Combinatorial evolution of site- and enantioselective catalysts for polyene epoxidation

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Abstract

Selectivity in the catalytic functionalization of complex molecules is a major challenge in chemical synthesis. The problem is magnified when there are several possible stereochemical outcomes and when similar functional groups occur repeatedly within the same molecule. Selective polyene oxidation provides an archetypical example of this challenge. Historically, enzymatic catalysis provided the only precedents. Although nonenzymatic catalysts are now known that meet some of these challenges, a comprehensive solution has remained elusive. Here, we describe low molecular weight, peptide-based catalysts discovered through a combinatorial synthesis and screening protocol that exhibit site- and enantioselective oxidation of certain positions of various isoprenols. This diversity-based approach, which exhibits features remiscent of the directed evolution of enzymes, delivers catalysts that compare favourably to the state-of-the-art for asymmertric oxidation of these compounds. Moreover, the approach culminated in catalysts that exhibit alternate site-selectivity in comparison to previously described oxidation catalysts.

Subject terms

chemistry; catalysis; epoxidation; chemical evolution; biomimetic

Analysis of biosynthetic pathways reveals that functional group selectivity in enzymatic reactions is a persistent issue. For example, olefins may undergo oxidation in favor of C-H bonds, or instead of alcohols. And different enzymes are engaged to reverse the order of events. Likewise, the derivation of strategies for the chemical synthesis of complex structures requires constant consideration of selectivity. The complexity of the situation is amplified when identical functional groups occur repeatedly within the same molecule. Now, for example, catalysts may be called upon to oxidize a singular olefin in the presence of others, and different catalysts may be needed to target alternative sites. Even so,

selectivity for one of the multiple sites represents but one aspect of the chemical challenge, since many functional groups may also react in ways that yield new isomers, adding stereochemical diversity to the positional selectivity outcomes. Thus, the selective oxidation of polyenes presents a profound challenge.

For many years, the selective functionalization of similarly reactive olefins within polyenes was primarily observed through the study of enzymes[1]. Today, small molecule catalysts are known that also meet some of these challenges. Yet, catalyst types that deliver substantially different site-selectivity are not well established, nor are generalizable protocols for their discovery. Venerable catalysts provide solutions to problems that enable allylic olefin epoxidation[2,3,4,5], oxidation of subterminal olefins of polyenes[6,7], and selective oxidation of conjugated systems[8,9]. Some strategies provide access to internal epoxides of polyenes using stoichiometric, auxiliary-based, "template-directed" strategies[10,11,12]. In this article, we describe low molecular weight, peptide-based catalysts[13,14], discovered through a combinatorial synthesis and screening protocol, that exhibit site- and enantioselective oxidation of various isoprenols. The diversity-based approach[15,16] delivers catalysts that compare favorably to the state-of-the-art for asymmetric oxidation of certain isoprenols, such as geraniol, farnesol, and geranylgeraniol. Moreover, the approach leads to catalysts that offer alternate site-selectivity in ways that are not known with previously described oxidation catalysts.

We have previously developed a catalytic cycle employing an aspartatic acid-based carboxylic acid and have applied this methodology to the asymmetric oxidation of allylic urethanes[17] and indoles[18]. During the course of the catalytic cycle, the carboxylic acid side chain of aspartic acid is activated with a carbodiimide, generating a transient peracid upon reaction with hydrogen peroxide (Fig. 1a). Catalytic turnover of this cycle is enhanced through addition of common nucleophilic co-catalysts such as 1-hydroxybenzotriazole (HOBt) and *N,N*-dimethylaminopyridine (DMAP). Notably, this approach is distinct from enzymatic "epoxidases"[19], which often exploit cofactors. When applied to farnesol (**1**), this catalytic cycle, employing propionic acid (**2**) as the catalyst, furnishes the same product selectivity as the textbook-level reagent *meta*-chloroperbenzoic acid (*m*-CPBA), which is used as a stoichiometric reagent (Fig. 1b).

The work began with an assessment of peptide-embedded aspartates to realize site-selective oxidation of farnesol (**1**). Could aspartate-based catalysts be found that would oxidize farnesol with high levels of site-selectivity and ultimately enantiocontrol? Would catalysts be found that would functionalize the allylic alkene[20], or other olefins of **1**? Oxidation of **1** with (+) or (−)-DIPT/Ti(O*i*-Pr)$_4$ under the conditions of Sharpless has been reported over the years to deliver **3** with excellent selectivity (82–95% ee; Fig 1c)[21,22,23,24,25,26]. However, we lacked a clear structure-based hypothesis for how peptide-based catalysts might achieve our goals. Therefore, we applied an on-bead screening protocol for discovering peptide oxidation catalysts[27] for selective oxidation of **1** (Fig. 1d).

Results and Discussion

Our approach employs the one-bead-one-compound approach to library design[29], which capitalizes on split-pool combinatorial synthesis[30,31]. This approach also allows for an evolutionary approach to catalyst optimization[32], in analogy to the strategy employed in the directed evolution of enzymes[33,34,35].

For our initial screening, we made a number of strategic decisions. For example, we elected to run reactions to low conversion initially, allowing preliminary assessment of catalyst k_{rel} values. We define k_{rel} as the relative rate of epoxidation at each olefinic site of **1** (2,3-position to give **3**; 6,7-position, **4**; 10,11-position, **5**). It is well appreciated that reaction conversion (i.e., the extent of overoxidation) can influence product distributions and muddle the extraction of meaningful k_{rel} comparisons among catalysts[36]. Thus, we planned to validate the best "hit" catalysts at a later stage under synthetically meaningful conditions, with sufficient stoichiometry of terminal oxidant to achieve good yields of products (*vide infra*).

We began with evaluation of a small library of resin-bound peptide catalysts we had synthesized in a parallel fashion, as well as a diverse split-and-pool library for the oxidation of **1**. (The data from initial screen is in the Supplementary Information.) Notably, both the parallel synthesis library and many members of the unbiased split-pool library displayed some selectivity, mostly toward the 2,3-position of **1** (Table 1, selected results). These observations were auspicious, given that many catalysts exhibited significant differences from the product distribution observed with catalytic propionic acid (**5:4:3** = 2.6:2.2:1.0; Fig. 1b). Catalyst **6** displayed the most significant deviation from the distribution afforded by propionic acid (**5:4:3** = 1.0:1.0:3.9; Table 1, entry 1).

To enhance selectivity for **3**, a small one-bead-one-compound (OBOC) combinatorial library was synthesized and biased toward peptide **6**, with four variable positions adjacent to the catalytic aspartic acid (Fig. 2a). Biasing of the library was accomplished as follows: at each variable position, approximately 50% of the functionalized polystyrene beads were coupled to the parent residue (corresponding to catalyst **6**) at each position, while the remaining 50% of the beads were evenly distributed for coupling to either seven different residues (positions $i+1$ and $i+2$) or six different residues ($i+3$ and $i+4$ positions; Fig. 2). The resulting library possesses a theoretical size of about 3,000 unique peptide sequences ($n = 7 \times 7 \times 8 \times 8$; see Supplementary Information). Upon completion, the theoretical distribution of peptides in this library follows the histogram shown in Fig. 2b: 6% of beads contain the parent sequence (**6**), 25% contain a single substitution, 38% contain two substitutions, *etc*.

Approximately 228 beads from this library were tested, including catalyst **7**, which favored epoxide **3** with approximate doubling of selectivity (**5:4:3** = 1.3:1.0:8.2, Table 1, entry 2). This result represented a further departure of the performance of a peptide-based catalyst from the control catalyst **2**. This data stimulated the evaluation of a second biased library.

For the new OBOC library, now biased toward **7**, we fixed the $i+1$ residue as Pro and $i+2$ position as Asn(Trt), hypothesizing that each was important for selectivity. The identities of

three adjacent residues (*i*+3, *i*+4 and *i*+5) were then varied. Biased library 2 (Table 1, entries 3 and 4) produced catalysts **8a** and **9a**, which exhibited even greater site-selectivity, again nearly doubling the preference for **3**. Catalyst **8a** demonstrated a **5:4:3** ratio of 1.2:1.0:18.0. Catalyst **9a** produced a ratio of 1.2:1.0:14.6. In contrast, truncated analog **10** exhibits reduced selectivity of 1.3:1.0:5.0 (entry 5).

We then assessed the performance of the catalysts upon resynthesis and evaluation in solution, and under synthetically meaningful conditions (e.g., 1.0 theoretical equiv oxidant, controlled by stoichiometry of DIC). Two points are of note. First, as noted below, we observe that catalysts provide better results when they are dissolved in solution, as opposed to when they are immobilized on beads. Second, and perhaps of greater significance, was our observation of significant enantioselectivity in these experiments. We had hypothesized that catalyst-dependent regioselectivity might also be coupled to the observation of enantioselectivity for a given monoepoxide isomer. The notion that the same intrinsic transition state organization associated with a specific regioselectivity might also translate into the organization required for enantioselectivity finds analogy in our previous rate-based selections for catalysts that also turned out to be enantioselective[29]. With these considerations in mind, we resynthesized **8a** and **9a** as their corresponding *C*-terminal methyl esters, **8b** and **9b**. When examined under conditions designed to lead to full conversion to monoepoxide (1.0 theoretical equiv oxidant: 1.0 equiv DIC, 2.0 equiv H_2O_2), methyl esters **8b** and **9b** both provide excellent overall selectivity. Catalyst **8b** delivers **3** with essentially total regioselectivity, and in 82% ee (Table 2, entry 1a). Catalyst **9b** also exhibits high regioselectivity, with **3** showing a slightly improved 86% ee (entry 1b). Strikingly, these peptide-based catalysts exhibit site- and enantioselectivities that compare favorably to the Sharpless catalyst for oxidation of **1** (Equation 1, above).

Given the analogy, we also evaluated catalyst **9b** for asymmetric epoxidation of other terpenes to assess its generality toward allylic alcohols. Peptide **9b** maintains excellent site-selectivity for geraniol (Table 2, entry 2; >100:1 site-selectivity, 87% ee). Nerol shows somewhat better enantioselectivity (entry 3, 93% ee) while retaining site-selectivity for the 2,3-*cis*-allylic position. Prenol also gives a favorable result, with the corresponding epoxide formed with 92% ee (entry 4). Catalyst **9b** shows no selectivity for the 2,3-olefin of farnesyl methyl ether, supporting a hydroxyl directed mechanism.

Initial evaluation of the libraries also revealed catalyst **11**, which exhibited a surprising, albeit modest, selectivity for the 6,7-position of **1**, to favor **4** (Table 1, entry 6; **5:4:3** = 1.5:1.9:1.0). Catalyst **11**, with D-Pro in the *i*+1 position, was unique in that we had not observed even this modest level of alternate regioselectivity previously. We therefore prepared a new library, biased toward the structure of **11**, wherein the *i*+1 D-Pro was fixed and the adjacent three residues were varied. Therein, we found catalyst **12a**, which displayed improved selectivity for **4** (Table 1, entry 7; **5:4:3** = 1.5:2.9:1.0). When resynthesized as the *C*-terminal methyl ester **12b**, and evaluated in $CHCl_3$, this catalyst yielded a product distribution of up to 1.0:4.1:1.3 (**5:4:3**). We then evaluated a second-generation library deliberately targeting 6,7-selective catalysts. Intriguingly, this study did not reveal catalysts that exhibited dramatically improved selectivity for **4**, although several comparable catalysts

were present (see Supplementary Table S1). The structures of the peptides unveiled by this library prompted us to prepare analogs of catalyst **12** in a hypothesis-driven manner. We projected that a *C*-terminal amide might better resemble the catalysts in their on-bead screening form (wherein peptides are linked to the resin as the amide). Catalysts **12c** (with *C*-terminal *n*-Bu amide) and **12d** (with *C*-terminal Gly-OMe) were examined. While both catalysts favored production of epoxide **4**, catalyst **12d** exhibited greater selectivity (**5:4:3** = 1.2:8.0:1.0) under optimized conditions (Fig. 3a). Furthermore, this product ratio simplifies an otherwise difficult separation of products by chromatography, allowing isolation of **4** in 43% yield as a >94:6 mixture of **4:5** isomers.

The appreciable, catalyst-dependent selectivity for the 6,7-position of farnesol is quite unusual. As in the case of the 2,3-selective catalysts, experiments with farnesyl methyl ether exhibited little to no selectivity (data not shown). Thus, the observed 6,7-selectivity may be largely the result of a hydroxyl-directed mechanism, despite the distance of the hydroxyl, six bonds away from the reacting olefin. Yet, unlike the results obtained with the best 2,3-selective catalysts, the production of **4** through the action of catalyst **12d** occurs with only modest, but measurable 10% ee. The observation that both high site-selectivity and enantioselectivity can track together qualitatively with 2,3-selective catalysts, but do not appear as tightly coupled with 6,7-selective catalysts is intriguing. This may be due to the higher number of rotatable bonds between the directing hydroxyl group and the preferred site of oxidation. We have observed, in our screening studies (in correspondingly unoptimized experiments), catalysts that give somewhat lower site-selectivity (Supplementary Table S2, **5:4:3**, ~1:3–4:1), but slightly higher enantioselectivity (~20–30% ee). Yet, our data sets are not large, and this concept requires further study for a full assessment. Even so, the observed 6,7-site-selectivity exhibited by catalyst **12d** greatly contrasts with the product distribution induced by *m*-CPBA, and any other reported catalyst, to our knowledge (Fig. 3b).

That catalyst **12d** shows a preference for the 6,7-position of **1** stimulated its assessment for oxidation of a more complex polyene, geranylgernaniol (**13**), which presents four competing olefins for functionalization. Fig. 3 shows the raw data for the oxidation of **1** (Fig. 3b) and **13** (Fig. 3c) with *m*-CPBA and with the catalysts described above. With geranylgernaniol (**13**; Fig. 3c), the results are quite striking. With *m*-CPBA, the four monoxides are formed essentially without control. Catalyst **9b**, as with the oxidation of **1**, exhibits high regioselectivity to give the 2,3-monooxide of geranylgeraniol (**14**). Catalyst **12d** again exhibits striking site-selectivity for an internal alkene, which we have assigned as the 6,7-monooxide **15**. The monoepoxide **14**, as well as the other monoepoxides (*i.e.*, the 10,11- and 14,15-epoxides; see Supplementary Information) are also produced in the reaction, but as minor products. The catalytic, site-selective epoxidation of geranylgeraniol (or farnesol), at a position other than the 2,3-olefin, has not previously been reported to our knowledge. The challenge of the task is manifest in the nearly equivalent reactivity of the 14,15-, 10,11- and 6,7-olefins. Thus, the observations shown in Figure 3 may represent an important step forward, enabled by the iterative peptide-based catalyst screening approach.

Conclusions

In summary, we have discovered different peptide catalysts capable of furnishing different isomers of epoxyfarnesol and related compounds that are minor products of generic peracid-based olefin epoxidation. Peptide **9b** appears to operate via a hydroxyl-directing mechanism, analogous to Sharpless' asymmetric epoxidation and with comparable selectivity. Peptide **12d**, provides unprecedented selectivity for a catalyst for the internal olefin of farnesol, with substantial site-selectivity, but modest enantioselectivity. Discovery of catalysts for complex selectivity situations, wherein myriad possible products may be formed as a function of a catalytic mechanism of bond formation, is a state-of-the-art challenge for asymmetric catalysis endeavors. The application of diversity-based approaches, such as that described above, may prove fruitful, and may also offer some analogy to the directed evolution strategies employed by natural and engineered enzymatic systems.

Methods

General procedure for epoxidation of allylic alcohols with peptide 9b

To a test tube with stir bar and Teflon-lined screw cap was added peptide **9b** (0.1 equiv); allylic alcohol (1.0 equiv.); a freshly prepared/sonicated solution containing HOBt•H_2O (0.1 equiv.) and DMAP (0.1 equiv.) in DCM (to a concentration of 0.2 M of substrate); and 30% aqueous H_2O_2 (2.0 equiv.). The test tube was placed in ice (if running reaction below room temperature) and allowed to chill before adding DIC (1.0 equiv.). The reaction tube was then sealed with a screw-cap under ambient conditions (without exclusion of air) and brought to a cold room (4 °C) where the reaction was stirred vigorously. After 7 h following addition of DIC, the reaction was quenched with a saturated aqueous solution of Na_2SO_3 and stirred for a few moments, sitting in ice, before allowing to warm to room temperature. Saturated aqueous $NaHCO_3$ and hexane or EtOAc were added, the mixture was vortexed, allowed to settle, and then the organic layer was removed, followed by two to three additional hexane or EtOAc extracts, peformed similarly. If site-selectivity was to be determined, an aliquot of the combined organics was removed, diluted with additional hexanes and analyzed by GC. The GC sample was returned to the organics, which were then concentrated *in vacuo* or under a stream of N_2, and purified by flash silica gel column chromatography.

General procedure for epoxidation of 6,7-olefin in polyisoprenols with peptide 12d

To a test tube with stir bar and Teflon-lined screw cap was added peptide **12d** (0.1 equiv); substrate (1.0 equiv.); about two thirds of a freshly prepared/sonicated solution containing HOBt•H_2O (0.1 equiv.) and DMAP (0.1 equiv.) in $CHCl_3$ (the $CHCl_3$ was passed through basic alumina prior to mixing with DMAP/HOBt); and 30% aqueous H_2O_2 (2.0 equiv.). The test tube was placed in ice and allowed to chill before adding DIC (1.0 equiv.) followed by the remaining $CHCl_3$ solution (to a concentration of 0.2 M of substrate), rinsing down the sides of the reaction tube. The reaction tube was then sealed with a screw-cap under ambient conditions without exclusion of air and placed in an ^{i}PrOH bath maintained at −12 °C to −18 °C by cryostat. The reaction was then stirred vigorously. 25–48 h following addition of DIC, the reaction was quenched with a saturated aqueous solution of Na_2SO_3 and stirred for a few moments, still at low temperature, before allowing to warm to room temperature. Saturated

aqueous $NaHCO_3$ and EtOAc were then added and the mixture was vortexed, allowed to settle, and then the organic layer was removed, along with three additional EtOAc extracts, peformed similarly. An aliquot of the combined organics was removed, diluted with additional EtOAc, and analyzed by GC. The organics were concentrated *in vacuo* and then purified by flash column chromatography.

Additional experimental procedures, descriptions of compounds, and analytical methods may be found in the Supplementary Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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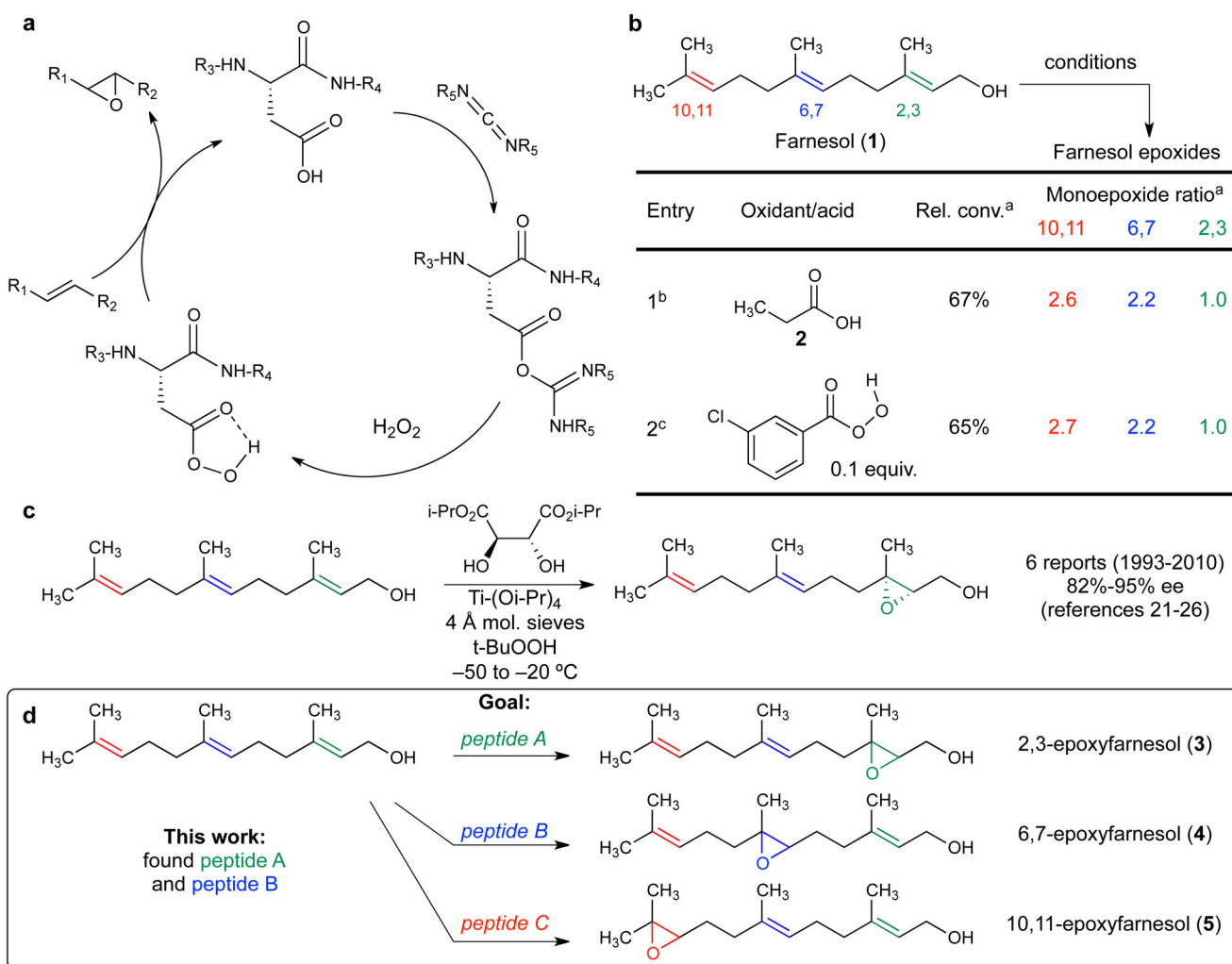
Figure 1. Precedent and goal of catalytic oxidation of farnesol

a, Catalytic cycle of aspartic acid-mediated epoxidations. **b**, Site-selectivity of *m*-CPBA and propionic acid. [a]Determined by uncalibrated GC integrations (see Supplementary Information for details). [b]1.0 equiv. *m*-CPBA, Na$_2$HPO$_4$ (2.0 equiv.), DCM, H$_2$O. [c]10 mol% acid, HOBt (10 mol%), DMAP (10 mol%), 1.0 equiv. *N,N'*-diisopropylcarbodiimide (DIC), 2.0 equiv. H$_2$O$_2$. **c**, Precedents of the Sharpless epoxidation of **1**. **d**, The goal of the present study.

a

*Split-and-Pool
Library Synthesis*

Boc-Asp-Pro-D-Thr(OtBu)-Asn(Trt)-Leu-◯

Initial Hit: **6**

Boc-Asp-Xaa-Xaa-Xaa-Xaa-linker-◯

1st Generation Library

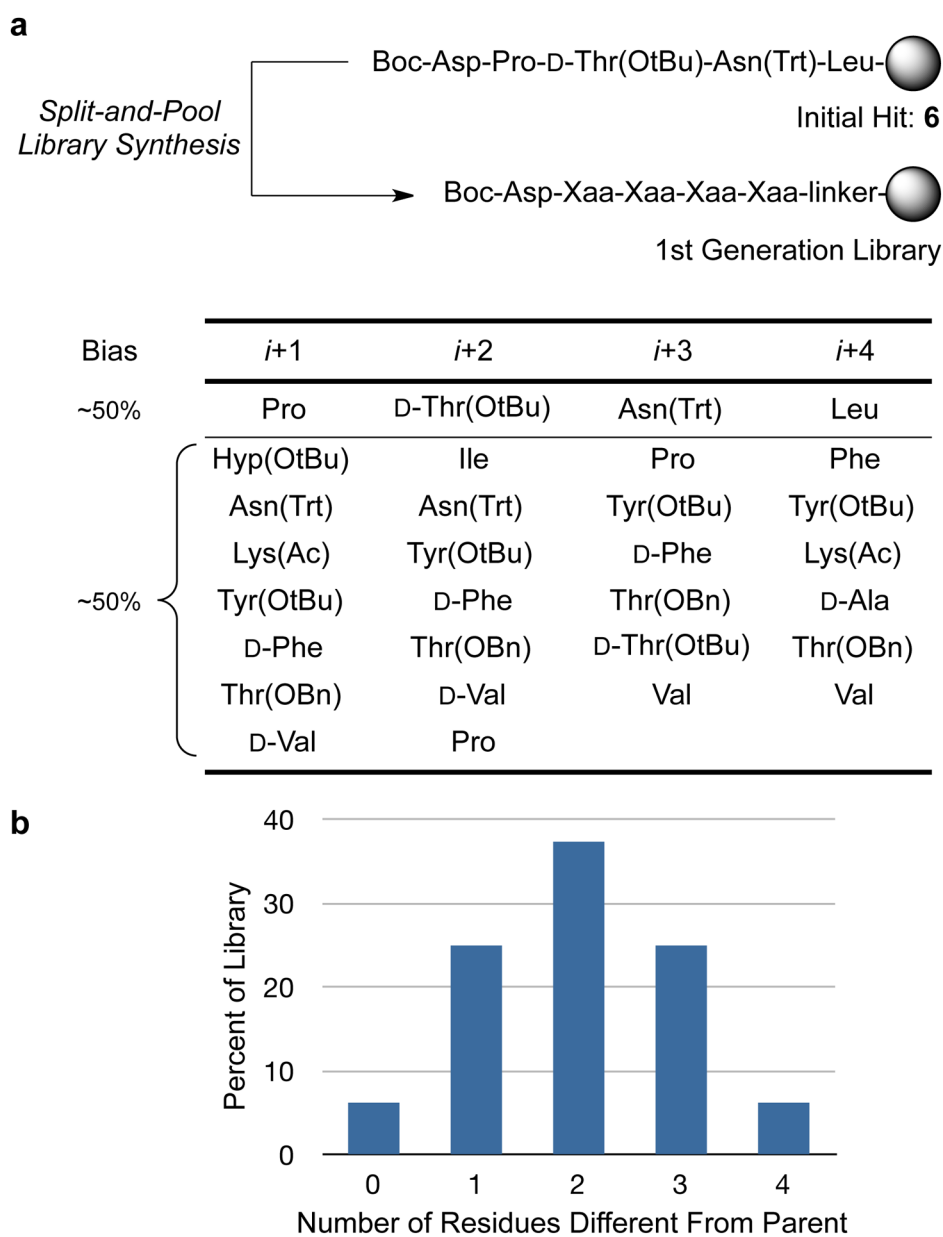| Bias	*i*+1	*i*+2	*i*+3	*i*+4
~50%	Pro	D-Thr(OtBu)	Asn(Trt)	Leu
~50%	Hyp(OtBu)	Ile	Pro	Phe
	Asn(Trt)	Asn(Trt)	Tyr(OtBu)	Tyr(OtBu)
	Lys(Ac)	Tyr(OtBu)	D-Phe	Lys(Ac)
	Tyr(OtBu)	D-Phe	Thr(OBn)	D-Ala
	D-Phe	Thr(OBn)	D-Thr(OtBu)	Thr(OBn)
	Thr(OBn)	D-Val	Val	Val
	D-Val	Pro		

b

Figure 2. Catalyst library design by split and pool synthesis via the one-bead-one-compound library method

a, Design of first directed library. **b**, Histogram of theoretical library composition.
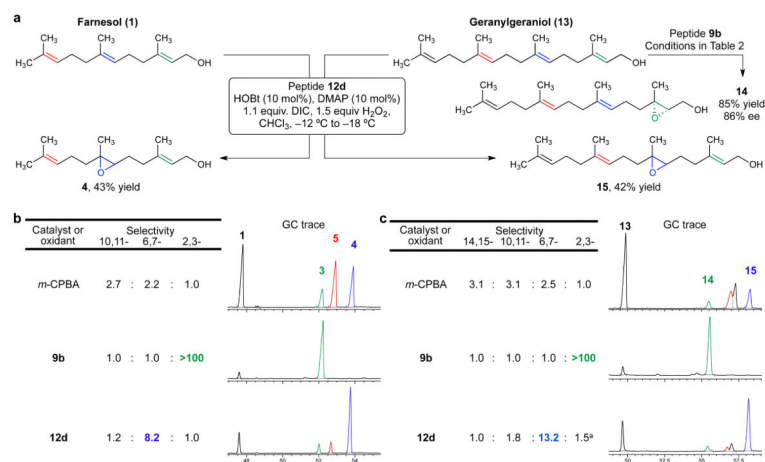
Figure 3. Results and raw data for the site-selective oxidation of farnesol and geranylgeraniol with *m*-CPBA, 2,3-selective catalyst 9b and 6,7-selective catalyst 12d

a, Optimized oxidation conditions with **12d**. Comparison of product ratios and GC spectra from crude reaction mixtures with *m*-CPBA, catalyst **9b**, and catalyst **12d**. Relevant area of GC spectra with, **b,** farnesol and, **c,** geranylgeraniol. [a]Peak has a shoulder that is integrated, overestimating the area. Conditions for *m*-CPBA reactions found in Fig. 1b; for reactions with catalyst **9b**, in Table 2; for reactions with catalyst **12d**, in **a**.

Table 1

Results from on-bead screening

Reactions for 2,3-selective catalysts were run in triplicate from trials run at the same time with the same batch of reagents. Conversions and monoepoxide ratios are estimated by GC. See Supplementary Information for details.
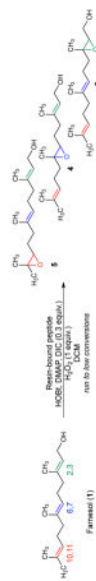
Entry	i	i+1	i+2	i+3	i+4	i+5	Catalyst	Approx. conv.	10,11- (5)	6,7- (4)	2,3- (3)	Std. dev.	Library
1	Boc-Asp	Pro	D-Thr(OtBu)	Asn(Trt)	Leu	–	**6**	13%	1.0	1.0	3.9	0.1	Initial screening
2	Boc-Asp	Pro	Asn(Trt)	D-Phe	Thr(OBn)	–	**7**	13%	1.3	1.0	8.2	0.1	Directed library #1
3	Boc-Asp	Pro	Asn(Trt)	D-Phe	Thr(OBn)	Asn(Trt)	**8a**	15%	1.2	1.0	18.0	0.5	Directed library #2
4	Boc-Asp	Pro	Asn(Trt)	D-Phe	Pro	Asn(Trt)	**9a**	12%	1.2	1.0	14.6	0.7	Directed library #2
5	Boc-Asp	Pro	Asn(Trt)	D-Phe	–	–	**10**	14%	1.3	1.0	5.0	0.0	
6	Boc-Asp	D-Pro	Thr(OBn)	Leu	–	–	**11**	14%	1.5	1.9	1.0	0.0	Truncated directed library #2
7	Boc-Asp	D-Pro	Thr(OBn)	Asn(Trt)	Tyr(OtBu)	–	**12a**	16%	1.5	2.9	1.0	0.0	Directed library #3

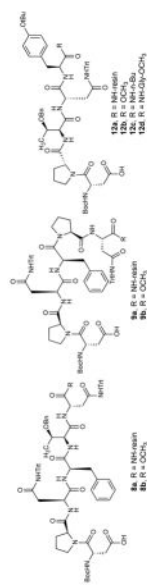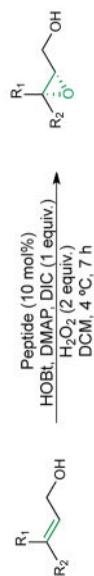Variations of selective peptide catalysts:

Table 2

Selectivity of peptide 9b with different linear terpenoid alcohols.

Entry	Substrate	Catalyst	Site-selectivity			Yield	ee	Product[a]
			10,11-	6,7-	2,3-			
1a		8b	1.0	1.0	>100	n.d	82%	
1b		9b	1.0	1.0	>100	81%	86%	
2		9b	1.0	1.0	>100	80%	87%	
3		9b	1.0	1.0	>100	79%	93%	
4		9b				75%[b]	92%	

[a] See supplementary information for details of absolute configuration assignment.

[b] GC yield.