Supporting Information

**Stereoselective Directed Cationic Cascades Enabled by Molecular Anchoring in Terpene Cyclases**

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Supporting Fig. 1 Established cyclization pathways vs. desired cyclization pathways of terpenes.

(A) Halonium-, Lewis acid- or Brønsted acid- induced cyclization of geranylgeraniol\(^1\), geranyllinalool\(^2\), nerolidol\(^3\) and geranyl acetone\(^4\) result mainly in polycyclic products by intramolecular quenching. (B) Cyclizations under cascade control potentially result in value-added products e.g. Aplysin 20\(^5\), Vitamin A precursor, Sclareol, γ-Snyderol\(^6\), γ-Dihydroionone \(^6\) and Tectoionol B\(^7\).
Supporting Fig. 2 Molecular anchors (red) in catalytic and material sciences. (A) α-halo carbonyl compound anchored by amino acids in the active site of ‘ene’-reductases. (B) Remote site-selective C-H activation directed by molecular anchoring at bifunctional template. (C) Computationally designed Diels-alderase with substrates anchored in the active site. (D) Ni-catalyst anchored on CdS nanocrystals for photocatalytic CO₂ reduction. (E) Cholesterol based anchors for phospholipid bilayers and for model biological membranes. (F) Anchoring of porphyrin wires on graphene electrodes.
Supporting Fig. 3 Natural reaction of squalene-hopene cyclase from *Alicyclobacillus acidocaldarius*. After proton-induced cationic cyclization and rearrangement the resulting carbocation is either deprotonated to form hopene (dark red) or attacked by water to form hopanol (green) with outstanding precision of 1:512 isomers\(^\text{[14]}\).
Supporting Fig. 4 Stereoselective multi-step synthesis of 3. (A) Titanium-catalyzed enantioselective and (B) enantioenriched synthesis of α-Ambrinol 7. Protected (−)-3 can potentially be deprotected carefully to result (−)-3 prior to cyclize to 7 or another protection group for 1t can be used e.g. dithianes [16].
Supporting Fig. 5 Ambrein and its photooxidative degradation compounds. (+)-γ-dihydroionone 3 (red square) can be used as a starting material to synthesize all compounds except of (−)-ambrox[6]. Ambrein is the main component of ambergris, which is formed in the intestines of the sperm whale[17].
Supporting Fig. 6 Biotransformations with geranyl acetone 1t and neryl acetone 1c. (A) The E-isomer 1t was almost fully converted to the bicyclic product 2t (red scaffold) and the Z-isomer 1c to bicyclic product 2c as well as monocyclic products 4 (grey) and 3 (blue). Relative conversion rates are given below the products. Colors correspond to the pie charts in (C). (B) Docking mode with the highest binding energy of E-geranyl acetone 1t (purple) in the active site wild-type SHC. Relevant residues are shown in sticks: Protonating D376 (red); hydrogen-bond donating Y420 (blue); steric repulsion by I261 with C1-Methyl group of the substrate 1t shown in dots. (C) Biotransformations of E-geranyl acetone 1t by AacSHC variants with substitutions at position I261 and Y420 demonstrate the relevance of these residues for productive substrate binding. Reduced steric bulk (I→V,A) at position I261 reduces the steric interaction of the C1-methyl group of the substrate 1t for tight binding. Therefore, relative conversion is reduced. Disabling the hydrogen-bond at position 420 and the steric interaction at position I261 (Y420F/I261A) kills enzymatic activity on the substrate 1t. Product selectivities shown as pie charts above the columns (for detailed numbers see Table S8)
Supporting Fig. 7 Site-directed mutagenesis studies at position G600 and L607. (A) Comparison of position G600 variants sorted by overall relative conversion. Selectivity towards the three products (Fig. S6A, top reaction) shown above in pie charts. The results demonstrate how small and polar amino acids drive the monocyclization (blue pie chart). (B) Biotransformations with L607 variants demonstrate that smaller substitutions compared to leucine are beneficial for the monocyclization reaction. Product selectivities are given above the corresponding variant in pie charts (for detailed numbers see Table S8). Colors correspond to the right structures in (B).
Supporting Fig. 8 Comparison of co-crystallized 2-Azasqualene in *AacSHC WT* (left) with best result of docked substrate 1t in *AacSHC WT* (right). Protonating aspartate shown in red sticks. Key mutated positions shown in grey sticks. 2-Azasqualene and geranyl acetone 1t shown in magenta sticks. The comparison shows the very similar pre-folding of both substrates in the active site.
Supporting Fig. 9 Stereoview of the two major pre-folding states in the docking of Z-geranyl acetone 1c in the active site of variant G600T (II). Protonating aspartate shown in red sticks. Hydrogen-bond donors shown in orange sticks. (Top) Pre-folding state 1, then 90° rotated in the xy-plane and additional 90° in the xz-plane. (Bottom) Pre-folding state 2, then 90° rotated in the xy-plane and additional 90° in the xz-plane.
Supporting Fig. 10 SDS-PAGE analysis of AacSHC variants. (A) After thermolysis (see (6)) and extraction the supernatant and the cell debris contain no AacSHC protein anymore. 2d extraction was sufficient in extracting all of the enzyme from the cell debris. (B+C) Extraction of lyophilized whole cell batches containing the specific variants. Triplikates demonstrate the almost equal extraction among all whole cell pellets.
Supporting Fig. 11 Chiral analysis of (−)-γ-dihydroionone 3. (A) The raw (−)-γ-dihydroionone 3 was converted with 2N sulfuric acid to (+)-α-ambrinol 7. (B) GC-MS chromatograms of raw (−)-γ-dihydroionone 3 before (top) and after treatment with sulfuric acid (middle) compared with the racemic α-ambrinol 7 standard. (C) Measurement over chiral GC demonstrates the excellent enantioselectivity of the enzyme.
Supporting Fig. 12 Stereoview of the final pre-folding state in the docking of Z-geranyl acetone 1c in the active site of variant V. Protonating aspartate shown in red sticks. Hydrogen-bond donors shown in orange sticks. (Top) Pre-folding state 1 30° rotated in the xy-plane and additional 90° in the xz-plane. (Bottom) Pre-folding state 2 90° rotated in the xy-plane and additional 90° in the xz-plane.
Supporting Fig. 13 Standard procedure for the upscaling reactions. After seven days of reaction the product can be isolated from the aqueous phase with diethyl ether.
Supporting Fig. 14 Time course of the biotransformation of E/Z-geranyl acetone 1 (60:40) with the variant V. The variant V preferably converts the Z-geranyl acetone 1c (green). Ambrinol 7 is obtained as a side product by acid-catalyzed cyclization (pH=6.0) of the product (−)-γ-dihydroionone 3.
**Table S1 |** List of buffers used in this work.

| Buffer                     | Ingredients                                                                 |
|----------------------------|-----------------------------------------------------------------------------|
| 10x phosphate buffer (KP\textsubscript{i}-buffer) | 0.17 M KH\textsubscript{2}PO\textsubscript{4}, 0.72 M K\textsubscript{2}HPO\textsubscript{4}, pH=7.4 |
| Whole cell buffer          | 100 mM Citric acid, 0.1 % SDS, pH=6.0                                       |
| Cyclodextrin (CD) buffer   | 0.2 % SDS, 10mM (2-Hydroxypropyl)-\textbeta- cyclodextrin, pH=6.0            |
| Lysis buffer               | 200 mM Citric acid, 0.1% EDTA, pH=6.0                                       |
| Extraction buffer          | 100 mM Citric acid, 1% CHAPS, pH=6.0                                       |
| Medium                                | Ingredients                                      |
|--------------------------------------|--------------------------------------------------|
| Lysogeny broth                      | 10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract |
| Auto-induction medium*(T-DAB)        | 12 g/L tryptone, 24 g/L yeast extract, 2.9 g/L   |
|                                      | glucose, 11.1 g/L Glycerol, 7.6 g/L Lactose       |

*(based on [18]*)
Table S3 | List of primers used in this work.

| Entry | Name          | Sequence (5'→3')                     | Forward/Reverse |
|-------|---------------|--------------------------------------|-----------------|
| 1     | AacSHC_G600C | CCGTATTATACCGGACACTTTCCGGGCG         |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 2     | AacSHC_G600D | CCGTATTATACCGGACCGATTCCGGGCG         |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 3     | AacSHC_G600E | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 4     | AacSHC_G600F | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 5     | AacSHC_G600G | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 6     | AacSHC_G600H | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 7     | AacSHC_G600I | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 8     | AacSHC_G600J | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 9     | AacSHC_G600K | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 10    | AacSHC_G600L | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 11    | AacSHC_G600M | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 12    | AacSHC_G600N | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 13    | AacSHC_G600O | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 14    | AacSHC_G600P | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 15    | AacSHC_G600Q | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 16    | AacSHC_G600R | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 17    | AacSHC_G600S | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 18    | AacSHC_G600T | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 19    | AacSHC_G600U | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 20    | AacSHC_G600V | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 21    | AacSHC_G600W | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 22    | AacSHC_G600X | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 23    | AacSHC_G600Y | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 24    | AacSHC_G600Z | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 25    | AacSHC_G600[ | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |
| 26    | AacSHC_G600\ | CCGTATTATACCGGACCGATTTCCGGGCG       |                 |
|       |               | CCATTTCCGGCGTATATACCGG               |                 |

Note: The table includes primers for various positions and sequences, as indicated in the entries.
| 27 | AacSHC_A306X | GCGGCTGGATGGTTTCAGNDTAGCATTAGCCCCGTG/ | GCGGCTGGATGGTTTCAGVHGAGCATTAGCCCCGTG/ | GCGGCTGGATGGTTTCAGTGGAGCATTAGCCCCGTG/ | CTGAAACATCCAGCCGCAATAATCCAGTCCACGCCCATAACAG |
| Name                  | Rate [°C/min] | Temperature [°C] | Hold [min] |
|----------------------|--------------|-----------------|-----------|
| Dihydroion_long (1)  | 120          | 0.1             |           |
|                      | 2            | 145             | 0.6       |
| Dihydroion_short (2) | 120          | 0.1             |           |
|                      | 2            | 137             | 0.6       |
| Calmusal (3)         | 110          | 0.1             |           |
|                      | 2            | 135             | 0.6       |
| General (4)          | 50           |                 | 3         |
|                      | 6            | 120             | 0         |
|                      | 10           | 150             | 0         |
|                      | 15           | 170             | 0         |
|                      | 20           | 200             | 0         |
|                      | 25           | 250             | 0         |
|                      | 30           | 310             | 1         |
| Chiral (5)           | 70           |                 | 3         |
|                      | 140          |                 | 0         |
|                      | 8            | 180             | 2         |
| substance                               | volume [µl] | final concentration |
|----------------------------------------|-------------|---------------------|
| ddH₂O                                  | 29          |                     |
| DMSO                                   | 2.5         |                     |
| KOD Hot Start Buffer (10x)             | 5           | 1x                  |
| dNTPs (2 mM each)                      | 5           | 250 µM (each)       |
| MgSO₄ (25 mM)                          | 4.5         | 2 mM                |
| Template DNA                           | 1           | 0.5-5 ng/ µl        |
| Primer forward (10 µM)                 | 1           | 0.2 µM              |
| Primer reverse (10 µM)                 | 1           | 0.2 µM              |
| KOD Hot Start DNA Polymerase           | 1           |                     |
**Table S6** PCR temperature profile.

| step            | Temperature [°C] | time [s] | cycles |
|-----------------|------------------|----------|--------|
| Initial denaturation | 95              | 120      | 1      |
| Denature        | 95              | 30       |        |
| Annealing       | 60              | 30       | 30     |
| Extension       | 70              | 210      |        |
| Final extension | 72              | 420      | 1      |
Table S7 Relative conversion \([\text{Area}_{\text{product}}/(\text{Area}_{\text{product}}+\text{Area}_{\text{substrate}})\times 100\text{ in }\%]\) of substrate mixture 1 \(t/c\) and isolated 1\(t\) and 1\(c\) with the wild-type enzyme and the variant G600R and the corresponding product selectivities (bicyclization vs. monocyclization) in brackets.

|                | \((E/Z)\)-Ger 1  | \((E)\)-Ger 1\(t\) (bi:mono) | \((Z)\)-Ger 1\(c\) (bi:mono) |
|----------------|------------------|--------------------------------|--------------------------------|
| **WT**         | 23.2 ± 4.1       | 29.0 ± 2.5                     | 0.7 ± 0.2                      |
|                | (100:0)          | (67:33)                        |                               |
| **G600R**      | 80.4 ± 5.1       | 95.7 ± 0.8                     | 68 ± 4.3                       |
|                | (100:0)          | (91:9)                         |                               |

Reaction conditions: *E. coli* whole cells harboring AacSHC variant resuspended in whole-cell buffer (0.1 M citric acid, 0.1% SDS, pH = 6.0) with an OD\(600=20\), 20h, 30°C, 4.4 mM substrate (=1 µl in 1ml cell suspension). Reactions were performed in technical triplicates.
Table S8. Relative conversion \([\text{Area}_{\text{product}}/(\text{Area}_{\text{product}}+\text{Area}_{\text{substrate}})\times 100\text{ in }\%]\) of the \(E/Z\)-geranyl acetone \(1t/c\) with the wild-type enzyme and the variants I261V, Y420F, I261A and I261A/Y420F.

|        | bi (2t+c) | mono (3+4) | overall relative conv. | error |
|--------|-----------|------------|------------------------|-------|
| WT     | 23.2      | 0.0        | 23.2                   | 0.9   |
| I261V  | 18.0      | 0.0        | 18.0                   | 1.2   |
| Y420F  | 14.0      | 0.6        | 14.6                   | 0.4   |
| I261A  | 5.0       | 0.0        | 5.0                    | 0.7   |
| I261A/Y420F | 0.0   | 0.0        | 0.0                    | 0.0   |

**Reaction conditions:** *E. coli* whole cells harboring AacSHC variant resuspended in whole-cell buffer (0.1 M citric acid, 0.1% SDS, pH = 6.0) with an \(\text{OD}_{600} = 20\), 20h, 30°C, 4.4 mM substrate (=1µl in 1ml cell suspension). Reactions were performed in technical triplikates.
Table S9 Relative conversion \(\frac{\text{Area}_{\text{product}}}{\text{Area}_{\text{product}} + \text{Area}_{\text{substrate}}} \times 100\) in % of neryl acetone 1c with all variants at position 600 and the corresponding product selectivities.

|     | chromene 2c | γ-dihydroionone 3 | α-dihydroionone 4 | overall relative conv. | error r |
|-----|-------------|-------------------|-------------------|------------------------|---------|
| G600R | 26.2        | 1.7               | 0.7               | 28.5                   | 0.6     |
| G600M | 25.5        | 0.7               | 0.6               | 26.7                   | 1.1     |
| G600T | 8.8         | 7.9               | 3.8               | 20.4                   | 0.4     |
| G600L | 16.1        | 0.4               | 0.3               | 16.8                   | 0.3     |
| G600N | 9.9         | 1.0               | 1.6               | 12.4                   | 1.2     |
| G600Q | 9.6         | 0.4               | 0.5               | 10.4                   | 2.0     |
| G600Y | 8.1         | 0.6               | 0.3               | 9.0                    | 0.2     |
| G600C | 6.8         | 1.5               | 0.7               | 9.0                    | 0.4     |
| G600S | 4.6         | 2.7               | 1.2               | 8.5                    | 0.5     |
| G600K | 6.6         | 0.7               | 0.3               | 7.5                    | 1.2     |
| G600D | 5.4         | 1.2               | 0.7               | 7.3                    | 2.5     |
| G600E | 5.8         | 1.1               | 0.3               | 7.2                    | 1.8     |
| G600V | 5.4         | 0.2               | 0.1               | 5.7                    | 2.3     |
| G600A | 2.9         | 0.9               | 0.3               | 4.2                    | 1.0     |
| G600F | 3.4         | 0.3               | 0.1               | 3.8                    | 2.1     |
| G600I | 3.4         | 0.1               | 0.0               | 3.6                    | 0.5     |
| G600  | 2.6         | 0.4               | 0.2               | 3.1                    | 0.3     |
| W    | 1.1         | 0.1               | 0.1               | 1.3                    | 0.8     |
| WT   | 0.5         | 0.1               | 0.1               | 0.7                    | 0.4     |
| G600P| 0.1         | 0.0               | 0.0               | 0.1                    | 0.0     |

**Reaction conditions:** *E. coli* whole cells harboring AacSHC variant resuspended in whole-cell buffer (0.1 M citric acid, 0.1% SDS, pH = 6.0) with an \(\text{OD}_{600} = 22\), 20h, 30°C, 8.8mM substrate (=2 µl in 1ml cell suspension). Reactions were performed in technical triplikates.
Table S10 Relative conversion $\left[\frac{\text{Area}_{\text{product}}}{\text{Area}_{\text{product}} + \text{Area}_{\text{substrate}}} \right] \times 100$ in % of the substrate neryl acetone 1c with the variants at position 607 and the corresponding product selectivities.

|          | chromene | γ-dihydroionone | α-dihydroionone | overall relative conv. | erro | r  |
|----------|----------|----------------|----------------|----------------------|------|----|
| L607S    | 8.8      | 6.3            | 1.8            | 16.9                 | 1.2  |
| L607M    | 10.5     | 0.4            | 0.3            | 11.2                 | 0.5  |
| L607A    | 5.5      | 3.3            | 0.8            | 9.5                  | 0.6  |
| L607V    | 3.8      | 1.8            | 0.5            | 6.1                  | 0.7  |
| L607G    | 1.2      | 0.4            | 0.2            | 1.7                  | 0.2  |
| L607Y    | 0.00     | 0.00           | 0.00           | 0.00                 | 0.0  |
| L607S/G600 T | 2.1     | 28             | 1              | 31.1                 | 3.1  |
| L607A/G600 T | 2.3   | 38             | 1.1            | 43.4                 | 1.5  |

Reaction conditions: *E. coli* whole cells harboring AacSHC variant resuspended in whole-cell buffer (0.1 M citric acid, 0.1% SDS, pH = 6.0) with an $\text{OD}_{600} = 20$, 20h, 30°C, 8.8 mM substrate (=2γl in 1ml cell suspension). Reactions were performed in technical triplikates.
Table S11 Relative conversion $[\text{Area}_{\text{product}}/(\text{Area}_{\text{product}}+\text{Area}_{\text{substrate}})]\times100$ in % of neryl acetone 1c, corresponding selectivities and total turnover numbers of the wild-type enzyme and the engineered variants I-V.

| Enzymconc. in g/l | 1.54 | 1.32 | 1.5 | 1.54 | 1.64 | 1.38 |
|-------------------|------|------|-----|------|------|------|
| Enzymconc. in mol/l | 2.15569E-05 | 1.848E-05 | 2.1E-05 | 2.156E-05 | 2.296E-05 | 1.932E-05 |
| MW = 71439 g/mol |     |      |     |      |      |      |
| WT | G600R (I) | G600T (II) | (III) (+L607A) | (IV) (+Y420F) | (V) (+306V) |
| chromene 2c | 0.5 | 21.9 | 6.4 | 4.4 | 1.8 | 1.0 |
| γ-dihydroionone 3 | 0.1 | 1.5 | 7.4 | 22.2 | 63.8 | 95.2 |
| α-dihydroionone 4 | 0.1 | 0.7 | 1.1 | 1.4 | 2.2 | 2.1 |
| overall relative conversion | 0.7 | 24.1 | 14.9 | 28.0 | 67.8 | 98.3 |
| error relative conversion | 0.2 | 3.0 | 0.5 | 3.2 | 0.9 | 2.4 |
| TTN | 2.9 | 114.8 | 62.4 | 114.4 | 260.0 | 447.8 |
| error TTN | 0.8 | 14.3 | 2.1 | 13.1 | 3.4 | 10.9 |

Reaction conditions: 10 mg lyophilized E. coli whole cells harboring AacSHC variant (18-22 µM, Fig. S9) resuspended in 1 mL CD buffer (0.2% SDS, 10mM 2-Hydroxypropyl)-β-cyclodextrin, pH = 6.0), 24h, 30°C, 8.8 mM substrate. Reactions were performed in technical triplikates.
Table S12. Relative conversion \( \frac{\text{Area}_{\text{product}}}{(\text{Area}_{\text{product}}+\text{Area}_{\text{substrate}})} \times 100 \) in % of the substrate neryl acetone 1c with the variants IV, Y420F/G600T, Y420F/L607A, Y420F/G600T/L607A/Y609F and Y420F/Y609F and the corresponding product selectivities.

| | chromene 2c | γ-dihydroi | α-dihydroi | overall relative conv. | error |
|---|-------------|------------|------------|------------------------|-------|
| IV =ABC (Y420F/G600T/L607A) | 1.4 | 58 | 1.8 | 60.2 | 5.4 |
| Y420F/G600T = AC | 1.2 | 11.6 | 0.7 | 14.3 | 2.4 |
| Y420F/L607A = BC | 2.6 | 8.2 | 0.5 | 12 | 1.1 |
| Y420F/G600T/L607A/Y609F | 1 | 1 | 0.2 | 2.2 | 0.1 |
| Y609F = ABCD | 0 | 0 | 0 | 0 | 0 |
| Y420F/Y609F = BCD | 0 | 0 | 0 | 0 | 0 |

**Reaction conditions:** *E. coli* whole cells harboring AacSHC variant resuspended in whole-cell buffer (0.1 M citric acid, 0.1% SDS, pH = 6.0) with an OD_{600} = 22, 20h, 30°C, 8.8 mM substrate (=2\( \mu l \) in 1ml cell suspension). Reactions were performed in technical triplicates.
Table S13 Relative conversion \( \frac{\text{Area}_{\text{product}}}{(\text{Area}_{\text{product}} + \text{Area}_{\text{substrate}})} \times 100 \) in % of the substrate neryl acetone \( 1c \) and substrate analogs \( 8, 10, 12 \) with the wildtype AacSHC and the variant G600R. Product selectivities (bicyclization vs. monocyclization) given in brackets.

|        | Ger 1t/c (bi:mono) | Ger-OH 8 (bi:mono) | Cal 10 (bi:mono) | Cal-OH 12 (bi:mono) |
|--------|--------------------|--------------------|------------------|---------------------|
| **WT** | 23.1 ± 4.1 (100:0) | 16 ± 0.6 (100:0)  | 0                | 0                   |
| **G600R** | 80.4 ± 5 (95:5)     | 65.4 ± 3.8 (100:0) | 35.4 ± 3.1 (98:2) | 4.8 ± 0.9 (99:1)   |

**Reaction conditions:** *E. coli* whole cells harboring AacSHC variant resuspended in whole-cell buffer (0.1 M citric acid, 0.1% SDS, pH = 6.0) with an OD \( \text{OD}_{600} = 20 \), 20h, 30°C, 4.4 mM substrate. Reactions were performed in technical triplicates.
Table S14 Relative conversion \([\text{Area}_{\text{product}}/(\text{Area}_{\text{product}}+\text{Area}_{\text{substrate}}) \times 100 \text{ in } \%]\) of the substrate analogs 8, 10, 12 with variants G600T, G600T/L607A, G600N/L607A and variant V.

|                | Ger-OH 8 | Cal 10 | Cal-OH 12 |
|----------------|-----------|--------|-----------|
|                | bicyclic  | monocyclic | bicyclic | monocyclic | bicyclic | monocyclic |
| G600T          | 15        | 0      | 40        | 16        | 3        | 0         |
| G600T/L607A    | 0         | 0      | 10        | 0.9       | 0        | 0         |
| V              | 1.4       | 0      | 11.2      | 1.6       | 0        | 0         |
| G600N/L607A    | 2.1       | 5.3    | 14        | 4.2       | 0.3      | 0.6       |

Reaction conditions: \(E. \text{ coli}\) whole cells harboring AacSHC variant resuspended in whole-cell buffer (0.1 M citric acid, 0.1% SDS, pH = 6.0) with an \(\text{OD}_{600} = 18\), 20h, 30°C, 4.4 mM substrate. Reactions were performed in technical triplicates.
I. Materials

(1) Chemicals. The chemicals used for syntheses, molecular biology and biochemical work have been purchased from Carl-Roth (Karlsruhe, DE), VWR (Pennsylvania, US), Sigma-Aldrich (St. Louis, US) and Alfa-Aesar (Ward Hill, US). The substrates (E/Z)-geranyl acetone 1 t/c from VWR (A19184.14), Calmusal 10 from ambinter (18445-88-0), ambrinol 7 (690993) from Symrise. All the other substrates for biocatalytic purposes were chemically synthesized and analyzed by $^1$H-NMR, $^{13}$C-NMR and GC/MS.

(2) Molecular biological kits. The molecular biological kits for DNA-purification (Zymoclean DNA Clean & Concentrator Kit), Agarose gel-extraction (Zymoclean Gel DNA Recovery Kit) and plasmid isolation (Zyppy™Plasmid Miniprep Kit) were purchased from ZymoResearch (Irvine, US).

(3) Buffers & Media
All list of all buffer and media ingredients is provided in table S1 and S2.

(4) Primer
All list of all primers is provided in table S3.

II. General analytics.

(1) Nuclear Magnetic Resonance
$^1$H- und $^{13}$C-NMR spectra were recorded on a Bruker Avance 500 Spectrometer at 500.15 MHz for $^1$H- and 125 MHz for $^{13}$C. The chemical shifts δ are referred to tetramethylsilane (=TMS) in ppm set to 0. All substances were dissolved in CDCl$_3$ and recorded at room temperature.

(2) Circular dichroism
The specific optical rotation of the compounds were measured on a Perkin Elmer Polarimeter 241. Therefore the substance was dissolved in CHCl$_3$ (c=0.5 mg/ml) and the specific rotation was measured with a sodium and a mercury spectral lamp.

(3) Gas chromatography
GC analyses were performed using an Agilent GC 7820A equipped with a mass spectrometer MSD 5977B and a HP-5MS capillary column (Agilent, 30 m x 250 μm x 0.25 μm) and helium as carrier gas with a constant pressure of 14.168 ψ. Injections (1 μL) were performed in split mode (10:1). Relative conversion rates were calculated directly from GC-MS spectra by integration-quotient of substrates and products. Chiral GC analysis was performed on a Shimadzu GC-2010 equipped with a CP ChiraSil-Dex CB capillary column (Agilent, 25 m x 250 μm x 0.25 μm) and hydrogen as carrier gas with constant
velocity (linear velocity: 33.1 cm/s). Injections (1 µL) were performed in split mode (5:1). Temperature programs are listed in table S4.

III. Chemical synthesis.

(1) Synthesis of geranyl isopropanol 8

![Geranyl isopropanol synthesis](image)

For the reduction reaction geranyl acetone 1 t/c (0.50 ml, 2.34 mmol 1.00 eq.) was dissolved in ethanol (10 ml). Sodium borohydride (0.088 g, 2.34 mmol, 1.00 eq.) was then added carefully and the reaction mixture was stirred at room temperature for 1 h. After the reaction was complete, the mixture was quenched with 0.5 N HCl (2 ml) and stirred again for 30 min. Then distilled water (50 ml) was added and the aqueous phase was extracted three times with DCM. The combined organic phases were dried over CaCl₂ and the geranyl isopropanol 8 was obtained as a clear oil (0.49 ml, 2.04 mmol, 87%).

¹H-NMR (CDCl₃, 500 MHz): δ (ppm) 1.19 (d, J = 2.9 Hz, 3H), 1.50 (quart, J = 7.7 Hz, 2H) 1.6 (s, 3H), 1.62 (s, 3H), 1.68 (s, 3H), 1.88-1.92 (t, J = 7.3 Hz, 2H), 2.04-2.12 (m, 4H), 3.77-3.84 (sept, J = 17.43 Hz, 1H), 5.05-5.10 (t, J = 6.7 Hz, 1H), 5.12-5.17 (t, J = 6.8 Hz, 1H). ¹³C-NMR (CDCl₃, 125 MHz): δ (ppm) 16.50 (1C), 16.66 (1C), 22.44-25.63 (4C), 38.15-38.70 (2C), 66.97 (1C), 75.67 (1C), 122.22-123.24 (2C), 134.9 (1C). MS (EI): m/z (%) = 196 (0.3), 153 (32), 135 (21), 109 (58), 95 (21), 82 (19), 81 (21), 69 (100), 68 (13), 67 (44). The data is consistent with the literature[19].

(2) Synthesis of 6,10-dimethylundeca-5,9-dien-2-ol 12

![6,10-dimethylundeca-5,9-dien-2-ol synthesis](image)

The reaction was carried out analog to synthesis (1). The product was obtained as a clear oil (0.21 ml, 1.04 mmol, 43%).
1H-NMR (CDCl3, 500 MHz): δ (ppm) 1.19-1.34 (m, 2H), 1.55-1.59 (m, 2H) 1.61(s, 3H), 1.62-1.65 (m, 1H), 1.68 (s, 3H), 1.69-1.71 (m, 2H), 2.04-2.12 (m, 4H), 3.62-3.67 (t, J = 6.6 Hz, 2H), 5.03-5.19 (m, 2H). The data is consistent with the literature [20].

(3) Sulfuric acid catalyzed cyclization of (−)-γ-dihydroionone 3

For the cyclization reaction (−)-γ-dihydroionone 3 (400 µL, 1.8 mmol) was dissolved in THF (15 mL) in a 50 mL Schott-bottle. 2N sulfuric acid (5 mL) was then added and the reaction mixture was shaken at 37 °C for 24h. The reaction was quenched by addition of water (20 mL) and extracted with Diethylether (3 x 30 mL). The combined organic phases were dried over MgSO4 and purified via silica chromatography (10:1, hexane: ethyl acetate) to yield the slightly yellowish liquid (+)-α-ambrinol 7 (350 µL, 1.5 mmol, 88 % yield); ([α]D20 = +84.6; Lit. = 81.8[15]).

1H-NMR (CDCl3, 500 MHz): δ (ppm) 0.87 (s, 3H), 0.91 (s, 3H) 1.14 (m, 1H), 1.22 (s, 3H), 1.24-1.40 (m, 3H), 1.45-1.51 (m, 2H), 1.67-1.74 (m, 2H), 1.98-2.02 (m, 2H), 2.06-2.17 (m, 2H), 5.45 (t, J = 3.84Hz, 1H). 13C-NMR (CDCl3, 125 MHz): δ (ppm) 22.6 (1C), 23.8 (1C), 25.05 (1C), 26.02 (1C), 28.07 (1C), 29.24 (1C), 31.11 (1C), 28.95(1C), 47.25 (1C), 49.82 (1C), 70.28 (1C), 122.04 (1C), 137.39 (1C). The data is consistent with the literature[6].

(+) α-ambrinol MS (EI): m/z (%) = 194 (5), 176 (40), 161 (30), 136 (100), 121 (66), 120 (40), 109 (28), 105 (31), 95 (49), 93 (28).

Side product: (−)-β-ambrinol: MS (EI): m/z (%) = 194 (6), 176 (55), 161 (100), 136 (40), 121 (84), 107 (43), 106 (46), 105 (60), 93 (52), 91 (42).
IV.  General Methods

(1) Plasmid isolation

Isolation of the plasmid proceeded following to the standard protocol of Zyppy™ Plasmid Miniprep Kit by ZymoResearch. For the photometric determination of the plasmid DNA concentration, 1 μL was measured on a Nanodrop 1000 (Agilent, Santa Clara, US) at a wavelength of 260 nm.

(2) Site-saturation/-directed mutagenesis

The gene encoding for AacSHC (UniProt: P33247) or a variant based on this gene was cloned into a pET-22b(+) vector system (Merck, Darmstadt, Germany). SacI and NdeI were used as restriction sites. Cloning followed the standard protocol of Novagen’s KOD Hot Start DNA Polymerase. The composition of the PCR mixture and the temperature profile are described in table S5 and S6.

Site-saturation libraries were generated employing the “22c-trick” method. PCR products were digested with 1 μL DpnI for 4h at 37 °C, purified by agarose gel electrophoresis and ligated into the pET22b(+) vector by Gibson assembly. After purification using the DNA Clean & Concentrator™-5 kit the plasmids were transformed via heat-shock method.

(3) Plasmid transformation

Chemically competent cells based on rubidium chloride were produced for the transformation of the plasmid DNA. The transformation was carried out under sterile conditions. For site saturation libraries 3 μL of the purified PCR product was added to 25 μL XL1-blue competent cells and incubated for 30 min on ice, followed by a heat shock at 42 °C for 105 s with subsequent ice cooling for 3 min. After adding 500 μl of LB medium, the cells were incubated for 40min at 37 ° C and used for inoculation of a 5 mL LB medium (Ampicillin, cend= 100 μg/ml) pre-culture overnight. After isolation of the plasmid, transformation into 50 μL BL21 (DE3) was performed using the heat shock method. After regeneration 150 μL were streaked out on an agar plate (Ampicillin, cend= 100 μg/ml) and incubated at 37 °C overnight. For quality control the plasmid was isolated from another 150 μL and sent for sequencing. For site-directed mutants the PCR product was digested with DpnI overnight and afterwards transformed into XL1-blue competent cells. After regeneration 300 μL were streaked out on an agar plate for single clone picking.

(4) Expression of AacSHC libraries in 96-DW plates

Individual colonies were picked from generated agar plates and cultivated in 500 μL LB medium (Ampicillin, cend= 100 μg/ml) for 18-20h at 37 °C, 800 rpm. Expression cultures were inoculated with 10
µL of the pre-culture into 1 mL of T-DAB autoinduction medium (Ampicillin, $c_{\text{end}} = 100 \mu g/ml$) with lactose as the inductor. The cultures were incubated for 20h at 37 °C, 800 rpm and harvested afterwards (4000 x g, 20 min).

(5) Expression in 24 DW-plates

Individual colonies were picked from generated agar plates and cultivated in 2 mL LB medium (Ampicillin, $c_{\text{end}} = 100 \mu g/ml$) for 18-20h at 37 °C, 180 rpm. Expression cultures were inoculated with 40 µL of the pre-culture into 4 mL of T-DAB autoinduction medium (Ampicillin, $c_{\text{end}} = 100 \mu g/ml$) with lactose as the inductor. The cultures were incubated for 20h at 37 °C, 600 rpm and harvested afterwards (4000 x g, 20 min).

(6) Thermolysis purification\cite{24,25}

Harvested or lyophilized cells were resuspended in 1 mL \textit{Lysis buffer} and incubated for 60 min at 70 °C. The cell suspension was centrifuged (14000 x g, 1 min) and the supernatant was discarded. As the enzyme is membrane-bound 1 mL 1%-CHAPS buffer was added to extract it from the cell pellet by shaking at room temperature for 2d, 600 rpm. After subsequent centrifugation (14000 x g, 1 min) the supernatant containing the AacSHC was transferred to a new tube followed by SDS-PAGE analysis and determination of enzyme concentration by using the \textit{Nanodrop 1000} (Agilent, Santa Clara, US). Therefore the “Protein A280” mode was chosen with MW= 71439 Da and molar extinction coefficient $\varepsilon = 185180$ as protein specific data.

(7) SDS-PAGE

After protein purification and extraction 20 µl of of the enzyme preparation was mixed with 10 µl SDS loading buffer and heated to 95 ° for 10min. Afterwards 10 µl of the preparation was loaded on the pre-prepared SDS-PAGE (Expedeon).

(8) Screening of AacSHC libraries via GC-MS

Harvested pellets were resuspended in 396 µL \textit{whole cell buffer} and transferred to another 96-DW plate equipped with 1.2 mL glass inlets. Afterwards 4 µL substrate/DMSO stock solution (substrate $c_{\text{end}}$= 2mM) was add directly into the cell suspension, the plates were sealed and shaken for 20h at 30 °C, 600 rpm. In order to stop the reaction 600 µL cyclohexene/o-xylol (1:1) was added and the mixture was inverted for 10 min and incubated for 30min. The plates were centrifuged (4000 x g, 5 min), sealed using PP-sealings and a GC-MS equipped with a PAL-Sampler was used to inject directly from the organic phase. Quantification was made directly from the Total Ion Count chromatogramm by quotient $\text{AREA}_{\text{product}}$/
(AREA\text{substrate} + AREA\text{product}) \times 100$. In total 90 variants per plate were screened. Promising variants were rescreened by expression in 24 DW-plates.

(9) Verification of promising hits

Promising candidates from the 96-DW screening were taken for inoculation of a 5 mL LB pre-culture. Afterwards the plasmids were isolated and transformed for single colony picking. The single colonies were expressed in 24 DW-plates and after harvesting the OD$\text{_{600}}$ was set to 20 in whole cell buffer substrate was added ($c_{\text{end}}$=4.4 mM) The reactions were carried out at least in technical duplicates. Reactions were stopped by adding dichloromethane. After two extractions with cyclohexane: ethyl acetate (1:1) the resulting organic phase was measured directly over GC-MS. Quantification was made directly from the Total Ion Count chromatogramm by quotient $\frac{\text{AREA}_{\text{product}}}{(\text{AREA}_{\text{substrate}} + \text{AREA}_{\text{product}}) \times 100}$.

(10) Determination of Total Turnover Number

After expression and harvesting in 24 DW-plates the cell pellets were frozen at -80°C overnight. Afterwards the frozen pellets were lyophilized in a Christ alpha 2-4 LD plus overnight. For the reaction setup 10 mg of the E. coli whole cells were resuspended in 1 ml cycloextrin buffer and 2 µl ($c_{\text{end}}$=8.8 mM) of substrate was added to the suspension and the reaction was stirred for 20h at 30°C. The reaction was stopped by addition of DCM. The reaction was extracted two times, 2mM 1-Undecanol was added and the combined organic phases were measured over GC-MS. Quantification was made by 1-Undecanol as internal standard. The protein concentration was determined by extracting the enzyme from 10 mg for each lyophilized whole cell batch in triplikates via thermolysis (see (6)). Verification and quality control was done by SDS-PAGE (see Fig. S10).

(11) Up-scaling reactions

In order to isolate and determine the structure of the products, upscaling of the biotransformation were performed. Therefore the corresponding variant was expressed and the harvested cell pellets were lyophilized. Afterwards 3g of lyophilized whole cells were resuspended in 200 mL CD-buffer and 200 µl substrate was added. The reactions were carried out in closed 250 mL flasks at 30 °C and 250 rpm for seven days (system can be accelerated by fine tuning rotation speed, detergent concentration or cell amount as exemplified by Eichhorn et al.[26]). The crude product was centrifuged to get rid of the cell
debris. The aqueous phase containing the product encapsulated by cyclodextrin was extracted with diethyl ether three times (cyclodextrin stays in aqueous phase), reduced under vacuum, dried over MgSO$_4$, purified over column chromatography (petroleum ether: ethyl acetate; 50:1→10:1) and evaluated via NMR and GC/MS.

For Z-geranyl acetone 1c conversion 10g of lyophilized whole cells were used in 1l cyclodextrin (CD) buffer and 2 g (2.24 ml) substrate was added.

\[
\begin{align*}
\text{a) } & \quad \text{E-geranyl acetone 1t with G600R} \\
& \quad \text{Colorless oil, 0.167 ml, 0.77 mmol, 85 % yield. (4S,8S)-2,5,5,8-tetramethyl-4,5,6,7,8,8-hexahydro-4H-chromene 1t: } ^1\text{H-NMR (CDCl}_3, 500 \text{ MHz): } \delta \text{ (ppm): 0.81 (s, 3H), 0.91 (s, 3H) 1.17 (s, 3H), 1.21-1.29 (m, 1H), 1.4-1.6 (m, 5H), 1.68 (s, 3H), 1.72-1.94 (m, 3H), 4.4-4.5 (m, 1H).} \\
& \quad \quad \text{13C-NMR (CDCl}_3, 125 \text{ MHz): } \delta \text{ (ppm): 19.07 (1C), 19.21 (1C), 19.82 (1C), 20.51 (1C), 20.77 (1C) 30.31 (1C), 32.25 (1C), 39.99 (1C), 41.65 (1C), 48.37 (1C), 76.48 (1C), 94.97 (1C), 147.97 (1C). The data is consistent with the literature[27].} \\
\text{b) } & \quad \text{Z-geranyl acetone 1c with G600R} \\
& \quad \text{Colorless oil, 0.098 ml, 0.45 mmol, 49 % yield. (4R,8S)-2,5,5,8-tetramethyl-4,5,6,7,8,8-hexahydro-4H-chromene 2c: } ^1\text{H-NMR (CDCl}_3, 500 \text{ MHz): } \delta \text{ (ppm): 0.85 (s, 3H), 0.87 (s, 3H) 1.16 (s, 3H), 1.32-1.39 (m, 1H), 1.54 (s, 3H), 1.6-1.66 (m, 3H), 1.68 (s, 3H), 1.72-1.97 (m, 3H), 2.14-2.27 (m, 1H), 4.4-4.5 (d, } \end{align*}
\]
2.6 Hz, 1H). $^{13}$C-NMR (CDCl$_3$, 125 MHz): δ (ppm) 18.13 (1C), 19.79 (1C), 20.54 (1C), 21.19 (1C), 26.50 (1C), 32.46 (1C), 33.73 (1C), 39.66 (1C), 41.99 (1C), 44.00 (1C), 74.71 (1C), 94.56 (1C), 148.76 (1C).

c) Z-geranyl acetone 1c with V

\[
\begin{align*}
\text{Z-geranyl acetone 1c} & \xrightarrow{V} \text{3} \\
\end{align*}
\]
Colorless oil, 1.97 ml, 9.1 mmol, 89% yield. (−)-γ-dihydroionone 3: $^1$H-NMR (CDCl$_3$, 500 MHz): δ (ppm) 0.87 (s, 3H), 0.92 (s, 3H), 1.10-1.30 (m, 2H), 1.42-1.62 (m, 2H), 1.66-1.70 (m, 1H), 1.76-1.83 (m, 1H), 1.97-2.04 (m, 2H), 2.11 (s, 3H), 2.22-2.45 (m, 2H), 4.50-4.51 (d, $J = 1.03$ Hz, 1H), 4.75-4.77 (m, 1H). $^{13}$C-NMR (CDCl$_3$, 125 MHz): δ (ppm) 20.31 (1C), 22.62 (1C), 23.52 (1C), 26.5 (1C), 28.3 (1C), 30.20 (1C), 32.00 (1C), 34.83 (1C), 42.38 (1C), 53.40 (1C), 109.5 (1C), 149.09 (1C), 209.52 (1C). The data is consistent with the literature[6].

d) E/Z-geranyl isopropanol 8 with G600N/L607S

Yellowish oil, 0.020 ml, 0.9 mmol, 10% yield. $^1$H-NMR (CDCl$_3$, 500 MHz): δ (ppm) 2S,4S,8S-Tetrahydroedulane 14: 0.81 (s, 3H), 0.89 (s, 3H), 1.14-1.15 (d, $J = 3.1$, 3H), 1.23 (s, 3H), 1.28 (s, 1H), 1.33 (s, 1H), 1.42-1.53 (m, 5H), 1.56 (s, 2H), 1.62-1.77 (m, 4H), 3.97-4.04 (m, 1H). 2R,4S,8S-Tetrahydroedulane 15: 0.74 (s, 3H), 0.87 (s, 3H), 1.09-1.10 (d, $J = 3.2$, 3H), 1.23 (s, 3H), 1.28 (s, 1H), 1.33 (s, 1H), 1.42-1.53 (m, 5H), 1.56 (s, 2H), 1.62-1.77 (m, 4H), 3.72-3.79 (m, 1H). $^{13}$C-NMR (CDCl$_3$, 125 MHz): 2R,4S,8S-Tetrahydroedulane 15: δ (ppm) 19.54 (1C), 19.59 (1C), 20.19 (1C), 20.78 (1C), 22.72 (1C), 32.11 (1C), 33.37 (1C), 35.61 (1C), 40.75 (1C), 41.67 (1C), 53.30 (1C), 65.51 (1C), 74.83 (1C). The data is consistent with the literature[28].
4-((R)-2,2-dimethyl-6-methylenecyclohexyl)butan-2-ol 9: Characteristic methylene signals at $^1$H-NMR (CDCl$_3$, 500 MHz): δ (ppm) 4.53 (d, $J = 1.25$ Hz, 1H) and 4.75 (t, $J = 1.25$ Hz, 1H). From the chiral GC data (see N15) and the enantiopure monocyclization of 1c we assume the stereocenter here to be $R$.

e) E/Z-6,10-dimethylundeca-5,9-dien-2-al 10 with G600T

Due to the instability of the products 16 and 11 the products could not successfully be separated and therefore no yield could be determined. Nevertheless the structures can be guessed from the crude NMR data:

In order to obtain product 16 solely substrate 10 was converted with AacSHC_G600R, which favors bicyclization (see G9). Characteristic signals at $^1$H-NMR (CDCl$_3$, 500 MHz): δ (ppm) 0.70 (s, 3H), 0.74 (s, 3H), 0.90 (s, 3H) and 6.81-6.84 (m, 1H) = C2-H.

Product mixture 16 + 11 crude NMR data:

Characteristic C7-methylene signals for 11 at $^1$H-NMR (CDCl$_3$, 500 MHz): 4.65 (d, $J = 1.25$ Hz, 1H) and 4.68 (d, $J = 1.25$ Hz, 1H). From and the enantiopure monocyclization of 10c we assume the stereocenter here to be $R$.

Characteristic C4-methylene signals for 16 at $^1$H-NMR (CDCl$_3$, 500 MHz): 4.66 (d, $J = 1.10$ Hz, 1H).

f) 6,10-dimethylundeca-5,9-dien-2-ol 12 with G600N/L607S

Characteristic C7-methylene signals for 13 at $^1$H-NMR (CDCl$_3$, 500 MHz): 4.55 (d, $J = 1.00$ Hz, 1H) and 4.75 (t, $J = 1.30$ Hz, 1H). From the enantiopure monocyclization of 1c we assume the stereocenter here to be $R$. 
V. Supplementary Text

(1) Substrate binding based on Chen et al. studies\textsuperscript{[29]}

Here the authors describe water as a permanent competing coordinator and highlight that the nature of a hydrogen-bond is more significant than its strength. In the present case this means, that the oxygen´s lone pairs of the substrate $1c$ with an acceptor capability of 10.4 kJ/mol are coordinated by water molecules that can occur in this binding area having a donor capability of 7.02 kJ/mol. T600 with an imminent capability of 6.3 kJ/mol is therefore only able to generate a weak hydrogen bond (strong acceptor and weak donor). Y609 on the other hand is able to outcompete water with a donor capability of 10.4 kJ/mol and therefore can coordinate the substrate tightly (strong acceptor and strong donor).

(2) Possible explanations for substrate analoga conversion

\textit{E/Z}-mixtures of substrate analoga were used for the biotransformations. Interestingly neryl acetone $1c$ was the only substrate, which was best converted by variant V in terms of monocyclization (Fig. 3, compound 3). The substrates containing a hydroxy-moiety instead of a carbonyl showed better conversion towards monocyclic compounds with the variant G600N/L607A (Fig. 3, compound 8 \rightarrow 9 and 12 \rightarrow 13). This is presumably because asparagine’s and hydroxyl-moiety binding capabilities match better than that of threonine and a hydroxyl-moiety (cf. ref. 29). The monocyclization towards compound 11 turned out be better with variant G600T (II) than G600T/L607A. As this substrate lacks the keto-methyl group, it should not be dependent on less steric bulk at position L607 (Fig. 3, compound 10) (for detailed information see table S13 & S14).
VI. Supporting chromatograms

G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11
VII. Mass spectra
VIII. NMR spectra
IX. Computational methods

Docking studies were performed using YASARA, which uses Autodock and VINA algorithms for the calculation of defined ligand-receptor interactions\cite{30}. \textit{In silico} mutations were introduced by changing the specific amino acid in the sequence based on the AacSHC WT (PDB: 2SQC), which homology structure was modeled by Swiss-Model\cite{31}. The resulting binding energies of 25 runs were clustered and are given below. The most likely structures (=highest binding energies) were always chosen for visualization.

(1) Geranyl acetone 1t in the AacSHC WT

| Cluster | Bind.energy [kcal/mol] |
|---------|------------------------|
| 1       | 7.534                  |
| 2       | 7.238                  |
| 3       | 7.099                  |
| 4       | 6.947                  |
| 5       | 6.914                  |
| 6       | 6.785                  |

(2) Neryl acetone 1c in II (G600T)

| Cluster | Bind.energy [kcal/mol] |
|---------|------------------------|
| 1       | 7.369                  |
| 2       | 7.164                  |
| 3       | 6.984                  |
| 4       | 6.975                  |
| 5       | 6.521                  |
| 6       | 5.865                  |

(3) Neryl acetone 1c in V

| Cluster | Bind.energy [kcal/mol] |
|---------|------------------------|
| 1       | 7.044                  |
| 2       | 6.987                  |
| 3       | 6.748                  |
| 4       | 6.68                   |
| 5       | 6.588                  |
X. Amino acid sequences

AacSHC wildtype

MAEQLVEAPAYARTLDRAVEYLLSCQKDEGYWWGPLLSVNTMEAEXYVLLCHILRDVRDDRMEKIR
LLHEQREDGTWALLYPGGPPDLTTIEAYVALYIMGRDEEMQKALRFIQSQGGIESSRFTRMWL
ALVGEYPWEKVMVPPEIMFLGKRMLNIYEFGSWARATVVALSIVMSRQPVFPLPERARPVELYETD
VPPRRGAKGGGGWIFDALDRALHGYQKLSVHPFRRAAEIRALDWERQAGDGSSWGGGIQPWYA
LIALKILDMTQHPAKWEGLELYGVELDYYGWMFQASIPVWDTSGLAVLARAGLAPHDRLVKA
GEWLLDQIPTVPGDWAVKRPNLKPGFFAFQFDNYYPDVDDTAVVVALNTLRPLPERRRRDAMTK
GFRWVGQSNGGWGYVDNTSPLNPFPFCDFGEVTDPPSEDVTAVLECFSGFYGDDAWKVIR
RAVEYLKREKPDGSGFWGRGVNYLYGTGAVVSALKAVGIDTREPYIQKALDWSAEIQHNPDDGGWG
EDCRSYEDPAYAKKGASTPSQTAWALMAIAGRAESEAARRGVQYLVEQTQRPDGGWDPEYYTGT
RFPGDFYLGYTMYRHVFPLALGTYRKYQAIERR

AacSHC Variant I (G600R)

MAEQLVEAPAYARTLDRAVEYLLSCQKDEGYWWGPLLSVNTMEAEXYVLLCHILRDVRDDRMEKIR
LLHEQREDGTWALLYPGGPPDLTTIEAYVALYIMGRDEEMQKALRFIQSQGGIESSRFTRMWL
ALVGEYPWEKVMVPPEIMFLGKRMLNIYEFGSWARATVVALSIVMSRQPVFPLPERARPVELYETD
VPPRRGAKGGGGWIFDALDRALHGYQKLSVHPFRRAAEIRALDWERQAGDGSSWGGGIQPWYA
LIALKILDMTQHPAKWEGLELYGVELDYYGWMFQASIPVWDTSGLAVLARAGLAPHDRLVKA
GEWLLDQIPTVPGDWAVKRPNLKPGFFAFQFDNYYPDVDDTAVVVALNTLRPLPERRRRDAMTK
GFRWVGQSNGGWGYVDNTSPLNPFPFCDFGEVTDPPSEDVTAVLECFSGFYGDDAWKVIR
RAVEYLKREKPDGSGFWGRGVNYLYGTGAVVSALKAVGIDTREPYIQKALDWSAEIQHNPDDGGWG
EDCRSYEDPAYAKKGASTPSQTAWALMAIAGRAESEAARRGVQYLVEQTQRPDGGWDPEYYTGT
RFPGDFYLGYTMYRHVFPLALGTYRKYQAIERR

AacSHC Variant II (G600T)

MAEQLVEAPAYARTLDRAVEYLLSCQKDEGYWWGPLLSVNTMEAEXYVLLCHILRDVRDDRMEKIR
LLHEQREDGTWALLYPGGPPDLTTIEAYVALYIMGRDEEMQKALRFIQSQGGIESSRFTRMWL
ALVGEYPWEKVMVPPEIMFLGKRMLNIYEFGSWARATVVALSIVMSRQPVFPLPERARPVELYETD
VPPRRGAKGGGGWIFDALDRALHGYQKLSVHPFRRAEIRALDWLLERQAGDGGSWGGIQPPW FYA LIALKILDMTQHPAFIKGWEGLELYGVELDYGGWMFQASISPVWDTGLAVLALRAAGLPADHDRLVK A GEWLLDRQITVPGDWAVKRPNLKGPGFAFQFDNVYYPDVDTAVVWALNTRLPDERRR RDAMTK GFRWIVGMQSSNGGWGAYVDNNTSDLPNHIPFCDFGEVTDPPSEDVTAHVLECFGFSFGY DDAWKVI RRAVEYLKREQKPDGSWFGRWGVNVLYGTVGAVSVALKAVGIDTREPIQKALDWVEQHQN PDGGW GEDCRS YE D P AY AGKGASTPSQ TAWALM ALIA GGREA SE A Arr GVQYLVETQRPDG G WDEP Y YT G TF PGDFYLG YT MYR HV FPT L A LG R YK QAIERR

AacSHC Variant III (G600T_L607A)

MAEQLVEAPAYARTLDRAVEYLLSCQKDEGYWWGPLLNSVTMEAEYVLLCHLDRVD RDR MEKIRYLLHEQREDGTWALYPGPDLTTIEAYVALKYIGMSRDEEPMQKALRFIQSQ GGIESSRVFRMWLALGYPEKVPMPVEIMFLGKRMLNIYEFGSWARATVVALSI V MSRQPVFPLPERARVPELYETDVPPRRGAGKGGGGWIFDALDRALHGYQKLSVHP RRAA EIRALDWLLERQAGDGGSWGGIQPPW FYA LIALKILD TQHPAFIKGWEGLELYGVELDYG GWMFQASISPVWDTGLAVLALRAAGLPADHDRLVKAGEWLLDRQITVPGDWAVKRPNLKP GGFAFQFDNVYYPDVDTAVVWALNTRLPDERRRDAMTKGFRWIVGMQSSNGGWGAYVD NTS DLPNHIPFCDFGEVTDPPSEDVTAHVLECFGFSFGYDDAWKVI RRAVEYLKREQK PDGSWFGRWGVNVLYGTVGAVSVALKAVGIDTREPIQKALDWVEQHQNPDGGWGE DCRS YEDPAY AGKGASTPSQTA W A LM ALIAG GREA SE A Arr GVQYLVETQRPDG G WDEP Y YT G TF PGDFYAGYT MYR HVFPT L A LG R YK QAIERR
AacSHC Variant IV (Y420F_G600T_L607A)

MAEQLVEAPAYARTLRAVEYLLSCQKDEGYWWGPLLNSVTMEAAYVLLCHILDRVDRDR
MEKIRYLLHEQREDGTWALYPGPPDLTTIEAYVALKYIGMSRDEEPMQKALRFIQSQ
GGIESSRVFRMMLALVGEPWEKVMVPEIMFLGKRMPLNYEFGSWARATVALSIV
MSRQPVFPPLERARVPELEYTDVPGRRAAKGGGWIFDALRHALHGYQKLSVHPFRRAA
EIRALDWLLERQAGDSGSGGIQPPWYALIALKILDMTQHPAFIKGWELEGLEYGVELDYG
GWMFQASISPVWDTGLAVLRAAQLPADHDRALKAGEWLLRDQITVPGDWAVKRPNLKP
GGFAFQFDNVYYPDVVDTAVVVALNTRLDPERRRDAMTKGFRWIVGMQSSNGGWGAFDVNT
SDLPNHIPFDCDFEVTDDPSEDVTAHVLECFGSFYDDAWKVIRRAVEYLKREQK
PDGSWFGRGWGVNYLYTGAIVSATKAVGITREPYIQKALDWVEQHONPDGGWEDCRSAYEDPAY
AGKGASTPSQTAWALMALIAAGGRAESEARRGVQYLVETQRPDGGWDEPYYTGTTFPGDFYAAGYT
MYRHVFPRTLALGRYKQAIERR

AacSHC Variant V (A306V_Y420F_G600T_L607A)

MAEQLVEAPAYARTLRAVEYLLSCQKDEGYWWGPLLNSVTMEAAYVLLCHILDRVDRDR
MEKIRYLLHEQREDGTWALYPGPPDLTTIEAYVALKYIGMSRDEEPMQKALRFIQSQ
GGIESSRVFRMMLALVGEPWEKVMVPEIMFLGKRMPLNYEFGSWARATVALSIV
MSRQPVFPPLERARVPELEYTDVPGRRAAKGGGWIFDALRHALHGYQKLSVHPFRRAA
EIRALDWLLERQAGDSGSGGIQPPWYALIALKILDMTQHPAFIKGWELEGLEYGVELDYG
GWMFQVSISPVWDTGLAVLRAAQLPADHDRALKAGEWLLRDQITVPGDWAVKRPNLKP
GGFAFQFDNVYYPDVVDTAVVVALNTRLDPERRRDAMTKGFRWIVGMQSSNGGWGAFDVNT
SDLPNHIPFDCDFEVTDDPSEDVTAHVLECFGSFYDDAWKVIRRAVEYLKREQK
PDGSWFGRGWGVNYLYTGAIVSATKAVGITREPYIQKALDWVEQHONPDGGWEDCRSAYEDPAY
AGKGASTPSQTAWALMALIAAGGRAESEARRGVQYLVETQRPDGGWDEPYYTGTTFPGDFYAAGYT
MYRHVFPRTLALGRYKQAIERR
