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SUPPORTING INFORMATION

Title: γ-Aminoadamantanecarboxylic Acids Through Direct C–H Bond Amidations
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Solid phase peptide synthesis (SPPS) of ^{A}Gly containing peptides

1. Synthesis of ^{A}Gly Oligomers H-(^{A}Gly)$_n$ with n = 5, 7 (44a, b)

General remarks.

HBTU was purchased from MultiSynTech (Witten, Germany). 2-Chloro-trityl chloride resin (loading: 1.6 mmol/g) was obtained by Senn-Chemicals (Dielsdorf, Switzerland). HOBt, DIPEA, trifluoroacetic acid (TFA), and α-cyano-4-hydroxycinnamic acid were from Fluka (Taufkirchen, Germany). Triethylsilane (TES), acetic anhydride and methanol were obtained from Merck (Darmstadt, Germany). The peptide-synthesis-grade reagents piperidine, 1-methyl-2-pyrrolidinone (NMP), N,N-dimethylformamide (DMF), dichloromethane (DCM) and diethylether, HPLC-grade acetonitrile and TFA for UV-spectroscopy were purchased from Biosolve (Valkenswaard, the Netherlands). 2,2,2-Trifluoroethanol (TFE) was obtained from Acros (Geel, Belgium). Plastic syringes (2 mL and 5 mL volume) equipped with polyethylene frits (pore size: 35 µm) were purchased by Roland Vetter Laborbedarf (Ammerbuch, Germany). Analytical and preparative reverse-phase HPLC was performed on Agilent equipment (Böblingen, Germany) by using the following columns: Luna C18(2), 3 µm, 4.60 x 150 mm, and Luna C18(2), 10 µm, 90 Å, 21.2 x 250 mm (Phenomenex, Aschaffenburg, Germany). The binary solvent system (A/B) was as follows: (A) 0.012 % (v/v) TFA in water, and (B) 0.01 % (v/v) TFA in acetonitrile. The flow rate was 1 mL/min and 21 mL/min for the analytical and preparative HPLC runs, respectively. The absorbance was detected at 220 nm. The molecular weights were determined by using ESI-MS (Thermoquest, Finnigan) and MALDI-TOF-MS (GSG, Bruchsal, Germany).

1. Solid-Phase Synthesis of the Heptamer H-(^{A}Gly)$_7$-OH (44b)

Loading of the 2-Chloro-Trityl Chloride Resin with Fmoc-^{A}Gly-OH. 108.9 mg of 2-chloro-trityl chloride resin were swollen in 1.5 mL dry DCM for 30 min. After removal of the excess solvent, 68.2 mg (0.163 mmol, 0.94 eq.) of Fmoc-^{A}Gly-OH dissolved in 1.1 mL DCM/DMF (3:1 v/v) were added to the swollen resin, followed by 55.8 µL (0.326 mmol, 1.87 eq) DIPEA.

After shaking for 3 h at rt, the resin was filtered off, washed several times with DMF, DCM, diethylether, and finally dried u.v. for 4 h.

The amino acid loading was determined spectrophotometrically by measuring the absorbance at 300 nm of the fluorene-piperidine adduct obtained by treating a small portion (4 – 6 mg) of the dried resin with 20% piperidine in DMF for 30 min. A loading of 0.89 mmol/g was calculated from the absorbance at 300 nm, using the extinction coefficient of 7800 M^{-1} cm^{-1}. The remaining free linker groups were capped by washing the resin five times with the mixture DCM/MeOH/DIPEA (17:2:1 v/v).

Chain Assembly. Fmoc-cleavage was performed by shaking the previously swelled resin in 800 µL of 40 % piperidine in DMF/NMP (4:1 v/v) for 5 min, and then in 800 µl of 20 % piperidine (2 x 5 min). Single couplings were carried out for 2.5 h by using Fmoc-^{A}Gly-OH/HOBt/HBTU/DIPEA in the ratio of 3:3:3:6 eq with respect to the resin loading, in DMF/NMP (4:1 v/v). After each coupling a capping step was carried out with acetic anhydride/DIPEA (each 0.75 equiv. with respect to the resin loading) in DMF/NMP (5 min).

Control of the Chain Growth by HPLC. Some beads were subjected to peptide cleavage before Fmoc-deprotection of the trimer, tetramer and hexamer. The beads were shaken in 92 µl of the mixture TFA/DCM/TES in the ratio of 25:20:1 (v/v) for 40 min. Afterwards the mixture was reduced to a minimum volume and the cleaved Fmoc-derivative was recovered by precipitation from ice-cold water and centrifugation. The residue was dissolved in MeOH and characterized by analytical HPLC and MALDI-TOF-MS (Table I and Fig. 1).

Table I. Analytical Data of the Fmoc-protected Trimer, Tetramer and Hexamer

Product	**HPLC-gradient**	**t_R (min)**	**% CH_3CN**	**MW_{calc} (Da)**	**MW_{found} (Da)**
Fmoc-(^{A}Gly)$_3$-OH	5 – 95% B in 55 min	43.85	76.75	771.42	793.9 ($M+Na^+$)
Fmoc-(^{A}Gly)$_4$-OH	30 – 95% B in 40 min	31.46	81.12	948.54	970.8 ($M+Na^+$)
Fmoc-(^{A}Gly)$_6$-OH	30 – 95% B in 40 min	36.69	89.62	1302.77	1324.8 ($M+Na^+$); 926.1 (pentamer+Na^+); 791.0 (Ac-tetramer+Na^+); 613.8 (Ac-trimer+Na^+)

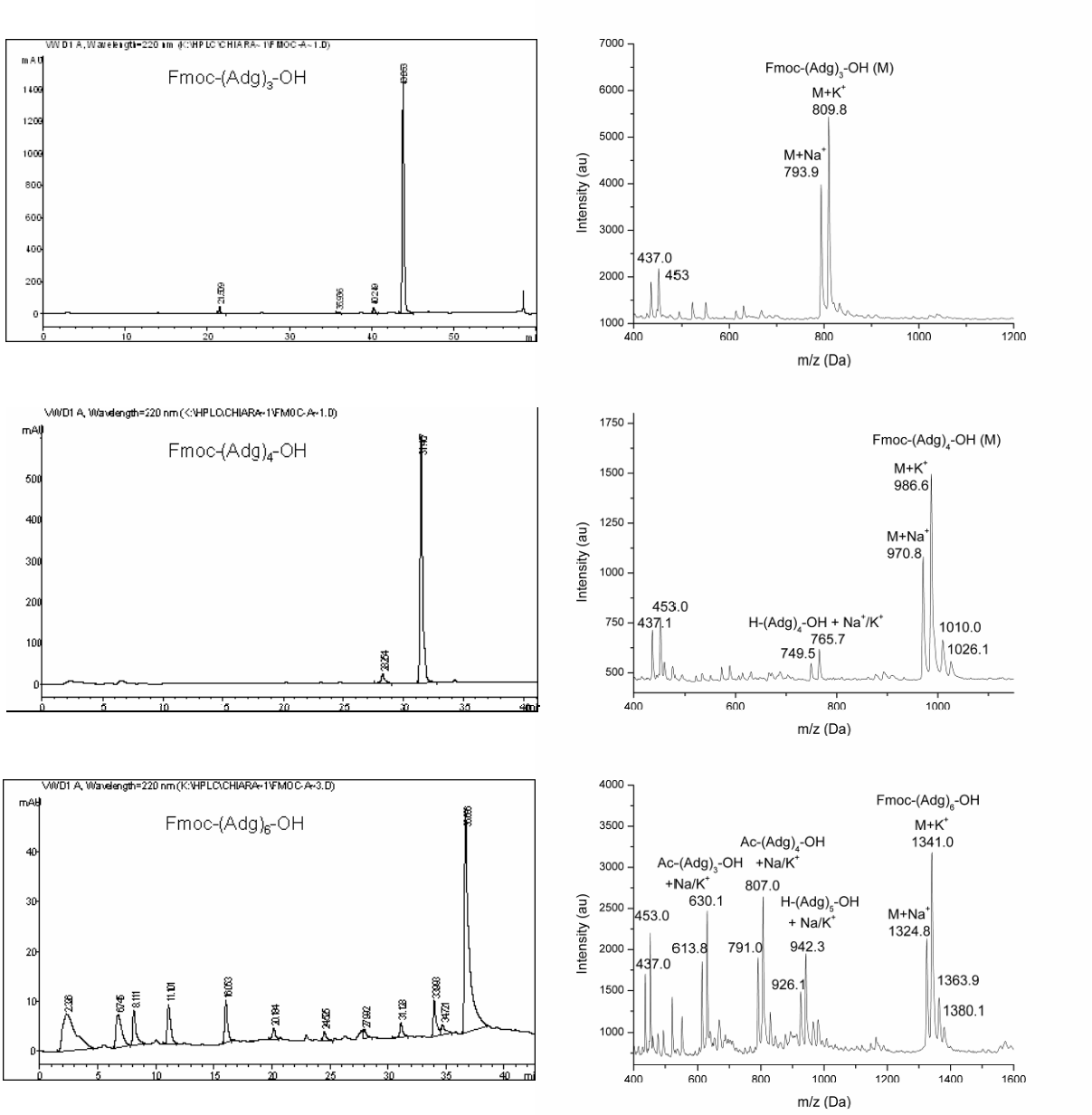

Figure 1. Analytical HPLCs and MALDI-TOF-MS spectra of Fmoc-(^{A}Gly)n-OH (Adg = ^{A}Gly). The following gradients were used: (n=3) 5-95 % B in 55 min; (n=4, 6) 30-95 % B in 40 min.

Total Cleavage of H-(AGly)$_7$-OH. The Fmoc-deprotected heptamer was removed from the resin by treatment with TFA/DCM/TES in the ratio of 40:10:1 (v/v) for 40 min. Afterwards the resin was filtered off and washed with TFA and DCM, the filtrate was reduced to a minimum volume and the cleaved heptamer was recovered by precipitation from ice-cold ether and centrifugation. The residue (22 mg) was insoluble in MeOH but completely soluble in TFE, where it was characterized by analytical HPLC, MALDI-TOF-MS and LC-ESI-MS (Fig. 2).

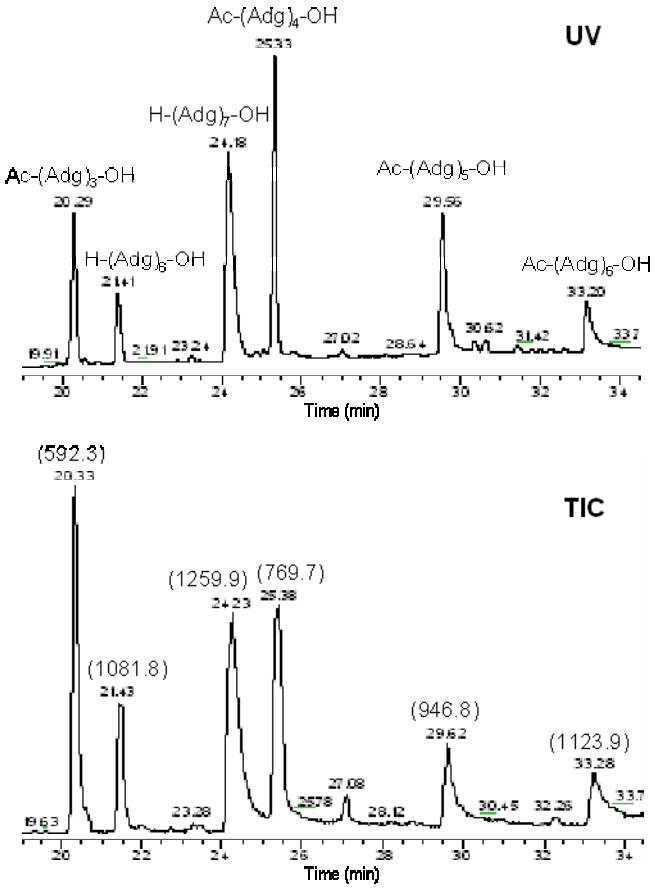

Figure 2. LC-ESI-MS of the crude product of the solid-phase synthesis (Adg = ^{A}Gly). Top: UV profile. Bottom: total ion current (TIC) profile (the m/z values corresponding to each peak are in the brackets).

Purification of H-(AGly)$_7$-OH. The heptamer was dissolved in TFE and purified by preparative HPLC by using a C18 column and the gradient 25 – 85% B in 67 min, with the elution system consisting of (A) 0.0059 % TFA in water (w/w) and of (B) acetonitrile. The fraction containing the desired compound was lyophilized, and the dry product (2 mg) was then characterized by HPLC and MALDI-TOF-MS (Fig. 3): (M+H$^+$)found 1259.0 Da (MW$_{calc}$ 1257.82 Da). HPLC gradient: 25-85 % B in 40 min, 85-95 % B in 5 min, 95 % for 10 min: t_R 18.52 min (elution at 52.78 % acetonitrile); 95.5 % purity.

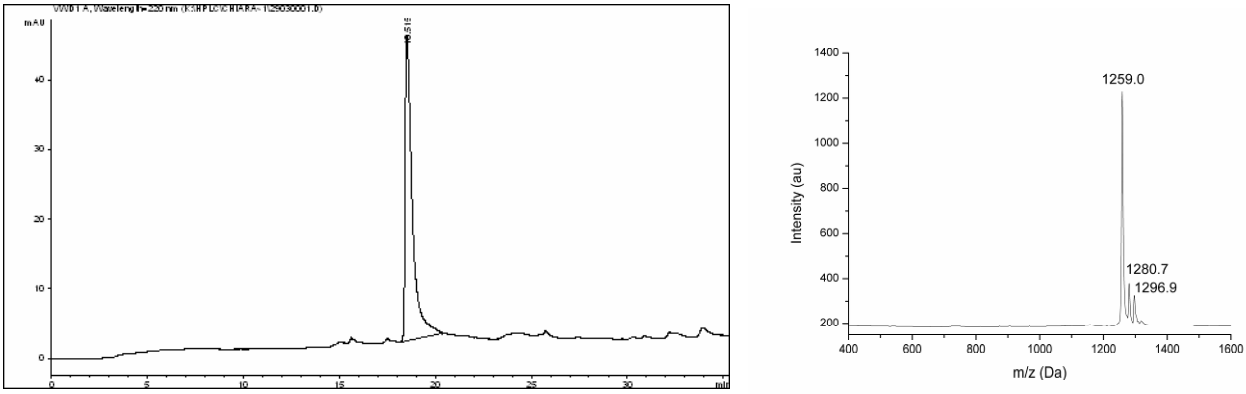

Figure 3. Analytical HPLC (left) and MALDI-TOF-MS (right) of the purified H-(^{A}Gly)$_7$-OH.

2. Solid-Phase Synthesis of the Pentamer H-(^{A}Gly)$_5$-OH (44a)

Chain Assembly. 54.6 mg of Fmoc-^{A}Gly-trityl resin (loading: 0.72 mmol/g) were swollen in DMF. Fmoc cleavage was performed by shaking the resin in 800 µL of 40 % piperidine in DMF/NMP (4:1 v/v) for 7 min, and then in 800 µL of 20 % piperidine (3 x 7 min). A single-coupling procedure was used for the attachment of the 2nd and 3rd ^{A}Gly unit: the acylation mixture was Fmoc-^{A}Gly-OH/HOBt/HBTU/DIPEA in the ratio of 3.7:3.7:3.7:7.4 equiv. with respect to the resin loading, and the reaction time was 3 h. After each coupling a capping step was carried out with acetic anhydride/DIPEA in the ratio of 1:1 (4 equiv. with respect to the resin loading) in DMF/NMP (10 min). For the attachment of the 4th and 5th ^{A}Gly unit a double-coupling procedure was applied with 4 and 5 equiv. of the amino acid, respectively.

Total Cleavage of H-(^{A}Gly)$_5$-OH. The pentamer was removed from the resin by treatment with TFA/H_2O/TES in the ratio of 20:1:1 (v/v) for 40 min. Afterwards the resin was filtered off and washed with TFA and DCM, the filtrate was reduced to a minimum volume and the cleaved product was recovered by precipitation from ice-cold ether and centrifugation. The dried precipitate (26 mg) was then dissolved in TFE and characterized by analytical HPLC and MALDI-TOF-MS (Fig. 4). $(M+H^+)_{found}$ 904.2 Da (MW_{calc} 903.59 Da). HPLC gradient: 25 – 85% B in 40 min, 85 – 95% B in 5 min, 95 % B for 10 min: t_R 12.53 min (elution at 43.8 % acetonitrile); 87 % purity.

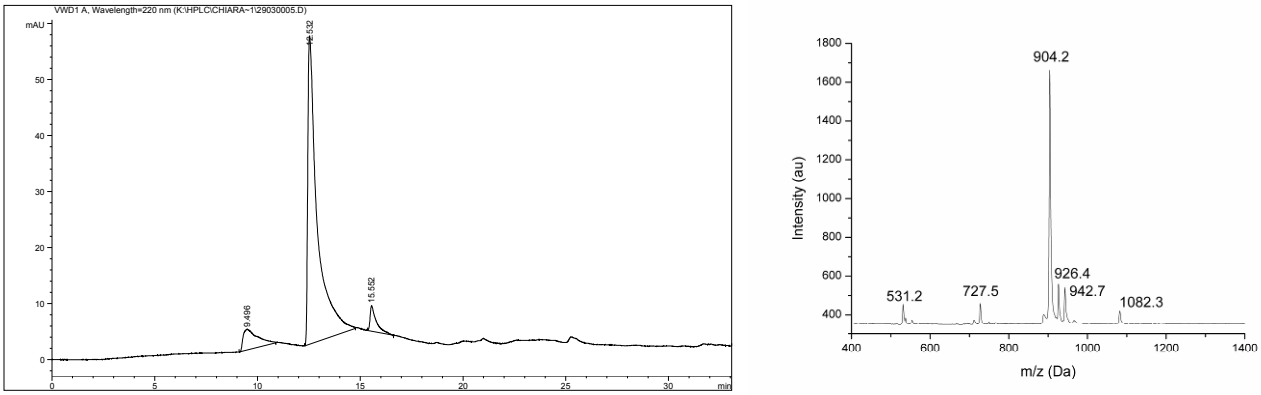

Figure 4. Analytical HPLC (left) and MALDI-TOF-MS (right) of H-(^{A}Gly)$_5$-OH in TFE.

3. Solid Phase Synthesis of Boc-His(π-Me)-^{A}Gly-Phe-OMe (46)

The trimer was synthesized on solid support using commercially available Wang polystyrene resin endcapped and preloaded with Fmoc-protected L-phenylalanine (Novabiochem). Fmoc cleavage was performed by shaking the resin twice in 25% piperidine in DMF (v/v). The

resin was washed 5 times each with DMF, dichloromethane and DMF. Chain elongation with Fmoc-^{A}Gly-OH (**39a**) was performed by a double coupling procedure using Fmoc-^{A}Gly-OH, HBTU, and DIPEA (3 : 3: 6 and 2 : 2 : 4 equiv., respectively). After washing and cleavage of the Fmoc-protective group as described above, the peptide was elongated using Boc-His(π-Me)-OH, HBTU and DIPEA in the same stoichiometric ratio as given above. After washing (5 times each with DMF, dichloromethane and diethylether), the trimer was cleaved from the resin by shaking 5 days with methanol, triethylamine and THF (9 : 1 : 1, v/v). The resin was filtered off and washed several times with THF. The collected solutions were concentrated and the residue was purified by flash chromatography eluting with dichloromethane / methanol (95 : 5). The peptide was characterized by ESI-MS and proton NMR; the analytical data are in accordance with those obtained from a sample of the same peptide prepared by solution phase synthesis (**46**).