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Promotion of cyclization of linear pentapeptides and heptapeptide by different univalent metal ions

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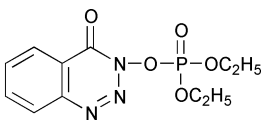
Univalent metal ions such as Na^+, K^+ and Cs^+ can enhance not only the cyclization yields of some linear pentapeptides and heptapeptide but also their cyclization rates while some bivalent and trivalent metal ions such as Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, Ni^{2+} and Cr^{3+} elevate neither the cyclization yields nor the cyclization rates and some of them prevent the cyclization.

Cyclic peptides, being constrained conformationally, have been applied as synthetic targets for potential drug leads, building blocks for the synthesis of macromolecules, conformational analysis and synthesis of peptide libraries.[1] Conventional methods for peptide cyclization generally involve a partially or fully protected linear precursor which is then cyclized in organic solvents through various combinations of orthogonal protecting groups and on- or off- resin cyclization schemes.[2] Recently, the thioester method[3] has been successfully applied to the cyclization of cyclic peptides and proteins—protected peptides as linear precursors are not necessary and coupling reagent is not required. The LiCl–DMF solvent system has been reported for driving the cyclization.[4] Cyclic peptides can also be obtained by enzymatic cyclization.[5]

We focused our efforts on starting with partially protected peptides as linear precursors to synthesize cyclic products by using an organophophorus coupling reagent, 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT)[6] (Scheme 1).

DEPBT can be used under mild peptide coupling conditions. A number of coupling reactions have been carried out using DEPBT.[7] It is notable that it is not necessary to protect the hydroxy of the amino component (such as tyrosine, serine *etc*.). It is also remarkable that amide bond forming reactions mediated by DEPBT are so strongly resistant to racemization, even when a base as strong as DIEA is employed.[8] Recently DEPBT has been used successfully for amide bond formation in the total synthesis of (−)-tamandarin B[9] and Teicoplanin Aglycon[10] respectively.

A cyclopentapeptide, c(Gly-Pro-Tyr-Leu-Ala) (**I**) and a cycloheptapeptide, c(Gly-Tyr-Gly-Gly-Pro-Phe-Pro) (**II**), which were isolated and identified from *Pseudostellaria heterophylla*[11] and *Stellaria yunnanensis* Franch (M)[12] respectively, were chosen as the model peptides to evaluate DEPBT as the coupling reagent for synthesis of cyclopeptide by solution method. Three linear peptide precursors, H-Tyr-Leu-Ala-Gly-ProOH (**I-1**), H-Ala-Gly-Pro-Tyr-LeuOH (**I-2**) and H-Gly-Tyr-Gly-Gly-Pro-Phe-ProOH (**II-1**) were selected for our experiments. They were cyclized[13] successfully by DEPBT in dilute DMF (10^{-3} or 2×10^{-3} M) at room temperature. The reaction yields were monitored by HPLC from 1 to 24 h. After 24 h, the cyclization yields were 22, 67 and 60% respectively. In order to improve the yields of cyclization, we used different metal ions as additive in addition to the coupling reagent DEPBT and the base DIEA. It is known that several natural cyclic peptides, *e.g*. Valinomycin, Anatamanide, Enniatine *etc*., act as ionphores *in vivo*. They can form stable ion complexes with metal ions. Furthermore, crown ethers were synthesized successfully by their corresponding metal ion complexes. In the current study, we investigated whether metal ions have any effect on the cyclization of penta- and heptapeptide.



Scheme 1 Structure of DEPBT.

Firstly, we used nine metal ions, Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+} and Cr^{3+} to study their effect on the cyclization of linear pentapeptides **I-1** and **I-2**. The reaction yields were monitored by HPLC. The results are shown in Table 1.

In the presence of Na^+, the cyclization yields of linear pentapeptides **I-1** and **I-2** were enhanced from 22 to 39% and 67 to 80% after 24 h. At the same time, the reaction rates were increased from 7 to 12 percentage points with Na^+, K^+ or Cs^+ after 2 and 4 h for compound **I-1**. Interestingly we could not detect any cyclopentapeptide by HPLC when Zn^{2+} and Cr^{3+} were added to the reaction. The influence of Na^+ concentration was also studied. With 10, 5 and 2 eq. of Na^+, the cyclization yields of **I-2** were 81, 80 and 76% respectively.

Secondly, in the case of the linear heptapeptide **II-1**, eleven metal ions, Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Fe^{2+}, Ni^{2+} and Cr^{3+}, were used. The results are shown in Table 2. Consistent with the results obtained with pentapeptides **I-1** and **I-2**, univalent metal ions, Li^+, Na^+, K^+ and Cs^+, could not only enhance the cyclization yields of **II-1** from 60 to 68%, 60 to 71%, 60 to 71%, and 60 to 78% respectively after 24 h, but also increase the cyclization rates. Bivalent metal ions, Mg^{2+} and Ca^{2+}, decreased the cyclization yields of **II-1** from 60 to 29% and 60 to 41% respectively after 24h, while the cyclization yields of **II-1** were decreased drastically when Zn^{2+} or Ni^{2+} was added. Furthermore, we could not detect any cycloheptapeptide by HPLC when Fe^{2+} or Cr^{3+} was added to the reaction. The influence of different concentrations of Cs^+ was also studied.

Table 1 Cyclization yields (%) of **I-1** and **I-2** by different metal ions

	Reaction time (h)		
Metal ions[a]	2	4	24
Li^+	8	17	37
Na^+	10	20	39 (80)[b]
K^+	12	19	38 (69)[b]
Cs^+	12	21	36 (69)[b]
Mg^{2+}	6	8	22
Ca^2	9	14	27
Ba^{2+}	10	11	31
Zn^{2+}	—	—	—
Cr^{3+}	—	—	—
None	5	9	22 (67)[b]

[a] Concentration of each metal ion was 5 eq. [b] Cyclization yields of **I-2**. '—' Cyclopentapeptide (**I**) was not found. HPLC analysis conditions: Microsorb column, C_{18} (150 × 3.9 mm) with a 6 min isocratic gradient of 15% CH_3CN in H_2O containing 0.1% TFA and a 35 min linear gradient of 15–80% CH_3CN in H_2O containing 0.1% TFA at a flow rate of 0.5 ml min^{-1} at 275 nm.

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The results are shown in Table 3: 5 eq. Cs^+ was the optimum concentration.

Preliminary molecular modelling studies were carried out to estimate the ring size of the cyclic pentapeptide and heptapeptide. Insight II (Accelrys, San Diego, USA) was used in the study with the CFF91 forcefield. A random conformation of the cyclic peptide was built and fully energy minimized. Then, molecular dynamics of 100 ns was performed at 298 K. During the calculations, a distance dependent dielectric constant was used (4.*r*). Various distances between the backbone atoms across the cyclic structures were measured and averaged using the trajectories saved every 1ns from the molecular dynamics simulations. The backbone of the cyclopentapeptide is a circle with a diameter of 6.0 ± 0.5 Å (standard deviation) while the cyclic heptapeptide is a rectangle or ellipse with dimensions 6.3 ± 0.5 and 9.0 ± 0.3 Å. The diameter of Na^+ is 1.96 Å while the diameter of Cs^+ is 3.30 Å. The van der Waals radius of an oxygen or a nitrogen atom is about 1.5 Å. With such size of ring structure, one hypothesis is that the oxygen of the amide carbonyl or the terminal carboxylate together with the nitrogen of the terminal amino group in the linear peptide coordinate the metal ion to form a ring structure. With other possible additional effects, such as the neighbouring effect between the carboxylate and the amino groups, the univalent metal ions, such as Na^+, K^+ and Cs^+, are able to facilitate the cyclization. On the other hand, bivalent and trivalent metal ions, such as Zn^{2+}, Fe^{2+} and Cr^{3+}, which can coordinate strongly to the carboxy and the amino groups in the linear peptide, block both groups to form a cyclic structure. Therefore, the yields decreased or no reaction occurred when bivalent or trivalent metal ions were introduced.

In conclusion, univalent metal ions (Na^+, K^+ and Cs^+) can enhance not only the cyclization yields of some linear peptides but also the cyclization rates. The smaller Na^+ is suitable for the cyclization of linear pentapeptides **I-1** and **I-2** while the bigger Cs^+ is better for the cyclization of linear heptapeptide **II-1**. Further study is under way to investigate the mechanisms of the interactions between the linear peptide precursors and the different metal ions.

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Table 2 Cyclization yields (%) of **II-1** by different metal ions

	Reaction time (h)			
Metal ions[a]	1	3	5	24
Li^+	21	39	52	68
Na^+	36	57	58	71
K^+	34	55	66	71
Cs^+	39	66	73	78
Mg^{2+}	2	10	12	29
Ca^{2+}	6	12	21	41
Ba^{2+}	22	38	51	64
Zn^{2+}	—	—	2	4
Ni^{2+}	—	2	3	12
Fe^{2+}	—	—	—	—
Cr^{3+}	—	—	—	—
None	20	38	49	60

[a] Concentration of each metal ion was 5eq. '—' Cycloheptapeptide (**II-1**) was not found. HPLC analysis condition: Microsorb column, C_{18} (150 × 3.9 mm) with a 30 min linear gradient of 5–50% CH_3CN in H_2O containing 0.1%TFA at a flow rate of 1 ml min^{-1} at 275nm.

Table 3 Influence of different Cs^+ concentrations on the cyclization yields (%) of **II-2**

	Reaction time (h)			
Cs^+	1	3	5	24
5 eq.	39	66	73	78
2 eq.	22	45	56	67
0.5 eq.	18	41	49	62
None	20	38	49	60

HPLC analysis condition: Microsorb column, C_{18} (150 × 3.9 mm) with a 30 min linear gradient of 5–50% CH_3CN in H_2O containing 0.1% TFA at a flow rate of 1 ml min^{-1} at 275 nm.

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