Syntheses and Properties of Resin-Bound Oligopeptides. 2.[1] Infrared Spectroscopic Conformational Analysis of Cross-Linked Polystyrene Resin Bound Oligoleucines in the Swollen State

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ABSTRACT: In order to elucidate the hydrogen-bonding behavior of cross-linked resin-bound peptides in the swollen state, conformational properties of cross-linked polystyrene resin bound oligoleucines were studied by infrared spectroscopy. The conformational analysis of H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) swollen in methylene chloride revealed that the peptide chains had various conformations depending on the chain length: n = 3, essentially an unordered structure; n = 6 and 9, mainly the β-sheet structure; and n = 15, predominantly the α-helical structure. The formation of the β-sheet structure indicates that cross-linked resin-bound peptides in the swollen state can easily interact with each other through intermolecular hydrogen bonds even at a low loading of peptide chains and that the intraresin site separation of the peptide chains is not achieved even when the peptide chain length is not so long. Effects of solvent polarity on conformational transformations of H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) were also examined. Cross-linking effects on the conformational properties of cross-linked H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) were further investigated by comparison with the conformational properties of soluble H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 20).

The conformations of monodisperse oligopeptides (including homooligoleucines) in solution[2-4] and in the solid state[2,5-8] have been extensively studied and various fruitful results have been obtained. The conformational properties of poly(ethylene glycol)-bound oligopeptides have also been examined in solution by circular dichroism, nuclear magnetic resonance, and infrared (IR) spectroscopy and in the solid state by IR spectroscopy.[9,10] However, very few investigations[11] have been reported of the conformational analysis of cross-linked polystyrene resin bound oligopeptides in the swollen state although it is of great interest in connection with solid-phase peptide synthesis.

Since the introduction of solid-phase peptide synthesis in 1963,[12] this new synthetic method has been successfully applied to peptides of increasing size.[13] However, physicochemical properties of peptide-containing cross-linked resins have been little investigated. Solvation and swelling properties of peptide-containing cross-linked polystyrenes were recently studied by Sarin et al.[14] In the investigation, it was concluded that the space availability for peptide chain growth within swollen resin beads is not a limiting factor in solid-phase peptide synthesis. However, this conclusion would only be applied for the special cases that intermolecular hydrogen bonds within swollen resin beads are broken in favor of solvent–peptide hydrogen bonds. In fact, the model peptides used in the study[14] were assembled with the repeating segments having the sequence Leu-Ala-Gly-Val-(oxymethyl)phenylacetyl. The small segments separated by the ester linkages would be efficiently solvated in polar solvents such as methylene chloride and *N*,*N*-dimethylformamide, which were used in the study, and have a randomly coiled structure within swollen resin beads.

Recently we introduced the methodological and conformational concept of "peptide segment separation" by the insertion of tertiary peptide bonds into a peptide chain at suitable intervals[15-18] and showed its usefulness for synthesis, purification, and homogeneity assessment of protected large peptides and proteins by preparing a series of sequential peptides having the following general formula: Boc-Leu$_3$-(Pro$_2$-Gly-Leu$_3$)$_n$-Pro$_2$-Gly-OBzl (n = 0, 1, 3, 5, 7, 9, and 11).[16] The small peptide segment having the sequence Pro$_2$-Gly-Leu$_3$ indeed had a randomly coiled structure in favor of solvent–chain hydrogen bonds in polar solvents,[16] and peptide molecules assembled with the small peptide segments had excellent solubility and high reactivity in the usual polar solvents used for peptide synthesis.

In a previous paper,[19] we reported the solid-state conformations of cross-linked polystyrene resin bound oligoleucines. Here, we report conformations of cross-linked resin-bound oligoleucines in the swollen state and present convincing evidence that intraresin site separation of peptide chains is not achieved even in the swollen state by virtue of aggregation through intermolecular hydrogen bonds.

Experimental Section

Materials. Copoly(styrene–1% divinylbenzene) beads of 200–400 mesh, Bio-Beads S-X1, were purchased from Bio-Rad Laboratories. Soluble and cross-linked resin-bound test oligoleucines having the general formula H-Leu$_n$-Phe-resins [n = 3, 6, 9, and 15 (except the soluble one)] were those prepared in the previous paper,[19] in which the step-by-step couplings of Boc-Leu$_3$-OH were performed on soluble and cross-linked H-Phe-((*p*-(oxymethyl)phenyl)acetamido)methylated polystyrene using dicyclohexylcarbodiimide (DCC) as a coupling reagent in the presence of 1-hydroxy-1*H*-benzotriazole (HOBt). The reaction using DCC and HOBt as coupling reagents has been well-known to be nearly free from racemization.[20] Amino acid analyses of acid hydrolysates of resin-bound oligoleucines showed that the mole ratios of the component amino acid were close to the values calculated [ratios of Leu/Phe: 3.0 (n = 3), 6.0 (n = 6), 8.9 (n = 9), and 13.4 (n = 15) for cross-linked resin-bound oligoleucines; 3.1 (n = 3), 5.9 (n = 6), and 8.6 (n = 9) for soluble resin-bound oligoleucines]. Soluble H-Leu$_{20}$-Phe-resin was prepared in a similar manner[19] using Boc-Leu$_5$-OH as a carboxyl component (amino acid ratio of Leu/Phe: 17.7).

IR Measurements. The IR absorption spectra of the cross-linked H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) and the soluble H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 20) were measured with a Jasco Model DS-701G spectrometer. IR measurements of the resins in the swollen state were performed by holding the samples between sodium chloride windows after these were swollen in solvents overnight. Those of the soluble H-Leu$_n$-Phe-resins in solution were recorded by employing 0.10-mm path length cells with potassium bromide windows. The concentration of the solution was kept near 50 mg/mL.

Results and Discussion

Oligoleucines Bound to Cross-Linked Resins and Soluble Polymers. The copoly(styrene–1% divinylbenzene) resin support (Bio-Beads S-X1), used as a

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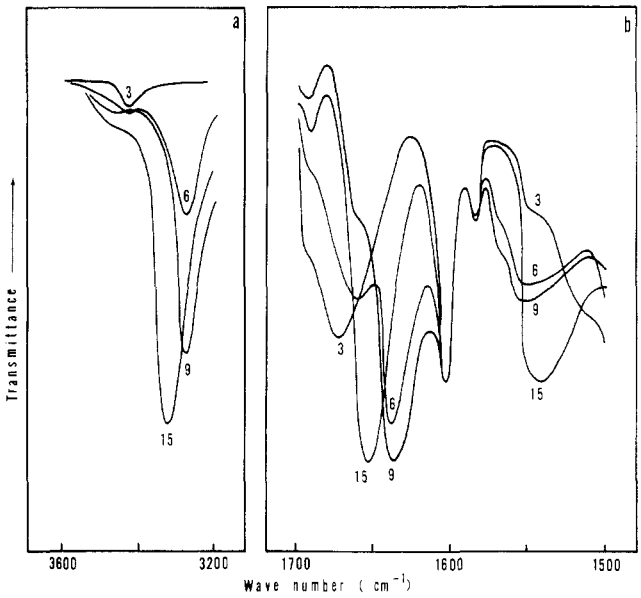

Figure 1. IR absorption spectra in the amide A region (a) and in the amide I and amide II regions (b) of the cross-linked H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) swollen in methylene chloride. The absorption bands at 1603 and 1585 cm^{-1} are due to aromatic rings of the polystyrene supports.

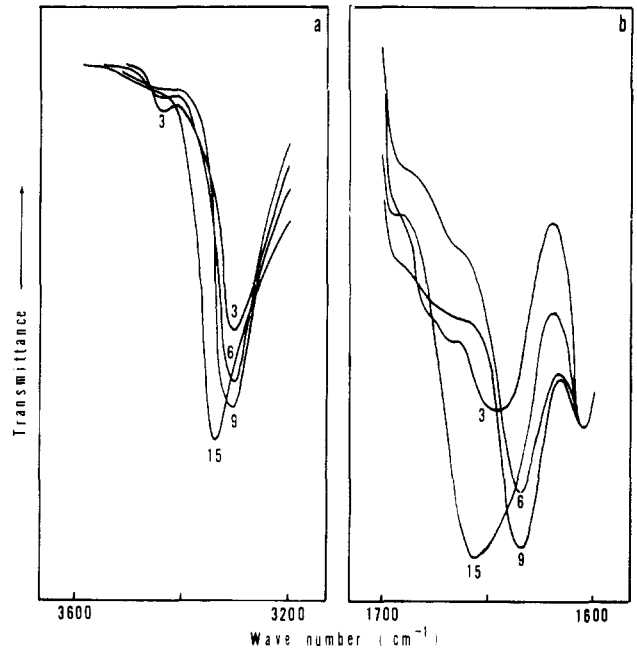

Figure 2. IR absorption spectra in the amide A region (a) and in the amide I region (b) of the cross-linked H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) swollen in carbon tetrachloride. For assignment of the absorption band at 1603 cm^{-1}, see Figure 1.

starting resin bead, has commonly been used for solid-phase peptide synthesis, and the aminomethylated form is expected to have a uniform distribution of aminoethyl groups, based on the reaction mechanism of aminomethylation.[21] Soluble aminomethylated polystyrene was prepared by the copolymerization of styrene and a mixture of m- and p-(pthalimidomethyl)styrene, followed by hydrazinolysis.[22] The aminomethyl groups are also thought to be randomly distributed on the basis of the copolymerization curve. Attachment of the first residue, Phe, of the peptide chain to the resin support was through a ((*p*-(hydroxymethyl)phenyl)acetamido)methyl group,[21] and Phe was used as an internal marker. The Phe contents were 81 μmol/g of resin (ca. 1 mol % per styrene unit) for the cross-linked H-Phe-resin and 104 μmol/g of resin for the soluble H-Phe-resin, respectively, according to amino acid analyses of the acid hydrolysates of the resins.[19] These initial Phe contents were selected in order to get rational information for peptide chain interactions in the conventional solid-phase method.

Conformational Analysis of the Cross-Linked Resin-Bound Oligoleucines Swollen in Methylene Chloride. As a typical example, the IR absorption spectra of the resin-bound oligoleucines swollen in methylene chloride, a common solvent in conventional solid-phase peptide synthesis, are presented in Figure 1 in the most significant spectral regions for conformational assignments (3600–3200 cm^{-1}, amide A; 1700–1600 cm^{-1}, amide I; 1600–1500 cm^{-1}, amide II). The results indicate the following: (1) The H-Leu$_3$-Phe-resin exhibits an essentially unordered conformation, characterized by appearance of a weak band at 3427 cm^{-1} and a strong band at 1684 cm^{-1}.[4,23] (2) The conformational preference within H-Leu$_n$-Phe-resins (n = 6 and 9) is clearly identified to be the β-sheet structure, characterized by the appearance of medium and strong bands at 3272 and 1635 cm^{-1}, respectively, accompanied with a weak or medium band at 1660 cm^{-1}, which is assigned to an unordered conformation.[4,23] (3) The H-Leu$_{15}$-Phe-resin forms the thermodynamically favorable α-helical structure, assigned by appearance of medium and strong bands at 3323 and 1653 cm^{-1}, respectively, and disappearance of bands at 3272 and 1635 cm^{-1}.[4,23] These results in the swollen state were the same as those obtained in the solid state.[19] The resin samples for measurements in the solid state were prepared by soaking in methylene chloride overnight, filtering, and drying under vacuum at 45 °C. The formation of the β-sheet structure on the H-Leu$_n$-Phe-resins (n = 6 and 9) signifies that resin-bound oligoleucines in the swollen state can interact with each other through intermolecular hydrogen bonds as easily as oligoleucines in the solid state free from a macromolecular C-terminal protecting group.[15] The intermolecular hydrogen bonding of resin-bound oligopeptides should result in additional cross-linking and influence the swelling and reactivity of terminal amino group in solid-phase peptide synthesis. In fact, it has recently been reported that internal aggregation of peptide chains bound to cross-linked polyamide supports, due to hydrophobicity of side-chain protecting groups, results in additional cross-linking and reduces accessibility to reagents and solvents of the reactive groups within the resin matrix.[24]

Solvent Effects on Conformational Transformations of Cross-Linked Resin-Bound Oligoleucines in the Swollen State. In order to investigate the influence of solvent polarity on intraresin aggregation of the peptide chains, IR spectroscopy was performed on the cross-linked H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) swollen in carbon tetrachloride, benzene, toluene, chloroform, dioxane, tetrahydrofuran, and hexamethylphosphoramide. The cross-linked resins had swollen sufficiently in these solvents. As a typical example for measurements in the nonpolar solvents carbon tetrachloride, benzene, and toluene, the IR absorption spectra of the resin-bound oligoleucines swollen in carbon tetrachloride are illustrated in Figure 2 in the most significant spectral regions for conformational assignments (3600–3200 cm^{-1}, amide A; 1700–1600 cm^{-1}, amide I). Here, the result of the H-Leu$_3$-Phe-resin was found to be noticeable in comparison with that of the resin obtained in methylene chloride. The H-Leu$_3$-Phe-resin swollen in carbon tetrachloride formed a β-like structure, in which peptide chains tie loosely through intermolecular hydrogen bonds,[4] characterized by

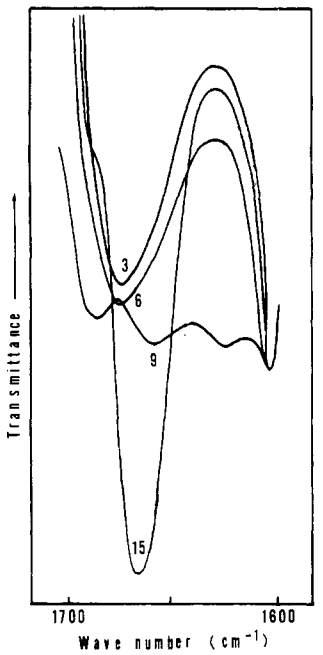

Figure 3. IR absorption spectra in the amide I region of the cross-linked H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) swollen in hexamethylphosphoramide. For assignment of the absorption band at 1603 cm^{-1}, see Figure 1.

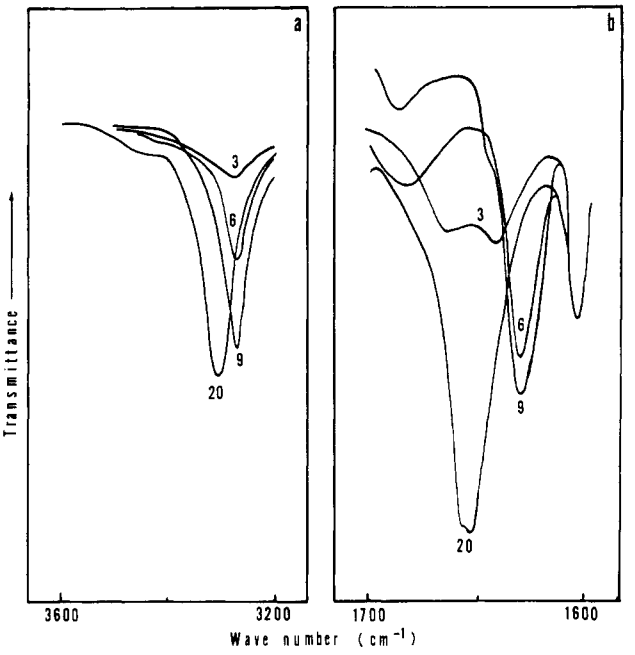

Figure 4. IR absorption spectra in the amide A (a) and in the amide I region (b) of the soluble H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 20) in carbon tetrachloride. For assignment of the absorption bands at 1603 cm^{-1}, see Figure 1.

the appearance of a sharp band at 3290 cm^{-1} and a strong band at 1640 cm^{-1}. The conformational behavior of the H-Leu$_3$-Phe-resin swollen in benzene and toluene was essentially the same as that observed in carbon tetrachloride. These results indicate that intraresin site separation of the peptide chains in the nonpolar solvents is not achieved even when the peptide chain is short and its loading on the resin is very sparse (ca. 1 mol % per styrene unit). In the IR spectra of H-Leu$_n$-Phe-resins (n = 6 and 9) swollen in the nonpolar solvents, the IR absorption band at 1660 cm^{-1}, which is assigned to an unordered structure,[4,23] was weaker than that observed in methylene chloride, indicating that the intermolecular hydrogen bonding within H-Leu$_n$-Phe-resins (n = 6 and 9) is more favorable in inert solvents than in methylene chloride. The conformational behavior of H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) swollen in the medium proton-donor solvent chloroform and the medium proton-acceptor solvents tetrahydrofuran and dioxane was essentially the same as that observed in methylene chloride.

A strong proton-acceptor or -donor solvent having high polarity is thought to lead to disruption of the intermolecular hydrogen bonding, which is of practical importance in solid-phase peptide synthesis by fragment condensation. Hexamethylphosphoramide has solvation properties for both cross-linked polystyrene and pendant peptide chains, and no abosorption band is observed in the amide A (3400–3200 cm^{-1}) and amide I regions. It is, therefore, of great interest to examine the conformational behavior of resin-bound oligoleucines in hexamethylphosphoramide. IR absorption spectra of H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) swollen in hexamethylphosphoramide are presented in Figure 3 in the amide I region. In comparison with the results of H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 15) swollen in carbon tetrachloride and methylene chloride, remarkable conformational changes were observed for the samples of H-Leu$_n$-resins (n = 6 and 9). Conformational preference of H-Leu$_6$-Phe-resin was clearly identified to be an unordered structure (1674 cm^{-1}, amide I). For the H-Leu$_9$-Phe-resin, the structures are probably unordered (1684 cm^{-1}, amide I), unordered and/or α-helical (1657 cm^{-1}, amide I), and β-sheet (1633 cm^{-1}, amide I). The H-Leu$_{15}$-Phe-resin swollen in hexamethylphosphoramide as well as other solvents showed α-helical structure (1660 cm^{-1}, amide I). These results mean that in hexamethylphosphoramide the intermolecular hydrogen bonding of the hexapeptide is efficiently destroyed by solvation of the pendant peptide chains, while for the nonapeptide, an equilibrium among unordered, α-helical, and β-sheet structures exists; for the pentadecapeptide, intramolecular hydrogen bonding stabilizes the α-helical structure.

Conformational Analysis of the Soluble Resin-Bound Oligoleucines in a Variety of Organic Solvents. In order to investigate the effect of cross-linking on the conformational properties of resin-bound peptides, IR measurements of soluble H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 20) were performed in solution or in the swollen state. IR absorption spectra of typical soluble polymer-bound oligoleucines in carbon tetrachloride and methylene chloride are shown in Figures 4 and 5, respectively, in the amide A and amide I regions. Comparison of the absorption bands at 3290 and 1646 cm^{-1} for soluble H-Leu$_3$-Phe-polymer with those of the cross-linked analogue shows that the amount of the β-like structure is much more within cross-linked H-Leu$_3$-Phe-resin than for the soluble version. These results indicate that cross-linking has a concentrating effect in the nonpolar solvents. The conformational behavior of the other soluble polymer-bound oligoleucines in a variety of organic solvents such as carbon tetrachloride, benzene, methylene chloride, chloroform, and tetrahydrofuran were essentially the same as that of the swollen cross-linked materials. On the other hand, in hexamethylphosphoramide, which has the highest polarity of the solvents examined, significant conformational change was observed for H-Leu$_9$-Phe-resin. IR absorption spectra of H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 20) in hexamethylphosphoramide are illustrated in Figure 6 in the amide I region together with the amide A region for H-Leu$_{20}$-Phe-resin. The conformational behaviors of the soluble H-Leu$_n$-Phe-polymers (n = 3, 6, and 20) were essentially the same as those of the corresponding resin-bound oligoleucines, respectively, and intermolecular hy-

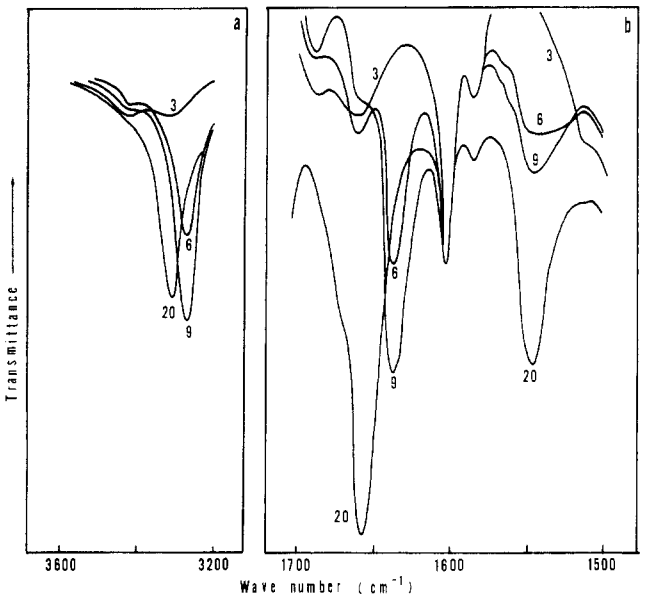

Figure 5. IR absorption spectra in the amide A (a) and amide I and amide II regions (b) of the soluble H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 20) in methylene chloride. For assignment of the absorption bands at 1603 and 1585 cm^{-1}, see Figure 1.

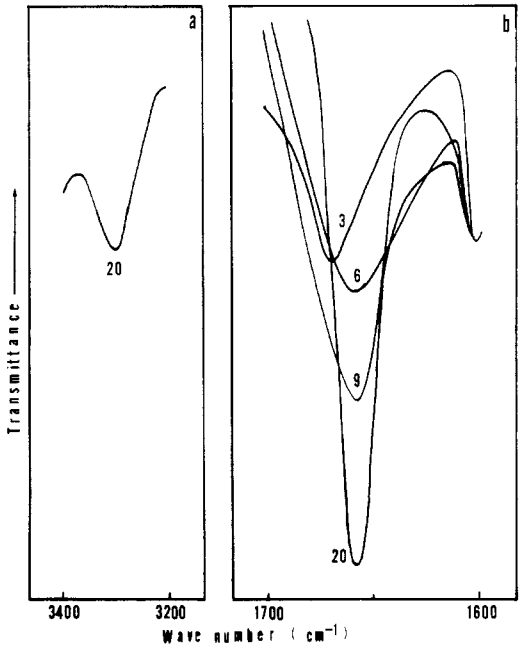

Figure 6. IR absorption spectra in the amide A (a) (n = 20) and in the amide I region (b) of the soluble H-Leu$_n$-Phe-resins (n = 3, 6, 9, and 20) in hexamethylphosphoramide. For assignment of the absorption band at 1603 cm^{-1}, see Figure 1.

drogen bonds were broken in favor of solvent–chain hydrogen bonds (n = 3 and 6) or in favor of intramolecular hydrogen bonds (n = 20). Meanwhile, the Leu$_9$ segment of the soluble H-Leu$_9$-Phe-resin was more efficiently solvated than that of the cross-linked H-Leu$_9$-Phe-resin.

In conclusion, oligoleucines bound to soluble polymers or to swollen cross-linked resins exhibit unordered, β-sheet, or α-helical structures depending on the peptide chain length and solvent polarity. The formation of the β-sheet structure indicates that oligoleucines bound to a swollen cross-linked resin can easily interact with each other through intermolecular hydrogen bonds even at low loading of peptide chains (ca. 1 mol % per styrene unit). Comparison of soluble polymer-bound oligoleucines (n = 3 and 9) with their cross-linked counterparts, in carbon tetrachloride and hexamethylphosphoramide, shows that cross-linking has no hyperentropic efficacy but a concentrating efficacy. This is in contrast to the assumption that the high dilution principle would be achieved in solid-phase organic syntheses.[25]

References and Notes

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