Bioconjugation of actinides using a peptoid scaffold

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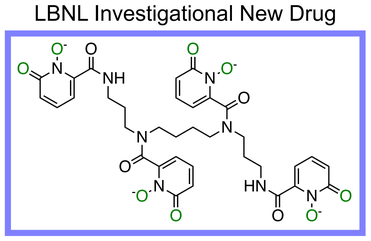
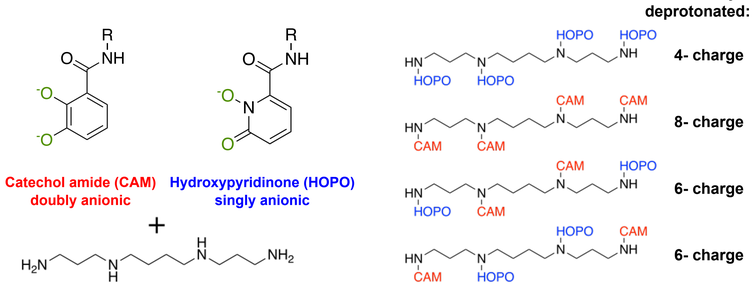
CURRENT LEAD COMPOUND This IND strongly chelates actinides and lanthanides, and is used for f-block metal decorporation. The synthesis permits limited variation, and no secondary functionality

HHHH Ligand, 3,4,3-LI(1,2-HOPO)
LBNL Investigational New Drug

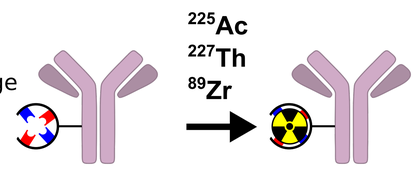




NEXT GENERATION CHELATOR Desirable to include bifunctionality, and optimization for specific metal ions

GOALS:

- Facile incorporation of orthogonal conjugation moieties
- Interchangable conjugation / click chemistries
- Unit-by-unit control of coordination functional group & ligand charge
- Maintain non-macrocyclic structure for rapid metal complexation
- Do not adversely impact complex stability with ligand redesign

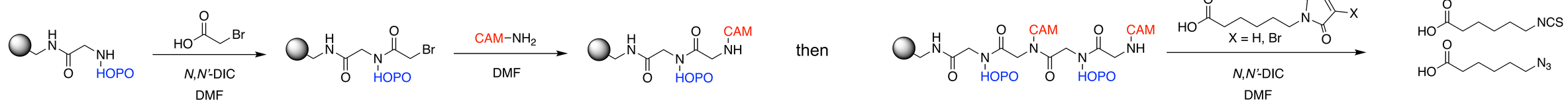


New design uses a biopolymer scaffold to construct the chelator via solid-phase synthesis

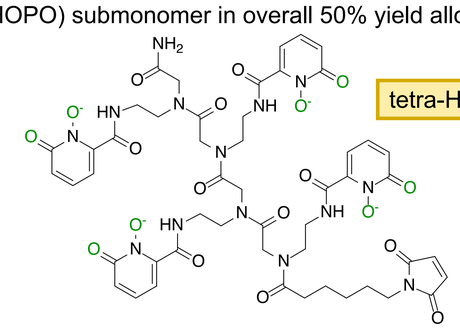
PEPTOID SYNTHESIS Solid-phase synthesis on Rink amide resin allows stepwise selection of HOPO or CAM submonomers. Four submonomer units and two chelating functional groups generate a library of 16 peptoids. The placement of HOPO and CAM units, as well as the overall charge of the peptoid, can be optimized for every radionuclide of interest.

- **Highly reproducible synthesis**
- **Readlily GMP compliant**
- **Immune to proteolysis**

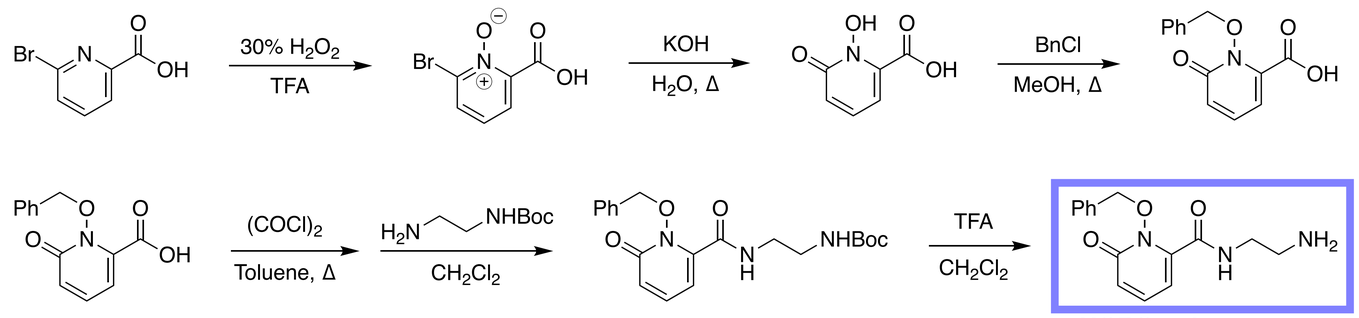
Functionalization of the terminal secondary amine with moieties suitable for conjugation to proteins or other targeting vehicles provides versatile, bifunctional chelators.



SUBMONOMER SYNTHESIS Synthesis of primary-amine-terminated 1-hydroxy-2-pyridinone (HOPO) submonomer in overall 50% yield allows facile incorporation into peptoid biopolymer.

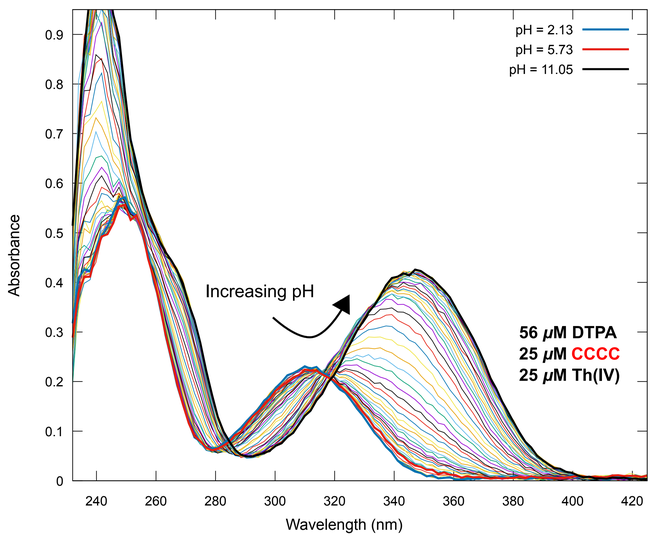


tetra-HOPO peptoid with thiol-reactive maleimide



HOPO submonomer

SOLUTION THERMODYNAMICS Stability constants for Ln^{n+} and An^{n+} complexes of the spermine-based chelators and the synthesized peptoids are determined by spectrophotometric titrations. Measurement of ligand pK_as and inclusion of metal hydrolysis products yields cumulative formation constants β_{mlh}



$$\beta_{mlh} = \frac{[M_m L_l H_h]}{[M]^m [L]^l [H]^h}$$

$$mM + lL + hH \rightleftharpoons [M_m L_l H_h]$$

[Ligand]$^{4-}$

Species	mlh	log β_{mlh}
HHHH Ligand		
$[EuL]^-$	110	20.2
$[EuLH]$	111	24.8
$[ZrL]$	110	43.1
$[ThL]$	110	40.1

[Ligand]$^{6-}$

Species	mlh	log β_{mlh}
CCHH Peptoid		
$[EuL]^{3-}$	110	33.71 ± 1.10
$[EuLH]^{2-}$	111	41.24 ± 0.82
$[EuLH_2]^-$	112	45.83 ± 0.76
CHHC Ligand		
$[EuL]^{3-}$	110	35.97 ± 0.06
$[EuLH]^{2-}$	111	45.69 ± 0.29
$[EuLH_2]^-$	112	51.50 ± 0.14
HCCH Ligand		
$[EuLH]^{2-}$	111	43.34 ± 0.49[b]

[Ligand]$^{8-}$

Species	mlh	log β_{mlh}
CCCC Peptoid		
$[EuL]^{5-}$	110	29.30 ± 0.30
$[EuLH]^{4-}$	111	38.94 ± 0.63
$[EuLH_2]^{3-}$	112	45.03 ± 0.62
$[ThL]^{4-}$	110	51.11 ± 0.63[a]
$[ThLH]^{3-}$	111	58.58 ± 0.49[a]
CCCC Ligand		
$[EuL]^{5-}$	110	29.65 ± 0.65
$[EuLH]^{4-}$	111	41.75 ± 0.06
$[EuLH_2]^{3-}$	112	46.79 ± 0.14
$[ZrL]^{4-}$	110	57.26 ± 0.20
$[ZrLH]^{3-}$	111	64.25 ± 0.32
$[ThL]^{4-}$	110	47.71 ± 0.08
$[ThLH]^{3-}$	111	55.36 ± 0.09

Data from three independent titrations.
[a]Data from two independent titrations
[b]Data from fluorimetric batch titrations

Speciation simulation performed using the HySS software showing the competition for Th^{4+} between the CCCC peptoid (Pep) and the CCCC ligand (CAM). At physiological pH, the peptoid is a better ligand.

SUMMARY The step-wise synthesis of the peptoid scaffold imparts flexibile design control. Peptoids can be optimized for therapeutic isotopes (e.g. ^{225}Ac, ^{153}Sm, ^{177}Lu), theranostic isotopes (e.g. ^{161}Tb), or theranostic "pairs" (e.g. ^{227}Th + ^{89}Zr) by sequence alteration

Peptoids are versatile chelators for many isotopes, with flexible incorporation of protein conjugation functionalities

Rees, Abergel et al. Patent Pending

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