Pergamon

Tetrahedron Letters, Vol. 38, No. 42, pp. 7325-7328, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01779-6

Solid Phase Synthesis of Sec-Amides and Removal from the Polymeric Support Under Mild Conditions

D. Sarantakis* and J.J. Bicksler

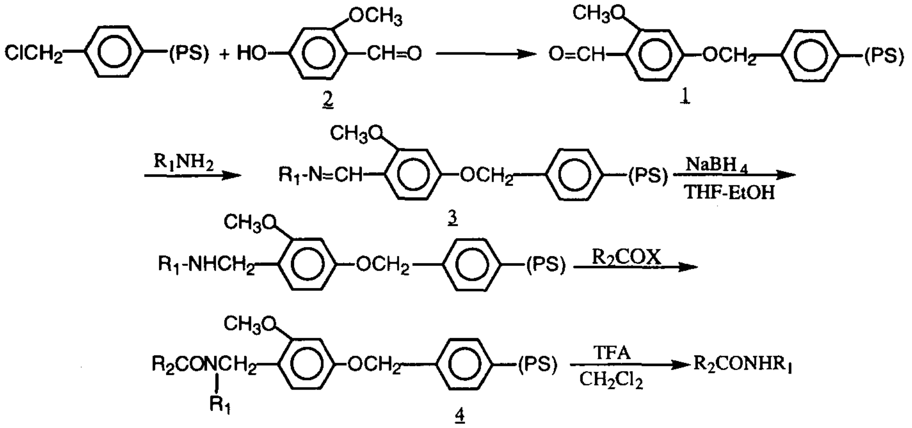
Medicinal Chemistry Department, Chemical Sciences,
Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543 USA.

Abstract: C-terminal sec-amides of peptidic and non-peptidic carboxylic acids can be prepared using 2-methoxy-4-benzyloxy-polystyrene aldehyde resin (MALDRE). The polymer bound sec-amide is removed from the resin by treatment with 10-50% trifluoroacetic acid in methylene dichloride for 30 minutes. © 1997 Elsevier Science Ltd.

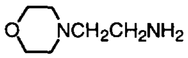
The solid phase synthesis of C-terminal sec-peptide amides has been addressed early with the introduction of N-substituted benzylhydrylamine resin.[1] N-ethyl peptide amides were prepared by the BOC-chemistry and were removed from the polymeric support by liquid HF in the presence of anisole. The alternative procedure of aminolysis of a polymeric benzyl ester or an activated oxime ester by a primary amine has been used extensively for the formation of secondary C-terminal amides.[2] This strategy suffers from the requirement for reactive amines and possible racemization. Also, the exposure of the peptidic chains often containing aspartyl or glutamyl residues to rather harsh basic conditions has resulted in the formation of imides or unwanted side chain amidation. Recently, polystyrene bound 4-hydroxy-2,6-dimethoxy benzaldehyde has been employed for the reductive amination of the construct and further acylation to provide sec-amides of peptides[3] and benzodiazepines.[4] In another approach reductive alkylation of 9-amino-xanthen-3-yloxymethyl poly (styrene) was applied to produce sec-alkyl amides by the FMOC/t-Bu methodology.[5] Both of the above methods rely on the TFA acidolysis of the final polymer bound sec-amide for removal from the resin. Severe steric hindrance and expensive intermediates are a serious restraint for wide application of these methods in large scale preparations. In this communication we report a general method for the solid phase synthesis of sec-amides, which avoids the use of liquid HF or the exposure of the constructed peptidic chains to an excess of amines. The liberation of the desired sec-amide is achieved by brief treatment of the polymeric amide with low concentration of TFA in CH_2Cl_2.

Our method uses the 2-methoxy-4-benzyloxy polystyrene aldehyde resin (MALDRE) (1) which can be prepared very conveniently from Merrifield chloromethylated polystyrene and 4-hydroxy-2-methoxy benzaldehyde[6] (2). A suspension of the Merrifield resin in DMF is treated with three equivalents of the aldehyde (2) and three equivalents of sodium methoxide under nitrogen at 60-70°C for 20 hours. Washing of the polystyrene bound aldehyde with DMF, MeOH, water, MeOH, CH_2Cl_2, and MeOH provides the aldehyde (1) in quantitative yield, based on the Cl-content of the Merrifield resin (Scheme 1).

SCHEME 1



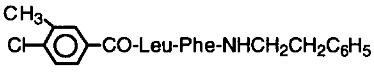
$R_1 = C_6H_5(CH_2)_2NH_2$, $C_6H_5(CH_2)_4NH_2$, $CH_3CH_2NH_2$,
$C_2H_5OCH_2CH_2NH_2$, $CH_3O_2C(CH_2)_3NH_2$, $CH_3(CH_2)_4NH_2$



R_2 = FMOC aminoacid or a carboxylic acid
X = -OH or -Cl

In order to form the amine-resin (4) the aldehyde-resin (1) is suspended in THF and stirred with a five-fold excess of a primary amine at room temperature for 18 hours. One equivalent of methyl orthoformate as a dehydrating agent has proved to be helpful to complete the reaction. Filtration of the imine-resin and washing with the THF, absolute MeOH, CH_2Cl_2 and MeOH provides the imine-resin (3).[7] This resin is suspended in a mixture of THF-EtOH, 3:1, v/v, and treated with 10 equivalents of $NaBH_4$ for 6 to 8 hours. Washing of the polymeric amine is accomplished with THF, EtOH, water, EtOH, THF and MeOH.[8] A variety of primary amines has been employed successfully, to provide the resin bound sec-amines. The sec-amine resin (4) can be acylated with FMOC-amino acids or other carboxylic acids by the DIC-HOBT method,[9] with symmetrical anhydrides[10] or acyl chlorides in the presence of pyridine or triethylamine to provide the polymer bound sec-amide of the corresponding acid. The method has been used to synthesize a number of peptides by the FMOC-chemistry. The sensitivity of the anchor to acidic conditions precludes the use of BOC-chemistry. Removal of the peptide sec-amide from the polymeric support is carried out by stirring the peptide-resin three times with portions of 30% TFA in CH_2Cl_2 containing appropriate scavengers for 10 minutes each time. The enkephalin (6a) and the peptides (6 b-e) (Table 1) were prepared in good yields after HPLC purification.

Table 1

	Peptide*	**Yield****
6a	Tyr-Gly-Gly-Phe-Leu-$NHCH_2CH_2CH_2CH_2C_6H_5$	44%
6b	$(CH_3CH_2CH_2)_2CHCO$-Gly-Tyr-$NHCH_2CH_2CH_2CH_2C_6H_5$	84%
6c	FMOC-Phe-Leu-$NHCH_2CH_2OCH_2CH_3$	51%
6d	FMOC-Leu-Cha-$NHCH_2CH_2C_6H_5$	80%
6e		63%

* Structure was confirmed by Mass Spec., NMR Spec., and anal. HPLC.
** Yield is calculated based on the N-content of the peptide resin.
Peptides were isolated by HPLC and yields determined based on HPLC isolated material.

It has been reported[11] that acylated benzylamines substituted at 2,4- or 3,4-positions with methoxy group provide primary amides in poor yield after treatment with 70% TFA in CH_2Cl_2. We have found that our sec-amide anchors are sensitive to even 10% TFA-CH_2Cl_2 although the yields of the liberated substances are not as satisfactory as with 30% TFA-CH_2Cl_2. The higher sensitivity of our sec-amide-resin bond to TFA in comparison to the primary-amides may be due to the increased basicity of the sec-amide bond which encourages easier protonation in the acidic deprotection medium. The 2,4-dimethoxy groups have been employed[12] for the temporary protection of central amide bonds in polypeptide sequences which tend to aggregate due to hydrogen bonding and have been removed by treatment with TFA solutions. Work towards exploitation of the full potential of our methodology for the synthesis of sec-amide peptidic or non-peptidic compounds is in progress.

Acknowledgements

We thank Dr. Magid Abou-Gharbia for encouragement and also the Analytical Department of Wyeth-Ayerst Research for carrying out MS, NMR and HPLC determinations.

References and Notes

1. Kornreich W.; Anderson H.; Porter J.; Vale W.; Rivier J.; Int.; J. Pept. Protein Res. (1985) 25 414.
2. Coy D.H.; Coy E.J.; Schally A.V.; Vipchez-Martinez J.A.; Debeljuk, L.; Cowter W.H.; Arimura A.; Biochemistry (1974) 13 323.
3. Jensen K.J.; Songster M.F.; Vagner J.; Alsina J.; Albericio F.; Barany G.; Peptides: Chem. Str. Biology Eds. Pravin T.P.; Kaumaya and Robert S. Hodges; Mayflower Scientific Ltd. (1996) p. 30.
4. Boojamra C.G.; Burow K.M.; Thompson L.A.; Ellman J.A.; J. Org. Chem. (1997) 62 1240.
5. Chan W.C.; Mellor S.L.; J. Chem. Soc., Chem. Commun. (1995) 1475.
6. 4-Hydroxy-2-methoxy benzaldehyde is commercially available from Lancaster Synthesis Inc.
7. IR spectrum shows a sharp absorption band at 1630 cm^{-1}.

8. Reduction of the imine double bond to the sec-amine can be ascertained by the disappearance of the ir band at 1630 cm^{-1}.

9. Sarantakis D.; Teichman J.; Lien E.L.; Fenichel R.L.; Bioch. Biophys. Res. Commun. (1976) 73 336.

10. Chen F.M.F.; Kuroda K.; Benoiton N.L.; Synthesis (1978) 928.

11. Albericio F.; Barany G.; Int. J. Pept. Protein Res. (1987) 30 206.

12. Johnson T.; Quibell M.; Sheppard R.C.; J. Pept. Science (1995) 1 11.

(*Received in USA* 29 *July* 1997; *revised* 18 *August* 1997; *accepted* 19 *August* 1997)