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Problem of aspartimide formation in Fmoc-based solid-phase peptide synthesis using Dmab group to protect side chain of aspartic acid

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Abstract: The sequence-dependent, acid- or base-catalysed aspartimide formation is one of the most serious side reactions in solid-phase synthesis of peptides containing aspartic acid. In the present work, we investigated the susceptibility of 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl (Dmab), an aspartic acid β-carboxy side-chain protecting group, for aspartimide formation. As a model, 15-amino acid-residue galanin fragment analogue containing the Asp-Ala motif was used during Fmoc-based solid-phase synthesis. Our study showed a strong tendency of Dmab-protected peptide to form aspartimide with unusual high efficiency. Furthermore, to investigate the susceptibility of Asp-Ala motif for aspartimide formation during the synthesis using Asp(ODmab), a 5-amino acid-residue galanin fragment LGPDA, different types of resin linkers, variety of Fmoc-deprotection conditions and coupling methods were applied. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: aspartic acid; aspartimide formation; Dmab; solid-phase peptide synthesis

INTRODUCTION

Aspartimide (Asi) formation is the best documented side reaction occurring during the synthesis of aspartic acid-containing peptides [1–21]. This problem concerns both Fmoc- and Boc-based solid-phase peptide synthesis (SPPS) [1–5]. The sequence-dependent cyclization of Asp residue may be catalysed by acids as well as by bases [6]. Sterically hindered β-carboxy side-chain protecting groups, such as *t*-Bu, do not fully prevent this side reaction [4,18]. In Fmoc-based SPPS, treatment with base (e.g. piperidine) necessary for Fmoc removal leads to Asi formation [3]. Further by-products (such as β-peptides and α/β-piperidides) arise from Asi-ring opening by nucleophiles and racemization of the imide derivatives.

In recent years, several strategies to avoid Asi formation have been developed [6–15], including methods based on Hmb or peptoid amide backbone protection [6–9], the use of resin linkers cleavable under neutral conditions [10,11] or additives to the deprotection mixture (such as HOBt) [12,13], as well as application of several new protecting groups to Asp side chain [14–20] or α-amino group [21]. However, employing of these methods does not fully prevent Asi formation and may cause certain difficulties during synthesis, or may not be suitable for the 'on-resin' synthesis of cyclic or branched peptides. For example, owing to the sterical hindrance of the amide backbone protecting groups (such as Hmb) the 'on-resin' synthesis of cyclic or branched peptides is inefficient (low purity/yield of final products is observed) [6–9]. Furthermore, peptoid methodology based on the preparation of *N*-substituted glycines using bromoacetic acid is restricted to the Asp-Gly motif only [9].

The recent introduction of the hydrazine-labile Dmab for protecting the β-carboxy side-chain group of Asp and subsequent commercial availability of the Fmoc–Asp(ODmab)–OH amino acid has made it possible to achieve a three-dimensional orthogonal protection strategy [22]. This additional level of protection, provided by the Dmab side-chain protecting group, may be applied to the 'on-resin' synthesis of cyclic (head-to-tail or side-chain-to-side-chain) and branched peptides (e.g. glycopeptides). Dmab group is stable to 20% piperidine and to TFA but quantitatively cleaved (without deprotection of other acid-labile side-chain protecting groups, such as *t*-Bu, Boc, Trt or Pbf) with 2% hydrazine monohydrate in DMF within minutes [22]. Recently, we investigated the possibility of application of Fmoc–Asp(ODmab)–OH in the syntheses of cyclic (head-to-side-chain) and branched (containing different structures in the side chain) analogues of 1–15 galanin

Abbreviations: Abbreviations: The symbols of the amino acids, peptides and their derivatives are in accordance with the 1983 'Recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature' [*Eur. J. Biochem.* 1984; **138**: 9] and 'Abbreviations and symbols in peptide science: a revised guide and commentary' [*J. Pept. Sci.* 2006; **12**: 1–12]; Other abbreviations: Asi, aspartimide; GAL, galanin; Dmab, 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl.

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fragment (GWTLNSAGYLLGPDA) modified in position 14 with Asp residue. Unfortunately, only aspartimide-containing GAL (1–15) analogue was found as the main product of synthesis (Table 1, synthesis of **1a**).

In the present paper, we report the problem of Asi formation during Fmoc-based SPPS of peptides containing Asp-Ala motif using Dmab group to protect β-carboxy side-chain group of Asp residue. Furthermore, to investigate the susceptibility of the Asp-Ala motif for Asi formation during SPPS using Asp(ODmab) a shorter galanin fragment (LGPDA) modified with Asp(ODmab) was synthesized and several different conditions shown in Table 1 were applied (syntheses of **2a–j**). In this work, different types of resin linkers, variety of Fmoc-deprotection conditions and coupling methods were studied. We intended to identify the factors affecting Asi formation and thus to find a simple and efficient synthesis protocol, which applied to SPPS, allows for significantly minimizing the process of Asi formation when Asp(ODmab) is used.

MATERIALS AND METHODS

Reagents

HOBt, TBTU and amino acid derivatives: Fmoc – Ala–OH, Fmoc–Asp(*t*-Bu)–OH, Fmoc–Asn(Trt)–OH, Boc–Gly–OH, Fmoc–Gly–OH, Boc – Leu–OH, Fmoc–Leu–OH, Fmoc–Pro–OH, Fmoc–Ser(*t*-Bu)–OH, Fmoc–Thr(*t*-Bu)–OH, Fmoc–Trp(Boc)–OH, Fmoc–Tyr(*t*-Bu)–OH were purchased from Peptides International (Louisville, USA). Fmoc–Asp-(ODmab)–OH derivative was obtained from Bachem (Bubendorf, Switzerland). TentaGel S RAM, TentaGel S PHB and chloro(2′-chloro)trityl polystyrene resins were purchased from Rapp Polymere (Tübingen, Germany). DBU, *N*,*N*′-diisopropylcarbodiimide (DIC), DIPEA, hydrazine monohydrate, phenol, piperazine, piperidine, TFA and triisopropylsilane (TIPS) were Sigma-Aldrich (Schnelldorf, Germany) products. Acetonitrile (ACN), DCM, methanol, DMF and *N*-methylpyrrolidone (NMP) were obtained from Labscan (Dublin, Ireland).

Peptide Synthesis and Analysis

Peptides were synthesized by SPPS on Labortec AG model SP 650 peptide synthesizer (Bubendorf, Switzerland) with the use of standard Fmoc-based protocol (a 3-fold molar excess of reagents in DMF/NMP solution for 1.5 h) [23–25] and conditions shown in Table 1. A base-labile Fmoc group was used to protect the α-amino function of the amino acids. Other side-chain groups were blocked by the acid-labile groups: Boc for tryptophan, Trt for asparagine, *t*-Bu for tyrosine, serine, threonine and aspartic acid (in case of syntheses of **1b, 2f**) or by the hydrazine-labile Dmab group for aspartic acid (syntheses of **1a, 2a–e, g–j**). As hydrazine will remove Fmoc group, peptides were protected by direct incorporation of the *N*-terminal residues (Gly or Leu) as Boc-protected amino acids (syntheses of **1a, 2a–j**). After synthesis of the peptide backbone had been completed, the Dmab groups were removed with 2% hydrazine monohydrate

Table 1 Conditions applied during peptide syntheses and relative yields of main products of syntheses determined by HPLC peak-area integration

No.	Protecting group of Asp	Type of resin linker	Coupling method (molar ratio 1 : 1 : 1)	Fmoc deprotection (for 5 + 15 min)	Relative yield (%)		
					Asp	Asi	Asp(O Me)
1a	ODmab[a]	Rink amide[c]	AA : TBTU : HOBt[f]	20% piperidine/DMF	11	72	17
1b	O*t*-Bu[b]	Rink amide[c]	AA : TBTU : HOBt[f]	20% piperidine/DMF	100	—	—
2a	ODmab[a]	Rink amide[c]	AA : TBTU : HOBt[f]	20% piperidine/DMF	46	32	22
2b	ODmab[a]	Rink amide[c]	AA : DIC : HOBt	20% piperidine/DMF	62	38	—
2c	ODmab[a]	Rink amide[c]	AA : TBTU : HOBt[f]	2% DBU/DMF	—	52	48
2d	ODmab[a]	Rink amide[c]	AA : TBTU : HOBt[f]	20% piperidine/DMF + 0.1 mM HOBt	44	35	21
2e	ODmab[a]	Rink amide[c]	AA : TBTU : HOBt[f]	6% piperazine/DMF + 0.1 mM HOBt	80	20	—
2f	O*t*-Bu [a]	Rink amide[c]	AA : TBTU : HOBt[f]	20% piperidine/DMF	100	—	—
2g	ODmab [b]	Rink amide[c]	AA : TBTU : HOBt[f]	20% piperidine/DMF	—	100	—
2h	ODmab[a]	Wang[d]	AA : TBTU : HOBt[f]	20% piperidine/DMF	66	34	—
2i	ODmab[a]	Cl-(2′-Cl)Trt[e]	AA : TBTU : HOBt[f]	20% piperidine/DMF	91	9	—
2j	ODmab[a]	Cl-(2′-Cl)Trt[e]	AA : DIC : HOBt	6% piperazine/DMF + 0.1 mM HOBt	95	5	—

Entries **1a** and **1b** refer to GAL (1–15) sequence and entries from **2a** to **2j** refer to truncated sequence of GAL(11–15).
Asp, aspartyl peptide; Asi, aspartimide peptide; Asp(OMe), α/β-aspartyl methyl ester peptides.
[a] Peptide treated with 2% hydrazine monohydrate in DMF (7 + 12 min) before cleavage from the resin.
[b] Peptide cleaved from the resin without hydrazine treatment.
[c] 4-(2′,4′-dimethoxyphenyl-Fmoc-aminomethyl)phenoxyacetamido polyethylene glycol resin (TentaGel S RAM resin, capacity 0.25 mmol/g).
[d] *O*-[4-(hydroxymethyl)phenyl]polyethylene glycol resin (TentaGel S PHB resin, capacity 0.31 mmol/g).
[e] Chloro(2′-chloro)trityl polystyrene resin (capacity 1.39 mmol/g).
[f] Coupling method with addition of 2-molar excess of DIPEA to DMF/NMP (1 : 1) solution.

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in DMF (7 + 12 min). Peptides were cleaved from resins with TFA/phenol/TIPS/H_2O (88 : 5 : 2 : 5) mixture for 2 h (syntheses of **1a–b, 2a–h**) or with 30% TFA in DCM for 1 h (syntheses of **2i, j**).

Crude peptides, thus obtained, were analysed by reverse phase high-performance liquid chromatography (RP-HPLC) on an analytical Beckman 'System Gold' chromatograph (Solvent Module 126 and Detector Module 166, Beckman Instruments Inc., Fullerton, USA) with a Vydac C18 column (4.6 × 250 mm, 5 μm particle size, flow rate 1 ml/min, detection at $\lambda = 226$ nm) or Beckman Ultrasphere ODS column (10 × 250 mm, 5 μm particle size, flow rate 3 ml/min, detection at $\lambda = 226$ nm) and several isocratic systems and linear gradients of 0.1% TFA in ACN. Products of synthesis were identified by MALDI-TOF mass spectrometry (Bruker BIFLEX III, Bruker Daltonics, USA). Relative yields of synthesis products were determined by HPLC peak-area integration. Table 2 summarizes some physicochemical properties of the synthesized peptides.

RESULTS AND DISCUSSION

In the present work, 15-amino acid-residue galanin fragment analogues, modified in position 14 with Asp were synthesized using Dmab group (synthesis of **1a**) and *t*-Bu group (synthesis of **1b,** as a reference) to protect side chain of Asp residue. Our observations showed that in our model Asi-formation problem is only restricted to Dmab-protecting group. Analysis of products of synthesis of **1a,** when galanin 1–15 fragment modified with Asp(ODmab) was synthesized using standard Fmoc-based protocol, has shown the presence of Asi-containing peptide (72%) and aspartyl methyl ester peptide (17%) as the main products of synthesis (Figure 1(a)). Aspartyl peptide was found in about 11%. However, these side products were not observed when the standard acid-labile *t*-Bu group was used to protect the side chain of Asp residue instead of the Dmab group (synthesis of **1b**). In this case, only the desired aspartyl peptide was found as the main synthesis product (Figure 1(b)).

To investigate the susceptibility of the Asp-Ala motif for Asi formation during SPPS using Asp(ODmab), a shorter galanin fragment (LGPDA) and different conditions shown in Table 1 were studied (syntheses of **2a–j**). During these syntheses (except for the synthesis of **2f**), a strong (or moderate) tendency of Dmab-protected peptides to yield Asi and Asi-related by-product formation was observed. Analysis of the products of synthesis of **2a**, when the standard HOBt/TBTU/DIPEA (1 : 1 : 2) coupling method was used, showed the presence of aspartyl peptide, Asi-containing peptide and aspartyl methyl ester peptide in quantities of about 46, 32 and 22%, respectively (Figure 2(a)). However, application of a protocol based on the HOBt/DIC (1 : 1) coupling method (synthesis of **2b**) showed the presence of aspartyl peptide and Asi-containing peptide in amounts of about 62 and 38%, respectively (Figure 2(b)). These observations suggest that the use of HOBt/TBTU coupling method involving a tertiary amine (DIPEA) in excess seems to lead to Asi formation more efficiently than a base-free DIC/HOBt coupling method.

Our studies showed also strong influence of Fmoc-deprotection conditions on Asi formation. The use of a base stronger than piperidine, such as DBU, to Fmoc removal strongly promote Asi formation (synthesis

Table 2 Comparison of some physicochemical properties of synthesized peptides

No.	Products found	Molecular mass		RP-HPLC t_R (min)
		Calculated	Found	
1a	[Asi^{14}]GAL(1–15)NH_2	1515.7	1515.7	15.77[a]
	[Asp^{14}]GAL(1–15)NH_2	1533.7	1533.7	15.01[a]
	[Asp(OMe)^{14}]GAL(1–15)NH_2	1547.7	1547.7	14.28[a]
1b	[Asp^{14}]GAL(1–15)NH_2	1533.7	1534.6	14.95[a]
2a,d	[Asi^{14}]GAL(11–15)NH_2	452.5	453.2	20.21[b]
	[Asp^{14}]GAL(11–15)NH_2	470.5	471.2	17.79[b]
	[Asp(OMe)^{14}]GAL(11–15)NH_2	484.5	485.2	19.03[b]
2b,e	[Asi^{14}]GAL(11–15)NH_2	452.5	453.2	20.21[b]
	[Asp^{14}]GAL(11–15)NH_2	470.5	471.2	17.79[b]
2c	[Asi^{14}]GAL(11–15)NH_2	452.5	453.2	20.21[b]
	[Asp(OMe)^{14}]GAL(11–15)NH_2	484.5	485.2	19.03[b]
2f	[Asp^{14}]GAL(11–15)NH_2	470.5	471.2	17.79[b]
2g	[Asi^{14}]GAL(11–15)NH_2	452.5	453.2	20.21[b]
2h–j	[Asi^{14}]GAL(11–15)OH	453.5	454.2	20.01[b]
	[Asp^{14}]GAL(11–15)OH	471.5	472.1	17.52[b]

[a] Gradient elution 20–60% of ACN for 30 min (Vydac C18 column, 4.6 × 250 mm, running at a flow rate of 1 ml/min).
[b] Gradient elution 0–50% of ACN for 40 min (Beckman Ultrasphere C18 column, 10 × 250 mm, running at a flow rate of 3 ml/min).

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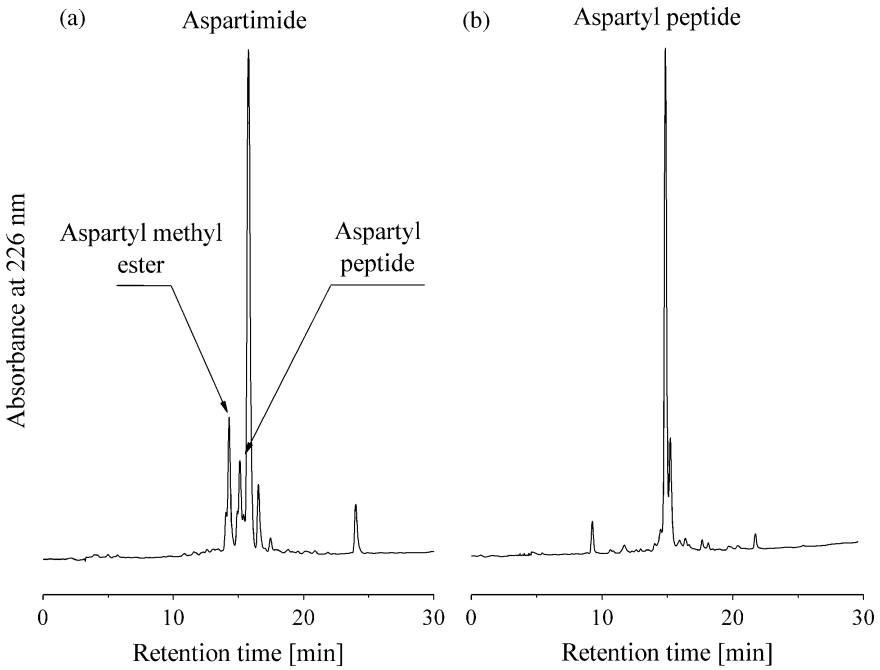

Figure 1 HPLC profiles of the crude GWTLNSAGYLLGPDA synthesized using: (a) Asp(ODmab) and (b) Asp(Ot-Bu); HPLC conditions: gradient elution 20–60% of ACN for 30 min, Vydac C18 column (250 × 4.6 mm) running at a flow rate 1 ml/min.

of **2c**). In this case, desired aspartyl peptide was not found at all. Only side products Asi-containing peptide and aspartyl methyl ester peptide were found in about 52 and 48%, respectively (Figure 2(c)). We have shown that the addition of HOBt (0.1 mM), reported as a partial suppressor of Asi formation [13], to a piperidine-containing Fmoc-deprotection mixture did not affect Asi-formation process (synthesis of **2d**). The relative content of products of synthesis of **2d** was similar to that occurred during the synthesis of **2a** (Figure 2(a)). However, applying Fmoc-deprotection mixture containing a base weaker than piperidine, such as 6% piperazine with addition of 0.1 mM HOBt (synthesis of **2e**), considerably decreased of Asi formation. In this case, the content of aspartyl peptide reached to about 80% (Figure 3(a)).

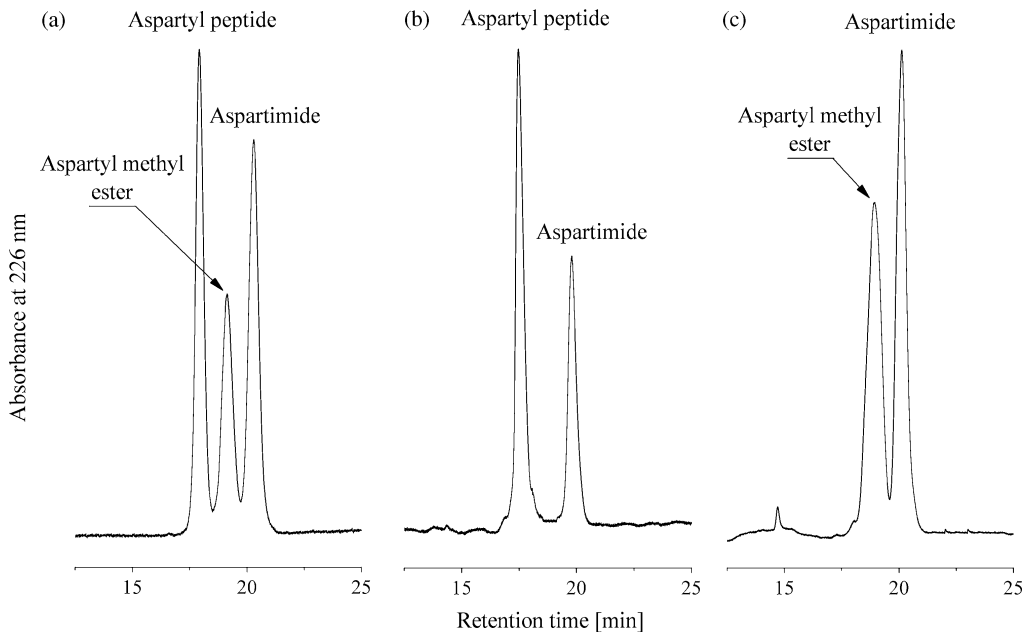

Figure 2 HPLC profiles of LGPDA: (a) syntheses of **2a, d**, (b) synthesis of **2b**, (c) synthesis of **2c**; HPLC conditions: gradient elution 0–50% of ACN for 40 min, Beckman C18 column (250 × 10 mm) running at a flow rate of 3 ml/min.

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Furthermore, to research the impact of hydrazine treatment (needed for Dmab-group removal) on Asi formation an LGPDA fragment was synthesized using *t*-Bu group to protect the side chain of Asp and treated with 2% hydrazine monohydrate (7 + 12 min) before TFA cleavage from the resin (synthesis of **2f**). In this case, only desired aspartyl peptide was found as the main product of the synthesis (Figure 3(b)). On the other hand, the TFA cleavage from the resin of the Dmab-protected LGPDA without hydrazine treatment yielded only the Asi-containing peptide (synthesis of **2g**, Figure 3(c)). Unexpectedly, the Dmab-protected peptide was not found at all. These observations suggest that the Asi-formation process depends rather on Fmoc-deprotection conditions than on hydrazine treatment used during the Dmab-group removal.

Our studies showed also a strong influence of the type of resin linker used during the synthesis on Asi formation. Application of the same synthesis conditions to TentaGel S RAM resin bearing Amide rink linker (synthesis of **2a**) and TentaGel S PHB resin bearing Wang linker (synthesis of **2h**) gave aspartyl peptides in quantities of about 46 (Figure 2(a)) and 66% (Figure 4(a)), respectively. However, employing mildly acid-sensitive chloro(2′-chloro)trityl polystyrene resin (synthesis of **2i**) considerably decreased Asi formation. In this case, the aspartyl peptide was observed in about 91% (Figure 4(b)). Finally, we synthesized LGPDA using chloro(2′-chloro)trityl polystyrene resin, Fmoc-deprotection mixture containing piperazine and the HOBt/DIC coupling method (synthesis of **2j**). In this case, we observed an increase in the content of aspartyl peptide by about 4% (Figure 4(c)) in comparison to synthesis of **2i**. However, we suggest that the proximity of Asp residue to chloro(2′-chloro)trityl moiety may be a decisive factor preventing Asi formation.

Moreover, we observed that the extensive use of methanol as a washing solvent during synthesis (syntheses of **1a** and **2a,c,d**), in the presence of small amounts of base, such as DIPEA – used during coupling reactions, lead to Asi-ring opening and quantitative formation of α/β-aspartyl methyl ester peptides (Figures 1(a), 2(a), (c)) [26,27]. To avoid this side-chain by-product formation, we proposed to completely eliminate methanol from the washing protocol (syntheses of **2e–j**) or eliminate DIPEA from the coupling protocol by employing, for example, the DIC/HOBt coupling method (synthesis **2b**). It's worth noting that α/β-piperidides, reported as the main by-products resulting from Asi-ring opening by piperidine [3–5], were not found in our studies. Furthermore, recently reported formation of *C*-terminal *N*-alkylated peptide amides occurring as a result of the Rink amide linker decomposition during TFA cleavage of the peptides were not observed in our studies [28].

CONCLUSIONS

In the present work, we have investigated the susceptibility of Dmab, an aspartic acid β-carboxy side-chain protecting group, for Asi formation. Our study showed a strong (or moderate) tendency of Dmab-protected peptide to form Asi during Fmoc-based SPPS of peptides containing the Asp-Ala motif when Dmab group is used to protect the β-carboxy side-chain group

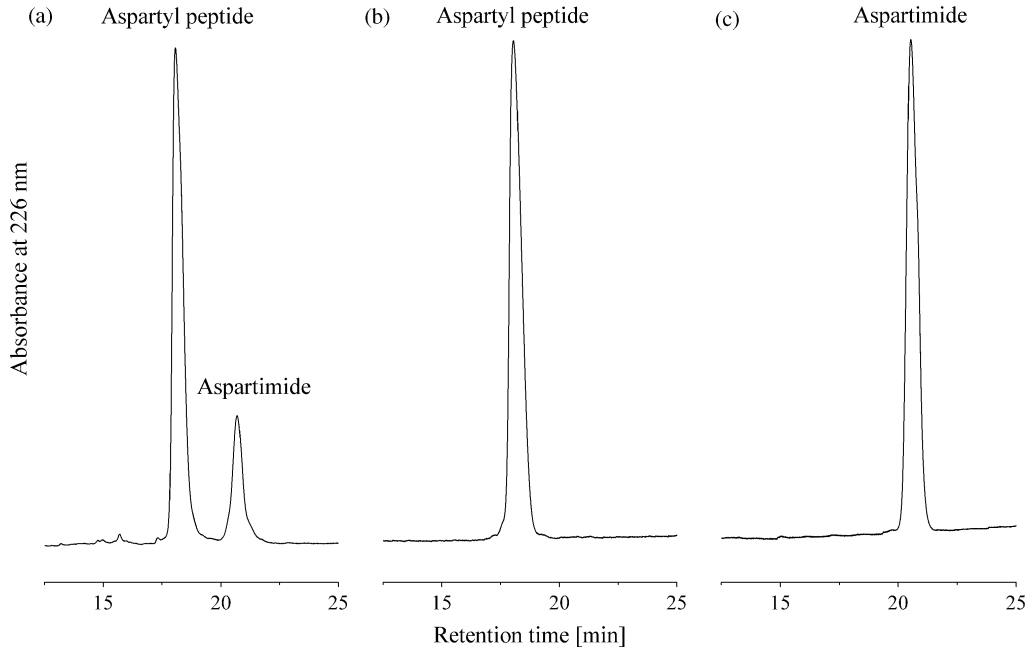

Figure 3 HPLC profiles of LGPDA: (a) synthesis of **2e**, (b) synthesis of **2f**, (c) synthesis of **2g**; HPLC conditions: gradient elution 0–50% of ACN for 40 min, Beckman C18 column (250 × 10 mm) running at a flow rate of 3 ml/min.

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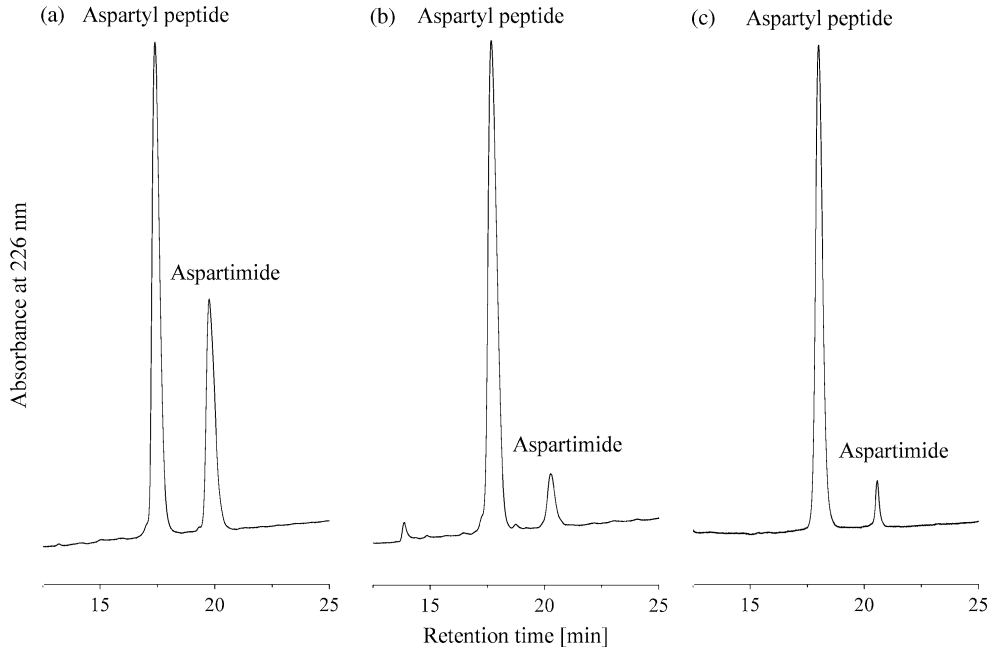

Figure 4 HPLC profiles of LGPDA: (a) synthesis of **3h**, (b) synthesis of **3i** and (c) synthesis of **3j**; HPLC conditions: gradient elution 0–50% of ACN for 40 min, Beckman C18 column (250 × 10 mm) running at a flow rate of 3 ml/min.

of Asp residue. Studies involving shorter GAL fragment analogues modified with Asp(ODmab) showed that Asi formation may strongly depends on the Fmoc-deprotection conditions, coupling method and also type of resin linker employed during synthesis. We obtained best results when chloro(2′-chloro)trityl resin linker or Fmoc-deprotection mixture containing piperazine or HOBt/DIC coupling method were employed (syntheses of **2b,e,i**). Employing a protocol based on the conditions mentioned above significantly reduces the process of Asi formation (5%) during Fmoc-based peptide synthesis when Asp(ODmab) is used (synthesis of **2j**). However, we suggest that the high content of desired aspartyl peptide (greater than 90%) observed for **2i,j** syntheses results rather from the proximity of Asp-Ala sequence to chloro(2′-chloro)trityl linker which prevents Asi formation. High susceptibility of the Dmab-protecting group for Asi formation may result from the presence of *p*-aminobenzyl group within the Dmab structure. The benzyl group (Bzl), extensively used in Boc methodology to protect β-carboxy side chain of Asp, is well-known as a group strongly promoting Asi formation [1–3]. We concluded that the Dmab group cannot be used as Asp β-carboxy side-chain protecting group in Fmoc-based SPPS without other precautions, such as amide backbone protection (e.g. with Hmb group).

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REFERENCES

1. Tam JP, Riemen MW, Merrifield RB. Mechanisms of aspartimide formation: the effects of protecting groups, acid, base, temperature and time. *Pept. Res.* 1988; **1**: 6–18.
2. Nicolás E, Pedroso E, Giralt E. Formation of aspartimide peptide in Asp-Gly sequences. *Tetrahedron Lett.* 1989; **30**: 497–500.
3. Yang Y, Sweeney WV, Schneider K, Thörnqvist S, Chait BT, Tam JP. Aspartimide formation in base-driven 9-fluorenylmethoxycarbonyl chemistry. *Tetrahedron Lett.* 1994; **35**: 9689–9692.
4. Dölling R, Beyermann M, Haenel J, Kernchen F, Krause E, Franke P, Brudel M, Bienert M. Pipreridine-mediated side product formation for Asp(Bu^t)-containing peptides. *J. Chem. Soc., Chem. Commun.* 1994; 853–854.
5. Lauer JL, Fields CG, Fields GB. Sequence dependence of aspartimide formation during 9-fluorenylmethoxycarbonyl solid-phase peptide synthesis. *Lett. Pept. Sci.* 1995; **1**: 197–205.
6. Quibell M, Owen D, Packman LC, Johnson T. Supresion of piperidine-mediated side product formation for Asp(OBu^t)-containing peptides by the use of *N*-(2-hydroxy-4-methoxybenzyl) (Hmb) backbone amide protection. *J. Chem. Soc., Chem. Commun.* 1994; 2343–2344.
7. Packman LC. *N*-2-2hydroxy-4-methoxybenzyl (Hmb) backbone protection strategy prevents double aspartimide formation in a 'difficult' peptide sequence. *Tetrahedron Lett.* 1995; **36**: 7523–7526.
8. Nicolás E, Pujades M, Bacardit J, Giralt E, Albericcio F. A new approach to Hmb-backbone protection of peptides: synthesis and reactivity of N^{α}-Fmoc-N^{α}-(Hmb)amino acids. *Tetrahedron Lett.* 1997; **38**: 2317–2320.
9. Zahariev S, Guarnaccia C, Pongor CI, Quaroni L, Eemaž M, Pongor S. Synthesis of 'difficult' peptides free of aspartimide and related products, using peptoid methodology. *Tetrahedron Lett.* 2006; **47**: 4121–4124.
10. Rabanal F, Pasto JJ, Nicolás E, Albericio F, Giralt E. Synthesis of aspartimide-free protected peptides on base-labile functionalized resins. *Tetrahedron Lett.* 2000; **41**: 8093–8096.
11. Wagner M, Dziadek S, Kunz H. The (2-phenyl-2-trimethylsilyl) ethel-(PTMSEL)-linker in the synthesis of glycopeptide partial

DOI: 10.1002/psc

structures of complex cell surface glycoproteins. *Chemistry* 2003; **9**: 6018–6030.
12. Martinez J, Bodanszky M. Side reactions in peptide synthesis. IX. Suppression of the formation of aminosuccinyl peptides with additives. *Int. J. Pept. Protein Res.* 1978; **12**: 277–283.
13. Wade JD, Mathieu MN, Marcis M, Tregear GW. Base-induced side reactions in Fmoc-solid phase peptide synthesis: minimization by use of piperazine as N^{α}-deprotection reagent. *Lett. Pept. Sci.* 2000; **7**: 107–112.
14. Karsltröm AH, Undén AE. The β-2,4-dimethyl-3-pentyl ester as a new protecting group for aspartic acid that prevents base-catalysed aspartimide formation on solid phase peptide synthesis. *Tetrahedron Lett.* 1995; **36**: 3909–3912.
15. Karsltröm AH, Undén AE. A new protecting group for aspartic acid that minimizes piperidine-catalysed aspartimide formation in Fmoc solid phase peptide synthesis. *Tetrahedron Lett.* 1996; **37**: 4243–4246.
16. Karsltröm AH, Undén AE. Design of protecting groups fort the β-carboxylic group of aspartic acid that minimize base-catalysed aspartimide formation. *J. Pept. Protein Res.* 1996; **48**: 305–311.
17. Vigil-Cruz SC, Aldrich JV. Unexpected aspartimide formation during coupling reactions using Asp(OAl) in solid-phase peptide synthesis. *Lett. Pept. Sci.* 1999; **6**: 71–75.
18. Mergler M, Dick F, Sax B, Weiler P, Vorherr T. The aspartimide problem in Fmoc-based SPPS. Part I. *J. Pept. Sci.* 2003; **9**: 36–46.
19. Mergler M, Dick F, Sax B, Stähelin C, Vorherr T. The aspartimide problem in Fmoc-based SPPS. Part II. *J. Pept. Sci.* 2003; **9**: 518–526.
20. Mergler M, Dick F. The aspartimide problem in Fmoc-based SPPS. Part III. *J. Pept. Sci.* 2005; **11**: 650–657.
21. Isidro-Llobet A, Guasch-Camell J, Álvarez M, Alberico F. *p*-Nitrobenzyloxycarbonyl (pNZ) as a temporary N^{α}-protecting group in orthogonal solid-phase peptide synthesis – avoiding diketopiperazine and aspartimide formation. *Eur. J. Org. Chem.* 2005; 3031–3039.
22. Chan WC, Bycroft BW, Evans DJ, White PD. A novel 4-aminobenzyl ester-based carboxy-protecting group for synthesis of atypical peptides by Fmoc-Bu^{t} solid-phase chemistry. *J. Chem. Soc., Chem. Commun.* 1995; 2209–2210.
23. Chan WC, White PD. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*. Oxford University Press: Oxford, 2000.
24. Ruczyński J, Konstański Z, Korolkiewicz R, Petrusewicz J, Rekowski P. Galanin and its analogues: a structure-activity relationship studies in rat isolated gastric smooth muscles. *Lett. Pept. Sci.* 2002; **9**: 91–99.
25. Ruczyński J, Konstański Z, Cybal M, Petrusewicz J, Wójcikowski C, Rekowski P, Kamińska B. Synthesis and biological properties of new chimeric galanin analogue GAL(1–13)-[$Ala^{10,11}$]ET-1(6–21)-NH_2. *J. Physiol. Pharmacol.* 2005; **56**: 273–285.
26. Kostidis S, Stathopoulos P, Chondrogiannis NI, Sakarellos C, Tsikaris V. Aspartyl methyl ester formation *via* aspartimide ring opening: a proposed modification of the protocols used in Boc- and Fmoc-based solid-phase peptide synthesis. *Tetrahedron Lett.* 2003; **44**: 8673–8676.
27. Stathopoulos P, Papas S, Kostidis S, Tsikaris V. α- and β- Aspartyl peptide ester formation *via* aspartimide ring opening. *J. Pept. Sci.* 2005; **11**: 658–664.
28. Stathopoulos P, Papas S, Tsikaris V. *C*-terminal *N*-alkylated peptide amides resulting from the linker decomposition of the Rink amide resin. A new cleavage mixture prevents their formation. *J. Pept. Sci.* 2006; **12**: 227–232.

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