Pergamon

Tetrahedron Letters, Vol. 37, No. 44, pp. 8045-8048, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01821-7

A Method for the Synthesis of Hydroxamic Acids on Solid Phase

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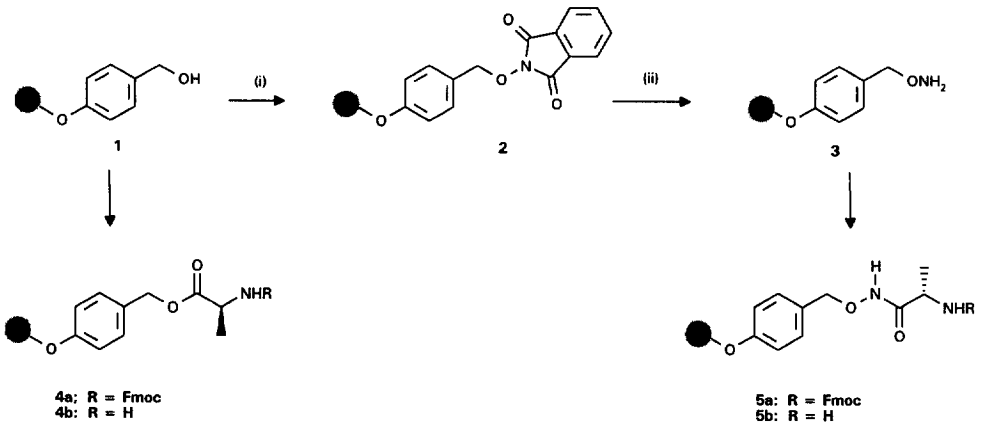
Wang resin **1** was modified using a Mitsunobu reaction to give resin bound *O*-hydroxylamine **3**. This resin was acylated and the adduct cleaved from the resin as a hydroxamic acid on treatment with TFA. Series of tripeptide, and sulfonamido **9** hydroxamic acids which act as inhibitors of metalloproteinases have been prepared. Resins more sensitive to acid cleavage can also be modified to simplify the work-up procedure.

Hydroxamic acid derivatives have a variety of pharmaceutical properties. Of particular recent interest has been the use of peptide and pseudopeptide hydroxamic acids as inhibitors of the matrix metalloproteinases (MMPs) for the modulation of a variety of inflammatory and cell proliferative conditions[1]. As part of our ongoing interest into the design and synthesis of these inhibitors we have been developing effective routes to the preparation of such compounds on solid phase; in particular we have examined ways of generating the hydroxamic acids directly on release from resin supports. In principle hydroxylamine derivatives can directly cleave suitable resin ester linkages; this has been reported in the preparation of some derivatives of tepoxalin[2]. In solution this is an established procedure, with or without Lewis acid assistance[3]. However we have not been able to use this method to give reproducible yields of product using direct cleavage of resin bound esters with hydroxylamine.

We chose instead to look at an acidic cleavage method which might overcome these problems and also reduce much of the post cleavage work-up and purification steps. This is particularly important since we wanted to use a solid phase method to generate libraries of hydroxamic acids, either containing compound mixtures *via* split-mix techniques or arrays of single compounds. The acid cleavable resins based on hydroxymethylphenoxy linkers, developed for peptide synthesis, have long been used to give reproducible yields of peptide acids[4]. We sought to modify these resins and then use them to generate hydroxamic acids in a manner analogous to the synthesis of carboxylic acids.

Wang resin **1** was converted to the N-hydroxyphthalimide derivative **2** using Mitsunobu conditions. The formation of **2** was confirmed by the infra-red spectrum (ν_{max} 1770 and 1726 cm^{-1}). Further treatment of **2** with hydrazine in THF- ethanol removed the phthalimido group to leave the hydroxylamine resin **3**. This resin did not give a positive Kaiser test and not suprisingly the ir spectrum of **3** differed little from the starting resin **1**. Both the resin **1** and **3** were coupled to an excess of Fmoc-alanine to give the protected alanine derivatives **4a** and **5a** (confirmed by the presence of the Fmoc group in the ir) from which the Fmoc group was removed with 25% piperidine in DMF (Scheme 1). The resulting resin bound derivatives could now be clearly differentiated using ir spectroscopy. Resin **4b** derived from Wang resin **1** exhibited a clear ester stretch at 1725 cm^{-1} whereas the modified resin only showed an amide carbonyl at 1681 cm^{-1} [5].



(i) PPh_3, N-hydroxyphthalimide, DEAD (3 eq each), THF, 0°-rt, o/n. (ii) hydrazine, THF-ethanol, 0°-rt, 16 hr

Scheme 1

In order to establish the utility of the modified resin the tripeptide Z-Pro-Leu-Ala-NHOH was chosen as a synthetic test. This tripeptide hydroxamic acid has been reported as a lead member of a series of transition state inhibitors of the MMPs in which the peptide side chains mimic the N-terminal ('left hand') side of the cleavage site of the substrate[6]. The tripeptide was constructed on the resin **3** using standard Fmoc protocols[7]. Cleavage from the resin (70% TFA in dichloromethane (containing ~2% water)) gave the desired hydroxamic acid as the only peptidic product. No carboxylic acid or racemised product was detected by reverse phase Hplc.

Following flash chromatography to remove some minor fast running impurities (presumably derived from TFA mediated resin breakdown) the tripeptide (> 80% yield) was submitted for biological assay and gave results[8] similar to those reported[6b]. Direct assay of the crude cleavage product also gave comparable results which indicated that the resin could be of use in the rapid generation of libraries of inhibitors for assay.

Table 1: Representative tripeptides prepared using resin (3)

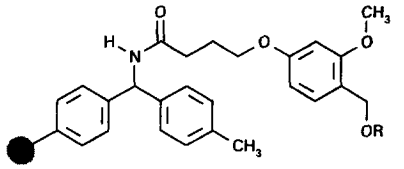
Z-Pro-hPhe-Nle-NHOH	33	Z-Pro-Phe-Ile-NHOH	78
Z-Pro-Nle-Nle-NHOH	76	Z-Pro-Nva-Ile-NHOH	81
Z-Pro-Cha-Lys(Z)-NHOH	25	Z-Pro-Pro-^{D}Leu-NHOH	78
Z-Pro-Nor-Lys(Z)-NHOH	52	Z-Asp-Pro-^{D}Leu-NHOH	69
Z-Pro-Nle-Ile-NHOH	84	Z-Phe-Pro-^{D}Leu-NHOH	79
Z-Pro-Asp-Ile-NHOH	57	Z-Leu-Pro-^{D}Leu-NHOH	88
Z-Pro-Lys(Z)-Ile-NHOH	50	Z-Ala-Pro-^{D}Leu-NHOH	85
Z-Pro-hPhe-Ile-NHOH	51	Z-thioPro-Nle-Ala-NHOH	81
Z-Pro-Ala-Ile-NHOH	81	Z-thioPro-Nle-Ile-NHOH (**8**)	73

percentage yields reported are for recovery of purified compounds and are unoptimised

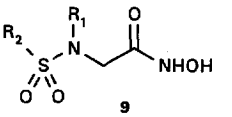
We have used the resin to produce a series of these tripeptides (Table 1) in fair to good yields. All the compounds gave single hydroxamic acid components which were readily purified by flash column or automated reverse phase Hplc. However, we did notice that the amount of non-peptidic contaminant did vary from batch to

batch of resin. In order to overcome these problems we investigated a more acid sensitive resin which allowed us to use greatly reduced concentrations of TFA in the cleavage step.

HMPB-MBHA resin **6** was treated as above to give the equivalent hydroxylamine derivative **7**. Z-ThioPro-Nle-Ile-NHOH **8** was constructed on the resin using standard TBTU coupling conditions[7]. Cleavage with 1% TFA in dichloromethane in the presence of triisopropylsilane gave the desired hydroxamic acid **8** which was detectable in the cleavage cocktail after less than 90 seconds. The nmr spectrum (500 MHz) was assigned to the desired product and indicated that it was uncontaminated with any resin cleavage product. Hplc comparison of this tripeptide with that prepared on the Wang-derived resin **3** showed a single peptidic product free from the small amount of fast running impurity obtained with resin **3**. The yield of the purified peptide rose from 71 to 90%.

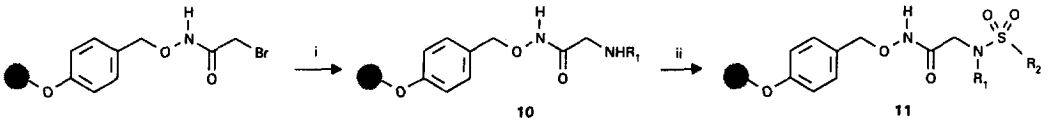


6; R = H
7; R = NH_2



9

We have also used the solid phase method to explore a series of sulfonamide hydroxamic acid derivatives **9** that we and others have reported as MMP inhibitors[9]. The resin **3** was loaded with bromoacetic acid and the bromine displaced with an amine to give the secondary amine species **10** using the same conditions that have been reported for non-modified resins[10,11]. Compound **10** was then treated with the appropriate sulfonyl chloride to give the sulphonamide **11** (Scheme 2). Cleavage as above gave the desired derivative **9**.



10 **11**

i) 2M R_1NH_2 in DMSO, 2h, quantitative conversion (ii) R_2SO_2Cl/py/DMF, 0.5M, 2-18 hr

Scheme 2

We found that this reaction pathway was not as efficient as the preparation of the simple tripeptides. Investigation of each step indicated that this was a consequence of the sulfonylation step which varied according to the nature of the sulfonyl chloride. Whereas the reaction proceeded fairly smoothly with aromatic sulfonyl chlorides variable yields were obtained with aliphatic species (Table 2). Fortunately the crude reaction species generally gave distinct product fractions with reverse phase Hplc which allowed separation on an automated apparatus. We have prepared several series of these sulphonamides **9** using arrays with differing R_1 and R_2 groups. Syntheses have been carried out manually or with an automated pipetting robot (MultiSynTech SyRo).

We are using these and other O-hydroxylamine resins in the automated combinatorial synthesis[12] of matrix metalloproteinase inhibitors and will report separately on the biological evaluation of these libraries.

Table 2: Representative sulfonamides 9 prepared as in Scheme 2

R_1 \ R_2	$C_{10}H_{21}$	$Cl(CH_2)_2$	4-$MeOC_6H_4$	4-MeC_6H_4
$PhCH_2$	22	30	68	64
3,4-$(OCH_2O)C_6H_4CH_2$	13	40	75	81
2-furanylmethy	12	64	83	78
2-thienylmethyl	16	63	85	77
$Me(CH_2)_2O(CH_2)_2$	21	52	67	65

percentage yields are for compounds (>97% pure) obtained following reverse phase Hplc

Acknowledgements: we wish to thank Wendy Cooper, Laura Harnett and Beth Charles for able technical assistance.

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5. *4-(Hydroxymethyl)phenoxymethyl-copoly(styrene-1% divinylbenzene)-resin (100-200 mesh) ("Wang" resin) (1.83 g, 0.7 mmol/g loading, 1.28 mmol) was suspended in dry THF (20 cm^3) and gently agitated for 30 minutes under a blanket of argon. N-Hydroxyphthalimide (624 mg, 3.82 mmol) and triphenylphosphine (1.005 g, 3.82 mmol) were added and the mixture was agitated until these reagents dissolved. DEAD (721 mg, 3.82 mmol) was added by cannula to give a bright red solution and the mixture was shaken. Most of the colour dissipated from the reaction mixture after 45 minutes. After 18 h the resin was collected by filtration, washed successively with THF, DMF, CH_2Cl_2, methanol and finally thoroughly with CH_2Cl_2 and then dried in vacuo; ν_{max} (KBr) 1726(vs), 1687, 1593 cm^{-1}. The resin from above was suspended in ethanol/THF (10 cm^3) cooled to 0°C and hydrazine (~100%)(1.5 cm^3) was added. The pale yellow solution was warmed to room temp over 1h and then gently agitated overnight. The resin was filtered, washed with DMF (2 x 15 cm^3), CH_2Cl_2 (2 x 15 cm^3), methanol (20 cm^3) and finally with CH_2Cl_2 (3 x15 cm^3) and dried. Ir (KBr) showed the absence of any carbonyl containing substituent confirming hydrazinolysis of the phthalimide. Elemental analysis of the resin confirmed the presence of nitrogen and suggested a loading of 0.68 mmol/g: C 89.17, H 7.70, N 0.95%. [The loading obtained varied for different batches of the resin due to variability in the loading of the starting resin, but in each case elemental analysis indicated that quantative conversion was achieved.]*
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11. The efficiency of the conversion was measured by loading the resin with excess bromoacetic acid. Elemental analysis (C, N, Br) established a loading value comparable to that for the starting resin.
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(*Received in UK* 21 *August* 1996; *accepted* 13 *September* 1996)