New Opportunities For Small Molecules

David L. Waller
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Frontiers Of Chemistry
Bondmaking: Then and Now

- The first preparation of an organic substance (1828)...

\[ \text{AgOCN} + \text{NH}_4\text{Cl} \rightarrow \text{H}_2\text{O} \rightarrow \text{H}_2\text{N} - \text{C} = \text{O} - \text{NH}_2 \]

“I must tell you that I can make urea without the use of kidneys”
F. Wöhler (1828)

… is still tactically and strategically in use today.

\[ \text{OTBDMS-} \text{NH}_2 \underset{\text{Ag(OCN)}}{\rightarrow} \text{NH} \text{COCl} \rightarrow \text{MeO} - \text{MeO} - \text{NH} - \text{CO} \text{MeO} \text{OTBDMS} \text{OTBDMS} \]


**Where is chemical synthesis going, and where can it take us?**

Friedrich Wöhler
Ordinary Professor of Chemistry
University of Göttingen, 1836 - 1882

David Waller @ Wipf Group
I. Into The Genome: DNA-Templated Discovery
DNA-Templated Synthesis: Fundamental Reactions and Regimes

- Pairing of complimentary DNA oligonucleotides: “substrate (template)” and “reagent”
- Pair in either in end-on mode (E) or hairpin mode (H)

Reaction Conditions:
P pH 7.5, 25 °C, 250 mM NaCl
Templates in 1:1 stoichiometry at 60 nM

- Designed “mismatches” in base pairing fail to undergo reaction.

- Reactions rates were similar between H and E architectures ($K \approx 10^5$ M$^{-1}$s$^{-1}$)


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DNA Templated Synthesis: Functional Group Compatibility

- H or E Template
- Thiol (S) or amine (N) nucleophile
- Matched (M) or mismatched (X) pairing

- $S_N$, $\alpha,\beta$-additions, and vinyl sulfone addition compatible with technology.

- Matched cases only proceeded to products efficiently, despite large differences in transition states, steric hindrance and conformational flexibility.

- Reactions with a single mismatch were 200 fold slower and could be eliminated by heating reaction above the estimated melting temperature.

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DNA-Templated Reactions: Distance Effects

- Distance of reacting components is not important.

- Designed reactants with 2-30 bases between reacted 10^4 – 10^5 times faster than for untemplated reactions.

- At a 30 base distance, product formation proceeds through a transition state resembling a 200-membered ring.

- Decreasing the concentration of the reactants dramatically slowed the reactions, indicating that DNA annealing is rate limiting.
DNA-Templated Synthesis: “Coding” for Multistep Synthesis

- Each “codon” on the template compliments a region on the reagent strand.
- Excess reacted/unreacted reagent is removed by biotin/streptavidin affinity removal.
- Each step yields between 52 and 85%.
- As little as $10^{-18}$ mol of product can be amplified by PCR (bottom right), allowing, in theory, “selection”.

![Diagram of DNA-Templated Synthesis](image-url)
DNA-Templated Synthesis: The “Evolution” of Small Molecules

Liu and Co-workers Science 2004, 305, 1601
DNA-Templated Synthesis: The “Evolution” of Small Molecules

- Engineer each substrate with a DNA sequence that codes for the order or type of reagent to react with.

- pH changes effect “deprotection” to enable reagent by-products to be removed.

- Final macrocyclization is Wittig olefination.

- “Selection” would give amplifiable DNA whose sequence would reveal structure of active binding molecule.
DNA-Templated Synthesis: “Selection” By Protein Affinity

A 65 member library of macrocycles is generated from the possible combinations of building blocks and reagents.

-100 fmol of each is assayed for binding to carbonic anhydrase, a well-characterized protein.

-Carbonic anhydrase is immobilized and incubated with the library (x2).

-Following “selection”, the DNA corresponding to the bound molecules is amplified and sequenced to reveal the identity of the binding molecule.

-In this case, 8e uniquely binds with carbonic anhydrase and was selected from a 65 member library.

DNA-Templated Synthesis: Reaction Discovery

-DNA-templating technology can, in principle, be used for reaction discovery.
-The ultra-small scale and amplification technologies can make the process extremely compact.

DNA-Templated Synthesis: Reaction Discovery

DNA-Templated Reaction Discovery

-A nanomole of substrate allows for evaluation of more than 168,000 reaction conditions.

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DNA-Templated Synthesis: Reaction Discovery


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DNA-Templated Synthesis: Reaction Discovery

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Green/red fluorescence ratios</th>
<th>DNA-templated yields (%)</th>
<th>Product consistent with observed mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°C</td>
<td>25°C</td>
<td>37°C</td>
</tr>
<tr>
<td>A5</td>
<td>2.7</td>
<td>3.7</td>
<td>35</td>
</tr>
<tr>
<td>A5</td>
<td>3.5</td>
<td>3.1</td>
<td>28</td>
</tr>
<tr>
<td>A5</td>
<td>1.6</td>
<td>1.9</td>
<td>36</td>
</tr>
<tr>
<td>A5</td>
<td>2.6</td>
<td>2.7</td>
<td>45</td>
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<tr>
<td>A4</td>
<td>3.0</td>
<td>2.8</td>
<td>57</td>
</tr>
<tr>
<td>A8</td>
<td>1.8</td>
<td>&lt;1.2</td>
<td>30</td>
</tr>
<tr>
<td>A8</td>
<td>1.8</td>
<td>&lt;1.2</td>
<td>19</td>
</tr>
<tr>
<td>A7</td>
<td>3.6</td>
<td>&lt;1.2</td>
<td>39</td>
</tr>
</tbody>
</table>


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DNA-Templated Synthesis: Reaction Discovery

-DNA-templated reaction proceeds well on large scale and is catalytic in Pd with the presence of an oxidant.

-Represents a fmol conversion on DNA to a scalable, mild bond formation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Metal(s)</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Isolated yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1 equiv. Na₂PdCl₄</td>
<td>1 M NaCl in H₂O</td>
<td>25 °C, 15 h</td>
<td>86%</td>
</tr>
<tr>
<td>b</td>
<td>5 mol% Na₂PdCl₄, 1 equiv. CuCl₂</td>
<td>100 mM NaCl in H₂O</td>
<td>25 °C, 2 h</td>
<td>90%</td>
</tr>
<tr>
<td>c</td>
<td>5 mol% Na₂PdCl₄, 1 equiv. CuCl₂</td>
<td>9:1 THF: H₂O</td>
<td>25 °C, 4 h</td>
<td>91%</td>
</tr>
<tr>
<td>d</td>
<td>15 mol% Na₂PdCl₄, 1 atm O₂</td>
<td>9:1 THF: H₂O</td>
<td>25 °C, 14 h</td>
<td>73%</td>
</tr>
<tr>
<td>e</td>
<td>1 equiv. CuCl₂</td>
<td>100 mM NaCl in H₂O</td>
<td>25 °C, 4 h</td>
<td>0%</td>
</tr>
<tr>
<td>f</td>
<td>1 equiv. CuCl₂</td>
<td>100 mM NaCl in H₂O</td>
<td>25 °C, 4 h</td>
<td>0%</td>
</tr>
</tbody>
</table>


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II. Into the Genome: Engineering Polyketide Synthase
Polyketide Biosynthesis: Signal-Driven Modular Synthesis in a Cell

-About 10,000 polyketide structures have been identified to date.

Dictyostatin

Discodermolide

Epothilones

-Theoretical analysis of all variables involved in polyketide biosynthesis suggest that there are more than 1,000,000,000 possible structures.

-Aldol methodology has been extensively developed to generate polyketides, but new bondmaking regimes are defining the edge of this field.
Polyketide Biosynthesis: Signal-Driven Modular Synthesis in a Cell

- Polyketides are generated in assembly-line type fashion.
- Modular sections of enzyme (polyketide synthase, PKS) direct attachment and manipulation of each ketide unit.
- Polyketides are generally biologically active at numerous biological targets.

ACP: Acyl Carrier Protein
KR: Ketoreductase
DH: Dehydrase
ER: Enoylreductase
-epimerase, transferase, cyclase, thioesterase, etc.

Polyketide Biosynthesis: Signal-Driven Modular Synthesis in a Cell

- 6-deoxyerythronolide B (DEBS) PKS was one of the early targets for deciphering and mutation.

- 3 proteins carry two extender modules each.

- Specific domains were added or deleted resulting in different levels of processing and/or chain elongation.

- This work culminated in a 50-member library synthesis, obtained by tedious genetic manipulation.
- A survey of PKS genes revealed key conservation which could serve as restriction domains and lend “authenticity” to the gene.

- Variable DNA was constructed for various sequencing of domains.

- 14 modules were generated and could be used as cassettes and were paired in unnatural ways (11 x 14 = 154 possible ketide lactones) and placed into E. Coli.
Engineering Polyketide Synthesis: Choosing The Signals

- After incubation for 72 h, LC/MS was used to study production of the triketide lactones.

- 0.02 – 23 mg/L were produced in 72/154 combinations.

- MS revealed that all combinations functioned as a donor or acceptor at least once, indicating that each is “catalytically competent”.

- Proof-of-concept study identifying flexibility and promiscuity in module pairing.

- Use of common restriction sites enables rapid gene production in modular fashion.

- 1.5 million possible base pairs in PKS genes were analyzed.
Bondmaking At The Edge: Fusing Natures Code With Synthesis

Friedrich Wöhler
Ordinary Professor of Chemistry
University of Göttingen, 1836 - 1882

177 Years

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10/29/2005
Code Breaking: Crack the Code – Win the War

-Can breaking the biological signal code help us win the war against disease?
I. Speaking The Language of Infection: Targeting HIV Signaling
HIV Therapy: Protease and Reverse Transcriptase Inhibitors

-HIV protease (pictured) and reverse transcriptase inhibitors have been key in the modern treatment of HIV.

-Most, if not all, are substrates for P-glycoprotein (Pgp) efflux from cells.

-Concerns exist about the long term use of these drugs due to the development of resistant strains of the disease.

-Regardless, these types of inhibitors cannot destroy HIV in infected patients.

-Continued development of these types of therapies will not cure HIV.

-With the conventional therapies failing, a new method of disrupting HIV infection is needed.

HIV Therapy: Targeting the Infection Mechanism

- To enter cells, HIV must bind the CCR5 receptor through a poorly understood signal path.
- Binding must occur in an orchestrated fashion with CD4 and surface glycoproteins.
- HIV binding is accomplished using glycoprotein gp120 and the CD4 protein.

-Early work focused on the characterization of the proteins involved in this mechanism.

-Hendrickson *Nature* 1998, 393, 648
Small Molecule Disruption: CCR5 Antagonists As Anti-HIV Therapy

-The pre-entry complex structure was determined by X-ray analysis.

-All factors involved in the binding process are not known.

-There is substantial reorganization of gp120 once bound to CD4.

-CCR5 binding then occurs and allows access to the cell.

-The CCR5 pathway constitutes an excellent target for disrupting binding and/or signalling.

-Hendrickson Nature 1998, 393, 648
Small Molecule Disruption: CCR5 Antagonists As Anti-HIV Therapy

-The first non-peptidic CCR5 antagonist reported was TAK-779.

-TAK-779 inhibits HIV replication with an IC\textsubscript{50} of 10 nM.

-TAK-779 is not orally bioavailable. TAK-220 (analogue shown) is orally bioavailable with comparable activity.

Maraviroc is extremely potent against a wide range of HIV strains by inhibiting entry via CCR5 binding.

Maraviroc has an IC₉₀ of <10 nM and does not interfere with other entry sites on cells.

Well tolerated in humans (up to 300 mg/day).

After short regimens (10 day), viral load remained suppressed for about 10 days.

Maraviroc is currently in Phase III clinical trials.

-Ridgway and Co-workers *Nature Medicine* Advanced Online Publication
II. Speaking The Language of Cellular Treason: Cancer Signaling
Cellular Signaling: Receptor Tyrosine Kinases (RTKs)

- RTKs are membrane spanning proteins and mediate a very wide variety of cellular events—differentiation, growth, metabolism, apoptosis, etc.

- Kinase activity is one component of a complex signal cascade within the cell, which is always initiated with binding at the receptor.

- Kinases act by phosphorylating specific tyrosine residues in target proteins using ATP, which continues the signal cascade.

- Eventually, the signals reach the nucleus and the encoded cellular event can then be carried out.
# Cellular Signaling: Receptor Tyrosine Kinases (RTKs)

<table>
<thead>
<tr>
<th>Transmembrane Receptor PTKs</th>
<th>PTK Enzyme Family</th>
<th>PTKs</th>
<th>Involvement in Cellular Signaling (Disease States)</th>
<th>Representative References</th>
</tr>
</thead>
</table>
Cellular Signaling: The ErbB Signal Network

- Many tumors contain genes that encode for RTK which are mutated, amplified, or the proteins are overexpressed.

- Often, the kinases in tumor and healthy cells are different, and can therefore be selectively targeted, much unlike traditional chemotherapy.

- Epidermal growth factor receptor (EGFR or ErbB) is disregulated in 60% of solid tumors.

- A multibillion dollar industry is to be had in this area.

- We will focus on the erbB family of RTKs and how small molecule manipulation of this signal pathway may revolutionize medicine.

Cellular Signaling: The ErbB Signal Network
Cellular Signaling: ErbB Signal Network Inhibitors

Phorbol Esters
*Euphorbiae*
Potent Tumor Promoter

Staurosporin

Geldanamycin

Purealidin J

Clavilactone CD

…and many others, including Bistramide A


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Parke-Davis (Pfizer) is credited with pioneering the development of EGFR inhibitors.

- **PD 0069896**
  - IC$_{50}$: 2 µM
  - 1.5 µM (solid tumor)
  - Reversible, ATP Competitive
  - EGFr selective

- **PD 0153035**
  - 29 pM, 14 nM (cellular)
  - Reversible, ATP competitive, selective
  - No other effects on GFs until 10 µM

- **PD 0158780**
  - 8 pM
  - Reversible, ATP competitive, selective


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Cellular Signaling: ErbB Signal Network Inhibitors

PD 0158780

- The extreme potency of these compounds (pM) and long lifetime in the binding pocket (>4 h) has been attributed to deep, tight binding followed by hydrophobic collapse.

- Solubility was a severe problem in animal testing, so hydrophilic adjustments were made resulting in the final reversible inhibitor (Tarceva). This would end the reversible inhibitor hunt at Pfizer.

Tarceva
10 – 20 nM
Water soluble, orally available

PD 0158780 bound into the EGFr active site.
Cellular Signaling: ErbB Signal Network Inhibitors

- Completely suppresses tumor growth in mice at 10 mg/kg/day.
- Iressa can be dosed at 800 mg/day before limiting toxicity sets in.
- At plasma levels of >200nM, complete tumor suppression and some shrinkage occurs in humans.
- Higher dosing (above minimum can result in a 2-4 fold increase in apoptosis).
- Both monotherapy and combination therapy is effective.

Iressa (Gefitinib), AstraZeneca
EGFR/ErbB1 Inhibitor (1 - 9 nM)
Orally available (1 pill a day)
Cellular Signaling: ErbB Signal Network Inhibitors

Iressa, AstraZeneca
Non-Small Cell Lung Cancer

Tarceva, OSI/Roche/Genentech
Non-Small Cell Lung Cancer

Novartis

GlaxoSmithKline
ErbB1/ErbB2 Inhibitor

-Most are active against breast, colon, blood, and digestive cancers and are still in clinical trials.
-Additionally, a number of monoclonal antibodies have been approved for use (Heceptin, Erbitux).
Update - Cellular Signaling: EGFR Axon Regeneration

- Myelin/Chondroitin Sulfate Proteoglycans inhibit axon (neutrite) regeneration in the adult CNS. These molecules are natural at CNS injury sites.

- This inhibition mechanism is not understood.

- Suppressing the EGFR/ErbB1 function (PD168393) blocks the inhibition of axon regeneration and promotes nerve fiber regeneration.

- This study also reveals that the regeneration inhibitors trigger EGFR phosphorylation, and that it is calcium dependent.

- This could be a promising treatment for CNS injury.

Cellular Signaling: Interference and Therapeutics

-Learning the signal pathways of disease can enable a new form of directed therapeutics for all types of diseases.

-Is this the new direction of drug discovery?

-Are we nature’s Bletchley Park?

Iressa
The Road Ahead