Supplementary Note

This section contains a detailed description of the chemical procedures and the characterization of products. The text is followed by a reaction scheme explaining the synthetic strategies used, original 1H- and 13C-NMR spectra to prove the identity and purity of final products and of original UV-spectra that prove the presence of the intact diazirine ring in each of the final products.

Chemical synthesis
(see also reaction scheme, bold underlined numbers in this text refer to the bold underlined numbers in the scheme)

Photo-Met

DL-2-Amino-5,5’-azi-hexanoic acid (1)
The compound was obtained by Strecker synthesis. Caution: Handling of NaCN is dangerous.
To a concentrated aqueous solution of 1.75 g of ammonium chloride were added 6 ml of conc. aqueous NH₃ and 1.61 g of NaCN. The mixture was cooled on ice and 3.45 g of 4,4’-azi-pentanal¹ were added dropwise under stirring, which was continued for 5 h at room temperature. Then, most of the water was evaporated under reduced pressure and, in a well-ventilated hood, 30 ml of concentrated HCl were added (Caution, possible release of highly toxic HCN gas). The mixture was stirred at 85°C for 6 h followed by evaporation of the water at reduced pressure. The dry residue was extracted with 40 ml hot methanol and the extract neutralized with N,N-dimethylethylamine. Upon standing for 2 days at –32°C a precipitate formed which was isolated and re-crystallized twice from 75% ethanol to yield 925 mg of pure 1.

DL-2-Acetamino-5,5’-azi-hexanoic acid (2)
1 (800 mg) was dissolved in 20 ml 1N NaOH. Ice (15 g) was added and 1.43 ml acetic anhydride was added under stirring, which was continued for 5 min. The
mixture was acidified to pH 2.5 with HCl and extracted with ethyl acetate. Evaporation of the solvent yielded 800 mg of pure 2.

1H-NMR (D₂O): 0.91 ppm (s, 3H, 6-CH₃), 1.20 – 1.67 (m, 4H, 3,4-CH₂), 1.90 (s, 3H, acetyl-CH₃), 4.03 (dd, 1H, 2-CH)

13C-NMR (D₂O): 18.42 ppm (6-C), 21.76 (acetyl-CH₃), 25.99 and 30.10 (3-C and 4-C), 26.02 (5-C), 54.49 (2-C), 173.48 and 178.65 (1-C and acetyl-CO)

L-2-amino-5,5’-azi-hexanoic acid (2, Photo-Met)

2 (1.5 g) was dissolved in 150 ml of water. The pH was adjusted to 7.5 by addition of NH₄OH, and 60 mg of porcine kidney acylase I (Sigma No. A-8376) were added. After stirring for 3 h at 30°C, the mixture was acidified with HCl, extracted with ether and filtered. The aqueous phase was loaded on a Dowex 50WX8 cation exchange resin (H⁺-form, 30 ml). Flow-through and ether extract were kept for isolation of D-2-amino-5,5’-azi-hexanoic acid (see below). The column was washed with 10 bed volumes of water and eluted with 2 N NH₄OH. Product was obtained by lyophilization and crystallized from 70 % ethanol to yield 400 mg pure photo-Met (3).

UV: 1.9 mg/ml in water: 349.1 nm ε = 55

in 50 mM HCl: 348.5 nm ε = 55

in 150 mM NaOH: 350.7 nm ε = 55

1H-NMR (D₂O): 1.04 ppm (s, 3H, 6-CH₃), 1.47 (m, 2H, 4-CH₂) and 1.77 (m, 2H, 3-CH₂), 3.70 (t, 1H, 2-CH)

13C-NMR (D₂O): 18.76 ppm (6-C), 25.37 and 29.79 (3-C and 4-C), 26.45 (5-C), 54.44 (2-C), 174.47 (1-C)

D-2-amino-5,5’-azi-hexanoic acid (D-Photo-Met)

The flow-through of the above cation exchange column was supplemented with 20 g of NaCl and extracted with 2 x 100 ml ethyl acetate. The extracts were combined with the above ether extract, dried and evaporated. The residual crude D-2-Acetamino-5,5’-azi-hexanoic acid was subjected to a second round of acylase digestion and separated from free L-amino acid to give 650 mg of D-2-Acetamino-5,5’-azi-hexanoic acid. The D-2-Acetamino-5,5’-azi-hexanoic acid was treated with 40 ml of 7 N HCl at 75°C for 16 h, followed by lyophilization and crystallization from 70 % ethanol to yield 300 mg pure product.
UV: 1.9 mg/ml in water: 349.5 nm $\varepsilon = 56$

in 50 mM HCl: 348.3 nm $\varepsilon = 56$

in 150 mM NaOH: 350.7 nm $\varepsilon = 56$

DL-[1-$^{14}$C]-2-amino-5,5'-azi-hexanoic acid (4, [1-$^{14}$C]Photo-Met)

A concentrated aqueous solution of 11 mg NH$_4$Cl was mixed with 40 µl conc. NH$_4$OH, 10 mg NaCN and 1 mCi [1-$^{14}$C]NaCN (52 mCi/mmol, about 1 mg). 40 µl 4,4'-Azipentanal$^1$ were added and the mixture was shaken at 33°C for 16 h. Free ammonia and part of the water were removed in a speed-vac at reduced pressure. 200 µl of conc. HCl were added and the mixture was shaken at 85°C for 7 h. 1.2 ml of water were added, the mixture was shaken at 50°C for 10 min and centrifuged. The supernatant was loaded onto a Dowex 50W8 cation exchange column (2 ml). The column was washed with 10 ml of water and eluted with 2 N NH$_4$OH. Fractions containing radioactivity were pooled to yield 520 µCi of raw 4. UV-spectroscopy of the pooled fractions showed the intact diazirine ring (348 nm, $\varepsilon = 50$). For further purification, the material was lyophilized and re-crystallized from 3 ml 70% ethanol to yield 250 µCi (53 µmol, 7.3 mg) product of 96% radiochemical purity as determined by chiral TLC (Fig. 1, Macherey & Nagel chiralplate, methanol/water/acetonitrile 1/1/4) or paper chromatography (Whatman 3 MM Chr, acetic acid/water/butanol 1/1/4, data not shown). In both assays, the substance behaved identical to authentic non-radioactive DL-2-amino-5,5'-azi-hexanoic acid 2.

Photo-Leu

DL-2-Bromo-4,4'-azi-pentanoic acid (5)

The compound was obtained by bromination of 4,4'-azi-pentanoic acid according to the procedure described by Harrp et al$^2$. Briefly, 4,4'-azi-pentanoic acid$^4$ (3 g, 23 mmol), CCl$_4$ (3 ml) and thionyl chloride (7 ml, 96 mmol) were heated to 65°C for 30 min. N-Bromosuccinimid (4.8 g, 27 mmol), CCl$_4$ (10 ml) and 0.1 ml of 48% HBr were added and the mixture was stirred at 55°C for 4 h. The solvent and free bromine were removed under reduced pressure and the residue was extracted with 50 ml CCl$_4$. The solvent was removed and the crude product (2-Bromo-4,4'-azi-pentanoyl chloride) dissolved in acetone and hydrolyzed with aq NaHCO$_3$. The crude
brominated free acid was obtained upon acidification with HCl and extraction with dichloromethane. The solvent was removed and the product filtered through silica gel in isohexane/ethyl acetate 9/1 followed by removal of the solvent.

UV 3.4 mg/ml in EtOH: 342.9 nm (ε = 48)
1H-NMR (CDCl₃): 1.12 ppm (s, 3H, 5-CH₃), 2.07 (dd, 1H, 3-CH₂), 2.25 (dd, 1H, 3-
CH’₂), 4.07 (t, 1H, 2-CH), 10 (broad, 1H, COOH)
13C-NMR (CDCl₃): 19.87 ppm (5-C), 24.09 (4-C), 38.23 (2-C), 39.81 (3-C), 174.36 (1-C)

DL-2-amino-4,4’-azi-pentanoic acid (6)
Aminolysis of (5) was performed in 100 ml ammonia-saturated methanol and 20 ml 25% aq ammonia for 5 days at 55°C. After evaporation of the ammonia, 20 ml of concentrated HCl were added followed by evaporation of the water at reduced pressure. The dry residue was extracted with 20 ml hot methanol and the extract neutralized with N,N-dimethylethylamine. Upon standing for 2 days at –32°C a precipitate formed which was isolated and re-crystallized twice from 70% ethanol to yield 450 mg pure 6.

L-2-amino-4,4’-azi-pentanoic acid (8, Photo-Leu)
6 was acetylated as described above (see 2) to obtain acetylation DL-2-acetamino-
4,4’-azi-pentanoic acid (7) followed by enzymatic deacetylation as described above to give pure 8.

UV: 1.9 mg/ml in water: 345.1 nm ε = 64
     1.9 mg/ml in 50 mM HCl: 343.5 nm ε = 64
     1.9 mg/ml in 150 mM NaOH: 348.7 nm ε = 64
1H-NMR (D₂O): 1.11 ppm (s, 3H, 5-CH₃), 1.74 (dd, J 8.2 Hz, 15.5 Hz, 1H, 3-CH₂),
     2.07 (dd, J 5.5 Hz, 15.5 Hz, 1H 3-CH’₂), 3.74 (dd, J 5.5 Hz, 8.2 Hz, 1H, 2-CH)
13C-NMR (D₂O): 18.75 ppm (5-C), 24.05 (4-C), 36.43 (3-C), 51.38 (2-C), 174.03 (1-
C)
Photo-Ile

3-R,S-Methyl-4,4’-azi-pentanoic acid (10)

10 was obtained from 3-R,S-Methyl-4-keto-pentanoic acid (9) by the procedure of Church and Weiss1.

UV: 2.3 mg/ml in EtOH: 346.2 nm ε=55
1H-NMR (CDCl3): 0.86 ppm (d, 3H, 3-methyl-CH3), 0.98 (s, 3H, 5-CH3), 1.93 (m, 1H, 3-CH), 2.03 (dd, 1H, 2-CH), 2.25 (dd, 1H, 2-CH’), 11 ppm (broad, 1H, COOH)
13C-NMR (D2O): 14.93 ppm (3-methyl-C), 17.88 (5-C), 28.29 (4-C), 33.89 (3-C), 36.56 (2-C), 178.38 (1-C)

2R,S-Amino-3-R,S-methyl-4,4’-azi-pentanoic acid (12, Photo-Ile)

Bromination (see 5) of 10 gave 2R,S-Bromo-3-R,S-methyl-4,4’-azi-pentanoic acid (11), which was subjected to aminolysis with excess ammonia according to the procedure described above for photo-Leu, to give low yields of pure 12 (80 mg). No attempts were made to resolve the 2S,3R-form that would correspond to the configuration of natural isoleucin (2S,3S).

UV: 2.2 mg/ml in water: 344.6 nm ε=50
2.2 mg/ml in 50 mM HCl: 342.9 nm ε=50
2.2 mg/ml in 150 mM NaOH: 348.6 nm ε=50
1H-NMR (D2O): 0.82 ppm (d, 3H, 3-methyl-CH3), 1.06 (s, 3H, 5-CH3), 1.96 (m, 1H, 3-CH), 3.65 (d, 1H, 2-CH)
13C-NMR (D2O): 12.03 ppm (3-methyl-C), 17.38 (5-C), 27.27 (4-C), 39.14 (3-C), 56.59 (2-C), 173.18 (1-C)

References

**Reaction Scheme**

(i) 5h at r.t., then conc HCl at 85°C for 6h. (ii) NaOH, acetic anhydride. (iii) kidney acylase 1 (iv) NH₃, NH₂OSO₃H, I₂. (v) SOCl₂, NBS + HBr, aq. NaHCO₃. (vi) conc. aq. NH₃, 55°C, 3-7 d
Photo-Leu 1H-NMR in D2O (500 MHz)
Photo-Leu 13C-NMR in D2O 125 MHz
Photo-Ile 13C-NMR in D2O 125 MHz

ppm

173.181

56.586

39.187

27.274

17.381

12.029
UV-Spectra

Photo-Met, 2.4 mg/ml in water

Photo-Leu, 2.8 mg/ml in water

Photo-Ile, 2.2 mg/ml in water