# Synthesis and Reactivity of a Bis-Sultone Cross-Linker for Peptide Conjugation and [$^{18}$F]-Radiolabelling *via* Unusual "Double Click" Approach

Thomas Priem,[a,b] Cédric Bouteiller,[*a] David Camporese,[a] Anthony Romieu,[b,c] and Pierre-Yves Renard[b,c,d]

[a]*Advanced Accelerator Applications, 20 Rue Diesel, 01630 Saint-Genis-Pouilly, France*
*Fax: +33-4-50-99-30-71*
*Phone: +33-4-50-99-30-70*
*E-mail: cedric.bouteiller@adacap.com*
*Web site: http://www.adacap.com*

[b]*Equipe de Chimie Bio-Organique, COBRA-CNRS UMR 6014 & FR 3038, rue Lucien Tesnière, 76131 Mont-Saint-Aignan, France*
*Lab homepage: http://ircof.crihan.fr*

[c]*Université de Rouen, Place Emile Blondel, 76821 Mont-Saint-Aignan, France*

[d]*Institut Universitaire de France, 103 Boulevard Saint-Michel, 75005, Paris, France*

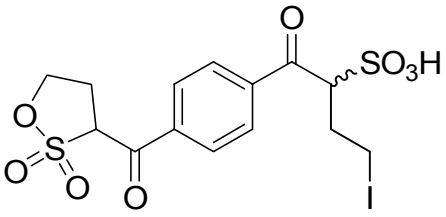
# SUPPORTING INFORMATION

**Abbreviations**

The following abbreviations are used throughout the text of the ESI file: ESI, electrospray ionisation; RP-HPLC, reversed-phase high performance liquid chromatography; rt, room temperature; TFA, trifluoroacetic acid.

**Experimental procedures**

**Mono-iodo-propanesultone**




Bis-propanesultone **3** (52.4 mg, 0.14 mmol, 1 equiv.) was dissolved in dry $CH_3CN$ (5 mL). NaI (21 mg, 0.14 mmol, 1 equiv.) in solution in dry $CH_3CN$ (8 mL) was then slowly added. The resulting reaction mixture was stirred at rt and the reaction was checked for completion by RP-HPLC (system A). After 6 h, the reaction was stopped to avoid significant formation of the non-desired bis-iodo derivative. The crude product was then dissolved with aq. TFA and purified by RP-HPLC (system B, 1 injection, $t_R$ = 35.0-41.5 min). The product-containing fractions were lyophilised to give the mono-iodo-propanesultone as an orange solid (33.1 mg, yield 49%, mixture of two racemic diastereomers). $\delta_H$ (300 MHz, $CD_3OD$) 8.29 (d, *J* 8.7, 2H), 8.23 (d, *J* 8.3, 2H), 5.65 (ddd, *J* 1.9, 6.8, 8.5, 1H), 5.21-5.16 (m, 1H), 4.68-4.54 (m, 2H), 3.25-3.15 (m, 2H), 2.83-2.74 (m, 2H), 2.72-2.60 (m, 2H); $\delta_C$ (75.5 MHz, $CD_3OD$) 195.5, 189.5, 142.2, 139.9, 130.7, 129.9, 69.8, 68.1, 61.5, 34.1, 28.0, 3.4; MS (ESI-): *m/z* 500.93 $[M - H]^-$, calcd for $C_{14}H_{15}IO_8S_2$ 501.93; HPLC (system A): $t_R$ = 22.8 and 23.0 min (two racemic diastereomers, purity 90%); $\lambda_{max}$(recorded during the HPLC analysis)/nm 265.

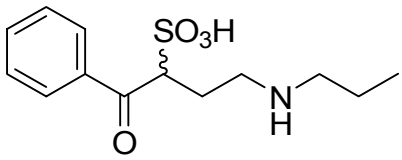
**Mono-fluoro-oxyamine-peptide conjugate.** The same protocol (synthesis and purification) than described for peptide conjugate **6** was used. An aminooxy-dodecapeptide (its sequence is confidential and not disclosed within this article but it contains an aminooxyacetic acid residue) was used as starting material. MS (ESI+): *m/z* 885.33 $[M + 2H]^{2+}$, 1769.53 $[M + H]^+$, calcd for $C_{73}FH_{117}N_{22}O_{24}S_2$ 1768.80; HPLC

(system A): $t_R$ = 20.9 min (four diastereomers, purity 68%); $\lambda_{max}$(recorded during the HPLC analysis)/nm 269.

**General procedure for the nucleophilic ring-opening of monosultones 1 and 2.** In a round bottom flask were introduced one equivalent of the nucleophile (halide, amine, alcohol, thiol or amino-acid derivatives) dissolved in $CH_3CN$ (with a small amount of deionised water or DMF if complete solubility in $CH_3CN$ was not obtained). The mixture was stirred at room temperature and one equiv. of monosultone (**1** or **2)** in solution in $CH_3CN$ was then added. The reaction was checked for completion by RP-HPLC (system A), quenched by dilution with aq. TFA and $CH_3CN$ and purified by RP-HPLC (system B, 1 injection).

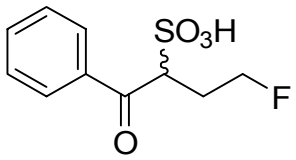
- For potassium halide salts, 1.1 equivalent of Kryptofix[K222] was added,
- For amino acid hydrochloride derivatives, 1.1 equivalent of $K_2CO_3$ (or $Cs_2CO_3$) was added.

**1-Oxo-1-phenyl-4-(propylamino)butane-2-sulfonic acid**



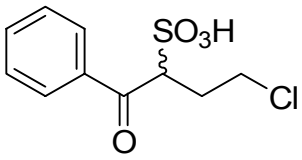

$\delta_H$ (300 MHz, $CD_3OD$) 7.73-7.63 (m, 5H), 5.07 (t, *J* 8.6, 1H), 4.63-4.56 (m, 1H), 4.37-4.29 (m, 1H), 3.90-3.84 (m, 2H), 2.87-2.77 (m, 2H), 1.93-1.84 (m, 2H), 0.96 (t, *J* 7.3, 3H); $\delta_C$ (75.5 MHz, $CD_3OD$) 184.4, 134.6, 130.2, 130.1, 128.4, 73.3, 60.6, 54.6, 25.2, 21.8, 11.1; MS (ESI-): *m/z* 284.20 $[M - H]^-$, calcd for $C_{13}H_{19}NO_4S$ 285.10; HPLC (system A): $t_R$ = 12.1 min (purity 94%); $\lambda_{max}$(recorded during the HPLC analysis)/nm 254.

**4-Fluoro-1-oxo-1-phenylbutane-2-sulfonic acid**



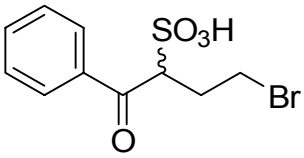

$\delta_H$ (300 MHz, $CD_3OD$) 8.10 (d, *J* 7.3, 2H), 7.62 (t, *J* 7.5, 1H), 7.51 (t, *J* 7.2, 2H), 5.14 (dd, *J* 4.1, 9.4, 1H), 4.70-4.28 (m, 2H), 2.76-2.44 (m, 2H); $\delta_C$ (75.5 MHz, $D_2O$) 196.8, 139.1, 134.4, 129.6, 128.8, 72.0, 64.7, 55.1; MS (ESI-): *m/z* 245.20 $[M - H]^-$, calcd for $C_{10}H_{11}FO_4S$ 246.04; HPLC (system A): $t_R$ = 14.1 min (purity 94%); $\lambda_{max}$(recorded during the HPLC analysis)/nm 254.

**4-Chloro-1-oxo-1-phenylbutane-2-sulfonic acid**



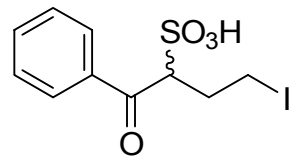

$\delta_H$ (300 MHz, $CD_3OD$) 8.11 (d, *J* 7.5, 2H), 7.62 (t, *J* 7.3, 1H), 7.55 (t, *J* 7.4, 2H), 5.20 (dd, *J* 4.5, 8.9, 1H), 3.76-3.47 (m, 2H), 2.74-2.66 (m, 1H), 2.66-2.51 (m, 1H); $\delta_C$ (75.5 MHz, $CD_3OD$) 196.5, 139.0, 134.5, 130.3, 129.6, 64.2, 43.7, 33.4; MS (ESI-): *m/z* 261.07 $[M - H]^-$, calcd for $C_{10}H_{11}ClO_4S$ 262.01; HPLC (system A): $t_R$ = 17.3 min (purity 93%); $\lambda_{max}$(recorded during the HPLC analysis)/nm 254.

**4-Bromo-1-oxo-1-phenylbutane-2-sulfonic acid**



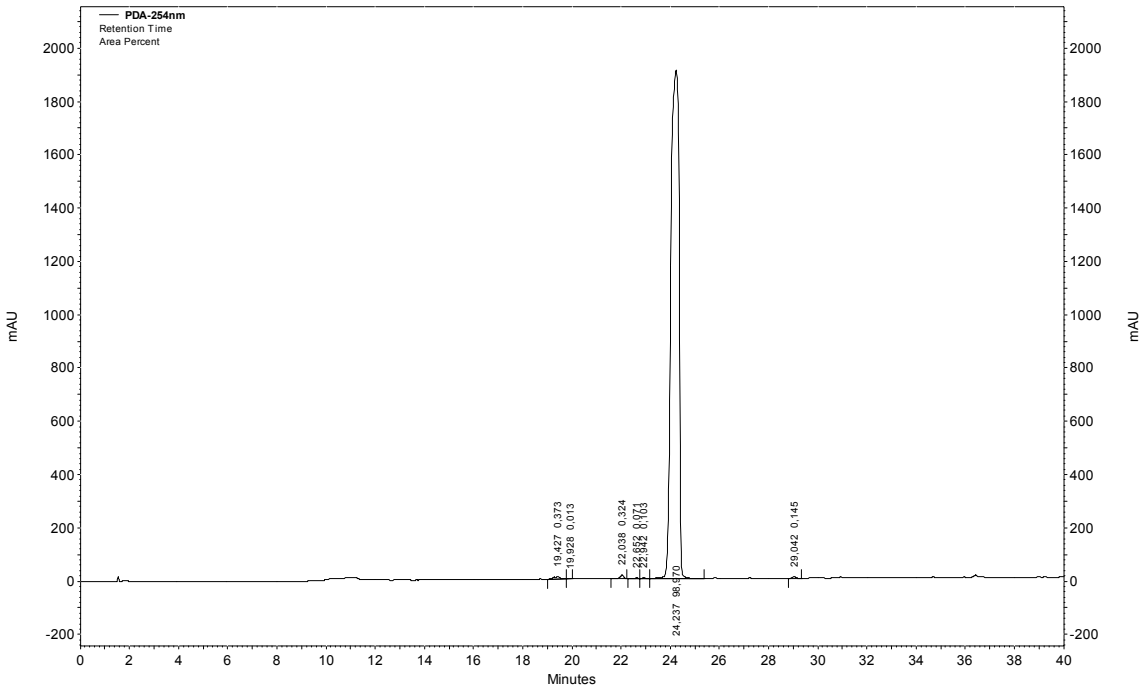

$\delta_H$ (300 MHz, $CD_3OD$) 8.10 (d, *J* 7.5, 2H), 7.62 (t, *J* 7.3, 1H), 7.50 (t, *J* 7.9, 2H), 5.21 (dd, *J* 4.5, 8.9, 1H), 3.64-3.57 (m, 1H), 3.44-3.36 (m, 1H), 2.84-2.74 (m, 1H), 2.70-2.60 (m, 1H); $\delta_C$ (75.5 MHz, $CD_3OD$) 196.1, 139.0, 134.4, 130.3, 129.5, 65.3, 33.4, 31.9; MS (ESI-): *m/z* 304.93 $[M - H]^-$, 418.80 $[M + TFA - H]^-$, calcd for $C_{10}H_{11}BrO_4S$ 305.96; HPLC (system A): $t_R$ = 17.8 min (purity 84%); $\lambda_{max}$(recorded during the HPLC analysis)/nm 254.

**4-Iodo-1-oxo-1-phenylbutane-2-sulfonic acid**

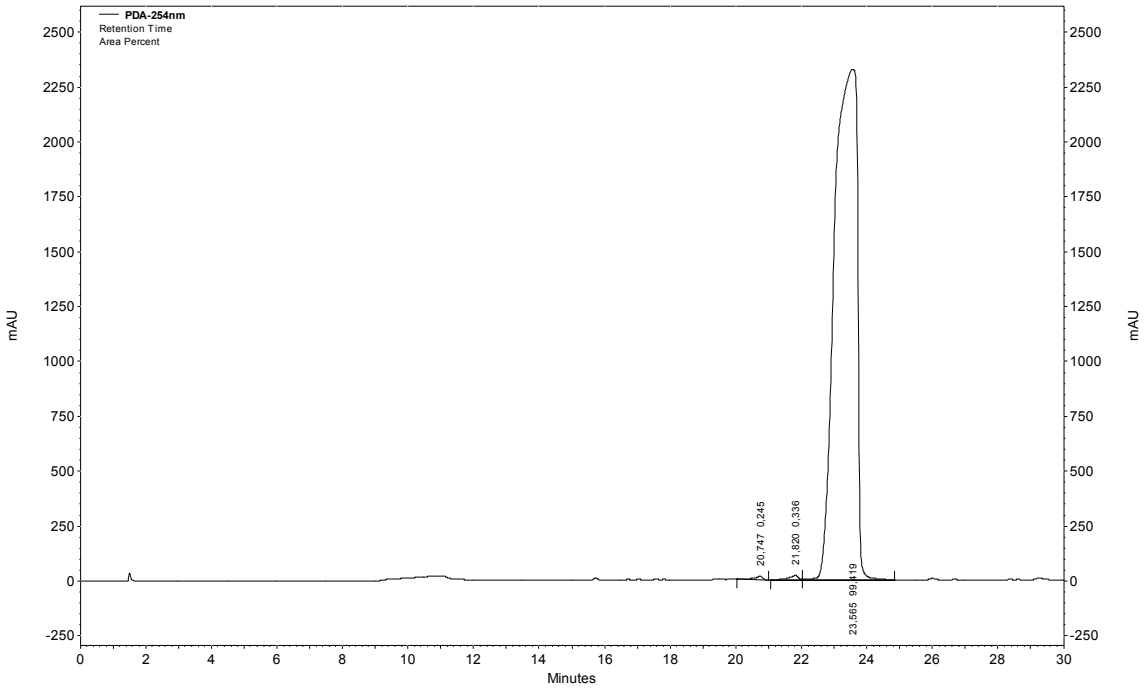



$\delta_H$ (300 MHz, $CD_3OD$) 8.02 (d, *J* 7.1, 2H), 7.63 (t, *J* 7.5, 1H), 7.50 (t, *J* 7.7, 2H), 5.16 (dd, *J* 4.5, 8.7, 1H), 3.40-3.14 (m, 2H), 2.82-2.63 (m, 2H); $\delta_C$ (75.5 MHz, $CD_3OD$) 196.6, 139.0, 134.5, 130.5, 129.6, 67.3, 34.4, 3.4; MS (ESI-): *m/z* 352.87 $[M - H]^-$, calcd for $C_{10}H_{11}IO_4S$ 353.94; HPLC (system A): $t_R$ = 19.7 min (purity 98%); $\lambda_{max}$(recorded during the HPLC analysis)/nm 254.

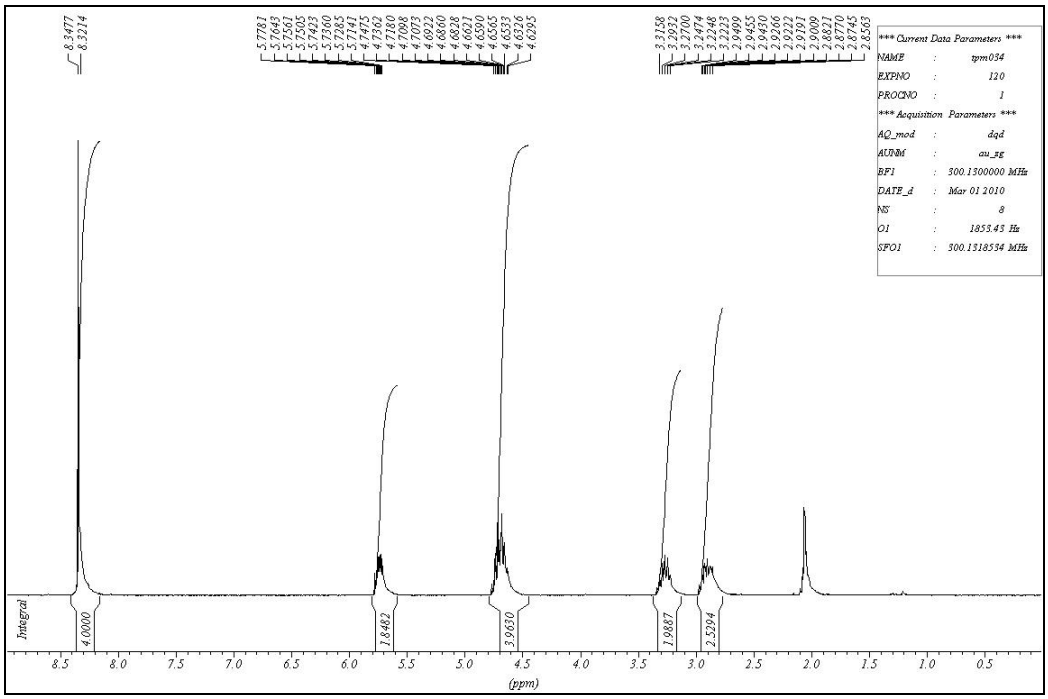
**RP-HPLC elution profile (system A) of 1-phenylacyl-1,3-propanesultone 1.**



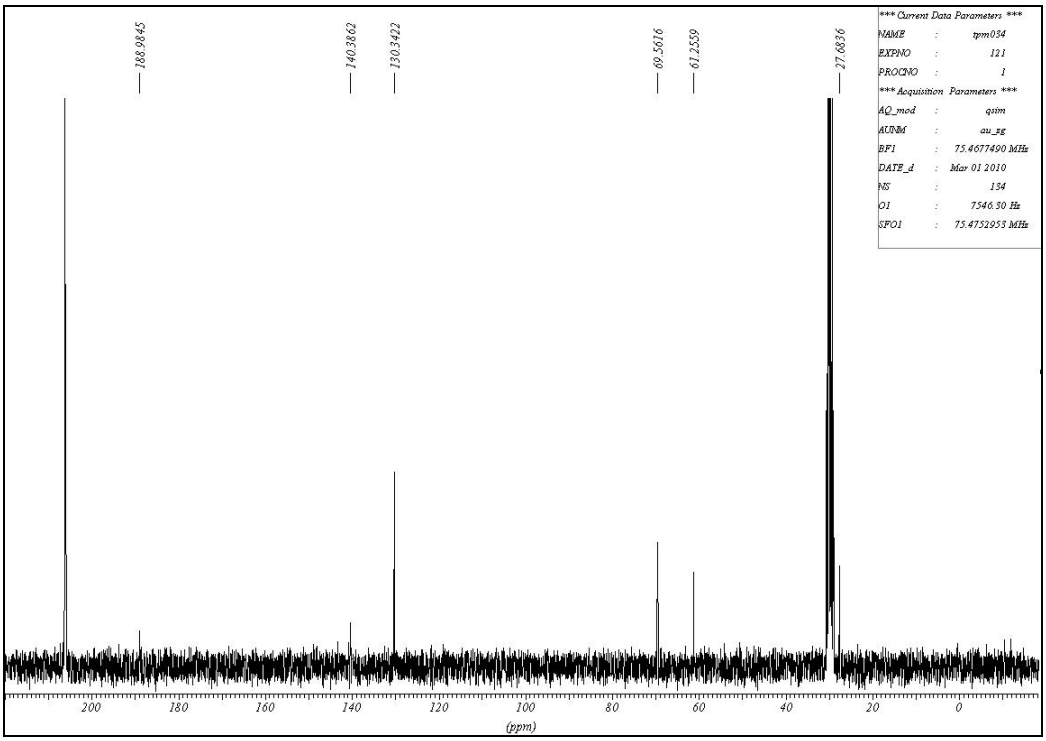

**RP-HPLC elution profile (system A) of 1-benzyl-1,3-propanesultone 2.**



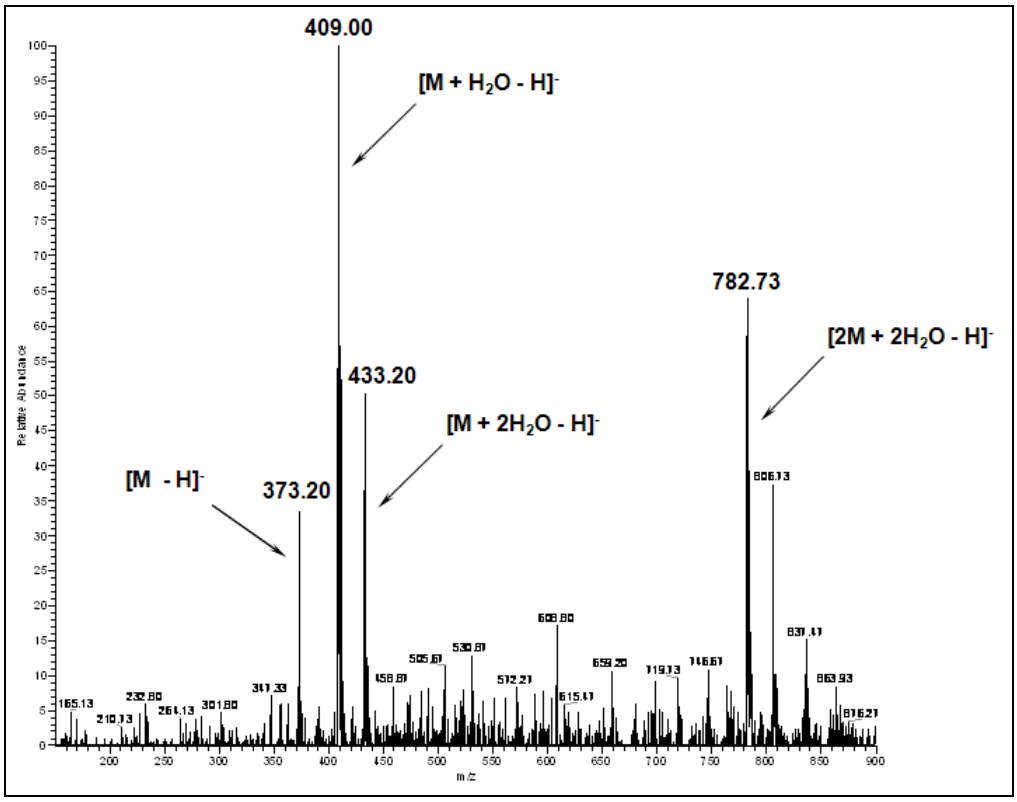
**$^{1}$H NMR spectrum of bis-propanesultone 3 recorded in acetone-$d_6$ at 300 MHz.**




**$^{13}$C NMR spectrum of bis-propanesultone 3 recorded in acetone-$d_6$ at 75.5 MHz.**

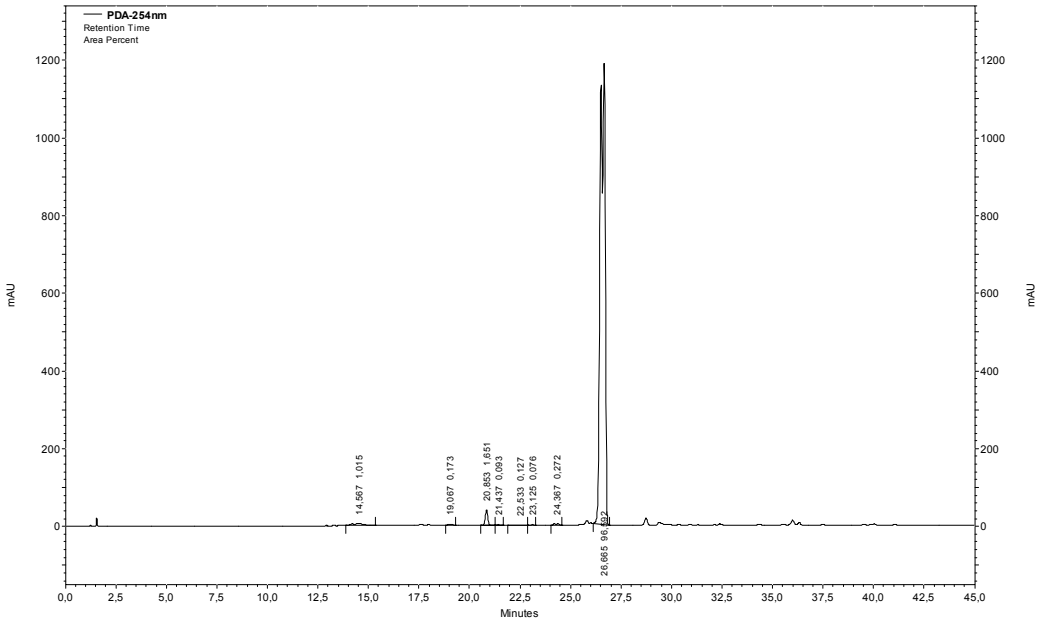


## ESI mass spectrum of bis-sultone 3 recorded in the negative mode.[a]



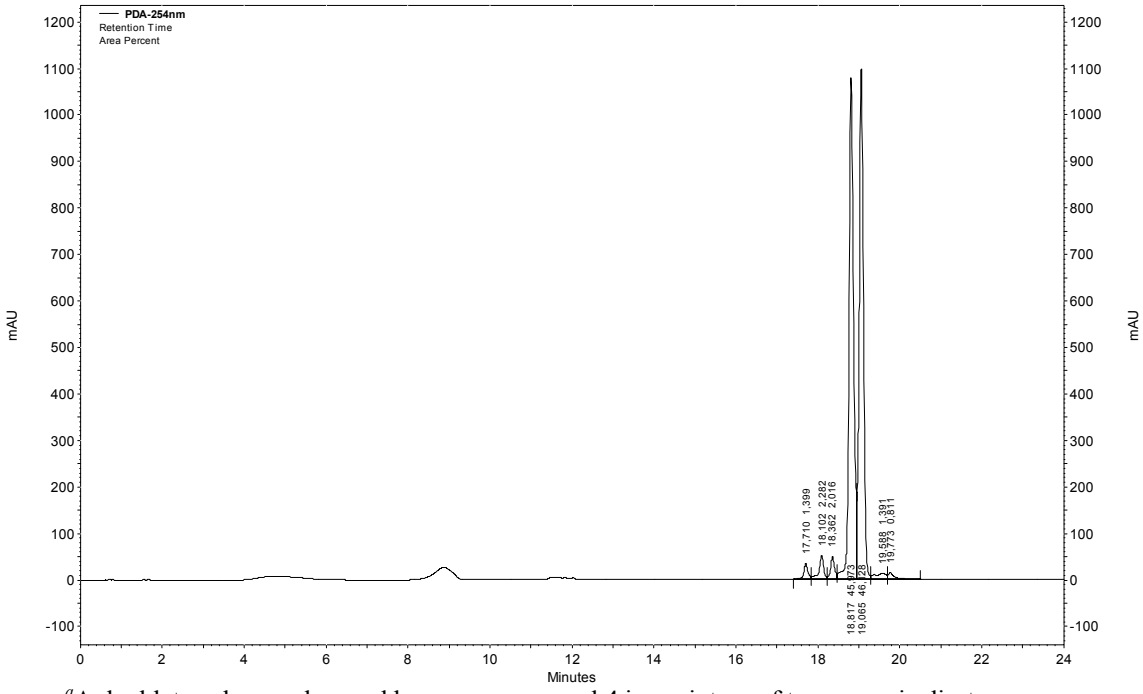

[a]hydration of sultone moieties was occurred during the ionisation process.

## RP-HPLC elution profile (system A) of bis-sultone 3.[a]



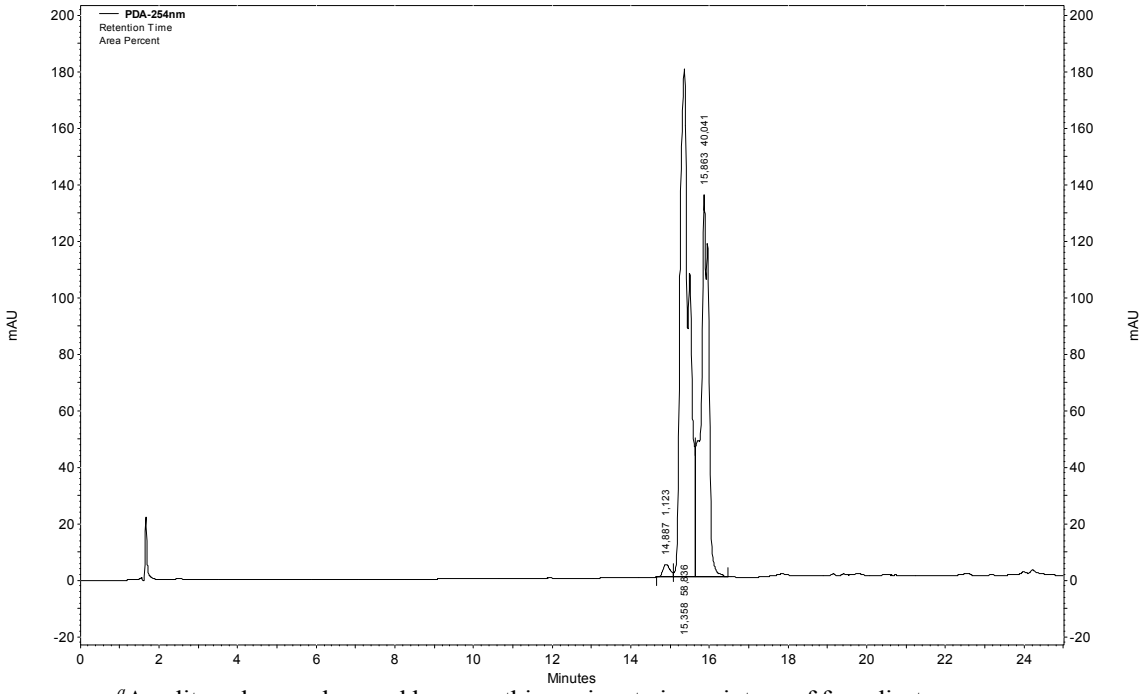

[a]A doublet peak was observed because compound **3** is a mixture of two racemic diastereomers.

## RP-HPLC elution profile (system A) of mono-fluoro-propanesultone 4.[a]



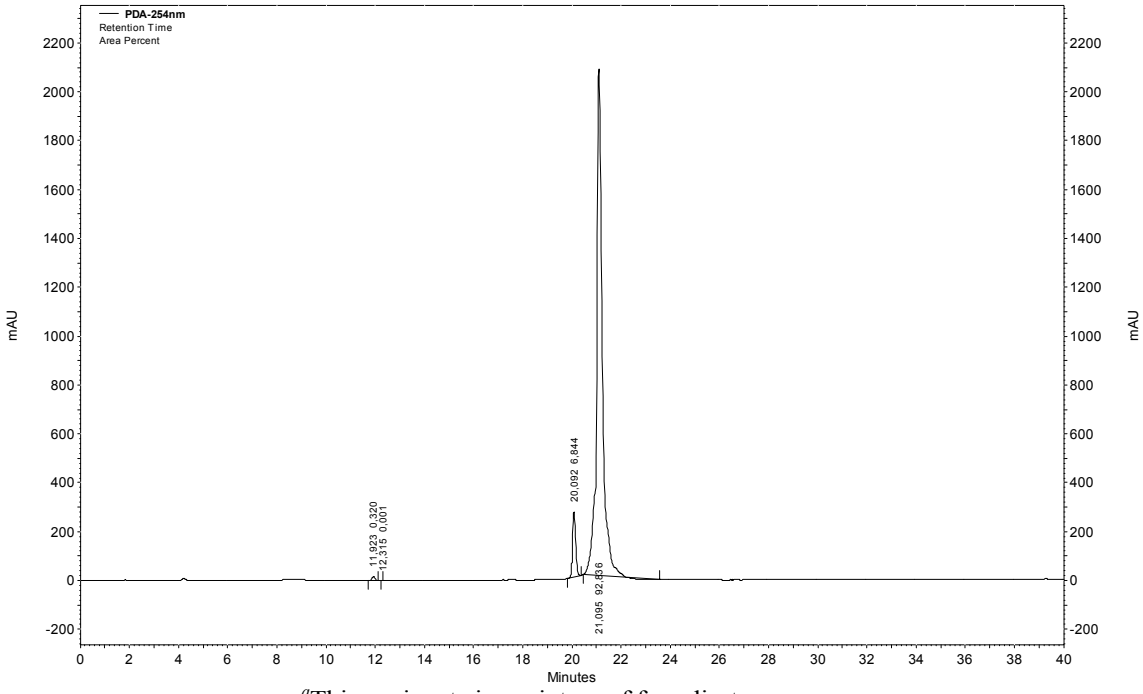

[a]A doublet peak was observed because compound **4** is a mixture of two racemic diastereomers.

## RP-HPLC elution profile (system A) of mono-fluoro-Boc-L-lysine-$NH_2$ conjugate 5.[a]



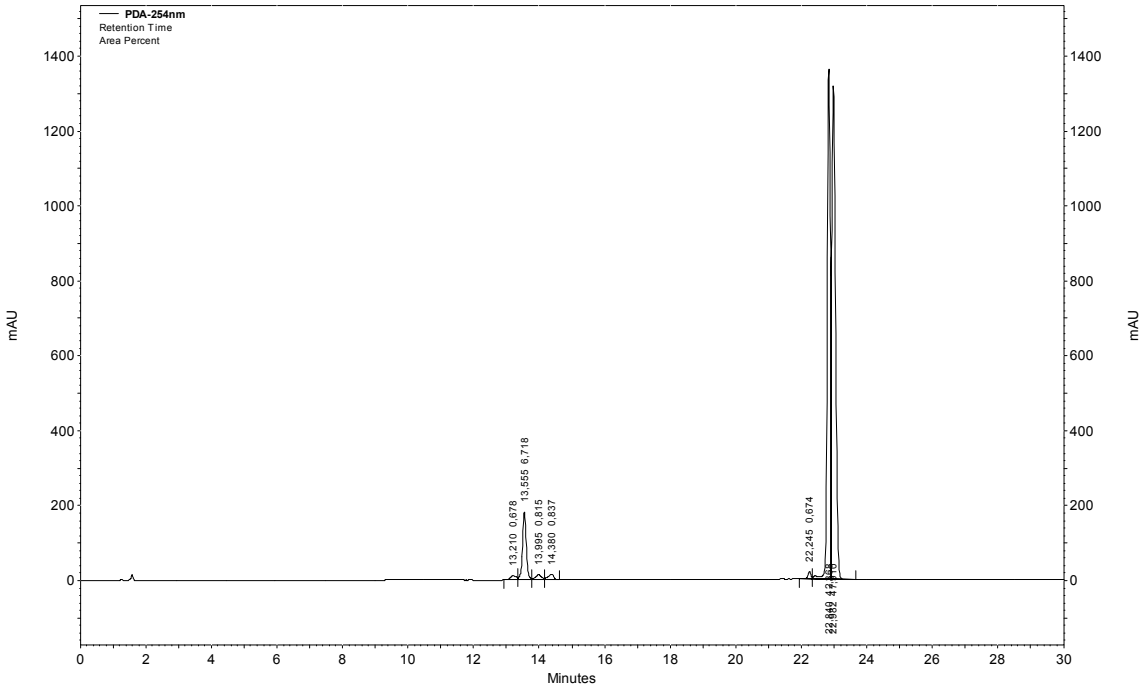

[a]A split peak was observed because this conjugate is a mixture of four diastereomers.

## RP-HPLC elution profile (system A) of mono-fluoro-peptide conjugate 6.[a]



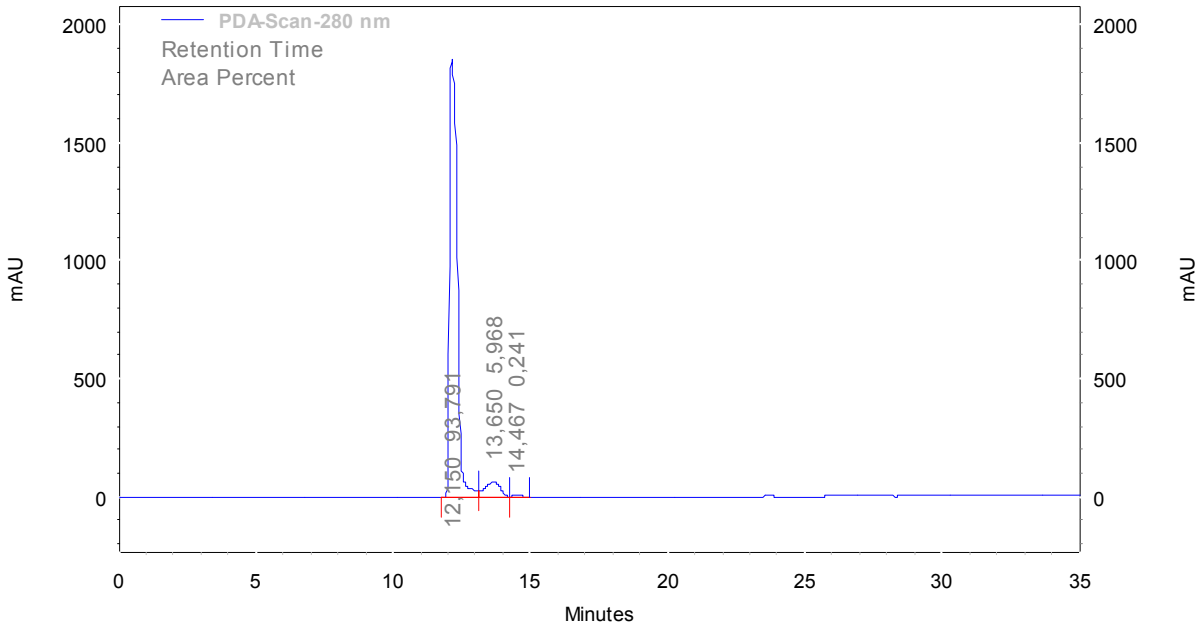

[a]This conjugate is a mixture of four diastereomers.

## RP-HPLC elution profile (system A) of mono-iodo-propanesultone.[a]

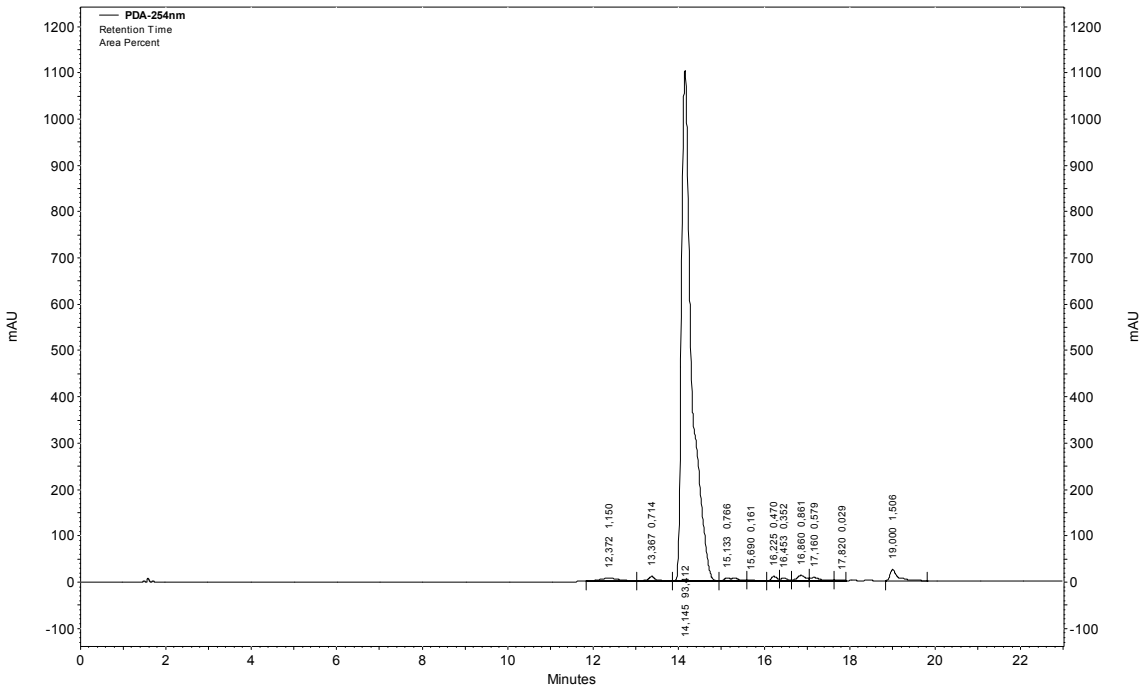



[a]A doublet peak was observed because this compound is a mixture of two racemic diastereomers.

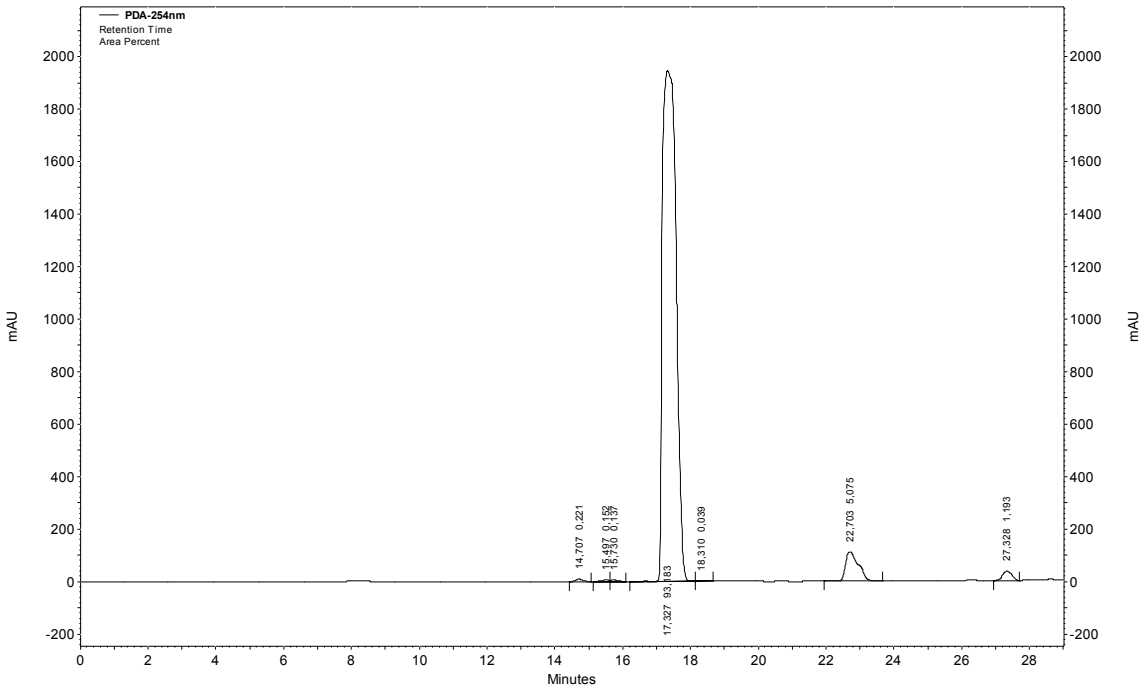
**RP-HPLC elution profile (system A) of 1-oxo-1-phenyl-4-(propylamino)butane-2-sulfonic acid.**



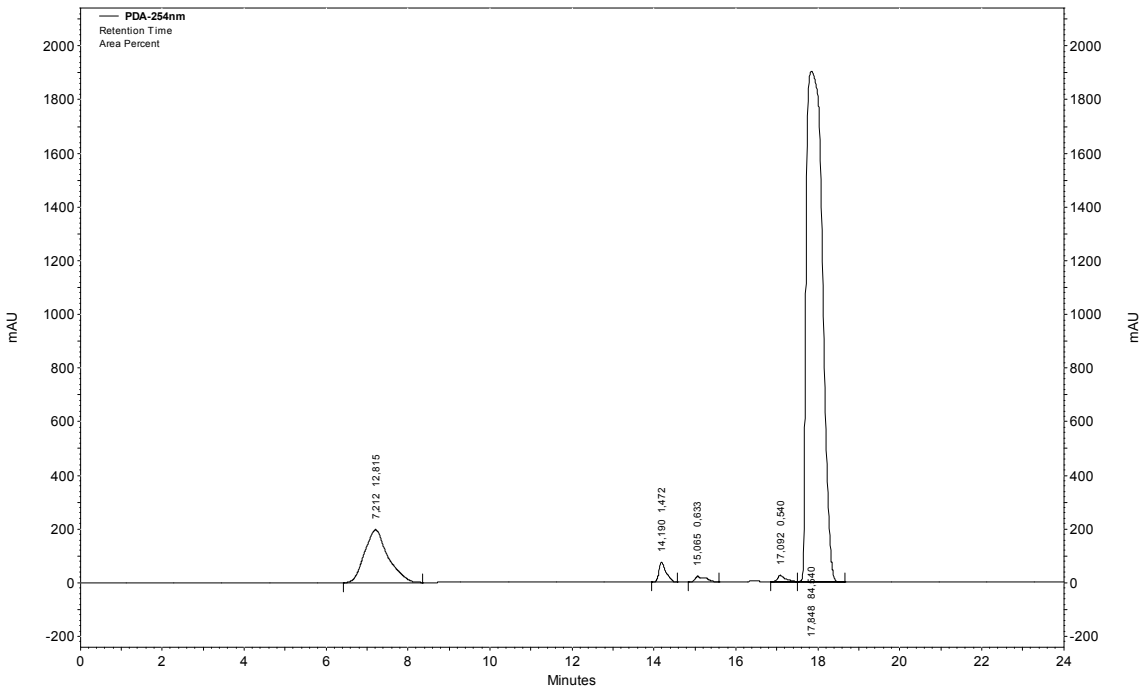

**RP-HPLC elution profile (system A) of 4-fluoro-1-oxo-1-phenylbutane-2-sulfonic acid.**



**RP-HPLC elution profile (system A) of 4-chloro-1-oxo-1-phenylbutane-2-sulfonic acid.**




**RP-HPLC elution profile (system A) of 4-bromo-1-oxo-1-phenylbutane-2-sulfonic acid.**



**RP-HPLC elution profile (system A) of 4-iodo-1-oxo-1-phenylbutane-2-sulfonic acid.**