

AD _____

Award Number: W81XWH-05-1-0593

TITLE: Prostate Cancer Evaluation: Design, Synthesis, and Evaluation of Novel Enzyme-Activated Proton MRI Contrast Agents

PRINCIPAL INVESTIGATOR: Jian-Xin Yu, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas
Southwestern Medical Center at Dallas
Dallas, TX 75390-9058

REPORT DATE: October 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 01-10-2006		2. REPORT TYPE Annual		3. DATES COVERED 15 Sep 2005 – 14 Sep 2006	
4. TITLE AND SUBTITLE Prostate Cancer Evaluation: Design, Synthesis, and Evaluation of Novel Enzyme-Activated Proton MRI Contrast Agents				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-05-1-0593	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jian-Xin Yu, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas Southwestern Medical Center at Dallas Dallas, TX 75390-9058				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES Original contains colored plates: ALL DTIC reproductions will be in black and white.					
14. ABSTRACT The lacZ gene encoding E. coli beta-gal has already been recognized as the most commonly used reporter system in cancer gene therapy. Moreover, prostate-specific membrane antigen (PSMA) has been identified as an ideal antigenic target in prostate cancer. We propose to develop a novel class of Gd(III)-based MRI contrast agents for in vivo detection of beta-gal or PSMA activity. This new concept of the GD(III)-based MRI contrast agents is composed of three moieties: (A) a signal enhancement group, such as Gd-DOTA or Gd-PCTA; (B) an Fe(III) chelating group; (C) beta-D-galactose or glutamate. Following cleavage by lacZ transgene or PSMA in prostate cancer cells, the released, activated aglycone Fe(III)-ligand will spontaneously trap endogenous Fe(III) at the site of enzyme activity forming a highly stable complex, to restrict motion of the GD(III) chelates enhancing relaxivity and providing local contrast accumulation. We plan to synthesize 8 novel MRI contrast agents for imaging beta-gal or PSMA activity in prostate cancer cell culture, explore the feasibility of applying the most promising analogies to cells grown in vivo in mice and rats.					
15. SUBJECT TERMS Prostate Cancer Evaluation, Contrast Agent Synthesis, MRI Gene Expression, Gene Therapy, in vivo Cancer Imaging, lacZ Gene, beta-Galactosidase, PSMA, NAALADase					
16. SECURITY CLASSIFICATION OF:			UU	18. NUMBER OF PAGES 14	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	9
Reportable Outcomes.....	9
Conclusions.....	9
References.....	10
Appendices.....	14

INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer and the second most common cause of cancer death in men in the United States.[1,2] Gene therapy has emerged as a potentially promising strategy for treatment of prostate cancer.[3-15] The prostate is particularly amenable to gene therapy.[11-16] However, there are major issues in terms of assessing the delivery to target tissue, assessing the uniformity (versus heterogeneity) of biodistribution and determining whether the genes are expressed.[15-33] A viral construct is often readministered on successive occasions, but this should optimally be timed to coincide with loss of expression. Inevitably gene therapy has associated risks, and thus non-invasive *in vivo* determining the duration of gene expression in an individual tumor could greatly enhance the viability of the approach.

Gene expression now is commonly monitored by *in situ* hybridization techniques or by introducing a marker gene to follow the regulation of a gene of interest. Since β -galactosidase (β -gal) activity is readily assessed by histology or in culture, in hosts as evolutionarily diverse as bacteria, yeast, and mammals, its introduction has become a standard means of assaying clonal insertion, transcriptional activation, protein expression, and protein interaction, *lacZ* gene encoding *E. coli* β -gal has already been recognized as the most commonly used reporter system.[34] However, the well-established chromogenic or fluorogenic substrates, relying on the hydrolysis by β -gal to release colorful compounds are limited to histology or *in vitro* assays.[35-39] Non-invasive *in vivo* detecting of transgene expression would be of considerable value in many ongoing and future clinical gene therapy trials.

Magnetic resonance imaging (MRI) techniques recently have obtained spectacular image resolutions (voxel resolutions of about $10\ \mu\text{m}^3$ *in vitro* and about $50\ \mu\text{m}^3$ *in vivo*), opening the realm of imaging at very high resolutions in small animals during development and in clinical practice.[40-44] Additionally, a new emerging generation of responsive MRI contrast agents holds great promise in the gene therapy arena.[45,46] The abilities of these contrast agents to relax water protons is triggered or enhanced greatly by recognition of a particular biomolecule opening up the possibility of developing MRI tests specific for biomarkers indicative of particular disease states and aiding in the early detection and diagnosis of tumors. Desreux *et al* [42,47] demonstrated that, by chelating Gd(phen)HDO3A with Fe(II) to form a highly stable tris-complex, as shown in **Figure 1**, the relaxivity increased 145% at 20MHz and 37°C from $5.1\text{mM}^{-1}\text{s}^{-1}$ per Gd(III) in Gd(phen)HDO3A form to $12.2\ \text{mM}^{-1}\text{s}^{-1}$ in the tris-complex. Desreux *et al* [42,47] also synthesized another iron-sensitive MRI contrast agent with a tris-hydroxamate (**Figure 2**). After the tris-hydroxamate groups formed a chelate with Fe(III), free rotation at the Gd(III) centers was restricted, thereby increasing relaxivity by 57% from 5.4 to $8.5\text{mM}^{-1}\text{s}^{-1}$ at 20 MHz.

Iron is a critically important metal ion for a wide variety of cellular events.[48] Tumor cells, as compared with their normal counterparts, frequently exhibit increased uptake and utilization of iron, as evidenced by an increase in transferrin receptors at the cell surface.[49-51] Additionally, cancer cells are

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ^1H MRI Contrast Agents

sensitive to the effects of iron chelators because of the critical requirement for iron in proteins that play essential roles in DNA synthesis and energy production.[52,53] Such studies have led to iron chelation therapy to clinically treat some tumors.[54-58]

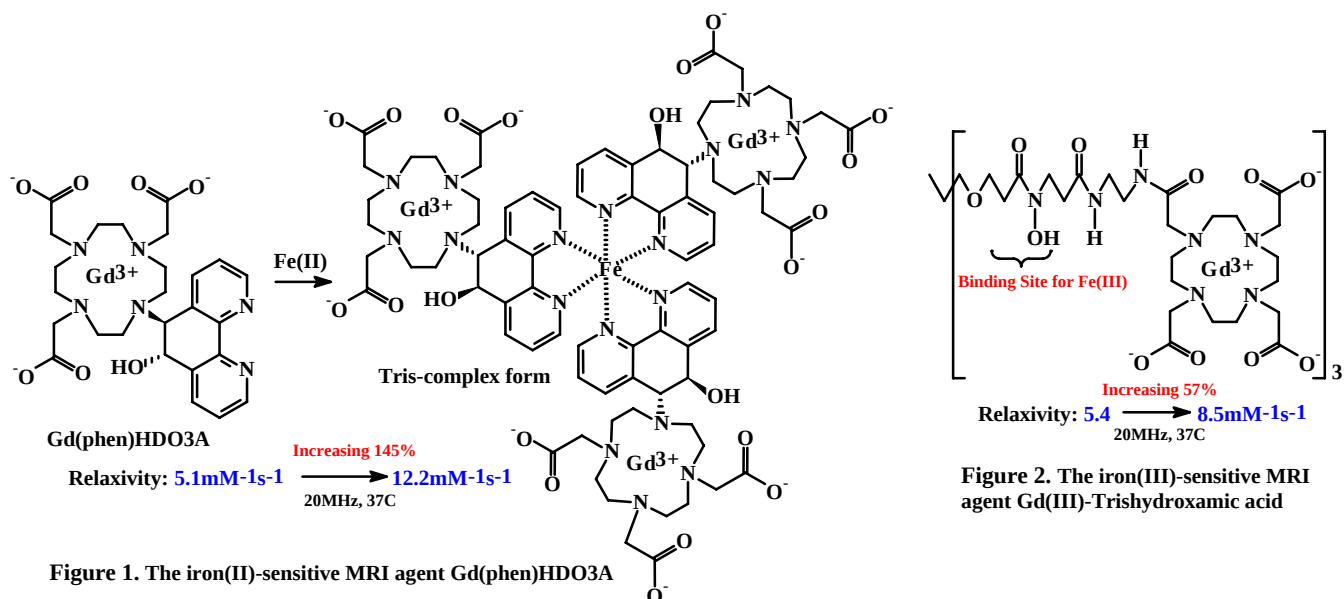


Figure 1. The iron(II)-sensitive MRI agent $\text{Gd}(\text{phen})\text{HDO3A}$

Figure 2. The iron(III)-sensitive MRI agent $\text{Gd(III)-Trishydroxamic acid}$

Based on the MRI contrast agents findings and the biologic features of tumor, we have proposed in this project a novel class of enzyme activated Gd^{3+} -based MRI contrast agent for *in vivo* detection of β -gal activity, in which we try to combine all means of reaching the highest possible relaxivities.[42,47] **Figure 3** depicts the mechanism for *in vivo* detection of *lacZ* gene expression through β -gal activated *in situ* Fe^{3+} -trapped MRI contrast agent formation.

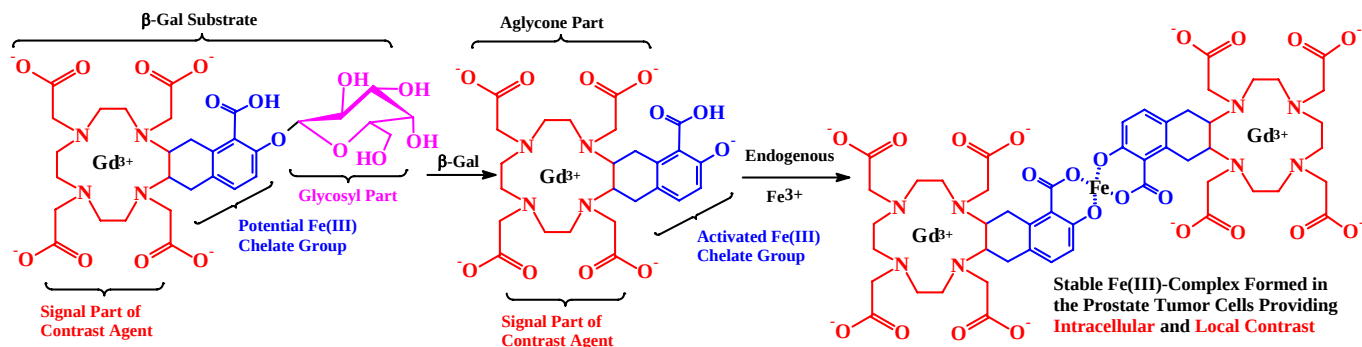


Figure 3. Mechanism of proposed new platform for *in vivo* detection of *lacZ* gene expression through β -gal activated *in situ* Fe^{3+} -trapped MRI contrast agent formation.

Additionally, prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein with enzymatic activities: N-acetylated α -linked L-amino dipeptidase (NAALADase) and γ -glutamyl carboxypeptidase (folate hydrolase).[59-61] Studies with the monoclonal antibodies have demonstrated that PSMA is the most well-established, highly restricted prostate cancer cell surface antigen, it is expressed at high density on the cell membrane of all prostate cancers.[62-64] The high prostate tissue specificity of PSMA has been identified as an ideal therapeutic and diagnostic target for prostate cancer,

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ^1H MRI Contrast Agents

this potential was exemplified by the recent FDA approval of an ^{111}In -labeled PSMA monoclonal antibody (Prostascint[®]) for diagnostic imaging of prostate cancer.[65-67] Furthermore, phase I and II trials have begun using immunotherapy directed against PSMA.[68-70] By introducing γ -glutamate residue instead of D-galactose in our proposed above new mechanism diagram, we intend to develop a novel class of Gd(III)-based MRI contrast agents for *in vivo* imaging prostate tumor through PSMA activated *in situ* Fe^{3+} -trapped MRI contrast agent formation (Figure 4).

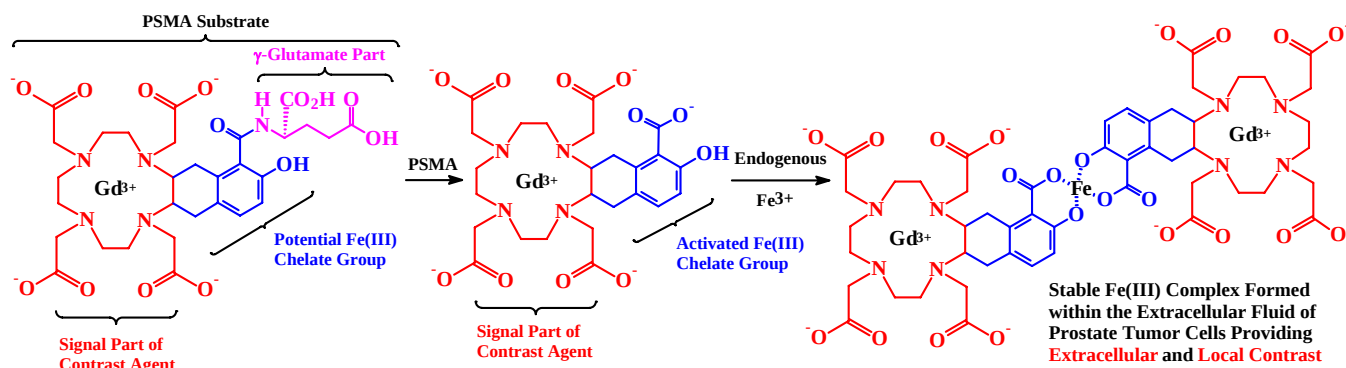


Figure 4. Proposed new mechanism for *in vivo* imaging prostate tumor through PSMA activated *in situ* Fe^{3+} -trapped MRI contrast agent formation.

Especially, PSMA has a large extracellular domain,[70] so the expression of PSMA tethered to the surface of the prostate cancer cells makes that the novel peptide-based MRI contrast agents can be targeted for activation within the extracellular fluid of prostate cancers [71] and overcomes the need for a peptide-based MRI contrast agent to penetrate the tumor cell membrane, thus, providing *in vivo* prostate cancer imaging through an **extracellular** MRI approach. The concern of permeability is one of the greatest challenges in the development of *in vivo* MRI contrast agents.[72]

Accordingly, depending upon the enzyme sources either being the *lacZ* transgene or the PSMA from prostate tumors, this new platform could provide *in vivo lacZ* gene expression assay or *in vivo* prostate cancer imaging (in particular, through **extracellular** contrast agents), with combining all the approaches of reaching the highest possible relaxivities.[42,47,72] Furthermore, this new class of responsive MRI contrast agent is composed of three functional moieties, in which the signal enhancing and Fe^{3+} chelating parts are flexible allowing modification in a search for ideal Fe^{3+} -trapped MRI contrast agents. Importantly, the combination of three functional moieties is based on the clinically applied strategies on cancer therapy. These facts strongly suggest the potential of the proposal to future clinical application.

Most recently, Merbach *et al* [73-76] also observed the remarkably high T_1 relaxivity gain by Fe(II) complex formation from $(\text{tpy-DTTA})\text{Gd}(\text{H}_2\text{O})$ with $7.3\text{mM}^{-1}\text{s}^{-1}$ to $\{\text{Fe}^{\text{II}}[\text{Gd}^{\text{III}}_2(\text{tpy-DTTA})_2(\text{H}_2\text{O})_4]_3\}^{4-}$ with $15.7\text{mM}^{-1}\text{s}^{-1}$ at 20MHz and 37°C, significantly, their detailed studies on structure and dynamics of the trinuclear complex $\{\text{Fe}^{\text{II}}[\text{Gd}^{\text{III}}_2(\text{tpy-DTTA})_2(\text{H}_2\text{O})_4]_3\}^{4-}$ indicate that the heterometallic self-assemblies attain high T_1 relaxivities by influencing three factors: water exchange, rotation, and electron

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ^1H MRI Contrast Agents

relaxation, which are fully consistent with the expecting results shown as above in **Figures 3** and **4**, the effectiveness of contrast agents can be increased by restricting the motion of Gd(III) chelates by linking them rigidly to macromolecules through covalent or non-covalent bonds, by an improvement of their intrinsic relaxivity or by attaching several paramagnetic entities to biological or synthetic oligomers. Obviously, these comprehensive investigations as relevant evidences strongly support for our current proposal.

STATEMENT OF WORK

Specific Aim 1 Design and synthesize model “smart” MRI contrast agents to report β -gal or PSMA activities with the ability of trapping Fe^{3+} ion.

Task 1 Design and optimization of synthetic strategies for reporter molecules. (**Months 1-18**)

Task 2 Structural characterizations of the synthesized molecules. (**Months 4-20**)

Specific Aim 2 Test the properties of molecules in solution and *in vitro* with cultured prostate cancer cells.

Task 3 Evaluation the basic properties of the agents **in solution**. (**Months 20-22**)

Task 4 Evaluation of the properties of the optimal molecules *in vitro* **with cultured prostate cancer cells**. (**Months 23-25**)

Specific Aim 3 Scale up synthesis of the most promising MRI contrast agent(s) and apply to animal investigations.

Task 5 Scale up synthesis of the most promising ^1H MRI contrast agent(s). (**Months 26-28**)

Task 6 Apply the most promising ^1H MRI contrast agent(s) to assess β -gal transfection efficiency, *lacZ* gene expression (spatial and temporal) in prostate tumors *in vivo* (48 mice + 48 rats). (**Months 29-35**)

Task 7 Test dosing protocols, timing, MR detection protocols (48 mice) (**Months 29-35**)

Task 8 Prepare manuscripts and final report (**Month 36**)

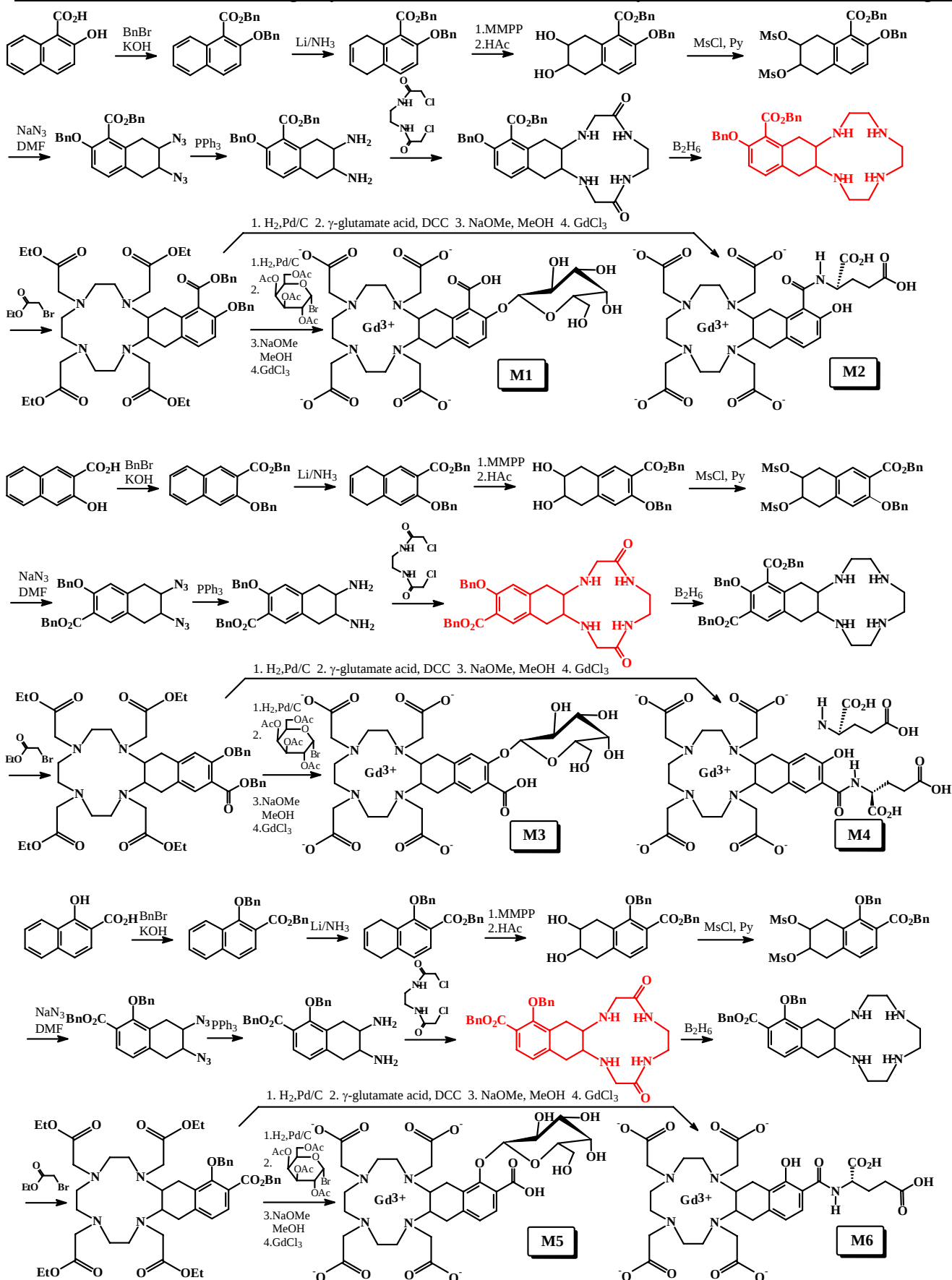
PROGRESS

In this first supported year, our work is totally focused on: **Task 1** Design and optimization of synthetic strategies for reporter molecules, and **Task 2** Structural characterizations of the synthesized molecules, strictly followed the research plan of the approved proposal W81XWH-05-1-0593.

For the designed molecules **M₁** and **M₂**, our syntheses have carried out according to the approaches as shown in **Figure 9** of the proposal. Through a series of reactions, we have built the key structure (*see the red structure*) of Gd^{3+} and Fe^{3+} chelators. In the next six months, we are going to stereo- and regioselectively couple with D-galactose or γ -glutamate acid.

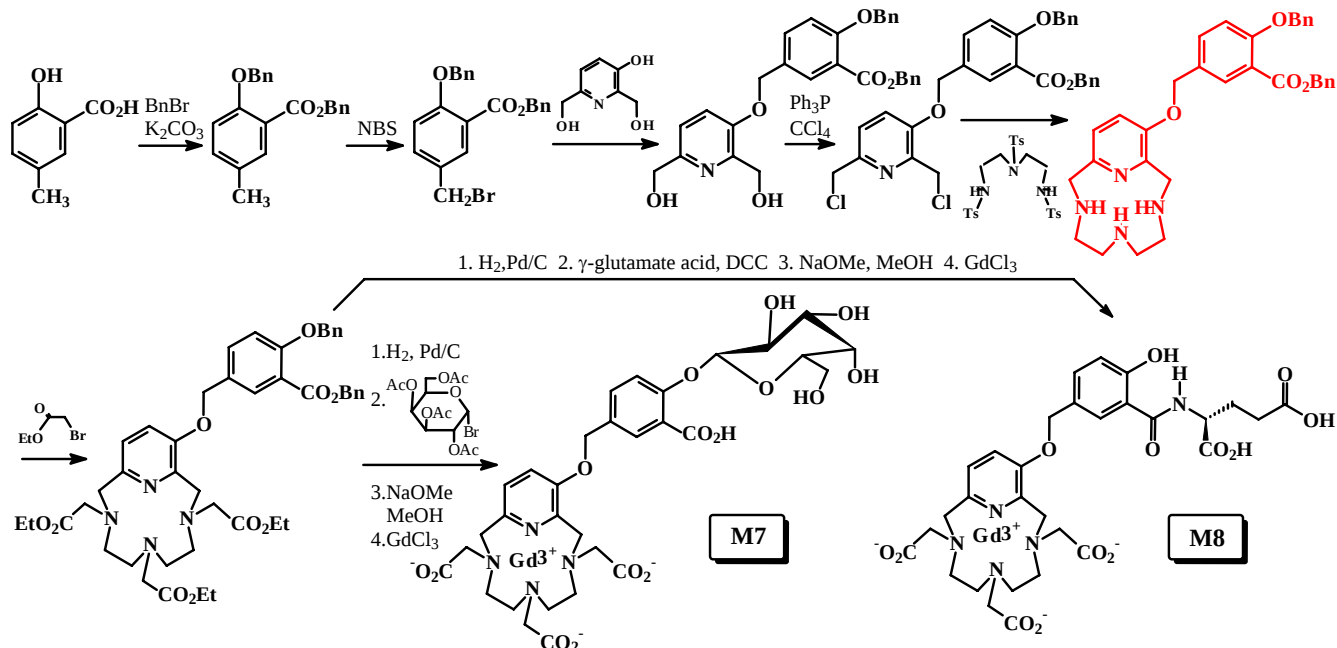
Similarly, the syntheses of **M₃** - **M₆** have reached to the skeleton structures (*see the red structures*) of Gd^{3+} and Fe^{3+} chelators.

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ^1H MRI Contrast Agents



Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ^1H MRI Contrast Agents

Also, the syntheses of **M**₇ - **M**₈ by constructing 3, 6, 9, 15-tetraazabicyclo[9.3.1]pentadeca-1(15), 11, 13-triene-3, 6, 9-triacetic acid as an alternative signal enhancement group through a much different route have arrived at the key structure (see the red structure) of Gd^{3+} and Fe^{3+} chelators.



In each step of the multiple reactions, products were purified by chromatography or recrystallization and characterized by acquisition of ^1H , ^{13}C , DEPT, ^1H - ^1H COSY NMR techniques.

KEY RESEARCH ACCOMPLISHMENTS

All syntheses for the target molecules **M**₁ - **M**₈ have accomplished the construction of the important key structures of Gd^{3+} and Fe^{3+} chelators, and their structures all are verified by NMR data, providing the solid foundation for the further syntheses. Meanwhile, we have accumulated relevant experience, and gotten some expertise for efficient synthesis and separation of these intermediates, which will greatly benefit for the scale-up synthesis of the most promising ^1H MRI contrast agent(s) in **Task 5**.

REPORTABLE OUTCOMES

A series of intermediates related the target molecules **M**₁ - **M**₈ have achieved.

CONCLUSIONS

Prostate cancer is the most commonly diagnosed cancer and the second most common cause of cancer death in men in the United States. The advent of effective screening measures can sharply decrease the mortality of prostate cancer through detecting this disease at an earlier stage. However, the evidence for mortality benefit from prostate cancer screening has been disappointing to date. Expanding knowledge of prostate cancer biology with combination of imaging technologies would be of considerable value in many ongoing and future clinical prostate cancer diagnosis and gene therapy trials.

Based on the biologic features of prostate cancer, we proposed in this project a new approach for *in vivo* lacZ gene expression assay or *in vivo* prostate cancer imaging (in particular, through **extracellular**

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ^1H MRI Contrast Agents

contrast agents). The ultimate objective is to demonstrate the utility and reliability of this new approach to measure β -gal or PSMA activities *in vivo*. We have accomplished the construction of the important key structures of Gd^{3+} and Fe^{3+} chelators, and verified by NMR data. We are now focusing on stereo- and regioselectively coupling with D-galactose or γ -glutamate acid to accomplish the designed molecules **M₁ - M₈**, anticipating to identify 1-2 as of the most promising MRI contrast agents for testing the sequence of tests with prostate cancer *in vitro* and *in vivo*.

REFERENCES:

1. American Cancer Society, Cancer Facts and Figures, 2004. (www.cancer.org).
2. Jemal A, Thomas A, Murray T, Thun M, 2002 Cancer statistics, 2002, *CA Cancer J. Clin.*, **52**, 23-47.
3. (a) Eastham JA, Hall SJ, Sehgal I, Wang J, Timme TL, Yang G, Connell-Crowley L, Elledge SJ, Zhang WW, Happer JW, 1995, *In vivo* gene therapy with p53 or p21 adenovirus for prostate cancer, *Cancer Res.*, **55**, 5151-5155; (b) Eastham JA, Chen SH, Sehgal I, Yang G, Timme TL, Hall SJ, Woo SL, Thompson TC, 1996, Prostate cancer gene therapy: Herpes simplex virus thymidine kinase gene transduction followed by ganciclovir in mouse and human prostate cancer models. *Hum. Gene Ther.*, **7**, 515-523.
4. Dorai T, Olsson CA, Katz AE, Buttyan R, 1997, Development of a hammerhead ribozyme against bcl-2. I. Preliminary evaluation of a potential gene therapeutic agent for hormonerefractory human prostate cancer, *Prostate*, **32**, 246-258.
5. Vieweg J, Rosenthal FM, Bannerji R, Heston WD, Fair WR, Gansbacher B, Gilboa E, 1994, Immunotherapy of prostate cancer in the Dunning rat model: Use of cytokine gene modified tumor vaccines. *Cancer Res.*, **54**, 1760-1765.
6. Sokoloff MH, Tso CL, Kaboo R, Taneja S, Pang S, Dekernion JB, Belldegrun AS, 1996, In vitro modulation of tumor progression-associated properties of hormone refractory prostate carcinoma cell lines by cytokines, *Cancer*, **77**, 1862-1872.
7. Simons JW, Mikhak B, Chang JF, Demarzo AM, Carducci MA, Lim M, Weber CE, Baccala AA, Goemann MA, Clift SM, Ando DG, Levitsky HI, Cohen LK, Sanda MG, Mulligan RC, Partin AW, Carter HB, Piantadosi S., Marshall FF, Nelson WG, 1999, Induction of immunity to prostate cancer antigens: Results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocytemacrophage colony-stimulating factor using *ex vivo* gene transfer. *Cancer Res.*, **59**, 5160-5168.
8. Belldegrun A, Tso CL, Zisman A, Naitoh J, Said J, Pantuck AJ, Hinkel A, Dekernion J, Figlin R, 2001, Interleukin 2 gene therapy for prostate cancer: Phase I clinical trial and basic biology, *Hum. Gene Ther.*, **12**, 883-892.
9. Blackburn RV, Galoforo SS, Corry PM, Lee YJ, 1998, Adenoviral-mediated transfer of a heat-inducible double suicide gene into prostate carcinoma cells. *Cancer Res.*, **58**, 1358-362.
10. Pantuck AJ, Matherly J, Zisman A, Nguyen D, Berger F, Gambhir SS, Black ME, Belldegrun A, Wu L, 2002, Optimizing Prostate Cancer Suicide Gene Therapy Using Herpes Simplex Virus Thymidine Kinase Active Site Variants, *Hum. Gene Ther.*, **13**, 777-789.
11. Igawa T, Lin FF, Rao P, Lin MF, 2003, Suppression of LNCaP prostate cancer xenograft tumors by a prostate-specific protein tyrosine phosphatase, prostatic acid phosphatase, *Prostate*, **55**, 247-258.
12. Steiner MS, Gingrich JR, 2000, Gene therapy for prostate cancer: Where are we now? *J. Urol.*, **164**, 1121-1136.

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ¹H MRI Contrast Agents

13. Harrington KJ, Spitzweg C, Bateman AR, Morris JC, Vile RG, 2001, Gene therapy for prostate cancer: Current status and future prospects, *J. Urol.*, **166**, 1220-1233.
14. Morris MJ, Scher HI, 2000, Novel strategies and therapeutics for the treatment of prostate carcinoma, *Cancer*, **89**, 1329-1348.
15. Gardner TA, Sloan J, Raikwar SP, Kao C, 2002, Prostate Cancer Gene Therapy: Past Experiences and Future Promise, *Cancer and Metastasis Reviews*, **21**, 137-145.
16. Shalev M, Thompson TC, Kadmon D, Ayala G, Kernen K, Miles BJ, 2001, Gene therapy for prostate cancer, *Urology*, **57**, 8-16.
17. Gyorffy S, Palmer K, Gauldie J, 2001, Adenoviral vector expressing murine angiostatin inhibits a model of breast cancer metastatic growth in the lungs of mice, *Am. J. Path.*, **159**, 1137-47.
18. Yazawa, K, Fujimori M, Nakamura T, Sasaki T, Amano J, Kano Y, Taniguchi S, 2001, Bifidobacterium longum as a delivery system for gene therapy of chemically induced rat mammary tumors, *Cancer Res. Treat.*, **66**, 165-170.
19. Sato M, Johnson M, Zhang L, Zhang B, Le K, Gambhir SS, Carey M, Wu L, 2003, Optimization of adenoviral vectors to direct highly amplified prostate-specific expression for imaging and gene therapy, *Mol. Ther.*, **8**, 726-737.
20. Wu L, Sato M, 2003, Integrated, molecular engineering approaches to develop prostate cancer gene therapy, *Curr. Gene Ther.*, **3**, 452-467.
21. Bastide C, Maroc N, Bladou F, Hassoun J, Maitland N, Mannoni P, Bagnis C, 2003, Expression of a model gene in prostate cancer cells lentivirally transduced in vitro and in vivo, *Prostate Cancer and Prostatic Diseases*, **6**, 228-234.
22. Stanbridge LJ, Dussupt V, Maitland NJ, 2003, Baculoviruses as vectors for gene therapy against human prostate cancer, *J. Biomed. Biotech.*, **6**, 79-91.
23. Igawa T, Lin F, Rao P, Lin M, 2003, Suppression of LNCaP prostate cancer xenograft tumors by a prostate-specific protein tyrosine phosphatase, prostatic acid phosphatase, *Prostate*, **55**, 247-258.
24. Nasu Y, 2002, Prostate cancer gene therapy: current status of clinical trial, *Igaku no Ayumi*, **203**, 323-327.
25. Pantuck AJ, Berger F, Zisman A, Nguyen D, Tso C, Matherly J, Gambhir SS, Belldegrin AS, 2002, CL1-SR39: a noninvasive molecular imaging model of prostate cancer suicide gene therapy using positron emission tomography, *J. Urology*, **168**, 1193-1198.
26. Kaminski JM, Nguyen K, Buyyounouski M, Pollack A, 2002, Prostate cancer gene therapy and the role of radiation, *Cancer Treatment Rev.*, **28**, 49-64.
27. Gdor Y, Timme TL, Miles BJ, Kadmon D, Thompson TC, 2002, Gene therapy for prostate cancer, *Expert Review of Anticancer Therapy*, **2**, 309-321.
28. Pantuck AJ, Matherly J, Zisman A, Nguyen D, Berger F, Gambhir SS, Black ME, Belldegrin A, Wu L, 2002, Optimizing prostate cancer suicide gene therapy using herpes simplex virus thymidine kinase active site variants, *Human Gene Therapy*, **13**, 777-789.
29. Zhang L, Adams JY, Billick E, Ilagan R, Iyer M, Le K, Smallwood A, Gambhir SS, Carey M, Wu L, 2002, Molecular engineering of a two-step transcription amplification (TSTA) system for transgene delivery in prostate cancer, *Mol. Ther.*, **5**, 223-232.
30. Voelkel-Johnson C, King DL, Norris JS, 2002, Resistance of prostate cancer cells to soluble TNF-related apoptosis-inducing ligand (TRAIL/Apo2L) can be overcome by doxorubicin or adenoviral delivery of full-length TRAIL, *Cancer Gene Therapy*, **9**, 164-172.

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ¹H MRI Contrast Agents

31. Pramudji C, Shimura S, Ebara S, Yang G, Wang J, Ren C, Yuan Y, Tahir SA, Timme TL, Thompson TC, 2001, *In situ* prostate cancer gene therapy using a novel adenoviral vector regulated by the caveolin-1 promoter, *Clinical Cancer Research*, **7**, 4272-4279.
32. Li Y, Okegawa T, Lombardi DP, Frenkel EP, Hsieh JT, 2002, Enhanced transgene expression in androgen independent prostate cancer gene therapy by taxane chemotherapeutic agents, *J. Urology*, **167**, 339-346.
33. Hsieh CL, Chung LWK, 2001, New perspectives of prostate cancer gene therapy: molecular targets and animal models, *Critical Reviews in Eukaryotic Gene Expression*, **11**, 77-120.
34. Serebriiskii IG, Golemis EA, 2000, Uses of *lacZ* to Study Gene Function: Evaluation of β -Galactosidase Assays Employed in the Yeast Two-Hybrid System, *Anal. Biochem.*, **285**, 1-15.
35. James AL, Perry J D, Chilvers K, Robson IS, Armstrong L, Orr KE, 2000, Alizarin- β -D-galactoside: a new substrate for the detection of bacterial beta-galactosidase, *Lett. Appl. Microbiol.*, **30**, 336-340.
36. Heuermann K, Cosgrove J, 2001, S-GalTM: An Autoclavable Dye for Color Selection of Cloned DNA Inserts, *Biotechniques*, **30**, 1142-1147.
37. Serebriiskii IG, Toby GG, Golemis EA, 2000, Streamlined yeast colorimetric reporter activity assays using scanners and plate readers, *Biotechniques*, **29**, 278-288.
38. Duttweiler HM, 1996, A highly sensitive and non-lethal beta-galactosidase plate assay for yeast, *Trends Genet.*, **12**, 340-341.
39. Timmons L, Becker J, Barthmaier P, Fyrberg C, Shearn A, Fyrberg E, 1997, Green fluorescent protein/ β -galactosidase double reporters for visualizing *Drosophila* gene expression patterns, *Dev. Genet.*, **20**, 338-347.
40. (a) Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, Basilion JP, 2000, *In vivo* magnetic resonance imaging of transgene expression, *Nature Medicine*, **6**, 351-354; (b) Weissleder R, Mahmood U, 2001, Molecular Imaging, *Radiology*, **219**, 316-333.
41. Caravan P, Ellison JJ, McMurry TJ, Lauffer RB, 1999, Gadolinium(III) chelates as MRI contrast agents: Structure, dynamics, and applications, *Chem. Rev.*, **99**, 2293-2352.
42. Comblin V, Gilsoul D, Hermann M, Humblet V, Jacques V, Mesbahi M, Sauvage C, Desreux JF, 1999, Designing new MRI contrast agents: A coordination chemistry challenge, *Coordination Chem. Rev.*, **185-186**, 451-470.
43. Lauffer RB, Parmelee DJ, Dunham S, Ouellet HS, Dolan RP, Witte S, McMurry TJ, Walovich RC, 1998, MS-325: albumin-targeted contrast agent for MR angiography, *Radiology*, **207**, 529-538.
44. Rudin M, Mueggler T, Allegrini PR, Baumann D, Rausch M, 2003, Characterization of CNS disorders and evaluation of therapy using structural and functional MRI, *Anal. Bioanal. Chem.*, **377**, 973-981.
45. Bell JD, Taylor-Robinson SD, 2000, Assessing gene expression *in vivo*: magnetic resonance imaging and spectroscopy, *Gene Therapy*, **7**, 1259-1264.
46. (a) Louie AY, Hüber MM, Ahrens ET, Rothbächer U, Moats R, Jacobs RE, Fraser SE, Meade TJ, 2000, *In vivo* visualization of gene expression using magnetic resonance imaging, *Nature Biotechnology*, **18**, 321-325; (b) Jacobs RE, Ahrens ET, Meade TJ, Fraser SE, 1999, Looking deeper into vertebrate development, *Cell Biology*, **9**, 73-76; (c) Moats RA, Fraser SE, Meade TJ, 1997, A "smart" magnetic resonance imaging agent that reports on specific enzyme activity, *Angew. Chem. Intl. Edn. Engl.*, **36**, 726-728.
47. (a) Jacques V, Desreux JF, 2002, New classes of MRI contrast agents, *Topics Curr. Chem.*, **221**, 123-164; (b) Jacques V, Desreux JF, 2001, Synthesis of MRI contrast agents. II. Macrocyclic ligands, *Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, 157-191.
48. Zu D, Hider RC, 2002, Design of iron chelators with therapeutic application, *Coordinat. Chem. Rev.*, **232**, 151-171.

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ¹H MRI Contrast Agents

49. Abeysinghe RD, Greene BT, Haynes R, Willingham MC, Turner J, Planalp RP, Brechbiel MW, Torti FM, Torti SV, 2001, p53-independent apoptosis mediated by tachpyridine, an anti-cancer iron chelator, *Carcinogenesis*, **22**, 1607-1614.
50. Faulk WP, His BL, Stevens PJ, 1980, Transferrin and transferrin receptors in carcinoma of the breast, *Lancet*, **2**, 390-392.
51. Seymour GJ, Walsh MD, Lavin MF, Strutton G, Gardiner RA, 1987, Transferrin receptor expression by human bladder transitional cell carcinomas, *Urol. Res.*, **15**, 341-344.
52. Becker EM, Lovejoy DB, Greer JM, Watts R, Richardson DR, 2003, Identification of the di-pyridyl ketone isonicotinoyl hydrazone (PKIH) analogues as potent iron chelators and anti-tumour agents, *Br. J. Pharmacol.*, **138**, 819-830.
53. Hershko C, 1994, Control of disease by selective iron depletion: a novel therapeutic strategy utilizing iron chelators, *Baillière's Clin. Haematol.*, **7**, 965-100.
54. Hoffbrand AV, Ganeshaguru K, Hooton JW, Tattersall MHN, 1976, Effect of iron deficiency and desferrioxamine on DNA synthesis in human cells, *Br. J. Haematol.*, **33**, 517-526.
55. Bergeron RJ, Cavanaugh PF Jr, Kline SJ, Hughes RG Jr, Elliot GT, Porter CW, 1984, Antineoplastic and antiherpetic activity of spermidine catecholamide iron chelators, *Biochem. Biophys. Res. Commun.*, **121**, 848-854.
56. Hoyes KP, Hider RC, Porter JB, 1992, Cell cycle synchronization and growth inhibition by 3-hydroxypyridin-4-one iron chelators in leukemic cell lines, *Cancer Res.*, **52**, 4591-4599.
57. Seligman P, Schleicher RB, Siriwardana G, Domenico J, Gelfand EW, 1993, Effects of agents that inhibit cellular iron incorporation on bladder cell proliferation, *Blood*, **82**, 1608-1617.
58. Torti SV, Torti FM, Whitman SP, Brechbiel MW, Park G, Planalp RP, 1998, Tumor cell cytotoxicity of a novel metal chelator, *Blood*, **92**, 1384-1389.
59. Horoszewicz JS, Kawinski E, Murphy GP, 1987, Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients, *Anticancer Res.*, **7**, 927-935.
60. Israeli RS, Powell CT, Fair WR, Heston WD, 1993, Molecular cloning of a complementary DNA encoding a prostate-specific membrane antigen, *Cancer Res.*, **53**, 227-230.
61. Schmittgen TD, Teske S, Vessella RL, True LD, Zakrajsek BA, 2003, Expression of prostate specific membrane antigen and three alternatively spliced variants of PSMA in prostate cancer patients, *Int. J. Cancer*, **107**, 323-329.
62. Carter RE, Feldman AR, Coyle JT, 1996, Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase, *Proc. Natl. Acad. Sci. USA*, **93**: 749-753.
63. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C, 1997, Prostate-specific membrane antigen expression in normal and malignant human tissues, *Clin. Cancer Res.*, **3**, 81-85.
64. Israeli RS, Powell CT, Corr JG, Fair WR, Heston WD, 1994, Expression of the prostate-specific membrane antigen, *Cancer Res.*, **54**, 1807-1811.
65. Babaian RJ, Sayer J, Podoloff DA, Steelhammer LC, Bhadkamkar VA, Gulfo JV, 1994, Radioimmunoscinigraphy of pelvic lymph nodes with ¹¹¹indium-labeled monoclonal antibody CYT-356, *J Urol.*, **152**, 1952-1955.
66. Kahn D, Williams RD, Manyak MJ, Haseman MK, Seldin DW, Libertino JA, Maguire RT, 1998, ¹¹¹Indium-capromab pendetide in the evaluation of patients with residual or recurrent prostate cancer after radical prostatectomy, *J. Urol.*, **159**, 2041-2047.

Prostate Cancer Evaluation: Design, Synthesis and Evaluation of Novel Enzyme-Activated ^1H MRI Contrast Agents

67. Kahn D, Williams RD, Seldin DW, Libertino JA, Hirschorn M, Dreicer M, Weiner GJ, Bushnell D, Gulfo J, 1994, Radioimmunoscinigraphy with $^{111}\text{indium}$ -labeled CYT-356 for the detection of occult prostate cancer recurrence, *J. Urol.*, **152**, 1490-1495.
68. Tjoa BA, Simmons SJ, Bowes VA, Ragde H, Rogers M, Elgamal A, Kenny GM, Cobb OE, Ireton RC, Troychak MJ, Salgaller ML, Boynton AL, Murphy GP, 1998, Evaluation of phase I/II clinical trials in prostate cancer with dendritic cells and PSMA peptides, *Prostate*, **36**, 39-44.
69. Murphy GP, Tjoa BA, Simmons SJ, Jarisch J, Bowes VA, Ragde H, Rogers M, ElgamalA, KennyGM, Cobb OE, Ireton RC, Troychak MJ, Salgaller ML, Boynton AL, 1999, Infusion of dendritic cells pulsed with HLA-A2-specific prostate-specific membrane antigen peptides: A phase II prostate cancer vaccine trial involving patients with hormone-refractory metastatic disease, *Prostate*, **38**, 73-78.
70. Huang X, Bennett M, Thorpe PE, 2004, Anti-tumor effects and lack of side effects in mice of an immunotoxin directed against human and mouse prostate-specific membrane antigen, *Prostate*, **61**, 1-11.
71. Mhaka A, Gady AM, Rosen DM, Lo KM, Gillies SD, Denmeade SR, 2004, Use of Methotrexate-Based Peptide Substrates to Characterize the Substrate Specificity of Prostate-Specific Membrane Antigen (PSMA), *Cancer Biol. Ther.*, **3**, 551-558.
72. Louie AY, Meade TJ, 2000, Recent advances in MRI: Novel contrast agents shed light on *in-vivo* biochemistry, *TiBS*, 7-11.
73. (a) Ruloff R, van Koten G, Merbach AE, Novel heteroditopic chelate for self-assembled gadolinium(III) complex with high relaxivity, *Chem. Comm.*, **2004**, (7), 842-843; (b) Costa J, Ruloff R, Burai L, Helm L, Merbach AE, Rigid $\text{M}^{\text{II}}\text{L}_2\text{Gd}_2^{\text{III}}$ ($\text{M} = \text{Fe}, \text{Ru}$) Complexes of a Terpyridine-Based Heteroditopic Chelate: A Class of Candidates for MRI Contrast Agents, *J. Am. Chem. Soc.*, **2005**, 127, 5147-5157.
74. (a) Livramento JB, Toth E, Sour A, Borel A, Merbach AE, Ruloff R, High relaxivity confined to a small molecular space: A metallostar-based, potential MRI contrast agent, *Angew. Chem.*, **2005**, 44, 1480-1484; (b) Torres S, Martins JA, Andre JP, Geraldes CFGC, Merbach AE, Toth E, Supramolecular assembly of an amphiphilic GdIII chelate: Tuning the reorientational correlation time and the water exchange rate, *Chem. Eur. J.*, **2006**, 12, 940-948;
75. Livramento JB, Sour A, Borel A, Merbach AE, Toth E, A starburst-shaped heterometallic compound incorporating six densely packed Gd^{3+} ions, *Chem. Eur. J.*, **2006**, 12, 989-1003.
76. Lothar H, Merbach AE, Inorganic and bioinorganic solvent exchange mechanisms, *Chem. Rev.*, **2005**, 105, 1923-1959.

APPENDICES NONE