Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
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Spin-Labelled Diketopiperazines and Peptide-Peptoid Chimera by Ugi-Multi-Component-Reactions

Haider N. Sultani[a], Haleh H. Haeri[b], Dariush Hinderberger[b,*], and Bernhard Westermann[a,c*]

a. Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle (Saale), Germany
b. Martin-Luther-Universität Halle-Wittenberg, Institute of Chemistry, Von-Danckelmann-Platz 4, 06120 Halle (Saale), Germany
c. Martin-Luther-Universität Halle-Wittenberg, Institute of Chemistry, Kurt-Mothes-Str. 2, 06120 Halle, Germany
bwesterm@ipb-halle.de

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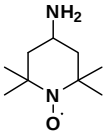
General remarks

All commercially available reagents were used without further purification. Dichloromethane has been dried before use following conventional procedures. Convertible isocyanides 2-isocyano-2-methylpropyl phenyl carbonate **7a**, IPB **7b**, and PEG isocyanide were prepared following reported procedures[1-3]. HPLC grade methanol was used in all Ugi reactions. Analytical thin layer chromatography (TLC) was performed using silica gel 60 F254 aluminum sheets (Merck, Germany) and the visualization of the spots has been done under UV light (254 nm) or by developing with a solution of ninhydrin (0.2% in *n*-butanol with 1% acetic acid and heating). Flash column chromatography was performed using silica gel (0.040 – 0.063 mm). ^{1}H and ^{13}C NMR spectra were recorded in solutions on a 400 MHz Varian MERCURY-VX 400 at 22°C at 400 MHz and 100MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H-NMR) and to the solvent signal (^{13}C NMR spectra). Note: due to the paramagnetism of nitroxide moiety, NMR cannot provide information useful for structural elucidation of nitroxides. The positive-ion high-resolution ESI mass spectra were obtained with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany) equipped with an HESI electrospray ion source (positive spray voltage 4 kV, capillary temperature 275 °C, source heater temperature 80 °C, FTMS resolution 60000). Nitrogen was used as sheath gas. The instrument was externally calibrated using the Pierce LTQ Velos ESI positive ion calibration solution (product number 88323, Thermofisher Scientific, Rockford, IL, 61105 USA). The data were evaluated using the software Xcalibur 2.7 SP1.

Instrumental details-EPR: X-Band (9.43 GHz) room temperature CW EPR measurements were performed on a Magnettech MiniScope MS400 benchtop spectrometer (Magnettech, Berlin, Germany). Spectra were recorded with a microwave power under the saturation limit (varied between -1-3mW), 100 KHz modulation frequency, modulation amplitude of 0.1mT and 4096 points. The lowest sample concentrations was 300μM. Contribution of solvent to spectra was examined using water and acetonitrile. Since it is difficult to evaluate fully resolved hyperfine- and g-tensors at x-band frequencies, only the isotropic values are reported.

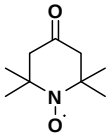
Q-band (33.9 GHz) room temperature CW EPR measurements were conducted on a Bruker EMX-plus spectrometer, using an ER5106QT resonator. A microwave power of 1mW, 100 KHz modulation frequency, modulation amplitude of 0.1mT and 2000 points were used during measurements.

4-Amino-2,2,6,6-tetramethylpiperidine-N-oxyl (2) [4]



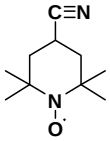
4-Acetamido-TEMPO (2.0 g, 9.40 mmol, available from TCI, Germany) was heated to reflux with KOH (6.32 g, 0.11 mol) in MeOH (5 mL) and water (18 mL) for 4 d. Subsequently, the product was extracted with Et_2O and the combined organic phases were dried over $MgSO_4$ and filtered. Removal of the solvents under reduced pressure afforded amine **2** (1.40 g, 8.17 mmol, 88 %) as a red oil that forms crystals when stored at cold temperatures 2-5°C. HRMS (ESI): m/z = calcd. for $C_9H_{19}N_2OH^+$ $[M+H]^+$172.1570; found 172.1566.

Synthesis of 2,2,6,6-Tetramethylpiperidin-N-oxyl-4-one [5]



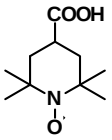
2,2,6,6-Tetramethyl-4-piperidone (2.0 g, 12.9 mmol) was dissolved in a mixture methanol/H_2O (3:2, 40 mL). $Na_2WO_4{\times}2H_2O$ (700 mg, 2.18 mmol) and H_2O_2 (8.80 mL, 77.4 mmol) were added and the reaction mixture was stirred for 5 d at rt. A catalytic amount of $Na_2WO_4{\times}2H_2O$ was added daily. The reaction completion was verified by TLC, upon which the reaction mixture was saturated with K_2CO_3 and extracted with Et_2O (3×20 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (ethyl acetate/hexane 3:2), and the product was obtained (1.90 g, 11.2 mmol, 86%) as a red solid**.** The compound was used immediately for the next step.

4-Cyano-2,2,6,6-tetramethyl-piperidin-N-oxyl [6]



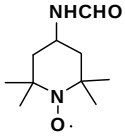
2,2,6,6-Tetramethylpiperidin-N-oxyl-4-one (1.0 g, 5.88 mmol) was dissolved in DME (50 mL) and tosyl methylisocyanide (1.20 g, 6.18 mmol, 1.05 eq) was added at 0 °C, after which a solution of t-BuOK (1.32 g,11.8 mmol) in DME/*t*-butanol (1:1, 20 mL) was added to the reaction mixture. The reaction mixture was stirred for 45 min at 0 °C and for further 1h at rt. The reaction was stopped by the addition of H_2O (70 mL) and the product was extracted with Et_2O (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The product was obtained (1.0 g, 5.51 mmol, 94%) as a red solid and was used without further purification.

4-Carboxy-2,2,6,6-tetramethyl-piperidin-N-oxyl (6) [6]



4-Cyano-2,2,6,6-tetramethyl-piperidin-N-oxyl (1.00 g, 5.51 mmol) was dissolved in methanol (15 mL) and a mixture of $Ba(OH)_2 \times 8H_2O$ (6.50 g, 20.8 mmol) and NaOH (0.348 g, 8.70 mmol) in H_2O (50 mL) was added. The reaction mixture was refluxed for 24 h. After cooling to rt, the mixture was extracted with $CHCl_3$ (3 × 60 mL). The aqueous layer was acidified with HCl (aq. 10%) to pH 2 and extracted with $CHCl_3$ (3 × 60 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The product **6** was isolated (0.95 g, 4.74 mmol, 87%) as a red solid and was used without further purification. HRMS (ESI) m/z calcd for $C_{10}H_{19}NO_3$ $[M + Na]^+$201.1359, found 201.1347.

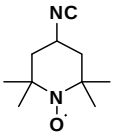
4-Formamido-2,2,6,6-tetramethylpiperidine-N-oxyl [7]



N-oxyl **2** (5.0 g, 25.1 mmol) was refluxed with excess of ethyl formiate (70mL) overnight. The formation of the product was monitored by TLC (ethyl acetate/hexane 9:1), after which removal of the solvents under reduced pressure yielded (4.0 g, 20.1 mmol, 80 %) as red oil and used without further purification.

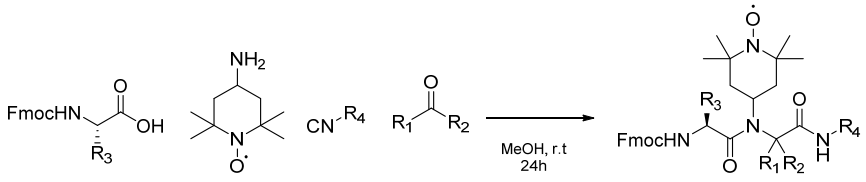
4-Isocyano-2,2,6,6-tetramethylpiperdine-N-oxyl (5) [7]

*The formation of isonitrile **5** is originally described in [7] using phosgene as the dehydrating agent. The protocol has be changed to the Appel procedure providing higher yields under mild reaction conditions.*



4-Formamido-2,2,6,6-tetramethylpiperidine-N-oxyl (1.99 g, 10.0 mmol), carbon tetrachloride (1.0 mL, 10.0 mmol), TEA (1.44 mL, 10.0 mmol) and triphenyl-phosphane (2.60 g, 10.0 mmol) are dissolved in chloroform (12 mL) and heated to 60° for 4 h. The reaction was allowed to cool to rt, then washed with sat. $NaHCO_3$-solution, the aqueous layer was extracted using chloroform and subsequently dried over $MgSO_4$ and filtered. Removal of the solvents under reduced pressure afforded crude product which was purified by column chromatography (ethyl acetate/hexane 3:7) to obtain isonitrile **5** (0.80 g, 4.41 mmol, 44%) as red solid. HRMS (ESI): m/z = calcd. for $C_{10}H_{18}N_2OH^+$ $[M+2H]^+$183.1492, found 183.1488.

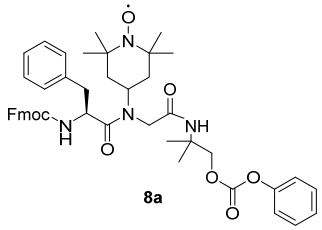
General procedure for the Ugi-4CR



Scheme S1 **8a-8m**

To a stirred solution of a N-oxyl amine **2** (1.0 mmol) in MeOH (2.5 mL) was added paraformaldehyde (1.0 mmol) and the mixture was stirred for 2 h. After this time the Fmoc-amino acid (1.0 mmol) and isonitrile (1.0 mmol) were added, before stirring was continued for 18 h. The solvent was removed under reduced pressure and the crude material purified by column chromatography to afford the desired products.

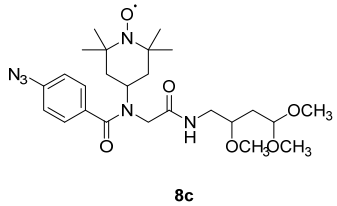
(9H-Fluoren-9-yl)methyl (S)-(1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-((2-methyl-1-((phenoxycarbonyl)oxy)propan-2-yl)amino)-2-oxoethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (8a)



8a

Obtained using the general method, 2-isocyano-2-methylpropyl phenyl carbonate (**7a**), paraformaldehyde, FMOC-*L*-phenyl alanine and N-oxyl amine **2** were used, the crude reaction product was purified by silica gel column chromatography (ethyl acetate/hexane 1:1) to yield peptoid **8a** (0.50 g, 0.63 mmol, 55%) as red solid. R_F 0.23 (ethyl acetate/hexane 1:1). HRMS (ESI) *m/z* calcd for $C_{46}H_{53}N_4O_8$ $[M + H]^+$ 790.3936, found 790.3927.

4-Azido-N-(1-oxyl-2,2,6,6-tetramethylpiperidinyl)-N-(2-oxo-2-((2,4,4-trimethoxybutyl) amino) ethyl)benzamide (8c)

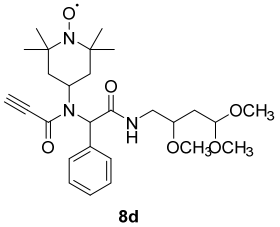


8c

Obtained using the general method, IPB isonitrile **7b**, N-oxyl amine **2**, *p*-azido benzoic acid and paraformaldehyde were used. The crude material purified by silica gel column chromatography (ethyl acetate /hexane 7:3) to give peptoid **8c** (0.35 g, 0.67mmol, 66%) as red

solid. R_F 0.5 (ethyl acetate), HRMS (ESI) *m/z* calcd. for $C_{25}H_{39}N_6O_6Na$ $[M + Na]^+$542.2823, found 542.2809.

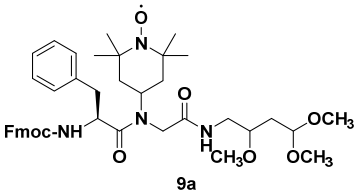
N-(2,2,6,6-Tetramethylpiperidin-4-yl)oxyl-N-(2-oxo-1-phenyl-2-((2,4,4-trimethoxybutyl) amino)ethyl)propiolamide (8d)



8d

Obtained using the general method, IPB isontirile **7b**, propiolic acid, benzaldehyde and N-oxyl amine **2** were used. The crude material was purified by silica gel column chromatography (ethyl acetate) to yield peptoid **8d** (0.28 g, 0.55 mmol, 55%) as red solid. R_F 0.46 (ethyl acetate), HRMS (ESI) *m/z* calcd for $C_{27}H_{40}N_3O_6Na$ $[M + Na]^+$ 525.2809, found 525.2796.

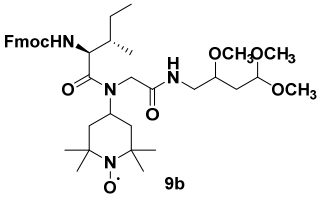
(9H-Fluoren-9-yl)methyl ((2S)-1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-oxo-2-((2,4,4-trimethoxybutyl)amino)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (9a)



9a

Obtained using the general method, IPB isonitrile **7a**, Fmoc-*L*-phenyl alanine, paraformaldehyde and N-oxyl amine **2** were used. The crude material purified by silica gel column chromatography (ethyl acetate) to give compound **9a** (0.34 g, 0.45 mmol, 46%) as red solid R_F 0.37 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{42}H_{56}N_4O_8$ $[M + H]^+$ 744.4093, found 744.4072.

(9H-Fluoren-9-yl)methyl ((2S,3S)-1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-oxo-2-((2,4,4-trimethoxybutyl)amino)ethyl)amino)-3-methyl-1-oxopentan-2-yl)carbamate (9b)

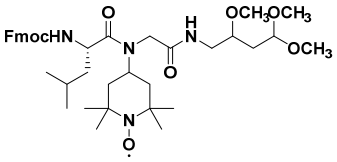


9b

Obtained using the general method, IPB isonitrile **7a**, Fmoc-*L*-isoleucine, paraformaldehyde and N-oxyl amine **2** were used. The crude material was purified by silica gel column chromatography (ethyl acetate) to give peptoid **9b** (0.35 g, 0.49 mmol, 50%) as red

solid. R_F 0.45 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{39}H_{58}N_4O_8$ $[M + H]^+$ 710.4249, found 710.4222.

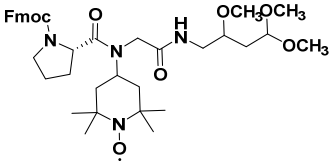
(9H-Fluoren-9-yl)methyl ((2S)-1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-oxo-2-((2,4,4-trimethoxybutyl)amino)ethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (9c).



9c

Obtained using the general method, IPB isonitrile **7a**, Fmoc-*L*-leucine, paraformaldehyde and N-oxyl amine **2** were used. The crude material purified by silica gel column chromatography (ethyl acetate) to give compound **9c** (0.43 g, 0.60 mmol, 61%) as red solid. R_F 0.50 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{39}H_{58}N_4O_8$ $[M + H]^+$ 710.4249, found 710.4221.

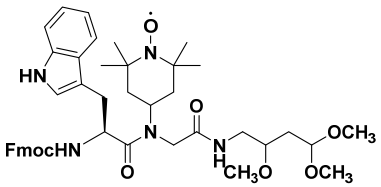
(9H-Fluoren-9-yl)methyl (2S)-2-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-oxo-2-((2,4,4-trimethoxybutyl)amino)ethyl)carbamoyl)pyrrolidine-1-carboxylate (9d)



9d

Obtained using the general method, IPB isonitrile **7a**, Fmoc-*L*-proline, paraformaldehyde and N-oxyl amine **2** were used. The crude reaction product was purified by silica gel column chromatography (ethyl acetate) to give peptoid **9d** (0.43 g, 0.62 mmol, 62%) as red solid. R_F 0.28 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{38}H_{54}N_4O_8$ $[M + H]^+$ 694.3936, found 694.3912.

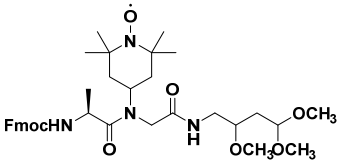
(9H-Fluoren-9-yl)methyl ((2S)-1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-oxo-2-((2,4,4-trimethoxybutyl)amino)ethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamate (9e)



9e

Obtained using the general method, IPB isonitrile **7a**, Fmoc-*L*-tryptophane, paraformaldehyde and N-oxyl amine **2** were used. The crude reaction product was purified by silica gel column chromatography (ethyl acetate) to yield peptoid **9e** (0.25 g, 0.32 mmol, 32%) as red solid. R_F 0.35 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{44}H_{57}N_5O_8$ $[M + H]^+$ 783.4202, found 783.4194.

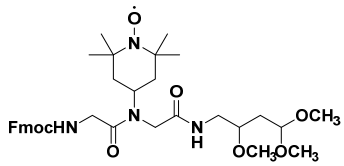
(9H-Fluoren-9-yl)methyl ((2S)-1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-oxo-2-((2,4,4-trimethoxybutyl)amino)ethyl)amino)-1-oxopropan-2-yl)carbamate (9f)



9f

Obtained using the general method, IPB isonitrile **7a**, Fmoc-*L*-alanine, paraformaldehyde and N-oxyl amine **2** were used. The crude reaction product was purified by silica gel column chromatography (ethyl acetate) to yield peptoid **9f** (0.37 g, 0.55 mmol, 55%) as red solid. R_F 0.32 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{36}H_{52}N_4O_8$ $[M + H]^+$668.3780, found 668.3760.

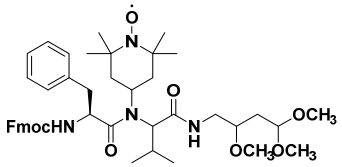
(9H-Fluoren-9-yl)methyl (2-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-oxo-2-((2,4,4-trimethoxybutyl)amino)ethyl)amino)-2-oxoethyl)carbamate (9g)



9g

Obtained using the general method, IPB isonitrile **7a**, Fmoc-glycine, paraformaldehyde and N-oxyl amine **2** were used. The crude reaction product was purified by silica gel column chromatography (ethyl acetate) to yield peptoid **9g** (0.39 g, 0.59 mmol, 60%) as red solid. R_F 0.45 (ethyl acetate). HRMS (ESI+) *m/z* calcd for $C_{35}H_{50}N_4O_8$ $[M + H]^+$ 654.3623, found 654.3593.

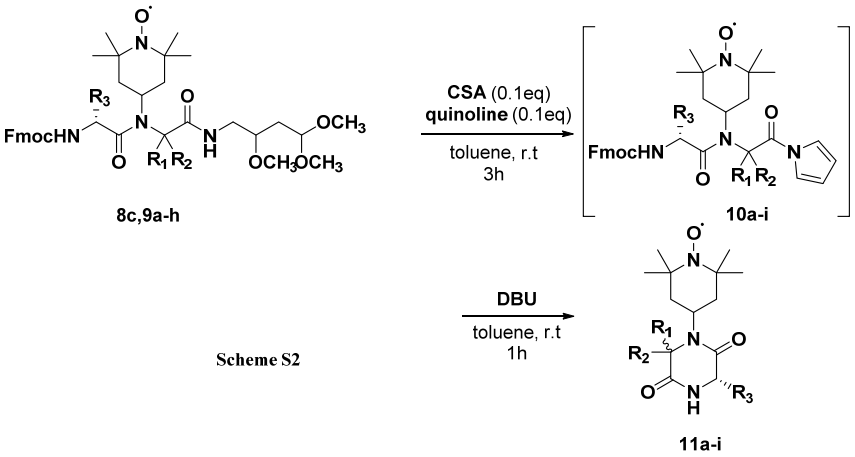
(9H-Fluoren-9-yl)methyl ((2S)-1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(3-methyl-1-oxo-1-((2,4,4-trimethoxybutyl)amino)butan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl) carbamate (9h)



9h

Obtained using the general method, IPB isonitrile **7a**, Fmoc-*L*-phenyl alanine, isobutyric aldehyde and N-oxyl amine **2** were used. The crude reaction product was purified by silica gel column chromatography (ethyl acetate) to yield peptoid **9h** (0.39 g, 0.49 mmol, 59%) as red soild. R_F 0.48 (ethyl acetate). HRMS (ESI+) *m/z* calcd for $C_{45}H_{62}N_4O_8$ $[M + H]^+$ 786.4562, found 786.4546.

General procedure for the conversion of peptoids 7 d-l to spinllabelled diketopiperazines 9a-i via *N*-acylpyrroles (Scheme S2).

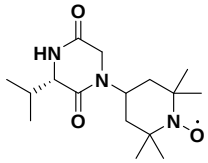


Scheme S2

To a solution of linear Ugi products **8c, 9a-h** (0.5 mmol) in toluene (10 mL) was added 10-camphorsulfonic acid (10 mol%) and quinoline (10 mol%). The mixture was stirred for 1 min at rt and then refluxed for at least 30 min, until TLC showed complete conversion. The mixture was cooled to rt, transferred to a separatory funnel and washed with 1M aqueous HCl (2 × 30 mL). The acidic aqueous phase was further extracted with ethyl acetate (1 × 20 mL). The organic layers were combined, washed with $NaHCO_3$ and brine (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to obtain the *N*-acyl pyrrole derivative which was used in the next step without further purification.

The *N*-acylpyrroles (0.12 mmol) were dissolved in toluene (2 mL) and DBU (20 mol %) was added. The resulting reaction mixture was stirred at rt until completion of the reaction (determined by TLC). Subsequently the reaction was concentrated under reduced pressure. The residual material was purified by silica gel column chromatography to give the desired product.

(S)-1-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-3-isopropylpiperazine-2,5-dione (11a)

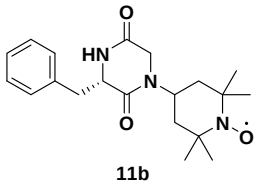


11a

Obtained using the general procedure starting from **8c**, the crude material was purified by silica gel column chromatography (ethyl acetate /methanol 95:5) to give diketopiperazine **11a** (28 mg, 0.09 mmol, 75%) as red solid. R_F 0.54 (ethyl acetate/methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{16}H_{29}N_3O_3$ [M +

H]$^+$ 311.2203, found 311.2195, $[\alpha]_D^{20}$ = –5.2 (*c* 0.2, MeOH). IR (ATR) λ_{max} cm^{-1}: 3246, 2970, 2931, 1639, 1460–1347.

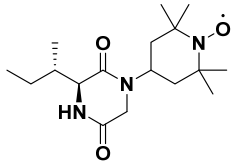
(S)-3-Benzyl-1-(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)piperazine-2,5-dione (11b)



11b

Obtained using the general procedure starting from **9a**, the crude material was purified by silica gel column chromatography (ethyl acetate /methanol 95:5) to give compound **11b** (27 mg, 0.07 mmol, 61%) as red solid. R_F 0.51 (ethyl acetate/ methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{20}H_{29}N_3O_3$ [M + H]$^+$ 359.2203, found 359.2194, $[\alpha]_D^{20}$ = –14.0 (*c* 0.2, MeOH). IR (ATR) λ_{max} cm^{-1}: 3252, 2975, 2930, 1641, 1456–1334.

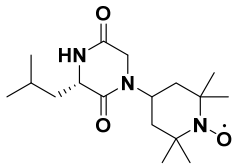
(S)-3-((S)-sec-Butyl)-1-(1oxyl-2,2,6,6-tetramethylpiperidin-4-yl)piperazine-2,5-dione (11c)



11c

Obtained using the general procedure starting from **9b**, the crude material was purified by silica gel column chromatography (ethyl acetate/ methanol 95:5) to give compound **11c** (27 mg, 0.08 mmol, 70%) as red solid. R_F 0.56 (ethyl acetate/ methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{17}H_{31}N_3O_3$ [M + H]$^+$ 325.2360, found 325.2354. IR (ATR) λ_{max} cm^{-1}: 3248, 2970, 2931, 1638, 1455-1376.

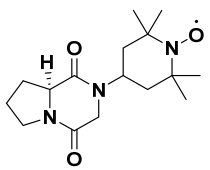
(S)-1-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-3-isobutylpiperazine-2,5-dione (11d)



11d

Obtained using the general procedure starting from **9c**, the crude material was purified by silica gel column chromatography (ethyl acetate/ methanol 95:5) to give compound **11d** (25 mg, 0.07 mmol, 65%) as red solid. R_F 0.61 (ethyl acetate/ methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{17}H_{31}N_3O_3$ [M + H]$^+$ 325.2360, found 325.2339, $[\alpha]_D^{20}$ = –8.0 (*c* 0.2, MeOH). IR (ATR) λ_{max} cm^{-1}: 3248, 2970, 2931, 1645, 1457–1345.

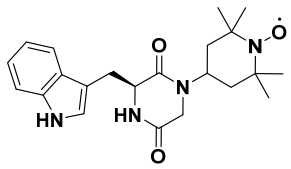
(S)-2-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (11e)



11e

Obtained using the general procedure starting from **9d**, the crude material was purified by silica gel column chromatography (ethyl acetate/ methanol 95:5) to give compound **11e** (20 mg, 0.06 mmol, 53%) as red solid. R_F 0.31 (ethyl acetate/ methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{16}H_{27}N_3O_3$ $[M + H]^+$ 309.2047, found 309.2041, $[\alpha]_D^{20}$ = –71.9 (*c* 0.2, MeOH). **IR** (ATR) λ_{max} cm^{-1}: 2974, 2934, 1651, 1447–1364.

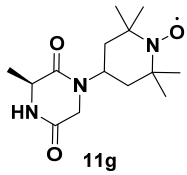
(S)-3-((1*H*-Indol-3-yl)methyl)-1-(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)piperazine-2,5-dione (11f)



11f

Obtained using the general procedure starting from **9e**, the crude material was purified by silica gel column chromatography (ethyl acetate/ methanol 95:5) to give compound **11f** (24 mg, 0.06 mmol, 51%) as red solid. R_F 0.45 (ethyl acetate/ methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{22}H_{30}N_4O_3$ $[M + H]^+$ 398.2312, found 398.2301, $[\alpha]_D^{20}$ =121.6 (*c* 0.2, MeOH). IR (ATR) λ_{max} cm^{-1}: 3403, 3242, 2974, 2920, 1648, 1458–1342.

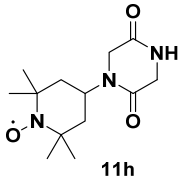
(S)-1-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-3-methylpiperazine-2,5-dione (11g)



11g

Obtained using the general procedure starting from **9f**, the crude material was purified by silica gel column chromatography (ethyl acetate/ methanol 95:5) to give compound **11g** (23 mg, 0.08 mmol, 66%) as red solid. R*F* 0.31 (ethyl acetate/ methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{14}H_{25}N_3O_3$ $[M + H]^+$ 283.1890, found 283.1883 , $[\alpha]_D^{20}$ =–11.8 (*c* 0.2, MeOH). IR (ATR) λ_{max} cm^{-1}: 3246, 2974, 2931, 1650, 1465–1374.

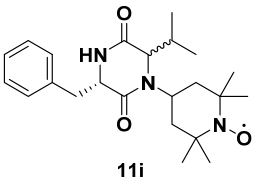
1-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)piperazine-2,5-dione (11h)



11h

Obtained using the general procedure starting from **9g**, the crude material was purified by silica gel column chromatography (ethyl acetate/ methanol 95:5) to give compound **11h** (16 mg, 0.06 mmol, 67%) as red solid. R_F 0.22 (ethyl acetate/ methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{13}H_{23}N_3O_3$ $[M + H]^+$ 269.1734 found 269.1725. IR (ATR) λ_{max} cm^{-1}: 3292, 2997, 2937, 1648, 1460–1352.

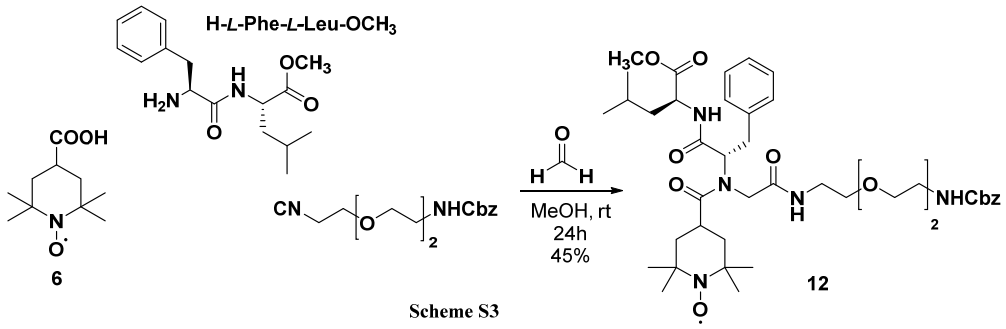
(3S)-3-Benzyl-1-(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-6-isopropylpiperazine-2,5-dione (11i)



11i

Obtained using the general procedure starting from **9h**, the crude material was purified by silica gel column chromatography (ethyl acetate /methanol 95:5) to give compound **11i** (29 mg, 0.07 mmol, 60%) as red solid. R_F 0.22 (ethyl acetate/methanol 95:5). HRMS (ESI+) *m/z* calcd for $C_{23}H_{35}N_3O_3$ [M + H]$^+$ 401.2673, found 401.2662**,** $[\alpha]_D^{20}$=–58.4 (*c* 0.2, MeOH). IR (ATR) λ_{max} cm^{-1}: 3241, 2972, 2932, 1639, 1466-1386 .

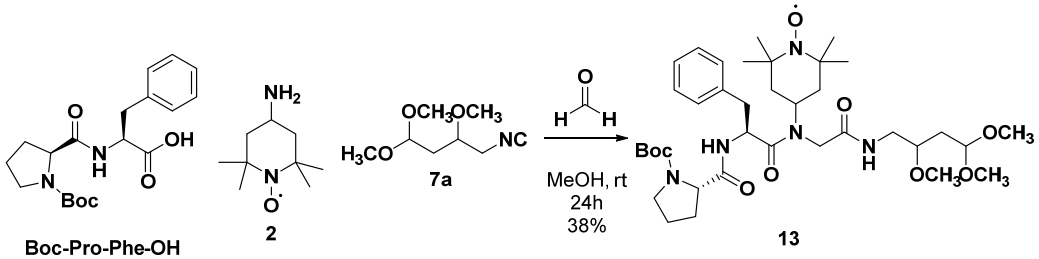
Methyl ((S)-14-benzyl-13-(1-oxyl-2,2,6,6-tetramethylpiperidine-4-carbonyl)-3,11-dioxo-1-phenyl-2,7-dioxa-4,10,13-triazapentadecan-15-oyl)-L-leucinate (12)



Scheme S3

To a stirred solution of dipeptide H-L-Phe-L-Leu-OCH_3 (290 mg, 1.0 mmol) in MeOH (2.5 mL) was added paraformaldehyde (30 mg, 1.0 mmol) and the mixture was stirred at rt for 4 h. After this time TEMPO-derived carboxylic acid **6** (200 mg, 1.00 mmol) and PEG-isocyanide (290 mg, 1.0 mmol)[3] were added, before stirring was continued for 18 h. The solvent was removed under reduced pressure and the crude material was purified by silica gel column chromatography (ethyl acetate) to afford **12** (365 mg, 0.45 mmol, 45%) as red solid. R_F 0.45 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{42}H_{63}N_5O_{10}$ $[M + H]^+$ 797.4569, found 797.4575. $[\alpha]_D^{20} = -72.7$ (*c* 0.2, MeOH).

tert-Butyl (2S)-2-(((2S)-1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)(2-oxo-2-((2,4,4-trimethoxybutyl)amino)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (13)

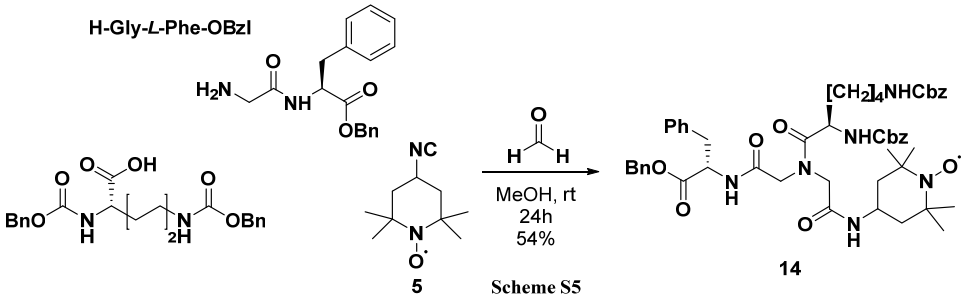


Scheme S4

To a stirred solution of TEMPO-derived amine **2** (170 mg, 1.0 mmol) in MeOH (2.5 mL) was added paraformaldehyde (30 mg, 1.0 mmol) and the mixture was stirred for 4 h at rt. After this time Boc-L-Pro-L-Phe-OH (360 mg, 1.0 mmol) and IPB isocyanide **7a** (170 mg, 1.0 mmol) were added, before stirring was continued for 18 h. The solvent was removed under reduced

pressure and the crude material purified by silica gel column chromatography (ethyl acetate) to afford **13** (270 mg, 0.37 mmol, 38%) as red solid. R_F 0.18 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{37}H_{61}N_5O_9$ $[M + H]^+$ 719.4464, found 719.4429. $[\alpha]_D^{20} = -18.5815$ (*c* 0.2, MeOH)

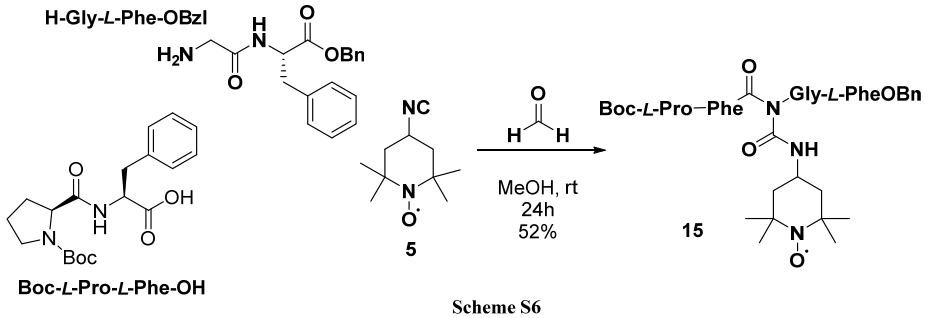
Benzyl N-(N2,N6-bis((benzyloxy)carbonyl)-L-lysyl)-N-(2-((1-oxyl-2,2,6,6-tetramethyl piperidin-4-yl)amino)-2-oxoethyl)glycylphenylalaninate (14)



Scheme S5

To a stirred solution of dipeptide H-Gly-L-Phe-OBn (310 mg, 1.0 mmol) in MeOH (2.5 mL) was added TEA (0.14 mL, 1.0 mmol) and paraformaldehyde (30 mg, 1.0 mmol). The mixture was stirred for 4 h at rt. After this time the amino acid Cbz-Lys(z)-OH (410 mg, 1.0 mmol) and TEMPO-derived isocyanide **5** (180 mg, 1.0 mmol) were added, before stirring was continued for 24 h. The solvent was removed under reduced pressure and the crude material was purified by silica gel column chromatography (ethyl acetate) to afford **14** (494 mg, 0.53 mmol, 54%) as red solid. R_F 0.64 (ethyl acetate). HRMS (ESI) *m/z* calcd for $C_{51}H_{64}N_6O_{10}$ $[M + H]^+$ 920.4678, found 920.4675, $[\alpha]_D^{20} = 7.88$ (*c* 0.2, MeOH)

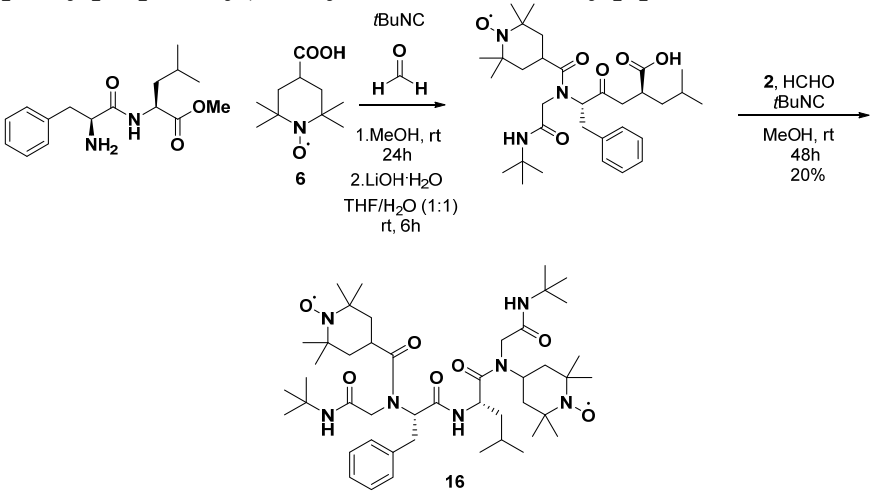
tert-butyl (S)-2-(((S)-1-((2-(((S)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxo-ethyl)(2-((1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-oxoethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (15)



Scheme S6

To a stirred solution of amine H-Gly-L-Phe-OBn (31 mg, 1.0 mmol) in MeOH (2.5 mL) was added TEA (0.14 mL, 1.0 mmol) and paraformaldehyde (30 mg, 1.0 mmol) and the mixture was stirred for 4 h. After this time the amino acid Boc-L-Pro-L-Phe-OH (360 mg, 1.0 mmol) and TEMPO-derived isocyanide **5** (180 mg, 1.0 mmol) were added, before stirring continued for 18 h at rt. The solvent was removed under reduced pressure and the crude material was purified by silica gel column chromatography (ethyl acetate/formic acid 0.3%) to afford **15** (450 mg, 0.53 mmol, 52%) R_F 0.2 (ethyl acetate/formic acid 98:2). HRMS (ESI) *m/z* calcd for $C_{48}H_{64}N_6O_9$ [M + H]$^+$ 868.4729, found 868.4728, $[\alpha]_D^{20} = 24.5$ (*c* 0.2, MeOH).

N-(2-(tert-butylamino)-2-oxoethyl)-N-((S)-1-(((S)-1-((2-(tert-butylamino)-2-oxoethyl)(1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-1-oxyl-2,2,6,6-tetramethylpiperidine-4-carboxamide (16)



Scheme S7

To a stirred solution of dipeptide H-L-Phe-L-Leu-OMe (290 mg, 1.0 mmol) in MeOH (2.5 mL) was added TEA (0.14 mL, 1.0 mmol) and paraformaldehyde (30 mg, 1.0 mmol) and stirred for 4 h at rt. After this time carboxy-derived TEMPO **6** (200 mg, 1.0 mmol) and t-butyl isocyanide (83 mg, 1.0 mmol) were added, before stirring was continued for 24 h. A solution of LiOH.H_2O (126 mg, 3.0 mmol) in THF:H_2O (1:1, 80 mL) was added in one portion at 0 ^oC. After stirring for 6 h, the mixture was transferred to a seperatory funnel. The solution was acidified to pH 3 using a saturated $NaHSO_4$ solution. Brine (20 mL) was added. The contents were extracted with ethyl acetate (3 × 40 mL). The organic layer was dried over Na_2SO_4 and the

solvent was removed under reduced pressure after filtration to afford the first Ugi intermediate which was subjected to the second Ugi reaction using preformed imine from amino-deived TEMPO **2** (170 mg, 1.0 mmol) and paraformaldehyde (30 mg, 1.0 mmol) followed by tert butyl isocyanide (83 mg, 1.0 mmol) and the reaction is stirred for 48h at rt. The solvent was removed under reduced pressure and the crude material was purified by silica gel column chromatography (ethyl acetate/hexane 8:2) to afford **16** (170 mg, 0.20 mmol, 20%) as dark red solid. HRMS (ESI) *m/z* calcd for $C_{46}H_{79}N_7O_7$ $[M + H]^+$ 840.5957, found 840.5948 $[\alpha]_D^{20} = -24.0$ (*c* 0.2, MeOH).

TableS1. Simulated CW EPR spectra details at 9.4 GHz frequency*

Compound Nr. (as in text)	Line width (mT)	Hyperfine tensor A (MHz)	Correlation time (τc /s)
UGI 8c (acetonitrile)	0.08,0.4	21.5,21.8,90.8	3e-11
DKP 11a (acetonitrile)	0.08,0.5	20.5,20.8,90.8	3e-12
DKP 11a (water)	0.2,0.04	28.0,24.0,91.5	9e-11
DKP 11b (acetonitrile)	0.08,0.48	20.5,22.2,90.3	5e-12
Peptide 12 (water)	0.19,0.05	24.0,26.0,92.5	1.87e-10
Peptide 13 (water)	0.19,0.05	24.0,26.0,93.0	2.67e-10
Peptide 14 (Methanol)	0.08,0.4	21.0,24.0,91.8	1.47e-10
Peptide 15 (water)	0.19,0.05	24.0,26.0,92.5	4.6e-11

*For all simulation the g tensor [2.008 2.006 2.0020] with an isotropic value of 2.005 is used. Line width is a combination of two Gaussian and Lorentzian line shapes.

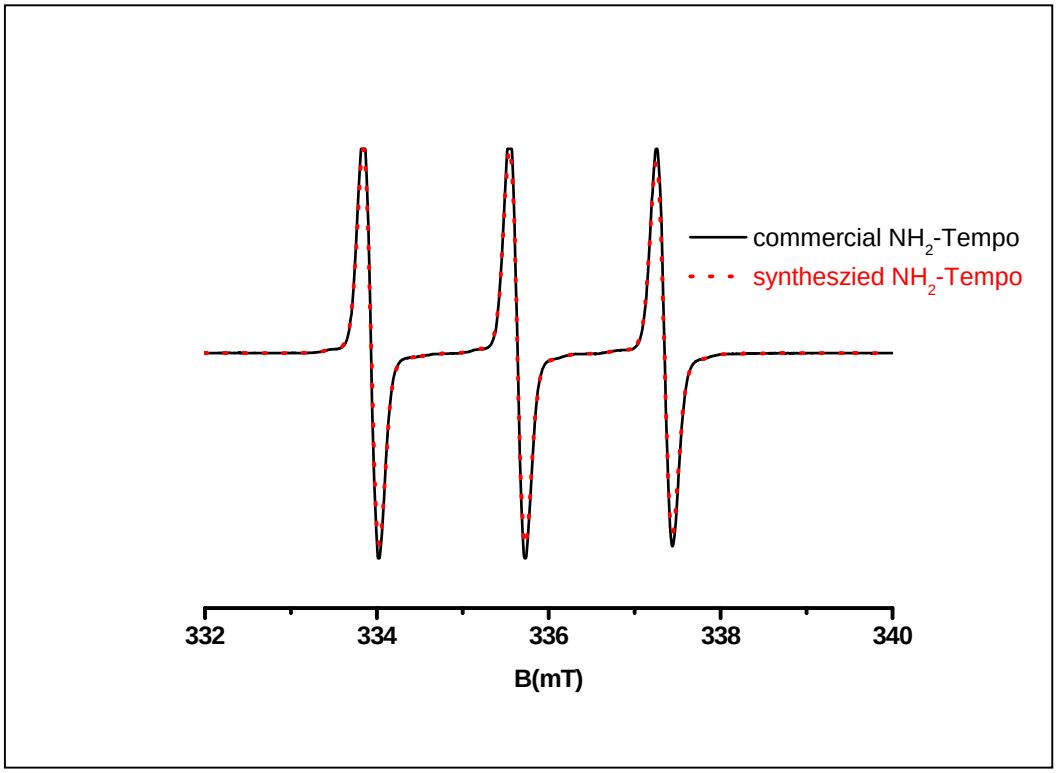


Figure S1- Comparison between synthesized and commercial 4-NH_2 TEMPO **2** in water

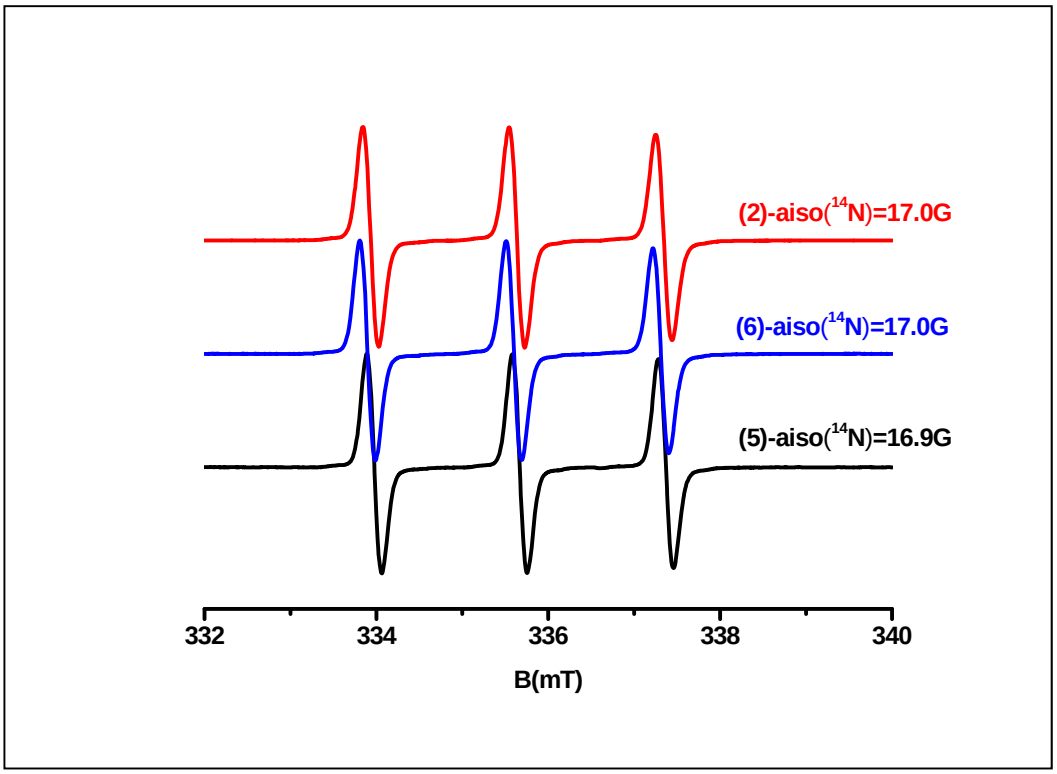


Figure S2- Room temperature CW-EPR spectra of TEMPO derivatives (in water) used in this research; 4-NH_2-TEMPO **2**, CN-TEMPO **5** and carboxy-TEMPO **6**.

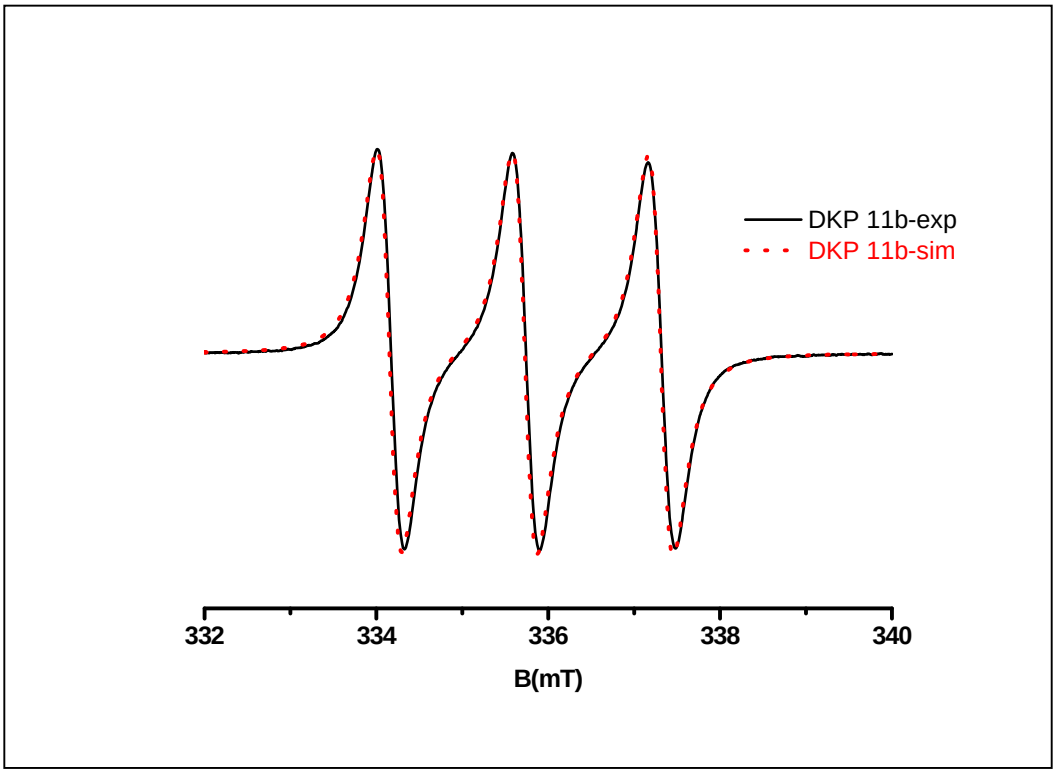


Figure S3- Experimental and simulated CW-EPR spectra of DKP **11b** in acetonitrile solvent.

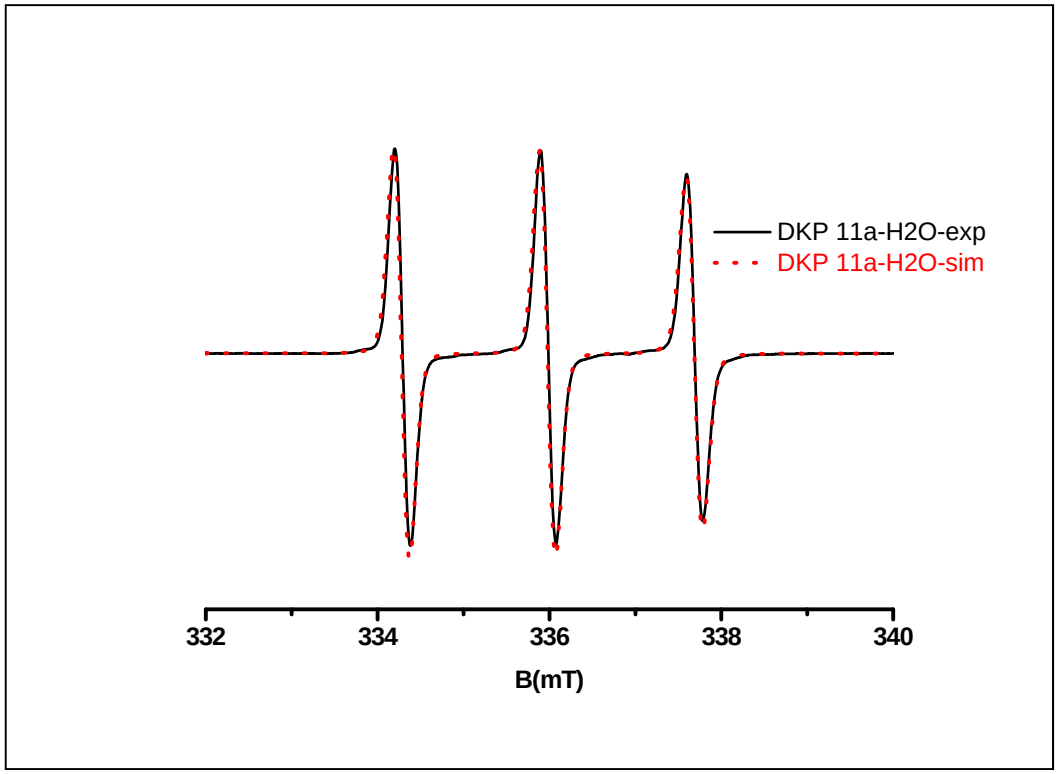


Figure S4- Experimental and simulated CW-EPR spectra of DKP **11a** in water solvent.

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