Highly Pure, Multi-Epitopic Lipopeptide Vaccine Delivery System: Synthesis and Investigation

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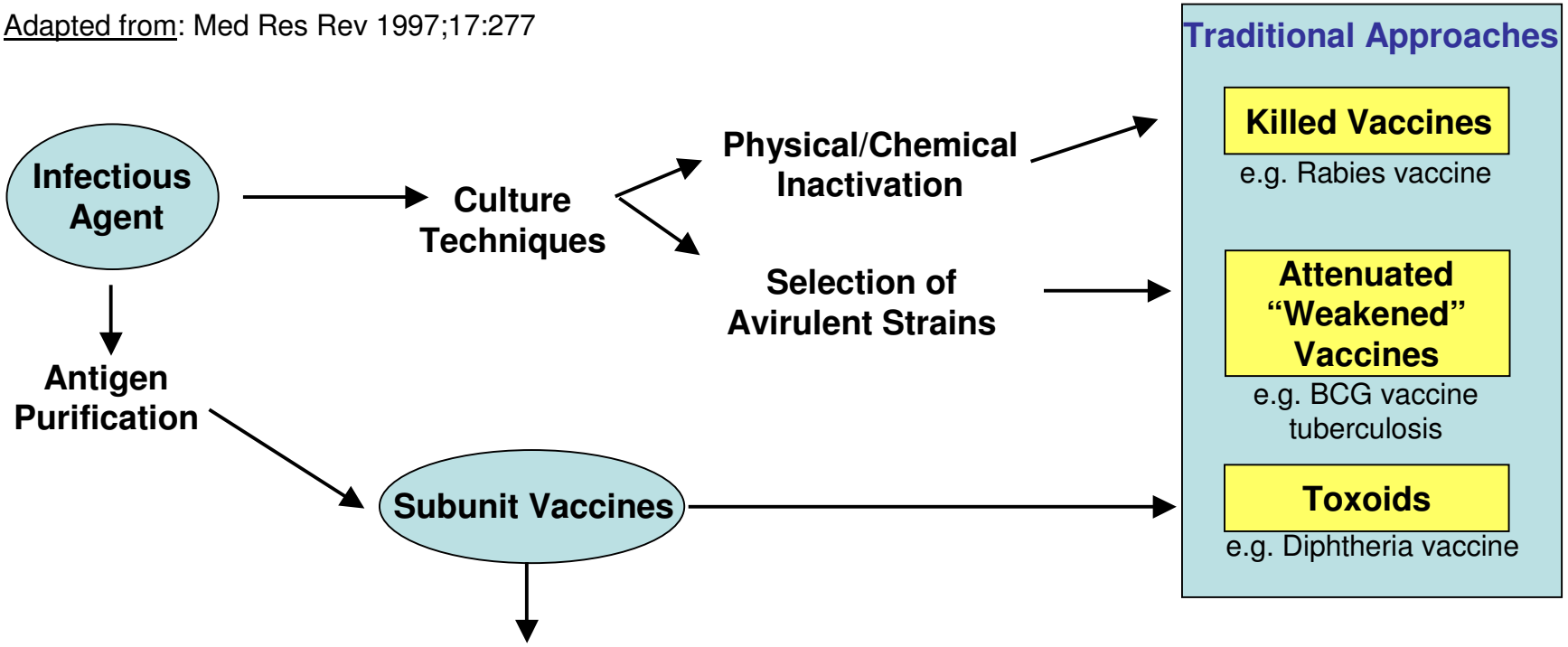
Introduction

Vaccination is the most effective/cost-effective public health intervention

• Disease prevention • Reduces health care costs • Reduces lost work time due to sickness

Adapted from: Med Res Rev 1997;17:277



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Introduction

Subunit Vaccines

Contain the minimal microbial components necessary to stimulate an appropriate immune response

- Vaccines are administered to healthy individuals (normally children).
- These people are being asked to take a medication when they are well.
- Therefore adverse effects must be minimal.

Advantage

- Removing unnecessary components, reduces the risk of auto-immune diseases and adverse effects.
- Not infectious; No reversion to virulence.
- Can customise the vaccine components to tailor an appropriate immune response.

Problem

- Removing unnecessary components often removes danger signals.
- Need strong adjuvant ('immune stimulating agent').
- In the case of peptides:
    - Small molecular weight limits their capacity to elicit immune responses.
    - Peptides lack the T-helper epitopes required for efficacy in an outbred population.

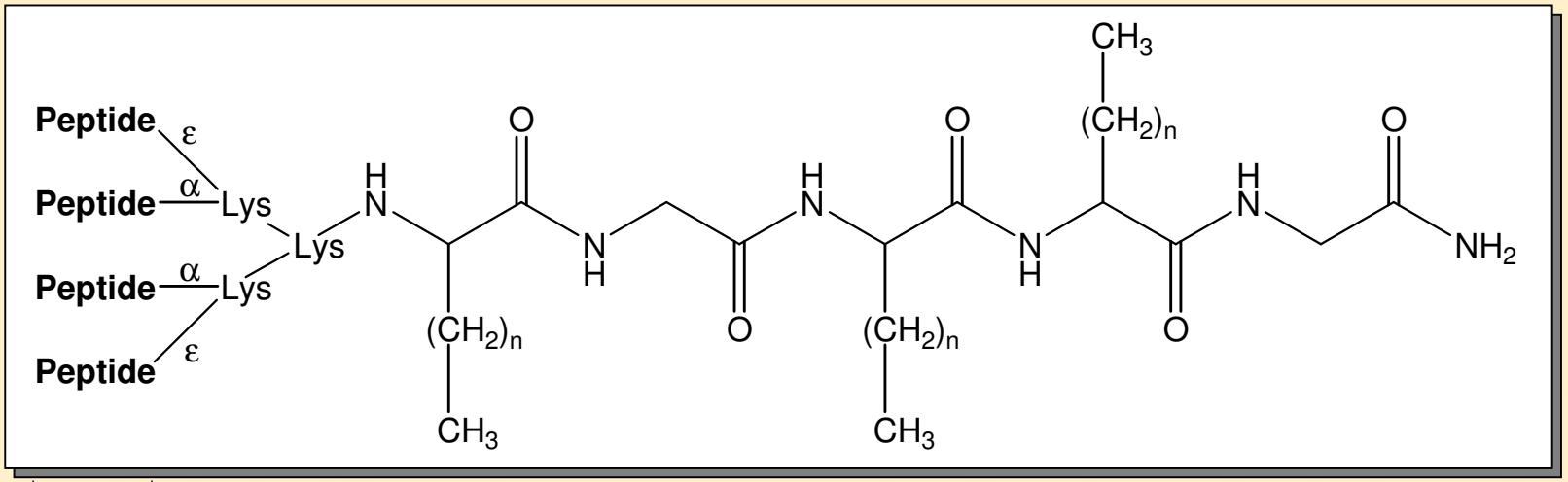
} **Carrier Molecule**

The Lipid-Core Peptide (LCP) System

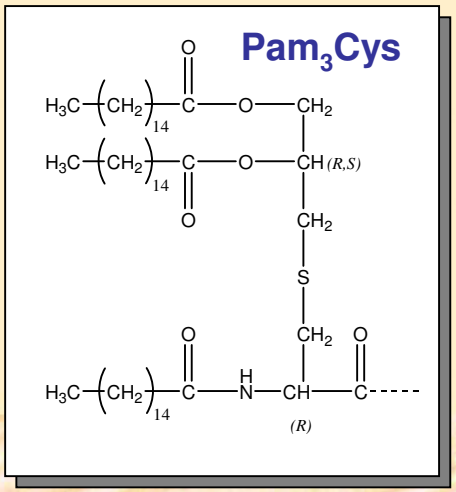
Poly-lysine
Multiple Antigen Peptide (MAP) System
(Carrier)
PNAS 1988;85:5409

Tetrahedron Lett 1993;34:3925

Lipoamino acid
Liebigs Ann Chem 1990;(12):1175



Peptides
(Antigen)



Lipid Core
(Adjuvant)
*** Mimics Pam_3Cys**

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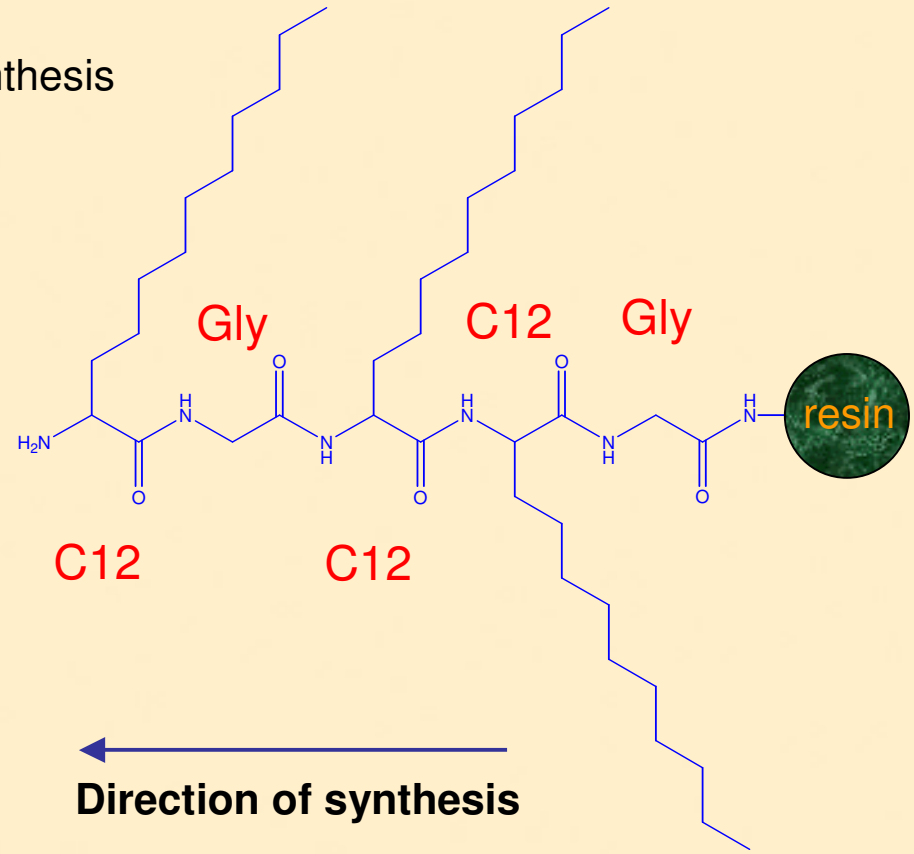
LCP Synthesis

1. <u>Synthesize LCP Lipid Core</u>

- Using stepwise solid-phase peptide synthesis



C12: 2-amino-D,L-dodecanoic acid (Liebigs Ann Chem 1990;(12):1175)

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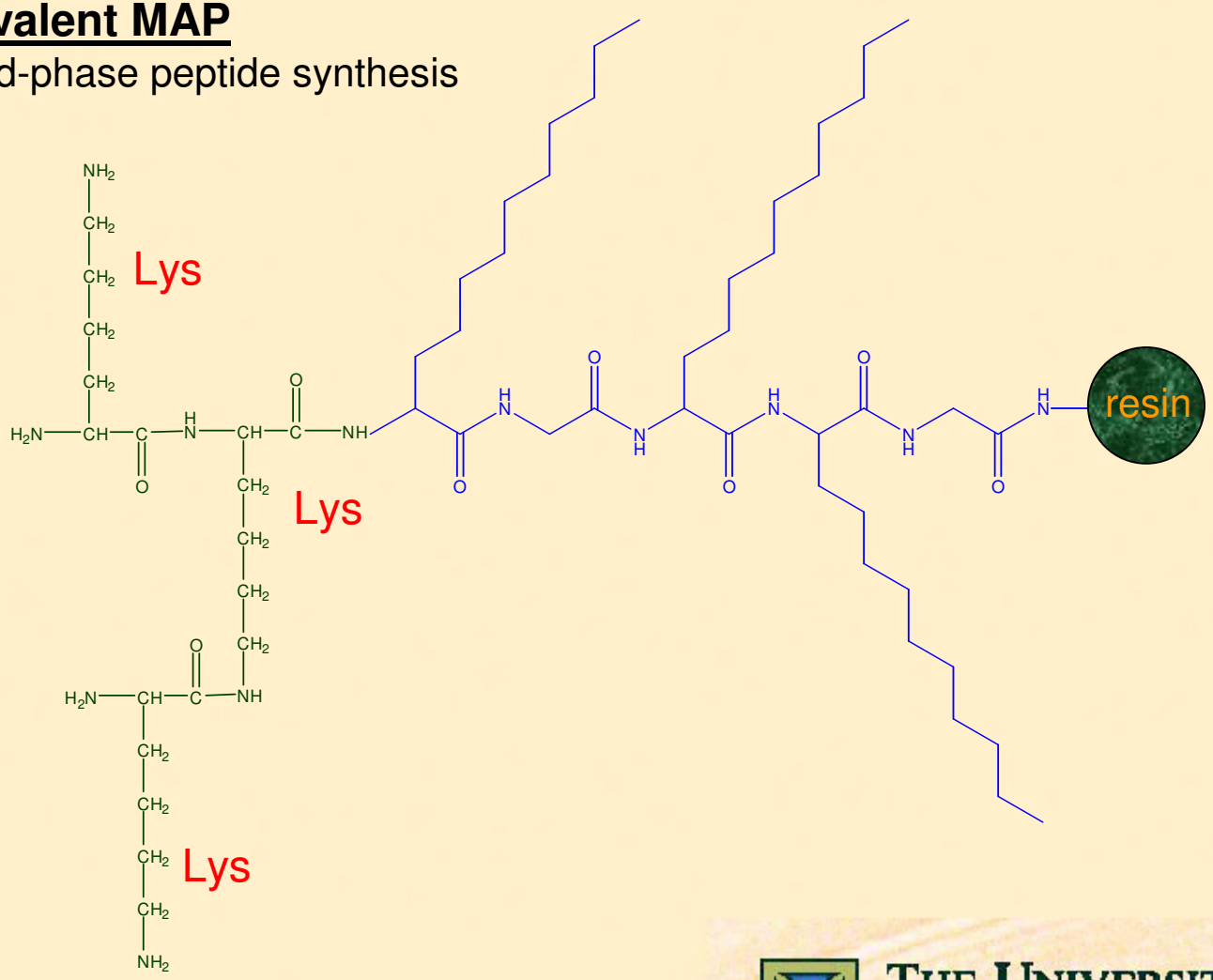
LCP Synthesis

2. <u>Synthesize Tetravalent MAP</u>

- Using stepwise solid-phase peptide synthesis



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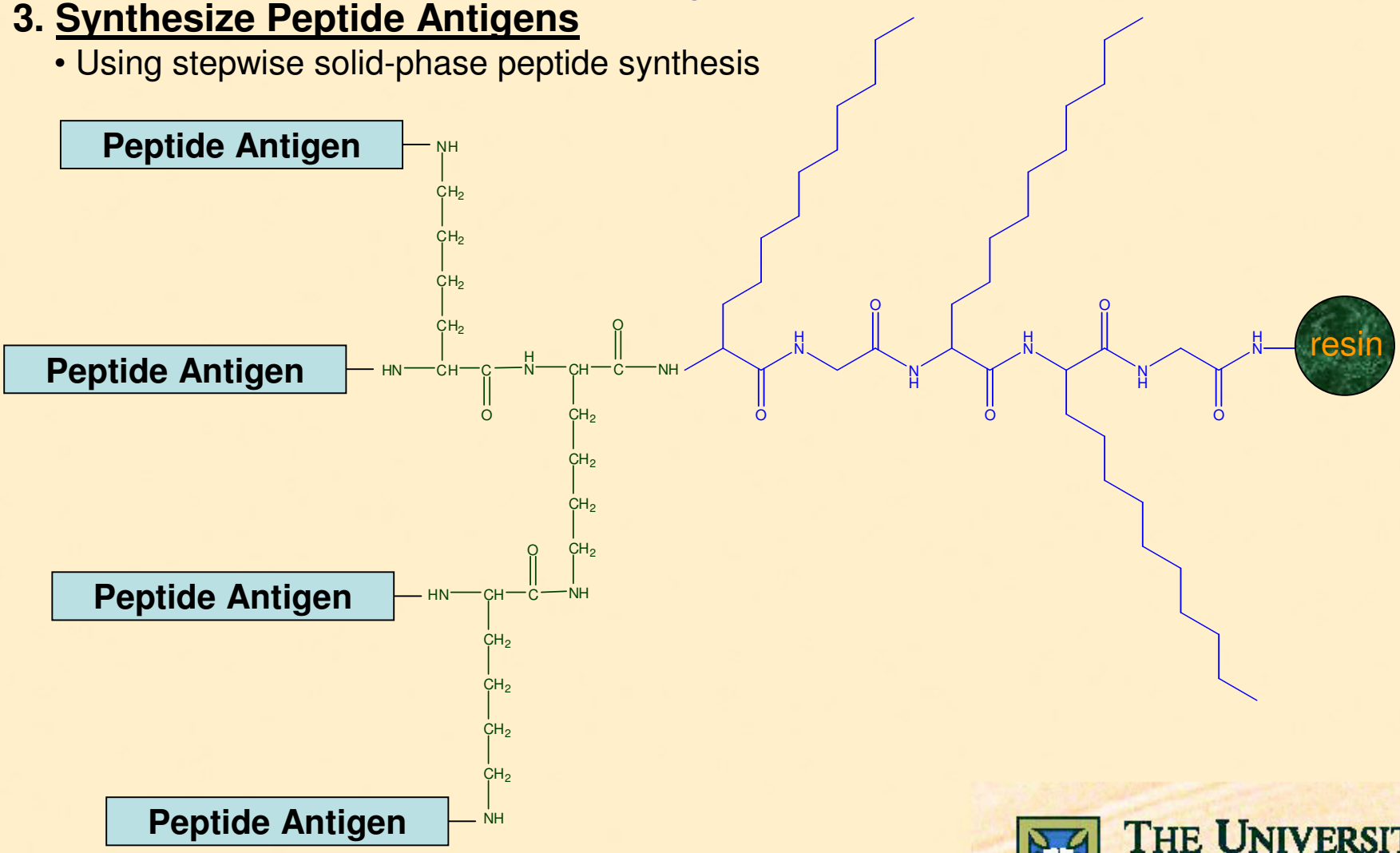
LCP Synthesis

3. <u>Synthesize Peptide Antigens</u>

- Using stepwise solid-phase peptide synthesis



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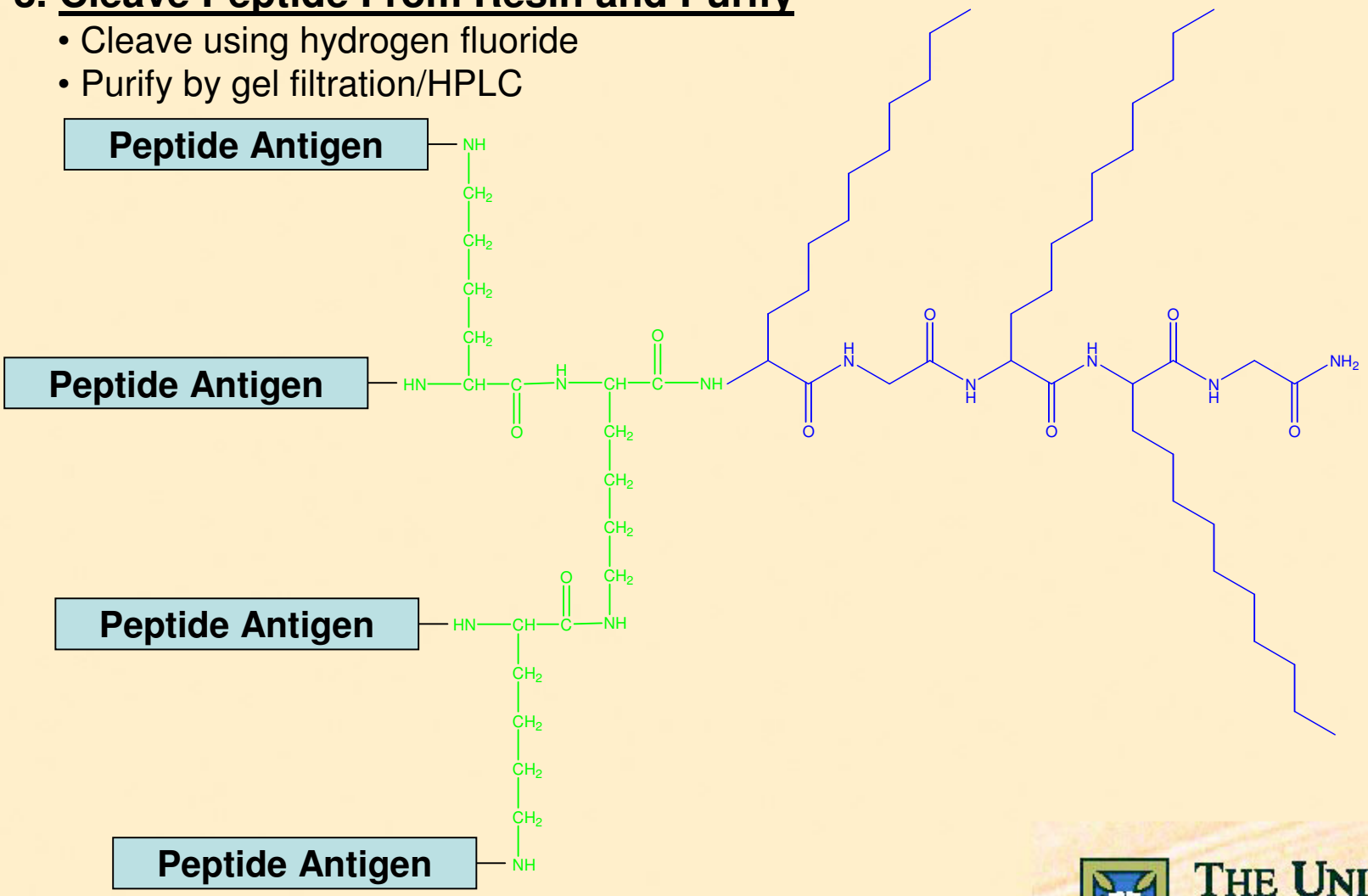
LCP Synthesis

3. <u>Cleave Peptide From Resin and Purify</u>

- Cleave using hydrogen fluoride
- Purify by gel filtration/HPLC



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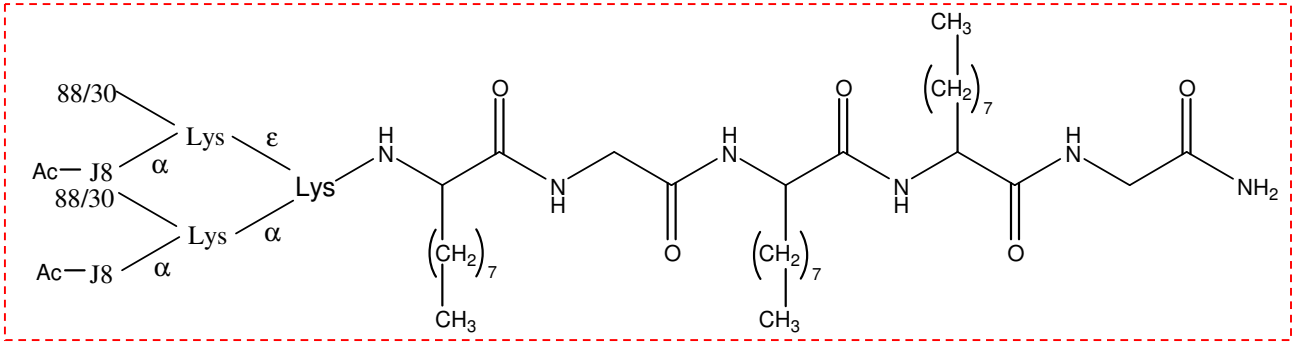
LCP-88/30-J8

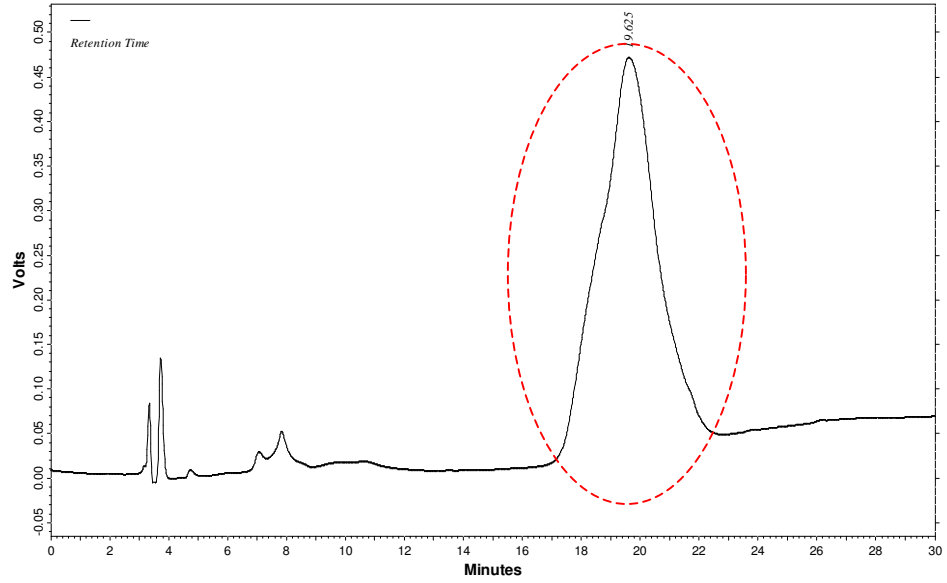
$C_{536}H_{918}N_{164}O_{166}S_2$

12380.16g/mol



J8: QAEDK VKQSR EAKKQ VEKAL KQLED KVQ (28mer)

88/30: DNGKA IYERA RERAL QELGP C (21mer)



A/ 0.1% TFA/H_2O

B/ 90% IPA/0.1% TFA/H_2O

Gradient:	0-100%B over 30min
Flowrate:	1mL/min
Detection:	214nm
Column:	Vydac 214TP54 (5µm; 250 × 4.6mm)
t_R:	19.625 min

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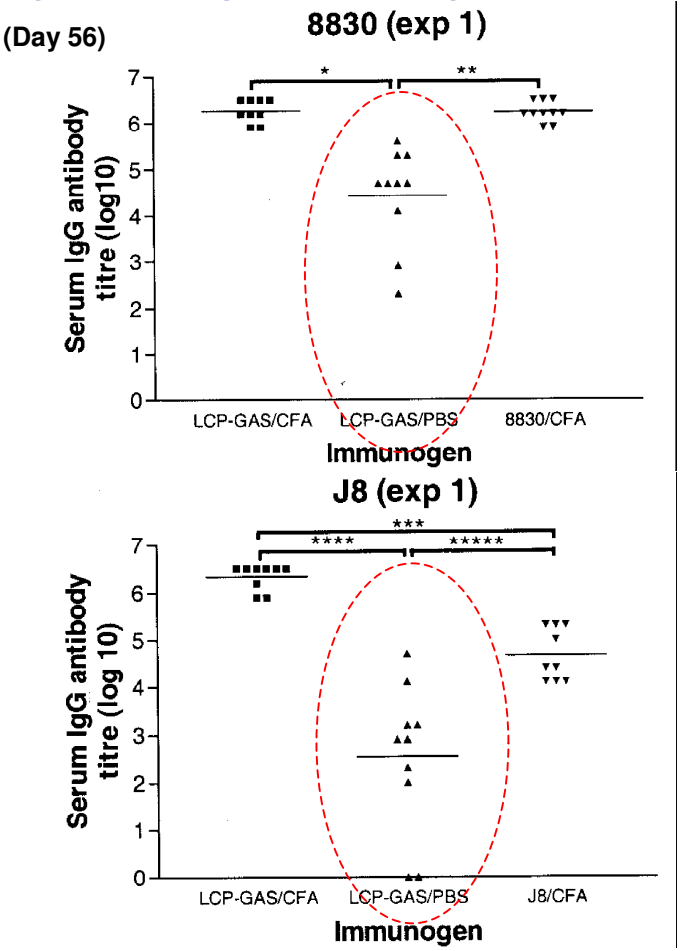
Infect Immun 2003;71:2373

LCP-88/30-J8

Systemic IgG Antibody Titers (ELISA)



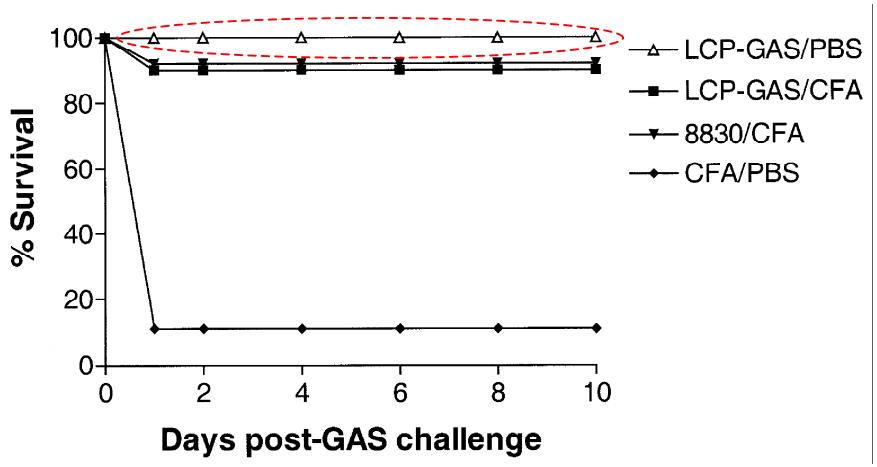
*, *P* 0.0001; **, *P* 0.0001; ***, *P* 0.0001; ****, *P* 0.0001; *****, *P* 0.002; ******, *P* 0.001.

Subcutaneous Immunization

B10.BR (H-2^K) mice (n=10) 4-6 week old female
Prime: 30µg LCP-88/30-J8 either 1:1 in CFA or in 50µL PBS
Boost: 3µg in PBS, days 21, 28, 35, 42, & 49

Intra-peritoneal Challenge

400µL (1 × 10^5 CFU/mL 88/30 GAS)



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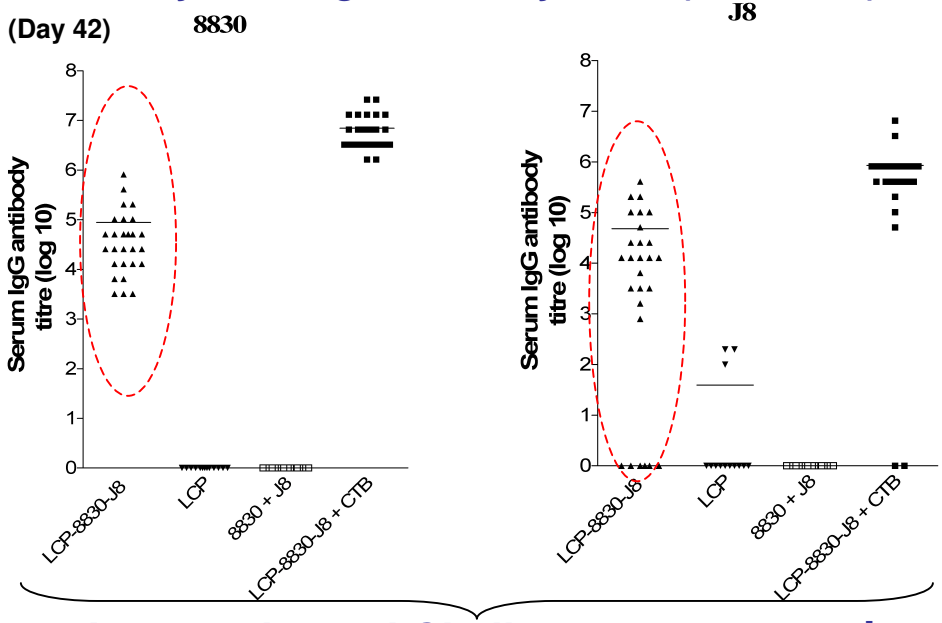
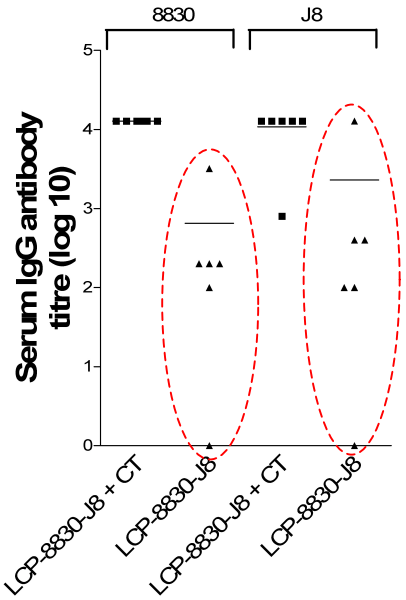
JID 2006;194:316

LCP-88/30-J8

Systemic IgG Antibody Titers (Intranasal)

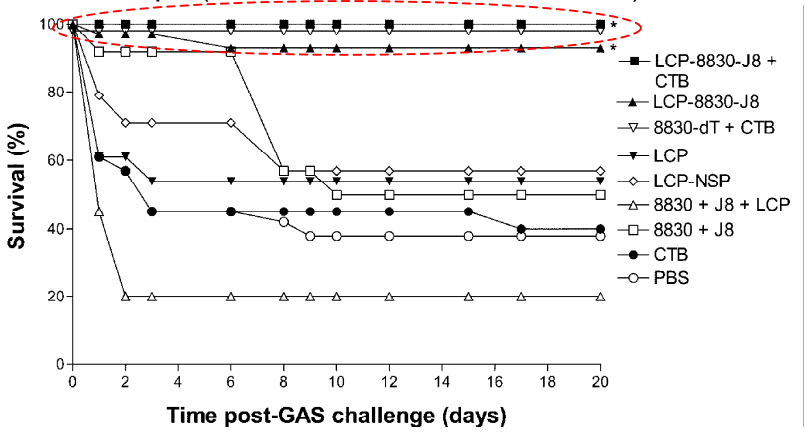


Systemic IgG Antibody Titers (Oral)



Intra-peritoneal Challenge

400µL (1-2 × 10^5 CFU/mL 88/30 GAS)



Intranasal Immunization

B10.BR (H-2^K) mice 4-6 week old female

Prime: 30-60µg LCP-88/30-J8
±10µg cholera toxin B-subunit in 20µL PBS

Boost: days 7, 14, 21, 28, 35

Oral Immunization

Prime: 100µg LCP-88/30-J8
±10µg cholera toxin in 0.2M sodium bicarbonate

Boost: weekly intervals; 5 (with CT) or 7 (without CT)

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Project Aims

Lipid Core Peptide System

Advantages:

- **High antibody (IgG) titers against attached peptides**
- **Comparable with the highly toxic adjuvant complete Freund's adjuvant (CFA)**
- **Potentially safe (non-toxic) for use in humans**

Disadvantages:

- **Difficult to purify**
- **∴not suitable for use in human clinical trials**

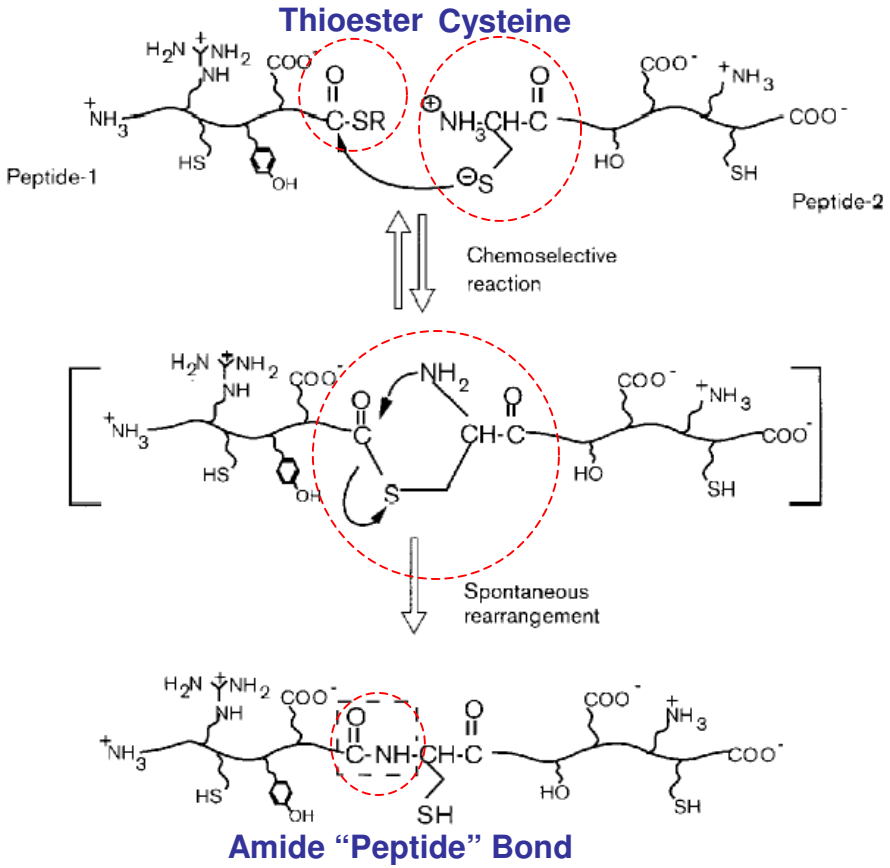
Project Aim:

- To develop a method to enable the synthesis of highly pure, easily characterized analogues of the lipid core peptide system

- Techniques to be assessed:
  - Solution- and solid-phase native chemical ligation
  - Fragment condensation

Native Chemical Ligation (NCL)

- Formation of “Native” peptide bond



Curr Opin Biotech 1998;9:412

C-terminal Peptide
- Contains N-terminal Cysteine

N-terminal Peptide
- Contains C-terminal Thioester

- Aqueous denaturing conditions
  - 6M Gdn.HCl
  - Urea
  - Phosphate buffer

- Performed at pH 7-8
  - Minimal side reactions

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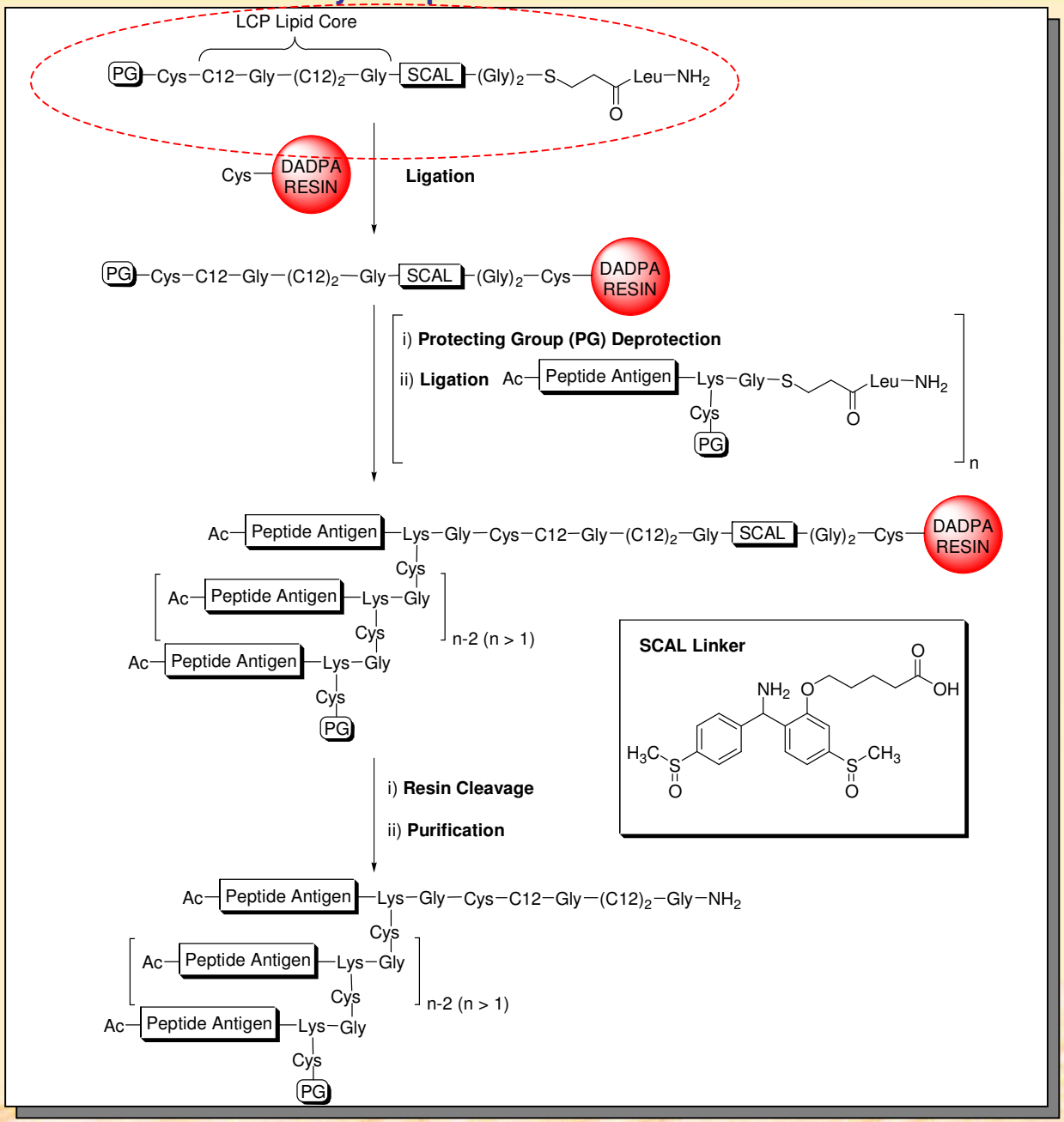
Poor solubility in aqueous buffers



Based on:

J Org Chem 2000;65(12):3829

Resin:

Diaminodipropylamine (DAPDA) derivatized 4% crosslinked agarose beads (16µmol NH_2/mL; Pierce Biotechnology, Rockford IL)

SCAL Linker:

Tetrahedron Lett 1991;32(31):3891

Boc-safety-catch acid labile linker (CSPS pharmaceuticals, San Diego CA)

<u>In oxidised (SO) form</u>:
Stable to TFA, HF, 50% piperidine, Pd(0)

<u>In reduced (S) form</u>:
Cleaved by 50% TFA

<u>Reducing agent</u>:
$SiCl_4$

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Problems with Solid-Phase NCL

Problems:

- **Poor solubility of lipid adjuvant in aqueous buffers**

- **Addition of organic solvents (e.g. TFE, MeCN, DMF, dioxane)**
  - **Solubilizes lipidic adjuvant**
  - **Ligation does not occur**

- **Need excess of thioester peptide to push ligation to completion (wasteful)**

- **Monitoring of ligation reactions and protecting group removals difficult**
  - **RP-HPLC provides some quantitative data**

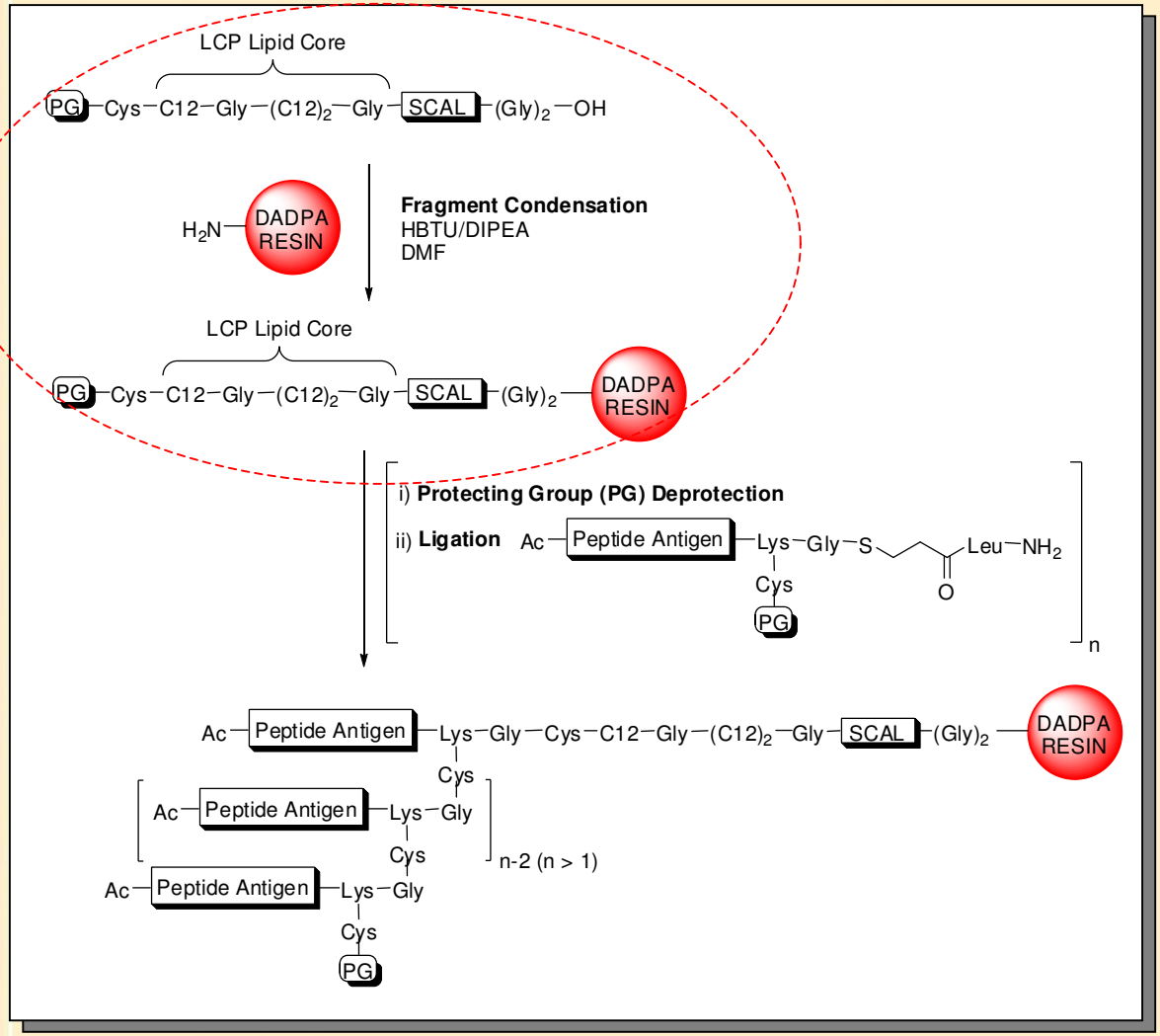
- **Cleavage of product from the resin is problematic**

- **The resin is not completely stable to the conditions used for ligation and protecting group removal**

Possible Solution to Solubility Issue:

- **Use fragment condensation to couple lipid adjuvant to resin, then use NCL to ligate immunogenic peptides**

Fragment Condensation



Fragment Condensation:

- Solubility in DMF of lipid adjuvant good.
- Coupling took over 24 hours

Native Chemical Ligation:

- Only 33% complete despite using 2eq thioester peptide
- Subsequent ligations worse

Conclusion:

- Difficult, expensive, and wasteful
- Use solution phase ligation

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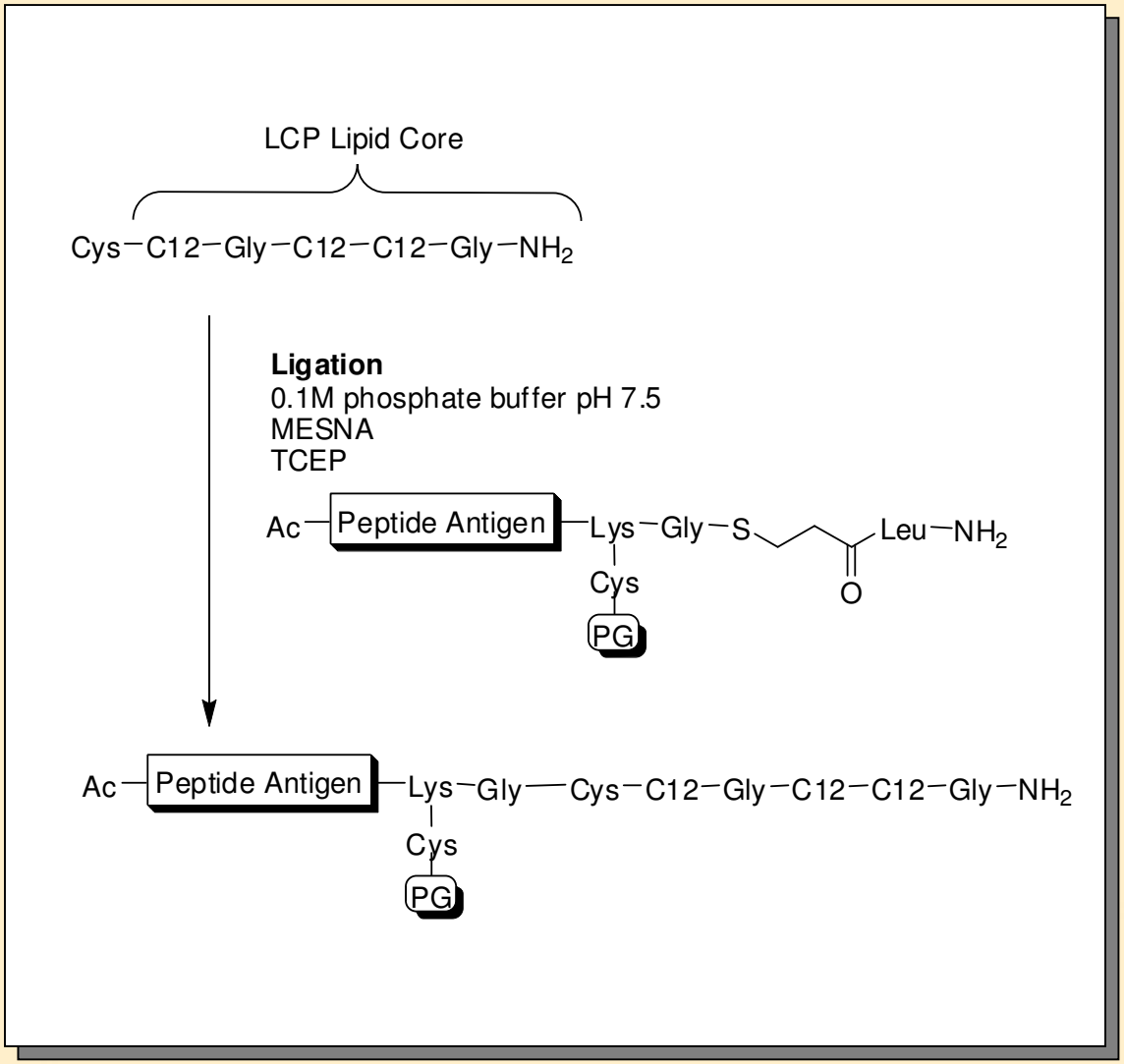
Solution-Phase NCL



Problems:

Poor solubility of lipid adjuvant in aqueous buffers

Ligation does not occur

Addition of organic solvents (e.g. TFE, MeCN, DMF, dioxane)

- Solubilizes lipidic adjuvant
- Ligation does not occur

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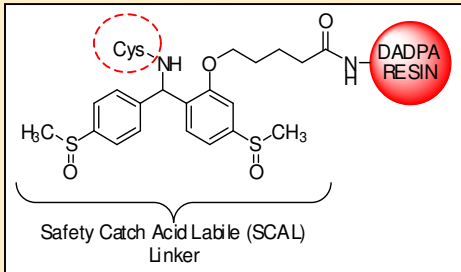
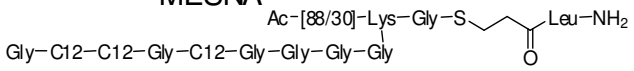
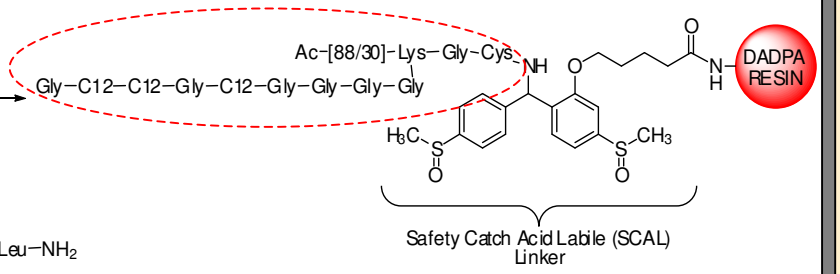
NCL + SDS



LIGATION

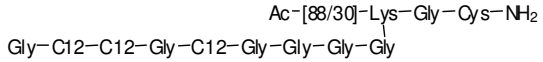
1% (w/v) SDS
0.1M phosphate buffer pH 7.5
TCEP
MESNA

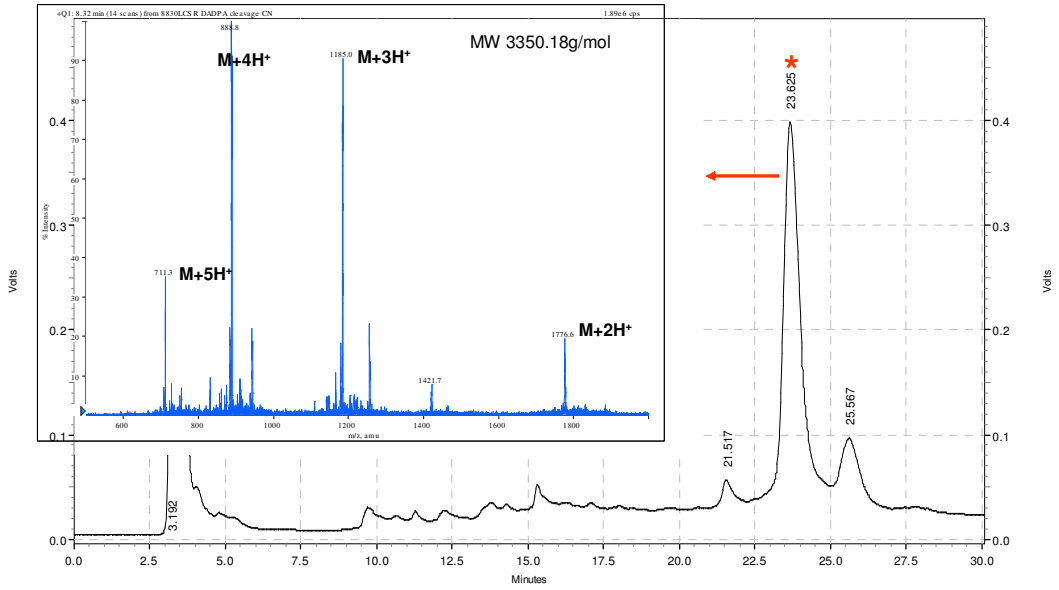




CLEAVAGE

TFA
Thioanisole
p-cresol
EDT
$SiCl_4$





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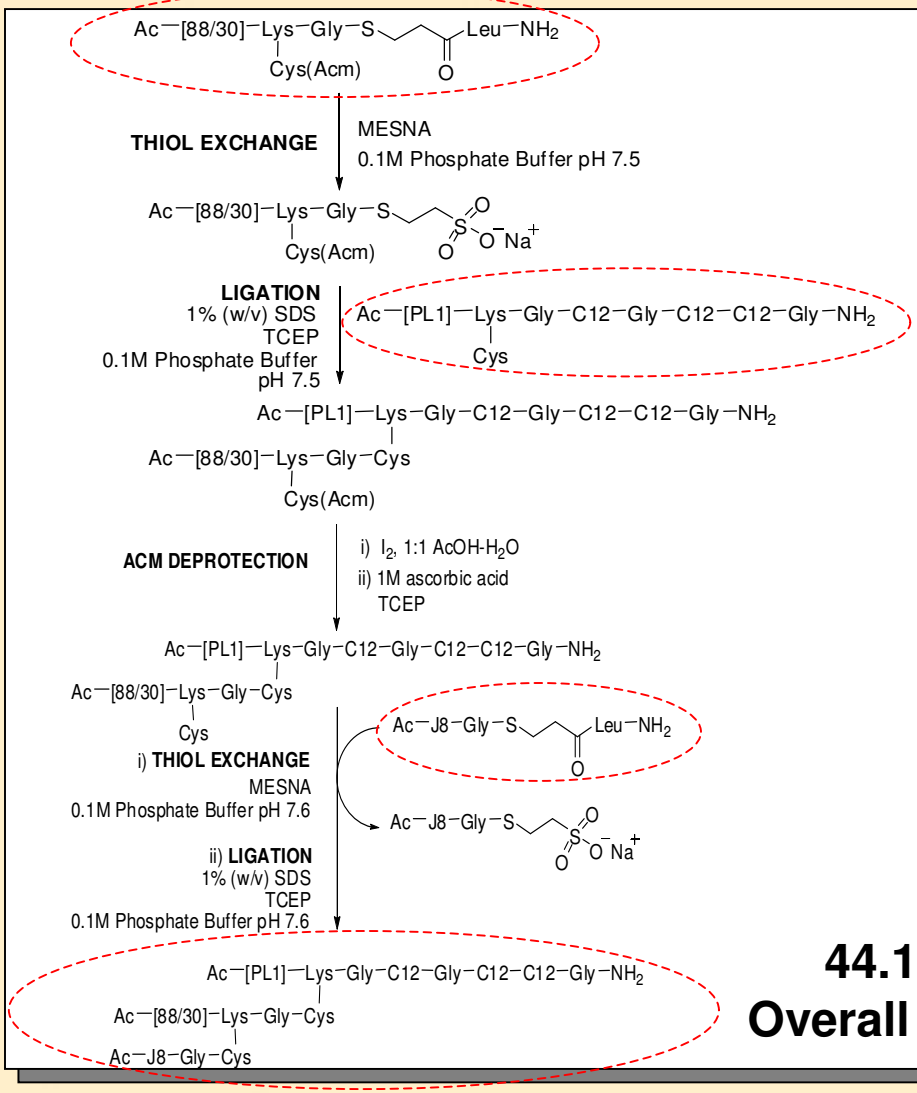
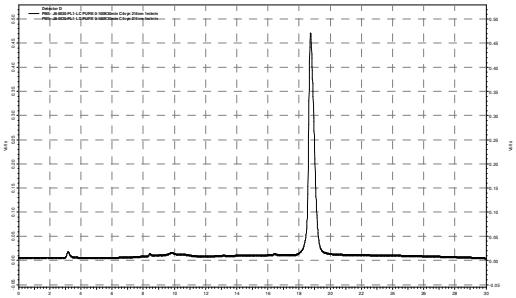
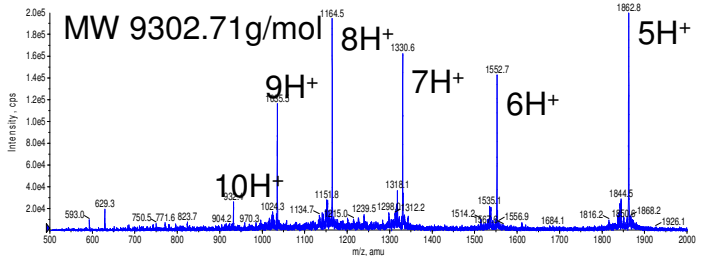
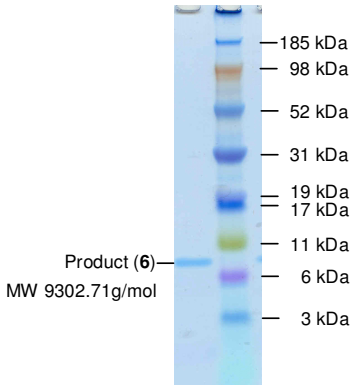
Synthesis of a Highly Pure LCP-Analogue









44.1%
Overall Yield

RP-HPLC: Solvent A: 0.1% TFA/H_2O; Solvent B: 90% ACN/0.1% TFA/H_2O; Flowrate: 1mL/min; Column: Vydac C4 (214TP54; 300Å; 5µm, 4.6 x 250mm); Gradient: 0% to 100% B over 30 min; Detection: 214nm; t_R: 18.733 min; Purity: 97.7%.

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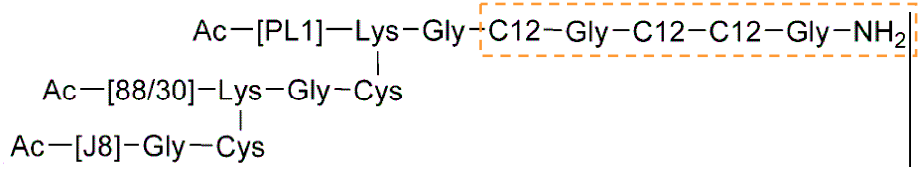
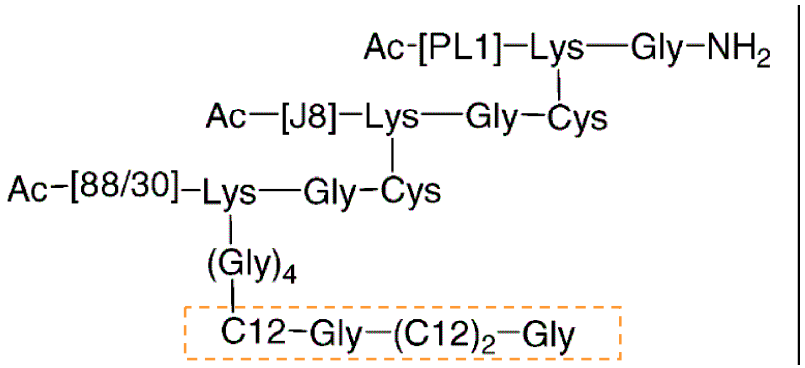
LCP-analogue 1



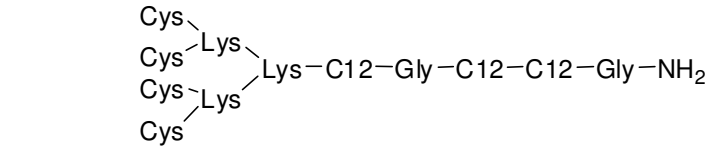
J Med Chem 2006;49(21):6364

LCP-analogue 2



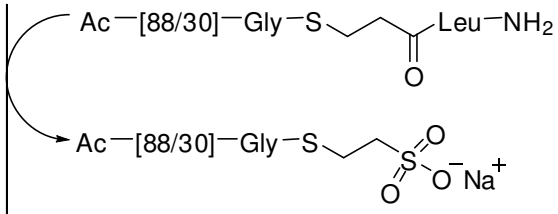
J Org Chem 2006;71(18):6846

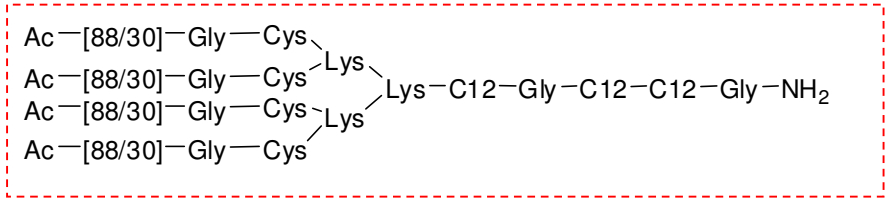
LCP-system

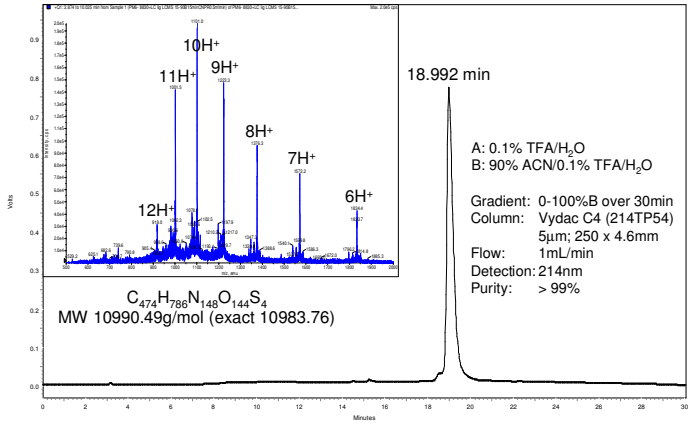


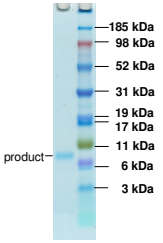
i) **THIOL EXCHANGE**
MESNA
0.1M Phosphate Buffer pH 7.6

ii) **LIGATION**
1% (w/v) SDS
TCEP
0.1M Phosphate Buffer pH 7.6

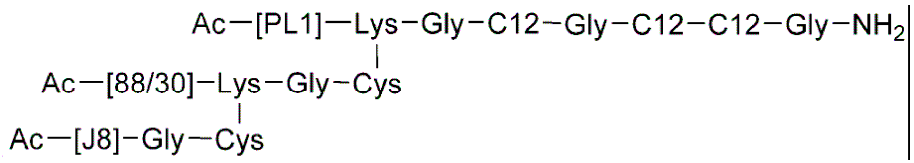


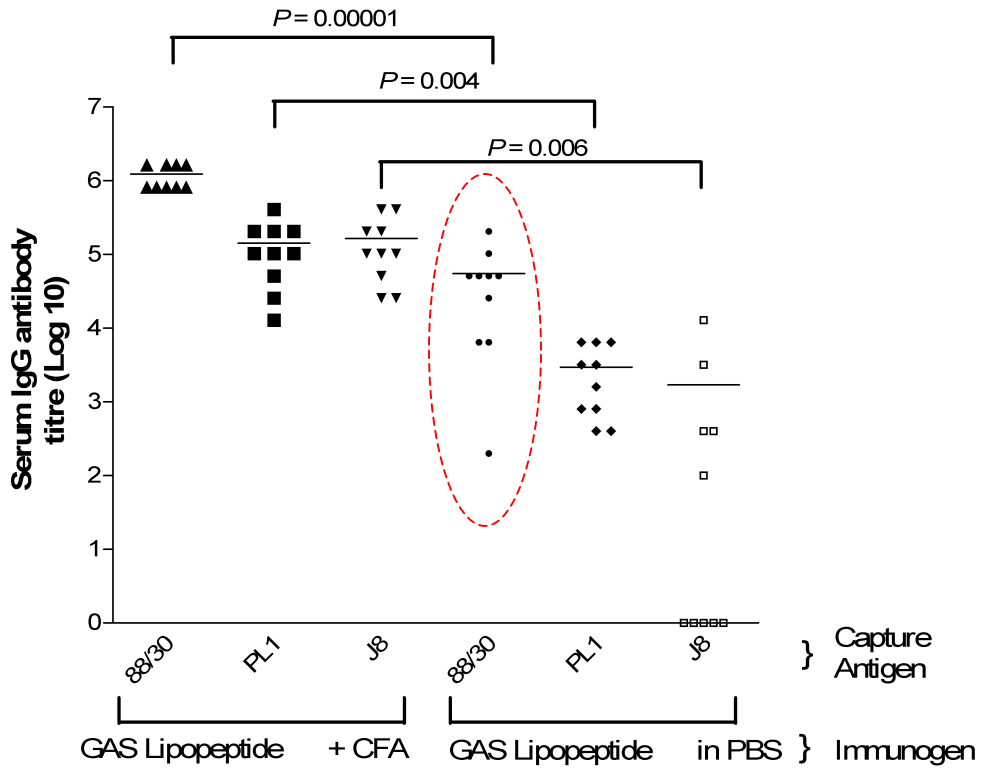


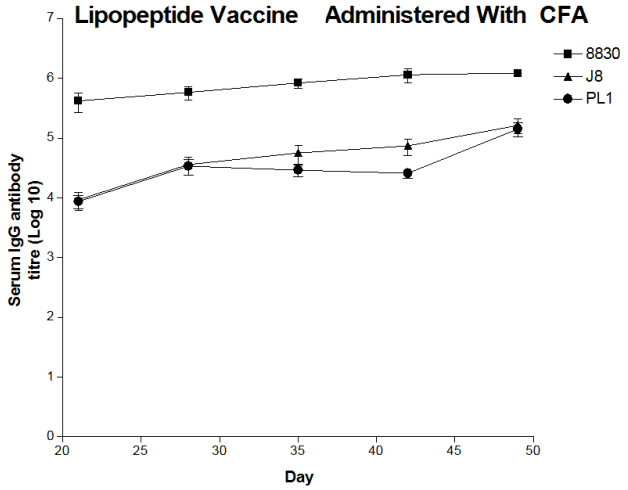


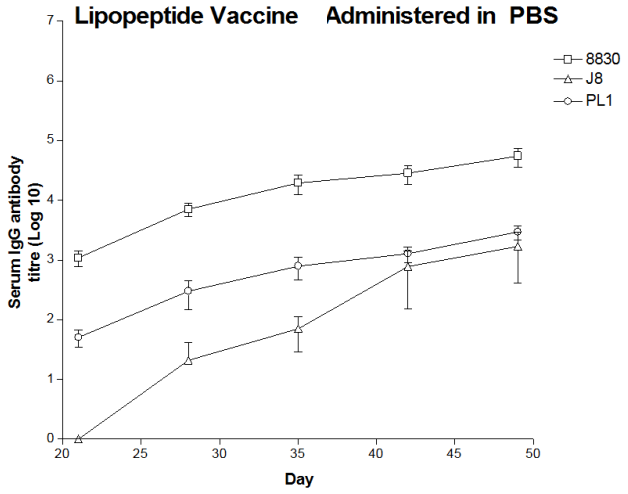


Subcutaneous Immunisation









Mice: 4-6 week old ♀ B10.BR (H-2^k)
Immunised at the tail base
1°: 30µg in 50µL PBS or 1:1 CFA
Boosts: 3µg in PBS (days 21, 28, 35, 42, & 49)

Conclusions

Photo of Brisbane River Skyline

- Demonstrated a method for the synthesis of highly pure, multi-epitopic, self-adjuvanting lipopeptide vaccines.
  - Required the use of SDS
- May prove useful for the synthesis of multi-epitopic vaccines against diseases caused by other microorganisms.

Acknowledgements

Queensland Institute of Medical Research

Dr Colleen Olive
Prof Michael Good.
Immunology experiments.

The University Of Queensland

Prof Istvan Toth
Dr Joanne Blanchfield
Toth group

Australian Government
National Health and Medical Research Council

Research Funding $$$

The Prince Charles Hospital Foundation

Research Funding $$$

Heart Foundation

Research Funding & Travel Grant $$$

Queensland Government

Research Funding $$$

This research is proudly supported by the Queensland Government's Growing the Smart State PhD Funding Program. The State of Queensland accepts no responsibility for decisions or actions resulting from any information supplied. The views and information contained in the research do not necessarily represent the views or opinions of the Queensland Government and carry no endorsement by the Queensland Government.