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A convenient parallel synthesis of low molecular weight hydroxamic acids using polymer-supported 1-hydroxybenzotriazole

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A convenient two-step procedure for the parallel synthesis of hydroxamic acids from carboxylic acids and hydroxylamine in good to high yields is reported. It involves the formation of a polymer-bound HOBt active ester and subsequent reaction with *O*-protected or free hydroxylamine. The hydroxamates are isolated with high purities by simple evaporation of volatile solvents. The use of free hydroxylamine leads to increased yields while maintaining high purities. Recycling of the spent resin to produce the same or a different hydroxamic acid has been achieved by a three-step protocol which is easily amenable to automation and cost-economical. The method presented here is well suited to the preparation of the title compounds and can be used effectively to synthesise large molecules containing a hydroxamic acid group.

Introduction

The hydroxamic acid functionality, –C(=O)NROH, is a key structural constituent of many biomolecules, some of which such as siderophores (pseudobactin, desferrioxamines, ferrichromes *etc.*) are naturally occurring,[1] and others, such as the peroxidase, matrix metalloproteinase and urease inhibitors,[2] are of synthetic origin. Hydroxamic acids can also act as anticancer, anti-fungal, anti-tuberculous and hypotensive agents.[1–3] Recent studies undertaken in our laboratories have shown that hydroxamic acids are nitric oxide donors,[4] and that acetylated hydroxamate derivatives can act as effective aspirin analogues by prostaglandin H_2 synthase inhibition.[5] The versatile biological activity of hydroxamates is due to their strong metal ion chelating ability,[1,6] their NO-releasing properties,[4] their ability when ionized to form salt linkages in their complexes with proteins,[5] or when unionized to engage in key hydrogen bonding interactions,[2b] and to provide sites for acylation.[5] To optimise the lead compounds identified, we have sought a method for the generation of chemical libraries of small molecule hydroxamic acids by parallel synthesis and describe herein a convenient resin-recyclable synthesis, the first to be reported for these compounds.

Two different approaches for the solid-phase synthesis of hydroxamates have previously been described. These involve acidic cleavage and release from insoluble polymers of acylated hydroxylamine, *O*-tethered,[7] or *N*-tethered,[8] to the resin support, or nucleophilic cleavage of resin bound esters,[9] or thioesters,[10] with hydroxylamine derivatives. A solution phase synthesis using polymer-supported reagents and scavengers has also been employed to generate an array of hydroxamic acids.[11]

All these methods suffer an inherent limitation for the synthesis of low molecular weight hydroxamic acids in that their preparation on a multi-milligram scale requires large quantities of the solid support. To overcome this constraint, we have investigated methods that would allow recycling of the resin. Using a cyclic three-step procedure involving activation, coupling and regeneration, we aimed at developing a new method affording high reaction rates for the loading and coupling steps.

To accomplish this we chose to exploit the high reactivity of polystyrene-supported (PS) HOBt (1-hydroxybenzotriazole) esters toward *N*-nucleophiles, which has previously been used for the synthesis of peptides,[12] and amides,[13] including lactams,[13c] and Weinreb amides.[13a] In these reactions, the insoluble, polymer-bound HOBt acts as a catalyst and is therefore easily recyclable.[14] We now report the successful application of this polymeric reagent to the synthesis of low molecular weight hydroxamic acids.

Results and discussion

The *N*-acylation of hydroxylamine derivatives was carried out according to a two-step procedure developed for the synthesis of amides,[15] and was not optimised.

Synthesis using *O*-protected hydroxylamine

The method was first validated using *O*-protected hydroxylamine (Scheme 1). Commercially available carboxylic acids **2** were esterified with the polymeric HOBt **1** using a soluble activating reagent 1,3-diisopropylcarbodiimide (DIC). The resin-bound HOBt esters **3** were then reacted with substoichiometric quantities of *O*-*tert*-butyldimethylsilylhydroxylamine ($TBDMSONH_2$), yielding the silyl hydroxamates **4** as the only products in solution. These were isolated by filtration and concentration of the reaction solution and the corresponding hydroxamic acids **5** were obtained by cleavage of the silyl ether with trifluoroacetic acid. Percentage yields, purities and mass spectroscopic data are reported in Table 1, cycle 1.

The insoluble polymer was recycled by washing and reused to activate the same carboxylic acid. The repetitive synthesis of hydroxamic acids was accomplished without loss of yield or purity, Table 1, cycle 2.

Synthesis using free hydroxylamine

Avoiding the cleavage step, Scheme 1, iii, of the hydroxamate protecting group would facilitate the automation of the method. We therefore investigated the reaction of the PS-HOBt active esters with unprotected,[9c] hydroxylamine (Scheme 2).

The carboxylic acids were loaded on the resin, using new batches (cycle 1) or reused (cycles 2–4) ones with the same acid,

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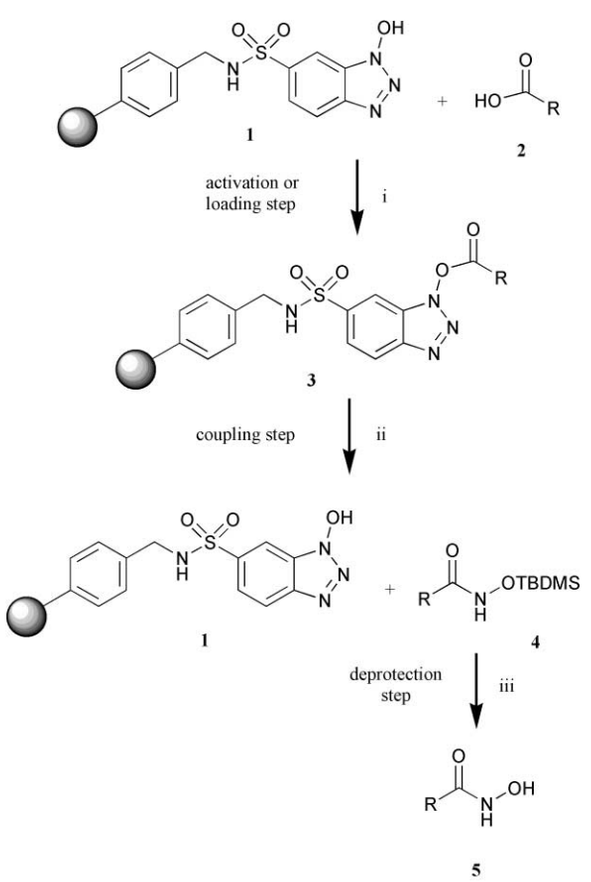
Table 1 Hydroxamic acid synthesis using PS-HOBt and protected hydroxylamine

Hydroxamic acid	Cycle[a]	Yield[b] (%)	Purity[c] (%)	[MH$^+$] Calcd/Found
Benzohydroxamic acid	1	55	94	138.14/138.12
Benzohydroxamic acid	2	70	95	138.14/138.12
2,4-Dimethoxybenzohydroxamic acid	1	97	90	198.33/198.33
2,4-Dimethoxybenzohydroxamic acid	2	Quant.	98	198.33/198.33
2,4-Dichlorobenzohydroxamic acid	1	58	93	206.13/206.03
2,4-Dichlorobenzohydroxamic acid	2	57	93	206.13/206.03
2-Naphthohydroxamic acid	1	60	98	188.18/188.23

[a] Number of use of the same batch of PS-HOBt. [b] Calculated from the amount of protected hydroxylamine used (0.5 equivalent relative to the loading of the PS-HOBt resin); based on the amount of hydroxamic acid recovered after cleavage of the silyl group. [c] Determined by reversed-phase HPLC analysis with detection at 235 and 280 nm; based on the area percent of the hydroxamic acid relative to the total area of all UV-absorbing components – hence reported purities are approximate since the relative responses of the various components are neglected (although the carboxylic acids, the main impurities in the present work, are expected to have similar responses to the corresponding hydroxamic acids).

by the DIC–DMAP coupling chemistry. The nucleophilic partner (hydroxylamine) was used again as the limiting reagent at 0.5 to 0.8 equivalents, relative to the loading of the PS-HOBt resin. In all cases, the hydroxamic acids are isolated in high purities (Table 2) by simple evaporation of the filtrate. The main by-product determined by reversed-phase HPLC is the carboxylic acid, together with traces of diisopropylurea and 4-(dimethylamino)pyridine (DMAP), as confirmed by LCMS, the last two compounds originating from the activation step and their quantities can therefore be minimised by a rigorous washing procedure. The quantities of carboxylic acids detected (2–10%) are likely to result from degradation of the polymeric ester reagents, and can therefore be controlled by optimising the coupling reaction time.[13a]



Scheme 1 *Reagents and conditions:* i, PS-HOBt (1 equiv.), carboxylic acid (1.5 equiv.), DIC (4.5 equiv.), DMAP (0.6 equiv.), 1 : 1 DCM–DMF, 3 h; ii, *O-tert*-butyldimethylsilylhydroxylamine (0.5 equiv., based on the loading of PS-HOBt resin), THF, 5 h; iii, 95 : 5 TFA–H_2O, 15 h.

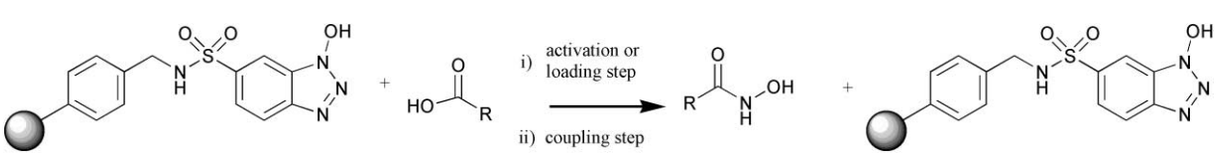
Further exploitation of the method was achieved by recycling the resin to produce a different hydroxamic acid. For example, the spent resin from the synthesis of the phenylacetohydroxamic acid (cycle 2) was reacted with an excess of isopropylamine,[14] then extensively washed and used to activate 2,4-dimethoxybenzoic acid. The corresponding hydroxamic acid was obtained in 64% yield (0.6 equivalents of hydroxylamine relative to the loading of the PS-HOBt resin) with 89% purity. The impurities identified by RP-HPLC analysis were DMAP (4%) and 2,4-dimethoxybenzoic acid (6%).

Advantages of the method

Among the methods for the synthesis of hydroxamic acids by reaction of polymer-bound esters with hydroxylamine derivatives,[9,10] this procedure offers the advantage of requiring fewer equivalents of reagents for both the loading and coupling steps, together with shorter reaction times even without optimisation. It is noteworthy that polymer-supported 1-hydroxybenzotriazole has been successfully used for the synthesis of peptides with reaction times as short as 15–20 min at 0 °C, using DCC as the activating reagent, and 20 min at room temperature, for the activation and coupling steps respectively.[12a] PS-HOBt was prepared from macroporous polystyrene and lacks the sulfonamide electron-withdrawing group which is expected to increase the reactivity of the polymeric coupling reagent.[13a]

In addition, the high reactivity of the PS-HOBt active esters allows the use of hydroxylamine in sub-stoichiometric quantities. Consequently, a simple evaporation of volatile solvents affords the hydroxamic acids. This protocol therefore allows the rapid generation of arrays and is easily amenable to automation.

In contrast to a closely related method which employs an oxime resin,[9b] the procedure presented here is suited to the preparation of aromatic hydroxamic acids. Recycling of the resin is advantageous if substantial quantities of materials are required for further derivatisation which cannot be undertaken on the solid support. The large quantities of spent polymeric reagent can be economically recycled. The regeneration step is carried out by reacting the used resin with isopropylamine and



Scheme 2 *Reagents and conditions:* i, PS-HOBt (1 equiv.), carboxylic acid (1.5 equiv.), DIC (4.5 equiv.), DMAP (0.6 equiv.), 1 : 1 DCM–DMF, 3 h; ii, hydroxylamine (0.6–0.8 equiv.), THF, 5 h.

Table 2 Hydroxamic acid synthesis using PS-HOBt and unprotected hydroxylamine

Hydroxamic acid	Cycle[a]	Yield[b] (%)	Purity[c] (%)	[MH$^+$] Calcd/Found
Benzohydroxamic acid	1	84[d]	84[g]	138.14/138.13
Benzohydroxamic acid	2	91[d]	87[g]	138.14/138.13
Benzohydroxamic acid	3	87[e]	83[h]	138.14/138.22
Benzohydroxamic acid	4	78[f]	85[h]	138.14/138.22
2,4-Dimethoxybenzohydroxamic acid	1	84[e]	96[h]	198.19/198.33
Phenylacetohydroxamic acid	1	55[d]	91[g]	152.16/152.23
Phenylacetohydroxamic acid	2	34[d]	90[g]	152.16/152.23
2-Naphthohydroxamic acid	1	76[e]	96[h]	188.18/188.23
N-Fmoc-L-Phe-NHOH	1	79[e]	98[h]	403.45/403.11

[a] Number of use of the same batch of PS-HOBt. [b] Calculated from the weight of hydroxamic acid isolated and based on the amount of hydroxylamine used relative to the loading of the PS-HOBt resin (see footnotes d, e and f). [c] Determined by reversed-phase HPLC analysis. [d] 0.5 Equiv. hydroxylamine. [e] 0.6 Equiv. hydroxylamine. [f] 0.8 Equiv. hydroxylamine. [g] Detection at 235 and 280 nm (see Table 1 footnote c). [h] Detection at 220 and 235 nm (see Table 1 footnote c).

washing with common solvents, is completely automated and complete in 4 hours. On the other hand, for amino acid derivatives, the same batch of PS-HOBt resin can be used to protect the amino group,[16] and introduce, after resin recycling, the hydroxamic acid functionality.

Conclusion

This method describes a convenient synthesis of hydroxamic acids combining the advantages inherent to both solid phase synthesis and polymer assisted solution phase synthesis.[17] Designed for the synthesis of low molecular weight hydroxamic acids, it is also suitable for the preparation of hydroxamates of higher molecular weight, by multi-step synthesis when the sequence of reactions does not involve an *N*-nucleophile, or by generating the hydroxamic acid functionality in the last step,[18] by taking advantage of the mild reaction conditions.

Experimental

Materials and methods

The 1-hydroxybenzotriazole-6-sulfonamidomethyl polystyrene resin (PS-HOBt, High Loading) was purchased from Argonaut Technologies. All other reagents and solvents were purchased from Aldrich and used without further purification.

The experiments were carried out on a Quest 210 ASW Argonaut Technologies Organic Synthesiser. All reactions were performed under an atmosphere of nitrogen. All percentage yields in Tables 1 and 2 were estimated from the isolated weight of each hydroxamic acid and are based on the amount of *O*-*tert*-butyldimethylsilylhydroxylamine or hydroxylamine used. Chromatographic analysis was performed on a PerSeptive Biosystems BioCAD SPRINT Perfusion Chromatography Workstation using POROS 20R2 Reversed Phase Perfusion Chromatography packing (column: 4.6 mm d/100 mm L, self-packed, A mobile phase: 0.1% TFA in water; B mobile phase: 0.1% TFA in acetonitrile – gradient: 2 to 60% B in 18 column volumes at 7 ml min^{-1} flow rate). Purities were determined from the area percent of the hydroxamic acid relative to the total area of all UV absorbing components. While this is routine practice in combinatorial chemistry, the reported purities are approximate since the relative responses of the various components are neglected (although the carboxylic acids, the main impurities in the present work, are expected to have similar responses to the corresponding hydroxamic acids). Mass spectrometry was performed on a PE Sciex API 3000 LC/MS/MS in electrospray mode.

Synthesis of hydroxamic acids

In a typical experiment, the PS-HOBt (150 mg, 1.41 mmol g^{-1} loading, 0.2 mmol) was pre-swollen with DMF. A solution of DMAP in 1 : 1 DMF–DCM (2.42 ml, 0.05 M) and a solution of carboxylic acid in DMF (853 µl, 0.38 M) were then added to the resin and the mixture was agitated for 2 min. A solution of DIC in DCM (565 µl of a 1.65 M) was then added to the resin and the resulting mixture was agitated for 3 h. The resin was collected by filtration, washed successively with DMF, DCM, DMF and THF (3 × 5 ml aliquots of each) and dried under nitrogen.

To the resin-bound esters was added a solution of *O*-*tert*-butyldimethylsilylhydroxylamine in THF (3 ml, 0.5–0.8 equivalents relative to the loading of the PS-HOBt resin) or a solution of anhydrous hydroxylamine (3 ml, prepared from hydroxylamine hydrochloride and sodium methoxide[9c]) in 2.3 : 1 THF–MeOH, and the mixture was agitated for 5 h. The solution was filtered, the resin washed with THF (3 ml) and the combined organic solutions evaporated under reduced pressure.

The cleavage of the *tert*-butyldimethylsilyl protecting group was carried out by dissolution in 95 : 5 TFA–H_2O (3 ml) and stirring for 15 h. The solution was then concentrated with nitrogen and the resulting solid was dried *in vacuo*.

Recycling of the PS-HOBt

The recycling of the solid phase to synthesise the same hydroxamic acid was achieved by washing the spent resin with THF (3 × 5 ml), DCM (3 × 5 ml), DMF (3 × 5 ml), DCM (3 × 5 ml), DMF (3 × 5 ml) and THF (3 × 5 ml).

Recycling of the solid phase to produce a different hydroxamic acid was carried out as follow: the resin was washed with DMF, then reacted with isopropylamine (5 equivalents relative to the loading of the PS-HOBt resin) in DCM, and washed with DCM (3 × 5 ml), DMF (3 × 5 ml), DCM (3 × 5 ml), NMP (3 × 5 ml), DCM (3 × 5 ml), DMF (3 × 5 ml), THF (3 × 5 ml) and dried under nitrogen.

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