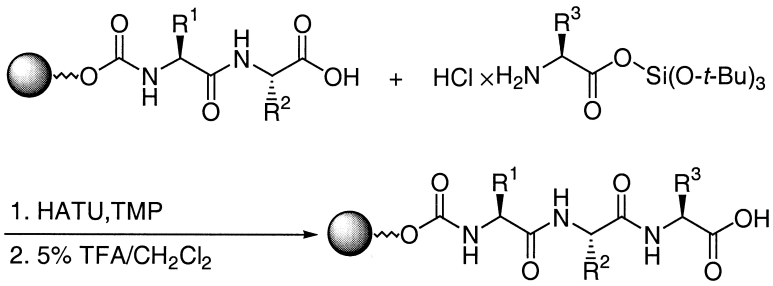
JOURNAL OF combinatorial CHEMISTRY

Article

An Improved Procedure for N- to C-Directed (Inverse) Solid-Phase Peptide Synthesis

Anja Johansson, Eva kerblom, Karolina Ersmark, Gunnar Lindeberg, and Anders Hallberg

J. Comb. Chem., **2000**, 2 (5), 496-507• DOI: 10.1021/cc000022h • Publication Date (Web): 09 August 2000



More About This Article

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

- Supporting Information
- Links to the 3 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

An Improved Procedure for N- to C-Directed (Inverse) Solid-Phase Peptide Synthesis

Anja Johansson, Eva Åkerblom, Karolina Ersmark, Gunnar Lindeberg, and Anders Hallberg*

Department of Organic Pharmaceutical Chemistry, Uppsala University, BMC, Box 574, SE-751 23 Uppsala, Sweden

Received March 20, 2000

A method for solid-phase peptide synthesis in the N- to C-direction that delivers good coupling yields and a low degree of epimerization is reported. The optimized method involves the coupling, without preactivation, of the resin-bound C-terminal amino acid with excess amounts of amino acid tri-*tert*-butoxysilyl (Sil) esters, using HATU as coupling reagent and 2,4,6-trimethylpyridine (TMP, collidine) as a base. For the amino acids investigated, the degree of epimerization was typically 5%, except for Ser(*t*-Bu) which was more easily epimerized (ca. 20%). Five tripeptides (AA_1-AA_2-AA_3) with different properties were used as representative model peptides in the development of the synthetic method: Asp-Leu-Glu, Leu-Ala-Phe, Glu-Asp-Val, Asp-Ser-Ile, and Asp-D-Glu-Leu. The study used different combinations of HATU and TBTU as activating agents, *N*,*N*-diisopropylethylamine (DIEA) and TMP as bases, DMF and dichloromethane as solvents, and cupric chloride as an epimerization suppressant. The epimerization of AA_2 in the coupling of AA_3 was further reduced in the presence of cupric chloride. However, the use of this reagent also resulted in a decrease in loading onto the resin and significant cleavage between AA_1 and AA_2. Experiments indicated that the observed suppressing effect of cupric chloride on epimerization in the present system merely seemed to be a result of a base-induced cleavage of the oxazolone system, the key intermediate in the epimerization process. Consequently, the cleavages were most pronounced in slow couplings. An improved synthesis of fully characterized amino acid tri-*tert*-butoxysilyl (Sil) ester hydrochloride building blocks is presented. The amino acid Sil esters were found to be stable as hydrochlorides but not as free bases. Although only a few peptides have been used in this study, we believe that the facile procedure devised herein should provide an attractive alternative for the solid-phase synthesis of short (six residues or less) C-terminally modified peptides, e.g., in library format.

Introduction

The introduction of solid-phase peptide synthesis (SPPS) by Merrifield in 1963 was of great importance for the development of peptide synthesis.[1] One reason for the tremendous impact of this method was the fact that it essentially allowed peptides to be synthesized without racemization when amino acids with N^{α}-protecting groups of the urethane (carbamate) type were coupled in a stepwise fashion. SPPS is still executed almost entirely in the C- to N-direction. Even C-terminally modified peptides are frequently prepared in this way. The use of appropriately designed linkers, which may be attached to specific amino acid side chains[2−4] or to the peptide backbone,[5,6] allows C-terminal incorporation of desired moieties at a suitable stage of the synthesis. Other ways of approaching the C-terminal modification are based on linker structures that deliver specific end groups upon cleavage[7−9] or that can be activated and/or cleaved with different nucleophiles.[10−12] Recently, internal resin capture techniques where the point of attachment to the resin is shifted from the C-terminal to the N-terminal end of the peptide have been applied successfully.[13]

Considering the importance of C-terminally modified peptides, for example, as protease inhibitors serving as starting points in the subsequent conversion to nonpeptidic drug-like entities, it is remarkable that relatively few reports have been devoted to the development of a direct method for peptide assembly in the inverse direction.[14−27] However, the advantages of this approach have long been recognized. Felix and Merrifield used protected amino acid hydrazides as building blocks for the C-terminal elongation of peptides in an attempt to circumvent the problems of epimerization.[23] Deprotection and subsequent reaction of the liberated hydrazide function with nitrite allowed the next building block to be coupled by the azide method, which at that time was believed to proceed without racemization. However, the procedure was elaborate, requiring activation and coupling at low temperature with moderate yields. Later investigations, mainly by Bayer and co-workers, have included the use of base labile amino acid 9-fluorenylmethyl esters, which were coupled with *N*-hydroxybenzotriazole (HOBt)/ diisopropylcarbodiimide (DIC) or *N*-[(1*H*-benzotriazole-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (TBTU)/*N*-methylmorpholine (NMM).[19] In

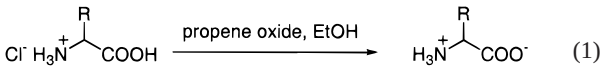
10.1021/cc000022h CCC: $19.00
Published on Web 08/09/2000

both cases, a 30-min preactivation step with a large excess of activator (8 equiv) was included before addition of the amino acid ester. Using TBTU/NMM extensively racemized products were obtained, whereas coupling using HOBt/DIC gave moderate racemization but led to significant formation of other byproducts.

In conjunction with our medicinal chemistry program aimed at identifying orally available protease inhibitors,[28,29] we became aware of a communication by Sharma et al. who described a few C-terminally modified tetrapeptide HIV-1 protease inhibitors, generated in the inverse direction.[14] We desired a robust and general method that permitted the attachment of a variety of functional groups, including electrophilic, at the C-terminal of small peptides without the need for special linkers. The simplicity of Sharma's approach,[25−27] which relied on coupling of amino acid tri-*tert*-butoxysilyl (Sil) esters[30] on solid phase, was attractive, and we foresaw a large potential of the method in automated parallel synthesis. Prior to library construction we wanted to evaluate the method in our system with special focus on yield and racemization. We report on the influence of factors such as coupling reagents, bases, solvents, racemization suppressing additives, and the use of preactivation on the yield and optical purity of a set of tripeptide models and describe an improved procedure for N- to C-directed solid-phase peptide synthesis. We also report on an improved method for the preparation of the fully characterized amino acid Sil ester building blocks.[31]

Results

Synthesis of Amino Acid Tri-*tert*-butoxysilyl (Sil) Ester Building Blocks. Our approach to the amino acid tri-*tert*-butoxysilyl (Sil) ester building blocks utilized a one-step generation of the hydrochloride salts of the amino acid Sil esters from the zwitterions of the amino acids. The amino acids that were not commercially available as zwitterions were prepared from the corresponding *N*-Boc protected derivatives. Boc removal was accomplished by treatment with 1 M HCl/ethyl acetate (EtOAc) to yield the hydrochlorides of H-Asp(OFm)-OH **1**, H-Glu(OFm)-OH **2**, and H-D-Glu(OFm)-OH **3**. The hydrochlorides **1**−**3** and the hydrochloride of H-Cha-OH **4** were then treated with propene oxide to deliver the zwitterions **5**−**8** (eq 1).[32−34]



(1)

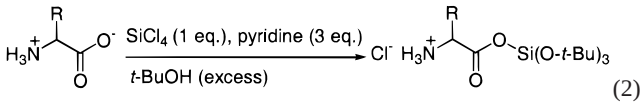
1	R= -CH_2COOFm	**5**	
2	R= -CH_2CH_2COOFm	**6**	
3	R= -(D)-CH_2CH_2COOFm	**7**	
4	R= -$CH_2C_6H_{11}$	**8**	

The synthesis of the amino acid Sil ester building blocks was effected by a modification of the protocol reported previously by Gruszecki[30,35] and Sharma.[25] The zwitterionic amino acids **5**−**20** were treated with tetrachlorosilane and three equiv of pyridine in 2-methyl-2-propanol (*t*-BuOH), rather than the four equiv, used in the original procedure, to give the hydrochlorides of the Sil esters **21**−**36** in acceptable yields, provided that dry conditions were maintained (eq 2 and Table 1). In previous reports the Sil esters were isolated as free amines by basic aqueous extraction.[25] However, we experienced fast ester hydrolysis under these conditions. Furthermore, attempted isolation of the hydrochlorides by conventional silica chromatography produced the free amines, which decomposed on evaporation of the solvent. Although less practical, the free amines could be converted to the hydrochlorides by the addition of an equimolar amount of HCl prior to evaporation. The use of excess acid caused fast hydrolysis of the ester, as did RP-HPLC in 0.1% aqueous TFA−acetonitrile. Mérette and co-workers have recently reported similar problems.[24] The isolation of the hydrochlorides **21**−**36** was finally accomplished by extraction of the crude residues with pentane or carbon tetrachloride which dissolved the hydrochlorides of the amino acid Sil esters but not pyridine hydrochloride. The remaining insoluble pyridine hydrochloride and unreacted amino acid were subsequently removed by filtration through Celite. By using 3 instead of 4 equiv of pyridine, no excess of (unprotonated) pyridine had to be removed. The hydrochlorides were highly pure as judged by elemental analysis and were stable for years in the freezer. In addition, no racemization was observed in the synthesis of the Sil esters, which was also verified by reconverting Ser(*t*-Bu)-OSil to the parent zwitterion (unpublished result).

Table 1. Yields of Amino Acid Sil Ester Hydrochlorides

amino acid (H-AA-OH)		H-AA-OSil × HCl	yield (%)
	Nonpolar		
9	Gly	**21**	82
10	Ala	**22**	68
11	Val	**23**	92
12	Leu	**24**	91
13	Ile	**25**	62
14	Pro	**26**	95
8	Cha[a]	**27**	97
	Aromatic		
15	Phe	**28**	67
	Polar		
16	Ser	**29**	91
17	Ser(*t*Bu)	**30**	87
	Basic		
18	Arg(Pbf)	**31**	39
	Acidic		
19	Asp(O*t*Bu)	**32**	77
5	Asp(OFm)	**33**	95
20	Glu(O*t*Bu)	**34**	82
6	Glu(OFm)	**35**	97
7	D-Glu(OFm)	**36**	95

[a] Cha = cyclohexylalanine.

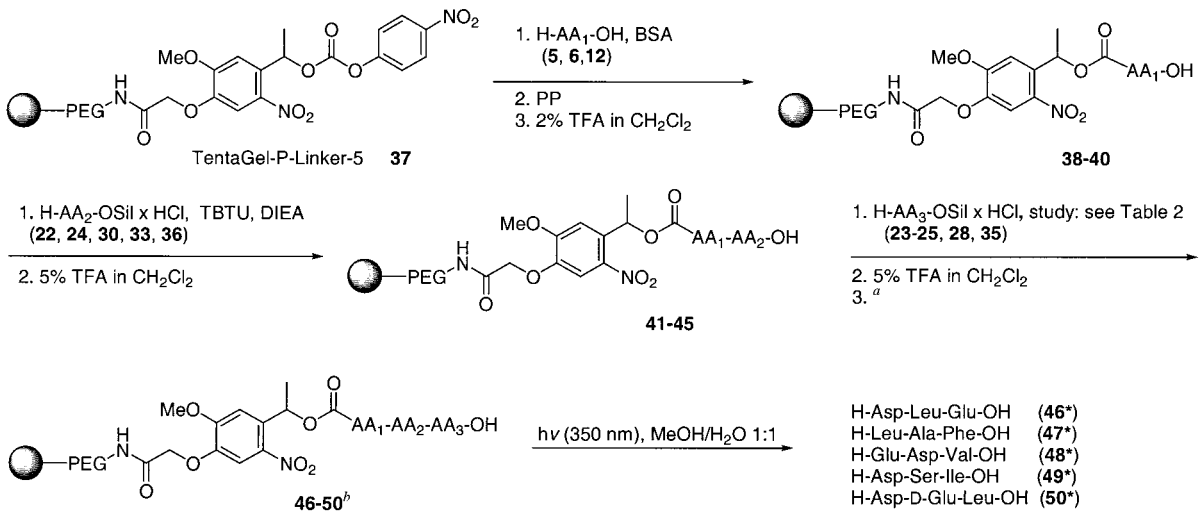


(2)

5-20 (Table 1) **21-36 (Table1)**

Loading of the First Amino Acid (Scheme 1). Two methods were used for loading of AA_1 onto the solid support. We used TentaGel-P-Linker-5, **37**, as the solid support with

Scheme 1



[a] Removal of the Fm group on Asp and Glu in **46** and **48**−**50** was performed with 25% piperidine/DMF, followed by removal of the piperidine salt with 5% TFA/CH_2Cl_2. The *t*-Bu ether on Ser in **49** was cleaved in 25% TFA/CH_2Cl_2. [b]Final tripeptides still attached to the solid support. Used in amino acid analysis.

a photolabile linker.[36,37] In the first approach, the zwitterionic amino acids H-Asp(OFm)-OH **5**, H-Glu(OFm)-OH **6**, and H-Leu-OH **12** were first silylated with TMS groups in order to achieve sufficient solubility, using the reagent *N*,*O*-bis-(trimethylsilyl)acetamide (BSA),[38] and then mixed with the resin **37** in the presence of 4-pyrrolidinopyridine (PP). Only 1 equiv of the base was employed to avoid Fm deprotection. 4-Pyrrolidinopyridine rendered a much higher reaction rate than *N*,*N*-diisopropylethylamine (DIEA), and loading onto the TentaGel resin was complete within 20 min to 2 h whereas 48 h was required for the related polystyrene resin with (dimethylamino)pyridine (DMAP) as a base.[38] Subsequent desilylation in 2% TFA/CH_2Cl_2 furnished **38**−**40** (Scheme 1). In the second approach, the first amino acid was added to the resin as the Sil ester in the presence of PP followed by desilylation in 5% TFA/CH_2Cl_2 (see Cleavage of the Sil Protecting Group).

Coupling of the Amino Acid Sil Esters (Scheme 1). The coupling of the Sil esters was performed without preactivation. The resins **38**−**40** were first mixed with the appropriate amino acid Sil ester hydrochloride **22**, **24**, **30**, **33**, or **36**, and TBTU. Thereafter, DIEA was added to initiate the reaction. After complete coupling the Sil protecting groups were cleaved in 5% TFA (see Cleavage of the Sil Protecting Group) to give the resin-bound dipeptides **41**−**45**. Essentially the same method was applied for the coupling of the next amino acid Sil ester **23**−**25**, **28**, or **35** except that different coupling reagents, bases, additives, and solvents were used (see Investigation of Coupling Conditions). The protecting groups were chosen to suit this method. The carboxylic acid side chains of Asp and Glu were protected throughout the synthesis as base labile fluorenylmethyl esters.[39] The hydroxyl functionality of Ser was protected as the acid labile *t*-Bu ether. Final deprotection was achieved with 25% piperidine in DMF and with 25% TFA in CH_2Cl_2, respectively. After each step, the products were examined by ^{13}C NMR to verify complete cleavage of the Sil group (Figure 1). In addition, the resin-bound peptides were examined by amino acid analysis. The final tripeptides **46***−**50*** were liberated from the linker by photolysis at 350 nm in 50% aqueous MeOH and analyzed by RP-HPLC and plasma desorption mass spectrometry (PDMS). We found the Sil protecting group to be well suited for peptide synthesis. Double couplings, which might result from the premature cleavage of the Sil esters during the reaction, were not observed.

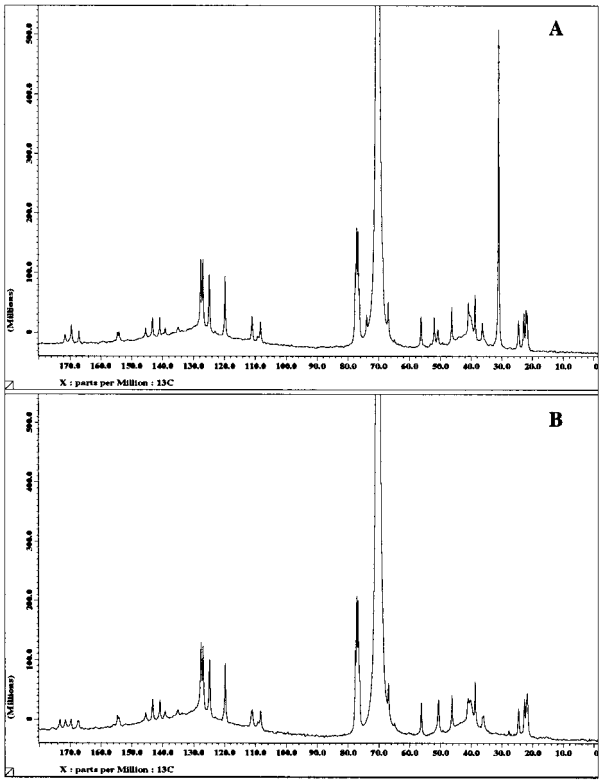
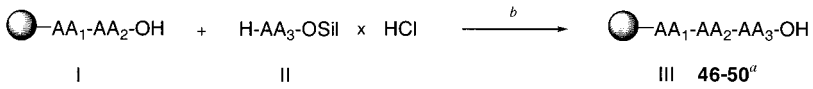


Figure 1. Cleavage of the Sil ester monitored by ^{13}C NMR spectroscopy. Complete cleavage indicated by total disappearance of the $-Si(OC(CH_3)_3)_3$ carbon at δ 31 ppm of TentaGel-P-linker-Asp(OFm)-Leu-OH (**41**) (spectrum B). Reference: TentaGel-P-linker-Asp(OFm)-Leu-OSil (spectrum A). JEOL EX270 at 67.8 MHz, 60 mg in $CDCl_3$, transients = 81 000, pulse width = 4 μs, acquisition time = 0.410 s, relaxation delay = 0.05 s, total time = 10.3 h.

Table 2. N- to C-Directed Synthesis of the Tripeptides **46**−**50**:[a] Effect of Various Coupling Conditions on Coupling Yield, Epimerization, Loading, and Stability of the Preformed AA_1-AA_2 Bond



entry	coupling conditions[b] reagent, base, additive	change in loading (%)[c]					change in AA_2/AA_1(%)[d]					yield, AA_3/AA_2 (%)[e]					LDL- epimer (%)[f]				
		46	**47**	**48**	**49**	**50**	**46**	**47**	**48**	**49**	**50**	**46**	**47**	**48**	**49**	**50**	**46***	**47***	**48***	**49***	**50***[g]
1	TBTU, DIEA	−2	+3	−11	−5	+2	±0	−1	+3	−3	−2	100	94	91	90	96	10	13	5	14	11
2	TBTU, DIEA, $CuCl_2$	−8	−10	−18	−4	−10	−8	−6	−2	−9	−10	95	94	96	85	102	4	6	1	11	6
3	TBTU, TMP	−1	+2	−6	−3	+2	+1	−1	+2	−4	±0	90	94	91	85	95	22	16	23	30	30
4	TBTU, TMP, $CuCl_2$	−30	−19	−30	−26	−26	−35	−7	−29	−44	−34	84	92	104	97	107	4	2	3	19	6
5	HATU, DIEA	−1	+1	−10	−1	−1	±0	−1	+5	−1	±0	100	90	92	81	102	5	4	4	14	13
6	HATU, DIEA, $CuCl_2$	−8	−4	−13	−4	−8	−8	−1	±0	−2	−14	100	91	96	83	98	4	1	≤1	13	5
7	HATU, TMP	−1	+1	−10	−2	−2	±0	−1	+6	±0	+5	99	92	91	86	101	5	3	5	17	9
8	HATU, TMP, $CuCl_2$	−12	−3	−20	−9	−9	−3	−1	+9	−14	−4	98	92	86	87	104	4	1	≤1	14	3

[a] **46** = Asp-Leu-Glu, **47** = Leu-Ala-Phe, **48** = Glu-Asp-Val, **49** = Asp-Ser-Ile, **50** = Asp-D-Glu-Leu. [b] See Experimental Section for general procedure (**46**−**50**); 2 h coupling in DMF/CH_2Cl_2 (5:2) without preactivation. The molar ratios of I, II, reagent, base, and $CuCl_2$ (if used) were 1:5:4:9:1, respectively. [c] Change in loading of AA_1 on III compared to the loading of AA_1 on I, calculated from amino acid analysis of I and III. The loading values (expressed as nmol per mg resin + linker) were corrected for the weight increase of AA_3 and for the actual proportions of the amino acids included. [d] $((AA_2/AA_1)^{III} - (AA_2/AA_1)^{I})/(AA_2/AA_1)^{I} \times 100$. Calculated from amino acid analysis of I and III. [e] Calculated from amino acid analysis of III. [f] Determined by RP-HPLC of III after release from the solid support (**46***−**50***, * = released from the solid support). [g] LLL-epimer.

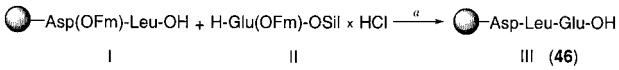
Investigation of Coupling Conditions. Our objective was to find conditions that made it possible to synthesize peptides in the N- to C-direction in high yields and with low epimerization. It is well-known that activated *N*-acylated amino acids easily form 2-alkyl-5(4*H*)-oxazolones, which are prone to epimerization.[19,40−44] Five peptides of varying composition (**46**−**50**, Scheme 1) were selected as representative targets for evaluation of epimerization and coupling yield under various conditions. Val and Ile which, due to the β-branching of the side chain, are sterically hindered and Ser, which is more readily racemized than most amino acids,[45−47] were included in the study in order to evaluate the limitations of the method. The N-terminal amino acid (AA_1) was attached to the linker as a carbamate in order to suppress potential racemization upon its activation. Thus, only the epimerization of AA_2 during the coupling of the third amino acid (AA_3) had to be considered. TBTU and *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) were examined as coupling reagents. The latter has been reported to provide higher optical yields and is recommended for the coupling of sterically hindered amino acids.[44,47,48] DIEA and 2,4,6-trimethylpyridine (TMP) were employed as bases. Of these bases, TMP is reported to cause less epimerization.[44,46,47,49,50] Addition of cupric chloride has proven beneficial to suppress epimerization in certain peptide couplings in solution,[51−54] but its application in solid-phase peptide synthesis has not, to our knowledge, been examined previously. This additive was included in the present study. The results of couplings in DMF/CH_2Cl_2 (5:2) are presented in Table 2.

As shown, the coupling yields (AA_3/AA_2) were satisfactory. With respect to epimerization, HATU seemed to be the better coupling reagent (entries 5−8, see % LDL epimer). It is noted that the combination TBTU/TMP resulted in extensive epimerization in all test peptides (entry 3). Coupling of Ile to Ser(*t*-Bu) also gave a high level of epimerization (sequence **49**). The addition of cupric chloride suppressed the level of epimerization in all reactions studied (entries 2, 4, 6, and 8). However, amino acid analysis of the crude resin-bound peptides revealed that although the degree of epimerization was lower in the cases where cupric chloride was used, the loading was also decreased considerably. In addition, the AA_2/AA_1 ratio was reduced, suggesting partial cleavage between AA_1 and AA_2. Entry 4 (TBTU, TMP, $CuCl_2$) provides the most striking example with ca. 25% reduction of the resin loading and equally pronounced cleavage (ca. 30%) between AA_1 and AA_2.

The ambiguous results with the cupric chloride additive prompted us to examine the coupling yield, epimerization, change in loading, and change in the AA_2/AA_1 ratio as a function of the amount of cupric chloride added and of the reaction times. The peptide Asp-Leu-Glu **46** was selected for this study. The results are shown in Tables 3 and 4. Reduction of the amount of cupric chloride resulted in decreased cleavage, but increased epimerization (Table 3). The reaction time had a minor effect on the outcome of the reactions, except for those given in entries 20−22 of Table 4 in which both yield and cleavage increased with time, which suggested that the undesired cleavage occurs in parallel with the coupling process.

Dichloromethane has been employed successfully as solvent for peptide coupling, for example, in fragment condensations where high levels of epimerization can also be expected.[47,50,55−58] In addition, a better swelling of our resins was observed in dichloromethane compared to DMF.

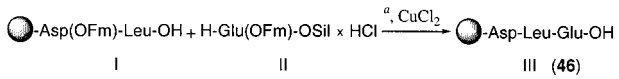
Table 3. N- to C-Directed Synthesis of Asp-Leu-Glu (**46**):[a] Effect of Various Equivalents of $CuCl_2$ on Coupling Yield, Epimerization, Loading, and Stability of the Preformed Asp-Leu Bond



	coupling conditions[a]			change in loading (%)[b]	change in Leu/Asp (%)[c]	yield, Glu/Leu (%)[d]	LDL-epimer (%)[e]
entry	reagent	base	$CuCl_2$ (equiv)				
9	TBTU	DIEA	1	−8	−8	95	4
10	TBTU	DIEA	0.25	−3	−4	101	12
11	TBTU	TMP	1	−31	−27	77	10
12	TBTU	TMP	0.25	−13	−18	87	14
13	HATU	DIEA	1	−8	−8	100	4
14	HATU	DIEA	0.25	−3	−3	102	9
15	HATU	TMP	1	−12	−3	98	4
16	HATU	TMP	0.25	−10	−1	100	5

[a] See Experimental Section for general procedure (**46**); 2 h coupling in DMF/CH_2Cl_2 (5:2) without preactivation. The molar ratios of I, II, reagent, and base were 1:5:4:9, respectively. [b−e] See corresponding footnotes in Table 2 (c−f).

Table 4. N- to C-Directed Synthesis of Asp-Leu-Glu (**46**) in the Presence of $CuCl_2$:[a] Effect of Coupling Times on Coupling Yield, Epimerization, Loading, and Stability of the Preformed Asp-Leu Bond



	coupling conditions[a]			change in loading (%)[b]	change in Leu/Asp (%)[c]	yield, Glu/Leu (%)[d]	LDL-epimer (%)[e]
entry	reagent	base	time (h)				
17	TBTU	DIEA	2	−8	−8	95	4
18	TBTU	DIEA	1	−10	−12	92	5
19	TBTU	DIEA	0.5	−10	−11	93	6
20	TBTU	TMP	2	−30	−35	84	4
21	TBTU	TMP	1	−25	−34	72	7
22	TBTU	TMP	0.5	−24	−29	61	11
23	HATU	DIEA	2	−8	−8	100	4
24	HATU	DIEA	1	−8	−9	99	4
25	HATU	DIEA	0.5	−10	−9	94	5
26	HATU	TMP	2	−12	−3	98	4
27	HATU	TMP	1	−11	−4	100	2
28	HATU	TMP	0.5	−9	−6	98	2

[a] See Experimental Section for general procedure (**46**). Coupling in DMF/CH_2Cl_2 (5:2) without preactivation. The molar ratios of I, II, reagent, base, and $CuCl_2$ were 1:5:4:9:1, respectively. [b−e] See corresponding footnotes in Table 2 (c−f).

Two of the peptides, **46** and **49**, were resynthesized using a 6:1 mixture of CH_2Cl_2/DMF (Table 5). A small amount of DMF had to be included in order to dissolve the coupling reagents. The extent of epimerization with Asp-Leu-Glu **46** was slightly decreased in all four couplings (c.f. Table 2 and Table 5), and the yields were high. Notably, the couplings with HATU and TMP gave *less than 1%* epimerization. However, with Asp-Ser-Ile **49**, the most easily epimerized and the most sterically hindered peptide in the present study, no improvements were observed.

Cleavage of the Sil Protecting Group. The Sil ester is reported to be stable at pH 4−8 and cleaved both under acidic and basic conditions.[25,30,35] We found that treatment with 5% TFA/CH_2Cl_2 for 20 min was adequate for deprotection in all cases studied. The extent of deprotection was determined by ^{13}C NMR (Figure 1). Neither of the basic mixtures, 2% DBU with 2% piperidine/DMF and 25% piperidine/DMF, nor saturated K_2CO_3/DMF was sufficient for deprotection whereas 0.2 M tetrabutylammonium fluoride (TBAF) in THF effected complete removal of the Sil group. However, the *tert*-butyl esters of Asp and Glu were also cleaved in the TBAF system.

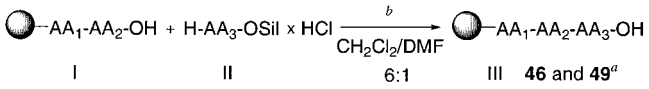
We also examined the stability of the carbamate linkage between P-linker-5 and the first amino acid and found that even upon treatment with 95% TFA/CH_2Cl_2 for 3 h only 5% cleavage was observed. On the other hand, the corresponding carbamate linkers with ArgoGel Wang and TentaGel PHB resins were not stable in 5% TFA/CH_2Cl_2, which was used for removal of the Sil esters (unpublished results).

Discussion

In the past, few reports have thoroughly addressed the importance of coupling conditions and amino acid sequence on yield and epimerization in N- to C-directed SPPS. Henkel et al., using TBTU and NMM with preactivation, observed levels of epimerization ranging from 27.5 to 49.9% in [Leu^5]enkephalin and in fragment 64−71 of HIV-1 protease for amino acids located at positions where oxazolone formation is likely to occur.[19] The considerably lower degree of epimerization observed in our system (Table 2 and Table 5) can most probably be attributed to the avoidance of preactivation, since conditions that shorten the lifetime of the activated intermediate (which can be expected to be the case when activation is carried out in the presence of the amino component) are known to suppress epimerization.[47,50,59−61] Accordingly, the use of a more efficient activating agent should also have a beneficial influence on the degree of epimerization. Such a tendency is indeed observed in our experiments when HATU (entries 5 and 7) is compared to the more slowly reacting TBTU (entries 1 and 3).[44,62] The effect is particularly large when TMP is used as base (entries 3 and 7). Most likely the high epimerization level (16−30%) observed for the combination TBTU/TMP can again be explained by a low coupling rate. TMP, although claimed to yield less epimerization than DIEA and NMM,[44,46,47,49,50] may be too weak a base to deprotonate the hydrochloride of the Sil ester efficiently. With TBTU, the low concentration of available amine leads to slow reaction, whereas the HATU-activated intermediate reacts more rapidly, probably due to the beneficial neighboring group effect of the nitrogen in the aromatic moeity.[44] In fact, HATU seems to give the best results overall, particularly in combination with TMP.

The use of cupric chloride as an additive for peptide couplings in *solution* results in very low levels of epimerization (<0.1%) but frequently also in low total yields.[51−54] Also in our system epimerization is suppressed efficiently, but analysis of the product composition after synthesis of the five model peptides strongly suggests that undesired cleavage of the AA_1-AA_2 and linker bonds occur with the coupling of AA_3 in the presence of cupric chloride (entries 2, 4, 6, and 8). Although no experimental data is available, it is reasonable to assume that similar side reactions could

Table 5. N- to C-Directed Synthesis of the Tripeptides **46** and **49**:[a] Effect of Dichloromethane on Coupling Yield, Epimerization, Loading, and Stability of the Preformed AA_1-AA_2 Bond

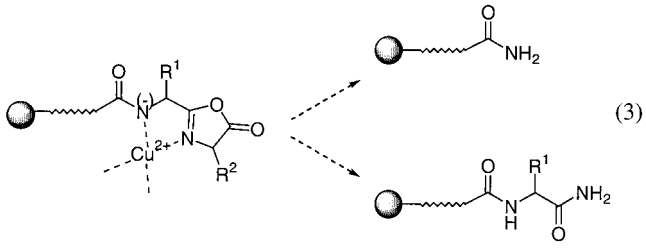


	coupling conditions[b]		change in loading (%)[c]		change in AA_2/AA_1(%)[d]		yield, AA_3/AA_2 (%)[e]		LDL- epimer (%)[f]	
entry	reagent	base	**46**	**49**	**46**	**49**	**46**	**49**	**46**	**49**
29	TBTU	DIEA	+2	−4	−1	−1	99	87	6	21
30	TBTU	TMP	±0	−7	−2	+1	98	88	16	26
31	HATU	DIEA	+2	−6	±0	−1	98	89	3	23
32	HATU	TMP	+2	−3	±0	−2	97	89	<1	22

[a,c−f] See corresponding footnotes in Table 2 (a, c−f). [b] See Experimental Section for general procedure (**46** and **49**); 2 h coupling without preactivation. The molar ratio of I, II, reagent, and base was 1:5:4:9, respectively.

also account for the lower yields reported for the related peptide syntheses in solution. As described above, the most pronounced effect on the epimerization is observed with TBTU/TMP. In the presence of cupric chloride, the epimerization levels for the same combination are considerably reduced, in most cases to less than 5%, whereas ca. 30% decrease of the AA_2/AA_1 ratio and ca. 25% decrease in the loading are observed. A higher concentration of cupric chloride leads to increased cleavage and decreased epimerization (Table 3). However, the presence of cupric chloride is not by itself sufficient for cleavage. Incubation with cupric chloride for 24 h left the resin-bound peptide Asp(OFm)-Leu unaffected. Furthermore, the effect of the coupling time is negligible except for TBTU/TMP/$CuCl_2$ where both the yield and extent of cleavage increases with time (Table 4). These results suggest that the undesired cleavage actually precedes amide bond formation and is a consequence of a slow coupling process.

Taken together, the data show that a long lifetime of the activated intermediate will allow side reactions to compete successfully with the peptide bond formation. In the absence of cupric chloride, epimerization via the oxazolone route seems to be the major complication. In the presence of cupric chloride, bond cleavage occurs. To confirm this hypothesis, the synthesis of the peptide Asp-Leu-Glu **46** was repeated, but this time a 90 min preactivation step with TBTU/TMP or TBTU/TMP/$CuCl_2$ was included before addition of the last amino acid. As expected, the effect of the extreme preactivation was considerable and resulted in 35% epimerization in the first case and in a 35% decrease in loading and a 47% decrease of the Leu/Asp ratio in the second case. To investigate if any specific intermediate or combination of reactants could account for the cleavage in the presence of cupric chloride, some key experiments with an unactivated peptide, an activated peptide (an OBt-ester of a proline-peptide, which is prevented from forming the corresponding oxazolone), and a peptide with a preformed oxazolone were performed (see Experimental Section). Clearly, cleavage by cupric chloride only occurred when the oxazolone and base were present at the same time. Formation of a tetrapeptide with TBTU/TMP/$CuCl_2$ showed that the cleavage is restricted to the amino side of the last (activated) and the penultimate amino acid residue, suggesting the formation of an intermediate copper complex between the two involved residues (eq 3).[63] Analysis of the product composition revealed that the corresponding primary amides had been formed (eq 3).



Apparently, the suppressing effect of cupric chloride on epimerization in the present system is a result of a base-induced cleavage of the oxazolone intermediate. Consequently, the use of a fast coupling method without preactivation, rather than the addition of cupric chloride, is the better way to suppress epimerization.

Dichloromethane was also tried as a coupling medium since it was observed that this solvent was able to swell our resin somewhat more effectively than was DMF. It was anticipated that the better swelling would lead to a higher coupling rate. However, the effects of changing the solvent composition from DMF/CH_2Cl_2 (5:2) to DMF/CH_2Cl_2 (1:6) were not conclusive (Table 5).

Conclusion

In summary, a significantly improved method for the solid-phase synthesis of short peptides in the inverse (N- to C-) direction that provides good yields and low degree of epimerization has been developed. The optimized method involves the coupling, without preactivation, of the resin-bound C-terminal amino acid with excess amounts of amino acid tri-*tert*-butoxysilyl (Sil) esters, using HATU as coupling reagent and 2,4,6-trimethylpyridine (TMP, collidine) as a base. Levels of epimerization were considerably lower than those reported for other N- to C-methods, usually ca. 5% and occasionally even below 1%. However, in unfavorable cases, as in the coupling of Ile to Ser, the level was higher (ca. 20%). The low epimerization is probably due to the omission of preactivation and the use of reagents that allow fast coupling reactions. The observed suppressing effect of cupric chloride on epimerization in the present system merely

seems to be a result of a base-induced cleavage of the oxazolone system, the key intermediate in the epimerization process.

Although only a few peptides have been included in this study, we believe that the facile procedure devised herein should provide an attractive alternative for the solid-phase synthesis of short (six residues or less) C-terminally modified peptides, e.g., in library format.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 spectrometer at 270 (67.8) MHz or a JEOL JNM-EX400 at 400 (100.58) MHz at ambient temperature. Chemical shifts are reported as δ values (ppm) indirectly referenced to TMS by the solvent signal ($CHCl_3$) δ 7.27, ($CDCl_3$) δ 77.23, (methanol-d_3) δ 3.31, (methanol-d_4) δ 49.15, and (DMSO-d_5) δ 2.50. Thin-layer chromatography (TLC) was performed using aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm, E. Merck). Samples were visualized by UV light and spraying with an ethanolic solution of ninhydrin (2%) followed by heating. IR spectra were recorded on a Perkin-Elmer model 1605 FT-IR and are reported as υ_{max} (cm^{-1}). To obtain IR spectra of solid-phase samples, the IR spectrometer was equipped with a Microfocus beam condenser with ZnSe lenses in a Diasqueeze Plus Diamond compressor cell (Graseby Specac Inc., Smyrna, U.S.A.). Column chromatography was executed on Riedel-de Haen silica gel S (32−63 μm) or Merck silica gel 60 (40−63 μm). Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter at ambient temperature. Specific rotations ($[\alpha]_D$) are reported in deg/dm, and the concentration (c) is given in g/100 mL in the specified solvent. Mass spectroscopy was carried out on an Applied Biosystem (Uppsala, Sweden) BIOION 20 plasma desorption mass spectrometer. LC-MS was performed on a HP 1100 LC/MSD with an analytical Zorbax SB C18 column (0.46 × 7.5 cm, 3.5 μm particles) and with APCI. Compounds were separated by gradient elution; 10 to 90% of solvent B (1% HCOOH /CH_3CN) in solvent A (1% HCOOH [aqueous]), over 40 min; flow rate 1.5 mL/min; UV and MS detection. Melting points were determined in open glass capillaries in an electrothermal melting point microscope and are uncorrected. Amino acid analyses were performed at the Department of Biochemistry, Biomedical Centre, Uppsala, Sweden, on 24 h hydrolyzates with an LKB 4151 alpha plus analyzer using ninhydrin detection. Cleavage of the peptides from the resin was performed in a RPR-200 Rayonet chamber reactor equipped with the Rayonet Merry-Go-round model-RMA-500 (22 samples) working as a photoreactor. All samples were rotated past the light source at a speed of 5 rpm. The stirring in each vial was carried out using a rebuilt magnetic stirrer placed underneath the reactor. The photoreactor was equipped with 16 25 W lamps emitting 350 nm UV light. The distance to the sample was 1.7 cm. RP-HPLC was performed on an analytical LiChrosphere C18 column (0.4 × 25 cm, 5 μm particles, 100 Å pores) or on an analytical Vydac C18 column (0.46 × 15 cm, 10 μm particles, 300 Å pores). The Quest 210 organic synthesizer (Argonaut Technologies) was utilized in the peptide syntheses. The photolabile solid support was protected from light during handling and storage.

Materials and Abbreviations. The resin used in the solid-phase syntheses was the photolabile TentaGel-P-linker-5 (**37**) (loading capacity: 0.21 mmol/g), prepared by coupling of 4-acetyl-2-methoxy-5-nitrophenoxy acetic acid to TentaGel S NH_2, followed by reduction and coupling of 4-nitrophenyl chloroformate.[36,37] Amino acids **9**−**20** and Boc-Asp(OFm)-OH, Boc-Glu(OFm)-OH, Boc-D-Glu(OFm)-OH, and H-Cha-OH × HCl (**4**) were purchased from Bachem (Bubendorf, Switzerland). *N*-[(1*H*-benzotriazole-1-yl)-(dimethylamino)-methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (TBTU) and *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) were obtained from Richelieu Biotech and Millipore, respectively. Bases used were *N*,*N*-diisopropylethylamine (DIEA) (Aldrich, 99%), 2,4,6-trimethylpyridine (TMP, collidine) (Merck, p.a), 4-pyrrolidino-pyridine (PP) (Aldrich, 98%), and pyridine (Sigma-Aldrich, 99.9%, stored over molecular sieves, 4 Å). DMF (Aldrich, 99.9+%, HPLC grade) was stored over molecular sieves (4 Å), dichloromethane (Riedel-deHaën) was freshly distilled over benzophenone, and 2-methyl-2-propanol (*t*-BuOH) (Sigma-Aldrich, 99.5%) was used as received. Copper(II) chloride (99.999%) and silicon tetrachloride ($SiCl_4$) were obtained from Aldrich, and *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (97%) from Fluka.

General Procedure for Preparation of Compounds 1−3. The *N*-Boc-protected amino acid was stirred in a solution of 1.4 M HCl in EtOAc (10 equiv) for 3 h. Precipitated product was isolated by filtration, washed with EtOAc, and dried in vacuo to yield pure **1**,[64−66] **2**,[64,66] and **3**.

D-Glutamic acid γ-9-fluorenylmethyl ester hydrochloride [H-D-Glu(OFm)-OH × HCl] (3): white solid, yield 0.82 g (96%); mp 181−185 °C; IR (KBr) 1739 cm^{-1}; $[\alpha]_D$ = −11.9° (c = 1.0, DMSO); ^{1}H NMR (CD_3OD) δ 7.84−7.78 (m, 2H), 7.66−7.60 (m, 2H), 7.45−7.28 (m, 4H), 4.52 (dd, J = 6.9, 10.9 Hz, 1H), 4.47 (dd, J = 6.3, 10.9 Hz, 1H), 4.25 (m, 1H), 3.96 (m, 1H), 2.70−2.50 (m, 2H), 2.26−2.01 (m, 2H); ^{13}C NMR (CD_3OD) δ 173.7, 171.4, 145.2, 142.7, 129.0, 128.3, 126.1, 121.1, 67.7, 53.2, 48.2, 30.7, 26.7; PDMS (MW 325.4) 326.5 (M + H$^+$). Anal. Calcd for $C_{19}H_{20}ClNO_4$: C, 63.1; H, 5.6; N, 3.9. Found: C, 62.7; H, 5.8; N, 4.0.

General Procedure for Preparation of the Zwitterions 5−8. Propene oxide (11 equiv) was added to a solution of the hydrochloride salt **1**−**4** in absolute EtOH (ca. 6 mL/mmol) in a round-bottomed flask fitted with a condenser. The reaction mixture was heated to 50 °C and stirred for 4 h. The precipitated zwitterions **5**,[67] **6**,[67] **7**, and **8**[68−70] were isolated by filtration, washed with EtOH, and dried in vacuo at 38 °C.

L-Glutamic acid γ-9-fluorenylmethyl ester [H-Glu(OFm)-OH] (6): white solid, yield 2.59 g (96%); mp 179−180 °C; IR (KBr) 1714, 1690−1490 cm^{-1}; $[\alpha]_D$ = +10.0° (c = 0.5, DMSO); ^{1}H NMR (DMSO-d_6) δ 7.94−7.86 (m, 2H), 7.70−7.63 (m, 2H), 7.47−7.29 (m, 4H), 4.41−4.34 (m, 2 H), 4.30−4.22 (m, 1H), 3.19 (m, 1H), 2.64−2.37 (m, 2H,

overlap with solvent signal, DMSO-d_5), 2.04−1.74 (m, 2H); PDMS (MW 325.4) 326.6 (M + H^+). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.1; H, 5.9; N, 4.3. Found: C, 69.8; H, 6.1; N, 4.4 [lit.[67] mp 156−158 °C]; $[\alpha]_D = +8.0°$ ($c = 0.5$, DMSO); ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.76 (d, $J = 7.2$ Hz, 2H), 7.65 (d, $J = 7.2$ Hz, 2H), 7.4−7.2 (m, 4H), 4.85 (br s, 2 H), 4.2−4.0 (m, 4H), 2.5−2.0 (m, 4H)].

D-Glutamic acid γ-9-fluorenylmethyl ester [H-D-Glu-(OFm)-OH] (7): white solid, yield 2.63 g (97%); mp 174−175 °C; IR (KBr) 1716, 1690−1490 cm^{-1}; $[\alpha]_D = -16.0°$ ($c = 0.5$, DMSO); ^{1}H NMR (DMSO-d_6) showed a mixture of two conformations (A & B) A: δ 7.94−7.86 (m, 2H), 7.70−7.63 (m, 2H), 7.47−7.29 (m, 4H), 4.41−4.34 (m, 2 H), 4.30−4.22 (m, 1H), 3.20 (m, 1H), 2.64−2.37 (m, 2H, overlap with solvent signal, DMSO-d_5), 2.04−1.74 (m, 2H). B: δ 7.91−7.82 (m, 2H), 7.70−7.63 (m, 2H), 7.42−7.26 (m, 4H), 4.09−3.95 (m, 2H), 3.77−3.70 (m, 2H), 2.42−2.21 (m, 1H), 2.16−2.07 (m, 2H) 2.04−1.74 (m, 1H); PDMS (MW 325.4) 326.7 (M + H^+). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.1; H, 5.9; N, 4.3. Found: C, 69.7; H, 6.0; N, 4.5.

General Procedure for Preparation of the Amino Acid Sil Ester Hydrochlorides 21−36. Dry conditions were of importance in this synthesis. The amino acid **5**−**20** was suspended in *t*-BuOH in a dried round-bottom flask. Pyridine (3 equiv, stored over molecular sieves) was added, and the flask was then fitted with a rubber septum and filled with N_2. $SiCl_4$ (1 equiv) was added dropwise via a syringe to the stirred reaction mixture at room temperature. An exothermic reaction resulted in a clear solution during or immediately after the addition. Stirring was continued for 4 h during which time pyridine hydrochloride precipitated in most of the cases. The reaction was monitored by TLC (eluent: MeOH/$CHCl_3$ (1:9)). Thereafter, pentane or CCl_4 was added to enhance the precipitation of pyridine hydrochloride. The precipitate was removed by filtration and the filtrate concentrated under reduced pressure (40 °C). The crude product was suspended in pentane or CCl_4 and filtered by gravity through Celite (height: 3−4 cm, eluent: pentane or CCl_4) to remove all pyridine hydrochloride. The filtrate was concentrated and dried in vacuo to yield the pure product **21**−**36** as a white/yellow solidified foam or viscous oil. ^{1}H NMR of the Sil esters in $CDCl_3$ frequently resulted in broad signals, due to proton exchange (−NH). Narrow signals were obtained in DMSO-d_6. However, $CDCl_3$ was retained as solvent since the water content in DMSO-d_6 partially hydrolyzed the product. Measurement of optical rotations could not be performed since the Sil esters interact with the glass surface.

Glycine tri-*tert*-butoxysilyl ester hydrochloride [H-Gly-OSil × HCl] (21): solidified foam, yield 2.93 g (82%), from glycine **9** (751 mg, 10.0 mmol), pyridine (2.43 mL, 30.0 mmol), $SiCl_4$ (1.15 mL, 10.0 mmol), *t*-BuOH (10 mL), and CCl_4 workup; IR (KBr) 1759 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.73 (br s, 3H), 3.89 (s, 2H), 1.32 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 164.4, 74.6, 41.5, 31.2; PDMS (MW 321.5) no molecular ion but typical fragmentation pattern. Anal. Calcd for $C_{14}H_{32}ClNO_5Si \times {}^1/_2\ H_2O$: C, 45.8; H, 9.1; N, 3.8. Found: C, 45.9; H, 9.1; N, 3.8.

L-Alanine tri-*tert*-butoxysilyl ester hydrochloride [H-Ala-OSil × HCl] (22): solidified foam, yield 2.84 g (68%), from alanine **10** (1.00 g, 11.2 mmol), pyridine (2.72 mL, 33.6 mmol), $SiCl_4$ (1.28 mL, 11.2 mmol), *t*-BuOH (10 mL), and CCl_4 workup; IR (KBr) 1751 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.80 (br s, 3H), 4.11 (br s, 2H), 1.74 (d, $J = 7.1$ Hz, 3H), 1.32 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 167.3, 74.7, 50.2, 31.4, 16.1; PDMS (MW 335.5) 336.4 (M + H^+). Anal. Calcd for $C_{15}H_{34}ClNO_5Si \times {}^1/_3\ H_2O$: C, 47.7; H, 9.2; N, 3.7. Found: C, 47.5; H, 8.9; N, 3.7.

L-Valine tri-*tert*-butoxysilyl ester hydrochloride [H-Val-OSil × HCl] (23): solidified foam, yield 2.46 g (92%), from valine **11** (785 mg, 6.70 mmol), pyridine (1.62 mL, 20.1 mmol), $SiCl_4$ (0.768 mL, 6.70 mmol), *t*-BuOH (10 mL), and pentane workup; IR (KBr) 1752 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.84 (br s, 3H), 3.92 (br s 1H), 2.67−2.42 (m, 1H), 1.32 (s, 27H), 1.25 (d, $J = 6.9$ Hz, 3H), 1.15 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.4, 74.7, 59.4, 31.4, 29.8, 18.8, 17.9; PDMS (MW 363.6) 363.8 (M + H^+). Anal. Calcd for $C_{17}H_{38}ClNO_5Si \times {}^1/_2\ H_2O$: C, 49.9; H, 9.6; Cl, 8.7; N, 3.4. Found: C, 49.7; H, 9.7; Cl, 8.4; N, 3.6.

L-Leucine tri-*tert*-butoxysilyl ester hydrochloride [H-Leu-OSil × HCl] (24): oil, yield 2.52 g (91%), from leucine **12** (879 mg, 6.70 mmol), pyridine (1.62 mL, 20.1 mmol), $SiCl_4$ (0.768 mL, 6.70 mmol), *t*-BuOH (10 mL), and pentane workup; IR (neat) 1751 cm^{-1}; ^{1}H NMR ($CDCl_3$) 8.85 (br s, 3H), 3.94 (br s, 1H), 2.23−1.77 (m, 3H), 1.30 (s, 27H), 1.04−0.97 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 167.3, 74.7, 52.7, 39.6, 31.4, 24.5, 22.9, 22,1; PDMS (MW 377.6) 378.8 (M + H^+). Anal. Calcd $C_{18}H_{40}ClNO_5Si \times {}^1/_4\ H_2O$: C, 51.7; H, 9.8; N, 3.4. Found: C, 51.4; H, 10.1; N, 3.4.

L-Isoleucine tri-*tert*-butoxysilyl ester hydrochloride [H-Ile-OSil × HCl] (25): oil, yield 3.45 g (62%), from isoleucine **13** (1.76 g, 13.4 mmol), pyridine (3.25 mL, 40.2 mmol), $SiCl_4$ (1.54 mL, 13.4 mmol), *t*-BuOH (20 mL), and pentane workup; IR (neat) 1746 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.70 (br s, 3H), 3.99 (br s, 1H), 2.24 (m, 1H), 1.62−1.41 (m, 2H), 1.32 (s, 27H), 1.21 (d, $J = 6.9$ Hz, 3H), 1.02 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 166.5, 74.7, 58.6, 36.5, 31.4, 25.1, 15.6, 12.0; PDMS (MW 377.6) 378.6 (M + H^+). Anal. Calcd for $C_{18}H_{40}ClNO_5Si \times {}^2/_3\ H_2O$: C, 50.7; H, 9.7; N, 3.2. Found: C, 50.7; H, 9.8; N, 3.3.

L-Proline tri-*tert*-butoxysilyl ester hydrochloride [H-Pro-OSil × HCl] (26): solidified foam, yield 2.53 g (95%), from proline **14** (771 mg, 6.70 mmol), pyridine (1.62 mL, 20.1 mmol), $SiCl_4$ (0.768 mL, 6.70 mmol), *t*-BuOH (10 mL), and CCl_4 workup; IR (KBr) 1750, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.46 (br s, 1H), 8.00 (br s, 1H), 4.46 (br s, 1H), 3.74−3.40 (m, 2H), 2.58−2.32 (m, 1H), 2.31−1.86 (m, 3H), 1.30 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 166.6, 74.9, 60.2, 46.4, 31.3, 28.9, 23.8; PDMS (MW 361.6) 362.4 (M + H^+). Anal. Calcd for $C_{17}H_{36}ClNO_5Si \times {}^1/_2\ H_2O$: C, 50.2; H, 9.2; N, 3.4. Found: C, 50.1; H, 9.0; N, 3.7.

L-Cyclohexylalanine tri-*tert*-butoxysilyl ester hydrochloride [H-Cha-OSil × HCl] (27): solidified foam, yield 2.57 g (97%), from cyclohexylalanine **8** (1.00 g, 5.84 mmol), pyridine (1.42 mL, 17.5 mmol), $SiCl_4$ (0.669 mL, 5.84 mmol), *t*-BuOH (12 mL), and pentane workup; IR (KBr) 1748 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.80 (br s, 3H), 4.00 (br s, 1H), 2.16−1.58 (m, 7H), 1.50−0.83 (m, 6H), 1.32 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 167.4, 74.7, 52.1, 38.1, 33.3, 33.2, 32.9,

31.4, 26.5, 26.0, 25.9; PDMS (MW 417.6) 418.9 ($M + H^+$). Anal. Calcd for $C_{21}H_{44}ClNO_5Si \times {}^1/_2\ H_2O$: C, 54.5; H 9.8; N 3.0. Found: C, 54.4; H, 9.8; N, 2.9.

L-Phenylalanine tri-*tert*-butoxysilyl ester hydrochloride [H-Phe-OSil × HCl] (28): oil, yield 2.01 g (67%), from phenylalanine **15** (1.11 g, 6.70 mmol), pyridine (1.62 mL, 20.1 mmol), $SiCl_4$ (0.768 mL, 6.70 mmol), *t*-BuOH (10 mL), and pentane workup; IR (neat) 1751 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.62 (br s, 3H), 7.49−7.12 (m, 5H), 4.33 (br s, 1H), 3.52 (dd, $J = 14.5$, 5.6 Hz, 1H), 3.40 (dd, $J = 14.5$, 4.9 Hz, 1H), 1.32 (s, 27H). ^{13}C NMR ($CDCl_3$) δ 166.1, 133.3, 130.3, 129.2, 128.0, 74.8, 54.8, 35.6, 31.4; PDMS (MW 411.6) 412.8 ($M + H^+$). Anal. Calcd for $C_{21}H_{38}ClNO_5Si$: C, 56.3; H, 8.6; Cl, 7.9; N 3.1. Found: C, 56.1; H, 8.4; Cl, 7.6; N, 3.2.

***O*-Tri-*tert*-butoxysilyl-L-serine tri-*tert*-butoxysilyl ester hydrochloride [H-Ser(Sil)-OSil × HCl] (29):** solidified foam, yield 5.81 g (92%), from serine **16** (1.05 g, 10.0 mmol), pyridine (5.66 mL, 70.0 mmol), $SiCl_4$ (2.29 mL, 20.0 mmol), *t*-BuOH (20 mL), and pentane workup; IR (KBr) 1760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.68 (br s, 3H), 4.38−4.17 (m, 3H), 1.32 (s, 27 H), 1.31 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 165.2, 74.8, 74.7, 61.1, 56.3, 31.4; PDMS (MW 597.9) 598.8 ($M + H^+$). Anal. Calcd for $C_{27}H_{60}ClNO_9Si_2 \times {}^1/_2\ H_2O$: C, 50.4; H, 9.6; N, 2.2. Found: C, 50.8; H, 9.7; N, 2.2.

***O*-*tert*-Butyl-L-serine tri-*tert*-butoxysilyl ester hydrochloride [H-Ser(*t*-Bu)-OSil × HCl] (30).** The starting material **17** contained $^1/_2$ mole of H_2O per mole and was therefore dissolved in MeOH and dried with molecular sieves (4 Å) overnight. The filtrate was concentrated in vacuo (50 °C) and the residue used in the synthesis: solidified foam, yield 4.50 g (87%), from H-Ser(*t*-Bu)-OH **17** (1.87 g, 11.6 mmol), pyridine (2.81 mL, 34.8 mmol), $SiCl_4$ (1.33 mL, 11.6 mmol), *t*-BuOH (10 mL), and pentane workup; IR (KBr) 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.59 (br s, 3H), 4.23 (br s, 1H), 4.07 (br d, $J = 9.5$ Hz, 1H), 1.02 (br d, $J = 9.5$ Hz, 1H), 1.32 (s, 27H), 1.21 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.2, 74.7, 74.5, 59.5, 54.6, 31.4, 27.5; PDMS (MW 407.6) 408.6 ($M + H^+$). Anal. Calcd for $C_{19}H_{42}ClNO_6Si \times {}^1/_2\ H_2O$: C, 50.4; H, 9.6; N, 3.1. Found: C, 50.1; H, 9.6; N, 3.0.

***N*^{G}-(2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine tri-*tert*-butoxysilyl ester hydrochloride [H-Arg(Pbf)-OSil × HCl] (31).** The starting material **18** contained 1 mole of H_2O per mole and was therefore dried as described for **17**: solidified foam, yield 0.26 g (39%), from H-Arg(Pbf)-OH **18** (400 mg, 0.938 mmol), pyridine (0.227 mL, 2.81 mmol), $SiCl_4$ (0.107 mL, 0.938 mmol), and *t*-BuOH (5 mL). Heating (50 °C) was required. The workup was performed in *t*-BuOH/pentane (1:9). IR (KBr) 1747 cm^{-1}; ^1H NMR ($CDCl_3$) significantly broad signals were obtained δ 9.6−7.6 (br m, 4H), 7.2−6.0 (br m, 2H), 4.22 (br s, 1H), 3.64−3.12 (m, 2 H), 3.13−2.80 (m, 2H), 2.75−1.50 (m, 13H), 1.47 (s, 6H), 1.30 (s, 27H); ^{13}C NMR ($CDCl_3$) very weak signals are reported in brackets δ 167.1, (158.9, 156.3, 138.7, 134.3, 132.6, 124.7, 117.6), 86.5, 74.9, 53.8, 43.5, 31.5, 28.8, 24.9, 19.8, 18.2, 12.6; PDMS (MW 673.0) 673.2 ($M + H^+$). Anal. Calcd for $C_{31}H_{57}ClN_4O_8SSi \times 2\ H_2O$: C, 49.9; H, 8.2; N, 7.5. Found C, 49.8; H, 8.0; N, 7.6.

L-Aspartic acid β-*tert*-butyl ester α-tri-*tert*-butoxysilyl ester hydrochloride [H-Asp(O*t*-Bu)-OSil × HCl] (32): oil, yield 3.62 g (77%), from H-Asp(O*t*Bu)-OH **19** (1.89 g, 10.0 mmol), pyridine (2.43 mL, 30.0 mmol), $SiCl_4$ (1.15 mL, 10.0 mmol), *t*-BuOH (15 mL), and pentane workup; IR (neat) 1751 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.72 (br s, 3H), 4.27 (br s, 1H), 3.40 (br d, $J = 18.5$ Hz, 1H), 3.05 (br d, $J = 18.5$ Hz, 1H), 1.46 (s, 9H), 1.32 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 169.5, 165.6, 83.2, 74.8, 50.5, 34.5, 31.4, 28.2; PDMS (MW 435.7) 436.8 ($M + H^+$). Anal. Calcd for $C_{20}H_{42}ClNO_7Si \times 1\ H_2O$: C, 49.0; H, 9.1; Cl, 7.2; N, 2.9. Found: C, 48.7; H, 8.9; Cl, 7.0; N, 2.8.

L-Aspartic acid β-9-fluorenylmethyl ester α-tri-*tert*-butoxysilyl ester hydrochloride [H-Asp(OFm)-OSil × HCl] (33): solidified foam, yield 3.63 g (95%), from H-Asp(OFm)-OH **5** (2.00 g, 6.42 mmol), pyridine (1.56 mL, 19.3 mmol), $SiCl_4$ (0.736 mL, 6.42 mmol), *t*-BuOH (40 mL), and CCl_4 workup; IR (KBr) 1751 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.92 (br s, 3H), 7.79−7.67 (m, 2H), 7.65−7.48 (m, 2H), 7.45−7.20 (m, 4H), 4.68−4.08 (m, 4H), 3.84−3.14 (m, 2H), 1.30 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 170.2, 165.3, 143.5, 143.3, 141.1, 127.7, 127.2, 125.4, 119.9, 74.7, 67.9, 50.7, 46.4, 34.1, 31.2; PDMS (MW 557.8) 559.2 ($M + H^+$). Anal. Calcd for $C_{30}H_{44}ClNO_7Si \times 2^1/_4\ H_2O$: C, 56.8; H, 7.7; N, 2.2. Found: C, 56.7; H, 7.5; N, 2.2.

L-Glutamic acid γ-*tert*-butyl ester α-tri-*tert*-butoxysilyl ester hydrochloride [H-Glu(O*t*-Bu)-OSil × HCl] (34): solidified foam, yield 2.33 g (82%), from H-Glu(O*t*Bu)-OH **20** (1.19 g, 5.86 mmol), pyridine (1.42 mL, 17.6 mmol), $SiCl_4$ (0.671 mL, 5.86 mmol), *t*-BuOH (**10** mL), and pentane workup; IR (neat) 1749, 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.80 (br s, 3H), 4.27 (br s, 1H), 2.78−2.21 (m, 4H), 1.44 (s, 9H), 1.32 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 172.3, 166.7, 81.5, 74.9, 53.3, 31.4, 31.0, 28.2, 25.2; PDMS (MW 449.7) 450.8 ($M + H^+$). Anal. Calcd for $C_{21}H_{44}ClNO_7Si \times 1^1/_2\ H_2O$: C, 49.2; H, 9.2; Cl, 6.9; N, 2.7. Found: C, 49.2; H, 8.9; Cl, 6.5; N, 2.7.

L-Glutamic acid γ-9-fluorenylmethyl ester α-tri-*tert*-butoxysilyl ester hydrochloride [H-Glu(OFm)-OSil × HCl] (35): solidified foam, yield 3.64 g (97%), from H-Glu(OFm)-OH **6** (2.01 g, 6.18 mmol), pyridine (1.50 mL, 18.5 mmol), $SiCl_4$ (0.708 mL, 6.18 mmol), *t*-BuOH (20 mL), and CCl_4 workup; IR (KBr) 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.97 (br s, 3H), 7.80−7.67 (m, 2H), 7.65−7.50 (m, 2H), 7.45−7.22 (m, 4H), 4.46−4.08 (m, 4H), 3.09−2.25 (m, 4H), 1.31 (s, 27H); ^{13}C NMR ($CDCl_3$) δ 172.5, 166.7, 144.0, 143.8, 141.3, 127.9, 127.4, 125.5, 120.0, 74.9, 67.2, 53.4, 46.8, 31.5, 29.9, 25.4; PDMS (MW 571.8) 572.6 ($M + H^+$). Anal. Calcd for $C_{31}H_{46}ClNO_7Si \times 2^1/_4\ H_2O$: C, 57.4; H, 7.9; N, 2.2. Found: C, 57.1; H, 7.6; N, 2.1.

D-Glutamic acid γ-9-fluorenylmethyl ester α-tri-*tert*-butoxysilyl ester hydrochloride [H-D-Glu(OFm)-OSil × HCl] (36): solidified foam, yield 4.46 g (95%), from H-D-Glu(OFm)-OH **7** (2.50 g, 7.68 mmol), pyridine (1.86 mL, 23.0 mmol), $SiCl_4$ (0.880 mL, 7.68 mmol), *t*-BuOH (15 mL), and CCl_4 workup; IR (KBr) 1744 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.95 (br s, 3H), 7.80−7.69 (m, 2H), 7.65−7.51 (m, 2H), 7.45−7.21 (m, 4H), 4.46−4.12 (m, 4H), 3.09−2.30 (m, 4H), 1.32 (s, 27H), ^{13}C NMR ($CDCl_3$) δ 172.5, 166.8, 144.0,

Table 6. Stability of Resin-Bound Compounds toward Cupric Chloride and Additives

TentaGel-P-linker-sequence	conditions(equiv)	time (h)	result
-Asp(OFm)-Leu-OH	$CuCl_2$ (1)	24	no cleavage
-Asp(OFm)-Gly-Asp(OFm)-Ser(*t*-Bu)-Pro-OH	$CuCl_2$ (1), TMP (4)	21	no cleavage
-Asp(OFm)-Gly-Asp(OFm)-Ser(*t*-Bu)-Pro-OBt[a]	$CuCl_2$ (1), TMP (4)	21	no cleavage
-Asp(OFm)-Leu-Glu(OFm)-OH	$CuCl_2$ (1), TBTU (4), TMP (9), Val-OSil (5)	2	cleavage of the Asp-Leu bond and Leu-Glu bond[b]
oxazolone of -Leu-Ala[c]	$CuCl_2$ (1)	2	no cleavage
oxazolone of -Leu-Ala[c]	$CuCl_2$ (1), TMP (4)	2	cleavage of the P-linker-Leu bond and Leu-Ala bond

[a] The OBt-ester of Pro was formed with TBTU and TMP. Because of the secondary nitrogen, Pro-OBt is prevented from forming the corresponding oxazolone. [b] LC-MS analysis of the products released from the solid support showed three major peaks corresponding to Asp(OFm)-NH_2 (mass 310.1, found M + H^+ 310.9), Asp(OFm)-Leu-NH_2 (mass 423.2, found M + H^+ 424.0), and Asp(OFm)-Leu-Glu(OFm)-Val-OH (mass 830.4, found M + H^+ 831.7). High NH_3 levels in the amino acid analysis also supported the amide formation. [c] The oxazolone was formed by treating the TentaGel-P-linker-5-Leu-Ala-OH sequence with TBTU and TMP for 30 min. The oxazolone formation/disappearance was monitored by IR using its characteristic peak at 1822 cm^{-1}.[43]

143.8, 141.4, 127.9, 127.4, 125.5, 120.0, 74.9, 67.2, 53.5, 46.8, 31.4, 30.0, 25.5; PDMS (MW 571.8) 572.7 (M + H^+). Anal. Calcd for $C_{31}H_{46}ClNO_7Si \times 1^1/_2\ H_2O$: C, 58.6; H, 7.8; N, 2.2. Found: C, 58.9; H, 7.6; N, 2.2.

Loading of the First Amino Acid (AA_1) for the Preparation of TentaGel-P-linker-Asp(OFm)-OH (38), TentaGel-P-linker-Leu-OH (39), and TentaGel-P-linker-Glu(OFm)-OH (40). Method I. Dry conditions were of importance in this synthesis. Aliquots of the vacuum-dried TentaGel-P-linker-5 **37** (0.10 g, 21 μmol) were swollen in DMF (1 mL) for 20−30 min in columns equipped with polypropylene frits or in Quest 210 columns. A solution of silylated amino acid was made by adding BSA (51.5 μL, 210 μmol) to the amino acid **5**, **6**, or **12** (105 μmol) in DMF (0.6 mL), in N_2 atmosphere, followed by heating. The solution was thereafter added to the resin followed by PP (3.11 mg, 21.0 μmol) dissolved in DMF (0.1 mL). The column was covered with aluminum foil and placed in an overhead mixer or was magnetically stirred in the Quest 210 for 2 h for product **39** but only 20 min for product **38** and **40**, to minimize hydrolysis of the Fm ester. The resin was washed with DMF (5 × 3 mL) and CH_2Cl_2 (4 × 3 mL). Removal of the TMS groups was accomplished by washing the resin two times with 2% TFA/CH_2Cl_2, followed by stirring for 2 min in 2% TFA/CH_2Cl_2 (3 mL). The TFA solution was removed by filtration, and the resin was subsequently washed with CH_2Cl_2 (5 × 3 mL) and MeOH (7 × 3 mL). The resin was dried in vacuo and analyzed by amino acid analyses and ^{13}C NMR.

Method II. Preparation of TentaGel-P-linker-Leu-OH (39). Essentially the same procedure as was used above, but H-Leu-OSil × HCl **24** (43.5 mg, 105 μmol) dissolved in DMF (0.5 mL) was added, instead of the TMS-silylated amino acid, to the swollen resin **37** (0.10 g, 21 μmol) followed by PP (18.6 mg, 126 μmol) dissolved in DMF (0.2 mL). The reaction was stirred for 35 min. Removal of the Sil group was performed with 5% TFA/CH_2Cl_2 for 20 min.

Preparation of the Resin-Bound Dipeptides: TentaGel-P-linker-Asp(OFm)-Leu-OH (41), TentaGel-P-linker-Leu-Ala-OH (42), TentaGel-P-linker-Glu(OFm)-Asp-(OFm)-OH (43), TentaGel-P-linker-Asp(OFm)-Ser(tBu)-OH (44), and TentaGel-P-linker-Asp(OFm)-D-Glu(OFm)-OH (45). Aliquots of the resins **38**−**40** (0.10 g, 21 μmol) were swollen in DMF (0.9 mL) for 20−30 min in columns equipped with polypropylene frits or in Quest 210 columns. A freshly prepared solution of the amino acid Sil ester hydrochloride **22**, **24**, **30**, **33**, or **36** (105 μmol) in CH_2Cl_2 (0.4 mL) was added to the swollen resin followed by a solution of TBTU (27.0 mg, 84.0 μmol) in DMF (0.1 mL). Finally, DIEA (32.3 μL, 189 μmol) was added. The column was covered with aluminum foil and placed in an overhead mixer or was magnetically stirred in the Quest 210 for 2 h. The resin was washed with DMF (5 × 3 mL) and CH_2Cl_2 (4 × 3 mL). Removal of the Sil group was accomplished by washing the resin two times with 5% TFA/CH_2Cl_2, followed by stirring for 20 min in 5% TFA/CH_2Cl_2 (4 mL). The TFA solution was removed by filtration, and the resin was subsequently washed with CH_2Cl_2 (5 × 3 mL) and MeOH (7 × 3 mL). The final resins were dried in vacuo and analyzed by amino acid analyses and ^{13}C NMR.

Preparation of the Resin-Bound Tripeptides: TentaGel-P-linker-Asp-Leu-Glu-OH (46), TentaGel-P-linker-Leu-Ala-Phe-OH (47), TentaGel-P-linker-Glu-Asp-Val-OH (48), TentaGel-P-linker-Asp-Ser-Ile-OH (49), and TentaGel-P-linker-Asp-D-Glu-Leu-OH (50). Aliquots of the resins **41**−**45** (45 mg, 9.5 μmol) were swollen in DMF (0.3 mL-0.4 mL, Tables 2−4) or CH_2Cl_2 (0.4 mL, Table 5) for 20−30 min in columns equipped with polypropylene frits or in Quest 210 columns. A freshly prepared solution of the amino acid Sil ester hydrochloride **23**−**25**, **28**, or **35** (47.3 μmol) in CH_2Cl_2 (0.2 mL) was added to the swollen resin followed by a solution of HATU or TBTU (37.8 μmol) in DMF (0.1 mL), and $CuCl_2$ (1 or 0.25 equiv, Tables 2−4) in DMF (0.1 mL) if used. Finally, DIEA or TMP (85.1 μmol) was added. The column was covered with aluminum foil and placed in an overhead mixer or magnetically stirred in the Quest 210 for 2 h. The resin was washed with DMF (5 × 3 mL) and CH_2Cl_2 (4 × 3 mL). Subsequent workup procedure was somewhat different for each tripeptide: Removal of the Sil group was accomplished by washing the resin two times with 5% TFA/CH_2Cl_2, followed by stirring for 20 min in 5% TFA/CH_2Cl_2 (4 mL). Removal of the Fm group was accomplished by washing the resin two times with 25% piperidine/DMF, followed by stirring in 25% piperidine/DMF for 20 min. The resin was washed with DMF (5 × 3 mL) and CH_2Cl_2 (4 × 3 mL), followed by removal of the piperidine salt by washing the resin with 5% TFA/CH_2Cl_2 (4 × 3 mL). Cleavage of the *t*-Bu ether was accomplished

by washing the resin two times with 25% TFA/CH_2Cl_2, followed by stirring in 25% TFA/CH_2Cl_2 (4 mL) for 20 min. After final acidic treatment, each resin-bound tripeptide was filtered off and washed with CH_2Cl_2 (5 × 3 mL) and MeOH (7 × 3 mL). The resins were dried in vacuo and analyzed by amino acid analyses and ^{13}C NMR.

Release of the Tripeptides (46*−50*) from the Resin. The resins **46**−**50** (10 mg each) were swollen in MeOH/H_2O (1:1) (0.5 mL) under N_2 atmosphere in Eppendorf tubes equipped with septa. The samples were placed in the photoreactor, and the magnetically stirred samples were irradiated for 3 h. The solid support was removed by filtration. The solutions were analyzed directly by RP-HPLC.

Determination of the LDL-Epimer Content in 46*−49* (LLL-Epimer for Sequence 50*). The epimeric tripeptides were separated by RP-HPLC by gradient elution; 10 to 20% of Solvent B (0.085% TFA/CH_3CN) in Solvent A (0.1% TFA [aqueous]), over 32 min; flow rate 0.7 mL/min; detection 220 nm. The epimer content was determined by integration of the peak areas ($A^{LDL}/(A^{LLL} + A^{LDL})$). The separated products were analyzed by PDMS. For sequence **50** the corresponding LLL-peptide was synthesized as a reference**.**

Stability of Resin-Bound Compounds toward Cupric Chloride and Additives. The general outline for the key experiments was identical to that described for previous couplings. The particular conditions used are indicated in Table 6. After being washed with DMF and CH_2Cl_2 the resins were analyzed by amino acid analysis and IR.

Acknowledgment. We gratefully acknowledge support from the Swedish Foundation for Strategic Research (SSF) and Medivir AB, Huddinge, Sweden. We also thank Carl-Johan Glans for assistance in the synthetic work.

Supporting Information Available. ^{13}C NMR of compounds **21**−**36** and of resin-bound compounds **46**−**50**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Merrifield, R. B. Solid-Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.
(2) Attwood, M. R.; Bennett, J. M.; Campbell, A. D.; Canning, G. G. M.; Carr, M. G.; Conway, E.; Dunsdon, R. M.; Greening, J. R.; Jones, P. S.; Kay, P. B.; Handa, B. K.; Hurst, D. N.; Jennings, N. S.; Jordan, S.; Keech, E.; O'Brien, M. A.; Overton, H. A.; King-Underwood, J.; Raynham, T. M.; Stenson, K. P.; Wilkinson, C. S.; Wilkinson, T. C. I.; Wilson, F. X. The Design and Synthesis of Potent Inhibitors of Hepatitis C Virus NS3-4A Proteinase. *Antiviral Chem. Chemother.* **1999**, *10*, 259−273.
(3) Chan, W. C.; White, P. D.; Beythien, J.; Steinauer, R. Facile Synthesis of Protected *C*-Terminal Peptide Segments by Fmoc/Bu^t Solid-Phase Procedures on *N*-Fmoc-9-amino-xanthen-3-yloxymethyl Polystyren Resin. *J. Chem. Soc., Chem. Commun.* **1995**, 589−592.
(4) Page, P.; Bradley, M. Solid-Phase Synthesis of Tyrosine Peptide Aldehydes. Analouges of (*S*)-MAPI. *J. Org. Chem.* **1999**, *64*, 794−799.
(5) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vágner, J.; Albericio, F.; Barany, G. Backbone Amide Linker (BAL) Strategy for Solid-Phase Synthesis of C−Terminal-Modified and Cyclic Peptides. *J. Am. Chem. Soc.* **1998**, *120*, 5441−5452.
(6) Bourne, G. T.; Meutermans, W. D. F.; Smythe, M. L. The Development of Solid Phase Protocols for a Backbone Amide Linker and Its Application to the Boc-Based Assembly of Linear Peptides. *Tetrahedron Lett.* **1999**, *40*, 7271−7274.
(7) Songster, M. F.; Barany, G. Handles for Solid-Phase Peptide Synthesis. In *Methods in Enzymology, Solid-Phase Peptide Synthesis*; Fields, G. B., Ed.; Academic Press: New York, 1997; Vol. 289, pp 126−174.
(8) Salvino, J. M.; Mervic, M.; Mason, H. J.; Kiesow, T.; Teager, D.; Airey, J.; Labaudiniere, R. Parallel Synthesis of Aldehydes and Ketones Facilitated by a New Solid-Phase Weinreb Amide. *J. Org. Chem.* **1999**, *64*, 1823−1830.
(9) Zheng, A.; Shan, D.; Wang, B. A Redox-Sensitive Resin Linker for Solid-Phase Synthesis of C−Terminal Modified Peptides. *J. Org. Chem.* **1999**, *64*, 156−161.
(10) Lelièvre, D.; Chabane, H.; Delmas, A. Simple and Efficient Solid-Phase Synthesis of Unprotected Peptide Aldehyde for Peptide Segment Ligation. *Tetrahedron Lett.* **1998**, *39*, 9675−9678.
(11) Kaljuste, K.; Tam, J. P. A Novel On-Resin Synthesis of *C*-Terminally Amidated Peptides. *Tetrahedron Lett.* **1998**, *39*, 9327−9330.
(12) Wang, P.; Shaw, K. T.; Whigham, B.; Ramage, R. Synthesis of Peptide C−Terminal Derivatives Using the Transfer Active Ester Condensation Technique. *Tetrahedron Lett.* **1998**, *39*, 8719−8720.
(13) Davies, M.; Bradley, M. Internal Resin Capture − A Self-Purification Method for the Synthesis of C-Terminally Modified Peptides. *Tetrahedron* **1999**, *55*, 4733−4746.
(14) Broadbridge, R. J.; Sharma, R. P.; Akhtar, M. First Efficient Synthesis of α-MAPI. *Chem. Commun.* **1998**, 1449−1450.
(15) Bayer, E.; Henkel, B.; Nicholson, G. Investigation of Racemization in N-to-C Direction (Inverse) Solid-Phase Peptide Synthesis. In *Peptides: Chemistry, Structure and Biology*, Proceedings of the 14th American Peptide Symposium, Columbus, Ohio, USA, June 18−23, 1995; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd.: England, 1996; pp 132−133.
(16) Bayer, E.; Henkel, B.; Zhang, L.; Clausen, N.; Goldammer, C.; Panhaus, G. New Approach to Peptide Chain Elongation in the N−C-Direction in Solid-Phase Synthesis ("Inverse Synthesis"). In *Peptides 1994*, Proceedings of the Twenty-Third European Peptide Symposium, Braga, Portugal, September 4−10, 1994; Maia, H. L. S., Ed.; ESCOM: Leiden, 1995; pp 201−202.
(17) Bleicher, K. H.; Wareing, J. R. PhFI Acetic Acid: A New Linker for Solid-Phase Organic Synthesis. *Tetrahedron Lett.* **1998**, *39*, 4591−4594.
(18) Bordusa, F.; Ullmann, D.; Jakubke, H.-D. Protease-Catalyzed Peptide Synthesis from N- to C-Terminus: An Advantageous Strategy. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1099−1101.
(19) Henkel, B.; Zhang, L.; Bayer, E. Investigation on Solid-Phase Peptide Synthesis in N-to-C Direction (Inverse Synthesis). *Liebigs Ann./Recueil.* **1997**, 2161−2168.
(20) Léger, R.; Yen, R.; She, M. W.; Lee, V. J.; Hecker, S. J. N-Linked Solid-Phase Peptide Synthesis. *Tetrahedron Lett.* **1998**, *39*, 4147−4174.
(21) Letsinger, R. L.; Kornet, M. J.; Mahadevan, V.; Jerina, D. M. Reactions on Polymer Supports. *J. Am. Chem. Soc.* **1964**, *85*, 5163−5165.
(22) Letsinger, R. L.; Kornet, M. J. Popcorn Polymer as a Support in Multistep Synthesis. *J. Am. Chem. Soc.* **1963**, *85*, 3045−3046.
(23) Felix, A. M.; Merrifield, R. B. Azide Solid-Phase Peptide Synthesis. *J. Am. Chem. Soc.* **1970**, *92*, 1385−1391.
(24) Mérette, S. A. M.; Green, D.; Patel, G.; Scully, M. F.; Kakkar, V. V.; Deadman, J. J. A General Method for *N* to *C* Terminal Solid-Phase Peptide Synthesis Applied to Bioactive Peptidic Boronates. In *Solid-Phase Synthesis & Combinatorial Libraries*, Proceedings of the Fifth International Symposium, London, England, U.K., September 2−6, 1997; Epton, R., Ed.; Mayflower Scientific Ltd.: Birmingham, England, U.K., 1999; pp 349−350.
(25) Sharma, R. P. Preparation of Peptides by a Solid-Phase Synthesis and Intermediates Therefor. WO 93/05065, March 18, 1993.
(26) Sharma, R. P.; Jones, D. A.; Corina, D. L.; Akhtar, M. A Novel Method of Solid-Phase Peptide Synthesis. In *Peptides: Chemistry, Structure and Biology*, Proceedings of the 13th American Peptide Symposium, 1993; Hodges, R. S., Smith, J., Eds.; ESCOM: Leiden, 1994; pp 127−129.
(27) Sharma, R. P.; Jones, D. A.; Broadbridge, R. J.; Corina, D. L.; Akhtar, M. A Novel Method of Solid-Phase Synthesis of Peptide Analogues. In *Innovation and Perspectives in Solid-Phase Synthesis*, Proceedings of the Third International Symposium, Oxford, England U.K., 1994; Epton, R., Ed.; Mayflower Worldwide Ltd.: Birmingham, U.K., 1994; pp 353−356.

(28) Alterman, M.; Andersson, H. O.; Garg, N.; Ahlsén, G.; Lövgren, S.; Classon, B.; Danielsson, U. H.; Kvarnström, I.; Vrang, L.; Unge, T.; Samuelsson, B.; Hallberg, A. Design and Fast Synthesis of C-terminal Duplicated Potent C_2−Symmetric P1/P1′-Modified HIV-1 Protease Inhibitors. *J. Med. Chem.* **1999**, *42*, 3835−3844.

(29) Hultén, J.; Andersson, H. O.; Schaal, W.; Danielsson, H. U.; Classon, B.; Kvarnström, I.; Karlén, A.; Unge, T.; Samuelsson, B.; Hallberg, A. Inhibitors of the C_2-Symmetric HIV-1 protease: Nonsymmetric Binding of a Symmetric Cyclic Sulfamide with Ketoxime Groups in the P2/P2′Side Chains. *J. Med. Chem.* **1999**, *42*, 4054−4061.

(30) Gruszecki, W.; Gruszecka, M.; Bradaczek, H. Stable Tri-*t*-Butoxysilyl Esters of Aspartic and Glutamic Acid as a New Protection of Their Carboxyl Group. In *Peptides 1990*, Proceedings of the Twenty-first European Peptide Symposium; Giralt, E., Andreu, D., Eds.; ESCOM: Leiden, 1991; pp 27−28.

(31) Johansson, A.; Åkerblom, E.; Lindeberg, G.; Hallberg, A. Solid-Phase Peptide Synthesis in the Inverse Direction. Hepatitis C Virus Protease Inhibitors. *Abstract of the Sixth International Symposium*, Solid-Phase Synthesis & Combinatorial Chemical Libraries, York, England, U.K., Aug 31−Sep 4, 1999, P 18, p 51.

(32) Gibson, S. E.; Guillo, N.; Middleton, R. J.; Thuilliez, A.; Tozer, M. J. Synthesis of Conformationally Constranied Phenylalanine Analogues via 7-, 8- and 9-*endo* Heck Cyclisations. *J. Chem. Soc., Perkin Trans. 1* **1997**, *1*, 447−455.

(33) Fink, W. Notiz zur Darstellung von N-Trimethylsilylmethyl-Glycin und Einiger Seiner Derivate. (Note on the Preparation of *N*-Trimethylsilylmethyl-Glycine and some of its Derivatives.) *Helv. Chim. Acta* **1974**, *57*, 1042−1044.

(34) E. Lilly & Co. Preparation of Amino Acids from Their Salts. U.S. Patent 2,404,503, July 23, 1946; *Chem. Abstr.* **1946**, 40,7233[2].

(35) Gruszecki, W.; Gruszecka, M.; Bradaczek, H. Protection of the Hydroxyl Group with Sil-Ester. In *Peptides 1992*, Proceedings of the Twenty-Second European Peptide Symposium, Interlaken, Switzerland, September 13−19, 1992; Schneider, C. H., Eberle, A. N., Eds.; ESCOM: Leiden, 1993; pp 200−201.

(36) Åkerblom, E. B.; Nygren, A. S.; Agback, K. H. Six New Photolabile Linkers for Solid-Phase Synthesis. 1. Methods of Preparation. *Mol. Diversity* **1998**, *3*, 137−148.

(37) Åkerblom, E. B. Six New Photolabile Linkers for Solid-Phase Synthesis. 2. Coupling of Various Building Blocks and Photolytic Cleavage. *Mol. Diversity* **1999**, *4*, 53−69.

(38) Dressman, B. A.; Spangle, L. A.; Kaldor, S. W. Solid-Phase Synthesis of Hydantoins Using a Carbamate Linker and a Novel Cyclization/Cleavage Step. *Tetrahedron Lett.* **1996**, *37*, 937−940.

(39) Bednarek, M. A.; Bodanszky, M. 9-Fluorenylmethyl Esters. *Int. J. Pept. Protein Res.* **1983**, *21*, 196−201.

(40) Chen, F. M. F.; Kuroda, K.; Benoiton, N. L. A Simple Preparation of 5-Oxo-4,5-dihydro-1,3-oxazoles (Oxazolones). *Synthesis* **1979**, 230−232.

(41) Benoiton, N. L.; Chen, F. M. F. 2-Alkoxy-5(4H)-Oxazolones from *N*-Alkoxycarbonylamino Acids and Their Implication in Carbodiimide-Mediated Reactions in Peptide Synthesis. *Can. J. Chem.* **1981**, *59*, 384−389.

(42) Bodanszky, M. *Peptide Chemistry*; Springer-Verlag: Berlin, Heidelberg, 1988; pp 115−118, 127−31.

(43) DeTar, D. F.; Silverstein, R.; Rogers, F. F. Reactions of Carbodiimides. III. The Reactions of Carbodiimides with Peptide Acids. *J. Am. Chem. Soc.* **1966**, *88*, 1024−1030.

(44) Albericio, F.; Carpino, L. A. Coupling Reagents and Activation. In *Methods in Enzymology, Solid-Phase Peptide Synthesis*; Fields, G. B., Ed.; Academic Press: New York, 1997; Vol. 289, pp 104−126.

(45) Fenza, A. D.; Tancredi, M.; Galoppini, C.; Rovero, P. Racemization Studies of Fmoc-Ser(tBu)-OH during Stepwise Continuous-Flow Solid-Phase Peptide Synthesis. *Tetrahedron Lett.* **1998**, *39*, 8529−8532.

(46) Romoff, T. T.; Goodman, M. Urethane-Protected *N*-Carboxyanhydrides (UNCAs) as Unique Reactants for the Study of Intrinsic Racemization Tendencies in Peptide Synthesis. *J. Pept. Res.* **1997**, *49*, 281−292.

(47) Carpino, L. A.; El-Faham, A.; Albericio, F. Racemization Studies during Solid-Phase Peptide Synthesis using Azabenzotriazole-Based Coupling Reagents. *Tetrahedron Lett.* **1994**, *35*, 2279−2282.

(48) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Alberico, F. Advantageous Applications of Azabenzotriazole (Triazolopyridine)-based Coupling Reagents to Solid-Phase Peptide Synthesis. *J. Chem. Soc., Chem. Commun.* **1994**, 201−203.

(49) Carpino, L. A.; El-Faham, A. Effect of Tertiary Bases on *O*-Benzotriazolyluronium Salt-Induced Peptide Segment Coupling. *J. Org. Chem.* **1994**, *59*, 695−698.

(50) Han, Y.; Albericio, F.; Barany, G. Occurrence and Minimization of Cysteine Racemization during Stepwise Solid-Phase Peptide Synthesis. *J. Org. Chem.* **1997**, *62*, 4307−4312.

(51) Miyazawa, T.; Donkai, T.; Yamada, T.; Kuwata, S. Effect of Copper(II) Chloride on Suppression of Racemization in Peptide Synthesis by the Mixed Anhydride and Related Methods. *Int. J. Pept. Protein Res.* **1992**, *40*, 49−53.

(52) Miyazawa, T.; Otomatsu, T.; Fukui, Y.; Yamada, T.; Kuwata, S. Effect of Copper(II) Chloride on Suppression of Racemization in Peptide Synthesis by the Carbodiimide Method. *Int. J. Pept. Protein Res.* **1992**, *39*, 237−244.

(53) Miyazawa, T.; Otomatsu, T.; Fukui, Y.; Yamada, T.; Kuwata, S. Simultaneous Use of 1-Hydroxybenzotriazole and Copper(II) Chloride as Additive for Racemization-Free and Efficient Peptide Synthesis by the Carbodiimide Method. *Int. J. Pept. Protein Res.* **1992**, *39*, 308−314.

(54) Rydanov, M. G.; Klimenko, L. V.; Mitin, Y. V. Suppression of Epimerization by Cupric (II) Salts in Peptide Synthesis using Free Amino Acids as Amino Components. *J. Pept. Res.* **1999**, *53*, 322−328.

(55) Benoiton, N. L.; Lee, Y. C.; Steinaur, R.; Chen, F. M. F. Studies on Sensitivity to Racemization of Activated Residues in Coupling of *N*-Benzyloxycarbonyldipeptides. *Int. J. Pept. Protein Res.* **1992**, *40*, 559−566.

(56) Haver, A. C.; Smith, D. D. Effect of Solvent on Racemization in Carbodiimide Mediated Solid-Phase Fragment Condensations. *Tetrahedron Lett.* **1993**, *34*, 2239−2242.

(57) Miyazawa, T.; Otomatsu, T.; Yamada, T.; Kuwata, S. Racemization Studies in Peptide Synthesis Through the Separation of Protected Epimeric Peptides by Reverse-Phase High Performance Liquid Chromatography. *Int. J. Pept. Protein Res.* **1992**, *39*, 229−236.

(58) Steinaur, R.; Chen, F. M. F.; Benoiton, N. L. Studies on Racemization Associated with the use of Benzotriazol-1-yl-tris (dimethylamino)-phosphonium hexafluorophosphate (BOP). *Int. J. Pept. Protein Res.* **1989**, *34*, 295−298.

(59) Shieh, W. C.; Carlson, J. A.; Shore, M. E. A Novel Kinetically-Controlled Peptide Synthesis − Dramatic Increase of Chemical Yield with Retention of Chiral Integrity. *Tetrahedron Lett.* **1999**, *40*, 7167−7170.

(60) Humphrey, J. M.; Chamberlin, A. R. Chemical Synthesis of Natural Product Peptides: Coupling Methods for the Incorporation of Noncoded Amino Acids into Peptides. *Chem. Rev.* **1997**, *97*, 2243−2266.

(61) Carpino, L. A.; El-Faham, A. The Diisopropylcarbodiimide/1-Hydroxy-7-azabenzotrizole System: Segment Coupling and Stepwise Peptide Assembly. *Tetrahedron* **1999**, *55*, 6813−6830.

(62) Carpino, L. A. 1-Hydroxy-7-azabenzotriazole. An Efficient Peptide Coupling Additive. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

(63) Sigel, H.; Martin, R. B. Coordination Properties of the Amide Bond. Stability and Structure of Metal Ion Complexes of Peptides and Related Ligands. *Chem. Rev.* **1882**, *82*, 385−426.

(64) Al-Obeidi, F.; Sanderson, D. G.; Hruby, V. J. Synthesis of β- and γ-Fluorenylmethyl Esters of Respectively N^{α}-Boc-L-Aspartic Acid and N^{α}-Boc-L-Glutamic Acid. *Int. J. Pept. Protein Res.* **1990**, *35*, 215−218.

(65) Al-Obeidi, F.; Castrucci, A. M. de L.; Hadley, M. E.; Hruby, V. J. Potent and Prolonged Acting Cyclic Lactam Analogues of α-Melanotropin: Design Based on Molecular Dynamics. *J. Med. Chem.* **1989**, *32*, 2555−2561.

(66) Albericio, F.; Nicolàs, E.; Rizo, J.; Ruiz-Gayo, M.; Pedroso, E.; Giralt, E. Convenient Synthesis of Fluorenylmethyl-Based Side Chain Derivatives of Glutamic and Aspartic Acids, Lysine, and Cysteine. *Synthesis* **1990**, 119−122.

(67) Belshaw, P. J.; Adamson, J. G.; Lajoie, G. A. Single Step Synthesis of ω-9-Fluorenylmethyl Esters of Aspartic and Glutamic Acids. *Synth. Commun.* **1992**, *22*, 1001−1005.

(68) Hoekstra, W. J.; Sunder, S. S.; Cregge, R. J. Large-Scale Synthesis of Anticoagulant Decapeptide MDL 28050. *Tetrahedron* **1992**, *48*, 307−318.

(69) Ishida, K.; Matsuda, H.; Murakami, M. Micropeptins 88-A to 88-F, Chymotrypsin Inhibitors from the Cyanobacterium *Microcystis Aeruginosa* (NIES-88). *Tetrahedron* **1998**, *54*, 5545−5556.

(70) Schuda, P. F. A Short and Efficient Synthesis of (3S,4S)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-3-hydroxypentanoic Acid Ethyl Ester. *J. Org. Chem.* **1988**, *53*, 873−875.

CC000022H