Journal of Peptide Science, Vol. 1, 80–87 (1995)

Multiple Synthesis by the Multipin Method as a Methodological Tool

ANDREW M. BRAY, ROBERT M. VALERIO, ANGELA J. DIPASQUALE, JOY GREIG and N. JOE MAEJI

Chiron Mimotopes Pty Ltd, Victoria, Australia

Received 13 May 1994
Accepted 24 May 1994

Abstract: The multipin method of peptide synthesis is demonstrated as a potent methodological tool, where large numbers of comparative studies can be performed concurrently. Two studies are presented. In each study, the test peptides were simultaneously synthesized, and the products examined by high throughput ion spray mass spectrometry and reverse-phase HPLC. In the first study, comprising 24 experiments, peptides **1** (AELFSTHYLAFKEDYSQ-NH_2) and **2** (LKDFRVYFREGRDQLWKGPG-NH_2) were prepared using Fmoc-Axx/BOP/HOBt/NMM (100:100:100:150 mM) and Fmoc-Axx/HATU/HOAt/NMM (100:100:100:150 mM) with 60, 90 and 120 min coupling times. The two reagent combinations were found to give comparable results. The second study compared the N-terminal coupling of Fmoc-Asn-OH, Fmoc-Asn(Mbh)-OH, Fmoc-Asn(Mtt)-OH, Fmoc-Asn(Tmob)-OH and Fmoc-Asn(Trt)-OH in the synthesis of seven test peptides: **3**, NVQAAIDYIG-cyclo(KP); **4**, NTVQAAIDYIG-cyclo(KP); **5**, NRVYVHPFNL; **6**, NRVYVHPFHL; **7**, NEAYVHDAPVRSLN; **8**, NQLVVPSEGLYLIYSQVLFK; **9**, NPNANPNANPNA. A total of 33 experiments were performed. Peptides **3** and **4** were selected to highlight the effect of steric bulk of each Asn derivative on coupling efficiency. Reagent efficiency, as measured by target peptide purity, was as follows: Fmoc-Asn(Tmob)-OH > Fmoc-Asn-OH > Fmoc-Asn(Mtt)-OH = Fmoc-Asn(Trt)-OH > Fmoc-Asn(Mbh)-OH.

Keywords: Multiple synthesis, optimizing peptide synthesis, N-terminal asparagine, BOP, HATU

Abbreviations

ACP, acyl carrier protein; Axx, amino acid; BOP, benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; DIC, diisopropylcarbodiimide; DKP, diketopiperazine; EDT, ethanedithiol; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, 2-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU, 2-(benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HEMA, hydroxyethyl methacrylic acid; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; Mbh, 4,4′-dimethoxybenzhydryl; MeCN, acetonitrile; MeOH, methanol; MS, ion spray mass spectrometry; Mtt, 4-methyltrityl; NMM, *N*-methylmorpholine; PhOMe, anisole; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Tmob, 2,4,6-trimethoxybenzyl; Trt, trityl.

INTRODUCTION

The ability to simultaneously synthesize many copies of a given compound while varying many parameters gives rise to a rapid, yet powerful, methodological tool

Author for correspondence: Dr. A. M. Bray, Chiron Mimotopes Pty Ltd, 11 Duerdin Street, Clayton, Victoria 3168, Australia.

CCC 1075-2617/95/010080-08

[1]. A parallel rather than serial optimization strategy allows for concurrent variation of several parameters. This is preferable to methodological studies where each parameter under examination is treated as being independent. If an 'optimized' approach is intended to be a general one, several targets should be included to allow for statistical evaluation. All of these considerations contrast with methodological studies where conclusions have been reached with relatively few syntheses.

The multipin method [2, 3] is particularly well suited to multiparameter methodological studies as it enables large numbers of compounds to be handled with relative ease. This is because the discrete solid supports upon which synthesis is performed are arranged in an 8×12 matrix format on a polypropylene holder. While unique reactions can be readily performed on each individual pin (in wells that match the 8×12 format), common reactions can be performed simultaneously in large baths, hence under identical conditions.

In this paper, two studies utilizing parallel handling for methodological development are presented. In the first, coupling agents BOP [4] and HATU [5, 6] are compared. Recently, Carpino [5] reported a potent coupling combination based on HOAt, an aza-analogue of the well-established auxiliary nucleophile, HOBt [7], and HATU, the corresponding aza-analogue of HBTU [8]. HATU and HOAt have already attracted much interest, being espoused for the synthesis of 'difficult' sequences. Consequently, we compared this reagent combination with BOP/HOBt, which is generally used in our laboratory.

In the second study, the issue of the most appropriate Asn derivative for N-terminal peptide capping was explored. Although Fmoc-Asn(Trt)-OH [9] has found general acceptance in Fmoc-based solid-phase synthesis, it has been noted that the rate of Trt deprotection can be unsatisfactory when Asn with an unblocked α-amino function is located at the N-terminus of a peptide [10, 11]. This seems to be due to the close proximity of the protonated α-amino function and so is not encountered with capped or non-N-terminal Asn residues. In order to overcome the problem, the more labile methyltrityl derivative, Fmoc-Asn(Mtt)-OH [10], was developed. When we have used this derivative in our laboratory, we have found instances where coupling is incomplete. Incomplete incorporation had also been encountered with Fmoc-Asn(Trt)-OH. Consequently, a comprehensive study was undertaken to determine the best conditions for introducing N-terminal Asn residues. The study included Fmoc-Asn-OH, Fmoc-Asn(Tmob)-OH [12] and Fmoc-Asn(Mbh)-OH [12].

Our laboratory regularly uses the multipin approach to assess new reagents for peptide synthesis. Generally, a new reagent would be assessed under a range of conditions, and with a selection of target compounds. These studies are performed concurrently, with variation being limited only to those steps where the new reagent is being employed. In the two studies presented here, 57 experiments were performed. The comprehensive data sets obtained from these studies enables unambiguous choices to be made in the selection of optimized reaction conditions.

MATERIALS AND METHODS

Peptides (see Table 1) were prepared on polyethylene pins with detachable heads (crowns), which had been radiation grafted with HEMA [3]. The comparative studies between BOP and HATU studies were performed on crowns that were functionalized with the Rink amide-forming handle [13, 14] (loading: 1 μmol/crown). The N-terminal Asn studies were performed on crowns functionalized with Fmoc-protected amino acid esters of hydroxymethylphenoxyacetic acid [13, 15] (5 μmol/crown) or DKP-forming handle [13, 16] (1 μmol/crown). Fmoc-protected amino acids were used; those requiring side-chain protection were as follows: Arg (Pmc), Asn (Trt), Asp(O*t*Bu), Gln (Trt), Glu (O*t*Bu), His (Boc), Lys (Boc), Ser (*t*Bu), Thr (*t*Bu), Tyr (*t*Bu) and Trp (Boc). The following Fmoc protected Asn derivatives were used for the final coupling in the N-terminal Asn study: Asn, Asn (Mbh), Asn (Mtt), Asn (Tmob) and Asn (Trt). Couplings were performed in distilled DMF using Fmoc-Axx/BOP/HOBt/NMM (100:100:100:150 mM) for 1 μmol crowns (150 μl/crown), and Fmoc-Axx/BOP/HOBt/NMM (139:139:139:209 M) for 5 μmol crowns (450 μl/crown). HATU-mediated couplings used the following conditions: Fmoc-Axx/HATU/HOAt/NMM (100:100:100:150 mM). Fmoc-Asn-OH was coupled using Fmoc-Asn-OH/DIC/HOBt (100:100:120 mM). Reagents were mixed 10 min prior to coupling. Couplings were performed in 1 ml Beckman deep well microtitre trays (polypropylene). Unless otherwise stated, couplings were performed at 25 °C for 2 or 16 h. Bromophenol blue [17] (50 μM)

Table 1. Test Peptides Used in This Study: General Characterization Data

	Sequence	HPLC retention time	MS data		Notes
			MW (obs.) (g/mol)	MW (calcd) (g/mol)	
1	AELFSTHYLAFKEDYSQ-NH_2	17.53	2048.0	2048.2	
2	LKDFRVYFREGRDQLWKGPG-NH_2	17.82	2466.9	2466.8	
3	NVQAAIDYIG-cyclo(KP)[a]	17.51	1270.4	1270.5	ACP 65-74 derived
4	NTVQAAIDYIG-cyclo(KP)[a]	18.00	1371.7	1371.6	ACP 64-74 derived
5	NRVYVHPFNL-OH	18.08	1258.4	1258.5	Angiotensin I (salmon)
6	NRVYVHPFHL-OH	17.85	1281.5	1281.5	Angiotensin I (goosefish)
7	NEAYVHDAPVRSLN-OH	15.30	1584.7	1584.7	IL-1β converting enzyme substrate
8	NQLVVPSEGLYLIYSQVLFK-OH	20.83	2310.6	2310.7	TNF α 46–65
9	NPNANPNANPNA-OH	12.83	1207.2	1207.2	Circumsporozoite protein derived

[a] cyclo (KP) = –Lys-Pro–

was included in all coupling reactions to monitor coupling efficiency. The Fmoc deprotection cycle was as follows: 20% piperidine/DMF (20 min), DMF (2 min), MeOH (3 × 2 min) and air drying.

Peptide cleavage by acidolysis was performed using either TFA/PhOMe/EDT (95:2.5:2.5, v/v/v) or TFA/PhOMe/H_2O/PhSMe/EDT (82.5:5:5:5:2.5, v/v/v/v/v) for 2.5 h at 25 °C. The cleavage solutions were evaporated, the products triturated with ethyl ether/petrol (1:2), the solutions centrifuged and decanted, and the trituration process repeated.

Peptide cleavage by DKP formation was preceded by side-chain deprotection with TFA/PhOMe/EDT (95:2.5:2.5, v/v/v) for 2.5 h. The crowns were then soaked in 0.1% AcOH in 50% MeOH (aq.) for 60 min, and then H_2O for 5 min. Cleavage was then performed in 0.1 M $(NH_4)HCO_3$ in 40% MeCN (aq.), (800 µl/crown) for 1 h under the action of high-power sonication. The resulting solutions were then dried under vacuum.

Analytical HPLC was performed on a Waters chromatography system using a Merck Hibar LiChrosphere 100RP-18 column (250 × 4 mm, 5 µm). The following conditions were used: solvent A = 0.1% TFA (aq.); solvent B = 0.1% TFA in 60% MeCN (aq.); linear gradient A to B from 5 to 20 min; flow rate = 1.5 ml min^{-1}. Absorbances were recorded at 214 and 254 nm. HPLC purities were determined by peak area at 214 nm.

MS analysis was performed on a Perkin Elmer Sciex API III ion spray mass spectrometer. The data were processed by software developed at Chiron Mimotopes Pty Ltd [18].

RESULTS

Comparison of BOP/HOBt and HATU/HOAt

Peptides **1** and **2** were selected to compare BOP/HOBt and HATU/HOAt over a range of coupling times. Peptide **1** is relatively difficult to assemble, with truncated 9-mer (LAFKEDYSQ-NH_2) being a major by-product. The peptides were assembled using 60, 90 and 120 min coupling times; coupling concentrations were fixed at 100 mM and NMM was used as base. Each synthesis was performed in duplicate, with 24 syntheses in all. Following cleavage, the peptides were analysed by MS and analytical HPLC. The data are presented in Tables II and III.

In the case of peptide **1**, the HATU/HOAt combination consistently gave a higher purity product by both HPLC and MS than did BOP/HOBt. The differences were especially marked with the 60 min coupling time; the results suggesting faster coupling rates with HATU/HOAt. The 60 min coupling with HATU/HOAt gave better results than did any of the BOP/HOBt couplings. Nevertheless, with longer coupling times, peptide quality improved with both reagent combinations. For a given coupling time, BOP generally yielded more specific deletion products than did HATU. This was most notable for

Table 2. Comparison of BOP vs. HATU in the Synthesis of Peptide **1**: Target Purity, and Identity and % Composition of Minor Peptidic By-products

Reagent	Coupling time (min)	% Purity		% Composition of minor components by ion spray MS							
				Truncation products		Deletion products					
		HPLC[e]	MS	9 mer[d]	10 mer[e]	Y	T	E	H	H&Y	H&T
BOP/HOBt[a]	60	21.1	30.7	9.0	3.2	7.3	5.5	4.6	2.0	2.1	2.3
		24.8	33.2	6.8	2.4	7.1	4.2	5.5	2.0	1.9	2.8
	90	29.0	30.3	8.0	3.9	7.9	5.2	4.0	2.4	2.4	2.7
		30.2	33.1	8.1	3.2	8.2	4.4	4.9	2.7	2.0	2.4
	120	33.5	36.3	7.6	2.6	7.5	3.9	4.5	2.6	1.6	2.3
		34.3	35.1	7.5	3.6	7.5	3.8	4.4	2.2	1.6	2.9
HATU/HOAt[b]	60	35.1	36.5	6.9	2.9	7.1	4.7	3.4	1.9	2.1	2.0
		36.0	39.0	6.6	2.2	7.2	4.6	5.2	1.8	1.5	1.5
	90	32.8	35.4	7.4	3.8	7.4	3.7	3.8	2.0	2.1	1.8
		35.0	36.2	8.2	3.9	7.1	4.5	4.3	1.5	1.7	1.3
	120	38.6	38.6	5.7	3.2	5.6	3.8	3.8	1.9	ND	1.7
		37.8	39.7	6.6	3.5	7.0	3.3	4.0	1.8	1.6	1.8

[a] Fmoc-Axx/BOP/HOBt/NMM (100:100:100:150mM) in DMF, 25°C
[b] Fmoc-Axx/HATU/HOAt/NMM (100:100:100:150mM) in DMF, 25°C
[c] Determined by peak area at 214 nm.
[d] LAFKEYSQ-NH_2.
[e] YLAFKEDYSQ-NH_2.

Table 3. Comparison of BOP vs. HATU in the Synthesis of Peptide: Target Purity, and Identity and % Composition of Minor Peptidic By-products

Reagent	Coupling time (min)	% Purity		% Composition of by products by MS Deletion products		
		HPLC[e]	MS	V	R	Y
BOP/HOBt[a]	60	67.2	63.6	2.0	0.7	[d]
		69.3	63.6	2.1	3.4	3.9
	90	70.1	65.4	1.6	2.7	3.3
		69.6	65.1	1.7	2.7	3.3
	120	75.4	70.6	1.2	1.7	2.2
		78.9	74.1	1.5	1.6	2.2
HATU/HOAt[b]	60	64.5	62.7	1.3	2.0	2.6
		64.4	63.3	1.5	2.4	2.9
	90	67.1	66.9	1.0	0.9	1.3
		65.7	63.3	1.5	2.4	2.9
	120	71.4	67.9	1.1	1.3	2.1
		70.3	66.0	[d]	1.3	1.9

[a] Fmoc-Axx/BOP/HOBt/NMM (100:100:100:150mM) in DMF, 25°C
[b] Fmoc-Axx/HATU/HOAt/NMM (100:100:100:150mM) in DMF, 25°C
[c] Determined by peak area at 214 nm.
[d] Not detected.

the 9-mer truncation product formed in the synthesis of **1**, where BOP gave rise to 2–3% more than did HATU for the three coupling times. In most cases, however, the differences were small.

This general trend was also true in the case of **2**, even though in this instance BOP consistently yielded the higher purity product. Many low-level by-products observed in the MS were not identified; in the case of **2**, these were more prevalent when HATU had been used.

Incorporation of N-Terminal Asn Residues

Seven N-terminal Asn peptides, **3–9**, were selected for this model study. The N-terminal residues were incorporated using five Asn derivatives: Fmoc-Asn(Mbh)-OH, Fmoc-Asn(Mtt)-OH, Fmoc-Asn(Tmob)-OH and Fmoc-Asn(Trt)-OH were coupled with BOP/NMM/HOBt. To avoid nitrile formation [12] Fmoc-Asn-OH was coupled with DIC/HOBt. Fmoc-Asn(Mbh)-OH was not used in the synthesis of **3** or **4**. HPLC and MS data are presented in Tables 4 and 5 respectively.

Peptides **3** and **4** were selected to study the effect of the steric bulk of the Asn derivatives on coupling efficiency. Peptide **3** is based on the ACP 65–74 sequence [15], which is well known for the difficulty of couplings following Gln. Peptide **4** includes the native Thr residue, which further increases the difficulty of subsequent couplings. The Asn residue which occurs at position 73 in the native sequence was deleted to simplify analysis. The efficiency of assembly of peptides **3** and **4** appeared to depend on the degree of steric bulk peculiar to each of the Asn derivatives used in the study. As shown in Tables 4 and 5, the degree of incorporation of Asn into **3** was greatest when Fmoc-Asn-OH was used; HPLC indicated 90% purity and truncation product was not observed by MS. In contrast, Fmoc-Asn(Mtt)-OH and Fmoc-Asn(Trt)-OH gave HPLC purities of 71% and 62% respectively, with MS revealing substantial amounts of truncation product. The less-sterically demanding Fmoc-Asn(Tmob)-OH gave a product of intermediate quality (85%). Peptide **4** yielded similar results, although truncated peptide was observed in all cases. In this instance Fmoc-Asn-OH was only marginally superior to Fmoc-Asn(Tmob)-OH, as assessed by MS, and equivalent by HPLC. Products arising from dehydration of the Asn carboxamide moiety were present at levels <1% in all cases, including those where Fmoc-Asn-OH was employed.

To further compare the reagent in general peptide synthesis, a range of N-terminal Asn peptides (**5–9**) were selected for study. Fmoc-Asn(Mbh)-OH consistently gave low-purity products due to incomplete side-chain deprotection, while the other four derivatives were generally comparable. When compared across the set of seven peptides (**3–9**) Fmoc-Asn(Tmob)-OH gave the overall superior performance with an average HPLC purity of 77 ± 11% being achieved. It is interesting to note that the low cost Fmoc-Asn-OH compared very favourably with the far more expensive side-chain protected species, delivering an average HPLC purity of 75 ± 12%. Two

Table 4. HPLC Purities of Test Peptides **3–9**: Comparison of N-Terminal Asn Incorporation Efficiency for a Range of Fmoc-Asn(X)-OH

Peptide	Purity[a] (%)				
	X = H	X = Mbh[b]	X = Mtt	X = Tmob	X = Trt
3	90	ND[c]	71	85	62
4	66	ND	49	66	59
5	76	43	79	78	78
6	74	44	85	87	81
7	80	31	84	82	82
8	52	22	50	56	49
9	86	41	82	83	84

[a] Determined by peak integration at 214 nm.
[b] Incomplete Mbh deprotection was observed with all peptides prepared using Fmoc-Asn(Mbh)-OH.
[c] ND: not done.

Table 5. MS Purities [18] of Test Peptides **3–9**. Comparison of N-Terminal Asn Incorporation Efficiency for a Range of Fmoc-Asn(X)-OH

Peptide	Purity[a] (%)				
	X = H	X = Mbh[b]	X = Mtt	X = Tmob	X = Trt
3	80	ND[d]	60 (20)	74 (7)	52 (22)
4	51 (14)	ND	30 (25)	45 (20)	49 (21)
5	67 (2)	51 (1)	69 (2)	70 (2)	69 (2)
6	81 (2)	52 (1)	85 (1)	83 (1)	85 (2)
7	80	48	80	80	80
8	60 (4) [1]	42 (3)	63	66 (4)	66 (5)
9	66 [9]	47 [2]	63 [4]	69 [4]	70 [4]

[a] Figures in parentheses () are % composition of the respective des-Asn by product. Figures in square brackets [] are % dehydration and deammonation product observed. Unless otherwise indicated values < 1% obtained.
[b] Incomplete Mbh deprotection was observed with all peptides prepared using Fmoc-Asn(Mbh)-OH.
[c] 13% truncated 4-mer (PFNL) observed in all cases.
[d] ND: not done.

instances of significant dehydration were observed with Fmoc-Asn-OH, the most significant being **9** which contained 5% dehydration product over and above the 4% observed across the set for this Asn-rich peptide. In the second case, crude **8** contained 1% dehydration product. Although the Trt and Mtt derivatives performed well with peptides **6**, **7** and **9**, respective overall mean HPLC purities of 71 ± 13% and 71 ± 14% were achieved with these reagents. Based on these results, reagent efficiency, as measured by target peptide purity, was as follows: Fmoc-Asn(Tmob)-OH > Fmoc-Asn-OH > Fmoc-Asn (Mtt)-OH=Fmoc-Asn(Trt)-OH > Fmoc-Asn(Mbh)-OH.

DISCUSSION

Coupling studies using BOP/HOBt and HATU/HOAt in the preparation of **1** and **2** demonstrated that both coupling combinations gave comparable results: HATU gave a superior result for **1**, while BOP gave the best result for **2**. The level of deletion and truncation products was generally lower with HATU, although in the case of **2** a greater number of non-assignable, low-level products were observed. We had previously settled on BOP as our primary coupling reagent after studies of a range of coupling reagents that are in common use (not reported). Although reagent performance is comparable, cost considerations currently favour the BOP/HOBt combination.

In the study of Asn derivatives, Fmoc-Asn(Tmob)-OH was found to give the overall best performance. This is most likely attributable to its relatively small steric bulk when compared with the trityl-based derivatives. Furthermore, masking of the carboxamide function allows for the use of BOP/HOBt, a generally more efficient coupling combination than DIC/HOBt. A potential drawback for the general use of Fmoc-Asn(Tmob)-OH is that the unprotected Trp indole function is prone to electrophilic attack by the trimethoxybenzyl cation during side-chain deprotection [12]. This alkylation, however, is unlikely where Fmoc-Trp(Boc)-OH is used in peptide assembly, as the -Trp(CO_2H)- intermediate generated during side-chain deprotection is highly resistant to attack by electrophiles [19].

Each methodological study presented in this paper comprised a significant number of discrete syntheses: 24 in the study comparing BOP/HOBt and HATU/HOAt, and 33 in the study on N-terminal Asn coupling. Using the multipin method, it was possible to perform all experiments within a given study concurrently. Without access to simultaneous multiple synthesis, these potentially laborious studies would probably be performed with fewer than the 57 syntheses reported here, or over a longer time period. Small-scale multiple synthesis not only minimizes the time, labour and cost associated with methodological studies, but also increases the confidence in the results of these studies due to the

numbers being handled. Furthermore, there is the opportunity to vary conditions more systematically, or over a more closely spaced range of concentrations, etc.

The methodological implications of multiple synthesis have been previously pointed out [1]. In principle, such studies can be performed by many of the methods of multiple peptide synthesis [20] now available. Large numbers are accessible with resin-based robotic multiple peptide synthesizers, although design and programming limitations restrict the chemistries that could be performed. The high degree of flexibility available with a robot designed for a wide range of chemistries as in the 'diversomer' approach [21] is appealing but probably beyond the budget of most laboratories. To be of general utility, comprehensive methodological studies require low-cost equipment which can be handled in a flexible manner, yet with the ability to cope with large numbers simultaneously. These criteria are met by the multipin approach. An additional concern is the compatibility, or otherwise, of the chemistries of interest and the support. For example, cellulose paper [22] and cotton [23] are not suited to 'sub-monomer' chemistry [24], a method able to access many unusual peptides. In this instance, ester linkages within the support would not tolerate the nucleophilic amines used in the method. This is an important general consideration as an increasing amount of novel peptide chemistry and non-peptide chemistry is being explored. As a wide range of polymers can be grafted to polyethylene pins [2, 3, 25, 26], the multipin system can be applied to a diverse range of chemistries.

The multipin method was initially developed for mass biological screening purposes [2]. By employing similar concepts of mass handling, synthetic methodological studies involving large ranges of conditions or reagents could be tackled, opening the way for multiple 'reaction screening'. By coupling multipin synthesis with high throughput analytical methods, in this case MS and reverse phase HPLC, this becomes a possibility. Of the high throughput analytical methods that we have assessed, MS has the greatest potential. Workers at our laboratory have developed new software for the API III ion spray mass spectrometer, allowing the rapid identification and quantification of all peptidic products [18]. During the development of the MS method, over 3000 peptides were compared by MS and HPLC.

The results presented here demonstrate the viability of mass methodological studies by the multipin method. Although the method is presented here in the context of peptide chemistry, multipin optimization strategies are equally valid for other forms of solid phase organic chemistry [24, 27].

Acknowledgements

The authors thank Liana Lagniton, Philip Rea, Michael Fitzgerald and Swee Smart for their excellent technical support. The MS program was written by Dr Tom Mason and Paul Bennell. We thank the Millipore Corporation for a gift sample of HATU and HOAt.

REFERENCES

1. D. Hudson (1988). Methodological implications of simultaneous solid-phase peptide synthesis. 1. Comparison of different coupling procedures. *J. Org. Chem. 53*, 617–624.
2. H. M. Geysen, R. H. Meloen and S. J. Barteling (1984). Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proc. Nat. Acad. Sci. USA 81*, 3998–4002.
3. R. M. Valerio, A. M. Bray, R. A. Campbell, A. DiPasquale, C. Margellis, S. J. Rodda and H. M. Geysen (1993). Multipin peptide synthesis at the micromole scale using 2-hydroxyethyl methacrylate grafted polyethylene supports. *Int. J. Peptide Protein Res. 42*, 1–9.
4. B. Castro, J. R. Dormoy, G. Evin and C. Seive (1975). Reactifs de couplage petidique IV (1)–1'hexafluorophosphate de benzotriazolyl *N*-oxytrisdimethylamino phosphonium (B.O.P.). *Tetrahedron Lett.* 1219–1222.
5. L. A. Carpino (1993). 1-Hydroxy-7-azabenzotriazole (HOAt). An efficient peptide coupling additive. *J. Am. Chem. Soc. 115*, 4397–98.
6. L. A. Carpino and A. El-Faham (1994). The effect of tertiary bases on *o*-benzotriazolyluronium salt-induced peptide segment coupling. *J. Org. Chem. 59*, 695–698.
7. W. Konig and R. Geiger (1973). *N*-Hydroverbindungen als Katalysatoren für die Aminolyse aktivierter Ester. *Chem. Ber. 106*, 3626–3635.
8. R. Knorr, A. Trzeciak, W. Bannwarth and D. Gillessen (1989). New coupling reactions in peptide chemistry. *Tetrahedron Lett. 30*, 1927–1930.
9. P. Sieber and B. Riniker (1991). Protection of carboxamide functions by the trityl residue. Application to peptide synthesis. *Tetrahedron Lett. 32*, 739–742.
10. B. Sax, F. Dick, R. Tanner and J. Gosteli (1992). 4-Methyltrityl (Mtt): A new protecting group for the side chain protection of Asn and Gln in solid-phase peptide synthesis. *Peptide Res. 5*, 245–246.

11. M. Friede, S. Denery, J. Neimark, S. Kieffer, H. Gausepohl and J. P. Briand in: *Peptides 1992*, C. H. Schneider and A. N. Eberle, Eds., p. 253–254, ESCOM Science Publishers B.V., Leiden 1993.
12. H. Gausepohl, M. Kraft and R. Frank (1989). Asparagine coupling in Fmoc solid phase peptide synthesis. *Int. J. Peptide Protein Res. 34*, 287–294.
13. R. M. Valerio, A. M. Bray and N. J. Maeji (1994). Multiple peptide synthesis on acid-labile handle derivatized polyethylene supports. *Int. J. Peptide Protein Res.*, in press.
14. H. Rink (1987). Solid-phase synthesis of protected peptide fragments using a trialkoxydiphenyl–methylester resin. *Tetrahedron Lett. 28*, 3878–3790.
15. E. Atherton, C. J. Logan and R. C. Sheppard (1981). Peptide synthesis. Part 2. Procedures for solid-phase synthesis using N^{α}-fluorenylmethoxycarbonylaminoacids on polyamide supports. Synthesis of substance P and acyl carrier protein 65-74 decapeptide. *J. Chem. Soc., Perkin Trans. I*, 538–546.
16. A. M. Bray, N. J. Maeji, R. M. Valerio, R. A. Campbell and H. M. Geysen (1991). Direct cleavage of peptides from a solid support into aqueous buffer. Application in simultaneous multiple peptide synthesis. *J. Org. Chem. 56*, 6659–6666.
17. V. Krchnak and J. Vagner (1990). Color-monitored solid-phase multiple peptide synthesis under low-pressure continuous-flow conditions. *Peptide Res. 3*, 182–193.
18. S. S. Smart, T. J. Mason, P. S. Bennell, N. J. Maeji and H. M. Geysen. Synthetic peptide purity and characterisation by mass spectrometry. Submitted.
19. P. White in: *Peptides, Chemistry and Biology*, J. A. Smith and J. E. Rivier, Eds., p. 537–538, ESCOM Science Publishers B.V., Leiden 1992.
20. G. Jung and A. G. Beck-Sickinger (1992). Multiple peptide synthesis methods and their applications. *Angew. Chem. 31*, 367–383.
21. S. H. DeWitt, J. S. Kiely, C. J. Stankovic, M. C. Schroeder, D. M. Reynolds Cody and M. R. Pavia (1993). 'Diversomers': An approach to nonpeptide, nonoligomeric chemical diversity. *Proc. Nat. Acad. Sci. USA 90*, 6909–6913.
22. R. Frank and R. Doring (1988). Simultaneous multiple peptide synthesis under continuous flow conditions on cellulose paper discs as segmental solid supports. *Tetrahedron*, 6031–6040.
23. J. Eichler, M. Bienert, A. Stierandova and M. Lebl (1991). Evaluation of cotton as a carrier for solid-phase peptide synthesis. *Peptide Res. 4*, 296–307.
24. R. N. Zuckermann, J. M. Kerr, S. B. H. Kent and W. H. Moos (1992). Efficient method for the preparation of peptoids [oligo(*N*-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc. 114*, 10646–10647.
25. R. H. Berg, K. Almdal, W. B. Pedersen, A. Holm, J. P. Tam and R. B. Merrifield (1989). Long-chain polystyrene-grafted polyethylene film matrix: A new support for solid-phase peptide synthesis. *J. Am. Chem. Soc. 111*, 8024–8026.
26. N. J. Maeji, R. M. Valerio, A. M. Bray, R. A. Campbell and H. M. Geysen (1994). Grafted supports used with the multipin method of peptide synthesis. *Reactive Polymers*, in press.
27. B. A. Bunin and J. A. Ellman (1992). A general and expedient method for the solid-phase synthesis of 1,4-benzodiazepine derivatives. *J. Am. Chem. Soc. 114*, 10997–10998.