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Solid-Phase Organic Synthesis: Bicyclic Peptides and Purine-Derived Small Molecules

by

Tuomas Karskela

TURUN YLIOPISTO
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From the Department of Chemistry
University of Turku
Turku, Finland

Custos
Professor Harri Lönnberg
Department of Chemistry
University of Turku
Turku, Finland

Reviewers
Professor Knud Jensen
Department of Chemistry
University of Copenhagen
Copenhagen, Denmark

Professor Roger Strömberg
Department of Biosciences and Nutrition
Karolinska Institutet
Stockholm, Sweden

Opponent
Professor Doctor Oliver Seitz
Department of Chemistry
Humboldt University Berlin
Berlin, Germany

Cover illustration: A stereomicroscope image of dry aminomethyl copoly(styrene–1% divinylbenzene) particles used in solid-phase synthesis. (by Tuomas Karskela)

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Preface

This thesis is based on the experimental work carried out at the Laboratory of Organic Chemistry, Department of Chemistry at the University of Turku between May 2000 and March 2011. In the beginning the funding came from the TEKES National Drug 2000 technology program, and thereafter from the University of Turku and the Ministry of Education and Culture.

The path that led to this thesis has been long and had its dead ends. Sometimes these wrong tracks have been regrettably lengthy and really tested the motivation and faith. I admire Professor Harri Lönnberg's enormous patience from which the support and guidance given through these years are a good example of. I am deeply grateful to him for giving me the opportunity to work and study under his supervision. In addition to study for the PhD degree, I have been given a chance to teach undergraduate students and work for several years as the coordinator of the National Doctoral Program of Organic Chemistry and Chemical Biology (OCCB). Although these assignments may have postponed my graduation, they have been rewarding and taught me a lot.

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Turku September 2013

Tuomas Karskela

Contents

List of Original Publications

This thesis is based on the following publications:

I Katajisto, J; Karskela, T; Heinonen, P; Lönnberg, H. An orthogonally protected α,α -bis(aminomethyl)- β -alanine building block for the construction of glycoconjugates on a solid support. *J. Org. Chem.* **2002**, *67*, 7995–8001.

II Karskela, T; Heinonen, P; Virta, P; Lönnberg, H. Solid-supported synthesis of bicyclic peptides containing three parallel peptide chains. *Eur. J. Org. Chem.* **2003**, 1687–1691.

III Karskela, T; Lönnberg, H. Solid-phase synthesis of 7-substituted 3*H*-imidazo[2,1-*i*]purines. *Org. Biomol. Chem.* **2006**, *4*, 4506–4513.

IV Karskela, T; Lönnberg, H. Solid-phase synthesis of 4(5),1′,5′-trisubstituted 2,4′-biimidazoles. *J. Org. Chem.* **2009**, *74*, 9446–9451.

V Karskela, T; Klika K, D; Lönnberg, H. Synthesis of 7-substituted 3-*β*-D-ribofuranosyl-3*H*-imidazo[2,1-*i*]purines, *Collect. Czech. Chem. Commun.* **2011**, *76*, 1043–1054.

Abbreviations

Ac	acetyl
Acm	acetamidomethyl
Ala	alanine
All	allyl
Alloc	allyloxycarbonyl
AM-CM	Aminomethyl ChemMatrix
Asn	asparagine
Asp	aspartic acid
ATP	adenosine-5'-triphosphate
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BTC	triphosgene
Bz	benzoyl
CatK	cathepsin K, a cysteine protease
Cdk	cyclin-dependent kinase
CLEAR	poly(ethylene glycol) cross-linked ethoxylate acrylate resin
CML	chronic myeloid leukaemia
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholinocarbenium hexafluorophosphate
CPG	controlled pore glass
CRH	corticotropin-releasing hormone
dA-CE phosphoramidite	N^6-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 3′-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)
DCC	*N,N*′-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DCM	dichloromethane
DIC	diisopropylcarbodiimide
DIEA	ethyldiisopropylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMTr	4,4′-dimethoxytrityl
DNA	deoxyribonucleic acid
DNP	2,4-dinitrophenyl
DNPH	2,4-dinitrophenylhydrazine/one
DVB	divinylbenzene
DVB-PS	polystyrene cross-linked with divinylbenzene
EDAC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EGFR	epidermal growth factor receptor
EST	oestrogen sulfotransferase
Fmoc	9-fluorenylmethyloxycarbonyl

Gly	glycine
GPCR	G-protein coupled receptor
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide
HBTU	1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium hexafluorophosphate 3-oxide
HCTU	1-[bis(dimethylamino)methylene]-5-chloro-1*H*-benzotriazolium hexafluorophosphate 3-oxide
HDMC	*N*-[(5-Chloro-3-oxido-1*H*-benzotriazol-1-yl)-4-morpholinyl-methylene]-*N*-methylmethanaminium hexafluorophosphate
HMBA	4-(hydroxymethyl)benzoic acid (linker)
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
HSP90	heat-shock protein-90
Ibu	isobutyryl
IP3K	inositol-1,4,5-triphosphate-3-kinase
LC-ESI-Q	liquid chromatography-electrospray ionization-quadrupole (mass spectrometry)
Leu	leucine
MALDI-TOF	matrix-assisted laser desorption/ionisation-time of flight (mass spectrometry)
MBHA	methylbenzhydrylamine (linker)
MMTr	4-methoxytrityl
MS	mass spectrometry
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
PAM	phenylacetamidomethyl
PASP	polymer-assisted solution-phase synthesis
PDE	phosphodiesterase
PEG	poly(ethylene glycol)
PEGA	polyacrylamide cross-linked with poly(ethylene glycol)
PEG-PS	polystyrene poly(ethylene glycol) graft copolymer
Phe	phenylalanine
Phth	phthaloyl
PK	protein kinase
pol	polymerase
PS	polystyrene
PS-RCS	polymer-supported reagents, catalysts, and scavengers

PyBOP	(Benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate
PyClock	(6-chloro-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate
RNA	ribonucleic acid
RP HPLC	reversed-phase high-performance liquid chromatography
SASRIN	super acid sensitive resin (linker)
SCAL	safety-catch amide linker
Ser	serine
SPE	solid-phase extraction
SPOS	solid-phase organic synthesis
SPPS	solid-phase peptide synthesis
SPS	solid-phase synthesis
Src	sarcoma (tyrosine kinase)
TBTU	1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium tetrafluoroborate 3-oxide
t-Bu	*t*-butyl
Tekes	Finnish Funding Agency for Technology and Innovation
TFA	trifluoroacetic acid
TFFH	tetramethylfluoroformamidinium hexafluorophosphate
TFMSA	trifluoromethanesulfonic acid
TG	Tentagel
TG S RAM	Rink amide Tentagel
THF	tetrahydrofuran
Trp	tryptophan
Trt	trityl
Val	valine
β-Ala	β-Alanine
β-AST	β-arylsulfotransferase

1. Introduction

During the last decades, solid supported chemistry methods have played a significant role in chemical biology and especially in drug discovery. Solid-phase synthesis (SPS) is the method of choice for chemical synthesis of biologically active oligomers like peptides, DNA, RNA, and PNA. It is also well suited for the production of chemical compound libraries. In drug discovery, large masses of chemical compounds contained in these libraries have been tested against biological targets to find novel active compounds for further development. Although currently about 100 million compounds of the hundreds of billions of possibly active organic small molecules have been synthetized, it still seems to be difficult to develop new small molecule drugs.[1,2] Moreover, it seems that the chemical space the synthesized molecular libraries cover is relatively small, as is the number of utilized molecular frameworks.[3] With considerations akin to above in mind, we decided to put an effort to develop new methods for library synthesis of compounds that could be of value for medicinal chemistry. This thesis summarizes some of that work.

1.1 Solid-Phase Synthesis

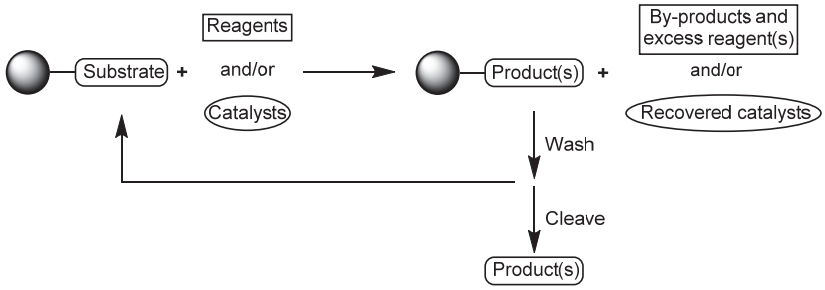
By writing a notebook entry "A New Approach to the Continuous, Stepwise Synthesis of Peptides" in 1959 Bruce Merrifield started a development that revolutionized the field of synthetic organic chemistry.[4] At first, the concept of solid-phase synthesis generated strong resistance among the scientific community. In traditional synthetic organic chemistry, isolation and characterization of reaction intermediates were needed as evidence supporting the chemical structure of the product. Isolation of the intermediates was often required for practical reasons as well: accumulation of the side products from consecutive synthesis steps could lead to a complex product mixture hard to fractionate into pure products. By contrast, in solid-phase chemistry a large excess of reagents are often used to force the reactions as close to completion as possible. In addition, multiple reaction steps are performed successively with only a simple washing off of excess reagents from the solid support between the steps. As a result, good planning and optimization of the synthesis route is necessary and furthermore, the products cleaved from the solid support require careful purification and characterization. The breakthrough of SPS would not have been feasible without concomitant advances in both purification techniques and analytic methods, such as HPLC, capillary electrophoresis, mass spectrometry, NMR spectroscopy, and automated amino acid analysis. Only a decade after the preparation of the first compound, namely tetrapeptide Leu-Ala-Gly-Val, produced on a solid-phase,[5] and after several improvements and extensions, the SPS method started to gain general acceptance. The subsequent years brought about a large variety of linkers, protecting groups, coupling reagents, solid supports, peptide synthesizers, as well as extensions to other areas of organic chemistry, for example to synthesis of depsipeptides[6], polyamides[7], oligonucleotides[8–11], oligosaccharides[12–14], peptide nucleic acids[15,16], and to solid-phase organic synthesis (SPOS)[17–21]

Moreover, the SPS methodology started the era of combinatorial chemistry in 1980s[22–28]. The SPS method is scalable from detection limit to metric ton scale industrial applications. For example, there are currently about thirty approved peptide pharmaceuticals that are manufactured in industrial scale by solid-phase peptide chemistry (SPPS).[29]

1.2 Advantages and Disadvantages of Using Insoluble Polymer Support

The idea of the SPS is simple: the substrate is attached to a polymer support, the intended chemical reaction is carried out and the excess reagents are washed off. This reaction-wash cycle is repeated until the synthesis is ready and the product is cleaved from the support (**Scheme 1**).

Anchoring of the substrate to an insoluble matrix has several advantages.[30] The most obvious and important advantage is that reaction work-up is simplified because excess reagents and soluble by-products can be removed by resin washing and filtration. Hence, it may be possible to avoid exposure of the reaction products to water (extraction) or to avoid chromatographic purification of the intermediate products. There is usually no need to dry the resin between washing and reaction steps which increases the speed of the synthesis. The ease of separation allows one to use a large excess of reagents, in case this leads to better reaction yields. Moreover, the process is highly applicable to automation. This is important especially for the industrial and combinatorial chemistry applications. SPS is particularly suitable for synthesis of oligomeric and polymeric compounds where automation of repetitive synthesis steps in a sealed environment brings about speed, reproducibility and higher yields compared to manual laboratory work.



Scheme 1. The main principle of solid-phase chemistry.

In many applications, especially on using polymer-supported catalysts, reagents and scavengers (*vide infra*), it is reasonable to recover, regenerate and reuse the solid-support.[20] Hence, polymers with complex functionalities that are economic or ecologic to use compared to their low-molecular weight analogues can be designed. A further advantage is that polymer bound functionalities, which would be significantly toxic or noxious as low molecular weight compounds, show reduced toxicity and are odourless

making handling of the material easier. The solid-supported chemistry can also be adapted to flow systems for both industrial and laboratory scale applications.[31]

Reactions are heterogeneous when performed on insoluble polymer matrix.[32] This leads to differences between reactions on a solid-support and in solution. The main types of differences are: effects resulting from the need for the soluble reagent to gain access to the supported reactants (i.e. diffusion), microenvironmental effects, and site–site interactions or site-isolation. The first may result in significant size selectivity or slower reactions, the second difference in polarity of the microenvironment of the substrate compared to the bulk of solvent, and the third either higher or lower intraresin reactivity[33]. The heterogeneity of the solid-supported reaction system is most often seen as a disadvantage although, for example, site-isolation has been successfully utilized in synthesis of cyclic peptides already during the early days of SPPS.[34]

It is crucial that reactions on solid-supported substrates proceed in very high yields with few side reactions because purification of intermediates is not possible. Formation of side-products may lead to a complex product mixture which severely complicates the purification of the desired products. For example, synthesis of a 40 amino acid peptide with a 99% average yield per deprotection-coupling cycle gives theoretically 67% correct product (**Table 1.**). The rest of the product mixture consists of diverse truncated and deletion sequences[a] or false products that have suffered side-reactions. These side-products may be very similar to the desired product and may possess similar chromatographic properties as well, making the purification difficult. The yield limitation restricts the size of the oligomers that are feasibly synthesized by stepwise coupling reactions. The practical limit for peptide sequence length is currently between 50 to 100 amino acid residues. For shorter syntheses, lower yields per step may produce acceptable results if the end product is sufficiently pure or easy to purify.

Table 1. Yields of final product in a multistep synthesis as a function of number of steps and yield per step.

	Number of steps								
Yield per step	**2**	**3**	**4**	**5**	**10**	**20**	**30**	**40**	**50**
99%	98%	97%	96%	95%	90%	82%	74%	67%	61%
95%	90%	86%	81%	77%	60%	36%	21%	13%	8%
90%	81%	73%	66%	59%	35%	12%	4%	1%	1%
85%	72%	61%	52%	44%	20%	4%	1%	0%	0%
80%	64%	51%	41%	33%	11%	1%	0%	0%	0%

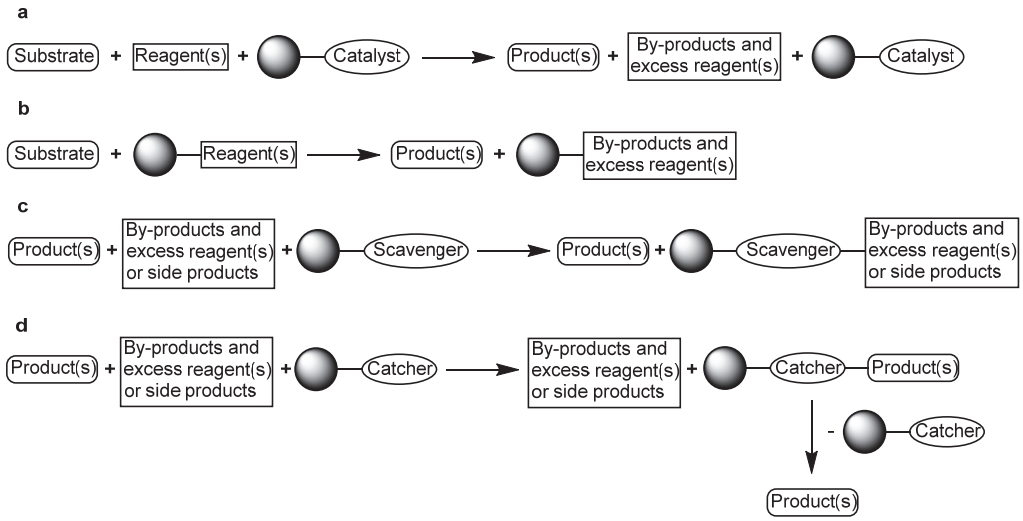
a) A truncated peptide is a peptide which becomes unavailable for reaction at some stage in the synthesis and does not add any further amino acids. A deletion sequence is a truncated peptide which resumes growth at some later stage in the synthesis.

Owing to the requirement of high yield and clean reactions, every synthesis step has to be carefully optimized. This can be quite a demanding and time consuming process. Optimization is laborious for solid-supported substrates in particular, because monitoring of the reaction progress and outcome is often impossible without cleaving a sample from the resin and analysing it. Czarnik has summarized some of the issues that one needs to take into account when planning a solid-phase organic synthesis, the main point being that a gel type solid-support is like a solvent.[35] Thus, the swollen resin together with the added solvent is the actual reaction solvent, although very viscose one.[36] Naturally, the selected solvent has an influence on reaction outcome and the resin should be chosen bearing this in mind.[37,38] The commonly used microporous resins need to swell in the reaction solvent for the reagents to gain access to the supported reactant or reagent.[39] In addition to the above mentioned, different batches of resins with similar specifications may behave differently in solid-phase synthesis.[40] Although the situation is better than 20 years ago when Pugh and co-workers reported[41] that the indicated cross-linking, mesh size range, and substitution are frequently inaccurate on commercial resins, the problem still exists[40]. Also age of the resin influences its performance.[35,42] The loading of a resin may decrease over the time since the reactive groups are not inert to storage and handling. Resins may contain entrapped impurities that can hamper the synthesis or if not reacting, they at least bias the determination of the yield and reaction monitoring.[43] For these reasons, it is advisable to optimize the reaction conditions and carry out the actual synthesis with the same batch of resin, prewash it well before use, and determine its swelling capacity as a simple measure of resin performance.[40]

1.3 Polymer-Assisted Solution-Phase Synthesis

In parallel with the solid-phase synthesis, evolved the chemistry of polymer-supported reagents, catalysts, and scavengers (PS-RCS).[44,45] The origins of the polymer-supported catalysts actually trace back to the development of ion-exchange resins in forties and fifties so that several reviews of the ion-exchange catalysis already existed by the advent of SPS[46,47]. PS-RCS methods have attracted only modest interest until last ten or fifteen years. The need to simplify work-up and reaction procedures in parallel synthesis and high-throughput screening has changed the situation. After initial popularity of SPOS in combinatorial synthesis, many chemists have preferred solution synthesis as a method of choice for preparation of combinatorial libraries.[48–50] The reasons include time consuming and expensive method development, slower reactions on solid support, low quantities of products produced, difficulties in reaction monitoring, and a need for an attachment point to the support.[51] On the other hand, high throughput parallel synthesis in solution has one major disadvantage, namely purification. Many solutions to address the problem have appeared.[52–54] One solution has been to use in combination solution-phase synthesis and purification techniques that utilize solid-support. This polymer-assisted solution-phase (PASP) synthesis method can be

divided into two main approaches: PS-RCS (**Scheme 2a-c**) and catch-and-release approaches (**Scheme 2d**).[55] In case of polymer-supported catalysts (Scheme 2 a), the catalyst is separated from the reaction mixture and after regeneration reused. If a reagent is bound to the support, the reaction product is purified by filtering off the bound by-products and excess reagent (Scheme 2 b). Scavenger resins can be used to pick out a reaction component after reaction to facilitate the purification (Scheme 2 c). For example, aminomethylated polystyrene can be used to remove electrophiles like acid chlorides, sulfonyl chlorides, isocyanates, and isothiocyanates.[56] A similar method is solid-phase extraction (SPE). While scavenger resins form a covalent bond with the impurities, SPE is based on noncovalent interactions. In catch-and-release or resin capture approach (Scheme 2 d), the product is purified by fishing it out from the reaction mixture with a polymer support and after filtration released to solution. In a variation of this method, a reactant is attached to the resin via activated bond which is then cleaved by conjugate reactant.



Scheme 2. The principle of using polymer-supported catalysts (a), reagents (b), scavengers (c), and resin capture method (d).

There are some advantages in PASP synthesis compared to SPOS in library production.[53] Firstly, in PS-RCS methods, synthesis is two steps shorter because the substrate is not attached and detached from the support. For the same reason, there is no need for a functionality to attach the substrate orthogonally[57] to the polymer either. Secondly, the utilized polymer can be chosen or tailored for the best reactivity for each step in question unlike in SPOS where the substrate remains bound to chosen polymer through the whole synthesis. Thirdly, because substrate and products are in solution, reaction can be monitored by traditional methods.[31] One important difference is that it is possible to purify products from synthesis steps which do not proceed cleanly to completion. Also, the use of convergent synthesis strategies is easier in PASP synthesis. Final-

ly, it is possible to perform simultaneously successive reactions in same solution by using a mixture of solid-supports.[58–60] Limitations of PASP synthesis include high cost and in some cases rapid deterioration of product quality in multistep synthesis.[61] Additionally many of the problems associated with heterogeneous reaction system may apply also for PASP methods, that is, slow diffusion, microenvironmental effects and intraresin reactivity.[62] A more thorough comparison between solution- and solid-phase combinatorial chemistry can be found in Carmen Baldino's perspective article[61]. It can be concluded that both methods are suitable for combinatorial library production and that the method or their combination should be chosen by application and available resources. For the synthesis of oligo- or polymeric peptides and nucleic acids SPS remains the method of choice in most cases.

1.4 Resins for Solid-Phase Organic Synthesis

There are two types of solid supports: gel type microporous resins and macroporous resins. In addition there are soluble polymer supports[63–66] which are not covered in this book. While there are numerous different solid supports developed for SPOS, it is beyond the scope of this thesis to review them thoroughly. The most commonly used resins in SPOS have been (examples in parentheses) polystyrene cross-linked with divinylbenzene[5] (DVB-PS) or 1,4-bis(vinylphenoxy)butane[67] (JandaJel), polystyrene poly(ethylene glycol) graft copolymers[68,69] (PEG-PS, Tentagel or TG, Argogel, Novagel), polyacrylamides[70, 71] (Pepsyn), polyacrylamides cross-linked with PEG[72] (PEGA), PEG cross-linked ethoxylate acrylate resin[73] (CLEAR), cross-linked PEG[74] (ChemMatrix), and Silica[75,76] (controlled pore glass, i.e. CPG).[63,77] The support material is mostly in bead form in either 100–200 mesh (75–152 μm) or 200–400 mesh (37–75 μm) range. In some cases (e.g. CPG and Pepsyn K) the shape of the particles is irregular. In addition to small particular form, the support can be grafted on variety of surfaces, for example on films, membranes, polyethylene pins, crowns or discs, or glass.[78] Cotton and cellulose has also been used as a solid support. Some properties of the common support types are summarized in **Table 2** and structures in **Figure 1**.

Table 2. Properties of some solid supports.

Resin	Loading mmol/g	Comments
Microporous DVB-PS	0.3 – 4.0	Widely used for peptide synthesis and SPOS in general. Good chemical stability. May suffer from osmotic shock.
JandaJel	0.45 – 1.2	Because of more flexible cross-linker, swells better than DVB-PS. Reactions are more homogenous on JandaJel which may lead to better rates and yields in SPOS.
Tentagel	0.1 – 0.6	Prepared by polymerization of ethylene oxide on PS. Useful for synthesis of oligomeric biomolecules.
Novagel	0.6 – 0.8	Like TG but made by partial derivatization of AM-PS with Methyl-PEG_{2000}-p-nitrophenylcarbonate. Unlike in TG where linker is attached to the end of PEG chains, the linker is attached to PS core. Has higher loading than TG.
PEGA	0.2 – 0.4	Hydrophilic resin for SPPS and SPOS. PEGA swells extensively and is permeable to macromolecules.
CLEAR	0.2 – 0.6	Highly cross-linked PEG with no PS core. A very polar resin with good swelling characteristics. For synthesis of difficult peptide sequences.
ChemMatrix	0.3 – 1.2	A PEG resin with good physical and chemical properties. It has higher loadings than other PEG resins. Especially good for synthesis of difficult peptide sequences.
CPG	0.02 – 0.05 (0.44)	Mostly used in oligonucleotide synthesis. Low loading with or without spacers. Partial negative charge on surface even with coating. Unsuitable for reactions with fluorides or highly corrosive chemicals.

1.4.1 Microporous Resins

Gel type resins are polymers that are lightly cross-linked (typically 0.5–2% divinylbenzene for polystyrene). In dry state, their amorphous polymer chain network is collapsed and the polymer chains are in molecular contact with each other resulting in a very low surface area.[39] Diffusion of any molecule through this glass state is very slow. When polymer gets into contact with a solvent that has similar polarity as the polymer, it starts to imbibe the solvent and swell. Individual polymer chains migrate further apart within the limits of cross-links and entanglements while the solvent fills the swelling bead from the outside inwards. Degree of cross-linking determines how

much a polymer can swell. Low level of cross-linker (under 1%) yields exceedingly swellable mechanically weak resin networks, while highly cross-linked polymers, though mechanically stronger, do not swell much even in good solvents (except CLEAR resin). In microporous resins, functional groups are evenly distributed through the polymer network, which means that they are mostly located inside the resin bead.[79] Therefore good swelling is crucial for the reagents to gain access to the supported reactant or reagent. There exists no universal polarity scale that would accurately interpret all solvent effects[80] and thus performance of a solvent has to be assessed experimentally. Experimental swelling properties of solid supports in different solvents are listed in literature.[81–83] **Table 3** represents swelling of six resins in eleven common solvents that are in decreasing polarity order.

Table 3. Swelling of six resins in eleven solvents (volume in mL/g). Dark grey colour indicates poor swelling, light grey moderate, and white good swelling. For Tentagel lower limits were used because of lower overall swelling and loading resulting from the PEG substitution.

Solvent[a]	Wang-PS[b]	MBHA-PS, neutralized[b]	MBHA-PS, HCl Salt[b]	TG S RAM[b]	AM-CM, HCl Salt[c]	$PEGA_{800}$[d]
TFA	2	4	4.5	6.4	16.2	[e]
water	1.6	2.2	1.0	3.6	11.0	16.0
Methanol	1.6	1.2	5.0	3.6	9.0	13.0
MeCN	2.0	2.8	2.0	4.0	6.2	[e]
DMSO	4.2	2.2	8.3	3.8	8.0	[e]
DMF	5.2	5.6	6.5	4.4	8.5	11.0
NMP	6.4	7.2	8.6	4.4	9.0	–
DCM	5.4	7.6	6.0	5.6	11.5	13.0
Pyridine	6.0	7.0	4.8	4.6	–	–
Et_2O	2.8	3.4	2.0	2.0	–	[f]
heptane	1.6	1.6	2.0	1.6	–	–

a) Solvents are in decreasing polarity order by Dimroth–Reichardt $E_T(30)$ parameter, except TFA, for which $E_T(30)$ parameter cannot be determined.
b) PS-Wang = Wang polystyrene (1% DVB, 0.6 mmol/g), MBHA-PS = methylbenzhydrylamine polystyrene (2% DVB, 0.8 mmol/g), TG S RAM = Rink amide Tentagel (1% DVB, 0.3 mmol/g)[82]
c) AM-CM = Aminomethyl ChemMatrix (0.4 mmol/g)[84]
d) $PEGA_{800}$ = Amino substituted PEGA (molecular weight of PEG chains is 800 g/mol, 0.4 mmol/g)[85]
e) Similar swelling as in DMF or DCM[86]
f) No swelling[87]

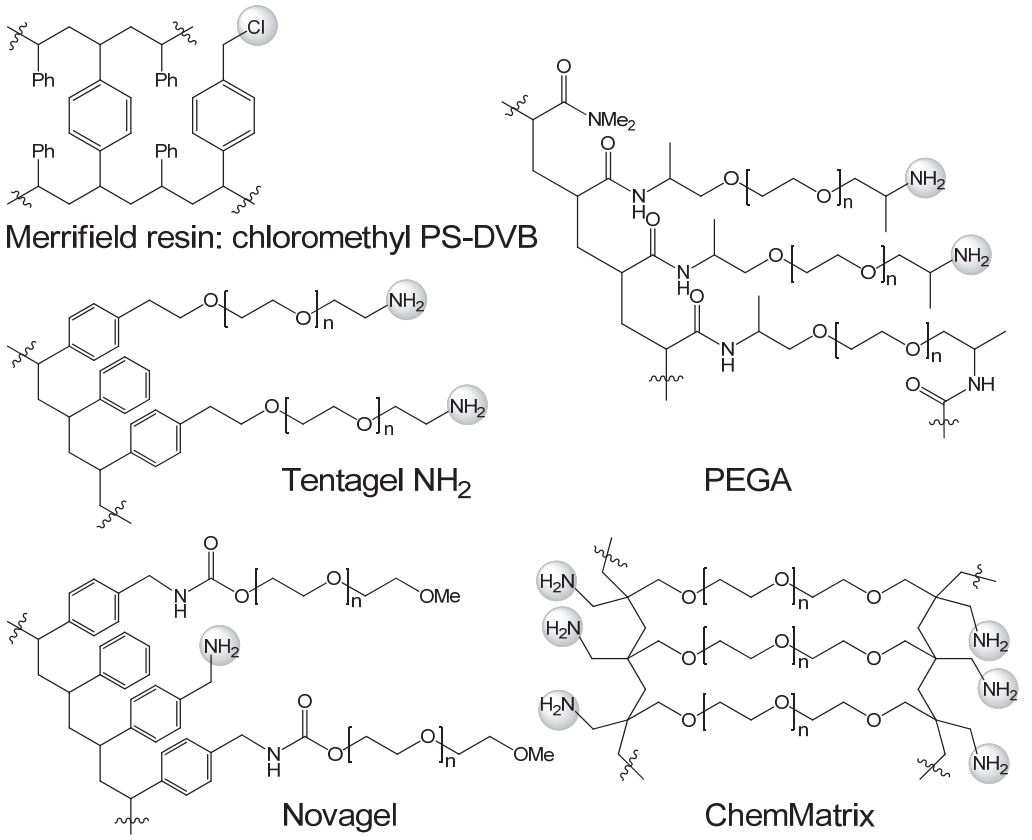


Figure 1. Structures of some common resins.

In addition to the solvent, the support bound molecules and their possible ionization have a major influence on swelling behaviour and reaction kinetics. For example, chloromethylated polystyrene resin swells well in toluene and not at all in water. Its reaction with trimethylamine to form benzyltrimethylammonium chloride residues yields a resin that is collapsed in toluene while swollen in water.[39] Hence, in a multi-step synthesis on a support, swelling behaviour may vary considerably. The extent of swelling can also markedly increase during the synthesis which has to be taken into account when choosing the synthesis vessel. To study this effect in SPPS, Sarin, Kent, and Merrifield synthesized a 60 residue long peptide on aminomethyl copoly(styrene–1% divinylbenzene) with 0.95 mmol/g initial loading.[88] After the synthesis, peptide content of the resin was 81% by weight. The volume of dry resin beads increased five-fold and beads swelled in DMF over 26-fold compared to dry unloaded beads. While the resin initially imbibed 2.3 mL DMF/g resin, the peptide resin imbibed 21.3 mL respectively. The results indicate that against common sense, the volume available for growing peptide chains increased inside the swollen bead as the synthesis progressed because of the mutual solubilizing effect of the resin and the peptide. Then again, in

case of increased holdup volume, either the concentration of reagents decreases or increased molar amounts of reagents are required if the concentration is kept high for optimum reaction rates. More solvent is also needed for efficient washing of the resin.

1.4.2 Macroporous Resins

Macroporous resins are highly cross-linked (>8% cross-linker) and they have a permanent porous structure and functionalization on the surface of a bead.[39,89] They don't swell markedly in any solvent making the choice of solvent less critical for functionality of the support. Non-swelling supports with good loading and accessibility allow high volumetric yield and lower solvent consumption which benefits large scale automated SPOS.[90] Because of the rigid open pore structure, diffusion of reagents to reaction sites is rapid. Likewise, removal of excess reagents and by-products in washing steps is quick. Also removal of the solvent from the resin is fast.[78] Macroporous resins are resistant to osmotic shock.[39] They have been used especially as ion-exchange resins which was their first application.[91,92] Other applications include solid-phase extraction, scavenger resins, and heterogeneous catalysis.[93] Macroporous resins have gained limited popularity in SPOS or SPPS with the exception of oligonucleotide synthesis. A comparative study performed in late nineties revealed that gel-type resins are superior to macroporous resins in SPPS in both product yield and purity.[78]

1.5 Linkers

The substrate is attached to the functionalized solid support with a fragment called a linker. Currently there are hundreds of different linkers developed for a wide range of applications.[94] Only few examples will be discussed below. Majority of the linkers can be regarded as support bound protecting groups, i.e. the substrate containing a functional group is attached to the linker moiety by a cleavable bond that is stable under synthesis conditions. In addition, there are so called traceless linkers which at the cleavage give a new C-H or C-C bond at the attachment point of the linker.[95]

The linkers can be classified in several ways, especially by the cleavage chemistry[96] (electrophilic, nucleophilic, photolysis, metal-assisted, reductive, oxidative, cycloaddition or cycloreversion) and by the functional group obtained after cleavage.[95,97,98] An ideal linker should fulfil several criteria[95], the primary properties being high yielding and easy attachment of the starting material, stability under a variety of synthesis conditions, and efficient cleavage reaction under conditions that do not damage the products. In addition, particularly in production of large libraries, the linker should be readily available and cheap, the work-up should be easy and cleavage should not introduce impurities that are difficult to remove.

Usually the substrate is attached to a preformed linker on the solid support. In some cases, though, it is favourable to attach the substrate to the linker in solution phase, purify this conjugate and attach it to the support.[99] The latter method can be used, for

example, if the preformed linker is unstable in storage, the loading reaction is disfavoured because of steric or electronic reasons or the loading reaction leads to unwanted side products which would contaminate the end products.[95]

Additional atoms that may be located between the polymer and the linker moiety constitute a spacer. Spacers can be introduced to increase mobility or solvation of the substrate. An example of increased mobility is offered by narrower line widths in NMR spectra of Tentagel bound molecules compared to polystyrene resin without a PEG spacer.[100] However, the mobility of the pendant group on a properly swollen polystyrene resin may well be sufficiently high to warrant good reaction kinetics.[38,101] For example, in peptide synthesis, the distance from the support has generally no significant influence on the synthetic efficiency.[102] Another reason for using a spacer may be the need to modify steric or electronic environment.[103–105]

1.5.1 Acid Labile Linkers

The chloromethylpolystyrene resin (i.e. Merrifield resin) has been used for the attachment of carboxylic acids since the early days of SPS.[5,106] The benzyl ester linkage formed requires a strong acid, such as HBr in TFA, TFMSA[107] or HF[108] for cleavage reaction. Because it was found out that the benzyl ester linkage is not fully stable in 50% TFA in DCM[109] used for deprotections in *tert*-butyloxycarbonyl/benzyl (Boc/Bn) peptide chemistry (*vide infra*), PAM (phenylacetamidomethyl) linker with electron withdrawing acetamidomethyl group *para* to the peptidyloxymethyl was introduced.[110] The foregoing demonstrates the fact that as the stability of the cation formed upon the cleavage reaction decreases, the acid stability of the linker increases. Thus electron withdrawing groups increase stability and electron donating groups increase lability of benzyl type linkers in acid (**Figure 2**).

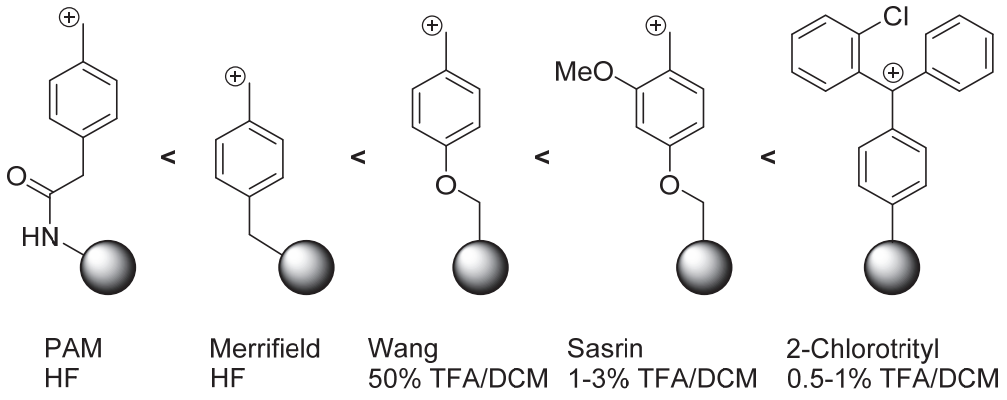
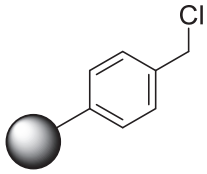
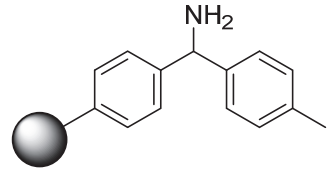


Figure 2. Stability of cations formed upon cleavage of some acid labile linkers. Lability of the corresponding linker increases from left to right.

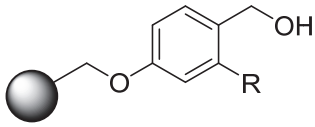
Wang or *para*-alkoxybenzyl alcohol linker (**Figure 3**) that has an electron donating group in *para* position is cleaved in milder conditions, i.e. 50% TFA in DCM.[111] SASRIN (Super acid sensitive resin)[112] or 2-chlorotrityl linkers[113] are very labile towards acids: peptide acids can be cleaved from both with 20% hexafluoro-2-propanol in DCM.[114] One advantage of 2-chlorotrityl linker compared to the alkoxybenzyl alcohol linkers is that the linker is resistant to nucleophilic attack while especially Wang linker is labile towards nucleophiles which can be a problem in certain reactions. Another important mild acid cleavable linker is Rink[115] in its different forms. It is used especially for synthesis of peptide amides by 9-fluorenylmethyloxycarbonyl/*tert*-butyl (Fmoc/*t*-Bu) protection strategy.[116] An example of an amide linker for milder cleavage conditions is Sieber amide linker[117] which is cleavable by 1% TFA in DCM while 4-methylbenzhydrylamine or MBHA resin[118], which has sufficient stability towards TFA, is used in Boc/Bn SPPS. Although only carboxylic acids and amides are mentioned here, these linkers allow release of a wide variety of leaving groups.



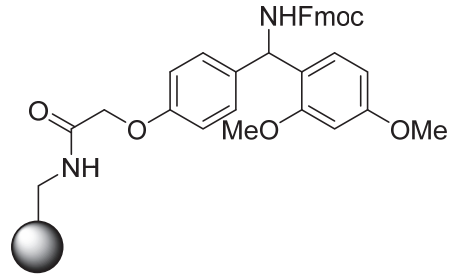
Merrifield resin, HF



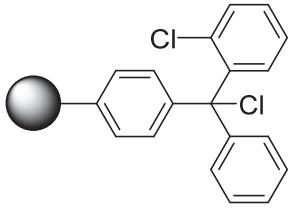
4-methylbenzhydrylamine resin, HF



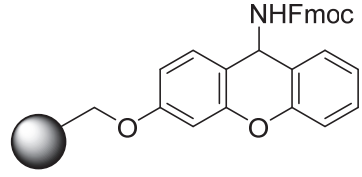
R = H, Wang resin, 50% TFA/DCM
R = OMe, SASRIN resin, 1-3% TFA/DCM



Rink amide AM resin, 20-95% TFA



2-Chlorotrityl resin
0.5-1% TFA/DCM

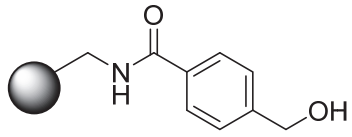


Sieber amide resin, 1% TFA/DCM

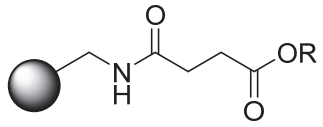
Figure 3. Examples of acid labile linkers and their typical cleavage conditions.

1.5.2 Linkers Cleaved by Nucleophilic Displacement or Other Methods

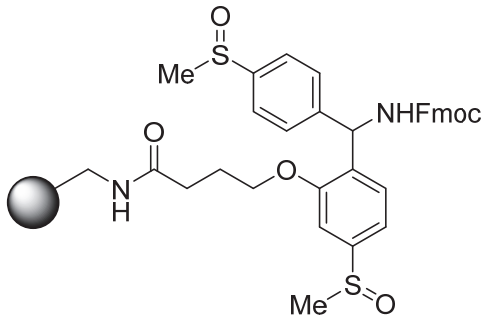
While some of the aforementioned benzyl ester linkers can be cleaved with nucleophiles, more appropriate ones for this purpose have been designed. The versatile 4-(hydroxymethyl)benzoic acid (HMBA) linker[119] (**Figure 4**) withstands acids but can be cleaved with a variety nucleophiles, e.g. 0.1 mol/L NaOH/dioxane, NH_3/MeOH[119], 4% $MeNH_2/H_2O$, MeOH/TEA[120] or NH_2NH_2/DMF, producing diverse end groups. Another example of nucleophilically cleaved linkers is succinyl linker,[121,122] common in oligonucleotide synthesis.



HMBA AM resin, NaOH (aq.); NH_3/MeOH; R^1NH_2; $NaBH_4$/EtOH; MeOH/TEA; NH_2NH_2/DMF



Succinyl linker in loaded form, NH_3 (aq.)

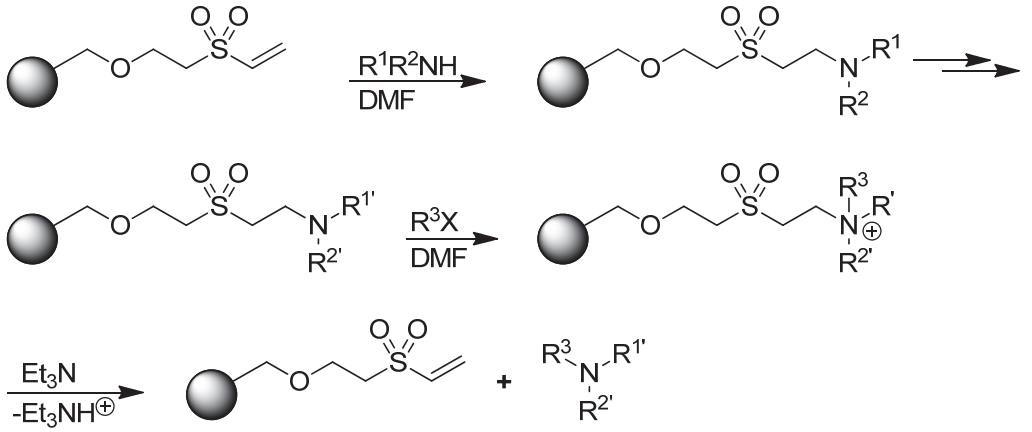


SCAL (safety-catch amide linker),
1) 1 mol/L PPh_3/Me_3SiCl/DCM,
2) TFA/H_2O (95/5); 1 mol/L Me_3SiBr/thioanisole/TFA

Figure 4. HMBA, succinyl and safety-catch amide linkers and their typical cleavage conditions.

A safety-catch linker[123] requires a two-step cleavage process: the linker is first activated and then cleaved. The main advantage of using safety-catch linkers is that reaction conditions that would otherwise cleave the linker can be used in the synthesis prior to activation. Vinyl sulfone linker[124] can be thought of as a safety-catch linker since a separate activation reaction is necessary to make the linking bond susceptible to cleavage reaction. The linker releases tertiary amines by β-elimination (**Scheme 3**) in basic conditions. The starting material, a secondary amine, is attached to a vinyl sulfone support by Michael addition forming a tertiary amine that is stable to a wide variety of reaction conditions. In the final steps of the synthesis, the amine is quaternarized and a short base treatment releases the tertiary amine to solution by Hoffman elimination. Another safety-catch linker is SCAL (**Figure 4**) that releases amides upon reductive acidolysis.[125] In oxidized form, it is very stable towards acids and bases but reduction of the sulfoxide groups turns it acid labile. The activation can be made in a separate

step with 1 mol/L PPh_3/Me_3SiCl/DCM or together with the acidolysis (1 mol/L Me_3SiBr/thioanisole/TFA).[126]



Scheme 3. Synthesis of tertiary amines using vinyl sulfone linker.

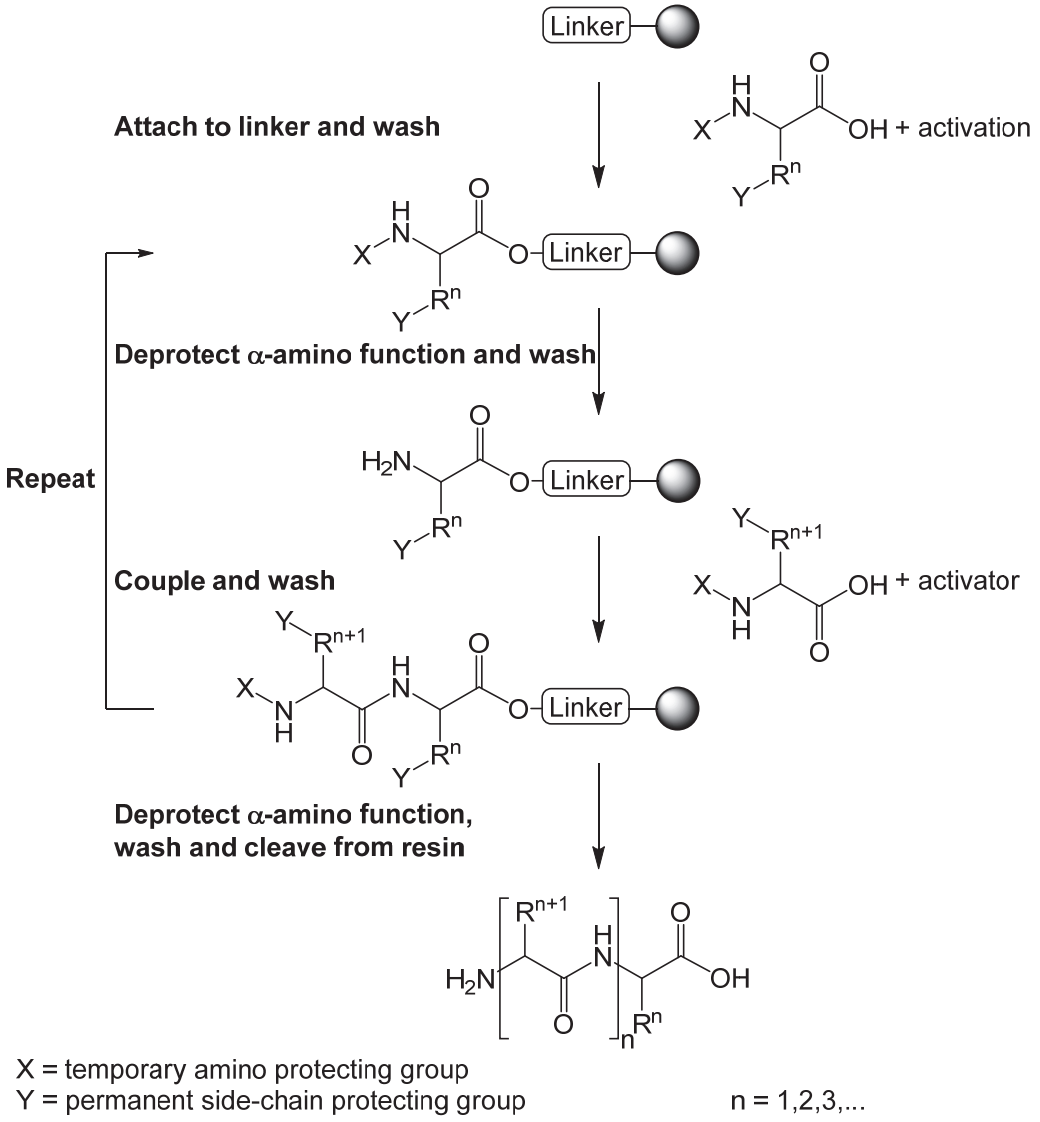
1.6 Solid-Phase Peptide Synthesis (SPPS)

Because of the wide range of biological functions of peptides and proteins[127], it is easy to understand their importance in scientific research and thus the significance of workable peptide synthesis methods. Although peptides are constructed from relatively simple amino acids joined by peptide bonds, development of efficient synthesis methods has been a huge and still on-going process. There are several side-reactions that hamper the synthesis: epimerization, diketopiperazine formation, aspartimide formation, side-chain alkylation and incomplete deprotections to mention few. In addition, there are difficult sequences caused by aggregation of peptide chains, insoluble peptides or peptides that are difficult to purify.[128] When the chain length increases, the yield limitation discussed in chapter 1.2 has to be taken into account. To tackle these problems, numerous protection[129], coupling[130], cleavage[131], ligation[132,133] etc. methods have been developed.

1.6.1 Synthesis Principle and Protection Strategies

In solid-phase peptide synthesis, a peptide is constructed by successive deprotection, coupling and washing steps (**Scheme 4**). Before the coupling step, there is often a preactivation step for the entering protected amino acid. The main protection strategies used in SPPS are Boc/Bn and Fmoc/*t*-Bu[134] methods. In the first method, Boc serves as a temporary protection for the α-amino group. It is removed by an acid (50% TFA in DCM) treatment in every synthesis cycle. In the standard method, there is a neutralization step between TFA deprotection and coupling steps. Alternative simultaneous neutralization and coupling can, however, give better results and can help to reduce peptide chain aggregation.[135] The side-chains of amino acids are protected permanently with benzyl or other protecting groups that are cleavable with strong acid at the end of

the synthesis. Additionally, there can be semi-permanent side-chain protections for on-resin modification of the side-chains (e.g. cyclization) or permanent orthogonal protections that endure the detachment from the solid support. In an orthogonal protection scheme, any of the protecting groups can be selectively removed without affecting the other protecting groups present.[57]



Scheme 4. The generalized solid-phase peptide synthesis principle. Boc/Bn chemistry contains additionally a neutralization step after deprotection.

Unlike Boc/Bn scheme, the Fmoc/*t*-Bu protection scheme is intrinsically orthogonal (**Figure 5**). The temporary Fmoc protection is removed with a base (20% piperidine in DMF) and the permanent *t*-butyl and Boc side-chain protections with acid (TFA) during detachment from the solid support. Third orthogonal dimension is often obtained by allyl and allyloxycarbonyl protections which can be cleaved by palladium chemistry. In some cases, a quasiorthogonal protection strategy is selected, i.e. a protecting group can be removed selectively in milder conditions than other protecting groups in the system. For example, trityl group can be removed from serine, threonine, and tyrosine side-chains with 1% TFA in DCM leaving the linker as well as *t*-butyl and Boc protections intact. While the more flexible Fmoc/*t*-Bu method is by vast more popular currently, Boc/Bn SPPS is still being used and developed.[105]

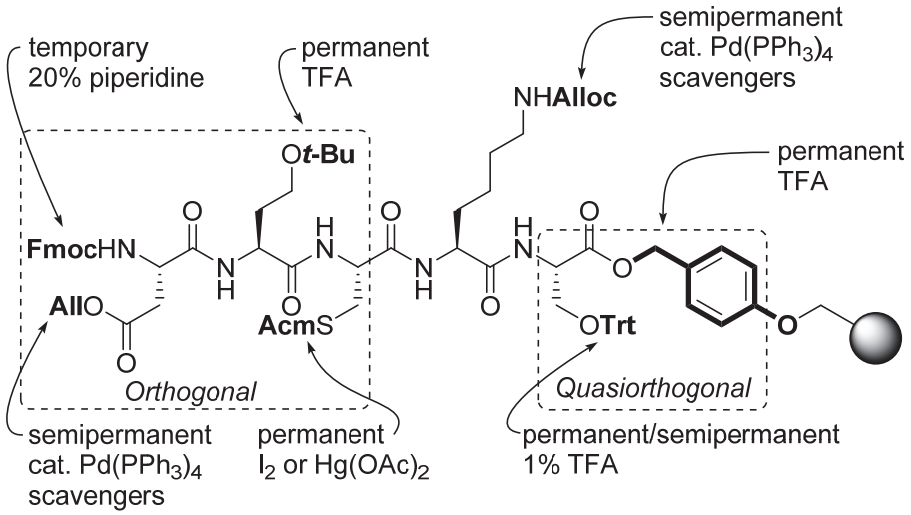
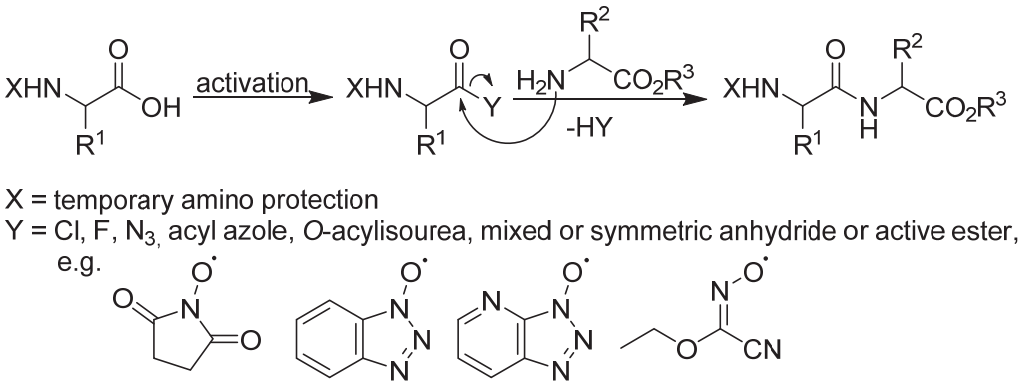


Figure 5. An imaginary example of Fmoc/t-butyl protection strategy with two additional orthogonal dimensions (Allyl and acetamidomethyl or Acm) on Wang linker.

1.6.2 Peptide Bond Formation

A peptide or amide bond is normally formed by a so called coupling reaction between carboxylic acid and amine (**Scheme 5**). This unification does not occur without activation at room temperature and high temperature required for direct condensation reaction between the two would often lead to an undesired product mixture. There are numerous peptide coupling reagents and methods in use[130,136,137], starting from acid chlorides by Fischer[138].



Scheme 5. Peptide coupling reaction.

Active ester method using coupling reagents (for the sake of readability the full names of the coupling reagents are only in the Abbreviations section) like DCC/HOBt[139,140] or HBTU[141] is currently the most popular. Among the wide variety of coupling reagents, HATU[142] has been considered to be the most efficient, while there are currently several comparable alternatives like COMU[143], HCTU[144], HDMC[145], and PyClock[146,147].

During the activation and coupling reaction, there is a major risk of racemization of the activated entering amino acid or peptide.[130] The racemization takes place base catalytically by direct enolization or through 5(4*H*)-oxazolone formation. For amino acids, the α-amino protecting group has an influence. Carbamates are less prone to form oxazolones than acyl groups and protecting groups with electron-withdrawing moieties increase enolization. The base used in coupling reaction also influences the racemization. Thus, using collidine, 2,3,5,6-tetramethylpyridine or octahydroacridine instead of ethyldiisopropylamine (DIEA) or *N*-methylmorpholine may facilitate to suppress racemization.[148,149] König and Geiger introduced in 1970 1-hydroxybenzotriazole (HOBt) additive for efficient racemization suppression.[139] Later Carpino introduced an even better, although expensive, suppressant 1-hydroxy-7-azabenzotriazole (HOAt).[142] Because HOBt and especially HOAt have explosive properties which may restrict their transportation, 2-cyano-2-(hydroxyimino)acetate (Oxyma) has been introduced.[150] Oxyma has similar or better coupling efficiency and ability to suppress racemization than HOAt or HOBt. The majority of the currently used coupling reagents are based on these suppressants or 6-chloro-1-hydroxybenzotriazole[151] which is a cheaper alternative to HOAt.

Acid halides have long had only limited use in peptide chemistry, because of the hydrolytic instability of acid chlorides and frequent side reactions (e.g. racemization and cleavage of protecting groups). During last two decades they have gained some popularity, much due to the work of Carpino and his group.[152] Acid halides are especially suitable for peptide coupling reactions of extremely hindered amino acids or couplings

with weak amino nucleophiles.[153,154] In situ activation using triphosgene (BTC)[155,156] or tetramethylfluoroformamidinium hexafluorophosphate (TFFH)[157] helps to tackle stability and racemization issues. On the other hand, acid fluorides do not suffer from many of the problems related to acid chlorides. They are shelf stable, they are compatible with most of the protecting groups and because they do not form readily oxazolones, couplings do not suffer from racemization.

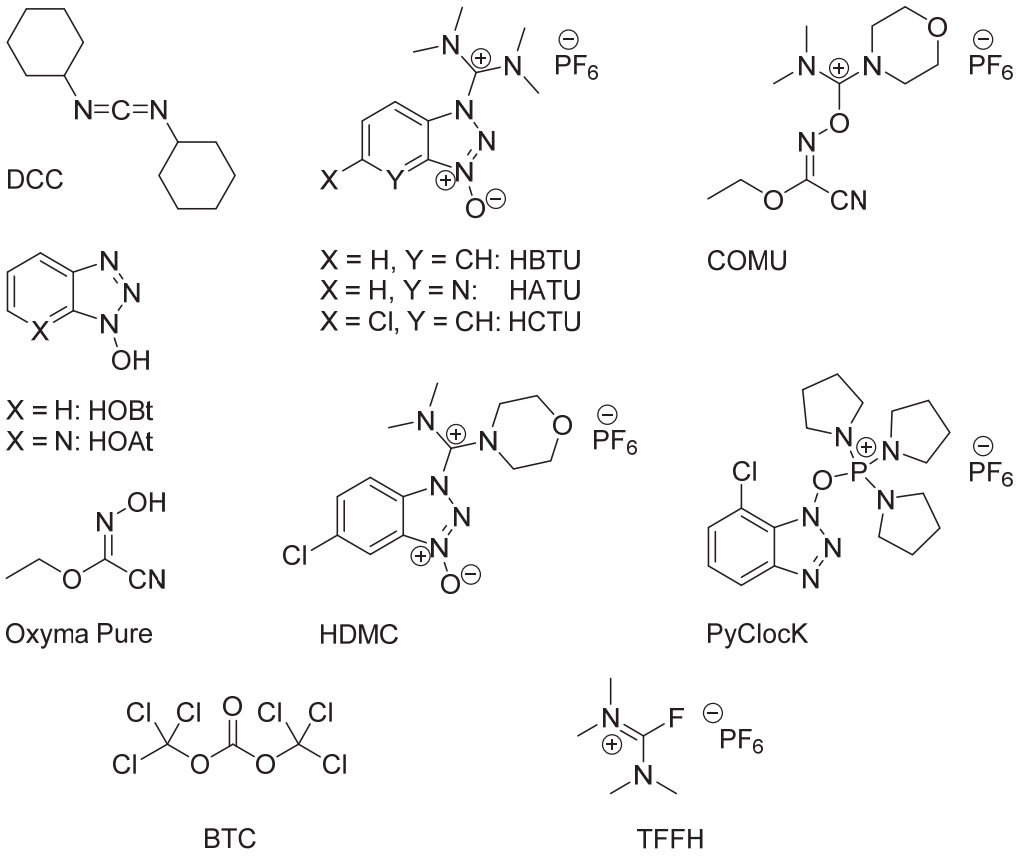


Figure 6. Some commonly used (DCC/HOBt, HOAt or Oxyma Pure, HBTU) and most efficient (HATU, HCTU, COMU, HDMC, PyClock, BTC, TFFH) coupling reagents.

1.6.3 Peptide Synthesizers

A variety of peptide synthesizers from micromole to industrial manufacturing scale are commercially available.[158] There are single channel instruments and instruments capable of parallel synthesis.[159] Especially the latter are often based on a robotic platform. Some of the modern peptide synthesizers can use microwave heating to facilitate the synthesis.[160] In batch reactors, the support is in a vessel that is agitated during the reaction. Addition of solvents and reagents occur either by applying a positive pressure on

the solvent or reagent flask for a preset time or by volumetric dispensing using digital injection pumps. In a batch reactor synthesis proceeds in discontinuous fashion: reagents are added, reaction takes place, reagents are removed and resin washed. By contrast, in continuous flow system, the fluid flows through the column continuously and the reactant mixture can be recirculated until the reaction is ready. Because only part of the support is in contact with the concentrated reagent at a moment, smaller amounts of reagents are needed. The deprotection and acylation reactions can be continuously followed for example by UV absorbance of the effluent flow in Fmoc/*t*-Bu method. Currently batch synthesizers are considerably more popular than continuous flow synthesizers.

1.6.4 Cyclic Peptides

Cyclization is a general way to increase the conformational constrain to otherwise quite flexible peptide chain.[161–163] It may lead to greater conformational integrity, increased agonist or antagonist potency, prolonged biological activity, increased enzymatic stability, increased specificity for a particular receptor[164] or increased cell-permeability[165]. The biological activities that have been utilized include, for example, antibacterial, immunosuppressive, and anti-tumour activities.[166] Many natural cyclic peptide hormones, such as calcitonin, oxytocin, somatostatin and vasopressin, are cyclic. Although macrocycles are promising drug molecules they have been relatively little exploited because they don't fulfil the Lipinski rule of 5 of drug-likeness[167] and their synthesis and screening has been considered challenging.[168] Currently highly stable cyclotides[169] and lasso peptides[170] are considered as promising templates for drug design. An important application of peptide cyclization is conformational studies that include explorations of protein folding and determination of biologically active conformations of peptides.[162–164] During the decades of peptide synthesis, several reviews of synthetic methods to prepare cyclic peptides have been published. [163,171–177]

1.7 Solid-Phase Organic Synthesis and Combinatorial Chemistry

Combinatorial chemistry is a technique to synthesize rapidly a large set of compounds, i.e. a library, under similar reaction conditions from a set of building blocks.[178] Typically the library is prepared on synthesizers which contain an array of reactors. The syntheses can be performed either in parallel fashion to obtain discrete compounds in separate reaction vessels or by so-called split-pool method pioneered by Furka,[22,179] to obtain product mixtures. Initially the combinatorial chemistry libraries consisted of peptides, but the publication of Ellman's benzodiazepine library[21] showed that synthesis of small molecule combinatorial libraries could be adapted to a solid support. After the successful application to combinatorial chemistry, the solid-phase organic synthesis started to stimulate wider interest although it had been studied already from the '60s. Its initial dominance in combinatorial chemistry, measured by published articles, was overtaken by solution phase synthesis after a decade.[180] A large portion of organic

transformations were adapted to solid support already by the end of '90s[181–185] and complex natural product derived libraries were developed[186–188]. Although majority of transformations were between carbon and heteroatom, many of the carbon–carbon bond forming reactions important for synthetic organic chemistry were also transferred to a solid support.[189,190] Currently, even radical reactions have been utilized on a solid support.[191]

1.7.1 Purine Based Compounds in Medicinal Chemistry

Purine is the most abundant N-heterocycle in the nature. Besides being a constituent of DNA and RNA, natural purine derivatives have a very wide range of biological functions.[192] Approximately 4–7% of all proteins encoded by the genome depend on purine nucleotides as co-factors or co-substrates for their function.[193] These include G-protein coupled receptors and protein kinases which comprise the two most studied drug target classes currently.[194,195] Figure **7** summarizes the potential applications of purine based drugs. Apart from being ubiquitous in nature, purine bicycle with its seven sites for substitution and well-established chemistry lends itself well for chemical manipulation.[193] Thus, it is an ideal scaffold for chemical library synthesis.

A large and diverse family of G-protein coupled receptors (GPCRs) consists of transmembrane receptors that sense ligands outside the cell and activate G-protein inside the cell. G-protein activates then the corresponding inside signal transduction pathways and thus cellular responses. Adenosine receptors constitute a class of GPCRs that are activated by adenosine which is a major local regulator of tissue function.[196] The effects of activation tend to be cytoprotective and aim at adapting the tissue or organ to a particular physiological stress condition taking place (e.g. hypoxia). Adenosine receptor drugs are being developed, for example, against neurological disorders, autoimmune diseases, chronic pulmonary diseases, asthma, and cardiovascular disorders.[196–198] Selective antagonists and agonists for all the four human adenosine receptor subtypes have been developed. Currently only one selective adenosine receptor drug, namely Lexiscan (Regadenoson) for radionuclide myocardial perfusion imaging, has been approved for clinical use but many others are undergoing clinical trials.[196]

Protein kinases (PK) mediate and regulate the majority of the signal transduction in cells. The 538 human protein kinases control most of the important biological processes which makes them a very attractive target for drug development.[199] The short of twenty regulatory approved PK inhibitor drugs are mostly used in treatment of different cancers. PKs exert their function by catalysing the transfer of the terminal phosphate group of ATP to the hydroxyl group of serine, threonine or tyrosine residue of their protein substrate. The intracellular ATP concentration is high and the ATP-binding site is highly conserved among protein kinases, which makes discovery of selective and potent PK inhibitors difficult. There are less conserved areas in the ATP

binding pocket that do not participate in ATP recognition, and this can be used to increase the selectivity. In addition, inhibitors that bind the inactive conformation of PK or bind outside ATP-pocket have been developed to tackle the problem.[200] Although majority of the known kinase inhibitors are ATP-competitive, purine or xanthine core is relatively infrequently utilized.

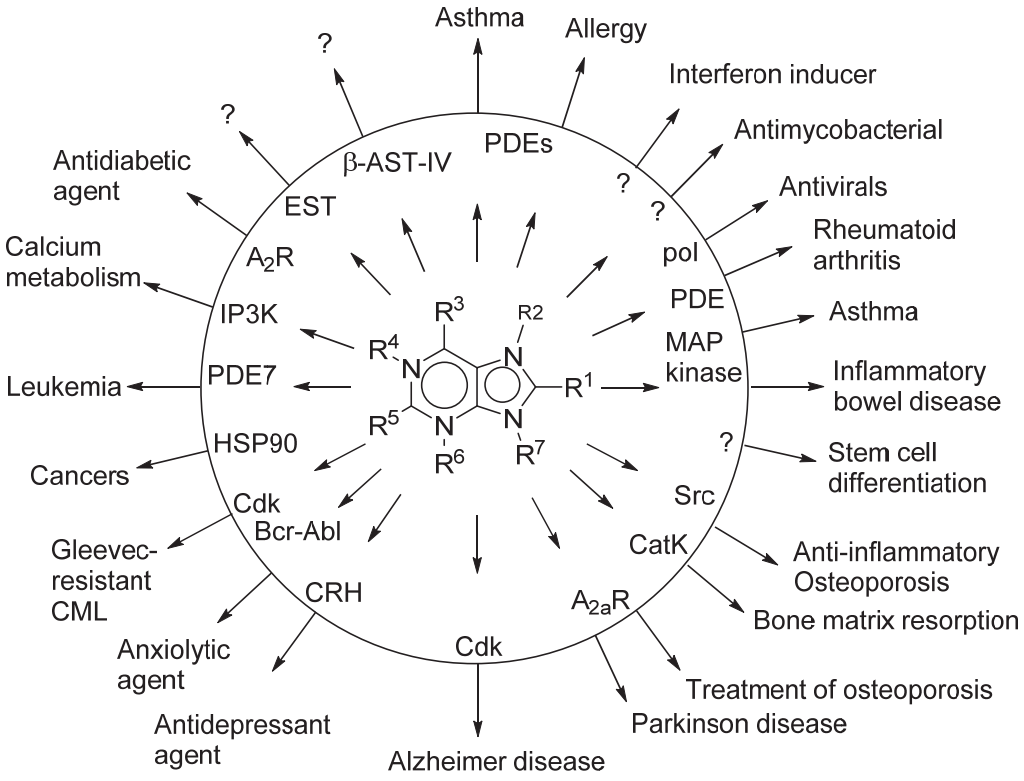


Figure 7. Potential applications of purine derivatives. Combinations of various substituents (1, 2, 3, 4…) on the different positions (R^1–R^7) of the purine ring lead to inhibitors of the different primary targets located inside the circle (PDEs, phosphodiesterase; pol, DNA or RNA polymerase; Src, Src tyrosine kinase; CatK, cathepsin K = cysteine protease; $A_{2A}R$, adenosine receptorA_{2A}; Cdk, cyclin-dependent kinase; CRH, corticotropin-releasing hormone; HSP90, heat-shock protein-90; IP3K, inositol-1,4,5-triphosphate-3-kinase; EST, oestrogen sulfotransferase; β-AST, β-arylsulfotransferase). Some potential therapeutic applications are noted at the outside of the circle (CML, chronic myeloid leukaemia). The question mark indicates that either the primary target or the possible application(s) is (are) not known. The figure is reworked from reference[193].

2. Aims of the Thesis

The studies included in this thesis have their origins in the national Tekes (the Finnish Funding Agency for Technology and Innovation) Drug 2000 technology program. The objectives of the subproject was to i) develop solid-supported methods to produce libraries of bicyclic peptides, bioconjugates, and biimidazoles, ii) prepare the libraries, and iii) study the use of bicyclic peptides as artificial receptors or drug carrier molecules and biimidazoles as protein kinase inhibitors and finally to transfer the research results to designated pharmaceutical companies. The biimidazole library method development spawned additionally synthesis methods of 7-substituted 3*H*-imidazo[2,1-*i*]purines and 7-substituted 3-*β*-D-ribofuranosyl-3*H*-imidazo[2,1-*i*]purines that were targeted towards adenosine receptors. In addition to studies included here, the research group focused on solid-supported synthesis of various branched[201] or cyclic[202–204] peptide structures and peptide[205,206] or oligonucleotide[207,208] glycoconjugates.

3. Results and Discussion

3.1 Bicyclic Peptides with Three Parallel Peptide Chains (II)

The underlying idea of the cyclic peptides discussed here was that they contained three dissimilar parallel peptide chains and that the peptides were homodetic, i.e. all covalent linkages between the amino acids are peptide bonds. This meant that two branching units to cap the amino and carboxy termini of the chains were needed. Moreover, because the synthesis was to take place on a solid support and a possibility for further conjugation was required, a functional group for anchoring or conjugation was needed in one of the branching units. Hence, orthogonally protected α,α-bis(aminomethyl)-*β*-alanine (**1**, **Figure 8**) and *N*-succinyliminodiacetic acid (**2**) were selected for the purpose and synthesized. Although **1** is chiral, no attempt to separate the enantiomers or to devise a route that produced optically pure compound was taken. Instead, it was thought that having both enantiomers present gave a better chance for success in the second cyclization because the three dimensional structure of the monocyclic precursors were unknown.

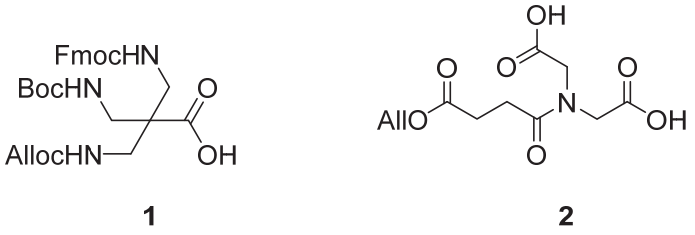
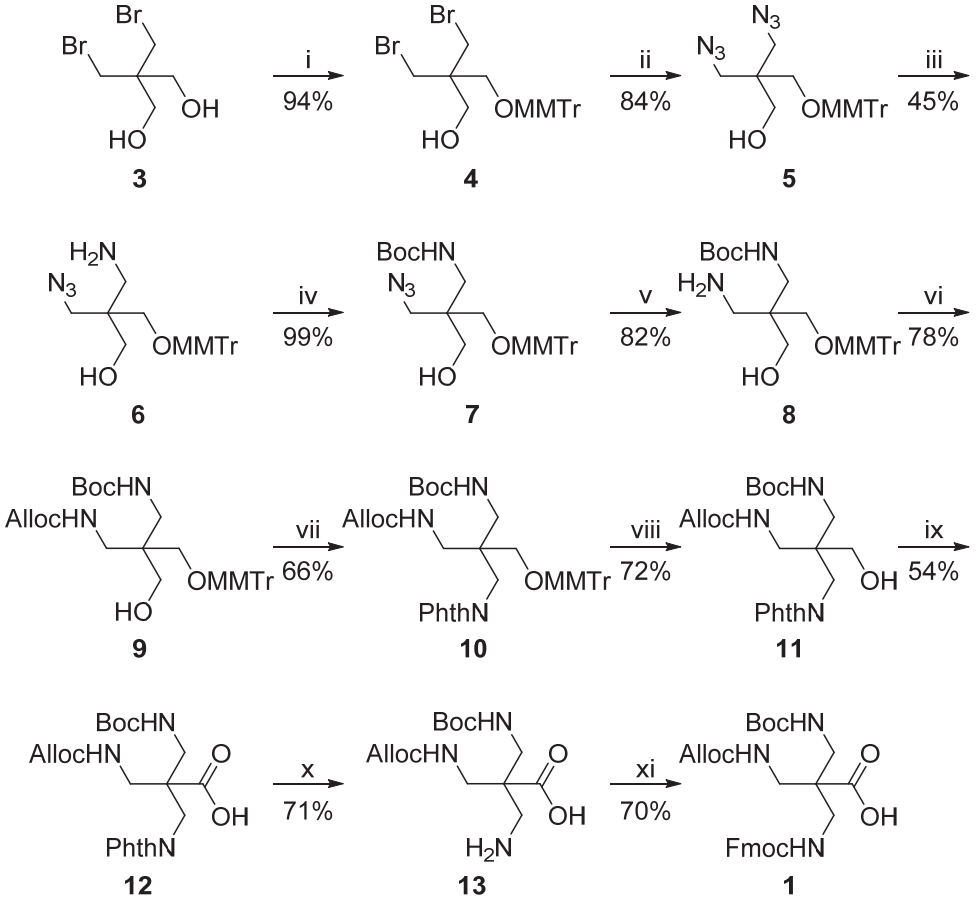


Figure 8. Branching units used in the synthesis of bicyclic peptides.

3.1.1 Synthesis of the Orthogonally Protected Branching Unit 1 (I)

The orthogonally protected branching unit **1** (3-{[(allyloxy)carbonyl]amino}-2-({[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}methyl)-2-({[(*tert*-butoxy)carbonyl]amino}-methyl)propanoic acid) was initially synthesized in low yield by a fifteen step time consuming route from pentaerythritol. After several different approaches based on different starting materials, a somewhat shorter and much faster route was devised (**Scheme 6**). The branching unit was synthesized from 2,2-bis(bromomethyl)-1,3-propanediol (**3**) in eleven steps in about three per cent overall yield. One of the hydroxyl groups of the starting material was first 4-methoxytritylated (MMTr). Only half equivalent of 4-methoxytrityl chloride was used to maximize the yield of the monoprotected product. Nucleophilic substitution of the bromo substituents with azide ion gave bis(azidomethyl) compound **5** which was reduced to **6** with sodium borohydride (1.2 equiv) and 1,3-propanedithiol (0.1 equiv)[209]. This selective reduction of only one azido group was the key step of the synthesis. Because longer reaction times or higher excess of reducing agents gave product mixtures, the reaction was stopped after 45% formation of the monoamino product. The starting material was recycled. After Boc protection of the amino group, the remaining azido group was subjected to Staudinger

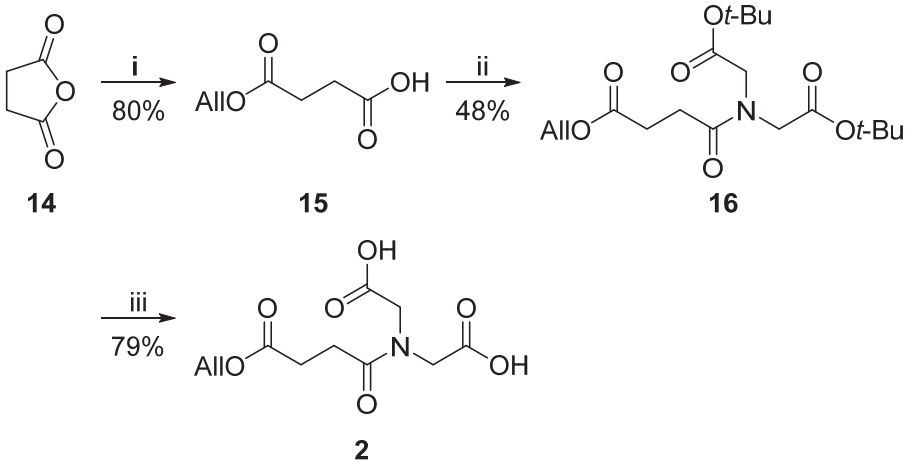
reduction[210] and the formed amino group was protected with allyloxycarbonyl (Alloc) chloride giving **9**. The free hydroxyl function was replaced with phthalimidoyl group by Mitsunobu reaction[211,212]. Removal of the MMTr group under mild conditions by 1% iodine in methanol[213] exposed the second hydroxyl function, which was oxidized in moderate yield to carboxylic acid (**12**) with Jones reagent[214]. In early studies, compound **12** was used as the branching unit in the SPPS. The removal of phthaloyl protection on solid support[215] was, however, slow, requiring elevated temperatures and in some cases reiterations. Thus, a more readily removable Fmoc protection was introduced. Phthaloyl protection was removed by hydrazinolysis in the presence of allyl alcohol to protect the Alloc group,[216] and the Fmoc group was introduced to yield **1**.



Scheme 6. Synthesis of *N*-Alloc-*N′*-Fmoc-*N″*-Boc-α,α-bis(aminomethyl)-*β*-alanine from 2,2-bis(bromomethyl)-1,3-propanediol. Reagents and conditions: i) MMTrCl, pyridine, ii) NaN_3, LiCl, DMF, pyridine, iii) $NaBH_4$, $HS(CH_2)_3SH$, NEt_3, *i*PrOH, iv) Boc_2O, NaOH, MeCN, v) PPh_3, NH_3, aq, vi) AllocCl, NEt_3, dioxane, vii) phthalimide, PPh_3, DEAD, THF, viii) I_2, MeOH, DCM, ix) CrO_3, H_2SO_4, H_2O, acetone, x) NH_2NH_2, allyl alcohol, DMF, dioxane, xi) FmocCl, K_2CO_3, H_2O, MeCN.

3.1.2 Synthesis of the Orthogonally Protected Branching Unit 2

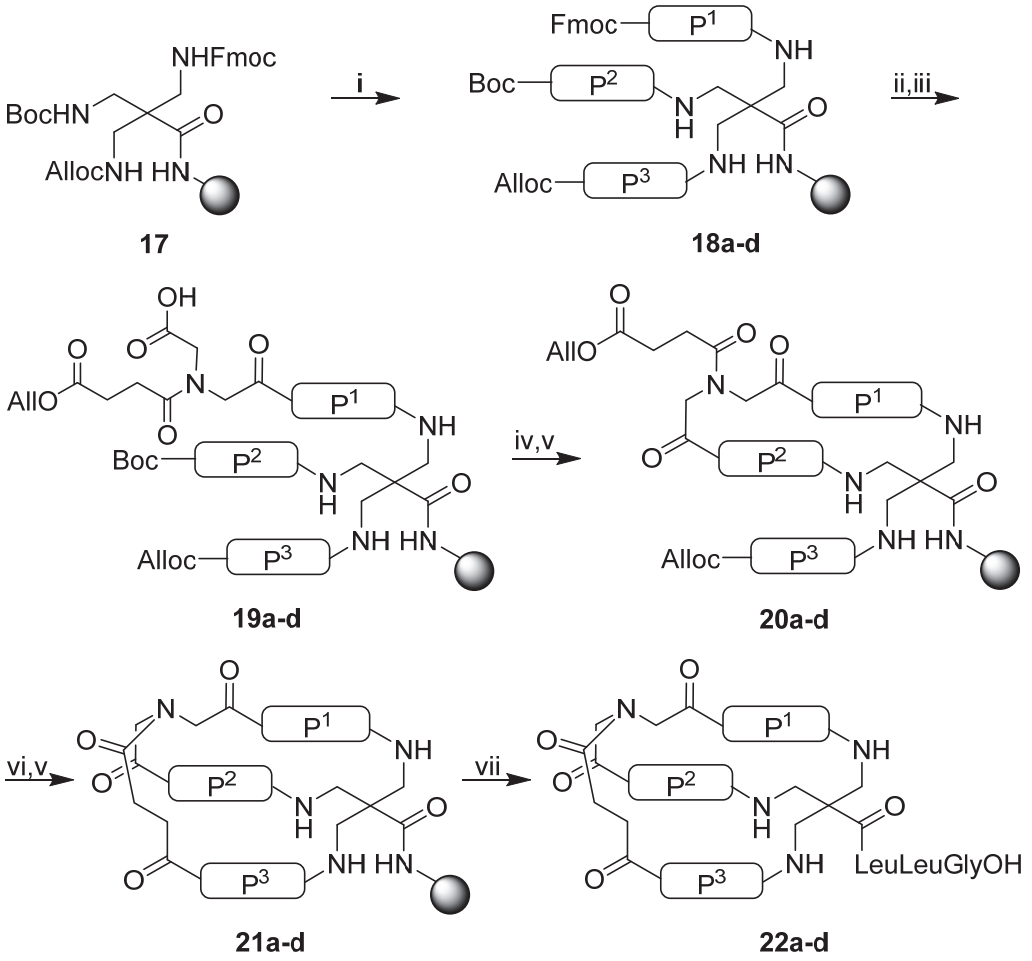
Synthesis of the second branching unit *N*-(4-allyloxy-4-oxobutanoyl)iminodiacetic acid **2** was more straightforward. Because **2** was to be coupled to the peptide as a cyclic anhydride, only one protecting group, namely allyl ester, was required. Accordingly, succinic anhydride was monoallylated in allyl alcohol–pyridine mixture giving **15** (**Scheme 7**). Di-*tert*-butyl iminodiacetate and **15** were condensed by HOAt/DCC activation. In the third step, *tert*-butyl protections were removed with TFA giving **2**.



Scheme 7. Synthesis of *N*-(4-allyloxy-4-oxobutanoyl)iminodiacetic acid **2**. Reagents and conditions: i) allyl alcohol, pyridine, ii) HOAt, DCC, di-*tert*-butyl iminodiacetate, pyridine, DMF, iii) TFA:DCM 2:1.

3.1.3 Synthesis of the Bicyclic Peptides

Hydroxymethyl polystyrene was first derivatized with an H-Leu-Leu-Gly-OH spacer.[217] Accordingly, glycine was attached to the resin by the symmetrical anhydride method, and the chain was elongated either manually or on a peptide synthesizer. The branching unit **1** was then coupled by HATU/DIEA activation to obtain tetrapeptide **17** (**Scheme 8**) bearing three orthogonally protected amino groups. The protections were removed one at a time and the corresponding peptide chains were constructed either by coupling of dipeptide segments (**18a** and **18b**) or by stepwise synthesis (**18c** and **18d**) In the latter case**,** the chains on the Fmoc and Boc protected amino groups were assembled by stepwise coupling using Fmoc and Boc protected amino acids, respectively. The third, Alloc protected Phe-β-Ala branch, was introduced as a segment in all peptides. Both approaches gave satisfactory coupling yields. The second branching unit (**2**) was converted to a cyclic anhydride with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) activation in DMF and coupled to the deprotected peptide chains P^1. After the Boc protection was removed from the chains P^2, the supported peptides were cyclized by HATU/DIEA activation in 5 hours. Because **2** is not chiral, there is no risk of epimerization even during such a slow cyclization reactions.[218]



Entry	P^1	P^2	P^3
18–22a	β-Ala-Gly	Gly-β-Ala	Phe-β-Ala
18–22b	Ser-β-Ala	Gly-β-Ala	Phe-β-Ala
18–22c	Ala-Gly	Asn-Gly	Phe-β-Ala
18–22d	Gly-Ala-Gly	Gly-Asn-Gly	Phe-β-Ala

= LeuLeuGly-hydroxymethyl-polystyrene

Scheme 8. Synthesis of bicyclic peptides. Reagents and conditions: i) SPPS, ii) piperidine, DMF, iii) *N*-(4-allyloxy-4-oxobutanoyl)iminodiacetic anhydride, DMF, iv) TFA, DCM, v) HATU or PyBOP, DIEA, DMF, vi) $Pd(OAc)_2$, PPh_3, Bu_3SnH, AcOH, DCM, vii) 30% HBr/AcOH, thioanisole, pentamethylbenzene, TFA

While the cyclizations of **19a** and **19b,** containing a flexible β-alanine residue in both of the participating peptide chains, proceeded well, the corresponding reaction with **19c** and **19d** consisting only of α-amino acids yielded a more complex product mixtures. The Alloc and allyl ester protecting groups were removed simultaneously from the monocyclic peptides using the palladium-tributyltin hydride chemistry.[219] The second cyclization of compounds **20a** and **20b** was then carried out by the HATU/DIEA chemistry (16 h) as before. By contrast, a phosphonium coupling reagent (PyBOP[220]) was used for the second cyclization of **21c** and **21d** because 1,1,3,3-tetramethylguanidino derivatives[221] could be detected among the side products. In addition, the solvent was changed from DMF to a 1:4 mixture of DMSO and NMP, and the reaction time was extended to 22 h. The cyclization reaction of **21d** was repeated since all the starting material had not disappeared. Despite these changes, the second cyclization reaction of **21c** and **21d** led to a complex reaction mixture and markedly decreased yield, probably due to polymerization on the support. Acid-catalysed cleavage from the support gave **22a**-**d** as free carboxylic acids.

All four peptides were obtained in pure state by HPLC, but in low yield. Only the most flexible peptide **22a** was obtained as a reasonably pure crude product. The compound appeared as a pair of diastereomers that were separated by HPLC (**Figure 9**). Isomerism originates from the racemic chiral branching unit **1**. Diastereomers of peptides **22b–d** could not be separated with the HPLC system used. In addition, two unsuccessful attempts to synthesize more rigid bicyclic peptides having a smaller ring size and no β-alanine residues were made. Accordingly, the described methodology allows the preparation of some homodetic bicyclic peptides on a solid support, but its applicability to library synthesis is severely limited.

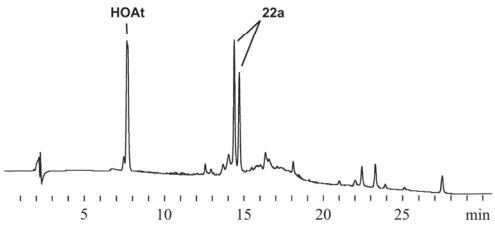
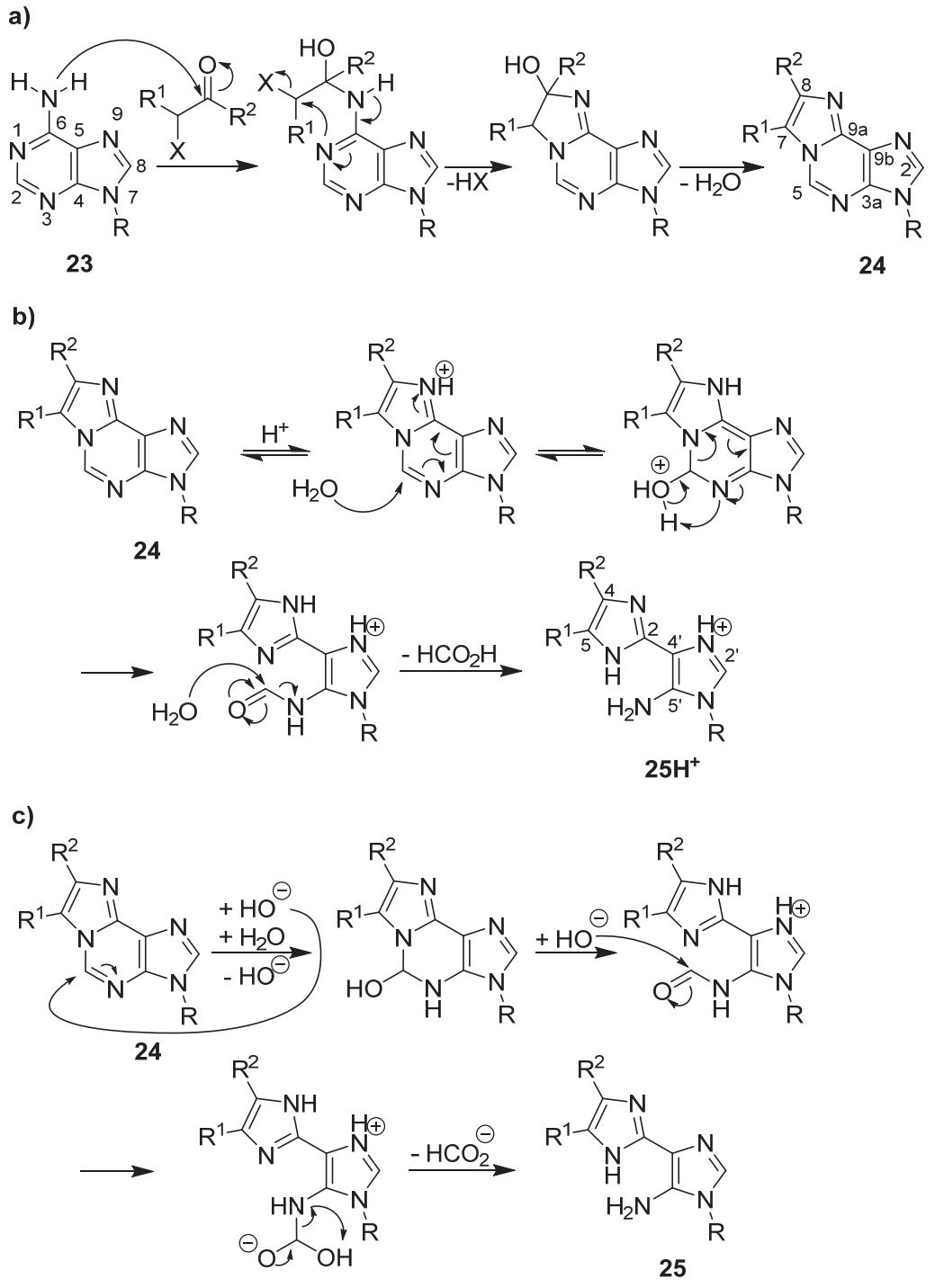


Figure 9. HPLC trace of the crude diastereomeric pair of **22a** cleaved from the resin (RP HPLC 0–100% acetonitrile, 0.1% TFA, λ = 215 nm).

3.2 7-Substituted 3*H*-Imidazo[2,1-*i*]purines (III)

The amidine-like moiety of adenine derivatives is known to undergo a cyclization reaction with α-halocarbonyl compounds (**Scheme 9**).[222–228] The reaction forms ring-extended 3*H*-imidazo[2,1-*i*]purines (**24**) which are also called 1,N^6-etheno adducts.



Scheme 9. Proposed mechanisms of cyclization of adenine derivatives with α-halocarbonyl compounds[229] (**a**) and intervening pyrimidine ring acid[230] (**b**) or base[231] (**c**) catalysed hydrolysis.

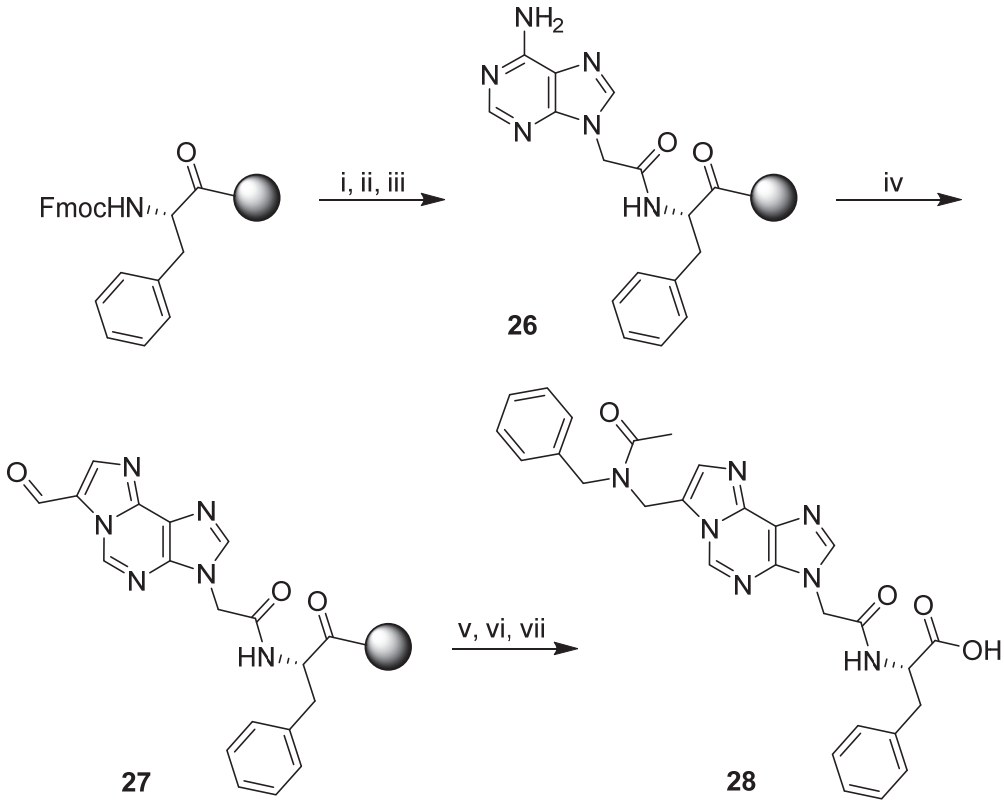
This bridging makes the intervening pyrimidine ring susceptible to acid[232] or base[233] hydrolysis which converts 3*H*-imidazo[2,1-*i*]purines to 5′-amino-2,4′-biimidazoles (**25**). These reactions have been utilized, for example, for the synthesis of fluorescent adenine derivatives[234] and 2-deuterated[235] or 2-substituted[236] adenine derivatives when combined with pyrimidine or 1,2,3-triazine ring closing[233] and etheno adduct removal[237]. The synthesis of biimidazole library was planned to utilize these techniques transferred to a solid support. Adaptation of the pyrimidine ring opening reaction to a mixed-phase reaction, however, turned out to be challenging. It was difficult to find reaction conditions that yielded sufficiently pure products. Therefore, it was decided to concentrate first on preparation of 3*H*-imidazo[2,1-*i*]purines which are by themselves interesting molecules for drug discovery.

Bromomalonaldehyde[238] is known to react with adenine derivatives in aqueous and organic solvents yielding 1,N^6-formyletheno adducts.[224] In aqueous solvents, the reaction mainly produces 1,N^6-etheno adducts because of the competing hydrolytic deformylation that takes place before cyclization of the carbinolamine intermediate.[229] Under the most favourable conditions, when pH is below 3.5, the two products are formed in about equimolar ratio. Then again in DMF, low yields have been earlier reported.[239] To optimize the reaction and the subsequent reductive amination on a polystyrene solid support, *N*-[(adenin-9-yl)acetyl]phenylalanine-Wang resin was chosen as the model polymer. Commercial N^{α}-Fmoc-phenylalanine-Wang resin was deprotected and loaded with [N^6-(4-methoxytrityl)adenin-9-yl]acetic acid[240] (**Scheme 10**). The 4-methoxytrityl protection was removed with 3% dichloroacetic acid in DCM. Thereafter, the polymer was neutralized with pyridine in DCM, washed and dried. The polymer was divided into portions and tested for the cyclization reaction under various conditions (**Table 4.**). In unbuffered DMF, the reaction proceeded slowly (entries 10, 15, 16, 19, and 20). During the first three hours, product was formed in 55% yield on using five equivalents of bromomalonaldehyde, but doubling the reaction time increased the yields only by few per cent. Similarly, doubling the concentration of bromomalonaldehyde did not much improve the situation. Basic conditions decelerated (entries 1–7, and 9) and moderately acidic conditions accelerated the reaction (entries 8, 11-14, 17, 18, 21, and 22). While addition of triethylamine to the reaction mixture was disadvantageous, 2,6-lutidine/formic acid mixture worked better. The reaction rate was relatively insensitive to the ratio of formic acid and 2,6-lutidine. Several different compositions of the mixture of formic acid and 2,6-lutidine has been successfully utilized since. For the synthesis of **27** 1:1 ratio was used.

Table 4. Optimization of formyletheno adduct formation on polystyrene in DMF at 60 °C using five equivalents of bromomalonaldehyde.

Entry	Buffer	Equiv.[a]	t /min.	Product[b] /%
1	Acetic acid / triethylamine	10/10	330	*14*
2	Formic acid / triethylamine	10/10	330	56
3	Formic acid / triethylamine	10/10	1320	*19*
4[c,d]	Formic acid / triethylamine	20/20	270	*0*
5[e]	Formic acid / triethylamine	20/20	270	45
6	Formic acid / triethylamine	28/10	270	32
7[f]	Formic acid / triethylamine	10/10	270	*18*
8	Formic acid / 2,6-lutidine	10/10	270	**95**
9	2,6-lutidine	0/10	65	47
10	—		65	51
11	Formic acid / 2,6-lutidine	50/10	65	53
12	Formic acid	50/ 0	65	55
13	Formic acid / 2,6-lutidine	100/10	65	68
14[g]	Formic acid / 2,6-lutidine	150/10	65	58
15	—		180	55
16[c]	—		180	64
17	Formic acid / 2,6-lutidine	30/10	180	**87**
18[c]	Formic acid / 2,6-lutidine	60/20	180	**92**
19	—		360	62
20[c]	—		360	68
21	Formic acid / 2,6-lutidine	30/10	360	**93**
22[c]	Formic acid / 2,6-lutidine	60/20	360	**92**

a) Compared to support bound adenine
b) Calculated from the HPLC chromatogram peak areas: A(product)/A(product)+A(starting material)×100%.
c) 10 equiv. of bromomalonaldehyde
d) Reaction at room temperature
e) 15 equiv. of bromomalonaldehyde added in three batches.
f) Solvent was DMSO/NMP 1/4.
g) According to mass spectrum of the reaction mixture, some product and starting material were cleaved during the reaction.



Scheme 10. Synthesis of the model 3*H*-imidazo[2,1-*i*]purine **28** for reaction optimization. Reagents and conditions: i) piperidine, DMF, ii) [N^6-(4-methoxytrityl)adenin-9-yl]acetic acid, TBTU, DIEA, DMF, iii) 3% dichloroacetic acid, DCM, iv) bromomalonaldehyde, HCO_2H, 2,6-lutidine, DMF, v) $BnNH_2$, $NaCNBH_3$, HCO_2H, DMF, MeOH, vi) Ac_2O, DCM, vii) 1:1 TFA, DCM.

Reductive amination of the support-bound 3*H*-imidazo[2,1-*i*]purine-7-carbaldehyde (**27**) with either sodium triacetoxyborohydride or sodium cyanoborohydride was slow when acetic acid was used as a catalyst. After 24 hours, 30% of **27** remained unreacted on using ten equivalents of benzylamine and sodium cyanoborohydride in DMF acidified with 4% acetic acid. The reaction was even slower on using corresponding amount of sodium triacetoxyborohydride. Replacing acetic acid with formic acid accelerated the amination so that it was completed in 5 hours. When amination with a sterically hindered amine, such as diisopropylamine or diethylamine, was attempted, the starting aldehyde persisted throughout the reaction. Reduction to 7-hydroxymethyl-3*H*-imidazo[2,1-*i*]purine was not detected.[241,242] Sodium cyanoborohydride in DMF containing 4% formic acid and 6% methanol was, hence, used for the subsequent reductive aminations. The secondary amines obtained by reductive amination may react further with another carbonyl compound forming bisalkylated products.[241] To prevent this

side-reaction, excess of amine and a sufficient pre-equilibration for rate-determing imminium formation can be used. Although 10 equivalents of benzylamine and a short 30 minutes reaction time for imine formation prior to sodium cyanoborohydride addition were used, about 6% of bisalkylated product was formed through an intraresin reaction.

After suitable reaction conditions for the solid-phase synthesis of the model compound **28** were found, a test synthesis was performed. No further adjustments to reaction conditions were made except that the reaction time for the ring forming reaction was 4 hours. Solid-supported *N*-benzyl(imidazo[2,1-*i*]purin-7-yl)methylamine moiety was acetylated with acetic anhydride in DCM. Acidolytic release from the support with 50% TFA in DCM gave *N*-{[7-(*N*-benzylacetamidomethyl)imidazo[2,1-*i*]purin-3-yl]acetyl}phenylalanine (**28**) in 29% overall yield after HPLC purification. Figure **10** shows the HPLC trace of the crude product mixture. The trace shows that the reactions, except acetylation, were not completed and, aside from bisalkylated product formation during reductive amination, no significant side-reactions were present.

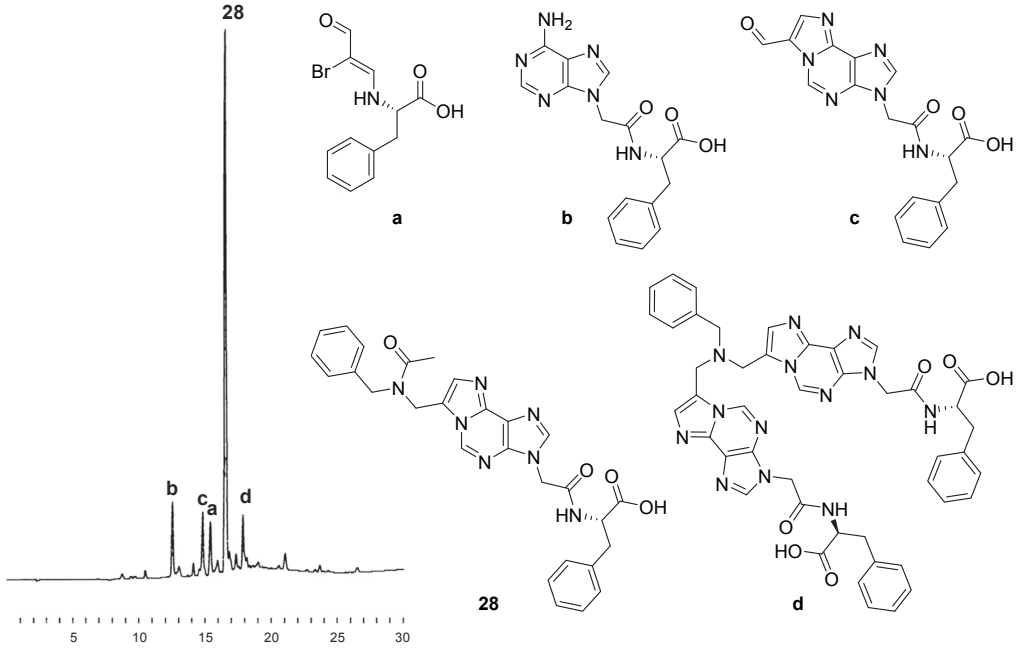
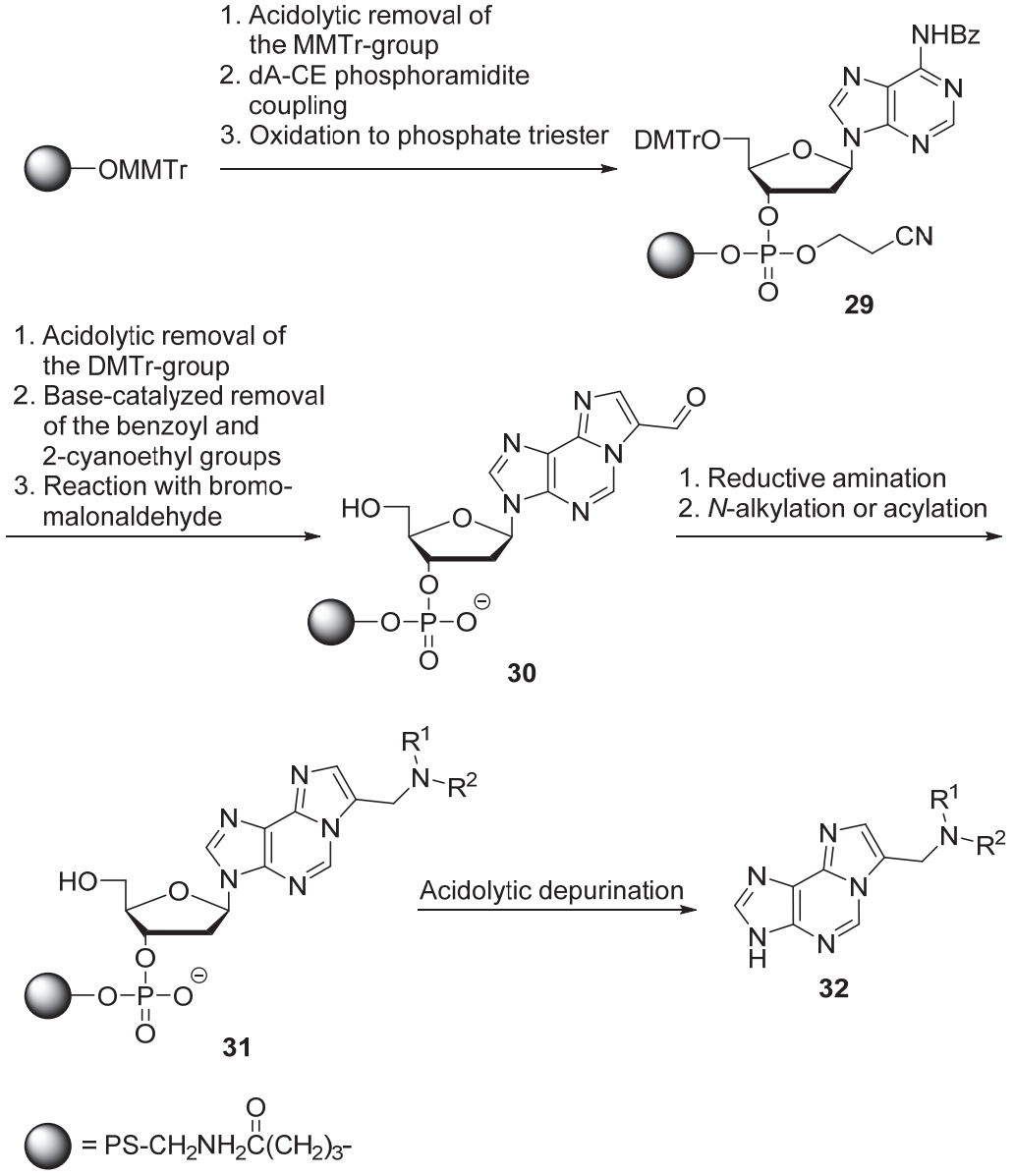


Figure 10. HPLC trace of crude *N*-{[7-(*N*-benzylacetamidomethyl)imidazo[2,1-*i*]purin-3-yl]acetyl}phenylalanine (**28**) (RP HPLC, 0–100% acetonitrile, 0.1% TFA, λ = 220 nm). The products were identified mass spectrometrically.

A small library of (imidazo[2,1-*i*]purin-7-yl)methylamines **32** was then synthesized on a solid-support utilizing the method described above (Scheme **11**). Commercial deoxyadenosine phosphoramidite building block was attached via the 3′-phosphate group to

the support. Unlike the stable phosphodiester bond, the *N*-glycosidic bond between 2′-deoxyribose and ethenoadenosine ring system can be cleaved by relatively mild acid treatment, i.e. with 5% TFA in DCM in one hour. The *N*-glycosidic bond nevertheless withstands the formic acid treatments applied to generate the additional imidazole ring and subsequent reductive amination of the formyl group.



Scheme 11. Synthesis of *N*-substituted (imidazo[2,1-*i*]purin-7-yl)methylamines.

A (4-methoxytrityloxy)butyric acid spacer was first attached to aminomethylpolystyrene resin using diisopropylcarbodiimide (DIC)/HOBt activation. After acidolytic deprotection, N^6-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine 3′-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) was coupled to the exposed hydroxy functions by 4,5-dicyanoimidazole activation and oxidized to a phosphate triester (**29**) with aqueous iodine. The 4,4′-dimethoxytrityl group was removed with 3% dichloroacetic acid in DCM to avoid the risk that partly detaching dimethoxytrityl cation could be engaged in some side-reaction during the following synthesis steps. The *N*-benzoyl and *O*-(2-cyanoethyl) protections were cleaved by refluxing the resin in THF containing aqueous sodium hydroxide. The exposed 5′-hydroxy and phosphodiester groups did not disturb the subsequent reactions. The deprotected support-bound 2′-deoxyadenosine was then treated with bromomalonaldehyde under the conditions described in the foregoing. The desired support-bound 3-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[2,1-*i*]purine-7-carbaldeyde (**30**) was formed in an almost quantitative yield, as seen from the HPLC trace of the acidolytically released crude product (**Figure 11**).

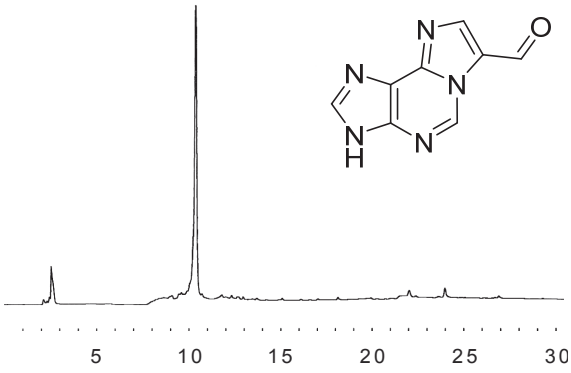


Figure 11. HPLC trace of crude 3*H*-imidazo[2,1-*i*]purine-7-carbaldehyde cleaved from the resin **30** (RP HPLC, 0–100% MeCN, 0.1% TFA, λ = 220 nm).

The scope of reductive amination of **30** was tested with a set of nine amines with varying steric hindrance or substituent inductive effects (**32a–32i**, **Figure 12**). It was observed from the HPLC traces that after one hour imine formation and subsequent four hours reduction step, aniline (**32a**) and unhindered primary amines (entries **32b**–**d**) had reacted well producing 84–100% product, while sterically hindered primary amines, *t*-butylamine and tris(hydroxymethyl)aminomethane (**32e**,**f**), gave only a 35% yield. Acyclic secondary amines, diethylamine and diisopropylamine (**32g**,**h**) did not react according to HPLC traces, although a clear product peak of **32g** was observed by MALDI-TOF mass spectrometry. By contrast, among cyclic secondary amines, piperidine (**32i**), reacted smoothly, giving a 73% yield. Benzylamine (**32b**) and *t*-butyl glycine acetate (**32d**) both gave additionally 4% of bisalkylated products. The method is

thus suitable for unhindered amines, but sterically more demanding amines give lower or non-existent yields.

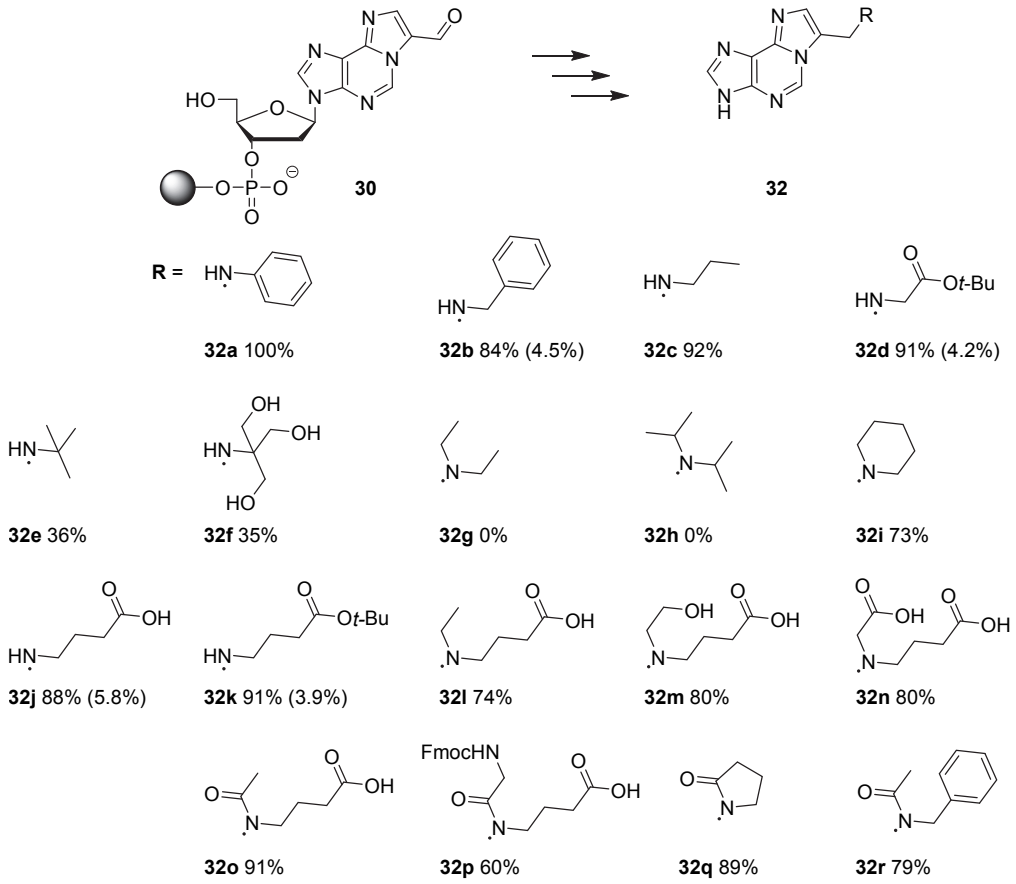


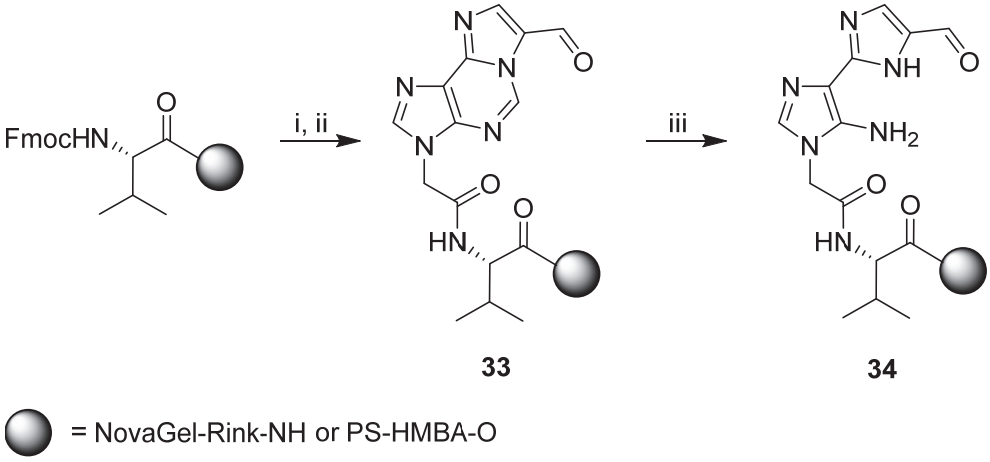
Figure 12. The synthesized library of (imidazo[2,1-*i*]purin-7-yl)methylamines **32**. The yields are calculated from RP HPLC chromatograms. Amount of bisalkylated products are in parentheses.

Support **30** was then aminated with 4-aminobutyric acid or 4-aminobutyric acid *t*-butyl ester. Besides the expected **32j** and **32k**, 4–6% of bisalkylated side products were formed. In case of 4-aminobutyric acid, 7% of a lactamized product was additionally obtained, in all likelihood by cyanoborohydride activation.[243-245] The lactam formation was verified by HPLC spiking and comparison of the mass spectra of the side product with 1-[(3*H*-imidazo[2,1-*i*]purin-7-yl)methyl]pyrrolidin-2-one (**32q**), synthesized by HBTU/DIEA mediated cyclization of **32j**.

Reductive alkylations of **32j** with acetaldehyde, glycolaldehyde dimer or glyoxylic acid monohydrate gave compounds **32l**–**n**. Sodium cyanoborohydride and formic acid were used like in the reductive aminations discussed above. With ten equivalents of aldehyde, three hours reaction time was sufficient to drive the reactions to completion. Compound **32k** was, in turn, acylated with acetic anhydride and with Fmoc protected glycine by TBTU/DIEA activation to obtain **32o** and **32p**, respectively. Both reactions were completed in one hour. Likewise, the above mentioned lactamization was completed in one hour. *N*-Benzyl-*N*-[(3*H*-imidazo[2,1-*i*]purin-7-yl)methyl]acetamide (**32r**) was synthesized as **28**, except that the reductive amination was repeated before the acylation.

3.3 4(5),1´,5´-Trisubstituted 2,4´-Biimidazoles (IV)

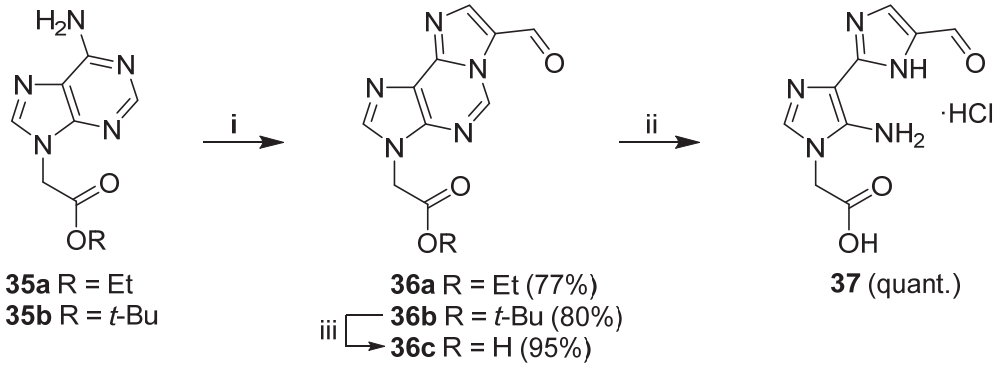
After the method for the synthesis of 7-substituted 3*H*-imidazo[2,1-*i*]purines was devised, the opening of the intervening pyrimidine ring was re-examined. Many problems in the base catalysed hydrolysis were encountered, the main ones being side-reactions, problems in isolating the product and most importantly the lack of reliable detection of the products formed. Shifting from MALDI-TOF to LC-ESI-Q mass spectrometry facilitated the studies so that methods for both base and acid catalysed hydrolysis on solid support were developed (**Scheme 12**).



Scheme 12. Intervening pyrimidine ring opening of 7-formyl 3*H*-imidazo[2,1-*i*]purine on a solid support. Reagents and conditions: i) piperidine, DMF, ii) 2-(7-formyl-3*H*-imidazo[2,1-*i*]purin-3-yl)acetic acid **36c**, TBTU or HATU, DIEA, DMF, iii) 0.08 mol/L NaOH, 1:1 H_2O/THF 55 °C for base hydrolysis or 3.6 mol/L HCl in dioxane, 10% H_2O for acid hydrolysis.

For the base catalysed hydrolysis, aminomethyl NovaGel resin was loaded with Rink amide linker giving a 0.5 mmol/g loading. It had been observed earlier that Wang linker did not withstand the conditions required for the hydrolysis. NovaGel was used be-

cause it has better swelling properties than polystyrene in the high water content solvent mixture used in the hydrolysis step. The resin was capped and derivatized consecutively with an amino acid (Fmoc-valine) and 2-(7-formyl-3*H*-imidazo[2,1-*i*]purin-3-yl)acetic acid (**36c**, **Scheme 13**). Coupling of **36c** turned out to be somewhat tricky. In spite of limited solubility, the compound could still be dissolved in a mixture of DMF and DIEA. After adding the coupling reagent, the active ester started to precipitate which was problematic for a solid-phase reaction. The precipitate filled the pores of the resin and clogged the frit of the synthesis column. Thus, the reaction rate was retarded and washing of the resin was difficult. Initially, TBTU was used as the coupling reagent, and it turned out to be advantageous to add the dissolved **36c** to the resin before the coupling reagent that was suspended in a small amount of DMF. Later, more efficient HATU was used instead of TBTU, although, very long reaction times were still needed. Extensive washing was required to remove the surplus reagents from the system. The hydrolysis step required overnight reaction at 55 °C when 0.08 mol/L sodium hydroxide in a 1:1 mixture (*v/v*) of THF and water was used.



Scheme 13. Synthesis of 2-(7-formyl-3*H*-imidazo[2,1-*i*]purin-3-yl)acetic acid (**36c**) and 2-(5′-amino-5-formyl-1*H*,1′*H*-2,4′-biimidazol-1′-yl)acetic acid (**37**) Reagents and conditions: i) $HCBr(CHO)_2$, HCO_2H, 2,6-lutidine, DMF, ii) HCl (aq.), iii) TFA/DCM

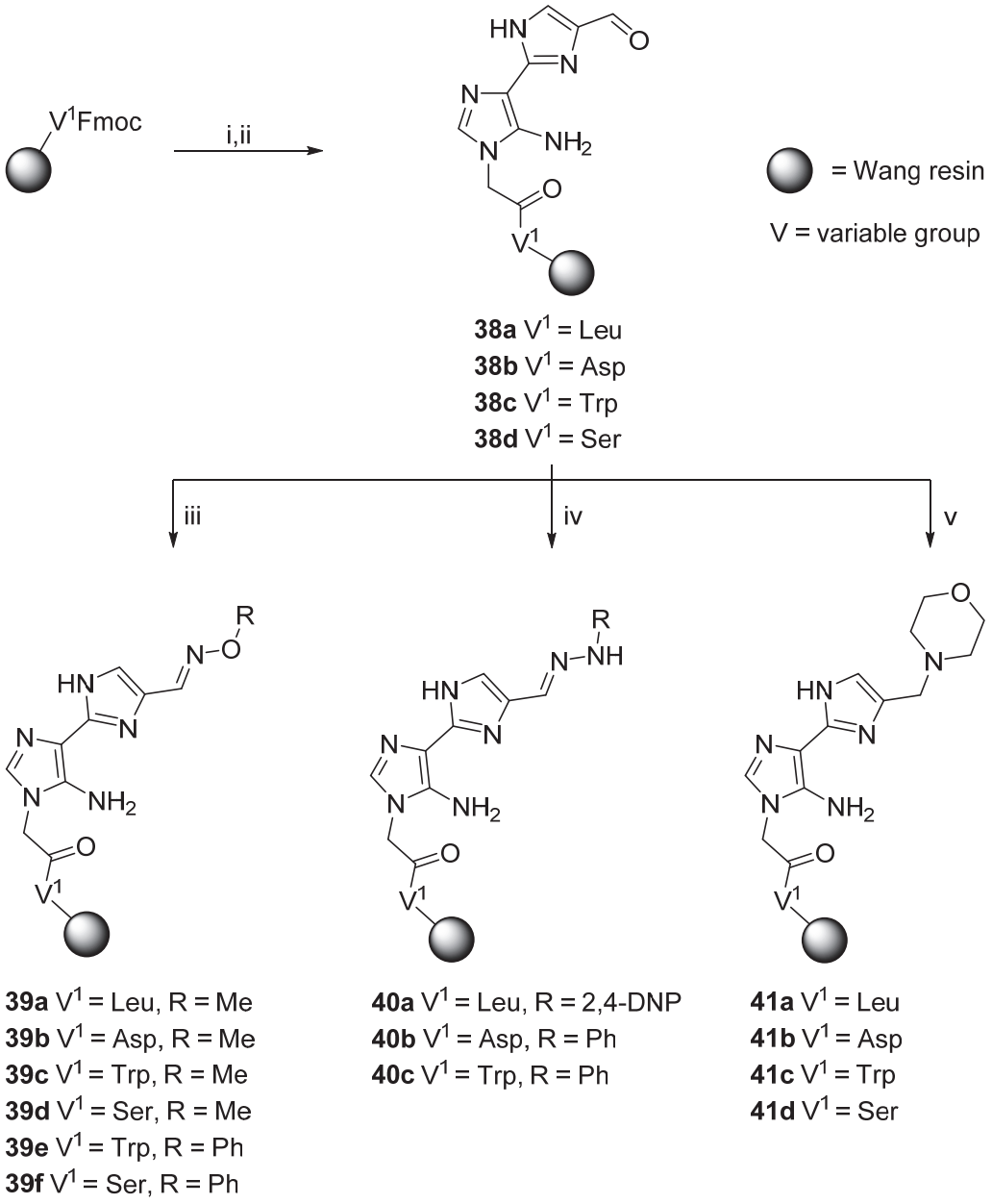
HMBA polystyrene resin was used for the acid-catalysed hydrolysis. The resin was derivatized in the same way as the Rink amide resin. The hydrolysis was carried out as an overnight reaction at room temperature using 3.2 mol/L HCl in a 9:1 mixture (*v/v*) of dioxane and water. The conditions were harsh enough to cleave part of the material from the resin during the hydrolytic ring-opening. While the solid-supported hydrolysis makes it possible to exploit 3*H*-imidazo[2,1-*i*]purines that have different substituents at 7 or 8 positions, we decided to concentrate on 7-formyl-3*H*-imidazo[2,1-*i*]purine in the library synthesis. Therefore, it was sensible to perform the pyrimidine ring hydrolysis in solution and couple the formed 2-(5′-amino-5-formyl-1*H*,1′*H*-2,4′-biimidazol-1′-yl)acetic acid (**37**, **Scheme 13**) to commercially available supports loaded with various amino acids. In this way, convergence kept the number of synthesis steps on support

lower and parallel synthesis of sub-libraries was facilitated. The core structure **37** of the library was synthesized in two-steps from ethyl 2-(adenin-9-yl)acetate (**35a**) and bromomalonaldehyde. The straightforward route did not require chromatographic purifications because both products of the route precipitated from the reaction mixture in a sufficiently pure state. Thus, *N*1 and *N*6 of the adenine moiety of **35a** (purine numbering) were bridged with a formyletheno group under the conditions developed for the solid support synthesis (see 3.2). The pyrimidine ring of the resulting 3*H*-imidazo[2,1-*i*]purine derivative (**36a**) was opened and the ethyl ester protection was removed simultaneously with 2 mol/L hydrochloric acid giving a white or yellowish hydrochloride product that precipitated from the solution. Attempted base-catalysed hydrolysis led to a dark brown product mixture.

A small sample library consisting of 22 members was then synthesized, in a parallel manner, on Wang polystyrene resins loaded with Fmoc protected amino acids. 2-(5′-Amino-5-formyl-1*H*,1′*H*-2,4′-biimidazol-1′-yl)acetic acid **37** was utilized as a trivalent scaffold. The carboxylic acid group was diversified first by attaching it to leucine, aspartic acid, tryptophan, or serine resin. The second diversification point was the formyl group which was derivatized either by oximation, hydrazone formation or reductive amination. The third point, 5′-amino group, was acylated. After syntheses the products were cleaved from the resin and purified by RP HPLC for the analysis.

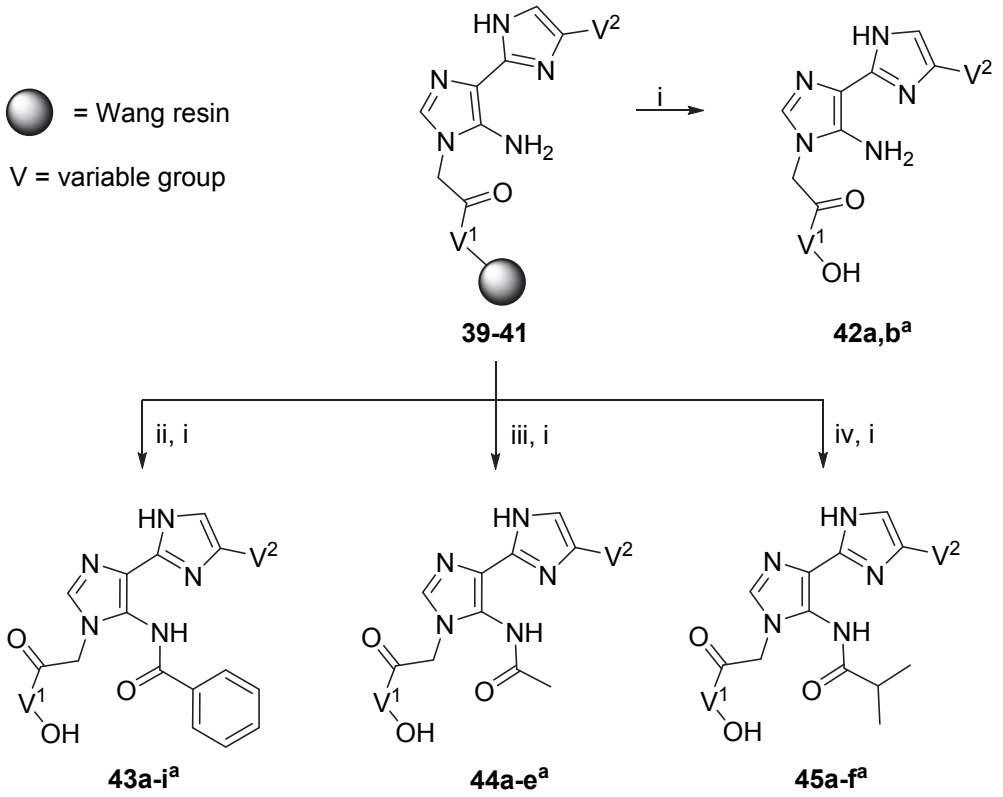
Coupling of **37** to the deprotected resin required some adjustments to the standard coupling methods, because **37** and its 1-hydroxybenzotriazole ester are sparingly soluble in DMF or DMF/DIEA. Thus, **37** was dissolved in warm dry pyridine and HBTU in DMF. The combined solutions were added onto the solid support (**Scheme 14**) and the reaction was shaken for seven hours to ensure completion of the coupling. Extensive washing was required as with the coupling of **36c**. Each of the supports obtained, **38a-d**, was divided into three portions. The first portions were oximated with methoxyamine in pyridine. The hydrazone formation of the second portion was tested with 2,4-dinitrophenylhydrazine (DNPH) and phenylhydrazine. Hydrazones are much more reactive than oximes[246, 247] and, hence, there was an increased risk of undesired side reactions during subsequent synthesis steps. Unfortunately, this turned out to be the case. While 2,4-dinitrophenylhydrazone **40a** reacted cleanly to single main products (**43b**, **45a**) upon subsequent acylations of the 5′-amino function, the phenylhydrazone gave a product mixture. In contrast to the 2,4-dinitrophenyl (DNP) derivative, which was deactivated by the strongly electron-withdrawing nitro groups, the sp^3-hybridized nitrogen atom of the phenylhydrazone was sufficiently nucleophilic to react with isobutyryl and benzoyl chlorides. The *N*-acylated phenylhydrazones formed were partially decomposed to the corresponding formylbiimidazoles and 1-phenylhydrazines during acidic cleavage from the support. An attempt to isobutyrate the 5′-amino function be-

fore hydrazone formation did not improve the situation, because the isobutyryl group was cleaved off under the conditions needed for the hydrazone formation.



Scheme 14. Reagents and conditions: i) 20% piperidine in DMF, ii) **3**, HBTU, pyridine, DMF, iii) $R^1ONH_2 \cdot HCl$, pyridine, iv)R^2NHNH_2, AcOH, DMF, heating, v) morpholine, HCO_2H, $NaCNBH_3$, MeOH, DMF.

Half of the remaining **38c** and **38d** were oximated with phenoxyamine instead. Oximation circumvented the acylation problem still yielding an analogous structure. The third portion of **38a-d** was aminated with morpholine by using a method developed earlier (see 3.2).



Scheme 15. Reagents and conditions: i) 2.5% H_2O, 2.5% triisopropylsilane in TFA, ii) BzCl, pyridine, iii) Ac_2O, pyridine, iv) isobutyryl chloride, pyridine. a) For annotation of variable groups see **Table 5**.

The 5′-amino group, which was the third diversification point of the biimidazole scaffold, was challenging to derivatize. It is relatively unreactive, and if a substitution reaction takes place, there is a risk of further reactions, e.g. cyclization to the outer imidazole ring. In addition, there are other reactive groups in the molecules, which limit the selection of available chemistries. Fmoc loadings and HLPC-MS data from preliminary experiments carried out with active esters or anhydride activated Fmoc protected amino acids revealed that coupling efficiency was not sufficient. More reactive acyl halides seemed to work better. Therefore acyl halides were used in the library synthesis. Acetic anhydride also exhibited the required reactivity. The products **39-41** were divided into portions and acylated either with benzoyl chloride, acetic anhydride or isobutyryl chloride (**Scheme 15**). Acyl halides reacted partially twice, but the second sub-

stituent was easily removed by a short ammonia treatment. Intention of having compounds with unsubstituted 5′-amino group as library members was abandoned because they turned out to be labile (**42a**, **42b**).

Table 5. Members of the synthesized 4(5),1′,5′-trisubstituted 2,4′-biimidazole test library and isolated overall yields.

Compound	V^1	V^2	V^3	Isolated yield (%)[a,b]
42a	Leu	CHNOMe	-	52
42b	Trp	CHNNHPh	-	n.p.
43a	Leu	CHNOMe	Bz	44
43b	Leu	DNPH	Bz	38
43c	Leu	morpholine	Bz	33
43d	Asp	CHNOMe	Bz	57
43e	Asp	CHNNHPh	Bz	6
43f	Asp	morpholine	Bz	17
43g	Trp	CHNOMe	Bz	8
43h	Ser	CHNOMe	Bz	53
43i	Ser	morpholine	Bz	44
44a	Leu	morpholine	Ac	29
44b	Asp	morpholine	Ac	21
44c	Trp	CHNOMe	Ac	8
44d	Ser	CHNOMe	Ac	71
44e	Ser	CHNOPh	Ac	47
45a	Leu	DNPH	Ibu[c]	36
45b	Asp	CHNOMe	Ibu	45
45c	Asp	CHNNHPh	Ibu	n.p.
45d	Trp	CHNOPh	Ibu	7[d]
45e	Trp	morpholine	Ibu	12
45f	Ser	morpholine	Ibu	25

a) According to ^{13}C NMR all as TFA salts except **43b**,**43e**,**44c**, and **45e**, b) n.p. = not purified, c) Ibu = isobutyryl, d) Combined yield of *E* and *Z* isomers

The library members **42–45** (**Table 5**) were cleaved from the support with a 2.5:2.5:95 mixture of water, triisopropylsilane and TFA. In most cases, the products were relatively pure after cleavage but they were still purified by HPLC for the analyses (see **IV** supporting information for the HRMS and NMR analyses[248]). **Figure 13** shows four illustrative HPLC traces of the crude products obtained. The isolated yields of the tryptophan-derived compounds (**43g, 44c, 45d,e)** were low, although scavengers were used. Oximated products existed as inseparable *E/Z* isomers. Only the phenoxyamine analogue **45d** could be separated by RP HPLC. The isomeric ratio of the methoxyami-

nated products was approximately 1:2 according to the ^{1}H NMR integrals. The isomers were identified by recording a nondecoupled ^{13}C spectrum of **44d** and measuring the $^{1}J_{CH}$ coupling constants of oxyimino carbons of the isomers (**Figure 14**). It is known that for a hydrogen *syn* to the imine nitrogen lone-pair a larger $^{1}J_{CH}$ coupling constant is expected than for *anti*.[249,250] The $^{1}J_{CH}$ coupling constants of the oxyimino carbons of the isomers were 173 Hz (δ = 137.8 ppm) and 181 Hz (δ = 133.9 ppm). Therefore it was concluded that the carbon with 181 Hz coupling constant belonged to the *Z* isomer, which was also the major isomer. Phenoxyamine analogues **45d** and **44e** formed in approximately 1:1 isomeric ratio. Prototropic tautomerism and hindered rotation about the C4′-C2 bond adjoining the imidazole rings[251,252] broadened the ^{13}C and ^{1}H NMR signals of the imidazole rings in most of the products (**Figure 15**). Some imidazole ring carbon signals were weak or even missing in several samples. The chemical shifts of those signals were assigned from the 2D spectra. In addition, signals referring to the morpholino group and methine and methylene bridges connecting the outer imidazole ring to the second diversity group were broadened.

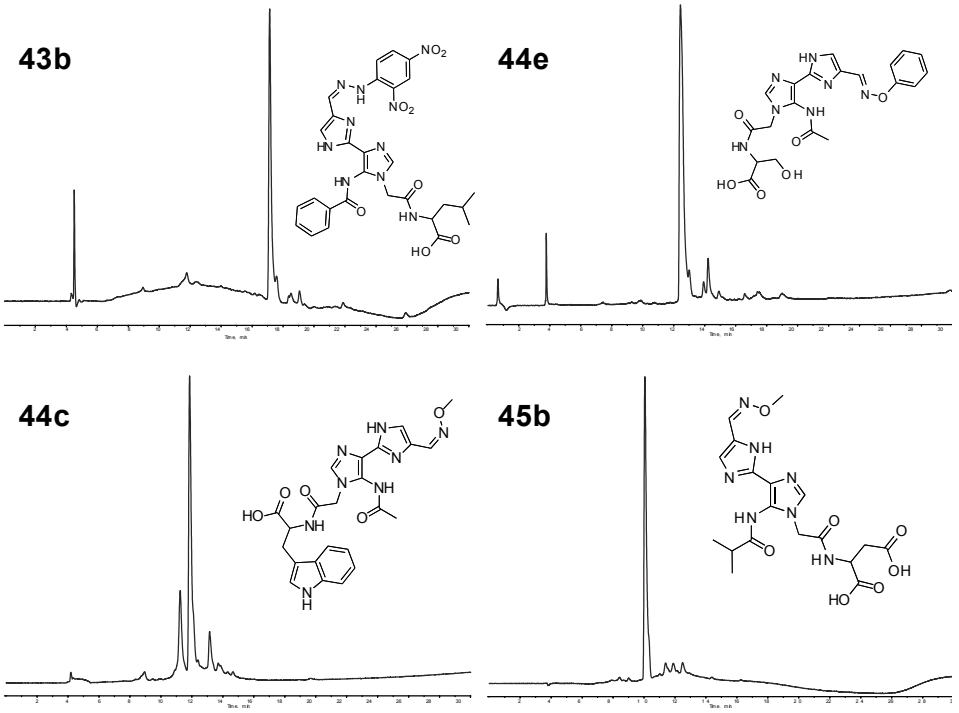


Figure 13. Some examples of HPLC traces of crude products cleaved from the resin (RP HPLC, 0–100% MeCN, 0.1% TFA, λ = 220 nm).

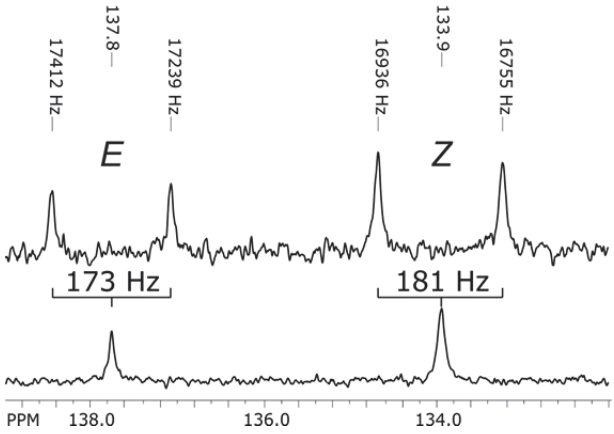


Figure 14. Decoupled (lower) and nondecoupled (upper) ^{13}C spectra of the *E/Z* isomers of oxyimino carbons of **44d**. The $^{1}J_{CH}$ coupling constants 173 Hz (δ = 137.8 ppm) and 181 Hz (δ = 133.9 ppm) show that the major isomer has *Z* conformation.

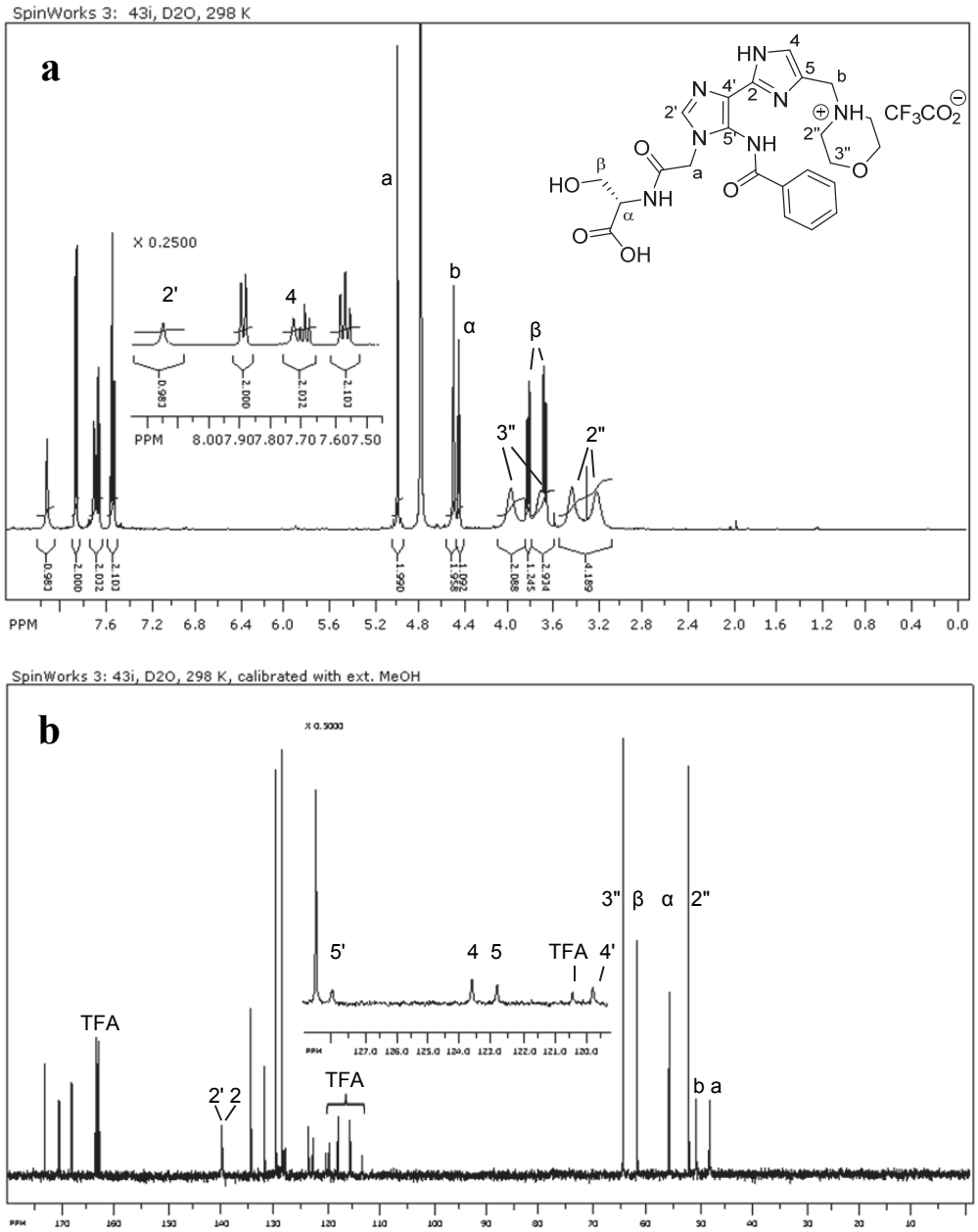
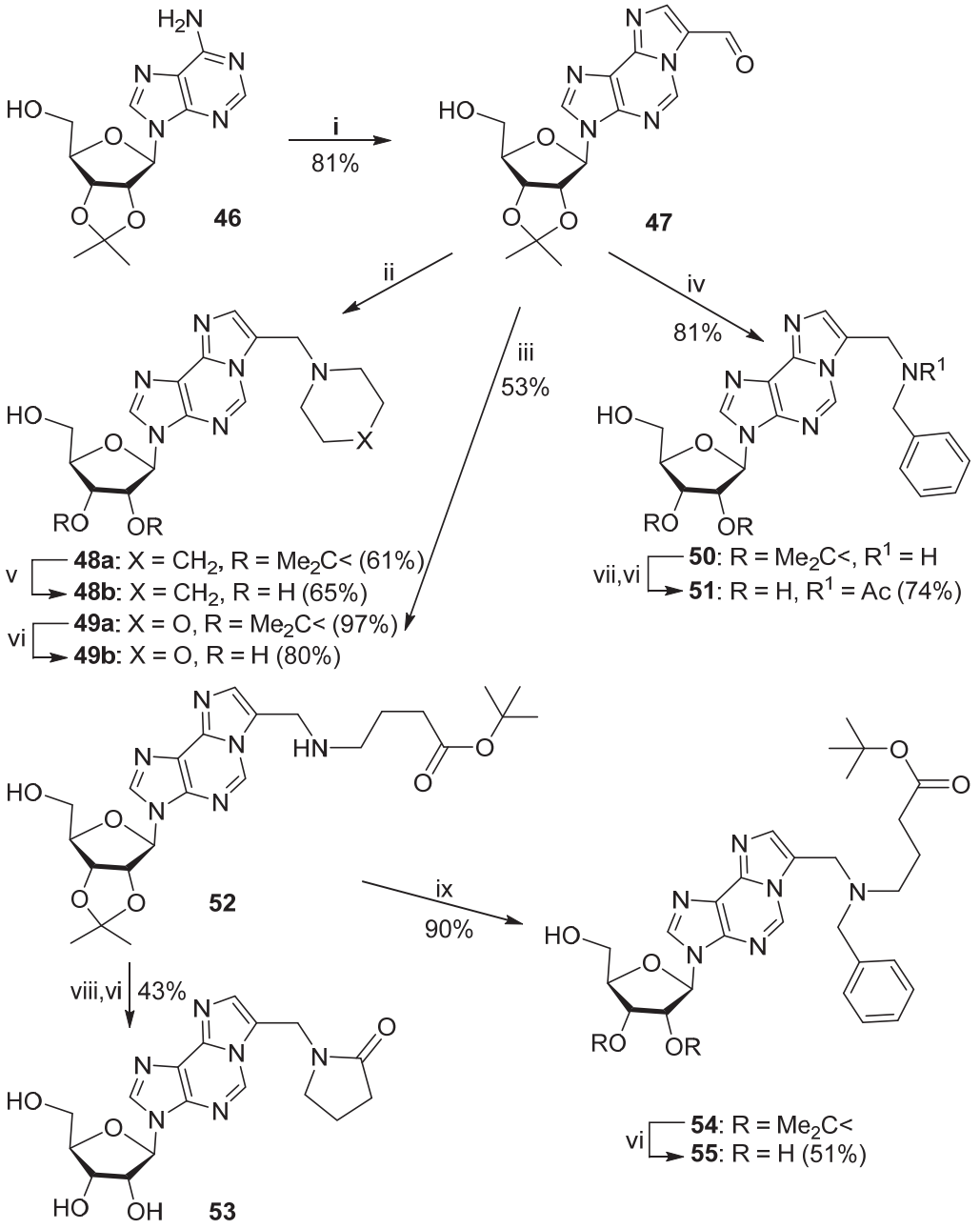


Figure 15. A sample of NMR spectra of the products: a) ^{1}H and b) ^{13}C spectra of **43i**.

3.4 7-Substituted 3-*β*-D-Ribofuranosyl-3*H*-imidazo[2,1-*i*]purines (V)

While in the two earlier studies some well-known synthetic transformations were adapted to a solid-support, in this last study the situation was reversed. The reactions optimized for the solid-phase synthesis were utilized in solution-phase production of 7-substituted 3-*β*-D-ribofuranosyl-3*H*-imidazo[2,1-*i*]purines. The swap of the reaction environment brought up some unexpected difficulties. The bridging of N^1 and N^6 of adenine with bromomalonaldehyde had already been successfully used both in solid- and solution-phase synthesis. When we tried to apply the same chemistry to unprotected adenosine, the result was a thick dark product mixture that was difficult to purify. Therefore, the 2' and 3' hydroxyl groups of the ribose moiety were protected with an isopropylidene group which seemed to have suitable stability characteristics. The bridging reaction was cleaner with 2',3'-*O*-isopropylideneadenosine (**46, Scheme 16**) and 81% yield was collected. Another advantage of the protecting group was that the solubility of the synthesis intermediates was enhanced, which facilitated the silica gel chromatographic purifications. Cleavage of the protection at the end of the syntheses turned out be challenging as will be shown below. Another reaction that needed readjustments was the reductive amination of the formyl group. The required conditions for successful transformation on a solid-support were too harsh for solution-phase synthesis. Thus, attempted reductive amination with piperidine, sodium cyanoborohydride and 1% formic acid in THF was found to give reduced starting material as the main product. Although a weaker acid catalyst, acetic acid, provided better yields, some reduction of the formyl group was still apparent. Although compound **50** was synthesized by this procedure, cleaner products were generally obtained by using sodium triacetoxyborohydride in 1,2-dichloroethane. Having acceptable reaction conditions established, the formyl group in **47** was reductively aminated, either with piperidine (**48a**), morpholine (**49a**), benzylamine (**50**) or *tert*-butyl 4-aminobutanoate (**52**). The secondary amine of compound **50** was acetylated with acetic anhydride in pyridine. During the reaction, the 5′-hydroxyl function was esterified as well. This extra acetyl group was selectively removed by sodium methoxide treatment yielding, after acidolytic deprotection, the desired nucleoside **51**. The other compound containing secondary amine group, **52**, was either lactamized after selective removal of the *tert*-butyl with 50% TFA in DCM, or alkylated reductively with benzaldehyde. The benzylation of secondary amine group of **52** with benzaldehyde and $NaBH(OAc)_3$ or $NaCNBH_3$ in 1,2-dichloroethane was found to be very slow and addition of acetic or formic acid brought about formation of acylated and lactamized side products.[243] Much improved selectivity and excellent yield was, however, obtained by using zinc-modified $NaCNBH_3$ as the reducing agent.[253]



Scheme 16. Reagents and conditions: i) $BrCH(CHO)_2$, HCO_2H, 2,6-lutidine, DMF, ii) piperidine (for **48a**), morpholine (for **49a**), $NaBH(OAc)_3$, AcOH, DCE, iii) Cl^- $^+H_3N(CH_2)_3CO_2tBu$, $NaBH(OAc)_3$, DCE, iv) $BnNH_2$, $NaCNBH_3$, AcOH, THF, v) $FeCl_3 \cdot 6\ H_2O$, MeOH, DCM, vi) 0.1 mol/L aq. HCl/THF, vii) 1. Ac_2O, Pyridine 2. NaOMe, MeOH, viii) 1. 1:1 TFA/ DCM, 2. HBTU, DIEA, DMF, ix) PhCHO, $ZnCl_2$, $NaCNBH_3$, MeOH.

The last step of all the syntheses, the removal of the 2',3'-*O*-isopropylidene protection, turned out to be problematic, because the compounds contained relatively acid labile moieties and the protecting group was unexpectedly stable. Of the methods attempted, 80% AcOH, 25 °C[254], I_2/MeOH[255], $PdCl_2(CH_3CN)_2$[256], ceric ammonium nitrate[257], ceric ammonium nitrate on silica[258], and hydrolysis at pH 2 were found to be too inefficient while harsher treatments such as 80% aqueous AcOH at reflux[254] or a 1:1 mixture of 1 mol/L aq. HCl and THF[259], resulted in side reactions such as depurination or even pyrimidine ring opening. The best results were obtained using a 1:1 mixture of 0.1 mol/L aq. HCl (aq.) and THF at 55 °C or $FeCl_3 \cdot 6H_2O$ in a 1:19 mixture of MeOH and DCM at elevated temperature[260]. The Brønsted acid-catalysed reaction was still accompanied by some depurination, while the cleaner Lewis acid-catalysed reaction was hampered by a more laborious work up that ultimately resulted in reduced yields. Because the methods were approximately equally yielding, the more convenient and faster former method was mainly used.

3.5 Rough Computational Evaluation of Bioactivity of the Compounds

The last three papers, included in this thesis, focused on creating scaffolds useful for drug discovery. At this stage of the development, obeying the rules of thumb of drug-likeness was considered less important. The applied chemistry merely displays some of the possibilities that the chosen scaffolds give. Nevertheless, some calculations of the potential bioactivity of the synthesized compounds were performed with free online services. GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor likeness were predicted with Molinspiration bioactivity scores calculation service v2011.06.[261] The service uses a fragment-based model which is developed by comparing active and inactive sets of molecules using Bayesian statistics. The results collected for the synthesized sample libraries are in **Table 6**. It can be seen that the 3*H*-imidazo[2,1-*i*]purines (**28**, **32**) which are small, structurally simple and quite planar molecules gather generally higher scores than the 2,4′-biimidazoles (**43**–**45**). The 3*H*-imidazo[2,1-*i*]purines have relatively high scores in GPCR ligand, kinase inhibitor, and enzyme inhibitor likeness while they are predicted not to bind well in nuclear receptors or proteases. The synthesized 2,4′-biimidazoles, which are larger and contain more polar atoms, show lower overall scores in this screen, as could be expected. Especially they seem to be poor ion channel blockers and nuclear receptor ligands. The lower overall scores can mean that they are possibly more selective, if a suitable target is found. Finally, the 3-*β*-D-ribofuranosyl-3*H*-imidazo[2,1-*i*]purines (**48**–**55**), while having a relatively large polar surface area alike, are predicted to be good GPCR ligands or enzyme inhibitors. The scores predict also functionality as ion channel blockers or kinase inhibitors.

Table 6. Molinspiration bioactivity scores of the synthesized sample compounds. Dark grey colour indicates high, light grey moderate, and white low probability of activity.

3*H*-imidazo[2,1-*i*] purines	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
28	0.48	-0.01	0.13	-0.33	0.32	0.30
32a	0.41	0.29	0.73	-0.83	-0.22	0.50
32b	0.44	0.28	0.62	-0.77	-0.11	0.48
32c	0.45	0.40	0.55	-1.22	-0.29	0.54
32d	0.50	0.34	0.49	-0.64	0.17	0.53
32d – *t*-Bu	0.49	0.39	0.48	-0.90	-0.03	0.67
32e	0.43	0.24	0.56	-1.13	-0.18	0.42
32f	0.51	0.19	0.65	-0.87	-0.02	0.53
32g	0.43	0.34	0.57	-1.08	-0.37	0.47
32h	0.53	0.36	0.58	-0.90	-0.12	0.46
32i	0.54	0.40	0.63	-0.92	-0.20	0.53
32j	0.67	0.43	0.59	-0.67	0.10	0.71
32k	0.59	0.40	0.55	-0.49	0.24	0.58
32l	0.69	0.36	0.56	-0.51	0.06	0.59
32m	0.67	0.34	0.63	-0.46	0.12	0.59
32n	0.67	0.35	0.53	-0.35	0.21	0.59
32o	0.67	0.27	0.53	-0.54	0.18	0.54
32p	0.50	-0.11	0.12	-0.42	0.43	0.29
32p – Fmoc	0.75	0.40	0.61	-0.59	0.35	0.64
32q	0.47	0.22	0.40	-1.05	-0.11	0.40
32r	0.46	0.08	0.45	-0.71	-0.02	0.35
2,4′-biimidazoles	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
42a	0.58	0.05	0.32	-0.04	0.67	0.67
42b	0.40	-0.12	0.15	-0.47	0.29	0.37
43a	0.41	-0.08	0.21	-0.01	0.48	0.43
43b	-0.16	-1.06	-0.68	-1.01	-0.02	-0.35
43c	0.42	0.00	0.19	-0.13	0.47	0.38
43d	0.44	-0.09	0.24	-0.04	0.44	0.45
43e	0.23	-0.37	-0.08	-0.48	0.23	0.20
43f	0.45	0.00	0.22	-0.16	0.44	0.40
43g	0.46	-0.32	0.23	-0.15	0.39	0.29
43h	0.43	-0.10	0.32	-0.09	0.44	0.48

2,4′-biimidazoles	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
43i	0.45	0.07	0.30	-0.20	0.44	0.45
44a	0.48	0.18	0.23	-0.12	0.51	0.45
44b	0.51	0.17	0.25	-0.15	0.47	0.48
44c	0.54	-0.02	0.37	-0.02	0.42	0.46
44d	0.50	-0.07	0.37	-0.08	0.48	0.56
44e	0.44	-0.02	0.24	-0.14	0.48	0.49
45a	0.01	-0.76	-0.44	-0.71	0.06	-0.10
45b	0.46	-0.13	0.22	-0.08	0.51	0.47
45c	0.24	-0.28	-0.08	-0.39	0.23	0.27
45d	0.36	-0.52	-0.01	-0.28	0.41	0.15
45e	0.48	-0.21	0.18	-0.20	0.41	0.28
45f	0.46	0.05	0.26	-0.15	0.46	0.47
3-*β*-D-ribofuranosyl-3*H*-imidazo[2,1-*i*] purines	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
48b	0.97	0.52	0.64	-0.76	0.16	0.86
49b	0.90	0.45	0.67	-0.80	0.12	0.82
51	0.77	0.27	0.43	-0.73	0.11	0.67
53	0.93	0.40	0.48	-0.85	0.22	0.77
55	0.73	0.19	0.33	-0.49	0.21	0.60
55 – *t*-Bu	0.82	0.38	0.44	-0.50	0.18	0.73

Potential protein targets for the compounds were searched from DrugBank[262] (4645 chemical structures), Binding database[263] (364221 structures), and PDB[264] (7072 structures) with ChemMapper[265266,267] using a similarity threshold of 1.2 (60% similarity). ChemMapper is a free web service which integrates pharmacophore matching and volumetric similarity approaches to find compounds with high 3D similarity to known active compounds with available pharmacology annotation. The probability of interaction is then calculated for the query structures with proteins that associated with the highest scoring database compounds. With exception of molecules from PDB, ChemMapper uses several low-energy conformations for the database compounds for the 3D similarity calculation. While this increases coverage and accuracy, having only one conformer of the query molecule limits it a lot. For the screening, the energies of 3D conformations of the compounds were first minimized with MM2 calculations and MOPAC2009[268] (PM6 method) using Chem3D Pro version 13.0.0.3015[269]. Because ChemMapper uses only one conformation of the query molecule in target navigator mode, both imidazole tautomers of the 2,4′-biimidazoles (**43**–**45**) were calculated. Both

Z and *E* isomers of the phenoxyaminated compounds were used in the screening, while only *Z* isomers of the methoxyaminated compounds were screened.

Majority of the 3*H*-imidazo[2,1-*i*]purines (**32**) returned hits (**Table 7**) and generally the number of targets per compound was high in the screened databases while only few 2,4′-biimidazoles (**43**–**45**) and 3-*β*-D-ribofuranosyl-3*H*-imidazo[2,1-*i*]purines (**48**–**55**) returned hits. The most popular targets for **32** include human EGFR tyrosine kinase, dihydrofolate reductase, sodium channel protein type 5 subunit α, inducible nitric oxide synthase, adenosine receptors A1 and A2A, and cyclin-dependent kinase 2. 3-Methyladenine DNA glycosylase (MAGIII) of *Helicobacter pylori* gathered several high scoring hits in PDB because it binds 3*H*-imidazo[2,1-*i*]purine which is the parent structure of the first library. Aurora kinase A and cyclin-dependent kinase 2 were the most frequently encountered targets for compounds **42**–**45**. Compounds **49b** and **53** were the only hitters in the last group. Their targets include for example adenosine receptor A1 and metabotropic glutamate receptor 1 which belong to G-protein coupled receptors.

Table 7. Number of compounds with hits in databases screened with ChemMapper.

Compound	DrugBank	Binding database	PDB
28,**32**	11	16	19
42–**45**	2	6	2
48–**55**	0	3	2

4. Epilogue

The beginning of the Results and Discussion chapter focused on development and application of methods for solid-phase peptide synthesis. While four homodetic bicyclic peptides were successfully synthesized on a solid support, the approach described in the second work turned out to have limited value for the intended use. Because of the difficulties encountered in the syntheses and changes in the project funding, this project was not continued further.

For the synthetic part, the aims of the thesis were met. Unfortunately, most of the compounds were not tested or used for the intended purpose. Some of the compounds were screened by a local pharmaceutical company against targets unknown to the author. The possible biological activity of the compounds from the last three papers was roughly evaluated by using free online virtual screening services. As could be expected, the compounds **32** which follow better the rules of drug-likeness got higher bioactivity scores than compounds from the papers **IV** and **V**. More targets for **32** were also found in molecular shape superposition and chemical feature matching of ChemMapper. This does not mean that they are better candidates but merely that their structure resembles more closely the ligand structures contained in the databases screened. It is known that the chemical space that combinatorial libraries cover is small.[3] Therefore, one of our aims was to develop scaffolds that would widen that space. The types of virtual screening that was executed cannot efficiently find hits for such compounds, hence in vitro screening would be required to determine the biological activity.

5. Summary

In the first work, an orthogonally protected *N*-Alloc-*N*′-Fmoc-*N*″-Boc-α,α-bis(aminomethyl)-*β*-alanine branching unit (**1**) was synthesized. The scaffold is spatially small and lends itself for synthesis of molecules with three or four different moieties that are connected through carboxylic acid or amine functions. The usefulness of the scaffold was demonstrated by SPS of glycoclusters having three different fully acetylated glycopyranosyl groups (**I**) and by SPS of bicyclic peptides having three parallel peptide chains (**II**). Four homodetic bicyclic peptides were successfully synthesized on a solid support. Peptide chemistry methods were used to aid the solid-supported synthesis and to bring diversity to the synthesized libraries of *N*,*N*-disubstituted (3*H*-imidazo[2,1-*i*]purin-7-yl)methylamines (**III**) and 5,1′,5′-trisubstituted 2,4′-biimidazoles (**IV**) as well. In addition to reactions familiar from the peptide chemistry, some other reactions commonly used on a solid support, namely oximation, hydrazine formation and reductive amination, were utilized. The libraries were built on a 7-formyl-3*H*-imidazo[2,1-*i*]purine scaffold which was constructed either in solution or on a solid support. 2′-Deoxyadenosine phosphoramidite reagent was introduced as a convenient quasi-traceless acid-labile linker. It was used to produce a library of *N*,*N*-disubstituted (3*H*-imidazo[2,1-*i*]purin-7-yl)methylamines. *N*3-linked derivatives of similar compounds can be prepared by the alternative method that was used to synthesize compound *N*-{[7-(*N*-benzylacetamidomethyl)imidazo[2,1-*i*]purin-3-yl]acetyl}phenylalanine (**28**). In the fourth paper a small library of 5,1′,5′-trisubstituted 2,4′-biimidazoles was synthesized on amino acid functionalized solid supports. The method is applicable for production of larger libraries by increasing the selection of the diversity reagents used, namely the resin, *O*-substituted hydroxylamine, amine and acid halide. Finally the method to produce 7-substituted 3*H*-imidazo[2,1-*i*]purines on solid support was successfully adapted to solution chemistry to produce 7-substituted 3-*β*-D-ribofuranosyl-3*H*-imidazo[2,1-*i*]purines (**V**).

6. Experimental

The synthetic and characterization methods used are described in the original publications (**I-V**) appended to this thesis. An exception is the intervening pyrimidine ring opening of 7-formyl 3*H*-imidazo[2,1-*i*]purine (**33**) on a solid support by base or acid catalysed hydrolyses which are described below. Outcome of the reactions described below was analysed by HPLC-MS method described in **IV** supporting information.

Base catalysed hydrolysis of 2-(7-formyl-3*H*-imidazo[2,1-*i*]purin-3-yl)acetic acid on a solid-support. Aminomethyl NovaGel resin (0.100 g, 0.77 mmol/g) was preswollen with DMF (200 µL). Fmoc Rink linker (124.6 mg, 0.231 mmol), TBTU (74.2 mg, 0.231 mmol) and DIEA (48.5 µL, 0.277 mmol) were dissolved in DMF (500 µL) and after 5 minutes activation added to the resin. The reaction mixture was shaken for 5 hours, filtered, washed successively with DMF, DMF/MeOH, MeOH, DMF, DCM, DCM/MeOH, MeOH, and Et_2O and then dried in a vacuum desiccator. The resin substitution was according to Fmoc loading assay 0.50 mmol/g. The Fmoc Rink amide NovaGel resin was then capped with a 30 min treatment with a solution of acetic anhydride (100 µL), 2,6-lutidine (100 µL), and *N*-methylimidazole (160 µL) in THF (1.64 mL). The resin was washed with THF, THF/MeOH, THF, DCM, MeOH, and Et_2O and then dried in a vacuum desiccator. Fmoc protection was removed from the resin with 20% piperidine in DMF in 20 minutes. Resin was successively washed with DMF, DCM, MeOH, and Et_2O and dried in vacuum. Fmoc-valine (78.4 mg, 0.231 mmol) was coupled to the resin as described for Fmoc Rink linker except that the reaction was repeated (Fmoc loading 0.540 mmol/g). Fmoc protection was removed from the resin as before. Compound **36c** (56.6 mg, 0.231 mmol), TBTU (74.2 mg, 0.231 mmol), DIEA (48.5 µL, 0.277 mmol) and DMF (1000 µL) were mixed and added onto the deprotected resin. The reaction suspension was kept in 80 °C for 3 hours and shaken at room temperature overnight. According to ninhydrin test, there were free amino groups left in the support. One equivalent of DIEA was added (40 µL, 0.231 mmol) and the reaction mixture was heated at 55 °C for a day and at room temperature overnight. The apparent amount of precipitate had decreased and the ninhydrin test was clear. The mixture was filtered and washed with DMF, DMF/MeOH, DMSO, DMSO/MeOH, MeOH, DMF, DMF/water, DMF, DCM, MeOH, THF, MeOH, and Et_2O and dried in a vacuum desiccator. Washing with DIEA/DMF mixture would have been beneficial for washing process. The dried resin was swollen in THF, filtered and a mixture of THF (250 µL) and 0.16 mol/L aqueous NaOH (250 µL) was added. The reaction mixture was agitated for 3 hours 30 minutes at 55 °C and at room temperature overnight. The resin was washed with THF, THF/water, water, THF, and MeOH and then dried in a vacuum desiccator.

Acid catalysed hydrolysis of 2-(7-formyl-3*H*-imidazo[2,1-*i*]purin-3-yl)acetic acid on a solid-support. HMBA-AM resin (125 mg, 0.80 mmol/g) was swollen in DMF. Fmoc-valine (339.4 mg, 1.00 mmol) was dissolved in dry DCM with the aid of few drops of DMF. DIC (80 μL, 0.50 mmol) was added to the solution and the mixture stirred for 20 minutes in a closed flask. DCM was evaporated with a rotary evaporator. The residue was dissolved in DMF and added to the swollen resin. Likewise dissolved dimethylaminopyridine (1.2 mg, 0.01 mmol) was added and the reaction mixture was shaken for 6 hours. The resin was filtered and washed with DMF, DCM, DCM/MeOH, and MeOH, and dried in a vacuum desiccator yielding Fmoc-Val-HMBA resin (loading 0.623 mmol/g). The resin was then capped with acetic anhydride and Fmoc protections removed (see above). Compound **36c** (73.6 mg, 0.300 mmol) and DIEA (104.5 μL, 0.600 mmol) were dissolved in DMF (1000 μL) and added onto the deprotected resin. HATU (114.1 mg, 0.300 mmol) was suspended in DMF (200 μL) and added onto the mixture. The reaction was shaken 24 hours, filtered and washed with DMF, DMF/MeOH, DMF, water, pyridine, DMF, DIEA/DMF, DMF, DCM, DCM/MeOH, MeOH, AcOH, DCM, MeOH and dried in vacuum. The resin was capped as before. For the pyrimidine ring hydrolysis, the resin was shaken 18 hours in a mixture of 4 mol/L HCl in dioxane (1.80 mL) and water (0.20 mL). The dioxane solution turned to yellowish green during the reaction and the solid support dark green. The resin was filtered and washed with dioxane, water/dioxane, water, dioxane, MeOH, DCM, 2% TEA/DCM, MeOH/DCM, DCM, dioxane, water, MeOH, DCM, MeOH

7. References

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