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Solid-Phase Synthesis of Cyclic Analogues Related to the Hypoglycaemic Peptide hGH(6–13): Comparison of Two $i \rightarrow i+4$ Lactam Cyclization Procedures

VITTORIA CAVALLARO†, PHILIP E. THOMPSON‡ and MILTON T.W. HEARN*

Department of Biochemistry and Molecular Biology, Monash University, Clayton, Australia

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Abstract: The use of 1,3-diisopropylcarbodiimide (DIC) for the synthesis of cyclic analogues of the hypoglycaemic peptide fragment derived from the *N*-terminus of human growth hormone (hGH), namely hGH[6–13], is described. Different strategies were examined to achieve improved yields for the on resin side-chain to side-chain cyclization of the corresponding linear peptides containing reverse β-turn motifs. When compared with the more reactive Castro's reagent, the results confirm that DIC in the presence of HOBt can be successfully employed to minimize the formation of intermolecular oligomeric by-products associated with the preparation of cyclic hGH[6–14] peptide analogues based on an $i \rightarrow (i+4)$Lys→Glu or Glu→Lys cyclization strategy. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: cyclic peptides; 1,3-diisopropylcarbodiimide; Fmoc-deprotection; hypoglycaemic analogues

INTRODUCTION

Determination of the relationship between peptide structure and biological activity is often aided by reduction in the diversity of the possible conformational states that a linear peptide can adopt through generation of cyclic analogues [1]. Besides the greater conformational constraint that can be introduced, biologically active cyclic peptides specifically designed and prepared by a linear→cyclic peptide approach have been found to possess several additional attributes including: (i) increased agonist or antagonist potency; (ii) prolonged biological activity and extended pharmacokinetics; (iii) increased stability to enzymatic degradation; and (iv) increased specificity for a particular receptor [2].

Similar considerations have been found previously to prevail with the hypoglycaemic peptide fragment derived from the *N*-terminus of human growth hormone (hGH), namely hGH[6–13], H-Leu-Ser-Arg-Leu-Phe-Asp-Asn-Ala-OH. The discovery in our laboratories that the relatively unstable α-aminosuccinimido (Asu) fragment of hGH, Asu^{11}-hGH[6–13], could be replaced to generate analogues that also act as potentiators of insulin action *in vivo* and *in vitro* [3] led us to initiate a programme involving the synthesis and biological evaluation of linear and cyclic peptide libraries related to this amino acid sequence. Various Asu^{11}-hGH[6–13] analogues were thus prepared in an attempt to improve the molecular stability of the lead pharmacophore, as the Asu^{11} structure is particularly susceptible to hydrolytic cleavage, with the α-Asp^{11}- and β-Asp^{11}-hGH[6–13] analogues displaying no biological activity [3–6]. In addition, recent studies with peptides derived from the *N*-terminal hGH sequence by Ohkura and Hori [7] have also attempted to decipher how these peptides influence insulin action. These investigators have hypothesized that the hGH[8–13] peptide

* Correspondence to: Department of Biochemistry and Molecular Biology, Centre for Bioprocess Technology, Monash University, Clayton, Victoria, Australia 3168; e-mail: milton.hearn@med.monash.edu.au
† Current address: Division of Livestock Industries, CSIRO, Private Bag 24, Geelong, Victoria, Australia 3220.
‡ Current address: Department of Medicine, Thrombogenix Pty Ltd., Box Hill Hospital, Box Hill, Victoria, Australia 3128.

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modulates insulin action by stabilizing the cell membrane but does not itself directly act on pathways associated with insulin-induced glucose metabolism. In our previous studies [3,4] the *in vitro* and *in vivo* active analogues D-Ala^{11}-Pro^{12}-hGH[6–13], D-Pro^{11}-hGH[6–13] and β-Ala^{11}-hGH[6–13] were modified by the incorporation of a Lys$\leftrightarrow$Glu macrocyclic lactam bridge spanning residues Phe^{10}... Gly^{13} or Leu^{9}... Ala^{14} of the hGH[6–14] sequence, thus stabilizing the type-II′ β-turn pharmacophore, whilst maintaining the presence and orientation of the Arg^{8} and Phe^{10} residues that are critical for activity [3,8].

While side-chain to side-chain cyclization of peptide sequences has been successful in many instances [1,9–11], a number of factors are known to significantly influence the efficiency of the cyclization reaction and the yield of the desired cyclic peptide product. Previously, we have examined [12] the solid phase synthesis of various $i \rightarrow i+4$ cyclic peptides containing reverse turn motifs. These studies demonstrated that Castro's reagent [benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate, BOP] was effective for the synthesis of some of these peptides, although the desired reaction products were frequently contaminated to varying extents by side reaction products associated with concomitant oligomer formation at low levels in some cases but very high levels in others. These findings lead us to the conclusion that the BOP reagent may not be the preferred reagent for use in the generation of cyclic peptide libraries related to the hGH[6–13] sequence. In other studies [12,13] we have shown that an alternative reagent, 1,3-diisopropylcarbodiimide (DIC), was less reactive but generally gave a lower proportion of oligomers. Here, we describe the solid-phase synthesis of cyclic analogues of hGH[6–13] (Table 1), based on the successful utilization of DIC as a cyclization agent for these peptides.

MATERIALS AND METHODS

Reagents and General Experimental Procedures

Peptides were manually synthesized in plastic columns with plastic sinters at the base for solvent removal under suction. Unless otherwise stated, solvents were of analytical grade. Thioanisole, 1,2-ethanedithiol, trifluoromethanesulfonic acid (TFMSA) and DIC were obtained from Aldrich (Milwaukee, WI, USA), Castro's reagent (BOP), *N*,*N*-diisopropylethylamine (DIEA), *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluoro-phosphate (HBTU), *N*,*N*-dimethylformamide (DMF), 1-hydroxybenzotriazole (HOBt), 4-methyl-benzhydrylamine (4-MBHA) resin (0.82 mmol eq/g) and all protected amino acids were obtained from Auspep Pty (Melbourne, Australia). HPLC was performed on a Waters Associates (Milford, MA, USA) liquid chromatography system consisting of a model 600 solvent delivery pump, a Wisp model 712 sample processor and automated gradient controller. Peptide samples (ca. 10 μg/10 μl per injection) were analysed using TSK-ODS-120T columns (150 × 4.6 mm I.D., Tosoh Corp., Yamaguchi, Japan) equilibrated in 0.1% (v/v) aqueous TFA. The peptides were eluted using a linear gradient from 0 to 60% (v/v) or 0 to 50% (v/v) acetonitrile–water containing 0.1% (v/v) TFA over 30 min at a flow rate of 1 ml/min. The eluate was monitored by UV detection at 214 nm. The cyclic hGH analogues (60–80 mg/ml per injection) were purified on a semi-preparative TSK-ODS-120T column (300 × 21.55 mm I.D., Tosoh Corp., Yamaguchi, Japan) equilibrated in 0.1% (v/v) TFA, using a linear gradient from 0 to 60% (v/v) acetonitrile–water containing 0.1% (v/v) TFA over 60 min and a flow rate of 6.0 ml/min. The eluate was monitored at 214 nm. Peptide fractions were collected, lyophilized and analysed by ESI-MS, amino acid analysis and analytical RP-HPLC as described previously [14]. The purity of the synthetic cyclic

Table 1 Sequences of the Cyclic hGH[6-14] Analogues Synthesized

Peptide no.	Sequence
1	*cyclo*(Lys^{9}, Glu^{13})-[H-Leu-Ser-Arg-Lys-Phe-D-Ala-Pro-Glu-Gly-NH_2]
2	*cyclo*(Lys^{9}, Glu^{13})-[H-Leu-Ser-Arg-Lys-Phe-D-Pro-Asn-Glu-Gly-NH_2]
3	*cyclo*(Lys^{9}, Glu^{13})-[H-Leu-Ser-Arg-Lys-Phe-β-Ala-Asn-Glu-Gly-NH_2]
4	*cyclo*(Glu^{9}, Lys^{13})-[H-Leu-Ser-Arg-Glu-Phe-D-Ala-Pro-Lys-Gly-NH_2]
5	*cyclo*(Glu^{9}, Lys^{13})-[H-Leu-Ser-Arg-Glu-Phe-D-Pro-Asn-Lys-Gly-NH_2]
6	*cyclo*(Glu^{9}, Lys^{13})-[H-Leu-Ser-Arg-Glu-Phe-β-Ala-Asn-Lys-Gly-NH_2]

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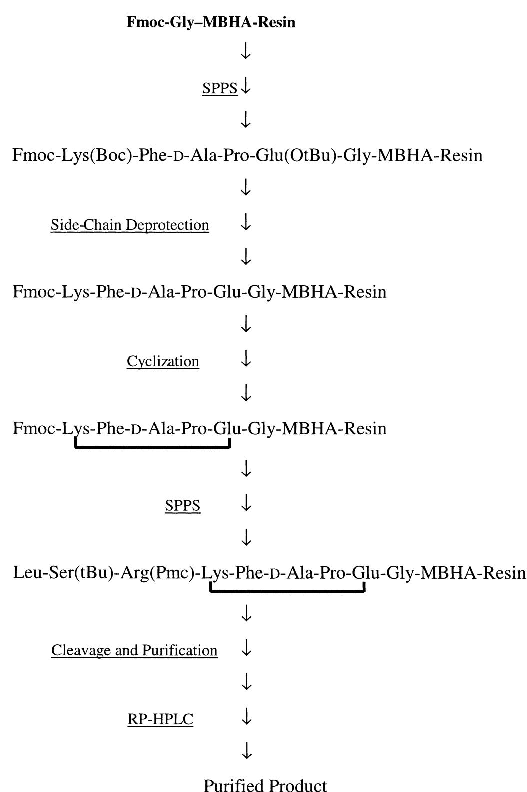

Figure 1 General scheme illustrating the approach employed for the synthesis of the cyclic peptides **1**–**6**, with peptide **1** used as an example.

peptides was typically $\geq 95\%$ as assessed by these analytical methods.

Solid-Phase Peptide Synthesis (SPPS)

Peptides were synthesized using Fmoc-based protocols developed previously in this laboratory with the MBHA-resin [12,13] and adapted from methods previously described by Fields and Noble [15] (Figure 1). Couplings were carried out in DMF using HOBt/HBTU as the activation agents (2 eq relative to the peptide concentration on the resin). Deprotection was achieved using 20% (v/v) piperidine in DMF for times up to 10 min.

Side-Chain Deprotection

Cleavage of side-chain protecting groups, prior to cyclization, from the precursor peptide resin was achieved by either of the two different methods that follow, depending on the sequence of the peptide:

Method I: For peptides **1** and **4** the peptide-resin was subjected to a 50% (v/v) TFA/dichloromethane (DCM) mixture for 30 min before the resin was washed with DCM (3×5 ml) and dried under reduced pressure (ca. 5 torr) prior to cyclization.

Method II: For peptides **2**, **3**, **5** and **6** the ice-cooled peptide-resin was suspended in an ice-cooled mixture of double distilled water (dH_2O, 0.25 ml) 1,2-ethanedithiol (0.25 ml) and TFA (9.5 ml). The resultant mixture was stirred at room temperature for 2 h before the resin was filtered away from cleavage solution. The collected resin was washed with DCM (3×5 ml) and vacuum dried prior to cyclization.

Cyclization Procedures

Following side-chain deprotection, the peptides were cyclized *in situ* on the resin by treatment with (A) BOP/DIEA or (B) DIC/HOBt (Table 2). For peptides **2**–**4**, two portions of the resins were cyclized separately by methods (A) and (B). The peptide-resin was swollen in a large volume of DMF before the addition of BOP/DIEA (3 eq each) or DIC/HOBt (3 eq for each for experiments extending to 6 days and 6 eq thereafter for each of the experiments

Table 2 Representative Details of the Conditions Used for the Synthesis of the Cyclic hGH[6–14] Peptide Analogues Indicating the Type of Methodology Employed for the Cyclization, the Total Reaction Time and the Yield of the Cyclic Peptide Product of $> 99.5\%$ Purity

Peptide no.	Cyclization method	Reaction time (h)	Yield (%)	Cyclization method	Reaction time (days)	Yield (%)
1	A	7	22			
2	A	24	1.5	B	9	13
3	A	24	1.6	B	9	14
4	A	24	1.6	B	9	14
5				B	9	8
6				B	9	8

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extending to 9 days). For method A the reagents were removed from the peptide-resin by filtration and replaced by fresh reagents twice at about 12-hourly intervals after the initial set-up permitting the cyclization reaction to be monitored over a total reaction time of 24 h. Similar procedures were used with method B to replace the reagents daily over a total reaction time of 9 days.

Continuation of SPPS after Cyclization

Following mid-synthesis cyclization of each peptide-resin (**1**–**6**), completion of the synthesis of the remaining sequence was achieved by initially deprotecting the *N*-terminal Fmoc group, as described above, followed by further coupling of 2,2,5,7,8-pentamethyl-chroman-6-sulphonyl (Pmc) side-chain protected Fmoc-arginine [Fmoc-Arg-(Pmc)-OH], *tert*-butyl (*t*Bu) side-chain protected Fmoc-serine [Fmoc-Ser(*t*Bu)-OH] and Fmoc-Leu-OH. The Fmoc group was removed from the completed peptide-resin with 20% (v/v) piperidine, and the peptide resin was then washed with DMF (2 × 10 ml) and dried before cleavage.

Cleavage with TFA/TFMSA and Peptide Purification

The ice-cooled peptide-resin (100 mg) was treated with thioanisole (100 μl) and 1,2-ethanedithiol (50 μl) followed by TFA (1.0 ml), with the mixture stirred at room temperature for 10 min. The mixture was cooled to −5°C and TFMSA (100 μL) added drop-wise with stirring. The mixture was then stirred at room temperature for 2 h. Ice-cold diethyl ether (50 ml) was added to the mixture, which was stirred vigorously for 1 min prior to filtration of the suspension. The peptide was extracted into TFA (3 × 3 ml), the solution concentrated under reduced pressure and cold diethyl ether (50 ml) added to precipitate the peptide. The solid was recovered by filtration, taken up into aqueous acetonitrile (50% v/v) and lyophilized. After cleavage each crude peptide was purified by RP-HPLC. The collected products were analysed and characterized by RP-HPLC (Figures 2 and 3). Details of the electro-spray ionization mass spectrometry (ESI-MS) and amino acid analysis are given in Table 3.

RESULTS

In this study comparing two different condensation reagent conditions (BOP and DIC), the cyclic hGH[6–14] peptide analogues **1**–**6** (Table 1) were

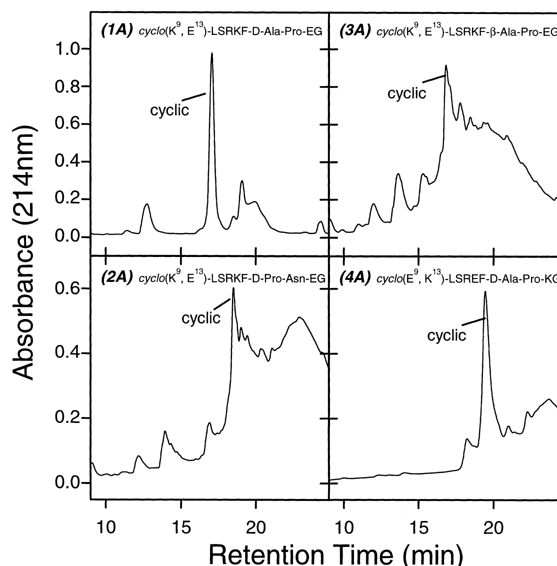

Figure 2 Analytical RP-HPLC elution profiles obtained after cleavage of the synthetic cyclic peptides **1**–**4** using the BOP reagent for mid-synthesis cyclization. A linear gradient from water–0.1% TFA (v/v) to 50% (v/v) acetonitrile–water containing 0.09% TFA over 30 min at a flow rate of 1.0 ml/min was employed with a TSK-ODS-120T column (150 × 4.6 mm I.D.) and UV detection at 214 nm.

prepared from their linear peptide counterparts using Fmoc-based SPPS techniques with HBTU/HOBt as the coupling agents. Cyclizations were performed on-resin and mid-synthesis after side-chain deprotection was achieved using 50% (v/v) TFA/DCM for peptides **1** and **4**, whilst a TFA/1,2-ethanediothiol cleavage method was employed to remove the protecting groups for the synthesis of peptides **2**, **3**, **5** and **6** due to the presence of the trityl(Trt) protecting group on the Asn residue in the sequence of these analogues.

The cyclization approach employed in these investigations was based either on the procedure described by Felix *et al.* [1], whereby the peptide-resin was reacted with BOP/DIEA (method A), or alternatively adapted from conditions used in our previous studies with DIC/HOBt in DMF (method B). Because of the slower reaction rate of the DIC/HOBt conditions, reaction times of up to 9 days at 25°C (Table 3) were based on previous observations [1,12] on the $i \rightarrow i+4$ cyclization of various model peptides with BOP/DIEA or DIC/HOBT. Following cyclization, SPPS was continued until the sequence was complete. The peptides were recovered by cleavage from the resin using a one-step TFMSA/TFA method with the crude products analysed and purified by RP-HPLC. Representative analytical RP-HPLC results

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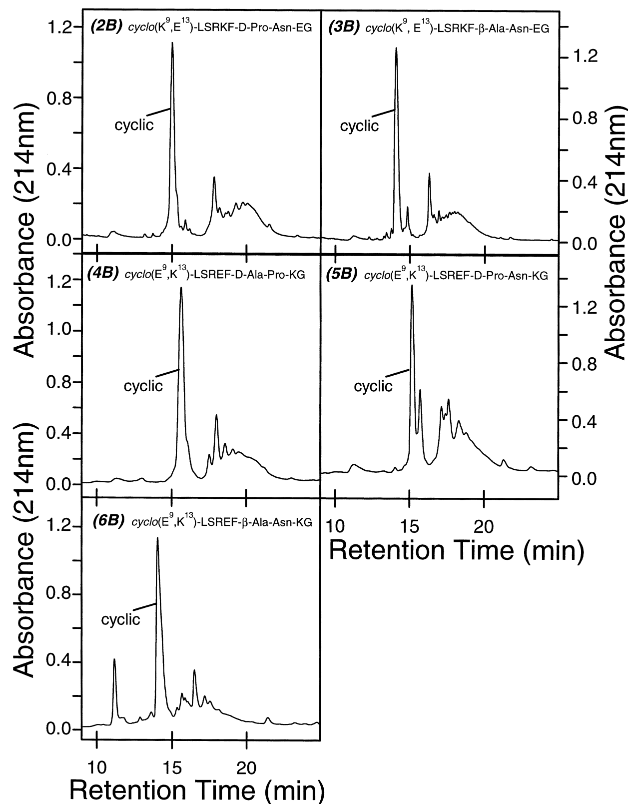

Figure 3 Analytical RP-HPLC elution profiles obtained after cleavage of the synthetic cyclic peptides **2**–**6** using the DIC reagent for mid-synthesis cyclization. A linear gradient from water–0.1% TFA (v/v) to 60% (v/v) acetonitrile–water containing 0.09% TFA over 30 min at a flow rate of 1.0 ml/min was employed with a TSK-ODS-120T column (150 × 4.6 mm I.D.) and UV detection at 214 nm.

for the crude products from the syntheses of the cyclic peptides **1**–**4** using the BOP reagent for mid-synthesis cyclization are shown in Figure 2, while the analytical RP-HPLC profiles, generated with relatively steep, but convenient linear gradient elution conditions, for the crude synthetic cyclic peptides **2**–**6** prepared using the DIC/HOBT conditions for mid-synthesis cyclization are shown in Figure 3. As noted previously [13], care must be taken when steep linear gradients, i.e. $>2\%$ per min, are employed for the analytical RP-HPLC separation of crude synthetic peptide mixtures, in terms of the reproducibility of gradient formation per se and the potential for the progressive loss of the more volatile acetonitrile when vigorous purging of the solvent reservoirs is carried out with an inert gas, such as helium. Both effects can lead to slight increases in elution times of the peptides over a period of several hours, as illustrated in Figure 2. The yields for the purified cyclic peptides based on BOP/DIEA cyclizations varied between 1 and 23% for an initial resin loading of ca. 0.8 mmol eq/g of the first amino acid. In comparison, the yields for the purified peptides using the DIC/HOBT method were consistently between 8 and 45% (Table 3). Of particular note was the almost complete absence of polymeric products when the DIC/HOBt method was used. On the basis of comparative RP-HPLC elution behaviour and ESI-MS analysis, the remainder of the crude reaction mixture largely corresponded to the linear (i.e. uncyclized) peptide with only very small amounts of intermolecular oligomeric products present.

DISCUSSION

Previous investigations [12] reported from this laboratory related to the ability of hexapeptides to cyclize using the activating agents BOP, HBTU or DIC provided an interesting insight into the role of the sequence-dependency and its dramatic effect on the outcome of the cyclization reaction. The findings from these previous studies showed that: (i) cyclic peptides with side chains linked from Lys (*N*-terminal) to Glu (*C*-terminal) typically were obtained in higher yields than the reverse lactam (Glu → Lys), and (ii) BOP could be used as a cyclization reagent but was prone to increased formation of higher molecular weight oligomers depending on the nature of the amino acid sequence. The results of the present investigation related to the synthesis of peptides **1**–**6** followed a similar pattern, with the BOP/DIEA conditions providing a faster reaction but greater amounts of by-products, as apparent from the RP-HPLC profiles of the crude peptide products. In contrast, the DIC/HOBT conditions were clearly slower in terms of affecting cyclization, but generated less by-products and a cleaner product for comparable reaction times.

Predicting the rate of peptide cyclization from the linear sequence counterpart has been the subject of several recent studies [16,17] including the use of computer-simulated techniques to model the 3D structure of the linear sequence before cyclization. The basic assumption under-lying the computational prediction of cyclization rates is that the rate of conversion of each linear precursor is proportional to the time that the molecule spends in cyclization-prone conformation(s). The inference drawn by most investigators is that cyclization yields of linear pentapeptides or hexapeptides will generally be enhanced by the presence of

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Table 3 Characterization Data for the Synthetic Cyclic hGH[6–14] Peptides **1**–**6** after Purification Using RP-HPLC Including Amino Acid Analysis and Molecular Masses Determined by ESI-MS. The Data also show the Cyclization Method Employed, i.e. BOP (Method A) or DIC (Method B)

Peptide	Amino acid analysis[a]	Molecular mass (expected)	Molecular mass (found)
Method A			
1	E: 0.96 (1); S: 1.1 (1); G: 1.1 (1); R: 1.0 (1); A: 1.1 (1); P: 1.1 (1); L: 1.2 (1); F: 1.1 (1); K: 0.83 (1)	985.3	985.6
2	D: 0.8 (1); E: 1.0 (1); S: 0.9 (1); G: 1.6 (1); R: 0.8 (1); P: 1.1 (1); L: 1.0 (1); F: 0.74 (1); K: 0.8 (1)	1028.3	1027.8
3	D: 0.8 (1); E: 1.0 (1); S: 0.95 (1); G: 1.5 (1); β-Ala: 1.1. (1); R: 0.75 (1); L: 0.9 (1); F: 0.8 (1); K: 0.8 (1)	1002.3	1001.6
4	E: 1.0 (1); S: 1.0 (1); G: 1.4 (1); R: 1.2 (1); A: 1.2 (1); P: 0.8 (1); L: 0.7 (1); F: 0.6 (1); K: 0.7 (1)	985.3	984.8
Method B			
2	D: 0.9 (1); E: 0.9 (1); S: 1.0 (1); G: 0.9 (1); R: 1.0 (1); P: 1.0 (1); L: 0.9 (1); F: 0.9 (1); K: 0.9 (1)	1028.3	1028.3
3	D: 1.1 (1); E: 1.2 (1); S: 1.1 (1); G: 1.2 (1); β-Ala: 1.4 (1); R: 1.0 (1); L: 0.9 (1); F: 0.9 (1); K: 0.8 (1)	1002.3	1002.2
4	E: 0.9 (1); S: 0.8 (1); G: 0.9 (1); R: 0.8 (1); A: 1.0 (1); P: 0.9 (1); L: 0.96 (1); F: 1.0 (1); K: 0.84 (1)	985.3	985.2
5	D: 0.73 (1); E: 0.7 (1); S: 1.0 (1); G: 0.95 (1); R: 1.0 (1); P: 1.0 (1); L: 1.25 (1); F: 1.1 (1); K: 1.0 (1)	1028.3	1028.1
6	D: 1.05 (1); E: 1.1 (1); S: 1.0 (1); G: 1.1 (1); β-Ala: 1.2 (1); R: 0.9 (1); L: 0.9 (1); F: 0.9 (1); K: 0.9 (1)	1002.3	1002.2

[a] The amino acid analyses were carried out using the Picotag procedure [14], which has been found with some hydrolysed peptide samples to result in high glycine values as evident for the analysis of the purified peptide obtained following synthesis **2A** and **3A**.

β-turn-inducing amino acids [18] or the ability to form *cis*-peptide bonds [19,20]. Experimental data and calculations provided by Besser *et al.* [17] lend further support to the conclusion that the nature of the conformational space of the linear precursor is crucial in determining the cyclization propensities for a specific peptide sequence.

Our initial attempts related to the synthesis of side-chain-to-side-chain cyclic hGH[6–14] analogues, using the BOP reagent, were highly variable and dependent upon the amino acid sequence, with yields ranging from 23% to below 1% (Figure 2). The low yields were invariably associated with an increased formation of oligomeric species. Results from our earlier studies with other peptides [12] have showed that, although DIC/HOBt was sluggish in its reactivity, this combination, however, resulted in much lower levels of oligomer formation. Based on these findings, it was concluded that extended cyclization times with this reagent mixture may improve yields with the hGH[6–14] analogues. Indeed, much cleaner product profiles were obtained, as apparent from the analytical RP-HPLC and ESI-MS analyses of these cyclic hGH[6-14] peptides with DIC/HOBt mediated cyclization reactions extending up to 9 days, and manifested as nearly 10-fold improvements in the recovered yield of the purified products when compared with the products obtained with the BOP reaction condition for the more difficult to prepare sequences **2**–**4**. Moreover, the DIC reaction conditions resulted in very little oligomer formation, with the second most abundant component in the reaction mixture corresponding to the uncyclized linear peptide. With DIC/HOBt the cyclic peptides containing the Lys $\rightarrow$ Glu lactam were obtained in higher yields as purified products than the corresponding cyclic peptides containing the reverse Glu $\rightarrow$ Lys lactam. This yield effect associated with the directional orientation of the macrocyclic lactam has also been observed [12] when BOP was employed for $i \rightarrow i + 4$ lactam cyclization.

Peptide libraries offer many practical advantages in the elucidation of structure–activity relationships. However, the successful development and

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application of combinatorial libraries depends upon reliable, reproducible, high yield synthesis of the various peptide members within the library. Our earlier studies with such sets of peptides related to hGH[6–13], synthesized using the BOP reagent, fell short of this requirement. However, as documented above as well as reported elsewhere [12,14], use of DIC/HOBt has provided products of much higher quality. Mid-synthesis cyclization with DIC/HOBt, while the peptide was still resin bound, was found to have several advantages, such as the ease of refreshing the reagents, the routine use of subsequent SPPS methods that do not require specialist side-chain protection reagents, and not least the observed importance of the Fmoc group, attached to the precursor sequence, in promoting the desired cyclization [12]. The approach described above has wide applicability, although clearly not every amino acid sequence will be compatible with this procedure, due to the need to use TFA or other cleavage reagents, which may affect the side-chain characteristics of other amino acid residues already in the sequence, during the deprotection of the carboxyl and amino side-chain groups prior to cyclization. The protected amino acid Asn(Trt) used for the synthesis of the peptide sequences **2**, **3**, **5** and **6** was such an example, but fortunately the unprotected Asn was compatible with the remaining SPPS after cyclization. However, as documented elsewhere, such potential synthesis difficulties, which arise due to the nature of the amino acid sequence, can be minimized by using a combination of *tert*-butyloxycarbonyl (Boc)- and Fmoc-based protecting groups [1,10,12,14].

Cyclic analogues exhibit numerous advantages as templates of bioactive peptides, but their synthesis has not always proved successful or straightforward. Research into methods to cyclize peptide sequences has been intense over the past several years, underpinning the importance of a range of metabolically stable, bioactive cyclic peptides. Methodologies for peptide cyclization have been improved by resin design [21] and the development of coupling reagents [22]. Consistent with the results from our earlier studies [12], the propensity of a specific sequence to undergo cyclization is conditional on the inherent differences in reactivity of the cyclization reagents. Minor changes in peptide sequences can significantly affect cyclization, reflecting both the relevance of the specific peptide in terms of its underlying structure–function relationships as well as the effect on the forming lactam. The results obtained for the cyclic hGH[6–14] analogues show that a compromise of longer reaction times with use of DIC/HOBt provide the benefit of a cleaner crude product and more consistent yields for all purified cyclic peptides in a comparison with BOP. Whether this attribute is also manifested in terms of generation of a more 'bioactive' conformation of other classes of cyclic peptides that are also relevant to better receptor occupancy and higher activity is currently being evaluated. The inability of the BOP reagent to provide a general approach to the synthesis of such peptides, however, illustrates the profound effect of the sequence and conformation of the linear peptide analogue in the Lys → Glu or Glu → Lys condensation reaction.

Acknowledgements

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