Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Tetrahedron Lett. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

Tetrahedron Lett. 2018 August 1; 59(31): 3026–3028. doi:10.1016/j.tetlet.2018.06.063.

Amidation Strategy for Final-Step α-Hydroxytropolone Diversification

Alex J. Berkowitz[a,b], **Rudolf G. Abdelmessih**[a], and **Ryan P. Murelli**[a,b,*]

[a]Department of Chemistry, Brooklyn College, The City University of New York, 2900 Bedford Avenue, Brooklyn, NY, 11210, United States

[b]Ph.D. Program in Chemistry, The Graduate Center, The City University of New York, 365 Fifth Avenue, New York, NY, 10016, United States

Abstract

α-Hydroxytropolones (αHTs) are excellent metalloenzyme-inhibiting fragments that have been the basis for the development of potent inhibitors of various therapeutically important enzymes. The following manuscript describes a final-step amidation approach for αHT diversification. The method takes advantage of a scalable, chromatography-free synthesis of a carboxylic acid-appended αHT, and in the present manuscript we describe the synthesis of eight amide-containing αHTs, three of which we envision using as chemical probes. We expect that the general strategy will find widespread usage in both chemical biology and medicinal chemistry studies on αHTs.

Keywords

Tropolones; Metal-Binding Fragment; Amidation; Oxidopyrylium Cycloaddition; Chemical Probe Synthesis

α-Hydroxytropolones (αHTs, Scheme 1A) are a class of heavily oxygenated aromatic compounds featuring a cycloheptatrienone ring and two free hydroxyls, with all three exocyclic oxygen atoms in a contiguous array. This unique arrangement of oxygen atoms has been shown to make αHTs potent inhibitors of a variety of therapeutically-relevant dinuclear metalloenzymes (Scheme 1A).[1] Our lab has developed an oxidopyrylium cycloaddition/ring-opening approach to the class of molecules (Scheme 1C)[2,3] that has proven to be useful in a broad range of structure function and optimization-driven medicinal chemistry studies.[4] One example of this activity is **6** (Scheme 1B), which has potent and selective cellular antiviral activity against hepatitis B,[5] and which recently demonstrated

*Corresponding author. rpmurelli@brooklyn.cuny.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

[a] HBTU and EtOAc used place of PyBOP and THF

[b] Run at room temperature for 4–7 days instead of under microwave irradiation

[c] Second step run at 95°C for 30 minutes under microwave irrdiation

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

modest but statistically significant antiviral activity in an FRG Human Liver Chimeric Mouse model. [6]

Despite the method's advantages, one of the major limitations is the harsh ring-opening (**3**→**4**) and demethylation (**4**→**5**) conditions, which prevents the incorporation of many valuable acid-labile substituents. While a recently described 3-component oxidopyrylium cycloaddition allowed for the incorporation of base-labile groups in place of the methyl ether,[7] the route suffered from lower yields and does not bypass the acid-mediated ringopening step of the sequence. We recently exploited this 3-component method in a fluorous-phase synthesis of αHTs, and through these studies found that amide-coupling could be performed on the free tropolone prior to cleavage of a fluorous tag.[8] However, cleavage of the tag also required similarly harsh conditions.

In the current manuscript, we demonstrate that a similar amidation can be performed on a scalable, fully non-protected αHT, thus providing a means to access various substituted amide-containing αHTs in the final step. We demonstrate the practicality of this method to access various tropamides with acid-sensitive groups, including molecular probes for future proteiomics studies.

Carboxylic acid αHT **5a** had been previously described,[9] and in the present manuscript we present a scale-up of the procedure that eliminates chromatography**.** Methyl ester oxabicycle **3a** was synthesized in large-scale quantities via a 1,3-dipolar cycloaddition with oxidopyrylium dimer **7** and an excess of methyl propiolate (**2b**). After removal of the solvent and propiolate *in vacuo*, **3a** can be immediately subjected to triflic acid-promoted ring-opening conditions. This process provided over 5 g of penultimate intermediate **4a** that could be demethylated/hydrolyzed using hydrobromic acid in acetic acid and water to generate the carboxylic acid-containing αHT, **5a**. This latter step has been accomplished successfully on a moderately large scale to generate 260 mg of the carboxylic acid **5a**, which at the current time is a convenient scale for our studies. Efforts to provide gram scale synthesis of this compound are currently underway.

Our amidation conditions were closely derived from those used previously in our fluorous-phase synthesis,[8] and was performed as follows: Acid **5a** was mixed with 2,6-lutidine and PyBOP[10] in THF under argon for 15 minutes at room temperature in order to activate the carboxylate for nucleophilic attack (**5a**→**8**, Scheme 3). The amine was then added to the reaction vessel, at which point the vessel was sealed and heated in a microwave reactor at 85°C for 10 minutes. Following column chromatography on C18-capped silica, the amide products were recovered in moderate to good yields. We believe these short reaction times, in addition to the scalability of the intermediates, should allow for rapid αHT library synthesis moving forward.

Amides derived from benzylamine (**9c**) and piperidine (**9a**) were synthesized in 60% and 78%, respectively, demonstrating the ability for primary and secondary amide synthesis. The coupling of 4-phenylbenzylamine was successfully completed using a slightly varied approach that overcame co-elution of PyBOP-based byproduct tris(pyrrolidinophosphine) oxide with non-polar substrates on reverse phase silica. HBTU couplings[11] feature a mildly

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

water-soluble byproduct, tetramethyl urea, which was easily separable via column chromatography. The solvent was also changed to EtOAc due to HBTU's improved solubility in it as compared to THF. Using this method we were able to obtain biphenyl amide αHT **9d** in 50% yield. A fluorescent substrate, **9h**, was also synthesized, which we envision could be useful, for example, to study αHT localization in cells,[12] or in the development of a fluorescence polarization assay for screening ligands against various dinuclear metalloenzymes.[13]

Another benefit to the route was its effectiveness at generating αHTs with acid-sensitive functional groups. For example, N-Boc piperazine-derived amide **9b** was synthesized in 53% yield. Notably, the acid-labile Boc group remained intact during the reaction and subsequent column chromatography. It was easily removed in a quantitative manner via microwave irradiation with trifluoroacetic acid in CH_2Cl_2 for 2 minutes at 70°C to yield the trifluoroacetate salt **10** (Scheme 4).

The method also provides access to two αHT probes of interest for proteiomics experiments that have acid-labile appendages that are vital to their efficacy as useful probes. Biotinylated probes such as **9f** require the use of a PEGylated spacer in order to allow ternary complex formation between avidan/streptavidid and the protein of interest.[14] The diazirine in probe **9g** is used for photochemical, covalent modification of enzyme targets in live cells, while the alkyne in **9g** is then used to label the enzyme with a reporter group through copper-catalyzed azide-alkyne cycloaddition.[15] Both of these compounds are unattainable via our previously described αHT synthetic routes due to their incompatibility with the harsh acidic environment of the demethylation.

Using the new route, we were able to obtain these molecules, albeit in <40% yields. A lower yield was also observed with aniline **9e**, which was obtained in only 38%. However, we found that allowing the reaction to run at room temperature over several days improved yields substantially in the cases of the aniline (38% to 72%) and the diazirine (35 to 61%). Less significant yield increases were found in the case of the biotinylated αHT **9f**, which we suspect may be due to solubility. Running reactions at room temperature is a practical method for parallel library synthesis, which could have strategic value moving forward.

In conclusion, we have disclosed conditions for the chromatography free, scalable synthesis of a carboxylic acid-containing αHT, **5a**, and have shown that amidation couplings can be carried out under either thermal or room temperature conditions. This method provides a reliable way to incorporate previously inaccessible acid-sensitive functional groups onto the αHT core, and has led to the synthesis of three αHT chemical probes, **9f-h**. The divergent approach is also a promising method for facile tropamide library synthesis, and current efforts are underway to leverage this in a variety of medicinal chemistry studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Acknowledgments

The authors are grateful for financial support from the National Institutes of Health (SC1GM111158).

References and Notes

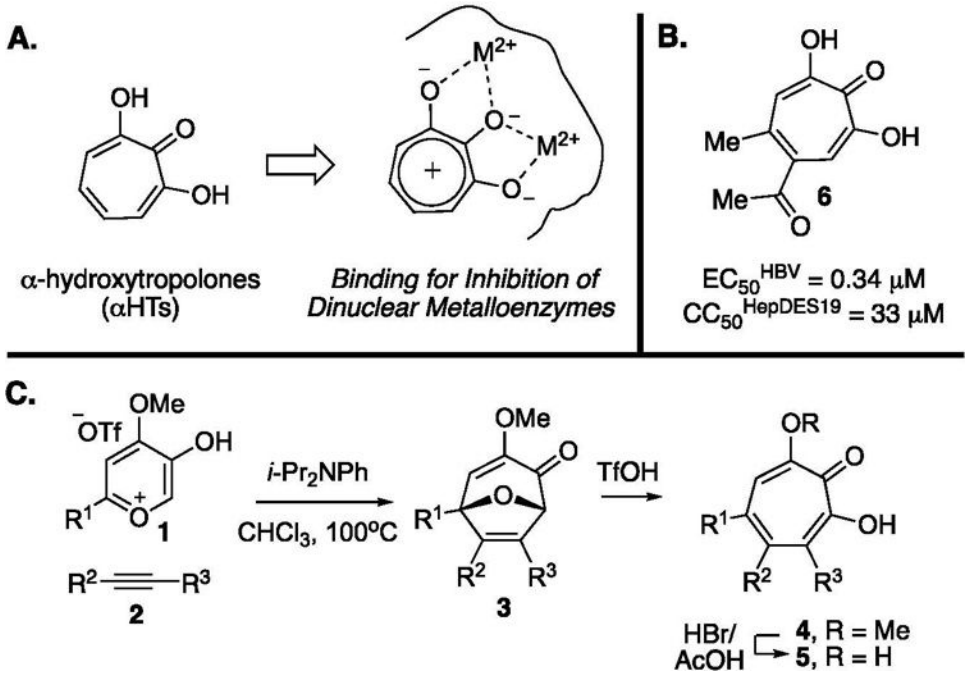
1. Meck C; D' Erasmo MP; Hirsch DR; Murelli RP Med. Chem. Commun 2014, 5, 842–852.
2. Meck C; Mohd N; Murelli RP Org. Lett, 2012, 14, 5988–5991. [PubMed: 23167954]
3. Williams YD; Meck C; Mohd N; Murelli RP J. Org. Chem, 2013, 78, 11707–11713 [PubMed: 24171600]
4. For lead examples, see: Hirsch DR; Cox GC; D'Erasmo MP; Shakya T; Meck C; Mohd N; Wright GD; Murelli RP Bioorg. Med. Chem. Lett 2014, 24, 4943–4947. [PubMed: 25283553] (b) Murelli RP; D'Erasmo MP; Hirsch DR; Meck C; Masaoka T; Wilson JA; Zhang B; Pal RK; Gallicchio E; Beutler JA; Le Grice SF J. Med. Chem. Commun 2016, 9, 1783–8.(c) Ireland PJ; Tavis JE; D'Erasmo MP; Hirsch DR; Murelli RP; Cadiz MM; Patel BS; Gupta AK; Edwards TC; Korom M; Moran EA; Morrison LA Antimicrob. Agents Chemother, 2016, 60, 2140–9. [PubMed: 26787704] For other examples of synthetic chemistry-driven medicinal chemistry studies on hydroxytropolones not using alternative approaches, see: (d) Chung S; Himmel DM; Jiang J-K; Wojtak K; Bauman JD; Rausch JW; Wilson JA; Beutler JA; Thomas CJ; Arnold E; Le Grice SFJ J. Med. Chem 2011, 54, 4462–4473. [PubMed: 21568335] (e) Didierjean J; Isel C; Querré F; Mouscadet J-F; Aubertin A-M; Valnot J-V; Piettre SR; Marquest R Antimicrob. Agents Chemother 2005, , 49, 4884–4894. [PubMed: 16304149] (f) Piettre SR; André C; Chanal MC; Ducep JB; Lesur B; Piriou F Raboisson P; Rondeau JM; Schelcher C;P Zimmermann P Ganzhorn AJ J. Med. Chem, 1997, 40, 4208–4221. [PubMed: 9435892] (g) Sennari G; Saito R; Hirose T; Iwatsuki M; Ishayama A; Hokari R; Otoguro K; Omura S; Sunazuka T Sci. Rep 2017, 7, 7259. [PubMed: 28775291]
5. Lomonosova E; Daw J; Garimallaprabhakaran AK; Agyemang NA; Ashani Y; Murelli RP; Tavis JE Antiviral Res 2017, 144, 164–172. [PubMed: 28633989]
6. Long KR; Lomonosova E; Li W; Ponzar NL; Villa JA; Touchette E; Rapp S; Liley RM; Murelli RP; Grigoryan A; Buller RM; Wilson L; Bial J; Sagartz JE; Tavis JE Antiviral Res 2018, 149, 41–47. [PubMed: 29129708]
7. D'Erasmo MP; Meck C; Lewis CA; Murelli RP J. Org. Chem 2016, 81, 3744–51. [PubMed: 27018974]
8. D'Erasmo MP; Murelli RP J. Org. Chem 2018, 83, 1478–1485. [PubMed: 29338231]
9. Donlin MJ; Zunica A; Lipnicky A; Garimallaprabhakaran AK; Berkowitz AJ; Grigoryan A; Meyers MJ; Tavis JE; Murelli RP Antimicrob. Agents Chemother, 2017, 61, e02574–16. [PubMed: 28167553]
10. Coste J; Le-Nguyen D; Castro B Tetrahedron Lett, 1990, 31, 205–208.
11. Dourtoglou V; Ziegler J-C; Gross B Tetrahedron Lett 1978, 19, 1269–1272.
12. (a) Alonso D; Vazquez-Villa H; Gamo AM; Martinez-Esperon MF; Tortosa M; Viso A; de la Pradilla RF; Junquera E; Aicart E; MartinFontecha M; Benhamu B; Lopez-Rodriquez ML; Ortega-Gutierrez S; ACS. Med. Chem. Lett 2010, 1, 249–53. [PubMed: 24900203] (b) Anees M; Benbow EW; J. Enzyme Inhib 1996, 10, 195–301. [PubMed: 8835945] (c) Wulff JE; Siegrist R; Myers AG J. Am. Chem. Soc 2007, 129, 14444–51. [PubMed: 17958425] (d) Niemann A; Baltes J; Elsasser H-P J. Histochem. Cytochem 2001, 49, 177–85. [PubMed: 11156686]
13. Lea WA; Simeonov A Expert Opin. Drug Discov 2011, 6, 17–32. [PubMed: 22328899]
14. (a) Diamandis EP; Christopoulos TK Clin. Chem, 1991, 37, 625–636 [PubMed: 2032315] (b) Gretch DR; Suter M; Stinski MF Anal. Biochem, 1987, 163, 270–277. [PubMed: 3039869]
15. (a) Li Z; Hao P; Li L; Tan CYJ; Cheng X; Chen GYJ; Sze SK; Shen H-M; Yao SQ Angew. Chem. Int. Ed, 2013, 52, 8551–8556(b) Dubinsky L; Krom BP; Meijler MM Bioorg. Med. Chem, 2012, 20, 554–570. [PubMed: 21778062]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Highlights

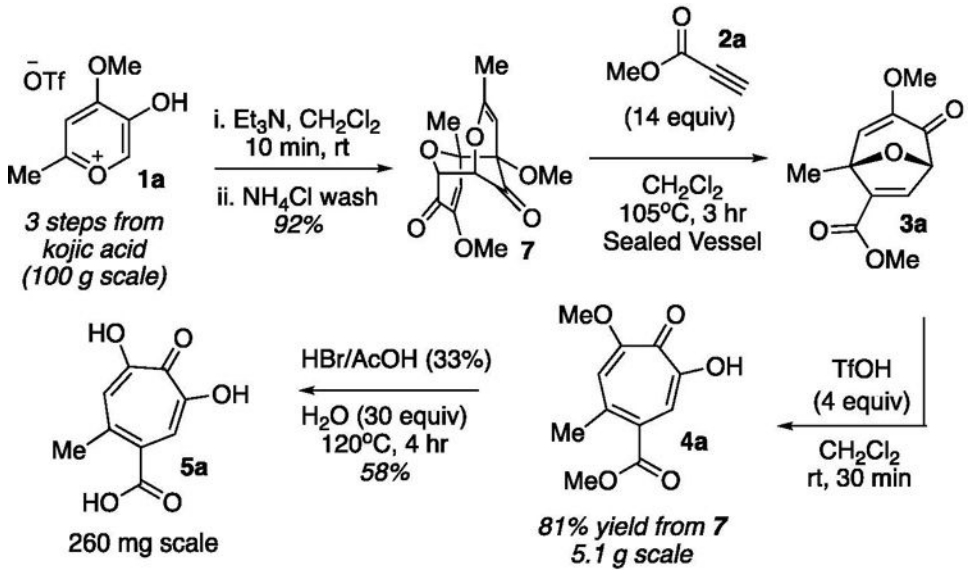
1. 7 step, single chromatography synthesis of amide-containing hydroxytropolones
2. Amidation is the final step of the synthesis, providing rapid diversification
3. Chromatography free, gram scale synthesis of penultimate intermediate
4. Successful synthesis of primary, secondary, and aniline-based tropamides.
5. Synthesis of new and previously inaccessible probes for proteomics.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



Scheme 1. α-Hydroxytropolone overview.

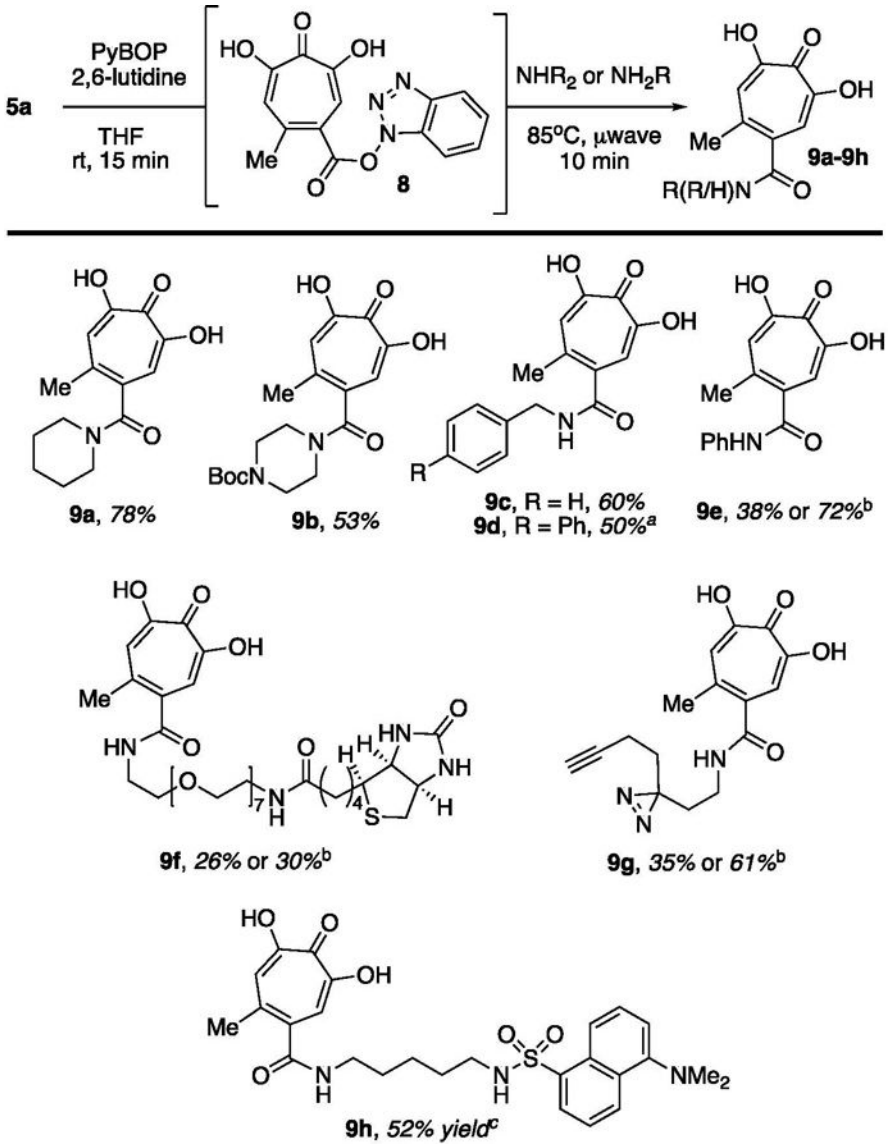
(A) α-Hydroxytropolone (αHT), redrawn in its dianionic tropylium form to illustrate dinuclear metalloenzymes inhibition. (B) A synthetic αHT and its anti-hepatitis B activity and cytotoxicity against the non-infected viral host cell. (C) Overview of oxidopyrylium cycloaddition/ring-opening route to αHTs.



Scheme 2.

Chromatography-free synthesis of **5a**

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

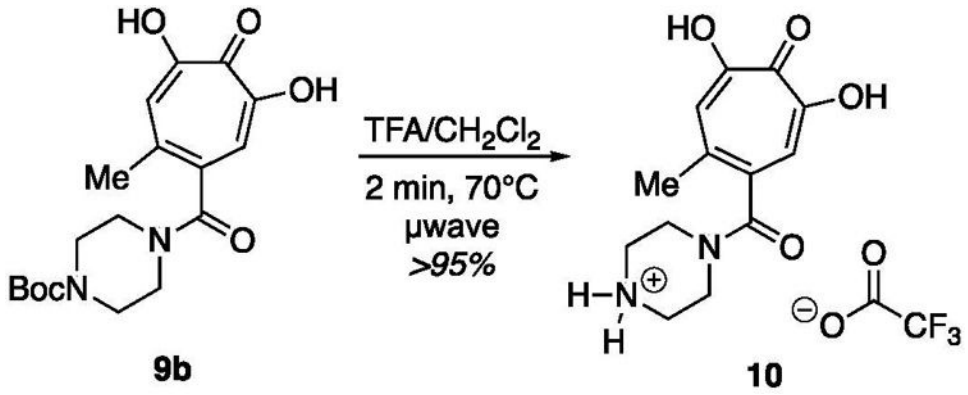


Scheme 3.

Amidation of tropolone carboxylate **5a**

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



Scheme 4.
Deprotection of αHT **9b**