The first metal-free water-soluble cryptophane-111

Ténin Traoré,[a] Guillaume Clavé,[b] Léa Delacour,[a] Naoko Kotera,[a] Pierre-Yves Renard,[b,c,d] Anthony Romieu,[b,c] Patrick Berthault,[d] Céline Boutin,[d] Nawal Tassali,[d] and Bernard Rousseau*[a]

[a]*CEA, iBiTecS, Service de Chimie Bioorganique et de Marquage, Gif sur Yvette, F-91191, France*
Fax: (+33) 1-69-08-79-91
Tel: (+33) 1-69-08-54-93
E-mail: bernard.rousseau@cea.fr

[b]*Equipe de Chimie Bio-Organique, COBRA-CNRS UMR 6014 & FR 3038, rue Lucien Tesnière, 76131 Mont-Saint-Aignan, France*
Fax (+33) 2-35-52-29-71
Tel (+33) 2-35-52-24-27 (or 24 76)
E-mail: anthony.romieu@univ-rouen.fr or pierre-yves.renard@univ-rouen.fr
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*

[e]*CEA, IRAMIS, SIS2M, Laboratoire Structure et Dynamique par Résonance Magnétique, Gif sur Yvette, F-91191, France*
Fax: +33 1-69-08-98-06
Tel: +33 1-69-08-54-93
E-mail: patrick.berthault@cea.fr

Supporting Information

Abbreviations

The following abbreviations are used throughout the text of the ESI file: ATR, attenuated total reflectance; PyBOP®, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; ESI, electrospray ionisation; RP-HPLC, reversed-phase high performance liquid chromatography; LC-MS, liquid chromatography coupled to mass spectrometry; NHS, *N*-hydroxysuccinimidyl; rt, room temperature; NMP, *N*-methylpyrrolidone; TFA, trifluoroacetic acid; TOF, time of flight; TSTU, *O*-(*N*-succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate.

Experimental Section

General methods.

Unless otherwise noted, all other commercially available reagents and solvents were used without further purification. DMF was dried through distillation over BaO. 2.0 M solution of DIEA in peptide synthesis grade NMP was purchased from Applied Biosystems. TLC were carried out on Merck DC Kieselgel 60 F-254 aluminium sheets. The spots were visualised by illumination with UV lamp (λ = 254 nm) and/or staining with ninhydrin solution (0.2% w/v in absolute ethanol). Purifications of reaction products were carried out by flash-chromatography using Geduran® Si 60 silica gel (40-63 mm) from Merck. *N*-Fmoc α-sulfo-β-alanine (Fmoc-β-Ala(SO_3H)-OH) was prepared from β-alanine by using the synthetic procedure recently reported by us[1] and is now commercially available from Iris Biotech GmbH (#FAA1915). 2-Aminoethane-1,1-disulfonic acid was readily synthesised through a modified Ritter reaction.[2] The HPLC-gradient grade acetonitrile (CH_3CN) and methanol (CH_3OH) were obtained from VWR. Aq. mobile-phases for HPLC were prepared using water purified with a Milli-Q system (purified to 18.2 MΩ.cm). Ion-exchange chromatography (for desalting trisulfonated linker **4**) was performed with an Econo-Pac® Disposable chromatography column (Bio-Rad, #732-1010) filled with an aq. solution of Dowex® 50WX8-400 (Alfa Aesar, *ca.* 10 g, 15 × 70 mm bed), regenerated using aq. 10% HCl solution and equilibrated with deionised water. NMR spectra (^1H and ^{13}C) were recorded either on a Bruker DPX 300 spectrometer or on a Bruker Avance 400 MHz spectrometer (Bruker, Wissembourg, France). Chemical shifts are reported in parts per million (ppm) downfield from residual solvent peaks (D_2O (δ_H = 4.79) or DMSO-d_6 (δ_H = 2.50, δ_C = 39.52))[3] and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), broad singlet (bs), broad doublet (bd), doublet (d). Splitting patterns that could not be interpreted or easily visualised are designated as multiplet (m or bm). ^{13}C substitutions were determined with JMOD experiments, differentiating signals of methyl and methine carbons pointing "up" (+) from methylene and quaternary carbons pointing "down" (-).[4] Infrared (IR) spectra were recorded with an universal ATR sampling accessory on a Perkin Elmer FT-IR Spectrum 100 spectrometer. Analytical HPLC was performed on a Thermo Scientific Surveyor instrument equipped with a PDA detector. Mass spectra were obtained with either a Finnigan LCQ Advantage MAX (ion-

[1] A. Romieu, D. Brossard, M. Hamon, H. Outaabout, C. Portal and P.-Y. Renard, *Bioconjugate Chem.*, 2008, **19**, 279-289.
[2] A. Romieu, D. Tavernier-Lohr, S. Pellet-Rostaing, M. Lemaire and P.-Y. Renard, *Tetrahedron Lett.*, 2010, **51**, 3304-3308.
[3] (a) G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176-2179, (b) H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512-7515.
[4] C. Le Cocq and J. Y. Lallemand, *J. Chem. Soc., Chem. Commun.*, 1981, 150-152.

trap), or a LCQ Duo (ion-trap) or an ESI/TOF Mariner apparatus equipped with an ESI source.

Production of laser-polarized xenon

Our home-built optical pumping setup using a 5W Titanium:Sapphire laser was described in the following references (Desvaux, H.; Gautier, T.; Le Goff, G.; Pétro, M.; Berthault, P. *Eur. Phys. J. D* **2000**, *12*, 289-296. Berthault, P.; Huber, G.; Desvaux, H. Prog. *NMR Spectrosc.* **2009**, *55*, 35-60). It provides an average xenon polarization of 40% (measured on the gas phase in the spectrometer). Hyperpolarized xenon frozen into a cold finger was transported inside a 3 kG solenoid. The transfer from this cold finger to the NMR tube was made via a simple Joule-Gay Lussac expansion in the fringe field of the magnet in order to preserve polarization. Vigorous shaking of the tube followed by a 10s waits time before each hyperpolarized ^{129}Xe NMR experiment ensured homogenization of the sample after disappearance of the bubbles.

NMR Spectroscopy

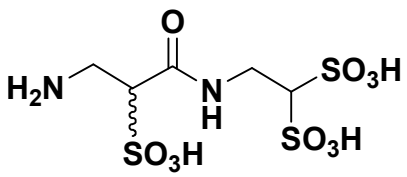
The ^{129}Xe and ^{1}H NMR experiments designed to study the interaction between xenon and the cryptophane were carried out on a Bruker Avance II 500 spectrometer equipped with 5 mm HNX and Broadband inverse probeheads. Accurate calibration of the temperature was made using a methanol sample. Prior the introduction of the hyperpolarized noble gas, the solutions were degassed through helium bubbling or using several freeze-pumpthaw cycles.

HPLC separations.

Several chromatographic systems were used for the analytical experiments and the purification steps:

- System A: RP-HPLC (Thermo Hypersil GOLD C_{18}, 5 µm, 4.6 × 150 mm) with CH_3OH and aq. 0.1% trifluoroacetic acid (aq. TFA 0.1%, pH 2.0) as eluents [90% TFA (5 min), then linear gradient from 10 to 90% (40 min) of CH_3OH] at a flow rate of 1.0 mL min^{-1}. UV detection with the "Max Plot" (*i.e.*, chromatogram at absorbance maximum for each compound) mode (220-400 nm).
- System B: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 2.1 x 50 mm) with CH_3CN and aq. TFA as eluents [80% aq. TFA (5 min), linear gradient from 20 to 100% of CH_3CN (53 min)] at a flow rate of 0.2 mL min^{-1}. Triple UV detection was achieved at 220, 260 and 288 nm and ESI-MS detection in the negative mode.
- System C : RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 10 x 100 mm) with the same eluents and gradient of system B, at a flow rate of 2.5 mL min^{-1}. Triple UV detection was achieved at 220, 260 and 288 nm.

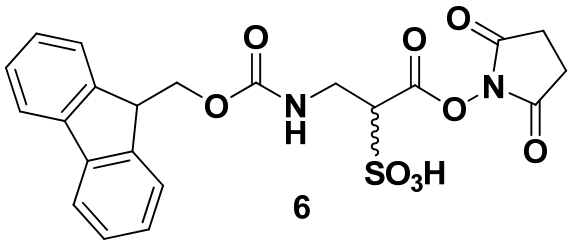
Trisulfonated linker (4).



4

(a) Schotten-Baumann reaction:

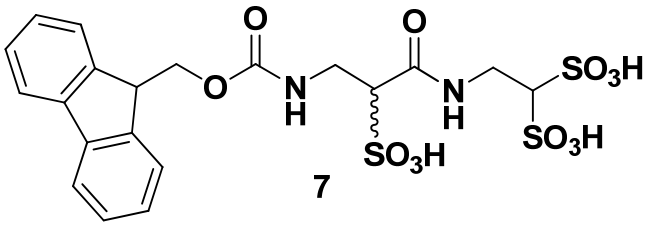
Fmoc-β-Ala(SO_3H)-OH (500 mg, 1.3 mmol) was dissolved in dry DMF (5 mL). DIEA (442 µL, 2.55 mmol, 2 equiv.) and TSTU reagent (390 mg, 1.3 mmol, 1 equiv.) were sequentially added at 4 °C. The resulting reaction mixture was stirred at rt for 30 min. The quantitative conversion to active NHS ester **6** was checked by ESI-MS. This active ester was used in the next coupling step without purification.



6

(ESI-): *m/z* 487.13 $[M - H]^-$, calcd $C_{22}H_{20}N_2O_9S$ 488.48.

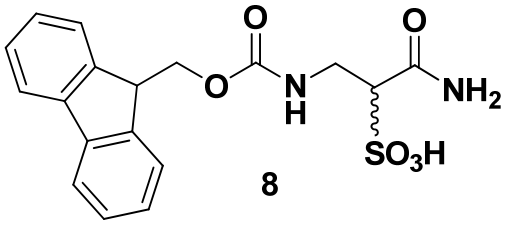
2-Aminoethane-1,1-disulfonic acid (525 mg, 2.55 mmol, 2 equiv.) was dissolved in deionised water (8 mL) and solid $NaHCO_3$ (646 mg, 7.7 mmol, 6 equiv.) was added. The solution was cooled to 0 °C with a ice/NaCl bath. The crude mixture containing NHS ester **6** was then slowly over 10 min and the resulting reaction mixture was stirred at rt for 1 h. The reaction was checked for completion by RP-HPLC (system A) and the mixture was acidified to pH ~ 2.0 with 10% aq. HCl. Thereafter, the reaction mixture was evaporated under reduced pressure. The resulting residue was purified on a KP-C18-HS SNAP cartridge (60 g, Biotage®) by means of an automated flash purification system (Biotage® Isolera One), and by using a linear gradient of CH_3OH (0-100%) in aq. TFA as the mobile phase (flow rate 50 mL min^{-1} and UV detection at 260 nm). The product-containing fractions were lyophilised to give *N*-Fmoc trisulfonated linker **7** as a glassy solid (mono DIEA salt, 316 mg, yield 42%).



7

ν_{max}/cm^{-1} 740, 1013, 1147, 1149, 1533, 1658, 1719; δ_H(300 MHz; DMSO-d_6) 8.18 (1H, bs, N*H*), 7.87 (2H, d, *J* 7.2, Ph-Fmoc), 7.70 (2H, d, *J* 7.0, Ph-Fmoc), 7.64 (1H, bs, N*H*), 7.42-7.31 (4H, m, Ph-Fmoc), 7.17 (1H, bs, N*H*), 4.21-4.13 (3H, m, C*H*$_2$-Fmoc & C*H*(SO_3H)$_2$), 3.90 (1H, bd, *J* 7.8, C*H*-β-Ala(SO_3H)), 3.65-3.28 (7H, m, C*H*-Fmoc, C*H*$_2$-β-Ala(SO_3H), C*H*$_2$-CH(SO_3H)$_2$, C*H*$_2$-DIEA), 3.12 (2H, m, 2 × C*H*-DIEA), 1.26-1.20 (15H, m, 5 × C*H*$_3$-DIEA); δ_C(75.5 MHz; DMSO-d_6) 12.5 (1 × CH_3), 16.8 (2 × CH_3), 18.1 (2 × CH_3), two CH_2 masked by DMSO signal, 41.9 (CH_2), 46.6 (CH), 53.6 (2 × CH), 65.7 (CH_2), 65.8 (CH), 71.7 (CH), 75.0 (CH), 120.1 (2 × CH), 125.6 (2 × CH), 127.3 (2 × CH), 127.7 (2 × CH), 140.7 (2 × Cq), 143.9 (2 × Cq), 155.9 (C=O), 166.2 (C=O); HPLC (system A): t_R = 23.1 min (broad peak), purity > 98%; (ESI-): *m/z* 577.33 $[M - H]^-$, calcd mass for $C_{20}H_{22}N_2O_{12}S_3$ 578.60. This compound proved to be too hygroscopic for suitable elemental analysis.

The other main product of this reaction was identified as the carboxamide derivative of *N*-Fmoc-α-sulfo-β-alanine (Fmoc-β-Ala(SO_3H)-NH_2 **8** , off-white amorphous powder, 375 mg, yield 50%) and its formation was assumed as the following: an aq. base-catalysed β-elimination reaction leading to the release of 1,1-ethenedisulfonic acid (*i.e.*, methylenemethionic acid).

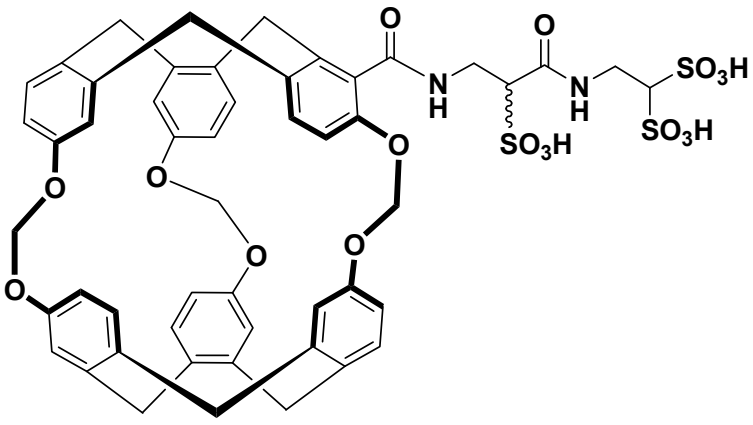


8

ν_{max}/cm^{-1} 735, 1042, 1163, 1250, 1442, 1537, 1659, 1694, 3200, 3349; δ_H(300 MHz; DMSO-d_6) 7.88 (2H, d, *J* 7.5, Ph-Fmoc), 7.68 (2H, d, *J* 6.6, Ph-Fmoc), 7.41 (2H, t, *J* 7.4, Ph-Fmoc), 7.32 (2H, t, *J* 7.4, Ph-Fmoc), 7.16 (1H, bs, N*H*), 6.97 (2H, bs, NH_2), 4.26-4.21 (3H, bm, CH_2-Fmoc & C*H*-β-Ala(SO_3H)), 3.48-3.33 (3H, m, C*H*-Fmoc & CH_2-β-Ala(SO_3H); δ_C(75.5 MHz; DMSO-d_6) one CH_2 masked by DMSO signal, 46.6 (CH_2), 64.7 (CH), 65.5 (CH), 120.0 (2 × CH), 125.2 (2 × CH), 127.1 (2 × CH), 127.6 (2 × CH), 140.6 (2 × Cq), 143.8 (2 × Cq), 155.7 (C=O), 169.1 (C=O); HPLC (system A): t_R = 30.7 min, purity 87%; (ESI-): *m/z* 389.20 [M - H]$^-$, calcd mass for $C_{18}H_{18}N_2O_6S$ 390.40. This compound proved to be too hygroscopic for suitable elemental analysis.

(b) Fmoc removal:
N-Fmoc derivative **8** (194 mg, 0.33 mmol) was dissolved in DMF (3 mL) and the resulting solution was cooled to 4 °C. Then, diethylamine (1 mL, 9.6 mmol, 29 equiv.) was added dropwise and the resulting reaction mixture was stirred at rt for 2 h. The reaction was checked for completion by RP-HPLC (system A). Thereafter, the reaction mixture was evaporated under reduced pressure without warming. The resulting residue was dissolved in deionised water (30 mL) and washed with CH_2Cl_2 (3 × 30 mL). The aq. phase was evaporated under high vacuum and at rt to avoid trans-amidification reaction of the pseudo-peptidyl bond involved between the two sulfonated units and the resulting yellow residue was rapidly desalted by ion-exchange chromatography (to remove diethylammonium salts) and lyophilised to give the acid form of trisulfonated linker **4** as a yellow hygroscopic amorphous powder (97 mg, yield 83%). δ_H(300 MHz, D_2O) 4.22 (1H, m, 1 × CH_2-CH(SO_3H)$_2$), 4.13 (2H, m, 1 × CH_2-CH(SO_3H)$_2$ & C*H*-β-Ala(SO_3H)), 3.74 (1H, dd, *J* 14.3, *J* 5.7, CH_2-C*H*(SO_3H)$_2$), 3.53 (2H, m, CH_2-β-Ala(SO_3H)); δ_C(75.5 MHz, D_2O) 37.4 (CH_2), 38.3 (CH_2), 61.4 (CH), 73.5 (CH), 165.8 (C=O); (ESI-): *m/z* 355.13 [M - H]$^-$,calcd mass for $C_5H_{12}N_2O_{10}S_3$ 355.99. Compound **4** proved to be too hygroscopic for suitable IR and elemental analysis.

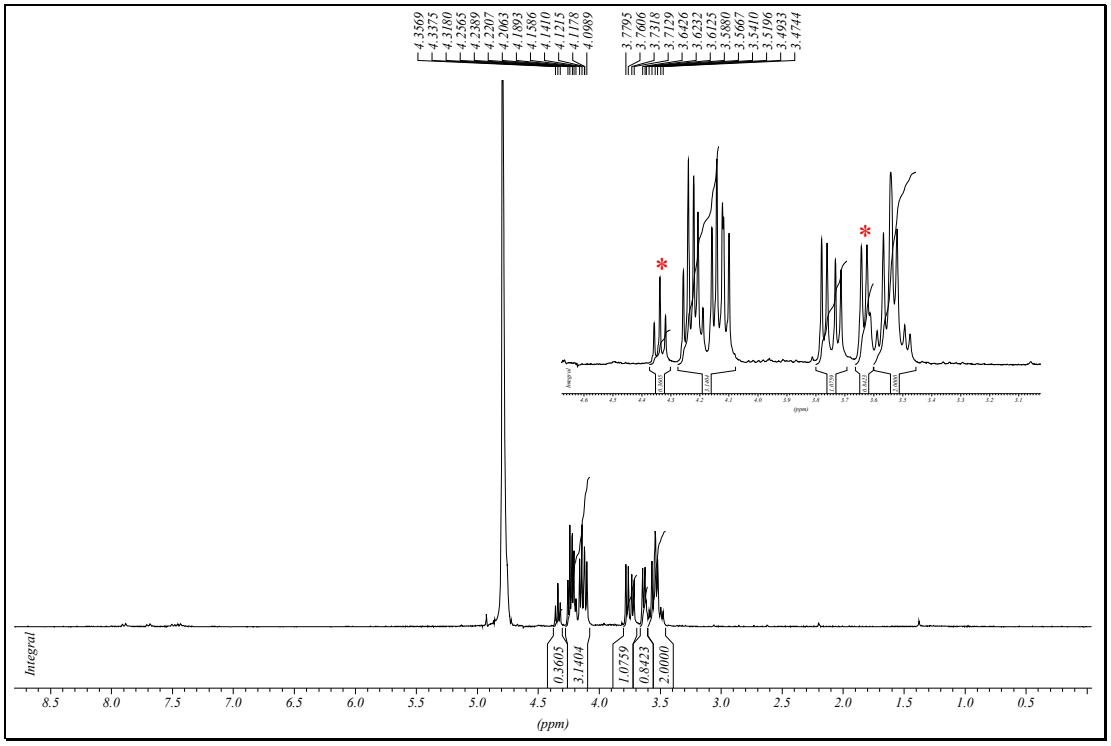
Water-soluble cryptophane (3).



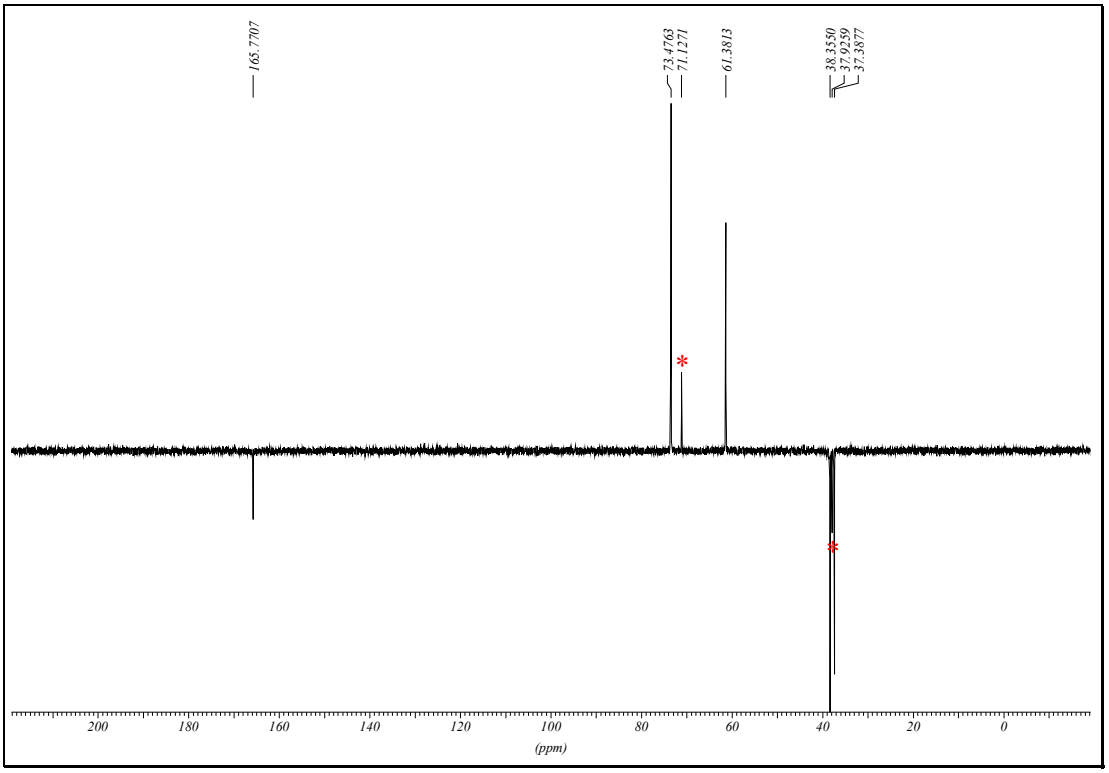
3

Cryptophane carboxylic acid **5** (4.0 mg, 5.6 µmol) was dissolved in dry DMSO (200 µL). Trisulfonated amino Linker **4** (8.4 mg, 16.8 µmol, 3 equiv.) and PyBOP® coupling reagent (8.7 mg, 16.8 µmol, 3 equiv.) were sequentially added. The resulting reaction mixture was stirred at rt for 1 min until the complete dissolution of **5** was observed. Then, a 2.0 M solution of DIEA in NMP (56 µL, 112 µmol, 20 equiv.) was added dropwise. The reaction was checked for completion by LC-MS (system B). Further amount of PyBOP® coupling reagent (8.7 mg, 16.8 µmol, 3 equiv.) and 2.0 M DIEA solution were added (56 µL, 112 µmol, 20 equiv.) and the reaction was finally quenched by dilution with aq. TFA 0.1% (~ 3 mL) after stirring at rt for 4 h. Purification was performed by RP-HPLC (system C, 2 injections). The product-containing fractions were lyophilised to give water-soluble cryptophane **3** as a white amorphous powder (2.5 mg, 2.4 µmol, yield 43%). δ_H(D_2O, 400 MHz) *vide infra*; HPLC (system B): t_R 34.9 min; (ESI-) *m/z* 1053.20 [M - H]$^-$, calcd for $C_{51}H_{46}N_2O_{17}S_3$ 1054.19.

^{1}H NMR spectrum of trisulfonated linker 4 recorded in D_2O at 300 MHz.

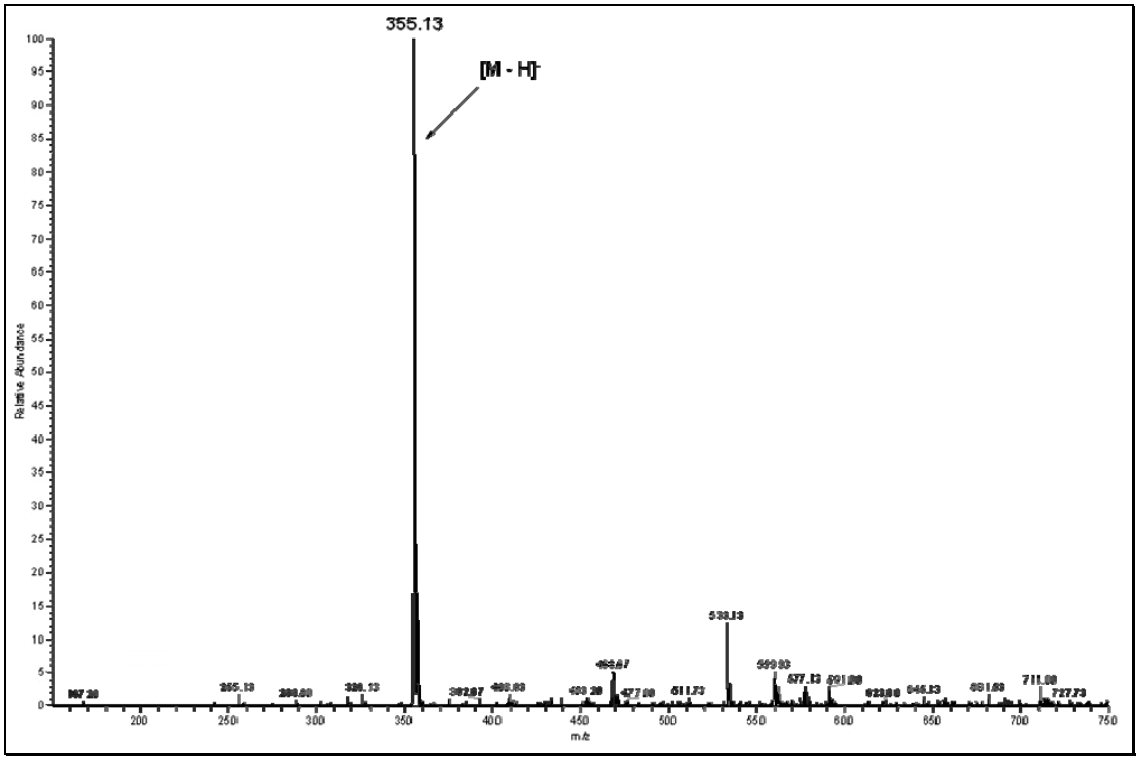


^{13}C NMR spectrum (JMOD) of trisulfonated linker 4 recorded in D_2O at 75.5 MHz.

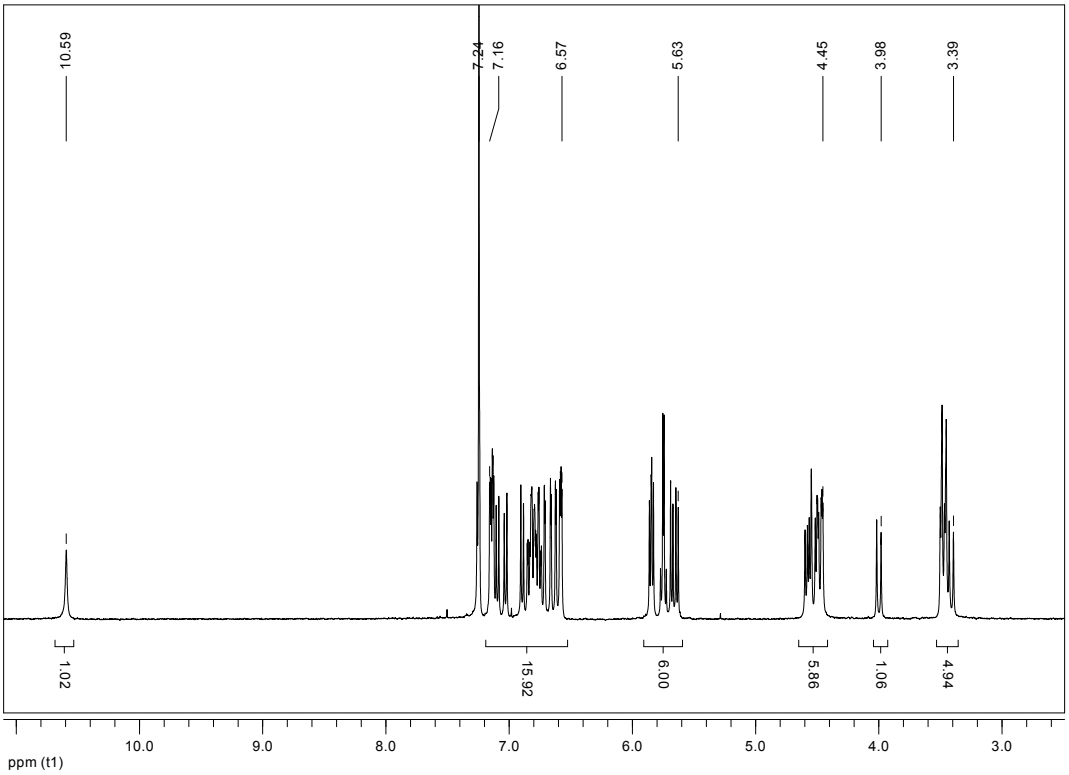


peaks assigned to residual amount of 2-aminoethane-1,1-disulfonic acid formed through acid hydrolysis of trisulfonated linker **4, during the course of ion-exchange chromatography over Dowex H^+ resin.*

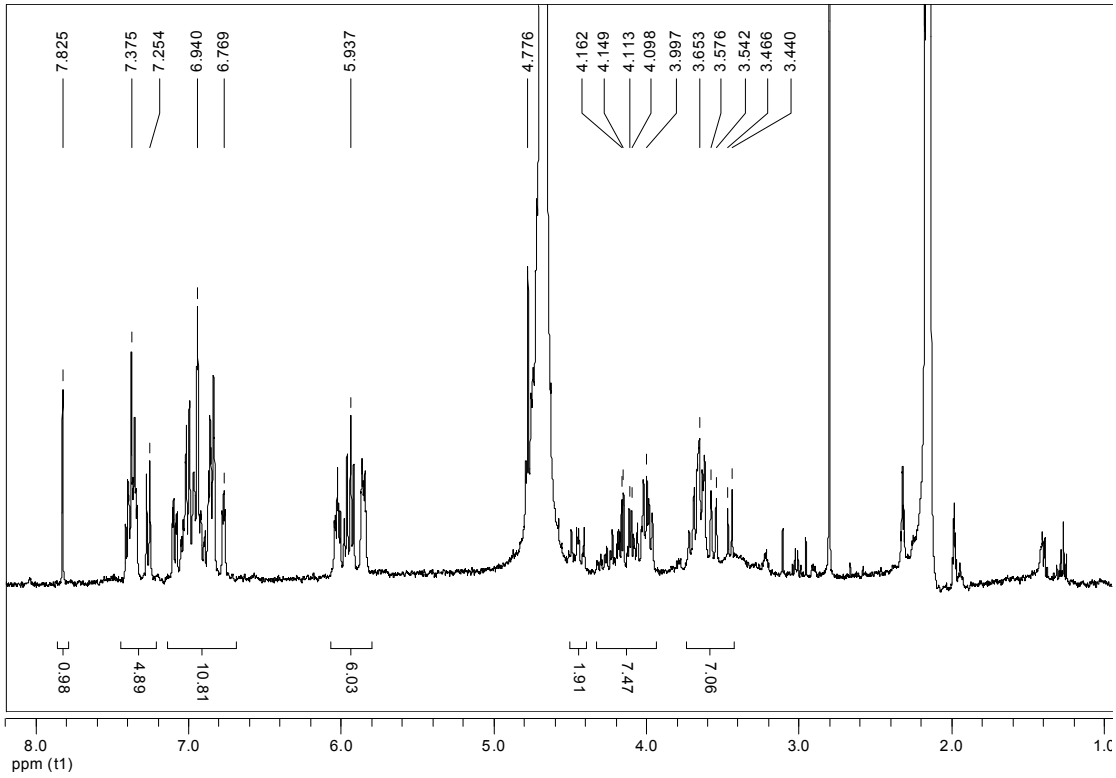
ESI mass spectrum of trisulfonated linker 4 recorded in the negative mode.



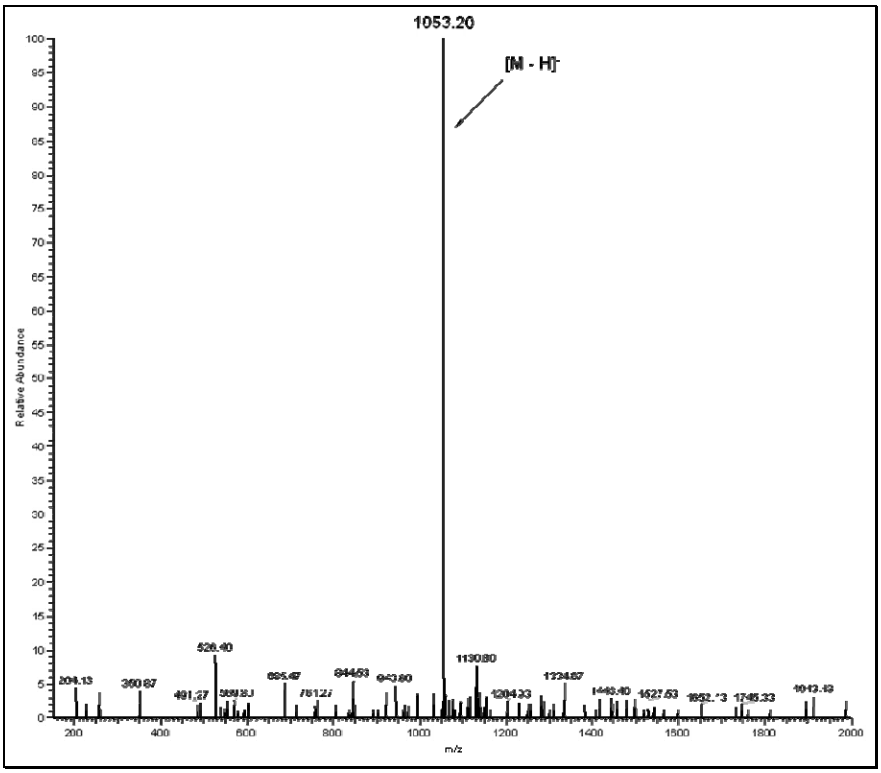
^{1}H NMR spectrum of cryptophane 5 recorded in $CDCl_3$ at 400 MHz.



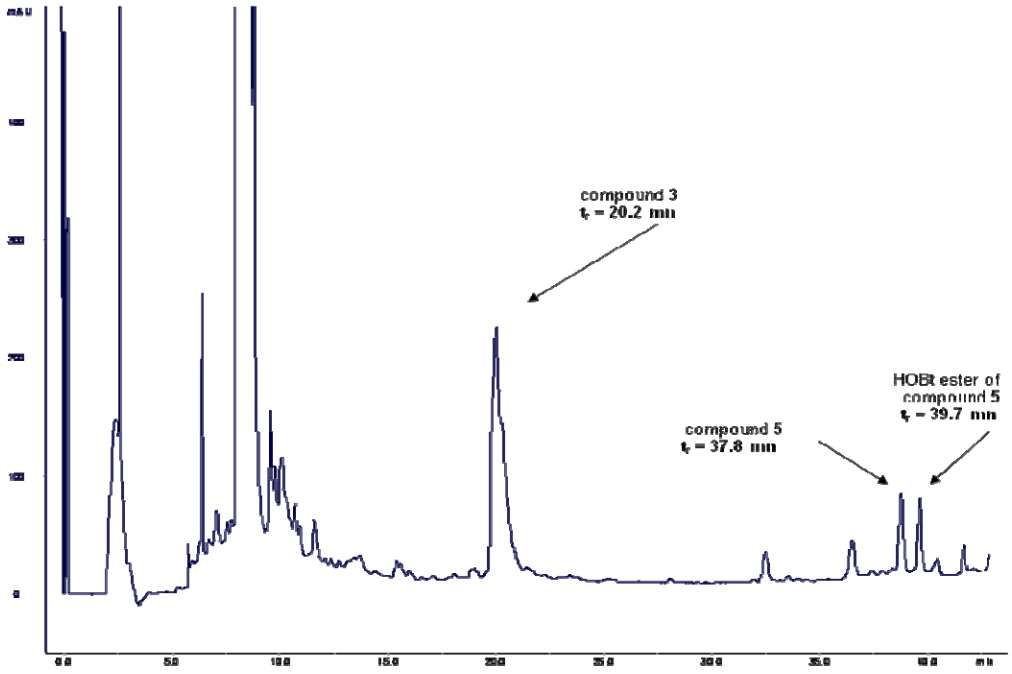
^{1}H NMR spectrum of water-soluble cryptophane 3 recorded in D_2O at 400 MHz.

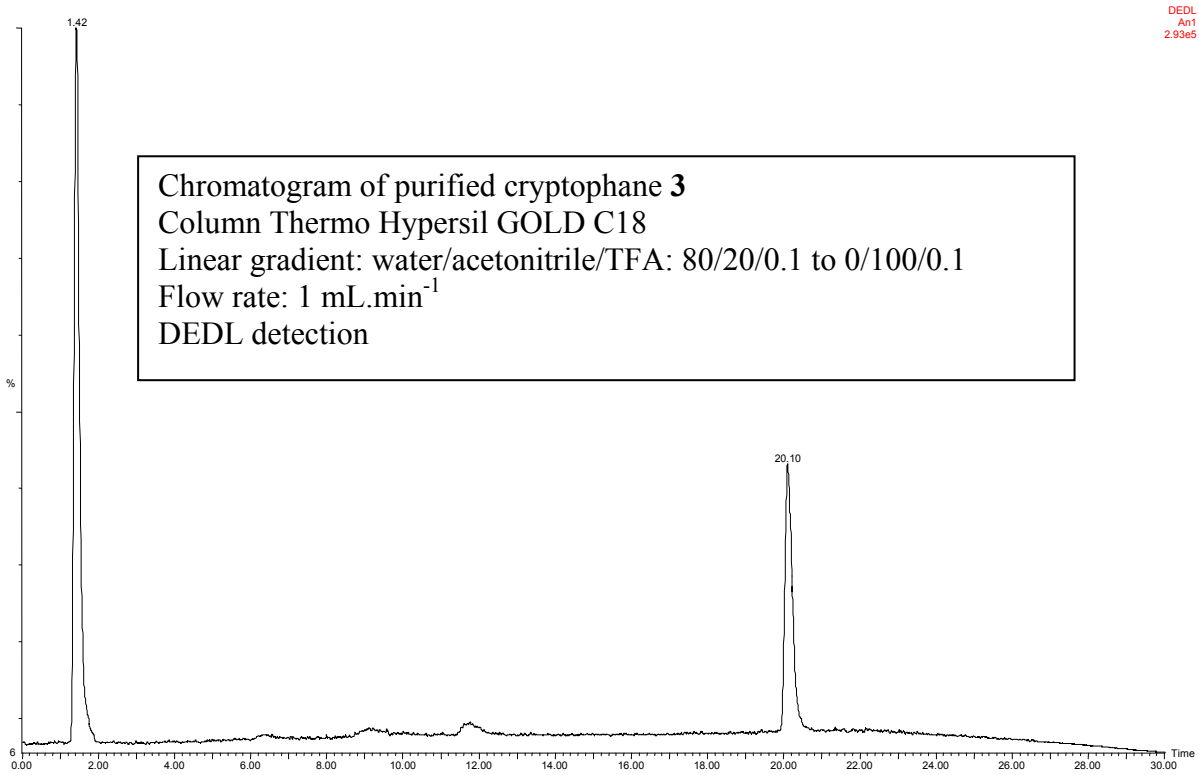


ESI mass spectrum of water-soluble cryptophane 3 recorded in the negative mode.



HPLC purification chromatogram of compound 3 (UV detection at 300 nm)





Chromatogram of purified cryptophane **3**
Column Thermo Hypersil GOLD C18
Linear gradient: water/acetonitrile/TFA: 80/20/0.1 to 0/100/0.1
Flow rate: 1 $mL.min^{-1}$
DEDL detection