Supporting Information

Chiral Amplification of Prebiotic Peptide Synthesis Induced by Chemical-Physical Interactions on Calcite Surfaces

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1 Materials and Methods

1.1 Materials

L-^{15}N-Leu, L-^{15}N-Ala, L-^{15}N-Val, L-^{15}N-Ile, L-^{15}N-Phe were obtained from Shanghai Research Institute of Chemical Industry CO., LTD., China. D-Leu, D-Ala, D-Val, D-Ile, D-Phe were obtained from Shanghai Macklin Biochemical Co., Ltd., China. P_3m were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd., China. Ultrapure water (18.2 MΩ cm) from a Milli-Q water purification system (Millipore, Bedford, MA) was used to prepare solutions and the mobile phase. The white and transparent Calcite was obtained from Lianyungang, Jiangsu Province, China. The surface area was used in this work was approximately 32.2 cm^2.

1.2 The method of elution of Calcite

The reaction surface was cleaned followed the order of deionized water, methanol, dichloromethane, methanol, and deionized water for a total of 2 cycles. The surface was soaked in the above solvents and was shook for 2 min during the cleaning procedure. Finally, the reaction surface was corroded with 0.02M HCl for 1 min.

1.3 Physical adsorption experiments

Place the cleaned calcite surface in a glass-bottom Petri dish with a diameter of 10 cm and add 10 mL of a 0.01 M racemic amino acid solution (pH 7 ± 0.2). In the experiment, L-type amino acids were labeled using 15N isotopic labeling technology to enable subsequent quantitative detection via LC-MS. This volume ensures the complete immersion of the calcite bottom surface in the solution while avoiding contact of any other surfaces with the liquid. The system undergoes dry-wet cycles. During the dry phase, the system is placed at 60 °C for 11.5 hours; during the wet phase, 10 mL of deionized water is added to the Petri dish at room temperature, ensuring that the dried amino acids on the calcite surface dissolve again. After standing for 0.5 hours, the next cycle begins at 60 °C. After completing 7 dry-wet cycles, the calcite is removed, and its bottom surface is immersed in deionized water to remove any unadsorbed amino acids. Once dried, the bottom surface is immersed in 10 mL of 0.02 M hydrochloric acid for 30 seconds. Subsequently, 0.02 M hydrochloric acid is applied to the surface using a pipette for another 20 seconds to ensure complete elution of adsorbed amino acids. Finally, the acidic eluate is collected and freeze-dried. The obtained dried powder is dissolved in 0.5 mL of deionized water and analyzed using LC-MS to quantify the ee values of amino acids. Due to the use of L-15N-AA, which shows a significant mass difference of 1 Dalton (Da) compared to D-AA in mass spectrometry analysis, rapid differentiation between the two is achieved.

1.4 Chemical-physical adsorption experiments

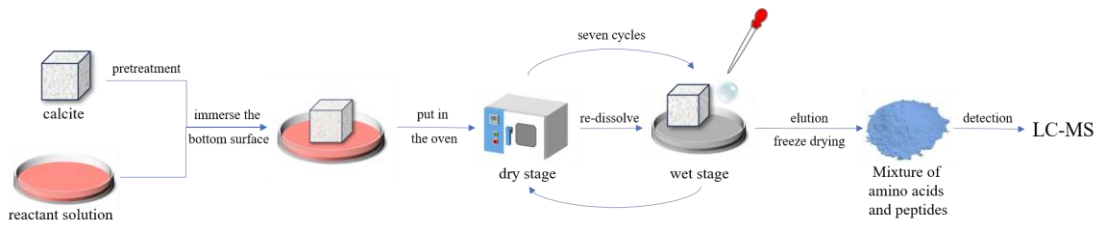
This study selected five amino acids—Ala, Leu, Val, Ile, and Phe—as the research subjects. The calcite used in the reactions was white and transparent, with dimensions of 62 × 52 × 77 mm and an approximate surface area of 32.2 cm^2 for adsorption studies.

After treating the calcite samples using the cleaning method to remove surface impurities, as shown in Figure S1, the samples were placed in a glass Petri dish with a diameter of 10 cm. Then, 10 mL of reaction solution containing 0.01 M racemic amino acids and 0.01 M P_3m (pH 10 ± 0.2, a pH closer to the prebiotic environment favorable for peptide formation) was added to the dish, initiating the dry-wet cycling process. During the dry phase, the setup was incubated in an oven at 60 °C for 11.5 hours. During the wet phase, 10 mL of deionized water was added at room temperature to dissolve the dried substrates and products on the calcite surface, completing the phase transition after 0.5 hours of standing.

After completing seven dry-wet cycles, the calcite was removed, and its bottom surface was immersed in deionized water to eliminate any unabsorbed amino acids and dipeptides. Once dried, the bottom surface was immersed in 10 mL of 0.02 M hydrochloric acid for 30 seconds. To ensure the complete elution of adsorbed amino acids, 0.02 M hydrochloric acid was further applied to the surface using a pipette for an additional 20 seconds. The acidic eluate was then collected and freeze-dried. The obtained dried powder was dissolved in 0.5 mL of deionized water and quantitatively analyzed using LC-MS to determine the ee values of amino acids and dipeptides.

For the tripeptide chirality study, a reaction solution containing 10 mL of 0.05 M L-type dipeptides, 0.05 M racemic amino acids (where the L-type components were labeled with 15N isotopes), and 0.01 M P_3m was used. The same dry-wet cycling process described above was applied to the calcite surfaces, and the eluates were quantitatively analyzed post-cycling.

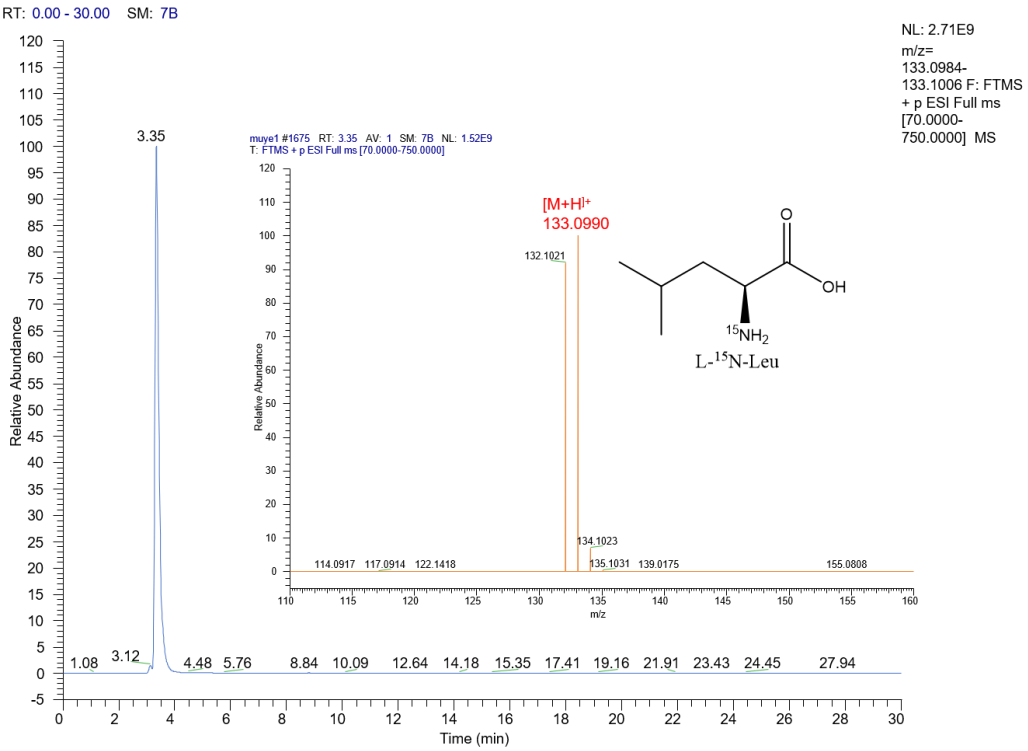
In the quantitative analysis of dipeptides, ^{15}N labeling technology was employed. The dipeptide products L-^{15}N-AA_2 and D-AA_2 exhibited a significant mass difference of 2 Da in mass spectrometry analysis. Similarly, for tripeptide analysis, a mass difference of 1 Da was observed between L-AA_2-L-^{15}N-AA, L-^{15}N-AA-L-AA_2 and L-AA_2-D-AA, D-AA-L-AA_2, enabling rapid differentiation and analysis of these enantiomeric pairs.



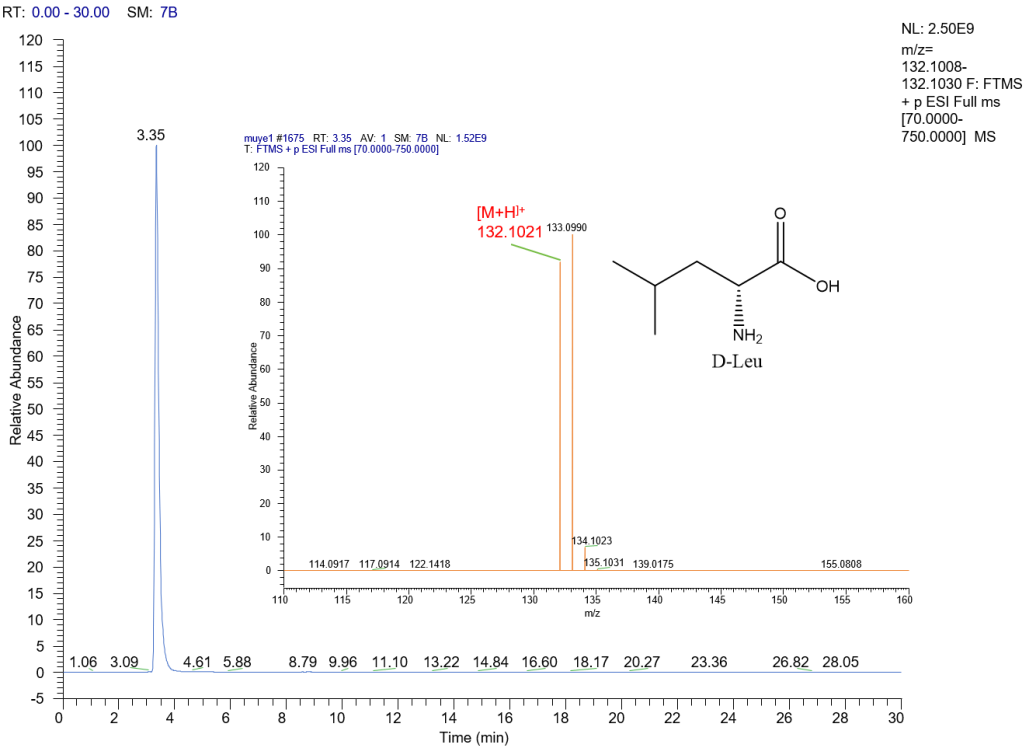
Supplementary Fig. 1 Experimental schematic diagram of chiral amplification on the surface of calcite through chemical- physical interactions.

2 Supplementary Figures of Chemical-Physical Interactions

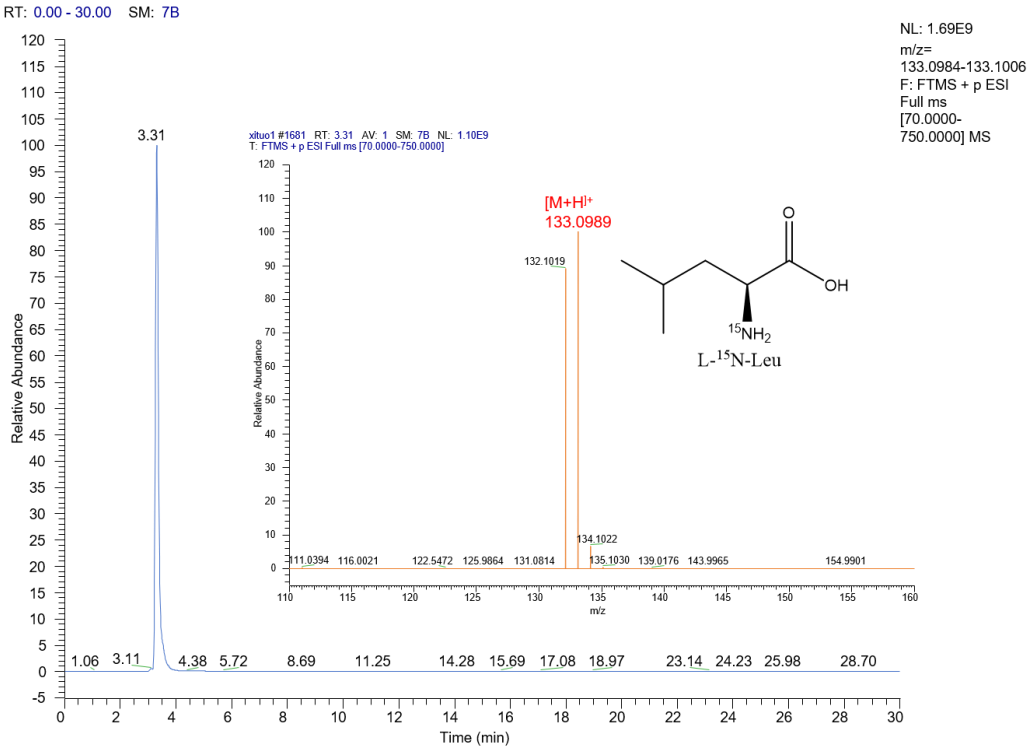
2.1 Chiral selection of Leucine dipeptide on calcite surface. (Figs. S2-S7)



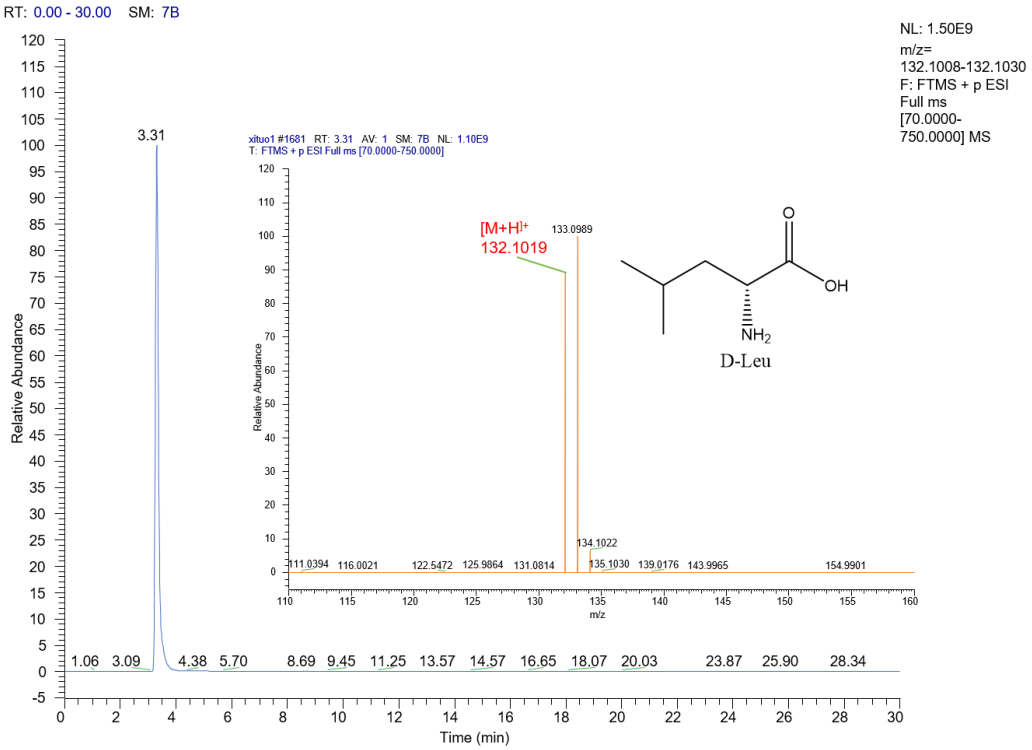
Supplementary Fig. 2 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 133.0990 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Leu with a retention time of 3.35 min.



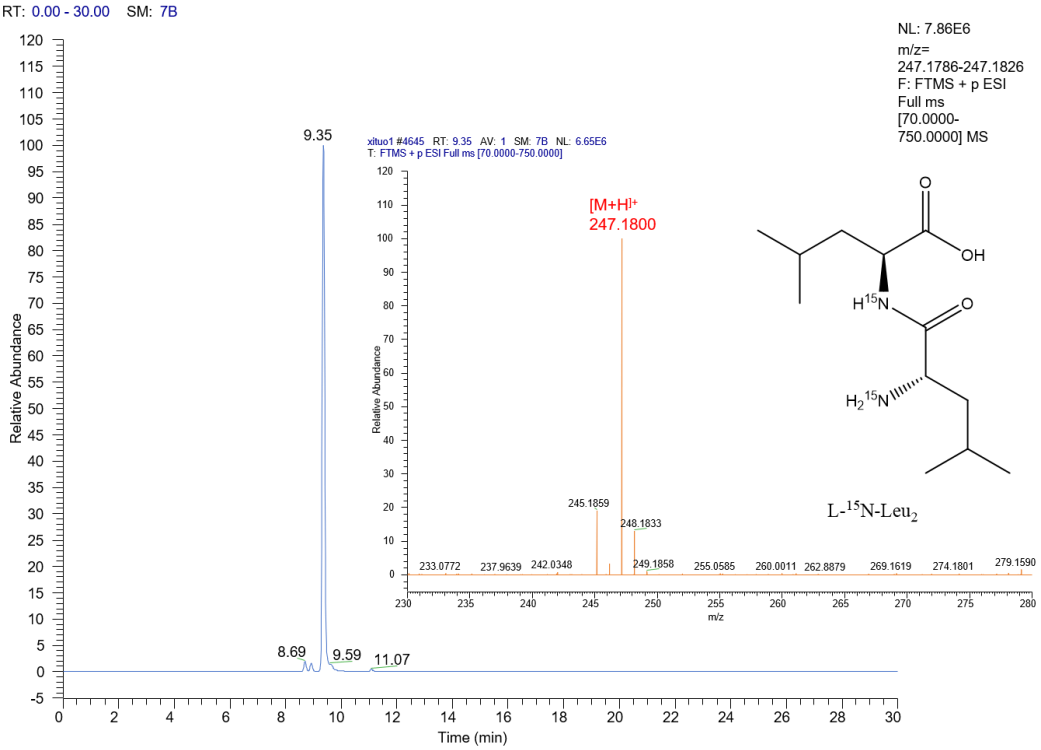
Supplementary Fig. 3 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 132.1021 was analyzed, and its EIC corresponded to the amino acid D-Leu with a retention time of 3.35 min.



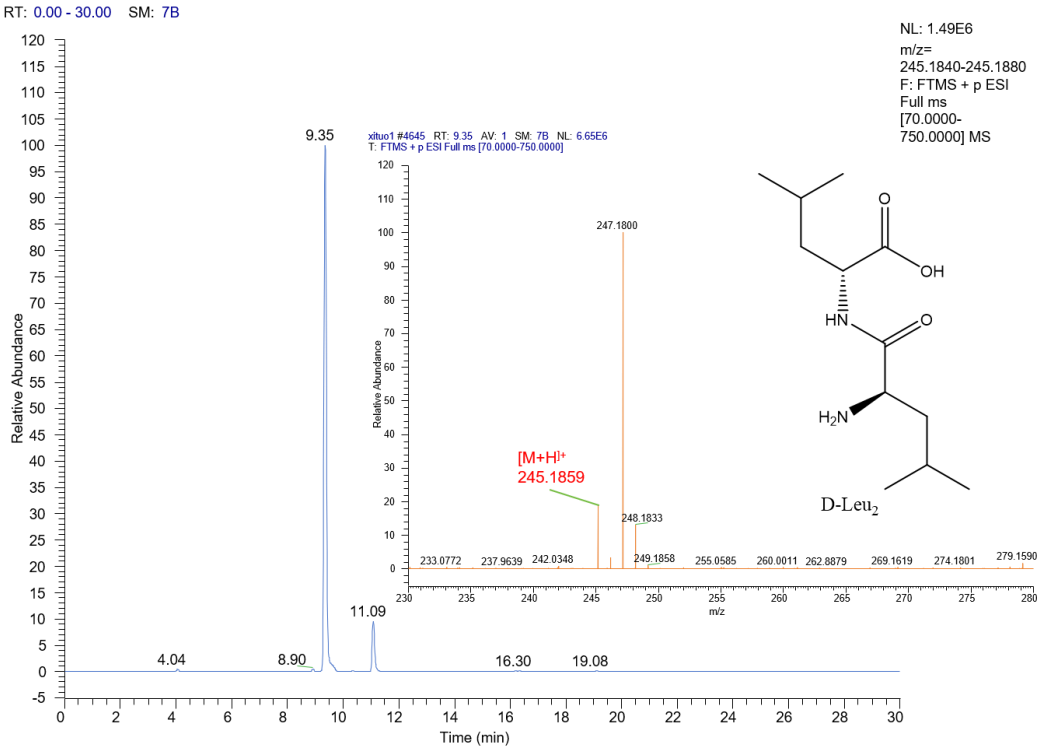
Supplementary Fig. 4 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 133.0989 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Leu with a retention time of 3.31 min.



Supplementary Fig. 5 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 132.1019 was analyzed, and its EIC corresponded to the amino acid D-Leu with a retention time of 3.31 min.

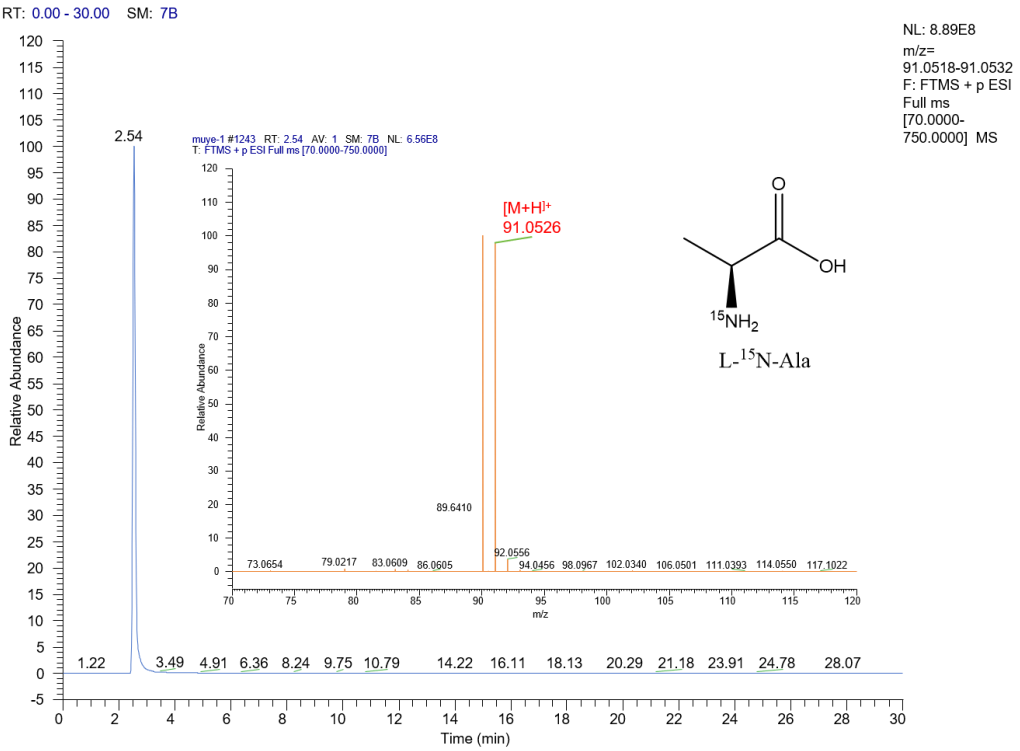


Supplementary Fig. 6 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 247.1800 was analyzed, and its EIC corresponded to the dipeptide L-^{15}N-Leu$_2$ with a retention time of 9.35 min.

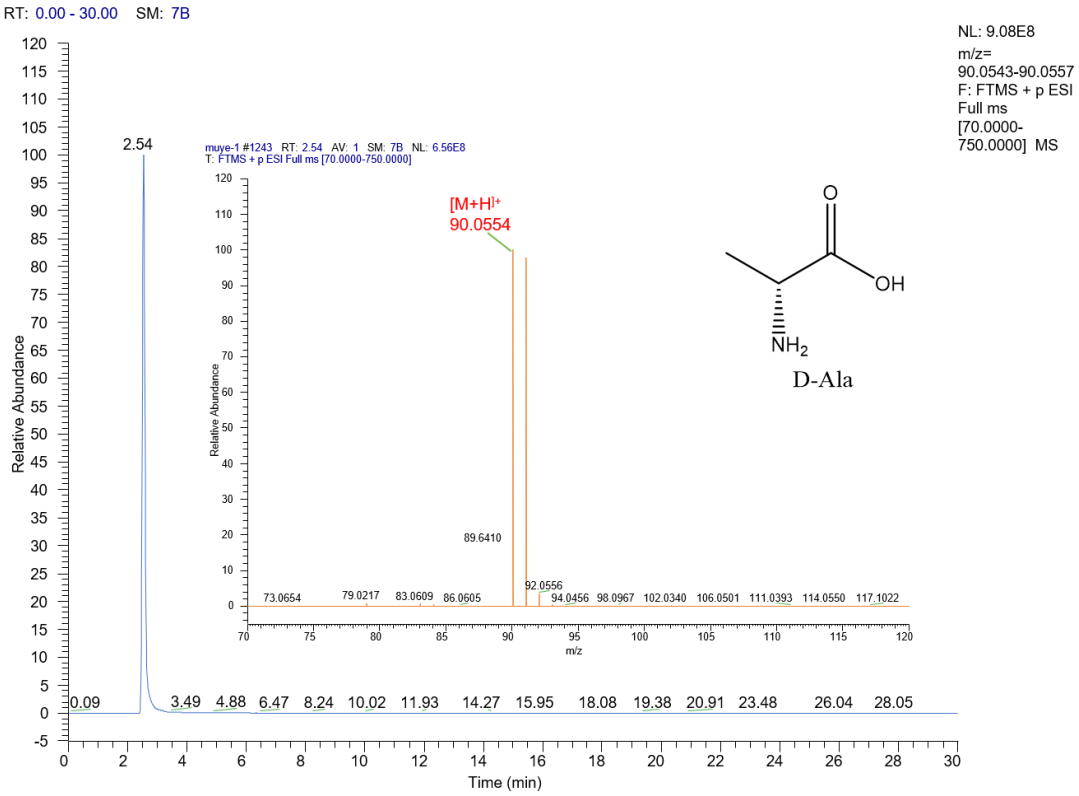


Supplementary Fig. 7 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 245.1859 was analyzed, and its EIC corresponded to the dipeptide D-Leu$_2$ with a retention time of 9.35 min.

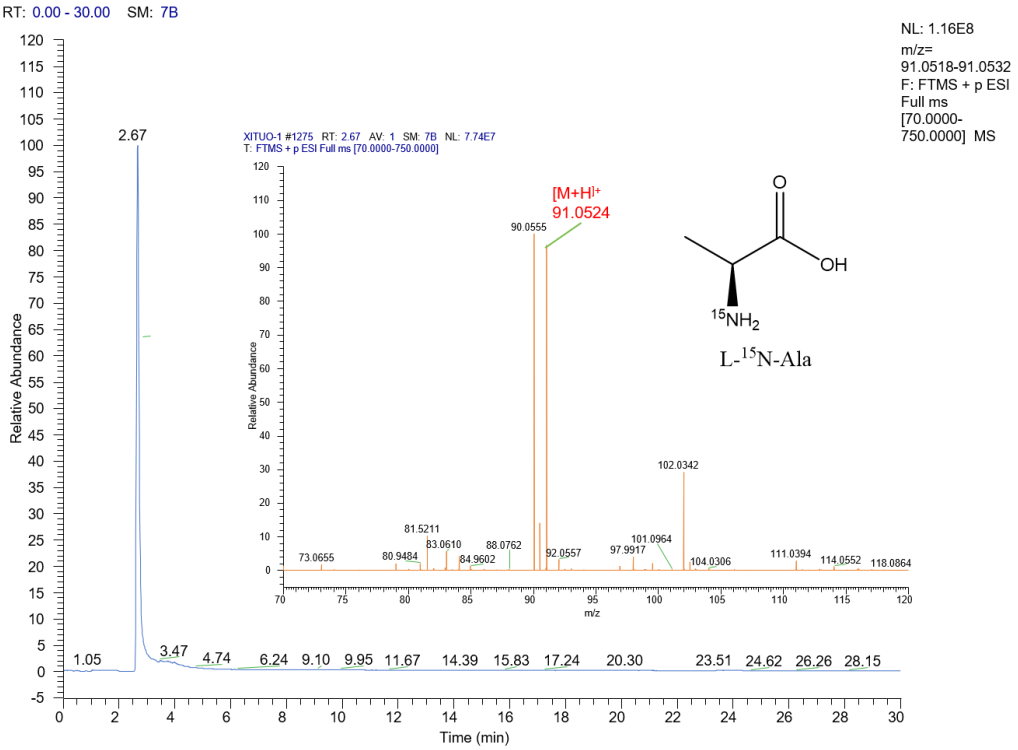
2.2 Chiral selection of Alanine dipeptide on calcite surface. (Figs. S8-S13)



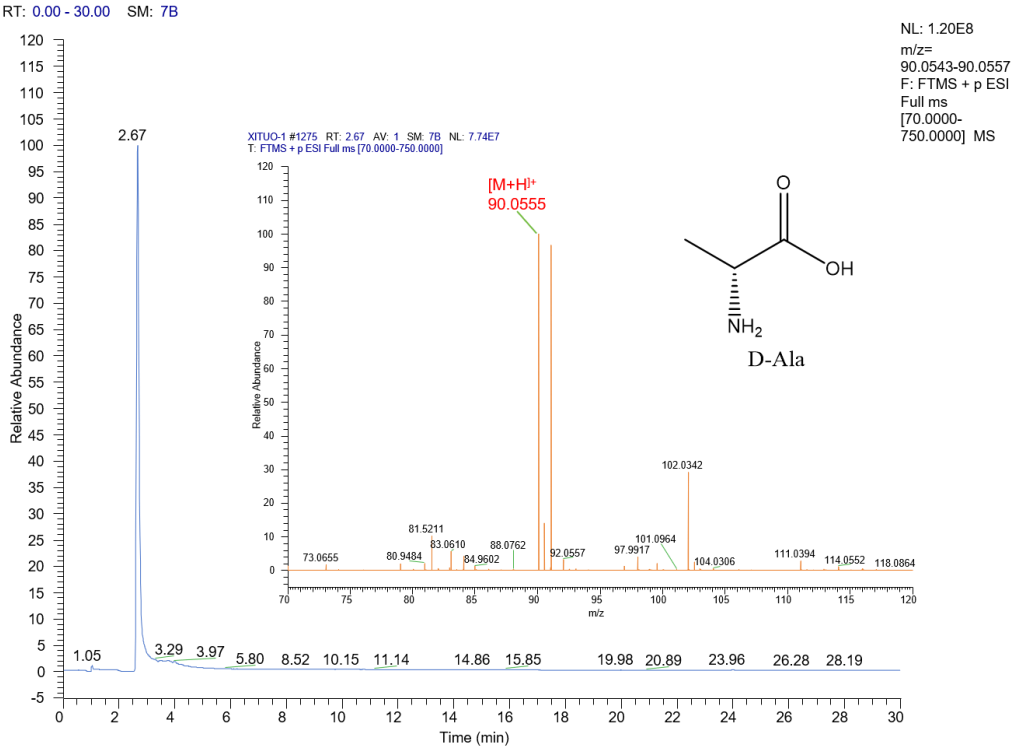
Supplementary Fig. 8 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 91.0526 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ala with a retention time of 2.54 min.



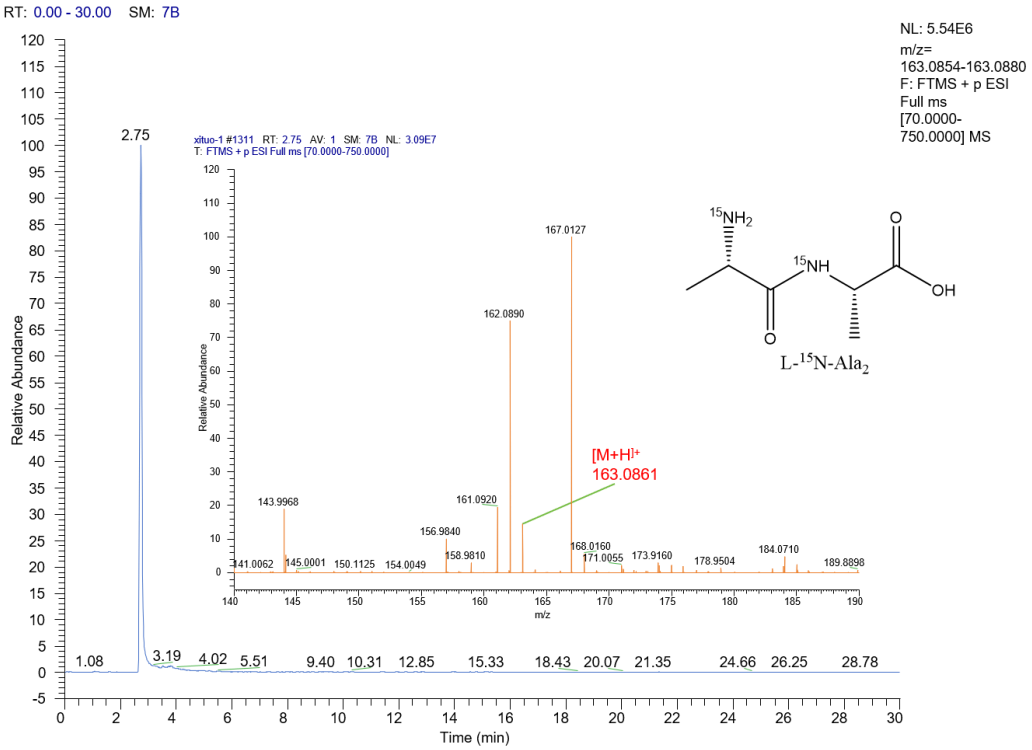
Supplementary Fig. 9 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 90.0554 was analyzed, and its EIC corresponded to the amino acid D-Ala with a retention time of 2.54 min.



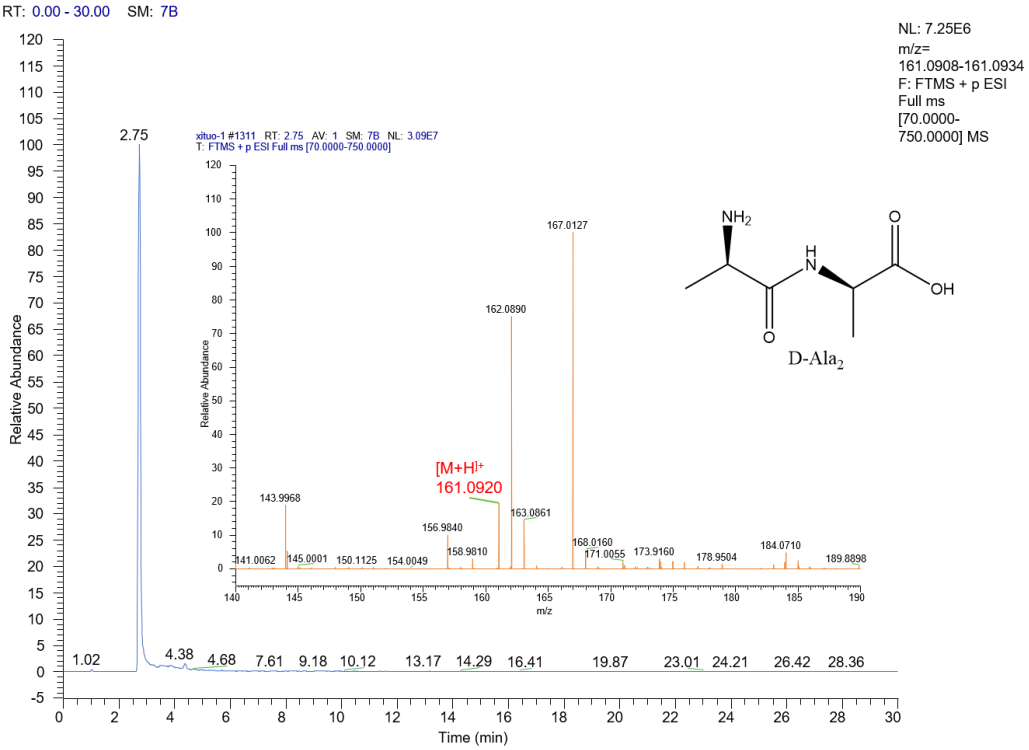
Supplementary Fig. 10 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 91.0524 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ala with a retention time of 2.67 min.



Supplementary Fig. 11 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 90.0555 was analyzed, and its EIC corresponded to the amino acid D-Ala with a retention time of 2.67 min.

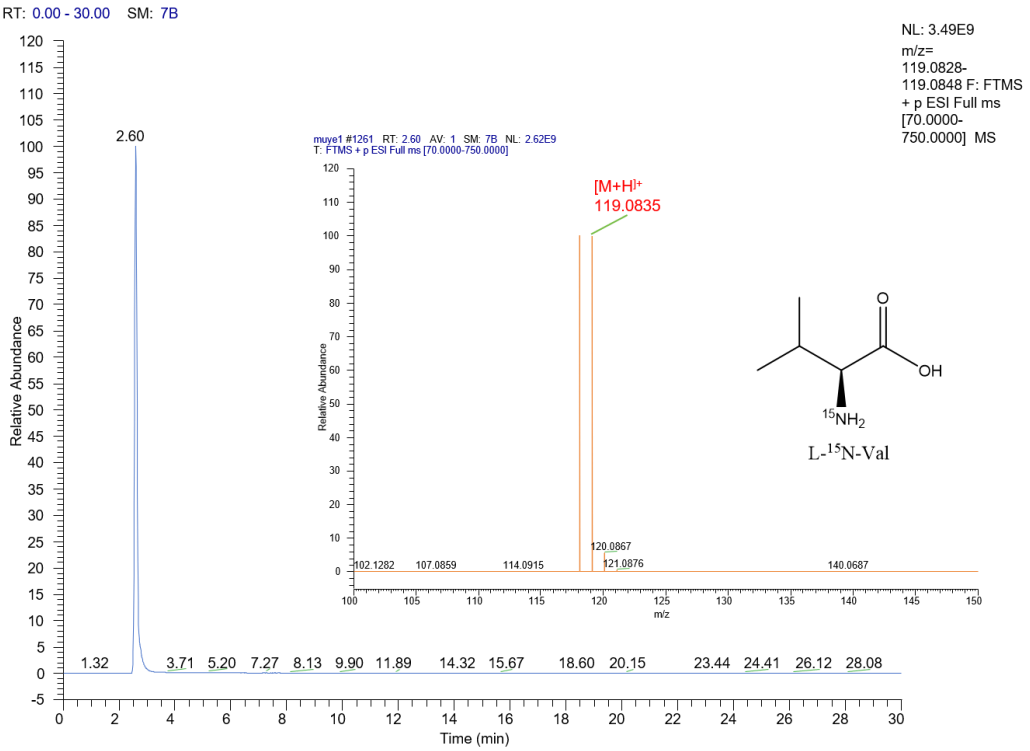


Supplementary Fig. 12 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 163.0861 was analyzed, and its EIC corresponded to the dipeptide L-^{15}N-Ala$_2$ with a retention time of 2.75 min.

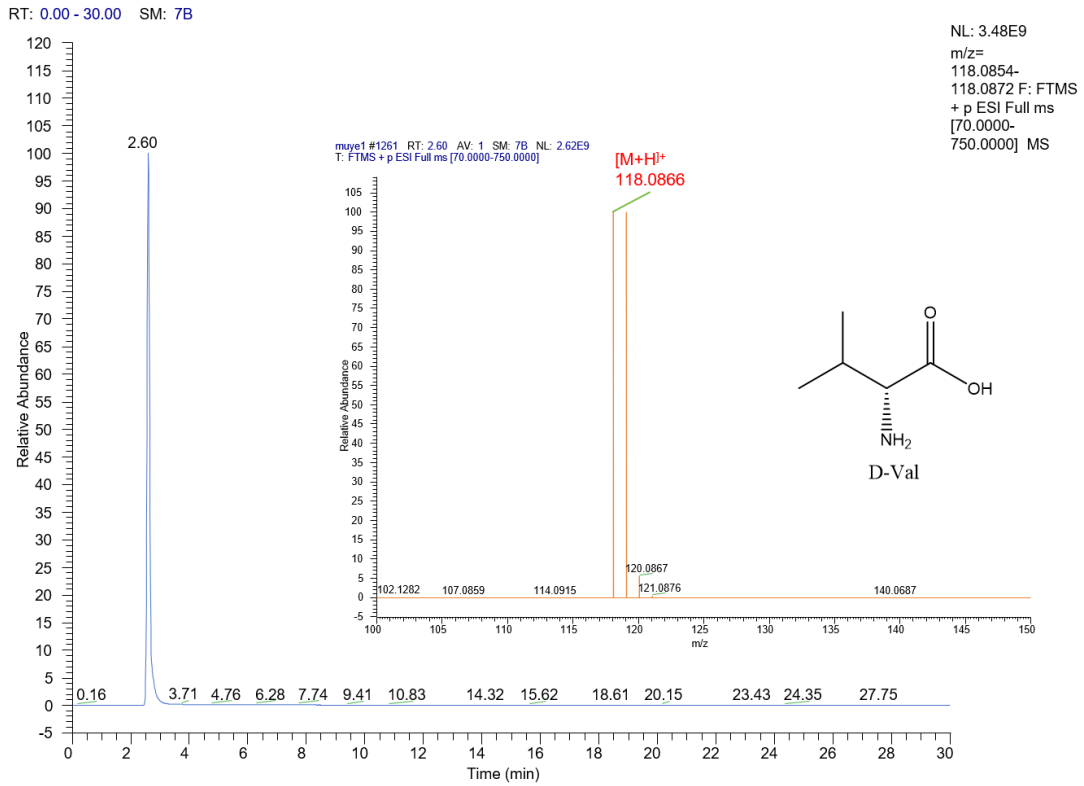


Supplementary Fig. 13 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 161.0920 was analyzed, and its EIC corresponded to the dipeptide D-Ala_2 with a retention time of 2.75 min.

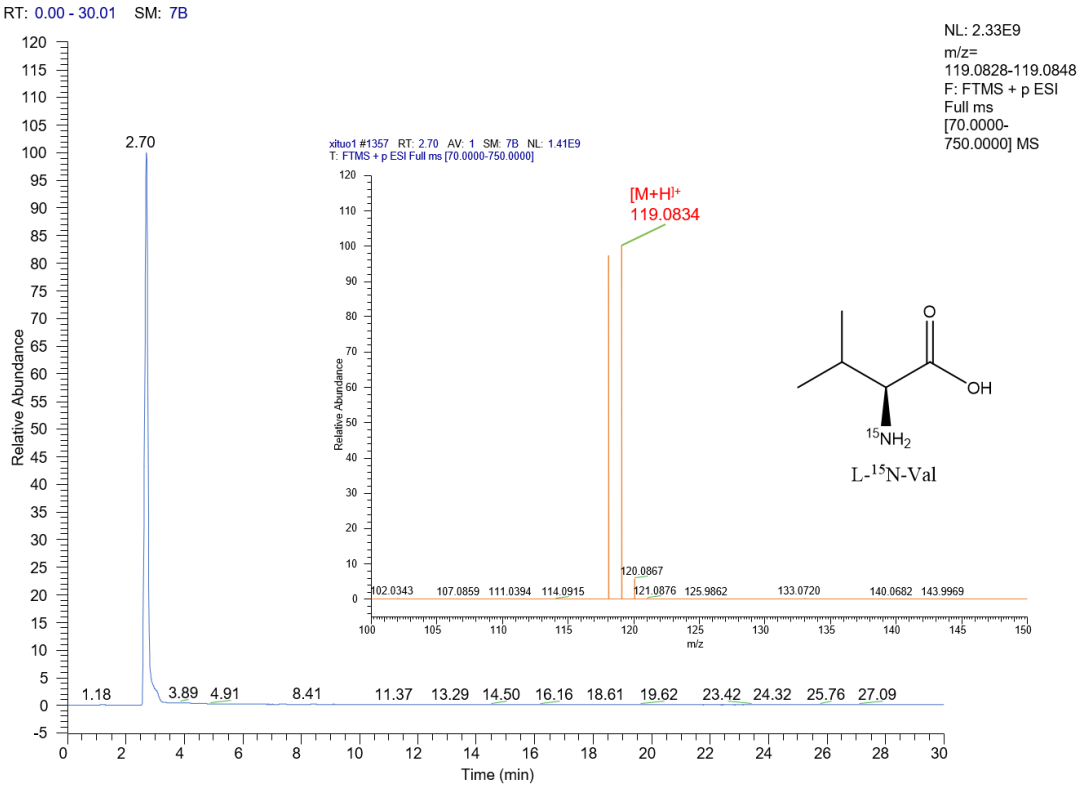
2.3 Chiral selection of Valine dipeptide on calcite surface. (Figs. S14-S19)



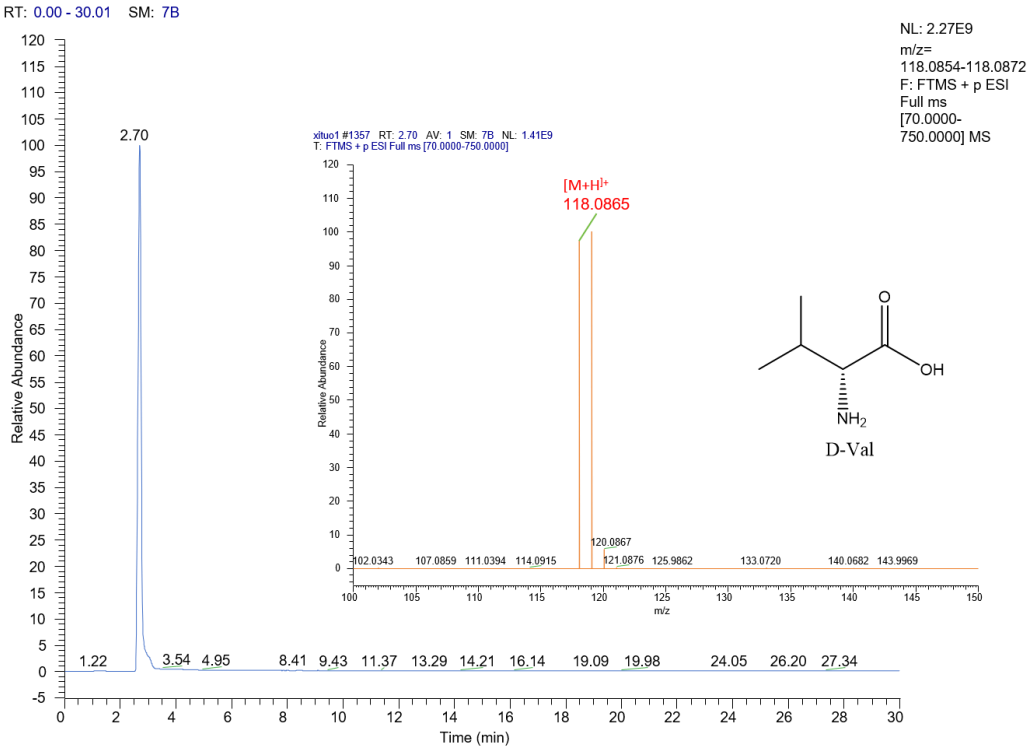
Supplementary Fig. 14 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 119.0835 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Val with a retention time of 2.60 min.



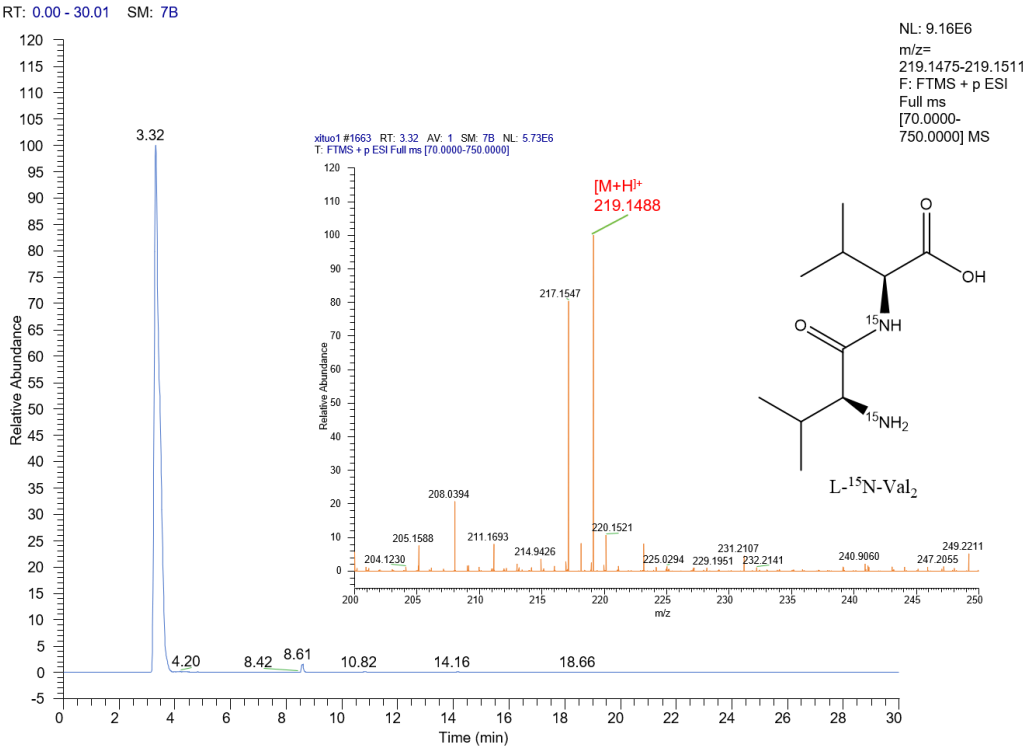
Supplementary Fig. 15 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 118.0866 was analyzed, and its EIC corresponded to the amino acid D-Val with a retention time of 2.60 min.



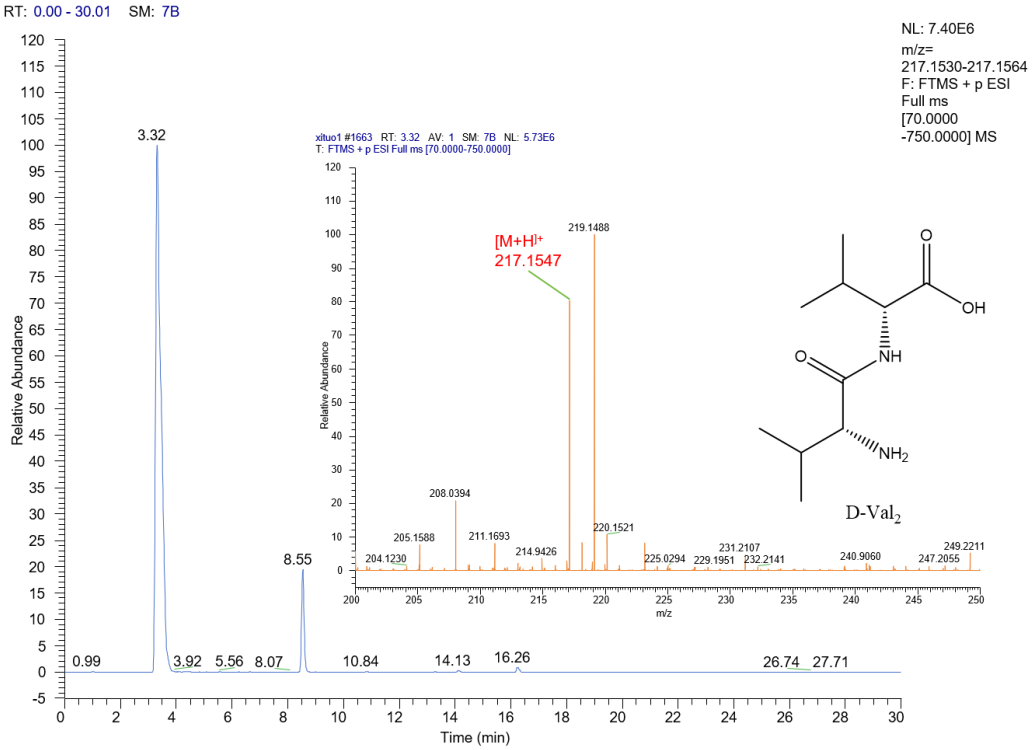
Supplementary Fig. 16 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 119.0834 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Val with a retention time of 2.70 min.



Supplementary Fig. 17 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 118.0865 was analyzed, and its EIC corresponded to the amino acid D-Val with a retention time of 2.70 min.

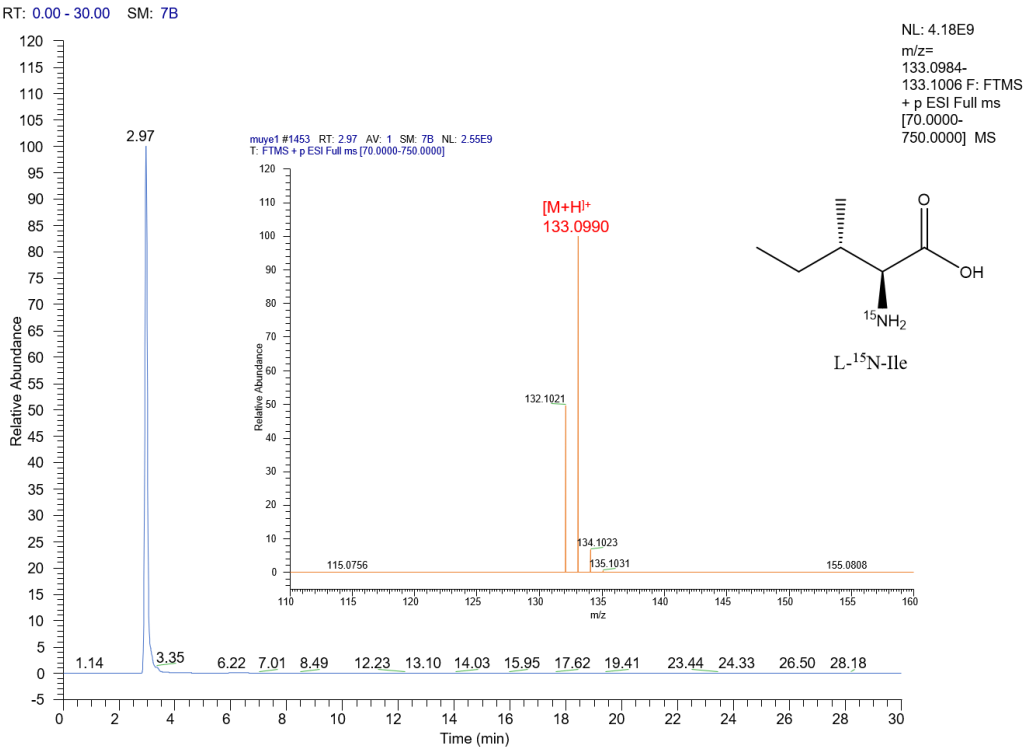


Supplementary Fig. 18 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 219.1488 was analyzed, and its EIC corresponded to the dipeptide L-^{15}N-Val$_2$ with a retention time of 3.32 min.

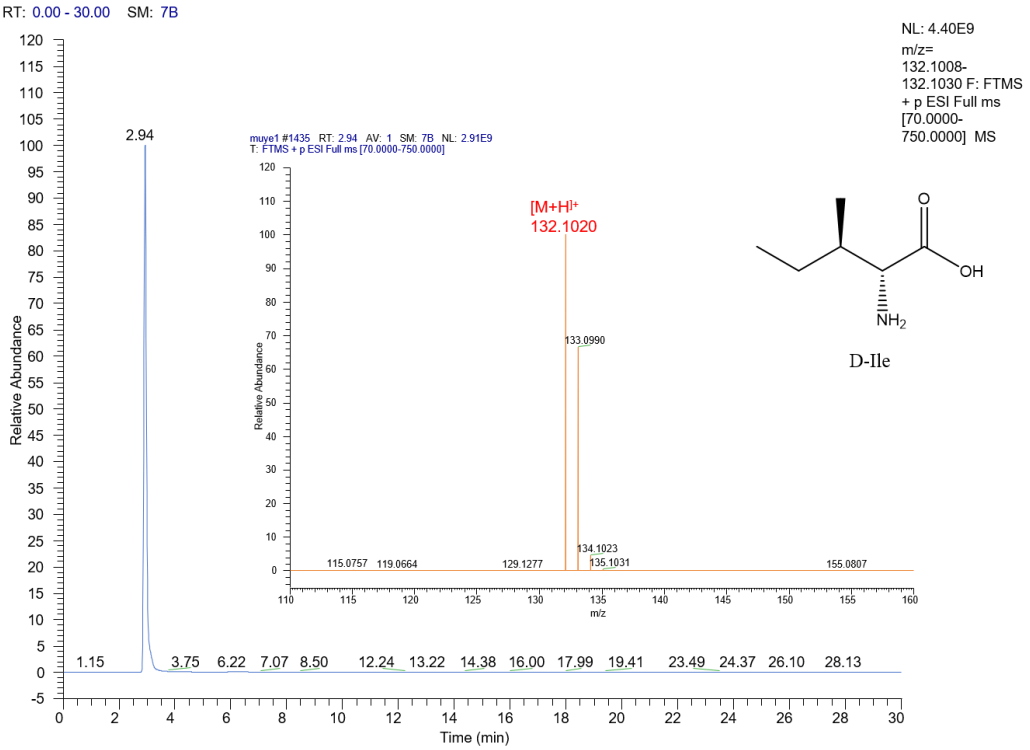


Supplementary Fig. 19 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 217.1547 was analyzed, and its EIC corresponded to the dipeptide D-Val_2 with a retention time of 3.32 min.

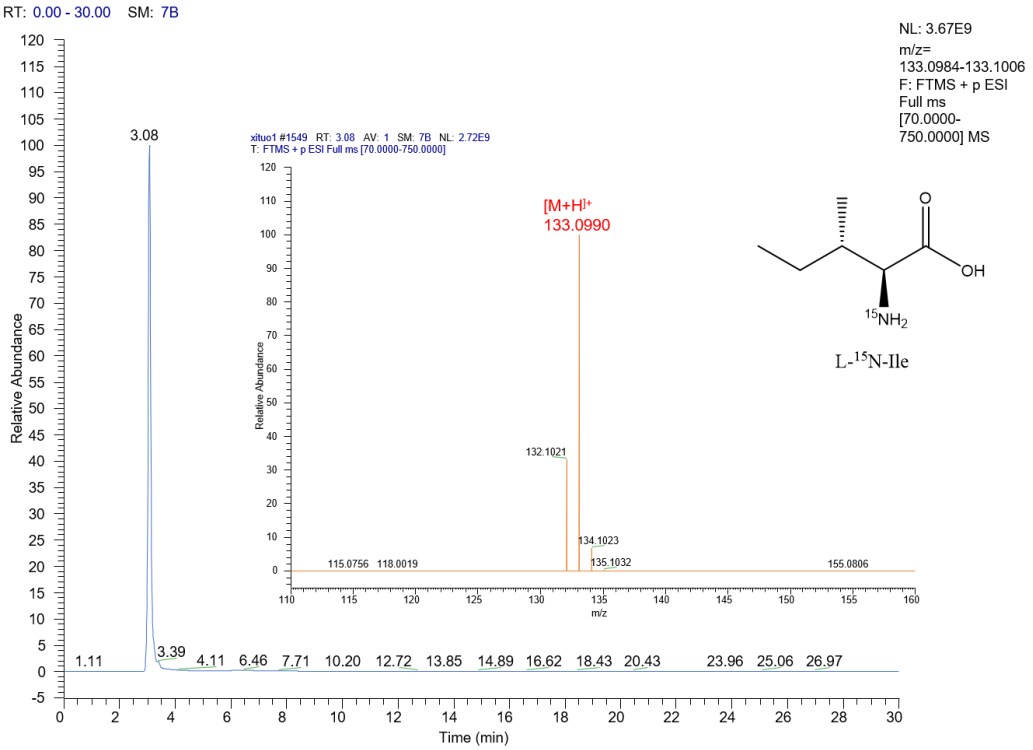
2.4 Chiral selection of Isoleucine dipeptide on calcite surface. (Figs. S20-S25)



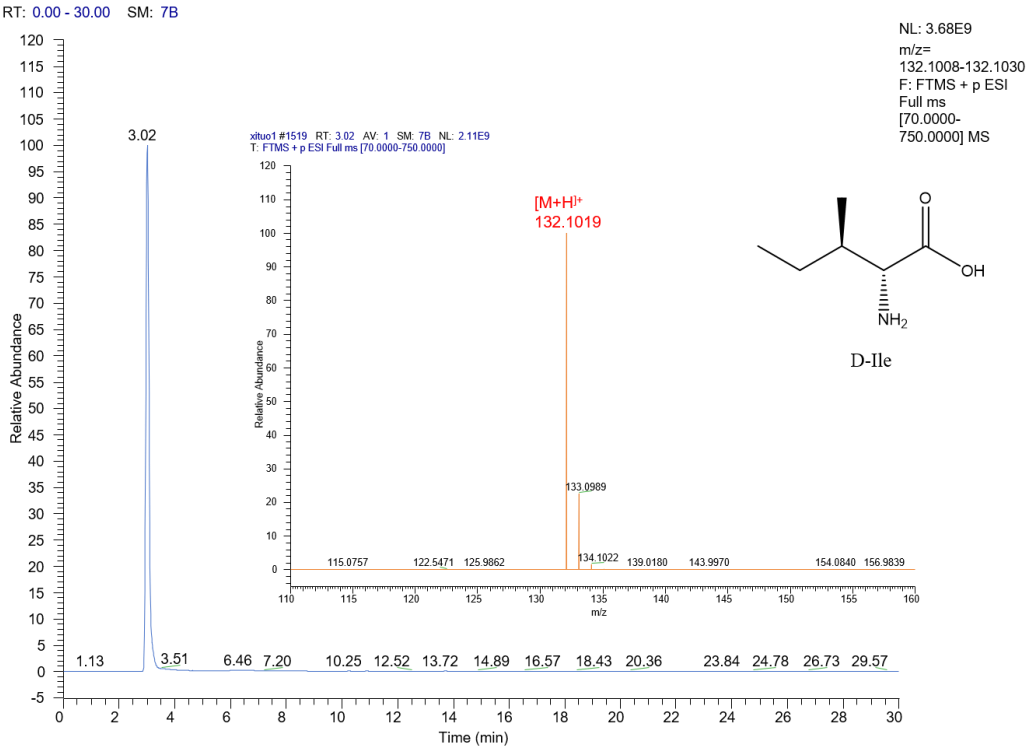
Supplementary Fig. 20 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 133.0990 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ile with a retention time of 2.97 min.



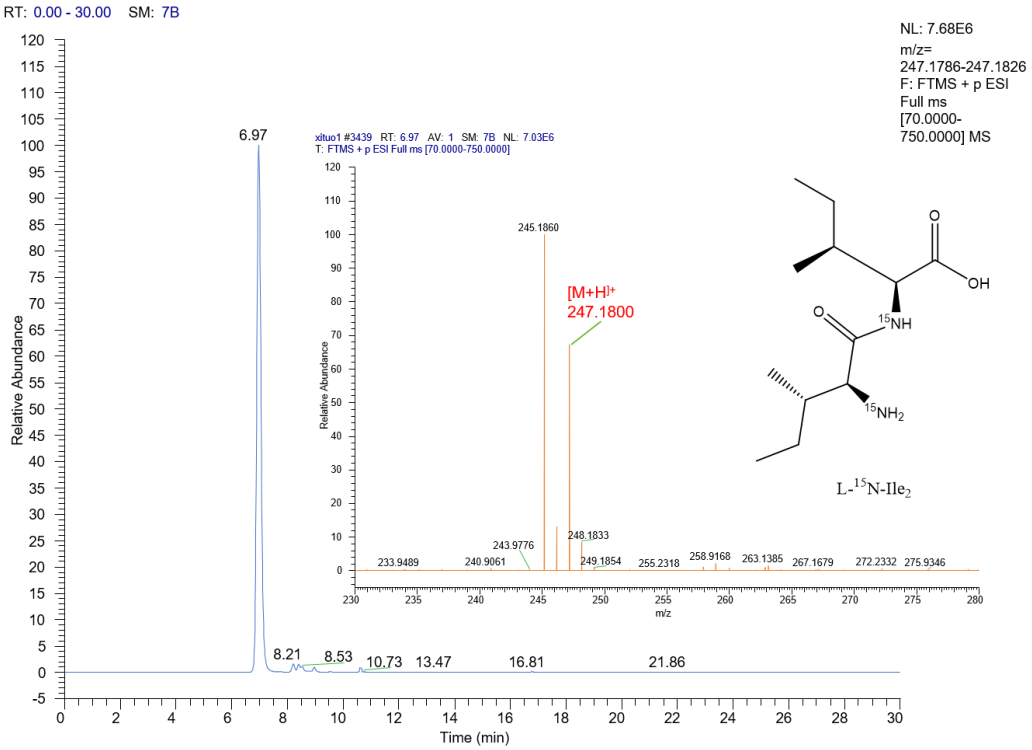
Supplementary Fig. 21 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 132.1020 was analyzed, and its EIC corresponded to the amino acid D-Ile with a retention time of 2.94 min.



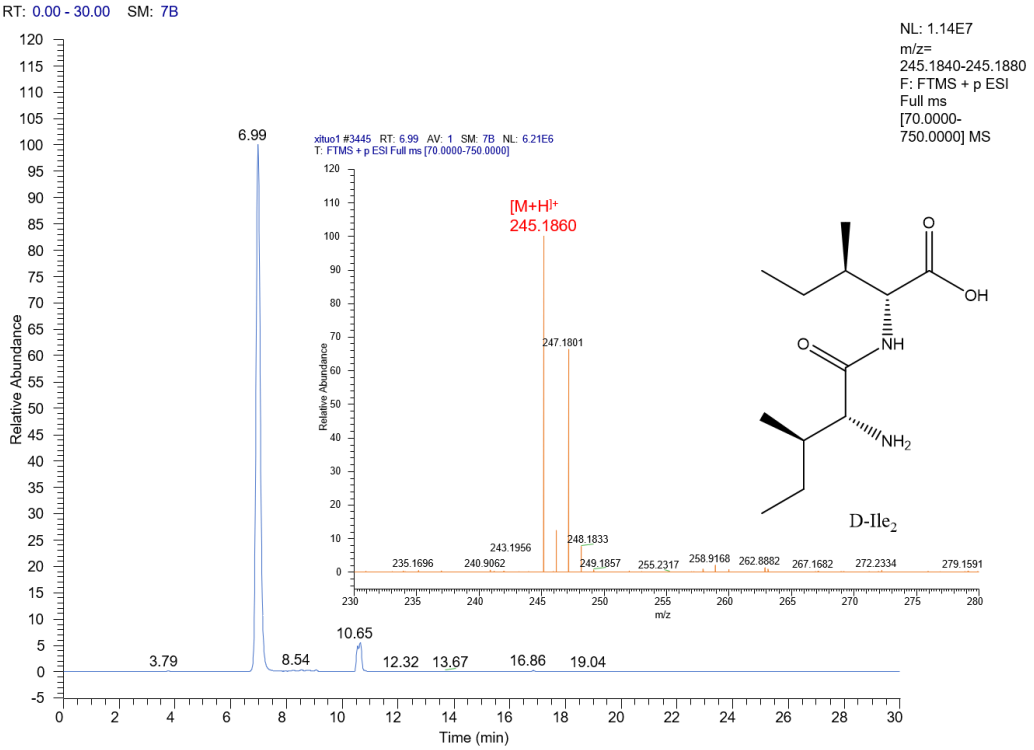
Supplementary Fig. 22 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 133.0990 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ile with a retention time of 3.08 min.



Supplementary Fig. 23 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 132.1019 was analyzed, and its EIC corresponded to the amino acid D-Ile with a retention time of 3.02 min.

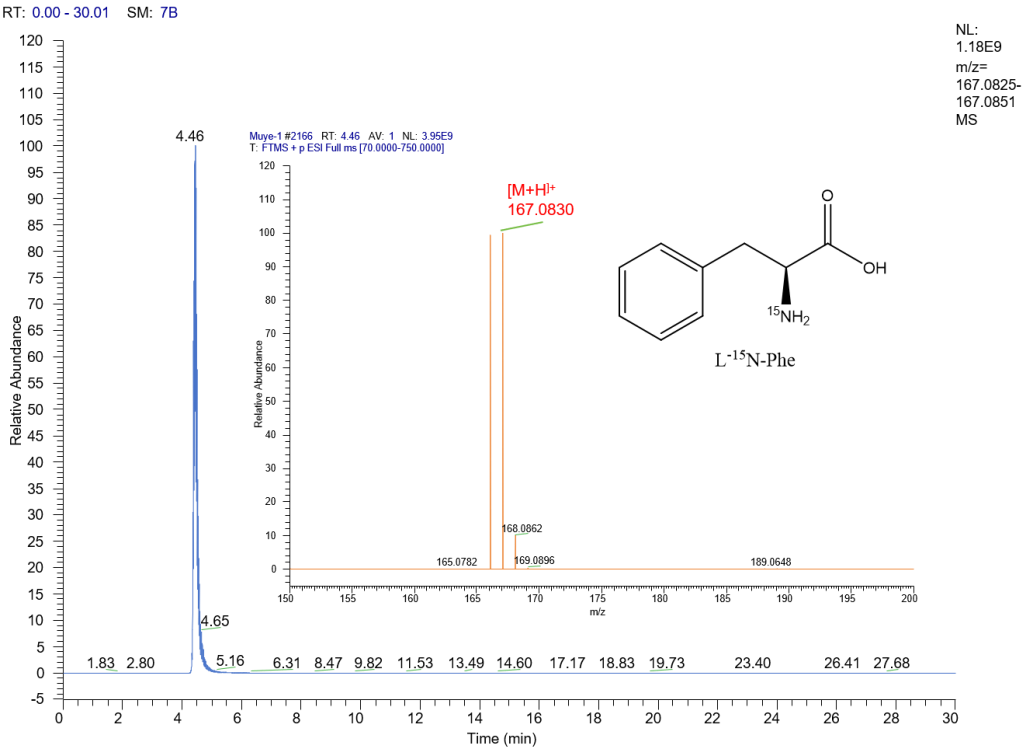


Supplementary Fig. 24 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 247.1800 was analyzed, and its EIC corresponded to the dipeptide L-^{15}N-Ile$_2$ with a retention time of 6.97 min.

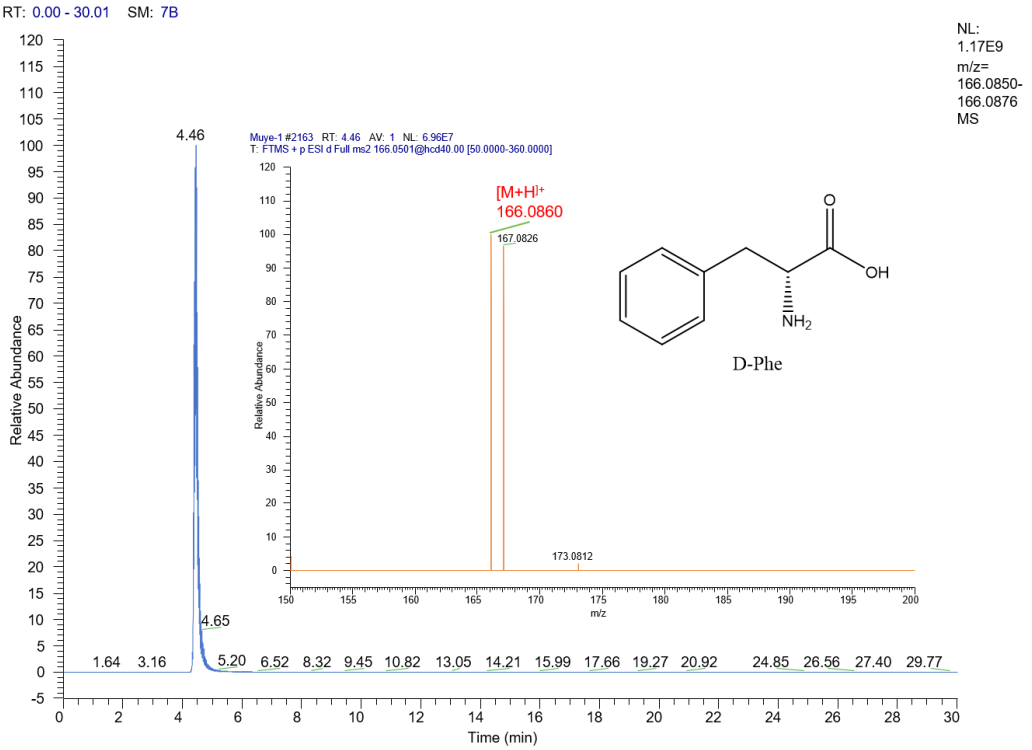


Supplementary Fig. 25 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 245.1860 was analyzed, and its EIC corresponded to the dipeptide D-Ile$_2$ with a retention time of 6.99 min.

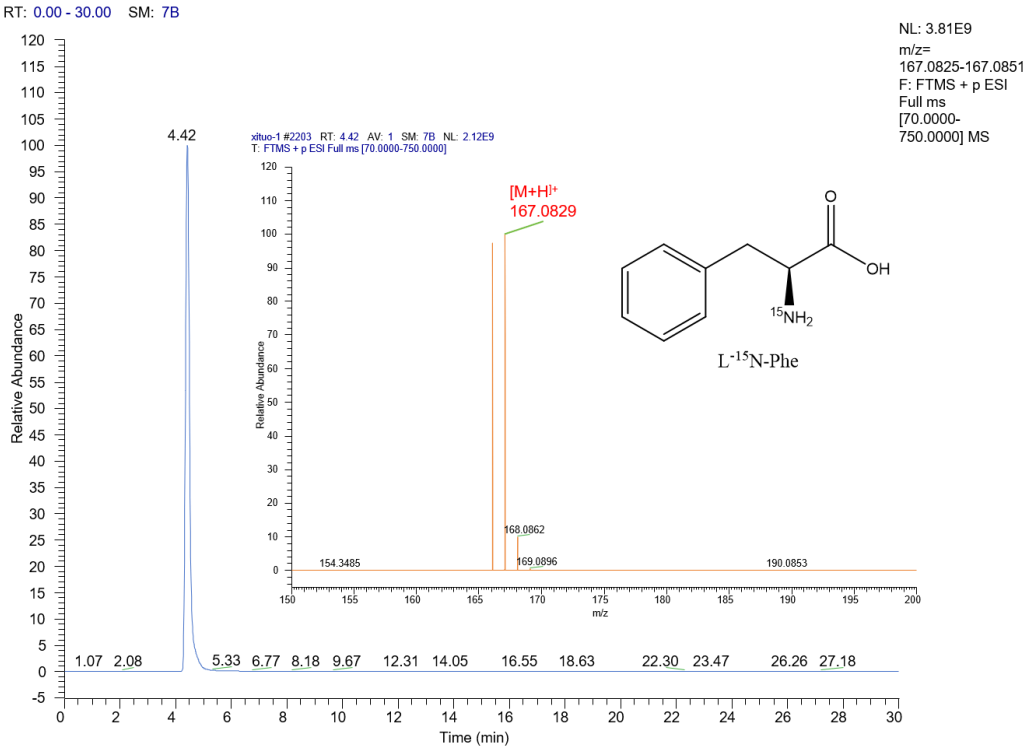
2.5 Chiral selection of Phenylalanine dipeptide on calcite surface. (Figs. S26-S31)



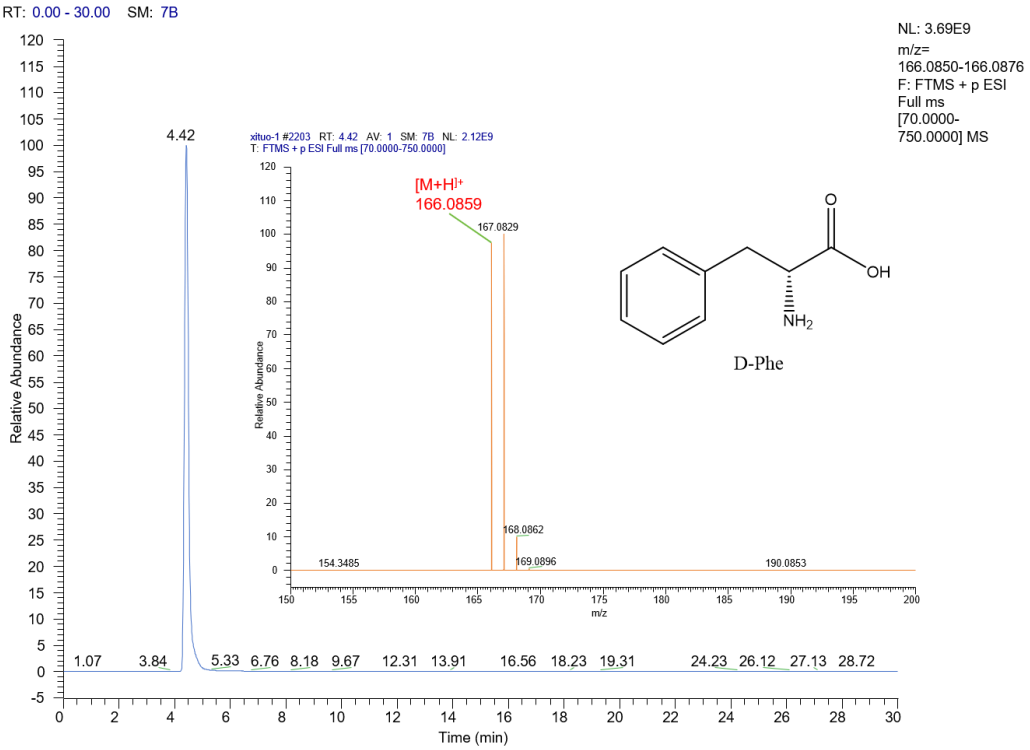
Supplementary Fig. 26 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 167.0830 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Phe with a retention time of 4.46 min.



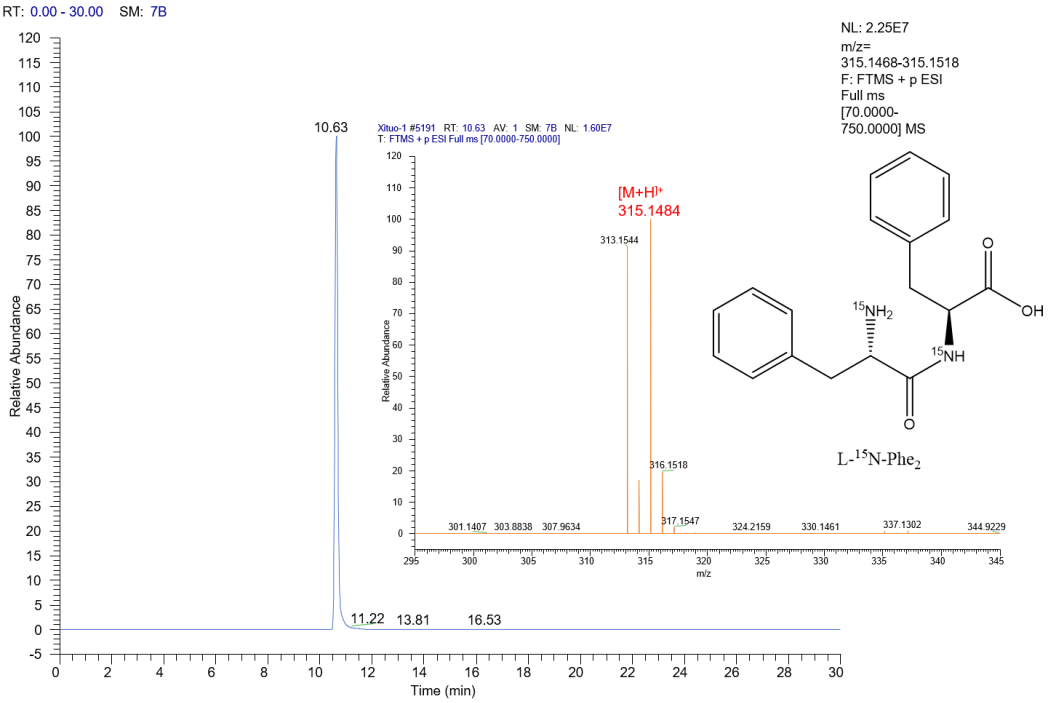
Supplementary Fig. 27 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 166.0860 was analyzed, and its EIC corresponded to the amino acid D-Phe with a retention time of 4.46 min.



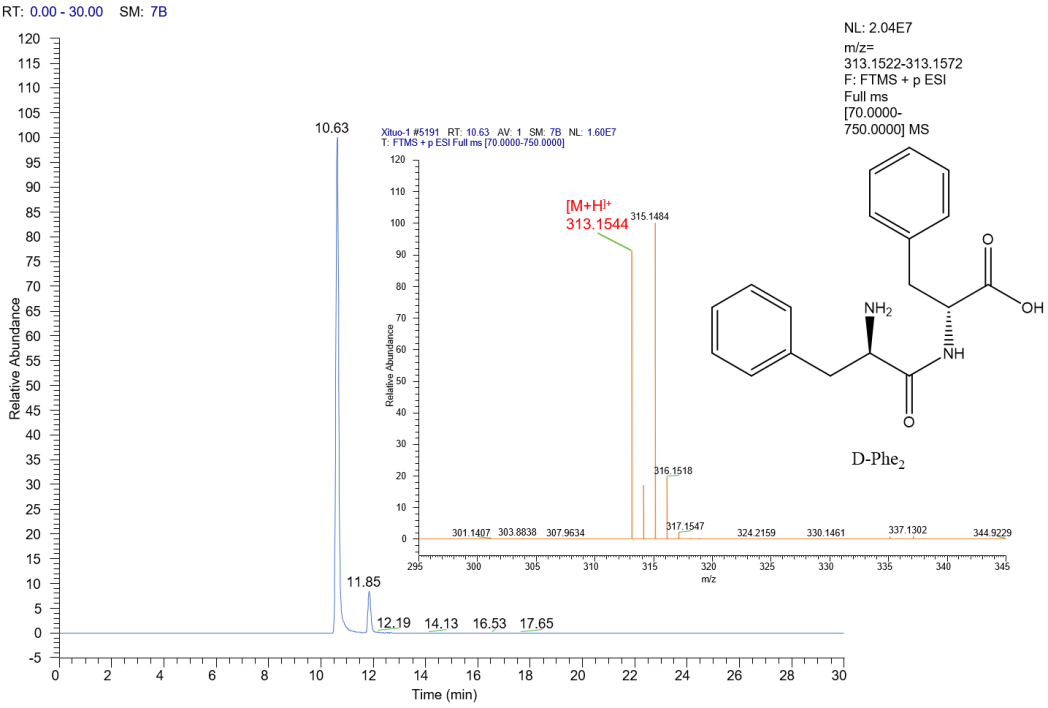
Supplementary Fig. 28 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 167.0829 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Phe with a retention time of 4.42 min.



Supplementary Fig. 29 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 166.0859 was analyzed, and its EIC corresponded to the amino acid D-Phe with a retention time of 4.42 min.

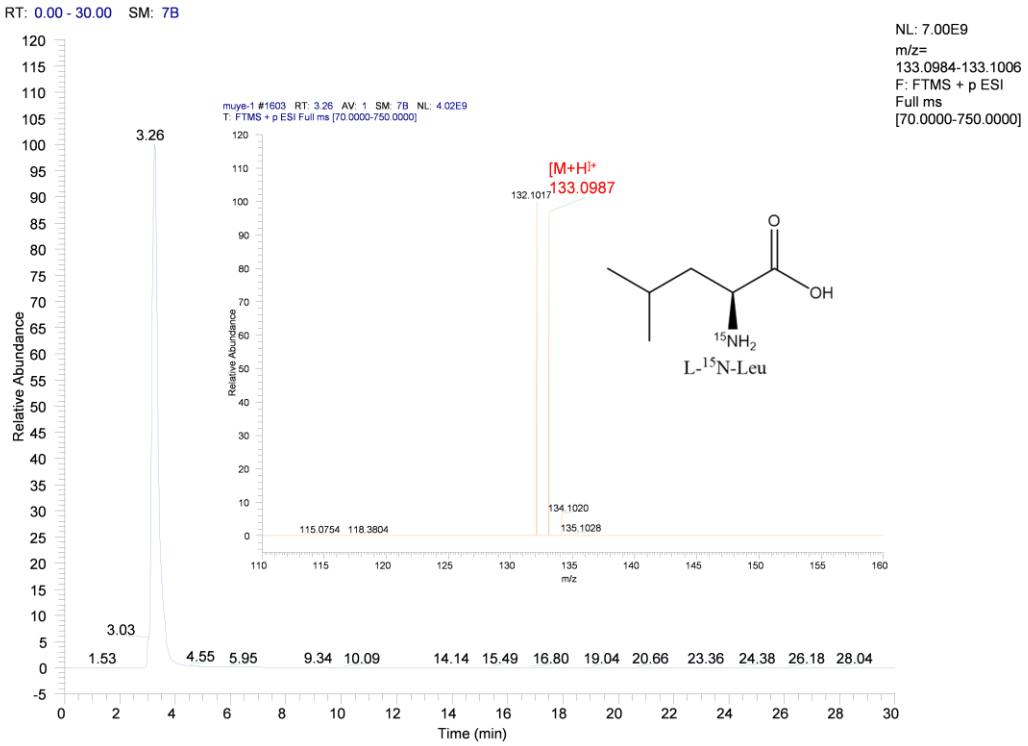


Supplementary Fig. 30 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 315.1484 was analyzed, and its EIC corresponded to the dipeptide L-^{15}N-Phe$_2$ with a retention time of 10.63 min.

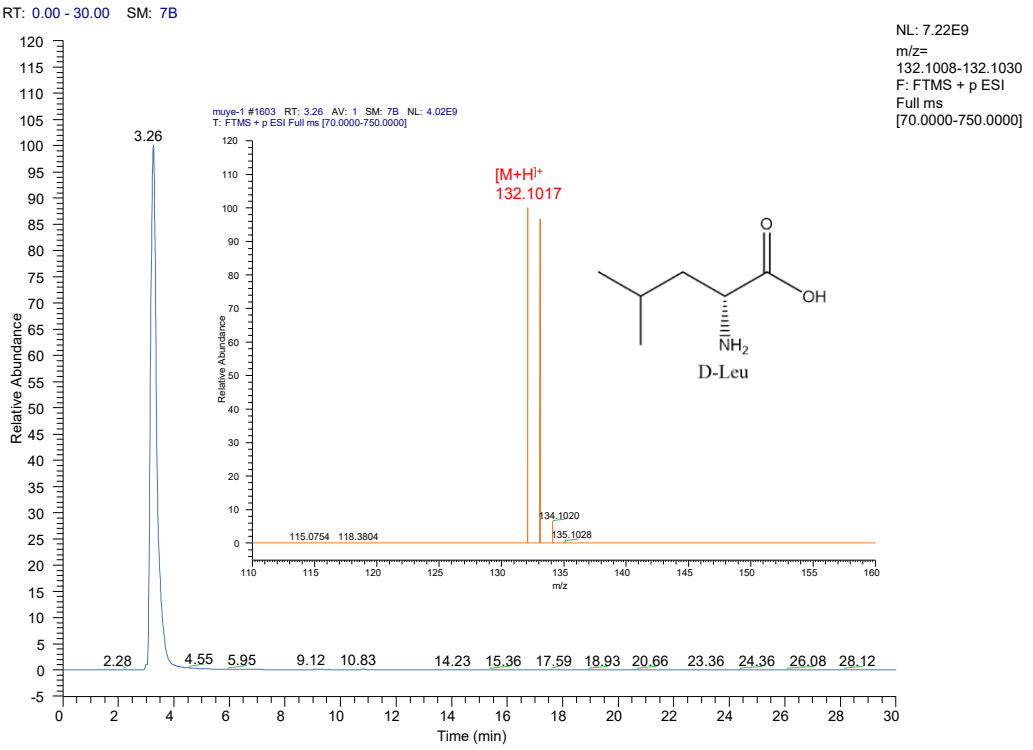


Supplementary Fig. 31 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 313.1544 was analyzed, and its EIC corresponded to the dipeptide D-Phe$_2$ with a retention time of 10.63 min.

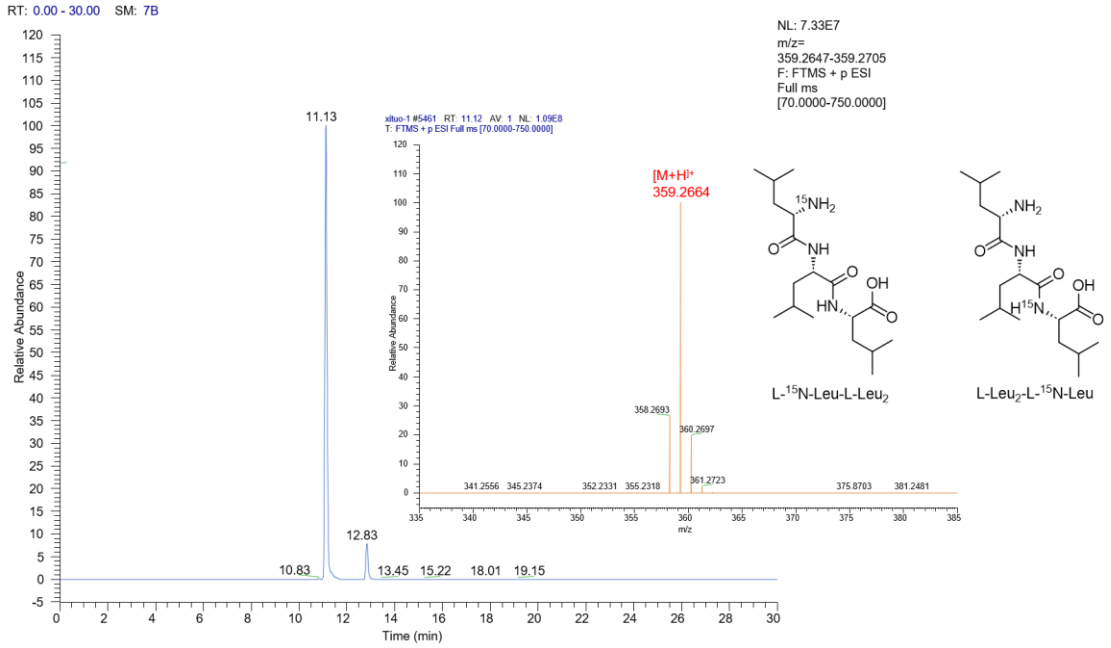
2.6 Chiral selection of tripeptides generated from L-Leucine dipeptides and racemic Leucine as substrates. (Figs. S32-S35)



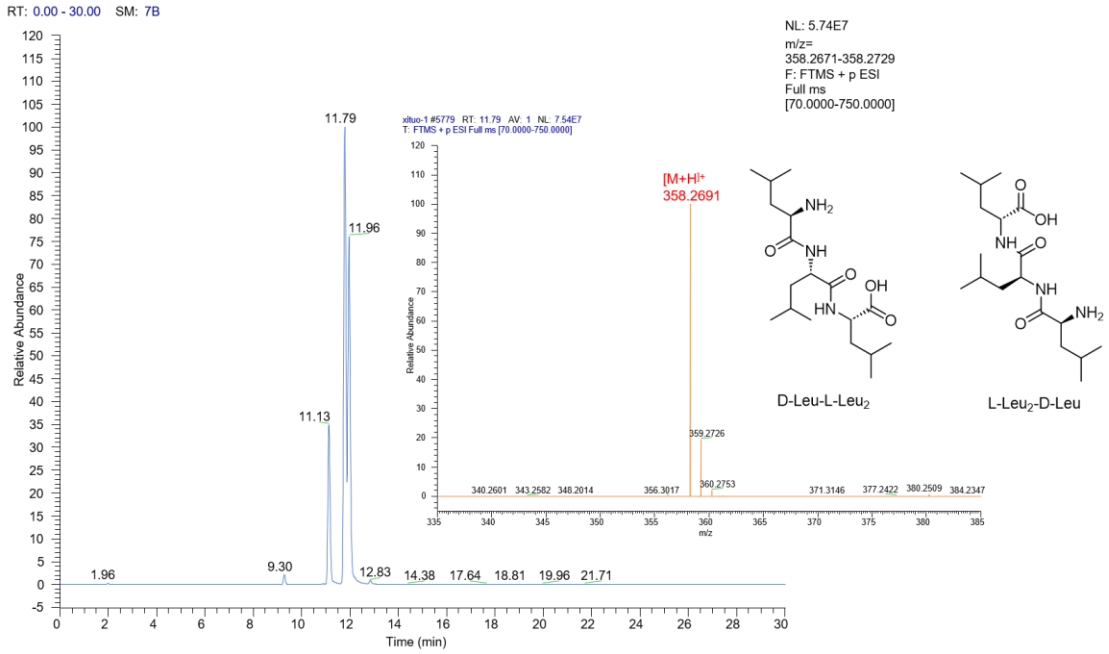
Supplementary Fig. 32 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 133.0987 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Leu with a retention time of 3.26 min.



Supplementary Fig. 33 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 132.1017 was analyzed, and its EIC corresponded to the amino acid D-Leu with a retention time of 3.26 min.

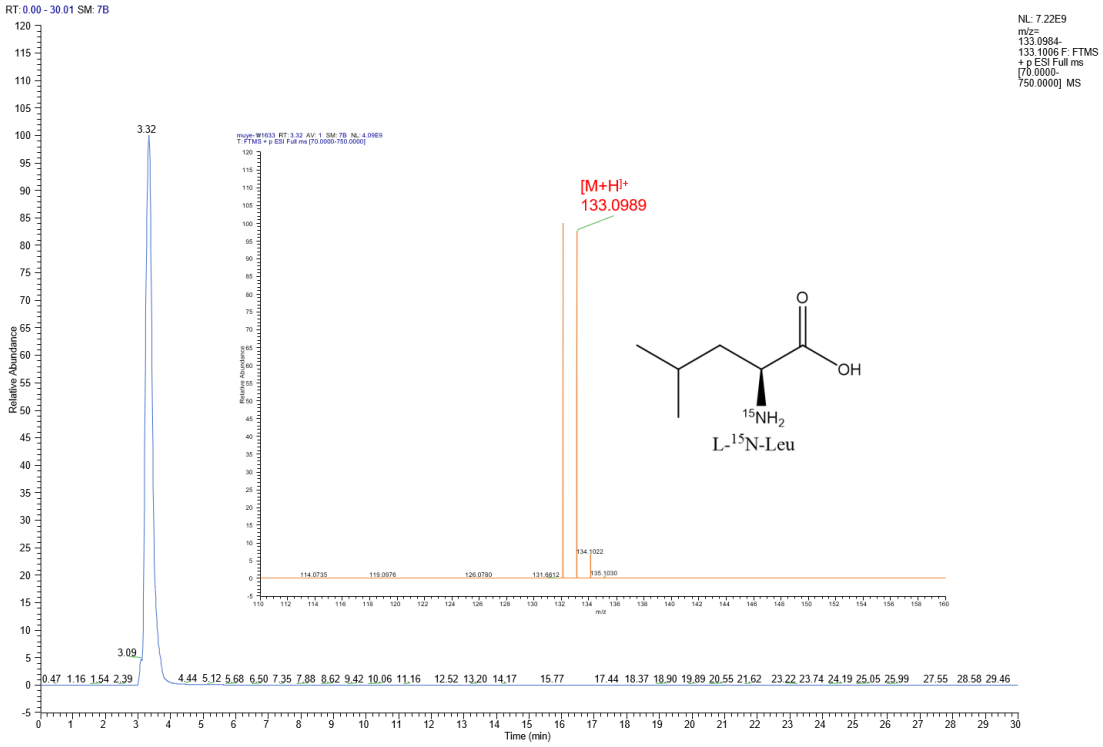


Supplementary Fig. 34 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 359.2664 was analyzed, and its EIC corresponded to the tripeptides L-^{15}N-Leu-L-Leu$_2$ and L-Leu$_2$-L-^{15}N-Leu with a retention time of 11.13 and 12.83 min.

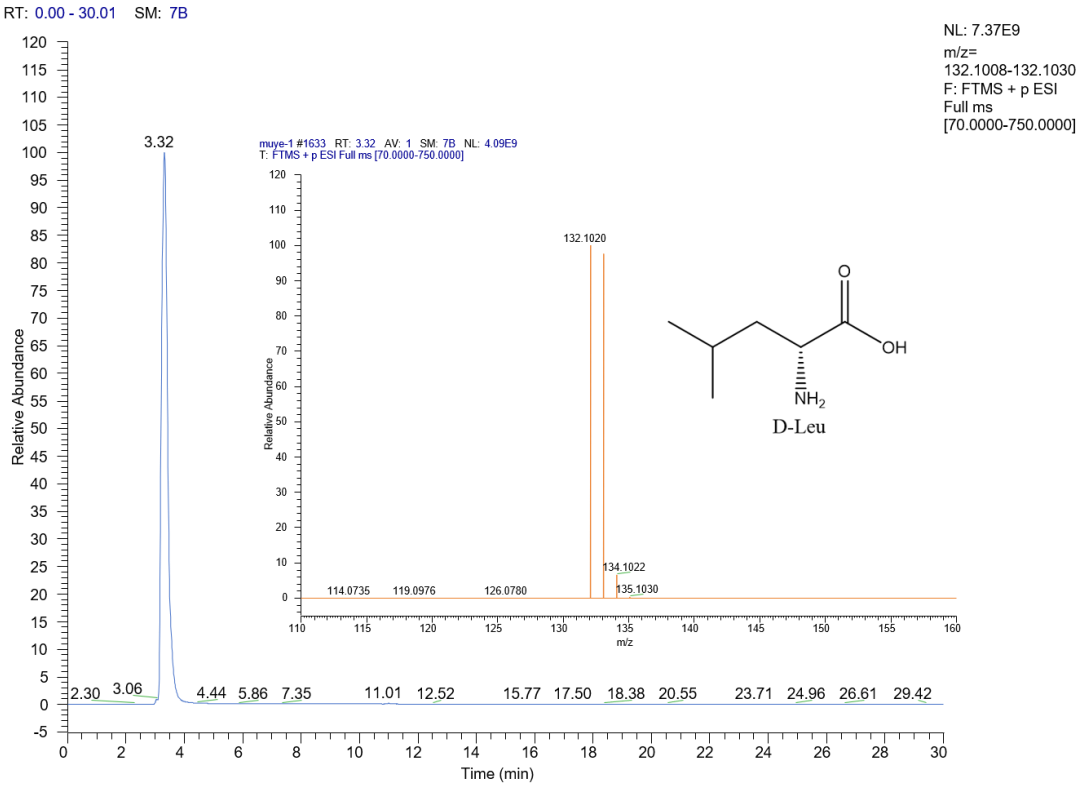


Supplementary Fig. 35 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 358.2691 was analyzed, and its EIC corresponded to the tripeptides D-Leu-L-Leu_2 and L-Leu_2-D-Leu with a retention time of 11.79 and 11.96 min.

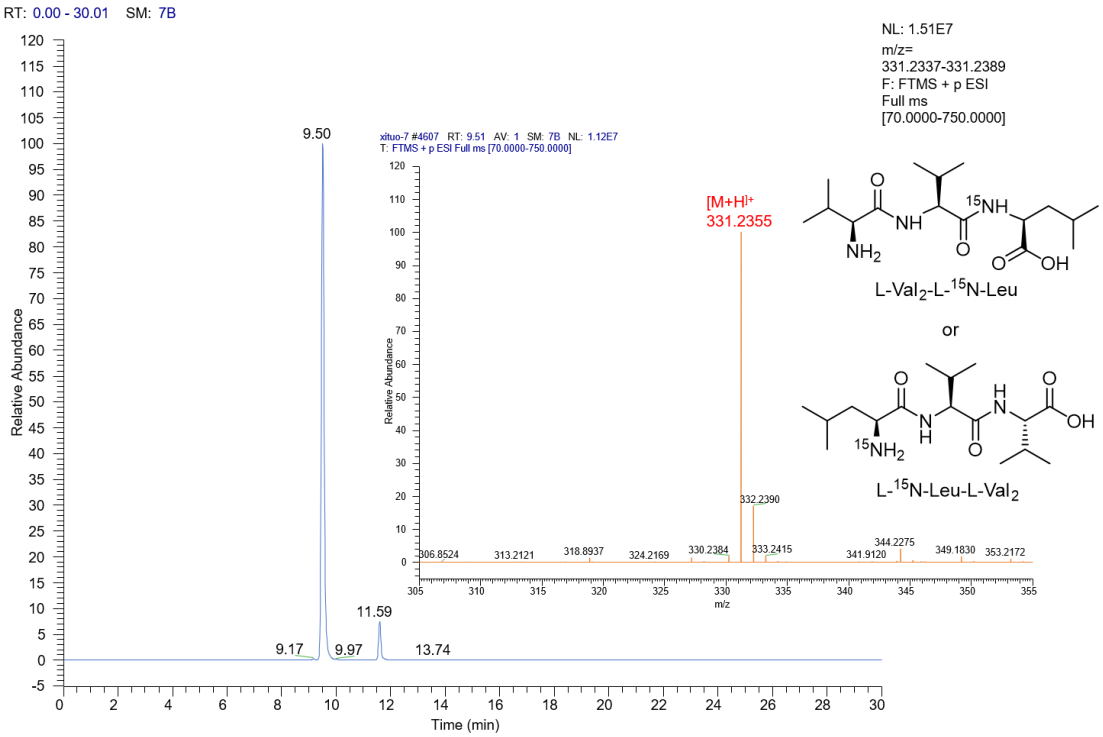
2.7 Chiral selection of tripeptides generated from L-Valine dipeptides and racemic Leucine as substrates. (Figs. S36-S39)



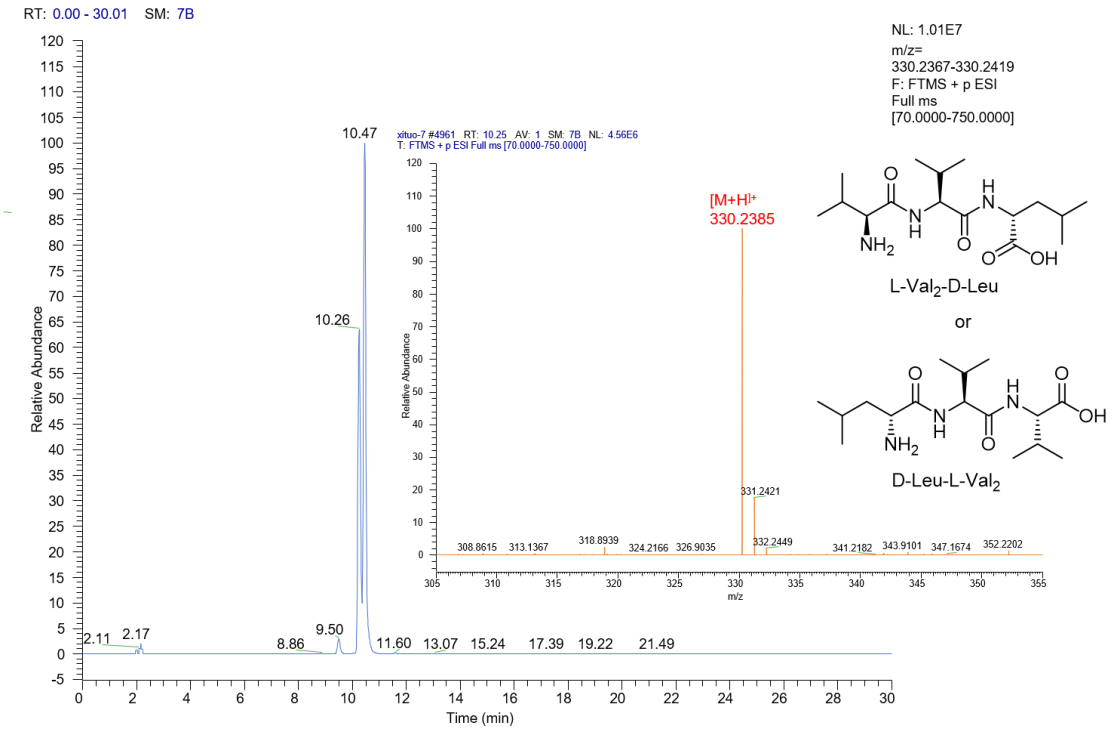
Supplementary Fig. 36 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 133.0989 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Leu with a retention time of 3.32 min.



Supplementary Fig. 37 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 132.1020 was analyzed, and its EIC corresponded to the amino acid D-Leu with a retention time of 3.32 min.

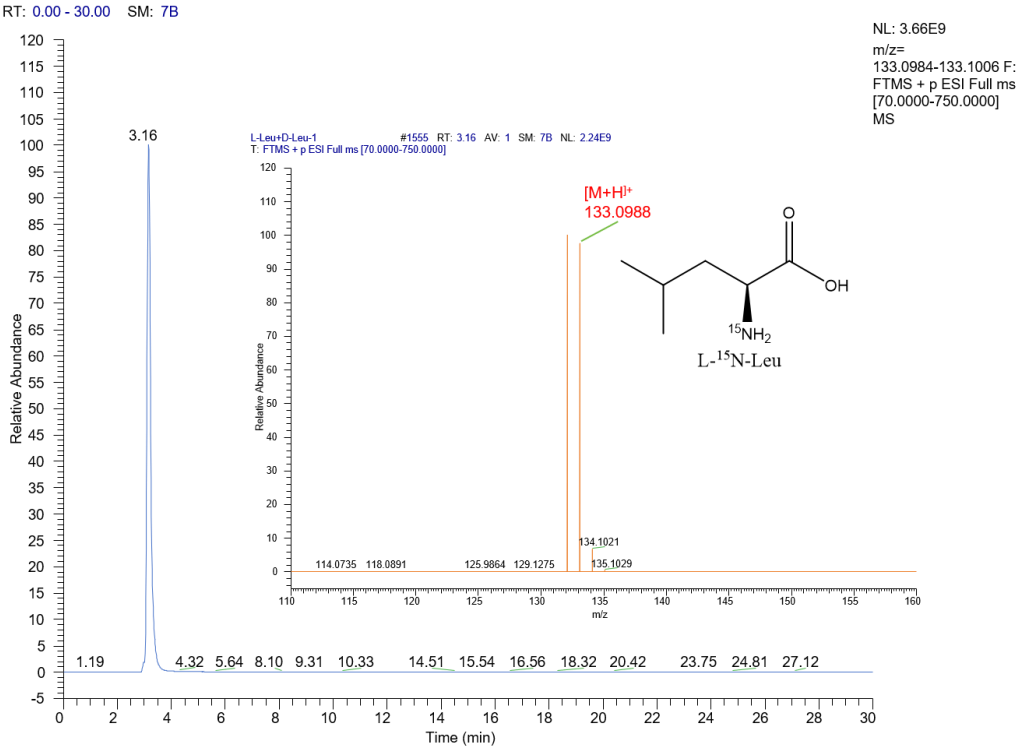


Supplementary Fig. 38 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 331.2355 was analyzed, and its EIC corresponded to the tripeptides L-^{15}N-Leu-L-Val$_2$ and L-Val$_2$-L-^{15}N-Leu with a retention time of 9.50 and 11.59 min.

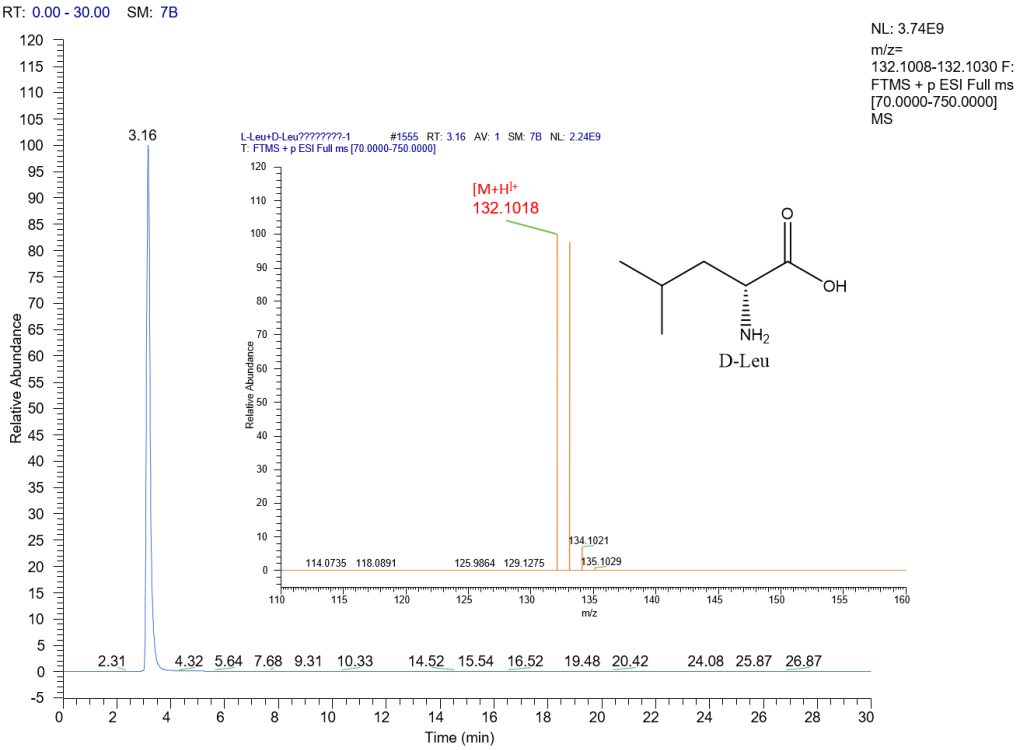


Supplementary Fig. 39 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 330.2385 was analyzed, and its EIC corresponded to the tripeptides L-Val_2-D-Leu and D-Leu-L-Val_2 with a retention time of 10.26 and 10.47 min.

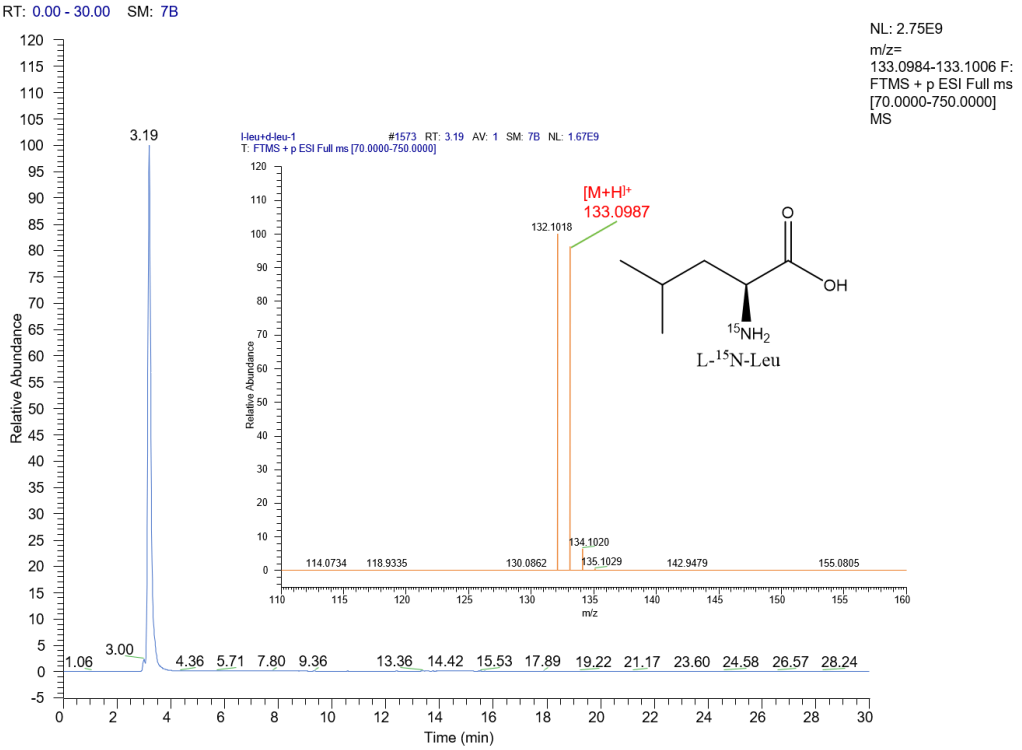
2.8 Chiral selection of Leucine after only chemical reactions. (Figs. S40-S46)



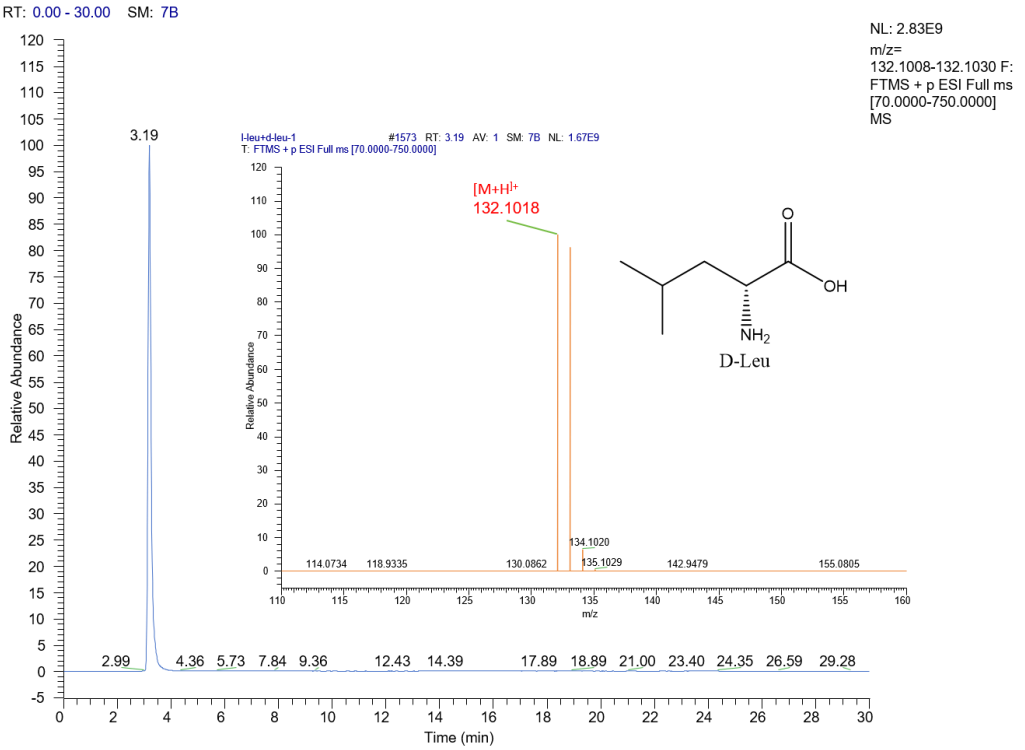
Supplementary Fig. 40 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 133.0988 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Leu with a retention time of 3.16 min.



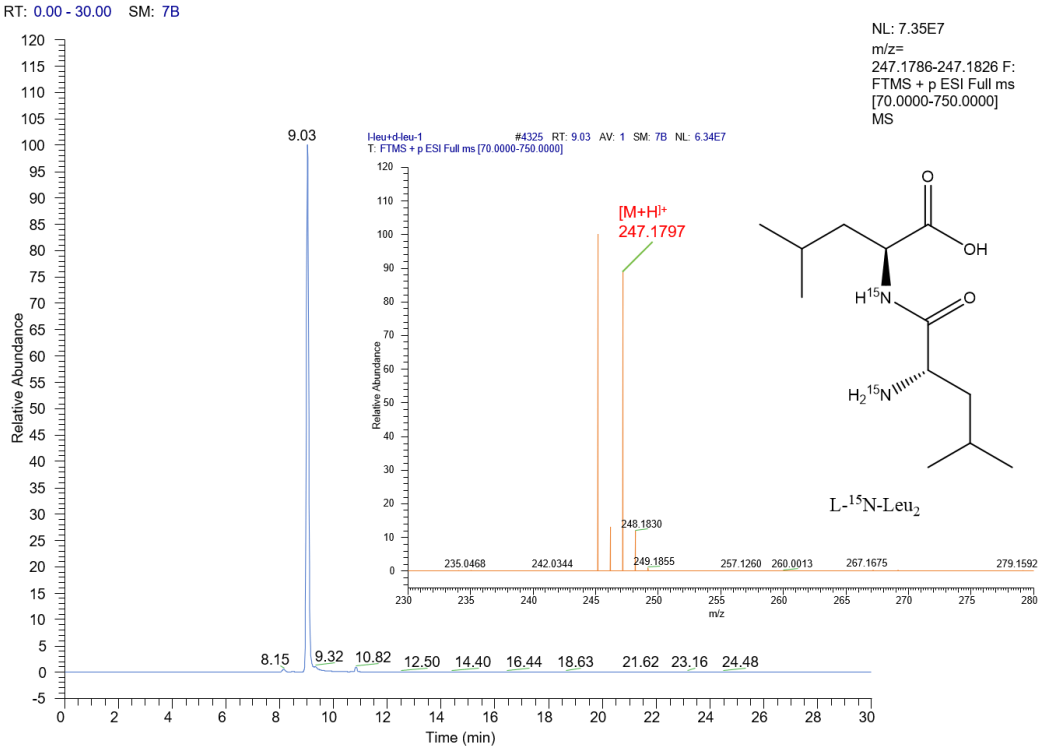
Supplementary Fig. 41 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 132.1018 was analyzed, and its EIC corresponded to the amino acid D-Leu with a retention time of 3.16 min.



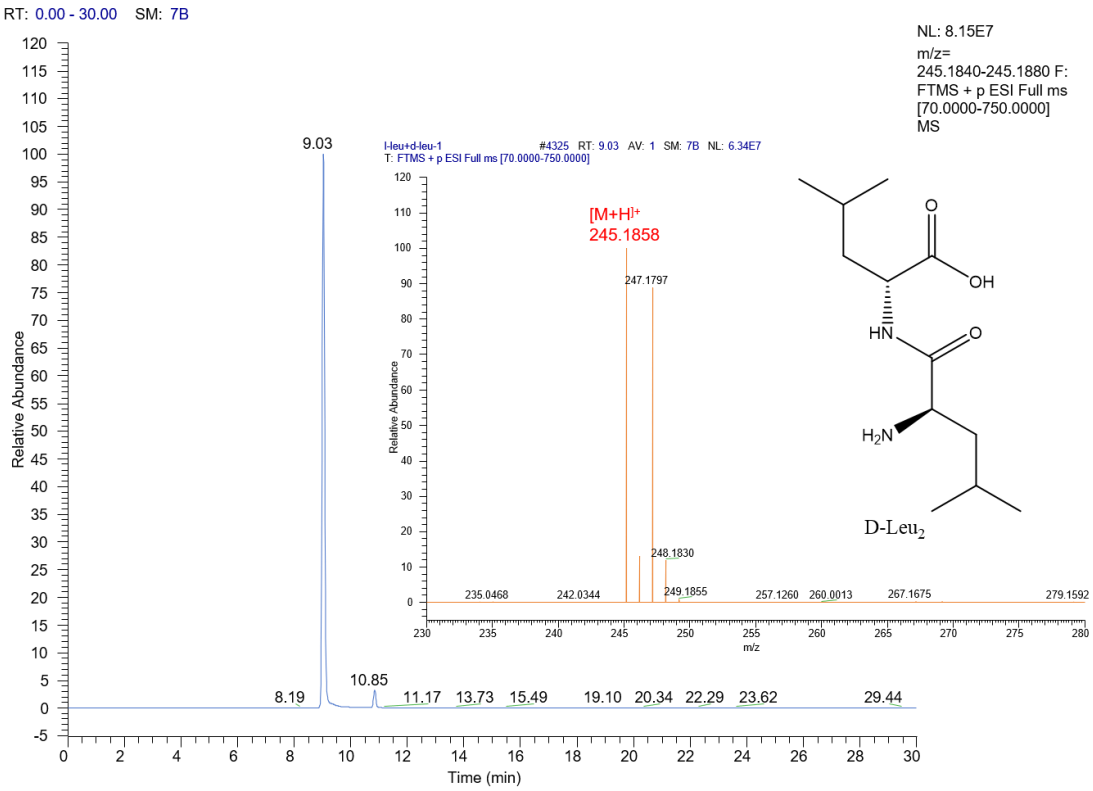
Supplementary Fig. 42 Extraction ion chromatography-mass spectrometry of the sample. The EIC of m/z 133.0987 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Leu with a retention time of 3.19 min.



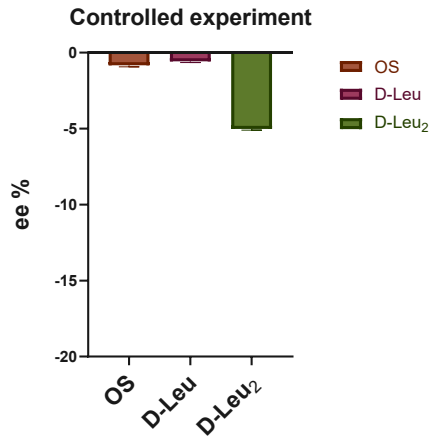
Supplementary Fig. 43 Extraction ion chromatography-mass spectrometry of the sample. The EIC of m/z 132.1018 was analyzed, and its EIC corresponded to the amino acid D-Leu with a retention time of 3.19 min.



Supplementary Fig. 44 Extraction ion chromatography-mass spectrometry of the sample. The EIC of m/z 247.1797 was analyzed, and its EIC corresponded to the dipeptide L-^{15}N-Leu$_2$ with a retention time of 9.03 min.

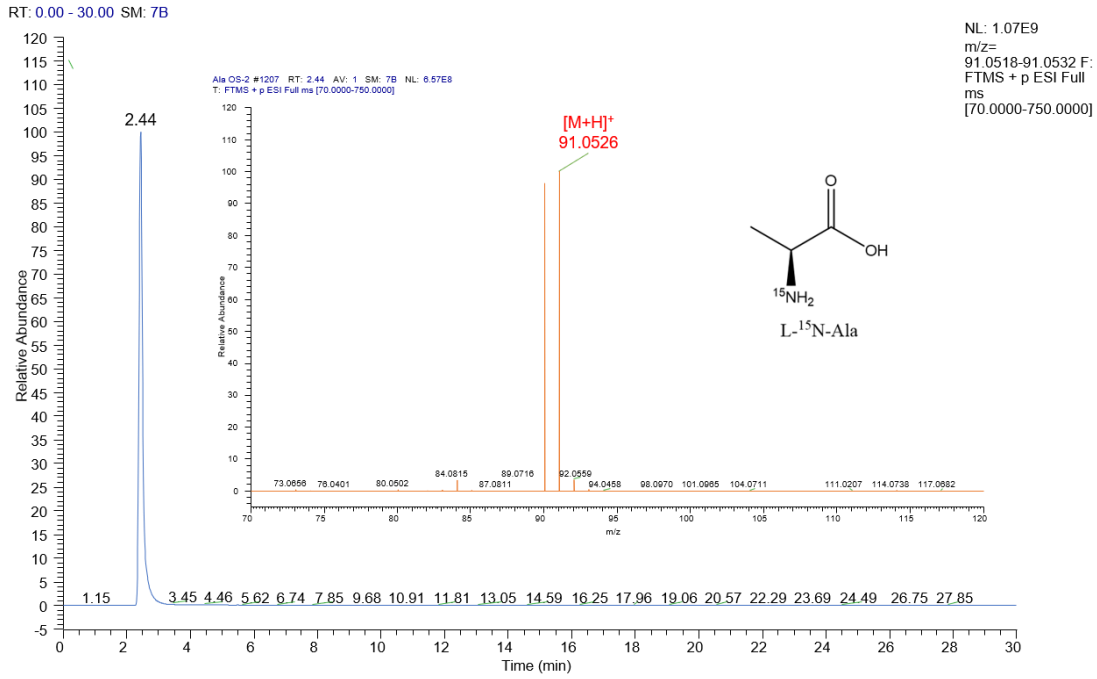


Supplementary Fig. 45 Extraction ion chromatography-mass spectrometry of the sample. The EIC of m/z 245.1858 was analyzed, and its EIC corresponded to the dipeptide D-Leu$_2$ with a retention time of 9.03 min.

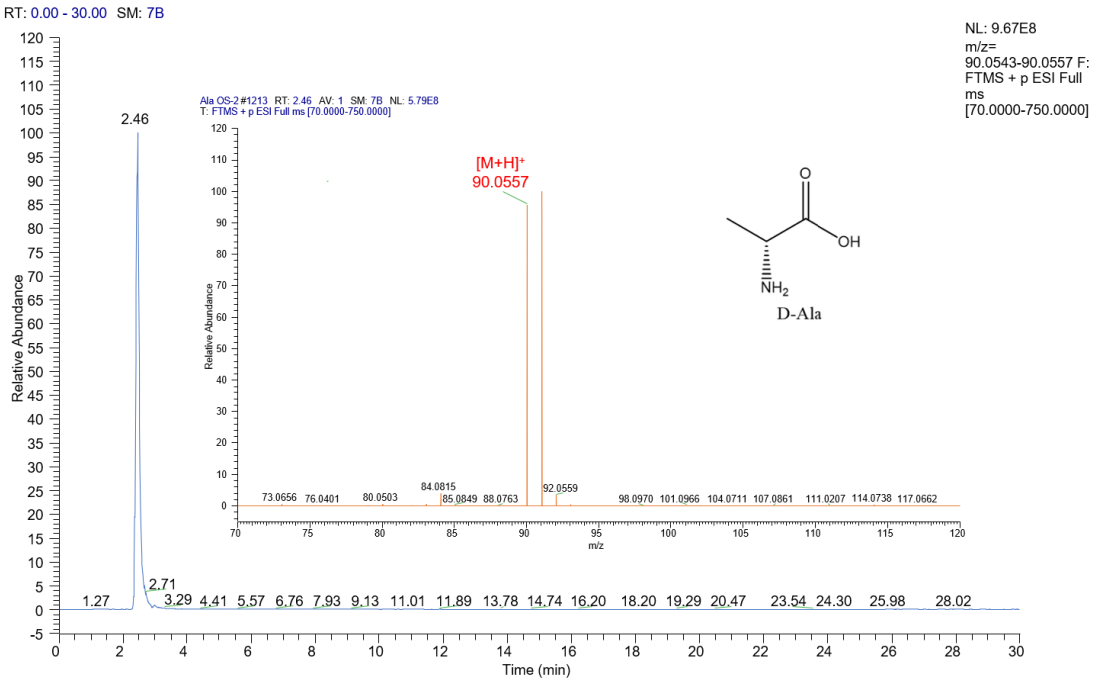


Supplementary Fig. 46 Chiral selectivity of Leu$_2$ formation in the presence of trimetaphosphate (P_3m). ee values for D-Leu/D-Leu$_2$ before and after the chemical synthesis process. OS: ee values (0.8%) of D-Leu in the original solution; D-Leu: ee values (0.6%) of D-Leu in the solution after the reaction; D-Leu$_2$: ee values (5.0%) of D-Leu$_2$ in the solution after the reaction.

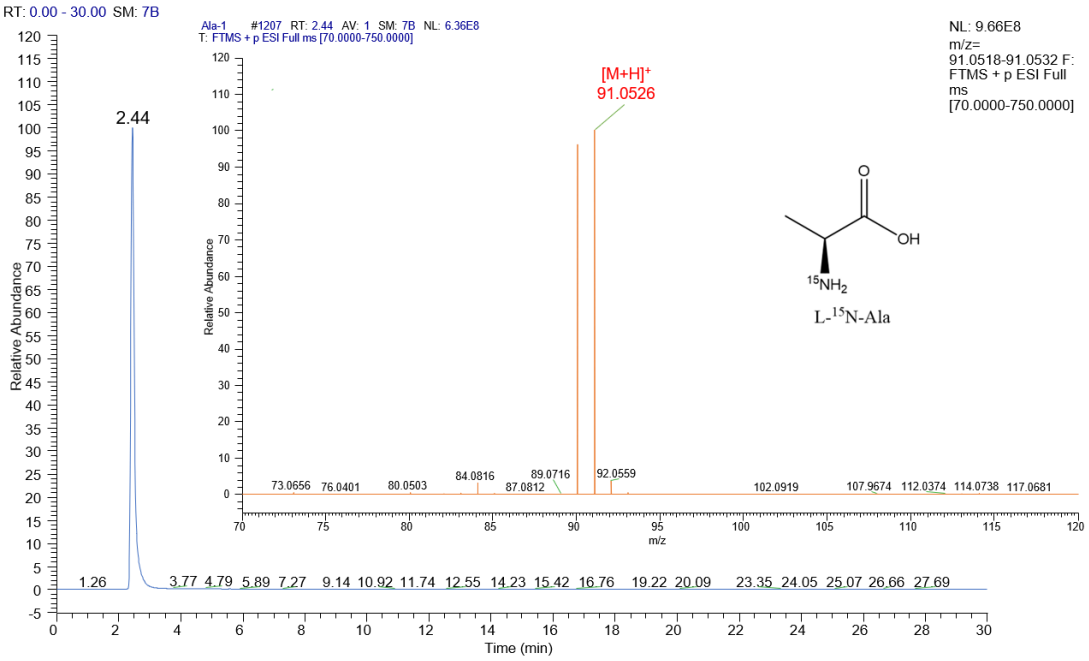
2.9 Chiral selection of Alanine after only chemical reactions. (Figs. S47-S53)



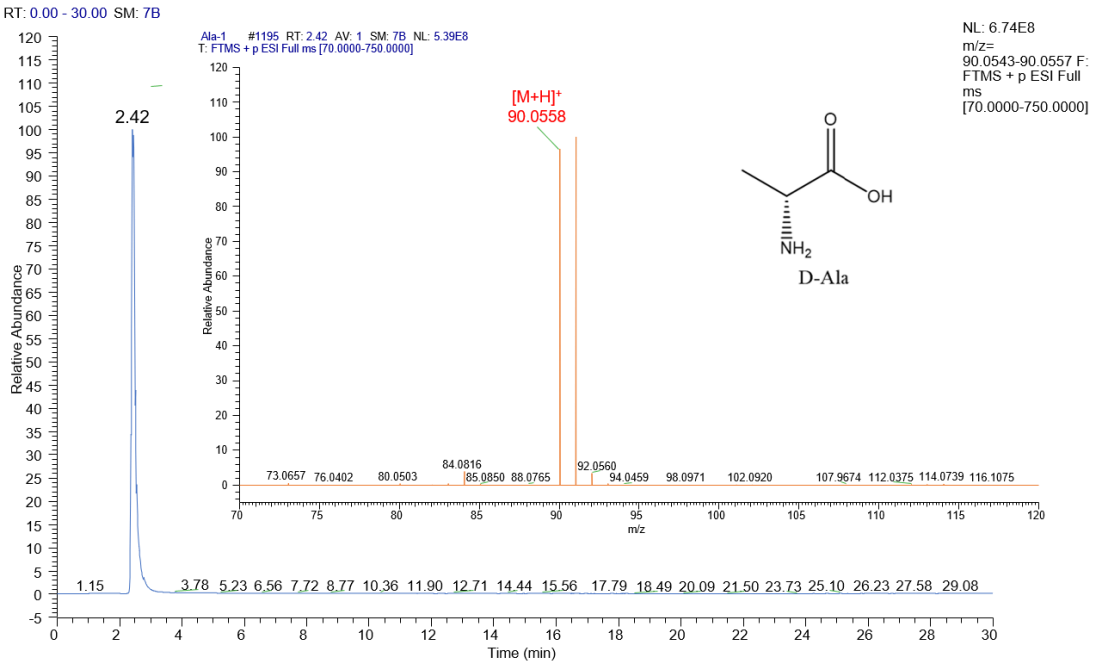
Supplementary Fig. 47 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 91.0526 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ala with a retention time of 2.44 min.



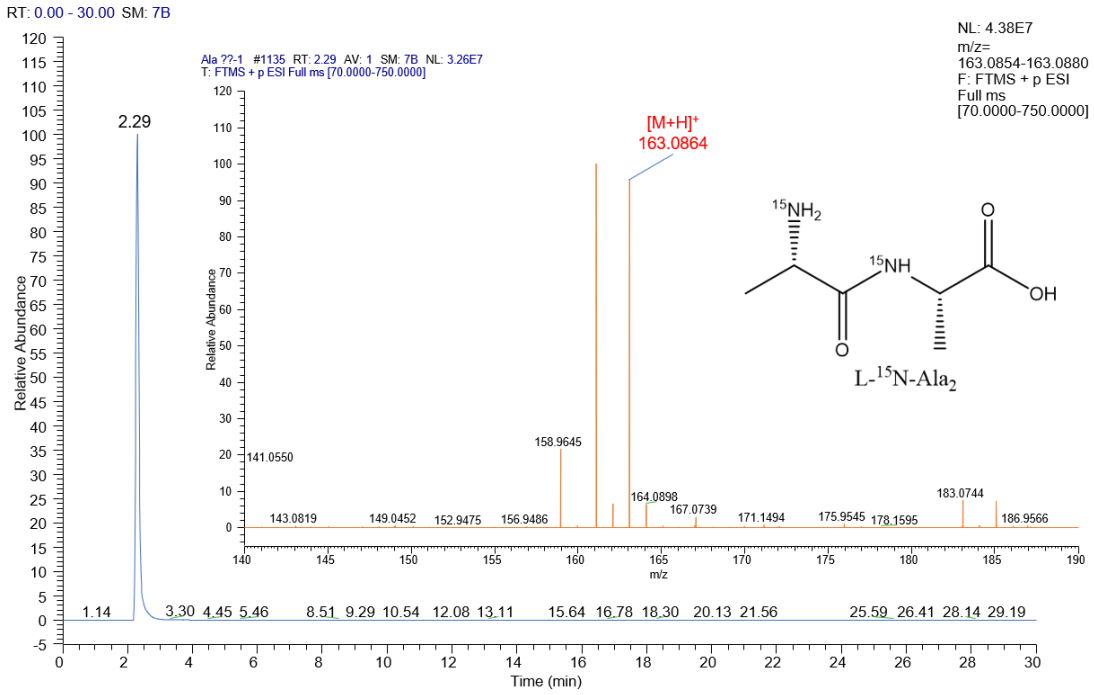
Supplementary Fig. 48 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 90.0557 was analyzed, and its EIC corresponded to the amino acid D-Ala with a retention time of 2.46 min.



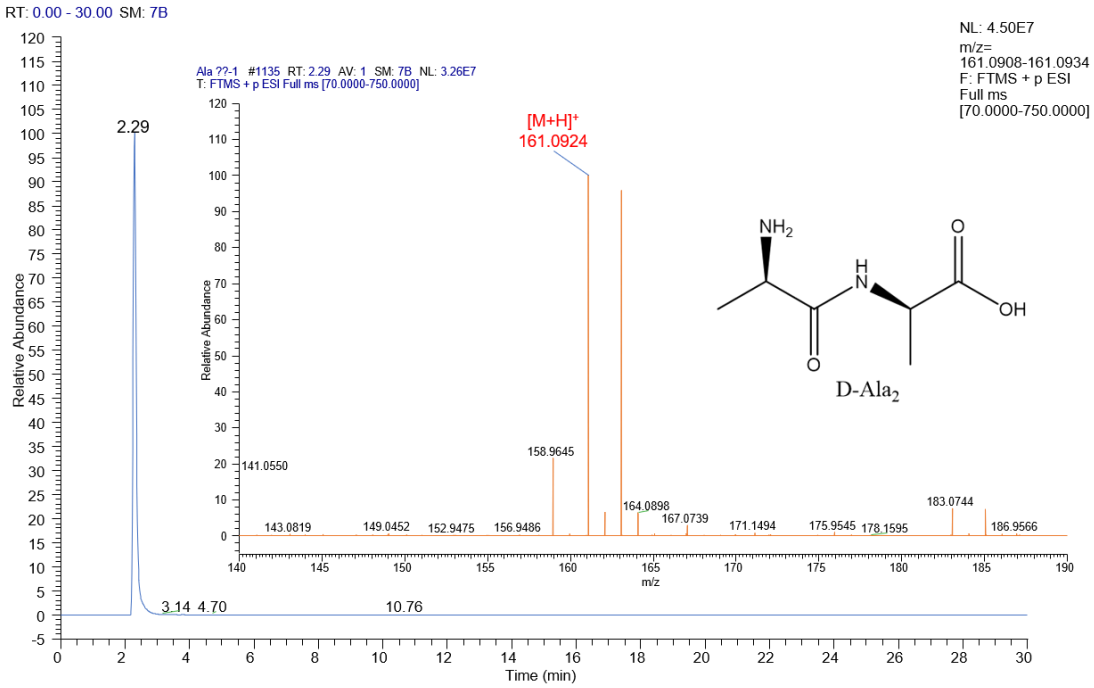
Supplementary Fig. 49 Extraction ion chromatography-mass spectrometry of the sample. The EIC of m/z 91.0526 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ala with a retention time of 2.44 min.



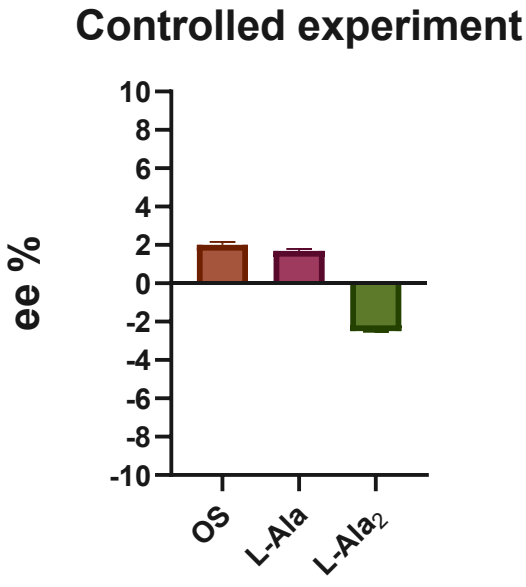
Supplementary Fig. 50 Extraction ion chromatography-mass spectrometry of the sample. The EIC of m/z 90.0556 was analyzed, and its EIC corresponded to the amino acid D-Ala with a retention time of 2.42 min.



Supplementary Fig. 51 Extraction ion chromatography-mass spectrometry of the sample. The EIC of m/z 163.0864 was analyzed, and its EIC corresponded to the dipeptide L-^{15}N-Ala$_2$ with a retention time of 2.29 min.



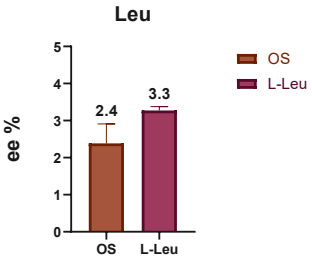
Supplementary Fig. 52 Extraction ion chromatography-mass spectrometry of the sample. The EIC of m/z 161.0924 was analyzed, and its EIC corresponded to the dipeptide D-Ala_2 with a retention time of 2.29 min.



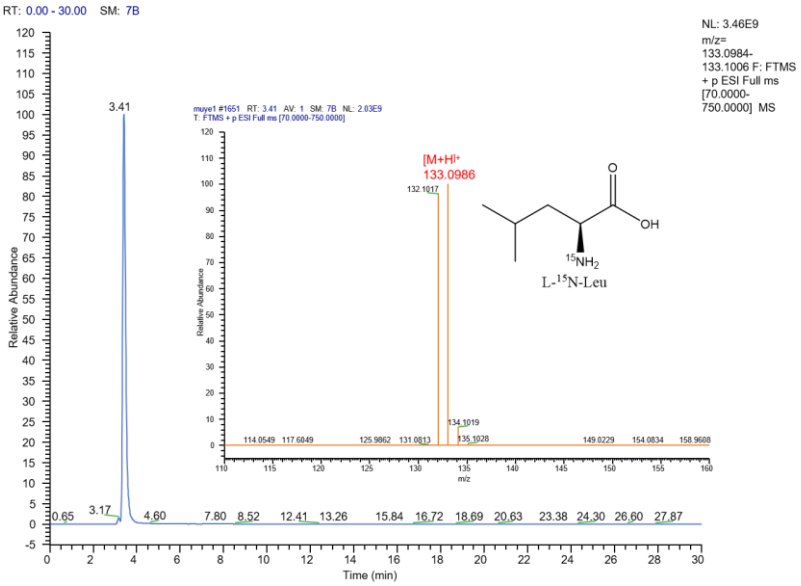
Supplementary Fig. 53 Chiral selectivity of Ala_2 formation in the presence of trimetaphosphate (P_3m). ee values for L-Ala/D-Ala_2 before and after the chemical synthesis process. OS: ee values (2.0%) of L-Ala in the original solution; L-Ala: ee values (1.7%) of L-Ala in the solution after the reaction; L-Ala_2: ee values (-2.5%) of L-Ala_2 in the solution after the reaction.

3 Supplementary Figures of Physical Adsorption

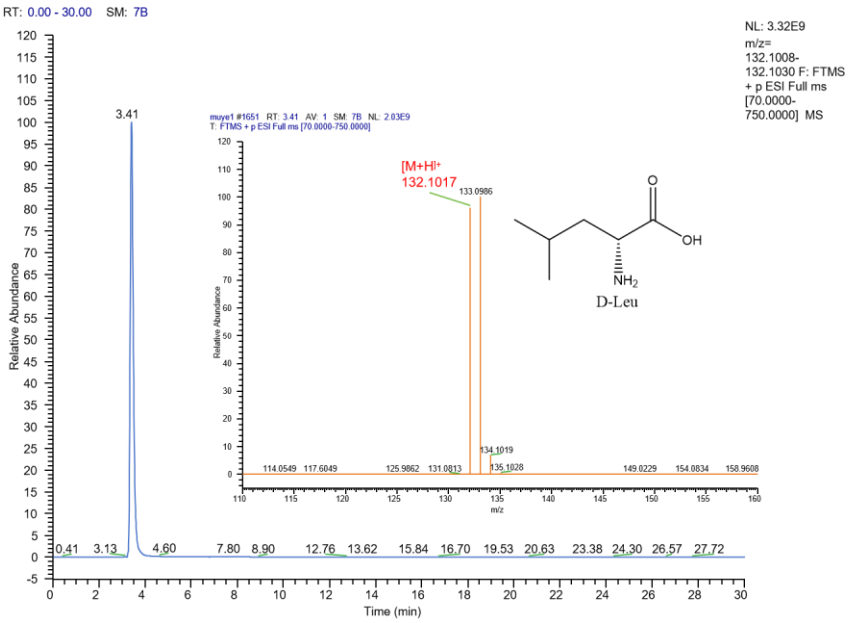
3.1 Chiral selection of Leu on calcite surface. (Figs. S54-S58)



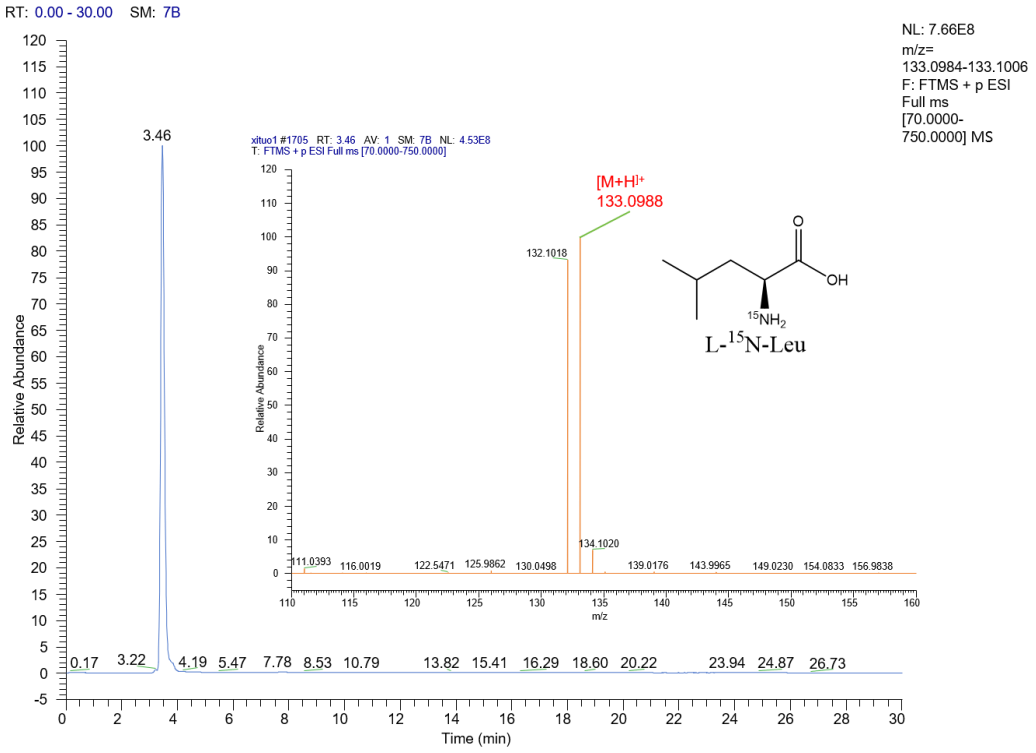
Supplementary Fig. 54 Investigation of chiral selection in Leu solutions before and after physical adsorption on calcite. OS: ee values in the original solution; L-Leu: ee values the solution after the reaction. The value of ee % > 0 represents the excess of L-enantiomer.



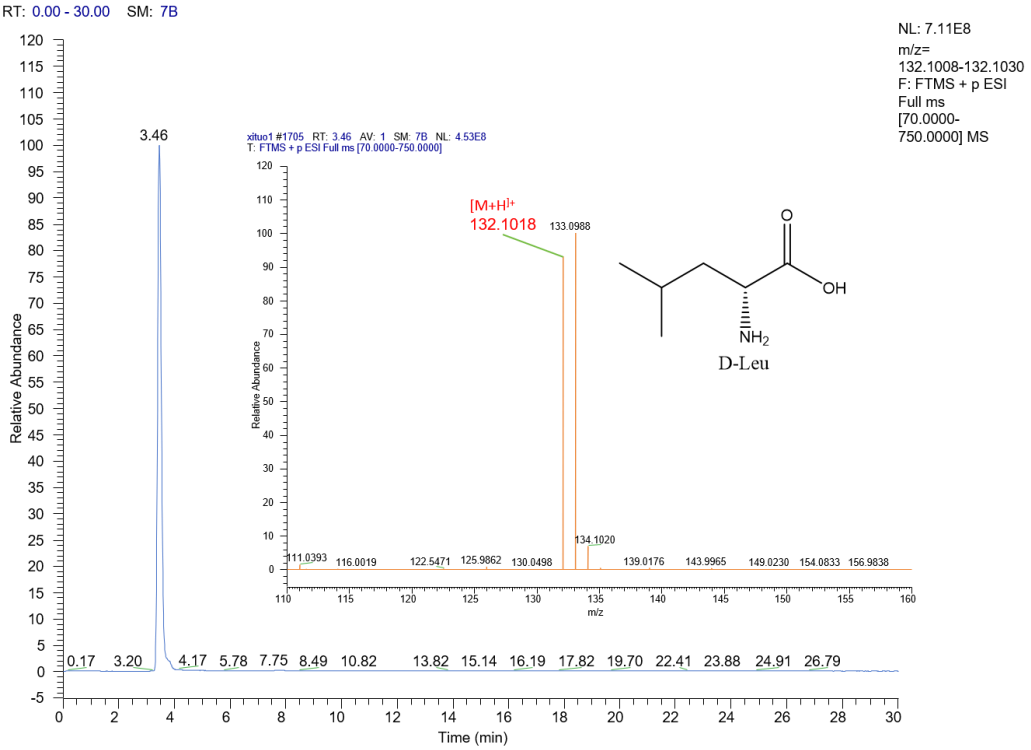
Supplementary Fig. 55 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 133.0986 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Leu with a retention time of 3.41 min.



Supplementary Fig. 56 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 132.1017 was analyzed, and its EIC corresponded to the amino acid D-Leu with a retention time of 3.41 min.

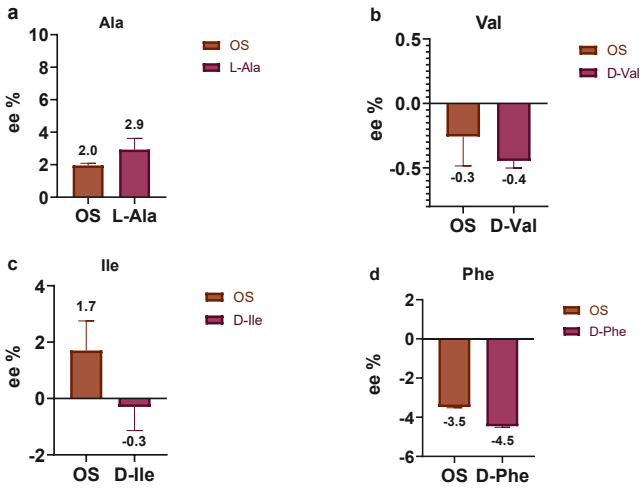


Supplementary Fig. 57 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 133.0988 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Leu with a retention time of 3.46 min.

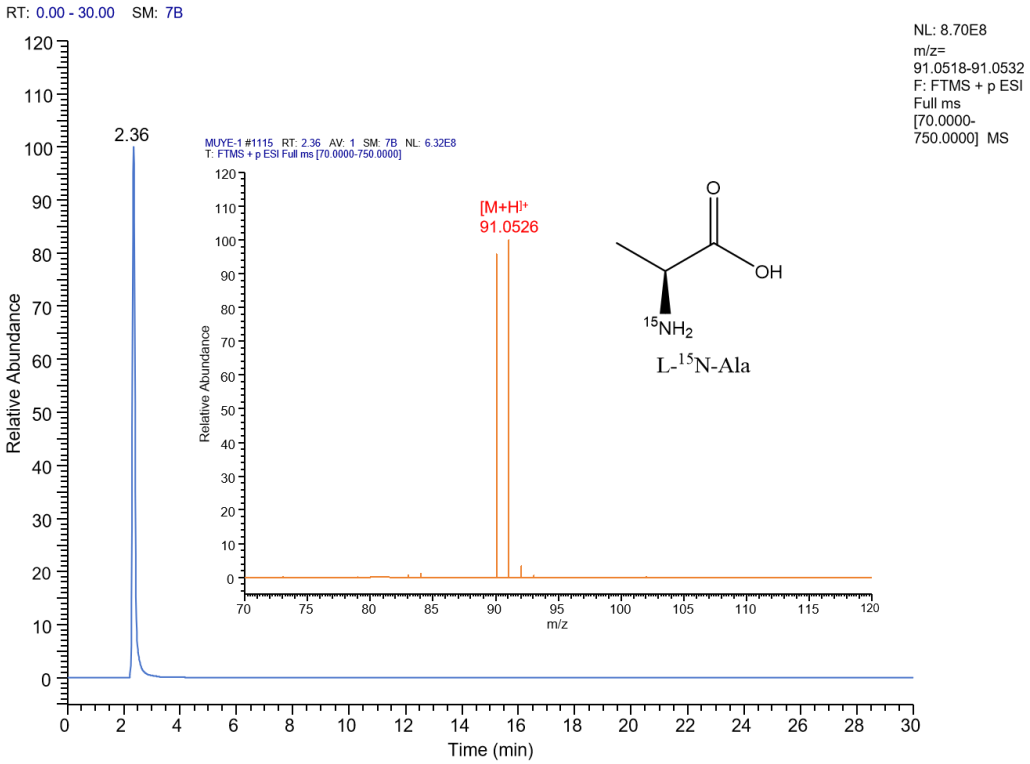


Supplementary Fig. 58 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 132.1018 was analyzed, and its EIC corresponded to the amino acid D-Leu with a retention time of 3.46 min.

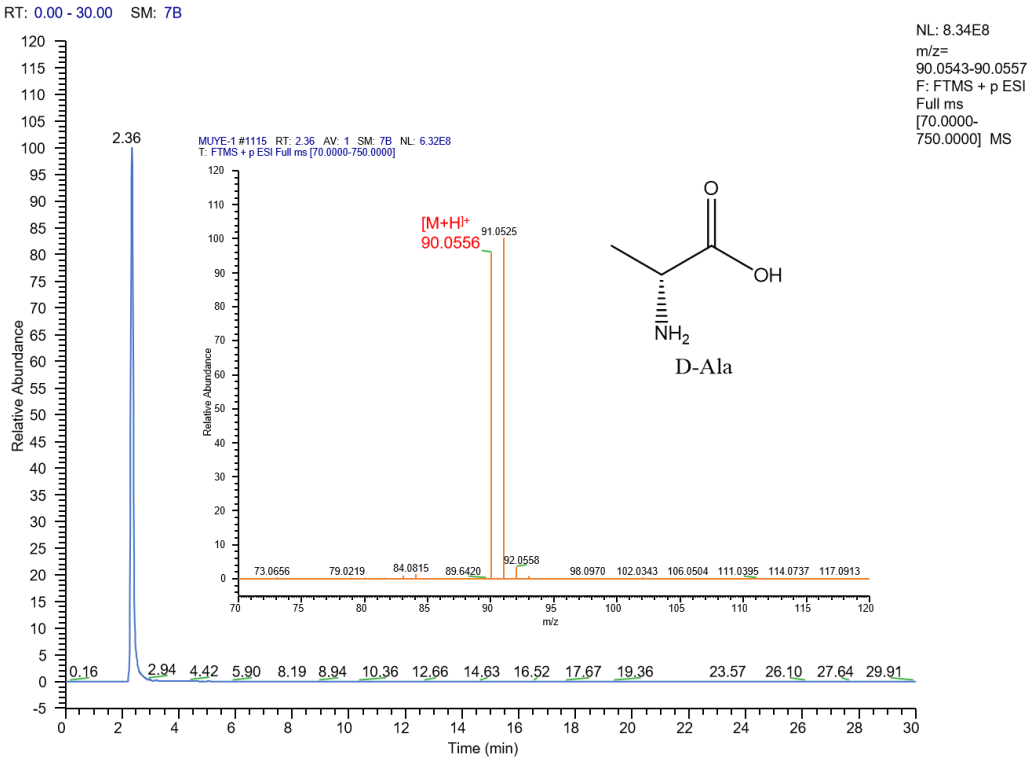
3.2 Chiral selection of Ala, Val, Ile and Phe on calcite surface. (Figs. S59-S75)



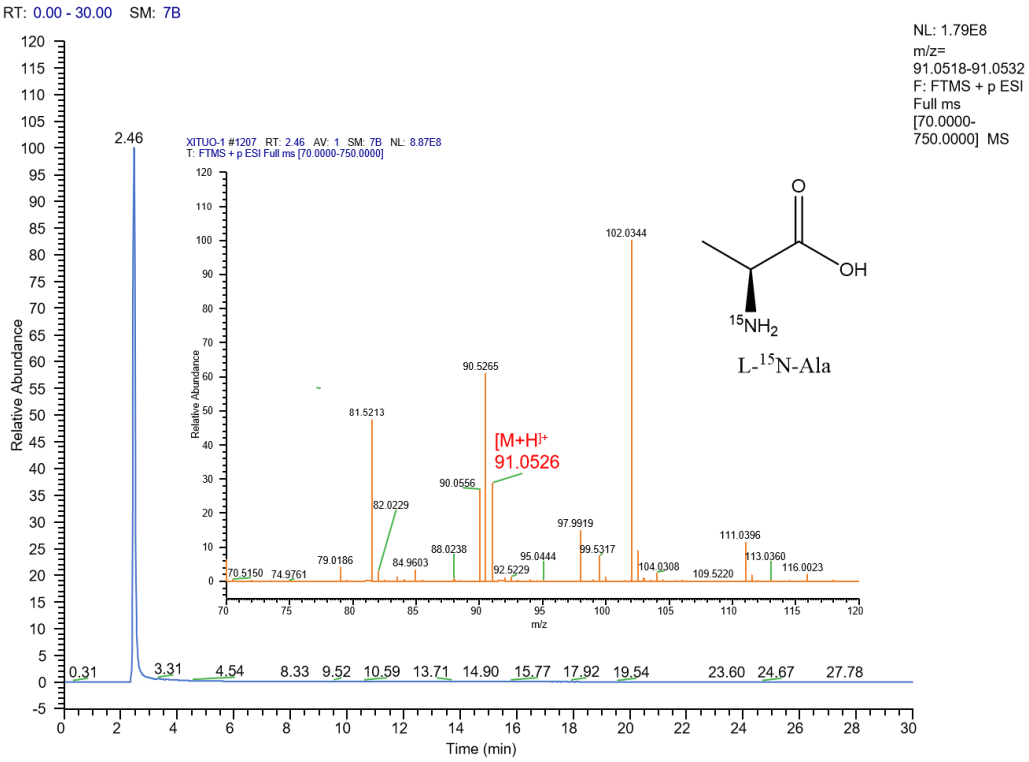
Supplementary Fig. 59 Investigation of chiral selection in four amino acids solutions before and after physical adsorption on calcite. (a) ee values for the solution before and after the physical adsorption of L-Ala; (b) ee values for the solution before and after the physical adsorption of D-Val; (c) ee values for the solution before and after the physical adsorption of L/D-Ile; (d) ee values for the solution before and after the physical adsorption of D-Phe. The value of ee % > 0 represents the excess of L-enantiomer, while the excess of D-enantiomer was represented by the value of ee % < 0.



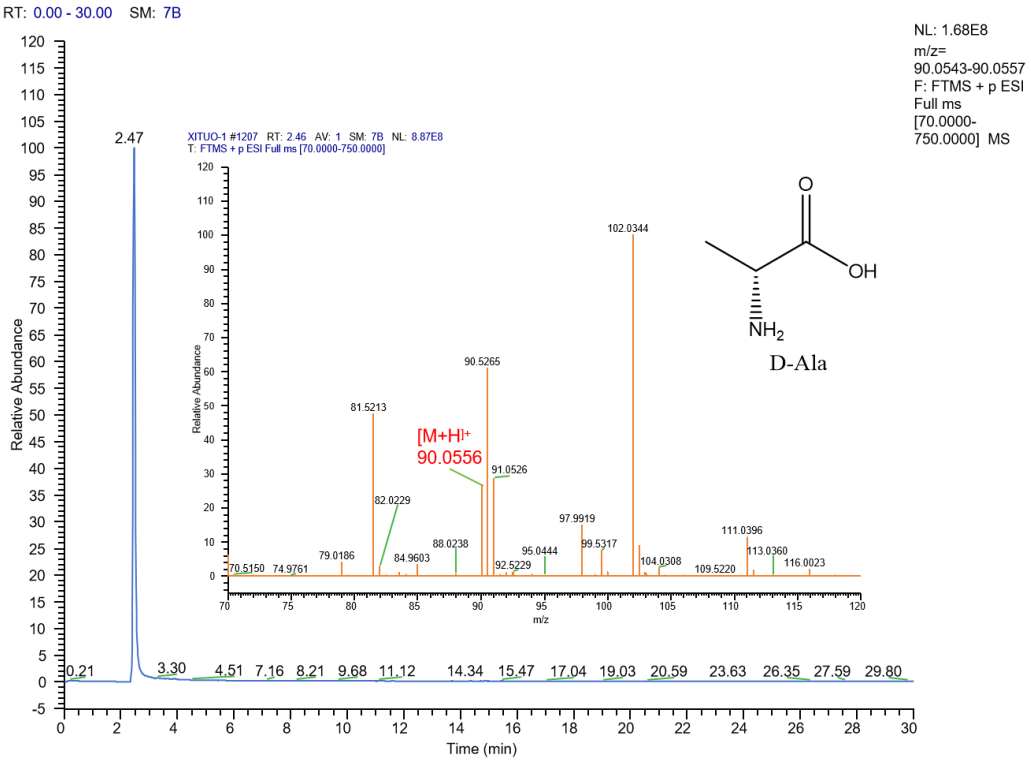
Supplementary Fig. 60 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 91.0526 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ala with a retention time of 2.36 min.



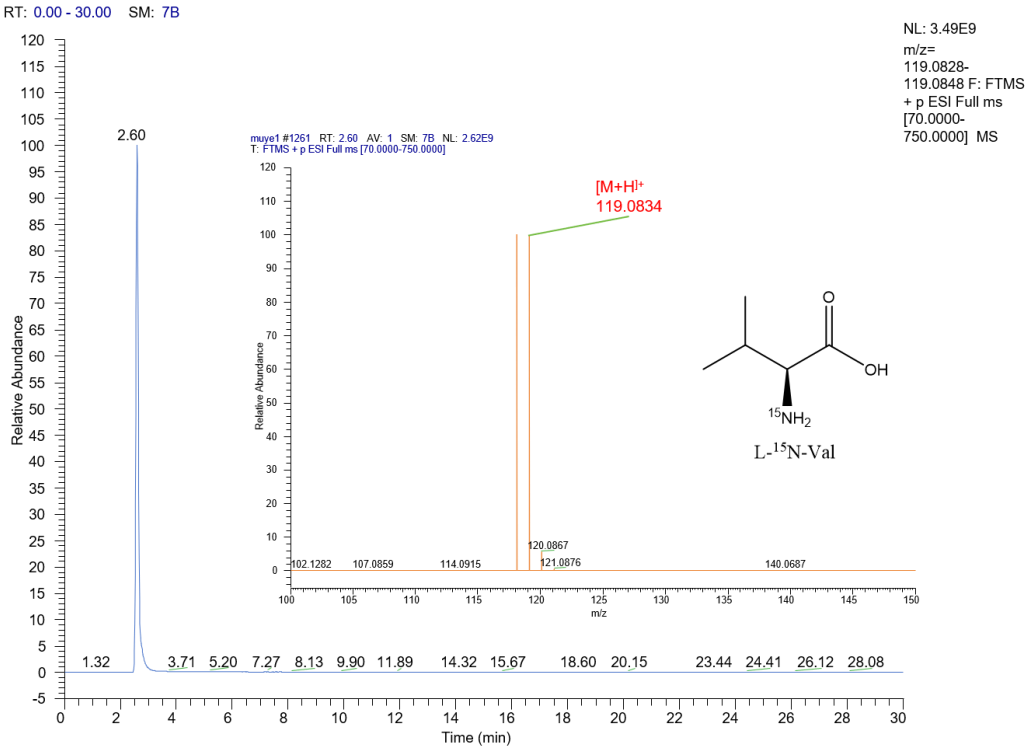
Supplementary Fig. 61 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 90.0556 was analyzed, and its EIC corresponded to the amino acid D-Ala with a retention time of 2.36 min.



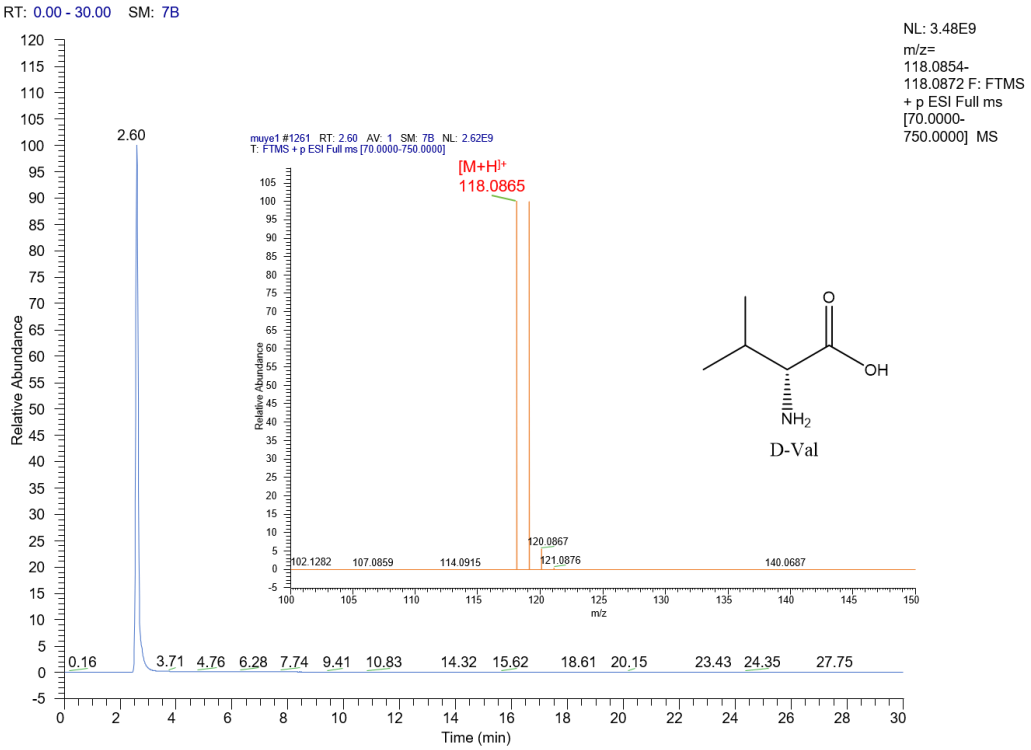
Supplementary Fig. 62 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 91.0526 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ala with a retention time of 2.46 min.



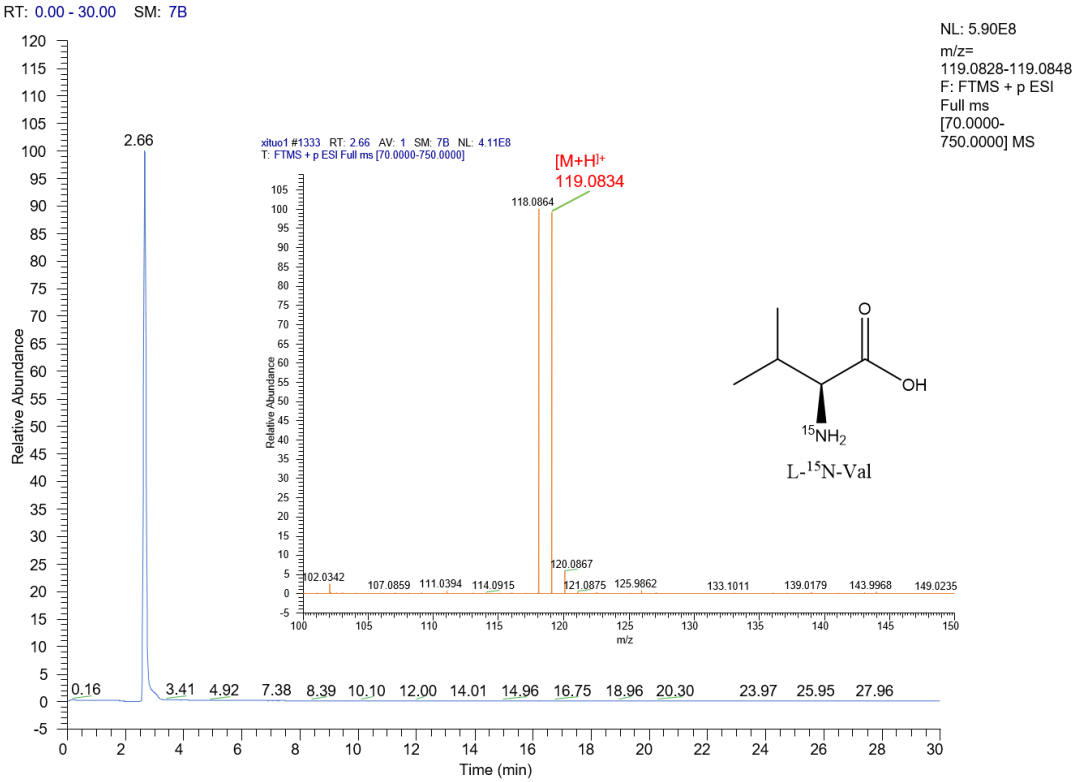
Supplementary Fig. 63 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 90.0556 was analyzed, and its EIC corresponded to the amino acid D-Ala with a retention time of 2.47 min.



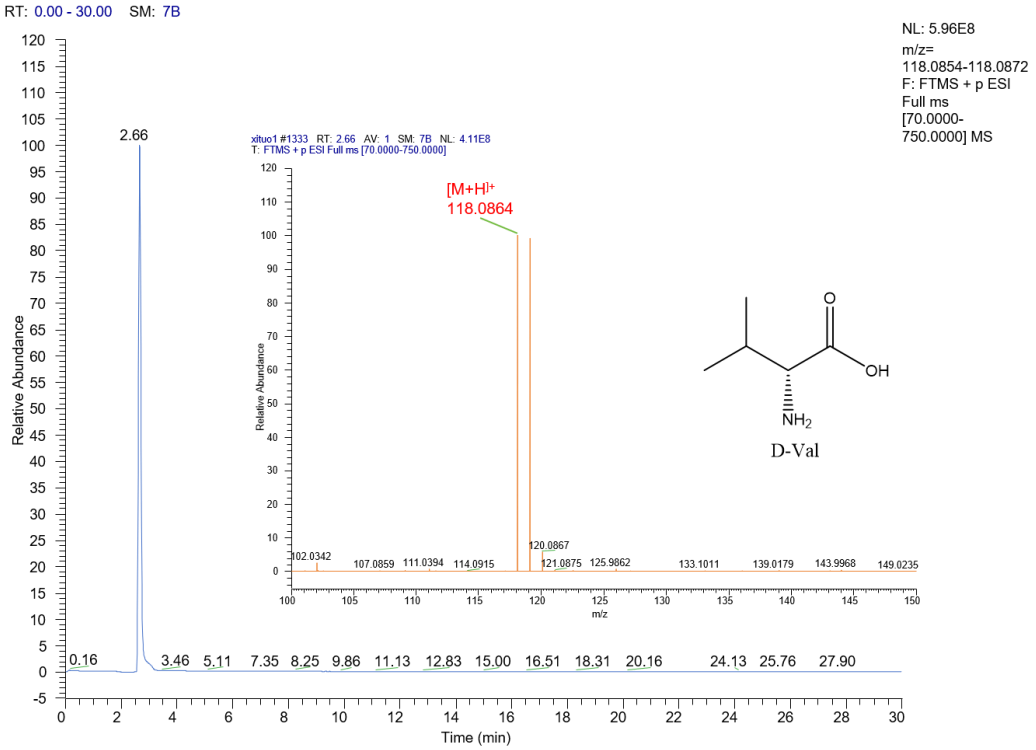
Supplementary Fig. 64 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 119.0834 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Val with a retention time of 2.60 min.



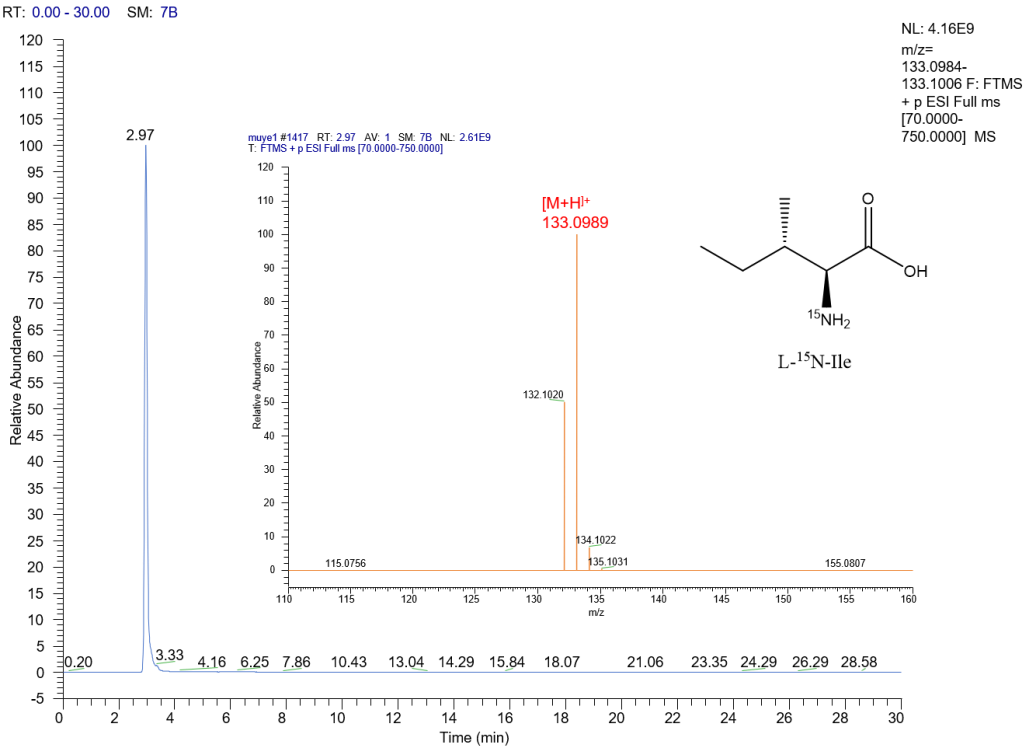
Supplementary Fig. 65 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 118.0865 was analyzed, and its EIC corresponded to the amino acid D-Val with a retention time of 2.60 min.



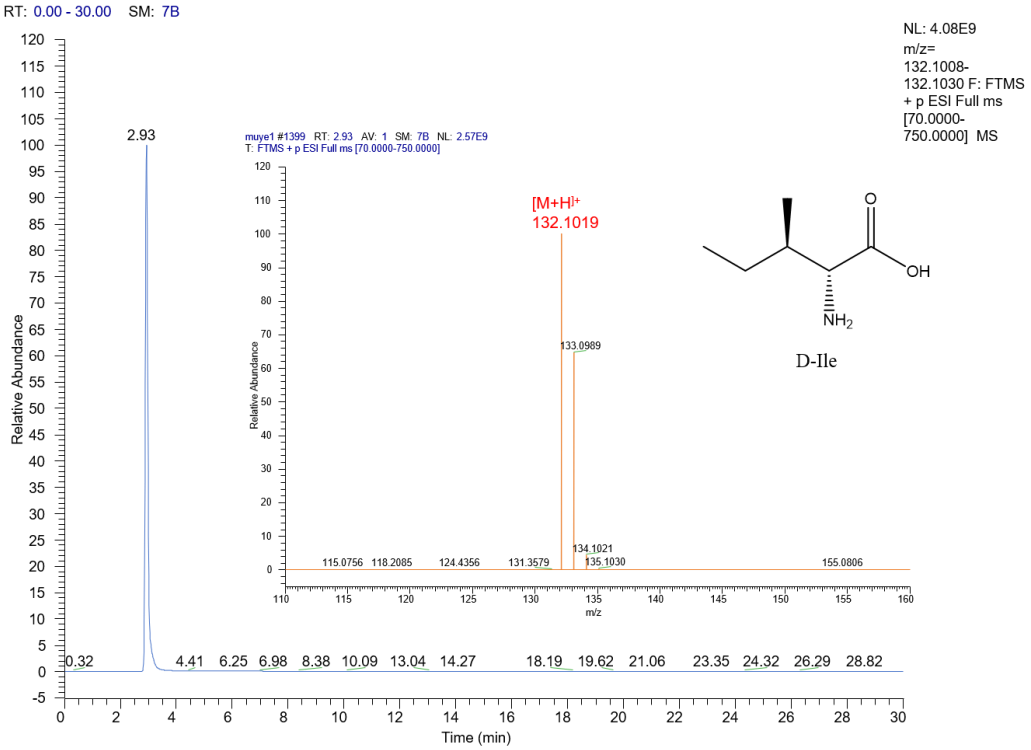
Supplementary Fig. 66 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 119.0834 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Val with a retention time of 2.66 min.



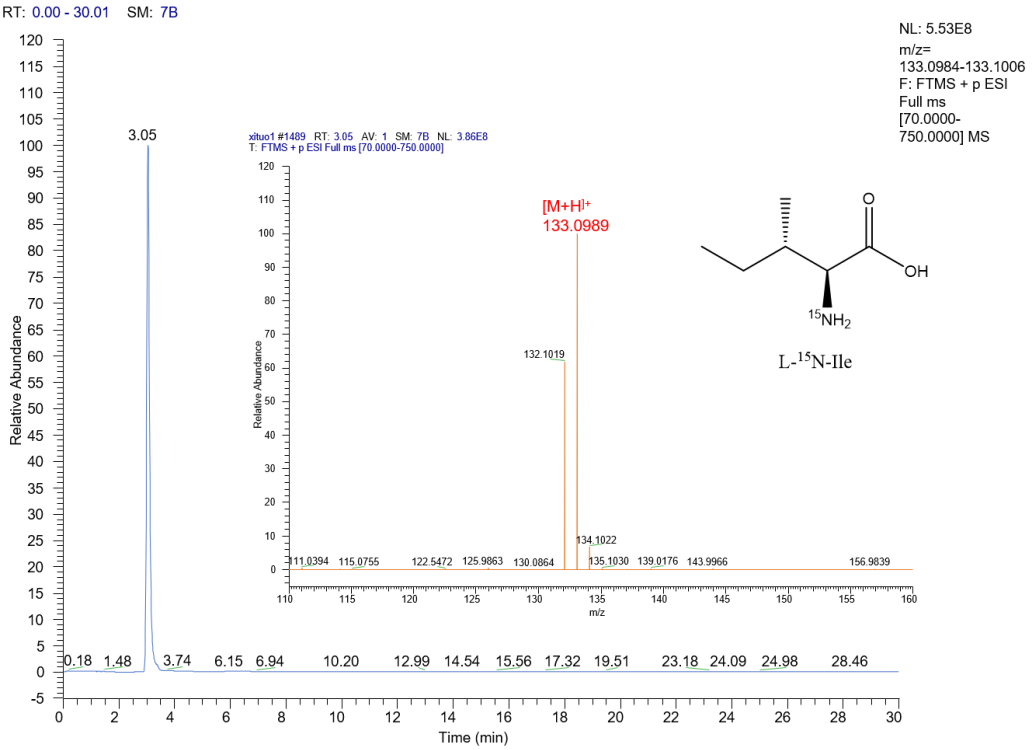
Supplementary Fig. 67 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 118.0864 was analyzed, and its EIC corresponded to the amino acid D-Val with a retention time of 2.66 min.



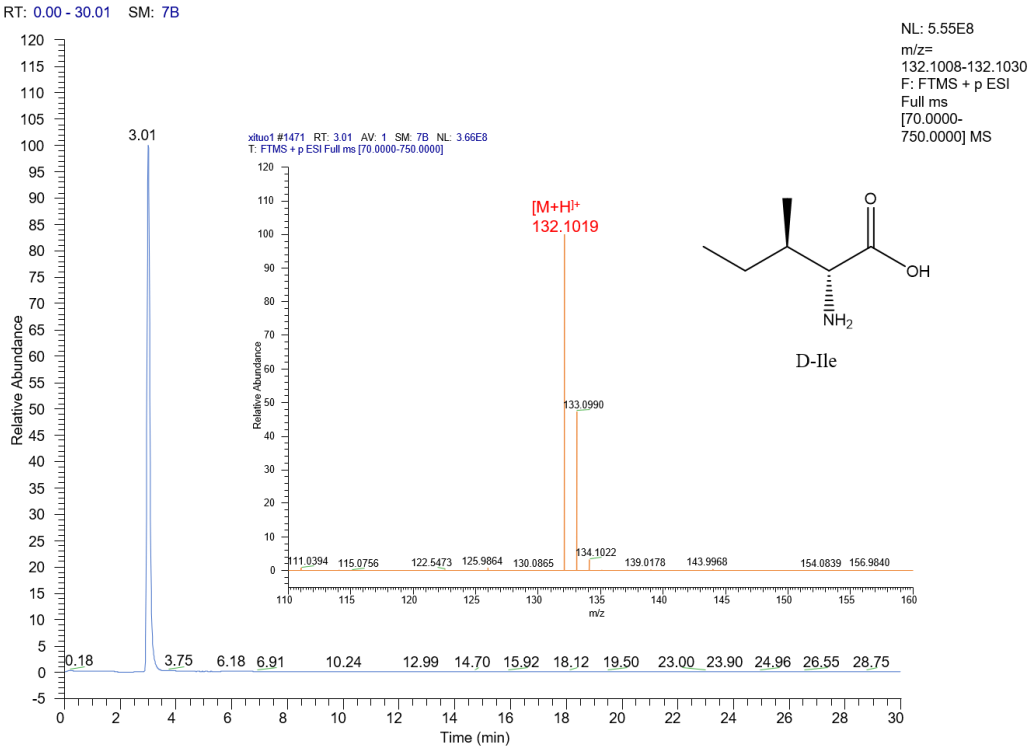
Supplementary Fig. 68 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 133.0989 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ile with a retention time of 2.97 min.



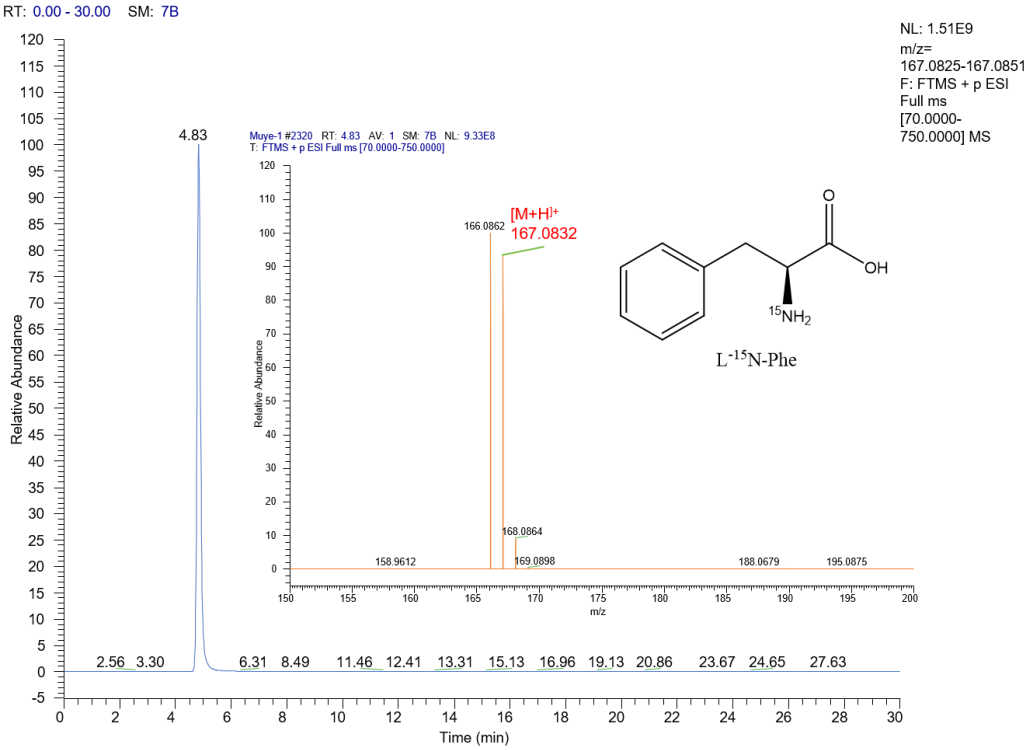
Supplementary Fig. 69 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 132.1019 was analyzed, and its EIC corresponded to the amino acid D-Ile with a retention time of 2.93 min.



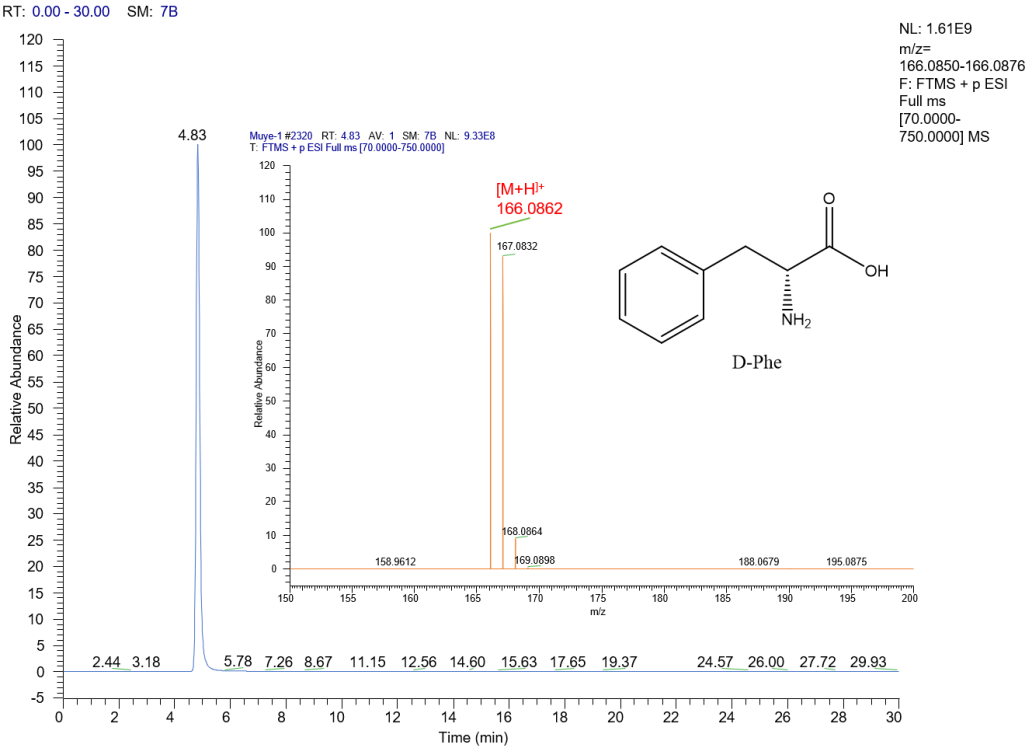
Supplementary Fig. 70 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 133.0989 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Ile with a retention time of 3.05 min.



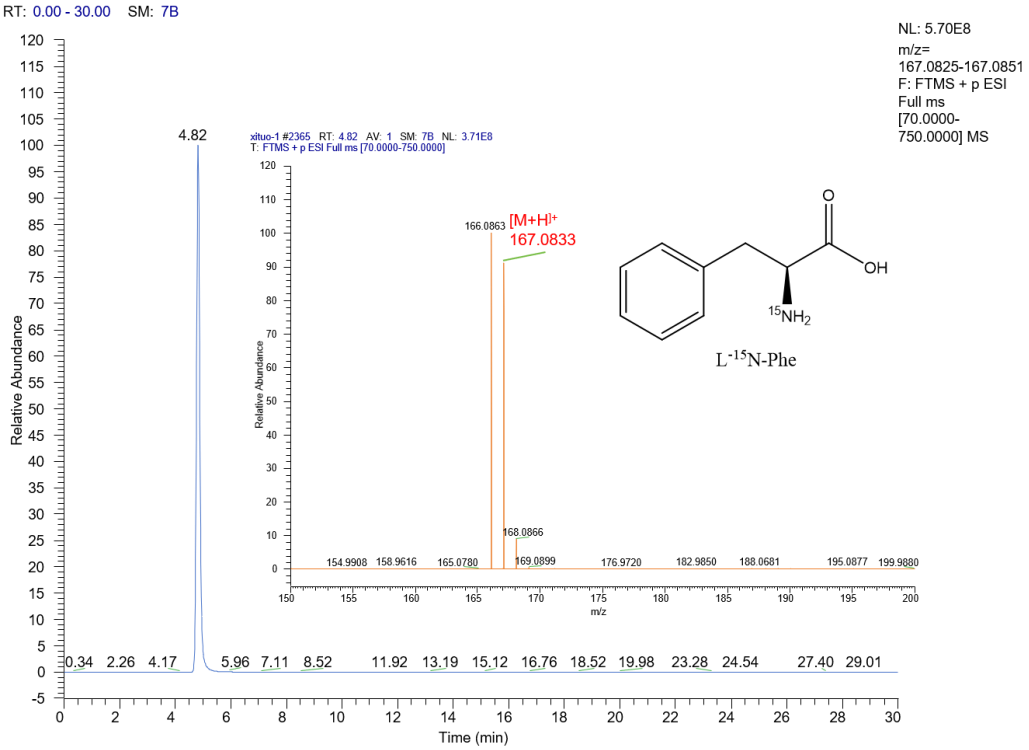
Supplementary Fig. 71 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 132.1019 was analyzed, and its EIC corresponded to the amino acid D-Ile with a retention time of 3.01 min.



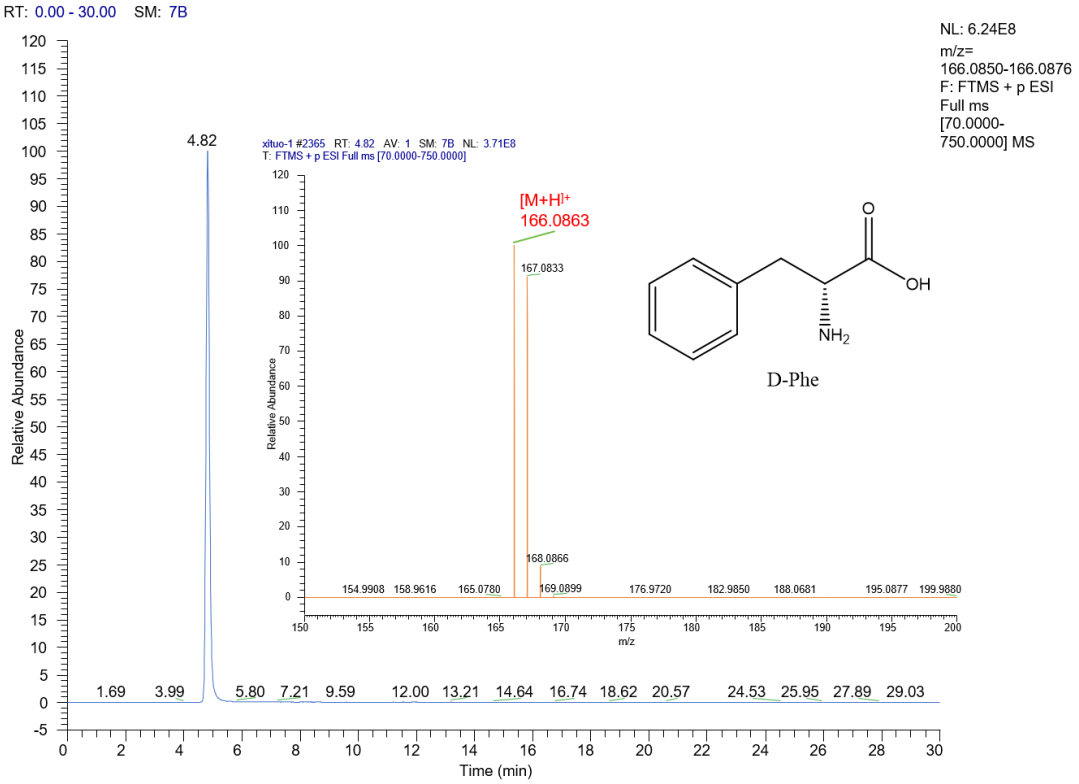
Supplementary Fig. 72 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 167.0832 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Phe with a retention time of 4.83 min.



Supplementary Fig. 73 Extraction ion chromatography-mass spectrometry of the original solution. The EIC of m/z 166.0862 was analyzed, and its EIC corresponded to the amino acid D-Phe with a retention time of 4.83 min.



Supplementary Fig. 74 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 167.0833 was analyzed, and its EIC corresponded to the amino acid L-^{15}N-Phe with a retention time of 4.82 min.



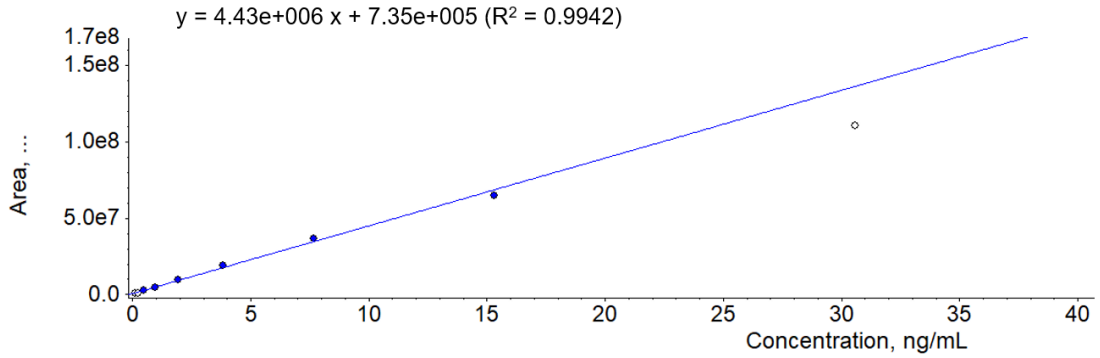
Supplementary Fig. 75 Extraction ion chromatography-mass spectrometry of the eluent. The EIC of m/z 166.0863 was analyzed, and its EIC corresponded to the amino acid D-Phe with a retention time of 4.82 min.

4 Comparison of L-Leu$_2$ yields under calcite and non-calcite conditions

4.1 Experimental conditions

A reaction solution containing 0.01 M L-Leu and 0.01 M P_3m (pH = 10 ± 0.2) was prepared, and 10 mL of this solution was transferred into a Petri dish with a diameter of 10 cm. In the experimental group, calcite was placed in the dish, whereas no treatment was applied in the control group. The system was then subjected to a wet–dry cycling process. During the drying phase, the dishes were incubated in an oven at 60 °C for 11.5 h. In the wetting phase, 10 mL of deionized water was added at room temperature to dissolve the dried substrates and products on the calcite surface, followed by a 0.5 h equilibration to complete the phase transition. After seven cycles, the reaction solutions were collected, and the products were analyzed by mass spectrometry to determine the yield.

4.2 Comparison of the yield of L-Leu with/without calcite conditions (Figs. S76)



Sample	Peak area	Concentration / (ng/mL)	Yield
EG	2.25e+007	4.90	4.02‰
CG	1.21e+007	2.56	2.11‰

Supplementary Fig. 76 Standard calibration curve and comparison of product yields between the experimental group (EG, with calcite) and the control group (CG, without calcite). The calibration curve was established using solutions of known concentrations of L-Leu dipeptide, showing a strong linear correlation (R^2 value). The accompanying table summarizes the detected product concentrations under identical conditions. The results indicate that the yield in the experimental group was higher than that in the control group, suggesting that calcite may play a promoting role in the formation of L-Leu dipeptides.