is not necessarily a significant factor in the ground-state structures[14] of other dilithiobiaryls in which the parent hydrocarbon has an appreciable barrier to internal rotation. One important, practical consequence is that many dilithiobiaryls will be obtainable in optically active form and can serve as novel intermediates in the synthesis of chiral, bidentate ligands.[8]

The existence of a substantial rotational barrier for 2,2′-dilithio-1,1′-binaphthyl also has significant mechanistic and theoretical applications, since the relative energies of the symmetrical bridged structure (i.e., the transition state for internal rotation) and the unsymmetrical ground-state structure can be studied by simple kinetic techniques as a function of solvent, coordinating ligands, metal, and biaryl. Such measurements would be of interest in elucidating the relative importance of electrostatic[15] and covalent interactions[1-3,16] to the bonding, structure, and reactivity of bridged metal-carbon compounds.

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Registry No. 2,2′-Dilithio-1,1′-binaphthyl, 92218-53-6.

(14) The fact that dilithiobinaphthyl can be obtained in optically active form rules out the symmetrically bridged planar structure III as the ground state. The actual ground-state structure is unknown at present and could be similar to the "classical" structure IIc, where each lithium atom replaces one of the hydrogens at the 2,2′ positions, and the aryl rings are rotated out of planarity to some degree (e.g., ~90°). An alternative is a distorted bridge structure where each lithium spans the 2,2′ positions in unsymmetrical fashion, which may also allow twisting of the naphthalene rings with respect to each other. Other structures can also be imagined. The calculations by Nengebauer, Kos, and Schleyer[2] estimate the bridging stabilization energy (16.8 kcal) from the energy difference between the twisted, classical structure IIc (C_2 symmetry) and III (C_{2v} symmetry). Even though the MNDO calculations place the C_2 structure of dilithiobiphenyl at an energy minimum, it is possible that the ground state of dilithiobinaphthyl may not correspond exactly to the classical, twisted structure (C_2 symmetry) and may be shifted toward an unsymmetrically bridged structure, where the naphthalene rings are twisted out of coplanarity. This possibility may alter the present estimates of the stabilization to be gained by symmetrical bridging of the two lithiums to give structure III, since the MNDO estimates and the experimental estimates may refer to different ground-state structures. However, since the steric interactions associated with rotation around the 1,1′ bond are substantially higher for dilithiobinaphthyl than for dilithiobiphenyl, and since the lithium/carbon and lithium/lithium interactions are similar (or possibly reduced in magnitude for dilithiobinaphthyl), it is likely that the energy-minimized C_2 structure for dilithiobinaphthyl exhibits a comparable (or higher) degree of asymmetry in lithium-carbon bond distances and a comparable (or higher) degree of twisting between the aryl rings compared to the analogous structure of dilithiobiphenyl.

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A General Approach to the Quantitation of Synthetic Efficiency in Solid-Phase Peptide Synthesis as a Function of Chain Length[⊥]

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Abstract: A model peptide, Leu-Ala-Gly-Val, was synthesized by solid-phase methods at increasing distances from a 1% cross-linked polystyrene resin support. The efficiency of the synthesis was evaluated by quantitatively measuring the amounts of the deletion peptides Leu-Ala-Val and Leu-Gly-Val that were produced during the synthesis of the tetrapeptide. By inserting an oxymethylphenylacetyl group between this test peptide and the peptide chains used to provide spacers from the support, it was possible to selectively evaluate the quality of the tetrapeptide without interference by the spacer. Low and constant levels of deletion peptides were found. No significant effect of distance from the support or of peptide loading on the synthetic efficiency could be detected up to a chain length of 60 residues and a peptide-to-resin weight ratio of 4:1.

The essential feature of solid-phase peptide synthesis[1] is the covalent attachment of the growing peptide chain to an insoluble solid support during the course of the chain assembly. This has obvious advantages which include rapid purification of the product peptide at each step by simple filtration and washing and low handling losses. However, since the early days of the solid-phase method it has frequently been assumed that a price is paid for this speed and simplicity in the form of a negative influence of the insoluble resin support on reaction kinetics, leading to incomplete reaction at each step, with the consequent accumulation of resin-bound peptide byproducts and generation of a complex product mixture after cleavage from the resin.[2-4] In particular, there has been a general feeling that there must be resin-imposed steric limitations to stepwise solid-phase peptide synthesis.[2] Some workers feel that reactions will be less efficient close to the polymer backbone,[3] while others feel that there will be significant declines in yields as the peptide is elongated, due to temporary steric occlusion of peptide chains within the polymer network.[4]

To resolve such conflicting views, we have determined experimentally the efficiency of solid-phase synthesis as a function of peptide chain length. Previous attempts to address this question have either been indirect or poorly defined and controlled. We report here a general experimental approach that allows direct

⊥ This paper is dedicated to the memory of our friend and colleague, Dr. Balz Gisin.
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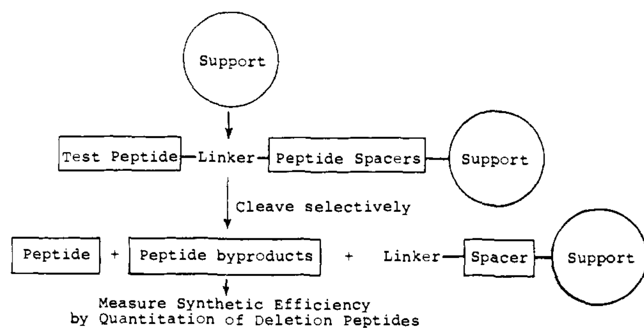

Figure 1. General scheme for measuring synthetic efficiency as a function of distance from the solid support.

testing of the synthetic efficiency.

The General Approach

The design of the experiments involved the synthesis of a well-characterized model peptide held at increasing distances from the solid support by intervening peptide chains and the determination of the efficiency of each synthesis by measuring the amount of deletion peptide byproducts arising from incomplete reactions during the assembly of the test peptide, as outlined in Figure 1. Thus, the same peptide is examined each time, but it is synthesized at different distances from the support by increasing the length of the spacer. Furthermore, the end of the spacer to which the test peptide is attached is the same each time. The key to this approach was the linker holding the test peptide to the spacer peptide. It must be designed so that the byproducts of the test peptide can be selectively detected in the presence of products from the spacer peptide. Two specific versions of this quite general approach have been examined: one in which the peptide spacer was released from the support as a series of blocked tetrapeptides upon cleavage by HF and another in which the spacer peptides were not released.

The model peptide selected for this study was Leu-Ala-Gly-Val (LAGV)[5] because a good chromatographic system has been worked out for the separation of all the possible delection and termination peptides from the parent peptide.[6] Each of these peptides can be quantitated at the 0.1% level in the presence of 99.9% of LAGV.

Series I. Assembly of the Test Peptide onto Resin-Bound Spacers Made by Stepwise Synthesis of Leu-Ala-Gly-Val-(oxymethyl)-phenylacetic Acid Units. The peptides were synthesized on aminomethylcopoly(styrene-1%–divinylbenzene) resin support[7] (0.95 mmol/g for series IA and 0.22 mmol/g for series IB). The C-terminal valine residue of the peptide was first converted to (*tert*-butyloxycarbonyl)valyl(oxymethyl)phenylacetic acid (Boc-Val-OMPA)[8] and then coupled to the derivatized resin to give Boc-valyl(oxymethyl)*p*henyl*a*cetamido*m*ethyl-resin (Boc-Val-OCH_2-Pam-resin)[9]. The subsequent amino acids were added stepwise by normal dicyclohexylcarbodiimide activation to yield Boc-Leu-Ala-Gly-Val-OCH_2-Pam-resin. Additional units of LAGV-OMPA were then assembled by the normal stepwise synthesis to produce Boc-(LAGV-OMPA)$_n$-$NHCH_2$-resin (n = 1–12) as shown in Figure 2.

The important feature of this experimental design was the OMPA group. The benzyl ester bond anchoring the peptide to the oxymethylphenylacetamidomethyl-resin is very stable to prolonged treatment with TFA and almost no loss of chains occurs during the assembly of a long peptide chain. However, this ester can be cleaved by treatment with HF at 0 °C under conditions

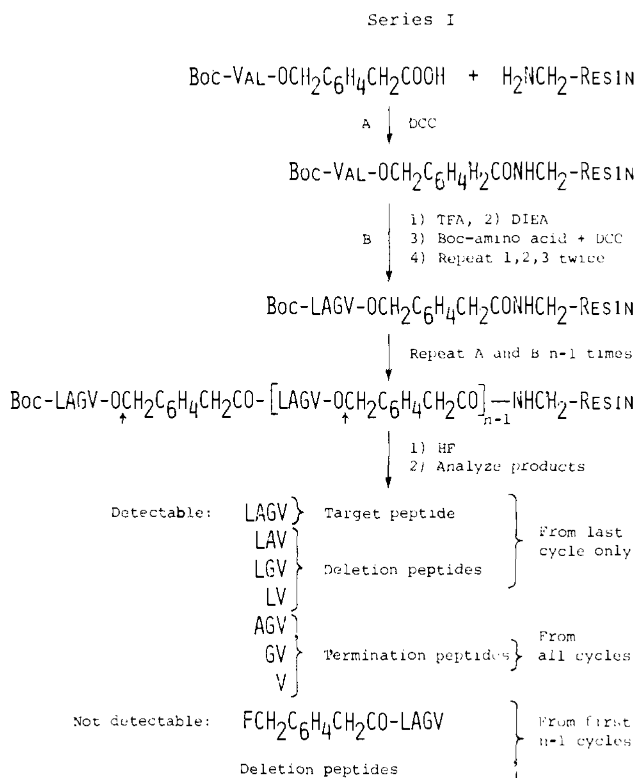

Figure 2. Specific scheme for measuring synthetic efficiency as a function of distance from the solid support—series I peptides.

where the amide bond between OMPA and the aminomethyl-resin or the α-amino group of a peptide chain is completely stable. Therefore, the HF cleavage of Boc-(LAGV-OMPA)$_n$-$NHCH_2$-resin will give rise to free LAGV and its deletion peptides arising from the *last* tetrapeptide unit, but not from the earlier internal units. The latter will be blocked by the $FCH_2C_6H_4CH_2CO$ group and will not be detected in the product analysis. Thus, the last tetrapeptide is our test peptide, and all the earlier tetrapeptide units serve as spacers to hold the test peptide away from the polymer support. In this way the same peptide is assembled at ever-increasing distances from the resin backbone, and the product distribution can be determined by direct analysis at each stage. For estimating synthetic efficiency the single deletion peptides were most useful, and of these LGV and LAV were preferred. They were obtained only from the last tetrapeptide (the test peptide). Termination peptides are also produced but because they will be obtained from all tetrapeptide cycles of this series, they were not used as a measure of synthetic efficiency.

It was known from earlier work that if the coupling reactions are forced toward completion by high concentrations of reactants and by multiple coupling, the levels of deletion peptides can be reduced to very low values (<0.05%). Therefore, in order to have sufficient amounts to give satisfactory comparative data, nonforcing conditions were used. Only single couplings for 1 h with 0.08 *M* Boc-amino acids were used for n = 1–6, and from n = 7 to 12 the couplings were carried out in a 4 times more dilute solution (0.02 *M*) in order to slow the reaction rate and to exagerate the amount of deletion peptides even more.

Series II. Assembly of the Test Peptide onto Resin-Bound Spacers Made by Fragment Condensation of the Tetrapeptide Boc-Leu-Ala-Gly-Val-OH. An alternative approach to study the variation of synthetic efficiency with distance was to assemble spacers of different desired lengths that were composed only of amino acid residues and then to build the model test peptide LAGV-OMPA as shown in Figure 3. This approach had the advantage that it avoided the intervening oxymethylphenylacetyl unit in the peptide backbone of the spacers and therefore did not disrupt the normal conformation of these peptide chains. Also, the use of Boc-LAGV-OH to build the peptide chain by fragment condensation reduced the number of synthetic steps involved in

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Table I. Analyses of Boc(LAGV-OMPA)$_m$-(LAGV)$_n$-F-NHCH$_2$-Resins

peptide-resin	initial NH_2CH_2 substitution, mmol/g	amino acid analysis (mmol/g of peptide-resin)					peptide content, %	
		Leu	Ala	Gly	Val	Phe	found[a]	calcd[b]
Boc-(LAGV)$_1$-F-NHCH$_2$-R	0.18	0.17	0.17	0.18	0.17	0.17	10.0	9.5
Boc-(LAGV)$_2$-F-NHCH$_2$-R	0.18	0.30	0.29	0.31	0.29	0.15	13.9	13.9
Boc-(LAGV)$_5$-F-NHCH$_2$-R	0.18	0.64	0.64	0.63	0.65	0.12	25.1	26.3
Boc-LAGV-OMPA-(LAGV)$_5$-F-NHCH$_2$-R	0.18	0.69	0.68	0.66	0.72	0.12	28.7	29.5

[a] Average amino acid analysis (mmol/g of peptide-resin = $^1/_2([\{(\text{Leu} + \text{Ala} + \text{Gly} + \text{Val})/4\}/(m + n)] + \text{Phe})$. [b] Peptide content found = average amino acid analysis × M wt × 100.

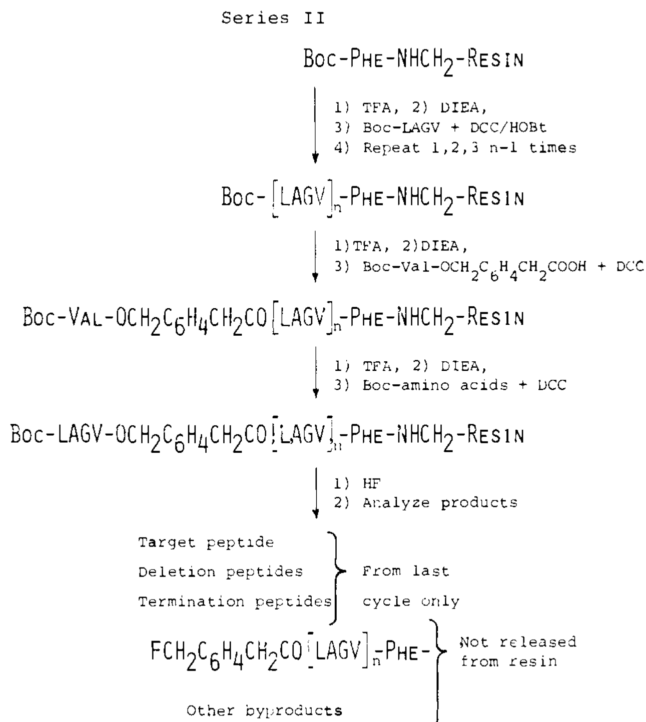

Figure 3. Specific scheme for measuring synthetic efficiency as a function of distance from the solid support—series II peptides.

assembling peptide chains of different lengths on the polymeric supports. The spacers were held by an acid-stable bond and were not released during the HF cleavage reaction. Detectable termination peptides were therefore restricted to the final LAGV-OMPA test peptide. A C-terminal phenylalanine residue was introduced by attaching it directly to aminomethyl-resin. This amino acid aided in the assessment of the length and purity of peptides assembled on the support. Boc-(LAGV)$_n$-F-NHCH$_2$-resins, $n = 0$ and 5, were prepared as follows. Boc-Phe was coupled to aminomethyl-copoly(styrene-1%-divinylbenzene)-resin beads by normal DCC activation to yield Boc-Phe-NHCH$_2$-resin. The N$^\alpha$-protecting group was removed and the tetrapeptide fragment, Boc-LAGV-OH, was coupled to the resin with use of the DCC/HOBt reaction. Deprotection and fragment coupling of Boc-LAGV-OH was continued until the desired peptide-resin Boc-(LAGV)$_5$-F-NHCH$_2$-resin was obtained. All coupling reactions were monitored to completion by ninhydrin analysis.[10] Coupling reactions were carried out with use of 2.5 times excess of Boc-LAGV-OH and 2.5 equiv each of DCC and HOBt in CH_2Cl_2/DMF.

After addition of 0 or 5 Boc-LAGV-OH units, a 100-mg sample of peptide-resin was taken out, the N$^\alpha$-protecting group was removed, and Boc-Val-OMPA was added with the standard DCC coupling procedure. Then, the model test peptide LAGV was completed by standard stepwise synthesis by addition of Gly, Ala, and Leu with use of 0.08 M Boc-amino acids (Figure 3).

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Results

Analyses of Peptide Resins. The peptide content of the series I peptide-resins was determined by amino acid analysis of acid hydrolysates. The data[11] showed that the ratios of the component amino acids were very close to unity at every stage of the synthesis. The average deviation was ±4%, which is within the limits of the automated ninhydrin analysis. The agreement between the duplicate analysis of separate hydrolysates was also within this limit. Therefore, the amino acid analysis is a good representation of the amount of peptide present and hence the length of the growing peptide chain at each stage of the peptide synthesis. There was a discrepancy of about 4% between the percentage of peptide found experimentally by amino acid analysis and that expected from the initial substitution and the number of LAGV-OMPA units added. This is primarily due to a low level of termination at each step of synthesis, which was confirmed for the $n = 6$ sample by picric acid titration[12] and by coupling Boc-Phe to an aliquot of the peptide-resin and determining the amount of Phe per gram of polystyrene.

The tetrapeptide Boc-LAGV-OH needed for the preparation of the series II peptide-resins was synthesized by using classical solution chemistry. It was recrystallized from ethyl acetate/petroleum ether and found to be homogenous on TLC by using two different solvent systems and by elemental analysis. A small amount of the tetrapeptide was deprotected and analyzed on an ion-exchange column under highly overloading conditions.[6] No traces of peptide impurities (<0.1%) could be detected.

The data in Table I show that the ratios of the component amino acids were close to unity at each stage of the synthesis. The average deviation between the calculated and found peptide content was ±3%. Solid-phase preview analysis by Edman degradation was used to estimate the length and quality of the peptide in H-(LAGV)$_5$-F-NHCH$_2$-resin.[13] Since this sample was prepared by a series of fragment condensations of the pure tetrapeptide unit, Boc-LAGV-OH, any deletion peptides would be shorter by a multiple of 4 amino acid residues. The premature appearance of Phe was an indication of a tetrapeptide deletion, and the ratio of PTH-Phe to PTH-Leu gave a quantitative measure of the effect. The N$^\alpha$-Boc protecting group was removed from the Boc-(LAGV)$_5$-F-NHCH$_2$-resin, which was then subjected to 21 cycles of standard solid-phase Edman degradation. Eluates from cycles 1, 5, 9, 13, 17, and 21 were analyzed for PTH-amino acids by HPLC. PTH-Phe was present at cycle 21 as it should be, but no PTH-phenylalanine ($\ll$1%) was detected in the earlier cycles, demonstrating that there were no significant amounts of deletion peptides in the peptide-resin Boc-(LAGV)$_5$-F-NHCH$_2$-resin sample.

Quantitative Analysis of HF-Treated Peptide Resins. After assembly of each test peptide a 50-mg sample of peptide-resin was washed with CH_2Cl_2, HOAc:CH_2Cl_2 (1:1), HOAc, 2-propanol, and CH_2Cl_2 and dried in vacuo. It was then treated with HF

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Table II. Quantitative Analysis of HF-Treated Boc-(LAGV-OMPA)$_n$-NHCH$_2$-Resins

series	initial substitution, mmol/g	coupling concn, M	n	deletion peptides, %		
				LAV	LGV	av
IA	0.95	0.08	1	0.1	0.1	0.1
			2	0.16	0.29	0.22
			3	0.27	0.27	0.27
			4	0.35	0.49	0.42
			5	0.11	0.28	0.19
			6	0.32	0.33	0.32
						av 0.25 ± 0.09
IA	0.95	0.02	7	0.93	1.00	0.96
			8	0.60	0.84	0.85
			9	0.36	1.68	1.02
			10	0.59	1.00	0.79
			11	0.78	0.70	0.74
			12	0.92	1.18	1.05
						av 0.89 ± 0.24
IB	0.22	0.08	1	0.35	0.19	0.27
			2	0.20	0.26	0.23
			3	0.45	0.67	0.56
			4	0.34	0.46	0.40
			5	0.47	0.57	0.50
			6	0.45	0.49	0.47
						av 0.41 ± 0.11
IB	0.22	0.08[b]	1	<0.10	<0.05	<0.05

[a] % = {(deletion of peptide (M))/(LAGV (M) + total deletion of peptides (M))}100. [b] Coupled twice under the same conditions.

Table III. Quantitative Analysis of HF-Treated Boc-LAGV-OMPA-[LAGV]$_n$-F-NHCH$_2$-Resin

series	initial substitution, mmol/g	n	deletion peptides, %		
			LAV	LGV	av
II	0.18	0	0.31	0.15	0.23
	0.18	5	0.13	0.57	0.35

containing 10% anisole at 0 °C for 60 min. After evaporation at 0 °C, the resin was washed with ether to remove anisole, and then the liberated peptide was extracted by washing with 4 × 5 mL of 10% HOAc and 3 × 5 mL of 20% HOAc. The aqueous washes were combined and evaporated to dryness. The crude tetrapeptide and all deletion and termination peptides were separated on an ion-exchange column and quantitated by the ninhydrin reaction. Note that the internal tetrapeptide spacer units of series I gave FCH$_2$-C$_6$H$_4$-CH$_2$CO-LAGV-OH, without a free amino group, and were not detected, while those of series II were not released from the resin. Therefore, the analyses were limited to the terminal test peptide. The results of such analyses for Boc-(LAGV-OMPA)$_n$-NHCH$_2$-resins IA$_{1-12}$ and IB$_{1-6}$ are shown in Table II and Figures 4 and 5. Data for Boc-LAGV-OMPA-(LAGV)$_n$-F-NHCH$_2$-resins II$_{0,5}$ are shown in Table III.

The deletion peptides LAV and LGV obtained in series IA, n = 1–6, averaged 0.22% and 0.29%, respectively, with an overall average of 0.25 ± 0.09%. Because of the very low levels being measured, a variability of 0.1% is not unreasonably large. The lower limit of detection was about 0.05%. Thus, the two diagnostic deletion peptides were produced in essentially equal amounts in the terminal test peptide in each sample of the series. Except for an unusually low value for n = 1, there was no significant trend in deletion level for either peptide as the value of n increased.

The peptides IA$_{7-12}$ were purposely synthesized under more limiting conditions (0.02 M Boc-amino acid), and the data of Table II show a corresponding increase in the levels of deletion peptides to 0.89 ± 0.24%. There also was a marginally greater deletion of Ala than Gly. There was, however, no significant trend for either deletion peptide to increase as the length of the peptide spacer increased from n = 7 to 12. The deletion peptides IB$_{1-6}$ averaged 0.41 ± 0.11%, and they also did not show a significant effect of chain length. In this series, substitution of peptide chains was only 20% of that in series I.

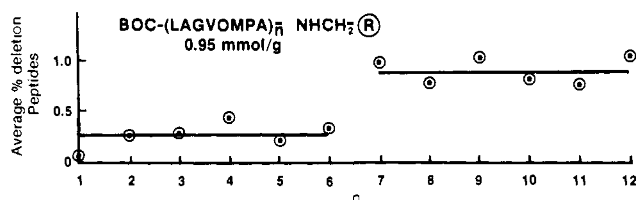

Figure 4. Deletion peptides as a function of distance from the solid support. The concentration of peptide chains was 0.95 mmol/g of resin. n = the number of LAGV-OMPA units. The average of the quantity of LAV and LGV is expressed as the mole percent of total free peptides detected. The concentration of Boc-amino acids in the coupling reaction was 0.08 M for n = 1–6 and 0.02 M for n = 7–12.

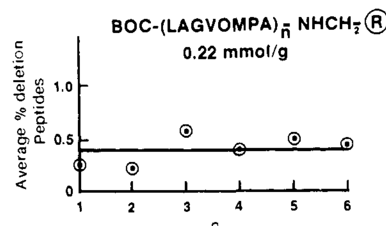

Figure 5. Deletion peptides as a function of distance from the solid support. The substitution of peptide chains was 0.22 mmol/g of resin. The concentration of Boc-amino acids was 0.08 M.

The final entry in Table II shows that when the test peptide was synthesized under optimal conditions the level of deletions was reduced below the detection limit of the assay method.[6]

For peptide series II, where the terminal test peptide contained the (oxymethyl)phenylacetic group but the spacer peptides did not, the results (Table III) were similar to those of series I, but they were less exact. The level of LGV increased when n went from 0 to 5, while the level of LAV decreased. However, the averages of the deletion peptides at the two values of n were low and approximately equal within our experimental error.

Discussion

Here, we have sought to answer the question are there inherent limitations to the efficiency of stepwise solid-phase peptide synthesis resulting from the length of the peptide chain or the loading of the resin support? This was examined by measuring the ef-

ficiency of the synthesis of a well-characterized peptide, Leu-Ala-Gly-Val, at increasing distances from the resin support (Figures 2 and 3). The spacers to provide the variable distances were either [Leu-Ala-Gly-Val-(oxymethyl)phenylacetyl]$_n$ or [Leu-Ala-Gly-Val]$_n$. The test peptide was linked to the spacers by an oxymethylphenylacetyl group so that it could be released and distinguished from the peptides derived from the spacer. When the peptide-resins were cleaved with HF, the tetrapeptide and all other free peptides formed during the *last* cycle of synthesis were detectable in the quantitative product analysis on an ion-exchange column. Terminal amino groups of peptides formed in earlier synthetic cycles of series I were permanently blocked by the oxymethylphenylacetyl group, which is stable to HF, and therefore were not detected during the above analysis using the ninhydrin reaction. This was an essential requirement for studying reliably the variation of synthetic efficiency with distance from the resin.

There are three general classes of byproducts produced in stepwise solid-phase synthesis: **deletion** peptides, which arise when the peptide chain is temporarily unavailable for reaction but subsequently takes part in the synthesis again; **termination** peptides, which arise when the peptide chain permanently stops growing at some cycle of the synthesis; and **modification** peptides, which arise from a variety of chemical side reactions during chain assembly, cleavage, and workup. The deletion peptides contain both the C-terminal and N-terminal amino acids but have one or more internal residues missing. Terminated peptides consist of chains containing the correct C-terminal residues but missing residues from the N-terminus. Modified peptides may have the correct number of residues but their structures have been altered in some way.

The termination peptides are permanently removed from participation in further steps of the synthesis. They can be produced by several known mechanisms[14,15] and, following cleavage and workup, are found either with a free amino group[15] or with the terminating group still present. Neither termination nor modification peptides are helpful in evaluating the synthetic efficiency resulting from effects of the solid phase.

By synthetic efficiency we mean the extent of the synthetic cycle: the deprotection, neutralization, and coupling steps. This can be measured with high sensitivity by separating and quantitating the deletion peptides formed by omission of an amino acid residue. For example, if Gly did not couple to Val on some of the chains the tripeptide Leu-Ala-Val would result. If Ala failed to couple, Leu-Gly-Val would be present. The appearance of Ala-Gly-Val could result either from a deletion of Leu or from a temporary termination during the synthesis and, therefore, was not a suitable indicator of synthetic efficiency. Failure of Val-OMPA to couple would lead to LAGLAGV in the cleavage mixture of series I samples and, although it could be identified, it was not used as a diagnostic peptide. In series II the resulting LAG(LAGV)$_{n-1}$ was not released from the resin. The double-deletion peptide IV was less likely to form and was not detected. Therefore, the most reliable measures of synthetic efficiency were LAV and LGV, both of which could be fully separated from other peptides and readily quantitated at the 0.1% level by ion-exchange chromatography. In these experiments it was the *extent* of the reaction under a particular set of conditions that was measured, not the reaction rate.

These syntheses were purposely conducted under less than optimal conditions so that sufficient levels of deletion peptides would be present for our comparisons. Depending on the severity of the coupling conditions chosen for a particular series of experiments, the levels of deletion peptides varied from 0.25% to 0.89%, and with forcing conditions they were reduced to undetectable levels (<0.05%). The important point was whether or not the level of deletion peptides within a series of test peptides was a function of the length of the peptide spacers. The data showed that **there was no significant change in synthetic efficiency with increasing distance of the growing peptide chain from the polymeric backbone.** In the extreme case (peptide IA$_{12}$) the chain length of the spacer was 48 amino acid residues plus 12 OMPA residues. It should be noted that these experiments covered a wide range of resin loading such that the final peptide-resins contained from 11 to 81% by weight of peptide (89 to only 19% polystyrene). **Synthetic efficiency was not affected by that range of substitution.**

Any study of this kind must be based on a single or small number of model test peptides, each of which has its individual chemical and physical properties, and other test peptides may behave differently. The first spacer used here contained a series of tetrapeptide-(oxymethyl)phenylacetic acid units. It could be argued that this did not give true peptides and that the results obtained are unrepresentative. The oligomers could be expected to have conformations different from polypeptides of the same amino acid composition. On the other hand, the spacers of the second series were assembled from tetrapeptide units alone and can therefore be considered typical polypeptides. Since the synthetic efficiency as a function of distance was similar for the two series, we believe they were adequate to answer the question posed.

The principle described here is quite general, however, and this system could be readily extended to further measures of synthetic efficiency in which different spacers and different test peptides are used.

The experimental results are in accord with our recent model for solvation and swelling behavior of peptide-resins during solid-phase peptide synthesis.[11] Solvation and hence swelling of the peptide-resin is influenced by both the polystyrene backbone and the peptide chain. They exert a complementary effect on each other and thereby keep the peptide-resin solvated throughout the synthesis. It was found that there was no spatial limitation to the growth of a peptide chain in lightly cross-linked polystyrene resin beads with a very high content of peptide. In fact, the space available for the growing peptide chain on the highly loaded swollen peptide-resin was larger than the initial volume of the swollen unsubstituted resin bead. Favorable solvation and the availability of sufficient space for the growing peptide chains within a swollen peptide-resin bead explain the high synthetic efficiency observed throughout the range of peptide synthesis in this study using copoly(styrene-1%-divinylbenzene) resin supports. The observation of high synthetic efficiency even up to 60 residues and 81% peptide clearly demonstrates the lack of intrinsic limitations to stepwise solid-phase synthesis over an extreme range of peptide loading. We believe the poor synthetic results obtained in certain instances reported previously have chemical rather than resin-related physical explanations.

Experimental Section

Copoly(styrene-1%-divinylbenzene) beads 200–400 mesh were purchased from Bio-Rad Laboratories. Boc-amino acids were purchased from Chemical Dynamics. Methylene chloride was distilled over sodium carbonate. DMF was MCB spectroquality and stored over 4 Å molecular sieves. Hydrolysis of peptide-resins was carried out with 12 N HCl: propionic acid (1:1)[16] with use of norleucine as the internal standard. Ion-exchange chromatography was performed on a Beckman amino acid analyzer (Model 121). TLC was on silica gel plates in the solvent system $CHCl_3$:MeOH:HOAc (85:10:5).

Chromatographic analysis of LAGV and its deletion and termination peptides was carried out on a Beckman 120B amino acid analyzer with a 0.9 × 60 cm column of AA-15 sulfonated copoly(styrene-1%-divinylbenzene) resin beads. The buffer pH 3.49 sodium citrate (0.2 M in Na^+) pumped at 66 mL/h at 56 °C, and the ninhydrin reagent was run at 33 mL/h. Absorbance was measured at 570 nm. The peptides were compared with pure synthetic standards.

Aminomethyl-resins of desired substitution were prepared according to the published procedure of Mitchell et al.[7] Boc-valyl-4-(oxy-

(14) Kent, S. B. H.; Mitchell, A. R.; Engelhard, M.; Merrifield, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2180–2184.

(15) Kent, S. B. H.; Merrifield, R. B. *Int. J. Peptide Protein Res.* **1983**, *22*, 57–65. A principal source of termination peptides was recently shown to be due to low levels of *sec*-Boc impurities in some commercial samples of the *tert*-Boc-amino acid reagents. The group is relatively stable to TFA, but it is removed by HF and gives rise to termination peptides with free amino groups.

(16) Westall, F. C.; Scotchler, J.; Robinson, A. B. *J. Org. Chem.* **1972**, *37*, 3363–3364.

methyl)phenylacetic acid (Boc-Val-OMPA) was prepared as the free acid according to the published procedure[8] and was recrystallized from ethyl acetate/petroleum ether to give a pure white solid: yield, 76%; mp 72–74 °C. This material was coupled to aminomethyl-resin by the standard DCC coupling procedure to yield Boc-valyl-4-(oxymethyl)phenylacetamidomethyl-resin (Boc-Val-OCH_2-Pam-resin).

Synthesis of Boc-(LAGV-OMPA)$_n$*NHCH$_2$*-Resin. The following protocol was used for the synthesis of the desired peptide-resin. Boc-Val-OCH_2-Pam-resin (1 g) was placed in a reaction vessel and for the introduction of each amino acid was treated with shaking with the following reagents for the times shown, followed by filtration: (1) 20 mL of CH_2Cl_2, 3 × 1 min; (2) 20 mL of trifluoroacetic acid:CH_2Cl_2 (1:1, v/v), 1 × 1 min; (3) 20 mL of trifluoroacetic acid:CH_2Cl_2 (1:1, v/v), 1 × 20 min; (4) 20 mL of CH_2Cl_2, 6 × 1 min; (5) 20 mL of 5% diisopropylethylamine in CH_2Cl_2, 1 × 5 min; (6) 20 mL of CH_2Cl_2, 3 × 1 min; (7) 20 mL of 5% diisopropylethylamine in CH_2Cl_2, 1 × 5 min; (8) 20 mL of CH_2Cl_2, 3 × 1 min; (9) Boc-Gly-OH (4 equiv) in 15 mL of CH_2Cl_2 for 5 min, without filtration; followed by (10) DCC (4 equiv) in 5 mL of CH_2Cl_2 for 30 min; (11) 20 mL of CH_2Cl_2, 6 × 1 min. This synthetic cycle was repeated with Boc-Ala-OH and then with Boc-Leu-OH. In a double-coupling synthesis, steps 7–11 were repeated in each cycle. Successive LAGV-OMPA units were assembled by the above procedure.

Valine Methyl Ester Hydrochloride. Dry HCl gas was bubbled through a suspension of L-valine (20 g, 0.17 mol) in methanol until all the material dissolved. The solvent was removed under reduced pressure, and the material was recrystallized from alcohol: ether to yield 13.6 g (48%).

***tert*-Butyloxycarbonylglycylvaline Methyl Ester.** Valine methyl ester hydrochloride (5.03 g, 0.03 mol) and *tert*-butyloxycarbonylglycine *p*-nitrophenyl ester (8.87 g, 0.03 mol) were dissolved in 35 mL of methylene chloride and 6.47 mL (0.06 moles) of triethylamine was added. The reaction mixture was stirred overnight at room temperature, and the solvent was evaporated under reduced pressure. The residue was dissolved in 50 mL of ethyl acetate and washed with bicarbonate, 2 N HCl, and water. After the residue was dried over Na_2SO_4, the solvent was removed under reduced pressure to yield 8.25 g of the desired product. It was recrystallized from ethyl acetate and petroleum ether to give 8.05 g (93%) of the pure product (TLC R_f 0.58).

***tert*-Butyloxycarbonyl-L-alanylglycyl-L-valine Methyl Ester.** The N^α-protecting group was removed from the depeptide ester prepared above by treatment with 1 N HCl in HOAc for 30 min. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was triturated to a white solid with hexane. The glycyl-L-valine methyl ester hydrochloride (3.58 g, 0.016 mol) was dissolved in 65 mL of distilled dimethoxyethane, and Boc-Ala-OSu (4.58 g, 0.016 mol) and triethylamine (2.26 mL, 0.016 mol) were added with 4 h of stirring. The solvent was removed under reduced pressure, and the residue was taken up in 75 mL of ethyl acetate. The organic solution was washed with bicarbonate, 2 N HCl, and water, dried over Na_2SO_4, and evaporated. The product was recrystallized from ethyl acetate/petroleum ether to yield 5.22 g (91%) (TLC R_f 0.64).

***tert*-Butyloxycarbonyl-L-leucyl-L-alanylglycyl-L-valine Methyl Ester.** L-Alanylglycyl-L-valine methyl ester hydrochloride (4.43 g, 0.015 mol) was dissolved in 105 mL of distilled dimethoxyethane, and Boc-L-Leu-OSu (4.19 g, 0.015 mol) and triethylamine (2.12 mL, 0.015 moles) were added with overnight stirring at room temperature. The solvent was evaporated to dryness, and the residue was dissolved in 100 mL of ethyl acetate. The organic solution was washed, dried, and evaporated. The product was recrystallized from ethyl acetate/petroleum ether to yield 5.8 g (82%) (TLC R_f 0.61). Anal. ($C_{22}H_{40}N_4O_7$) C, H, N. NMR ($DCCl_3$) δ 0.70–1.13 (distorted doublet, 12 H, 4CH_3), δ 1.13–1.80 (singlet with a multiplet underneath, 14 H, 3 CH_3, 1 CH_3, 1 CH_2), 2.04 (s, 2 H, CH_2), 3.74 (S, 3 H, CH_3), 3.87–5.00 (m, 5 H, 3 CH 2 CHC_2), 5.44–5.74 (br d, 1 H, NH) 7.34–8.0 (m, 3 H, 3 N*H*).

***tert*-Butyloxycarbonyl-L-leucyl-L-alanylglycyl-L-valine.** The N^α-protected tetrapeptide methyl ester was saponified at 25 °C, 1 h, with 1.8 mL of 5 N NaOH in 16.5 mL ethanol to yield 3.2 g (82%) of the desired tetrapeptide. It was found to be one spot on TLC when visualized both by ninhydrin and chlorine/toluidine, R_f 0.5. Anal. ($C_{21}H_{38}N_4O_7$) C, H, N.

Synthesis of Boc(LAGV)$_n$-F-NHCH$_2$-Resin. Boc-Phe-OH was coupled to the aminomethyl-resin (0.18 mmol/g of styrene) by the standard DCC coupling procedure. The Boc-Phe-$NHCH_2$-resin (1 g) was treated according to the following protocol: (1) 20 mL of CH_2Cl_2, 3 × 1 min; (2) 20 mL of trifluoroacetic acid in CH_2Cl_2 (1:1, v/v), 1 × 1 min; (3) 20 mL of trifluoroacetic acid in CH_2Cl_2 (1:1, v/v), 1 × 20 min; (4) 20 mL of CH_2Cl_2, 6 × 1 min; (5) 20 mL of 5% diisopropylethylamine in CH_2Cl_2, 1 × 5 min; (6) 20 mL of CH_2Cl_2, 3 × 1 min; (7) 20 mL of 5% diisopropylethylamine in CH_2Cl_2, 1 × 5 min; (8) 20 mL of CH_2Cl_2, 3 × 1 min; (9) Boc-LAGV-OH (2.5 equiv) in 10 mL of CH_2Cl_2:DMF (1:1, v/v) for 2 min, without filtration; followed by (10) DCC (2.5 equiv), HOBt (2.5 equiv) in 5 mL of CH_2Cl_2:DMF (1:1, v/v), 40 h; (11) 20 mL of CH_2Cl_2, 6 × 1 min. At this stage, the completeness of the coupling reaction was monitored by the qualitative ninhydrin reaction.[10] If needed, steps 7–11 were repeated. This cycle of steps 1–11 was repeated until the Boc-$(LAGV)_5$-F-$NHCH_2$-resin was assembled.

Synthesis of Boc-LAGV-OMPA-F-NHCH$_2$-Resin. Boc-Phe$NHCH_2$-resin (100 mg) was treated in a reaction vessel according to the following protocol: (1) 2 mL of CH_2Cl_2, 3 × 1 min; (2) 2 mL of trifluoroacetic acid in CH_2Cl_2 (1:1 v/v), 1 × 1 min; (3) 2 mL of trifluoroacetic acid in CH_2Cl_2 (1:1 v/v), 1 × 20 min; (4) 2 mL of CH_2Cl_2, 6 × 1 min; (5) 2 mL of 5% diisopropylethylamine in CH_2Cl_2, 1 × 5 min; (6) 2 mL of CH_2Cl_2, 3 × 1 min; (7) 2 mL of 5% diisopropylethylamine in CH_2Cl_2, 1 × 5 min; (8) 2 mL of CH_2Cl_2, 3 × 1 min; (9) Boc-Val-OMPA (4 equiv) in 1 mL of CH_2Cl_2 for 5 min, without filtration; followed by (10) DCC (4 equiv) in CH_2Cl_2, 2 h; (11) 2 mL of CH_2Cl_2, 6 × 1 min. The completeness of coupling reaction was monitored by the qualitative ninhydrin reaction. If needed, steps 7–11 were repeated. This cycle of synthetic steps was repeated with Boc-Gly-OH, Boc-Ala-OH, and then Boc-Leu-OH.

Synthesis of Boc-LAGV-OMPA(LAGV)$_5$-F-NHCH$_2$-Resin. The peptide-resin Boc-$(LAGV)_5$-F-$NHCH_2$-resin (100 mg) was treated according to the previous protocol to assemble the desired peptide resin Boc-LAGV-OMPA-$(LAGV)_5$-F-$NHCH_2$-resin.

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