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Cyclic RGD peptide by ring-closing metathesis

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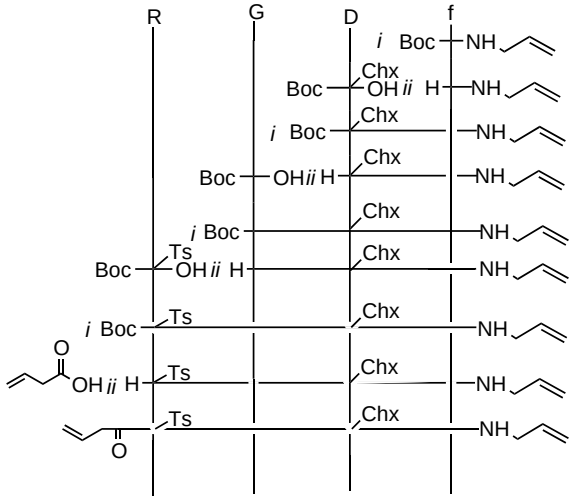
Abstract—A cyclic pseudopeptide containing the RGD sequence has been synthesized in high yield by ring-closing metathesis using a Grubbs' catalyst in tetrahydrofuran. Conformational analysis has been correlated to structure modeling and preliminary biological activity as an integrin binding inhibitor has been evaluated.

Conformationally restricted structures obtained by cyclizing aminoacid sequences often present relatively to their linear precursors several advantages such as increased metabolic stability, bioavailability and receptor selectivity.[1,2] True cyclic peptides resulting from head-to-tail cyclization have been used as inhibitors of integrin $\alpha_v\beta_3$[3] or of pancreatic trypsin,[4] synthetic immunogens,[5] antigens for Herpes Simplex Virus.[6] Those obtained by side chain-to-side chain lactamization have been applied to improve receptor selectivity in RGD-dependent adhesion proteins,[7] enkephalin,[8] cholecystokinin,[9] tachykinin.[10]



Scheme 1. Linear peptide precursor synthesis.

Since the recent discovery of efficient and robust catalysts,[11] cyclic pseudopeptides obtained by ring-closing metathesis (RCM) start to become more popular due to the smoothness of the catalysis conditions and the tolerance for functional groups.[12] In that way, cyclic dithiopeptides can be modified and stabilized by replacement of the S–S link for a C–C bond.[12] Moreover, the olefin metathesis reaction should be considered as a new way for introducing the dehydrocarba surrogate of the peptide bond.[13a] This enzymatically-resisting lipophilic isosteric mimic increases the whole biodisponibility of the target molecule.[13b]

Membrane adhesion proteins belonging to the integrin superfamily are involved in different major diseases such as: thrombosis, osteoporosis, diabetic retinopathy, angiogenesis (reviewed in Refs. 14 and 15). Since many integrins bind to their natural putative ligands of the extracellular matrix mainly through the same RGD sequence thus, their binding specificity must rely on the spatial arrangement of the lateral chain charges of this tripeptide. Increasing the selectivity of their inhibitors is currently a challenging objective.

Starting from the c(RGDfV) peptide identified by Kessler and Coll.[3] as a potent cyclopeptide highly specific of the $\alpha_v\beta_3$ versus $\alpha_{IIB}\beta_3$ integrin binding inhibition, we designed a dehydrocarba analog of the parent peptide prepared by ring-closing metathesis of the corresponding diolefinic linear precursor containing the

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same RGDf sequence and bearing an allylic substitution at the N- and C-terminal positions. The linear peptide was synthesized using a step by step coupling strategy in solution as depicted in Scheme 1.

The Boc group was used as temporary protection and HF-cleavable protection chosen for the side chain of arginine (Ts) and aspartic acid (Chx). The linear protected peptide was obtained in nine steps from allylamine to the butenoyl grafting with a global yield of 7.5%. Coupling reactions were carried out in the presence of BOP and DIEA in DMF as solvent and Boc deprotection obtained with a CH_2Cl_2/TFA mixture.

The protected precursor has been submitted to RCM in conventional conditions as it may be seen in Scheme 2. Reactions were carried out at a concentration of 2 mM and cyclization was monitored by LC/MS. Poor results were firstly obtained using the first Grubbs' catalyst **I** in CH_2Cl_2 (entry 1, Table 1) until the solvent and catalyst were changed towards more polar media and the second Grubbs' catalyst **II**. In THF, rarely used until now[16] for RCM, a quantitative cyclization yield (entry 6, Table 1) was hopefully obtained. This good result is probably related to a better solubility of the peptide and may also be due to a higher conformational freedom allowing a favorable position of the two olefinic groups. One cannot also exclude a positive interaction of THF oxygens with the catalyst, releasing it from possible unproductive chelates due to the presence of complexing functional groups of the peptidic structure.[17,18]

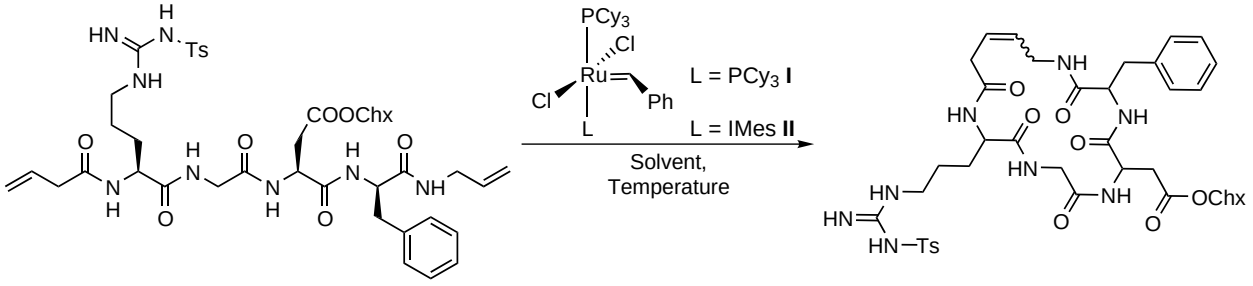
After HF deprotection, the cyclopseudopeptide was purified and analyzed.[19] ^{1}H NMR analysis[19] showed a high amount (more than 90%) of the *cis*-olefin stereomer and two out of five possible intramolecular hydrogen bonds [NH-Gly and NH-Apa (5-aminopent-3-enoic acid residue)] as judged by a ^{1}H NMR temperature effect study (Table 2). The chemical shifts of the amide proton showed a linear upfield shift as the temperature increased, indicating that no major conformation changes occurred over this temperature range in DMSO-d_6. Temperature coefficients of $>[5\times10^{-3}]$ ppm/K are indicative of freely exchanging amide protons, whereas values of $<[3\times10^{-3}]$ ppm/K are generally considered to be involved in an intramolecular hydrogen bond.

A modeling study using the Genmol program[20] gave as the most stable conformation, a structure including the two intramolecular NH bonds indicated above but presenting a shorter distance between Cα Arg and Cα Asp than that obtained with c(RGDfV) (0.49 nm versus 1.16 nm). Preliminary biological studies of this peptide were performed in a cell adhesion assay using CHO cell clones expressing recombinant human $\alpha_v\beta_3$ integrins.[21] The dehydrocarba cyclopeptide was shown to inhibit $\alpha_v\beta_3$-dependent cell adhesion to immobilized fibrinogen with an IC100% at 0.25 mM.

In summary, a cyclic pseudopeptide containing an olefinic moiety was successfully prepared by a catalytic RCM performed in THF. This methodology would be easily applied to other dehydrocarba analogs contain-

Table 2. Chemical shift assignments and temperature coefficients of NH of *cyclo*-(R-G-D-f-Apa) in DMSO-d_6

Entry	NH proton	δ_{NH}	$\Delta\delta_{NH}$ (ppb)/ΔT (K)
1	Asp	8.73	−4.9
2	Phe	8.50	−5.4
3	Arg	8.35	−4.7
4	Apa	7.47	−1.8
5	Gly	7.42	−1.7



Scheme 2. Peptide cyclization by olefin metathesis.

Table 1. Reaction conditions for the ring-closing metathesis

Entry	Catalyst (% mol)	Solvent	Temperature (°C)	Time (h)	Conversion (%)
1	**I** (10)	CH_2Cl_2	Reflux	24	0
2	**II** (20)	CH_2Cl_2	Reflux	3	59
3	**II** (20)	CH_2Cl_2/THF (2/1)	Reflux	3	74
4	**II** (20)	CH_2Cl_2/THF (1/2)	Reflux	3	89
5	**II** (20)	THF	Room temperature	3	82
6	**II** (20)	THF	Reflux	3	100

ing the RGD sequence designed to increase the $\alpha_v\beta_3$ binding inhibitor activity.

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19. All new compounds gave satisfactory analytical data: ^{1}H and ^{13}C NMR analyses were performed with a 400 MHz NMR spectrometer. The HPLC analyses were carried out on a Waters Millennium with a photodiode array detector 996, wavelength 214 nm, using as a reversed phase Nucleosil C18 column, 5μ, (250×10 mm) with a flow rate of 1 ml/min, eluants (solvent A: 0.1% TFA in H_2O, solvent B: 0.1% TFA in CH_3CN).
Butenoyl-L-Arg(Ts)-Gly-L-Asp(Cy)-D-PheNH-Allyl: R_f 0.5 (EtOAc 100%). ^{1}H NMR (CD_3OD) δ 1.25–1.90 (m, 16H), 2.40 (s, 3H), 2.60–2.80 (m, 2H), 2.85–3.10 (m, 3H), 3.10–3.35 (m, 8H), 3.80 (d, $J=5.0$ Hz, 2H), 3.85 (s, 3H), 4.25 (t, 1H), 4.50–4.60 (m, 1H), 4.70 (t, $J=46.0$ Hz, 3H), 5.0–5.30 (m, 5H), 5.65–4.05 (m, 2H), 7.15–7.40 (m, 5H), 7.70–7.80 (d, $J=9.0$ Hz, 2H). ^{13}C NMR (CD_3OD) δ 20.41, 23.20, 23.70, 25.40, 28.60, 29.67, 31.45, 31.50, 36.21, 37.60, 40.48, 41.76, 43.00, 50.14, 54.24, 55.80, 73.65, 115.25, 117.91, 126.13, 126.77, 128.51, 129.30, 129.32, 131.70, 134.12, 137.65, 141.16, 142.55, 157.65, 170.61 (2C), 171.61, 172.12, 173.47, 174.31. ESI-MS 837.4 (M+H$^+$). HRMS calcd 837.3970, found 837.3969. HPLC 12.07 min. IR cm^{-1} 1130.7, 1253.2, 1450.4, 1542.0, 1638.6, 1720.2, 2931.8, 3290.8.
***cyclo*-(L-Arg(Ts)-Gly-L-Asp(Cy)-D-Phe-*cis*-Apa)**: Linear peptide (270 mg, 0.32 mmol) was dissolved in THF (160 ml) then Grubbs' catalyst **II** (55 mg, $6.4.10^{-2}$ mmol) was added to the solution. The mixture was stirred at reflux overnight. THF was evaporated and residue was purified over preparative HPLC to yield the title compound (60%). ^{1}H NMR (CD_3OD) δ 1.25–1.95 (m, 15H), 2.40 (s, 3H), 2.50–2.55 (m, 1H), 2.80–2.95 (m, 2H), 3.10 (q, $J=6.5$ Hz, 1H), 3.15–3.25 (sl, 2H), 3.35 (dd, $J=4.5$ Hz, $J=14.0$ Hz, 1H), 3.80–3.95 (m, 4H), 4.35 (q, $J=4.5$ Hz, 1H), 4.45 (t, $J=6.5$ Hz, 1H), 4.60 (dd, $J=4.5$ Hz, $J=10.5$ Hz, 1H), 4.65–4.75 (m, 1H), 5.65–5.85 (m, 2H), 7.20–7.35 (m, 7H), 7.75 (d, $J=8.5$ Hz, 2H). ^{13}C NMR (CD_3OD) δ 20.42, 23.66, 25.40, 27.96, 31.45, 35.29, 36.74, 39.75, 41.34, 41.99, 51.49. 53.43, 55.00, 73.60, 125.74, 126.16, 126.75, 128.49, 129.11, 129.26, 129.34, 137.93, 169.96, 170.14, 171.93, 172.15, 172.69, 173.15. HPLC 11.175 min. HRMS calcd 809.3665, found 809.3665.
***cyclo*-(L-Arg-Gly-L-Asp-D-Phe-*cis*-Apa)**: Cyclic peptide (0.040 g, 0.049 mmol) was added to a solution of anhydric fluorhydric acid at 0°C for 1 h in the presence of anisole. The mixture was heated to 20°C and HF was evaporated. The residue was precipitated in ether then purified over preparative HPLC to yield 0.020 g (60%) of the title compound. ^{1}H NMR (DMSO-d_6) δ 1.15 (sl, 1H), 1.30–1.50 (m, 4H), 1.75 (sl, 1H), 2.20–2.30 (m, 2H), 2.60–2.75 (m, 2H), 2.90–3.10 (m, 3H), 3.60–3.80 (m, 4H), 4.00–4.10 (m, 1H), 4.10–4.20 (m, 1H), 4.25–4.35 (m, 1H), 5.50–5.70 (m, 2H), 7.05–7.20 (m, 5H), 7.35 (sl, 1H), 7.40 (sl, 1H), 8.25 (d, $J=8.5$ Hz, 1H), 8.40 (d, $J=9.0$ Hz, 1H), 8.65 (d, $J=5.0$ Hz, 1H), 12.35 (sl, 1H). ^{13}C NMR (DMSO-d_6) δ 26.16, 28.61, 35.85, 37.03, 41.73, 42.34, 51.87, 52.99, 54.75, 126.87, 127.03, 128.94, 129.53, 139.33, 157.51, 170.02, 171.07, 171.29, 171.79. ESI-MS m/z 573.2 (M+H$^+$). HRMS calcd for $C_{26}H_{37}O_7N_8$ 573.2785, found 573.2778. HPLC 7.312 min.
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