materials

Conjugates of Copper Alginate with Arginine-Glycine-Aspartic Acid (RGD) for Potential use in Regenerative Medicine

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General Methods for Solid-Phase Peptide Synthesis

Method 1a. Attachment of C-Terminal Amino Acid to the 2-Chlorotrityl Chloride Resin

At room temperature, 1 g of 2-chlorotrityl chloride resin (CTC) with maximum loading of 1 mmol/g was preswollen for 30 min in dry dichloromethane (DCM). Subsequently, 3 eq. of the appropriate Fmoc-Asp(tBu)-OH was dissolved in DCM, to which 6 eq. *N*,*N*-diisopropylethylamine (DIPEA) was added. This loading solution was added to 2-chlorotrityl chloride resin and the suspension was shaken for 2 h. After this time, three washings steps with DCM were performed. To cap unreacted active groups on the 2-chlorotrityl chloride resin, a solution of DCM:MeOH:DIPEA (17:2:1) was prepared and added to the previously washed and drained resin. This suspension was shaken for 30 min at room temperature. The capping solution was then filtered, and the resin was washed three times with *N*,*N*-dimethylformamide (DMF) and three times with DCM.

Method 1b. Attachment of C-Terminal Amino Acid to the Rink Amide Resin

The resin (0.5 g) was swollen in DMF and a solution of 20% piperidine in DMF is added to cleave the resin-bound Fmoc groups. The reaction mixture is shaken for 20 min at room temperature. The resin is drained and washed with DMF (5x). A solution of 3.0 equiv of amino acid (relative to resin capacity) in DMF is added to the resin. The reaction mixture is shaken at room temperature for 5 min. A solution of 3.0 equiv DMT/NMM/TosO$^-$ in DMF was added to the reaction mixture, followed by 6.0 equiv of DIPEA. The reaction mixture is shaken for 45 min at room temperature. The resin was drained and washed with DMF (3x), DCM (3x), MeOH (3x). Small quantities of resin are tested for unreacted amine with Kaiser test.

Standard Coupling Procedure (Method 2)

DMF solution of 3 equiv. of amino acid, 3 equiv. of coupling reagent (DMT/NMM/TosO$^-$) and 6 equiv. of NMM was added to the resin and shaken for 1-2h or overnight. The progress of the reaction was monitored by the Kaiser test.

Deprotection of Fmoc group (Method 3)

The Fmoc group was removed by treating of 25% piperidine in DMF (15-20 min).

Kaiser test (Method 4)

Solution 1: 5 g ninhydrin in 100 mL EtOH. Solution 2: 80 g PhOH and 20 mL EtOH. Solution 3: 2 mg of KCN in 100 mL pyridine. To a resin beads 2-3 drops of each solution was added. The suspension was heat for 2-3 min at 100°C.

Cleavage from the Resin (Method 5)

Peptides were cleaved from the resin using TFA/TIS/H2O (95:2.5:2.5; ca. 2ml/0.1g resin). Cleavage was achieved during 4 h. After precipitation and liophilization The final product was obtained as TFA salt.

Synthesis of H-Arg-Gly-Asp-OH

Fmoc-Asp(OtBu)-OH (0.617 g, 1.5 mmol) was attached to 2-chlorotrityl chloride resin (500 mg, 1.0 mmol/g) according to Method 1a. Starting materials: 2-chlorotrityl chloride resin (0.5 g, 1.0 mmol/g, 0.5 mmol), Fmoc-Asp(OtBu)-OH (0.617 g, 1.5 mmol), DIPEA (540 μL, 3.0 mmol), Fmoc-Gly-OH (0.446 g, 1.5 mmol), Fmoc-Arg(Pbf)-OH (0.973 g, 1.5 mmol), DMT/NMM/$TosO^-$ (0.619 g, 1.5 mmol) and NMM (330 μL, 3.0 mmol). The peptide was cleaved from the resin according to Method 5. Product: H-Agr-Gly-Asp-OH-OH. Anal. RP-HPLC (20–60% B in 45 min): t_R 2.15 min, purity 99.4%. LC/MS: 347.1807 corresponding to $[M+H]^+$ (M^+ for $C_{12}H_{22}N_6O_6^+$; calc. 346.3397).

Synthesis of H-Arg-Gly-Asp-NH_2

Fmoc-Asp(OtBu)-OH (0.617 g, 1.5 mmol) was attached to Rink Amide resin (500 mg, 1.0 mmol/g) according to Method 1b. Starting materials: Rink Amide resin (0.5 g, 1.0 mmol/g, 0.5 mmol), Fmoc-Asp(OtBu)-OH (0.617 g, 1.5 mmol), DIPEA (540 μL, 3.0 mmol), Fmoc-Gly-OH (0.446 g, 1.5 mmol), Fmoc-Arg(Pbf)-OH (0.973 g, 1.5 mmol), DMT/NMM/$TosO^-$ (0.619 g, 1.5 mmol) and NMM (330 μL, 3.0 mmol). The peptide was cleaved from the resin according to Method 5. Product: H-Agr-Gly-Asp-NH_2. Anal. RP-HPLC (20–60% B in 45 min): t_R 2.09 min, purity 99.7%. LC/MS: 346.1963 corresponding to $[M+H]^+$ (M^+ for $C_{12}H_{23}N_7O_5^+$; calc. 345.1601).

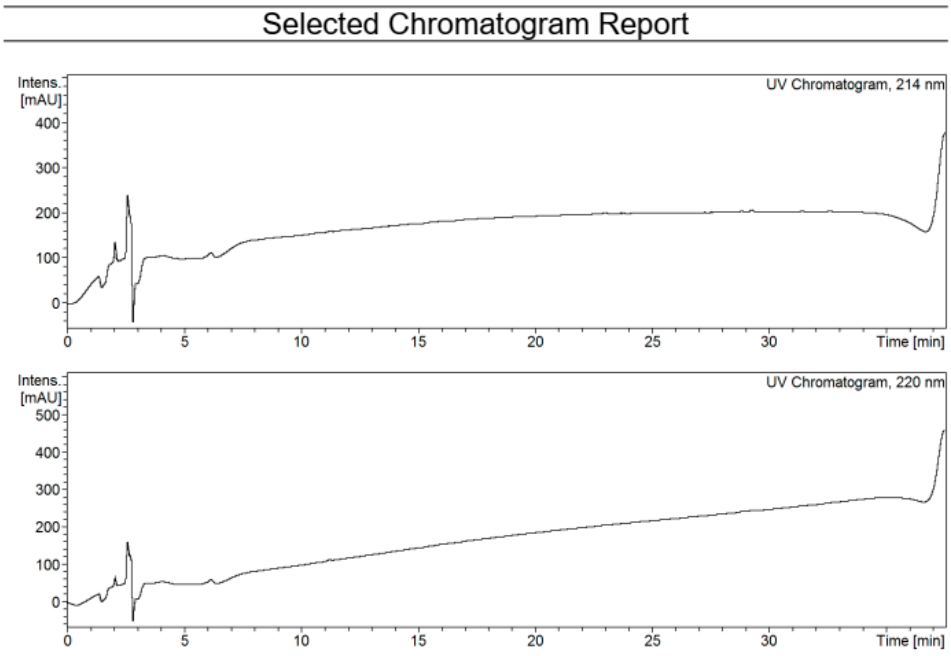


Figure S1. HPLC of H-Arg-Gly-Asp-OH.

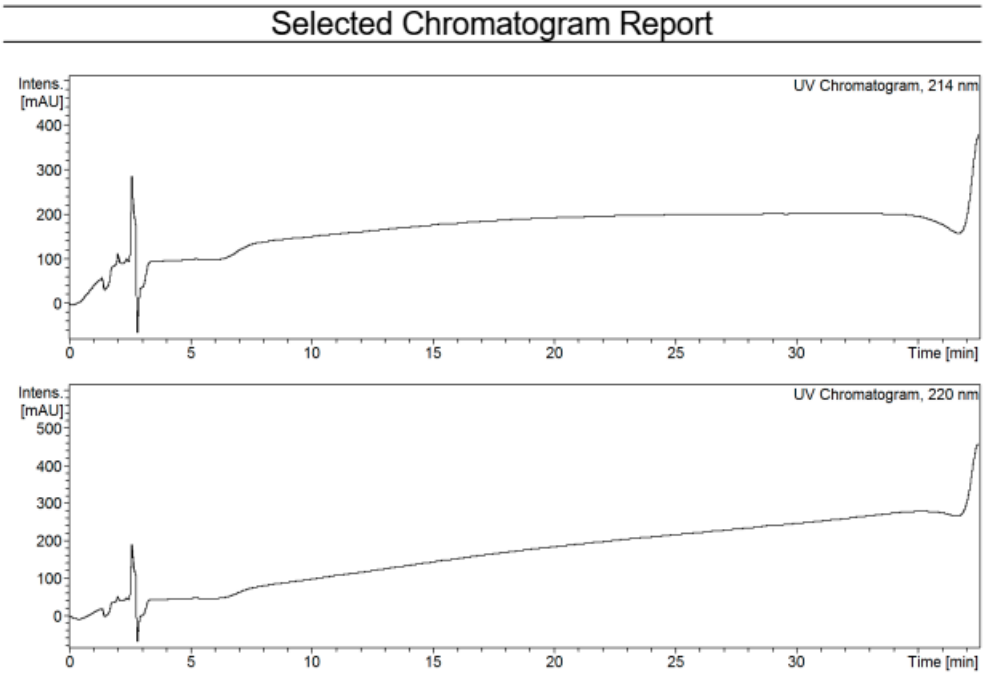
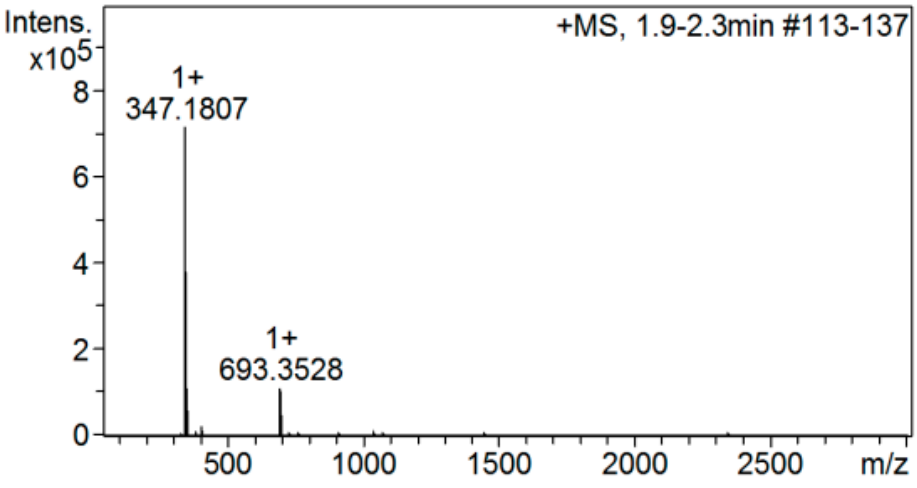
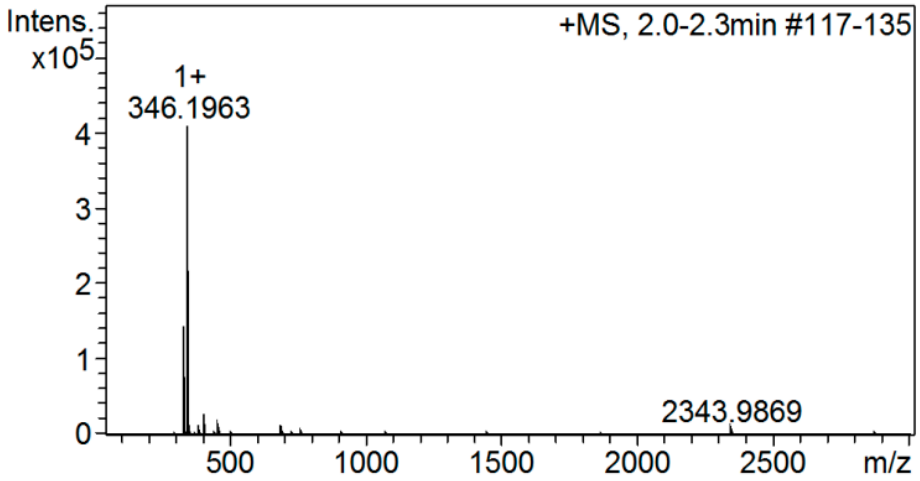


Figure S2. HPLC of H-Arg-Gly-Asp-NH_2.



#	m/z	I
1	347.1807	715184
2	348.1817	112393
3	349.1823	16481
4	385.1234	8190
5	387.2104	5989
6	404.1995	21989
7	693.3528	112918
8	694.3551	30930
9	763.0614	5807
10	1039.5228	9165

Figure S3. MS spectrum of H-Arg-Gly-Asp-OH.



#	m/z	I
1	329.1680	143408
2	330.1681	18830
3	346.1963	408751
4	347.1956	61597
5	348.1954	8046
6	384.1427	13983
7	386.2084	13645
8	403.2193	27552
9	460.2431	11440
10	691.3811	11704

Figure S4. MS spectrum of H-Arg-Gly-Asp-NH_2.