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N-Fmoc-aminooxy-2-chlorotrityl polystyrene resin: A facile solid-phase methodology for the synthesis of hydroxamic acids[1]

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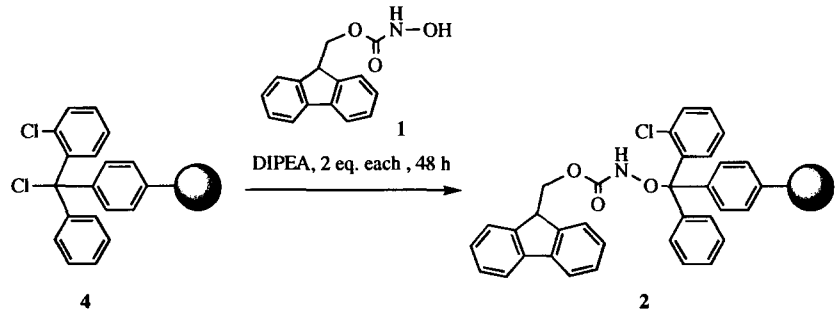
Abstract: Using the new compound *N*-Fmoc-hydroxylamine **1** we have generated a facile route to a high loading, acid labile solid-phase resin bearing a hydroxylamine linker. The novel *N*-Fmoc-aminooxy-2-chlorotrityl polystyrene **2** showed generic utility for the construction of hydroxamic acids, including peptidyl hydroxamic acids. © 1997 Elsevier Science Ltd.

Peptidyl[2] and 2-phenyloxazoline[3] hydroxamic acids have recently been shown to be potent enzyme inhibitors, and hence are legitimate targets as potential therapeutic agents. The use of combinatorial chemistry as a tool for drug discovery has highlighted the need to generate such compounds on a solid-phase resin. More recently it has been demonstrated that it is possible to generate a resin bearing a hydroxylamine linker.[4] We now wish to report a facile method for the production of *N*-Fmoc-aminooxy-2-chlorotrityl polystyrene **2**, a highly functionalised solid-phase resin, which has the distinct advantage of being cleaved by mild acidolysis to yield hydroxamic acids.

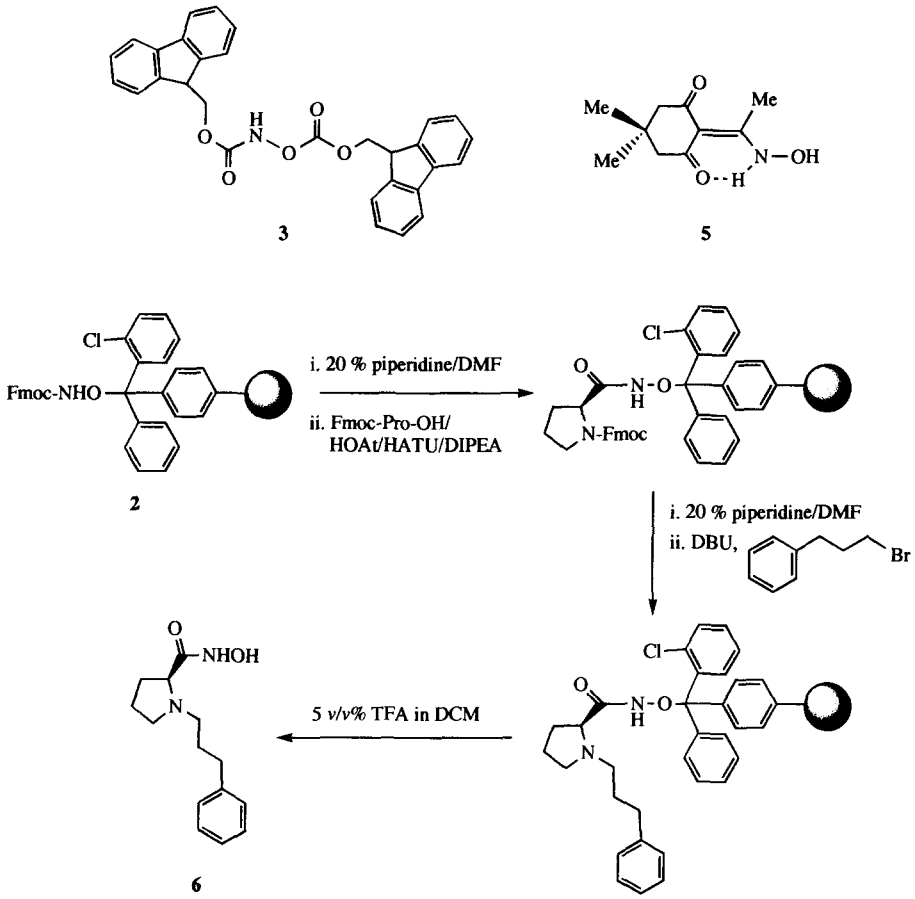


4 **2**

Scheme 1

Our strategy was to prepare the novel compound *N*-Fmoc-hydroxylamine **1**, and use the hydroxyl functionality for attachment to the resin *via* a simple nucleophilic displacement (Scheme 1). The key component, *N*-Fmoc-hydroxylamine **1**, was synthesised in excellent yields by reacting hydroxylamine hydrochloride with stoichiometric amount of Fmoc-Cl under basic conditions for 3-4 h.[5] Here, the use of an excess acylating reagent and/or stronger basic conditions typically promote significant formation of the undesired *bis*-protected compound *N,O-bis*-Fmoc-hydroxylamine **3**.

Using the high loading 2-chlorotrityl chloride polystyrene resin **4**[6], the *N*-Fmoc-hydroxylamine was attached to the resin by stirring in the presence of 2 eq. of DIPEA in dichloromethane for 48 h. Unreacted chloride sites were then "capped" by stirring with methanol (1 ml) for 30 min. The resulting *N*-Fmoc-aminooxy-2-chlorotrityl polystyrene resin **2** exhibited loadings[5] which were typically >0.8 mmol g^{-1}, indicating a reaction efficiency >80%. During the course of our studies, *N*-[1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl]hydroxylamine **5** (Dde-NHOH)[7] was also successfully exploited in a similar manner.



Scheme 2

In order to demonstrate that the new resin could be used for the facile production of peptidyl hydroxamic acids, a heptamer H-Arg-Arg-Arg-Trp-Trp-Arg-Phe-NHOH was synthesised. Following Fmoc-deprotection with 20% piperidine/DMF, coupling of the first amino acid, Fmoc-Phe-OH, was achieved with 94% efficiency[5] using 4 eq. of the acylating mixture Fmoc-Phe-OH:HOAt:HATU[8]:DIPEA (1:1:1:2). Subsequent amino acids were sequentially coupled using the milder HOBt:TBTU activation conditions to give the peptide in ~80% yield. Acidolysis using 90% TFA, necessary for side chain deprotection, also effected cleavage of the peptide from the resin, and subsequent analysis using RP-HPLC allowed us to estimate product[9] purity at >90%.

To further demonstrate the resin **2** utility in the production of small molecule libraries Fmoc-proline was loaded on to the resin with 81% efficiency[5] using the HOAt:HATU coupling procedure (Scheme 2). Investigations into the acid lability of this resin showed that it was possible to release Fmoc-Pro-NHOH from the resin using either 50 or 5 *v/v*% TFA in dichloromethane. Critical analysis by RP-HPLC indicated that using a higher acid concentration only served to decrease the purity of product released.

Following Fmoc-deprotection of the proline residue it was further possible to achieve *N*-alkylation by adding 4 equivalents of the non-nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), followed by 4 equivalents of 3-phenylpropyl bromide. Treatment with 5 *v/v*% TFA in dichloromethane released the alkylated proline hydroxamic acid, (Php)Pro-NHOH[9] **6** from the resin in excellent yield and purity.

We anticipate that *N*-Fmoc-aminooxy-2-chlorotrityl polystyrene resin **2** will prove an indispensable solid support for the synthesis of hydroxamic acids by multiple and combinatorial approaches. Not only is its production both simple and efficient, but effective acylation can be achieved using standard solid-phase methodology and cleavage accomplished by mild acidolysis, making it invaluable for the synthesis of both peptidyl hydroxamic acids and small molecule libraries alike.

ACKNOWLEDGMENTS

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REFERENCES AND NOTES

1. A preliminary account was presented at the 24th European Peptide Symposium, Edinburgh, UK, 8-13 September 1996, P120.
Abbreviations: Fmoc, 9-fluorenylmethoxycarbonyl; HOAt, 1-hydroxy-7-azabenzotriazole; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIPEA, *N,N*-diisopropylethylamine; HOBt, 1-hydroxybenzotriazole; TBTU, *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid.
Amino acid derivatives used for peptide synthesis were Fmoc-Trp(Boc)-OH and Fmoc-Arg(Pmc)-OH.

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5. A typical procedure for the synthesis of Fmoc-NHOH **1**:
A mixture of an aqueous solution of sodium hydrogen carbonate (6.6 mmol, 10 ml) and ethyl acetate (~20 ml) was added to hydroxylamine hydrochloride (3.0 mmol) and cooled to 5°C. Fmoc-Cl (3.0 mmol), dissolved in ethyl acetate (1 ml), was then added dropwise to the rapidly stirred hydroxylamine solution over a period of 30 min. Following addition, the biphasic mixture was allowed to reach ambient temperature and vigorously stirred for a further 3-4 h. The reaction was monitored by TLC (ethyl acetate: hexane (1:1), Fmoc-NHOH $R_f = 0.14$). The reaction mixture was then separated and the

organic phase washed with saturated aqueous potassium hydrogen sulphate (3 x 30 ml) and brine (30 ml). The organic extract was dried and evaporated to dryness *in vacuo* to afford, following trituration with hexane, Fmoc-NHOH (0.686 g, 90%) as a white crystalline solid.

M.p. 164.5-167.5°C. Electrospray(ES)-MS, *m/z* 278.3 ($M+Na^+$).

δ_H (250 MHz, $CDCl_3$) 4.21 (1H, t, *J* 6.9 Hz, Fmoc C*H*), 4.32 (2H, d, *J* 6.7 Hz, Fmoc CH_2), 7.28-7.43, 7.68, 7.86 (8H, m, Fmoc Ar. C*H*), 8.77 (1H, s, N*H*), 9.75 (1H, br s, O*H*).

δ_C (62.90 MHz, $CDCl_3$) 47.49 (Fmoc *C*H), 66.44 (Fmoc *C*H_2), 120.86, 126.00, 127.85, 128.53 (Fmoc Ar. *C*H), 141.57, 144.52 (Fmoc Ar. *C*), 158.46 (*C*=O).

Fmoc-NHO-Fmoc **3**: m.p. 159.5-161°C; ES-MS, *m/z* 478.4 (MH^+); TLC (ethyl acetate:hexane, 1:1) $R_f = 0.64$.

The resin loading is based on spectrophotometric determination of the Fmoc-derived chromophore liberated upon treatment with 20% piperidine/DMF using $\varepsilon_{290} = 5\ 253\ M^{-1}\ cm^{-1}$, which was used to calculate % efficiency.

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7. Dde-NHOH **5** was prepared, in 51% yield, by reacting 2-acetyldimedone[10] with hydroxylamine in MeOH/THF at 5°C for 3 h, followed by recrystallisation from ice-cold hexane.

   M.p. 116-118°C; ES-MS, *m/z* 198.5 (MH^+); TLC (ethyl acetate:hexane, 3:1) $R_f = 0.27$.

   δ_H (250 MHz, $CDCl_3$) 1.04 (6H, s, -C(CH_3)$_2$), 2.35 (4H, s, 2 -CH_2-), 2.57 (3H, s, =CCH_3), 10.9, 15.0 (2 x 1H, 2 x br s, O*H*, N*H*).

   Fmoc-N(Me)OH was also synthesised using the procedure outlined above[5] from *N*-methylhydroxylamine hydrochloride in 86% yield; m.p. 116-117°C, ES-MS, *m/z* 292.4 ($M+Na^+$). Similarly, Fmoc-N(Me)OH was readily condensed with 2-chlorotrityl chloride polystyrene to afford *N*-Fmoc-methylaminooxy-2-chlorotrityl polystyrene of a respectable loading (0.64 mmol g^{-1}). However, following Fmoc-deprotection, it is worth noting that we were unable to acylate the resin-bound secondary amine to any significant level; a raft of activation chemistry was evaluated. We concluded that this observation is the result of severe steric constraint.

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   The dried resin **2** was allowed to swell overnight in dichloromethane prior to use. Furthermore, acylation of the Fmoc-deprotected **2** was carried out at r.t. for 24 h.

9. H-Arg-Arg-Arg-Trp-Trp-Arg-Phe-NHOH, ES-MS calc. MH^+ 1178.4, found 1177.9; (Php)Pro-NHOH, calc. MH^+ 249.3, found 249.2.

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