Electronic Supplementary Information

A Novel Strategy for Site Selective Spin-Labeling to Investigate Bioactive Entities by DNP and EPR Spectroscopy

Kevin Herr[a+], Max Fleckenstein[b+], Martin Brodrecht[a], Mark V. Höfler[a], Henrike Heise[c,d], Fabien Aussenac[e], Torsten Gutmann[a], Michael Reggelin[b*] and Gerd Buntkowsky[a]*

[a] Institute of Physical Chemistry, Technical University Darmstadt, Alarich-Weiss-Straße 8, D-64287 Darmstadt, Germany

[b] Institute of Organic Chemistry, Technical University Darmstadt, Alarich-Weiss-Straße 4, D-64287 Darmstadt, Germany

[c] Institute of Complex Systems, Structural Biochemistry (ICS-6), Forschungszentrum Jülich, D-52425 Jülich, Germany.

[d] Institut für Physikalische
Biologie, Heinrich-Heine-Universität Düsseldorf, D-40225 Düsseldorf, Germany

[e] Bruker France SAS, 34 rue de l'industrie , F-67160 Wissembourg, France

[+] These authors contributed equally to this work.

* eMail: gerd.buntkowsky@chemie.tu-darmstadt.de
re@chemie.tu-darmstadt.de

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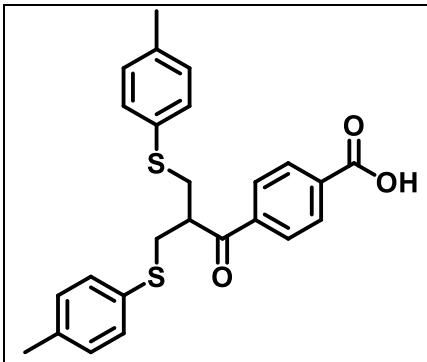
1.1 Bisthioether 7



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1.1.1 ^{1}H liquid NMR spectrum

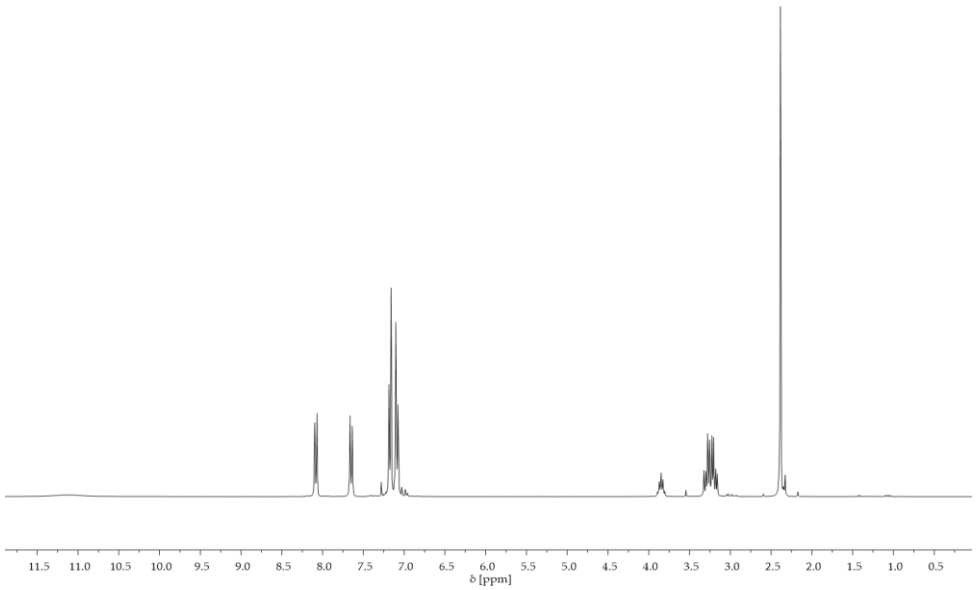


Figure 1: ^{1}H-NMR spectrum of bisthioether **7** in $CDCl_3$ at 301.2 K and 300 MHz.

1.1.2 ^{13}C liquid NMR spectrum

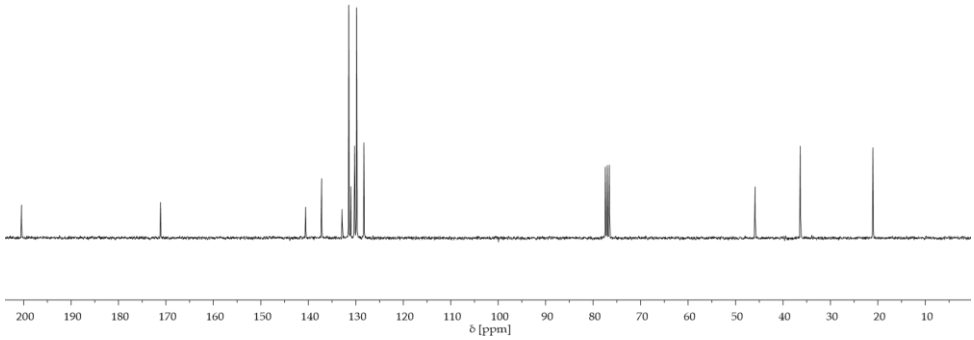
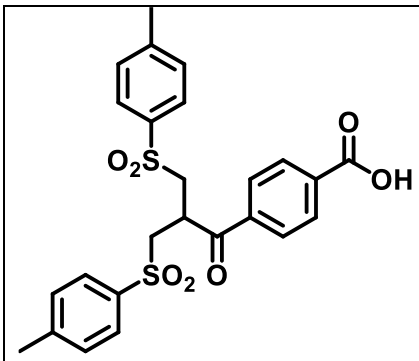


Figure 2: ^{13}C-NMR spectrum of Bisthioether **7** in $CDCl_3$ at 301.2 K and 75 MHz.

1.2 Bis-sulfone 8



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1.2.1 ^{1}H liquid NMR spectrum

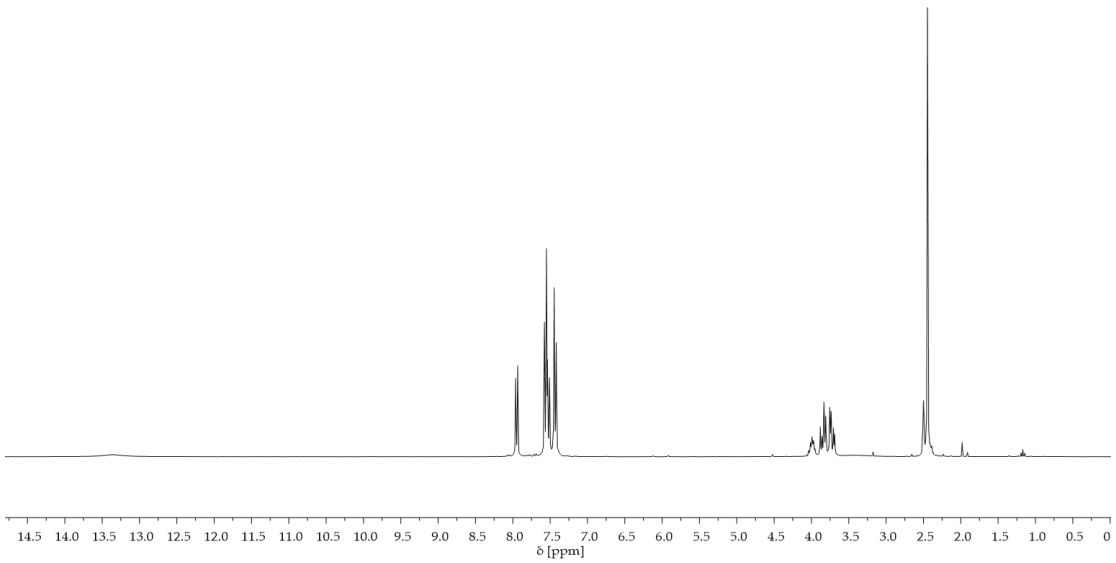


Figure 3: ^{1}H-NMR spectrum of bis-sulfone **8** in DMSO-d_6 at 303 K and 300 MHz.

1.2.2 ^{13}C liquid NMR spectrum

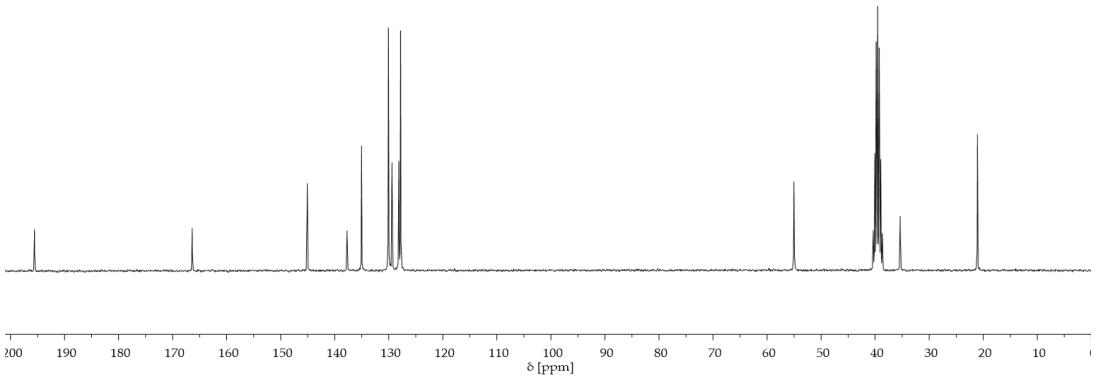
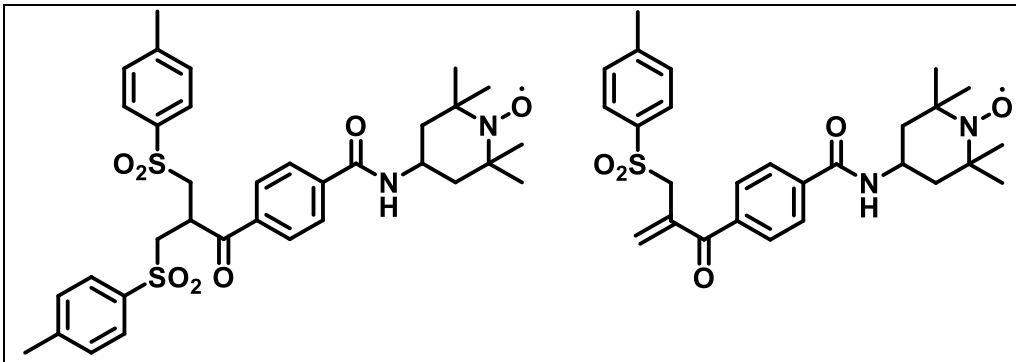


Figure 4: ^{13}C-NMR spectrum of bis-sulfone **8** in DMSO-d_6 at 303 K and 75 MHz.

1.3 Bis-sulfone based spin label 4 and 5



4	5

1.3.1 ^{1}H liquid NMR spectrum

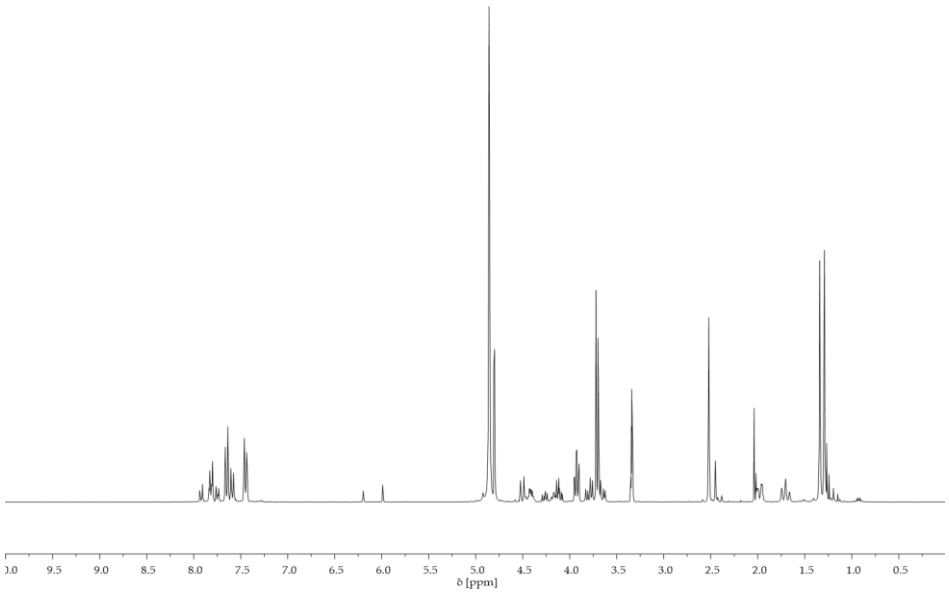


Figure 5: ^{1}H-NMR spectrum of the mixture of bis-sulfone based spin labels **4** and **5** (after addition of ascorbic acid) in MeOH-d_4 at 303 K and 300 MHz.

1.3.2 ^{13}C liquid NMR spectrum

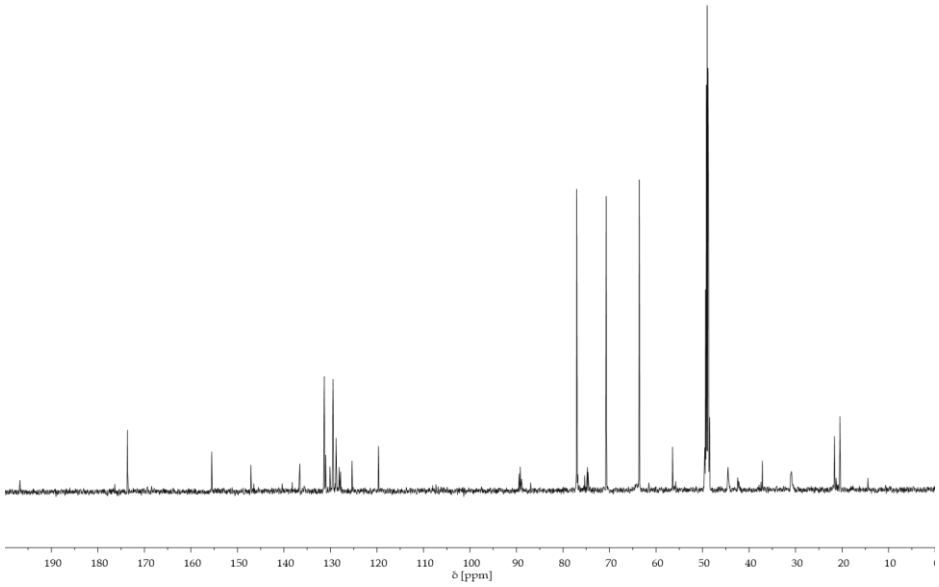


Figure 6: ^{13}C-NMR spectrum of the mixture of bis-sulfone based spin labels **4 and 5** (after addition of ascorbic acid) in MeOH-d_4 at 303 K and 75 MHz.

1.3.3 HPLC chromatogram

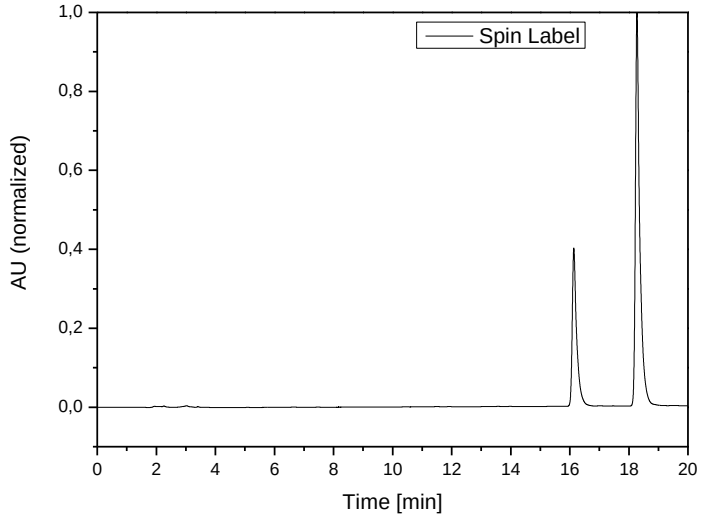


Figure 7: HPLC chromatogram of bis-sulfone based spin labels **4 (**t_R = 18.3 min.) and **5** (t_R = 16.1 min.) at 214 nm, with gradient of acetonitril in water from 20% to 80% with 0.1% TFA for 20 minutes.

1.3.4 EPR spectrum

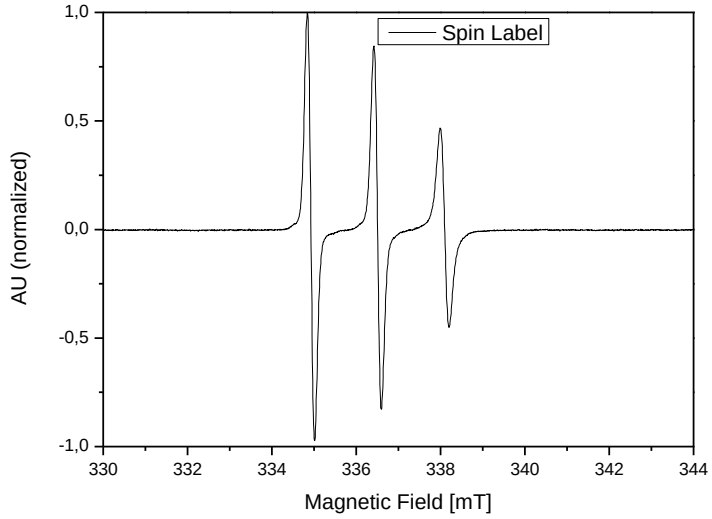


Figure 8: ESR spectrum of ca. 15 mM bis-sulfone based spin label **4** and **5** at 20 °C.

1.3.3 HPLC-MS chromatogram

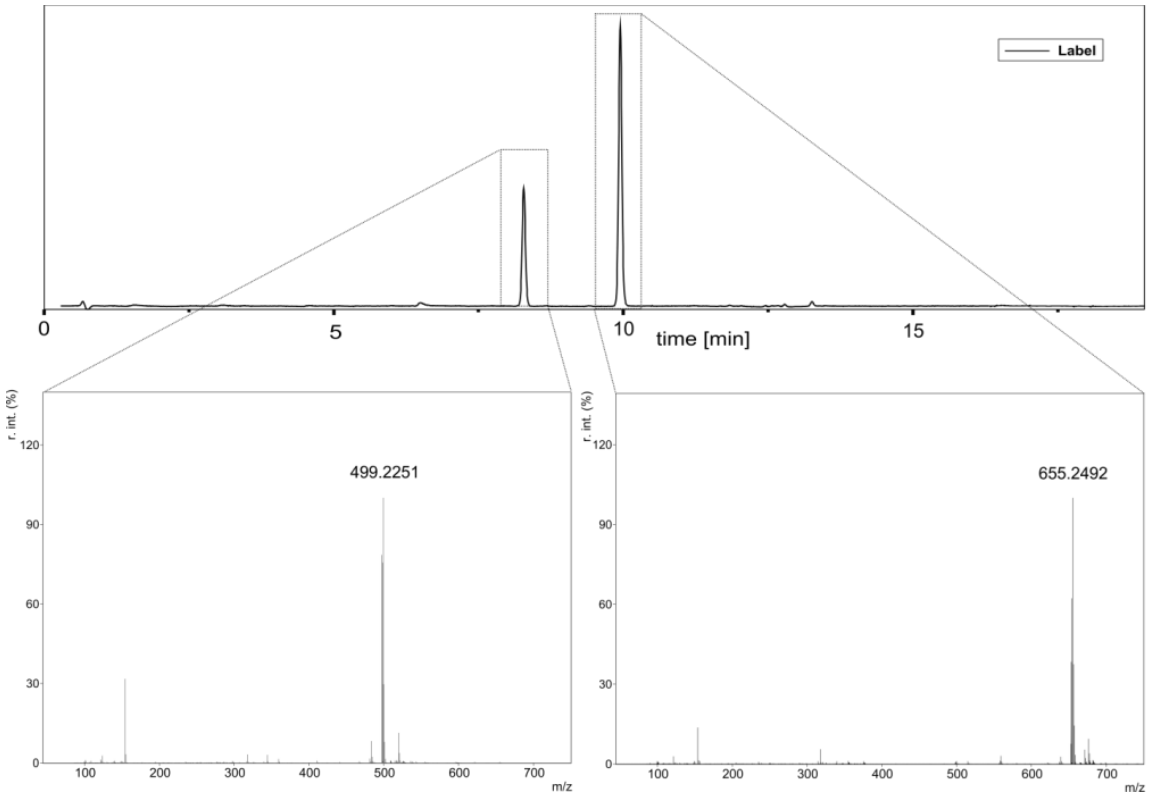
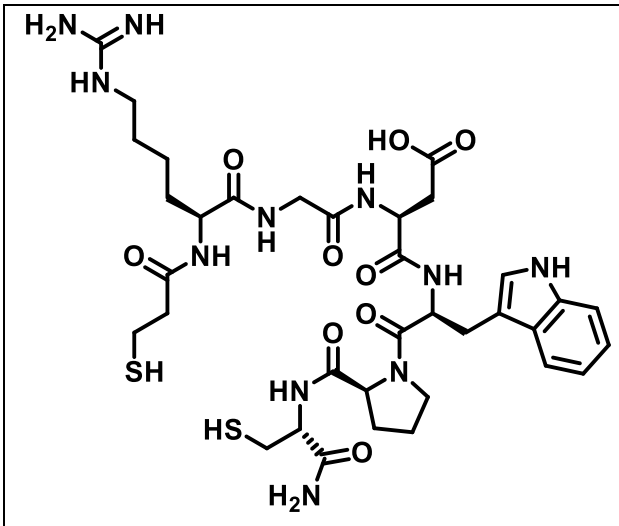


Figure 9: HPLC-MS chromatogram of bis-sulfone based spin label **4** and **5** at 180-400 nm, with gradient of acetonitril in water from 20% to 90% with 0.1% formic acid for 19 minutes.

2. Synthesis of spin labeled eptifibatide

2.1 Reduced eptifibatide 2



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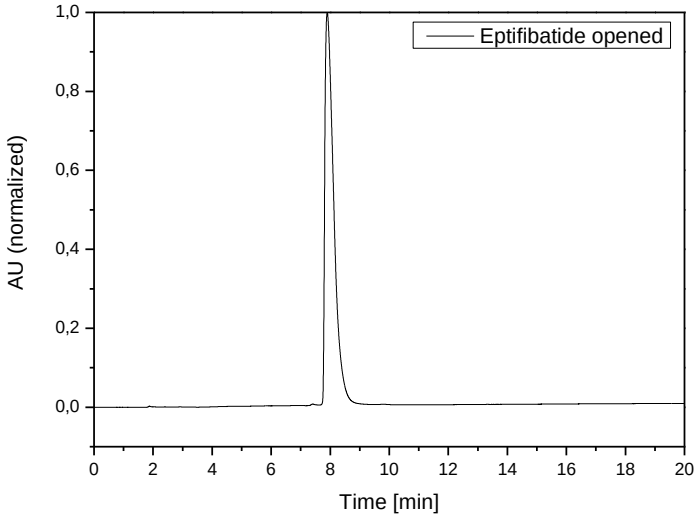


Figure 10: HPLC chromatogram of reduced eptifibatide **2** detected at 214 nm, with gradient of acetonitril in water from 20% to 80% with 0.1% TFA for 20 minutes.

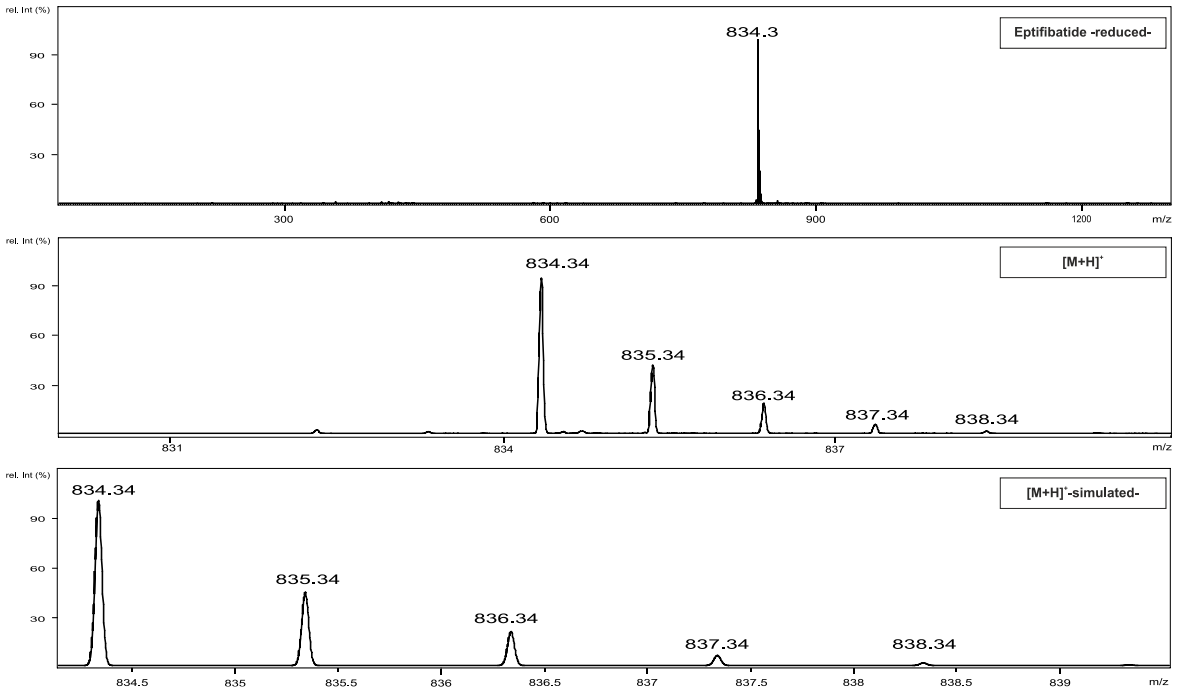
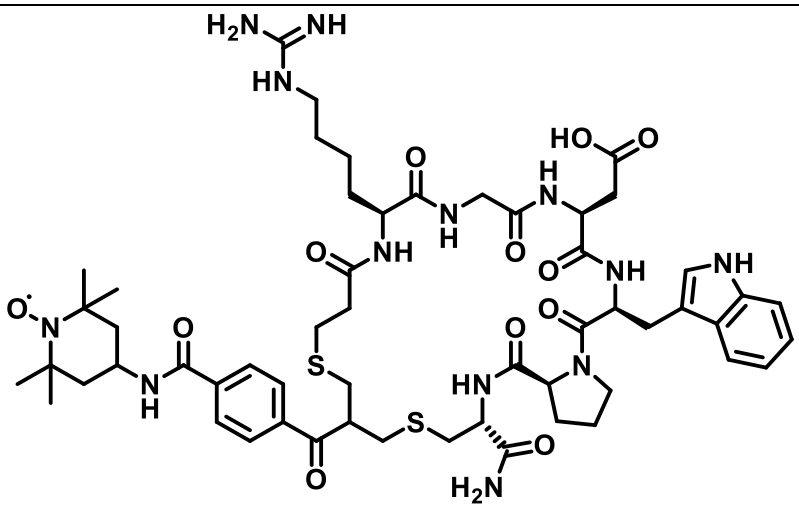


Figure 11: ESI-MS spectrum of reduced eptifibatide **2** with zoom into the area of $[M+1H]^+$ and the simulation for $[M+1H]^+$.

2.2 Spin labeled eptifibatide 3



3

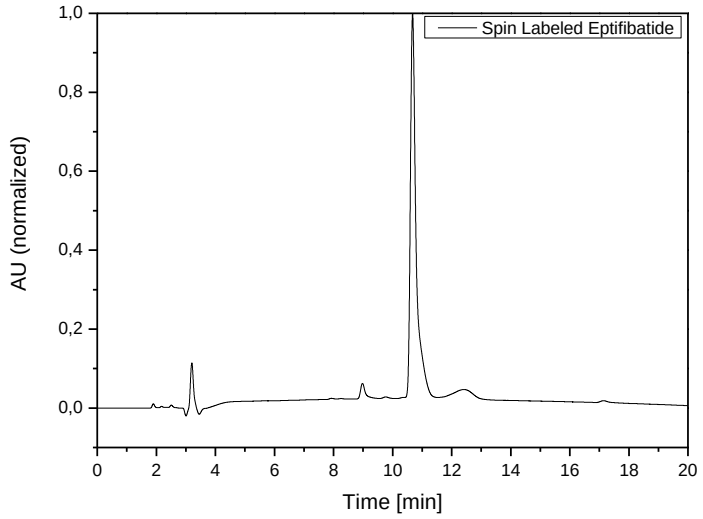


Figure 12: HPLC chromatogram of labeled eptifibatide **3** detected at 214 nm, with gradient of acetonitril in water from 20% to 80% with 0.1% TFA for 20 minutes.

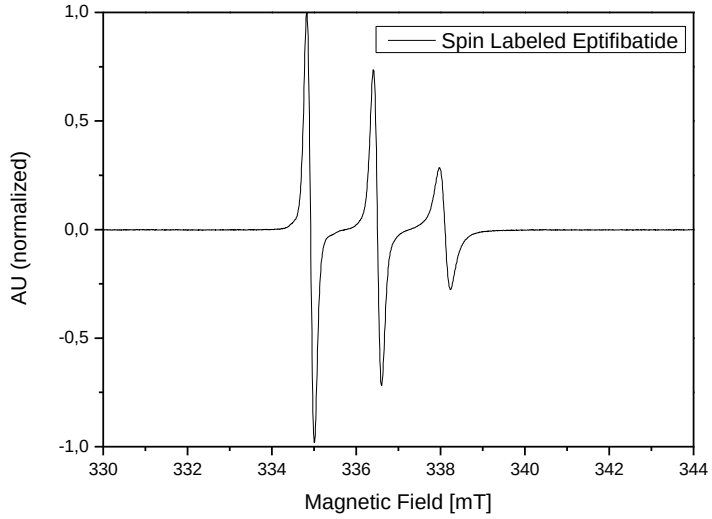


Figure 13: ESR spectrum of ca. 15 mM spin labeled eptifibatide **3**.at 20 °C.

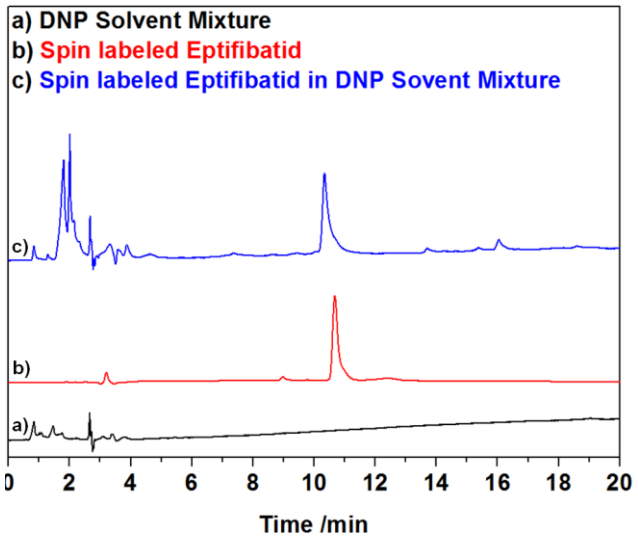


Figure 14: HPLC traces (214 nm, with gradient of acetonitril in water from 20% to 80% with 0.1% TFA for 20 minutes) illustrating the DNP solvent mixture (a), spin labeled eptifibatide **3** (b) and spin labeled eptifibatide **3** in DNP solvent mixture (c).