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Supporting Information

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Chemoselective Peptide Cyclization via Induced Traceless Staudinger Ligation

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General. All reagents, amino acids, and solvents were purchased from commercial suppliers and used without further purification. Dry DMF was purchased from ACROS ORGANICS, dry ethyl ether was obtained from a dry solvent system (BRAUN). Phosphinothiol derivatives **4** and **7** were synthesized by published protocols,[1] azidoglycine (**5**) and benzyl azide by nucleophilic substitution of the corresponding bromide compound with NaN_3 in DMSO.[2,3]
HPLC and HRMS spectra were recorded on an Agilent 6210 TOF LC/MS system, Agilent Technologies, Santa Clara, CA, USA. Spray voltage was set to 4 kV. Drying gas flow rate was set to 25 psi. Seperation of the sample was performed on a Agilent Eclipse XDB-C_{18} column (5 µm, 4.6*150 mm) at a flow rate of 0.5 mL/min. HPLC analysis and purification for the cyclic peptide **1** was preformed on a JASCO LC-2000 Plus system using a C_{18} column (5 µm, 4.6*250 mm with a flow rate of 1 mL/min for analytical and 25*250 mm with a flow rate of 12 mL/min for preparative separation). Specific gradients are given in the synthetic procedures.

Peptide Synthesis. Peptides were synthesized on an ABI 433A peptide synthesizer using standard amide coupling conditions HBTU/HOBt (Fast-moc protocol). As the solid support, the TGT®-Resin (Novabiochem) was used with the first amino acid (Gly) already attached to the resin.

Synthesis and NMR-analysis of Diphenylphosphino(borane)methanethiol acetate (4). Compound **4** was synthesized as described previously. Detailed ^{1}H-, ^{31}P- and ^{11}B-NMR-analysis revealed the presence of the borane protecting group on the phosphorous atom, which showed a characteristic ^{11}B-NMR chemical shift for the tetracoordination of the borane.[4]

^{1}H-NMR [^{31}P] (500 MHz, DMF-d_7): δ = 7.88-7.86 (m, 4H), 7.70-7.63 (m, 5H), 4.06 (s, 2H), 2.38 (s, 3H), 1.33-0.77 (m, 3H)

^{1}H-NMR [^{11}B] (500 MHz, DMF-d_7): δ = 7.88-7.85 (m, 4H), 7.69-7.63 (m, 5H), 4.06 (d, J = 7.2 Hz, 2H), 2.38 (s, 3H), 1.03 (d, J = 15.0 Hz, 3H)

^{31}P-NMR (202 MHz, DMF-d_7): δ = 18.34 (m)

^{31}P-NMR [^{1}H] (202 MHz, DMF-d_7): δ = 18.34 (d, J = 59.1 Hz)

^{11}B-NMR (160.5 MHz, DMF-d_7): δ = –26.42 (m) (see Figure S1)

^{11}B-NMR [^{1}H] (160.5 MHz, DMF-d_7): δ = –26.29 (d, J = 45.9 Hz) (see Figure S2)

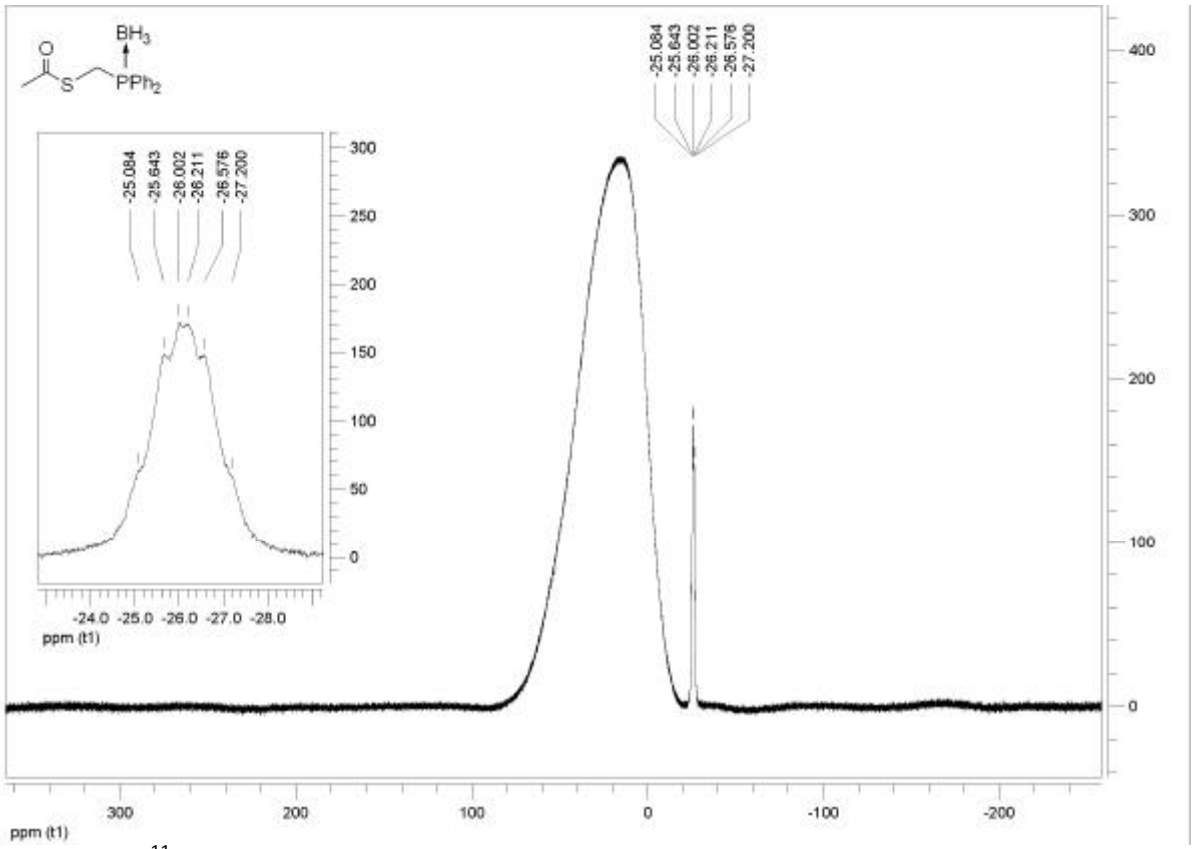


Figure S1. ^{11}B-NMR (160.5 MHz, DMF-d_7) of compound **4**.

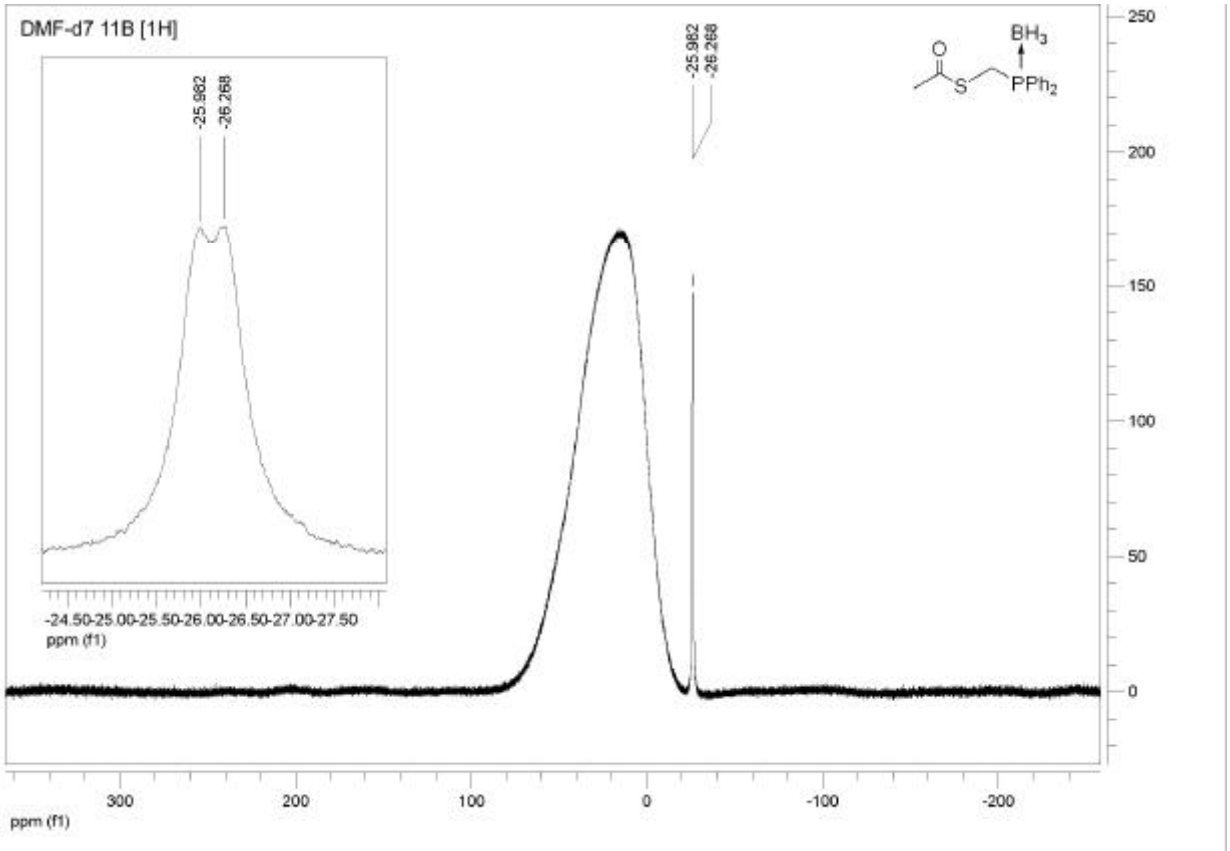


Figure S2. ^{11}B-NMR [^{1}H] (160.5 MHz, DMF-d_7) of compound **4**.

TFA-deprotection and NMR-analysis of Diphenylphosphino(borane)methanethiol acetate (4). 92 mg of **4** (0.32 mmol) were treated with 0.5 mL of a TFA-solution containing 2.5 % triisopropylsilane (TIS)

for 1 h. After removing the TFA via high vacuum over 1 h the crude deprotected phosphine **9** is dissolved in 0.7 mL anhydrous DMF-d_7. The solution is degassed by 3 cycles of vacuum and argon. To this solution 72 mg DABCO (0.64 mmol, 2 eq.) were added to scavange residual amounts of TFA.
Detailed ^{1}H-, ^{31}P- and ^{11}B-NMR-analysis revealed borane deprotection of **4** and the presence of a three-valent borate species as shown by a characteristic ^{11}B-NMR chemical shift.[4]

^{1}H-NMR (500 MHz, DMF-d_7): d = 7.58-7.54 (m, 5H), 7.49-7.48 (m, 8H), 3.70 (d, J = 3.4 Hz, 2H), 2.39 (s, 3H)

^{1}H-NMR [^{31}P] (500 MHz, DMF-d_7): d = 7.57-7.55 (m, 5H), 7.49-7.48 (m, 8H), 3.70 (s, 2H), 2.39 (s, 3H)

^{1}H-NMR [^{11}B] (500 MHz, DMF-d_7): d = 7.58-7.55 (m, 5H), 7.49-7.48 (m, 8H), 3.70 (d, J = 3.6 Hz, 2H), 2.39 (s, 3H)

^{31}P-NMR [^{1}H] (202 MHz, DMF-d_7): d = –10.39

^{11}B-NMR (160.5 MHz, DMF-d_7): d = 7.56 (q, J = 96.2 Hz) (see Figure S3)

^{11}B -NMR [^{1}H] (160.5 MHz, DMF-d_7): d = 7.56 (s) (see Figure S4)

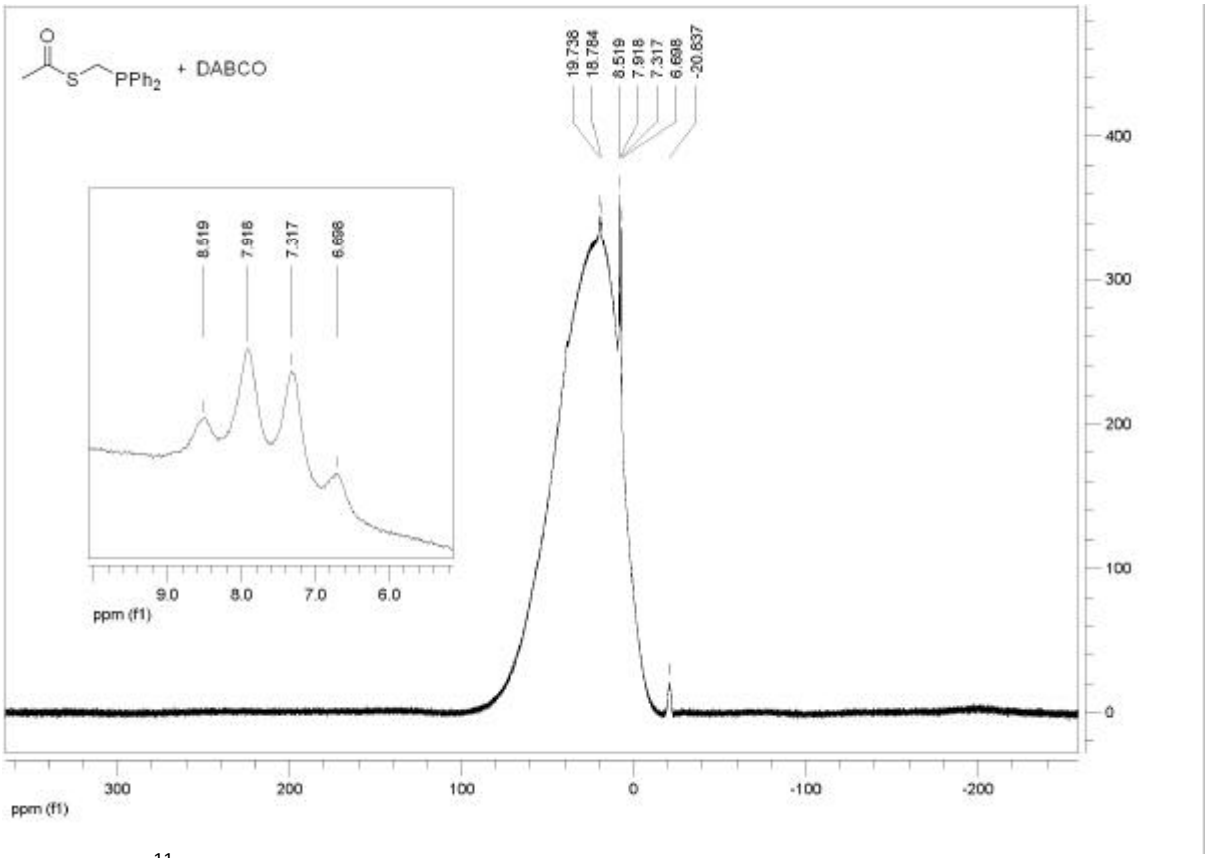


Figure S3. ^{11}B-NMR (160.5 MHz, DMF-d_7) of compound **8**.

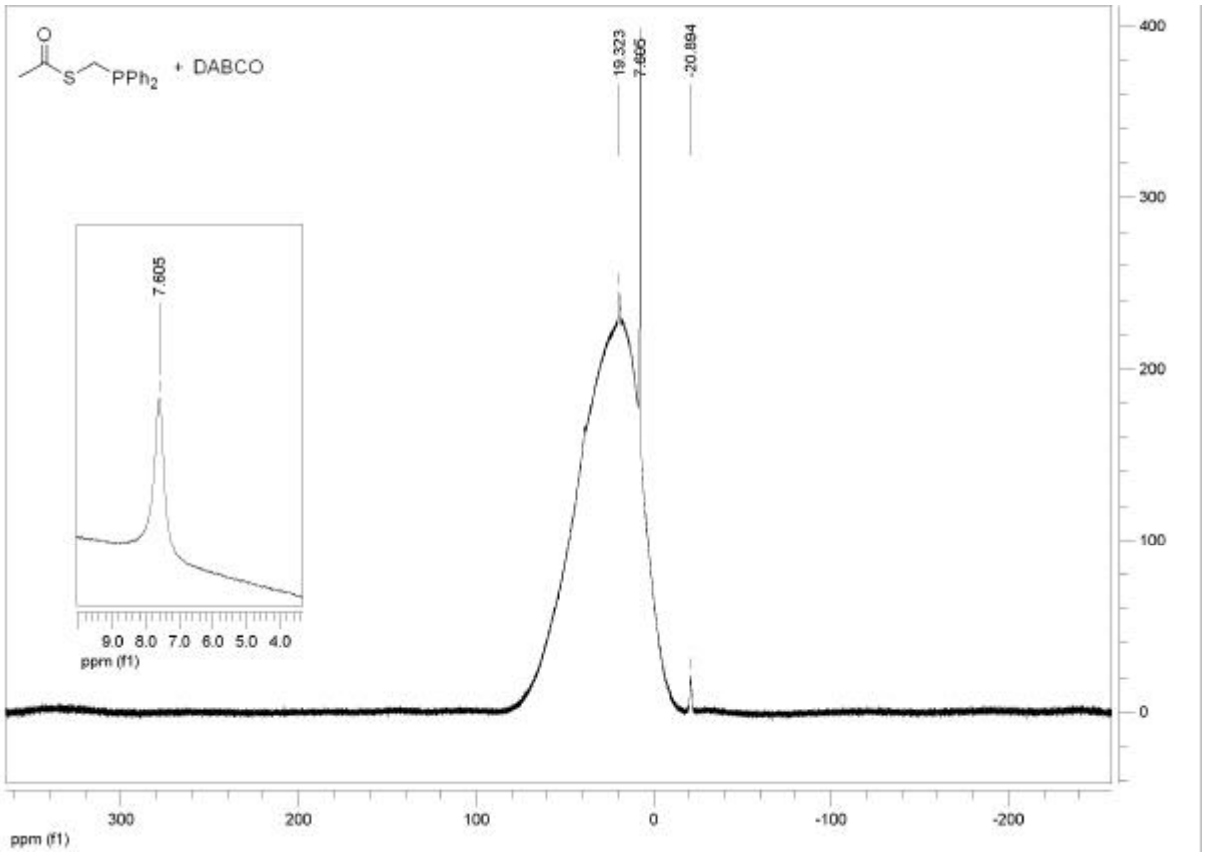


Figure S4. ^{11}B-NMR [^{1}H] (160.5 MHz, DMF-d_7) of compound **8**.

Staudinger ligation of deprotected phosphinothiol 8 with benzyl azide. The deprotected phosphinothiol derivate **8** was prepared by TFA-deprotection as described above. To a solution of **8** (which was obtained from 0.32 mmol **4**) in 0.7 mL anhydrous DMF-d_7 43 mg benzyl azide (0.32 mmol, 1 eq.) and 72 mg DABCO (0.64 mmol, 2 eq.) were added. The reaction was stirred under argon for 12 h. ^{1}H-NMR-analysis (see Figure S5) revealed full conversion of the benzyl azide to the corresponding N-benzylacetamide (**9**). The analytical data was in accordance with the commercially available N-benzylacetamide (**9**) (TCI).

^{1}H-NMR (500 MHz, DMF-d_7): d = 7.99-7.95 (m, 4H), 7.64-7.60 (m, 8H), 7.37-7.36 (m, 5H), 7.28 (m, 1H), 4.42 (d, J = 5.9 Hz, 2H), 2.01 (s, 3H) (see Figure S5)

^{13}C-NMR (125 MHz, DMF-d_7): d = 171.25, 141.97, 130.17, 129.33, 128.61, 44.50, 24.16

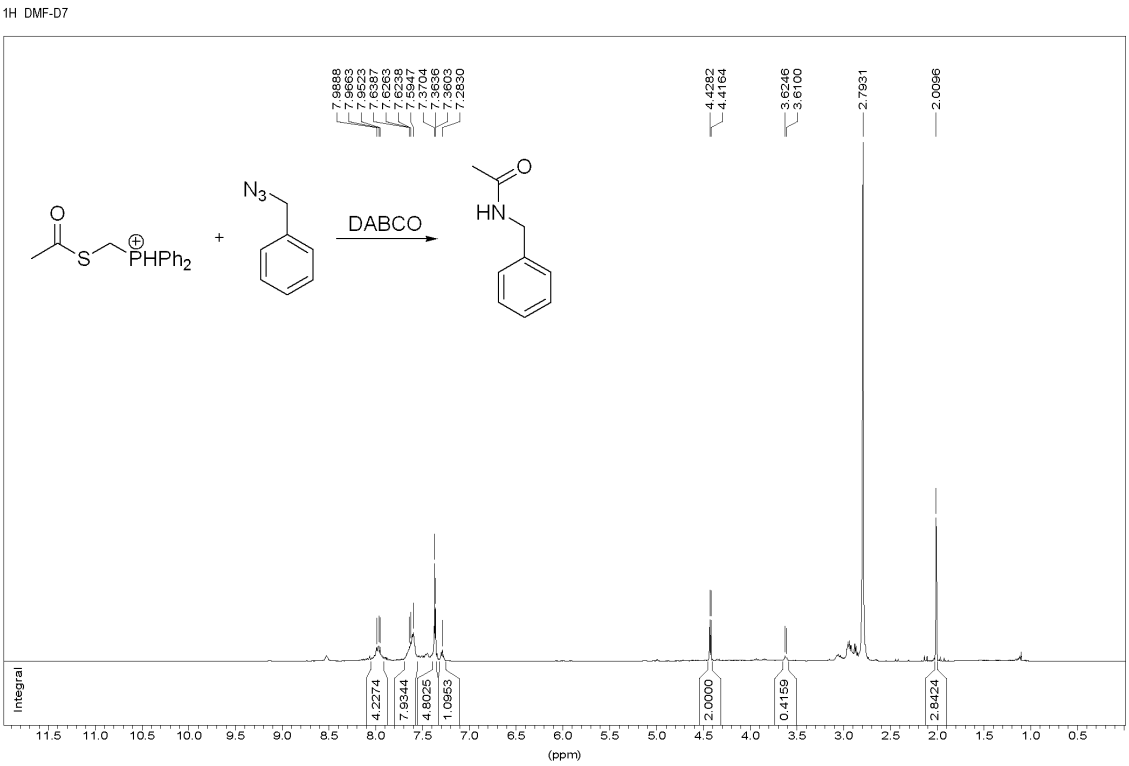
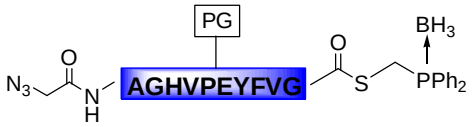


Figure S5. ^{1}H-NMR (500 MHz, DMF-d_7) of crude reaction mixture for the Staudinger ligation of **8** with benzyl azide.

Base-induced Staudinger cyclization of the azido-peptide-phosphinothioester 2.

a) Synthesis of the azido-peptide-phosphinothioester **2**.



2

The azido-peptide-phosphinothioester **2** was prepared as described in the experimental part using DIC/DMAP activation in CH_2Cl_2. The conversion of **2** was determined via LC/MS analysis (C_{18}-column, constant flow: 3 min at 3 % CH_3CN (with 1 % AcOH), gradient: 3 % to 100 % CH_3CN (with 1 % AcOH) over 19 min), in which **2** eluted at 21.88 min (see Figure 1A and Figure S6).

HRMS (ESI-TOF): m/z = 1740.8377 $[M+H]^+$ (calcd.: m/z = 1740.8372)

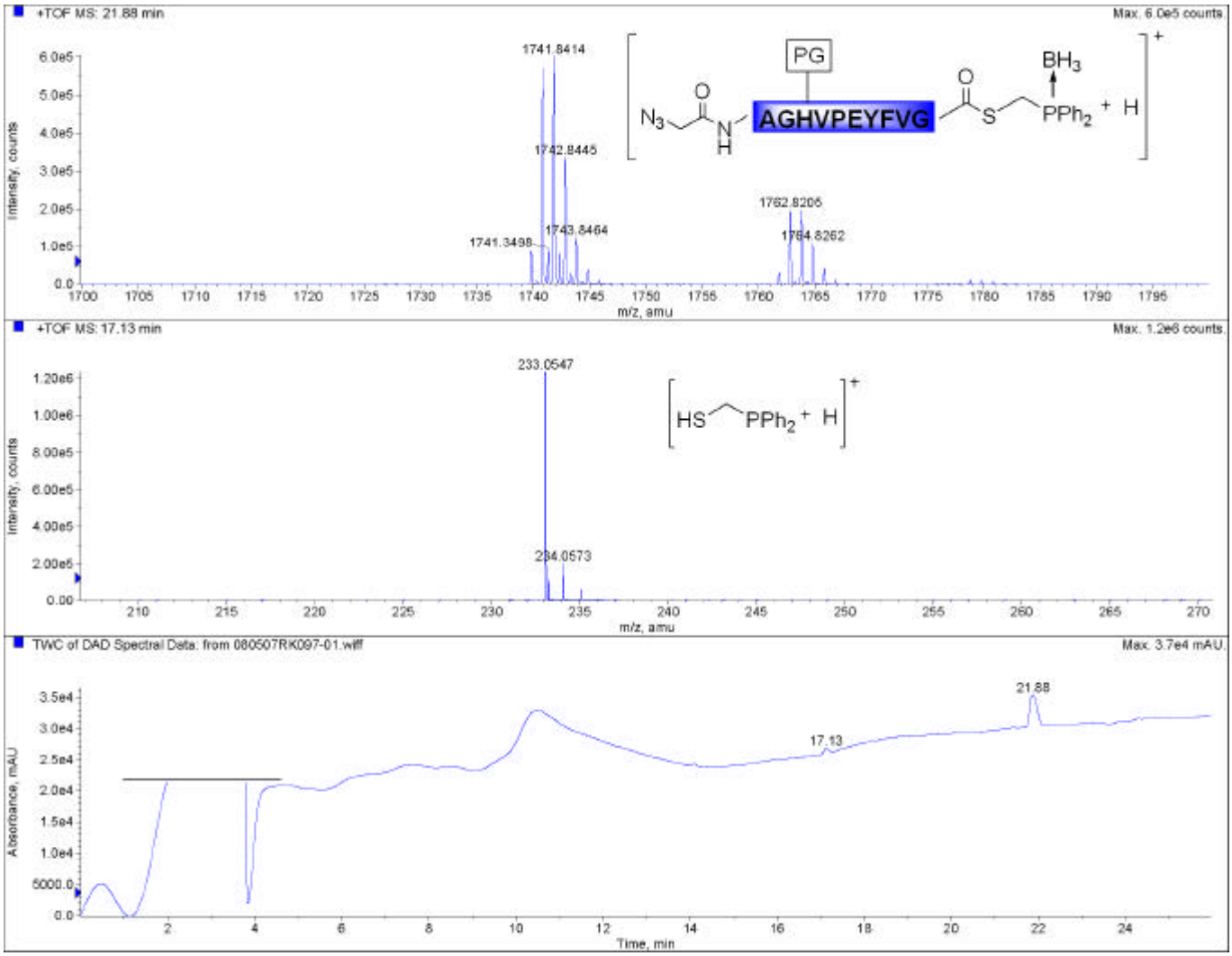
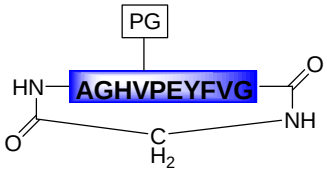


Figure S6. HPLC (bottom) and HRMS (top and middle) analysis of the azido-peptide-phosphinothioester **2** (for HPLC conditions see text).

b) Base induced cyclization of **2** to yield the protected cyclic peptide **3**.



3

After removal of the solvent (CH_2Cl_2), dry DMF (5 mL/0.033 mmol) and 3 eq. DABCO were added. The reaction mixture was stirred at 40 °C for 4 h and for additional 36 h at 25 °C. The crude reaction mixture was analyzed via LC/MS (C_{18}-column, constant flow: 3 min at 3 % CH_3CN (with 1 % AcOH), gradient: 3 % to 100 % CH_3CN (with 1 % AcOH) over 19 min), in which the cyclized peptide **3** eluted at 20.28 min (see Figure S7).

HRMS (ESI-ToF): m/z = 1490.7580 $[M+Na]^+$ (calcd.: m/z = 1490.7483)

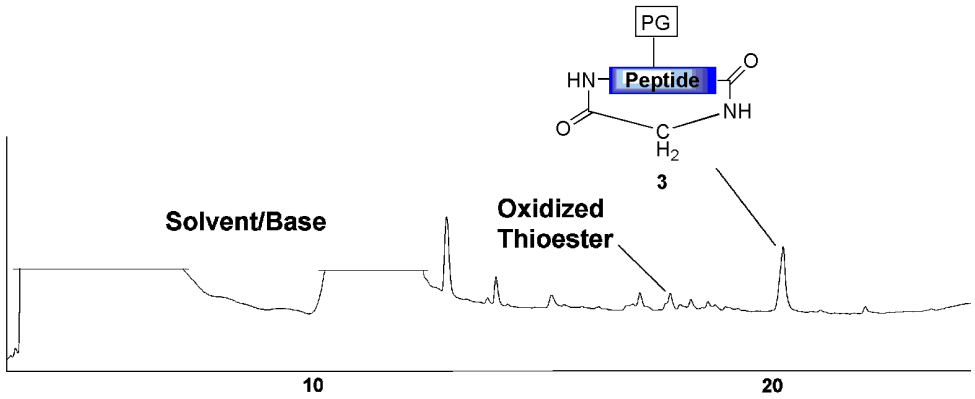


Figure S7. HPLC analysis for the base induced cyclization of **2** to yield the protected cyclic peptide **3** (for HPLC conditions see text. It is important to note that the reactions was performed with peptide **2** directly obtained from synthetic transformations without intermediate HPLC purification).

TFA-deprotection of the azido-peptide-phosphinothioester 2 and subsequent Staudinger cyclization.

a) TFA-deprotection of the azido-peptide-phosphinothioester **2**.

10

The azido-peptide-phosphinothioester **2** was globally deprotected with a cleavage cocktail of 97.5 % TFA and 2.5 % TIS and precipitated from dry ethyl ether as described in the experimental part. The deprotected phosphinothioester **10** was analyzed via LC/MS analysis (C_{18}-column, constant flow: 3 min at 3 % CH_3CN (with 1 % AcOH), gradient: 3 % to 100 % CH_3CN (with 1 % AcOH) over 19 min), in which **10** eluted at 16.35 min (see Figure 1B and Figure S8).

HRMS (ESI-TOF): m/z = 1372.5649 $[M+H]^+$ (calcd.: m/z = 1372.5697).

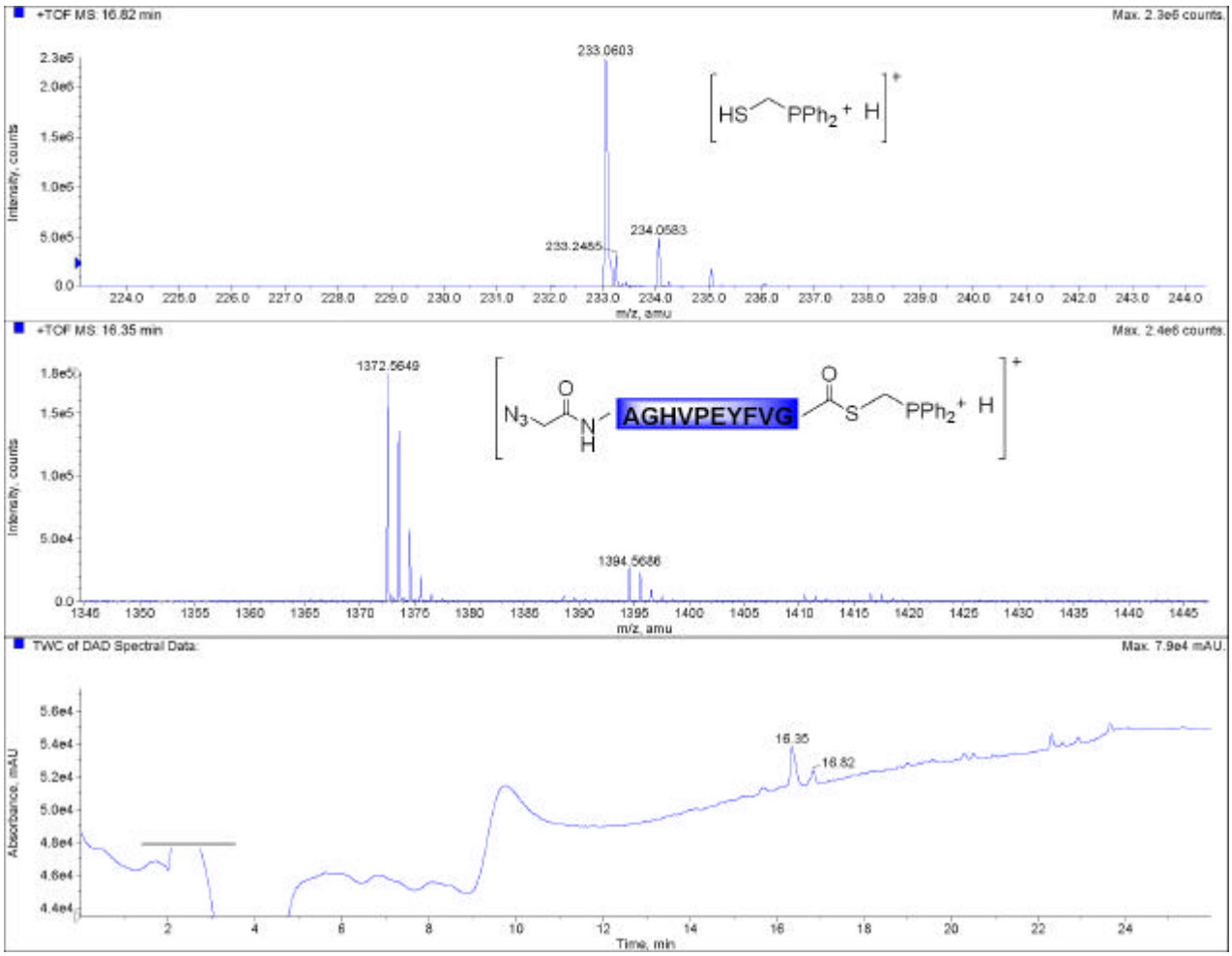
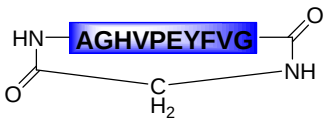


Figure S8. HPLC (bottom) and HRMS (top and middle) analysis of the deprotected azido-peptide-phosphinothioester **10** (for HPLC conditions see text).

b) Staudinger cyclization of the deprotected azido-peptide-phosphinothioester **10** to cyclic peptide **1a**.



1a

The azido-peptide-phosphinothioester **10** was cyclized as described in the experimental part. The conversion to the cyclic peptide **1a** was analyzed via HPLC analysis (C_{18}-column, constant flow: 5 min at 7 % CH_3CN (with 0.1 % TFA), gradient: 7 % to 95 % CH_3CN (with 0.1 % TFA) over 30 min), in which **1a** eluted at 19.78 min (Figure S9). After preparative purification of 50 % of the crude peptide under analogous HPLC conditions, 1.2 mg (1.05 µmol) of the cyclic peptide **1a** were isolated, which corresponds to 36 % overall yield starting from the Fmoc-Gly-OH loaded TGT®-resin (0.006 mmol scale).[6] The purified peptide **1a** was analyzed by HPLC and HRMS (Figure 1C). Further analysis demonstrated the oxidized phosphinothioester peptide with an intact azide at the *N*-terminus (**11a**) as a minor peptidic side product.[5]

HRMS (ESI-TOF): m/z = 1114.5316 $[M+H]^+$ (calcd.: m/z = 1114.5322)

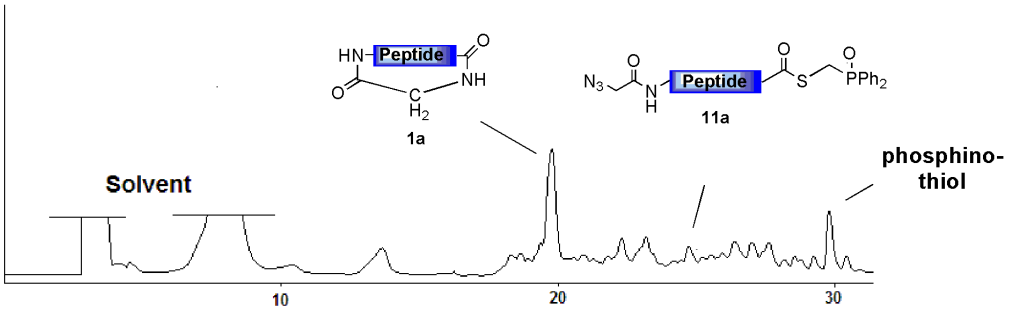


Figure S9. Crude HPLC analysis for the cyclization of **10** to yield the protected cyclic peptide **1a** (for HPLC conditions see text. It is important to note that the reaction was performed with peptide **10** without intermediate HPLC purification).

c) Synthesis of cyclic peptides **1b** and **1c**

Peptides **1b** (cyclo-GGIVPQFYSAG) and **1c** (cyclo-GIGTPISFYGG) were synthesized in analogy to peptide **1a** starting from the Fmoc-Gly-OH loaded TGT®-resin (0.012 mmol scale). The corresponding azido-peptide-phosphinothioesters were cyclized as described in the experimental part. After preparative HPLC purification of 50 % of the crude reaction mixture, (C_{18}-column, constant flow: 5 min at 7 % CH_3CN (with 0.1 % TFA), gradient: 7 % to 95 % CH_3CN (with 0.1 % TFA) over 30 min) 1.9 mg (1.76 µmol, 31% yield, retention time 20.3 min) of the cyclic peptide **1b** and 1.2 mg (1.14 µmol, 20% yield, retention time 19.0 min) of the cyclic peptide **1c** were isolated and analyzed (Figure S10).

HRMS of **1b** (ESI-TOF): m/z = 1050.5244 $[M+H]^+$ (calcd.: m/z = 1050.5365)

HRMS of **1c** (ESI-TOF): m/z = 1077.5364 $[M+H]^+$ (calcd.: m/z = 1077.5474)

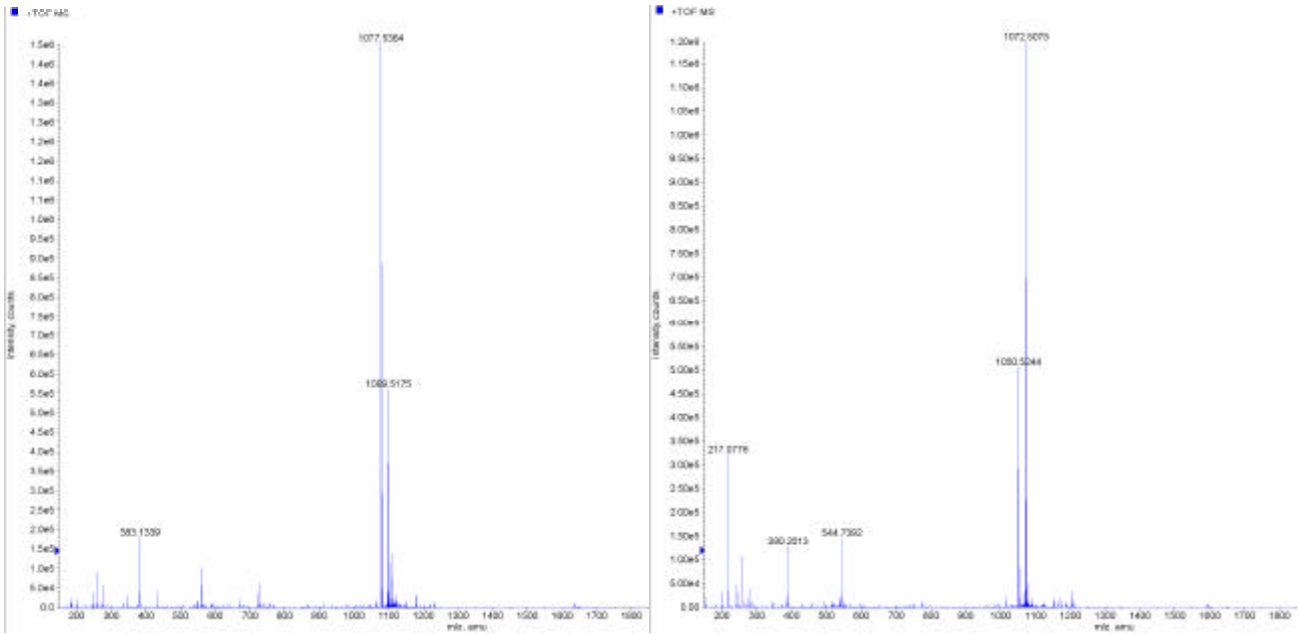


Figure S10. HRMS analysis for the cyclic peptides **1b** and **1c**.

References:

[1] M.B. Soellner, B.L. Nilsson, R.T. Raines, *J. Org. Chem.* **2002**, *67*, 4993-4996.

[2] S.G. Alvarez, M.T. Alvarez, *Synthesis* **1997**, 413-414.

[3] **IMPORTANT REMARK**: Although the azides reported here did not show any instability, we strongly recommend caution and the use of appropriate protection during the handling of azides, especially with compounds of low molecular weight and during heating and/or concentrating steps. See also: S. Bräse, C. Gil, K. Knepper, V. Zimmermann, *Angew. Chem.* **2005**, *117*, 5320-5374; *Angew. Chem. Int. Ed.* **2005**, *44*, 5188-5240.

[4] a) H. Nöth, B. Wrackmeyer in *NMR Spectroscopy of Boron Compounds*, Springer, Berlin-Heidelberg-New York, **1978**. b) For an overview of ^{11}B-NMR chemical shifts see: http://www.chemistry.sdsu.edu/research/BNMR.

[5] It is important to note that **11a** has a stronger UV signal than cyclic peptide **1** due to the presence of the $O{=}PPh_2$ moiety.

[6] Yield was calculated based on an isolation of 35% of peptide material after cleavage from the TGT®-resin (R. Kleineweischede, C. P. R. Hackenberger, *unpublished results*).