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Synthesis of Nitrogen-Containing Five-Membered Heterocycles. 1,3-Dipolar Cycloadditions, Solid-Phase Techniques, and Parallel Methods

by

Kirsi Harju

ACADEMIC DISSERTATION

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Kirsi Harju

ABSTRACT

Five-membered heterocycles are widely present in nature and in drugs. Of the several methods to prepare them in the laboratory, 1,3-dipolar cycloaddition has proven particularly useful.

In these studies, five-membered nitrogen-containing heterocycles were prepared via 1,3-dipolar cycloadditions. 1,2,3-Triazoles, pyrazolopyridines, and pyrazoles were prepared on solid support, and a library of pyrroles was prepared in solution in a parallel fashion. In the synthesis of the pyrazoles and pyrroles, reactions were facilitated by microwave irradiation. Parallel reactions, evaporations, isolations, and purifications were performed. The 1,2,3-triazoles, pyrazolopyridines, pyrazoles, and pyrroles were prepared from various dipoles and dipolarophiles. 1,2,3-Triazoles were prepared from resin-bound azides and alkynes or enamine, and pyrazolopyridines were obtained from resin-bound alkynes and azomethine imines. Mesoionic dipoles were used for the synthesis of pyrazoles and pyrroles. Pyrazoles were synthesized from resin-bound sydnones and alkynes, and pyrroles were obtained in the 1,3-dipolar cycloaddition reaction between münchnones or azlactones and alkynes. Cleavage of the compounds from solid support was studied. 1,2,3-Triazoles and pyrazoles were cleaved from the 2-methoxy-substituted resin in a traceless manner. Pyrazolopyridines, which were linked to the resin with an ester linkage, were cleaved from it as carboxylic acids or methyl esters.

Structurally related compounds were prepared by taking advantage of combinatorial methods. Several alkynes were used in the cycloadditions. Additionally, pyridine derivatives were employed as building blocks for the synthesis of pyrazolopyridines, and amino acids for pyrazoles and pyrroles. The dipoles and dipolarophiles differed in their reactivity: pyrazolopyridines could be prepared at room temperature, whereas 1,2,3-triazoles required prolonged heating, and pyrazoles and pyrroles were prepared under microwave irradiation. All products were isolated, purified, and fully characterized. The overall yields of the products varied from moderate to high. Regiochemical outcome of the 1,3-dipolar cycloadditions was studied by NMR techniques and crystal structure analysis. The regioselectivity was good in most reactions between the dipoles and electron-withdrawing alkynes, but occasionally a mixture of regioisomers was obtained.

In summary, a range of techniques were applied to the synthesis of five-membered nitrogen-containing heterocycles. Five-membered heterocycles have many important applications, making new synthetic techniques of great significance.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Harju K.; Vahermo M.; Mutikainen I.; Yli-Kauhaluoma J. Solid-phase synthesis of 1,2,3-triazoles via 1,3-dipolar cycloaddition *J. Comb. Chem.* **2003**, *5*, 826–833.

II Harju K.; Kylänlahti I.; Paananen T.; Polamo M.; Nielsen J.; Yli-Kauhaluoma J. Solid-phase synthesis of pyrazolopyridines from polymer-bound alkyne and azomethine imines *J. Comb. Chem.* **2006**, *8*, 344–349.

III Harju K.; Vesterinen J.; Yli-Kauhaluoma, J. Solid-phase synthesis of amino acid derived *N*-unsubstituted pyrazoles via sydnones *Org. Lett.* **2009** 11, 2219–2221.

IV Harju K.; Manevski N.; Yli-Kauhaluoma, J. Microwave-assisted synthesis of pyridylpyrroles from *N*-acylated amino acids, *submitted*

The publications are referred to in the text by their Roman numerals.

ABBREVIATIONS

ATR	attenuated total reflection
t-Boc	*tert*-butyloxycarbonyl
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DEPT	distortionless enhancement by polarization transfer
DFT	density functional theory
DIPEA	*N*-ethyldiisopropylamine
DMAD	dimethyl acetylenedicarboxylate
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DVB	divinylbenzene
FMO	frontier molecular orbital
Fmoc	fluorenylmethoxycarbonyl
FT–IR	Fourier transform infrared spectroscopy
GC	gas chromatography
HMBC	heteronuclear multiple bond correlation
HOMO	highest occupied molecular orbital
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
LC	liquid chromatography
LUMO	lowest unoccupied molecular orbital
MALDI	matrix assisted laser desorption/ionization mass spectrometry
MAS	magic angle spinning
MS	mass spectrometry
MW	microwave
Mw	molecular weight
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser enhancement spectroscopy
PEG	polyethylene glycol
PS	polystyrene
rt	room temperature
SASRIN	super acid-sensitive resin
TBAF	tetrabutylammonium fluoride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
UV	ultraviolet

1 INTRODUCTION

Combinatorial chemistry has had a revolutionary impact on drug discovery. An article recounting the history of combinatorial chemistry in the first issue of the *Journal of Combinatorial Chemistry*,[1] launched in 1999, describes anything but a smooth beginning, however. Parallel personal comments on the pioneering publications show that exploring the new area was tedious and the opposition was vociferous. Long referee reading times, refusals of ground-breaking articles by leading journals, and sharp arguments over terminology were typical. Only after several years of uphill struggle were combinatorial chemistry and the synthesis of compound libraries accepted as fruitful avenues of research. The power of the new approach is now unquestioned, and drug discovery proceeds today in a radically different manner than earlier.

Five-membered nitrogen-containing heterocycles occur in a diversity of natural products and drugs and are of great importance in a wide variety of applications. Aromatic nitrogen-containing five-membered heterocycles include pyrroles, pyrazoles, imidazoles, 1,2,3-triazoles, 1,2,4-triazoles, and tetrazoles with one to four nitrogen atoms in the ring (Figure 1). Additionally, aromatic nitrogen heterocycles may contain another heteroatom, such as the oxygen in isoxazoles, oxazoles, 1,3,4-oxadiazoles, and 1,2,4-oxadiazoles; or the sulfur in isothiazoles and thiazoles. Non-aromatic nitrogen-containing heterocycles include partially saturated "-olines" (preferably named with the prefix dihydro-) and completely saturated "-olidines". Partially or totally reduced heterocycles are of less interest in drug discovery because of their probable instability and chirality.

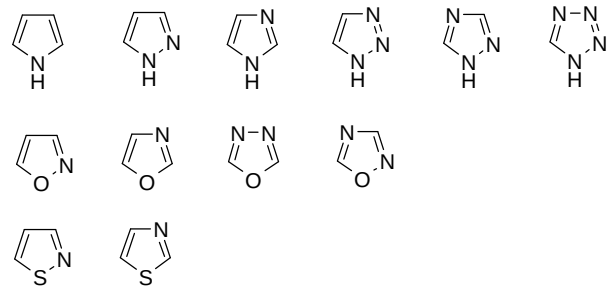


Figure 1. *Aromatic five-membered heterocycles with nitrogen atoms*

Among the drugs containing aromatic five-membered nitrogen heterocycles are cholesterol-reducing atorvastatin, anti-inflammatory celecoxib, antiulcerative cimetidine, β-lactamase inhibitory tazobactam, antifungal fluconazole, and antihypertensive losartan (Figure 2).

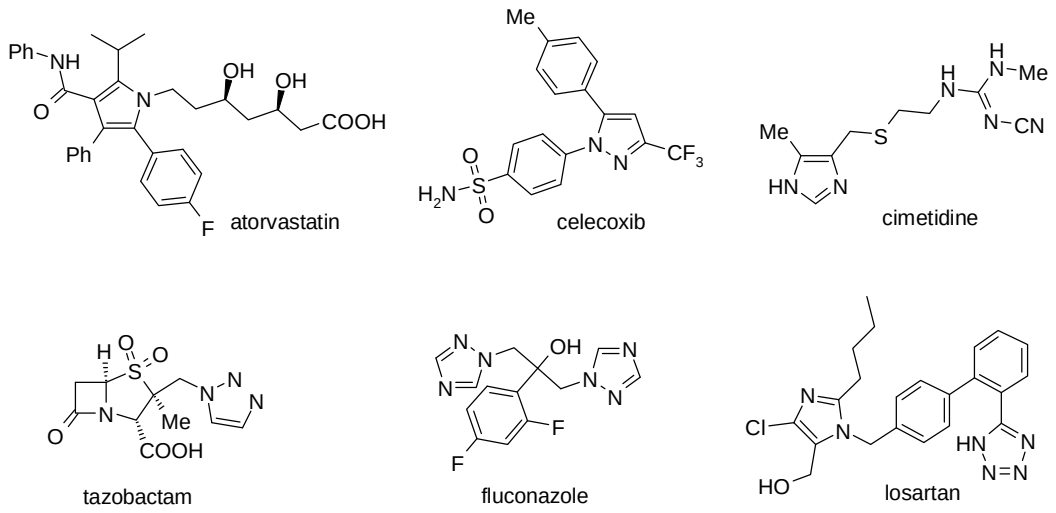


Figure 2. *Drugs containing aromatic five-membered nitrogen heterocycles*

The methods of synthesizing nitrogen-containing heterocycles vary widely. One of the main approaches to the synthesis of five-membered heterocycles is 1,3-dipolar cycloaddition. Rolf Huisgen[2] carried out the major pioneering studies of 1,3-dipolar cycloadditions in the 1960s. The facile copper-catalyzed 1,2,3-triazole synthesis was discovered in 2002,[3,4] and since then the number of publications concerning 1,3-dipolar cycloadditions has increased dramatically. The introduction of the copper catalyst solved many problems, including long reaction times, high reaction temperatures, and poor regioselectivity, and provided a new way to produce the heterocyclic ring under mild reaction conditions.

The continuing need for new drugs has led to the development of solid-phase methods. Solid-phase methods were first used in peptide synthesis, and later applied to the solid-phase synthesis of small organic compounds. Solid-phase methods enable the use of excess reagents and fast isolation of the products. Various approaches have been described to link a compound to a resin and then cleave the product from it. Microwave-assisted reactions, parallel systems, and automation have been developed for high-throughput synthesis. These methods have proven to be excellent tools for speeding up reactions and obtaining a wide variety of compounds in the same time frame. Today they command a central place in drug discovery.

Combinatorial chemistry has now reached a turning point and, according to the *Journal of Combinatorial Chemistry* in 2009, the new era of *distributed drug discovery* (D^3) has begun.[5,6,7] The concept of *distributed drug discovery* was first formulated in Indiana University–Purdue University Indianapolis. Research problems, computational analysis, synthesis, and biological screening are divided into small units and are handled in a distributive fashion in separate university laboratories. Global sharing of information and sources produces a virtual catalog that is available to all through open access. Sharing of

information assists the discovery of inexpensive drugs that can be used to treat diseases in developing countries as well.

The review of the literature that follows in chapter 2 covers the history and basic principles of 1,3-dipolar cycloadditions, solid-phase chemistry, and high-throughput synthesis, the three approaches applied in the practical work. 1,3-Dipolar cycloadditions on solid supports are discussed in terms of linker strategy. The results of the work, reported in publications I–IV, are summarized in chapter 5. The results cover the synthesis of small compound libraries of nitrogen-containing five-membered heterocycles via 1,3-dipolar cycloadditions, solid-phase techniques, parallel systems, and microwave-assisted reactions. Although most of the products have been tested in a wide variety of applications for potential bioactivity, the main emphasis of the work was the development of the combinatorial techniques, not drug discovery. 1,3-Dipolar cycloaddition was chosen as reaction type because of its utility to prepare various nitrogen heterocycles in an atom-economical way from simple starting materials. Aromatic products were favored due to their stability and greater practicality in drug discovery. Solid-phase reactions were carried out with polystyrene resin because of the availability, low cost, high loading, and applicability to FT–IR analyses. Various linker strategies were studied with the ultimate aim of traceless cleavages, and several resin types were tested. One part of the project was the construction of parallel and automation facilities in our laboratory. Parallel reactions were conducted either on solid support or in solution. Solid-phase reactions showed more utility especially in the isolation and purification steps, but they also required more effort in development of methods and cleavage strategies. After purchase of a microwave reactor for the laboratory, conventional heating of the cycloaddition reactions was replaced with microwave irradiation. The prepared products were fully characterized, and the regiochemistry of the cycloadducts was explored.

2 REVIEW OF THE LITERATURE

2.1 1,3-Dipolar cycloadditions

1,3-Dipolar cycloaddition reactions belong to the family of thermally occurring pericyclic reactions, and lead to five-membered heterocyclic rings.[8] The other well-known thermal pericyclic reaction is Diels-Alder cycloaddition, which gives a six-membered ring.[9] A 1,3-dipole is a reactive compound with a distributed charge, which forms a five-membered ring with a dipolarophile (Figure 3). The most common dipolarophiles are alkenes, alkynes, imines, enamines, and nitriles.

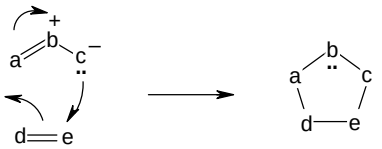


Figure 3. *Cycloaddition reaction mechanism*[8]

1,3-Dipoles have resonance structures that allow them to react as both nucleophiles and electrophiles. In the octet structures the free electron pair is delocalized over the two termini of the dipole, and in the sextet structures allylic π electrons are localized at the center atom (Figure 4). Despite their positive and negative charges, most of the 1,3-dipoles are not polar.

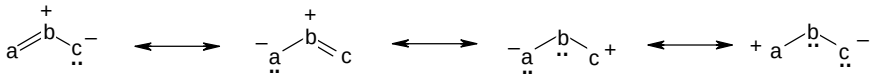


Figure 4. *Resonance structures of the 1,3-dipole*[8]

The dipoles can be divided into two groups: propargyl–allenyl-type dipoles and allyl-type dipoles. Propargyl–allenyl-type dipoles are linear, whereas allyl-type dipoles are bent (Figure 5).

Figure 5. *Propargyl–allenyl-type and allyl-type 1,3-dipoles*[8]

There are a wide variety of propargyl-type (Figure 6) and allyl-type (Figure 7) 1,3-dipoles. The most useful nitrogen-containing dipoles are propargyl-type nitrile ylides, nitrile imines, nitrile oxides, diazoalkanes, and azides and allyl-type azomethine ylides,

azomethine imines, and nitrones. Although some 1,3-dipoles, such as the azides and diazoalkanes, are stable, most are formed *in situ* during the cycloaddition. The principal difference between the propargyl- and allyl-type dipoles is the type of heterocycle formed in cycloaddition. Reactions of propargyl-type dipoles with triple bonds give directly aromatic products, whereas the cycloadducts obtained from allyl-type dipoles need further oxidation to aromatic products. The wide diversity of dipoles is of great consequence to drug discovery. Dipoles capable of bearing several substituents, such as azomethine ylides and imines, are of greater importance than unsubstituted dipoles, such as nitrous oxide and ozone.

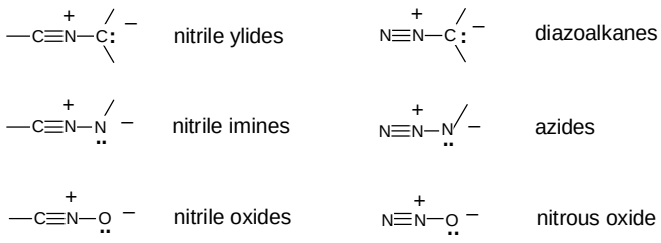


Figure 6. *Propargyl-type dipoles*[8]

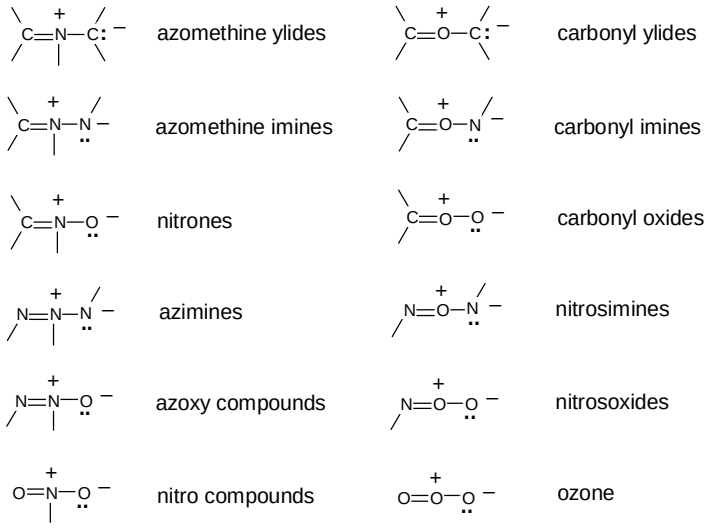


Figure 7. *Allyl-type dipoles*[8]

Mesoionic compounds are relatively stable cyclic compounds that react as dipoles in 1,3-dipolar cycloadditions (Figure 8). Among the most common mesoionic compounds are azomethine ylide-type münchnones and azlactones and azomethine imine-type sydnones. The 1,3-dipolar cycloaddition of münchnones,[10] azlactones,[11] and sydnones[12] with alkynes was first reported by Huisgen and co-workers. Münchnones are obtained from *N*-acylated secondary amino acids with a dehydrating agent such as acetic anhydride, and they are highly reactive toward alkynes. Azlactones, which are obtained from *N*-acylated primary

amino acids, require a tautomeric proton shift before the cycloaddition.[13] Sydnones are obtained from *N*-nitrosated secondary amino acids with dehydrating agent. During 1,3-dipolar cycloadditions, mesoionic compounds release carbon dioxide. Mesoionic dipoles are more stable than the corresponding non-cyclic dipoles and so are easier to handle in parallel synthesis.

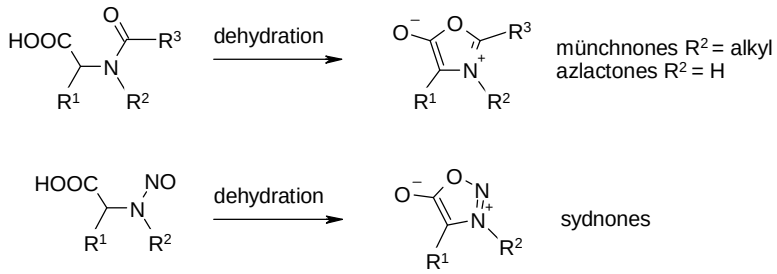


Figure 8. *Mesoionic münchnones, azlactones, and sydnones*

1,3-Dipoles and 1,3-dipolar cycloadditions were first described more than one hundred years ago, with dipoles such as ethyl diazoacetate[14] and diazomethane[15] among the first to be reported. About the same time, the 1,3-dipolar cycloadditions of ethyl diazoacetate[16] and azide[17] were published. The first 1,3-dipolar cycloadditions of nitrones, diazo compounds, and azides were reviewed by Smith in 1938.[18] However, the methods were limited, and the structures of the 1,3-dipoles and the products could not be properly characterized. The field of 1,3-dipolar cycloadditions was enormously extended by Huisgen in the 1960s.[2] The concept of 1,3-dipolar cycloadditions was sharpened, a large number of studies were published, and 1,3-dipolar cycloaddition became the standard method to prepare five-membered heterocycles. Cycloadditions in general were classified and defined by Huisgen[19] at an early stage of the work.

The mechanism of 1,3-dipolar cycloaddition has been intensively investigated. The first mechanistic study was published by Huisgen in 1963.[20] A few years later, Woodward and Hoffman[21] defined the concepts of pericyclic reactions and orbital symmetry and developed the interacting π electron model. Fukui[22] discovered that the chemical reactivity can be explained in terms of interacting frontier molecular orbitals: the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). The transition state of the cycloaddition reaction has been thoroughly studied and reviewed.[23] Whether the mechanism of the cycloaddition is a stepwise diradical mechanism[24] or a concerted mechanism sparked considerable debate.[25,26] In the end, it was concluded that the mechanism may be an asynchronous concerted reaction mechanism in which the forming bonds in the transition state are of unequal length.[27] The aromatic transition state of 1,3-dipolar cycloaddition has been considered as evidence for the concerted reaction mechanism.[28] Likewise, stereospecificity of the cycloaddition reaction has been cited as supporting the concerted reaction: 1,3-dipolar cycloaddition to *trans*- and *cis*-alkenes produces stereospecifically diastereomeric cycloadducts (Figure 9). Huisgen's group has

nevertheless reported exceptions to the concerted reaction mechanism: the first two-step[29] and the first nonstereospecific 1,3-dipolar cycloadditions of sulfur-containing dipoles,[30] which react via zwitterionic intermediates.

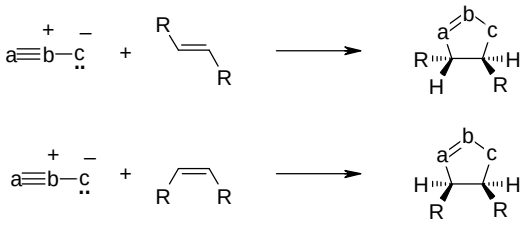


Figure 9. *Stereochemistry of 1,3-dipolar cycloaddition to trans and cis alkenes*[8]

The reactivity of 1,3-dipoles and dipolarophiles varies, and the variation has been explained with a frontier molecular orbital (FMO) model.[31,32] 1,3-Dipolar cycloadditions are HOMO–LUMO controlled reactions where the reactivity depends on the nature of the dipole and dipolarophile and the energy gap between the HOMO and LUMO orbitals (Figure 10). The overlap of the HOMO and LUMO orbitals is maximized during the cycloaddition.

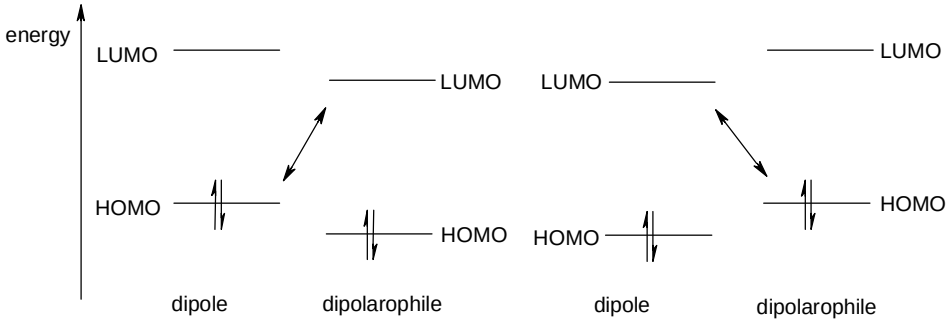


Figure 10. *Molecular orbital control of 1,3-dipolar cycloaddition*[33]

Regiochemistry of the reaction depends on which frontier molecular orbital interaction is dominant.[33] If the energy difference between the HOMO and LUMO reactions is small, a mixture of regioisomers is formed (Figure 11). Substituents affect atomic orbital coefficients, and thereby the regiochemistry of the cycloaddition.

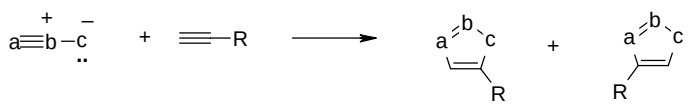


Figure 11. *Regiochemistry of the cycloaddition*[8]

Recently, more accurate density functional theory (DFT) has been applied in the mechanistic studies on 1,3-dipolar cycloadditions,[34] and the reactivity and regiochemistry of the cycloadditions have been predicted on the basis of electron densities derived from quantum mechanical calculations.

The recent advances in 1,3-dipolar cycloadditions were reviewed in 2004.[35] The most important innovation has been the copper-catalyzed cycloaddition of azides and alkynes and the application of this in click chemistry. The concept of click chemistry was introduced by the Sharpless group in 2001[36] and rapidly attracted wide attention. Click chemistry was developed for purposes of drug discovery and was first considered as a viable alternative to solid-phase synthesis. Its benefits include modularity, high yields, stereospecificity, readily available starting materials, simple reaction conditions, and easy isolation and purification of the reaction products. Demko and Sharpless[37,38] reported 1,3-dipolar click reactions of nitriles and azides yielding tetrazoles. Nowadays, the concept of click chemistry is mostly reserved for reactions over copper(I) catalyst yielding 1,2,3-triazoles. The copper catalyst was discovered simultaneously by two independent groups. The Meldal group[3] reported copper(I) iodide-catalyzed solid-phase synthesis of peptidotriazoles (Figure 12), while the Sharpless group[4] used copper(II) sulfate as copper(I) source to synthesize a wide variety of 1,2,3-triazoles. During the reaction, copper(II) was reduced to copper(I) with sodium ascorbate. The whole procedure was performed at room temperature, and 4-substituted 1,2,3-triazoles were formed regioselectively (Figure 12).

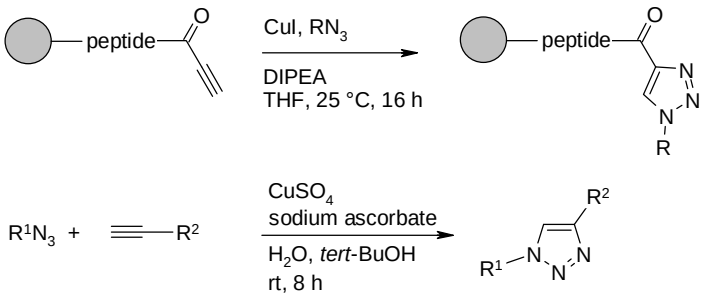


Figure 12. *Copper-catalyzed 1,2,3-triazole synthesis*[3,4]

In study of the mechanism of the copper-catalyzed cycloaddition, the results were proposed to be consistent with a stepwise mechanism involving copper complexes.[39] The excellence of the copper-catalyzed reaction in 1,2,3-triazole synthesis is the total regioselectivity, in contrast to previous reactions that, without the catalyst, mostly yielded mixtures of regioisomers. Additional advantages are mild reaction conditions, higher reactivities, reduced reaction temperatures, and shorter reaction times. Copper catalyst facilitates the reaction by lowering the activation energy.[40] The dramatic improvement of the original Huisgen reaction has now indeed been well demonstrated. Many patents were applied for and numerous papers published following the introduction of click chemistry.

The whole field of click chemistry has recently been extensively reviewed.[41] Click chemistry has been exploited in applications as diverse as drug discovery and medicinal chemistry,[42,43] the modification of peptides[44,45] and other biomacromolecules,[46,47,48] and polymer and materials science.[49,50,51] Copper-mediated reactions are not without disadvantages, however. Since copper may be toxic to biological systems, copper-free systems such as strain-promoted cycloaddition of cyclooctynes have also been developed.[52,53,54]

Exploration of the regioselectivity of the 1,3-dipolar cycloaddition has led to the regioselective synthesis of 1,5-disubstituted 1,2,3-triazoles, as well. Bromomagnesium acetylides have been reported to give 1,5-disubstituted 1,2,3-triazoles after hydrolysis of the 4-metallotriazole intermediate.[55] Recently, a new ruthenium catalyst that yields regioselectively 5-substituted 1,2,3-triazoles was introduced.[56] Ruthenium-catalyzed reactions with internal alkynes have also been studied, but the regioselectivity is lower.[57]

In summary, there can be no doubt about the significance of 1,3-dipolar cycloadditions, which have found use in many applications already. With the discovery of new catalysts and the development of facile regioselective and enantioselective reactions at reduced temperatures, the power of the reactions would increase still more. The next section discusses solid-phase methods and how they have been exploited in 1,3-dipolar cycloaddition reactions.

2.2 Solid-phase methods

Solid-phase methods were initially developed for peptide synthesis. Use of a solid support afforded a fast and robust method to synthesize peptides, with easier isolation and automation. Since 1963, when Merrifield[58] first introduced solid-phase synthesis and reported the synthesis of a tetrapeptide, the chemistry has been substantially developed. Attesting to the novelty and significance of the method, Merrifield was awarded the Nobel Prize in chemistry in 1984.[59] Merrifield's original idea was to attach the carboxyl end of a nitrogen-protected amino acid to a solid support with benzyl ester linkage, and then let the peptide chain grow gradually by repeated deprotection and coupling steps (Figure 13).

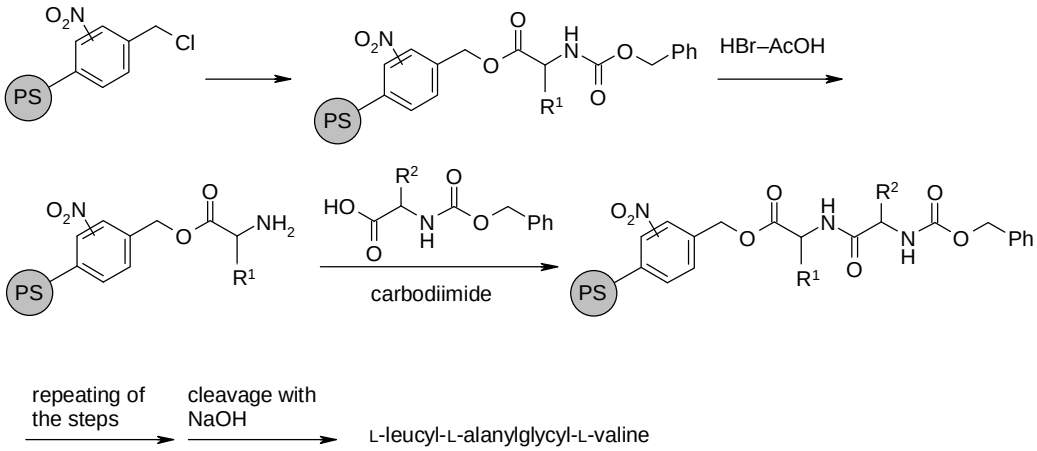


Figure 13. *Merrifield's original solid-phase synthesis of a tetrapeptide*[58]

Excess of reagents and the by-products were removed by filtering and washing of the resin, and the products could be obtained in high purity without crystallization. Yields were also good because none of the compound was lost during the isolation and purification steps. The details of the process were subsequently improved. The protecting benzyloxycarbonyl group was soon changed to the *tert*-butyloxycarbonyl group (*t*-Boc) to obtain milder conditions for the deacylation and to prevent cleavage of the peptide from the resin during the reaction steps (Figure 14).[60] Later, the base-labile fluorenylmethoxycarbonyl (Fmoc) group became popular in the peptide synthesis because it is stable to acids but can easily be removed under mildly basic, non-hydrolytic conditions (Figure 14).[61] When the Fmoc strategy is used, the side-chains of the amino acids can be protected with acid-cleavable groups, and cleavage from the resin is carried out under mildly acidic conditions. Strong UV absorbance of the fluorenyl group allows facile monitoring of the reactions.[62]

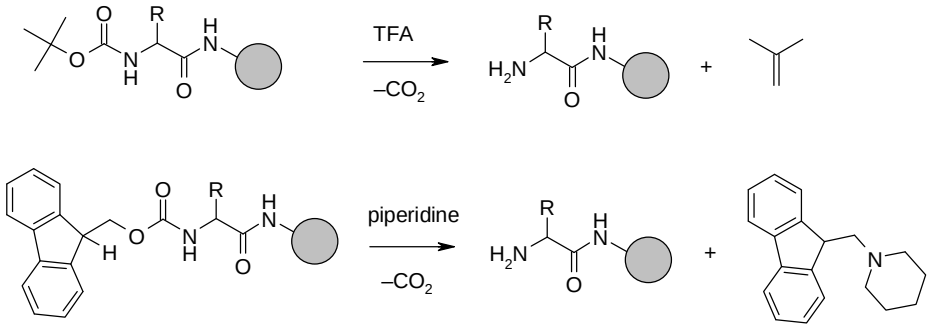


Figure 14. *t-Boc and Fmoc protecting groups of amines*

Merrifield's method was used for the synthesis of several oligopeptides and polypeptides, including nonapeptide bradykinin,[63] octapeptide angiotensin,[64] bovine insulin with 30 amino acids,[65] and ribonuclease A with 124 amino acids.[66] In time, the peptide method was extended to other simple oligomers and polymers such as depsipeptides,[67] oligonucleotides,[68] and oligosaccharides.[69] However, the first methods suffered from purity problems and from other difficulties such as failures in sequences, incomplete couplings, and poor analytical methods.[70,71] Nowadays solid-phase peptide synthesis is automated and the reactions are well developed.[72,73]

Solid-phase synthesis of small organic compounds, on the other hand, is still challenging owing to the diversity of reactions, reagents, and reaction conditions. Peptide synthesis proceeds through amide bond formation and use of protecting groups for amino acids, whereas solid-phase organic synthesis of small compounds relies on a variety of reaction methods. Solid-phase organic synthesis was introduced in the 1970s. Leznoff[74] was the first to undertake systematic and diverse studies of solid-phase organic synthesis, though some miscellaneous solid-phase reactions, such as acylation[75] and alkylation[76] of esters, Dieckmann condensation,[77] and Wittig reaction,[78] had been published in preceding years. In the early stage, the solid support was used for protecting symmetrical difunctional compounds such as diols,[79] dialdehydes,[80] diacid chlorides,[81] and diamines.[82] The methods were applied to multistep reactions, such as synthesis of stilbenes,[83] insect pheromones,[84] and carotenoids.[85] Benzodiazepines were the first drugs to be synthesized on solid support, as early as 1974.[86] The progress of the early stage of solid-phase organic synthesis is well reviewed.[87,88,89,90]

A new era of solid-phase organic synthesis commenced in the 1990s with the extensive studies on solid-phase synthesis of benzodiazepine derivatives by Ellman's group[91] and others.[92,93,94] Additionally, reactions such as the synthesis of ureas;[95] 1,3-dipolar cycloadditions of nitrile oxides;[96,97] Heck,[98] Stille,[99] and Suzuki[100] coupling reactions; and Mitsunobu reactions[101] were being performed on solid support. Nicolaou *et al.* announced the first syntheses of complex natural products such as epothilone[102] and sarcodictyin[103] on solid support. Since then more and more complex natural product-derived compounds have been synthesized on solid support.[104,105,106] Since the 1990s the growth of solid-phase

organic synthesis has been rapid and extensive and the history of solid-phase synthesis has been widely reviewed.[107,108,109,110]

2.2.1 Types and properties of resins

Typically, the symbol of the resin is a gray sphere regardless of the actual material. Thus the symbol tells nothing of the properties of the resin. It is like not mentioning the solvent of a reaction.[111] The resin is an insoluble material with functional groups attached to it via a linker. It should be inert to the reaction conditions, permeable to reagents, and have reaction sites available. The swelling of the resin defines its behavior in diverse solvents, and it affects the permeability of the reagents. Mechanical, chemical, and thermal stabilities are also necessary for good resins. Merrifield describes the background of the resin development in Hudson's review.[112] The materials that were then commercially available, like cellulose, polyvinyl alcohol, and ion-exchange resins, were not directly applicable for solid-phase synthesis. In the end, 1–2% divinylbenzene (DVB) cross-linked polystyrene turned out to be the best choice. Chemical stability of the matrix, the availability of functional groups, and proper size and porosity of the resin particles were the focus when methods were being developed. Further studies of the resins followed, and the history of the resin development is well reviewed.[113,114,115,116] This section describes the common resins in solid-phase organic synthesis.

As already noted, the most popular resin in solid-phase organic synthesis is 1–2% DVB cross-linked polystyrene (Figure 15). The amount of the cross-linkage affects the reaction kinetics, and the diffusion of the reagents could become rate-limiting with higher cross-linking.[117] Polystyrene resin features high loading capacity, low price, and good sustainability for a range of reaction conditions. Although it is highly useful when nonpolar solvents are used, the swelling is poor in polar solvents.

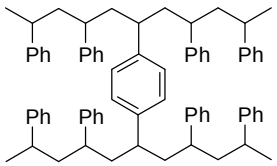


Figure 15. *Divinylbenzene cross-linked polystyrene*

Polyethylene glycol (PEG) resin was developed for peptide synthesis (Figure 16).[118] It is soluble in water and most organic solvents but can be precipitated with hexane, diethyl ether, or *tert*-butyl methyl ether. Homogeneous reaction conditions can be obtained because of its good solubility. Because it is water soluble, however, inorganic material and organometallic reagents are difficult to remove. Indeed, because it is soluble, PEG resin does not in the strict sense qualify as a solid support. PEG resins have gained little popularity in solid-phase organic synthesis.

Figure 16. *Polyethylene glycol PEG and methylated MeOPEG resins*

Grafting of PEG to divinylbenzene cross-linked polystyrene improves the handling, isolation, and purification of PEG. PEG-grafted resins exhibit good swelling in polar solvents. Reaction sites are further away from the polystyrene core, and the swelling of the resin is less critical than for pure polystyrene resin. TentaGel, a PEG-grafted polymer developed by Bayer *et al.*,[119] has benzylic ether linkage, which means that the leakage of PEG impurities is a problem in acidic conditions (Figure 17). High temperatures may also cleave impurities from the resin.[120] Moreover, the loading of the resin is low. HypoGel resins from Rapp Polymere GmbH are related PEG-grafted resins that have a more acid-stable ethyl ether linker (Figure 17). Yet, another PEG-grafted resin, ArgoGel, was developed by Argonaut Technologies (Figure 17).[121] The aliphatic linkage in ArgoGel offers good acid stability, and PEG impurities are not leaked from the resin as easily as from TentaGel. Because of the bifunctional linkage, the loading is higher than that of TentaGel resin. PEG-based resins allow good diffusion of the reagents in polar solvents. The hydrophilic properties of PEG-grafted resins are not always best for organic reactions, however. JandaJel is a polystyrene resin with tetrahydrofuran-derived cross-linkers, which provides good swelling in organic solvents because the cross-linkers are more "organic solvent-like" (Figure 17).[122] Swelling in solvents such as tetrahydrofuran, dichloromethane, and *N*,*N*-dimethylformamide is nearly twice as great as that of the corresponding DVB cross-linked polystyrene.[123]

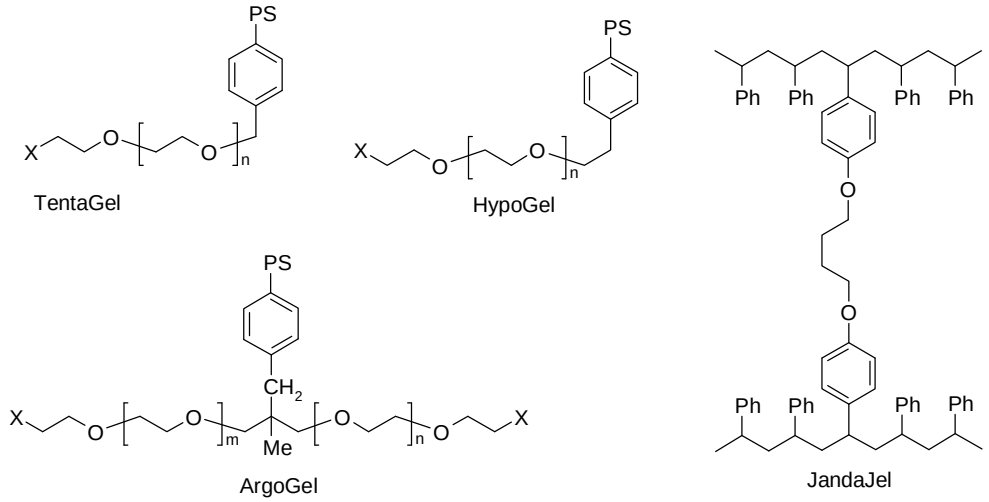


Figure 17. *TentaGel, HypoGel, ArgoGel, and JandaJel resins*

PEGA resin, a highly hydrophilic polyacrylamide–polyethylene glycol copolymer with good swelling in polar solvents, was developed for peptide synthesis (Figure 18).[124] The reactions can be monitored by spectrophotometric methods without interference from the resin in the aromatic region.

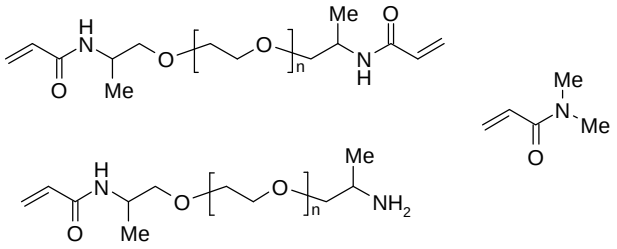


Figure 18. *The monomers of PEGA resin*

The most common resins and their properties are listed in Table 1.

Table 1. *Some typical resins and their properties*

Resin	***Properties***	***Loading***
Polystyrene-divinylbenzene resin (PS–DVB) 1–2% cross-linked	high loading capacity high stability poor swelling in polar solvents	~1–2 mmol/g
TentaGel (PEG–PS/DVB)	good swelling in polar solvents acid-labile benzylic linker	~0.15–0.3 mmol/g
ArgoGel (PEG–PS/DVB	good swelling in polar solvents acid-stable linker	~0.3–0.5 mmol/g
JandaJel (pTHF–PS/DVB) 2% cross-linked	tetrahydrofuran-based cross-linkers good swelling properties	~0.5–1 mmol/g

The loading of the resin is important; it affects the amount of the reagents, the yields, and the reaction kinetics. Other measurable properties of the resins include cross-linkage, mesh size, and swelling properties. Typically the divinylbenzene cross-linkage is 1–2%. Mesh size of the resin indicates the particle size of the resin beads and is measured with sieves. The larger the mesh the smaller is the diameter of the beads. Typically resins are 100–200 mesh, which means that the diameter is 75–150 μm.[112] Swelling of the resin promotes the reactivity. The swelling properties of selected resins, as listed in Table 2, show the better swelling of PEG-grafted resins in polar solvents. Recently it was discovered that resins with identical specifications do not necessarily exhibit the same swelling, and it was highly recommended that resin suppliers would include swelling capacity as one of the specification parameters for a batch of resin.[125]

Table 2. *Swelling of selected resins in various solvents*[126]

Resin	***Swollen volume mL/g dry resin***					
	THF	$CHCl_3$	DMF	DMSO	MeCN	MeOH
PS–1% DVB	7.9	7.8	5.0	2.7	2.6	2.2
ArgoGel, 0.4 mmol/g	6.4	8.8	6.8	6.5	6.5	6.0
TentaGel, 0.2 mmol/g	4.0	4.5	4.0	3.7	3.7	3.5

In summary, the material of the resin affects the reactions that can be carried out on it. In addition to the material, the linkage of the compound to the resin is important. The next section deals with the most common linkers, and section 2.2.3 with the most common analytical methods for the solid-phase reactions. Later, in section 2.2.4, solid-supported 1,3-dipolar cycloadditions are discussed on the basis of the linker strategy.

2.2.2 Linkers

There are more than 200 linkers today.[127] Linkers need to withstand broad ranges of reagents and allow selective cleavage of the product. Several reviews have appeared on linker strategy.[128,129,130]

The Merrifield resin, named after its discoverer, is a simple resin with only a chloromethyl group attached to DVB cross-linked polystyrene (Figure 19). In the beginning, Merrifield's peptides were coupled to the Merrifield resin as benzyl esters, and harsh cleavage of the compounds was required, with hydrobromic or hydrofluoric acid.

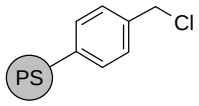


Figure 19. *Merrifield resin*

The introduction of the Wang resin with *p*-alkoxybenzyl alcohol linker in 1973 dramatically improved cleavage of the products (Figure 20).[131] Attached compounds can be released from the Wang resin with trifluoroacetic acid. Other functional groups, such as chloro or bromo substituents, can be introduced to the resin in place of the hydroxy group. SASRIN (super acid-sensitive) resin with 2-methoxy group is more acid-labile, and the products can be cleaved with 0.5–1% trifluoroacetic acid (Figure 20).[132] Peptides with *t*-Boc protected side-chains are released without deprotection of the side-chains due to the differences in acid labilities.[133] A related resin with aldehyde group is AMEBA (acid-sensitive methoxy benzaldehyde) (Figure 20).[134] Amines can be attached to the aldehyde group with a reductive amination. Acid-cleavable Rink resin was developed for peptide synthesis (Figure 20).[135] The resin is highly acid-sensitive, and cleavage of the peptide has been performed with highly diluted trifluoroacetic acid.

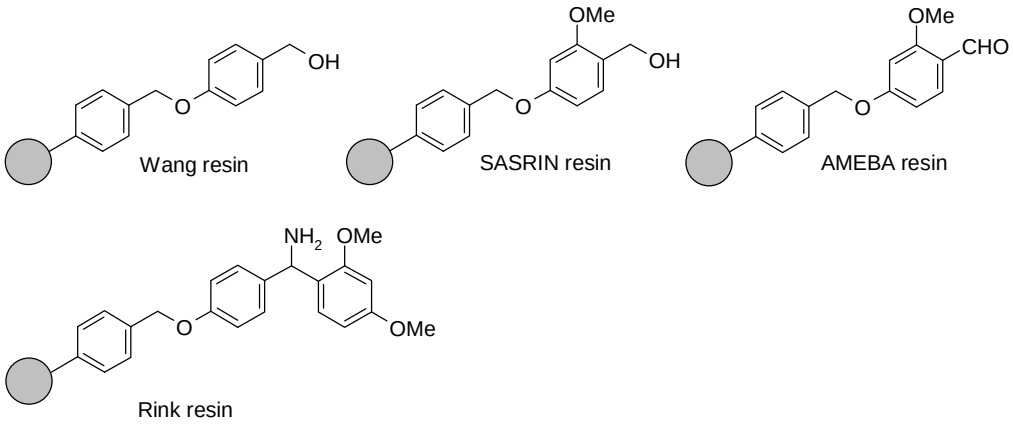


Figure 20. *Wang, SASRIN, AMEBA, and Rink resins*

An intermediate carbocation is formed during cleavage of the product from the resin in acidic conditions. The more stable the cation, the easier is the cleavage (Figure 21).

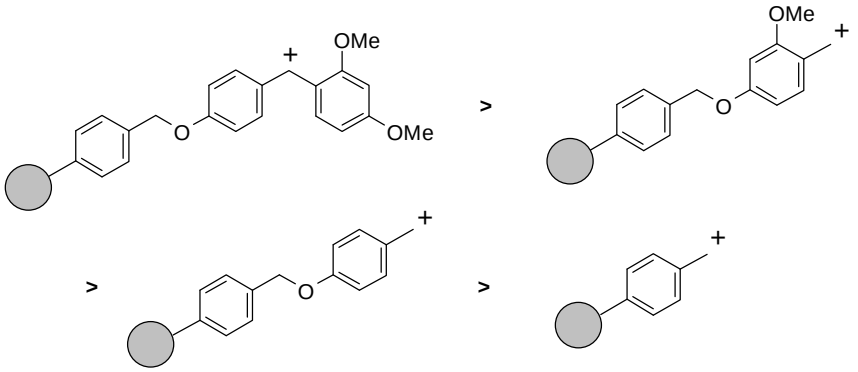


Figure 21. *Relative stabilities of the carbocations upon cleavage*

Trityl linkers were developed for the selective protection of polyhydroxy alcohols.[136] There are several related trityl resins with varying acid sensitivity (Figure 22). The trityl carbocation is a tertiary carbocation, which is highly stabilized by the delocalization of the positive charge over the phenyl rings. An electron-donating substituent in the phenyl ring increases the stability of the carbocation and facilitates the acidic cleavage of the product.

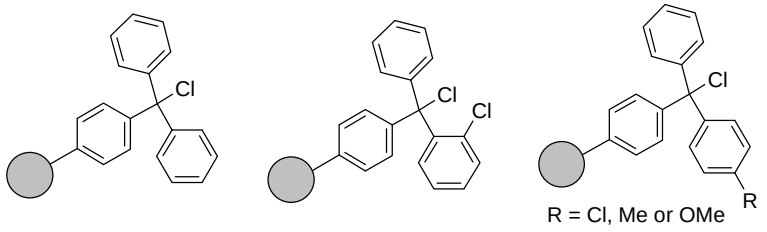


Figure 22. *Trityl resins*

2.2.3 Analysis of solid-phase reactions

Monitoring of the progress of reactions is important for the proper completion of the reactions, optimizing of the reaction conditions, and determining purities and yields. A special challenge of solid-phase synthesis is to determine what is attached to the resin and to what extent. While conventional solution-phase analytical techniques cannot be applied to polymer beads as such, small samples can be cleaved from the resin and analyzed by such conventional techniques as chromatography. Analytical methods suitable for solid supports have been studied intensively and extensively reviewed.[137,138,139,140] Only the most important and useful of these are discussed below.

Infrared (IR) spectroscopy can be used for the monitoring of functional groups, though it is suitable only for compounds with strong recognizable bands and gives limited quantitative information. At an early stage, IR technique was employed to monitor the functional groups in the solid-phase synthesis of oligosaccharides.[69] Various FT–IR methods have been applied in solid-phase synthesis. IR samples have long been analyzed as KBr pellets, though more sophisticated, rapid, and non-destructive methods are now available. Single-bead FT–IR microspectroscopy was developed especially for the real-time monitoring of organic reactions on solid support, the spectra being obtained with high signal-to-noise ratio from a single bead isolated fast from the reaction mixture.[141] In the attenuated total reflection (ATR) method, the FT–IR spectrum is obtained as a reflection of the IR beam on the resin bead.[142] ATR measurement is rapid, but the quality of the spectrum is poorer than that of spectra obtained by single-bead microspectroscopy.[143]

Although NMR analyses are highly informative, there are certain difficulties in the analysis of solid-phase samples. The heterogeneity of the resin means that sensitivity is poor and measuring times are long. Gel-phase ^{13}C NMR measurements of swollen resins give relatively low resolution spectra with broad linewidths and poor sensitivity. Improved signal-to-noise ratio is obtained with resins with ^{13}C-enriched building blocks.[144] The fluorine-containing linker in TentaGel resin enables ^{19}F NMR monitoring of reactions: the chemical shifts of fluorine are of wide range, and the structural transformations affect the position of the fluorine signal so that the reactions are easily monitored.[145] Magic angle spinning (MAS) NMR[146] was specifically developed for solid samples and has the advantage that it reduces the line broadening caused by restricted rotation of the sample, the inhomogeneous magnetic field, dipolar interactions, and chemical shift anisotropy.[147] The resolution of the spectra is improved as a result. Fitch *et al.*[148] reported the first nanoprobe MAS ^1H NMR measurement of an organic compound bound to TentaGel resin. MAS ^1H NMR was later found to be suitable for other resins, including the Merrifield resin.[149] The various NMR methods applied in solid-phase synthesis have been reviewed.[150]

Mass spectrometric measurements are more sensitive than IR or NMR measurements. One of the methods is matrix assisted laser desorption/ionization (MALDI) mass spectrometry, which was first applied to an acid cleavable Rink linker to analyze small compounds directly on bead.[151,152] Later the method was applied to real-time monitoring of organic reactions on solid supports with an ionizable and photocleavable linker.[153] MALDI analyses also give good structural information about compounds.[154] The mass spectrometric methods used in solid-phase synthesis have been thoroughly reviewed.[155,156,157]

Color tests are appropriate for fast qualitative detection, but quantitation is difficult. Colorimetric tests are available for several organic compounds, including amines, aldehydes, alcohols, thiols, and carboxylic acids.[158,159] Because of the original application of solid-phase synthesis to peptides, amino groups are probably the most common functionalities analyzed on solid supports. Kaiser's ninhydrin test[160] is a major method for determining amines.

Yan *et al.*[161] studied the suitability of combustion elemental analysis for C, H, N, Cl, S, and Br in solid-phase organic synthesis. The results were accurate and quantitative. Since small amounts of impurities in the resin affect the results, resins must first be carefully washed and dried.

The most common analytical methods in solid-phase synthesis are summarized in Table 3.

Table 3. *Positive (+) and negative (–) features of the most common analytical methods in solid-phase synthesis*

Method	***Comments***
IR spectroscopy	+ most common method
	– limited quantitative information
NMR spectroscopy	+ highly informative, non-destructive
	– inhomogeneous samples
MS spectroscopy	+ sensitive, rapid, gives structural information
	– selective cleavage method necessary
colorimetric tests	+ fast
	– nonspecific, difficult to quantitate
elemental analyses	+ quantitative
	– interference from impurities

2.2.4 1,3-Dipolar cycloadditions on solid supports

The first 1,3-dipolar cycloaddition on a solid support was reported in 1980 by Yedidia and Leznoff.[162] 1,3-Dipolar cycloadditions on solid supports have been reviewed, with coverage of the literature up to December 2003.[163] The review was divided into three parts

treating cycloadditions to resin-bound dipolarophiles, cycloadditions to resin-bound dipoles, and intramolecular cycloadditions. 1,3-Dipolar cycloadditions on solid supports are now discussed below on the basis of linkers and cleavages.

As noted above, the most common resin in solid-phase 1,3-dipolar cycloadditions is polystyrene, and roughly 90% of solid-phase 1,3-dipolar cycloaddition reactions have been performed with polystyrene-based resin. In addition, soluble PEG resin has been used in the synthesis of isoxazoles,[164] MeOPEG resin in the synthesis of pyrrolines[165] and 1,2,3-triazoles,[166,167] ArgoGel resin in the synthesis of pyrrolidines[168] and imidazoles,[169] and TentaGel resin in the synthesis of pyrrolidines[170] isoxazolidines,[171] and 1,2,3-triazoles.[172] PEGA resin has been used for the copper-catalyzed synthesis of peptidotriazoles.[3]

The ester linkage is widely exploited in 1,3-dipolar cycloaddition reactions. Various products, such as pyrrolidines, 1,2,3-triazoles, isoxazolines, and isoxazolidines have been cleaved from the resin as acids or esters (Table 4). SASRIN- and trityl resin-bound esters can be cleaved with highly diluted trifluoroacetic acid.

Table 4. *Ester linkage in 1,3-dipolar cycloadditions on solid supports*

Resin	***Dipolarophile + Dipole → product***	***Cleavage conditions and product***	***Reference***
Wang	alkene (maleimide) + resin-bound azomethine ylide → pyrrolidine	50% TFA–DCM cleavage as carboxylic acid	[173,174]
Wang	resin-bound alkene (acrylate) + azomethine ylide → pyrrolidine	50% TFA–DCM cleavage as carboxylic acid $NaCN/Et_3N/THF/MeOH$ cleavage as methyl ester	[175]
SASRIN	resin-bound alkene (maleimide) + azomethine ylide → pyrrolidine	0.5% TFA–DCM cleavage as carboxylic acid	[176]
Wang	alkyne + resin-bound azide → 1,2,3-triazole	50% TFA–DCM cleavage as carboxylic acid	[177]
Wang	alkene/resin-bound alkene + resin-bound nitrile oxide/nitrile oxide → isoxazoline	20% TFA–DCM cleavage as carboxylic acid	[178,179]
2-Chlorotrityl resin	resin-bound alkene (acrylate) + nitrone → isoxazolidine	5% TFA–DCM cleavage as carboxylic acid	[180]

Rink linker is typically used to attach amides to the solid support (Table 5). Cleavage of the products as amides can then be achieved with trifluoroacetic acid. Phenolic ether linkage has been used for the synthesis of pyrrolidines, isoxazoles, isoxazolines, and isoxazolidines on Wang and trityl resins (Table 6). Treatment with trifluoroacetic acid gives the products as phenols.

Table 5. *Amide linkage in 1,3-dipolar cycloadditions on solid supports*

Resin	***Dipolarophile + Dipole → product***	***Cleavage conditions and product***	***Reference***
Rink	alkyne + resin-bound azomethine ylide (münchnone) → pyrrole	15–20% TFA–DCM cleavage as amide	[181]
Rink	alkyne + resin-bound azomethine ylide (münchnone) → pyrrole	20% TFA–DCM cleavage as amide	[182]
Rink	resin-bound alkene (vinyl sulfone) + azomethine imine → pyrroline	25% TFA–DCM cleavage as amide	[183]
Rink	resin-bound alkene/alkyne + nitrile oxide → isoxazoline/isoxazole	20% TFA cleavage as amide	[97]
Rink	alkene + resin-bound nitrone → isoxazolidine	95% TFA– H_2O cleavage as amide	[180]

Table 6. *Ether linkage in 1,3-dipolar cycloadditions on solid supports*

Resin	***Dipolarophile + Dipole → product***	***Cleavage conditions and product***	***Reference***
Wang	resin-bound alkene + azomethine ylide → pyrrolidine	50% TFA–DCM cleavage as phenol	[184]
Wang	alkene (maleimide) + resin-bound azomethine ylide → pyrrolidine	50% TFA–DCM cleavage as phenol	[185]
Wang	alkene + resin-bound nitrile oxide → isoxazoline	10% TFA–DCM cleavage as phenol	[186]
Wang	alkene + resin-bound nitrile oxide → isoxazoline	20% TFA–DCM cleavage as phenol	[179]
Chlorotrityl resin	alkene/alkyne + resin-bound nitrile oxide → isoxazoline/isoxazole	1% TFA–DCM cleavage as phenol	[187]
2-Chlorotrityl resin	alkene + resin-bound nitrone → isoxazolidine	5% TFA–DCM cleavage as phenol	[180]

Alcohols have been cleaved from the resin with a reductive cleavage (Figure 23).[167] Alkyne-functionalized alcohol was attached to the resin with an oxalyl chloride linkage, cycloaddition of alkyne with carbohydrate-derived azides gave resin-bound 1,2,3-triazoles, and reductive cleavage with sodium borohydride freed the products as alcohols.

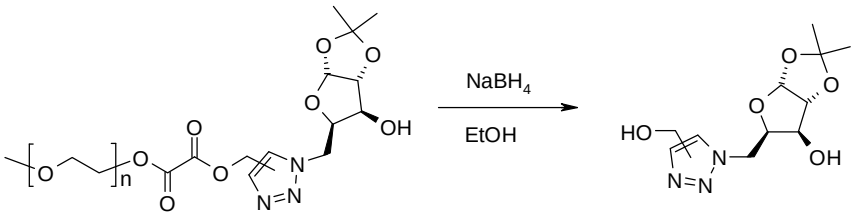


Figure 23. *Reductive cleavage of 1,2,3-triazoles*[167]

Alcohols have also been attached to the resin with a tetrahydropyranyl linker (Figure 24).[188] Resin-bound nitrile oxide was reacted with various alkynes, and cleavage with diluted trifluoroacetic acid gave isoxazoles as alcohols in a traceless manner.

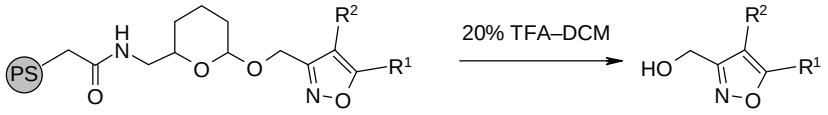


Figure 24. *Tetrahydropyranyl-linked isoxazole*[188]

Traceless cleavage is highly desirable in solid-phase synthesis, and much effort has gone into the development of traceless methods. Removal of peptides from the solid support is straightforward, as the amide bond is easily cleaved. Cleavage of diversely linked small organic compounds from solid supports presents challenges of a different order. In the worst case the linker of the resin accompanies the cleaved product. Originally the resin was used as a protecting group for a specific functionality; nowadays more sophisticated traceless methods to cleave organic compounds from the resin are available.[189] Many traceless cleavages of cycloadducts have been reported. Heterocycles have commonly been linked to the resin with a benzylic C–N bond. SASRIN resin has a 2-methoxy group that facilitates C–N bond cleavage, and it has been used in a variety of applications. Resin-bound imidazoles have been obtained in cycloadditions with resin-bound münchnones and imines and the products then cleaved in a traceless manner with acetic acid (Figure 25).[169]

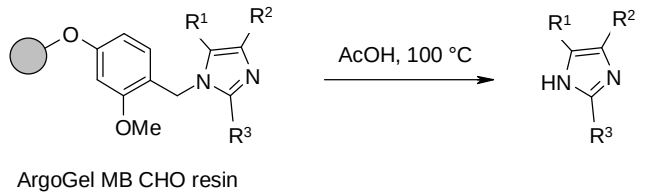


Figure 25. *Traceless cleavage of imidazoles*[169]

Related cycloaddition of resin-bound münchnones with N=N double bond gave 1,2,4-triazoles that could be cleaved from the resin with 30% trifluoroacetic acid (Figure 26).[190]

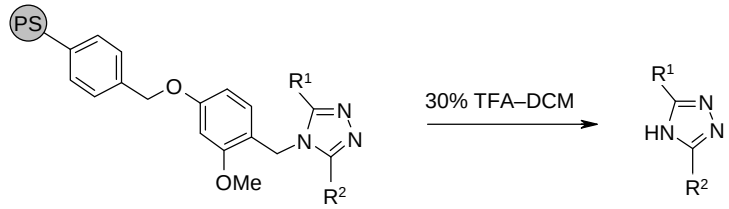


Figure 26. *Traceless cleavage of 1,2,4-triazoles*[190]

2-Methoxy-substituted resin has also been used for the synthesis of imidazoles on solid support. In this case, the cleavage occurs with the linker because the imidazole core is attached directly to the aryl ring with a carbon–carbon bond (Figure 27).[191]

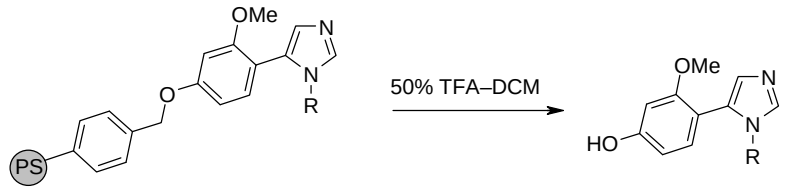


Figure 27. *Cleavage of imidazoles with a linker*[191]

Oxidative cleavage of benzylic C–N bond has been achieved with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) with release of isoxazolines in a traceless manner (Figure 28).[192] Cleavage of benzylic C–N bond has also been obtained with acyl chlorides in the presence of potassium iodide (Figure 29).[193] Here pyrrolidines were released from the resin as amides.

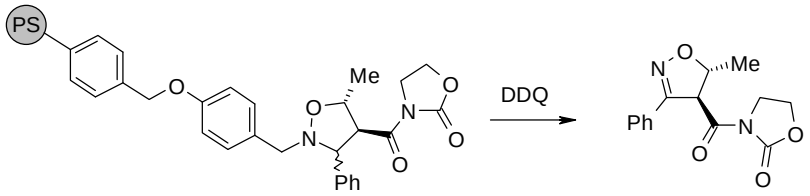


Figure 28. *Oxidative C–N bond cleavage*[192]

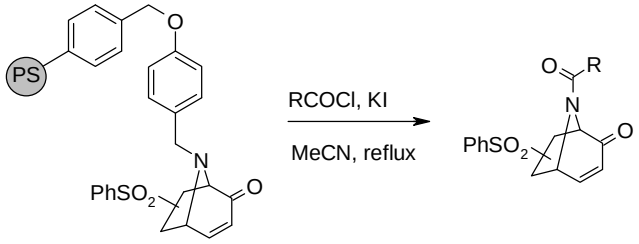


Figure 29. *Acylative C–N bond cleavage*[193]

Trityl resin is highly acid-sensitive and has been used in 1,3-dipolar cycloadditions with various linkers. Traceless cleavage of the C–N bond between the resin and the cycloadduct was achieved with 50% TFA–DCM (Figure 30).[194]

Figure 30. *Traceless C–N bond cleavage from trityl resin*[194]

Traceless cleavage of products from the resin can also be achieved through elimination reaction. Aromatization of the product is the driving force for the elimination. Elimination of the piperazine linker with highly diluted trifluoroacetic acid yielded pyrazoles in a traceless manner (Figure 31).[195]

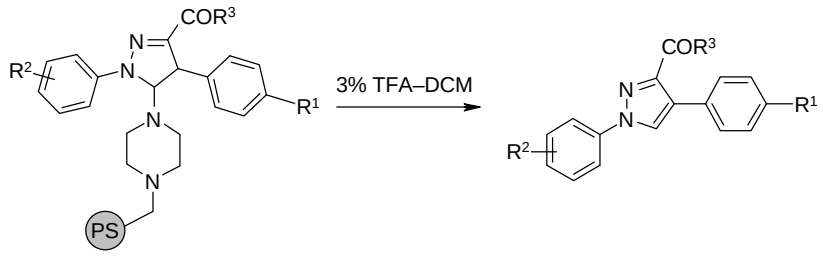


Figure 31. *Traceless cleavage of pyrazoles*[195]

Isoxazoles, too, have been obtained through elimination reaction (Figure 32).[196] Cycloaddition of nitrile oxide to resin-bound vinyl ether gave resin-bound isoxazoline, which aromatized to isoxazole after the elimination.

Figure 32. *Traceless cleavage of isoxazoles*[196]

Resin-bound isoxazolines have been obtained by reaction of resin-bound alkene with nitrile oxides (Figure 33).[197,198] Traceless cleavage was then carried out under alkaline conditions.

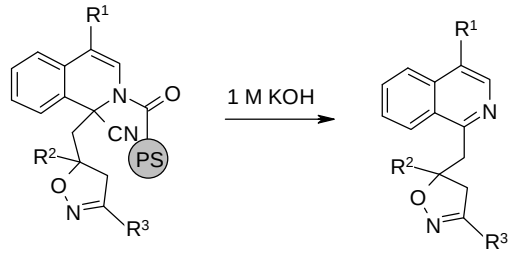


Figure 33. *Traceless cleavage of isoxazolines*[197,198]

Selenium linker has been used for the preparation of various nitrogen-containing heterocycles. Cycloaddition of resin-bound alkyne to nitrile oxide gave resin-bound isoxazoles, and traceless oxidative cleavage of the isoxazoles was obtained via elimination of the resin with hydrogen peroxide (Figure 34).[199] A related method has been applied for the synthesis of various nitrogen heterocycles. Reaction of resin-bound alkyne or alkene with azides, nitrile oxides, or azomethine ylides yielded heterocycles such as 1,2,3-triazoles, isoxazoles, isoxazolines, and pyrrolines.[200,201,202,203,204]

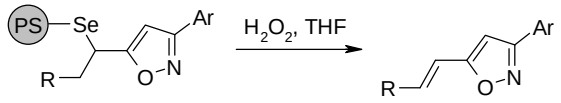


Figure 34. *Traceless selenium linker*[199]

Traceless cleavage can also be obtained with a sulfone linker. Resin-bound vinyl sulfone was reacted with sodium azide under microwave irradiation, and 1,2,3-triazoles were obtained in a traceless manner (Figure 35).[205] Traceless cleavage with a sulfone linker has also been utilized in the solid-phase synthesis of isoxazolines[206] and isoxazoles.[207]

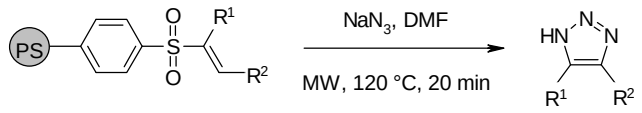


Figure 35. *Traceless sulfone linker*[205]

Silyl linkage has been applied in the traceless synthesis of pyrrolidines. Acidic or alkylative cleavage of the N–Si bond (Figure 36)[208] and cleavage of the C–Si bond with tetrabutylammonium fluoride (TBAF) (Figure 37)[209] released pyrrolidines from the resin in a traceless manner.

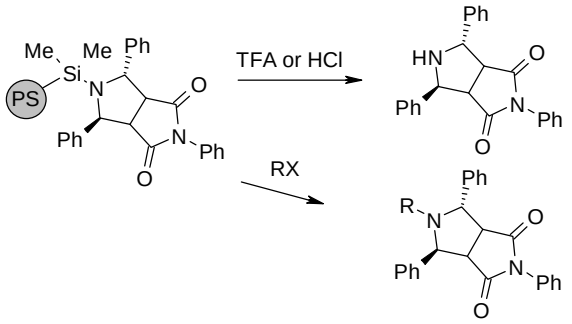


Figure 36. *Traceless cleavage of N–Si bond*[208]

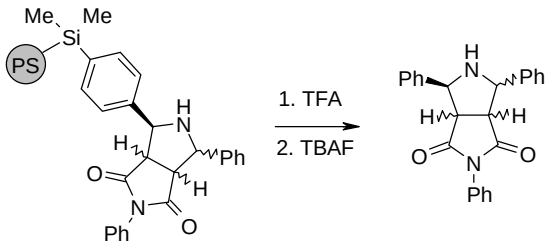


Figure 37. *Traceless cleavage of C–Si bond*[209]

Cyclization is also known to release resin-bound compounds without the linker. Isoxazoles were cleaved from the resin via thiohydantoin[210] or hydantoin[211] formation (Figure 38), and 1,2,3-triazoles were released with lactone formation (Figure 39).[212]

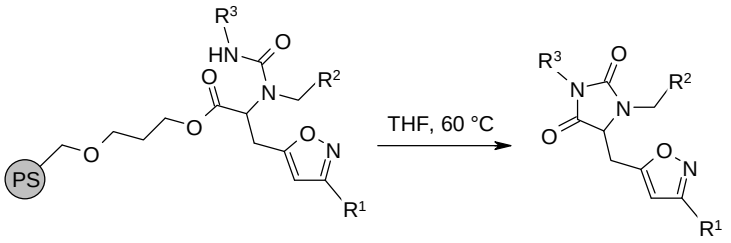


Figure 38. *Traceless cyclization cleavage of isoxazoles*[211]

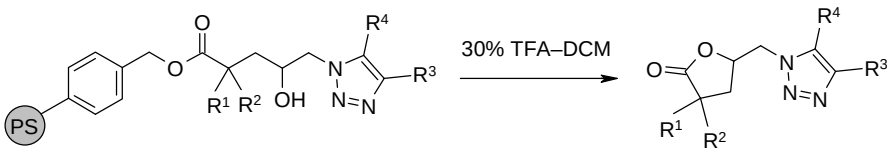


Figure 39. *Traceless cyclization cleavage of 1,2,3-triazoles*[212]

Tornøe *et al.*[3] reported the first solid-phase copper-catalyzed formation of peptidotriazoles. Since then the copper-catalyzed reaction has been employed in various solid-phase syntheses of peptides and oligonucleotides coupled with 1,2,3-triazoles. 1,3-Dipolar cycloadditions of peptides have been performed with either alkyne[213,214] or azide[215] attached to the resin. Several intramolecular reactions leading to cyclic peptides have also been reported.[216,217,218,219,220] Moreover, peptidomimetics,[221] oligonucleotides,[222] peptide–oligonucleotide conjugates,[223] and cyclic oligonucleotides[224] linked with 1,2,3-triazole ring have been prepared on solid supports via copper-catalyzed 1,3-dipolar cycloadditions. 1,2,3-Triazole linkage has also been used in block copolymers.[225] Additionally, a 1,2,3-triazole-based solid-phase click linker has been developed (Figure 40).[226] Several related studies have been reported where 1,2,3-triazole acts as a linker on solid support.[227,228,229]

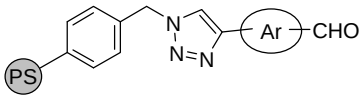


Figure 40. *1,2,3-Triazole click linker*[226]

To summarize, 1,3-dipolar cycloadditions on solid supports have been widely studied. Various resins, linkers, and cleavage strategies have been exploited for the synthesis of nitrogen-containing heterocycles. Esters, amides, and ethers are the most common linkers. Additionally, many traceless cleavages of heterocycles have been reported. Solid-phase synthesis allows faster isolation and purification of the intermediates. Other methods besides solid-phase synthesis used to enhance reactions make use of parallel reactions, automation, and microwave reactors, and these are discussed in the following section.

2.3 High-throughput synthesis

The automated synthesis of peptides was first reported by Merrifield in 1965.[230,231,232] Parallel methods and combinatorial chemistry were developed soon thereafter, and Frank *et al.*,[233] Geysen *et al.*,[234] and Houghten[235] announced the principles of library synthesis in the 1980s. Chiron's synthesizer (1990, Chiron Corporation),[236] the Diversomer apparatus (1993, ChemGlass, Inc.),[237] and the Nautilus™ 2400 instrument (1996, Argonaut Technologies)[238] were among the first commercial devices for parallel organic synthesis. As listed in Table 7, a wide variety of instruments are now commercially available for parallel evaporations, parallel reactions, microwave-assisted synthesis, and automated purifications. Several reviews of automated methods,[239,240,241] combinatorial chemistry,[242,243,244] and the synthesis of compound libraries have been published.[245,246]

Table 7. *Some commercially available instruments for high-throughput synthesis*

Instrument	***Purpose***	***Manufacturer***
Genevac series	parallel evaporations	Genevac Ltd (Ipswich, UK)
SpeedVac systems	parallel evaporations	Thermo Fisher Scientific Inc. (Watham, MA, USA)
TurboVap® concentration workstation	parallel evaporations	Caliper Life Sciences (Hopkinton, MA, USA)
Greenhouse evaporator	parallel evaporations	Radleys (Essex, UK)
Syncore® polyvap system	parallel evaporations	Büchi (Flawil, Switzerland)
MicroDancer evaporator	parallel evaporations	Zinsser Analytic GmbH (Frankfurt, Germany)
Radleys carousel reaction station	parallel reactions	Radleys (Essex, UK)
Greenhouse plus parallel synthesizer	parallel reactions	Radleys (Essex, UK)
Mettler MiniBlock rack	parallel reactions	Mettler-Toledo Inc. (Columbus, OH, USA)
Syncore® reactor	parallel reactions	Büchi (Flawil, Switzerland)
CEM microwave systems	microwave reactions	CEM Corporation (Matthews, NC, USA)
Milestone MultiSYNTH labstation	microwave reactions	Milestone Inc. (Monroe, CT, USA)
Biotage Initiator microwave synthesizer	microwave reactions	Biotage (Uppsala, Sweden)
CombiFlash® flash chromatography system	automated purification	Teledyne Isco Inc. (Lincoln, NE, USA)
FlashMaster flash chromatography system	automated purification	Biotage (Uppsala, Sweden)
Biotage Isolera flash purification system	automated purification	Biotage (Uppsala, Sweden)

Domestic microwave ovens first became available in the 1970s and the first microwave-assisted organic reactions were performed by Gedye *et al.*[247] and Giguere *et al.*[248] in 1986. Since then, a large number of publications have demonstrated the utility of microwaves, and microwave reactions have been widely reviewed.[249,250,251,252] Advantages of microwaves include faster reactions, reduced solvent amounts, better yields, and higher purities. Both temperature- and pressure-controlled reactions are easily monitored and reproduced. Microwave reactors are set to operate at a specific frequency (2.45 GHz) in order to avoid interference with telecommunications equipment.[253] Molecular motion and rotation of the dipoles in an alternating electric field causes friction that produces heat.[254] Superheating of a solvent is possible, allowing the temperature to rise over its boiling point. Solvents, or at least reagents, should be polar. Solvents without a dipole moment, such as benzene, 1,4-dioxane, and tetrachloromethane, are transparent to microwaves.[253] Additionally, reaction vessels are prepared from borosilicate glass, quartz, or Teflon, which are not heated under microwave irradiation. Today, dedicated microwave reactors are common laboratory equipment and microwaves can be regarded as a viable alternative to conventional heating.

Microwave-assisted reactions are now widely utilized in solid-phase synthesis. The first microwave-assisted solid-phase peptide coupling was reported by Yu *et al.* in 1992.[255] Stille couplings and Suzuki couplings[256] were among the first solid-phase organic reactions to be facilitated with microwave irradiation. Polystyrene-based Wang resin has been found to be stable under microwave irradiation even at 200 °C.[257] Microwave-assisted sequential,[258] parallel,[259,260] and continuous flow[261,262] reactions have also been reported. Microwave-assisted synthesis of heterocycles has recently been reviewed.[263] Cycloadditions often need long reaction times at elevated temperatures, and the benefit of microreactors is clear. The first microwave-assisted syntheses involved Diels-Alder, Claisen, and ene reactions.[248] Microwaves have been used to enhance several 1,3-dipolar cycloadditions.[264,265,266] Various microwave-assisted syntheses of 1,2,3-triazoles and tetrazoles have been reported to replace the long reaction times required in conventional cycloadditions of azides with alkynes and nitriles (Table 8).

Table 8. *Reaction conditions in microwave-assisted synthesis of 1,2,3-triazoles and tetrazoles via 1,3-dipolar cycloadditions compared with reaction conditions in conventional heating*

Product	***MW irradiation***	***Conventional heating***	***Reference***
1,2,3-triazole	MW 120–200 °C, 5–10 min	120 °C, >24 h	[267]
1,2,3-triazole	MW 55–85 °C, 30 min	110 °C, 12 h	[268]
1,2,3-triazole	MW 120–210 °C, 15 min	180 ºC, 48 h, no reaction	[269]
1,2,3-triazole	MW 55–100 °C, 0.5–1 h	60 °C, 18 h	[270]
tetrazole	MW 100 °C, 1 h	150 °C, 12 h	[271]
tetrazole	MW 200 °C, 15–25 min	105 °C, 10–12 h	[272]
tetrazole	MW 140 °C, 8 h	105 °C, 72 h	[273]
tetrazole, $ZnBr_2$ catalyst	MW 80 °C, 15–45 min	100 °C, 12–48 h	[274,275]
tetrazole, Cu catalyst	MW 80 °C, 2 h	20 °C, 4–48 h	[276]

Altogether, high-throughput synthesis offers a wide variety of methods to prepare compound libraries. Instruments and equipment specifically designed for organic high-throughput synthesis are now commercially available, and the advantages of automation, parallel reactions, and microwave-assisted reactions are well appreciated. High-throughput synthesis is now an essential part of drug discovery.

With the history and recent progress of 1,3-dipolar cycloadditions, solid-phase techniques, parallel methods, and microwave-assisted reactions now reviewed, the following chapters turn to the practical work summarized in this thesis. Chapter 3 sets out the aims of the study, chapter 4 describes the materials and methods, and chapter 5 summarizes the results. The conclusions are presented in a final chapter.

3 AIMS OF THE STUDY

The aim of the study was to synthesize five-membered nitrogen-containing heterocycles via 1,3-dipolar cycloaddition reactions with use of solid-phase and parallel techniques.

The more specific aims of the research were

- to study linker strategies and develop traceless syntheses (I, III)
- to apply parallel reaction techniques for the preparation of compound libraries (II, III, IV)
- to facilitate reactions with the use of microwaves (III, IV)

4 MATERIALS AND METHODS

The solvents, resins, and reagents used in the study are listed in Tables 9, 10, and 11, respectively, and materials and instrumentation in Table 12. Unless otherwise stated, the solvents (Table 9) were of analytical or HPLC grade and were obtained from more than one supplier. The solvents and reagents were purchased from the following sources: Acros Organics (Geel, Belgium), Altia Oyj (Rajamäki, Finland), Bachem (Weil am Rhein, Germany), Chem-Impex International (Wood Dale, IL, USA), Ega Chemie (Steinheim, Germany), Fisher Scientific International (Loughborough, Leicestershire, UK), Fluka (Buchs, Switzerland), J. T. Baker (Deventer, Holland), Lab-Scan Analytical Sciences (Gliwice, Poland), Lancaster (Morecambe, Lancashire, UK), Merck (Darmstadt, Germany), Novabiochem (Läufelfingen, Switzerland), Rathburn (Walkerburn, Scotland), Riedel-de Haën (Seelze, Germany), Sigma-Aldrich (Steinheim, Germany), Oy Woikoski Ab (Voikoski, Finland).

Table 9. *Solvents used in the study*

Solvents	***Publication***
acetonitrile	II, III, IV
benzene, Fluka	III
chloroform	II
chloroform, deuterated ($CDCl_3$)	III, IV
dichloromethane	I, II, III, IV
1,4-dioxane, Lab-Scan Analytical Sciences	III
N,*N*-dimethylformamide	I, II, IV
dimethyl sulfoxide, deuterated (DMSO–d_6)	I, II, III, IV
ethanol (96%), Altia Oyj	II
ethyl acetate	I, II, III, IV
n-hexane	I, II, III, IV
methanol	I, II, III, IV
methanol, deuterated (CD_3OD)	I
2-propanol	II
tetrahydrofuran	I, II, III
toluene	I, III, IV
1,1,1-trifluorotoluene, Sigma-Aldrich	IV

Table 10. *Resins used in the study*

Resin	***Publication***
4-(bromomethyl)phenoxymethyl polystyrene, Novabiochem 01-64-0186 100–200 mesh, 1% cross-linked with divinylbenzene, loading 0.76–1.7 mmol/g	I, II
4-hydroxy-2-methoxybenzyl alcohol, polymer-bound, Sigma-Aldrich 54,073-0 50–90 mesh, 1% cross-linked with divinylbenzene, loading 0.4 mmol/g	I
4-hydroxy-2-methoxybenzaldehyde, polymer-bound (Ameba resin), Aldrich 51,644-9 100–200 mesh, 1% cross-linked with divinylbenzene, loading 1.2 mmol/g	III
N-benzyl-*N*′-cyclohexylcarbodiimide, polymer-bound, Aldrich 561843 100–200 mesh, 1% cross-linked with divinylbenzene, loading 1.7 mmol/g	IV

Table 11. *Reagents used in the study*

Reagent, [CAS], producer, purity	***Publication***
acetic acid, [64-19-7], Merck, p.a.	II
acetic anhydride, [108-24-7], Riedel-de Haën, puriss p.a., ≥99%	III, IV
acetylenedicarboxylic acid, [142-45-0], Fluka, purum, ≥94.0%	II
DL-alanine methyl ester hydrochloride, [13515-97-4], Sigma	III
L-2-aminobutyric acid methyl ester hydrochloride, [15399-22-1], Bachem, >99%	III
L-2-amino-3-cyclohexylpropionic acid methyl ester hydrochloride [17193-39-4] Novabiochem, ≥98%	III
L-2-aminohexanoic acid methyl ester hydrochloride, [3844-54-0], Bachem, >99%	III
1-aminopyridinium iodide, [6295-87-0], Aldrich, 97%	II
L-2-aminovaleric acid methyl ester hydrochloride, [56558-30-6], Bachem, >99%	III
ammonium acetate, [631-61-8], Fluka, 99.995%	I, II
benzyl alcohol, [100-51-6], Aldrich, >99%	IV
2-butyne-1,4-diol, [110-65-6], Fluka, purum, ≥98.0%	I
2-butynoic acid, [590-93-2], Aldrich, 98%	II
calcium hydride, [7789-78-8], Fluka, puriss	III, IV
cesium iodide, [7789-17-5], Riedel-de Haën, 99.5%	II
N,*N*'-dicyclohexylcarbodiimide, [538-75-0], Novabiochem	IV
N,*N*'-diisopropylcarbodiimide, [693-13-0], Aldrich, 99%	IV
dimethyl acetylenedicarboxylate, [762-42-5], Fluka, purum, ≥96.0%; Aldrich, 99%	I, III, IV
N-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride, [25952-53-8], Fluka, purum, ≥98.0%	III, IV
N-ethyldiisopropylamine, [7087-68-5], Fluka, purum, ≥98.0%	II
ethyl phenylpropiolate, [2216-94-6], Merck, ≥98.0%	I, III
ethyl propiolate, [623-47-2], Fluka, purum, ≥99.0%; Aldrich, 99%	I, III
ethyl-3-(1-pyrrolidinyl)acrylate, [65651-80-1], Lancaster	I
formic acid, [64-18-6], Riedel-de Haën, 98–100%	III, IV
glycine methyl ester hydrochloride, [5680-79-5], Fluka, puriss, ≥99.0%	III

Table 11. *Reagents used in the study (continued)*

Reagent, [CAS], producer, purity	***Publication***
hydriodic acid, [10034-85-2], Fluka, puriss, ≥67%; Sigma-Aldrich, 57%	II
hydrochloric acid, [7647-01-0], J.T. Baker, 37–38%	III
hydrogen gas, [1333-74-0], Oy Woikoski Ab	IV
hydroxylamine-*O*-sulfonic acid, [2950-43-8], Acros Organics, 97%	II
3-(hydroxymethyl)pyridine, [100-55-0], Acros Organics, 98%	II
L-isoleucine methyl ester hydrochloride, [18598-74-8], Bachem	III
isonicotinic acid, [55-22-1], Fluka, purum, ≥99.0%	IV
isoquinoline, [119-65-3], Acros Organics, 97%	II
lepidine (4-methylquinoline), [491-35-0], Aldrich, 99%	II
L-leucine, [61-90-5], Fluka, >99%	IV
L-leucine methyl ester hydrochloride, [7517-19-3], Aldrich, 98%	III
L-methionine methyl ester hydrochloride, [2491-18-1], Bachem, ≥99.0%	III
methyl isonicotinate, [2459-09-8], Acros Organics, 98%	II
methyl propiolate, [922-67-8], Aldrich, 99%	I, III, IV
nicotinic acid, [59-67-6], Ega Chemie, 99%	IV
4-nitro-L-phenylalanine methyl ester hydrochloride, [17193-40-7], Bachem, >99%	III
oxalyl chloride, [79-37-8], Fluka, puriss, ≥99.0%	IV
palladium, 10 wt. % on activated carbon, Aldrich	IV
phenylacetylene, [536-74-3], Fluka, purum, ≥97.0%	I
L-phenylalanine methyl ester hydrochloride, [7524-50-7], Bachem, >99%	III
N_6-[(phenylmethoxy)carbonyl]-L-lysine methyl ester hydrochloride [27894-50-4], Bachem >98%	III
S-(phenylmethyl)-L-cysteine methyl ester hydrochloride, [16741-80-3], Bachem, >99%	III
O-(phenylmethyl)-L-serine methyl ester hydrochloride, [19525-87-2], Bachem, >98%	III
O-(phenylmethyl)-L-tyrosine methyl ester hydrochloride, [34805-17-9], Bachem, >99%	III
phenylpropargyl aldehyde, [2579-22-8], Aldrich, 96%	I
phenylpropiolic acid, [637-44-5], Aldrich, 99%	I, II
2-picoline, [109-06-8], Acros Organics, 98%	II
3-picoline, [108-99-6], Fluka, purum, ≥98.0%	II
DL-pipecolinic acid, [535-75-1], Aldrich, 98%	IV
potassium bromide, [7758-02-3], Sigma-Aldrich, FT–IR grade, ≥99%	I, II, III, IV
potassium carbonate, [584-08-7], Aldrich, ≥99%; Merck, 99%	II
propargylamine, [2450-71-7], Aldrich, 98%	I
propiolic acid, [471-25-0], Acros Organics, 98%; Aldrich, 95%	I, II
1-pyrrolidino-1-cyclohexene, [1125-99-1], Acros Organics, 95%	I
1-pyrrolidino-1-cyclopentene, [7148-07-4], Acros Organics, 97+%	I
quinoline, [91-22-5], Acros Organics, 99%	II
sarcosine benzyl ester *p*-toluenesulfonate, [54384-06-4] Chem-Impex International, Inc., >99%	IV
sodium azide, [26628-22-8], Riedel-de Haën	I

Table 11. *Reagents used in the study (continued)*

Reagent, [CAS], producer, purity	***Publication***
sodium hydrogen carbonate, [144-55-8], J. T. Baker	IV
sodium hydroxide, [1310-73-2], J. T. Baker	I, III
sodium methoxide, [124-41-4], unspecified source	II
sodium nitrite, [7632-00-0], Ph. Nord., unspecified source	I, III
sodium sulfate anhydrous, Fisher Scientific International ≥99%	IV
sodium triacetoxyborohydride, [56553-60-7], Aldrich, 95%	III
98% sulfuric acid, [7664-93-9], unspecified source	I
thionyl chloride, [7719-09-7], Merck ≥99.0%	I
p-toluenesulfonic acid monohydrate, [6192-52-5], Aldrich, 98.5%	IV
triethylamine, [121-44-8], Riedel-de Haën, purum, ≥99%; Sigma-Aldrich, ≥99%	III
trifluoroacetic acid, [76-05-1], Riedel-de Haën, 99%; Fluka, purum, ≥98.0%	I, II, III
trimethylsilylacetylene, [1066-54-2], Fluka, purum, ≥98.0%	I
L-valine benzyl ester *p*-toluenesulfonate, [16652-76-9] Chem-Impex International, Inc.	IV
D-valine methyl ester hydrochloride, [7146-15-8], Fluka, purum, ≥99.0%	III

Table 12. *Materials and instrumentation used in the study*

Material or instrument	***Publication***
Radleys 12-place carousel reaction station (Essex, UK)	II, III, IV
Heidolph MultiReax shaker (Schwabach, Germany)	II, III
B. Braun syringes 2 mL (4606027V), 10 mL (4606108V), and 20 mL (4616200V) with self-made polyethylene filters (Melsungen, Germany)	II, III
Isolute IST VacMaster-10 manifold for filtration (Hengoed, UK)	II, III
Biotage Microwave Initiator EXP EU (Uppsala, Sweden)	III, IV
Genevac HT-4 series II evaporator (Ipswich, UK)	I, II
Merck TLC aluminum sheets coated with silica gel 60 F_{254} (Darmstadt, Germany)	I, II, III, IV
Merck silica gel 60, 0.040–0.063 mm (Darmstadt, Germany)	I
Celite 545, Filter Agent, Sigma-Aldrich (Steinheim, Germany)	IV
Biotage SP4 Flash chromatography purification system (Charlottesville, VA, USA)	II
Biotage SP1-A2C Flash chromatography purification system (Charlottesville, VA, USA)	III, IV
Biotage 12+M (FPK0-1107-15046) or Biotage 25+M (FPK0-1107-16046) silica cartridges (Uppsala, Sweden)	II, III, IV
Bibby Stuart Scientific SMP melting point apparatus (UK)	I
Electrothermal IA9100 digital melting point apparatus (Essex, UK)	II, III, IV
Varian Unity 500 NMR spectrometer (Palo Alto, CA, USA)	I
Varian Mercury 300 Plus NMR spectrometer (Palo Alto, CA, USA)	I, II, III, IV
Bruker Avance 300 NMR spectrometer (Ettlingen, Germany)	II
Perkin Elmer FT–IR spectrometer 1725X (Waltham, MA, USA)	I
Bruker Vertex 70 FT–IR spectrometer (Ettlingen, Germany)	II, III, IV

Table 12. *Materials and instrumentation used in the study (continued)*

Material or instrument	***Publication***
Agilent HP 1100 series instrument (Waldbronn, Germany) with API 3000 triple quadrupole mass spectrometer (MDS Sciex, Concord, Canada)	I, II
Agilent HP 1100 series instrument (Waldbronn, Germany) with Esquire-LC Bruker Daltonik ion trap mass spectrometer (Bremen, Germany)	III, IV
Waters Micromass Q-Tof Micro quadrupole time-of-flight mass spectrometer (Manchester, UK)	II
MilliQ water system (Millipore, MA, USA)	I, II, III, IV
Merck Chromolith Speed ROD RP-18 (50 × 4.6 mm) column (Darmstadt, Germany)	I
Waters XTerra MS RP18 (4.6 × 30 mm, 2.5 µm) column (Milford, MA, USA)	II, III, IV

5 RESULTS AND DISCUSSION

This chapter describes the various techniques that were used in the synthesis of the five-membered nitrogen-containing heterocycles (section 5.1), the reactivity of the 1,3-dipoles and dipolarophiles (section 5.2), the solid-phase methods that were employed (section 5.3), regiochemistry of the 1,3-dipolar cycloadditions (section 5.4), and the analytical methods that were applied (section 5.5). Detailed information about the methods and the results can be found in the original articles (I–IV).

5.1 Techniques used in the synthesis of five-membered heterocycles

Nitrogen-containing five-membered heterocycles were synthesized via 1,3-dipolar cycloadditions with use of solid-phase techniques, parallel methods, and microwave irradiation. The prepared compounds were 1,2,3-triazoles from polymer-bound azides and alkynes or enamine (I), pyrazolopyridines from polymer-bound alkynes and azomethine imines (II), pyrazoles from polymer-bound sydnones and alkynes (III), and pyrroles from münchnones or azlactones and alkynes (IV). 1,2,3-Triazoles were cleaved from the resin along with the linker or in a traceless manner (I). Solid-phase techniques were carried out with either the dipole (I, III) or dipolarophile (II) attached to the resin. Polymer-bound reagent was tested as well (IV). The core structures of the final products are presented in Figure 41 and the strategies of the synthesis in Table 13.

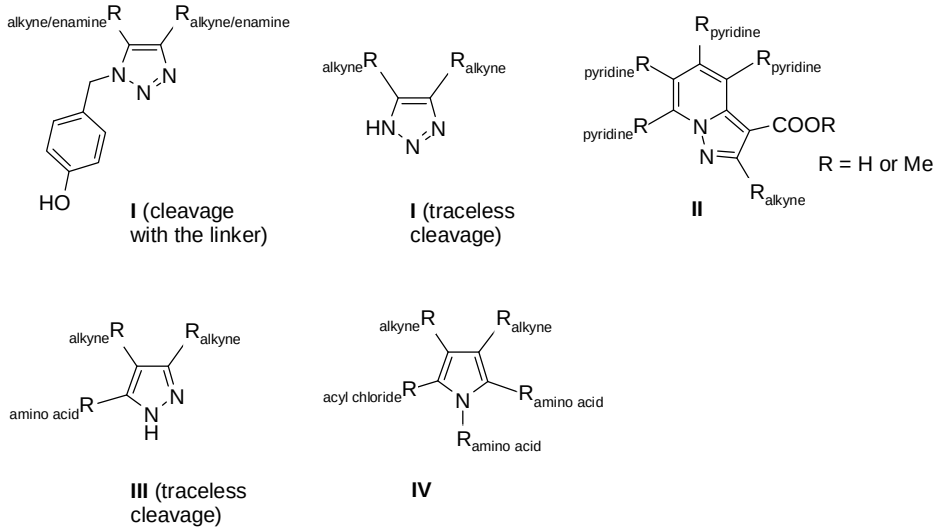


Figure 41. *Core structures of the five-membered nitrogen-containing heterocycles (I–IV). Roman numerals refer to the publications.*

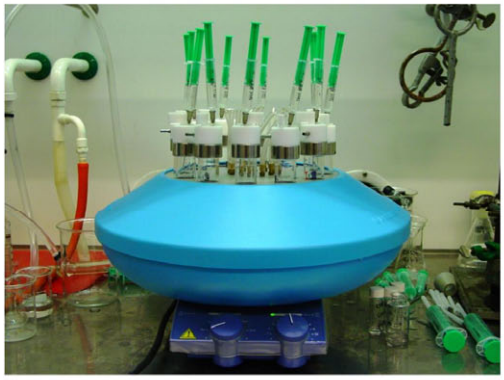
Table 13. *Strategies employed in the synthesis of nitrogen-containing five-membered heterocycles*

Ref.	***Dipole***	***Dipolarophile***	***Product***	***Cleavage***
I	polymer-bound azide	alkyne enamine	1,2,3-triazole	with the linker
I	polymer-bound azide	alkyne	1,2,3-triazole	traceless
II	azomethine imine	polymer-bound alkyne	pyrazolopyridine	as carboxylic acid or methyl ester
III	polymer-bound sydnone (azomethine imine)	DMAD ethyl propiolate methyl propiolate	pyrazole	traceless
IV	münchnone/azlactone (azomethine ylide)	DMAD methyl propiolate	pyrrole	–

The techniques applied in the syntheses are listed in Table 14 and the corresponding equipment is presented in Figure 42. All products were obtained via 1,3-dipolar cycloadditions where an alkyne (I–IV) or enamine (I) was used as dipolarophile. Aromatic products were obtained either directly or after spontaneous aromatization. In studies I–III, cycloadditions were performed on solid support, with excess of reagents to push the reaction equilibrium toward the products. Combinatorial syntheses were carried out by varying the components of the synthesis (II–IV). Parallel methods (I–IV) and microwave irradiation (III, IV) were exploited to facilitate the reactions. Parallel reactions were performed with Radleys 12-place reaction station or by shaking reaction mixtures in sealed syringes (II–IV). Cooling, heating, refluxing, and also hydrogenation were likewise done in a parallel fashion. Microwave-assisted reactions were done in microwave reaction tubes with a Biotage Microwave Initiator, with fixed reaction time and temperature. Polar 1,1,1-trifluorotoluene was used as a cosolvent with toluene in order to achieve the high temperatures required. Parallel isolation and washing of the resins were easily done in syringes (II, III). The filtrates were evaporated in a parallel fashion in small vials with a Genevac evaporator (I, II). Sequential purifications were done with pre-packed Biotage cartridges and automated collection of the fractions with UV detection (II–IV). All products were fully characterized, and the regiochemistry of the cycloadditions was carefully studied by NMR techniques (I–IV). Crystal structure analysis was carried out for confirmation of the regiochemistry (I, II).

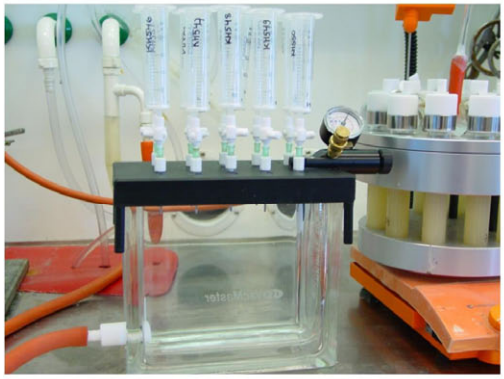
Table 14. *Techniques used in the synthesis of five-membered heterocycles*

Technique	***Publication***
1,3-dipolar cycloaddition	I, II, III, IV
solid-phase synthesis	I, II, III
use of polymer-bound reagent	IV
combinatorial chemistry	II, III, IV
parallel reactions	II, III, IV
microwave reactions	III, IV
parallel washing of resins	II, III
parallel evaporations	I, II
automated purification	II, III, IV
analysis of the regiochemistry by NMR techniques	I, II, III, IV
determination of the regiochemistry by crystal structure analysis	I, II



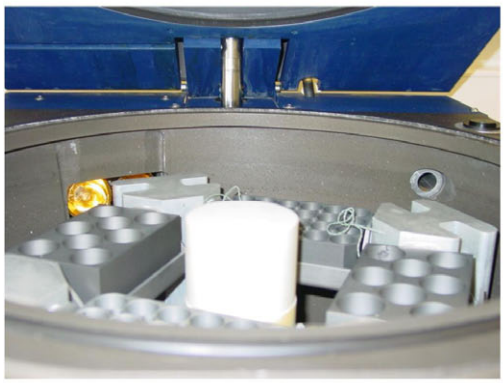
A.

B.

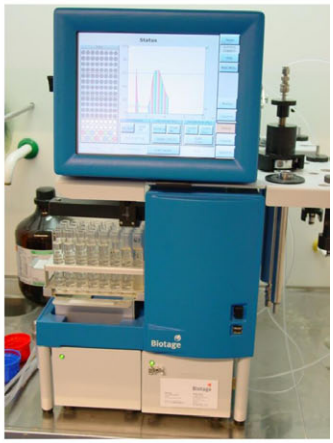


C.

D.



E.



F.

Figure 42. *Equipment used in the synthesis of five-membered heterocycles*
A. Parallel N-nitrosation under cooling with slow addition of sodium nitrite (II)
B. Parallel hydrogenation of benzyl esters of amino acids (IV)
C. Parallel washing of the resins (II, III)
D. Parallel cleavage of products in syringes (II, III).
E. Parallel evaporations (I, II)
F. Sequential purifications (II, III, IV)

5.2 1,3-Dipolar cycloadditions

Reactivity of the 1,3-dipoles and dipolarophiles varied. Synthesis of 1,2,3-triazoles (I) required heating, and prolonged reaction times were necessary for alkynes that lacked electron-withdrawing substituents. Microwave irradiation (Table 8) and copper catalysts[3,4] were not available at the time the work on 1,2,3-triazoles was done, but since then they have dramatically improved the reactivity of alkynes toward azides.

Pyrazolopyridines (II) were formed at room temperature from resin-bound alkynes and azomethine imines. However, some azomethine imines were difficult to prepare from the pyridine reagents, and some did not react in 1,3-dipolar cycloaddition. Only the successful cycloadditions are reported in the original publication (II). 1-Amino-4-methoxycarbonyl pyridinium iodide was obtained in less than 50% purity and as it could not be isolated it was used as a mixture. Solid-phase technique was very helpful in this case, allowing the use of excess of reagent and easy removal of unreacted starting material.

Mesoionic compounds are known to be reactive in cycloadditions. However, heating was needed, and the reactions were facilitated with microwave irradiation (III, IV). In the case of sydnones (III), test was also made of other alkynes, but only the electron-withdrawing alkynes reacted under the same reaction conditions. Cycloaddition of münchnones and azlactones (IV) was studied only with DMAD and methyl propiolate; other alkynes were not tested. The substituents had an effect on the reactivity of münchnones and azlactones, and yields of the 1,3-dipolar cycloadditions varied. However, for practical reasons reaction conditions were kept fixed for both münchnones and azlactones. Reaction conditions and the yields of the products are listed in Table 15.

Table 15. *Reaction conditions and yields of the 1,3-dipolar cycloadditions*

Product	***Reaction conditions***	***Yield (%)***	***Publication***
1,2,3-triazoles	80–120 °C, 3–111 h	10–58	I
pyrazolopyridines	rt, 20–40 h	11–79	II
pyrazoles	MW irradiation, 150 °C, 30 min	12–60	III
pyrroles	MW irradiation, 130 °C, 5 min	21–85	IV

5.3 Solid-phase methods

The function of the resin in the 1,3-dipolar cycloadditions varied (Figure 43). In the preparation of 1,2,3-triazoles, the solid support was used as a carrier of the azide (I). Sodium azide is toxic and forms explosive compounds with heavy metals and dichloromethane. Under acidic conditions, it liberates toxic hydrazoic acid gas. It was reasoned, therefore, that a polymeric support would provide a safer medium for the azide reactions. When the cycloadditions of azides were studied in 2002 and 2003, only a few studies concerning solid-phase synthesis of 1,2,3-triazoles had been undertaken, and these with either dipolarophile[3,167,277,278] or azide[166,212,278,279] attached to the resin. The discovery of copper-catalyzed cycloadditions has since led to a dramatic increase in the study of reactions leading to 1,2,3-triazoles.

In the preparation of pyrazolopyridines and pyrazoles, the polymer acted as a protecting group for the reactions as well as a carrier (II, III) (Figure 43). When the alkyne with carboxyl group was attached to the resin with an ester linkage, the resin probably protected the carboxylic acid from decarboxylation (II). Related pyrazolopyridines with free carboxyl group had earlier been prepared via 1,3-dipolar cycloaddition with acetylenic ester, with subsequent hydrolysis of the ester group.[280,281] The preparation of polymer-bound sydnones would not have been possible without the formation of secondary amino acids (III). Here the resin acted as a protecting group for primary amino acid because primary amino acids are diazotized, not *N*-nitrosated, in the presence of sodium nitrite and hydrochloric acid. Recently sydnones have been utilized in several 1,3-dipolar cycloadditions yielding *N*-substituted pyrazoles.[282,283,284,285,286] The technique developed within these studies now offers a new and facile route to *N*-unsubstituted pyrazoles via sydnones.

In the preparation of münchnones and azlactones from *N*-acylated amino acids, polymer-bound reagent, carbodiimide, was used as a dehydrating agent (IV) (Figure 43). The resin-bound side-product, urea, was easily separated by simple filtration. Yields were poor, however, when the polymer-bound reagent was used as a dehydrating agent for cycloaddition, and acetic anhydride was chosen as a dehydrating agent instead. A small library of pyrroles was then prepared in solution. Because attachment and cleavage strategies were not needed, the available diversity was wider and development of the method was easier. A number of problems were nevertheless encountered in solution-phase parallel synthesis. Excess of alkynes gave better yields, but products were then more difficult to isolate from the solution. Additionally, sequential isolation of the products was more tedious when the compounds were not attached to a solid support.

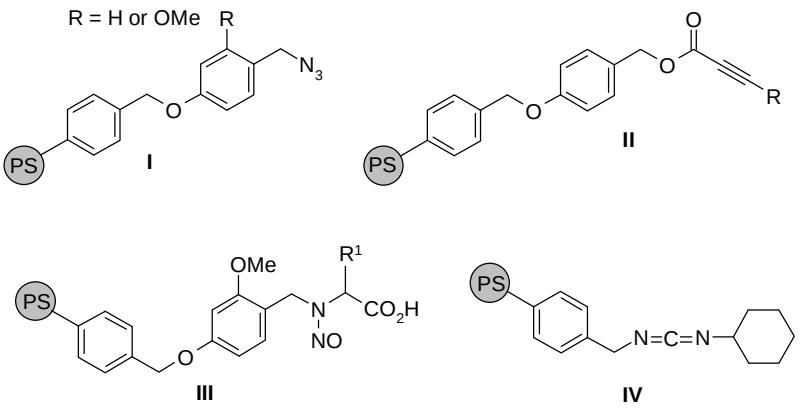


Figure 43. *Function of the solid support in 1,3-dipolar cycloadditions. Roman numerals refer to the publications.*

In study of the cleavage of products from the resin, the most important finding was the facilitating effect of the 2-methoxy group on the traceless cleavage of nitrogen heterocycles. 1,2,3-Triazoles were obtained with 4-hydroxybenxyl linker from the Wang resin (R = H) or as *N*-unsubstituted 1,2,3-triazoles in a traceless cleavage from the SASRIN resin (R = OMe) (Figure 44). The 2-methoxy group stabilizes the positive charge of the benzylic carbocation and makes the traceless cleavage easier (Figure 21). Minor amounts of *N*-unsubstituted 1,2,3-triazoles were also cleaved from the Wang resin.

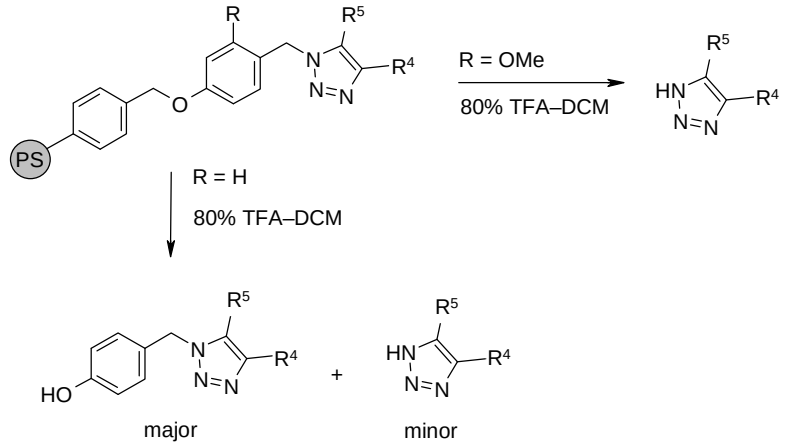


Figure 44. *Cleavage of 1,2,3-triazoles from the resin (I)*

Related traceless C–N bond cleavage was found to occur in the case of the pyrazoles (Figure 45). 2-Methoxy-substituted resin has previously been reported to facilitate the traceless cleavage of imidazoles[169] and 1,2,4-triazoles.[190,287]

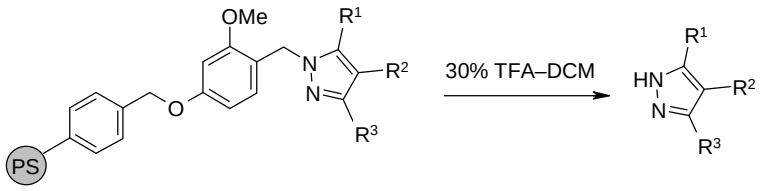


Figure 45. *Cleavage of pyrazoles from the resin (III)*

Pyrazolopyridines were attached to the resin with an ester linkage, and the products were cleaved either as carboxylic acids with trifluoroacetic acid or as methyl esters with sodium methoxide (Figure 46). Ester linkage is widely used in solid-phase 1,3-dipolar cycloadditions, and typically the products are cleaved as carboxylic acids with trifluoroacetic acid (Table 4).

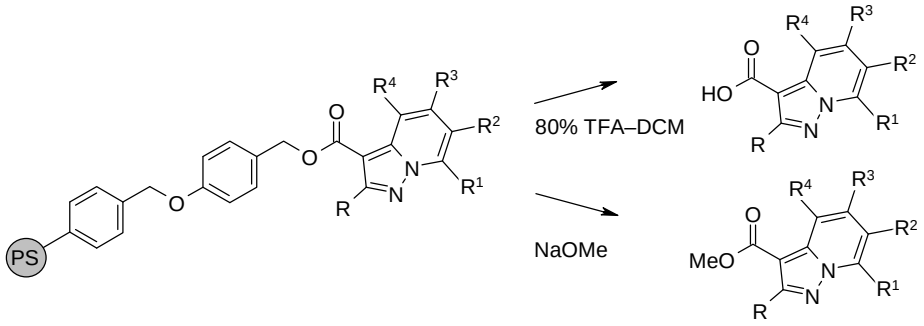


Figure 46. *Cleavage of pyrazolopyridines from the resin (II)*

5.4 Analysis of the regiochemistry

The regiochemistry of the cycloadditions was investigated in several ways. As shown in Figure 47, the ^{1}H NMR chemical shift of the aromatic nitrogen heterocyclic proton varies with the position of the proton.

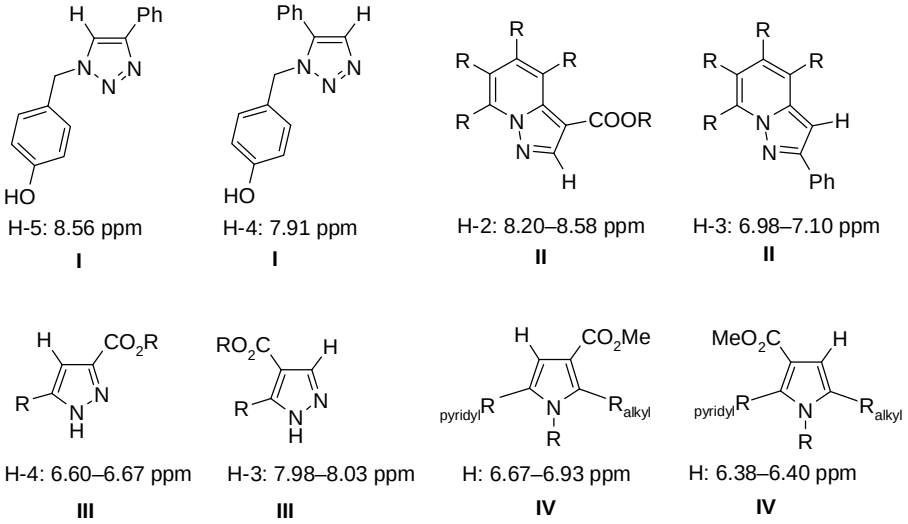


Figure 47. *^{1}H NMR shifts of aromatic protons in the nitrogen heterocycles (I–IV). Roman numerals refer to the publications.*

3-Substituted *N*-aminopyridine salts may give two separate regioisomers depending on the site of the ring formation (II). ^{1}H NMR coupling patterns revealed the regiochemistry of the ring formation (Figure 48). The 6-substituted regioisomer showed two ortho couplings and one singlet, whereas the 4-substituted regioisomer showed ortho couplings for all three protons in the pyridine ring.

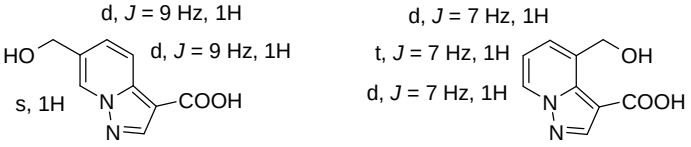
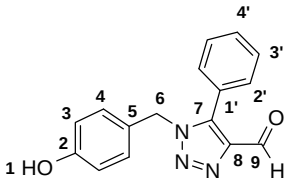


Figure 48. *Coupling patterns of 6-hydroxymethylpyrazolo[1,5-a]pyridine-3-carboxylic acid and 4-hydroxymethylpyrazolo[1,5-a]pyridine-3-carboxylic acid regioisomers (II)*

Additional information about the regiochemistry was obtained from heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) NMR experiments. HSQC experiments showed the connection between carbons and protons over one bond and were used for the assignment of atoms (Table 16, Figure 49). HMBC experiments revealed the coupling over longer distances (typically over two or three bonds).

Table 16. *Assignment of ^{1}H and ^{13}C NMR ppm values of 1-(4-hydroxybenzyl)-5-phenyl-1H-[1,2,3]triazole-4-carbaldehyde in DMSO-d_6 (I)*



Number	*Atom*	*Chemical shift (ppm)*	*Chemical shift (ppm)*
1	O*H*	9.51 (s, 1H)	–
2	Ar*C*OH	–	157.2
3	Ar*CH*	6.62 (d, *J* = 8.5 Hz, 2H)	115.4
4	Ar*CH*	6.80 (d, *J* = 8.5 Hz, 2H)	129.0
5	Ar*C*	–	125.1
6	CH_2	5.47 (s, 2H)	51.0
7	Triazole *C*	–	140.6
8	Triazole *C*	–	143.1
9	*CH*O	9.91 (s, 1H)	184.2
1’	Ph*C*	–	124.7
2’–4’	Ph*CH*	7.49–7.59 (m, 5H)	130.4, 129.8, 128.9

Aldehyde proton C*H*O (9.91 ppm) couples to both triazole carbons over two or three bonds (140.6 and 143.1 ppm), whereas phenyl protons (7.49–7.59 ppm) and CH_2 protons (5.47 ppm) couple to one and the same triazole carbon (140.6 ppm) over three bonds (Figure 50). These couplings reveal the positions of the aldehyde and phenyl groups. Confirmation of the regiochemistry was obtained in a crystal structure analysis. Other 1,2,3-triazole regioisomers were analyzed in the same manner (I).

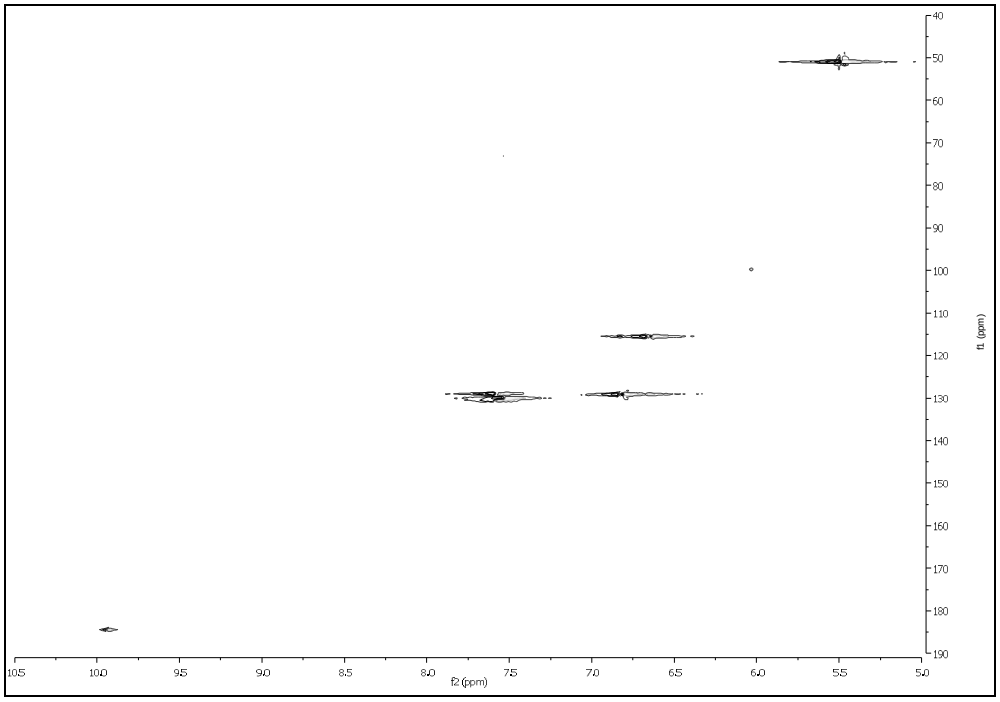


Figure 49. *HSQC spectrum of 1-(4-hydroxybenzyl)-5-phenyl-1H-[1,2,3]triazole-4-carbaldehyde (I)*

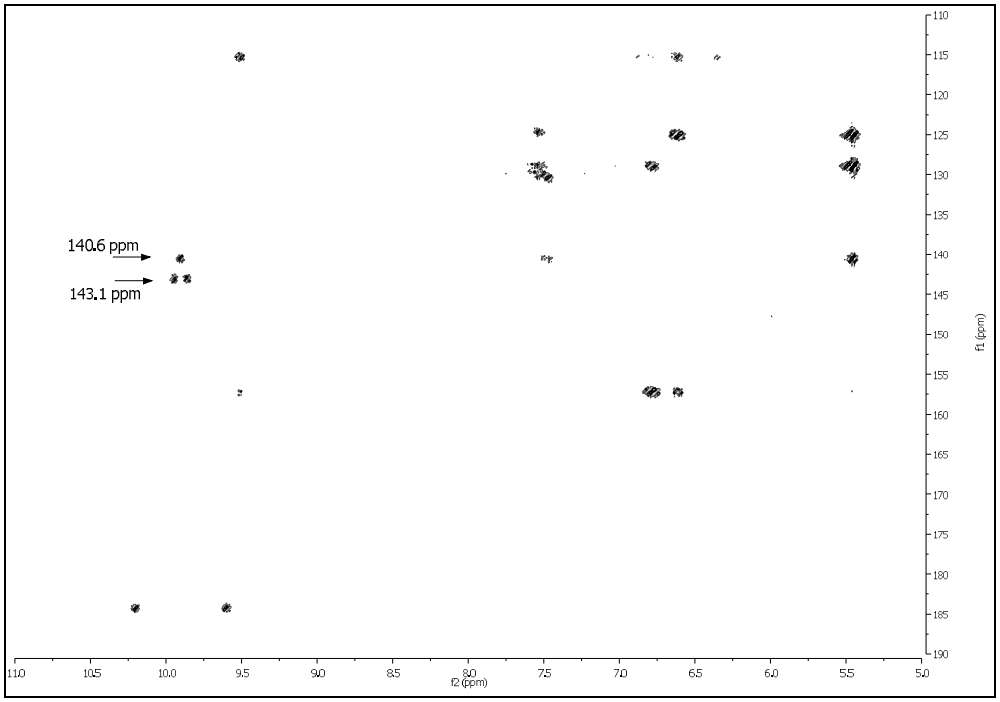


Figure 50. *HMBC spectrum of 1-(4-hydroxybenzyl)-5-phenyl-1H-[1,2,3]triazole-4-carbaldehyde (I)*

In a few cases nuclear Overhauser enhancement spectroscopy (NOESY), which shows couplings of protons through space, was used for further confirmation of the structure. NOESY of 2-phenylpyrazolo[1,5-*a*]pyridine revealed coupling between two protons (Figure 51), indicating the regiochemistry of the phenyl group (II).

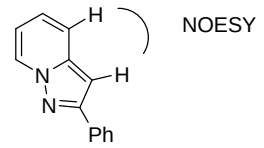


Figure 51. *NOESY coupling of 2-phenylpyrazolo[1,5-a]pyridine (II)*

The methyl protons in 2-methylpyrazolo[1,5-*a*]pyridine-3-carboxylic acid did not show any coupling to aromatic proton, but after decarboxylation in acidic conditions NOESY coupling between two aromatic protons in close proximity was detected (Figure 52). Detection of the coupling gave further confirmation of the regiochemistry of the methyl and carboxyl groups in 2-methylpyrazolo[1,5-*a*]pyridine-3-carboxylic acid (II).

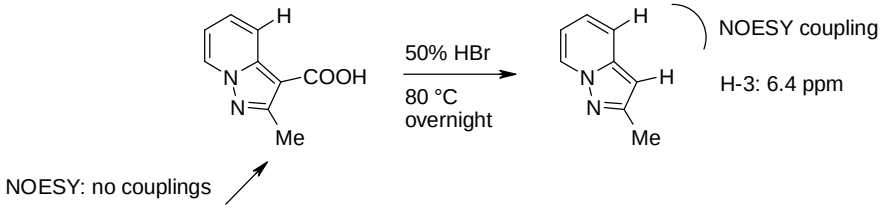


Figure 52. *NOESY couplings of 2-methylpyrazolo[1,5-a]pyridine-3-carboxylic acid and 2-methylpyrazolo[1,5-a]pyridine (II)*

NOESY experiments were also used for the regiochemical analysis of pyrazoles (III). As can be seen in Figures 53 and 54, the experiments revealed couplings through space between the alkyl protons and the aromatic pyrazole proton.

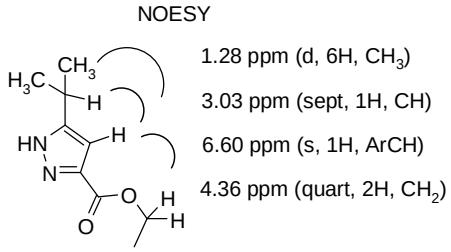


Figure 53. *NOESY couplings of 5-(1-methylethyl)-1H-pyrazole-3-carboxylic acid ethyl ester (III)*

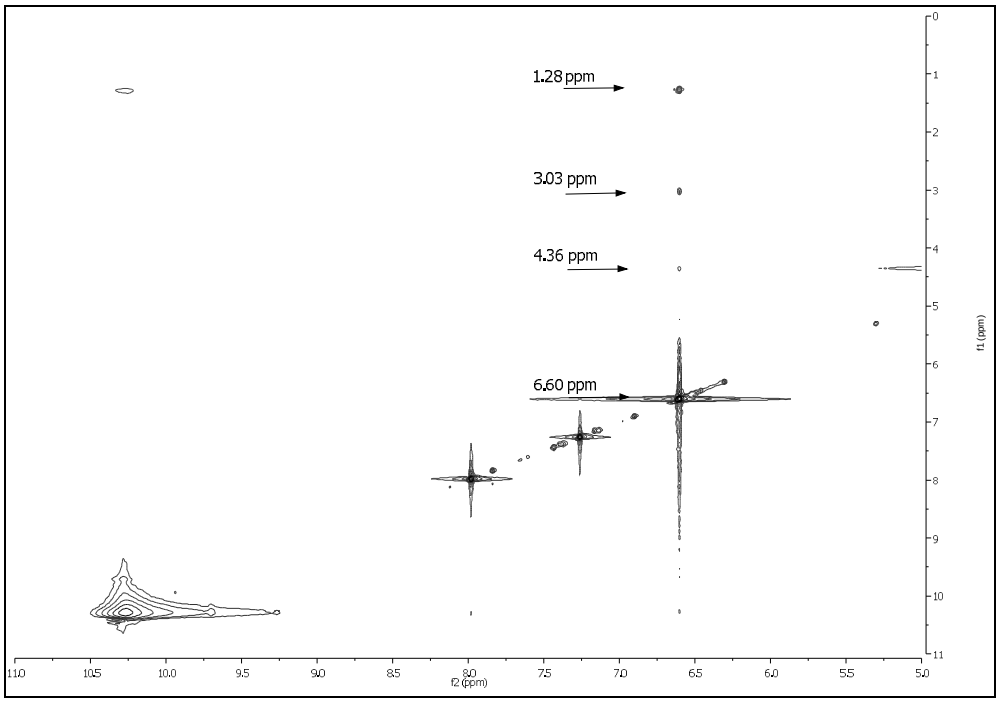


Figure 54. *NOESY spectrum of 5-(1-methylethyl)-1H-pyrazole-3-carboxylic acid ethyl ester (III)*

NOESY experiments were also used to analyze the regiochemistry of the pyrroles (IV). NOESY revealed the nearest environment of the aromatic pyrrole C*H* proton, between the nearest aromatic pyridyl protons and the pyrrole proton or between the closest alkyl protons and the pyrrole proton (Figures 55–57).

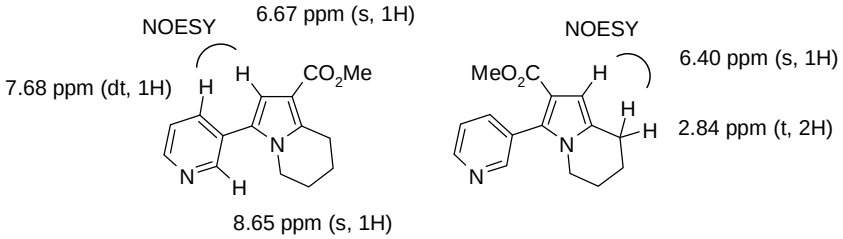


Figure 55. *Analysis of the regiochemistry of 3-(3-pyridinyl)-5,6,7,8-tetrahydroindolizine-1-carboxylic acid methyl ester and 3-(3-pyridinyl)-5,6,7,8-tetrahydroindolizine-2-carboxylic acid methyl ester with a NOESY experiment (IV)*

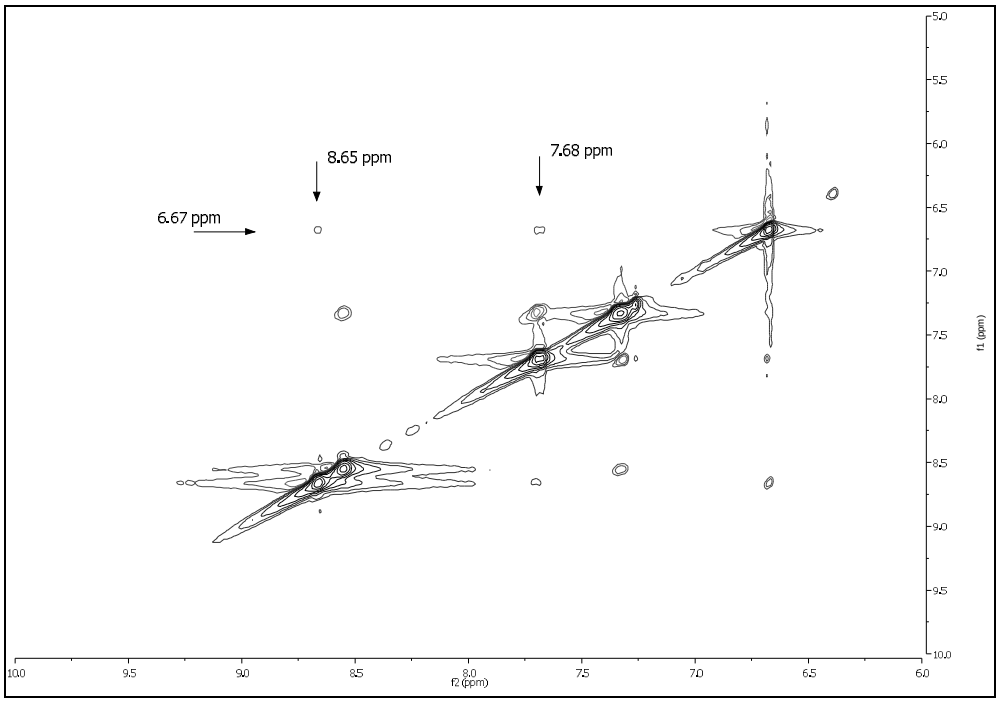


Figure 56. *NOESY spectrum of 3-(3-pyridinyl)-5,6,7,8-tetrahydroindolizine-1-carboxylic acid methyl ester (IV)*

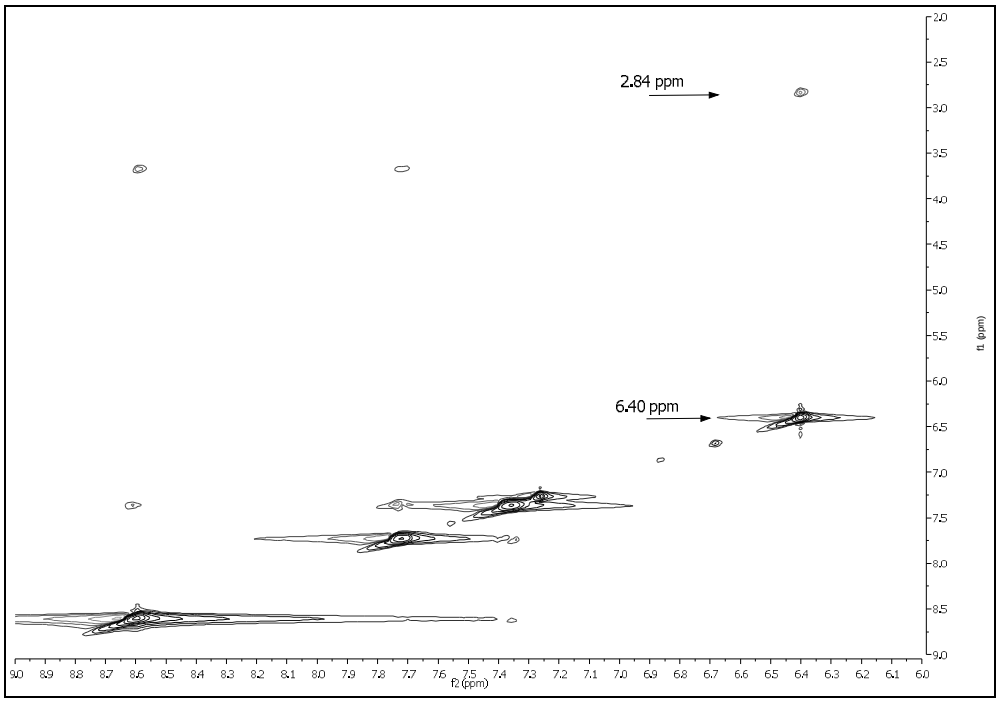


Figure 57. *NOESY spectrum of 3-(3-pyridinyl)-5,6,7,8-tetrahydroindolizine-2-carboxylic acid methyl ester (IV)*

In summary, the regiochemical outcome of the cycloadditions varied (Figure 58). Azides are known to yield 4-substituted regioisomers regioselectively with electron-withdrawing alkynes and a mixture of regioisomers with non-activated alkynes.[288] (Trimethylsilyl)acetylene gives a 4-substituted regioisomer, possibly for steric reasons.[289] Here, pyrazolopyridines were obtained regioselectively from azomethine imines and resin-bound alkynes (II). 3-Substituted *N*-aminopyridine salts gave two regioisomers (II). According to the literature, the ring formation occurs on the same side as the substituent unless sterically hindering groups are present.[290] In the case of hydroxymethyl substituent, a 2:1 mixture of regioisomers was formed and, possibly for steric reasons, the major regioisomer was the one with the ring formation on the opposite side of the substituent. Pyrazoles were obtained from polymer-bound sydnones and ethyl or methyl propiolate as roughly a 4:1 mixture of regioisomers (III). Pyrroles prepared from methyl propiolate and azlactones were obtained regioselectively, whereas pyrroles obtained from münchnones gave a mixture of regioisomers (IV).

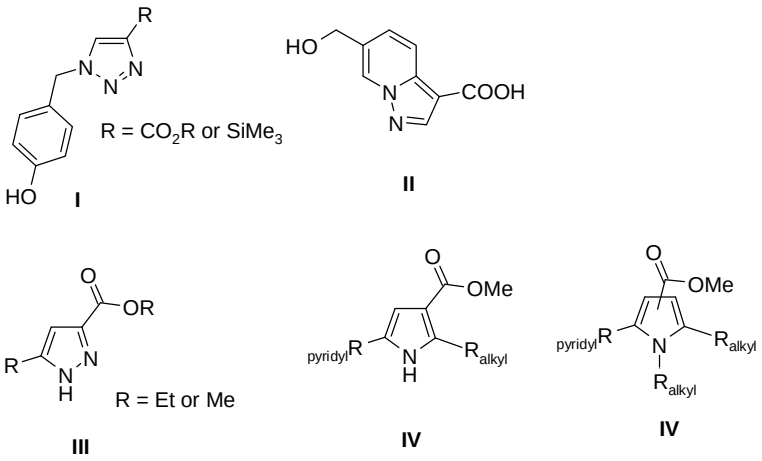


Figure 58. *Major regioisomers formed in 1,3-dipolar cycloadditions (I–IV). Roman numerals refer to the publications.*

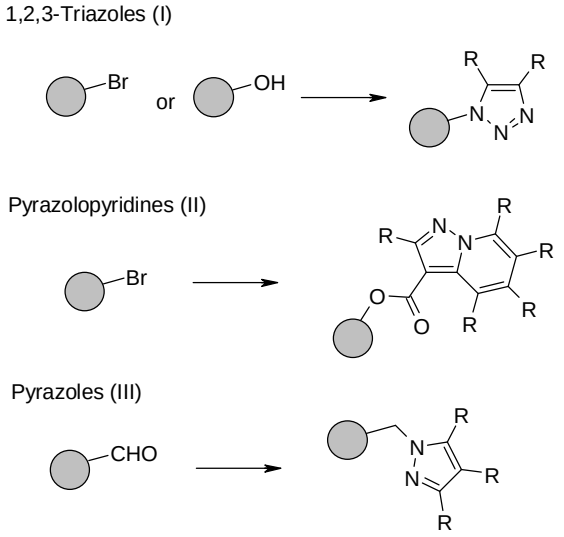
5.5 Analysis of compounds

A necessary step in any organic synthesis is the characterization of the products. Particularly important is to establish the identities and purities of new compounds. The methods used in the reported studies are now briefly discussed. The detailed results of the analyses can be found in the original publications (I–IV).

Yields

The yields of the products were reported for isolated and purified compounds (see Table 15 and the original publications). The yields of the solid-phase syntheses were expressed relative to the original loading of the resin (I) or to the theoretically corrected loading of the resin (II, III). Attachment of a large compound increases the weight of the resin and significantly decreases the loading (Table 17). Where the yield was expressed relative to the theoretically corrected loading, the increase in the molecular weight was taken into account.

Table 17. *Theoretically corrected loadings*



Original loading (mmol/g)	*Original group attached to the resin*	*Mw of the product (g/mol)*	*Corrected loading (mmol/g)*	*Publication*
1.2	Br	68.1–217.2[a]	1.0–1.2	I
0.4	OH	127.1–217.2	0.4	I
0.76	Br	176.2–208.3	0.7	II
1.7	Br	162.2–226.2	1.4–1.5	II
1.2	CHO	182.2–389.4	0.8–1.0	III

[a] The linker is disregarded

Purities

The purities of the products were evaluated on the basis of LC–MS results, elemental analyses, and NMR analyses. With UV detection, LC–MS purities were derived from ratios of the peak areas. Since products could contain impurities that absorb UV light better than the target compound, UV detection at wavelength 220 nm or lower is recommended by the *Journal of Combinatorial Chemistry*. Moreover, LC–MS analyses do not reveal possible polymeric material in the crude products cleaved from the resin because this material is not eluted through the column. Elemental analyses, for their part, do not reveal regioisomeric impurities. Additionally, impurities of very similar structures could give results within the acceptable limits (0.4%). Alternatively, chemically distinct impurities, such as residual solvents, easily change the results so that the measured values do not lie within the limits. The purities can also be roughly estimated from ^{1}H or ^{13}C NMR spectra with good resolution and high signal-to-noise ratio.

R_f values

The TLC retention factor (R_f value) is the distance moved by the compound divided by the distance moved by the eluent. The R_f values of the compounds were measured simultaneously with the same eluent that was used in column chromatography, and the average of two or three measurements was calculated.

Melting points

The melting points were first screened by fast method (10 °C/min), and then measured by slowly (1 °C/min) increasing the temperature near the approximately determined melting point. At least two accurate measurements were carried out for each compound. Since even small amounts of impurities decrease the melting points of compounds, melting points should, strictly speaking, be reported only for pure and crystallized compounds.

LC–MS analyses

The purities and identities of the compounds were determined by LC–MS, with retention times, UV purities, and molecular masses of the compounds reported. Accurate masses (HRMS) gave additional information about compound identities.

FT–IR analyses

FT–IR was the only direct method used for analysis of the resins. The resins had functionalities such as azides (I), alkynes (II), or carbonyl groups (I, II, III) that produce strong detectable bands, allowing easy monitoring of the solid-phase reactions. FT–IR was also used to analyze the final products. In publications I and II the five most intense bands of the products were listed, and in publications III and IV only the most significant bands.

NMR analyses

NMR spectroscopy provides the most informative methods for analyzing the compounds. ^{1}H NMR and ^{13}C NMR spectra were measured for all compounds, and HSQC, HMBC, and NOESY experiments were done to reveal the regiochemistry of the cycloadditions (I–IV). Additionally, HSQC was useful in a few cases where signals of two carbons were overlapping. The protonated carbons were assigned by DEPT analysis (III, IV).

X-ray crystal structure analyses

Crystal structures, determined at the Department of Chemistry, University of Helsinki, provided final confirmation of the regiochemistry of the cycloadditions. Results of the crystal determinations were consistent with the NMR data. Crystal structure information is given in the supporting information of articles (I, II).

6 SUMMARY AND CONCLUSIONS

New techniques to prepare five-membered nitrogen heterocycles were developed. A wide variety of 1,2,3-triazoles (I), pyrazolopyridines (II), pyrazoles (III), and pyrroles (IV) were synthesized via 1,3-dipolar cycloaddition reactions. The dipolarophiles were alkynes (I–IV) or enamine (I) and the dipoles were azides (I), azomethine imines (II, III), or azomethine ylides (IV).

Solid-phase syntheses applied in 1,3-dipolar cycloadditions were carried out with compounds attached to the resin with an ester linkage (II) or a benzylic C–N bond (I, III). The ester linkage was cleaved with trifluoroacetic acid or sodium methoxide, and the products were obtained as carboxylic acids or esters, respectively. The presence of 2-methoxy substituent in the resin had a significant effect on the cleavage of nitrogen-containing heterocycles. The cleavage of the benzylic C–N bond of resin-bound pyrazoles (III) occurred in a traceless manner, and no sign of the linker was detected. The effect of the 2-methoxy substituent was also clearly seen in the case of 2-methoxy-substituted resin-bound 1,2,3-triazoles (I): cleavage of the benzylic C–N bond released the 1,2,3-triazoles without the linker. In the case of the Wang resin, which lacks the 2-methoxy substituent, 1,2,3-triazoles were released along with a 4-hydroxybenzyl linker. This dramatic difference in the cleavages can be exploited in the solid-phase synthesis of other nitrogen-containing heterocycles in future.

Much effort was put into the development of the cleavage strategies. The results show that these efforts were worthwhile: reasonable yields and purities of the products were obtained from the solid supports. The advantages of solid-phase reactions are clear: isolation and purification of the resins is easy, the excess of reagents in solid-phase reactions probably helps in pushing the cycloaddition reactions toward the products, and it is easy to remove the impurities in solution. On the negative side, the required large excess of expensive or noncommercial reagents is wasteful, solid-phase reactions require two additional reaction steps, resins tend to be expensive and difficult to recycle, and monitoring of the reactions is relatively complicated.

Microwave methods were used to facilitate some of the reactions (III, IV). Conventional heating was tested as well as microwave irradiation. The results indicate that reaction times can be reduced from several hours to minutes (30 min, III; 5 min, IV). 1,1,1-Trifluorotoluene is a good co-solvent for nonpolar toluene to reach reaction temperatures over the boiling point of toluene. Moreover, microwave-assisted reactions are simple to carry out in small scale (~1–20 mL).

Compound libraries were prepared with parallel techniques (I–IV). Parallel reactions were easy to perform, and techniques such as heating, cooling, argon atmosphere, slow addition of reagents, and hydrogenation could be applied. Much time was saved by parallel isolation and washing of the resins in disposable syringes. Sequential filtration and

washing of the resins in conventional sintered disk filter funnels required more time, and after several filtrations the funnels tended to be blocked with the resin beads. Parallel evaporation in glass vials saved time by allowing many small samples to be evaporated at the same time. Sequential flash chromatographic purifications of the products with pre-packed Biotage cartridges also proved to be convenient. Automated collection of the fractions based on UV detection reduced the amount of the collected fractions, and TLC analyses of the fractions were not necessary. Manual packing of the columns and collection of the fractions had to be done separately, which was time-consuming.

The parallel methods also have certain limitations and drawbacks. Weighing of the starting materials takes time, and addition of the reagents is difficult to do simultaneously in a parallel fashion. The reactivity of the cycloadditions depends on the substituents of the reagents. Longer reaction times may be necessary for certain reagents, or fixed reaction conditions may affect the yields and purities of the products. Additionally, if the parallel reactions fail, they fail in a parallel fashion, so reaction conditions must always be tested separately before performing the parallel reactions. Sequential isolations of parallel solution-phase reactions by conventional methods are laborious and time-consuming, especially when the reactions include tedious steps such as extractions, filtrations, and evaporations. Monitoring of the solid-phase reactions was performed by FT–IR, and the preparation of tens of KBr pellets in between the reaction steps took time and slowed the reaction sequences. In all, more than 1000 reactions were performed during the studies. Only successful reactions were reported.

Regiochemistry of the cycloadditions was extensively studied by several techniques (I–IV). Analysis of the regiochemistry is routinely done by NMR methods. Crystal structure analysis of a crystallized compound is useful for confirmation of the regiochemistry, but sometimes, as in this work, crystals large enough for analysis are difficult to obtain and, in particular, a regioisomeric mixture may hinder the formation of good crystals. The formation of two regioisomers is usually an undesirable result. Development of regioselective reactions would expand the utility of 1,3-dipolar cycloadditions with unsymmetric dipolarophiles. The significant improvement in the preparation of 1,2,3-triazoles achievable with copper catalyst has recently been demonstrated.

In summary, 1,3-dipolar cycloadditions, solid-phase techniques, and parallel methods proved highly advantageous in the synthesis of nitrogen-containing five-membered heterocycles. Use of various building blocks such as alkynes, pyridine derivatives, and amino acids provided a diversity of compounds. The new methods that were developed will be valuable for the synthesis of related compounds in future.

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