Supplementary Material (ESI) for Chemical Communications
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Effects of Cucurbit[7]uril on Enzymatic Activity

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1. Materials and Methods

Trypsin (from bovine pancreas, 2500 U/mg) and chymotrypsin (CT, from bovine pancreas, 1500 U/mg) were purchased from AppliChem (Darmstadt, Germany), and leucine aminopeptidase (LAP, type IV-S, from porcine kidney microsomes, 24 U/mg) was from Sigma. The assay conditions for LAP, trypsin and CT were adjusted as reported.[1] Peptides were selected or designed according to known sequence-specific cleavage preferences of the individual enzymes.[1, 2] Peptides **1**-**3** were synthesized by Biosyntan GmbH (Berlin, Germany) in > 95 % purity; Fmoc-protected 2,3-diazabicyclo[2.2.2]oct-2-ene-labeled asparagine is commercially available (Assaymetrics, UK). The substrates **4** and **6** were from Fluka, **5** was commercially available from Sigma. Cucurbit[7]uril was synthesized as previously reported.[3-5]

Kinetic traces for the fluorescence assays were recorded with a Varian Eclipse spectrofluorometer (λ_{exc} = 365 nm, λ_{em} = 450 nm for **1** and λ_{exc} = 280 nm, λ_{em} = 350 nm for substrates **2**-**3**). Kinetic traces for the UV assays were performed on a Varian Cary 50 spectrophotometer. NMR-spectra were recorded on a JEOL JNM-ECX400. pH measurements were performed with a WTW 330i pH meter equipped with a WTW SenTix Mic glass electrode. Ultrapure water (Millipore) was used.

2. Enzyme Assays

The UV assays performed with trypsin for peptides **4**-**6** were described previously.[6, 7] The fluorescence assays with LAP for peptides **2** and **3** were based on the quenching of tryptophan fluorescence by the *N*-terminal and *C*-terminal residue (asparagine labeled with diazabicyclo[2.2.2]oct-2-ene), respectively. The quenching in these substrates occurs via fluorescence resonance energy transfer (FRET)[8] and a related assay has been described before by Latt et. al. using a dansyl group as a FRET quencher of tryptophan fluorescence.[9] The

fluorescence assays with trypsin and CT for peptide **1** employed conversely Dbo as FRET donor and 3-nitrotyrosine as acceptor. For all employed fluorescence assays, cleavage of the substrate separates donor and acceptor, such that fluorescence quenching by FRET no longer applies and the fluorescence increases (see Figure 1 of main text).

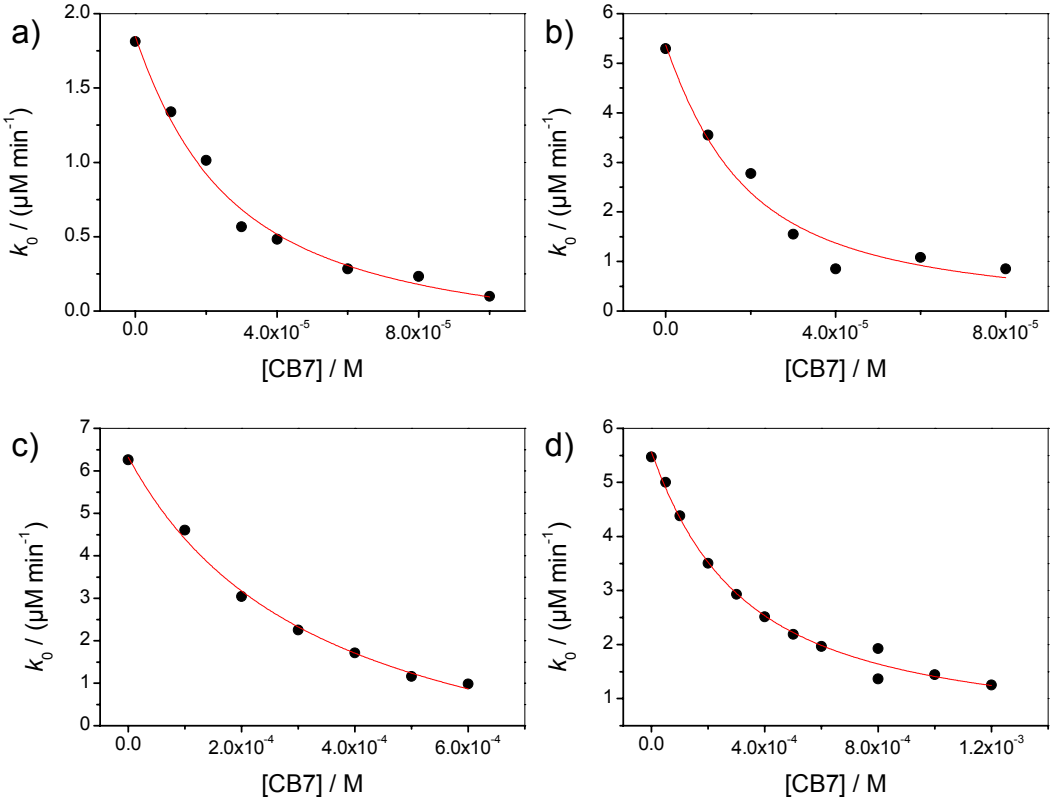

Figure S-1: Plots of initial cleavage rates (k_0) *versus* CB7 concentration for a) 10 µM peptide **2a** and LAP, b) 10 µM peptide **2b** and LAP, c) 10 µM peptide **3** and LAP, and d) 500 µM peptide **6** and trypsin. The red lines represent the curve fits according to the host-substrate binding model (eqs. 1 and 2 of main text).

3. Analysis of Enzyme Kinetics

In order to extract the apparent binding constants (K_a) and hydrolysis rates for the complexed substrates (Table 1 of main text) from the time profiles for enzyme cleavage (see Figure 1 of main text), we determined from the normalized spectroscopic traces (after subtraction of background and conversion to concentration) the initial rates (< 10% conversion) for proteolytic cleavage, k_0, at increasing CB7 concentration. The k_0 values were subsequently plotted *versus* the concentration of CB7 as shown in Figure S-1. The fitting for the trypsin

activity towards peptide **1** showed a larger scatter at low CB7 concentrations, presumably due to deviations from the presumed 1:1 binding stoichiometry, which allowed only a lower limit for K_a to be estimated.

Independently, we assessed the rates of proteolytic cleavage of substrates **1**-**3** from the pseudo-first order rate constants (k_{cat}/K_M). This method, which is valid for substrate concentrations lower than the Michaelis-Menten constant K_M,[1, 10, 11] does not require the normalization procedure and restriction to the initial rate as employed in the first method but utilizes instead the entire spectroscopic traces (see Figure 1 of main text) for fitting. This method afforded the same binding constants, within error, as the former method.

4. NMR Spectroscopy

The ^{1}H NMR spectra for peptides **4** and **6** in D_2O in the absence and presence of CB7 (5 mM) are shown in Figure S-2. The complexation-induced shifts are qualitatively indicated by arrows; note the downfield shift for the arginine methylene protons, indicative of portal binding (association or exclusion complex), and the upfield shifts for the aromatic protons, characteristic for complexes of the inclusion type.

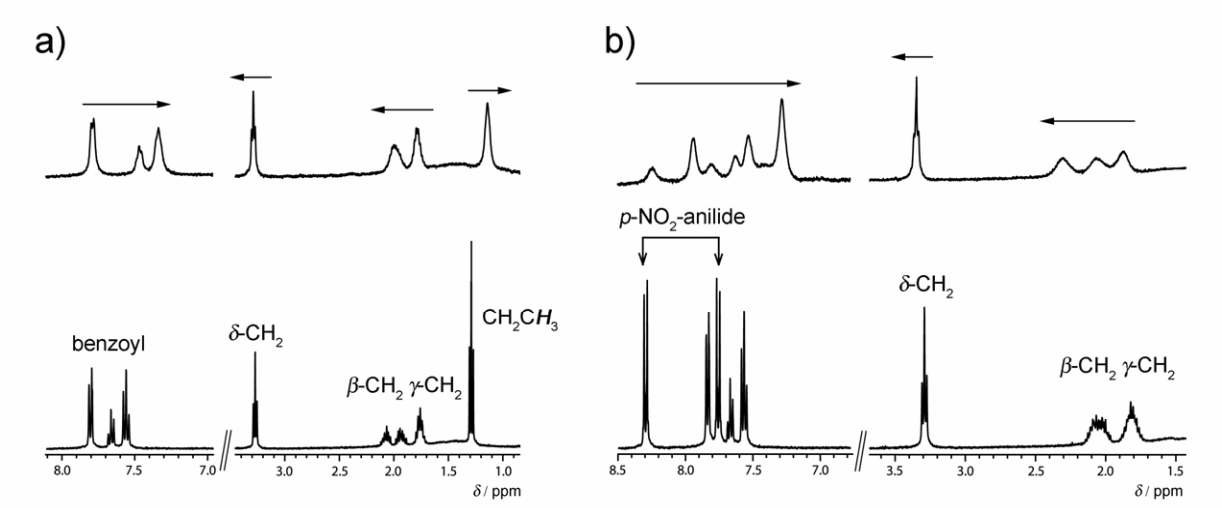

Figure S-2: NMR spectra in unbuffered D_2O of a) N_α-benzoyl-L-arginine ethylester (**4**, 2 mM) and b) N_α-benzoyl-L-arginine *p*-nitroanilide (**6**, 2 mM) in their free form (bottom) and complexed with CB7 (top, [CB7] = 5 mM).

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5. References

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