ISCHIA SEPTEMBER 2006 ROCHE LECTURE

BETTER SCIENCE THROUGH SYNTHESIS:

NEW MEDICINAL LEADS BASED ON PHARMACOPHORE TARGETING OF NEW REACTIONS AND ON NOVEL DRUG DELIVERY SYSTEMS

WENDER GROUP - STANFORD UNIVERSITY

Dept. of Chemistry(H&S); Dept of Molecular Pharmacology (Medical School); Molecular & Genetic Medicine; Cancer Biology; Clinical Oncology Quantitative Chemical Biology; Neurobiology; Epithelial Biology; Imaging Institute
SOME RELEVANT LEAD REFERENCES


Erin A. Clark, Bradley S. Davidson, Paul A. Wender, Susan L. Mooberry “Lauimalide and Synthetic Lauimalide Analogs are Synergistic with Paclitaxel and 2-Methoxyestradiol” Molecular Pharmaceutics 2006, 457-467.


Elena Goun, Rajesh Shinde, Karen Dehnert, Angie Adams-Bond, Paul Wender, Chris H. Contag, Benjamin L. Franc “Intracellular Cargo Delivery by an Octaarginine Transporter Adapted to Target Prostate Cancer Cells Though Cell Surface Protease Activation” Bioconjugate Chem. 2006, 17(3), 787-796.


Jonathan B. Rothbard, Sarah Garlington, Qin Lin, Thorsten Kirschberg, Erik Kreider, P. Leo McGrane, Paul A. Wender and Paul A. Khavari, Conjugation of Arginine Oligomers to Cyclosporin A Facilitates Topical Delivery and Inhibition of Inflammation, Nature Medicine 2000, 6, 1253-1257.
OUR RESEARCH FOCUSES ON...

*NOVEL STRUCTURES...
AS INSPIRATION FOR NEW REACTIONS,
CATALYSTS, STRATEGIES

*UNIQUE AND POTENT ACTIVITY, FUNCTION...
AS INSPIRATION FOR NEW THERAPEUTIC LEADS,
MODES OF ACTION, MATERIALS

*INTEGRATION OF CHEM, BIO, MED

**DESIGN**

NEW THERAPEUTIC LEADS
NEW MODES OF ACTION

**FUNCTION**

NEW REACTIONS,
CATALYSTS, STRATEGIES

**SYNTHESIS**

NEW DRUG DELIVERY SYSTEMS

**INPUT**

**OUTPUT**

APOPTOLIDIN

HIV TAT 9-MER

PHORBOL

TAXOL

BRYOSTATIN

RTX
TWO MAJOR PRIORITIES IN CHEMISTRY

FUNCTION ORIENTED SYNTHESIS

• MEDICINAL AGENTS
• MATERIALS
• CATALYSTS
• PROBES
• IMAGING TOOLS
• DIAGNOSTICS
• SENSORS
• NANODEVICES
• ENERGY COLLECTION
• ENERGY STORAGE
• ENERGY CONVERSION
• ENVIRONMENTAL

Wender, P.A.; Baryza, J.L.; Brenner, S.E.; Clarke, M.O.; Craske, M.L.; Horan, J.C.; Meyer, T.

• OVER 200 TOTAL SYNTHESES / YEAR IN ACS JOURNALS ALONE
• MAKING MOLECULES IS NO LONGER THE BIGGEST CHALLENGE IN SYNTHESIS
• THE MAJOR CHALLENGES NOW ARE TARGET DESIGN & SELECTION & ADVANCING SYNTHESIS TO MAKE SUCH TARGETS …

IN A PRACTICAL IF NOT IDEAL FASHION

Wender, Wright, Handy, *"Toward the Ideal Synthesis"* *Chemistry & Industry, 1997, 765.*
THE IDEAL SYNTHESIS AND STEP ECONOMY

- ONE STEP, 100 % YIELD
- READILY AVAILABLE STARTING MATERIALS
- OPERATIONALLY SIMPLE, SAFE AND ENVIRONMENTALLY SOUND
- RESOURCE (COST, TIME, MATERIAL, PERSONNEL) EFFECTIVE


WHY IS STEP ECONOMY SO IMPORTANT?

A 70 step synthesis, even if 100% selective & efficient, is still a 70 step synthesis. 70 steps take time, add cost, deplete resources, generate waste (solvent, etc)...

STEP ECONOMY:

REDUCES LENGTH, WASTE (SOLVENT, ATOM LOSS!!), ENVIRONMENTAL IMPACT, DEVELOPMENT & EXECUTION TIME, SEPARATION SCIENCE, EFFORT, COST;

IMPROVES YIELD, SPEED, SCIENTIFIC ADVANCEMENT, SAFETY, RETURN ON INVESTMENT, AND MOST IMPORTANTLY HUMAN ECONOMY

Wender, P.A.; Baryza, J.L.; Brenner, S.E.; Clarke, M.O.; Craske, M.L.; Horan, J.C.; Meyer, T.
**BRYOSTATIN: NOVEL STRUCTURE, UNIQUE FUNCTION**

**Underwater Treasures**
Doctors Searching for Potential Cancer Cures Beneath the Sea
*John McKenzie abc NEWS*
“A treasure chest of potential medicine lies in the sponges, algae and coral that live beneath the sea. And doctors think some of them may even help cure cancer…”

**Louis Piaroulli…**
his cancer was spreading through his bones and lymph nodes. Traditional chemotherapy had failed. Then, doctors tried again with the same chemotherapy drug, but they added **bryostatin**. Before adding **bryostatin**, Piaroulli's bones were riddled with cancer.

*Five months after the treatment, there was no trace of the disease.*

"The response was exceptional and dramatic, and we would not have anticipated this response from chemotherapy alone," says Schwartz (Memorial Sloan-Kettering).

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**BRYOSTATIN 1**

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BEYOND NATURAL PRODUCTS: DESIGN A BETTER TARGET

SIMPLER BUT FUNCTIONALLY SUPERIOR TARGETS = SHORTER (STEP ECONOMICAL) SYNTHESES

TOWARD A PRACTICAL (MANUFACTURING) SYNTHESIS OF “BYROLOGS”


(a) NaH, TBDMSCI, THF, rt; (b) (COCl)$_2$, DMSO, Et$_3$N, CH$_2$Cl$_2$, -78°C; (c) 4-chlorobutanol, MeMgCl, -78°C; Mg, THF, reflux; -78°C, THF; 75% 3 steps; (d) (COCl)$_2$, DMSO, Et$_3$N, CH$_2$Cl$_2$, -78°C; 75%; (e) 5 mol% Ti(Oi-Pr)$_4$, 10 mol% (R)-BINOL, B(Ome)$_3$, allylSnBu$_3$, 4 Å sieves, MePh; RT; 80%; (g) MMPP, NaHCO$_3$, CH$_2$Cl$_2$, MeOH, 0°C; 75%; (h) 0.1 eq. TPAP, 3 eq. NMO, 4 Å sieves, CH$_2$Cl$_2$, RT; 81%; (i) K$_2$CO$_3$, MeOH, (MeO)(OH)CHCO$_2$Me, -78°C; 80%; (j) CeCl$_3$$\cdot$7H$_2$O, NaBH$_4$, MeOH, -30°C; (k) C$_7$H$_5$CO$_2$H, DIC, DMAP, CH$_2$Cl$_2$, RT; 79% 2 steps; (l) HF/pyridine, THF, RT.; (m) Dess-Martin periodinane, NaHCO$_3$, CH$_2$Cl$_2$, RT; 79% 2 steps; (n) (DHQD)$_2$PYR, K$_2$OsO$_2$(OH)$_4$, K$_2$CO$_3$, K$_3$Fe(CN)$_6$, t-BuOH, H$_2$O, 0°C; 90%; (o) TESCl, pyr, CH$_2$Cl$_2$, rt; (p) 1-Br, 2-EtO-ethene, t-BuLi, ZnMe$_2$, -78°C; (q) HF, CH$_3$CN, H$_2$O, rt; (r) 1:3 TBSCl:imidazole, 9:1 CH$_2$Cl$_2$: DMF; 40% 4 steps.
TOWARD A PRACTICAL (MANUFACTURING) SYNTHESIS OF “BRYOLOGS”

Paul A. Wender, Alexander V. M. Mayweg, Christopher L. VanDeusen Organic Lett. 2003, 277-279

10 steps, 25% overall yield

\[ \text{Reagents and conditions: (a) LDA, 4-benzyloxy-2-butane, } -78 \, ^\circ\text{C, 10 min, 68%; (b) Ru-(S)-BINAPCl}_2, \text{ MeOH, H}_2, (95 \text{ atm}), 30 \, ^\circ\text{C, 78 h, 92% (97% BORSM); (c) silica, PhMe, 12 h, reflux, 95%; (d) TBDPSCl, imidazole, DMF, 2 h, 85%; (e) ethylacetateacetate, LDA (2 equiv), } -78 \, ^\circ\text{C; (f) Et}_3\text{SiH, TFA, } -30 \, ^\circ\text{C, 4 h, 70% over two steps; (g) Ru-(R)-BINAPCl}_2, \text{ EtOH, H}_2 \text{ (78 atm), } 96 \, ^\circ\text{C, 91%; (h) H}_2, \text{ Pd(OH)}_2\text{, Et}_2\text{O, 1 h, then LiBH}_4, \text{ 1 h, 96%; (i) 2,2-dimethoxypropane, TsOH, DMF, then silica, DCM, 4 h, 93%; (j) TEMPO, NaOCl, NaClO}_2, \text{ MeCN, 50 } ^\circ\text{C, 4 h, 92%}. \]
A PRACTICAL (MANUFACTURING) SYNTHESIS OF DESIGNED TARGET

**SPACER DOMAIN**

**RECOGNITION DOMAIN**

- LATE STAGE CONVERGENCY
- MILD MACROACETALIZATION
- COMBINATORIAL SYNTHESIS

BRYOSTATIN

Scale up in progress
BRYOLOG ~ 25 STEPS
(BRYOSTATIN > 70 STEPS)

REAL TIME EVALUATION OF PKC TRANSLOCATION

inactive (cytosol)  
translocation releases pseudosubstrate and activates protein  
active (membrane)

1. encode plasmid for each isozyme  
2. express each plasmid independently

plasmid  
transfection  
expression  
activation/translocation

Newton, Chem. Rev. 2001

J. Baryza, S. Brenner, M. Craske, T. Meyer, P. Wender  
Chemistry & Biology 2004, 1261.

508 nM  
488 nM

J. Baryza, S. Brenner, M. Craske, T. Meyer, P. Wender  
Chemistry & Biology 2004, 1261.
GROWTH INHIBITION IN HUMAN CANCER CELL LINES

Stanford / National Cancer Institute USA (Ven Narayanan)

Cell Sensitivity

SOME ANALOGS $10^{2-3}$ BETTER

log(bryoGI50) - log(analogGI50)
FUNCTION ORIENTED SYNTHESIS: BETTER, AVAILABLE LEADS

STEP ECONOMY

>70 STEPS  \rightarrow  <30 STEPS


- **BRYOLOG AND BRYOSTATIN HAVE SIMILAR VIRTUAL STRUCTURES, SOLUTION STRUCTURES, PKC AFFINITIES**
- **BRYOLOGS ARE GENERALLY MORE POTENT (10-100 FOLD) THAN BRYOSTATIN IN HUMAN CANCER CELL GROWTH INHIBITION**
- **BRYOLOGS ARE COMPARABLE OR BETTER THAN BRYOSTATIN IN ANIMAL ASSAYS**
- **PICOLOG SYNTHESIS < 30 STEPS, ~ $3K/gm (BRYO $2.3M/ gm); NOW IN SCALE UP**
  
  
  **PRE-CLINICAL DEVELOPMENT CANDIDATE**
STEP ECONOMY AND FUNCTION ORIENTED SYNTHESIS:


---

**NEW RXNS**

---

**TARGET COMPLEXITY [VALUE]**

---

**# OF STEPS**

---

**IMPRACTICAL**

---

**PRACTICAL**

---

**IDEAL**

---

**FOS**

---

**DESIGNED FUNCTIONAL ANALOG**

---

**target**

---

**WTARGET**

---

**O**

---

**N**

---

**NEW RXNS**

---

**WILLSTÄTTER & WASER 1911**

---

**Ni (0) cat**

---

**70%**

---

**REPPE & TEOPEL 1940**

---

**13 steps**

---

**1-2%**

---

**NOW…COMBINE NEW REACTIONS AND FOS…**

---

**INVENT NEW FUNCTIONAL TARGETS WITH NEW RXNS**
PHARMACOPHORE TARGETING WITH NEW REACTIONS

The virtual medicinal chemist

>70 steps

<25 steps

DESIGN
HIGH
THROUGHPUT
IN SILICO

FUNCTION
H-T
SCREENS

SYNTHESIS
COMBI
CHEM

INPUT

OUTPUT

SELECTIVE INHIBITORS AS MEDICINAL LEADS

Real time cell & animal assays

New reactions novel structures

• STEP ECONOMY TO KNOWN SCAFFOLDS
• STEP ECONOMY TO NEW SCAFFOLDS

NEXT LEVEL?

SIMPLER FASTER

• FOS
• FOS

NEXT LEVEL?
DESIGNING NEW REACTIONS FOR UNMET NEEDS

THE DIELS-ALDER CYCLOADDITION: A POWERFUL PROCESS FOR 6-MEMBERED RINGS

\[ \text{[4+2]} \rightarrow \text{[5+2]} \]

**DESIGNING NEW REACTIONS: THE [5+2] CYCLOADDITION**


* THERMAL REACTION DOES NOT WORK

![HEAT ALONE](image)

Eₐ > 50 kcal/mol

* THEREFORE, SELECT SUITABLE METAL TO MEDIATE \[\pi\]-SYSTEM ACTIVATION (MANY METALS REACT WITH CPs and / or VCPs; must suppress 2+2+2, VCP to CP)

![Diagram of reaction pathway](image)

A NEW REACTION: TRANSITION METAL CATALYZED [5+2] CYCLOADDITIONS


\[
\begin{align*}
\text{R} & \rightarrow \text{O} \\
\text{R} & = \text{H, Me, CO}_2\text{Me, TMS, Ph} \\
\end{align*}
\]

1-10 mol % Rh(PPh\(_3\))\(_3\)Cl
1-10 mol % AgOTf

R= H, Me, CO\(_2\)Me, TMS, Ph

80-95 %


\[
\begin{align*}
\text{MeO}_2\text{C} & \rightarrow \text{MeO}_2\text{C} \\
\text{MeO}_2\text{C} & \rightarrow \text{MeO}_2\text{C} \\
\text{R} & = \text{H, Me} \\
\end{align*}
\]

0.1 mol % RhCl(PPh\(_3\))\(_3\)
0.1 mol% AgOTf

1.0M, PhMe, 110°C, 17h

R= H, Me

86-91%, only cis
(exo selective)

* ALLENES (Wender; Glorius; Husfeld; Langkopf; Love J. Am. Chem. Soc., 1999, 5348; Organic Lett. 2000, 2323)

\[
\begin{align*}
\text{E} & \rightarrow \text{E} \\
\text{E} & \rightarrow \text{E} \\
\text{t-Bu} & \rightarrow \text{H} \\
\text{H} & \rightarrow \text{H} \\
\end{align*}
\]

1 mol % CAT

PhCH\(_3\), 100 °C
3 h

91% ee

96%, only cis

92% ee
**IT IS REMARKABLY GENERAL** (1-3hr @ 25-40°C for most alkynes)

**ONE MOLE SCALE**

5 mol % [Rh (CO)₂Cl]₂, CDCl₃, 40 °C; H⁺

**ALKYNYL ESTERS**

93 %

**INTERNAL AND TERMINAL ALKYNES**

88 %

**ALKYNYL KETONES**

88 %

**EVEN ACETYLENE**

79 %
CC ACTIVATION: FROM VCPs to VCBs & A NEW REACTION


FIRST [6+2] CYCLOADDITIONS

\[ \text{catalyst} \]

110°C, tol

R1=R2=H  
95%  A, 3h  10 mol % RhCl(PPh₃)₃, 10 mol % AgOTf

R1=H, R2=Me  
78%  B, 20h  10 mol % RhCl(CO)(PPh₃)₂, 10 mol % AgOTf

NEW REACTIONS INSPIRING OTHER NEW REACTIONS:

Mechanism: Wender; Correa; Sato; Sun J. Am. Chem. Soc. 2000, 7815:

\[ \text{5 mol % [Rh(CO)₂Cl]₂, 10 mol % P(n-Bu)₃, 10 mol % AgOTf} \]

86%  
-8%

8%
A NEW THREE COMPONENT CYCLOADDITION:
TRANSITION METAL CATALYZED [5+2+1] CYCLOADDITIONS

THUS FAR, [4+4], [4+2], [5+2], AND [6+2] CYCLOADDITIONS, OVERALL [M+N] CYCLOADDITIONS. COULD A 3rd COMPONENT OF 1, 2, etc ATOMS BE ADDED TO ACHIEVE [M+N+O] CYCLOADDITIONS?

NEW REACTIONS BEGET OTHER NEW REACTIONS

[5+2]

[6+2]
A NEW, THREE COMPONENT CYCLOADDITION:
TRANSITION METAL CATALYZED [5+2+1] CYCLOADDITIONS


1-5 mol% [Rh(CO)₂Cl]₂, dioxane, 60°C, 1-2 atm CO, conc. up to 0.5M;
Works with esters, amides, aldehydes, ketones; yields good to excellent; high regioselectivity

NEW SCAFFOLDS

1 mol% [Rh(I) cat.]

100%
Representative New TM Catalyzed Reactions...New Strategies...New Structures...
Intermolecular [5+2]/[4+2] cycloadditions (2001)
Intra/intermolecular [5+2]/[4+2] cycloadditions
Intermolecular [5+2+1] cycloaddition of VCPs, alkenes, alkenes (2003)
Intermolecular [5+1+2+1] cycloaddition of VCPs, alkynes, 2 CO (2005)
Carbonylative ring expansion [4+2+1]/[6+1] of allenylcyclobutanes (2006)
The allenyl Pauson-Khand [2+2+1] cycloaddition of bis-alkenes, CO (2006)
Intermolecular [3+2] cycloadditions (unpub)

Can we exploit these new reactions to rapidly assemble new or novel FUNCTIONAL molecules
GRAND CHALLENGES: THE BARRIER PROBLEM

- Genomics (blueprint)
- Proteomics (targets)
- Systems Biology (pathways)
- Chemogenomics (ligands)

A CHALLENGE AHEAD...
THERAPY DEPENDS ON BREACHING BARRIERS...

DRUG / PROBE TRANSPORTER

BREACHING BIOLOGICAL BARRIERS

THE PROBLEM: MOST DRUGS MUST BE BOTH WATER & LIPID SOLUBLE FOR PASSIVE DIFFUSION INTO TISSUES AND CELLS

THINKING OUT OF THE log P BOX AND PHYSICAL PROPERTY HOMOGENEITY

TOO POLAR (LIPID BILAYER PROBLEMS)
e.g., RNAi

TOO NON-POLAR (WATER SOLUBILITY PROBLEMS)
e.g., TAXOL

JUST RIGHT

WATER SOLUBLE
MEMBRANE IMPERMEABLE

THE LOG P BOX

BARRIER PROBLEMS ARE A MAJOR CAUSE OF HIT-TO-LAUNCH ATTRITION
A POTENTIAL FIX: MOLECULAR TRANSPORTERS
HIV tat and Antennapedia are transcription factors that cross biological membranes

\[
\text{MEPVDPRLEPWKHPGSQPKTACTTCYCKKCCFHCQVCFTTKALG}
\]

\[
\text{ISYG}^{\text{RKKRRQRRR}} \text{PPQGSQTHQVSLSKQPTSQP}
\]

\[
\text{RGDPTGPKE*KKKVERETETDPFD}
\]

* HIV tat 49-57 is required for translocation (Frankel, Pabo 1988)

SIGNIFICANTLY, IT IS CHARGED BUT EXHIBITS FACILITATED UPTAKE

arginine-lysine-lysine-arginine-arginine-glutamine-arginine-arginine-arginine-arginine

* This works for the HIV tat protein, the “gold standard” in research
  - it would have limited use in therapy (cost of goods, metabolism, etc) and
  - it is not necessarily an optimized system

* Design a better transporter; what’s required in tat?
  Stanford (Engleman, Fathman, Kiley, Rothbard, Wender) -> CellGate
CHEMICAL CODES FOR CELLULAR UPTAKE

Uptake of Peptide & Peptoid-aca-FITC conjugates into Human Jurkat T Cells

SUPERIOR SYSTEMS AND 
COST OF GOODS*

CONFOCAL ANALYSIS 
HEAD GROUP TYPE & 
TRANSPORTER LENGTH

BACKBONE STEREOCHEMISTRY 
& POSITION (PEPTOIDS)

Mean Fluorescence

NOT SIMPLY CHARGE

0 1000

Tat 49-57

K9 R5 R6 R7 R8 R9 R15 R20 R25 R30

UPTAKE

Mean Fluorescence

0 D

Tat 49-57 R7 R9 r7 r9 N-hex7 N-hex8 N-hex9

UPTAKE

* Wender, Jessop, Pattabiraman, Pelkey, VanDeusen Organic Letters 2001, 322
STEP ECONOMY THROUGH SYNTHESIS AND FUNCTION GUIDED DESIGN


**LINEAR:** TARGET OF n UNITS REQUIRES 2n STEPS; THUS EVERY 2 STEPS ADD 1 UNIT

1. Activate
2. Couple

Repeat:

7. Make acid
8. Make amine
9. Couple

16 OPERATIONS OVERALL

\[
\begin{align*}
&\text{G} \quad \text{G} \quad \text{G}_2 \quad \text{G}_3 \quad \text{G}_4 \quad \text{G}_5 \quad \text{G}_6 \quad \text{G}_7 \quad \text{G}_8 \\
&\text{G} \quad \text{G}_2 \quad \text{G}_3 \quad \text{G}_4 \quad \text{G}_5 \quad \text{G}_6 \quad \text{G}_7 \quad \text{G}_8
\end{align*}
\]

**SEGMENT DOUBLING:** A TARGET OF 2^n UNITS REQUIRES 3n STEPS
THUS AN 8-MER (n=3) REQUIRES ONLY 9 STEPS; THUS EVERY 3 STEPS DOUBLE THE SIZE!

\[
\begin{align*}
&\text{1. } \text{Activate} \\
&\text{2. } \text{Couple} \\
&\text{3. } \text{BocNHRCO}_2\text{H} \\
&\text{4. } \text{BocNHRCONHRCONHRCO}_2\text{H} \\
&\text{5. } \text{HCl•NH}_2\text{RCO}_2\text{Bn} \\
&\text{6. } \text{BocNHRCONHRCONHRCO}_2\text{Bn} \\
&\text{7. Make acid} \\
&\text{8. Make amine} \\
&\text{9. Couple}
\end{align*}
\]

9 OPERATIONS OVERALL
NO RESIN, SOLN. SCALABLE
COST SAVINGS >100FOLD;
GMP SCALED IN US PHARMA

MOLECULAR TRANSPORTERS FOR CELLULAR UPTAKE

OLIGOARGININE TRANSPORTERS
*Nature Medicine* 2000, 1253

PEPTOID TRANSPORTERS
*Proc. Natl. Acad. Sci. USA* 2000, 13003

OLIGOCARBAMATE TRANSPORTERS

OLIGOARGININE TRANSPORTERS

**RXRXRXXRXXRXR**

SPACED ARG-TRANSPORTERS

ARBOREAL DENDRIMER TRANSPORTERS
*Organic Lett.* 2005, 4815

AND OTHERS...
The bilayer provides the basic structure of the membrane and serves as a relatively impermeable barrier to the flow of water soluble molecules. Variations of this statement are found in most standard textbooks.
WHY ARGinine AND NOT LYSINE?

ARGININE

LYSINE

EXTRACELLULAR POLAR MILIEU

LESS POLAR COMPLEX

EXTRACELLULAR POLAR MILIEU

LESS STABLE COMPLEX


ARE HYDROGEN BONDS THAT IMPORTANT?
TRANSPORTER-PEPTIDE CARGO UPTAKE INTO HEART TISSUE

PRECONDITIONING: BRIEF EPISODES OF ISCHEMIA DECREASE NECROSIS DURING PROLONGED ISCHEMIA
RACK PEPTIDES SIMULATE PRECONDITIONING WHEN INJECTED INTO CELLS BUT CANNOT ENTER BY DIFFUSION

PEPTIDE DELIVERY TO PREVENT ISCHEMIC DAMAGE
L. Chen, L. Wright, C-H. Chen, S. F. Oliver, P. A. Wender,* D. Mochly-Rosen*
CHEMISTRY & BIOLOGY, 2001, 1123

\[
\text{RACK-TRANSPORTER CONJUGATE} \quad \xrightarrow{\text{HO}_2\text{C-DYGIPDAHC-SS-CR}_7\text{-CONH}_2} \quad \text{RACK PEPTIDE} \quad \text{TRANSPORTER}
\]

\[
\text{HO}_2\text{C-DYGIPDAHC-SH} + \text{HS-CR}_7\text{-CONH}_2
\]

- THE RACK PEPTIDE ITSELF MUST BE MICROINJECTED INTO CELLS
- TRANSPORTER RACK CONJUGATE WORKS WITHOUT INJECTION

BASIS FOR NEW COMPANY, 2003

L. Chen, L. Wright, C-H. Chen, S. F. Oliver, P. A. Wender,* D. Mochly-Rosen*
CHEMISTRY & BIOLOGY, 2001, 1123
**TRANSPORTER ENABLED DRUG UPTAKE IN HUMAN SKIN**

Paul Wender, Dennis Mitchell, Kanaka Pattabiraman, Erin Pelkey, Lawrence Steinman, Jonathan Rothbard

UPTAKE OF BIOTINYLATED CYCLOSPORIN (A) AND BIOTINYLATED CYCLOSPORIN TRANSPORTER CONJUGATE (C) IN HUMAN SKIN GRAFTED ON IMMUNE DEFICIENT MICE. PANELS B AND D ARE CONTROLS USING PROPIDIUM IODIDE TO VISUALIZE ALL CELLS.

HUMAN TRIALS ESTABLISHED SAFETY AND THERAPEUTIC LEVELS OF CONJUGATE: T-L-CsA -> T-L + CsA (pH based release)

**ASSAY NEEDED TO QUANTIFY RELEASE IN ANIMALS**
REAL TIME QUANTIFICATION OF TISSUE PENETRATION, CELL UPTAKE, RELEASE & INTRACELLULAR FUNCTION IN TRANSGENIC MICE


Luc-RL-Trans (Luciferin-Releasable Linker-Transporter)

- Stratum corneum
- Animals can be reused!
- Luc-RL-Trans
  - Cell membrane
  - Intracellular release
- Luc + RL + Trans
  - Luciferase
  - Light

![Bioluminescence and Fluorescence Images](image)

Animals can be reused!
DESIGN STRATEGY FOR REAL TIME DERMAL UPTAKE

SYNTHESIS OF NEW BIO-RELEASABLE CONJUGATES


R8-CARBONATE 5 SYNTHESIS: 3 STEPS, OVERALL YIELD 40%

1. 2-aldithiol, MeOH, 97%; 2. i) Phosgene, pyr, tol, ii) Luciferin, NaOH, H₂O, 70%; 3. AcHN-Cys-arg8-CONH₂, DMF, 59%

• C₆₇H₁₂₄N₃₆O₁₅S₄
• NO PROTECTING GROUPS
• GENERAL AND SCALABLE
REAL TIME QUANTIFICATION OF CELL UPTAKE IN TRANSFECTED PC3M CELLS

**Luc-RL(SS)-Trans**
- **Cell Entry**
- **GS (in cell)**
- **Luc + RLSH + HS-Trans**
- **Luciferase**
- **Light**

**POINTS OF IMPORTANCE:**
- **UPTAKE & RELEASE ARE RAPID**
- **UPTAKE/RELEASE DOSE DEPENDENT**
- **UPTAKE IS INHIBITED BY K+**
- **UPTAKE/RELEASE MAX. IN MINUTES**
- **SIGNAL FROM CONJUGATE IS ~ ALL FROM BIORELEASE**
- **UPTAKE/RELEASE IS REPRODUCIBLE**
- **UPTAKE OF LUCIFERIN SHOWS DIFFERENT KINETICS**

---

**Luciferin-RL(SS)-conjugate**

- **Flux (photons/second)**
- **Time (seconds)**

**Luciferin**

- **Flux (photons/second)**
- **Time (seconds)**

FUNCTION ORIENTED SYNTHESIS

• MEDICINAL AGENTS  • IMAGING TOOLS  • ENERGY COLLECTION
• MATERIALS  • DIAGNOSTICS  • ENERGY STORAGE
• CATALYSTS  • SENSORS  • ENERGY CONVERSION
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