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Tetrahedron Letters 46 (2005) 1707–1710
Tetrahedron Letters

Synthesis of sidechain adapted β-turn mimics for modifying the C-terminus of substance P

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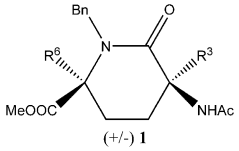
Received 23 November 2004; accepted 12 January 2005
Available online 29 January 2005

Abstract—Sidechain adapted β-turn mimics of type **1** characterised by a fixed *cis*-conformation of the peptide chain in the aminopiperidinonecarboxylate scaffold have been synthesised from pyrazinones in order to perform a β-turn scan of the messenger region of substance P. The synthesis of a substance P peptide analogue is also described.

1. Introduction

The synthesis of constrained peptidomimetics is an important field in synthetic organic chemistry. Incorporation of rigidified scaffolds in a peptide sequence and further assessment of its biological activity can provide information about the bioactive conformation of the system. We recently published a general synthesis for systems of type **1** (Fig. 1) and classified them as 'type-VI-like' β-turn mimetics.[2,1] In these aminopiperidinonecarboxylate (APC) scaffolds, the peptide bond is fixed in a *cis*-conformation and different sidechain functionalities can be incorporated in these systems. So far however, we only synthesised model compounds, which are not suitable for incorporation into the peptide chain. In this paper, we report the synthesis of functionalised APC scaffolds, which enables one to do a β-turn/*cis*-peptide bond scan of the C-terminal sequence of the neuropeptide substance P (SP). Our approach is illustrated by the synthesis of a first APC modified substance P analogue.



(+/-) **1**

Figure 1. Aminopiperidinonecarboxylate.

Substance P (SP) is the undecapeptide neurotransmitter Arg[1]-Pro[2]-Lys[3]-Pro[4]-Gln[5]-**Gln[6]-Phe[7]-Phe[8]-Gly[9]-Leu[10]-Met[11]-NH$_2$**.[12] It has been implicated in several diseases including arthritis, asthma, inflammatory bowel disease and depression.[8,7] According to ^{1}H NMR experiments, a β-turn is present in the C-terminal hexapeptide sequence Gln[6]-Phe[7]-Phe[8]-Gly[9]-Leu[10]-Met[11]-NH$_2$. This C-terminal hexapeptide sequence (messenger region) shows similar activity as the native sequence, however it lacks its selectivity. A 'type VI-like turn' containing a *cis*-peptide bond was observed at the Phe[7]-Phe[8] position of a bioactive [pGlu[6], NMe-Phe[8], Aib[9]] (6–11) analogue of substance P.[6,9] In this context, replacement of the Phe[7]-Phe[8] residues with a turn inducing substructure that contains a type VI-like, constrained *cis*-peptide bond, seems an interesting research goal. Also other turn types centered at Phe[7]-Phe[8], Phe[8]-Gly[9] and Gly[9]-Leu[10] residues have been proposed by various authors.[10,13,3] Interestingly, application of our APC scaffold methodology would enable one to perform a *cis*-peptide bond/β-turn scan of the whole C-terminal region of the peptide. An important feature of our methodology in this regard is that the sidechain of the corresponding amino acid residue (through which binding to the receptor might occur) can be retained in the

Keywords: *cis*-Peptide bond; β-Turn; Peptidomimetics; Substance P; Pyrazinones.
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doi:10.1016/j.tetlet.2005.01.056

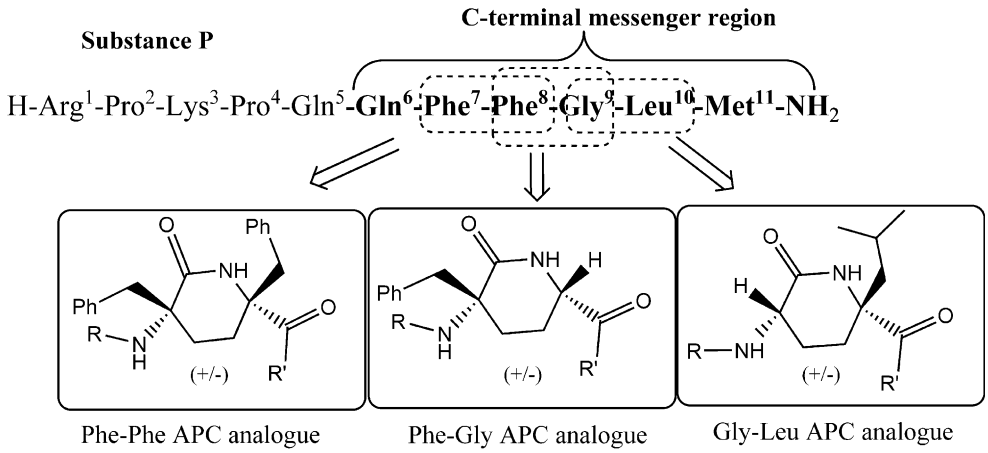


Figure 2. β-Turn scanning of the messenger region of substance P.

scaffolds (principle shown in Fig. 2). Boc and Fmoc protection strategies have been elaborated to render these scaffolds amenable for peptide synthesis.

2. Synthesis of bicyclic precursors

The bicyclic precursors **5a**–**c** for the APC systems, with correct sidechain functionality in place, were synthesised using our established method. This implies functionalisation of dichloropyrazinones **2a**–**c**, followed by cycloaddition of **3a**–**c** with ethene at high pressure. The C_2-bridge introduced in the Diels–Alder reaction eventually serves as a conformational restriction in the APC systems. Finally, the reactive imidoyl chloride adducts **4a**–**c** were hydrolysed to give the bislactam systems **5a**–**c** (Scheme 1). As the R^1 substituent, a PMB group was chosen instead of the benzyl group that was used previously because the latter could not be removed.

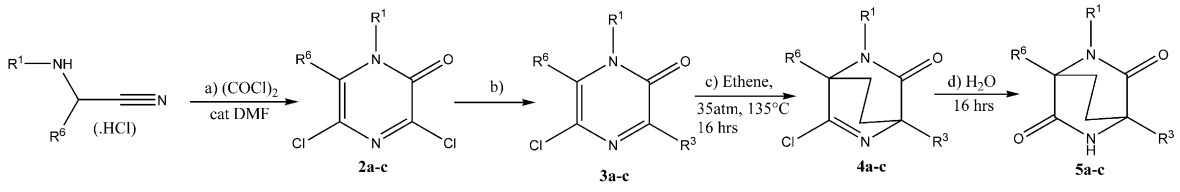
3. Methanolysis of bislactam systems and formation of Boc/Fmoc protected scaffolds

The strained bislactam systems **5a**–**c**, when treated with an HCl–saturated methanol solution, open selectively at the secondary lactam position to yield hydrochloride salts **6a**–**c** (Scheme 2).[11,2] The corresponding amines were trapped by subsequent reaction with a tertiary amine and Boc_2O/Fmoc-Cl or an acid chloride to yield compounds **7a**–**d**. NMR and mass spectral analysis of these compounds confirm the structures proposed. At this stage, the N-terminus of the Boc and Fmoc derivatives **7a**–**d** is already suitably protected, but the methyl ester and the PMB function still have to be removed in order to make the analogues suitable for peptide work. Model compound **7e** was initially used to study these subsequent deprotection reactions.

4. Removal of the PMB group and cleavage of the methyl ester, a test case

To deblock the carboxyl function of model compound **7e**, a simple saponification was tried using aqueous sodium hydroxide or lithium hydroxide (Scheme 3). The desired compound **8e** was formed only in minor amount. The main product isolated was the cyclised bislactam **5b**. This means that the cleavage of the amide was the faster reaction (and not the hydrolysis of the hindered ester) resulting in regeneration of cyclic compound **5b**. Treatment of methyl ester **7e** with lithium iodide in EtOAc however led to a very clean reaction with the formation of only the desired product **8e** in a good yield.[4] The soft nucleophile iodide selectively attacks the methyl group. It also allows cleavage of the ester in the presence of base labile groups like Fmoc.

Different conditions were tried on the model compound **7e** to remove the PMB group (Scheme 4). Treating **7e** with trifluoroacetic acid at reflux temperature nor a reductive procedure using H_2/Pd–C (10%) gave satisfactory yields of the desired compounds. However



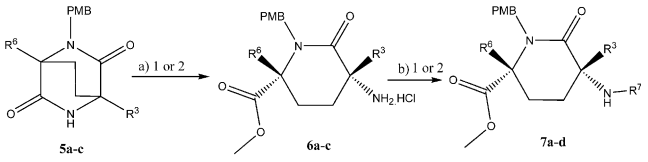
	R^1	R^6	Yield (%)
2a	PMB	Bn	40
2b	PMB	H	61
2c	PMB	Isobutyl	55

	R^1	R^3	R^6	b) Reagents & conditons	Yield (%)
3a	PMB	Bn	Bn	BnMgBr, THF, -78°C	64
3b	PMB	Bn	H	BnMgBr, THF, -78°C	72
3c	PMB	H	Isobutyl	$Pd(PPh_3)_4$, HCOONa, DMF, 110°C	55

	R^1	R^3	R^6	Yield (%)
5a	PMB	Bn	Bn	63
5b	PMB	Bn	H	81
5c	PMB	H	Isobutyl	60

PMB = *p*-methoxybenzyl

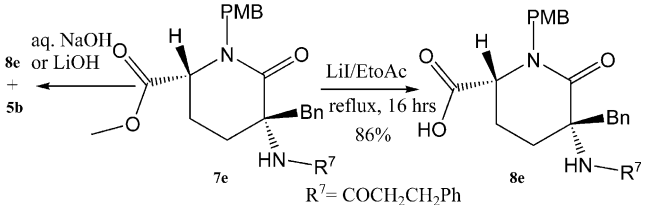
Scheme 1. Synthesis of bicyclic precursors.



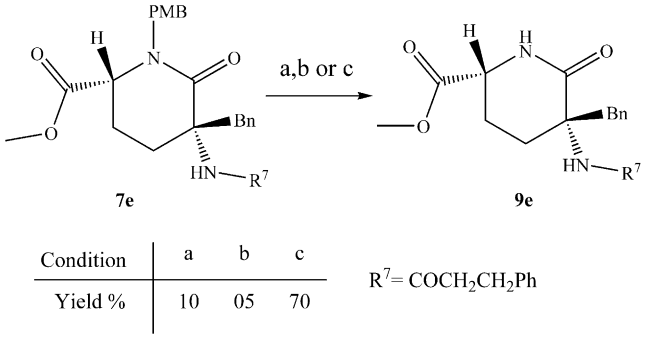
	R^3	R^6	R^7	Conditions Step: a	b	Yield (%)
7a	Bn	Bn	Fmoc	1	1	45
7b	Bn	H	Fmoc	2	1	68
7c	Bn	H	Boc	2	2	72
7d	H	Isobutyl	Fmoc	1	1	65
7e	Bn	H	$CO(CH_2)_2Ph$	2	3	70

PMB = *p*-methoxybenzyl

Scheme 2. Methanolysis of bicyclic adducts. Reagents and conditions: (a) (1) MeOH/HCl, 50 °C, 16 h; (2) MeOH/HCl, rt, 2 h; (b) (1) Fmoc-Cl, DIEA, DMAP, $CHCl_3$, 2 h; (2) Boc_2O, Et_3N, $CHCl_3$, 1.5 h; (3) $Ph(CH_2)_2COCl$, Et_3N, $CHCl_3$, 1 h.



Scheme 3. Deprotection of the carboxyl function, a test case.



Condition	a	b	c
Yield %	10	05	70

R^7= $COCH_2CH_2Ph$

Scheme 4. Removal of PMB group. Reagents and conditions: (a) TFA, reflux, 16 h; (b) H_2/Pd–C (10%), ethanol, 16 h; (c) CAN, acetonitrile, −15 °C–rt.

oxidative cleavage by ceric ammonium nitrate (CAN) was very successful.

5. Removal of the PMB group and cleavage of the methyl ester in the APC systems

Based on the work performed on the model compound **7e**, demethylation of the methyl ester and removal of the PMB group in turn was effected for the Boc and Fmoc protected APC systems (Scheme 5). In general, the oxidative PMB removal gives satisfactory results for all the Fmoc protected analogues. We soon found that CAN also is an effective reagent to remove a Boc protective group,[5] so the selective oxidative removal of PMB on Boc protected compound **9c** was not successful.

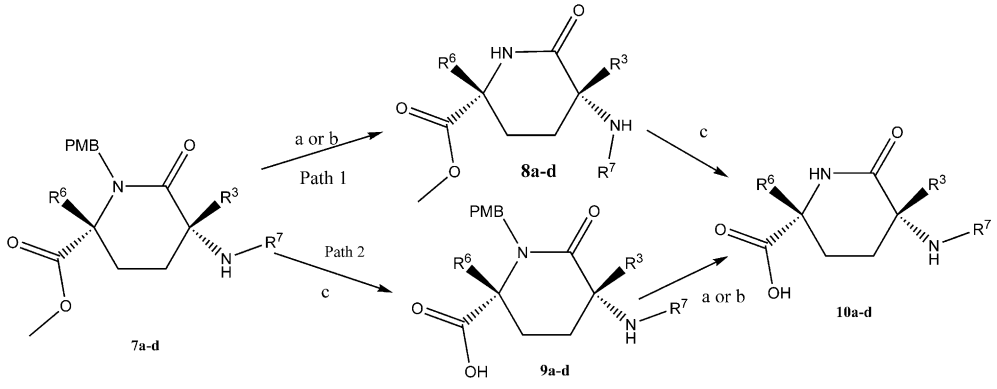
In contrast to our test case **7e**, we were able to remove the PMB group from the APC systems with R^6 = isobutyl using trifluoroacetic acid (TFA) at reflux temperature. This TFA procedure does not work in any of the other cases.

As shown in Scheme 5 and the accompanying table we tried two different sequences: first removal of the PMB group followed by demethylation and vice versa. The yield of the final products in both sequences are comparable, path 1 was more practical for **10b** and path 2 for **10d** (Table 1).

As can be seen from the table, we managed to synthesise all the Fmoc-protected derivatives **10a**,**b** and **10d** that were initially proposed for the modification of SP. The synthesis of the Boc analogue **10c** was not successful, due to the unselective nature of the CAN deprotection. The partially deprotected intermediate **9c** obtained by demethylation of methyl ester **7d** however is also expected to be useful in Boc-based peptide synthesis.

6. Incorporation of APC systems into the C-terminal hexapeptide region of SP, a first example

A substance P peptide analogue Gln^6-Phe^7-Phe^8-$(Gly\text{-}Leu)_{APC}$-Met^{11}-NH_2 was synthesised using solid-phase peptide techniques (Scheme 6). Rink amide resin was

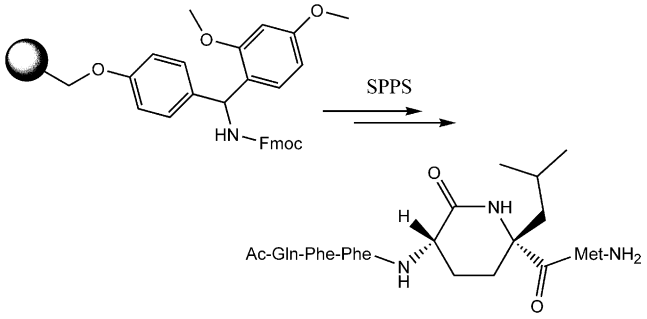


Scheme 5. Synthesis of fully deprotected APC analogues. Reagents and conditions: (a) CAN/water, acetonitrile, 0 °C to rt, 2 h; (b) TFA, reflux, overnight; (c) LiI, ethyl acetate, reflux, overnight.

Table 1. Deprotection of the scaffolds

R^3	R^6	R^7	Path 1				Path 2			
			Product	Yield^{method} (%)	Product	Yield (%)	Product	Yield (%)	Product	Yield^{method} (%)
Bn	Bn	Fmoc	**8a**	67[a]	**10a**	52	**9a**	42	**10a**	—
Bn	H	Fmoc	**8b**	66[a]	**10b**	68	**9b**	71	**10b**	70[a]
Bn	H	Boc	**8c**	—	**10c**	—	**9c**	90	**10c**	00[a]
H	Isobutyl	Fmoc	**8d**	55[a] 70[b]	**10d**	64	**9d**	69	**10d**	60[a] 73[b]

[a] See method a in Scheme 5.
[b] See method b in Scheme 5.



Scheme 6. Synthesis of SP (6–11) APC analogue.

used in order to obtain the C-terminal carboxamide. Successive couplings with the common *N*-Fmoc protected amino acids (used in standard threefold excess) were accomplished by reaction with HOBT/DIC in DMF and were completed within 2 h. However, attachment of the more hindered Gly-Leu APC scaffold **10d** (twofold excess) required activation with TBTU in DMF and took 16 h for completion (ninhydrin test). The N-terminal Gln residue (with a trityl protected sidechain) was capped with acetic anhydride. Finally, the peptide was cleaved from the resin and detritylated using TFA/water/ethylene-dithiol/triisopropylsilane (95:2:2:1). Following purification by reverse-phase-HPLC (C18, acetonitrile–water gradient), the peptide was obtained with 26.5% yield and 99% purity. Due to the incorporation of the racemic APC system, the peptide consists of a mixture of two diastereoisomers, as confirmed by the presence of two peaks in the chromatogram, which could both be characterised by LC–MS (electrospray ionisation, MH^+ ion observed at *m*/*z* 809). The amino acid sequence of the peptide was confirmed by further MS/MS analysis of the MH^+ ion, which resulted in specific cleavages of the amide linkages to form mainly fragment ions of the type H_3N^+-chain-$MetNH_2$ retaining the C-terminus, for example, *m*/*z* 639 and 492.

7. Conclusion

In summary, we have elaborated the concept of synthesising sidechain adapted APC scaffolds, which are suitable to perform a *cis*-peptide bond scan of the β-turn containing region of a peptide. The synthesis of both Fmoc and Boc protected derivatives was tried, but the Fmoc based scaffolds seem to be the more feasible ones. A first peptide analogue has been prepared demonstrating that the APC-scaffold can be introduced into the peptide sequence with an appropriate coupling reagent and using a longer coupling time. The synthesis of the other substance P analogues and biological testing is under current investigation.

Acknowledgements

The authors thank the F.W.O. (Fund for Scientific Research–Flanders (Belgium)) and the Johnson and Johnson Pharmaceutical Research Foundation for financial support. W.M.D.B. (Postdoctoral Fellow of the F.W.O.) and V.M. (Research Assistant of the F.W.O.) thank the F.W.O. for the fellowship received.

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