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cis-Apa: A Practical Linker for the Microwave-Assisted Preparation of Cyclic Pseudopeptides via RCM Cyclative Cleavage

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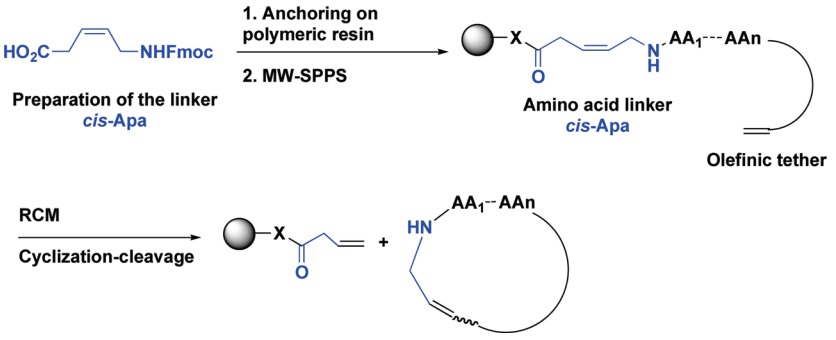
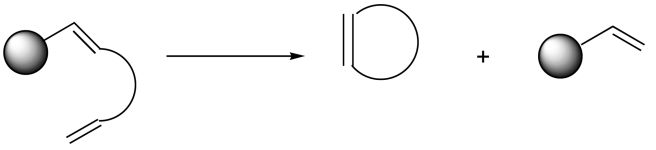


A new linker *cis*-5-aminopent-3-enoic acid (*cis*-Apa) was prepared for the synthesis of cyclic pseudopeptides by cyclization−cleavage by using ring-closing methatesis (RCM). We developed a new synthetic pathway for the preparation of the *cis*-Apa linker that was tested in the cyclization−cleavage process of different RGD peptide sequences. Different macrocyclic peptidomimetics were prepared by using this integrated microwave-assisted method, showing that the readily available *cis*-Apa amino acid is well adapted as a linker in the cyclization−cleavage process.

Introduction

In the search for new bioactive molecules, chemists and biologists have shown a major interest in cyclic peptides. For comparison to their linear precursors, these conformationally restricted structures present several advantages including better bioavailability, metabolic stability, and receptor selectivity.[1] The construction of cyclic peptides is still a challenge for the chemist. One attractive approach is the cyclization−cleavage strategy, which has become a practical method using a solid-phase synthesis on polymeric support, but mostly for the preparation of small cyclic molecules.[2] The major advantage of this method is the simultaneous cyclization/release of the expected product from the support with good purity.[2c] Generally, this concept involves the formation of a carbon−heteroatom bond rather than carbon−carbon bond formation.[2g] One exception is the synthesis of cyclic compounds by cyclization−cleavage using ring-closing metathesis (RCM). This process (Scheme 1) involves a carbon−carbon bond formation in mild reaction conditions and remarkable functional group tolerance due to the use of ruthenium-based catalysts. To our knowledge, only two reports describe the cyclization−cleavage strategy via RCM as a method for the preparation of macrocyclic peptides.[2c,h]

SCHEME 1. Cyclization−Cleavage Process via RCM



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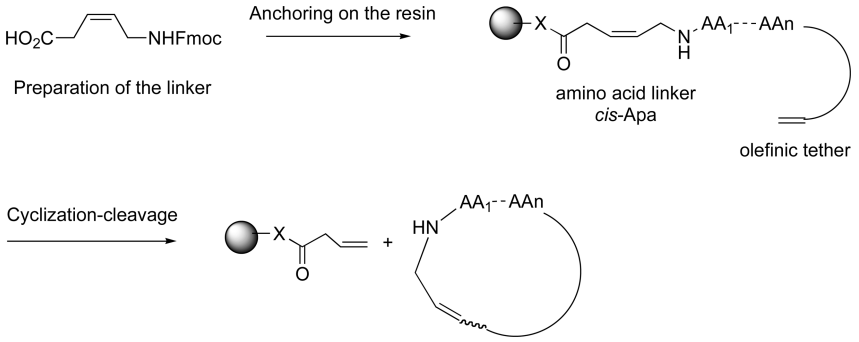
SCHEME 2. Method for the Preparation of Cyclic Pseudopeptides



We report herein the development of a practical and efficient integrated strategy for the preparation of cyclic pseudopeptides including (i) synthesis of a new linker suitable for solid phase synthesis and the cyclization−cleavage step, (ii) microwave-assisted peptide synthesis on a solid support, and (iii) cyclization−release using ring-closing metathesis (RCM) (Scheme 2).

The first step of the project consisted of the preparation of a linker suitable for the cyclization-release strategy and easily introduced in an automated solid-phase peptide synthesis (SPPS) using a Fmoc strategy. This strategy was illustrated in the preparation of cyclic RGD peptides, well-known to be highly potent and in some cases selective $\alpha_v\beta_3$ integrin antagonists.[3]

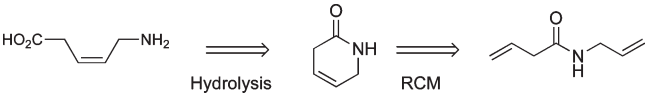
Results and Discussion

Synthesis of the *cis*-5-Aminopent-3-enoic Acid (*cis*-Apa) Linker. The choice of the amino acid linker depended on specific criteria such as the presence of an olefin for the RCM, the accessibility in large quantities, and the possibility to use a Fmoc protection of the amino group for the introduction in SPPS. The simplest structure fitting these conditions is the acyclic unsaturated amino acid 5-aminopent-3-enoic acid (Apa). Moreover we were attracted to its cis isomer since Grubbs and co-workers have demonstrated that the cis isomer of bulky internal olefins reacts faster than the trans counterpart in ruthenium-catalyzed metathesis.[4]

Few methods for preparing the *cis*-Apa are described[5] and they typically require an amination step from the corresponding diacid, made from sequential oxidations of 1,4-cyclohexadiene.[5c] A different synthesis proposes the formation of the diacid in two steps from the hex-3-yne-1,6-diol;[5a] the yields for the corresponding hydrogenation and reduction are not reported.

We have investigated a new pathway for the synthesis of *cis*-Apa based on a ring-closing/ring-opening strategy of the corresponding diethylenic amide (Scheme 3).

SCHEME 3. Retrosynthetic Analysis of the *cis*-Apa



Guibé and co-workers[6] reported on the synthesis of (*Z*)-ethylenic δ amino acids using an RCM step. They prepared enantiopure *Z* alkene analogues of various dipeptides starting from the corresponding diethylenic amides bearing a protecting group on the nitrogen atom.

On the basis of this study, we proposed an original synthesis of the protected *cis*-Apa in four steps (Scheme 4).

Various reaction conditions were studied for the synthesis of the *N*-allyl-3-butenamide **3**, since the formation of an undesired isomer was observed especially with classical coupling agents such as BOP, HBTU, and HATU. Using the symmetrical anhydride method (formation of the anhydride of **1** with DCC and filtration of DCU before reaction with allylamine **2**) suppressed the formation of isomer **4**. However, these mild and efficient conditions present a major limitation since the maximum theoretical yield is 50%.[7] Liebeskind and co-workers[8] prepared **3** from the corresponding acid chloride and allylamine **2** in 52% yield. We improved these conditions, with the EDC as coupling reagent in the presence of HOBt,[7] as additive, at low temperature, to afford the expected product in 75% yield. Microwave activation[9] had also been described for amidation on different substrates. These conditions applied to our synthesis led only to the formation of the undesired isomer in 27% yield.

As a first approach, the Boc protection step of **3** was achieved before the ring-closing metathesis step, to ensure a sufficient proportion of the s-cis rotamer versus the s-trans rotamer to allow the cyclization step.[6b] Protected amide **5** was isolated in 87% yield, then ring-closing metathesis was achieved with Grubbs' II catalyst **10** (Figure 1) under microwave activation to yield **6** (62%).

RCM could be performed directly on the unprotected substrate by using Grubbs' I catalyst of the first generation, in CH_2Cl_2 for 12 h, in 80% yield according to Liebeskind.[8b] In our hands, we achieved this reaction employing microwave activation in CH_2Cl_2, for 10 min in 66% yield.

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SCHEME 4. Synthesis of the *cis*-Apa Linker

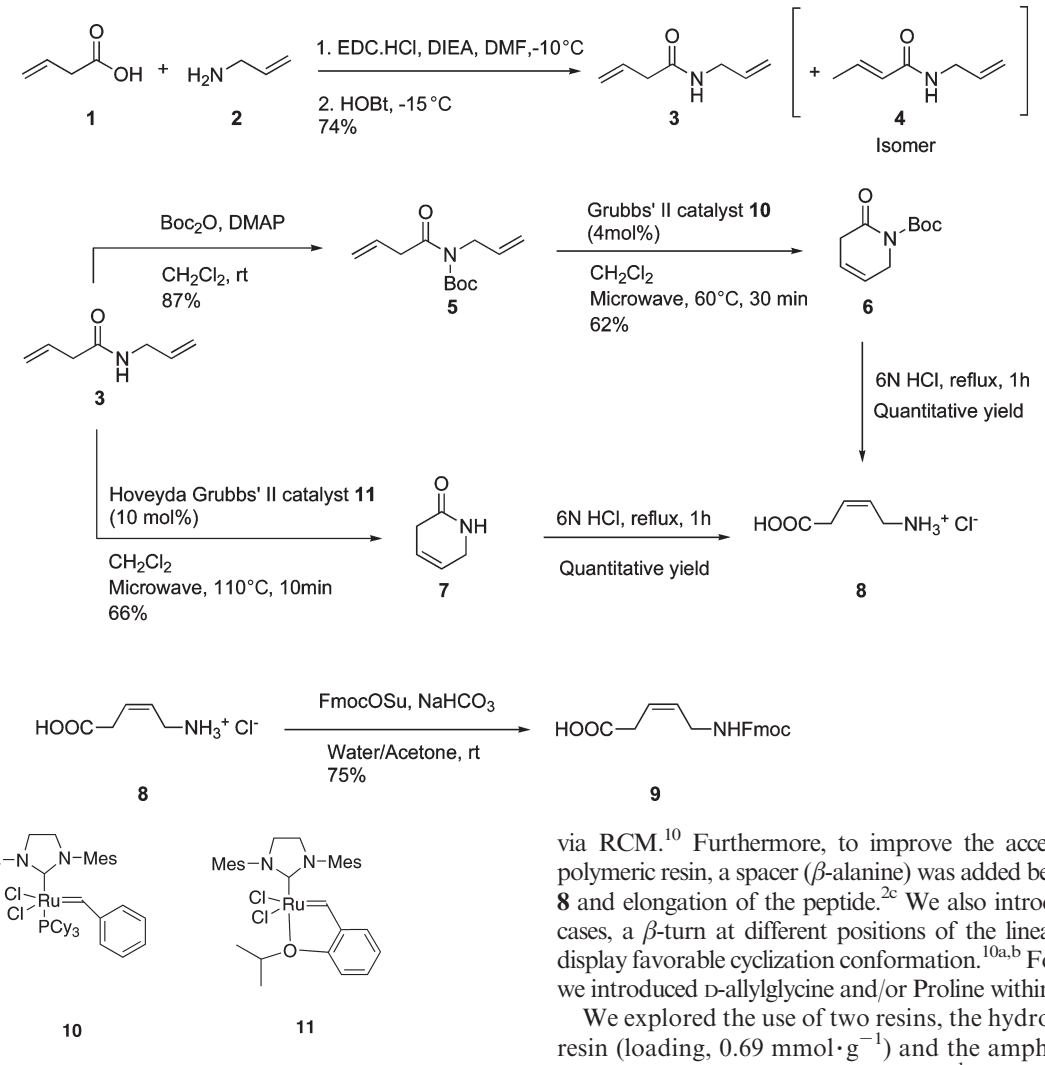

FIGURE 1. Metathesis catalysts.

No isomerization of the double bond was observed. Subsequent acidic hydrolysis provided the amino acid **8** in quantitative yield without further purification. The protection step with FmocOSu reagent provided protected *cis*-Apa **9** in 76% yield (37% overall yield for the four steps), suitable for use in peptide synthesis.

Preparation of Linear Pseudopeptides. We chose as a model peptides derived from the RGD sequence (Figure 2). The strategy involves the anchor of the *cis*-Apa on a solid support to perform the solid-phase synthesis of the linear pseudopeptides. Since another olefin was needed to perform the RCM, we introduced at the *N*-terminus of every sequence an allylglycine, used very often in the preparation of cyclic peptides via RCM.[10] Furthermore, to improve the accessibility of the polymeric resin, a spacer (β-alanine) was added before anchoring **8** and elongation of the peptide.[2c] We also introduced, in some cases, a β-turn at different positions of the linear precursor to display favorable cyclization conformation.[10a,b] For this purpose, we introduced D-allylglycine and/or Proline within the sequence.

We explored the use of two resins, the hydrophobic Wang resin (loading, 0.69 mmol·g^{-1}) and the amphiphilic ChemMatrix resin (loading, 0.47 mmol·g^{-1}), for the synthesis of the peptides. Eight different linear precursors (**12a**/**b**−**15a**/**b**) were synthesized on an automatic microwave peptide synthesizer (Figure 3). To evaluate the efficiency of the solid-phase synthesis, for each sequence, an aliquot was subjected to acidic cleavage conditions (TFA/H_2O/TIS solution (95:2.5:2.5 v/v/v)), filtered, and evaporated and the residue was analyzed by LC/MS. Since the method developed herein will be applied to potentially bioactive peptides, the most important criteria was purity (over yield) to allow the use of these molecules in biological tests. The purity was evaluated by HPLC/UV analyses and was superior to 90%. No deletion byproduct was formed during the synthesis. Yields of the linear peptides were evaluated and are in the range of 40−60%.

Cyclization−Cleavage Studies. RCM cyclative cleavage experiments were performed on a semiautomated organic synthesizer with mechanical stirring at room temperature for 48 h with Grubbs' II **10** and Hoveyda Grubbs' II **11** as catalyst (Scheme 5). Thirty mol % of the catalyst (classical conditions for RCM on a solid support) was used in each experiment.[3b] Results are presented in Table 1.

In each case, the cyclic pseudopeptide (Figure 4) was detected and analyzed by LC/MS analysis. To evaluate the

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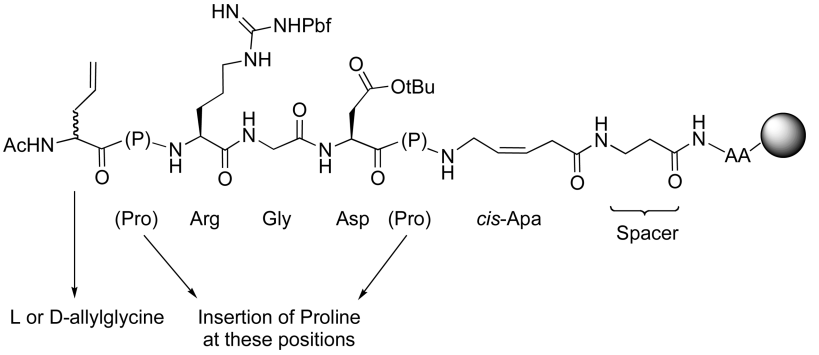


FIGURE 2. RGD-based linear model.

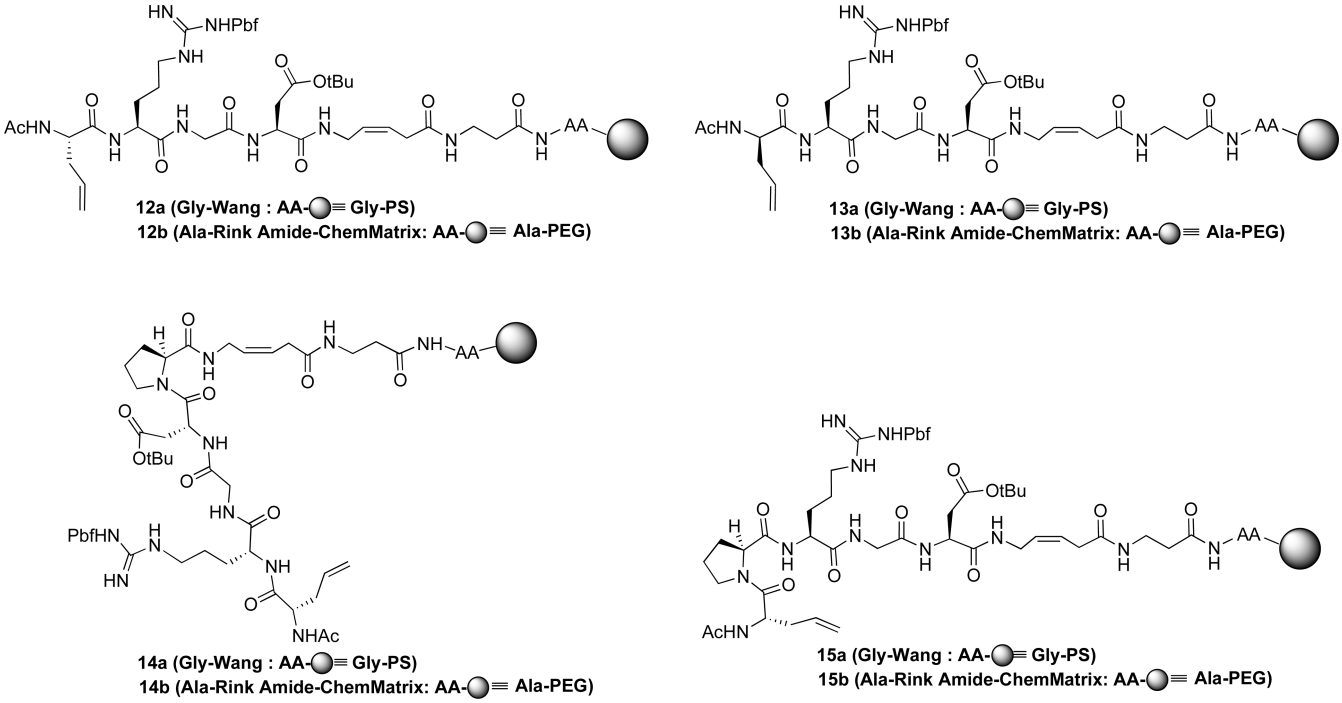
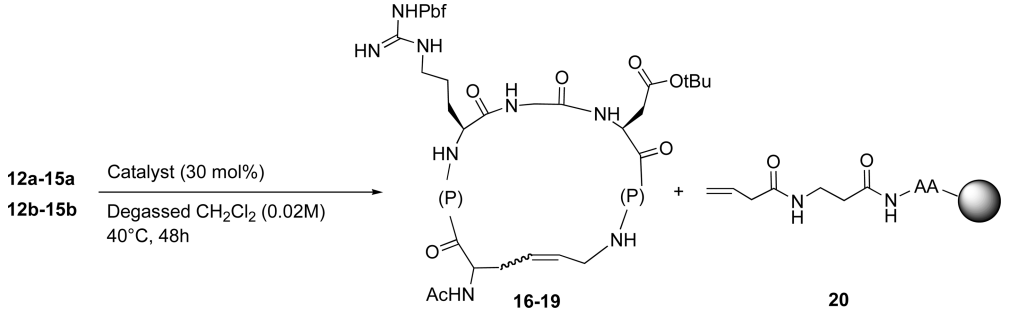


FIGURE 3. Linear precursors.

SCHEME 5. RCM Cyclative Cleavage Conditions



quantity of recovered peptide, the crude mixture was weighed: 20−30 mg (35 to 53% crude yield) was recovered for the synthesis performed with ChemMatrix resin and 30−50 mg (29 to 57% crude yield) for the synthesis performed on the Wang resin. This is in agreement with the higher loading of the Wang resin. After the RCM, the presence of unreacted linear peptides on the resin was evaluated. For this purpose the corresponding resins were subjected to acidic cleavage conditions (TFA/H_2O/TIS solution (95:2.5:2.5 v/v/v)), filtered, and evaporated and the residues were analyzed by LC/MS. No linear peptide was detected.

The purity was evaluated by HPLC/UV analyses. For both resins, the better results were obtained with the Grubbs'

TABLE 1. Results of Cyclization−Cleavage of Linear Precursors 12a/b−15a/b to Macrocycles 16−19

entry	linear SM	product	catalysts (30 mol %)	purity (%)	cyclic dimer (%)
			Wang resin		
1	**12a**	**16**	**10**	40	0
2	**13a**	**17**	**10**	39	6
3	**14a**	**18**	**10**	31	10
4	**15a**	**19**	**10**	58	5
5	**12a**	**16**	**11**	25	0
6	**13a**	**17**	**11**	25	0
7	**14a**	**18**	**11**	20	15
8	**15a**	**19**	**11**	24	10
			ChemMatrix resin		
9	**12b**	**16**	**10**	50	5
10	**13b**	**17**	**10**	57	7
11	**14b**	**18**	**10**	39	13
12	**15b**	**19**	**10**	65	9
13	**12b**	**16**	**11**	36	0
14	**13b**	**17**	**11**	37	4
15	**14b**	**18**	**11**	25	11
16	**15b**	**19**	**11**	40	13

II catalyst **10** (entries 1−4 and 9−12). Furthermore, the nature of the resin also had an influence on the outcome of the reaction, the ChemMatrix giving improved purities whatever the catalyst (entries 9−16). ChemMatrix resin is well-known to perform efficiently, as compared to PS resins, in the solid-phase synthesis of hydrophobic, highly structured, and sterically hindered peptides.[11]

In most of the cases, formation of a cyclic dimer was detected by mass spectrometry (Figure 5). One possible structure of this dimer is shown in Figure 5 but detailed analysis of these compounds was not performed. No other byproduct could be detected by LC/MS.

The formation of the dimer may arise from the opening of the desired cyclic peptide, dimerization of the so-formed linear peptide through cross-metathesis, and again cyclization (RCM). We tested this hypothesis by reacting, after synthesis through our method and isolation, cyclic peptide **19** with catalyst **10** under microwave activation. No dimer could be detected. But this does not completely deter the hypothesis presented above since in the original conditions, the presence of the solid support bearing the linear peptide may help in the reaction. Indeed, cyclic **19** could open in solution, then react through a cross-metathesis on the resin-supported linear peptide, followed by a RCM which would provide the cyclic dimer.

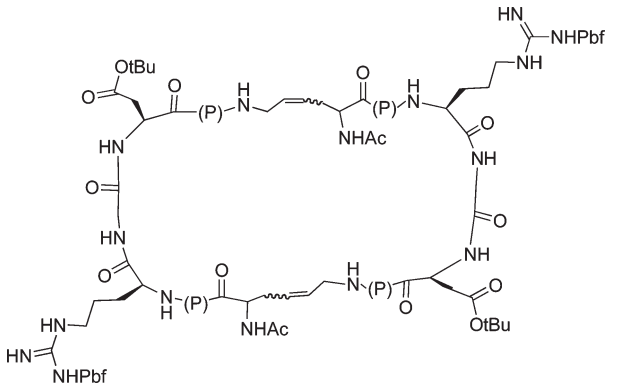


FIGURE 5. Possible structure of the dimer.

Cyclization occurred for each sequence, even in the absence of a β-turn inducing component. Switching from L-allylglycine to D-allylglycine (entries 1, 5, 9, 13 vs entries 2, 6, 10, 14, respectively) did not provide better cyclization. The presence of a proline on the C-terminus of the RGD sequence did not bring much improvement either (entries 3, 7, 11, 15 vs entries 1, 5, 9, 13, respectively). In contrast, the introduction of a proline on the N-terminus of the linear peptide gave the best results (entries 4, 12, 16 vs entries 1, 9, 13, respectively). According to these different experiments, the cyclization−cleavage on the linear peptide incorporating the proline on the N-terminus extremity, synthesized on ChemMatrix resin and using Grubbs' II **10** as catalyst, led to the macrocycle **19** with an acceptable purity (entry 12).

Using these best conditions, microwave activation was also tested for the cyclization of precursor **15b** and results are presented in Table 2. Using Grubbs' II catalyst **10** at 60 °C

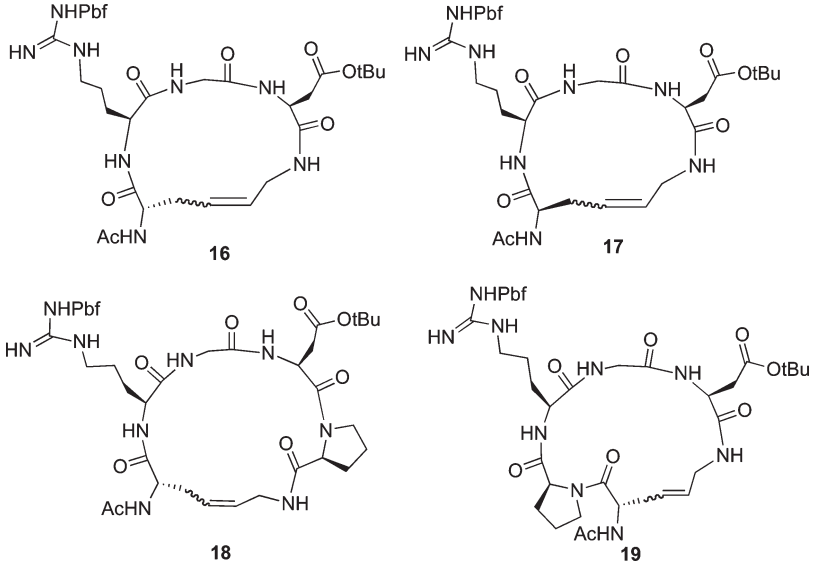


FIGURE 4. Macrocyclic pseudopeptides.

TABLE 2. Results of Cyclization−Cleavage of Linear Precursor 15b to Macrocycle 19 under Microwave Activation[a]

entry	conditions	additive	purity (%)	cyclic dimer (%)
1	60 °C, 5 h		73	8
2	60 °C, 2 h		47	8
3	100 °C, 1 h	LiCl (10 mol %)	56	6
4	100 °C, 1 h	styrene (1 equiv)	15	2
5	100 °C, 30 min	**10** added in three portions	64	7
6	100 °C, 30 min	**10** added in three portions LiCl (10 mol %)	55	0

[a]Catalyst **10** (30 mol %) was used in CH_2Cl_2.

for 5 h, a good purity was obtained (entry 1). Reducing reaction time decreased the amount of cyclic pseudopeptide formed (entry 2). Since it is known that the use of various additives could improve RCM on a solid support, reactions including LiCl[10f,h] (templating effect) (entry 3) or styrene[2d,12] (recovery of resin sequestered catalyst) (entry 4) were performed. Styrene gave very poor results while LiCl had no positive effect on conversion. Adding the catalyst in three portions[3b] in the absence (entry 5) or presence (entry 6) of LiCl did not provide better results.

In comparison to conventional heating, microwave activation led to a better conversion for a shorter reaction time with the initial conditions (entry 1). These efficient conditions made this approach appropriate to the preparation of cyclic pseudopeptides with acceptable purity. For analytical purposes, macrocycle **19**, synthesized using the initial conditions (Table 2, entry 1), was purified by reverse-phase chromatography. The two *E* and *Z* configurational isomers could be separated. Twelve mg of ***E*-19** and 4 mg of ***Z*-19** were obtained. This corresponds to a 25% overall yield of **19** (starting from the resin) and a 58% cyclization yield from **15b**. The assignment of the configuration was performed by analyzing the products by ^{1}H NMR at 600 MHz by irradiating the adjacent methylene groups. The coupling constants of the olefinic protons, in these experiments, were 14.7−15.9 Hz (which corresponds to the *E*-isomer) and 5.9−6.2 Hz (which corresponds to the *Z*-isomer).[10c,e]

Conclusion

We have described above the synthesis of a new amino acid linker *cis*-Apa for the construction of cyclic pseudopeptides using an RCM cyclative−cleavage method. A new pathway was developed and led efficiently to the Fmoc-protected amino acid in a four-step synthesis. This linker was incorporated in a Solid phase Peptide Strategy (SPPS) to obtain a set of cyclic peptides. On the basis of RGD sequences, the objective was to obtain a practical and efficient integrated method of cyclative cleavage release. Different sequences, including β-turn inducing amino acids such as proline or D-allylglycine, at different positions on the peptide chain, have been synthesized on two different resins. Peptides built from ChemMatrix resin led to better purities. We have demonstrated that, using microwave activation, the incorporation of the proline at the N-terminal extremity facilitates RCM cyclative cleavage. Four different macrocyclic peptidomimetics were prepared by using an efficient and practical method, showing that the readily available *cis*-Apa amino acid is well adapted as a linker in the cyclization−cleavage process.

Experimental Section

***N*-Allylbut-3-enamide (3).**[8b] To a solution of EDC·HCl (489 mg, 2.55 mmol) in 13 mL of DMF was added DIEA (197 μL, 2.55 mmol) slowly at 0 °C. The mixture was stirred at 0 °C for 5 min then 3-butenoic acid (200 mg, 2.32 mmol) and HOBt (426 mg, 2.78 mmol) were added at −10 °C and the solution was stirred 30 min. The mixture was cooled to −10 °C, allylamine (197 μL, 2.55 mmol) was added, and the solution was stirred overnight at room temperature, then evaporated. The residue was diluted with AcOEt (50 mL) and washed successively with 10% $KHSO_4$ (2 × 10 mL), NaCl (2 × 10 mL), and $NaHCO_3$ (2 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to afford 290 mg of the amide **3** (74% yield) as a colorless oil: R_f 0.23 (cyclohexane−EtOAc: 2/1); ^{1}H NMR ($CDCl_3$, ppm) δ 5.99−5.74 (m, 2H), 5.24−5.12 (m, 4H), 3.86 (m, 2H), 3.01 (br d, J = 7.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 170.3, 134.3, 131.3, 120.2, 116.6, 42.0, 41.7; ESIMS m/z 125.9 $(M + H)^+$.

***tert*-Butyl Allylbut-3-enoylcarbamate (5).** To a solution of *N*-allyl-3-butenamide **3** (1 g, 8 mmol) in 50 mL of anhydrous CH_2Cl_2 were added Boc_2O (5.24 g, 24 mmol) and DMAP (97.6 mg, 0.8 mmol) under nitrogen. The mixture was stirred at room temperature for 24 h. The mixture was then extracted with CH_2Cl_2 (100 mL), and the organic layer was washed successively with 10% $KHSO_4$ (2 × 20 mL), NaCl (2 × 20 mL), and $NaHCO_3$ (2 × 20 mL), then dried over $MgSO_4$ and concentrated under reduced pressure. Column chromatography (silica gel, cyclohexane−EtOAc 9/1) afforded 1.56 g of the protected amide **5** (87% yield) as a colorless oil: R_f 0.57 (cyclohexane−EtOAc: 9/1); IR 2980, 1735, 1694, 1364, 1351, 1139 cm^{-1}; ^{1}H NMR ($CDCl_3$, ppm) δ 6.05−5.94 (m, 1H), 5.84−5.73 (m, 1H), 5.16−5.10 (m, 4H), 4.28 (d, J = 7.0 Hz, 2H), 3.66 (d, J = 6.7 Hz, 2H), 1.51 (s, 9H); ^{13}C NMR ($CDCl_3$, ppm) δ 173.4, 152.7, 133.0, 131.3, 117.6, 116.1, 82.8, 46.2, 42.6, 27.7. ESIMS m/z 226.1 $(M + H)^+$; HRMS (ESI) calcd for $C_{12}H_{20}NO_3$ $[M + H]^+$ 226.1443, found 226.1440.

***tert*-Butyl 5,6-Dihydro-6-oxopyridine-1(2*H*)-carboxylate (6).** A solution of **5** (1.1 g, 4.89 mmol) and Grubbs' II catalyst **10** (166 mg, 0.20 mmol) in 14 mL of anhydrous CH_2Cl_2 was heated under microwave at 60 °C (initial power 400 W) during 30 min. After cooling the mixture was evaporated and column chromatography (silica gel, cyclohexane−EtOAc, 9/1 to 7/3) afforded 594.6 mg of **6** (62% yield) as a brown oil: R_f 0.52 (cyclohexane−EtOAc: 1/1); IR 2981, 1773, 1716, 1369, 1240, 1139 cm^{-1}; ^{1}H NMR ($CDCl_3$, ppm) δ 5.81−5.72 (m, 2H), 4.25−4.21 (m, 2H), 3.11−3.07 (m, 2H), 1.53 (s, 9H); ^{13}C NMR ($CDCl_3$, ppm) δ 168.5, 151.9, 122.2, 121.5, 83.4, 47.0, 35.1, 28.1; ESIMS m/z 198.1 $(M + H)^+$; HRMS (ESI) calcd for $C_{10}H_{16}NO_3$ $[M + H]^+$ 198.1130, found 198.1121.

1,6-Dihydropyridin-2(3*H*)-one (7).[8b] A solution of **3** (2 g, 16 mmol) and Hoveyda Grubbs' II catalyst **11** (1 g, 1.6 mmol) in 72 mL of anhydrous CH_2Cl_2 was heated under microwave at

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110 °C (initial power 400 W) during 10 min. After cooling the mixture was evaporated and column chromatography (silica gel, EtOAc) afforded 971 mg of **7** (63% yield) as a white solid: R_f 0.12 (EtOAc: 1); mp 103−104 °C; ^{1}H NMR (CDCl3, ppm) δ 5.77 (br s, 2H), 4.01−3.89 (m, 2H), 2.96−2.92 (m, 2H); ^{13}C NMR (CDCl3, ppm) δ 169.2, 121.9, 120.3, 43.6, 31.0; ESIMS m/z 98.9 $(M + H)^+$.

(*Z*)-5-Aminopent-3-enoic Acid (8).[5a] A solution of **7** (594.6 mg, 3.03 mmol) in 16 mL of 6 N HCl was stirred at reflux for 1 h. The mixture was then evaporated to afford 568 mg of **8** (quantitative yield) as a white solid: mp 95−97 °C; ^{1}H NMR (D_2O, ppm) δ 6.04−5.87 (m, 1H), 5.77−5.64 (m, 1H), 3.66 (br d, J = 7.1 Hz, 2H), 3.25 (br d, J = 6.6 Hz, 2H); ^{13}C NMR (D_2O, ppm) δ 173.1, 125.9, 121.5, 33.6, 29.8; ESIMS m/z 116.1 $(M + H)^+$.

(*Z*)-5-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]pent-3-enoic Acid (9). To a solution of amino acid **8** (562.4 mg, 3.02 mmol) in 20 mL of a 1:1 mixture of water/acetone was added $NaHCO_3$ (277 mg, 9.97 mmol) then FmocOSu (1.1 g, 3.02 mmol) in 4 mL of acetone. The mixture was stirred overnight at room temperature and then evaporated. The residue was diluted with AcOEt (50 mL) and acidified with 1 N HCl (10 mL) at 0 °C to pH 1. The aqueous phase was extracted three times with AcOEt (3 × 20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was recrystalized in CH_2Cl_2 to afford 763 mg of **9** (75% yield) as a white solid: mp 134−135 °C; IR 3329, 3041−2961, 1686, 1534, 1257 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, ppm) δ 7.88 (d, J = 7.4 Hz, 2H), 7.67 (d, J = 7.3 Hz, 2H), 7.41−7.30 (m, 4H), 5.63−5.43 (m, 2H), 4.31−4.21 (m, 3H), 3.62 (t, J = 5.8 Hz, 2H), 3.08 (br d, J = 6.7 Hz, 2H); ^{13}C NMR ($(CD_3)_2SO$, ppm) δ 172.5, 156.3, 144.1, 141.1, 129.3, 127.8, 126.8, 125.4, 124.0, 120.3, 65.6, 46.9, 37.6, 32.7, 25.3; ESIMS m/z 338.1 $(M + H)^+$, 360.1 $(M + Na)^+$; HRMS (ESI) calcd for $C_{20}H_{20}NO_4$ $[M + H]^+$ 338.1392, found 338.1396.

General Protocol for the Synthesis of Peptides. The linear peptides were synthesized on 0.25 mmol scale on Wang-Gly resin **12a**−**15a** (0.69 mmol/g loading) and on Rink Amide-ChemMatrix resin **12b**−**15b** (0.47 mmol/g loading), using an automated microwave peptide synthesizer. Deprotections were performed with a 20% piperidine in DMF solution. All coupling reactions were performed with 5 equiv of HBTU in DMF (0.5 M), 5 equiv of amino acids in DMF (0.2 M), and 10 equiv of DIPEA in NMP solution (2 M). Each deprotection and coupling reaction was performed with microwave energy and nitrogen bubbling. The microwave cycle was characterized by two deprotection steps, the first one lasting 30 s, the second one lasting 180 s. All coupling reactions lasted 300 s. When the sequence was completed, the N-terminal Fmoc group was removed, and the N-terminal amine was acetylated with a mixture of 7 mL of acetic anhydride/DMF (2:8 v/v) for 30 min at room temperature. The resin was rinsed with DMF, CH_2Cl_2, methanol, and diethyl ether and then dried under vacuum to obtain finely divided powder.

General Protocol for RCM Cyclization−Cleavage of Peptides (12a−15a). To a suspension of the dry resin Wang-Gly **12a**−**15a** (150 mg, 0.104 mmol) in 5.2 mL of degassed CH_2Cl_2 (5.2 mL) was added Grubbs' II catalyst **10** (25.5 mg, 0.03 mmol) or Hoveyda-Grubbs' II catalyst **11** (19 mg, 0.03 mmol) and the suspension was heated at reflux for 48 h under Ar. The mixture was then filtered. The resin was washed with CH_2Cl_2 (5 × 3 mL). The filtrate was evaporated and analyzed by LC/MS.

General Protocol for RCM Cyclization−Cleavage of Peptides (12b−15b). To a suspension of dry resin ChemMatrix **12b**−**15b** (150 mg, 0.071 mmol) in 3.6 mL of degassed CH_2Cl_2 was added Grubbs' II catalyst **10** (18.1 mg, 0.02 mmol) or Hoveyda-Grubbs' II catalyst **11** (13 mg, 0.02 mmol) and the suspension was heated at reflux for 48 h under Ar. The mixture was then filtered. The resin was washed with CH_2Cl_2 (5 × 3 mL). The filtrate was evaporated and analyzed by LC/MS.

Ac-c(Ag-Arg(Pbf)-Gly-Asp(OBu-*t*)-Apa) (16). LC/MS analyses of the isolated residue confirmed the formation of cyclic peptide **16**. ESIMS m/z 805.5 $(M + H)^+$, 375.2 $[M + 2H - t\text{Bu}]^{2+}$; HRMS (ESI) calcd for $C_{37}H_{57}N_8O_{10}S$ $[M + H]^+$ 805.3918, found 805.3909.

Ac-c(D-Ag-Arg(Pbf)-Gly-Asp(OBu-*t*)-Apa) (17). LC/MS analyses of the isolated residue confirmed the formation of cyclic peptide **17**. ESIMS m/z 805.5 $(M + H)^+$, 375.2 $[M + 2H - t\text{Bu}]^{2+}$; HRMS (ESI) calcd for $C_{37}H_{57}N_8O_{10}S$ $[M + H]^+$ 805.3918, found 805.3920.

Ac-c(Ag-Arg(Pbf)-Gly-Asp(OBu-*t*)-Pro-Apa) (18). LC/MS analyses of the isolated residue confirmed the formation of cyclic peptide **18**. ESIMS m/z 902.5 $(M + H)^+$, 423.7 $[M + 2H - t\text{Bu}]^{2+}$; HRMS (ESI) calcd for $C_{42}H_{64}N_9O_{11}S$ $[M + H]^+$ 902.4446, found 902.4466.

General Protocol for Microwave-Assisted RCM Cyclization−Cleavage of Peptide (15b). To a suspension of the dry resin **15b** (150 mg, 0.071 mmol) in 3.6 mL of degassed CH_2Cl_2 was added Grubbs' II catalyst **10** (25.5 mg, 0.03 mmol) and the suspension was heated under microwave at a specific temperature and time (initial power 400 W). After cooling the mixture was then filtered. The resin was washed with CH_2Cl_2 (5 × 3 mL). The filtrate was evaporated and analyzed by LC/MS.

(*E*)-Ac-c(Ag-Pro-Arg(Pbf)-Gly-Asp(OBu-*t*)-Apa) (19). After the resin was washed with CH_2Cl_2, pyridine oxide (50 mol %, 4 mg)[13] was added to the filtrate with stirring for 2 h. LC/MS analyses of the isolated residue confirmed the formation of cyclic peptide **19**. Purification by C_{18}, RP-HPLC, 30−40% MeCN (0.1% TFA) over 20 min (t_R = 1.934 min) yielded the cyclic peptide ***E*-19** (12 mg, 19% yield) and cyclic ***Z*-19** (4 mg, 6% yield) as white solids. Spectral data for ***E*-19**: ^{1}H NMR ($(CD_3)_2SO$, ppm) δ 8.65 (m, 1H), 8.55 (s, 1H), 8.18 (d, J = 7.5 Hz, 1H), 8.06 (d, J = 9.7 Hz, 1H), 7.70 (m, 1H), 6.41 (br s, 1H), 5.54−5.52 (m, 1H), 5.52−5.47 (m, 1H), 4.79−4.7 (m, 1H), 4.31 (m, 1H), 4.30 (m, 1H), 3.96 (m, 2H), 3.81 (m, 1H), 3.32−3.26 (m, 4H), 3.06 (m, 2H), 2.88 (s, 2H), 2.60−2.42 (m, 2H), 2.16 (m, 2H), 1.98 (s, 3H), 1.85−1.66 (m, 8H), 1.57 (m, 2H), 1.47−1.23 (m, 20H); ESIMS m/z 902.5 $(M + H)^+$, 423.7 $[M + 2H - t\text{Bu}]^{2+}$; HRMS (ESI) calcd for $C_{42}H_{64}N_9O_{11}S$ $[M + H]^+$ 902.4446, found 902.4449.

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Supporting Information Available: General remarks, copies of ^{1}H and ^{13}C NMR spectra for compounds **1**−**9**, ^{1}H and COSY spectra for compound ***E*-19**, and mass spectra for compounds **16**−**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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