Journal of Peptide Science, Vol. 4, 72–80 (1998)

Chemoselectively Addressable HCan Building Blocks in Peptide Synthesis: L-Homocanaline Derivatives

IRMTRAUD LANG, NADINE DONZÉ, PATRICK GARROUSTE, PASCAL DUMY and MANFRED MUTTER

Institute of Organic Chemistry, University of Lausanne, BCH-Dorigny, Lausanne, Switzerland

Received 18 April 1997
Accepted 29 May 1997

Abstract: (*S*-2-amino-5-(aminooxy)pentanoic acid (L-homocanaline, HCan), a structural analogue of lysine, contains a reactive alkyloxyamine side chain and is therefore considered to react chemoselectively with carbonyl compounds by forming a kinetically stable oxime bond. The chemical synthesis of L-homocanaline starting from protected glutamic acid derivatives is described. Two orthogonally protected homocanaline derivatives were synthesized and their use in standard SPPS procedures was exemplified for the synthesis of a chemoselectively addressable cyclic peptide for use in TASP design. Moreover, the wide range of applications of this unique building block was demonstrated for the chemoselective ligation of an unprotected disaccharide to a HCan containing model peptide resulting in a chimeric glycopeptide structure. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

J. Pep. Sci. 4: 72–80 (1998)

Keywords: L-Homocanaline; chemoselectively addressable amino acid; oxime bond; peptide synthesis; glycopeptide

INTRODUCTION

Recent progress in the methodology of peptide synthesis has stimulated the synthesis of large polypeptides including small proteins [1,2]. In addition, the construction of novel proteins exhibiting tailor-made structural and functional properties is becoming the realm of today's synthetic tools [3, 4]. Here, the recent introduction of chemoselective ligation procedures represents an elegant way to overcome some well-known limitations of convergent strategies, e.g. the low solubility of fully protected peptide segments, or the extensive need of orthogonal protecting groups for the preparation of large polypeptides or TASP molecules [5–7]

So far, the insertion of appropriate functionalities for chemoselective ligation into the peptide backbone or on chain ends comprises several additional chemical steps, often hampered by the complexity of functional side chains within a given peptide sequence. Consequently, the direct availability of amino acids featuring chemoselectively addressable side chains represents an important step for accessing the full potential of this methodology in peptide synthesis, protein design and protein modification. In this article, we elaborate synthetic routes for the aminooxy-containing building block 'homocanaline (HCan)' and its application in peptide synthesis.

Abbreviations: Ac_2O, acetic anhydride; Aloc, allyloxycarbonyl; ByBOP, benzotriazol-1-yl-oxy-tris-pyrralidino-phosponium-hexafluorphosphate; Bu_3SnH, tributyltinhydride; DCM, dichloromethane; DIEA, *N,N*-diisopropyl-ethylamine; DME, dimethoxyethane; Hex, hexane; HCan, homocanaline; IBCF, isobutylchloroformate; $Na_2S_2O_5$, sodium pyrosulfite; NMM, *N*-methyl-morphine; $Pd(PPH_3)_2CL_2$, bis(triphenylphosphine)palladium dichloride; *p*-TsOH, toluene-4-sulphonic acid.

Address for correspondence: Prof. Manfred Mutter, Institute of Organic Chemistry, University of Lausanne, BCH-Dorigny, CH-1015 Lausanne, Switzerland.

© 1998 European Peptide Society and John Wiley & Sons, Ltd.
CCC 1075-2617/98/010072–9

MATERIAL AND METHODS

Homocanaline Derivatives

The starting material *t*-butyloxycarbonyl-L-glutamic acid-*t*-butylester (Boc-L-Glu-OtBu) was obtained from Bachem Feinchemikalien AG, Bubendorf, Switzerland. The chemicals and solvents from Fluka, Buchs, Switzerland, were used without further purification. Precoated plates of silica gel F254 and silica gel 60 (Merck, Darmstadt, Germany) were respectively used for thin layer chromatography and for flash chromatography columns. ^{1}H-NMR and C^{13}-NMR spectra were recorded on a Bruker AMX-400 FT instrument in deuterated dimethylsulphoxide (Glaser, Basel, Switzerland). Molecular masses were determined in the chemical ionization mode using a Nermag R10-10C; infrared spectra were recorded in chloroform with a Perkin-Elmer FT-IR paragon 1000 instrument.

Peptide Synthesis

Fmoc amino acids and the coupling reagents PyBOP and HOBT were supplied from Novabiochem AG (Läufelfingen, Switzerland). The resin Fmoc-Ala Sarin® was from Bachem. Solid-phase peptide synthesis was performed semi-automatically using an Fmoc/tBu strategy. The intermediates and the final products were analysed by RP-HPLC using a combined system containing Waters TM controller, TM 486 tuneable absorbancy detector driven by a TM 600 pump. Retention times refer to a linear gradient rising within 30 min from 0 to 100% of water/acetonitrile (1:9) containing 0.09% TFA. The flow rate of the solvent mixture was adjusted to 1 ml per minute. Mass spectra were measured in a Finnigan MAT SSQ 710 C mass spectrometer using electrospray ionization.

Homocanaline Derivatives

Compounds **2–8** were characterized by TLC and RP-HPLC chromatography and by NMR-spectroscopy. The corresponding R_F (TLC) and R_t (RP-HPLC) values are presented in Table 1. H^1- and C^{13}-NMR data are presented in Tables 2 and 3. IR absorption is described as weak (w), medium (m) or strong (s).

***Boc-L-Nval(δ-OH)-OtBu* 2**. Some 34.61 mmol of IBCF were added at −10 °C to a stirred solution of Boc-L-Glu-OtBu **1** (32.96 mmol) and NMM (34.61 mmol) in anhydrous THF (100 ml). The mixture was stirred at −10 °C for 30 min and the formed precipitate was filtered off. The recovered solution was added to 1 l of a cooled 0.1 M solution of $NaBH_4$ in water/THF (1:5) and stirred overnight at room temperature. The solvent was evaporated, the pH of the reaction mixture adjusted to 3 by adding 10% citric acid and the aqueous layer extracted three times with 200 ml AcOEt. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give 32 mmol (97%) of a colourless oil. MS-CI: m/z, 290 $(M+1)^+$ (calc. M 289.36). FT-IR, $CHCl_3$ wavelength ν (cm^{-1}) (absorption): 3436 (w), 2982 (w), 2933 (w), 1704 (s), 1599 (w), 1496 (m), 1454 (m), 1367 (s), 1153 (s), 1098 (m), 972 (m).

***Boc-L-Nval(δ-OTS)-OtBu* 3**. 25.57 mmol of **2** was dissolved in 100 ml of pyridine and cooled to −10 °C, then 40.91 mmol of *p*-TsOH were added. The mixture was kept for 30 h at −5 °C, poured on crushed ice and extracted three times with 100 ml of AcOEt. The work-up followed the procedure for **2** including extraction with 5% ice-cold citric acid. Some 25.55 mmol (100%) of the desired product were isolated. MS-CI: m/z, 444 $(M+1)^+$ (calc. M, 443.56). FT-IR, $CHCl_3$ wavelength ν (cm^{-1}) (absorption): 3433 (w), 2983 (m), 2933 (w), 1704 (s), 1599 (w), 1496 (m), 1454 (m), 1367 (s), 1153 (s), 1098 (m), 972 (m).

***Boc-L-Nval(δ-I)-OtBu* 4**. Some 24.80 mmol of **3** and 100 mmol of sodium iodide were dissolved in 50 ml of DME and stirred in dark for one day at room temperature. After complete reaction, the solvent

Table 1 TLC R_F Values and RP-HPLC R_t Values for Compounds **2–8**

	2	**3**	**4**	**5**	**6**	**7**	**8**
R_F	0.17[a]	0.67[a]	0.86[a]	0.50[a]	0.49[b]	0.11[b]	0.51[b]
R_t[c]	20.99	28.53	31.48	27.61	24.26	19.49	16.93

[a] AcOEt: Hex (1:2). [b] $CHCl_3$:MeOH:AcOH (9:1:0:2). [c] Retention time in min. Column: Vydac C18 (4.6 × 250); gradient: 0–100% B in 30 min; B, CH_3CN/water (9:1), TFA 0.09%; flow, 1 ml/min.

© 1998 European Peptide Society and John Wiley & Sons, Ltd.

Table 2 ^{1}H-NMR Chemical Shift Assignment in p.p.m. for compounds **2–8** Measured in DMSO-d6 at 300 K

	Chemical shifts (δ)						
	2	**3**	**4**	**5**	**6**	**7**	**8**
OH	–	–	–	–	12.35	–	12.25
αH	3.75	3.72	3.80	3.76	3.96	3.77	3.95
βH_a	1.62	1.62	1.67	1.70	1.82	1.62	1.75
βH_b	1.54	1.50	1.60	1.62	1.65	1.55	1.60
$\gamma H_{a/b}$	1.51	1.60	1.80	1.57	1.60	1.50	1.58
$\delta H_{a/b}$	3.35	4.00	3.22	3.68	3.73	3.50	3.65
αNH	7.08	7.11	7.15	7.10	7.66	7.68	7.60
δNH	–	–	–	10.32	10.36	–	9.93

Table 3 C^{13}-NMR Chemical Shift Assignment in p.p.m. for Compounds **2–8** Measured in $DMSO_{d6}$ at 300 K

	Chemical shifts(δ)						
	2	**3**	**4**	**5**	**6**	**7**	**8**
CO-AA	172.4	171.8	171.9	172.1	174.3	175.5	174.3
αC	54.7	54.1	53.8	54.6	54.0	55.0	54.0
βC	60.6	71.0	8.2	65.2	75.3	75.5	75.3
βC	29.4	28.0	32.0	27.6	27.7	28.7	24.8
γC	27.9	27.1	30.0	24.7	24.8	25.7	23.8

was evaporated and the residue redissolved in AcOEt. The organic layer was extracted with 5% $Na_2S_2O_5$ and the work-up procedure for **2** was repeated yielding 21.57 mmol (87%) of sticky oil. MS-CI: m/z, 400 $(M+1)^+$ (calc. M 399.26). FT-IR, $CHCl_3$ wavelength ν (cm^{-1}) (absorption): 3434 (w), 2982 (w), 1704 (s), 1497 (m), 1455 (w), 1393 (m), 1368 (m), 1257 (w), 1153 (s).

***Boc-L-HCan(Aloc)-OtBu* 5**. Some 14.5 mmol of Aloc-NH-OH (*N*-allyloxycarbonyl hydroxylamine) was dissolved in 50 mmol anhydrous THF, cooled to −10 °C and 13 mmol of sodium hydride was added in portions. The reaction mixture was adjusted to 0 °C and added to a solution of **4** (5 mmol) in THF. After overnight stirring at room temperature the solvent was evaporated, the residue dissolved in 200 ml AcOEt, extracted with ice cold 1 M HCl, 5% $Na_2S_2O_5$ and brine, dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product was purified on flash chromatography (eluent AcOEt:Hex (1:4) and 3.05 mmol (61%) of a colourless sticky product were obtained. MS-CI: m/z 389 $(M+1)^+$ (calc. M 388.46). FT-IR, wavelength ν (cm^{-1}) (absorption): 3434 (w), 2982 (w), 1709 (s), 1498 (m), 1454 (m), 1368 (m), 1153 (m), 1112 (m).

***Fmoc-L-HCan(Aloc)-OH* 6**. Some 2.31 mmol of **5** was dissolved in a 1:1 mixture of TFA/DCM and stirred at room temperature until no starting material could be observed. Then the solvent was evaporated, yielding 2.3 mmol (100%) of H-HCan(Aloc)-OH. Some 10.29 mmol of this partially deprotected product and 2.5 eq. of $NaHCO_3$ were dissolved in dioxane/water (1:1), a solution of Fmoc-Cl (11.32 mmol) in 7 ml dioxane/water (1:1) was added and the mixture was stirred for 2 h at room temperature. The solvent was evaporated, the residue solved in water, neutralized with 1 M HCl and extracted with AcOEt. The organic layer was washed as described for **2** and the crude product purified on flash chromatography (eluent: AcOEt/AcOH 100:3) Some 6.48 mmol (63%) of a dry foam were isolated. MS-CI: m/z, 455 $(M+1)^+$ (calc. M, 454.48). FT-IR, $CHCl_3$ wavelength ν (cm^{-1}) (absorption): 3310 (w), 2952 (w), 1720 (m), 1509 (w), 1451 (w), 1334 (w), 1256 (w), 1114 (w).

***Fmoc-L-HCan-OH* 7**. Some 2.27 mmol of **6** was dissolved in 20 ml of a 3% AcOH/DCM solution. To this, 2.86 mmol of Bu_3SnH and 0.22 mmol of $Pd(PPh_3)_2Cl_2$ were added and the reaction mixture was stirred at room temperature. After 45 min, 2 ml of methanol were added, the solution was filtered

© 1998 European Peptide Society and John Wiley & Sons, Ltd.

and the solvent was evaporated. The product was precipitated with a DCM/Hex mixture yielding 1.8 mmol (82%) of desired product. MS-CI: m/z, 455 $(M+1)^+$ (calc. M, 454.48). FT-IR, wavelength ν (cm^{-1}) (absorption): 3430 (w), 3323 (w), 2952 (w), 1713 (m), 1584 (w), 1509 (w), 1450 (m), 1404 (w), 1339 (w), 1291 (w), 1178 (w), 1106 (w), 1078 (w), 1007 (w).

***Fmoc-L-HCan(Boc)-OH* 8**. Some 0.75 mmol of **7** and 1.87 mmol of $NaHCO_3$ were dissolved in water and 0.94 mmol of $(Boc)_2O$ was dissolved in dioxane. Both solutions were mixed and stirred for 3 h at 40 °C. As for compound **6** the product was isolated with successive extractions. The crude product was purified on flash chromatography (eluent: AcOEt:DCM:AcOH (4:8:0.2) yielding 0.45 mmol (60%) of pure product. MS-CI: m/z, 471 $(M+1)^+$ (calc. M, 470.52). FT-IR, $CHCl_3$ wavelength ν (cm^{-1}) (absorption): 3428 (w), 3369 (w), 2985 (m), 2934 (m), 1811 (m), 1721 (s), 1509 (w), 1478 (w), 1451 (m), 1396 (w), 1120 (s), 1075 (m), 842 (w).

Synthesis of a Cyclic Decapeptide

***c(Lys(Boc)-Pro-Gly-HCan(Aloc)-Ala-Lys(Boc)-Pro-Gly-HCan(Aloc)-Ala)* 10**. Starting from Fmoc-Ala-Sasrin® (0.5 g, 0.25 mmol) the peptide was assembled applying the Fmoc/tBu strategy. For Fmoc deprotection the resin was treated three times for 10 min with a solution of 20% piperidine in DCM. Complete deprotection was confirmed by UV absorption at 301 nm. For each coupling step a solution of 0.38 mmol of Fmoc-amino acid, 0.38 mmol of coupling reagent PyBOP and 0.75 mmol of DIEA in 10 ml of DMF were added to the resin and the suspension was shaken for 40 min. The completeness of each coupling was confirmed by the Kaiser test [8]. The peptide was cleaved from the resin by repetitive treatment with 1% TFA in DCM solution. After neutralization with 1% pyridine in DCM, 0.16 mmol (69%) of **9** was obtained by precipitation from DCM/Et_2O. The linear decapeptide was purified by HPLC and then 35 μmol reacted with 1 eq. of PyBOP in 30 ml DMF, adjusted to pH 9 with DIEA. The reaction mixture was stirred for 2 h at room temperature, the solvent evaporated, and the product was precipitated in Et_2O. Some 23 μmol (67%) of the cyclic decapeptide **10** was recovered.

***c{Lys(Ac)-Pro-Gly-HCan(C(CH$_3$)$_2$))-Ala-Lys(Ac)-Pro-Gly-HCan(C(CH$_3$)$_2$))-Ala}* 11**. Boc cleavage of 15 μmol of **10** was effected with 2 ml 50% TFA in DCM and 13 μmol of product were isolated by precipitation in Et_2O after evaporation of solvent. Subsequent reaction with Ac_2O/pyridine (1:2) for 30 min leads to the acyl substituted decapeptide. Some 10 μmol of the product were isolated after evaporation of the solvent and subsequent precipitation in Et_2O. The product was dissolved in 1 ml of DCM. To this, 0.3 ml of Bu_3SnH (0.1 M in DCM, 5% AcOH) and 10 μl of a solution of $Pd(PPh_3)Cl_2$ (0.1 M in DCM) were added. After 45 min stirring at room temperature, methanol was added and the reaction mixture was evaporated. Some 7 μmol of the product were precipitated in Et_2O, dissolved in 1 ml water/acetone (1:1) which was evaporated after 20 min. After precipitation in Et_2O, 5 μmol of **11** (33% overall yield from **10**) was recovered.

***Synthesis of H-Pro-Gly-HCan(mal)-Ala-OH* 13**. Fmoc-Ala-Sasrin® resin (0.2 g, 0.12 mmol) was used to assemble the linear protected tetrapeptide according to the described procedure for **9**. The crude product H-Pro-Gly-HCan(Boc)-Ala-OH **12** had to be purified by semi-preparative HPLC to yield 16.8 μmol (14%). This product was treated for 40 min with 1 ml of 50% TFA in DCM. When no starting material could be observed the solvent was removed and the deprotected tetrapeptide was precipitated in cold Et_2O. Subsequently it was dissolved in 1 ml of AcONa/AcOH buffer solution (pH = 4) and 1.6 mmol D(+)maltose monohydride were added. After stirring the mixture for 30 h at room temperature only little starting material could be detected and **13** was isolated on semi-preparative HPLC resulting in 11 μmol (65%) of the pure product.

RESULTS AND DISCUSSION

L-Homocanaline (L-HCan) Derivatives

The aminooxy-group containing non-proteinogenic amino acid (S)-2-amino-5-(aminooxy)pentanoic acid (L-homocanaline, L-HCan, Figure 1) may be considered as structural analogue of L-lysine. Its lower homologue, L-canaline, has been isolated from leguminous plants as enzymatic degradation product of L-canavanine [9]. The chemical synthesis of L-canaline starting from L-homoserine of L-methionine derivatives [10,11] and the isolation of aminooxy-containing amino acids in gram scale by extraction of biological material [12] have been reported.

© 1998 European Peptide Society and John Wiley & Sons, Ltd.

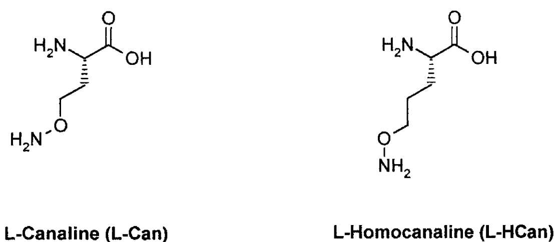
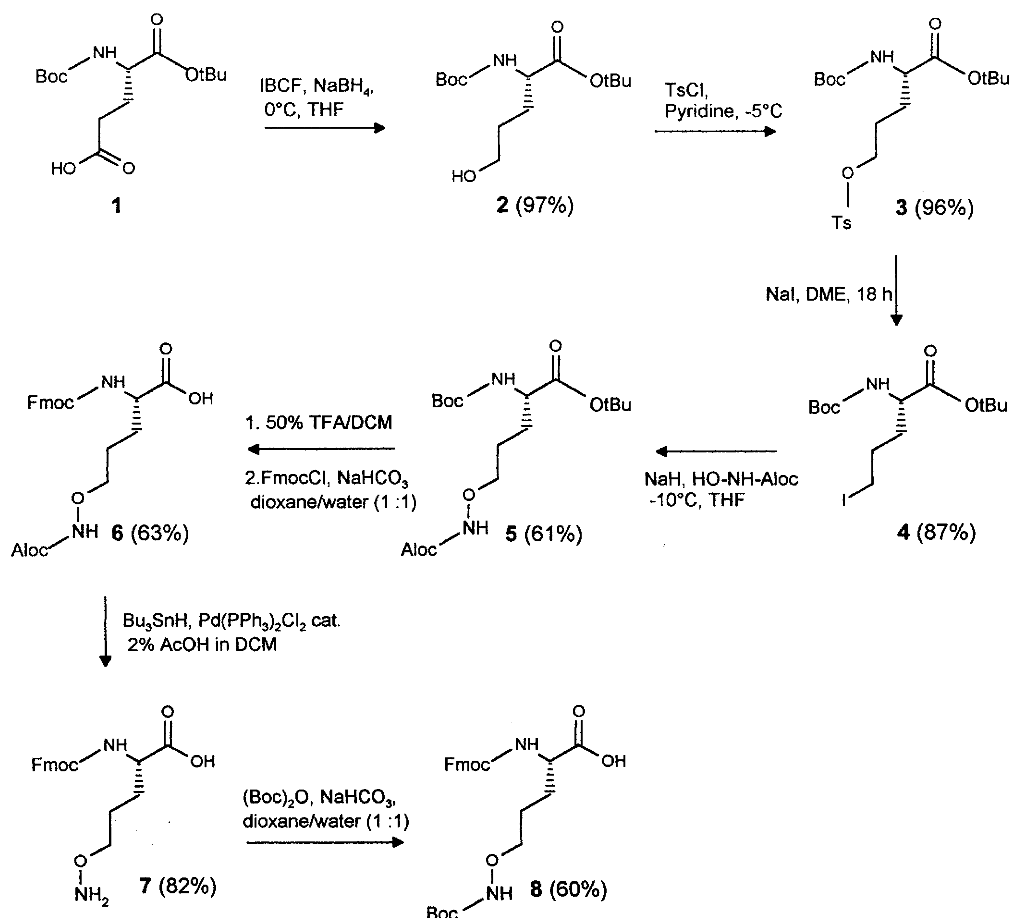


Fig. 1 Structure of L-canaline and L-homocanaline

Here we focus on the synthesis of the corresponding L-lysine analogue L-homocanaline and its derivative suitable for solid phase synthesis (SPPS) and chemoselective ligation chemistry. As depicted in Scheme 1, the γ-COOH group of the commercially available Boc-L-Glu-OtBu **1** was reduced to the alcohol **2** and transformed to the primary iodide **4** after activation via the tosylate **3**. For accessing orthogonally protected L-HCan derivatives, the key intermediate **4** (overall yield 81% from **1**) was reacted with the Aloc-protected hydroxylamine in THF at low temperature to give the fully protected Boc-L-HCan(Aloc)-OtBu derivative **5** in 61% yield. Depending on the synthetic strategy, derivative **5** can be readily transformed to versatile building blocks for peptide synthesis in solution or SPPS. For example, simultaneous removal of the t-butyl- and the Boc-group and subsequent introduction of the Fmoc-group results in Fmoc-L-HCan(Aloc)-OH **6** as a completely orthogonal building block useful in standard Fmoc-based SPPS [13]. This versatile Hcan derivative can be further transformed to the Fmoc-L-HCan(Boc)-OH **7** in applying conventional protection group chemistry. In summary, two completely orthogonally protected HCan derivatives needed for the incorporation into peptides have become accessible in good overall yields.

HCan in Peptide Synthesis

The formation of oxime bonds in chemoselective ligation procedures has become a most efficient tool for the synthesis of protein-like polypeptides [6] as



Scheme 1 Synthesis of HCan derivatives from protected glutamic acid.

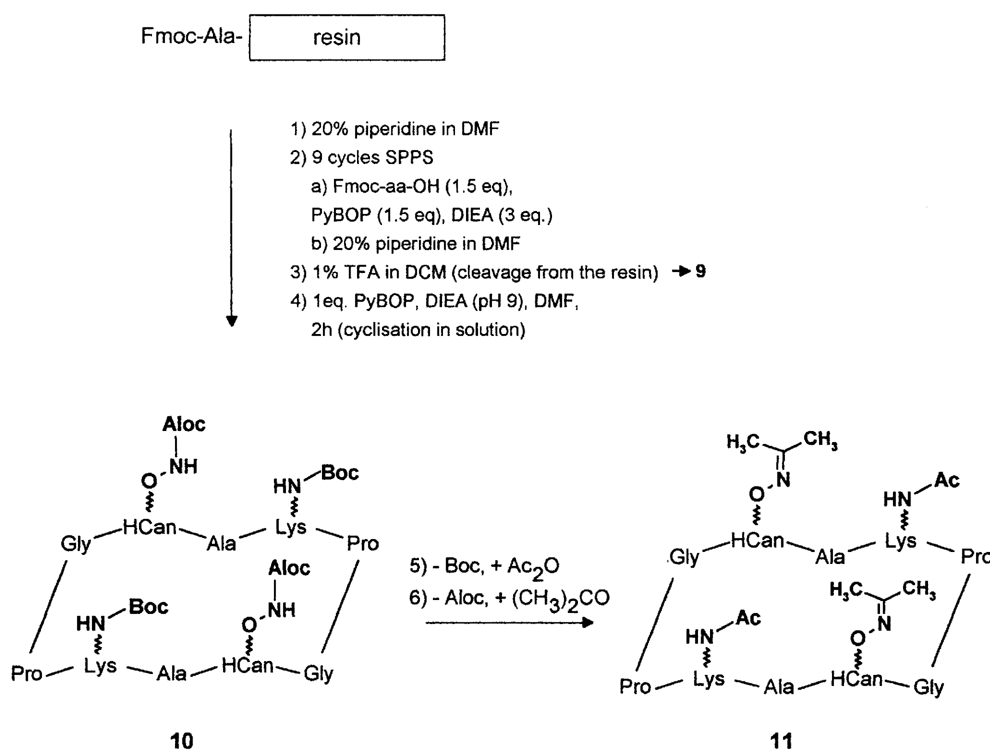
© 1998 European Peptide Society and John Wiley & Sons, Ltd.

well as in TASP design [5]. Here, carbonyl compounds are reacted under mild conditions, e.g. in aqueous buffer solution with aminooxy-group containing molecules to result in kinetically stable oxime bonds. The well-known α-effect [14] of the oxygen atom affects the nucleophilic and basic character of the neighbouring amino-group in a distinct way. Due to its physico-chemical properties, the aminooxy group reacts chemoselectively with aldehydes of ketones in the presence of the most common functional side chains encountered in peptides. On the other hand, owing to the increased acid character of protected aminooxy-derivatives, side reactions such as N-acylation may occur during the chain assembly of peptides containing this building block. For example substantial N-acylation during SPPS [15] was observed if Boc-protected aminooxy N^{ε}-acylated lysine derivatives were applied in standard Fmoc-SPPS. In contrast, the use of sterically demanding Trt-derivatives showed no detectable side reactions during the coupling [16].

In order to evaluate the feasibility for the newly prepared Hcan derivatives in SPPS, we applied compounds **6** and **8** for the synthesis of chemoselectively addressable peptide templates. To this end, a cyclic peptide useful in TASP design [17] was prepared according to Scheme 2. The linear peptide **9** was built up in Sasrine® resin applying the standard protocols of Fmoc-based SPPS. Interestingly the coupling of the newly synthesized HCan derivative **6** (1.5-fold excess) proceeded smoothly without detectable side reactions. In particular, no increase of fluorenyl absorption (being indicative for O-NH-Aloc acylation) could be observed [18].

Consequently, the linear decapeptide **9** (containing two Hcan residues) could be obtained in yield up to 90% according to analytical HPLC (Figure 2). After cyclization in solution, the orthogonally protected peptide template **10** was used as a prototype for the regioselective functionalization according to the TASP concept. Sequential cleavage of the Boc- (as N^{ε}-Lys side-chain protection) and Aloc- (as aminooxy protection in HCan) groups and subsequent condensation in solution of acetic anhydride and acetone resulted in high yields of the desired TASP model **11**.

As a second illustrative example for the use of HCan building blocks in chemoselective ligation chemistry, a glycopeptide as a prototype for chimeric molecules [19] was prepared (Scheme 3. In analogy to the linear decapeptide above, the tetrapeptide H-



Scheme 2 Synthesis of the cyclic decapeptide containing two Aloc-protected HCan residues showing the orthogonally addressable side chains.

© 1998 European Peptide Society and John Wiley & Sons, Ltd.

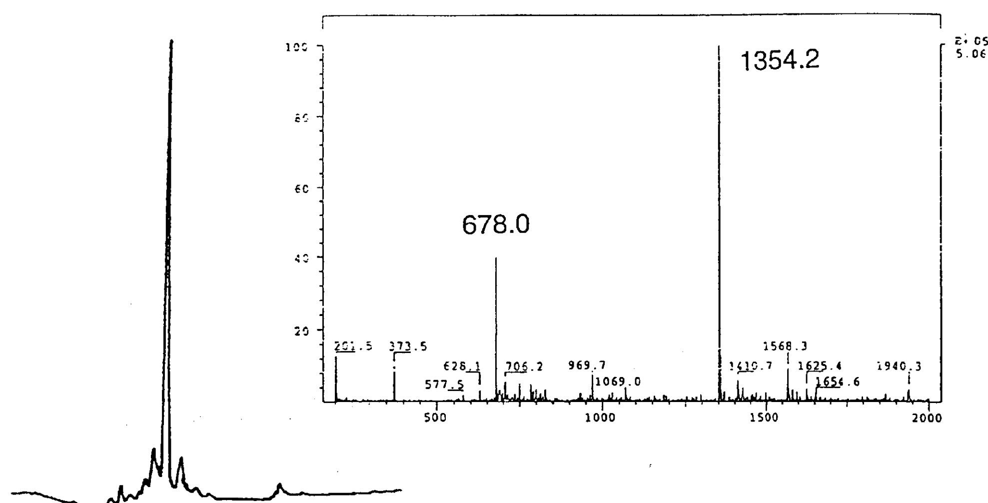


Fig. 2 RP-HPLC of crude H-Lys(Boc)-Pro-Gly-HCan(Aloc)-Ala-Lys(Boc)-Pro-Gly-HCan(Aloc)-Ala-OH (**9** in Scheme 2). Column Vydac C18 (4.6 × 250); gradient 0–100% B in 30 min; B CH_3CN/Water (9:1), TFA 0.09%; flow; 1ml/min. Retention time of the main peak was 22.42 min and purity was up to 90%. Insert: electrospray ionization mass spectrum of **9**. ES-MS obtained (calculated): $(M+)^+$=1354.2(1354.5), $(M/2+1)^+$ =678.0(677.8).

Pro-Gly-HCan(Boc)-Ala-OH **12** was built up by SPPS applying identical coupling and deprotection protocols. After removing the Boc protecting group of the HCan side chain, the completely unprotected disaccharide was reacted in aqueous NaOAc/HOAc buffer solution (pH = 4) with the chemoselectively addressable tetrapeptide to give the corresponding glycopeptide in good yields (Figure 3. As detected by ^{1}H-NMR (not shown), the maltose moiety was linked to the peptide via oxime bind formation (both *syn* and *anti*-forms are present), i.e. the attached sugar adopts a non-cyclic structure.

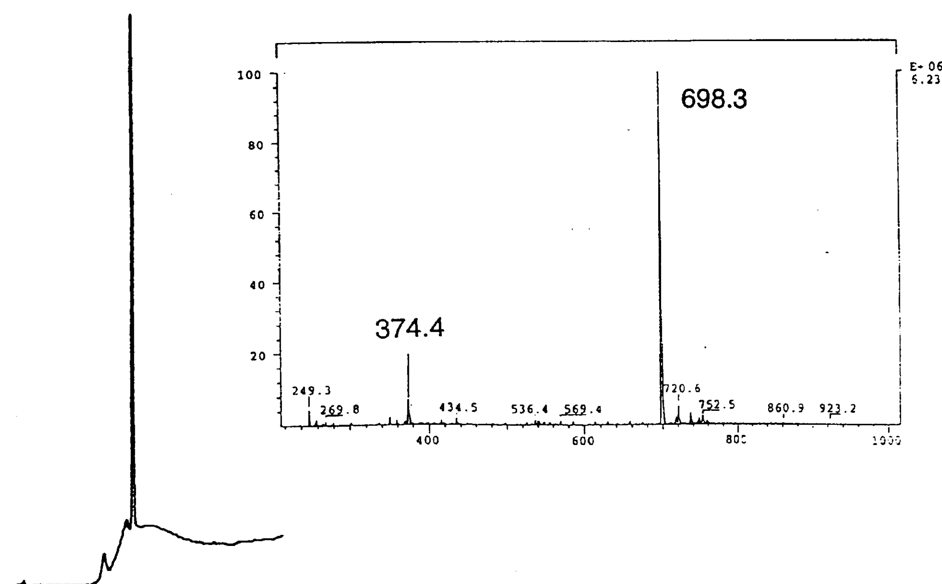
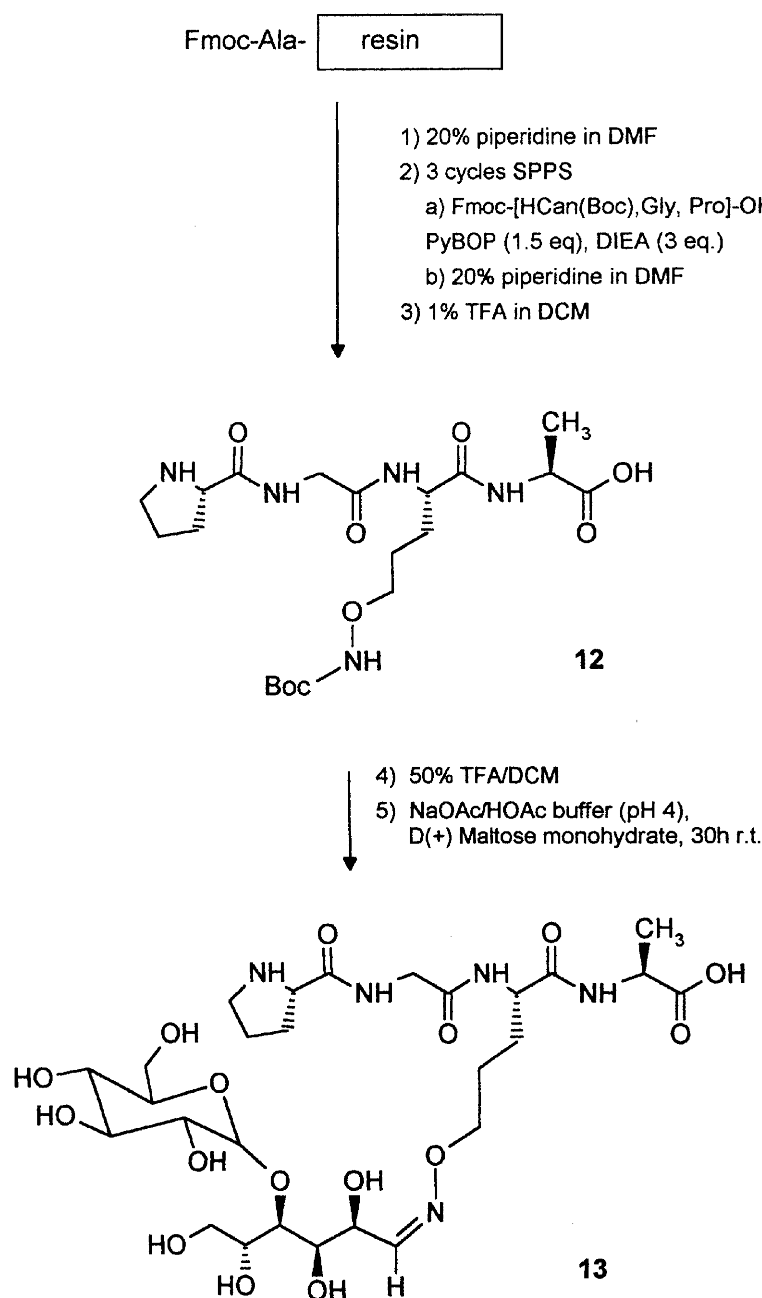


Fig. 3 RP-HPLC of crude H-Pro-Gly-HCan(mal)-Ala-OH (13 in scheme 3). Column; Vydac C18 (4.6 × 250); gradient 0–100% B in 30 min; B CH_3CN/water (9:1); TFA 0.09%, flow 1 ml/min. Retention time of the main peak was 10.2 min and purity was up to 97%. Insert: electrospray ionization mass spectrum **13**. ES-MS obtained (calculated): $(M+1)^+$ =698.3(698.63), (M-mal+1)$^+$=374.4 (374.2).

© 1998 European Peptide Society and John Wiley & Sons, Ltd.



Scheme 3 Synthesis of a glycopeptide applying chemselective ligation techniques.

CONCLUSIONS

Chemoselectively addressable building blocks derived from homocanaline (HCan) with a wide potential for applications in peptide synthesis, protein design and modification have been prepared in good overall yield. The orthogonally protected HCan-derivatives were successfully incorporated into growing peptide chains applying standard SPPS. Most notably, no side reaction were observed during the deprotection or coupling steps in HCan containing peptides. This opens the way for accessing chemoselectively addressable peptides as demonstrated for the construction of two prototypes relevant in peptide and protein design. The incorporation of HCan as chemoselectively addressable building block into proteins [20] represents a most fascinating perspective.

© 1998 European Peptide Society and John Wiley & Sons, Ltd.

Acknowledgements

This work was supported by the Swiss National Science Foundation.

References

1. P. E. Dawson, T. W. Muir, I. Clark-Lewis and S. B. H. Kent (1994). Synthesis of proteins by native chemical ligation. *Science 266*, 776–779.
2. K. Rose (1994). Formation of homogeneous artificial proteins. *J. Am. Chem. Soc. 116*, 30–33.
3. J. W. Bryson, S. F. Betz, H. S. Lu, D. J. Suich, H. X. Zhou, K. T. O'Neil and W. F. DeGrado (1995). Protein design: a hierarchic approach. *Science 270*, 935–941.
4. G. Tuchscherer and M. Mutter (1996). Template assisted protein *de novo* design. *Pure appl. Chem. 68*, 2153–2162.
5. G. Tuchscherer (1993). Template assembled synthetic proteins: condensation of a multifunctional peptide to a topological template via chemoselective ligation. *Tetrahedron Lett. 34*, 8419–8422.
6. J. P. Tam and J. Shao (1995). Unprotected peptides as building blocks for the synthesis of peptide dentrimers with oxime, hydrazone and thiazolidine linkages. *J. Am. Chem. Soc. 117*, 3893–3899.
7. M. Mutter, P. Dumy, P. Garrouste, C. Lehmann, M. Mathieu, C. Peggion, S. Peluso, A. Razaname and G. Tuchscherer (1996). Template assembled synthetic proteins (TASP) as functional mimetics of proteins. *Angew. Chem. Int. Ed. Engl. 35*, 1481–1485.
8. E. Kaiser, R. L. Colescott, C. D. Bossinger and P. I. Cook (1970). Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal. Biochem. 34*, 595–598.
9. G. A. Rosenthal (1978). The biological and biochemical properties of L-canaline, a naturally occurring structural analogue of L-ornithine. *Life Sci. 23*, 93–98.
10. K. Barlos, D. Papaioannou and D. Sanida (1986). Peptidsynthetisch interessante Derivate von L-Canalin. *Liebigs Ann. Chem.* 287–291.
11. A. J. Ozaniskas and G. A. Rosenthal (1986). Synthesis of L-canaline and δ-functional 2-aminobutyric acid derivatives. *J. Org. Chem. 51*, 5047–5050.
12. M. Bass, L. Harper, G. A. Rosenthal, S. N. Phuket and P. A. Crooks (1995). Large scale production and chemical characterisation of the protective higher plant alleochemicals-L-canavanine and L-canaline. *Biochemic. Systematics Ecol. 23*, 717–721.
13. J. M. Stewart and J. D. Young in: *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chemical Co., Rockford, IL 1984.
14. R. F. Hudson (1973). Perturbation treatment of chemical reactivity. *Angew. Chem. 85*, 63–84.
15. O. Nyanguile, 'La fonctionnalisation de motifs structuraux et la ligation chemoselective comme outils pour la synthèse des proteines artificielles', PhD Thesis, Université de Lausanne 1994.
16. F. Wahl and M. Mutter (1996) Analogues of oxytocin with an oxime bridge using chemoselectively addressable building blocks. *Tetrahedron Lett. 37*, 6861–6864.
17. P. Dumy, I. M. Egglestone, S. Cervigni, U. Sila, X. Sun and M. Mutter (1995). A convenient synthesis of cyclic peptides as regioselectively addressable functionalized templates (RAFT). *Tetrahedron Lett. 36*, 1255–1258.
18. J. Meienhofer, M. Waki, E. P. Heimer, T. J. Lambros, R. C. Makofske and C. D. Chang (1979). *Int. J. Peptide Protein Res. 13*, 35–42.
19. S. E. Cervigni, P. Dumy and M. Mutter (1996). Synthesis of glycopeptides and lipopeptides by chemoselective ligation. *Angew. Chem. Int. Ed. Engl. 35*, 1230–1232.
20. G. A. Rosenthal and D. L. Dahlmann (1991). Incorporation of L-canavanine into proteins and the expression of its antimetabolic effects. *J. Agric. Food Chem. 39*, 987–990.

© 1998 European Peptide Society and John Wiley & Sons, Ltd.