Published in final edited form as:

Tetrahedron Lett. 2017 April 12; 58(15): 1441–1444. doi:10.1016/j.tetlet.2017.02.056.

Efficient route to pre-organized and linear polyaminopolycarboxylates: Cy-TTHA, Cy-DTPA and mono/di-reactive, *tert*-butyl protected TTHA/Cy-TTHA

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Abstract

Pre-organized polyaminopolycarboxylate chelators Cy-TTHA and Cy-DTPA were synthesized via modular five-step syntheses from commercially available starting materials in ~ 62% and 47% overall yields, respectively. Furthermore, strategies are reported for the efficient preparation of mono- and di-reactive, *tert*-butyl-protected TTHA/Cy-TTHA to selectively functionalize central chelators' carboxylic acids.

Graphical Abstract

Keywords

Chelators; Lanthanides; Ligand Design

Diethylenetriamine pentaacetic acid (DTPA) and triethylenetetraamine hexaacetic acid (TTHA) are among the most widely employed acyclic chelators for the coordination of metal ions (Fig. 1).^{1–3} Due to their ability to provide high denticities via a hard base binding core (carboxylate ions and tertiary amines), their corresponding lanthanide complexes have been heavily studied.^{4–20} While linear polyaminopolycarboxylates form thermodynamically stable complexes, their relative kinetic instability with respect to many metals such as Gd(III) can limit their utility in biological or clinical environments.^{21–25} In contrast, the widely used macrocyclic polyaminocarboxylates DOTA and TETA (Fig. 1) form exceptionally stable complexes with a variety of clinically important radiometals used in

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Supplementary Material

Synthetic procedures for compounds and NMR spectra.

diagnostic imaging and therapy.^{26,27} However, macrocycles often require elevated temperature (60–95 °C) and extended reaction times (30–90 minutes) for efficient metal chelation.^{26,27} These labeling conditions can limit macrocycles' applications in heat sensitive experiments, such as antibody labeling, or when utilizing short half-life isotopes (e.g. ⁴⁴Sc, ⁶²Cu, ⁶⁸Ga, and ^{212/213}Bi).^{26,27} In order to improve the kinetic inertness of DTPA, Brechbiel incorporated a pre-organized cyclohexyl moiety into the chelator backbone.^{3,28–30} The structurally reinforced Cy-DTPA effectively formed thermodynamically stable and kinetically inert complexes with ¹¹¹In, ⁹⁰Y and ¹⁷⁷Lu.^{31–44} Similarly, Gestin reported elevated *in vivo* kinetic inertness for a bioconjugate of Cy-TTHA(¹¹¹In) with mouse immunoglobulin G (IgG) over 96 h.⁴⁵ This group subsequently reported exceptional *in vitro* stability for dimethanephosphonic Cy-TTHA(¹⁵³Sm) when challenging it against high excess of optimal ¹⁵³Sm chelator EDTMP.^{46,17}

Recently, we prepared a series of luminescent Tb(III) and Eu(III) complexes based on Cy-TTHA (**6**) that exhibited exceptional quantum yields of emission in aqueous solution.²⁰ We also demonstrated that Cy-TTHA analogues were substantially more kinetically inert than their linear counterparts, as evidenced by an EDTA challenge assay. In order to prepare brightly emissive complexes, we needed an efficient synthetic approach that permitted selective mono-substitution of chromophoric sensitizer moieties at designated pendant positions. Previously reported syntheses of Cy-TTHA (**6**) and Cy-DTPA (**11**) suffer from low overall yields (.18% for Cy-DTPA and .29% for Cy-TTHA).^{28,45} Furthermore, with the installation of all pendant carboxylic groups in one step, there is no easy way to further electronically or coordinatively modify the chelator scaffolds.^{12,13} Frequently, selective conjugation of DTPA or TTHA terminal carboxylic acids to biomolecules has been performed by preparing a dianhydride analogue,^{47,48} followed by an amidation reaction with an amino group on the appropriate biomolecules. However, dianhydrides can cross-link to form undesired dimers.^{21,49} Alternatively, mono-anhydride analogues can be prepared by treatment of DTPA with isobutyl chloroformate/trimethylamine, but this approach gives a mixture of terminal/central activated-carboxylic acids as well as small amounts of linear dianhydride analogue.^{50,51} Various approaches have been developed both in solution^{52–58} and in solid phase⁵⁹ that exploit differential protection of carboxylic acids followed by orthogonal deprotection so as to conjugate mono-reactive pro-ligands under standard amide coupling conditions.

Spurred by our interest in developing luminescent lanthanide complexes for bioanalytical and imaging applications,^{10,11,60–63} we sought to develop more efficient strategies for preparing Cy-TTHA (**6**) and Cy-DTPA (**11**) derivatives. Herein, we report a modular, five-step syntheses of Cy-TTHA (**6**) and Cy-DTPA (**11**) with good overall yields. Moreover, our approach makes it possible to prepare novel mono- and di-reactive TTHA and Cy-TTHA analogues that may be selectively functionalized at their central pendant carboxylic acids.

Previously reported syntheses required purification of Cy-TTHA (**6**), Cy-DTPA (**11**) and their derivatives by reversed-phase HPLC and ion-exchange.^{3,28,45} In order to avoid these methods, we prepared *tert*-butyl-protected pro-ligands by leveraging the oft-employed,^{64,57} orthogonal benzyl and *tert*-butyl protecting groups. Cy-TTHA (**6**) was synthesized with a general reaction scheme that follows reductive amination of benzaldehyde, dialkylation of

the corresponding secondary amine with di-*tert*-butyl 2,2'-((2-bromoethyl)azanediyl)diacetate, catalytic debenzylation by Pd/C followed by a second alkylation with *tert*-butyl bromoacetate. The *tert*-butyl deprotection of the pro-ligand **5** afforded Cy-TTHA (**6**) in high overall yield of 62% (Scheme 1).

Taking advantage of an established synthetic method for Py3CA by Caravan⁶⁵ we prepared the key intermediate **9** with few modifications (Scheme 2). Subsequent alkylation with di-*tert*-butyl 2,2'-((2-bromoethyl)azanediyl)diacetate followed by *tert*-butyl deprotection afforded Cy-DTPA (**11**) in a good cumulative yield of 47%. Most notably, our strategy permits using modified alkylating groups in order to develop carbon-functionalized derivatives hence precluding concomitant reduction of the aryl nitro group in the original synthesis of CHX-A''-DTPA and its derivatives.³

Selective incorporation of a chromophore or conjugation handle at a single pendant arm position may be required when preparing, for example, emissive lanthanide complexes or bio-reactive chelators. Synthesis of monocarboxylate-functionalized analogues **19** and **20** began with mono-alkylation of secondary amines **2** and **12** by *tert*-butyl bromoacetate followed by a second alkylation with benzyl bromoacetate that provided differently alkylated compounds **15** and **16**, which were subsequently debenzylated with Pd/C to afford key intermediates **17** and **18**, respectively (Scheme 3). The target molecules **19** and **20** were readily obtained by a dialkylation reaction with di-*tert*-butyl 2,2'-((2-bromoethyl)azanediyl)diacetate in 43% and 46% overall yields respectively.

Di-reactive analogues were accessed by an initial dialkylation of precursors **2** and **12** with di-*tert*-butyl 2,2'-((2-bromoethyl)azanediyl)diacetate and subsequent quantitative hydrogenation by Pd/C yielded compounds **4** and **22**. These intermediate substrates were then readily dialkylated with benzyl bromoacetate followed by catalytic debenzylation to afford di-reactive, *tert*-butyl protected TTHA (**27**) and Cy-TTHA (**28**) in 54% and 45% overall yields (Scheme 4).

In summary, the five-step synthesis of Cy-TTHA (**6**) and Cy-DTPA (**11**) are reported. Both synthesis are straightforward and provide the desired products in an overall yield of 62% and 47%, respectively. A stepwise alkylation-based strategy allows control over the choice of chelator scaffolds as well as coordination groups. Using analogous conditions, we prepared mono- and di-reactive *tert*-butyl protected TTHA and Cy-TTHA so as to selectively functionalize central carboxylic arms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health (R01GM081030).

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Fig 1.
Structures of chelators discussed in this work

Scheme 1.**Synthesis of Cy-TTHA**

Reagents and conditions: (a) PhCHO (2.0 equiv), MeOH, reflux then NaBH₄ (4.3 equiv), 0 °C- rt (b) Na₂CO₃ (3.0 equiv), di-*tert*-butyl 2,2'-((2-bromoethyl)azanediyl)diacetate (2.1 equiv), MeCN, 60 °C. (c) Pd/C (10 wt %), H₂, MeOH, rt. (d) *tert*-butyl bromoacetate (2.0 equiv), Na₂CO₃ (1.0 equiv), MeCN, 60 °C. (e) TFA/CH₂Cl₂/ iPr₃SiH, 5:5:1.8, v/v, rt.

Scheme 2.

Synthesis of Cy-DTPA

Reagents and conditions: (a) benzyl bromide (0.1 equiv), MeCN, rt. (b) *tert*-butyl bromoacetate (3.1 equiv), Na₂CO₃ (3.1 equiv), MeCN, rt. (c) Pd/C (10 wt %), H₂, MeOH, rt. (d) di-*tert*-butyl 2,2'-((2-bromoethyl)azanediyl)diacetate (1.1 equiv), Na₂CO₃ (1.1 equiv), MeCN, 60 °C. (e) TFA/CH₂Cl₂/ iPr₃SiH, 5:5:1.8, v/v, rt.

Scheme 3.

Modular synthesis of mono reactive *tert*-butyl protected TTHA and Cy-TTHA

Reagents and conditions: (a) *tert*-butyl bromoacetate (0.25 equiv), Na_2CO_3 (1.0 equiv), MeCN, rt. (b) benzyl bromoacetate (1.0 equiv) Na_2CO_3 (1.0 equiv), MeCN, 60 °C. (c) Pd/C (10 wt %), H_2 , MeOH, rt. (d) di-*tert*-butyl 2,2'-((2-bromoethyl)azanediyl)diacetate (2.1 equiv), Na_2CO_3 (3.0 equiv), MeCN, 60 °C.

Scheme 4.

Modular synthesis of di reactive *tert*-butyl protected TTHA and Cy-TTHA

Reagents and conditions: (a) di-*tert*-butyl 2,2'-((2-bromoethyl)azanediyl)diacetate (2.1 equiv), Na₂CO₃ (3.0 equiv), MeCN, 60 °C. (b) Pd/C (10 wt %), H₂, MeOH, rt. (c) benzyl bromoacetate (2.1 equiv), Na₂CO₃ (3.0 equiv), MeCN, 60 °C.