4-[2,4-Dimethoxyphenyl(*N*-fluoren-9-ylmethoxycarbonyl-*N*-alkylaminooxy)-methyl]phenoxymethyl polystyrene: a multiple solid-phase approach to *N*-alkylhydroxamic acids

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***N*-Fluoren-9-ylmethoxycarbonyl-*N*-alkylhydroxylamines, prepared *via* K_2CO_3-catalysed *N*-alkylation of *N*,*O*-bis(*tert*-butoxycarbonyl)hydroxylamine, undergo a highly efficient condensation with 4-[2,4-dimethoxyphenyl(chloro)methyl]-phenoxymethyl polystyrene (PS) to afford 4-[2,4-dimethoxyphenyl(*N*-fluoren-9-ylmethoxycarbonyl-*N*-alkylaminooxy)methyl]phenoxymethyl PS, which is used for the facile synthesis of structurally diverse *N*-substituted hydroxamic acids.**

Peptide hydroxamic acids, *e.g.* actinonin, foroxymithine and matlystatin B, isolated from microorganisms are known to be potent and selective inhibitors of many important metalloproteases.[1] The ability of the hydroxamic acid functionality to form a bidentate chelate with the zinc atom in the enzyme's active site is considered to be an important functional feature.[1] Recently, exploitation of this concept has led to the design and synthesis of α-alkylmalonyl and 2-phenyloxazoline hydroxamic acids as potent inhibitors of endothelin-converting enzyme and *E. coli* uridine 5′-diphosphate-3-*O*[3*R*)-3-hydroxymyristoyl]-*N*-acetyl-glucosamine deacetylase, respectively.[2]

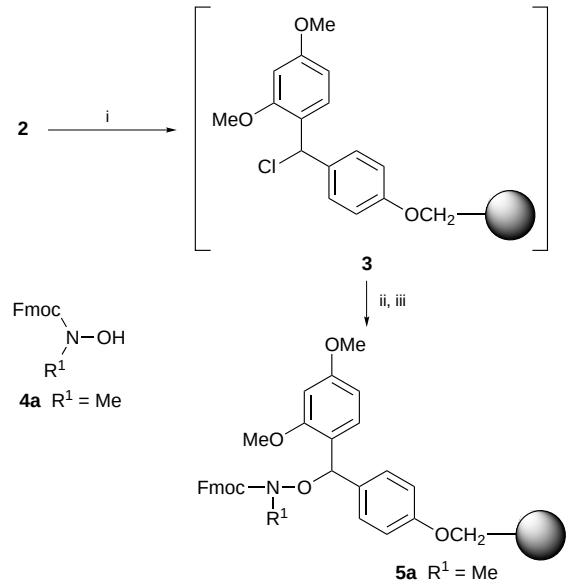
The implication of combinatorial chemistry for high throughput generation of structurally diverse hydroxamic acids is self evident. Recently, several solid phase approaches for the synthesis of hydroxamic acids have been reported.[3,4] For example, we outlined a facile solid phase procedure using the high-loading resin, *N*-fluoren-9-ylmethoxycarbonylaminooxy-2-chlorotrityl polystyrene **1**.[3] However, these strategies are evidently not applicable for the synthesis of *N*-alkyl hydroxamic acids, which is necessary in order to provide a further dimension in chemical diversity, in a manner analogous to our previously reported[5] peptidyl *N*-alkyl amides. We report herein a novel parallel solid phase strategy to *N*-alkyl hydroxamic acids. The key feature of our strategy is the multiple synthesis of *N*-fluoren-9-ylmethoxycarbonyl-*N*-alkyl hydroxylamines **4**, followed by *O*-alkylation by the unique 4-[2,4-dimethoxyphenyl(chloro)methyl]phenoxymethyl PS **3** to afford 4-[2,4-dimethoxyphenyl(*N*-fluoren-9-ylmethoxycarbonyl-*N*-alkyl-aminooxy)methyl]phenoxymethyl PS **5**.

Preliminary investigations suggested that existing solid phase strategies[3,4] could not be adapted for the synthesis of *N*-alkyl hydroxamic acids for two reasons: (i) inefficient (65–80%) reduction was observed with *O*-benzylhydroxylamine-derived oximes using either $NaBH_3CN$ in 30–50% AcOH in MeOH–THF or BH_3·THF in THF, and (ii) negligible acylation (< 5%) of NH(Me)O-2-chlorotrityl PS[3] was achieved as a result of considerable steric effect. In view of these results, we focused our efforts in the development of a new linker resin.

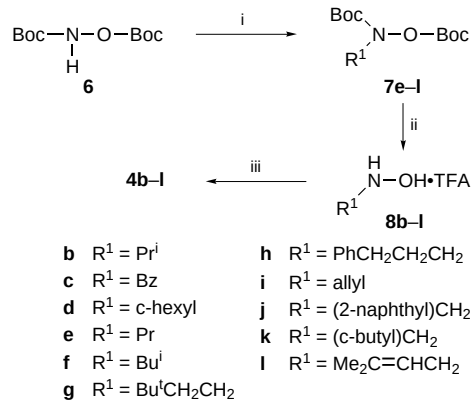
The acidolysis of 4-[2,4-dimethoxyphenyl(hydroxy)methyl]-phenoxymethyl PS **2** and its esters is well documented,[6] and since the linker group in **2** has significantly less steric hindrance than 2-chlorotrityl, we have established a facile solid phase procedure to the new linker resin **5** starting with commercially available **2**† (Scheme 1). Following evaluation of a series of chlorinating reagents and conditions, the conversion of **2** to the chloride **3** is best achieved by a simple S_N1 approach. Thus, treatment of **2** with 1% HCl in CH_2Cl_2–THF under continuous flow conditions gave **3** with typically *ca.* 70% efficiency.‡ In initial model experiments, the condensation of **3** with FmocN-(Me)OH[3] **4a** proceeded smoothly to afford **5a** with *ca.* 95% efficiency (0.38 mmol g^{-1}).† Any remaining reactive sites on the linker resin were 'eliminated' by treatment with MeOH and Ac_2O. We were then able to successfully acylate the Fmoc-deprotected **5a** with Fmoc-Phe-OH, carboxy-activated using HATU-based[7] method, with 89% efficiency, which on treatment with 1–5% TFA in CH_2Cl_2 yielded within 5 min the desired hydroxamic acid, Fmoc-Phe-N(Me)OH, in excellent purity (> 95%).§

Furthermore, using commercially available **8b**–**d**·HCl, the Fmoc-derivatives **4b**–**d** were synthesized (Scheme 2) using our previously reported procedure[3] in excellent yields (71–86%), which on condensation with **3** yielded **5b**–**d** with good efficiencies (58–92%, 0.20–0.37 mmol g^{-1}). As expected, **4b** and **4d** were observed to react with lower efficiencies (58 and 73%, respectively) due to steric hindrance.

In order that the above strategy could be used as a generic solid phase approach to *N*-substituted hydroxamic acids, we went on to investigate synthetic methodology to chemically diverse Fmoc-N(R^1)OH **4**. Following a systematic study, we have established a facile method to compounds **4** *via* *N*-alkylation of *N*,*O*-bis(*tert*-butoxycarbonyl) hydroxylamine[8,9] **6** in predominantly good overall yields (Scheme 2).¶ The alkylation of **6** was accomplished using the appropriate bromides in the presence of K_2CO_3 to furnish **7e**–**l** in typically 78–90% yields. In addition, we have also achieved alkylation of **6** using 3 equiv.



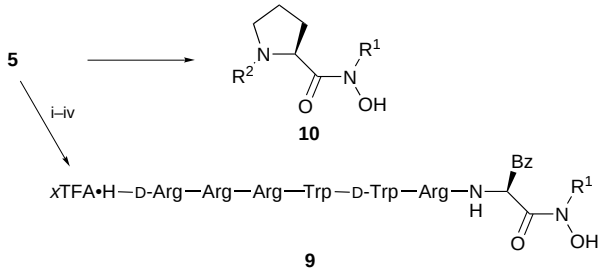
Scheme 1 *Reagents and conditions*: i, 1% w/v HCl in CH_2Cl_2–THF; ii, **4** (1.5 equiv.), Pr^i_2EtN (1.5 equiv.), CH_2Cl_2, 18 h; iii, MeOH–CH_2Cl_2, 30 min, then Ac_2O, Pr^i_2EtN, DMF, 4 h



Scheme 2 *Reagents and conditions*: i, R′Br, K_2CO_3, DMF, 18 h; ii, 30% v/v TFA in CH_2Cl_2; iii, FmocCl, aq. $NaHCO_3$, 4 h

of a chloride, $Me_3C(CH_2)_2Cl$ in the presence of K_2CO_3 (1.25 equiv.) in DMF at 55 °C for 72 h, to give **7g** in 68% yield. The key building blocks **4e**–**l** were then readily obtained by, firstly, deprotection of **7e**–**l** with 30% v/v TFA–CH_2Cl_2, followed by introduction of the *N*-Fmoc protecting group using FmocCl to yield crystalline **4e**–**l** (65–81%).¶ In all cases, we observed high reaction efficiencies (78–97%) between **4e**–**l** and **3** to afford our key linker resins **5e**–**l** (0.26–0.30 mmol g^{-1}; 0.23 mmol g^{-1} for **5j**.† In addition, the Fmoc-deprotected **5e**–**l** were readily acylated with HATU-activated Fmoc-Phe-OH (18 h) to yield the resin-bound Fmoc-Phe-(Fmoc-deprotected) **5e**–**l** in typically 93–100% yield, upon which 5% TFA-mediated cleavage afforded exclusively the desired hydroxamic acids Fmoc-Phe-N(R^1)OH (> 95% purity judged by RP-HPLC§).

The utility of **5** was further illustrated by the synthesis of the model heptapeptidyl *N*-alkyl hydroxamic acids **9** (Scheme 3), using standard solid phase peptide chemistry.[10] The assembled peptidyl-(Fmoc-deprotected) **5e**–**l** following a two-step TFA treatment yielded **9e**–**l** in excellent purity and yield (80–90%).§ In addition, **5** was used for the solid phase synthesis of a biased 'small molecule' library comprising the generic template N^1-alkyl/acyl imino acyl *N*-alkyl hydroxamic acids, *e.g.* **10**; the N^1-alkylation step was typically accomplished using 4 equiv. of an alkyl bromide in the presence of 1 equiv. of DBU.



Scheme 3 *Reagents and conditions*: i, 20% piperidine–DMF; ii, Fmoc-Phe-OH–HATU–HOAt–Pr^i_2EtN (1 : 1 : 1 : 2, 10 equiv., *ca*. 0.3 M in DMF], 18 h; iii, standard Fmoc/Bu^t solid-phase procedures (2.5 h acylation); iv, TFA–Et_3SiH–CH_2Cl_2 (5 : 1 : 94), 5 min, then TFA–Pr^i_3SiH–$HSCH_2CH_2SH$–H_2O (90 : 1 : 4 : 5), 4 h, 30 °C

In summary, we have outlined a facile solid phase approach to *N*-substituted hydroxamic acids *via* the new linker resin **5** prepared from readily accessible building blocks **4**. The strategy allows the cleavage of target molecules from the solid support under exceptionally mild acidic conditions. The synthesized compounds **9** and **10** are currently being evaluated for biological activity.

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Footnotes and References

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† Compound **2** has a chemically reactive hydroxy loading of either 0.54 or 0.64 mmol g^{-1}. The derivatised resin loading is based on spectrophotometric determination of the Fmoc-derived chromophore liberated upon treatment with 20% piperidine–DMF using ε_{290} = 5253 M^{-1} cm^{-1}.

‡ The following reaction was performed using oven-dried glasswares. A solution of *ca*. 1% w/v HCl was prepared by the addition of $SOCl_2$ (2.50 ml) to THF–CH_2Cl_2(1 : 3, 100 ml) followed by MeOH (1.25 ml). The resin **2** (0.5 mmol), suspended in CH_2Cl_2 in a glass column (15 × 150 mm), was treated with the 1% w/v HCl solution (100 ml) at *ca*. 4 ml min^{-1} and washed with CH_2Cl_2 (20 ml), THF–CH_2Cl_2 (5 ml) and CH_2Cl_2 (20 ml) to afford **3**, which was used immediately. To the suspension of **3** in CH_2Cl_2 (*ca*. 6 ml) was added **4** (0.75 mmol) followed by Pr^i_2EtN (0.13 ml, 0.75 mmol), and the resultant mixture was gently stirred at ambient temperature overnight to yield **5**. MeOH (0.2 ml) was added to the reaction mixture, stirred for 30 min and the resin was then washed with DMF (30 ml). The resin, now suspended in DMF, was treated with Ac_2O (5 mmol)–Pr^i_2EtN (0.5 mmol) for 4 h, after which the resin was washed with DMF, filtered off, washed repeatedly with CH_2Cl_2 and MeOH, and dried *in vacuo*. The chloride loading in **3** was determined using an acid–base back-titration procedure.

§ Crude hydroxamic acids **9** and **10** were analysed by RP-HPLC on a Hypersil Pep C_{18} columnn (4.6 × 150 mm). All purified synthetic compounds gave the expected ES-MS, *e.g.* **9d**: calc. M^+ 1259.52, found 1258.38; **9g**: calc. 1261.54, found 1261.34; **9h**: calc. 1295.54, found 1294.95; **10f** (R^2 = $CH_2CH_2CH_2Ph$): calc. MH^+ 305.43, found 305.3; **10k** [R^2 = CH_2(2-naphthyl)]: calc. MH^+ 339.44, found 339.5.

¶ A typical procedure for the synthesis of *N*-fluoren-9-ylmethoxycarbonyl-*N*-alkyl hydroxylamine FmocN(Php)OH **4h**: To a solution of **6** (5 mmol) in DMF (10 ml) was added K_2CO_3 (6.25 mmol) followed by portionwise addition of 1-bromo-3-phenylpropane (5.2 mmol), and the resultant mixture was vigorously stirred at room temperature overnight. Following a standard work-up procedure trituration with ice-cold pentane yielded **7h** (1.58 g, 90%) as a white crystalline solid [mp 75–76 °C; > 95% purity when analysed by RP-HPLC; ES-MS : MH^+ calc. 352.45, found 352.3].

A solution of **7h** (4 mmol) in TFA–CH_2Cl_2 (3 : 7, 10 ml) was left at ambient temperature for 3–4 h, and then evaporated to dryness to yield **8h** (1.05 g, 99%; mp 67–69 °C), which was dried *in vacuo* over KOH pellets and used without further purification.

Compound **4h** was obtained by treating a solution of **8h** in EtOAc–aq. $NaHCO_3$ (2.2 equiv.) with FmocCl (1.1 equiv.) using the procedure previously outlined (ref. 3) in 67% yield [mp 84–86 °C; δ_H($CDCl_3$, 250 MHz) 1.98 (2 H, m, Php $CH_2CH_2CH_2N$), 2.64 (2 H, t, *J* 7.8, Php $CH_2CH_2CH_2N$), 3.59 (2 H, t, *J* 7.0, Php $CH_2CH_2CH_2N$), 4.26 (2 H, t, *J* 6.7, Fmoc C*H*), 4.50 (2 H, d, *J* 6.7, Fmoc CH_2), 7.12–7.47, 7.56, 7.81 (13 H, Fmoc and Ph ArH); ES-MS : MH^+ calc. 374.45, found 374.4].

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