Amino Acids (2003) 24: 405–411
DOI 10.1007/s00726-002-0341-5

Amino
Acids
Printed in Austria

Optimized conventional synthesis of "RGD" and "RGDS" peptides and their sarcosine mimics as integrin GP IIb/IIIa antagonists*

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Received April 10, 2002
Accepted August 29, 2002
Published online December 18, 2002; © Springer-Verlag 2002

Summary. Synthetic arginyl-glycyl-α-aspartyl "RGD" and arginyl-glycyl-α-aspartyl-serinyl "RGDS" peptide sequences, which are originally located in matrix proteins, are confirmed to be as versatile integrin GP IIb/IIIa antagonists. Since integrins, as cell surface glycoprotein receptors are implicated in several physiological mechanisms, these peptides are recently specially considered in the design of new therapeutics.

Replacing glycine by sarcosine, as its more lipophilic isomer, in RGD peptides seemed, accordingly, interesting in revealing some structural/biological activity relationships.

To render "RGD" peptides more conveniently available, an ameliorated quasi-gram yield conventional synthesis in solution of the parent "RGD" and "RGDS" [**8**, Scheme 1A & **15**, Scheme 1B] and their sarcosine analogues, [**8′**, Scheme 1A & **15′**, Scheme 1B] respectively, is herein described.

A compilation of the mild hydrogenolysis removable Z and NO_2 groups and/or the acidiolytic removable Boc group were manipulated for the amino temporary protecting steps. Both the DCCI/HOBt and MA methodologies served well as peptide coupling methods.

Abbreviations: Boc, *tert*-butyloxycarbonyl; DCCI, N,N′-dicyclohexylcarbodiimide; EI-MS, electron ionization mass spectrum; ESI-MS, electron spray mass spectrum; HOBt, 1-hydroxy-benzotriazole; MA, mixed anhydride method of coupling; $MeCl_2$, methylene chloride; NMM, N-methylmorpholine; OBzl, benzyl ester; Sar, sarcosine; TEA, triethylamine; THF, tetrahyrofuran; TFA, trifluoroacetic acid; Z, benzyloxycarbonyl.

Keywords: RGD – RGDS – Peptides – Sarcosine – GP IIb/IIIa Antagonists

1 Introduction

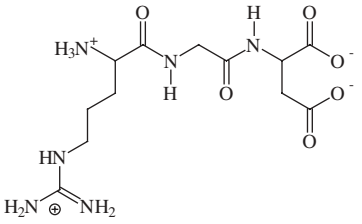
Extra-cellular matrix proteins exemplified by fibronectin, fibrinogen, vitronectin, collagen, von Willebrand factor and laminin are significant proteins implicated in several cell/cell or matrix protein/cell interactions (Robert, 2001; Sage, 2001). Numerous human physiological and pathological mechanisms are thus involved. The concerned targets were identified as cell surface glycoprotein (GP) receptors of the integrin family, exemplified by GP IIb/IIIa (Richard, 1987).

It was later confirmed that the integrins recognize their ligand matrix proteins through a common peptide sequence, namely arginyl-glycyl-α-aspartyl "RGD" or arginyl-glycyl-α-aspartyl-seryl "RGDS". The sequence is, consequently, considered as their minimum GP IIb/IIIa recognizable molecular segment (Pierschbacher et al., 1982; Puleo and Bizios, 1987).

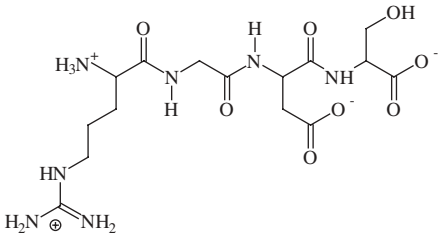
Interestingly, it was realized that synthetic [linear or cyclic] short peptides possessing "RGD" sequence or their peptido-mimics are competitively and antagonistically recognizable by the GP IIb/IIIa integrin cell receptors (Pierschbacher et al., 1983; Shashidhar, 1992; Houseman and Mrksich, 2001). Interference with some pathological mechanisms as thrombus formation, cell invasion, migration and proliferation could, thereby, be achieved.

"RGD" peptides become, consequently, attractive therapeutic candidates representing, new generations of anti-thrombotics, anti-angiogensis and anti-cancer

*A preliminary report was presented in "The 6th International Congress on Amino Acids", August 3–7, 1999, Bonn Germany.



Arginyl-glycyl-α-aspartyl. "RGD"



Arginyl-glycyl-α-aspartyl-seryl. "RGDS"

metastasis (Pierschbacher and Ruoslahti, 1984; Yamada and Kennedy, 1991; Nichols et al., 1992; Peter et al., 1992; Marcus et al., 1997; Alma et al., 1999; Shosuke et al., 1999).

Replacing glycine by sarcosine, as its more lipophilic N-methyl positional isomer, in both "RGD" and its serine homologue "RGDS" seemed, accordingly, significant in several structural/biological activity relationships and drug design considerations (Akira, 1987; Rudraksh et al., 2001).

In such context, it was assumed plausible to re-approach simplified methodologies for potentiated syntheses that render these expensive and interesting peptides more practically and conveniently available for the concerned studies.

2 Materials and methods

A General methodology

Otherwise stated, the general methodology followed is identical with that sited in our previous work (Abo-Ghalia et al., 1999). In addition mass spectra (EI) were run on "Finnigan SSQ 700 Spectrometer". Ion spray mass spectra (ESI-MS) were determined on a Sciex Mass Spectrometer TSQ 70, Mat 711A (Germany). Amino acid Analysis of the peptide was carried out on amino acid analyzer (Biotronik System LC 6000 E).

B Preparation of the starting compounds

β-benzylaspartate (**9**, Scheme 1B) was prepared according to Benoiton (1962). Boc-β-benzyl-α-Aspartate (**10**, Scheme 1B) was prepared similar to the method of Polzhofer (1969). Nito-Arginine (**1**, Scheme 1A and 1B), dienzyloxycarbonylnitroarginine (**2**, Scheme 1A and 1B) and dibenzylaspartate-*p*-toulenesulfonate (Scheme 1A) were prepared according to Hofmann et al. (1956).

C Peptide Synthesis

tert-Butyloxycarbonyl-β-benzylaspartylserine methyl ester.

[Boc-α-Asp(β-OBzl)-Ser-OMe, **12**, Scheme 1B]

A cold (~0°C) THF solution (20 ml) of *tert*-Butyloxycarbonyl-β-benzylaspartate (**10**, 1.62 g, 5 mmoles) was neutralized with NMM (0.56 ml, 5 mmoles). The mixture was then cooled (−15°C), then ethylchloroformate (0.57 ml, 6 mmoles) was added while stirring. After ~15 minutes, a pre-cooled (−15°C) solution of serine methyl ester hydrochloride (**11**, 0.78 g, 5 mmoles) and NMM (0.56 ml, 5 mmoles) were added. Stirring was continued at (−15°C) for 45 minutes, then for two hours at room temperature. The solvent was removed under reduced pressure and the residue was re-dissolved in chloroform (~100 ml), washed with 1 N HCl, water, 0.5 M $KHCO_3$, again with water and dried. The solvent was evaporated under reduced pressure and the obtained residue was triturated with pet. ether (b.p. 40–60°C), and left at 4°C for two days. The formed white crystals were filtered off, washed with pet. Ether and air dried to give 4.5 g (90%) of the protected dipeptide **12**, M.p.: 104–105°C, $[\alpha]^{25}_D$: +20 (C = 1, chloroform). Calculated Mwt for $C_{20}H_{28}N_2O_8$: (424.59). EI-mass (m/z): 425 (M^+).

Benzyloxycarbonylnitroarginylglycine ethyl ester

[Z-Arg(NO_2)Gly-OEt, **4**, Scheme 1B]

A dry dioxane solution (40 ml) of benzyloxycarbonylnitroarginine (**2**, 4.21 g, 12 mmoles), was neutralized while stirring with NMM (1.32 ml, 12 mmole). The reaction mixture was cooled (~8–10°C) then ethylchloroformate (1.14 ml, 12 mmoles) was added and the mixture was kept at (~8–10°C). After ~15 min., a pre-cooled (~10°C) dioxane solution of glycine ethyl ester hydrochloride (1.67 g, 12 mmoles) and NMM (12 mmoles) was added. Stirring was continued for 30 min. then for 45 min. at room temperature. Dioxane was removed under reduced pressure and the obtained residue was re-dissolved in chloroform, thoroughly washed with 1 N HCl, water, 1 N Na_2CO_3, water and then dried. Chloroform was removed under reduced pressure till dryness and the residue was triturated with pet. ether (b.p. 40–60°C) to give 4.85 g (95.5%) of **4** as a white powder. M.p 120–122°C, $[\alpha]^{36}_D$: −l0 (C = 1, chloroform), Lit.: (Gibian and Schroder, 1961, M.p.: 118–120°C, $[\alpha]^{25}_D$: −13 (methanol). Calculated M.wt. for $C_{18}H_{26}N_7O_5$ (420.45), EI-MS (m/z): 420 (M^+).

Benzyloxycarbonylnitroargingylglycine

[Z-Arg(NO_2)-Gly-OH, **5**, Scheme 1B]

Benzyloxycarbonylnitroarginylglycine ethyl ester (**4**, 7.62 g, 17.39 mmoles) was suspended in NaOH (0.5 N, 55 ml). The mixture was stirred at room temperature for 30 min then was extracted with ethylacetate, and the pre-cooled aqueous layer was acidified to pH 2 with concentrated HCl. The resulting crystalline product was collected by filtration, washed with water. Re-crystallization from aqueous ethanol followed by air drying under suction afforded 5.7 g (79.8%) of the peptide as white powder, M.p 114–117°C, $[\alpha]^{36}_D$: −15 (C = 1, methanol), Lit.: (Hofmann et al., 1956) M.P.: 111–113°C, $[\alpha]^{28}_D$: −16.8 (C = 1, methanol), Calculated M.wt. for $C_{16}H_{22}N_6O_7$: (410.4). El-MS (m/z): 411.1 ($M^+ + 1$).

Benzyloxycarbonylnitroarginylglycyldibenzylaspartate

[Z-Arg(NO_2)-Gly-α-Asp (β-OBzl) OBzl, **7**, Scheme 1A]

A cold (−5°C) and stirred THF solution (10 ml) of benzyloxycarbonylnitro-arginylglycine (**5**, 0.41 g, 1 mmoles) and HOBt (0.135 g, 1 mmole) was added to a pre-cooled (−5°C) THF solution (10 ml) of dibibenzylaspartate-*p*-toluenesulfonate (0.485 g, 1 mmole) containing TEA (0.173 ml, 1.2 mmoles). A THF solution (5 ml) of DCCI (0.247 g, 1.2 mmoles) was then dropwise added through ~15 min. The cold (−5°C) reaction mixture was stirred for additional two hours, kept at ~4°C overnight, then for three hours at room temperature. Dicyclohexylurea was removed by filtration and the solvent was evaporated under reduced pressure. The obtained syrupy residue was re-dissolved in chloroform (100 ml), filtered re-washed with a saturated solution of $NaHCO_3$ water, 1 N HCl and water. The solvent was evaporated under reduced pressure to give 0.69 g (97%) of the a crude protected tripeptide. Purification by preparative TLC and crystallization from methanol/ether afforded 0.49 g. of **7** (70%). M.p.: 135–137°C, $[\alpha]^{36}_D$: −12.5 (C = 0.8, DMF). Calculated/Found for $C_{34}H_{29}N_7O_{10}$ (695.64): ESI-MS(m/z): 696 (M^+).

Benzyloxycarbonylnitroarginylglycyl-β-benzyl-α-aspartylserine' methyl ester

[Z-Arg(NO_2)-Gly-α-Asp-(β-OBzl)-Ser-OMe, **14**, Scheme 1B]

A methylene chloride$_2$ solution of *tert*-Butyloxycarbonyl-β-benzyl-aspartylserine methyl ester (**12**, 10 ml, 1.34 g, 3.16 mmoles) was stirred with TFA (5 ml) at room temperature for 30 min. The solvent was removed under reduced pressure and another portion of $MeCl_2$ was added and removed several times, then a pre-cooled solution of N-methylmorpholine (1.5 ml, pH 7.5) in THF (5 ml) was added. A cold (−15°C) THF solution of each of benzyloxy-carbonylnitroarginylglycine (1.29 g, 3.16 mmoles), NMM (**5**, 0.347 ml, 3.16 mmoles) and ethylchloroformate (0.30 ml, 3.16 mmoles) was stirred for 10 minutes, then a cold (−15°C, 10 ml) of the amino free dipeptide ester in the same solvent was added. Stirring was continued for 45 min at (−15°C), and for one hour at room temperature. The solvent was removed under reduced pressure to give an oily residue which was re-dissolved in chloroform (100 ml), washed with 1N HCl, water, 1 N Na_2CO_3, water and dried. Chloroform was removed under reduced pressure to give 1.52 g (67%) crude oily protected tetrapeptide which upon purification by preparative TLC to give 1.23 g (54%) of the pure protected tetrapeptide **14**, M.p.: 178–181°C, $[\alpha]^{35}_D$: −34.97 (C = 0.28, DMF). Calculated M.wt. for $C_{31}H_{40}N_8O_{12}$: (716.68). ESI-MS (m/z): 717(M^+, 100%).

Benzyloxycarbonylnitroarginylsarcosine ethyl ester

[Z-Arg($NO)_2$-Sar-OEt, **4′**, Scheme 1A]

To a THF solution (~0°C, 30 ml) of benzyloxycarbonylnitroarginine (**2**, 0.77 g, 0.5 mmoles) and HOBt (0.081 g, 0.5 mmoles), a pre-cooled THF (~0°C) suspension (5 ml) of sarcosine ethyl ester hydrochloride (0.5 mmoles) and TEA (0.07 ml) was added while stirring and cooling. A THF solution of DCCI (5 ml, 0.206 g, 1 mmoles) was added through 15 minutes. The reaction mixture was stirred at (~0°C) for two hours, kept at 4°C overnight and stirred for additional 3 hours at room temperature. After the dicyclohexylurea was removed by filtration, the solvent was evaporated affording an oily residue, which was dissolved in chloroform (30 ml), washed by 1 N HCl, water, 5% $NaHCO_3$ and water. The chloroform solution was dried, evaporated under reduced pressure to afford 0.157 g (69.6%) of white powder, M.p. 139–140°C, $[\alpha]^{35}_D$: −15 (C = 1, chloroform). Calculated Mwt $C_{19}H_{28}N_6O_7$: (452.46). ESl-MS (m/z): 453 (M^+ +1).

Benzyloxcarbonylnitroarginylsarcosine ethyl ester

[Z-Arg(NO_2)-Sar-OEt, **4′** Scheme 1A, MA Method]

A cold (~10°C) dry Dioxane solution (30-ml) of benzyloxycarbonylnitroarginine (3.54 g, 10 mmoles) and NMM (1.0 ml, 10 mmoles) was stirred with ethylchloroformate (0.95 ml, 10 mmoles). After ~15 min. a pre-cooled (10–12°C) solution of sarcosine ethyl ester hydrochloride (1.54 g, 10 mmoles) and NMM (1.1 ml, 10 mmoles) was added. Stirring was continued for additional 45 min at the same temperature, for two hours at room temperature, then the solvent was evaporated to dryness. The residue was dissolved in chloroform, washed with 1 N HCl, water, 5% $NaHCO_3$, and water. The solvent was removed to give 3.39 g (75%) of title protected dipeptide ester which had identical physical data with that obtain by the DCCI/HOBt method of coupling.

Benzyloxycarbonylnitroarginylsarcosine

[Z-Arg(NO_2)-Sar-OH, **5′**, Scheme 1A]

Benzyloxycarbonylnitroarginylsarcosine ethyl ester (**4′**, 3.0 g, 6.64 mmoles) was suspended in NaOH (0.5 N, 16 ml) and the mixture was stirred at room temperature for 45 min. It was extracted with ethylacetate and the pre-cooled aqueous layer was acidified (pH 2–3) with 5% $KHSO_4$. The mixture was kept cold (~0°C) overnight to give a precipitate which was collected by filtration and air dried to give 2.3 g (80%) of **5′**, M.p: 120–123°C, $[\alpha]^{36}_D$: +50 (C = 0.4, THF). Calculated M.Wt. for $C_{14}H_{24}N_6O_4$ (430.4). EI-MS(m/z): 430 (M^+).

Benzyloxycarbonylnitroarginylsarcosyldibenzyl-α-aspartate

[Z-Arg(NO_2)-Sar-α-Asp (α-OBzI)OBzl, **7**, Scheme 1A]

A cold (−15°C) THF solution (30 ml) of benzyloxycarbonylnitro-arginylsarcosine (**5′**, 4.24 g, 10 mmole) and NMM (1.1 ml, 10 mmoles} was stirred with ethylchlororformate (0.95 ml, 10 mmoles). After 15 min. a pre-cooled (−15°C) THF solution (20 ml) of dibenzylasparlate *p*-toluenesulfonate (4.85 g, 10 mmole) and NMM (1.1 ml, 10 mmoles) was added. Stirring was continued for additional 45 min at (−15°C), then for two hours at room temperature. The solvent was removed under reduced pressure to give an oily residue was dissolved in chloroform (50 ml) washed with 1 N HCl, water, 5% $NaHCO_3$, water and dried The solvent was evaporated to give an oily resinous material 5.95 g, (82.7%) which upon purification by preparative TLC 4.82 g, (67%) of a pure protected tripeptide. M.P.: 315°C (decomposition), $[\alpha]_D^{25}$: +25 (C = 1, methanol). Calculated Mwt for $C_{35}H_{41}N_7O_{10}$ (719.75). ESI-MS (m/z): 720 (M+).

Benzyloxycabonylnitroarginylsarcosyl-β-benzyl-a-aspartyl-serine methyl ester

[Z-Arg(NO_2)-Sar-α-Asp (OBzl)-Ser-OMe, **14′**, Scheme 1B]

A methylene chloride solution (5 ml) of *tert*-butyloxycarbonyl-β-benzyl-α-aspartylserine methyl ester (**12**, 1.14 g, 3 mmoles) was stirred with TFA (5 ml) at room temperature for 30 minutes. The

solvent was removed under reduced pressure and additional $MeCl_2$ (10 ml) was added and evaporated several times. A pre-cooled solution of NMM (1.5 ml) in THF (10 ml, −15°C) was then added thus affording the free dipeptide ester **13**. A cold (−15°C) solution of benzyloxycarbonyl-nitroarginylsarcosine (**5′**, 1.27 g, 3 mmoles), NMM (0.33 ml, 3 mmoles) and ethylchlororformate (0.292 ml, 3 mmoles) was stirred for 10 min. The Pre-cooled (−15°C) THF solution of the free amino dipeptide ester was then added. Stirring of the reaction mixture was continued for 45 minutes at (−15°C) and for one hour at room temperature. The solvent was removed to give an oily residue, which was re-dissolved in chloroform (50 ml), washed by 1 N HCl, water, 5% $NaHCO_3$, water and then dried. The solvent was evaporated under reduced pressure to give 2 g (90 %) of an oily residue of the crude protected tetrapeptide **14′**, which upon purification by preparative TLC gave 1.4 g, (63.9%) of a pure product, M.p. 185–188°C, $[\alpha]^{35}_D = +50$ (C = 0.4, DMF). Calculated M wt for $C_{32}H_{42}N_8O_{12}$: (730.7). ESI-MS(m/z): 731 (M^+).

Deprotection of the synthesized peptides **7**, **7′** (Scheme 1A) and **14**, **14′** (Scheme 1B)

(Catalytic transfer hydrogenation)

The deprotection was principally carried out similar to the methodology followed by El-Amin et al., 1979 and Sivanandaiah et al., 1979. Under nitrogen atmosphere, a suspension of Pd-black in formic acid/methanol (5 ml, 4.4%) was added, to a stirred solution of one of the protected peptides (100 mg) in the same solvent (5 ml). Stirring was continued 12–24 hrs, after which the reaction mixture was filtered off and the solvent was evaporated till dryness affording an oily residue, which upon addition of pet. ether a white precipitate of the fully deprotected peptides **8**, **8′**, **15** and **15′** was obtained. Repeated dissolution and precipitation afforded the peptides in a pure form.

Table 1 presents the obtained physical data.

Spectral and amino acid analytical data of the final products

Arg-Gly-α-Asp (Formate) [**8**, Scheme 1A]

Negative ESI-MS showed m/z at 345.2 [M-HCOO-H] as a base peak. The value is compatible with the calculated molecular formula and weight [C_{13},H_{24},N_6O_8, 392.34]. Equally, the positive ESI-MS showed m/z at 347 that corresponds to [M-HCOOH+H].

IR (λ Cm^{-1}): 3600–3100 (b, s, overlap ν OH, ν NH, COOH) 2954 (w. ν CH. aliphatic). 1665 (s, ν CO, amide I). 1560 (s, CO, amide II). 1393 (s, ν carboxylate, symmetrical stretching).

^{1}H-NMR (DMSO, δ, ppm): 8.31 (s, 1H, α-NH, Gly), 8.03 (s, 1H, α-NH, Asp), 7.41 (b.s, 2H, α-NH_2, Arg), 4.29 (m, 1H, α-CH, Asp), 3.9 (m, 1H, α-CH, Arg), 3.58–3.36 (b.s, 2H, CH_2, Gly), 3.15 (t, 2H, δ-CH_2, Arg). 2.83–2.23 (complex, 2H, β-CH_2, Asp), 1.81–1.55 (b. complex, m, 4H, β-CH_2, Arg, γ-CH_2, Arg).

The amino acid analysis for Arg, Gly and Asp indicated a calculated/found of 1/0.89 : 1/0.90 : 1/0.94.

Arg-Sar-α-Asp (Formate) [**8′**, Scheme 1A]

Positive ESl-MS showed m/z at 361[M-HCOOH+ H] and m/z 343 (base peak, [M-HCOOH-OH]). These data are compatible with the calculated molecular formula and weight ($C_{14}H_{26}N_6O_8$, 406.39).

1R (λ Cm^{-1}): 3500–3100 (b, s, overlap, v OH, v NH), 2930 (w, ν CH. aliphatic), 1680 (s, ν CO, amide I), 1645 (s, ν CO, *tert*-amide, CO-N-CH_3), 1515 (s, CO, amide II).

^{1}H-NMR (DMSO, δ, ppm): 8.31 (s, 1H, α-NH, Asp), 7.68 (s, 2H, α-NH_2, Arg), 7.05 (b.s, 2H, ε-NH_2, Arg), 4.08–3.73 (complex, m, 2H, α-CH, Asp, α-CH, Arg), 3.07 (t, 2H,CH_2, Arg), 2.83 (s, 3H, CH_3-NCO, Sar), 2.68 (m, 2H, β-CH_2, Asp), 1.84–1.45 (complex, m, 4H, β-CH_2, Arg, γ-CH_2, Arg).

The amino acid analysis for Arg, Sar and Asp indicated a calculated/found of 1/1 : 1/0.90 : 1/0.86

Arg-Gly-α-Asp-Ser OMe (Formate) [**15**, Scheme 1B]

Positive ESl-MS indicated m/z at 448[M-HCOOH+H] as a base peak, which is compatible with the calculated molecular formula and weight ($C_{17}H_{31}N_7O_{10}$, 493.47), 1R(λ Cm^{-1}): 3600–3100 (b.s, overlapped, ν OH. ν NH), 2980 (w. ν CH aliphatic), 1740 (s, ν CO, ester) 1654 (s, ν CO, amide I) 1556 (s, CO, amide II).

^{1}H-NMR (DMSO, δ, ppm): 10.20 (s, 1H, β-COOH, Asp), 8.84 (s, 1H, α-NH, Gly), 8.41 (s, 1H, α-NH, Asp), 8.08 (s, 2H, α-NH_2, Arg), 7.54 (d, 1H, α-NH, Ser), 7.22 (s, 2H, ε NH_2, Arg), 4.37 (m, 1H, α-NH, Asp), 4.1–3.47 (complex, m, 6H, β-CH_2, Ser, α-CH, Arg, α-CH.Ser, 2H, CH_2, Gly.), 3.65 (s, 3H, CH_3-O, ester), 3.10 (b.s, 2H, γ-CH_2, Arg), 2.19–2.10 (two, d, 2H, β-CH_2, Asp), 1.87–1.50 (b.m., 4H, β-CH2, Arg, γ-CH_2, Arg).

The amino acid analysis for Arg, Gly, Asp, and Ser indicated a calculated/found of 1/0.85 : 1/0.90 : 1/0.96 : 1/0.89.

Arg-Sar-α-Asp-Ser OMe (Formate) [**15′**, Scheme 1B]

Positive ES1-MS showed m/z at 462 [M-HCOOH+H] which is compatible with the calculated molecular formula and weight ($C_{18}H_{33}N_7O_{10}$, 507.50).

IR (λCm^{-1}): 3600–3050 (b,s, overlapped ν OH. NH), 2960 (w, ν CH, aliphatic), 1737 (s, ν CO. ester), 1642 (s, ν CO, amide I), 1685 (s. ν CO, *tert*-amide CH_3-NCO), 1520 (s, CO, amide II).

^{1}H-NMR (DMSO, δ, ppm): 8.85 (s, 1H, α-NH, Asp), 8.45 (s, 1H, α-NH, Asp), 7.95 (s, 2H, α-NH_2, Arg), 4.85 (b.s. OH, Ser), 4.38 (b.m, 1H, α-CH, Asp), 4.10–3.75 (complex, m, 6H, β-CH_2, Ser. α-CH, Arg, α-CH_2, Ser), 3.65 (s, 3H,CH_3-O ester), 3.18 (s, 1, α-CH_2, Sar), 3.15

Table 1. Physical data of the synthesized peptides

Compound	Yield %	M.P.°C	$[\alpha]_D^{36}$
Arg-Gly-Asp (Formate)	76	200–203	+50, C = 0.2, H_2O
Arg-Sar-Asp (Formate)	65	Dec. 298	+55, C = 0.2, H_2O
Arg-Gly-Asp-Ser OMe (Formate)	61	158–161	+50, C = 0.9, H_2O
Arg-Sar-Asp-Ser OMe (Formate)	63	oil	+60, C = 0.2, MeOH

(m, 2H, δ-CH_2, Arg), 2.80 (s, 3H, CH_3-NCO, Sar), 2.41 (m, β-CH_2, Asp), 1.85–1.52 (m, 4H, β-CH_2, Arg, γ-CH_2, Arg).

The amino acid analysis for Arg, Sar, Asp and Ser indicated a calculated/found of 1/0.86 : 1/0.90 : 1/0.96 : 1/0.92.

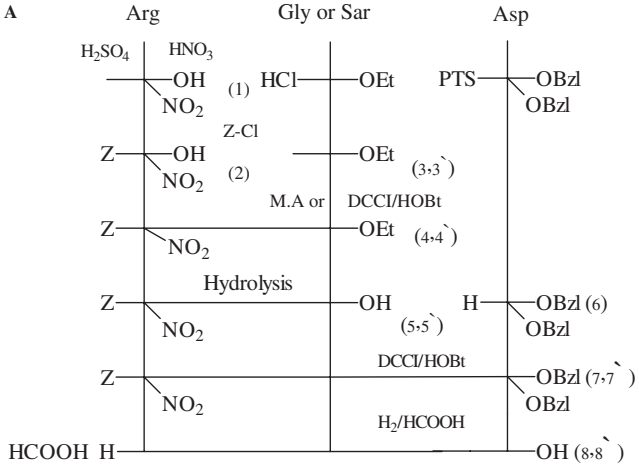
3 Results and discussion

The increasing interest in the biological applications of the crucial "RGD" peptides is frequently hindered by the difficulty of a convenient obtainment of the required assayed sequences. Solid phase technique of peptide synthesis, despite less laborious than the classical conventional methodologies in solution, yet generally provided insufficient quantities of the peptides in question (Yoshiaki et al., 1991).

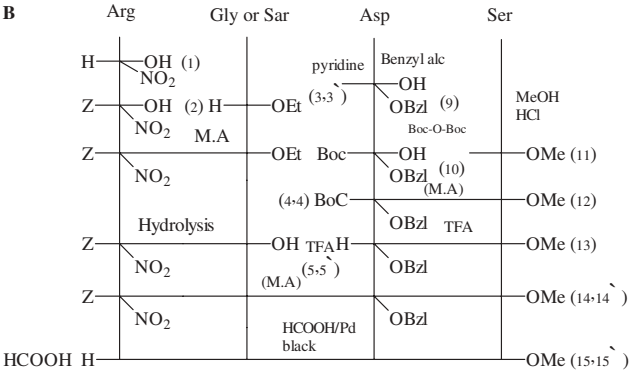
According to the herein followed conventional methodology, the core tripeptide sequence arginyl-glycyl-α-aspartic acid "RGD" (**8**, Scheme 1A) and its sarcosine congener arginyl-sarcosyl-α-aspartic acid (**8′**, Scheme 1A), as well as the corresponding methylserinate tetrapeptides, namely, arginylglycyl-α aspartylserine methyl ester (**15**, Scheme 1B) and arginyl-sarcosyl-α-aspartylserine methyl ester (**15′** Scheme 1B) were optimally synthesized, purified and fully characterized.

The synthesis of the intermediate required functionalized amino acids (**1**, **2**, **6**, Scheme 1A and **9**, **10**, **11**, Scheme 1B) and the intermediate peptides (**4**, **4′**, **5**, **5′**, **7**, **7′**, Scheme 1A and **11**, **12**, **13**, **14**, Scheme 1B), was generally planned to be mastered in gram quantities. Synthetically a convenient supply for these crucial sequences is thereby described. Equally, facilitation for the obtainment of longer peptide homologues, when required, by segment or stepwise condensation strategies and the corresponding "RGD" peptidomimics in which the peptide linkage is replaced by either NHCO, CSNH, CH_2NH, $COCH_2$, NHCONH, CH = CH or CH_2S groups seemed, accordingly, to be more practically realizable.

The selection of sarcosine as the N-methyl positional lipophilic isomer of glycine, to be its substitute in both a tripeptide **8′** and a tetrapeptide **15′** could be rationalized by the continuously reported literature interest in the investigation of the biological consequences of such structure modulation. The replacement of these N-methyl amino acid isomers, as inserts, in biologically active amino acid derivatives and peptides has, generally, an interesting investigatable biological impacts (Akira, 1987; Rudraksh et al., 2001; Abo-Ghalia et al., 1997). Solubility, receptor selectivity, transport properties, enzymatic degradation



Scheme 1A. Synthesis of Arg-Gly-α-Asp and Arg-Sar-α-Asp



Scheme 1B. Synthesis of Arg-Gly-α-Asp-Ser-OMe and Arg-Sar-α-Asp-Ser-OMe

patterns, are frequently favorably altered. This rational is particularly supported by what was reported that, the Sarcosine tetrapeptide analogue of RGDS (Akira, 1987) had a promising cancer metastatic inhibitory activity (pulmonary cancer, melanoma B_{16}-F_{10}). The synthesis of that peptide, however, was ambiguously described by a solid phase methodology, employing N-*tert*-butyloxycarbonyl amino acids and 4-(hydroxymethyl)phenyl-acetamido-methyl resin.

The general features of the herein-followed synthetic strategies represented by Scheme 1A and 1B, implicated a concise compilation of two principles namely:

1- The amino and carboxyl terminal temporary protection was planned to be achieved via the

benzyloxycarbonyl function and the benzyl ester respectively. Both, in addition to the nitro group utilized for the protection of Arginine guanidino function, are cleavable by mild hydrogenolysis. Unlikely, the intermediate dipeptide aspartyl-serine (Scheme 1B) was conveniently obtained as its N-*tert*-butyloxycarbonyl and C-methyl ester cleavable by the acidiolytic treatment and mild saponification, respectively. Such strategy, permits, for the synthesized peptides, to be elongated, after the appropriate de-protection, from either its N-terminal (HBr/AcOH treatment) or its C-terminal (alkaline hydrolysis or hydrazinoloysis, if the azide method of coupling was the method of choice.

2- Both the mixed anhydride method of coupling, frequently furnishing the desired peptide as the sole product and the potentiated, non-racemizing DCCl/HOBt coupling were selected as peptide bond forming methods. Particularly for the mixed anhydride, the experimental variants, namely the used coupling agent, the tertiary base, solvent medium, time required for the formation of the mixed anhydride and time of coupling of the second, amino acid were deliberately studied.

Analytical and spectral details of the obtained intermediates are included in the material and methods section. Synthetic routes are however outlined in Scheme 1A and 1B.

4 Conclusion

An ameliorated simplified conventional synthetic protocol for the crucial RGD and RGDS and their sarcosine analogues were described. A quasi-gram peptide yield could be obtained for the intermediate peptide and functionalized amino acids, thus rendering practical, the availability of these biologically interesting sequences.

Acknowledgment

Amino acid analysis and ion spray mass spectra were realized in the Organic Chemistry Institute, Tübingen University, Germany. The co-operation of the staff members and particularly, Prof. E. Bayer is highly appreciated.

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