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Electronic supplementary information

A Rapid and Highly Enantioselective C-^{11}C Bond Formation of L-[^{11}C]Phenylalanine via Chiral Phase-Transfer Catalysis

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Abbreviations‡

AA: Amino acid

Cat 1: *O*-Allyl-*N*-(9-anthracenylmethyl) cinchonidinium bromide

Cat 2: (11b*S*)-(–)-4,4-Dibutyl-4,5-dihydro-2,6-bis(3,4,5-trifluorophenyl)-3H-dinaphth[2,1-c:1′,2′-e]azepinium bromide

Cat 3: (11b*R*)-(+)-4,4-Dibutyl-4,5-dihydro-2,6-bis(3,4,5-trifluorophenyl)-3H-dinaphth[2,1-c:1′,2′-e] azepinium bromide

Cat 4: (*R*,*R*)-3,4,5-fluorophenyl-NAS Bromide

Cat 5: *O*,*O*′-Diallyl-*N*,*N*′-(2,7-naphthalenediyldimethyl) bis(hydrocinchonidinium) dibromide

$CDCl_3$: Deuterated chloroform

DCM: Dichloromethane

DMSO: Dimethyl sulfoxide

EOB: End of bombardment

GMP: Good manufacturing practice

HCl: Hydrochloric acid

He: Helium

HI: Hydroiodic acid

ppm: Parts per milion

PTC: Phase-transfer catalysis

RCC: Radiochemical conversion

RCY: Radiochemical yield

SA: Specific activity

SD: Standard Deviation

T: Temperature

TBAF: *tetra-n*-butylammonium fluoride

TBAB: *tetra*-n-butylammonium bromide

TBAHS: *tetra*-n-butylammonium hydrogen sulfate

THF: Tetrahydrofurane

Experimental details

General

Chemicals were obtained commercially from Sigma-Aldrich (Zwijndrecht, the Netherlands), Fluorochem (Derbyshire, United Kingdom), Bachem (Bubendorf, Switzerland) and Wako Chemicals GmbH (Neuss, Germany) and used without further purification. Solvents were purchased from Biosolve (Valkenswaard, the Netherlands) and used as received unless stated otherwise. Reactions were performed at ambient temperature unless stated otherwise. Reactions were monitored by thin layer chromatography on pre-coated silica 60 F_{254} aluminum plates (Merck, Darmstadt, Germany). Spots were visualized by UV light, ninhydrine and bromcrezol green. Solvents were evaporated under reduced pressure using a rotary evaporator (Rotavapor® R II, Flawil, Switzerland). Flash column chromatography was performed on a Büchi (Flawil, Switzerland) Sepacore system (comprising a C-620 control unit, a C-660 fraction collector, two C-601 gradient pumps and a C-640 UV detector) equipped with Büchi Sepacore pre-packed flash columns.

Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker (Billerica, MA, USA) Avance 250 (250.13 MHz for ^{1}H) or a Bruker Avance 500 (500.23 MHz for ^{1}H and 125.78 MHz for ^{13}C) with chemical shifts (δ) reported in parts per million (ppm) relative to the solvent ($CDCl_3$, ^{1}H 7.26 ppm, ^{13}C 77.16 ppm). Splitting patterns are indicated as s:

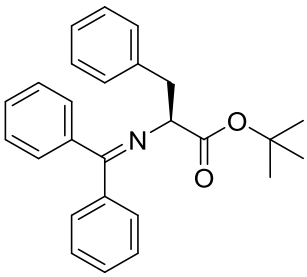
singlet, d: doublet, t: triplet, q: quartet, m: multiplet and br: broad peak, and *J*, coupling constant in Hz.

Electrospray ionization-high resolution mass spectrometry (ESI-HRMS) was carried out using Bruker microTOF-Q instrument in a positive ion mode (capillary potential of 4500 V).

Analytical isocratic high pressure liquid-chromatography (HPLC) was performed on a Jasco (Easton, MD, USA) PU-2089 Plus station with a Alltima C18 5 µm (250 x 4.6 mm) column (Grace, Breda, the Netherlands) (A), ReproSil 100 Chiral-AA 8 µm (250 x 4.0 mm) column (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) (B), and Chiralcel OJ 10 µm (250 x 4.6 mm) (Chiral Tecnologies Europe, Illkirch, France) (C) with Jasco UV-2075 Plus UV detector (254 nm and 210 nm) and NaI radioactivity detector (Raytest, Straubenhardt, Germany) at ambient temperature. Acetonitrile (CH_3CN) (D), methanol (MeOH) (E), water (F), n-Hexane (G), 2-propanol (H) and buffer 1 (4 mM sodium formate and 4 % dimethylformamide) (I) were used as mobile phases. Chromatograms were acquired with Raytest GINA Star software (version 5.8).

Synthesis

N-(diphenylmethylene)-L-*phenylalanine tert-butyl ester*

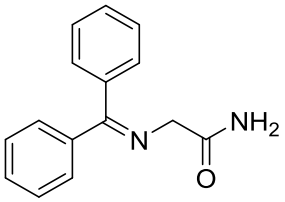


Reference compound was synthesized and characterized according to O'Donnell *et al.*[1,2]

Benzophenone imine (1 eq, 1.94 mmol, 354 mg), dissolved in 2 mL of dichloromethane (DCM), was added to (*S*)-phenylalanine *tert*-butyl ester (1 eq, 1.94 mmol, 499 mg), dissolved

in 2 mL of DCM. The mixture was stirred for 48 h at RT, filtered and evaporated to dryness on a rotary evaporator. Ether (20 mL) was added to the residue and the resulting solution was washed with water (3 x 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude compound was purified using flash column chromatography to obtain pure product in 74.6 % yield (557 mg, 1.45 mmol). ^{1}H NMR (250.13 MHz, $CDCl_3$, ambient *T*): δ 7.77 -7.67 (m, 2 H), 7.61 -7.55 (m, 2 H), 7.49 – 7.35 (m, 7 H), 7.22 - 7.19 (m, 2 H), 7.05 – 7.02 (m, 2 H), 6.67 (s, 1H), 3.12 (d, *J*= 7.5 Hz, 1 H), 3.04 (d, *J*= 7.5 Hz, 1 H), 1.36 (s, 9 H) ppm.

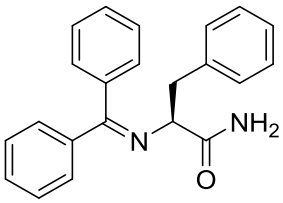
*N-(diphenylmethylene)-glycine amide (**9**)*



Reference compound **9** was synthesized according to O'Donnell *et al.*[1] and characterized according to O'Donnell *et al.*[3]

Benzophenone imine (1 eq, 13.5 mmol, 2.50 g), dissolved in 10 mL of DCM, was added to glycine amide (1 eq, 13.5 mmol, 1.00 g), dissolved in 20 mL of DCM. The mixture was stirred for 16 h at RT, filtered and evaporated to dryness on a rotary evaporator. After addition of small amount of EtOAc and n-Hexane, precipitation occurred and white crystals were filtered to obtain **9** in 6.31 % yield (203 mg, 851 µmol). No further isolation was performed with the remaining reaction mixture. ^{1}H NMR (250.13 MHz, $CDCl_3$, ambient *T*): δ 7.61 -7.56 (m, 2 H), 7.44 – 7.30 (m, 6 H), 7.08 (m, 2 H), 5.63 (s, 1 H), 3.91 (s, 2 H) ppm. ^{13}C NMR (125.78 MHz, $CDCl_3$, ambient *T*): δ 173.51, 170.34, 138.74, 129.05, 128.90, 57.06 ppm. HRMS (m/z) calculated for $C_{15}H_{16}N_2O$ 239.12; found 239.1216 [M+H$^+$], calculated for $C_{15}H_{14}N_2NaO$ 261.10; found 261.1033 [M+Na]$^+$.

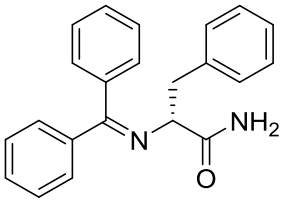
N-(diphenylmethylene)-L-*phenylalanine amide*



Reference compound was synthesized according to O'Donnell *et al.*[1] and characterized according to Hyett *et al.*[4]

Benzophenone imine (1 eq, 1.22 mmol, 221 mg), dissolved in 7 mL of DCM, was added to L-phenylalanine amide (1 eq, 1.22 mmol, 200 mg), dissolved in 14 mL of DCM and triethylamine (1 eq, 1.22 mmol, 170 µL) at 40 °C. The mixture was stirred for 16 h at RT, evaporated to dryness on a rotary evaporator and purified by flash chromatography to yield **10** in 13.0 % yield (52.0 mg, 15.9 µmol). ^{1}H NMR (250.13 MHz, $CDCl_3$, ambient *T*): δ 7.53 – 7.50 (m, 2 H), 7.38 – 7.10 (m, 11 H), 6.98 – 6.94 (m, 2 H), 6.36 (d, *J*= 5.0 Hz, 2 H), 5.43 (s, 1 H), 4.12 – 4.04 (m, 1 H), 3.18 – 3.11 (m, 1 H), 3.03 – 2.94 (m, 1 H) ppm. ^{13}C NMR (125.78 MHz, $CDCl_3$, ambient *T*): δ 175.56, 170.23, 139.05, 137.85, 130.59, 128.61, 128.32, 127.34, 126.37, 67.63, 41.52 ppm. HR-MS (m/z) calculated for $C_{22}H_{21}N_2O$ 329.17; found 329.1670 [M+H$^+$], calculated for $C_{22}H_{20}N_2NaO$ 351.15; found 351.1477 [M+Na]$^+$.

N-(diphenylmethylene)-D-*phenylalanine amide*



Reference compound was synthesized according to O'Donnell *et al.*[1] and characterized according to Hyett *et al.*[4]

Benzophenone imine (1 eq, 1.00 mmol, 181 mg), dissolved in 5 mL of DCM, was added to D-phenylalanine amide (1 eq, 1.00 mmol, 164 mg), dissolved in 5 mL of 2-propanol. The mixture was stirred for 16 h at RT, evaporated to dryness on a rotary evaporator and purified by flash chromatography to yield **11** in 29.2 % yield (96.0 mg, 29.2 µmol). ^{1}H NMR (250.13 MHz, $CDCl_3$, ambient *T*): δ 7.53 – 7.50 (m, 2 H), 7.38 – 7.10 (m, 11 H), 6.98 – 6.94 (m, 2 H), 6.36 (d, *J*= 5.0 Hz, 2 H), 5.41 (s, 1 H), 4.12 – 4.04 (m, 1 H), 3.18 – 3.11 (m, 1 H), 3.03 – 2.94 (m, 1 H) ppm. ^{13}C NMR (125.78 MHz, $CDCl_3$, ambient *T*): δ 177.95, 171.13, 139.16, 137.77, 130.17, 128.61, 128.30, 128.15, 127.37, 126.44, 67.79, 41.57 ppm. HR-MS (m/z) calculated for $C_{22}H_{21}N_2O$ 329.17; found 329.1634 [M+H$^+$], calculated for $C_{22}H_{20}N_2NaO$ 351.15; found 351.1460 [M+Na]$^+$.

Radiosynthesis

*[^{11}C]Benzyl iodide (**5**)*

[^{11}C]Benzyl iodide was synthesized according to the procedure in our previous paper.[5]

[^{11}C]CO_2 was produced by the ^{14}N(*p*,α)^{11}C nuclear reaction performed in a 0.5 % O_2/N_2 gas mixture using IBA Cyclone 18/9 (IBA, Louvain-la Neuve, Belgium). After trapping of [^{11}C]CO_2 from the target in a stainless trap dispensed in liquid N_2, [^{11}C]CO_2 was transferred with a He flow of 10 mL·min^{-1} into 100 µL of 1 M phenylmagnesium bromide in tetrahydrofuran (THF) at 35 °C. After obtaining the maximum radioactivity in the vessel, the reaction mixture was stirred using He flow (10 mL·min^{-1}) for 1 min and additional 1 min at a He flow of 50 mL·min^{-1}. 100 µL 1 M $LiAlH_4$ in THF was added and immediately evaporated to dryness by heating the reaction vial to 130 °C under a He flow (50 mL·min^{-1}) for 90 s. Subsequently, 100 µL of 57 % HI in water was added at 0 °C and the mixture was left to react for 2 min at 120 °C to yield [^{11}C]benzyl iodide. The reaction mixture has been first cooled down to RT or 0 °C, dissolved in 2.5 mL of toluene or DCM and subsequently passed through

5 x 0.5 cm column filled with potassium carbonate and magnesium sulfate (approx. 8/2 by volume) into a second reaction vial, containing one KOH pellet (96 ± 9 mg (n=20)), which has been precooled to 0 °C, when the reactions were performed with the aid of phase-transfer catalyst.

*Racemic [^{11}C]Phenylalanine (**8**)*

→ Reactions in presence of a strong base: tetra-*n*-butylammonium fluoride (TBAF)

1 M Tetra-*n*-butylammonium floride (TBAF) in tetrahydrofuran (THF) (2 eq, 34 µL, 34 µmol), dissolved in 200 µL of dimethyl sulfoxide (DMSO), was added in the reaction vessel containing precursor *N*-(diphenylmethylene)glycine *tert*-butyl ester (1 eq, 5.0 mg, 17 µmol), dissolved in 100 µL of dichloromethane (DCM) and 100 µL of dimethyl sulfoxide (DMSO), 30 s prior addition of **5**. [^{11}C]Benzyl iodide in DCM (300 µL) was slowly added to the reaction vessel and stirred for 10 min at 45 °C with He flow (10 mL·min^{-1}). Afterwards 200 µL of 37 % HCl was added to the reaction vessel and heated to 120 °C for 2 min. The reaction mixture was cooled down to ambient temperature and quenched with 1.0 mL of 0.1 M solution of ammonium phosphate dibasic. The identity of the product was confirmed with analytical HPLC by co-injection of the product and authentic reference of D- *or* L-Phenylalanine (column B, solvents E/EF, 70/30, *v/v*, wavelength 210 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 5.0 min for L-phenylalanine and Rt 6.4 min for D-Phenylalanine).

In addition, the identity of the synthetic intermediate **7** was confirmed with analytical HPLC by co-injection of the corresponding racemic intermediate **7** and authentic reference of *N*-(diphenylmethylene)-L-phenylalanine *tert*-butyl ester (column A, solvents D/I, 80/20, *v/v*, wavelength 254 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 12.5 min).

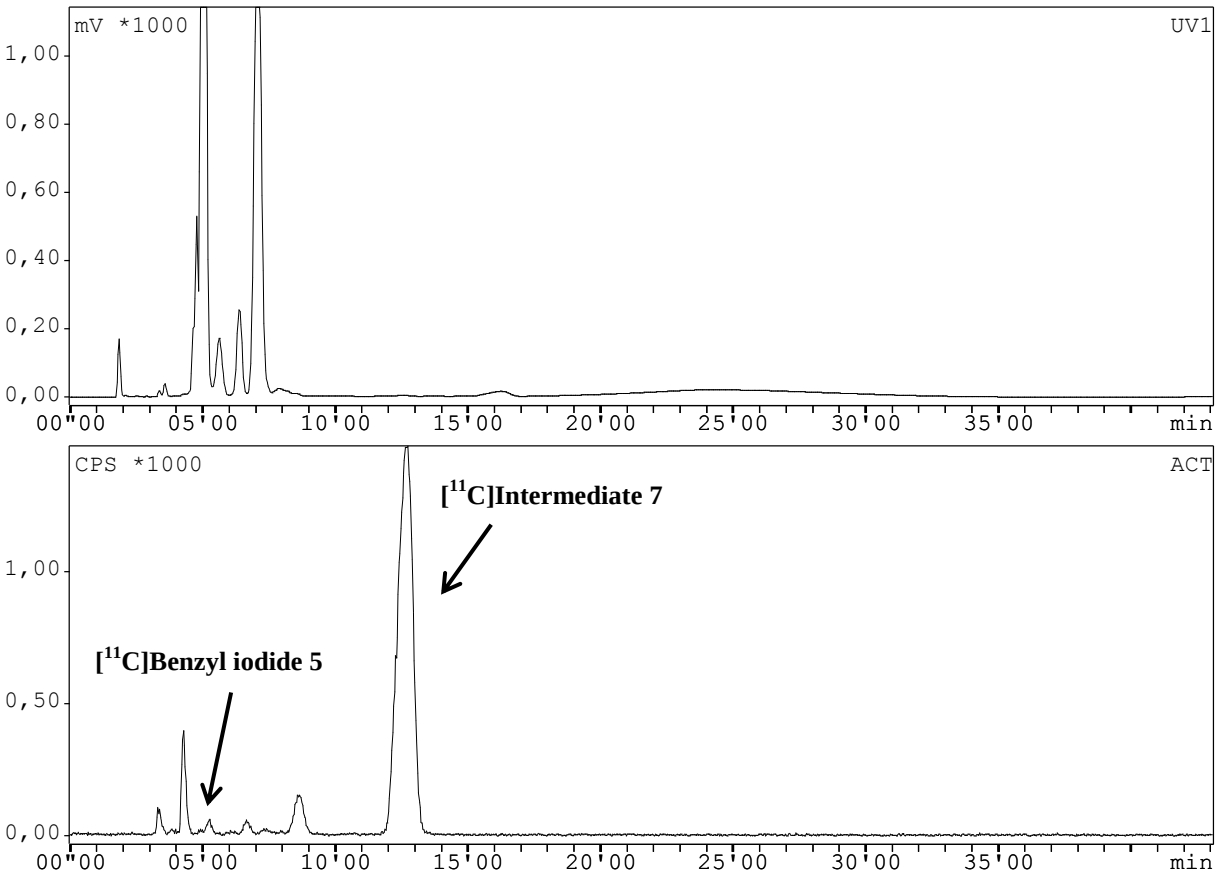


Figure 1: HPLC UV and radioactivity profile of the crude reaction mixture after [^{11}C]benzylation: Separation of [^{11}C]benzyl iodide **5** and racemic intermediate **7** to determine RCC %.

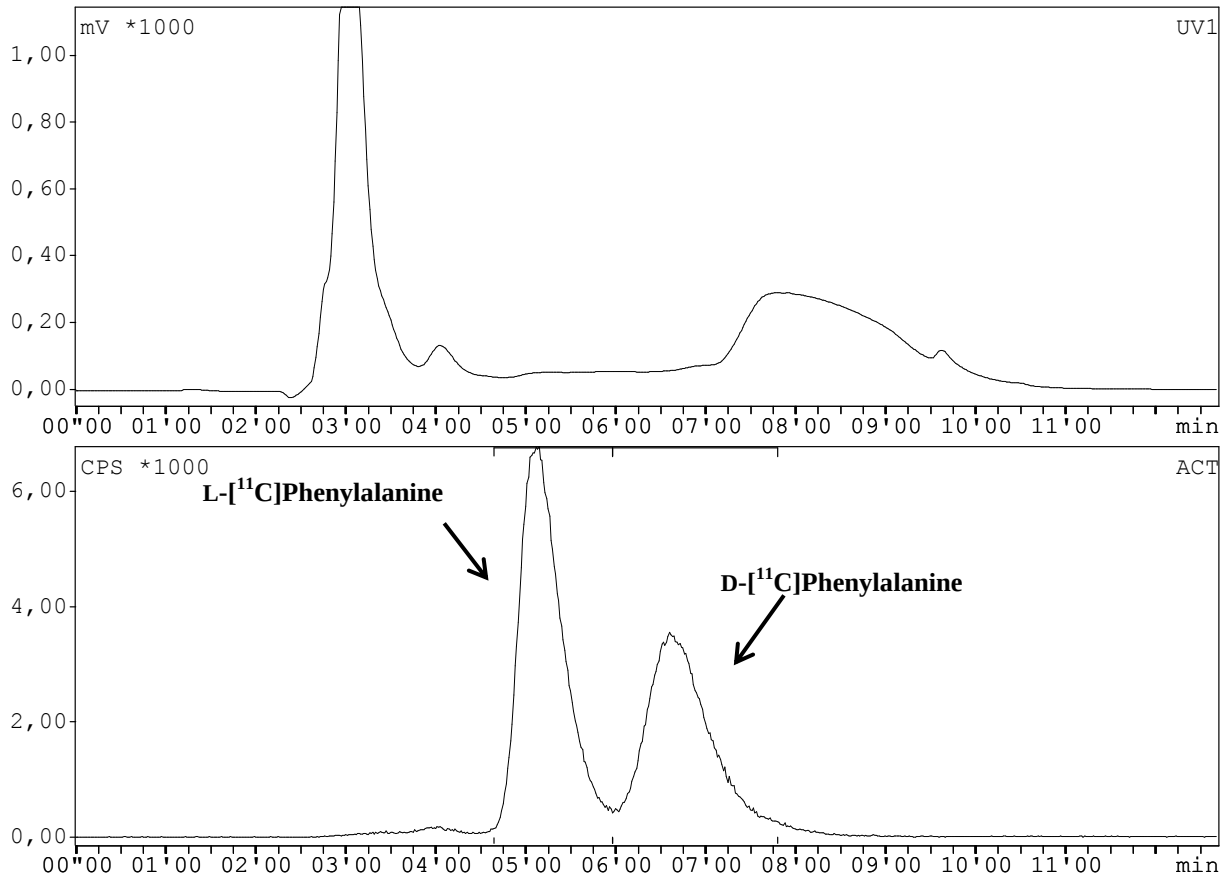


Figure 2: HPLC UV and radioactivity profile of the crude reaction mixture: Separation of L- and D-[^{11}C]phenylalanine on chiral column to determine ee %.

*Racemic [^{11}C]Phenylalanine amide (**10**)*

→ Reactions in presence of a strong base: tetra-*n*-butylammonium fluoride (TBAF)

1 M Tetra-*n*-butylammonium floride (TBAF) in tetrahydrofuran (THF) (2 eq, 30 µL, 30 µmol), dissolved in 200 µL of dimethyl sulfoxide (DMSO), was added in the reaction vessel containing precursor *N*-(diphenylmethylene)glycine amide **9** (1 eq, 3.5 mg, 15 µmol), dissolved in 100 µL of dichloromethane (DCM) and 100 µL of dimethyl sulfoxide (DMSO), 30 s prior addition of **9**. [^{11}C]Benzyl iodide in DCM (300 µL) was slowly added to the reaction vessel and stirred for 10 min at 45 °C with He flow (10 mL·min^{-1}). The reaction mixture was cooled down to ambient temperature and quenched with 1.0 mL of mobile phase.

The identity of the product was confirmed with analytical HPLC by co-injection of the product and authentic reference of Ph_2C=L-Phe-NH_2 (column A, solvents D/I, 70/30, *v/v*, wavelength 254 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 15.3 min).

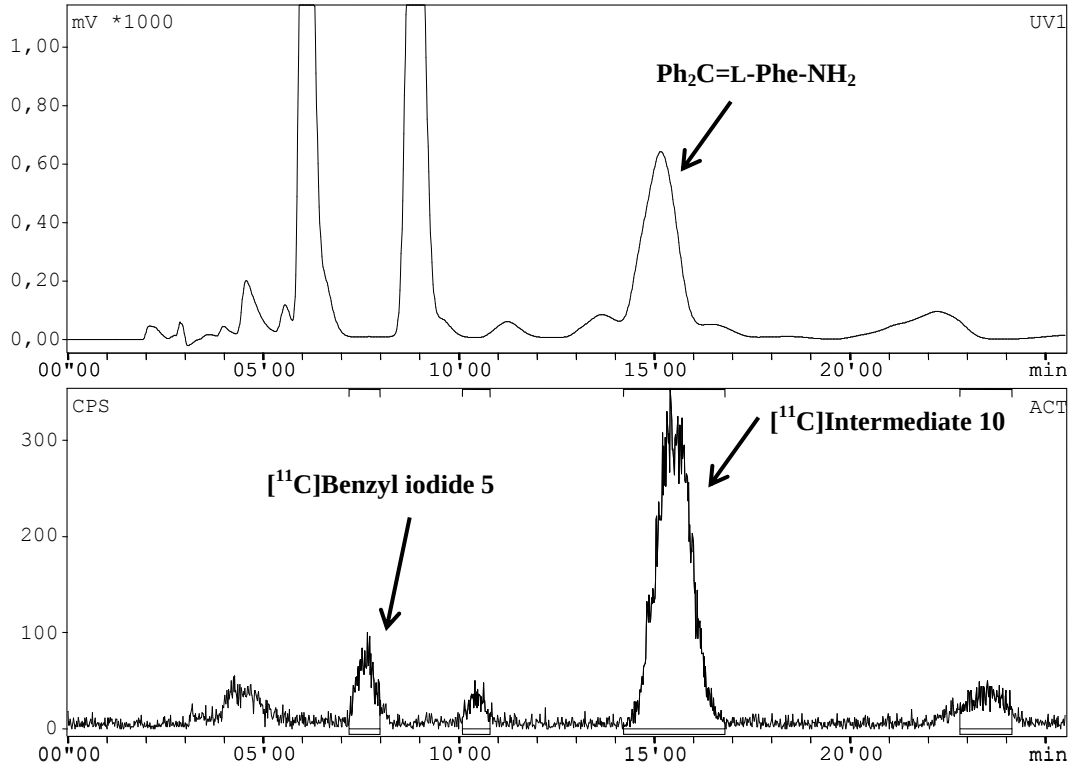


Figure 3: HPLC UV and radioactivity profile of the crude reaction mixture after [^{11}C]benzylation with co-injection of the cold standard Ph_2C=L-Phe-NH_2: Separation of [^{11}C]benzyl iodide **5** and racemic intermediate **10** to determine RCC %.

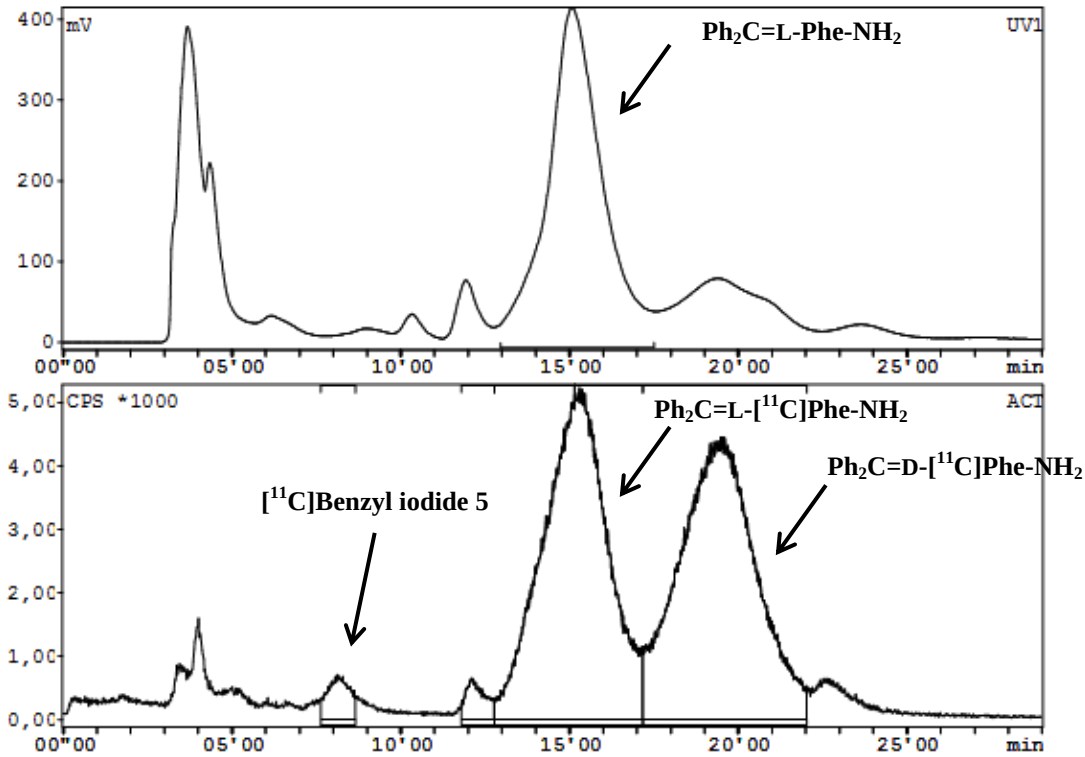


Figure 4: HPLC UV and radioactivity profile of the crude reaction mixture with co-injection of the cold standard Ph_2C=L-Phe-NH_2: Separation of Ph_2C=D- and Ph_2C=L-Phe-NH_2 on chiral column.

D- *or* L-*[^{11}C]Phenylalanine* ***(8)***

→ Reactions in presence of the chiral phase-transfer catalyst: **Cat 1-5**

[^{11}C]Benzyl iodide in toluene (400 µL) was slowly transferred in the reaction vessel containing *N*-(diphenylmethylene)glycine *tert*-butyl ester (1 eq, 5.0 mg, 17 µmol), cesium hydroxide monohydrate (40 eq, 114 mg, 679 µmol) and catalyst **2** or **3** (0.1 eq, 1.30 mg, 1.74 µmol) and stirred for 7 min at 0 °C with He flow (25 mL·min^{-1}). Afterwards 200 µL of 37 % HCl was added to the reaction vessel and heated to 120 °C for 2 min. The reaction mixture was cooled down to ambient temperature and quenched with 1.0 mL of 0.1 M solution of ammonium phosphate dibasic. The identity of the product was confirmed with analytical HPLC by co-injection of the product and authentic reference of D- or L-Phenylalanine

(column B, solvents E/F, 90/10, *v/v*, wavelength 210 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 6.1 min for L-Phenylalanine and Rt 9.2 min for D-Phenylalanine).

In addition, the identity of the synthetic intermediate **7** was confirmed with analytical HPLC by co-injection of the corresponding intermediate and authentic reference of *N*-(diphenylmethylene)-L-phenylalanine *tert*-butyl ester (column A, solvents D/I, 80/20, *v/v*, wavelength 254 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 12.9 min).

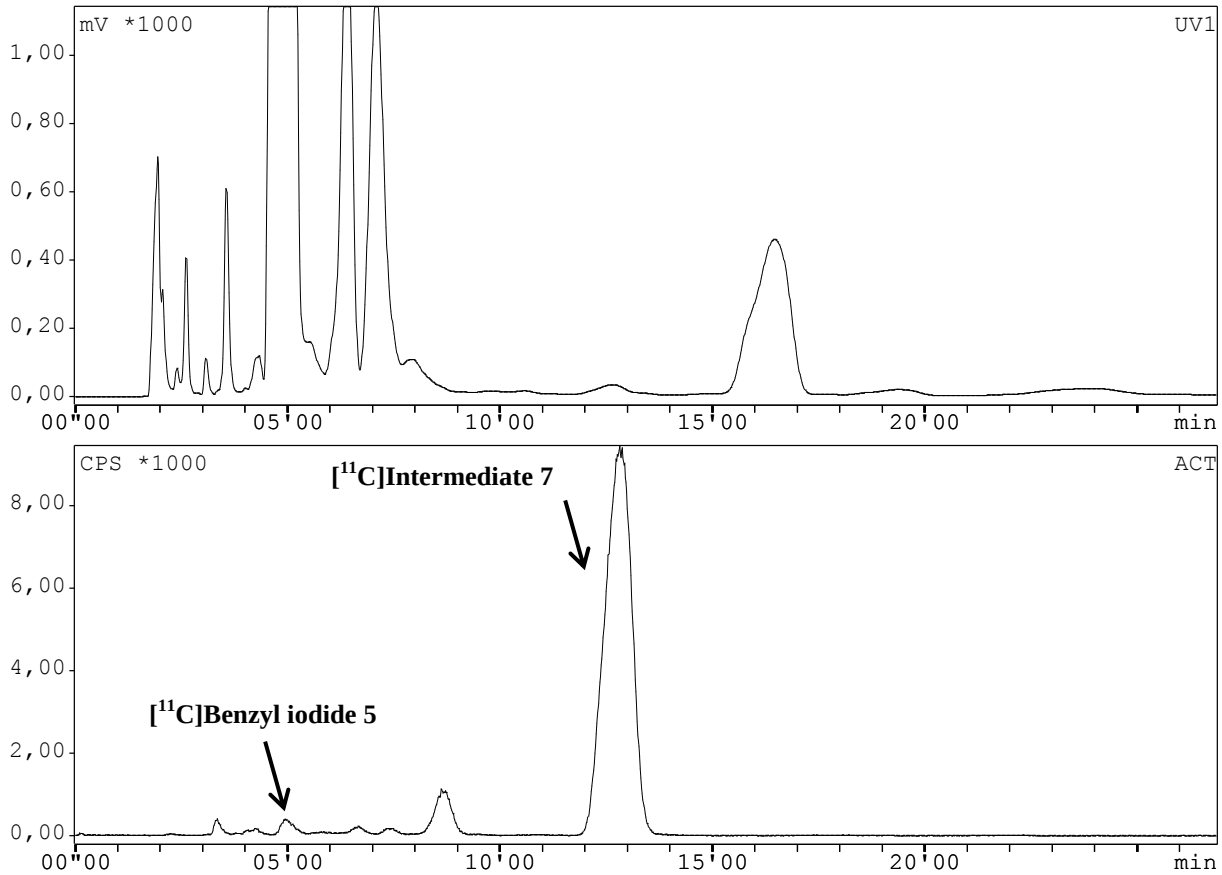


Figure 5: HPLC UV and radioactivity profile of the crude reaction mixture after [^{11}C]benzylation: Separation of [^{11}C]benzyl iodide **5** and intermediate **7** to determine RCC %.

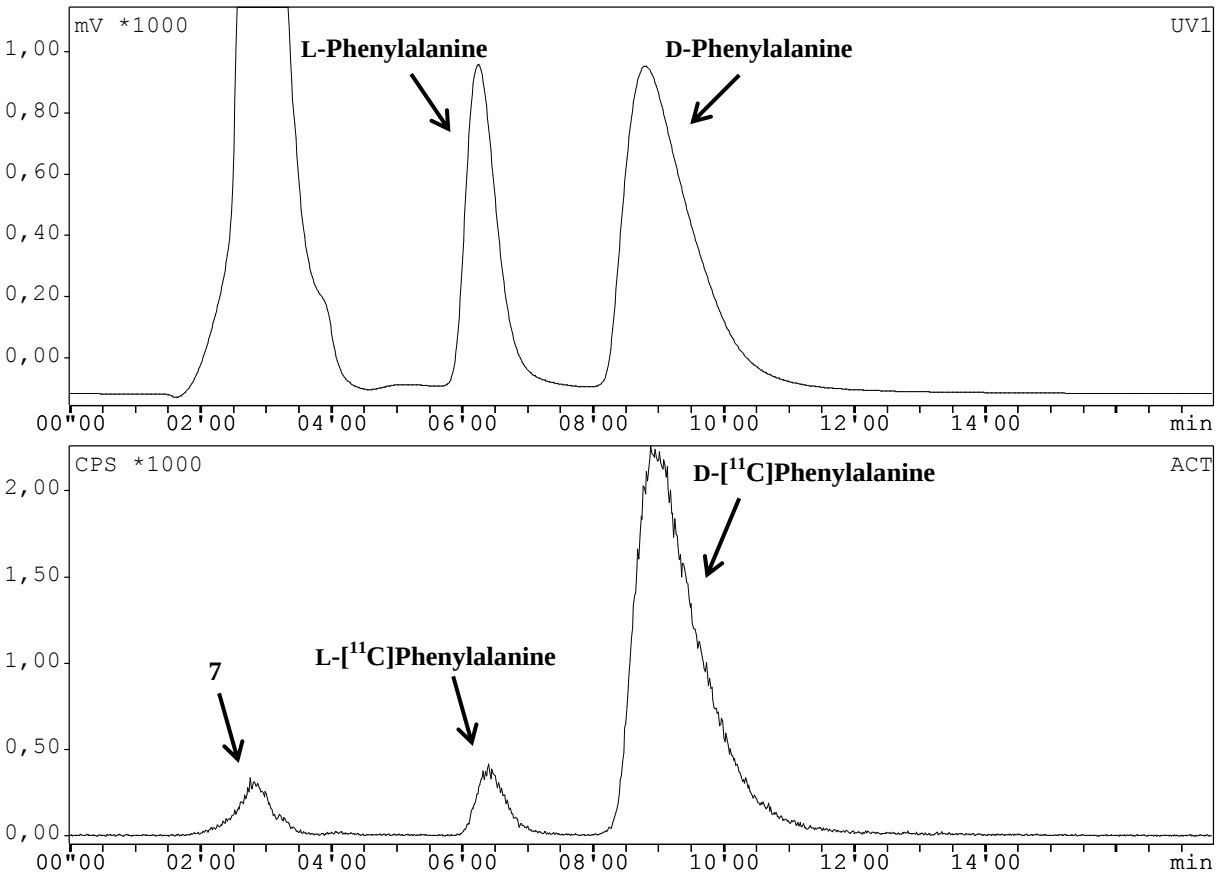


Figure 6: HPLC UV and radioactivity profile of the crude reaction mixture with the aid of **Cat 2** with co-injection of the cold standard L- and D-phenylalanine: Separation of L- and D-[^{11}C]phenylalanine on chiral column to determine ee %.

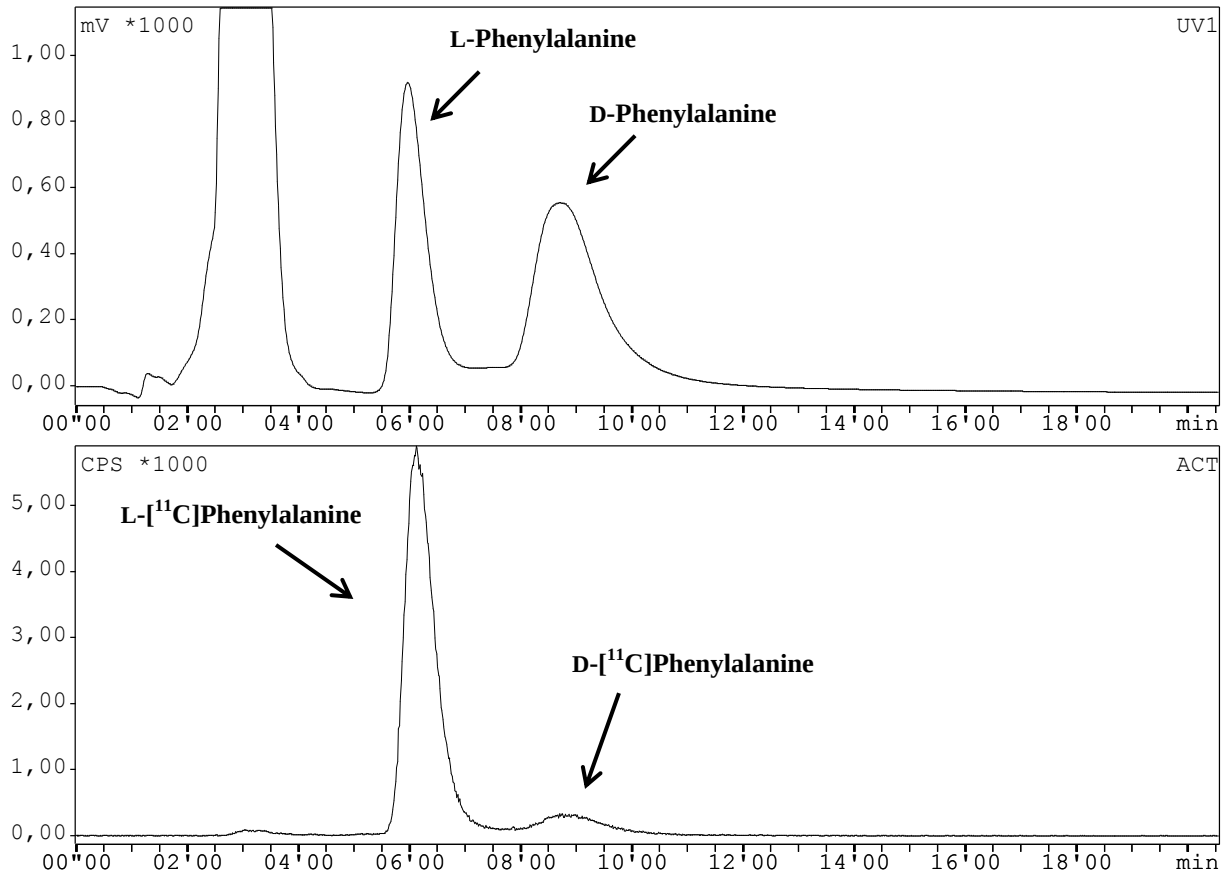


Figure 7: HPLC UV and radioactivity profile of the crude reaction mixture with the aid of **Cat 3** with co-injection of the cold standard L- and D-phenylalanine: Separation of L- and D-[^{11}C]phenylalanine on chiral column to determine ee %.

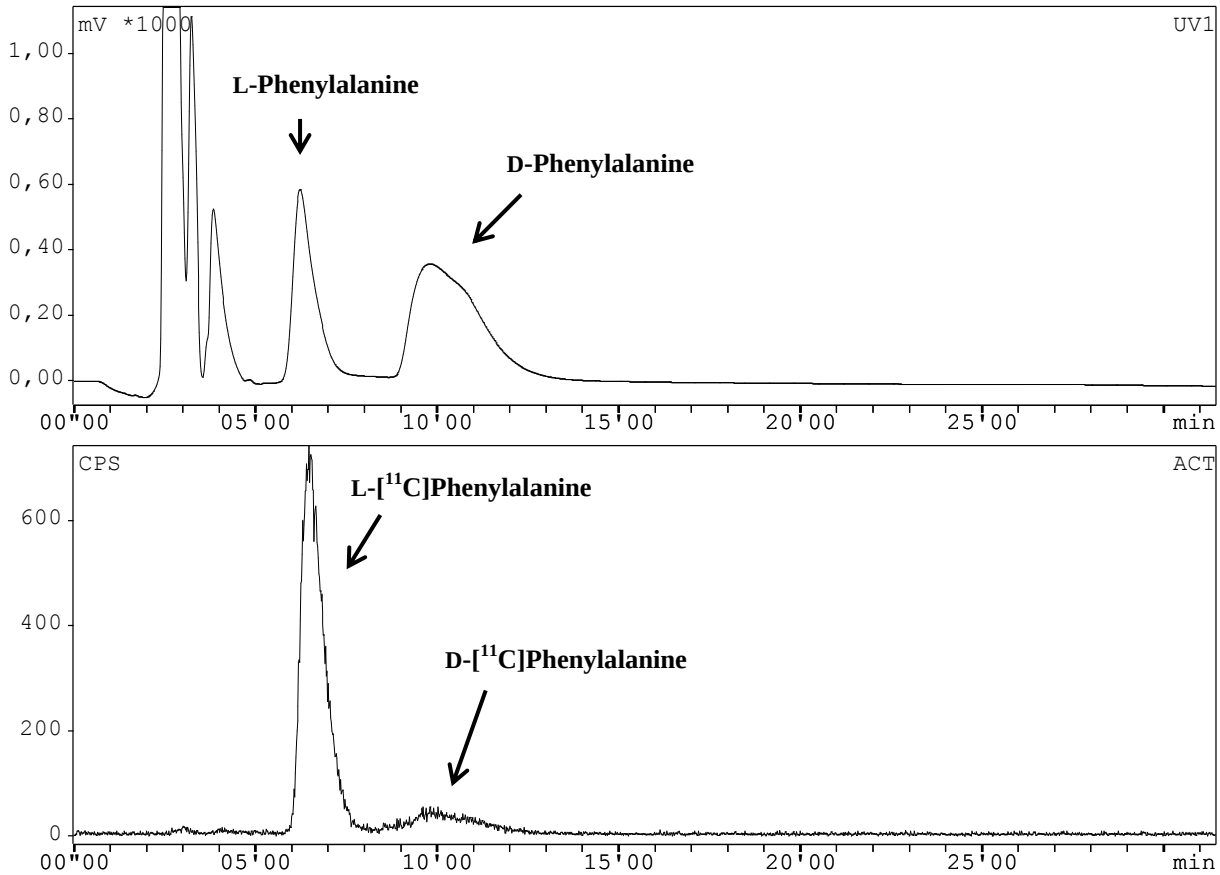


Figure 8: HPLC UV and radioactivity profile of the crude reaction mixture with the aid of **Cat 1** with co-injection of the cold standard L- and D-phenylalanine: Separation of L- and D-[^{11}C]phenylalanine on chiral column to determine ee %.

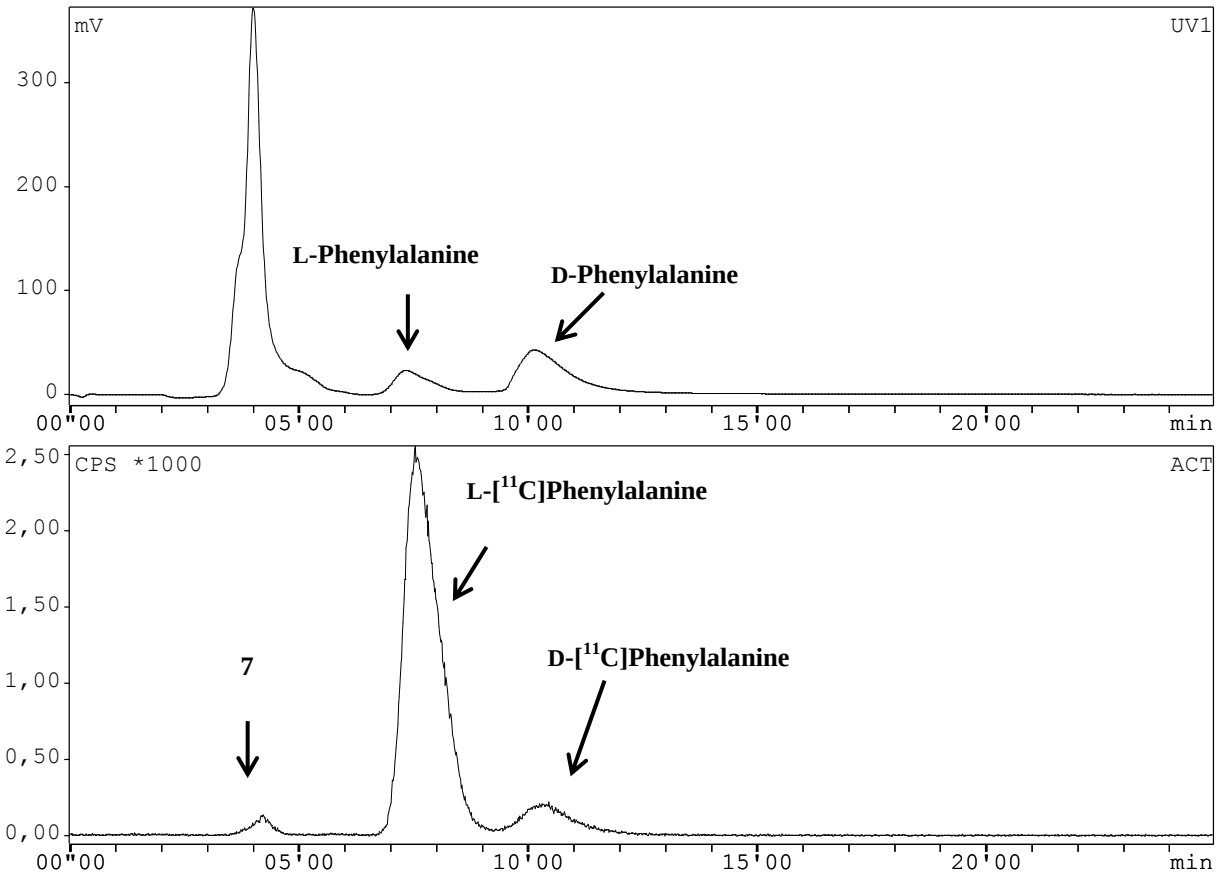


Figure 9: HPLC UV and radioactivity profile of the crude reaction mixture with the aid of **Cat 5** with co-injection of the cold standard L- and D-phenylalanine: Separation of L- and D-[^{11}C]phenylalanine on chiral column to determine ee %.

→ Reaction performed on Vortex mixer in presence of the chiral phase-transfer catalyst: **Cat 3**

[^{11}C]Benzyl iodide in toluene (400 µL) was slowly transferred in the reaction vessel containing *N*-(diphenylmethylene)glycine *tert*-butyl ester (1 eq, 5.0 mg, 17 µmol), cesium hydroxide monohydrate (40 eq, 114 mg, 679 µmol), catalyst **3** (0.1 eq, 1.30 mg, 1.74 µmol) and magnetic stirrer, precooled for 20 min at 0 °C, and stirred for 40 s at ambient temperature using Vortex mixer. Afterwards 200 µL of 37 % HCl was added to the reaction vessel and heated to 120 °C for 2 min. The reaction mixture was cooled down to ambient temperature and quenched with 1.0 mL of 0.1 M solution of ammonium phosphate dibasic. The identity of the product was confirmed with analytical HPLC by co-injection of the product and authentic

reference of D- or L-Phenylalanine (column B, solvents E/F, 90/10, *v/v*, wavelength 210 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 5.2 min for L-Phenylalanine and Rt 8.3 min for D-Phenylalanine).

In addition, the identity of the synthetic intermediate **7** was confirmed with analytical HPLC by co-injection of the corresponding intermediate and authentic reference of *N*-(diphenylmethylene)-L-phenylalanine *tert*-butyl ester (column A, solvents D/I, 80/20, *v/v*, wavelength 254 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 15.1 min).

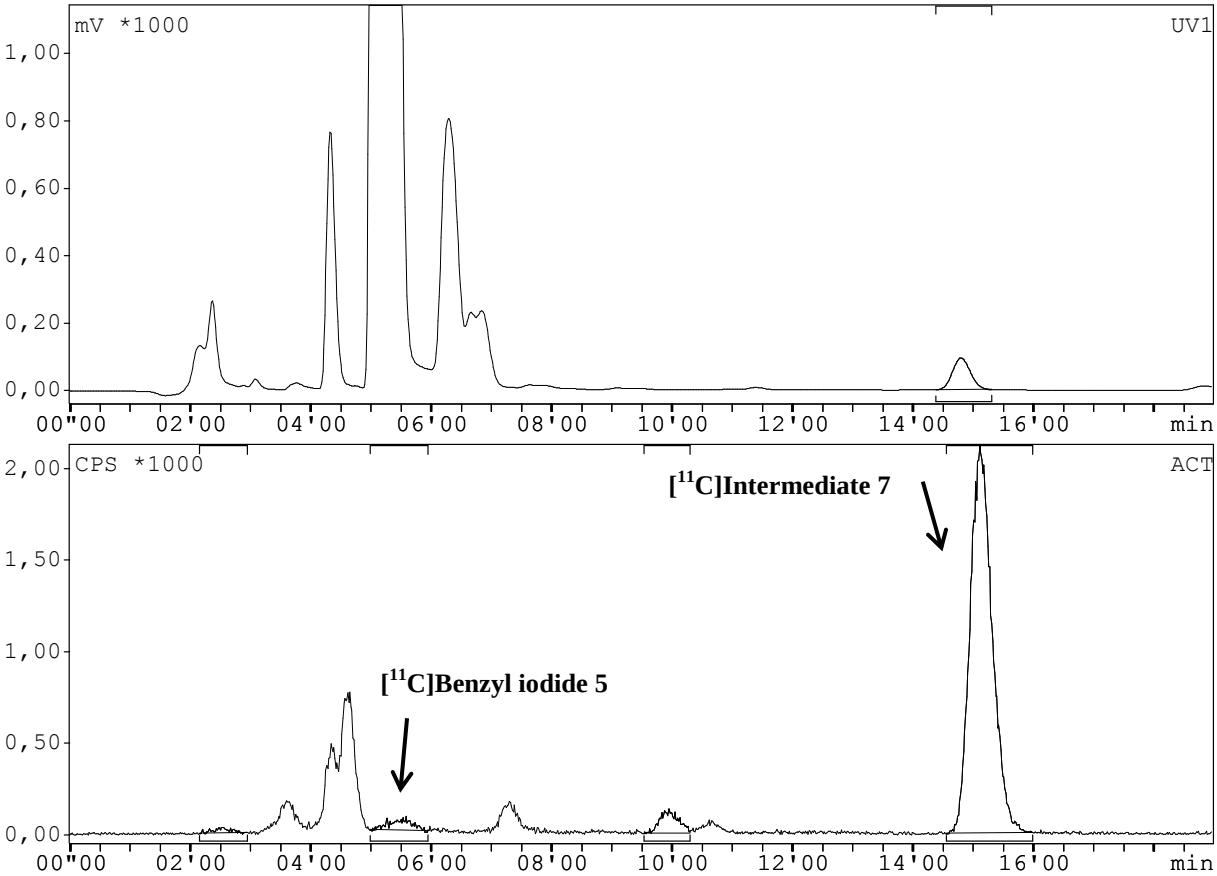


Figure 10: HPLC UV and radioactivity profile of the crude reaction mixture after [^{11}C]benzylation: Separation of [^{11}C]benzyl iodide **5** and intermediate **7** to determine RCC %.

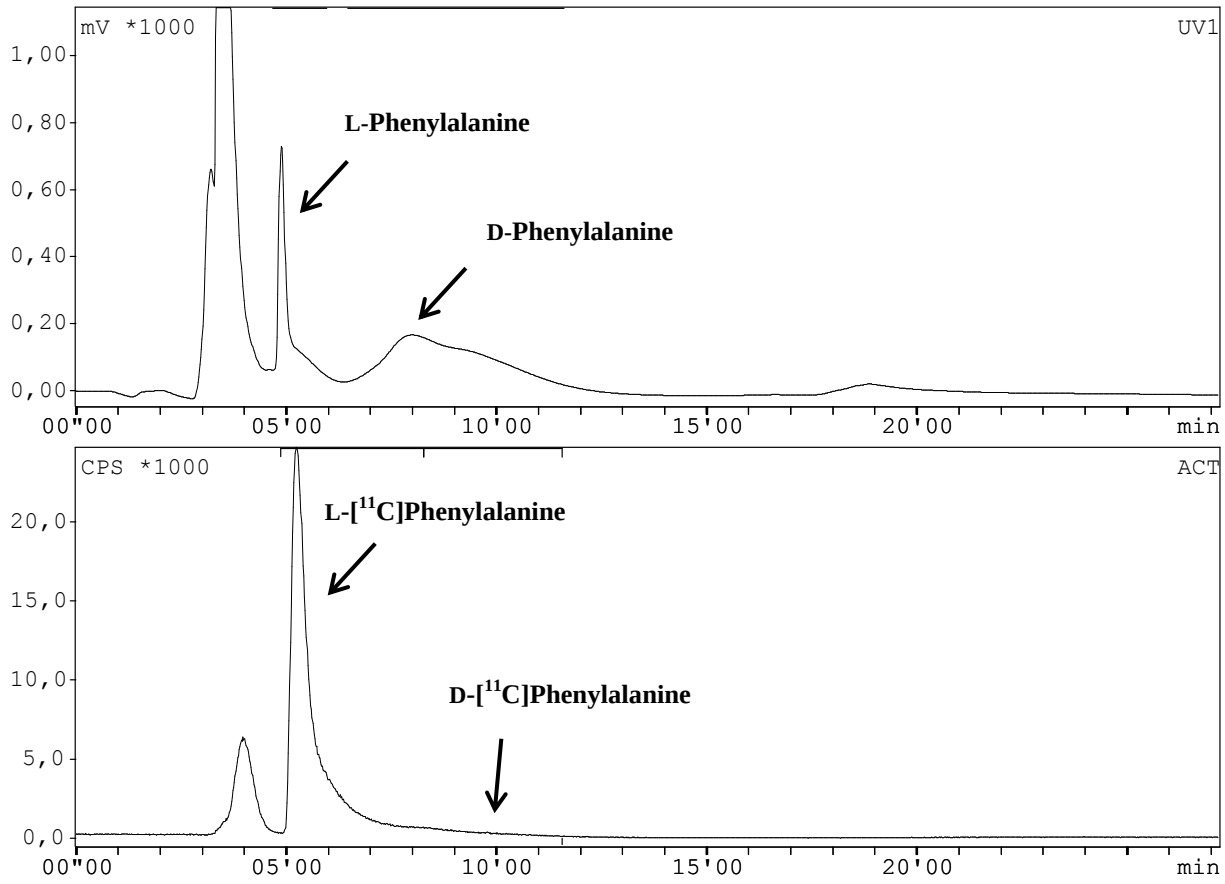


Figure 11: HPLC UV and radioactivity profile of the crude reaction mixture with the aid of **Cat 3** with co-injection of the cold standard L- and D-phenylalanine: Separation of L- and D-[^{11}C]phenylalanine on chiral column to determine ee %.

D- *or* L-*[^{11}C]Phenylalanine amide (**11**)*

→ Reactions in presence of the phase-transfer catalyst: **Cat 1**, **3** and **4**

[^{11}C]Benzyl iodide in toluene or DCM (400 µL) was slowly transferred in the reaction vessel containing *N*-(diphenylmethylene)glycine amide **9** (1 eq, 3.3 mg, 15 µmol), cesium hydroxide monohydrate (205 eq, 516 mg, 3.08 mmol) and catalyst **1**, **3** or **4** (0.1 eq) and stirred for 10 min at 0 °C with He flow (25 mL·min^{-1}). The reaction mixture was cooled down to ambient temperature and quenched with 1.0 mL of mobile phase. The identity of the product was confirmed with analytical HPLC by co-injection of the product and authentic reference of Ph_2C=D- and Ph_2C=L-Phe-NH_2 (column C, solvents G/H, 97/3, *v/v*, wavelength 220 nm,

column temperature – ambient, flow 1 mL·min^{-1}, Rt 15.0 min for Ph_2C=L-Phe-NH_2 and Rt 19.2 min for Ph_2C=D-Phe-NH_2). In addition, the radiochemical conversion was determined by analytical HPLC by co-injection of the corresponding intermediate and authentic reference Ph_2C=L-Phe-NH_2 (column A, solvents D/I, 70/30, *v/v*, wavelength 254 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 15.3 min).

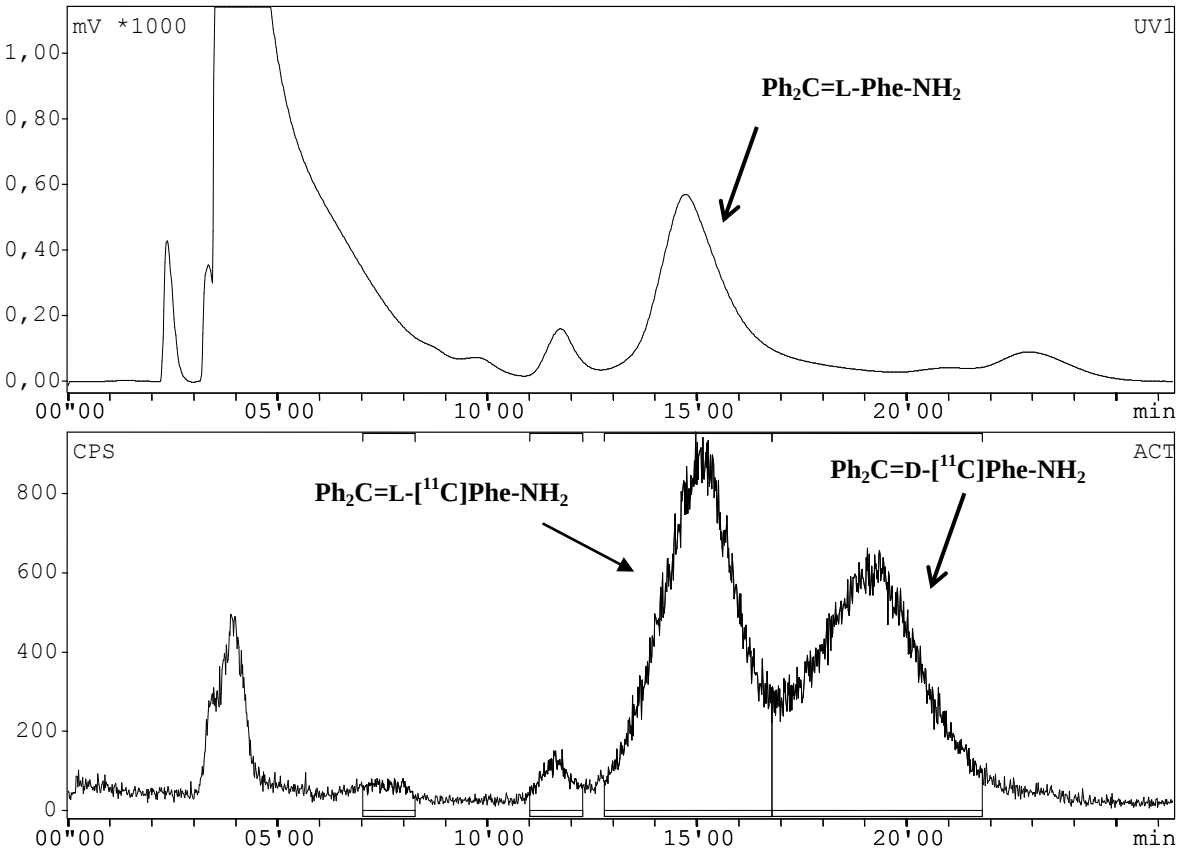


Figure 12: HPLC UV and radioactivity profile of the crude reaction mixture with the aid of **Cat 1** with co-injection of the cold standard Ph_2C=L-Phe-NH_2: Separation of Ph_2C=D- and Ph_2C=L-Phe-NH_2 on chiral column.

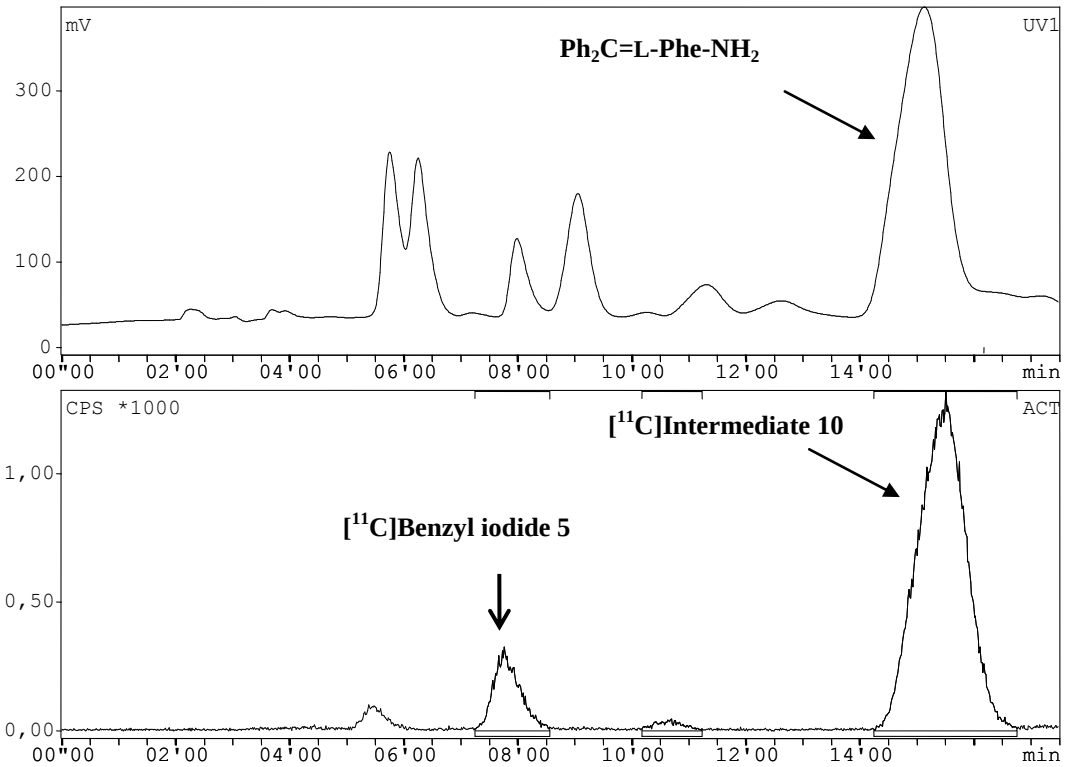


Figure 13: HPLC UV and radioactivity profile of the crude reaction mixture after [^{11}C]benzylation with the aid of **Cat 3** with co-injection of the cold standard Ph_2C=L-Phe-NH_2: Separation of [^{11}C]benzyl iodide **5** and intermediate **10** to determine RCC %.

Radiochemical Conversion Calculation

Radiochemical conversion (RCC) was determined by HPLC analysis as the percentage of converted [^{11}C]benzyl iodide to desired product (intermediate) in the crude reaction mixture using an analytical HPLC method described in the radiochemistry section under each procedure (see Figure 1). Before each RCC analysis, sample of [^{11}C]benzyl iodide was injected on the analytical HPLC using the same method, to determine purity of the [^{11}C]alkylating agent and possible contaminants, which were not accounted for the RCC calculation.

Radiochemical Yield Calculation

Radiochemical yield (RCY) is the amount of radioactivity in the product expressed as the percent of related starting radioactivity used in the corresponding synthesis. RCY was calculated as the quotient of measured activity at the end of the synthesis (EOS) in the vessel (non-isolated) and the measured activity at the end of the cyclotron bombardment (EOB) in the vessel at the beginning of the synthesis.. RCY has been corrected for decay from the end of the cyclotron bombardment (EOB). No corrections have been made for material losses (activity withdrawn for analysis, potentially volatile radioactive species, or residual activity in the vial, tubes and syringes).

Enantiomeric Excess Calculation

Enantiomeric excess (ee) of D- or L-[^{11}C]phenylalanine was determined by HPLC analysis of the free amino acid in the crude reaction mixture using a chiral column (column B, solvents D/E, 90/10, *v/v*, wavelength 210 nm, column temperature – ambient, flow 1 mL·min^{-1}, Rt 6.1 min for L-Phenylalanine and Rt 9.2 min for D-Phenylalanine). For the calculations following formula has been used: ee (%) = ((D-L)/(D+L)*100 ; D+L=1 for preferential D-[^{11}C]alkylation and ee (%) = ((L-D)/(D+L)*100 ; D+L=1 for preferential L-[^{11}C]alkylation.

Specific Activity Calculation

Specific activity (SA) of the radioactive products was determined by measurement of the UV absorbance of a known amount of radioactivity under identical analytical chiral HPLC conditions used to generate a calibration curve for the corresponding nonradioactive standard.

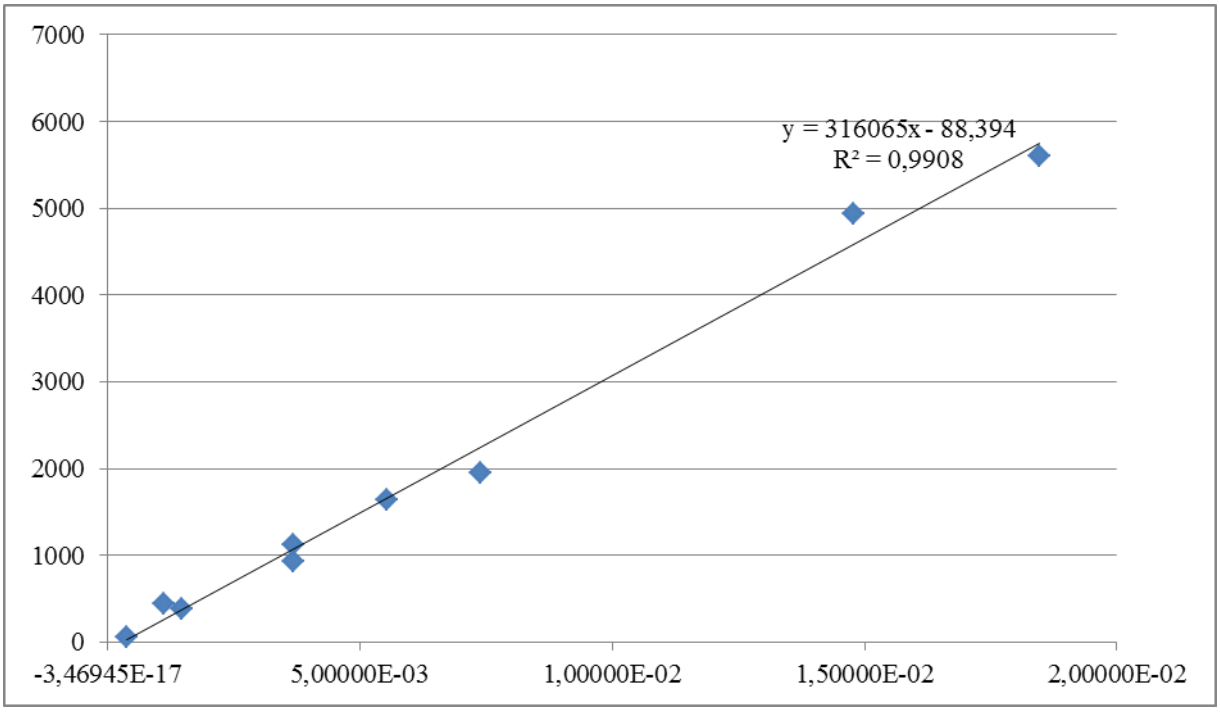
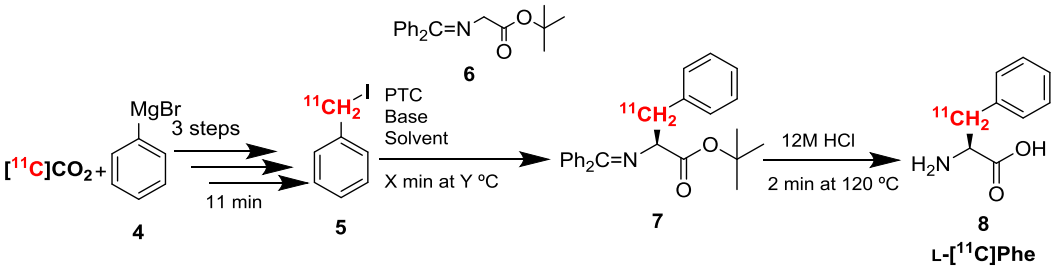


Figure 14: Calibration curve of UV absorbance vs. injected amount of L-Phenylalanine (µmol). For raw data used to construct the curve, see supplementary Table 1.

Table 1: Data for UV calibration of L-Phenylalanine.

Entry	L-Phenylalanine µmol injected	UV Absorbance (mV*s) 210 nm
1	1.84636E-02	5598.202
2	1.47709E-02	4934.7867
3	7.38543E-03	1946.836
4	5.53908E-03	1641.259
5	3.69272E-03	924.925
6	3.69272E-03	1130.394
7	1.47709E-03	382.2692
8	1.10782E-03	442.1582
9	3.69272E-04	60.8696

Full Optimization of Asymmetric Synthesis of L-[^{11}C]Phenylalanine



Scheme 1: Fully automated radiosynthesis of L-[^{11}C]phenylalanine **8** with **Cat 3**.

Keypoints:

- Excellent incorporation (85±6%) of [^{11}C]benzyl iodide to intermediate has been achieved using 5 mg of precursor **6**, 2 eq of TBAF[6], compared to **6**, 100 µL of DCM and 300 µL of DMSO at 45 ^{0}C for 10 min, as deducted from Table 2.

Table 2: Screening the [^{11}C]alkylation conditions with TBAF.

Entry	Precursor Concentration [mM]	Eq of base[a]	Solvent	T [^{0}C]	Time [min]	Stirring[b]	RCC 7 [%][c]
1	20	2	DCM	45	10	He flow	**<1**
2	45	2	DCM	45	10	He flow	**4**
3	50	2	DCM	45	10	He flow	**4**
4	60	2	DCM	45	10	He flow	**<1**
5	70	2	DCM	45	10	He flow	**5** and **87**
6	70	3	DCM	45	10	He flow	**4**
7	55	2	DCM	35	10	He flow	**1**
8	55	2	DCM	25	10	He flow	**3**
9	40	2	DCM+DMSO	45	10	He flow	**21**
10	55	2	DCM+DMSO	45	10	He flow	**85±6%**
11	60	2	DCM+DMSO	45	10	He flow	**86±0%**
12	55	2	DCM+DMSO	45	5	He flow	**30±2%**
13	55	2	DCM+DMSO	45	10	Stirrer	**78±3%**

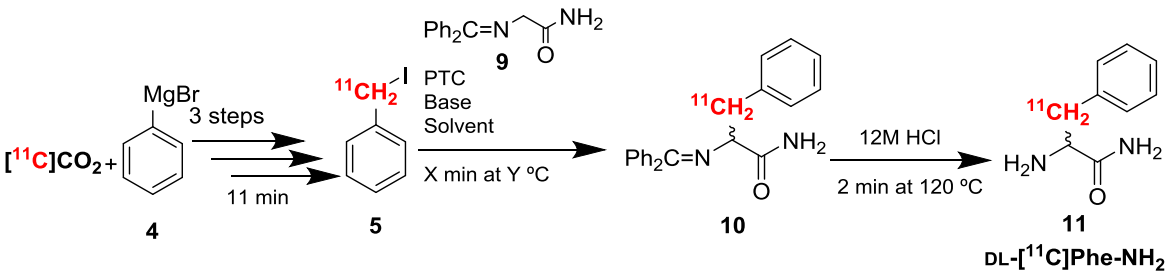
[a]1M TBAF solution in THF, compared to **6**. [b]Helium flow of 25 mL·min^{-1}. [c]Radiochemical conversions were determined by HPLC.

- Strong base such as TBAF, especially in combination with DMSO as solvent, deprotonated α-carbon of the Schiff base precursor **6** resulting in the corresponding enolate, observed as a yellow color in the reaction mixture.

- As a side effect, these conditions also promote cleavage of the diphenylmethylene group on *N*-terminus[6], crucial for alkylation of the Schiff base. In order to reduce decomposition of **6**, we proposed that the base was added to **6** shortly before alkyl iodide **5**.
- In addition to the right sequence, the optimal amount of glycine precursor has been increased from 5 to 17 µmol in the alkylation reactions.
- Our solid-liquid PTC conditions resulted in accelerated and higher incorporation, but lower ee than reported by Corey and co-workers (23 h at -78 ^{0}C; ee of 94%)[7]. Extrapolation of these conditions to radiochemistry, especially with short-lived isotope carbon-11, is not feasible as the synthesis time must be kept as short as possible.

- Between the different batches of commercially available PTC (**Cat 2** and **Cat 3**.) that were used during this study, different results with respect to the obtained ee of the product were observed. These different batches of the PTC used, were inevitably slightly different with respect to HPLC purity and optical rotation angle. Nonetheless, even a minor difference in optical purity can change the ee for a few %, as ee is defined as an absolute difference between each enantiomer. Notable is also the difference in HPLC purity and specific rotation of **Cat 2** and **Cat 3**, where the certified specific rotation is always higher for **Cat 3**. Therefore, the catalyst with higher optical purity should always give higher ee of the desired product.
- Our results with **Cat 5** (Table 2; main paper) might be explained by the temperature influence, since Park achieved high enantioselectivitie of 99% (*S*) for **Cat 5** at -40 ^{0}C over 20 h.[11] As argued for **Cat 1**, in radiochemical reactions with carbon-11 we proposed reactions at 0 ^{0}C for smooth automation and the required shorter reaction times.

Full Optimization of Asymmetric Synthesis of L-[^{11}C]Phenylalanine Amide



Scheme 2: Radiosynthesis of [^{11}C]phenylalanine amide **11** with the aid of chiral PTC.

Table 3: Screening the solid-liquid PTC conditions for L-[^{11}C]phenylalanine amide (n>3).

Entry	Base	PTC	T [°C]	Solvent	RCC[b] [±SD;%]	*Er*[b] (*S*:*R*)[c] [%]
1	$CsOH{\cdot}H_2O_{(s)}$	Cat 1	25	Toluene	**84±9**	**47:53**
2	$CsOH{\cdot}H_2O_{(s)}$	Cat 1	0	Toluene	**74±10**	**59:41**
3	$CsOH{\cdot}H_2O_{(s)}$	Cat 3	25	DCM	**93±4**	**46:54**
4	$CsOH{\cdot}H_2O_{(s)}$	Cat 3	0	DCM	**94±4**	**54:46**
5	$CsOH{\cdot}H_2O_{(s)}$	Cat 4	0	DCM	**99**	**54:45**
6	$CsOH{\cdot}H_2O_{(s)}$	/[a]	0	DCM	**99**	**46:54**

All reactions were conducted with 15 µmol **9**, 205 eq of base, 400 µL of solvent and 10% of PTC. Eq of base and % of Cat is compared to **9**. [a]Chiral catalyst has not been employed. [b]Radiochemical conversion and enantiomeric ratio (er) were determined by chiral HPLC. [c]Configuration at α-position.

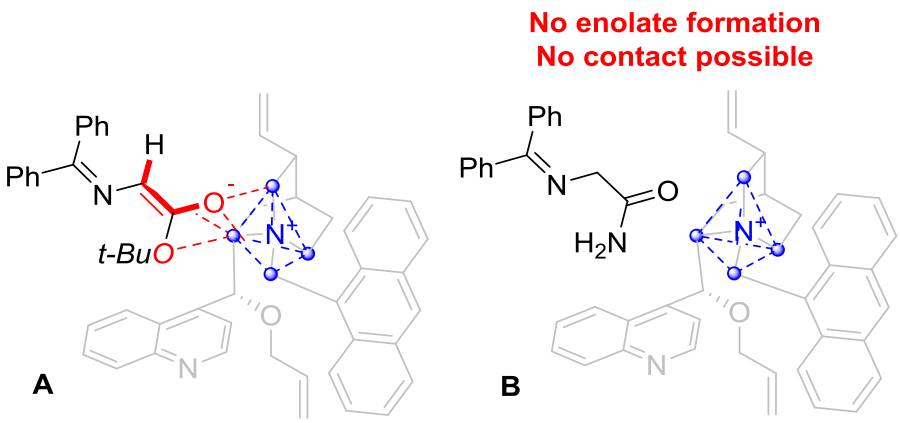


Figure 15: Tetrahedron selectivity model for **Cat 1** and the proposed contact between enolate anion (A: glycine ester **6**; B: glycinamide **9**) and the ammonium cation. Three of the faces of this tetrahedron are blocked by the quinuclidine, quinoline, and anthracenyl group, while the remaining face is sufficiently open to allow the stabilizing $R_3N^+\cdots^-O$-C=C hydrogen bonds.[14]

Full Optimization of Deprotection of L-[^{11}C]Phenylalanine and of L-[^{11}C]Phenylalanine Amide

Last step of the synthesis, deprotection of *tert*-butyl ester and/or diphenylmethylene moiety, has been studied from the beginning of our experiments, since alkylated product had to be deprotected quantitatively to [^{11}C]phenylalanine **8** and [^{11}C]phenylalanine amide **11** to determine the exact stereochemical outcome of the alkylation reaction using chiral radioHPLC.

Table 4: Screening the acidic deprotection conditions for L-[^{11}C]phenylalanine and L-[^{11}C]phenylalanine amide (n>3).

Entry	Alkylated Intermediate	Acid	T [°C]	Time [min]	RCC[b] [±SD;%]
1	7	6M $HCl_{(aq)}$	120	2	**25±5%**
2	7	12M $HCl_{(aq)}$	120	2	**>96%**
3	10	12M $HCl_{(aq)}$	120	2	**>98%**

All reactions were conducted with 200 µL of $HCl_{(aq)}$. [b]Radiochemical conversion was determined by HPLC.

Automated synthesis of L-[^{11}C]Phenylalanine

Previously described synthesis procedure was first performed manually (whole 5 step radiosynthetic procedure) in the research dedicated hot-cells and has been later on automated using adjustable home-build synthesis units (Figure 16) and performed according to state-of-the-art GMP standards.

Figure 16: Adjustable home-build synthetic units, made by our dedicated team at Radionuclide Center Amsterdam, in the GMP compliant laboratory.

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction performed in a 0.5 % O_2/N_2 gas mixture using IBA Cyclone 18/9 (IBA, Louvain-la Neuve, Belgium). After trapping of [^{11}C]CO_2 from the target in a stainless trap dispensed in liquid N_2, [^{11}C]CO_2 was transferred with a He flow of 10 mL·min^{-1} to the production unit/module into the reaction vessel. The synthesis units were operated in the following sequence (Figure 17):

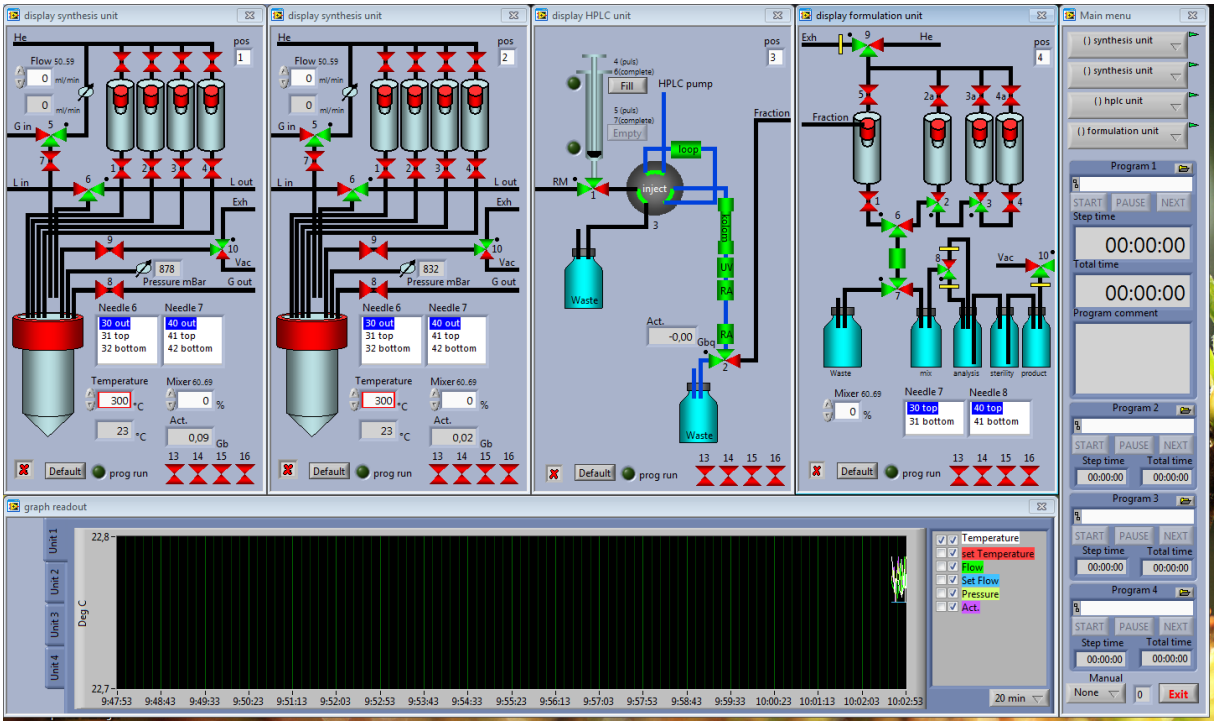


Figure 17: Synthesis units were operated using above program.

Unit	Time	Command	Value	Comment
1		Unit select	Synthesis unit	
2		Unit select	Synthesis unit	
1		default	1	
2		default	1	
				Synthesis [11C]Benzyl iodide
1	5:00:00	valve 9	0	Click NEXT to start synthesis
		valve 7	1	Trapping [11C]CO2
	0:00:01	valve 5	1	Trapping [11C]CO2
		valve 9	1	Trapping [11C]CO2
		temp	35	Trapping [11C]CO2
		flow	10	Trapping [11C]CO2
	5:00:00	needle 6	bottom	Trapping [11C]CO2. Click NEXT when activi reaction vessel 1 is max.
	0:00:05	needle 6	top	
		valve 5	0	
	0:01:00	temp	35	Reaction to benzoic acid with He 10mL/min
	0:01:00	flow	50	Blowing out unreacted [11C]CO2
	0:00:10	Needle 6	out	Addition of LiAlH4
		flow	10	Addition of LiAlH4
	0:00:08	temp	130	Setting temp to 130°C
		needle 6	bottom	
	0:01:00	temp	130	Evaporating THF at 130°C

2		temp	0	Preparing for purification [11C]Benzyl iodide
1	0:01:20	flow	50	Adjusting He flow to 50ml/min
		flow	0	Cooling the unit for HI
		needle 6	out	Cooling the unit for HI
	0:01:50	temp	0	Cooling the unit for HI
	0:00:03	valve 2	1	Pressurising HI vial for 3 second
		valve 2	0	Pressurising HI vial for 3 second
	0:00:08	valve 1	1	Adding HI
		valve 1	0	Adding HI
	0:00:02	valve 9	0	Closing Valve 9
	0:02:45	temp	120	Iodination with HI + Adjusting temp to 120 (4
	0:02:00	temp	0	Cooling the unit for purification
2		temp	0	Preparing for purification [11C]Benzyl iodide
	0:00:04	valve 9	1	Open exhaust unit 2
1	0:00:06	needle 7	bottom	Preparing for purification [11C]Benzyl iodide
	0:02:00	Valve 4	1	Diluting reaction mixture (reservoir 4)
		valve 4	0	Click NEXT when act. in reaction vessel 2 is
	0:00:06	needle 7	out	Needle 7 to out position
2	0:00:04	Mixer	90	Stirring
1		default	1	
2	0:01:00			Click NEXT when activity in reaction vessel l max. Place needles on position
2	0:00:02	Valve 7	1	Open valve 7
		flow	25	He flow 25ml/min
	0:07:00	temp	0	Stirring
2	0:00:02	Mixer	0	stop mixing
	0:00:10	Needle 6	out	Needle 6 out
		flow	0	Stop He
		valve 7	0	close valve 7
	0:00:08	valve 1	1	HCl addition (reservoir 1)
		valve 1	0	Close reservoir 1
	0:01:20	temp	120	Increase T
	0:02:00	temp	120	Deprotection step
	0:01:00	temp	20	Cooling the unit
2		default	1	[11C]phenylalanine synthesis finished
1		default	1	[11C]phenylalanine synthesis finished
		end	end	

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