Pergamon

Tetrahedron Letters, Vol. 37, No. 40, pp. 7259-7262, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01661-9

Synthesis Of Hydrazinopeptides Using Solid Phase N-Amination. Application To Chemical Ligation

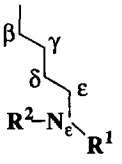
Christine Klinguer, Oleg Melnyk*, Estelle Loing, Hélène Gras-Masse

Laboratoire de Chimie des Biomolécules associé au CNRS, Institut Pasteur de Lille
1 rue du Professeur Calmette BP 245, 59019 Lille, France

Abstract: A hydrazinopeptide was synthesized using the solid phase N-electrophilic amination with N-Boc-3-(4-cyanophenyl)oxaziridine and we describe its reactivity towards cyclohexanecarboxaldehyde. Copyright © 1996 Elsevier Science Ltd

Recently, we have observed that the modification of a peptide by a simple aliphatic group was sufficient to insure its transport into the cytoplasm of intact cells [1] and to stimulate the immune system.[2] Chemical ligation between a peptide and a variety of lipidic moieties through hydrazone formation seems to be a versatile method to produce a series of analogues to be tested for their ability to deliver the peptide to the cytoplasm.[3] In this context, we describe the synthesis of hydrazone **3** (figure 1), where a lipophilic aldehyde was ligated with a totally deprotected peptide bearing a hydrazine group at the end of a lysine side chain. The selected peptide is a pseudosubstrate sequence for intracellular targets (PKC α,β isoforms), and is able to inhibit the corresponding enzymes.

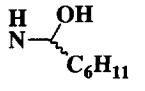
Ac-Lys-Asp-Val-Ala-Asn-Arg-Phe-Ala-Arg-Lys-Gly-Ala-Leu-Arg-Gln-NH_2



1: $R^1 = NH_2$, $R^2 = H$

2: $R^1 = Ac$, $R^2 = H$

3: $R^1 =$  $R^2 = H$

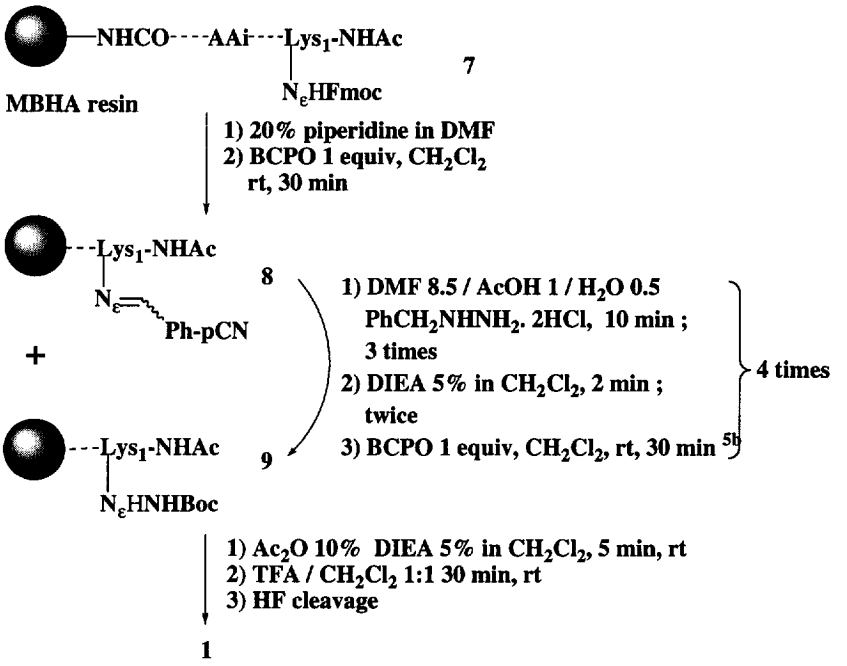
4: $R^1 =$  $R^2 = H$

5: $R^1 = Ac$, $R^2 = NH_2$

figure 1

The side chain of the N-terminal lysine was modified on the solid phase as shown in scheme 1. The peptide was elaborated by conventional solid phase peptide synthesis using the Boc / benzyl strategy. The side chain primary amino group of the N-terminal lysine was reacted with the commercially available N-Boc-3-(4-cyanophenyl)oxaziridine (BCPO) [4] after removal of the Fmoc protecting group.

Vidal *et al.* showed that primary amines led to incomplete reactions due to imine formation with the liberated p-cyanobenzaldehyde **6**. Indeed, the treatment of the resin with DMF 8.5 / AcOH 1 / H_2O 0.5 released **6** in the washing mixture. Preliminary experiments showed that a few cycles composed of acidic washes, neutralisation with DIEA and reaction with BCPO did not permit to avoid the formation of a large quantity of N_ε-acetylated peptide **2** after capping. The equilibrium between the amine and the imine was displaced by adding benzylhydrazine dihydrochloride to the washing mixture.[5a] Four cycles as described in scheme 1 led to a nice crude product where no N_ε-acetylated peptide **2** was detected. The purification by RP-HPLC gave 25% overall yield of the hydrazinopeptide **1** which was characterized by TOF-PDMS (MH^+ = 1786) and 2D-NMR.[6]



scheme 1

The capping step of scheme 1 merits further comments. As expected, a short capping time caused no problems due to the low nucleophilic character of the N_ε-nitrogen atom of the hydrazide **9**.[7] On the other hand, long acetylation times led to an appreciable amount of hydrazide **5** (**1** / **5** = 1 after 24h, for **5** MH^+ = 1828 by TOF-PDMS). This should allow an easy access to complex peptide hydrazides useful for chemical ligation.

The hydrazinopeptide **1** in hand, we studied the hydrazone formation with a model lipophilic aldehyde : cyclohexanecarboxaldehyde. A water / acetonitrile : 1 / 2 mixture allowed the solubilisation of both peptide and aldehyde.[8] The reaction mixture showed the formation of the carbinolamine **4** and the hydrazone **3**. The pH of the medium (adjusted with aqueous 1N NaOH) had a pronounced effect on the kinetics and the yields of **4** and **3** (table 1). A side product of molecular weight 1991 by TOF-PDMS was present in significant amounts (14-18%) for entries 1 and 6 but in minor amounts for the other entries.[9]

Table 1. pH effect on the yields of peptides **3** and **4**

		4 (yield %) / **3** (yield %)			
Entry	**pH**	**3h**	**22h**	**47h**	**72h**
1	4.44	13 / 26	14 / 30	9 / 23	7 / 23
2	4.66	nd	17 / 4	17 / 43	10 / 38
3	4.79	nd	11 / 5	nd	12 / 25
4	5.02	nd	11 / 8	13 / 23	15 / 24
5	5.13	nd	14 / 13	15 / 36	13 / 32
6	5.24	nd	13 / 24	12 / 26	10 / 26

a) the yields were estimated using the peak areas obtained by C-18 RP-HPLC. Eluent A: 0.05% TFA in water; Eluent B: 0.05% TFA in acetonitrile-water (80 / 20). Gradient : 10% to 100% of eluent B in 30 min, 1 mL/min., detection at 215 nm. b) not determined.

Entry 2 shows the best conditions for the synthesis of the hydrazone **3**. Both peptides **3** and **4** were purified by preparative C-18 RP-HPLC in acidic buffer (pH 2, water-acetonitrile gradient containing 0.05% TFA). The HPLC fractions were rapidly neutralized with ammonia to avoid their hydrolysis into peptide **1**. **3** was stable in water at pH 7.7 for 2 weeks and during lyophilisation despite the low boiling point of cyclohexanecarboxaldehyde. Only one isomer was detected by ^{1}H-NMR [10] and a strong NOE contact between H_1 and $N_\varepsilon H$ agreed well with the trans isomer (figure 2). The weak NOE observed between H_1 and H_2 showed that these two protons were probably anti to each other, in a conformation which minimized the steric interaction between the cyclohexyl moiety and the hydrazone linkage.

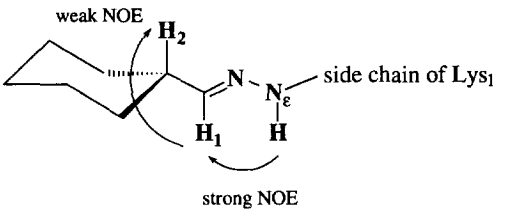


figure 2

The carbinolamine **4** was stable enough to be lyophilized and analysed by TOF-PDMS (for **4** MH^+ = 1898) and 2D-NMR[11], but it decomposed within few days at pH 7.7 and rt.

In conclusion, we have developed a new and flexible synthesis of hydrazinopeptides by using solid phase N-amination. The alkylhydrazine group reacts with cyclohexanecarboxaldehyde to give the corresponding hydrazone and should be useful for other applications. Moreover, this solid phase synthesis of alkylhydrazines and hydrazides should allow an access to new combinatorial chemical libraries.

Acknowledgments: We thank Gérard Montagne and Jean-Michel Wieruszeski for NMR experiments and useful discussions. We gratefully acknowledge financial support from Institut Pasteur de Lille.

References and Notes

1 Loing, E.; Delanoye, A.; Sergheraert, C., Tartar, A.; Gras-Masse, H. *Pep. Research*, submitted.

2 Déprez, B.; Sauzet, J.-P.; Boutillon, C.; Martinon, F.; Tartar, A.; Sergheraert, C.; Guillet, J.-G.; Gomard, E.; Gras-Masse, H. *Vaccine* **1996**, *14*, 375-382.

3 King, T.E.; Zhao, S.W.; Lam, T. *Biochemistry* **1986**, *25*, 5774-5779.
Gaertner, H.F.; Rose, K.; Cotton, R.; Timms, D.; Camble, R.; Offord, R.E. *Bioconjugate Chem.* **1992**, *3*, 262-268.

4 a) Vidal, J.; Drouin, J.; Collet, A. *J. Chem. Soc. Chem. Commun.* **1991**, 435-437.
b) Vidal, J.; Guy, L.; Stérin, S.; Collet, A. *J. Org. Chem.* **1993**, *58*, 4791-4793.
c) Aubry, A.; Mangeot, J.-P.; Vidal, J.; Collet, A.; Zerkout, S.; Marraud, M. *Int. J. Peptide Protein Res.* **1994**, *43*, 305-311.

5 For 0.25 mmol of 4-methylbenzhydrylamine resin (0.57 mmol / g). a) Hydrolysis of the imine **8** with 5 mL of DMF 8.5 / AcOH 1.5 / H_2O 0.5 containing 50 mg of $PhCH_2NHNH_2$. 2HCl (controlled by TLC CH_2Cl_2 / AcOEt : 80 / 20). b) The N-amination was performed with 60 mg of BCPO dissolved in 4 mL of CH_2Cl_2. For the last cycle, 100 µL of DIEA were added after 15 min to neutralize acidic impurities.

6 Hydrazino peptide **1** 4.4 mM in H_2O / CD_3CN : 1 / 1 at pH 7.7 and 20 °C (sodium 3-(trimethylsilyl) propionate used as the internal reference, chemical shifts expressed in ppm). For the modified Lys_1 : $N_\alpha H$ 8.21; H_α 4.13; H_ε 2.80; H_β 1.73; $H_{\beta'}$ 1.68, H_δ 1.51, H_γ 1.36.

7 a) Schmidt, U.; Riedl, B. *Synthesis* **1993**, 809-814.
b) Hosmane, R.S.; Bhadti, V.S.; Lim, B.B. *Synthesis* **1990**, 1095-1100.

8 2 mg of peptide **1** were dissolved in 300 µL of H_2O / CH_3CN : 1 / 2 containing 18 µL of cyclohexanecarboxaldehyde. The pH of the medium was adjusted with aqueous NaOH 1N and the solution stirred at rt.

9 This compound of molecular weight 1991 could arise from the reaction of the Lys_{10} side chain of peptide **3** with cyclohexanecarboxaldehyde to give the corresponding hemiaminal. Another side product was discovered at the occasion of experiments 1 and 6 (MH^+ = 1768 by TOF-PDMS, co-eluting by HPLC with the starting peptide **1**).

10 Peptide **3** in H_2O / CD_3CN : 1 / 1 at pH 7.7 and 20 °C. For the modified Lys_1: $N_\alpha H$ 8.05; H_α 4.23; H_ε 2.94; H_β 1.81; $H_{\beta'}$ 1.75; H_δ 1.63; H_γ 1.43; H_γ 1.38; $N_\varepsilon H$ 6.75; HC=N 7.74; CH-C=N 4.23; methylene protons of the cyclohexyl moiety 1.40. MH^+ = 1880 by TOF-PDMS.

11 Peptide **4** in H_2O / CD_3CN : 1 / 1 at pH 7.7 and 20 °C. For the modified Lys_1: $N_\alpha H$ 8.16; H_α 3.86; H_ε 2.71; H_β 1.69; H_δ and H_γ 1.40; C<u>H</u>-C(OH)NH 2.11; methylene protons of the cyclohexyl moiety 1.71-1.63-1.35-1.29-1.20.

(*Received in France* 2 *July* 1996; *accepted* 20 *August* 1996)