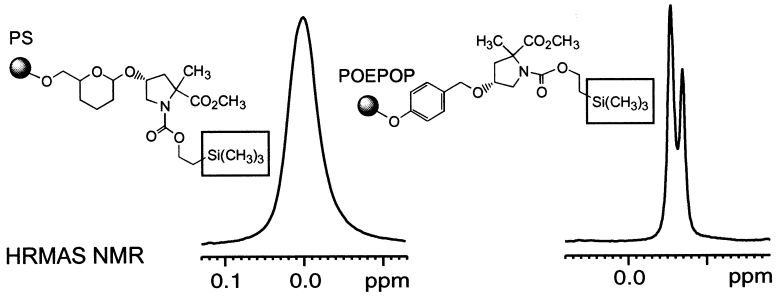
JOURNAL OF combinatorial CHEMISTRY

Article

Multistep Synthesis of 2,5-Diketopiperazines on Different Solid Supports Monitored by High Resolution Magic Angle Spinning NMR Spectroscopy

Alberto Bianco, Julien Furrer, David Limal, Gilles Guichard,
Karim Elbayed, Jsus Raya, Martial Piotto, and Jean-Paul Briand

J. Comb. Chem., **2000**, 2 (6), 681-690• DOI: 10.1021/cc0000489 • Publication Date (Web): 21 October 2000



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Combinatorial Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Multistep Synthesis of 2,5-Diketopiperazines on Different Solid Supports Monitored by High Resolution Magic Angle Spinning NMR Spectroscopy

Alberto Bianco,*[,†] Julien Furrer,[‡] David Limal,[§] Gilles Guichard,[†] Karim Elbayed,[‡] Jésus Raya,[‡] Martial Piotto,[||] and Jean-Paul Briand[†]

Institut de Biologie Moléculaire et Cellulaire, Laboratoire de Chimie Immunologique, UPR 9021 CNRS, Strasbourg, France, Institut de Chimie, UMR 7510 CNRS-Bruker, Université Louis Pasteur, Strasbourg, France, Laboratoire de Chimie Macromoléculaire, ENSCMu, Mulhouse, France, and UMR 7510 CNRS-Bruker, Wissembourg, France

Received June 14, 2000

The solid-phase synthesis of 2,5-diketopiperazines containing the *trans*-4-hydroxy-L-proline amino acid residue (Hyp) was performed on Ellman polystyrene, polyoxyethylene-polyoxypropylene (POEPOP), polystyrene-polyoxyethylene NovaSyn, and Wang resins, respectively. The reaction pathway allowed the introduction of different functional groups around the bicyclic scaffold in a combinatorial approach, and it generated mixtures of isomers. A detailed characterization of the single reaction steps by high resolution magic angle spinning (HRMAS) NMR spectroscopy was performed. The NMR spectral resolution of the resin-bound intermediates and final products was greatly influenced by the polymer matrix. The POEPOP resin permitted to obtain HRMAS NMR spectra with a resolution comparable with that of the spectra of the molecules in solution. Moreover, configurational and conformational isomers formed during the solid-phase reaction steps could be detected and easily assigned. Therefore, the combination of the HRMAS NMR technique with the use of nonaromatic resins may become an extremely powerful tool in solid-phase organic synthesis. This approach will allow the monitoring of multistep reactions and the conception of on-bead structural studies either on small molecules or on natural and/or synthetic oligomers.

Introduction

Solid-phase organic synthesis (SPOS) and combinatorial techniques are more and more attracting the interest of chemists in relation with the high demand of new drugs.[1−3] Among the broad range of templates, heterocyclic scaffolds represent the most promising molecules as lead structures for the discovery of potential pharmacophores.[4] In particular, 2,5-diketopiperazines (DKPs), present in the core of many natural products, display interesting therapeutic properties. Such compounds have been shown to inhibit several enzymes, as well as to recognize, modulate, and control the activity of many receptors.[5] The preparation of DKPs on a solid support can be performed following two strategies: (i) by cleavage-induced cyclization of linear dipeptides[6] and (ii) by on-bead cyclization before the final release from the resin.[7] Very recently, hydroxyproline-based 2,5-diketopiperazines have appeared as a new class among these heterocyclic molecules.[7a] The solid-phase parallel synthesis of these molecules was carried out on Ellman polystyrene resin[8] and conceived to enable a sequence of hydroxyproline $^{\alpha}$C-alkylation, N-acylation, cyclization, and final amide bond alkylation. The series of subsequent reactions generated mixtures of isomers, fully characterized after the final cleavage of the molecules from the resin.

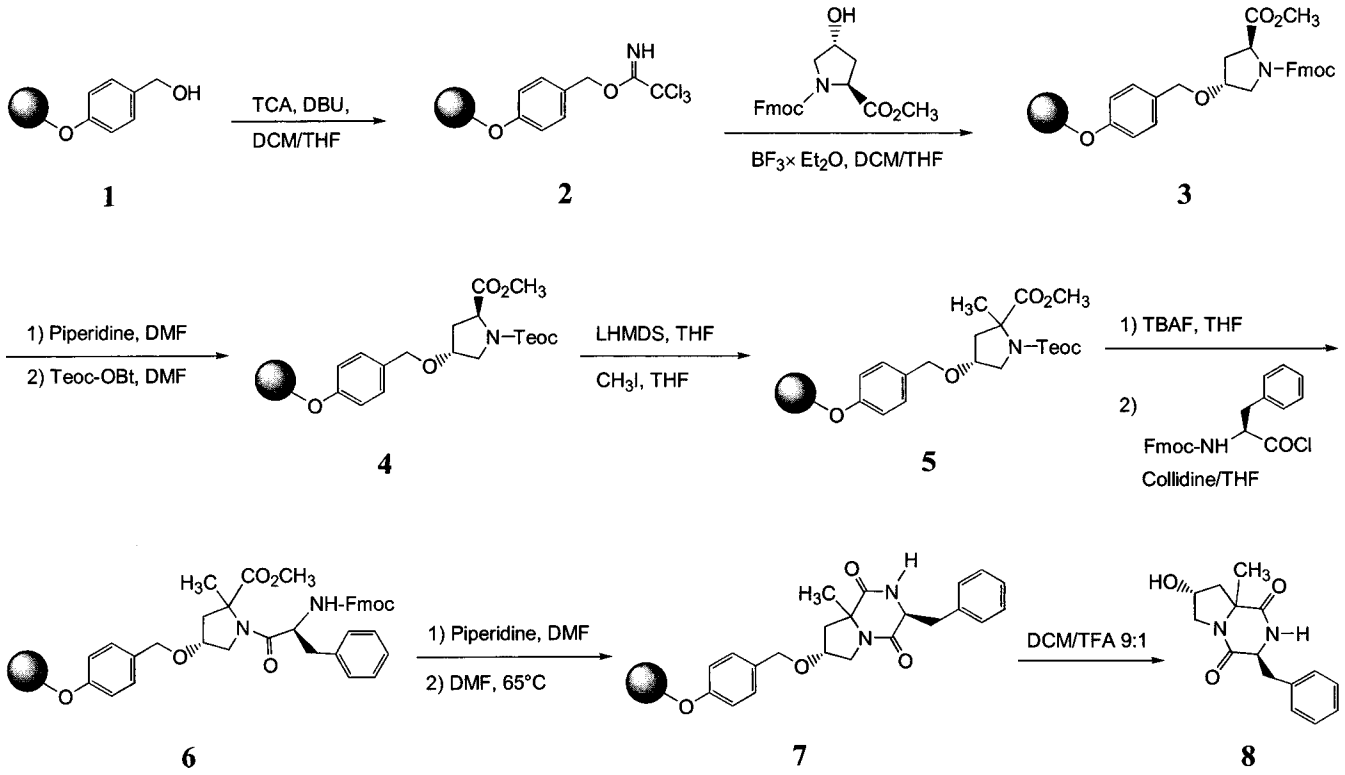
Solid-phase organic synthesis, which is a well-established strategy, presents the drawback of a limited use of analytical techniques for monitoring the intermediates still bound to the solid support all along the synthesis. Between the different analytical methods, including infrared spectroscopy[9] and mass spectrometry,[10] HRMAS (high resolution magic spinning angle) NMR spectroscopy has recently received much attention.[11] This nondestructive technique can be classified at the interface of the solid state and solution NMR, and it allows the study of small molecules and oligomers while linked to an insoluble carrier. Multidimensional HRMAS NMR has been used to control the amino acid difficult couplings during solid-phase peptide synthesis, as well as to elucidate the aggregation properties and the secondary structure of peptides.[12] In the combinatorial synthesis of small molecules, HRMAS permits quantification and step by step monitoring of solid supported organic reactions.[13] However, a major difficulty in the interpretation of simple 1D NMR spectra is due to the presence of signals originated from the resin.[14] Moreover, a problem intrinsic to the use of polystyrene-based resins lies in the increased line width of the bound molecules due to the anisotropic magnetic susceptibility induced by the aromatic rings which constitute the matrix.[15] These issues can be addressed using nonaromatic resins, such as polymers of polyamide and/or poly(ethylene glycol) origin.[16]

In this paper, we discuss the solid-phase synthesis of 2,5-diketopiperazines containing *trans*-4-hydroxy-L-proline resi-

† Institut de Biologie Moléculaire et Cellulaire.
‡ Institut de Chimie, Université Louis Pasteur.
§ Laboratoire de Chimie Macromoléculaire, ENSCMu.
|| Bruker.

10.1021/cc0000489 CCC: $19.00
Published on Web 10/21/2000

Scheme 1



due using four different resins, namely Ellman and Wang polystyrene resins, polystyrene-polyoxyethylene NovaSyn resin, and polyoxyethylene-polyoxypropylene (POEPOP)[17] resin, and present a detailed characterization of the reaction steps by HRMAS NMR. In particular, we compare the NMR spectral quality of the resin-bound intermediates and final products, and we demonstrate the influence of the carriers on the resolution of the spectra, which, in the case of the resin based on cross-linked poly(ethylene glycol) derivatives, are comparable with those obtained in solution.

Result and Discussion

Synthesis of DKPs on the Different Resins. We have recently reported the synthesis of a series of highly substituted DKPs containing the *trans*-4-hydroxy-L-proline amino acid residue (Hyp) bound to the Ellman's dihydropyran resin through the hydroxyl function.[7a] The cyclo-L,D-(αMe)Hyp-L-Phe **8** (Scheme 1) has been chosen as a representative compound to repeat the synthesis on POEPOP, NovaSyn TG HMP (TentaGel hydroxymethylphenoxy), and Wang resins, using a slightly modified reaction pathway.

For this purpose, we initially prepared a new PEG (poly(ethylene glycol)) cross-linked POEPOP resin using a PEG 1000 monomer as described by Renil and Meldal.[17] POEPOP was subsequently derivatized with the HMP linker using a Mitsunobu reaction.[18] The bifunctional HMP was selectively protected with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) at the benzyl alcohol function, allowing the free aromatic hydroxyl to react under the Mitsunobu conditions with the primary and secondary hydroxyl groups of the POEPOP resin (Figure 1). The resin with the protected linker moiety was characterized by HRMAS NMR. ^{1}H NMR spectrum of the derivatized $POEPOP_{1000}$, swollen in $CDCl_3$, confirmed the

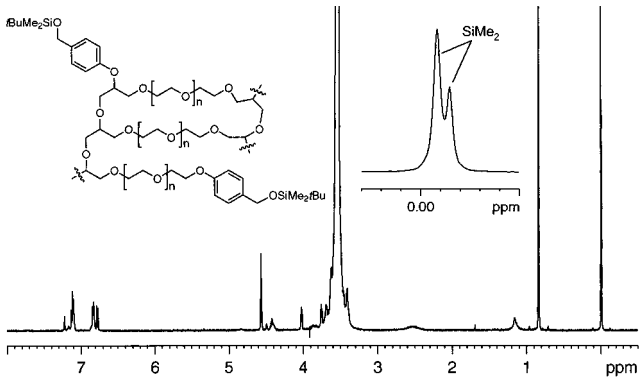


Figure 1. Molecular structure and proton HRMAS spectrum of POEPOP-HMP-TBDMS resin swollen in $CDCl_3$. Inset: partial ^{1}H NMR spectrum showing the two different methyl signals of the *tert*-butyldimethylsilyl protecting group, due to the primary and secondary POEPOP hydroxyl functions.

presence of the two types of hydroxyl groups, as already described for the $POEPOP_{1500}$ (Figure 1, inset).[16]

TBDMS protecting group was removed with tetrabutylammonium fluoride (TBAF), and POEPOP-HMP resin (**1**) was activated as trichloroacetimidate (**2**) in order to link the N-Fmoc-protected hydroxyproline methyl ester through its hydroxyl function to finally obtain the resin-bound derivative **3** (Scheme 1).[19] After cleavage of the Fmoc group and reprotection of the free Hyp nitrogen with Teoc (trimethylsilylethoxycarbonyl), activated as a benzotriazole ester, the $^{\alpha}$CH atom of the hydroxyproline residue was methylated (**5**) using methyl iodide and LHMDS [lithium bis(trimethylsilyl)-amide] in THF as a base. Following the release of Teoc with TBAF, pyrrolidine nitrogen was acylated with Fmoc-Phe-OH, activated in turn as a chloride with triphosgene in the presence of collidine (**6**).[20] This method of coupling was

faster than the method of fluorides and that of the azido acids reported before.[7a] Moreover, this method has the advantage of preparing in situ the active chloride and does not require further workup.[21,22] The on-bead formation of the 2,5-diketopiperazine (DKP) **7** was initiated immediately during the cleavage of the Fmoc group and continued overnight by heating the resin in DMF. DKP **8** was finally liberated from the resin with a mixture of trifluoroacetic acid in dichloromethane.

The same procedure was followed to prepare DKP **8** on the commercially available Wang and NovaSyn TG HMP resins.

Compound **8**, obtained on the different solid supports, was characterized by RP-HPLC and MALDI-TOF mass spectrometry. Interestingly, when using POEPOP-HMP resin the ratio between the two diastereoisomers deriving from the alkylation of the chiral hydroxyproline α-carbon atom was in favor of the isomer with the α-methyl group in configuration *R* (major/minor 1:0.85), instead of the other stereoisomer *S* as obtained on the Ellman's resin (major/minor 1:0.6).[7a] Similarly, the ratio between Hyp α-methyl in configuration *R* and the chiral center in configuration *S* was 1:0.85 for the molecules synthesized on NovaSyn support and 1:0.75 for those prepared on Wang resin. A slightly lower stereochemical control was achieved by the hydroxymethylphenoxy linker with respect to the dihydropyran anchor which is likely more sterically hindered and less symmetrical.

Synthesis of DKP Intermediates in Solution. To compare the quality of the different HRMAS NMR spectra with the NMR spectra obtained in solution, a N-Teoc-L,D-(αMe)-Hyp(Bzl)-OMe couple of diastereoisomers was prepared by methylation of the $^{\alpha}$CH carbon atom of the fully protected hydroxyproline residue. The benzyl group on the γ-hydroxyl function was introduced to mimic the linker of the Wang, NovaSyn TG, and POEPOP resins. The alkylation is nonstereoselective, and the two isomers N-Teoc-D-(αMe)Hyp-(Bzl)-OMe (**11a**) and N-Teoc-L-(αMe)Hyp(Bzl)-OMe (**11b**), isolated in a 1:0.9 ratio, were fully characterized by 2D NMR experiments.[23]

HRMAS NMR Characterization of DKPs on Ellman Polystyrene Resin. We initially focused our attention on the DKP **7a** bound to the Ellman polystyrene resin through the diyhdropyran linker (Figure 2) and recorded a series of 1D and multinuclear 2D HRMAS NMR spectra for the total configurational assignment of the solid-supported bicyclic molecule. Two main difficulties were evident during the interpretation of the proton spectrum, namely the presence of broad resonances and the overlapping of the aromatic and aliphatic signals of the resin with those of the bound molecule.

Intrinsically, the heterocyclic DKP system was complicated by the formation of four diastereoisomers: (i) two of them, formed in almost equimolar ratio, were due to the generation of the chiral center at the dihydropyran anchor, upon attachment of hydroxyl function of hydroxyproline derivative; and (ii) the other two were generated during the methylation of the $^{\alpha}$CH carbon of the Hyp pyrrolidine ring in the ratio 1:0.6. Additional bidimensional experiments were necessary to correlate the chemical shifts of the different

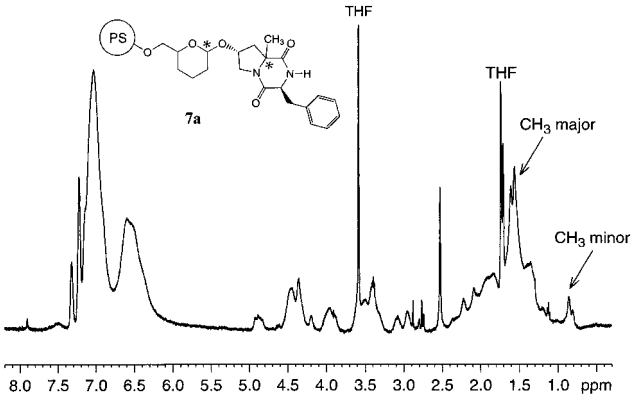


Figure 2. Molecular structure and ^{1}H HRMAS NMR spectrum of the Ellman polystyrene resin-bound 2,5-diketopiperazine **7a** swollen in THF-d_8, recorded at 500 MHz. The chiral centers generated during the synthesis are labeled with a star.

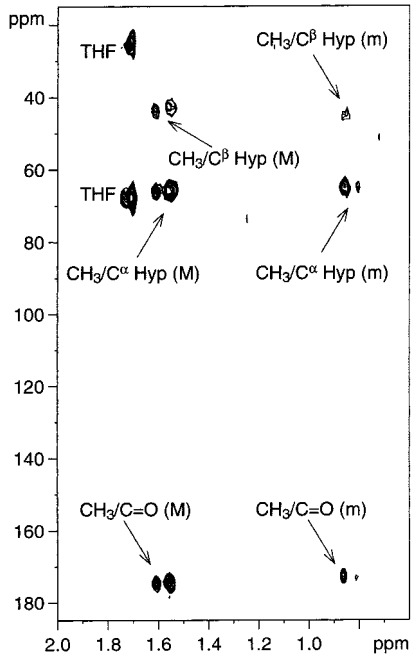


Figure 3. Partial 500 MHz HMBC HRMAS NMR spectrum of the four diastereoisomers of compound **7a** swollen in THF-d_8. The most important ^{1}H-^{13}C correlations are labeled (M: major isomers; m: minor isomers).

diastereoisomers. ROESY,[24] HMQC,[25] and HMBC[26] permitted the attribution of all the proton and carbon signals and the assignment of the absolute configuration of the four diastereoisomers. In particular, the HMBC spectrum clearly displayed the presence of the couple of isomers due to the chiral center generated by the addition of the hydroxyproline to the dihydropyran moiety (Figure 3).

The proton and carbon resonances of the hydroxyproline ring, which are closer to the chiral center of the linker, were doubled. Table 1 reports the ^{1}H and ^{13}C chemical shifts of **7a** swollen in deuterated tetrahydrofuran. By nuclear Overhauser effect we could understand the relative spatial relationship of the hydrogens and functional groups around the cyclic backbone. In the case of the two major isomers, two strong ROE correlations were due to the spatial proximity between the α-methyl groups at 1.55 and 1.60 ppm, respectively, and the $^{\alpha}$CH proton of L-phenylalanine

Table 1. HR MAS NMR Chemical Shifts of **7a** on DHP-Resin Swollen in THF-d_8

^{1}H	major isomer	minor isomer	^{13}C	major isomer	minor isomer
$^{\alpha}CH_3$ Hyp	1.55[a] 1.60[b]	0.80[a] 0.86[b]	C methyl	24.5 24.5	26.2 26.2
$^{\beta 1,2}CH_2$ Hyp	2.09[a] 2.22[b]	2.30/1.46[b] 1.96/1.86[a]	$^{\beta}$C Hyp	43.7 42.3	44.7 41.7
$^{\gamma}$CH Hyp	4.35[a] 4.37[b]	4.52	$^{\gamma}$C Hyp	73.5 73.5	73.5
$^{\delta 1,2}CH_2$ Hyp	4.04/3.60[a] 3.90/3.55[b]	3.96/3.43	$^{\delta}$C Hyp	52.7 52.1	50.5
$^{\alpha}$CH Phe	4.38	4.22	$^{\alpha}$C Phe	56.7	59.6
$^{\beta 1,2}CH_2$ Phe	3.42/2.96	3.10	$^{\beta}$C Phe	37.5	41.6
CH aromatic	7.33−7.15	7.33−7.15	CH aromatic	139.9−128.3	139.9−128.3
NH	nd[c]	7.50	$^{\alpha}$C Hyp	66.7	65.4
			C═O Hyp	174.4 173.7	172.6 172.3
			C═O Phe	nd[c]	nd[c]

[a,b] Resonances belonging to the same isomer. [c] Not determined.

at 4.38 ppm. ROE peaks due to contact of the α-methyl at 1.55 and 1.60 ppm with $^{\beta}CH_2$ at 2.09 and 2.22 ppm and $^{\delta}CH_2$ around 3.60 ppm were also visible, indicating the same orientation of the functional groups with respect to the pyrrolidine plane. For the minor diastereoisomers, ROE interactions between $^{\beta}CH_2$ (2.30 ppm) and $^{\gamma}$CH (4.52 ppm) protons of the hydroxyproline and the methyl group at the Hyp α-carbon atom were indicative of a *R* configuration of the latter. A further cross-peak arose from the spatial vicinity of the minor α-methyl with the $^{\beta}CH_2$ protons of the Phe residue at 3.10 ppm. In these two isomers, the folding of the aromatic group over the 2,5-diketopiperazine plane also created a strong ring current shift on the α-methyl groups, which appeared at higher fields (0.80 and 0.86 ppm), as described for the same compound analyzed by NMR in solution.[7a] The corresponding signal for the major isomers were located, as expected, around 1.6 ppm (Table 1). Instead, it was not possible to detect the relative orientation of the couples of the major and of the minor isomers with respect to chiral proton of the dihydropyran linker.

It was challenging to perform the HRMAS NMR analysis on this complicated system containing several diastereoisomers. Nevertheless, these experiments would have been very time-consuming and not conceivable for a more practical monitoring and characterization of the intermediates and products of a multiple-step solid-phase organic synthesis. It has been recently reported that the multiple-step synthesis of trisubstituted amines on a polystyrene-acrylate resin via final Hoffman elimination can be followed using different analytical techniques. A time inexpensive protocol, based on the combination of FT-IR, ^{1}H and ^{13}C HRMAS spectroscopies, has been optimized.[13e] Nevertheless, in the case of more complex reactions on polystyrene resins, monodimensional HRMAS spectra do not afford conclusive information on the single steps.

HRMAS NMR Characterization of DKP Intermediates on Different Resins. Until now, HRMAS spectra of different solid supports functionalized only with simple aromatic moieties or with one amino acid residue have been compared.[14,16] Moreover, step-by-step HRMAS analysis of solid-phase organic reactions was carried out mainly on polystyrene-based resins. Since our multistep synthesis of 2,5-diketopiperazines affords a mixture of isomers, we were interested

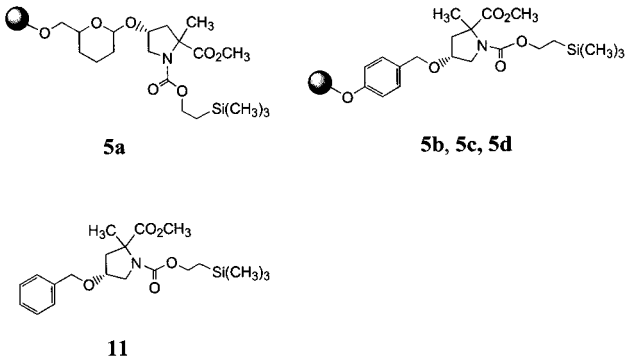


Figure 4. Molecular structures of the intermediate 5 linked to Ellman polystyrene resin (**5a**), Wang polystyrene resin (**5b**), NovaSyn TG (**5c**), and POEPOP resin (**5d**), respectively, and of N-Teoc-(αMe)Hyp(Bzl)-OMe (**11**).

to find a carrier that would have permitted a direct and easy analysis by HRMAS of the resin-bound diastereoisomeric mixture immediately after the alkylation reaction of the Hyp derivative. This is also strictly related to the possibility of modifying the reaction conditions in order to induce a better stereoselectivity.

The influence of the polymeric matrix on the resolution of the HRMAS spectra was therefore emphasized by analyzing the resin-bound intermediates **5a**−**5d**, deriving from the $^{\alpha}$CH Hyp alkylation reaction, on Ellman, Wang, NovaSyn TG, and POEPOP resins, respectively, and comparing the different HRMAS spectra with those collected in solution for compound **11** (Figure 4).

The trimethylsilyl moiety of the Teoc protecting group could be considered as a helpful probe since the trimethyl proton chemical shift appears at high field (about zero ppm), far from all the signals of the resin. Figure 5 displays the ^{1}H NMR partial spectra of this group for the solid and solution systems. Line widths measured at half-height of the peak were 24.5 Hz (Figure 5A) and 18 Hz (Figure 5B) for the two polystyrene-based resins, and 13.5 Hz for the TentaGel-based NovaSyn solid support (Figure 5C). In the case of POEPOP resin, the mean line widths were, after deconvolution, 4.4 and 3.7 Hz for the higher and the smaller peak, respectively (Figure 5D), comparable with the values of the molecule in solution (3.4 Hz for the higher peak and 2.6 Hz for the lower peak; Figure 5E).

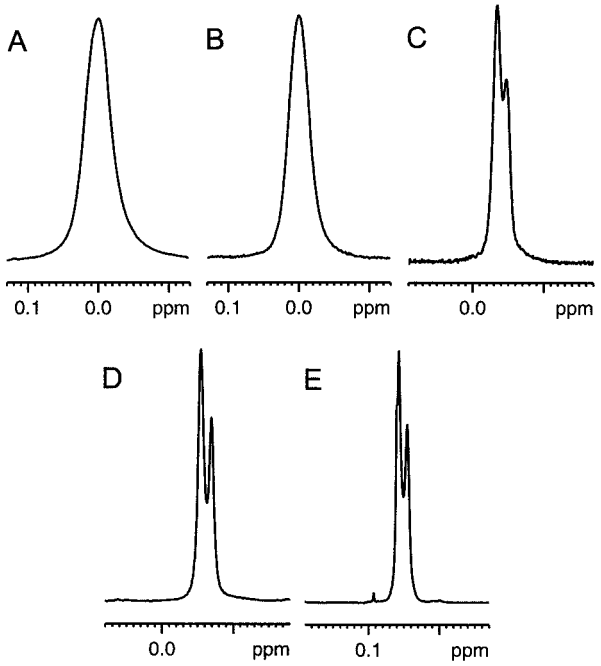


Figure 5. Partial 500 MHz ^{1}H HRMAS NMR spectra of resin-bound intermediates **5a** (A, Ellman's dihydropyran resin), **5b** (B, Wang resin), **5c** (C, NovaSyn TG resin), and **5d** (D, POEPOP resin) swollen in $CDCl_3$, and partial 400 MHz ^{1}H NMR spectrum of compound **11** (E, solution) in $CDCl_3$, showing the trimethylsilyl group chemical shift.

Moreover, the high resolution of the spectrum relative to the intermediate **5d** allowed the separation of two conformers due to the *cis*−*trans* equilibrium about the hydroxyproline tertiary amide bond, which are instead embedded in the broad NMR peak in the case of Ellman and Wang resins (Figure 5A,B).[27] In fact, the two peaks collapsed when the NMR spectrum was recorded at higher temperature. The PEG tether of NovaSyn TG resin also allowed a partial resolution of the peak, showing the presence of the rotational isomers (Figure 5C). The trimethylsilyl group did not permit the separation of the two diastereoisomers generated by the methylation reaction since, located at the end of the flexible Teoc protecting group, it is far from the α-chiral center. It must be underlined that the method of Hannessian[19] for anchoring hydroxyl functions to the solid support does not generate diastereoisomers as detected for the derivatization of the resin with the DHP linker. Therefore, Wang-, NovaSyn TG-, and POEPOP-bound intermediate **5** was constituted of only two configurational isomers instead of four. The effect of splitting the NMR signals by the primary and secondary hydroxyl functions of the POEPOP resin was not observed. Figure 6 shows the total ^{1}H spectrum of derivative **5d** on the POEPOP resin swollen in $CDCl_3$ and permitted the unambiguous detection of all the protons of the molecule.

By analyzing the region of the NMR spectrum relative to the α-methyl group, it was possible to ascertain that four resolved signals relative to the configurational and conformational isomers were present. The assignment of which couple of peaks was due to the diastereoisomers and which one derived from the conformers was done by a NOESY experiment. Figure 7 (bottom) clearly displays the exchange peaks of the rotational process at room temperature. The *cis*−*trans* exchange equilibrium was also confirmed by experiments at increased temperatures in $CDCl_3$ and other solvents (data not shown). Surprisingly, in deuterated acetonitrile and dimethyl sulfoxide the spectra recorded at 300 K showed that the conformers coalescence temperature was already

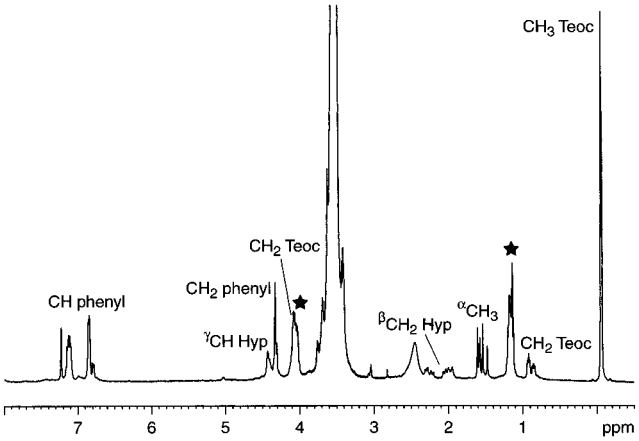


Figure 6. ^{1}H HRMAS NMR spectrum of the POEPOP resin-bound derivative **5d** in $CDCl_3$, recorded at 500 MHz. Peaks labeled with a star correspond to impurities linked to the resin and generated during the Mitsunobu and the alkylation reactions.[32]

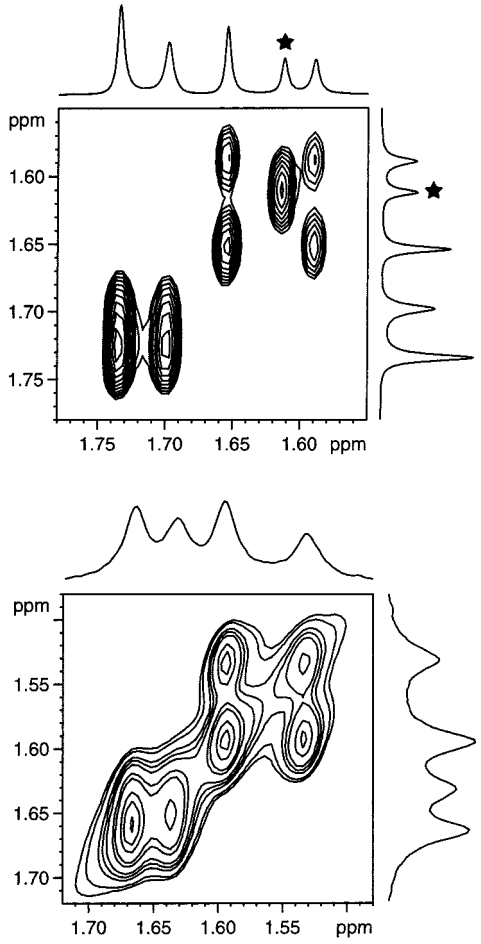


Figure 7. Partial 500 MHz NOESY (t_m = 500 ms) HRMAS NMR spectrum of POEPOP-bound intermediates **5d** (bottom) swollen in $CDCl_3$, and partial 400 MHz NOESY (t_m = 650 ms) NMR spectrum of compound **11** (top) in $CDCl_3$. The exchange peaks relative to the *cis*−*trans* interconversion are shown. Peak labeled with a star is due to water.

reached. Only the methyl resonance relative to the *cis*−*trans* interconversion for the minor isomer displayed a broad signal, while the major peak was sharp. The same behavior for the equilibrium of isomerization was also observed for N-Teoc-(αMe)Hyp(Bzl)-OMe **11** analyzed in solution (Figure 7, top). The sharpness and separation of the peaks on the carrier-linked intermediate **5d** were, therefore, sufficient for a clear quantification of the diastereomeric ratio, which was in favor of the compound with α-methyl in configuration *R*, immediately after the alkylation reaction. Moreover, a simple change of solvent may allow a direct integration, on the monodimensional spectrum, of the separated α-methyl signals giving the diastereoisomeric ratio, without further experiments at different temperatures. The same observations could be made in the case of the PEG-tethered NovaSyn TG resin. Again, the HRMAS spectrum of the intermediate **5c** showed four resolved peaks relative to the α-methyl group due to the presence of the two configurational and two conformational isomers. Nevertheless, the aliphatic region is complicated by the multiple broad resonances arising from the polystyrene matrix. Presaturation of the PEG signal around 3.5 ppm did not reduce the aromatic and aliphatic signals as it was described for a series of TentaGel resins derivatized with a protected amino acid residue.[14] The aliphatic background peaks were instead eliminated by presaturation of the aromatic region.

So far, only one example of solid-phase aldol reactions on hydrophilic resins forming couples of diastereoisomers has been described and analyzed by proton HRMAS NMR.[28] In another study, diastereoisomers have been prepared to measure the enantiomeric excess during the asymmetric dihydroxylation of olefins linked to Wang or TentaGel resins. For this purpose, it was necessary, however, to acquire a series of ^{13}C HRMAS spectra.[13c]

Although in the past few years NMR multidimensional experiments were implemented to improve the HRMAS technique,[29] we believe that the easiest way to follow reactions on a solid support resides in the use of resins that allow solution-like spectra. Indeed, we have demonstrated in this work that the multiple-step synthesis of 2,5-diketopiperazines, generating couples of configurational and conformational isomers, could be easily monitored by HRMAS experiments on a cross-linked polyoxyethylene glycol-based resin, without use of additional techniques, such as infrared spectroscopy, or without cleavage of each intermediate from the solid support for a classical characterization in solution.

Conclusions

In summary, the synthesis of hydroxyproline-based 2,5-diketopiperazines via on-bead cyclization has been performed on four different resins, namely Ellman polystyrene, Wang polystyrene, NovaSyn TG, and POEPOP. The multistep reaction, which affords a mixture of diastereisomers, could be monitored by HRMAS technique. The intermediates and the final products were characterized acquiring a series of 1D and 2D HRMAS spectra. In particular, the analysis of the resin-bound intermediates deriving from the α-carbon atom alkylation reaction of Hyp residue permitted the demonstration of how the resolution of the HRMAS spectra was influenced by the type of resin. In the case of Ellman and Wang polystyrene-based resins, it was not possible to obtain conclusive information immediately after the alkylation step by a direct analysis of the mondimensional spectrum. A better resolution of the peaks could be obtained with TentaGel-based NovaSyn resin, although residual aliphatic and aromatic resonances of the polymer cover part of the signals of the solid-supported molecule. Instead, the poly(ethylene glycol)-based POEPOP matrix allowed a resolution of the proton signals comparable with those of the intermediate molecules in solution. Moreover, configurational and conformational isomers generated during the Hyp alkylation reaction could be easily assigned. Therefore, the combination of HRMAS NMR spectroscopy with the use of nonaromatic resins may become an extremely powerful tool in solid-phase organic synthesis.

Experimental Section

General. All reagents and solvents were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF), dichloromethane (DCM), and 1,2-dichloroethane (1,2-DCE) were carefully distilled prior to use. 3,4-Dihydro-2*H*-pyran-2-ylmethoxymethyl polystyrene (DHP-HM resin, Ellman's dihydropyran resin) and NovaSyn TG HMP were purchased from Novabiochem (Läufelfingen, Switzerland) and loaded with Fmoc-L-Hyp-OMe as reported.[8] Wang resin was obtained from Neosystem (Strasbourg, France). HCl·H-Hyp(Bzl)-OMe was purchased from Bachem (Bubendorf, Switzerland). POEPOP resin (substitution, 0.82 mmol/g) was prepared as described by Renil and Meldal,[17] using poly(ethylene glycol) 1000 (PEG_{1000}) furnished by Fluka. WANG-TCA, NovaSyn TG HMP-TCA, and POEPOP-HMP-TCA resins were prepared as described in the literature, using a mixture of dry THF/DCM or dry cyclohexane/DCM as solvents.[19] The alkylations of the hydroxyproline CH^{α} atom were done under argon on a glass tube, and the cyclization of DKPs were then continued manually in small plastic syringes fitted with a frit. Teoc-OBt was synthesized as previously described.[30] RP-HPLC analysis was done on a C_{18} column (5 μm, 150 × 4.6 mm) using a linear gradient of A: 0.1% TFA in water and B: 0.08% TFA in acetonitrile, 0−100% B and/or 30−100% B in 20 min at 1.2 mL/min flow rate. Chromatograms were recorded at 210 nm wavelength. MALDI-TOF mass analysis was performed on a linear MALDI-TOF Bruker instrument using α-cyano-4-hydroxycinnamic acid and/or 2,5-dihydroxybenzoic acid as matrixes. 1D and 2D liquid-state NMR spectra were recorded on a Bruker AC 200 MHz and Avance DPX 400 MHz spectrometers. The samples were dissolved in $CDCl_3$ and/or DMSO-d_6. HRMAS 1D and 2D NMR spectra were obtained on a Bruker Avance DSX 500 MHz spectrometer equipped with a 4 mm double MAS probe. The samples were packed into a 4 mm HRMAS rotor, and solvents were added to the resin directly inside the rotor. In all experiments, samples were spun at 4 or 5 kHz. The spectra were acquired at a temperature of 300 K. All 2D spectra were recorded in pure phase mode using time proportional phase incrementation method. Homonuclear spectra were recorded with 2048

data points in t2 and 256 increments in t1. Typically 8 or 16 scans per increment were accumulated. A spectral width of 4629.63 Hz was used for the proton. Through space dipolar connectivities were obtained from NOESY[23a] and ROESY[24] spectra using mixing times from 150 to 800 ms. A spinlock field of 2.5 kHz was employed in ROESY experiments. Heteronuclear spectra were recorded with 2048 data points in t2 and 128 increments in t1. Either 128 scans or 256 scans per increment were added. Sweep widths for ^{1}H and ^{13}C dimensions were 4629.63 and 35461 Hz, respectively. BIRD pulse was used to suppress the unwanted proton signals bonded to ^{12}C in HMQC sequence.[25] Direct ^{1}H-^{13}C correlations were obtained from BIRD-HMQC experiment, whereas long-range couplings were obtained from HMBC experiment.[26] The samples were swollen in $CDCl_3$, CD_3CN, DMSO-d_6, and/or THF-d_8.

Abbreviations. Symbols and abbreviations for amino acids and peptides are in accord with the recommendations of the IUPAC−IUB Commission on Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). Other abbreviations used are as follows: SPOS, solid-phase organic synthesis; DKP, 2,5-diketopiperazine; Bt, benzotriazole; Bzl, benzyl; DHP, dihydropyran; DIAD, diisopropylazodicarboxylate; DIC, diisopropylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DME, dimethoxyethane; Fmoc, fluorenylmethyloxycarbonyl; HMP, 4-hydroxymethylphenoxy; HOBt, 1-hydroxybenzotriazole; LHMDS, lithium bis(trimethylsilyl)amide; Me, methyl; MSNT, 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole; -OMe, methoxy; PEG, poly(ethylene glycol); TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; TCA, trichloroacetimidate; Teoc, trimethylsilylethoxycarbonyl; TFA, trifluoroacetic acid; TG, TentaGel; NOE, nuclear Overhauser effect.

Synthesis of the Derivatives. 4-*tert*-Butyldimethylsilyloxymethyl Phenol (9). A solution of *tert*-butyldimethylchlorosilane (2.50 g, 16.6 mmol) in DCM (30 mL) was added dropwise to a solution of 4-hydroxybenzyl alcohol (2.48 g, 20 mmol) in pyridine (30 mL). The mixture was stirred for 2 h at room temperature and concentrated. The residue was dissolved in DCM, and the organic phase was washed with 1 N HCl, water, 10% $NaHCO_3$, and water and dried over $MgSO_4$. Flash chromatography (SiO_2, eluant: ethyl acetate/*n*-hexane 1:4) afforded **9** as a colorless oil. Yield: 77%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.18 (d, $J = 8.4$ Hz, CH phenyl), 6.77 (d, $J = 8.4$ Hz, CH phenyl), 5.61 (s, OH), 4.67 (s, CH_2), 0.95 (s, CH_3 Si*t*Bu), 0.11 (s, CH_3 SiMe). ^{13}C NMR (50 MHz, $CDCl_3$) δ 154.75 133.40, 127.98, 115.25, 64.98, 26.06, 18.50, −5.09. HPLC t_R 17.5 min (linear gradient, 0−100% B).[31]

N-Teoc-Hyp(Bzl)-OMe (10). To a solution of HCl·H-Hyp(Bzl)-OMe (272 mg, 1.0 mmol) in acetonitrile (10 mL), neutralized with DIEA (191 μL, 1.1 mmol), was added Teoc-OBt (279 mg, 1.0 mmol). The mixture was stirred for 16 h and the solvent evaporated. The crude compound dissolved in AcOEt was washed with 1 N $KHSO_4$, water, $NaHCO_3$ 10%, and water and dried with Na_2SO_4. Following evaporation, the desired product was isolated as a colorless oil. Yield: 97%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.28 (m, CH phenyl), 4.48 (m, CH_2 Bzl, $^{\gamma}CH$ Hyp), 4.17 (m, $^{\alpha}CH$ Hyp, CH_2 Teoc), 3.71 and 3.68 (2s, CH_3 OMe *cis*−*trans*), 3.58 (m, $^{\delta}CH_2$ Hyp), 2.38 (m, $^{\beta}CH_2$ Hyp), 2.09 (m, $^{\beta}CH_2$ Hyp), 0.96 (m, CH_2 Teoc), 0.00 and −0.01 (2s, CH_3 $SiMe_3$ *cis*−*trans*). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.18, 137.67, 128.46, 127.81, 127.61, 127.51, 76.77, 75.89, 71.13, 63.72, 57.86, 57.69, 52.22, 51.66, 51.60, 36.78, 35.57, 17.77, −1.48, −1.57. HPLC t_R: 14.6 min (linear gradient: 30−100% B). $[\alpha]_D^{20} = -48°$ (*c* 0.5, methanol). MALDI-MS (MW = 379.52) $m/z = 402.13$ $[M + Na]^+$.

N-Teoc-(αMe)Hyp(Bzl)-OMe (11). To a solution of N-Teoc-Hyp(Bzl)-OMe (155 mg, 0.41 mmol) in dry 1:1 DME/THF (4 mL), under argon, was added MeI (308 μL, 12 equiv) at room temperature, followed by 1 M solution of LHMDS in THF (1.64 mL, 4 equiv) at room temperature. After 6 h, the excess of base was hydrolyzed with a saturated solution of NH_4Cl and extracted with AcOEt. The organic phase was washed with $NaHCO_3$ 10% and water and dried with Na_2SO_4. Following evaporation, the crude product was purified by flash chromatography (SiO_2, eluant, *n*-hexane/AcOEt 4:1) and isolated as a colorless oil (mixture of two diastereoisomers, ratio **11a/11b** 1:0.9). Yield: 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, CH phenyl), 4.54 (m, CH_2 Bzl, $^{\gamma}CH$ Hyp **b**), 4.51 (m, CH_2 Bzl **a**), 4.18 (m, CH_2 Teoc), 4.18 (m, $^{\gamma}CH$ Hyp **b**), 4.16 (m, $^{\gamma}CH$ Hyp **a**), 3.85 (m, $^{\delta}CH_2$ Hyp **a**), 3.78 (m, $^{\delta}CH_2$ Hyp **b**), 3.75, 3.71, 3.70 and 3.68 (4s, CH_3 OMe *cis*−*trans*), 3.69 (m, $^{\delta}CH_2$ Hyp **b**), 3.58 (m, $^{\delta}CH_2$ Hyp **a**), 2.44 (m, $^{\beta}CH_2$ Hyp **a**), 2.36 (m, $^{\beta}CH_2$ Hyp **b**), 2.16 (m, $^{\beta}CH_2$ Hyp **b**), 2.12 (m, $^{\beta}CH_2$ Hyp **a**), 1.74 and 1.70 (2s, $^{\alpha}CH_3$ *cis*−*trans* **b**) 1.66 and 1.59 (2s, $^{\alpha}CH_3$ *cis*−*trans* **a**) 1.00 (m, CH_2 Teoc), 0.06 and 0.05 (2s, CH_3 $SiMe_3$ *cis*−*trans*). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.88, 174.75, 174.39, 174.13, 154.66, 154.56, 137.71, 137.64, 128.40, 128.33, 127.71, 127.51, 127.45, 76.28, 75.49, 74.74, 74.05, 71.36, 71.20, 71.10, 65.17, 64.64, 63.46, 63.27, 63.20, 53.20, 52.81, 52.61, 52.35, 52.25, 45.47, 45.01, 44.38, 43.89, 24.13, 23.40, 23.14, 22.42, 17.86, 17.67, −1.51, −1.67. NOE main contacts, **11a**: H($^{\gamma}CH$−Hyp):H($^{\beta 1}CH_2$−Hyp); H($^{\beta 1}CH_2$−Hyp):H($^{\alpha}CH_3$); H($^{\gamma}CH$−Hyp):H($^{\alpha}CH_3$). **11b**: H($^{\gamma}CH$-Hyp):H($^{\beta 1}CH_2$−Hyp); H($^{\beta 2}CH_2$-Hyp):H($^{\alpha}CH_3$); H($^{\delta 2}CH_2$-Hyp):H($^{\alpha}CH_3$). HPLC t_R: 15.6 min (**11a**) and 15.8 min (**11b**) (linear gradient: 30−100% B). MALDI-MS (MW = 393.55) $m/z = 416.06$ $[M + Na]^+$.

Preparation of POEPOP-HMP Resin. (a) POEPOP-HMP-TBDMS. A solution of **9** (489 mg, 2.05 mmol) in DCM (5 mL) was added under Ar to a suspension of $POEPOP_{1000}$ resin (0.82 mmol/g) (500 mg, 0.41 mmol) in DCM (10 mL) containing PPh_3 (538 mg, 2.05 mmol). The mixture was cooled to 0 °C, and a solution of DIAD (436 μL, 2.05 mmol) in DCM (5 mL) was added dropwise under Ar along 1 h. The reaction mixture was stirred for 48 h, and the resin was filtered, washed with DCM, DMF, water, methanol, DCM, and diethyl ether, and dried under high vacuum for 12 h.

(b) POEPOP-HMP. POEPOP-HMP-TBDMS (590 mg, 0.40 mmol) was stirred in a solution of THF (3.5 mL) containing TBAF (8 equiv) for 1 h. This step was repeated twice. The resin was extensively washed with THF, DCM, DMF, water, and methanol and dried with diethyl ether.

(c) Loading Determination. To POEPOP-HMP resin (30 mg, theoretical 21 μmol), a solution of Fmoc-Gly-OH (3

equiv), MSNT (3 equiv), and *N*-methylimidazole (2.5 equiv) in 600 μL of dry DCM was added. The coupling was repeated two times for 1.5 h. Following washings, Fmoc group was removed with 25% piperidine in DMF (700 μL, 2 × 15 min) and recovered for the determination of the UV absorbance at 306 nm. Loading: 0.47 mmol/g.

General Procedure for Preparation of 2,5-Diketopiperazines. POEPOP-HMP-TCA or Wang-TCA or NovaSyn TG HMP-TCA resin (100 μmol) was swollen in 1:1 dry DCM/THF or 1:1 dry DCM/cyclohexane (5 mL), under argon. Fmoc-L-Hyp-OMe (5 equiv) was added followed by $BF_3 \cdot Et_2O$ (7.5 μL), and the mixture was stirred 10 min under argon. The resin was extensively washed and treated with 3 mL of 25% piperidine in DMF (2 × 15 min). The new loading (0.32 mmol/g) was calculated as described above. After washings with DMF and DCM, a solution of Teoc-OBt (3 equiv) in DMF (3 mL) was added to the resin, and the mixture was stirred for 15 h at room temperature. The solution was eliminated, and the resin washed with DMF, DCM, and Et_2O and dried under vacuum. The resin was suspended, under argon, in dry THF (4 mL), and after 5 min MeI (15 equiv) was added. The mixture was stirred 5 min, and a 1 M solution of LHMDS in THF (15 equiv) was added at room temperature. After 6 h, the excess of base was hydrolyzed with a saturated solution of NH_4Cl, and the resin was extensively washed with water, methanol, DMF, and DCM. Teoc was removed using a fresh 1 M solution of TBAF (3 mL) in THF for 1 h. After washing, the resin was coupled with Fmoc-Phe-Cl (5 equiv), prepared in turn by solubilizing Fmoc-Phe-OH (5 equiv) in dry THF (3 mL) and adding subsequently triphosgene (1.65 equiv) and 2,4,6-collidine (14 equiv)[20] for 1 h. Following several washings with DCM and DMF, the Fmoc group was removed with 25% piperidine in DMF (3 mL, 2 × 15 min), which immediately started the cyclization to DKP. This step was monitored by Kaiser test. After elimination of the base, the resin was swollen in DMF and heated at 65 °C for 18 h. The DKP was finally cleaved from the resin using a 9:1 mixture of DCM/TFA (3 mL, 30 min) and recovered as an oily compound after evaporation of the acidic solution under reduced pressure.

Supporting Information Available. 500 MHz HRMAS TOCSY, ROESY, HMQC, and HMBC spectra of resin-bound DKP **7a**. 400 MHz COSY and NOESY spectra of compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Houghten, R. A.; Pinilla, C.; Appel, J. R.; Blondelle, S. E.; Dooley, C. T.; Eichler, J.; Nefzi, A.; Ostresh, J. M. Mixture-based synthetic combinatorial libraries. *J. Med. Chem.* **1999**, *42*, 3743−3778.

(2) Giannis, A.; Kolter, T. Peptidomimetics for receptor ligands − Discovery, development, and medical perspectives. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267.

(3) (a) Jung, G. *Combinatorial Chemistry*; Wiley-VCH: Weinheim, 1999. (b) Booth, S.; Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. Solid-phase organic reactions III: a review of the literature Nov 96-Dec 97. *Tetrahedron* **1998**, *54*, 15385−15443. (c) Thompson, L. A.; Ellman, J. A. Synthesis and applications of small molecule libraries. *Chem. Rev.* **1996**, *96*, 555−600.

(4) (a) Franzén, R. G. Recent advances in the preparation of heterocycles on solid support: a review of the literature. *J. Comb. Chem.* **2000**, *2*, 195−214. (b) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. The current status of heterocyclic combinatorial libraries. *Chem. Rev.* **1997**, *97*, 449−472.

(5) (a) Cody, W. L.; Augelli-Szafran, C. E.; Berryman, K. A.; Cai, C.; Doherty, A. M.; Edmunds, J. J.; He, J. X.; Narasimhan, L. S.; Penvose-Yi, J.; Plummer, J. S.; Rapundalo, S. T.; Rubin, J. R.; Van Huis, C. A.; Leblond, L.; Winocour, P. D.; Siddiqui, M. A. The design of potent and selective inhibitors of thrombin utilizing a piperazinedione template: part 1. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2497−2502. (b) Cui, C.-B.; Kakeya, H.; Osada, H. Novel mammalian cell inhibitors, spirotryprostatins A and B, produced by *Aspergillus fumigatus*, which inhibit mammalian cell cycle at G2/M phase. *Tetrahedron* **1996**, *52*, 12651−12666. (c) Alvarez, M. E.; Houck, D. R.; White, C. B., Brownell, J. E., Bobko, M. A., Rodger, C. A., Stawicki, M. B., Sun, H. H., Gillum, A. M.; Cooper, R. Isolation and structure elucidation of two new calpain inhibitors from *Streptomyces griseus*. *J. Antibiot.* **1994**, *47*, 1195−1201. (d) Charlton, P. A.; Faint, R. W.; Bent, F.; Bryans, J.; Chicarelli-Robinson, I.; Mackie, I.; Machin, S.; Bevan, P. Evaluation of a low molecular weight modulator of human plasminogen activator inhibitor-1 activity. *Thromb. Haemost.* **1996**, *75*, 808−815. (e) Baures, P. W.; Ojala, W. H.; Costain, W. J.; Ott, M. C.; Pradhan, A.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. Design, synthesis, and dopamine receptor modulating activity of diketopiperazine peptidomimetics of L-prolyl-L-leucylglycinamide. *J. Med. Chem.* **1997**, *42*, 3594−3600.

(6) (a) Szardenings, A. K.; Antonenko, V.; Campbell, D. A.; 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. (b) Szardenings, A. K.; Harris, D.; Lam, S.; Shi, L.; Tien, D.; Wang, Y.; Patel, D. V.; Navre, M.; Campbell, D. A. Rational design and combinatorial evaluation of enzyme inhibitor scaffolds: identification of novel inhibitors of matrix metalloproteinases. *J. Med. Chem.* **1998**, *41*, 2194−2200. (c) Goodfellow, V. S.; Laudeman, C. P.; Gerrity, J. I.; Burkard, M.; Strobel, E.; Zuzack, J. S.; McLeod, D. A. Rationally designed non-peptides: variously substituted piperazine libraries for the discovery of bradykinin antagonists and other G-protein-coupled receptors. *Mol. Diversity* **1996**, *2*, 97−102. (d) Smith, R. A.; Bobko, M. A.; Lee, W. Solid-phase synthesis of a library of piperazinediones and diazepinediones via Kaiser oxime resin. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2369−2374. (e) Szardenings, A. K.; Burkoth, T. S.; Lu, H. H.; Tien, D. W.; Campbell, D. A. A simple procedure for the synthesis of diketopiperazine and diketomorpholine derivatives. *Tetrahedron* **1997**, *53*, 6573−6593. (f) Kowalski, J.; Lipton, M. A. Solid-phase synthesis of a diketopiperazine catalyst containing the unnatural amino acid (S)-norarginine. *Tetrahedron Lett.* **1996**, *37*, 5839−5840. (g) Gordon, D. W.; Steele, J. Reductive alkylation on a solid phase: synthesis of a piperazinedione combinatorial library. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 47−50. (h) Scott, B. O.; Siegmund, A. C.; Marlowe, C. K.; Pei, Y.; Spear, K. L. Solid-phase organic synthesis (SPOS): a novel route to diketopiperazines and diketomorpholines. *Mol. Diversity* **1995**, *1*, 125−134.

(7) (a) Bianco, A.; Sonksen, C. P.; 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. (b) Del Fresno, M.; Alsina, J.; Royo, M.; Barany, G.; Albericio, F. Solid-phase synthesis of diketopiperazines, useful scaffold for combina-

torial chemistry. *Tetrahedron Lett.* **1998**, *39*, 2639−2642. (c) Sabatino, G.; Chelli, M.; Mazzucco, S.; Ginanneschi, M.; Papini, A. M. Cyclization of histidine containing peptides in the solid-phase by anchoring the imidazole ring to trityl resins. *Tetrahedron Lett.* **1999**, *40*, 809−812.

(8) Thompson, L. A.; Ellman, J. A. A straightforward and general method for coupling alcohols to solid support. *Tetrahedron Lett.* **1994**, *35*, 9333−9336.

(9) (a) Haap, W. J.; Walk, T. B.; Jung, G. FT-IR Mapping − A new tool for spatially resolved characterization of polymer-bound combinatorial compound libraries with IR microscopy. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 3311−3314. (b) Yan, B.; Kumaravel, G.; Anjaria, H.; Wu, A.; Petter, R. C.; Jewell, C. F.; Wareing, J. R. Infrared spectrum of a single resin bead for real-time monitoring of solid-phase reactions. *J. Org. Chem.* **1995**, *60*, 5736−5738.

(10) (a) Brummel, C. L.; Lee, I. N. W.; Zhou, Y.; Benkovic, S. J.; Winograd, N. A mass spectrometric solution to the address problem of combinatorial libraries. *Science* **1994**, *264*, 399−402. (b) Metzger, J. W.; Kempter, C.; Wiesmüller, K.-H.; Jung, G. Electrospray mass spectrometry and tandem mass spectrometry of synthetic multicomponent peptide mixtures: determination of composition and purity. *Anal. Biochem.* **1994**, *219*, 261−277.

(11) (a) Keifer, P. A. High-resolution NMR techniques for solid-phase synthesis and combinatorial chemistry. *Drug Discovery Today* **1997**, *2*, 468−478. (b) Shapiro, M. J.; Wareing, J. R. NMR methods in combinatorial chemistry. *Curr. Opin. Chem. Biol.* **1998**, *2*, 372−375.

(12) (a) Dhalluin, C.; Boutillon, C.; Tartar, A.; Lippens, G. Magic angle spinning nuclear magnetic resonance in solid-phase peptide synthesis. *J. Am. Chem. Soc.* **1997**, *119*, 10494−10500. (b) Jelinek, R.; Valente, A. P.; Valentine, K. G.; Opella, S. J. Two-dimensional NMR spectroscopy of peptides on beads. *J. Magn. Res.* **1996**, *125*, 185−187. (c) Warrass, R.; Wieruszeski, J.-M.; Boutillon, C.; Lippens, G. High-resolution magic angle spinning NMR study of resin-bound polyalanine peptides. *J. Am. Chem. Soc.* **2000**, *122*, 1789−1795. (d) Gotfresden, C. H.; Grøtli, M.; Willert, M.; Meldal, M.; Duus, J. Ø. Single-bead structure elucidation. Requirements for analysis of combinatorial solid-phase libraries by nanoprobe MAS NMR spectroscopy. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1167−1171.

(13) (a) Warrass, R.; Lippens, G. Quantitative monitoring of solid-phase organic reactions by high-resolution magic angle spinning NMR spectroscopy. *J. Org. Chem.* **2000**, *65*, 2946−2950. (b) Sefler, A. M.; Gerritz, S. W. Using one- and two-dimensional NMR techniques tocharacterize reaction products bound to Chiron SynPhase crowns. *J. Comb. Chem.* **2000**, *2*, 127−133. (c) Riedl, R.; Tappe, R.; Berkessel, A. Probing the scope of the asymmetric dihydroxylation of polymer-bound olefins. Monitoring by HRMAS NMR allows for reaction control and on-bead measurement of enantiomeric excess. *J. Am. Chem. Soc.* **1998**, *120*, 8994−9000. (d) Ruhland, T.; Andersen, K.; Pedersen, H. Selenium-linking strategy for traceless solid-phase synthesis: direct loading, aliphatic C−H bound formation upon cleavage and reaction monitoring by gradient MAS NMR spectroscopy. *J. Org. Chem.* **1998**, *63*, 9204−9211. (e) Luo, Y.; Ouyang, X.; Armstrong, R. W.; Murphy, M. M. A case study of employing spectroscopic tools for monitoring reactions in the developmental stage of a combinatorial chemistry library. *J. Org. Chem.* **1998**, *63*, 8719−8722. (f) Chin, J.; Fell, B.; Shapiro, M. J.; Tomesch, J.; Wareing, J. R.; Bray, A. M. Magic angle spinning NMR for reaction monitoring and structure determination of molecules attached to multipin crowns. *J. Org. Chem.* **1997**, *62*, 538−539. (g) Sarkar, S. K.; Garigipati, R. S.; Adams, J. L.; Keifer, P. A. An NMR method to identify nondestructively chemical compounds bound to a single solid-phase-synthesis bead for combinatorial chemistry applications. *J. Am. Chem. Soc.* **1996**, *118*, 2305−2306. (h) Pop, I. E.; Dhalluin, C. F.; Déprez, B. P.; Melnyk, P. C.; Lippens, G. M.; Tartar, A. L. Monitoring of a three-step solid-phase synthesis involving a Heck reaction using magic angle spinning NMR spectroscopy. *Tetrahedron* **1996**, *52*, 12209−12222. (i) Wehler, T.; Westman, J. Magic angle spinning NMR: a valuable tool for monitoring the progress of reactions in solid-phase synthesis. *Tetrahedron Lett.* **1996**, *37*, 4771−4774. (j) Fitch, W. L.; Detre, G.; Holmes, C. P.; Shoorlery, J. N.; Keifer, P. A. High-resolution ^{1}H NMR in solid-phase organic synthesis. *J. Org. Chem.* **1994**, *59*, 7955−7956.

(14) Keifer, P. A. Influence of resin structure, tether length, and solvent upon the high-resolution ^{1}H NMR spectra of solid-phase-synthesis resins. *J. Org. Chem.* **1996**, *61*, 1558−1559.

(15) Elbayed, K.; Bourdonneau, M.; Furrer, J.; Richert, T.; Raya, J.; Hirshinger, J.; Piotto, M. Origin of the residual NMR line width of a peptide bound to a resin under magic angle spinning. *J. Magn. Res.* **1999**, *136*, 127−129.

(16) Grøtli, M.; Gotfresden, C. H.; Rademann, J.; Buchardt, J.; Clark, A. J.; Duus, J. Ø.; Meldal, M. Physical properties of poly(ethylene glycol) (PEG)based resins for combinatorial solid-phase organic synthesis: a comparison of PEG-*cross*-linked and PEG-grafted resins. *J. Comb. Chem.* **2000**, *2*, 108−119.

(17) Renil, M.; Meldal, M. POEPOP and POEPS: inert poly(ethylene glycol) cross-linked polymeric supports for solid synthesis. *Tetrahedron Lett.* **1996**, *37*, 6185−6188.

(18) (a) Mitsunobu, O. The use of diethyl azodicaboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, 1−28. (b) Hughes, D. L. The Mitsunobu Reactions. In *Organic Reactions*; Paquette, L. A., Ed.; Wiley and Sons: New York, 1992; Vol. 42, pp 335−656.

(19) (a) Hanessian, S.; Xie, F. Polymer-bound *p*-alkoxybenzyl trichloroacetimidates: reagents for the protection of alcohols as benzyl ethers on solid-phase. *Tetrahedron Lett.* **1998**, *39*, 733−736. (b) Hanessian, S.; Xie, F. Exploring functional and molecular diversity with polymer-bound *p*-alkoxybenzyl ethers − Scope and applications of preparatively useful organic reactions. *Tetrahedron Lett.* **1998**, *39*, 737−740.

(20) 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. Pept. Res.* **1999**, *53*, 507−517.

(21) Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. Peptide synthesis via amino acid halides. *Acc. Chem. Res.* **1996**, *29*, 268−274.

(22) Meldal, M.; Juliano, M. A.; Jansson, A. M. Azido acids in a novel method of solid-phase peptide synthesis. *Tetrahedron Lett.* **1997**, *38*, 2531−2534.

(23) (a) Jeneer, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. Investigation of exchange processes by two-dimensional NMR spectroscopy. *J. Chem. Phys.* **1979**, *71*, 4546−4553. (b) Piantini, U.; Sørensen, O. W.; Ernst, R. R. Multiple quantum filters for elucidating NMR coupling networks. *J. Am. Chem. Soc.* **1982**, *104*, 6800−6801.

(24) Bax, A.; Davis, D. G. Practical aspects of two-dimensional transverse NOE spectroscopy. *J. Magn. Res.* **1985**, *63*, 207−213.

(25) (a) Bax, A.; Griffey, R. H.; Hawkins, B. L. Correlation of proton and nitrogen-15 chemical shifts by multiple quantum NMR. *J. Magn. Res.* **1983**, *55*, 301−315. (b) Garbow, J. R.; Weitkamp, D. P.; Pines, A. Bilinear rotation decoupling of homonuclear scalar interactions. *Chem. Phys. Lett.* **1982**, *93*, 504−509.

(26) Bax, A.; Summers, M. ^{1}H and ^{13}C Assignments from sensivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.

(27) Rose, G. G.; Gierash, L. M.; Smith, J. A. Turns in peptides and proteins. *Adv. Protein Chem.* **1985**, 1−109.
(28) Graven, A.; Grøtli, M.; Meldal, M. Towards peptide isostere libraries: aqueous aldol reactions on hydrophilic solid supports. *J. Chem. Soc., Perkin Trans. 1* **2000**, 955−962.
(29) (a) Chin, J.; Chen, A.; Shapiro, M. J. SPEEDY: Spin−echo enhanced diffusion filtered spectroscopy. A new tool for high-resolution MAS NMR. *J. Comb. Chem.* **2000**, *2*, 293−296. (b) Warrass, R.; Wieruszeski, J.-M.; Lippens, G. Efficient suppression of solvent resonances in HR-MAS of resin-supported molecules. *J. Am. Chem. Soc.* **1999**, *121*, 3787−3788. (c) Chin, J.; Fell, B.; Pochapsky, S.; Shapiro, M. J.; Wareing, J. R. 2D SECSY NMR for combinatorial chemistry. High-resolution MAS spectra for resin-bound molecules. *J. Org. Chem.* **1998**, *63*, 1309−1311. (d) Meissner, A.; Bloch, P.; Humpfer, E.; Spraul, M.; Sørensen, O. W. Reduction of inhomogeneous broadening in two-dimensional high-resolution MAS NMR spectra of molecules attached to swelled resins in solid-phase synthesis. *J. Am. Chem. Soc.* **1997**, *119*, 1787−1788. (e) Shapiro, M. J.; Chin, J.; Marti, R. E.; Jarosinski, M. A. Enhanced resolution in MAS NMR for combinatorial chemistry. *Tetrahedron Lett.* **1997**, *38*, 1333−1336. (f) Garigipati, R. S.; Adams, B.; Adams, J. L.; Sarkar, S. K. Use of spin−echo magic angle spinning ^{1}H NMR in reaction monitoring in combinatorial organic chemistry. *J. Org. Chem.* **1996**, *61*, 2911−2914. (g) Maas, W. E.; Laukien, F. H.; Cory, D. G. Gradient, high resolution, magic angle sample spinning NMR. *J. Am. Chem. Soc.* **1996**, *118*, 13085−13086.
(30) Shute, R. E.; Rich, D. H. Synthesis and evaluation of novel activated mixed carbonate reagents for the introduction of the 2-(trimethylsilyl)ethoxycarbonyl(Teoc)-protecting group. *Synthesis* **1987**, 346−349.
(31) Partial cleavage of TBDMS protecting group affording 4-hydroxybenzyl alcohol was observed due to the low percentage of TFA (0.1%) in the HPLC aqueous mobile phase.
(32) Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J. A mechanistic study of the Mitsunobu esterification reaction. *J. Am. Chem. Soc.* **1988**, *110*, 6487−6491.

CC0000489