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A Synergistic Combinatorial and Chiroptical Study of Peptide Catalysts for Asymmetric Baeyer–Villiger Oxidation

Michael W. Giuliano,[a] Chung-Yon Lin,[b] David K. Romney,[a] Scott J. Miller,[a,*] and Eric V. Anslyn[b,*]

[a] Department of Chemistry, Yale University, 225 Prospect Street, New Haven, Connecticut 06520-8107, USA
Fax: (+1)-203-436-4900; phone: (+1)-203-432-9885; e-mail: scott.miller@yale.edu
[b] Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712, USA
Fax: (+1)-512-471-8696; phone: (+1)-512-471-0068; e-mail: anslyn@austin.utexas.edu

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Dedicated to Prof. Stephen L. Buchwald on the occasion of his 60th birthday.

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Abstract: We report an approach to the asymmetric Baeyer–Villiger oxidation utilizing bioinformatics-inspired combinatorial screening for catalyst discovery. Scaled-up validation of our on-bead efforts with a circular dichroism-based assay of alcohols derived from the products of solution-phase reactions established the absolute configuration of lactone products; this assay proved equivalent to HPLC in its ability to evaluate catalyst performance, but was far superior in its speed of analysis. Further solution-phase screening of a focused library suggested a mode of asymmetric induction that draws distinct parallels with the mechanism of Baeyer–Villiger monooxygenases.

Keywords: Baeyer–Villiger oxidation; catalysis; circular dichroism; peptides

Introduction

Over the course of the storied evolution of asymmetric catalysis, certain families of catalysts have proven to be applicable to many different organic reactions.[1] While a universal understanding of why such 'privileged' catalyst scaffolds are able to translate from one reaction to another remains elusive, it is noteworthy that many catalysts operate in a similar fashion: restriction of the orientations of reacting molecules in solution in the key bond-forming steps. However, there are many asymmetric transformations in which the initial bond-forming steps of a reaction are not stereodetermining, and thus these scaffolds and/or rational design-based approaches have not been particularly successful. One such reaction is the venerable Baeyer–Villiger (B–V) oxidation,[2] which remains a formidable challenge to asymmetric catalysis.[3]

Our laboratory has employed a combinatorial approach to develop aspartic acid-containing peptide-based oxidation catalysts to address chemical transformations that are often recalcitrant to hypothesis-driven catalyst design.[4] The catalysts operate *via in situ* generation of a peracid from the reaction of a DIC-activated aspartic acid side-chain with hydrogen peroxide (Figure 1a). While initially developed for asymmetric epoxidation reactions,[5] we have also applied this catalytic cycle to the B–V oxidation of a variety of cyclic ketones.[6] Very recently, our group reported the application of this combinatorial approach to the discovery of a peptide-based B–V oxidation catalyst.[6b] This catalyst was proficient at overcoming the substrates' inherent regioselectivity biases by means of directing group interactions (Figure 1b).[7] Catalyst-substrate interactions predicated on hydrogen-bonding seem to be at the heart of the observed high selectivity in our earlier studies, but substrates that lack functionality for the same type of phenomena present a special challenge. Herein we report the synergistic use of combinatorial screening, rational library design, HPLC analysis, and a recently reported chiroptical assay that involves a multi-component assembly, all of which lays the foundation for the use of peptide-based B–V catalysts with substrates that lack directing groups (Figure 1c). The chiroptical assay[8] played a crucial role in evaluating catalyst performance, and provided stereochemical information about the oxidation products that will inform our

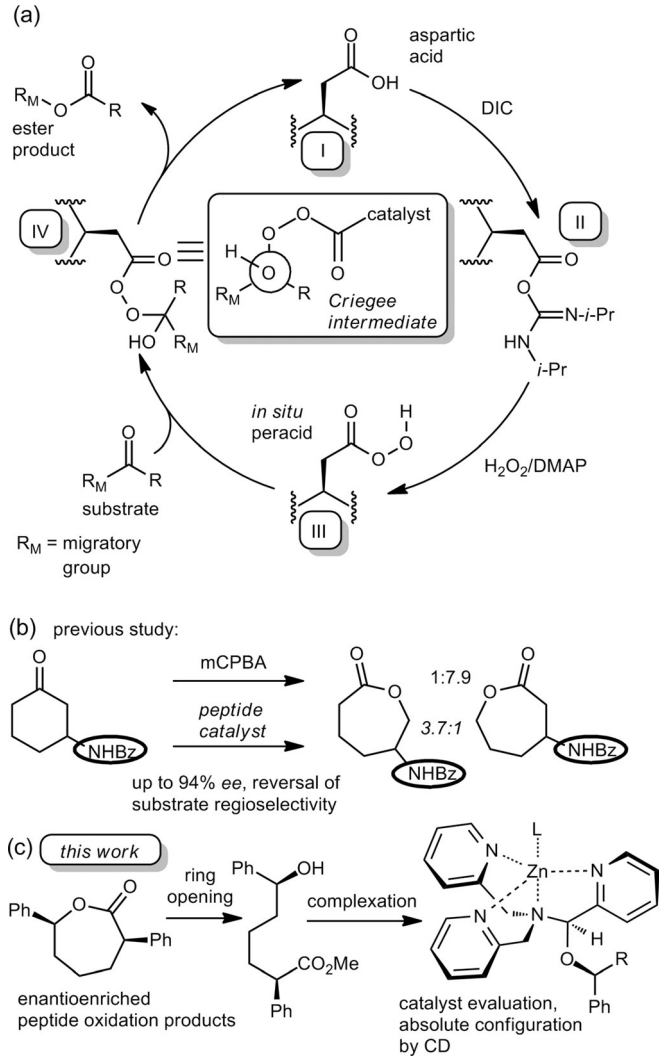


Figure 1. (a) Catalytic cycle for peracid-mediated B–V oxidations based on DIC/H_2O_2 activation of aspartic acid. Inset – Newman projection of the Criegee intermediate. (b) Inversion of inherent substrate regioselectivity preferences *via* use of a combinatorially discovered peptide catalyst and an amide directing group (ovals).[6b] (c) Work-up and assembly used herein to measure *ee* values *via* CD spectroscopy.

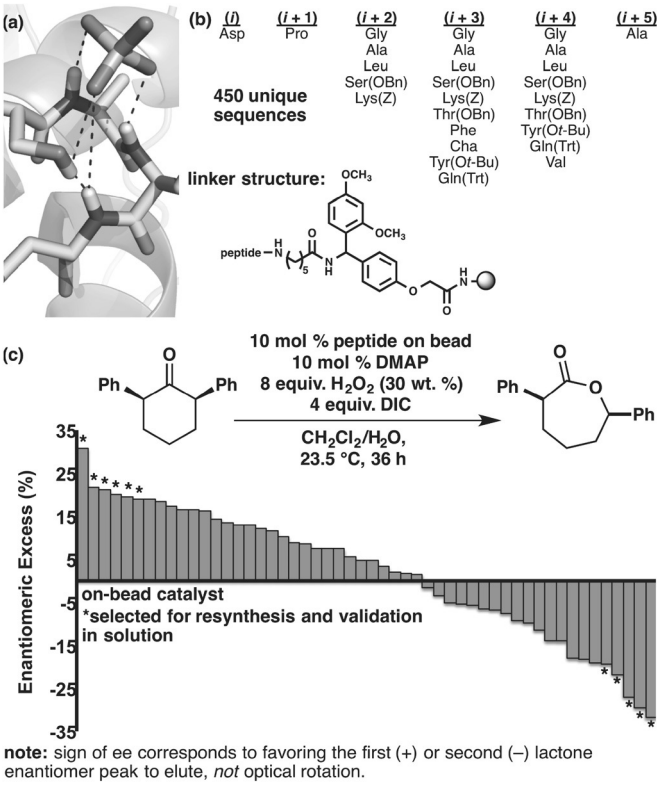


Figure 2. (a) Tetrahedral sulfate anion bound by helical protein loop. PDB: 1YCC. (b) Library composition informed by bioinformatics analysis of protein binding loop sequences. (c) Screening results using 50 beads from combinatorial peptide library. Reactions were 0.1 M in ketone substrate on a scale of 0.69 μmol ketone. Each bead contained 69 nmol peptide catalyst, corresponding to a loading of 10 mol%. H_2O_2 was added from a stock solution at a concentration of 3.0 M.

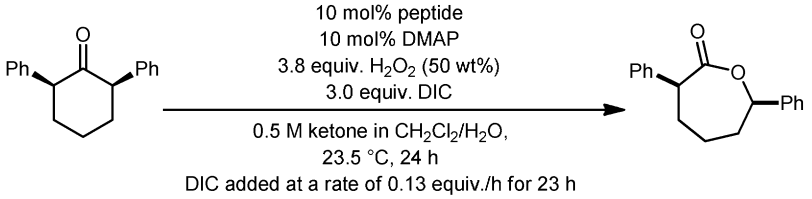
future efforts in catalyst development for the B–V oxidation. Its successful implementation has established an important benchmark toward our future goal of realizing ultra-high throughput screening, with, potentially, hundreds of *ee* values determined per hour.[9]

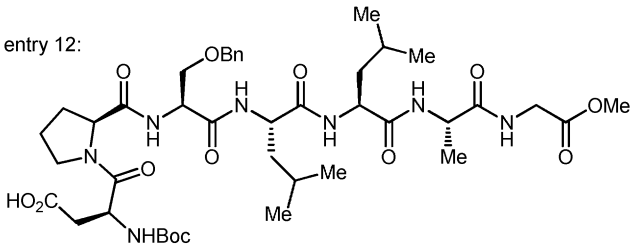
Results and Discussion

Our attention was drawn to a body of literature concerning protein-anion interactions.[10] Specifically, a number of different protein loop sequences have been observed to interact with phosphate, sulfate, and other anions (e.g., Figure 2a).[10b,c] We wondered if this sequence space could be reappropriated for the purposes of a peptide-catalyzed asymmetric B–V oxidation, owing to the structural similarities between tetrahedral anions and Criegee intermediates (*cf.* **IV** in Figure 1a). We prepared a combinatorial library based on a bioinformatic analysis of a so-called CαNN′ motif,[10b,c] in which the first two variable residues were biased toward helix-promoting amino acids and the last position incorporated Val to accommodate β-strand torsion angles. Alanine was chosen as the C-terminal residue of the library to account for the helical preferences of a number of the anion-binding protein loops.[10c] The N-terminal residues consisted of the catalytically active Asp followed by an L-Pro residue, preempting the possibility of aspartimide rearrangement[11] under the reaction conditions. This library comprised 450 unique sequences immobilized onto Rink linker-functionalized polystyrene macrobeads (Figure 2b).

Our on-bead screening commenced with the B–V oxidation of the sterically challenging ketone substrate, *cis*-2,6-diphenylcyclohexanone (Figure 2c); the previously reported peptide B–V catalyst was unable

Table 1. Solution-phase validation and focused library screening of peptide catalysts for the B–V oxidation.





Entry	Sequence[a] (i+2, i+3, i+4)	Conversion[c,d] [%]	*ee*[c,d] [HPLC, %]	*ee*[c,e] [CD, %]
1	**Leu**-Phe-Tyr(O-*t*-Bu)	75	46	–[g]
2	**Leu**-Phe-Thr(OBn)	63	42	43
3	Lys(*Z*)-Lys(*Z*)-Ala	70	24	22
4	**Leu**-Lys(*Z*)-Thr(OBn)	59	39	36
5	**Leu**-Leu-Thr(OBn)	74	33	29
6	**Leu**-Cha-Ala	77	40	36
7	Boc-Asp(OH)-OBn	> 99	−2	–[g]
8	**Ser(OBn)**-Gly-Val	55	−35	−32
9	**Ser(OBn)**-Lys(*Z*)-Ser(OBn)	76	−41	−42
10	**Ser(OBn)**-Cha-Tyr(O-*t*-Bu)	82	−37	−42
11	**Ser(OBn)**-Leu-Ser(OBn)	77	−47	−44
12	**Ser(OBn)**-Leu-Leu	75	−51	–[g]
Entry	Sequence[b] (i+2, i+3)	Conversion[c,d] [%]	*ee*[c,d] [HPLC, %]	*ee*[c,e] [CD, %]
13	Pro-Ser(O-*t*-Bu)	82	−8	−9
14	Hyp(O-*t*-Bu)-Ser(O-*t*-Bu)	90	9	8
15	Pip-Ser(O-*t*-Bu)	90	5	3
16	Ala-Ser(O-*t*-Bu)	59	4	1
17	Pro-Ser(OH)	85	−10	−5
18	Hyp(O-*t*-Bu)-Ser(OH)	41	−6	−6
19	Pip-Ser(OH)[f]	47	−3	−4
20	Ala-Ser(OH)	20	1	1
21	Pro-Thr(OBn)	88	−10	−8
22	Hyp(O-*t*-Bu)-Thr(OBn)	91	1	0

[a] Parent sequence of library hits: Boc-Asp-Pro-Xaa-Xaa-Xaa-Ala-Gly-OMe.
[b] Parent sequence of focused library: Boc-Asp-Xaa-Xaa-Leu-Leu-Ala-Gly-OMe.
[c] Average of two runs. Note: sign of *ee* corresponds to favoring the first (+) or second (−) lactone enantiomer to elute on an HPLC assay, not optical rotation.
[d] Determined from HPLC traces of worked up reactions.
[e] Determined from CD intensity at 270 nm of complex that incorporated lactone-derived secondary alcohol. All runs carried out on a scale of 0.3 mmol ketone.
[f] Single run due to limited catalyst material.
[g] Catalyst used to develop CD calibration curve.

to provide a positive result under on-bead conditions.[6] After screening only fifty beads, we observed a number of catalysts that seemed to converge into two groups. Each favored opposite enantiomers of the lactone product up to approximately 30% *ee*, despite the library being composed solely of amino acids with the L configuration. MS/MS sequencing indicated that the two groups of sequences had distinct preferences at the (i+2) position. One group universally had an *O*-benzyl serine (i+2) residue while the other group favored an (i+2) leucine residue (Table 1, entries 1–12).

To determine the absolute configuration and the *ee* of these two distinct stereochemical preferences, we turned to a recently described CD assay (Figure 3a).[8] Incorporation of chiral secondary alcohols into a tren-like ligand creates a dynamically assembled zinc(II) complex that exhibits characteristic Cotton effects at 270 nm.[8] The intensity of this signal varies linearly with the *ee* of the incorporated alcohol, while its sign correlates to the *M* or *P* twist of the pyridyl ligands about the zinc(II) center. In turn, this twist is indicative of the absolute configuration, *S* or *R*, respectively, of the enantiomer of alcohol that is in excess. Our plan was to utilize this assay in the screening of peptide catalysts in solution to develop models of catalyst–Criegee intermediate interactions. Furthermore, we were intrigued to compare the accuracy of this very rapid CD assay with our HPLC assay.

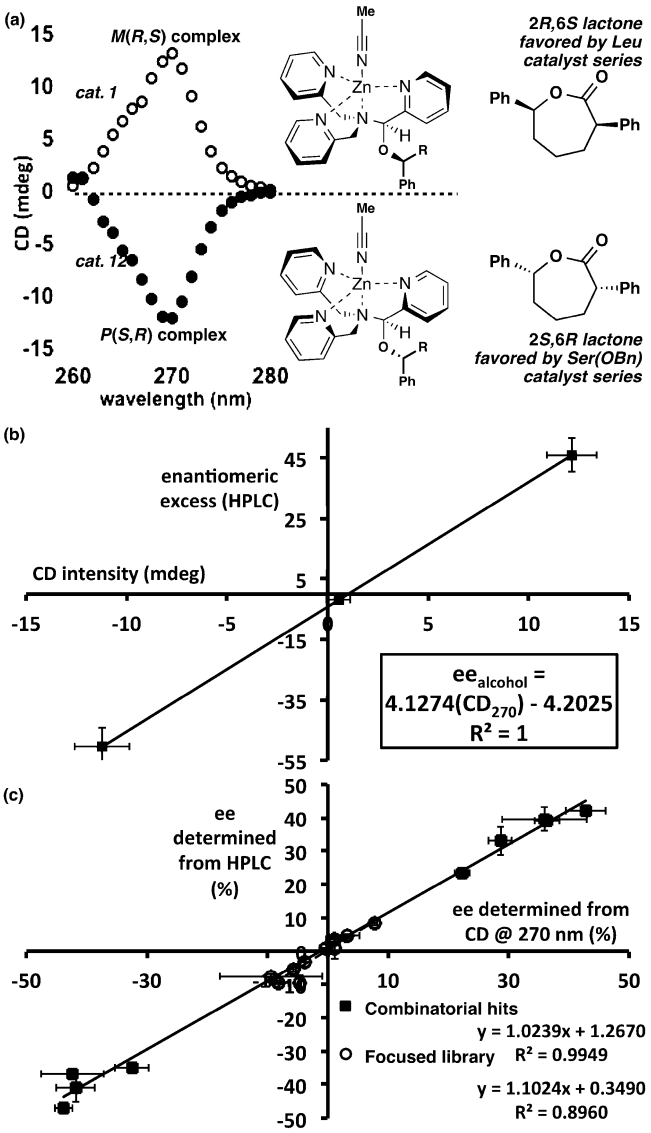


Figure 3. (a) Derivation of lactone absolute configuration from CD spectra of complexed lactone-derived 2° alcohols with catalysts **1** and **12** (*cf.* Table 1; open and filled circles, respectively). (b) Calibration curve of *ee vs.* CD intensity using catalysts **1**, **7**, and **12** (Table 1). (c) Comparison of *ee* by HPLC and *ee* by CD for duplicate screened combinatorial hits and focused library. Error bars in b and c indicate standard deviation from the average of duplicate runs.

We resynthesized 11 peptide hits from our combinatorial screen, except with a glycine methyl ester residue replacing the aminohexanoic acid linker used in solid phase screening. These peptides were contrasted to *N*-Boc aspartic acid benzyl ester as a negative control catalyst. Catalyst performance in solution was markedly improved compared to the on-bead sequences, with *ee* for the best catalysts increasing to 46% for the Leu series (Table 1, entry **1**) and 51% for the Ser(OBn) series (Table 1 entry **12**); such improvements are a common phenomenon in bead-based optimization of catalyst architecture.[4]

Because we were in the advantageous situation of having catalysts that favored opposite enantiomers, we used a catalyst of each stereochemical preference and *N*-Boc aspartic acid benzyl ester to establish a three-point calibration curve. The curve relates CD signal intensity at 270 nm to the *ee* of the alcohols derived from our product lactones *via* methanolysis (Figure 3b; Table 1, entries **1**, **7**, and **12**).[12] The y-intercept of the calibration curve corresponded to approximately a 4% error in *ee* (Figure 3b, from the expected value of 0 for racemic), which is clearly useable for a rapid screening method. In addition, we observed equivalent performance of the CD-assay when compared to HPLC for the remaining 9 combinatorial hits (Figure 3c, black trend line). Alcohols derived from the lactones produced by peptide catalysts with $(i+2)$ Ser(OBn) residues favored negative CD signals, while those with $(i+2)$ Leu residues favored positive CD signals (Figure 3a). We thus assign the stereochemistry of the lactones produced by Ser(OBn) catalysts as (2*S*,6*R*) and that produced by Leu catalysts as (2*R*,6*S*).[8] The excellent correlation we observe between the CD and HPLC data is key to another feature of the CD assay – the additional stereocenter in the lactone-derived alcohols does not interfere because the assembly is only responsive to alcohol functional groups. Furthermore, all of the alcohol samples were analyzed without the need for removal of ketone starting material, which remains a spectator; oxidation and methanolysis-related by-products/reagents were removed *via* a simple silica plug.

Previous study of peptide-catalyzed epoxidations of the terpene natural product farnesol led to the discovery of a remotely directed catalyst that implicated an $(i+2)$ ether side-chain in its mode of stereochemical induction.[4c] We were intrigued by a possible parallel observation in the Ser(OBn) series of catalysts. Additionally, we were curious if any changes to the $(i+1)$ L-Pro residue might alter the stereochemical outcome.

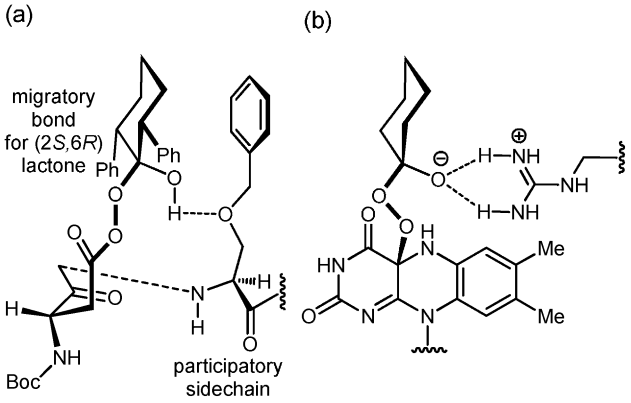


Figure 4. (a) Putative H-bonding between Criegee intermediate and Ser(OBn) side-chain. (b) Drawing of an Arg side-chain interacting with FAD-bound Criegee intermediate, the key stereodetermining interaction in B–V monooxygenases.[13]

We screened ten additional peptide catalysts targeted to address these questions (Table 1, entries **13**–**22**), maintaining the ($i+3$) and ($i+4$) Leu residues of the best performing sequence of the Ser(OBn) series. We found that, once again, the CD and HPLC assays of product *ee* were equivalent in performance, with a strong linear correlation between the *ee* values obtained with each method (Figure 3c, dashed trend line).

We were intrigued by catalysts **13**, **17**, and **21** (Table 1), which, relative to peptide **12**, varied the ($i+2$) residue to Ser(O*t*Bu), Ser(OH), and Thr(OBn), respectively. In all three cases, we observed lower product *ee*, which directly implicates this side-chain in the enantiodetermining C–C bond migration. In light of these results, and based on the aforementioned precedent involving ether-containing peptide oxidation catalysts,[4c] we entertained the possibility of the ($i+2$) side-chain acting as a hydrogen bond acceptor. Based on this hypothesis, it would seem that increased steric bulk about the side-chain oxygen, as for **13** and **21**, decreases the ability of this atom to act as an H-bond acceptor. This putative H-bond between the Criegee intermediate and the ($i+2$) side-chain would also be weakened if the Lewis basicity of the oxygen were reduced. This may be the case for peptide **17** although we cannot discount potentially deleterious interference of a free hydroxy group with the catalytic cycle.

Peptides with non-Pro ($i+1$) residues *and* altered ($i+2$) residues were not drastically changed relative to peptides **13**, **17**, and **21**. The nature of these effects, although small, may suggest that a determinant of asymmetric induction in the directing group-free peptide-catalyzed B–V oxidation is interaction between the aspartic peracid-bound Criegee intermediate and the side-chain of the ($i+2$) Ser(OBn) catalyst series. While speculative, some thoughts about this series include the possibility of an H-bond between the O-atom of the ($i+2$) Ser(OBn) residue and the OH of the catalyst-bound Criegee intermediate, potentially directing migration of the pro-*R* C–C bond (Figure 4a). Hence, there is a potential parallel with the mode of action of B–V monooxygenases, which direct flavin-bound Criegee intermediate rearrangement *via* interaction with an active site arginine side-chain (Figure 4b).[13] Structural characterization of these catalysts is a focus of our ongoing efforts to explore this intriguing possibility.

Conclusions

We have reported two families of peptide catalysts that induce enantioselective B–V oxidation by virtue of what we believe to be direct peptide–Criegee intermediate interactions. Both series of catalysts readily oxidize a highly encumbered ketone, which is itself a minimal model of the encumbered ketones found in terpene and polyketide natural products. The design principles used in our combinatorial library, namely targeting sequence space involved in the recognition of moieties similar to the key intermediate of our reaction, led to the discovery of two distinct catalyst families by screening only fifty beads. Our previous B–V and epoxidation reaction efforts based on *de novo* sequence discovery each required the screening of hundreds of beads to yield hits.[4,6b]

The CD assay we employed proved crucial in our analysis of the stereochemical course of reactions as it yielded the absolute configuration of the lactone products. We find that its ability to evaluate catalyst performance is equivalent to HPLC, even for samples with low *ee*. The assay provided enormous time-savings in the analysis of alcohol samples, reducing the time from ~30 min to just a few seconds per sample. The added configurational information, available even for samples with low levels of enantioenrichment, combined with the operational simplicity and per-sample speed of the assay itself make a case for wide implementation of this method in the synthetic chemistry community.

Experimental Section

Materials and Instrumentation

Methylene chloride, diethyl ether, and *N*,*N*-dimethylformamide (DMF) solvents were purified using a Seca Solvent Purification System by Glass Contour. Ethyl acetate, pentane, and methanol were used as obtained in glass bottles from Pharmaco-Aapger, Brand-Nu, and JT Baker, respectively. Biotech-grade *N*-methylpyrrolidone (NMP), *N*-methylmorpholine, *N*,*N*-diisopropylethylamine, and 2,2,2-trifluoroacetic

acid were used as received from Acros Organics. 2-Chlorotrityl resin for combinatorial hit and focused library synthesis was obtained from Peptides International while aminoethyl polystyrene resin macrobeads (Polystyrene A RAM) used in library synthesis were obtained from Rapp Polymere. Amino acids were obtained from Peptides International, Novabiochem (EMD), Advanced ChemTech (Creosalus), and Chem-Impex. HCTU [*O*-(1*H*-6-chlorobenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate] and EDCI [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide] were obtained from Chem-Impex and 6-chloro-HOBt (1-hydroxybenzotriazole) was obtained from Advanced ChemTech Creosalus). Dipicolylamine and zinc(II) triflate were obtained from TCI. All other reagents were used as received from Sigma–Aldrich.

^{1}H NMR spectra were obtained on 400 MHz, 500 MHz, or 600 MHz Agilent spectrometers. ^{1}H chemical shifts are reported in ppm (δ) with respect to tetramethylsilane (TMS) at 0.00 ppm. Spectra were referenced to the solvent residual peak for $CDCl_3$ at 7.26 ppm. Data are reported as: chemical shift (δ) [multiplicity, coupling constants (Hz), integration]. Multiplicities are abbreviated according to the following convention: singlet (s); doublet (d); triplet (t); quartet (q); pentet (p); doublet of doublets (dd); doublet of triplets (dt); doublet of doublet of doublets (ddd), broad singlet (bs), multiplet (m). ^{13}C NMR spectra were obtained on 400 MHz (100 MHz), 500 MHz (125 MHz), or 600 MHz (150 MHz) Agilent spectrometers. ^{13}C chemical shifts are reported in ppm (δ) with respect to TMS at 0.00 ppm. Spectra are referenced to the solvent residual peak for $CDCl_3$ at 77.16 ppm. Normal-phase HPLC data were collected on an Agilent 1100 series chromatograph equipped with a photodiode array detector and a Chiralpak IC column or a Chiralcel OD-H column. Data at 210 nm were used for integrations to yield *ee* and conversions. IR data were obtained using a Nicolet 6700 FT-IR and a partial list of peaks in ν (cm^{-1}) are reported according to convention.

Peptide sequencing was obtained with MS^E (MS/MS) data obtained on a Waters XEVO instrument equipped with ESI, a QToF mass spectrometer, and a photodiode array detector using a Waters Acquity UPLC® BEH C8 column (1.7 μm, 2.1 × 100 mm). Sequences were determined from b and y ion series.[14] High-resolution mass spectrometry (HR-MS) used electrospray ionization (ESI) and was conducted by the Mass Spectrometry Laboratory at the University of Illinois at Urbana-Champaign.

Hit peptides and the focused library sequences were purified using reverse phase chromatography on a Biotage Isolera 1 system using KP-C18-HS cartridges. Analytical thin layer chromatography (TLC) was obtained using EMD Millipore silica gel 60 plates coated with F254 ultraviolet indicator. Spots were visualized for ketone/lactone/alcohol mixtures using the UV-indicator and ceric ammonium molybdate (CAM) stain. Peptides were visualized using phosphomolybdic acid stain.

High resolution mass spectrometry (HR-MS) data was obtained *via* the mail-in service at the Mass Spectrometry Laboratory at the University of Illinois at Urbana-Champaign for solid samples of all tested catalysts and characterized compounds. Data were obtained for small molecules on a Waters Synapt G2-Si ESI mass spectrometer and data for peptides were obtained on a Waters Q-Tof Ultima ESI mass spectrometer. The Q-Tof Ultima mass spectrometer was purchased in part with a grant from the National Science Foundation, Division of Biological Intrastrcture (DBI-0100085).

Preparation of *cis*-2,6-Diphenylcyclohexanone (Adapted from ref.[15])

A mixture of the *cis* and *trans* isomers of 2,6-diphenylcyclohexanone (5.0 g, 20 mmol, 1.0 equiv.) was slurried in 150 mL 2:1 (v/v) CH_3OH:H_2O at 23.5 °C (room temperature). Pyrrolidine (30 drops; quantity was adapted from the cited procedure which stated that 3 drops were added on a scale of 2.0 mmol ketone) was added from 12-gauge needle. The reaction vessel was equipped with a water reflux condenser, heated, and held at reflux (oil bath temperature of ~95–105 °C) for thirty minutes. The reaction vessel was removed from heat and allowed to slowly cool to room temperature without stirring. Over this time a large quantity of colorless/pale yellow crystals (needles) formed. The flask was sealed with a septum and cooled for 12 h in a 4 °C refrigerator to maximize product crystallization. Crystals were isolated onto a filter paper in a porcelain Büchner funnel and washed with ~10 mL ice-cold 2:1 (v/v) CH_3OH:H_2O. Product was transferred to a tared vial and dried under high vacuum; isolated yield: 2.3072 g (46%). R_f (3:1 v/v pentane:Et_2O) = 0.55; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.32 (t, J = 7.5 Hz, 4H), 7.25 (t, J = 7.5 Hz, 2H), 7.18 (d, 7.2 Hz, 4H), 3.82 (dd, J = 5.4 Hz, 13.2 Hz, 2H), 2.41 (m, 2H), 2.16 (m, 3H), 2.09 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 208.3, 138.6, 128.9, 128.3, 127.0, 58.1, 36.5, 26.2; HR-MS (calculated/found for $C_{18}H_{19}O^+$; $[M+H^+]$): 251.1436/251.1443.

Crystallization

cis-2,6-Diphenylcyclohexanone (4 mg) was weighed into a 4 mL (1 dram) glass vial equipped with a Teflon-lined cap. H_2O (1 mL) was added to the vial and the mixture was heated to boiling with a heat gun. CH_3OH was added dropwise *via* Pasteur pipet until the sample became a homogenous solution. The vial was set on the bench-top and capped, vented only slightly to air. Colorless needle-like crystals were observed within 18 h of slow evaporation. Crystals were submitted to the Yale CBIC for structure determination by Brandon Q. Mercado. See section VII of the Supporting Information for full structural data. An image of the crystal structure (ORTEP) is shown in Figure S1 in the Supporting Information.

Additionally, CCDC 1052232 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Procedure for Peptide-Catalyzed Baeyer–Villiger Oxidation with an On-Bead Catalyst (Combinatorial Library Screening, *cf.* Figure 2c)

Because of the small scale of the reactions (0.069 μmol peptide on bead, 0.69 μmol ketone substrate), reagents were prepared into two solutions. *Solution 1*: 12.5 mg *cis*-2,6-diphenylcyclohexanone, 0.6 mg 4-dimethylaminopyridine

(DMAP), and 32 μL of *N,N*-diisopropylcarbodiimide (DIC) were dissolved in 436 μL CH_2Cl_2. *Solution 2*: 3.06 mL of 30 wt% H_2O_2 (aq.) was diluted to a volume of 10 mL with H_2O to afford a 3.0 M solution of aqueous hydrogen peroxide. Individual beads from the combinatorial library were sorted with tweezers into 250 μL glass inserts in 2 mL glass HPLC vials equipped with septa. 6.9 μL of solution 1 was added to the reaction vessel, adding ketone substrate (0.69 μmol, 1.0 equiv.), DIC (2.76 μmol, 4.0 equiv.), and DMAP co-catalyst (0.069 μmol, 0.1 equiv.). 1.9 μL of solution 2 (H_2O_2, 5.52 μmol, 8.0 equiv.) were then carefully added directly into the reagent solution in the reaction vessel. The small reaction volumes did not necessitate either stirring or any other agitation. The reactions were carried out standing at 23.5 °C (room temperature) for 36 h prior to quenching.

Quench and assay: Reactions were quenched by addition of 20 μL saturated aqueous Na_2SO_3. 200 μL HPLC-grade hexanes were added to each vial and the layers were mixed. The organics were removed, leaving the beads behind, and passed through a solid mixture of Na_2SO_4 and oxalic acid (roughly 1:1 by solid volume) in a cotton-plugged Pasteur pipet. The filter was washed with 300 μL additional hexanes. Samples were analyzed *via* normal-phase HPLC with a chiral stationary phase. 5 μL of reaction extract were injected and analyzed on a Chiralpak IC column at a flow rate of 1 mL min^{-1}, eluting with 20% (v/v) EtOH in hexanes at ambient temperature over a period of 17 min. A representative HPLC trace is shown in Figure S2 of the Supporting Information. Library design and full on-bead screening data are summarized in section II of the Supporting Information.

Procedure for Peptide-Catalyzed Baeyer–Villiger Oxidation in Solution (Screening of Hit Peptides and Focused Library) and Ring Opening to Secondary Alcohol (*cf.* Table 1, Figure 3)

Peptide catalyst (0.03 mmol, 0.1 equiv.), *cis*-2,6-diphenylcyclohexanone (75 mg, 0.3 mmol, 1.0 equiv.) and DMAP (3.7 mg, 0.03 mmol, 0.1 equiv.) were weighed into a 4-mL vial equipped with a Teflon-coated magnetic stirbar. The mixture was dissolved in 0.6 mL CH_2Cl_2 and H_2O_2 was added [50 wt% (aq.); 64.8 μL, 1.14 mmol, 3.8 equiv.]. The vials were sealed with Teflon tape and septum caps and the contents stirred at 23.5 °C (room temperature). DIC (135 μL, 0.9 mmol, 3.0 equiv.) was added *via* syringe pump at a rate of 0.13 equiv. per hour (5.9 μL h^{-1}) over a period of 23 h. The reactions were stirred one hour past this time for a total reaction time of 24 h, over which time white precipitate formed.

Quench and work-up: Reactions were quenched with 200 μL saturated aqueous Na_2SO_3. The mixtures were transferred to a separatory funnel quantitatively by rinsing several times with a total volume of 10 mL ethyl acetate (EtOAc). The mixture was diluted to a total volume of 30 mL with EtOAc and washed twice each (15 mL each wash) with saturated aqueous Na_2SO_3 and saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and sampled for HPLC analysis (~100 μL from the worked up solution). Samples were analyzed *via* HPLC using the same method as used for analyzing the reactions from the combinatorial screen (*vide supra*).

Lactone opening: The worked up reaction mixtures were concentrated to afford a solid white residue by rotovap. This residue was pushed through a plug of 10–15 mL (dry volume) silica in a 2.5 cm diameter column that was packed with 3:1 (v/v) pentane:Et_2O. Ketone/lactone mixtures were fully eluted with a total volume of 150–175 mL 3:1 (v/v) pentane:Et_2O. The plug was necessary to remove peptide, DMAP, and DIC-related reaction by-products that could coordinate and potentially interfere with the CD assay. We note that ketones and esters do not interfere with the assay, which is designed specifically for alcohol functional groups. The eluent was concentrated, transferred to a 24-mL glass vial, and fully concentrated to a white residue. The residue was rinsed into the bottom of the vial with 2–3 mL CH_2Cl_2 and this mixture was allowed to evaporate to dryness in the fume hood. This step was necessary as in the course of our study we discovered that the racemate of the lactone product was substantially less soluble than the enriched material. If all solid was not dissolved in the ring-opening step, the information obtained by either HPLC or the CD assay led to falsely high *ee* values. The dried residue was then ring opened (we note that other common lactone-opening methods led to full or partial epimerization of the C-2 stereocenter, confounding the analysis of our data). According to a modification of the procedure of Seebach,[12] a solution in CH_3OH of 0.1 M DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) and 1.0 M LiBr was prepared in a volumetric flask. An aliquot of this solution was added to the lactone sample to afford a reaction mixture that was 0.2 M in lactone (1.0 equiv.), 0.1 M in DBU (0.5 equiv.), and 1.0 M in LiBr (5.0 equiv.). The mixture was sonicated for 1 min and then stirred for 30 min at room temperature with occasional sonication to break up agglomerated solids. At 30 min, 1–2 mL CH_2Cl_2 were added to the mixture and the reaction was stirred 15 min further over which time all solids dissolved. The reaction was quenched with 1 mL of 1 N HCl (aq.). 1 mL brine was added and the mixture was extracted three times with 3 mL (each extract) CH_2Cl_2. Extracts were passed through a Pasteur pipet filter containing silica and Na_2SO_4 into a 24-mL glass vial. Solvent was removed *via* rotovap and the remaining residue for the calibration curve samples was analyzed by HLPC. All samples were dried under high vacuum and then evaluated in the CD assay for *ee* (see below and section IV of the Supporting Information).

HPLC sample preparation: <1 mg of each ring-opened product mixture was dissolved in ~250 μL of 15% (v/v) EtOH in hexanes. Samples were analyzed on a Chiralcel OD-H column, eluting with 3% (v/v) EtOH in hexanes at a flow rate of 1 mL min^{-1} at ambient temperature over a period of 32 min. HPLC analysis of lactones and their corresponding alcohols is summarized with representative traces in Figure S3 of the Supporting Information. Section IV of the Supporting Information contains tabulated HPLC integrations and CD_{270} values for all runs used to generate Table 1 and Figure 3.

Baeyer–Villiger Oxidation of *cis*-2,6-Diphenylcyclohexanone with *m*CPBA (3-Chloroperoxybenzoic Acid) and Ring Opening to Alcohol (Racemic Standard Preparation and Characterization)

cis-2,6-Diphenylcaprolactone: *cis*-2,6-Diphenylcyclohexanone (500 mg, 2.0 mmol, 1.0 equiv.) was dissolved in 4 mL CH_2Cl_2 at 23.5 °C (room temperature) in a 100-mL round-bottom flask equipped with a Teflon-coated magnetic stir-bar. *m*CPBA (1.425 g, 6.0 mmol, 3.0 equiv.) was added and the reaction mixture was stirred under an N_2 atmosphere for 36 h at 23.5 °C (room temperature).

Quench and work-up: The reaction mixture was quenched by careful addition of 5 mL saturated aqueous Na_2SO_3 and stirred for 15 min at 23.5 °C (room temperature) open to air. The mixture was diluted with 50 mL EtOAc and transferred quantitatively to a separatory funnel. The reaction mixture was then washed twice with 30 mL (each wash) saturated aqueous $NaHCO_3$ and once with 30 mL brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated to afford the crude product mixture as a white solid. The solid was purified by flash column chromatography on silica using a 3.5 cm diameter glass column and ~200 mL silica (dry volume) packed with 9:1 (v/v) pentane:Et_2O. The crude product was loaded with minimal 9:1 (v/v) pentane:Et_2O with small amounts of CH_2Cl_2 to aid in solubility and eluted with 4 column volumes (~150 mL solvent) of 9:1 (v/v) pentane:Et_2O, followed by 3:1 (v/v) pentane:Et_2O until all lactone product was observed to elute by TLC. TLC plates were developed with 3:1 (v/v) pentane:Et_2O and visualized with CAM stain. The product was isolated as a white solid; yield: 177 mg (33%). R_f (3:1 v/v pentane:Et_2O) = 0.42; ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 7.46 (d, J = 7.2 Hz, 2H), 7.39 (t, J = 7.2 Hz, 2H), 7.37 (t, J = 6.8 Hz, 2H), 7.30 (t, J = 6.4 Hz, 4H), 5.54 (d, J = 9.2 Hz, 1H), 4.02 (d, J = 8.8 Hz, 1H), 2.16 (m, 5H), 2.01 (m, 1H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ = 174.69, 140.84, 140.39, 128.75, 128.57, 128.57, 128.36, 127.41, 126.17, 81.88, 49.60, 37.19, 31.75, 28.63; HR-MS (calculated/found for $C_{18}H_{19}O_2^+$; [M+H$^+$]): 267.1385/267.1384.

Methyl 6-hydroxy-2,6-diphenylhexanoate (lactone opening): Lactone (177 mg, 0.67 mmol, 1.0 equiv.) was slurried in CH_3OH at 23.5 °C. LiBr (286.7 mg, 3.3 mmol, 5.0 equiv.) was added and some of the solids dissolved upon sonication of the mixture. DBU was added (49 μL, 0.33 mmol, 0.5 equiv.) and the reaction mixture was stirred for ~30 min at 23.5 °C with TLC monitoring (1:1 v/v Et_2O:pentane). The reaction mixture became entirely soluble over this time. We note that this procedure is simpler than that described above. We suspect that this was due to the lack of other reaction-related materials and the fact that the opening of the products of catalytic runs could yield false positives in the absence of the described rigor with respect to ensuring lactone solubility.

Work-up: The reaction was quenched with 5 mL 1N HCl and the mixture was diluted with 25 mL brine. The mixture was extracted three times with 50 mL CH_2Cl_2 (each extract). The organics were combined, dried over Na_2SO_4, filtered and concentrated to afford a pale yellow oil as crude product. The product was purified *via* flash column chromatography in a 2.5 cm diameter glass column with ~100 mL silica (dry volume). The column was eluted with an Et_2O:pentane gradient as follows: 1 CV (column volume) 1:9 (v/v); 1 CV 1:4; 1 CV 3:7; 1 CV 2:3, 1 CV 1:1. The product was isolated as a clear, colorless oil; isolated yield: 144 mg (72%; conversion complete by TLC). R_f (3:1 v/v pentane:Et_2O) = 0.12: ^{1}H NMR: (400 MHz, CD_3CN): δ = 7.30 (m, 10H), 4.54 (dt, J = 7.8 Hz, 4.8 Hz, 1H), 3.58 (s, 3H), 3.57 (t, J = 7.5 Hz, 1H), 3.13 (d, J = 4.2 Hz, 1H), 2.01 (m, 1H), 1.75 (m, 1H), 1.68 (m, 1H), 1.62 (m, 1H), 1.33 (m, 1H), 1.17 (m, 1H); ^{13}C NMR: (100 MHz, CD_3CN): δ = 175.52, 146.83, 140.56, 129.56, 129.08, 128.83, 128.11, 127.87, 126.76, 74.00, 52.38, 52.02, 39.82, 34.11, 24.54; HR-MS (calculated/found for $C_{19}H_{22}O_3^+$; [M$^+$]): 298.1569/298.1567; IR: ν = 3394 (broad), 2917, 1732, 1602, 1494, 1454, 1206, 1165, 1068, 1028, 734, 698 cm^{-1}.

Circular Dichroism Assay

CD spectra were obtained on a Jasco J-815 CD Spectrometer with Starna Type 1 GL14-S 10 mm quartz cells at 25 °C. The assembly stock solution was prepared by mixing 2-pyridinecarboxaldehyde (1 equiv.), 2,2′-dipicolylamine (1.2 equiv.), $Zn(OTf)_2$ (1 equiv.), and 4-(2-chloroethyl)morpholine hydrochloride (1 equiv.) in acetonitrile at 50 mM with respect to 2-pyridinecarboxaldehyde. The stock solution was then added to a sample containing 3–5 equiv. methyl 6-hydroxy-2,6-diphenylhexanoate of unknown enantioenrichment with molecular sieves (3 Å) and left at room temperature (20 °C) for 12–16 h. The alcohol assembly (see Figure S8 in the Supporting Information) was then diluted to 0.175 mM, with respect to 2-pyridinecarboxaldehyde, before taking CD measurements. Note: This arrangement of testing allowed for the blind testing of samples by CD, which, as discussed in the main text, provided equivalent data to HPLC with added information regarding the absolute configuration of the incorporated alcohol stereocenter. Calibration curve derivation and all CD_{270} data are summarized in section IV of the Supporting Information.

Peptide Synthesis

All peptides for on-bead and solution phase reactions were synthesized using standard Fmoc solid phase synthesis techniques on Rink amide or 2-chlorotrityl-functionalized polystyrene resins, respectively. Sequences were purified using a combination of normal-phase flash chromatography on silica and reverse phase chromatography on a Biotage Isolera 1 system. Explicit details for these procedures, MS/MS sequencing protocols, full characterization for peptides **1** and **12**, and ^{1}H NMR and HRMS data for all other solution phase peptides are summarized in sections II, III, and VI of the Supporting Information. Characterization data for peptides **1** and **12** are shown below:

Peptide 1: isolated yield: 202 mg (71%). Note: ^{1}H NMR data show highly broadened resonances, suggesting peptide aggregation, which precludes full assignment of multiplets in many cases. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.43 (broad, 1H), 7.32 (d, J = 7.2 Hz, 1H), 7.23 (m, 2H). 7.19 (m, 2H), 7.12 (d, J = 7.8 Hz, 2H), 7.03 (broad, 2H), 6.92 (d, J = 8.4 Hz, 2H), 4.95 (d, J = 8.4 Hz, 1H), 4.74 (m, 1H), 4.48 (m 2H), 4.33 (m, 1H), 4.21 (m, 1H), 4.16 (q, J = 7.2 Hz, 1H), 3.90 (broad, 4H), 3.69 (s, 3H) – the next three signals belong to three different ABX patterns, but the resonances are not resolved sufficiently for proper analysis – 3.19 (m,

1H), 3.04, (m, 2H), 2.94 (m, 1H), 2.64 (m, 1H), 2.58 (m, 1H), 2.35 (broad, 2H), 2.04 (broad, 4H), 1.60 (broad, 2H), 1.46 (s, 9H), 1.42 (d, $J=7.2$ Hz, 3H), 1.31 (s, 9H), 0.91 (d, $J=6$ Hz, 3H), 0.88 (m, 3H), 0.84 (d, $J=6$ Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta=175.03$, 173.81, 173.83, 171.56, 170.57, 154.89, 154.36, 136.75, 132.11, 129.82, 128.77, 128.65, 126.97, 124.44, 80.88, 78.56, 66.01, 62.06, 56.69, 55.85, 53.29, 52.43, 49.72, 49.42, 48.35, 48.19, 41.42, 38.53, 37.35, 36.04, 35.86, 34.27, 30.75, 29.97, 28.98, 28.47, 25.20, 25.07, 23.08, 22.49, 21.28, 17.77, 17.19, 15.42, 14.22. HR-MS (calculated/observed for $C_{48}H_{70}N_7O_{13}^+$): 952.5032/952.5035.

Peptide 12: isolated yield: 176 mg (67%). Note: ^{1}H NMR data shows highly broadened resonances, suggesting peptide aggregation, which precludes full assignment of multiplets in many cases. ^{1}H NMR (600 MHz, $CDCl_3$): δ07.43 (broad, 1H), 7.37 (d, $J=4.8$ Hz, 1H), 7.30 (m, 7H), 7.23 (d, $J=7.8$ Hz, 1H), 7.18 (d, $J=6.6$ Hz, 1H), 5.04, (d, $J=9$ Hz, 1H), 4.82 (m, 1H), 4.56 (m, half of an AB quartet, other half resolved poorly, 1H), 4.47 (m, half an AB quartet overlapped with an additional resonance, 2H), 4.37 (m, 1H), 4.29 (m, 2H), 4.19 (m, 1H), 4.07 (m, half an ABX pattern; other half unresolved, $J_{AB}=12{,}6$ Hz, $J_{AX}=6.0$ Hz, 1H), 3.95 (m, 3H), 3.87 (m, 1H), 3.78 (m, 1H), 3.69 (s, 3H), 2.72 (m, 2H), 2.34 (m, 1H), 2.00 (m, 4H), 1.70 (m, 7H), 1.43 (s, 9H), 1.43 (d, unresolved, 3H), 0.97 (d, $J=6$ Hz, 3H), 0.94 (d, $J=6.6$ Hz, 3H), 0.91 (d, $J=6$ Hz, 3H), 0.89 (d, $J=6$ Hz, 3H), ^{13}C NMR (150 MHz, $CDCl_3$): $\delta=174.13$, 173.52, 173.30, 173.09, 171.92, 171.39, 170.36, 154.90, 137.07, 128.72, 128.42, 128.29, 127.73, 80.91, 73.68, 68.40, 62.25, 56.23, 54.26, 53.37, 52.27, 49.38, 48.59, 48.35, 41.39, 39.74, 39.47, 37.32, 29.83, 28.41, 25.24, 25.12, 25.97, 23.47, 23.32, 21.30, 21.02, 17.30, HR-MS (calculated/observed for $C_{42}H_{66}N_7O_{13}^+$): 876.4719/876.4736.

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