COMBINATORIAL CHEMISTRY 2

Combinatorial Chemistry
Synthesis, Analysis, Screening
Edited by Günther Jung

Dynamic Combinatorial Chemistry
Edited by Joost N.H. Reek and Sijbren Otto
The "One-bead-one-compound" Combinatorial Library Method

The Base Technology: OBOC

In 1991, the one-bead-one-compound (OBOC) combinatorial library method was introduced through the recognition that via the "split-mix" scheme of synthesis, a unique synthetic compound were displayed on each bead, thus the "one-bead one-compound" concept. Using this method, millions of compounds can be generated within days and screening can be achieved within days as well.

There are several criteria that can be applied to classify synthetic combinatorial libraries:
(i) design of the library,
(ii) novelty of structures,
(iii) type of chemistry used for synthesis,
(iv) Structural features of library members,
(v) Chemical strategy, etc.
1. ACYCLIC LIBRARIES

- PS: Polystyrene/DVB
- TG: Tentagel
- S: Sasrin resin
- M: Merrifield resin
- W: Wang resin
- Me: Methyloxy carbonyl
- Ph: 9-fluorenyl

Chemical structures and reactions:

1. Acyclic Libraries
2. Structures of commonly used linkers. The symbols introduced here are used in the rest of the text.
4. (i) Piperidine in DMF
   (ii) Fmoc amino acids
   (iii) R₃-SO₂Cl, R₃-NCO, R₃-COOH, or R₃-COCl

Additional notes:
- Polystyrene/DVB is a type of resin commonly used in organic synthesis.
- Tentagel is another type of resin often used for peptide synthesis.
- Sasrin, Merrifield, and Wang resins are used for attaching amino acids to a solid support.
- Methyloxy carbonyl (Me) and 9-fluorenyl (Ph) are used as protecting groups in peptide synthesis.

[Diagram showing chemical structures and reactions]
2. LIBRARIES ON PREFORMED SCAFFOLDS

(i) MeOH/DCM (1:1), 24 h, rt

N-acyl amino alkyl amides

Ugi four-component condensation reaction.

3-amino-5-hydroxyl benzoic acid
(Rink resin)

(i) Piperidine in DMF
(ii) R₂COCl, DIEA in DCM
(iii) R₃COOH, HBTU, HOAt, DIEA in NMP/DMF, 12 h
(iv) R₄X, DBU in DMSO
(v) R₅SO₂Cl, DIEA in DCM

Scheme of biphenyl library synthesis.

Scheme of scaffold-based library synthesis.

(i) TEA in DCM, 12, rt
(ii) 20% piperidine in DCM, 12 h
(iii) preformed betaine in DCM, alcohol in toluene, 3 days
(iv) HOAc, TBAF, 12 h

Tetrasubstituted biphenyls
(scaffold system)
(i) xylene, reflux under Ar, 19h
(ii) Boc-ethylenediamine, DMF, 6h, rt
(iii) DCC, DCM
(iv) Fmoc-ethylenediamine.TFA, DIEA, DMF
(v) AA-β-Ala-Gly-β-Ala-Gly-O-TG, BOP, DMF
(vi) 20% piperidine in DMF, 10 min
(vii) R\textsubscript{2}(3)-COOH, DCC (2:1), 30 min
(viii) 50% TFA in DCM (1:1), 1h, followed by DIEA

Synthesis of a library based on Kemp’s triacid.

\[ 75000 \]
The library was screened against a model target, **carbonic anhydrase**, and a 4 nM active compound, \([N-(4-sulfamoylbenzoyl)-L-leucyl]-Piperidine-3-carboxylic acid\), was identified.

(i) Carbonates prepared in solution, then coupled to TentaGel
(ii) Piperidine in DMF
(iii) Fmoc amino acids
(iv) \(R_3\)-SO\(_2\)Cl, \(R_3\)-NCO, \(R_3\)-COOH, or \(R_3\)-COCl

**Acylpiperidine library synthesis.**
12000 triazines

(i) DIEA in DCM, 1h, rt
(ii) R2-NH2 in DCM, 2h, rt
(iii) R3-NH2 in dioxane, 2h, 90 °C
(iv) thioanisol, ethanediethiol, H2O in TFA, 2.5h, rt
(v) BrCN, HCl in H2O, 12h, rt

Synthesis of library based on gradual substitution of trichlorotriazine.
3. HETEROCYCLIC LIBRARIES

**β-LACTAMS**

[2+2] cycloaddition reaction of ketenes with resin bound imines

(i) Piperidine/NMP, 45 min
(ii) 0.8 M R₂-CHO in DCM/CH(OMe)₃, 3 h
(iii) 0.8 M R₃-CH₂-COCl, 1.1 M TEA in DCM, 0 °C, 16 h

→ β-Lactam library synthesis.

**PYRROLES**

1,3-dipolar cycloaddition reaction

(i) Piperidine/DMF
(ii) 1 M Ar-CHO in CH(OMe)₃, 4 h
(iii) Ac₂O, DIEA, 15 min
(iv) 1 M olefin, 1 M AgNO₃, 1 M TEA in MeCN, 8 h
(v) mercaptoacetyl chlorides

→ 1,2,4- trisubstituted pyrrolidines
**THIAZOLIDINE**

(i) 0.25 M aldehyde, THF, mol sieves, 70 °C, 2 h
(ii) 0.5 M α-mercapto acid, THF, 70 °C, 2 h
(iii) 0.5 M β-mercapto acid, THF, 70 °C, 2 h

**Figure 22.** Thiazolidinone and metathiazanone library synthesis.

**BENZIMIDAZOLES**

(i) KHMDS
(ii) Bu₃SnH, Pd(PPh₃)₄
(iii) TentaGel S NH₂, DIC, HOBr
(iv) R₁-NH₂
(v) NaBH₄-Cu(acac)₂
(vi) n-BuOH/DMF, 90 °C, 24 h

**Figure 23.** Benzimidazole library synthesis.
**HYDANTOINS**

(i) TFMSA anhydride, lutidine in DCM, -78 - 0 °C  
(ii) O-benzylhydroxylamine, 0 °C  
(iii) Ar-NCO, DCE, reflux 24 h  
(iv) t-BuOK, R₂-OH, rt

**BENZOPYRANS**

(i) PPh₃, DEAD in THF, rt  
(ii) N-Boc aminoketone  
(iii) TFA in DCM  
(iv) R₃-SO₂Cl, R₃-NCO, R₃-COOH, or R₃-COCI

**Figure 25.** Alkoxydantoin library synthesis.

**Figure 26.** Dihydrobenzopyran library synthesis.
**Figure 27.** Tetrahydroisoquinoline library synthesis.

(i) HC(OMe)$_3$ in DMF, 3h, rt
(ii) DIEA in DMF, 18h, rt
(iii) HATU in DMF, 20 min. rt followed by R$_3$-NH$_2$, rt, overnight

**Figure 28.** Diketopiperazine library synthesis.

(i) Piperidine/DMF
(ii) Ar-CHO, NaBH(OAc)$_3$ in DCM
(iii) Boc-amino acid, PyBrop
(iv) TFA
(v) Toluene, reflux 5h
Figure 29. Synthesis of diketopiperazine library based on spontaneous iminodiacetic acid monomethyl ester intramolecular cyclization.

(i) DIC, HOBr in DMF
(ii) piperidine in DMF
(iii) Fmoc amino acid, DIC, HOBr in DMF

Figure 30. Diketopiperazine and diketomorpholine library synthesis.
BENZODIAZEPINES

(i) Piperidine in DMF
(ii) Fmoc-amino acid fluoride
(iii) 5% AcOH in DMF
(iv) lithiated 5-(phenylmethyl)-2-oxazolidinone in THF followed by alkylation agent in DMF

Benzodiazepine library synthesis.

Cyanuric fluoride

Fmoc-protected amino acid fluorides

2-aminobenzophenones

1,4-benzodiazepines
Combinatorial chemistry has played an increasingly important role in the field of drug discovery for identification and optimization of drug leads. Combinatorial methods provide rapid access to large numbers of compounds for testing. Shown is a general schematic of a combinatorial library prepared from a small set of building blocks.
McCarthy and coworkers reported an early and elegant example for the synthesis of triazine-based corticotropin-releasing factor receptor antagonists by the sequential addition of anilines and amines to dichlorotriazine (Scheme 1).

The symmetrical nature of the triazine starting material combined with the differential nucleophilicity of the aniline and amine reagents yields products that are of high purity following acid extraction to remove excess amine reagents. Clearly, many reactions are not amenable to straightforward isolation procedures, placing increased emphasis on high-throughput purification methods.
Solid-Phase Synthesis

The power of solid-phase synthesis has been amply demonstrated by the rapid and automated parallel synthesis of peptides and oligonucleotides.

Examples of this strategy are the attachment of alcohols to support using silyl or tetrahydropyranyl linkages as well as the attachment of amines to support via a carbamate linkage (Figure 2).

**Figure 2.** Examples of linkers which protect a specific functional group by attachment to solid support.

\[ R = \text{Ph or } i-\text{Pr} \]
Scheme 2. Traceless linkers for the solid-phase synthesis of (a) pyridine-based tricycles and (b) isoquinolines.
Scheme 3. Cyclative cleavage strategies for the synthesis of (a) benzodiazepines and (b) hydantoins.
Scheme 4. Use of diversification linkers for the synthesis of (a) amides, (b) amines, (c) aromatic, and (d) heteroaromatic compounds.
Support-Bound Reagents and Scavengers

Figure 3. General examples of polymer-supported (a) reagents, (b) catalysts, and (c) scavengers.

(a) \[ A \xrightarrow{X-B} A-B + X \xrightarrow{\text{filter}} A-B \]

(b) \[ A + B \xrightarrow{X} A-B + X \xrightarrow{\text{filter}} A-B \]

(c) \[ A + B \xrightarrow{X} A-B + Y \xrightarrow{\text{filter}} A-B + X-Y \xrightarrow{\text{filter}} A-B \]
Scheme 5. A library of functionalized ureas synthesized using support-bound reagents and scavengers.

Scheme 6. Multi-step synthesis of pyrroles employing support-bound reagents and scavengers.
Discrete Compound versus Mixture Libraries

Figure 4. A general schematic depicting the split and mix synthesis concept.
Library Design

Lead Optimization Libraries

$K_i = 0.05 \text{ nM (sstr2 selective)}$

$K_i = 24 \text{ nM (sstr3 selective)}$

$K_i = 100 \text{ nM (sstr2)}$

$K_i = 1.4 \text{ nM (sstr1 selective)}$

$K_i = 0.4 \text{ nM (sstr5)}$

$K_i = 3.3 \text{ nM (sstr1)}$

**Figure 5.** Potent somatostatin inhibitors produced by combinatorial lead optimization.
Somatostatin (also known as growth hormone-inhibiting hormone (GHIH)) is a peptide hormone that regulates the endocrine system and affects neurotransmission and cell proliferation via interaction with G protein-coupled somatostatin receptors and inhibition of the release of numerous secondary hormones. Somatostatin inhibits insulin and glucagon secretion.
Fig. 1. Molecular modeling of the somatostatin pharmacophore. The three-dimensional model of L-363,377 was used as a probe in a search of the Merck sample collection database. The coloration of the molecules depicted in two dimensions schematically highlights the superposition between the residue side chains with the correspondingly colored moieties of L-264,930. Substitutions of the aromatic group are shown in blue, of the tryptophan in green, and of the diamine in red. The search strategy is described in greater detail elsewhere (11).
Fig. 2. Subtype-selective nonpeptide agonists of the somatostatin receptor. Color coding of sst receptor–selective compounds illustrates the relationship of various parts of each molecule to the original lead structure L-264,930. Colors are as in Fig. 1. L-797,591, L-779,976, L-796,778, L-803,087, and L-817,818 are selective for the sst1, sst2, sst3, sst4, and sst5 receptors, respectively.
Prospecting Libraries

The **Ugi reaction** is a multi-component reaction in organic chemistry involving a ketone or aldehyde, an amine, an isocyanide and a carboxylic acid to form a bis-amide.
Combination of reaction components

The usage of bifunctional reaction components greatly increases the diversity of possible reaction products. Likewise, several combinations lead to structurally interesting products. The Ugi reaction has been applied in combination with an intramolecular Diels-Alder reaction in an extended multistep reaction.
Scheme 8. Tandem Ugi-Diels–Alder reaction.
Scheme 9. Examples of azomethine ylide cycloaddition by (a) Affymax and (b) Bartlett and coworkers.
Multicomponent One-Step Mixture Synthesis

- Libraries of single compounds
- 4 components
  - 20 structural variants/input
- 160,000 compounds generated

DIEA: $N,N$-Diisopropylethylamine

HBTU: (2-[(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)

NMP: $N$-Methyl-2-pyrrolidone

DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene

HOAt: 1-Hydroxy-7-azabenzotriazole

BOP: (Benzotriazol-1-yl)oxy)tris(dimethylamino)phosphonium hexafluorophosphate

Betaine: [trimethylglycine]

TBAF: tetra-n-butylammonium fluoride

HMPA: Hexamethylyphosphoramidate