Pergamon

Tetrahedron Letters, Vol. 38, No. 9, pp. 1511-1514, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00143-3

Solid Phase Synthesis of Peptide Hydroxamic Acids

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Abstract: The synthesis of peptide hydroxamic acids has been performed on a solid support. A carboxyl group of a peptide synthesized on *para*-methylbenzhydrylamine (pMBHA) resin was converted to a hydroxamate functional group by condensing with NH_2OBzl, which was found preferable to NH_2OtBu or NH_2OTrt. The hydroxamate benzyl protecting group was removed subsequently during HF cleavage of the peptide resin. Five peptide hydroxamic acids were prepared according to this new method. © 1997 Elsevier Science Ltd. All rights reserved.

Peptide hydroxamic acids possess a great variety of biological activities and have been used as enzyme inhibitors and metal chelators.[1] These compounds have generally been prepared using solution phase chemistry via acylation of hydroxylamines with activated carboxylic acid derivatives.[2] Our interest in hydroxamate libraries led us to develop a new approach for the synthesis of peptide hydroxamic acids using solid phase methods. The general route of our synthesis is outlined in Figure 1. First a desired precursor molecule is prepared on para-methyl benzhydrylamine (pMBHA) resin, with a residue bearing a carboxylate moiety introduced by using a suitably protected amino acid precursor, such as Fmoc-Asp(OtBu)-OH. The carboxyl group is exposed by TFA deprotection. We found that succinimide formation, a common problem with aspartyl derivatives, could be overcome by pre-neutralizing the resin with diisopropylethylamine (DIEA) prior to the condensation with the protected hydroxylamine. Next, in a reversal of the usual condensation direction, the O-protected amine, NH_2OBzl is coupled with the carboxyl side chain to produce the peptide hydroxamate on the solid support. This methodology eliminates the tedious separation of solution phase methods and should be applicable to the rapid and simultaneous multiple synthesis of peptide and non-peptide hydroxamates.

Selection of a suitable O-protected hydroxylamine and a convenient O-deprotection method after synthesis were important issues. We found that trityl protected hydroxylamine suffered from steric hindrance while the t-butyl group resulted in a product overly volatile following release from its HCl salt. Among commercially available O-protected variants, O-benzyl hydroxylamine can be easily recovered from its HCl salt and has been widely used in hydroxamic acid synthesis. Even though the O-benzyl group is usually removed by catalytic hydrogenation,[3] it appeared that deprotection can also be effected by boron trifluoroacetate as well as liquid HF.[4] Our studies indicated that the benzyl protecting group on hydroxamate was cleanly removed during routine peptide cleavage with anhydrous liquid HF.[5] No N-O dissociation or other significant side reactions were detected within the limits of our analysis. Since the benzyl protecting group is stable to both acid and base treatments, this method should be compatible with both Boc and Fmoc methods of synthesis.

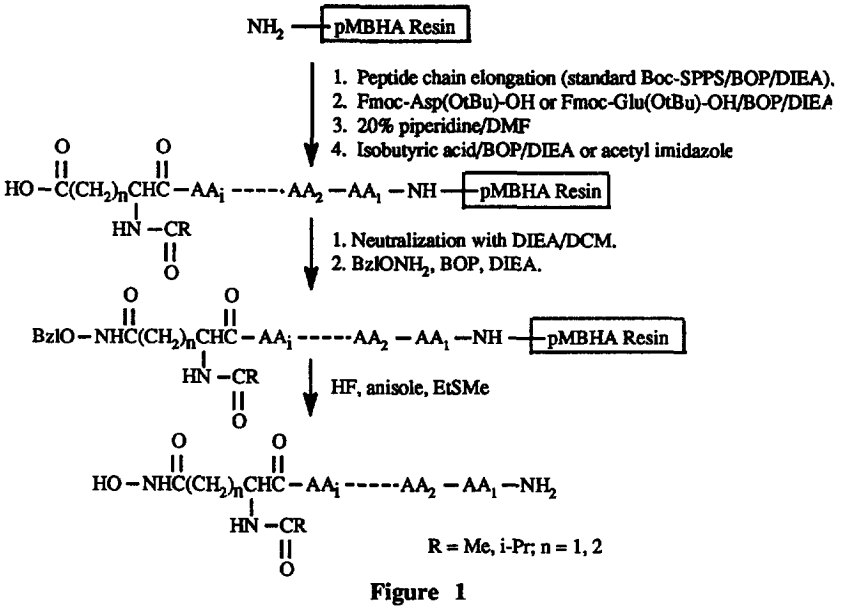

Figure 1

To select the best coupling reagents and solvent conditions for forming hydroxamates, several inverse coupling protocols[6] were investigated using the synthesis of the model peptide hydroxamic acid, Ac-Asp(NHOH)-Phe-Ala-NH_2 (**1**).[7] The traditional condensing reagents, benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and diisopropylcarbodiimide (DIC) were

Table 1. Comparative Studies with Ac-Asp(NHOH)-Phe-Ala-NH_2 (1)

Reagent[a]	Molar ratio of NH_2OBzl/ Coupling reagent	Solvent	Peptide Content in Crude Product (%)[b]	Yield (%)[c]
PyAOP	3	CH_2Cl_2	88	67
DIC/HOBt	2	DMF	90	71
BOP	3	CH_2Cl_2	92	69
BOP	3	DMF	89	64
BOP	1	DMF	91	66

a. 4 eq. of coupling reagent were used relative to the peptide substitution level.
b. Determined by RP-HPLC at 220 nm.
c. Calculated after RP-HPLC purification according to the initial substitution level (0.69 meq/g) on the pMBHA resin.

compared in our study. 7-Azabenzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP),[8] was also evaluated. The coupling results were assessed by reversed phase-HPLC (RP-HPLC) analysis of crude products and are summarized in Table 1. The results suggest that peptide hydroxamates could be efficiently prepared on the solid phase support using a variety of coupling reagents. No severe succinimide

formation was detected within our analysis limits. The studies also suggest that solvent and excess amounts of NH_2OBzl do not have a significant effect on the purity or yield of the final products.

In addition to the model peptide, four other peptide hydroxamic acids were prepared according to the method described above (Table 2). All gave satisfactory yields and purities. The synthesis of **5**, i-PrCO-D-Glu(NHOH)-Nal-Asp-Ala-NH_2, demonstrates that this method is also compatible with standard ester protection on side chain carboxyl groups and can be used to prepare fully protected peptide hydroxamates on a solid support. Our studies also suggest that this method can be applied to solid phase synthesis using a benzyl ester linkage.[9] We are currently using this approach for the synthesis of peptide libraries containing hydroxamate functional groups as potential metalloprotease inhibitors.[10]

Table 2. Peptide Hydroxamic Acids Prepared by SPPS

Compound	Compound	Yield (%)[c]
1[a]	Ac-Asp(NHOH)-Phe-Ala-NH_2	69
2[a]	Ac-Glu(NHOH)-Phe-Ala-NH_2	63
3[a]	i-PrCO-D-Asp(NHOH)-Nal-Ala-NH_2	59
4[a]	i-PrCO-D-Glu(NHOH)-Nal-Ala-NH_2	52
5[b]	i-PrCO-D-Glu(NHOH)-Nal-Asp-Ala-NH_2	72

a Prepared using 9 eq of NH_2OBzl. 3 eq of BOP and 3 eq of DIEA in CH_2Cl_2.
b. Prepared using 4.5 eq NH_2OBzl, 3 eq DIC and 3 eq HOBt in DMF. Boc-Asp(OcHex)-OH was used when incorporating Asp residue.
c. Calculated using the initial substitution level of the pMBHA resin. Products were purified by semi-preparative RP-HPLC and gave satisfactory RP-HPLC, ^{1}H-NMR and MS results.

ACKNOWLEDGEMENTS

This research is supported by the Department of Immunological Diseases of Boehringer Ingelheim Pharmaceuticals, Inc.(Ridgefield, Connecticut). ESI-MS data were provided by the Protein and Carbohydrate Facility of the University of Michigan.

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10. While this manuscript was in preparation, Floyd et al. (*Tetrahedron Lett.* **1996**, *37*, 8045-8048) described the solid phase synthesis (Fmoc approach) of hydroxamic acids in which this group is directly attached to the support, facilitating the synthesis of C-terminal peptide hydroxamates.

(*Received in USA* 2 *December* 1996; *revised* 15 *January* 1997; *accepted* 16 *January* 1997)