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Bachem – Insights into Peptide Chemistry Achievements by the World's Leading Independent Manufacturer of Peptides

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Abstract: The Swiss fine chemicals company Bachem, pioneer and specialist in the chemical synthesis of peptides, has also become an internationally leading manufacturer of peptide active pharmaceutical ingredients (APIs). In response to increasing demands in scale and purity, Bachem's research efforts centered on the chemistry involved in solid-phase peptide synthesis and the production of the required amino acid derivatives with the aim of a continuous improvement of the technology. The resulting optimized protocols together with high-throughput equipment enabled us to manufacture long peptide APIs and, more recently, even pharma-grade glycoproteins in industrial scale.

Keywords: Active pharmaceutical ingredients · Large-scale peptide manufacturing · Process development · Side reactions · Solid-phase peptide synthesis

Introduction

More than 40 years of experience allows us to highlight research and development activities in the field of peptide chemistry by Bachem. Achievements in the field of liquid- and solid-phase synthesis, with special focus on side reactions, will be discussed chronologically, starting from first R&D work, leading to current hot topics.

Bachem was founded as a producer of amino acid derivatives and peptides as research chemicals by Peter Grogg in 1971 in Liestal. The company grew rapidly and started with the manufacturing of peptide active pharmaceutical ingredients (APIs) under cGMP (current Good Manufacturing Practice) conditions in the 1980s after having moved to a larger facility in Bubendorf. The immunomodulatory pentapeptide thymopentin (TP-5) was the first peptide drug substance synthesized in bulk amounts at Bachem and is still in our portfolio of more than 50 generic APIs.

This achievement triggered a further significant growth: Processes for manufacturing of luteinizing hormone-releasing hormone (LHRH), LHRH agonists, and even large peptides such as salmon calcitonin consisting of 32 amino acids were developed *via* solution synthesis during the 1980s, because this was the superior method for preparing bulk quantities of desired purity for use as drug substances compared to solid-phase peptide synthesis (SPPS) with its limitations in purification and scalability at that time.

Shortly after the introduction of the N^{α}-Fmoc-based strategy,[1] Bachem developed the syntheses of APIs, *e.g.* glucagon and endothelin and subsequently, SPPS became the preferred choice for production of peptides of any scale such as octreotide, aprotinin (bovine pancreatic trypsin inhibitor), and, more recently, liraglutide, a drug substance inducing insulin secretion from the pancreatic β-cells. Currently, the vast majority of peptide APIs are manufactured by chemical means including SPPS and a few by recombinant methods.[2] Solution synthesis still has its place at Bachem for specific applications, *e.g.* the synthesis of short to medium-sized peptide APIs in large scale by optimized processes in one batch.

Of special interest in R&D activities at Bachem is the field of large peptides: Recently our efforts resulted in a pioneering breakthrough in the chemical synthesis of interferon β-1a,[3] a 166 amino acid glycoprotein, which has been developed in collaboration with GlyTech Inc., resulting in a process suitable for industrial scale.

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Peptide Synthesis at Bachem

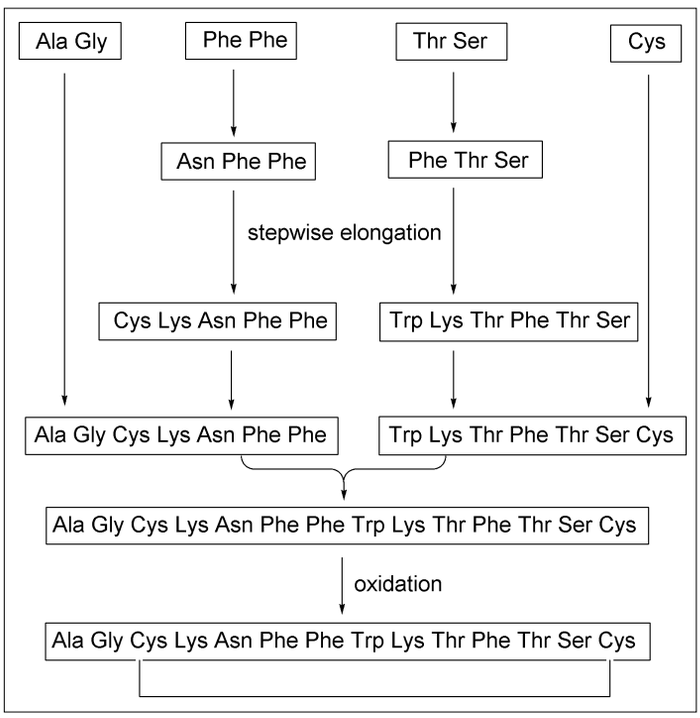
The 'classical' solution synthesis still has its place at Bachem for manufacturing short peptides for research purposes or, adhering to cGMP guidelines, for generic APIs. Over the years, our synthesis processes have been thoroughly optimized so that kilograms of pure peptide drug substance can be produced in a single batch.

LHRH (GnRH, gonadorelin) and LHRH agonists such as goserelin or leuprolide are synthesized by solution-phase methods, which also enabled us to obtain longer peptide APIs such as somatostatin or calcitonin on large scale.[4] The synthesis of somatostatin represents a typical example for this methodology (Scheme 1).

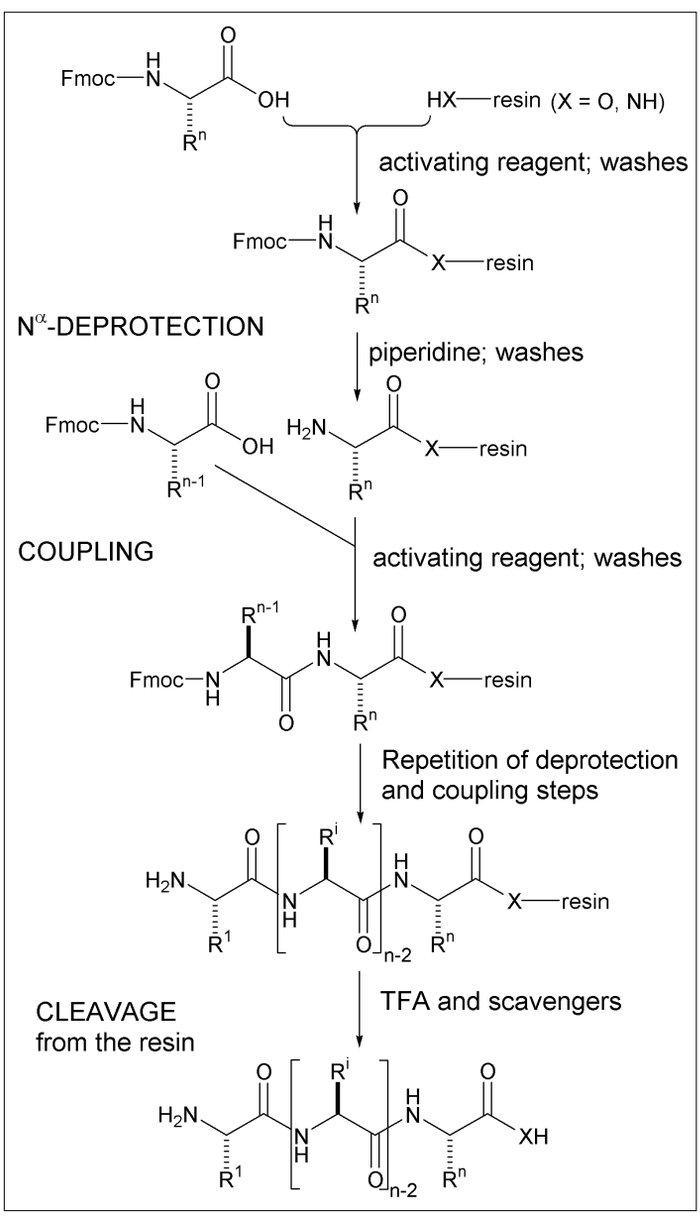
Nevertheless, in the manufacture of peptide APIs by chemical means, the future belongs to solid-phase approaches. Since the 1990s, Fmoc in combination with tBu-type protecting groups has become the preferred choice for N^{α}/side-chain protection for SPPS of any scale, not only at Bachem.[5,6] The development of the large-scale synthesis of the HIV fusion inhibitor enfuvirtide (T-20 or FUZEON®),[7] a 32 amino acid peptide, *via* an SPPS/solution-phase hybrid approach can most probably be considered as the main breakthrough of the Fmoc strategy.[6]

Whereas the 'classical' solution approach usually follows a convergent strategy, the peptides are assembled stepwise from C- to N-terminus on the resin, SPPS consists of repetitive N^{α}-deprotection, washing and coupling steps (Scheme 2).

Over the years, our capacity for solid-phase synthesis, subsequent purification by preparative HPLC, and lyophilization



Scheme 1. Convergent solution-phase synthesis of somatostatin. Protecting groups have been omitted for clarity.



Scheme 2. Fmoc-based solid-phase peptide synthesis. The side chains R^i may contain functionalities, which have to be protected during the assembly of the peptide. These protecting groups are removed during the final cleavage step.

of peptides has been increased from grams to kilograms of final product. Our 1000 L SPPS-reactor (Fig. 1) allows us to produce multiple kilograms of crude peptide on up to 30–50 kg of resin. As much as 3 kg of the crude product can be purified by preparative HPLC in a single run employing our recently acquired 60 cm column (Fig. 2).

Bachem is not only an expert in solution and solid-phase methodologies. We also developed resin derivatives such as Sasrin™ (4-(4-hydroxymethyl-3-methoxyphenoxymethyl) poly(styrene-co-1% divinylbenzene),[8] which allows for the synthesis of fully tBu-type protected peptide fragments, and 'Sasrin chloride' for the low-racemization coupling of Fmoc-amino acids to the carrier *via* nucleophilic substitution[9] since the 1980s. In the meantime, 2-chlorotrityl chloride resin[10] has become an excellent alternative to benzyl alcohol-type resins.

Fig. 1. 1000 L stainless steel reactor for SPPS.

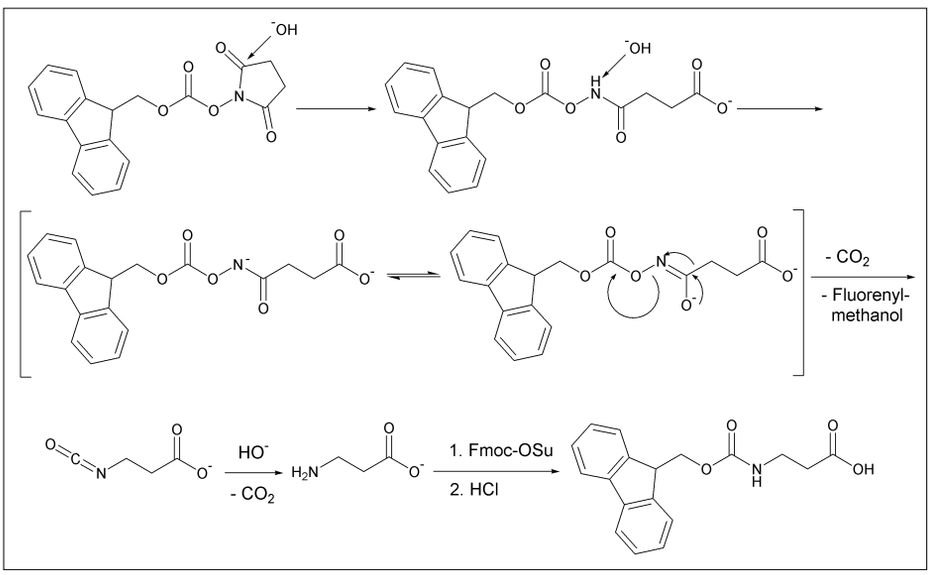
Fig. 2. Base part of our 60 cm column for preparative HPLC (dynamic axial compression module). Total height (frame, column cylinder, and hydraulics) 3.5 m.

Currently, a large number of our generic APIs and the vast majority of our research peptides are produced by Fmoc/tBu-SPPS. As the production of peptides used in drugs has become the main business area of Bachem, developing methods for increasing their purity is the most important part of our research activities. Higher purity can be achieved by complementary approaches:

i) Improvement of the quality of starting materials as amino acid derivatives, carrier resin, solvents: Not only did highly sensitive methods for analyzing our reactants have to be developed, we also had to evaluate the synthesis of the Fmoc amino acid derivatives, our building blocks.

ii) Improvement of the synthetic method: The second approach prompted our researchers to scrutinize the reactions taking place during SPPS. Time and money for these studies were well invested, as improving the quality of the crude product and thus simplifying purification also means increasing the final yield. The following chapters give an overview of these achievements.

iii) Improvement of purification methods: We continuously increased the efficiency of purification by using the latest reversed-phase column media and applying state-of-the-art equipment for preparative HPLC. In parallel, the maximum throughput had to be multiplied due to increasing demand.[11]



Scheme 3. Putative mechanism for the formation of Fmoc–β-Ala–OH from Fmoc–OSu *via* Lossen rearrangement.

Quality of Starting Materials

Impurities in Fmoc amino acids such as non-inert residual solvents or the recently detected Fmoc-β-alanine[12,13] will cause difficulties during downstream processes.

Fmoc-β-alanine can be generated *via* Lossen rearrangement of Fmoc-OSu, when preparing Fmoc-amino acids by the standard method using this reagent in the presence of base (Scheme 3). Even Fmoc-β-Ala dipeptides could be detected in such contaminated derivatives.

Whereas other impurities can lead to undesired truncation, the peptide still can be elongated after incorporation of Fmoc-β-Ala-OH yielding a failure sequence, a contaminant possibly difficult to remove. The formation of the β-Ala analogs is promoted when coupling sterically demanding Fmoc amino acid derivatives.

Another long-known side reaction during the preparation of Fmoc-amino acids, formation of the corresponding Fmoc-dipeptide, still cannot be completely suppressed.

Pseudoproline dipeptides constitute a highly useful tool for obtaining long or difficult peptides by SPPS.[14] The requirements of high purity also apply to these compounds, but, even though they have long been commercially available, it took some time until their quality could meet our standards.

Only after optimization of the synthesis of Fmoc-amino acids to minimize the side reactions and the development of sensitive analytical methods for detecting the impurities mentioned above and others, would Bachem attempt the stepwise solid-phase synthesis of very long peptides in the development of active ingredients.

The quality of the resin is of utmost importance for the outcome of the synthesis.

Whereas the building blocks, reagents and solvents used in SPPS have been thoroughly analyzed before use, the resin remains a 'black box'. Quite remarkably, the crosslinked polystyrene already used by Merrifield[15] (though soon after Merrifield's pioneering work, poly[styrene-co-1% divinylbenzene] replaced the less swellable polymer crosslinked with 2% divinylbenzene and became standard) is still the most popular carrier resin for SPPS. Certainly, the resin is not the optimal carrier for the synthesis of all kinds of peptides, and a considerable number of alternatives has been developed. The more polar polystyrene-derived Tentagel® resin[16] can be a valuable alternative when the desired peptide cannot be obtained on the standard resin.

Nevertheless, crosslinked polystyrene seems to be a good compromise regarding the various requirements of SPPS, *e.g.* mechanical stability, inertness, and sufficient swelling.

It has to be kept in mind that the resin used in SPPS has been subjected to several chemical modifications including coupling the C-terminal Fmoc-amino acid before the actual peptide synthesis is started. These conversions are accompanied by side reactions as well. The esterification ('loading') of the C-terminal Fmoc-amino acid to Wang resin or Sasrin™ is accompanied by varying degrees of concomitant racemization. The conversion tends to be incomplete, even though large excesses of amino acid derivative are used.

Remaining free hydroxyl groups have to be blocked to prevent their conversion during a subsequent coupling step. An excess of benzoyl chloride in the presence of pyridine is the standard reagent used in this so-called capping step[17] (Fig. 3). Otherwise, C-terminally truncated peptides can be obtained as by-products. $^{1}H,^{13}C$-HSQC HR-MAS NMR, a variant of magic angle spinning (MAS) NMR, is one of the best methods applicable to resins to shed some light into the black box of SPPS.[18] The resonances of the benzylic protons of the Wang linker, which strongly depend on the chemical environment, are measured. The method can be calibrated, so it allows, *e.g.* the extent of capping by quantifying residual hydroxyl moieties to be monitored (Fig. 4) and the loading to be determined without further sample treatment.

We extended the use of this method to monitor the conversion of other resins, *e.g.* loading of 2-chlorotrityl resins. Such NMR data can be supplemented by the SPPS of test peptides. Trial syntheses may be more elaborate, but they yield further significant data for the characterization of a resin.

Side Reactions During SPPS

By-products which accumulate during SPPS often cannot be removed completely by preparative HPLC. Their number and

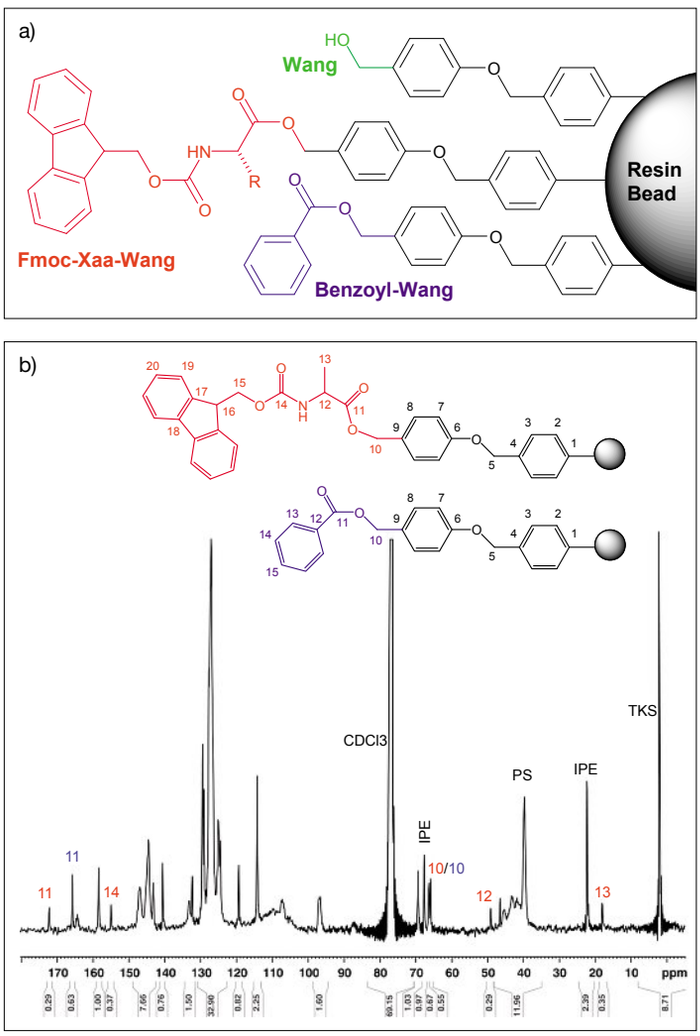


Fig. 3. a) Structure of loaded and capped (benzoylated) Wang resin. b) ^{1}D ^{13}C MAS NMR spectrum of Fmoc-Ala-Wang resin with benzoyl endcapped residual Wang sites recorded at 100.6 MHz (Bruker Avance 400, MAS rate 2 kHz) with assignment of characteristic signals.

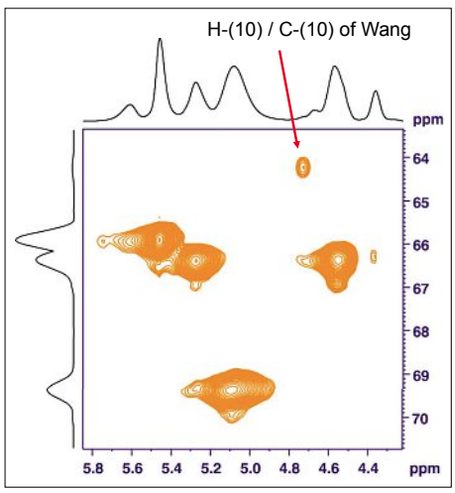


Fig. 4. Expansion of the $^{1}H,^{13}C$-HSQC HR-MAS NMR spectrum of benzoyl endcapped Fmoc-Ala-Wang spiked with 2% Wang (Bruker Avance 400, MAS rate 4 kHz).

amount depend on the sequence of the peptide and can increase with its length. The by-products due to impurities in reactants add to those resulting from side reactions. Only low amounts of contaminants are tolerated in peptide APIs and these impurities have to be identified.

For this reason, Bachem's research efforts were also devoted to the side reactions occurring during SPPS aiming at minimizing or even avoiding them. Common side reactions such as aspartimide formation and racemization of amino acid derivatives during coupling were successfully tackled.

Aspartimide Formation and Similar Reactions

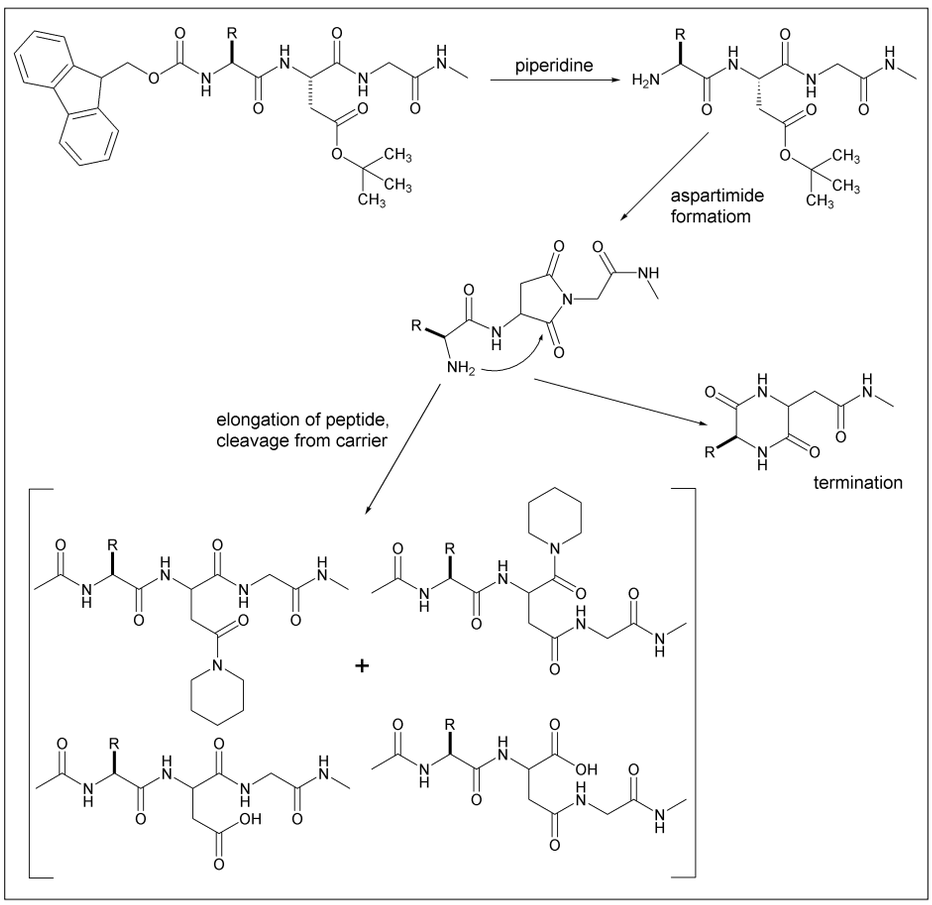
Base-catalyzed aspartimide formation is one of the most notorious side reactions during Fmoc-SPPS. Peptides containing the Asp-Gly motif are especially prone to base-driven cyclization and subsequent reopening of the ring (Scheme 4). Due to its complex mechanism, which may even involve participation of the preceding amino acid resulting in chain termination,[19] this reaction leaves an array of by-products. Though the most efficient protocol for suppressing aspartimide formation is blocking the Asp(OtBu)-Xaa bond by N-alkylation with 2-hydroxy-4-methoxybenzyl (Hmb)[20–22] or 2,4-dimethoxybenzyl (Dmb)[23] (Fig. 5), replacement of the Asp β-t-butyl ester by the sterically more demanding 3-methylpentyl ester (OMpe)[24] can suffice (Table 1, which also lists values obtained with unhindered β-carboxy protecting groups). Further substituent crowding (*e.g.* 2,3,4-trimethylpentyl) increases the resistance of the lateral ester to cyclization even more, as expected, but such derivatives are not readily available.[25] On the other hand, only in the case of Xaa = Gly could the backbone protecting group be split off smoothly under standard conditions.[22]

We also evaluated the sensitivity of the Glu(OtBu)-Xaa motif, which turned out

Table 1. Extent of formation of aspartimide and other by-products during syntheses of the model peptide H-Val-Lys-Asp-Gly-Tyr-Ile-OH. Fmoc removal was performed with piperidine/DMF (1 : 4), 5 and 10 min, at room temperature. Percentages of desired product and by-products were determined by RP-HPLC.[21]

Protecting group[a]	Desired product	Desired product [%]	Aspartimide (D and L) [%]	α-Piperidide [%]	β-Piperidide [%]	By-product [%][b]
OtBu	VKDGYI	91.1	2.3	1.5	nd	nd
OAll	VKD(OAll)GYI	nd[c]	49.6	12.0	2.9	25.0
OPp	VKDGYI	80.7	9.0	1.1	0.3	1.5
OBzl	VKD(OBzl)GYI	1.5	63.6	12.3	2.0	14.2
OMpe	VKDGYI	93.9	0.7	nd	nd	nd
OtBu/Hmb	VKDGYI	94.0	nd	nd	nd	nd

[a] OAll allyl ester, OBzl benzyl ester (unhindered); OPp 2-phenyl-2-propyl ester (sterically hindered); [b] The molecular mass of this by-product indicates a chain termination by diketopiperazine formation[19]; [c] nd: not detected



Scheme 4. Aspartimide formation and subsequent reactions.

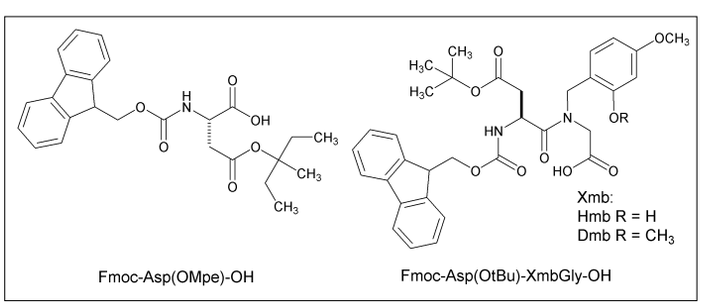


Fig. 5. Mpe ester and backbone protection.

to be much more resistant to base-catalyzed cyclization than the Asp(OtBu)-Xaa motif.[26]

Racemization of Histidine

As the racemization of activated histidine derivatives is catalyzed by the π-nitrogen of the lateral imidazole moiety (Scheme 5), blocking this heteroatom is the most efficient way for preserving the optical purity during coupling.[27] Fmoc-His(π-Bum)[28,29] and the recently described Fmoc-His(π-Mbom)[30] fulfil this need perfectly (Fig. 6).

The long-known Bum derivative was accessible only by alkylation with the highly volatile and toxic t-butoxymethyl chloride.[28] So we had to find an alternative and opted for optimizing the coupling conditions for the racemization-prone standard

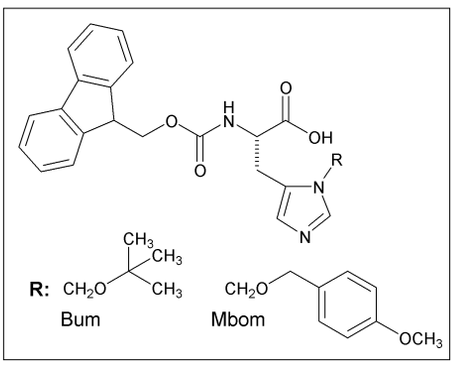
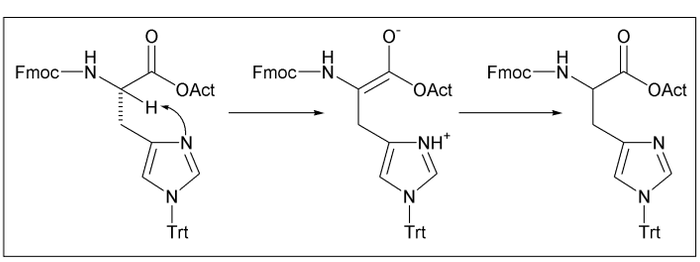


Fig. 6. N^{π}-protected His derivatives.

derivative Fmoc-His(τ-Trt). 3-(Diethoxyphosphoryloxy)-1,2,3-benzo[d]triazin-4(3H)-one (DEPBT) turned out to be the superior coupling reagent for this His derivative (Table 2).[31] In our hands, this slight change of protocol generated a marked increase in purity of crude His-containing peptides. It should be mentioned that acetal-type protecting groups such as Bum or Mbom yield formaldehyde during acidolytic cleavage, which has to be scavenged efficiently *e.g.* by addition of methoxyamine hydrochloride, whereas the rather inert Trt cation can be easily trapped by scavengers. This slight disadvantage will probably not prevent Fmoc-His(π-Mbom)-OH from becoming the derivative of choice for synthesizing His-containing peptides.

Racemization of Cysteine

Normally, racemization of an amino acid during coupling yields a D-epimer of the resulting peptide. In the case of cys-



Scheme 5. Racemization of histidine during activation.

Table 2: Extent of epimerization of the model peptide Z-Ala-His-Pro-OH during coupling of Fmoc-His(τ-Trt)-OH as determined by HPLC.[31]

Coupling reagent	Base	D-His Peptide [%]
DCC/HOBt	–	2.8
DEPBT	DIEA	0.8
DEPBT	collidine	0.8
TBTU	DIEA	4.5
TBTU	collidine	4.1
PyBOP	DIEA	12.7
PyBOP	collidine	4.4

teine racemization,[32] the situation can become much more complex. In bioactive peptides, cysteines are usually part of disulfide bridges stabilizing the active conformation. Hence, this amino acid comes in pairs. Disulfide bridge formation relies on the proper stereoisomerism of the cysteines, the D-Cys epimers could yield oligomers or, in case of a multiple disulfide bond-containing peptide, undesired connectivities. When synthesizing complex disulfide-bridged peptides such as aprotinin, success or failure will depend on the extent of Cys racemization in the linear precursor.

Activated cysteine derivatives tend to racemize in the presence of bases (Table 3a). The extent of racemization also depends on the polarity of the solvent (Table 3b).

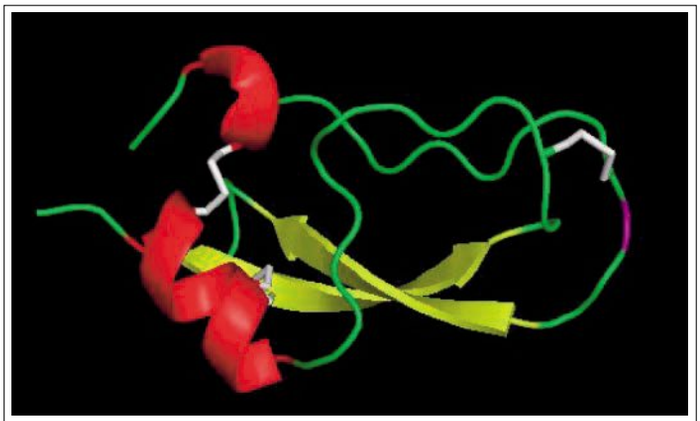
Owing to our optimized coupling protocol of Fmoc-Cys(Trt)-OH[33] and further improvements of the process, Bachem succeeded as early as 2002/2003 in the kg-scale manufacturing of pharma-grade aprotinin for subsequent market supply. By then, it was the largest and most complex peptide drug substance obtained by SPPS and, to the best of our knowledge, it still is. The 58 amino acid serine protease inhibitor aprotinin (Fig. 7), also known as bovine pancreatic trypsin inhibitor (BPTI),[34] contains three disulfide bridges, which are formed simultaneously by oxidative folding of the purified linear peptide.

Table 3. Racemization of Fmoc-Cys(PG)-OH during coupling as a function of activation reagent and solvent polarity. Model peptide Z-Ala-Cys(PG)-Pro-OH, PG Acm or Trt.[33]

3a) Effect of activation reagent[a]			
Reagent	Base/ Additive	PG[b]	Epimer [%]
TBTU	DIEA	Trt	2.6
TBTU	DIEA	Acm	4.6
TBTU	Collidine	Trt	2.5
TBTU	Collidine	Acm	0.6
DCC	HOBt	Trt	0.6
DIC	HOBt	Trt	0.9
DIC	HOBt	Acm	0.7

3b) Effect of solvent[c]	
Solvent	Epimer [%]
DMF	0.9
DMF/DCM	0.2
DMF/toluene	0.3
NMP	0.4
NMP/DCM	0.3
NMP/toluene	0.3

[a]Coupling in DMF; [b]PG: protecting group; [c]PG = Trt, coupling with DIC/HOBt.



RPDFCLEPPYTGPCKARIIRYFYNAKAGLCQTFVYGGCRAKRNNFKSAEDCMRTCGGA

(Disulfide bonds between Cys^{5} and Cys^{55}, Cys^{14} and Cys^{38}, Cys^{30} and Cys^{51})

Fig. 7. Sequence and schematic presentation of the tertiary structure of aprotinin (magenta: binding site, white: disulfide bonds, red: α-helical domains, yellow: antiparallel β-sheets, green: other).

Further Side Reactions

Deletion peptides can be formed due to incomplete Fmoc-cleavage. To reduce this risk we have developed a method for detecting residual Fmoc. Harsher Fmoc cleavage conditions allow complete deblocking, though at the risk of base-induced side reactions.[22,35] Incomplete couplings lead to deletions as well, but they can usually be driven to completion by optimizing the choice of coupling reagent, auxiliary, solvent, and other parameters.

Numerous undesired reactions of the peptide can occur during the final TFA treatment. Most of them can be suppressed by optimizing the composition of the cleavage cocktail. Nevertheless, the contact with TFA should be kept as short as possible. For peptides containing N-terminal Asn or Gln, the standard Trt side chain protection is split off only sluggishly. Incorporation of Fmoc-Asn(Mtt) or Fmoc-Gln(Mtt), which were developed at Bachem,[36] allowed reducing the cleavage time considerably.[37]

Each of the changes we implemented in our SPPS protocols may improve the purity of a peptide on its own, but in combination they allow us to obtain crude long peptides of excellent purity. We have, for example, increased the HPLC-purity of crude glucagon almost threefold (Fig. 8).

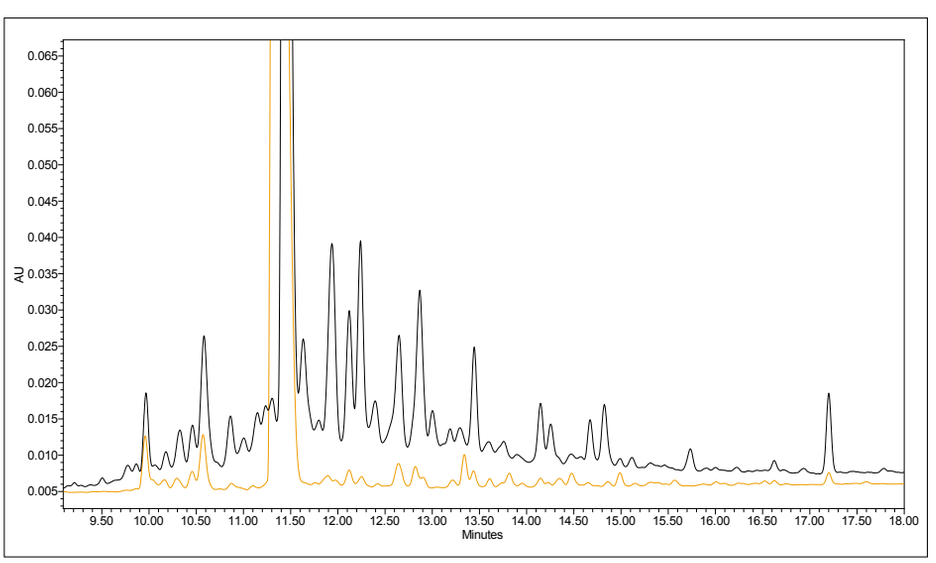


Fig. 8. Increased purity of crude glucagon by modifications of the SPPS protocol. Analytical HPLC profiles: First generation process (black trace, glucagon 27%) and second generation process (orange trace, glucagon 76%).

During the years, Bachem has advanced from the synthesis of short peptide APIs such as thymopentin or CCK-4 (cholecystokinin tetrapeptide) to the large-scale manufacture of miniproteins such as aprotinin. Recently, even more complex structures have been brought within the

reach of chemical synthesis. Our efforts in developing syntheses of peptide APIs culminated in the synthesis of a 166 amino acid glycoprotein, interferon β-1a,[38] in collaboration with GlyTech, Inc., Kyoto.

Conclusions and Outlook

During his Nobel Price lecture in 1984, Bruce Merrifield stated that "I cannot emphasize enough how important it is to be attentive to even the smallest of details if one expects to synthesize a peptide of high quality".[39] We have always kept this statement in mind.

Our attention to the small details of peptide synthesis, which drives all of our efforts in-house or in collaboration with research institutions in Switzerland such as the University of Basel, the Swiss Federal Institute of Technology Zurich (ETHZ), the Swiss Federal Laboratories for Materials Science and Technology (EMPA), and various Universities of Applied Science (FH), has allowed us to obtain numerous long and difficult peptides by chemical means in excellent yield and quality. We have demonstrated that large amounts of long peptides for use as active ingredients can be obtained by solid-phase synthesis in a quality which makes chemical synthesis the method of choice.

List of Abbreviations

Acm	Acetamidomethyl
API	Active Pharmaceutical Ingredient
cGMP	current Good Manufacturing Practice
tBu	t-Butyl
Bum	t-Butoxymethoxy
DCC	Dicyclohexyl carbodiimide
DCM	Dichloromethane
DEPBT	3-(Diethoxy-phosphoryloxy)-1,2,3-benzo[d]triazin-4(3H)-one
DIC	Diisopropyl carbodiimide
DIEA	N,N-Diisopropylethylamine
Dmb	2,4-Dimethoxybenzyl
Fmoc	9-Fluorenylmethoxycarbonyl
Hmb	2-Hydroxy-4-methoxybenzyl
HOBt	1-Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
HR-MAS	High Resolution Magic Angle Spinning
HSQC	Heteronuclear Single Quantum Coherence
LHRH	Luteinizing Hormone-Releasing Hormone, also called Gonadotropin-Releasing Hormone, GnRH
Mbom	4-Methoxybenzoxymethoxy
Mtt	4-Methyltrityl
NMP	N-Methyl-2-pyrrolidone
OMpe	3-Methylpentyl Ester
OSu	N-Hydroxysuccinimide Ester
PG	Protecting Group
PyBOP	Benzotriazol-1-yloxy-tripyrrolidino-phosphonium hexafluorophosphate
SPPS	Solid Phase Peptide Synthesis
TBTU	N-[(1H-Benzotriazol-1-yl)(dimethylamino)-methylene]-N-methylmethanaminium tetrafluoroborate N-oxide
TFA	Trifluoroacetic Acid
Trt	Trityl
Xaa	Unspecified Amino Acid

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[1] a) E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, B. J. Williams, *J. Chem. Soc., Chem. Commun.* **1978**, 537; b) C.-D. Chang, J. Meienhofer, *Int. J. Pept. Protein Res.* **1978**, *11*, 246.

[2] J. Reichert, P. Pechon, A. Tartat, M. K. Dunn, 'Development trends for peptide therapeutics: A comprehensive quantitative analysis of peptide therapeutics in clinical development', Peptide Therapeutics Foundation, San Diego, **2010**.

[3] Press release of Bachem AG and GlyTech, Inc., July 10th **2013**.

[4] T. Vorherr, F. Dick, B. Sax, *Chimia* **2005**, *59*, 25.

[5] T. Vorherr, *Chim. Oggi* **2010**, *28*, 22.

[6] B. L. Bray, *Nat. Rev. Drug Discov*. **2003**, *2*, 587.

[7] Registered trademark of Hoffmann-La Roche, Inc.

[8] a) M. Mergler, R. Nyfeler, R. Tanner, J. Gosteli, P. Grogg, *Tetrahedron Lett.* **1988**, *29*, 4009; b) M. Mergler, R. Tanner, J. Gosteli, P. Grogg, *Tetrahedron Lett.* **1988**, *29*, 4005; c) M. Mergler, J. Gosteli, P. Grogg, R. Nyfeler, R. Tanner, *Chimia* **1999**, *53*, 29.

[9] a) M. Mergler, R. Nyfeler, J. Gosteli, *Tetrahedron Lett.* **1989**, *30*, 6741; b) M. Mergler, R. Nyfeler, J. Gosteli, R. Tanner, *Tetrahedron Lett.* **1989**, *30*, 6745.

[10] a) K. Barlos, D. Gatos, I. Kallitsis, D. Papaioannou, P. Sotiriou, *Liebigs Ann. Chem.* **1988**, 1079; b) K. Barlos, D. Gatos, J. Kallitsis, G. Papaphotiu, P. Sotiriu, Y. Wenqing, W. Schäfer, *Tetrahedron Lett.* **1989**, 3943.

[11] T. Vorherr, *Speciality Chemicals* **2007**, *27*, 40.

[12] M. Obkircher, C. Stähelin, F. Dick, *J. Pept. Sci.* **2008**, *14*, 763.

[13] A. Isidro-Llobet, X. Just-Baringo, A. Ewenson, M. Álvarez, F. Albericio, *Biopolymers (Pept. Sci.)* **2007**, *88*, 733.

[14] M. Mutter, A. Nefzi, T. Sato, X. Sun, F. Wahl, T. Wöhr, *Pept. Res.* **1995**, *8*, 145.

[15] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149.

[16] Registered trademark of Rapp Polymere GmbH.

[17] J. W. van Nispen, J. P. Polderdijk, H. M. Greven, *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 99.

[18] D. Rentsch, C. Stähelin, M. Obkircher, R. Hany, M. Simeunovic, D. Samson, G. Loidl, F. Dick, *ACS Comb. Sci.* **2012**, *14*, 613.

[19] D. Samson, G. Loidl, in 'Peptides Building Bridges: The Proceedings of the 22nd American Peptide Symposium', Ed. M. Lebl, Prompt Scientific Publishing, San Diego, and Prague, **2012**, p. 58.

[20] a) L. C. Packman, *Tetrahedron Lett.* **1995**, *36*, 7523; b) M. Quibell, D. Owen, L. C. Packman, T. Johnson, *J. Chem. Soc. Chem. Commun.* **1994**, *20*, 2343.

[21] M. Mergler, F. Dick, B. Sax, P. Weiler, T. Vorherr, *J. Pept. Sci.* **2003**, *9*, 36.

[22] M. Mergler, F. Dick, B. Sax, C. Stähelin, T. Vorherr, *J. Pept. Sci.* **2003**, *9*, 518.

[23] V. Cardona, I. Eberle, S. Barthélémy, J. Beythien, B. Doerner, P. Schneeberger, J. Keyte, P. D. White, *Int. J. Pept. Res. Ther.* **2008**, *14*, 285.

[24] A. H. Karlström, A. E. Undén, *Tetrahedron Lett.* **1995**, *36*, 3909.

[25] M. Mergler, F. Dick, *J. Pept. Sci.* **2005**, *11*, 650.

[26] M. Mergler, F. Dick in 'Peptides 2004: Proceedings of the 3rd International and 28th European Peptide Symposium', Eds. M. Flegel, M. Fridkin, C. Gilon, J. Slaninova, KENES International, Geneva, **2005**, p. 343.

[27] J. H. Jones, W. I. Ramage, M. J. Witty, *Int. J. Pept. Protein Res.* **1980**, *15*, 301.

[28] R. Colombo, F. Colombo, J. H. Jones, *J. Chem. Soc. Chem. Commun.* **1984**, 292.

[29] M. Mergler, F. Dick, B. Sax, J. Schwindling, T. H. Vorherr, *J. Pept. Sci.* **2001**, *7*, 502.

[30] H. Hibino, Y. Nishiuchi, *Tetrahedron Lett.* **2011**, *52*, 4947.

[31] M. Mergler, F. Dick, T. Vorherr in 'Innovation and Perspectives in Solid Phase Synthesis and Combinatorial Libraries: Proceedings of the 7th International Symposium', Ed. R. Epton, Mayflower International, Southampton, **2002**, p. 235.

[32] Y. Han, F. Albericio, G. Barany, *J. Org. Chem*. **1997**, *62*, 4307.

[33] G. Loidl, F. Dick, M. Mergler, R. O. Schoenleber, *Adv. Exp. Med. Biol.* **2009**, *611*, 163.

[34] a) J. P. Vincent, M. Lazdunski, *Biochemistry* **1972**, *11*, 2967; b) H. Fritz, G. Wunderer, *Arzneimittelforschung* **1983**, *33*, 479.

[35] J. L. Lauer, C. G. Fields, G. B. Fields, *Lett. Pept. Sci.* **1995**, *1*, 197.

[36] B. Sax, F. Dick, R. Tanner, J. Gosteli, *Pept. Res.* **1992**, *5*, 245.

[37] M. Friede, S. Denery, J. Neimark, S. Kieffer, H. Gausepohl, J. P. Briand, *Pept. Res.* **1992**, *5*, 145.

[38] I. Sakamoto, K. Tezuka, K. Fukae, K. Ishii, K. Taduru, M. Maeda, M. Ouchi, K. Yoshida, Y. Nambu, J. Igarashi, N. Hayashi, T. Tsuji, Y. Kajihara, *J. Am. Chem. Soc.* **2012**, *134*, 5428.

[39] R. B. Merrifield, *Angew. Chem. Int. Ed.* **1985**, *24*, 799.