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New methodology for the preparation of 3-hydroxy-2-pyridinone (3,2-HOPO) chelators - Reaction of amines with a novel electrophilic 3,2-HOPO precursor

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Abstract

The preparation of the new electrophilic iminium ester mesylate salt **5** and its reaction with primary and secondary amines have been investigated. Aniline, *t*-butylamine, and secondary amines react with **5** via ring opening to give the corresponding HOPO derivatives in high yields. The usefulness of this methodology has been demonstrated by the preparation of two new di-HOPO derivatives **19** and **21**. This method allows the introduction of the HOPO ligand onto a variety of amine platforms without the concomitant formation of an amide bond and provides access to HOPO chelators of increased water solubility.

Keywords

Hydroxypyridinones; synthesis; metal ion chelators; MRI contrast agents; iron overload diseases

Chelators carrying the 3-hydroxy-2-pyridinone (3,2-HOPO) ligand, as well as its positional isomers (1,2-HOPO and 3,4-HOPO) have been of interest due to their strong Fe(III) binding properties and potential applications in the treatment of patients suffering from iron overload diseases (β -thalassemia also known as Cooley's Anemia).¹ Polyhydroxypyridinone derivatives also form strong and stable complexes with Gd(III) that make them potentially useful as relaxation agents in magnetic resonance imaging (MRI) applications.² Several hydroxypyridinones, including di, tri and tetra HOPO derivatives have also been examined for their ability to achieve *in vivo* clearance of actinide ions.³ Such agents may be useful for the treatment of patients exposed to actinides such as Pu(IV). The incorporation of 3-hydroxy-2-pyridinone ligands into calix[4]arenes has been shown to yield useful Th(IV) extractants.⁴ Chelating resins with hydroxypyridinone ligands have been investigated for their ability to achieve the separation of actinides present in radioactive waste streams.⁵

For both therapeutic and imaging applications, synthetic methods leading to poly HOPO derivatives that have the requisite water solubility and ability to form strong complexes with the target cation are needed. The most common method for the preparation of 3,2- as well as 1,2-HOPO derivatives involves the coupling of an amine with an activated carboxylic acid linker on or attached to the pyridinone ring system.^{2a,6} One drawback of this approach is that the resultant HOPO derivatives usually have limited aqueous solubility due to the presence of the polyamide linkages. The direct alkylation of 2,3-dihydroxypyridine on a trimesylate has been reported to yield a tri 3,2-HOPO derivative in low yields.⁷ This reaction requires harsh conditions and the highly polar product was difficult to purify. In

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conjunction with our program to develop selective chelators⁸ for actinides and trivalent cations such as Fe(III) and Gd(III), we desired a new method that would allow the easy incorporation of the 3,2-HOPO ligand onto a variety of platforms. The initial goal was to develop an electrophilic reagent that would allow the ready incorporation of 3,2-HOPO groups onto amines without formation of an amide linkage in the coupling step, in turn resulting in chelators with improved water solubility. In this paper, we disclose a new convenient method for the preparation of a variety of 3,2-HOPO derivatives using such a strategy.

The cesium fluoride (10 mol%) assisted Michael reaction of 2,3-dihydroxypyridine (2,3-DHP) **1** with ethyl acrylate in refluxing acetonitrile gave the corresponding ester **2** in good yields (Scheme 1).⁹ Very little of the desired product **2** was formed when 2,3-DHP was refluxed with excess ethyl acrylate under neutral or acetic acid catalysis. Subsequent *O*-benzylation of **2** using standard conditions (K₂CO₃/acetonitrile/reflux) followed by reduction of the ester moiety (BH₃·THF, rt) gave alcohol **3** in 90% yield after chromatography. Treatment of the alcohol **3** with methanesulfonic anhydride in dichloromethane in the presence of triethylamine led directly to the formation of the desired cyclic salt **5** (~85–90%) along with some of the intermediate mesylate **4** (~10–15%) as determined by ¹H NMR spectral analysis. Complete conversion to the cyclic salt **5** could be achieved by stirring the crude product mixture from the mesylation in chloroform at room temperature. After trituration with hot ethyl acetate, the salt **5** was isolated as a pale white solid in 92% yield in high purity.¹⁰

The reaction of the mesylate salt **5** with primary and secondary amines was then investigated. At the outset, there was concern that the desired ring opening reaction of the salt (path A) with amines to yield HOPO derivatives may compete with attack at the iminium center (path B) yielding undesired amidines (Scheme 2). This concern proved quite real. When salt **5** was reacted with excess amylamine (neat) at room temperature (Condition a, Table 1, Entry 1), only one product was isolated in high purity from the reaction after an aqueous workup. Careful spectral analysis showed that the isolated product was the amidine, **7**, formed via path B and not the desired amino HOPO derivative, **6**, expected from path A. For structural verification, a sample of **6** was prepared via an alternate route.¹¹ The amidine **7**¹² could be readily distinguished from the amine derivative **6** by chemical shift differences in their ¹H NMR spectra. A number of other experimental conditions were tried to alter the mode of the ring opening of **5** with amylamine but with no success. For example, treatment of salt **5** with 3 equiv. of amylamine in acetonitrile also gave the amidine product in 93% yield (Condition b, Table 1).

The reaction of salt **5** was then examined with several primary amines to understand the effects of electronic and steric factors on the preferred mode of nucleophilic attack (path A or B), Table 1. The reaction of salt **5** with benzylamine (Conditions a or b) was similar to the reaction with amylamine and gave only the amidine **8** (Table 1, Entry 2). In general, the neat conditions (Condition a) gave amidines of higher purity and hence the products were easier to purify.

However, it was found that some primary amines were capable of nucleophilic attack on the salt **5** via path A to give the desired HOPO derivatives. The sterically hindered *t*-butylamine reacted with **5** to give the corresponding HOPO derivative, **9**, (Table 1, Entry 3) in high yield. The reaction of salt **5** with aniline also followed path A and gave high yields of HOPO derivative **10**, but required heating at 60°C (Table 1, Entry 4).

The reaction of salt **5** with secondary amines proceeded via path A to give the corresponding HOPO derivatives in high yields (Table 1, Entries 5–8). In fact ring opening of salt **5** was

quite efficient and could be effected using 1.2 equiv. of the desired amine in the presence of triethylamine in acetonitrile at 60°C.¹³ Even sterically hindered dibenzylamine (Table 1, Entry 7) and *N*-methylaniline (Table 1, Entry 8) were effective nucleophiles and gave the desired products in good yields.

To enlarge the synthetic usage of salt **5**, its reaction with azide anion (ammonia equivalent) has also been investigated. Ring opening of salt **5** with azide followed by reduction should lead to a HOPO derivative having a primary amino group suitable for further attachment to biomolecules. The reaction of **5** with 1.2 equiv. of sodium azide in acetonitrile in the presence of 2 equiv. of triethylamine gave the desired azido derivative **15** in 88% yield (eqn 1). Treatment of azide **15** with triphenylphosphine in THF at room temperature gave **16** in excellent yields after an aqueous workup. Amine **16** was deprotected using 1:1 conc. HBr/glacial acetic acid to give HOPO, **17**, as its hydrobromide.^{2b}

(1)

Finally, the utility of our new methodology has been demonstrated by the preparation of two new dihydroxypyridinone chelators **19** and **21**, which are HOPO analogs of the well known dihydroxamate siderophore rhodotorulic acid.¹⁴ Treatment of piperazine (1 equiv.) with the mesylate salt **5** (3 equiv.) in triethylamine (4 equiv.) in acetonitrile (55 °C) led to the formation of **18** in 93% yield after purification. Debenzylation with HBr/AcOH (1:1) gave the dihydroxypyridinone **19** in 93% yield as its hydrobromide salt (eqn 2).^{2b} Similarly, the diHOPO chelator **21** was prepared in good yields from *N,N'*-dimethylpropanediamine in two steps (eqn 3). As predicted, both chelators **19** and **21** exhibit good water solubility over a wide pH range.

(2)

(3)

In conclusion, the mesylate salt **5** is a convenient reagent for the incorporation of the 3,2-HOPO moiety onto a variety of amine platforms. This methodology leads to HOPO derivatives without concomitant formation of an amide linkage and hence leads to chelators with better aqueous solubility. It is clear that the electronic and steric nature of the primary amine determine the site of the reaction of **5** (path A vs path B) while secondary amines react predictably via path A leading to the desired HOPO derivatives. The commercial

availability of a number of aromatic polyamines and secondary polyamines should allow access to a structurally diverse group of HOPO chelators using this strategy. The preparation of two new dihydroxypyridinone analogs **19** and **21** clearly demonstrates the potential of this methodology.

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10. Methanesulfonic anhydride (2.5 g, 14.4 mmol) was added portionwise to a solution of HOPO alcohol **3** (2.5 g, 9.6 mmol) in dry CH₂Cl₂ (10 mL) and triethylamine (1.95 g, 19.3 mmol) at 0°C under N₂. The reaction was stirred at 0°C for 1 h and then at rt for 1 h. The CH₂Cl₂ was removed under reduced pressure. The residue was dissolved in CHCl₃ and stirred overnight at rt. The solvent was removed *in vacuo* and the residue was triturated with hot ethyl acetate (4 × 25 mL). The resulting solid was dried *in vacuo* to give **5** as a pale white solid (3.0 g, 92%): mp 108–110°C; IR (neat) 3436, 1639 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 2.52 (m, 2H), 2.69 (s, 3H), 4.87 (t, *J*=5.5 Hz, 2H), 4.98 (t, *J*=6.0 Hz, 2H), 5.21 (s, 2H), 7.28 (t, *J*=7.4 Hz, 1H), 7.37–7.44 (m, 5H), 7.62 (dd, *J*=8.3 and 1.2 Hz, 1H), 8.22 (dd, *J*=6.6 and 1.3 Hz, 1H); Anal. calc'd for C₁₆H₁₉NO₅S: C, 56.96; H, 5.68; N, 4.15; Found: C, 56.63; H, 5.56; N, 4.19.

11. An authentic sample of **6** was prepared by conversion of the alcohol **3** to the corresponding chloride (PPh₃, CCl₄) followed by its reaction with neat amylamine at rt. Spectral data for **6**: ¹H NMR (200 MHz, CDCl₃) δ 0.89 (t, *J*=6.7 Hz, 3H), 1.22–1.39 (m, 4H), 1.42–1.58 (m, 2H), 1.90–2.04 (m, 2H), 2.39 (br s, 1H), 2.61 (unres q, 4H), 4.07 (t, *J*=6.8 Hz, 2H), 5.11 (s, 2H), 6.02 (t, *J*=7.2 Hz, 1H), 6.64 (dd, *J*=7.4 and 1.6 Hz, 1H), 6.95 (dd, *J*=6.9 and 1.7 Hz, 1H), 7.29–7.46 (m, 5H).
12. Spectral data for **7**: ¹H NMR (200 MHz, CDCl₃) δ 0.82 (t, *J*=6.8 Hz, 3H), 1.10–1.30 (m, 4H), 1.44–1.62 (m, 2H), 1.78–1.90 (m, 2H), 3.47 (t, *J*=5.4 Hz, 2H), 3.78 (t, *J*=7.6 Hz), 4.06 (t, *J*=5.9 Hz, 2H), 4.88 (s, 2H), 5.65 (t, *J*=7.1 Hz, 1H), 6.25 (dd, *J*=7.0 and 1.2 Hz, 1H), 6.64 (dd, *J*=6.9 and 1.4 Hz, 1H), 7.34–7.41 (m, 5H).
13. A solution of salt **5** (0.206 g, 0.610 mmol), N-methylbenzylamine (0.089 g, 0.732 mmol) and triethylamine (0.123 g, 1.22 mmol) in acetonitrile (3 mL) was heated at 60 °C for 2 d under N₂. The reaction was then diluted with dichloromethane (50 mL) and washed with saturated NaHCO₃ (50 mL). The aqueous layer was extracted once more with dichloromethane (25 mL). The combined organic extracts were dried (Na₂SO₄), the solvent removed *in vacuo* and the excess N-methylbenzylamine removed via vacuum distillation to give **12** as a colorless oil (0.206 g, 93%).: IR (neat) 1606, 1652 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 1.91–2.05 (m, 2H), 2.20 (s, 3H), 2.41 (t, *J*=6.6 Hz, 2H), 3.48 (s, 2H), 4.04 (t, *J*=6.9 Hz, 2H), 5.11 (s, 2H), 5.94 (t, *J*=7.1 Hz, 1H), 6.62 (dd, *J*=7.4 and 1.6 Hz, 1H), 6.85 (dd, *J*=6.9 and 1.7 Hz, 1H), 7.24–7.46 (m, 10H); Analysis calc'd for C₂₃H₂₆N₂O₂: C, 76.20; H, 7.15; N, 7.73; Found: C, 75.82; H, 7.23; N, 7.59.
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Scheme 1.

Scheme 2.

Table 1

Reaction of amines with mesylate salt **5**

Entry	Amine	Conditions	Product	Yield %
1		<i>a</i>		97 ^d
		<i>b</i>		93
2		<i>a</i>		91 ^d
		<i>b</i>		89
3		<i>a</i>		89 ^e
		<i>b</i>		84
4		<i>a</i> (60 °C)		100 ^f
		<i>b</i> (60 °C)		80
5		<i>c</i>		79 ^g
6		<i>c</i>		93 ^d
7		<i>c</i>		66 ^f
8		<i>c</i>		88 ^e

^a 20–25 equiv. amine (neat), rt^b 3.0 equiv. amine, CH₃CN, rt^c 1.2 equiv. amine, 2 equiv. Et₃N, CH₃CN, 60 °C

^d purified by Kugelrohr distillation

^e purified by chromatography on basic alumina

^f purified by silica gel chromatography

^g isolated as HCl salt