Supporting Information:

A Selective Carborane-Functionalized Gastrin-Releasing Peptide Receptor Agonist as Boron Delivery Agent for Boron Neutron Capture Therapy

Paul Hoppenz,[†] Sylvia Els-Heindl,[†] Martin Kellert,[‡] Robert Kuhnert,[‡] Stefan Saretz,[‡] Hans-Georg Lerchen,[§] Johannes Köbberling,[§] Bernd Riedl,[§] Evamarie Hey-Hawkins,[‡] and Annette G. Beck-Sickinger[,†]*

[†]Institute of Biochemistry, Faculty of Life Sciences, Leipzig University, Brüderstrasse 34, 04103 Leipzig, Germany
[‡]Institute of Inorganic Chemistry, Leipzig University, Johannisallee 29, 04103 Leipzig, Germany
[§]Bayer AG, Aprather Weg 18A, Wuppertal, Germany

*corresponding author: Annette G. Beck-Sickinger; E-mail: abeck-sickinger@uni-leipzig.de. Telephone: +49 341 97-36900. Telefax: +49 341 97-36909.

Table of Content

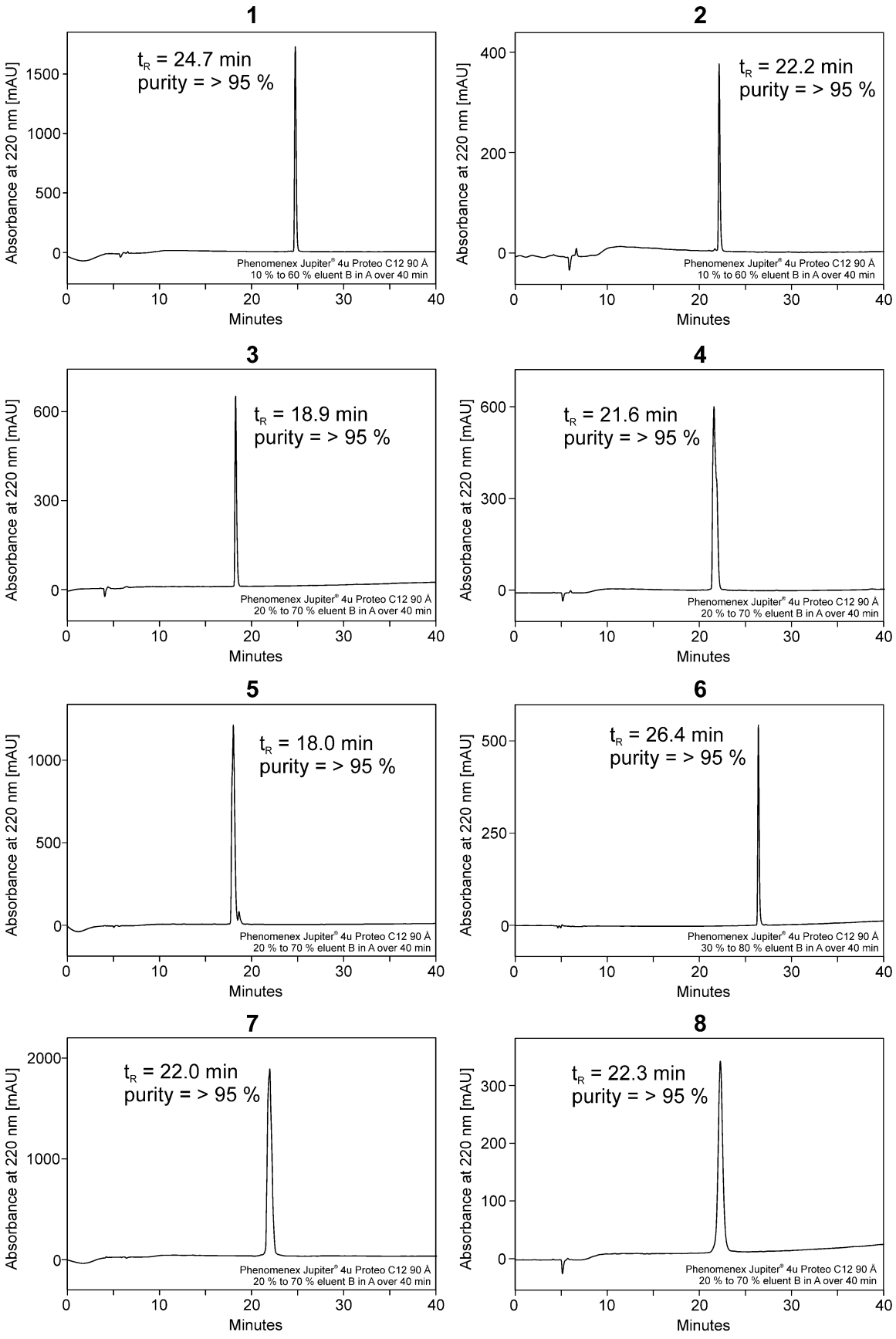

Figure S1: RP-HPLC pure analytics of peptide **1-8** using a Phenomenex Jupiter® 4u Proteo C12 90 Å column and applying linear gradients of eluent B (ACN + 0.08 % TFA (v/v)) in eluent A (H_2O + 0.1 % TFA (v/v)) over 40 min.

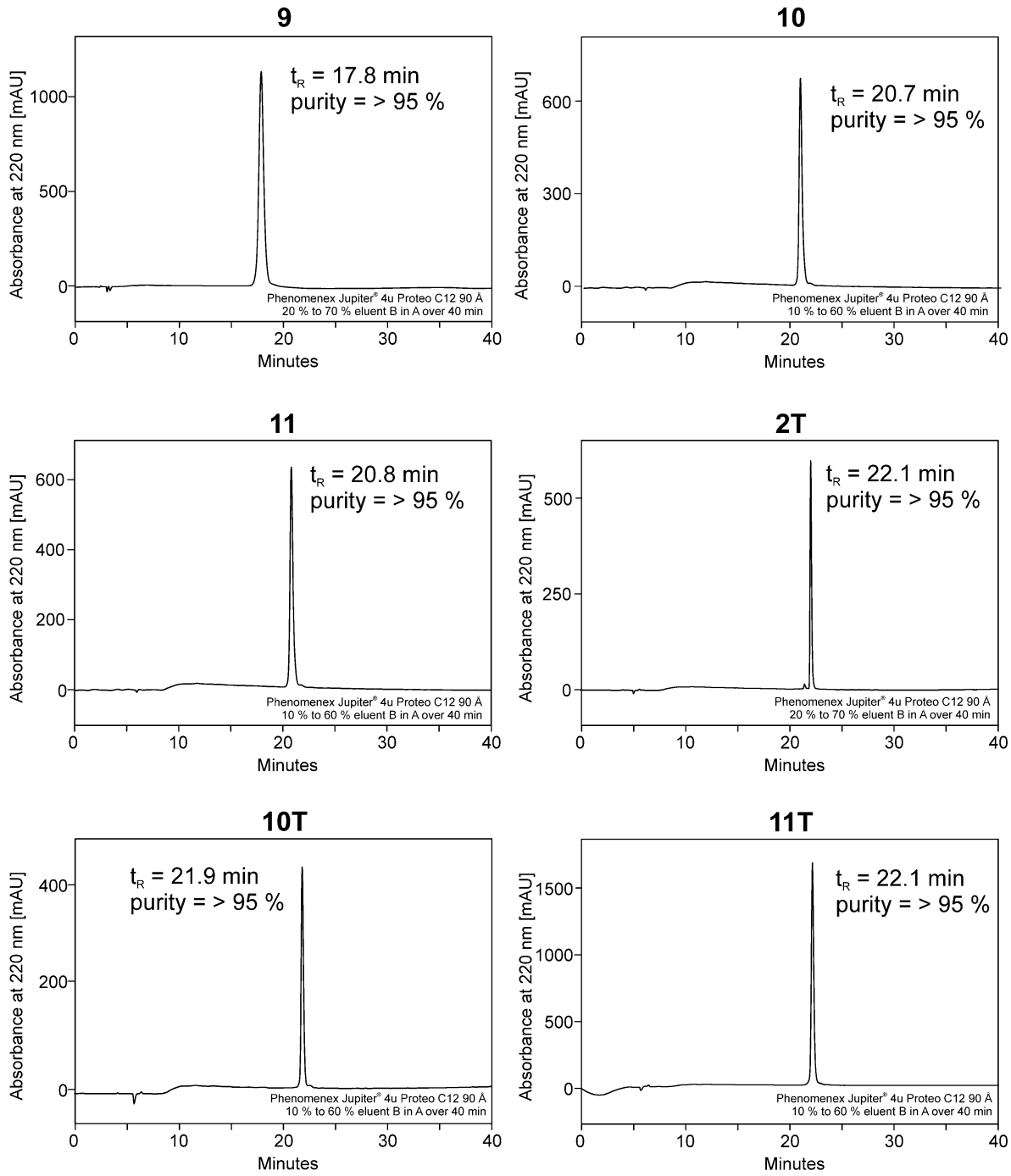

Figure S2: RP-HPLC pure analytics of peptide **9-11, 2T, 10T** and **11T** using a Phenomenex Jupiter® 4u Proteo C12 90 Å column and applying linear gradients of eluent B (ACN + 0.08 % TFA (v/v)) in eluent A (H_2O + 0.1 % TFA (v/v)) over 40 min.

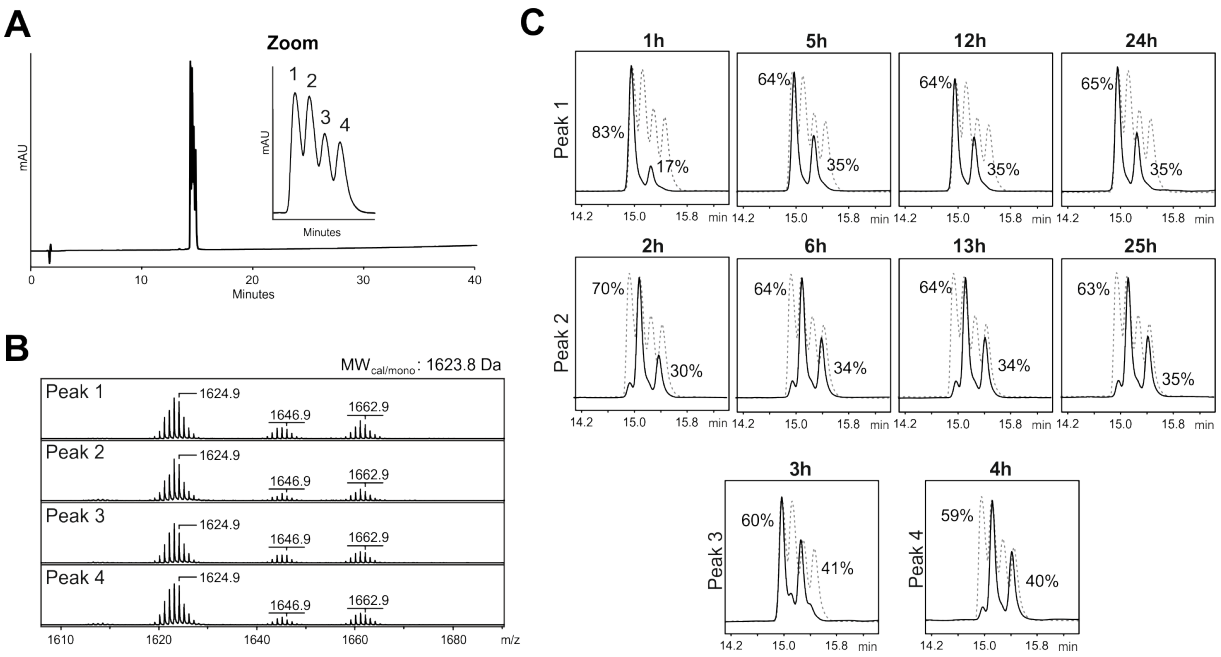

Figure S3: Detailed characterization of conjugate 4 by mass spectrometry and RP-HPLC. (A) Pure RP-HPLC chromatogram of conjugate **4** on a Kinetex XB-C_{18} column (Phenomenex, C18, 5 µm, 100 Å) using a linear gradient of 20 % to 70 % (*v/v*) eluent B (0.08 % TFA in ACN) in eluent A (0.1 % TFA in water) in 40 min. **(B)** MALDI-ToF mass spectra of each separated peak obtained from RP-HPLC analysis of conjugate **4**. **(C)** RP-HPLC analysis of each separated peak after indicated incubation times at room temperature using a Kinetex XB-C_{18} column (Phenomenex, C18, 5 µm, 100 Å) and a linear gradient of 20 % to 70 % (*v/v*) eluent B (0.08 % TFA in ACN) in eluent A (0.1 % TFA in water) in 40 min.

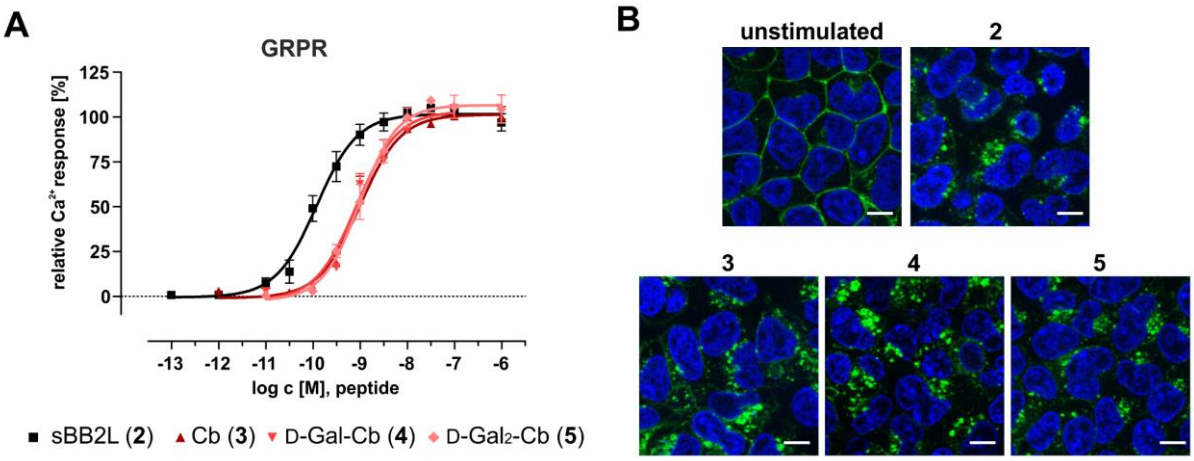

Figure S4: ***In vitro*** **characterization of mono and bis-Gal-cluster conjugates 3-5 (A)** Concentration-response curves of conjugate **3-5** obtained from Ca^{2+}-mobilization assay using stably transfected HEK293_GRPR-tGFP cells. Data were normalized to Uni response (bottom value = 0 %, top value = 100 %) and the standard error of mean (SEM) is indicated at each data point, which represents the mean, obtained in at least two independent experiments, each performed in duplicate. **(B)** Receptor internalization studies of HEK293 cells stably expressing the GRPR fused to tGFP (green). Nuclei were stained with Hoechst33342 (blue) and cells were stimulated with 100 nM for 1 h at 37 °C. Scale bar = 10 µm

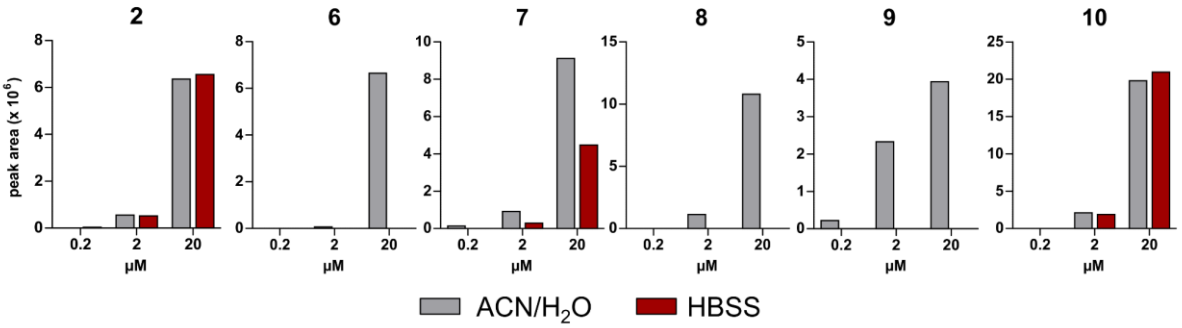

Figure S5: Kinetic solubility assay investigating the aggregation properties of conjugate 2 and 6-10.