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Traceless Solid-Phase α-Hydroxytropolone Synthesis

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α-Hydroxytropolones are established inhibitors of several therapeutically relevant binuclear metalloenzymes, and thus lead drug targets for various human diseases. We have leveraged a recently-disclosed three-component oxidopyrylium cycloaddition in the first solid-phase synthesis of α-hydroxytropolones. We also showed that, while minor impurities exist after cleavage and aqueous wash, the semi-crude products display activity in HIV RT-associated RNaseH enzymatic and cell-based assays consistent with pure molecules made in solution phase. These proof-of-principle studies demonstrate the feasibility of solid-phase α-hydroxytropolone synthesis and its potential to serve as a powerful platform for α-hydroxytropolone-based drug discovery and development.

α-Hydroxytropolones are a promising class of compounds that have been implicated as therapeutic leads for several human diseases.[1] The activity is often attributed to the three contiguous oxygen atoms, which each have high negative charge character at physiological pH and are well positioned to bind to and inhibit bridged dinuclear metalloenzymes (Figure 1A).[2] Our lab has been studying an oxidopyrylium cycloaddition/ring-opening strategy that has enabled the synthesis of a variety of structurally diverse α-hydroxytropolones (Figure 1B).[3,4] Medicinal chemistry studies associated with antibiotic resistance,[5] hepatitis B,[6] herpes simplex virus,[7] and HIV are on-going in our lab that use this strategy. The following describes the adaptation of this method to a solid-support format.

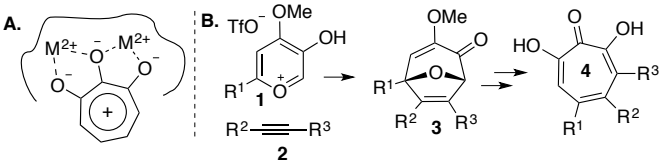
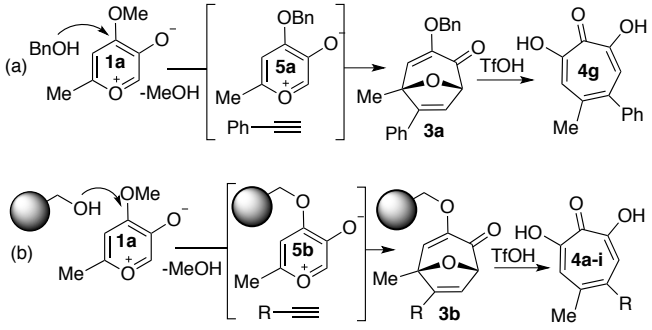


Figure 1. (A) α-Hydroxytropolone drawn in the tropylium resonance and dianionic form, illustrating its favourable metal group-binding features and (B) Overview of oxidopyrylium cycloaddition/ring-opening route to α-hydroxytropolones.

The key advance that has allowed for this modification is a 3-component oxidopyrylium cycloaddition recently described by our lab that facilitates alcohol incorporation into oxabicyclic products (**1a→3a**, Scheme 1a).[8,9] Furthermore, benzyl alcohol-derived oxabicyclic products made through this strategy can be directly converted into α-hydroxytropolones using triflic acid (**3a → 4g**, Scheme 1a). Given the prevalence of polystyrene-derived solid-supports in chemical synthesis, we became intrigued by the possibility adapting this approach to solid-support using benzyl alcohol on polystyrene (Scheme 2b). A substantial advantage to benzyl alcohol polystyrene as support over widely used derivatives such as Wang resin is that after cleavage, no additional components would exist in solution, and the process could thus lead to assay-ready compounds without the need for chromatography.

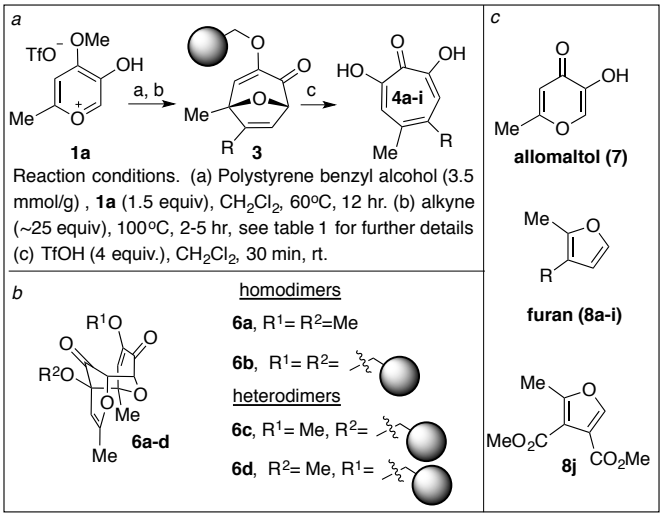
Scheme 1. (a) Previously described 3-component oxidopyrylium cycloaddition and application to α-hydroxytropolone synthesis, and (b) an overview of the solid-phase platform described in the current manuscript.



A procedure that we deemed practical for parallel synthesis is described in Figure 2a, and yields are shown in Table 1. First, in a sealed vessel, a solution of **1a** and base was stirred overnight at 60°C in the presence of benzyl alcohol polystyrene beads. We hypothesize that this generates an oxidopyrylium heterodimer (**6c** and/or **6d**, Figure 2b) based upon known rapid dimerization of oxidopyrylium ylides, although additional

cross-linking homodimer **6b** cannot be ruled out currently.[3a] Following the overnight stirring, alkynes were added, and the reactions were heated to 100°C to facilitate the *on-bead* cycloaddition (see Table 1 for results). In instances in which the alkynes were liquids, it was advantageous to remove the solvent and its solutes prior to addition of alkyne. Solid alkyne was directly added to the reaction without removing the solvent and solutes. Optimal cycloaddition reaction times were dependent upon reactivity of the alkyne, and insufficient reaction times led to increased amounts of allomaltol (**7**). Reactions with electronically poor ynones and propiolates were generally complete within 2 hours, whereas aromatic alkynes took ~5 hours to maximize yields. Aside from allomaltol and trace baseline impurities, the only other significant byproducts occasionally observed were furan impurities (**8a-i**), which, as expected, were the major product when dimethylacetylene dicarboxylate was used (**8j**, entry 20).[3b]

Figure 2. (a) Overview of operation through which α-hydroxytropolones were made through the use of solid-phase intermediate. (b) Relevant oxidopyrylium heterodimers and homodimers. (c) Common impurities observed along with products made on solid-phase



Between 1-5 mg of various α-hydroxytropolones were made from 33 mg of the benzyl alcohol polystyrene and 50 mg of the oxidopyrylium salt, or approximately 5-10% yield based upon the polystyrene resin (Table 1). The greatest mass used was the alkyne which, although 400 mg - 500 mg was needed, is readily available in large quantities. Most importantly, though, the process is highly time efficient by circumventing the need for chromatography. As many as 8 reactions have been run in parallel in a single 24 hour period during the course of our studies, and enough material was generated to confirm product formation, assess purity level by ^{1}H NMR, and carry out several bioassays. Thus, although improvements are desirable, particularly related to yields, this procedure or variants of it has the potential to serve an important role in rapid library synthesis for screening purposes.

Table 1. Select Results from Solid-Phase Synthesis

Entry	Alkyne	Time[b]	Yield (**4:7:8**)
1	R = COMe	1.5 hr	8% **4a** (9:1:0)
2	R = COMe	2 hr	7% **4a**
3[c]	R = COMe	2 hr	4% **4a** (9:1:0)
4[a]	R = COCy	2 hr	11% **4b** (15:1:4)
5[a]	R = COPh	2 hr	22% **4c** (1:0:1)
6[a,c]	R = COPh	2 hr	6% **4c** (5:0:1)
7[a]	R = CO(4-Ph)Ph	3 hr	9% **4d** (1:0:1)
8	R = CO_2Et	1.5 hr	7% **4e**
9	R = CO_2Et	1.5 hr	9% **4e**
10[c]	R = CO_2Et	1.5 hr	4% **4e**
11	R = CO_2Me	1.5 hr	6% **4f**
12[c]	R = CO_2Me	1.5 hr	6% **4f**
13	R = Ph	4.5 hr	7% **4g** (9:1:0)
14	R = Ph	5.5 hr	13% **4g** (20:1:0)
15[c]	R = Ph	5.5 hr	6% **4g** (5:1:0)
16	R= 4-CF_3Ph	4.5 hr	11% **4h** (4:1:0)
17	R = 4-CF_3Ph	5.5 hr	6% **4h** (4:1:0)
18	R = 1-Npth	4.5 hr	7% **4i** (5:1:0)
19	R = 1-Npth	5.5 hr	6% **4i** (15:1:0)
20	DMAD[d]	1 hr	10% **8j**

[a] Dichloromethane and solutes not removed prior to addition of alkyne. [b] Time for step 2. [c] Reaction run at twice the scale of prior run. [d] DMAD = Dimethyl acetylenedicarboxylate.

One of the most promising targets for α-hydroxytropolone therapeutics is the HIV RT-associated RNaseH, against which the natural products β-thujaplicinol and manicol have demonstrated IC_{50} values of 200 nM and 600 nM respectively.[10] RNaseH remains a promising enzymatic target for HIV that is untargeted clinically, and major efforts are underway to identify clinically viable inhibitors of this enzymatic function.[11] The solid-phase platform represents a powerful tool to increase the throughput of new α-hydroxytropolone-based leads for HIV RT RNaseH therapeutic development, and thus we sought to evaluate our solid-phase library experimentally in HIV RT RNaseH-associated assays in order to test the assay-ready nature of the compounds (Table 2).

We first measured inhibitory activity using a FRET endpoint to monitor RNaseH activity of HIV-1 RT.[12] Consistent inhibitory activity was observed for the majority of the molecules, with typical IC_{50} values between 200 nM and 1 μM. IC_{50} values of the same compounds made through solid-phase versus solution phase were all within 2-3 fold difference, and are mostly within standard deviation. We next tested the molecules' ability to stabilize the enzyme against thermal denaturation using differential scanning fluorimetry (Thermofluor).[13] All molecules typically increased the melting point of the enzyme by 2-3°C, regardless of associated impurities, and those with lower values (**4d, 4h, 4i**) did so regardless of whether prepared in solid or solution phase. Finally, we assessed the molecules' protective effects of the synthetic α-hydroxytropolones in HIV-associated cellular assays.[14] Only three of the molecules showed protective effects, and this trend was consistent across solution and solid-phase

synthesized compounds. Collectively, these assays show that minor impurities seen in the samples made via the solid-phase platform do not detrimentally impact the enzymatic or cell-based assays, thus demonstrating the assay-ready nature of the molecules and highlighting the advantages in future screening approaches.

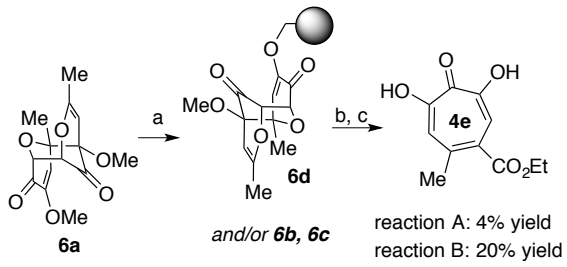
Table 2. Synthetic α-Hydroxytropolones in HIV RT RNaseH-associated assays.

Compound		HIV RT RNaseH Assays Inhibition IC_{50} (μM)	HIV RT RNaseH Assays ThermoFluor ΔT_m (°C)	Cell-Based Assays Antiviral EC_{50} (μM)	Cell-Based Assays Cytotoxicity CC_{50} (μM)
β-thujaplicinol		0.20 (± 0.03)	2.34	n.p.	2.3
manicol		0.6	n.a.	n.p.	13.6
4a	(Solid)	0.57 (± 0.07)	2.3 (± 0.8)	<50%, 2.8, 5.2	7.7, 8.2, 24
	(Solution)	0.29 (± 0.11)	2.9 (± 0.2)	6.5, 7.3	20, 21
4b	(Solid)	0.52 (± 0.22)	2.0 (± 0.4)	n.p., n.p.	2.3, 3.1
	(Solution)	0.21 (± 0.14)	2.4 (± 0.1)	n.p., n.p.	2.7, 7
4c	(Solid)	0.32 (± 0.12)	2.5 (± 0.1)	n.p., n.p., n.p.	2.6, 6.6, 8.8
	(Solution)	0.22 (± 0.07)	2.9 (± 0.1)	<50%., n.p.	7.2, 8.8
4d	(Solid)	1.0 (± 0.4)	1.7 (± 0.1)	n.p., n.p.	0.14, 0.97
	(Solution)	0.59 (± 0.20)	1.9 (± 0.7)	n.p., n.p.	0.1, 0.83
4e	(Solid)	0.54 (± 0.13)	2.9 (± 0.3)	<50%, <50%	8.5, 9.7
	(Solution)	0.25 (± 0.07)	2.4 (± 0.1)	4.8, 5.1	22, 22
4f	(Solid)	0.44 (± 0.24)	2.9 (± 0.4)	2.2, <50%, 4.2	5.4, 6.2, 11
	(Solution)	0.16 (± 0.06)	2.7 (± 0.4)	7.7, 7.2	15, 15
4g	(Solid)	0.25 (± 0.08)	2.2 (± 0.3)	n.p., n.p., n.p.	6.4, 7, 5.6
	(Solution)	0.39 (± 0.04)	1.5 (± 0.2)	n.p., n.p.	2.5, 3.7
4h	(Solid)	0.80 (± 0.30)	0.2 (± 0.2)	n.p., n.p., n.p.	2.3, 7.1, 2.7
	(Solution)	1.1 (± 0.7)	0.9 (± 0.6)	n.p., n.p.	2.2, 6.7
4i	(Solid)	0.56 (± 0.19)	1.0 (± 0.8)	n.p., n.p., n.p.	2.1, 2.4, 5.4
	(Solution)	0.26 (± 0.06)	1.5 (± 1.0)	n.p., n.p.	3.3, 6.7

Natural product assay data, with exception of Thermofluor, reported previously (ref 10a). 'Solid' refers to molecules made on solid-phase without any purification other then an aqueous wash, and 'Solution' refers to higher purity molecules made through solution-phase synthesis. Enzymatic assays of synthetic α–hydroxytropolones shown as the average of 2 or 3 triplicate runs ± standard deviation. Cell-based assays shown as duplicate runs using α–hydroxytropolones made on solid-support, run alongside the pure molecules made in solution phase. '<50%' indicates observed protective effects that can't be assigned EC_{50} values because they don't exceed 50% cell viability. For objectivity, these are defined as those that display 35% - 50% cell viability at optimal concentrations. 'n.p.' indicates no protective effects (<35% cell viability throughout assay). 'na' indicates not available. For select examples, α–hydroxytropolones made on solid-support from a separate trial run were tested to assess batch variability, and are the third set of data where applicable

While these proof-of-principle synthetic and biological studies demonstrated the extremely time efficient nature of the process and assay-ready purity of the compounds made, improvements with the system are desirable. For example, while the yields of the process were deemed practical for screening purposes, they are still exceedingly low overall. This can be at least tied to some inefficiency with the 3-component oxidopyrylium cycloaddition, where even in solution phase the top yields achieved to date are in 60-70% range, owing largely to incomplete alcohol exchange. In the solid-phase work, this challenge seems to be is exacerbated due to the heterogeneous nature of the reaction. As an example, whereas previous solution-phase work witnessed considerable increases when purified oxidopyrylium dimer was used as the source of the ylide, no yield increases were observed in the solid-phase studies (Scheme 2, reaction A vs. Table 1, entry 8/9).

Scheme 2. Solid-phase synthesis employing purified oxidopyrylium dimer **6a**.



Reaction conditions. (a) **Reaction A.** Polystyrene benzyl alcohol, **6a** (0.75 equiv, 1.5 equiv based on monomer, 66% recovered), CH_2Cl_2, 60°C, 12 hr. **Reaciton B.** Polystyrene benzyl alcohol, **6a** (4 equiv, 8 equiv based on monomer, 87% recovered), CH_2Cl_2, 60°C, 12 hr. (b) Ethyl propiolate (~25 equivalents), neat, 100°C, 1.5 hr. (c) TfOH (4 equiv.), CH_2Cl_2, rt, 30 min.

On the other hand, the use of purified oxidopyrylium dimer during the first step did allow us to easily recover it by simply removing the supernatant and evaporating the solvent. In addition to helping re-obtain the unreacted starting material, this feature also provided a means to effectively monitor incorporation. In reaction A in scheme 1, for example, 16 of the 24 mg of the starting material dimer was re-isolated. Assuming that the remaining 8 mg of material exists on bead as the heterodimer, only about half of that material would then be available for subsequent solid-supported cycloaddition and cleavage. Driven by these results, we increased dimer concentrations to 4 equivalents (8 equivalents based upon monomer), and allowed the incorporation step of the reaction to stir over 9 days at room temperature (Scheme 2, reaction B). We were able to recover 87% of the dimer (104 mg of the 120 mg used), and increase our yields based upon bead loading to 20%. These provide some directions for future optimization work.

In summary, we have leveraged a 3-component oxidopyrylium cycloaddition in the first solid-phase synthesis of α-hydroxytropolones. Given the potential of α-hydroxytropolones as therapeutics for a variety of different diseases, this method will find wide usage in therapeutic development. Continued efforts are underway to improve upon this methodology so that it can meet its full potential.

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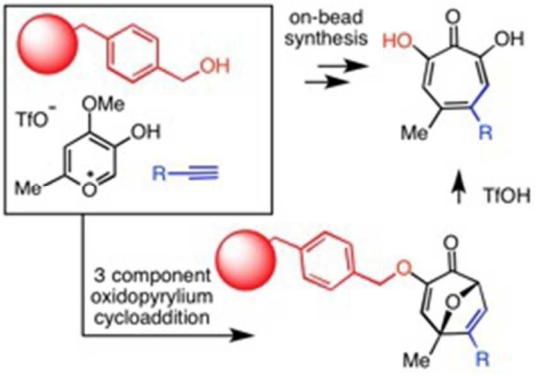

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