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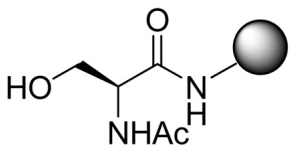

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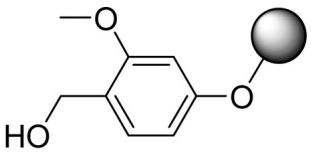

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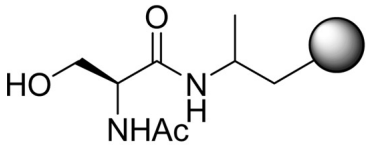

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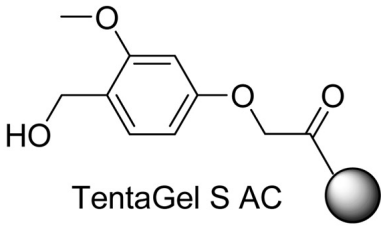
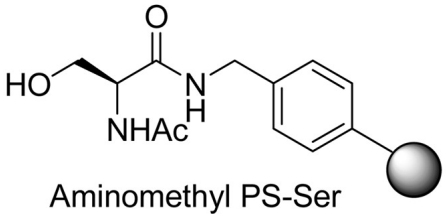


TentaGel S AC



Aminomethyl PS-Ser

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Microwave-Assisted Solid-Phase Synthesis of 2,5-Diketopiperazines: Solvent and Resin Dependence

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Solid-phase synthesis of diketopiperazines (DKPs) was preformed using various combinations of resins (polystyrene, TentaGel, ArgoGel, and PEGA) and solvents (toluene, *tert*-butyl alcohol, water, and toluene/2-butanol (1:4, v/v). The DKPs were synthesized from solid-phase bound dipeptides via intramolecular aminolysis. Both thermal and microwave-assisted solid-phase synthesis of DKPs gave high yields of products independently of resin and organic solvent used; however, only the PEGA resin resulted in high yields of DKPs in water independent of heating method. The short reaction times, high yields, and the possibility to run reactions in water when an appropriate resin is used makes the microwave-assisted solid-phase synthesis the method of choice. The method should be suitable for solid-phase synthesis of diketopiperazine-based libraries.

Introduction

Diketopiperazines (DKPs) are the smallest cyclic peptides, derived from the folding head-to-tail of linear dipeptides.[1] The DKPs are common naturally occurring structures[2] and have received continuous interest because of their broad spectrum of pharmacological activities. Such compounds have been shown to inhibit several enzymes as well as to recognize, modulate, and control the activity of many receptors.[3]

Several synthetic procedures of 2,5-diketopiperazines have been reported, both solution- and solid-phase reactions.[4] The most common strategies for the solid-phase synthesis of DKPs are based on cleavage-induced cyclization of linear dipeptides[5] or on-bead cyclization before final release from the resin.[6] Other methods have also been reported,[4] including the use of the Ugi reaction.[5a,5e,7] Recently, microwave-assisted, solvent-free synthesis of DKPs has been reported.[8]

DKP formation is strongly dependent on the nature and the sequence of the amino acids.[9] The cyclization reaction is favored in dipeptides containing at least one amino acid, which can easily adopt a *cis*-amide conformation (for example, Gly or Pro). In addition, combinations of one L- and one D-amino acid in the dipeptide also favor the formation of DKPs, due to the minimal steric interference between the two side chains during cyclization. Furthermore, the nature of the peptide−resin linkage also influences the rate of DKP formation in the solid-phase mode.[9]

DKP formation via solid-phase intramolecular cyclization is well-represented in the literature. The majority of these reports describe the synthesis of specific DKPs, such as hydroxyproline-containing derivatives.[6a] Various reaction conditions have been used, and the yields have varied from excellent to poor. So far, however, there have been no reports describing microwave-assisted, solid-phase synthesis of DKPs.

In this paper, we present a study of classic thermal and microwave-assisted, solid-phase synthesis of different DKPs using various combinations of resins (polystyrene, TentaGel, ArgoGel, and PEGA) and solvents (toluene, *tert*-butyl alcohol, water, and toluene/2-butanol (1:4)). In particular, we compare the results with our previous solution-phase study[10] and demonstrate the differences between solution- and solid-phase reactions regarding solvent dependence of DKP formation. In that study, we found that microwave-assisted heating for 10 min using water as solvent proved, by far, to be the most efficient method of cyclization, giving moderate to excellent yields (63−97%) of DKPs independent of the amino acid sequence (yields determined by HPLC using an internal standard). In the present method, the PEGA-Ser resin gave high yields of 2,5-diketopiperazines in water with microwave-assisted heating for 30 min. The method should, therefore, be useful for synthesis of diketopiperazine libraries.

Results and Discussion

Resins and Linkers. The success of solid-phase chemistry is critically dependent on the chemical composition and physical properties of the polymer matrix.[11] Although the development of a universal support that possesses features ideally suited for all applications is unlikely, many polymers have proven to be effective for specific uses.[12] Classically, polystyrene (PS)-based solid supports have been the most widely used.[13] However, the hydrophobicity of PS limits its use for some applications, thus prompting the evaluation of more hydrophilic supports. Several polyethylene glycol (PEG)−PS resins have been developed[14−20] that provide a combination of a hydrophobic PS core with hydrophilic PEG chains in the same support. Due to the unique conformational

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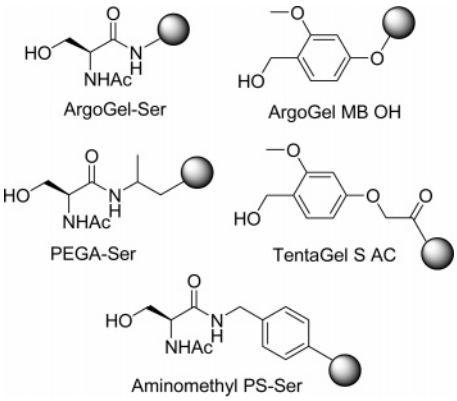


Figure 1. The different resins and linkers used in the synthesis of DKPs.

flexibility of PEG chains, PEG−PS resins are compatible with both polar and nonpolar solvents.[21] More hydrophilic PEG-based resins have also been developed, including the polyamine-containing PEGA resin[22,23] and the totally PEG-based resins, such as POEPOP,[24] SPOCC,[25] and ChemMatrix.[26] The amphiphilic nature of PEG ensures that the resin is well-solvated in both polar and nonpolar solvents.[21] The objective of this investigation was to compare the influence of the polymer matrix on DKP formation using four different commercially available resins (PEGA, TentaGel S AC, ArgoGel MB OH, and PS).

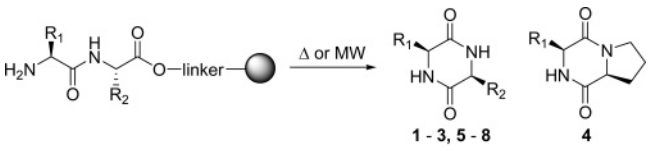
We reasoned that an ester linkage between the peptide and the resin would be suitable for cyclizations via intramolecular aminolysis. Two different types of linkers were selected: the 4-(4-hydroxymethyl-3-methoxyphenoxy)-linker and the N-acetylated serine linker (Figure 1). Acylation of ArgoGel-NH_2, PEGA, and PS with Fmoc-protected Ser(OtBu) was accomplished using TBTU and DIPEA in DMF. Fmoc-deprotection (20% piperidine) followed by N-acetylation with acetic anhydride and removal of the *tert*-butyl protection group with TFA/CH_2Cl_2-furnished ArgoGel-Ser, PEGA-Ser, and aminomethyl PS-Ser (Figure 1).

After attachment of the Fmoc-protected serine on PEGA, ArgoGel, and aminomethyl PS, the loading grade was calculated by measuring the UV absorbance of the adduct of dibenzofulvene and piperidine formed upon treatment of a weighed polymer sample with 20% piperidine in DMF.

To compare the performance of the two linkers during synthesis of DKPs, cyclization of Phe−Trp on ArgoGel-Ser and ArgoGel-MB OH (see Choice of Amino Acids) was attempted. Head-to-tail cyclization was carried out in toluene/2-butanol (1:4 v/v) at 80 °C for 35 h using thermal heating. Compound **1** (c(PheTrp)) was obtained in comparable yields (84 and 83%, respectively) on the two resins (the yields were determined by HPLC analyses; see Experimental Section). Therefore, the linkers were considered to be equally suitable for the solid-phase synthesis of DKPs.

Choice of Amino Acids and Synthesis of Dipeptides. Eight different DKPs were synthesized using the solid-phase method: c(PheTrp) (**1**), c(PheNle) (**2**), c(PheLeu) (**3**) c(PhePro) (**4**), c(LeuTyr) (**5**), c(TrpSer) (**6**), c(TrpAsn) (**7**), and c(ValPhe) (**8**). DKPs **1**−**7** were used in our previous solution-phase study, thus allowing a direct comparison of yields.[10] Phenylalanine or tryptophane was incorporated into all DKPs to facilitate UV detection in the HPLC analyses. Compounds **1**−**3** and **5**−**7** were chosen because they have previously proved difficult to synthesize in solution using thermal heating.[10] Compound **4** was synthesized as a reference compound because it has been shown that proline facilitates the formation of DKPs.[9] Compound **8** was chosen to investigate if the position (N- or C-terminal) of phenylalanine in the dipeptide affects the cyclization reaction.

Table 1. The Head-to-Tail Cyclization Resulting in 2,5-Diketopiperazine Formation



compd	R_1	R_2
1	benzyl	(3-indolyl)-CH_2
2	benzyl	butyl
3	benzyl	isobutyl
4	benzyl	
5	isobutyl	4-OH-benzyl
6	(3-indolyl)-CH_2	CH_2OH
7	(3-indolyl)-CH_2	CH_2CONH_2
8	isopropyl	benzyl

The solid-phase synthesis of dipeptides was performed using standard Fmoc chemistry (see Tables 3 and 4 in the Experimental Section). To ensure efficient coupling, each reaction was repeated twice. The hydroxyl group of the linker was derivatized by esterification with an Fmoc-protected amino acid activated by MSNT[27] (4 equiv) in the presence of NMI (3.9 equiv) in DMF for 2 h. After Fmoc-deprotection, one cycle of solid-phase peptide synthesis using TBTU[27] and DIPEA furnished the solid-phase bound dipeptide.

Solid-Phase Synthesis of DKPs. The general procedure for the solid-phase synthesis of DKPs is shown in Table 1. The reaction occurs via a head-to-tail cyclization with the release of the corresponding DKP from the resin. This procedure facilitates the purification considerably because only the desired product is present in the solution. No traces of the linear dipeptide could be detected in the reaction medium. The limited swelling of the resins, especially in water, could trap the DKPs within the resin and result in a low recovery. To minimize this problem, the resins were washed sequentially with the reaction solvent and *tert*-butyl alcohol after the cyclization reaction.

Choice of Solvents and Temperature for the Cyclization Reaction. Four solvents were chosen for the cyclization reactions: water, an excellent solvent for solution-phase microwave-assisted synthesis of DKPs;[10] *tert*-butyl alcohol and toluene because they gave high yields in the thermally heated solution-phase reactions; and the toluene/2-butanol (1:4 v/v) mixture because it has previously proved to be suitable for the synthesis of DKPs.[10,29] The overall yields of DKPs were determined by HPLC using tryptophan (6.5 mM) as the internal standard.

To find a suitable temperature for the cyclization reaction, the synthesis of **1** on ArgoGel-MB OH resin in toluene/2-butanol (1:4 v/v) was carried out at five different tempera-

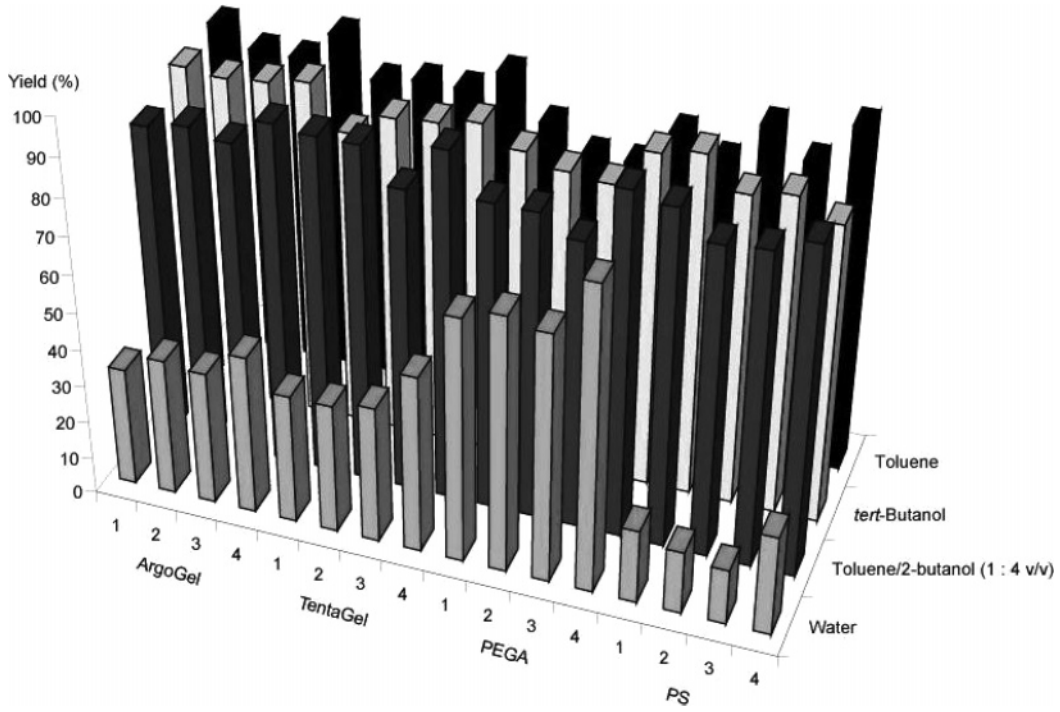


Figure 2. Yields of DKPs **1**−**4** obtained in solid-phase reactions using classical thermal heating at 80 °C for 35 h (HPLC analysis). In each reaction, 50 mg of the resin (7−22 μmol) was used. Specific yields are reported in Table S1 in the Supporting Information.

tures (20, 50, 80, 100, and 120 °C). The reaction at room temperature required more than a week before the reaction was completed. At 50 °C, the reaction was finished after 60 h, and at 80 °C, the reaction time was reduced to 35 h. At 100 °C, the reaction was completed after 8 h, but it resulted in side-products. An increase in temperature to 120 °C gave only a 15% yield of **1** together with significant amounts of side-products. As a consequence, the thermally heated solid-phase syntheses of **1**−**4** described below were carried out at both 50 °C (60 h) and 80 °C (35 h) to see if any differences in yields could be detected; however, only small changes in yields were observed (see Table S1 in the Supporting Information). The syntheses of compounds **5**−**8** by thermal heating were therefore studied only at 80 °C.

Synthesis of DKPs 1−8 Using Thermal Heating. DKPs **1**−**8** were synthesized using ArgoGel MB OH, TentaGel S AC, PEGA-Ser, and aminomethyl PS-Ser in the four different solvents (Figures 2 and 3). The proline-containing DKP (**4**) was obtained in slightly higher yields, as compared to **1**−**3** and **5**−**8**, in all of the reactions. The HPLC analysis of the reaction mixtures showed no traces of linear dipeptide.

The yields in the water-based reactions were dependent on the washing procedure used, which facilitated the release of the product from the resin. The yields without an additional washing step (*tert*-butyl alcohol (3 × 5 min)) were 40−50% lower, depending on the resin used in the reaction. Specific yields are found in Table S1 and S2 in the Supporting Information.

Reactions on the ArgoGel MB OH resin in toluene/2-butanol (1:4 v/v), *tert*-butyl alcohol and toluene gave high yields of all eight DKPs (80−92%). However, the cyclization reaction in water provided **4** in only 41−43% yields and **1**−**3** and **5**−**8** in 29−37% yields. Thus, although ArgoGel MB OH swells in polar solvents,[20] the yields were surprisingly low. TentaGel S AC gave a comparable result with ArgoGel MB OH, which could be expected since both resins are PEG-grafted polystyrene supports.

Interestingly, the high-PEG-containing PEGA-Ser support in combination with water as solvent gave considerably higher yields of all eight DKPs (65−82%). This resin was also highly compatible with the organic solvents because toluene/2-butanol (1:4 v/v), *tert*-butyl alcohol, and toluene gave high yields of all DKPs (71−92%).

As expected, the combination of aminomethyl PS-Ser and water gave low yields of all DKPs (15−27%). Due to the hydrophobic nature of this polymer, polar solvents may fail to solvate the matrix.[30] The other three solvents used gave DKPs in high yields (76−94%).

The solid-phase synthesis of DKPs **1**−**3** and **5**−**8** in toluene/2-butanol (1:4 v/v), *tert*-butyl alcohol, and toluene gave considerably higher yields as compared to the solution-phase synthesis of the same DKPs (72−94% compared to 5−62%).[10] However, the solid-phase reaction times in, for example, *tert*-butyl alcohol (35 h at 80 °C) were considerably longer than those used in the solution-phase study (12 h at 80 °C).

Microwave-Assisted Synthesis of DKPs on Solid Phase. It has previously been shown that microwave-assisted, solution-phase synthesis of DKPs offers significant advantages over classic thermal heating.[10] To study if this is also true for solid-phase synthesis, DKPs **1**−**8** were synthesized in a microwave reactor using the same solvents as in the thermally heated reactions.

Optimization of Temperature and Reaction Time for the Cyclization Reactions Using Microwave-Assisted Heating. To find the optimal temperature and time for the microwave-assisted reactions, a series of test reactions were performed using the ArgoGel-MB OH resin for the synthesis

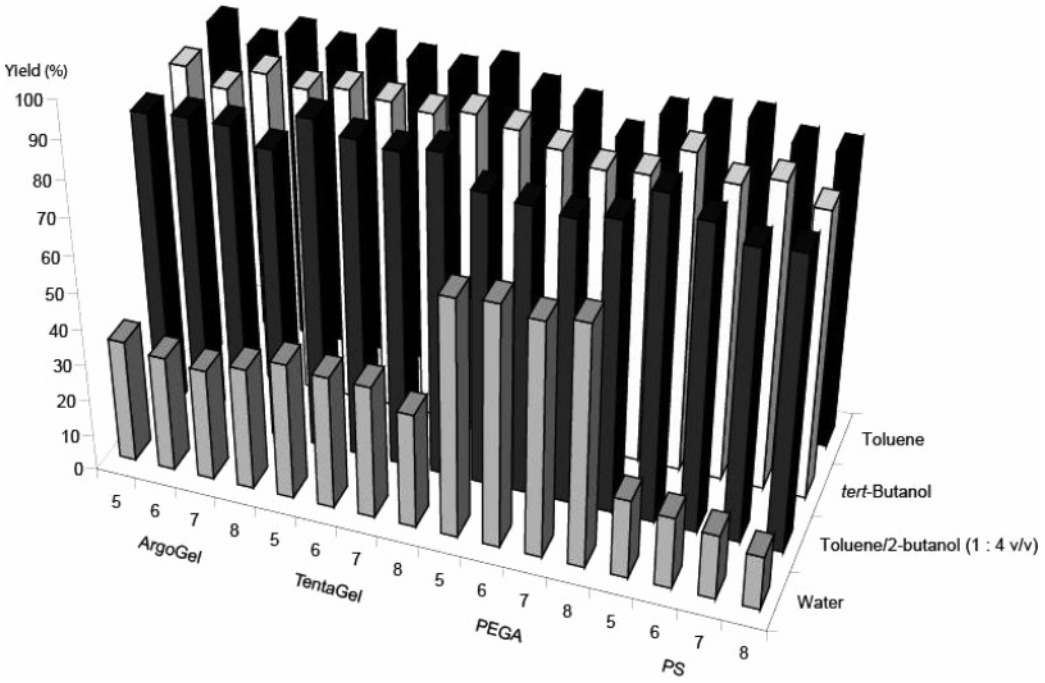


Figure 3. Yields of DKPs **5**−**8** obtained in solid-phase reactions using classical thermal heating at 80 °C for 35 h (HPLC analysis). In each reaction, 50 mg of the resin (7−22 μmol) was used. Specific yields are reported in Table S2 in the Supporting Information.

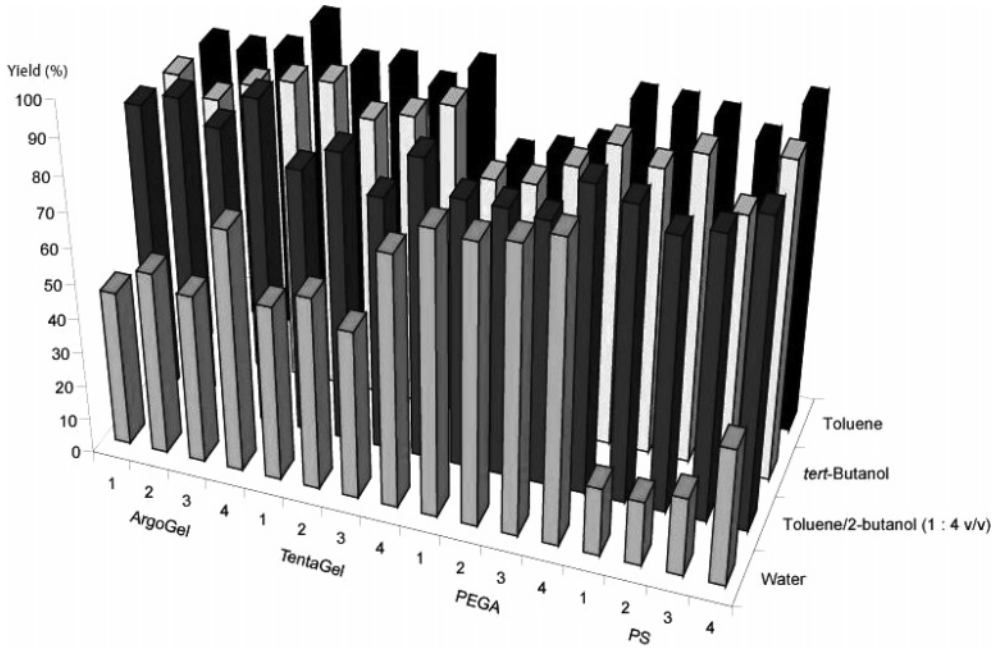


Figure 4. Yields of DKPs **1**−**4** obtained in solid-phase reactions using microwave-assisted heating (HPLC analysis). In each reaction, 50 mg of resin (7−22 μmol) was used. The reaction temperature was 120 °C, and the reaction time was 30 min. Specific yields are reported in Table S3 in the Supporting Information.

of **1**. Reactions in toluene/2-butanol (1:4 v/v) at 80 °C for 4 h gave **1** in 48% yield. Increasing the reaction time to 8 h resulted in 67% yield of **1**. When the temperature was set to 100 °C for 1 h, the yield improved to 77%, and a further increase in yield (85%) was obtained when the reaction was run for 30 min at 120 °C. Higher temperatures did not give any additional improvements. When using *tert*-butyl alcohol as solvent, the yield of **1** was 80% at 110 °C, 82% at 120 °C, and 77% at 130 °C. For both solvents, reaction times longer than 30 min did not improve the yield. As a consequence, the microwave-assisted, solid-phase syntheses of DKPs described below were carried out at 120 °C for 30 min.

Synthesis of DKPs 1−8 using Microwave-Assisted Heating. The results from the microwave-assisted, solid-phase synthesis of DKPs are presented in Figures 4 and 5. The DKPs were obtained in high yields (73−93%) from the PEG-grafted supports (ArgoGel MB OH and TentaGel S AC) in *tert*-butyl alcohol, toluene/2-butanol (1:4 v/v), or toluene. The yields were comparable to those obtained with classic

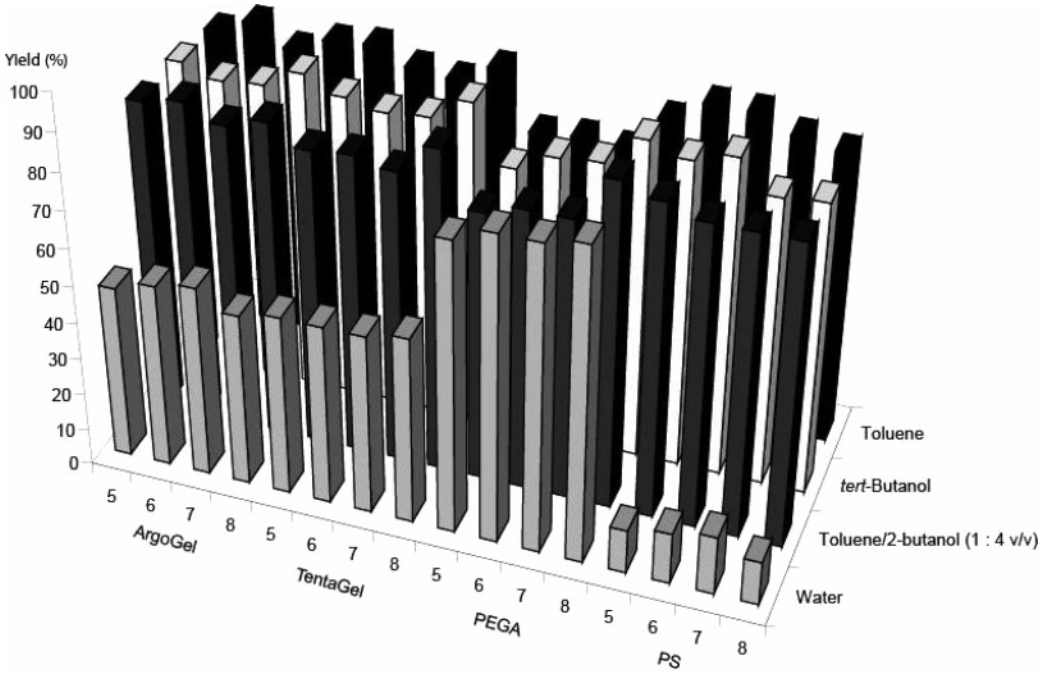


Figure 5. Yields of DKPs **5**−**8** obtained in solid-phase reactions using microwave-assisted heating (HPLC analysis). In each reaction, 50 mg of resin (7−22 μmol) was used. The reaction temperature was 120 °C, and the reaction time was 30 min. Specific yields are reported in Table S4 in the Supporting Information.

Table 2. Isolated Yields of **1**−**8** Obtained in the Cyclization Reactions[a]

		yield (%)	
DKP	resin	Δ	MW
1	ArgoGel-MB OH[b]	85	84
2	TentaGel S AC[c]	84	84
3	PEGA-Ser[d]	80	75
4	PS-Ser[e]	88	89
5	ArgoGel-MB OH[b]	81	86
6	TentaGel S AC[c]	91	80
7	PEGA-Ser[d]	79	74
8	PS-Ser[e]	82	80

[a] The classic thermally heated reactions were run for 35 h at 80 °C, and the microwave-assisted reactions were run for 30 min at 120 °C. PEGA Ser(OtBu) and TentaGel resins were used on a 0.5 g scale (70−100 μmol) in each reaction. PS and ArgoGel resins were used on a 0.2 g scale (80−88 μmol) in each reaction. [b] Loading grade (PEGA) 0.2 mmol/g. [c] Loading grade (PS) 0.85 mmol/g. [d] Loading grade (PEGA Ser(OtBu)) 0.14 mmol/g. [e] Loading grade (PS Ser(OtBu)) 0.4 mmol/g.

thermal heating. Performing the reaction in water resulted in moderate to good yields for both resins, typically in the range of 45−73%. Aminomethyl PS-Ser gave high yields of DKPs (74−93%) in all solvents except water. PEGA-Ser in toluene/2-butanol (1:4 v/v) or *tert*-butyl alcohol resulted in high yields of all eight products; however, in toluene, **1**−**3** and **5**−**8** were obtained in slightly lower yields (64−79%) as compared to the other resins (79−86%). A considerable increase in yields was observed for all DKPs when using PEGA-Ser in water, as compared to using other resins. As found for classic thermal heating, the proline-containing DKP (**4**) was formed in higher yields, as compared to **1**−**3** and **5**−**8**, independent of the resin used.

Compounds **1**−**8** were synthesized on a 70−88-μmol scale in toluene/2-butanol (1:4, v/v) as solvent when using both classic thermal and microwave-assisted heating to obtain isolated yields (Table 2). The compounds were dried under vacuum, and yields were calculated on the basis of the weight. NMR and elementary analysis confirmed the high purity of the obtained compounds (see Supporting Information). The isolated yields (74−91%) were comparable to those determined by HPLC analysis.

Conclusion

Using classic thermal heating for the solid-phase synthesis of DKPs gave high yields of products independently of resin and solvent, with the exception of water. However, the reaction times were rather long: 60 h at 50 °C and 35 h at 80 °C. The yields in water were considerably improved by using the more polar PEGA-Ser resin.

Table 3. Reagents and Equivalents Used in the Addition of Linkers to the PEGA and PS Resin

sequence	reagents
PEGA (0.20 g; 40 μmol)[a]	FmocSer(OtBu) (61 mg; 0.16 mmol)/TBTU (51 mg; 0.16 mmol), DIPEA (27 μL; 0.16 mmol)
PS (0.20 g; 0.17 mmol)[b]	FmocSer(OtBu) (0.26 g; 0.68 mmol)/TBTU (0.22 g; 0.68 mmol), DIPEA (0.12 mL; 0.66 mmol)
PEGA-Ser(OtBu) (0.20 g; 28 μmol)[c]	acetic anhydride (53 μL; 0.56 mmol)/pyridine (45 μL; 0.56 mmol)
PS-Ser(OtBu) (0.20 g; 80 μmol)[d]	acetic anhydride (53 μL; 0.56 mmol)/pyridine (45 μL; 0.56 mmol)
PEGA *N*-Ac-Ser (OtBu)	5% TFA in CH_2Cl_2 (1 mL)
PS *N*-Ac-Ser (OtBu)	5% TFA in CH_2Cl_2 (1 mL)

[a] Loading grade (PEGA) 0.2 mmol/g. [b] Loading grade (PS) 0.85 mmol/g. [c] Loading grade (PEGA-Ser(OtBu)) 0.14 mmol/g. [d] Loading grade (PS-Ser(OtBu)) 0.4 mmol/g.

Table 4. Reagents and Equivalents Used in the Synthesis of Polymer-Bound **1**−**8**

resin	first amino acid (4.0 equiv)	coupling reagent (4.0 equiv)	base (3.9 equiv)	second amino acid (4.0 equiv)	coupling reagent (4.0 equiv)	base (3.9 equiv)
ArgoGel MB (50 mg; 22 μmol)[a]	FmocTrp (38 mg; 88 μmol)	MSNT (26 mg; 88 μmol)	NMI (6.8 μL; 86 μmol)	FmocPhe (34 mg; 88 μmol)	TBTU (28 mg; 88 μmol)	DIPEA (15 μL; 86 μmol)
ArgoGel MB (50 mg; 22 μmol)[a]	FmocNle (31 mg; 88 μmol)	MSNT (26 mg; 88 μmol)	NMI (6.8 μL; 86 μmol)	FmocPhe (34 mg; 88 μmol)	TBTU (28 mg; 88 μmol)	DIPEA (15 μL; 86 μmol)
ArgoGel MB (50 mg; 22 μmol)[a]	FmocLeu (31 mg; 88 μmol)	MSNT (26 mg; 88 μmol)	NMI (6.8 μL; 86 μmol)	FmocPhe (34 mg; 88 μmol)	TBTU (28 mg; 88 μmol)	DIPEA (15 μL; 86 μmol)
ArgoGel MB (50 mg; 22 μmol)[a]	FmocPro (30 mg; 88 μmol)	MSNT (26 mg; 88 μmol)	NMI (6.8 μL; 86 μmol)	FmocPhe (34 mg; 88 μmol)	TBTU (28 mg; 88 μmol)	DIPEA (15 μL; 86 μmol)
TentaGel S AC (50 mg; 10 μmol)[b]	FmocTrp (17 mg; 40 μmol)	MSNT (12 mg; 40 μmol)	NMI (3.1 μL; 39 μmol)	FmocPhe (16 mg; 40 μmol)	TBTU (13 mg; 40 μmol)	DIPEA (6.8 μL; 39 μmol)
TentaGel S AC (50 mg; 10 μmol)[b]	FmocNle (14 mg; 40 μmol)	MSNT (12 mg; 40 μmol)	NMI (3.1 μL; 39 μmol)	FmocPhe (16 mg; 40 μmol)	TBTU (13 mg; 40 μmol)	DIPEA (6.8 μL; 39 μmol)
TentaGel S AC (50 mg; 10 μmol)[b]	FmocLeu (14 mg; 40 μmol)	MSNT (12 mg; 40 μmol)	NMI (3.1 μL; 39 μmol)	FmocPhe (16 mg; 40 μmol)	TBTU (13 mg; 40 μmol)	DIPEA (6.8 μL; 39 μmol)
TentaGel S AC (50 mg; 10 μmol)[b]	FmocPro (13 mg; 40 μmol)	MSNT (12 mg; 40 μmol)	NMI (3.1 μL; 39 μmol)	FmocPhe (16 mg; 40 μmol)	TBTU (13 mg; 40 μmol)	DIPEA (6.8 μL; 39 μmol)
PEGA-Ser (50 mg; 7.0 μmol)[c]	FmocTrp (12 mg; 28 μmol)	MSNT (8.3 mg; 28 μmol)	NMI (2.2 μL; 27 μmol)	FmocPhe (11 mg; 28 μmol)	TBTU (9.0 mg; 28 μmol)	DIPEA (4.8 μL; 27 μmol)
PEGA-Ser (50 mg; 7.0 μmol)[c]	FmocNle (10 mg; 28 μmol)	MSNT (8.3 mg; 28 μmol)	NMI (2.2 μL; 27 μmol)	FmocPhe (11 mg; 28 μmol)	TBTU (9.0 mg; 28 μmol)	DIPEA (4.8 μL; 27 μmol)
PEGA-Ser (50 mg; 7.0 μmol)[c]	FmocLeu (10 mg; 28 μmol)	MSNT (8.3 mg; 28 μmol)	NMI (2.2 μL; 27 μmol)	FmocPhe (11 mg; 28 μmol)	TBTU (9.0 mg; 28 μmol)	DIPEA (4.8 μL; 27 μmol)
PEGA-Ser (50 mg; 7.0 μmol)[c]	FmocPro (9.4 mg; 28 μmol)	MSNT (8.3 mg; 28 μmol)	NMI (2.2 μL; 27 μmol)	FmocPhe (11 mg; 28 μmol)	TBTU (9.0 mg; 28 μmol)	DIPEA (4.8 μL; 27 μmol)
PS-Ser (50 mg; 20 μmol)[d]	FmocTrp (34 mg; 80 μmol)	MSNT (24 mg; 80 μmol)	NMI (6.2 μL; 78 μmol)	FmocPhe (31 mg; 80 μmol)	TBTU (26 mg; 80 μmol)	DIPEA (14 μL; 78 μmol)
PS-Ser (50 mg; 20 μmol)[d]	FmocNle (28 mg; 80 μmol)	MSNT (24 mg; 80 μmol)	NMI (6.2 μL; 78 μmol)	FmocPhe (31 mg; 80 μmol)	TBTU (26 mg; 80 μmol)	DIPEA (14 μL; 78 μmol)
PS-Ser (50 mg; 20 μmol)[d]	FmocLeu (28 mg; 80 μmol)	MSNT (24 mg; 80 μmol)	NMI (6.2 μL; 78 μmol)	FmocPhe (31 mg; 80 μmol)	TBTU (26 mg; 80 μmol)	DIPEA (14 μL; 78 μmol)
PS-Ser (50 mg; 20 μmol)[d]	FmocPro (27 mg; 80 μmol)	MSNT (24 mg; 80 μmol)	NMI (6.2 μL; 78 μmol)	FmocPhe (31 mg; 80 μmol)	TBTU (26 mg; 80 μmol)	DIPEA (14 μL; 78 μmol)
ArgoGel MB (0.20 g; 22 μmol)[a]	FmocTyr (36 mg; 88 μmol)	MSNT (26 mg; 88 μmol)	NMI (6.8 μL; 86 μmol)	FmocLeu (31 mg; 88 μmol)	TBTU (28 mg; 88 μmol)	DIPEA (15 μL; 86 μmol)
ArgoGel MB (0.20 g; 22 μmol)[a]	FmocSer (29 mg; 88 μmol)	MSNT (26 mg; 88 μmol)	NMI (6.8 μL; 86 μmol)	FmocTrp (38 mg; 88 μmol)	TBTU (28 mg; 88 μmol)	DIPEA (15 μL; 86 μmol)
ArgoGel MB (0.20 g; 22 μmol)[a]	FmocAsn (31 mg; 88 μmol)	MSNT (26 mg; 88 μmol)	NMI (6.8 μL; 86 μmol)	FmocTrp (38 mg; 88 μmol)	TBTU (28 mg; 88 μmol)	DIPEA (15 μL; 86 μmol)
ArgoGel MB (0.20 g; 22 μmol)[a]	FmocPhe (34 mg; 88 μmol)	MSNT (26 mg; 88 μmol)	NMI (6.8 μL; 86 μmol)	FmocVal (30 mg; 88 μmol)	TBTU (28 mg; 88 μmol)	DIPEA (15 μL; 86 μmol)
TentaGel S AC (50 mg; 22 μmol)[b]	FmocTyr (16 mg; 40 μmol)	MSNT (12 mg; 40 μmol)	NMI (3.1 μL; 39 μmol)	FmocLeu (14 mg; 40 μmol)	TBTU (13 mg; 40 μmol)	DIPEA (6.8 μL; 39 μmol)
TentaGel S AC (50 mg; 22 μmol)[b]	FmocSer (13 mg; 40 μmol)	MSNT (12 mg; 40 μmol)	NMI (3.1 μL; 39 μmol)	FmocTrp (17 mg; 40 μmol)	TBTU (13 mg; 40 μmol)	DIPEA (6.8 μL; 39 μmol)
TentaGel S AC (50 mg; 22 μmol)[b]	FmocAsn (14 mg; 40 μmol)	MSNT (12 mg; 40 μmol)	NMI (3.1 μL; 39 μmol)	FmocTrp (17 mg; 40 μmol)	TBTU (13 mg; 40 μmol)	DIPEA (6.8 μL; 39 μmol)
TentaGel S AC (50 mg; 22 μmol)[b]	FmocPhe (15 mg; 40 μmol)	MSNT (12 mg; 40 μmol)	NMI (3.1 μL; 39 μmol)	FmocVal (14 mg; 40 μmol)	TBTU (13 mg; 40 μmol)	DIPEA (6.8 μL; 39 μmol)
PEGA-Ser (50 mg; 7.0 μmol)[c]	FmocTyr (11 mg; 28 μmol)	MSNT (8.3 mg; 28 μmol)	NMI (2.2 μL; 27 μmol)	FmocLeu (9.9 mg; 28 μmol)	TBTU (9.0 mg; 28 μmol)	DIPEA (4.8 μL; 27 μmol)
PEGA-Ser (50 mg; 7.0 μmol)[c]	FmocSer (9.2 mg; 28 μmol)	MSNT (8.3 mg; 28 μmol)	NMI (2.2 μL; 27 μmol)	FmocTrp (12 mg; 28 μmol)	TBTU (9.0 mg; 28 μmol)	DIPEA (4.8 μL; 27 μmol)
PEGA-Ser (50 mg; 7.0 μmol)[c]	FmocAsn (9.9 mg; 28 μmol)	MSNT (8.3 mg; 28 μmol)	NMI (2.2 μL; 27 μmol)	FmocTrp (12 mg; 28 μmol)	TBTU (9.0 mg; 28 μmol)	DIPEA (4.8 μL; 27 μmol)
PEGA-Ser (50 mg; 7.0 μmol)[c]	FmocPhe (11 mg; 28 μmol)	MSNT (8.3 mg; 28 μmol)	NMI (2.2 μL; 27 μmol)	FmocVal (9.5 mg; 28 μmol)	TBTU (9.0 mg; 28 μmol)	DIPEA (4.8 μL; 27 μmol)
PS-Ser (50 mg; 20 μmol)[d]	FmocTyr (32 mg; 80 μmol)	MSNT (24 mg; 80 μmol)	NMI (6.2 μL; 78 μmol)	FmocLeu (28 mg; 80 μmol)	TBTU (26 mg; 80 μmol)	DIPEA (14 μL; 78 μmol)
PS-Ser (50 mg; 20 μmol)[d]	FmocSer (26 mg; 80 μmol)	MSNT (24 mg; 80 μmol)	NMI (6.2 μL; 78 μmol)	FmocTrp (34 mg; 80 μmol)	TBTU (26 mg; 80 μmol)	DIPEA (14 μL; 78 μmol)
PS-Ser (50 mg; 20 μmol)[d]	FmocAsn (28 mg; 80 μmol)	MSNT (24 mg; 80 μmol)	NMI (6.2 μL; 78 μmol)	FmocTrp (34 mg; 80 μmol)	TBTU (26 mg; 80 μmol)	DIPEA (14 μL; 78 μmol)
PS-Ser (50 mg; 20 μmol)[d]	FmocPhe (31 mg; 80 μmol)	MSNT (24 mg; 80 μmol)	NMI (6.2 μL; 78 μmol)	FmocVal (27 mg; 80 μmol)	TBTU (26 mg; 80 μmol)	DIPEA (14 μL; 78 μmol)

[a] Loading grade 0.44 mmol/g. [b] Loading grade 0.2 mmol/g. [c] Loading grade 0.14 mmol/g. [d] Loading grade 0.4 mmol/g.

Microwave-assisted, solid-phase synthesis of DKPs in toluene/2-butanol (1:4 v/v), *tert*-butyl alcohol, or toluene at 120 °C for 30 min resulted in high to excellent yields of all DKPs, independent of the solid support used. However, in water, the yields were low or moderate when using the more lipophilic resins (45−73%); only the synthesis of DKPs on PEGA-Ser in water using microwave-assisted heating gave high yields of products (79−87%). The water-based reactions required an additional washing step with organic solvents to release the water-insoluble products out of the polymer matrix.

The results from the solid-phase synthesis of DKPs clearly demonstrate that microwave-assisted heating offers a fast and efficient way to produce DKPs in high yields. Similar results can be obtained with classic thermal heating, but much longer reaction times are required. In contrast to our previous

solution-phase study, the DKPs are in general obtained in higher yields in organic solvents; however, more environmentally benign conditions can be used if the proper combination of solid phase and solvent is chosen since reactions run on the PEGA-Ser resin in water gave products in high yields.

Importantly, with microwave-assisted heating, the reaction times decreased to 30 min, thus making this the method of choice, which should prove highly practical for combinatorial solid-phase synthesis of DKPs.

Experimental Part

TentaGel (0.2 mmol/g) was purchased from Rapp Polymer (Tübingen, Germany); PEGA resin (0.2 mmol/g) was purchased from Polymer Laboratories (Amherst, MA); and polystyrene (0.82 mmol/g) and ArgoGel (0.44 g/mol) were purchased from NovaBioChem (Laüfelfingen, Switzerland) and Argonaut Technologies Inc. (San Carlos, CA), respectively. Solid-phase peptide chemistry was performed in plastic syringes. Flat-bottom PE syringes were equipped with sintered Teflon filters (50-μm pores), Teflon tubing, and valves for applying suction to the syringes from below. Commercially available reaction vials with septa and screw caps were employed (Reacti-Vial, Pierce, Rockford, IL) for the classic thermally heated reactions. The microwave reactions were carried out in a Biotage Initiator instrument with a fixed hold time. Resin loadings were determined by Fmoc cleavage and optical density measurements at 290 nm and were calculated employing a calibration plot. The structural characterization data for **1**−**8** are given in the Supporting Information.

General Procedure for Peptide Coupling. The coupling reactions were performed using standard Fmoc chemistry with either TBTU or MSNT as coupling reagents together with DIPEA or NMI, respectively, in DMF. The equivalents of reagents are shown in Tables 3 and 4. The Fmoc-protected amino acid and TBTU/MSNT were dissolved in DMF, followed by addition of DIPEA/NMI. The solution was immediately added to the resin and reacted for 2 h. The resin was washed with DMF, and the Fmoc group was removed by 20% piperidine solution and washed again. The coupling reaction was repeated twice for every addition of amino acid.

General Procedure for Cyclization Reactions. The resin (50 mg; 7−22 μmol) containing the dipeptide was added to a Reacti-Vial in 3 mL of the solvent (toluene/2-butanol (1:4 v/v), H_2O, *tert*-butyl alcohol, or toluene) and heated to 50 or 80 °C for 60 and 35 h, respectively. Using microwave-assisted heating with a fixed hold time (Biotage Initiator), the temperature was set to 120 °C using toluene/2-butanol (1:4 v/v), H_2O, *tert*-butyl alcohol or toluene (4 mL) as solvent. After the reaction was finished, the polymer was filtered off, and the resin was washed with the reaction solvent. When using water, the resins were washed with *tert*-butyl alcohol (3 × 5 min) to ensure a full recovery of product. The same procedure was used for the reactions run on a 70−100-μmol scale in 20 mL of solvent.

General Procedure for HPLC Analysis. The chromatographic analysis was performed by analytical reversed-phase HPLC with a Genesis 4-μm C-8 column (length, 15 cm; diameter, 4.6 mm) on a Varian 9012 Solvent Delivery System equipped with a Varian 9050 detector and the Varian LC Star Workstation software. Analyses were performed using a linear gradient of 100% of A (water and acetonitrile in a 95:5 ratio) to 100% B (acetonitrile) for 20 min at a flow rate of 1 mL/min. A solution of tryptophan in A (6.5 mM) was used as an internal standard. The chromatograms were recorded at 450 nm. A linear calibration plot for the method of standard additions was set up for each DKP and the internal standard. The reaction solvent was evaporated, the internal standard and solvent A (50 μL) were added, and the solution was analyzed on HPLC.

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Supporting Information Available. Tables of yields obtained in the synthesis of DKPs **1**−**4** using classic thermal heating at 50 °C (60 h) and 80 °C (35 h) and for **5**−**8** using classic thermal heating at 80 °C (35 h). Tables of yields obtained in the synthesis of DKPs **1**−**8** using microwave-assisted heating at 120 °C for 30 min. Structural characterization of compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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