THE UNIVERSITY OF EDINBURGH

The Development of New Tools for High-Throughput-Synthesis and High-Throughput-Screening

by

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Doctor of Philosophy

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High-throughput-synthesis has become a fundamental part of the drug-discovery process, allowing the rapid synthesis of hundreds to potentially millions of compounds. There are many strategies used in high-throughput-synthesis and one technique, based on the use of polymer-supported reagents in solution-phase, has evolved considerably over the past decade. However, only a limited number of efficient polymer-supported coupling reagents have been reported and many of them offer serious drawbacks, unlike their solution-phase equivalents. The available solid-phase supported coupling reagents fall into this category, with many available but few proving really reliable. A comparison between common coupling reagents (IIDQ, HATU, PyAOP and BOP-CI) was therefore carried out and revealed that IIDQ was the reagent of choice in the absence of activation step. Polymer-supported IIDQ was therefore targeted. This reagent performed better than commercially available HATU and all supported carbodiimides. Scope and limitation studies proved that the reagent was a versatile tool for the synthesis of amides from carboxylic acids and amines, including hindered substrates, secondary amines and anilines.

A new strategy for the high-throughput-screening of small-molecules was developed. Microarrays are a powerful method to test quickly a wide range of small molecules against biological targets. In parallel, fragment-based drug discovery has boomed in the past decade. The combination of the two principles gave birth to the new concept of dual fragment microarrays. The technology was developed and evaluated using fragments known to bind to the human FK506-binding protein. These fragments were synthesised in solution-phase, then linked to a PNA tag synthesised on solid-phase, followed by release into stock solution and hybridisation onto DNA microarrays. Screening the arrays of mixtures of two fragments revealed that the expected strongest “dual” interaction was detected as the most intense spot, and thus validated the concept of dual fragment microarrays.
DECLARATION

I declare that:

• This thesis has been composed by myself
• The work presented in this thesis is my own
• No part of this thesis has been previously submitted at these or any other University for any other degree or professional qualification.

Eric Valeur
3rd November 2006
The research described in this thesis was carried out under the supervision of Prof. Mark Bradley at the University of Southampton (Oct. 2002 – Jan. 2005) and the University of Edinburgh (Feb. 2005 – Jan. 2006).

Part of the work presented here has been published or in the process of being published:

- "PS-IIDQ: an efficient amide coupling reagent"

- "PS-IIDQ: A supported coupling reagent for efficient and general amide bond formation"
  Valeur, E.; Bradley, M. *To be submitted*

- "Efficient, mild, parallel and purification-free synthesis of aryl ethers via the Mitsunobu reaction"
  Valeur, E.; Bradley, M. *To be submitted*
ACKNOWLEDGMENTS

I would like firstly to thank my supervisor, Prof. Mark Bradley for all his help and support during these three years. I am very grateful to my advisor in Southampton, Dr. Bruno Linclau, for all his advices and constructive critics! I would also like to thank all the members of Prof. Bradley's research group, especially the members of the Combinatorial Centre of Excellence: Luciano, Alessandra, Gianluca, Christophe and Delphine. In particular, I would like to thank Christophe and Luciano for their friendship and their dedication to make the lab run as efficiently as possible.

I am also very grateful to Juanjo for all his help and time for the microarray project, and to Delphine for her great advices on the PNA synthesis!

Finally, I would like to thank my family for their continual support all along my studies and during my PhD, especially my wife Nicola and my son Antoine. Having to move to Edinburgh in the middle of my PhD while you were still in Southampton was a real shock and nightmare, but we finally surmounted this difficult time, and I dedicate this work to you.
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# Abbreviations

## Aminoacids

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<tr>
<td>Aib</td>
<td>Aminoisoasbyric acid</td>
</tr>
<tr>
<td>Ala</td>
<td>Alanine</td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
</tr>
<tr>
<td>Asn</td>
<td>Asparagine</td>
</tr>
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<td>Asp</td>
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</tr>
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<tr>
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<td>Hip</td>
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## General

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<td>Ac</td>
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<tr>
<td>ACP</td>
<td>Acyl carrier protein decapeptide 65-74</td>
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<tr>
<td>ADDP</td>
<td>1,1'-(azodicarbonyl) dipiperidine</td>
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<tr>
<td>Alloc</td>
<td>Allyloxycarbonyl</td>
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<tr>
<td>Anal.</td>
<td>Elemental Analysis</td>
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<tr>
<th>Abbreviation</th>
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<td>2-tert-Butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorin</td>
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<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butoxycarbonyl</td>
</tr>
<tr>
<td>br</td>
<td>Broad</td>
</tr>
<tr>
<td>Bz</td>
<td>Benzoyl</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-Coupled Device</td>
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<tr>
<td>cHex</td>
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<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>CPG</td>
<td>Controlled-Pore Glass</td>
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<td>d</td>
<td>doublet</td>
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<tr>
<td>Da</td>
<td>Dalton</td>
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<tr>
<td>DABCO</td>
<td>Bicyclo[2,2,2]-1,4-diazaoctane</td>
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<td>DAP-DP</td>
<td>(p-dimethylaminophenyl)-diphenylphosphine</td>
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<td>DBAD</td>
<td>Di-tert-butyl azodicarboxylate</td>
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<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
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<td>DEAD</td>
<td>Diethylazodicarboxylate</td>
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<tr>
<td>DEPT</td>
<td>Distortionless Enhanced Polarization Transfer</td>
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<tr>
<td>DHTD</td>
<td>4,7-dimethyl-3,5,7-hexahydro-1,2,4,7-tetraazocin-3,8-dione</td>
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<td>DIAD</td>
<td>Diisopropylazodicarboxylate</td>
</tr>
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<td>N,N-Dimethylformamide</td>
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<td>DMPU</td>
<td>Dimethylpropyleneurea</td>
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<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DNAD</td>
<td>Bis(5-norbornen-2-ylmethyl) azodicarboxylate</td>
</tr>
<tr>
<td>DPPE</td>
<td>1,2-bis(diphenylphosphino)ethane</td>
</tr>
<tr>
<td>ELSLD</td>
<td>Evaporative Light Scattering Detector</td>
</tr>
<tr>
<td>ES</td>
<td>Electrospray</td>
</tr>
<tr>
<td>FKBP</td>
<td>FK-506 Binding Protein</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-Fluorenlymethoxycarbonyl</td>
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<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<tr>
<td>HMPA</td>
<td>Hexamethylphosphoramidate</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
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<td>HR</td>
<td>High Resolution</td>
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HSQC  Heteronuclear Single Quantum Correlation Spectra
HTS  High-Throughput-Screening
iBuOH  Isobutanol
IBX  2-Iodoxybenzoic acid
LCMS  Liquid Chromatography – Mass Spectrometry
LHRH  Luteinising Hormone Releasing Hormone
m  Multiplet (NMR) or medium (IR)
MAS  Magic Angle Spinning
MMt  Monomethoxytrityl
Mp  Melting Point
MS  Mass Spectrometry
MTBD  7-Methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene
MW  Molecular Weight
NMM  N-methyl-morpholine
NMP  N-Methyl-2-Pyrrolidone
NMR  Nuclear Magnetic Resonance
PBS  Phosphate Buffered Saline
P.E.  Petroleum Ether
PEG  Polyethyleneglycol
Ph  Phenyl
PhG  Phenylglycine
PMA  Phosphomolybdic acid
PNA  Peptide Nucleic Acid
PS  Polystyrene
RCM  Ring Closure Metathesis
RNA  Ribonucleic acid
s  Singlet (NMR) or strong (IR)
SAM  Self-Assembled Monolayer
SDS  Sodium n-Dodecyl Sulfate
SPOS  Solid Phase Organic Synthesis
SPE  Solid Phase Extractor
SPR  Surface Plasmon Resonance
TAP  Tris(dimethylamino)phosphine
TBAF  Tetra-n-butylammonium fluoride
TBD  1,5,7-Triazabicyclo[4.4.0]dec-5-ene
TBS  Tert-butyl-dimethyl-silyl
TEA  Triethylamine
TES  Triethylsilane
TFA  Trifluoroacetic acid
THF  Tetrahydrofuran
TIS  Triisopropylsilane
<table>
<thead>
<tr>
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<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>TMAD</td>
<td>( N, N, N', N')-Tetramethyldiazodicarbonamide</td>
</tr>
<tr>
<td>TMU</td>
<td>Tetramethylurea</td>
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<td>TPAP</td>
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<td>TPP</td>
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<td>( t_r )</td>
<td>Retention time</td>
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<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>w</td>
<td>weak</td>
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<td>Z</td>
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### Coupling Reagents and Additives

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<td>Chloro-1,1,3,3-tetramethyluronium hexachloroantimonate</td>
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<td>AOMP</td>
<td>5-(7-azabenzotriazol-1-yl)oxytris(dimethylamino) phosphonium hexachloroantimonate</td>
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<tr>
<td>AOP</td>
<td>(7-azabenzotriazol-1-yl)-oxygen-(dimethylamino) phosphonium hexachloroantimonate</td>
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<td>BBC</td>
<td>Benzotriazolyl-bis(pyridylidino)-carbonium hexafluorophosphate</td>
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<tr>
<td>BDMP</td>
<td>5-(1H-benzotriazol-1-yl)-oxo-3,4-dihydro-1-methyl-2H-pyrrrolium hexachloroantimonate</td>
</tr>
<tr>
<td>BEMT</td>
<td>2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate</td>
</tr>
<tr>
<td>BEP</td>
<td>2-bromo-1-ethylpyridinium tetrafluoroborate</td>
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<td>BEPH</td>
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<td>4,5-B(HATU)</td>
<td>( N)-[(dimethylamino)(3H-1,2,3-triazolo[4,5-c]isoquinolin-3-yloxy)-N-methylmethanaminium hexafluorophosphate</td>
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<td>BIODPP</td>
<td>Diphenyl benzo[d]isoxazol-3-ylphosphonate</td>
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<td>BMMP</td>
<td>1-(1-(1H-benzotriazol-1-yl)oxy-1,3,5-triazol-1-yl)oxyethyldiene)pyrrolidinium hexachloroantimonate</td>
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<td>2-bromo-3-methyl-4-methyl thiazolium bromide</td>
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<td>BOI</td>
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<tr>
<td>BPMMP</td>
<td>1-(1H-benzotriazol-1-yl)oxy-phenyl-methylene pyrrolidinium hexachloroantimonate</td>
</tr>
<tr>
<td>BroP</td>
<td>Bromotris(dimethylamino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>BTFFH</td>
<td>Bis(tetramethylene)fluoroformamidinium hexafluorophosphate</td>
</tr>
<tr>
<td>CBDO</td>
<td>2-chlorobenzo[d][1,3]dioxol-1-ium hexachloroantimonate</td>
</tr>
<tr>
<td>CBMIT</td>
<td>1,10-carbonylbis(3-methyl-imidazolium)-triflate</td>
</tr>
<tr>
<td>CDI</td>
<td>Carbonyldimidazole</td>
</tr>
<tr>
<td>CDMS</td>
<td>Chlorodimethylsulfonium hexachloroantimonate</td>
</tr>
<tr>
<td>CDMT</td>
<td>2-chloro-4,6,6-dimethoxy-1,3,5-triazine</td>
</tr>
<tr>
<td>CDTP</td>
<td>2-chloro-1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-1-ium perchlorate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>CIP</td>
<td>2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate hexafluorophosphate</td>
</tr>
<tr>
<td>Clop</td>
<td>Chlorotris(dimethylamino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>CMMM</td>
<td>Chloro(4-morpholino)methylene morpholinium hexafluorophosphate</td>
</tr>
<tr>
<td>CPMA</td>
<td>(Chlorophenylthiomethylene)dimethylaminium chloride</td>
</tr>
<tr>
<td>CPDT</td>
<td>2-chloro-5-phenyl-1,3-dithiol-1-ium hexachloroantimonate</td>
</tr>
<tr>
<td>DAST</td>
<td>Diethylaminosulfur-trifluoride</td>
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<td>DCIH</td>
<td>1,3-dimethyl-2-chloro-4,5-dihydro-1H-imidazolium hexafluorophosphate</td>
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<tr>
<td>DCMT</td>
<td>2,4-Dichloro-6-methoxy-1,3,5-triazine</td>
</tr>
<tr>
<td>DEPC</td>
<td>Diphenyl phosphorochloridate</td>
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<tr>
<td>DEPBT</td>
<td>3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one</td>
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<tr>
<td>DepOA</td>
<td>3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl diethyl phosphate</td>
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<td>DepODhbt</td>
<td>Diethyl 4-oxobenzo[d][1,2,3]triazin-3(4H)-yl phosphate</td>
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<td>DFIH</td>
<td>1,3-dimethyl-2-fluoro-4,5-dihydro-1H-imidazolium hexafluorophosphate</td>
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<td>DIC</td>
<td>Diisopropylcarbodiimide</td>
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<tr>
<td>DmppOA</td>
<td>1-(2,8-dimethylphenoxophosphinoyloxy)-7-azabenzotriazole</td>
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<td>DMTMM</td>
<td>4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholinium chloride</td>
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<td>DOMP</td>
<td>5-(3',4'-dihydro-4'-oxo-1',2',3'-benzotriazin-3'-yloxy)-3,4-dihydro-1-methyl 2H-pyrrolium hexachloroantimonate</td>
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<tr>
<td>Dpop-Cl</td>
<td>Diphenyl phosphorochloridate</td>
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<tr>
<td>DpopOA</td>
<td>1-(diphenoxophosphoryloxy)-7-azabenzotriazole</td>
</tr>
<tr>
<td>DpopODhbt</td>
<td>3-(diphenoxophosphinoyloxy)-3,4-dihydro-4-oxo-1,2,3-benzotriazene</td>
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<tr>
<td>DPP</td>
<td>Diphenylphosphate</td>
</tr>
<tr>
<td>DPPA</td>
<td>Diphenylphosphoryl azide</td>
</tr>
<tr>
<td>Dpp-Cl</td>
<td>Diphenylphosphinic chloride</td>
</tr>
<tr>
<td>DtpOA</td>
<td>1-[di(O-tolyl)-phosphinoyloxy]-7-azabenzotriazole</td>
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<tr>
<td>DtpOBt</td>
<td>1-[di(O-tolyl)phosphinoyloxy]benzotriazole</td>
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<tr>
<td>DtpODhbt</td>
<td>3-di(O-tolyl)phosphinoyloxy]-3,4-dihydro-4-oxo-1,2,3-benzotriazene</td>
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<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide</td>
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<td>EEDQ</td>
<td>N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline</td>
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<tr>
<td>ENDPP</td>
<td>Phosphoric acid 3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-en-4-yl ester diphenyl ester</td>
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<td>FDMP</td>
<td>3,5-bis(trifluoromethyl)phenyl diphenylphosphinate</td>
</tr>
<tr>
<td>FDPP</td>
<td>Pentfluorophenyl diphenyl phosphinate</td>
</tr>
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<td>FEP</td>
<td>2-fluoro-1-ethylpyridinium tetrafluoroborate</td>
</tr>
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<td>FEPH</td>
<td>2-fluoro-1-ethylpyridinium hexachloroantimonate</td>
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<tr>
<td>FOMP</td>
<td>5-(pentfluorophenyl)oxy]-3,4-dihydro-1-methyl-2H-pyrrolium hexachloroantimonate</td>
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<td>HAMDU</td>
<td>O-(7-azabenzoazol-1-yl)-1,3-dimethyl-1,3-dimetyleneuronium hexafluorophosphate</td>
</tr>
<tr>
<td>HAMTU</td>
<td>O-(7-azabenzoazol-1-yl)-1,1,3,3-bis(pentamethylene)uronium hexafluorophosphate</td>
</tr>
</tbody>
</table>
HAPipU $O$-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(pentamethylene) uronium hexafluorophosphate

HAPyTU $S$-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)thiouonium hexafluorophosphate

HAPyU $1$-(1-pyrrolidinyl-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene)pyrrolidinium hexafluorophosphate $N$-oxide

HATU $O$-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyliuronium hexafluorophosphate

HBMDU $O$-(benzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorophosphate

HBMP $1H$-benzo[d][1,2,3]triazol-1-ylmethanesulfonate

HBpipU $O$-(benzotriazol-1-yl)-1,1,3,3-bis(pentamethylene)uroniumhexafluorophosphate

HBSP $1H$-benzo[d][1,2,3]triazol-1-ylbenzenesulfonate

HBTU $O$-(benzotriazol-1-yl)-1,1,3,3-tetramethyliuronium hexafluorophosphate

HCTU $1H$-Benzotriazolium-1-{bis(dimethylamino)methylene} -Schlоро-hexafluorophosphatе (1-),3-oxide

HCSCP 6-chloro-$1H$-benzo[d][1,2,3]triazol-1-yl 4-chlorobenzenesulfonate

HCSP 6-chloro-$1H$-benzo[d][1,2,3]triazol-1-yl benzenesulfonate

HDATU (bis(dimethylamino)methyl)(4-oxopyrido[3,2-d][1,2,3]triazin-3(4H)-yl)oxonium hexafluorophosphate

HDADU (bis(dimethylamino)methyl)(4-oxopyrido[3,2-d]pyrimidin-3(4H)-yl)oxonium hexafluorophosphate

HDAPyU 1-((4-oxopyrido[3,2-d][1,2,3]triazin-3(4H)-yloxy)(pyrrolidin-1-yl)methylene)pyrrolidinium hexafluorophosphate

HDPyU 1-((4-oxobenzo[d][1,2,3]triazin-3(4H)-yloxy)(pyrrolidin-1-yl)methylene)pyrrolidinium hexafluorophosphate

HDTU $O$-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyliuronium hexafluorophosphate

HOAt 1-Hydroxy-7-Azabenzotriazole

HOBt 1-Hydroxy-$1H$-Benzotriazole

HODhbt 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine

HODT $S$-(1-oxido-2-pyridinyl)-1,3-dimethyl-1,3-trimethylenethiouronium

HONB 2-(5-norbornene-2,3-dicarboximide)

HOPfp Pentafluorophenol

HPyOPfp $N$, $N$, $N'$, $N'$-bis(tetramethylene)-$O$-pentafluorophenyluronium hexafluorophosphate

HPySPfp 1-((perfluoro phenylthio)(pyrrolidin-1-yl)methylene)pyrrolidinium hexafluorophosphate

HOSu $H$-Hydroxysuccinimide

HOTT $S$-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethyliuronium hexafluorophosphate

IDDQ $N$-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline

NDPP Norborn-5-ene-2,3-dicarboximidophenylphosphate

PEC Phenylethylcarbodiimide

PFNB Perfluorophenyl 4-nitrobenzenesulfonate

PIC Phenylisopropylcarbobodiimide

PTOC pyridine-2-thione-$N$-oxycarbonyl

PyAOP [(7-azabenzotriazol-1-yl)oxy]tris-(pyrrolidino)-phosphonium hexafluorophosphate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Chemical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>PyBOP</td>
<td>Benzotriazol-1-yl-oxytri(pyrrolidino)-phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>PyBrop</td>
<td>Bromo-tri(pyrrolidino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>PyClop</td>
<td>Chloro-tri(pyrrolidino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>PyCloP</td>
<td>Chlorobispyrrolidinophenyl-phosphonium hexachloroantimonate</td>
</tr>
<tr>
<td>PyFlO P</td>
<td>Fluorotri(pyrrolidino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>PyCIU</td>
<td>Chloro-1,1,3,3-bis(tetramethylene)-formamidinium hexafluorophosphate</td>
</tr>
<tr>
<td>PyDAOP</td>
<td>(4-oxopyrido[3,2-d][1,2,3]triazin-3(4H)-yl-oxy)tripyrrolidin-1-ylphosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>PyDOP</td>
<td>[(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]tris-(pyrrolidino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>PyDPP</td>
<td>Diphenyl 2-oxopyridin-1(2H)-ylphosphonate</td>
</tr>
<tr>
<td>PyFOP</td>
<td>[(6-(trifluoromethyl)benzotriazol-1-yl)oxy]tris(pyrrolidino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>PyNOP</td>
<td>[(6-nitrobenzotriazol-1-yl)oxy]tris-(pyrrolidino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>PyPOP</td>
<td>(perfluorophenoxy)tripyrrolidin-1-ylphosphonium</td>
</tr>
<tr>
<td>PyTOP</td>
<td>(pyridyl-2-thio)tris(pyrrolidino)-phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>SDPP</td>
<td>2,5-dioxopyrrolidin-1-yl diphenyl phosphate</td>
</tr>
<tr>
<td>SMDOP</td>
<td>4-oxobenzo[d][1,2,3]triazin-3(4H)-yl methanesulfonate</td>
</tr>
<tr>
<td>SPDOP</td>
<td>4-oxobenzo[d][1,2,3]triazin-3(4H)-yl benzenesulfonate</td>
</tr>
<tr>
<td>SOMI</td>
<td>5-(succinimidylxylo)-N,N-dimethyl-methaniminium hexachloroantimonate</td>
</tr>
<tr>
<td>SOMP</td>
<td>5-(succinimidylxylo)-3,4-dihydro-1-methyl 2H-pyrrolium hexachloroantimonate</td>
</tr>
<tr>
<td>TATU</td>
<td>O-(7-azabenzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate</td>
</tr>
<tr>
<td>TAPipU</td>
<td>1-(1-pyridinyl-1H,1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene)pyrrolidinium tetrafluoroborate N-oxide</td>
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<td>TBFH</td>
<td>N,N,N',N'-Tetramethyldibromoformamidinium-hexafluorophosphate</td>
</tr>
<tr>
<td>TBTU</td>
<td>O-benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate</td>
</tr>
<tr>
<td>TCFH</td>
<td>N,N,N',N'-Tetramethylchloroformamidinium-hexafluorophosphate</td>
</tr>
<tr>
<td>TCTU</td>
<td>1 H-Benzotriazolium, 1- [bis (dimethylamo) methylene] - 5-chloro-, tetrafluoroborate (1-), 3-oxide</td>
</tr>
<tr>
<td>TDBTU</td>
<td>2-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate</td>
</tr>
<tr>
<td>TFMS-DEP</td>
<td>Diethylphenyl(trifluoromethylsulfonyl)phosphoramidate</td>
</tr>
<tr>
<td>TFFH</td>
<td>Tetramethylfluoroformamidinium hexafluorophosphate</td>
</tr>
<tr>
<td>TNTU</td>
<td>2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborate</td>
</tr>
<tr>
<td>TOPT</td>
<td>S-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethylthiouuronium tetrafluoroborate</td>
</tr>
<tr>
<td>TODT</td>
<td>S-(1-oxido-2-pyridinyl)-1,3-dimethyl-1,3-trimethyleneithiouronium tetrafluoroborate</td>
</tr>
<tr>
<td>TPTU</td>
<td>1-((dimethylamino)(dimethyliminio)methoxy)-2-hydroxypyrindinium tetrafluoroborate</td>
</tr>
<tr>
<td>TSTU</td>
<td>2-succinimido-1,1,3,3-tetramethyluronium tetrafluoroborate</td>
</tr>
</tbody>
</table>

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Chapter I
Introduction to High-Throughput-Synthesis

1.1. Solid-Phase chemistry and library synthesis
Since the genesis of modern organic chemistry, classical organic reactions have been performed in solution-phase, so-called because of the use of a solvent, which usually then imply a workup and purification in order to isolate the expected product and separate it from any unreacted starting materials, reagents or by-products. These steps can be particularly time consuming and even the evaporation of the reaction solvent can give rise to difficulties. In order to simplify purification and solubility issues associated with peptide synthesis, Merrifield introduced the concept of solid-phase chemistry.\(^1\) The concept was based on the immobilisation of a reactant via a chemical functionality present on a so-called solid support. The advantage of the technique relies on the simplification of the purification process to a filtration step and washing of the support, so that any unreacted reactant is washed away after reaction (Figure 1.1). The process comes into its own when reactions are repeated again and again, to afford pure and complex molecules without time-consuming purification of the intermediates.

![Diagram](image_url)

**Figure 1.1**: Principle of Solid-Phase Organic Synthesis
The method radically changed peptide synthesis, and was applied by Merrifield to the total synthesis of Ribonuclease A on a solid support, an enzyme of 124 amino acid residues. The concept of solid-phase synthesis was further developed with the development of high throughput and automated techniques, which has facilitated the screenings of hundreds of thousands of compounds. This increase in the screening flow and the ever increasing number of therapeutic targets revealed the need to rapidly synthesise large collections of compounds.

In order to make these collections possible, the concept of combinatorial chemistry was proposed, where compounds are systematically assembled by combining pools of building blocks. As an example, a range of monomers $A_n$, carrying a certain functionality, such as a carboxylic acid can be reacted with another range of monomers $B_n$, carrying another type of functionality, such as an amine. In the example shown below, an array of $5 \times 5 = 25$ amides could be obtained simultaneously (Figure 1.2).

Traditional Organic Synthesis

$$A + B \rightarrow A-B$$

Combinatorial chemistry

$$A_1 + B_1, A_1 + B_2, ..., A_5 + B_5 \rightarrow A_1B_1, A_1B_2, ..., A_5B_5$$

**Figure 1.2**: Comparison between traditional and combinatorial chemistry

If the molecules $B_n$ carry another functionality such as an aromatic halogen, the $n^2$ compounds could be reacted with a group of 5 boronic acid $C_n$, thus forming at the end 125 compounds. This example illustrates how a growing number of molecules can be rapidly synthesised. Synthesis of each combination can be carried out either in separate reaction vessels (parallel synthesis) or simultaneously to generate a mixture. Typically libraries synthesised in parallel contain hundreds of members, often taking
advantage of 96- or 384-well plate formats. The synthesis of such libraries using traditional solution-phase techniques would require carefully chosen reaction conditions in order to minimise the formation of by-products. However, by taking advantage of the pioneering work of Merrifield, solid-phase methodology facilitates the removal of reagents and by-products.

1.2. Supports for Solid-Phase Organic Synthesis (SPOS)\(^3\)

Virtually, any inert solid material can be used as an anchor for solid-phase organic synthesis, but the choice of support for a given reaction can have a dramatic effect on the outcome of the reaction.\(^4\) Support-types include the following categories.

1.2.1. Hydrophobic supports

The most common supports are hydrophobic polystyrene beads 1.3, which consist of polystyrene cross-linked with 1 or 2% divinylbenzene 1.2 (Scheme 1.1). The support swelling depends on the type of solvent. Indeed, PS beads swell significantly in apolar solvents while they do not swell in polar protic solvents such as alcohols and water. Therefore reactions involving PS beads supports are not usually carried out in these polar media as only 1 to 2% of the bead’s reactive sites are located on the surface.

![Scheme 1.1: Synthesis of cross-linked polystyrene](image)

1.2.2. Hydrophilic supports

Some supports are hydrophilic and, usually, consist of PEG chains grafted onto a polystyrene core. These supports (TentaGel 1.5, ArgoGel 1.6, PEGA 1.10) are entirely insoluble but exhibit very good swelling properties in polar solvents such as DMF or water, offering a suitable support for reactions based in these solvents (Figure 1.3).
However these supports usually have low loadings (0.2-0.3 mmol/g for TentaGel to 0.4-0.6 mmol/g for ArgoGel), and are often limited to specific uses.\textsuperscript{5} PEGA \textsuperscript{1.10} resin is used for the synthesis of peptides (Figure 1.4).\textsuperscript{6}

![Figure 1.3: General structure of TentaGel and ArgoGel resins](image)

1.2.3. Macroporous supports

Another class of supports consist of macroscopic pores embedded in a rigid structure that does not allow swelling of the matrix. One of the most used supports in this class are 20-40\% cross-linked polystyrene.\textsuperscript{5} Macroporous supports do not normally swell, which make them suitable for most organic solvents. Other supports include silica-based supports with a porous structure and controlled-pore glass (CPG).

1.2.4. Miscellaneous supports

Geysen developed functionalised pins for parallel synthesis in a 96-well plate format.\textsuperscript{7} This allowed manipulation of a large number of compounds through many
synthetic steps. Chiron extended the concept by developing so-called lanterns with transponder-encoding for large scale library synthesis.\(^8\)

![Diagram showing synthetic steps and structures](image)

**Scheme 1.2**: Examples of derivatisation on Crown supports

Traditional polystyrene beads have been handled loose. However they have also been immobilised in a high density polyethylene matrix forming so-called resin "plugs".\(^9,10\)

Other types of support have been reported, such as cellulose,\(^11\) cotton\(^12\) and various polymeric membranes.\(^13\) These supports offer however low loadings (from 0.5-0.6 \(\mu\text{mol/g}\) for cellulose to 0.1 \(\text{mmol/g}\) for cotton). Glass has also been used due to its chemical inertness.\(^14\)

### 1.3. Polymer-assisted solution-phase synthesis\(^15\)

#### 1.3.1. Limitations of SPOS

Although the solid-phase synthesis approach allows the preparation of pure compounds, there are several limitations with this technique. Firstly, the tethering of compounds to the support requires a functionality for attachment. Furthermore, in order to get a pure product after cleavage, the reactions have to be carried out with high conversion and therefore a time-consuming optimisation of the reaction conditions needs to be performed. Practically, the monitoring of reactions on solid-phase is an issue as only a limited range of techniques (FTIR, Gel-phase/MAS NMR) are available to analyse immobilised compounds, although elegant techniques, such as self-indicating resins, have been develop to allow an "on-bead" monitoring.\(^16\) All
these issues have lead to the development of new solution-phase strategies while keeping all the benefits of solid-phase chemistry. So-called polymer-assisted solution-phase chemistry has other advantages over traditional solution-phase chemistry. The technique can be easily automatised, and toxicity and odours of supported species are reduced compared to the free species, while polymer-assisted solution-phase chemistry can be based on the use of immobilised scavengers, reagents or catalysts.15

1.3.2. Scavengers

Scavengers are functionalised polymers which can sequester a compound from a reaction mixture (Figure 1.5). This strategy was applied by Kaldor for the synthesis of amides using acid chlorides in excess.17 After reaction, the excess acid chloride was scavenged with aminomethyl-polystyrene (Scheme 1.3).

**Figure 1.5**: Principle of the use of scavengers

**Scheme 1.3**: An example of the use of a supported amine to scavenge acid-chlorides
Scavengers of many common functionalities have been developed. Thus immobilised electrophiles logically scavenge nucleophiles, while immobilised nucleophiles sequestrate electrophiles. Ketones and aldehydes can be scavenged using supported amines and hydrazines. Reciprocally polymer-supported aldehydes are used to scavenge amines, hydrazines and keto esters, while polymer-supported isocyanates allow selective sequestration of secondary amines in the presence of tertiary amines.

An application of immobilised scavengers is the so-called Solid-Phase Extractors which are particularly advantageous in terms of time and ease of use for parallel synthesis and represent one of the main uses of scavengers, especially acid and base resins. They consist of a syringe filled with a scavenger. Purification occurs by eluting the solution mixture through the SPE. For example, to purify amides obtained from carboxylic acids and amines, the amide solution can be eluted through a syringe containing a mixture of acid and basic ion-exchange resin. The unreacted amine and carboxylic are retained on the resin and the filtrate containing the pure amide is collected (Figure 1.6). It has to be noted that the use of supported-scavengers offer the advantages of using reagents that would normally be incompatible in solution. Indeed, in the example of amide synthesis explained above, the “mixed bed” of scavenger allows purification via removal of the acidic and basic unreacted species in a single step.

Figure 1.6: Principle of purification via SPE
A broad range of Solid-Phase Extractors have been made available to organic chemists, including more recently thiol-based macroporous SPE for scavenging metal-species after cross-coupling reactions.21

1.3.3. Catch-and-release

Another strategy for purification consists of catch and release of the expected compound. After reaction, a polymer "fishes out" the targeted compound and impurities and unreacted species are washed away. The expected compound is then released using a suitable reagent (Figure 1.7). This strategy was used by Porco to synthesise 1,2,3-thiadiazoles in 48-98% yield and 71-100% purity (Scheme 1.4).22

\[
\begin{align*}
\text{Reactant 1} & \quad \text{Reactant 2} \\
\text{(in excess)} & \\
\text{Expected compound} & + \text{Excess of one reactant} \\
& + \text{By-products}
\end{align*}
\]

\[
\begin{align*}
\text{Filtration} & \\
\text{"Fishing out"} & \\
\text{Release} & \\
\end{align*}
\]

**Figure 1.7**: Principle of catch & release strategy

\[
\begin{align*}
\text{1.23} & \quad \text{1.24} \\
\text{1.25} & \quad \text{1.26}
\end{align*}
\]

**Scheme 1.4**: An example of a catch-and-release process
Chapter 1

The supported Weinreb amide 1.23 was first displaced using a Grignard reagent followed by hydrolysis using sulfonic acid resin to form the supported ketones 1.24. The desired ketones were then "caught" with a supported hydrazide via imine formation. The compounds were then released with thionyl chloride via cyclisation to form 1,2,3-thiadiazoles 1.26.

1.3.4. Reagents

Another strategy for polymer-assisted solution-phase synthesis involves the use of polymer-supported reagents. The reagent can be attached either covalently or through ionic interactions. Many polymer-supported reagents have been reported and embrace many of the areas obtainable with traditional solution-phase synthesis.23 The reagent can be used in large excess in order to obtain quantitative transformation of the reactants into the desired product. The reacted and unreacted reagent is then easily filtered off to yield pure products (Figure 1.8).

![Figure 1.8: Principle of polymer-supported reagents](image)

Polymer-supported reagents can trace their origin to 1946,24 and have seen further development in particular by Cainelli,25 Leznoff,26 Sherrington,27 and Hodge28 in the 1970's and 1980's. However, at that time, these reagents were considered as too expensive to be broadly used.29 The need for cleaner and faster methodologies to synthesise collections of compounds requested by the pharmaceutical industry announced the huge development of immobilised reagents in the late 1990's. A comprehensive review has been carried out by Ley29 and only representative examples, most of them commercially available, of the different classes of reagents will therefore be given herein.
**Immobilised Oxidants**

Many immobilised oxidants have been developed, essentially those based on quaternary ammonium ion-exchange resins and RuO₄⁺, ClO⁻ or HCrO₄⁻ ions.¹⁵ Thus oxidation of alcohols can be realised via the use of an immobilised TPAP 1.27 on ion-exchange resin.³⁰ Polymer-supported IBX 1.28 has also been used successfully to oxidise primary and secondary alcohols to the corresponding aldehydes and ketones.³¹

![Figure 1.9: Polymer-supported oxidants](image)

Based on the Swern oxidation protocol, an immobilised sulfoxide 1.29 was used for the oxidation of alcohols in 91-99% yield.³² Advantageously, this soluble polymer-bound sulfoxide was readily recycled after reaction with sodium periodate (Scheme 1.5). Other examples of immobilised oxidants include polymer-supported peracids which were successfully used to oxidise sulfides to sulfoxides and sulfones.³³

![Scheme 1.5: Swern oxidation using a supported sulfoxide](image)
Immobilised reducing agents

The principal examples of immobilised reducing agents are ion-exchange resins anchoring cyanoborohydride 1.31 or borohydride 1.32 ions, and these reagents can be used in reductive amination reactions and reduction of ketones and aldehydes to alcohols.\textsuperscript{34, 35} Interestingly, polymer-supported borohydride has the additional advantage compared to solution-phase sodium borohydride of reducing $\alpha,\beta$-unsaturated carbonyl compounds to the corresponding $\alpha,\beta$-unsaturated alcohols without competing reduction of the double bond.\textsuperscript{35}

![PS-cyanoborohydride](image1)

**Figure 1.10**: Polymer-supported borohydrides

Like in solution-phase synthesis, immobilised borohydrides can be doped by using transition metal salts. Indeed when using a catalytic amount of nickel acetate, the immobilised reducing agent is able to reduce moieties such as nitro,\textsuperscript{36} azido,\textsuperscript{37} and aryloximes groups to the corresponding primary amines.\textsuperscript{38} Ley took advantage of this possibility in his strategy for the total synthesis of (±)-epibatidine 1.35 using exclusively supported reagents, where the transformation of 1.33 into 1.34 proved to be problematic under standard hydrogenation protocols to the observed dechlorination (Scheme 1.6).\textsuperscript{39} In addition the use of the polymer-supported borohydride in conjunction with NiCl\textsubscript{2} proved to be superior to NaBH\textsubscript{4} with NiCl\textsubscript{2}.

![Scheme 1.6](image2)

**Scheme 1.6**: An example of use of immobilised borohydride with NiCl\textsubscript{2} for the reduction of a nitro group
Another type of immobilised reagent with reductive properties are polymer-supported organotin reagents, especially tin hydrides. These reagents were targeted in order to avoid contamination of products with tin species in medicinal chemistry, and have been used occasionally for the reduction of alkyl halides.\textsuperscript{40, 41}

**Immobilised bases**

Many organic tertiary bases have been reported and include morpholinomethyl-polystyrene \textsuperscript{1.36} and piperidino methyl-polystyrene \textsuperscript{1.37}.\textsuperscript{20} Other bases such as polymer-supported TBD \textsuperscript{1.38} have been reported and can advantageously used for the alkylation of amines or phenols.\textsuperscript{42}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{polymer-supported_bases.png}
\caption{Polymer-supported organic bases}
\end{figure}

Globally many of the supported bases reported were obtained readily from Merrifield resin such as PS-DBU\textsuperscript{43} \textsuperscript{1.39} and PS-DMAP\textsuperscript{44} \textsuperscript{1.40}. Even Schwesinger’s phosphazene base has been immobilised (PS-BEMP \textsuperscript{1.41}) without loss of basicity.\textsuperscript{45} This supported base was successfully used to mediate the alkylation of 2\textit{H}-ptalazin-1-one with an \textalpha{}-bromo ketone (Scheme 1. 7).
Chapter 1

1.41

1.42

1.43

1.44

Scheme 1.7: Alkylation reaction using PS-BEMP

Immobilised bases are not limited to organic ones, and hydroxide and hydrogencarbonate ion-exchange resins are frequently used, although their applications mainly take place in scavenging acids or neutralising amines. A basic ion-exchange resin has successfully been used to promote an intramolecular Claisen-type condensation during the synthesis of 4-hydroxyquinolin-2(1H)-ones (Scheme 1.8).

1.45

1.46

Scheme 1.8: Intramolecular Claisen-type condensation promoted by hydroxide ion-exchange resin

**Immobilised acids**

Only a few supported acids have been reported and these are often simply used as nucleophile scavengers and for catch-and-release. However, a polymer-supported version of toluene-sulfonic acid 1.47 has been used to promote reactions requiring strong acid catalysis, and this resin was also used for the deprotection of Boc groups.

1.47

Figure 1.12: Sulfonic acid-PS


Reagents for halogenation reactions

Most of these reagents are based on ion-exchange resins. Indeed Amberlyst-26 in the perbromide form has been used to brominate a wide range of organic substrates. Saturated aldehydes and ketones are easily brominated, while α,β-unsaturated ketones give dibrominated products.\(^{52}\) Perbromide ion-exchange resins can also be used to perform 1,2-addition to alkenes and alkynes.\(^ {53}\)

Although many reagents have been developed for bromination reactions, no direct reagent for chlorination has been reported, and such reactions are often carried out with a supported triphenylphosphine and a source of chlorine. Indeed PS-TPP and \(\text{CCl}_4\) have been used for the chlorination of primary alcohols under mild conditions.\(^ {54}\) Similarly, PS-TPP and \(\text{I}_2\) have been used for the iodination of electron-rich aromatic systems.\(^ {55}\)

Miscellaneous immobilised reagents

In order to simplify the complex purification step in the Mitsunobu reaction, a supported diethylazodicaboxylate \(1.48\) was developed.\(^ {56}\) Polymer-supported triphenyl-phosphine \(1.49\) can also be used very advantageously in Mitsunobu reactions as triphenylphosphine oxide that is formed during the reaction is usually very difficult to separate from the product.\(^ {57}\)

Polymer-supported coupling reagents have also been reported but these are discussed in detail in Chapter II.


\[\text{Figure 1.13: Example of other polymer-supported reagents}\]
Application of immobilised reagents

Examples of complex compounds synthesised in solution-phase using polymer-supported reagents and scavenger are numerous, and include natural products. One of the first example of the synthesis of a fairly complex organic product using polymer-supported reagents used sequentially was reported in 1974 by Rebek, who used a polymer-supported cyclobutadiene iron complex and a polymer-supported succinimide to generate the diamide 1.54 (Scheme 1.9).

![Scheme 1.9: First synthesis of a complex organic product using polymer-supported reagents](image)

One of the advantages of using immobilised reagents is the ability to use in a single step reagents that are mutually incompatibles. This principle was highlighted by Cainelli’s work on the synthesis of unsaturated nitriles. Using, simultaneously, Amberlyst 15-H 1.47 and Amberlyst-26-Phosphate 1.56, the nitriles 1.57 was obtained via acetal deprotection and Wittig-Horner reaction (Scheme 1.10).

![Scheme 1.10: Synthesis of unsaturated nitriles using simultaneously two immobilised reagents](image)
Using usually incompatible reagents is remarkable when unstable products are generated. Thus Bessodes reported a one-pot cleavage of 1,2-diols to primary alcohols. In this work, the unstable dialdehyde generated in-situ via a supported periodate was readily reduced by a supported borohydride (Scheme 1.11).

![Scheme 1.11: One-pot cleavage of diols](image)

The “one-pot” use of immobilised reagents was also illustrated by Parlow who used three polymer-supported reagents at the same time for the synthesis of substituted pyrazoles (Scheme 1.12).

![Scheme 1.12: An example of simultaneous use of three polymer-supported reagents](image)
1.3.5. Catalysts

Polymer-supported catalysts use the same principle as polymer-supported reagents. A wide range of supported-catalysts has been reviewed by de Miguel and a review emphasising the advantages of recyclable supported catalysts and reagents was performed by Bradley. Immobilised catalysts allow high purities in reactions where the catalyst is difficult to separate from the reaction mixture. Some of the first immobilised catalysts were reported in 1975 by Pittman, who used nickel, ruthenium and rhodium catalysts in particular for hydroformylation reactions. Polymer-entrapped palladium catalysts have been used advantageously in cross-coupling reactions, while ruthenium complex bound to a resin, have efficiently mediated ring closure metathesis reactions. The supported catalyst is a polystyrene-immobilised equivalent of the second generation Grubbs Ruthenium complex. Cycles of various sizes were synthesised in excellent yields using this catalyst (Scheme 1.13).

Scheme 1.13: RCM using a polymer-supported Grubbs catalyst

1.4. Conclusion

A wide range of polymer-supported reagents have been reported, but if one looks more closely, many of them tend to be too specific or too sensitive, or even too difficult to synthesise. A trend in the pharmaceutical industry is for polymer-assisted solution-phase chemistry in order to obtain quickly, collections of few hundred compounds, which illustrates the need to identify and synthesise new polymer-supported reagents in order to achieve faster high-throughput-synthesis of potentially biologically active molecules.
Chapter II
Amide-bond formation in organic chemistry

2.1. Importance of amide-bond formation

2.1.1. Molecules containing amide-bonds

**Peptides**
Peptides are small molecules consisting of sequences of amino-acids. They have many biological activities, including actions such as hormones (e.g. angiotensin I^{66} 2.1 and gastrin^{67} 2.2), first messengers in neurotransmission (vasopressin^{68} 2.3), local mediators in cardiovascular diseases (endothelin I^{69} 2.4) and antibiotics such as berninamycin A^{70} 2.5 or fungicides such as cecropin A 2.6^{71}

![Figure 2.1: biologically active peptides](image-url)

**Angiotensin I 2.1**  Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

**Gastrin 2.2**  Pyr-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH₂

**Vasopressin 2.3**  Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH₂

**Endothelin I 2.4**  Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp

**Berninamycin A 2.5**

Synthesising peptides therefore holds many interests. Indeed, it allows a study of biochemical or biological processes, in particular for receptors. It is possible to develop new drugs based on peptides: for example the modified natural product cyclosporin A is an immunosuppressive agent, and direct synthesis therefore presents an interest. On the other hand some natural products such as didemnins are active but generate many side-effects, and the synthesis of analogues is desirable. Peptides drugs have been developed in many fields, and include Insulin and analogues such as Insuline Glargine (glycemic control in diabetes type I), goserelin (breast cancer), luteineizing hormone-releasing hormone (LHRH-analogues have applications in many cancers), and antibiotics such as gramicidin A and bacitracin A. Very recently a new antiretroviral drug, Enfuvirtide, became the first drug to inhibit the entry of HIV-1 into host cells. Enfuvirtide is particularly challenging in terms of synthesis for a commercial drug as it consisted of a total sequence of 36 aminoacids.

**Figure 2.2: biologically active peptides**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRH</td>
<td>Pyr-Glu-His-Pro-NH₂</td>
</tr>
<tr>
<td>Didemnin A</td>
<td>Hip-Leu-Pro-Me₂Tyr-Thr-Ist</td>
</tr>
<tr>
<td>Goserelin</td>
<td>Glu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-AzGly-NH₂</td>
</tr>
<tr>
<td>LHRH</td>
<td>Pyr-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂</td>
</tr>
<tr>
<td>Bacitracin A</td>
<td>His-D-Asp-Asn-Lys-D-Orn-Ile-D-Phe</td>
</tr>
</tbody>
</table>

Peptides have also applications as vaccines. They are applied to the treatment or prevention of allergic and autoimmune diseases, infectious diseases and malignant disorders, where synthetic peptides represent pathogenic T cell epitopes.
to generate an immune response. Although peptides vaccines present the advantages of being easily synthesised via classic organic synthesis, they suffer from a lack of immunogenicity.  

*Other molecules containing amide-bond*

The presence of amide-bonds is not limited to peptides, and many drugs on the market contain the amide moiety. For example, Lipitor (Atorvastatin 2.14, manufactured by Pfizer), the top selling drug worldwide in 2003, blocks the production of cholesterol and contains an amide bond (Figure 2.3).  

![2.14 Atorvastatin](image)

![2.15 Lisinopril](image)

![2.16 Valsartan](image)

![2.17 Diltiazem](image)

![2.18 Zolpidem](image)

*Figure 2.3: examples of top drugs containing amide bond*

Other major drugs containing an amide bond include Lisinopril 2.15 and analogues (inhibitors of angiotensin converting enzyme), Valsartan 2.16 (blockade of angiotensin-II receptors), Diltiazem 2.17 (calcium channel blockers in the treatment of angina and hypertension), Zolpidem 2.18 and analogues (hypnotic, anxiolytic, anticonvulsant and muscle relaxant agents acting at the benzodiazepine receptor). These examples are just a small representation of the presence of amide-bonds in valuable organic compounds and illustrate the importance of this functional group.
2.1.2. Basic principles of amide-bond formation

Amides are synthesised from carboxylic acids and amines; however, the coupling between these two kinds of functional groups does not occur spontaneously at ambient temperature. At higher temperatures (e.g. >200°C) it will take place with elimination of water, but these conditions are often incompatible with the stability of the substrate.\textsuperscript{91} At room temperature, when added together, the only reaction occurring is a simple acid-base reaction yielding a salt. For this reason, it is necessary to firstly activate the carboxylic acid thus increasing the electrophilicity of the carbonyl group, a process that usually takes place by using a good leaving group (Scheme 2.1).

\begin{center}
\textbf{Scheme 2.1} Principle of the activation process for amide-bond formation
\end{center}

Acids are usually activated as acid chlorides, (mixed) anhydrides, carbonic anhydrides or active esters. Many reviews on coupling reagents have been performed but are often incomplete.\textsuperscript{92-98}

2.1.3. Racemisation issues when coupling amino acids

2.1.3.1. Epimerisation via an oxazolone intermediate

Epimerisation generally occurs through an oxazolone 2.19 intermediate, which can be formed via an internal amide once the carboxylic acid has been activated (Scheme 2.2).

\begin{center}
\textbf{Scheme 2.2:} Mechanism of epimerisation via oxazolone formation
\end{center}
Enolisation of the oxazoline results in the formation of an sp\(^2\) carbon, inducing a loss of stereochemistry. All this has dramatic consequences on coupling amino-acids: if the aminoacid is protected as an amide, or if the activated carboxylic acid is part of a dipeptide, then racemisation will be observed. If the amine is protected as a carbamate, then there is usually very little epimerisation. However, even if carbamates are used as protecting groups, epimerisation can be observed if the kinetics of the coupling reaction are poor, which provides some of the interest in finding efficient coupling reagents.

2.1.3.2. Practical evaluation of the epimerisation occurring with coupling reagents

Many methods have been investigated for the evaluation of epimerisation during both aminoacid and peptide segment coupling. For general couplings, Young's test is predominantly used and consists of coupling N-Benzoyl-Leucine with ethyl glycinate. Analysis of the optical rotation of the mixture of enantiomers after coupling allows the determination of the extent of racemisation by comparison with the optical rotation of the L-isomer ([\(\alpha\])\(_D\) = -20.8°).

Weinstein reported a test which consisted in coupling N-Acetyl-Phenylalanine with alanine methyl ester and checking the extent of epimerisation by NMR, while Bodansky’s test consisted of coupling N-Acetyl-isoLeucine and Glycine ethyl ester, followed by hydrolysis with HCl and analysis with an aminoacid analyser for determination of racemisation (chiral HPLC).

Racemisation during segment-coupling is usually evaluated with Anteuni's test, via the coupling of Z-Gly-Phe-OH with H-Val-OMe. The extent of epimerisation can be determined either by HPLC or by NMR. Due to the presence of the phenyl group, the dipeptide is particularly subject to epimerisation and therefore Anteuni’s test represents a very sensitive method for the detection of epimerisation.

Other tests such as Anderson's (Z-Gly-Phe-OH and H-Gly-OEt, followed by fractional crystallisation of the racemic D/L and pure L-tripeptide), or Izumiya's (Z-Gly-Ala-OH and H-Leu-OBz, followed by hydrogenation and HPLC analysis), have been reported but are not widely used. The various methods, used by different research groups reporting new coupling reagents, make the comparison between all coupling reagents difficult to carry out.
2.2. Coupling using carbodiimides

2.2.1. Dicyclohexylcarbodiimide
Carbodiimides were the first coupling reagents to be synthesised and dicyclohexylcarbodiimide (DCC, 2.20) has been used for coupling reactions since 1955.\textsuperscript{105, 106} The mechanism for coupling carboxylic acids to amines is shown in Scheme 2.3.

The first step involves the reaction of the carboxylic acid with DCC to form the \textit{O}-acylurea 2.21. This intermediate can then yield different products:

- The amide via direct coupling with the amine. (The by-product formed, dicyclohexylurea (DCU, 2.22), is usually insoluble in the reaction solvent and can be removed via filtration).
- Formation of an \textit{N}-acylurea 2.23 by-product that is unreactive
- Formation of the anhydride of the carboxylic acid which subsequently yields the amide by reaction with the amine.
As indicated earlier, formation of the oxazolone can take place after formation of the O-acylurea and racemisation is usually high when using DCC.\(^{103}\)

### 2.2.2. Use of additives

In order to reduce the racemisation level when using carbodiimides as coupling reagents, Koenig and Geiger used 1-Hydroxy-1H-Benzotriazole (HOBt) \(^ {2.24}\) as an additive.\(^ {107-109}\) They proved that when using this acid, the yields were higher and racemisation levels lower. For example, when coupling Z-Gly-Phe-OH to H-Val-OMe, the epimerisation levels dropped from 35% to only 1.5%.

HOBt \(^ {2.24}\) reacts with the O-acylurea \(^ {2.21}\) to give the OBt active ester \(^ {2.25}\). HOBt lowers the epimerisation by enhancing the reactivity of the carbonyl group and stabilising the attack of the amine (Scheme 2. 4). However, using HOBt can sometimes yield other by-products. Indeed, it catalyses the formation of diazetidine \(^ {2.26}\) (Scheme 2. 5).\(^ {110}\)

\[
\text{Scheme 2. 4: Action of HOBt when used as an additive with DCC}
\]

In 1994, Carpino reported a similar additive, 1-Hydroxy-7-Azabenzotriazole (HOAt) \(^ {2.27}\) (Figure 2. 4), which is even more efficient than HOBt \(^ {2.24}\) in terms of yield,
kinetics and racemisation levels. For example epimerisation during coupling of Bz-Val-OH and H-Val-OMe using DCC 2.20 dropped from 41.9% with HOBt 2.24 to 14.9% with HOAt 2.27, while when coupling Z-PheVal-OH to H-Ala-OMe using EDC and NMM, it dropped from 4.1% with HOBt 2.24 to under 2% with HOAt 2.27.

\[
\text{Figure 2. 4: Structure of HOAt}
\]

Much work has been carried out on the benefit of using additives. In particular, Carpino studied various isomers of HOAt concluding that the 7-isomer was the most efficient. Albericio also showed that Copper(II) complexes with HOAt or HOBt are efficient additives in lowering the epimerisation level.

### 2.2.3. Other carbodiimides

Since the application of DCC to peptide synthesis, many carbodiimides, including DIC 2.28 (diisopropylcarbodiimide), have been reported and the field has been reviewed on many occasions. In particular, attention has focused on so-called water-soluble carbodiimides, as the ureas formed when using DCC 2.20 or DIC 2.28 can sometimes be difficult to remove. Sheehan investigated several derivatives 2.29-2.32, and concluded that coupling was more efficient when using tertiary amine carbodiimides compared to quaternary derivatives. Sheehan also reported other water-soluble carbodiimides, but no comparison with these reagents was given.

Carpino compared DIC 2.28 to EDC 2.35 and analogues 2.33-2.34, and also compared it to some unsymmetrical alkyl/aryl carbodiimides such as phenyl ethyl carbodiimide (PEC 2.36) and phenyl isopropyl carbodiimide (PIC 2.37). Overall, when using HOAt as an additive, DIC gave the best results for peptide segment coupling.
2.3. Coupling reagents based on 1-\textit{H}-benzotriazole

2.3.1. Uronium/aminium salts

Many coupling reagents are based on the HOBt system and uronium/aminium salts, but have since been surpassed by those based on HOAt because of its increased efficiency.\textsuperscript{121, 122} Uronium \textit{2.38} and aminium \textit{2.39} isomers of these reagents have been structurally identified (Figure 2. 6).\textsuperscript{123} Indeed, coupling reagents based on uronium salts were first reported as being obtained as an \textit{O}-isomer (2.38). However, Carpino proved by X-ray crystallography that HATU \textit{2.40a} and HBTU \textit{2.40b} were in fact obtained as an \textit{N}-isomer (2.39).\textsuperscript{123}
Figure 2.7: Uronium/aminium type coupling reagents
These reagents react with carboxylic acids to form OAt/OBt active esters, which then react with amines (Scheme 2.6).

![Scheme 2.6: Activation process using uronium/aminium type reagents](image)

The uronium salts included HBTU\(^{124}\) 2.40b and TBTU\(^{125}\) 2.41b which had similar efficiency (Figure 2.7). However, after Carpino showed that the best results were obtained with HOAt, many coupling reagents started to be based on this additive such as HATU 2.40a and TATU 2.41a.\(^{111}\) It has been proven that coupling reagents based on HOAt, compared to HOBt, give faster, more efficient couplings with less epimerisation.\(^{126}\) Much work has been carried out with variation of the substituents, yielding HAPyU 2.42 (also named BBC by Chen\(^{127}\)) and TAPipU 2.43 with relatively little influence on the outcome of couplings.\(^{128}\) Other modifications include HAPipU 2.44a,\(^{122}\) HBpipU 2.44b,\(^{129}\) HAMDU 2.45a,\(^{122}\) HBMDU 2.45b (also named BOI),\(^{122}\) and HAMTU 2.46.\(^{122}\) Overall the structural differences between these reagents did not appear to be based on rational considerations and were merely a screening of different substituents. These reagents 2.44-2.46 gave poor coupling results because the reagents were too reactive and degraded before coupling could take place.

Carpino tried to modify the HOAt ring to form 5,6- Benzo 2.47 and 4,5-Benzo 2.48 derivatives.\(^{130}\) The rational was based on the postulated fact that HOAt is more reactive than HOBt because the seven-ring anchimeric effect involving the nitrogen atom of the pyridine ring (HOAt) in the intermediate active ester 2.49 is supposed to
be more effective in promoting reactivity than the six-ring effect in HOBt 2.50 (Figure 2. 8).\textsuperscript{111} The derivatives synthesised were supposed to be more basic than the reagents synthesised before and therefore more reactive. The difference in racemisation levels were modest but 4,5-B(HATU) 2.48 decreased the reaction half-time for hindered amino-acids (Z-Aib-OH) compared to HATU.

![Figure 2. 8: Ring-effect in HOBt and HOAt](image)

More recently, other derivatives based on 6-chloro-HOBt were developed by Albericio, HCTU 2.51a and TCTU 2.51b,\textsuperscript{131, 132} and by scientists at Argonaut, ACTU 2.51c,\textsuperscript{133} but these reagents have not been directly compared to other coupling reagents. Papini used HCTU 2.51a, TCTU 2.51b and HBTU 2.40b in the solid-phase synthesis of cyclopeptides, and the 6-Cl-HOBt based reagents reacted faster and gave higher yields than HBTU.\textsuperscript{134}

Although many of these reagents couple efficiently amines to carboxylic acid, a side-reaction can often take place with the amine reacting with the coupling reagent to form a guanidinium by-product 2.52 (Scheme 2. 7).\textsuperscript{96}

![Scheme 2. 7: Guanidinium formation with aminium/uronium type coupling reagents](image)
2.3.2. Phosphonium salts

Another family of coupling reagents based on HOBt/HOAt is where the uronium/aminium system is replaced by a phosphonium system. The first phosphonium salt introduced was BOP 2.52b,\textsuperscript{135,136} but its use has been limited due to the carcinogenicity and respiratory toxicity associated with HMPA generated when BOP is used in coupling reactions. Instead, the pyrrolidino derivative PyBOP 2.53b was developed.\textsuperscript{136-138} Carpino prepared AOP\textsuperscript{122} 2.52a and PyAOP\textsuperscript{122,139} 2.53a and compared them to BOP 2.52b and PyBOP 2.53b. The aza-derivatives proved to be more reactive both for activation and coupling. Interestingly he showed that phosphonium salts were less stable than the corresponding aminium/uronium derivatives in the absence of a base. However, in the presence of a base, the phosphonium species were slightly more stable, and as a base is required for efficient coupling, the phosphonium salts appear to be more efficient. The phosphonium salts also have the advantage of not yielding guanidinium by-products via reaction of the coupling reagent with amines. In order to achieve the synthesis of thioamides, Hoeg-Jensen et al. developed phosphonium coupling reagents based on the 6-nitro HOBt ring.\textsuperscript{140} PyNOP 2.54, PyFOP 2.55 and NOP 2.56 were used successfully for the formation of thioamides, with good thioamide/amide selectivity but their solubility in organic solvents was poor.

![Phosphonium type coupling reagents](image)

Figure 2.9: Phosphonium type coupling reagents
2.3.3. Immonium salts

More recently, Li designed and synthesised new immonium type coupling reagents, such as BOMI 2.57, BDMP 2.58, BMMP 2.60, and AOMP 2.61. In particular, these reagents were used to carry out the total synthesis of Cyclosporine O, an immunosuppressive agent.

![Structures of immonium salts]

Figure 2. 10: immonium type coupling reagents

2.3.4. Other reagents

Carpino reported DepOAt 2.62. The reaction half-lives were similar to common reagents such as HATU but proved to be much more efficient in terms of epimerisation than any of the other HOAt/HOBt coupling reagents. He tested also named DpopOAt 2.63, as well as DmppOAt 2.64, DtpOAt 2.65a and DtpOBt 2.65b, which were targeted to be more stable toward moisture in the air than the corresponding phosphate esters. In term of half-lives for the formation of Z-Aib-OXt, DpopOAt 2.63 gave similar results to DepOAt 2.62 (< 2 min.), but DtpOBt 2.65b gave poor comparison (65-70 min.). A racemisation study for the coupling of Z-Phe-Val-OH and H-Pro-NH₂ showed that DmppOAt 2.64 (3.6% of LDL isomer) and DtpOAt 2.65a (2.9%) were giving less epimerisation than HATU 2.40a (5.0%), while DtpOBt 2.65b was worse (11.4%), but no explanation for these results was given by Carpino.
Chapter II

A thio-analogue of HAPyU 2.42,128 HAPyTU 2.66, was tested by Klose but proved to be unsuccessful as yields were lower and epimerisation higher than HAPyU 2.42.150

Another type of reagent based on sulfonates was developed by Itoh.151 These reagents 2.67-2.70 were designed with a HOBt and HOCt (6-chloro-HOBt) ring with different substituents on the sulfonate. The best results were obtained with HCSCP 2.70, as the effects of the chlorine atoms enhanced the reactivity of the reagent. However, the reagents were not compared directly to each other as they were tested on different reactions. Compared to DCC 2.20 (without using HOBt), these reagents gave less side-reactions and the by-products were easily removed during aqueous workup. According to the authors, racemisation was lower than with DCC, but no proof was provided.
2.4. Reagents generating acid halides

2.4.1. General reagents used in organic chemistry and triazine-type reagents
Fischer reported the first synthesis of a dipeptide (Gly-Gly) in 1901 and developed the acid chloride method of coupling. The general approach consists of using thionyl chloride or phosphorus pentachloride to generate the acid chloride which reacts quickly with amines to form amides. However this method is quite harsh and not compatible with many protecting groups. It has however been used by Carpino to synthesise peptides via an Fmoc strategy. Triphosgene has also been reported to generate amino-acids acid chloride, especially useful for hindered substrates. Similarly, acid cyanides and azides have been used to synthesise amides. Cyanuric fluoride 2.71 can be used to synthesise acid fluorides (Scheme 2.8), which couple N-methylated amino-acids very efficiently.

![Scheme 2.8: Use of cyanuric fluoride to generate acid fluorides](image)

A variety of other reagents have been reported for the formation of acid fluoride, and include deoxo-fluoro 2.72 and DAST 2.73 (Figure 2.12), with which amides have been synthesised in good yields, but many side-reactions occurred with hindered amines. In addition, these two reagents are expensive, and purification by chromatography is required after reaction.

![Figure 2.12: Structure of deoxo-fluoro and DAST](image)
Part of this category of reagents is based on triazines (cyanuric fluoride, chloride and derivatives) and has been reviewed in details by Kaminski.\textsuperscript{161} In particular CDMT 2.74 and DCMT 2.75 (2,4-dichloro-6-methoxy-1,3,5-triazine) have been successfully applied to the synthesis of anhydrides.\textsuperscript{162}

![Figure 2.13: coupling reagents based on triazines](image)

DMTMM 2.76 is another triazine derivative, which has the particular advantage of promoting amide synthesis in alcohols or aqueous media, without ester formation and with selectivity comparable to DCC and EDC.\textsuperscript{163,164} Recently, a new generation of reagents based on DMTMM was developed by Kaminski (Scheme 2.9).\textsuperscript{165}

![Scheme 2.9: Exchange of counter anion on DMTMM](image)

Analogues using different tertiary bases have been synthesised and the derivative including DABCO proved to give the best synthesis yield. However a full study was carried out on the N-methylmorpholine derivative 2.77, because of its lower production cost. The reagent proved to be particularly efficient with high yields and low epimerisation levels. For the synthesis of the 65-74 segment of ACP, each coupling went faster (15 min.) than with TBTU (45 min.) or HATU (30 min.) and gave better purities (84 %) than TBTU (69%).\textsuperscript{165}
2.4.2. Halo-uronium, halo-phosphonium and halo-sulfonium type reagents

TFFH 2.78a,\textsuperscript{166} BTFFH 2.79,\textsuperscript{166, 167} and DFIH\textsuperscript{166} 2.80a have been used with mixed success to generate acid fluorides since cyanuric fluoride failed in some cases (such as protected histidine and arginine), while PyFloP 2.81a did not yield any acid fluoride. Interestingly, TFFH 2.78a (100% coupling after 10 min.) gave better results than the chloro- and bromo- analogues, TCFH 2.78b (86%) and TBFH 2.78c (79%), for the coupling of Fmoc-Val-OH to H-Ile-PEG-PS.\textsuperscript{166} Overall, BTFFH 2.79 gave the best conversions.\textsuperscript{167}

Reagents aimed at generating acid chlorides or bromides under milder conditions than thionyl chloride, for example, have been targeted. Brop 2.82a was first synthesised by Coste,\textsuperscript{168} and later PyBrop 2.81b and PyClop 2.81c.\textsuperscript{138, 169, 170} These reagents were more efficient that PyBOP 2.53b in coupling N-methyl amino acids. However, PyBroP 2.81b and PyCloP 2.81c can give N-carboxyanhydrides when coupling Boc-amino acids. PyCIU 2.83, also synthesised by Coste, gave high yields when coupling hindered amino acids.\textsuperscript{169} DCIH 2.80b (named CIP originally) gave comparable results to PyBroP and PyCloP.\textsuperscript{171} One of the drawbacks of PyBrop, PyCloP and DCIH is the established formation of mixtures of symmetric anhydrides and oxazolones. Clop 2.82b was reported by Castro and shown to give low levels of racemisation via Young's test.\textsuperscript{172}

An analogue of PyCloP 2.81c, PyCloP 2.84, was reported by Li, in an attempt to increase reactivity by replacing a pyrolidine ring by a phenyl group. The reagent was reported as being efficient for hindered peptide synthesis, but no results were given to illustrate this fact.\textsuperscript{141}

BOP-Cl 2.85 is a reagent that has been widely used in peptides synthesis,\textsuperscript{173} and was in particular reported as being suitable for coupling hindered substrates,\textsuperscript{174} but it has the major drawback of capping primary amines.\textsuperscript{175}

Other reagents include CDTP\textsuperscript{176} 2.86 and CMIM 2.87,\textsuperscript{173} but these reagents, like the one detailed above, usually give high racemisation during coupling. CMIM 2.87 has been compared to other reagents such as FEP 2.93b, and gave poor results with coupling times of over 2h and racemisation over 30% with the Anteuni's test.\textsuperscript{141}
Chapter II

2.4.3. Halo-sulfonium, halo-dioxolium, and halo-dithiolium coupling reagents

Li synthesised other types of coupling reagents, including CDMS 2.88, CBDO 2.89 and CPDT 2.90. However these reagents were far too reactive and decomposed in solution before activation could take place!

![Halo-sulfonium, halo-dioxolium and halo-dithiolium type reagents](image)
2.4.4. Halo-thiazolium and halo-pyridinium type reagents

Li designed reagents based on thiazolium and 2-halopyridinium salts. Their design was based on the fact that, in halouronium type coupling reagents, the carbocation is well stabilised via the lone electron pairs of the nitrogen atoms. Therefore, the carbocation shares a relatively high electron density and the uronium salt demonstrates a relatively low reactivity in the nucleophilic reaction involved in peptide synthesis. For this reason Li attempted to replace one nitrogen atom with other atoms without electron lone pairs or more electronegative atoms with lone electron pairs to enhance the reactivity of the reaction-mediated carbocations. The first attempt to replace one nitrogen with sulphur yielded a thiazolium reagent, BEMT 2.91, with high efficiency.\textsuperscript{177} The same type of reagent, BMTB 2.92, was proposed by Wischnat (Scheme 2.10).\textsuperscript{178} BMTB 2.92 performed better than HATU in coupling Boc-MeIle to MeIle-OBn. However BMTB 2.92 was not compared to BEMT 2.91.

\begin{center}
\textbf{Scheme 2.10: Synthesis of BEMT and BMTB}
\end{center}

\begin{center}
\textbf{Figure 2.16: Other halo-thiazolium and halo-pyridinium type reagents}
\end{center}
Li reported 2-halopyridinium salts like BEP 2.93a, FEP 2.93b, BEPH 2.94a and FEPH 2.94b. Mukaiyama has used extensively 2-chloro- and 2-bromo-pyridinium iodides 2.95 to synthesise esters, lactones and amides, but the conditions used were unsuitable for peptide synthesis, as reactions had to be performed at reflux in DCM due to the poor solubility of the reagents. For this reason Li used tetrafluoroborate and hexachloroantimonate counter anions to improve the solubility, and chose the fluoro-analogues for higher reactivity. A mechanism of the coupling reaction when using these reagents is indicated in Scheme 2.11. The efficiency of these reagents proved to be higher than PyBrop 2.81b, PyClU 2.83, BTFFH 2.79 or
BOP-Cl \(2.85\). However these reagents might be a bit too reactive as the base used during the coupling had to be added very slowly to avoid the coupling reagents reacting too violently.

### 2.5. Other coupling reagents

#### 2.5.1. Reagents generating carbonic anhydrides

Another coupling reagent, EEDQ \(2.96\), was originally developed in 1967.\(^{181, 182}\) EEDQ offers several advantages over most coupling reagents, as the reaction with an amine cannot yield a guanidinium salt, a typical side reaction observed with uronium type coupling reagents. In addition, the carbonic anhydride is formed slowly but consumed rapidly, which avoids its accumulation and therefore minimises the possibility of side-reactions such as epimerisation.\(^{182}\) EEDQ \(2.96\) has been used to efficiently couple hindered aromatic carboxylic acids with tris(hydroxymethyl)aminomethane.\(^{183}\) This coupling reagent was also described in the synthesis of a D-lactosyl cluster nucleotide conjugate,\(^{184}\) and for the formation of benzimidazoles\(^{185}\) and numerous other amide derivatives.\(^{185, 186}\) Analogues of EEDQ \(2.96\) have also been successfully investigated such as IIDQ \(2.97\), and a number of unsymmetrical reagents.\(^{187}\)

![Figure 2.17: Structure of EEDQ and IIDQ](image)

#### 2.5.2. Pentafluorophenol (HOPfp)-based coupling reagents

These types of reagents are based on the traditional pentafluorophenol leaving group and the generation of active esters. They usually require the addition of HOAt as the level of racemisation is quite high: when coupling Z-Phe-Val-OH to H-Pro-NH\(_2\), 33.7% of the DL isomer was observed in solution phase when using HPyOPfp \(2.98a\),
while epimerisation dropped to 1.7% when adding HOAt to the reaction mixture. The use of a thiophenol-analogue, HPySPfp $2.98b$ did not change the outcome of the coupling reactions.\textsuperscript{150} Consequently these reagents are not ideal in solution-phase chemistry as the use of an additive means that this would have to be removed from the reaction mixture after coupling.

Li described a pentafluorophenyl immonium type reagent FOMP $2.99$,\textsuperscript{144} but this reagent was not as efficient as the other immonium type reagents, based on HOBt/HOAt.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{reagents.png}
\caption{coupling reagents based on pentafluorophenol}
\end{figure}

A reagent, PFNB $2.100$, was made by Pudhom, but Boc-Gly-OH reacted slowly and incompletely and it was necessary to add HOBt to get good conversion.\textsuperscript{188} In order to synthesise thioamides, Hoeg-Jensen synthesised PyPOP $2.101$, but this reagent was not as efficient as PyNOP $2.54$ or PyFOP $2.55$.\textsuperscript{140} Other reagents include FDPP $2.102$, which gave lower epimerisation levels than HBTU $2.40b$, BOP $2.52b$ and DCC $2.20$.\textsuperscript{189}

2.5.3. Reagents based on 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HODhbt)

HODhbt was first mentioned in 1970 by Koenig who investigated over 30 N-hydroxy compounds as additives for peptide synthesis.\textsuperscript{109} HOBt gave excellent results but HODhbt proved to be generally superior. However Koenig pointed out that HODhbt
is limited due to inherent side reactions, in particular the formation of a azido-benzoyl derivative 2.103 (Figure 2.19).

![Figure 2.19: side-product formed when using HODhbt as additive](image)

Knorr proposed the generation a HODhbt based coupling reagent, synthesising HDTU 2.104a. Although HDTU 2.104a gave little epimerisation, it was noticed that its use is recommended only in critical cases because of the risk of side-reactions. A similar reagent TDBTU 2.105, where the hexafluorophosphonium counter anion is replaced by tetrafluoroborate has also been reported with comparable results to HDTU 2.104a. Another disadvantage of HDTU 2.104a is its poor stability in DMF compared to classic reagents such as HATU as after 5h HDTU 2.104a had totally decomposed compared to less than 1% for HATU. 122

Carpino compared organophosphorus reagents to commonly used coupling reagents, and showed that DpopODhbt 2.106 was comparable to HATU in terms of reaction times for the formation of the active ester of Z-Aib-OH (< 2 min.) but DepODhbt 2.107 (also named DEPBT by Ye190, 191) was not as efficient (7-8 min.). Another reagent, DtpODhbt 2.108 gave more epimerisation (4.3% of LDL isomer) than DepODhbt 2.107 (3.5%) but less than HATU (5.0%) when carrying out the coupling of Z-Phe-Val-OH and H-Pro-NH₂. The synthesis of the ACP decapptide (H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH₂) was used to show that DepODhbt gave poor results (<1% yield) compared to HATU (85%).

Li also based an immonium type reagents on HOBhbt, but DOMP 2.109 showed very poor results for the coupling between Z-Gly-Phe-OH and H-Val-OMe with only 5.6% yield after 2h compared to 95% for BDMP for example. 144 PyDOP 2.110 was targeted for the synthesis of thioamides, but proved to be surpassed by PyNOP or PyFOP. 140
More recently, Carpino developed coupling reagents based on aza-analogues of HODhbt, and successfully synthesised HDATU, HDADU, HDPyU and PyDAOP. As expected, derivatives of HODAhbt were more reactive than their HODhbt analogue. Thus, HDATU gave better results than HDTU, but was still less reactive than HATU. Moreover, results were more random for segment coupling as they depended on the system studied. However, in many cases, HDATU proved to be better than HATU for the solid-phase synthesis of ACP.
Itoh developed sulfonate reagents based on HODhbt. The two reagents synthesised, SMDOP 2.114 and SPDOP 2.115 were however not as efficient as the other sulfonate reagents that this group synthesised, such as HCSCP 2.70.

2.5.4. Reagents based on 2-hydroxysuccinimide (HOSu) and 2-(5-norbornene-2,3-dicarboximide) (HONB)

Only a few reagents incorporating the hydroxysuccinimide leaving group have been synthesised. Knorr developed TSTU 2.116 and its succinimide analogue TNTU 2.117, which showed high epimerisation levels without the use of additive.

Other examples are SOMP 2.118 and SOMI 2.119 developed by Li, and similar other immonium type reagents, like DOMP 2.109, but they gave poor results.

Phosphate-based succinimide coupling reagents such as NDPP 2.120 and SDPP 2.121 have also been developed. The use of ENDPP 2.122 proved to be a better method than the isobutylchloroformate method because it could be performed at
room temperature, but no other comparisons were reported. Similarly, SDPP was only reported as being a "more convenient method" to use than DCC.

2.5.5. Phosphonium and phosphate type reagents (not based on HOAt, HOBt, -OPfP, -OSu, and -ODhbt)

PyTOP 2.123 was developed by Hoeg-Jensen for the formation of thioamides but the reagent gave poorer selectivities than PyNOP and PyFOP.\textsuperscript{140}

The possibility of using using Dpp-Cl 2.124 was first investigated with success by Jackson,\textsuperscript{195} who claimed that racemisation studies via NMR proved that no epimerisation was observed with this method,\textsuperscript{196} although this result is quite surprising, as racemisation is usually high when acid chloride are generated.

Other derivatives have also been synthesised and include the azide analogue DPPA 2.125\textsubscript{a},\textsuperscript{197,198} and cyano analogue DEPC 2.126, which gave good coupling yields but
with many side-reactions via the cyanide.\textsuperscript{198, 199} Dpop-Cl \textsuperscript{2.125b} was also tested but poor results were observed without the use of an additive.\textsuperscript{149} More recently, Yasuhara reported TFMS-DEP \textsuperscript{2.127} as a successful coupling reagent as Young's test gave only 2% racemisation in 85% yield.\textsuperscript{200}

Other reagents include FDMP \textsuperscript{2.128}, which gave poor results (2% yield compared to 84% yield for BEMT when coupling Z-Gly-Phe-OH to NH$_2$-Val-OMe),\textsuperscript{141} and BIODPP \textsuperscript{2.129}, which gave amides in good yields but were not compared to any other coupling reagent.\textsuperscript{201} Finally, PyDPP \textsuperscript{2.130} was reported as giving low epimerisation rates, but was not compared to other coupling reagents.\textsuperscript{202}

2.5.6. Miscellaneous reagents

CPMA \textsuperscript{2.131}, a reagent based on a chloroimmonium salt (Figure 2.23), mediated the esterification of carboxylic acids,\textsuperscript{203, 204} and in terms of amide bond formation, the reagent was efficient but only two examples were reported.

![Figure 2.23: structure of CPMA](image)

Figure 2.23: structure of CPMA

![Scheme 2.12: Synthesis of HOTT and TOTT from 2-mercaptopyridone](image)

2-mercaptopyridine-1-oxide \textsuperscript{2.132} was used as a starting material to generate a cheaper and new type of uronium coupling reagent HOTT \textsuperscript{2.133} and TOTT \textsuperscript{2.134} (Scheme 2.12).\textsuperscript{205, 206} Both reagents gave better results that CIP or PyBrop and were comparable to HATU, and the dipeptide Z-MeVal-Aib-OMe was obtained in 80% yield (89% for HATU). The racemisation level was evaluated via Young's test and
the use of TOTT resulted in only 3.7% racemisation compared to BOP (20%), PyBOP (15%), or HATU (20%). HOTT and TOTT have also been successfully used to synthesise a primary amide from carboxylic acids using ammonium chloride.\textsuperscript{206}

Najera also synthesised two analogues of HOTT/TOTT, forming HODT \textsuperscript{2.135} and TODT \textsuperscript{2.136} (Figure 2.24).\textsuperscript{207} These two reagents gave higher yields in SPPS, but were less efficient than HOTT/TOTT, giving more racemisation, which was explained by the fact that the DMPU derivatives are less stable than the TMU derivatives.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{hodt_todt.png}
\caption{structure of HODT and TODT}
\end{figure}

A reagent similar to the ones based on 2-mercapto-pyridine oxide was proposed by Knorr but TPTU \textsuperscript{2.137}, based on 2-carbonyl-pyridine-oxide gave high racemisation level when used without an additive.\textsuperscript{125}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{tptu.png}
\caption{structure of TPTU}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scheme_2_13.png}
\caption{Mechanism of the coupling reagents using substituted \textit{O}-hydroxybenzenesulfonyl chlorides}
\end{figure}
An original coupling reagent has been based on the rearrangement of carboxylic-sulfonic mixed anhydrides. Substituted O-hydroxybenzenesulfonyl chlorides 2.138 were used as condensation reagents via the mechanism detailed in Scheme 2. 13. Various peptides were obtained using this method with good yields. The epimerisation level was only assessed through optical purity, and no comparison was made with any common coupling reagent. Another coupling reagent 2.139 was produced by activating diethylphosphate with trifluoromethanesulfonalide. Itoh investigated the possibility of using sulfonate-based coupling reagents, and developed 2-methanesulfonyloximino-2-cyanoacetate 2.140, which proved however to be outperformed by HCSCP 2.70.

Figure 2.26: Structure of other miscellaneous reagents

Carbonyl-diimidazole (CDI 2.141) has been used to generate amide bonds, but more recently, Saha reported the use of an analogue (CBMIT 2.142). He obtained good yields and low racemisation but these were not evaluated on standard tests and are therefore difficult to compare to other results. Very recently, diphenylphosphite (DPP 2.143), and tetrakis(pyridine-2-yloxy)silane 2.144 have been used to synthesise amides. DPP 2.143 forms a phosphonic-carboxylic mixed anhydride, while tetrakis(pyridine-2-yloxy)silane yields silyl ester intermediates 2.145 (Scheme 2. 14). These reagents afforded amides in good yields but were not compared to other coupling reagents.
2.6. Other methods of N-acylation

2.6.1. Mixed anhydrides

The formation of mixed anhydrides is a classic method of amide bond formation. It is important to note that many mixed anhydrides can be generated using some of the coupling reagents reported in this chapter. The mixed anhydride method was first reported by Vaughan, who tested many acid chloride derivatives and concluded that the success of the amide-bond formation was governed by steric and inductive effects. Isovaleryl chloride proved to give the best results. However, as reported by many research groups, this method has a tendency to generate symmetrical anhydrides by reaction of a second carboxylic acid molecule on the mixed anhydride (Scheme 2. 15). In addition regioselectivity is a major issue, as the amine can potentially react on either carbonyle. These drawbacks can sometimes be minimised by carrying out the coupling reactions at low temperature.

2.6.2. Chloroformates

The use of chloroformates for amide-bond formation was first reported by Vaughan and was based on the mixed anhydride method. In the presence of a base, the reaction between a carboxylic acid and a chloroformate yields a mixed
carbonic anhydride, which reacts quickly with amines to form amides. Vaughan's study highlighted slightly better results when using \( s \)-butylchloroformate compared to isobutylchloroformate.\(^{214} \) The method was "reinvestigated" by Anderson,\(^{215} \) who tested several different chloroformate, and whose conclusions suggested that isobutylchloroformate was the most efficient reagent.

### 2.6.3. Direct preparation of active esters

The direct formation of active esters has often attracted a lot of attention due to stability of many of them, which allows storage. Many example of active esters have therefore been reported and include -O-succinimides,\(^{216} \) -OBt and derivatives,\(^{107} \) \( p \)-nitrophenol,\(^{217} \) OPfP,\(^{218} \) HODhbt,\(^{219} \) an PTOC.\(^{220} \) However, in order to form these active esters, reagents such as DCC have to be used. Sometimes, harsher conditions such as SOCl\(_2\) are required, making the direct active ester formation strategy poorly attractive.

### 2.7. Polymer-supported coupling reagents

#### 2.7.1. Immobilised carbodiimides

Only a few polymer-supported coupling reagents are commercially available, probably because coupling reagents are mainly used in peptide synthesis, which is usually carried out on solid phase, the coupling reagent being in solution. However DCC\(^{221} \), DIC\(^{222} \) and EDC\(^{223} \) have been successfully immobilised. These reagents have been applied to the synthesis of hapten active esters,\(^{224} \) acylsulfonamides,\(^{225} \) and obviously amides.\(^{226} \) However, these carbodiimides maintain the same drawbacks as their solution-phase equivalents, in particular in terms of racemisation in the absence of an additive. In addition the synthesis seems to be difficult to monitor and to lack reproducibility. Furthermore, one can wonder at the interest of PS-EDC in comparison to PS-DCC as EDC was originally designed and synthesised to be water soluble. Having the "extractable" moiety on a polystyrene support appears to be pretty bizarre! In addition, the ionic part of EDC in solution-phase has proven to be counterproductive regarding the coupling reaction rate compared to DIC.\(^{119} \) A polyhexamethylene-carbodiimide has also been reported.\(^{227} \)
2.7.2. **Immobilised additives and reagents based on the HOBt ring**

Some coupling reagents in solution can in rare cases be extracted after reaction in relative simple ways (e.g. EDC). However, the use of an additive is often required to limit racemisation, and this additive has also to be separated from the reaction mixture. Therefore polymer-supported HOBt has been reported many times in different guises.²²⁸,²²⁹ PS-HOBt has also been used as a core for synthesising supported reagents for the preparation of \( N \)-hydroxysuccinimide active esters.²³⁰

The idea of using PS-HOBt to form an immobilised HOBt-based coupling reagent was first exploited by Chinchilla, who synthesised a polymer-supported TBTU.²³¹ The idea was also applied by Filip for the synthesis of polymer-supported BOP.²³² These reagents present however the same drawbacks as TBTU and BOP in solution, while the structure of the reagent means that part of it will end up in solution after the coupling, clearly an undesirable occurrence for a supported reagent.

2.7.3. **Other immobilised reagents**

Triazine-based coupling reagents have been widely used in solution-phase. In 1999, Taddei reported a polymer-supported chlorotriazine ².¹⁴⁶²³³ Although amides were synthesised in moderate to good yield using this reagent, the \(^1\text{H}\) NMR of the crude compounds revealed the presence of 5 to 10% of by-products.

![Figure 2.27: Structure of polymer-supported chlorotriazine](image)

Chinchilla et al. developed some reagents based on polymeric succinimides such as P-TSTU ².¹⁴⁷ and P-HSTU ².¹⁴⁸,²³⁴ and ammonium salts ².¹⁴⁹ (Figure 2. ²⁸ and Figure 2. ²⁹).²³⁵ The results were good for classic aminoacids but the yields were moderate to low when coupling hindered aminoacids. Globally these reagents did not really add any benefit to the range of coupling reagents available, and, like PS-BOP and PS-TBTU, part of the reagent ended up in solution.

More recently, Convers reported an immobilised Mukaiyama reagent ².¹⁵⁰ (Figure 2. ³⁰).²³⁶ However, Crosignani investigated this new reagent and reported that the
synthesis was poorly reproducible, and thus recently developed another route (Figure 2.3). This reagent 2.151 appeared to work very efficiently for the synthesis of esters and amides including hindered substrates, secondary amines and anilines. Finally, another type of supported-coupling reagent has been reported recently in a patent but no comparison is available.

\[
\text{Ph} \quad \underset{\text{O}}{\underset{\text{N}}{\text{Ph}}} \quad \underset{\text{O}}{\underset{\text{NMe}}{\text{Me}}}
\]

**Figure 2.28: Structure of P-TSTU and P-HSTU**

\[
\text{Ph} \quad \underset{\text{O}}{\underset{\text{NMe}}{\text{Me}}} \quad \underset{\text{OTf}}{\text{Cl}}
\]

**Figure 2.30: Structure of immobilised Mukaiyama reagent by Convers.**

**Figure 2.29: Structure of ammonium salts of P-succinimide**

**Figure 2.29: Structure of ammonium salts of P-succinimide**

**Figure 2.29: Structure of ammonium salts of P-succinimide**

**Figure 2.29: Structure of ammonium salts of P-succinimide**

**Figure 2.29: Structure of ammonium salts of P-succinimide**

2.7.4. Conclusion on available coupling reagents

Li has produced a wide range of new reagents. However, most of these reagents are probably too reactive (the base needs to be added slowly at low temperature) and the use of antimonate salts is problematic in terms of toxicity. Some reagents, such as PyNOP or PyFOP, have solubility issues. Many of the coupling reagents reported have not been compared to others, making the judgement about their efficiency tricky. As many groups have reported "new" reagents as being wonderful and better than others, the organic chemist looking at the field of coupling reagent can be completely lost and this fact is illustrated by the title of a recent poster presented at an ACS national meeting: "Peptide coupling experiments: Understanding the role of coupling reagents and amino acid sequence". Unless new reagents are systematically tested against commonly considered "top coupling reagents", such as
HATU, and traditional methods, it is likely that most new coupling reagents will have an application limited to the original publication by their authors.

2.8. "In-house" comparison of some common coupling reagents

2.8.1. The quest for a universal coupling reagent

The huge choice of coupling reagents available provides the chemist with a lot of possibilities for general and more challenging amide bond formation. However, many of the coupling reagents reported have drawbacks or are not efficient. In addition, many of those reagents are targeted for peptide synthesis, which involves primary amines. In the area of synthesising potential drugs, the medicinal chemist faces the challenges of finding a method to couple efficiently, anilines, secondary amines and bulky substrates. However, none of the reagents reported to date can achieve this with good conversions.

Overall, most of coupling reagents have the following drawbacks:

- The structure of the reagent means that side-reactions will occur in the presence of nucleophiles. This is particularly the case with uronium-type reagents which can yield guanidinium species in the presence of amines.
- A pre-activation step is necessary: the carboxylic acid has to be added in the presence of the coupling reagent before addition of the amine. This has particularly an impact particularly in parallel synthesis.
- Many reagents require the use of a base in order to deprotonate first the carboxylic acid. However many reagents are poorly stable in the presence of a base. For example, only 36% of HATU remains in a solution of DMF after 1 h in the presence of 1 equiv. of DIPEA.\textsuperscript{122}

For all these reasons, the ideal coupling reagent should have the following characteristics:

- no pre-activation step needed
- all the reactants should be added at the same time
- no particular specificity to amino-acids, the reagent should be used for general amide bond formation
- no other additive, base etc. should be needed to get an efficient coupling
- no racemisation when coupling aminoacids
2.8.2. Comparison of IIDQ and EEDQ

EEDQ is an interesting, under-used coupling reagent which presents the advantage of being inert towards amines (no-preactivation needed) and also contains a base, which makes the use of a base redundant. The remaining desirable characteristic that EEDQ should have in order to be an "ideal" coupling reagent would be the capability to perform well in general amide-bond formation. A study and comparison to other couplings reagents was therefore targeted. Anderson demonstrated that isobutyl chloroformate was superior to any other chloroformate, when using this strategy of amide bond formation,\textsuperscript{214,215} therefore, due to the analogy between the chloroformate method and the EEDQ method, this could potentially mean that IIDQ could be more efficient than EEDQ. As a consequence, a comparison between IIDQ and EEDQ was also performed. The reactivity of EEDQ and IIDQ was compared by testing them on three amines including an aniline (tert-butylaniline) and a secondary amine (morpholine) and two carboxylic acids (benzoic acid and phenylacetic acid). The results are presented in Table 2.1. Yields were good including when coupling anilines, but conversion was only moderate when using morpholine. Those results proved that IIDQ was only slightly more efficient than EEDQ, and IIDQ was retained for a test against other common coupling reagents.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amide</th>
<th>Amine</th>
<th>Acid</th>
<th>IIDQ Yield</th>
<th>Purity</th>
<th>EEDQ Yield</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.152</td>
<td>tert-Bu-aniline</td>
<td>Phenylacetic acid</td>
<td>96</td>
<td>100</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>2.153</td>
<td>benzylamine</td>
<td>Phenylacetic acid</td>
<td>91</td>
<td>100</td>
<td>87</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>2.154</td>
<td>morpholine</td>
<td>Phenylacetic acid</td>
<td>38</td>
<td>100</td>
<td>32</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>2.155</td>
<td>tert-Bu-aniline</td>
<td>Benzoic acid</td>
<td>88</td>
<td>100</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>2.156</td>
<td>benzylamine</td>
<td>Benzoic acid</td>
<td>85</td>
<td>100</td>
<td>66</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>2.157</td>
<td>morpholine</td>
<td>Benzoic acid</td>
<td>50</td>
<td>99</td>
<td>41</td>
<td>95</td>
</tr>
</tbody>
</table>

Average: 76, 100, 67, 98

\textsuperscript{a} isolated yield; \textsuperscript{b} purity determined by ELSD

Table 2.1: Comparison of the efficiency of EEDQ and IIDQ in terms of yield and purity over 6 reactions
2.8.3. Comparison of IIDQ with HATU, PyAOP and BOP-Ci

In order to compare the efficiency of IIDQ with three other coupling reagents during amide bond formation without pre-activation, four amines and three carboxylic acids were tested. The synthesis was carried out in CH$_3$CN with 1 equiv. of each reactant/reagent. CH$_3$CN was chosen as the reaction solvent because of its good solubility ability of carboxylic acids and its ease of evaporation in parallel synthesis. DMF or NMP, two of the usual solvents for amide-bond formation were not selected, as the evaporation of the solvent after reaction is not ideal in parallel synthesis. Importantly, all the reactants were added at the same time. After reaction, the reaction mixtures were filtrated through a SPE cartridge containing a mixed bed of sulfonic acid / quaternary ammonium hydroxide ion-exchange resins, in order to scavenge any unreacted materials. The results are reported in Table 2.2.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amide</th>
<th>Amine</th>
<th>Acid</th>
<th>Purity$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IIDQ</td>
<td>PyAOP</td>
</tr>
<tr>
<td>1</td>
<td>2.158</td>
<td>tert-Butyl-aniline</td>
<td>Z-Ala-OH</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>2.159</td>
<td>benzylamine</td>
<td>Z-Ala-OH</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>2.160</td>
<td>1,2,3,4-Tetrahydro-1-naphthalen-1-ylamine</td>
<td>Z-Ala-OH</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>2.161</td>
<td>morpholine</td>
<td>Z-Ala-OH</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>2.152</td>
<td>tert-Butyl-aniline</td>
<td>Phenylacetic acid</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>2.153</td>
<td>benzylamine</td>
<td>Phenylacetic acid</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>2.162</td>
<td>1,2,3,4-Tetrahydro-1-naphthalen-1-ylamine</td>
<td>Phenylacetic acid</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>2.154</td>
<td>morpholine</td>
<td>Phenylacetic acid</td>
<td>87</td>
</tr>
<tr>
<td>9</td>
<td>2.155</td>
<td>tert-Butyl-aniline</td>
<td>Benzoic acid</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>2.156</td>
<td>benzylamine</td>
<td>Benzoic acid</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>2.163</td>
<td>1,2,3,4-Tetrahydro-1-naphthalen-1-ylamine</td>
<td>Benzoic acid</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>2.157</td>
<td>morpholine</td>
<td>Benzoic acid</td>
<td>39</td>
</tr>
</tbody>
</table>

$^a$ purity determined by ELSD

Table 2.2: comparison of IIDQ with three other coupling reagents

The average purities clearly showed that IIDQ yielded generally highly pure products. It was interesting to note that quinoline was surprisingly removed after
filtration through the SPE. For the other coupling reagents, their products of decomposition remained in the reaction mixture yielding less pure products. However, HATU performed surprisingly well considering its usual poor stability, but this could be explained by the absence of tertiary base during coupling. In fact, in terms of purities HATU performed as well as IIDQ.

Due to the high purities obtained when using IIDQ or HATU, the yields obtained when using these coupling reagents were compared (Table 2.3). The poor purities obtained when using PyAOP or BOP-Cl made the investigation of the yields with these coupling reagents meaningless.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amide</th>
<th>Amine</th>
<th>Acid</th>
<th>Yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.158</td>
<td>tert-Butyl-aniline</td>
<td>Z-Ala-OH</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>2.159</td>
<td>benzylamine</td>
<td>Z-Ala-OH</td>
<td>86</td>
</tr>
<tr>
<td>3</td>
<td>2.160</td>
<td>1,2,3,4-Tetrahydro-naphthalen-1-ylamine</td>
<td>Z-Ala-OH</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>2.161</td>
<td>morpholine</td>
<td>Z-Ala-OH</td>
<td>69</td>
</tr>
<tr>
<td>5</td>
<td>2.152</td>
<td>tert-Butyl-aniline</td>
<td>Phenylacetic acid</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>2.153</td>
<td>benzylamine</td>
<td>Phenylacetic acid</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>2.162</td>
<td>1,2,3,4-Tetrahydro-naphthalen-1-ylamine</td>
<td>Phenylacetic acid</td>
<td>59</td>
</tr>
<tr>
<td>8</td>
<td>2.154</td>
<td>morpholine</td>
<td>Phenylacetic acid</td>
<td>33</td>
</tr>
<tr>
<td>9</td>
<td>2.155</td>
<td>tert-Butyl-aniline</td>
<td>Benzoic acid</td>
<td>89</td>
</tr>
<tr>
<td>10</td>
<td>2.156</td>
<td>benzylamine</td>
<td>Benzoic acid</td>
<td>88</td>
</tr>
<tr>
<td>11</td>
<td>2.163</td>
<td>1,2,3,4-Tetrahydro-naphthalen-1-ylamine</td>
<td>Benzoic acid</td>
<td>67</td>
</tr>
<tr>
<td>12</td>
<td>2.157</td>
<td>morpholine</td>
<td>Benzoic acid</td>
<td>48</td>
</tr>
</tbody>
</table>

| Yield average | 70 | 44 |

*a*isolated yield

Table 2.3: Comparison of the yields obtained when using IIDQ and HATU

Overall, IIDQ performed better than HATU in terms of yield. The moderate yields obtained with HATU could be explained by the absence of tertiary base which makes the activation process more difficult as the carboxylic acid does not get deprotonated. Most of the substrates chosen were coupled in good yield but morpholine proved to give lower conversion, emphasising the usual difficulty of coupling with secondary amines. This study showed that IDDQ could potentially be used as a coupling reagent for efficient, general amide-bond formation.
Chapter III
Preparation and evaluation of PS-IIDQ as a novel polymer-supported coupling reagent

3.1. IIDQ as coupling reagent

3.1.1. Advantages of IIDQ
The study of coupling reagents in Chapter II showed the lack of a universal coupling reagent, suitable for hindered or unhindered substrates. Amongst the existing coupling reagents, IIDQ has been proven to be of great interest (Chapter II). IIDQ offers several advantages over most coupling reagents: for example, reaction with an amine cannot yield a guanidinium salt, a typical side reaction observed with uronium type coupling reagents. Another advantage of IIDQ is that there is no need to have an activation step: the acid, the amine, and the reagent can be introduced at the same time. This is different to most coupling reagent that require a pre-activation time with the coupling reagent and the carboxylic acid added before the introduction of the amine.

3.1.2. Activation process using IIDQ and related issue
The mechanism of activation using IIDQ is given in Scheme 3.1.241 The reaction between IIDQ 3.1 and the carboxylic acid yields a carbonic anhydride 3.2 that is very reactive to amines. The activation process also shows that unlike many coupling reagents, IIDQ does not require the use of a base.
Although IIDQ offers many advantages, it has not been widely used in amide bond formation. This can be easily explained by the fact that the by-product of the reaction, quinoline 3.3, is usually hard to separate from the product.
Scheme 3.1 Activation process using IIDQ as coupling reagent

3.1.3. Interest of a polymer-supported IIDQ and choice of synthesis

Immobilising IIDQ on a resin would immobilise the by-product obtained during the activation process, quinoline. A simple filtration would therefore yield the pure product and isobutanol (boiling point: 108°C) that can be removed in vacuo.

3.2. Synthesis of the solution-phase equivalent of PS-IIDQ

3.2.1. Synthesis of the intermediate

The aim of synthesising the solution-phase equivalent of supported IIDQ, 6-benzyloxy-2-isobutoxy-1-(isobutoxycarbonyl)-1,2)-dihydroquinoline, was to determine the best conditions for the solid-phase synthesis of PS-IIDQ, and to ensure that 6-hydroxy modification did not alter the reactivity of IIDQ.

Scheme 3.2: synthesis of 6-benzyloxyquinoline

The first step of the synthesis consisted of coupling 6-hydroxyquinoline 3.4 with benzyl chloride 3.5 (Scheme 3.2). The nitrogen atom of quinoline did not need to be
protected as Moody had reported the coupling of 6-hydroxyquinoline to 4-nitrobenzylbromide in 77% yield,\(^{242}\) while Perranti reported the synthesis of 6-benzyloxyquinoline in 78% yield.\(^{243}\) However these conditions were not reproducible, and other conditions were examined (Table 3.1).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Base (equiv.)</th>
<th>Other conditions</th>
<th>Conversion(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH</td>
<td>KOH (1.1)</td>
<td>-</td>
<td>total (^b)</td>
</tr>
<tr>
<td>2</td>
<td>('BuOH</td>
<td>KOH (1.1)</td>
<td>-</td>
<td>incomplete</td>
</tr>
<tr>
<td>3</td>
<td>DMF</td>
<td>KOH (1.1)</td>
<td>-</td>
<td>total (^b)</td>
</tr>
<tr>
<td>4</td>
<td>DMSO</td>
<td>KOH (1.1)</td>
<td>-</td>
<td>incomplete</td>
</tr>
<tr>
<td>5</td>
<td>CH(_3)CN</td>
<td>K(_2)CO(_3) (1.1)</td>
<td>-</td>
<td>incomplete</td>
</tr>
<tr>
<td>6</td>
<td>DMF</td>
<td>K(_2)CO(_3) (1.1)</td>
<td>-</td>
<td>incomplete</td>
</tr>
<tr>
<td>7</td>
<td>CH(_3)CN</td>
<td>K(_2)CO(_3) (1.1)</td>
<td>1.1 equiv. KI</td>
<td>incomplete</td>
</tr>
<tr>
<td>8</td>
<td>DMF</td>
<td>K(_2)CO(_3) (1.1)</td>
<td>1.1 equiv. KI</td>
<td>incomplete</td>
</tr>
<tr>
<td>9</td>
<td>DMF</td>
<td>K(_2)CO(_3) (5)</td>
<td>-</td>
<td>total (^b)</td>
</tr>
</tbody>
</table>

\(^a\) determined by TLC analysis, eluant Et\(_2\)O/P.E. 8/2, after 24 hours; \(^b\) all starting material had been consumed, but insoluble impurities were present

Table 3.1: comparison of the reaction conditions for the synthesis of 6-benzyloxyquinoline

Conversion proved to be complete with potassium hydroxide in MeOH or in DMF (entries 1 and 3). As MeOH does not swell polystyrene resin, DMF was more suitable. The choice of KOH for solid-phase synthesis was not ideal but potassium carbonate (1.1 equiv.) in DMF gave incomplete reaction (entry 6). When potassium carbonate was used in large excess (5 equiv., entry 9), conversion was complete. Therefore potassium carbonate in DMF was chosen for the attachment of 6-hydroxyquinoline onto the resin.
### 3.2.2. Synthesis of 6-benzyloxy-IIDQ

After purification, 6-benzyloxyquinoline [3.6] was transformed into 6-benzyloxy-IIDQ [3.7] (Scheme 3.3). Although the synthesis of IIDQ had been reported, the adaptation of the reaction conditions to the synthesis of 6-benzyloxy-IIDQ did not go to completion. According to $^1$H NMR data, 6-benzyloxy-IIDQ [3.7] was successfully synthesised using the conditions indicated in Scheme 3.3. However, it was not possible to isolate the product because of its apparent sensitivity which resulted in its conversion back to 6-benzyloxyquinoline.

### 3.3. Optimisation and characterisation in solid-phase

#### 3.3.1. Coupling onto Merrifield resin

Coupling of 6-hydroxyquinoline [3.4] onto Merrifield resin [3.7] succeeded using the same conditions as in solution as indicated on Scheme 3.4 with 96% conversion according to nitrogen elemental analysis.

The kinetics of formation of PS-quinoline [3.8] were evaluated using nitrogen elemental analysis (Table 3.2). High conversion was obtained after six hours.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Time (h)</th>
<th>Conversion (^a) (%)</th>
<th>Loading (mmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>94</td>
<td>1.07</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>98</td>
<td>1.12</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>98</td>
<td>1.12</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>96</td>
<td>1.09</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>97</td>
<td>1.11</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>100</td>
<td>1.16</td>
</tr>
</tbody>
</table>

\(^a\) determined by nitrogen elemental analysis

Table 3.2: kinetics of formation of PS-quinoline

However, when using a high loading resin (Polymer Labs, 3.99 mmol/g), the conversion using identical conditions proved to be only 66% according to nitrogen elemental analysis. As the percentage of the remaining chlorine was low (0.15 mmol/g), the reduced conversion could be explained by cross-linking via the nitrogen of the quinoline ring, or by hydrolysis. Another explanation would be that Merrifield resin reacts with the decomposition products of DMF, as reported by Alexandratos,\(^{245}\) capping some of the chlorine reactive sites. In order to solve this potential issue, dimethylacetamide (DMA) was used as a replacement of DMF. However, although this change of solvent permitted a complete conversion of Merrifield resin into PS-quinoline, it was necessary to carry out the reaction for 5 days at 65°C in order to observe complete disappearance of the chlorine band seen via IR.

In order to reduce the reaction time, the possibility of carrying out the reaction at reflux was investigated (Scheme 3.5). After 6h, the IR spectra revealed almost completed conversion, while leaving the reaction mixture for a longer reaction time was not advantageous as insoluble compounds, which could not be identified, started to be formed. Nitrogen and chlorine elemental analysis of the resin after 6h revealed a 98% conversion with a loading of 2.74 mmol/g (starting from 3.99 mmol/g).
3.8 was confirmed by Magic-Angle Spinning NMR and the spectrum obtained was compared to the $^1$H NMR spectra of 6-benzyloxyquinoline 3.6 (Figure 3.1).

**Figure 3.1: MAS-NMR of PS-Quinoline and $^1$H NMR of 6-Benzylxloxyquinoline**

**3.3.2. Conversion into supported IIDQ**

The reaction of isobutyl chloroformate with the supported quinoline 3.8 in the presence of Hünig's base yields a highly reactive intermediate 3.9 which can be quenched with isobutanol to form polymer-supported IIDQ 3.10 (Scheme 3.6). The transformation appeared to be successful by IR (Figure 3.2) and was confirmed by MAS-NMR.
Figure 3.3 shows the $^1$H NMR spectra of IIDQ 3.1 in solution and the MAS-NMR spectra of polymer-supported IIDQ 3.10. The comparison of these spectra shows that
polymer-supported IIDQ 3.10 was successfully synthesised. $^1$H-$^1$H COSY spectra confirmed the correlation between the protons H-12 (and H-16) and the proton H-13 (and H-17 respectively).

![NMR spectra showing evidence of the synthesis of PS-IIDQ](image)

**Figure 3. 3:** NMR spectra showing evidence of the synthesis of PS-IIDQ

3.3.3. Optimisation of the reaction conditions

The transformation of PS-quinoline 3.8 into PS-IIDQ 3.10 was carried out with and without DIPEA in DCM. In the absence of DIPEA (entry 1, Table 3. 3) the reaction failed proving that the presence of a base was necessary. Several other bases were tested, using 3 equiv. of base and 3 equiv. of isobutylchloroformate in DCM (Table 3. 3). Conversion was evaluated by IR, via integration of the carbamate band and taking the integration of one of the resin backbone band at 1490 cm$^{-1}$ as reference. 100% was assigned to the base giving the highest integration ratio. DIPEA (entry 2) gave the best conversion while all the other bases performed quite poorly.
### Table 3.3: Optimisation of the base for the synthesis of PS-IIDQ

Several solvents were evaluated (Table 3.4), but little difference was observed in the IR spectra in terms of conversion. Dioxane gave the best result (entry 3) but DCM also gave good conversion (entry 1), especially considering the error margin due to the determination of conversion by integration of bands in the IR spectra. Therefore DCM was chosen because of its good swelling properties for the resin compared to dioxane.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Base</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCM</td>
<td>DIPEA</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>THF</td>
<td>DIPEA</td>
<td>94</td>
</tr>
<tr>
<td>3</td>
<td>Dioxane</td>
<td>DIPEA</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>CH$_3$CN</td>
<td>DIPEA</td>
<td>93</td>
</tr>
<tr>
<td>5</td>
<td>DMSO</td>
<td>DIPEA</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>DIPEA*</td>
<td>DIPEA</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>TEA*</td>
<td>TEA</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>DMF</td>
<td>DIPEA</td>
<td>81</td>
</tr>
</tbody>
</table>

*the base was used as the reaction solvent

### Table 3.4: Optimisation of the solvent for the synthesis of PS-IIDQ from PS-Quinoline
3.3.4. Determination of the loading
As only carbon, hydrogen and oxygen atoms were added during the conversion of quinoline into IIDQ, C, H, N elemental analysis was not a suitable method for determination of the final loading. Therefore, the coupling between phenylacetic acid and benzylamine was chosen for loading determination using 1 g of resin. The product formed was isolated and the yield of the reaction was related to the loading. Using this method, a loading of 1.68 mmol/g was found for the batch of PS-IIDQ synthesised, corresponding to a conversion of >90% for the supported quinoline into the supported IIDQ.

3.3.5. Optimisation of the coupling conditions
The coupling between benzylamine and phenylacetic acid was optimised with different solvents. DCM was the first solvent tested and this proved to work efficiently as shown in Table 3.5 (entry 1). However the choice of DCM was not optimal, because many carboxylic acids are insoluble in it, while it is too volatile for parallel synthesis use.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Yield&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCM</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>DMF</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>DMA</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;CN</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>THF</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>Dioxane</td>
<td>87</td>
</tr>
<tr>
<td>7</td>
<td>DCE</td>
<td>94</td>
</tr>
</tbody>
</table>

<sup>a</sup> isolated yield

Table 3.5: Optimisation of the solvent for the coupling reaction via the coupling of benzylamine (1 equiv.) and phenylacetic acid (1 equiv.) in the presence of PS-IIDQ (3 equiv.) for 24h.

In order to solve these problems, DMF, DMA, CH<sub>3</sub>CN, THF, dioxane and DCE were also evaluated, and the results presented in Table 3.5 showed that DMF (entry 2) or DMA (entry 3) gave clearly worse yields/purities than DCM (entry 1). DCE (entry 7) gave similar results to DCM, but its choice was again not optimal in terms of solubility. THF (entry 5) was not efficient enough, while dioxane (entry 6) also gave acceptable results. The best compromise found was acetonitrile which gave a
comparable conversion to DCM but with better solubility and less volatility. Therefore acetonitrile was chosen as the coupling solvent, although it is known generally as having poor swelling properties for polystyrene supports. The quantity of coupling reagent required for an efficient coupling was also investigated. 1.5 equiv. of PS-IIDQ only gave 67% yield, while 2, 2.5 or 3 equiv. gave 80-85%.

3.4. Comparison of PS-IIDQ with IIDQ in solution

PS-IIDQ was tested on 3 amines and 3 carboxylic acids (Table 3.6) in CH$_3$CN for 24h at room temperature, and the yields and purities were compared to IIDQ in solution. Synthesis comparing PS-IIDQ and IIDQ was carried out using the optimised conditions and, after reaction, a quick aqueous work-up was carried out in order to remove any unreacted amine and carboxylic acid.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amide</th>
<th>Amine</th>
<th>Acid</th>
<th>PS-IIDQ</th>
<th>IIDQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yield (%)$^a$ Purity(%)$^b$</td>
<td>Yield (%)$^a$ Purity (%)$^b$</td>
</tr>
<tr>
<td>1</td>
<td>3.13</td>
<td>4-tBu-aniline</td>
<td>Boc-Aib-OH</td>
<td>69</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>3.14</td>
<td>Benzylamine</td>
<td>Boc-Aib-OH</td>
<td>66</td>
<td>92</td>
</tr>
<tr>
<td>3</td>
<td>3.15</td>
<td>H-PhG-OMe</td>
<td>Boc-Aib-OH</td>
<td>64</td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>3.16</td>
<td>4-tBu-aniline</td>
<td>Phenylacetic acid</td>
<td>60</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>3.11</td>
<td>Benzylamine</td>
<td>Phenylacetic acid</td>
<td>80</td>
<td>74</td>
</tr>
<tr>
<td>6</td>
<td>3.17</td>
<td>H-PhG-OMe</td>
<td>Phenylacetic acid</td>
<td>61</td>
<td>84</td>
</tr>
<tr>
<td>7</td>
<td>3.18</td>
<td>4-tBu-aniline</td>
<td>Benzoic acid</td>
<td>89</td>
<td>78</td>
</tr>
<tr>
<td>8</td>
<td>3.19</td>
<td>Benzylamine</td>
<td>Benzoic acid</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>9</td>
<td>3.20</td>
<td>H-PhG-OMe</td>
<td>Benzoic acid</td>
<td>75</td>
<td>81</td>
</tr>
</tbody>
</table>

$^a$ Isolated yield, $^b$ Purity determined by ELSD.

Table 3.6: Coupling using PS-IIDQ or IIDQ

The different amides were in most cases obtained in acceptable yield and very high purity. The coupling with the sterically hindered building block L-PhG-OMe (entries 3, 6 and 9) was successful even when coupling to hindered carboxylic acids such as Boc-Aib-OH acid. Interestingly, the coupling using an aniline succeeded in good
yield (entries 1, 4 and 7). Generally the results were slightly improved in terms of purity when using PS-IIDQ. This illustrates the advantage of PS-IIDQ over the classic solution-phase reagent IIDQ, where an intensive work up is necessary to remove all the quinoline generated during coupling.

3.5. Comparison of PS-IIDQ with commercial coupling reagents

PS-IIDQ was compared to some commercially available coupling reagents: HATU and two polymer-supported carbodiimides PS-EDC and PS-DCC (Table 3.7). The coupling was realised using optimised conditions and an excessive coupling time of 24h was used in order to enable difficult substrates to react. This contrasts with many coupling reagents, which usually have very high reactivity but are unstable in solution with most of the reagent (or active HOBT ester) being degraded after an hour, a characteristic which is unsuitable for hindered substrates or if the coupling is slow. Considering this problem, PS-IIDQ offers a good balance between reactivity and stability.

HATU (5 min. pre-activation) gave lower yields than PS-IIDQ. In particular coupling with HATU using aminoisobutyric acid (entries 1, 2, 3) and/or anilines (entries 1, 4, 7) were significantly lower. This result is not surprising as HATU decomposes rapidly in solution. Thus, hindered substrates can couple poorly, as they need a longer activation time.

Results were quite poor for the supported carbodiimides. PS-EDC gave an average yield of 41% while PS-DCC gave only 26%, in comparison to the average 71% yield obtained with PS-IIDQ. This can be explained by the usually poor quality of commercial supported-carbodiimides, and in-house synthesis of these supported reagent is usually necessary to achieve good conversions. Both supported-carbodiimides were less efficient than PS-IIDQ, making PS-IIDQ a reagent of choice for parallel solution-phase libraries.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Amide</th>
<th>PS-IIDQ</th>
<th>HATU&lt;sup&gt;c&lt;/sup&gt;</th>
<th>PS-EDC</th>
<th>PS-DCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yield&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Purity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Yield&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Purity&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Image" /></td>
<td>69</td>
<td>100</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Image" /></td>
<td>66</td>
<td>100</td>
<td>45</td>
<td>93</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Image" /></td>
<td>64</td>
<td>100</td>
<td>48</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Image" /></td>
<td>60</td>
<td>100</td>
<td>43</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Image" /></td>
<td>80</td>
<td>100</td>
<td>69</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6.png" alt="Image" /></td>
<td>61</td>
<td>100</td>
<td>77</td>
<td>92</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7.png" alt="Image" /></td>
<td>89</td>
<td>100</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td><img src="image8.png" alt="Image" /></td>
<td>85</td>
<td>100</td>
<td>68</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td><img src="image9.png" alt="Image" /></td>
<td>75</td>
<td>100</td>
<td>54</td>
<td>100</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>72</td>
<td>100</td>
<td>55</td>
<td>98</td>
</tr>
</tbody>
</table>

<sup>a</sup>Isolated yield, <sup>b</sup>Purity determined by ELSD, <sup>c</sup>a 5 min. activation time was allowed for this coupling reagent

**Table 3. 7:** Comparison of PS-IIDQ with some commercial coupling reagents
3.6. Scope and limitation of PS-IIDQ

The library reported in table 3.7 proved that Polymer-Supported IIDQ was efficient even when using hindered substrates. A more comprehensive study of the scope and limitations was carried out, by testing the same carboxylic acids used above on six different amines, and another two carboxylic acids on the same selection of 8 amines (Table 3.9). All building blocks were commercially available, except H-Aib-OMe 3.21, which was prepared according to the literature. Coupling was realised using the optimised conditions and the unreacted amine and carboxylic acid were removed either by ion-exchange resins (Amberlyst 15 and 26) or by a quick aqueous workup (entries 3, 9, 15, 21, 26, 29, 34), depending on the presence of acid/basic labile protecting groups on the amine and/or carboxylic acid. PS-IIDQ succeeded, in almost all cases, to couple the amine to the acid in excellent purities. Yields were good even when coupling hindered substrates such as aminoisobutyric acid, to any of the different acids (entries 3, 9, 15, 21, 29). Similarly, H-PhG-OMe was coupled to Z-Ala-OH and phenoxyacetic acid in high yield (entries 26 and 34). In addition secondary amines were successfully coupled including morpholine (entries 1, 7, 13, 19 and 27) and Proline (entries 6, 12 and 18). However, PS-IIDQ failed to couple 4-nitroaniline to any of the acids, which is not surprising as nitroanilines are some of the hardest amines to couple in view of the electron withdrawing nitro group.

The yield was very low when coupling aminoisobutyric acid to Proline (entry 6). Only 3% of the expected amide product was isolated after column chromatography, which was needed to isolate the amide. Similarly, the amide obtained by coupling Proline to phenylacetic acid and benzoic acid needed purification. However the global library was successful as two thirds of the substrates gave the expected amides in yields over 67%, with a general average yield of 73% for the reactions yielding the expected amides.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Amide</th>
<th>Amine</th>
<th>Carboxylic acid</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.22</td>
<td>0H</td>
<td>Z(\text{H}_{3}\text{N}-\text{CO}\text{OH})</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>3.23</td>
<td>0NH</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.24</td>
<td>0NH</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.25</td>
<td>0NH</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.26</td>
<td>0NH</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3.27</td>
<td>0NH</td>
<td>3</td>
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<td>7</td>
<td>3.12</td>
<td>0NH</td>
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<td></td>
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<tr>
<td>18</td>
<td>3.38</td>
<td>0NH</td>
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Table 3.8 - Part 1: Scope & limitations of PS-IIDQ
<table>
<thead>
<tr>
<th>Entry</th>
<th>Amide</th>
<th>Amine</th>
<th>Carboxylic acid</th>
<th>Yield</th>
</tr>
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<td>0</td>
<td>Z-NH OH</td>
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<td>0</td>
<td>N-H2</td>
<td>71</td>
</tr>
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<td>21</td>
<td>3.41</td>
<td>0</td>
<td>O-C-NH2</td>
<td>82</td>
</tr>
<tr>
<td>22</td>
<td>3.42</td>
<td>0</td>
<td>O2-N-C-NH2</td>
<td>0</td>
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<td>3.43</td>
<td>0</td>
<td>C-NH2</td>
<td>64</td>
</tr>
<tr>
<td>24</td>
<td>3.44</td>
<td>0</td>
<td>t-Bu-C-NH2</td>
<td>70</td>
</tr>
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<td>25</td>
<td>3.45</td>
<td>0</td>
<td>C-NH2</td>
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<td>0</td>
<td>O-C-NH2</td>
<td>83</td>
</tr>
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<td>3.47</td>
<td>0</td>
<td>C-NH2</td>
<td>80</td>
</tr>
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<td>0</td>
<td>N-H2</td>
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<td>0</td>
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<td>70</td>
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<td>0</td>
<td>C-NH2</td>
<td>74</td>
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<td>0</td>
<td>t-Bu-C-NH2</td>
<td>83</td>
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<td>0</td>
<td>C-NH2</td>
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<tr>
<td>34</td>
<td>3.54</td>
<td>0</td>
<td>O-C-NH2</td>
<td>82</td>
</tr>
</tbody>
</table>

Table 3.9 - Part 2: Scope & limitations of PS-IIDQ
3.7. Side-reactions observed when coupling proline

The formation of a carbamate by-product can occur during the coupling reaction once the carbonic anhydride has been generated. The amine can in fact react at two electrophilic centres, either on the carbon of the carbonic anhydride itself, yielding a carbamate specie 3.55, or on the carbon of the carboxylic acid, yielding the expected amide (Scheme 3.7). Proline was coupled to 15 carboxylic acids with various groups in the α and β positions (Table 3.9). The expected amide and the carbamate by-product 3.55 were separated by column chromatography after acidic/basic workup of the reaction mixtures. When the α-carbon of the acid was not sterically too hindered, that is to say, when it was linked to one bulky group and two hydrogen atoms, the major product obtained was the expected amide (entry 14). On the other hand when the α-carbon was much more hindered (such as two methyl groups) the carbamate 3.55 was almost the exclusive product (entry 13) because the approach of the Proline to the electrophilic carbon was disfavoured. Benzoic acid since it was not so hindered gave intermediate results as the steric hindrance of the α-carbon does not disfavour the formation of the amide as much as the aminoisobutyric acid (entry 15). The presence of bulky groups in the α position clearly increased the quantity of by-product (entry 5) compared to phenylacetic acid (entry 14). One of the predominant effects seemed to be the presence of a methyl group in the α position, which increased dramatically the by-product generation (entries 9 and 13), although two phenyl groups had little effect (entry 4).

![Scheme 3.7: Side reaction observed when coupling proline using PS-IIDQ.](image-url)

3.55
<table>
<thead>
<tr>
<th>Entry</th>
<th>Amide</th>
<th>Carboxylic Acid</th>
<th>Carbamate Yield</th>
<th>Amide Yield</th>
<th>Relative percentage</th>
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<td>24</td>
<td>64 / 36</td>
</tr>
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<td>3.57</td>
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<td>76</td>
<td>13</td>
<td>85 / 15</td>
</tr>
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<td>3</td>
<td>3.58</td>
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<td>18</td>
<td>74</td>
<td>20 / 80</td>
</tr>
<tr>
<td>4</td>
<td>3.59</td>
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<td>20</td>
<td>71 / 29</td>
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<td>51</td>
<td>41 / 59</td>
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<td>3.62</td>
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<td>37</td>
<td>56 / 44</td>
</tr>
<tr>
<td>8</td>
<td>3.63</td>
<td><img src="image8.png" alt="Image" /></td>
<td>57</td>
<td>17</td>
<td>77 / 23</td>
</tr>
<tr>
<td>9</td>
<td>3.64</td>
<td><img src="image9.png" alt="Image" /></td>
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<td>24</td>
<td>59 / 41</td>
</tr>
<tr>
<td>10</td>
<td>3.65</td>
<td><img src="image10.png" alt="Image" /></td>
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<td>55</td>
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</tr>
<tr>
<td>11</td>
<td>3.66</td>
<td><img src="image11.png" alt="Image" /></td>
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<td>26</td>
<td>68 / 32</td>
</tr>
<tr>
<td>12</td>
<td>3.67</td>
<td><img src="image12.png" alt="Image" /></td>
<td>49</td>
<td>47</td>
<td>51 / 49</td>
</tr>
<tr>
<td>13</td>
<td>3.27</td>
<td><img src="image13.png" alt="Image" /></td>
<td>3</td>
<td>38</td>
<td>8 / 92</td>
</tr>
<tr>
<td>14</td>
<td>3.32</td>
<td><img src="image14.png" alt="Image" /></td>
<td>60</td>
<td>4</td>
<td>94 / 6</td>
</tr>
<tr>
<td>15</td>
<td>3.38</td>
<td><img src="image15.png" alt="Image" /></td>
<td>34</td>
<td>53</td>
<td>39 / 61</td>
</tr>
</tbody>
</table>

Table 3.9: Test of acids for the side-reaction observed with H-Pro-OBn
However, some results were quite surprising. Indeed, when removing a phenyl group in the β position (entries 1 and 2), the percentage of the carbamate increased. When passing from a cyclohexyl- to a cyclopentyl- the percentage changed significantly (entries 10 and 11). This study showed a limitation of PS-IIDQ but no real rule could be established for the influence of groups on the carboxylic acid. The formation of carbamate could presumably be reduced by using another alcohol (e.g. 2-hydroxy-3-methyl-butane) in order to increase the steric hindrance at the carbonate position.

### 3.8. Racemisation studies: Anteuni's test

The above examples proved that PS-IIDQ worked efficiently for general amide bond formation. In order to apply PS-IIDQ for coupling amino-acids, the racemisation by epimerisation during segment coupling had to be evaluated. The classic method to achieve this is to carry out Anteuni’s test (Scheme 3.8). This test is particularly important as amide cyclisation to give the oxazolone is a real issue for dipeptides. Coupling between Z-Gly-L-Phe-OH 3.68 and H-L-Val-OMe 3.69 gave the expected tripeptide 3.70 in 65% yield without any D/L diastereoisomer detected by $^1$H NMR (400 MHz). In order to make sure that the signal of the L/L and D/L isomers in the NMR spectra did not overlap, the D/L isomer was synthesised separately and the NMR spectra was compared to the L/L isomer obtained with PS-IIDQ. Unambiguously it confirmed the absence of epimerisation (Figure 3.4).

![Scheme 3.8: Anteuni's test](image-url)
Figure 3.4: Evidence of the absence of epimerisation by NMR at 400 MHz. The signal shown corresponds to the CH group of the Phenylalanine.

3.9. Recycling of PS-IIDQ

The possibility of recycling/regenerating PS-IIDQ was investigated. Thus the resin used was washed extensively with various solvents (THF/H₂O 1:1, MeOH, DCM, Et₂O). Then the procedure used previously to transform PS-quinoline into PS-IIDQ was applied, and the loading evaluated by coupling phenylacetic acid to benzylamine. The same regeneration process was carried out three times with little variation in loading levels (Table 3.10).

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Loading (mmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≥ 1.68 mmol/g</td>
</tr>
<tr>
<td>2</td>
<td>≥ 1.71 mmol/g</td>
</tr>
<tr>
<td>3</td>
<td>≥ 1.65 mmol/g</td>
</tr>
<tr>
<td>4</td>
<td>≥ 1.64 mmol/g</td>
</tr>
</tbody>
</table>

Table 3.10: Recycling of PS-IIDQ

Interestingly, no loss of activity was observed after recycling, every evaluation of the loading giving results within the same range with a margin error of ± 0.1 mmol/g.
3.10. Conclusion

PS-IIDQ is a high loading, efficient polymer-supported coupling reagent for general amide bond formation, including hindered substrates and anilines. Its use can also be suitable for amino-acids coupling as the epimerisation level was low. PS-IIDQ proved to be more efficient than other commercially available polymer-supported coupling reagents (PS-EDC, PS-DCC) and also better than the powerful and widely used HATU. Only one limitation of this new reagent was observed, but this was noticed only with proline. Stability, ease of recycling, convenient procedures for parallel amide bond formation, good yields and high purities make PS-IIDQ a reagent of choice for N-acylation in organic chemistry.

*PS-IIDQ is now commercially available from Novabiochem (01-64-0469) and Polymer Laboratories*
Chapter IV

A new approach to the screening of small-molecules on microarrays

4.1. Fragment-based drug discovery

4.1.1. Introduction

The time usually spent in the pharmaceutical industry between the identification of an active compound in HTS and lead/hit optimisation represents, still, a significant part of the drug discovery process. Attempts to reduce this time have therefore been investigated thoroughly. Jencks first developed the idea of fragment-based drug discovery by showing that drug-like molecules can be regarded as the combination of two or more individual binding residues. Leads are usually derived from hits obtained via screening of large collections of compounds and the usual optimisation carried out allows the activity to be typically increased from the micromolar to the nanomolar range. Big pharmaceutical companies tend to screen large libraries of highly functionalised compounds in order to identify a significant number of hits. However, although high-throughput-chemistry has provided industry with powerful tools to synthesise millions of compounds, no collection of compounds comes anywhere near the complete exploration of chemical space. As the number of possible drug-like molecules decreases exponentially with their size, it is more efficient to screen smaller molecules, i.e. fragments, with fragments being defined as “small, relatively simple molecules that have the ability to bind to a target and serve as substrates for further medicinal chemistry”. Screened fragments are small (typically MW 120-250), have less functionality and also weaker affinity than most usual HTS hits, with typical binding affinities in the millimolar to 10 micromolar range.

In principle, fragment-based strategies can sample a larger theoretical “diversity space” than is practical through standard screening methods. Globally the probability
of finding a good ligand-target match decays exponentially as the size of the ligand increases due to the fact that as the complexity of the ligand increase, there are far more chances to obtain a mismatch rather than a match. All the advantages of fragments are illustrated by the development of companies such as Abbott (NMR), Astex (HT X-ray crystallography), Triad Therapeutics (NMR) and Graffinity (surface plasmon resonance). Despite the theoretical advantages, fragment-based drug discovery can however be difficult in practice.

4.1.2. Techniques for finding fragments

4.1.2.1. General considerations for fragment design

Like traditional target-oriented drug discovery, the fragment-based approach requires first the design of libraries of compounds. Lipinski’s rules of five are traditionally applied to the design of libraries, and more recently a new rule was added, which limits to a maximum of seven the number of rotatable bonds. However all these considerations are not necessary the best ways to find a good lead compound. Indeed, leads usually have lower molecular weight, lower lipophilicity, fewer aromatic rings and fewer hydrogen-bond acceptors than their clinical candidates. Similarly, libraries of compounds with molecular weight ranging from 100 to 350 and a clogP ranging from 1 to 3 are usually superior for finding leads. This is easily explained by the fact that the lead optimisation process typically increases the molecular weight by 80 Da and the lipophilicity by 1 log unit. Therefore when a lead already contains good drug-like properties in terms of oral availability, the final clinical candidate typically presents poorer drug-like properties because its properties are further away from Lipinski’s rules of five. With all these issues in mind, Jhoti elaborated adapted rules for fragment libraries due to their smaller size and potential for optimisation.

- Compounds should have a molecular weight lower than 300 Da
- The lipophilicity cLogP should be lower than 3
- The number of hydrogen bond donors and acceptors should be lower than 3

Analyses of drugs have led to the synthesis of privileged libraries of fragments containing scaffolds present in drugs, and Fesik in particular synthesised fragments that bind to proteins frequently. Once the design of fragments has been
achieved and the libraries synthesised, several techniques have been elaborated in order to identify active fragments.

4.1.2.2. Functional or direct binding assays at high concentrations

Usually fragments have relatively low binding affinities and are therefore difficult to detect in HTS where low concentrations are used. In order to overcome these difficulties, Ellman used a strategy based instead on high concentrations for the identification of an inhibitor of the kinase c-Src with nanomolar activity. A similar approach was used at Graffinity using surface plasma resonance and fragments attached to microarrays. In this method, fragments are synthesised on solid-phase and carry a common linker for immobilisation onto the array. The result of the printing is an array containing 10000 fragments displayed on top of a self assembled monolayer (SAM). A thin layer of gold provides support for SAM formation and surface plasmon resonance (SPR). The use of SAM offers the advantage of presenting an anchor point for immobilisation of the fragment. After incubation with a target protein, detection of fragment-protein interactions is carried out by SPR.

Both approaches have the following advantages:

- Fewer molecules have to be synthesised
- The technique does not rely on the knowledge of the structure of the biological target, or established leads
- Functional screening is potentially more likely to provide a functionally relevant inhibitor rather than a molecule that binds to but does not inhibit the target.

However many drawbacks can be foreseen:

- The technique requires that two fragments bind to adjacent but non-overlapping sites
- The optimisation of linking fragments in the absence of any structural knowledge can be difficult
- Functional screening (especially at high concentrations) is subject to a number of pitfalls.
4.1.2.3. NMR-based screening

The principle was first developed by Fesik and consists of screening a fragment library against a $^{15}$N-labelled biological target. When compounds bind, the resulting changes in amide chemical shifts around the binding site are detected by Heteronuclear Single Quantum Correlation Spectra (HSQC). If the structure is known and the NMR fully assigned, this allows identification where in the protein a compound binds. Ligands that bind to adjacent sites on the protein are then selected and optimised. Finally the best ligands are linked together in order to identify a ligand with very high affinity (Figure 4.1).

With this approach, Fesik identified aminophenol amides derivatives 4.1-4.7 which were binding with a millimolar affinity to the FK-506 Binding Protein (FKBP). Another ligand 4.8, based on piperolic acid, a moiety which was reported previously as having a micromolar affinity to FKBP, was also identified as binding to FKBP. After analysing the position of the binding between FKBP and the different fragments, Fesik linked the best ligand of the series 4.7 (based on p- aminophenol) to 4.8. To achieve the link between these two fragments, Fesik used
different chain length and an impressive affinity of 19 nM was found for the resulting best ligand 4.9 (Figure 4.2).

![Chemical structure](image)

**Figure 4.2: The discovery of a high affinity ligand by Fesik**

Although the initial assays by Fesik were successful,\textsuperscript{259} the technique presents some drawbacks. In particular, it requires significant quantities of pure \textsuperscript{15}N-labelled protein (usually over 200 mg). In addition, ligands need to be soluble at high concentrations (> 0.2 mM) and in order to obtain maximum structural information, the protein backbone residues need to be assigned. However the sensitivity of the method has recently been improved in particular by \textsuperscript{13}C labelling amino acids residues and by instrumental improvements such as cryogenic NMR probes,\textsuperscript{265} allowing the screening of ligands against bigger proteins (100 kDa).
4.1.2.4. Mass Spectrometry-based methods

The possibility of using MS for fragments screening was first explored by Swayze.\textsuperscript{266-268} The principle consisted in tuning the ionisation and desolvation processes in order to detect the binding of noncovalent weak binding fragments by ESMS. After linkage between the best members of different series, the resulting compounds proved to be more potent but still quite poor (micromolar range).\textsuperscript{269, 270}

An approach developed by Erlanson, called tethering,\textsuperscript{271, 272} was based on the site-directed modification of the targeted protein close to the active site (mutagenesis) or more simply using a cysteine residue, if there were any close to the pocket. This creates an anchoring point for fragments that contain a thiol functionality, and the screening of a library of hundreds of thiol fragments allows the detection of the fragment forming a disulfide bond with the highest affinity for the protein (Figure 4.2).

![Figure 4.3: Principle of the Tethering method (adapted from: www.sunesis.com)](image)

Although the tethering method gives some information on the binding mode, the amount of information obtained by MS is relatively limited, and crystallography is therefore used after MS-screening, in order to get wider information about the binding. All this makes the technique relatively tedious, as libraries need to be designed in a certain way in order to incorporate a thiol moiety and MS-screening needs ideally to be followed by crystallography, which results in relatively high quantity of protein being required (0.5 to 5mg). Finally, it requires precise knowledge of the biological target in order to assess the presence of the cysteine group or to modify the protein.
4.1.2.5. Crystallography-based techniques

Another technique involves the use of crystallography to identify fragments. The concept is based on the analysis of the electron-density map of crystals.\(^{273}\) The changes in density are correlated with binding to a specific part of the biological target and the method can be used with dynamic libraries.\(^ {274}\) This technique was illustrated by the discovery of nanomolar inhibitors of Src SH\(_2\), a domain of the Src protein involved in signal transduction.\(^ {275, 276}\)

Fragment-based lead discovery by X-ray crystallography has been developed by Astex Technology in the U.K. and many companies in the U.S.A.\(^ {277}\) The approach has been made possible with the help of robotics and automatic data processing software. The strategy involves soaking a cocktail of fragments into preformed crystals of the target protein. In particular, the technique was applied to the discovery of novel protein kinase inhibitors using the same technique.\(^ {278}\)

Although crystallography is the technique giving the most binding information, it requires precise knowledge of the protein structure. In addition, the technique is still relatively low-throughput, and weak binding ligands do not show electron density.

4.1.2.6. Conclusion regarding the current methods

Most of the techniques presented show the same drawbacks. They are usually very time-consuming. Often the techniques require relatively high quantities of protein. In that respect, the Graffinity method is particular interesting as it is using tiny amounts of protein because fragments are attached to microarrays. This also brings the advantage of being suitable for high-throughput compared to most other methods. However, fragments needs to be linked after screening, and the possibility to have many fragments binding in the same portion of the protein pocket is high resulting in a difficult hit optimisation process.

4.2. Small molecules microarrays: principle and applications

4.2.1. Introduction

The microarray concept has raised much interest in the past decade, as it allows a multitude of high-throughput experiments. Technologies based on micro-arrays have in particular been applied to the high-throughput analysis of biomolecules.\(^ {279}\) This
include DNA, RNA and oligonucleotides, proteins, membrane proteins, peptides, carbohydrate and tissue, live cells, and small molecules. Since small-molecules microarrays were introduced by Schreiber in 1999, the field has grown significantly, and has been reviewed. Biological evaluation of small molecules represent a major part of the drug discovery process making the application of a robust screening tool essential for the identification of possible drug candidates. Difficulties of signal detection due to non-specific protein-binding to the slide surface are however a common drawback.

4.2.2. Immobilisation of small molecules on microarrays

4.2.2.1. Covalent immobilisation

Most of the reported examples of small-molecules microarrays involved prior synthesis on solid-phase resins before release and immobilisation on the array surface. Schreiber used this strategy for his pioneering work on small-molecules microarrays. Three known ligands of biological targets were derivatised to carry a thiol group and were successfully immobilised onto a maleimide-derivatised glass slide via a Michael addition (Figure 4.4). The derivatised ligands were spotted onto the array to obtain 10800 spots in total on a single slide (3600 per ligand) with a 200-250 μm diameter. The assays showed that the proteins were binding specifically to their ligand and that the intensity of fluorescent spots correlated well with the affinity of the protein for the immobilised compounds.

Figure 4.4: Preparation of maleimide-derivatised glass slides by Schreiber
Chapter IV

After validation of the concept, Schreiber’s group reported many examples of small-molecules microarrays, mainly in correlation with diversity oriented synthesis. They successfully activated glass slides as a chlorinated surface for the attachment of alcohols (Figure 4.5), and applied the concept one bead – one stock to dissect a glucose signalling pathway. They synthesised 3780 1,3-dioxane compounds and an hydroxyl group was exposed after cleavage from the resin providing a suitable tethering point for attachment onto the chlorinated surface (Scheme 4.1).

![Diagram of immobilisation of alcohols via chlorinated glass slides](image)

**Figure 4.5: Immobilisation of alcohols via chlorinated glass slides**

**Scheme 4.1: Generation of diversity oriented synthesis “one bead – one stock” for dissecting a glucose signalling pathway**
Schreiber again used the combination of three hydroxyl-containing compounds libraries for immobilisation of 12396 species on chlorinated glass slides.\(^{296}\) In order to expand the functional group compatibility, Schreiber's group developed a new linker for the attachment of small molecules to the array surface. The use of a diazabenzyldiene moiety 4.16 on the derivatised glass slide allowed the linkage of phenol-containing compounds (Figure 4.6).\(^{297-299}\) Interestingly the linker does not react with alcohols while it captures compounds containing acidic protons such as phenols, carboxylic acids and sulfonamides, making this strategy a versatile tool and complementary to chlorinated glass slides.

Waldmann developed another chemoselective method via the use of mild conditions.\(^{300}\) Compounds are synthesised on solid-phase with a Kenner-type safety-catch linker and released into stock solution. The compounds contain after cleavage an azido group which can then be used to carry out a Staudinger reaction on a phosphane derivatised glass slide (Figure 4.7).
Other examples of immobilisation of small molecules have been reported. Chang chose a 1,3,5-triazine template, with a 2,2’[1,2-ethanediyl-bis(oxy)]bisethanamine spacer in order to immobilise a triazine library onto slides activated with N-hydroxysuccinimide esters. They identified with this small-molecule microarrays new ligands of Human IgG.

Figure 4.7: Immobilisation of small-molecules on microarrays via the Staudinger ligation strategy

Figure 4.8: Immobilisation on hydrazide-containing molecules onto epoxide-coated slides
Chapter IV

The immobilisation of hydrazide-containing substances onto epoxide-coated surfaces was proposed by Lee and Shin.\textsuperscript{302} The strategy was applied to microarrays of small molecules, carbohydrates and peptides (Figure 4. 8). The hydrazides are incorporated into the small molecules while they are attached to a solid support and bind to the surface selectively, giving a wide functional group compatibility (alcohols, amines, carboxylic acids and mercapto groups did not bind to the slide under the conditions applied at pH 3-5, according to the selectivity tests carried out).

4.2.2.2. Hybridisation onto DNA microarrays

Another strategy developed by Schultz involves the indirect attachment of small molecules to DNA arrays.\textsuperscript{303} Small molecules are linked and encoded through a PNA tag, complementary to DNA sequences on the array.\textsuperscript{304} The compounds are linked to the surface via PNA/DNA hybridisation. With this technique no particular functional group is required after cleavage for immobilisation onto the array. However a particular functional group is still required for attachment to the PNA tag.

This technique has been used to profile protein function,\textsuperscript{305-308} and was also reported by Bradley at the same time (Figure 4. 9).\textsuperscript{309}

Figure 4. 9 : Protein profiling using PNA-peptides conjugates on DNA microarrays
4.2.2.3. Photoactivation strategies

Mrksich reported the synthesis of small-molecules microarrays by photoactivation of self-assembled alkanethiolate monolayers using photomasks.\textsuperscript{310} The principle is based on nitroveratryloxycarbonyl-protected hydroquinone, coated onto a gold surface, which are first deprotected using light and then oxidised to the corresponding benzoquinone. The later can then react with small molecules containing a cyclopentadiene tag via a Diels-Alder reaction.

![Diagram](image)

**Figure 4.10:** Randomised immobilisation of small-molecules on microarrays (adapted from Kanoh\textsuperscript{311})

In order to avoid the need to synthesise small-molecules with a particular functional group for immobilisation, Kanoh used an innovative technique.\textsuperscript{311} Small-molecules are first spotted onto a surface coated with a diazirin-based photo-affinity linker. After UV irradiation, the small molecules react with the linker in a functional group independent manner, allowing therefore a randomised presentation of molecules with multiple facets exposed for interaction (Figure 4.10). Immunosuppressive agents such as FK506, rapamycin and cyclosporin A were immobilised on an array using this strategy and were successfully recognised by their target protein.

4.2.2.4. In situ Synthesis

Some of the first examples of chemical synthesis carried out directly on the surface were reported by Fodor (light-directed photolithography),\textsuperscript{312} and by Frank on membranes (cellulose, polypropylene etc.).\textsuperscript{313} Frank synthesised peptides under mild...
conditions by spotting a solution of reagents on the membrane ("SPOT-synthesis"),
constituting an array of spot reactors. With this technique, peptides were
successfully synthesised and tested either directly on the membrane or by releasing
them in solution.
Germeroth attempted to extend the strategy to other small molecules. The surface
was first derivatised at specific locations (spots) with a linker containing a terminal
amino group. The first building block was then linked via the amine functionality,
which was then reacted with cyanuric chloride. After substitution of the other
chlorine atoms on the triazine ring, the 1,3,5-derivatised triazines were cleaved and
adsorbed on to the membrane, conserving the spatially addressed format. Although
attractive, this strategy suffers from poor purity of the final compounds synthesised
meaning bioassays being jeopardised.

4.2.2.5. Recent developments for improved detection
One of the disadvantages of microarrays is the rigidity of the bound compounds due
to their attachment to a surface. Indeed during the assays, the screening method
varies from the in vitro / in vivo processes where ligands and protein are free to
approach each other in a "solution-phase" manner. Gosalia decided to retain the high
throughput concept of arrays but changed the approach. Indeed, ligands are printed
in glycerol (to avoid evaporation). The solution of enzyme to be tested is then
sprayed onto the surface as an aerosol. The concept means that each drop constitutes
a micro reactor where neither the biological target nor the ligand are immobilised.
The approach is therefore closer to an in vitro assay and is potentially easier to carry
out as ligands do not need to be designed with a particular functionality for
attachment onto the array.
A common issue when screening microarrays is the signal to noise ratio which can
be quite low. In order to improve the sensitivity of the surfaces, Puskas tested six
different surfaces involving dendrimer and triamino linker systems on acrylic and
epoxy surfaces. These surfaces successfully increased immobilisation efficiency
and reduced the background noise.
4.3. The use of PNA tags for Micro-arrays

4.3.1. Peptide Nucleic Acids (PNA): Concept and properties

Peptide nucleic acids were first described by Nielsen, replacing the deoxyribose phosphate backbone of DNA by a poly(N-(2-aminoethyl)glycine)amide carrying the nucleobases via a methylenecarbonyl linker (Figure 4.11). The DNA mimic obtained is of particular interest due to its remarkable properties, and has been reviewed on several occasions.

As all intramolecular distances and the configuration of nucleobases are similar to those in DNA, specific hybridisation occurs between PNA and cDNA or RNA sequences. In addition, the uncharged nature of PNA is responsible for the thermal stability of PNA-DNA duplexes compared with the DNA-DNA equivalents, and single-base mismatches are considerably more destabilising. Finally, PNAs are resistant to nucleases and proteases. All these properties have made PNAs a great
tool for DNA microarrays. Indeed, compounds carrying a PNA tag can be hybridised on DNA microarrays containing complementary DNA sequences.\textsuperscript{303, 305, 306, 309}

4.3.2. Synthesis of PNA tags

4.3.2.1. Strategies

Several synthesis strategies have been developed for the synthesis of PNA monomers and oligomers. The first method involved the use of the Boc group to protect the primary amino group of the backbone and the use of the Z group to protect the exocyclic amino groups of the nucleobases.\textsuperscript{322} The rationale for using acid labile groups was based on the liability of the N-terminal nucleobase to conduct a rearrangement to the primary amino group of the backbone under alkaline conditions.\textsuperscript{323} However Thomson successfully used the Fmoc group as an alternative to the Boc group without observing the rearrangement, probably because this process is slow.\textsuperscript{324} More recently, Bialy developed another strategy based on Dde-protection which was fully orthogonal to the Fmoc group.\textsuperscript{325, 326} This strategy allowed the direct synthesis of PNA-peptide conjugates. Aminoacids can be coupled to one arm of the supported lysine 4.17 using a Fmoc strategy, with PNA tag synthesis carried out on the other arm of lysine using a Dde strategy (Figure 4.12). Alternate coupling of amino-acids/PNA monomers allows encoding of the combinatorial library. The use of Alloc-protected aminoacids and Fmoc-protected PNA monomers has also been mentioned,\textsuperscript{308} although this strategy is poorly efficient.\textsuperscript{309}

\textbf{Figure 4.12:} Strategy developed by Diaz-Mochon to encode aminoacid libraries
4.3.2.2. Synthesis of the backbone

The synthesis of the backbone was optimised by Bialy.\textsuperscript{326} The first step consisted in reacting ethylene diamine 4.18 with chloroacetic acid yielding 4.19 in 85\% yield, which was then esterified with thionyl chloride in methanol to form 4.20

\[ \text{H}_2\text{N-}\text{-NH}_2 \xrightarrow{\text{ClCH}_2\text{CO}_2\text{H}} \text{H}_2\text{N-}\text{CO}_2\text{H} \]

\[ \text{4.18} \xrightarrow{0^\circ\text{C then rt}} \text{4.19} \]

\[ \text{Dde-OH (4.21)} \xrightarrow{\text{DiPEA, DCM}} \text{H}_2\text{N-}\text{N} \text{-OH} \]

\[ \text{4.22} \xrightarrow{0^\circ\text{C then reflux 18h}} \text{4.20} \]

\[ \text{H}_2\text{N-}\text{CO}_2\text{Me} \]

Scheme 4. 2: Synthesis of the backbone

HPLC and NMR spectra showed that some ethylene diamine dihydrochloride was present in the mixture. Therefore extensive purification was required because the impurity would react with Dde-OH 4.21 in the next step. 4.20 was purified by recrystallisation yielding 45\% of almost pure product. The primary amine of 4.20 was then selectively protected with Dde-OH 4.21 (previously synthesised from dimedone)\textsuperscript{327} in the presence of DIPEA to yield 4.22 in moderate yield (35\%).

4.3.2.3. Synthesis of the adenine monomer

The exocyclic amino group of adenine requires protection in order to avoid chain extension from this position and the introduction of the protecting group is carried out after alkylation. Reaction of adenine 4.23 with ethylbromoacetate in the presence of NaH yielded 4.24 in 67\% yield and was followed by protection of the amino group with MMt-Cl in presence of 4-ethylmorpholine and saponification with KOH to afford the adenine building block 4.25 in 72\% yield over two steps. Once the adenine building-block 4.25 was synthesised, it was coupled to the backbone 4.22 using HBTU as coupling reagent and then saponified to afford the adenine monomer 4.25 (Scheme 4. 3). The yield of 68\% for the last two steps has to be balanced with
the very high purity of the adenine derivative obtained which is an absolute requirement for the synthesis of PNA oligomers.

Scheme 4.3: Synthesis of the adenine monomer

4.3.2.4. Synthesis of the cytosine monomer

In the case of cytosine, protection of the exocyclic amino group is carried out prior to alkylation, partly to increase the solubility of cytosine in organic solvents. Cytosine 4.27 was first reacted with MMt-Cl in presence of 4-ethylmorpholine yielding 4.28 in 45% yield. The low yield is easily explained by the poor solubility of cytosine in DCM/Pyridine making the reaction difficult due to the heterogeneous conditions. Alkylation of 4.28 with ethylbromoacetate and NaH followed by in situ saponification yielded the cytosine building block 4.29 in 68% yield. The building block 4.28 was then coupled to the backbone 4.22 and saponified in a similar way to the adenine monomer 4.30 (Scheme 4.4) to afford the cytosine monomer in 51% yield.
Scheme 4. 4: Synthesis of the cytosine monomer

4.4. High-Throughput-Screening using Dual Fragments Microarrays

4.4.1. Principle of the proposed new concept to fragment-based drug discovery

4.4.1.1. Current Method for Fragment Microarrays

Fragment Microarrays have only been reported by Graffinity Pharmaceuticals so far. However, a key point is the difficulty to identify weak binding, in particular because fragments can bind very weakly to a biological target and the interaction can "disappear" when the protein solution is washed away (Figure 4. 13). Therefore the technique does not appear to be particularly attractive at the present time and new strategies have to be developed.
4.4.1.2. Novel approach to Fragment-Microarrays: Dual Fragment Microarrays

A common step in Fragment-based drug discovery techniques is the need at some point to link active fragments in order to obtain a high affinity compound. The linkage process can be difficult, in particular in the case where no information is available on the binding sites of the two fragments. Indeed two active fragments can potentially bind to the biological target in the same area. With that issue in mind, the best solution is to be able to screen at least two fragments at the same time. Spotting two fragments on the same location of a microarray could potentially fulfil this requirement (Figure 4. 14). After addition of the protein solution, weak interactions between fragments and the target will be “amplified” by the binding at the same time of the other fragment to the target. This strategy would allow direct identification of active fragments that bind to the target with direct cooperative effects. The generation of lead compounds could therefore be speeded up as the anchoring point for the two fragments is known and optimisation of the linkage could be started immediately after screening the library of fragments.
Classic screening of separate fragments can sometimes be unsuccessful for optimisation as the binding of one fragment can change the conformation of the binding pocket, making the binding of another positive fragment not as strong as originally screened. The proposed strategy suppresses this drawback as cooperative fragments are screened at the same time.

### 4.4.1.3. Choice of biological target and fragments to validate the concept

In order to validate the concept, assays need to be based on a well established target and fragments. The best example of fragment-based drug discovery is probably the technique developed by Fesik.\(^{259}\) The FK506-binding protein (also known as FKBP12) has been used as a biological target on many occurrences, due to its role in immune response. Ligands of this protein and more generally of immunophilins have therefore attracted a lot of interest. Examples of immunosuppressive agents are numerous and include Rapamycin \(^{4.38}\), FK506 \(^{4.39}\) and Cyclosporin A \(^{4.40}\) (Figure 4.15).\(^{328\text{-}330}\) Therefore FKBP represents an attractive target to study.

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**Figure 4.14**: Principle of the proposed Dual Fragment Microarrays
In addition the fragments synthesised by Fesik have proved to validate the fragment-based drug discovery concept. Therefore these fragments would be suitable to validate the new concept of Dual Fragments Microarrays. Fragments could be linked to various spacers in order to study at the same time the impact of the spacer type/length on the results that would be obtained with Dual Fragment Microarrays. The template ”fragment-spacer” would have to be linked to the microarray surface. The use of the PNA tag strategy developed by Diaz-Mochon appears to be attractive. Indeed although no complex library is initially required to validate the concept of dual fragment microarrays, the PNA tag could potentially be used later on to encode fragment libraries.

4.4.2. Synthesis of the PNA tag

The Fmoc-Rink linker was first bound to PEGA resin using PyBOP as coupling reagent, followed by Fmoc-deprotection. Fmoc-Lys(Dde)-OH 4.42 was then coupled to the resin to afford the starting point for the PNA tag synthesis 4.43 (Scheme 4. 5).
Leaving the Fmoc-protected arm free, the resin 4.43 was deprotected using conditions described by Diaz-Mochon.\textsuperscript{325} and successive cycles of coupling/deprotection afforded the PNA tag 4.44 with the following chosen sequence: N-T-C-A-A-C-T-T-C-A-T-A-C (Scheme 4. 6). Although the use of PyBOP was reported for coupling the Dde-PNA monomers, HATU was successfully
used allowing shorter reaction times (1.5h instead of 6h) and smaller quantities of monomer to be used (3 equiv. instead of 5.5 equiv.). These conditions are particularly attractive when synthesising a tag on a large scale and was therefore applied to the target tag.

4.4.3. Synthesis of the fragments
4.4.3.1. Synthesis of fragment F₁

The synthesis of the first fragment was adapted from Taylor. 331 3',4',5'-trimethoxyacetophenone 4.45 was first oxidised using selenium dioxide to form the α-keto-acid 4.46 in 81% yield. Separately, (S)-piperidine-1,2-dicarboxylic acid-1-tertbutyl ester 4.46 was esterified with thionyl chloride/MeOH in quantitative yield (Scheme 4.7). The choice of 4.47 was made due to the lower cost of the material compared to the unprotected amine.

The fragments were first coupled using DCC/DMAP, which however resulted in poor conversion (less than 5%). In comparison, EDC gave 40% yield but regular addition of the reagent was necessary to achieve this. Attempts to transform the acid into the acid chloride using thionyl chloride resulted in degradation of the fragment and were therefore unsuitable. The possibility to use PS-IIDQ was not investigated as the piperidine scaffold was similar to proline, which proved to yield carbamate by-product (see Chapter III). The best results of coupling were obtained with HBTU but
the coupling was still a moderate 48% yield (Scheme 4.7). After coupling, the protected fragment 4.49 was easily saponified with lithium hydroxide in 93% yield to afford fragment F₁ 4.50.

4.4.3.2. Synthesis of Fragments F₂ to F₅

The other fragments reported by Fesik were based on aminophenols.²⁵⁹ Coupling between 2-, 3- and 4-aminophenols (4.51a-c) and 4-hydroxybenzoic acid 4.52 using IIDQ resulted in mixtures of the expected amides 4.53a-c and the corresponding esters 4.54a-c (Scheme 4.8).

Although the esters 4.54a-c could be easily cleaved, the possibility of protecting the phenol previous to the coupling was chosen. Indeed, this protection would insure the selective attachment of the fragment to the spacer through the hydroxyl group of the acid. Therefore 2-, 3- and 4-aminophenol 4.51a-c were protected with TBS-Cl using classical conditions (Scheme 4.8).³³²

The protected aminophenols 4.55a-c obtained failed however to couple to 4-hydroxybenzoic acid 4.52 under various conditions (IIDQ, EDC and DCC). For this
reason, 4-acetoxybenzoic acid 4.56 was used as a replacement of 4-hydroxybenzoic acid 4.52. The coupling succeeded using IIDQ in moderate yield (35%, 45% and 49% over two steps for the ortho 4.57a, meta 4.57b and para 4.57c derivatives respectively) (Scheme 4.10).

Various conditions of saponification of the meta isomer 4.57b were studied and lithium hydroxide and potassium carbonate both gave complete conversion after 1h and three TBS-protected fragments 4.58a-c were obtained. However, the ortho isomer 4.58a decomposed quickly under these conditions although only 5 min. were required for complete cleavage of the ester and the product had to be purified by column chromatography.

Another fragment (F5) 4.61, synthesised from aniline 4.59 and 4-acetoxybenzoic acid 4.56 was also obtained with this method in 85% overall yield (Scheme 4.11).
4.4.4. Methodology for parallel solution-phase Mitsunobu reactions

4.4.4.1. General considerations

Many spacers could be used in the project, but diols and glycols appeared to be attractive to use for the phenol-based fragments (F2-F5). Indeed the Mitsunobu reaction provides an efficient synthetic tool for the synthesis of aryl ethers (Scheme 4.12).333-336 The reaction occurs under mild, essentially neutral reaction conditions (0°C to room temperature), and tolerates a variety of functional groups. The pKa of the nucleophilic component (in this case, the phenol) has to be lower than the betaine intermediate pKa (about 13). Some non-polar solvents accelerate the conversion, and therefore THF, diethylether, DCM and toluene are often preferred as solvents, though ethyl acetate, acetonitrile and DMF are sometimes used.

![Scheme 4.12: Synthesis of aryl-alkyl ethers via the Mitsunobu reaction](image)

However, the reagents used in this reaction are often hard to remove, although some newer methods have been reported.337 For this reason the possibility of developing a simplified process was investigated, and fragment F3 (meta isomer) and 1,3-propanediol were chosen as test-substrates.

Traditional conditions using solid-supported phosphine (Novabiochem) or triphenylphosphine in solution, and DIAD were first investigated. The triphenylphosphine oxide was difficult to separate from the reaction mixture. Immobilised-triphenylphosphine gave poor conversion.
4.4.4.2. Alternative coupling reagents

Other Mitsunobu coupling reagents were investigated. DEAD 4.62 and DIAD 4.63, which are commonly used in Mitsunobu reactions, can usually be readily removed from the product mixture by column-chromatography. However, this is not suitable for parallel synthesis, and new coupling reagents have been reported (Figure 4.16).

ADDP 4.64 is a useful alternative azodicarbonyl equivalent, which can be readily filtered off from the reaction mixture (along with the corresponding hydrazine by-product) after dilution of the completed reaction mixture with hexanes. Some other diazocarbonyl equivalents derived from morpholine 4.65 and N-methylpiperazine 4.66 have also been reported but are not commercially available. Another reagent was developed by Tsunoda: DHTD 4.67 can be synthesised easily but is also not commercially available. Di-tert-butylazodicarboxylate 4.68 (DBAD) has also been
used in order to avoid chromatographic separation.\textsuperscript{341} DNAD 4.69 has been proposed to enable selective derivatisation by polymerisation of by-products using a norbornene tag.\textsuperscript{342} After reaction, ruthenium-catalysed polymerisation of olefin-containing compounds (ring opening metathesis) leads to insoluble by-products. Finally, TMAD 4.70 has been reported as being an efficient Mitsunobu reagent and is commercially available.\textsuperscript{343, 344} One could mention the availability of a polymer-supported DEAD 4.71.\textsuperscript{345} However, this reagent is extremely expensive and has to be used in conjunction with a phosphine in solution.

4.4.4.3. Alternative phosphines
Removal of the by-product triphenylphosphine oxide as well as excess triphenylphosphine 4.72 has been the most problematic facet of Mitsunobu chemistry. In some cases, the by-product may be filtered from the reaction mixture, but often the by-products must be removed by a chromatographic separation. Tri-\textit{n}-butylphosphine 4.73 has been reported as being a useful improvement for this method with little or no effect on the reaction yield.\textsuperscript{346} In this case, the by-product tributylphosphine oxide is water soluble and may be largely removed by an aqueous workup. Excess tributylphosphine is volatile and is usually removed during removal of the reaction solvent. More generally alkylphosphines are suitable for the Mitsunobu reaction.\textsuperscript{347} Other methods have been developed to increase the ease of workup of Mitsunobu chemistry, and a great deal of effort has been put toward the development of triarylphosphines. Modification of the phosphine portion has generated reagents that are easily removed from the completed reaction mixture, along with the corresponding phosphine oxide by-products (Figure 4. 17). O’ Neil showed that DPPE 4.74 is a convenient replacement for triphenylphosphine in the Mitsunobu reaction.\textsuperscript{348} Due to the greater polarity of the resulting bis-triphosphine oxide by-product, this component is usually insoluble to the point at which it can be easily removed from the reaction mixture by a simple filtration prior to workup and purification. Other phosphine equivalents include DAP-DP 4.75,\textsuperscript{349} Ph$_2$P-Py 4.76,\textsuperscript{341} and TAP 4.77.\textsuperscript{350} These phosphines present the advantage of containing basic functionalities, making the extraction through an acidic workup possible.
Solid-supported triphenylphosphine 4.78 has become a standard procedure for Mitsunobu reactions. In particular it has been used along with DBAD for parallel synthesis of alkyl aryl ethers. However as explained before, DBAD is not suitable for the targeted substrates.
Similarly to the strategy used with the coupling reagent DBAD, Flynn used a tert-butyl-masked, carboxy-tagged alkyldiphenyolphosphate 4.79 which was deprotected with TFA. Yoakim also used a silyl-masked phosphine 4.80, which was unmasked with TBAF (Figure 4.18). Other examples of tagged-phosphine include 15-crown-5 tagged triphenyolphosphate 4.81, which can be scavenged via an ammonium-functionalised resin, and anthracene-modified phosphine 4.82, which can be removed via a Diels-Alder reaction with a polymer-bound maleimide.

4.4.4.4. Conclusion on suitable reagents
By selecting only the commercially available reagents and eliminating any reagent requiring strongly acidic conditions, a list of three coupling agents (DIAD 4.63, TMAD 4.70 and ADDP 4.64) and six phosphines (PPh3 4.72, PBu3 4.73, DPPE 4.74, PyPPh2 4.76, DAP-DP 4.75 and PS-PPh2 4.78) was created. Although triphenyolphosphate and diisopropylazodicarboxylate are known to require purification, they remained in the list of reagents to screen as a comparison due to their well-established properties as mediating well Mitsunobu reactions.

4.4.4.5. Screening of coupling reagents and phosphines
The combination of suitable and commercially available coupling reagents (DIAD, TMAD, ADDP) and phosphines (PPh3, PBu3, DPPE, PyPPh2, DAP-DP and PS-PPh2) was first investigated in parallel. The conditions were adapted from a reported synthesis of alkyl aryl ethers, and in particular alkyl aryl ethers involving diols.
An excess of diol (1.5 equiv.) to the phenol (1 equiv.) was used in order to prevent a double Mitsunobu reaction, and the phosphine and coupling reagents were used accordingly (1.5 equiv.) followed by an aqueous workup. A mixture of DCM and THF was used and the coupling reagent was added portion-wise over 2h. After reaction, an aqueous workup was carried out. In the case of ADDP and TMAD, the hydrazine by-products formed during the reaction were filtered off prior to workup.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Coupling reagent</th>
<th>Phosphine</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DIAD</td>
<td>PPh₃</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>PBu₃</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Py-PPh₂</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>DAP-DP</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>DPPE</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>PS-PPh₂</td>
<td>100 a</td>
</tr>
<tr>
<td>7</td>
<td>TMAD</td>
<td>PPh₃</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>PBu₃</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Py-PPh₂</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>DAP-DP</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>DPPE</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>PS-PPh₂</td>
<td>100 b</td>
</tr>
<tr>
<td>13</td>
<td>ADDP</td>
<td>PPh₃</td>
<td>51</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>PBu₃</td>
<td>36</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>Py-PPh₂</td>
<td>72</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>DAP-DP</td>
<td>21</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>DPPE</td>
<td>11</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>PS-PPh₂</td>
<td>100</td>
</tr>
</tbody>
</table>

a determined by ELSD; b DIAD by-products were not detected by ELSD

Table 4.1: Screening of coupling reagents and phosphines followed by SPE purification

The mixtures including the expected product 4.83 were analysed by LCMS with attention focused on the presence of phosphine oxide or coupling reagent by-product. Unfortunately, none of the "water-extractable" phosphines proved to be removed efficiently from the reaction mixtures. However, it was noticeable that TMAD and
ADDP did not leave any trace in the mixture in comparison to DIAD. Polymer-supported triphenylphosphine (purchased from Fluka) again proved to give poor conversion. As removal of the "water-extractable" phosphines was surprisingly only partial, the combination of all phosphines and coupling reagents was investigated again with a new approach to the workup. After reaction, the mixtures were filtered through SPE extractors containing a mixed bed of acidic/basic macroporous ion exchange resin, and the mixtures were analysed by LCMS and ELSD (Table 4.1). Polymer-supported triphenylphosphine (Polymer Lab) was tested again. The solid-phase extractors failed to entirely remove the phosphines oxides, making all the "water-extractable" phosphines unsuitable for library synthesis. However, the supported triphenylphosphine gave excellent results in that case. When used in combination with TMAD or ADDP, no coupling reagent or phosphine, were present in the mixture at the end of the reaction (Figure 4.19).

Figure 4.19: Comparison of the product purity by HPLC (ELSD) with (a) PS-PPh₃/TMAD, (b) DAP-DP/ADDP and (c) DIAD/PPh₃
4.4.5. Initial choice of spacers

Six spacers were chosen initially for the validation of the Dual-Fragment Microarrays concept (Scheme 4.13). All of them contained a hydroxyl group on one side for attachment to the fragment and either an acid or hydroxyl group on the other side for attachment to the PNA tag. Fragments were planned to be coupled to the spacers via the Mitsunobu process developed above and then linked to the resin either via a carbamate (spacer $S_1$-$S_4$) or amide bond (spacers $S_5$-$S_6$).

![Scheme 4.13: Spacers used for phenol-based fragments](image)

Before synthesising the library of spacer-fragment conjugates, the feasibility of a carbamate linkage to the resin was evaluated. Urethane formation (excluding protecting groups) has been reported on solid-phase but with the hydroxyl group being on the resin.\textsuperscript{361}

The fragment-spacer conjugate synthesised (4.83) was coupled to $H_2N$-Lys(Dde)-Rink-PEGA 4.43 (Scheme 4.14).

![Scheme 4.14: Target reaction to study the feasibility of the carbamate linkage](image)
Many conditions were tested involving the use of carbonyldiimidazole (CDI) and 4-nitrophenylchloroformate. After reaction, the resin was washed and cleaved with TFA/DCM/TIS (90/5/5). The conditions tested are reported in Table 4.2. As PEGA resin has a tendency to retain moisture, the resin was washed many times with dry THF prior to reaction.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent</th>
<th>Order of activation</th>
<th>Solvent</th>
<th>Base</th>
<th>Additive</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CDI</td>
<td>Resin first</td>
<td>DCM</td>
<td>DiPEA</td>
<td>-</td>
<td>no reaction</td>
</tr>
<tr>
<td>2</td>
<td>4-NO₂-OC(O)Cl</td>
<td>Resin first</td>
<td>DCM</td>
<td>DiPEA</td>
<td>-</td>
<td>4.85</td>
</tr>
<tr>
<td>3</td>
<td>CDI</td>
<td>Alcohol first</td>
<td>DCM</td>
<td>DiPEA</td>
<td>-</td>
<td>no reaction</td>
</tr>
<tr>
<td>4</td>
<td>4-NO₂-OC(O)Cl</td>
<td>Alcohol first</td>
<td>DCM</td>
<td>DiPEA</td>
<td>-</td>
<td>no reaction</td>
</tr>
<tr>
<td>5</td>
<td>4-NO₂-OC(O)Cl</td>
<td>Alcohol first</td>
<td>DCM</td>
<td>DiPEA</td>
<td>DMAP</td>
<td>no reaction</td>
</tr>
<tr>
<td>6</td>
<td>4-NO₂-OC(O)Cl</td>
<td>Alcohol first</td>
<td>THF</td>
<td>DiPEA</td>
<td>DMAP</td>
<td>no reaction</td>
</tr>
<tr>
<td>7</td>
<td>CDI</td>
<td>Alcohol first</td>
<td>DCM</td>
<td>DiPEA</td>
<td>DMAP</td>
<td>no reaction</td>
</tr>
</tbody>
</table>

Table 4.2: Conditions tried for carbamate-bond formation

Figure 4.20: Cross-linking product formed

None of the conditions tried afforded the expected carbamate compound. Either no new product was observed, or the product observed 4.85 corresponded to the crosslinking on the resin via the activating agent (Figure 4.21). Other reagents could have been tried such as triphosgene, but this would have required extensive purification of each fragment-spacer conjugate after reaction, while reaction of triphosgene on the resin could have yielded cross-linking. Another strategy was therefore developed.
4.4.6. Extension of the chain on the resin

The amino group of the amino acid was extended with adipic acid monoester (Scheme 4. 15) (confirmed by MS analysis after cleavage of a few beads). A $\text{C}_6$ chain was chosen because shorter chains can sometimes yield cyclic by-products after coupling.

\[ \text{H}_2\text{N} - \text{CO} - \text{NH} - \text{H}_2\text{N} \quad \rightarrow \quad \text{H}_2\text{NCO} - \text{(CH}_2\text{)}_6\text{-CO}_2\text{H}, \text{HATU, DiPEA, rt, 1h} \]

\[ \text{Dde} \quad \text{NH} \quad \quad \rightarrow \quad \text{MeO} \quad \text{Dde} \quad \text{NH} \]

\[ \text{LiOH} \quad \text{THF/water} \quad \text{rt, 4h} \]

\[ \text{HO} - \text{N} - \text{MeO} \quad \quad \rightarrow \quad \text{HO} - \text{N} - \text{NH} \]

\[ 4.43 \quad \rightarrow \quad 4.86 \quad \rightarrow \quad 4.87 \quad \rightarrow \quad 4.88 \]

\( \text{Scheme 4.15: "Extension" of the resin with adipic acid monoester} \)

Hydrolysis of the methyl ester via lithium hydroxide in THF / water was successful, however, under these conditions partial deprotection (about 20%) of the Dde group was observed. This could be easily corrected by capping the amino group with an acetyl group. These conditions were applied to the resin 4.44 synthesised previously to afford resin 4.89 (Figure 4. 21).

\[ \text{Fmoc} \quad \text{HN} - \text{-Tag} \quad \text{NH} \quad \rightarrow \quad \text{HO} - \text{N} - \text{-Tag} \quad \text{NH} \]

\[ \text{HN} - \text{-Tag} \quad \text{NH} \quad \rightarrow \quad \text{HN} - \text{-Tag} \quad \text{NH} \]

\[ 4.44 \quad \rightarrow \quad 4.89 \]

\( \text{Figure 4.21: Synthesis of resin 4.89} \)
4.4.7. Coupling of spacers and fragments to the resin

4.4.7.1. Spacers for phenol-type fragments

Based on the spacers that were going to be used via a Mitsunobu reaction ($S_1$-$S_6$), three new spacers were selected ($S'_1$-$S'_3$, Figure 4.22), with an amino group on one end for coupling to the resin.

\[
\begin{align*}
S_1 & \quad \text{HO-OH} \quad \text{H}_2\text{N-} \quad \text{OH} \\
S'_1 & \quad \text{HO-OH} \quad \text{H}_2\text{N-} \quad \text{OH} \\
S_2 & \quad \text{HO-OH} \quad \text{H}_2\text{N-} \quad \text{OH} \\
S'_2 & \quad \text{HO-OH} \quad \text{H}_2\text{N-} \quad \text{OH} \\
S_3 & \quad \text{HO-OH} \quad \text{H}_2\text{N-} \quad \text{OH} \\
S'_3 & \quad \text{HO-OH} \quad \text{H}_2\text{N-} \quad \text{OH}
\end{align*}
\]

Figure 4.22: Choice of new spacers

The three spacers were coupled to resin 4.89 using DIC/HOBt, which has been reported to couple amino-alcohols selectively\textsuperscript{362} giving resins 4.90, 4.91 and 4.92 (Scheme 4.16), while this selectivity has not been investigated using IIDQ.

\[
\begin{align*}
\text{HO (i R N} & \quad \text{DIC/HOBt} \\
\text{2) } S'_1 \text{ or } S'_2 \text{ or } S'_3 & \quad \text{HN-Tag NH} \\
\text{(Dde)} & \quad \text{HN-Tag'} \\
\text{(Dde)} & \quad \text{HN-Tag} \\
\text{4.89} & \quad \text{4.90 R = HO } \\
\text{4.91 R = HO} & \quad \text{4.92 R = HO}
\end{align*}
\]

Scheme 4.16: Coupling of spacers $S'_1$-$S'_3$ to the resin
4.4.7.2. **Coupling of fragments F₂-F₅ to the resin**

Fragments F₂-F₅ were coupled to the resin via classic Mitsunobu reaction conditions using PPh₃ and DIAD in THF/DCM for 16h (Scheme 4.17) (the conditions previously optimised for Mitsunobu reaction could not be applied to this strategy because the final product are immobilised and purification is not thus an issue). 4-trifluoromethyl-phenol 4.93 (named fragment F₆) and phenol 4.94 (named fragment F₇) and were also coupled to the resin in order to study capping effects during the assays.

![Diagram of coupling of fragments F₂-F₅ to the resin](image)

**Scheme 4.17: Coupling of fragments F₂-F₅ to the spacers via Mitsunobu reaction**

Cleavage of the resin with TFA / DCM / TES (90:5:5) allowed the release of the tagged fragments. The strongly acidic conditions also resulted in deprotection of the
TBS group. After precipitation with ether, the 18 tagged spacer-fragments conjugates were obtained on a 1-3 mg scale (~ 0.5 μmol).

4.4.7.3. Spacers for Fragment F₁

In order to couple fragment F₁ to the resin 4.90, the spacers required an amino-group on both ends. However, diamines risk crosslinking. In addition during the capping step, the free amino-group would also be capped. For these reasons, Fmoc-monoprotected fragments S''₁-S''₃ were selected (Figure 4.23).

![Figure 4.23: Spacers selected for Fragment F₁](image)

These spacers were coupled to the resin using DCC/HOBt, and the resulting resins were then capped using AcOH /HATU/ DIPEA, followed by Fmoc-deprotection to afford resins 4.95, 4.96 and 4.97 (Scheme 4.18).

![Scheme 4.18: Coupling of spacers S''₁-S''₃ to the resin](image)
4.4.7.4. Coupling of fragment $F_1$ to the resin

Fragment $F_1$ was easily coupled to resins 4.95, 4.96 and 4.97 with HATU/DIPEA (Scheme 4.19). Rhodamine was also coupled to the same resins for detection of successful hybridisation.

Following the same cleavage conditions used for the other fragments, the 3 tagged spacer-$F_1$ conjugates and 3 tagged spacer-rhodamine conjugates were obtained on a 1-3 mg scale (~ 0.5 μmol).
4.4.8. Preparation of the microarrays

4.4.8.1. Printing of the oligonucleotide sequences on aldehyde and agarose slides

The complementary sequence of the PNA tag previously synthesised was purchased from Sigma-Genosys. This sequence was 3'-amino-modified in order to link the oligonucleotides to aldehyde-derivatised slides via reductive amination. In addition the sequence was preceded with six thymine units, to act as a spacer. The same 3'-modified nucleotide was also purchased with an additional 5' modification with fluorescein in order to check successful printing on the surface.

Two different surfaces were selected: standard aldehyde glass-slides presenting a planar two dimensional surface, and glass-slides covered with a layer of activated agarose gel forming a 3D texture. Potentially, agarose slides can immobilise more DNA chains due to the 3D architecture and therefore increase the fluorescence signal resulting in better signal to noise ratio. It was indeed successfully used for DNA and protein microarrays. Agarose coated slides are now commercially available and are activated to present aldehyde functionalities for attachment of amino-derivatised DNA.

![Fluorescence detection after DNA printing on an aldehyde slide using a FITC filter](image)

Figure 4.24: Fluorescence detection after DNA printing on an aldehyde slide using a FITC filter

After printing, the slides were washed (including a reducing washing solution containing NaBH₄) and scanned using a FITC filter (aldehyde slide, Figure 4.24). The expected fluorescein-derivatised DNA pattern on the array was detected proving...
that printing had been successful. Scanning of agarose slides interestingly showed higher fluorescence intensity compared to the aldehyde slides. However when zooming onto the pattern, the difference was more difficult to appreciate (Figure 4.25)

![agarose slide vs aldehyde slide](image-url)

**Figure 4.25**: Comparison of fluorescence intensity after DNA printing on an agarose (average: 8.8 $10^5$) and an aldehyde slide (average : 5.1 $10^5$)

### 4.4.8.2. Printing of the tagged fragments

Hybridisation of PNA encoded libraries onto DNA microarrays is usually performed with a hybridiser which consists in passing the solution of the mixture onto the slide at 90°C in order to make sure that each tagged-molecules can access its corresponding complementary site on the array and that uncomplementary duplexes are separated. Direct hybridisation of a PNA-tagged molecule on to the complementary DNA sequence by spotting a stock solution onto the array has not been reported before. In order to allow some tolerance with the positioning, each stock solutions was spotted 5 times, making therefore a bigger drop onto the array and increasing the chance of superposition on the DNA spot (Figure 4.26).

![DNA printing vs stock solutions](image-url)

**Figure 4.26**: Illustration of the importance of a multiple printing
Successful hybridisation could be determined by comparing the printing pattern of rhodamine-containing tags and the image obtained by scanning the slides using a Cy3 filter (Figure 4.27).

![Figure 4.27: Fluorescence detection after hybridisation using a Cy3 filter](image)

The comparison clearly showed the presence of the right patterns confirming that hybridisation had been successful. The elaborated procedure represents therefore a new way to obtain small-molecules microarrays. Indeed small-molecules can be synthesised in a parallel approach, linked to a PNA-tag and hybridised onto a DNA microarray. Although direct printing of small-molecules has been reported, this approach offers the advantage of controlling printing of small-molecules as shown by the rhodamine control.

4.4.9. Screening of the microarrays against FKBP

4.4.9.1. Methodology of the assays

The strategy chosen was to use a protein complementary to an antibody. After incubation of the protein, the corresponding antibody can be added. After incubation of this primary antibody, a fluorescent secondary antibody can be used to detect binding between the ligands and the protein by fluorescence (Figure 4.28).
RGS-tagged FKBP 12 was kindly provided by Prof. Stuart Schreiber and Dr. Angela Koehler from Harvard University. This protein is recognised by Qiagen's RGS mouse antibody. All arrays printed previously were first incubated with RGS-FKBP12, then incubated with Alexa 488® anti-mouse and scanned using a FITC filter. The mapping of the spotted stock solutions (Figure 4.29) was correlated with the intensity of fluorescence (Figure 4.30).
4.4.9.2. Analysis of the aldehyde slides

First Aldehyde slide

The intensity of the spots was analysed using the FIPS software (LaVision BioTech). Globally, the background was high. The average of the two patterns gave four hits (Figure 4.31). In the case of single fragments, fragment $F_1$ gave the highest intensity when associated with spacer $S_3$. This result was anticipated as $F_1$ is the ligand with the best affinity according to Fesik’s experiment. The two other hits corresponded to 4-trifluoromethyl-phenol associated with spacer $S_2'$ and $S_3'$. This result is not surprising as the trifluoromethyl group is known as being an excellent pharmacophore to many biological targets including FKBP. Results with spacer $S_1$ were very similar for each fragment and the intensity of fragment $F_1$ was quite low. This could indicate that spacer $S_1$ is too short for efficient binding to the protein.
Figure 4.31: Fluorescence intensities obtained on the first aldehyde slide
In the case of dual fragments, only one mixture was higher than the others. This was obtained with spacer 2 and corresponded to the mixture of fragment F1 and fragment F4. This corresponded exactly to the results from Fesik! However the difference of intensity between the single fragment F1 and the mixture F1+F4 was not huge. In addition it was surprising not to observe a similar result with spacer S'3.

Second aldehyde slide

Results were slightly different on the second aldehyde slide. Indeed fragment F2, F3 and F5 associated with spacer S'1 gave surprisingly high intensities (Figure 4.32). For mixtures of fragments, an impressive result was observed as the intensity of the mixture F1 + F4 associated with spacer S'2 was much higher that any other spots. This result corresponded once again to the affinities observed by Fesik.

Figure 4.32: Fluorescence intensities obtained on the second aldehyde slide
4.4.9.3. Analysis of the agarose slides

Analysis did not allow many conclusions to be drawn as too many hits came up. Analysis (Figure 4.33) for single fragments showed that more hits were detected when using spacer $S''_3$. This tendency was also observed with mixtures of fragments. However, while the association of fragment $F_1$ and $F_3$ proved to be the highest with spacers $S''_1$ and $S''_2$, the same association showed the worse intensity that any of the other mixtures when associated with spacer $S''_3$. Across the slide, 4-trifluoromethylphenol was detected as a hit.

![Fluorescence intensity graph](image)

**Figure 4.33**: Fluorescence intensities obtained on the agarose slides

4.4.9.4. Conclusion of the screening

Although showing higher average intensities than the aldehyde slides, the agarose slides showed many spots with similar fluorescence intensities and therefore failed to identify any hit.

Whatever the surface was, high background noise made the results difficult to interpret. However, although variations from one slide to the other were observed (average intensities: $4.1 \times 10^5$ and $5.9 \times 10^5$), the results obtained with aldehyde slides were encouraging. Indeed, on both slides the mixture of fragment $F_1$ with fragment $F_4$ gave a hit, as expected from Fesik’s results. In particular the second aldehyde
slide showed a significant difference between the intensity of this mixture ($7.2 \times 10^5$) and any other single fragment/mixture (average: $5.8 \times 10^5$). However, the difference of intensities for different spots in terms of percentages was quite low, which suggests that the results obtained need to be considered with caution.

In order to evaluate the influence of the spacer, the average intensities over the two aldehydes slides were investigated (Figure 4.34). For single fragments, the spacer did not seem to have a noticeable influence as the average intensities were in the similar range ($5.01 \times 10^5$ to $5.03 \times 10^5$). However, the spacer type had a major impact on dual fragments. Indeed, intensities obtained with spacer $S_2'$ ($5.21 \times 10^5$) were higher than the one obtained with spacer $S_1'$ ($4.94 \times 10^5$) and $S_3'$ ($4.97 \times 10^5$) gave lower intensities. Again, the difference in terms of percentage was low.

![Figure 4.34: Influence of the spacer on the fluorescence intensity (average intensities over the two aldehyde slides)](image)

**4.5. Conclusion**

The first results of screening of Dual Fragment Microarrays were promising and the tendency to detect significantly the best association expected (fragment $F_1 + F_4$) was encouraging. Moreover, the results tended to show that spacer $S_2'$ was the most suitable for Dual Fragment Microarrays. These primary results revealed that many parameters would need to be optimised. Indeed the background noise was high, making clear conclusions difficult to undertake. Therefore, incubation parameters of
the protein, primary and secondary antibodies would probably need to be adapted. In order to reduce the background, the use of a secondary antibody labelled with a different fluorophore would also be interesting to investigate. All this study partially proved that the concept of Dual Fragment Microarrays is valid but much optimisation is still required.
5.1. General Section

The reagents commercially available were used without further purification. Solvents were not dried or distilled except where specified. All solution-phase reactions were stirred magnetically, unless otherwise stated, and followed by HPLC or thin-layer chromatography (TLC) where appropriate, using aluminium-coated Silica Gel 60 (Macheray Nagel: 0.20 mm layer). TLC visualisation was performed using short wavelength UV light (254 nm) and/or PMA oxidation. NMR spectra were recorded on Bruker DPX 400 and 300, or ARX 250 spectrometers in the solvents indicated at 298 K. Chemical shifts are reported on the δ scale in ppm and are referenced to residual non-deuterated solvent resonances. Signals of $^1$H Magic Angle Spinning-NMR spectra were assigned by comparison with the $^1$H NMR spectra obtained with the solution-phase equivalent of the supported compound. All $^{13}$C NMR experiments were supported with DEPT. IR spectra were obtained on a Thermo Mattson Satellite FTIR spectrometer or Bruker Tensor 27 Spectrometer, with 16 scans, at a resolution of ± 4 cm$^{-1}$. The FTIR spectrometers were fitted with a Specac single reflection diamond ATR Golden Gate, and neat compounds were used for analysis. Frequencies are reported in cm$^{-1}$ and only frequencies corresponding to significant functional groups are reported. LC-Mass spectra were recorded either on a water ZMD single quadrupole MS, with a 2700 Autosampler and a 600 Pump, or an Agilent Technologies LC/MSD Series 1100 Quadrupole Mass Spectrometer (QMS), both with an electrospray ion source. HPLC spectra were recorded using an Agilent 1100 Series coupled to a Polymer Lab 100 ES Evaporative Light Scattering Detector (ELSD), with a Phenomenex Luna C18, 5μm, 10cm column (column 1), a Phenomenex Gemini C18, 5μm, 10cm column (column 2), or a Phenomenex Luna C18, 5μm, 15cm column (column 3).
HPLC grade water, MeOH (or CH₃CN) with 0.1 % formic acid were used as eluants, at a flow rate of 1 mL/min., with samples prepared to a concentration of about 30 μg.mL⁻¹ (ZMD) or 1 mg.mL⁻¹ (Agilent) and filtered prior to injection. The following methods were used:

Method A (column 1, H₂O/MeOH, 12 min.): 0 min. (95/5), 7 min. (5/95), 9 min. (5/95), 9.05 (95/5).

Method B (column 2, H₂O/MeOH, 10 min.): 0 min. (95/5), 7 min. (5/95), 9 min. (5/95), 9.05 (95/5).

Method C (column 3, H₂O/MeOH, 15 min.): 0 min. (95/5), 12 min. (5/95), 14 min. (5/95), 14.05 (95/5).

Method D (column 1, H₂O/CH₃CN, 6 min.): 0 min. (95/5), 3 min. (5/95), 4 min. (5/95), 4.05 (95/5).

Melting points (Pyrex capillaries) are uncorrected.

Specific optical rotations were recorded with PolAAr 3001 at 589 nm.

HRMS analyses were performed by the Mass Spectrometry Service of the University of Southampton, U.K. Elemental analysis were carried out by Medac Ltd, U.K.
5.2. Experimental for Chapter II

5.2.1. Synthesis of the library for the comparison of IIDQ and EEDQ

Preparation of mother solutions

The following solutions were prepared:

**Amines:**
- tert-butyl aniline: 207 μL (0.5 mmol) in 5 mL CH$_3$CN (0.1 mmol/mL)
- benzylamine: 143 μL (0.5 mmol) in 5 mL CH$_3$CN (0.1 mmol/mL)
- morpholine: 114 μL (0.5 mmol) in 5 mL CH$_3$CN (0.1 mmol/mL)

**Acids:**
- phenyl acetic acid: 95 mg (0.7 mmol) in 7 mL CH$_3$CN (0.1 mmol/mL)
- benzoic acid: 85 mg (0.7 mmol) in 7 mL CH$_3$CN (0.1 mmol/mL)

**Coupling reagents:**
- IIDQ: 212 mg (0.7 mmol) in 7 mL CH$_3$CN (0.1 mmol/mL)
- EEDQ: 172 mg (0.7 mmol) in 7 mL CH$_3$CN (0.1 mmol/mL)

Procedure

In each vial, a solution of an amine (1 mL, 0.1 mmol, 1 equiv.) and of a carboxylic acid (1 mL, 0.1 mmol, 1 equiv.) were added, followed by the addition of a solution of a coupling reagent (1 mL, 0.1 mmol, 1 equiv.). The 12 vials were shaken at room temperature for 16h. The contents of the vials were then filtered through a SPE cartridge containing a mixed bed of MP acidic/basic ion-exchange resins (Polymer Lab, 500mg). The filtrates were concentrated *in vacuo* to afford the compounds listed below.
N-(4-tert-Butyl-phenyl)-2-phenyl-acetamide (2.152)

The named compound was obtained as an off-white solid (25 mg, 96%).

HPLC (method B): $t_R = 7.32$ min.

Purity (ELSD): 100%

$^1$H NMR (250 MHz, CDCl$_3$): 7.48 (s, 1H, NH), 7.39-7.25 (m, 9H, Ar-H), 3.69 (s, 2H, CH$_2$), 1.28 (s, 9H, C(CH$_3$)$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): 169.16 (C=O), 147.32 (C-Ar), 135.02 (C-Ar), 134.61 (CH-Ar), 129.38 (CH-Ar), 129.02 (CH-Ar), 127.42 (CH-Ar), 125.63 (CH-Ar), 119.71 (CH-Ar), 44.60 (CH$_2$), 34.27 (C(CH$_3$)$_3$), 31.27 (C(CH$_3$)$_3$).

FTIR (neat): 3285 (m), 1655 (s).

Mp: 146-148°C (methanol)

HRMS (ES): calc. for C$_{18}$H$_{14}$N0: 268.1696 [M+H]$^+$ Found: 268.1696.

N-Benzyl-2-phenyl-acetamide$^{365}$ (2.153)

The named compound was obtained as a white solid (20 mg, 91%).

HPLC (method A): $t_R = 7.64$ min.

Purity (ELSD): 96%

$^1$H NMR (300 MHz, CDCl$_3$): 7.26-7.08 (m, 10H, Ar-H), 5.71 (br s, 1H, NH), 4.32 (d, $J = 5.7$ Hz, 2H, NH-CH$_2$), 3.53 (s, 2H, CH$_2$).

$m/z$ (ESMS): 226 [M+H]$^+$ (85%), 248 [M+Na]$^+$ (100%)
1-Morpholin-4-yl-2-phenyl-ethanone \(^{366}\) (2.154)

The named compound was obtained as a colourless oil (8 mg, 38%).

**HPLC (method C):** \(t_R = 8.11\) min.

**Purity (ELSD):** 100%

**\(^1\)H NMR (250 MHz, CDCl\(_3\)):** 7.36-7.23 (m, 5H, Ar-H), 3.74 (s, 2H, Ar-CH\(_2\)), 3.65-3.46 (m, 8H, CH\(_2\)).

**m/z (ESMS):** 206 [M+H]\(^+\) (46%), 228 [M+Na]\(^+\) (100%).

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\(N\)-(4-tert-Butyl-phenyl)-benzamide \(^{367}\) (2.155)

The named compound was obtained as a white solid (22 mg, 88%).

**HPLC (method A):** \(t_R = 8.88\) min.

**Purity (ELSD):** 100%

**\(^1\)H NMR (250 MHz, CDCl\(_3\)):** 7.96 (br s, 1H, NH), 7.87-7.84 (m, 2H, OC-C-CH), 7.59-7.35 (m, 7H, Ar-H), 1.32 (s, 9H, C(CH\(_3\))\(_3\)).

**m/z (ESMS):** 254 [M+H]\(^+\) (65%), 276 [M+Na]\(^+\) (61%), 529[2M+Na]\(^+\) (100%).
**N-Benzyl-benzamide**\(^{368}\) (2.156)

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The named compound was obtained as a white solid (18 mg, 85%).

**HPLC (method A):** \(t_R = 7.52\) min.

**Purity (ELSD):** 100%

\(^1\)H NMR (300 MHz, CDCl\(_3\)): 7.74-7.71 (m, 2H, OC-C-CH), 7.46-7.19 (m, 8H, Ar-H), 6.51 (br s, 1H, NH), 4.56 (d, \(J = 5.7\) Hz, 2H, CH\(_2\)).

\(m/z\) (ESMS): 212 [M+H]\(^+\) (72%), 234 [M+Na]\(^+\) (100%).

**Morpholin-4-yl-phenyl-methanone**\(^{369}\) (2.157)

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The named compound was obtained as a white solid (9 mg, 50%).

**HPLC (method C):** \(t_R = 7.59\) min.

**Purity (ELSD):** 100%

\(^1\)H NMR (300 MHz, CDCl\(_3\)): 7.36-7.31 (m, 5H, Ar-H), 3.85-3.32 (m, 8H, CH\(_2\)).

\(m/z\) (ESMS): 192 [M+H]\(^+\) (100%).
5.2.2. Synthesis of the library for the comparison of IIDQ, PyAOP, BOP-Ci and HATU

Preparation of mother solutions

The following solutions were prepared:

**Amines:**
- tert-butyl aniline: 207 μL (1.3 mmol) in 13 mL CH₃CN (0.1 mmol/mL)
- benzylamine: 143 μL (1.3 mmol) in 13 mL CH₃CN (0.1 mmol/mL)
- 1,2,3,4-Tetrahydro-naphthalen-1-ylamine: 137 μL (1.3 mmol) in 13 mL CH₃CN (0.1 mmol/mL)
- morpholine: 114 μL (1.3 mmol) in 13 mL CH₃CN (0.1 mmol/mL)

**Acids:**
- Z-Ala-OH: 469 mg (2.1 mmol) in 21 mL CH₃CN (0.1 mmol/mL)
- phenyl acetic acid: 285 mg (2.1 mmol) in 21 mL CH₃CN (0.1 mmol/mL)
- benzoic acid: 257 mg (2.1 mmol) in 21 mL CH₃CN (0.1 mmol/mL)

**Coupling reagents:**
- IIDQ: 485 mg (1.6 mmol) in 16 mL CH₃CN (0.1 mmol/mL)
- PyAOP: 836 mg (1.6 mmol) in 16 mL CH₃CN (0.1 mmol/mL)
- BOP-Ci: 407 mg (1.6 mmol) in 16 mL CH₃CN (0.1 mmol/mL)
- HATU: 608 mg (1.6 mmol) in 16 mL CH₃CN (0.1 mmol/mL)

**Procedure**

In each vial, a solution of an amine (1 mL, 0.1 mmol, 1 equiv.) and of a carboxylic acid (1 mL, 0.1 mmol, 1 equiv.) were added, followed by the addition of a coupling reagent (1 mL, 0.1 mmol, 1 equiv.). The 48 vials were shaken at room temperature for 16h. The contents of the vials were then filtered through a SPE cartridge containing a mixed bed of MP acidic/basic ion-exchange resins (Polymer Lab, 500mg). The filtrates were concentrated in vacuo to afford the compounds listed below.
[(S)-1-(4-tert-Butyl-phenylcarbamoyl)-ethyl]-carbamic acid benzyl ester (2.158)

The named compound was obtained as a pale orange solid (33 mg, 93%).

**HPLC (method C):** \( t_R = 10.96 \text{ min} \).

**Purity (ELSD):** 100%

**\(^1\)H NMR (250 MHz, CDCl\(_3\)):**
- 8.37 (br s, 1H, Ar-NH), 7.43-7.28 (m, 9 H, Ar-H),
- 5.61 (br d, \( J = 7.5 \) Hz, 1H, CH-NH), 5.12 (AB-d, \( J = 12.25 \) Hz, 2H, O-CH\(_2\)),
- 4.49-4.38 (m, 1H, CH), 1.45 (d, \( J = 7.2 \) Hz, 3H, CH\(_3\)), 1.29 (s, 9H, C(CH\(_3\))\(_3\)).

**\(^{13}\)C NMR (75 MHz, CDCl\(_3\)):**
- 170.49 (C=O), 156.38 (C=O), 147.44 (C-Ar), 136.03 (C-Ar), 135.00 (C-Ar), 128.58 (CH-Ar), 128.26 (CH-Ar), 128.02 (CH-Ar), 126.14 (CH-Ar), 125.74 (CH-Ar), 119.81 (CH-Ar), 67.25 (CH\(_2\)), 51.25 (CH), 34.36 (C(CH\(_3\))\(_3\)), 31.35 (C(CH\(_3\))\(_3\)), 18.29 (CH\(_3\)).

**m/z (ESMS):** 355 [M+H]\(^+\).

**FTIR (neat):** 3294 (w), 3264 (w), 1689 (s), 1661 (s).

**Mp:** 85-88°C (methanol)


\([\alpha]_D^{23} = -30.4\)
Chapter V

((S)-1-Benzylcarbamoyl-ethyl)-carbamic acid benzyl ester (2.159)

The named compound was obtained as a white solid (27 mg, 86%).

HPLC (method C): $t_R = 9.77$ min.

Purity (ELSD): 100%

$^1$H NMR (300 MHz, CDCl$_3$): 7.38-7.21 (m, 10H, Ar-H), 6.61 (br s, 1H, NH), 5.46 (br d, $J = 7.2$ Hz, 1H, NH), 5.03 (AB-d, $J = 12.5$ Hz, 2H, O-CH$_2$), 4.40 (dd, $J = 2.2$ Hz, 5 Hz, 2H, NH-CH$_2$), 4.35-4.22 (m, 1H, CH), 1.39 (d, $J = 7.0$ Hz, 3H, CH$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): 172.44 (C=O), 156.08 (C=O), 137.99 (C-Ar), 136.15 (C-Ar), 128.68 (CH-Ar), 128.54 (CH-Ar), 128.20 (CH-Ar), 127.99 (CH-Ar), 127.59 (CH-Ar), 127.47 (CH-Ar), 66.97 (OCH$_2$), 50.62 (CH), 43.45 (NHCH$_2$), 18.81 (CH$_3$).

FTIR (neat): 3297 (w), 3279 (m), 1684 (s), 1640 (s).

Mp: 129-131°C (methanol)

HRMS (ES): calc. for C$_{18}$H$_{20}$N$_2$O$_3$: 313.1547 [M+H]$^+$ Found: 313.1547.

[$\alpha$]$_D^{23} = -10.2$
[(S)-1-(1,2,3,4-Tetrahydro-naphthalen-1-ylcarbamoyl)-ethyl]-carbamic acid benzyl ester (2.160)

The named compound was obtained as a white solid (25 mg, 71%).

**HPLC (method C):** $t_R = 10.55$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (300 MHz, CDCl$_3$):** 7.27-6.97 (m, 10H, Ar-H), 6.37 (br s, 1H, NH), 5.40 (br s, 1H, NH), 5.04 (m, 1H, NHCH$_{naphtyl}$), 4.91 (s, 2H, OCH$_2$), 4.17 (m, 1H, CH$^*$), 2.67 (m, 2H, Ar-CH$_2$ naphtyl), 1.93 (m, 1H, CH$_{naphtyl}$), 1.71 (m, 3H, CH$_{naphtyl}$), 1.32 (dd, $J = 6.9$ Hz, 3.3Hz, 3H, CH$_3$).

**$^{13}$C NMR (75 MHz, CDCl$_3$):** 171.55 (CO), 155.90 (C=O), 137.55 (C-Ar), 136.37 (C-Ar), 136.15 (C-Ar), 129.19 (CH-Ar), 128.52 (CH-Ar), 128.44 (CH-Ar), 128.19 (CH-Ar), 128.04 (CH-Ar), 127.35 (CH-Ar), 127.31 (CH-Ar), 126.30 (CH-Ar), 66.95 (OCH$_2$), 50.72 (CH naphtyl), 47.59 (CH$^*$), 30.14 (CH$_2$), 29.20 (CH$_2$), 20.03 (CH$_2$), 18.98 (CH$_3$).

**FTIR (neat):** 3289 (m), 1687 (s), 1639 (s).

**Mp:** 130-132°C (methanol)

**HRMS (ES):** calc. for C$_{21}$H$_{24}$N$_2$O$_3$: 353.1865 [M+H]$^+$ Found: 353.1862

$[\alpha]^D_{23} = -9.2$
The named compound was obtained as a white solid (20 mg, 69%).

**HPLC (method C):** $t_R = 8.72$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (300 MHz, CDCl$_3$):** 7.34-7.29 (m, 5H, Ar-H), 5.83 (d, $J = 6.9$ Hz, 1H, NH), 5.09 (s, 2H, Ar-CH$_2$), 4.64 (quint., $J = 6.0$ Hz, 1H, CH), 3.73-3.43 (m, 8H, CH$_2$ morpholine), 1.32 (d, $J = 6.9$ Hz, 3H, CH$_3$).

**$^{13}$C NMR (75 MHz, CDCl$_3$):** 170.94 (C=O), 155.56 (C=O), 136.40 (C-Ar), 128.51 (CH-Ar), 128.11 (CH-Ar), 128.00 (CH-Ar), 66.77 (OCH$_2$ morpholine), 66.51 (OCH$_2$Ph), 46.53 (CH), 45.92 (N-CH$_2$), 19.23 (CH$_3$).

**FTIR (neat):** 3307 (w), 3289 (w), 1711 (s), 1638 (s).

**Mp:** 134-136°C (ethanol)

**HRMS (ES):** calc. for C$_{15}$H$_{20}$N$_2$O$_4$: 293.1496 [M+H]$^+$ Found: 293.1494

[$\alpha$]$_D^{23} = -13.7$
2-Phenyl-N-(1,2,3,4-tetrahydro-naphthalen-1-yl)-acetamide (2.162)

The named compound was obtained as a white solid (16 mg, 59%).

**HPLC (method C):** $t_R = 10.36$ min.

**Purity (ELSD):** 100%

$^1$H NMR (400 MHz, CDCl$_3$): 7.33-7.04 (m, 9H, Ar-H), 5.63 (br s, 1H, NH), 5.20-5.15 (m, 1H, NHCH$_{\text{naphthyl}}$), 3.62 (s, 2H, Ar-CH$_2$-CO), 2.74-2.72 (m, 2H, Ar-CH$_2$$_{\text{naphthyl}}$), 2.07-2.00 (m, 1H, CH$_{\text{naphthyl}}$), 1.82-1.63 (m, 3H, CH$_{\text{naphthyl}}$).

$^{13}$C NMR (75 MHz, CDCl$_3$): 170.35 (C=O), 137.54 (C-Ar), 136.59 (C-Ar), 134.91 (C-Ar), 129.33 (CH-Ar), 129.13 (CH-Ar), 129.01 (CH-Ar), 128.21 (CH-Ar), 127.33 (CH-Ar), 127.20 (CH-Ar), 126.22 (CH-Ar), 47.71 (CH), 44.03 (Ph-CH$_2$), 30.21 (CH$_2$), 29.18 (CH$_2$), 20.15 (CH$_2$).

$m/z$ (ESMS): 266 [M+H]$^+$ (43%), 288 [M+Na]$^+$ (100%).

**FTIR (neat):** 3264 (m), 1631 (s).

**Mp:** 124-125°C (methanol).

The named compound was obtained as a white solid (18 mg, 67%).

**HPLC (method C):** t<sub>R</sub> = 10.33 min.

**Purity (ELSD):** 100%

**<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):** 7.71-7.68 (m, 2H, OC-C-CH), 7.43-7.03 (m, 7H, Ar-H), 6.30 (br s, 1H), 5.32 (m, 1H, NHCH<sub>naphthyl</sub>), 2.74 (m, 2H, Ar-CH<sub>2</sub>naphthyl), 2.06 (m, 1H, CH<sub>naphthyl</sub>), 1.91-1.77 (m, 3H, CH<sub>naphthyl</sub>).

**<sup>1</sup>C NMR (75 MHz, CDCl<sub>3</sub>):** 166.70 (C=O), 137.74 (C-Ar), 136.68 (C-Ar), 134.70 (C-Ar), 131.46 (CH-Ar), 129.27 (CH-Ar), 128.78 (CH-Ar), 128.57 (CH-Ar), 127.39 (CH-Ar), 126.95 (CH-Ar), 126.37 (CH-Ar), 47.99 (CH), 30.22 (CH<sub>2</sub>), 29.30 (CH<sub>2</sub>), 20.09 (CH<sub>2</sub>).

**FTIR (neat):** 3301 (m), 1632 (s).

**Mp:** 122-125°C (ethanol).

**HRMS (ES):** calc. for C<sub>17</sub>H<sub>17</sub>NO: 274.1202 [M+H]<sup>+</sup> Found: 274.1206.
5.3. **Experimental for Chapter III**

6-benzyloxyquinoline (3.6)

6-hydroxyquinoline (5 g, 1 equiv., 34.4 mmol) was dissolved in DMF (30 mL). K₂CO₃ (23.8 g, 5 equiv., 172 mmol) was introduced and the reaction mixture was stirred for 1 h. Benzyl chloride (4 mL, 1 equiv., 34.4 mmol) was added and the mixture was stirred at 80°C for 24 h. DMF was evaporated under reduced pressure yielding a dark brown solid. This solid was suspended in water (150 mL) and DCM (250 mL) and the insoluble impurities were filtered off. The water phase was extracted three times with DCM (50 mL). The combined organic phases were washed with brine (100 mL), dried over Na₂SO₄ and evaporated in vacuo. The brown solid obtained was extracted with petroleum ether at 50°C. Evaporation of petroleum ether yielded 6-benzyloxyquinoline as a pale yellow powder (3.78 g, 47%).

**HPLC (method B):** \( t_R = 7.05 \text{ min.} \)

**Purity (ELSD):** 98%

\(^1\text{H} \text{ NMR (400 MHz, CDCl}_3\):} 8.79 (dd, 1 H, \( J = 4.4 \text{ Hz and 1.5 Hz, N-CH} \)), 8.05 (dd, 1H, \( J = 8.1 \text{ Hz and 1.5 Hz, N-CH-CH-CH} \)), 8.04 (d, 1H, \( J = 8.8 \text{ Hz, N-C-CH} \)), 7.52-7.34 (m, 7H, Ar-H), 7.17 (d, 1H, \( J = 2.9 \text{ Hz, O-C-CH-C} \)), 5.20 (s, 2H, OCH₂).

\(^{13}\text{C} \text{ NMR (100 MHz, CDCl}_3\):} 157.3 (O-C-Ar), 148.5 (N-CH-Ar), 144.9 (C-Ar), 136.9 (C-Ar), 135.3 (CH-Ar), 131.4 (CH-Ar), 129.7 (CH-Ar), 129.1 (C-Ar), 128.6 (CH-Ar), 127.9 (CH-Ar), 123.0 (CH-Ar), 121.8 (CH-Ar), 107.0 (CH-Ar), 70.7 (OCH₂).

\( m/z \) (EIMS): 235.13 [M⁺] (100%).

**FTIR (neat):** 1618 (m), 1225 (m), 1019 (m).

**Mp:** 66-68°C (ethanol).

**Anal.:** calc. for C₁₆H₁₃NO: C: 81.68; H: 5.57; N: 5.95. Found C: 81.46; H: 5.63; N: 5.95.
Polymer-Supported Quinoline (3.8)

Merrifield resin (25 g, Polymer Lab, 3.99 mmol/g, 99.8 mmol, 1 equiv.) was swollen in DMA (250 mL). K$_2$CO$_3$, (69 g, 499 mmol, 5 equiv), 6-hydroxyquinoline (36.2 g, 249 mmol, 2.5 equiv.) and a catalytic amount of KI were added and the reaction mixture was heated at reflux under mechanical agitation for 6h. The resin was filtered and washed successively with THF/H$_2$O (1:1, 3 x 250 mL), THF (3 x 250 mL), DCM (3 x 250 mL), MeOH (3 x 250 mL), DCM (3 x 250 mL), MeOH (3 x 250 mL), Et$_2$O (3 x 250 mL).

$^1$H MAS-NMR + $^1$H-$^1$H COSY (400 MHz, CDCl$_3$): 8.75 (N-CH), 8.05-7.96 (N-CH-CH-CH and N-C-CH), 5.04 (OCH$_2$).

FTIR (neat): 1622 (w), 1223 (m), 1016 cm$^{-1}$ (w)

Loading of Polymer-supported Quinoline: determined by Nitrogen Elemental Analysis (C: 88.6, H: 7.18, N: 1.54, Cl: 0.20): 2.74 mmol/g (98%).
Polymer-supported IIDQ (3.10)

PS-Quinoline (20 g, 2.74 mmol/g, 54.8 mmol, 1 equiv.) was swollen in dry DCM (200 mL). DIPEA (27 mL, 164 mmol, 3 equiv.) was added and the mixture was mechanically stirred and cooled to 0°C. Isobutyl chloroformate (21.4 mL, 164 mmol, 3 equiv.) was added dropwise to the reaction mixture. After 3 h., isobutanol (100 mL) was added, the mixture allowed to warm up to room temperature and stirred mechanically for 16 h. The resin was then filtered and washed successively with 3 cycles of DCM, DCM/Et₂O, Et₂O (200 mL each).

¹H MAS-NMR (400 MHz, CDCl₃): 7.80 (N-C-CH), 6.22 (N-CH-CH), 4.80 (OCH₂-PS), 4.00 (CO₂CH₂), 3.32 (OCH₂CH), 1.97 (CO₂CH₂CH(CH₃)₂), 1.74 (OCH₂CH(CH₃)₂), 0.95 (CO₂CH₂CH(CH₃)₂), 0.83 (OCH₂CH(CH₃)₂).

FTIR (neat): 1709 (s), 1265 (s), 1018 (m).

Determination of IIDQ loading:
Phenylacetic acid (765 mg, 5.62 mmol, 3 equiv.) and benzylamine (614 µL, 5.62 mmol, 3 equiv.) were dissolved in DCM (5 mL). PS-IIDQ (maximum loading 1.87 mmol/g, 1.0 g, 1.87 mmol, 1 equiv.) was added and the reaction mixture was shaken at room temperature for 16 h. The resin was removed by filtration and washed with DCM/MeOH (3 cycles of 5 mL). The filtrates were combined and concentrated in vacuo. The residue was taken up in EtOAc (50 mL), washed with 1N HCl (3 x 20 mL), 1N NaHCO₃ (3 x 20 mL), brine (1 x 20 mL), dried over MgSO₄ and concentrated in vacuo to give 2-phenyl-benzylacetamide as a white powder (370 mg, 90%). The yield of the reaction was related to the loading of the resin (Loading = Yield x Therotical Loading = 0.88 x 1.87 = 1.68 mmol/g)
General Procedure for using PS-IIDQ as coupling reagent
To a solution of amine (1 equiv.) and carboxylic acid (1 equiv.) in CH₃CN was added PS-IIDQ (2 equiv.). The reaction mixture was shaken at room temperature for 24h. The resin was then filtered off and washed with DCM/MeOH (3 cycles). The filtrates were concentrated in vacuo and the unreacted amine/carboxylic acid were removed either by an aqueous workup (EtOAc, 1N HCl, 1N NaHCO₃) or by using SPE cartridges containing a mixed bed of acidic/basic MP ion-exchange resin (Polymer Lab, 500 mg).

[1-(4-tert-Butyl-phenylcarbamoyl)-1-methyl-ethyl] carbamic acid tert-butyl ester (3.13)

![Chemical structure](image)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (62 mg, 69%).

**HPLC (method A):** $t_R = 9.13$ min.

**Purity (ELSD):** 100%

**¹H NMR (250 MHz, CDCl₃):** 7.47-7.30 (m, 4H, Ar-H), 4.93 (br s, 1H, NH), 1.55 (s, 6 H, C(CH₃)₂), 1.44 (s, 9H, Ar-C(CH₃)₃), 1.30 (s, 9H, OC(CH₃)₃).

**¹³C NMR (62.5 MHz, CDCl₃):** 172.55 (C=O), 155.27 (C=O), 146.93 (C-Ar), 135.54 (C-Ar), 125.72 (CH-Ar), 119.53 (CH-Ar), 80.27 (OC(CH₃)₃), 57.64 (NH-C(CH₃)₂), 34.32 (Ar-C(CH₃)₃), 31.35 (Ar-C(CH₃)₃), 28.25 (OC(CH₃)₃), 25.73 (C(CH₃)₂).

**FTIR (neat):** 3315 (m), 1686 (s), 1596 (m).

**Mp:** 148-151°C (methanol)

(1-Benzylcarbamoyl-1-methyl-ethyl)-carbamic acid tert-butyl ester (3.14)

![Chemical Structure](image)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (52 mg, 66%).

**HPLC (method B):** $t_R = 7.47$min.

**Purity (ELSD):** 99%

$^1H$ NMR (300 MHz, CDCl₃): 7.26-7.17 (m, 5H, Ar-H), 6.70 (br s, 1H, NH), 4.86 (br s, 1H), 4.37 (d, $J = 5.4$ Hz, 1H, CH₂), 1.44 (s, 6H, C(CH₃)₂), 1.33 (s, 9H, C(CH₃)₃).

$^{13}$C NMR (75 MHz, CDCl₃): 174.49 (C=O), 154.73 (C=O), 138.40 (C-Ar), 128.60 (CH-Ar), 127.65 (CH-Ar), 127.33 (CH-Ar), 80.27 (OC(CH₃)₃), 56.87(C(CH₃)₂), 43.72 (CH₂), 28.26 (OC(CH₃)₃), 25.80(C(CH₃)₂).

**FTIR (neat):** 3350 (m), 3292 (m), 1682 (s), 1654 (s).

**Mp:** 135-138°C (methanol)

(R)-(2-tert-Butoxycarbamylamino-2-methyl propionylamino)-phenyl-acetic acid methyl ester (3.15)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (60 mg, 64%).

**HPLC (method A):** $t_R = 8.02 \text{ min.}$

**Purity (ELSD):** 100%

$^1$H NMR (300 MHz, CDCl$_3$): 7.43 (br s, 1H, NH), 7.32-7.22 (m, 5H, Ar-H), 5.47 (d, $J = 7.2 \text{ Hz}$, 1H, CH), 4.85 (br s, 1H, NH), 3.64 (s, 3H, CH$_3$), 1.42 (d, $J = 6.0 \text{ Hz}$), 6H, C(CH$_3$)$_2$), 1.33 (s, 9H, C(CH$_3$)$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): 173.99 (C=O), 171.30 (C=O), 154.68 (C=O), 136.61 (C-Ar), 128.86 (CH-Ar), 128.37 (CH-Ar), 127.20 (CH-Ar), 80.32 (OC(CH$_3$)$_3$), 56.78 (C(CH$_3$)$_2$), 56.55 (CH), 52.66 (OCH$_3$), 28.20 (OC(CH$_3$)$_3$), 25.34 (C(CH$_3$)$_3$).

FTIR (neat): 3320 (m), 1748 (m), 1684 (s), 1657 (s).

Mp: 104-108°C (methanol)

HRMS (ES): calc. for C$_{18}$H$_{26}$N$_2$O$_5$: 351.1915 [M+H]$^+$ Found: 351.1918. $[\alpha]_D^{23} = +90.7$
The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (46 mg, 61%).

**HPLC (method B):** \( t_R = 6.50 \text{ min} \).

**Purity (ELSD):** 100%

**\( ^1H \) NMR (300 MHz, CDCl\(_3\)):** 7.31-7.15 (m, 10H, Ar-H), 6.41 (d, \( J = 6.3 \text{ Hz} \), 1H, NH), 5.48 (d, \( J = 7.2 \text{ Hz} \), 1H, CH), 3.60 (s, 3H, CH\(_3\)), 3.53 (s, 2H, CH\(_2\)).

**\( ^{13}C \) NMR (75 MHz, CDCl\(_3\)):** 170.21 (C=O), 169.24 (C=O), 135.35 (C-Ar), 133.42 (C-Ar), 128.35 (CH-Ar), 127.96 (CH-Ar), 127.90 (CH-Ar), 127.48 (CH-Ar), 126.40 (CH-Ar), 126.10 (CH-Ar), 55.42 (CH), 51.74 (OCH\(_3\)), 42.41 (CH\(_2\)).

**FTIR (neat):** 3303 (m), 1740 (s), 1647 (s).

**Mp:** 96-98°C (methanol)

**HRMS:** calc. for C\(_{17}\)H\(_{17}\)NO\(_3\): 284.1281 [M+H]\(^+\) Found: 284.1283.

\([\alpha]_D^{23} = +130.4\)
(S)-Benzoylamino-phenyl-acetic acid methyl ester\(^{370}\) (3.20)

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\begin{array}{c}
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\]

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (54 mg, 75%).

**HPLC (method C):** \(t_R = 9.38\) min.

**Purity (ELSD):** 100%

**\(^1\)H NMR (250 MHz, CDCl\(_3\)):** 7.85-7.80 (m, 2H, OC-C-CH), 7.55-7.30 (m, 8H, Ar-H), 7.19 (br d, \(J = 6.3\) Hz, 1H, NH), 5.78 (d, \(J = 7.2\) Hz, 1H, CH), 3.77 (s, 3H, CH\(_3\)).

**\(m/z\) (ESMS):** 270 [M+H]\(^+\) (27%), 292 [M+Na]\(^+\) (100%).

Amino-isobutyric acid methyl ester hydrochloride\(^{371}\) (3.21)

To a solution of Aminoisobutyric acid (5 g, 48.5 mmol, 1 equiv.) in MeOH (50 mL) was added dropwise at 0°C thionyl chloride (5.5 mL, 242 mmol, 5 equiv.). After completion of the addition, the solution was allowed to warm up to room temperature and stirred for 5h. The volatiles were then evaporated in vacuo. The residue was taken up in MeOH and crystallised upon addition of Et\(_2\)O. The solid was filtered off, washed with Et\(_2\)O and dried in vacuo to afford the named compound as white crystals (6.46g, 87%).

**\(^1\)H NMR (300 MHz, CDCl\(_3\)):** 8.70 (br s, 1H, NH\(_2\)), 3.76 (s, 3H, CH\(_3\)), 1.63 (s, 6H, C(CH\(_3\))\(_2\))

**\(m/z\) (ESMS):** 118 [M+H]\(^+\) (100%)
(1,1-Dimethyl-2-morpholin-4-yl-2-oxo-ethyl)-carbamic acid benzyl ester (3.22)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (40 mg, 44%).

HPLC (method B): $t_R = 7.91$ min.

Purity (ELSD): 100%

$^1$H NMR (250 MHz, CDCl$_3$): 7.36-7.30 (m, 5H, Ar-H), 5.49 (br s, 1H, NH), 5.06 (s, 2H, Ar-CH$_2$), 3.64-3.42 (m, 8H, CH$_2$ morpholine), 1.52 (s, 6H, C(CH$_3$)$_2$).

$^{13}$C NMR (62.5 MHz, CDCl$_3$): 171.17 (C=O), 154.18 (C=O), 136.20 (C-Ar), 128.47 (CH-Ar), 128.35 (CH-Ar), 128.27 (CH-Ar), 66.53 (CH$_2$OCH$_2$, Ar-CH$_2$-O), 56.76 (C(CH$_3$)$_2$), 45.59 (N-CH), 25.98 (C(CH$_3$)$_2$)

FTIR (neat): 3306 (w), 3265 (m), 1714 (s), 1605 (s)

Mp: 135-141°C (methanol)

[1-(Cyclohexylmethyl-carbamoyl)-1-methyl-ethyl]-carbamic acid benzyl ester (3.23)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (58mg, 61%).

**HPLC (method C):** $t_R = 10.32$ min.

**Purity (ELSD):** 100%

$^1$H NMR (400 MHz, CDCl$_3$): 7.27 (m, 5H, Ar-H), 6.03 (s, 1H, NH), 5.26 (s, 1H, NH), 5.01 (s, 2H, Ar-CH$_2$), 3.64 (m, 1H, CH), 1.74-0.99 (m, 10H, CH$_2$ cHex), 1.43 (s, 6H C(CH$_3$)$_2$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 172.64 (C=O), 154.47 (C=O), 135.74 (C-Ar), 127.93 (CH-Ar), 127.58 (CH-Ar), 127.52 (CH-Ar), 66.08 (OCH$_2$), 56.28 (C(CH$_3$)$_2$), 47.65 (CH), 32.19 (CH$_2$), 24.94 (C(CH$_3$)$_2$), 24.46 (CH$_2$), 24.08 (CH$_2$).

$m/z$ (ESMS): 319 [M+H]$^+$ (30%), 341 [M+Na]$^+$ (100%).

**FTIR (neat):** 3341 (w), 3296 (m), 1699 (s), 1649 (s).

**Mp:** 121-122°C (methanol).

**Anal.:** calc. for $C_{18}H_{26}N_2O_3$: C: 67.90, H: 8.23, N: 8.79. Found: C: 67.78, H: 8.28, N: 8.78.
2-(2-Benzoxycarbonylamino-2-methyl-propionylamino)-2-methyl-propionic acid methyl ester\textsuperscript{372} (3.25)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (67 mg, 67%).

\textbf{HPLC (method C)}: $t_R = 9.35$ min.

\textbf{Purity (ELSD)}: 100\%

$^1$H NMR (300 MHz, CDCl$_3$): 7.27-7.23 (m, 5H, Ar-H), 6.83 (br s, 1H, NH), 5.26 (br s, 1H, NH), 5.03 (s, 2H, CH$_2$), 3.64 (s, 3H, OCH$_3$), 1.44 (s, 12H, C(CH$_3$)$_2$).

$m/z$ (ESMS): 337 [M+H]$^+$ (20\%), 359 [M+Na]$^+$ (100\%).
[1-Methyl-1-(1,2,3,4-tetrahydro-naphthalen-1-ylcarbamoyl)-ethyl]-carbamic acid benzyl ester (3.26)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (81 mg, 74%).

HPLC (method C): $t_R = 10.63\text{ min}$.

Purity (ELSD): 100%

$^1$H NMR (300 MHz, CDCl$_3$): 7.32-7.08 (m, 9H, Ar-H), 6.40 (br d, $J = 7.8$ Hz, 1H, NH-CH), 5.34 (br s, 1H, NH), 5.14-5.09 (m, 1H, NHCH$_{\text{naph}}$), 5.07 (s, 2H, OCH$_2$), 2.84-2.68 (m, 2H, Ar-CH$_2$$_{\text{naph}}$), 2.02-1.99 (m, 1H, CH$_{\text{naph}}$), 1.80-1.69 (m, 3H, CH$_{\text{naph}}$), 1.55 (s, 6H, C(CH$_3$)$_2$).

$^{13}$C NMR (75 MHz, CDCl$_3$): 173.48 (C=O), 154.90 (C=O), 137.65 (C-Ar), 136.67 (C-Ar), 136.19 (C-Ar), 129.14 (CH-Ar), 128.55 (CH-Ar), 128.20 (CH-Ar), 128.13 (CH-Ar), 127.22 (CH-Ar), 126.28 (CH-Ar), 66.76 (OCH$_2$), 56.67 (C(CH$_3$)$_2$), 47.71 (CH), 30.04 (CH$_2$), 29.28 (CH$_2$), 25.57 (C(CH$_3$)$_2$), 20.08 (CH$_2$).

FTIR (neat): 3351 (w), 3286 (m), 1694 (s), 1645 (s).

Mp: 119-121°C (ethanol).

(S)-1-(2-Benzoxycarbonylamino-2-methyl-propionyl)-pyrrolidine-2-carboxylic acid benzyl ester (3.27)†

The named compound was prepared according to the general procedure of coupling with PS-II:DQ and separated from the carbamate by-product by column chromatography (Hexane/EtOAc 6:4) and was obtained as a colourless oil (4 mg, 3%).

**HPLC (method B):** \( t_R = 6.32 \text{ min} \).

**Purity (ELSD):** 100% 

**FTIR (neat):** 3277 (m), 1739 (s), 1702 (s), 1621 (s).

**HRMS (ES):** calc. for \( \text{C}_{21}\text{H}_{30}\text{N}_{2}\text{O}_{5} \): 391.2228 [M+H]+ Found: 391.2231

† In the case of amides derived from proline, the presence of rotamers made the NMR spectra poorly significant to report. However compound 3.55 was fully characterised using NMR at higher temperature.
**N-Cyclohexylmethyl-2-phenyl-acetamide (3.28)**

![Chemical Structure](image)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (29 mg, 44%).

**HPLC (method C):** \( t_R = 9.96 \text{ min} \)

**Purity (ELSD):** 100%

\( ^1H \) NMR (300 MHz, CDCl\(_3\)): 7.27-7.16 (m, 5H, Ar-H), 5.22 (br s, 1H, NH), 3.73-3.63 (m, 1H, CH), 3.46 (s, 2H, Ar-CH\(_2\)), 1.79-0.88 (m, 10H, CH\(_2\) cHex).

\( ^{13}C \) NMR (75 MHz, CDCl\(_3\)): 169.98 (C=O), 135.21 (C-Ar), 129.34 (CH-Ar), 128.94 (CH-Ar), 127.22 (CH-Ar), 48.16 (Ph-CH\(_2\)), 44.01 (CH), 32.89 (CH\(_2\)), 25.46 (CH\(_2\)), 24.68 (CH\(_2\)).

**FTIR (neat):** 3267 (m), 1665 (s).

**Mp:** 125-127°C (methanol)

**HRMS (ES):** calc. for C\(_{14}\)H\(_{19}\)NO: 218.1540 \([M+H]^+\) Found: 218.1540.

**2-methyl-2-phenylacetylamino-propionic acid methyl ester\(^{374}\) (3.29)**

![Chemical Structure](image)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (51 mg, 73%).

**HPLC (method C):** \( t_R = 8.66 \text{ min} \)

**Purity (ELSD):** 100%

\( ^1H \) NMR (300 MHz, CDCl\(_3\)): 7.31-7.19 (m, 5H, Ar-H), 5.93 (br s, 1H, NH), 3.64 (s, 3H, OCH\(_3\)), 3.47 (s, 2H, CH\(_2\)), 1.42 (6H, C(CH\(_3\))\(_2\)).

**m/z (ESMS):** 236 \([M+H]^+\) (33%), 258 \([M+Na]^+\) (100%).
(S)-1-Phenylacetyl-pyrrolidine-2-carboxylic acid benzyl ester (3.32)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and separated from the carbamate by-product by column chromatography (Hexane/EtOAc 6:4) and was obtained as a colourless oil (52 mg, 60%).

**HPLC (method A):** $t_R = 8.19$ min.

**Purity (ELSD):** 100%

**FTIR (neat):** 1740 (s), 1642 (s).

**HRMS (ES):** calc. for C$_{20}$H$_{21}$NO$_3$: 324.1594 [M+H]$^+$ Found: 324.1592.

$N$-Cyclohexylmethyl-benzamide$^{375}$ (3.34)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (51 mg, 84%).

**HPLC (method C):** $t_R = 9.77$ min.

**Purity (ELSD):** 100%

$^1$H NMR (300 MHz, CDCl$_3$): 7.77-7.75 (m, 2H, OC-C-CH), 7.51-7.39 (m, 3H, OC-C-CH-CH-CH), 6.09 (br s, 1H, NH), 4.05-3.92 (m, 1H, NHCH), 2.06-2.01 (m, 2H, CH$_{naphtyl}$), 1.80-1.59 (m, 3H, CH$_{naphtyl}$), 1.51-1.34 (m, 2H, CH$_{naphtyl}$), 1.32-1.14 (m, 3H, CH$_{naphtyl}$).

$m/z$ (ESMS): 204 [M+H]$^+$ (100%).
2-Benzoylamino-2-methyl-propionic acid methyl ester (3.35)

![Chemical Structure](image)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (60 mg, 91%).

**HPLC (method C):** $t_R = 8.35$ min.

**Purity (ELSD):** 100%

$^1$H NMR (400 MHz, CDCl₃): 7.70 (d, $J = 6.8$ Hz, 2H, OC-CH), 7.42-7.33 (m, 3H, OC-CH-CH-CH), 6.74 (br s, 1H, NH), 3.71 (s, 3H, OCH₃), 1.46 (s, 6H, C(CH₃)₂).

$^{13}$C NMR (100 MHz, CDCl₃): 176.81 (C=O), 168.12 (C=O), 136.05 (C-Ar), 133.05 (CH-Ar), 130.04 (CH-Ar), 128.46 (CH-Ar), 58.44 (C(CH₃)₂), 54.26 (OCH₃), 26.26 (C(CH₃)₂).

**FTIR (neat):** 3223 (m), 1732 (s).

**MP:** 116-119°C (ethanol).

**HRMS (ES):** calc. for C₁₂H₁₅NO₃: 244.0944 [M+Na]⁺ Found: 244.0943.

(S)-1-Benzoyl-pyrrolidine-2-carboxylic acid benzyl ester (3.38)

![Chemical Structure](image)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and separated from the carbamate by-product by column chromatography (Hexane/EtOAc 6:4) and was obtained as a colourless oil (26 mg, 34%).

**HPLC (method A):** $t_R = 7.88$ min.

**Purity (ELSD):** 100%

**FTIR (neat):** 1740 (s), 1629 (s).

**HRMS (ES):** calc. for C₁₉H₁₄NO₃: 310.1438 [M+Na]⁺ Found: 310.1434
[(S)-1-(Cyclohexylmethyl-carbamoyl)-ethyl]-carbamic acid benzyl ester (3.40)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (69 mg, 71%).

HPLC (method C): $t_R = 10.20$ min.

Purity (ELSD): 100%

$^1$H NMR (300 MHz, CDCl$_3$): 7.26 (m, 5H, Ar-H), 5.96 (br s, 1H, NH), 5.39 (br s, 1H, NH), 5.03 (s, 2H, Ar-CH$_2$), 4.10 (m, 1H, CH*), 3.64 (m, 1H, CH cHex), 1.86-0.97 (m, 10H, CH$_2$ cHex), 1.26 (d, $J = 7.2$ Hz, 3H, CH$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): 171.23 (C=O), 155.97 (C=O), 136.25 (C-Ar), 128.53 (CH-Ar), 128.18 (CH-Ar), 128.01 (CH-Ar), 66.95 (OCH$_2$), 50.65 (CH*), 48.26 (CH cHex), 32.88 (CH$_2$), 25.47 (CH$_2$), 24.73 (CH$_2$), 18.79 (CH$_3$).

FTIR (neat): 3277 (m), 1688 (s), 1642 (s).

Mp: 153-156°C (methanol)

HRMS (ES): calc. for C$_{17}$H$_{24}$N$_2$O$_3$: 327.1679 [M+H]$^+$ Found: 327.1677.

$[\alpha]_D^{23} = -10.4$
The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (79 mg, 82%).

**HPLC (method C):** $t_R = 9.24$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (300 MHz, CDCl$_3$):** 7.30-7.21 (m, 5H, Ar-H), 6.65 (br s, 1H, NH), 5.37 (br s, 1H, NH), 5.04 (s, 2H, CH$_2$), 4.17-4.13 (m, 1H, CH), 3.64 (s, 3H, OCH$_3$), 1.44 (d, $J = 2.7$ Hz, 6H, C(CH$_3$)$_2$), 1.29 (d, $J = 6.9$ Hz, 3H, CH-CH$_3$).

**m/z (ESMS):** 345 [M+Na]$^+$ (100%).
(S)-(S)-2-Benzyloxycarbonylamino-propionylamino)-phenyl-acetic acid methyl ester (3.46)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (92 mg, 83%).

HPLC (method C): $t_R = 9.76$ min.

Purity (ELSD): 100%

$^1$H NMR (300 MHz, CDCl$_3$): 7.24 (m, 10H, Ar-H), 7.11 (br s, 1H, NH), 5.46 (d, $J = 7.2$ Hz, 1H, CH-Ph), 5.32 (br s, 1H, NH), 4.99 (s, 2H, Ar-CH$_2$), 4.27 (m, 1H, CH-CH$_3$), 3.63 (s, 3H, OCH$_3$), 1.31 (d, $J = 6.9$ Hz, 3H, CH-CH$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): 171.69 (C=O), 171.05 (C=O), 155.93 (C=O), 136.17 (C-Ar), 136.11 (C-Ar), 128.99 (CH-Ar), 128.60 (CH-Ar), 128.53 (CH-Ar), 128.17 (CH-Ar), 128.06 (CH-Ar), 127.21 (CH-Ar), 67.03 (OCH$_2$), 56.49 (CH-Ph), 52.82 (OCH$_3$), 50.35 (CHCH$_3$), 18.61 (CH$_3$).

$m/z$ (ESMS): 371 (M+H)$^+$.  

FTIR (neat): 3301 (m), 1734 (s), 1692 (s), 1650 (s).  

Mp: 121-123°C (methanol).


$[\alpha]_D^{23} = +65.1$
1-Morpholin-4-yl-2-phenoxy-ethanone (3.47)

![Morpholin-4-yl-2-phenoxy-ethanone](image)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (53 mg, 80%).

**HPLC (method C):** $t_R = 8.21$ min.

**Purity (ELSD):** 100%

$^1$H NMR (300 MHz, CDCl$_3$): 7.20 (m, 2H, O-C-CH-CH), 6.89 (m, 3H, O-C-CH-CH-CH), 4.62 (s, 2H, Ar-O-CH$_2$), 3.57 (m, 8H, CH$_2$ morpholine).

$m/z$ (ESMS): 222 [M+H]$^+$ (23%), 244 [M+Na]$^+$ (100%).

$N$-Cyclohexylmethyl-2-phenoxy-acetamide (3.48)

![N-Cyclohexylmethyl-2-phenoxy-acetamide](image)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (36 mg, 52%).

**HPLC (method C):** $t_R = 10.36$ min.

**Purity (ELSD):** 100%

$^1$H NMR (300 MHz, CDCl$_3$): 7.26-7.22 (m, 2H, O-C-CH-CH), 6.95 (t, $J = 7.5$ Hz, 1H, O-C-CH-CH-CH), 6.85 (d, $J = 7.8$ Hz, 2H, O-C-CH), 6.35 (br s, 1H, NH), 4.40 (s, 2H, Ar-O-CH$_2$), 3.80 (m, 1H, CH), 1.88-1.04 (4m, 10H, CH$_2$ cHex).

$^{13}$C NMR (75 MHz, CDCl$_3$): 167.20 (C=O), 157.26 (C-Ar), 129.77 (CH-Ar), 122.10 (CH-Ar), 114.74 (CH-Ar), 67.47 (OCH$_2$), 47.83 (CH), 32.98 (CH$_2$), 25.45 (CH$_2$), 24.77 (CH$_2$).

FTIR (neat): 3335 (m), 1647 (s).

Mp: 75-78°C (methanol)

**HRMS (ES):** calc. for C$_{14}$H$_{19}$NO$_2$: 256.1308 [M+Na]$^+$ Found: 256.1305.
2-Methyl-2-(2-phenoxy-acetamido)-propionic acid methyl ester (3.49)

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\text{\begin{center}
\includegraphics[width=0.3\textwidth]{compound.png}
\end{center}
}
\]

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (53 mg, 70%).

**HPLC (method C):** $t_R = 9.14$ min.

**Purity (ELSD):** 100%

$^{1}H$ NMR (300 MHz, CDCl$_3$): 7.25 (t, $J = 7.5$ Hz, 2H, O-C-CH-CH), 7.08 (br s, 1H, NH), 6.95 (t, $J = 7.5$ Hz, 1H, O-C-CH-CH-CH), 6.87 (d, $J = 7.8$ Hz, 2H, O-C-CH), 4.37 (s, 2H, CH$_2$), 3.69 (s, 3H, OCH$_3$), 1.53 (s, 6H, C(CH$_3$)$_2$).

$^{13}$C NMR (75 MHz, CDCl$_3$): 174.70 (C=O), 167.52 (C=O), 157.23 (C-Ar), 129.74 (CH-Ar), 122.14 (CH-Ar), 114.81 (CH-Ar), 67.53 (OCH$_2$), 56.43 (C(CH$_3$)$_2$), 52.73 (OCH$_3$), 24.71 (C(CH$_3$)$_2$).

$m/z$ (ESMS): 252 [M+H]$^+$ (22%), 274 [M+Na]$^+$ (100%).

FTIR (neat): 3209 (w), 1738 (s), 1649 (s).

**Mp:** 61-62°C (methanol).

**Anal.:** calc. for C$_{13}$H$_{17}$NO$_4$: C: 62.14, H: 6.82, N: 5.57. Found: C: 61.98, H: 6.85, N: 5.52.
The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (62 mg, 74%).

**HPLC (method C):** $t_R = 10.76$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (400 MHz, CDCl$_3$):** 7.31 (t, $J = 8.8$ Hz, 2H, O-C-CH-CH$\text{H}$), 7.22-7.10 (m, 4H, Ar-H$_\text{naphyl}$), 7.02 (t, $J = 7.2$ Hz, 1H, O-C-CH-CH-CH$\text{H}$), 6.90 (d, $J = 8.0$ Hz, 2H, O-C-CH), 6.79 (d, $J = 7.6$ Hz, 1H, NH), 5.32-5.28 (m, 1H, NHCH$\text{H}$), 4.57 (AB-d, $J = 14.8$ Hz, 2H, OCH$_2$), 2.87-2.74 (m, 2H, Ar-CH$_2$$_\text{naphyl}$), 2.14-2.09 (m, 1H, CH$_\text{naphyl}$), 1.87-1.77 (m, 3H, CH$_\text{naphyl}$).

**$^{13}$C NMR (75 MHz, CDCl$_3$):** 167.67 (C=O), 157.17 (C-Ar), 137.64 (C-Ar), 136.21 (C-Ar), 129.77 (CH-Ar), 129.23 (CH-Ar), 128.42 (CH-Ar), 127.39 (CH-Ar), 126.33 (CH-Ar), 122.14 (CH-Ar), 114.72 (CH-Ar), 67.43 (OCH$_2$), 47.25 (CH), 30.28 (CH$_2$), 29.11 (CH$_2$), 20.15 (CH$_2$).

**m/z (ESMS):** 304 [M+Na]$^+$ (100%)

**FTIR (neat):** 3309 (m), 1649 (s).

**Mp:** 79-82°C (ethanol).

**Anal.:** calc. for C$_{18}$H$_{19}$NO$_2$: C: 76.84, H: 6.81, N: 4.98. Found: C: 76.69, H: 6.81, N: 4.97.
The named compound was prepared according to the general procedure of coupling with PS-IIDQ and obtained as a white solid (70 mg, 83%).

**HPLC (method C):** $t_R = 11.09$ min.

**Purity (ELSD):** 100%

**$^{1}$$H$ NMR (300 MHz, CDCl₃):** 8.16 (br s, 1H, NH), 7.43-7.39 (m, 2H, HN-C-CH), 7.30-7.23 (m, 2H, HN-C-CH-CH, 2H, O-C-CH-CH) , 6.99-6.89 (m, 3H, O-C-CH-CH-CH), 4.51 (s, 2H, CH₂), 1.23 (s, 9H, C(CH₃)₃).

**$^{13}$$C$ NMR (75 MHz, CDCl₃):** 166.23 (C=O), 157.10 (C-Ar), 147.93 (C-Ar), 134.21 (C-Ar), 129.91 (CH-Ar), 125.92 (CH-Ar), 122.44 (CH-Ar), 120.02 (CH-Ar), 114.89 (CH-Ar), 67.69 (OCH₂), 34.43 (C(CH₃)₃), 31.36 (C(CH₃)₃).

**m/z (ESMS):** 306 [M+Na]$^+$ (47%), 589 [2M+Na]$^+$ (100%).

**FTIR (neat):** 3264 (w), 1657 (s).

**Mp:** 80-82°C (methanol).

N-Benzyl-2-phenoxy-acetamide\textsuperscript{378} (3.53)

![Chemical Structure](image)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (59 mg, 82%).

**HPLC (method C):** \( t_R = 9.85 \) min.

**Purity (ELSD):** 100%

\(^1\)H NMR (300 MHz, CDCl\textsubscript{3}): 7.26-7.18 (m, 7H, Ar-H), 6.94 (t, \( J = 7.5 \) Hz, 1H, O-C-CH-CH-CH), 6.82 (d, \( J = 7.5 \) Hz, 2H, O-C-CH), 4.46 (d, \( J = 5.7 \) Hz, 2H, NH-CH\textsubscript{2}), 4.46 (s, 2H, OCH\textsubscript{2}).

\( m/z \) (ESMS): 242 [M+H]\textsuperscript{+} (22%), 264 [M+Na]\textsuperscript{+} (100%).

\((S)-(2-Phenoxy-acetylamino)-phenyl-acetic\textsuperscript{379} (3.54)

![Chemical Structure](image)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ and obtained as a white solid (73 mg, 82%).

**HPLC (method C):** \( t_R = 9.94 \) min.

**Purity (ELSD):** 99%

\(^1\)H NMR (300 MHz, CDCl\textsubscript{3}): 7.58 (d, \( J = 6.6 \) Hz, 1H, NH), 7.37-7.29 (m, 7H, Ar-H), 7.06-7.01 (m, 1H, O-C-CH-CH-CH), 6.97-6.92 (m, 2H, O-C-CH), 5.67 (d, \( J = 7.5 \) Hz, 1H, CH) 4.53 (AB-d, \( J = 15 \) Hz, 2H, CH\textsubscript{2}), 3.74 (s, 3H, CH\textsubscript{3}).

\( m/z \) (ESMS): 300 [M+H]\textsuperscript{+} (19%), 322 [M+Na]\textsuperscript{+} (100%).
Isobutylichloroformate (54 µL, 0.41 mmol, 1 equiv.) was added to a mixture of praline benzyl ester hydrochloride (100 mg, 0.41 mmol, 1 equiv.) and DIPEA (137 µL, 0.82 mmol, 2 equiv.) in DCM (10 mL). The mixture was stirred at room temperature for 2h. and concentrated in vacuo. The residue was taken up in EtOAc (50 mL) and washed with 1N NaHCO₃ (3 x 25 mL), 1N HCl (3 x 25 mL), brine (1 x 25 mL), dried over MgSO₄, filtered, and concentrated in vacuo to give the named compound as a pale yellow oil (109 mg, 85%).

**HPLC (method A):** $t_R = 9.02$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (400 MHz, 373K, $d^6$-DMSO):** 7.38-7.33 (m, 5H, Ar-H), 5.15 (s, 2H, Ar-CH₂), 4.34 (dd, $J = 3.5$ Hz and 8.5 Hz, 1H, CH*), 3.78 (d, $J = 6.4$ Hz, 2H, OCH₂CH), 3.44 (m, 2H, N-CH₂), 2.26 (m, 1H, CH(CH₃)₂), 1.94-1.84 (m, 4H, CH*CH₂CH₂), 0.87 (d, $J = 6.75$ Hz, 6H, CH(CH₃)₂).

**$^{13}$C NMR (100 MHz, 373K, $d^6$-DMSO):** 172.01 (C=O), 154.00 (C=O), 135.9 C-Ar, 128.23 (CH-Ar), 127.84 (CH-Ar), 127.56 (CH-Ar), 70.60 (Ph-CH₂), 65.85 (OCH₂CH), 58.75 (CH*), 46.28 (CH₂), 29.80 (CH₂), 27.44 (CH(CH₃)₂), 23.28 (CH₂), 18.56 (CH(CH₃)₂).

**FTIR (neat):** 1745 (m), 1702 (s).

**HRMS (ES):** calc. for C$_{17}$H$_{23}$NO$_4$: 306.1700 [M+H]$^+$ Found: 306.1699.  
[α]$_D^{23}$ = -50.1
(S)-1-(3,3-Diphenyl-propionyl)-pyrrolidine-2-carboxylic acid benzyl ester (3.56)

\[
\begin{align*}
\text{The named compound was prepared according to the general procedure of coupling} \\
\text{with PS-IIDQ, separated from the carbamate by-product by column chromatography} \\
\text{(Petroleum ether/ EtOAc 6:4), and obtained as pale yellow oil (48 mg, 43%).} \\
\text{HPLC (method A): } t_R = 9.44 \text{ min.} \\
\text{Purity (ELSD): 94%} \\
\text{FTIR (neat): 1740 (s), 1645 (s).} \\
\text{HRMS (ES): calc. for } C_{27}H_{27}NO_3: 414.1907 \text{ [M+H]}^+ \text{Found: 414.1905.}
\end{align*}
\]

(S)-1-(3-Phenyl-propionyl)-pyrrolidine-2-carboxylic acid benzyl ester (3.57)

\[
\begin{align*}
\text{The named compound was prepared according to the general procedure of coupling} \\
\text{with PS-IIDQ, separated from the carbamate by-product by column chromatography} \\
\text{(Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (69 mg, 76%).} \\
\text{HPLC (method A): } t_R = 8.72 \text{ min.} \\
\text{Purity (ELSD): 100%} \\
\text{FTIR (neat): 3222 (w), 1739 (s), 1642 (s).} \\
\text{HRMS (ES): calc. for } C_{21}H_{23}NO_3: 338.1751 \text{ [M+H]}^+ \text{Found: 338.1758.}
\end{align*}
\]
(S)-1-(3,3,3-Triphenyl-propionyl)-pyrrolidine-2-carboxylic acid benzyl ester (3.58)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (24 mg, 18%).

HPLC (method B): $t_R = 8.22$ min.

Purity (ELSD): 100%

FTIR (neat): 3218 (w), 1738 (s), 1643 (s).


(S)-1-Diphenylacetyl-pyrrolidine-2-carboxylic acid benzyl ester (3.59)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (54 mg, 50%).

HPLC (method A): $t_R = 9.19$ min.

Purity (ELSD): 100%

FTIR (neat): 1740 (s), 1645 (s).

HRMS: calc. for $C_{26}H_{25}NO_3$: 400.1907 $[M+H]^+$ Found: 400.1905.
(S)-1-[1-(4-Chloro-phenyl)-cyclopropanecarbonyl]-pyrrolidine-2-carboxylic acid benzy1 ester (3.60)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (44 mg, 43%).

HPLC (method A): $t_R = 9.31$ min.

Purity (ELSD): 96%.

FTIR (neat): 1740 (s), 1637 (s).

HRMS: calc. for $C_{22}H_{22}NO_3Cl$: 384.1361 [M+H]$^+$ Found: 284.1362.

(S)-1-(2-Cyclohexyl-acetyl)-pyrrolidine-2-carboxylic acid benzy1 ester (3.61)

The named compound was prepared according to the general procedure of coupling with PS-IIIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (58 mg, 66%).

HPLC (method A): $t_R = 9.26$ min.

Purity (ELSD): 100%

FTIR (neat): 1742 (s), 1642 (s).

(S)-1-(2-Adamantan-1-yl-acetyl)-pyrrolidine-2-carboxylic acid benzyl ester (3.62)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (49 mg, 48%).

HPLC (method A): $t_R = 10.10$ min.

Purity (ELSD): 100%.

FTIR (neat): 1742 (s), 1638 (s).


(S)-1-(3-Phenyl-butyryl)-pyrrolidine-2-carboxylic acid benzyl ester (3.63)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (54 mg, 57%).

HPLC (method B): $t_R = 7.14$ min.

Purity (ELSD): 100%

FTIR (neat): 1742 (s), 1644 (s).

(S)-1-(2-Phenyl-propionyl)-pyrrolidine-2-carboxylic acid benzyl ester (3.64)

![Chemical structure of (S)-1-(2-Phenyl-propionyl)-pyrrolidine-2-carboxylic acid benzyl ester](image)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (32 mg, 35%).

**HPLC (method B):** $t_R = 7.11$ min.

**Purity (ELSD):** 99%

**FTIR (neat):** 1742 (s), 1644 (s).

**HRMS (ES):** calc. for C$_{21}$H$_{23}$NO$_3$: 338.1751 [M+H]$^+$ Found: 338.1748.

(S)-1-Cyclohexanecarbonyl-pyrrolidine-2-carboxylic acid benzyl ester (3.65)

![Chemical structure of (S)-1-Cyclohexanecarbonyl-pyrrolidine-2-carboxylic acid benzyl ester](image)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/ EtOAc 6:4), and obtained as a pale yellow oil (25 mg, 30%).

**HPLC (method A):** $t_R = 8.80$ min.

**Purity (ELSD):** 100%

**FTIR (neat):** 1741 (s), 1639 (s).

(S)-1-Cyclopentanecarbonyl-pyrrolidine-2-carboxylic acid benzyl ester (3.66)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/EtOAc 6:4), and obtained as a pale yellow oil (44 mg, 55%).

HPLC (method A): $t_R = 8.42$ min.

Purity (ELSD): 100%

FTIR (neat): 1741 (s), 1639 (s).


Boc-Pro-Pro-OBn$^{373}$ (3.67)

The named compound was prepared according to the general procedure of coupling with PS-IIDQ, separated from the carbamate by-product by column chromatography (Petroleum ether/EtOAc 6:4), and obtained as a pale yellow oil (53 mg, 49%).

HPLC (method A): $t_R = 8.46$ min.

Purity (ELSD): 100%

FTIR (neat): 3222 (w), 1741 (m), 1693 (s), 1656 (s).

To a solution of H-Val-OMe hydrochloride (29 mg, 0.175 mmol, 1 equiv.), DIPEA (29 μL, 0.175 mmol, 1 equiv.) and Z-Gly-Phe-OH (60 mg, 0.175 mmol, 1 equiv.) in CH₃CN (5 mL) was added PS-IIDQ (250 mg, 0.350 mmol, 2 equiv.). The reaction mixture was shaken at room temperature for 24h. The resin was then filtered off and washed with DCM/MeOH (3 cycles of 5 mL). The filtrates were concentrated in vacuo and the unreacted amine/carboxylic acid were by using a SPE cartridge containing a mixed bed of acidic/basic MP ion-exchange resin (Polymer Lab, 500 mg). The named compound was obtained as a pale yellow oil (82 mg, 65%).

HPLC (method A): tᵣ = 7.12 min.

Purity (ELSD): 100%

¹H NMR (300 MHz, CDC₃): 7.33-7.15 (m, 10 H, Ar-H), 6.82 (br s, 1 H, NH), 6.46 (br s, 1H, NH), 5.48 (br s, 1H, NH), 5.09 (s, 2H, Ph-CH₂-O), 4.74-4.69 (m, 1H, CH*phe), 4.40 (dd, J = 8.4 Hz and 5.2 Hz, 1H, CH*val), 3.83 (m, 2H, NH-CH₂), 3.67 (s, 3H, OCH₃), 3.03 (d, J = 4.4 Hz, 2 H, CHCH₂Ph), 2.10-2.02 (m, 1H, CH(CH₃)₂), 0.83 (d, J = 6.8 Hz, 3 H, CH₃), 0.80 (d, J = 6.8 Hz, 3H, CH₃).

m/z (ESMS): 470 [M+H]+ (100%).
Z-Gly-D-Phe-Val-OMe\textsuperscript{372} (3.71)

The named compound was prepared according in analogy to compound 3.70 and obtained as a pale yellow oil (78 mg, 63%).

**HPLC (method A):** $t_R = 7.01$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (300 MHz, CDCl\textsubscript{3}):** 7.32-7.15 (m, 10 H), 6.66 (br s, 1 H, NH), 6.31 (br s, 1H, NH), 5.37 (br s, 1H, NH), 5.08 (s, 2H, Ph-CH\textsubscript{2}-O), 4.70-4.65 (m, 1H, CH*\textsubscript{phe}), 4.38 (dd, $J = 8.4$ Hz and 5.2 Hz, 1H, CH*\textsubscript{val}), 3.83 (m, 2H, NH-CH\textsubscript{2}), 3.66 (s, 3H, OCH\textsubscript{3}), 3.08-2.98 (m, 2 H, CH\textsubscript{CH\textsubscript{2}}-Ph), 2.09-2.01 (m, 1H, CH(CH\textsubscript{3})\textsubscript{2}), 0.82 (d, $J = 6.8$ Hz, 3 H, CH\textsubscript{3}), 0.79 (d, $J = 6.8$ Hz, 3H, CH\textsubscript{3}).

**$m/z$ (ESMS):** 470 [M+H]\textsuperscript{+} (100%).
5.4. Experimental for Chapter IV

(2-Amino-ethylamino)-acetic acid methyl ester dihydrochloride\textsuperscript{382} (4.20)

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} \quad \text{O} \\
\text{OCH}_3 & \quad \text{0.2 HCl}
\end{align*}
\]

Chloroacetic acid (90.0 g, 952 mmol, 1 equiv.) was added portionwise at 0\(^\circ\)C to ethylene diamine (636 mL, 9.52 mol, 10 equiv.). After completion of the addition, the reaction mixture was stirred at room temperature for 48h. Ethylene diamine was evaporated \textit{in vacuo}, and DMSO was added to the residue occurring precipitation of a white solid. Out of the 85g of the solid obtained, 80 g (0.52 mol, 1 equiv.) was suspended in MeOH (1.5 L). The mixture was cooled to 0\(^\circ\)C and thionyl chloride (188 mL, 2.58 mol, 5 equiv.) was added dropwise. The mixture was then stirred at room temperature for 18h. The solid was filtered off and recrystallised from MeOH to afford the named compound as white crystals (74g, 38% over two steps).

\textbf{HPLC (method C):} \textit{t}_R = 1.44 \text{ min}.

\textbf{Purity (ELSD):} 100\%

\textbf{\textsuperscript{1}H NMR (250 MHz, d\textsuperscript{6}-DMSO):} 8.55 (br s, 3H, NH), 4.06 (s, 2H, CH\textsubscript{2}CO), 3.74 (s, 3H, CH\textsubscript{3}), 3.30-3.20 (m, 4H, CH\textsubscript{2}-CH\textsubscript{2})

\textbf{m/z (ESMS):} 133 [M+H]\textsuperscript{+} (100\%)
Dde-OH$^{383}$ (4.21)

To a solution of dimedone (23 g, 164 mmol, 1 equiv.) in DMF (350 mL) was added acetic acid (9.52 mL, 164 mmol, 1 equiv.), DCC (34 g, 164 mmol, 1 equiv.) and DMAP (20 g, 164 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 48h. The solution was filtered (elimination of DCU) and concentrated in vacuo. The residue was taken up in EtOAc and washed with 1N KHSO$_4$ (3 x 100 mL), brine (1 x 100 mL), dried over MgSO$_4$ and concentrated in vacuo. The residue was taken up in EtOAc, filtered (elimination of DCU) and concentrated in vacuo to afford the named compound as an orange oil (29.9 g, 84%).

**HPLC (method B):** $t_R = 7.13$ min.

**Purity (UV, 254 nm):** 91%

**$^1$H NMR (250 MHz, CDCl$_3$):** 2.59 (s, 3H, CH$_3$), 2.52 (s, 2H, CH$_2$), 2.34 (s, 2H, CH$_2$), 1.06 (s, 6H, C(CH$_3$)$_2$).

**m/z (ESMS):** 183 [M+H]$^+$ (100%)
Methyl N-{2-[1-(4,4-dimethyl-2,6-dioxo-cyclohexyliden)-ethylamino]-ethyl}glycinate$^{382}$ (4.22)

To a stirred solution of 4.20 (10 g, 48.7 mmol, 1 equiv.) and DiPEA (16.1 mL, 97.5 mmol, 2 equiv.) in DCM/MeOH (1:1, 200 mL) was added portionwise Dde-OH (8.88 g, 48.7 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 18h. After evaporation of the solvent in vacuo, the crude was taken up in EtOAc (150 mL), and extracted with 1M KHSO₄ (3 x 100 mL). The aqueous extracts were combined and brought to pH 9 with NaHCO₃ and extracted with EtOAc (3 x 100 mL). The organic phase were combined, washed with brine (1 x 100 mL), dried over MgSO₄ and concentrated in vacuo to give the named compound as a dark orange oil (4.5 g, 31%).

HPLC (method B): t_R = 1.43 min.

Purity (ELSD): 100%

$^1$H NMR (250 MHz, CDCl₃): 13.48 (br s, 1H, NH-Dde), 3.71 (s, 3H, OCH₃), 3.43-3.47 (m, 4H, NH(CH₂)₂), 2.92 (t, J = 6.0 Hz, 2 H, NHCH₂CO), 2.54 (s, 3H, CCH₃), 2.33 (s, 4H, CH₂-Dde), 1.87 (br s, 1H, NH), 0.99 (s, 6H, C(CH₃)₂).

m/z (ESMS): 297 [M+H]$^+$ (100%).
(6-Amino-purin-9-yl)-acetic acid ethyl ester\(^{384}\) (4.24)

![Chemical Structure](image)

To a suspension of adenine (25 g, 185 mmol, 1 equiv.) in DMF was added NaH (60% dispersion in mineral oil, 8.88 g, 222 mmol, 1.2 equiv.), and the resulting mixture was stirred at room temperature for 1h. The reaction mixture was then cooled to 0°C and methyl-2-bromoacetate (41 mL, 370 mL, 2 equiv.) was added dropwise. The mixture was stirred at room temperature for 18h and the solvent was removed in vacuo. Water was added to the residue, which resulted in crystallisation of a solid. The solid was filtered off and recrystallised from EtOH to give the named compound as white crystals (16.85 g, 41%).

**HPLC (method C):** \(t_R = 4.99\) min.

**Purity (ELSD):** 100%

\(^1\)H NMR (250 MHz, \(d^6\)-DMSO): 8.14 (s, 1H, CH), 8.12 (s, 1H, CH), 7.32 (s, 2H, NH\(_2\)), 5.09 (s, 2H, CH\(_2\)), 3.70 (s, 3H, OCH\(_3\))

\(m/z\) (ESMS): 222 [M+H]\(^+\) (100%)
[N^6-(4-Methoxytrityl)-adenin-9-yl]-acetic acid \(^{382}\) (4.25)

To a stirred suspension of 4.24 (5 g, 22.6 mmol, 1 equiv.) in DCM/Pyridine (1:1, 200 mL) was added N-ethylmorpholine (2.87 mL, 22.6 mmol, 1 equiv.) and 4-monomethoxytrityl-chloride (10.5 g, 33.9 mmol, 1.5 equiv.). The mixture was heated to 40°C and stirred for 18h. After evaporation of the solvents in vacuo, the residue was taken up in EtOAc (150 mL), washed with 1N aq. KHSO\(_4\) (3 x 50 mL), 1M aq. NaHCO\(_3\) (3 x 50 mL), brine (1 x 50 mL), and dried over MgSO\(_4\). The solvent was removed in vacuo and the residue was purified by chromatography (EtOAc) to give a buff coloured solid.

The previous solid was suspended in 1N aqueous NaOH (100 mL) and the mixture was stirred at reflux for 2h. The solution was then cooled to 0°C. 1N aq. KHSO\(_4\) (120 mL) was added and the mixture was stirred at 0°C for 30 min. The resulting precipitate was collected by filtration and washed with water. The brown solid obtained was taken up in DCM and the insoluble particles were filtered off. Et\(_2\)O was added to the solution and the resulting precipitate was collected, affording the named compound as a buff coloured solid (9.21 g, 87% over 2 steps).

**HPLC (method B):** \(t_R = 8.52\) min.

**Purity (ELSD):** 98%

\(^1\)H NMR (250 MHz, \textit{d}^6-DMSO): 8.20 (s, 1H, CH\(_{\text{pur}}\)), 7.91 (s, 1H, CH\(_{\text{pur}}\)), 7.34-7.20 (m, 12H, CH\(_{\text{Mt}}\)), 6.85 (d, \(J = 9.0\) Hz, 2H, CH\(_{\text{Mt}}\)), 4.96 (s, 2H, CH\(_2\)), 3.71 (s, 3H, OCH\(_3\)).

\(m/z\) (ESMS): 466 [M+H]\(^+\) (100%)
Chapter V

\[ N'\text{-}[N'^{6}\text{-}(4\text{-Methoxytrityl})\text{-adenin-9-yl})\text{-acetyl}]\text{-}N'\text{-}[1\text{-}(4,4\text{-dimethyl}-2,6\text{-dioxo-cyclohexyliden})\text{-ethyl-amino}]\text{-ethyl}]\text{-glycine}^{382} (4.26) \]

To a solution of 4.25 (3 g, 6.44 mmol, 1 equiv.) in CH$_3$CN (50 mL) was added HBTU (2.93 g, 7.73 mmol, 1.2 equiv.) and DIPEA (1.06 mL, 6.44 mmol, 1 equiv.). The mixture was stirred for 3 min. and added to a solution of 4.22 (1.91 g, 6.44 mmol, 1 equiv.) in CH$_3$CN (50 mL). The resulting solution was stirred at room temperature for 45 min. and the solvent was removed in vacuo. The residue was taken up in EtOAc (150 mL), washed with 1 M KHSO$_4$ (3 x 50 mL), 1 M NaHCO$_3$ (3 x 50 mL), brine (1 x 50 mL), dried over MgSO$_4$ and concentrated in vacuo to give a buff coloured solid. The previous solid was suspended in a 2:1 mixture of MeOH and 2N Cs$_2$CO$_3$ (30 mL) and stirred for 1.5 h. The mixture was evaporated to dryness and taken up in water (150 mL), washed with DCM (2 x 50 mL), acidified with 1 M aq. KHSO$_4$ and extracted with DCM (3 x 100 mL). The organic phases were combined, washed with brine (1 x 100 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was dissolved in a minimum amount of EtOAc and precipitated with hexanes. The named compound was obtained as a pale yellow solid (3.2 g, 68% over 2 steps).

**HPLC (method B):** $t_R = 8.66$ min.

**Purity (ELSD):** 98%

$^1$H NMR (250 MHz, CDCl$_3$) two rotamers: 13.25 and 13.16 (s, 1H, Dde-NH), 8.09 and 8.08 (s, 1H, CH$_{pur}$), 7.87 (s, 1H, CH$_{pur}$), 7.31-7.18 (m, 12H, CH$_{Mmt}$), 6.85 (d, $J = 8.75$ Hz, 2H, CH$_{Mmt}$), 5.24 and 5.06 (s, 2H, CH$_2$CO$_2$), 4.40 and 4.03 (s, 2H, CH$_2$CO),
3.77 (m, 2H, CH₂N), 3.71 (s, 3H, OCH₃), 3.53 (m, 2H, CH₂N), 2.56 and 2.46 (s, 3H, CCH₃), 2.29 and 2.26 (s, 4H, CH₂-Dde), 0.93 (s, 6H, CH₃-Dde).

m/z (ESMS): 728 [M-H]⁻ (100%)

(4-Amino-2-oxo-2H-pyrimidin-1-yl)-acetic acid ethyl ester³⁸⁵ (4.28)

To a stirred suspension of cytosine (5g, 45 mmol, 1 equiv.) in DCM / Pyridine (1:1, 200 mL) was added N-ethylmorpholine (5.75 mL, 45 mmol, 1 equiv.) and 4-monomethoxytrityl-chloride (20.8 g, 67.5 mmol, 1.5 equiv.). The mixture was heated to 40°C and stirred for 24h. The mixture was cooled down to room temperature and water (200 mL) was added. The precipitate formed was filtered off, washed with water, THF and dried in vacuo. The named compound was obtained as a white solid (11.2 g, 65%).

HPLC (method D): tᵣ = 3.19 min.

Purity (ELSD): 95%

m/z (ESMS): 273 [Mmt]+ (100%), 767 [2M+H]+ (20%)

(The named compound is insoluble in common deuterated solvents and therefore cannot be analysed by NMR as reported in the literature for a Z-protected cytosine³⁸⁴).
[N^4-(4-Methoxytrityl)-cytosin-1-yl]-acetic acid \(382\) (4.29)

To a suspension of 4.28 (5.0 g, 13 mmol, 1 equiv.) in DMF (50 mL) was added NaH (60% in mineral oil, 520 mg, 13 mmol, 1 equiv.) and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was then cooled to 0°C and ethyl-2-bromoacetate (1.73 mL, 15.6 mmol, 1.2 equiv.) was added dropwise. The mixture was stirred at room temperature for 18 h and the solvent was removed \textit{in vacuo}. The resulting gum was precipitated with water and collected by filtration. The solid was washed with water and a small portion of CH\(_3\)CN, and dried \textit{in vacuo}.

The previous solid was suspended in 2N aqueous NaOH (50 mL) and the reaction mixture was stirred at reflux for 2 h. After cooling to room temperature, the solution was acidified with 2N aqueous HCl (70 mL) and the resulting precipitate was collected by filtration. The solid was washed with water and dried \textit{in vacuo} to afford the named compound as a white solid (3.93 g, 66% over 2 steps).

\textbf{HPLC (method B)}: \(t_R = 7.81\) min.

\textbf{Purity (ELSD)}: 98%

\textbf{\(^1\)H NMR (250 MHz, \(d^6\)-DMSO)}: 8.36 (br s, 1H, NH), 7.45 (d, \(J = 6.75\) Hz, 1H, CH\(_{\text{Cyt}}\)), 7.25-7.15 (m, 12H, CH\(_{\text{Mnt}}\)), 6.87-6.82 (m, 2H, CH\(_{\text{Mnt}}\)), 6.18 (d, \(J = 6.75\) Hz, 1H, CH\(_{\text{Cyt}}\)), 4.23 (s, 2H, CH\(_2\)), 3.72 (s, 3H, CH\(_3\)).

\textbf{m/z (ESMS)}: 440 [M-H]\(^-\) (100%)
The named compound was synthesised from 4.29 (3.0 g, 6.79 mmol) in analogy to 4.26 and obtained as a buff coloured solid (2.9 g, 61% over two steps).

**HPLC (method B):** $t_R = 8.26$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (250 MHz, CDCl$_3$)** two rotamers: 13.16 and 13.14 (s, 1H, NH-Dde), 8.34 (s, 1H, NH-Mmt), 7.51-7.23 (m, 10H, CH$_{Mmt}$, 1H, CH$_{Cyt}$), 7.17 (d, $J = 8.5$ Hz, 2H, CH$_{Mmt}$), 6.84 (d, $J = 8.0$ Hz, 2H, CH$_{Mmt}$), 6.18 (d, $J = 5.25$ Hz, 1H, CH$_{Cyt}$), 4.50 and 4.36 (s, 2H, CH$_2$CO$_2$), 4.26 and 3.99 (s, 2H, CH$_2$CO), 3.73 (s, 3H, OCH$_3$), 3.78-3.63 (m, 4H, CH$_2$-CH$_2$), 2.50 and 2.47 (s, 3H, CCH$_3$), 2.28 (s, 4H, CH$_2$-Dde), 1.00 and 0.94 (s, 6H, CH$_3$-Dde).

**m/z (ESMS):** 704 [M-H]$^-$ (100%)
The Fmoc-Rink linker (1.14 g, 2.11 mmol, 5 equiv.), PyBOP (800 mg, 2.11 mmol, 5 equiv.) and DiPEA (697 μL, 4.22 mmol, 10 equiv.) were premixed in DMF (10 mL) and added to PEGA resin (Polymer Lab, 0.4 mmol/g, 7.1 g wet) and the mixture was shaken for 3h at room temperature. Completion of the reaction was confirmed by ninhydrin test. The resin was filtered, washed with DMF (3 x 20 mL), DCM (3 x 20 mL), MeOH (3 x 20 mL), DCM (3 x 20 mL) and MeOH (3 x 20 mL).
To a suspension of Fmoc-Lysine (16.2 g, 43.9 mmol, 1 equiv.) in ethanol (250 mL), was added Dde-OH 4.21 (18.0 g, 98.8 mmol, 2 equiv.) and TFA (630 μL, 8.48 mmol, 0.2 equiv.). The mixture was refluxed for 72h and concentrated in vacuo. The residue was taken up in EtOAc (350 mL), washed with 1N aq. KHSO₄ (3 x 100 mL), brine (1 x 100 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The resulting oil was triturated in Hexane to afford a yellowish solid, which recrystallised from DCM / Hexane to yield the named compound as an off-white solid (9.54 g, 82%).

**HPLC (method C):** $t_R = 11.12$ min.

**Purity (ELSD):** 99%

$^1$H NMR (250 MHz, CDCl₃): 13.32 (br s, 1H, N$^\text{H}$), 8.03 (br s, 1H, CO$_2$H), 7.74 (d, $J = 7.5$ Hz, 2H, CH$_{\text{Fmoc}}$), 7.58 (t, $J = 6.5$ Hz, 2H, CH$_{\text{Fmoc}}$), 7.37 (t, $J = 7.0$ Hz, 2H, CH$_{\text{Fmoc}}$), 7.28 (d, $J = 7.25$ Hz, 2H, CH$_{\text{Fmoc}}$), 5.84 (d, $J = 8.25$ Hz, 1H, N$^\text{H}$), 4.50-4.47 (m, 1H, CH), 4.35 (d, $J = 7.25$ Hz, 2H, CH$_2$ Fmoc), 4.19 (t, $J = 7.0$ Hz, 1H, CH-Fmoc), 3.39 (m, 2H, C$^\text{O}$H$_2$ Lys), 2.53 (s, 3H, CH$_3$ Dde), 2.35 (s, 4H, CH$_2$ Dde), 1.99-1.89 (m, 2H, C$^\text{O}$H$_2$ Lys), 1.87-1.63 (m, 2H, C$^\text{O}$H$_2$ Lys), 1.61-1.45 (m, 2H, C$^\text{O}$H$_2$ Lys), 1.00 (s, 6H, C(CH$_3$)$_2$ Dde).

$m/z$ (ESMS): 533 [M+H]$^+$ (100%)
Fmoc-Lys(Dde)-Rink-PEGA\(^{382}\) (4.43)

Fmoc-(Dde)LysOH 4.42 (1.13 g, 2.11 mmol, 5 equiv.), PyBOP (800 mg, 2.11 mmol, 5 equiv.) and DiPEA (697 µL, 4.22 mmol, 10 equiv.) were premixed in DMF (10 mL) and added to Rink-PEGA resin 4.41 (previously deprotected with 20% piperidine in DMF) and the mixture was shaken for 5h at room temperature. Completion of the reaction was confirmed by ninhydrin test. The resin was filtered, washed with DMF (3 x 20 mL), DCM (3 x 20 mL), MeOH (3 x 20 mL), DCM (3 x 20 mL) and MeOH (3 x 20 mL).
PNA tag synthesis (4.44)

General procedure for the deprotection of the Dde group

The resin (3.5 g wet, 0.2 mmol) was pre-swollen in DMF (5 mL). A 20% solution of imidazole/hydroxylamine (1:0.75 equiv) in NMP/DMF (5:1, 10 mL) was added to the resin and the resulting mixture was shaken for 1 h at room temperature. The resin was then filtered and washed with DMF (3 x 10 mL), DCM (3 x 10 mL) and MeOH (3 x 10 mL).

General procedure for coupling PNA monomers

The PNA monomer (Adenine: 438 mg, Cytosine: 424 mg or Thymine: 269 mg, 0.6 mmol, 3 equiv.) was premixed with HATU (228 mg, 0.6 mmol, 3 equiv.) and DIPEA (99 μL, 0.6 mmol, 3 equiv.) in DMF (2 mL). This solution was added to the deprotected resin (pre-swollen in 5 mL DMF) and shaken for 1.5 h at room temperature. Ninhydrin confirmed complete conversion and the resin was filtered and washed with DMF (3 x 10 mL), DCM (3 x 10 mL) and MeOH (3 x 10 mL).

PNA tag characterisation

A small sample of resin was cleaved using TFA/DCM/TIS (90:5:5) for 1.5 h. m/z (ESMS): calc. for C_{159}H_{198}N_{67}O_{41} : 3703 (M+H)^+ Found: 742 (M+5H)^{5+}/5.
Oxo-(3,4,5-trimethoxy-phenyl)-acetic acid^387 (4.46)

![Structure of Oxo-(3,4,5-trimethoxy-phenyl)-acetic acid](image)

To a solution of 3',4',5'-trimethoxyacetophenone (5.0 g, 23.8 mmol, 1 equiv.) in pyridine (50 mL) was added selenium dioxide (9.9 g, 89.2 mmol, 3.75 equiv.). The mixture was stirred at 110°C for 16h and the solvent was removed _in vacuo_. The residue was taken up in 5% NaOH (100 mL), filtered through celite, and extracted with Et₂O (2 x 50 mL). The aqueous phase was then acidified with conc. HCl and the resulting precipitate was collected by filtration. The solid was redissolved in EtOAc (150 mL), washed with brine (1 x 50 mL), dried with Na₂SO₄ and concentrated _in vacuo_ to afford the named compound as pale yellow crystals (4.62 g, 81%).

_HPLC (method C): t_R = 7.20 min._

_Purity (ELSD): 100%_

^1H NMR (250 MHz, d₆-DMSO): 7.21 (s, 2H, Ar-H), 3.86 (s, 6H, OCH₃ meta), 3.80 (s, 3H, OCH₃ para)

m/z (ESMS): 239 [M-H]⁻ (100%)
Piperidine-2-carboxylic acid methyl ester hydrochloride \(^{388}\) (4.48)

\[
\begin{align*}
\text{HCl}
\end{align*}
\]

To a solution of \(N\)-Boc piperidine-2-carboxylic acid (5.0 g, 21.8 mmol, 1 equiv.) in MeOH (150 mL) was added at 0°C thionyl chloride (15.9 mL, 218 mmol, 10 equiv.). After addition, the mixture was refluxed for 3h and the solvent was removed \textit{in vacuo}. The residue was taken up in a minimum amount of MeOH and precipitated with \(\text{Et}_2\text{O}\). The solid obtained was collected by filtration and washed with \(\text{Et}_2\text{O}\). The named compound was obtained as white crystals (3.87 g, 98 %).

\textbf{HPLC (method C):} \(t_R = 1.92\) min.

\textbf{Purity (ELSD):} 100%

\(^1\text{H NMR (250 MHz, CDCl}_3\):} 4.05 (dd, \(J = 3.5\) Hz, 11.5 Hz, 1H, \(\text{CH}^*\)), 3.83 (s, 3H, \(\text{OCH}_3\)), 3.53-3.45 (m, 1H, \(\text{CH}_{\text{pip}}\)), 3.11-3.00 (m, 1H, \(\text{CH}_{\text{pip}}\)), 2.32-2.26 (m, 1H, \(\text{CH}_{\text{pip}}\)), 1.92-1.57 (m, 5H, \(\text{CH}_{\text{pip}}\)).

\(m/z\) (ESMS): 144 [\text{M+H}]^+ (100\%)
To a 2 min. premixed solution of **4.46** (1.34 g, 5.57 mmol, 1 equiv.), HBTU (2.32 g, 6.12 mmol, 1.1 equiv.) and DiPEA (1.84 mL, 11.1 mmol, 2 equiv.) in CH$_3$CN (50 mL), was added **4.48** (1.0 g, 5.57 mmol, 1 equiv.). The mixture was stirred for 3h at room temperature and the solvent was removed *in vacuo*. The residue was taken up in EtOAc (100 mL), washed with 1N HCl (3 x 50mL), 1N NaHCO$_3$ (3 x 50mL), brine (1 x 50mL), dried over Mg$_2$SO$_4$ and concentrated *in vacuo* to afford the named compound as a pale brown solid (977 mg, 2.67 mmol, 1 equiv.). To a solution of the previous brown solid in MeOH (20 mL) was added LiOH (168 mg, 4.01 mmol, 1.5 equiv.) and the mixture was stirred at room temperature for 1h. The solvent was evaporated *in vacuo* and the residue was taken up in water (50 mL), washed with DCM (2 x 25 mL), and acidified with conc. HCl. The resulting precipitate was collected by filtration and washed with water. The solid was dissolved in EtOAc (50 mL), washed with brine (1 x 25 mL), dried over Na$_2$SO$_4$, filtered and concentrated *in vacuo* to afford the named compound as an off-white solid (874 mg, 44% over two steps).

**HPLC (method B):** t$_R$ = 7.10 min.

**Purity (ELSD):** 100%

**$^1$H NMR (250 MHz, CDCl$_3$) two rotamers:** 9.17 (br s, 1H, OH), 7.33 and 7.30 (s, 2H, Ar-H), 5.49 (m, CH*), 3.97 and 3.96 (s, 3H, OCH$_3$ para), 3.93 and 3.92 (s, 6H, OCH$_3$ meta), 3.66 and 3.55 (d, $J = 13.25$ Hz, 1H, CH$_{p1}$), 3.26 and 3.03 (dt, $J = 3.0$ and 12.25 Hz, 1H, CH$_{p1}$), 2.43 and 2.27 (d, $J = 13.25$ Hz, 1H, CH$_{p1}$), 1.91-1.43 (m, 5H, CH$_{pro}$).
\(^{13}\)C NMR (62.5 MHz, CDCl\(_3\)): 190.47 (C=O), 175.53 (C=O), 167.82 (C=O), 153.50 (C-Ar), 144.03 (C-Ar), 127.95 (C-Ar), 106.81 (CH-Ar), 61.00 (OCH\(_3\) para), 56.28 (OCH\(_3\) meta), 51.45 (CH\(^*\)), 44.42 (CH\(_2\)), 26.07 (CH\(_2\)), 24.68 (CH\(_2\)), 21.13 (CH\(_2\)).

Mp: 172-175°C (methanol)

FTIR (neat): 1734 (s), 1678 (s), 1127 (s).

HRMS (ES): calc. for C\(_{17}\)H\(_{21}\)NO\(_7\): 374.1210 [M+Na]\(^+\) Found: 374.1218.
Acetic acid 4-[2-(tert-butyl-dimethyl-silyloxy)-phenylcarbamoyl]-phenyl ester (4.57a)

The procedure followed for the protection step was reported by Swenton.\textsuperscript{389}

To a solution of 2-aminophenol (5.0 g, 45.8 mmol, 1 equiv.) and imidazole (4.68 g, 68.7 mmol, 1.5 equiv.) in THF (100 mL) was added TBS-Cl (8.97 g, 59.5 mmol, 1.3 equiv.) under vigorous agitation. A heavy white precipitate was formed quickly. After 30 min., the mixture was poured into water (250 mL) and extracted with \( \text{Et}_2\text{O} \) (2 x 100 mL). The organic phase were combined, washed with water (2 x 50 mL), brine (1 x 50 mL), dried over \( \text{MgSO}_4 \) and concentrated \textit{in vacuo} to afford an orange oil. The previous oil (9.22 g, 41.3 mmol, 1 equiv.) was dissolved in \( \text{CH}_3\text{CN} \) (150 mL). 4-acetoxybenzoic acid (7.43 g, 41.3 mmol, 1 equiv.) and IIDQ (12.52 g, 41.3 mmol, 1 equiv.) were added and the mixture was stirred at room temperature for 18 h and the solvent was removed \textit{in vacuo}. The residue was taken up in \( \text{EtOAc} \) (200 mL), washed with 1N HCl (3 x 100 mL), 1N NaHCO\textsubscript{3} (3 x 100 mL), brine (1 x 100 mL), dried over \( \text{MgSO}_4 \) and concentrated \textit{in vacuo}. The residue was redissolved in DCM and solid impurities filtered off. Evaporation of DCM under reduced pressure afforded the named compound as a white solid (6.18 g, 35% over two steps).

\textbf{HPLC (method B)}: \( t_R = 8.74 \text{ min.} \)

\textbf{Purity (ELSD):} 100%

\( ^1\text{H NMR (250 MHz, CDCl}_3\):} 8.54-8.50 (m, 1H, NH, 1H, HN-C-CH), 7.93 (d, \( J = 8.75 \text{ Hz, 2H OC-CH} \)), 7.27-7.22 (m, 2H, AcO-C-CH), 7.07-6.96 (m, 1H, TBSO-C-CH-CH, 1H, HN-C-CH-CH), 6.92-6.87 (m, 1H, TBSO-C-CH), 2.35 (s, 3H, CH\textsubscript{3}CO), 1.03 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}), 0.30 (s, 6H, Si(CH\textsubscript{3})\textsubscript{2}).

\( ^{13}\text{C NMR (62.5 MHz, CDCl}_3\):} 168.88 (C=O), 164.05 (C=O), 153.28 (C-Ar), 144.27 (C-Ar), 132.68 (C-Ar), 129.68 (CH-Ar), 128.33. (CH-Ar), 123.81 (CH-Ar), 121.90 (CH-Ar), 121.83 (CH-Ar), 120.20 (CH-Ar), 117.41 (CH-Ar), 25.80 (C(CH\textsubscript{3})\textsubscript{3}), 21.15 (CH\textsubscript{3}CO), 18.18 (C(CH\textsubscript{3})\textsubscript{3}), -4.20 (Si(CH\textsubscript{3})\textsubscript{2}).

\textbf{Mp:} 108-110°C (methanol)
FTIR (neat): 3424 (m), 1757 (s), 1669 (s).
HRMS (ES): calc. for C$_{21}$H$_{27}$NO$_4$Si: 386.1782 [M+H]$^+$ Found: 386.1783.

Acetic acid 4-[3-(tert-butyl-dimethyl-silanyloxy)-phenylcarbamoyl]-phenyl ester (4.57b)

![Chemical Structure](image)

The named compound was prepared in analogy to 4.57a. The residue obtained was purified by column chromatography (Hexane/EtOAc 7:3) to give 4.57b as a colourless sticky oil (7.93 g, 45% over two steps).

HPLC (method B): $t_R = 8.59$ min.

Purity (ELSD): 100%

$^1$H NMR (250 MHz, CDCl$_3$): 7.87-7.82 (m, 1H, NH, 2H, OC-C-CH), 7.31 (t, $J = 2.25$ Hz, 1H, HN-C-CH-C-OTBS), 7.23-7.11 (m, 4H, Ar-H), 6.64 (ddd, $J = 1.5$ Hz, 2.25 Hz, 7.5 Hz, TBSO-C-CH), 2.33 (s, 3H, CH$_3$), 0.99 (s, 9H, C(CH$_3$)$_3$), 0.23 (s, 6H, Si(CH$_3$)$_2$).

$^{13}$C NMR (62.5 MHz, CDCl$_3$): 169.03 (C=O), 164.84 (C=O), 156.26 (C-Ar), 153.22 (C-Ar), 138.91 (C-Ar), 132.63 (C-Ar), 129.65 (CH-Ar), 128.46 (CH-Ar), 121.91 (CH-Ar), 116.30 (CH-Ar), 112.89 (CH-Ar), 112.06 (CH-Ar), 25.67 (C(CH$_3$)$_3$), 21.13 (CH$_3$CO), 18.19 (C(CH$_3$)$_3$), -4.41 (Si(CH$_3$)$_2$).

FTIR (neat): 3306 (w), 1758 (s), 1650 (s).

HRMS (ES): calc. for C$_{21}$H$_{27}$NO$_4$Si: 386.1782 [M+H]$^+$ Found: 386.1785.
Acetic acid 4-[4-(tert-butyl-dimethyl-silanyloxy)-phenylcarbamoyl]-phenyl ester (4.57c)

The named compound was prepared in analogy to 4.57a. The residue was crystallised from EtOAc / Hexane to give white crystals (8.64 g, 49% over two steps).

**HPLC (method B):** $t_R = 8.58$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (250 MHz, CDCl$_3$):** 7.84 (d, $J = 8.75$ Hz, 2H, OC-C-CH), 7.81 (br s, 1H, NH), 7.46 (d, $J = 8.75$ Hz, 2H, HN-C-CH), 7.17 (d, $J = 8.5$ Hz, 2H, AcO-C-CH), 6.83 (d, $J = 8.75$ Hz, 2H, TBSO-C-CH), 2.33 (s, 3H, CH$_3$CO), 0.98 (s, 9H, C(CH$_3$)$_3$), 0.19 (s, 6H, Si(CH$_3$)$_2$).

**$^{13}$C NMR (62.5 MHz, CDCl$_3$):** 169.04 (C=O), 164.76 (C=O), 153.13 (C-Ar), 152.59 (C-Ar), 132.68 (C-Ar), 131.44 (C-Ar), 128.43 (CH-Ar), 121.89 (CH-Ar), 121.83 (CH-Ar), 120.41 (CH-Ar), 25.67 (C(CH$_3$)$_3$), 21.13 (CH$_3$CO), 18.20 (C(CH$_3$)$_3$), -4.45 (Si(CH$_3$)$_2$).

**Mp:** 136-138°C (methanol)

**FTIR (neat):** 3263 (w), 1756 (s), 1641 (s).

**HRMS (ES):** calc. for C$_{21}$H$_{27}$NO$_4$Si: 386.1782 [M+H]$^+$ Found: 386.1785.
To a solution of 4.57a (1.5 g, 3.89 mmol, 1 equiv.) in MeOH (50 mL) was added LiOH (171 mg, 4.08 mmol, 1.05 equiv.). The mixture was stirred for 5 min. and concentrated in vacuo. The residue was taken up in water (100 mL), acidified with 1N HCl and extracted with DCM (3 x 50 mL). The organic extracts were combined and washed with brine (1 x 50 mL), dried with Na₂SO₄ and concentrated in vacuo. The oil obtained was purified by column chromatography (Hexane/EtOAc 6:4) to give the named compound as an off-white solid (1.07 g, 80%).

**HPLC (method B):** \( t_R = 8.95 \text{ min.} \)

**Purity (ELSD):** 100%

**¹H NMR (250 MHz, CDCl₃):** 8.51 (br s, 1H, NH), 8.47-8.43 (m, 1H, HN-C-CH), 7.76 (d, \( J = 8.75 \text{ Hz} \), 2H, OC-CCH), 7.67 (br s, 1H, OH), 7.02-6.86 (m, 5H, Ar-H), 1.00 (s, 9H, C(CH₃)₃), 0.28 (s, 6H, Si(CH₃)₂).

**¹³C NMR (62.5 MHz, CDCl₃):** 165.75 (C=O), 159.94 (C-Ar), 144.49 (C-Ar), 129.52 (C-Ar), 128.95 (CH-Ar), 126.45 (C-Ar), 123.98 (CH-Ar), 121.82 (CH-Ar), 120.41 (CH-Ar), 117.54 (CH-Ar), 115.79 (CH-Ar), 25.77 (C(CH₃)₃), 18.16 (C(CH₃)₃), -4.24 (Si(CH₃)₂).

**Mp:** 146-149°C (methanol)

**FTIR (neat):** 3418 (m), 3297 (m), 1651 (s).

**HRMS (ES):** calc. for C₁₉H₂₅NO₃Si: 344.1676 [M+H]⁺ Found: 344.1680.
The named compound was prepared in analogy to 4.58a. The residue was crystallised from EtOAc / Hexane to give white crystals (1.23 g, 92%).

**HPLC (method B):** \( t_R = 8.84 \text{ min} \).

**Purity (ELSD):** 99%

**\(^1\text{H NMR (250 MHz, } d^6\text{-DMSO)}:** 10.13 (br s, 1H, NH), 9.93 (s, 1H, OH), 7.83 (d, \( J = 8.5 \text{ Hz} \), 2H, OC-C-CH), 7.43-7.37 (m, 2H, HN-C-CH-CH), 7.17 (t, \( J = 8.25 \text{ Hz} \), 1H, HN-C-CH-C-OTBS), 6.85 (d, \( J = 8.75 \text{ Hz} \), 2H, HO-C-CH), 6.54 (ddd, \( J = 1.0 \), 2.25 Hz, 8.25 Hz, 1H, TBSO-C-CH-CH), 0.96 (s, 9H, C(CH\(_3\))\(_3\)), 0.20 (s, 6H, Si(CH\(_3\))\(_2\)).

**\(^{13}\text{C NMR (62.5 MHz, } d^6\text{-DMSO):**} 165.01 (C=O), 160.46 (C-Ar), 155.08 (C-Ar), 140.59 (C-Ar), 129.61 (CH-Ar), 129.19 (CH-Ar), 125.31 (C-Ar), 114.79 (CH-Ar), 114.55 (CH-Ar), 113.11 (CH-Ar), 111.50 (CH-Ar), 25.49 (C(CH\(_3\))\(_3\)), 17.85 (C(CH\(_3\))\(_3\)), -4.58 (Si(CH\(_3\))\(_2\)).

**Mp:** 109-112°C (methanol)

**FTIR (neat):** 3271 (m), 1646 (s).

**HRMS (ES):** calc. for C\(_{19}\)H\(_{25}\)NO\(_3\)Si: 344.1676 [M+H]\(^+\) Found: 344.1682.
The named compound was prepared in analogy to 4.58a. The residue was crystallised from EtOAc / Hexane to give white crystals (1.19 g, 91%).

**HPLC (method B):** $t_R = 8.67$ min.

**Purity (ELSD):** 100%

**$^1$H NMR (250 MHz, CDCl$_3$):**
- 10.07 (s, 1H, NH), 9.88 (s, 1H, OH), 7.83 (d, $J = 8.75$ Hz, 2H, OC-C-CH), 7.61 (d, $J = 9.0$ Hz, 2H, HN-C-CH), 6.85 (d, $J = 9$ Hz, 2H, HO-C-CH), 6.82 (d, $J = 9.25$ Hz, 2H, TBSO-C-CH), 0.95 (s, 9H, C(CH$_3$)$_3$), 0.18 (s, 6H, Si(CH$_3$)$_2$).

**$^{13}$C NMR (62.5 MHz, CDCl$_3$):**
- 164.64 (C=O), 160.28 (C-Ar), 150.78 (C-Ar), 133.18 (C-Ar), 129.46 (CH-Ar), 125.44 (C-Ar), 121.77 (CH-Ar), 119.52 (CH-Ar), 114.76 (CH-Ar), 25.52 (C(CH$_3$)$_3$), 17.87 (C(CH$_3$)$_3$), -4.61 (Si(CH$_3$)$_2$).

**Mp:** 144-147°C (methanol)

**FTIR (neat):** 3328 (m), 3108 (m), 1637 (s).

**HRMS (ES):** calc. for C$_{19}$H$_{25}$NO$_3$Si: 344.1676 [M+H]$^+$ Found: 344.1679.
Acetic acid 4-phenylcarbamoyl-phenyl ester (4.60)

The named compound was prepared in analogy to 4.57a and obtained as a white solid (832 mg, 90%).

**HPLC (method B):** $t_R = 7.06$ min.

**Purity (ELSD):** 99%

$^1$H NMR (250 MHz, CDCl$_3$): 10.28 (s, 1H, NH), 8.00 (d, $J = 8.75$ Hz, 2H, OC-C-CH), 7.77 (d, $J = 7.5$ Hz, 2H, HN-C-CH), 7.39-7.28 (m, 2H, AcO-C-CH, 2H, HN-C-CH-CH), 7.10 (t, $J = 7.25$ Hz, 1H, HN-C-CH-CH-CH), 2.31 (s, 3H, CH$_3$).

$^{13}$C NMR (62.5 MHz, CDCl$_3$): 168.71 (C=O), 164.53 (C=O), 152.62 (C-Ar), 138.86 (C-Ar), 132.25 (C-Ar), 128.92 (CH-Ar), 128.35 (CH-Ar), 123.42 (CH-Ar), 121.57 (CH-Ar), 120.05 (CH-Ar), 20.63 (CH$_3$).

**Mp:** 129-132°C (methanol)

**FTIR (neat):** 3354 (m), 1755 (s), 1656 (s).

**HRMS (ES):** calc. for C$_{15}$H$_{13}$NO$_3$: 256.0968 [M+H]$^+$ Found: 256.0969.

4-Hydroxy-N-phenyl-benzamide (4.61)

The named compound was prepared in analogy to 4.58a and obtained as a white solid (95%).

$^1$H NMR (250 MHz, CDCl$_3$): 10.11 (s, 1H, NH), 9.99 (s, 1H, OH), 7.86 (d, $J = 8.75$ Hz, 2H, OC-C-CH), 7.76 (d, $J = 7.5$ Hz, 2H, HN-C-CH), 7.33 (t, $J = 7.5$ Hz, 2H, HN-C-CH-CH), 7.06 (t, $J = 7.5$ Hz, 1H, HN-C-CHCHCH), 6.87 (d, $J = 8.75$ Hz, 2H, HO-C-CH).

$m/z$ (ESMS): 214 [M+H]$^+$
Synthesis of resin 4.89

Adipic acid monomethylester (52 mg, 0.3 mmol, 3 equiv.), HATU (122 mg, 0.3 mmol, 3 equiv.) and DiPEA (53 µL, 0.3 mmol, 3 equiv.) were premixed in DMA (1 mL) and added to resin 4.44 (0.1 mmol, previously deprotected with 20% piperidine in DMF) and the mixture was shaken for 2h at room temperature. Completion of the reaction was confirmed by ninhydrin test. The resin was filtered, washed with DMF (3 x 5 mL), DCM (3 x 5 mL), MeOH (3 x 5 mL), DCM (3 x 5 mL) and MeOH (3 x 5 mL). A 7:3 solution of 1 M aq LiOH in THF (5 mL) was added to the resin (pre-swollen in THF) and the mixture was shaken for 3h at room temperature. The resin was filtered, washed with THF/H₂O (1:1) (3 x 5 mL), THF/1M aq. KHSO₄ (3 x 5 mL), THF/H₂O (1:1) (3 x 5 mL), MeOH (3 x 5 mL), DCM (3 x 5 mL) and MeOH (3 x 5 mL).
Synthesis of resin 4.90

To resin 4.89 (0.025 mmol, 1 equiv.) pre-swollen in DMA was added a solution of DIC (32 μL, 0.2 mmol, 8 equiv.) and HOBt (31 mg, 0.2 mmol, 8 equiv.) in DMA (1.5 mL). The mixture was shaken at room temperature for 3 h. A solution of 3-aminopropanol (15 mg, 0.2 mmol, 8 equiv. in DMA (0.5 mL) was added and the mixture was shaken for a further 3 h. The resin was then filtered and washed with DMF (3 x 2 mL), DCM (3 x 2 mL), MeOH (3 x 2 mL), DCM (3 x 2 mL) and MeOH (3 x 2 mL).

Synthesis of resin 4.91

Resin 4.91 (0.025 mmol) was prepared in analogy with resin 4.90 using 5-aminopentanol (21 mg, 0.2 mmol, 8 equiv.).
Synthesis of resin 4.92

Resin 4.93 (0.025 mmol) was prepared in analogy with resin 4.90 using 2-(2-Aminoethoxy)-ethanol (21 mg, 0.2 mmol, 8 equiv.).
Synthesis of tagged spacer-fragment conjugates from Fragments F₂-F₇

Preparation of mother solutions

The following solutions were prepared:

**Fragments:**

- **Fragment F₂ (4.58a):** 75 mg (0.218 mmol) in 900 μL anhydrous DCM (0.24 mmol/mL)
- **Fragment F₃ (4.58b):** 75 mg (0.218 mmol) in 900 μL anhydrous DCM (0.24 mmol/mL)
- **Fragment F₄ (4.58c):** 75 mg (0.218 mmol) in 900 μL anhydrous DCM (0.24 mmol/mL)
- **Fragment F₅ (4.61):** 47 mg (0.218 mmol) in 900 μL anhydrous DCM (0.24 mmol/mL)
- **Fragment F₆ (4-hydroxybenzotrifluoride):** 35 mg (0.218 mmol) in 900 μL anhydrous DCM (0.24 mmol/mL)
- **Fragment F₇ (phenol):** 21 mg (0.218 mmol) in 900 μL anhydrous DCM (0.24 mmol/mL)

**Mediating agents:**

- **triphenylphosphine:** 349 mg (1.33 mmol) in 5.4 mL anhydrous THF (0.24 mmol/mL)
- **DIAD:** 252 μL (1.33 mmol) in 5.4 mL anhydrous THF (0.24 mmol/mL)

**Procedure for the library synthesis**

Each of the resin 4.90, 4.91 and 4.92 were divided into six equal quantities. Each batch of resin was then swollen in 200 μL anhydrous DCM. 300 μL of a fragment solution, 300 μL of DIAD solution and 300 μL of triphenylphosphine solution were then introduced as a 6x3 array and the mixtures were shaken at room temperature for 16h. The resins were then filtered and washed with DCM (3 x 1 mL), MeOH (3 x 1 mL), DCM (3 x 1 mL) and MeOH (3 x 1 mL).
Cleavage from the resin and precipitation

Each tagged spacer-fragment conjugate was then cleaved from the resin with TFA/DCM/TIS (90:5:5) for 1.5 h at room temperature. The resin was then filtered and washed with DCM (2 x 1 mL). The collected filtrates were concentrated in vacuo. Cold Et₂O was added to the residues resulting in precipitation of the PNAs. The solutions were then centrifuged for 5 min. and the supernatants discarded. The compounds were then dried in vacuo.

Synthesis of resin 4.95

To resin 4.89 (0.017 mmol, 1 equiv.) pre-swollen in DMA was added a solution of DIC (16 μL, 0.1 mmol, 6 equiv.) and HOBt (16 mg, 0.1 mmol, 6 equiv.) in DMA (0.5 mL). The mixture was shaken at room temperature for 3 h. A solution of mono-fmoc 1,3-diaminopropane hydrochloride (33 mg, 0.1 mmol, 6 equiv.) and DIPEA (17 μL, 0.1 mmol, 6 equiv.) in DMA (0.5 mL) was added and the mixture was shaken for a further 3 h. The resin was then filtered and washed with DMF (3 x 2 mL), DCM (3 x 2 mL), MeOH (3 x 2 mL), DCM (3 x 2 mL) and MeOH (3 x 2 mL). The resin was then swollen in 0.5 mL DMF. A pre-mixed solution of acetic acid (6 μL, 0.1 mmol, 6 equiv.), HATU (38 mg, 0.1 mmol, 6 equiv.) and DIPEA (17 μL, 0.1 mmol, 6 equiv.) in DMA (0.5 mL) was added to the resin and the mixture was shaken at room temperature for 3 h. The resin was then filtered and washed with DMF (3 x 2 mL), DCM (3 x 2 mL), MeOH (3 x 2 mL), DCM (3 x 2 mL) and MeOH (3 x 2 mL). The resin was then shaken in 20% piperidine in DMF (1 mL) for 25 min. at room temperature, filtered and washed with DMF (3 x 2 mL), DCM (3 x 2 mL), MeOH (3 x 2 mL), DCM (3 x 2 mL) and MeOH (3 x 2 mL).
Synthesis of resin 4.96

Resin 4.96 (0.017 mmol) was prepared in analogy with resin 4.95 using mono-fmoc 1,5-diaminopentane hydrobromide (41 mg, 0.1 mmol, 6 equiv.).

Synthesis of resin 4.97

Resin 4.97 (0.017 mmol) was prepared in analogy with resin 4.95 using mono-fmoc 2-(2-Amino-ethoxy)-ethylamine hydrochloride (36 mg, 0.1 mmol, 6 equiv.).
Coupling of fragment F₁ and rhodamine to resins 4.95, 4.96 and 4.97
Each resin was separated into two equal quantities (0.008 mmol). Then a pre-mixed solution of fragment F₁ 4.50 (18 mg, 0.05 mmol, 6 equiv.) or rhodamine (20 mg, 0.05 mmol, 6 equiv.), HATU (16 mg, 0.05 mmol, 6 equiv.) and DIPEA (9 µL, 0.05 mmol, 6 equiv.) in DMA (0.5 mL) was added to the resins as a 3x2 array. The mixtures were shaken at room temperature for 3 h. The resins were filtered and washed with DMF (3 x 1 mL), DCM (3 x 1 mL), MeOH (3 x 1 mL), DCM (3 x 1 mL) and MeOH (3 x 1 mL). The 3 spacer-fragment F₁ and 3 spacer-rhodamine conjugates were released in solution in analogy with the other spacer-fragment conjugates.

DNA microarray production
3’-amino modified DNA oligos were purchased from Sigma-Genosys, U. K. DNA printing was performed using a Robot Microarrayer (Genetix QMini, U.K.). 1 solid pin was used in order to print 3’-amino modified DNA 18mers on aldehyde-coated glass slides (Genetix) and agarose-coated glass slides keeping humidity at 70%. The same pattern was reproduced twice: a 5x4 subarray for 3’-amino modified 5'-fluoresceine derivatised DNA oligo and eight 5x4 subarrays for 3-amino modified DNA oligo. Each DNA was printed 5 times on each spot. Then, the microarrays were kept overnight in a chamber containing a 3M NaCl solution. The chips were then washed successively with SDS (2 x 2 min.), distilled water (2 x 2 min), Reductive solution of NaBH₄ (1 x 5 min.), distilled water (1 x 1 min.), and SDS (3 x 1 min.) and distilled water (2 x 1 min.). After spin-drying by centrifugation, the chips were stored in dark before hybridisation was carried out.

Hybridisation onto DNA microarrays
The PNA-tagged fragment-spacer conjugates were dissolved in DMSO at a concentration of 2 mM. Each solutions was then diluted with water to obtain 200μM solutions, which were then diluted with GenHYB buffer (Genetix) to afford stock solutions of single fragment at a concentration of 100 μM. Stock solutions of mixtures of two fragments were prepared in a similar manor to obtain printing solutions with a concentration of 50 μM for each fragment.
Each stock solution was printed in a 3 x 1 subarray format with a repetition of 5 printing onto the same spot. All the printing process was performed keeping humidity at 80%.

After printing, the microarrays were kept for 24 h in a chamber containing a 3M NaCl solution. The chips were then washed successively with PBS buffer (2 x 15 min.) and distilled water (2 x 2 min) and spin-dried by centrifugation.

**FKBP12 experiment**

RGS-His\textsubscript{4} tagged FKBP12 was donated by Prof. Stuart Schreiber and Dr. Angela Koehler, Harvard University, U.S.A.

300 µL PBST buffer (PBS buffer + 0.05 % Tween 20) containing 1 µg of RGS-His\textsubscript{4}-FKBP12 was deposited on each microarray and incubated for 30 min. at room temperature. The chips were then washed with PBST buffer (3 x 3 min.) and incubated with 300 µL of a 1:1000 dilution of RGS-His\textsubscript{4} mouse antibody (Qiagen) in PBST for a further 30 min. at room temperature. The microarrays were then washed with PBST buffer (3 x 3 min.) and incubated with 300 µL of a 1:1000 dilution of Alexa 488 anti-mouse (Molecular Probes) in PBS buffer containing 0.1 % of BSA (Sigma), for 30 min. at room temperature. The chips were then washed with PBST buffer (3 x 5 min.), PBS (1 x 2 min.) and spin-dried by centrifugation.

**Scanning**

Microarrays were scanned with a CCD based fluorescence scanner (Bioanalyser 4F, LaVision Biotech, Germany) using FITC filters. Analysis of the microarray images was made using FIPS software (LaVision Bio Tech).
### Analysis of the aldehyde slides

The following fluorescence intensities were measured (average over 3 spots):

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### Analysis of the agarose slides

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<td>Bailen, M. A.; Chinchilla, R.; Dodsworth, D. J.; Najera, C., Tetrahedron</td>
<td>2000</td>
<td>41</td>
<td>9809-9813</td>
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<td>Albericio, F.; Bailen, M. A.; Chinchilla, R.; Dodsworth, D. J.; Najera, C.</td>
<td>2001</td>
<td>57</td>
<td>9607-9613</td>
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<td>Yang, T.; Lin, C.; Fu, H.; Jiang, Y.; Zhao, Y. F., Bioorganic Chem.</td>
<td>2005</td>
<td>33</td>
<td>386-392</td>
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<td>Albertson, N. F., Org. Reac.</td>
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<td>12</td>
<td>157-355</td>
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<td>Zhang, M.; Vedanthan, P.; Flynn, D. L.; Hanson, P. R., J. Org. Chem.</td>
<td>2004</td>
<td>69</td>
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<td>Lannuzel, M.; Lamothe, M.; Perez, M., Tetrahedron Lett.</td>
<td>2001</td>
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<td>Wolman, Y.; Kivity, S.; Frankel, M., Chem. Commun.</td>
<td>1967</td>
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