行政院國家科學委員會專題研究計畫　期中進度報告

雙體鑭系稀土離子之大環多氨基酸配位化學及 DNA/RNA 切割劑之應用(1/3)

計畫類別：個別型計畫
計畫編號：NSC91-2113-M-009-021-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：國立交通大學生物科技研究所

計畫主持人：張正

報告類型：精簡報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 92 年 6 月 6 日

行政院國家科學委員會專題研究計畫成果報告

雙體鑭系稀土離子之大環多氨基酸配位化學及 DNA/RNA 切割劑之應用

計畫類別： 個別計畫 整合計畫

計畫編號： NSC 91-2113-M-009-021

執行期間： 91 年 08 月 01 日 至 92 年 07 月 31 日

個別型計畫: 計畫主持人： 張 正

共同主持人： N/A

整合型計畫： 總計畫主持人： N/A

子計畫主持人： N/A

註： 整合型計畫總報告與子計畫報告請分開編印各成一冊，

彙整一起繳送國科會。

處理方式： 可立即對外提供參考

一年候可對外提供參考

兩年候可對外提供參考

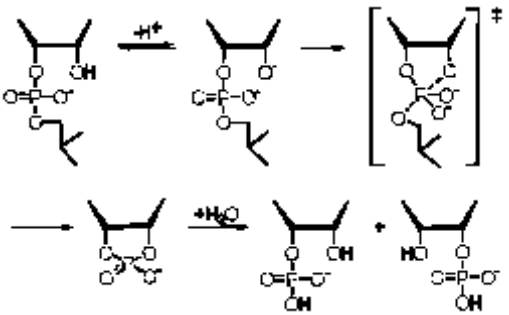
(必要時，本會得展延發表時限)

執行單位：交通大學生物科技研究所

中華民國 92 年 05 月 26 日

一　前言/背景敘述

早期有關人造限制脢的研究，主要著眼於 RNA 切割的研究上。RNA 在其五碳糖第二位置上有一氫氧基(2’-hydroxyl group;2’-OH)，在反應機構中被認為扮演極重要之角色。目前在水解 RNA 的領域中，較被廣泛接受的反應機構，包括轉酯和水解兩個步驟，如下圖所示；在第一個步驟轉酯反應中，RNA 五碳糖之 2’-OH 會先解離掉氫離子(deprotonation)，形成強攻擊基(alkoxide);此攻擊基攻擊五碳糖 3’端之磷酸酯鍵之磷原子，形成環磷酸酯(2’,3’-cyclic phosphate)；在第二個步驟中，此環磷酸酯再進一步水解成 2’-phosphate 及 3’-phosphate，完成整個水解反應。在轉酯過程中，可能有形成一雙三角椎(trigonal bipyrimide)之中間物或過渡態活化物。



在整個過程中，若有金屬離子的參與，將使轉酯和水解反應更容易進行；金屬離子可在反應中扮演以下幾種功能（如圖所示）；第一種功能是和水配位並解離形成金屬氫氧根離子（metal hydroxide），充當路易士鹼(Lewis base)來抓取 2’-OH 上之氫，使成為強攻擊基；第二種功能是配位在 2’-OH 的氧原子上，吸引其上的電子雲，使 2’-OH 之氫更容易解離，形成所需之攻擊基；第三種功能是可以和轉酯過程中所形成之中間物或過渡態活化物靜電吸引（正負相吸）提高其穩定度；第四種功能則是配位在離去基上(leaving group)，穩定離去基，使整個反應更容易朝水解方向來進行。另外一種在圖上沒有並值得一提的是配位在磷酸酯鍵的氧原子上，導引其上的電子雲，使磷原子更加裸露易被攻擊。

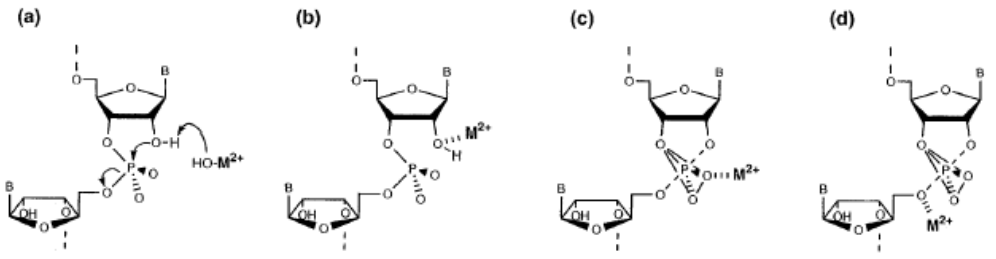


Figure 2. Possible roles for metal-catalyzed transesterifications or hydrolysis: (a) metal hydroxide as a general base, (b) metal stabilization of hydroxyl (or water), (c) metal stabilization of pentacoordinate transition state/intermediate, and/or (d) metal stabilization of leaving group.

可用來協助水解的金屬離子幾乎遍滿整個元素週期表;氫離子(protons)、氫氧根離子(hydroxide)、氨類(amines)及其他含氮化合物、鎂離子(Mg2+)、鈣離子(Ca2+)、鐵三價離子(Fe3+)、鎳二價離子(Ni2+)、銅二價離子(Cu2+)、鋅離子(Zn2+)、鉛離子 Pb2+)、三價鑭系金屬離子(trivalent lanthanides)、正二價二氧化鈾離子(UO22+)和釷鹽(Th salts)等等；當然酵素也包括在內；而上述所列僅包括了親核性攻擊反應的部分而已。所以自然界中存在的 RNA 常不穩定，很容易就分解掉。但相對地，要水解 DNA 就困難許多；和 RNA 比較，DNA 缺少 2'-OH，使其水解反應之親核性攻擊部分，必須仰賴外來的親核基；外來的親核基帶著負電，磷酸酯鍵也帶著負電，而且外來之親核基呈游離狀態，如此由於電荷排斥的原故，將使親核性攻擊的反應極不容易進行；所以 DNA 水解的半生期常在數萬年以上。

根據以上描述，我們知道若要能快速水解 DNA，考慮水解 RNA 反應之特性是一個不錯的方法。另一個參考的材料便是自然界中之酵素，如下圖所示，

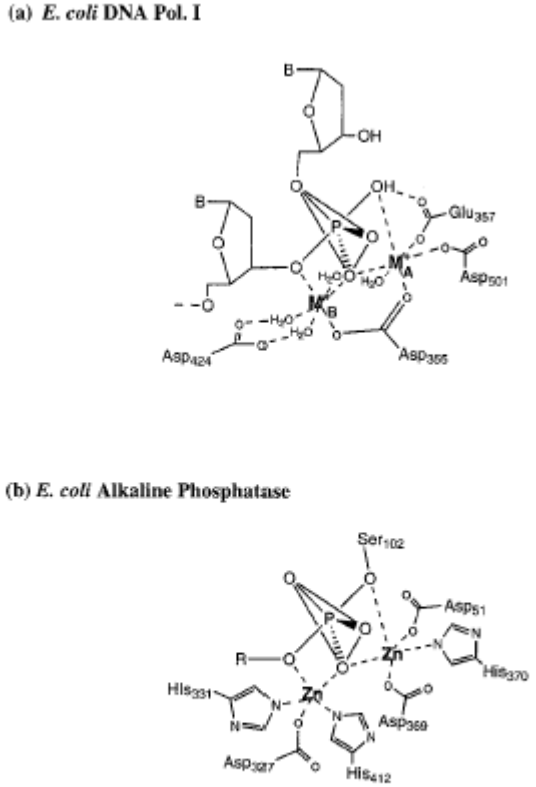


Figure 4. Proposed transition-state structures for protein-based phosphoryl transfer reactions as exemplified by (a) the exonuclease site of *E. coli* DNA polymerase I and (b) the active site of *E. coli* alkaline phosphatase.

上圖為 DNA 切割酵素水解磷酸酯鍵可能的過渡態活化物結構圖；以 E. coli DNA 聚合脢的外切脢部分和 E. coli alkaline phosphatase 為例，誠如上述有關金屬離子在 RNA 水解中所扮演的重要角色，金屬離子在 DNA 水解酵素中亦扮演

著極重要的角色，而且其功能大致相同，並有酵素本身在活化區之胺基酸所含的酸基及胺基，以路易士鹼的形態輔助其切割。在 E. coli DNA 聚合脢之外切脢部分的圖中，MA 和 MB 是被眾多胺基酸和水分子配位包圍，而形成的雙金屬活化中心；當受質(substrate)-DNA backbond 進到此活化中心，活化中心的 MB 和 MA 將分別和磷酸酯鍵上的氧鍵結，以固定此 DNA backbond，MA 上所鍵結之氫氧根離子並攻擊在磷原子上，形成此雙三角椎的結構。E. coli alkaline phosphatase 亦與前述相仿，只是攻擊磷原子的攻擊基是由 Ser102-OH 來取代，而不是金屬氫氧根離子。下圖則是兩個切割 RNA 可能之過渡態活化物結構圖，皆與以上所描述有關水解 RNA 之反應機構相符。再次強調的是，RNA 多半是以其五碳糖上的 2'-OH 作為攻擊基。

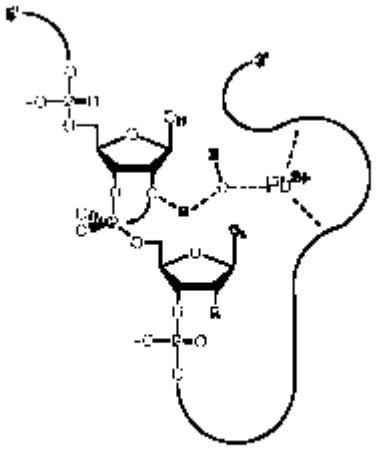


Figure 3. Possible transition state structure for the lead-based cleavage of $tRNA^{Phe}$ between dihydrouridine (D_{17}) and guanosine (G_{18}).[32,33]

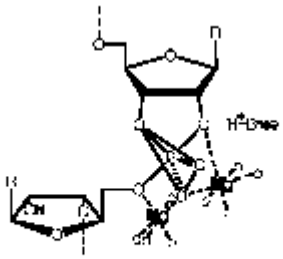
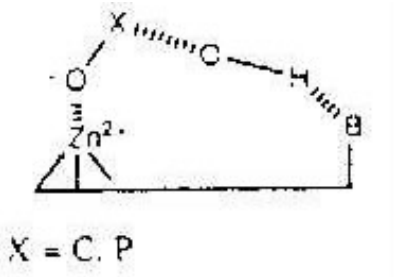


Figure 5. Possible transition-state structure for the hammerhead ribozyme showing two possible roles for the metal cofactors.

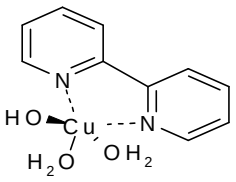
綜合言之，要能快速水解 DNA，大致上要考慮幾個要點；第一、需要金屬離子參與，作為路易士酸以活化反應；第二、需要路易士鹼的參與以活化反應；第三、金屬離子和鹼基之間之配位鍵結，使整個催化劑的構形能與水解時所需之反應機構相符。

的確，目前以人造化合物水解 DNA 的研究上，多半是根於上述要件，所設計出來的一些金屬及胺基的錯合物。這類人造酵素基本上就是透過金屬離子和鹼基的雙重活化，進行催化反應(bifunctional catalysis)。根於上述的反應機構，更仔細地來觀察，我們可以發現當反應進行時，電子流動方向大致上是從鹼基到金屬離

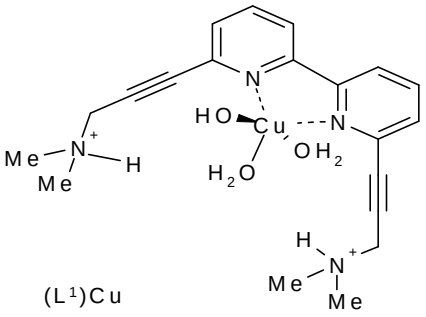
子。例如氫氧根離子(hydroxyl group)和磷酸酯的反應，若加入金屬離子和鹼基當催化劑，其催化反應過渡狀態(transition state)之示意圖如下(Zn2+為鋅二價金屬離子，X 為碳或磷原子中心，B 則為鹼基或路易士鹼)；電子像是從鹼基流到氫氧根離子之氫及氧原子，再到磷酸酯之磷及氧原子，最後到金屬離子上。



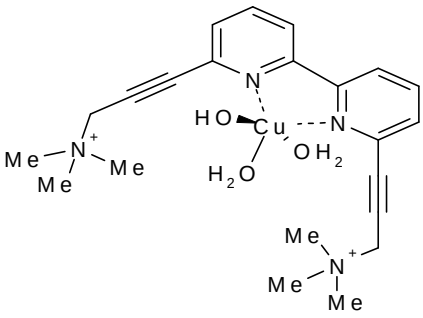
在 DNA 切割中，金屬離子和胺基酸側鏈之胺基，與磷酸酯(phosphate ester) 鍵結以固定 DNA，再進行水解。1996 年，Endre Kovari 和 Roland Kramer 模仿此反應機構，設計出兩個 ligand（如下圖所示）。其結構是由兩個 pyridine 環構成，並在 pyridine 環 2 的位置上接上 alkylamine 作為胺基酸的類比物。兩個 ligand 中，一個 amine 上有一個氫，另一個則完全被甲基取代，不具有氫。兩者均配位上二價銅離子形成錯合物。氫的作用在於和進入此 complex 之 phosphate ester 形成氫鍵以固定其構形，以方便進行切割，甚至可能活化磷酸酯之磷原子使切割更容易進行。銅離子則可提供配位空間供磷酸酯進行配位鍵結（此部分其另有 X-ray 晶體繞射之結果加以佐証）。兩個錯合物切割 BNPP (bis(4-nitrophenyl) phosphate) 之結果如下，其 kcat 值(L1)Cu（含-NMe2H+者）約為 4.4(0.4)× 10-3 s-1；(L2)Cu（含-NMe3 者）約為 4(1)× 10-6 s-1；L1Cu 約為 L2Cu 的 1000 倍。另外，(bpy)Cu 之 kcat 值約為 1.5(0.2)× 10-6 s-1，與(L2)Cu 相當。此結果似乎說明了 L1Cu 之所以具有較高的切割效率，與其上之 NMe2H group 有關，也提高了上述反應機構的可能性，即磷酸酯經由和金屬離子配位及和-NMe2H 形成氫鍵的雙重活化，再由銅離子上的 metal hydroxide 攻擊磷原子完成切割。



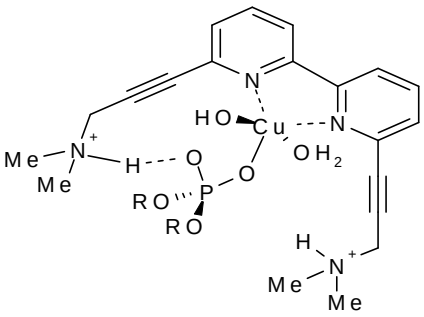
(bpy)Cu



$(L^1)Cu$



$(L^2)Cu$



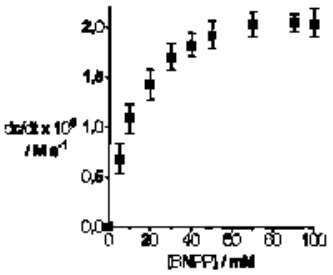


Figure 4. Saturation kinetics for the hydrolysis BNPP by $(\mathbf{L}^1)Cu^{4+}$ (5 mM) in ethanol−water 19:1. Conditions: pH 6.6(±0.1), 20(±0.5) °C, $I \approx 100$ mM, buffer: 2,4,6-trimethylpyridine (100 mM).

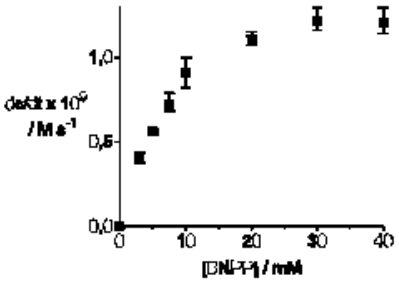
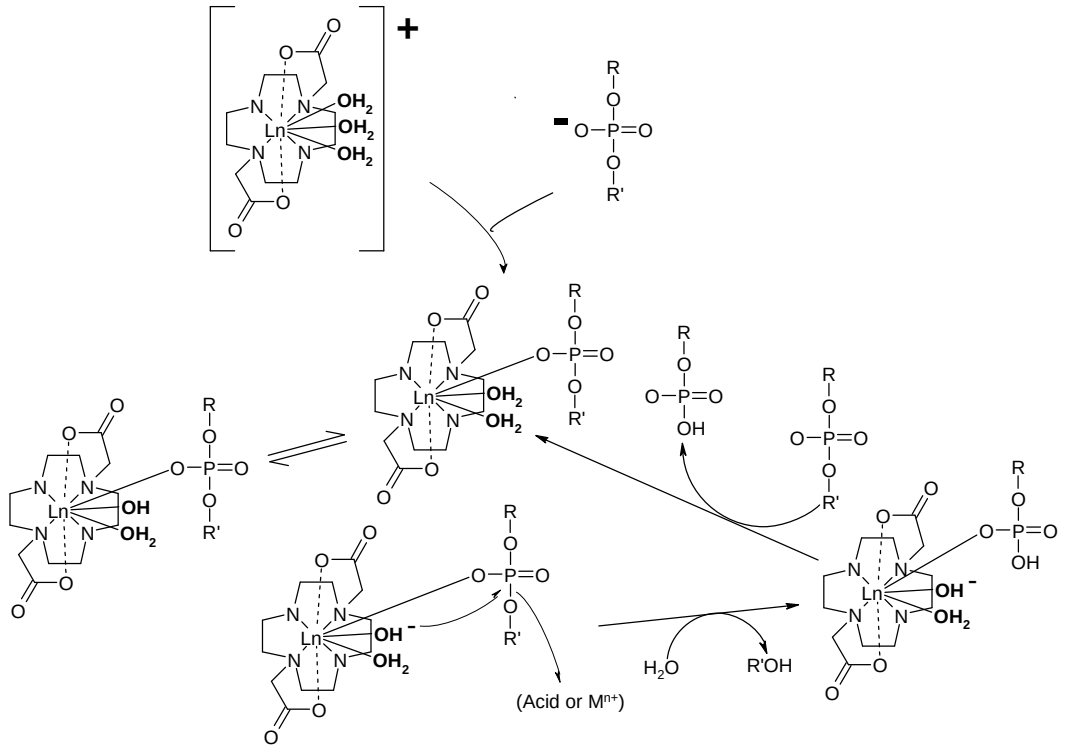


Figure 5. Saturation kinetics for the hydrolysis BNPP by $(bpy)Cu^{2+}$ (1 mM) in ethanol−water 19:1. Conditions: pH 6.6(±0.1), 20(±0.5) °C, $I \approx 100$ mM, buffer: 2,4,6-trimethylpyridine (100 mM).

Table 1. Observed and Relative Values of k_{cat} for BNPP Hydrolysis by Copper(II) Complexes[a]

catalyst	k_{cat} (s^{-1})	k_{rel}
$(\mathbf{L}^1)Cu$	$4.4(\pm0.4) \times 10^{-3}$	4×10^7
$(\mathbf{L}^2)Cu$	$4(\pm1) \times 10^{-6}$	4×10^4
(bpy)Cu	$1.5(\pm0.2) \times 10^{-6}$	1.4×10^4
$\mathbf{L}^1$	$1.2(\pm0.3) \times 10^{-10}$	1.1
none (k_{uncat})	$1.1(\pm0.3) \times 10^{-10}$	1

[a] Error limits are based on the reproducibility of kinetic measurements. For reaction conditions see Figure 5.



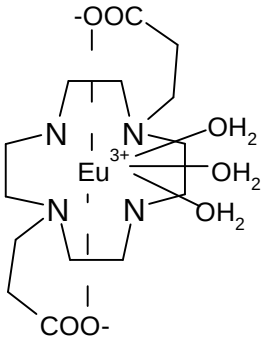
二　精簡報告

This is a research program originally started seven years ago (since 1995). Over the years, we have made considerable research progress as proposed and described previously. Recent progress shall be briefly discussed as follows:

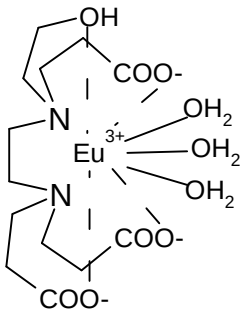
A. Comparisons of Various Lanthanide Complexes as Catalysts for BNPP Phosphodiester Bond Hydrolysis

We have been interested in the design, synthesis and characterization of artificial DNA/RNA cleavage agents employing macrocyclic lanthanide complexes. We have partially completed the study of the use of the europium complex, $EuDO2A^{+}$ (DO2A is 1,7-dicarboxymethyl-1,4,7,10-tetraazacyclododecane) and two other complexes, Eu(HEDTA) and $Eu(EDDA)^{+}$, as catalysts for the hydrolysis of phosphodiester bond of the model compound BNPP. At pH 11.0, the hydrolysis rate of BNPP in the presence of $EuDO2A^{+}$ is 100 times faster than that of EuHEDTA, presumably due the additional positive charge of $EuDO2A^{+}$. $EuEDDA^{+}$ with the greatest number of water-coordinated sites among the three hydrolyzes BNPP more efficiently at pH below 8. (At pH > 8, $EuEDDA^{+}$ solution becomes misty and precipitates form.) Plots of observed rate constants (25°C) vs. pH (9.35-11.0) for the BNPP (0.1 mM) - EuHEDTA (10-70 mM) reaction shows a titration-like curve, indicating that the first

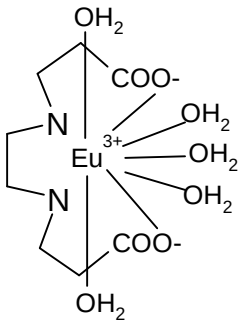
pK_h of the coordinated water molecule of EuHEDTA is around 10.5. On the other hand, an interesting second order dependence on [$EuDO2A^+$] for the BNPP hydrolysis reaction suggests that the dinuclear species of $EuDO2A^+$ is probably more reactive than the mononuclear species.



$EuDO2A^+$



EuHEDTA

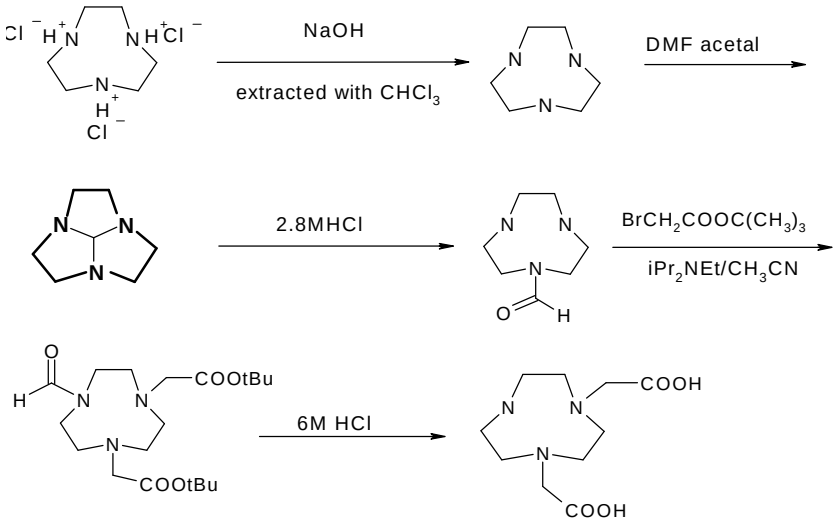


$EuEDDA^+$

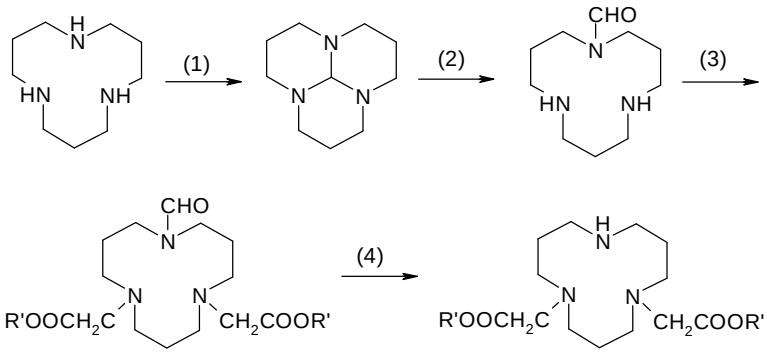
B. The Synthesis of Polyaminopolycarboxylate Macrocyclic Ligands NO2A and 3N12DA

The synthesis and characterization of trivalent lanthanide (Ln^{3+}) complexes of macrocyclic polyaminopolycarboxylate ligands are of recent interest for biomedical applications, including the use as magnetic resonance imaging (MRI) contrast agents and artificial DNA/RNA cleavage reagents. These complexes require high thermodynamic stability and low kinetic lability and macrocyclic polyaminopolycarboxylate ligands can serve the purpose. We have initiated the sunthesis of the macrocyclic ligands, NO2A and 1,5-di-(carbomethyl)-1,5,9-triazacyclododecane (3N12DA). The synthetic schemes are depicted below:

NO2A**:**



3N12DA**:**



(1) Me2NCH(OMe)2, benzene, reflux, 2-4 h (~100%)
(2)EtOH-H2O, room temp., 2 h (70~85%)
(3)BrCH2COOR', iPr2NEt, MeCN (~90%)
(4)H+

For NO2A, we have prepared the CHO-group protected macrocycle, the alkylation step will follow, after hydrolysis and purification, NO2A should be obtained. For 3N12DA, the 12-membered ring macrocycle has been made. The next protection and alkylation steps will be carried out immediately.

C. Molecular Structural Simulations of Ligands and Their Lanthanide(III) Complexes

Functional mimics of ribozymes are of current interest. Recently, we are interested in developing lanthanide complexes as artificial nucleases, includong the synthesis and characterization of 1,4-dicarboxymethyl-1,4,7- triazacyclononane (NO2A) and its lanthanide complexes. While the thermodynamic stability constants and formation and dissociation reaction rate constants are to be measured, we have initiated the molecular modeling studies of Ln(NO2A) and several structural

analogues, ie. 1,5-dicarboxymethyl-1,5,9-triazacyclododecane (3N12DA) and 1,7-dicarboxymethyl-1,4,7,10-tetraazacyclododecane (DO2A). The ligand systems studied include the following: (1) NO2A, NOTA; (2) 3N12DA, 3N12TA; (3) TE2A (3 isomers), TE3A, TETA; (4) DO2A (2 isomers), DO3A, DOTA.The lowest energy structures as well as possible protonation sites have been predicted based on these studies. The results reveal that all ligands except TETA related systems are pre-organized for lanthanide complexation. For 12-membered macrocyclic ligands (e.g. DO2A and 3N12DA), tertiary nitrogens are the preferred protonation sites. However, for 9-membered macrocyclic ligands (e.g., NO2A), one secondary nitrogen and it's adjacent tertiary nitrogen sites are more likely to be protonated.

References:

1. C. Allen Chang; *Proc. Natl. Sci. Counc. ROC(A)*, 1997, *21,* 1-13.
2. C. Allen Chang, Yuh-Liang Liu, Chang-Yuh Chen and Xiu-Mei Chou; *Inorg. Chem.*, 2001, *40*, 3448-3455.
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3. E.-R. Chan, **C.A. Chang**, T.-Z. Wu, and Y.-L. Lin, "Effects of Recombinant Lysostaphin on Cytotoxicity and Interleukin-8 Level in Normal Human Epidermal Keratinocites Cell Lines", *Biotech. Lett.,* 1998, **20**, 1053-1056.
4. E.-R. Chan, M.-R. Pan, **C.A. Chang**, T.-Z. Wu, and Y.-B. Kuo, "A Synthetic Complement C1q-like Peptide Selectively Interacts with Immune Complexes", *Biotech. Lett.,* 1998, **20**, 1119-1123.

5. **C.A. Chang,** H.-Y. Chen, and C.-Y. Chen**. "**Determination of Stability Constants of Metal Ion Complexes by Capillary Electrophoresis." *J. Chin. Chem. Soc. (Taipei)*, 1999, **46**, 519-528.
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8. K.-T. Chen, J.-D. Lin, T.-C. Chao, **C.A. Chang**, H.-F. Weng, and E.-C. Chan. "Quantitative Monitoring of Gene Expression Patterns in Metastatic and Follicolar Human Thyroid Carcinoma Using a Complementary DNA Array", *Thyroid,* 2001, **11**, 41-46.
9. **C.A. Chang**, Y.-L. Liu, C.-Y. Chen, X.-M. Chou, and J.-S. Ho. "Ligand Preorganization in Metal Ion Complexation: Molecular Mechanics/Dynamics, Kinetics and the Laser-Excited Luminescence Studies of Trivalent Lanthanide Complex Formation with Macrocyclic Ligands DOTA and TETA", *Inorg. Chem*, 2001, **40**, 3448-3455.
10. D.-L. Sheu, H.-A. Fan, K.-C. Hsu, **C.A. Chang**, Y.-S. Li, C.-C. Chiou, and E.-C. Chan. "Down-Regulation of Matrix Gla Protein Messenger RNA in Human Colorectal Adenocarcinomas", 2002, *Disease of the Colon and Rectum,* submitted.
11. **C.A. Chang** and P.-Y. Kuan. "Effects of pH on the Rates of Phosphate Diester Hydrolysis by Macrocyclic lanthanide Complexes as Artificial Nucleases". to be submitted.
12. **C.A. Chang** and C.-L. Chen. "Dissociation Kinetics of $Ln(DO2A)^+$." to be submitted.

We finally clarified several difficult-to-explain experimental results, and the last two papers listed above will be written and submitted shortly.

E. Recent Abstracts and Papers Presented at Scientific Meetings (2000 - 2003)

1. D.L. Sheu, **C.A. Chang,** and E.-C. Chan. "Expression of Cell Cycle Regulator Genes CDC25, Wee1HU, and Proto-Oncogen C-MYC in Human Colonrectal Cancer". *2000 The Fifthteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 25-26, 2000.
2. K.-T. Chen, **C.A. Chang,** and E.-C. Chan. "Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Array in Metastatic and Human Thyroid Follicular Tissues". *2000 The Fifthteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 25-26, 2000.
3. K.-T. Chen, J.D. Lin, M.J. Liou, **C.A. Chang,** C.C. Chiou, and E.-C. Chan. "Detection of Distinctive Gene Expression in Thyroid Follicular Carcinoma Cell

Line by Using cDNA Array Technology". *The Sixteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 24-25, 2001.

4. J.W. Hsieh, J.D. Lin, M.J. Liou, **C.A. Chang,** and E.-C. Chan. "A Rapid Modified Method of Polymerase Chain Reaction for Detection of Point Mutated-Iodide Transport Gene". *The Siteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 24-25, 2001.

5. Chih-Huai Chen, Wen-Joan Chiang, Pei-Lin Kang, Lie-Fen Shyur, Ning-Sun Yang, **C.A. Chang**, Chi-Meng Tzeng. "Establishment of Platform Technology of Pharmacogenomics for Herbal Medicine Validation. (Molecular pharmaco-mechanism of Chinese Herbal Medicine (CHM))". *IBC's Annual International Microtechnology Event*, San Diego, CA, U.S.A., Oct. 28-Nov. 1, 2001.

6 Chih-Huai Chen, Wen-Joan Chiang, Pei-Lin Kang, Lie-Fen Shyur, Ning-Sun Yang, **C.A. Chang**, Chi-Meng Tzeng. "Establishment of Platform Technology of Pharmacogenomics for Herbal Medicine Validation. (Molecular pharmaco-mechanism of Chinese Herbal Medicine (CHM))". *IBC's Annual International Microtechnology Event*, San Diego, CA, U.S.A., Oct. 28-Nov. 1, 2001.

7. **C.A. Chang**; Chia-Ling Chen. "Dissociation Kinetics of Lanthanide(III) Complexes of Macrocyclic Polyaza Polycarboxylate Ligand DO2A". *2001 Annual Meeting of the Chinese Chemical Society (Taipei)*, Tainan, Taiwan, December 29-30.

8. **C.A. Chang**, Pu-Yun Kuan, Chia-Ling Chen, Po-Hong Wu. "Macrocyclic Lanthanide Complexes as Artificial Nucleases：Hydrolysis of Phosphodiester Bonds by LnDO2A and LnK21DA". *2001 Annual Meeting of the Chinese Chemical Society (Taipei)*, Tainan, Taiwan, December 29-30.

9. **C.A. Chang**, Wen-Hung Wang, Chun-Chieh Lin, Bo-Hung Wu, Yen-Yun Kwan, and Chih-Cheng Lin. "Synthesis and Characterization of Macrocyclic Ligands to be Used for Lanthanide Artificial Nucleases and MRI Contrast Agents". *2001 Annual Meeting of the Chinese Chemical Society (Taipei)*, Tainan, Taiwan, December 29-30.

10. P.C. Lan and **C.A.Chang**. "Cloning of Recombinant Porcine Placental Lactogen in E. coli." *The Seventh Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 23-24, 2002.

11. C.F. Teng, M.C. Lin, and **C.A. Chang**. "Expression and Renaturation of Recombinant Human Placental Lactogen in E. coli." *The Seventh Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 23-24, 2002.

12. K.T. Chen, J.D. Lin, M.J. Liou, **C.A. Chang**, and E.C. Chan. "Characterization of Distinctive Gene Expression in Thyroid Follicular Carcinoma Cells." *The Seventh Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 23-24, 2002.

13. J.W. Hsieh, F.S. Lo, **C.A. Chang**, and E.C. Chan. "Development of a Method for a High Throughput Screening Gene Variation." *The Seventh Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 23-24, 2002.

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Chemical Society (Taipei), Taipei, Taiwan, October 25-27, 2002.

15. Y-Y. Kwan, **C.A. Chang**. "Molecular Structural Simulations of Lanthanide(III) Complexes of 9- and 12-membered Macrocyclic Polyamino Carboxylates". *2002 Annual Meeting of the Chinese Chemical Society (Taipei)*, Taipei, Taiwan, October 25-27, 2002.
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17. H-C. Chiu, P.-G. Lan, Y.-C. Hu, and **C.A. Chang**. "A Multi-species Correlation Model to Determine Orthologous Relationship." *The Eighteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 22-23, 2003.
18. P.-G. Lan, and **C.A. Chang**. "Construction of Porcine Placental cDNA Library Construction and Placental Functional Gene Cloning and Expression." *The Eighteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 22-23, 2003.
19. K.T. Chen, J.D. Lin, **C.A. Chang**, and E.C. Chan.. "An Aberrant Autocrine Activation of the Platelet-derived Growth Factor A-receptor in Follicular Thyroid Carcinoma Cell Line and Papillary Thyroid Carcinoma Cell Line." *The Eighteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 22-23, 2003.