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Supporting Information

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Supporting Information

for

Expanding the Scope of Protein *trans*-Splicing to Fragment Ligation of an Integral Membrane Protein: Towards Modulating Porin-Based Ion Channels by Chemical Modification

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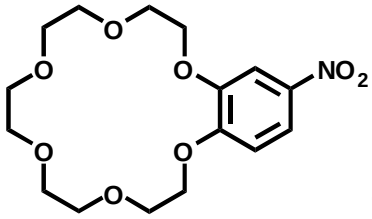
Contents

1 Experimental procedures

1.1 Synthesis of cysteine modifying reagent (6)

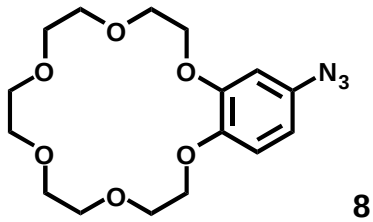
(4-Nitro)-benzo[18]crown-6-ether



7

Benzo[18]crown-6 (1 g, 3.2 mmol) was dissolved in $CHCl_3$ (100 mL) and cooled to 0 °C. $NO_2^+BF_4^-$ (0.425 g, 3.2 mmol), dissolved in acetonitrile (50 mL), was slowly added. The reaction mixture was vigorously stirred and allowed to warm up to room temperature over night. After completion (TLC: $CHCl_3$/MeOH 10:1) silica gel was added and the solvent was removed under reduced pressure. The resulting solid was purified by column chromatography with silica gel ($CHCl_3$/MeOH 100:10) yielding **7** (1.12 g, 3.13 mmol, 98%) as a yellowish solid. ^{1}H NMR ([D_6]DMSO, 300 MHz): δ = 7.93 (dd, *J* = 9.06, 2.64 Hz, 1H, H_{Ar}), 7.77 (d, *J* = 9, 2.64 Hz, 1H, H_{Ar}), 7.22 (d, *J* = 9.06 Hz, 1H, H_{Ar}), 4.27-4.23 (m, 4H, -CH_2-), 3.83-3.81 (m, 4H, -CH_2-), 3.65-3.56 (m, 12H, -CH_2-). ^{13}C NMR ([D_6]DMSO, 75 MHz): δ = 154.05, 147.85, 140.54, 117.59, 111.63, 102.26 (C_{Ar}), 69.93, 69.78, 69.64, 68.80, 68.61, 68.33, 68.27 (-CH_2-). HR-MS: (ESI) *m/z* for $C_{16}H_{23}NO_8$ [*M*+Na]$^+$ calcd.: 380.1321, found: 380.1320.

(4-Azido)-benzo[18]crown-6-ether



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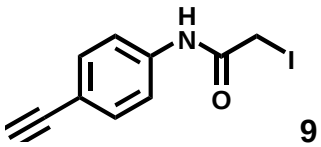
(4-Nitro)-benzo[18]crown-6-ether **7** (0.5 g, 1.4 mmol) was dissolved in glacial acid (25 mL). Fe-powder (0.78 g, 14 mmol) was added and the reaction mixture was vigorously stirred at room temperature until conversion was complete (TLC: $CHCl_3$/MeOH/ NH_3 10:1:0.1). The insoluble residue was filtered and the filtrate was cooled to 0 °C. $NaNO_2$ (0.106 g, 1.54 mmol) was added, the mixture was stirred for 15 min and subsequently NaN_3 (0.100 g, 1.54 mmol) was slowly added (***CAUTION***: N_2-formation).

After complete conversion (TLC: $CHCl_3$/MeOH/NH_3 10:1:0.1) the reaction mixture was extracted six times with 30 mL $CHCl_3$/*i*PrOH (5:1). The deep red raw material was purified by column chromatography ($CHCl_3$/MeOH 100:8) yielding azide **8** (0.49 g, 1.39 mmol, 99%) as a light yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.89 (d, *J* = 8.7 Hz, 1H, H_{Ar}), 6.62 (dd, *J* = 2.4, 8.7 Hz, 1H, H_{Ar}), 6.55 (d, *J* = 2.7 Hz, 1H, H_{Ar}) 4.17-4.14 (m, 4H, -CH_2-), 3.96-3.93 (m, 4H, -CH_2-), 3.79-3.70 (m, 12H, -CH_2-). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 146.31, 115.32, 115.27, 111.12, 105.48 (C_{Ar}), 70.99, 70.70, 70.49, 70.42, 70.14, 69.60, 69.54, 69.46, 69.43, 69.00, 68.59 (-CH_2-). HR-MS: (ESI) *m/z* for $C_{16}H_{23}N_3O_6$ [*M*+Na]$^+$ calcd.: 376.3601, found: 376.3611.

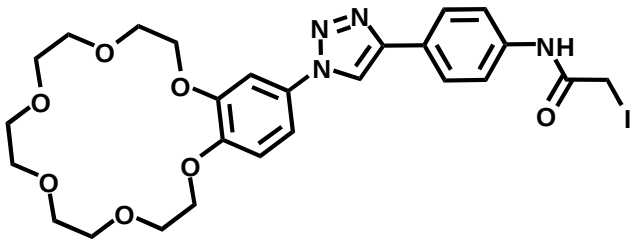
_N_-(4-Ethynyl-phenyl)-2-iodo-acetamide



9

4-Ethynyl-phenylamine (0.1 g, 0.85 mmol) was dissolved in $CHCl_3$ (60 mL) and iodo-acetic anhydride (0.36 g, 1 mmol) was added. The reaction mixture was stirred at room temperature for 3 h (TLC: $CHCL_3$/MeOH/NH_3 10:1:0.1). The solvent was evaporated and the crude product was purified by column chromatography ($CHCl_3$/MeOH 100:1) yielding **9** (0.23 g, 96%) as a white solid. ^{1}H NMR ([D_6]DMSO, 300 MHz): δ = 10.48 (s, 1H, NH), 7.59 (d, *J* = 7.65 Hz, 2H, H_{Ar}), 7.44 (d, *J* = 7.50 Hz, 2H, H_{Ar}), 4.10 (s, 1H, H_{Alkin}), 3.83 (s, 2H, -CH_2-I). ^{13}C NMR ([D_6]DMSO, 75 MHz): δ = 166.70 (C=O), 139.25, 132.33, 118.78, 116.40 (C_{Ar}), 83.31 (HC≡), 79.91 (≡C-), 1.23 (-CH_2-I). HR-MS: (ESI) *m/z* for $C_{10}H_8INO$ [*M*+Na]$^+$ calcd.: 307.9548, found: 307.9551.

{[(Benzo[18]crown-6-ether)-1H-[1,2,3]triazole-4-yl]-phenyl}-2-iodo-acetamide



6

Azide **8** (0.12 g, 0.35 mmol) and iodo-acetamide **9** (0.1 g, 0.35 mmol) were dissolved in EtOH/H_2O (4 mL, 1:1). Na-ascorbate (10 mol%) was added and the solution was

stirred for 15 min. $CuSO_4$ (1 M, 20 mol%) was added and the mixture was stirred over night. The reaction mixture was diluted with water, the precipitate was filtrated and washed with ice-cold water. Product **6** (0.14 g, 0.22 mmol, 63%) was isolated as a colourless solid. ^{1}H NMR ([D_6]DMSO, 300 MHz): δ = 10.47 (s, 1H, NH), 9.15 (d, s, 1H, CH-triazole), 7.89 (d, *J* = 8.7 Hz, 2H, H_{Ar}) 7.70 (d, *J* = 8.7 Hz, 2H, H_{Ar}), 7.51 (d, *J* = 2.4 Hz, 1H, H_{Ar}), 7.46 (dd, *J* = 8.4, 2.4 Hz, 1H, H_{Ar}), 7.18 (d, *J* = 9.0 Hz, 1H, H_{Ar}), 4.23-4.18 (m, 2H, -CH_2-), 4.17-4.15 (m, 2H, -CH_2-), 3.85 (s, 2H, -CH_2-I), 3.81-3.77 (m, 4H, -CH_2-), 3.63-3.53 (m, 12H, -CH_2-). ^{13}C NMR ([D_6]DMSO, 100 MHz): δ = 166.55 (C=O), 148.62 ($C_{Ar,q}$), 148.26 ($C_{triazole}$), 146.17, 138.66 ($C_{Ar,q}$), 129.99 ($C_{triazole}$), 125.93 ($C_{Ar,q}$), 125.75, 125.63 (C_{Ar}), 119.76 ($C_{Ar,q}$), 119.30, 119.08, 113.17, 112.05, 105.48 (C_{Ar}), 69.83, 69.72, 69.66, 68.51, 68.45, 68.40, 68.34 (-CH_2-), 1.45 (-CH_2-I). HR-MS: (ESI) *m/z* for $C_{26}H_{31}IN_4O_7$ [*M*+Na]$^+$ calcd.: 661.1130, found: 661.1120.

1.2 DNA cloning of expression plasmids

The DNA sequence encoding full-length OmpF of *E. coli* DH5α was obtained by polymerase chain reaction (PCR) using genomic DNA as the template and the oligonucleotides 5'-GGTAGGTCATGATGAAGCGCAATATTCTG-3' and 5'-GTAGGAATTC-AAGCTTATTA GAACTGGTAAACGATACCC-3' as primers. After cloning the PCR product into the vector pCR2.1-TOPO (Invitrogen), the *Bsp*HI-*Eco*RI fragment was excised and ligated with the vector pET28a (Novagen), which was treated with the same restriction enzymes, to give plasmid OmpF1. To create pSB40 for the expression of native OmpF without signal sequence (as reference protein for CD recordings and Figure S3), a PCR was performed with template plasmid OmpF1 and 5'-ATAAA-GCTTAGAACTGGTAAACGATACCC-3' and 5'-ATACATATGGCAGAAATCTATAAC-AAAGATGG-3'. The amplified sequence was cloned into the vector pI_{C3}P (ref. S1) exploiting the *Nde*I and *Hind*III restriction sites.

For the construction of the fusion proteins with the split *Psp*-GBD Pol intein, the sequence encoding the N-terminal intein half (aa 1-440, which was found to contain a L195F substitution in pMI_{N3}) was amplified by PCR using the template DNA pMI_{N3} (ref. S1) and the oligonucleotides 5'-ATAGAATTCATGCGTTTTCCTTTGAC-3' and 5'-ATAGGATCCTTAGTGGTGGTGGTGGTGGTGTCTGTATTCCGTAAACTT-AAGTTCC-3', which introduced a C-terminal hexahistidin sequence (His_6). The PCR product was cloned into the pCR2.1-TOPO vector, digested with EcoRI and BamHI,

and ligated into pMI_{N3} to yield pSB26. The C-terminal intein sequence, consisting of aa 441-537, was excised from $pI_{C3}P$ and cloned into pET16b (Novagen) downstream of the His_{10}-sequence using the restriction enzymes NdeI and HindIII, giving rise to pSB23. The plasmids pMI_{N3} and $pI_{C3}P$ were kindly provided by Maurice Southworth and Francine Perler (ref. S1).

OmpF fragment sequences were obtained by PCR from plasmid OmpF1. With the primers 5'-ATACATATGGCAGAAATCTATAACAAAGATGG-3' and 5'-ATAGGTACCCATTCTTCCGGTAAAATGCTGTTTTCACCGTTACCCTTGG-3' the sequence for OmpF (aa 1-30) could be amplified, while a PCR with the former oligonucleotide together with 5'-ATAGGTACCCATTCTTCCGGTAAAATGCTAGCCTCGAGACCCTTGGAAAAATAATGCAGACC-3' resulted in the DNA fragment encoding OmpF (aa 1-26)-LEA. Each PCR product was cloned into pSB26 using the NdeI and KpnI restriction sites to create the expression plasmids pSB27 for construct OmpF(1-30)-*Psp* Pol(1-440)-His_6 (**1**) and pSB28 for OmpF(1-26)-LEA-*Psp* Pol(1-440)-His_6 (**1a**). The DNA sequence coding for OmpF (aa 31-340) was confined by 5'-ATAAAGCTTAGAACTGGTAAACGATACCC-3' and 5'-ATAGGATCCCTCTATGCACATAATAGTTACGGTGGCAATGGCGAC-3', whereas the substitution of the latter with 5'-ATAGGATCCCTCTATGCACATAATTCAGGCCTC AACGGTGAAAACAGTTACGGTGG-3' gave rise to the sequence of the fragment SGL-OmpF (aa 27-340). Both PCR products were digested with BamHI and HindIII and ligated into pSB23, treated the same way, to give pSB24 and pSB37, encoding His_{10}-*Psp* Pol(441-537)-OmpF(31-340) (**2**) and His_{10}-*Psp* Pol(441-537)-SGL-OmpF(27-340) (**2a**), respectively.

Mutant plasmid pSB44 for the expression of OmpF(1-26)K16C-LEA-*Psp* Pol(1-440)C351A-His_6 (**1b**) was obtained by site-directed mutagenesis using *Pfu*Turbo Hotstart DNA Polymerase (Stratagene). At first the template DNA pSB28 was applied to amplification with reverse complement primers containing the C351A mutation (5'-CATCTTTGAGAGCCTTGCTGGGACTTTGGCAGAAAAC-3', 5'-GTTTTCTGCCAAAGTCCCAGCAAGGCTCTCAAAGATG-3') and subsequent template degradation was achieved by incubation with *Dpn*I. The mutated plasmid pSB43 was used for *E. coli* transformation. The K16C mutation was introduced the same way using pSB43 and the primers 5'-GTAGATCTGTACGGTTGCGCTGTTGGTCTGCAT-3' and 5'-ATGCAGACCAACAGCGCAACCGTACAGATCTAC-3' to yield pSB44. All plasmids were verified by DNA sequencing.

1.3 Protein expression and purification

All constructs were expressed using the porin deficient *E. coli* strain BL21(DE3)omp8 (ref S2). Cells were grown in LB medium containing 100 µg mL^{-1} ampicillin at 37 °C until an optical densitiy of 0.7 at 600 nm was reached. Overexpression was induced by adding IPTG (0.2 mM) and allowed to proceed for 4 h. Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM Tris, pH 7, 300 mM NaCl, 1 mM EDTA, 5 mM DTT) and stored at -20 °C. After cell rupture usin g an emulsifier, insoluble inclusion bodies and cell debris were pelleted. The brown pellet was washed four to five times with wash buffer (50 mM Tris, pH 8, 300 mM NaCl, 1 mM EDTA, 2 mM DTT, 1 M urea, 2% TritonX-100) and once with wash buffer lacking urea and detergent to yield white inclusion bodies, which could be stored at 4 °C. For each experiment inclusion bodies were solubilized by shaking over night at 4 °C in splice buffer (50 mM Tris, pH 7, 300 mM NaCl, 6 M urea). Protein concentrations were determined using a bicinchoninic acid (BCA) protein quantitation kit (VWR). Native OmpF was extracted from *E. coli* cell envelopes using a shortened protocol according to ref. S3.

1.4 Cysteine labeling reactions

Protein solutions of **1b** (40 µM) were treated at room temperature with TCEP (5 eq.) for 15 min before of iodoacetamide reagent **6** (20 eq.) was added. After 10 min reaction time the pH was checked and if required readjusted to 7-7.5. TCEP and labeling reagent were applied again and reacted for 2 h. Unreacted iodoacetamide functionalities were quenched with DTT (5 eq. relative to **6**) and removed by dialysis against splice buffer.

1.5 Protein splicing assay

Complementary intein constructs were combined at a concentration of 20-40 µM and incubated over night at 4 °C. Reactions on a prepar ative scale were carried out in a total volume of 3 to 5 mL. The protein splicing reaction was induced by shifting the temperature to 37 °C and allowed to proceed over ni ght. For purification the pH was adjusted to 8.0 and 1 mL Ni^{2+}-NTA material (GE Healthcare) per 5 mL of protein splicing reaction were added. After shaking 2 h at 4 °C the material was pelleted by centrifugation for 15 min at 500×g. The supernatant was concentrated using Vivaspin 4 concentrator spin columns (Sartorius, MWCO 5000) to 1/10 of the initial volume before refolding.

1.6 Protein refolding and OmpF trimer purification

Mixed small unilamellar lipid vesicles composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC, Alexis Biochemicals) and dodecyl-*β*-*D*-maltoside (DDM, Carl Roth) were prepared according to Surrey *et al.* (Ref S4). Shortly, DMPC (10 mg) were dissolved in $CHCl_3$ (200 µL) followed by solvent evaporation at 35 °C with 300 mbar and drying in vacuo for 1 h. 2 mL of 20 mM KPO_4, pH 7.3 buffer were added, sonicated for 30 min at 30 °C, and shaken over night at the same temperature. After addition of DDM (7.6 mg) and sonication for 10 min at 30 °C, vesicles were incubated over night while shaking at 30 °C.

Concentrated protein splicing mixtures or recombinant OmpF samples were diluted 1:5 or 1:10 into suspensions of small unilamellar lipid vesicle in a volume of 0.5 to 2 mL. Refolding was performed by shaking over night at 37 °C and precipitated material was allowed to sediment at 4 °C. Trimeric OmpF was separated from residual monomeric protein and unreacted intein constructs by SDS-PAGE and subsequent gel extraction with 20 mM KPO_4, pH 7.3, 0.5%*n*-octylpolyoxyethylene (OPOE, Bachem) buffer by shaking over night at 37 °C. Alternatively, the supernatant of the refolding mixture was treated with trypsin at a ratio of 1:100 compared to protein content at 37 °C over night and loaded onto a HiTrap Q HP column (GE Healthcare) equilibrated with 20 mM Tris, pH 8.3, 1% OPOE buffer. Elution was accomplished by a linear gradient up to 1 M NaCl. Sample purity was always checked by SDS-PAGE and protein concentrations were determined using the BCA assay. Gel extracted samples still contained 20-50% starting material, whereas in trypsin digested samples only 5-10% impurities remained. Purified trimeric proteins were stored at 4 °C.

1.7 Black lipid membrane measurements

Planar lipid bilayer experiments were performed in a two chamber apparatus (cuvette: CP2A of polystyrene, bilayer chamber: BCH-22A, manufacturer: Warner Instruments). Both chambers of this unit were filled with a salt solution (150 mM KCl, 5 mM HEPES, pH 7.2) to the same height. Planar lipid membranes were prepared by painting a solution of a 4:1 mixture of L-α-phosphatidylcholine (Sigma P3556) and L-α-phosphatidylethanolamin (Sigma P8193) in *n*-decane (25 mg mL^{-1}) over the aperture of a polystyrene cuvette with a diameter of 0.20 mm or by rising the surface of the solution up and down over the aperture. A volume of 1 to 5 µL of the ion channel containing micelle solution (0.1 to 1 mg ion channel/mL) was added to the *trans*-side

of the cuvette, the membrane was formed again, and a voltage of +140 mV was applied. Current detection and recording were performed with a patch-clamp amplifier Axopatch 200B, a Digidata 1200B A/D converter and pClamp 9 software (Axon Instruments, Foster City, CA, USA). The acquisition frequency was 5 kHz. The data were filtered with a digital filter at 200 Hz for further analysis, applying the pClamp 9.2 software.

A typical behaviour of membrane incorporated OmpF-trimers is the opening as a trimer (all monomers open at the same time), followed by three separate monomer closings (O3 → O2 → O1 → C). For our analysis these trimer-monomer events were identified and the current alterations representative of the monomer closings were measured. Average and standard deviation were calculated on the base of at least 10 monomer closings. Results achieved this way showed a higher reproducibility and a narrower distribution than those obtained from measuring opening or closing events that were not part of a trimer-monomer event.

1.8 Circular dichroism spectroscopy

CD spectra were recorded on a Jasco J-715 spectropolarimeter at 8 ℃ using a cuvette with 0.1 mm path length for the recombinant control (0.25 mg mL^{-1}) and a 1 mm cuvette for the OmpF splice product **3** (0.03 mg mL^{-1}). The wavelength range of 190-260 nm was scanned with 1 nm increments and 20 accumulations. Spectra of elution buffer from ion exchange chromatography were used for background corrections.

2 Supplementary results

2.1 Preparation of OmpF with the insertion of residues LEASGL

For the construction of a second set of intein fusion proteins (**1a** and **2a**), OmpF was split after residue 26, which is located in the center of loop L1 (Figure S1C). As the protein splicing activity of most inteins is impaired by the nature of their flanking extein residues, we introduced the aa LEA and SGL directly adjacent to the intein (Figure S1 A). In this context the *Psp*-GBD Pol intein was reported to mediate efficient protein *trans*-splicing (Ref. S1). The protein splicing reaction of **1a** and **2a** yielded splice product **3a** (Figure S1 B), which represented the OmpF protein with the inserted LEA-SGL sequence, with a conversion of 30-40%. During the protein splicing reaction some protein precipitation was observed. However, splice product **3a** could be

puri-fied and concentrated to give refolded trimers at higher concentrations compared to splice product **3** with native primary sequence. The efficiency of trimer reconstitution was estimated to be ~80%. The reconstituted porin was positively tested for ion channel activity (data not shown), though full quantitative analysis was not carried out.

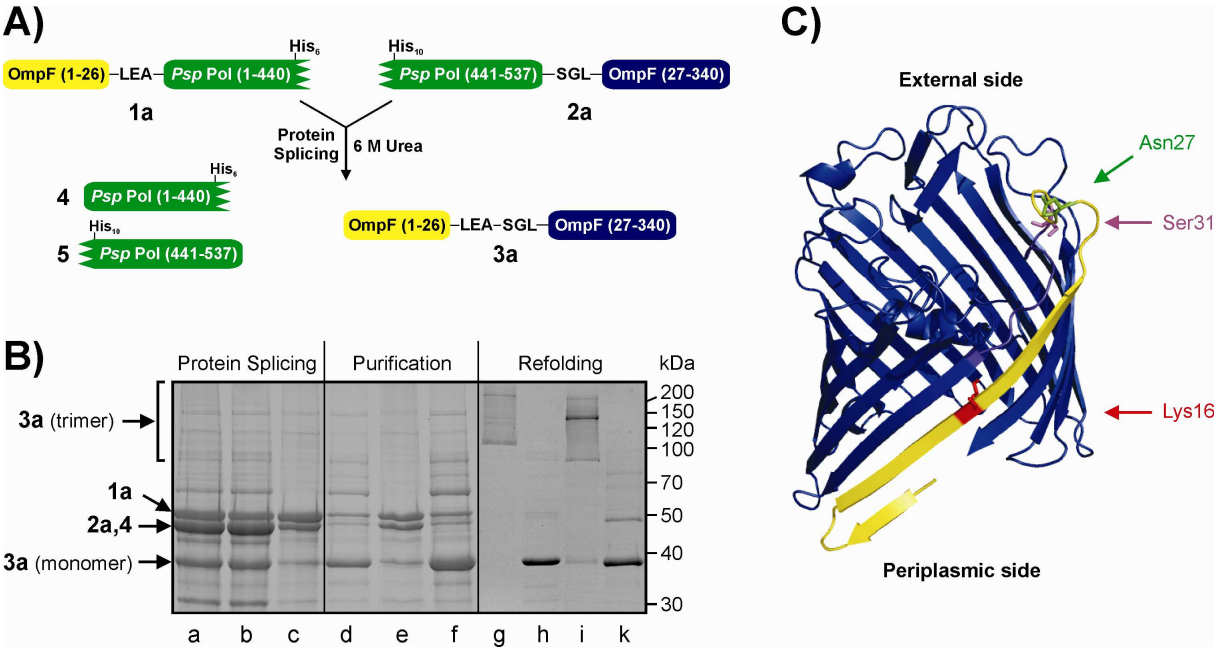


Figure S1: Preparation of the OmpF derivative **3a**. (A) Scheme of the protein splicing reaction. Flanked by the residues LEA and SGL the intein was known to promote efficient *trans*-splicing. (B) SDS-PAGE gel of preparation (a: protein splicing reaction of **1a** and **2a**, b: soluble fraction of the protein splicing reaction, c: precipitate during reaction, d: splice product **3a** separated by "negative" affinity purification, e: proteins bound to Ni^{2+}-NTA matrix, f: concentrated sample shown in d, g: refolded splice product **3a**, h: heat-denatured refolding mixture, i: gel extracted trimer, k: gel extracted trimer after heat-denaturation). (C) OmpF structure illustrating the significant positions used for ion channel engineering. Only one monomer is depicted.

2.2 Preparation of functionalized OmpF porin

The incorporation of crown ether building block **6** into the OmpF pore was accomplished through the reaction of the iodoacetamide moiety **6** with the cysteine side chain of construct **1b** to give **1b**-crown (Figure S2 A). The protein splicing reaction of **1b**-crown with **2a** produced modified product **3b**-crown (Figure S2 B), which could be purified, concentrated and reconstituted to finally give trimeric porin **3b**-crown (~60%

refolding efficiency) with the crown ether inside the pore lumen and the LEASGL sequence expanding the L1 loop. The band marked with an asterisk (Figure S2 B, lane g) arose from protein degradation, whereas the very faint band below that one of **3b-crown** represents remaining traces of unlabeled splice product **3b** (compare also lane e and g in Figure S3).

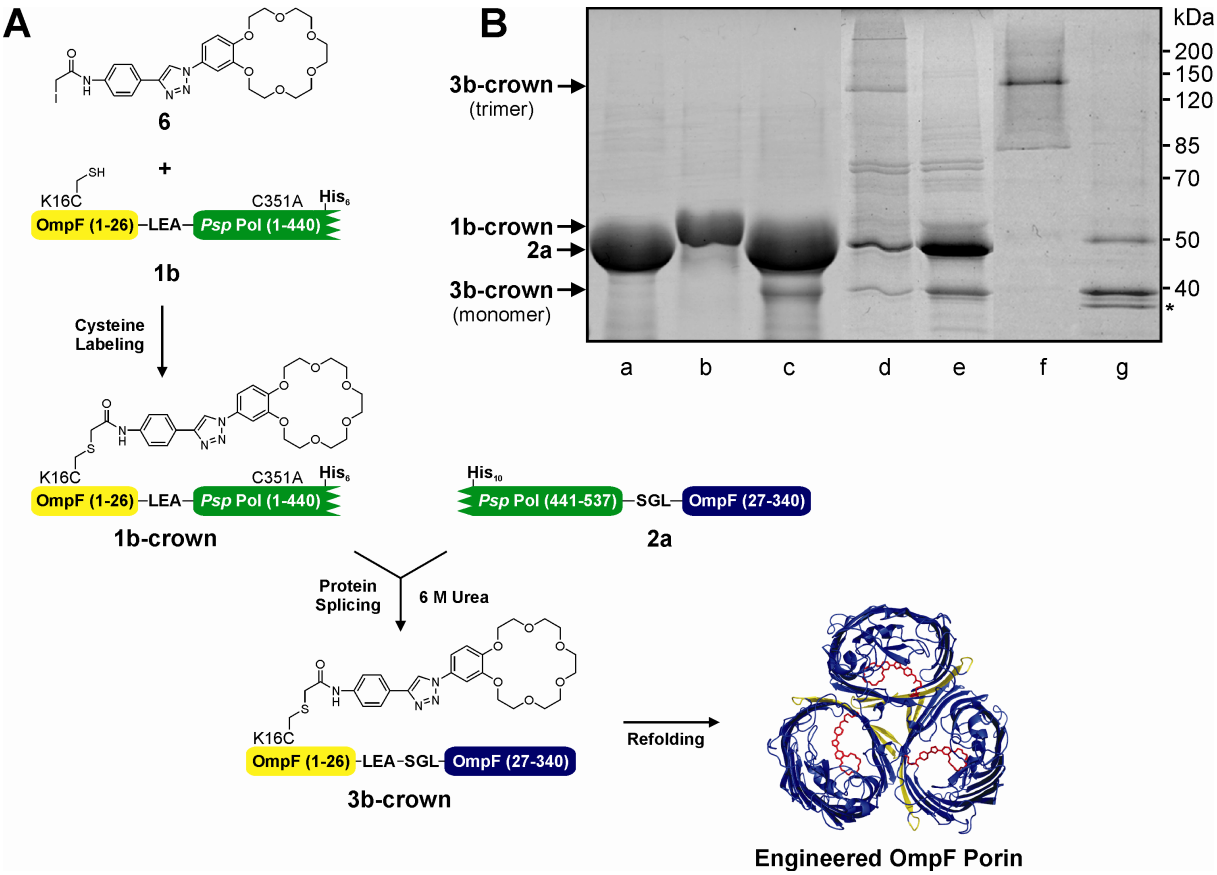


Figure S2: Preparation of the OmpF derivative **3b**-crown modified with a crown ether building block. A) Scheme illustrating the steps of the reaction sequence: cysteine labeling, protein *trans*-splicing under denaturing conditions and refolding of the modified trimer. B) SDS-PAGE gel of preparation (lane a: **2a**, b: **1b**-crown, c: protein splicing reaction of **1b**-crown and **2a**, d: refolding mixture of purified and concentrated splice product **3b**-crown, e: sample shown in d after heat denaturation, f: extracted trimer of **3b**-crown, g: denatured sample f, *degradation band).

2.3 Control experiment for protein splicing vs. C-terminal cleavage

We further confirmed that the band designated as the OmpF monomer (e.g. lane e in Figure 2; lane a in Figure S1; lane e in Figure S2) corresponded indeed to the ex-

pected splice product. Because of similar molecular weight, we wanted to rule out the possibility that this band arose from C-terminal cleavage, a common side reaction in protein splicing, where the peptide bond between intein and C-extein is broken before ligation with the N-extein sequence. To this end, we compared the electrophoretic mobility of the designated splice product with a control construct OmpF(27-340) N27C (**10**). This latter protein, which was kindly provided by S. Reitz and L.-O. Essen (ref. S5), lacks the first 26 aa of OmpF and thus closely resembles the predicted C-terminal cleavage products (see Tab. S1). Figure S3 shows that the band corresponding to **10** could clearly be resolved from all splice products. Thus, the absence of that band during splice product preparation (see Fig. 2 & S1 & S2) proves that C-terminal cleavage was negligible and the bands indicated indeed corresponded to full length OmpF monomer.

Table S1: Calculated molecular weights of splice products and potential C-terminal cleavage products.

Construct	Sequence	MW / kDa
3	OmpF (1-340)	37.1
3b	OmpF(1-26)-LEASGL-OmpF(27-340) K16C	37.6
3b-crown	OmpF(1-26)-LEASGL-OmpF(27-340) K16C-crown	38.1
C-cleavage of **1** + **2**	OmpF(31-340)	33.8
C-cleavage of **1b** + **2a**	SGL-OmpF(27-340)	34.5
10	OmpF(27-340) N27C	34.2

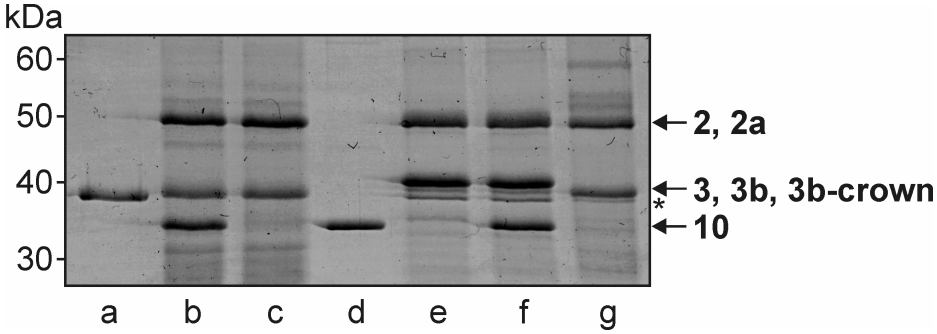


Figure S3: SDS-PAGE gel for comparison of splice products and approximated C-terminal hydrolysis product (a: recombinant OmpF standard; b: heat-denatured refolding mixture of splice product **3** mixed with **10**; c: denatured refolding mixture of **3** without added **10**; d: control protein **10**; e: denatured refolding mixture of **3b**-crown; f: sample shown in e mixed with **10**; g: refolding mixture of **3b** after heat-denaturation).

2.4 BLM recordings

Figure S4 shows representative current traces for samples analyzed by the BLM method in addition to Figure 3. The statistical analysis of current decrements for samples **3b** and **3b**-crown (see main text) revealed relatively broad standard deviations. This might be due to a mixture of oxidation states of the free cysteine in the case of **3b** and to different positions occupied by the synthetic crown ether moiety due to its conformational flexibility in case of **3b**-crown.

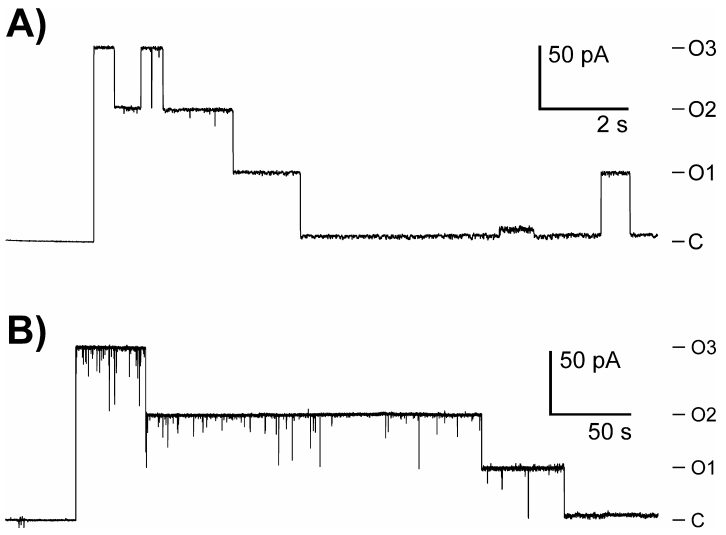


Figure S4: Current traces of single channel conductance recordings in a black lipid membrane (BLM). A) Native OmpF standard extracted from *E. coli* cell envelopes. B) OmpF splice product **3b** (decrement: 51.7 ± 13 pA, trimer conductance: 1.11 ± 0.28 nS).

3 References

S1. M. W. Southworth, E. Adam, D. Panne, R. Byer, R. Kautz, F. B. Perler, *EMBO J.* **1998**, *17*, 918-926.

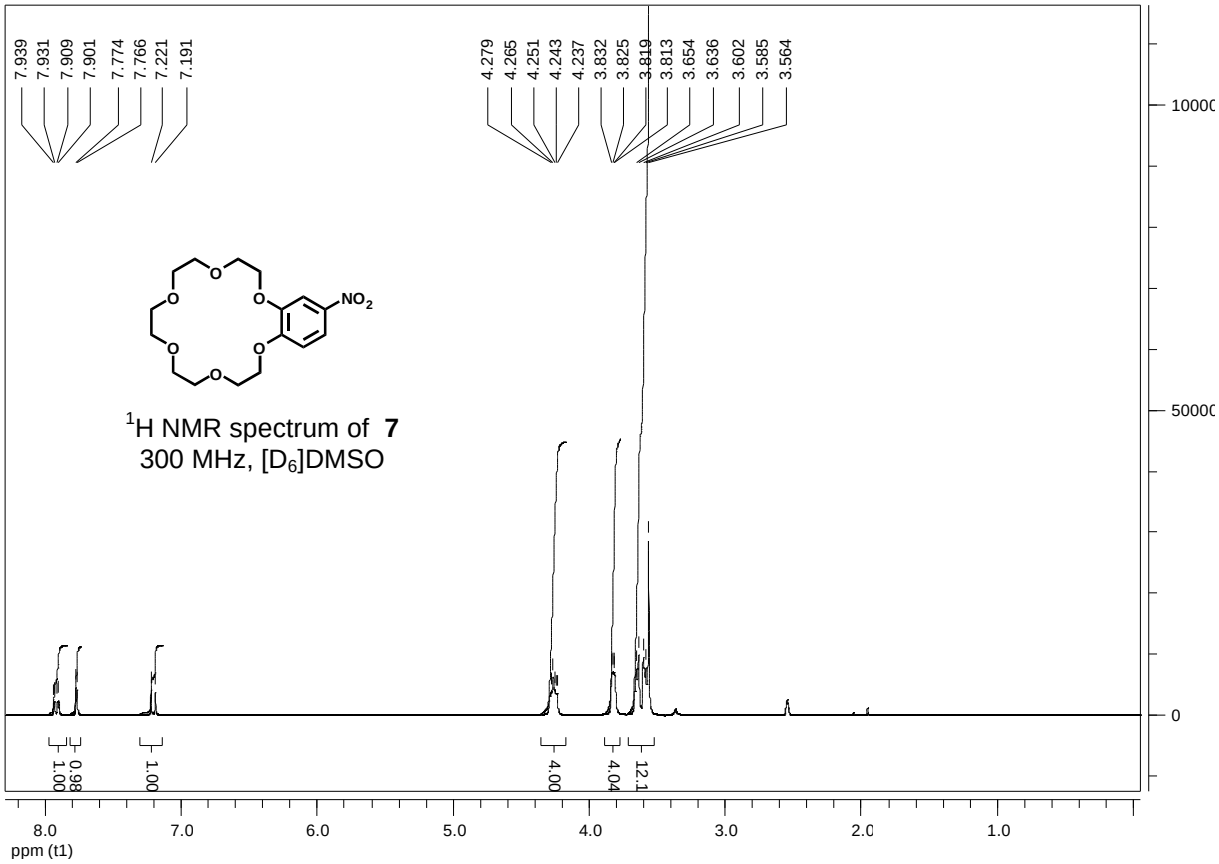
S2. A. Prilipov, P. S. Phale, P. Van Gelder, J. P. Rosenbusch, R. Koebnik, *FEMS Microbiol. Lett.* **1998**, *163*, 65-72.

S3. R. M. Garavito, J. P. Rosenbusch, *Methods Enzymol.* **1986**, *125*, 309-328

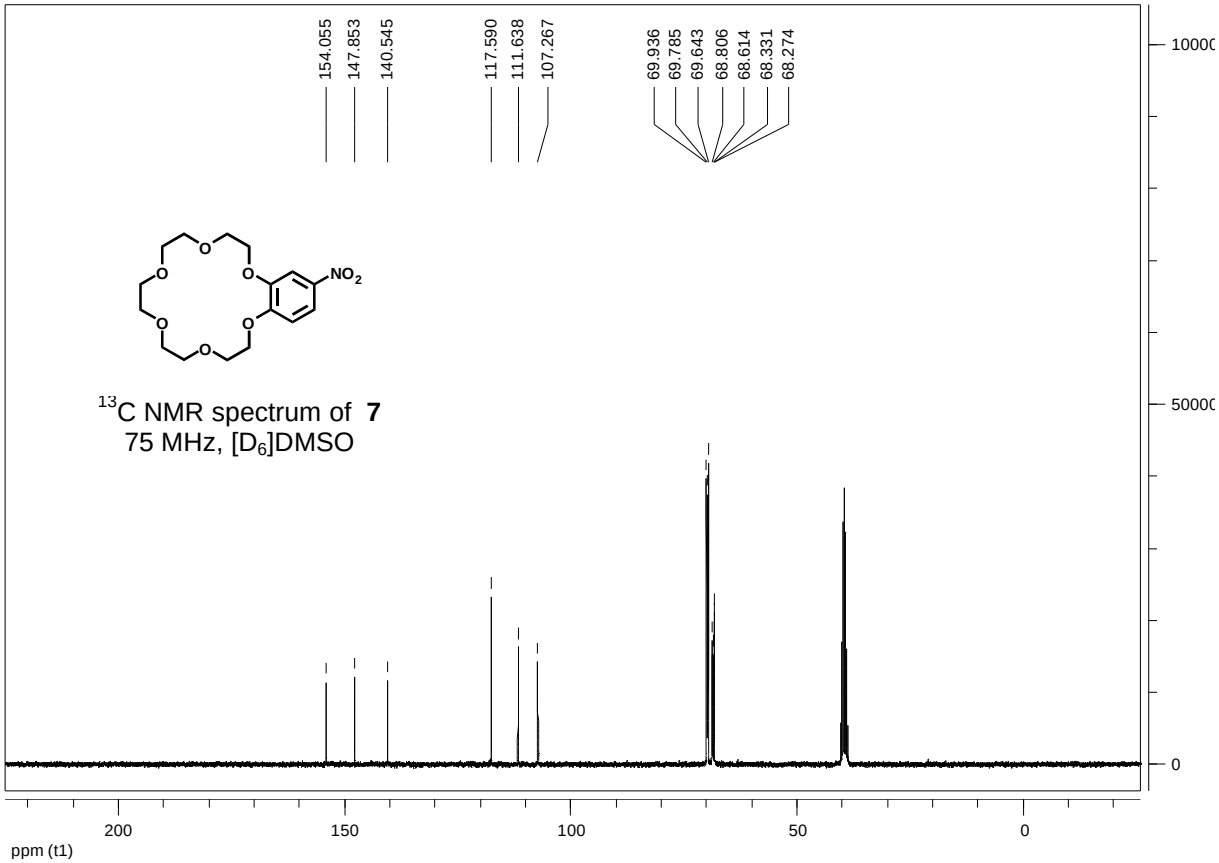
S4. a) T. Surrey, F. Jähnig, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7457-7461; b) T. Surrey, A. Schmid, F. Jahnig, *Biochemistry* **1996**, *35*, 2283-2288.

S5. S. Reitz, M. Cebi, P. Reiß, G. Studnik, U. Linne, U. Koert and L.-O. Essen, *Angew. Chem.* **2009**, *in press*.

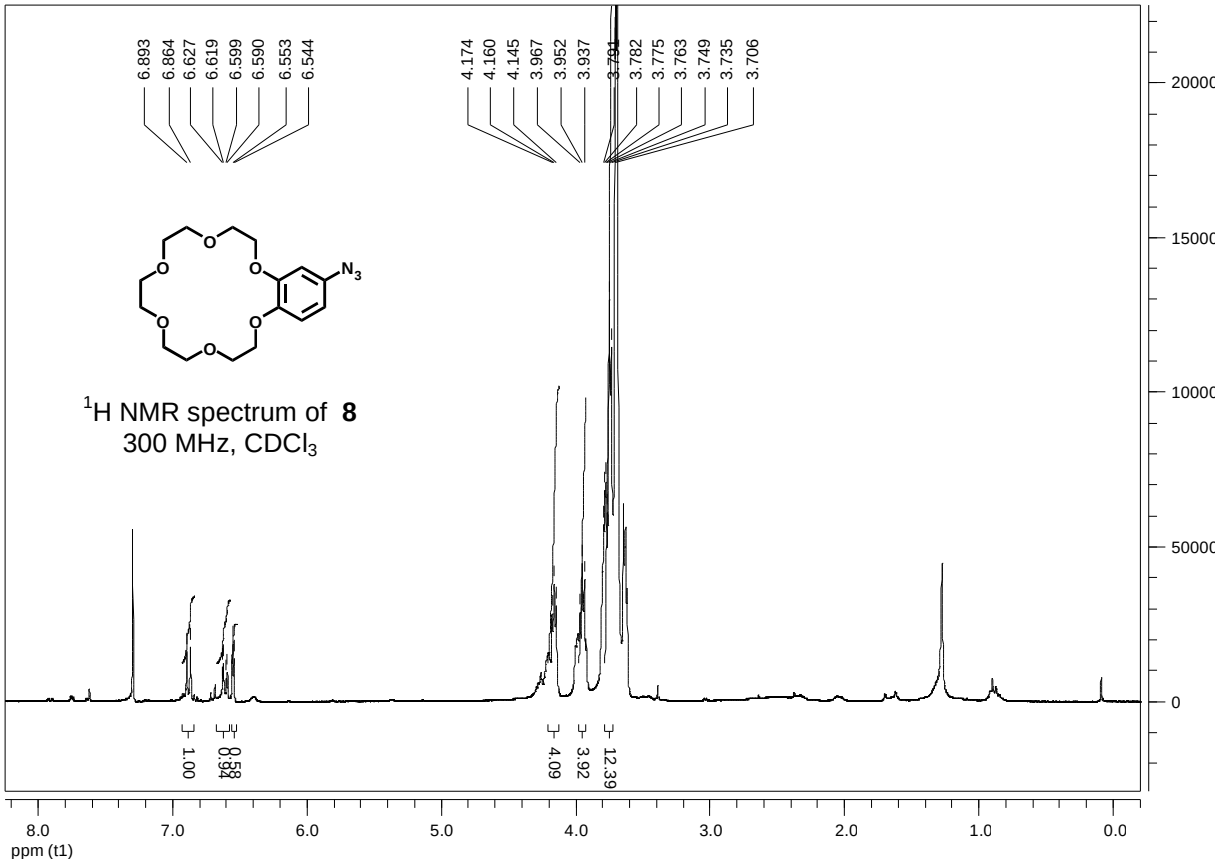
4 Appendix

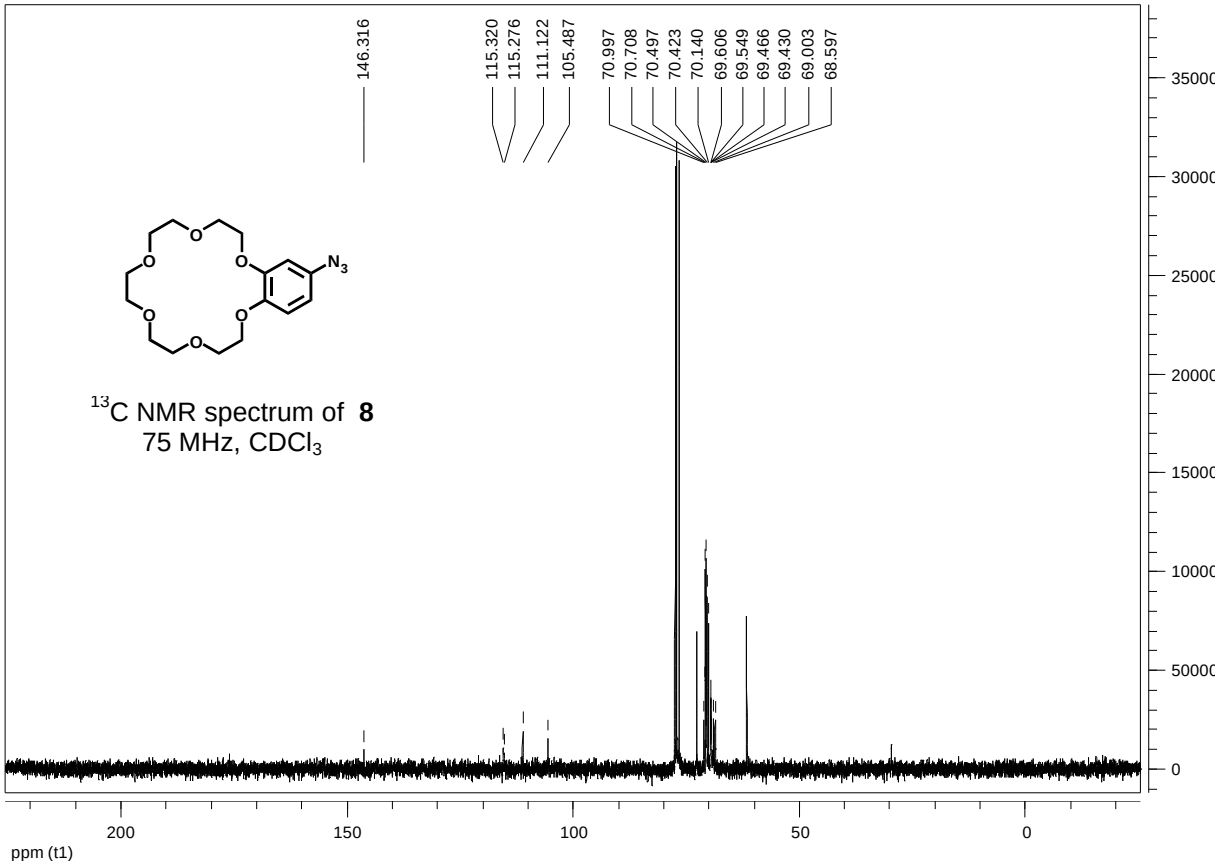


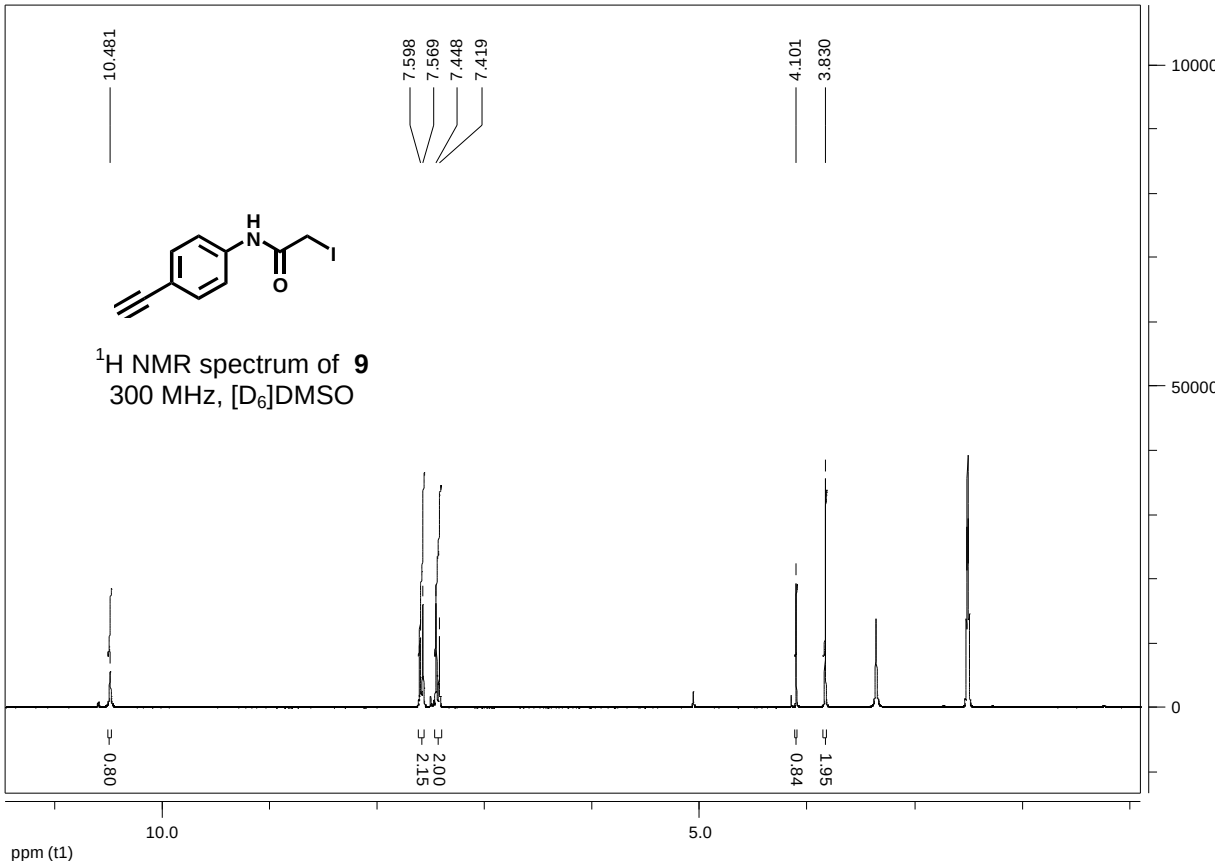
^{1}H NMR spectrum of **7**
300 MHz, [D_6]DMSO



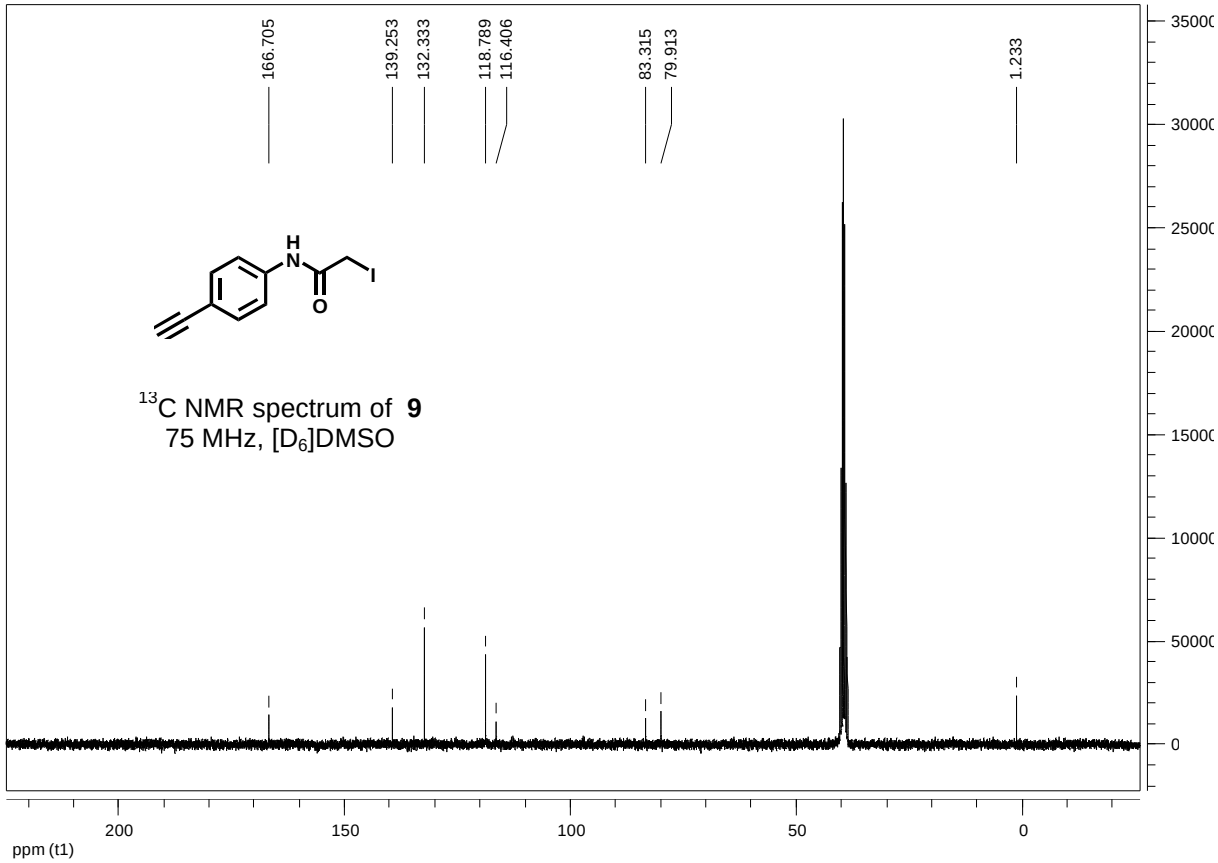
^{13}C NMR spectrum of **7**
75 MHz, [D_6]DMSO


6.893
6.864
6.627
6.619
6.599
6.590
6.553
6.544
4.174
4.160
4.145
3.967
3.952
3.937
3.791
3.782
3.775
3.763
3.749
3.735
3.706
O
O
O
O
O
O
N3
1H NMR spectrum of 8
300 MHz, CDCl3
1.00
0.98
0.58
4.09
3.92
12.39
20000
15000
10000
50000
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

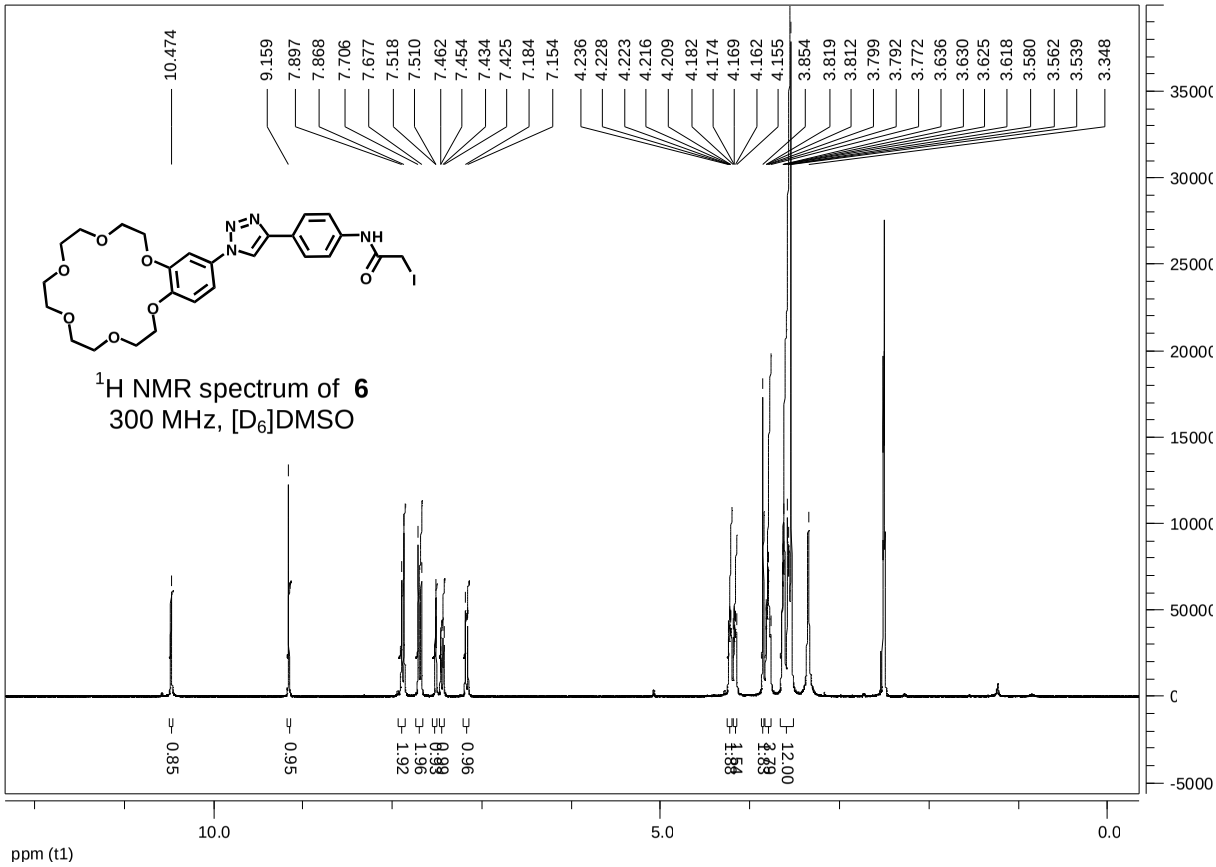

146.316
115.320
115.276
111.122
105.487
70.997
70.708
70.497
70.423
70.140
69.606
69.549
69.466
69.430
69.003
68.597
O
O
O
O
O
O
N3
13C NMR spectrum of 8
75 MHz, CDCl3
35000
30000
25000
20000
15000
10000
50000
0
200
150
100
50
0
ppm (t1)



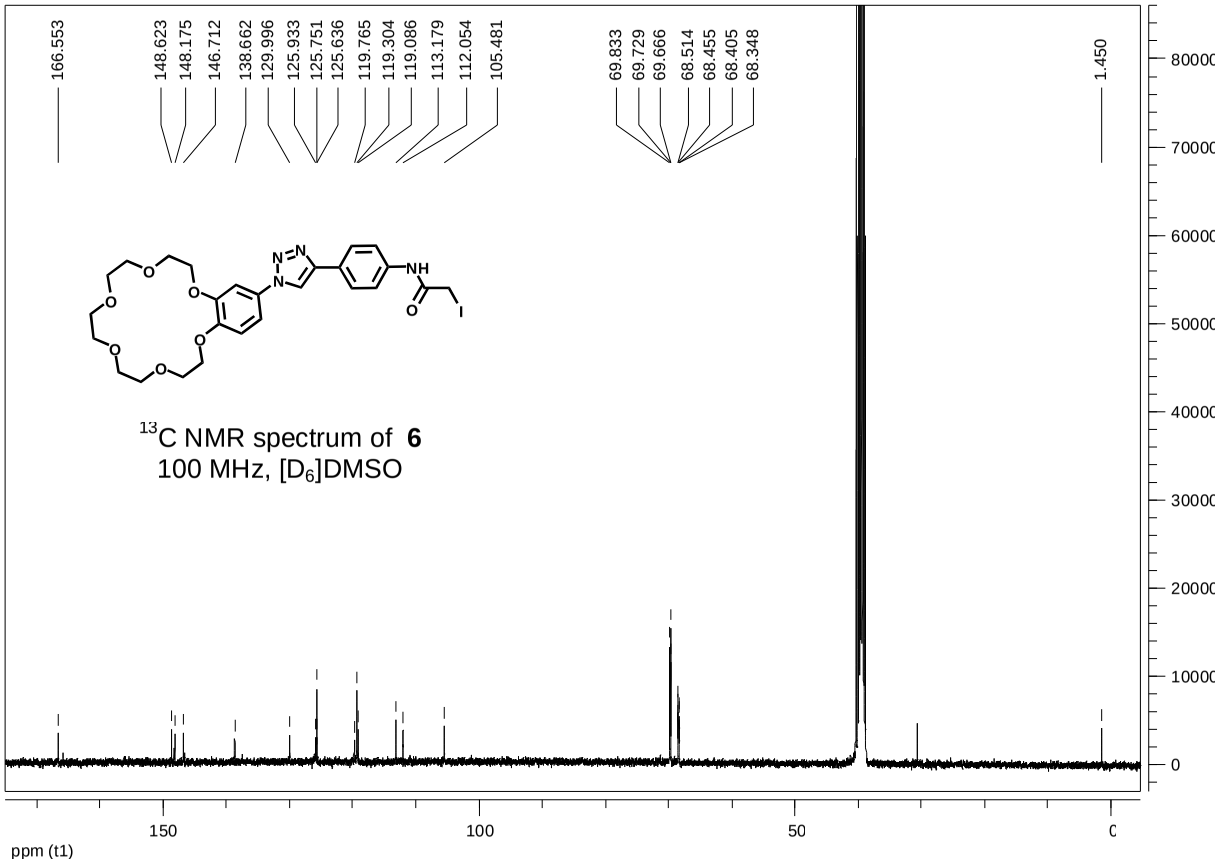
^{1}H NMR spectrum of **9**
300 MHz, [D_6]DMSO



^{13}C NMR spectrum of **9**
75 MHz, [D_6]DMSO



^{1}H NMR spectrum of **6**
300 MHz, [D$_6$]DMSO



^{13}C NMR spectrum of **6**
100 MHz, [D$_6$]DMSO