Supporting Information

Solid-Phase Synthesis of β-peptoids with Chiral Backbone Substituents Using Reductive Amination

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Experimental

General remarks:

Chemicals and solvents used in this study were purchased from commercial suppliers and used without further purification. Preparative HPLC was performed on a Prominence HPLC system (Shimazu) with a $5C_{18}$-MS-II column (Nacalai tesque, 10 mm I.D.×150 mm, 34355-91). Analytical HPLC was performed on a Prominence HPLC system with a $5C_{18}$-AR-II column (Nacalai tesque, 4.6 mm I.D.×150 mm, 38144-31). Elution was monitored by absorbance at 210 nm. HRMS data was obtained using micrOTOF II (Bruker Daltonics). MALDI-TOF MS analysis was performed on autoflex speed (Bruker Daltonics) using α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich) as matrix. Fmoc-protected β^3-amino acids were purchased from Watanabe Chemical Industries, LTD.

General procedure for β-peptoid synthesis:

RinkAmide MBHA resin was swelled with DMF in a spin column (GeneDesign, EP-31301) for 1 h. The resin was treated with 20% piperidine/DMF (3 min and 12 min) to remove Fmoc protecting group and washed with DMF three times. The resin was treated with DMF solution of Fmoc-β-amino acid (4 equiv, 0.2 M), HATU (4 equiv, 0.2 M), HOAt (4 equiv, 0.2 M) and DIPEA (8 equiv, 0.4 M) with continuous shaking for 5 h. After removing the solution, the resin was washed with DMF three times. After deprotecting Fmoc group, the resin was incubated with 1 M (20 equiv) of an aldehyde solution in anhydrous DMF. Aldehyde solution was filtered off and the resin was quickly washed with DMF and DCM. A freshly prepared suspension of $NaBH_4$ (20 equiv.) in 3/1 DCM/MeOH was added to the resin and the spin column was stood for 30 min with occasional shaking. The cap of the column was not tightly closed to prevent inner pressure is increased too much. The resin was washed with MeOH five times then with DCM and DMF three times each. The coupling and reductive amination procedure was repeated to afford β^3-peptoid oligomers on resin. The oligomers were cleaved by treating the resin with 95/2.5/2.5 TFA/triisopropylsilane/water solution for 2 h. The solution was transferred to a round bottom flask. Resin was washed with 1 mL of TFA twice and the washing solution was recovered to the round bottom flask. The TFA solution was removed under reduced pressure. The crude product was dissolved in acetonitrile and water and purified by a reversed phase column on HPLC using acetonitrile and water containing 0.1% TFA as solvents. Because peptides are cleaved from resin by TFA and purified by solvent containing TFA, all the products are obtained as TFA salts. Therefore, yield was calculated using molecular weight of each compound as TFA salt. The crude and purified product was analyzed on a reversed phase column by HPLC and ESI-TOF MS.

***N*-Benzyl-D-β^3-homoalanine amide (1):**

52.6 mg of RinkAmide MBHA resin (0.67 mmol/g, 35 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and benzaldehyde were used as submonomers. Yield was 7.0 mg (64%). ^{1}H NMR (CD_3CN, 400 MHz): δ 1.37 (d, 3H, J = 6.9 Hz), 2.60 (dd, 1H, J = 4.6, 16.9), 2.68 (dd, 1H, J = 8.2, 16.9), 3.48-3.56 (m, 1H), 4.11 (d, 1H, J = 13.3 Hz), 4.30 (d, 1H, J = 13.3 Hz), 6.13 (br, 1H), 6.68 (br, 1H), 7.39-7.50 (5H), 8.0-11.0 (br, 2H). ^{13}C NMR (CD_3CN, 400 MHz): δ 16.3, 36.4, 48.6, 52.1, 130.0, 130.2, 130.6, 132.9, 175.0. The ^{1}H NMR is shown in **Figure S3** and ^{13}C NMR spectra is shown in **Figure S4**. HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{11}H_{17}N_2O^+$ 193.1335; Found 193.1341.

***N*-Isobutyl-D-β^3-homophenylalanine amide (2):**

50.4 mg of RinkAmide MBHA resin (0.70 mmol/g, 35 μmol) was used for synthesis. Fmoc-D-β^3-homophenylalanine and isobutylaldehyde were used as submonomers. Yield was 6.7 mg (55%). ^{1}H NMR (CD_3CN, 400 MHz): δ 1.00 (d, 3H, J = 6.9 Hz), 1.01 (d, 3H, J = 6.4 Hz), 1.97-2.09 (m, 1H), 2.51 (2H), 2.82 (2H), 2.95-3.06 (br, 1H), 3.31 (dd, 1H, J = 4.6, 13.3 Hz), 3.59-3.70 (br, 1H), 6.14 (br, 1H), 6.58 (br, 1H), 7.23-7.39 (m, 5H), 9.08 (br, 1H), 9.70 (br, 1H). ^{13}C NMR (CD_3CN, 400 MHz): δ 20.0, 20.2, 26.9, 32.4, 37.0, 52.6, 58.4, 128.2, 129.8, 130.4, 137.1, 175.3. The ^{1}H NMR is shown in **Figure S5** and ^{13}C NMR spectra is shown in **Figure S6**. HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{14}H_{23}N_2O^+$ 235.1805; Found 235.1776.

***N*-Furfuryl-L-β^3-homoleucine amide (3):**

49.6 mg of RinkAmide MBHA resin (0.70 mmol/g, 35 μmol) was used for synthesis. Fmoc-D-β^3-homoleucine and furfural were used as submonomers. Yield was 8.4 mg (71%). ^{1}H NMR (CD_3CN, 400 MHz): δ 0.87 (d, 3H, J = 5.5 Hz), 0.93 (d, 3H, J = 5.5 Hz), 1.48-1.72 (m, 3H), 2.55 (dd, 1H, J = 8.2, 16.9 Hz), 2.69 (dd, 1H, J = 3.4, 17.2 Hz), 3.37-3.46 (m, 1H), 4.24 (d, 1H, J = 14.4 Hz), 4.33 (d, 1H, J = 14.8 Hz), 6.13 (br, 1H), 6.44-6.48 (m, 1H), 6.59 (d, 1H, J = 3.2 Hz), 6.63 (br, 1H), 7.56 (s, 1H), 9.46 (br, 2H). ^{13}C NMR (CD_3CN, 400 MHz): δ 21.3, 23.6, 25.3, 33.3, 39.4, 41.0, 54.5, 112.1, 113.3, 145.4, 146.4, 175.2. The ^{1}H NMR is shown in **Figure S7** and ^{13}C NMR spectra is shown in **Figure S8**. HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{12}H_{21}N_2O_2^+$ 225.1598; Found 225.1616.

***N*-Benzyl-D-β^3-homoalanine dimer (4):**

52.6 mg of RinkAmide MBHA resin (0.67 mmol/g, 35 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and benzaldehyde were used as submonomers. Yield was 8.2 mg (48%). ^{1}H NMR (CD_3CN, 400 MHz) spectrum is shown in **Figure S13**. The NMR spectrum was recorded at 5 mM. HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{30}N_3O_2^+$ 368.2333; Found 368.2321.

***N*-Benzyl-D-β^3-homoalanine trimer (5):**

19.5 mg of RinkAmide MBHA resin (0.67 mmol/g, 20 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and benzaldehyde were used as submonomers. Yield was 6.9 mg (51%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{33}H_{43}N_4O_3^+$ 543.3330; Found 543.3343.

***N*-Benzyl-D-β^3-homoalanine tetramer (6):**

30.3 mg of RinkAmide MBHA resin (0.67 mmol/g, 20 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and benzaldehyde were used as submonomers. Yield was 5.1 mg (30%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{44}H_{56}N_5O_4^+$ 718.4327; Found 718.4316.

***N*-Benzyl-D-β^3-homoalanine pentamer (7):**

30.3 mg of RinkAmide MBHA resin (0.67 mmol/g, 20 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and benzaldehyde were used as submonomers. Yield was 9.7 mg (47%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{55}H_{69}N_6O_5^+$ 893.5324; Found 893.5340.

Evaluation of racemization during synthesis:

N-Benzyl-β^3-homoalanine dimer *RR* (**4**) and *N*-Benzyl-β^3-homoalanine dimer *SR* (**8**) were synthesized on 30.0 mg of RinkAmide MBHA resin (0.70 mmol/g, 21 μmol). Fmoc-D-β^3-homoalanine, Fmoc-L-β^3-homoalanine and benzaldehyde were used as submonomers. Crude products were analyzed by HPLC. Full spectra are shown on **Figure S11**. Main peaks were corrected and analyzed by ESI-MS to confirm the formation of the objective compound. Observed mass values are described at the figure legend of **Figure S11**.

β-peptoid tetramer (9):

30.0 mg of RinkAmide MBHA resin (0.70 mmol/g, 21 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine, Fmoc-L-β^3-homoalanine, Fmoc-L-β^3-homophneylalanine, isobutylaldehyde, furfural and benzaldehyde were used as submonomers. Yield was 5.5 mg (32%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{42}H_{62}N_5O_5^+$ 716.4745; Found 716.4717.

β-peptoid tetramer (10):

30.0 mg of RinkAmide MBHA resin (0.70 mmol/g, 21 μmol) was used for synthesis. Fmoc-L-β^3-homoalanine, Fmoc-D-β^3-homoalanine, Fmoc-L-β^3-homophenylalanine, furfural, cyclohexanecarboxaldehyde, piperonal and isobutylaldehyde were used as submonomers. For imine formation with piperonal, resin was incubated for 2 h. Also, for piperonal, reductive amination was conducted twice. Yield was 4.2 mg (22%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{46}H_{66}N_5O_7^+$ 800.4957; Found 800.4949.

***N*-Isobutyl-D-β^3-homoalanine dimer (11):**

30.0 mg of RinkAmide MBHA resin (0.70 mmol/g, 21 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and isobutylaldehyde were used as submonomers. Yield was 5.0 mg (58%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{16}H_{34}N_3O_2^+$ 300.2646; Found 300.2626.

***N*-Isobutyl-D-β^3-homoalanine trimer (12):**

30.0 mg of RinkAmide MBHA resin (0.67 mmol/g, 21 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and isobutylaldehyde were used as submonomers. Yield was 5.0 mg (43%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{49}N_4O_3^+$ 441.3799; Found 441.3790.

***N*-Isobutyl-D-β^3-homoalanine tetramer (13):**

30.0 mg of RinkAmide MBHA resin (0.70 mmol/g, 21 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and isobutylaldehyde were used as submonomers. Yield was 3.7 mg (25%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for

$C_{32}H_{64}N_5O_4^+$ 582.4953; Found 582.4935.

_N_-Isobutyl-D-β^3-homoalanine pentamer (14):

30.0 mg of RinkAmide MBHA resin (0.70 mmol/g, 21 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and isobutylaldehyde were used as submonomers. Yield was 3.4 mg (20%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{40}H_{79}N_6O_5^+$ 723.6106; Found 723.6099.

Circular dichroism studies:

Solution of β-peptoids was prepared by dissolving lyophilized compounds in acetonitrile followed by diluting to give the desired concentration. CD spectra were acquired at 25 °C with a CD spectrometer (JASCO, J-1500) using 1 mm path length quartz cell (JASCO, 209J). Data pitch was set to 0.1 nm. The scanning speed was set to 100 nm/min and spectra were averaged from three scans. Spectral baseline was recorded using acetonitrile. All data points were baseline subtracted, converted to a uniform scale of molar elipticity per *N*-alkylamide bond, and plotted. Each plot was smoothed using nine data points per data point. For measurement of temperature dependence, temperature of sample holder was set to measurement temperature 1 min prior to the measurement.

ESI-MS analysis:

Solution of β-peptoids was prepared by dissolving lyophilized compounds in acetonitrile. All the compounds were measured using positive mode.

NMR spectroscopic studies of _N_-Benzyl-D-β^3-homoalanine dimer (4):

30.0 mg of RinkAmide MBHA resin (0.70 mmol/g, 21 μmol) was used for synthesis. Fmoc-D-β^3-homoalanine and benzaldehyde were used as submonomers. Yield was 8.0 mg (79%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{30}N_3O_2^+$ 368.2333; Found 368.2343. NMR spectra were recorded at 5 mM compound concentration on a JEOL ECS-400. Chemical shifts are reported in p.p.m relative to solvent peaks as internal standards (δH, CH_3CN 1.94 ppm; δC, CH_3CN 1.32 p.p.m). ^{1}H NMR (CD_3CN, 400 MHz) spectrum is shown in **Figure S13**. COSY spectrum was recorded with relaxation delay of 1.5 s and receiver gain of 50. NOESY spectrum was recorded with relaxation delay of 1.5 s, mixing time of 600 ms and receiver gain of 50. These 2D spectra are shown on **Figure S14**.

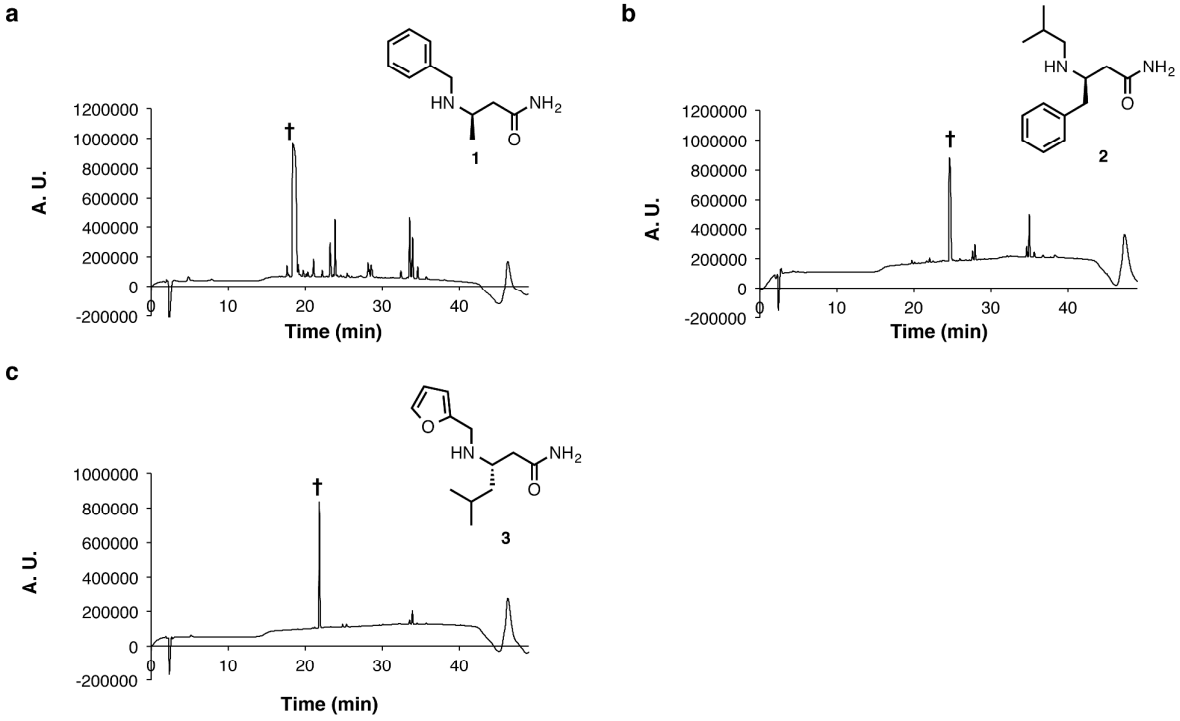

Figure S1. HPLC chromatograms of crude products of β-peptoid monomers **(a) 1**, **(b) 2** and **(c) 3**. The peak containing the desired product is labeled with † on each chromatogram.

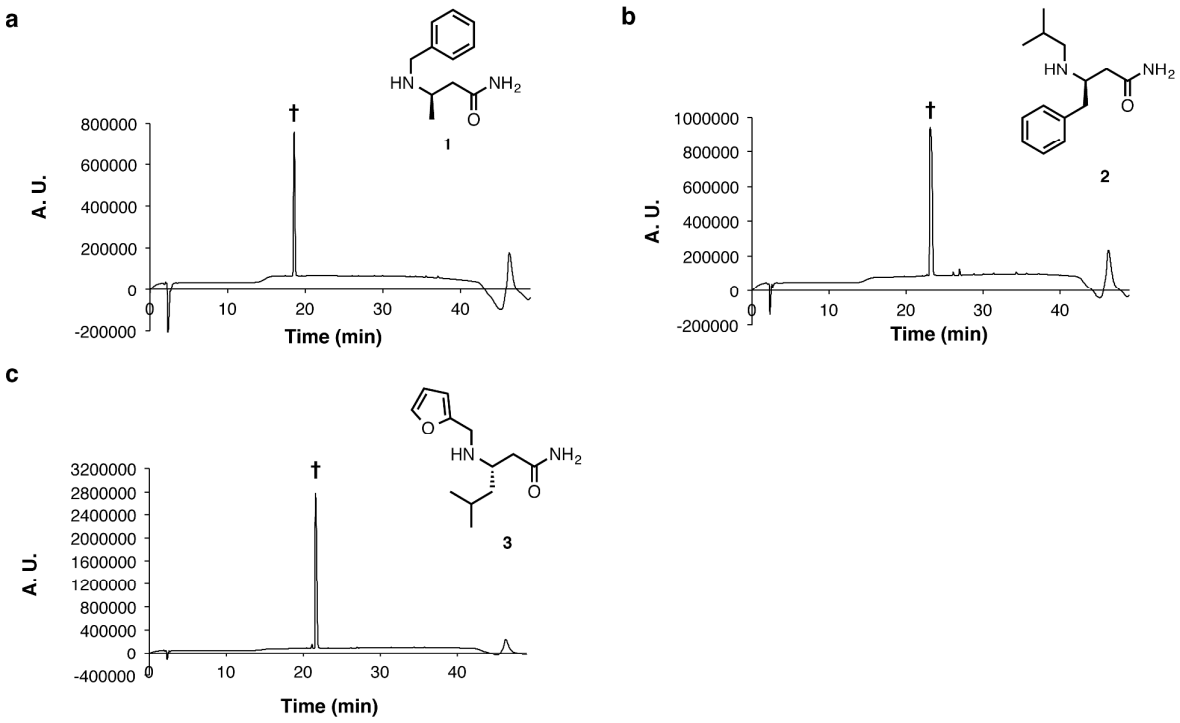

Figure S2. HPLC chromatograms of purified products of β-peptoid monomers **(a) 1**, **(b) 2** and **(c) 3**. The peak containing the desired product is labeled with † on each chromatogram.

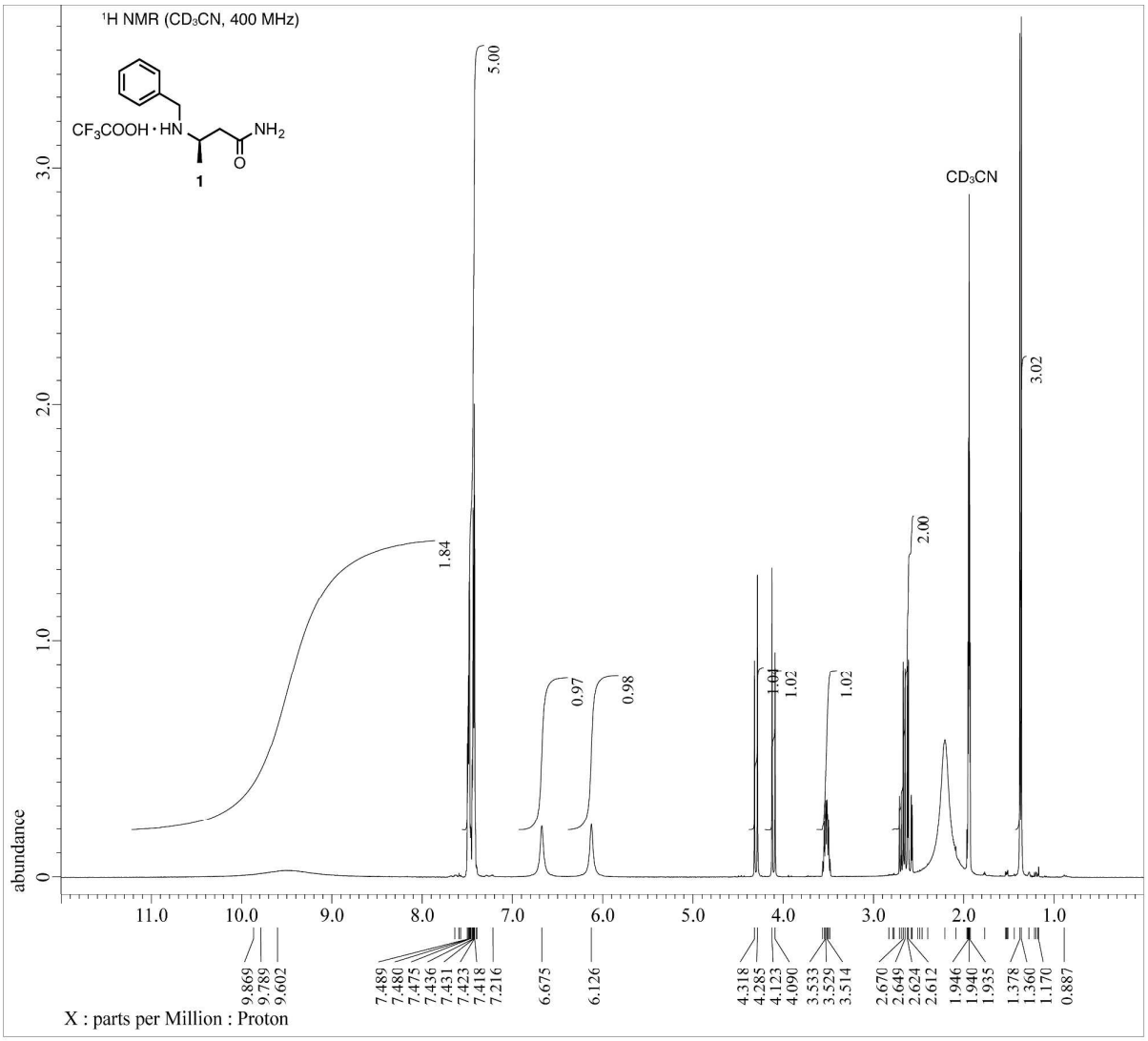

Figure S3. ^{1}H-NMR spectrum of *N*-Benzyl-D-β^{3}-homoalanine amide (**1**).

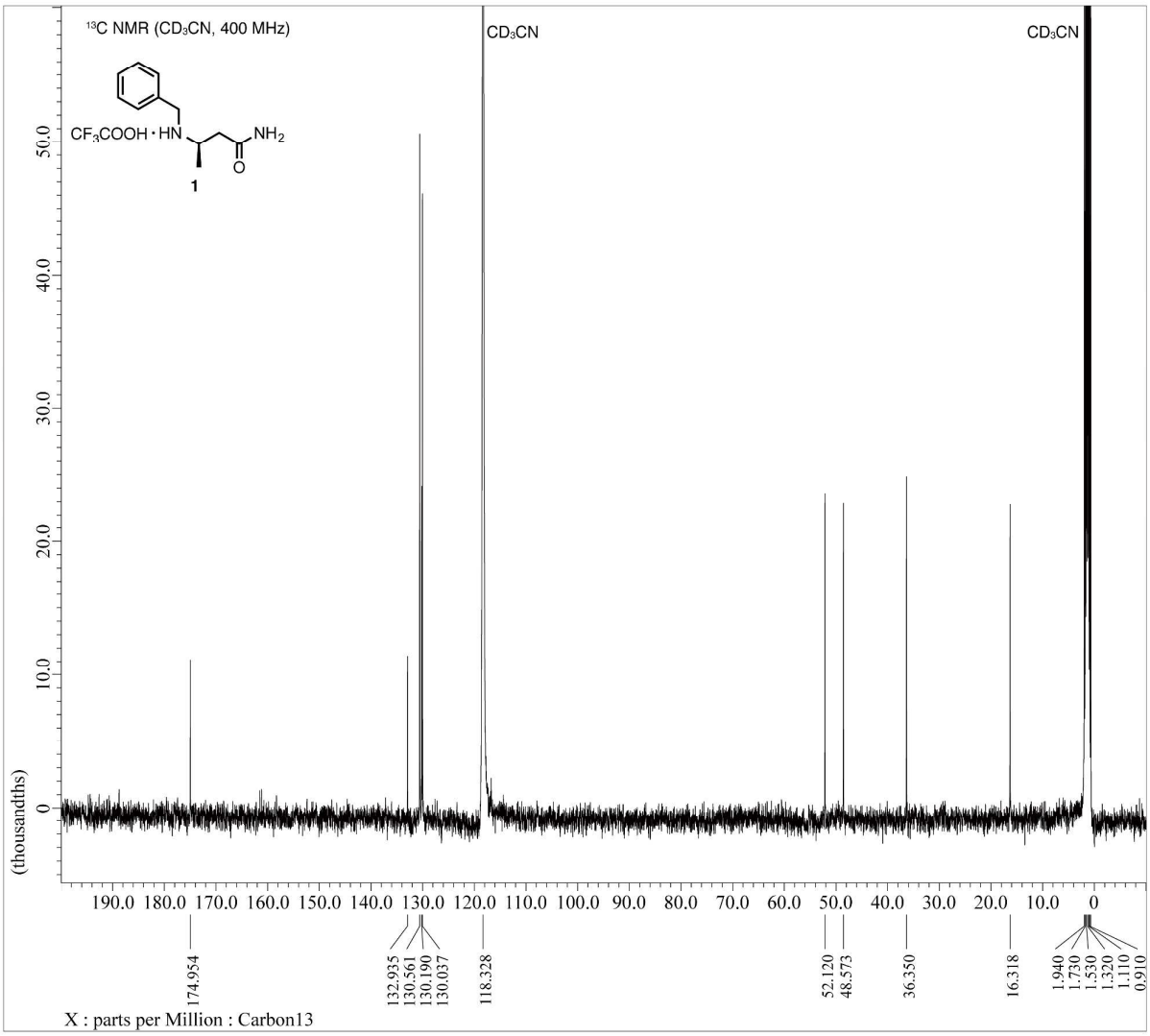

Figure S4. ^{13}C-NMR spectrum of *N*-Benzyl-D-β^3-homoalanine amide (**1**).

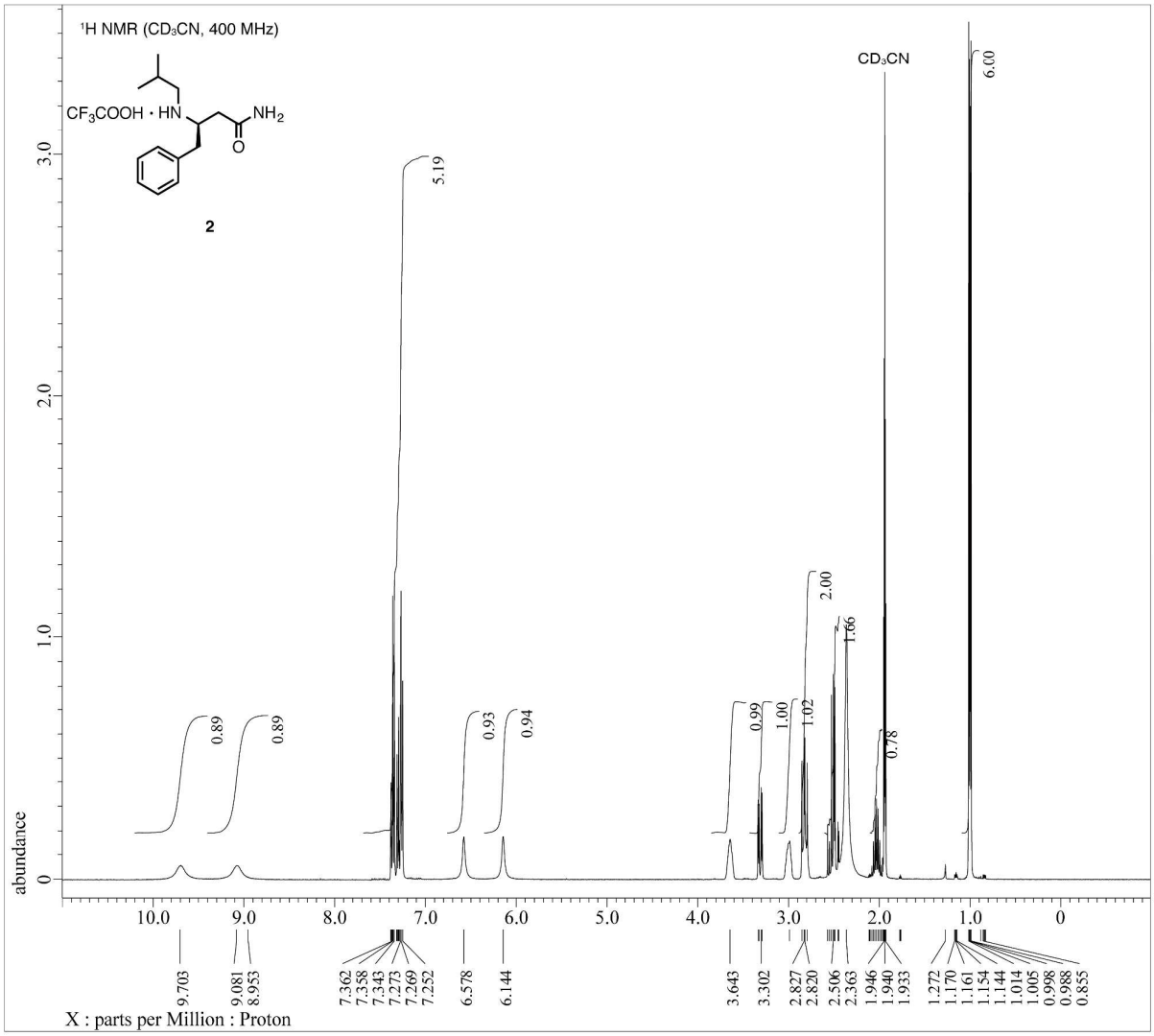

Figure S5. ^{1}H-NMR spectrum of *N*-Isobutyl-D-β^{3}-homophenylalanine amide (**2**).

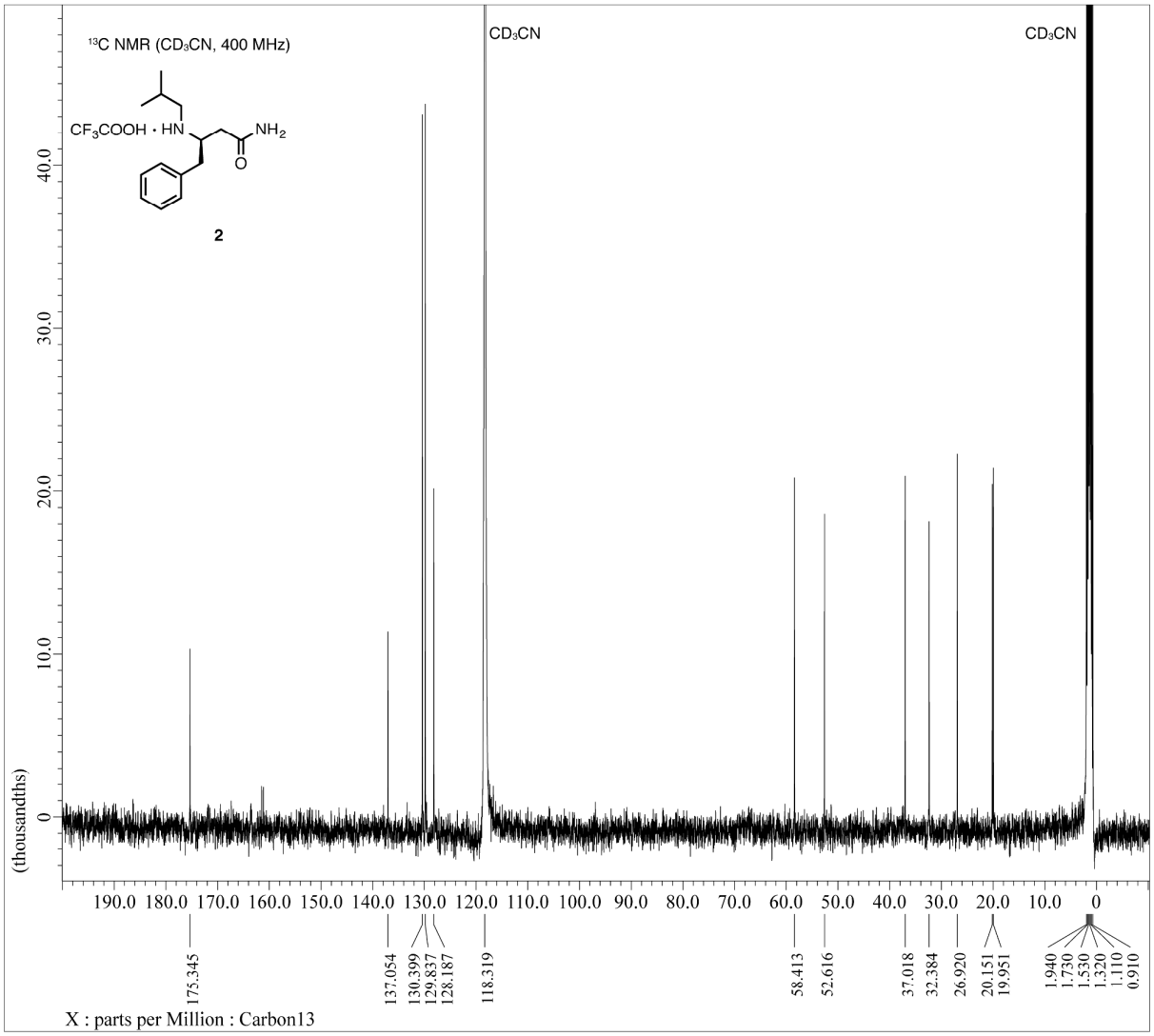

Figure S6. ^{13}C-NMR spectrum of *N*-Isobutyl-D-β^3-homophenylalanine amide (**2**).

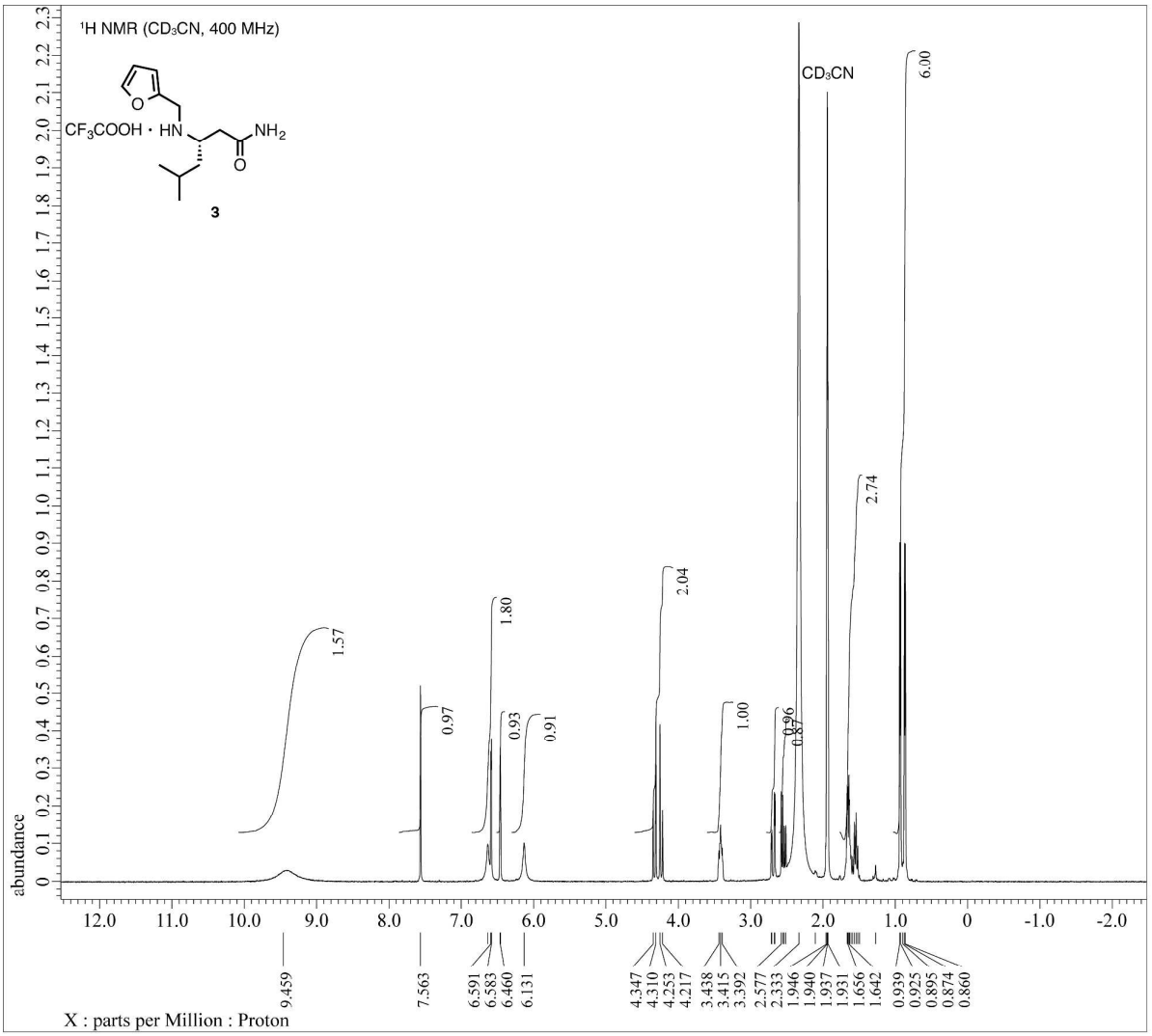

Figure S7. ^{1}H-NMR spectrum of *N*-Furfuryl-L-β^{3}-homoleucine amide (**3**).

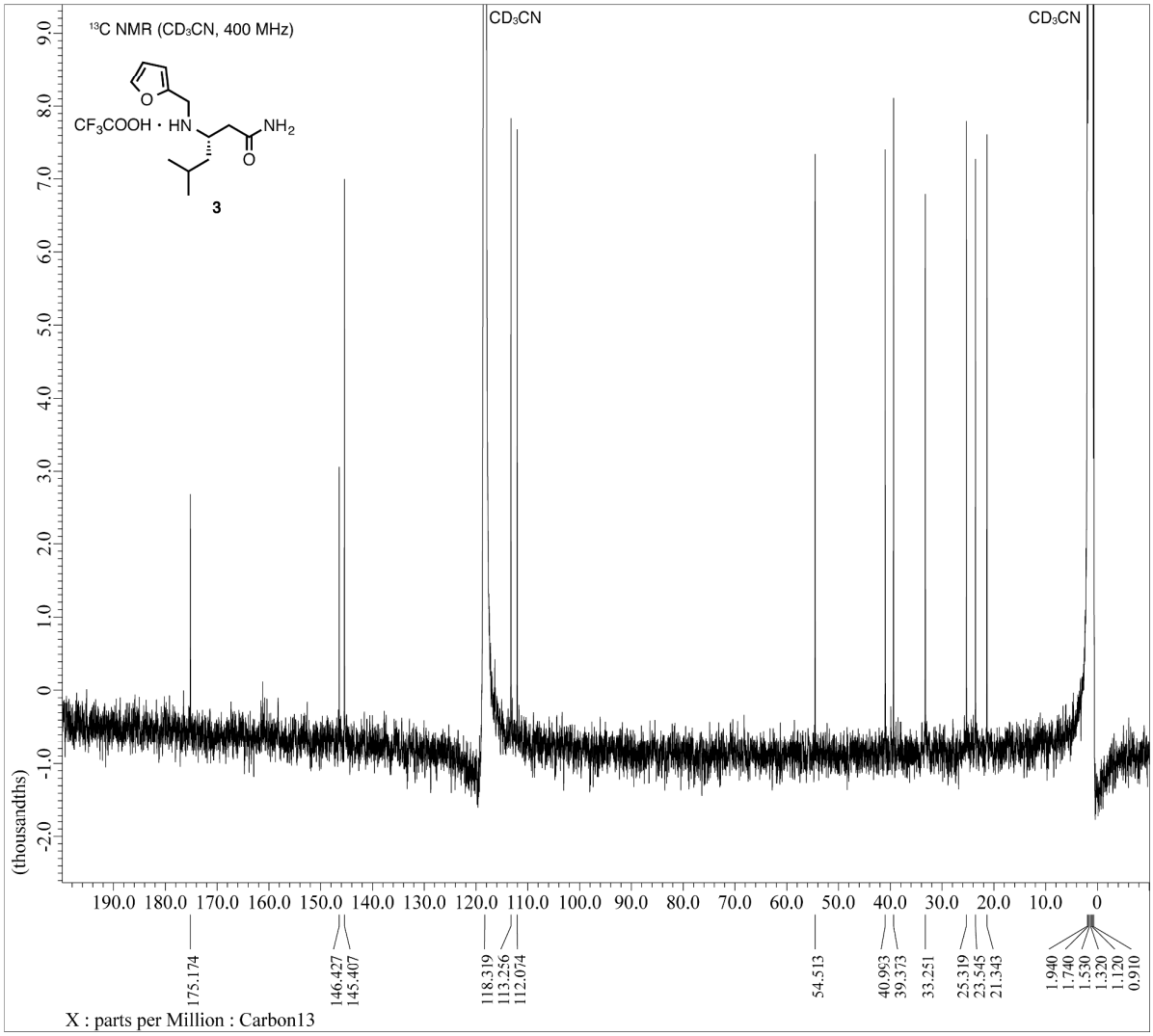

Figure S8. ^{13}C-NMR spectrum of *N*-Furfuryl-L-β^3-homoleucine amide (**3**).

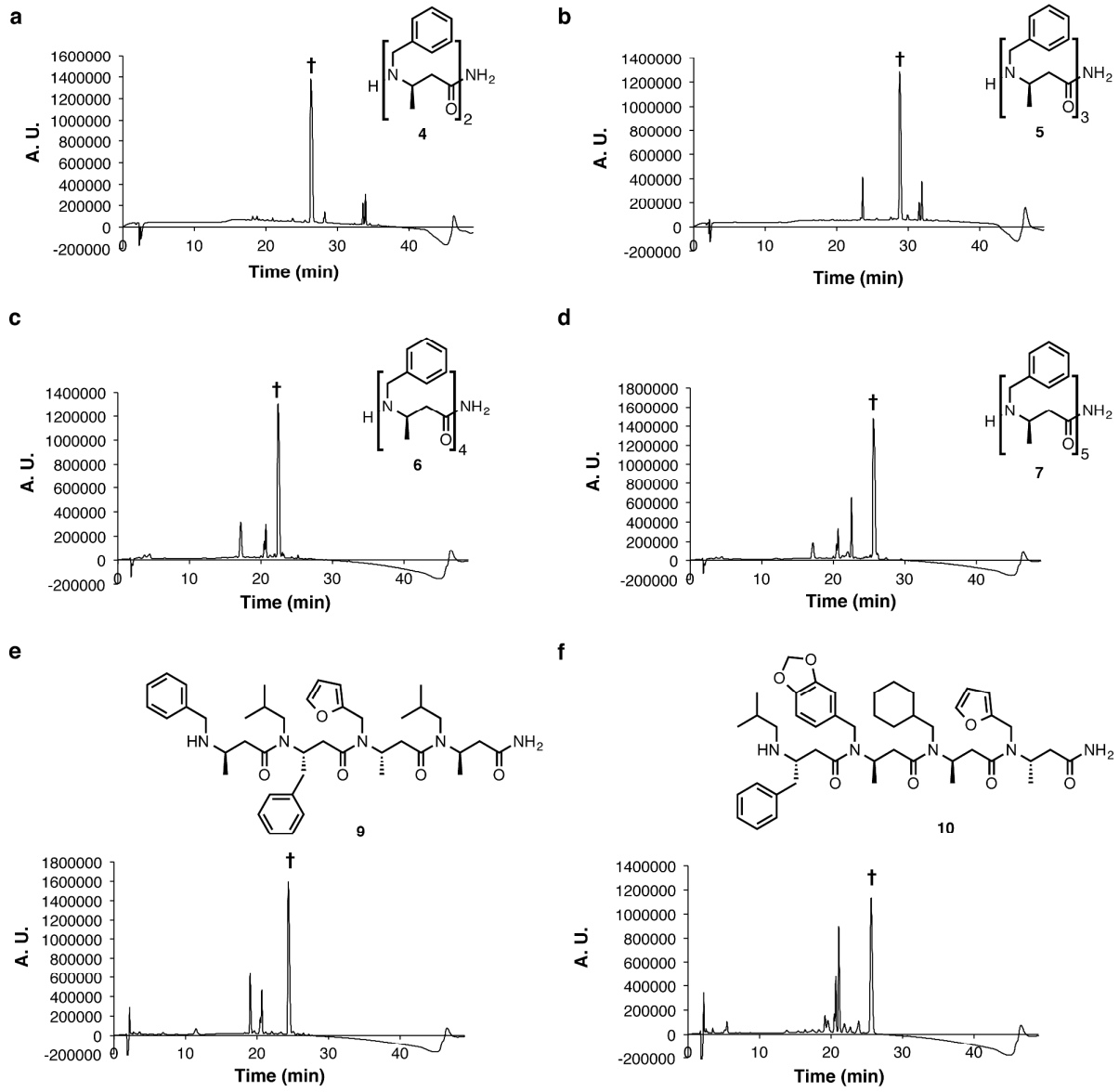

Figure S9. HPLC chromatograms of crude products of β-peptoid oligomers. **(a) 4**. **(b) 5**. **(c) 6**. **(d) 7**. **(e) 9**. **(f) 10**. The peak containing the desired product is labeled with † on each chromatogram.

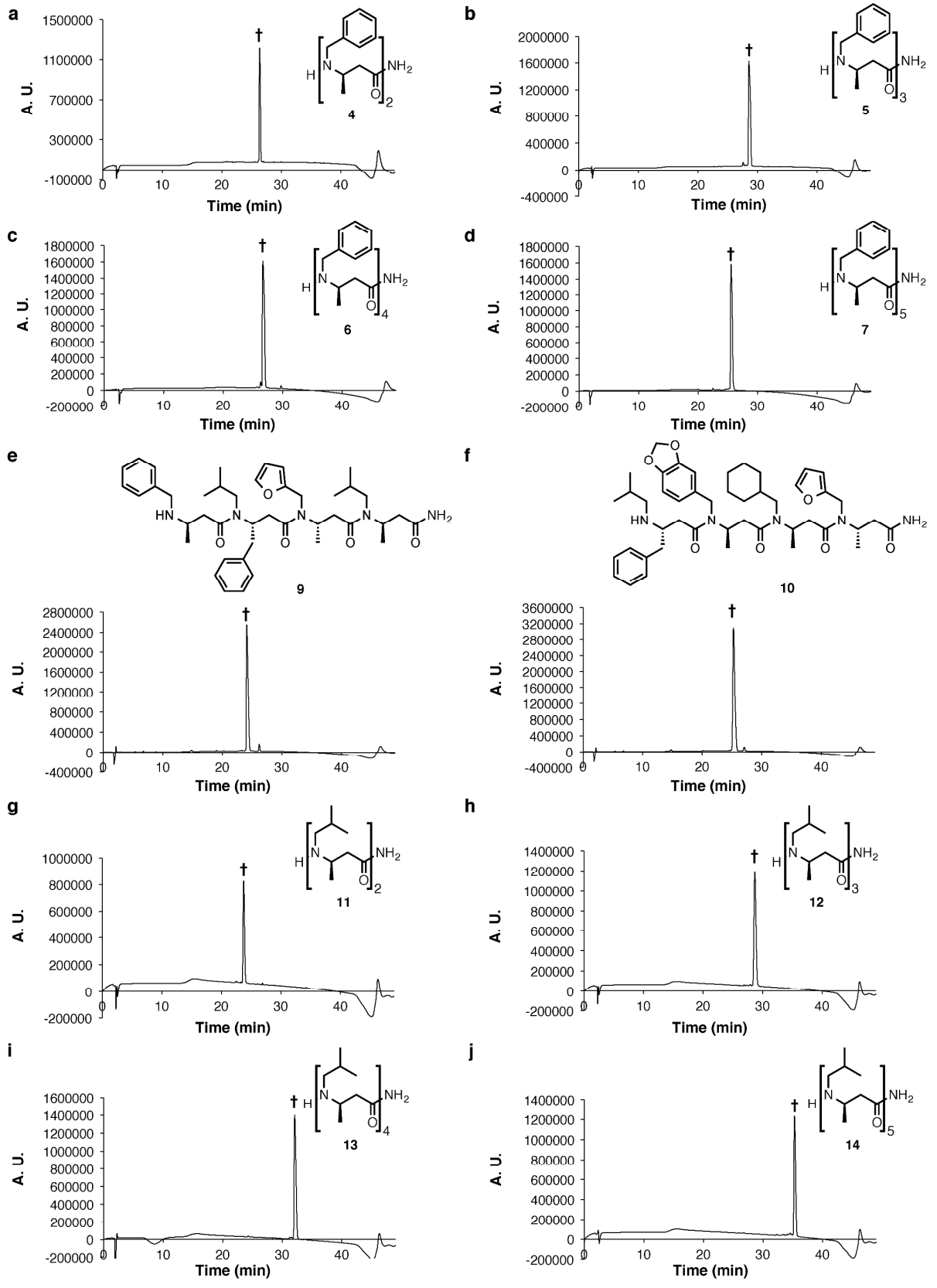

Figure S10. HPLC chromatograms of purified products of β-peptoid oligomers **(a) 4**. **(b) 5**. **(c) 6**. **(d) 7**. **(e) 9**. **(f) 10**. **(g) 11**. **(h) 12**. **(i) 13**. **(j) 14**. The peak containing the desired product is labeled with † on each chromatogram.

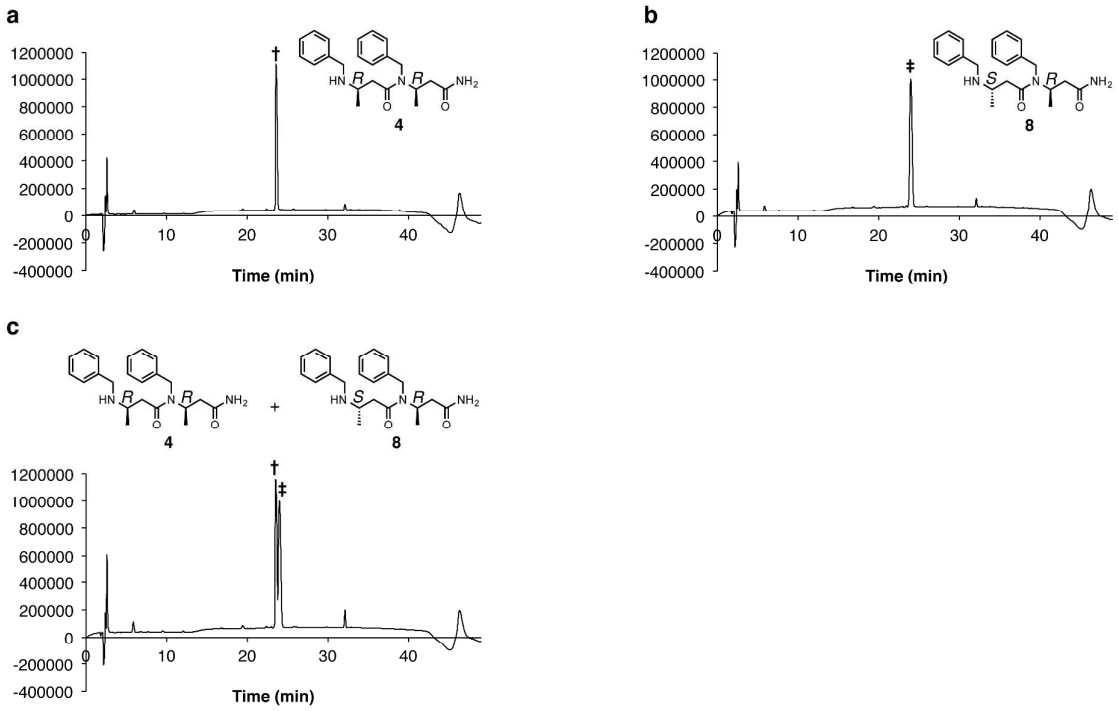

Figure S11. HPLC chromatograms of crude products of diastereomers **(a) 4** and **(b) 8** and **(c)** coinjection of **4** and **8**. Full spectra of **FIgure 2** are shown. Peaks corresponding to **4** and **8** are labeled with † and ‡, respectively. **(a)** HPLC chromatogram of **4**. The fraction † was analyzed by ESI-MS: $[M+H]^+$ Calcd. 368.2333, Found. 368.2343. **(b)** HPLC chromatogram of **8**. The fraction ‡ was analyzed by ESI-MS: $[M+H]^+$ Calcd. 368.2333, Found. 368.2353. **(c)** HPLC chromatogram of co-injection of **4** and **8**. The fraction † and ‡ were analyzed by ESI-MS: † $[M+H]^+$ Calcd. 368.2333, Found. 368.2356; ‡ $[M+H]^+$ Calcd. 368.2333, Found. 368.2355.

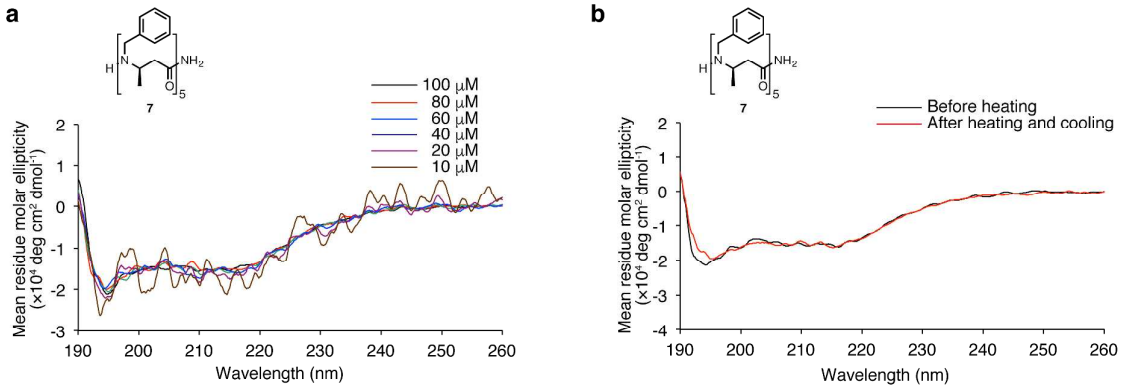

Figure S12. CD spectra of *N*-benzyl-β^3-homoalanine pentamer **7** with varied conditions. **(a)** CD spectra measured at 10-100 μM compound concentration. Spectra were measured at 25 °C. **(b)** CD spectra measured at 25 °C before and after heating to 65 °C. Spectra were measured at 100 μM compound concentration.

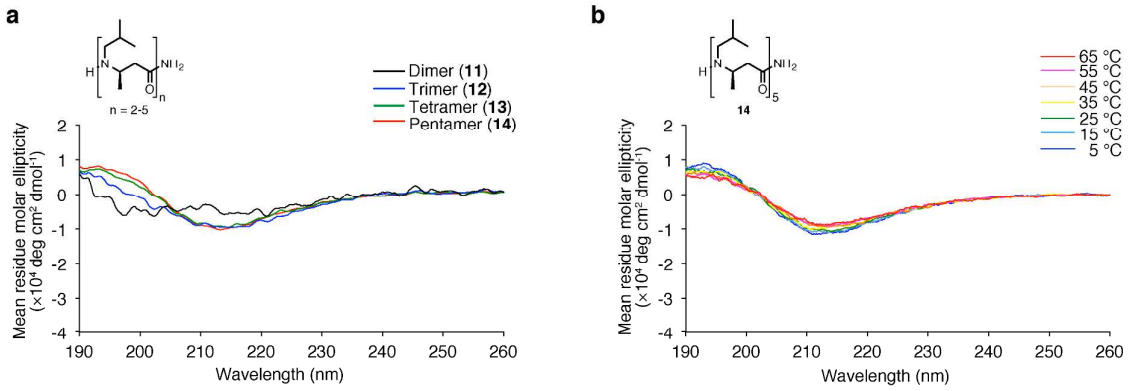

Figure S13. CD spectrum of *N*-isobutyl-β^3-homoalanine oligomers **11**–**14**. All spectra were obtained using 100 µM solution of the respective compound in acetonitrile. Y-axis was normalized to molar ellipticity per *N*-substituted amide bond. **(a)** CD spectra of dimer (**11**), trimer (**12**), tetramer (**13**) and pentamer (**14**) at 25 °C. **(b)** CD spectra of pentamer (**14**) at 5-65 °C.

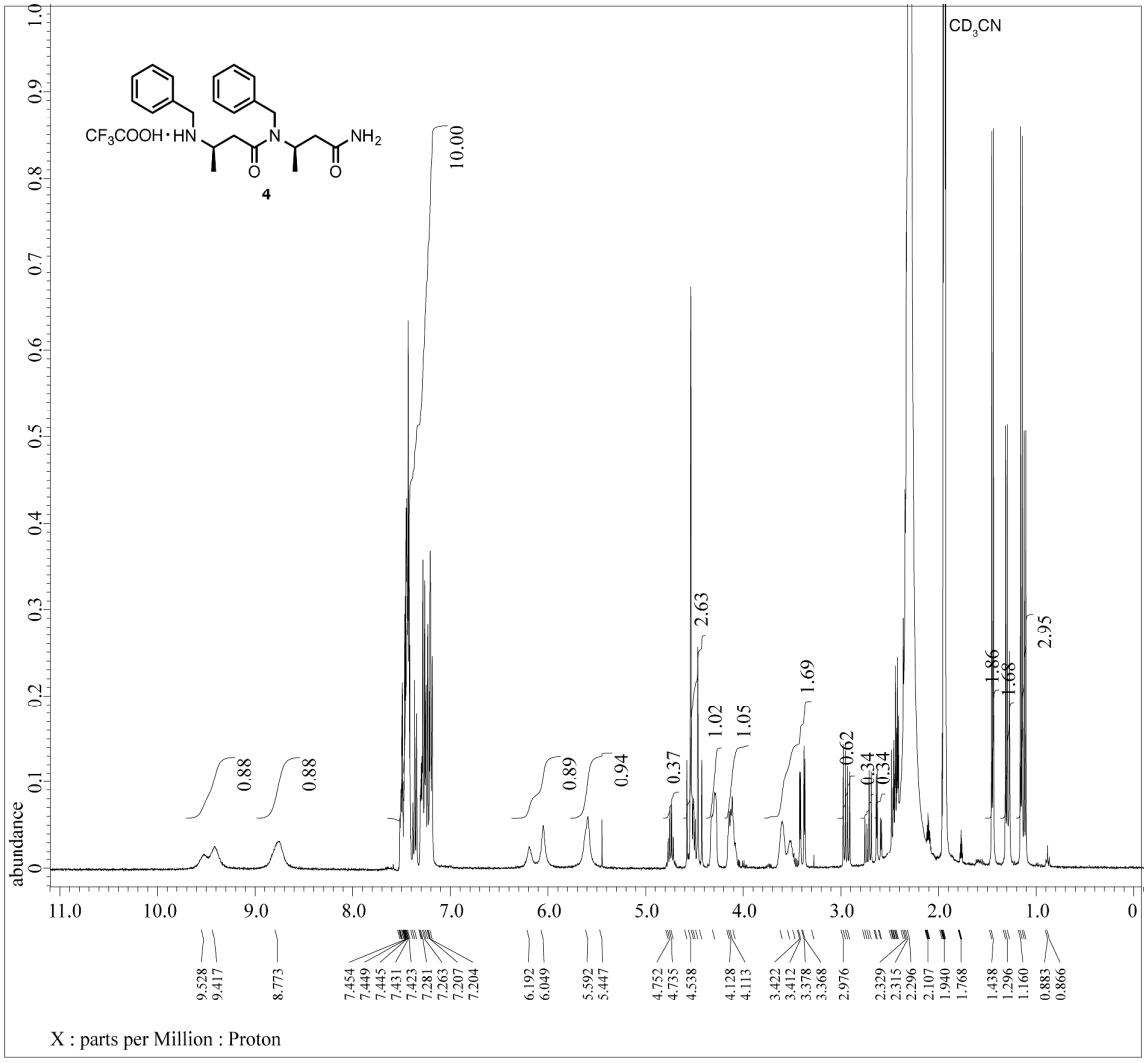

Figure S14. ^{1}H-NMR spectrum of *N*-benzyl-β^{3}-homoalanine dimer **4**.

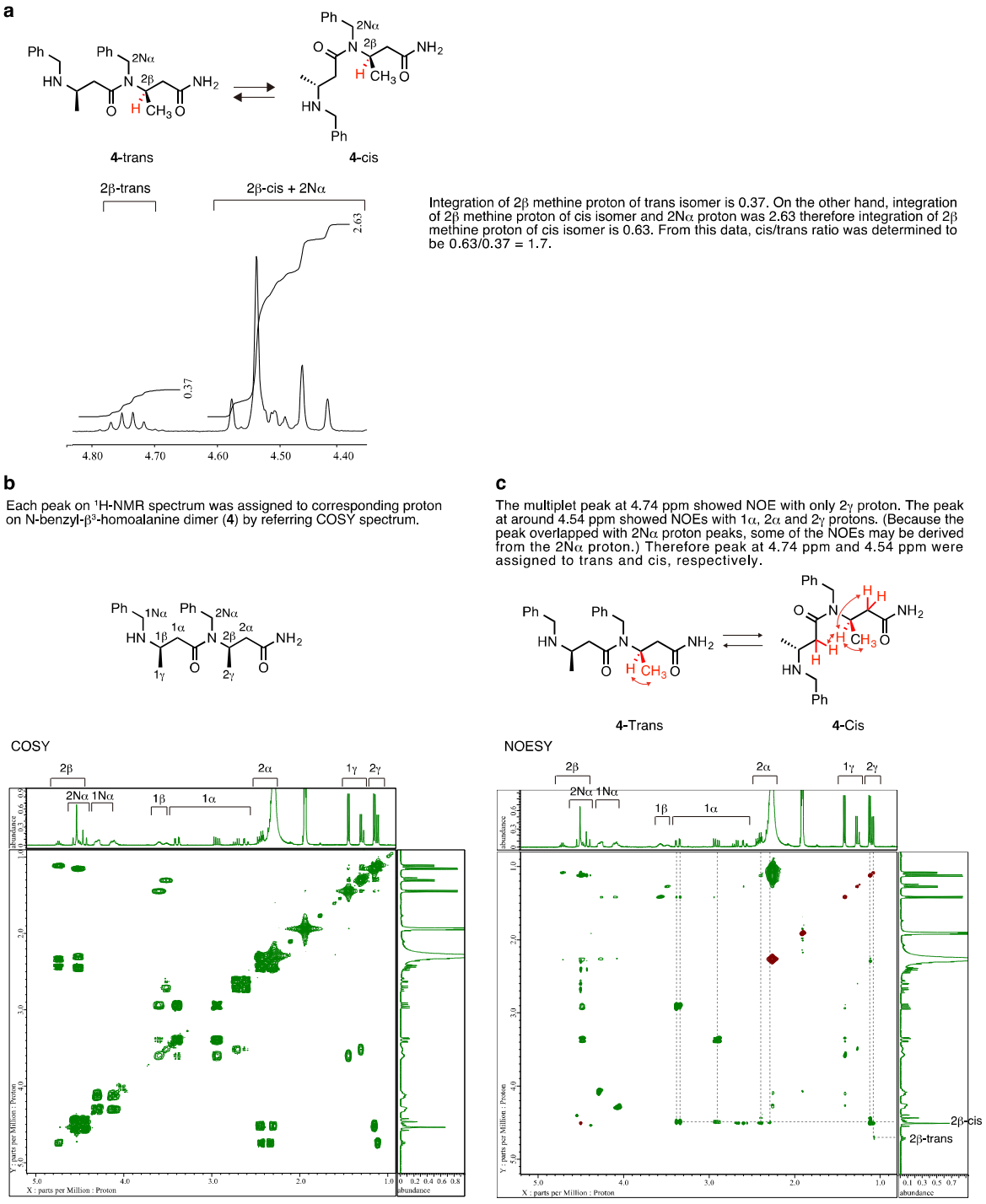

Figure S15. Estimation of cis/trans ratio of *N*-benzyl-β[3]-homoalanine dimer (**4**). Each proton was named as 1α, 1β, 1γ 1Nα, 2α, 2β, 2γ, 2Nα as shown on the structures. **(a)** ^{1}H-NMR spectrum. Region of β-methine proton of C-terminal residue is shown. **(b)** COSY spectrum. Each peak of ^{1}H-NMR spectrum was assigned to corresponding proton. **(c)** NOESY spectrum. Peaks at 4.74 ppm and around at 4.54 ppm were assigned to 2β methine proton of trans and cis isoform, respectively, from the NOEs.