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Review

Diketopiperazines in Peptide and Combinatorial Chemistry

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Abstract: Diketopiperazines (DKPs), the smallest cyclic peptides, represent an important class of biologically active natural products and their research has been fundamental to many aspects of peptide chemistry. The advent of combinatorial chemistry has revived interest in DKPs for two reasons: firstly, they are simple heterocyclic scaffolds in which diversity can be introduced and stereochemically controlled at up to four positions; secondly, they can be prepared from readily available α-amino acids using very robust chemistry. Here synthetic methods, conformation, as well as applications of DKPs are summarized and discussed critically.

Keywords: diketopiperazine; DKP; piperazine-2,5-dione; combinatorial chemistry; SPPS; SPOC; side reaction; peptide cyclization

INTRODUCTION AND HISTORICAL PERSPECTIVE

The purpose of the present literature review is to bring together information about all aspects of DKP chemistry, relevant to those working in the area of peptide chemistry. The subject has not been reviewed comprehensively. Earlier reviews were concerned predominantly with the natural occurrence of DKPs [1], as well as their structure [2] and reactivity [3]. More recently, DKPs have gained importance in drug discovery [4], as inhibitors of various enzymes, including e.g. topoisomerases, collagenase-1, as well as bradykinin antagonists, modulators of plasminogen activator inhibitor-1, and opioid receptor agonists and antagonists (see [5] and references cited therein).

It is perhaps ironic that DKP formation, today regarded by peptide chemists mostly as a troublesome side reaction during peptide chain assembly, represented the first successful attempt, over a century and a half ago, at linking two α-amino acids in a peptide bond. Heating glycine in a stream of CO_2 or HCl gas [6] provided a mixture whose chief component was *cyclo*-[Gly-Gly]. The first well-defined synthetic peptide was also obtained from this DKP: upon warming in aq HCl, it underwent partial hydrolysis and provided crystalline H-Gly-Gly-OH.HCl [7]. However, the earliest report of what was later found to be a DKP, viz. 'leucinimide',[†] dates back even further [8]. A number of DKPs, including examples containing unnatural amino acids [9], were reported in the literature of the late 19th century; but it was not until the turn of the next century that DKPs as a separate class of compounds were suggested explicitly. After spontaneous formation of *cyclo*-[Gly-Gly] from moist H-Gly-OEt had been observed [10], it was realized that DKPs could be synthesized efficiently from amino acid esters [11].

Abbreviations: Amino acid and peptide nomenclature conforms to IUPAC-IUB rules (*J. Peptide Sci.* 1999, **5**: 465–471).

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[†] In the early literature homogeneous DKPs are often termed in such a manner, i.e. leucinimide for *cyclo*-[Leu-Leu], lactimide for *cyclo*-[Sar-Sar], etc. The other terminology encountered frequently in early reports, e.g. leucylalanine anhydride for *cyclo*-[Leu-Ala], extends to unsymmetrical DKPs.

BIOSKETCH

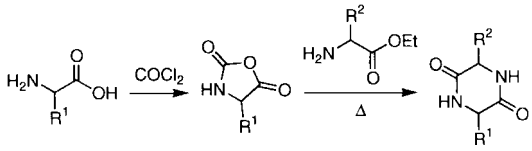
Peter Fischer is Head of Discovery Research at Cyclacel Limited, the cancer biotechnology company based in Dundee, Scotland, whom he has been with since its operational inception in 1997. Throughout his medicinal chemistry career in both industry and academia, peptide and peptidomimetic chemistry have played an important part and Dr Fischer has published numerous papers and patents in this field. Before his current appointment, he headed a medicinal chemistry group at Nycomed, Oslo, Norway. Dr Fischer originally hails from Switzerland but received his tertiary education in Australia, he holds science degrees from Macquarie University in Sydney and Deakin University in Geelong, Victoria.

Subsequently numerous symmetrical and unsymmetrical DKPs were prepared; many of these results were summarized in connection with amino acid polycondensation [12].

DKPs were not only important in early peptide synthesis, but they were also instrumental to the pioneering methods used to elucidate the primary structure of polypeptides. It was known that controlled partial acid hydrolysis of proteins could be made to stop at the dipeptide stage to a large extent and DKPs were obtained from the hydrolysates. This was useful because the crystalline DKPs could be isolated relatively easily and could be compared with authentic synthetic DKPs. Limited sequence information could then be gleaned from the composition of the DKPs obtained from the protein hydrolysates. In an extension of this method [13], the polypeptide to be sequenced was first refluxed in glacial AcOH to form a mixture of DKPs. These were then identified and the original polypeptide submitted to one cycle of Edman degradation [14], followed again by DKP formation. The possibility of stepwise sequencing from the *C*-terminus of a protein was foreshadowed by some indications that it might be possible to form terminal *N*-acyl-DKPs in peptides with the aid of diphenylphosphorazidate and 2-mercapto- or 2-hydroxypyridine, followed by removal of the terminal cyclic dipeptides with Bu^nNH_2 in EtOH [15].

SYNTHESIS OF DKPs

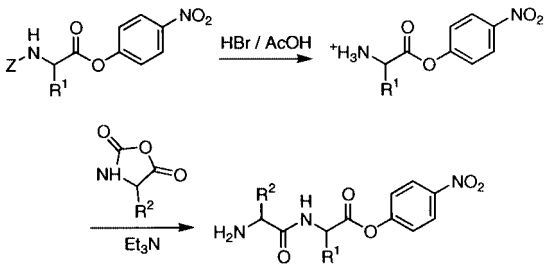
Most homogeneous DKPs can be prepared simply by heating the free amino acid methyl esters in a sealed tube and this method can even be effective for DKPs from amino acids with reactive side chains. However, it is generally advisable to use protected precursors since for example, Dab, Orn, and Lys derivatives can give rise to the corresponding pyrrolidone, piperidone and homopiperidone by-products [16]. Perhaps the oldest method of unsymmetrical DKP synthesis consists of dipeptide ester treatment with methanolic ammonia. The strongly basic conditions in this procedure can lead to epimerization and the same is true of other base-catalysed cyclization methods of dipeptide acids, esters and NCAs [17]. A method less prone to loss of chiral integrity consists of Boc-dipeptidyl methyl ester *N*-deprotection with formic acid, followed by reflux of the dipeptidyl ester formate salt in 2-butanol/toluene and removal of formic acid through azeotropic distillation [18]. However, other side reactions appear to be attendant with this procedure [19]. Heating of free dipeptide salts in phenol [20] or 2-naphthol [21] has also been proposed for the preparation of DKPs. For many DKPs, however, simple reflux of dipeptidyl methyl esters in low-boiling solvents, particularly methanol, is effective [22]. Alternatively, reflux of dipeptidyl methyl, ethyl, or benzyl esters in 2-butanol containing 0.1–2 M AcOH, is an efficient method of DKP preparation in a wide variety of cases [23]. It is also possible to obtain DKPs directly from *Z*-protected dipeptidyl methyl esters through simultaneous deprotection and cyclization, for example through the use of catalytic transfer hydrogenation at elevated temperature [24]. When free dipeptide esters are to be used for unsymmetrical DKP preparation, the former are conveniently prepared by reaction between appropriate NCAs and amino acid ethyl esters [25] (Scheme 1). The advantages of this method are the high reactivity of NCAs, the fact that no condensation by-products need to be removed, and the ready availability of NCAs from amino acids [26,27]. In some cases, one-pot procedures have been adopted [28].



Scheme 1

For the synthesis of many DKPs simple dipeptide alkyl esters are not sufficiently reactive. A number of suitable procedures, in which amino-deprotection in the presence of the activated carboxyl group is possible, are available: *In situ* generation of dipeptidyl succinimido esters by deprotection with TFA of the *N*-terminally Boc-protected precursors, followed by cyclization in pyridine, has been found to be effective [29]. A similar active ester procedure involves the acidolytic deprotection of Boc-dipeptidyl Pcp esters with TFA, followed by spontaneous cyclization under liberation of the trifluoroacetates with the aid of *N,N*-diethylaniline in hot benzene or CCl_4. Similarly [30], *N*-trityl dipeptidyl succinimido esters can be detritylated selectively with HCl in Et_2O, followed by cyclization in basic aqueous solution. Preparation of reactive esters from protected dipeptide acids is not always straightforward, however, not least because of the danger of epimerization during peptide esterification. Provided highly reactive intermediates are used for acylation, *p*-nitrophenyl esters (acylating agents themselves) can be used for temporary carboxyl protection in the 'backing-off' procedure [31] (Scheme 2). *N,N′*-Diaryl-DKPs can be obtained by self-cyclocoupling of 2-bromopropananilides; stereochemistry can be controlled to some extent by the choice of promoter, aryl substituent and solvent [32].

There are also available various solid-phase synthesis methods for DKP preparation. Amongst these methods are those employing linkers based on *o*-nitrophenyl [33], *p*-thiophenyl [34] (with oxidation of the thiol to the sulfone prior to cyclization), and 4-bromomethyl-3-nitrobenzoylaminobenzyl [35] (originally conceived as a photo labile linker) esters. Another example [36] is based on the use of the so-called Kaiser oxime resin. Even standard Merrifield peptide synthesis resin can, under optimized reaction conditions, be used to synthesize DKPs [37].

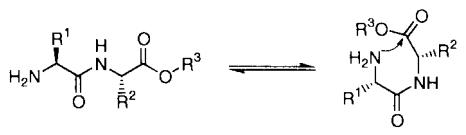


Scheme 2

MECHANISM OF DKP FORMATION

The planar backbone amide bonds in polypeptides are known to occur predominantly in the *trans* conformation. Planarity is maintained through a rotational energy barrier because of the partial double bond character of the peptide bond. On average, the energy difference between *trans* and *cis* peptide bond isomers is of the order of 2.5 kcal/mol [38]. Such isomerism is relevant to DKP formation because for a dipeptide derivative intramolecular attack of the amino group on the terminal carboxyl group is possible only from a folded conformation containing a *cis* peptide bond (Scheme 3).

The low abundance of *cis* peptide bonds in naturally occurring polypeptides (less than 2% overall) [39] is thought to be due mostly to steric conflict between neighbouring C^{α}-substituents in the *cis* conformation. Mechanistic studies suggest that large C^{α}-substituents should effectively prohibit isomerization [40]. However, the actual frequencies of occurrence of *cis* peptide bonds in known protein structures do not correlate well with residue side-chain bulk. Excepting Pro-containing dipeptides, the most frequent *cis* dipeptide units in proteins are Cys-Thr, Ser-Gln, Arg-Asp, and Thr-Thr; while for example, Gly-Gly *cis* units are less frequent and most combinations with insignificant side-chain bulk do not appear to occur in the *cis* conformation [41]. Similarly, the relative ease of DKP formation from a number of dipeptide derivatives cannot be explained sufficiently by steric interactions between the side chains in the *trans* and *cis* isomers (see below). Particularly noteworthy in this respect are dipeptides involving α-alkyl amino acid residues. For such compounds one would predict large energy differences between *trans* and *cis* isomers on the basis of steric crowding in the *cis* isomers, whereas in fact such peptides have been observed to cyclize quite readily. It is likely that the conformational constraints (on backbone torsional angles) introduced into a peptide by for example Aib residues result in amide bond isomerization/cyclization mechanisms with low energy barriers being favoured. Possibly such factors as overall proximity between terminal



Scheme 3

amino and carbonyl groups, as well as stabilizing intramolecular interactions may be involved [42].

Intramolecular aminolysis of dipeptide esters with formation of DKPs may be acid- [43] or base-catalysed [44]. For the acetic acid-catalysed DKP formation from H-Val-Pro-O-resin, a mechanism was postulated (Scheme 4).

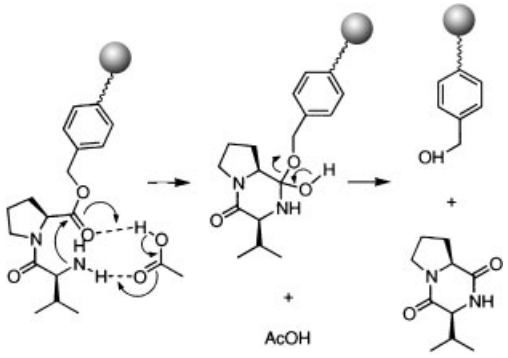
Sometimes DKPs are formed under the influence of nucleophilic reagents. The facile cyclization of spaglumic acid upon methylation with diazomethane is an example in point (Scheme 5) [45]. Apparently diazomethane here acts as a base in the aqueous layer of the two-phase system adopted. Acid-catalysed esterification did not give rise to the DKP but furnished the trimethyl ester as expected. The latter dipeptide could only be made to cyclize under forcing conditions.

Tryptic and chymotryptic peptides from protein digests have been found to be partially cyclized through DKP formation involving Asp α-carboxyl groups liberated during β-aspartyl shifts [46]. A related source of DKP formation is the rearrangement of β-alkyl-Asp-containing peptides to aspartimide peptides [47] (Scheme 6). Here intramolecular attack of the peptide amino terminus on one of the aminosuccinyl carbonyl groups is the mechanism.

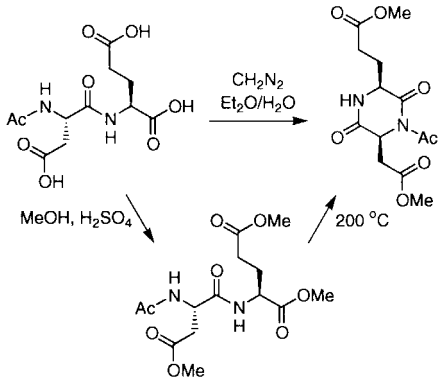
Intriguing cyclizations to DKPs, aza-cyclols and peptide thiolactones of *N*-terminal Cys di- and tripeptides were also reported [48]. The corresponding Ser-containing peptides undergo similar transformations with cyclodepsipeptide instead of thiolactone intermediates [49]. The involvement of thiol-amino acids in DKP formations has been known for some time and *cyclo*-[Cys-Cys], from which the dimer is obtained upon air oxidation, was reported [50]. Homocysteine lactone undergoes an interesting polymerization reaction on treatment with base (Scheme 7) [51].

Influence of the Leaving Group

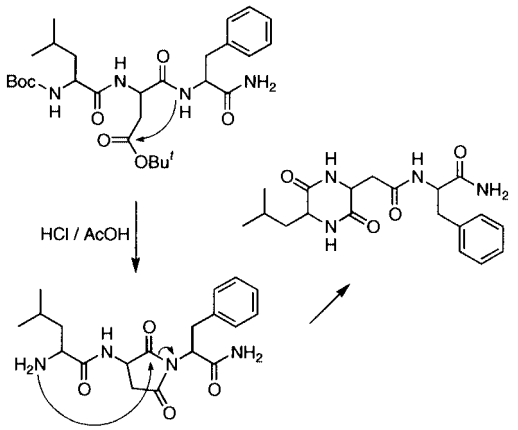
The fact that the nature of the alcohol portion of dipeptidyl esters has a major influence on the ease of DKP formation is indicated by the fact that amino



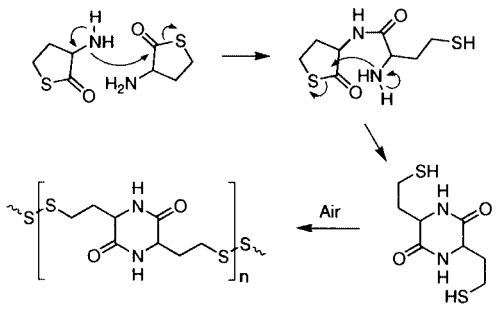
Scheme 4



Scheme 5



Scheme 6



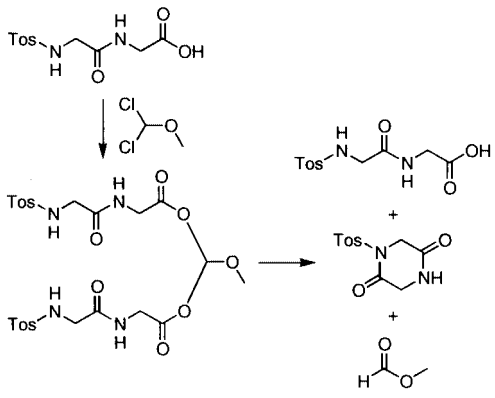
Scheme 7

acid methyl esters generally undergo cyclodimerization more readily than the corresponding ethyl esters. In a systematic study [52] using H-Gly-Gly-OR, it was found that the order of reactivity towards DKP formation depended on the steric bulk in R as follows: Me $\gg$ Am^n > Et > Am^i > Bu^n = Bu^i > Pr^n $\gg$ Bn > Pr^i. Some α-carboxyl protecting groups that have found widespread application in peptide synthesis are unsuitable from the point of view of the DKP side reaction. Chief amongst these are esters more prone to nucleophilic attack than ordinary alkyl esters. Thus substituted methyl esters in which the substituents are electron-withdrawing, such as is the case for examples 2,2,2-trichloroethyl [53] and phenacyl esters [54], are suspect in this regard. In fact immobilized phenacyl bromide has proven an effective starting point for the solid-phase synthesis of DKPs [55]. Phenacyl esters have attained major importance as a semi-permanent carboxyl-blocking group in convergent solution peptide synthesis [56]. Benzyl esters, which find very wide application in peptide synthesis, are less reactive, although still appreciably prone to nucleophilic attack. DKP cyclization of dipeptide benzyl esters may occur even when residues are present which are not normally observed to facilitate cyclization. For example, appreciable cyclization was observed upon piperidine deprotection of Fmoc-Tyr(Bu^t)-Ala-OBn [57]. Similar observations were made in connection with 4-(aminomethyl)piperidine deprotection of Fmoc-dipeptide benzyl esters [58]. In solution peptide synthesis based on 4-picolyl esters [59], special precautions also have to be taken to avoid DKP formation. Bu^t esters are commonly, and mistakenly, thought to be completely resistant to intramolecular nucleophilic attack as occurs in DKP formation, although they are usually the esters of choice for sequences known to be prone to DKP cyclization [60] and they are mandatory in really difficult cases, e.g. elongation of MeLeu-MeVal dipeptides [61]. A semi-quantitative study [62] with H-Glu(OBu^t)-Asp(OBu^t)-OR dipeptide esters demonstrated that benzyl and *p*-nitrobenzyl esters are prone to intramolecular nucleophilic attack, while the more acid-labile *p*-methoxy and particularly the *o,p*-dimethoxybenzyl esters were better in this respect. Of the alkyl esters studied, the order of reactivity was Me $\gg$ Pr^n = allyl $\gg$ Bu^t. Even with the Bu^t ester considerable DKP formation was observed under standard acylation conditions with DCC and HOBt [63]. Other esters commonly used as *C*-terminal protecting groups, which were incompletely resistant to DKP cyclization, were the 2,2,2-trichloroethyl [53] and the 2-(toluene-*p*-sulphonyl)-ethyl [64] esters. The 2-(2-pyridyl)ethyl ester [65,66], on the other hand, would appear to be particularly suitable for avoiding DKP formation from problematic dipeptide esters.

Reactive esters of dipeptides as used in the so-called 'backing-off' procedure of solution peptide synthesis are of course very much prone to cyclization. In fact DKP formation in such cases may occur even from the *N*-protected dipeptide ester. Thus Z-Gly-Pro-ONp ester undergoes cyclization with expulsion of *p*-nitrophenol and formation of the acyl-DKP [31]. *N*-Acyl DKPs have also been observed after treatment of *N*-blocked dipeptides with the condensing agent dichloromethyl methyl ether (which can be used to prepare NCAs from Z-protected amino acids) [67] (Scheme 8). Here the initially formed orthoester can undergo intramolecular acylation to form a DKP in the presence of tertiary base.

Influence of the Amino Acid Residues

The fact that the relative ease of DKP formation not only depended on the amino acid leaving group but was also influenced by residue structural features was seen very early. Thus polycondensation of H-Gly-OEt gave the DKP almost exclusively, whereas the same reaction with H-Ala-OEt did not appear to be accompanied by DKP formation [68]. The presence of Gly in dipeptide esters generally facilitates DKP cyclization since here the *cis* amide bond in the transition state to the DKP is more favourable than with other amino acids due to the absence of an interfering side chain (refer Figure 1a: one of $R^{1a}, R^{1b}, R^{2a}, R^{2b} <> H, R^3 = H$). The unfavourable effect of bulky amino acid side



Scheme 8

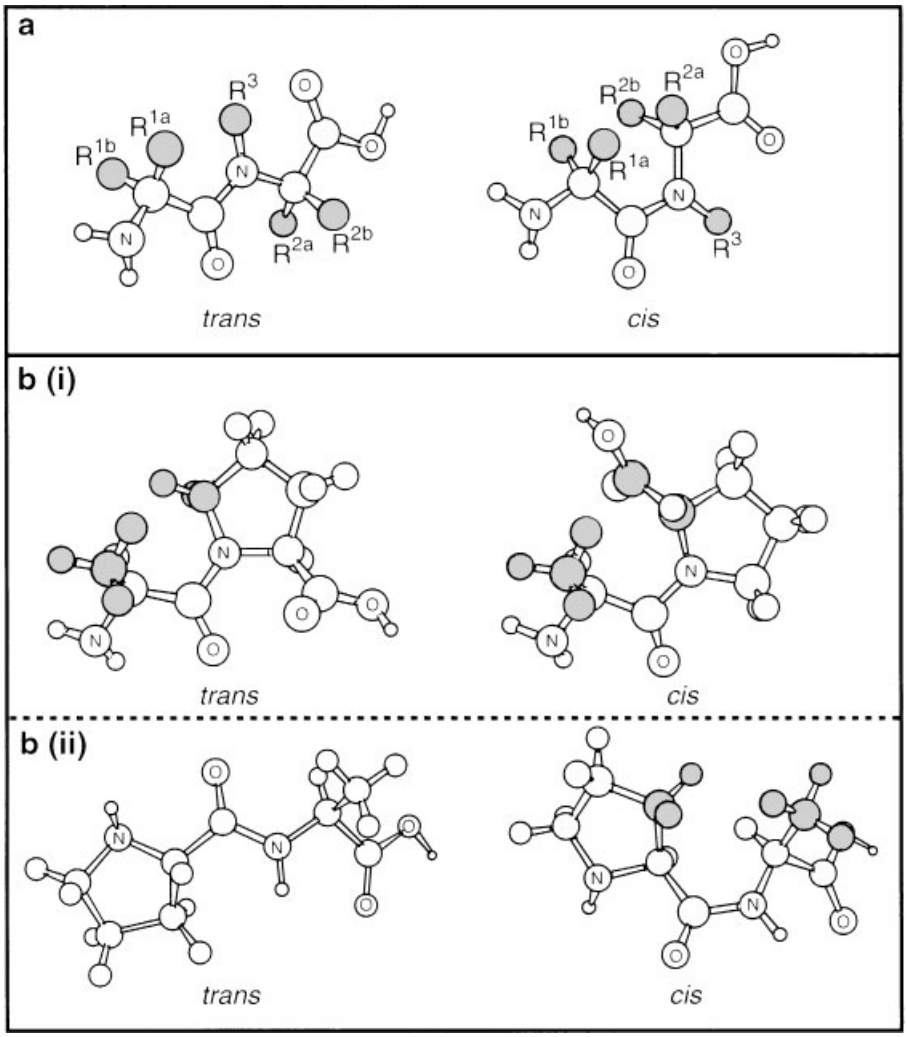


Figure 1 Steric relationship between C^{α}-substituents of amino-terminal residue (R^{1a}, R^{1b}) and C^{α} – (R^{2a}, R^{2b}) and *N*-(R^{3}) substituents of carboxyl-terminal residue in dipeptides adopting *trans* or *cis* amide bonds (a). Steric relationship between C^{β} groups in *trans* and *cis* Pro-containing dipeptides: H-Ala-Pro-OH, b (i) and H-Pro-Ala-OH, b (ii).

chains in the necessary *cis* dipeptide precursors leading to the DKP can be visualized using Figure 1a (for natural amino acids, the side chains correspond to R^{1a} and R^{2a}, the remaining substituents being H). However, steric bulk close to the α-carbon as for example in β-branched residues such as Ile, Val, and Thr, has a stronger influence on steric crowding in transition states leading to DKPs than steric bulk further away. This fact is exemplified by the successful preparation of numerous *cyclo*-[Tyr-Arg] analogues, many with side chains of exceptional steric bulk [69]. It is clear, however, that factors other than side chain steric bulk can be of importance. Thus for esters of certain bulky β-branched amino acids DKP formation still competes effectively with polycondensation, e.g. certain arylglycinates afford DKPs in appreciable yields upon heating to moderate temperature [70]. At least in DKPs containing two straight-chain alkyl side chains, UV spectroscopic experiments suggest that any interaction between the side chains (e.g. those of Met) can be ruled out [71]. Those amino acids which favour DKP cyclization in dipeptide derivatives exert their effect whether present *C*- or *N*-terminally, although the effect is less pronounced in the latter case [72].

Cyclizations involving dipeptides composed of residues with opposing configurations appear to be particularly facile, presumably due to the fact that in such cases *trans*-DKPs are formed [73]. Here steric

congestion in the *cis* dipeptide isomer is less severe (Figure 1a: if e.g. the amino- and carboxyl-terminal residues are L and D, respectively, then R^{1a} and R^{2b} correspond to the side chains, the remaining substituents being H). It is thus not surprising that DKP formation from mixed D/L-dipeptide methyl and ethyl esters is common [74]. An example is the tripeptide H-D-Val-Pro-Sar-OH, which upon storage in solution at ambient temperature yields the thermodynamically very favourable *cyclo*-[D-Val-Pro] [75].

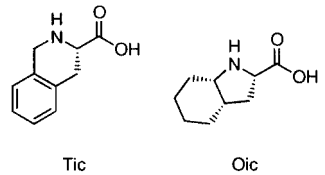
For geometric isomers of imide (e.g. Xaa-Pro) peptide bonds, the energy difference between *trans* and *cis* isomers is much smaller than with peptide bonds not involving cyclic amino acids (refer Figure 1b (i)), probably about 0.5 kcal/mol. It follows that the occurrence of *cis* imide bonds in polypeptides is much higher (*ca.* 10%–30%) [41]. Pro-Xaa dipeptides, on the other hand, are not significantly different from dipeptides without cyclic amino acids as far as steric congestion differences in the *trans* and *cis* isomeric states are concerned (refer Figure 1b (ii)). This accounts for the facts that Pro-Xaa *cis* amide bonds are not particularly abundant in proteins and that Pro-Xaa dipeptide sequences are far less prone to DKP formation than the Xaa-Pro sequences. Thus H-Gly-Pro-OEt upon standing *in vacuo* at ambient temperature overnight yielded the DKP at the exclusion of linear polymers [76]. DKP formation from this dipeptide ester was much faster than that from the inverted sequence H-Pro-Gly-OEt. This led to the realization that isomeric dipeptide esters, which cyclize to the same DKP, in general do so at vastly different rates.

Facile DKP formation when cyclic amino acids other than Pro (e.g. Tic and Oic; Figure 2) are present has also been observed. Peptides containing Tic residues, e.g. H-Tyr-Tic-Phe-Phe-NH_2, H-Tyr-Tic-Phe-OH, and H-Tyr-Tic-NH_2, while stable in aqueous solution at weakly basic pH, were reported to decompose spontaneously in DMSO or MeOH solution to yield *cyclo*-[Tyr-Tic] [77]. In the case of the dipeptide amide, complete cyclization was observed even in the solid state. In order to overcome this problem, peptide analogues containing reduced peptide bond Ticψ[CH_2-NH]Phe units were prepared [78]. However, even these structures underwent intramolecular rearrangements resulting in DKP-like structures [79]. In a different case it was found that a dipeptide containing a Tic analogue underwent cyclization with particular ease, despite the fact that nucleophilic attack had to occur on a comparatively unreactive carboxyl group [80]. Here DKP formation was particularly facile in aqueous solutions at or below pH 4 and again cyclization was observed in the lyophilized material.

Greater diversity of conformational states is available for peptides containing *N*-substituted amino acid residues. Comparison of the Ramachandran plots of, for example, Ac-Ala-NMe_2 with that of Ac-Sar-NMe_2 shows a greater number of energy minima for the latter, including minima arising from conformers containing a *cis* amide bond [81]. The unfavourable effect of a substituent R^3 in the *trans* rather than *cis* amide bond can be seen from Figure 1a. DKP formation from sequences containing *N*-substituted amino acid residues is thus favourable and has been observed with, for example, Sar-containing dipeptides [82].

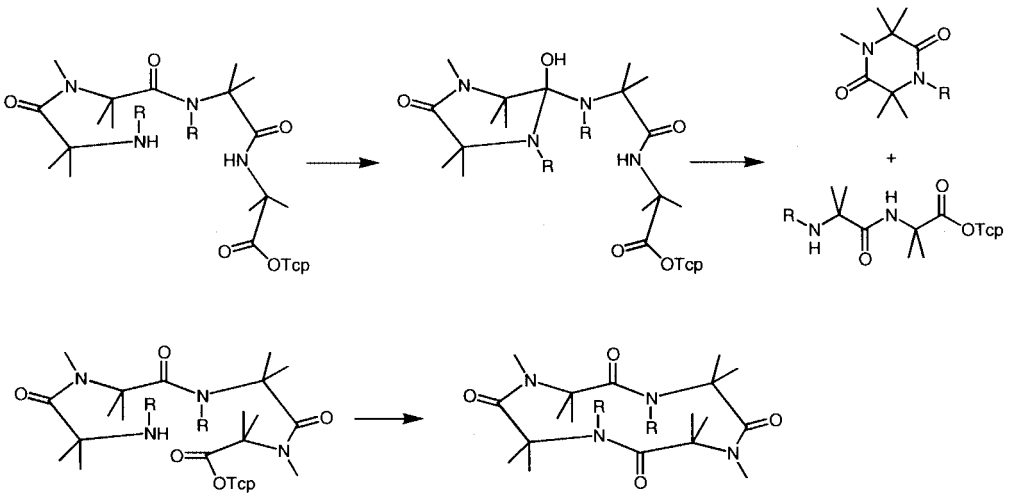
Interesting results were seen when cyclization of linear tetrapeptide Tcp esters were attempted [83] (Scheme 9). Depending on the amino acid residues in the peptide, DKP formation was a serious side reaction. This was particularly the case when the second residue was Sar. In this case the free amino group can approach readily the second carbonyl group due to the equal likelihood of *cis* and *trans* amide bonds between residues 1 and 2, resulting in cyclol formation and rearrangement to DKP. The liberated dipeptide ester subsequently cyclizes to another DKP. If the fourth residue is an *N*-methyl amino acid, then the free amino group may also react readily with the activated carbonyl group thus giving the cyclic tetrapeptide apart from the DKPs. If the second residue is Gly or Ala, on the other hand, then a *trans* peptide bond is preferred and cyclol formation is effectively suppressed.

Similarly, upon neutralization of the TFA salts of dipeptidyl resins containing MePhe, DKP formation was observed [84]. Under identical reaction conditions around 80% resin cleavage took place for the Pro-MePhe and Ala-MePhe derivatives, whereas the corresponding Pro-Pro and Ala-Pro derivatives gave around 40% to 60% cyclization. Even very bulky *N*-substituents in amino acids



Tic Oic

Figure 2 Structures of L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) and (7*S*,8*S*)-*endo*-*cis*-octahydroindole-2-carboxylic acid (Oic).



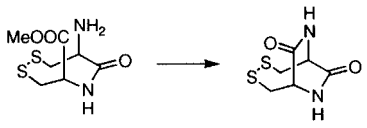
Scheme 9

can give rise to DKPs. Thus an attempt to synthesize *N*,*N*′-ditritylglycinamide from *N*-tritylglycine and tritylamine with the aid of DCC yielded *N*,*N*′-ditrityldiketopiperazine instead, which was also formed as the main product in the absence of tritylamine [85].

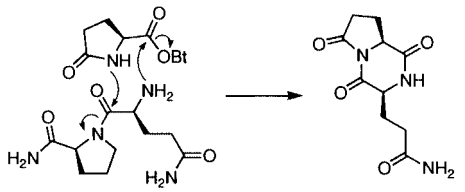
Dipeptides containing α,α-disubstituted amino acid residues [86] have been observed to cyclize very easily. For example, Boc-Val-Aib-OMe and Z-Aib-Ala-OMe cyclized quantitatively upon acidolysis and transfer hydrogenation, respectively [87]. Even H-Aib-Aib-OMe was reported to afford the DKP slowly upon standing in solution, although this finding stands in contrast to the reported difficulty of forming *cyclo*-[Aib-Aib] from the dipeptide Tcp ester, i.e. an activated species [88]. Furthermore, alkyl esters of α-aminoisobutyric acid and α-amino-α-methylbutyric acid have been reported to be much more resistant to both intermolecular condensation and cyclization than natural amino acids [89]. The presence of a *gem*-dialkyl group would be expected to disfavour heavily a *cis*-amide bond but imposes considerable conformational restrictions about the C^{α}-C' and *N*-C^{α} bonds [90], resulting in stable folded conformations for Aib-containing sequences characteristic of 3_{10}- and α-helices. In such helical structures the terminal amino and carbonyl groups of dipeptide units are typically only *ca.* 4.5 Å apart as opposed to distances of 5.5–6 Å in common extended conformations (based on calculations from average backbone dihedral angles of highly populated minima of the four-dimensional Ramachandran map [91]). Such proximity may be even higher for Aib-containing dipeptide units since it has been found that Aib was a suitable surrogate for a *cis*-Pro in certain bradykinin analogues [92]. In any case proximity between the groups involved in DKP amide bond formation, as well as possible forced distortion of the favoured *trans*-amide bond, may help to facilitate DKP cyclization in Aib-containing peptides. Diacylated *cyclo*-[Aib-Aib] may also form, presumably through rearrangement of symmetrical anhydrides formed during attempted couplings of *N*-protected Aib-containing dipeptides with H-Aib-OMe using DCC. Furthermore, attempted cyclizations of tri- and tetrapeptide acid chlorides consisting of Aib residues can result in formation of imidazolones [93,94].

A *cis* peptide bond lock is also provided in cyclic cystine derivatives and such compounds thus undergo DKP cyclization readily (Scheme 10), e.g. the hydroformate salt of cyclic cystine methyl ester converts to the DKP in MeOH solution at 45 °C [95]. Cystine-containing DKPs have also been suspected during SPPS of unsymmetrical dimeric peptides [96].

While cyclizations of glutamine derivatives to pyrrolidones and glutarimides are well known, a glutamine-containing dipeptide, *viz.* Boc-Pro-Gln-NH_2, was found to undergo quite a different side reaction upon acidolysis and attempted coupling with active esters of Glp, i.e. DKP rearrangement



Scheme 10



Scheme 11

to *cyclo*-[Glp-Gln] (Scheme 11) [97], rather than the more expected pyroglutamylamidopiperidine-2,6-dione or *N*-acylpiperazine-2,5-dione cyclizations. The proposed mechanism for the observed reaction involves nucleophilic attack of the Glp amide nitrogen on the Gln C^{α}-carbonyl with peptide bond formation between Glp and Gln residues and elimination of the Pro residue.

In this context it is interesting to note that terminal Glp residues in peptides can sometimes lead to rather unexpected decomposition through DKP formation. *Cyclo*-[Glp-His] was reported to be formed as a by-product of 2-mercaptoethanol-mediated imidazole deprotection of the tripeptide Glp-His(Dnp)-Pip-OMe [98].

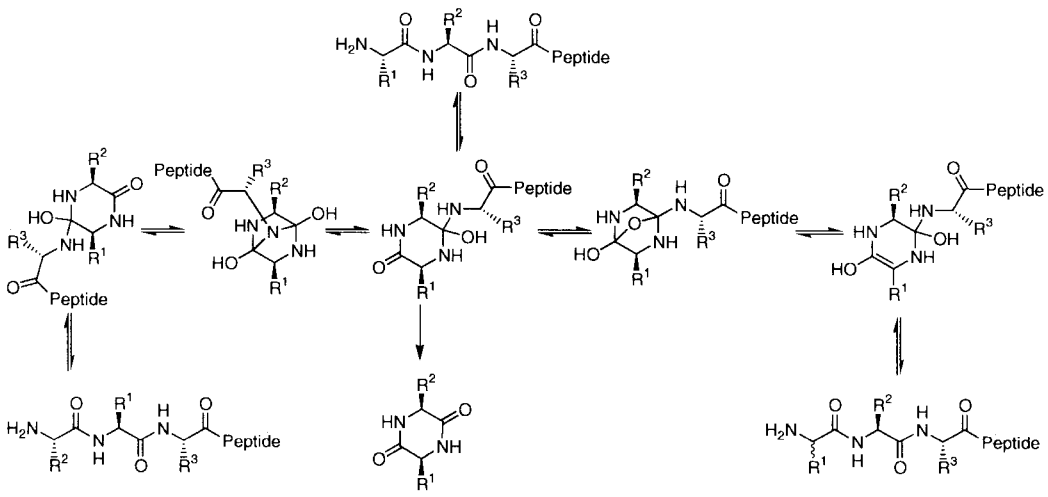
Tripeptides may also decompose spontaneously, particularly if they contain Gly and Pro residues. Thus very facile *cyclo*-[Aib-Pro] formation has been observed [99]. Stereopopulation control has been invoked to explain this finding: the combination of the *gem*-dimethyl group in the Aib residue, together with the Pro ring, apparently force the free amino group very close to the carboxyl group, thus strongly favouring DKP formation at this position with expulsion of the following Trp residue. Another well-documented example of DKP formation from tripeptides is H-His-Pro-Phe-OH, which in weakly acidic solution cleaves to *cyclo*-[His-Pro] and H-Phe-OH [100]. Both anchimeric assistance of the His imidazole side chain, as well as the presence of the *cis*-amide bond in the Pro residue are thought to contribute to the particular ease of cyclization. Interestingly, the tripeptidyl methyl ester requires much more vigorous conditions before undergoing the same cleavage reaction. This is attributed to a rate-enhancing mechanism *via* an H-bonded intermediate possible for the peptide acid but not for an ester.

Finally DKP formation may even occur from longer peptides, particularly if these contain certain Pro-containing sequences. In fact DKP formation at the amino terminus of polypeptides may be an important process in the abiotic decomposition of proteins in fossils [101]. An *N*-terminally truncated product, formed through Phe-Pro DKP formation, was isolated from preparations of both full-length recombinant human growth hormone, as well as an *N*-terminal tryptic fragment [102]. The degradation of substance P (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) upon storage was shown to proceed through two subsequent DKP cyclizations [103]. Evidently the extreme lability of this peptide must be due to its particular conformation rather than sequence effects only: the susceptibility of the Pro^2-Lys^3 amide bond to intramolecular attack did not appear to be caused by the vicinity of the two basic amino acid side chains, since the model peptide H-Arg-Pro-Lys-NHMe was far more stable to DKP formation. It is also interesting to note that peptide salts containing acetate were far more prone to DKP formation than the corresponding hydrochlorides or trifluoroacetates. This observation is in keeping with findings made elsewhere that weak carboxylic acids are particularly good catalysts for DKP-forming reactions.

EPIMERIZATION

When DKPs are exposed to epimerization-inducing conditions they are found to epimerize at similar rates as the corresponding dipeptides, although some DKPs epimerize particularly readily, e.g. *cyclo*-[Pro-Phe] [104]. Upon epimerization-forcing conditions, the rate of DKP epimerization is fast initially but soon slows, presumably due to hydrolysis to the dipeptide. It was shown that under these conditions for Gly-Ala dipeptides equilibria between H-Ala-Gly-OH, DKP and H-Gly-Ala-OH, as well as between the individual epimerization rates exist [105]. The decomposition and epimerization of aspartame (H-Asp-Phe-OMe) in the DKP and peptide products as a function of pH and temperature has been studied in detail [106]. Under conditions where there is no amide bond hydrolysis, on the other hand, DKPs appear to be more stable to epimerization than corresponding dipeptide derivatives, presumably since DKPs cannot give rise to azlactones. When there exists an enhanced tendency for α-proton abstraction, however, epimerization can of course occur, as is the case for example in piperazine-2,5-onothiones and -2,5-dithiones possessing facile thione → thiol tautomerism [107].

It has been observed that in aqueous solution peptides containing a Gly residue in position 3 may undergo rearrangement *via* DKP intermediates involving sequence inversion of the first two residues



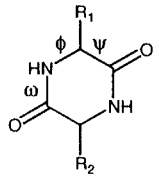
Scheme 12

and epimerization at position 1 [108] (Scheme 12). For peptides containing amino acids other than Gly at position 3, on the other hand, epimerization appears to be preferred to rearrangement. The mechanism proposed for these experimental observations includes attack of the *N*-terminal amino group on the carbonyl group of the second residue of the polypeptide sequence. The resulting tetrahedral DKP-like intermediate may either decompose to the DKP proper with chain scission; alternatively, it can form a bicyclic structure by transannular attack on the first carbonyl group by the newly formed amino group, resulting in rearranged products, or it may form a bicyclic structure by attack of the newly formed hydroxy group on the carbonyl group leading to epimerized products.

STRUCTURE OF DKPs

Due to the fact that the DKP ring contains two *cis* peptide bonds [109], it follows that it must be nearly planar, although very flat twist forms of boats and chairs are also possible. Avoidance of steric interaction between side chains of the DKP ring appears to influence strongly DKP ring conformation [110]. In general, if the dihedral bond angles between α-carbons and the carbonyl and amino groups are termed ψ and ϕ, respectively, as well as the amide *N-C* bond ω, then the general constraints shown in Figure 3 apply.

The molecular rigidity of the DKP ring was suggested early [111] and this was borne out by optical rotatory dispersion experiments [112]. Crystal



$0° < |\phi| \approx |\psi| < 50°; 0° < |\omega| < 8°; |\phi| \approx |\omega + \psi|$

Figure 3 DKP ring conformation.

structures of DKPs with sterically insignificant side chains also show a planar conformation [113]. DKPs with aromatic side chains have ring conformations that are influenced by the tendency of the aromatic substituents to overlap with the DKP ring, a phenomenon suggested by various physical methods [114], notably ^{1}H-NMR [115]. The interaction between the DKP and aromatic rings in such systems is of a short-range nature, the force involved probably being of the dipole-induced dipole kind, the amide groups providing the dipole and the aromatic ring supplying a polarizable π-electron cloud. In the case of *cis* DKPs, various combinations of aromatic and non-aromatic ring substituents lead to DKPs whose conformations can be rationalized by three basic conformations (Figure 4).

For *trans* DKPs only the planar ring conformation is likely to be of importance. Aromatic ring stacking is also highly favoured in DKPs where this is possible [116]. Thus a ring conformation with negative degree of folding and two pseudo-axial substituents was observed for *cyclo*-[Trp-Phe] [117]. A similar situation exists in *cyclo*-[Tyr-Tic], however, here the DKP ring is nearly planar [118]. Folding of

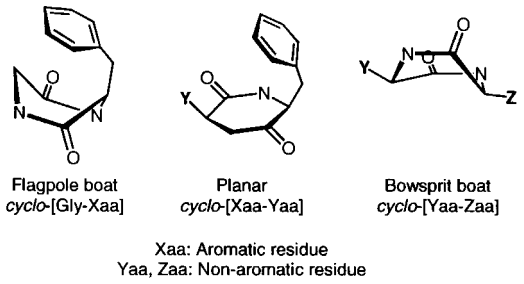


Figure 4 The three basic ring conformations found in *cis* DKPs.

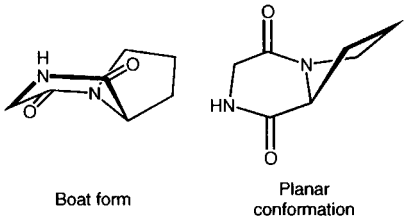


Figure 5 DKP conformations involving Pro residues.

aromatic side chains on to the DKP ring was also observed for DKPs containing two aromatic residues (Phe, naphthylAla) and here planar or nearly planar bowsprit boat-type DPK ring conformations were found [119].

In Pro-containing DKPs [120] the planar DKP ring conformation (Figure 5) is energetically unfavourable because it requires that the five-membered Pro ring be severely twisted. The strongly favoured near-planar Pro ring conformation is only possible if the DKP ring assumes a boat-like conformation. An even stronger stabilization of the DKP boat form would be expected in the case of *cyclo*-[Pro-Pro] because here the $C^{\alpha} - C^{\beta}$ bonds of the two Pro residues are in pseudoequatorial positions. For *cyclo*-[Pro-D-Pro], on the other hand, only the planar DKP ring conformation is possible, with the two Pro rings taking up half-chair conformations.

DKPs IN SOLID-PHASE PEPTIDE SYNTHESIS

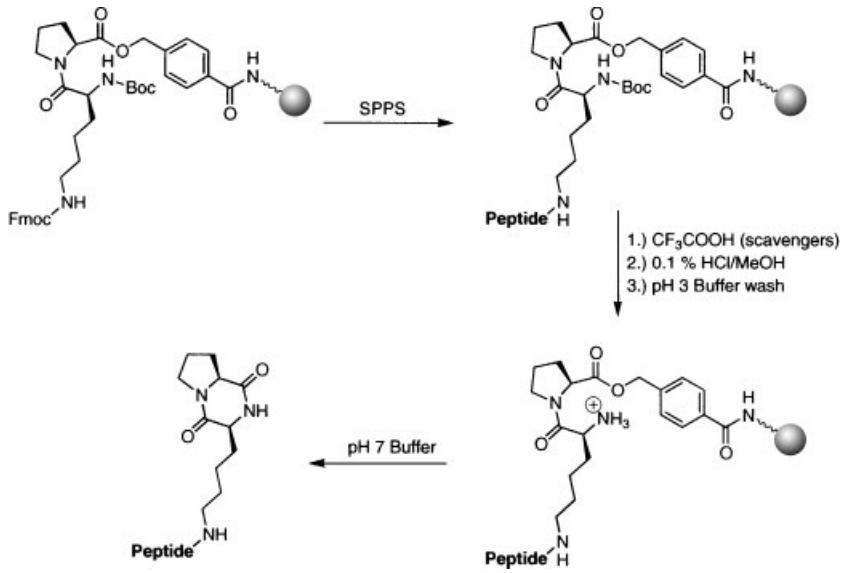
SPPS Linkers Based on DKP Formation

In multiple simultaneous peptide synthesis it is of advantage to be able to detach peptides directly from the solid support into aqueous medium for testing, without the need for acidolysis, work-up, etc. A linker was developed which permits such a procedure. In the original version [121] an orthogonally protected Boc-Lys(Fmoc)-Pro dipeptide was elaborated on the side-chain hydroxyl group of a solid phase-esterified *N*-acetyl Ser residue. Peptides were then built up using Fmoc chemistry after deprotection of the Lys side-chain amino group. Once assembly was complete, the Lys N^{α}-Boc group, together with other acid-labile amino side chain protecting groups, was removed. When transferred into aqueous conditions at mildly basic pH, DKP cyclization was triggered, thus releasing the peptides from the synthesis supports. Improvements in lability towards DKP formation could be achieved by altering the ester bond between the Pro residue and the solid support. When glycolamido and 4-(oxymethyl)benzamide esters of Lys-Pro were substituted for the Ser side chain ester, cleavage could be induced with physiological buffers directly [122] (Scheme 13).

Combinatorial peptide libraries based on the principle of biochemical screening of very large numbers of discrete peptidyl resin beads were developed for the purpose of new lead generation in drug research [123]. This method has been extended to library screening in solution and entails orthogonal release of predetermined amounts of peptide from peptidyl resin beads for convergent selection of 'active' sequences [124]. Various orthogonal linkage-cleavage chemistry multi-detachable protection schemes have been reviewed [125]. In such strategies quantities of peptide e.g. binding assays in solution can be detached by successive treatment with acidic and basic reagents. DKP formation from a Boc-Glu-Pro ester unit has been incorporated as one of the selective detachment steps. The cyclization is set up by first removing the Boc group from the Glu residue, followed by treatment with alkaline buffer solution.

Assessment of Linkers in Terms of Ease of DKP Formation

In general the acid-labile linkers that give rise to peptide amides [126,127] would be considered less prone to DKP formation at the dipeptide stage than dipeptidyl ester resins. This is due to the fact that an anchoring amide bond is generally less reactive towards nucleophiles than an ester. Furthermore, the amide linkers in these systems usually possess considerable steric bulk. On the other hand peptide amides may be obtained by ammonolysis from special linkers, e.g. based on *p*-carboxy-substituted benzyl esters [128]. Such systems with enhanced lability to nucleophiles are of course inadvisable in cases where DKP formation might be a problem.



Scheme 13

An ever-increasing number of linkers, mostly based on acid-labile esters, is being proposed for the synthesis of peptide acids by SPPS. Some of these have been tabulated in recent review articles [129–131]. In principle the same observations as were made above for *C*-terminal peptide esters as used in solution chemistry apply, since the majority of anchoring methods in SPPS are based on ester bonds between the growing peptide chain and the bifunctional linker to the solid support. Additionally, in solid-phase syntheses, loss of dipeptides through DKP formation gives rise to reactive sites on the polymer (e.g. hydroxyl sites) [132], which themselves may represent a source for other side reactions. Many of these esters are derived from benzyl alcohol and are thus more or less prone to nucleophilic attack. In the original version of SPPS [133], growing peptide chains are anchored *via* *p*-alkylbenzyl alcohol ester linkages to the insoluble support. Using this synthesis method, premature peptide loss from the resin at the stage of dipeptidyl deprotection and tripeptidyl acylation stages was observed early [82,96]. Resins based on *p*-acetamidobenzyl alcohol and *o*-nitro-*p*-carboxamidomethylbenzyl alcohol are even more susceptible [134]. In general with linkers of the benzyl alcohol type it can be expected that the danger of DKP formation through intramolecular dipeptide ester aminolysis will be related to the electron-donating effect of any phenyl ring substituents. Thus particularly acid-sensitive linkers based on for example methoxy-substituted benzyl alcohol [135–137] will be less prone to this side reaction than the corresponding unsubstituted ones.

SPPS based on the Fmoc protecting group generally utilizes *p*-alkoxybenzyl alcohol linkers [138]. Little attention has apparently been paid to DKP side reactions in this synthesis strategy. Unfortunately piperidine, the reagent of choice for Fmoc deprotection, is quite an efficient catalyst for DKP cyclizations. It was shown that the loss of dipeptide in Fmoc-based syntheses can be suppressed very effectively if a peptide-resin linker derived from *t*-butyl alcohol is employed [139]. It was shown that with such a linker peptides containing the very difficult *C*-terminal Pro-Pro, D-Val-Pro and Tyr-Pro sequences could be obtained with less than 5% loss due to DKP formation. Using conventional *p*-alkoxybenzyl alcohol resins, DKP formation in these cases is practically quantitative during standard Fmoc-deprotection cycle with piperidine/DMF. It should be kept in mind that conventional esterification methods (e.g. the DMAP-catalysed esterification with Fmoc-amino acid anhydrides [140]) for *C*-terminal amino acid anchoring fail with this linker and the very reactive Fmoc-amino acid chlorides or fluorides have to be resorted to in order to effect resin substitution [141]. In the so-called liquid-phase peptide synthesis method [142], the advantages to be gained in terms of resistance to nucleophilic attack by using Bu^t-based linkers were also suggested and suitable functionalization of polyethylene glycol is in fact possible.

In convergent syntheses protected peptides with *C*-terminal Gly and Pro are often desirable, since the problem of epimerization during segment condensation can thus be circumvented. However, dipeptides with these terminal residues are more prone to DKP formation than most others and this fact must be taken into account when protected peptide segments are synthesized. The use of resins with trityl linkers has recently gained much importance for the synthesis of protected segments by the Fmoc strategy [143] (Figure 6). The original trityl linker, when introduced into standard styrene-divinylbenzene copolymer by Friedel-Crafts acylation with benzoyl chloride, followed by Grignard phenylation [144], was found to result in peptide-linker ester bonds which were too acid-sensitive and the corresponding 2-chlorotrityl resin has thus been advocated [145]. When the trityl linker was introduced into modified styrene-ethyleneglycol copolymers through acylation with *p*-carboxyl-trityl alcohol [146], on the other hand, the ester bonds between trityl linker and the carboxyl groups of the *C*-terminal residue were sufficiently stable during peptide synthesis [147]. In both cases the resin-bound trityl alcohol moieties are converted to trityl chlorides, which in turn can then be used for very mild and efficient anchoring of *C*-terminal Fmoc-amino acids. Protected peptides can be cleaved under very mild conditions with dilute AcOH, in the case of the 2-chlorotrityl linker with dilute hexafluoroisopropanol [148]. It was shown that dipeptidyl resins containing *C*-terminal Pro residues are not prone to DKP formation when trityl resins are used [149]. This stability to cyclization was attributed to the extreme steric hindrance imposed by the bulky trityl ester function.

Silicon-based fluoridolysable linkers [150,151], whose underlying principles can be traced back to the trimethylsilylethyl group for carboxyl protection [152], have also gained some importance. In general it can be expected that these linkers, since they are based on secondary alcohols and contain bulky alkylsilyl substituents, are less prone to DKP formation than for example conventional benzyl alcohol-based linkers. At least in one case, where the linker 4-[1-hydroxy-2-(trimethylsilyl)ethyl]benzoic acid was utilized [153], this has been confirmed. The recently reported (2-phenyl-2-trimethylsilyl)ethyl linker, which is suitable for the synthesis of protected peptides and glycopeptides, is apparently very resistant to DKP formation for steric reasons [154].

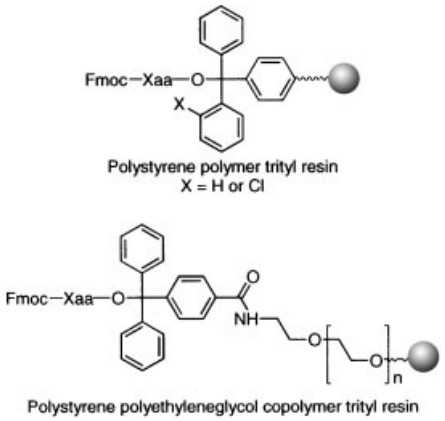


Figure 6 Trityl alcohol-based linkers for SPPS.

The *o*-nitrobenzyl resin has been suggested as a useful tool for convergent syntheses since protected peptide segments can be detached from this linker photochemically, leaving even very acid-labile amino acid protecting groups intact [155]. A problem with this method is incorporation of the third residue due to DKP formation at the dipeptide stage; here this side-reaction is more pronounced than with ordinary benzyl esters [156] (refer synthesis section).

The glycolamide ester linker [157] was proposed as a base-labile peptide anchoring method, permitting detachment under aqueous conditions. It was found later that the activated methyl ester nature of this linker leads to dipeptide cyclization [158].

A *p*-nitrobenzophenone polystyrene resin now commonly referred to as the 'Kaiser oxime' resin, was developed for the preparation of protected peptide segments for mixed solid-phase/solution convergent peptide synthesis [159]. It offers the possibility of removing the fully protected peptides after assembly using a variety of mild nucleophiles. This fact has been exploited to prepare head-to-tail cyclized peptides directly on the resin [160]. Due to the fact that the peptide-oxime resin is quite susceptible to nucleophilic attack, it is not surprising that intramolecular attack after dipeptide *N*-deprotection can be a serious problem here. In fact the usual DCC/HOBt coupling chemistry applied with Boc-amino acid derivatives may not be efficient enough to compete with DKP formation [161]. For solid-phase segment condensation strategies using the oxime resin it has been suggested that segments are chosen in such a manner as to avoid the 'slow couplers' Asn and Gln in the third position from the *C*-terminus [162].

Another resin useful for preparation of protected peptide segments, Boc-aminoacyl-oxyacylpolystyrene, from which peptides can be liberated by

hydrazinolysis or with Crown ether/KCN, is sensitive to the DKP side reaction for the same mechanistic reasons discussed above for dipeptide phenacyl esters [163].

When planning multiple simultaneous peptide syntheses, a technique that has gained much interest in the last few years in connection with combinatorial chemistry approaches, it would be highly advisable to take into consideration the fact that DKP formation can be a problem. Thus if for example a standard assembly chemistry on a benzyl alcohol-type resin is used and all possible tripeptides are the target, one would expect an appreciable number of sequences to be under-represented or missing completely from the peptidyl resin mixture due to loss through DKP formation. Monitoring techniques sometimes applied in simultaneous multiple synthesis (e.g. bromophenol blue indicator [164] or conductance measurement [165]) would not be expected to reveal such problems. The situation could be further aggravated since manipulations of the peptidyl resins, e.g. through pooling and recombination, could result in prolonged intervals between deprotection and acylation cycles, providing additional time for cyclization to occur. In simultaneous multiple solid-phase syntheses choice of resins resistant to DKP formation is thus of particular importance, as has been pointed out [166].

Strategies Employed to Prevent DKP Formation in Solution and SPPS

Since DKP formation may be acid- or base-catalysed, it is advisable in potentially problematic cases to choose dipeptide N^{α}-deprotection and acylation strategies that may proceed under neutral conditions. One solution to this problem is application of Z-protection, removable by hydrogenolysis, and chain extension with the very reactive Pfp esters, again without the need for the addition of tertiary base [167]. In Fmoc-based SPPS, as a general precaution, abbreviated deprotection and wash cycles at the dipeptide stage are advisable [168], although for particularly prone dipeptide sequences such a protocol (e.g. 50% piperidine/DMF for 5 min) may still be problematic and further shortening of the reaction time will lead to incomplete deprotection [169]. A possible solution to such sequences is introduction of the second amino acid residue as the hyper-acid labile 2-(4-biphenylyl)isopropyloxycarbonyl (Bpoc) derivative [170]. More recently it has been shown that in Fmoc-based SPPS replacement of the piperidine with TBAF [171] may be useful. Thus deblocking of Fmoc-Lys(Boc)-Pro-(*p*-alkoxybenzyl alcohol resin) with 20 mM TBAF in DMF was reported to suppress substantially DKP formation from this particularly prone dipeptide [172]. However, similar treatment of a different dipeptidyl resin (Pro-Tyr) did not effectively suppress DKP formation and caused almost complete epimerization at the Tyr residue [173]. Addition of up to 2% EtOH (i.e. conditions which moderate the nucleophilicity of the fluoride ion) to the TBAF/DMF mixture drastically reduced both cyclization and epimerization. A different alternative Fmoc-deprotection reagent, namely the sterically hindered tertiary base DBU has recently found application in solid-phase syntheses of phospho- and glycopeptides. Apparently it does not, however, reduce the DKP side reaction [174].

In syntheses employing the N^{α}-Boc protecting group it has been found useful to employ *in situ* neutralization combined with acylation mediated by Castro's reagent [175], and reagents derived from it, as a precautionary measure against the DKP side reaction [176]. Apparently the nature of the tertiary base in the acylation mixture is important, e.g. DIEA performing better than NMP in terms of DKP-suppression [177]. The advantage of *in situ* neutralization methods lies in the fact that they can easily be incorporated into computer-programmed automated synthesis protocols. This is in contrast to the better-established Suzuki procedures [178] for effective suppression of DKP formation. These methods are based on substituting 4 M HCl in dioxane in place of TFA for acidolysis of the Boc group. The *N*-terminal amine hydrochlorides, which of course are non-nucleophilic and thus do not cyclize, are not neutralized prior to the next acylation cycle. The latter is carried out with excess NMM salt of the next Boc-amino acid in the desired sequence, followed by DCC (or in reversed order); alternatively Boc-amino acid reactive esters, followed by 1.1 equivalents of NMM, may be used. Under these conditions even the very labile Pro-Pro peptidyl resins could be elongated without problems. A variation of these methods consists in conversion of the dipeptide hydrochloride derivative emanating from HCl/dioxane deprotection with a large excess of pyridinium acetate to the acetate salt, followed by acylation with preformed active esters in complete absence of auxiliary base [96]. A method of DKP suppression through acylation without prior neutralization of the deprotected dipeptidyl resin, which is compatible with Fmoc/Bu^t solid-phase strategy, has also been reported [179]. Here the

second amino acid is introduced in *N*-Trt-protected form. The Trt group is then removed using 0.2% TFA and 1% H_2O in CH_2Cl_2 and the resulting dipeptidyl TFA salt is acylated directly with excess of the next Fmoc-amino acid derivative, PyAOP [180], and DIEA. The method is applicable to the synthesis of free peptides using resins with the 3-(4-hydroxymethylphenoxy)propionic acid linker (modified Wang resin) [181], as well as to synthesis of protected peptides with the linker 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid (HMPB) [135]. Synthesis of the tripeptide H-Lys-D-Val-Pro-OH using these two resins and standard Fmoc chemistry resulted in 89% and 67% DKP side reaction, respectively, whereas with the modified Trt-procedure only 0 and 5% loss through DKP formation was encountered.

N-methyl amino acids are particularly prone to cyclization. Thus the extreme case represented by D-(*N*-Me)Phe-Pro peptidyl resin required particular precautions in order to avoid DKP formation [182]. The dipeptide amine salt obtained by HCl/dioxane Boc-deprotection was reacted with the appropriate Fmoc-amino acid chloride for several minutes at low temperature, followed by the addition of DIEA to 'pH 8'. The very high reactivity of the Fmoc-amino acid chloride, together with control of temperature and base strength was necessary to counteract the extremely short half-life (15 min) of the neutralized dipeptide resin with respect to DKP formation.

A conceptually particularly attractive way of preventing DKP formation in peptide synthesis was reported [183]. It consists of entrapment of the amine nucleophile resulting from dipeptide deprotection by *in situ* acylation. Such an approach requires that the acylating agent be *N*-protected and carboxyl-activated in such a way as to be compatible with the method of *N*-deprotection of the susceptible dipeptide derivative. If the dipeptide carries a Z protecting group to be removed by hydrogenolysis, then Su and Pfp active esters, as well as the Boc- and 2-(trimethylsilyl)ethoxycarbonyl (Teoc) [184] amino-protecting groups fulfil these criteria. Thus hydrogenolysis of a mixture of Z-Ala-D-Pro-OMe in dioxane with Pd/C catalyst in the presence of a slight excess of Teoc-D-Ala-OPfp resulted in an 80% yield of the tripeptide product.

As a last resort it is of course possible to circumvent the problem of DKP formation at the dipeptidyl resin stage altogether by coupling a protected dipeptide segment to the amino acyl resin. The disadvantage is the usual danger of epimerization, depending on the nature of the *C*-terminal residue of the protected dipeptide and the coupling chemistry applied.

APPLICATION OF DKPs IN COMBINATORIAL CHEMISTRY

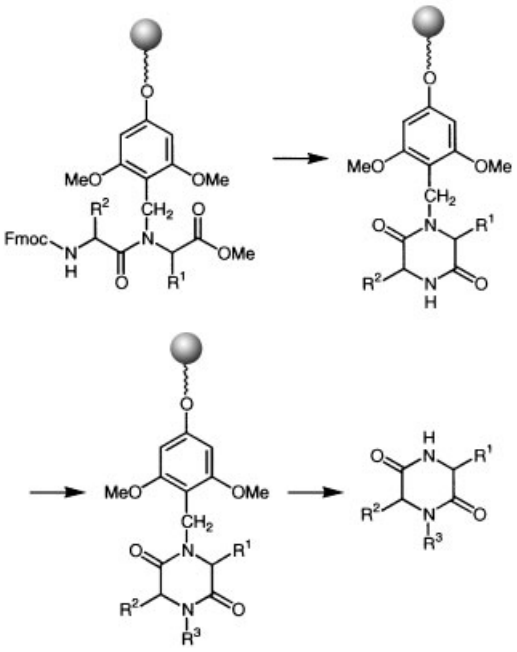
An early example of the exploitation of facile DKP formation is that reported by Gordon and Steele [185], who prepared a combinatorial library of 1000 DKPs. Fmoc-amino acids were immobilized on Wang resin, deprotected, and reductively alkylated with aldehydes and $NaBH(OAc)_3$. The resulting 2° amines were acylated with Boc-amino acids (PyBrOP/DIEA coupling). After deprotection of the Boc group with neat TFA, cyclization with concomitant release from the support was achieved by reflux in toluene for 5 h. In a related study [186], 2500 DKPs and piperazines with three centres of diversity were prepared combinatorially: 10 Fmoc-amino acids immobilized on Wang resin were Fmoc-deprotected, reductively alkylated with 12 aldehydes or ketones, and the resulting 2° amines bromoacetylated, followed by amination with 12 different amines. Finally DKP cyclization was accomplished by heating in 2 M AcOH in 2-butanol. The resulting DKPs were further transformed into piperazines ($LiAlH_4$ or BH_3 reduction). A similar approach was taken for the combinatorial synthesis of tetrasubstituted DKPs from α-bromocarboxylic acids and amines [187]. Yet another DKP-based combinatorial approach starts from side-chain protected *cyclo*-[Glu-Glu], which is *N*-alkylated with α-bromoacetate esters and thus gives rise to a DKP scaffold, which, upon judicious choice of ester protecting groups, offers up to four individually accessible points for combinatorial diversity [188].

In a similar approach [189], based on the 'diversomer' principle elaborated for the combinatorial synthesis of hydantoins and benzodiazepines [190], the polyethyleneglycol (Tentagel) or 4-hydroxymethyl-phenylacetamidomethyl (PAM) linkers of peptide synthesis resins were esterified with protected amino acids. Depending on the amino acids, standard DIC/DMAP, Mitsunobu, or acyl fluoride esterification methods were used. Following deprotection, the amino group was reductively alkylated with aliphatic or aromatic aldehydes. The imine was formed on the solid support using trimethylorthoformate, $NaCNBH_3$ served as the reducing agent and an additional proton source

(AcOH or MeOH) was required for complete conversion to the 2° amine. This was then followed by acylation with Boc-protected amino acids (using HATU, DMFP, DIC/HOAt, or symmetrical anhydride coupling chemistry). Deprotection with TFA removed the terminal Boc group, as well as acid-labile side-chain protecting groups, without release of the *N*-alkylated dipeptides from the support. Cyclization was then induced by treatment with toluene/EtOH mixtures under acidic (1% AcOH; 8–12 h) or basic (4% TEA, 2–5 h) conditions. Apparently very pure DKPs were obtained with this method, since by-products, including those from incomplete alkylation and acylation, were not released from the support. Furthermore, the synthesis method was applied to the preparation of soluble DKP libraries *via* the split-and-pool method [191].

A strategy particularly suitable for the combinatorial preparation of resin-bound DKP libraries was reported [192]. In this method masked (as cyclic *N*-Boc *N,O*-acetals) aldehyde carboxylic acids are immobilized *via* the carboxyl group. The aldehyde group is then unmasked (95 : 5 TFA/H_2O; 3 min) and aminated reductively with amino acyl Bu^t esters and $NaBH(OAc)_3$ in DMSO/DCM/AcOH (50 : 50 : 1), followed by acylation of the 2° amino group with Fmoc-amino acids under various coupling conditions, amongst which *in situ* generation of the acyl chloride with the aid of bis(trichloromethyl)carbonate [193] and coupling in the presence of 2,4,6-collidine at 50–55 °C was the most successful. Cyclization was found to take place readily upon final Fmoc-deprotection.

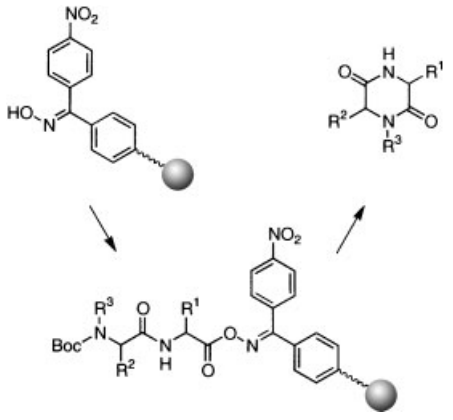
A backbone amide linker based on the tris(alkoxy)-benzylamine system was developed for the synthesis of *C*-terminally modified and cyclic peptides [194]. It was noticed, however, that Fmoc-dipeptidyl allyl esters immobilized *via* the peptide amide nitrogen were prone to cyclization upon piperidine-mediated Fmoc deprotection (dipeptidyl allyl esters have been found to be prone to DKP formation elsewhere [195]). This propensity of the tertiary amide-containing dipeptide esters was then exploited for the intentional synthesis of various DKPs (Scheme 14) [196]. The starting materials were obtained by amidation of 5-(4-formyl-3,5-dimethoxyphenoxy) valeric acid with an amino resin, followed by reductive amination of the aldehyde function with amino acyl methyl esters and acylation with Fmoc-amino acids. In all cases investigated, standard Fmoc deprotection at this stage led to quantitative DKP formation. The fact that here the DKP is not displaced from the solid support, as is the case with most other systems,



Scheme 14

is an advantage since further on-resin modification, e.g. alkylation of the primary amide as shown, or elaboration of orthogonally protected functional side chains R^1 and R^2, is possible. The suitability of this system for combinatorial synthesis approaches has also been demonstrated [197].

As mentioned earlier, the Kaiser oxime resin can be used to prepared DKPs [36]. This approach was applied to the automated synthesis of a DKP library [198] (Scheme 15). Treatment of the Boc-deprotected resins with DIEA (2.2 eq) / AcOH (5 eq) in DCM for 16 h afforded the DKPs in most cases. However, the apparent lack of certain DKPs (e.g. those including Pro and Tic residues) was probably



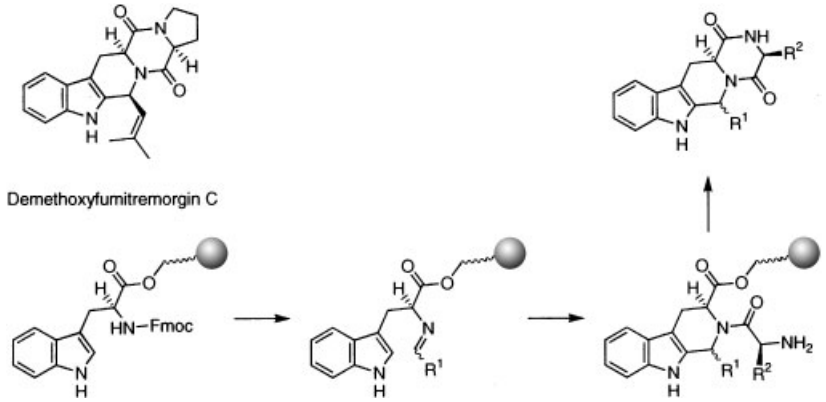
Scheme 15

due to premature cyclization during Boc-dipeptide deprotection (25% TFA/DCM; 20 min).

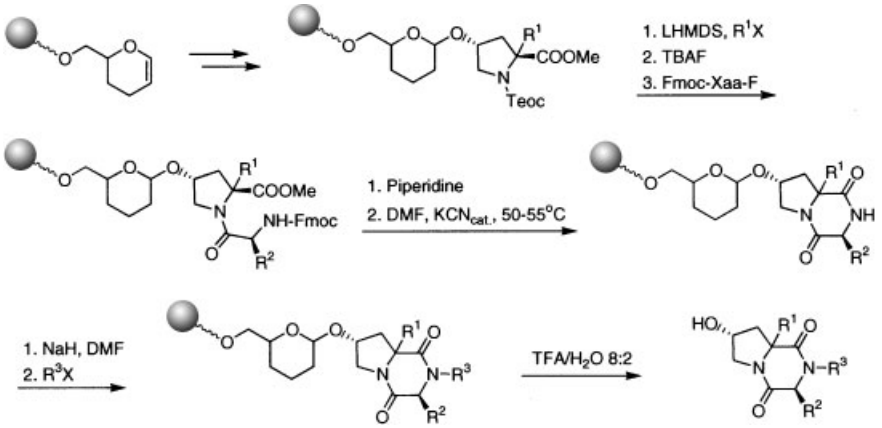
Demethoxyfumitremorgin C (Scheme 16) is the most active of a series of prenylated indole alkaloids with anti-proliferative properties. It contains a tetrahydro-β-carboline system with an embedded Pro DKP ring. The originally devised synthetic route was adapted in order to devise a solid-phase method permitting combinatorial synthesis of analogues [199]. Fmoc-Trp was immobilized on Wang resin and reacted, after deprotection, with various aldehydes to afford the intermediate imines. These were then subjected to *N*-acyliminium Pictet-Spengler condensation using a variety of Fmoc-amino acid chlorides. Upon Fmoc deprotection with piperidine the DKP rings were formed in high yield.

The synthesis of highly functionalized DKP libraries based on Hyp is shown in Scheme 17 [200]. Fmoc-Hyp-OH was immobilized on a dihydropyran-functionalized resin [201], Fmoc-deprotected and Teoc-reprotected. The latter protecting group was stable to the alkylation conditions in the next step. After removal of the Teoc group with fluoride ions, the pyrrolidine nitrogen was acylated with Fmoc-Xaa-F in the presence of *N,O*-bis(trimethylsilyl)acetamide [141]. The formation of the DKP was initiated immediately during cleavage of the Fmoc group and was completed by heating in DMF in the presence of a catalytic amount of KCN. The backbone amide function was finally alkylated and the DKPs removed through mild hydrolysis. A range of different insoluble polymer supports was investigated using this chemistry [202]. It was found that polyoxyethylene-polyoxypropylene (POE-POP) resins [203] were particularly suitable, since they permitted analysis of resin-bound intermediates (by high-resolution magic angle spinning NMR spectroscopy) at a resolution similar to that with corresponding molecules in solution.

An efficient solution-based library approach to the synthesis of DKPs was adopted by Hulme



Scheme 16

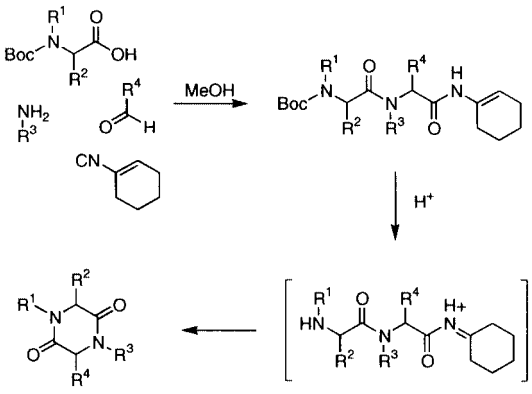


Scheme 17

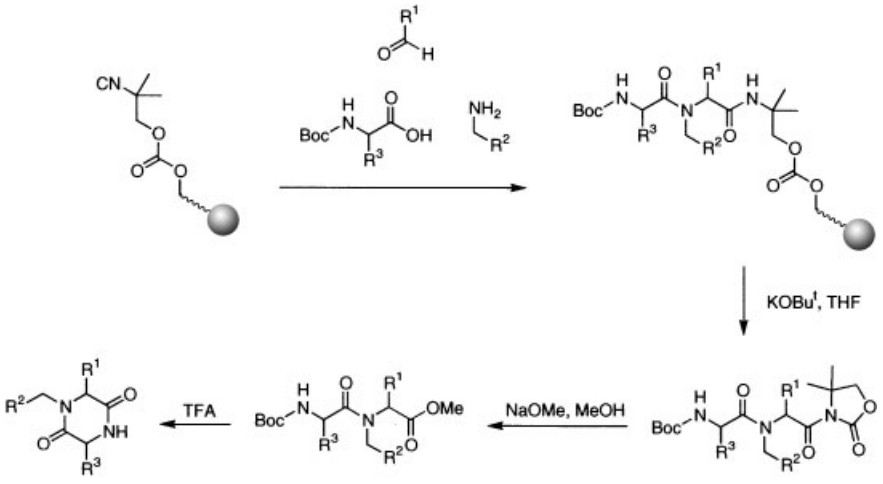
et al. [204] Here the Ugi 4-component condensation (4CC) reaction [205] was employed combinatorially, i.e. reaction between different Boc-amino acids, aldehydes, amines, and isonitriles. When the so-called convertible isonitrile (cyclohexenyl isonitrile) [206], as shown in Scheme 18, was employed, it was found that the intermediate dipeptidyl amides cyclized to the corresponding DKPs with extraordinary ease. This is due to the fact that during TFA-mediated Boc deprotection the cyclohexenyl amide is converted to the *N*-acyliminium ion, which is very prone to nucleophilic attack. A related method, in which ethyl glyoxalate in place of a simple aldehyde was used in the Ugi 4CC reaction, has also been used to prepare DKP libraries [207]. In this case facile cyclization after Boc-deprotection of the terminal amino group, by reaction with the ethyl ester function, was achieved.

Solid-phase versions for the combinatorial synthesis of DKPs *via* the Ugi 4CC route have also been reported [208,209]. Thus collagenase-1 inhibitory DKPs containing Cys residues were prepared [210]. Instead of the simple amine components of the usual Ugi input, immobilized amino acid esters were used. A traceless linker based on a convertible isonitrile was used very recently for the synthesis of a DKP library [211]. The resin-bound carbonate convertible isonitrile was employed in a standard (Scheme 19) Ugi 4CC procedure. The intermediate immobilized Boc-dipeptidyl amides were then converted to the corresponding *N*-acyloxazolidones, with concomitant cleavage from the resin. These did not undergo cyclization and were therefore converted to the methyl esters, which underwent DKP formation upon Boc deprotection, as expected. Apparently this methodology permitted the high-yield parallel preparation in 80-well plates of a 4000-member DKP library.

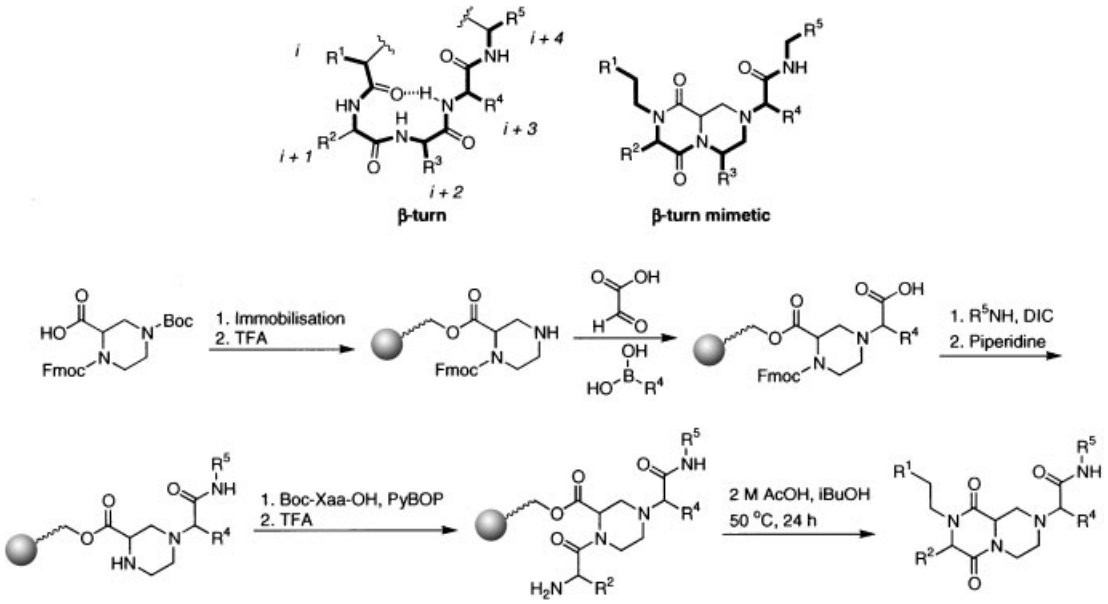
A different multi-component condensation strategy based on the Petasis reaction [212] was used in the high-throughput synthesis of bicyclic DKP-containing protein β-turn mimetics (Scheme 20) [213]. Such turns contain a 10-membered H-bonded ring and are frequent in protein structures [214]. The synthesis started with orthogonally protected piperazinic acid, which was immobilized and Boc-deprotected. Condensation with arylboronic acids in the presence of glyoxylic acid gave the N^{β}-substituted intermediates. These were aminated, then N^{α}-deprotected and further acylated at that position. Finally cyclization was achieved by heating in AcOH/Bu^{i}OH. It was found that practically all of the commercially available arylboronic acids could be used in this procedure.



Scheme 18



Scheme 19



Scheme 20

3-Ylidene-DKPs, i.e. DKPs containing didehydroamino acid residues, are particularly versatile organic substrates and this subject has been reviewed recently [215]. Combinatorial syntheses, both in solution [216] and using solid-phase methods [217], have recently been reported.

CONCLUSION

As shown in this review, DKPs have been the peptide chemists' constant companions for a long time and we have amassed a great deal of knowledge about them, which has been applicable to the understanding of the chemistry of polypeptides in general. The well-understood synthetic methods for the preparation of DKPs are now being exploited in combinatorial chemistry strategies aimed at accelerating the drug discovery process, particularly in the pharmaceutical industry.

REFERENCES

1. Sammes PG. Naturally occurring 2,5-dioxopiperazines and related compounds. In *Fortschritte der Chemie Organischer Naturstoffe*. Springer-Verlag: Vienna, 1975; 51–118.
2. Anteunis MJO. The cyclic dipeptides. Proper model compounds in peptide research. *Bull. Soc. Chim. Belg.* 1978; **87**: 627–650.
3. Rajappa S, Natekar MV. Piperazine-2,5-diones and related lactim ethers. In *Advances in Heterocyclic Chemistry*. Academic Press: San Diego, CA, 1993; 187–289.
4. Krchnak V, Weichsel AS, Cabel D, Flegelova Z, Lebl M. Structurally homogeneous and heterogeneous synthetic combinatorial libraries. *Mol. Div.* 1996; **1**: 149–164.
5. Acharya AN, Ostresh JM, Houghten RA. Solid-phase synthesis of substituted imidazoline-tethered 2,3-diketopiperazines, cyclic ureas, and cyclic thioureas. *J. Comb. Chem.* 2001; **3**: 612–623.
6. Kohler A. Ueber eine neue Verwandlung des Leucins. *Liebigs Ann. Chem.* 1865; **134**: 367–372.
7. Fischer E, Forneau E. Ueber einige Derivate des Glykocolls. *Chem. Ber.* 1901; **34**: 2868–2877.
8. Bopp F. Einiges ueber Albumin, Casein und Fibrin. *Liebigs Ann. Chem.* 1849; **69**: 16–37.
9. Abenius PW, Widman O. Ueber das Bromoacetortholuid und einige daraus erhaltene Verbindungen. *Chem. Ber.* 1888; **21**: 1662–1664.
10. Curtius T. Ueber das Glycocoll. *Chem. Ber.* 1883; **16**: 753–757.
11. Curtius T, Goebel F. Ueber Glycinanhydrid und seine Verbindungen. *J. Prakt. Chem.* 1888; **37**: 173–181.
12. Katchalski E. Poly-α-amino acids. *Adv. Protein Chem.* 1951; **6**: 123–185.
13. Albertson NF, McKay FC. Acid-catalyzed decarbobenzyloxylation. *J. Am. Chem. Soc.* 1953; **75**: 5323–5326.
14. Edman P, Begg G. A protein sequenator. *Eur. J. Biochem.* 1967; **1**: 80–91.
15. Yamada S, Yokoyama Y, Shioiri T. A method for sequencing peptides: a co-operation of diphenyl

phosphorazidate and 2-mercapto- or 2-hydroxypyridine for N-acyldiketopiperazine formation. *Experientia* 1976; **32**: 398–399.
16. Adamson DW. The anhydrides of basic amino-acids. *J. Chem. Soc.* 1943; 39–40.
17. Rosenmund P, Kaiser K. Diketopiperazines from Leuchs anhydrides. *Angew. Chem. Int. Ed. Engl.* 1970; **9**: 162–163.
18. Nitecki DE, Halpern B, Westley JW. Simple route to sterically pure dioxopiperazines. *J. Org. Chem.* 1968; **33**: 864–866.
19. Lee S, Kanmera T, Aoyagi H, Izumiya N. Cyclic peptides. VI. Asymmetric hydrogenation of dehydroalanine or dehydroaminobutanoic acid residue in cyclodipeptides. *Int. J. Peptide Protein Res.* 1979; **13**: 207–217.
20. Kopple KD, Ghazarian HG. Covenient synthesis of 2,5-piperazinediones. *J. Org. Chem.* 1968; **33**: 862–864.
21. Lichtenstein N. The behavior of peptides when heated in β-naphthol. *J. Am. Chem. Soc.* 1938; **60**: 560–563.
22. Ueda T, Saito M, Kato T, Izumiya N. Facile synthesis of cyclic dipeptides and detection of racemization. *Bull. Chem. Soc. Jpn* 1983; **56**: 568–572.
23. Suzuki K, Sasaki Y, Endo N, Mihara Y. Acetic acid-catalyzed diketopiperazine synthesis. *Chem. Pharm. Bull.* 1981; **29**: 233–237.
24. Iyer MS, Lipton MA. An improved synthesis of the diketopiperazine catalyst cyclo[(*S*)-His-(*S*)-Phe]. *Bioorg. Med. Chem. Lett.* 1993; **3**: 2061–2062.
25. Schoellkopf U. Enantioselective synthesis of non-proteinogenic amino acids via metallated bis-lactim ethers of 2,5-diketopiperazines. *Tetrahedron* 1983; **39**: 2085–2091.
26. Oya M, Katakai R, Nakai H. A novel synthesis of N-carboxy-α-amino acid anhydride. *Chem. Lett.* 1973; 1143–1144.
27. Daly WH, Poche D. The preparation of N-carboxyanhydrides of α-amino acids using bis(trichloromethyl)-carbonate. *Tetrahedron Lett.* 1988; **29**: 5859–5862.
28. González A, Vorob'eva SL, Linares A. Synthesis and structural study of piperazine-2,5-diones derived from (*R*)-cysteine. *Tetrahedron: Asymmetry* 1995; **6**: 1357–1366.
29. Bergeron RJ, Phanstiel O, Yao GW, Milstein S, Weimar WR. Macromolecular self-assembly of diketopiperazine tetrapeptides. *J. Am. Chem. Soc.* 1994; **116**: 8479–8484.
30. Plata DJ, Leanna MR, Morton HE. The stereospecific preparation of an hydroxyethylene isostere precursor via a novel piperidine-2,5-dione template. *Tetrahedron Lett.* 1991; **32**: 3623–3626.
31. Goodman M, Steuben KC. Peptide synthesis via amino acid active esters. II. Some abnormal reactions during peptide synthesis. *J. Am. Chem. Soc.* 1962; **84**: 1279–1283.
32. D'Angeli F, Marchetti P, Rondanin R, Bertolasi V. Stereoselective synthesis of N,N′-diaryl-2,5-dioxopiperazines from homochiral or racemic 2-bromopropananilides. *J. Org. Chem.* 1996; **61**: 1252–1255.
33. Fridkin M, Patchornik A, Katchalski E. A synthesis of cyclic peptides utilizing high molecular weight carriers. *J. Am. Chem. Soc.* 1965; **87**: 4646–4648.
34. Flanigan E, Marschall GR. Synthesis of cyclic peptides on dual function supports. *Tetrahedron Lett.* 1970; 2403–2406.
35. Giralt E, Eritja R, Josa J, Kuklinski C, Pedroso E. The use of Nbb resin in cyclic dipeptide ('diketopiperazine') synthesis. *Synthesis* 1985; 181–184.
36. Oesapay G, Bouvier M, Taylor JW. Peptide cyclization on oxime resin (PCOR). In *Techniques in Protein Chemistry II*. Academic Press: New York, 1991; 221–231.
37. Kowalski J, Lipton MA. Solid phase synthesis of a diketopiperazine catalyst containing the unnatural amino acid (*S*)-norarginine. *Tetrahedron Lett.* 1996; **37**: 5839–5840.
38. Ramachandran GN, Mitra AK. An explanation for the rare occurrence of *cis* peptide units in proteins and polypeptides. *J. Mol. Biol.* 1976; **107**: 85–92.
39. Radzicka A, Pedersen L, Wolfenden R. Influences of solvent water on protein folding: free energies of solvation of cis and trans peptides are nearly identical. *Biochemistry* 1988; **27**: 4538–4541.
40. Li Y, Garrell RL, Houk KN. Mechanism of *cis-trans* isomerization of amide and peptide excited states. *J. Am. Chem. Soc.* 1991; **113**: 5895–5896.
41. Stewart DE, Sarkar A, Wampler JE. Occurrence and role of *cis* peptide bonds in protein structure. *J. Mol. Biol.* 1990; **214**: 253–260.
42. Fischer S, Dunbrack RL, Karplus M. *Cis-trans* isomerization of the proline dipeptide. *J. Am. Chem. Soc.* 1994; **116**: 11 931–11 937.
43. Gisin RF, Merrifield RB. Carboxyl-catalyzed intramolecular aminolysis. A side reaction in solid-phase peptide synthesis. *J. Am. Chem. Soc.* 1972; **94**: 3102–3106.
44. Rothe M, Mazanek J. Nebenreaktionen bei der Festphasensynthese von Peptiden, IV. Intrachenare und interchenare aminolyse der Benzylesterverbindung zum polymeren Traeger und ihre Auswirkungen. *Liebigs Ann. Chem.* 1974; 439–459.
45. Reddy AV, Ravindranath B. Synthesis of α, β- and cyclic spaglumic acids. *Int. J. Peptide Protein Res.* 1992; **40**: 472–476.
46. Jornvall H. Blocked α-amino groups in peptides due to diketopiperazine formation. *FEBS Lett.* 1974; **38**: 329–333.
47. Schoen I, Kisfaludy L. Formation of aminosuccinyl peptides during acidolytic deprotection followed by their transformation to piperazine-2,5-dione derivatives in neutral media. *Int. J. Peptide Protein Res.* 1979; **14**: 485–494.

48. Zanotti G, Pinnen F, Lucente G. Cyclization under mild conditions of cysteine containing peptides. *Tetrahedron Lett.* 1985; **26**: 5481–5484.
49. Lucente G, Pinnen F, Zanotti G, Cerrini S, Fedeli W, Mazza F. Cyclols, cyclodepsipeptides, and N-acyldiketopiperazines from linear tripeptides. *Pept. Synth. Struct. Funct.* 1981; 93–96.
50. Greenstein JP. Studies of multivalent amino acids and peptides. VIII. The synthesis of bisanhydro-L-cystinyl-L-cystine and other diketopiperazines of cystine. *J. Biol. Chem.* 1937; **118**: 321–329.
51. du Vigneaud V, Patterson WI, Hunt M. Opening of the ring of the thiolactone of homocysteine. *J. Biol. Chem.* 1938; **126**: 217–231.
52. Abderhalden E, Suzuki S. Studien ueber den Einfluss der an der Bildung von Aminosaeureestern beteiligten Alkoholgruppe auf die Geschwindigkeit der Bildung von 2,5-Dioxopiperazinen und die Entstehung von Guanidinoverbindungen bei der Einwirkung von Guanidin auf verschiedene Aminosaeureester. *Hoppe-Seyler's Z. Physiol. Chem.* 1928; **176**: 101–108.
53. Woodward RB, Heusler K, Gosteli J, Naegeli P, Oppolzer W, Ramage R, Ranganathan S, Vorbrueggen H. Total synthesis of cephalosporin C. *J. Am. Chem. Soc.* 1966; **88**: 852–853.
54. Stelakatos GC, Paganou A, Zervas L. New methods in peptide synthesis. Part III. Protection of carboxyl group. *J. Chem. Soc. C.* 1966; 1191–1199.
55. Wang D-X, Liang M-T, Tian G-J, Lin H, Liu H-Q. A facile pathway to synthesize diketopiperazine derivatives. *Tetrahedron Lett.* 2002; **43**: 865–867.
56. Sakakibara S. Synthesis of large peptides in solution. *Biopolymers Pept. Sci.* 1995; **37**: 17–28.
57. Obeyesekere NU, La Croix J-N, Budde RJA, Dyckes DF, McMurray JS. Solid-phase synthesis of (tyrosyl-alanyl-glutamyl)n by segment condensation. *Int. J. Peptide Protein Res.* 1994; **43**: 118–126.
58. Beyermann M, Bienert M, Niedrich H, Carpino LA, Sadat-Aalaee D. Rapid continuous peptide synthesis via FMOC amino acid chloride coupling and 4-(aminomethyl)piperidine deblocking. *J. Org. Chem.* 1990; **55**: 721–728.
59. Pinker TG, Young GT, Elliott DF, Wade R. Amino acids and peptides. XXXIX. Synthesis of analogs of bradykinin with modifications in positions 1, 6, and 9. *J. Chem. Soc. Perkin Trans. 1* 1976; 220–228.
60. Pettit GR, Taylor SR. Synthesis of the marine sponge cycloheptapeptide stylopeptide 1. *J. Org. Chem.* 1996; **61**: 2322–2325.
61. Tung RD, Dhaon MK, Rich DH. BOP-Cl mediated synthesis of the cyclosporine A 8–11 tetrapeptide fragment. *J. Org. Chem.* 1986; **51**: 3350–3354.
62. Fischer PM, Solbakken M, Undheim K. Solution synthesis of a dimeric pentapeptide: diketopiperazine cyclisation of Glu-Asp dipeptide esters and Asp-racemisation during segment condensation. *Tetrahedron* 1994; **50**: 2277–2288.
63. Koenig W, Geiger R. Eine neue Methode zur Synthese von Peptiden: Aktivierung der Carboxylgruppe mit Dicyclohexylcarbodiimid und 3-Hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazin. *Chem. Ber.* 1970; **103**: 2034–2040.
64. Ludescher U, Schwyzer R. Synthese von [2,7-Cystin]-Gramicidin S, einem kuenstlichen homodet-heterodet-bicyclischen Decapeptid. *Helv. Chim. Acta* 1972; **55**: 2052–2060.
65. Kessler H, Becker G, Kogler H, Wolff M. 2-(2-Pyridyl-)ethyl esters. A new carboxyl protecting group in peptide synthesis. *Tetrahedron Lett.* 1984; **25**: 3971–3974.
66. Kunz H, Kneip M. 2-(Pyridyl)ethylester als sicherer und dennoch leicht abloesbarer polariphiler Carboxy-Schutz bei der Peptidsynthese. *Angew. Chem.* 1984; **96**: 702–704.
67. Poduska K, Gross H. Ueber α-Halogenäther, VII. Reaktionen von Dichlormethyl-alkyl-aethern mit Aminosaeurederivaten. *Chem. Ber.* 1961; **94**: 527–537.
68. Frankel M, Katchalski E. Poly-condensation of α-amino acid esters. II. Poly-condensation of alanine ethyl ester. *J. Am. Chem. Soc.* 1942; **64**: 2268–2271.
69. Sasaki Y, Akutsu Y, Matsui M, Suzuki K, Sakurada S, Sato T, Kisara K. Studies on analgesic oligopeptides. II. Structure-activity relationship among thirty analogs of a cyclic dipeptide, cyclo(-Tyr-Arg-). *Chem. Pharm. Bull.* 1982; **30**: 4435–4443.
70. Kolar P, Tisler M. Heterocyclic amino acids as synthons. Reactions with dicarbonyl compounds. *J. Heterocycl. Chem.* 1993; **30**: 1253–1260.
71. Ajo D, Granozzi G, Guantieri V, Tamburro AM. UV photoelectron spectra of peptide unit model compounds: methionyl-containing cyclic dipeptides. *J. Mol. Struct.* 1983; **96**: 369–372.
72. Barany G, Merrifield RB. Solid-phase peptide synthesis. In *The Peptides: Analysis, Synthesis, Biology*. Academic Press: New York, 1980; 1–284.
73. Bodanszky M, Martinez J. Side reactions in peptide synthesis. In *The Peptides, Analysis, Synthesis, Biology*. Academic Press: New York, 1983; 111–216.
74. Bodanszky M. Active esters in peptide synthesis. In *The Peptides: Analysis, Synthesis, Biology*. Academic Press: New York, 1979; 105–196.
75. Meienhofer J, Sano Y, Patel RP. Synthesis of actinomycin D (C1). In *Peptides: Chem. Biochem., Proc. Amer. Peptide Symp., 1st (1970), Meeting Date 1968*. Marcel Dekker: New York, 1970; 419–434.
76. Rydon HN, Smith PWG. Polypeptides. Part IV. The self-condensation of the esters of some peptides of glycine and proline. *J. Chem. Soc.* 1956; 3642–3650.
77. Marsden BJ, Nguyen TM-D, Schiller PW. Spontaneous degradation via diketopiperazine formation of peptides containing a tetrahydroisoquinoline-3-carboxylic acid residue in the 2-position of the peptide sequence. *Int. J. Peptide Protein Res.* 1993; **41**: 313–316.

78. Schiller PW, Nguyen TM-D, Weltrowska G, Wilkes BC, Lemieux C, Chung NN. TIPP opioid peptides: development of extraordinarily potent and selective D antagonists and observation of astonishing structure-intrinsic activity relationships. In *Peptides: Chemistry, Structure and Biology*. ESCOM: Leiden, 1994; 483–486.
79. Carpenter KA, Weltrowska G, Wilkes BC, Schmidt R, Schiller PW. Spontaneous diketopiperazine formation via end-to-end cyclization of a nonactivated linear tripeptide: an unusual chemical reaction. *J. Am. Chem. Soc.* 1994; **116**: 8450–8458.
80. Gu L, Strickley RG. Diketopiperazine formation, hydrolysis, and epimerization of the new dipeptide angiotensin-converting enzyme inhibitor RS-10085. *Pharm. Res.* 1987; **4**: 392–397.
81. Simon RJ, Kania RS, Zuckermann RN, Huebner VD, Jewell DA, Banville S, Ng S, Wang L, Rosenberg S, Marlowe C, Spellmeyer DC, Tan R, Frankel AD, Santi DV, Cohen FE, Bartlett PA. Peptoids: a modular approach to drug discovery. *Proc. Natl. Acad. Sci. USA* 1992; **89**: 9367–9371.
82. Rothe M, Mazanek J. Side-reactions arising on formation of cyclodipeptides in solid-phase peptide synthesis. *Angew. Chem. Int. Ed. Engl.* 1972; **11**: 293.
83. Titlestad K. Formation of cyclic dipeptides and bi- and tricyclic products from linear tetrapeptides. In *Chemistry and Biology of Peptides*. Ann Arbor Science Publishers, 1972; 59–65.
84. Khoshla MC, Smeby RR, Bumpus FM. Failure sequence in solid-phase peptide synthesis due to the presence of an N-alkylamino acid. *J. Am. Chem. Soc.* 1972; **94**: 4721–4726.
85. Goldberg I, Stein Z, Lin L-TW, Hart H. Structure of N,N′-ditrityl-2,5-diketopiperazine and its 1 : 1 inclusion complex with methylene chloride. *Acta Cryst.* 1985; **C41**: 1539–1543.
86. Toniolo C. $C^{\alpha},^{\alpha}$-symmetrically disubstituted glycines: useful building blocks in the design of conformationally restricted peptides. *Janssen Chim. Acta* 1993; **11**: 10–16.
87. Nagaraj R, Balaram P. Facile diketopiperazine formation from dipeptides containing α-aminoisobutyric acid. Conformational factors influencing cyclisation. *Heterocycles* 1977; **7**: 885–890.
88. Ali MY, Dale J, Titlestad K. Bi- and tricyclic products from tetra- and pentapeptides of α-methylalanine. *Acta Chem. Scand.* 1973; **27**: 1509–1518.
89. Jacobson RA. Esters of α-aminoisobutyric acid. *J. Am. Chem. Soc.* 1946; **68**: 2628–2630.
90. Paterson Y, Rumsey SM, Benedetti E, Nemethy G, Scheraga HA. Sensitivity of polypeptide conformation to geometry. Theoretical conformational analysis of oligomers of α-aminoisobutyric acid. *J. Am. Chem. Soc.* 1981; **103**: 2947–2955.
91. Perczel A, Kajtar M, Marcoccia JF, Csizmadia IG. The utility of the four-dimensional Ramachandran map for the description of peptide conformations. *J. Mol. Struct.* 1991; **232**: 291–319.
92. Vavrek RJ, Stewart JM. Bradykinin analogs containing α-aminoisobutyric acid (Aib). *Peptides* 1980; **1**: 231–235.
93. Jones DS, Kenner GW, Preston J, Sheppard RC. Peptides. XVII. Synthesis of peptides and polymers of some sterically hindered amino acids via oxazolone intermediates. *J. Chem. Soc.* 1965; 6227–6239.
94. Jones DS, Kenner GW, Preston J, Sheppard RC. Peptides. XIX. Isomerization of oxazolones derived from tripeptides. *Tetrahedron* 1965; **21**: 3209–3218.
95. Kamber B. Cystinpeptide aus (S-Acetamidomethylcystein)-peptiden durch Oxydation mit Jod: Die Synthese von cyclo-L-Cystin. *Helv. Chim. Acta* 1971; **54**: 927–930.
96. Lunkenheimer W, Zahn H. Peptide, LXXIX. Merrifield-Synthese symmetrischer Cystinpeptide. *Liebigs Ann. Chem.* 1970; **740**: 1–17.
97. Mazurov AA, Andronati SA, Korotenko TI, Gorbatyuk VY, Shapiro YE. Formation of pyroglutamylglutamine (or asparagine) diketopiperazine in 'non-classical' conditions: a side reaction in peptide synthesis. *Int. J. Peptide Protein Res.* 1993; **42**: 14–19.
98. Nutt RF, Holly FW, Homnick C, Hirschmann R, Veber DF. Synthesis of thyrotropin-releasing hormone analogues with selective central nervous systems effects. *J. Med. Chem.* 1981; **24**: 692–698.
99. Gerig JT, McLeod RS. Attempted synthesis of 2-methylalanyl-L-prolyl-L-tryptophan. An unexpected result. *J. Org. Chem.* 1976; **41**: 1653–1655.
100. Mazur RH, Schlatter JM. A novel peptide cleavage reaction. *J. Org. Chem.* 1963; **28**: 1025–1029.
101. Steinberg SM, Bada JL. Peptide decomposition in the neutral pH region via the formation of diketopiperazines. *J. Org. Chem.* 1983; **48**: 2295–2298.
102. Battersby JE, Hancock WS, Canova-Davis E, Oeswein J, O'Connor B. Diketopiperazine formation and *N*-terminal degradation in recombinant human growth hormone. *Int. J. Peptide Protein Res.* 1994; **44**: 215–222.
103. Kertscher U, Bienert M, Krause E, Sepetov NF, Mehlis B. Spontaneous chemical degradation of substance P in the solid phase and solution. *Int. J. Peptide Protein Res.* 1993; **41**: 207–211.
104. Ott H, Frey AJ, Hofmann A. Stereospecific cyclolization of *N*-(α-hydroxyacyl)-phenylalaninyl-proline lactams. *Tetrahedron* 1963; **19**: 1675–1684.
105. Smith GG, Baum R. First-order rate constants for the racemization of each component in a mixture of isomeric dipeptides and their diketopiperazines. *J. Org. Chem.* 1987; **52**: 2248–2255.
106. Gaines SM, Bada JL. Aspartame decomposition and epimerization in the diketopiperazine and dipeptide products as a function of pH and temperature. *J. Org. Chem.* 1988; **53**: 2757–2764.
107. Szokan G, Majer Z, Kollat E, Kajtar M, Hollosi M, Peredy-Kajtar M. Chromatographic studies on the

racemization of thiopeptides. *J. Liq. Chromatogr.* 1995; **18**: 941–964.
108. Sepetov NF, Krymsky MA, Ovchinnikov MV, Bespalova ZD, Isakova OL, Soucek M, Lebl M. Rearrangement, racemization and decomposition of peptides in aqueous solution. *Peptide Res.* 1991; **4**: 308–313.
109. Song S, Asher SA, Krimm S, Shaw KD. Ultraviolet resonance Raman studies of trans and cis peptides: photochemical consequences of the twisted π^* excited state. *J. Am. Chem. Soc.* 1991; **113**: 1155–1163.
110. Webb LE, Lin C-F. Conformations of cyclic dipeptides. Structure of cyclo-glycyl-L-tyrosyl (L-3-(4-hydroxybenzyl)-2,5-piperazinedione). *J. Am. Chem. Soc.* 1971; **93**: 3818–3819.
111. Corey RB. Crystal structure of diketopiperazine. *J. Am. Chem. Soc.* 1938; **60**: 1598–1604.
112. Balasubramanian D, Wetlaufer DB. Optical rotatory properties of diketopiperazines. *J. Am. Chem. Soc.* 1966; **88**: 3449–3450.
113. Benedetti E, Corradini P, Goodman M, Pedone C. Flexibility of supposed 'rigid' molecules: substituted 2,5-piperazinediones (diketopiperazines). *Proc. Natl. Acad. Sci. USA* 1969; **62**: 650–652.
114. Schlessinger J, Gafni A, Steinberg IZ. Optical rotatory power in the ground state and electronically excited state of diketopiperazines containing aromatic side chains. *J. Am. Chem. Soc.* 1974; **96**: 7396–7400.
115. Kopple KD, Ohnishi M. Conformations of cyclic peptides. II. Side-chain conformation and ring shape in cyclic dipeptides. *J. Am. Chem. Soc.* 1969; **91**: 962–970.
116. Basu G, Kubasik M, Anglos D, Kuki A. Spin-forbidden excitation transfer and heavy-atom induced intersystem crossing in linear and cyclic peptides. *J. Phys. Chem.* 1993; **97**: 3956–3967.
117. Maes CM, Potgieter M, Steyn PS. NMR assignments, conformation, and absolute configuration of ditryptophenaline and model dioxopiperazines. *J. Chem. Soc. Perkin Trans. 1* 1986; 861–866.
118. Ciajolo MR, Balboni G, Picone D, Salvadori S, Tancredi T, Temussi PA, Tuzi A. Solution and solid-state structure of the diketopiperazine of tyrosyl-tetrahydroisoquinoline-3-carboxylic acid. *Int. J. Peptide Protein Res.* 1995; **46**: 134–138.
119. Egusa S, Takagi J, Sisido M, Imanishi Y. Synthesis and conformation of aromatic cyclic dipeptides. Cyclo(phenylalanyl)$_2$, cyclo(1-naphthylalanyl)$_2$, and cyclo(2-naphthylalanyl)$_2$. *Bull. Chem. Soc. Jpn.* 1986; **59**: 2195–2201.
120. Siemion IZ. Die Konformation des Prolin-Ringes in Diketopiperazin-Systemen. *Liebigs Ann. Chem.* 1971; **748**: 88–95.
121. Maeji NJ, Bray AM, Geysen HM. Multi-pin peptide synthesis strategy for T cell determinant analysis. *J. Immunol. Methods* 1990; **134**: 23–33.
122. Bray AM, Maeji NJ, Valerio RM, Campbell RA, Geysen HM. Direct cleavage of peptides from a solid support into aqueous buffer. Application in simultaneous multiple peptide synthesis. *J. Org. Chem.* 1991; **56**: 6659–6666.
123. Lam KS, Salmon SE, Hersh EM, Hruby VJ, Kazmierski WM, Knapp RJ. A new type of synthetic peptide library for identifying ligand-binding activity. *Nature* 1991; **354**: 82–84.
124. Salmon SE, Lam KS, Lebl M, Kandola A, Khattri PS, Wade S, Pçtek M, Kocis P, Krchnçk V, Thorpe D, Felder S. Discovery of biologically active peptides in random libraries: solution-phase testing after staged orthogonal release from resin beads. *Proc. Natl. Acad. Sci. USA* 1993; **90**: 11 708–11 712.
125. Patek M. Multistep deprotection for peptide chemistry. *Int. J. Peptide Protein Res.* 1993; **42**: 97–117.
126. Bernatowicz MS, Daniels SB, Koester H. A comparison of acid labile linkage agents for the synthesis of peptide *C*-terminal amides. *Tetrahedron Lett.* 1989; **30**: 4645–4648.
127. Story SC, Aldrich JV. Preparation of protected peptide amides using Fmoc chemical protocol. Comparison of resins for solid phase synthesis. *Int. J. Peptide Protein Res.* 1992; **39**: 87–92.
128. Atherton E, Logan CJ, Sheppard RC. Peptide synthesis. Part 2. Procedures for solid-phase synthesis using N$^{\alpha}$-fluorenylmethoxycarbonylamino-acids on polyamide supports. Synthesis of substance P and acyl carrier protein 65–74 decapeptide. *J. Chem. Soc. Perkin Trans. 1* 1981; 538–546.
129. Barany G, Kneib-Cordonier N, Mullen DG. Solid-phase peptide synthesis: a silver anniversary report. *Int. J. Peptide Protein Res.* 1987; **30**: 705–739.
130. Benz H. The role of solid-phase fragment condensation (SPFC) in peptide synthesis. *Synthesis* 1994; 337–358.
131. Fields GB, Noble RL. Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int. J. Peptide Protein Res.* 1990; **35**: 161–214.
132. Kent SBH, Mitchell AR, Engelhard M, Merrifield RB. Mechanisms and prevention of trifluoroacetylation in solid-phase peptide synthesis. *Proc. Natl. Acad. Sci. USA* 1979; **76**: 2180–2184.
133. Merrifield RB. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* 1963; **85**: 2145–2154.
134. Giralt E, Eritja R, Pedroso E. Diketopiperazine formation in acetamido- and nitrobenzamido-bridged polymeric supports. *Tetrahedron Lett.* 1981; **22**: 3779–3782.
135. Floersheimer A, Riniker B. Solid-phase synthesis of peptides with the highly acid-sensitive HMPB linker. *Peptides 1990*. ESCOM: Leiden, 1991; 131–133.
136. Mergler M, Tanner R, Gosteli J, Grogg P. Peptide synthesis by a combination of solid-phase and solution methods. I. A new very acid-labile anchor for the solid phase synthesis of fully protected fragments. *Tetrahedron Lett.* 1988; **29**: 4005–4008.

137. Sheppard RC, Williams BJ. Acid-labile resin linkage agents for use in solid phase peptide synthesis. *Int. J. Peptide Protein Res.* 1982; **20**: 451–454.
138. Wang S-S. p-Alkoxybenzyl alcohol resin and p-alkoxybenzyloxycarbonylhydrazide resin for solid phase synthesis of protected peptide fragments. *J. Am. Chem. Soc.* 1973; **95**: 1328–1333.
139. Akaji K, Kiso Y, Carpino LA. Fmoc-based solid-phase peptide synthesis using a new *t*-alcohol type 4-(1′,1′-dimethyl-1′-hydroxypropyl)phenoxyacetyl handle (DHPP)-resin. *J. Chem. Soc. Chem. Commun.* 1990; 584–586.
140. Dhaon MK, Olsen RK, Ramasamy K. Esterification of *N*-protected α-amino acids with alcohol/carbodiimide/4-(dimethylamino)-pyridine. Racemization of aspartic and glutamic acid derivatives. *J. Org. Chem.* 1982; **47**: 1962–1965.
141. Carpino LA, Beyermann M, Wenschuh H, Bienert M. Peptide synthesis via amino acid halides. *Acc. Chem. Res.* 1996; **29**: 268–274.
142. Mutter M, Bayer E. The liquid phase method for peptide synthesis. In *The Peptides*. Academic Press: New York, 1979; 285–332.
143. Lloyd-Williams P, Albericio F, Giralt E. Convergent solid-phase peptide synthesis. *Tetrahedron* 1993; **49**: 11 065–11 133.
144. Frechet JMJ, Nuyens LJ. Use of polymers as protecting groups in organic synthesis. III. Selective functionalization of polyhydroxy alcohols. *Can. J. Chem.* 1976; **54**: 926–934.
145. Barlos K, Chatzi O, Gatos D, Stavropoulos G. 2-Chlorotrityl chloride resin. Studies on anchoring of Fmoc-amino acids and peptide cleavage. *Int. J. Peptide Protein Res.* 1991; **37**: 513–520.
146. Bayer E, Clausen N, Goldammer C, Henkel B, Rapp W, Zhang L. New polymer and strategy for the solid-phase synthesis of protected peptide fragments. In *Peptides: Chemistry, Structure and Biology*. ESCOM: Leiden, 1994; 156–158.
147. Rapp WE, Bayer E. Prediction and prevention of peptide conformations during synthesis. In *Peptides: Chemistry, Structure and Biology*. ESCOM: Leiden, 1994; 40–43.
148. Bollhagen R, Schmiedberger M, Barlos K, Grell E. A new reagent for the cleavage of fully protected peptides synthesised on 2-chlorotrityl chloride resin. *J. Chem. Soc. Chem. Commun.* 1994; 2559–2560.
149. Gruebler G, Zimmermann H, Echner H, Stoeva S, Bernardi E, Pourrias B, Voelter W. Solid phase syntheses (SPPS) of *C*-terminal proline peptides using an anchor system suppressing diketopiperazine (DKP) formation. In *Innovation and Perspective in Solid Phase Synthesis. Peptides, Proteins and Nucleic Acids. Biological and Biomedical Applications*. Mayflower Worldwide: Birmingham, 1994; 517–520.
150. Mullen DG, Barany G. A new fluoridolysable anchoring linkage for orthogonal solid-phase peptide synthesis: preparation and properties of the N-(3 or 4)-[[[(4-hydroxymethyl)-phenoxy-*t*-butylphenyl]silyl]phenyl]-pentanedioic acid, monoamide (PBS) handle. *Tetrahedron Lett.* 1987; **28**: 491–494.
151. Ramage R, Barron CA, Bielecki S, Thomas DW. Solid phase peptide synthesis: fluoride ion release of peptide from the resin. *Tetrahedron Lett.* 1987; **28**: 4105–4108.
152. Sieber P, Eisler K, Kamber B, Riniker B, Rittel W, Maerki F, de Gasparo M. Synthesis and biological activity of two disulphide bond isomers of human insulin: [A7-A11,A6-B7-cystine]- and [A6-A7,A11-B7- cystine]insulin (human). *Hoppe-Seyler's Z. Physiol. Chem.* 1978; **359**: 113–123.
153. Chao H-G, Bernatowicz MS, Reiss PD, Klimas CE, Matsueda GR. A novel and versatile silicon-derived linkage agent, 4-[1-hydroxy-2-(trimethylsilyl)ethyl]-benzoic acid, compatible with the Fmoc/*t*-Bu strategy for solid-phase synthesis of C-terminal peptide acids. *J. Am. Chem. Soc.* 1994; **116**: 1746–1752.
154. Wagner M, Kunz H. The (2-phenyl-2-trimethylsilyl)-ethyl(PTMSEL) linker — a novel linker for the solid-phase synthesis of protected peptides and glycopeptides cleavable with fluoride. *Angew. Chem. Int. Ed.* 2002; **41**: 317–321.
155. Giralt E, Albericio F, Pedroso E, Granier C, van Rietschoten J. Convergent solid phase peptide synthesis. II. Synthesis of the 1–6 apamin protected segment on a NBB-resin. Synthesis of apamin. *Tetrahedron* 1982; **38**: 1193–1201.
156. Lloyd-Williams P, Gairi M, Albericio F, Giralt E. Convergent solid-phase peptide synthesis. X. Synthesis and purification of protected peptide fragments using the photolabile Nbb-resin. *Tetrahedron* 1991; **47**: 9867–9880.
157. Calas B, Mery J, Parello J, Cave A. Solid-phase synthesis using a new polyacrylic resin. Synthesis of the fragment 14–21 of the amino acid sequence of histone H4. *Tetrahedron* 1985; **41**: 5331–5339.
158. David M-L, Sola R, Pascal R. Towards anchoring groups allowing the use of aqueous media in solid phase peptide synthesis. In *Peptides 1992*. Escom Science Publishers B.V.: Leiden, 1993; 271–272.
159. Kaiser ET, Mihara H, Laforet GA, Kelly JW, Walters L, Findeis MA, Sasaki T. Peptide and protein synthesis by segment synthesis-condensation. *Science* 1989; **243**: 187–192.
160. Oesapay G, Profit A, Taylor JW. Synthesis of tryocidine A: use of oxime resin for peptide chain assembly and cyclization. *Tetrahedron Lett.* 1990; **31**: 6121–6124.
161. Sasaki T, Findeis MA, Kaiser ET. Evaluation of the oxime resin based segment synthesis-condensation approach using RNase T1 as a model synthetic target. *J. Org. Chem.* 1991; **56**: 3159–3168.

162. Mihara H, Chmielewski JA, Kaiser ET. Synthesis of the 60 amino acid homeo domain and smaller fragments of the Drosophila gene regulatory protein Antennapedia by a segment synthesis-condensation approach. *J. Org. Chem.* 1993; **58**: 2209–2215.
163. Tam JP, Cunningham-Rundles WF, Erickson BW, Merrifield RB. Selective cleavage of protected amino acids and peptides from oxacyl resins by an 18-Crown-6 complex of potassium cyanide. *Tetrahedron Lett.* 1977; 4001–4004.
164. Krchnak V, Vagner J, Lebl M. Noninvasive continuous monitoring of solid-phase peptide synthesis by acid-base indicator. *Int. J. Peptide Protein Res.* 1988; **32**: 415–416.
165. McFerran NV, Walker B, McGurk CD, Scott FC. Conductance measurements in solid phase peptide synthesis. I. Monitoring coupling and deprotection in Fmoc chemistry. *Int. J. Peptide Protein Res.* 1991; **37**: 382–387.
166. Fox JE. Multiple peptide synthesis. *Mol. Biotechnol.* 1995; **3**: 249–258.
167. Szirtes T, Kisfaludy L, Palosi E, Szporny L. Synthesis of thyrotropin-releasing hormone analogues. 1. Complete dissociation of central nervous system effects from thyrotropin-releasing activity. *J. Med. Chem.* 1984; **27**: 741–745.
168. Cammish LE. Reduction of diketopiperazine formation in Fmoc-peptide synthesis. In *Polypept. Protein Drugs*. Horwood: London, 1991; 265–269.
169. Pedroso E, Grandas A, de las Heras X, Eritja R, Giralt E. Diketopiperazine formation in solid phase peptide synthesis using p-alkoxybenzyl ester resins and Fmoc-amino acids. *Tetrahedron Lett.* 1986; **27**: 743–746.
170. Sieber P, Iselin BM. Selective acidolytic cleavage of aralkyloxycarbonyl amino-protecting groups. *Helv. Chim. Acta* 1968; **51**: 614–622.
171. Ueki M, Amemiya M. Removal of 9-fluorenylmethyloxycarbonyl (Fmoc) group with tetrabutylammonium fluoride. *Tetrahedron Lett.* 1987; **28**: 6617–6620.
172. Freund J, Kapurniotu A, Holak TA, Lenfant M, Voelter W. Synthesis and two-dimensional ^{1}H and ^{13}C NMR investigations of an inhibitor of hematopoietic stem cell proliferation Ac-Ser-Asp- Lys-Pro-OH. *Z. Naturforsch.* 1992; **47b**: 1324–1332.
173. Kapurniotu A, Ungermann C, Voelter W. Optimized SPPS of the new stem cell proliferation inhibiting factor Ac-SDKP and derivatives. In *Innovation Perspect. Solid Phase Synthesis*. Intercept: Andover, UK, 1992; 319–329.
174. Wade JD, Bedford J, Sheppard RC, Tregear GW. DBU as an N^{α}-deprotecting reagent for the fluorenylmethoxycarbonyl group in continuous flow solid-phase peptide synthesis. *Peptide Res.* 1991; **4**: 194–199.
175. Castro B, Dormoy JR, Evin G, Selve C. Reactifs de couplage peptidique IV (1) - L'hexafluorophosphate de benzotriazole N-oxytrisdimethylamino phosphonium (B.O.P.). *Tetrahedron Lett.* 1975; 1219–1222.
176. Gairi M, Lloyd-Williams P, Albericio F, Giralt E. Use of BOP reagent for the suppression of diketopiperazine formation in Boc/Bzl solid-phase peptide synthesis. *Tetrahedron Lett.* 1990; **31**: 7363–7366.
177. Lloyd-Williams P, Gairi M, Cardenas F, Albericio F, Giralt E. Recent results in convergent solid-phase peptide synthesis. In *Peptides 1990*. ESCOM Science Publishers, B. V.: Leiden, 1991; 146–147.
178. Suzuki K, Nitta K, Endo N. Suppression of diketopiperazine formation in solid phase peptide synthesis. *Chem. Pharm. Bull.* 1975; **23**: 222–224.
179. Alsina J, Giralt E, Albericio F. Use of *N*-tritylamino acids and PyAOP for the suppression of diketopiperazine formation in Fmoc/^{t}Bu solid-phase peptide synthesis using alkoxybenzyl ester anchoring linkages. *Tetrahedron Lett.* 1996; **37**: 4195–4198.
180. Carpino LA, El-Faham A, Minor CA, Albericio F. Advantageous applications of azabenzotrazole (triazolopyridine)-based coupling reagents to solid-phase peptide synthesis. *J. Chem. Soc. Chem. Commun.* 1994; 201–203.
181. Albericio F, Barany G. Improved approach for anchoring N^{α}-9-fluorenylmethyloxycarbonylamino acids as p-alkoxybenzyl esters and solid-phase peptide synthesis. *Int. J. Peptide Protein Res.* 1985; **26**: 92–97.
182. Perlow DS, Erb JM, Gould NP, Tung RD, Freidinger RM, Williams PD, Veber DF. Use of N-Fmoc amino acid chlorides and activated 2-(fluorenylmethoxy)-5(4H)-oxazolones in solid-phase peptide synthesis. Efficient synthesis of highly N-alkylated cyclic hexapeptide oxytocin antagonists related to L-365,209. *J. Org. Chem.* 1992; **57**: 4394–4400.
183. Shute RE, Rich DH. Prevention of diketopiperazine formation in peptide synthesis by simultaneous deprotection-coupling procedure: entrapment of reactive nucleophilic species by *in situ* acylation. *J. Chem. Soc. Chem. Commun.* 1987; 1155–1156.
184. Wuensch E, Moroder L, Keller O. Synthesis of 2-(trimethylsilyl)ethyloxy-carbonylamino acids and their use in peptide chemistry. *Hoppe-Seyler's Z. Physiol. Chem.* 1981; **362**: 1289–1292.
185. Gordon DW, Steele J. Reductive alkylation on a solid phase: synthesis of a piperazinedione combinatorial library. *Bioorg. Med. Chem. Lett.* 1995; **5**: 47–50.
186. Goodfellow VS, Laudeman CP, Gerrity JI, Burkard M, Strobel E, Zuzack JS, McLeod DA. Rationally designed non-peptides: variously substituted piperazine libraries for the discovery of bradykinin antagonists and other G-protein-coupled receptor ligands. *Mol. Div.* 1996; **2**: 97–102.
187. Scott BO, Siegmund AC, Marlowe CK, Pei Y, Spear KL. Solid phase organic synthesis (SPOS): a novel route to diketopiperazines and diketomorpholines. *Mol. Div.* 1996; **1**: 125–134.
188. Falorni M, Giacomelli G, Porcheddu A, Taddei M. Solution-phase synthesis of mixed amide libraries by

simultaneous addition of functionalities (SPSAF) to a diketopiperazinetetracarboxylic acid scaffold monitored by GC analysis of isobutyl alcohol. *Eur. J. Org. Chem.* 2000; 1669–1675.
189. Szardenings AK, Burkoth TS, Lu HH, Tien DW, Campbell DA. A simple procedure for the solid phase synthesis of diketopiperazine and diketomorpholine derivatives. *Tetrahedron* 1997; **53**: 6573–6593.
190. Hobbs DeWitt S, Kiely JS, Stankovic CJ, Schroeder MC, Reynolds Cody DM, Pavia MR. 'Diversomers': an approach to nonpeptide, nonoligomeric chemical diversity. *Proc. Natl. Acad. Sci. USA* 1993; **90**: 6909–6913.
191. Furka A, Sebestyen F, Asgedom M, Dibo G. General method for rapid synthesis of multicomponent peptide mixtures. *Int. J. Peptide Protein Res.* 1991; **37**: 487–493.
192. Groth T, Meldal M. *N*-Terminal peptide aldehydes as electrophiles in combinatorial solid phase synthesis of novel peptide isosteres. *J. Comb. Chem.* 2001; **3**: 45–63.
193. Falb E, Yechezkel T, Salitra Y, Gilon C. *In situ* generation of Fmoc-amino acid chlorides using bis-(trichloromethyl)carbonate and its utilization for difficult couplings in solid-phase peptide synthesis. *J. Peptide Res.* 1999; **53**: 507–517.
194. Jensen KJ, Alsina J, Songster MF, Vagner J, Albericio F, Barany G. Backbone amide linker strategy for solid-phase synthesis of *C*-terminal-modified and cyclic peptides. *J. Am. Chem. Soc.* 1998; **120**: 5441–5452.
195. Sabatino G, Chelli M, Mazzucco S, Ginanneschi M, Papini AM. Cyclization of histidine containing peptides in the solid-phase by anchoring the imidazole ring to trityl resins. *Tetrahedron Lett.* 1999; **40**: 809–812.
196. del Fresno M, Alsina J, Royo M, Barany G, Albericio F. Solid-phase synthesis of diketopiperazines, useful scaffolds for combinatorial chemistry. *Tetrahedron Lett.* 1998; **39**: 2639–2642.
197. Royo M, Del Fresno M, Frieden A, Van Den Nest W, Sanseverino M, Alsina J, Kates SA, Barany G, Albericio F. Useful scaffolds and handles for creating diversity in the preparation of chemical libraries. *React. Funct. Polymers* 1999; **41**: 103–110.
198. Smith RA, Bobko MA, Lee W. Solid-phase synthesis of a library of piperazinediones and diazepinediones via Kaiser oxime resin. *Bioorg. Med. Chem. Lett.* 1998; **8**: 2369–2374.
199. Wang H, Ganesan A. The N-acyliminium Pictet-Spengler condensation as a multicomponent combinatorial reaction on solid phase and its application to the synthesis of demethoxyfumitremorgin C analogues. *Org. Lett.* 1999; **1**: 1647–1649.
200. Bianco A, Sonksen CP, Roepstorff P, Briand J-P. Solid-phase synthesis and structural characterization of highly substituted hydroxyproline-based 2,5-diketopiperazines. *J. Org. Chem.* 2000; **65**: 2179–2187.
201. Thompson LA, Ellman JA. Straightforward and general method for coupling alcohols to solid supports. *Tetrahedron Lett.* 1994; **35**: 9333–9336.
202. Bianco A, Furrer J, Limal D, Guichard G, Elbayed K, Raya J, Piotto M, Briand J-P. Multistep synthesis of 2,5-diketopiperazines on different solid supports monitored by high resolution magic angle spinning NMR spectroscopy. *J. Comb. Chem.* 2000; **2**: 681–690.
203. Renil M, Meldal M. POEPOP and POEPS: inert polyethylene glycol crosslinked polymeric supports for solid synthesis. *Tetrahedron Lett.* 1996; **37**: 6185–6188.
204. Hulme C, Morrissette MM, Volz FA, Burns CJ. The solution phase synthesis of diketopiperazine libraries via the Ugi reaction: novel application of Armstrong's convertible isonitrile. *Tetrahedron Lett.* 1998; **39**: 1113–1116.
205. Ugi I, Demharter A, Hoerl W, Schmid T. Ugi reactions with trifunctional α-amino acids, aldehydes, isocyanides and alcohols. *Tetrahedron* 1996; **52**: 11 657–11 664.
206. Armstrong RW, Combs AP, Tempest PA, Brown SD, Keating TA. Multiple-component condensation strategies for combinatorial library synthesis. *Acc. Chem. Res.* 1996; **29**: 123–131.
207. Hulme C, Cherrier MP. Novel applications of ethyl glyoxalate with the Ugi MCR. *Tetrahedron Lett.* 1999; **40**: 5295–5299.
208. Chucholowski A, Masquelin T, Obrecht D, Stadlwieser J, Villalgordo JM. Novel solution- and solid-phase strategies for the parallel and combinatorial synthesis of low-molecular-weight compound libraries. *Chimia* 1996; **50**: 525–530.
209. Moroder L, Lutz J, Grams F, Rudolph-Boehner S, Oesapay G, Goodman M, Kolbeck W. A new efficient method for the synthesis of 1,4-benzodiazepine-2,5-dione diversomers. *Biopolymers* 1996; **38**: 295–300.
210. Szardenings AK, Antonenko V, Campbell DA, DeFrancisco N, Ida S, Shi L, Sharkov N, Tien D, Wang Y, Navre M. Identification of highly selective inhibitors of collagenase-1 from combinatorial libraries of diketopiperazines. *J. Med. Chem.* 1999; **42**: 1348–1357.
211. Kennedy AL, Fryer AM, Josey JA. A new resin-bound universal isonitrile for the Ugi 4CC reaction: preparation and applications to the synthesis of 2,5-diketopiperazines and 1,4-benzodiazepine-2,5-diones. *Org. Lett.* 2002; **4**: 1167–1170.
212. Petasis NA, Zavialov IA. New reactions of alkenylboronic acids. *Adv. Boron Chem.* 1997; **201**: 179–182.
213. Golebiowski A, Klopfenstein SR, Chen JJ, Shao X. Solid supported high-throughput organic synthesis

of peptide β-turn mimetics via tandem Petasis reaction/diketopiperazine formation. *Tetrahedron Lett.* 2000; **41**: 4841–4844.
214. Ball JB, Hughes RA, Alewood PF, Andrews PR. β-Turn topography. *Tetrahedron* 1993; **49**: 3467–3478.
215. Liebscher J, Jin S. 3-Ylidenepiperazine-2,5-diones as versatile organic substrates. *Chem. Soc. Rev.* 1999; **28**: 251–259.
216. Loughlin WA, Marshall RL, Carreiro A, Elson KE. Solution-phase combinatorial synthesis and evaluation of piperazine-2,5-dione derivatives. *Bioorg. Med. Chem. Lett.* 2000; **10**: 91–94.
217. Li W-R, Yang JH. Solid-phase synthesis of unsaturated 3-substituted piperazine-2,5-diones. *J. Comb. Chem.* 2002; **4**: 106–108.