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A safety catch linker for Fmoc-based assembly of constrained cyclic peptides

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Abstract: A new safety-catch linker for Fmoc solid-phase peptide synthesis of cyclic peptides is reported. The linear precursors were assembled on a *tert*-butyl protected catechol derivative using optimized conditions for Fmoc-removal. After activation of the linker using TFA, neutralization of the *N*-terminal amine induced cyclization with concomitant cleavage from the resin yielding the cyclic peptides in DMF solution. Several constrained cyclic peptides were synthesized in excellent yields and purities.

Keywords: cyclic peptides; safety-catch linker; combinatorial chemistry

INTRODUCTION

Cyclic peptides represent a large class of biologically active molecules [1–6]. Compared with their linear counterparts, cyclic peptides possess greater enzymatic and proteolytic stability and greater absorption potential [7–11]. Some cyclic peptides are drugs in their own right. Examples include cyclosporin A [8] used as an immunosuppressant, tyrocidine A [9] and gramicidin S [10] as antibiotics and octreotide to induce labour [11].

The broad range of biological activities that may be accessed from cyclic peptides makes the combinatorial synthesis of these molecules quite appealing. Particularly, given the success in deducing pharmacophores using active analogues, and by extension inactive analogues, to drive a small molecule programme, possibly incorporating privileged-like structures [12,13]. Libraries of constrained cyclic peptides are therefore exceedingly useful as molecular toolkits [1–3]. Constraining a single set of pharmacophores into different conformational substates would provide a valuable library for probing various receptors. Such a library is 'optimally diverse', since it explores both the conformational and chemical elements of diversity.

In recent years, we have been interested in establishing solid-phase chemistries that allow the synthesis of such constrained libraries [14–18]. The safety catch linker **1** previously developed essentially fulfils this requirement, however, is complicated by harsh acidic conditions for activation (TFMSA) (Figure 1) [18]. This causes unwanted by-products including trifluoroacetylation [19], alkylated material, aspartimide formation [18,20] and/or incomplete deprotection [18].

To circumvent these problems, it was envisaged that an Fmoc-based strategy would be more suitable. Activation and deprotection could then be accomplished by a milder acid treatment than TFMSA. To our knowledge there are only few successful procedures employing a 'safety catch' linker for Fmoc-based cyclic peptide synthesis [21,22]. This is principally due to the demanding chemical requirements of stability during peptide synthesis but requiring an active ester for concomitant cleavage from resin after acid treatment.

The present paper reports on the development of a safety-catch linker with the general structure **2** (Figure 1) and associated chemistries for the synthesis of head-to-tail cyclic peptides containing organic constraints. This paper describes the effective assembly of modified cyclic-peptides using Fmoc-based peptide chemistry. This linker technology provides a new solid-phase avenue to access large arrays of cyclic peptide derivatives.

MATERIALS AND METHODS

Materials

Aminomethyl polystyrene resin (substitution value = 0.41 mmol/g) and all N_α-Fmoc-amino acids were peptide synthesis grade purchased from Auspep (Melbourne, Australia) or Novabiochem (San Diego). Dichloromethane, diisopropylethylamine, *N*,*N*-dimethylformamide, and trifluoroacetic acid were obtained from Auspep (Melbourne, Australia). Triisopropylsilane and trifluoromethanesulfonic acid were purchased from Aldrich (Sydney, Australia). HPLC grade acetonitrile was purchased from BDH (Brisbane, Australia). 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Richelieu Biotechnologies (Quebec, Canada). Parallel synthesis was carried out with the aid of Bohdan MiniBlock™. This instrument was purchased from Mettler Toledo Ltd.

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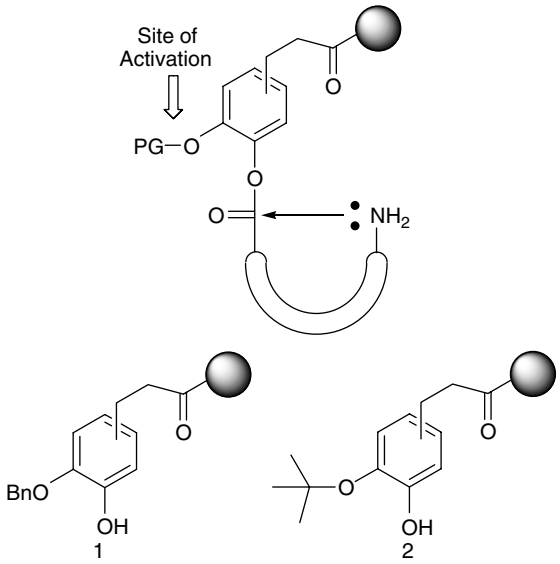

Figure 1 Concept and structural formulas of the safety catch linkers.

Analytical and Preparative HPLC

Analytical reversed-phase high-performance liquid chromatography was performed on a C-18, 5 μm, 0.46 cm × 25 cm column. Mass directed preparative reversed-phase high-performance liquid chromatography was performed on a C-18, 10 μm, 2.2 cm × 25 cm column. Separations were achieved using linear gradients of buffer B in A (A = 0.1% aqueous TFA; B = 90% CH_3CN, 10% H_2O, 0.09% TFA) at a flow rate of 1 ml/min (analytical) and 20 ml/min (preparative).

NMR Spectroscopy

NMR spectra were recorded on a Bruker Avance 600 or a Varian Gemini 300 spectrometer at 298K. COSY spectra were used to assign ^{1}H-NMR spectra.

Mass Spectrometry

High resolution mass spectra were acquired on a QSTAR Pulsar QqTOF mass spectrometer (SCIEX, Ontario, Canada), equipped with an ionspray (nebulizer assisted electrospray) atmospheric pressure ionization source. Samples (1 μl) were injected into a moving solvent (20 μl/min; 60/50 MeCN/0.1% formic acid (aq)), coupled directly to the ionization source via a fused silica capillary interface (50 μm i.d. × 50 cm length). Polypropylene glycol (ppg) solution was added at 50 pmole per μl concentration to use as the internal standard mass calibrant. Sample droplets were ionized at a positive potential of 5 kV and entered the analyser through an interface plate and subsequently through an orifice (100–120 μm diameter) at a declustering and focusing potential of 80V and 250V, respectively. Data were acquired over the mass range of m/z 350–2000 using two close internal calibration ions from the ppg solution for the accurate mass calibration. Data acquisition and processing was carried out using BioAnalyst QS software 1.1 (SCIEX, Canada).

Synthesis

Benzyl 1-(3-benzyloxy-4-hydroxyphenyl)propanoate and benzyl 1-(4-benzyloxy-3-hydroxyphenyl)propanoate. To a solution of 3,4-dihydroxyhydrocinnamic acid **4** (10.0 g, 54.9 mmol) and K_2CO_3 (19.1 g, 137.3 mmol) in DMF (300 ml) was added benzyl bromide (18.8 g, 109.8 mmol) and a catalytic amount of Bu_4NI. The mixture was stirred at room temperature overnight and then water (300 ml) and 5% aqueous HCl (50 ml) were added. The solution was extracted with diethyl ether (3 × 200 ml) and the combined organic phases were washed with water (200 ml) and brine (200 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, 5%–20% EtOAc in petroleum ether) to give a mixture of isomeric monobenzyl ethers as a colourless oil. (10.8 g, 54%). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.51–7.30 (16H, m, Ar), 6.89–6.79 (4H, m, Ar), 6.75–6.64 (2H, m, Ar), 5.13 (2H, s, CH_2Ph), 5.12 (2H, s, CH_2Ph), 5.08 (2H, s, CH_2Ph), 5.04 (2H, s, CH_2Ph), 2.92 (2H, t, $J = 7.3$ Hz, CH_2), 2.89 (2H, t, $J = 8.0$ Hz, CH_2), 2.65 (4H, t, $J = 7.8$ Hz, CH_2). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.5, 146.5, 146.4, 145.0, 137.0, 136.6, 134.8, 133.0, 129.4, 129.2, 129.1, 128.9, 128.6, 128.5, 122.0, 120.4, 115.5, 115.3, 113.1, 112.9, 71.9, 71.7, 67.0, 37.0, 36.7, 31.3, 31.1.

Benzyl 1-(3-benzyloxy-4-tert-butoxyphenyl)propanoate and benzyl 1-(4-benzyloxy-3-tert-butoxyphenyl)propanoate. The monobenzylated esters from the previous synthesis (10.8 g, 29.8 mmol) was dissolved in DCM (200 ml), cooled to −50 °C under N_2 and approximately 10 ml of isobutene was added to the solution. Trifluoromethanesulfonic acid (0.3 ml, 3.4 mmol) was added dropwise and the mixture was stirred for 3 h keeping the temperature at −40 °C. 1 ml of DIEA was added and the solution was allowed to come to room temperature and the solvent was carefully removed under reduced pressure. The residue was redissolved in DCM (200 ml) and washed with saturated aq. $NaHCO_3$ (2 × 100 ml) and water (100 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, 0–15% EtOAc in petroleum ether) to give a mixture of isomers as a colourless oil (7.6 g, 61%) together with recovered starting material (1.0 g, 9%). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.51–7.28 (16H, m, Ar), 6.97–6.69 (6H, m, Ar), 5.12 (4H, s, CH_2Ph), 5.06 (2H, s, CH_2Ph), 5.03 (2H, s, CH_2Ph), 2.92 (2H, t, $J = 7.2$ Hz, CH_2), 2.89 (2H, t, $J = 6.7$ Hz, CH_2), 2.65 (2H, t, $J = 7.3$ Hz, CH_2), 2.64 (2H, t, $J = 7.5$ Hz, CH_2), 1.34 (18H, s, *t*-Bu). ^{13}C-NMR (75 MHz, $CDCl_3$): 179.5, 153.9, 152.5, 145.9, 144.3, 138.3, 138.1, 137.1, 136.6, 133.9, 129.3, 129.1, 128.9, 128.4, 128.2, 128.0, 128.0, 126.6, 126.5, 124.5, 121.4, 115.8, 115.7, 80.6, 80.5, 71.8, 71.6, 67.0, 36.8, 36.7, 31.4, 30.9, 30.4, 30.4, 30.1, 29.5.

1-(3-hydroxy-4-tert-butoxyphenyl)propanoic acid and 1-(4-hydroxy-3-tert-butoxyphenyl)propanoic acid (4). A solution of the *tert*-butyl ethers (7.6 g, 16.8 mmol) in methanol (200 ml) was added to 10% Pd/C (0.4 g) and the mixture was placed under hydrogen at 35 psi for 6 h. Filtration through a pad of Celite and evaporation of the solvent under reduced pressure gave a mixture of isomers **4** as a yellow oil (4.0 g, 93%). ^{1}H-NMR (300 MHz, $CDCl_3$): 6.93–6.79 (5H, m, Ar), 6.61 (1H, dd, $J = 8.2$ Hz, 2.1 Hz, Ar), 2.86 (2H, t,

$J = 7.2$ Hz, CH_2), 2.85 (2H, t, $J = 7.7$ Hz, CH_2), 2.64 (2H, t, $J = 6.5$ Hz, CH_2), 2.63 (2H, t, $J = 8.2$ Hz, CH_2), 1.39 (18H, s, *t*-Bu). ^{13}C-NMR (75 MHz, $CDCl_3$): 178.0, 178.0, 149.5, 148.0, 141.7, 140.2, 136.3, 131.5, 128.2, 123.7, 122.2, 122.1, 119.2, 114.7, 80.6, 80.4, 35.8, 36.4, 30.0, 28.6, 28.6.

Fmoc-β-amino acid safety-catch linker resin (5). Aminomethyl polystyrene resin (5 g, 2.05 mmol) was derivatized with H-Gly-Leu-Leu using *in situ* neutralization/HBTU activation protocols for Boc chemistry. A mixture of 1-(3-hydroxy-4-*tert*-butoxyphenyl)propanoic acid and 1-(4-hydroxy-3-*tert*-butoxyphenyl)propanoic acid (0.98 g, 4.10 mmol) in a 0.2 M solution of HBTU (20.5 ml, 4.10 mmol) was added to the resin. This was followed by diisopropylethylamine (1.15 mL, 6.50 mmol) and the mixture was then shaken for 2 h. The resin was filtered and washed with DMF (3 × 30 ml) and DCM (3 × 30 ml) to give resin **2**. DIC (0.641 ml, 4.10 mmol) was added to a stirred solution of Fmoc-β-amino acids in DCM/DMF (4 : 1) and then added to the resin together with DMAP (50 mg, 0.41 mmol) and shaken for 16 h. The resin was filtered and washed with DMF (3 × 30 ml) and DCM (5 × 30 ml) and dried under vacuum overnight to give resin **5**.

Synthesis of cyclic peptides 8a–h. All reactions were performed on a Bohdan MiniBlock™ instrument using 1.5 ml of solvent for all reactions and washings. Resin **5** (100 mg, 30 μmol) was preswelled in DMF and filtered. Piperidine/DMF (1 : 4) was added and shaken for 1 min and the treatment repeated once. The linear peptide was assembled using double couplings of Fmoc amino acids and HBTU/DIEA in DMF for 10 min per coupling. Deprotections were performed using piperidine/DMF (1 : 4) for 2 × 1 min. After removal of the final Fmoc group, the resin was washed with DMF (3×) and DCM (3×) and then TFA/TIPS (97 : 3) was added for 2 × 30 min. The resin was washed with DCM (3×), DMF (3×) and DCM (5×) and then dried under vacuum for 4 h. Under a nitrogen atmosphere, 5% DIEA in DMF was added and the mixture shaken for 4 h. The resin was filtered and the filtrate collected. This procedure was repeated with fresh DIEA/DMF solutions for 48 h and again for 72 h. The filtrates were combined and evaporated under reduced pressure to give the crude cyclic peptide. Preparative HPLC followed by freeze-drying gave the pure peptide, typically as a white solid material. Table 1 describes the purities and isolated yields for the eight cyclic peptides, including both penta, hexa and hepta peptides. Table 2 assigns the NMR for all isolated peptides.

RESULTS AND DISCUSSION

Design and Synthesis of Linker

Central to the overall design of the safety-catch linker is the reactivity of *O*-hydroxyphenyl ester towards amines. In the alkylated form, the ester has previously been reported to be approximately three orders of magnitude less reactive than the unalkylated precursor for a description of ester cleavage of *O*-hydroxyphenyl esters by a nucleophilic base see [23]. Moreover, it was reported that *O*-benzyloxyphenyl esters are masked active esters in solution [24,25]. In addition, a study by Beech *et al.* showed that a *tert*-butyl protected catechol linker was relatively stable towards nucleophilic cleavage [26].

Another feature required in the design of the linker was a high yielding synthesis that contains relatively few steps. It was thus postulated that linker **2** would meet our chemical requirements. Synthesis of linker **2** was accomplished in a three-step process as shown in Figure 2. Initially the catechol **3** was treated with two equivalents of benzyl bromide resulting in two regio-isomers. The benzylation presumably occurred on the carboxyl moiety first, followed by one of two hydroxyls. The *tert*-butyl moiety was introduced on the remaining hydroxy group using excess isobutene and a catalytic amount of TFMSA in DCM at low temperature.

Subsequent hydrogenation removed both benzyl groups to give the mono *tert*-butyl protected linker **4** in a total yield of 31% for the two regio-isomers. All further chemistries were evaluated using the regio-isomeric mixture of linkers. Primarily this was driven by the fact that they co-eluted on preparative HPLC, but also because both regio-isomers were found in a preliminary study to be chemically equivalent in terms of stability and reactivity towards peptide assembly and cyclization.

Fmoc-removal Studies

To determine whether this approach was suitable for cyclic peptide production, different Fmoc-removal

Table 1 Purities, Isolated Yields and Accurate Mass of Cyclic Peptides **8a–h**

Entry	Cyclic peptide **8**	Purity[a]	Isolated yield[b]	ES-MS (calc)	ES-MS (found)
a	Cyclo-[Phe-(D-Trp)-Gly-Thr-βAla]	81%	23%	562.2534	562.2530
b	Cyclo-[Phe-(D-Trp)-Arg-Thr-βAla][15]	59%	28%	660.3330	660.3337
c	Cyclo-[Leu-Tyr-(D-Ala)-His-βAla]	87%	31%	555.2797	555.2793
d	Cyclo-[Gly-Asp-Ile-Ser-Pro-βAla][18]	55%	10%	540.2538	540.2553
e	Cyclo-[Tyr-Pro-Ala-Pro-Phe-βAla]	99%	19%	646.3109	646.3093
f	Cyclo-[Gly-Asp-His-Phe-Tyr-(D-Ala)-βAla]	73%	14%	761.3127	761.3117
g	Cyclo-[Leu-Tyr-(D-Ala)-His-(5-ACA)]	52%	20%	597.3269	597.3257
h	Cyclo-[Leu-Tyr-(D-Ala)-His-βhomoTrp]	64%	14%	684.3378	684.3395

[a] Determined by HPLC-UV_{210} peak integration.
[b] Purified by preparative HPLC. 5-ACA, 5-aminocaproic acid.

Table 2 NMR-data for New Cyclic Peptides. Spectra Recorded in DMSO-d_6 at 298K and 600 MHz. All Data are Chemical Shifts in ppm Relative to TMS. Compound **8e** was Present as two Conformers in the Ratio 4 : 6

Peptide	Residue	NH	H_α	H_β	Others
8a	βAla	7.17	2.25, 1.98	3.62, 3.00	
	Thr	7.28	4.12	4.33	H_γ 1.01, OH 4.70
	Gly	8.65	3.74, 3.55		
	D-Trp	8.57	4.26	3.07, 3.00	H_δ 7.17, H_ε 10.86
	Phe	7.66	4.70	2.81, 2.70	
	Aromatics				7.52, 7.35, 7.19–7.14, 7.10–7.06, 7.00
8b	β-Ala	6.84	2.28, 1.99	3.62, 2.96	
	Thr	7.50	4.04	4.34	H_γ 1.03, OH 4.78
	Arg	8.68	3.74	1.66, 1.35	H_γ 0.98, H_δ 2.92
	D-Trp	8.65	4.32	3.00	H_δ 7.16, H_ε 10.78
	Phe	7.82	4.66	2.87, 2.71	
	Aromatics				7.17–7.38, 7.06, 6.99
8c	β-Ala	7.53	2.38, 2.13	3.59, 3.02	
	His	7.86	4.46	3.33, 3.05	H_{Im} 9.19, 8.92, 7.22
	D-Ala	8.18	4.22	1.17	
	Tyr	8.32	4.13	3.13, 2.78	H_{Ar} 6.66, 6.94
	Leu	7.76	4.22	1.07, 1.34	H_γ 1.49, H_δ 0.83, 0.80
8e	β-Ala	6.96	2.58, 1.95	3.39, 3.12	
Conformation 1	Phe	8.34	4.16	2.67	H_{Ar} 7.24–7.08
	Pro		3.89	1.87	H_γ 2.14, H_δ 3.45
	Ala	8.90	4.41	1.31	
	Pro		3.55	1.77	H_γ 2.32, H_δ 3.48
	Tyr	7.61	4.29	3.25, 2.81	H_{Ar} 6.93, 6.67
8e	β-Ala	6.48	2.44, 2.10	3.31, 3.11	
Conformation 2	Phe	7.98	4.58	2.67	H_{Ar} 7.24–7.08
	Pro		4.47	1.61	H_γ 1.46, H_δ 3.22
	Ala	8.62	4.35	1.19	
	Pro		3.98	2.08, 1.68	H_γ 1.68, H_δ 3.26
	Tyr	8.41	4.08	3.14, 2.78	H_{Ar} 6.93, 6.67
8f	β-Ala	7.64	2.34, 2.27	3.33, 3.22	
	D-Ala	7.92	4.12	1.17	
	Tyr	7.21	4.19	2.89, 2.83	H_{Ar} 6.67, 6.98
	Phe	8.25	4.44	2.75, 2.60	H_{Ar} 7.31–7.12
	His	8.05	4.27	3.07, 2.99	H_{Im} 9.18
	Asp	7.92	4.32	2.92, 2.86	
	Gly	8.44	3.74, 3.55		
8g	5-ACA	7.29	2.16, 2.03	1.59, 1.42	H_γ 1.43, 1.31, H_δ 1.27, 1.11, H_ε 3.44, 2.87
	His	7.99	4.07	2.88, 2.79	H_{Im} 9.23, 8.95, 7.15
	D-Ala	8.18	3.92	0.97	
	Tyr	8.31	4.41	2.93, 3.32	H_{Ar} 6.65, 6.97
	Leu	8.28	4.07	1.30	H_γ 1.57, H_δ 0.81, 0.74
8h	β-Trp	7.45	2.66, 2.31	3.97	H_γ 3.12, 2.83, H_{Ar} 7.67, 7.34, 7.07, 7.00
	His	8.12	4.49	3.27, 2.97	H_{Im} 9.16, 8.96, 7.29
	D-Ala	8.34	4.12	1.08	
	Tyr	7.66	4.24	2.93, 2.69	H_{Ar} 6.92, 6.63, OH 7.17
	Leu	7.82	3.99	1.32, 1.17	H_γ 1.42, H_δ 0.79, 0.72

strategies were investigated. The linker **2** was attached to an acid labile trityl polystyrene resin and spacer and loaded with either Fmoc-phenylalanine or Fmoc-β-alanine. Hereafter different cleavage mixtures which have previously been reported to remove the fluorenylmethyloxycarbonyl group were added [27], including DBU [28,29], DBU/HOBT [30,31], TBAF [32] and employing various concentrations of piperidine, with different solvents and reaction times. After acetylation (10% Ac_2O in DMF) the trityl linker was cleaved from the resin and the amount of Fmoc-removal and cleavage of amino acid from the linker were estimated using HPLC. The use of DBU, DBU/HOBT or TBAF were all found to be unsatisfactory, either because of incomplete

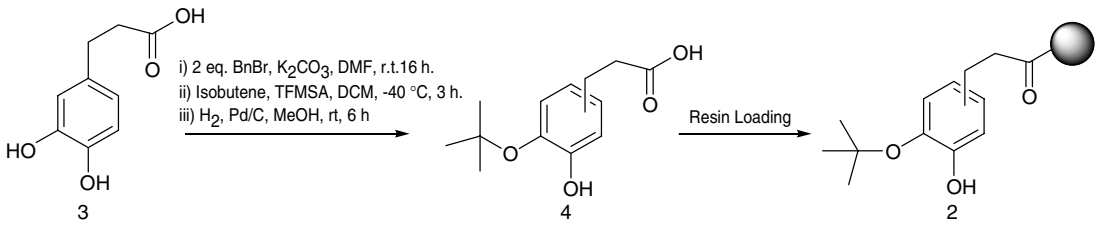

Figure 2 Synthesis of the *tert*-butyl safety catch linker **4** and subsequent loading on resin.

deprotection or from partial or even complete cleavage of the amino acid from the linker which would eventually result in low yields of the target peptide.

Orain had previously reported the use of piperidine/DMF solutions for very short reaction times to remove Fmoc groups in the presence of a phenyl ester [33]. For non-α-amino acids, the optimal conditions used for complete Fmoc removal and no detectable cleavage by HPLC was a 20% piperidine/DMF solution for 2×1 min at room temperature. Interestingly this ester group could withstand a 10 min treatment of the piperidine/DMF solution with no presence of aminolysis by HPLC. This contrasted to the α-amino acids where small unwanted nucleophilic cleavage was found to be problematic.

There is a significant amount of work dedicated towards deriving a unifying mechanism to explain varied results observed with ester hydrolysis [23, 34–40]. In our case, one likely explanation for α-amino ester lability is general base catalysis [23, 34–39]. The NH of the amino esters attached to the catechol may act as a relay for the action of reagents in solution [34]. As a consequence the amine of the α-amino-ester acts in a similar manner to the hydroxyl group of the safety catch linker. This effect can probably occur for non-α-amino acids as well, but was not observed due to the reaction times. The difference between amino-esters is small but significant, which results in α-amino acids to being partially cleaved while non-α-amino acids were shown to be stable under the described reaction conditions.

The outcome is thus based upon conformational and steric grounds. This idea is somewhat supported by Bruice *et al.* [40] who observed significant differences in the rates of hydrolysis of phenyl esters of γ-(*N*,*N*-dimethylamino)-butyric acid and δ-(*N*,*N*-dimethylamino)-valeric acid. This difference was shown by kinetic experiments to be solely due to a difference in entropy of activation.

Synthesis of Cyclic Peptides

Using the optimized conditions for Fmoc-removal, a small selection of modified cyclic peptides containing non-α-amino acids at the first position were synthesized. Essentially the synthesis begins with the linker **4** being attached to an aminomethyl polystyrene resin[†] (Figure 3). followed by the attachment of Fmoc amino acids using DIC and DMAP (Figure 3). The linear sequences were assembled using HBTU/DIEA and after the final Fmoc-removal, the linker was activated by simple removal of the *tert*-butyl group using TFA with triisopropylsilane as a scavenging agent. Addition of 5% DIEA in DMF promoted the cyclization and concomitant cleavage from the resin to yield cyclic peptides **8**. This final step was performed under a dry nitrogen atmosphere since the cyclization solution is hygroscopic. The presence of water causes base catalysed hydrolysis giving linear peptides, which can be considerable in cases were the cyclization is slow. After removal of the DIEA/DMF under reduced pressure, the peptides were purified by HPLC. Table 1 describes the purities and isolated yields for eight cyclic peptides, including both penta, hexa and hepta peptides.

Cyclic peptides **8a**–**8h** were obtained in varying yields and purities comparable to other methods [14–18,21,22,41]. For all cyclizations no linear peptide or oligerimization was detected. This is presumably due to the combination of the nitrogen atmosphere and the presence of the non-α-amino acids within the peptide motif allowing ease of cyclization. For tryptophan containing sequences only a small amount of *tert*-butylated product was observed and, compared with the BOC/TFMSA method, the products were easily purified on HPLC. For other sequences no alkylation or trifluoroacetylation was observed due to the mild activation/deprotection conditions.

CONCLUSIONS

In conclusion, an Fmoc-based safety catch linker was developed suitable for the synthesis of modified cyclic peptides. The overall process involves a suitable *tert*-butylether protecting group on the catechol group. Synthesis of linear peptides using Fmoc-based solid phase chemistries followed by TFA treatment and cyclization using a tertiary base gave the cyclic peptides. This has considerable advantages. Workup is simple solvent removal, allowing for the synthesis of very large

[†] It was noted several times that peptide assembly on resin is more effective when the linker is separated from the resin by a small peptide sequence. Therefore a tripeptide spacer (Gly-Leu-Leu) was introduced on the aminomethylated resin prior to attaching the linker.

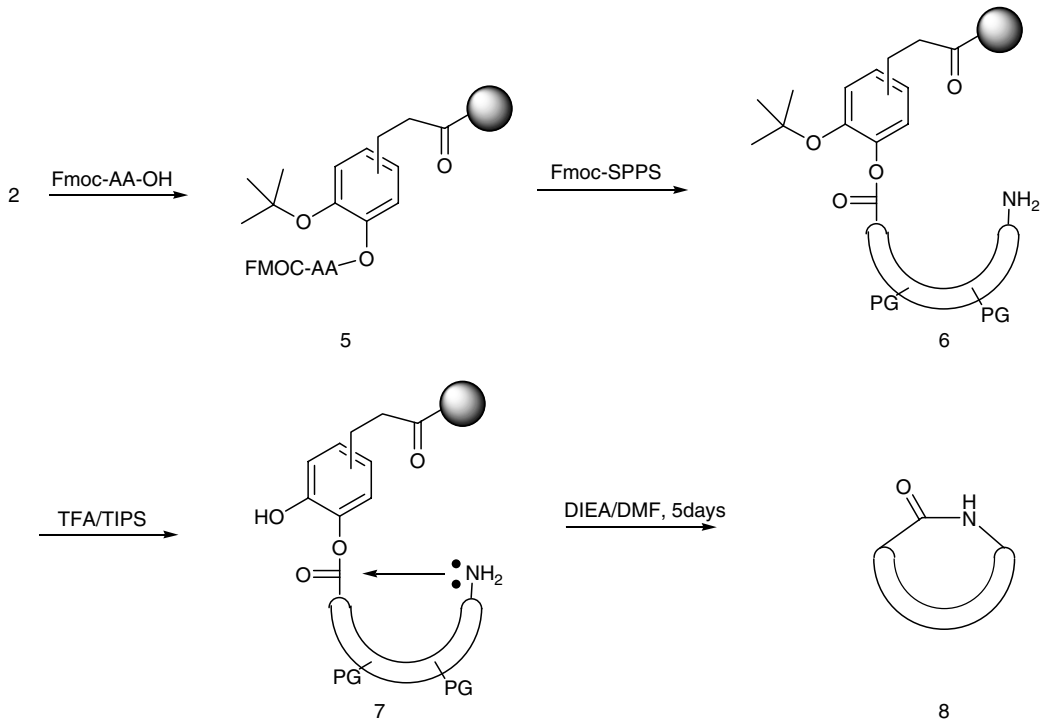

Figure 3 Synthesis of the constrained cyclic peptides **8** using the safety catch linker **2**.

numbers of compounds using the safety catch linker **2**. This is a significant improvement to our initial linker since it circumvents many of the problems associated with TFMSA treatment, such as alkylation, aspartimide formation and incomplete deprotection of side chain protecting groups present within Boc-chemistries. Consequently, crude purity and cyclization efficiencies are greatly enhanced. The only limiting aspect of this procedure is the requirement to use non-α-amino acids at the first position. However, from our perspective, we are interested in the synthesis of constrained cyclic peptide libraries that contain unnatural amino acids. The constraints, such as β-amino acids, are selected to stabilize different conformational substates of the peptide. By selecting different scaffolds and selecting different sequences, such a library is optimally diverse as it samples both the conformational and chemical elements of diversity. The safety catch linker reported here has significant advantages in the preparation of such a library over existing linkers and chemistries. Finally as with other handles, our linker is compatible with a wide range of functional supports that are currently in use in peptide synthesis.

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