Department of Chemistry and Pharmacy - Universität Regensburg

COMBINATORIAL CHEMISTRY AND SOLID PHASE SYNTHESIS: SEMINAR AND LABORATORY COURSE

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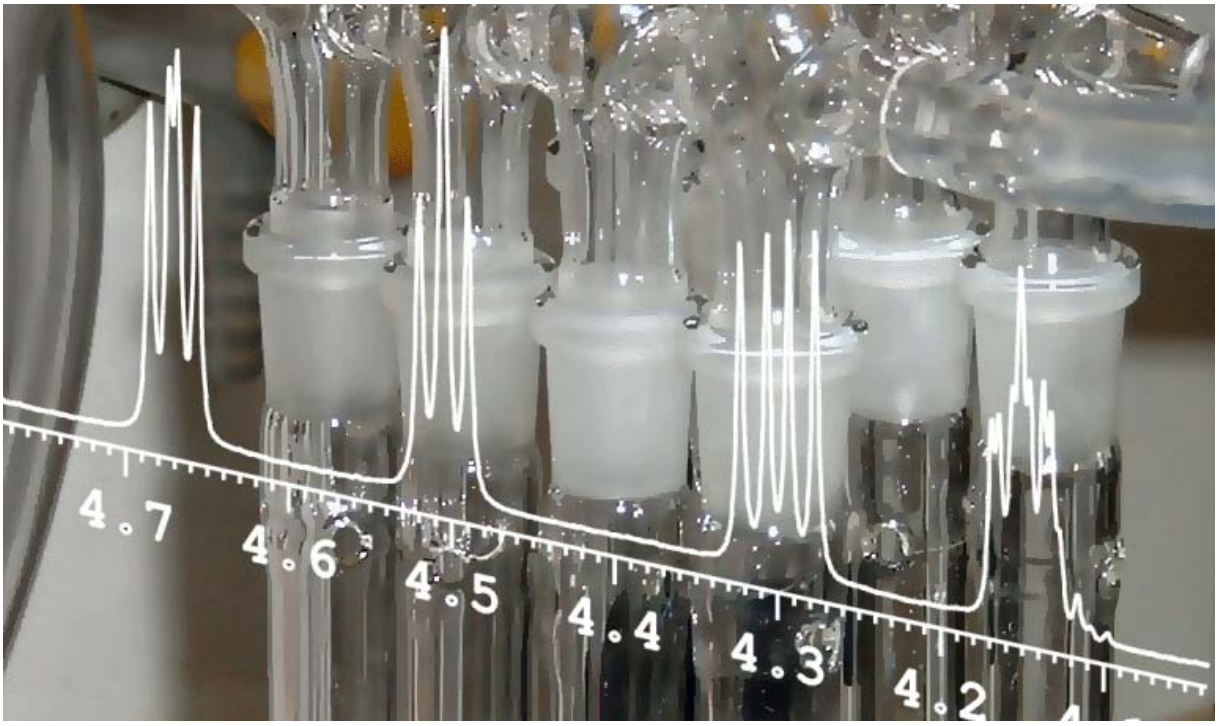

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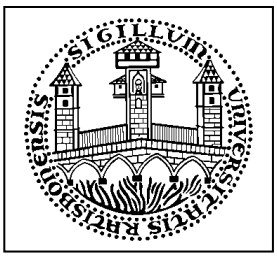

Foreword

Combinatorial chemistry concepts are changing the way in which academic research in many disciplines is conducted. The essence of this field is the rational and informed selection of diversity elements followed by their combinatorial association within a predefined framework to generate a chemical library. Two schools of thought emerged over the past few years regarding library design and synthesis: the first favours parallel synthesis and screening of relatively small target–oriented libraries. Conceptually, this strategy relies on retrosynthetic analysis and conventional organic synthesis to generate a chemical diversity space that targets a specific biological function or biochemical pathway, a target–oriented synthesis (TOS) approach. The second relies on the process of split–pool synthesis to generate small or large, encoded or non–encoded, spatially resolved (resin–supported) chemical libraries. The most recent application of this strategy is the diversity–oriented synthesis (DOS) approach developed specifically for the search and validation of novel chemical and therapeutic targets. Both TOS and DOS rely on similar theoretical and experimental descriptors to effectively explore the diversity space, structural complexity and lead–like nature of the targeted library.

While TOS offers the advantage of screening individual, well–characterized compounds obtained in multi–mg quantities, it is somewhat limited by the size of the library that can be produced and processed in a given period of time and is confined to a limited diversity space due to its target–oriented nature. DOS on the other hand takes advantage of split–pool synthesis on a polymeric support to dramatically reduce the synthetic effort and give rise to small or large libraries in which each compound is present in relatively small quantity (<1 μmol/compound using macrobeads). This approach must rely on firmly established chemistry because the synthetic intermediates can be numerous, structurally complex, in small quantity, and not amenable to routine purification and characterization techniques.

Encoded combinatorial chemistry emerged over the past decade as a strategy for tracking the chemical identity of individual compounds in a chemical library. The main goal being that large numbers of compounds can be tested simultaneously and only those with the desired properties would be decoded. There are two main approaches to accomplish this. The first relies on spatial segregation on a 2D matrix, wherein each library member is identified by its (x,y) coordinates. The second relies on microcarriers bearing each a unique compound along with its encoding element. While the first approach reached the market rapidly, its scope is

limited to a few classes of compounds and chemistries, namely DNA, protein and peptides, presynthesized small molecules, and inorganic/organic materials microarrays. The second approach benefits from the multitude of microcarriers available, their amenability to split–pool synthesis, and their compatibility with a broad spectrum of encoding/code readout strategies. The microcarriers can be encoded during library synthesis by adding a detectable chemical tag at each synthesis cycle that encodes for that particular step (parallel encoding approach). Alternatively, the microcarriers can be encoded before the synthesis (pre–encoding approach), in which case they must be decoded at each synthetic cycle in order to keep track of their chemical history (directed sorting strategy).

Parallel encoding requires the physical separation of the tags from the microcarrier followed by their analysis in order to uncover the chemical identity of the encoded material. Common molecular tags include oligonucleotide, halo–aryls, trityls, secondary amines, fluorescent dyes, or peptides. While the detection methods for the elucidation of the codes are generally difficult to automate, they include an array of techniques including mass spectrometry, high–resolution magic angle spinning and gel phase ^{1}H– and ^{13}C–NMR, ^{19}F–NMR, energy–dispersive x–ray spectroscopy, x–ray photoelectron spectroscopy, infrared and Raman spectroscopy, and fluorescence spectroscopy. Pre–encoding requires simply matching the microcarrier's preset code with the corresponding library member. The encoding methods in this case include optical, colloidal, organic and inorganic dye, radiofrequency, graphical, size and shape encoding.

The purpose of this manual is to provide advanced undergraduate students with a hands-on experience on several of the basic concepts that led to the current state-of-the-art in the field of combinatorial chemistry. The experiments were judiciously selected, carefully developed and clearly presented so that any student with basic training in organic chemistry could implement them.

The topics covered are currently practiced in advanced research laboratories in academia as well as in the pharmaceutical industry. They include combinatorial (split-pool) and parallel synthesis of resin supported peptide libraries, liquid phase synthesis of oligoamides on polyethylene glycol monomethylether, solution phase synthesis using polymer supported reagents (PASS*flow* technology), solution phase parallel synthesis and screening of transition metal catalysts for allylic alkylation, and finally, solution phase Fisher synthesis of an ester

library. This is a very rich combination of experiments that includes peptide coupling and protection/deprotection chemistry, oxidation of benzylic alcohols, reduction of nitroaromatics and aldehydes, reductive aminations, acylation reactions, transition metal catalyzed allylic alkylations, Fisher esterifications and dyes on solid support.

In terms of analytical and assay methods, this manual offers a broad array of opportunities for the students to learn state-of-the art-techniques as well as the means to develop creative and simple assay methods for chemical library screening. An excellent illustration of the latter is the colorimetric assay for catalyst discovery and the olfactive assay for the screening of aromatic esters (in the fragrance sense!). The students also learn to process and analyze complex NMR spectra and LC-MS data.

Overall, this manual is an excellent practical entry into combinatorial chemistry at the advanced undergraduate level. I recommended it highly to college professors interested in exploring this very timely and enabling field of the chemical sciences, combinatorial chemistry.

Hicham Fenniri

Combinatorial chemistry is both – a philosophy and a technique. The material contained in this manual has been used in the past years to teach advanced chemistry, biochemistry and pharmacy students (honours, master course level). The ten day intensive course consists of a seminar, in which the participants present and discuss essential facts from the list of topics provided. In groups of 2-3 students experimental tasks are addressed in the lab. Most of the experiments require more than one day. Depending on the level of experience of the participants, available equipment and help from teaching assistants experiments can be done in parallel. Some time is needed for analytical characterization of prepared products or libraries of compounds. The course is concluded by result presentations of the participants. Negative results and the discussion of the likely origin of failure are as important as perfect experiments. The positive feedback we got from participants over the last years encouraged us to publish this manual. It may facilitate by suitable adaptation of its content to develop an experimental teaching unit “Combinatorial Chemistry” at the university or college level. Experience and feedback from every course helped us to improve the experimental procedures and this will continue. The manual is not a final and fully optimized guide book, it is rather a current snapshot of a continuously developing teaching unit. We therefore encourage all users to participate in this process and give us feedback and suggestions for improvement.

Burkhard König
Regensburg, 2004

COMBINATORIAL CHEMISTRY SEMINAR

1. List of topics

The following list of topics is a brief journey through the main concepts and techniques of current combinatorial chemistry and solid phase synthesis. Each topic may be addressed by a short presentation (12-15 min) and discussed by the seminars participants.

1.) History of Combinatorial Chemistry
(ref.: XIII. pp.9 + citated literature)

2.) Principles of Combinatorial Chemistry
(ref.: I. pp. 1-34)

3.) Resins for Solid Phase Synthesis
(ref.: III. pp. 1-82; VIII/1. pp. 24-58; IX pp. 1-78; XII pp. 118-126)

4.) Anchors/Linkers for Solid-Phase Synthesis (General, Examples f. Functional Groups)
(ref.: I. pp. 167-228; II. pp. 47-98; V. pp. 465-510; VIII/1. pp. 59-153; XII pp. 127-137)

5.) Anchors/Linkers for Solid-Phase Synthesis (Traceless Linkers, Photolabile Linkers, Safety Catch, Enzymatic Cleavage)
(ref.: I. pp. 167-228; II. pp. 47-98; V. pp. 465-510; VIII/1. pp. 59-153; XII pp. 127-137)

6.) Solid Phase Peptide Synthesis, Strategies and Resins (Fmoc Strategy)
(ref.: VII. pp. 1-162; IX.; Novabiochem and Advanced ChemTech Catalog)

7.) Solid Phase Peptide Synthesis, Strategies, Resins (Boc Strategy) and Comparison with Fmoc Strategy (Advantages, Disadvanteges) (**Note:** Students for topic 6 and 7 should work together!)
(ref.: VII. pp. 1-162; IX.; Novabiochem and Advanced ChemTech Catalog)

8.) Different Techniques in Combinatorial and Solid Phase Chemistry
(ref.: XIII. pp. 34-38, 43-45, 71-73 + world wide web)

9.) Combinatorial Chemistry of Multicomponent Reactions
(ref.: I. pp. 125-165; VIII/1 pp. 685-705; IX. pp. 123-131; XII. pp. 287-302)

10.) Solution Phase Combinatorial Chemistry
(ref.: I. pp. 77-103; II. pp. 5-46)

11.) Purification Principles in High-Speed Solution Phase Chemistry
(ref.: I. pp. 106-119; II. pp. 21-45)

12.) Solid Phase Organic Reactions Part 1 - Cyclative Cleavage
(ref.: II. pp. 99-122; XII. pp. 139-262; I. pp. 35-76

13.) Solid Phase Organic Reactions Part 2 - C-C Bond Formation
(ref.: II. pp. 123-156; VI. pp. 25-80; XII. pp. 139-262

14.) Solid Phase Organic Reactions Part 3 - Heterocycles
(ref.: II. pp. 157-208, and 329-368; VI. pp. 25-80; XII. pp. 139-262

15.) Solid Phase Organic Reactions Part 4 – Reduction, Oxidation, Formation of Carbonyl Compounds and Derivatives Thereof
(ref.: I. pp. 35-76; VII/1. pp. 346-439; XII. pp. 139-262)

16.) Synthetic Libraries of Linear Oligomers
(ref.: I. pp. 257-290)

17.) Polymer Supported Organic Reactions/Reagents
(ref.: II. pp. 209-237; VI. pp. 149-194; III. pp. 83-156; IV. pp. 314-349)

18.) PASS*flow* Technique
(ref.: XVI.)

19.) Analytical Methods in Combinatorial Chemistry
(ref.: I. pp. 479-542; V. pp. 247-286; XII. pp. 263-286)

20.) Automation for Combinatorial Chemistry and Parallel Organic Synthesis
(ref.: XII. pp. 243-276; I. pp. 543-558)

21.) Encoding/Decoding
(ref.: VIII/1. pp. 170-187; XIII. pp. 82-99)

22.) Combinatorial Approaches to Chiral Catalyst Discovery
(ref.: XII. pp.433-455)

23.) Illustrative Syntheses (One Peptide and One Non-peptide)
(ref.: VII. pp. 163-191; VIII/2. pp. 613-642; X; XI)

24.) Computer-assisted Library design
(ref. II. Pp. 277-328)

25.) Industrial Concepts of Combinatorial Chemistry in Process Development
(ref.: VIII. pp 831-884, world wide web)

26.) High-throughput Screening
(ref.: VIII. pp 831-884, world wide web)

27.) Microwave-assisted Organic Synthesis for Combinatorial Chemistry
(ref.: XIV.)

28.) Steroids and Combinatorial Chemistry
(ref.: XV.)

29.) Fluorous Synthesis: Fluorous Mixture Synthesis
(ref.: XVII)

2. References

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II. Bannwarth W. and Felder E. (eds.): *Combinatorial Chemistry, A Practical Approach*, Wiley-VCH, Weinheim, **2000** (86/VK 5500 B219)

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XIII Beck-Sickinger A., Weber P., *Combinatorial Strategies in Biology and Chemistry*, Wiley-VCH, **2002** (86/VK 5500 B397 in German)

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XV Maltais R., Tremblay M. R., Ciobanu L. C., Poirier D., *Journal of Combinatorial Chemistry*, Vol. 6, Nr. 4, 444-456, **2004**

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4118-4120, **2001**; http://www.oci.uni-hannover.de/AK_Kirschning/images/Publications/Novel%20Microreactors%20for%20the%20Automated%20Synthesis%20in%20Solution.pdf

XVII Luo Z., Zhang Q., Oderaotoshi Y., Curran D.P., *Science*, 291, 1766-1769, **2001**

Experimental procedures

Safety in the chemical laboratory

To protect humans and the environment from possible hazards that may arise from chemicals or chemical apparatus laws, rules and guidelines exist. They may be a little different from country to country or between institutions. Every participant of a chemical teaching lab course must know about the basic regulations and rules to protect herself/himself, others and the environment. Therefore safety instructions are an important part of every practical instruction class. All lab and safety regulations and guidelines of your institution must be strictly followed. As a general advice: Always think about what you are doing when working in the lab - and what consequences may arise.

Solid phase peptide synthesis 1

Synthesis of a pentapeptide on Wang resin with Fmoc strategy

Peptide synthesis on solid support is a routine technique widely used in chemistry and biochemistry. The process can be run automatically in a peptide synthesizer to obtain oligo-peptides or small proteins. Depending on the amino acid sequence even more than 50 amino acid residues can be introduced. Even larger peptides are prepared from these fragments by native chemical ligation.

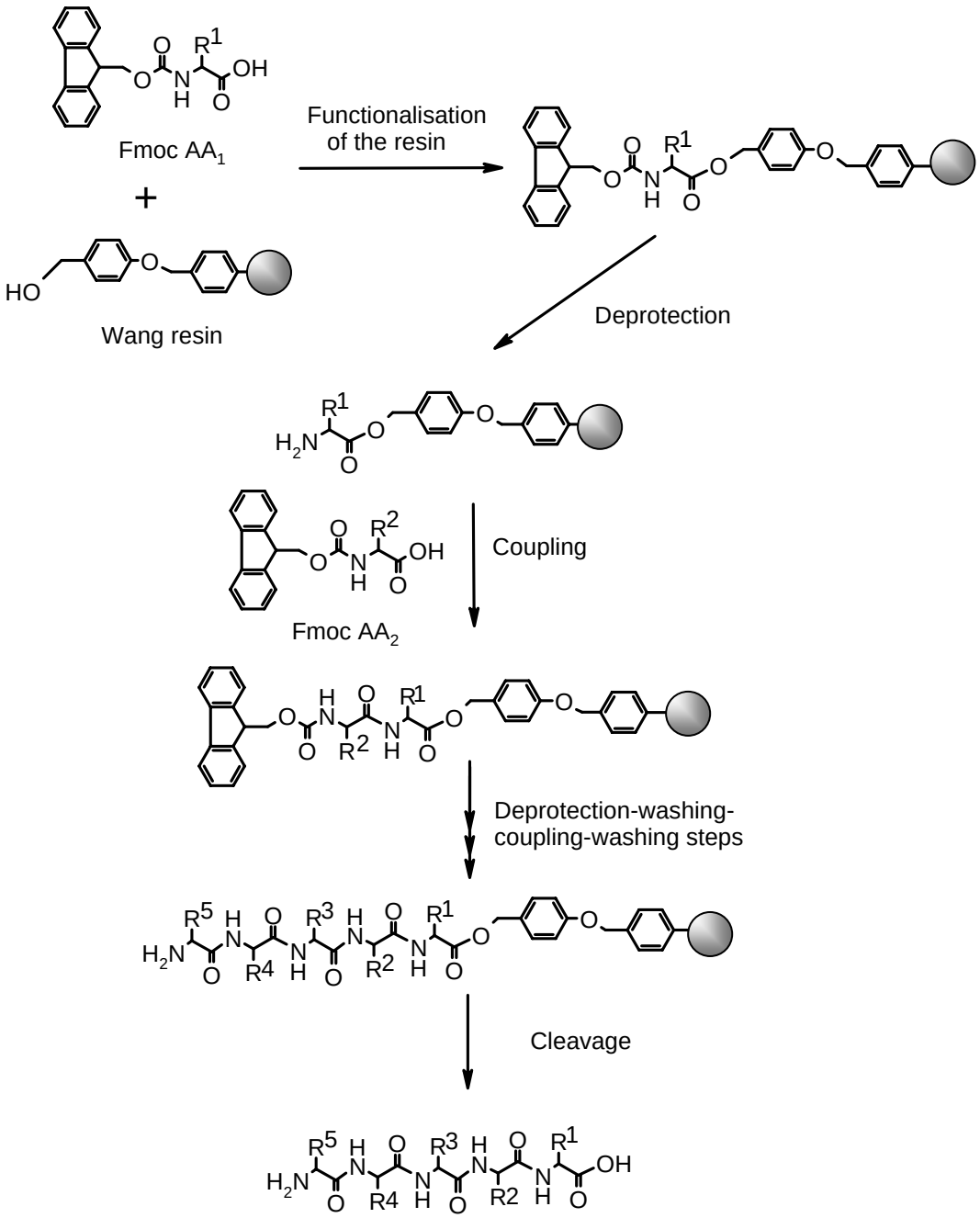


Figure 1. Synthesis of a pentapeptide

Safety considerations:

Lab coats and safety glasses should be worn at all times. Wear protective gloves whenever necessary. Be careful working with TFA! Working with DMF and DCM - keep them clean! Always obey all lab and safety rules of your university.

Mission:

You will synthesize a pentapeptide on Wang resin using Fmoc strategy. The performed manual SPPS involves functionalisation of the resin, condensation and deprotection reactions, resin test, peptide cleavage from the resin and precipitation protocol. Finally you will analyse your product by *NMR* and *LC-MS*.

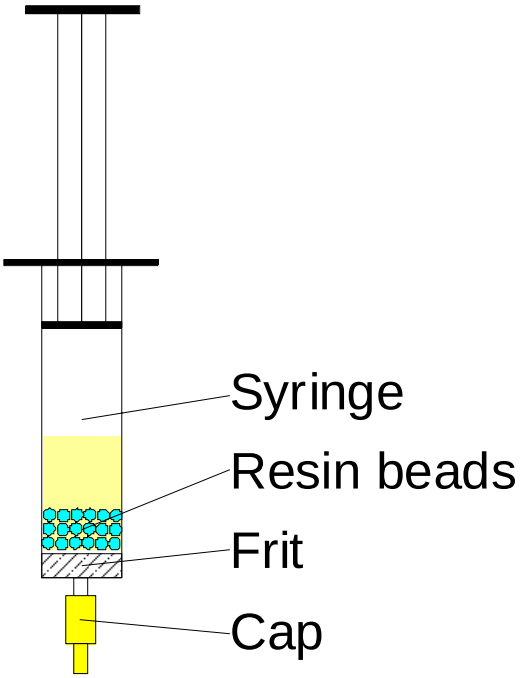


Figure2. Syringe for manual SPPS

Reactions:

Functionalisation of the resin

Let the Wang resin (0.1 mmol, for the loading see information provided by the supplier) swell for 1 h in 2 mL of DMF, then drain the solvent off. Dissolve FmocAA_1 (0.5 mmol, 5 eq.) and HBTU (0.49 mmol, 4.9 eq.) in 2 mL DMF then add DIPEA (1.0 mmol, 10 eq.) to the mixture. Wait for 2 min, then add the solution to Wang resin and shake the mixture for 3 h at room temperature. Drain the solvent and wash the resin with DMF (4 × 2 mL), then DCM (4 × 2 mL).

It is possible to control the loading by the following procedure (not performed during the practical course):

Take a sample of the dry resin (2 × about 1 mg), and determine the loading as follows:

Add 3 mL of piperidine/DMF (2/8) to each sample and mix for 4 min, then put the solvents into a UV cuvette (1 x 1 cm) and record the absorbance at 301 nm.

Calculate the yield using the following equation:

$mmol/g = (Abs_{solv} – Abs_{ref}) / 1.28 \times mg\ sample.$

If the yield is lower than 90% the coupling should be repeated.

If necessary preswell the resin in DCM, then add DCM (2 mL), pyridine (81 µL, 1.0 mmol) and Ac_2O (47 µL, 0.5 mmol) and shake the mixture for 30 min. Drain the solvent off and wash with DCM (2 × 2 mL) and DMF (4 × 2 mL). (During this step you will protect the unreacted -OH groups on the resin).*

Deprotection

Remove the Fmoc protecting group by treating the preswollen resin with a solution of piperidine/DMF (2/8, 2 mL) for 5 min then drain the solution and wash the resin with DMF (2 × 2 mL). Repeat this procedure once again, and wash the resin with DMF (4 × 2 mL) and DCM (4 × 2 mL). **Note**: Leftover piperidine may lead to premature deprotection which decreases the overall yield and purity. At no time during the deprotection should the resin be allowed to become dry.

Coupling with the second $FmocAA_2$

Dissolve $FmocAA_2$ (0.5 mmol, 5 eq.), HOBt (0.5 mmol, 5 eq.) and HBTU (0.49 mmol, 4.9 eq.) in DMF (2 mL) then add DIPEA (1.0 mmol, 10 eq.), mix and add to the resin (preswollen in DMF). Shake the mixture for 2 h then filter off the solution and wash the resin with DMF (4 × 2 mL), and DCM (4 × 2 mL). The reaction progress should be checked with the Kaiser test described below.*

Deprotection

Remove the Fmoc protecting group by treating the preswollen resin with a solution of piperidine/DMF (2/8, 2 mL) for 5 min then drain the solution and wash the resin with DMF (2 × 2 mL). Repeat this procedure once again, and wash the resin with DMF (4 × 2 mL) and DCM (4 × 2 mL). **Note**: Leftover piperidine may lead to premature deprotection which

decreases the overall yield and purity. At no time during the deprotection should the resin be allowed to become dry.

The cycle of coupling, washing, deprotecting and washing should be repeated until the desired sequence of amino acids is obtained.

Where can I stop?
After each coupling-washing procedure (*) the resin can be stored under DCM in the fridge till the next lab day.

Finally, after the last deprotection step, wash the resin with DMF (4 × 2 mL), DCM (4 × 2 mL), MeOH (1 × 2 mL), DCM (4 × 2 mL), then dry the resin under vacuum.

Cleavage
Add TFA/H_2O (95/5, 1.5 mL, prepared in advance) to the resin, shake for 2 h (follow and protocol the colour of the resin with attention), then drain the solvent and wash the resin with TFA/H_2O (95/5, 1 mL). Precipitate the peptide by addition of cold Et_2O. Filter off the solid and wash with cold Et_2O.
Dissolve the product in distilled water, lyophilize it, then perform the following analyses.

DO NOT DISCARD resin support or ether. Store them under nitrogen at 4^{o}C until peptide analysis is complete. If your peptide does not precipitate, evaporate all of the solvents, dissolve the peptide in a minimal volume of DCM and precipitate your peptide with hexane.
Note: The rotavap´s traps must be filled with KOH solution or NaOH solution to absorb and neutralize evaporated *TFA.*

Analysis: NMR spectrum (in DMSO-d6)
MS-ESI spectrum and, if possible, LC-MS spectrum should be recorded.
For the LC-MS spectrum: Dissolve 0.5 mg of your compound in MeOH p.a. (1 mL) then filter into an Eppendorf tube. Determine the molecular formula and the molecular weight.

Kaiser test:

Prepare the following solutions:

1: 0.5 g of Ninhydrin in 10 mL of ethanol.

2: 8 g of liquefied phenol in 2 mL of ethanol.

3: 0.2 mL of a 0.001 M aqueous solution of KCN in 9.8 mL of pyridine.

Place a few resin beads in a Eppendorf tube and rinse several times with ethanol. Add two drops of each of the solutions above. Mix well and heat to 100^{o}C for 4-6 min. Positive test is indicated by blue/purple resin beads which means that the coupling was not complete and that there are still uncoupled amines left on the beads. Negative test is indicated by pale yellow/brown colour.

References for this experiment: Novabiochem and Advanced ChemTech Catalog

Solid phase peptide synthesis 2

Parallel synthesis of tripeptide library on Wang resin using Fmoc peptide synthesis protocol

Solid phase peptide synthesis leads to libraries of similar peptides if done in parallel. Such peptide libraries are interesting to explore e.g. the affinity to biological molecules or generate first lead compounds for drug development.

This experiment is performed in a miniblock system as shown below.

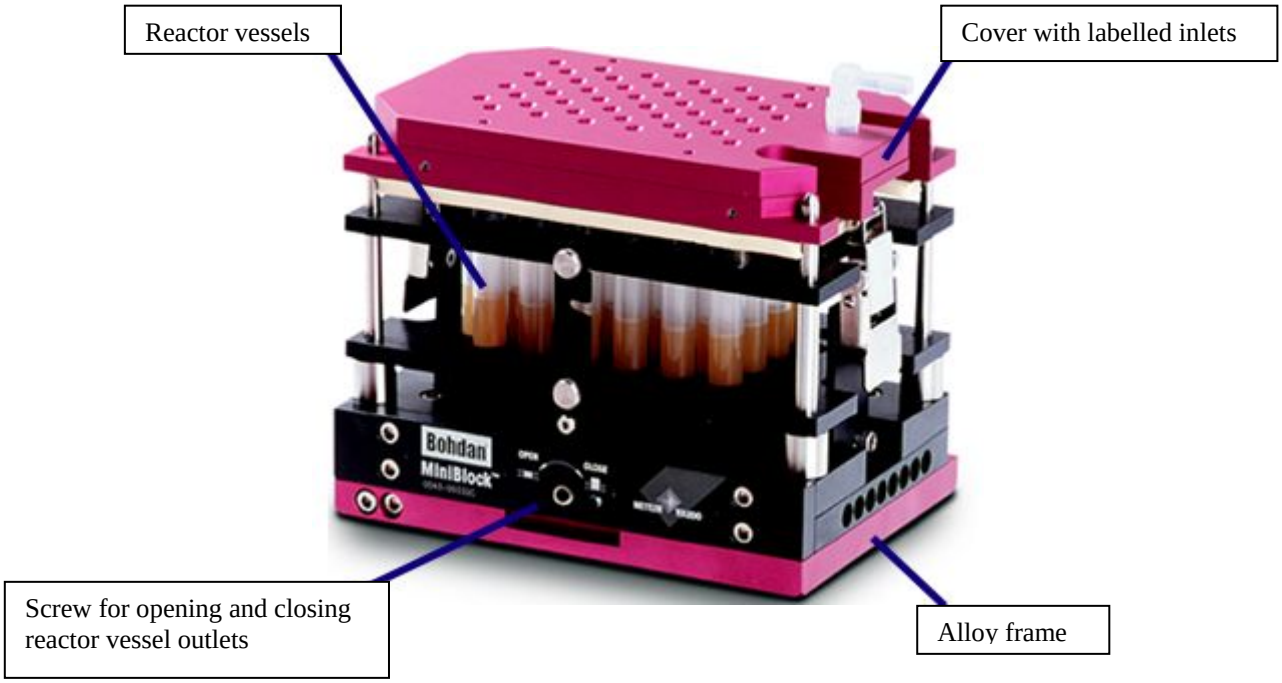


Figure 3. Miniblock system for parallel synthesis peptide libraries

Safety considerations:

Lab coats and safety glasses should be worn at all times. Use protective gloves when necessary. Be careful working with TFA! Working with DMF and DCM, please keep the solvents clean! After the experiment the miniblock reactors must be cleaned as soon as possible, TFA is extremely corrosive! Always obey all lab and safety rules of your university.

Mission:

You will synthesize a tripeptide library using parallel solid phase methods and Fmoc chemistry. The synthesis involves functionalisation of the resin, condensation and deprotection reactions and cleavage protocols. Finally, the resulting 27 different tripeptides will be analysed by NMR and LC-MS.

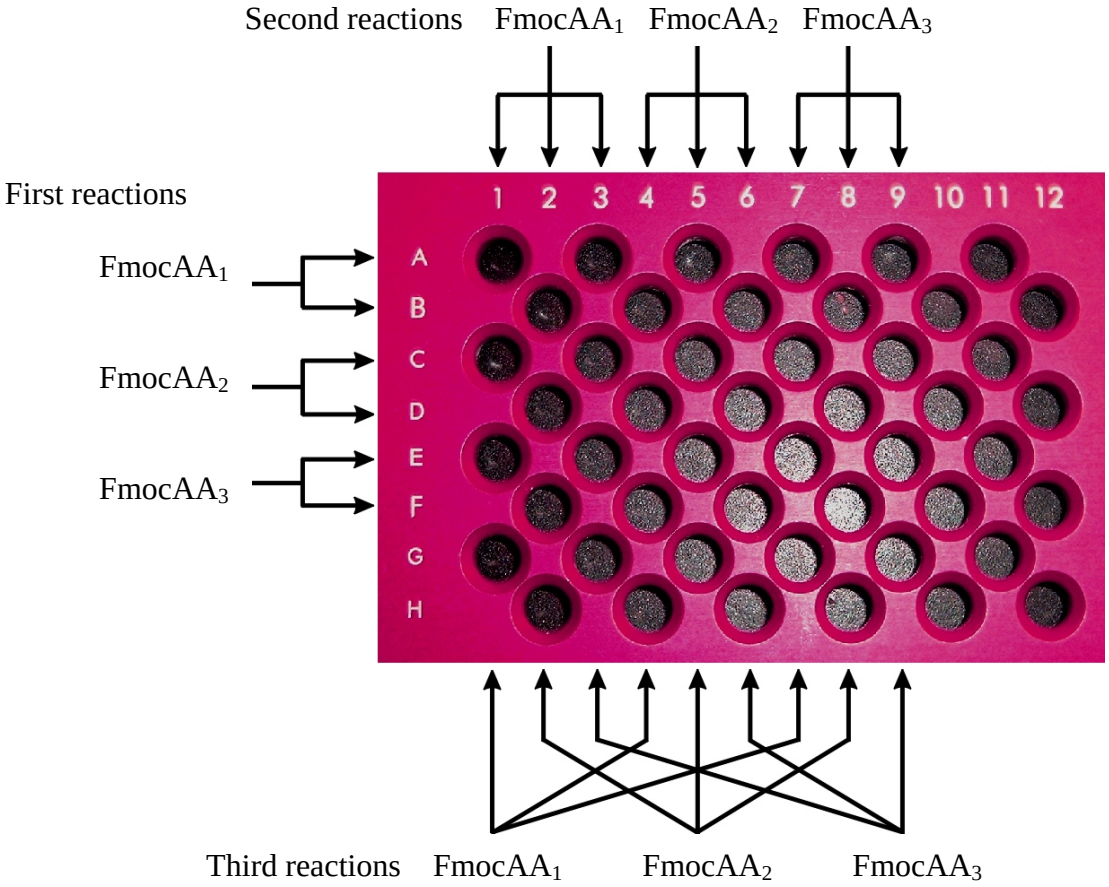


Figure 4. Preparation of a tripeptide library

Reactions:

Functionalisation of the resin

Place the Wang resin (0.065 mmol, for the loading see information provided by the supplier) in each of the 27 reaction vessel and preswell in DMF for 1 h, then drain the solvent.

Dissolve 9 × 0.25 mmol $FmocAA_1$ (3,75 eq.) and 9 × 0.25 mmol HBTU (3,75 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA to the mixture. Wait for 2 min, then distribute the solution equally over a third of the reaction vessels, e.g. A1, A3, A5, A7, A9, B2, B4, B6, B8. Dissolve 9 × 0.25 mmol $FmocAA_2$ (3,75 eq.) and 9 × 0.25 mmol HBTU (3,75 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA (3,75 eq.) to the mixture. Wait for 2 min, then distribute over the second third of the reaction vessels, e.g. C1, C3, C5, C7, C9, D2, D4, D6, D8. Dissolve 9 × 0.25 mmol $FmocAA_3$ (3,75 eq.) and 9 × 0.25 mmol HBTU (3,75 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA (3,75 eq.) to the mixture. Wait for 2 min, then distribute over the last third of the reaction vessels, e.g. E2, E4, E6, E8, F1, F3, F5, F7, F9). Shake all

the mixtures for 3 h at room temperature, drain the solvent and wash the resins with DMF (4 × 1 mL), then DCM (4 × 1 mL).*

If necessary preswell the resin in DCM and drain the solvent, then add DCM (1.5 mL), pyridine (41 µL, 0.25 mmol) and Ac_2O (24 µL, 0.25 mmol) to each vessel and shake the mixture for 30 min. Drain the solvent off and wash with DCM (2 × 2 mL) and DMF (4 × 2 mL). (During this step you will protect the unreacted -OH groups on the resin).*

Note: * These points are convenient to end the laboratory time for today. After each coupling-washing procedure the resin can be stored under DCM in the fridge till the next lab day.

Deprotection

Remove the Fmoc protecting group by treating the resin with a solution of piperidine/DMF (2/8, 2 mL) for 5 min then drain the solution and wash the resin with DMF (2 × 2 mL). Repeat this procedure once again, and wash the resin with DMF (4 × 2 mL) and DCM (4 × 2 mL). **Note**: Leftover piperidine may lead to premature deprotection which decreases the overall yield and purity. At no time during the deprotection should the resin be allowed to become dry.

Coupling of the second FmocAA

Dissolve 9 × 0.25 mmol $FmocAA_1$ (3,75 eq.), 9 × 0.25 mmol HOBt (3,75 eq.) and 9 × 0.24 mmol HBTU (3,6 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA (10 eq.) to the mixture. Wait for 2 min, then distribute the solution equally over a third of the reaction vessels, e.g. A1, A3, B2, C1, C3, D2, E1, E3, F2. Dissolve 9 × 0.25 mmol $FmocAA_2$ (3,75 eq.), 9 × 0.25 mmol HOBt (3,75 eq.) and 9 × 0.24 mmol HBTU (3,6 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA (10 eq.) to the mixture. Wait for 2 min, then distribute the solution equally over the second third of the reaction vessels, e.g. A5, B4, B6, C5, D4, D6, E5, F4, F6. Dissolve 9 × 0.25 mmol $FmocAA_3$ (3,75 eq.), 9 × 0.25 mmol HOBt (3,75 eq.) and 9 × 0.24 mmol HBTU (3,6 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA (10 eq.) to the mixture. Wait for 2 min, then distribute the solution equally over the last third of the reaction vessels, e.g. A7, A9, B8, C7, C9, D8, E7, E9, F8. Shake all the mixtures for 2 h at room temperature, drain the solvents and wash the resins with DMF (4 × 1 mL) then DCM (4 × 1 mL).*

Deprotection

Remove the Fmoc protecting group by treating the resin with a solution of piperidine/DMF (2/8, 2 mL) for 5 min then drain the solution and wash the resin with DMF (2 × 2 mL). Repeat this procedure once again, and wash the resin with DMF (4 × 2 mL) and DCM (4 × 2 mL). **Note**: Leftover piperidine may lead to premature deprotection and decreases the overall yield and purity. At no time during the deprotection should the resin be allowed to become dry.

Coupling of the third FmocAA

Dissolve 9 × 0.25 mmol $FmocAA_1$, (3,75 eq.), 9 × 0.25 mmol HOBt (3,75 eq.) and 9 × 0.24 mmol HBTU (3,6 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA (10 eq.) to the mixture. Wait for 2 min, then distribute the solution equally over a third of the reaction vessels, e.g. A1, A7, B4, C1, C7, D4, E1, E7, F4. Dissolve 9 × 0.25 mmol $FmocAA_2$ (3,75 eq.), 9 × 0.25 mmol HOBt (3,75 eq.) and 9 × 0.24 mmol HBTU (3,6 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA (10 eq.) to the mixture. Wait for 2 min, then distribute the solution equally over the second third of the reaction vessels, e.g. A5, B2, B8, C5, D2, D8, E5, F2, F8. Dissolve 9 × 0.25 mmol $FmocAA_3$ (3,75 eq.), 9 × 0.25 mmol HOBt (3,75 eq.) and 9 × 0.24 mmol HBTU (3,6 eq.) in 12 mL DMF and add 9 × 0.5 mmol DIPEA (10 eq.) to the mixture. Wait for 2 min, then distribute the solution equally over the last third of the reaction vessels, e.g. A3, A9, B6, C3, C9, D6, E3, E9, F6. Shake all the mixtures for 2 h at room temperature, drain the solvents and wash the resins with DMF (4 × 1 mL), then DCM (4 × 1 mL). *

Deprotection

Remove the Fmoc protecting group by treating of the resin with a solution of piperidine/*DMF* (2/8, 2 mL) for 5 min then drain the solution and wash the resin with DMF (2 × 2 mL). Repeat this procedure once again, and wash the resin with DMF (4 × 2 mL) and DCM (4 × 2 mL).

Finally, after the last deprotection step wash the resins with DMF (4 × 1 mL), DCM (4 × 1 mL), MeOH (1 × 1 mL), DCM (4 × 1 mL), and dry under vacuum.

Cleavage

Add TFA/H_2O (95/5, 0.75 mL, prepared in advance) to the resins and shake for 2h. Drain the solvents and wash the resins with TFA/H_2O (95/5, 0.5 mL). Place the filtrates in small vials, cover with parafilm, punch holes in the parafilm with a Pasteur pipette and dry the vials in a

vacuum desiccator filled with powdered KOH. Dissolve the resulting solid samples in distilled water (0.5 mL each), lyophilize them and analyze as follows:

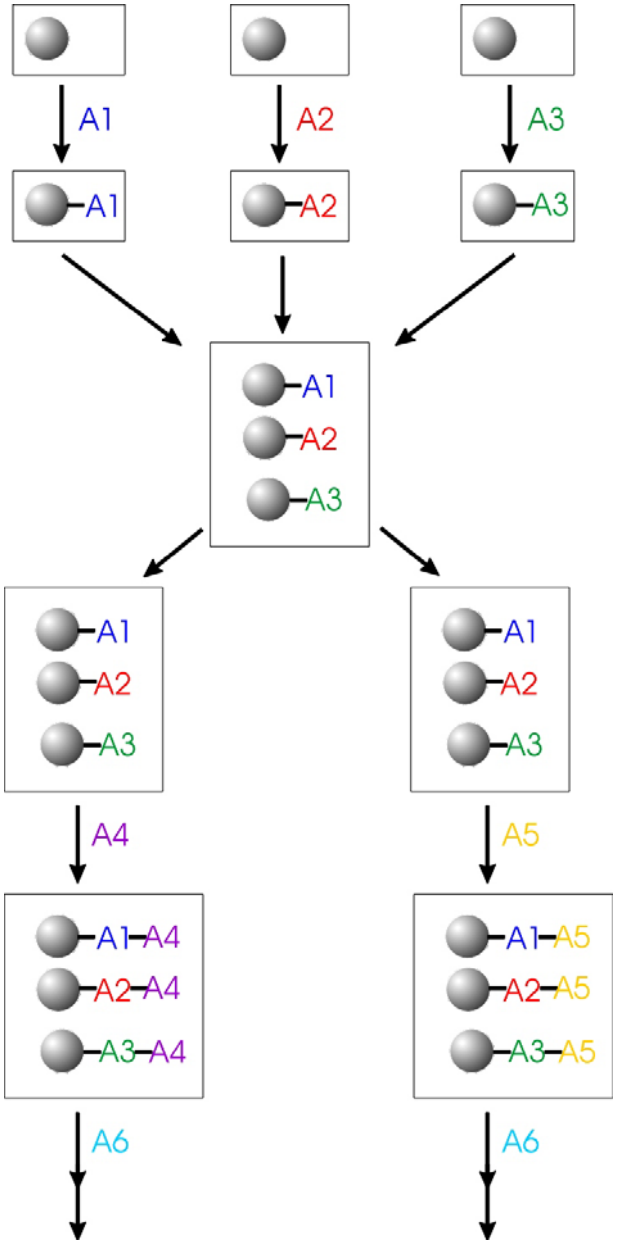
Analysis:
Approximately 10 samples should be selected for LC-MS, and the remaining should be analysed by NMR (in DMSO-d6).
For LC-MS dissolve 0.5 mg of compound in MeOH (1 mL) and filter into an Eppendorf tube. Determine the molecular formula and the molecular weight.

References for this experiment: Novabiochem and Advanced ChemTechCatalog

Solid phase peptide synthesis 3

Synthesis of split-mix tripeptide library on Wang resin using Fmoc peptide synthesis protocol

The split-mix protocol allows the rapid synthesis of larger libraries of compounds. On each polymer bead one type of compounds is prepared. The split and combine approach is one of the classic strategies in combinatorial chemistry.



1) Derivatisation of the resin with the first amino acid
2) Mixing of the resin beads
3) Distribution of the resin beads
4) Deprotection-coupling steps until the desired sequence is obtained
5) Cleavage from the resin and analysis of the compounds

Figure 5. Split-mix synthesis of a tripeptide library

Safety considerations:

Lab coats and safety glasses should be worn at all times. Use protective gloves when necessary. Be careful working with TFA! Working with DMF and DCM; keep the solvents clean! After the experiment the miniblock reactors must be cleaned as soon as possible, TFA is extremely corrosive! Always obey all lab and safety rules of your university.

Mission:

You will synthesize two tripeptide libraries using split-mix method and Fmoc chemistry. The synthesis involves functionalisation of the resin, condensation, deprotection reactions and cleavage protocols. Finally the tripeptide libraries will be analysed by LC-MS.

Reactions:

Functionalisation of the resin

Place the Wang resin (0.065 mmol, for the loading see information provided by the supplier) in each of the 27 reaction vessel and preswell in DMF for 1 h, then drain the solvent.

Dissolve FmocAA_1 (0.25 mmol, 3.75 eq.) and HBTU (0.25 mmol, 3.75 eq.) in 1.5 mL DMF and add DIPEA (0.5 mmol, 7.5 eq.) to the mixture. Wait for 2 min, then add the solution to the first reaction vessel. Dissolve FmocAA_2 (0.25 mmol, 3.75 eq.) and HBTU (0.25 mmol, 3.75 eq.) in 1.5 mL DMF and add DIPEA (0.5 mmol, 7.5 eq.) to the mixture. Wait for 2 min, then add the solution to the second reaction vessel. Dissolve FmocAA_3 (0.25 mmol, 3.75 eq.) and HBTU (0.25 mmol, 3.75 eq.) in 1.5 mL DMF and add DIPEA (0.5 mmol, 7.5 eq.) to the mixture. Wait for 2 min, then add the solution to the third reaction vessel. Shake all vessels for 3 h at room temperature, drain the solvent and wash the resins with DMF (4 × 1 mL), then DCM (4 × 1 mL).

Split and Mix

Combine the resins into one vessel and mix them. Then distribute it into two vessels.

If necessary preswell the resins in DCM (2 mL), add DCM (1.5 mL), pyridine (41 µL, 0.5 mmol) and Ac_2O (24 µL, 0.25 mmol) and shake the mixtures for 30 min. Drain the solvent and wash the resins with DCM (2 × 1 mL) and DMF (4 × 1 mL),

Deprotection

Remove the Fmoc protecting group by treating the resin with a solution of piperidine/DMF (2/8, 2 mL) for 5 min then drain the solution and wash the resin with DMF (2 × 2 mL). Repeat this procedure once again, and wash the resin with DMF (4 × 2 mL) and DCM (4 × 2 mL). **Note**: Leftover piperidine may lead to premature deprotection which decreases the overall yield and purity. At no time during the deprotection should the resin be allowed to become dry.

Coupling the second FmocAA

Dissolve $FmocAA_4$ (0.35 mmol, 3.5 eq.), HOBt (0.35 mmol, 3.5 eq.) and HBTU (133 mg, 0.34 mmol, 3.4 eq.) in 1.5 mL DMF and add DIPEA (0.70 mmol, 7 eq.) to the mixture (**Note**: We now need more coupling reagents, because all of the resin from three reaction vessels in the first step is now placed into two vessels). Wait for 2 min, then add the solution to the first reaction vessel. Dissolve $FmocAA_5$ (0.35 mmol, 3.5 eq.), HOBt (0.35 mmol, 3.5 eq.) and HBTU (0.34 mmol, 3.4 eq.) in 1.5 mL of DMF and add DIPEA (0.70 mmol, 7 eq.) to the mixture. Wait for 2 min, then add the solution to the second reaction vessel. Shake the mixtures for 2 h at room temperature, drain the solvent and wash the resins with DMF (4 × 1 mL), then DCM (4 × 1 mL).*

Deprotection

Remove the Fmoc protecting group by treating the resin with a solution of piperidine/DMF (2/8, 2 mL) for 5 min then drain the solution and wash the resin with DMF (2 × 2 mL). Repeat this procedure once again, and wash the resin with DMF (4 × 2 mL) and DCM (4 × 2 mL). **Note**: Leftover piperidine may lead to premature deprotection which decreases the overall yield and purity. At no time during the deprotection should the resin be allowed to become dry.

Coupling the third FmocAA

Dissolve 2 × 0.35 mmol $FmocAA_6$, 2 × 0.35 mmol HOBt and 2 × 0.34 mmol HBTU in 3 mL of DMF and add 2 × 0.70 mmol DIPEA (256 µL) to the mixture. Wait for 2 min, then distribute the solution equally into two reaction vessels. Shake the mixtures for 2 h at room temperature, drain the solvent and wash the resins with DMF (4 × 1 mL), then DCM (4 × 1 mL).*

Deprotection

Remove the Fmoc protecting group by treating the resin with a solution of piperidine/DMF (2/8, 2 mL) for 5 min then drain the solution and wash the resin with DMF (2 × 2 mL). Repeat this procedure once again, and wash the resin with DMF (4 × 2 mL) and DCM (4 × 2 mL).

Finally, after the last deprotection step, wash the resins with DMF (4 × 1 mL), DCM (4 × 1 mL), MeOH (1 × 1 mL), DCM (4 × 1 mL) and dry the resins under vacuum.

Cleavage

Add TFA/H_2O (95/5, 0.75 mL, prepared in advance) to the resins and shake for 2 h. Drain the solvents and wash the resins with TFA/H_2O (95/5, 0.5 mL). Place the filtrates in small vials, cover with parafilm, punch holes in the parafilm with a Pasteur pipette and dry the vials in a vacuum desiccator filled with powdered KOH. Dissolve the resulting solid samples in distilled water (0.5 mL each), lyophilize and analyze as follows.

Analysis: Analyze the libraries by LC-MS and compare the results with those of the **Solid phase peptide synthesis 2** experiment.

For the LC-MS prepare the samples as follows: dissolve 0.5 mg of compound in MeOH (1 mL), then filter into an Eppendorf tube. Determine the molecular formula and the molecular weight.

Note: * These points are convenient to end the laboratory time for today. After each coupling-washing procedure the resin can be stored under DCM in the fridge till the next lab day.

References for this experiment: Novabiochem and Advanced ChemTech Catalog

Solid supported synthesis of oligoamides

Liquid phase synthesis of oligoamides using PEG monomethylether as a solid support

Polyethylenglycol (PEG), a linear industrial polymer available in a range of molecular weights, is an alternative support material to polystyrene. PEG is soluble in DCM and water, but precipitates from ether or ethanol. This allows combining advantages of solid phase synthesis and reaction in homogeneous solution. However, the loading capacity of PEG is smaller when compared to functionalized polystyrene.

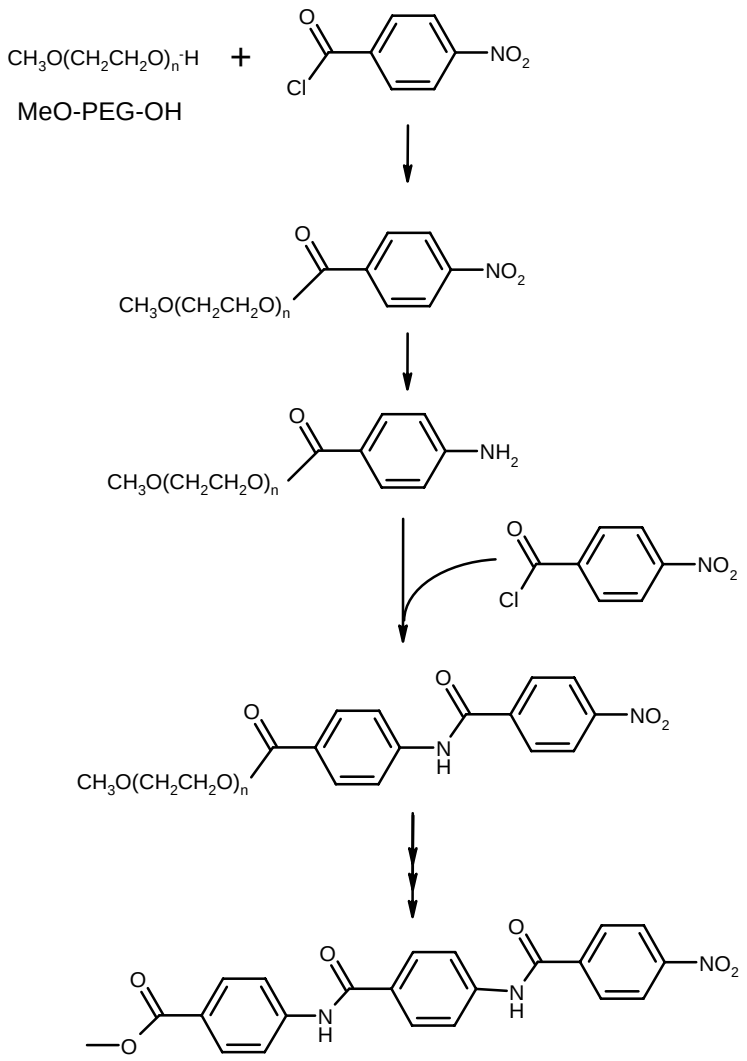


Figure 6. Liquid phase synthesis of aromatic oligoamides

Safety considerations:

Lab coats and safety glasses should be worn at all times. Use protective gloves when necessary. Working with DMF, DCM and MeOH, please keep the solvents clean! Always obey all lab and safety rules of your university.

Mission:

You will synthesize a diamide and a triamide using polyethylene glycol (PEG) as a solid support. This experiment involves condensation and reduction reactions, cleavage from the polymer, and special work up protocols for PEG coupled products. Finally, the resulting PEG-supported oligoamides will be analyzed by NMR and MS.

Reactions:

Preparation of the Diamide

Functionalisation of the PEG support

Dissolve 5g of PEG monomethylether (MW:~5000) in DCM (10 mL) in a beaker and add pyridine (2 ml). Dissolve 5 equiv. of *p*-nitro benzoyl chloride in DMF (2 mL) and add this solution to the PEG solution. Stir the mixture overnight at room temperature. Precipitate the PEG product with diethyl ether (400 mL) and filter off the solid. Dissolve the solid in DCM (10 ml of DCM per 1 g of precipitate), precipitate once again with diethyl ether (400 mL), filter, and dry the solid under vaccum.

Check the yield of the coupling by NMR as follows:

Dissolve 50-100 mg of the PEG product in $CDCl_3$ (0.7 mL), and compare the integral of the aromatic protons with the -OMe group of the PEG. The conversion of the reaction should be quantitative, otherwise the coupling must be repeated.

Reduction

Dissolve the functionalised PEG in MeOH (5-10 mL per 1g of the functionalized PEG, if necessary add a small amount of DCM). Add 40 equiv. of ammonium formiate and Pd/C (100 mg per 1 g of of the functionalized PEG) and stir the mixture vigorously overnight.

Filter the suspension over CELITE, and add diethyl ether (400 mL) to the filtrate. Isolate the resulting precipitate by filtration, redissolve in a minimum amount of DCM, filter through CELITE and wash the CELITE filter with DCM. Finally, precipitate the desired product from the combined filtrates with diethyl ether (400 mL) and determine the yield of the reaction by NMR as follows:

Dissolve 50-100 mg of the polymer in $CDCl_3$, and assess the yield using the chemical shift of the acylated CH_2 group (CH_2-group of the reduced compound is shifted downfield).

Amide coupling

Dissolve the reduced compound from the previous step in DCM (5-10 ml DCM per 1 g of compound) and pyridine (10 equiv.), then add 5 equiv. of *p*-nitro benzoyl chloride in DMF (5 mL per 1 g of chloride). Stir the mixture overnight at room temperature and isolate the reaction product by precipitation with diethyl ether (300 mL). Dissolve the precipitate in DCM (5-10 ml DCM per 1 g of precipitate) and repeat the precipitation/filtration procedure. The product is dried in vacuum and the yield of the coupling is determined by NMR:

Dissolve 50-100 mg of the polymer in $CDCl_3$, and compare the integral of the aromatic resonance signals with the one of the OMe group of the PEG, and check the chemical shift of the acylated CH_2 group (only one multiplet should be observed). The conversion of the reaction should be quantitative, otherwise the coupling must be repeated.

Cleavage

Procedure I yielding the methyl ester: Dissolve the compound in MeOH, and add NaOMe until pH 9 is reached. Stir the reaction mixture overnight, then dilute with water and EE, extract and isolate the organic phase, dry (Na_2SO_4), filter and concentrate.

Procedure II yielding the carboxylic acid: Dissolve the compound in 2 N KOH and stir the reaction mixture at room temperature for 2 h. Neutralize with HCl and add EE and water, extract and isolate the organic phase, dry (Na_2SO_4), filter and concentrate.

Analyse the diamide products by NMR and MS.

Preparation of a triamide

Repeat the reduction and the condensation step once more.

Reduction (Same as above)

Dissolve the functionalised PEG in MeOH (5-10 mL per 1 g of the functionalized PEG, if necessary add a small amount of DCM). Add 40 equiv. of ammonium formiate and Pd/C (100 mg per 1 g of the functionalized PEG) and stir the mixture vigorously overnight. Isolate the compound by precipitation in the same manner as described for the first reduction.

Determine the yield of the reaction by NMR as described above.

Amide coupling (Same as above)

Dissolve the reduced compound in DCM (10 ml DCM per 1 g of the reduced PEG) and pyridine (10 equiv.), then add 5 equiv. of *p*-nitro benzoyl chloride in DMF (5 mL per 1 g of chloride) and stir the mixture overnight at room temperature. The reaction product is isolated by precipitation with diethyl ether (250 mL). Dissolve the precipitated solid in DCM (5-10 ml DCM per 1 g of precipitate) and precipitate once again with diethyl ether (250 mL).

Determine the yield of the coupling by NMR as described above. The conversion of the reaction should be quantitative, otherwise the coupling must be repeated.

Cleavage

Procedure I yielding the methyl ester: Dissolve the compound in MeOH, and add NaOMe until pH 9 is reached. Stir the reaction mixture overnight, then dilute with water and EE, extract and isolate the organic phase, dry (Na_2SO_4), filter and concentrate.

Procedure II yielding the carboxylic acid: Dissolve the compound in 2 N KOH and stir the reaction mixture at room temperature for 2 h. Neutralize with HCl and add EE and water, extract and isolate the organic phase, dry (Na_2SO_4), filter and concentrate.

Analyse the triamide products by NMR (in DMSO-d_6) and MS.

References for this experiment: B. König, U. Papke, M. Rödel, *New J. Chem*. **2000**, *24*, 39 – 45.

Synthesis with polymer supported reagents

If, instead of substrates or starting materials, reagents are bound to polymer support, they can be used in excess and easily removed after the reaction. The reaction product stays in homogeneous solution and can be analyzed, tested or used for the subsequent transformation.

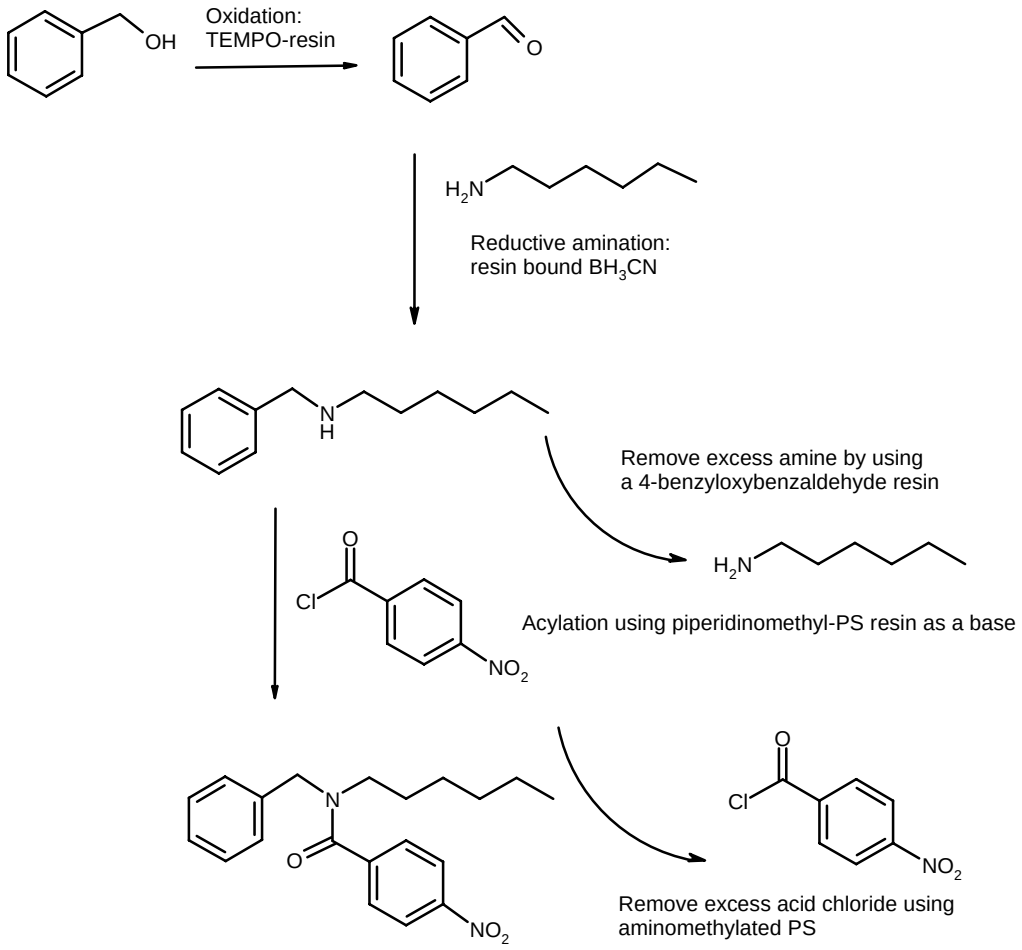


Figure 7: A simple reaction sequence with polymer supported reagents.

Safety considerations:

Lab coats and safety glasses should be worn at all times. Use protective gloves when necessary. Working with DMF, DCM and MeOH, please keep the solvents clean! Always obey all lab and safety rules of your university.

Mission:

You will synthesize an acylated secondary amine in solution phase. The synthesis involves the use of resins as reagents or scavengers for organic reactions. Your compound will be analyzed by NMR and MS. Because you will be working on very small scale, you will have to recover your entire compound after NMR characterization to carry out the subsequent transformations.

Reactions:

Oxidation with TEMPO polystyrene:

Preswell the TEMPO polystyrene (0.5 g, 0.87 mmol)in DCM for 30 min, then drain the solvent off. Dissolve *N*-chlorosuccinimide (520 mg, 3.9 mmol) in DCM containing 4 N HCl in dioxane (1.2 mL, 4.7 mmol). After 5 min add the solution to TEMPO polystyrene and shake the mixture for 15 min, filter and wash the resin with dry DCM. Add to this material a solution of benzyl alcohol (16 µL, 0.157 mmol) dissolved in 2 mL of DCM. Shake the suspension for 1.5 h at room temperature, then remove the resin by filtration and wash with DCM. Evaporate the combined filtrates to dryness to obtain the product benzaldehyde.

Record the ^{1}H-NMR spectrum of the compound in deuterated benzene. (**Note:** Recover the compound after the measurement.)

Reductive amination:

Dissolve *N*-hexylamine (41 µL, 0.31 mmol) in AcOH : DCM (1/10, 2 mL) and shake at room temperature. Add benzaldehyde from the previous step, followed by (polystyrylmethyl)trimethylammonium cyanoborohydride (0.4 mmol) and shake the reaction mixture for 16 h at room temperature. Filter off the resin, wash with DCM and evaporate the combined filtrates yielding the crude reaction mixture.

Record the ^{1}H NMR spectrum in $CDCl_3$. (**Note:** Recover the compound after the measurement.)

Remove the excess of amine by 4-benzyloxybenzaldehyde polystyrene:

Add the resin (3 equiv. with respect to the amine) to the crude reaction mixture in DCM. Stir the reaction mixture for 3 h at 50^{o}C. The reaction may be catalyzed by addition of AcOH. Remove the resin by filtration, wash with DCM (3x) and evaporate the filtrate to dryness.

Record the ^{1}H-NMR spectrum of the compound in $CDCl_3$. (**Note:** Recover the compound after the measurement.)

Acylation of the secondary amine using piperidinomethyl polystyrene as a base:
Stir the mixture of benzylhexylamine (the product of the previous step) and piperidinomethyl polystyrene (6 equiv.) in 2 mL of DCM. Add *p*-nitro benzoylchloride (3 equiv.) to the mixture and stir overnight. Remove the resin by filtration, wash with DCM (3x) and evaporate the combined filtrates to yield the crude reaction product.

Removing excess acyl chloride using aminomethyl polystyrene:
Add the amine resin (6 equiv. with respect to acyl chloride) to the crude reaction product of the previous step in 2 mL of DCM. Shake gently for 4 h at room temperature. Remove the resin by filtration, wash the resin with DCM (3 x 2 mL) and evaporate the combined filtrates to dryness.
Analyze your product by NMR (in $CDCl_3$) and MS.

References for this experiment: Novabiochem Catalog

Chemical Reactions in a PASS*flow*-apparatus

Bases of this new technology are monolithic microreactors, which allow for in-flow chemical reactions with immobilised reagents. The proprietary composites are loaded with reagents or catalysts (**C**). Chemical reactions (**A**= starting material) take place on the surface leaving used reagents in the reactor. The product (**B**) is collected at the end of the column without work up (e.g. without extraction, chromatography or filtration).

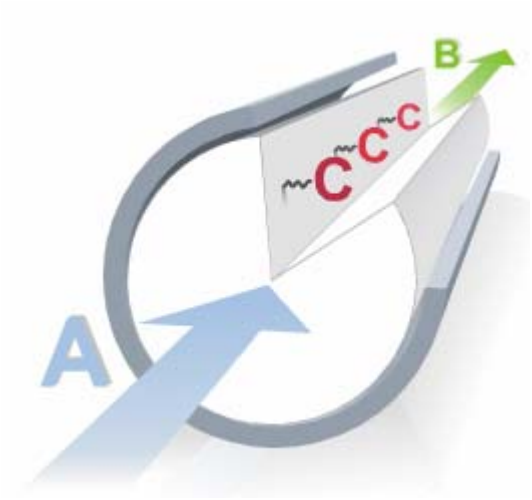


Figure 8. Schematic picture of a flow microreactor

Figure 9. A simple lab set up of a PASSflow microreactor apparatus

Safety considerations:

Lab coats and safety glasses should be worn at all times. Use protective gloves whenever necessary. Electric pump and pressurized solvents are used in these experiments, which may cause hazards. Always obey all lab and safety rules of your university.

Mission:

You will perform a chemical transformation (either a reductive amination or an aldehyde reduction) in a flow reactor. The reactor material is loaded with the reducing agent (hydride), which is partly consumed during the reaction. You will isolate the reaction product from solution and regenerate the reactor.

Reactions:

1) Reductive Amination with $NaBH_4$

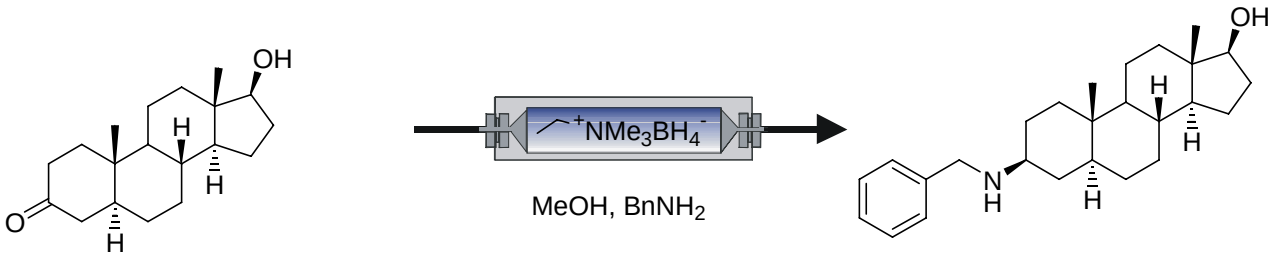


Figure 10. Reductive amination of 5α-androstan-17β-ol-3-one with benzyl amine

Microreactor Preparation

The microreactor has a total capacity of approximately 0.4 mmol. In order to generate the chloride form of the microreactor, the conditioning should follow the given washing procedure (flow rate 5 mL/min, check the flow direction):

1. ethanol (15 mL)
2. water (15 mL)
3. 1N NaOH (15 mL)
4. water (15 mL)
5. 1N HCl (15 mL)
6. water (15 mL)
7. ethanol (15 mL)

Loading of the microreactor with BH_4^-

Flush the microreactor (Cl^--form) with 50 mL of a 0.25 M aqueous solution of sodium borohydride (flow rate 7 mL/min). Then wash with water (10 mL) and methanol (15 ml).

Reaction

Pump a solution of 5α-androstan-17β-ol-3-one (41.6 mg, 0.125 mmol) and benzylamine (16.4 µL, 0.15 mmol) in methanol (40 mL) in a cycle mode through the BH_4^--loaded microreactor for 12 h at room temperature. After complete conversion, rinse the microreactor with methanol (20 mL). The combined organic solutions are concentrated under vacuum. Expected yield 85 %, (β/α = 4:1), small amounts of reduced alcohol may be present. Analyze your product by NMR.

Regeneration of the microreactor

The regeneration of the microreactor for the next reaction is done as described above (*Microreactor Preparation).*

2) Reduction of aldehydes with $NaBH_4$

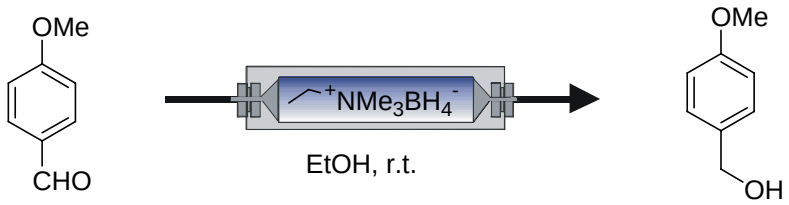


Figure 11. Reduction of *para*-methoxy benzaldehyde

Microreactor Preparation

The microreactor is conditioned and prepared for the reaction as described above.

Loading of the microreactor with BH_4^-

The loading of the microreactor with borate is done in the same way as described for the last experiment.

Aldehyde Reduction Reaction

Pump a solution of the aldehyde (0.25 mmol) in 10 mL of ethanol in a cycle mode through the BH_4^--loaded microreactor for 12 h at room temperature with a flow rate of 4 mL/min. After complete conversion rinse the microreactor with ethanol (20 mL), combine the organic solvents and concentrate under vacuum. Analyze your product by NMR.

Regeneration of the microreactor

The regeneration of the microreactor for the next reaction is done as described above (*Microreactor Preparation).*

References for this experiment: ref. XVI.

Discovery of novel catalysts for allylic alkylation using a visual colorimetric assay

Transition metal catalysed allylic alkylations represent a powerful tool for carbon-carbon bond formation reactions. Besides the rich chemistry of palladium complexes, other transition metals such as nickel, rhodium, iron, molybdenum, ruthenium etc. have shown promising catalytic activity. Therefore novel catalysts for allylic alkylation in neutral media using these metal ions should be discovered with the aid of fast, parallel colorimetric screening.

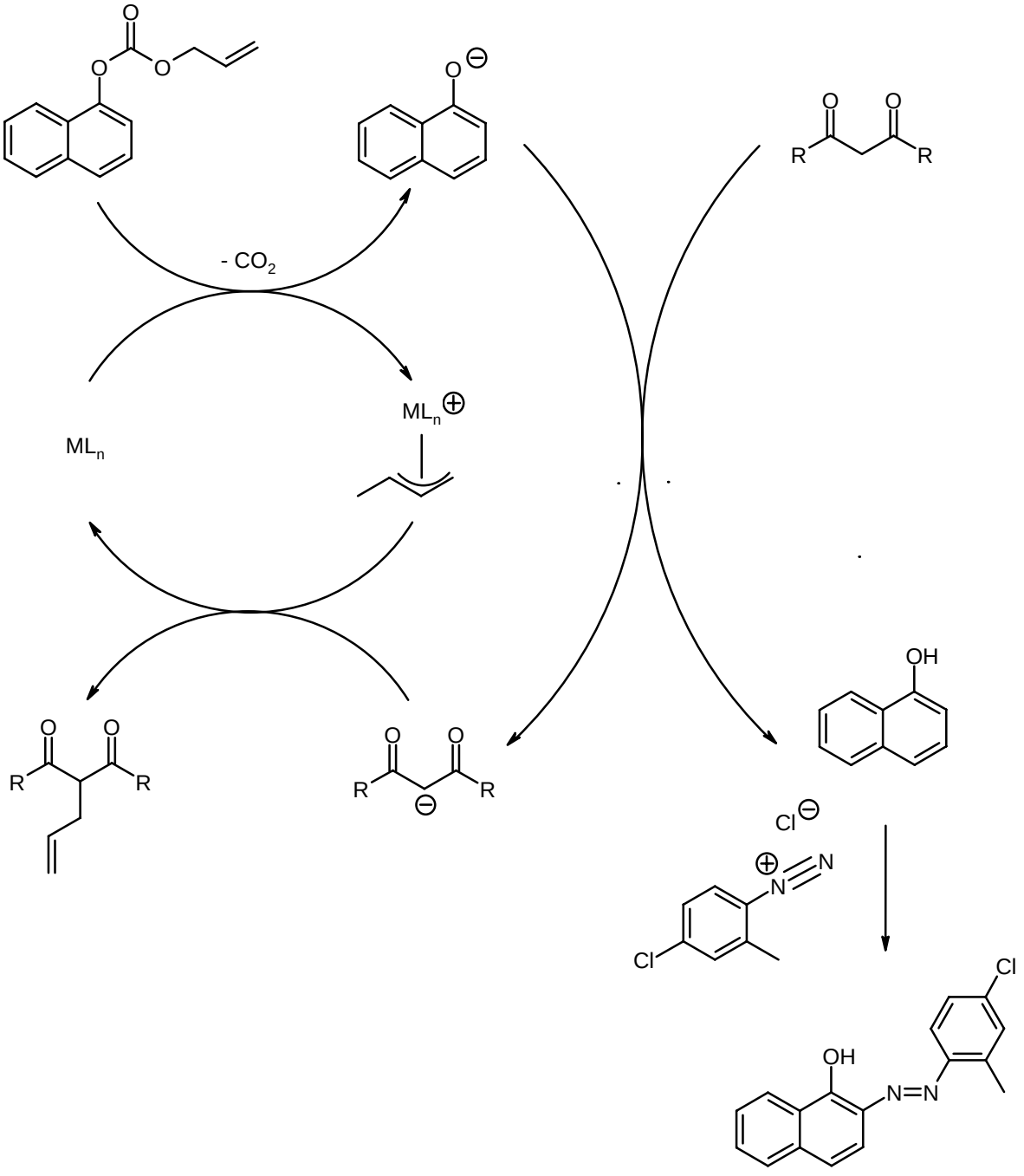


Figure 12. Catalytic allylic alkylation

Safety considerations: Lab coats and safety glasses should be worn at all times. Use protective gloves when necessary. Always obey all lab and safety rules of your university.

Mission:

You will study catalytic allylic alkylation reactions. The strategy for the detection of catalytic activity is based on release of colorless 1-naphtol which reacts with a diazonium salt to give a colored product. Under the present reaction conditions the starting allyl-naphtyl carbonate does not react with the Fast Red diazonium salt. You will use different substrates and allyl scavengers, and you have to find the right metal salt – ligand combination for this reaction.

Reactions:

Prepare the following solutions:

A:

25 mL 0.1 M naphtyl-allyl carbonate in THF.

B:

B1: 5 mL 0.4 M DABCO (1,4-diazabicyclo[2.2.2] octane) in THF.

B2: 5 mL 0.4 M dimedon (5,5-dimethyl-1,3-cyclohexanedione) in THF.

B3: 10 mL 0.4 M diethyl malonate in THF.

B4: 5 mL 0.4 M pyridine

C:

C1: 10 mL 0.001 M $FeCl_3$ in THF.

C2: 10 mL 0.001 M $NiCl_2$ in THF.

C3: 10 mL 0.001 M $Pd(OAc)_2$ in THF.

D:

D1: 10 mL 0.001 M PPh_3 in THF.

D2: 10 mL 0.001 M $P(OEt)_3$ in THF.

D3: 10 mL 0.001 M P(*o*-Tol)$_3$ in THF.

Solution of Fast Red: Dissolve 30 mg of Fast Red in THF (2 mL) and add water (0.1 mL)

At the end of this experiment you will find a matrix for each reaction to facilitate the screening process. Make a photocopy of the sheets and put the respective vial of your screening in the right square.

B1:

Take 9 small reaction tubes and add 0.5 mL of solution A and B1 into each tube. Then add 0.5 mL of C1 to the first three tubes, C2 to the second three tubes and C3 to the third three tubes, 0.5 mL of D1 to the first, 4th and 7th tube, D2 to the 2nd, 5th and 8th tube, D3 to the 3rd, 6th and 9th tube. Close the tubes and allow them to react for 2 h; keep them as dry as possible. Then add a solution of Fast Red in wet THF (THF/H_2O 20/1) and look for any change of the color.

B2:

Take 9 small reaction tubes and add 0.5 mL of solution A and B2 into each tube. Then add 0.5 mL of C1 to the first three tubes, C2 to the second three tubes and C3 to the third three tubes, 0.5 mL of D1 to the first, 4th and 7th tube, D2 to the 2nd, 5th and 8th tube, D3 to the 3rd, 6th and 9th tube. Close the tubes and allow them to react for 2 h; keep them as dry as possible. Then add a solution of Fast Red in wet THF (THF/H_2O 20/1) and look for any change of the color.

B3:

Take 9 small reaction tubes and add 0.5 mL of solution A and B3 into each tube. Then add 0.5 mL of C1 to the first three tubes, C2 to the second three tubes and C3 to the third three tubes, 0.5 mL of D1 to the first, 4th and 7th tube, D2 to the 2nd, 5th and 8th tube, D3 to the 3rd, 6th and 9th tube. Close the tubes and allow them to react for 2 h; keep them as dry as possible. Then add a solution of Fast Red in wet THF (THF/H_2O 20/1) and look for any change of the color.

B4:

Take 9 small reaction tubes and add 0.5 mL of solution A and B4 into each tube. Then add 0.5 mL of C1 to the first three tubes, C2 to the second three tubes and C3 to the third three tubes, 0.5 mL of D1 to the first, 4th and 7th tube, D2 to the 2nd, 5th and 8th tube, D3 to the 3rd, 6th and 9th tube. Close the tubes and allow them to react for 2 h; keep them as dry as possible. Then add a solution of Fast Red in wet THF (THF/H_2O 20/1) and look for change of the color.

6 Control samples should be prepared using 0.5 mL pure THF instead of using the solutions of metal salts or ligands. This is necessary to show that ligands or metal salts do not react with Fast Red to give colored products. For the preparation of the control samples it is sufficient to only use B3. (so prepare: “A+B3+C1-C3+THF” and “A+B3+THF+D1-D3”).

References for this experiment; adapted from: O. Lavastre, J. P. Morken, *Angew. Chem. Int. Ed.* **1999**, *38*, 3163 - 3165.

B1	**D1**	**D2**	**D3**
C1			
C2			
C3			

B2	**D1**	**D2**	**D3**
C1			
C2			
C3			

B3	**D1**	**D2**	**D3**
C1			
C2			
C3			

B4	**D1**	**D2**	**D3**
C1			
C2			
C3			

Parallel Combinatorial Esterification and Identification of Different Odors

The success of combinatorial experiments always depends on general and efficient reaction procedures. Simple and practical screening methods are also necessary to analyze the large number of synthesised compounds. Therefore simple biochemical analytical tests are often used to identify the molecule of interest in a very short and fast way.

Safety considerations:

Lab coats and safety glasses should be worn at all times. Wear protective gloves when it is necessary. Use the assigned syringe for each vessel. **Each syringe has to be cleaned with water and acetone after each use!** Always obey all lab and safety rules of your university.

Mission:

In this experiment you will learn how to create a small library of eight esters by sulfuric-acid catalyzed Fischer esterification in a parallel way. After a short work-up (also done in parallel) a distinct odor should be identified.

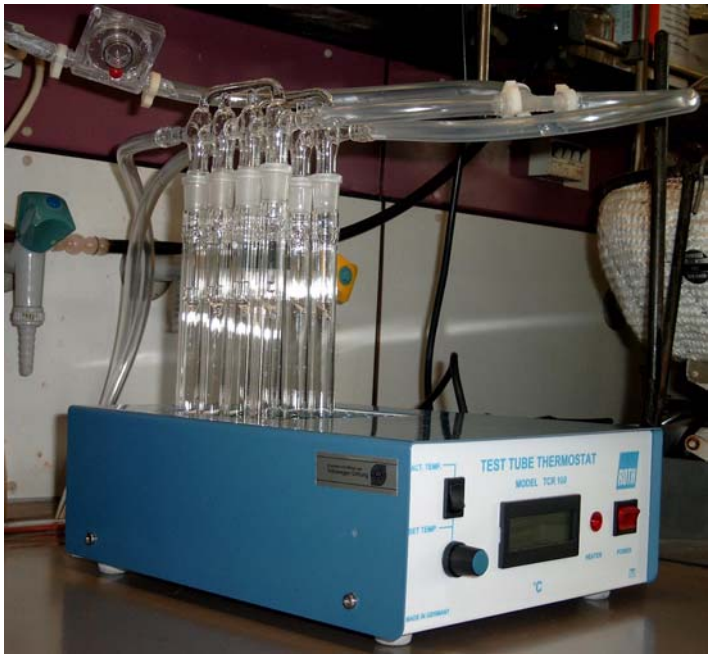


Figure 13. Parallel esterification using test tubes in a heating block

Reactions:

All esterifications are performed in a heating-block with a special cooling system.

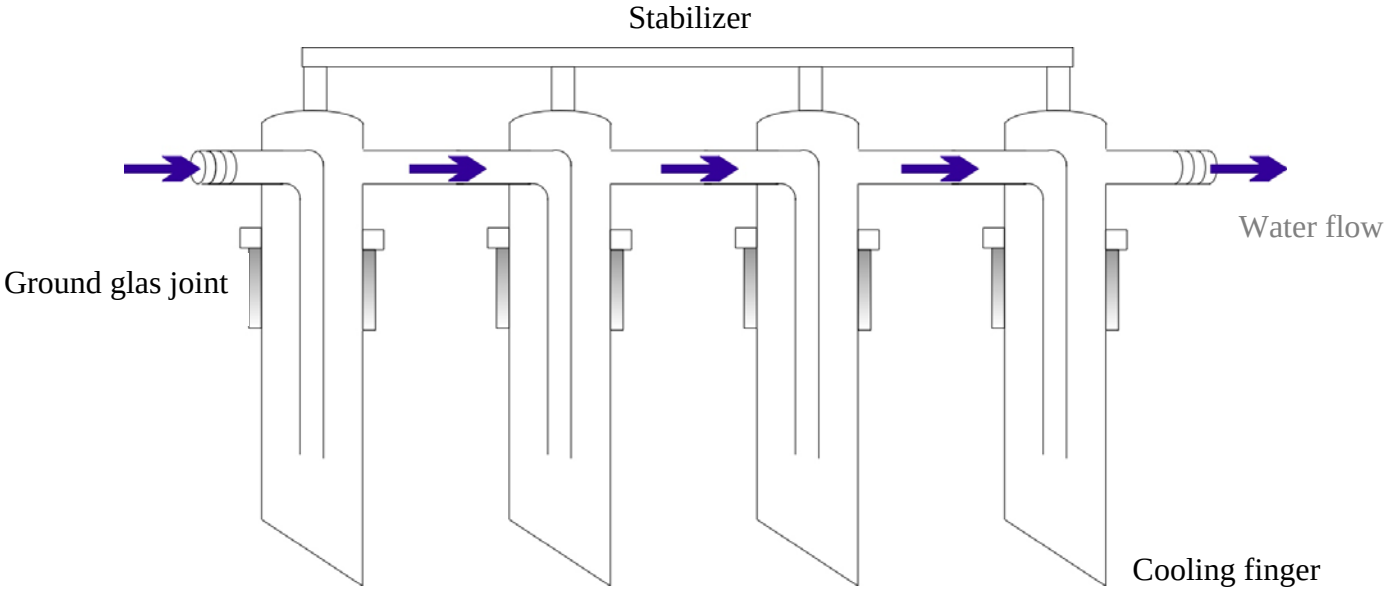


Figure14. Scheme of Cooling system

Eight parallel reactions are performed. To facilitate the identification of products, the reaction tubes should be labeled as shown in Figure 12.

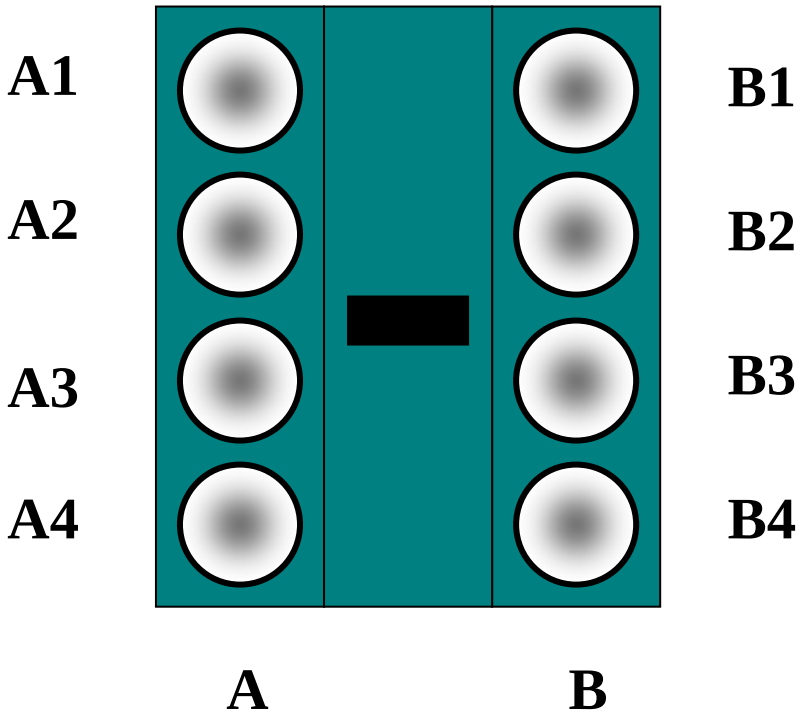


Figure 15. Labeling of the eight parallel reactions

Fill columns **A** and **B** with two different alcohols. **A1-A4** and **B1-B4** represent the acids used in the different reactions. Each reaction tube should be filled with the carboxylic acid first followed by the necessary amount of alcohol and sulfuric acid. If you have to work with **anthranilic acid** the whole mixing procedure should be performed in an ice bath (exothermic reaction!).

Once all of the reactants have been added, the cooling fingers have to be plugged into each tube to allow reflux. The heating block is turned on (maximum temperature setting; 100°C). Each reaction should reflux for approximately 30 min. Each group has to identify only one of the characteristic odors (wintergreen, banana or pear).

a) Identification of **Wintergreen** (tooth paste, chewing gum)

A:	**Methanol**		
A1:	6 mmol anthranilic acid	50 mmol MeOH	40 drops H_2SO_4
A2:	6 mmol benzoic acid	24 mmol MeOH	15 drops H_2SO_4
A3:	6 mmol salicylic acid	24 mmol MeOH	15 drops H_2SO_4
A4:	12 mmol propionic acid	24 mmol MeOH	15 drops H_2SO_4
B:	**Ethanol**		
B1:	6 mmol anthranilic acid	50 mmol EtOH	40 drops H_2SO_4
B2:	6 mmol benzoic acid	24 mmol EtOH	15 drops H_2SO_4
B3:	6 mmol salicylic acid	24 mmol EtOH	15 drops H_2SO_4
B4:	12 mmol propionic acid	24 mmol EtOH	15 drops H_2SO_4

b) Identification of **Banana**

A:	**1-Methylpropanol**		
A1:	12 mmol butyric acid	24 mmol 1-methylpropanol	15 drops H_2SO_4
A2:	6 mmol benzoic acid	24 mmol 1-methylpropanol	15 drops H_2SO_4
A3:	6 mmol salicylic acid	24 mmol 1-methylpropanol	15 drops H_2SO_4
A4:	24 mmol acetic acid	24 mmol 1-methylpropanol	15 drops H_2SO_4

B:	**3-Methyl-1-butanol**		
B1:	12 mmol butyric acid	24 mmol 3-methyl-1-butanol	15 drops H_2SO_4
B2:	6 mmol benzoic acid	24 mmol 3-methyl-1-butanol	15 drops H_2SO_4
B3:	6 mmol salicylic acid	24 mmol 3-methyl-1-butanol	15 drops H_2SO_4
B4:	24 mmol acetic acid	24 mmol 3-methyl-1-butanol	15 drops H_2SO_4

c) Identification of **Pear**

A:	**Propanol**		
A1:	12 mmol butyric acid	24 mmol propanol	15 drops H_2SO_4
A2:	6 mmol benzoic acid	24 mmol propanol	15 drops H_2SO_4
A3:	6 mmol salicylic acid	24 mmol propanol	15 drops H_2SO_4
A4:	24 mmol acetic acid	24 mmol propanol	15 drops H_2SO_4
B:	**Isopropanol**		
B1:	6 mmol anthranilic acid	50 mmol isopropanol	40 drops H_2SO_4
B2:	6 mmol benzoic acid	24 mmol isopropanol	15 drops H_2SO_4
B3:	6 mmol salicylic acid	24 mmol isopropanol	15 drops H_2SO_4
B4:	12 mmol propionic acid	24 mmol isopropanol	15 drops H_2SO_4

Work-up:

After the reaction mixtures have cooled down to room temperature, a 10 ml syringe, labeled according to the reaction tube, is used to suck the mixture out of the tube. The tube is washed with ether (3 mL), water (3 mL) and ether (3 mL) and all liquids are collected in the same syringe. This syringe is then used for extraction. The extraction procedure can be performed for all eight reactions at the same time.

The organic layer is extracted twice with 5% aqueous $NaHCO_3$ solution. Each ether phase is then transferred into a small labeled glass vial and closed with a plastic cap.

To identify the odor of each ester a piece of paper towel is dipped into the vial.

Note: You should be able to identify more than just the odor required!

Reference for this experiment; adapted from: D. Birney, S. Starnes, *J. Chem. Edu.* **1999**, *76*, 1560-1561.

Dyes on Solid support

Cyanine dyes have many applications. Their use as photosensitizers for colour photography, markers for flow cytometry or phototherapeutic agents make them attractive targets for chemical synthesis. Asymmetric cyanine dyes consist of two different heteroaromatic fragments conjugated by a mono- or polymethene chain. By varying the length of this chain, the photophysical properties of these dyes can be altered.

Safety considerations:

Lab coats and safety glasses should be worn at all times. Use protective gloves when necessary. Always obey all lab and safety rules of your university.

Mission:

In this experiment you synthesise different dyes on a solid support using a Rink amide MBHA resin.

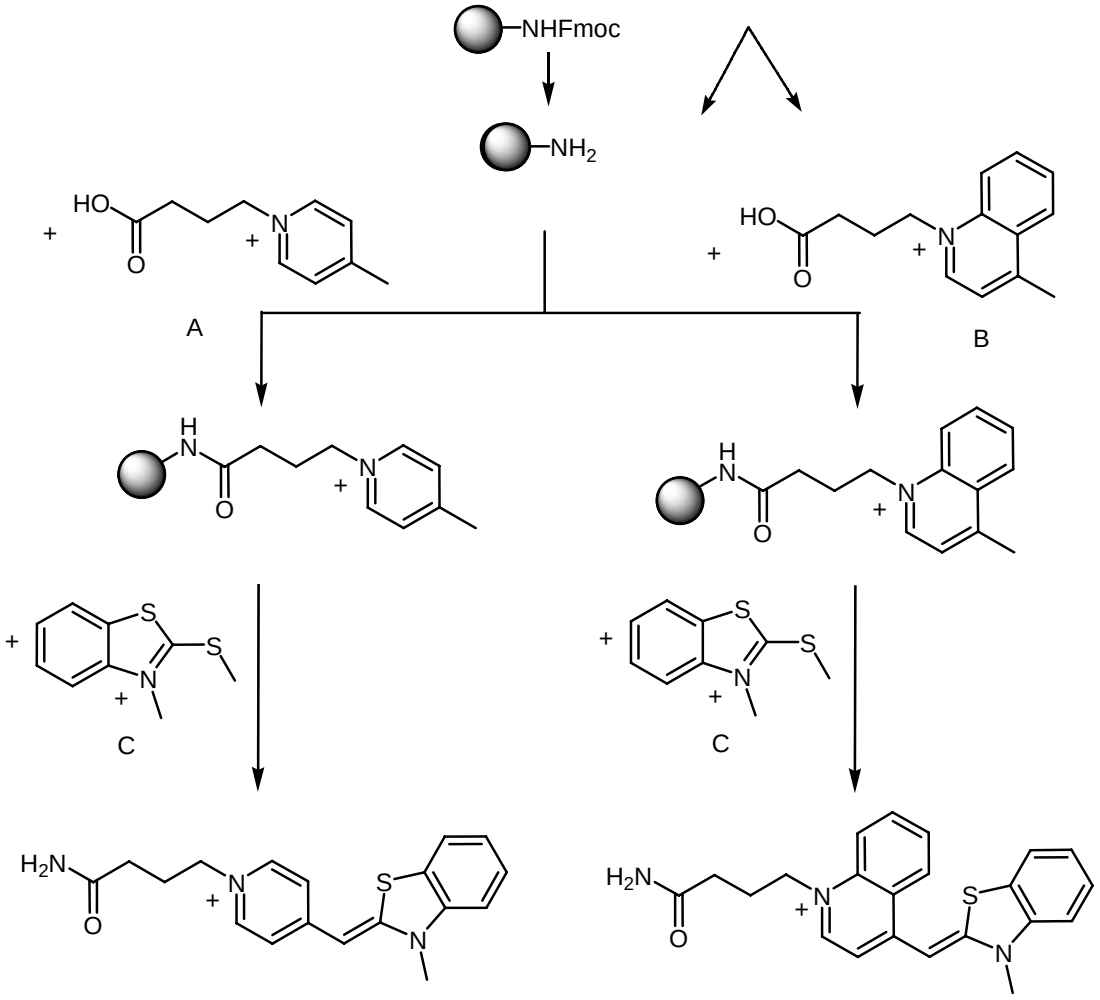


Figure 16. Dye synthesis

Reactions:

*Pyridine derivative **A**:*

4-Methylpyridine (picoline) (0.97 mL, 10 mmol) and 4-bromobutyric acid (1.67 g, 10 mmol) are mixed in a round bottom flask and refluxed for 8 h. After cooling, the mixture is poured into EE under vigorous stirring. The precipitate is filtered off and purified by recrystallisation from acetone.

*Chinoline derivative **B**:*

4-Methylchinoline (lepidine) (1.39 mL, 10 mmol) and 4-bromobutyric acid (1.67 g, 10 mmol) are mixed in a round bottom flask and refluxed for 8 h. After cooling the mixture is poured into EE under vigorous stirring. The precipitate is filtered off and purified by recrystallisation from acetone.

*Benzoethiazole salt **C**:*

2-Thiomethylbenzothiazole (1.81 g, 10 mmol) and p-methyl-toluenesulfonate (1.91mL, 10 mmol) are heated to 140 °C for 4 h. After cooling the mixture is poured into EE under vigorous stirring. The formed crystals are filtered off.

Synthesis of the dyes on a solid support:

The reactor tube (a syringe with frit) is filled with 25 mg Fmoc-protected Rink amide MBHA resin and preswollen twice in 1.0 mL of DMF for 30 min.

Deprotection: Piperidine (1.0 mL; 25% in DMF) is added. After 5 min shaking the resin is washed with DMF (3 x 2 mL). Repeat this step but shake for 30 min, then drain the solvent off and wash with DMF (3 x 2 mL) and DCM (3 x 2 mL).

1st Coupling:

First tube: Pyridine derivative **A** (14 mg, 0.05 mmol), HBTU (19 mg, 0.05 mmol) and 9 µL DPIEA are dissolved in 0.5 mL DMF:Pyridine (1/1). The mixture is added to the resin. After 2 h min of shaking, the solvent is drained and the resin is washed with DMF (3 x 2 mL) and DCM (3 x 2 mL).

Second tube: See first tube but **A** is substituted by Chinoline derivative **B** (19 mg, 0.05 mmol).

2nd Coupling:

After the addition of 0.75 mL of DCM to each reactor, Benzoethiazole salt **C** (16 mg, 0.04 mmol) and 28 µL NEt_3 are added to the resin. This mixture is shaken for 1.5 hours, the resin filtered off and washed with DCM (3 x 2 mL).

Cleavage: Each tube is filled with 0.5 mL of TFA:H_2O (95:5). Shaking for 1 hour completes the cleavage (Observe the colour!). Each solution is filtered into a new flask and the resin is washed with 0.5 mL of the TAF mixture. The solvent is evaporated to obtain the different dyes.

Characterize the dye by recording its UV spectrum. Postulate a possible reaction mechanism.

Reference for this experiment; adapted from: J. Isacsson, G. Westman, *Tetrahedron Lett.* **2001**, *42*, 3207-3210.

Abbreviations

DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMF	N,N-Dimethylformamide
HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	N-Hydroxybenzotriazole
TFA	Trifluoroacetic acid
MBHA	4-Methylbenzhydrylamine

Glossary

Cleavage

Process of releasing compound from solid support, thereby permitting assay or analysis of the compound by solution-phase methods. Dissolution of the compound following cleavage, rather than the cleavage step itself, may be rate-limiting.

Fischer esterification

Proton catalyzed formation of an ester from an alcohol and an acid.

Fmoc strategy

Usage of Fmoc groups (9-Fluorenylmethoxycarbonyl) in a peptide synthesis for protecting the N-terminal amines.

Kaiser test

Colorimetric test on free amines using ninhydrine.

Library

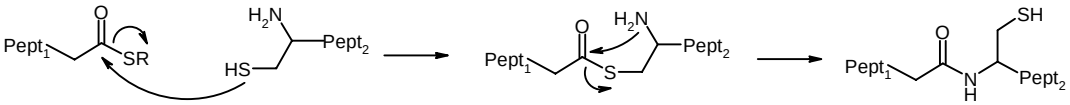
The set of compounds which would be created by reacting together all possible combinations of selections from lists of precursors. When more than one list is involved, all combinations of list entries are produced.

Loading

Characteristic property of a solid support which describes the amount of a specific chemical species per mass unit of the support.

Native chemical ligation

Linkage of two oligopeptides. One C-terminus is activated as a thioester, the other fragment must have a N-terminal cysteine.



Parallel synthesis

Strategy whereby sets of discrete compounds are prepared simultaneously in arrays of physically separate reaction vessels or microcompartments without interchange of intermediates during the assembly process.

PEG monomethylether

Poly(ethylene glycol) monomethylether. A linear industrial polymer available in a range of molecular weights, which is an alternative support material to poly styrene. PEG is soluble in DCM and water, but precipitates from ether or ethanol. This allows combining advantages of solid phase synthesis and reaction in homogeneous solution.

Peptide synthesizer

Machine for automated peptide synthesis.

Polymer bead

Normally spherical particle of polymer.

Preswell

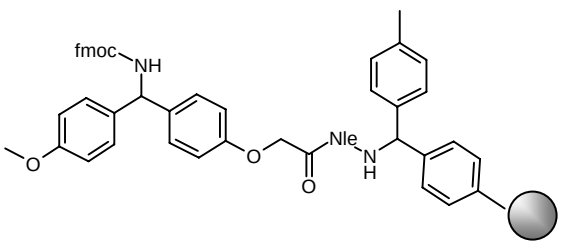
Swelling of the resin with solvents before each reaction.

Resin

Insoluble polymeric material which allows ready separation from liquid-phase materials by filtration; can be used to carry library members (i.e. solid support) or reagents, or to trap excess reagents or reaction by-products.

Rink amide MBHA resin

The Rink amide linker is attached via norleucine to MBHA resin.
Cleavage from this resin can be effected by a single step treatment with 95 % TFA.



Scavenger resins

Solid-supported reagent which will react with undesired materials (such as excess reagents) and remove them from solution.

Solid support

Insoluble, functionalized, polymeric material to which library members or reagents may be attached (often via a linker) allowing them to be readily separated (by filtration, centrifugation, etc.) from excess reagents, soluble reaction by-products, or solvents.

Split-mix protocol

Strategy for assembly of a combinatorial library. The solid support is divided into portions, each of which is subjected to reaction with a single building block. Pooling of these portions results in a single batch of solid support bearing a mixture of components. Repetition of the divide, couple, recombine processes results in a library where each discrete particle of solid support carries a single library member, and the number of members is equal to the product of the number of building blocks incorporated at each step.

Wang resin

(4-hydroxymethyl)phenoxymethyl polystyrene (see **Figure 1**).