rpm, 37 °C). When an optical density of OD_{600}=0.5–0.7 was reached, the SHC expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) with a final concentration of 1 mM. Expression was carried out for 4 h at 30 °C and 180 rpm. The cells were harvested by centrifugation (16,900×*g*, 14 min, 4 °C), frozen in liquid nitrogen and stored at −80 °C.

3.6. Enzyme purification

All enzyme purification steps were carried out on ice or at 4 °C. For enzyme isolation, the frozen cells were thawed and resuspended in lysis buffer (3 mL per gram cell pellet, 200 mM citrate buffer, pH 6.0, 1 mM EDTA), phenylmethanesulfonylfluoride (PMSF) with a final concentration of 1 mM and a pinch of DNaseI were added. The cells were disrupted by passing the suspension twice through a high-pressure homogenizer (EmulsiFlex C5, Avestin, 100 MPa). The broken cells were centrifuged (38,700×*g*, 45 min, 4 °C) and the pellet was resuspended in solubilization buffer containing 1% CHAPS as detergent (1 mL per gram pellet, 60 mM citrate buffer, pH 6.0). The mixture was incubated for 1 h at 4 °C and centrifuged again (38,700×*g*, 45 min, 4 °C). The *Aac*SHC containing supernatant was heated for 15 min to 60 °C to precipitate unstable *E. coli* proteins. The *Zmo*SHC1 containing supernatant was incubated at 30 °C to precipitate unstable *E. coli* proteins. The solutions were centrifuged (38,700×*g*, 45 min, 4 °C) again and the supernatant was used for biotransformations. SDS-PAGE was used for controlling successful expression and enzyme isolation (see Supplementary data). The protein concentration was determined according to the Bradford Ultra (Expedeon) method using bovine serum albumin (BSA)/CHAPS solution as standard.

3.7. General procedure for the enzymatic cyclization of substrates 11–14

For the biotransformations an emulsion of substrate **11**–**14** (10 μL, 200 mM DMSO stock) in reaction buffer (740 μL, 60 mM citrate buffer, pH 6.0) was prepared. Protein solution was added (250 μL, 2.4 mg/mL *Aac*SHC protein solution, 13.6 mg/mL *Zmo*SHC1 protein solution) resulting in a total volume of 1 mL (0.25% CHAPS final conc.). The biotransformations were performed in glass tubes and were shaken in a thermomixer (Eppendorf) at 1000 rpm and 60 °C (*Aac*SHC) and 30 °C (*Zmo*SHC1), respectively. After 20 h of incubation, 1-decanol (10 μL, 200 mM DMSO stock) was added as internal standard. The reaction was terminated by extraction with ethyl acetate (3×500 μL). The organic phases were combined, dried over Na_2SO_4, and analyzed by GC-FID. The biotransformations were performed in triplicates. As negative control substrate in reaction buffer, substrate in reaction buffer containing 0.25% CHAPS and preparations from cells harboring empty pET-22b(+)-vector were used.

3.8. General procedure for preparative enzymatic cyclization

For product isolation and characterization larger biotransformations (150–300 mg substrate) were performed in round-bottom flasks equipped with a magnetic stirrer. To afford a greater amount of cyclic products, the reaction mixtures were stirred for 3–6 days. The extract was filtered through silica and concentrated in vacuo. The products were separated via reversed phase HPLC. For detailed information regarding IR and NMR see Supplementary data.

3.8.1. Product ***15***. Isolated as a colorless oil. IR (KBr): 2925, 2866, 2360, 1605, 1579, 1488, 1447 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 0.93 (s, 3H, CH_3-16), 1.01 (s, 3H, CH_3-17), 1.28 (s, 3H, CH_3-15), 1.23–1.30 (m, 1H, H-3), 1.41 (dt, *J*=3.8, 13.1 Hz, 1H, H-1), 1.46–1.51 (m, 1H, H-3), 1.62–1.68 (m, 1H, H-2), 1.68 (dd, *J*=3.7, 12.1 Hz, 1H, H-5), 1.70–1.81 (m, 1H, H-2), 2.21–2.27 (m, 1H, H-1), 4.19 (dd, *J*=10.6, 11.9 Hz, 1H, H-6), 4.38 (dd, *J*=3.6, 10.5 Hz, 1H, H-6), 6.73–6.76 (m, 1H, H-14), 6.81–6.85 (m, 1H, H-12), 7.04–7.08 (m, 1H, H-13), 7.13 (dd, *J*=1.4, 7.7 Hz, 1H, H-11); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.9 (C-2), 21.9 (C-16), 24.5 (C-15), 32.0 (C-4), 33.0 (C-17), 34.7 (C-10), 37.2 (C-1) 41.9 (C-3), 47.8 (C-5), 64.0 (C-6), 116.3 (C-14), 119.8 (C-12), 124.3 (C-11), 127.0 (C-13), 135.7 (C-9), 152.6 (C-8); HREIMS *m*/*z* 230.1670 (calcd for $C_{16}H_{22}O$ 230.1671).

3.8.2. Product ***16***. Isolated as a colorless oil. IR (KBr): 2921, 2848, 1709, 1599, 1587, 1497, 1474, 1456 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 0.88 (s, 3H, CH_3-11), 0.89 (s, 3H, CH_3-13), 0.90 (s, 3H, CH_3-12), 1.10–1.23 (m, 2H, H-2 & H-6), 1.23–1.29 (m, 1H, H-4), 1.40–1.49 (m, 2H, H-1 & H-2), 1.51–1.62 (m, 1H, H-1), 1.71 (s, 3H, CH_3-22), 1.85–1.95 (m, 1H, H-7), 1.95–2.06 (m, 2H, H-6 and H-7), 2.19 (br s, 1H, H-10), 3.91 (dd, *J*=9.6, 6.2 Hz, 1H, H-14), 4.11 (dd, *J*=9.5, 3.0 Hz, 1H, H-14), 5.49–5.54 (m, 1H, H-8), 6.87–6.95 (m, 3H, H-17 and H-19 and H-21), 7.23–7.30 (m, 2H, H-18 and H-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.7 (C-13), 18.8 (C-1), 21.9 (C-22), 22.0 (C-12), 23.7 (C-7), 33.0 (C-3), 33.3 (C-11), 36.0 (C-5), 39.7 (C-6), 42.1 (C-2), 49.9 (C-4), 54.1 (C-10), 66.3 (C-14), 114.6 (C-17 & C-21), 120.4 (C-19), 123.2 (C-8), 129.4 (C-18 and C-20), 133.3 (C-9), 158.9 (C-16); HREIMS *m*/*z* 298.2295 (calcd for $C_{21}H_{30}O$ 298.2297).

3.8.3. Product ***17***. Isolated as a white solid. IR (KBr): 2923, 2867, 2360, 1734, 1606, 1581, 1488, 1446 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86 (s, 3H, CH_3-19), 0.88 (s, 3H, CH_3-20), 0.91–0.95 (m, 1H, H-4), 0.94 (s, 3H, CH_3-21), 1.05 (dt, *J*=12.9, 4.0 Hz, 1H, H-10), 1.17 (dt, *J*=13.2, 4.1 Hz, 1H, H-2), 1.29 (s, 3H, CH_3-22), 1.38–1.43 (m, 1H, H-2), 1.43–1.50 (m, 1H, H-1), 1.50–1.57 (m, 2H, H-5 and H-6), 1.62 (dd, *J*=11.7, 3.5 Hz, 1H, H-8), 1.62–1.67 (m, 1H, H-1), 1.70–1.78 (m, 2H, H-5 and H-10) 2.31–2.36 (m, 1H, H-6), 4.18 (dd, *J*=10.6, 11.6 Hz, 1H, H-11), 4.35 (dd, *J*=10.5, 3.5 Hz, 1H, H-11), 6.73 (dd, *J*=8.2, 0.9 Hz, 1H, H-18), 6.81–6.85 (m, 1H, H-16), 7.02–7.07 (m, 1H, H-17), 7.13 (dd, *J*=7.7, 1.2 Hz, 1H, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.8 (C-21), 18.4 (C-1), 18.9 (C-5), 21.4 (C-19), 25.9 (C-22), 33.2 (C-3), 33.3 (C-20), 35.0 (C-7), 36.6 (C-9), 39.2 (C-6), 39.7 (C-10), 41.9 (C-2), 52.2 (C-8), 56.3 (C-4), 63.3 (C-11), 116.2 (C-18), 119.9 (C-16), 214.5 (C-15), 126.9 (C-17), 135.9 (C-14), 152.4 (C-13); HREIMS *m*/*z* 298.2297 (calcd for $C_{21}H_{30}O$ 298.2297).

3.8.4. Product ***18***. Isolated as a white solid. IR (KBr): 2921, 2849, 2361, 1736, 1599, 1586, 1497, 1474 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 0.82 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 1.09–1.17 (m, 1H), 1.20 (dd, *J*=11.4, 5.4 Hz, 1H), 1.24–1.27 (m, 2H), 1.29 (dd, *J*=12.8, 3.7 Hz, 1H), 1.32–1.42 (m, 3H), 1.56–1.61 (m, 2H), 1.61–1.67 (m, 1H), 1.71 (s, 3H, CH_3), 1.87–2.01 (m, 2H), 2.04–2.09 (m, 1H), 2.18–2.23 (m, 1H), 3.91 (dd, *J*=9.5, 6.4 Hz, 1H), 4.10 (dd, 9.5, 2.9 Hz, 1H), 5.46–5.50 (m, 1H), 6.87–6.95 (m, 3H), 7.24–7.30 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.65, 15.7, 18.5,

18.8, 21.7, 21.7, 22.6, 33.1, 33.4, 36.1, 37.3, 39.9, 41.3, 41.9, 54.6, 54.9, 56.2, 66.2, 114.6 (2C), 120.4, 123.0, 129.4 (2C), 133.1, 158.9; HREIMS *m*/*z* 366.2930 (calcd for $C_{26}H_{38}O$ 366.2923).

3.8.5. Product ***19***. Isolated as a white solid. IR (KBr): 2928, 2866, 1645, 1599, 1586, 1496, 1474 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 0.81 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 1.11–1.19 (m, 2H), 1.32–1.37 (m, 2H), 1.37–1.45 (m, 4H), 1.51 (s, 1H), 1.57–1.63 (m, 2H), 1.66–1.74 (m, 2H), 1.85–1.89 (m, 1H), 2.09 (td, *J*=13.1, 4.7 Hz, 1H), 2.19–2.24 (m, 1H), 2.39–2.45 (m, 1H), 4.07–4.12 (m, 1H), 4.16 (dt, *J*=9.5, 3.7 Hz, 1H), 4.56 (d, *J*=1 Hz, 1H), 4.87 (d, *J*=1 Hz, 1H), 6.89–6.95 (m, 3H), 7.26–7.29 (m, 2H); HREIMS *m*/*z* 366.2925 (calcd for $C_{26}H_{38}O$ 366.2923).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2012.06.041.

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