CHEMBIOCHEM

# Supporting Information

CHEMBIOCHEM

# Supporting Information

for

# How Do Halogen Substituents Contribute to Protein Binding Interactions? A Thermodynamic Study of Peptide Ligands With Diverse Aryl Halides

Adnan Memic and Mark R. Spaller*

## 1.1 Materials

*Novabiochem*: preloaded Val-Wang resin (Fmoc-Val-Wang), Fmoc-protected standard amino acids, hydroxybenzotriazole (HOBt)

*PepTech Corporation*: Fmoc-protected halogenated Phe amino acids

*Fisher Scientific/Fisherbiochem*: diisopropylcarbodiimide (DICPI), diethyl ether, methanol, dichloromethane (DCM)

*Alfa Aesar*: trifluoroacetic acid (TFA), dimethyl formamide (DMF), piperidine, dimethyl sulfoxide (DMSO)

*TCI America:* ethanedithiol (EDT)

## 1.2 Methods

### 1.2.1 General Procedure for Linear Peptide Synthesis

1. A reaction vessel for peptide synthesis was loaded with Val–Wang resin (n mol). Dichloromethane (DCM) was used to swell the resin (~45 min) with mild shaking. DCM was then suctioned off by a weak vacuum, produced by water aspiration.
2. Piperidine/ DMF (dimethyl formamide) (10 x resin volume, 25% v/v) was added to the resin to deprotect the amino acids from the Fmoc group. After shaking for approximately 10 min, the piperidine/ DMF was suctioned off and the resin was treated with fresh piperidine/ DMF (10 x resin volume, 20% v/v) for another approximately 10 min with shaking. The solvent was removed, and the resin was washed with DMF (10 times, 10 x resin volume).
3. Three equivalents of amino acid precursor (3n mol), four equivalents of DIPCI (4n mol), six equivalents of HOBt (6n mol) and DMF (5 x resin volume) were combined into the synthesis vessel, followed by mild shaking for at least 2 h. A small amount of resin sample was then removed, placed in a small test tube and washed successively with DMF (3 x 1 mL), DCM (1 x 1 mL) and diethyl ether (2 x 1 mL). A sample of the resin was then submitted for the Kaiser test. Negative results indicated that the coupling was complete. If the reaction was not complete, an

additional one equivalent of DIPCI (n mol) was added and the vessel shaken for another 2 h. If the coupling was complete, the solution was suctioned off and the resin was washed with DMF (10 x resin volume).

4. Steps 2 and 3 were repeated until the desired peptide was synthesized. Step 2 was then used to remove the Fmoc group, from the *N*-terminus.
5. A TFA-EDT (95:5) solution (5 x resin volume) was added to the resin in order to cleave the peptide. After shaking for at least 2 h, the solution was collected in a test tube. The resin was washed with TFA (3 x 0.5 mL) that was added to the initial TFA solution. The solution was divided into two equal portions and put into two test tubes. Diethyl ether was added until the peptide precipitated. The precipitate was centrifuged and the supernatant was decanted. Fresh diethlyl ether was used to wash the peptide 1-3 times in the same way. Finally, the peptide was dissolved in water (5-10 mL) and lyophilized. A white peptide powder was obtained after lyopholization in about 24 h.
6. A small amount of the crude peptide (1 mg) was used for mass spectral analysis by MALDI or ESI to confirm correct peptide mass.

### 1.2.2 Purification and Analysis of Aryl Halide Peptides

A fraction of the lyophilized powder was used for the initial mass (by MALDI-TOF and ESI-MS) and purity (analytical RP-HPLC) characterization. For the latter, ca. 1.0 mg of the powder was dissolved in $H_2O$ (1 mL). For evaluating optimal HPLC separation conditions, several ratios for the acetonitrile:$H_2O$ (0.1% TFA) solvent system were used (C18 RP-HPLC Phenomenex column), and small injections (~20 µL) were made at different ratios. After an adequate solvent system was found, peptide purification was carried out at a preparative scale (20 mg/mL of crude peptide). The major single peak fractions of each run were collected and combined, frozen and lyophilized to a powdered solid. The purified material was subjected to additional mass spectral analysis, and an analytical scale RP-HPLC was done again to confirm a single peak. If the analysis resulted in more than one peak, a repurification was performed until a single peak was observed.

### 1.2.3 Isothermal Titration Calorimetry (ITC)

Isolation of the PDZ3 domain (non-GST fusion form) and standard ITC binding experiments were conducted with minimal deviations from the methods as previously described (*Biochemistry* (2007) *46*, 6340-52) using a VP-ITC microcalorimeter (MicroCal). In brief, PDZ3 subcloned from PSD-95 protein (residues 302-402) was expressed in *E. coli* BLR-Gold as a glutathione-*S*-transferase (GST) fusion protein, then purified after trypsin cleavage from the GST through chromatographic purification steps. After PDZ3 was extensively dialyzed (24 h) in the ITC buffer (20 mM MES, 10 mM NaCl, pH = 6.00), portions of the dialysis buffer were used to dissolve the ligands to be titrated (thereby insuring no buffer mismatch between samples). The pH of the PDZ3 sample was measured and the peptide ligand solution was adjusted to the same value (with an allowed difference of <0.02 units). The protein and peptide samples were individually degassed for 20-30 min under vacuum without stirring.

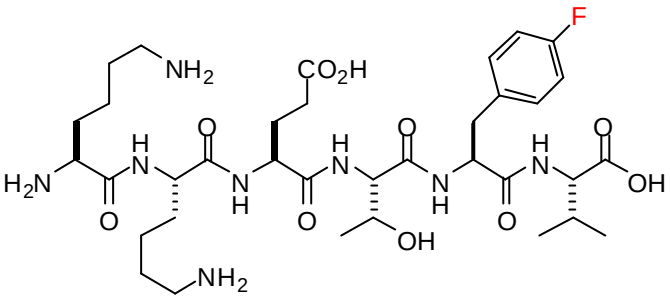
In a typical ITC experiment, 60-120 µM PDZ3 protein (1.4 mL) was loaded into the sample cell and 0.8-1.4 mM peptide (290 µL) was placed in the syringe. Typically 30 or 59 injections were programmed, with the first injection volume set at 1 µL (2 sec injection) and the remaining at 10 µL (in 30 injections of 20 sec each) or 5 µL (in 59 injections of 10 sec each). The spacing between injections was set to 180 sec. The reference power was set to 10 µcal/s with an initial delay of 2 min. All experiments were conducted at 25 °C with a stirring speed of 270 rpm. The raw data were collected and analyzed by ORIGIN software (version 5.0, Microcal). Thermodynamic parameters were determined by non-linear least squares fitting using a One Set of Binding Sites model.

## Appendix 1. Structures and Mass Spectral Data for Aryl Halide Peptides

**Compound 1 KKETFV** (unmodified parent peptide)

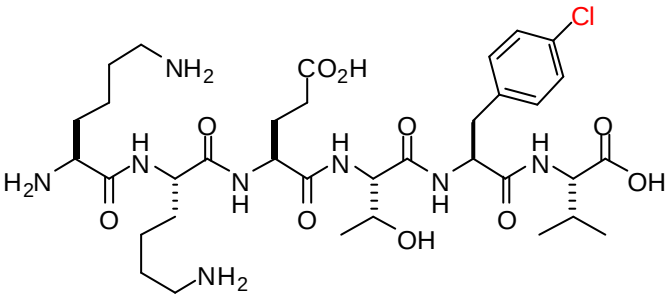
Calculated exact mass: 750.43; mass found (MALDI-TOF): 751.66 $(M+H)^+$, 772.88 $(M+Na)^+$,

**Compound 2** (4-fluorophenyl)



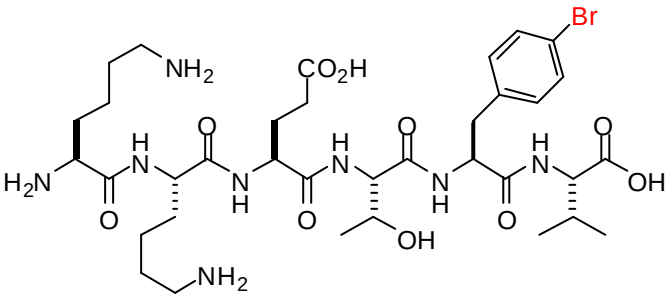

Calculated Mass: 768.42 Found mass (ESI): 769.5 $(M+H)^+$ 770.5 $(M+2H)^{++}$

**Compound 3** (4-chlorophenyl)



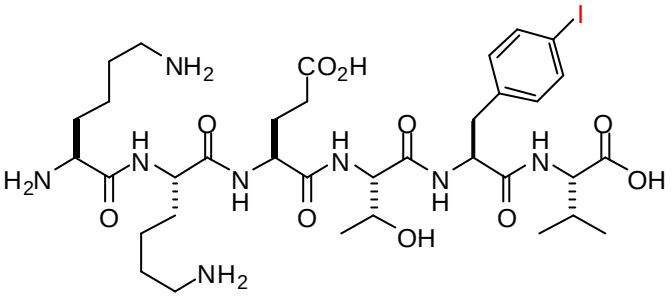

Calculated Mass: 784.39 Found mass (ESI): 785.5 $(M+H)^+$ 786.5 $(M+2H)^{++}$

**Compound 4** (4-bromophenyl)



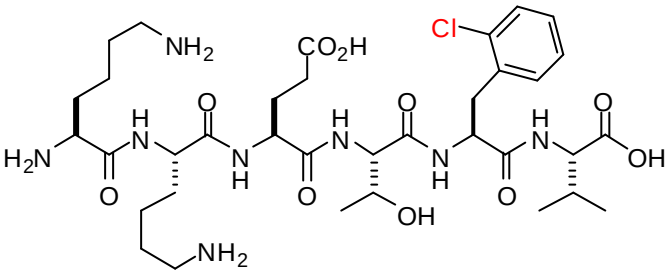

Calculated Mass: 828.34 Found mass (ESI): 829.4 $(M+H)^+$ 851.4 $(M+Na)^+$

**Compound 5** (4-iodophenyl)



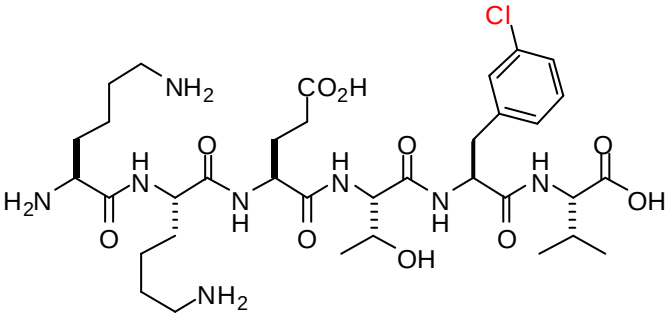
Calculated Mass: 876.32 Found mass (ESI): 877.4$(M+H)^+$ 878.5$(M+2H)^{++}$

**Compound 6** (2-chlorophenyl)



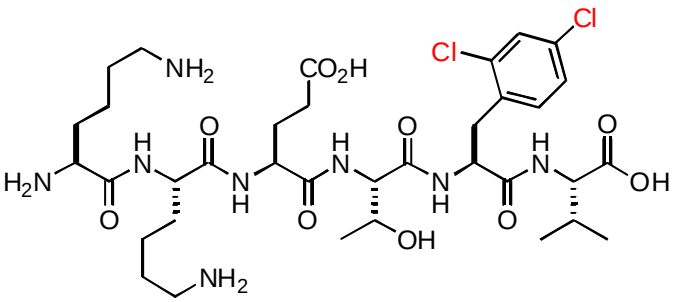
Calculated Mass: 784.39 Found mass (ESI): 785.4$(M+H)^+$ 807.4$(M+Na)^+$

**Compound 7** (3-chlorophenyl)



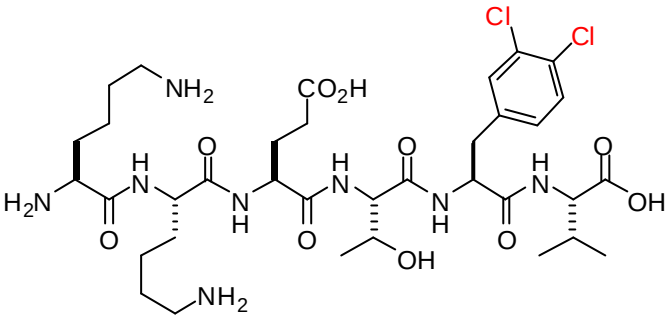
Calculated Mass: 784.39 Found mass (ESI): 785.3$(M+H)^+$ 807.3$(M+Na)^+$

**Compound 8** (2,4-dichlorophenyl)



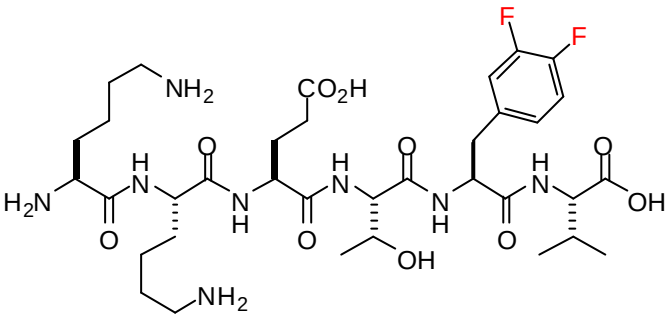
Calculated Mass: 818.35 Found mass (ESI): 819.3 $(M+H)^+$ 841.3 $(M+Na)^+$

**Compound 9** (3,4-dichlorophenyl)




Calculated Mass: 818.35 Found mass (ESI): 819.3 $(M+H)^+$ 841.3 $(M+Na)^+$

**Compound 10** (3,4-difluorophenyl)




Calculated Mass: 786.41 Found mass (ESI): 787.4 $(M+H)^+$ 809.4 $(M+Na)^+$