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Exploration of Structural Frameworks for Reactive and Enantioselective Peptide Catalysts by Library Screenings

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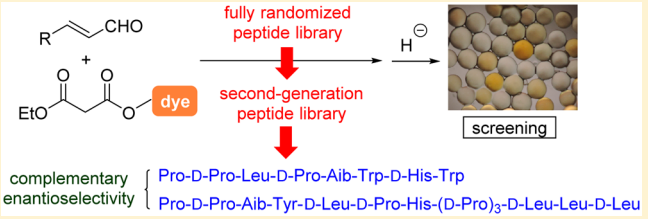
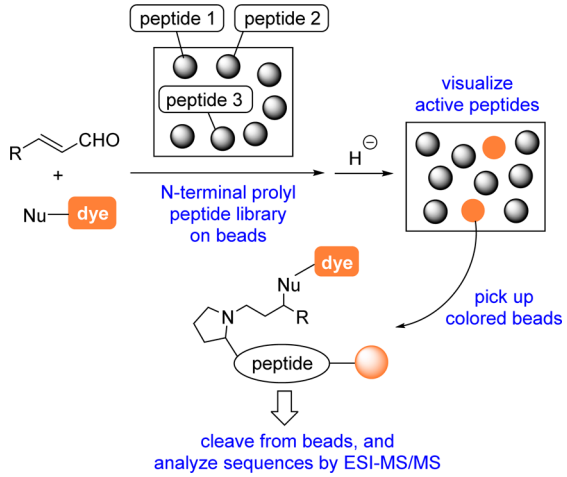
Supporting Information

ABSTRACT: By screening large-scale N-terminal L-prolyl peptide libraries, we explored efficient catalysts for asymmetric Michael addition of a malonate to an enal. The catalytically active peptides obtained by the screening could be categorized into two groups based on the similarity of amino acid sequences. One group of the peptides selectively gave an *S*-product, whereas the other gave an *R*-product, despite all of the peptides having a common N-terminal sequence, Pro-D-Pro. Further optimization by second-generation screenings afforded more reactive and enantioselective catalysts. It was found that the peptides having a histidine residue at the seventh position were good catalysts, and their reaction efficiencies were correlated with the abilities of entrapping a substrate into resin beads.



Peptides are promising candidates for efficient and selective organocatalysts.[1,2] One of the distinct features of peptide catalysis is that the structural diversity of peptides can be easily attained by changing amino acid sequences. While such a tunability of peptides assures the high potency for the development of capable catalysts, identifying an optimal sequence from numerous combinations of amino acids is difficult and time-consuming. A powerful solution to this problem is the screening of a peptide library in a high-throughput manner.[3,4] We have recently reported a novel high-throughput screening method,[5] in which resin-bound N-terminal prolyl peptides can be assayed for an amine-catalyzed Michael reaction[6] (Scheme 1). The procedure includes the following steps: (1) stirring a mixture of an enal, a dye-labeled nucleophile, and a peptide library on beads; (2) anchoring the dye-labeled product to the peptide by addition of a reducing agent; (3) collecting colored beads under the microscope; and (4) analyzing sequences by electrospray ionization tandem mass spectrometry (ESI-MS/MS) after the cleavage of peptides from the beads. Because active peptides can be colored through the in situ reductive amination, a premodification of a peptide library is not necessary. By using this method, we successfully obtained a histidine-containing peptide which had the function of enhancing the substrate binding to the catalyst.[5] In our previous study, however, peptide libraries were constructed based on a reported sequence; only a few residues were varied, while the main secondary structures such as a β-turn and a helix were retained. It was expected that new structural frameworks for more efficient catalysts could be found by screening peptide libraries with fully randomized residues, which would cause dramatic changes in enantioselectivity due to the diverse three-dimensional structures of screened peptides. In this paper, a pair of peptides with complementary enantioselectivity was developed based on the consensus sequences obtained by the screenings and was further optimized with second-generation libraries.

Scheme 1. Screening Procedure of a Prolyl Peptide Library for Michael Addition



Initially, two kinds of libraries consisting of N-terminal proline and the following variable six residues were constructed by a split-and-mix method (Figure 1).[7] At the C-terminus, octaethylene glycol and methionine were introduced to increase the sensitivity in ESI-MS/MS analyses and for the cleavage of peptides from resin beads by cyanogen bromide, respectively. The randomized part of library A contained six kinds of amino

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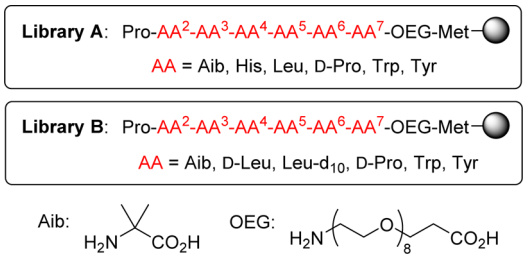
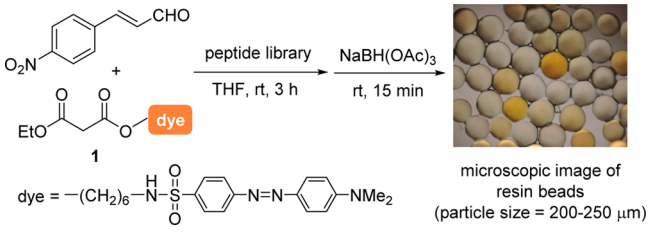


Figure 1. Peptide libraries with six randomized residues. AA = amino acid.

acids, which were the components of previously developed catalysts.[5] We expected that a histidine-containing peptide would be obtained, but upon screening this library with 4-nitrocinnamaldehyde and dye-labeled malonate **1** (Scheme 2),[8]

Scheme 2. Conditions for Initial Screenings



none of the detected sequences had a histidine residue. Thus, in order to access to a wider conformational space, D-leucine was employed in library B instead of histidine in library A.[9] The peptide sequences found by the screenings of both libraries are shown in Figure 2, and a number of observations are noteworthy: (i) all of the peptides have D-Pro at the AA^2 position; (ii) D-amino acids appeared in a higher frequency than

Library A

Sample	AA^2	AA^3	AA^4	AA^5	AA^6	AA^7	
1	D-Pro	Leu	D-Pro	Aib	Tyr	Aib	type I
2	D-Pro	Leu	D-Pro	Trp	Leu	D-Pro	
3	D-Pro	Leu	D-Pro	Tyr	Leu	Aib	
4	D-Pro	Tyr	D-Pro	Leu	Tyr	Aib	
5	D-Pro	Tyr	D-Pro	Trp	Leu	D-Pro	
6	D-Pro	Tyr	D-Pro	Trp	Trp	D-Pro	
7	D-Pro	Trp	D-Pro	Tyr	Trp	Aib	
8	D-Pro	Trp	D-Pro	Trp	Trp	D-Pro	
9	D-Pro	Leu	Aib	D-Pro	Leu	Trp	
10	D-Pro	D-Pro	D-Pro	D-Pro	Leu	Tyr	

Library B

Sample	AA^2	AA^3	AA^4	AA^5	AA^6	AA^7	
11	D-Pro	Leu	D-Pro	Aib	Tyr	D-Leu	type I
12	D-Pro	Tyr	D-Pro	Aib	Trp	D-Leu	
13	D-Pro	Tyr	D-Pro	Aib	Trp	D-Leu	
14	D-Pro	Trp	D-Pro	Aib	Trp	D-Leu	
15	D-Pro	Aib	Tyr	D-Leu	D-Pro	Tyr	type II
16	D-Pro	Aib	Tyr	D-Leu	D-Pro	Trp	
17	D-Pro	Aib	Trp	D-Leu	D-Pro	Leu	
18	D-Pro	Aib	Leu	D-Leu	D-Pro	D-Leu	
19	D-Pro	Leu	Trp	D-Leu	D-Pro	Tyr	
20	D-Pro	Trp	Tyr	D-Leu	D-Pro	Tyr	

Figure 2. Detected sequences by initial screenings. Consensus residues are boxed by blue and red lines.

statistically expected; (iii) all peptides found in library B possess D-Leu; (iv) the majority of the peptides detected from library A have a consensus sequence, Pro-D-Pro-X-D-Pro-X-X-{Aib or D-Pro}; (v) the similar sequences with D-Leu at the AA^7 position were also found in the peptides from library B; (vi) other peptides obtained from library B have a different consensus sequence, Pro-D-Pro-X-X-D-Leu-D-Pro-X; and (vii) the preserved parts in the consensus sequences from both libraries unexceptionally consisted of D-amino acids or Aib. Consequently, the detected peptides except for samples 9 and 10 could be classified as type I and type II according to the common sequences with D-amino acids (Figure 2).

Four type I peptides (samples 1, 4, 7, and 8) and two type II peptides (samples 15 and 19) were selected, and the corresponding resin-supported catalysts were prepared by a conventional solid-phase peptide synthesis. In the presence of these catalysts, asymmetric Michael addition of dimethyl malonate to 4-nitrocinnamaldehyde was conducted (Table 1).[10] This reaction hardly proceeded with the peptides obtained from colorless beads during the screening (Table 1, entries 1–3). The reaction was significantly enhanced by the type I peptides (Table 1, entries 4–7). The ee values were moderate, and the major isomer of the product had the *S*-configuration. From the fact that no amino acid with the L-form was found at the seventh residue in the type I peptides, D-amino acids seemed suitable at this position. When the seventh Aib of catalyst **2** was replaced with D-amino acids, the reactivity slightly increased (Table 1, entries 8–10), and in the cases with D-Ala and D-Trp, a higher enantioselectivity was also observed (entries 9 and 10). Next, type II peptides were examined. While the reactivity decreased somewhat compared to that of type I peptide **2**, the ee values were higher (Table 1, entries 11 and 12). The configuration of the major product was *R*, which was the opposite enantioselectivity of the case with the type I peptide. This is interesting because the two N-terminal residues of peptides **2** and **3** and even the three residues of peptides **2** and **4** are identical to each other, but the selectivities are completely altered by the subsequent residues.[3c] The replacement of D-Leu at the fifth residue in peptide **3** to L-Leu decreased the catalytic efficiency along with a dramatic loss in the enantioselectivity (Table 1, entry 13). Similar to the type I peptide, the seventh residue of peptide **3** also affected the reaction; the use of alanine instead of tyrosine lowered the conversion (Table 1, entry 14). In our previous study, it was found that a histidyl side chain allocated in proximity to an N-terminal proline enhances the reaction.[5] Accordingly, the seventh tyrosine of peptide **3** was replaced with histidine, which resulted in a marked improvement of the reaction efficiency (Table 1, entry 15).

Next, for a further enhancement of the catalytic capability, second-generation libraries were constructed based on the initial screenings and evaluations (Figure 3). For the type I peptide, an octapeptide library with four randomized residues was designed (library A-2nd). The variable positions constituted 10 kinds of amino acids, and the D-forms of amino acids were employed at the seventh residue. A different approach was employed for the type II peptide; additional six residues were introduced to the C-terminus of peptide **5** by using the same components of the initial library (library B-2nd). Such a stepwise introduction of the randomized block facilitates the screening of long peptide sequences by suppressing the scale of a library not to exceed a practically affordable number of beads. These second-generation libraries

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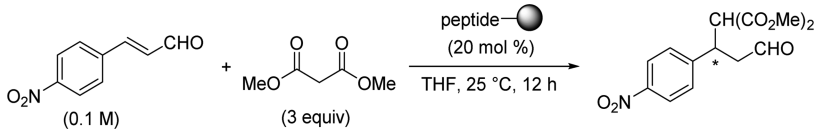
Table 1. Evaluation of Peptide Sequences for Asymmetric Michael Addition of a Malonate



entry	peptide	conversion (%)	ee (%)[a]
1	Pro-Tyr-His-(Trp)$_2$-D-Pro-Tyr (from colorless bead)	1	nd[b]
2	Pro-His-Leu-Tyr-Leu-Tyr-Leu (from colorless bead)	<1	nd[b]
3	Pro-(Tyr)$_3$-(Leu)$_2$-Trp (from colorless bead)	2	nd[b]
4	Pro-D-Pro-Leu-D-Pro-Aib-Tyr-Aib (**2**) (sample 1 in library A)	55	75 (*S*)
5	Pro-D-Pro-Tyr-D-Pro-Leu-Tyr-Aib (sample 4 in library A)	53	45 (*S*)
6	Pro-D-Pro-Trp-D-Pro-Tyr-Trp-Aib (sample 7 in library A)	56	61 (*S*)
7	Pro-D-Pro-Trp-D-Pro-Trp-Trp-D-Pro (sample 8 in library A)	57	52 (*S*)
8	Pro-D-Pro-Leu-D-Pro-Aib-Tyr-D-**Pro**	66	71 (*S*)
9	Pro-D-Pro-Leu-D-Pro-Aib-Tyr-D-**Ala**	74	85 (*S*)
10	Pro-D-Pro-Leu-D-Pro-Aib-Tyr-D-**Trp**	66	82 (*S*)
11	Pro-D-Pro-Aib-Tyr-D-Leu-D-Pro-Tyr (**3**) (sample 15 in library B)	40	96 (*R*)
12	Pro-D-Pro-Leu-Trp-D-Leu-D-Pro-Tyr (**4**) (sample 19 in library B)	30	82 (*R*)
13	Pro-D-Pro-Aib-Tyr-**Leu**-D-Pro-Tyr	25	12 (*R*)
14	Pro-D-Pro-Aib-Tyr-D-Leu-D-Pro-**Ala**	29	95 (*R*)
15	Pro-D-Pro-Aib-Tyr-D-Leu-D-Pro-**His** (**5**)	69	95 (*R*)

[a]The symbol in parentheses indicates the absolute configuration of the major enantiomer. [b]Not determined.

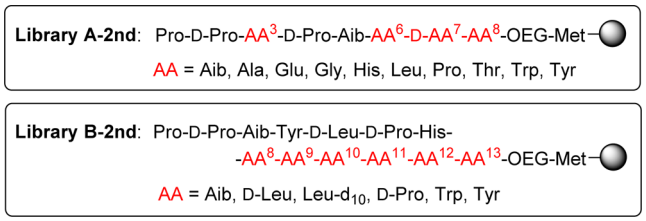
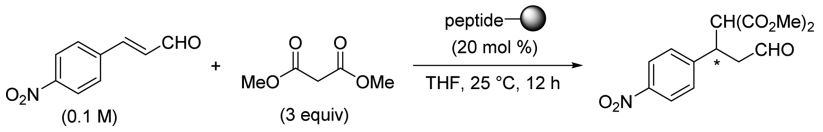


Figure 3. Design of second-generation libraries.

were screened, as well, but the reaction time for coloring was shortened to 10 min from 3 h for the initial screening. Although the detected sequences from library A-2nd did not show a noticeable tendency, the amino acids with bulky side chains such as tyrosine and tryptophan were frequently observed in all of the randomized positions (see Figure S1 in the Supporting Information). The peptides with these sequences significantly accelerated the Michael reaction (Table 2, entries 1 and 2), compared to peptide **2** (Table 1, entry 4). Among them, peptide **6** containing D-His at the seventh residue was most reactive, which was clearly indicated by the data with 10 mol % of catalyst loading (Table 2, entries 1 and 2, conversions in parentheses).[11] The importance of each amino acid of peptide **6** at the AA^6 to AA^8 positions was confirmed by alanine scanning (Table 2, entries 3–5). When the side chain of D-His at the AA^7 position was protected by a trityl group, the catalyst showed a lower reactivity (Table 2, entry 6). It is assumed that D-His of peptide **6** simply does not function as a bulky residue,

Table 2. Evaluation of Peptide Sequences from Second Screenings



entry	peptide	conversion (%)[a]	ee (%)[b]
1	Pro-D-Pro-Leu-D-Pro-Aib-Trp-D-His-Trp[c] (**6**)	91 (67)	82 (*S*)
2	Pro-D-Pro-Trp-D-Pro-Aib-Trp-D-Tyr-Trp[c]	90 (38)	81 (*S*)
3	Pro-D-Pro-Leu-D-Pro-Aib-**Ala**-D-His-Trp	(38)	84 (*S*)
4	Pro-D-Pro-Leu-D-Pro-Aib-Trp-D-**Ala**-Trp	(35)	81 (*S*)
5	Pro-D-Pro-Leu-D-Pro-Aib-Trp-D-His-**Ala**	(31)	76 (*S*)
6	Pro-D-Pro-Leu-D-Pro-Aib-Trp-D-**His(Trt)**-Trp	(23)	76 (*S*)
7	Pro-D-Pro-Leu-D-Pro-Aib-Trp-D-His-**Pya**[d] (**7**)	91 (65)	87 (*S*)
8	Pro-D-Pro-Aib-Tyr-D-Leu-D-Pro-His-(D-Pro)$_3$-D-Leu-Leu-D-Leu[e] (**8**)	74	99 (*R*)
9	Pro-D-Pro-Aib-Tyr-D-Leu-D-Pro-His-D-Leu-D-Pro-Aib-D-Pro-D-Leu-D-Leu[e]	76	98 (*R*)
10	Pro-D-Pro-Aib-Tyr-D-Leu-D-Pro-His-(D-Pro)$_3$-D-Leu	68	97 (*R*)
11	Pro-D-Pro-Aib-Tyr-D-Leu-D-Pro-His-(D-Pro)$_3$	59	98 (*R*)
12	Pro-D-Pro-Aib-Tyr-D-Leu-D-Pro-His-(D-Pro)$_2$	58	98 (*R*)

[a]The value in parentheses indicates the conversion with 10 mol % of peptide catalyst. [b]The symbol in parentheses indicates the absolute configuration of the major enantiomer. [c]Found by the screening of library A-2nd. [d]Pya indicates L-3-(1-pyrenyl)alanine. [e]Found by the screening of library B-2nd.

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but the imidazole group is actively engaged in a catalytic cycle. The residues at sixth and eighth positions are considered to have a role for fixing the histidyl side chain in an appropriate three-dimensional space. By exchanging the eighth Trp of peptide **6** with more bulky L-3-(1-pyrenyl)alanine (Pya), a comparable reactivity with an improved enantioselectivity was observed (Table 2, entry 7). Meanwhile, the screening of library B-2nd afforded the sequences highly enriched with D-Pro and D-Leu residues (see Figure S2 in the Supporting Information). The detected sequences showed slightly higher conversions and ee values for the Michael reaction (Table 2, entries 8 and 9), compared to peptide **5** (Table 1, entry 15). The enhancement of the reaction efficiency by the second screening is not so remarkable, and it seems that such a long peptide chain is unnecessary. However, when the C-terminal residues of peptide **8** were truncated, the conversion was gradually decreased as the peptide shortened (Table 2, entries 10–12).[12]

Thus, two types of catalysts, peptides **7** and **8**, with opposite enantioselectivities were successfully developed through the screenings and optimization. These peptides possess D- or L-histidine at the seventh residue, and this amino acid is considered to be important for promoting the reaction. In our previous study, it was suggested that a histidine-containing prolyl peptide had the ability to bind an α,β-unsaturated aldehyde through the formation of an iminium intermediate at the N-terminal proline followed by the Michael addition of the imidazole group.[5,13] In addition to the acceleration of the reaction by entrapping the substrate into resin beads, high enantioselectivity can be expected because one face of the substrate is covered by the histidyl residue. To examine such abilities of the present catalysts, the substrate aldehyde and the resin-supported peptides were mixed, and the amount of the aldehyde remaining in solution phase was monitored by ^{1}H NMR (Figure 4). The entrapment of the aldehyde into the resin beads was observed, and a more reactive peptide in the Michael addition showed a faster uptake of the substrate.

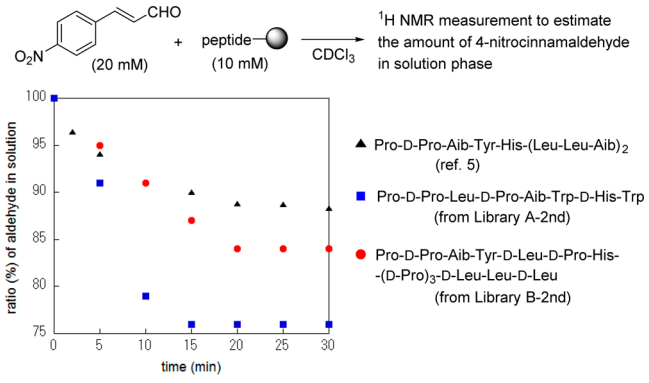
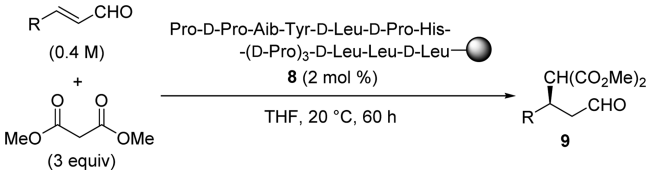


Figure 4. Evaluation of substrate-binding capability of peptides.

Finally, other substrates were tested for the asymmetric Michael addition of a malonate (Table 3). Peptide **8** was chosen for the examination due to the highest enantioselectivity. In the presence of 2 mol % of the catalyst, the reaction proceeded sufficiently and afforded the product with excellent enantioselectivity (Table 3, entries 1–6).

In conclusion, efficient and enantioselective peptide catalysts for Michael addition of a malonate were developed by the screening of fully randomized libraries followed by the optimization with second-generation libraries. Two types of peptides obtained in the screenings showed an opposite enantioselectivity to each other, although they possessed common N-terminal residues. A rational design for such sequences and optimization of them in a conventional way are considered to be difficult. This study demonstrates the potency of the present screening method, and further applications to other reactions are expected.

Table 3. Asymmetric Michael Addition of a Malonate with Peptide 8



entry	R	9	yield (%)	ee (%)
1	4-$NO_2C_6H_4$	**a**	84	99
2	4-ClC_6H_4	**b**	83	99
3	4-BrC_6H_4	**c**	80	98
4[a]	4-$MeOC_6H_4$	**d**	81	96
5	C_6H_5	**e**	81	98
6	2-naphthyl	**f**	81	97

[a]The concentration of the aldehyde was 0.6 M.

EXPERIMENTAL SECTION

Procedure for Constructing Peptide Libraries. For resin beads, TentaGel MB NH_2 (Rapp Polymere GmbH, product number: MB 250002, 0.23 mmol/g amine loading) was used. The coupling reaction of an amino acid was performed in an empty column with a filter at the bottom. Before the reaction, the resin was swollen with dichloromethane (DCM) for 20 min and washed with *N*,*N*-dimethylformamide (DMF). A typical procedure for the elongation of an amino acid was as follows. A DMF solution of an Fmoc-protected amino acid (3.0 equiv), *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 3.0 equiv), 1-hydroxybenzotriazole (HOBt, 3.0 equiv), and diisopropylethylamine (DIEA, 6.0 equiv) was added to the resin. The resulting mixture was stirred every 10 min with a vortex mixer. After 1 h, the resin was washed with DMF. To remove the Fmoc group, the resin was washed once with a DMF solution of piperidine (20 vol %), and this DMF/piperidine solution was added afresh to the resin. The resulting mixture was stirred in every 5 min with a vortex mixer. After 20 min, the resin was washed with DMF and used for the next coupling.

Preparation of Library A. After the coupling of methionine and the removal of its Fmoc group, Fmoc–NH–$(CH_2–CH_2–O)_8$–$(CH_2)_2$–CO_2H (Fmoc–OEG–OH, ChemPep, Inc., product number: 280112) was introduced. The coupling of this amino acid was performed with 2 equiv of Fmoc–OEG–OH, HBTU, and HOBt, and 4 equiv of DIEA for 4 h. After the removal of the Fmoc group, the resin beads were divided into six groups, and they were transferred into different columns. For these groups, Fmoc-protected Aib, His(Trt), Leu, D-Pro, Trp, and Tyr(*t*Bu) were attached. After the coupling reactions, all beads were combined together, and the terminal Fmoc protection was removed. The series of the above manipulations, splitting the beads, coupling amino acids, mixing the beads, and removing the Fmoc group, was repeated until six randomized residues were introduced. Finally, Fmoc–Pro–OH was attached to the combined mixture of the beads. After the removal of the terminal Fmoc group, the resin was washed with DCM and dried under reduced pressure. To remove the protecting groups of side chains, the resin was soaked in trifluoroacetic acid containing water (3 vol %), and the resulting mixture was stirred every 10 min with a vortex mixer. After 1 h, the resin was successively washed with DCM, DMF, triethylamine/DCM (1:1), DMF, and DCM and was dried under reduced pressure.

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Preparation of Library B. The same procedure for the preparation of library A was conducted using Aib, D-Leu, Leu-d_{10}, D-Pro, Trp, and Tyr(*t*Bu) as the constituent amino acids for the randomizing part.

Preparation of Library A-2nd. After the introduction of methionine and OEG and the removal of the Fmoc group according to the above procedure, the resin beads were divided into 10 groups, and they were coupled with Fmoc-protected Aib, Ala, Glu(O*t*Bu), Gly, His(Trt), Leu, Pro, Thr(*t*Bu), Trp, and Tyr(*t*Bu). All beads were combined together, and the terminal Fmoc protection was removed. For the randomized positions of the library, amino acids were introduced in this way. For the seventh residue from the N-terminus, Aib, Gly, and the D-forms of Ala, Glu(O*t*Bu), His(Trt), Leu, Pro, Thr(*t*Bu), Trp, and Tyr(*t*Bu) were used. The common sequences, Pro-D-Pro at the first and second residues and D-Pro-Aib at the fourth and fifth residues, were introduced by the coupling reaction to the combined mixture of the beads. After the introduction of all residues, the following procedure was the same as above.

Preparation of Library B-2nd. Instead of N-terminal proline of library B, the common six residues were introduced to the randomized part using Fmoc-protected His(Trt), D-Pro, D-Leu, Tyr(*t*Bu), Aib, D-Pro, and Pro, successively.

Procedure for Screening Peptide Libraries. To a round-bottom flask that contained a peptide library on resin beads (0.3 g, approximately 0.05−0.06 mmol of the terminal prolyl group), a solution of 4-nitrocinnamaldehyde and dye-labeled malonate **1** (10 mM for each reagent) in THF (5 mL) was added. After this mixture was stirred for 3 h in the initial screening, or for 10 min in the second screening, sodium triacetoxyborohydride (0.25 mmol) was added. After the resulting mixture was stirred for an additional 15 min, the resin beads were transferred to an empty column with a filter at the bottom and washed with DMF and DCM, successively. Then, the resin beads were put on a glass plate, and highly colored beads were picked up with a needle under a microscopic view. Each selected bead was put into an Eppendorf tube separately. To detach peptides from the beads, an aqueous solution (8 μL) of formic acid (70%) and cyanogen bromide (50 mg/mL) was added to the tube, and this was left in the dark at 25 °C for 24 h. The solvent was removed under a stream of nitrogen gas and completely dried under reduced pressure. The residue was dissolved in methanol (100 μL), and the solution was passed through a membrane filter (pore size: 0.45 μm). This sample was analyzed by an ESI-MS/MS measurement in a positive-ion mode.

Preparation of Resin-Supported Peptide Catalysts. A similar procedure with the preparation of peptide libraries was employed. For resin beads, TentaGel S NH_2 (AnaSpec, Inc., product number: 22798, 0.25 mmol/g amine loading) was used. Instead of HBTU and HOBt, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate and 1-hydroxy-7-azabenzotriazole were used. The reaction time for the coupling was 30 min with stirring every 5 min, and the completion of the reaction was checked by the Kaiser test. The coupling reaction to an N-terminal Aib, L-, or D-Pro residue was performed twice without the Kaiser test.

Procedure for the Evaluation of Peptide Catalysts (Tables 1 and 2). To a mixture of a resin-supported peptide (0.006 or 0.003 mmol of the terminal prolyl group), 4-nitrocinnamaldehyde (0.03 mmol), and THF (300 μL) was added dimethyl malonate (0.09 mmol). After the mixture was stirred at 25 °C for 12 h, the peptide catalyst was filtered off and washed with chloroform. The conversion was determined by ^{1}H NMR measurement of the crude mixture in $CDCl_3$. The ratio of the starting aldehyde and the product was used for the calculation. Then, the crude mixture was dissolved in *t*-butyl alcohol (200 μL), 1 M aqueous solution of sodium dihydrogen phosphate (200 μL), and 1 M aqueous solution of potassium permanganate (200 μL). After the mixture was stirred for 30 min, 1 M aqueous solution of hydrochloric acid was added. The mixture was extracted with ethyl acetate, and the organic layer was washed with water three times. After the removal of the solvent under reduced pressure, the residue was dissolved in methanol (500 μL) and toluene (200 μL). To this solution was added trimethylsilyldiazomethane (0.6 M solution in hexane, 200 μL). After the mixture was stirred for 10 min, the solvent was removed under reduced pressure. The residue was purified by preparative TLC (ethyl acetate/hexanes 1:1) to afford the corresponding methyl ester of the product.[10b] Enantiomeric excess of this compound was determined by HPLC analysis with Chiralpak IA (hexane/2-propanol 7:3, 0.8 mL min^{-1}).[5]

Procedure for the Peptide-Catalyzed Michael Addition of a Malonate (Table 3). To a mixture of resin-supported peptide **8** (8.7 mg, 0.0016 mmol of the terminal prolyl group), α,β-unsaturated aldehyde (0.08 mmol), and THF (200 μL, or 133 μL for 4-methoxycinnamaldehyde) was added dimethyl malonate (0.24 mmol). After the mixture was stirred at 20 °C for 60 h, the peptide catalyst was filtered off and washed with chloroform. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (ether/hexanes 3:7 to 9:1) to afford the Michael product. The spectral data of all products (**9a**,[10d] **9b**,[10c] **9c**−**e**,[10b] **9f**[5]) were reported. To determine the enantioselectivity, Michael products were converted to the corresponding methyl esters according to the previous report.[5,10b]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01591.

Results of second-generation screenings, ESI-MS/MS spectra of peptides, NMR spectra of Michael products, and HPLC traces (PDF)

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Notes

The authors declare no competing financial interest.

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